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Letter

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Synthesis and Structure–Activity Relationship Study of 1-Phenyl-1-(quinazolin-4-yl)ethanols as Anticancer Agents

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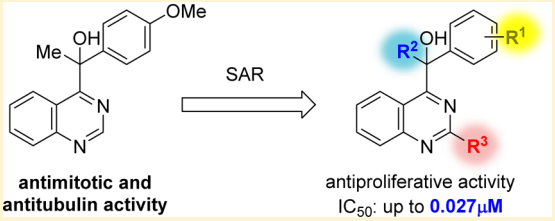
Supporting Information

ABSTRACT: A quinazoline derivative PVHD121 (**1a**) was shown to have strong antiproliferative activity against various tumor-derived cell lines, including A549 (lung), NCI-H460 (lung), HCT116 (colon), MCF7 (breast), PC3 (prostate), and HeLa (cervical) cells with IC_{50} values from 0.1 to 0.3 μM. A structure–activity relationship (SAR) study at the 2- and 4-position of the quinazoline core lead to the discovery of more potent anticancer agents (**14**, **16**, **17**, **19**, **24**, and **31**). The results of an *in vitro* tubulin polymerization assay and fluorescent-based colchicine site competition assay with purified tubulin indicated that **1a** inhibits tubulin polymerization by binding to the colchicine site.



KEYWORDS: *Quinazoline, anticancer, tubulin inhibitor, colchicine binding site, structure–activity relationship study*

Cancer is one of the most common causes of death worldwide.[1] According to the World Health Organization, cancer killed 7.6 million people in 2008, representing approximately 13% of all deaths. The number of cancer deaths is estimated to reach 13.1 million by 2030. Many bioactive compounds have been shown to possess anticancer activity.[2] However, their use is limited due to side effects or limited scope of activity. Thus, the identification of novel, potent drugs with fewer side effects and a broad spectrum of activity is desirable to improve cancer treatment. Among the available anticancer drugs, antimitotic agents, which interact with microtubules, are one of the most successful therapeutics.[3−8] Microtubules are a component of the cytoskeleton in eukaryotic cells and consist of tubulin protein polymers. These proteins can polymerize and depolymerize. Antimitotic agents interact with binding sites on tubulin, such as the colchicine site and the vinblastine site,[9] and inhibit or promote microtubule polymerization, thereby affecting their function.[3−8] A number of antimitotic compounds have been synthesized as promising drug candidates in many laboratories.[10]

In the course of random screening for anticancer agents, we discovered a quinazoline derivative, PVHD121 (**1a**), which had strong antiproliferative activity against the cervical cancer cell line, HeLa. Recently, there have been reports of quinazoline-based anticancer agents with amino groups at the 4-position of the core, rather than the 1-hydroxyethyl group in **1a**.[11−13] In the present study, we describe the results of a structure–activity relationship (SAR) study for the development of novel anticancer drugs and demonstrate the biological activity of **1a** and its derivatives for the first time.

The hit compound **1a** was originally prepared as a substrate in the development of a new synthetic catalysis.[14] According to the reported procedure, the synthesis of derivatives **1a–n**, which contain various aromatic rings on the substituents at the 4-position of the quinazoline nucleus, was initiated by NHC-catalyzed aroylation using an aldehyde, followed by a Grignard reaction with methylmagnesium bromide. Derivatives **4o–q**, which have methylamino, dimethylamino, and methylthio groups, were synthesized via nucleophilic aromatic substitution (S_NAr) of the fluoro group of **4b** by *N*- or *S*-nucleophiles and a subsequent Grignard reaction (Scheme 1).

The synthesis of compounds **5–8**, which have ethyl, benzyl, trifluoromethyl, and a hydrogen atom at the benzyl position instead of a methyl moiety, is shown in Scheme 2. The Grignard reaction between **4a** and ethylmagnesium iodide produced ethyl-substituted derivative **5**. Benzyl-substituted derivative **6** was similarly synthesized from **4a**. Trifluoromethylation of the keto group of **4a** using TMS-CF_3 and TBAF[15] yielded derivative **7**. The reduction of the keto group of **4a** using $NaBH_4$ afforded **8**.

Derivative **9**, which has a 1-(4-anisyl)-1-hydroxyethyl group at position 2 of the quinazoline core, instead of the 4-position, was also prepared from 2,4-dichloroquinazoline **13a** via substitutions of the 2- and 4-chloro groups and a Grignard reaction with methylmagnesium bromide (see Scheme S-1, Supporting Information).

The compounds having methyl, phenyl, trichloromethyl, and chloro groups at position 2 of the quinazoline (**14–17**) were synthesized using the same procedure as **1a–n** (Scheme 3). Specifically, NHC-catalyzed aroylation of 2-substituted 4-

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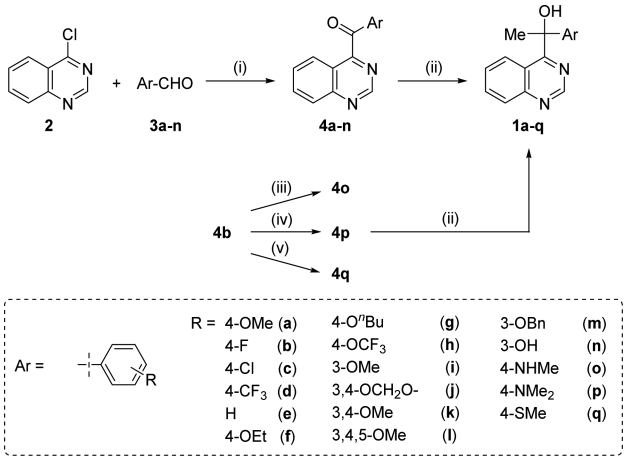
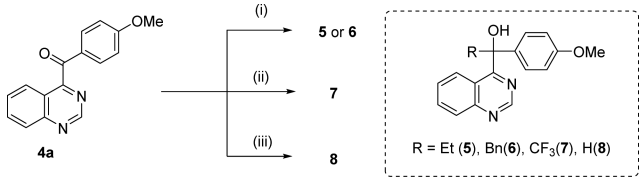
Scheme 1[a]



[a]Reagents and conditions: (i) *N*,*N*′-dimethylimidazolium iodide (catalyst), NaH, THF, reflux; (ii) MeMgBr, THF, rt; (iii) $MeNH_2$, 1,4-dioxane, reflux; (iv) Me_2NH, 1,4-dioxane, reflux; (v) NaSMe, 1,4-dioxane, reflux.

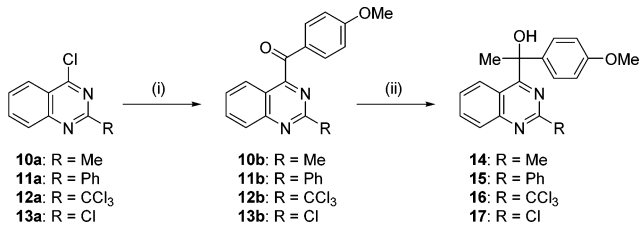
Scheme 2[a]



[a]Reagent and conditions: (i) RMgI, THF, rt; (ii) TMS-CF_3, TBAF, THF, 0 °C to rt; (iii) $NaBH_4$, ethanol, rt.

chloroquinazoline **10a**−**13a**[12,16−18] was performed, followed by Grignard reaction, yielding **14**−**17** (Scheme 3).

Scheme 3[a]



[a]Reagents and conditions: (i) *N*,*N*′-1,3-dimethylimidazolium iodide (catalyst), *p*-anisaldehyde, NaH, THF/DMF, reflux; (ii) MeMgI, THF, rt.

Synthesis of **18**−**34** from the 2-chloro derivative **17** was performed by S_NAr or Suzuki−Miyaura cross coupling (Scheme 4).[19,20]

Hit compound **1a** induced G2/M accumulation in human lung carcinoma A549 cells (Figure 1A). In addition, **1a** showed a broad spectrum of antiproliferative activity against various solid tumor-derived cell lines including A549 (lung), NCI-H460 (lung), HCT116 (colon), MCF7 (breast), PC3 (prostate), and HeLa (cervical) cells, with IC_{50} values ranging from 0.1 to 0.3 μM (see Figure S-1, Supporting Information).

The obtained derivatives **1b**−**q**, **5**−**9**, and **14**−**34**, along with both enantiomers of **1a** separated by chiral column chromatography were tested for cytotoxicity against the human lung cancer cell line A549 (Table 1). Growth inhibition by the quinazoline derivatives was evaluated by MTS assay.

Among the derivatives with various aromatic rings on the substituent at the 4-position of quinazoline, **1f** (containing a 4-ethoxyphenyl group, IC_{50} = 0.30 μM) and **1q** (containing a 4-methylthiophenyl group, IC_{50} = 0.34 μM) showed the same degree of antiproliferative activity as **1a** (IC_{50} = 0.27 μM). However, none of the derivatives had a higher antiproliferative activity than **1a** in A549 cells. Although their absolute configurations are not yet determined, (+)-**1a** showed stronger antiproliferative activity than (−)-**1a** (see Figure S-2, Supporting Information).

We found that several 2-substituted compounds had stronger antiproliferative activity against A549 than **1a**, including 2-methyl (**14**), 2-trichloromethyl (**16**), 2-chloro (**17**), 2-methoxy (**19**), 2-methythio (**24**), and 2-(3-thienyl) (**31**) substituted derivatives. In particular, the 2-chloro-substituted analogue **17** showed the highest activity (IC_{50} = 0.027 μM), which is ten times more effective than the hit compound **1a**. In constast, the introduction of 2-substituents, such as hydroxyl (the analogue with 2-hydroxyl group mainly exists as its tautomer **18**), propoxy (**21**), phenoxy (**23**), phenylamino (**26**), piperidin-1-yl (**27**), 4-methylpiperadin-1-yl (**28**), 4-(4-fluorophenyl)-piperadin-1-yl (**30**), and 3-chlorophenyl (**34**) significantly decreased activity.

We further investigated the biological target of the compounds. After A549 cells had been treated with three different concentrations of **1a** for 24 h, the cell cycle profiles were analyzed by flow cytometry. As shown in Figure 1A, **1a** clearly affected the cell cycle progression of A549 cells and accumulated cells in the G2/M phase in a concentration-dependent manner. We next performed immunofluorescent microscopy analysis of A549 cells treated with **1a**. Staining with DAPI and an anti-α tubulin antibody revealed that **1a** abrogated the microtubule networks like a well-known tubulin binder, colchicine (Figure 1B). We next assessed the effect of **1a** on *in vitro* tubulin polymerization using a fluorescence-based tubulin polymerization assay. As shown in Figure 1C, **1a** moderately inhibited the tubulin polymerization at 3 μM and more strongly inhibited it at 10 μM.

To gain further insight into the mechanism of microtubule destabilization, we investigated the binding site of **1a** on the tubulin protein using a fluorescent-based colchicine site competition assay with purified tubulin. Colchicine is known to show clear fluorescent properties when bound to tubulin (excitation at 350 nm and emission at 430 nm), although it is nonfluorescent by itself.[21] The fluorescence signal was robustly attenuated in the presence of nocodazole, which binds the colchicine-binding site (Figure 1D). In contrast, vinblastine, which preferentially binds to a noncolchicine binding site, did not diminish the fluorescence (Figure 1E). In this competition assay, the fluorescence was clearly attenuated by **1a** in a concentration-dependent manner (Figure 1D,E). These results suggest that **1a** has high affinity for the colchicine-binding site.

One of the derivatives (**1f**) had comparable antiproliferative activity to **1a** and showed similar inhibitory activity in the tubulin polymerization assay. The more potent inhibitor **15** inhibited tubulin polymerization more robustly than **1a** at 10 μM (see Figure S-3, Supporting Information). Furthermore, such correlation was also seen in the experiments using both enantiomers of **1a** (see Figure S-2, Supporting Information).

To develop a novel anticancer drug, various substituents at the 2- and 4-positions of quinazoline were synthesized via

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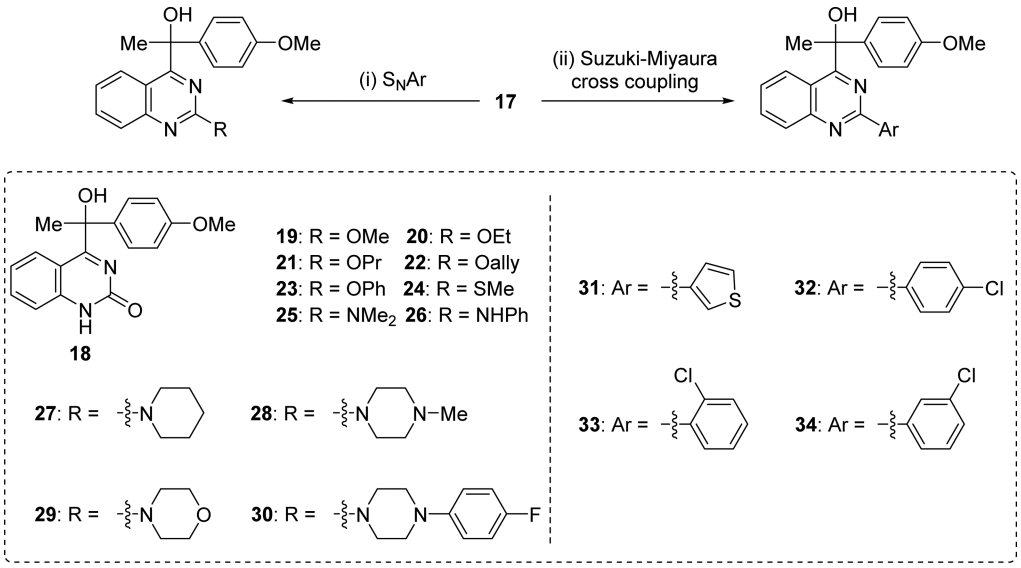
Scheme 4[a]



[a]Reagent and conditions: (i) NaOH, THF, reflux/R′ONa, MeOH, rt/MeSNa, 1,4-dioxane, reflux/amine, 1,4-dioxane, reflux (see Supporting Information for details); (ii) $ArB(OH)_2$, K_2CO_3, $Pd(OAc)_2$, 1,4-dioxane, reflux.

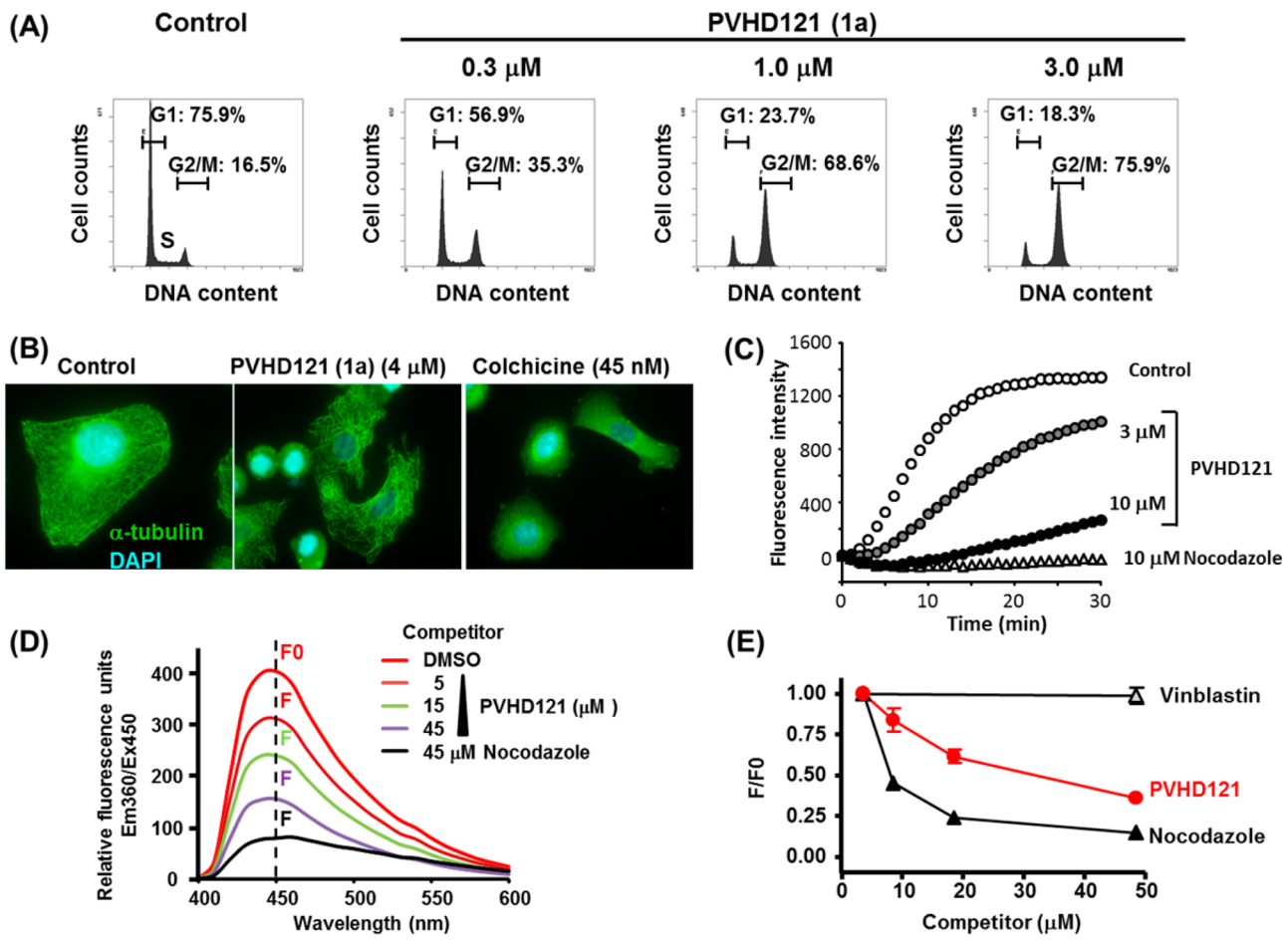


Figure 1. (A) Flow cytometric analysis of cell cycle distribution in A549 cells. (B) Immunomicroscopic analysis of tubulin destabilization in A549 cells. (C) *In vitro* tubulin polymerization assay. (D) Competitive displacement binding assay on the colchicine-binding site of tubulin. (E) Concentration-dependent binding of **1a** to the colchicine-binding site.

NHC-catalyzed aroylation. On the basis of the SAR studies, the 2-chloroquinazoline derivatives **14**, **16**, **17**, **19**, **24**, and **31** were shown to be the most potent analogues. In addition, our results suggest that this type of quinazoline derivative exhibits

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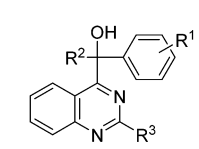
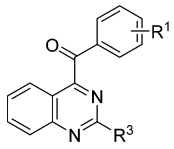
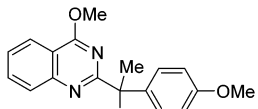
Table 1



1a-q, 5-8, 14-17,19-34



4a,4f



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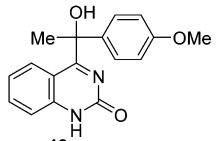

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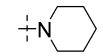
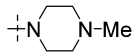
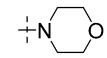
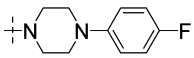
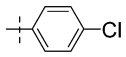
compound	R^1	R^2	R^3	IC_{50} (μM)	compound	R^1	R^2	R^3	IC_{50} (μM)
1a	4-OMe	Me	H	0.27	**16**	4-OMe	Me	CCl_3	0.038
1b	4-F	Me	H	>25	**17**	4-OMe	Me	Cl	0.027
1c	4-Cl	Me	H	>25	**18**				>25
1d	4-CF_3	Me	H	>25	**19**	4-OMe	Me	OMe	0.058
1e	H	Me	H	>25	**20**	4-OMe	Me	OEt	0.34
1f	4-OEt	Me	H	0.30	**21**	4-OMe	Me	OPr	1.2
1g	4-O^{n}Bu	Me	H	>25	**22**	4-OMe	Me	Oallyl	0.41
1h	4-OCF_3	Me	H	>25	**23**	4-OMe	Me	OPh	3.3
1i	3-OMe	Me	H	>25	**24**	4-OMe	Me	SMe	0.067
1j	3,4-OCH_2O-	Me	H	20	**25**	4-OMe	Me	NMe_2	0.21
1k	3,4-OMe	Me	H	21	**26**	4-OMe	Me	NHPh	2.7
1l	3,4,5-OMe	Me	H	>25	**27**	4-OMe	Me		2.7
1m	3-OBn	Me	H	>25					
1n	3-OH	Me	H	>25	**28**	4-OMe	Me		19
1o	4-NHMe	Me	H	4.1					
1p	4-NMe_2	Me	H	1.3	**29**	4-OMe	Me		0.17
1q	4-SMe	Me	H	0.34					
4a	4-OMe		H	20	**30**	4-OMe	Me		1.9
4f	4-OEt		H	>25					
5	4-OMe	Et	H	1.8	**31**	4-OMe	Me		0.035
6	4-OMe	Bn	H	9.8					
7	4-OMe	CF_3	H	1.1	**32**	4-OMe	Me		0.40
8	4-OMe	H	H	>25					
9				2.0	**33**	4-OMe	Me		0.78
14	4-OMe	Me	Me	0.053					
15	4-OMe	Me	Ph	0.1	**34**	4-OMe	Me		2.1

antiproliferative activity through binding to the colchicine-site of tubulin, thereby inhibiting microtubule polymerization in cancer cells. Further derivatization to enhance activity and the elucidation of the detailed mechanism of action are now under investigation.

■ ASSOCIATED CONTENT

Supporting Information

Scheme S-1, Figures S-1−3, experimental procedures, analytical data, ^1H and ^{13}C NMR charts, and chiral HPLC analysis charts. 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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