

Synthesis and biological evaluation of boronic acid-containing phenstatin analogues

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Dedicated to Professor Keith Smith on the occasion of his 65th anniversary

Abstract

A series of boronic acid-containing benzophenones was synthesized by introducing a boronic acid as an acceptor-type functional group into the aromatic ring B of the phenstatin skeleton. Among the compounds synthesized, **4c** in which a hydroxyl group on the aromatic ring B of the phenstatin was replaced by a boronic acid, exhibited significant cell growth inhibition and the GI₅₀ values toward B-16 and 1-87 cell lines are 0.013 μ M and 0.087 μ M, respectively.

Keywords: Boronic acid, phenstatin, cell growth inhibition, benzophenone

Introduction

Combretastatin A-4 (**1a**) was isolated by Pettit and coworkers from the stem wood of the South African tree *Combretum caffrum* in 1982.¹ This compound is one of the most potent antimetabolic agents and exhibits potent cytotoxicity against a wide variety of human cancer cells including multidrug resistant cancer cell lines.² Phenstatin (**2a**) is a synthetic tubulin assembly inhibitor developed by the same research group in 1998.³ This compound is a benzophenone-type molecule and the functional groups on the two phenyl rings are similar to combretastatin A-4.⁴ Based on the structures of both lead compounds, various benzophenone derivatives have been developed including aminobenzophenones (**2b-c**)^{5,6} and isocombretastatin A (**3**).⁷ These analogues have a donor-type functional group, as a common feature, substituted on the aromatic ring B (Figure 1). We are interested in introducing boronic acid as an acceptor-type functional group into the aromatic ring B in the phenstatin skeleton.

A boron atom has a vacant orbital and interconverts with ease between the neutral sp² and the anionic sp³ hybridization states, which generates a new stable interaction between a boron atom

and a donor molecule through a covalent bond.⁸ 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 might produce a potent biological activity.⁹

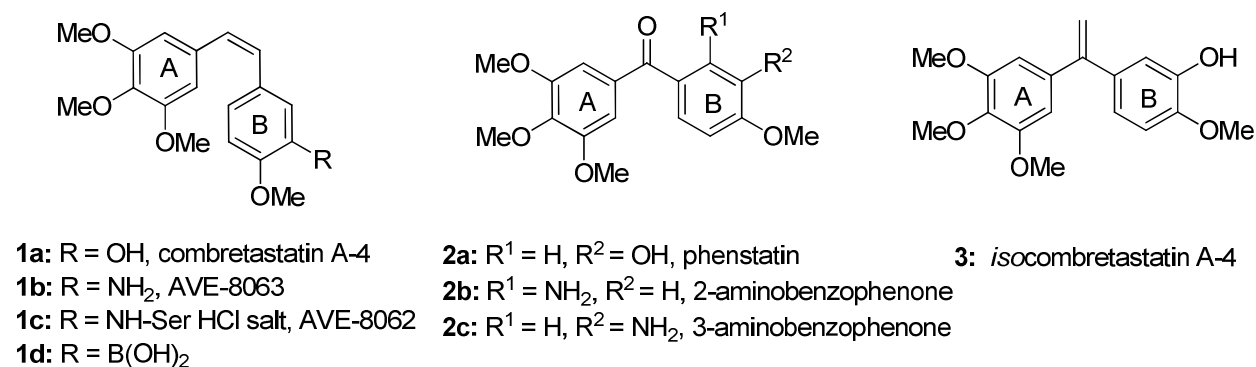


Figure 1. Structures of combretastatin and phenstatin derivatives **1-3**.

Our strategy for the design of boron compounds is based on the properties that make them different from conventional biologically active compounds.¹⁰⁻¹⁵ Previously, we reported the synthesis of boronic acid-containing combretastatin A-4 analogue **1d**.^{11,16} Herein, we report the synthesis and biological evaluation of boronic acid-containing phenstatin analogues (Figure 2).

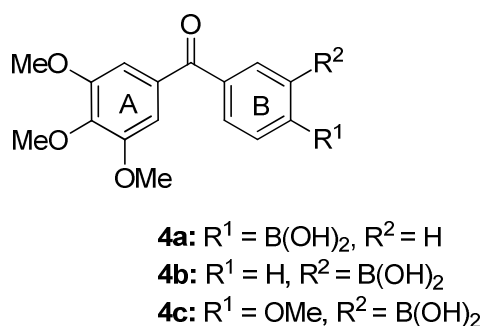
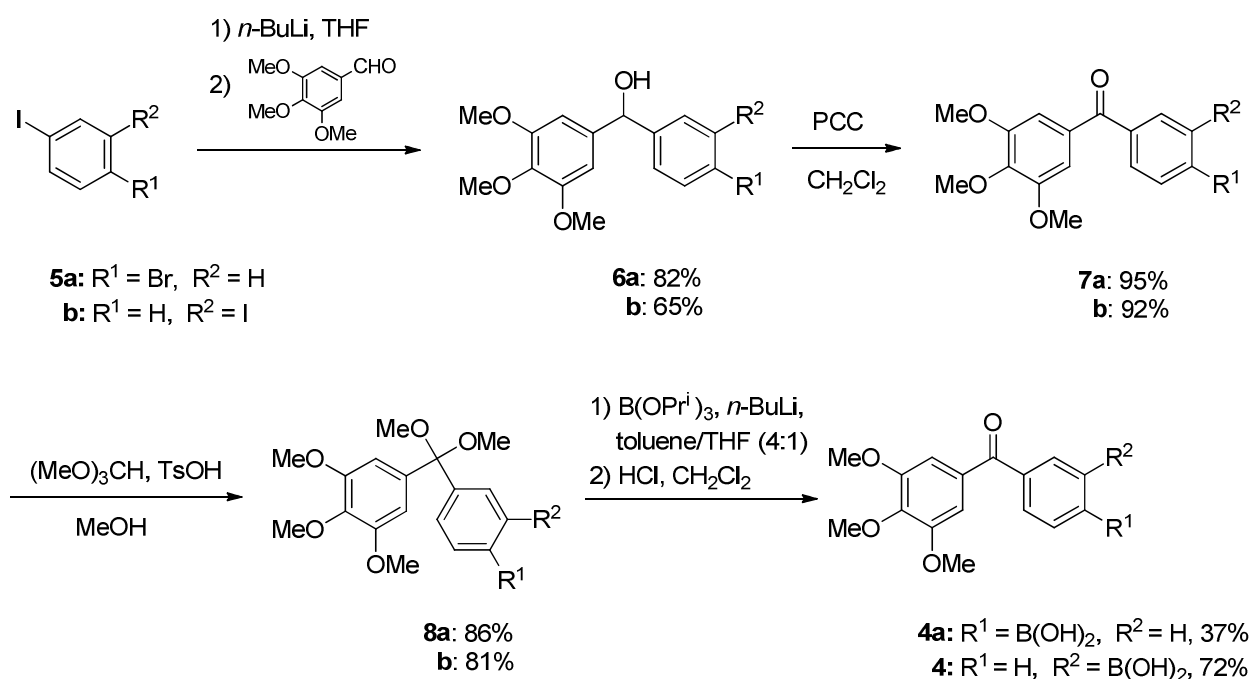


Figure 2. Design of boronic acid containing phenstatin analogues **4**.

Results and Discussion

The boronic acid-containing phenstatin analogues **4a** and **4b** were synthesized from 3,4,5-trimethoxybenzaldehyde and dihalobenzenes as shown in Scheme 1. The reaction of 1-bromo-4-iodobenzene **5a** with *n*-butyllithium in THF generated the 4-bromophenyllithium, which reacted with 3,4,5-trimethoxybenzaldehyde to give the corresponding carbinol **6a** in 82% yield.¹⁷ In a similar manner, carbinol **6b** was obtained from 1,3-diiodobenzene **5b** in 65% yield. The carbinols **6a** and **6b** were converted into the corresponding ketones **7a**¹⁷ and **7b** using pyridinium

chlorochromate (PCC) as an oxidizing agent and the ketal protection of the carbonyl groups was performed by treating with trimethyl orthoformate in methanol under acidic conditions to give **8a** and **8b** in 86% and 81% yields, respectively. Treatment of the ketals **8a** and **8b** with *n*-butyllithium and triisopropyl borate at $-78\text{ }^{\circ}\text{C}$, followed by acidic hydrolysis, afforded the boronic acid-containing phenstatin analogues **4a** and **4b** in 37% and 72% yields in two steps, respectively.

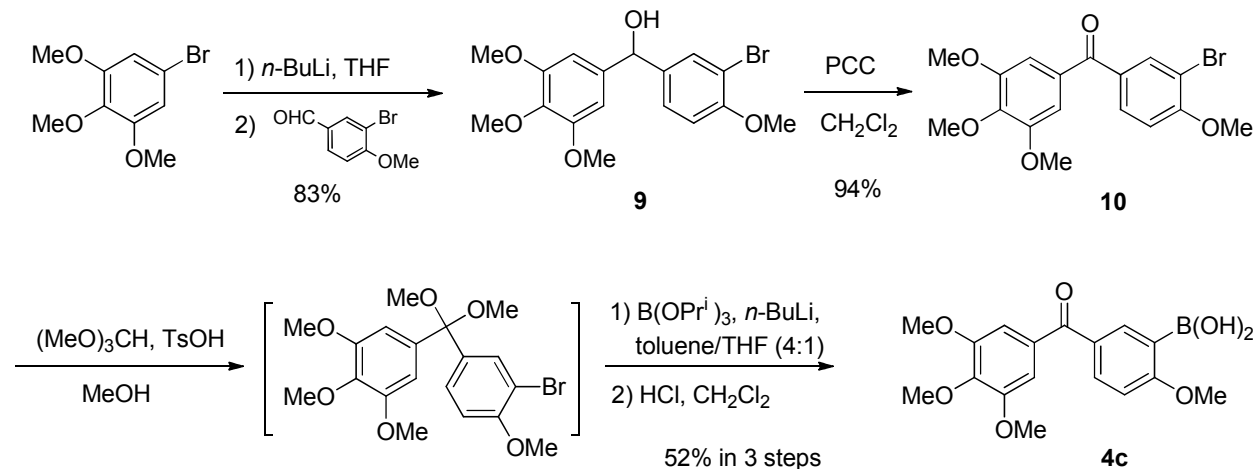


Scheme 1. Synthesis of boronic acid-containing phenstatin analogues **4a** and **4b**.

We also synthesized boronic acid-containing phenstatin analogue **4c** (Scheme 2). The reaction of 5-bromo-1,2,3-trimethoxybenzene with *n*-butyllithium in THF generated the corresponding aryllithium, which reacted with 3-bromo-4-methoxybenzaldehyde to give the carbinol **9** in 83% yield. The carbinol **9** underwent oxidation by treatment with PCC to give the corresponding ketone **10** in 94% yield. Protection of the carbonyl group in ketone **10** followed by introduction of boronic acid using *n*-butyllithium and triisopropyl borate at $-78\text{ }^{\circ}\text{C}$ and deprotection of the ketal group under acidic conditions gave phenstatin analogue **4c** in 52% yield in three steps.

We next examined inhibition of cell growth by the boronic acid analogues using B16 and 1-87 cell lines. The results are summarized in Table 1. The benzophenones **4a** and **4b** exhibited 50% inhibition of cell growth at 0.85 μM and 0.37 μM concentrations toward B16 cells and at 3.1 μM and 1.9 μM concentrations toward 1-87 cells, respectively. The benzophenone **4c**, which has a boronic acid group at the *meta* position and a methoxy group at the *para* position, exhibited higher inhibition of cell growth against both cell lines and the GI₅₀ values of **4c** toward B16 and

1-87 cells are 0.013 μM and 0.087 μM , respectively. The boronic acid-containing combretastatin A-4 analogue **1d**, which was previously synthesized as a tubulin polymerization inhibitor in our group¹¹ as well as Brown's group, independently,¹⁶ was more potent in cell growth inhibition. In all cases, B16 cells are more sensitive toward the synthesized boronic acids than 1-87 cells. ClogP (partition coefficient of a compound between octanol/water speculated by structure-based speculated program) values of each compound are also shown in Table 1. According to the ClogP values, one of the low inhibitory potency of **4a** and **4b** toward cell growth may be due to higher hydrophilicity, which could prevent the compounds from crossing the cell membrane.



Scheme 2. Synthesis of boronic acid-containing phenstatin analogue **4c**.

Table 1. Cell growth inhibition of boronic acid containing phenstatin analogues^a

compound	GI_{50} / μM		Clog P ^d
	B16 cell ^b	1-87 cell ^c	
4a	0.85	3.1	0.989
4b	0.37	1.9	0.989
4c	0.013	0.087	2.06
1d	0.0062	0.013	3.50

^a The compounds were assayed at least three times and the GI_{50} values reported here are mean of an average of three experiments. ^b Mouse B-16 melanoma cell line. ^c Human lung carcinoma 1-87 cell line. ^d Clog P values were speculated by using structure-based calculation program, ChemBioDraw Ultra ver. 12, PerkinElmer Inc.

We also examined the effects of the boronic acid-containing phenstatin analogues **4a-c** on *in vitro* polymerization of tubulin. The tubulin was purified from porcine brains according to the Shelanski protocol¹⁸ with modification.¹⁹ Although it was reported that phenstatin significantly

inhibits tubulin polymerization *in vitro* with an IC_{50} value of 1.1 μM , the boronic acid containing phenstatin analogues **4a-c** did not exhibit inhibition potency even at 100 μM . These results indicate that the current boronic acid containing phenstatin analogues, especially compound **4c**, possess significant inhibitory activity toward cell growth without interacting microtubule system.

Conclusions

We succeeded in synthesizing the boronic acid-containing phenstatin analogues **4a-4c**. The lithiation of the aromatic ring B of the phenstatin with *n*-butyllithium is essential for introduction of a boronic acid moiety in the molecules. Among the boronic acid-containing phenstatin analogues synthesized, compound **4c**, in which a hydroxyl group on the aromatic ring B of the phenstatin was replaced by a boronic acid, exhibited significant cell growth inhibition. Since the current synthesized compounds **4a-4c** did not show any inhibition potency toward tubulin polymerization process *in vitro*, they are considered to inhibit cell growth by different mechanisms other than inhibition of the microtubule system.

Experimental Section

General. ^1H NMR and ^{13}C NMR spectra were measured on a JEOL JNM-AL 300 (300 MHz) or VARIAN UNITY-INOVA 400 (400 MHz) spectrometers. Chemical shifts of ^1H NMR were expressed in parts per million downfield from CDCl_3 as an internal standard ($\delta = 7.24$) in CDCl_3 or from CD_3OD as an internal standard ($\delta = 3.35$). Chemical shifts of ^{13}C NMR were expressed in parts per million downfield from CDCl_3 as an internal standard ($\delta = 77.0$) in CDCl_3 or from CD_3OD as an internal standard ($\delta = 49.3$). Analytical thin layer chromatography (TLC) was performed on a glass plates (Merck Kieselgel 60 F₂₅₄, layer thickness 0.2 mm or RP-18 F_{254s}, layer thickness 0.2 mm). Visualization was accompanied by UV light (254 nm), I_2 and KMnO_4 . Column chromatography was performed on silica gel (Merck Kieselgel 70-230 mesh). All reactions were carried out under an argon atmosphere using standard Schlenk techniques. Most chemicals and solvents were analytical grade and used without further purification.

4'-Bromo-3,4,5-trimethoxybenzhydrol (6a). To a mixture of 1-bromo-4-iodobenzene (**5a**) (8.5 g, 30 mmol) in THF (90 mL) was added *n*-butyllithium (1.6 M in hexane, 19 mL, 30 mmol) at -80°C under argon, and the mixture was stirred for 3 h. A solution 3,4,5-trimethoxybenzaldehyde (5.9 g, 30 mmol) in THF (60 mL) was then added to the reaction mixture dropwise, and the reaction mixture allowed to warm to room temperature for 1 h with stirring. The reaction was quenched by saturated aqueous NH_4Cl solution and the mixture was extracted with dichloromethane, dried over anhydrous MgSO_4 , and concentrated. Purification by silica gel column chromatography with hexane/ethyl acetate (2:1) gave **6a** (8.7 g, 25 mmol, 82%) as a

colorless oil: ^1H NMR data were in agreement with those in the reported in the literature¹⁷: ^1H NMR (400 MHz, CDCl_3) δ 7.45 (d, $J = 8.8$ Hz, 2H), 7.25 (d, $J = 8.8$ Hz, 2H), 6.55 (s, 2H), 5.71 (d, $J = 3.2$ Hz, 1H), 3.81 (s, 6H), 3.81 (s, 3H), 2.19 (d, $J = 3.2$ Hz, 1H); ^{13}C NMR (75 MHz; CDCl_3): δ 153.1, 142.6, 139.1, 137.1, 131.3, 128.1, 121.2, 103.4, 75.4, 60.7, 55.9.

3'-Iodo-3,4,5-trimethoxybenzhydryl (6b). This compound was synthesized from 1,3-diiodobenzene (**5b**) (3.3 g, 10 mmol) and 3,4,5-trimethoxybenzaldehyde (2.1 g, 11 mmol) using the procedure described for **6a** to give **6b** (2.6 g, 6.5 mmol, 65%) as a colorless oil: ^1H NMR (400 MHz; CDCl_3) δ 7.73 (s, 1H), 7.57 (d, $J = 8.0$ Hz, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 7.04 (t, $J = 8.0$ Hz, 1H), 6.53 (s, 2H), 5.64 (d, $J = 2.8$ Hz, 1H), 3.80 (s, 6H), 3.80 (s, 3H), 2.49 (d, $J = 2.8$ Hz, 1H); ^{13}C NMR (75 MHz; CDCl_3) δ 153.3, 145.8, 138.8, 137.4, 136.6, 135.3, 130.1, 125.7, 103.5, 94.4, 75.5, 60.8, 56.1; HRMS (ESI, positive) m/z calcd. for $\text{C}_{17}\text{H}_{19}\text{BrO}_5$ $[\text{M}+\text{Na}]^+$: 405.0314, found: 405.0313

4'-Bromo-3,4,5-trimethoxybenzophenone (7a). To a mixture of pyridinium chlorochromate (PCC; 3.4 g, 16 mmol), sodium acetate (2.6 g) and MgSO_4 (2.6 g) in CH_2Cl_2 (50 mL) was added a solution of **6a** (3.7 g, 10 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at rt for 1 d. After removal of solvents under reduced pressure, the residue was purified by column chromatography on silica gel with hexane/ethyl acetate (5:1) to yield the benzophenone **7a** (3.5 g, 9.9 mmol, 95%) as a white solid: ^1H NMR data were in agreement with those in the reported in the literature 17: mp 101-103 °C; ^1H NMR (300 MHz; CDCl_3) δ 7.64 (s, 2H), 7.63 (s, 2H), 7.01 (s, 2H), 3.92 (s, 3H), 3.85 (s, 6H); ^{13}C NMR (75 MHz; CDCl_3) δ 194.5, 152.9, 142.2, 136.5, 132.0, 131.5, 131.3, 127.2, 107.6, 60.9, 56.2.

3'-Iodo-3,4,5-trimethoxybenzophenone (7b). This compound was synthesized from **6b** (2.5 g, 6.5 mmol) and PCC (2.1 g, 9.7 mmol) using the procedure described for **7a** to give **7b** (2.3 g, 5.8 mmol, 92%) as a white solid: mp 113-115 °C; ^1H NMR (400 MHz; CDCl_3) δ 8.11 (t, $J = 7.6$ Hz, 1H), 7.89 (dq, $J = 7.6, 1.6$ Hz, 1H), 7.71 (dq, $J = 7.6, 1.6$ Hz, 1H), 7.21 (t, $J = 7.6$ Hz, 1H), 7.01 (s, 2H), 3.93 (s, 3H), 3.86 (s, 6H); ^{13}C NMR (75 MHz; CDCl_3) δ 194.0, 153.0, 142.5, 141.0, 139.7, 138.6, 131.8, 129.9, 128.9, 107.8, 94.0, 61.0, 56.3; HRMS (ESI, positive) m/z calcd. for $\text{C}_{16}\text{H}_{17}\text{IO}_4$ $[\text{M}+\text{Na}]^+$: 423.0069, found: 423.0066

4'-Bromo-3,4,5-trimethoxybenzophenone dimethyl acetal (8a). To a mixture of **7a** (1.1 g, 3.1 mmol) and *p*-toluenesulfonic acid monohydrate (0.030 g, 0.16 mmol) in MeOH (15 mL) was added trimethoxymethane (0.40 mL, 3.7 mmol) and the mixture was stirred under reflux for 8 h. Sodium methoxide (28%w/w in methanol; 0.31 mL, 1.6 mmol) was added to the reaction mixture, which was stirred for further 5 min and then the solvents were evaporated. Saturated aq. NaHCO_3 solution was added and the mixture was extracted with CH_2Cl_2 , dried over anhydrous MgSO_4 , and concentrated. The resulting solid was washed with cold MeOH to afford **8a** (1.0 g, 2.6 mmol, 86%) as a white solid. This solid was pure enough to perform the next step: mp 125-127 °C; ^1H NMR (400 MHz; CDCl_3) δ 7.41 (d, $J = 8.4$ Hz, 2H), 7.35 (d, $J = 8.4$ Hz, 2H), 6.67 (s, 2H), 3.80 (s, 9H), 3.09 (s, 6H); ^{13}C NMR (75 MHz; CDCl_3) δ 152.8, 141.3, 137.4, 137.2, 131.0, 128.5, 121.6, 103.9, 102.3, 60.6, 56.0, 49.3; HRMS (ESI, positive) m/z calcd. for $\text{C}_{18}\text{H}_{21}\text{BrO}_5$ $[\text{M}+\text{Na}]^+$: 419.0470, found: 419.0472

3'-Iodo-3,4,5-trimethoxybenzophenone dimethyl acetal (8b). This compound was synthesized from **7b** (1.2 g, 3.0 mmol) and trimethoxymethane (0.39 mL, 3.6 mmol) using the procedure described for **8a** to give **8b** (1.1 g, 2.4 mmol, 81%) as a white solid: mp 113-115 °C; ¹H NMR (400 MHz; CDCl₃): δ 7.88 (s, 1H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.68 (s, 2H), 3.80 (s, 9H), 3.10 (s, 6H); ¹³C NMR (75 MHz; CDCl₃) : δ 152.9, 144.6, 137.3, 137.3, 136.6, 135.6, 129.8, 126.2, 104.0, 101.9, 94.1, 60.7, 56.1, 49.4; HRMS (ESI, positive) *m/z* calcd. for C₁₈H₂₁IO₅ [M+Na]⁺: 467.0331, found: 467.0331

4'-(Dihydroxylboryl)-3,4,5-trimethoxybenzophenone (4a). To a mixture of **8a** (1.0 g, 2.6 mmol) and triisopropyl borate (0.73 mL, 3.1 mmol) in THF (2 mL) and toluene (8 mL) was added *n*-butyllithium (1.6 M in hexane; 1.8 mL, 2.9 mmol) dropwise at –78 °C. The mixture was stirred for 1.5 h at –78 °C and allowed to warm to –20 °C for 30 min with stirring. The reaction was quenched by aqueous HCl solution (1 N) and the mixture was extracted with CH₂Cl₂, dried over anhydrous MgSO₄, and concentrated. Purification by column chromatography on silica gel with hexane/EtOAc (2/1) gave **8a**, which was dissolved in CH₂Cl₂ (4 mL) and conc. HCl (4 mL) was added. The mixture was stirred for 2 h and then diluted with water. The aqueous mixture was extracted with EtOAc and the organic layer was neutralized with saturated aqueous NaHCO₃ solution. The organic layer was separated, dried over anhydrous MgSO₄, and concentrated. The resulting white solid was washed with co-solvent of ether and hexane to afford **4a** (0.31 g, 0.97 mmol, 37 % in 2 steps) as a white solid: mp 134-136 °C; ¹H NMR (400 MHz; CD₃OD) : δ 7.93 (s, 2H), 7.77 (s, 2H), 7.13 (s, 2H), 3.90 (s, 3H), 3.89 (s, 6H); ¹³C NMR (75 MHz; CD₃OD) : δ 197.5, 154.0, 143.3, 140.0, 134.5, 133.6, 129.5, 108.8, 61.2, 56.7, 49.9. Anal. calcd for C₁₆H₁₇BO₆: C, 60.79; H, 5.42. found: C, 60.53; H, 5.29.

3'-(Dihydroxylboryl)-3,4,5-trimethoxybenzophenone (4b). This compound was synthesized from **8b** (0.60 g, 1.4 mmol) and triisopropyl borate (0.38 mL, 1.6 mmol) using the procedure described for **4a** to give **4b** (0.31 g, 0.98 mmol, 72%) as a white solid: mp 91-93 °C; ¹H NMR (400 MHz; CD₃OD) : δ 8.20 (bs, 1H), 8.05 (bd, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.13 (s, 2H), 3.91 (s, 3H), 3.89 (s, 6H); ¹³C NMR (75 MHz; CD₃OD) : δ 197.9, 154.3, 143.5, 138.9, 138.2, 136.4, 133.9, 132.4, 128.7, 109.0, 61.2, 56.7. Anal. calcd for C₁₆H₁₇BO₆: C, 60.79; H, 5.42. found: C, 60.57; H, 5.31.

3'-Bromo-3,4,4',5-tetramethoxybenzhydrol (9). To a mixture of 5-bromo-1,2,3-trimethoxybenzene (1.3 g, 5.1 mmol) in THF (15 mL) was added *n*-butyllithium (1.6 M in hexane, 3.3 mL, 5.3 mmol) at -80 °C under argon dropwise, and the mixture was stirred for 1.5 h. A solution 3-bromo-4-methoxybenzaldehyde (1.2 g, 5.3 mmol) in THF (10 mL) was then added to the reaction mixture dropwise for 1 h, and the reaction mixture allowed to warm to 0 °C for 1 h with stirring. The reaction was quenched by saturated aqueous NH₄Cl solution and the mixture was extracted with CH₂Cl₂, dried over anhydrous MgSO₄, and concentrated. Purification by silica gel column chromatography with hexane/EtOAc (2:1) gave **9** (1.6 g, 4.2 mmol, 83%) as a colorless oil: ¹H NMR (400 MHz; CDCl₃): δ 7.55 (d, *J* = 2.0 Hz, 1H), 7.24 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.55 (s, 2H), 5.68 (d, *J* = 3.2 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 6H), 3.81 (s, 3H), 2.23 (d, *J* = 3.2 Hz, 1H); ¹³C NMR (75 MHz; CDCl₃) : δ 155.0, 153.1, 139.2, 137.3,

137.0, 131.4, 126.6, 111.6, 111.4, 103.2, 75.0, 60.7, 56.1, 55.9; HRMS (ESI, positive) m/z calcd. for $C_{17}H_{19}BrO_5$ $[M+Na]^+$: 405.0314, found: 405.0313

3'-Bromo-3,4,4',5-tetramethoxybenzophenone (10). To a mixture of PCC (1.3 g, 5.9 mmol), sodium acetate (1.0 g) and $MgSO_4$ (1.0 g) in dichloromethane (16 mL) was added a solution of **9** (1.5 g, 3.9 mmol) in dichloromethane (16 mL) and the mixture was stirred at room temperature for 2 days. After removal of solvents under reduced pressure, the residue was purified by column chromatography on silica gel with hexane/ethyl acetate (2:1) to yield the benzophenone **10** (1.4 g, 3.7 mmol, 94%) as a white solid: mp 105-107 °C; 1H NMR (400 MHz; $CDCl_3$): δ 8.05 (d, J = 2.4 Hz, 1H), 7.76 (dd, J = 8.4, 2.4 Hz, 1H), 6.98 (s, 2H), 6.95 (d, J = 8.4 Hz, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 3.86 (s, 6H); ^{13}C NMR (75 MHz; $CDCl_3$): δ 193.1, 159.0, 152.7, 141.7, 135.3, 132.5, 131.2, 131.1, 111.6, 110.8, 107.4, 61.0, 56.5, 56.3; HRMS (ESI, positive) m/z calcd. for $C_{17}H_{17}BrO_5$ $[M+Na]^+$: 403.157, found: 403.0161

3'-(Dihydroxyboryl)-3,4,4',5-tetramethoxybenzophenone (4c). To a mixture of **10** (0.99 g, 2.6 mmol) and *p*-toluenesulfonic acid monohydrate (0.025 g, 0.13 mmol) in MeOH (13 mL) was added trimethoxymethane (0.40 mL, 3.7 mmol) and the mixture was stirred under reflux for 4 h. Sodium methoxide (28%w/w in MeOH; 0.31 mL, 13 mmol) was added to the reaction mixture, which was stirred for further 5 min and then the solvents were evaporated. Saturated aqueous $NaHCO_3$ solution was added and the mixture was extracted with CH_2Cl_2 , dried over anhydrous $MgSO_4$, and concentrated. The resulting dimethyl ketal was dissolved in THF (2.6 mL) and toluene (10 mL), and triisopropyl borate (0.73 mL, 3.1 mmol) was added. *n*-Butyllithium (1.6 M in hexane; 1.9 mL, 3.0 mmol) was added dropwise at -78 °C to this mixture, which was stirred for 1.5 h at -78 °C and allowed to warm to -20 °C for 30 min with stirring. The reaction was quenched by aqueous HCl solution (1 N) and the mixture was extracted with CH_2Cl_2 , dried over anhydrous $MgSO_4$, and concentrated. The resulting solid was dissolved in CH_2Cl_2 (4 mL) and conc. HCl (4 mL) was added. The mixture was stirred for 2 h and then diluted with water. The aqueous mixture was extracted with EtOAc and the organic layer was neutralized with saturated aqueous $NaHCO_3$ solution. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated. Purification by column chromatography on silica gel with CH_2Cl_2 /EtOH (100:1) gave **4c** (0.44 g, 1.3 mmol, 43% in 3 steps) as a white solid: mp 160-162 °C; 1H NMR (400 MHz; $CDCl_3$): δ 8.33 (d, J = 2.4 Hz, 1H), 7.99 (dd, J = 8.8, 2.4 Hz, 1H), 7.03 (s, 2H), 7.01 (d, J = 8.8 Hz, 1H), 5.76 (s, 2H), 4.00 (s, 3H), 3.92 (s, 3H), 3.86 (s, 6H); ^{13}C NMR (75 MHz; $CDCl_3$): δ 194.5, 167.5, 152.8, 141.8, 139.6, 135.3, 132.9, 130.8, 110.0, 107.6, 60.9, 56.3, 56.0. Anal. calcd for $C_{17}H_{19}BO_7$: C, 58.99; H, 5.53. found: C, 58.89; H, 5.45.

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References

1. Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. *Can. J. Chem.* **1982**, *60*, 1374.
2. McGown, A.; Fox, B. *Canc. Chemother. Pharmacol.* **1990**, *26*, 79.
3. Pettit, G. R.; Toki, B.; Herald, D. L.; Verdier-Pinard, P.; Boyd, M. R.; Hamel, E.; Pettit, R. *K. J. Med. Chem.* **1998**, *41*, 1688.
4. Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666.
5. Liou, J.-P.; Chang, C.-W.; Song, J.-S.; Yang, Y.-N.; Yeh, C.-F.; Tseng, H.-Y.; Lo, Y.-K.; Chang, Y.-L.; Chang, C.-M.; Hsieh, H.-P. *J. Med. Chem.* **2002**, *45*, 2556.
6. Liou, J.-P.; Chang, J.-Y.; Chang, C.-W.; Chang, C.-Y.; Mahindroo, N.; Kuo, F.-M.; Hsieh, H.-P. *J. Med. Chem.* **2004**, *47*, 2897.
7. Messaoudi, S.; Tréquier, B.; Hamze, A.; Provot, O.; Peyrat, J.-F.; De Losada, J. R.; Liu, J.-M.; Bignon, J.; Wdzieczak-Bakala, J.; Thoret, S.; Dubois, J. I.; Brion, J.-D.; Alami, M. *J. Med. Chem.* **2009**, *52*, 4538.
8. Koehler, K. A.; Lienhard, G. E. *Biochemistry* **1971**, *10*, 2477.
9. Trippier, P. C.; McGuigan, C. *Med. Chem. Comm.* **2010**, *1*, 1838.
10. Asano, T.; Nakamura, H.; Uehara, Y.; Yamamoto, Y. *ChemBioChem* **2004**, *5*, 483.
11. Nakamura, H.; Kuroda, H.; Saito, H.; Suzuki, R.; Yamori, T.; Maruyama, K.; Haga, T. *Chem. Med. Chem* **2006**, *1*, 729.
12. Ban, H. S.; Usui, T.; Nabeyama, W.; Morita, H.; Fukuzawa, K.; Nakamura, H. *Org. Biomol. Chem.* **2009**, *7*, 4415.
13. Nakamura, H.; Watanabe, M.; Ban, H. S.; Nabeyama, W.; Asai, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3220.
14. Nakamura, H.; Horikoshi, R.; Usui, T.; Ban, H. S. *Med. Chem. Comm.* **2010**, *1*, 282-286.
15. Shimizu, K.; Maruyama, M.; Yasui, Y.; Minegishi, H.; Ban, H. S.; Nakamura, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1453.
16. Kong, Y.; Grembecka, J.; Edler, M. C.; Hamel, E.; Mooberry, S. L.; Sabat, M.; Rieger, J.; Brown, M. L. *Chem. Biol.* **2005**, *12*, 1007.
17. Lee, J.; Kim, S. J.; Choi, H.; Kim, Y. H.; Lim, I. T.; Yang, H.-m.; Lee, C. S.; Kang, H. R.; Ahn, S. K.; Moon, S. K.; Kim, D.-H.; Lee, S.; Choi, N. S.; Lee, K. J. *J. Med. Chem.* **2010**, *53*, 6337.
18. Shelanski, M. L.; Gaskin, F.; Cantor, C. R. *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 765.
19. Haga, T.; Abe, T.; Kurokawa, M. *FEBS Lett.* **1974**, *39*, 291.