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Synthesis and Antiproliferative Activities of Ottelione A Analogues

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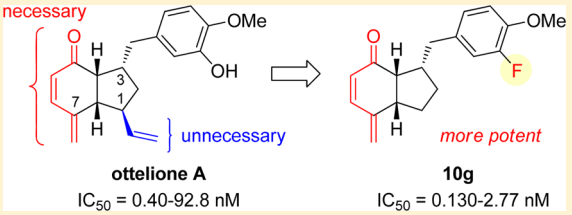
Supporting Information

ABSTRACT: Through the syntheses of its C-1 desvinyl, C-7 methylene, C-7 exocyclic ethylidene, and various C-3 phenylmethyl analogues, the structure−activity relationship of antimitotic ottelione A (**4**) against tubulin and various cancer cells was established. The results indicated that compound **4** was a colchicine-competitive inhibitor and that the C-1 vinyl group is unnecessary for its potency, whereas the C-7 exocyclic double bond is essential, possibly because of its irreversible interaction with tubulin. Further optimization of the substituents on the phenylmethyl group at the C-3 position generated compound **10g** with a 3′-fluoro-4′-methoxyphenylmethyl substituent, which was 6−38-fold more active against MCF-7, NCI-H460, and COLO205 cancer cells relative to **4**. Results from in vitro tubulin polymerization assay confirmed the potency of compounds **4**, **10g**, and **11a**.



KEYWORDS: *ottelione A, antimitotic, microtubule, tubulin, anticancer*

After the success of antimitotic taxanes and vinca alkaloids as anticancer drugs,[1,2] antimitotic agents that target the colchicine-binding site to inhibit tubulin polymerization have attracted much attention. Many structurally diverse compounds binding to the colchicine-binding site have been discovered from natural or synthetic sources.[3,4] Despite no compound of this class having been approved for clinical use, the prototype colchicine (**1**, Figure 1) has been used to treat gout for many years.[5] Combretastatin A-4 (**2**)[6−9] and ABT-751 (**3**)[10,11] in Figure 1 are representative examples of colchicine-competitive inhibitors currently in clinical trials to treat various cancers.

Ottelione A (**4**, Figure 1), isolated from the fresh water plant *Ottelia alismoides* collected in the Nile Delta,[12] is among the most potent natural products that possess in vitro antiproliferative activity. Its IC_{50} values lie in the pM−nM range against a panel of 60 human cancer cell lines through acting as a colchicine-competitive inhibitor.[12,13] Because of its promising antitumor activity and intriguing core structure with four stereogenic centers, several synthetic groups have investigated the total synthesis of **4**,[14−21] but no analogue has been reported and assayed for anticancer activity, perhaps because of the many steps in its preparation.

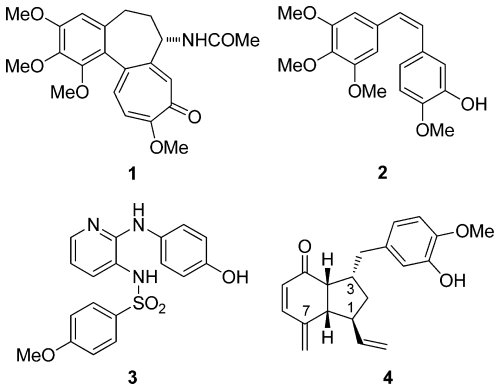


Figure 1. Structures of colchicine (**1**), combretastatin A-4 (**2**), ABT-751 (**3**), and ottelione A (**4**).

We already achieved an enantioselective total synthesis of **4** using our α-carbonyl radical cyclization method.[21] The bicyclo[4.3.0]nonane core structure was constructed via radical cyclization. The C-3 3′-hydroxy-4′-methoxyphenylmethyl group, C-1 vinyl group, and C-7 exocyclic double bond were then sequentially introduced. We thus envisaged that our scheme could be adaptable for the preparation of various analogues of **4**, which would be useful to establish its structure−activity relationship.

As shown in Scheme 1, we first prepared compounds **10a−g** and **11a−c** according to our scheme for the total synthesis of **4**. For economy, these compounds were all synthesized in their racemic forms. Compound **5**, prepared via α-carbonyl radical cyclization,[21] served as the starting material. Hydroboration of **5** with 9-borabicyclo(3.3.1)nonane (9-BBN) at its exocyclic double bond, followed by Suzuki−Miyaura coupling with various aryl iodides and subsequent removal of the *tert*-

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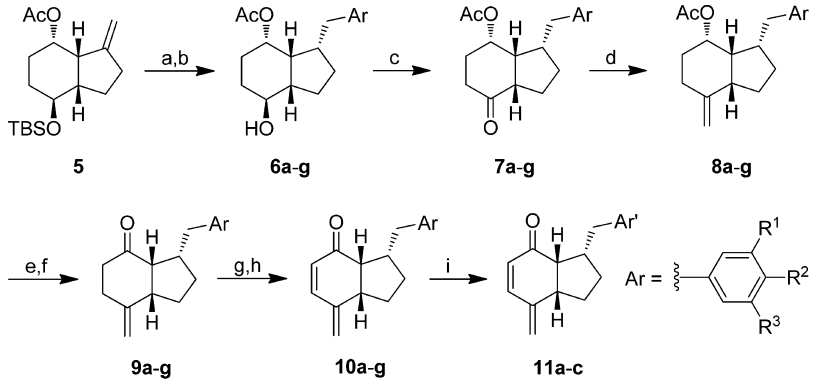
Scheme 1. Synthesis of Compounds 10a–g and 11a–c[a]



6a-10a R^1 = H, R^2 = OMe, R^3 = OMOM
6b-10b R^1 = H, R^2 = OMOM, R^3 = H
6c-10c R^1 = H, R^2 = OMOM, R^3 = F
6d-10d R^1 = R^2 = R^3 = H
6e-10e R^1 = R^2 = R^3 = OMe
6f-10f R^1 = H, R^2 = R^3 = F
6g-10g R^1 = H, R^2 = OMe, R^3 = F

11a R^1 = H, R^2 = OMe, R^3 = OH
11b R^1 = H, R^2 = OH, R^3 = H
11c R^1 = H, R^2 = OH, R^3 = F

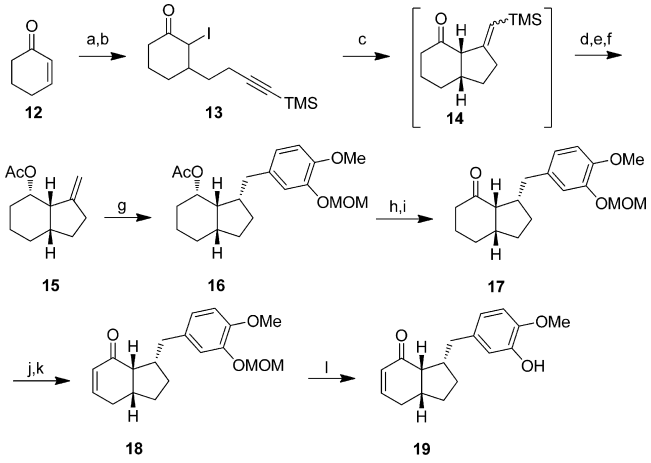
[a]Reagents and conditions: (a) 9-BBN, ArI, $PdCl_2$(dppf), NaOH (3 M), THF. (b) HF (50%), CH_3CN, 23–65% (from **5**). (c) PCC, CH_2Cl_2, 61–93%. (d) $Ph_3P^+CH_3Br^-$, ^{t}BuOK, benzene, 71–95%. (e) NaOH, MeOH. (f) PCC, CH_2Cl_2, 38–95% (from **8a–g**). (g) $LiN(SiMe_3)_2$, PhSeBr, THF. (h) H_2O_2, CH_2Cl_2, 45–74% (from **9a–g**). (i) TFA, THF, H_2O, 55–65% (from **10a–c**).

butyldimethysilyl (TBDMS) protective group, gave compounds **6a–g** in 23–65% yields. Oxidation of **6a–g** with pyridinium chlorochromate (PCC) in CH_2Cl_2 generated **7a–g** in 61–93% yields. Treatment of **7a–g** with $Ph_3P^+CH_3Br^-$ and ^{t}BuOK afforded compounds **8a–g** possessing a C-7 exocyclic double bond. Removal of the acetyl group from **8a–g** with NaOH followed by oxidation with PCC afforded compounds **9a–g** in 38–95% yields. To introduce the double bond at the C-5 and C-6 positions, we treated compounds **9a–g** first with $LiN(SiMe_3)_2$ and PhSeBr then *m*CPBA to give compounds **10a–g** in 45–74% yields. Removal of the methoxymethyl (MOM) protective group in **10a–c** provided compounds **11a–c** in 55–65% yields (Scheme 1).

Compound **19**, lacking a C-1 vinyl and C-7 exocyclic double bond, was prepared from 2-cyclohexen-1-one (**12**) according to our method similar to that for **11a–c** (Scheme 2).[22] Reaction of **12** with 4-(trimethylsilyl)-3-butynylmagnesium chloride in the presence of CuI and TMSCl followed by iodination with NaI and *m*CPBA gave α-iodoketone **13** in 96% yield. Radical cyclization of **13** followed by reduction with $NaBH_4$ and protection with Ac_2O afforded **15** through the key intermediate **14**. Compound **15** was converted to compound **16** by hydroboration and Suzuki–Miyaura coupling in 30% yield. Deprotection of **16** followed by oxidation gave ketone **17** in 74% yield. Compound **17** was converted to α,β-unsaturated enone **18** in 33% yield via treatment with $LiN(SiMe_3)_2$ and PhSeBr followed by oxidative elimination. Acidic hydrolysis of the MOM protecting group in **18** provided **19** in 63% yield.

To prepare C-7 exocyclic ethylidene derivative **23**, we treated compound **7a** with $Ph_3P^+CH_2CH_3Br^-$ and ^{t}BuOK to give compound **20** in 88% yield (Scheme 3). Removal of the acetyl group in **20** followed by oxidation gave **21** in 81% yield. Introduction of the double bond between C-5 and C-6 positions gave **22** in 63% yield. Compound **22** was subsequently hydrolyzed in acidic conditions to remove the MOM group to afford the desired exocyclic ethylidene derivative **23** in 80% yield. Compounds **20–23** were all

Scheme 2. Synthesis of C-7 Exocyclic Methylene Analogue 19[a]



[a]Reagents and conditions: (a) 4-(Trimethylsilyl)-3-butynylmagnesium chloride, CuI, HMPA, TMSCl, Et_3N. (b) NaI, *m*CPBA, THF, 96% (two steps). (c) Bu_6Sn_2, benzene, then Bu_3SnH, AIBN. (d) TFA, CH_2Cl_2. (e) $NaBH_4$, MeOH; (f) Ac_2O, pyridine, CH_2Cl_2, 56% (from **13**). (g) ArI, 9-BBN, $PdCl_2$(dppf), NaOH, THF, 30%. (h) NaOH, MeOH. (i) PCC, CH_2Cl_2, 74% (two steps). (j) $LiN(SiMe_3)_2$, PhSeBr, THF. (k) H_2O_2, CH_2Cl_2, 33% (two steps). (l) TFA, THF, H_2O, 63%.

obtained in *Z* and *E* mixtures and could not be separated by simple column chromatography; we therefore employed NOE experiments to determine the *E*/*Z* ratio of **23**. Two doublet peaks at 7.34 and 6.82 ppm, corresponding to the C-6 hydrogen with an integral ratio 1/3.4, were recorded in the ^{1}H NMR spectrum of **23**. Irradiation of the peak at 7.34 ppm showed a positive NOE effect with 7.58% enhancement at the signal of CH_3 at 1.83 ppm, indicating that the peak corresponded to (*E*)-**23**; irradiation of the peak at 6.82 ppm, however, showed no positive NOE effect, revealing it to

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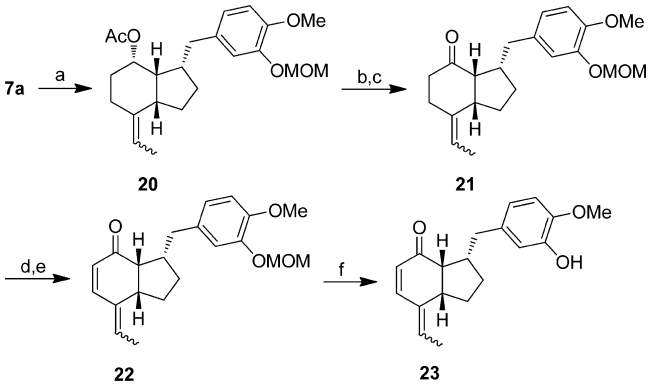
Scheme 3. Synthesis of C-7 Exocyclic Ethylidene Analogue 23[a]



[a]Reagents and conditions: (a) $Ph_3P^+CH_2CH_3Br^-$, ^{t}BuOK, benzene, 88%. (b) NaOH, MeOH. (c) PCC, CH_2Cl_2, 79% (two steps). (d) $LiN(SiMe_3)_2$, PhSeBr, THF. (e) H_2O_2, CH_2Cl_2, 63% yield (two steps). (f) $HClO_4$, CH_2Cl_2, 80%.

correspond to (*Z*)-**23**. The *E*/*Z* ratio of **23** was hence determined to be 1/3.4.

After these compounds were prepared, assays of the percentage of colchicine binding inhibition (CBI %) and antiproliferation against human cancer cells including KB (nasopharyngeal), MCF-7 (breast), NCI-H460 (lung), HCT-116 (colon), A375 (melanoma), and COLO205 (colon) were employed to evaluate their potencies. Compound **4** and its C-1 desvinyl derivative **11a**, C-7 methylene derivative **19**, and C-7 exocyclic ethylidene derivative **23** were assayed first (Table 1). Enantiomerically pure **4**, prepared according to our published method,[21] showed strong inhibition of the growth of the cancer cells with IC_{50} values of 0.40−92.8 nM; the results are consistent with the reported potency of **4** from natural[12] and synthetic sources.[17] The cells most sensitive toward **4** were HCT-116 and A375 cells, which were inhibited at subnanomolar concentrations (IC_{50}, 0.74 and 0.40 nM, respectively). Compound **4** also showed great CBI % with a near-plateau value of 98.3%, revealing its binding to the colchicine-binding site that contributed to its remarkable antiproliferative activities.

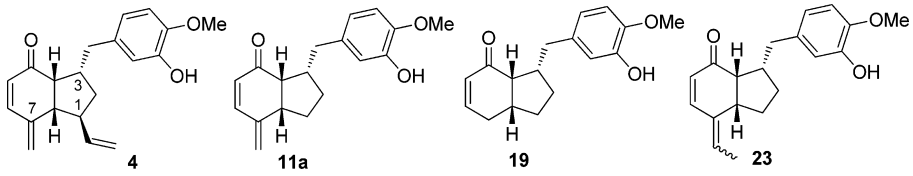
The C-1 desvinyl analogue **11a** was also active against the cancer cells with IC_{50} values ranging from 1.07 to 243.9 nM (Table 1). Although slightly less potent than **4**, compound **11a** also exhibited a satisfactory CBI % (95.8%). As a result, the C-1 vinyl might be less important for the potency of **4**. We subsequently prepared all analogues without the vinyl substituent. Compound **11a** also displayed a spectrum of activities against the cancer cells similar to that of **4**, being more active to inhibit HCT-116 and A375 cells but less potent against COLO205.

Unlike the C-1 vinyl group, the C-7 exocyclic double bond was crucial for the potency of **4**; the evidence came from the loss of the antimitotic (CBI %, 39.2%) and antiproliferative activities (IC_{50} > 1.0 μM) of **19** that did not bear the moiety (Table 1). As the C-7 exocyclic double bond is conjugated with the C-4 carbonyl, it might be susceptible to nucleophilic 1,6-addition[23] with amino acid residues in the binding site, for example, serine, threonine, and cysteine residues.[24] As a result, an irreversible inhibition of tubulin polymerization caused by the C-7 exocyclic double bond was hinted. To provide further evidence, we introduced a methyl substituent on the double bond to generate analogue **23** that was more hindered for nucleophilic addition. It showed decreased antiproliferative activities (1.5−8.6-fold) relative to **11a**, which indicated that the 1,6-addition was possible. The nucleophile would be a thiol, for T138067, a pentafluorobenzenesulfonamide that possesses an *N*-aryl group similar to that of **2**, is reported to covalently modifies tubulin at a conserved cysteine residue in the colchicine-binding site.[25]

After clarifying the roles of the C-1 and C-7 moieties, we examined the effect of substituents on the phenyl group of **4** on the antimitotic and antiproliferative activities. Compounds **10b−d**, **11b**, and **11c** were subjected to the in vitro assays; the results are summarized in Table 2. The unsubstituted **10d** (R^1 = R^2 = R^3 = H) was less potent against the cancer cells with IC_{50} values of 237−678 nM, which were 2.2−221-fold less potent than **11a**, especially on the sensitive HCT-116 and A375 cells (85- and 221-fold less potent, respectively). Its CBI % was also decreased to 43.2%. For **11b** bearing a *para*-hydroxyl group, the antiproliferative activities and CBI % were greater than unsubstituted **10d**. Blocking the *para*-hydroxyl in **11b** with a MOM group gave compound **10b** with decreased antimitotic and antiproliferative activities.

As the structure of **4** is similar to that of **2**, we synthesized compound **10e** with a trimethoxyphenyl substituent, but a complete loss of activity was observed (CBI % = 8.93% and IC_{50} > 1.0 μM). The 3′-hydroxyl-4′-methoxyphenyl in **4** evidently did not occupy the same space as the trimethoxylphenyl group in **2** in the colchicine-binding site.

Table 1. CBI % and Antiproliferative Activities of Compounds 4, 11a, 19, and 23



		antiproliferative IC_{50} (nM)[b]					
compd	CBI %[a]	KB	MCF-7	NCI-H460	HCT-116	A375	COLO205
4	98.3	1.59	1.17	14.49	0.74	0.40	92.8
11a	95.8	4.94	3.73	16.04	3.01	1.07	243.9
19	39.2	>10^3	>10^3	>10^3	>10^3	914	>10^3
23	96.1	22.6	26.3	24.6	14.0	9.2	739

[a]CBI %: percentage of colchicine-binding inhibition at a compound concentration of 10 μM, replicated. [b]The IC_{50} values were averaged from two independent dose−response curves; the variation was generally <15%.

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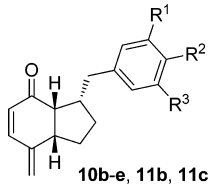
Table 2. Percentage of Colchicine-Binding Inhibition and Antiproliferative Activities of C-1 Desvinyl Compounds 10b−e, 11b, and 11c



10b-e, 11b, 11c

					antiproliferative IC_{50} (nM)[b]					
compd	R^1	R^2	R^3	CBI %[a]	KB	MCF-7	NCI-H460	HCT-116	A375	COLO205
10d	H	H	H	43.2	591	260	678	257	237	547
11b	H	OH	H	71.6	68.8	33.8	77.6	38.5	30.8	218.1
10b	H	OCH_2OCH_3	H	69.3	208	63.6	226	129	77.4	219
10e	OMe	OMe	OMe	8.93	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
10c	H	OCH_2OCH_3	F	68.9	76.6	77.2	106	77.4	40.0	75.7
11c	H	OH	F	37.3	156	147	229	126	98.4	3003
10f	H	F	F	61.7	478	233	511	235	222	428
10g	H	OMe	F	97.1	2.77	0.130	2.62	1.11	1.68	2.45

[a]CBI %: percentage of colchicine-binding inhibition at a compound concentration of 10 μM, replicated. [b]The IC_{50} values were averaged from two independent dose−response curves; the variation was generally <15%.

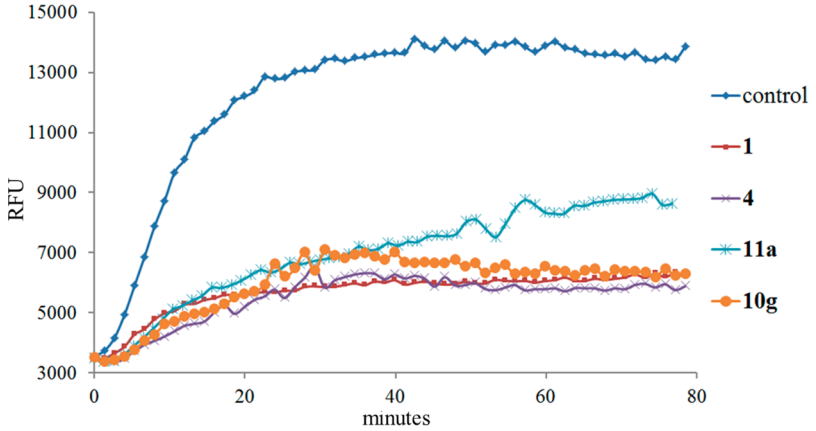


Figure 2. Effect of compounds **1**, **4**, **10g**, and **11a** (each 10 μM) on tubulin polymerization. The assays were conducted with a fluorescence-based assay kit (Cytoskeleton Inc.) using purified porcine neuronal tubulin. All compounds effectively inhibited the polymerization of tubulin; **10g** and **4** showed activities comparable to that of **1**.

Introduction of a *m*-fluoro group to **10b** gave compound **10c** with the retention of CBI % (69.3% for **10b** and 68.9% for **10c**; see Table 2), but **10c** showed increased antiproliferative activity against cancer cells relative to **10b**, except MCF-7. Removal of the MOM group in **10c** afforded compound **11c** with a ∼2-fold decrease of CBI % (37.3%) as well as its antiproliferative activities. Compound **10f** with 3′,4′-difluoro substituents in the phenyl ring was less active than **11c**, in both CBI % and antiproliferative activities. We eventually obtained compound **11g** with a 3′-fluoro-4′-methoxylphenyl substituent that showed a CBI % value, 97.1%, comparable with that of **4** and **11a**. It was most potent among the compounds in this work against various cancer cells, with IC_{50} values of 0.130−2.77 nM. Even on the least sensitive COLO205 cells, its activity was also remarkable, possessing an IC_{50} value of 2.45 nM, which was 38 times as active as **4**.

The change of the hydroxyl group in **2** to a fluoro atom was found to retain its antiproliferative activity against K562 cells.[26] In our work, the switch from OH to F in **11a** also provided **10g** with retained or improved potency. As a result, compound **4** might bind in tubulin in a position similar to that of compound **2**. The 3′-hydroxyl-4′-methoxylphenyl substituent in **2** and **4** would occupy the same space in the colchicine-binding site.

To confirm the drug target of the compounds in Tables 1 and 2, potent compounds **4**, **10g**, and **11a** were tested using in vitro tubulin polymerization assay at concentration of 10 μM with **1** as the positive control. As presented in Figure 2, all compounds significantly inhibited the polymerization of tubulin; **4** and **10g** showed potency similar to that of **1**. In combination with their CBI % values as shown in Tables 1 and 2, the results confirmed that the antiproliferative activity of the compounds arose from inhibiting tubulin polymerization through binding to the colchicine-binding site.

We summarize our work on the structure−activity relationship of **4** against various cancer cells in Figure 3. As **11a** showed activity comparable with that of **4**, the C-1 vinyl is unnecessary for the activity, but the C-7 exocyclic double bond is essential for the potency. The exocyclic double bond in conjugation with the C-4 carbonyl might make it susceptible to nucleophilic addition, thus irreversibly binding with tubulin. Among the hydrogen, hydroxyl, methoxy, and MOM groups at the para position of the phenyl group in **4**, only the methoxy group

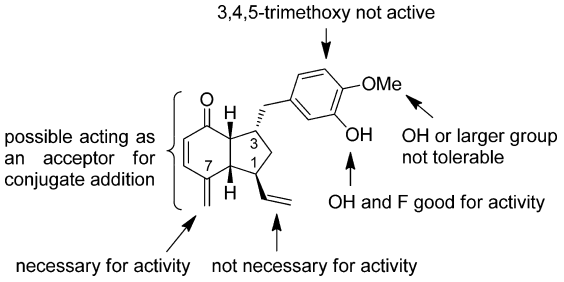


Figure 3. Structure–activity relationship of **4** against various cancer cells.

provided potent compounds. For the meta position, a fluoro or a hydroxyl group enhanced the potency, whereas trimethoxy substituents on the phenyl group deactivated compound **10e**.

In conclusion, we have established the structure–activity relationship of **4** focusing on the C-1 vinyl, C-7 exocyclic double bond, and the substituents on the phenyl ring to inhibit colchicine binding and the growth of cancer cells. Compound **10g**, a less complicated analogue of **4**, show potency comparable with, or superior to, that of **4**. Extensive syntheses and biological evaluation are now in progress in our laboratory. Using the information provided in this work, we are seeking more potent and druggable molecules for further development.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures for compounds **6a–g**, **7a–g**, **8a–g**, **9a–g**, **10a–g**, **11a–c**, and **13–23** and procedures for bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

9-BBN, 9-borabicyclo(3.3.1)nonane; CBI %, percentage of colchicine-binding inhibition; MOM, methoxymethyl; PCC, pyridinium chlorochromate; TBDMS, *tert*-butyldimethysilyl; TFA, trifluoroacetic acid

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