

# Supporting information

## ID-Checker technology for the highly selective macroscale delivery of anticancer agents to the cancer cells

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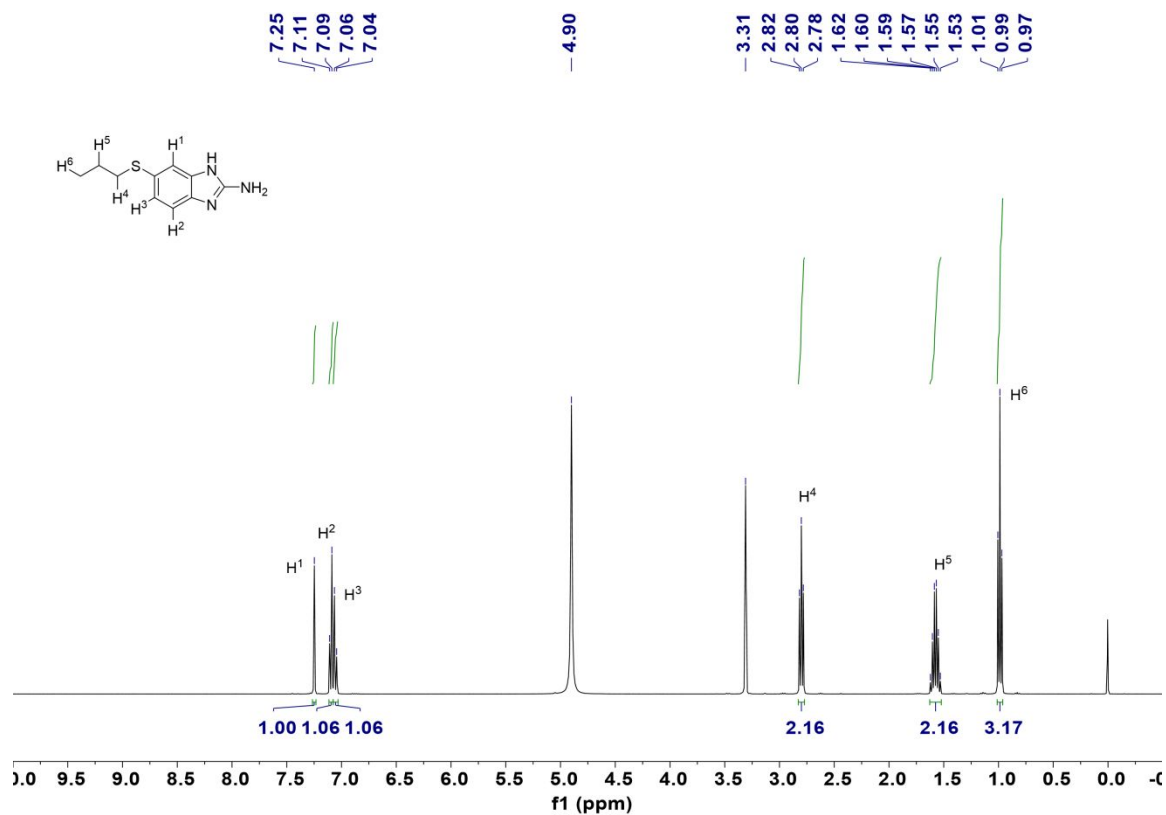


Figure S1. <sup>1</sup>H NMR spectrum of AI-NH<sub>2</sub> in CD<sub>3</sub>OD.

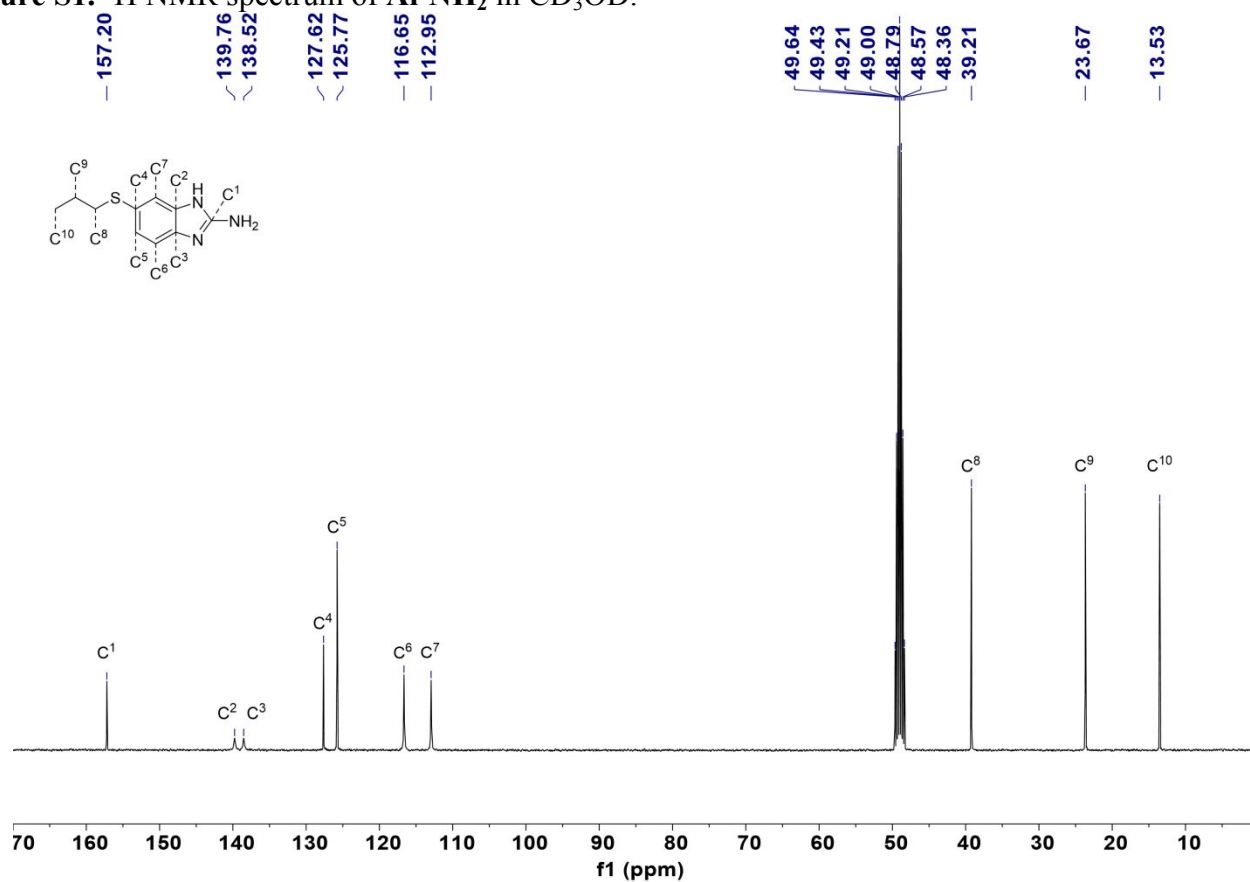


Figure S2. <sup>13</sup>C NMR spectrum of AI-NH<sub>2</sub> in CD<sub>3</sub>OD.

RT : 0.81 min Scan# : 22  
 Elements : C 10/0, H 21/0, N 3/0, S 1/0  
 Mass Tolerance : 1000ppm, 5mmu if m/z < 5, 50mmu if m/z > 50  
 Unsaturation (U.S.) : -0.5 - 20.0

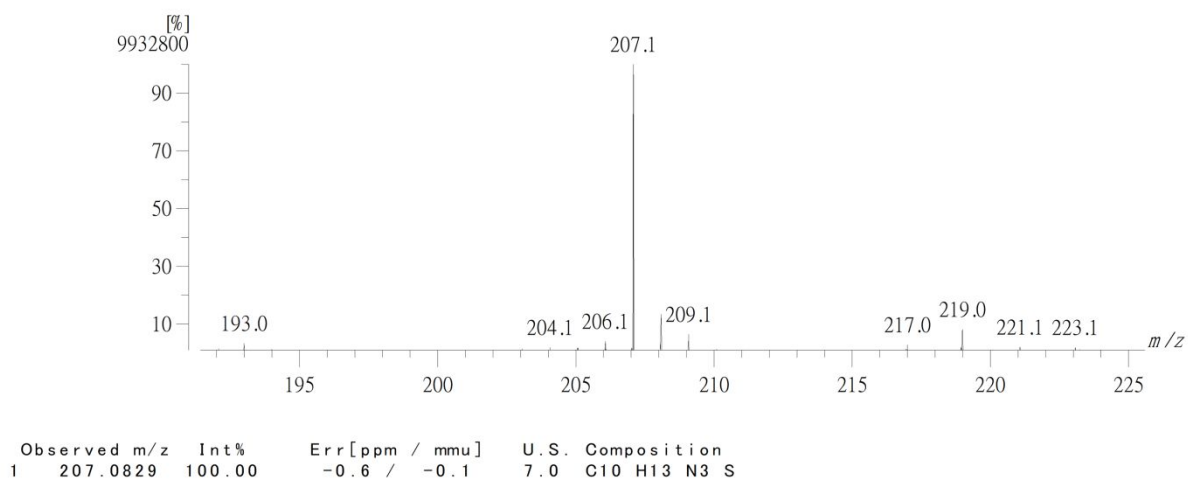
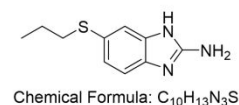


Figure S3. Mass spectrum of Al-NH<sub>2</sub>.

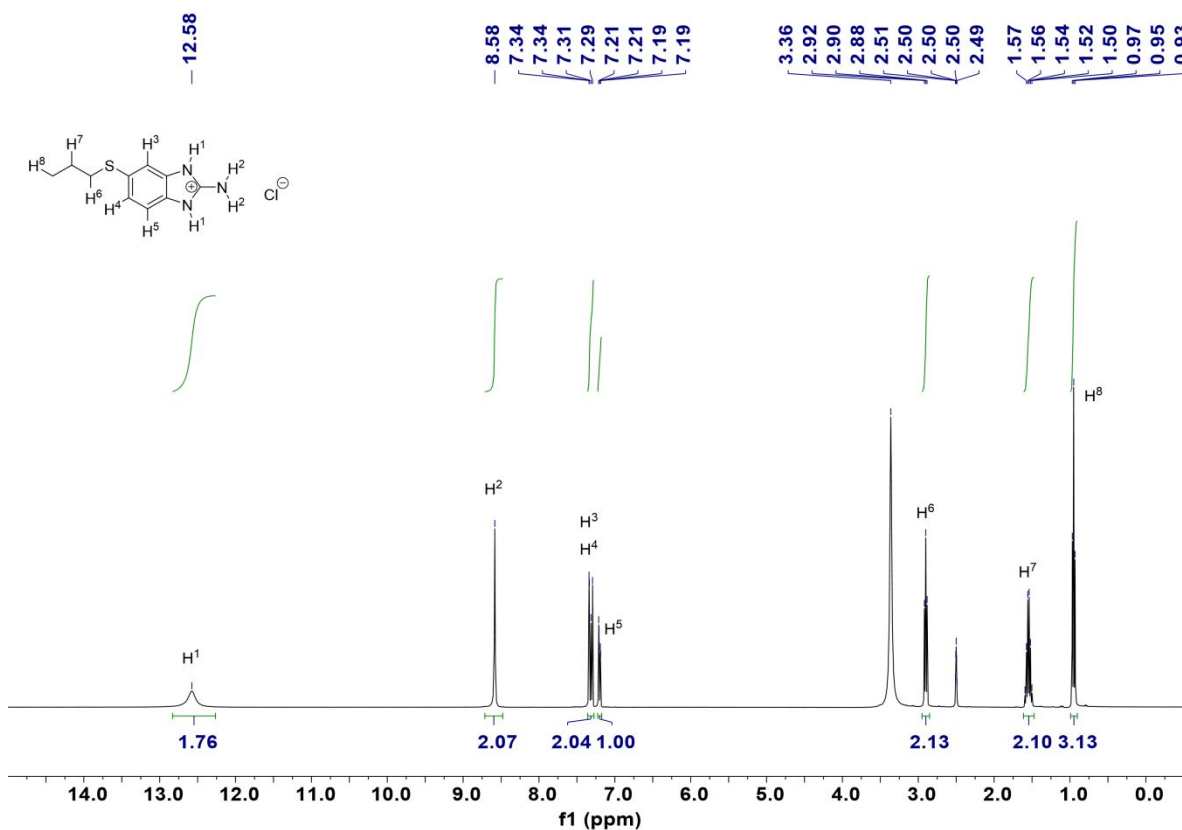


Figure S4. <sup>1</sup>H NMR spectrum of Al-NH<sub>2</sub>.HCl in DMSO-d<sub>6</sub>.

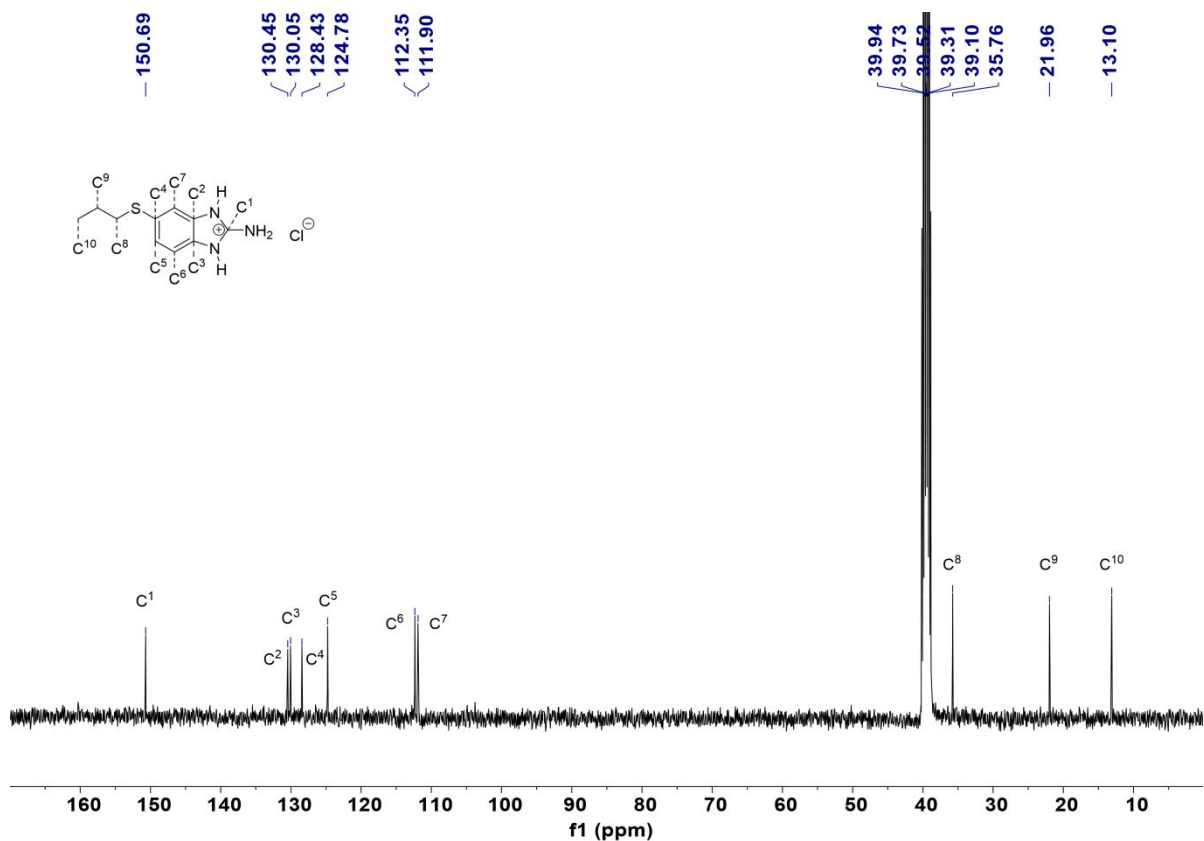


Fig. S5. <sup>13</sup>C NMR spectrum of Al-NH<sub>2</sub>.HCl in DMSO-d<sub>6</sub>.

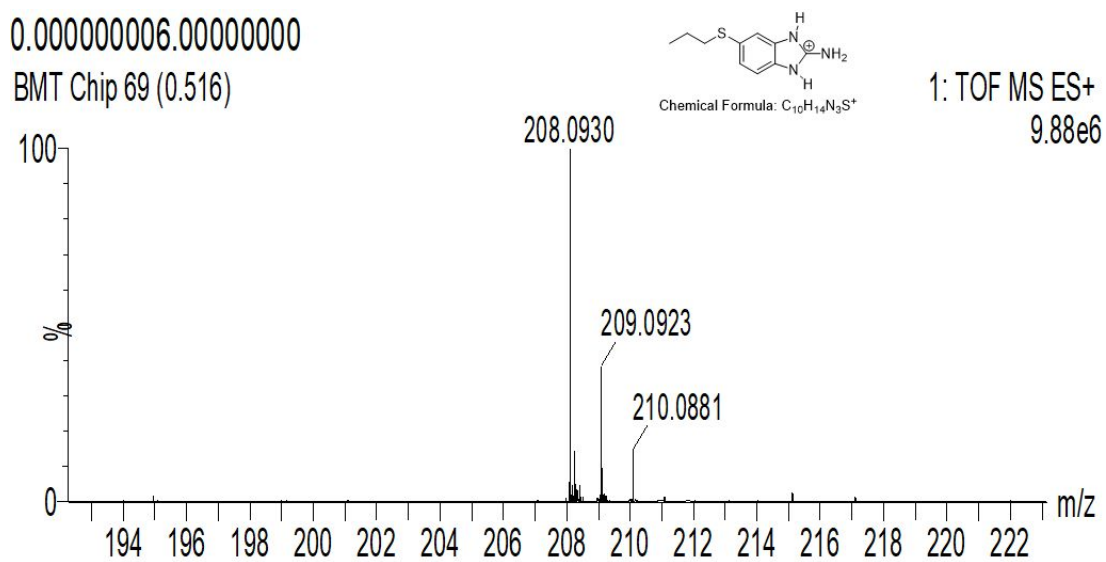


Fig. S6. Mass spectrum of Al-NH<sub>2</sub>.HCl

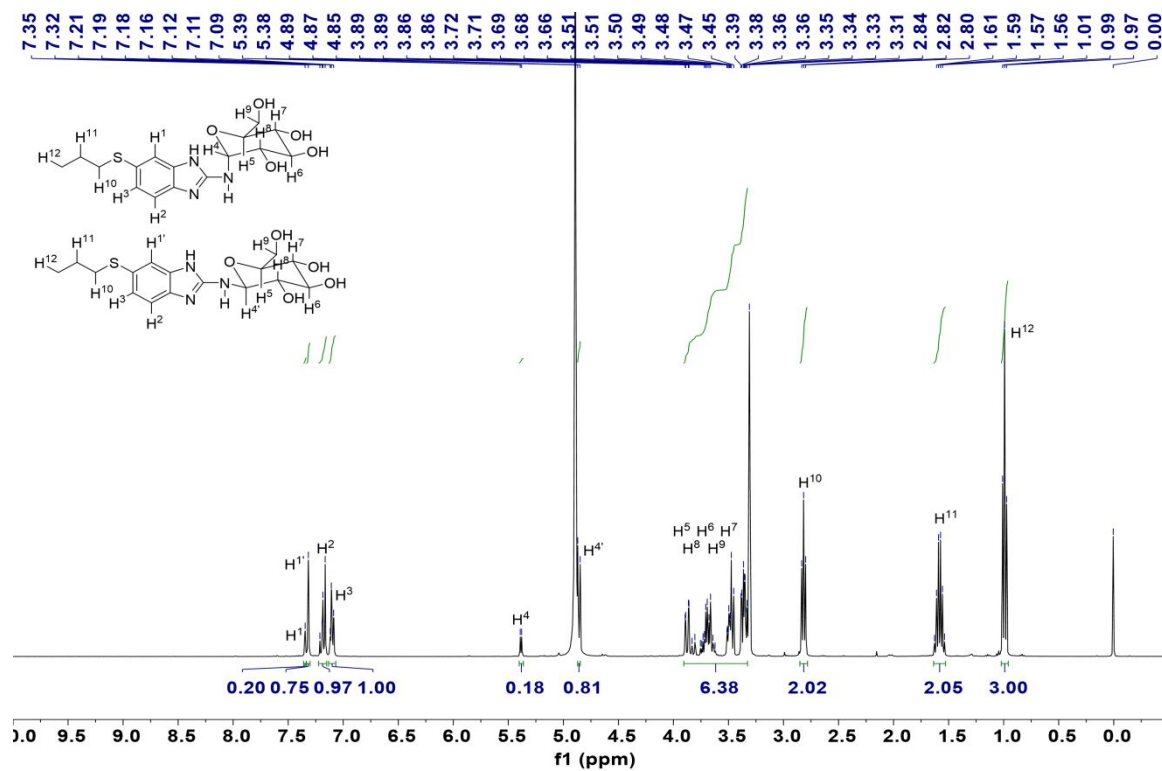


Fig. S7. <sup>1</sup>H NMR spectrum of Al-G in CD<sub>3</sub>OD.

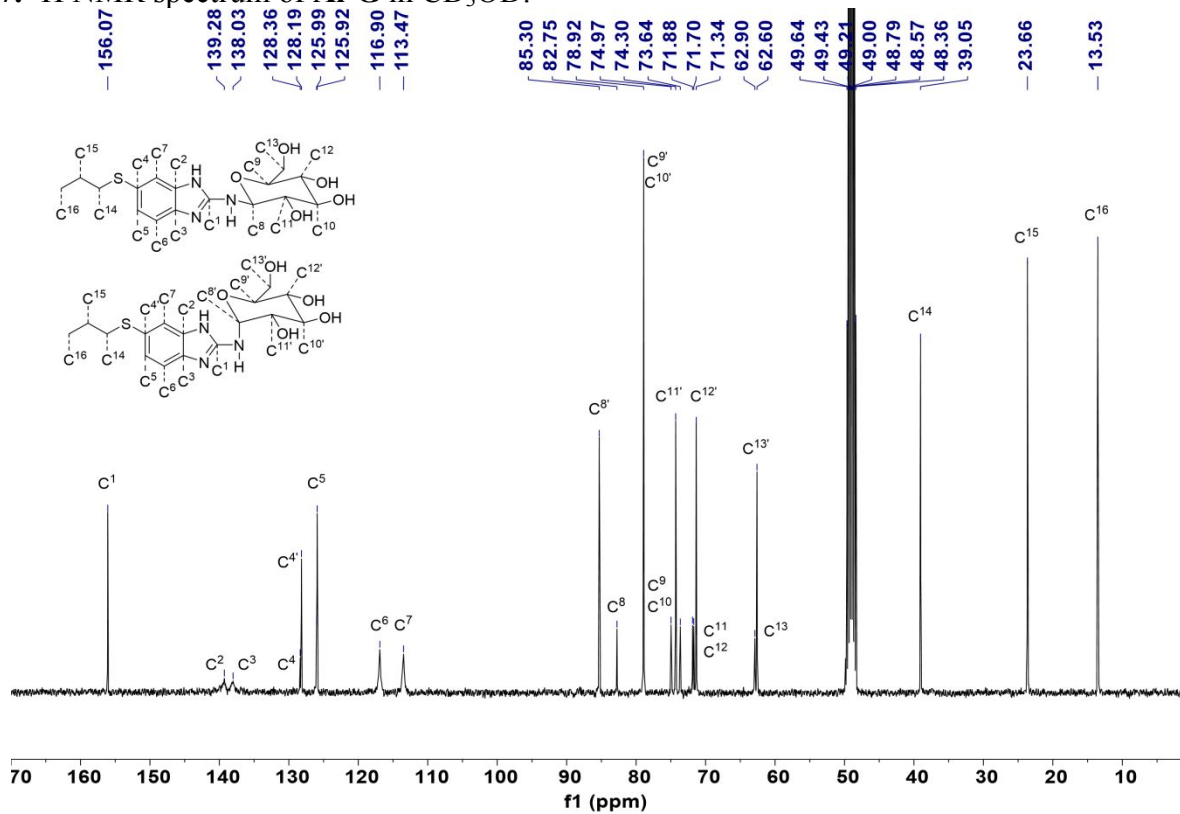
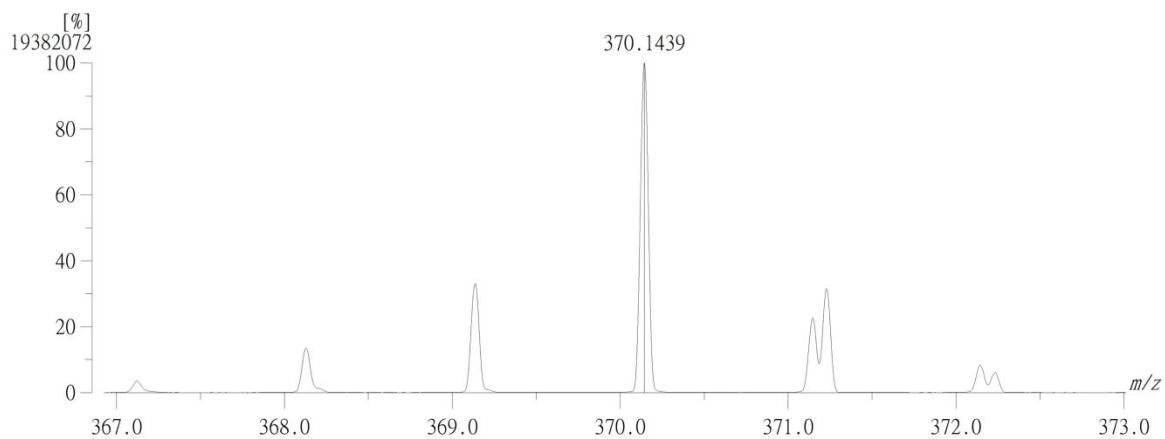
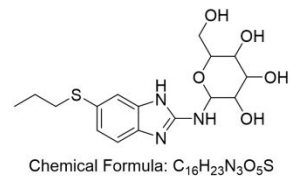


Fig. S8. <sup>13</sup>C NMR spectrum of Al-G in CD<sub>3</sub>OD.

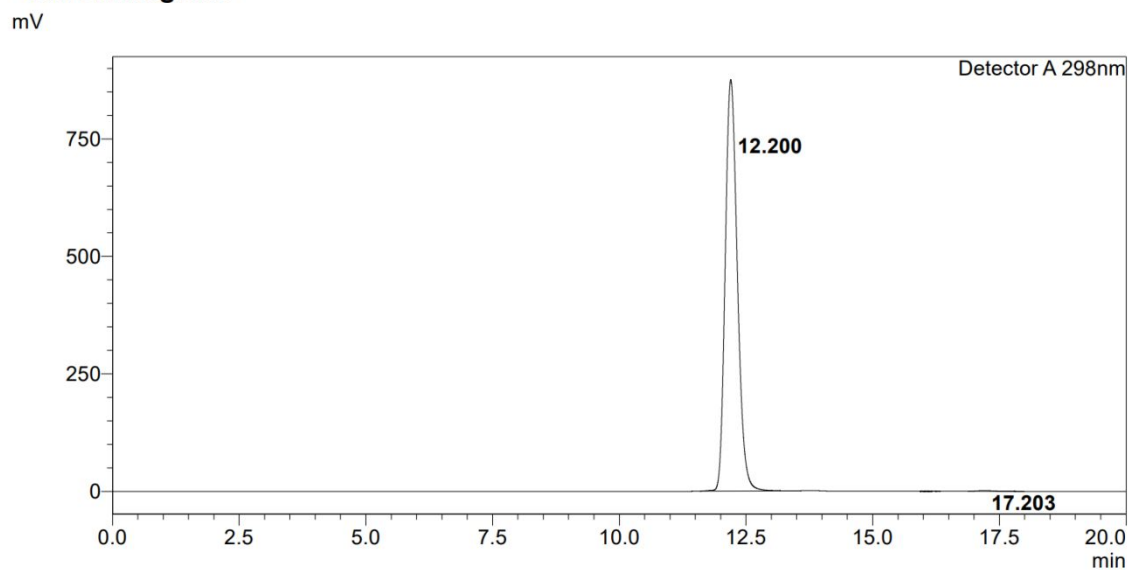
RT : 0.03 min Scan# : (238)  
 Elements : C 100/0, H 100/0, N 5/0, O 10/0, S 1/0  
 Mass Tolerance : 1000ppm, 5mmu if m/z < 5, 10mmu if m/z > 10  
 Unsaturation (U.S.) : 0.0 - 15.0



Observed m/z	Int%	Err [ppm / mmu]	U. S. Composition
1 370.1439	100.00	+0.6 / +0.2	7.5 C16 H24 N3 O5 S

**Fig. S9.** Mass spectrum of Al-G.

**<Chromatogram>**

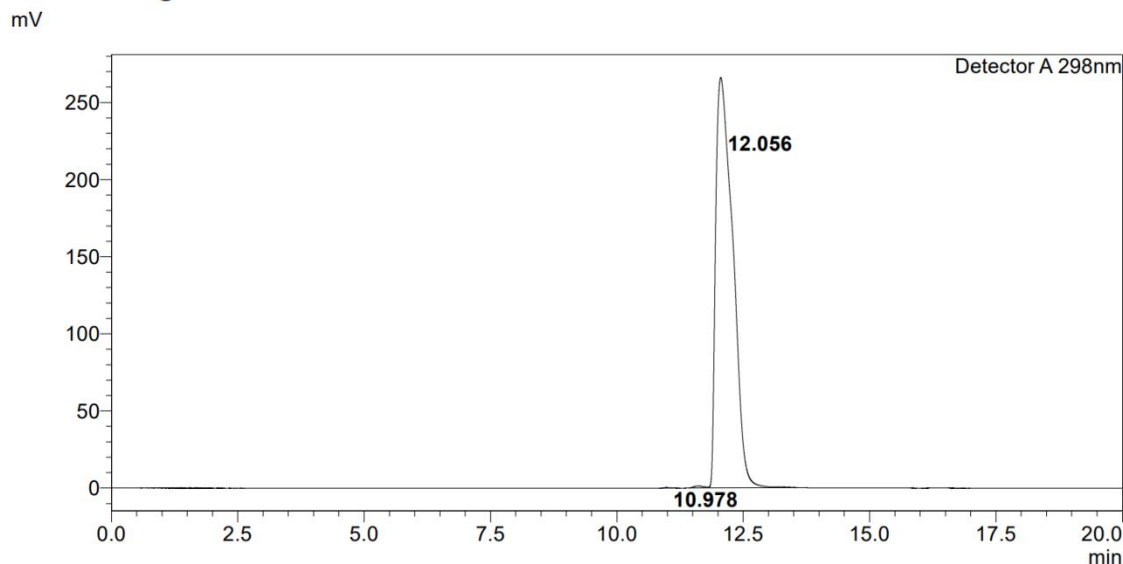


**<Peak Table>**

Detector A 298nm					
Peak#	Ret. Time	Height	Height%	Area	Area%
1	12.200	874500	99.838	14570874	99.770
2	17.203	1418	0.162	33606	0.230
Total		875918	100.000	14604480	100.000

**Fig. S10.** HPLC trace for 1mM Al-NH<sub>2</sub> in MeOH (Eluent, 100% MeOH; Pressure, 10 bar; Injection volume, 10 μL; Oven temp., 40 °C).

<Chromatogram>

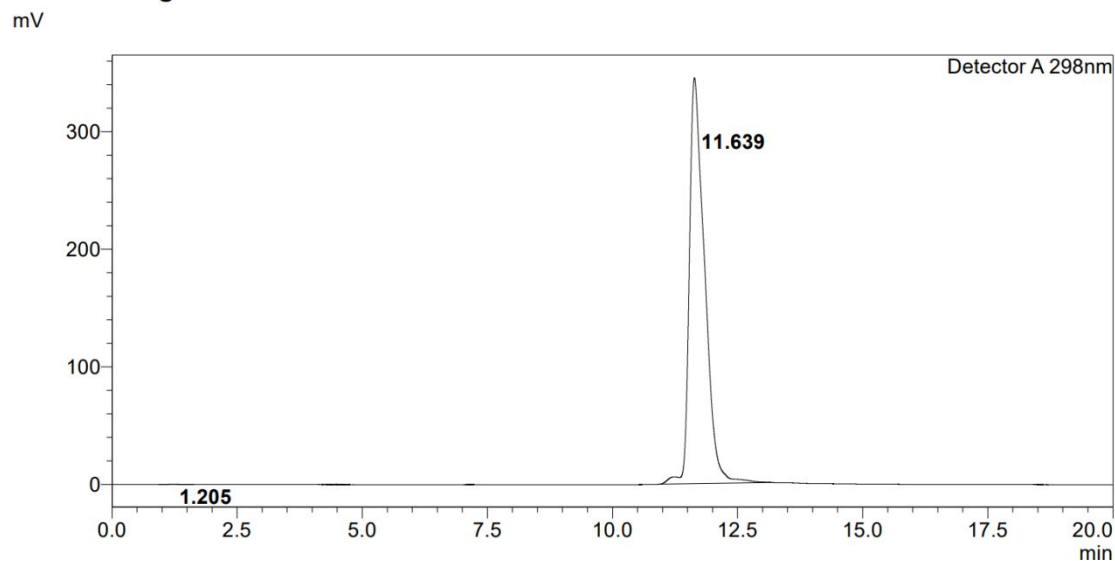


<Peak Table>

Detector A 298nm					
Peak#	Ret. Time	Height	Height%	Area	Area%
1	10.978	394	0.148	2776	0.044
2	12.056	266026	99.852	6274852	99.956
Total		266420	100.000	6277628	100.000

Fig. S11. HPLC traces for Al-NH<sub>2</sub>.HCl (Eluent, 100% MeOH; Pressure, 10 bar; Injection volume, 10 μL; Oven temp., 40 °C).

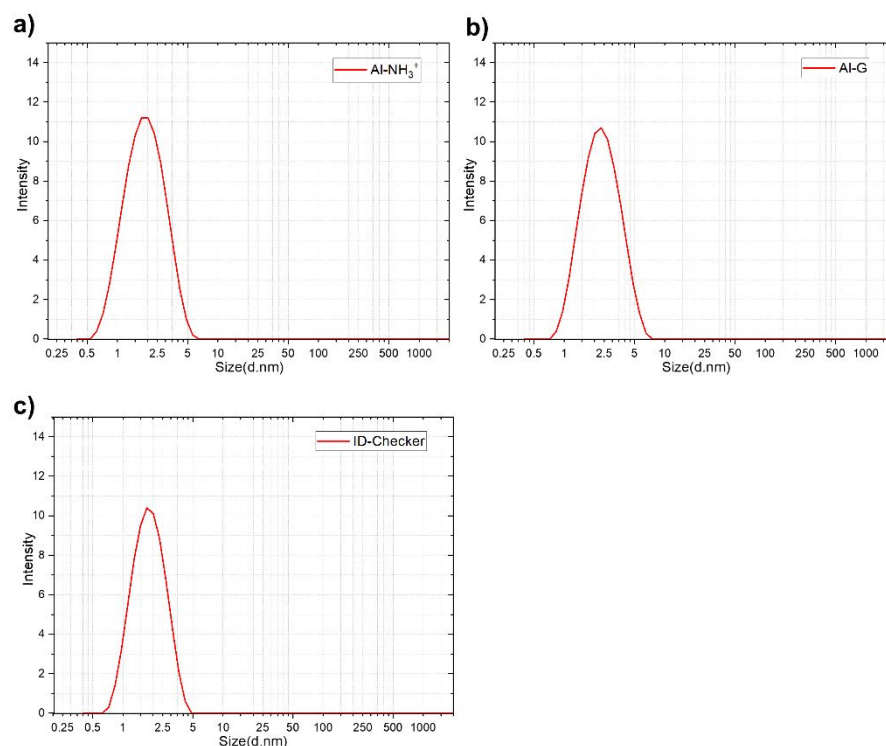
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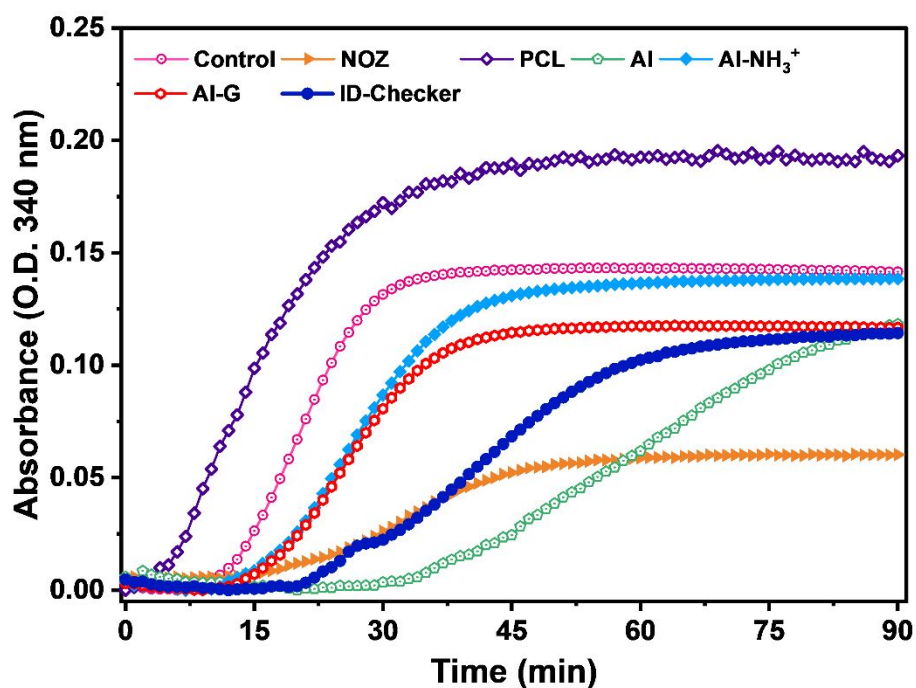
<Peak Table>

Detector A 298nm					
Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.205	192	0.055	3592	0.049
2	11.639	345059	99.945	7345757	99.951
Total		345251	100.000	7349348	100.000

Fig. S12. HPLC traces for Al-G (Eluent, 100% MeOH; Pressure, 10 bar; Injection volume, 10 μL; Oven temp., 40 °C).

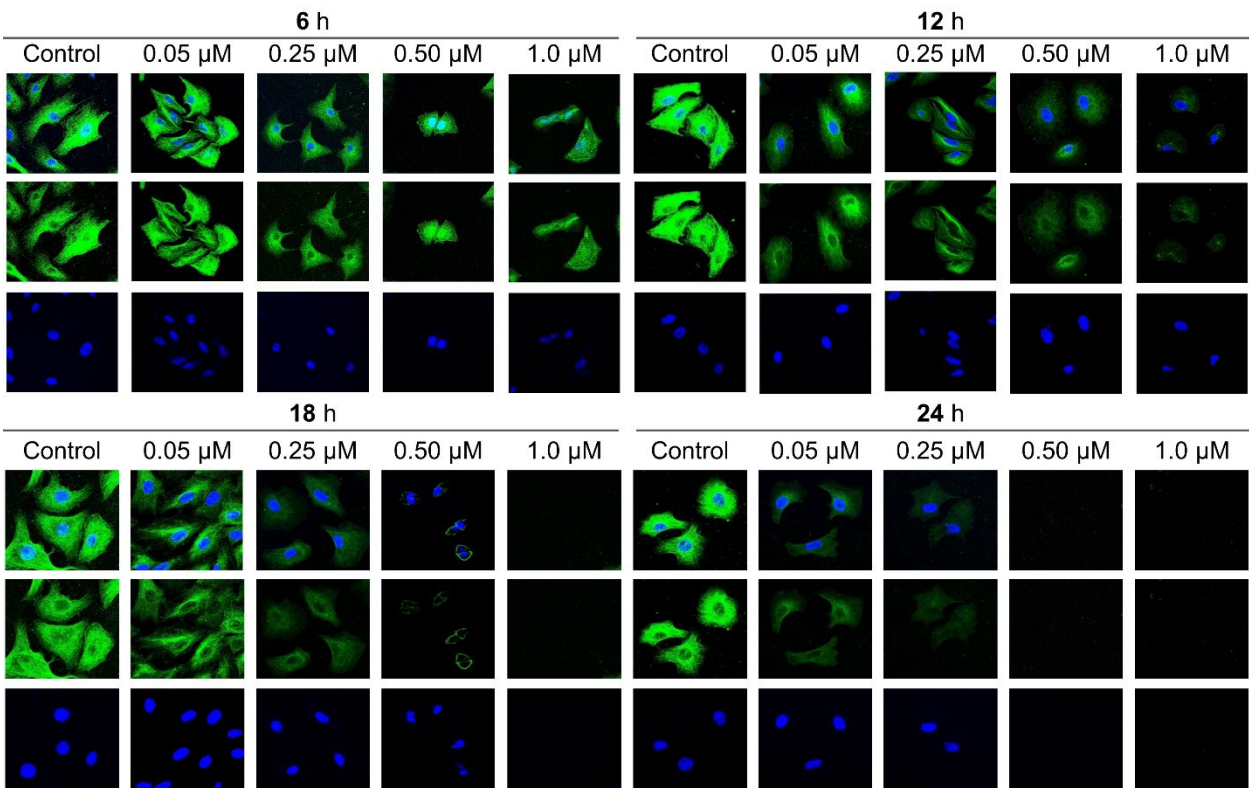


**Figure S13.** DLS data representing the particle size of a) Al-NH<sub>3</sub><sup>+</sup> aggregate, b) Al-G aggregate, and c) ID-Checker

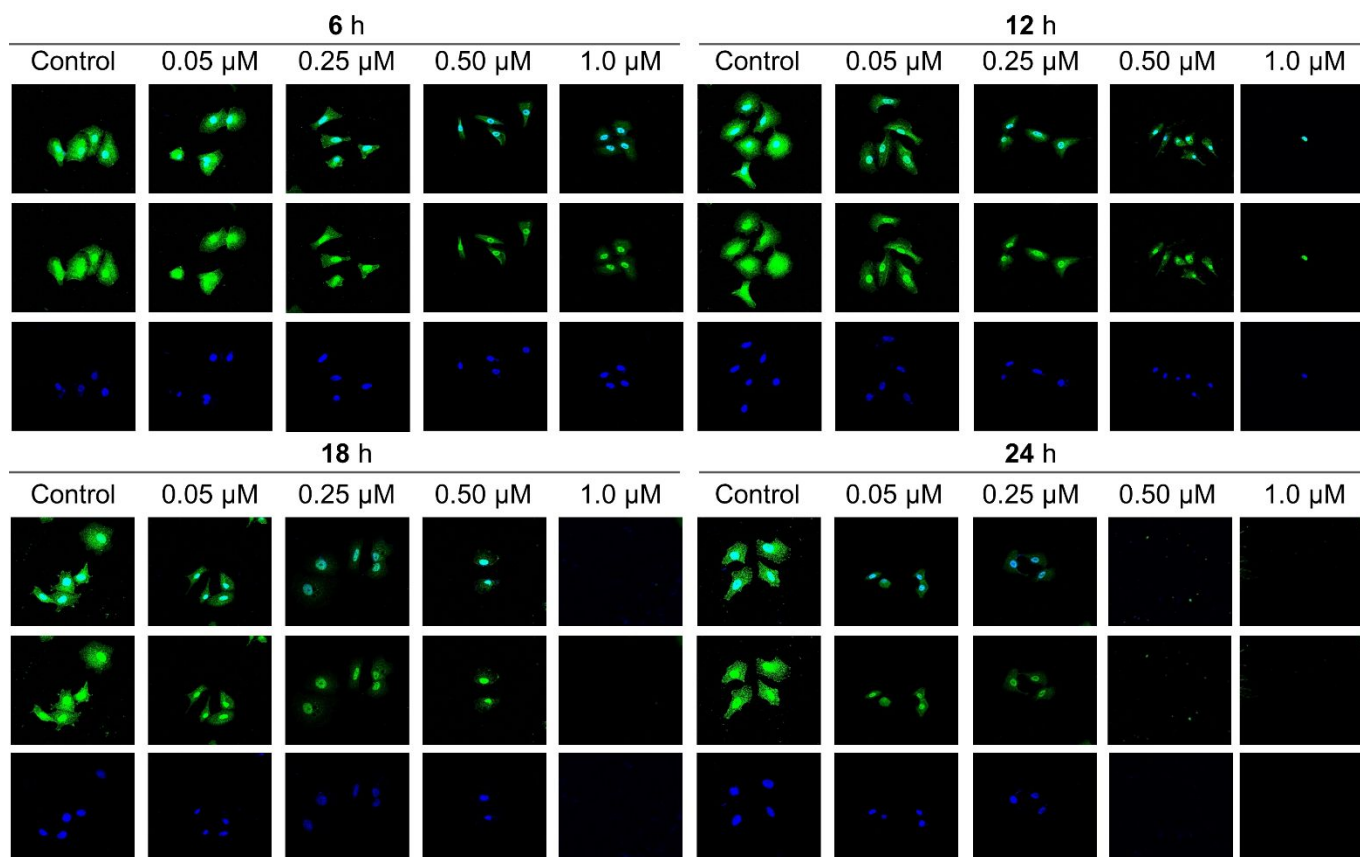


**Fig. S14.** Effects of NOZ, PCL, AI, Al-NH<sub>3</sub><sup>+</sup> aggregate, Al-G aggregate, and ID-Checker on tubulin polymerization. Effect of ID-Checker on tubulin polymerization. Polymerization of purified tubulin into microtubules was measured using spectrometer over 90 min at 1 min interval by incubating it at 37 °C in the absence (control) or presence of 0.20 μM ID-Checker, 20 μM NOZ, 20 μM PCL, 20 μM AI, 20 μM Al-NH<sub>3</sub><sup>+</sup> aggregate, and 20 μM Al-G aggregate.

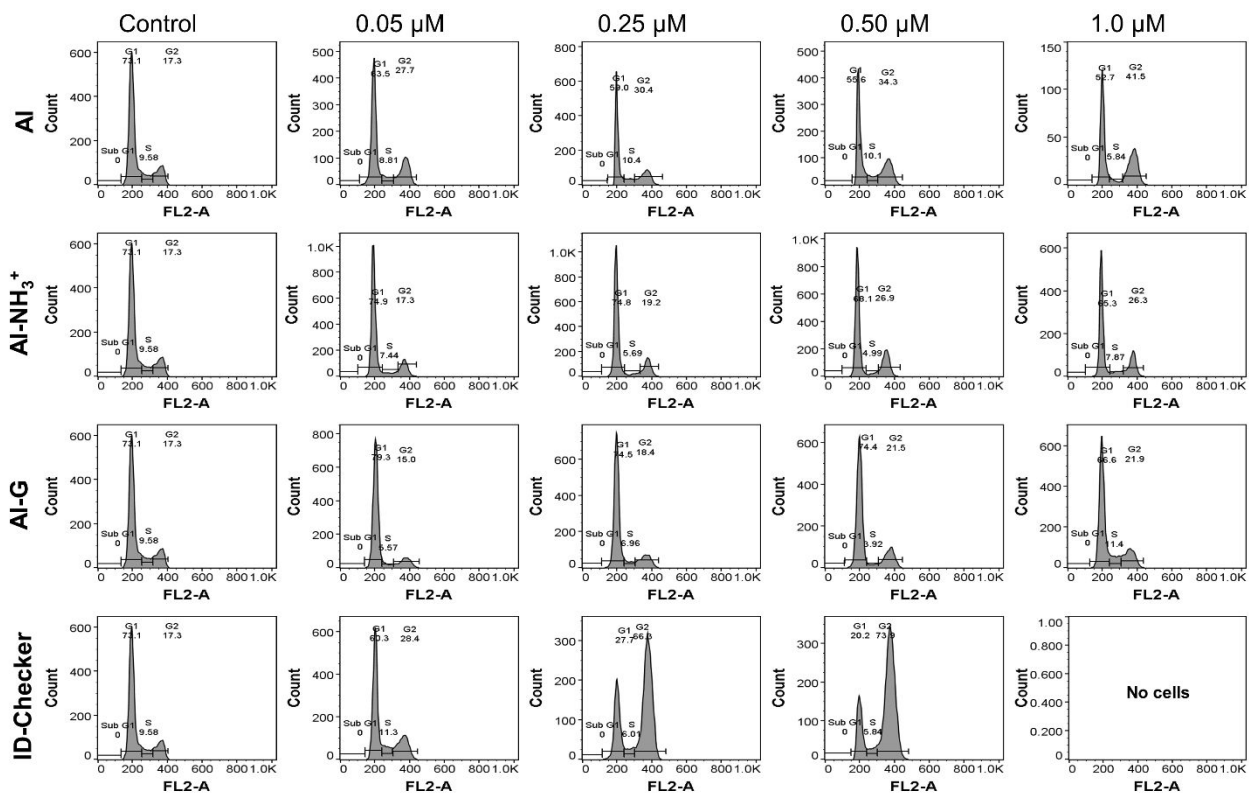




**Fig. S15.** Immunofluorescence staining of microtubules in A549 cells. Cells were treated with vehicle (0.9% saline), 0.05, 0.25, 0.50, and 1.0 μM **ID-Checker** for 6, 12, 18, and 24 h. Cells were incubated with anti- $\alpha$ -tubulin antibody followed by anti-mouse IgG/FITC antibody (green fluorescence) to stain the microtubules, and nuclei were stained by subsequent incubation of cells with (DAPI, blue fluorescence). Bottom, DAPI; middle, microtubule network; top, merged images observed by confocal microscopy.



**Fig. S16.** Immunofluorescence staining of GLUT1 channels in A549 cells. Cells were treated with vehicle (0.9% saline), 0.05, 0.25, 0.50 and 1.0  $\mu$ M **ID-Checker** for 6, 12, 18, and 24 h. Cells were incubated with rabbit anti-GLUT1 antibody followed by donkey anti-rabbit IgG/ALEXA488 antibody (green fluorescence) to stain the GLUT channels and nuclei were stained by subsequent incubation of cells with DAPI (blue fluorescence). Bottom, DAPI; middle, GLUT channels; top, merged images observed by confocal microscopy.



**Fig. S17.** Effects on the cell cycle distribution of MCF-7 cells upon treatment with AI, AI-NH<sub>3</sub><sup>+</sup>, AI-G, and ID-Checker at various concentrations (0, 0.05, 0.25, 0.50, and 1.0 μM) 24 h followed by staining with PI to determine the proportion of DNA by flow cytometry.

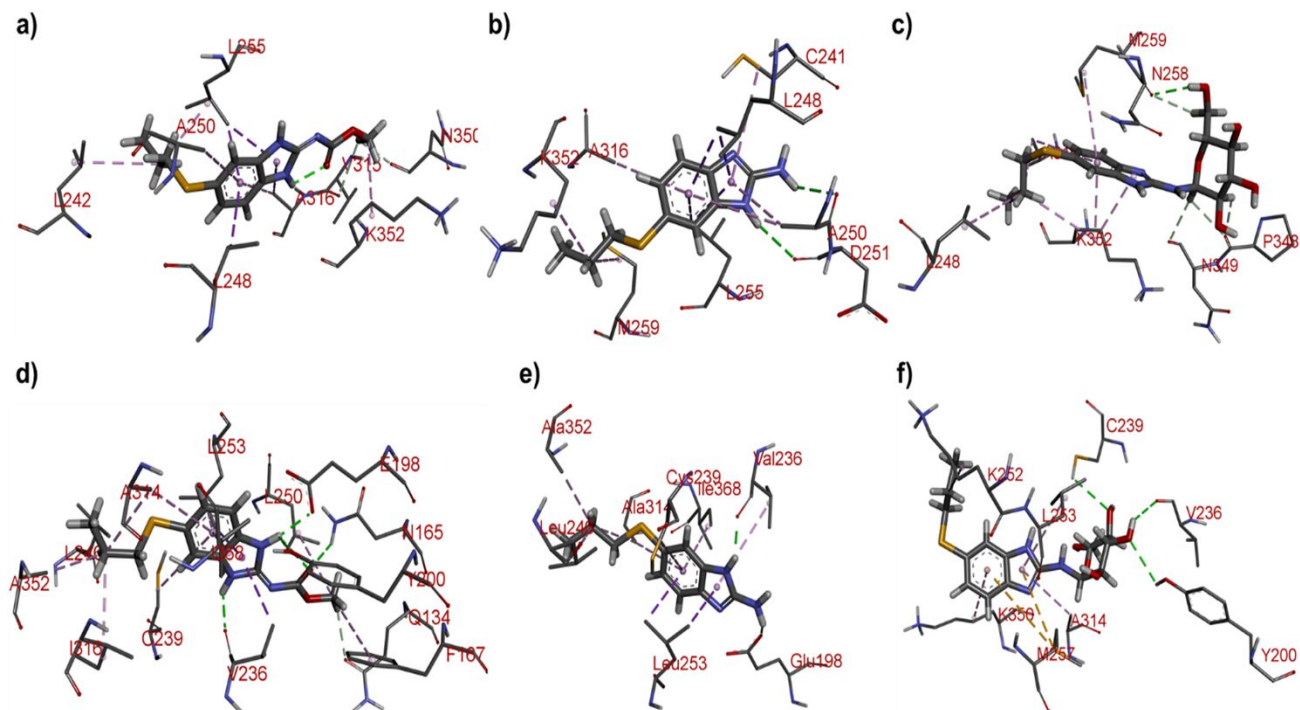
**Table S1.** Body weight of experimental animals (g)

Day	G1	G2	G3	G4
0	19.10 ± 0.16	19.00 ± 0.16	19.00 ± 0.16	19.00 ± 0.28
11	19.35 ± 0.20	19.15 ± 0.20	18.85 ± 0.18	19.00 ± 0.13

**Table S2.** Docking scores for AI, AI-NH<sub>3</sub><sup>+</sup>, and AI-G for binding with tubulin in the colchicine and nocodazole binding sites.

Compound	CBS		NBS	
	Glide Score (kcal mol <sup>-1</sup> )	Glide Energy (kcal mol <sup>-1</sup> )	Glide Score (kcal mol <sup>-1</sup> )	Glide Energy (kcal mol <sup>-1</sup> )
AI	-6.64	-49.0	-7.31	-46.9
AI-NH <sub>3</sub> <sup>+</sup>	-6.44	-33.3	-7.14	-30.7
AI-G	-6.20	-30.9	-6.61	-34.5

CBS, colchicine binding site; NBS, nocodazole binding site

**Fig. S18.** The docking poses of a) AI, b) AI-NH<sub>3</sub><sup>+</sup>, c) AI-G, in colchicine binding site (PDB ID: 4O2B) and d) AI, e) AI-NH<sub>3</sub><sup>+</sup>, f) AI-G in the nocodazole binding site of tubulin (PDB ID: 5CA1).