Supporting Information

Development of a novel class of tubulin inhibitor from desmosdumotin B with a hydroxylated bicyclic B-ring

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1. Synthetic Procedure for Benzo[*b*]thiophene Aldehydes

5-Methoxy-3-methylbenzo[*b*]thiophene (35) [1,2]**:** To a solution of 4-methoxybenzenethiol (**32**, 5.4 g, 38.9 mmol) in DMF (30 mL) was added K_2CO_3 (11.0 g, 79.7 mmol) and chloroacetone (3.4 mL, 42.7 mmol) at 0 ºC. The mixture was stirred for 0.5 h at 0 ºC, then allowed to warm to rt and stirred for 3 h. The reaction mixture was diluted with AcOEt and washed with water. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was heated with polyphosphoric acid (22 g) in toluene (60 mL) at 100 ºC for 18 h. The mixture was concentrated, carefully neutralized with K_2CO_3 at 0 ºC and extracted with AcOEt. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel with AcOEt–hexane as eluent to afford the target compound **35** (3.827 g) in 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.70 (d, 1H, *J* = 8.6 Hz, 7-*H*), 7.14 (d, 1H, *J* = 2.6 Hz, 4-*H*), 7.08 (br s, 1H, 2-*H*), 7.00 (dd, 1H, *J* = 8.6 and 2.6 Hz, 6-*H*), 3.90 (s, 3H, 5-OC*H*$_3$), 2.40 (d, 3H, *J* = 1.2 Hz, 3- C*H*$_3$).

4- and 6-Methoxy-3-methylbenzo[*b*]thiophene (36[3] **and 37**[4]**):** To a solution of 3-methoxybenzenethiol (**33**, 6.0 mL, 48.9 mmol) in DMF (30 mL) was added K_2CO_3 (14.3 g, 103.7 mmol) and chloroacetone (4.3 mL, 54 mmol) at 0 ºC. The mixture was stirred for 4 h allowed to warm to rt without heating. After the same work-up as described above, the residue was heated with polyphosphoric acid (35.3 g) in toluene (50 mL) at 100 ºC for 18 h. Work-up was performed as described above. The residue was chromatographed on silica gel with AcOEt–hexane as eluent to afford **36** (1.728 g) as a colorless solid in 20% yield and **37** (3.634 g) as a colorless oil in 50% yield. **36**: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.39 (d, 1H, *J* = 8.0 Hz, 7-*H*), 7.22 (dd, 1H, *J* = 8.0 and 7.8 Hz, 6-*H*), 6.86 (s, 1H, 2-*H*), 6.71 (d, 1H, *J* = 7.8 Hz, 5-*H*), 3.90 (s, 3H, 4-OC*H*$_3$), 2.61 (s, 3H, 3- C*H*$_3$). **37:** ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.58 (d, 1H, *J* = 8.8 Hz, 4-*H*), 7.32 (d, 1H, *J* = 7.8 Hz, 7-*H*), 7.01 (dd, 1H, *J* = 8.8 and 2.3 Hz, 5-*H*), 6.88 (d, 1H, *J* = 1.2 Hz, 2-*H*), 3.87 (s, 3H, 6-OC*H*$_3$), 2.40 (d, 3H, *J* = 1.2 Hz 3- C*H*$_3$).

7-Methoxy-3-methylbenzo[*b*]thiophene (38[5]**):** To a solution of 3-methoxybenzenethiol (**34**, 2.0 g, 14.4 mmol) in DMF (10 mL) was added K_2CO_3 (4.2 g, 30.4 mmol) and chloroacetone (1.3 mL, 16.3 mmol) at 0 ºC. The mixture was stirred for 3 h while warming to rt without heating. After the same work-up as described above, the residue was heated with polyphosphoric acid

(15.5 g) in toluene (25 mL) at 100 ºC for 21 h. Further work-up was as described above. The residue was chromatographed on silica gel with AcOEt–hexane as eluent to afford **38** (1.400 g) as a colorless solid in 55% yield. **38**: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.38-7.32 (m, 2H), 7.06 (br s, 1H), 6.82–6.76 (m, 1H), 4.00 (s, 3H, OC*H*$_3$), 2.43 (s, 3H, C*H*$_3$).

General synthetic procedures for 24–27: To a solution of methoxy-3-methylbenzo[*b*]thiophene (**35**, 1.244 g, 7.0 mmol) in CCl_4 (80 mL) was added *N*-bromosuccinimide (1.532 g, 8.6 mmol) and benzoyl peroxide (17 mg, 0.07 mmol). The reaction mixture was refluxed for 1.5 h. After filtration, the volatile solvent was removed *in vacuo*. The residue was dissolved in $CHCl_3$ (25 mL). Hexamethyltetramine (1.505 g, 10.8 mmol) was added, and the mixture was refluxed overnight. The volatile solvent was removed *in vacuo,* and 50% AcOH aq. (40 mL) was added to the residure. The mixture was refluxed for 3 h. After addition of water (20 mL) and $CHCl_3$ (4.0 mL), the mixture was refluxed for an additional 10 min, then allowed to stand for 3 h at rt. The mixture was diluted with water, and extracted with AcOEt. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel with AcOEt–hexane as eluent to afford the target compound (**24**, 0.521 g) in 39% yield.

5-Methoxybenzo[*b*]thiophene-3-carbaldehydes (24)[6,7]: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 10.11 (s, 1H, -C*H*O), 8.31 (s, 1H, *2-H*), 8.18 (d, 1H, *J* = 2.5 Hz, 4-*H*), 7.72 (d, 1H, *J* = 9.0 Hz, 7-*H*), 7.10 (dd, 1H, *J* = 9.0 and 2.2 Hz, 6-*H*), 3.92 (s, 3H, -OC*H*$_3$).

4-Methoxybenzo[*b*]thiophene-3-carbaldehydes (25)[3,7]: 25% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 10.65 (s, 1H, -C*H*O), 8.36 (s, 1H, *2-H*), 7.50 (d, 1H, *J* = 8.0 Hz, 5- or 7-*H*), 7.37 (t, 1H, *J* = 8.0 Hz, 6-*H*), 6.92 (d, 1H, *J* = 8.0 Hz, 5- or 7-*H*), 4.03 (s, 3H, -OC*H*$_3$).

6-Methoxybenzo[*b*]thiophene-3-carbaldehydes (26)[7]: 21% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 10.08 (s, 1H, -C*H*O), 8.55 (d, 1H, *J* = 9.0 Hz, 4-*H*), 8.15 (s, 1H, 2-*H*), 7.33 (d, 1H, *J* = 2.3 Hz, 7-*H*), 7.13 (dd, 1H, *J* = 9.0 and 2.3 Hz, 5-*H*), 3.90 (s, 3H, -OC*H*$_3$).

7-Methoxybenzo[*b*]thiophene-3-carbaldehydes (27): 13% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 10.15 (s, 1H, -C*H*O), 8.31 (s, 1H, 2-*H*), 8.24 (dd, 1H, *J* = 8.0 and 0.8 Hz, 4-*H*), 7.47 (t, 1H, *J* = 8.0 Hz, 5-*H*), 6.89 (d, 1H, *J* = 8.0 Hz, 5-*H*), 4.02 (s, 3H, -OC*H*$_3$).

2. Table S1.

cLogP and PSA Parameters of TEDB-TBs

	Ring-B	cLogP	PSA*
4	Benzo[*b*]thiophen-3'-yl	5.267	63.6
6-9	Hydroxyl benzo[*b*]thiophen-3'-yl	4.759	83.83
13	Benzo[*b*]thiophene-2'-yl	5.477	63.6
14	Hydroxyl benzo[*b*]thiophene-2'-yl	4.749	83.83
5	Naphthalen-1'-yl	5.396	63.6
15	4'-Hydroxyl naphtalen-1'-yl	4.835	83.83
16	2'-Hydroxyl naphtalen-1'-yl	4.535	83.83
18	Naphthalen-2'-yl	5.396	63.6
19	6'-Hydroxyl naphtalen-2'-yl	4.729	83.83

Calculated by ChemBioDraw 13 software

PSA: polar surface area

3. Table S2.

Standard Deviations for Table 1

	Cell line/IC_{50} (μM)										Inhibitory effects	
	KB	KB-VIN	PC-3	A549	Hep-G2	HCT-8	MDA-MB-231	SK-BR-3	MCF-7	ZR-75-1	ITA EC_{50} (μM)	ICB (%)
4	0.01	0.01	0.01	0.01	0.01	0.01	0.08	0.02	0.03	0.04	0.1	5
6	0.10	0.07	0.80	0.10	0.11	0.15	0.09	0.02	0.04	0.62	0.2	2
7	0.73	0.19	0.94	0.35	1.30	0.27	0.11	0.12	0.01	0.22	0.2	0.8
8	1.13	0.33	0.33	0.14	0.53	0.91	0.23	0.33	0.01	0.23	0.5	2
9	0.46	0.07	0.20	0.34	0.64	1.42	NT	NT	NT	NT	0.6	5
10	NA	0.52	NA	NA	NT[f]	NT	NT	NT	NT	NT	NT	NT
11	NA	NA	NA	NA	NT	NT	NT	NA	NT	NT	NT	NT
12	0.11	1.61	1.93	0.99	NT	NT	0.44	0.72	0.12	0.09	NT	NT
13	0.51	0.76	0.25	1.02	0.51	1.27	NT	NT	NT	NT	NT	NT
14	1.24	0.44	2.17	0.53	NT	NT	NT	2.10	NT	NT	NT	NT
5	-	-	-	-	-	-	NT	NT	-	NT	0.08	10
15	0.08	0.13	3.7	0.06	0.26	0.15	NT	0.10	NT	NT	0.3	5
16	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.4	3
17	0.10	0.07	0.07	0.05	0.05	0.22	NT	0.96	0.14	NT	0.3	3
18	0.05	0.08	0.10	0.10	0.03	0.05	NT	NT	0.05	NT	NO	0.4
19	1.26	0.78	1.89	0.99	1.58	1.55	NT	0.81	NT	NT	NT	NT
20	NA	NA	NA	NA	NA	NA	NT	NA	NT	NT	NT	NT
	Combretastatin A-4 (CA): Tubulin inhibitor										1.1	0.06

4. Figure S1.

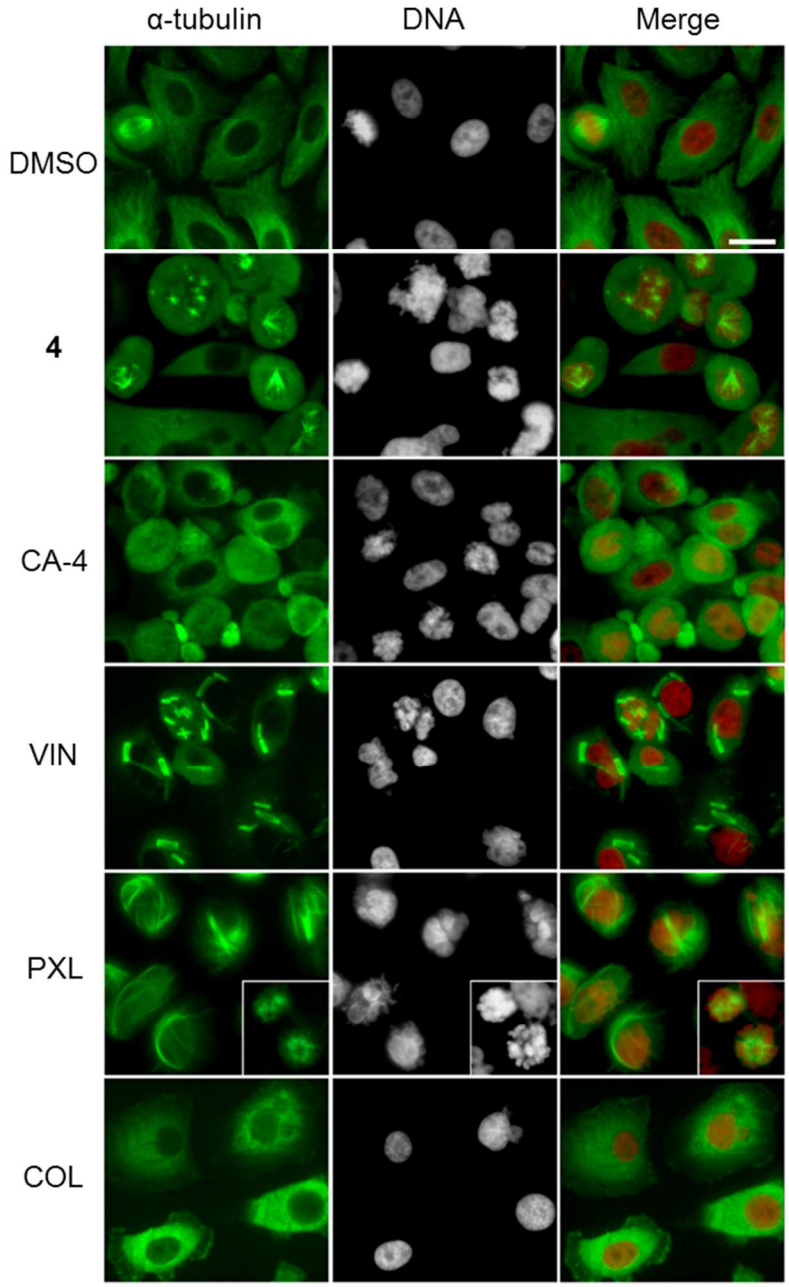
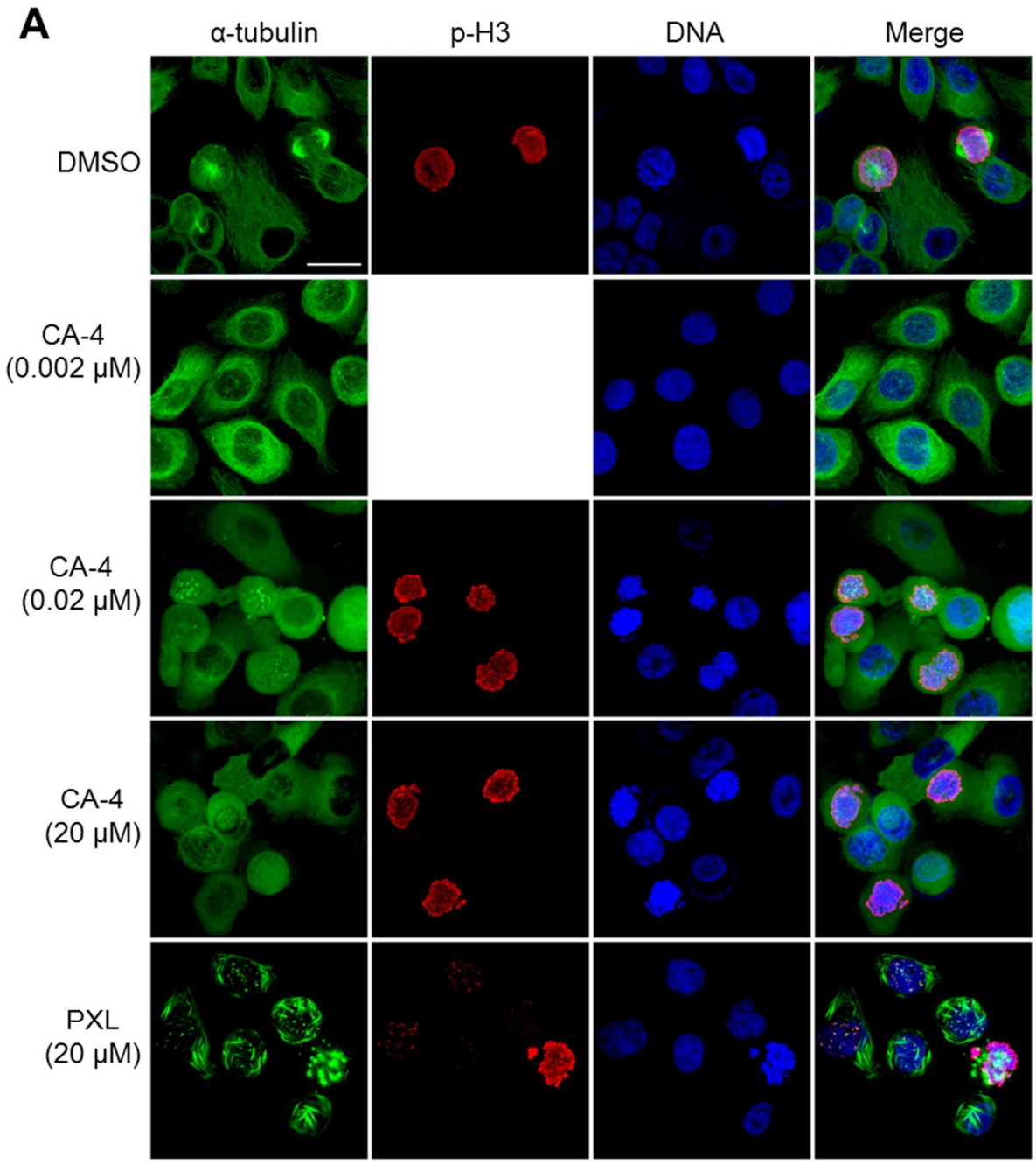
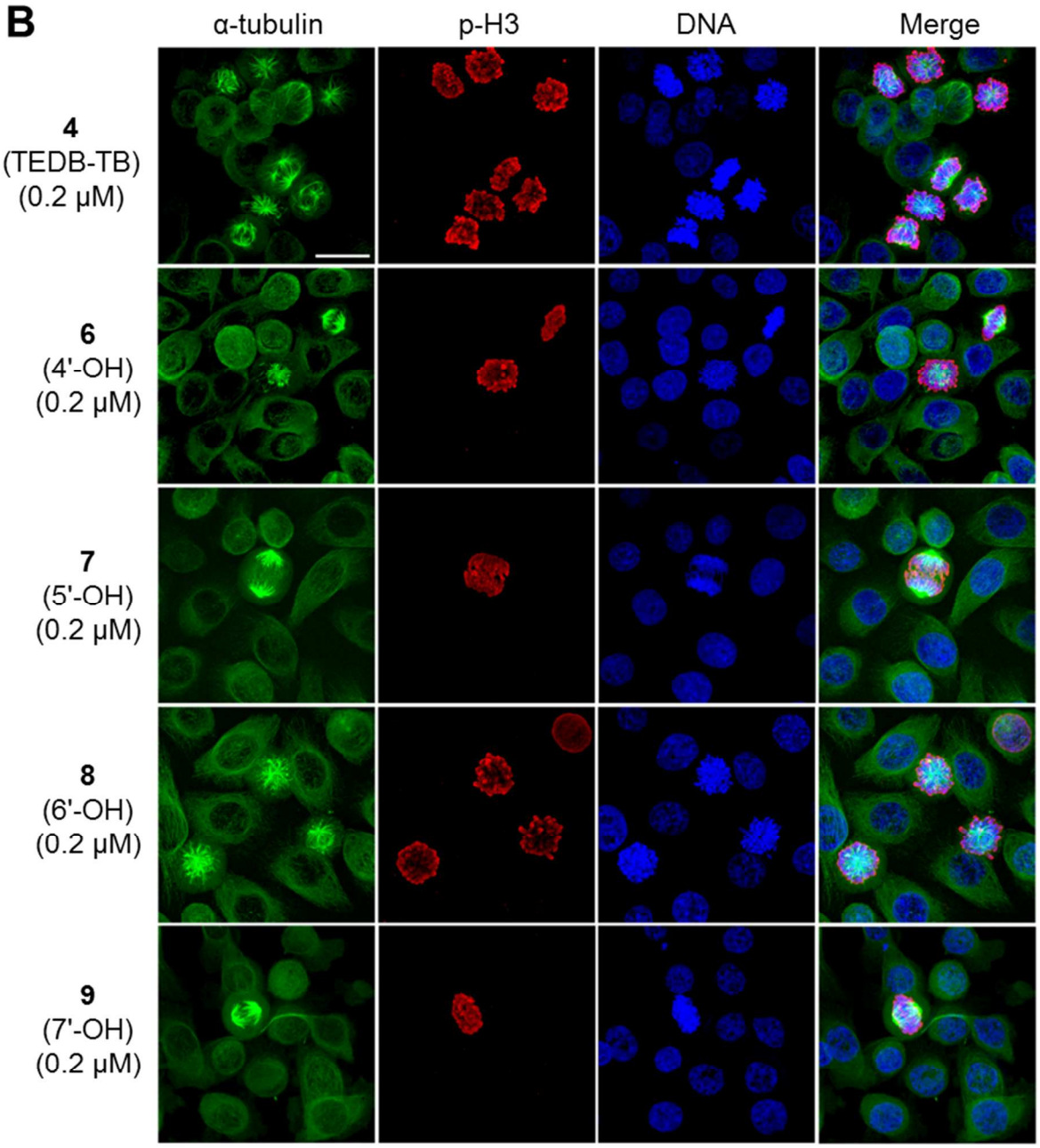
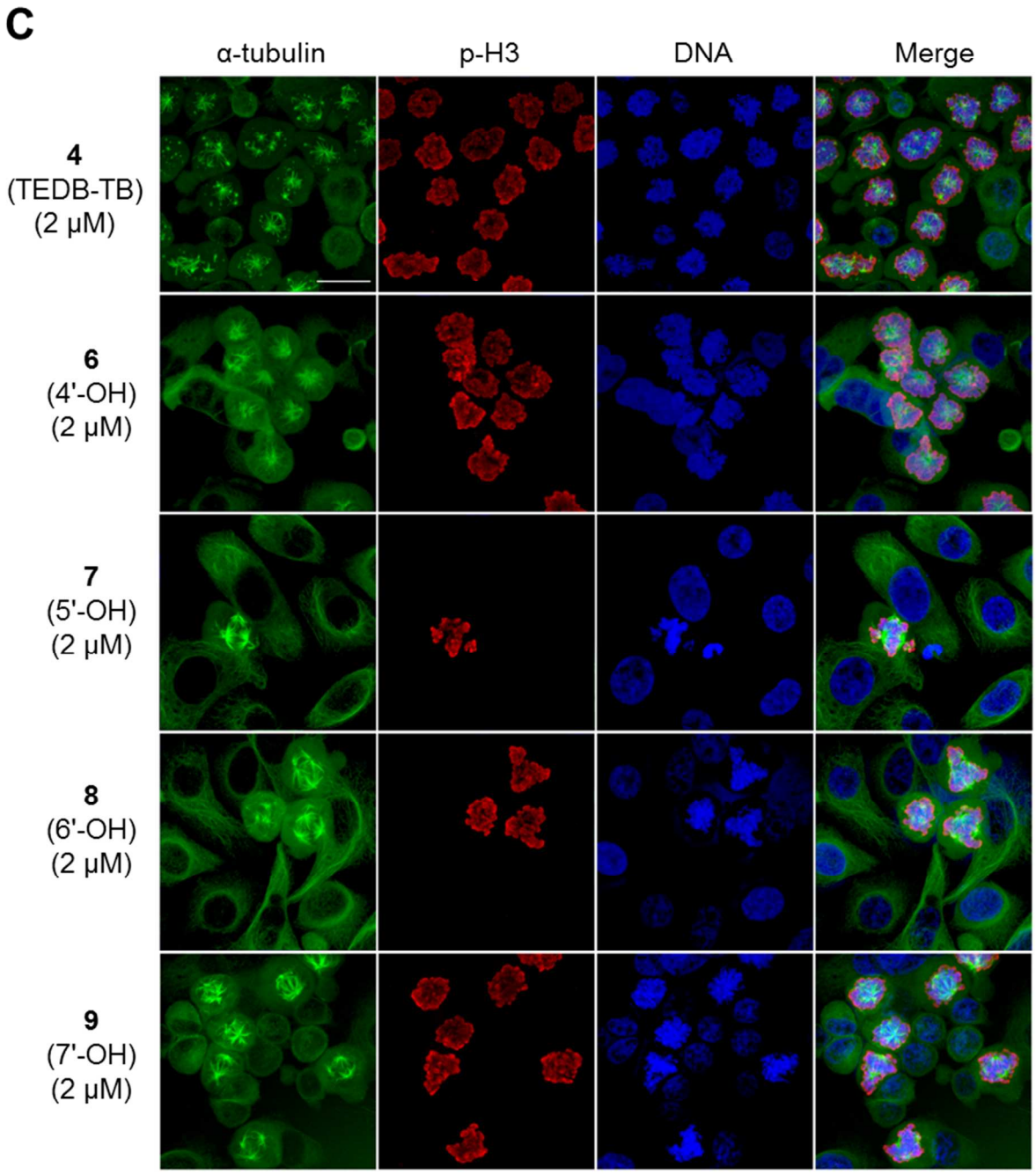


Figure S1. Effects of compounds on microtubule morphology in PC-3 cells. PC-3 cells were cultured and treated for 24 h with DMSO (control), 0.2 μM compound **4** (TEDB-TB), 0.2 μM CA-4 as a colchicine-like tubulin polymerization inhibitor, 5 μM VIN, 0.2 μM PXL as a microtubule hyper-stabilization agent, or 1 μM colchicine (COL). Cells were stained with monoclonal antibody to α-tubulin (green) and DAPI for DNA (white). Images were captured with an Olympus BX61 fluorescence microscope with CCD camera. Merged images were prepared using Adobe Photoshop. DNA was pseudo-colored in red the merged images. Bar, 25 μm.

5. Figure S2.


A
α-tubulin
p-H3
DNA
Merge
DMSO
CA-4
(0.002 μM)
CA-4
(0.02 μM)
CA-4
(20 μM)
PXL
(20 μM)


B
α-tubulin
p-H3
DNA
Merge
4
(TEDB-TB)
(0.2 μM)
6
(4'-OH)
(0.2 μM)
7
(5'-OH)
(0.2 μM)
8
(6'-OH)
(0.2 μM)
9
(7'-OH)
(0.2 μM)


C
α-tubulin
p-H3
DNA
Merge
4
(TEDB-TB)
(2 μM)
6
(4'-OH)
(2 μM)
7
(5'-OH)
(2 μM)
8
(6'-OH)
(2 μM)
9
(7'-OH)
(2 μM)

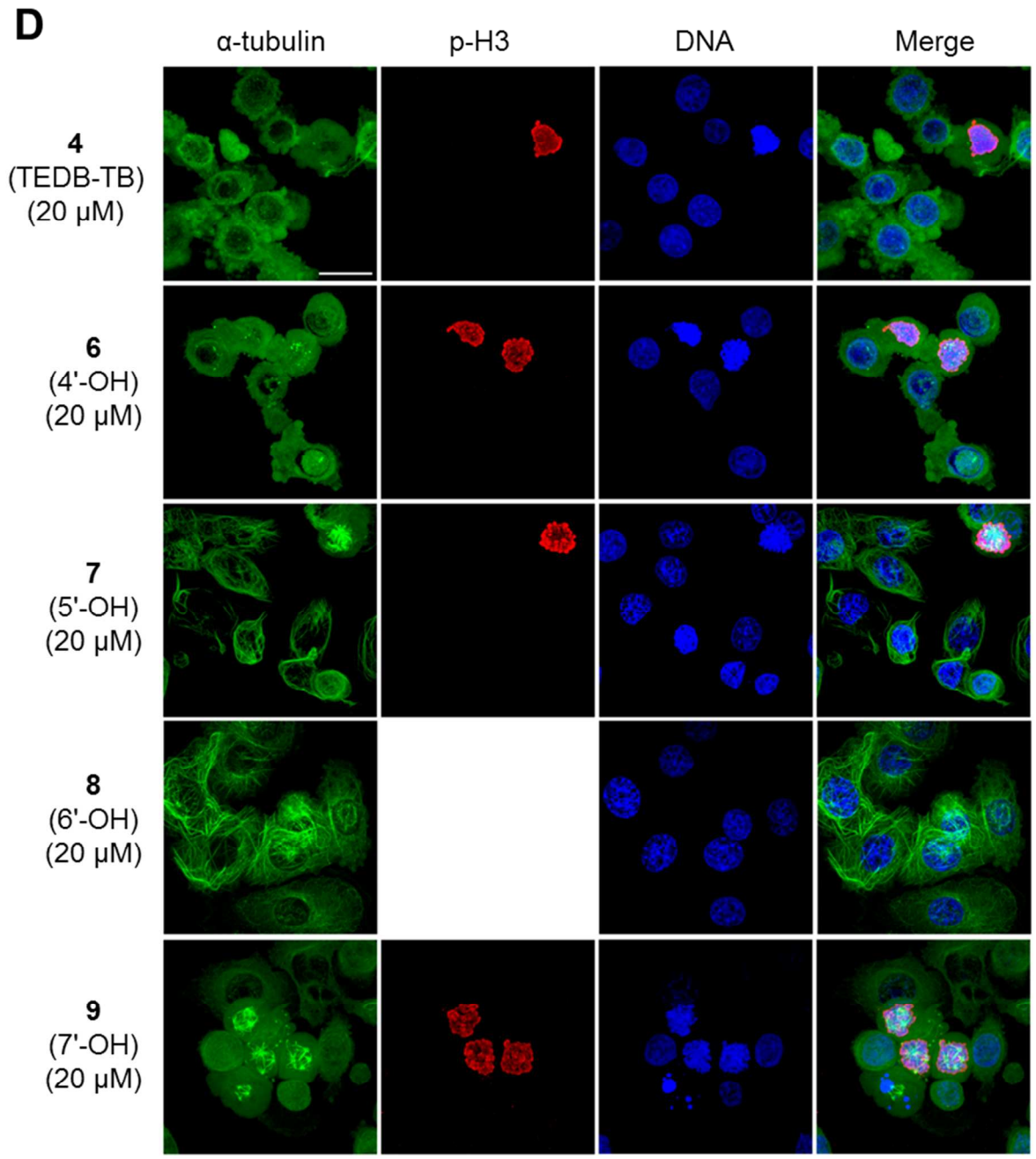


Figure S2. Dose-dependent effects of compounds on microtubule morphology in PC-3 cells. PC-3 cells were cultured and treated for 24 h with one of several concentrations of CA-4, PXL (A), or compounds **4**, **6**, **7**, **8** or **9** at 0.2 (B), 2 (C) or 20 μM (D). Cells were triple labeled with mouse monoclonal antibody to α-tubulin (green), rabbit polyclonal antibody to Ser10-phosphorylated histone H3 (p-H3) (red) as mitotic marker, and DAPI for DNA (blue). 10 to 20 confocal microscopic stacked images were reconstructed by ZEN software.

(A) Defects of microtubules following treatment with depolymerization agent CA-4 or microtubule stabilizing agent PXL. At the CA-4 concentration that inhibited microtubule polymerization, cells that were positive for accumulated p-H3 (Ser10) had no spindle formation, suggesting cell cycle arrest at prometaphase occurred in tandem with interphase microtubule depolymerization. Following treatment with 0.002 μM CA-4, no of p-H3 positive cells were observed in the field, indicated by the absence of an image. Bar, 25 μm.

(B-D) Dose dependent effects of **4** and its analogues on microtubule morphology in PC-3 cells. PC-3 cells were cultured and treated for 24 h with compound at 0.2 (B), 2 (C) or 20 μM (D), followed by triple-labeling of α-tubulin (green), p-H3 (red) and DNA (Blue). Confocal images were processed by ZEN software. No p-H3 (Ser10) positive cells were observed in the field with cells treated with 0.002 μM CA-4 (indicated by an absent image in A) or 20 μM compound **8** (indicated by an absent image in D). Bar, 25 μm.

6. Figure S3.

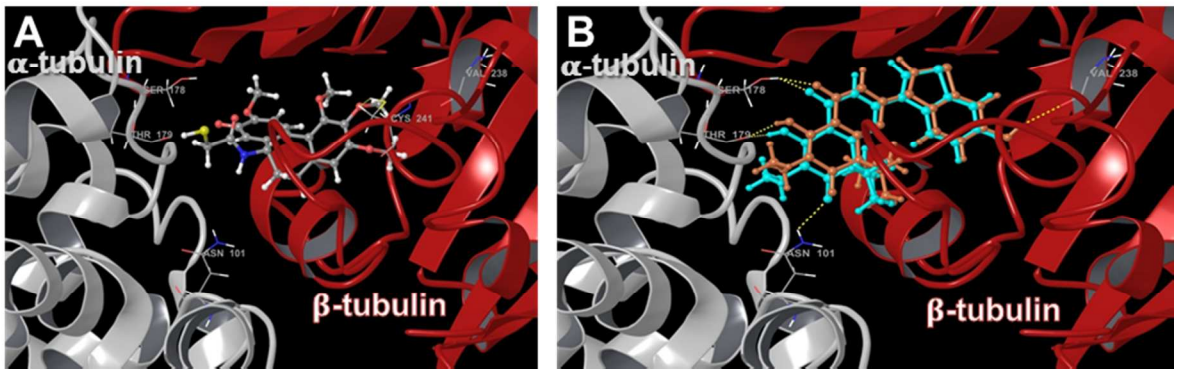


Figure S3. Predicted docking modes for compounds binding to tubulin.

(A) Detailed view of docking model of DAMA-colchicine in the tubulin crystal structure (PDB ID: 1SA0). Colchicine shows a H-bond-like interaction with the side chain of βCys241. (B) H-bonds calculated to be less than 3 Å between the protein and compounds **4** (blue) and **8** (brown) are represented by dashed lines.

7. Figure S4.

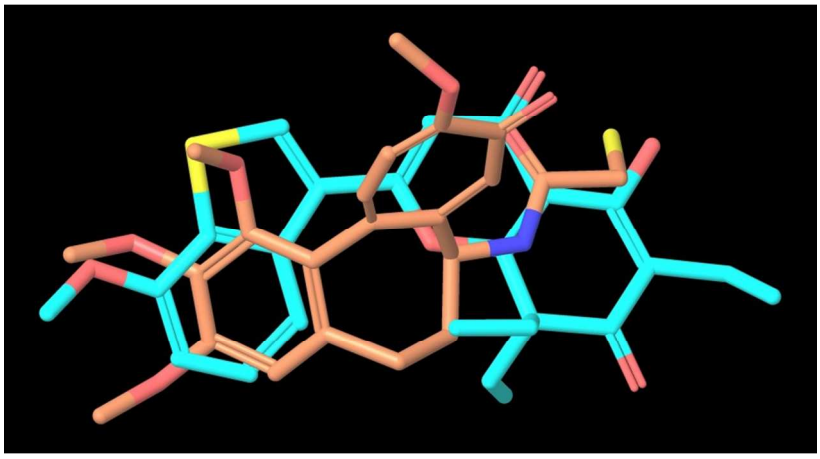


Figure S3. 3D models of **12** (blue skeleton) and DAMA-colchicine (brown skeleton) with oxygen in red, nitrogen in blue and sulfur in yellow.

8. References for Supporting Information

1. Plé, P.A.; Marnett, L.J, Synthesis of substituted benzo[*b*]thiophene by acid-catalyzed cyclization of thiophenylacetals and ketones. *J. Heterocyclic Chem.* **1988**, *25*, 1271-1272.
2. Matsunaga, N.; Kaku, T.; Itoh, F.; Tanaka, T.; Hara, T.; Miki, H.; Iwasaki, M.; Aono, T.; Yamaoka, M.; Kusaka, M.; Tasaka, A. $C_{17,20}$-Lyase inhibitors I. Structure-based de novo design and SAR study of $C_{17,20}$-lyase inhibitors. *Bioorg. Med. Chem.* **2004**, *12*, 2251-2273.
3. Campaigne, E.; Rpgers, R.B. Benzo[*b*]thiophene derivatives. XIX. Sulfur isosteres of psilocine and related isomers. *J. Heterocyclic Chem.* **1973**, *10*, 297-305.
4. Campaigne, E.; Dinner, A.; Neiss, E.S. Benzo[b]thiophene derivatives. XV. Preparation and electrophilic substitution of 6-methoxy-3-methylbenzo[b]thiophene. *J. Heterocyclic Chem.* **1970**, 70, 695-670.
5. Buchwald, S.L.; Fang, Q. An efficient one-pot method for the preparation of polysubstituted benzothiophenes. *J. Org. Chem.* **1989**, *54*, 2793-2797.
6. Buchheit, K.-H.; Gamse, R.; Giger, R.; Hoyer, D.; Klein, F.; Kloeppner, E.; Pfannkuche, H.; Mattes, H. The serotonin 5-HT4 receptor. 2. Structure-activity studies of the indole carbazimidamide class of agonists. *J. Med. Chem.* **1995**, *38*, 2331-2338.
7. Lena, G.; Trapani, J.A.; Sutton, V.R.; Ciccone, A.; Browne, K.A.; Smyth, M.J.; Denny, W.A.; Spicer, J.A. Dihydrofuro[3,4-*c*]pyridinones as inhibitors of the cytolytic effects of the pore-forming glycoprotein perforin. *J. Med. Chem.* **2008**, *51*, 7614-7624.