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# Optimization of *N*-aryl-6-methoxy-1,2,3,4-tetrahydroquinolines as tubulin polymerization inhibitors

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## Abstract

Thirteen new *N*-aryl 1,2,3,4-tetrahydroquinoline compounds (**4a–f**, **6a–c**, and **8a–d**) were synthesized and evaluated for antitumor activity and drug-like properties. Compound **4a** exhibited high inhibitory potency with low nanomolar $GI_{50}$ values of 16–20 nM in cellular assays, including excellent activity against the P-glycoprotein overexpressing cell line KBvin. Compound **4a** inhibited colchicine binding to tubulin and tubulin assembly with an $IC_{50}$ value of 0.85 μM, superior to the reference compound CA4 (1.2 μM) in the same assay. In addition, **4a** also exhibited highly improved water solubility (75 μg/mL) and a suitable log P value (3.43) at pH 7.4. With a good balance between antitumor potency and drug-like properties, compound **4a** could be a new potential drug candidate for further development. Current results on SAR studies and molecular modeling provided more insight about this class of compounds as tubulin polymerization inhibitors targeting the colchicine site.

## Graphical Abstract

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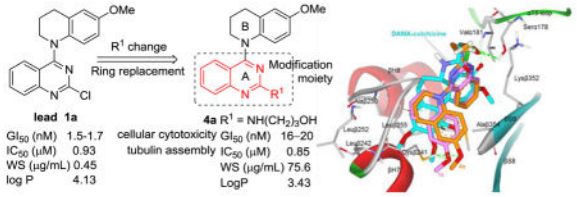


**Keywords**

*N*-Aryl 1,2,3,4-tetrahydroquinolines; Cytotoxic activity; Tubulin polymerization inhibitors; Colchicine binding inhibitors

## 1. Introduction

Microtubules play critical roles in cell division. They form the mitotic spindle, which directs and monitors the movement and attachment of chromosomes during mitosis and, thus, microtubules provide a target for anticancer drug development. The antitumor drugs paclitaxel and vinblastine, as well as related analogs, interfere with microtubule dynamics,[1] either stabilizing microtubules or inhibiting tubulin polymerization, respectively, and are widely used as first-line drugs in clinical treatments. However, high toxicity and the development of drug resistance greatly limit their effectiveness. Recently, diverse small molecules binding at the colchicine site (CS) of tubulin[2,3,4] have undergone extensive development as vascular-disrupting agents (VDAs).[5] VDAs with high cytotoxicity for human tumor cells target the vasculature induced by solid tumors, shut down blood flow to tumor cells, and cause cancer cell death via extensive necrosis and apoptosis, while blood flow in normal tissues remains relatively intact.[6] A dozen VDA drug candidates targeted at the CS are currently in clinical development as cancer treatments,[7,8] e.g., combretastatin A-4 (CA-4), its phosphate derivative CA-4P,[9] BNC105,[10] and verubulin (MPC-6827),[11] as shown in Figure 1, but none has an approved New Drug Application. Thus, continuous development of CS agents with novel structures or unique binding to the CS is needed to determine the optimal drug candidate.

In our prior studies on anticancer agents,[12,13,14] we discovered new *N*-aryl 6-methoxy-1,2,3,4- tetrahydroquinolines as potent tubulin polymerization inhibitors targeting the CS. We found high cytotoxicity in cellular assays and inhibitory potency in tubulin assembly and colchicine binding assays, resulting in two leads, *N*-(2′-substituted-quinazol-4′-yl)-6-methoxy-1,2,3,4- tetrahydroquinolines **1a** and **1b** (Figure 2). Subsequently, we performed structural optimizations on **1a**, modifying either the piperidine ring (B-ring) or the quinozoline ring (A-ring), to elucidate structure-activity relationship (SAR) and structure-property relationship (SPR) correlations aimed at developing potential new drug candidates. The B-ring modification studies revealed some correlative SARs and resulted in promising potential drug candidates with a new scaffold and high antitumor potency in vitro and in vivo.[15] Meanwhile, modifications on the quinazoline ring (A-ring) of **1a** were also carried out in parallel, and related results are reported herein. Our continued optimization focused first on the 2-Cl group in **1a** becuase of its chemical reactivity, which could lead to lower metabolic stability (**1a**, $t_{1/2}$ 10.6 min) and present potential liability for

further development. Because the previously synthesized 2-methylamino lead **1b** exhibited improved aqueous solubility and metabolic stability compared with **1a** (Figure 2),[11] we postulated that the 2-substituent on the quinazoline ring (A ring) might improve drug-like properties without loss of antitumor potency. Therefore, we introduced several substituted amino or alkoxy groups at the 2-position on the quinazoline, resulting in new compounds **4a–f**. Next, we replaced the quinazoline, a common moiety in many kinase inhibitors as well as the known VDA candidate MPC-6827, in **1a** (Figure 1), with other bioisomeric hetero-aromatic cyclic rings, such as unsubstituted and substituted purines (**6** series), quinoline, and isoquinoline (**8** series) (Figure 2), to explore new antitumor agents with different scaffold(s). Complementary to our prior structural optimizations,[12] our current results with three new series **4**, **6**, and **8** are reported herein, including chemical synthesis, antitumor activity in vitro, drug-like property evaluation, molecular modeling, and SAR analysis.

## 2. Chemistry

The designed new $N^1$-aryl-6-methoxy-1,2,3,4-tetrahydroquinolines **4a–f** and **6a–c** were synthesized as shown in Schemes 1 and 2. According to previous methods,[11] lead **1a** was prepared by coupling 6-methoxy-1,2,3,4-tetrahydroquinoline (**3**)[16] and 2,4-dichloroquinazoline (**2a**) in the presence of $NaHCO_3$ in anhydrous EtOH at reflux. Subsequently, the 2-chloro in **1a** was converted to a substituted amino group by nucleophile reaction with 3-aminopropanol, cyclopropylamine, or cyclopenylamine in ethanol under microwave (mw) irradiation at 150 ºC for 15 min to afford corresponding compounds **4a–c**, respectively, in yields ranging from 71 to 96%. Under the same reaction conditions, compound **4d** with a 2-dimethylamino group was obtained from **1a** and DMF, serving as both dimethylamine source[17] and solvent. Reaction of **1a** with NaOMe in methanol at reflux produced 2-methoxyquinazoline compound **4e** in 88% yield. While **1a** was obtained under alkaline conditions, compound **3** was coupled with commercially available 4-chloroquinazoline (**2b**) in the presence of HCl in *i*-PrOH at reflux to afford *N*-(4-quinazolyl)-6-methoxy-1,2,3,4- tetrahydroquinoline **4f**. Similarly, $N^1$-(6-purinyl)-6-methoxy-1,2,3,4-tetrahydroquinolines **6a–c** were synthesized from **3** and a 6-chloro-9*H*-purine (**5a, b** or **c**) (Scheme 2). Finally, compound **3** was coupled with a halo-quinoline or -isoquinoline (**7a**–**d)** in the presence of $Pd(OAc)_2$, X-phos (ratio 2%–4%), and $Cs_2CO_3$ in toluene at reflux to provide corresponding $N^1$-aryl substituted 6-methoxy-1,2,3,4-tetrahydroquinolines **8a–d**, respectively. All new target compounds **4**, **6**, and **8** were identified from $^1$H NMR and MS spectroscopic data, and their purities were greater than 95% as measured by HPLC.

## 3. Results and discussion

### 3.1. Evaluation of cytotoxicity and tubulin inhibition activity in vitro

The newly synthesized *N*-aryl substituted 6-methoxy-1,2,3,4-tetrahydroquinolines, series **4**, **6** and **8**, were first tested in cellular assays to evaluate in vitro antitumor activity. A panel of human tumor cell lines (HTCL) including A549 (lung carcinoma), KB (epidermoid carcinoma of the mouth), KBvin, a P-glycoprotein overexpressing multidrug-resistant cell line (vincristine-resistant KB),[18,19] and DU145 (prostate cancer) was used with paclitaxel as

a reference. The potency ($GI_{50}$) in vitro was determined using the established sulforhodamine B (SRB) method,[20] and data are shown in Tables 1 and 2.

All six series **4** compounds with a 2-substituted quinazoline B-ring system exhibited significant cytotoxicity against the growth of the tested HTCL (Table 1). Among them, compounds **4a**, **b**, **e**, and **f** showed low nanomolar $GI_{50}$ values ranging from 13 to 30 nM, comparable to lead **1b** but less potent than **1a** (1.5–1.7 nM). With low to sub-micromolar $GI_{50}$ values (0.14–1.42 μM), the 2-cyclopentylamino- (**4c**) and 2-*N*,*N*-dimethylamino (**4d**) compounds were at least 10 times less potent than **4a,b,e,f**, indicating that more bulk at the 2-position on the quinazoline was not favorable for cytotoxic activity. Furthermore, potency dropped dramatically when the quinazoline moiety was replaced by a purine without or with a substituent (**6a**–**c**, $GI_{50}$ > 17.53 μM) (Table 1). However, compounds **8a–c** with a quinoline or isoquinoline rather than quinazoline showed moderate cytotoxicity with $GI_{50}$ values of 0.16–2.08 μM, while **8d** was less potent ($GI_{50}$ 8.71–16.2 μM) in the cellular assay (Table 2). These results indicated that the quinazoline ring could be changed, but more investigations are warranted.

The data in Tables 1 and 2 also demonstrate that all newly synthesized compounds, unlike paclitaxel, have equivalent activities in the KB and KBvin cell lines. The latter overexpresses the drug transporter P-glycoprotein, resulting in a multidrug resistant phenotype.

Next, selected compounds from the cellular cytotoxicity assays were evaluated in tubulin assembly inhibition and colchicine binding assays for comparison with the control CA-4, a phase III clinical candidate. These data are shown in Table 3. As expected, the most cytotoxic compounds **4a**, **4b**, and **4f** also exhibited high potency in the tubulin assembly assay with low micromolar (1.3 μM, **4b** and **4f**) or sub-micromolar (0.85 μM, **4a**) $IC_{50}$ values, comparable with or better than the value obtained with CA4 ($IC_{50}$ 1.2 μM). The three compounds also inhibited colchicine binding to tubulin. In addition, the moderately potent compounds **4c**, **8a**, and **8b** ($GI_{50}$ values, 0.92–2.08 μM) and less active compound **8d** ($GI_{50}$ 8.71–16.2 μM) from the cellular cytotoxicity assay were also tested against tubulin assembly and colchicine binding in cell-free assays. Compared with **4a**, **4b**, and **4f**, compounds **4c**, **8a**, **8b** showed lower potency, with assembly $IC_{50}$ values ranging from 2.6 to 5.7 μM and less than 40% inhibition of colchicine binding at 5 μM, whereas **8d** did not display significant inhibitory activity in the assembly assay. The consistency of the results between the cellular and cell-free assays, as well as with our previous results, confirms that our new active compounds are tubulin polymerization inhibitors targeting the CS.

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### 3.2. Physicochemical property evaluations

Representative active compounds **4a** and **4b** were further evaluated for aqueous solubility, which is good drug-like property, especially for antitumor drugs given via intravenous administration, and logP parameters at pH 7.4 by using HPLC methodology.[21] As expected, compared with **1a** (0.45 μg/mL), compound **4a**, with a polar 2-hydroxypropylamino group on the quinazoline, showed greatly improved aqueous solubility (75.6 μg/mL), while compound **4b** with the more lipophilic 2-cyclopropylamino group displayed less improved aqueous solubility (2.55 μg/mL) (Table 4). Consistent with the solubility results, **4a** had a more suitable log P value (3.45) than **4b** (log P 4.13). Therefore, compound **4a** could be a potential anticancer drug candidate for further development by evaluating its in vivo activity.

### 3.3. Molecular modeling

In an attempt to better understand the molecular basis of **4a** as a tubulin inhibitor, we modeled compound **4a** into the CS in the tubulin dimer by using the CDOCKER program in the Discovery Studio 3.0 software with the tubulin crystal structure (PDB: 1SA0).[22] As shown in Figure 3, the binding conformation of **4a** (orange) displayed a lower energy (−46.11 kcal/mol) than lead **1a** (−38.68 kcal/mol) and superimposed well with **1a** (pink) and original ligand DAMA-colchicine (cyan) in the tubulin crystal structure. From literature reports,[23,24] Cys241 in β-H7 of tubulin is a key amino acid that anchors most inhibitors, and its side chain forms a hydrogen bond with the oxygen atom of the 2-$OCH_3$ on the phenyl ring (C-ring) of DAMA-colchicine. Like lead **1a**, compound **4a** also formed a hydrogen bond from the 6-$OCH_3$ group on the tetrahydroquinoline ring (B/C-ring) with Cys241. The tetrahydroquinoline ring in **4a** and **1a** overlapped well with the trimethoxyphenyl portion of DAMA-colchicine. Meanwhile, the cyclic linker in **4a** extended toward a lipophilic cavity surrounded with lipophilic amino acid residues, Leuβ242, Alaβ250, Leuβ252 and Leuβ255, but only Leuβ255 was close enough to the cyclic linker to be within distance of van der Waal's force. Thus, we suggest that the cyclic linker could be further modified to have more interactions with the lipophilic cavity to enhance the affinity of this compound type with the colchicine binding site of tubulin. Compared with **1a**, the terminal hydroxy group of the 2-substituent on the quinazoline ring of **4a** formed an additional hydrogen bond with Ser178 in the α-T5 loop of tubulin. Although these hydrogen bonds between tubulin and the thiol group of DAMA-colchicine were observed, intact colchicine may not form these hydrogen bonds. Additionally, we observed that the linear 2-hydroxypropylamino group in **4a** was oriented similarly to the mercaptoacetamido (−$HNCOCH_2SH$) group in DAMA-colchicine, and both superimposed well. Thus, the flexible 2-hydroxypropylamino group could attain a

suitable conformation to interact with amino acids of the tubulin binding site to enhance molecular affinity.

## 4. Conclusion

By modifying the quinazoline ring moiety of lead **1a**, we synthesized 13 new 1,2,3,4-tetrahydroquinoline compounds, **4a–f**, **6a–c**, and **8a–d**, with various *N*-aryl substituents. Compound **4a** showed high inhibitory potency in the tubulin polymerization assay ($IC_{50}$ 0.85 μM), targeting the CS, with greater potency than the reference compound CA-4 (1.2 μM). Compound **4a** also strongly inhibited the growth of human tumor cells with low nanomolar $GI_{50}$ values (16–20 nM). Compound **4a** was fully active in a multidrug resistant cell line overexpressing P-glycoprotein. Notably, active compound **4a** also exhibited highly improved water solubility (75 μg/mL) and a suitable log P value (3.43) at pH 7.4 that are better than those of lead **1a** and other new compounds. Thus, compound **4a** could be a potential drug candidate for further development. Meanwhile, the current results also revealed additional SAR information. (1) The 2-substituent on the quinazoline ring is modifiable to enhance or maintain molecular antitumor activity (**4a–b**, **4e–f)**, but a bulky group is unfavorable (**4c** and **4d**). (2) The quinazoline ring is a better moiety than purine (cf. **6** series, which exhibited much lower activity) for antitumor activity but is replaceable (cf. **8** series with quinoline or isoquinoline ring, which maintained low micromolar $GI_{50}$ values). (3) The polar hydroxypropylamino substituent is favorable due to its flexibility and H-bond forming ability, resulting in a good balance between potency and drug-like properties. Molecular modeling results provided additional insight on the interactions of **4a** with the tubulin colchicine site. These current results complement our prior studies and will help us to further optimize and develop new drug candidates for clinical studies as novel tubulin inhibitors targeting the CS.

## 5. Experimental section

### 5.1. Chemistry

All commercial chemical reagents were purchased from Beijing Chemical Works or Sigma-Aldrich, Inc. Proton nuclear magnetic resonance ($^1$H NMR) spectra were measured on a JNM-ECA-400 (400MHz) spectrometer using tetramethylsilane (TMS) as internal standard. The solvent used was $CDCl_3$ unless otherwise indicated. Mass spectra (MS) were measured on an API-150 mass spectrometer with an electrospray ionizer from ABI, Inc., and data are presented as intensity of ion peak (%). Melting points were measured by a SGW X-4 Micro-Melting point detector without correction. Microwave-assisted reactions were performed on a reactor from Biotage, Inc. Medium-pressure column chromatography was performed using a CombiFlash Companion system from ISCO, Inc. Thin-layer chromatography (TLC) was performed on silica gel GF254 plates. Aqueous solubility and log P of target compounds were determined by using an Agilent 1200 HPLC system with UV detector and an Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm), flow rate 0.8 mL/min, UV detection at 254 nm, and injection volume of 20 μL. The mobile elution is a mixture of solvents MeOH and water (80:20) contained 0.04% ammonium acetate. The purity of target compounds was also measured by HPLC using the above conditions, unless otherwise indicated. The preparation of **1a** was reported in our previous publication.[11]

**5.1.1. $N^1$-(2′-(3-Hydroxypropyl)aminoquinazol-4′-yl)-6-methoxy-1,2,3,4-tetrahydro- quinoline (4a)—**A mixture of **1a** (145 mg, 0.44 mmol) and 3-aminopropanol (0.1 mL, excess) in EtOH (2.5 mL) was stirred under mw irradiation at 150 ºC for 15 min. The mixture was poured into ice-water, extracted with EtOAc, and washed with water to neutral. After removal of solvent, crude product was purified on a silica column with gradient elution (MeOH/$CH_2Cl_2$ 0–5%) by a CombiFlash system to produce 137 mg of **4a** in 71% yield, yellow solid, mp 134~136 ºC; $^1$H NMR $\delta$ppm 1.77 (2H, t, $J$ = 7.2 Hz, $CH_2$), 2.07 (2H, m, 3-$CH_2$), 2.85 (2H, t, $J$ = 6.8 Hz, Ph$CH_2$), 3.67(4H, m, 2×$CH_2$), 3.79 (3H, s, $OCH_3$), 3.93(2H, t, $J$ = 6.8 Hz, 2-N$CH_2$), 6.56 (1H, dd, $J$ = 8.8 and 2.8 Hz, PhH-7), 6.70 (1H, d, $J$ = 8.8 Hz, PhH-8), 6.77 (1H, d, $J$ = 2.8Hz, PhH-5), 6.84 (1H, m, ArH-6), 7.25 (1H, d, $J$ = 8.0 Hz, ArH-5), 7.47 (2H, m, ArH-7,8), MS $m/z$ (%) 365 $(M + H)^{+;}$ $^{13}$C NMR $\delta$ppm 24.10, 27.11, 33.90, 37.01, 47.38, 55.39, 57.76, 111.86, 113.34, 120.76, 122.95, 124.99, 126.54, 132.16,, 132.79 (C×2), 135.67, 152.78, 155.87, 159.78, and 163.02; HPLC purity 98.9%.

**5.1.2. $N^1$-(2′-Cyclopropylaminoquinazol-4′-yl)-6-methoxy-1,2,3,4-tetrahydroquinoline (4b)—**Prepared in the same manner as **4a**. Starting with **1a** (50 mg, 0.15 mmol) and cyclopropanamine (0.1 mL, excess) in EtOH (2.5 mL) under mw irradiation at 150 ºC for 15 min to provide 51 mg of **4b** in 97% yield, yellow solid, mp 173~175 ºC; $^1$H NMR (DMSO-$d_6$) $\delta$ ppm 0.51 (2H, m, $CH_2$), 0.66 (2H, m, $CH_2$), 1.97 (2H, m, 3-$CH_2$), 2.81 (3H, m, Ph$CH_2$ and CH), 3.71 (3H, s, $OCH_3$), 3.82 (2H, t, $J$ = 6.4 Hz, 2-$CH_2$N), 6.56 (1H, dd, $J$ = 8.8 and 2.4 Hz, PhH-7), 6.68 (1H, d, $J$ = 8.8 Hz, PhH-8), 6.76 (1H, d, $J$ = 2.4 Hz, PhH-5), 6.84 (1H, m, ArH-6), 7.29 (1H, d, $J$ = 8.8 Hz, ArH-5), 7.45 (1H, m, ArH-7), 7.54(1H, d, $J$ = 8.8 Hz, ArH-8); MS $m/z$ (%) 347 $(M + H)^+$; HPLC purity 95.2%.

**5.1.3. $N^1$-(2′-Cyclopentyl)aminoquinazol-4′-yl)-6-methoxy-1,2,3,4-tetrahydroquinoline (4c)—**Prepared in the same manner as **4a**. Starting with **1a** (150 mg, 0.45 mmol) and cyclopentanamine (0.1 mL, excess) in EtOH (2.5 mL) under mw irradiation at 150 ºC for 15 min to afford 138 mg of **4c** in 80% yield, yellow solid, mp 178~180 ºC; $^1$H NMR (DMSO-$d_6$) $\delta$ ppm 1.54 (4H, m, 2×$CH_2$), 1.68 (2H, m, $CH_2$), 1.92 (2H, m, $CH_2$), 1.98 (2H, m, 3-$CH_2$), 2.81 (2H, t, $J$ = 6.8 Hz, Ph$CH_2$), 3.71 (3H, s, $OCH_3$), 3.82 (2H, t, $J$ = 6.8 Hz, N$CH_2$), 4.28 (1H, m, CH), 6.55 (1H, dd, $J$ = 8.8 and 2.4 Hz, PhH-7), 6.68 (1H, d, $J$ = 8.8 Hz, PhH-8), 6.78 (1H, d, $J$ = 2.4 Hz, PhH-5), 6.88 (1H, m, ArH-6), 7.24 (1H, d, $J$ = 8.8 Hz, ArH-5), 7.45 (1H, m, ArH-7), 7.54 (1H, d, $J$ = 8.8 Hz, ArH-8); $^{13}$C NMR $\delta$ppm 23.88 (C×2), 24.28, 27.38, 33.68 (C×2), 47.39, 53.01, 55.53, 111.86, 113.44, 120.21, 122.67, 125.84, 126.58, 131.86,, 132.44 (C×2), 136.38, 154.37, 155.57, 159.25, and 163.14; MS $m/z$ (%) 375 $(M + H)^+$; HPLC purity 99.2% (elution $CH_3CN$/water 70:30).

**5.1.4. $N^1$-(2′-Dimethylaminoquinazol-4′-yl)-6-methoxy-1,2,3,4-tetrahydroquinoline (4d)—**A solution of **1a** (50 mg, 0.15 mmol) in DMF (2 mL) in the presence of 0.1 mL of ammonium hydroxide ($NH_3 \cdot H_2O$) was heated with stirring under mw irradiation at 150 ºC for 15 min. The mixture was poured into ice-water, and a yellow solid was filtered and washed with water until neutral. The crude product was purified by a flash chromatography (gradiant elution: MeOH/ $CH_2Cl_2$ 0–5%) to provide 45 mg of **4d** in 88% yield, light yellow solid, mp 89~91 ºC; $^1$H NMR $\delta$ ppm 2.08 (2H, m, 3-$CH_2$), 2.85 (2H, t, $J$

= 6.8 Hz, $PhCH_2$), 3.27 (6H, s, 2×$NCH_3$), 3.78 (3H, s, $OCH_3$), 3.97 (2H, t, *J* = 6.8 Hz, $NCH_2$), 6.53 (1H, dd, *J* = 8.8 and 2.8 Hz, PhH-7), 6.65 (1H, d, *J* = 8.8 Hz, PhH-8), 6.77 (2H, m, PhH-5 and ArH-6), 7.23 (1H, d, *J* = 8.0 Hz, ArH-5), 7.42 (1H, m, ArH-7), 7.50 (1H, d, *J* = 8.0 Hz, ArH-8); MS *m/z* (%) 335 $(M + H)^+$; HPLC purity 97.5%.

**5.1.5. $N^1$-(2′-Methoxyquinazol-4′-yl)-6-methoxy-1,2,3,4-tetrahydroquinoline (4e)—**A mixture of **1a** (81 mg, 0.25 mmol) in anhydrous MeOH (4 mL) and NaOMe (34 mg, 0.62 mmol) was refluxed for 1 h. The mixture was poured into ice-water with stirring and neutralized with HCl (2 N). The solid product was collected on a filter, washed with water until neutral, and dried to obtain 71 mg of pure **4e**, 88% yield, pale yellow solid, mp 161–163 ºC; $^1$H NMR $\delta$ ppm 2.09 (2H, m, 3-$CH_2$), 2.85 (2H, t, *J* = 6.8 Hz, $PhCH_2$), 3.80 (3H, s, $OCH_3$), 4.03 (2H, t, *J* = 6.8 Hz, $NCH_2$), ), 4.09 (3H, s, $OCH_3$), 6.53 (1H, dd, *J* = 8.8 and 2.8 Hz, PhH-7), 6.67 (1H, d, *J* = 8.8 Hz, PhH-8), 6.79 (1H, *J* = 2.8 Hz, PhH-5), 6.98 (1H, m, ArH-6), 7.23 (1H, d, *J* = 8.4 Hz, ArH-5), 7.56 (1H, m, ArH-7), 7.68 (1H, d, *J* = 8.4 Hz, ArH-8); MS *m/z* (%) 322 $(M + H)^+$; HPLC purity 98.8%.

**5.1.6. 6-Methoxy-$N^1$-(4′-quinazolyl)-1,2,3,4-tetrahydroquinoline (4f)—**A mixture of 4-chloroquinazoline (**2b**, 170 mg, 1.04 mmol) and 6-methoxy-1,2,3,4-tetrahydroquinoline (**3**, 180 mg, 1.10 mmol) in *i*-PrOH (15 mL) with a drop of HCl (conc.) was refluxed for 1 h monitored by TLC until the reaction was completed. The mixture was poured into ice-water, adjusted to neutral pH with $NaHCO_3$, extracted with EtOAc three times, and dried over anhydrous $Na_2SO_4$. After removal of solvent, the product was purified on a silica chromatographic column with gradient elution ($MeOH/CH_2Cl_2$ 0–5%) to furnish 193 mg of **4f** in 65% yield, light yellow solid, mp 94~95 ºC; $^1$H NMR $\delta$ ppm 2.11 (2H, t, *J* = 6.8 Hz, 3-$CH_2$), 2.87 (2H, t, *J* = 6.8 Hz, 4-$CH_2$), 3.80 (3H, s, $OCH_3$), 4.05 (2H, t, *J* = 6.8 Hz, 2-$CH_2$), 6.56 (1H, dd, *J* = 8.8 and 2.8 Hz, PhH-7), 6.65 (1H, d, *J* = 8.8Hz, PhH-8), 6.79 (1H, d, *J* = 2.8 Hz, PhH-5), 7.21 (1H, t, *J* = 8.8 Hz, ArH-6), 7.49 (1H, d, *J* = 8.8 Hz, ArH-5), 7.68 (1H, t, *J* = 8.8 Hz, ArH-7), 7.89 (1H, d, *J* = 8.8 Hz, ArH-8), 8.86 (1H, s, ArH-2); MS *m/z* (%) 292.2 $(M + H)^+$; HPLC purity 98.4%.

**5.1.7. $N^1$-(6-Purin-9H-yl)-6-methoxy-1,2,3,4-tetrahydroquinoline (6a)—**A mixture of **3** (180 mg, 1.10 mmol) and 6-chloro-9*H*-purine (**5a**, 150 mg, 0.97 mmol) in *i*-propanol (15 mL) in the presence of 1 drop of HCl (conc.) was refluxed for 5 h. When the reaction was complete, the mixture was poured into ice-water, adjusted to neutral pH, extracted with EtOAc, and dried over anhydrous $Na_2SO_4$. After removal of solvent in vacuo, crude product was purified by flash silica column chromatography (gradient elution: $MeOH/CH_2Cl_2$, 0–5%) to produce 201 mg of **6a** in 74% yield, white solid, mp 189~191 ºC; $^1$H NMR $\delta$ ppm 2.10 (2H, m, 3-$CH_2$), 2.87 (2H, t, *J* = 6.4 Hz, $PhCH_2$), 3.82 (3H, s, $OCH_3$), 4.59 (2H, t, *J* = 6.4 Hz, $NCH_2$), 6.73 (1H, s, PhH-5), 6.77 (1H, dd, *J* = 8.8 and 2.4 Hz, PhH-7), 7.52 (1H, d, *J* = 8.8 Hz, PhH-8), M8.03 (1H, s, ArH-8), 8.48 (1H, s, ArH-2); MS *m/z* (%) 282 $(M + H)^+$; HPLC purity 97.2%.

**5.1.8. $N^1$-(2-Fluoro-9H-purin-6-yl)-6-methoxy-1,2,3,4-tetrahydroquinoline (6b)—**Prepared in the same manner as **6a**. Starting with 6-chloro-2-fluoro-9*H*-purine (**5b**, 110 mg, 0.64 mmol) and **3** (115 mg, 0.70 mmol) in *i*-propanol at reflux for 4 h to furnish 137 mg of

**6b** in 73% yield, white solid, mp 218–220 ºC; $^1$H NMR $\delta$ ppm 2.12 (2H, m, 3-$CH_2$), 2.86 (2H, t, $J$ = 6.8 Hz, Ph$CH_2$), 3.82 (3H, s, $OCH_3$), 4.59 (2H, t, $J$ = 6.8 Hz, $NCH_2$), 6.71 (1H, d, $J$ = 2.8 Hz, PhH-5), 6.77 (1H, dd, $J$ = 8.8 and 2.8 Hz, PhH-7), 7.54 (1H, d, $J$ = 8.8 Hz, PhH-8), 7.93 (1H, s, ArH-8); MS *m/z* (%) 300 $(M + H)^+$.

**5.1.9. $N^1$-(2-Aminopurin-9H-6-yl)-6-methoxy-1,2,3,4-tetrahydroquinoline (6c)—** Prepared in the same manner as **6a**. Starting with 2-amino-6-chloro-9*H*-purine (**5c**, 150 mg, 0.89 mmol) and **3** (160 mg, 0.98 mmol) in *i*-propanol for 4.5 h to obtain 157 mg of **6c** in 61% yield, white solid, mp 183~186 ºC; $^1$H NMR (DMSO-$d_6$) $\delta$ppm 1.85 (2H, m, 3-$CH_2$), 2.73 (2H, t, $J$ = 6.0 Hz, Ph$CH_2$), 3.69 (3H, s, $OCH_3$), 4.33 (2H, t, $J$ = 6.0 Hz, $NCH_2$), 5.72 (2H, bs, $NH_2$), 6.66 (2H, m, PhH), 7.32 (1H, $J$ = 8.8 Hz, PhH), 7.70 (1H, s, ArH), 12.22 (1H, s, NH). MS *m/z* (%) 297 $(M + H)^+$; HPLC purity 94.5% (elution $CH_3$CN/water 70:30).

**5.1.10. $N^1$-(1-Isoquinolin-1-yl)-6-methoxy-1,2,3,4-tetrahydroquinoline (8a)—**A mixture of 1-bromoisoquinoline (**7a**, 170 mg, 0.83 mmol) and **3** (150 mg, 0.92 mmol) in the presence of $Cs_2CO_3$ (2 equiv.), X-Phos (5% equiv), and $Pd(OAc)_2$ (4% equiv) in toluene (15 mL) was refluxed for 26 h. The mixture was added to EtOAc, unsoluble solid filtered out, and solvent removed under reduced pressure. The crude product was purified on a silica column with gradiant elution (EtOAc/petroleum ether, 0–30%) to provide 180 mg of **8a** in 75% yield, yellow solid, mp 121~122 ºC; $^1$H NMR $\delta$ ppm 2.12 (2H, t, $J$ = 6.0 Hz, 3-$CH_2$), 2.95 (2H, t, $J$ = 6.0 Hz, Ph$CH_2$), 3.74 (3H, s, $OCH_3$), 3.86 (2H, t, $J$ = 6.0 Hz, $NCH_2$), 6.33 (1H, d, $J$ = 8.8 Hz, PhH-8), 6.45 (1H, dd, $J$ = 8.8 an 2.0 Hz, PhH-7), 6.70 (1H, d, $J$ = 2.0 Hz, PhH-5), 6.99 (1H, d, $J$ = 8.0 Hz, ArH-4), 7.40 (1H, t, $J$ = 8.0 Hz, ArH-7), 7.61 (1H, t, $J$ = 8.0 Hz, ArH-6), 7.78 (1H, d, $J$ = 8.0 Hz, ArH-5), 7.93 (1H, d, $J$ = 8.0 Hz, ArH-8), 8.28 (1H, s, ArH-3); MS *m/z* (%) 291 $(M + H)^+$; HPLC purity 96.0%.

**5.1.11. $N^1$-(4-Isoquinolinyl)-6-methoxy-1,2,3,4-tetrahydroquinoline (8b)—** Prepared in the same manner as **8a**. Starting with 4-bromoisoquinoline (**7b**, 170 mg, 0.83 mmol) and **3** (150 mg, 0.92 mmol), $Cs_2CO_3$ (2 equiv), X-Phos (5% equiv), $Pd(OAc)_2$ (4% equiv) in toluene (15 mL) at relux for 14 h to produce 180 mg of **8b** in 76% yield, yellow solid, mp 86~88 ºC; $^1$H NMR $\delta$ ppm 2.11 (2H, t, $J$ = 6.0 Hz, 3-$CH_2$), 2.87 (2H, t, $J$ = 6.0 Hz, Ph$CH_2$), 3.74 (2H, t, $J$ = 6.0 Hz, $NCH_2$), 3.73 (3H, s, $OCH_3$), 6.13 (1H, d, $J$ = 8.8 Hz, PhH-8), 6.53 (1H, dd, $J$ = 8.8 and 2.8 Hz, PhH-7), 6.70 (1H, d, $J$ = 2.8 Hz, PhH-5), 7.65 (1H, dt, $J$ = 8.0 and 2.0 Hz, ArH-7), 7.67 (1H, dt, $J$ = 8.0 and 2.0 Hz, ArH-6), 7.95 (1H, dt, $J$ = 8.0 and 2.0 Hz, ArH-5), 8.05 (1H, dd, $J$ = 8.0 and 2.0 Hz, ArH-8), 8.41 (1H, s, ArH-3), 9.15 (1H, s, ArH-1); MS *m/z* (%) 291 $(M + H)^+$; HPLC purity 98.5%.

**5.1.12. 6-Methoxy-3,4-dihydro-2H-1,4′-biquinoline (8c)—**Prepared in the same manner as **8a**. Starting with 4-chloroquinoline (**7c**, 150 mg, 0.92 mmol) and **3** (165 mg, 1.01 mmol) in the presence of $Cs_2CO_3$ (2 equiv), X-Phos (0.05 equiv), $Pd(OAc)_2$ (0.04 equiv) in toluene (15 mL) at reflux for 14 h to furnish 188 mg of **8c** in 71% yield, yellow solid, mp 41~43 ºC; $^1$H NMR $\delta$ ppm 2.11 (2H, t, $J$ = 6.4 Hz, 3-$CH_2$), 2.87 (2H, t, $J$ = 6.4 Hz, Ph$CH_2$), 3.74 (2H, t, $J$ = 6.4 Hz, $NCH_2$), 3.77 (3H, s, $OCH_3$), 6.50 (1H, d, $J$ = 8.8 Hz, PhH-8), 6.53 (1H, dd, $J$ = 8.8 and 2.4 Hz, PhH-7), 6.72 (1H, d, $J$ = 2.4 Hz, PhH-5), 7.07 (1H, d, $J$ = 8.0 Hz, ArH-3), 7.44 (1H, t, $J$ = 8.0 Hz, ArH-6), 7.68 (1H, t, $J$ = 8.0 Hz, ArH-7), 7.95 (1H, d, $J$

= 8.0 Hz, ArH-5), 8.11 (1H, d, *J* = 8.0 Hz, ArH-8), 8.75 (1H, d, *J* = 8.0 Hz, ArH-2); MS *m/z* (%) 291 $(M + H)^+$; HPLC purity 95.6% (mobile elution MeOH/water 70:30).

**5.1.13. 6-Methoxy-3,4-dihydro-2H-1,8′-biquinoline (8d)—**Prepared in the same manner as **8a**. Starting with 8-chloroquinoline (**7d**, 550 mg, 3.37 mmol) and **3** (570 mg, 3.49 mmol) in the presence of $Cs_2CO_3$, X-Phos, and $Pd(OAc)_2$ in toluene (15 mL) at reflux for 12 h to provide 598 mg of **8d**, 61% yield, yellow solid, mp 86~88 ºC; $^1$H NMR $\delta$ ppm 2.00 (2H, t, *J* = 6.4 Hz, 3-$CH_2$), 2.93 (2H, t, *J* = 6.4 Hz, Ph$CH_2$), 3.74 (3H, s, $OCH_3$), 3.74 (2H, t, *J* = 6.4 Hz, $NCH_2$), 6.49 (1H, d, *J* = 8.0 Hz, PhH-8), 6.50 (1H, d, *J* = 8.0 Hz, PhH-7), 6.65 (1H, s, PhH-5), 7.39 (1H, t, *J* = 8.0 Hz, ArH-6), 7.41 (1H, t, *J* = 8.0 Hz, ArH-3), 7.45 (1H, d, *J* = 8.0 Hz, ArH-5), 7.56 (1H, d, *J* = 8.0 Hz, ArH-4), 8.15 (1H, d, *J* = 8.0 Hz, ArH-7), 8.75 (1H, d, *J* = 8.0 Hz, ArH-2); MS *m/z* (%) 291 $(M + H)^+$; HPLC purity 95.2%.

### 5.2. Antiproliferative activity assay

Target compounds were assayed by the SRB method for cytotoxic activity using a HTCL assay according to procedures described previously.[25,27] The cell line panel included human lung carcinoma (A-549), epidermoid carcinoma of the nasopharynx (KB), P-glycoprotein overexpressing epidermoid carcinoma of the nasopharynx (KBvin), and prostate cancer (DU145). The cytotoxic effects of each compound were expressed as $GI_{50}$ values, which represent the molar drug concentrations required to cause 50% tumor cell growth inhibition.

### 5.3. Tubulin assays

Tubulin assembly was measured by turbidimetry at 350 nm as described previously.[28] Assay mixtures containing 1.0 mg/mL (10 μM) of tubulin and varying compound concentrations were pre-incubated for 15 min at 30 ºC without guanosine 5′-triphosphate (GTP). The samples were placed on ice, and 0.4 mM GTP was added. Reaction mixtures were transferred to 0 ºC cuvettes, and turbidity development was followed for 20 min at 30 ºC following a rapid temperature jump. Compound concentrations that inhibited an increase in turbidity by 50% relative to a control sample were determined.

Inhibition of the binding of [$^3$H]colchicine to tubulin was measured as described previously.[29] Incubation of 1.0 μM tubulin with 5.0 μM [$^3$H]colchicine and 5.0 or 1.0 μM inhibitor took place for 10 min at 37 ºC, the time at which about 40 60% of maximum colchicine binding occurs in control samples.

### 5.4. Aqueous solubility studies

Solubility was measured at pH 7.4 by using an HPLC-UV method. A test compound was initially dissolved in DMSO at a concentration of 1.0 mg/mL. Ten microliters of this stock solution was slowly dropped into 990 μL of water at pH 7.4. The mixture was stirred at room temperature for 4 h, and then centrifuged at 3000 rpm for 10 min. The clear water phase was transferred to a vial for analysis by HPLC. For quantification, a model 1200 HPLC–UV (Agilent) system was used with an Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm), and the eluent was MeOH/$H_2O$ (80:20) with 0.04% acetamide. The flow rate was 0.8 mL/min, injection volume was 20 μL, and detection wavelength was 254 nm. Aqueous concentration was determined by comparison of the peak area of the saturated

solution with a standard curve plotted peak area versus known concentrations, which were prepared by solutions of test compound in ACN at 50, 12.5, 3.13, 0.78, and 0.20 μg/mL. Each sample was performed in triplicate.

### 5.5. Log P measurement

One to two milligrams of test compound were dissolved in 1.0–2.0 mL of *n*-octane to obtain a 1.0 mg/mL solution. Next, the same volume of water as *n*-octane was added to each vial. The mixture was stirred at rt for 24 h and left without stirring overnight. The aqueous and organic phases of each mixture were transferred to separate vials for HPLC analysis. The instrument and conditions were the same as those for water solubility determinations. The log P was calculated by the peak area ratio in *n*-octane and in water.

### 5.6. Molecular modeling studies

All molecular modeling studies were performed with Discovery Studio 3.0 (Accelrys). The crystal structure of tubulin in complex with DAMA-colchicine (PDB code: 1SA0) was downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/pdb) for use in the modeling study. CDOCKER was used to evaluate and predict *in silico* binding free energy of the inhibitors and for automated docking. The protein protocol was prepared by several operations, including standardization of atom names, insertion of missing atoms in residues and removal of alternate conformations, insertion of missing loop regions based on SEQRES data, optimization of short and medium size loop regions with the Looper algorithm, minimization of remaining loop regions, calculation of pK, and protonation of the structure. The receptor model was typed with the CHARMM force field. A binding sphere with a radius of 9.0 Å was defined through the original ligand (DAMA-colchicine) as the binding site for the study. The docking protocol employed total ligand flexibility, and the final ligand conformations were determined by the simulated annealing molecular dynamics search method set to a variable number of trial runs. Docked ligand **4a** or **1a** was further refined using in situ ligand minimization with the Smart Minimizer algorithm by standard parameters. The ligand and its surrounding residues within the above-defined sphere were allowed to move freely during the minimization, whereas the outer atoms were frozen. The implicit solvent model of Generalized Born with Molecular Volume (GBMV) was also used to calculate the binding energies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data associated with this this article can be found in the online version. These data include HPLC purity data of target compounds, representive $^{1}H$ and $^{13}C$ NMR spetra, and MOL files of the most important compounds described in this article.

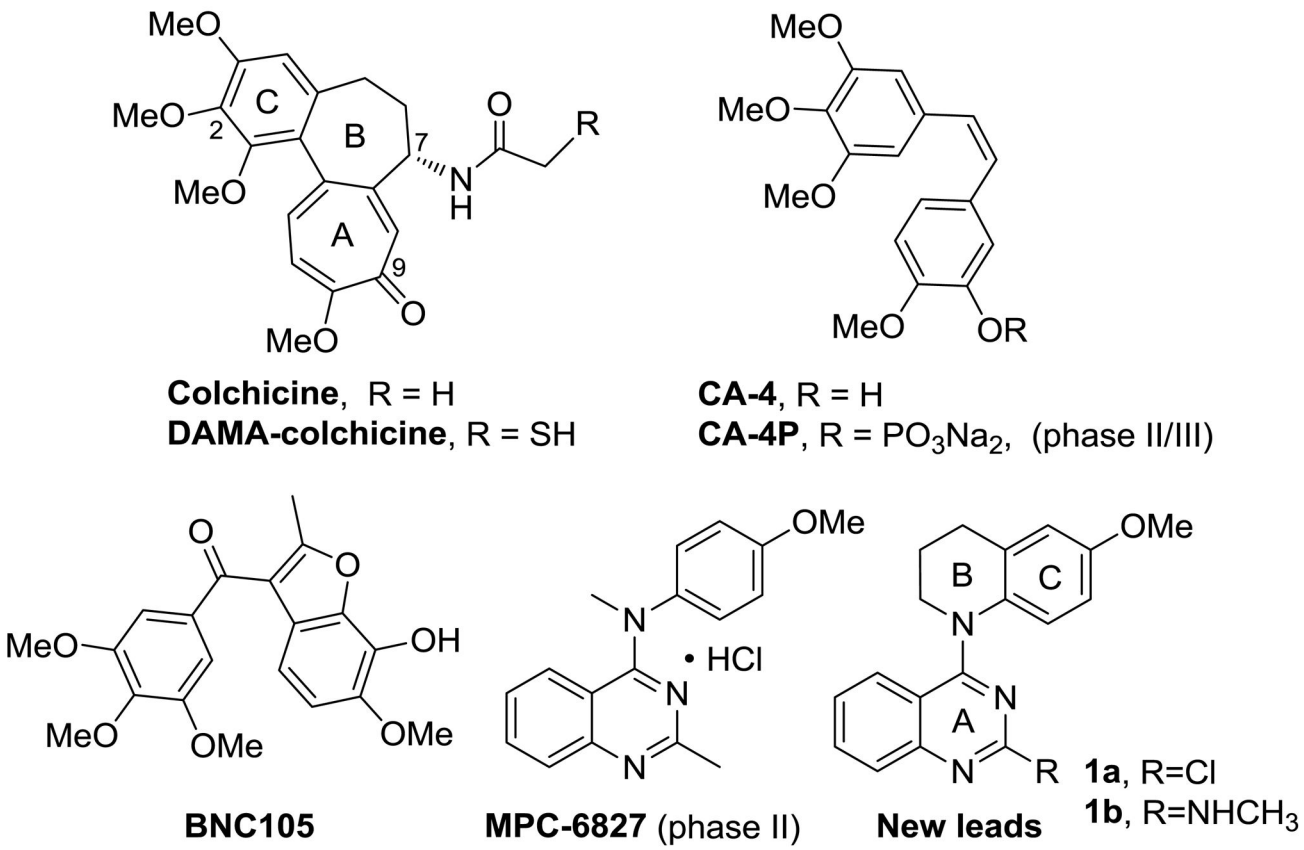



**Figure 1.**
Colchicine and analogs, drug candidates of VDAs, and our leads

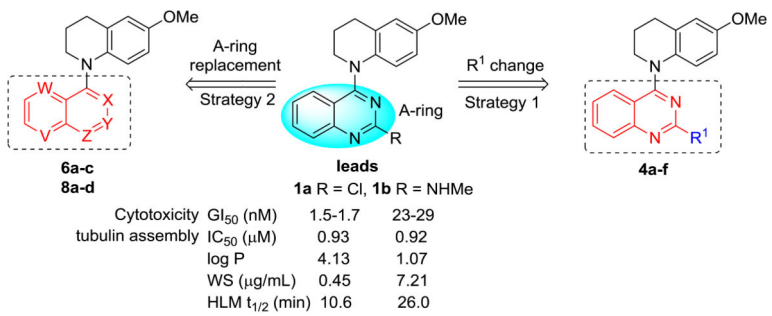



**Figure 2.**
Leads and optimization strategy

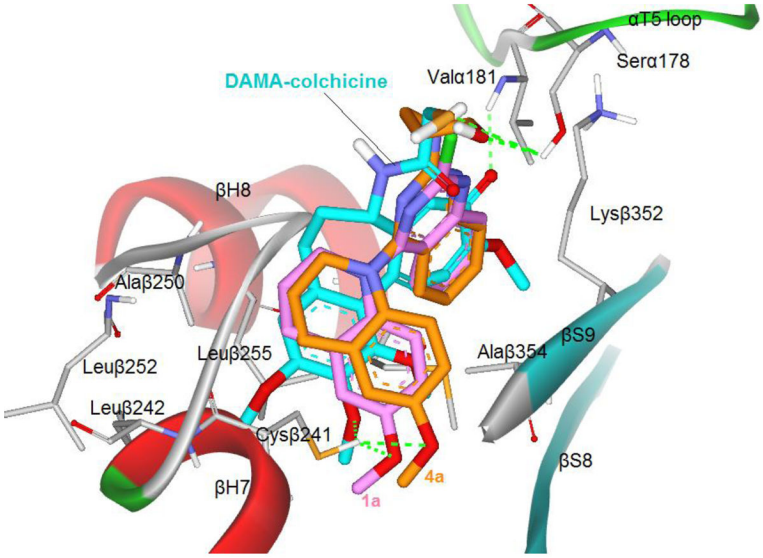
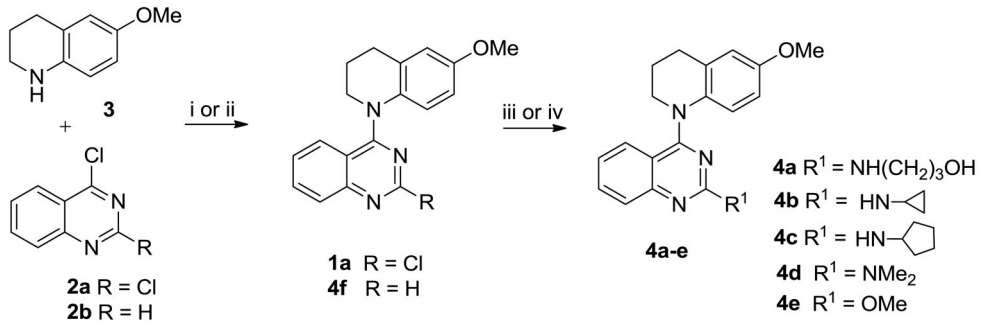


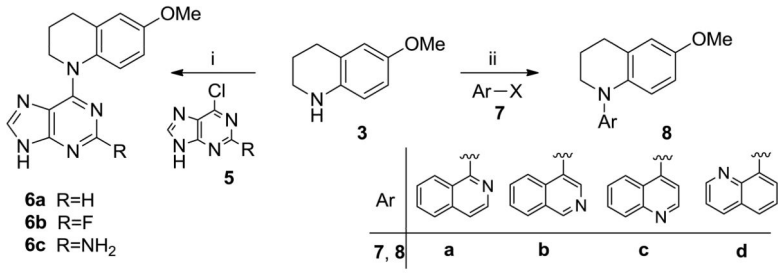

**Figure 3.**

Predicted modes for **4a** (orange stick for carbon atoms) binding with tubulin (PDB 1SA0) and overlapping with **1a** (pink stick for carbon atoms) and DAMA-colchicine (cyan for carbon atoms, the native ligand of 1SA0). Surrounding amino acid side chains are shown in gray stick format and labeled. Hydrogen bonds are shown by green dashed lines, and the distances between ligands and protein are less than 3 Å.




**Scheme 1.**

(i) **2a**, anhydrous EtOH/$NaHCO_3$, reflux, 3 h; (ii) **2b**, *i*-PrOH/*c*HCl, reflux, 1 h; (iii) alkylamine, in EtOH or DMF (for **4d**), mw, 150 ºC, 15 min; (iv) NaOMe/MeOH (for **4e**), reflux, 1 h.




**Scheme 2.**

(i) *i*-PrOH/*c*HCl, reflux; (ii) $Pd(OAc)_2$/X-phos (mole ratio 2 – 4%), $Cs_2CO_3$, in toluene reflux.

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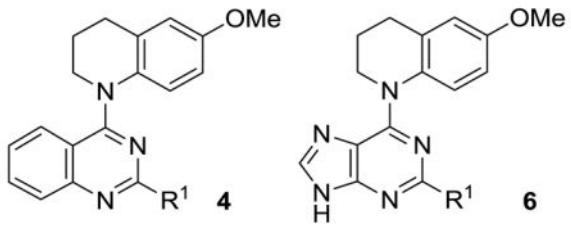
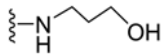
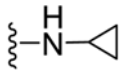
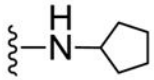
**Table 1**

Cytotoxicity of the **4** and **6** series against a HTCL panel




| Cmpd | R[1] | GI$_{50}$ (μM ± SD)[a] | | | |
|---|---|---|---|---|---|
| | | A549 | KB | KBvin | DU145 |
| **1a**[b] | -Cl | 0.0017 ± 0.0003 | 0.0017 ± 0.0008 | 0.0017 ± 0.0003 | 0.0015 ± 0.0003 |
| **1b**[b] | -NHMe | 0.027 ± 0.005 | 0.025 ± 0.002 | 0.029 ± 0.005 | 0.023 ± 0.003 |
| **4a** |  | 0.016 ± 0.001 | 0.018 ± 0.003 | 0.019 ± 0.002 | 0.020 ± 0.007 |
| **4b** |  | 0.016 ± 0.003 | 0.013 ± 0.002 | 0.013 ± 0.002 | 0.016 ± 0.002 |
| **4c** |  | 1.34 ± 0.10 | 1.19 ± 0.19 | 1.42 ± 0.02 | 1.29 ± 0.13 |
| **4d** | $NMe_2$ | 0.17 ± 0.01 | 0.14 ± 0.02 | 0.20 ± 0.02 | 0.15 ± 0.02 |
| **4e** | OMe | 0.024 ± 0.005 | 0.026 ± 0.006 | 0.030 ± 0.005 | 0.021 ± 0.002 |
| **4f** | H | 0.018 ± 0.002 | 0.014 ± 0.003 | 0.019 ± 0.001 | 0.014 ± 0.003 |
| **6a** | H | >35 | >35 | >35 | >35 |
| **6b** | F | 23.6 ± 2.36 | >33 | 27.10 ± 5.06 | 25.5 ± 0.47 |
| **6c** | $NH_2$ | 19.4 ± 1.99 | 17.5 ± 1.30 | 18.9 ± 5.12 | 18.0 ± 3.05 |
| CA-4[c] | | 0.0841 ± 0.006 | 0.0036 ± 0.0003 | 0.0012±0.0002 | NT[d] |
| Paclitaxel[c] | | 0.0076 ± 0.002 | 0.0064 ± 0.001 | 1.21 ± 0.19 | 0.006 ± 0.001 |

[a] Concentration of compound that inhibited human tumor cell growth by 50%, presented as mean ± standard deviation (SD), performed at least in triplicate.

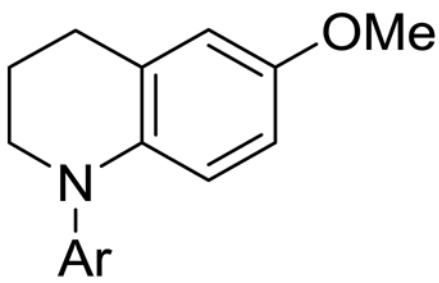
[b] Data from reference 14.

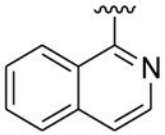
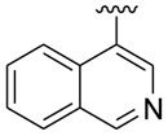
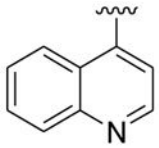
[c] Positive control.

[d] Not tested.

**Table 2**

Cytotoxicity of the **8** series against HTCLs




| Cmpd | Ar | $GI_{50}$ (μM ± SD) | | | |
|---|---|---|---|---|---|
| | | A549 | KB | KBvin | DU145 |
| **8a** |  | 2.08 ± 0.07 | 1.72 ± 0.45 | 1.64 ± 0.15 | 1.47 ± 0.06 |
| **8b** |  | 1.95 ± 0.31 | 1.48 ± 0.37 | 1.54 ± 0.06 | 0.92 ± 0.28 |
| **8c** |  | 0.20 ± 0.02 | 0.16 ± 0.02 | 0.18 ± 0.02 | 0.18 ± 0.03 |

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| Cmpd | Ar | $GI_{50}$ (μM ± SD) | | | |
|---|---|---|---|---|---|
| | | A549 | KB | KBvin | DU145 |
| 8d |  | 12.1 ± 1.18 | 16.2 ± 3.47 | 8.71 ± 1.41 | 12.8 ± 1.48 |

## Table 3

Inhibition of tubulin polymerization and colchicine binding to tubulin by selected compounds.

| Compound | Inhibition of tubulin assembly [a] | Inhibition of colchicine binding [b] | |
|---|---|---|---|
| | $IC_{50}$ (μM) ± SD | % Inhibition ± SD | |
| | | 5 μM | 1 μM |
| **1a**[c] | 0.93 ± 0.04 | 99 ± 0.6 | 95 ± 0.7 |
| **1b**[c] | 0.92 ± 0.06 | 95 ± 0.2 | 76 ± 0.1 |
| **4a** | 0.85 ± 0.07 | 78 ± 1 | 49 ± 5 |
| **4b** | 1.3 ± 0.04 | 89 ± 0.1 | 56 ± 5 |
| **4f** | 1.3 ± 0.02 | 91 ± 1 | 69 ± 4 |
| **4c** | 5.7 ± 0.1 | 29 ± 1 | NT[d] |
| **8a** | 2.6 ± 0.2 | 37 ± 2 | NT |
| **8b** | 3.3 ± 0.01 | 39 ± 4 | NT |
| **8d** | >20 | NT | NT |
| **CA-4**[e] | 1.2 ± 0.1 | 97 ± 0.4 | 84 ± 0.3 |

[a] Extent of assembly of 10 μM tubulin measured after 20 min at 30 °C.

[b] Tubulin: 1 μM, [$^3$H]colchicine: 5 μM, inhibitor 5 μM or 1 μM. Incubation was performed for 10 min at 37 °C.

[c] Data from reference 14.

[d] Not tested.

[e] Reference compound, a drug candidate in phase II/III clinical trials.

## Table 4

Aqueous solubility and lipophilicity of selected compounds [a]

| Compound | Solubility (μg/mL) | Log P |
|---|---|---|
| **4a** | 75.6 ± 0.89 | 3.43 ± 0.01 |
| **4b** | 2.55 ± 0.27 | 4.13 ± 0.03 |

[a] Measured at pH 7.4 and presented as mean from three separate experiments with standard deviation (±SD).