Supporting Information

Optimization of 4-(*N*-cycloamino)phenylquinazolines as a novel class of tubulin polymerization inhibitors targeting the colchicine site

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1. HPLC purity and conditions:

Purities of active target compounds were determined by using an Agilent HPLC-1200 with UV detector and an Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm), flow rate 0.8 mL/min, UV detection at 254 nm and an injection volume of 15 μL. Elution was performed with a mixture of solvents, acetonitrile (A) and water (B) (condition 1) or methanol (A) and water (B) (condition 2). For **4b**, **4c**, **5c**, **5e**, **5g**, and **5h**, solvent B contained 0.04% ammonium acetate in condition 1.

Table 1 HPLC purity data of active target compounds

Compounds	**Condition-1 (ACN/H_2O)**			**Condition-2 (MeOH/H_2O)**		
	Solvent Ratio	Retention time (min)	Purity %	Solvent Ratio	Retention time (min)	Purity %
4a	70/30	14.5	99.5	80/20	10.20	99.1
4b	70/30	7.08	100.0	85/15	6.85	99.6
4c	70/30	7.94	98.1	85/15	7.20	98.8
5a	60/40	4.22	96.9	70/30	6.62	96.8
5b	70/30	2.86	100.0	80/20	3.49	98.6
5c	60/40	2.35	98.8	75/25	3.43	97.9
5d	60/40	6.98	100.0	75/25	7.06	100.0
5e	60/40	4.42	100.0	75/25	6.22	100.0
5f	60/40	1.89	98.9	70/30	5.54	98.9
5g	60/40	3.17	98.8	75/25	4.40	98.1
5h	70/30	4.17	95.1	85/15	4.49	95.7
5i	70/30	5.22	100.0	85/15	4.25	100.0
5j	70/30	8.80	100.0	85/15	5.90	99.7
5k	70/30	8.40	98.5	80/20	8.74	96.2

2. Cell cycle analysis:

It was performed after treatment of cells with **5f** using a FACSCalibur (BD Biosciences, Mountain View, CA). A549 cells were treated with 1 nM and 3 nM **5f** for 24 h. Cell cycle distribution was analyzed by the standard propidium iodide procedure as mentioned under the Experimental Section.

Table 2 Cell cycle distribution data of A549 cells treated with **5f**

Compound (contribution)	% of cells		
	G0/G1 phase	S phase	G2/M phase
control	72.91	18.77	8.31
Colchicine (100 nM)	31.60	22.08	46.32
Colchicine (300 nM)	9.75	16.75	73.49
5f (1 nM)	56.59	21.52	21.89
5f (3 nM)	3.74	12.45	83.81