Combretastatin Inspired Heterocycles as Antitubulin Anticancer Agents

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Supporting Information

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Scanned NMR Spectra

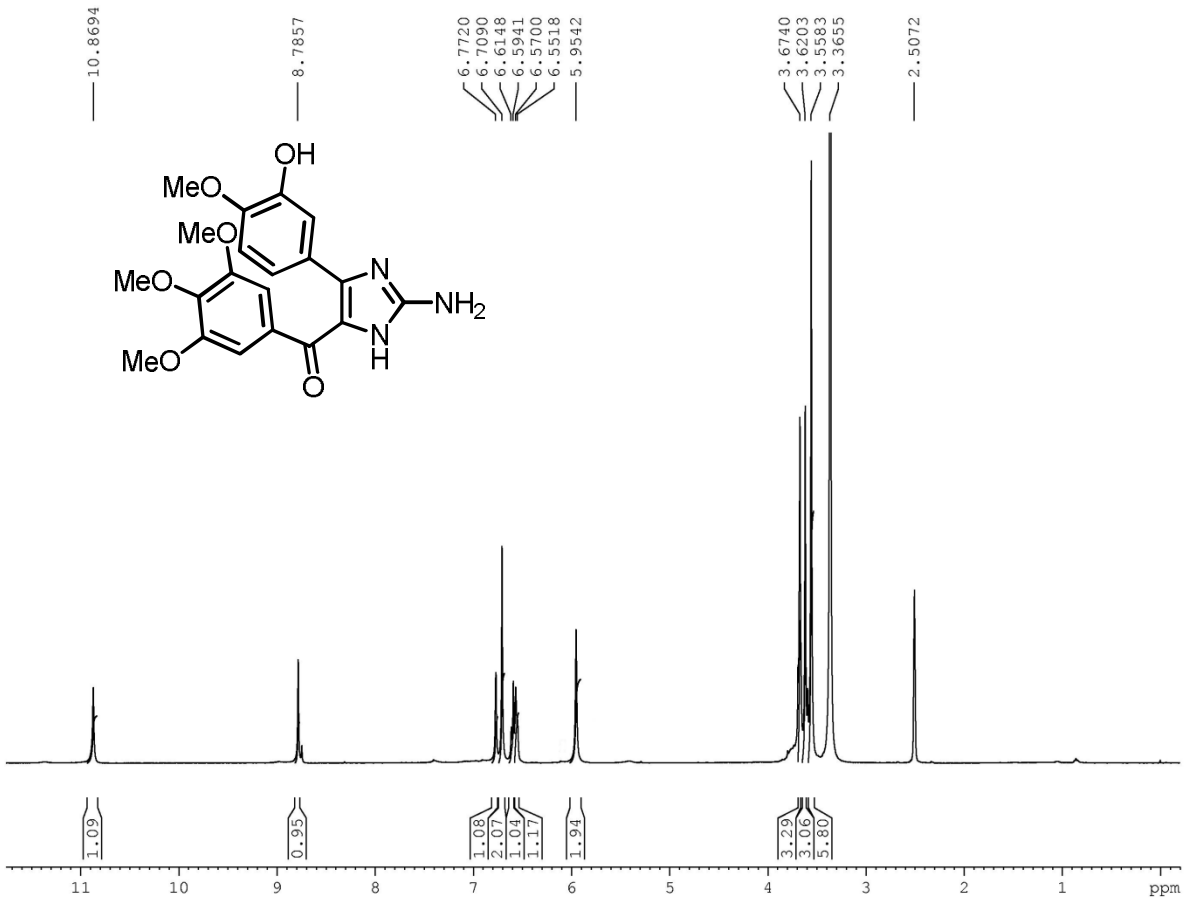

Figure S1. Compound 8:^{1}H NMR

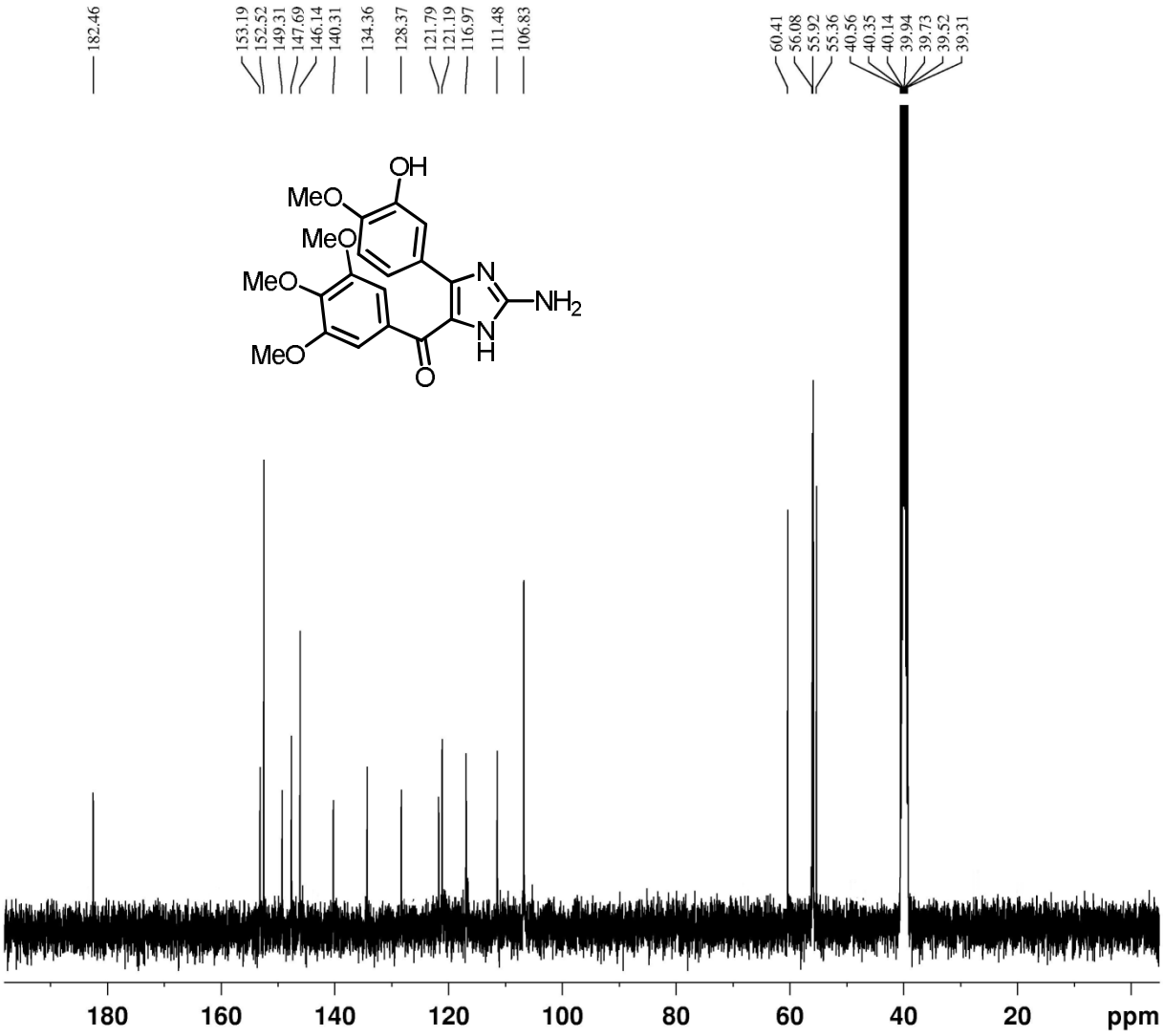

Figure S2. Compound 8:^{13}C NMR

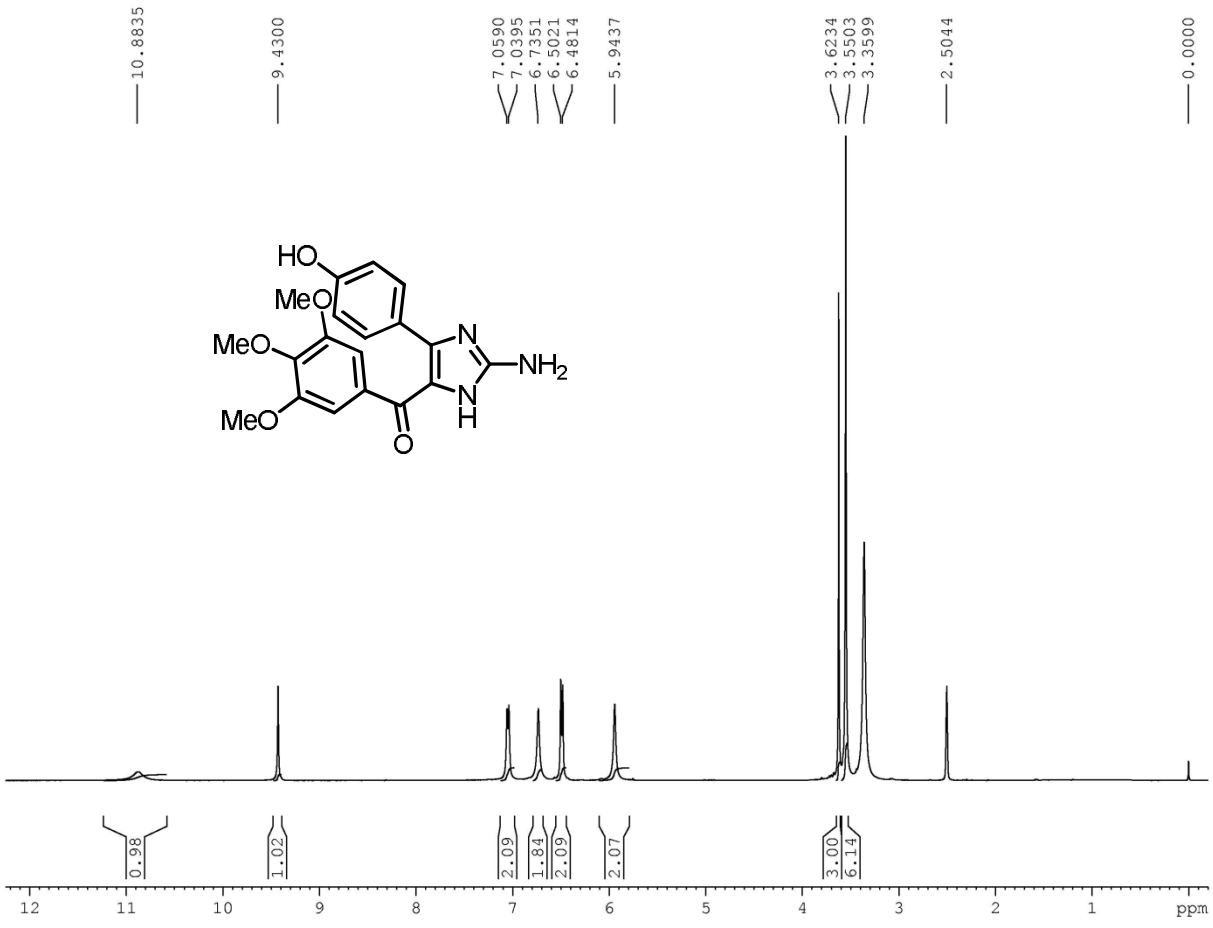

Figure S3. Compound 10:^{1}H NMR

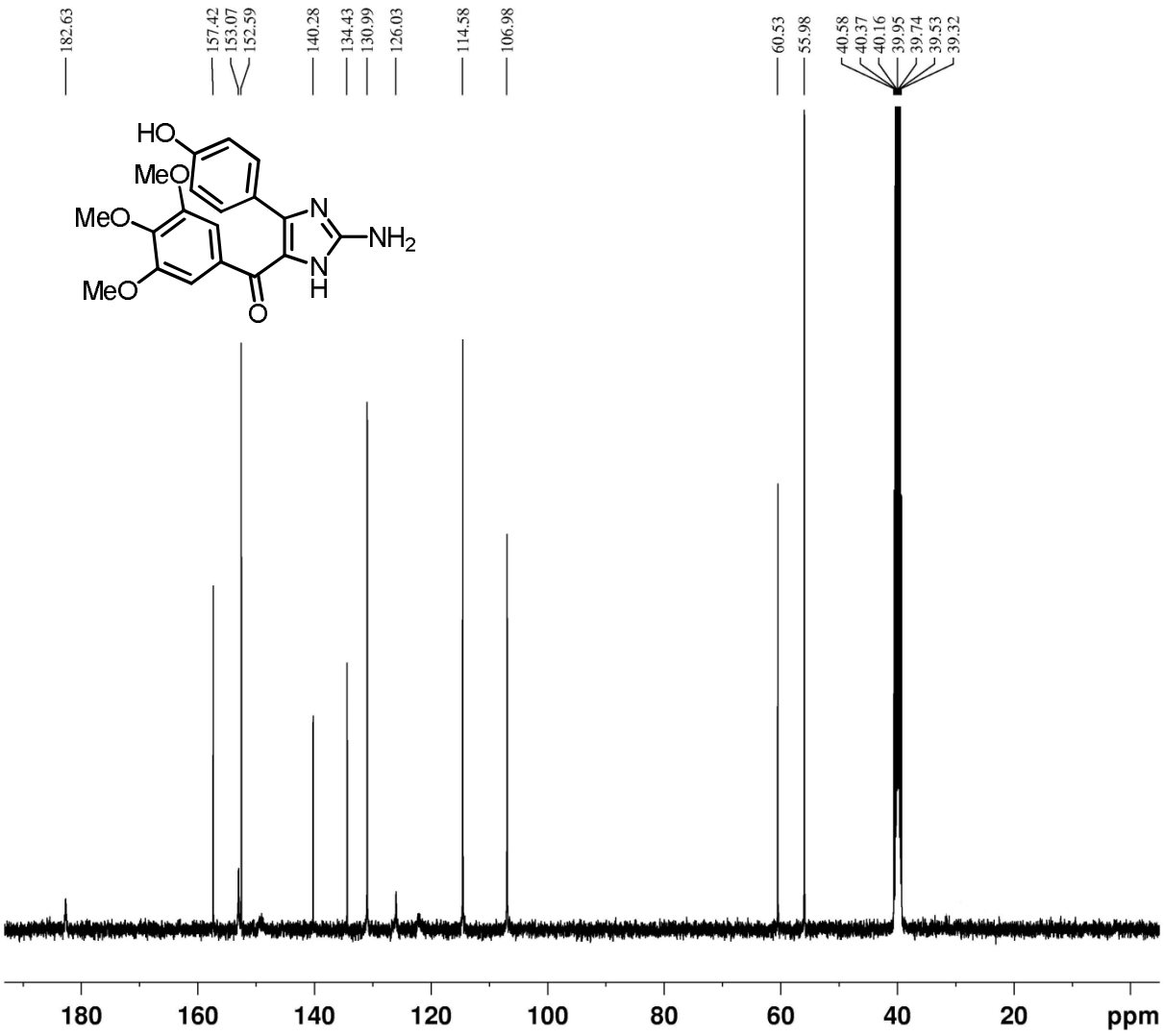

Figure S4. Compound 10:^{13}C NMR

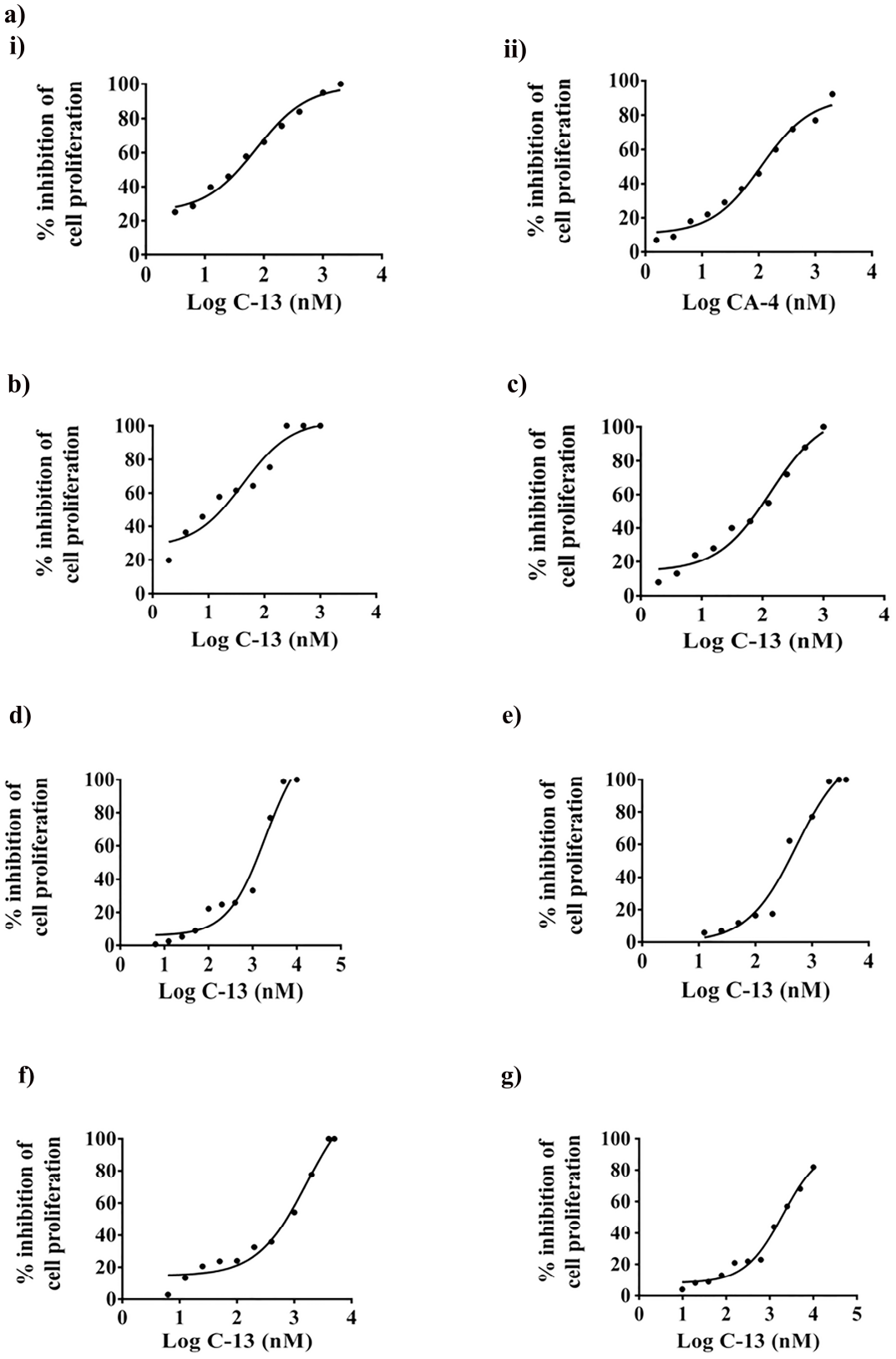

Figure S5. C-13 inhibited the proliferation of different cell lines. Effects of **C-13** (A-i) and CA-4 (A-ii) on A549 and effect of **C-13** on HeLa (B), MCF-7 (C), MCF 10A (D), B16F10 (E), L929 (F) and EMT6/AR1 (G) cell lines are shown.

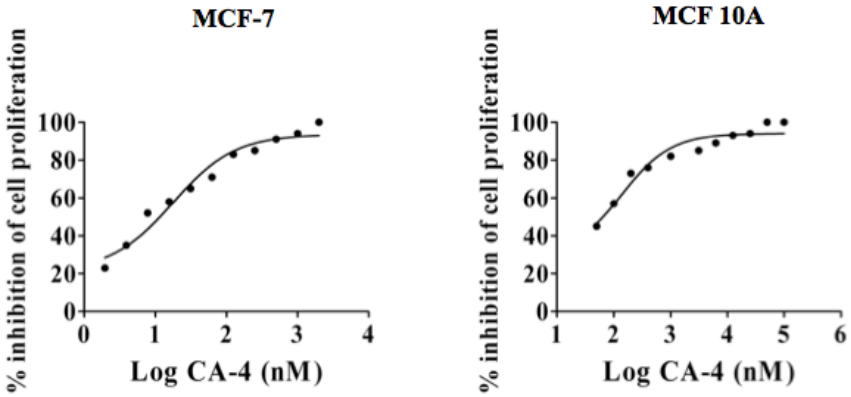

Figure S6. Determination of Half-maximal inhibitory concentration (IC_{50}) of CA-4 in MCF-7 and MCF 10A cells.

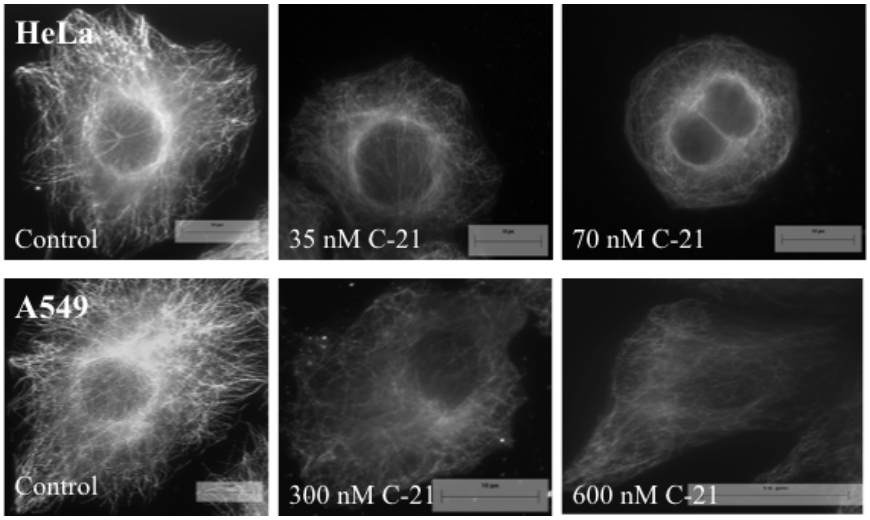

Figure S7. C-21 depolymerized interphase microtubules in HeLa and A549 cells. HeLa cells were treated without and with 35 and 70 nM of **C-21** and A549 cells were treated without and with 300 and 600 nM of **C-21** for 24 h and processed for immunostaining. Grayscale images are shown. The scale bar is 10 μm.

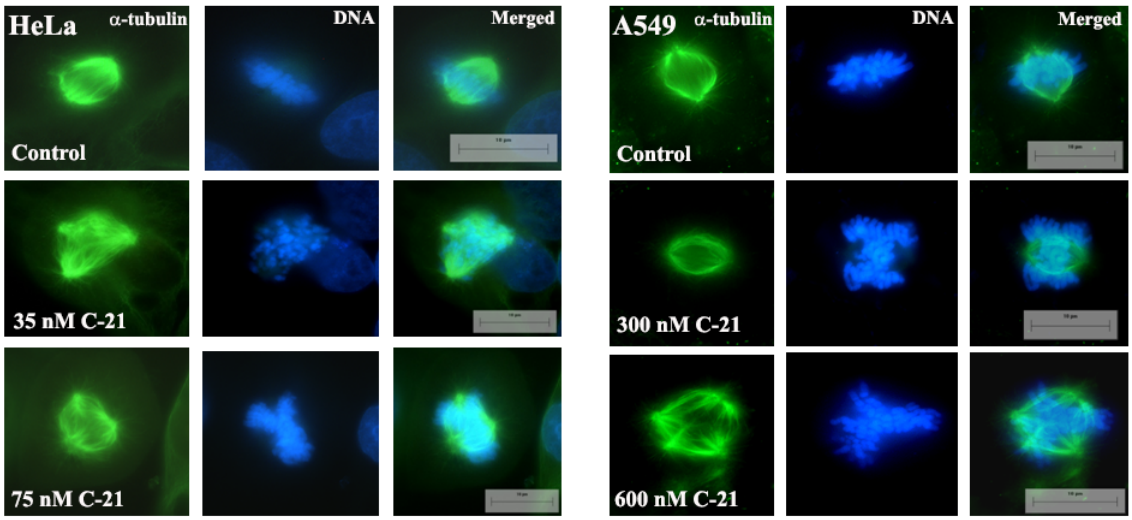

Figure S8. Effect of C-21 on mitotic spindles of HeLa and A549 cells. HeLa cells were treated without and with 35 and 70 nM of **C-21** and A549 cells were treated without and with 300 and 600 nM of **C-21** for 24 h. Cells were fixed and stained for α-tubulin (green) and nucleus (blue). The scale bar is 10 μm.

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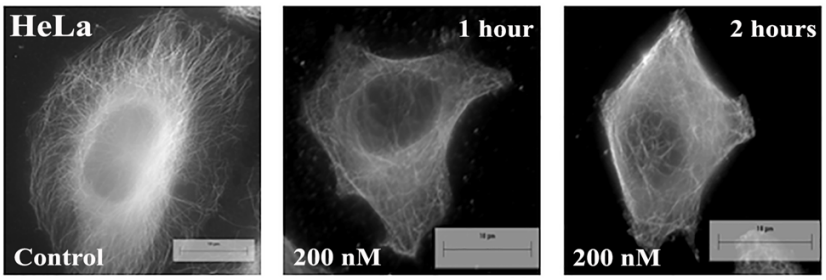
a)



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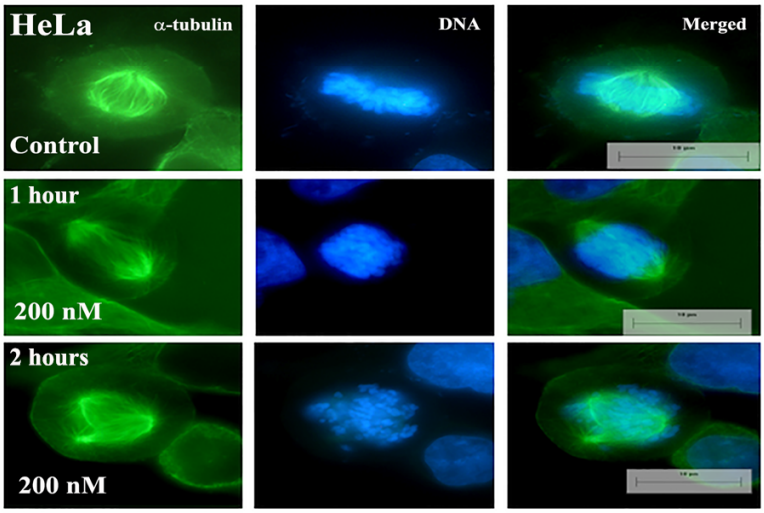


Figure S9. A short exposure of C-13 depolymerized interphase microtubules and formed multipolar spindle in mitotic cells of HeLa. HeLa cells were treated with 200 nM **C-13** for 1 and 2 h and processed for immunostaining. A) Interphase microtubules are shown in Grayscale. B) Mitotic cells are stained with α-tubulin (green) and nucleus (blue). The scale bar is 10 μm.

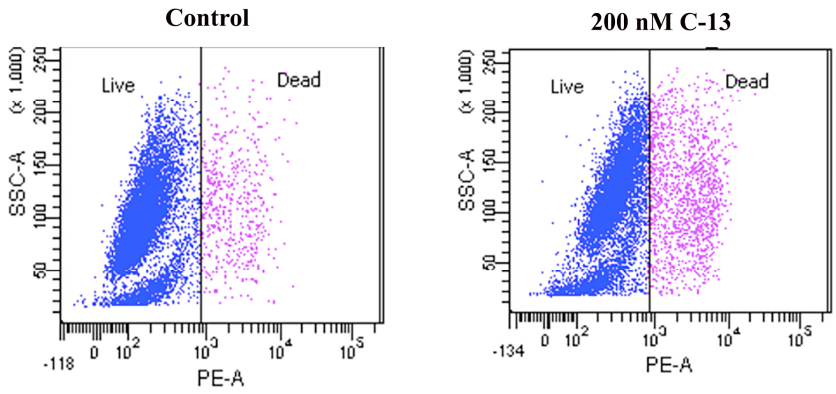
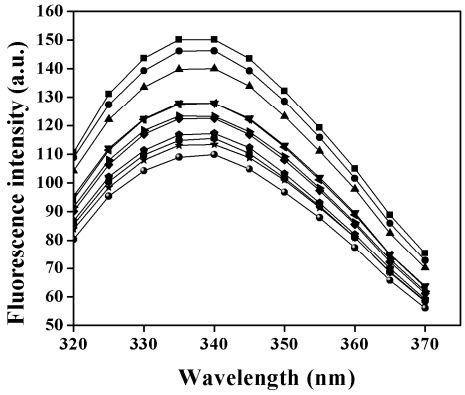


Figure S10. Reversibility of drug action. HeLa cells were treated with 200 nM **C-13** for 1 h. Later, fresh media was added and after 24 h live/dead cell assay was performed using Flow cytometer. Three independent sets of experiments were performed. One of the representative figures is shown.

A)



B)

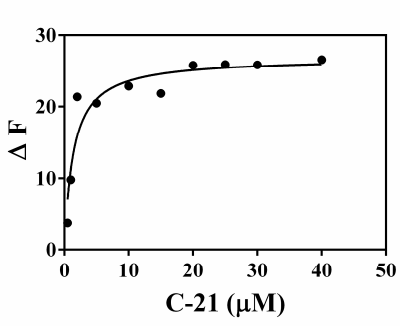


Figure S11. C-21 reduces the intrinsic tryptophan fluorescence of tubulin. A) Tubulin (2 μM) was incubated without (■) and with different concentrations 0.5 (●), 1 (▲), 2 (▼), 5 (◀), 10 (▶), 15 (◆), 20 (⬟), 25 (⬢), 30 (★) and 40 (◕) μM of **C-21** in PIPES buffer

(25 mM, pH 6.8) for 30 min at 25°C. Fluorescence spectra (320-370 nm) were monitored using excitation wavelength of 295 nm. B) Change in fluorescence intensity upon binding of **C-21** to tubulin was plotted. The experiment was done five times. One of the five sets is shown.

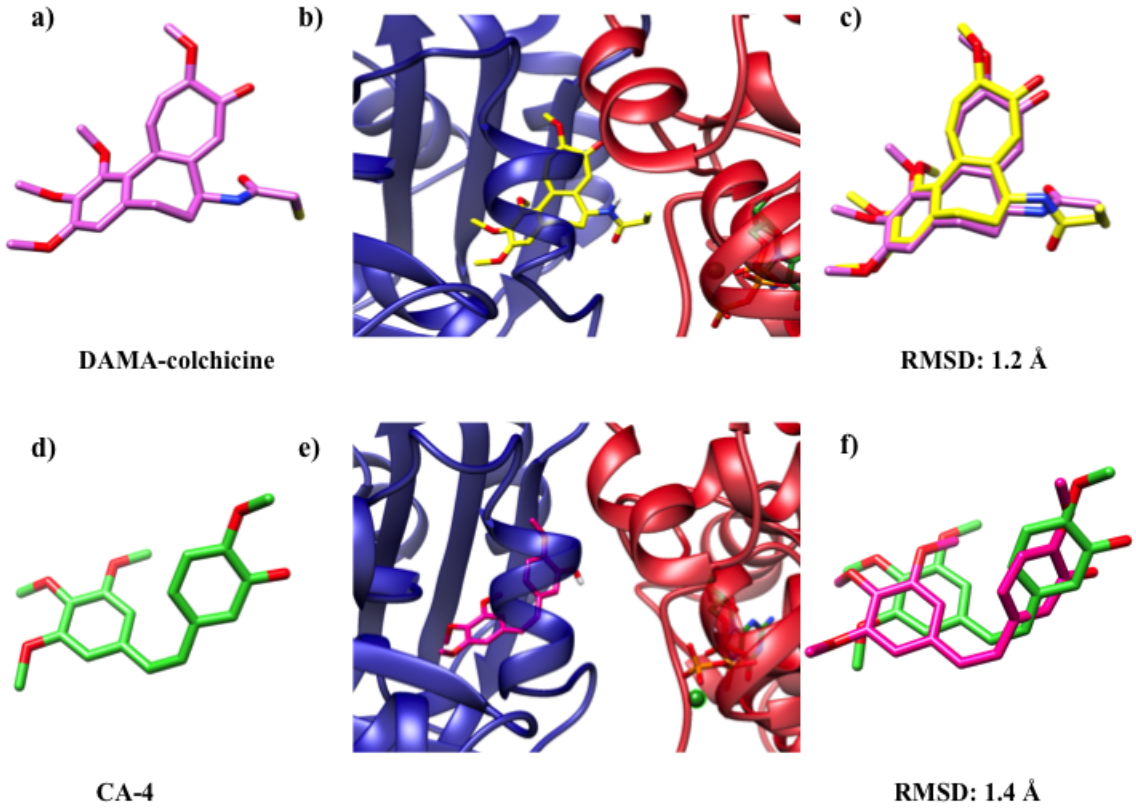


Figure S12. DAMA-colchicine and CA-4 docked on tubulin dimer interface. The color scheme for alpha tubulin is dark red, for beta tubulin is dark blue, for DAMA-colchicine (crystal structure) is orchid pink, for DAMA-colchicine (docked structure) is yellow, for CA-4 (crystal structure) is green, for CA-4 (docked structure) is deep pink. Tubulin is depicted in ribbon while compounds are in stick representations. Sulfur, hydrogen, oxygen, and nitrogen atoms are illustrated in yellow, white, red, and dark blue sticks, respectively. a) Crystal structure of DAMA-colchicine in stick representation. b) Docked conformation of DAMA-colchicine at the tubulin dimer interface. c) RMS deviation between the docked (yellow) and crystal structure (orchid pink) of DAMA-colchicine on overlapping of co-ordinates. d) Crystal structure of CA-4 in stick representation. e) Docked conformation of CA-4 at the tubulin dimer interface. f) RMS deviation between the docked (deep pink) and crystal structure (green) of CA-4 on overlapping of co-ordinates.

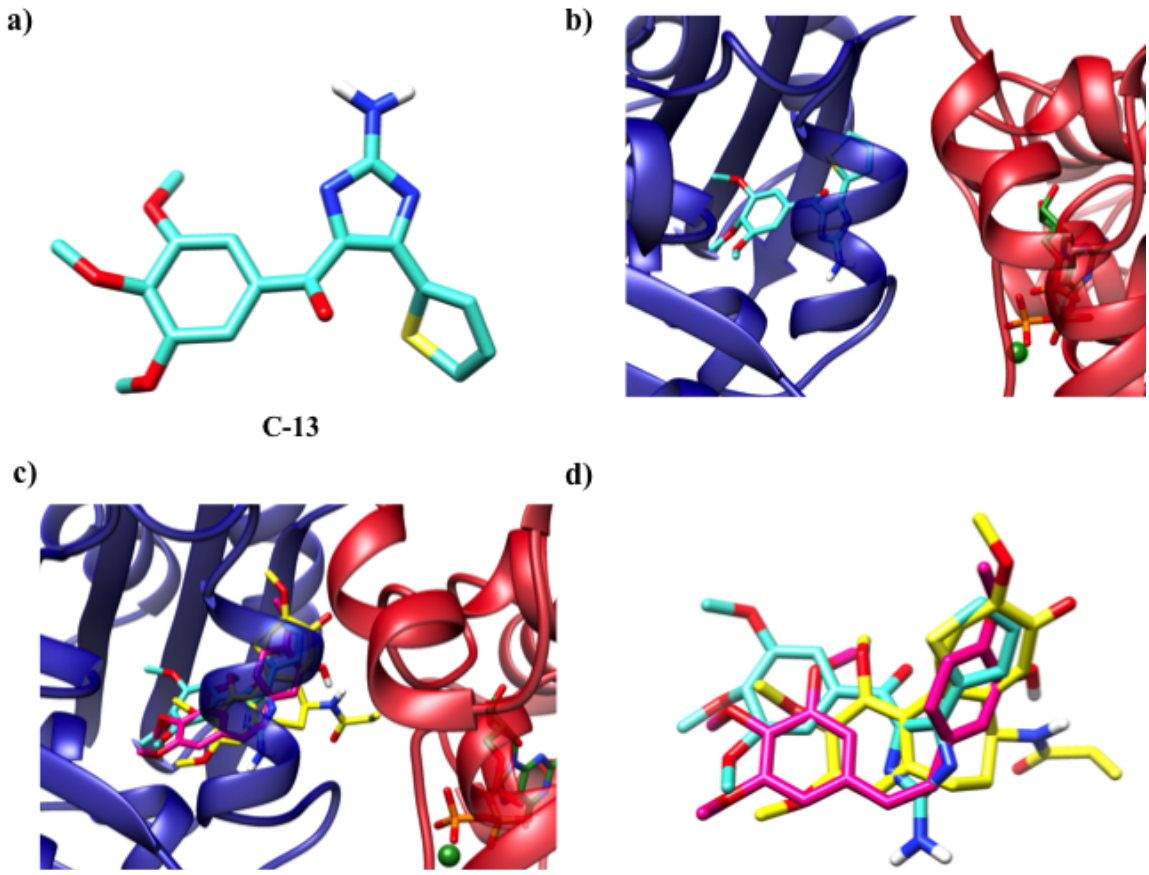


Figure S13. C-13 docked in colchicine binding pocket at the interface of tubulin dimer. The color scheme for alpha tubulin is dark red, for beta tubulin is dark blue, for DAMA-colchicine (docked structure) is yellow, for CA-4 (docked structure) is deep pink and for C-13 is turquoise. Tubulin is depicted in ribbon while compounds are in stick representations. Sulfur, hydrogen, oxygen, and nitrogen atoms are illustrated in yellow, white, red, and dark blue sticks, respectively. a) Structure of C-13 in stick representation. b) Docked conformation of C-13 at the tubulin dimer interface. c) Superimposition of docked structures of DAMA-colchicine (yellow), CA-4 (deep pink) and C-13 (turquoise) at the tubulin dimer interface indicating binding in the same pocket. d) Zoomed view of c) showing overlap of three docked molecules.

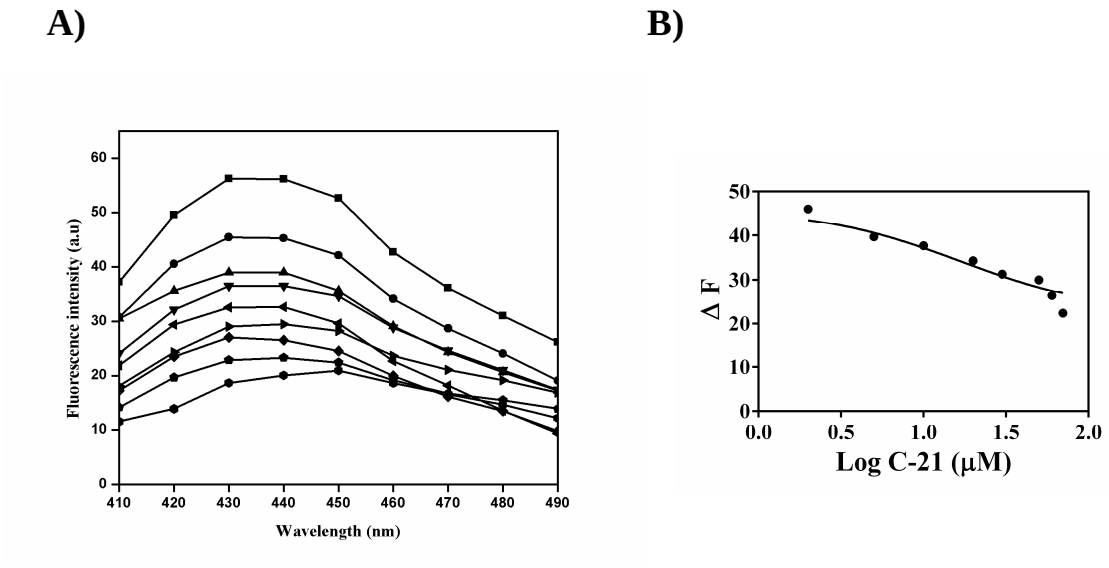


Figure S14. C-21 binds to colchicine binding site. A) Tubulin (5 μM) was incubated without (■) and with different concentrations 2 (●), 5 (▲), 10 (▼), 20 (◀), 30 (▶), 50 (◆), 60 (⬟) and 70 (⬢) μM of **C-21** in PIPES buffer (25 mM, pH 6.8) for 15 min at 37 °C. Colchicine (10 μM) was then added into the reaction mixture and incubated at 37°C for 45 min. Fluorescence spectra (410-500 nm) were monitored using excitation wavelength of 340 nm. The experiment was carried out four times. One of the four sets is shown. B) The change in fluorescence is plotted against different concentrations of **C-21**.

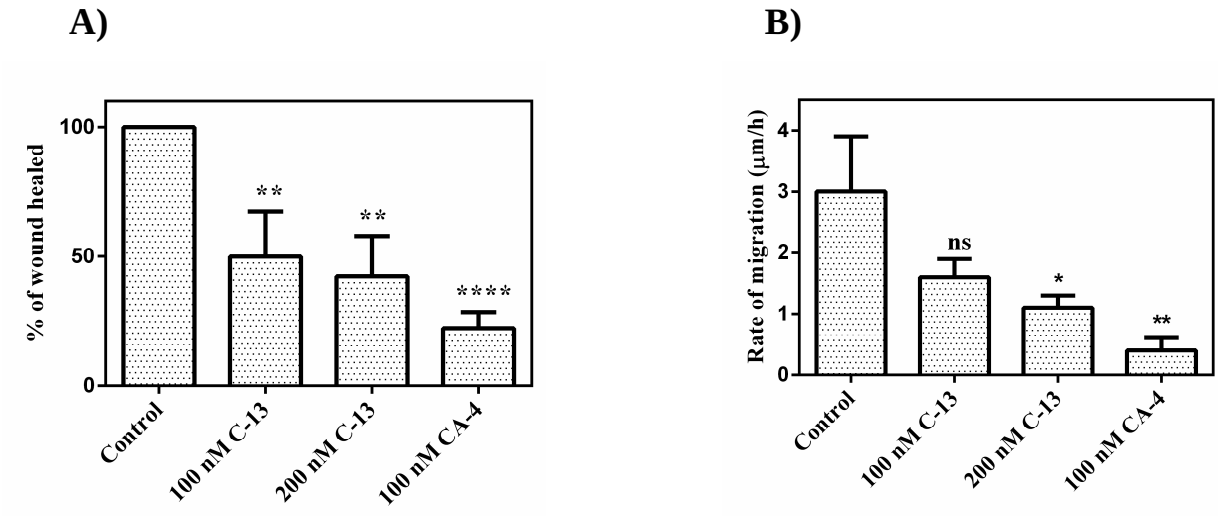


Figure S15. C-13 retarded the migration of A549 cells. A) Wound closure assay was performed. **C-13** was found to affect the migration of A549 cells. B) **C-13** also affected the rate of migration of A549 cells. Three sets of experiments were performed. Error bars represent standard deviation. (ns :$p > 0.05$; *: $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$)

	G1	S	G2/M
Control	54	21	26
75 nM C-13	8	22	70
200 nM C-13	5	17	78
20 nM CA-4	12	16	73

Table S1. Cell cycle analysis of HeLa cells using flow cytometry. Data is an average of two sets.

Docked compound	α -tubulin residues	β -tubulin residues
DAMA-colchicine	Asn (101), Thr (179), Ala (180), Val (181)	**Cys (241)**, Leu (242), **Leu (248)**, **Ala (250)**, **Lys (254)**, **Leu (255)**, **Asn (258)**, **Met (259)**, Thr (314), Val (315), **Ala (316)**, Val (318), Asn (350), **Lys (352)**, **Ile (378)**
CA-4	Thr (179)	Tyr (202), Gly (237), Val (238), **Cys (241)**, Leu (242), **Leu (248)**, **Ala (250)**, Asn (251), **Lys (254)**, **Leu (255)**, **Asn (258)**, **Met (259)**, Val (315), **Ala (316)**, Ile (318), **Lys (352)**, **Ile (378)**
C-13		Gly (237), Val (238), Thr (240), **Cys (241)**, **Leu (248)**, **Ala (250)**, Asn (251), **Lys (254)**, **Leu (255)**, **Asn (258)**, **Met (259)**, **Ala (316)**, Ala (317), Ile (318), **Lys (352)**, Thr (376), **Ile (378)**

Table S2. Tubulin residues within 4 Å of the docked compounds

Ligand	Binding energy (kcal/mol)	Hydrogen bonding interactions	
		residues involved	distances (Å)
DAMA-colchicine	-9.02	Thr179A	3.76
		Val181A	3.22
		Cys241B	3.09
CA-4	-7.87	Thr179A	2.23
		Lys352B	3.01
C-13	-8.44	Cys241B	3.09
		Asn251	2.54
			2.77
		Ile318	3.07

Table S3. Binding energies and hydrogen bonding interactions of the docked compounds with tubulin dimer