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Synthesis and Biological Evaluation of Aryloxazole Derivatives as Antimitotic and Vascular-Disrupting Agents for Cancer Therapy

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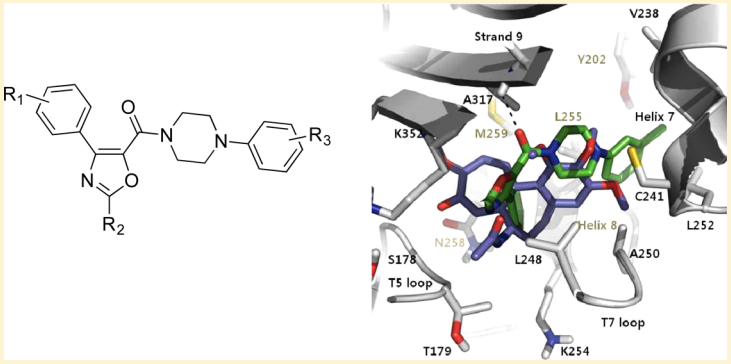
Supporting Information



ABSTRACT: A series of aryloxazole, thiazole, and isoxazole derivatives was synthesized as vascular-targeting anticancer agents. Antiproliferative activity and tumor vascular-disrupting activity of all of the synthesized compounds were tested in vitro using various human cancer cell lines and HUVECs (human umbilical vein endothelial cells). Several compounds with an arylpiperazinyl oxazole core showed excellent cytotoxicity and metabolic stability in vitro. Among this series, two representative compounds (**6-48** and **6-51**) were selected and tested for the evaluation of anticancer effects in vivo using tumor-bearing mice. Compound **6-48** effectively reduced tumor growth (42.3% reduction in size) at the dose of 100 mg/kg. We believe that compound **6-48** will serve as a good lead compound for antimitotic and vascular-disrupting agents; further investigation to improve the in vivo efficacy of this series is underway.

INTRODUCTION

Mitosis is a series of mechanical events that generate two new nuclei containing identical copies of DNA during cell division. Because of the crucial role of the mitotic spindle in mitosis, the spindle fiber has been a valuable drug target in cancer therapy for decades.[1] The discovery of natural products, such as taxol and vinca alkaloids, which interact with the spindle fibers during mitosis in malignant cell growth, has led scientists to explore diverse sets of natural compounds and synthetic small molecules as anticancer drugs. These mitotic spindle poisons halt cell cycle progression by two distinct mechanisms. One is preventing tubulins from polymerizing into microtubules, thereby inhibiting cellular division. For example, vinca alkaloids and colchicine are known to bind β-tubulins and to block the formation of functional microtubules. Taxol and their derivatives, however, bind to microtubules and disrupt their functions, halting cells in the mitotic state.[2] By the early 1990's, hundreds of effective mitosis inhibitors had been developed; unfortunately, the majority of spindle poisons failed in clinical trials because of their poor therapeutic index. Many compounds demonstrated inadequate efficacy or high toxicity for clinical use, which possibly derived from chemical instability and unrecognized interactions with multiple protein targets.[3,4]

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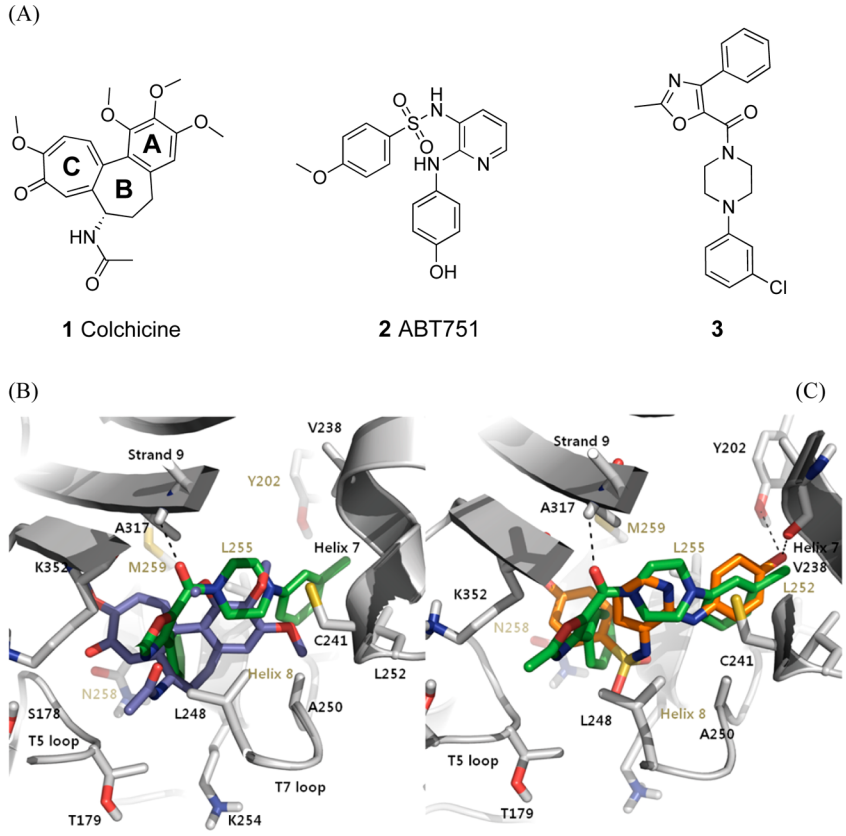

Figure 1. (A) Structures of **1** (colchicine), **2** (ABT751), and **3**. (B) Docked pose of compounds **3** (green) and **1** (purple) in the colchicine binding pocket (PDB code: 3UT5). (C) Docked pose of compounds **3** (green) and **2** (orange) in the colchicine binding pocket (PDB code: 3HKC).

Despite the early clinical failures of the first-generation spindle poisons, paclitaxel, a microtubule depolymerization inhibitor, has achieved huge success in the clinic and is known to be the most broadly effective and commercially successful anticancer agent. This success led to extensive efforts to develop other tubulin poisons with improved pharmacokinetic profiles and selectivity. Interestingly, these tubulin binders were also found to interact with tumor endothelial cells, leading to a rapid occlusion of tumor vasculature and resultant necrotic cell death.[5,6] These vascular-disrupting agents (VDAs) selectively block established tumor vasculature and prevent tumor progression rapidly, whereas conventional antiangiogenesis agents inhibit the formation of new blood vessels, thereby preventing tumor growth chronically. Currently, several notable VDAs including combretastatin A-4 phosphate (CA4P),[7] DMXAA,[8] ZD6126,[9] and BNC105[10] are undergoing clinical trials.

Considering the unique selectivity and effectiveness of tubulin binding VDAs, we aimed to develop a new class of anticancer drugs having dual effects: tumor vasculature disruption and mitotic arrest. In this article, we report the design, synthesis, and biological evaluation of aryl oxazole derivatives as dual-action anticancer agents and present a complete SAR study of the new core to identify essential structural properties required for dual effects. We designed and synthesized a series of heterocyclic small molecules containing oxazoles and isoxazoles based on the previous structure–activity relationship (SAR) study on the colchicine binding sites of tubulin.[11]

We evaluated biological activity of the newly synthesized compounds in vitro and in vivo. First, we incubated human leukemia cells (HL-60) with our compounds to evaluate their inhibitory effect on tumor cell growth in vitro. Second, we measured the vascular-disrupting effect of our compounds using human umbilical vein endothelial cells (HUVEC). Finally, we measured the inhibitory effect of our compounds against tubulin polymerization. Combined with microsomal stability test, we were able to identify two final compounds that are not only stable against metabolism but also inhibit tubulin polymerization and HUVEC growth at low concentrations. Anticancer activities of two selected compounds were evaluated in vivo using tumor-bearing mice.

■ COMPUTATIONAL DESIGN

Colchicine (**1**), a natural product from plants, is known to bind β-tubulins and to inhibit tubulin polymerization. Despite the high toxicity of colchicine, which is comparable to arsenic poisoning, its excellent binding affinity toward spindle fibers makes it an attractive lead compound as an anticancer agent. To date, most of the related studies have focused on developing small molecules targeting the colchicine binding site with low toxicity. One of the most notable examples is ABT-751 (**2**) (Figure 1A).[12] Compound **2** is a synthetic small molecule selected from random screening of antibacterial sulfonamides, and it recently demonstrated promising anticancer effects in phase II trials.[13] By taking a similar approach, we have tested an in-house library of compounds with *N*-phenylpyrazole, 4,5-hydropyrazole, and oxazole core for cytotoxicity against various tumor cell lines (details not shown).

Our preliminary results showed that some of these compounds demonstrated potent cytotoxicity against tumor cell lines as well as inhibited tubulin polymerization, which are

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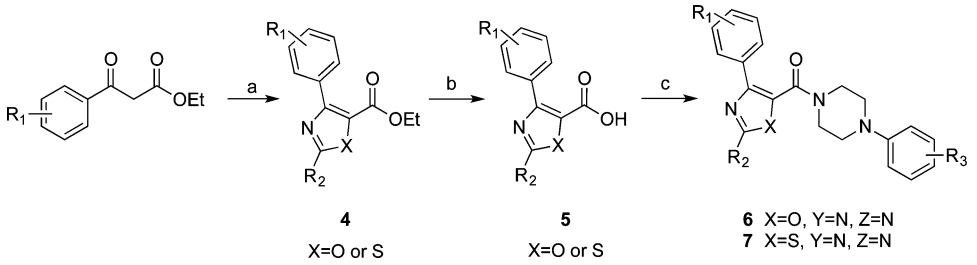
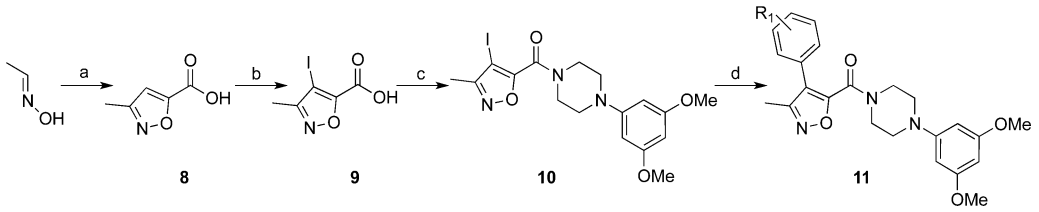
Scheme 1[a]



[a]Reagents and conditions: (a) (i) HDNIB, CH_3CN, reflux, 1 h, (ii) acetamide (or thioacetamide), microwave 80 W, 1 min; (b) (i) 1N NaOH, (ii) 1 N HCl, (c) arylpiperazine, EDCI, HOBt, NMM, CH_2Cl_2.

Scheme 2[a]



[a]Reagents and conditions: (a) (i) NEt_3, NCS, CH_2Cl_2, (ii) propargyl alcohol, NEt_3, THF, (iii) Jones reagent, acetone, 0 °C; (b) (i) *n*-BuLi, THF, −78 °C, then I_2, −30 °C; (c) 1-(3,5-dimethoxy- phenyl)piperazine, EDCI, HOBt, NMM, CH_2Cl_2; (d) arylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane.

required for dual effects (Table S1, Supporting Information). On the basis of these preliminary data, we hypothesized that these tested compounds might bind to tubulin in the same way as colchicine. Therefore, we further carried out molecular-modeling studies to design a series of analogs that occupy the colchicine binding domain of tubulin. We selected compound **3**, one of the most potent cytotoxic agents in the screening library, docked in the colchicine binding domain, and we superimposed this with colchicine and **2** in the crystal structures, as shown in Figure 1B,C (PDB codes 3UT5 and 3HKC, respectively). Compound **3** appeared to fit in the colchicine binding site and partially overlapped with a docked colchicine in the hydrophobic binding pocket, as shown in Figure 1B. The estimated binding energy of compound **3** (GScore = −4.88 kcal/mol) was much higher than that of colchicine **1** (GScore = −6.789 kcal/mol). It has been reported that the A ring with three methoxy groups serves as an anchor to place the C ring of colchicine in the proper orientation within the binding site.[14,15] Therefore, the apparent lack of this anchoring effect in **3** may lead to a slight perturbation inside the binding pocket, resulting in the higher binding energy. However, it should be noted that compound **3** has an extra carbonyl group, which provides an additional hydrogen bond with the amide nitrogen of Ala317 in the backbone of β-strand 9 compared to colchicine. Interestingly, the methyl group of the oxazole ring in **3** is pointed toward α-tubulin T5 loop, which also appears to partially overlap with the N-acetyl substituent of colchicine. Conformational changes of the T5 loop are known to be involved in the assembly of tubulin $\alpha\beta$ heterodimers, indicating that the methyl group of the oxazole moiety may be critical for its biological activity.[16,17]

When compound **3** was docked in the colchicine binding domain, **3** appeared to overlap very well with the bound **2**, as shown in Figure 1C. However, the estimated binding energy of compound **3** (GScore = −4.88 kcal/mol) was also higher than that of **2** (GScore = −6.89 kcal/mol). One possible explanation is that the hydroxyl group on the aniline moiety of **2** forms hydrogen bonds with Tyr202 and Val238, which is absent in **3**. However, the 3-chlorophenyl ring of **3** extended deeper to strand S6 of the N-terminal nucleotide binding domain; thus, it provided additional interactions with hydrophobic residues, such as Leu252, Leu242, Val238, Leu255, and Tyr202. On the basis of the binding mode analyses of compound **3**, the oxazole analogs fit in the colchicine binding domain and can be further optimized by additional structural modifications. Therefore, a series of analogs containing the various substituted aryl oxazole and thiazole group was designed and synthesized.

CHEMISTRY

Aryl oxazole and thiazole derivatives were prepared according to Scheme 1. Oxazole and thiazole cores were synthesized from commercially available ethyl 3-oxo-3-phenyl-propanoate in the presence of HDNIB ([hydroxy(2,4-dinitrobenzene-sulfonyloxy)iodo]benzene) in one pot. The reaction mixture first formed an α-sulfonyloxyl ketone as an intermediate, and upon the treatment of acetamide (or thioacetamide) in combination with a microwave irradiation at 80 W for 1 min, heterocyclic core **4** was obtained in over 20% yield. Hydrolysis of **4** afforded the acid fragment **5** in 80% yield. A peptide coupling reaction was carried out with various aryl piperazines using EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) to obtain the desired derivatives, series **6** and **7**, in over 60% yield. Most of the substituted arylpiperazines were commercially available, but 1-(3-ethoxyphenyl)piperazine, 1-(2,3-dichlorobenzyl)piperazine, 1-(benzo[*d*][1,3]dioxol-5-yl)-piperazine, 3-(piperazin-1-yl)benzonitrile, 2-(3-(piperazin-1-yl)phenyl)propan-2-ol, 1-(3,5-dimethoxyphenyl)piperazine, 1-(3,5-dichlorophenyl) piperazine, 1-(5-chloro-2-methylphenyl)-piperazine, and 1-(3,5-difluoro-phenyl)piperazine were synthesized by employing an S_N2 reaction with substituted anilines and bis(2-chloroethyl)amine (Scheme S1, Supporting Information).

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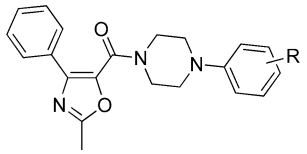
Table 1. Cytotoxic Effects of (2-Methyl-4-phenyloxazol-5-yl)(4-phenylpiperazin-1-yl)methanone Derivatives against Human Leukemia Cells (HL-60)



	R	IC_{50} (nM)[a]		R	IC_{50} (nM)[a]
3	3-chloro	39.2	**6-15**	4-pro-1-en-2-yl	NC
6-1	H	39.1	**6-16**	4-isopropanoyl	NC
6-2	3-methoxy	34.8	**6-17**	3-carbamoyl	71
6-3	3-ethoxy	151	**6-18**	benzo[1,3]dioxol-5-yl	251
6-4	3-cyano	39.6	**6-19**	2,6-dimethyl	146.1
6-5	4-fluoro	>1000	**6-20**	3,5-dimethoxy	19.2
6-6	3-fluoro	45	**6-21**	2,4-dimethyl	100.5
6-7	2-fluoro	146.8	**6-22**	3,4-dimethyl	43
6-8	4-trifluoromethyl	>1000	**6-23**	2,3-dimethyl	35
6-9	3-trifluoromethyl	152.9	**6-24**	2,5-dimethyl	31.3
6-10	2-trifluoromethyl	119.5	**6-25**	3,4-dichloro	124.3
6-11	2-chloro	124.1	**6-26**	3,5-dimethyl	34.5
6-12	4-methyl	>1000	**6-27**	3,5-dichloro	41.8
6-13	3-aminoacetyl	329	**6-28**	2,3-dichloro	133
6-14	4-acetyl	NC	**12** (CYT997)		112.3

[a]IC_{50} = compound concentration required to inhibit cell growth by 50% after cells were treated with compounds for 72 h. Data are expressed as the mean from at least three independent experiments.

Isoxazole derivatives with a methyl substituent were prepared according to Scheme 2. (*E*)-Acetaldehyde oxime was treated with triethylamine and *N*-chlorosuccinimide successively followed by the addition of propargyl alcohol to afford the isoxazole core. The primary alcohol group was then converted to a carboxylic acid via Jones oxidation to obtain the isoxazole fragment **8**. Iodination of intermediate **8** using *n*-BuLi and iodine produced compound **9** in 30% yield. It should be noted that this iodination step requires a constant temperature at −78 °C and absolute anhydrous conditions to obtain the desired product. After a peptide coupling reaction with 1-(3,5-dimethoxyphenyl)piperazine, intermediate **10** was obtained and further subjected to Suzuki cross-coupling with arylboronic acids to give a series of the isoxazole analogues, **11**, in over 60% yield.

RESULTS AND DISCUSSION

Structure−Activity Relationship and in Vitro Cytotoxicity. Cytotoxic effects of all of the synthesized derivatives were first evaluated by measuring cell viability of human leukemia cells (HL-60) upon being treated with each compound at different concentrations. CYT997[18] (**12**), a known cytotoxic vascular-disrupting agent, was also tested as a reference. Among the oxazole derivatives with a phenyl group (Table 1), the 3-substituted aryl piperazine derivatives (**3**, **6-2**, **6-4**, and **6-6**) showed good cytotoxic effects, having IC_{50} values in the low nanomolar range. Despite having substituents with different electronegativity values, all four compounds have similar IC_{50} values (35−45 nM), suggesting that electronegativity on the 3-position does not affect cytotoxicity significantly; however, when a fluoride was placed on either 2- or 4-position (**6-5** and **6-7**), the IC_{50} values increased markedly. It should be also noted that if the 3-position is substituted with slightly bulkier groups, such as trifluoromethyl (**6-9**) or aminoacetyl group (**6-13**), then the IC_{50} values increased up to 2- to 3-fold. Additionally, when these bulkier groups were placed on the 4-position, these compounds completely lost cytotoxicity, indicating that the size and the position of the substituents seem to be more important factors rather than electronegativity. Some of the derivatives with multiple substituents also showed excellent cytotoxicity, for example, 3,5-dimethoxy-substituted compounds (**6-20**) exhibited the lowest IC_{50} value (19.2 nM) among this series. Both 3,5-dimethyl- and -dichloro-substituted derivatives also demonstrated good cytotoxicity; however, when these substituents were moved to different positions, such as 2,6-, 2,4-, or 2,3-, these compounds became much less potent, again indicating that a steric factor is critical for the cytotoxicity of these derivatives.

To investigate the effect of the substituents on the phenyl ring directly linked to the oxazole core, we modified the phenyl ring with methoxy groups (**6-29**−**6-31**), hydrophilic groups (**6-32**−**6-37**), and halogen groups (**6-38**−**6-63**) and tested cytotoxicity as shown in Table 2. All of the derivatives with hydrophilic substituents and methoxy groups (**6-29**−**6-37**) became much less potent compared to their nonsubstituted counterparts, suggesting that van der Waals interaction may play an important role in this particular binding region. All fluoro-substituted derivatives with 3,5-dimethoxy on R_2 (**6-51**−**6-53**) exhibited excellent cytotoxic effect, with IC_{50} values ranging from 10.3 to 38.5 nM. When the 3,5-dimethoxy groups were replaced with a 3-chloro substituent (**6-57**−**6-59**), little or no effects were observed, confirming that electronegativity on this position does not affect overall binding. Interestingly, among all of the oxazole compounds in this series, only the derivatives with the 3,5-dimethoxy groups on the R_2 position showed slightly improved activity compared to the parent compound without any substituent (**6-1**, R_1 = R_2 = H), suggesting that hydrogen-bond interactions may exist in this region.

Next, we replaced the methyl group on the oxazole core with various alkyl groups to see if the bulkier substituent affects

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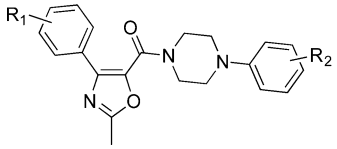
Table 2. Cytotoxic Effects of (2-Methyl-4-phenyloxazol-5-yl)(4-phenylpiperazin-1-yl)methanone Derivatives against Human Leukemia Cells (HL-60)



	R_1	R_2	IC_{50} (nM)[a]
6-29	4-methoxy	3-chloro	NC
6-30	3,4-dimethoxy	3-chloro	NC
6-31	3-methoxy	3-chloro	NC
6-32	3-hydroxy	3-chloro	104.9
6-33	3-sodium phosphate	3-chloro	>1000
6-34	4-hydroxy-3-methoxy	3-chloro	85.9
6-35	3-methoxy-4-sodium phosphate	3-chloro	303
6-36	3-hydroxy-4-methoxy	3-chloro	NC
6-37	3-aminoacetyl	3-chloro	208
6-38	2-fluoro	4-pro-1-en-2-yl	NC
6-39	4-fluoro	4-chloro	NC
6-40	4-chloro	3-isopropanoyl	NC
6-41	4-fluoro	2,5-dimethyl	358
6-42	2-fluoro	2,5-dimethyl	318.9
6-43	4-fluoro	4-isopropanoyl	NC
6-44	3-fluoro	2,5-dimethyl	204.2
6-45	2-chloro	2,5-dimethyl	>1000
6-46	3-chloro	2,5-dimethyl	345.2
6-47	4-chloro	2,5-dimethyl	NC
6-48	2-fluoro	3,5-dichloro	60.2
6-49	3-fluoro	3,5-dichloro	41.7
6-50	4-fluoro	3,5-dichloro	52.1
6-51	2-fluoro	3,5-dimethoxy	10.3
6-52	3-fluoro	3,5-dimethoxy	28.2
6-53	4-fluoro	3,5-dimethoxy	38.5
6-54	2-fluoro	3,5-difluoro	186.2
6-55	2-fluoro	5-chloro-2-methyl	228.7
6-56	2-fluoro	5-chloro-2-methoxy	152.9
6-57	2-fluoro	3-chloro	39.4
6-58	3-fluoro	3-chloro	33.7
6-59	4-fluoro	3-chloro	40.1
6-60	2-chloro	3-chloro	132.4
6-61	2-fluoro	3-methoxy	56.1
6-62	3-fluoro	3-methoxy	NC
6-63	4-fluoro	3-methoxy	112.3

[a] IC_{50} = compound concentration required to inhibit cell growth by 50% after cells were treated with compounds for 72 h. Data are expressed as the mean from at least three independent experiments.

cytotoxicity. All of the modified analogues (**6-64**–**6-84**) exhibited a marked decrease in cytotoxicity, as shown in Table 3. As the size of the substituent becomes bigger, these derivatives completely lost cytotoxicity, suggesting that the oxazole binding region can accommodate only a small substituent for favorable interaction. We also modified the oxazole core to thiazole (**7-1**–**7-2**) and isoxazole (**11-1**–**11-5**) to investigate the structural effects of the main core on cytotoxicity, as shown in Table 4. We chose to modify the oxazole core of compound **6-51**, which showed the lowest IC_{50} value (10.3 nM). Unfortunately, all of the derivatives with a thiazole or an isoxazole core became significantly less potent, showing 10–20-fold increased IC_{50} values, which indicates that having an oxazole core is critical for cytotoxicity. We also replaced the arylpiperazine group on the oxazole with various heterocycles including arylpiperidines and homopiperazines (Supporting Information, Tables S2 and S3); however, all of the modified compounds in this series completely lost cytotoxicity, which indicates that both of the nitrogen atoms in the piperazine contribute to possible hydrogen-bond interactions in this region. In addition, the spatial arrangement of the substituted aryl group also appears to be crucial for proper binding.

Additionally, we tested several selected compounds against various cancer cell lines including human lung cancer cells and colon cancer cells, as described in Table 5. Not surprisingly, all of the tested compounds showed moderate to potent cytotoxicity similar to what these compounds already demonstrated against HL-60 cells. It was also observed that compounds with low IC_{50} values against HL-60 (**6-51**–**6-53**) showed low IC_{50} values against other cancer cell lines,

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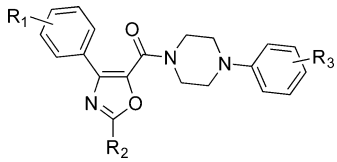
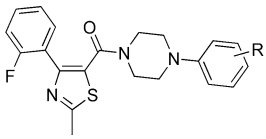
Table 3. Cytotoxic Effects of (2-Substituted-4-phenyloxazol-5-yl)(phenylpiperazin-1-yl)methanone Derivatives against Human Leukemia Cells (HL-60)

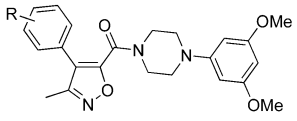


	R_1	R_2	R_3	IC_{50} (nM)[a]
6-64	2-fluoro	ethyl	3-chloro	138.8
6-65	2-fluoro	*t*-butyl	3-chloro	>1000
6-66	2-fluoro	cyclohexyl	3-chloro	NC
6-67	2-fluoro	trifluoromethyl	3-chloro	NC
6-68	3-fluoro	ethyl	3-chloro	317.3
6-69	3-fluoro	trifluoromethyl	3-chloro	NC
6-70	4-fluoro	ethyl	3-chloro	168.5
6-71	4-fluoro	trifluoromethyl	3-chloro	NC
6-72	2-chloro	ethyl	3-chloro	98.9
6-73	3-chloro	trifluoromethyl	3-chloro	NC
6-74	4-methoxy	ethyl	3-chloro	>1000
6-75	4-methoxy	propyl	3-chloro	NC
6-76	3,4-dimethoxy	ethyl	3-chloro	NC
6-77	3,4-dimethoxy	propyl	3-chloro	NC
6-78	2-fluoro	ethyl	3-methoxy	606.7
6-79	3-fluoro	ethyl	3-methoxy	200.5
6-80	3-fluoro	trifluoromethyl	3-methoxy	NC
6-81	*t*-butyl	ethyl	3-methoxy	NC
6-82	isopropyl	ethyl	3-methoxy	886.5
6-83	4-fluoro	ethyl	3-methoxy	410.6
6-84	4-fluoro	trifluoromethyl	3-methoxy	NC

[a]IC_{50} = compound concentration required to inhibit cell growth by 50% after cells were treated with compounds for 72 h. Data are expressed as the mean from at least three independent experiments.

Table 4. Cytotoxic Effects of (2-Methyl-4-phenylthiazol)-5-yl)methanone Derivatives and (3-Methyl-4-phenylisoxazol-5-yl)methanone Derivatives against Human Leukemia Cells (HL-60)





	R	IC_{50} (nM)[a]		R	IC_{50} (nM)[a]
7-1	3,5-dimethoxy	96.8	**11-1**	H	152.3
7-2	3,5-dichloro	156.8	**11-2**	2-fluoro	165.3
			11-3	3-fluoro	151.9
			11-4	4-fluoro	475.3
			11-5	2-furyl	191.4

[a]IC_{50} = compound concentration required to inhibit cell growth by 50% after cells were treated with compounds for 72 h. Data are expressed as the mean from at least three independent experiments.

confirming the potent antiproliferative activities of these compounds.

Vascular-Disrupting Activity (HUVEC Assay). To determine the vascular-disrupting effect of the synthesized compounds, we selected 18 compounds with low IC_{50} values ranging from 10 to 100 nM for further evaluation. We incubated human umbilical vein endothelial cells (HUVECs) with each compound at different concentrations to calculate the effective concentration to inhibit 50% of HUVEC growth (GI_{50}). In addition, the GI_{50} value for each compound was measured at the different densities of HUVECs (HUVEC distribution, ng/mL) where inhibition of cell growth was observed. The relative ratio of GI_{50} versus HUVEC distribution (normalized GI_{50}) was also calculated and is shown in Table 6.

Table 5. Cytotoxic Effects of Selected Compounds against Various Cancer Cell Lines[a]

	IC_{50} (nM)[b]		
	H-460	HCT-116	A-549
6-34	211	375	302
6-48	165	319	204
6-49	221	366	354
6-50	243	484	296
6-51	42	26	27
6-52	67	216	98
6-53	68	404	158
12	272	356	338

[a]H-460, human lung cancer cells; HCT-116, human colorectal carcinoma cell line; A-549, human lung adenocarcinoma cell line. [b]IC_{50} = compound concentration required to inhibit cell growth by 50% after cells were treated with compounds for 72 h. Data are expressed as the mean from at least three independent experiments.

Table 6. Effects of Selected Compounds on the Capillary Formation by Human Umbilical Vein Endothelial Cells (HUVECs)

	HUVEC distribution (ng/mL)[a]	GI_{50} (ng/mL)[b]	b/a
3	100	463	4.6
6-1	300	9,631	32.1
6-4	100	2,520	25.2
6-6	100	1052	10.5
6-20	30	319	10.6
6-22	300	3522	11.7
6-23	100	380	3.8
6-24	100	1732	17.3
6-26	100	356	3.6
6-27	100	191	1.9
6-32	1000		
6-34	300		
6-48	100	177	1.77
6-49	100	134	1.34
6-50	300		
6-51	30	118	3.93
6-52	100	126	1.26
6-53	300		
12	100	2098	20.98

[a]Data represents the starting concentrations of each compound for the disruption of capillary-like structure formation. [b]GI_{50} = effective concentration of the tested compounds to inhibit 50% of HUVEC growth.

Representative images of the HUVEC growth assay are shown in Figure 2 in comparison with **12** (CYT997, IC_{50} = 112.3 nM) as reference. The two most cytotoxic compounds, **6-20** (R_1 = H, R_2 = 3,5-dimethoxy, IC_{50} = 19.2 nM) and **6-51** (R_1 = 2-F, R_2 = 3,5-dimethoxy, IC_{50} = 10.3 nM) inhibited the growth of HUVECs at the lowest concentration, 30 ng/mL, whereas **6-32** (R_1 = 3-hydroxy, R_2 = 3-chloro, IC_{50} = 104.9 nM), which showed the highest IC_{50} among the selected compounds,

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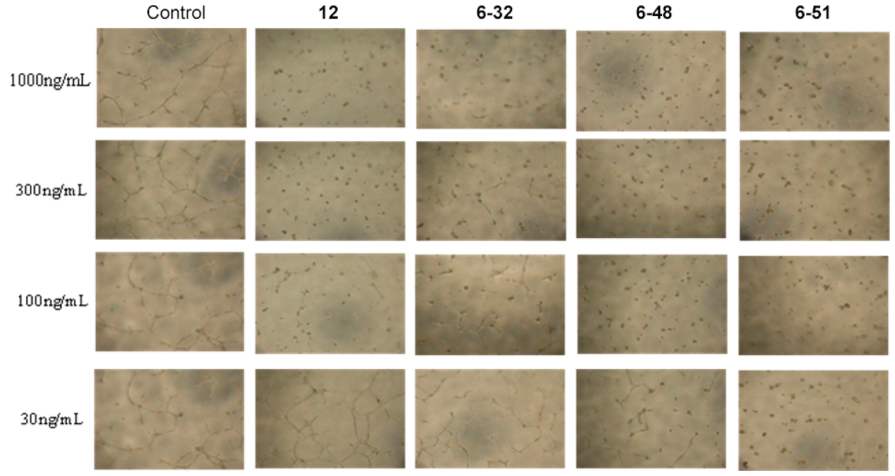

Figure 2. Representative images of HUVECs (human umbilical vein endothelial cells) in the vascular-disrupting activity assay. HUVECs were placed onto a thick layer of Matrigel in the presence of vehicle (control) or tested compounds at the indicated concentrations.

inhibited the growth of HUVECs at only 1000 ng/mL. All other compounds inhibited growth of HUVEC at 100−300 ng/mL. This result indicates that the in vitro cytotoxicity of these compounds correlates with their vascular-disrupting activity.

Tubulin Polymerization Inhibition (TPI Test). To examine the ability to block tubulin polymerization of the synthesized compounds, we tested several compounds with high cytotoxicity, as shown in Table 7. Interestingly, all tested compounds inhibited tubulin polymerization at lower concentrations compared to known polymerization inhibitor **13** (AC7739),[19] indicating that cytotoxicity and vascular-disrupting activity of these compounds are due to the inhibition of tubulin polymerization during mitosis.

Table 7. Inhibition of Tubulin Polymerization of Selected Compounds

	IC_{50} (μg/mL)[a]
6-1	0.62
6-23	0.53
6-27	0.67
6-48	0.73
6-49	0.77
6-51	0.71
13 (AC7739)	1.01

[a]The tubulin concentration was 4 mg/mL. Data are expressed as the mean from at least three independent experiments.

Microsomal Stability. To evaluate metabolic stability, we selected 14 compounds that showed good vascular-disrupting effects at the concentrations less than 100 ng/mL and measured microsomal stability using liver microsomes from human and mouse (Table 8). We incubated each compound in buffers containing liver microsomes and measured the quantity of remaining drugs after 1 h. Compounds **6-23** and **6-48** were the most stable, having 83.6 (in mouse liver microsomes) and 82.3% (in human liver microsomes) remaining, respectively. It was remarkable that over 70% of **6-34** was remaining in both microsomes (70.3% for human, 72.5% for mouse), and we believe that hydrophilic groups on the R_1 position may contribute to metabolic stability in liver microsomes despite having low cytotoxicity and weak vascular-disrupting ability.[20] The compounds with halide groups on R_2 (**6-48**−**6-50**, R_2 = 3,5-dichloro) seemed more stable in human liver microsomes than in mouse liver microsomes, and these compounds also showed much higher stability than the compounds with methoxy groups (**6-51**−**6-53**, R_2 = 3,5-dimethoxy). It was disappointing that compounds **6-51**−**6-53** demonstrated low metabolic stability because these compounds showed excellent cytotoxic effects and vascular-disrupting activity in vitro. Overall, we decided to move forward with compounds **6-48** (R_1 = 2-F, R_2 = 3,5-dimethyl) and **6-51** (R_1 = 2-F, R_2 = 3,5-dimethoxy) for further evaluation of their antitumor activity in vivo.

Table 8. Percentages of Remaining Drug after Incubation of Synthesized Compounds in the Presence of NADPH at 37 °C

	microsomal stability	
	human (%)	mouse (%)
3	13.5	2
6-4	8	1.6
6-20	15.8	0.7
6-23	29.8	83.6
6-24	33.8	1.4
6-26	25.6	12
6-27	66.3	43.4
6-34	70.3	72.5
6-48	82.3	49
6-40	75.9	40.7
6-50	71.8	31.1
6-51	15	16.6
6-52	11.3	11.6
6-53	12.9	5.6
12	54.6	87.5

Tumor Growth Inhibition in Vivo. Considering in vitro biological activity and metabolic stability, we selected **6-48** and **6-51** and evaluated antitumor activity in vivo using a simple tumor xenograft model (HCT-116, human colorectal carcinoma) (Figure 3). Tumor-bearing mice were injected intraperitoneally with each compound once a day for 14 days, and tumor volumes were determined by caliper measurements. After 5 days of the treatment with **6-48**, significant inhibition of tumor growth was observed at 50 and 100 mg/kg. When compared to untreated mice, the treatment with compound **6-**

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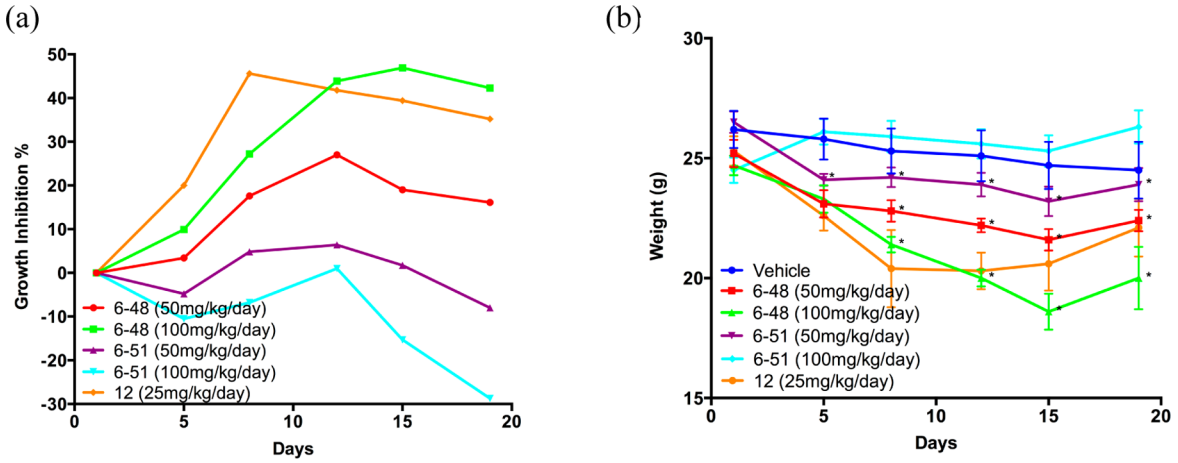

Figure 3. Antitumor effects of daily administration of compounds **6-48** and **6-51**: (a) tumor growth inhibition by **6-48** and **6-51** in HCT-116 colon cancer xenograft model (calculated on the basis of the vehicle control) and (b) changes in body weight over the course of the treatment.

48 reduced the size of the tumors to 42% at 100 mg/kg and 16.1% at 50 mg/kg, whereas 19% of body weight was lost at 100 mg/kg and 10.8% at 50 mg/kg. When we injected 200 mg/kg of **6-48**, tumor growth was inhibited markedly; however, two out of six mice died during the experiment, indicating that **6-48** is toxic at high concentrations. Unfortunately, despite having potent activity in vitro, the treatment of **6-51** did not affect tumor growth in vivo and did not seem to change body weight. On the basis of the microsomal stability test, we believe that because of poor metabolic stability **6-51** may have degraded during systemic circulation before the compound reached its therapeutic target.

CONCLUSIONS

We have developed a series of aryloxazole, thiazole, and isoxazole for antimitotic and vascular-disrupting agents. In vitro cytotoxicity was evaluated using various tumor cell lines, and tumor vascular-disrupting activity was also tested using a HUVEC assay. Several compounds with excellent cytotoxicity and vascular-disrupting activity were selected, and metabolic stability and inhibition of tubulin polymerization were examined. As a result, the two most potent compounds, **6-48** and **6-51**, were chosen for further in vivo evaluation of antitumor activity. Compound **6-51** (R_1 = 2-F, R_2 = 3,5-OMe) exhibited the highest cytotoxicity, with an IC_{50} value of 10.3 nM against HL-60, and excellent vascular-disrupting activity. Although compound **6-51** seemed only moderately stable in liver microsomes (15% for human, 16.6% for mice), compound **6-48** (R_1 = 2-F, R_2 = 3,5-Cl) demonstrated an outstanding microsomal stability (82.3% for human, 49% for mice), although it showed relatively lower cytotoxicity (IC_{50} = 60.5 nM) and vascular-disrupting activity compared to **6-51**. Both of these compounds inhibited tubulin polymerization at low concentrations, suggesting that their biological activity comes from tubulin binding. When injected into tumor-bearing mice, compound **6-48** effectively reduced tumor growth (42.3% in size) at 100 mg/kg; however, compound **6-51** did not affect tumor growth, apparently because of its poor metabolic stability.

We have developed a dual-action anticancer agent containing an aryloxazole moiety. Compound **6-48** showed an excellent antimitotic effect and vascular-disrupting activity in vitro and demonstrated promising antitumor activity in vivo, possibly because of its metabolic stability. It is interesting to note that **6-48** showed greater effects on tumor growth in vivo despite having a much lower in vitro potency than **6-51**. Considering that many tubulin binders are too toxic and have short half-lives, which prevents them from shutting down tumor vasculature effectively and thus possibly leaving an outer rim of viable cancer cells,[21] we believe that this compound will serve as an excellent lead to discover potent dual-acting agents.

EXPERIMENTAL SECTION

Docking Studies. The crystal structures of complexes (PDB codes 3UT5 and 3HKC) were initially prepared by adding hydrogen atoms at pH 7.4 and charges using the Protein Preparation Wizard in Maestro9.2. All waters were deleted during preparation. The ligand structures were drawn and prepared by ionizing at pH 7.4 using the LigPrep module in Maestro9.2. All ligand structures were minimized using the OPLS_2001 force field. The ligands were docked into the protein structures using Glide XP module in Maestro 9.2. A receptor grid was generated before docking the compounds. An outer box was used to generate the grid and accommodating ligands, and an inner box was used for ligand center site point search. The outer and inner boxes were set to lengths of less than 20 and 10 Å, respectively. For grid generation, the scaling factor for receptor atom van der Waals radius was set to 1.0.

Synthesis. *General Methods.* All chemicals were reagent grade and used as purchased. Moisture-sensitive reactions were performed under an inert atmosphere of dry nitrogen with dried solvents. Reactions were monitored by TLC analysis using Merck silica gel 60 F-254 thin-layer plates. Flash column chromatography was carried out on Merck silica gel 60 (230–400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker (AVANCE II) at 400 and 300 MHz for ^{1}H NMR and at 100 and 75 MHz for ^{13}C NMR. The coupling constants (J) are reported in Hz. High-resolution mass spectra (HRMS) were recorded on a LTQ Orbitrap (Thermo Electron Corporation). Microwave-mediated reactions were carried out in a Personal Chemistry (Biotage) microwave synthesizer. Purity was measured using a Waters e2695/2489 HPLC instrument (1 mL/min flow rate; H_2O/CH_3CN 90:10 → 0:100 in 15 min; 10 μL of injection volume; λ = 254, 280 nm). All test compounds exhibited >95% purity except fo compounds **6-30** and **6-34**, which were 94 and 90% pure, respectively.

Detailed synthetic procedures and characterization data for intermediates **4**, **5**, and aryl piperazine fragments can be found in the Supporting Information.

*Ethyl 2-Methyl-4-phenyloxazole-5-carboxylate (**4-1**).* Ethyl 3-oxo-3-phenylpropanoate (8 g, 41 mmol) was mixed with HDNIB (25 g, 54 mmol), prepared in advance, and 7.2 g of acetamide (0.12 mol) was

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added to the mixture. The reaction mixture was heated at 120 °C for 30 s under microwave irradiation (80 W). After being cooled to rt, the suspension was diluted with saturated $NaHCO_3$ solution (10 mL), extracted with EA, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash column chromatography on silica gel (EA/Hex 1:7) afforded desired product **1** (6.01 g, 26 mmol, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07−8.02 (m, 2H), 6.98−6.94 (m, 3H), 4.40 (q, J = 7.12 Hz, 2H), 2.57 (s, 3H), 1.43 (t, J = 7.14 Hz, 3H).

*2-Methyl-4-phenyloxazole-5-carboxylic Acid (**5**-**1**).* To a solution of **1** (1.74 g, 7.55 mmol) in ethanol (10 mL) was added 1 N NaOH solution (22.6 mL) at rt. The mixture was heated at 40 °C for 1 h. After cooling, the mixture was adjusted to pH 2 with 1 N HCl solution at 0 °C, and white solid was precipitated and filtered. The residue was extracted with EA (50 mL × 3), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. A white solid was obtained without purification as desired product **2** (1.22 g, 6.04 mmol, 80%). ^{1}H NMR (300 MHz, CH_3OD) δ 7.99 (q, J = 1.96 Hz, 2H), 7.42 (q, J = 1.80 Hz, 3H), 2.56 (s, 3H).

*General Procedure for Compounds **3** and **6**-**1** through **6**-**91**.* To a mixture of **5** (45.5 mg, 0.224 mmol), EDCI (90 mg, 0.448 mmol), HOBt (50 mg, 0.39 mmol), and NMM (40 μL, 0.39 mmol) in dry CH_2Cl_2 (10 mL) was added phenylpiperazine (40 μL, 0.27 mmol). After 1 h of stirring, water (10 mL) was added and extracted with CH_2Cl_2 (10 mL × 3), and the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/CH_3OH 40:1) to obtain the desired product.

*(4-(3-Chlorophenyl)piperazin-1-yl)(2-methyl-4-phenyloxazol-5-yl)methanone (**3**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75−7.73 (m, 2H), 7.41−7.36 (m, 3H), 7.15−7.13(m, 1H), 6.85−6.83 (m, 1H), 6.81(s, 1H), 6.80 (d, J = 2.04 Hz, 1H), 3.89 (br, 2H), 3.46 (br, 2H), 3.22 (br, 2H), 2.91 (br, 2H), 2.59 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.74, 159.86, 151.74, 140.58, 137.69, 135.05, 130.38, 130.20, 129.16, 128.80, 127.65, 120.33, 116.48, 114.52, 50.22, 48.80, 46.62, 42.13, 14.07. mp 191−193 °C. HPLC purity 99%. HR-MS m/z $[M + H]^+$ (ESI^+) calcd for $C_{21}H_{21}ClN_3O_2$, 382.1317; found, 382.1328.

*(2-Methyl-4-phenyloxazol-5-yl)(4-phenylpiperazin-1-yl)-methanone (**6**-**1**).* **6**-**1** was obtained in 48% yield (40 mg, 0.18 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.50 Hz, 2H), 7.44−7.37 (m, 3H), 7.27 (t, J = 7.91 Hz, 2H), 6.96−6.86 (m, 3H), 3.93 (br, 2H), 3.45 (br, 2H), 3.24 (br, 2H), 2.93 (br, 2H), 2.57 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.62, 159.87, 150.75, 140.38, 137.82, 130.43, 129.25, 129.06, 128.76, 127.61, 127.12, 120.72, 116.74, 50.22, 47.88, 14.03. mp 180−182 °C. HPLC purity 98%. HR-MS m/z $[M + H]^+$ (ESI^+) calcd for $C_{21}H_{22}N_3O_2$, 348.1707; found, 348.1723.

*(4-(3,5-Dichlorophenyl)piperazin-1-yl)(4-(2-fluorophenyl)-2-methyloxazol-5-yl)methanone (**6**-**48**).* ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (td, J = 7.51, 1.58 Hz, 1H), 7.41−7.34 (m, 1H), 7.27−7.22 (m, 1H), 7.15−7.09 (m, 1H), 6.84 (s, 1H), 6.71 (s, 2H), 3.86 (br, 2H), 3.53 (br, 2H), 3.22 (br, 2H), 3.03 (br, 2H), 2.65 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.64, 159.67 (d, J_{C-F} = 248.5 Hz), 159.43, 152.14, 140.02, 135.59, 135.10, 130.84 (d, J_{C-F} = 8.3 Hz), 130.30, 130.27, 124.60 (d, J_{C-F} = 3.4 Hz), 119.79, 118.85, 118.67, 115.92 (d, J_{C-F} = 21.7 Hz), 114.43, 48.39, 47.56, 14.02. mp 248−249 °C. HPLC purity 95%. FABMS (m/z): $[M^+ + H]$ calcd for $C_{21}H_{19}Cl_2FN_3O_2$. 434.0760; found, 434.0843.

*(4-(3,5-Dimethoxyphenyl)piperazin-1-yl)(4-(2-fluorophenyl)-2-methyloxazol-5-yl)methanone (**6**-**51**).* ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (td, J = 7.51, 1.27 Hz, 1H), 7.39−7.33 (m, 1H), 7.27−7.21 (m, 1H), 7.15−7.09 (m, 1H), 6.05 (s, 3H), 3.79 (br, 2H), 3.77 (s, 6H), 3.53 (br, 2H), 3.21−2.96 (br, 4H), 2.58 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.59, 161.54, 159.69 (d, J_{C-F} = 248.0 Hz), 159.40, 152.77, 140.19, 134.83, 130.73 (d, J_{C-F} = 8.5 Hz), 130.30, 130.26, 124.52 (d, J_{C-F} = 3.3 Hz), 118.94, 118.75, 115.92 (d, J_{C-F} = 21.7 Hz), 95.71, 92.34, 55.26, 49.30, 46.56, 42.14, 14.00. mp 221−222 °C. HPLC purity 95%. FABMS (m/z): $[M^+ + H]$ calcd for $C_{23}H_{25}FN_3O_4$, 426.1751; found, 426.1829. HR-MS m/z $[M + H]^+$ (ESI^+) calcd for $C_{23}H_{25}FN_3O_4$, 426.1824; found, 426.1841.

*3-Methylisoxazole-5-carboxylic Acid (**8**).* To a solution of acetaldehyde oxime (1 g, 16.9 mmol) in dry THF (15 mL) was added NCS (2.7 g, 20 mmol) at rt. After 2 h of stirring, a diluted solution of propargyl alcohol (1.42 g, 25 mmol) in dry THF (4 mL) was added slowly, and after 30 min, a diluted solution of TEA (2.78 mL, 20 mmol) in dry THF was added dropwise successively. After 1 h with vigorous stirring, water (10 mL) was added to the reaction mixture. The suspension was extracted with EA (20 mL × 3), and the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash column chromatography on silica gel (EA/Hex 1:1) afforded (3-methylisoxazol-5-yl)methanol (0.93 g, 8.28 mmol, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.09 (s, 1H), 4.73 (s, 2H), 2.52 (br, 1H), 2.34 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.09, 159.85, 102.53, 56.46, 11.37.

To a solution of (3-methylisoxazol-5-yl)methanol (0.5 g, 4.42 mmol) in acetone (19 mL) was added Jones reagent (2.21 mL) at 0 °C, and the mixture was stirred for 3 h. The reaction was monitored by TLC, and after termination of the reaction, the mixture was adjusted to pH 2 with 1 N HCl solution. The residue was extracted with EA (20 mL × 3), and the combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash column chromatography on silica gel (EA/Hex 1:1) afforded desired product **8** (0.63 g, 4.97 mmol, 100%). ^{1}H NMR (300 MHz, CH_3OD) δ 6.90 (s, 1H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, CH_3OD) δ 161.05, 160.82, 158.16, 109.46, 9.81.

*4-Iodo-3-methylisoxazole-5-carboxylic Acid (**9**).* To a solution of **8** (85 mg, 0.66 mmol) in dry THF (5 mL) was added *n*-BuLi (2.64 mL, 2 M in hexane) at −78 °C. After the mixture was stirred for 1 h, iodine (0.2 g, 0.8 mmol) in dry THF was added dropwise at −30 °C. After 5 h with vigorous stirring at −78 °C, 1 N HCl solution (2 mL) was added to the reaction mixture. This suspension was extracted with EA (5 mL × 3), and the combined organic layer was washed with Na_2SO_3 satd solution and brine water (5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash column chromatography on silica gel (CH_2Cl_2/CH_3OH 5:2) afforded desired compound **6** (48 mg, 0.19 mmol, 29%). ^{1}H NMR (300 MHz, CH_3OD) δ 2.34 (s, 3H). ^{13}C NMR (75 MHz, CH_3OD) δ 163.99, 159.88, 157.70, 67.76, 11.06.

*(4-(3,5-Dimethoxyphenyl)piperazin-1-yl)(4-iodo-3-methylisoxazol-5-yl)methanone (**10**).* In a flask, 4-iodo-3-methylisoxazole-5-carboxylic acid **6** (30 mg, 0.12 mmol), EDCI (33 mg, 0.18 mg), HOBt (23 mg, 0.18 mg), and NMM (0.02 mL, 0.18 mmol) were dissolved in 5 mL of dry CH_2Cl_2. The reaction mixture was stirred for 1 h. 1-(3,5-Dimethoxyphenyl)piperazine (32 mg, 0.14 mmol) was added in the reaction mixture and stirred for 1 h. Water (5 mL) was added to the mixture, the mixture was extracted with CH_2Cl_2, and the organic layer was washed with Na_2SO_3 satd solution (5 mL) and brine water (5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash column chromatography on silica gel (EA/Hex 3:5) afforded the desired compound (21 mg, 0.046 mmol, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.10 (q, J = 1.46 Hz, 3H), 3.92 (t, J = 4.64 Hz, 2H), 3.82 (s, 6H), 3.65 (t, J = 4.50 Hz, 2H), 3.28 (t, J = 5.16 Hz, 2H), 3.23 (t, J = 4.95 Hz, 2H), 2.18 (s, 3H).

*General Procedure for Compound **11**.* To a solution of **10** (21 mg, 0.5 mmol), phenyl-boronic acid (7 mg, 0.55 mmol), and $Pd(PPh_3)_4$ (44 mg, 3.5×10^{-7} mol) in dry dioxane (5 mL) was added sodium carbonate (8 mg, 0.75 mmol) in water (1.0 mL) at rt. The reaction mixture was heated at 100 °C for 10 h. After being cooled to rt, the mixture was filtered over a Celite pad, and the filtrate was evaporated to dryness. The residue was purified by flash column chromatography on silica gel (EA/Hex 1:2), affording the desired compound.

Biological Assays. *Cytotoxicity Assay.* The HL-60 (leukemia) cell line was obtained from American Type Culture Collection (ATCC, USA). HL-60 cells were grown in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) at 37 °C under a humidified 5% CO_2 atmosphere. All cells were seeded into 96-well plates, and the compound diluents were added. After a 72 h incubation at 37 °C in a humidified 5% CO_2 atmosphere, cell viability was determined by addition of MTT (final concentration 0.25 mg/mL).

HUVEC Tube-Disruption Assay. HUVECs were obtained from Modern Cell & Tissue Technologies (MCTT, Korea). HUVECs were cultured in endothelial cell growth medium (EGM-2) and used between passages 3 and 7. HUVECs were seeded on a Matrigel-coated 96-well plate. After capillary-like tube formation was observed, HUVECs were treated with DMSO or serial-diluted test compounds for 1 h.

Tubulin Polymerization Assay. Tubulin polymerization was carried out using a kit supplied by Cytoskeleton. A DMSO solution of the test compounds was prepared at varying concentrations (10, 3, 1, and 0.3 μg/mL). Compounds that interact with tubulin alter the polymerization of tubulin can be detected using a spectrophotometer (FlexStation, Molecular Devices, LLC). The absorbance at 340 nm over a period of 1 h at 37 °C was monitored. The experimental procedure of the assay was performed as described in version 8.2 of the tubulin polymerization assay kit manual.

Microsomal Stability. The reaction mixture consisted of potassium phosphate buffer (50 mM, pH 7.4), 2.5 mM magnesium chloride, NADPH-generating system (1.0 NADP, +1.0 NADPH, 2.5 glucose 6-phosphate, 1.0 U glucose 6-phosphate dehydrogenase), and testing compounds (final concentrations at 5 μM) in a total volume of 200 μL. The reaction mixture was incubated at 37 °C for 60 min in a shaking water bath, and the reaction was stopped by adding 200 μL of acetonitrile. The samples were then spun at 13 000 rpm for 5 min, and aliquots (10 μL) were analyzed by LC/MS. The metabolic stability of test compounds was evaluated by measuring the disappearance of each compound compared to the control (no NADPH and NADP+) sample.

Inhibition of Tumor Growth in Vivo. Male BALB/C nu/nu mice were obtained from the Central Lab Animal Inc. (Seoul, Korea). Procedures involving animals and their care were conducted in conformity with institutional guidelines, which are in compliance with the Korean Animal Welfare Act. Mice were used between 5 to 6 weeks of age. The antitumor activity of selected compounds was measured in a human colorectal carcinoma (HCT-116) xenograft model in male BALB/C nu/nu mice. HCT-116 cells were implanted subcutaneously in the flanks of nude mice. Mice bearing tumors of approximately the same size (100–200 mm^3) were dosed intraperitoneally with vehicle or testing compounds once a day for 14 days. Tumor volumes were determined twice per week by caliper measurements and were recorded along with body weights and mortality as an indicator of gross toxicity.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Initial screening hits and anti-proliferative effects against various tumor cell lines, cytotoxic effects of (2-methyl-4-phenyloxazol (or phenylthiazol)-5-yl) methanone derivatives and (3-methyl-4-phenylisoxazol-5-yl)methanone derivatives against human leukemia cells (HL-60), cytotoxic effects of (2-methyl-4-phenyloxazol-5-yl) (phenylhomopiperazin-1-yl)methanone derivatives against human leukemia cells (HL-60), changes in tumor volume of compounds **6-48** and **6-51** treated mice, and detailed synthetic procedures and characterization data of all test compounds. 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.

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■ ABBREVIATIONS USED

VDA, vascular-disrupting agents; HUVECs, human umbilical vein endothelial cells; SAR, structure–activity relationship; IC_{50}, inhibitory concentration 50%; HR-MS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography

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