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5-Amino-2-aroylquinolines as Highly Potent Tubulin Polymerization Inhibitors. Part 2. The Impact of Bridging Groups at Position C-2

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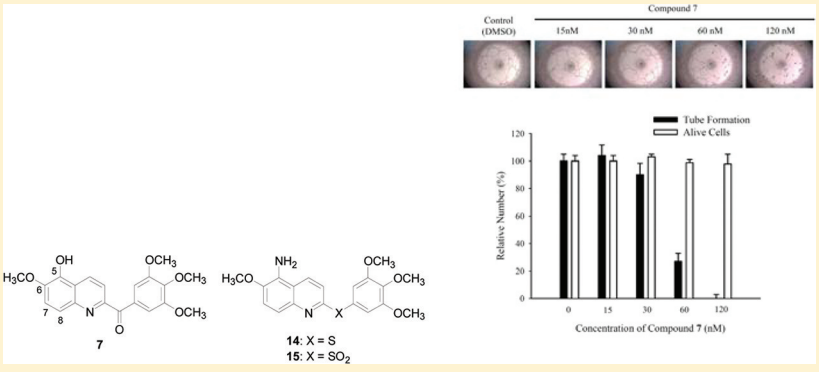
Supporting Information



ABSTRACT: A variety of studies on the modification of combretastatin A-4 triggered our interest in the impact of the linkers between the 3,4,5-trimethoxyphenyl ring and 5-amino-6-methoxyquinoline on biological activity. The replacement of the carbonyl group with bond, amine, ether, sulfide, and sulfone groups was evaluated in this study. The results showed that compounds **14** and **15** containing sulfide and sulfone groups between the 3,4,5-trimethoxyphenyl ring (A-ring) and 5-amino-6-methoxyquinoline exhibited substantial antiproliferative activity against KB, HT29, and MKN45 cells with mean IC_{50} values of 42 and 12 nM, respectively. **15** inhibited the tubulin polymerization with an IC_{50} value of 2.0 μM, similar to that with CA4. The continued work on the C-5 substituents of 3′,4′,5′-trimethoxybenzoyl-6-methoxyquinoline derivatives demonstrated that compound **7** possessing OH at C-5 exhibited excellent antiproliferative activity with mean IC_{50} values of 3.4 nM and microtubule destabilizing potency with an IC_{50} of 1.5 μM, comparable to that of CA4 (IC_{50} = 1.9 μM). It also exhibited substantial vascular disrupting effects. Compounds **7** and **15** exhibited significant efficacy against MDR/MRP-related drug-resistant cell lines (KB-vin10, KB-S15, and KB-7D) with mean IC_{50} values of 6.7 and 2.6 nM, respectively.

INTRODUCTION

Microtubules are recognized as crucial components involved in the formation of the mitotic spindle during the cell proliferative process; therefore, they become a target of cancer therapy, which attracts scientific interest for the development of anticancer agents.[1] Natural products such as colchicine (**1**) and combretastatin A-4 (CA4, **2**) exhibited potent tubulin polymerization inhibitory ability, and their distinctive structural features started numerous projects in the development of antimitotic agents. To date, a large portion of the interest in the development of antimitotic agents consistently focused on the modification of the naturally potent products, especially combretastatin A-4 that interacts with tubulin at the colchicine-binding site.[2] These research efforts brought many products into clinical trials;[3] for instance, the phosphate prodrug CA4P (**3**) with improved solubility compared to that of CA4 is in human clinical trials as a vascular disrupting agent (VDA).[4] Compound **4**, which has a 3′-OH group replaced with a 3′-NH_2 group, demonstrated potent anti-tubulin activity, and its serine prodrug (AVE8602, **5**)[5] is undergoing human clinical trials, as well.

On the basis of the development of various anti-tubulin agents, both hydroxy and amino substituents usually contributed to potent anti-tubulin activity and provided the opportunity to adjust the pharmaceutical formula. This phenomenon was also observed in our study of 1-aroylindoles as antimitotic agents; the hydroxy group at position C-4 of 5-methoxyindole exhibited better potency than the one bearing an amino group at the same position.[6] On the basis of these

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observations, the current study was designed to replace the amino group with a hydroxyl group (**7**) in 5-amino-2-aroylquinolines (compound **6**), which were shown to exhibit potent antiproliferative activity in our recent report.[7] In addition, halogen atoms (**8**–**10**) were investigated in this study.

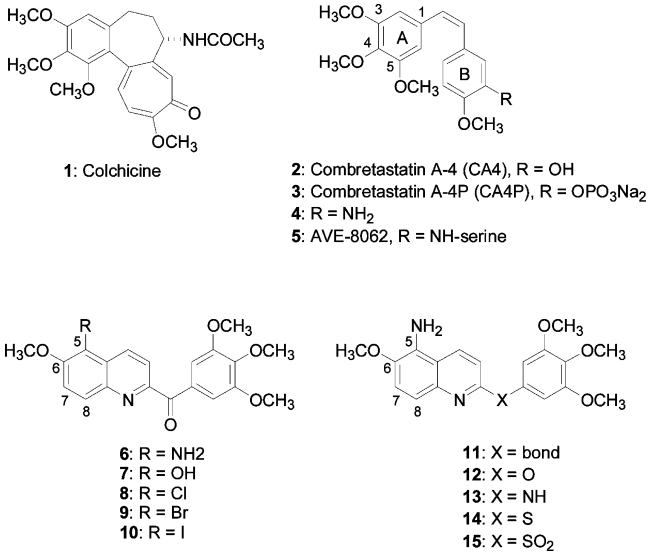


Figure 1. Natural anitmitotic agents and their derivatives and synthetic 2-substituted 6-methoxyquinolines.

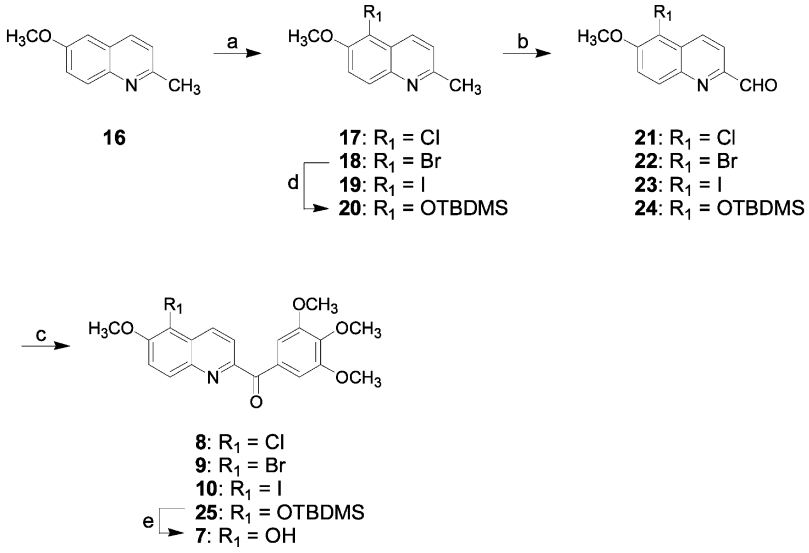
As the 3,4,5-trimethoxyphenyl ring (A-ring) was identified as a significant motif in the realm of antimitotic agents, the research efforts aimed at antimitotic agents by modification of combretastatin A-4 mainly focused on two sections: B-ring and *cis*-stilbene modifications. The work on B-ring modification principally focused on the exploitation of heterocyclic rings, such as indole,[8] thiophene,[9] benzothiophene,[10] and quinoline.[7] The modification of the (*Z*)-stilbene part intensively attracted attention and was mainly through the replacement with either functional groups or heterocyclic rings. With respect to the *cis*-stilbene modification, heterocyclic rings, including imidazole,[11] triazole,[12] lactam,[13] isoxazoline/isoxazole or pyridine,[14] or isoxazole and imidazole,[15] were comprehensively utilized to maintain the *Z* geometry. On the other hand, the (*Z*)-stilbene geometry was also modified by a variety of bridging groups, such as sulfide,[16] amine,[17] -X- (bond),[18] sulfonamide,[19] etc. Because all efforts resulted in high potency in the examples mentioned above, we focused on modification of the connecting part between 5-amino-6-methoxyquinoline and the 3,4,5-trimethoxyphenyl ring by using several linkers such as bond (**11**), ether (**12**), amine (**13**), sulfide (**14**), and sulfone (**15**) groups according to the literature cited above.

RESULTS AND DISCUSSION

Chemistry. Scheme 1 describes the synthesis of 2-(3′,4′,5′-trimethoxybenzoyl)-6-methoxyquinolines. To introduce halogens and a nitro group at C-5 of quinoline, we treated compound **16** with NCS, NBS, and NIS, yielding compounds **17**–**19**, respectively. The bromo atom of **18** was converted into the hydroxy group via palladium-mediated reaction followed by the protection with TBDMS to yield compound **20**. The conversion of C-2 methyl groups of compounds **17**–**20** into aldehydes was conducted with selenium dioxide (SeO_2), yielding compounds **21**–**24**, respectively. The resulting aldehydes were reacted with 3,4,5-trimethoxyphenylmagnesium bromide followed by oxidation by pyridinium dichromate to give compounds **8**–**10** and **25**. The deprotection of compound **25** with TBAF finally yielded compound **7**.

The synthetic approaches to compounds **11**–**15** are shown in Scheme 2. The starting **26** underwent nitration followed by chlorination via treatment with mCPBA and $POCl_3$ to yield compound **28**, which subsequently underwent C-2 substitution as follows. Compound **28** was reacted with 3,4,5-trimethoxyphenol, 3,4,5-trimethoxyaniline, and 3,4,5-trimethoxybenzenethiol

Scheme 1. Synthetic Approaches to Compounds 7–10[a]



[a]Reagents and conditions: (a) for **17**, NCS, acetonitrile, reflux, 76%; for **18**, NBS, acetonitrile, room temperature, 98%; for **19**, NIS, sulfuric acid, room temperature, 96%; (b) SeO_2, 1,4-dioxane, reflux, 69–92%; (c) (i) 3,4,5-trimethoxyphenylmagnesium bromide, 0–25 °C; (ii) pyridinium dichromate (PDC), CH_2Cl_2, molecular sieves, room temperature, 26–64%; (d) (i) pinacolborane, $Pd(PPh_3)_4$, TEA, 1,4-dioxane, microwave, 160 °C; (ii) $NH_2OH{\cdot}HCl$, NaOH, CH_3OH, room temperature, 38%; (iii) *t*-$Bu(CH_3)_2SiCl$, DIPEA, CH_2Cl_2, room temperature; (e) TBAF, THF, room temperature, 31%.

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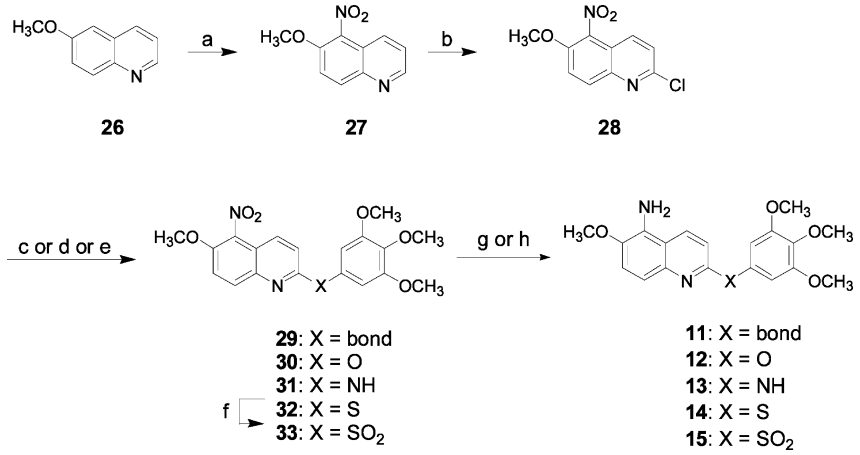
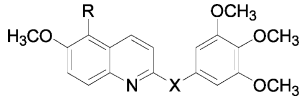
Scheme 2. Synthetic Approaches to Compounds 11−15[a]



[a]Reagents and conditions: (a) 65% HNO_3, H_2SO_4, 0−25 °C, 95%; (b) (i) mCPBA, CH_2Cl_2, room temperature; (ii) $POCl_3$, CH_2Cl_2, reflux, 78%; (c) 3,4,5-trimethoxyphenol or 3,4,5-trimethoxybenzenethiol, K_2CO_3, DMF, 120 °C, 45−63%; (d) 3,4,5-trimethoxyaniline, 200 °C, 19%; (e) 3,4,5-trimethoxyphenylboronic acid, $Pd(PPh_3)_4$, 2 M K_2CO_3(aq), toluene, ethanol, microwave, reflux, 47%; (f) mCPBA, CH_2Cl_2, 78%; (g) sodium sulfide nonahydrate, NaOH, ethanol, H_2O, reflux; (h) Fe, NH_4Cl, isopropyl alcohol, H_2O, reflux, 47−80%.

Table 1. IC_{50} Values of Compounds 7−15



			IC_{50} ± SD[a] (nM)		
compd	R	-X-	KB cells	HT29 cells	MKN45 cells
6[b]	NH_2	-CO-	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.2
7	OH	-CO-	2.8 ± 3	3.1 ± 1.2	4.5 ± 2.2
8	Cl	-CO-	45 ± 12	51 ± 15	60 ± 19
9	Br	-CO-	108 ± 9	195 ± 15	167 ± 63
10	I	-CO-	451 ± 46	1089 ± 158	819 ± 274
11	NH_2	bond	>5000	>5000	>5000
12	NH_2	-O-	31 ± 6	139 ± 71	61 ± 8
13	NH_2	-NH-	>5000	>5000	>5000
14	NH_2	-S-	59 ± 28	40 ± 1	28 ± 10
15	NH_2	$-SO_2-$	18 ± 8	16 ± 12	4.0 ± 2.5
colchicine	–	–	10.3 ± 0.9	16.2 ± 2.9	19.2 ± 1.0
CA4	–	–	2.0 ± 0.4	94 ± 26	4.8 ± 0.8

[a]SD, standard deviation. All experiments were independently performed at least three times. [b]Data from ref 7.

in the presence of potassium carbonate, yielding compounds **30**−**32**, respectively. The sulfide bond of compound **32** was oxidized by mCPBA to give the corresponding sulfone **33**. Under the Suzuki conditions, compound **28** reacted with 3,4,5-trimethoxyphenylboronic acid on exposure to microwaves to furnish compound **29**. Finally, the nitro groups of compounds **29**−**33** were reduced by sodium sulfide nonahydrate or iron powder, yielding compounds **11**−**15**, respectively.

Biological Evaluation. *In Vitro Cell Growth Inhibitory Activity.* In an attempt to evaluate the effect of various 6-methoxyquinolines on cancer cell inhibitory ability, we evaluated all synthesized compounds (**7**−**15**) and reference compounds, colchicine and CA4, for antiproliferative activity against three human cancer cell lines, cervical carcinoma KB cells, colorectal carcinoma HT29 cells, and stomach carcinoma MKN45 cells (Table 1).

The comparisons of compounds **8**−**10** demonstrated the effect of substituents (halogen atoms) at position C-5 of quinoline on cytotoxic activity. Compound **8**, which has a chloro atom, exhibited the most potent activity among those possessing halogen atoms, and it showed antiproliferative activity against KB, HT29, and MKN45 cells with IC_{50} values of 45, 51, and 60 nM, respectively. Compound **9** containing a bromo atom exhibited moderate antiproliferative activity that is ∼3-fold weaker than that of compound **8**, and the iodo atom of compound **10** caused the loss of activity. This result indicates that the order of influence of sequence of halogen atoms on the antiproliferative activity is as follows: Cl > Br > I. Compound **7** with an OH group at C-5 inhibited the growth of cancer cells with IC_{50} values in the range of 2−5 nM and, thus, was identified as the most potent compound in this study. The comparisons of compounds **11**−**15** showed the significant impact of bridging groups such as bond, amine, ether, sulfide, and sulfone groups linking the 3,4,5-trimethoxyphenyl ring to the 5-amino-6-methoxyquinoline on antiproliferative activity. The removal of the linkage, compound **11**, caused the dramatic loss of antiproliferative activity, which was shown in the

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Table 2. Growth Inhibition of Compounds 7, 12, and 15 against Drug-Resistant Cell Lines

		$IC_{50} \pm SD$[a]					
cell line	resistant type	vincristine[b]	paclitaxel[b]	VP-16[b]	7	12	15
KB	parental	0.4 ± 0.1 nM	3.3 ± 1.2 nM	1.1 ± 0.2 μM	2.8 ± 3.0 nM	31.0 ± 6.0 nM	18 ± 8.0 nM
KB-VIN10	MDR ↑	90.1 ± 7.4 nM	16500 ± 707 nM	23 ± 3 μM	5.8 ± 5.2 nM	78.8 ± 9.8 nM	2.8 ± 2.3 nM
KB-S15	MDR ↑	17.6 ± 2.2 nM	273 ± 15 nM	3.5 ± 0.3 μM	5.2 ± 6.1 nM	42.9 ± 6.3 nM	2.5 ± 2.5 nM
KB-7D	MRP ↑	1.2 ± 0.4 nM	7.9 ± 0. nM5	54 ± 3.5 μM	9.2 ± 10.1 nM	76.2 ± 21.8 nM	2.4 ± 2.2 nM

[a]SD, standard deviation. All experiments were independently performed at least three times. [b]Data from ref 20.

replacement with the amine group (**13**), as well. Compounds **12** and **14** possessing ether and sulfide linkages, respectively, exhibited moderate antiproliferative activity. The conversion from sulfide to sulfone (**15**) led to a significant improvement in the potency against a panel of human cancer cell lines with IC_{50} values from 4 to 18 nM. To improve our understanding of the efficacy against drug-resistant cell lines, compounds **7**, **12**, and **15** were evaluated for antiproliferative activity against a variety of resistant lines as shown in Table 2. Despite the high level of expression of drug-resistant efflux protein (MDR/P-gp or MRP) in KB-Vin 10, KB-S15, and KB-7D cells, compounds **7**, **12**, and **15** showed similar cytotoxic efficacy between parental cells and these resistant cell lines. Interestingly, resistant cells were more sensitive to compound **15** with the sulfone linker, which displayed a mean IC_{50} value of 2.6 nM.

Inhibition of Tubulin Polymerization and Colchicine Binding Activity. To investigate whether the activities of the synthetic products in this study were related to the interactions with a microtubule system, compounds **7**, **12**, and **15** and reference compound CA4 were evaluated for tubulin polymerization inhibitory activity as well as [^{3}H]colchicine binding activity (Table 3). Compound **7** showed the best tubulin polymerization assembly inhibition ability (IC_{50} = 1.5 μM), which was comparable to that of CA4. Compound **15** showed tubulin depolymerization with IC_{50} values of 2.0 μM and was slightly less potent than CA4; in addition, the tubulin polymerization assembly inhibition activity of compound **12** was 3-fold less potent than that of CA4. These results were correlated with that of an examination of antiproliferation and indicated that these compounds inhibited the growth of cancer cells through the inhibition of tubulin polymerization. In the [^{3}H]colchicine binding assay (Table 3), the results demonstrated that compounds **7** and **15** were bound to the colchicine-binding site. The binding affinity of compound **7** is similar to that of CA4, and compound **15** was bound to the colchicine-binding site in a dose-dependent manner as well even though it exhibited slightly weak binding with the colchicine-binding site. As shown in Figure 2, compounds **7** and **15** inhibited polymerization of pure MAP-rich tubulins in a concentration-dependent manner and disrupted tubulin assembly almost completely at 5.0 μM.

Table 3. Inhibition of Tubulin Polymerization and Colchicine Binding Inhibition by Compounds 7, 12, 15, and CA4

		colchicine binding[b] (%)	
compd	tubulin[a] IC_{50} (μM)	1 μM	5 μM
7	1.5	91	97
12	5.8	44	60
15	2.0	65	85
CA4	1.9	90	96

[a]Inhibition of tubulin polymerization.[21] [b]Inhibition of [^{3}H]colchicine binding.[7] The tubulin concentration was 1 μM; the [^{3}H]colchicine concentration was 5 μM.

Investigation of Vascular Disrupting Activity. In addition to tubulin polymerization inhibitory activity, CA4P (**3**) has been shown to cause vascular shutdown within solid tumors as a vascular disrupting agent (VDA).[4] Therefore, compound **7**, which displayed the most potent antiproliferative activity, was investigated for vascular disrupting activity. The HUVECs were plated on Matrigel and allowed to form capillary tubes in the presence of VEGF (20 ng/mL), followed by exposure to different concentrations of compound **7**.[22] As shown in Figure 3, compound **7** was capable of disrupting formed capillaries in a concentration-dependent manner without affecting cell viability.

CONCLUSION

The replacement of the C-5 amino group of compound **6** with a C-5 hydroxy group according to the equivalent effect of amino and hydroxy groups on the antimitotic activity was investigated; in addition, halogen atom substitution at position C-5 was also studied. Compound **7** bearing a C-5 hydroxy group exhibited potent antiproliferative activity against a panel of human cancer cell lines with IC_{50} values ranging from 2.8 to 4.5 nM. It also demonstrated potent microtubule destabilizing potency with IC_{50} values of 1.5 μM comparable to that of CA4. The examination of vascular disrupting activity showed that compound **7** caused the disruption of formed capillaries in a manner similar to that of CA4. The attempt to modify the (*Z*)-stilbene moiety of CA4 employed a variety of bridging groups, such as bond, ether, amine, sulfide, and sulfone groups, applied in this study on the 5-amino-6-methoxyquinoline derivatives. Compounds that possess ether (**12**), sulfide (**14**), and sulfone (**15**) bridges exhibited good cancer growth inhibitory activities. Compound **15** demonstrated potent antiproliferative activity with IC_{50} values in the range of 4.0−18.0 nM and overcame the drug-resistant cell lines (KB-Vin 10, KB-S15, and KB-7D) with IC_{50} values ranging from 2.4 to 2.8 nM. It also was identified as an inhibitor of tubulin polymerization, exhibiting an IC_{50} of 2.0 μM that was comparable to that of CA4. These results showed that the sulfur and oxygen atoms are preferred between the 3,4,5-trimethoxyphenyl ring and 5-amino-6-methoxyquinoline as compared with nitrogen atoms or a bond. Consequently, this work not only revealed potent 2-substituted 5-amino-6-methoxyquinolines but also revealed that the linkage part has a capacity for various bridging groups increasing the diversity of this series of antimitotic agents.

EXPERIMENTAL SECTION

Chemistry. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Bruker DRX-500 spectrometer (operating at 500 MHz),

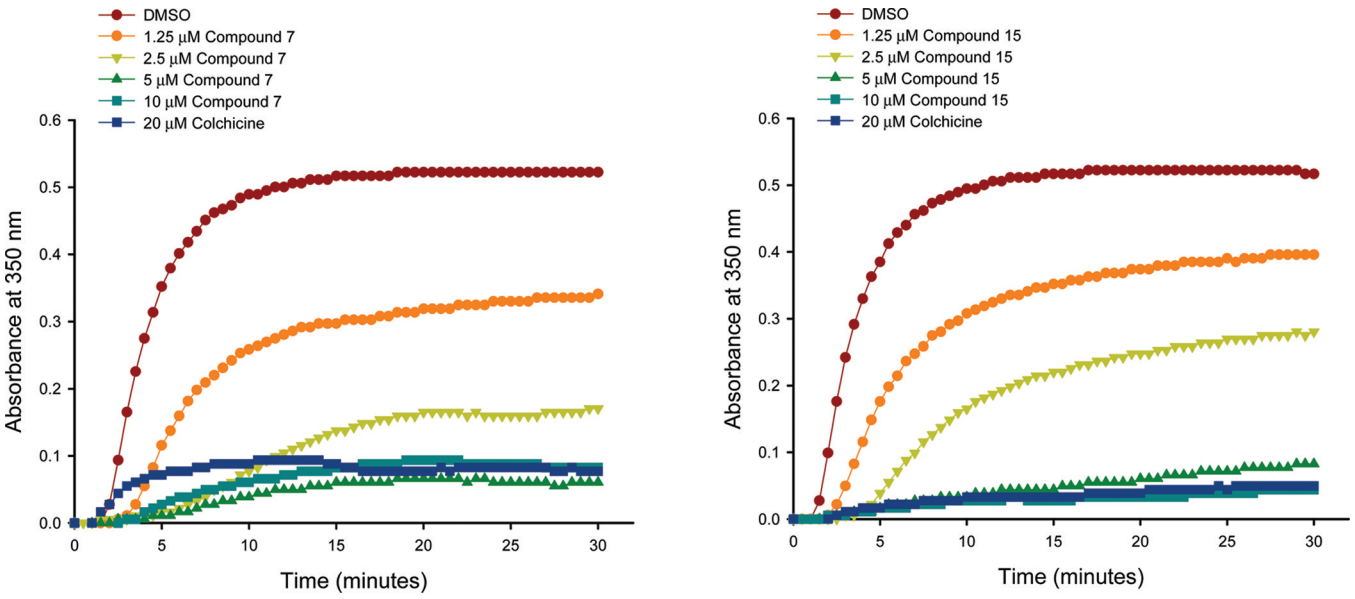

Figure 2. Effect of compounds **7** and **15** on in vitro tubulin polymerization. MAP-rich tubulins were incubated at 37 °C in the absence [dimethyl sulfoxide (DMSO) control] or presence of drugs (colchicine or serial concentrations of compounds **7** and **15**). The absorbance at 350 nm was measured every 30 s for 30 min and is presented as the increased polymerized microtubule.

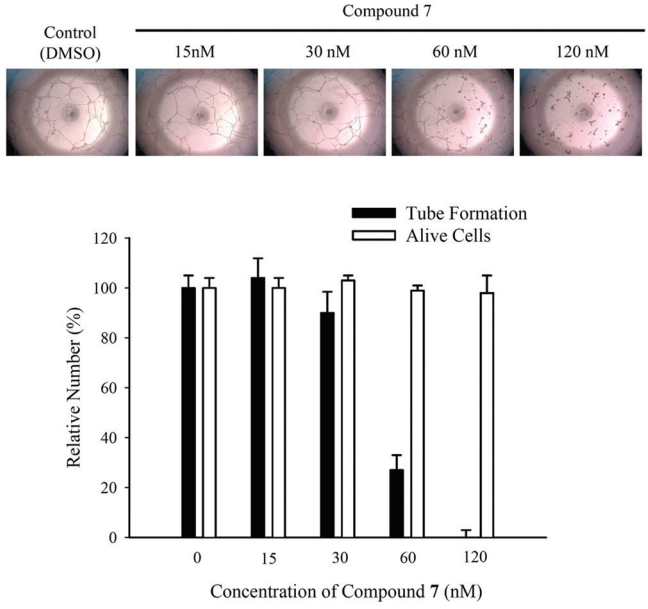

Figure 3. Investigation of the vascular disrupting activity of compound **7**. HUVECs were plated on Matrigel and allowed to form capillary tubes in the presence of VEGF (20 ng/mL) followed by exposure to different concentrations of compound **7**. Cultures were photographed, and the number of capillary tube networks was determined by counting under a microscope (original magnification of 100×). Data reflect the mean number of capillary tube networks to vehicle control group (DMSO) ± the standard deviation (SD) from three separate experiments.

with chemical shifts in parts per million (δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were recorded with a JEOL (JMS-700) electron impact (EI) mass spectrometer. Elemental analyses were performed on a Heraeus varioIII-NCH instrument. The purities of the final compounds were determined using an Agilent 1100 series HPLC system using a C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) and were found to be ≥95%. Flash column chromatography was conducted using silica gel (Merck Kieselgel 60, No. 9385, 230−400 mesh ASTM). All reactions were conducted under an atmosphere of dry nitrogen.

*5-Chloro-6-methoxy-2-methylquinoline (**17**).* A solution of compound **16** (0.3 g, 1.73 mmol) in acetonitrile (3 mL) was cooled to 0 °C followed by the addition of *N*-chlorosuccinimide (0.26 g, 1.9 mmol) over a period of 5 min. The resulting green slurry was heated to reflux for 3 h. The reaction was then quenched with 10% aqueous $NaHSO_3$ (0.36 mL). The reaction mixture was poured into 0.1 N aqueous NaOH (2.2 mL). The slurry was stirred at room temperature for 1 h, filtered, washed with water, and dried to give the title compound (76% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.73 (s, 3H), 4.04 (s, 3H), 7.35 (d, J = 8.7 Hz, 1H), 7.49 (d, J = 9.3 Hz, 1H), 7.97 (d, J = 9.3 Hz, 1H), 8.42 (d, J = 8.7 Hz, 1H).

*5-Bromo-6-methoxy-2-methylquinoline (**18**).* The title compound was obtained in 98% overall yield from compound **16** in a manner similar to that described for the preparation of **17**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.73 (s, 3H), 4.03 (s, 3H), 7.33 (d, J = 8.7 Hz, 1H), 7.46 (d, J = 9.2 Hz, 1H), 8.00 (d, J = 9.2 Hz, 1H), 8.40 (d, J = 8.7 Hz, 1H).

*5-Iodo-6-methoxy-2-methylquinoline (**19**).* The title compound was obtained in 96% overall yield from compound **16** in a manner similar to that described for the preparation of **17**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.73 (s, 3H), 4.03 (s, 3H), 7.29 (d, J = 8.7 Hz, 1H), 7.39 (d, J = 9.2 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 8.31 (d, J = 8.7 Hz, 1H).

*5-Chloro-6-methoxyquinoline-2-carboxaldehyde (**21**).* The title compound was obtained in 85% overall yield from compound **17** in a manner similar to that described for the preparation of **22**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.10 (s, 3H), 7.62 (d, J = 9.3 Hz, 1H), 8.06 (d, J = 8.7 Hz, 1H), 8.19 (d, J = 9.3 Hz, 1H), 8.64 (d, J = 8.8 Hz, 1H), 10.18 (s, 1H).

*5-Bromo-6-methoxyquinoline-2-carboxaldehyde (**22**).* To a suspension of SeO_2 (0.18 g, 1.59 mmol) and *p*-xylene (3 mL) was added dropwise a solution of compound **18** (0.2 g, 0.79 mmol) in *p*-xylene (4 mL) at room temperature. The solution was heated to reflux for 5 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was evaporated to give a residue that was purified by column chromatography to afford compound **22** (92% yield; 1:2 EtOAc/*n*-hexane): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.11 (s, 3H), 7.61 (d, J = 9.2 Hz, 1H), 8.07 (d, J = 8.9 Hz, 1H), 8.25 (d, J = 9.2 Hz, 1H), 8.66 (d, J = 8.7 Hz, 1H), 10.20 (s, 1H).

*5-Iodo-6-methoxyquinoline-2-carboxaldehyde (**23**).* The title compound was obtained in 69% overall yield from compound **19** in a manner similar to that described for the preparation of **22**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.10 (s, 3H), 7.55 (d, J = 9.3 Hz, 1H), 8.04 (d, J = 8.7 Hz, 1H), 8.28 (d, J = 9.2 Hz, 1H), 8.59 (d, J = 9.0 Hz, 1H), 10.23 (s, 1H).

*5-(tert-Butyldimethylsilyloxy)-6-methoxyquinoline-2-carboxaldehyde (**24**).* In a 10 mL glass tube were placed a magnetic stir bar, compound **18** (0.13 g, 0.52 mmol), pinacolborane (0.12 mL, 0.77 mmol), tetrakis(triphenylphosphine)palladium (0.02 g, 0.02 mmol), triethylamine (0.21 mL, 1.5 mmol), and 1,4-dioxane (2 mL). The vessel was sealed and placed in the microwave cavity. The reaction mixture was held at 160 °C for 15 min. After it was cooled to room temperature, the mixture was poured into water and then extracted with EtOAc and aqueous $NaHCO_3$. The combined organic extract was dried over $MgSO_4$ and evaporated to give a crude residue that was dissolved in ethanol (1.2 mL). Sodium hydroxide (0.04 g, 1.04 mmol) and hydroxylamine hydrochloride (0.05 g, 0.78 mmol) were added to the reaction mixture, and the mixture was stirred at room temperature for 16 h. The reaction mixture was poured into water and then extracted with EtOAc. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a residue, which was purified by column chromatography (2:3 EtOAc/*n*-hexane) to afford the corresponding hydroxyl product. A mixture of the resulting product (0.54 g, 2.85 mmol), *tert*-butylchlorodimethylsilane (1.76 g, 11.42 mmol), and diisopropylethylamine (1.89 mL, 11.42 mmol) in CH_2Cl_2 (17.2 mL) was stirred at room temperature for 18 h, poured into water, and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a residue that was purified by flash chromatography (1:3 EtOAc/*n*-hexane) to afford a residue. To the residue were added dropwise SeO_2 (1.3 g, 5.7 mmol) and *p*-xylene (14 mL) at room temperature. The solution was heated to reflux for 5 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was evaporated to give a residue that was purified by column chromatography to afford compound **24** (32% yield; 1:7 EtOAc/*n*-hexane): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.23 (s, 6H), 1.08 (s, 9H), 3.98 (s, 3H), 7.58 (d, J = 9.5 Hz, 1H), 7.91 (d, J = 9.0 Hz, 1H), 7.95 (d, J = 8.5 Hz, 1H), 8.59 (d, J = 8.5 Hz, 1H), 10.17 (s, 1H).

*5-Hydroxy-6-methoxy-2-(3′,4′,5′-trimethoxybenzoyl)quinoline (**7**).* A solution of 3,4,5-trimethoxyphenylmagnesium bromide (1.6 mL, 1.0 M in THF, prepared in advance) was added slowly to a solution of compound **24** (0.25 g, 0.32 mmol) in tetrahydrofuran (2.5 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for an additional 16 h. A saturated NH_4Cl solution was slowly added at 0 °C and extracted with EtOAc (2 × 15 mL) and CH_2Cl_2 (2 × 15 mL). The combined organic extract was dried over $MgSO_4$ and evaporated to give a crude residue. To the residue were added molecular sieves (4 Å, 0.60 g), pyridinium dichromate (0.60 g, 1.57 mmol), and CH_2Cl_2 (50 mL), and the mixture was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was evaporated to give a residue that was dissolved in THF (2 mL). Tetra-*n*-butylammonium fluoride (0.41 mL, 1.0 M in THF) was added to the reaction mixture while it was stirred at room temperature for 16 h. The residue was extracted with EtOAc and H_2O. The organic layers were combined and evaporated to give a residue that was purified by column chromatography to give compound **7** (31% yield; 2:3 EtOAc/*n*-hexane): mp 173−175 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.78 (s, 3H), 3.81 (s, 6H), 3.96 (s, 3H), 7.53 (s, 2H), 7.54 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 9.5 Hz, 1H), 8.05 (d, J = 9.0 Hz, 1H), 8.66 (d, J = 8.5 Hz, 1H); MS (EI) m/z 369 (100%); HRMS (EI) for $C_{20}H_{19}NO_6$ (M^+) calcd 369.1212, found 369.1215. Anal. Calcd for $C_{20}H_{19}NO_6$: C, 65.03; H, 5.18; N, 3.79. Found: C, 64.83; H, 5.24; N, 3.46.

*5-Chloro-6-methoxy-2-(3′,4′,5′-trimethoxybenzoyl)quinoline (**8**).* The title compound was obtained in 65% overall yield from compound **21** in a manner similar to that described for the preparation of **9**: mp 176−177 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.90 (s, 6H), 3.96 (s, 3H), 4.10 (s, 3H), 7.60 (d, J = 8.1 Hz, 1H), 7.61 (s, 2H), 8.13−8.19 (m, 2H), 8.70 (d, J = 8.8 Hz, 1H); MS (EI) m/z 387 (M^+, 13%), 334 (100%); HRMS (EI) for $C_{20}H_{18}ClNO_5$ (M^+) calcd 387.0874, found 387.0873.

*5-Bromo-6-methoxy-2-(3′,4′,5′-trimethoxybenzoyl)quinoline (**9**).* A solution of 3,4,5-trimethoxyphenylmagnesium bromide (5.4 mL, 1.0 M in THF, prepared in advance) was added slowly to the solution of compound **22** (0.96 g, 3.6 mmol) in tetrahydrofuran (5.4 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 48 h. A saturated NH_4Cl solution was slowly added at 0 °C and extracted with EtOAc (2 × 15 mL) and CH_2Cl_2 (2 × 15 mL). The combined organic extract was dried over $MgSO_4$ and evaporated to give a crude residue that was dissolved in CH_2Cl_2 (50 mL). Molecular sieves (4 Å, 2.7 g) and pyridinium dichromate (2.7 g, 7.2 mmol) were added to the reaction mixture, and the mixture was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was evaporated to give a residue that was purified by column chromatography to afford compound **9** (26% yield; 1:2 EtOAc/*n*-hexane): mp 163−165 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.90 (s, 6H), 3.96 (s, 3H), 4.10 (s, 3H), 7.58 (d, J = 9.4 Hz, 1H), 7.60 (s, 2H), 8.16−8.20 (m, 2H), 8.70 (d, J = 8.9 Hz, 1H); MS (EI) m/z 431 (M^+, 40%), 195 (100%); HRMS (EI) for $C_{20}H_{18}BrNO_5$ (M^+) calcd 431.0368, found 431.0367.

*5-Iodo-6-methoxy-2-(3′,4′,5′-trimethoxybenzoyl)quinoline (**10**).* The title compound was obtained in 26% overall yield from compound **23** in a manner similar to that described for the preparation of **9**: mp 202−204 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.90 (s, 6H), 3.97 (s, 3H), 4.10 (s, 3H), 7.51 (d, J = 9.2 Hz, 1H), 7.60 (s, 2H), 8.13 (d, J = 8.8 Hz, 1H), 8.20 (d, J = 9.2 Hz, 1H), 8.61 (d, J = 8.8 Hz, 1H); MS (EI) m/z 479 (100%); HRMS (EI) for $C_{20}H_{18}INO_5$ (M^+) calcd 479.0230, found 479.0229.

*6-Methoxy-5-nitroquinoline (**27**).* Compound **26** (1.0 mL, 7.24 mmol) was added to 65% nitric acid (4 mL) and 95% sulfuric acid (4 mL) at 0 °C in portions. After the mixture had been stirred for 1 h, the reaction was quenched with water, and the mixture was extracted with CH_2Cl_2. The combined organic layer was evaporated to give a residue that was purified by column chromatography to give **27** as a yellow solid (95% yield; 1:1 EtOAc/*n*-hexane): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.08 (s, 3H), 7.54 (dd, J = 4.2, 8.4 Hz, 1H), 7.60 (d, J = 9.4 Hz, 1H), 8.10 (d, J = 8.6 Hz, 1H), 8.30 (d, J = 9.4 Hz, 1H), 8.88 (d, J = 3.4 Hz, 1H).

*2-Chloro-6-methoxy-5-nitroquinoline (**28**).* To a mixture of compound **27** (1.40 g, 6.86 mmol) and CH_2Cl_2 (22 mL) was added *m*-chloroperbenzoic acid (1.77 g, 10.3 mmol) at 0 °C. The reaction mixture was stirred at ambient temperature overnight and washed with 10% sodium sulfite, saturated $NaHCO_3$, and saturated NaCl. The combined organic layer was collected and evaporated to afford an oily residue. To the residue were added CH_2Cl_2 (33 mL) and phosphoryl chloride (6.4 mL), and the mixture was heated to reflux overnight. The reaction mixture was concentrated in vacuo, and the residue was extracted with CH_2Cl_2. The combined organic layer was evaporated to give a residue that was purified by column chromatography to give **28** (78% yield; 1:3 EtOAc/*n*-hexane): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.07 (s, 3H), 7.50 (d, J = 8.9 Hz, 1H), 7.60 (d, J = 9.4 Hz, 1H), 8.03 (d, J = 8.9 Hz, 1H), 8.17 (d, J = 9.4 Hz, 1H).

*6-Methoxy-5-nitro-2-(3′,4′,5′-trimethoxyphenyl)quinoline (**29**).* A mixture of compound **28** (1.0 g, 4.2 mmol), tetrakis(triphenylphosphine)palladium (0.40 g, 0.36 mmol), 3,4,5-trimethoxyphenylboronic acid (2.70 g, 12.6 mmol), 2 M potassium carbonate (11.5 mL), toluene (120 mL), and ethanol (58 mL) was heated to reflux for 16 h in nitrogen. The reaction mixture was concentrated in vacuo, and the residue was extracted with CH_2Cl_2. The combined organic layer was purified by flash chromatography to give **29** (47% yield; 1:2 EtOAc/*n*-hexane): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.93 (s, 3H), 4.01 (s, 6H), 4.09 (s, 3H), 7.39 (s, 2H), 7.59 (d, J = 9.4 Hz, 1H), 7.95 (d, J = 9.0 Hz, 1H), 8.14 (d, J = 8.9 Hz, 1H), 8.37 (d, J = 8.7 Hz, 1H).

*6-Methoxy-5-nitro-2-(3′,4′,5′-trimethoxyphenoxy)quinoline (**30**).* The title compound was obtained in 45% overall yield from compound **28** in a manner similar to that described for the preparation of **31**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.81 (s, 6H), 3.83 (s, 3H), 4.03

(s, 3H), 6.48 (s, 2H), 7.20 (d, J = 9.1 Hz, 1H), 7.47 (d, J = 9.3 Hz, 1H), 7.97 (d, J = 9.3 Hz, 1H), 8.05 (d, J = 9.1 Hz, 1H).

6-Methoxy-5-nitro-2-(3′,4′,5′-trimethoxyphenylamino)quinoline (31). A mixture of 3,4,5-trimethoxyaniline (0.12 g, 0.63 mmol) and compound **28** (0.1 g, 0.42 mmol) was heated to 200 °C for 10 min. The residue was extracted with CH_2Cl_2 and aqueous $NaHCO_3$. The organic layers were combined and purified by flash chromatography to give compound **31** (19% yield; 2:3 EtOAc/*n*-hexane): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.89 (s, 9H), 3.93 (s, 3H), 6.52 (s, 2H), 7.51 (d, J = 9.0 Hz, 1H), 7.52 (d, J = 9.8 Hz, 1H), 7.92 (d, J = 9.6 Hz, 1H), 9.02 (d, J = 9.1 Hz, 1H), 9.95 (s, 1H).

6-Methoxy-5-nitro-2-(3′,4′,5′-trimethoxyphenylthio)quinoline (32). The title compound was obtained in 63% overall yield from compound **28** in a manner similar to that described for the preparation of **31**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 6H), 3.91 (s, 3H), 4.04 (s, 3H), 6.89 (s, 2H), 7.12 (d, J = 9.0 Hz, 1H), 7.51 (d, J = 9.5 Hz, 1H), 7.83 (d, J = 9.0 Hz, 1H), 8.08 (d, J = 9.5 Hz, 1H).

6-Methoxy-5-nitro-2-(3′,4′,5′-trimethoxyphenylsulfonyl)-quinoline (33). To a mixture of compound **32** (0.50 g, 1.25 mmol) and CH_2Cl_2 (100 mL) was added *m*-chloroperbenzoic acid (0.65 g, 3.75 mmol) at 0 °C. The reaction mixture was stirred at ambient temperature overnight and washed with 10% sodium sulfite, saturated $NaHCO_3$, and saturated NaCl. The combined organic layer was evaporated to give a residue that was purified by column chromatography to give **33** (78% yield; 1:1 EtOAc/*n*-hexane): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.87 (s, 3H), 3.91 (s, 6H), 4.10 (s, 3H), 7.32 (s, 2H), 7.67 (d, J = 9.5 Hz, 1H), 8.27 (dd, J = 9.0, 2.5 Hz, 2H), 8.36 (d, J = 9.5 Hz, 1H).

5-Amino-6-methoxy-2-(3′,4′,5′-trimethoxyphenyl)quinoline (11). The title compound was obtained in 80% overall yield from compound **29** in a manner similar to that described for the preparation of **14**: mp 222–223 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.91 (s, 3H), 4.00 (s, 6H), 4.00 (s, 3H), 4.25 (br, 2H), 7.37 (s, 2H), 7.45 (d, J = 9.1 Hz, 1H), 7.66 (d, J = 9.0 Hz, 1H), 7.74 (d, J = 8.9 Hz, 1H), 8.19 (d, J = 8.8 Hz, 1H); MS (EI) m/z 340 (100%); HRMS (EI) for $C_{19}H_{20}N_2O_4$ (M^+) calcd 340.1423, found 340.1423.

5-Amino-6-methoxy-2-(3′,4′,5′-trimethoxyphenoxy)quinoline (12). The title compound was obtained in 80% overall yield from compound **30** in a manner similar to that described for the preparation of **14**: mp 209–210 °C; ^{1}H NMR (500 MHz, DMSO) δ 3.66 (s, 3H), 3.72 (s, 6H), 3.83 (s, 3H), 5.45 (s, 2H), 6.53 (s, 2H), 6.91 (d, J = 9.0 Hz, 1H), 6.97 (d, J = 9.1 Hz, 1H), 7.33 (d, J = 9.0 Hz, 1H), 8.53 (d, J = 9.1 Hz, 1H); MS (EI) m/z 356 (100%); HRMS (EI) for $C_{19}H_{20}N_2O_5$ (M^+) calcd 356.1372, found 356.1375.

5-Amino-6-methoxy-2-(3′,4′,5′-trimethoxyphenylamino)-quinoline (13). The title compound was obtained in 68% overall yield from compound **31** in a manner similar to that described for the preparation of **14**: mp 222–223 °C; ^{1}H NMR (500 MHz, DMSO) δ 3.60 (s, 3H), 3.79 (s, 6H), 3.80 (s, 3H), 5.23 (s, 2H), 6.81 (d, J = 9.2 Hz, 1H), 6.91 (d, J = 8.8 Hz, 1H), 7.24 (d, J = 8.8 Hz, 1H), 7.40 (s, 2H), 8.20 (d, J = 9.2 Hz, 1H), 9.12 (s, 1H); MS (EI) m/z 355 (M^+, 85%), 340 (100%); HRMS (EI) for $C_{19}H_{21}N_3O_4$ (M^+) calcd 355.1532, found 355.1530.

5-Amino-6-methoxy-2-(3′,4′,5′-trimethoxyphenylthio)quinoline (14). A mixture of **32** (0.1 g, 0.25 mmol), iron powder (0.04 g, 0.75 mmol), and ammonium chloride (0.03 g, 0.5 mmol) in IPA (2.5 mL) and water (0.6 mL) was heated to reflux for 3 h. The reaction solvent was removed under reduced pressure, the reaction quenched with water, and the mixture extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and purified by column chromatography over silica gel to afford compound **14** (47% yield): mp 180–182 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.84 (s, 6H), 3.90 (s, 3H), 3.97 (s, 3H), 6.90 (s, 2H), 6.93 (d, J = 9.0 Hz, 1H), 7.39 (d, J = 9.0 Hz, 1H), 7.53 (br, 2H), 7.95 (d, J = 9.0 Hz, 1H); MS (EI) m/z 372 (100%); HRMS (EI) for $C_{19}H_{20}N_2O_4S$ (M^+) calcd 372.1144, found 372.1145.

5-Amino-6-methoxy-2-(3′,4′,5′-trimethoxyphenylsulfonyl)-quinoline (15). The title compound was obtained in 76% overall yield from compound **33** in a manner similar to that described for the preparation of **14**: mp 168–169 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.86 (s, 3H), 3.90 (s, 6H), 4.00 (s, 3H), 7.35 (s, 2H), 7.51 (d, J = 9.0 Hz, 1H), 7.69 (d, J = 9.5 Hz, 1H), 8.04 (d, J = 9.0 Hz, 1H), 8.32 (d, J = 9.0 Hz, 1H); MS (EI) m/z 404 (M^+, 26%), 325 (100%); HRMS (EI) for $C_{19}H_{20}N_2O_6S$ (M^+) calcd 404.1042, found 404.1045.

Biology. *Materials.* Regents for cell culture were obtained from Gibco-BRL Life Technologies (Gaitherburg, MD). Microtubule-associated protein (MAP)-rich tubulin was purchased from Cytoskeleton, Inc. (Denver, CO). [^{3}H]Colchicine (specific activity, 60–87 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA).

Cell Growth Inhibitory Assay. Human cancer cell lines (KB, MKN45, and HT29) used in this study were procured from American Type Culture Collection (Rockville, MD) and grown in Dulbecco's modified Eagle's medium, minimal essential medium, or RPMI 1640 medium. Resistant cell lines KB-Vin10, KB-7D, and KB-S15 were maintained in medium containing additional 10 nM vincristine, 7 μM VP16, and 50 nM paclitaxel, respectively. All cell cultures were supplemented with 10% fetal bovine serum, 2 μM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin and incubated in a humidified atmosphere (95% air and 5% CO_2) at 37 °C. KB-Vin10 and KB-S15 cell lines were resistant to vincristine and paclitaxel, respectively, and both overexpressed the MDR drug efflux protein. KB-7D cells were resistant to VP16 and overexpressed MRP. All resistant cell lines were incubated in drug-free medium for 3 days before being harvested for the growth inhibition assay. In vitro growth inhibition was assessed with the methylene blue assay.[23] In brief, exponentially growing cells were seeded into 24-well culture plates at a density of 8000–20000 cells per milliliter per well (depending on the doubling time of the cell line) and allowed to adhere overnight. Cells were incubated with various concentrations of drugs for 72 h. Then, we measured the A_{595} of the resulting solution from 1% *N*-lauroylsarcosine extraction. Fifty percent growth inhibition (IC_{50}) was calculated on the basis of the A_{595} of untreated cells (taken as 100%). The values shown are the means and standard errors of at least three independent experiments performed in duplicate.

Tubulin Polymerization in Vitro Assay.[21,24] Turbidimetric assays of microtubules were performed as described by Bollag et al.[25] In brief, microtubule-associated protein (MAP)-rich tubulin (from bovine brain, Cytoskeleton, Inc.) was dissolved in reaction buffer [100 mM PIPES (pH 6.9), 2 mM $MgCl_2$, and 1 mM GTP] in preparing the 4 mg/mL tubulin solution. The tubulin solution (240 μg of MAP-rich tubulin per well) was placed in a 96-well microtiter plate in the presence of test compounds or 2% (v/v) DMSO as a vehicle control. The increase in absorbance was measured at 350 nm in a PowerWave X Microplate Reader (BIO-TEK Instruments, Winooski, VT) at 37 °C and recorded every 30 s for 30 min. The area under the curve (AUC) was used to determine the concentration that inhibited tubulin polymerization by 50% (IC_{50}). The AUCs of the untreated control and 10 μM colchicine were set to 100 and 0% polymerization, respectively, and the IC_{50} was calculated by nonlinear regression in at least three experiments.

Tubulin Competition Binding Scintillation Proximity Assay.[26–28] This assay was performed in a 96-well plate. In brief, 0.08 μM [^{3}H]colchicine was mixed with the test compound and 0.5 μg of special long-chain biotin-labeled tubulin (0.5 μg) and then incubated in 100 μL of reaction buffer [80 mM PIPES (pH 6.8), 1 mM EGTA, 10% glycerol, 1 mM $MgCl_2$, and 1 mM GTP] for 2 h at 37 °C. Then 80 μg of streptavidin-labeled SPA beads was added to each reaction mixture. The radioactive counts were then directly measured with a scintillation counter.

Capillary Disruption Assays.[22] Capillary disruption assays were conducted in a 96-well plate format using human umbilical vein endothelial cells (HUVECs) plated at a density of 2×10^4 cells/well in 20% FBS M199 medium containing 20 ng/mL VEGF on a Matrigel layer (BD Biosciences). Capillaries were allowed to form over a 4 h period before the addition of test compound or vehicle control. Images were acquired immediately following addition of the compound and 4 h after exposure to the test compound. Tube formation was quantified by measuring the network number of capillary structures manually by counting under a microscope (original magnification of 100×).

■ ASSOCIATED CONTENT

Supporting Information

HPLC results for target compounds **8**–**15** and ^{1}H NMR spectra of compounds **7** and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

VDA, vascular disrupting agent; MDR, multi-drug-resistant; MRP, multi-drug-resistant-associated protein; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); NCS, *N*-chlorosuccinimide; NBS, *N*-bromosuccinimide; NIS, *N*-iodosuccinimide; mCPBA, *m*-chloroperoxybenzoic acid; TBDMS, *tert*-butylchlorodimethylsilane; TBAF, tetra-*n*-butylammonium fluoride; DIPEA, diisopropylethylamine; PDC, pyridinium dichromate.

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