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ARTICLE

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Antitumor Agents. 284. New Desmosdumotin B Analogues with Bicyclic B-Ring as Cytotoxic and Antitubulin Agents

Kyoko Nakagawa-Goto,*[,†] Pei-Chi Wu,[†] Chin-Yu Lai,[†] Ernest Hamel,[‡] Hao Zhu,[§] Liying Zhang,[§] Takashi Kozaka,[†] Emika Ohkoshi,[†] Masuo Goto,[∥] Kenneth F. Bastow,*[,⊥] and Kuo-Hsiung Lee*[,†,#]

[†]Natural Products Research Laboratories, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, United States

[‡]Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, United States

[§]Laboratory for Molecular Modeling, Division of Medicinal Chemistry and Natural Products, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States

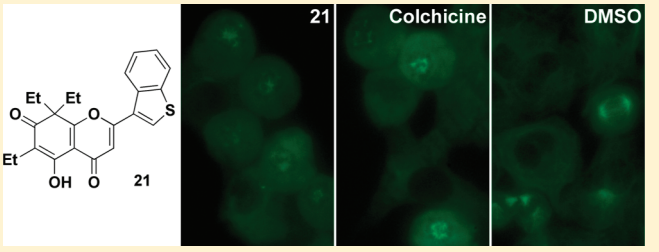
[∥]Cell and Developmental Biology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599, United States

[⊥]Division of Medicinal Chemistry and Natural Products, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, United States

[#]Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan

Supporting Information

ABSTRACT: We previously reported that the biological activity of analogues of desmosdumotin B (**1**) was dramatically changed depending on the B-ring system. A naphthalene B-ring analogue **3** exerted potent in vitro activity against a diverse panel of human tumor cell lines with GI_{50} values of 0.8−2.1 μM. In contrast, **1** analogues with a phenyl B-ring showed unique selective activity against P-glycoprotein (P-gp) overexpressing multidrug resistant cell line. We have now prepared and evaluated **1** analogues with bicyclic or tricyclic aromatic B-ring systems as in vitro inhibitors of human cancer cell line proliferation. Among all synthesized derivatives, **21** with a benzo[*b*]thiophenyl B-ring was highly active, with GI_{50} values of 0.06−0.16 μM, and this activity was not influenced by overexpression of P-gp. Furthermore, **21** inhibited tubulin assembly in vitro with an IC_{50} value of 2.0 μM and colchicine binding by 78% as well as cellular microtubule polymerization and spindle formation.



INTRODUCTION

We previously reported that desmosdumotin B (**1**, Figure 1) exerted selective inhibition of a P-glycoprotein (P-gp) overexpressing multidrug resistant (MDR) tumor cell line with significantly lower activity against non-MDR tumor cells.[1] The observed selectivity index [collateral sensitivity (CS),[2] activity ratio of MDR line versus non-MDR line] was greater than 20. This selective in vitro antitumor activity was further enhanced by replacing the three methyl groups at C-6 and C-8 with ethyl groups (**2**, Figure 1) and also by adding an alkyl group at the C-4′ position. During this study, we also found that analogues in which the phenyl B-ring was replaced with a naphthyl moiety (**3**, Figure 1) had dramatically different activity profiles, displaying strong cytotoxicity against multiple cancer cells, regardless of MDR expression, with GI_{50} values of 0.8−2.1 μM. Thus, placing a larger, more electron-rich aromatic B-ring at C-2 resulted in broader antiproliferative activity and loss of specific activity against the MDR cell line. These compounds also induced rapid cell rounding without immediate detachment, leading us to hypothesize antitubulin activity as a mechanism of action, which was tested and confirmed using biochemical assays. Compound **3**, therefore, represents a new scaffold for targeting tubulin assembly.

Design and synthesis of compounds targeting the microtubule constitute an attractive strategy for the discovery of new antitumor agents.[3] The microtubule network is an essential component of the cytoskeleton, and its timely depolymerization and repolymerization are critical for the cell to construct a functional mitotic spindle. Typically, cells arrested in apparent mitosis eventually undergo apoptosis. Antimitotic agents targeting tubulin are generally classified into two groups, compounds that either stimulate or inhibit microtubule assembly, depending on their effects on the tubulin-microtubule equilibrium. Taxoids and epothilones are well-known enhancers of microtubule polymerization.

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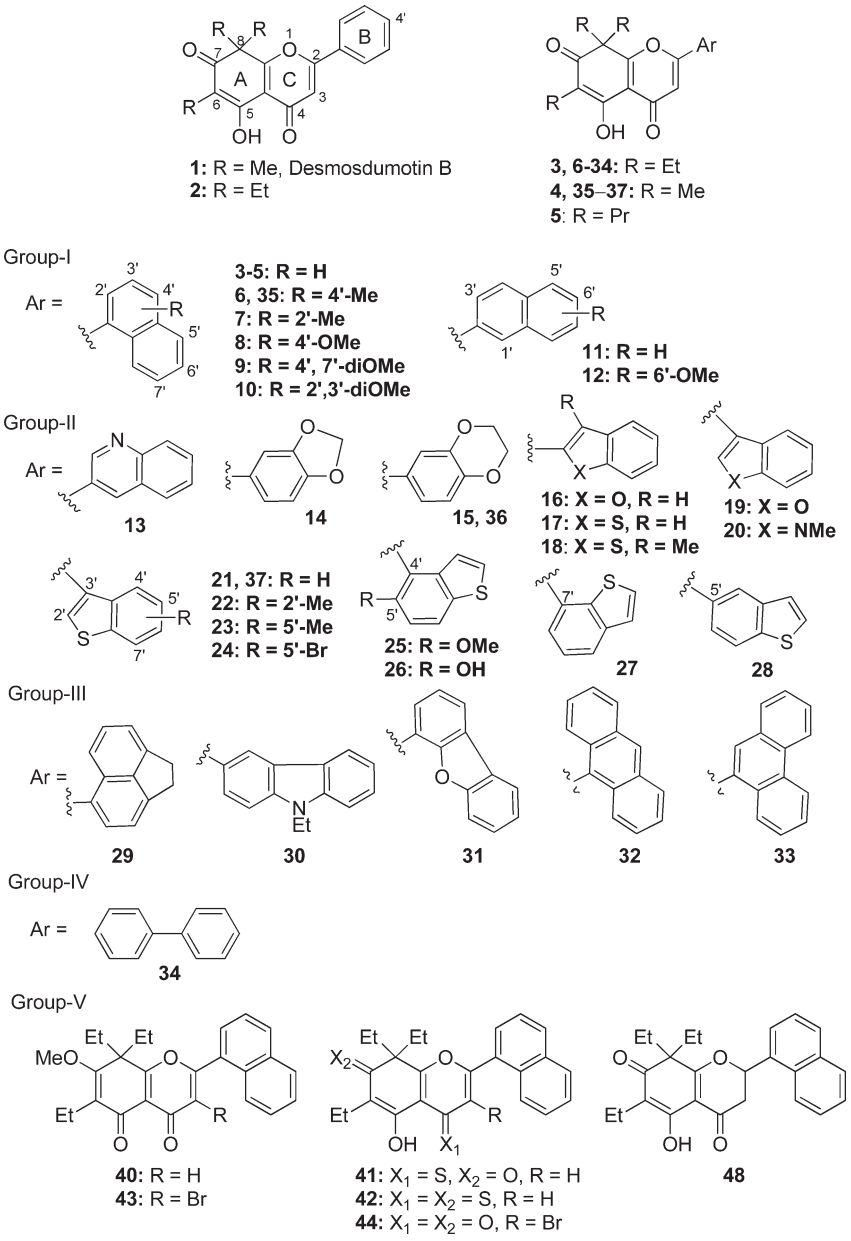

Figure 1. Structures of new analogues of **1**.

Colchicine and the vinca alkaloids are the best known inhibitors of microtubule assembly. All of these agents bind to β-tubulin to exert their antimitotic activity. The best characterized drug binding domains are known as the colchicine site, the vinca domain, and the taxoid site.

The vinca alkaloids and the taxoids are among the most useful drugs for cancer therapy, and numerous compounds targeting drug binding sites on tubulin have been developed. Unfortunately, most antitubulin agents that have entered clinical trials have failed because of adverse effects, such as limited therapeutic effects at maximally tolerated doses, perhaps because of drug resistance, high toxicity, or poor physicochemical properties involved in the absorption, distribution, metabolism, and excretion profile. Interestingly, thus far, no colchicine site drug has proved useful in cancer treatment. Despite its lack of utility in cancer therapy, colchicine is used for the treatment of gout, familial Mediterranean fever, secondary amyloidosis, and scleroderma. Moreover, colchicine has long been an important tool for the study of microtubule structure/function[4] and a key molecular model for structure−activity relationship studies. Previous research revealed that a two-ring aromatic system with the rings either directly bonded or separated by a one to three atom bridge is a common structural feature for binding to the colchicine site.[5] Combretastatin A-4 (CA-4) is an attractive natural product that targets tubulin polymerization via the colchicine site. CA-4 exerts strong cytotoxicity against multiple human tumor cell lines.[6] The discovery of CA-4 and its simple molecular structure stimulated research to identify new chemotypes that interact with the colchicine site.

Our interesting discovery of 2-naphthyldesmosdumotin B (**3**) as an antitubulin agent prompted us to prepare additional **3** analogues with bicyclic and tricyclic aromatic substituents at C-2. In the work presented here, we describe the syntheses and bioactivities of this compound series.

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CHEMISTRY

Thirty-four newly synthesized analogues, **4**−**37**, are depicted in Figure 1. They were synthesized from **38** (R = Et for **6**−**34**, R = Me for **4** and **35**−**37**, and R = Pr for **5**)[1] through the related chalcones (**39**) as shown in Scheme 1.[1,7] The chalcones (**39**) were prepared by the Claisen−Schmidt condensation of **38** with various commercially available aromatic aldehydes (ArCHO) except for **55** and **56**. In most cases, the condensation was carried out in the presence of 50% aqueous KOH in EtOH. However, under these conditions, the reaction with 3-quinoline aldehyde resulted in extremely low yields of product. Finally, the reactions were run with good yields in the presence of piperidine or $Ba(OH)_2 \cdot H_2O$. The iodine-catalyzed cyclization of **39**, followed by demethylation, provided **3**−**37**. The treatment of **3** (R = Et, Ar = naphthyl) with excess Lawesson's reagent[8] resulted in replacement of oxygen with sulfur to yield both the monothioketone **41** and the dithioketone **42**. The structure of **41** was determined from the ^{1}H NMR and MS spectra, which indicated the insertion of a sulfur atom. Compared to the ^{1}H NMR of the related compound **3**, the spectra of **41** showed a 0.79 ppm high field shift of a proton at the C-3 position and no shift for the ethyl protons at the C-6 and C-8 positions. There was, however, a high field shift for the ethyl protons of dithioketone **42** (Table 1). Bromination of **40a** (R = Et, Ar = naphthyl) with *n*-Bu_4NBr in the presence of $PhI(OAc)_2$[9] afforded 3-bromo derivative **43**, which was converted to **44** by demethylation with BBr_3. Meanwhile, flavanone analogue **48** was prepared by the treatment of **39** with HI in HOAc.

Aldehydes **55** and **56** were prepared as shown in Scheme 2. We originally expected that 5-methoxybenzothiophene and 5-methylbenzothiophene, **53**[10] and **54**,[11] could be obtained from the related 3-carboxyaldehydes through formylation.[12] While Rieche formylation of 5-methylbenzothiophene (**54**) using $SnCl_4$ provided the desired 3-carboxaldehyde **56** in good yield, the same treatment of 5-methoxybenzothiophene (**53**) unexpectively produced 4-formyl product **55**. The structure of **55** was identified by ^{1}H NMR, which showed two doublet signals with a coupling constant of 5.6 Hz at 8.39 and 7.65 ppm for the C-3 and C-2 protons, respectively. Furthermore, NOE cross-peaks were observed between the aldehyde and methoxy protons, the aldehyde and C-3 protons, as well as the methoxy and C-6 protons. The interesting difference in reactivity between the 5-methyl and 5-methoxy groups might be caused by a chelation effect of the Lewis acid with the oxygen atom of the methoxy group.

Newly synthesized analogues could be classified into the following five groups according to the C-2 substituent: (I) naphthalene group (**3**−**12** and **35**), (II) heterobicyclic aromatic group (**13**−**28**, **36**, and **37**), (III) tricyclic aromatic group (**29**−**33**), (IV) biphenyl group (**34**), and (V) naphthalane group with A- and/or C-ring modifications (**40a**−**44** and **48**).

RESULTS AND DISCUSSION

All new analogues were evaluated against seven human tumor cell lines, specifically, HCT-8 (colon adenocarcinoma), PC-3 (prostate cancer), A549 (lung carcinoma), MCF-7 (breast cancer) or DU145 (prostate cancer), HepG2 (hepatocellular carcinoma), KB (epidermoid carcinoma of the nasopharynx), and KB-VIN, which is an MDR (P-gp-overexpressing) KB subline selected using increasing concentrations of vincristine. The selected active compounds **3**, **6**, **8**, **11**, **15**, **21**, **22**, **26**, and **35**−**37** were also tested for inhibitory effects on tubulin assembly and inhibition of binding of [^{3}H]colchicine to tubulin, together with **2** for comparison. Table 2 lists the antiproliferative data for **1**−**48** and vincristine, the positive control. The tubulin study data are presented in Table 3.

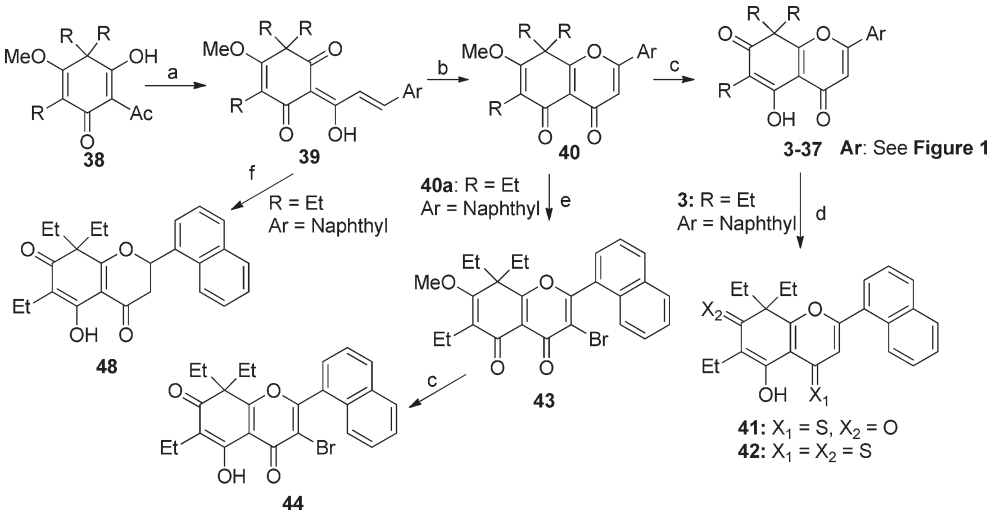
Monophenyl analogues **1** and **2** were clearly "nonactive" against all non-MDR tumor cells (**2** was also inactive in the tubulin assembly assay), while they were "active" against the P-gp overexpressing MDR tumor cell line KB-VIN. In comparison, most analogues with a bicyclic substituent at C-2 (groups I and II) showed in vitro activity against all tested cell lines, with GI_{50} values ranging from 0.1 to 20 μM, while three of the five analogues (**30**−**32**) with a tricyclic substituent at C-2 (group III) were inactive.

With respect to the alkyl groups at C-6 and C-8, the 6,8,8-tri-Me (**4**) and tri-Et (**3**) analogues showed similar growth inhibitory activity against the tested tumor cell lines, while the tripropyl analogue **5** was much less active.

Group II compound **21**, with a tri-Et A-ring and a benzo[*b*]thiophene B-ring at C-2, had dramatically increased antiproliferative activity, exhibiting GI_{50} values of 0.06−0.16 μM against the human tumor cell lines. Although the activity of **37**, with a tri-Me A-ring and a benzo[*b*]thiophene B-ring at C-2, was less than that of **21**, it still showed potent activity with GI_{50} values of 0.09−1.08 μM. In comparison, the related analogue **17**, with the chromene skeleton attached at the 2′-position of the benzo[*b*]thiophene system rather than at the 3′-position, showed considerally lower activity against all cell lines (GI_{50} = 3.8−19.0 μM), with significant activity only against PC-3. The replacement of sulfur (**21**) with oxygen (**19**) or *N*-methyl (**20**) led to reduced activity. Other relatively active analogues, with mostly submicromolar GI_{50} values, were tri-Et compounds **3**, **6**, **8**, **11**, and **15**, as well as the related tri-Me analogues **4** and **35**−**37**. All of these compounds had a naphthyl B-ring at C-2 except for **15** and **36**, which had a 2,3-dihydrobenzo[1,4]dioxine group at C-2. Quinoline **13**, benzofurans **16** and **19**, and indole **20**, each bearing a bicyclic heteroaromatic B-ring system, displayed only moderate activity against all tested cell lines. Analogue **14**, with a benzo[1,3]dioxolyl B-ring system, also moderately inhibited tumor cell growth, although it was about 10 times less active than **21**. These data demonstrated that although a 10π-electron B-ring system was optimal, it was not essential for activity. Among the group III compounds, those with the three rings arranged linearly were inactive while compound **29** (C-2 dihydroacenaphthyl) showed significant antiproliferative activity. Compounds **33** (C-2 phenanthryl) and **34** (C-2 biphenyl) showed only moderate potency.

When the ketone oxygen at C-7 was replaced with a methoxy group (**3** vs **40a**), there was a substantial loss of activity (GI_{50} values of 0.8−2.1 μM for **3** and 9.7−20.9 μM for **40a**). The latter compounds would lose the possibility of a hydrogen bond between the C-4 ketone carbonyl and the C-5 hydroxy group, which might account for the difference in potency. Activity also decreased when a bromide group was inserted at the C-3 position (**3** vs **44**), although the bromine atom had no effect when a C-7 methoxy group was present (cf. **40a** and **43**). Reduced activity also occurred when both oxygens in the C-4 and C-7 ketones were changed to sulfur (**3** vs **42**). However, monothioketone **41** retained relatively good activity, compared with **3**, against A549 and KB cells, with GI_{50} values of 1.86 and 1.41 μM, respectively. The saturation of the double bond between C-2 and C-3 also decreased activity (**3** vs **48**). Finally, it is pointed out that MDR

Scheme 1. Syntheses of New Analogues of 1[a]

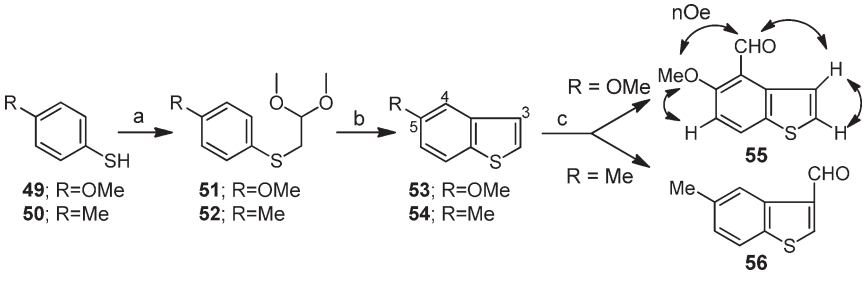


[a] Reagents and conditions: (a) ArCHO, base/solvent [piperidine/EtOH for 3-quinolinecarboxaldehyde, $Ba(OH)_2 \cdot 8H_2O$/MeOH for 4-dimethylamino-1-naphthaldehyde, and KOH/EtOH for others]; (b) I_2 (cat.), DMSO, H_2SO_4 (cat.), 90−95 °C, 1 h; (c) BBr_3, 0° C to room temp; (d) Lawesson's reagent, toluene, reflux; (e) $PhI(OAc)_2$, *n*-Bu_4NBr, room temp; (f) 45% HI, HOAc.

Table 1. ^{1}H NMR Chemical Shifts (ppm) of 3, 41, and 42

		C-6		C-8	
	C-3 H	-CH_2CH$_3$	-CH$_2$$CH_3$	-CH_2CH$_3$	-CH$_2$$CH_3$
3	6.82 (s, 1H)	2.49 (q, 2H)	1.07 (t, 3H)	2.26−2.12 (m, 2H) 1.98−1.84 (m, 2H)	0.73 (t, 6H)
41	7.62 (s, 1H)	2.52 (q, 2H)	1.07 (t, 3H)	2.31−2.07 (m, 2H) 2.01−1.86 (m, 2H)	0.74 (t, 6H)
42	7.62 (s, 1H)	2.99 (q, 2H)	1.10 (t, 3H)	2.58−2.42 (m, 2H) 2.30−2.16 (m, 2H)	0.64 (t, 6H)

Scheme 2. Syntheses of 5-Substituted Benzothiophenecarboxaldehyde[a]



[a] Reagents and conditions: (a) 2-chloro-1,1-dimethoxyethane, NaOMe, MeOH, xylene, 110 °C; (b) H_3PO_4, xylene, 110 °C; (c) Cl_2CHOMe, $SnCl_4$, CH_2Cl_2, room temp, 75% for **55**, 82% for **56**.

KB-VIN cells were as sensitive as the parental KB cells to all active compounds.

Because compound **21**, containing a benzo[*b*]thiophene system, showed potent antiproliferative activity, we investigated additional related derivatives **22**−**28**. While none of these compounds showed greater activity than **21**, some interesting facts were revealed. (1) The 2′-Me derivative (**22**) retained activity against all tested cell lines except PC-3. (2) 5′-Me (**23**) and 5′-Br (**24**) analogues lost activity. (3) Activity varied with the position of attachment of the benzo[*b*]thiophene system to the chromene skeleton. The rank order of overall activity was 3′ (**21**) > 7′ (**27**) > 2′ (**17**) >5′ (**28**). (4) Compound **28** displayed selective activity against the DU145 and KB cell lines. (5) Compound **26** (5′-OH) exhibited potent cytotoxicity against all tested cell lines, while **25** (5′-OMe) lost activity against most cell lines. (6) Compounds **24** and **27** possessed "collateral

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Table 2. Antiproliferative Activity of 1 and Analogues of 1[a]

		GI_{50} (μM)[b]						
Cmpd	HCT-8	PC-3	A549	DU145	MCF-7	HepG2	KB	KB-VIN
				Group I				
1	NA	NA	NA	NT	NA	NA	NA	13.51
2	NA	NA	NA	NT	NA	NA	NA	1.07
3	1.03	1.03	1.55	NT	1.55	2.06	1.55	0.77
4	1.16	1.45	1.73	NT	1.73	NT	0.29	0.58
5	23.26	17.44	23.26	NT	NA	NA	18.60	17.44
6	0.62	2.49	0.50	NT	0.75	1.24	0.47	0.27
7	17.41	12.44	17.41	NT	13.68	16.67	19.90	19.90
8	3.35	2.39	1.48	NT	3.59	1.48	1.67	1.56
9	NA	NA	NA	NT	NA	NA	NA	NA
10	NA	NT	NA	NT	NT	NT	NA	35.71
11	1.29	2.58	1.16	NT	1.42	1.55	1.03	1.16
12	11.96	4.78	16.75	NT	8.37	13.40	10.77	9.57
35	2.56	13.33	1.25	NT	NT	1.33	0.61	0.72
				Group II				
13	12.85	12.85	12.85	NT	19.28	14.91	15.42	14.91
14	13.09	5.24	26.18	NT	11.26	11.78	18.32	17.02
15	2.27	1.52	1.52	NT	1.89	1.52	1.26	1.14
16	5.82	5.82	17.20	NT	NA	15.08	14.81	14.55
17	19.04	3.81	13.96	NT	15.23	15.74	11.17	9.90
18	>25	>25	>25	>25	NT	>25	>25	>25
19	15.61	28.04	9.79	NT	NT	17.72	4.50	2.09
20	15.86	20.46	5.63	NT	17.39	21.74	18.67	2.81
21	0.13	0.16	0.06	NT	0.11	0.13	0.08	0.07
22	2.70	90.69	1.35	3.92	NT	2.03	0.61	0.76
23	93.14	>98	>98	>98	NT	>98	53.92	>98
24	42.19	37.97	42.19	31.65	NT	30.59	26.37	10.55
25	19.58	51.89	66.04	21.46	NT	24.76	11.32	11.08
26	3.66	21.46	3.17	1.12	NT	1.02	0.76	0.78
27	11.17	3.81	5.84	0.74	NT	1.90	2.49	0.94
28	>25	>25	>25	10.41	NT	>25	11.22	>25
36	1.30	1.50	0.76	NT	NT	23.73	0.76	0.65
37	1.08	0.91	0.23	NT	NT	0.57	0.11	0.09
				Group III				
29	2.42	6.04	6.04	NT	3.62	4.59	2.17	1.93
30	NA	NA	NA	NT	NA	NA	NA	NA
31	NA	NA	NA	NT	NA	NA	NA	NA
32	NA	NA	NA	NT	NA	NA	NA	NA
33	12.79	13.70	18.26	NT	9.13	NT	11.42	9.82
				Group IV				
34	NA	8.45	18.12	NT	15.70	19.32	18.12	12.08
				Group V				
40a	11.69	14.68	16.67	NT	20.90	20.65	11.19	9.70
41	8.17	16.58	1.86	NT	3.22	8.42	1.41	2.70
42	13.33	24.05	5.95	NT	14.05	24.05	7.38	5.95
43	7.68	10.79	4.77	NT	11.00	14.52	9.13	9.96
44	13.89	NA	25.64	NT	24.57	NA	6.41	11.97
48	9.28	12.63	7.99	NT	NT	9.02	5.93	3.87
VCR	0.036	0.018	0.007	0.018	0.018	NT	0.004	9.091

[a] NA: not active; test compound (20 μg/mL) did not reach 50% inhibition. NT: not tested. [b] Antiproliferative activity as GI_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay. Human colon adenocarcinoma (HCT-8), prostate cancer (PC-3 and DU145), lung carcinoma (A549), breast cancer (MCF-7), hepatocellular carcinoma (HepG2), epidermoid carcinoma of the nasopharynx (KB), and MDR line overexpressing P-glycoprotein (KB-VIN).

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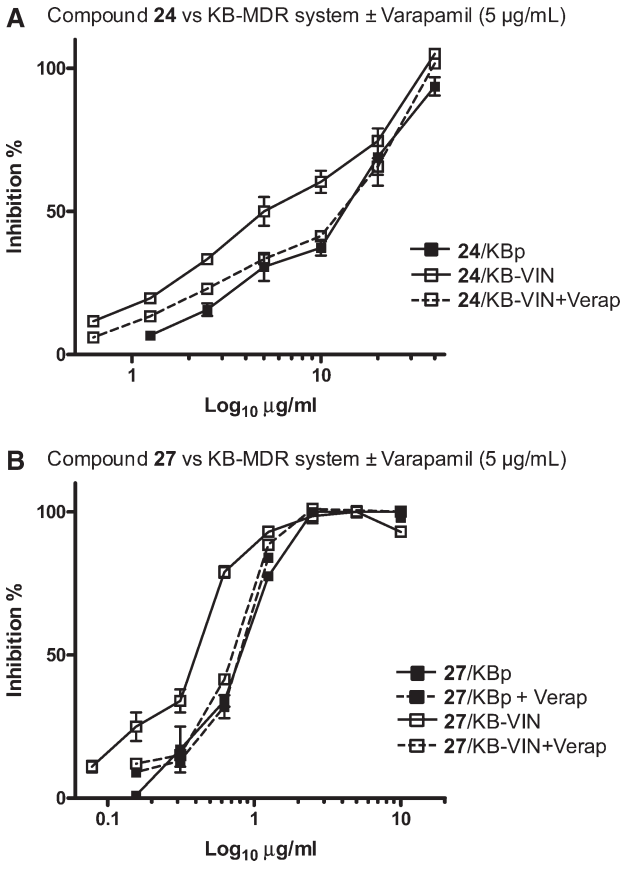

Figure 2. (A) Reversal of MDR selectivity of **24** by verapamil cotreatment. (B) Reversal of MDR selectivity of **27** by verapamil co-treatment.

sensitivity" (CS),[2] exhibiting over 2-fold greater cytotoxicity against the MDR line (KB-VIN) than against the parental line (KB). Figure 2 shows the reversal effects of co-treatment of **24** or **27** together with a nontoxic concentration of verapamil (VERAP), a known P-gp modulator. Co-treatment of **24** and **27** with VERAP partially reversed the cytotoxic activity, showing that the MDR selectivity was dependent in part on P-gp function and consistent with the effect on P-gp activity measured using the co-incubation treatment protocol. This result is consistent with data obtained with analogues of **1** containing a phenyl B-ring.[13]

We observed that cells treated with cytotoxic concentrations of these agents displayed a characteristic rounded shape that was morphologically similar to the cells treated with colchicine, when examined microscopically. We explored microtubules and spindles in A549 cells treated with **21**. The A549 cells were treated with **21**, colchicine, pacritaxel, doxorubicine, or DMSO for 24 h followed by the immunofluorescence staining of tubulin using monoclonal antibody to α-tubulin. As we expected, microtubule polymerization and spindle formation were significantly inpaired by treatment with **21**, which was similar to the effects of colchicine and clearly different from pacritaxel or doxorubicine (Figure 3). The antimicrotubule activity of **21** was seen within 24 h at 0.1 nM that was 1000-fold less than that of colchicine (100 nM).

We therefore evaluated selected active compounds for antitubulin activities, and the results are shown in Table 3. All cytotoxic compounds (GI_{50} values of less than 1 μg/mL) also exhibited potent inhibition of tubulin assembly. Analogue **21**, which showed the most potent cytotoxicity, strongly inhibited

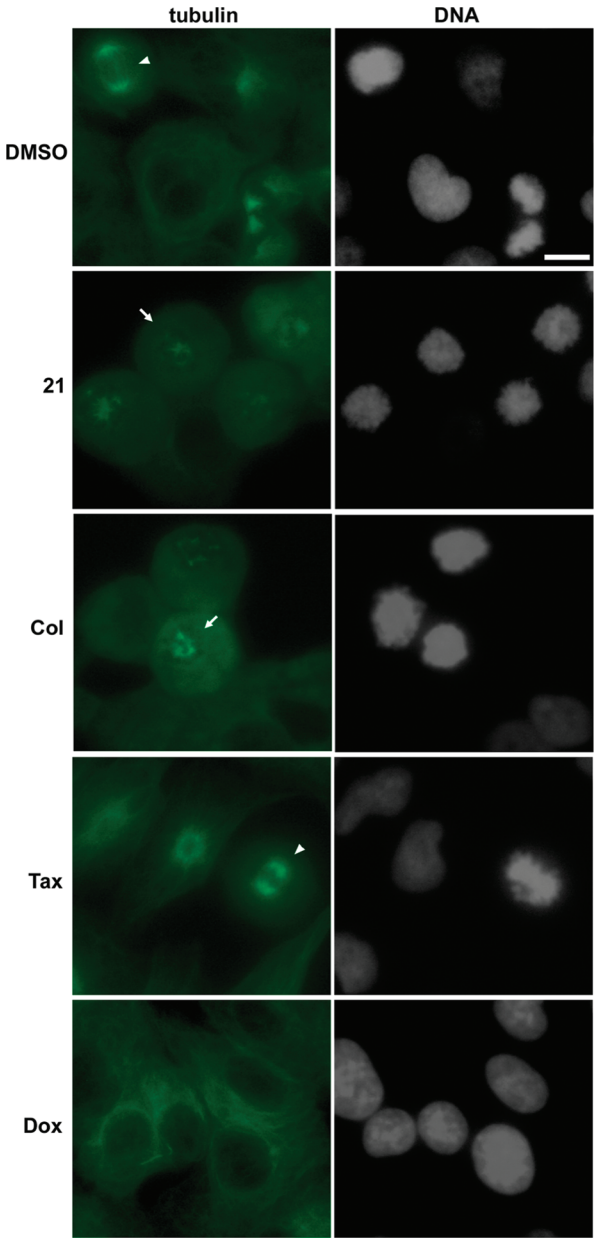

Figure 3. Effect of compound **21** on microtubule assembly. A549 cells were cultured and treated with agent for 24 h as indicated. Cells were labeled immunocytochemically using antibody to α-tubulin (left panels) and DAPI for DNA (right panels). The mitotic spindles were clearly seen in control (DMSO) and pacritaxel (Tax) treated cells (arrow heads) while undetectable in the cells treated with **21**, colchicine (Col), or doxorubicine (Dox). The aggregations of tubulin were seen in the prophase cells treated with **21** or colchicine (arrow). In addition, microtubules were undetectable in the cells treated with **21** or colchicine but detectable in cells treated with DMSO, pacritaxel, or doxorubicine. These observations clearly indicate that **21** inhibits tubulin polymerization in the human tumor cells. Bar, 10 μm.

tubulin assembly with an IC_{50} value of 2.0 μM. It was also the most active of these compounds as an inhibitor of colchicine binding to tubulin, inhibiting the binding reaction by 78%. Its trimethyl analogues **35**−**37** and cytotoxic analogues **3**, **6**, **8**, **21**, **22**, and **26** also inhibited tubulin assembly with IC_{50} values of 2.3−3.7 μM, although these compounds were less active than **21** as inhibitors of colchicine binding. Compounds **11** and **15** were

Table 3. Inhibition of Tubulin Assembly[a] and Colchicine Binding[b]

compd	IC_{50} ± SD (μM)	inhibition of colchicine binding ± SD (%)
	Group I	
2	NA	
3	2.3 ± 0.08	43 ± 10
6	2.7 ± 0.1	50 ± 0.4
8	2.7 ± 0.3	31 ± 3
11	not obtainable[c]	29 ± 0.4
35	2.5 ± 0.2	37 ± 5
	Group II	
15	6.5 ± 0.7	33 ± 2
21	2.0 ± 0.1	78 ± 5
22	3.4 ± 0.3	38 ± 4
26	3.4 ± 0.2	52 ± 0.6
36	3.7 ± 0.09	36 ± 4
37	2.4 ± 0.007	69 ± 3
CA-4	1.1 ± 0.1	99 ± 0.7

[a] The tubulin assembly assay measured the extent of assembly of 10 μM tubulin after 20 min at 30 °C. [b] Tubulin: 1 μM. [^{3}H]colchicine: 5 μM. Inhibitor: 5 μM. Incubation was for 10 min at 37 °C. [c] Partial inhibition observed and was maximal with 4 μM compound. Higher compound concentrations resulted in the same amount of inhibition observed with 4 μM, suggesting poor solubility of **11** in the reaction mixture.

even less active, and **2** had no significant interaction with tubulin at the highest concentration evaluated (40 μM). Thus, we concluded that **21** is a potent tubulin polymerization inhibitor.

In 2005, Nguyen et al. studied 15 colchicine site inhibitiors (CSIs) and proposed seven pharmacophoric points based on consistent structural features and recurring ligand interactions.[14] The seven points were three hydrogen bond acceptors (A1, A2, and A3), one hydrogen bond donor (D1), two hydrophobic centers (H1 and H2), and one planar group (R1). The H2 and R1 points represented the rigid portion of the molecular scaffold, while the other five points were important for binding specificity. Nguyen et al.[14] also mentioned that A2, H1, H2, and R1 emerged as essential features for inhibitory activity at the colchicines site. Most of the 15 CSIs contained five to six of the seven pharmacophoric points. For example, Figure 4A shows that the well-known tubulin polymerization inhibitors, colchicine and CA-4, map to five pharmacophoric points, A1, A2, D1, H2, and R1. Figure 4B overlays the structures of colchicine and CA-4 onto a 3D picture of the five pharmacophores. We used Molecular Operating Environment (MOE) software to search for the best-fit 3D conformations of three of our active compounds, **3**, **21** and **26**, with the five pharmacophore features. The superpositions are shown in Figure 5. The ring systems of **3**, **21**, and **26** matched well with the planar center (R1) and hydrophobic center (H2), which are critical portions for the molecular scaffold. In addition, the hydrogen acceptor (A1) and hydrogen bond donor (D1) pharmacophores overlapped with the oxygen at the 4-position and hydroxyl group at the 5-position, respectively, of **3**, **21**, and **26**. Compound **21** showed a better fit to the pharmacophore points R1 and H2 than compounds **3** and **26**, which could explain the greater inhibitory activity of **21** against the colchicine binding site. Another possible reason of its great potency is that the sulfur atom in **21** might interact positively with the binding site. Further structural modifications to enhance expression of one or more of

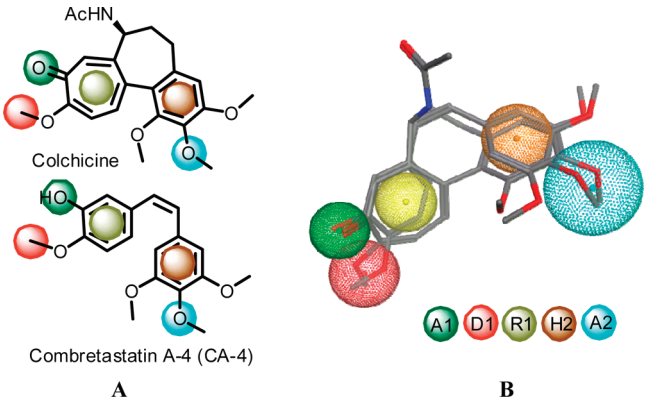

Figure 4. Common pharmacophores of colchicine and CA-4. (A) Five point pharmacophores, H2 (hydrophobic center), R1 (planar group), A1, A2 (hydrogen bond acceptors), and D1 (hydrogen bond donor), for colchicine and CA-4 by Nguyen.[14] (B) Structures of colchicine and CA-4 onto a 3D picture of the five pharmacophores by MOE. R1 is shown as a hydrophobic/aromatic center in this model.

the other three pharmacophore features, A2, A3, and H1, could increase the potency of these tubulin-destabilizing agents targeting the colchicine site.

In summary, we discovered that analogues of **1** with bicyclic aryl substituents at C-2 possessed promising antitumor activity, with significant antiproliferative effects against HCT-8, PC-3, A549, MCF-7, HepG2, KB, and KB-VIN tumor cells. Benzo[*b*]thiophene analogue **21** displayed the most potent inhibitory effects on tumor cell growth, with GI_{50} values of 0.06−0.16 μM. The activity of the compounds, including **21**, was not influenced by overexpression of P-gp (GI_{50} = 0.07 μM against KB-VIN vs 0.08 μM for the parental KB line). Furthermore, cytotoxicic analogues **3**, **6**, **8**, **11**, **15**, **21**, **22**, **26**, and **35**−**37**, which all possess a bicyclic B-ring at C-2, displayed significant antitubulin activities with 30−80% inhibition of binding to the colchicine site. Compounds **3**, **21**, and **26** overlapped with four out of seven pharmacophoric points that were derived from the binding models of 15 selected CSIs. Thus, they are a new class of CSIs, and further modifications would be useful to develop more potent antitubulin agents. However, analogues of **1** with a phenyl B-ring, including the natural product **1** itself, showed selective cytotoxicity against KB-VIN cells relative to KB cells. Thus, the results presented here indicate that the B-ring structure is critically important in the interaction of this compound class with P-gp. This distinction may well prove valuable in designing more effective chemotherapeutic agents.

EXPERIMENTAL SECTION

All chemicals and solvents were used as purchased. All melting points were measured on a Fisher-Johns melting point apparatus without correction. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 (300 MHz) or a Varian Inova (400 MHz) NMR spectrometer with TMS as the internal standard. All chemical shifts are reported in ppm. NMR spectra were referenced to the residual solvent peak. Chemical shifts δ are in ppm, and apparent scalar coupling constants J are in Hz. Mass spectroscopic data were obtained on a Shimazu LC-MS2010 instrument. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). Biotage Flash or Isco Companion systems were used for flash chromatography. All target compounds were characterized and determined as at least >95% pure by ^{1}H NMR, MS, and elemental analyses or analytical HPLC.

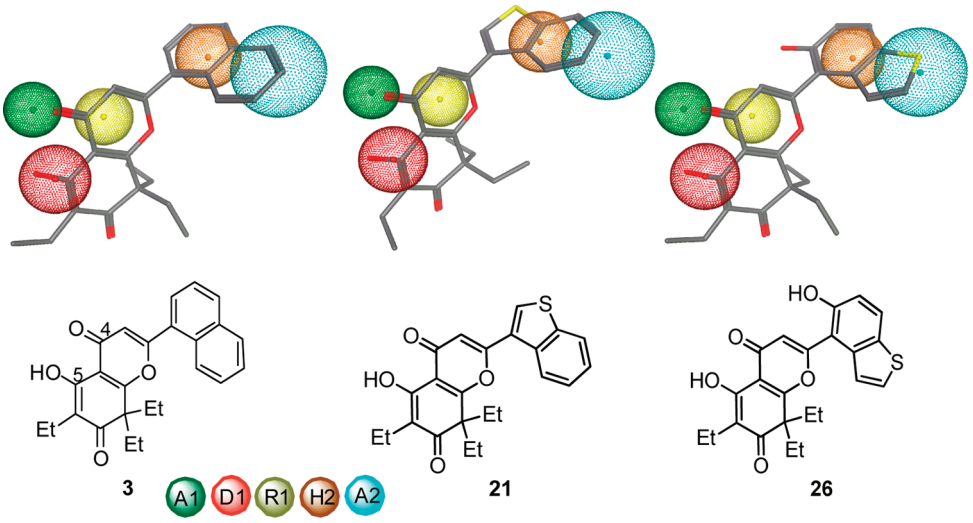

Figure 5. Alignment of pharmacophore and compounds **3**, **21**, and **26**: hydrophobic center (H2), planar group (R1), hydrogen bond acceptors (A1 and A2), hydrogen bond donor (D1).

General Synthetic Procedures for 31. A solution of **30** in EtOH−50% aqueous KOH (1:1, v/v) and an appropriate aromatic aldehyde (excess) was stirred at room temperature. After the reaction was complete by TLC analysis, the mixture was poured into ice-cold 1 N HCl and then extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with CH_2Cl_2−hexane as eluent to afford the target compound, which was crystallized from CH_2Cl_2−hexane.

General Synthetic Procedures for 3−37. Compound **39** was dissolved separately in 1% H_2SO_4 in DMSO, then I_2 (0.1 equiv mol) was added and the reaction mixture heated at 90 °C for 1 h. The reaction mixture was treated in the same manner as described above to afford compound **40**, which was dissolved in anhydrous CH_2Cl_2. BBr_3 (3 equiv mol, 1.0 M solution in CH_2Cl_2) was added to the solution at 0 °C, which was warmed to room temperature spontaneously and stirred overnight. After addition of water, the reaction mixture was extracted three times with CH_2Cl_2. The extracts were combined, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residues were chromatographed on silica gel, eluting with EtOAc−hexane (1:4) to obtain analogues **5**−**37**.

2-(Naphthalen-1′-yl)-6,8,8-tripropyldesmosdumotin B (5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.07 (s, 1H, chelated-O*H*), 8.09 (d, 1H, J = 7.9 Hz, 5′-*H*), 8.01−7.97 (m, 1H, 4′- or 8′-*H*), 7.92−7.86 (m, 1H, 4′ or 8′-*H*), 7.70−7.58 (m, 4H, J = 7.3 Hz, 2′-, 3′-, 6′-, and 7′-*H*), 6.80 (s, 1H, 3-*H*), 2.43 (t, 2H, J = 7.5 Hz, 6-CH_2CH$_2$CH$_3$), 2.12 (dt, 2H, J = 12.5 and 4.3 Hz, 8-CH_2CH$_2$CH$_3$), 1.83 (dt, 2H, J = 12.5 and 4.3 Hz, 8-CH_2-CH$_2$CH$_3$), 1.49 (q, 2H, J = 7.7 Hz, 6-CH$_2$CH_2CH$_3$), 1.24−1.11 (m, 2H, 8-CH$_2$CH_2CH$_3$), 1.10−0.50 (m, 2H, 8-CH$_2$CH_2CH$_3$), 0.96 (t, 3H, J = 7.7 Hz, 6-CH$_2$CH$_2$CH_3), 0.82 (t, 6H, J = 7.5 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 431 (M$^+$ + 1). Anal. ($C_{28}H_{30}O_4$) C, H, O.

2-(4′-Methylnaphthalen-1′-yl)-6,8,8-triethyldesmosdumotin B (6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.11 (s, 1H, chelated-O*H*), 8.17−8.12 (m, 1H, 5′-*H*), 7.97−7.92 (m, 1H, 8′-*H*), 7.70−7.56 (m, 2H, 6′- and 7′-*H*), 7.58 (d, 1H, J = 7.3 Hz, 2′-*H*), 7.46 (d, 1H, J = 7.3 Hz, 3′-*H*), 6.79 (s, 1H, 3-*H*), 2.80 (s, 3H, 4′-CH_3), 2.48 (q, 2H, J = 7.3 Hz, 6-CH_2CH$_3$), 2.26−2.12 (m, 2H, 8-CH_2CH$_3$), 1.96−1.84 (m, 2H, 8-CH_2CH$_3$), 1.06 (t, 3H, J = 7.3 Hz, 6-CH$_2$CH_3), 0.72 (t, 6H, J = 7.3 Hz, 8-CH$_2$CH_3 × 2). Anal. ($C_{26}H_{26}O_4 \cdot {}^1/_4H_2O$) C, H, O.

2-(2′-Methylnaphthalen-1′-yl)-6,8,8-triethyldesmosdumotin B (7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.05 (s, 1H, chelated-O*H*), 7.96 (d, 1H, J = 8.7 Hz, 4′-*H*), 7.94−7.88 (m, 1H, 5′-*H*), 7.58−7.48 (m, 3H, 6′-, 7′-, and 8′-*H*), 7.46 (d, 1H, J = 8.7 Hz, 3′-*H*), 6.64 (s, 1H, 3-*H*), 2.49 (q, 2H, J = 7.5 Hz, 6-CH_2CH$_3$), 2.46 (s, 3H, 2′-CH_3), 2.20−2.06 (m, 2H, 8-CH_2CH$_3$), 1.88−1.74 (m, 2H, 8-CH_2-CH$_3$), 1.07 (t, 3H, J = 7.3 Hz, 6-CH$_2$CH_3), 0.69 (t, 6H, J = 7.3 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 403 (M$^+$ + 1). Anal. ($C_{26}H_{26}$-$O_4 \cdot {}^1/_4H_2O$) C, H, O.

2-(4′-Methoxynaphthalen-1′-yl)-6,8,8-triethyldesmosdumotin B (8). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.19 (s, 1H, chelated-O*H*), 8.40 (dd, 1H, J = 7.3 and 2.2 Hz, 5′-*H*), 7.94 (dd, 1H, J = 7.3 and 2.2 Hz, 8′-*H*), 7.65 (d, 1H, J = 8.0 Hz, 2′-*H*), 7.65−7.56 (m, 2H, 6′ and 7′-*H*), 6.93 (d, 1H, J = 8.0 Hz, 3′-*H*), 6.78 (s, 1H, 3-*H*), 4.10 (s, 3H, -OCH_3), 2.48 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.24−2.12 (m, 2H, 8-CH_2CH$_3$), 1.99−1.84 (m, 2H, 8-CH_2CH$_3$), 1.06 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.72 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 419 (M$^+$ + 1). Anal. ($C_{26}H_{26}O_5$) C, H, O.

2-(4′,7′-Dimethoxynaphthalen-1′-yl)-6,8,8-triethyldesmosdumotin B (9). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.22 (s, 1H, chelated-O*H*), 8.31 (d, 1H, J = 10.0 Hz, 5′-*H*), 7.59 (d, 1H, J = 8.2 Hz, 2′-*H*), 7.24 (d, 1H, J = 10.0 Hz, 6′-*H*), 7.23 (s, 1H, 2′-*H*), 6.80 (d, 1H, J = 8.2 Hz, 3′-*H*), 6.77 (s, 1H, 3-*H*), 4.07 (s, 3H, -OCH_3), 3.89 (s, 3H, -OCH_3), 2.48 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.27−2.13 (m, 2H, 8-CH_2CH$_3$), 2.01−1.87 (m, 2H, 8-CH_2CH$_3$), 1.06 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.71 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 449 (M$^+$ + 1). Anal. ($C_{27}H_{28}O_6 \cdot {}^1/_4H_2O$) C, H, O.

2-(2′,3′-Dimethoxynaphthalen-1′-yl)-6,8,8-triethyldesmosdumotin B (10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.09 (s, 1H, chelated-O*H*), 7.81 (d, 1H, J = 8.2 Hz, 5′-*H*), 7.54−7.38 (m, 3H, 6′,7′ and 8′-*H*), 7.49 (s, 1H, 4′-*H*), 6.67 (s, 1H, 3-*H*), 4.05 (s, 3H, -OCH_3), 3.89 (s, 3H, -OCH_3), 2.49 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.20−2.06 (m, 2H, 8-CH_2CH$_3$), 1.92−1.78 (m, 2H, 8-CH_2CH$_3$), 1.06 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.69 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 449 (M$^+$ + 1). Anal. ($C_{27}H_{28}O_6 \cdot H_2O$) C, H, O.

2-(Naphthalen-2′-yl)-6,8,8-triethyldesmosdumotin B (11). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.10 (s, 1H, chelated-O*H*), 8.33 (d, 1H, J = 1.8 Hz, 1′-*H*), 8.04−7.96 (m, 2H, 5′- and 8′-*H*), 7.96−7.90 (m, 1H, 4′-*H*), 7.80 (dd, 1H, J = 1.8 and 8.5 Hz, 3′-*H*), 7.69−7.60 (m, 2H, 6′- and 7′-*H*), 7.04 (s, 1H, 3-*H*), 2.48 (q, 2H, J = 7.3 Hz, 6-CH_2CH$_3$), 2.38−2.24 (m, 2H, 8-CH_2CH$_3$), 2.15−2.01 (m, 2H, 8-CH_2CH$_3$), 1.06 (t, 3H, J = 7.3 Hz, 6-CH$_2$CH_3), 0.71 (t, 6H, J = 7.3 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 389 (M$^+$ + 1). Anal. ($C_{25}H_{24}O_4$) C, H, O.

2-(6′-Methoxynaphthalen-2′-yl)-6,8,8-triethyldesmosdumotin B (12). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.18 (s, 1H, chelated-O*H*), 8.24 (d, 1H, J = 1.8 Hz, 1′-*H*), 7.88 (d, 2H, J = 8.7 Hz, 4′ and 8′-*H*), 7.76 (dd, 1H, J = 8.7 and 1.8 Hz, 3′-*H*), 7.27 (dd, 1H, J = 8.7 and 1.8 Hz,

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7′-*H*), 7.19 (d, 1H, *J* = 1.8 Hz, 5′-*H*), 6.99 (s, 1H, 3-*H*), 3.98 (s, 3H, -OC*H*$_3$), 2.47 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.38−2.23 (m, 2H, 8-C*H*$_2$CH$_3$), 2.14−2.00 (m, 2H, 8-C*H*$_2$CH$_3$), 1.05 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.70 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). Anal. (C$_{26}$H$_{26}$O$_5$) C, H, O.

6,8,8-Triethyl-2-(quinolin-3′-yl)desmosdumotin B (13). ^{1}H NMR (300 MHz, CDCl$_3$): δ 12.88 (s, 1H, chelated-O*H*), 9.29 (d, 1H, *J* = 2.2 Hz, 2′-*H*), 8.56 (d, 1H, *J* = 2.2 Hz, 4′-*H*), 8.21 (d, 1H, *J* = 8.5 Hz, 5′-*H*), 8.00 (d, 1H, *J* = 7.4 Hz, 8′-*H*), 7.94−7.86 (m, 1H, 7′-*H*), 7.72 (dd, 1H, *J* = 8.5 and 7.4 Hz, 6′-*H*), 7.09 (s, 1H, 3-*H*), 2.48 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.39−2.25 (m, 2H, 8-C*H*$_2$CH$_3$), 2.12−1.99 (m, 2H, 8-C*H*$_2$CH$_3$), 1.06 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.71 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z*: 390 (M$^+$ + 1). Anal. (C$_{24}$H$_{23}$O$_4$N·^{1}/$_8$H$_2$O) C, H, O.

2-(Benzo[*d*][1′,3′]dioxol-5′-yl)-6,8,8-triethyldesmosdumotin B (14). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.13 (s, 1H, chelated-O*H*), 7.37 (dd, 1H, *J* = 8.2 and 1.8 Hz, 6′-*H*), 7.20 (d, 1H, *J* = 1.8 Hz, 4′-*H*), 6.97 (d, 1H, *J* = 8.2 Hz, 7′-*H*), 6.76 (s, 1H, 3-*H*), 6.12 (s, 2H, 2′-C*H*$_2$-), 2.45 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.32−2.17 (m, 2H, 8-C*H*$_2$CH$_3$), 2.03−1.90 (m, 2H, 8-C*H*$_2$CH$_3$), 1.04 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.67 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z*: 383 (M$^+$ + 1). Anal. (C$_{22}$H$_{22}$O$_6$·^{1}/$_4$H$_2$O) C, H, O.

2-(2′,3′-Dihydrobenzo[*d*][1′,4′]dioxin-6′-yl)-6,8,8-triethyldesmosdumotin B (15). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.17 (s, 1H, chelated-O*H*), 7.34−7.28 (m, 2H, 5′- and 7′-*H*), 7.02 (d, 1H, *J* = 9.2 Hz, 8′-*H*), 6.77 (s, 1H, 3-*H*), 4.40−4.30 (m, 4H, -OC*H*$_2$C*H*$_2$O-), 2.45 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.31−2.16 (m, 2H, 8-C*H*$_2$CH$_3$), 2.05−1.91 (m, 2H, 8-C*H*$_2$CH$_3$), 1.04 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.66 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z*: 397 (M$^+$ + 1). Anal. (C$_{23}$H$_{24}$O$_6$·^{1}/$_4$H$_2$O) C, H, O.

2-(Benzofuran-2′-yl)-6,8,8-triethyldesmosdumotin B (16). ^{1}H NMR (300 MHz, CDCl$_3$): δ 12.98 (1H, chelated-O*H*), 7.71 (d, 1H, *J* = 7.7 Hz, 4′-*H*), 7.60 (d, 1H, *J* = 7.9 Hz, 7′-*H*), 7.49 (dd, 1H, *J* = 7.9 and 7.2 Hz, 6′-H), 7.43 (s, 1H, 3′-*H*), 7.36 (dd, 1H, *J* = 7.7 and 7.4 Hz, 5′-H), 7.01 (s, 1H, 3-*H*), 2.46 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.34−2.18 (m, 2H, 8-C*H*$_2$CH$_3$), 2.06−1.92 (m, 2H, 8-C*H*$_2$CH$_3$), 1.04 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$C*H*$_3$), 0.69 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$C*H*$_3$ × 2). MS *m*/*z* 351 (M$^+$ − 1). MS (ESI$^+$) *m*/*z*: 379 (M$^+$ + 1). Anal. (C$_{23}$H$_{22}$O$_5$) C, H, O.

2-(Benzo[*b*]thiophen-2′-yl)-6,8,8-triethyldesmosdumotin B (17). ^{1}H NMR (300 MHz, CDCl$_3$): δ 12.96 (s, 1H, chelated-O*H*), 7.94−7.88 (m, 2H, 4′- and 7′-*H*), 7.91 (s, 1H, 3′-*H*), 7.52−7.46 (m, 2H, 5′- and 6′-*H*), 6.83 (s, 1H, 3-*H*), 2.46 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.34−2.21 (m, 2H, 8-C*H*$_2$CH$_3$), 2.10−1.96 (m, 2H, 8-C*H*$_2$CH$_3$), 1.05 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$C*H*$_3$), 0.70 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z*: 395 (M$^+$ + 1). Anal. (C$_{23}$H$_{22}$O$_4$S) C, H, O.

2-(3′-Methylbenzo[*b*]thiophen-2′-yl)-6,8,8-triethyldesmosdumotin B (18). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.00 (1H, chelated-O*H*), 7.91−7.82 (m, 2H, Ar-*H*), 7.55−7.45 (m, 2H, Ar-*H*), 6.82 (s, 1H, 3-*H*), 2.72 (s, 3H, 3′-C*H*$_3$), 2.46 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.32−2.20 (m, 2H, 8-C*H*$_2$CH$_3$), 2.07−1.95 (m, 2H, 8-C*H*$_2$CH$_3$), 1.05 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.70 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z* 409 (M$^+$ + 1). Anal. (C$_{24}$H$_{24}$O$_4$S) C, H, O.

2-(Benzofuran-3′-yl)-6,8,8-triethyldesmosdumotin B (19). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.03 (s, 1H, chelated-O*H*), 8.23 (s, 1H, 2′-H), 7.90−7.84 (m, 1H, 4′- or 7′-*H*), 7.68−7.63 (m, 1H, 4′- or 7′-*H*), 7.53−7.42 (m, 2H, 5′- and 6′-*H*), 6.89 (s, 1H, 3-*H*), 2.5−2.42 (m, 2H, 6-C*H*$_2$CH$_3$), 2.38−2.24 (m, 2H, 8-C*H*$_2$CH$_3$), 2.06−1.94 (m, 2H, 8-C*H*$_2$CH$_3$), 1.05 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.71 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z*: 379 (M$^+$ + 1). Elemental analysis results are not available because of limited quantity.

2-(1′-Methyl-1*H*-indol-3′-yl)-6,8,8-triethyldesmosdumotin B (20). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.61 (1H, chelated-O*H*), 8.00−7.93 (m, 1H, 4′-*H*), 7.70 (s, 1H, 2′-*H*), 7.48−7.34 (m, 3H, 5′-, 6′-, and 7′-*H*), 6.79 (s, 1H, 3-*H*), 3.94 (s, 3H, N-C*H*$_3$), 2.46 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.36−2.21 (m, 2H, 8-C*H*$_2$CH$_3$), 2.10−1.96 (m, 2H, 8-C*H*$_2$CH$_3$), 1.05 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$C*H*$_3$), 0.70 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z*: 392 (M$^+$ + 1). Anal. (C$_{24}$H$_{25}$O$_4$N) C, H, O.

2-(Benzo[*b*]thiophen-3′-yl)-6,8,8-triethyldesmosdumotin B (21). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.08 (s, 1H, chelated-O*H*), 8.12−8.06 (m, 1H, 4′-*H*), 8.07 (s, 1H, 2′-*H*), 8.02−7.96 (m, 1H, 7′-*H*), 7.61−7.48 (m, 2H, 5′- and 6′-*H*), 6.94 (s, 1H, 3-*H*), 2.47 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.36−2.08 (m, 2H, 8-C*H*$_2$CH$_3$), 2.07−1.93 (m, 2H, 8-C*H*$_2$CH$_3$), 1.06 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.71 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z*: 395 (M$^+$ + 1). Anal. (C$_{23}$H$_{22}$O$_4$S·^{1}/$_8$H$_2$O) C, H, O.

2-(2′-Methylbenzo[*b*]thiophen-3′-yl)-6,8,8-triethyldesmosdumotin B (22). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.08 (1H, chelated-O*H*), 7.86−7.82 (m, 1H, 4′-*H*), 7.72−7.68 (m, 1H, 7′-*H*), 7.48−7.38 (m, 2H, 5′- and 6′-*H*), 6.67 (s, 1H, 3-*H*), 2.97 (s, 3H, 2′-C*H*$_3$), 2.48 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.28−2.16 (m, 2H, 8-C*H*$_2$CH$_3$), 1.98−1.86 (m, 2H, 8-C*H*$_2$CH$_3$), 1.06 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.70 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z* 409 (M$^+$ + 1). HPLC.

2-(5′-Methylbenzo[*b*]thiophen-3′-yl)-6,8,8-triethyldesmosdumotin B (23). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.09 (1H, chelated-O*H*), 8.03 (s, 1H, 2′-*H*), 7.89 (br s, 1H, 4′-*H*), 7.84 (d, 1H, *J* = 8.4 Hz, 7′-*H*), 7.34 (dd, 1H, *J* = 0.98 and 8.4 Hz, 6′-*H*), 6.93 (s, 1H, 3-*H*), 2.54 (s, 3H, 5′-C*H*$_3$), 2.48 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.34−2.22 (m, 2H, 8-C*H*$_2$CH$_3$), 2.06−1.95 (m, 2H, 8-C*H*$_2$CH$_3$), 1.06 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$C*H*$_3$), 0.72 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z* 409 (M$^+$ + 1). Anal. (C$_{24}$H$_{24}$O$_4$S) C, H, O.

2-(5′-Bromobenzo[*b*]thiophen-3′-yl)-6,8,8-triethyldesmosdumotin B (24). ^{1}H NMR (400 MHz, CDCl$_3$): δ 12.97 (1H, chelated-O*H*), 8.25 (d, 1H, *J* = 1.9 Hz, 4′-*H*), 8.07 (s, 1H, 2′-*H*), 7.84 (d, 1H, *J* = 8.8 Hz, 7′-*H*), 7.61 (dd, 1H, *J* = 1.9 and 8.8 Hz, 6′-*H*), 6.88 (s, 1H, 3-*H*), 2.47 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.37−2.24 (m, 2H, 8-C*H*$_2$CH$_3$), 2.05−1.94 (m, 2H, 8-C*H*$_2$CH$_3$), 1.06 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.71 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z* 473 and 475 (M$^+$ + 1). Anal. (C$_{23}$H$_{21}$BrO$_4$S) C, H, O.

2-(5′-Methoxybenzo[*b*]thiophen-4′-yl)-6,8,8-triethyldesmosdumotin B (25). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.19 (1H, chelated-O*H*), 8.00 (d, 1H, *J* = 8.8 Hz, Ar-*H*), 7.63 (d, 1H, *J* = 5.6 Hz, Ar-*H*), 7.25 (d, 1H, *J* = 5.6 Hz, Ar-*H*), 7.16 (d, 1H, *J* = 8.8 Hz, Ar-*H*), 6.75 (s, 1H, 3-*H*), 3.92 (s, 3H, 5′-OC*H*$_3$), 2.47 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.20−2.10 (m, 2H, 8-C*H*$_2$CH$_3$), 1.94−1.85 (m, 2H, 8-C*H*$_2$CH$_3$), 1.06 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$C*H*$_3$), 0.69 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z* 425 (M$^+$ + 1). Anal. (C$_{24}$H$_{24}$O$_5$S) C, H, O.

2-(5′-Hydroxybenzo[*b*]thiophen-4′-yl)-6,8,8-triethyldesmosdumotin B (26). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.11 (1H, chelated-O*H*), 7.90 (d, 1H, *J* = 8.8 Hz, Ar-*H*), 7.82 (br s, 1H, 5′-O*H*), 7.63 (d, 1H, *J* = 5.6 Hz, Ar-*H*), 7.32 (d, 1H, *J* = 5.6 Hz, Ar-*H*), 7.14 (d, 1H, *J* = 8.8 Hz, Ar-*H*), 7.12 (s, 1H, 3-*H*), 2.49 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.25−2.14 (m, 2H, 8-C*H*$_2$CH$_3$), 2.02−1.91 (m, 2H, 8-C*H*$_2$CH$_3$), 1.07 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$C*H*$_3$), 0.70 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z* 411 (M$^+$ + 1). Anal. (C$_{23}$H$_{22}$O$_5$S) C, H, O.

2-(Benzo[*b*]thiophen-7′-yl)-6,8,8-triethyldesmosdumotin B (27). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.10 (1H, chelated-O*H*), 8.06 (dd, 1H, *J* = 7.8 and 0.8 Hz, 4′-*H*), 7.79 (d, 1H, *J* = 7.6 Hz 6′-*H*), 7.62 (d, 1H, *J* = 5.6 Hz, 2′-*H*), 7.57 (dd, 1H, *J* = 7.8 and 7.6 Hz, 5′-*H*), 7.50 (d, 1H, *J* = 5.6 Hz, 3′-*H*), 7.12 (s, 1H, 3-*H*), 2.48 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.34−2.18 (m, 4H, 8-C*H*$_2$CH$_3$ × 2), 1.06 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$C*H*$_3$), 0.70 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$C*H*$_3$ × 2). MS (ESI$^+$) *m*/*z* 395 (M$^+$ + 1). HPLC

2-(Benzo[*b*]thiophen-5′-yl)-6,8,8-triethyldesmosdumotin B (28). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.10 (1H, chelated-O*H*), 8.26 (d, 1H, *J* = 1.9 Hz, 4′-*H*), 8.05 (d, 1H, *J* = 8.5 Hz, 7′-*H*), 7.72 (dd,

1H, J = 1.9 and 8.5 Hz, 6′-*H*), 7.62 (d, 1H, J = 5.4 Hz, 2′-*H*), 7.49 (d, 1H, J = 5.4 Hz, 3′-*H*), 6.98 (s, 1H, 3-*H*), 2.46 (q, 2H, J = 7.3 Hz, 6-CH_2CH$_3$), 2.36−2.24 (m, 2H, 8-CH_2CH$_3$), 2.10−2.00 (m, 2H, 8-CH_2CH$_3$), 1.05 (t, 3H, J = 7.3 Hz, 6-CH$_2$CH_3), 0.70 (t, 6H, J = 7.3 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z 395 (M$^+$ + 1).

2-(1′,2′-Dihydroacenaphthylen-5′-yl)-6,8,8-triethyldesmosdumotin B (29). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.21 (1H, chelated-O*H*), 7.81 (d, 1H, J = 8.2 Hz, 8′-*H*), 7.72 (d, 1H, J = 7.5 Hz, 4′-*H*), 7.61 (dd, 1H, J = 8.2 and 7.5 Hz, 7′-H), 7.46 (d, 1H, J = 7.5 Hz, 3′ or 6′-*H*), 7.42 (d, 1H, J = 7.5 Hz, 3′- or 6′-*H*), 6.88 (s, 1H, 3-*H*), 3.49 (s, 4H, -CH_2- × 2), 2.48 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.30−2.15 (m, 2H, 8-CH_2CH$_3$), 2.04−1.90 (m, 2H, 8-CH_2CH$_3$), 1.06 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.72 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 415 (M$^+$ + 1). Anal. (C$_{27}$H$_{26}$O$_4$) C, H, O.

2-(9′-Ethyl-9′*H*-carbazol-2′-yl)-6,8,8-triethyldesmosdumotin B (30). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.31 (1H, chelated-O*H*), 8.47 (d, 1H, J = 1.8 Hz), 8.29 (d, 1H, J = 1.8 Hz), 7.91 (dd, 1H, J = 1.8 and 8.7 Hz), 7.65 (dd, 1H, J = 1.8 and 8.7 Hz), 7.55 (d, 1H, J = 8.7 Hz), 7.37 (d, 1H, J = 8.7 Hz), 6.97 (s, 1H, 3-*H*), 4.42 (q, 2H, J = 7.3 Hz, N-CH_2CH$_3$), 2.46 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.40−2.27 (m, 2H, 8-CH_2CH$_3$), 2.16−2.03 (m, 2H, 8-CH_2CH$_3$), 1.48 (t, 3H, J = 7.3 Hz, N-CH$_2$CH_3), 1.06 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.72 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 455 (M$^+$ + 1). Elemental analysis results are not available because of limited quantity.

2-(Dibenzo[*b*,*d*]furan-4′-yl)-6,8,8-triethyldesmosdumotin B (31). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.14 (s, 1H, chelated-O*H*), 8.18 (dd, 1H, J = 7.8 and 1.4 Hz, 9′-*H*), 8.03 (dd, 1H, J = 6.9 and 1.4 Hz, 1′-*H*), 7.94 (dd, 1H, J = 7.8 and 1.4 Hz, 6′-*H*), 7.73 (s, 1H, 3-*H*), 7.70 (d, 1H, J = 8.2 Hz, 3′-*H*), 7.62−7.42 (m, 3H, 2′-, 7′-, and 8′-*H*), 2.48 (q, 2H, J = 7.3 Hz, 6-CH_2CH$_3$), 2.38−2.24 (m, 2H, 8-CH_2CH$_3$), 2.18−2.04 (m, 2H, 8-CH_2CH$_3$), 1.07 (t, 3H, J = 7.3 Hz, 6-CH$_2$CH_3), 0.72 (t, 6H, J = 7.3 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 429 (M$^+$ + 1). Anal. (C$_{27}$H$_{24}$O$_5$·$^1/_2$H$_2$O) C, H, O.

2-(Anthracen-9-yl)- 6,8,8-triethyldesmosdumotin B (32). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.06 (1H, chelated-O*H*), 8.70 (s, 1H, 10′-*H*), 8.17−8.09 (m, 2H, 5′- and 8′-*H*, or 1′- and 3′-*H*), 7.83−7.75 (m, 2H, 5′- and 8′-*H*, or 1′- and 3′-*H*), 7.61−7.54 (m, 4H, 2′-, 3′-, 6′-, and 7′-*H*), 6.83 (s, 1H, 3-*H*), 2.51 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.18−2.04 (m, 2H, 8-CH_2CH$_3$), 1.84−1.70 (m, 2H, 8-CH_2CH$_3$), 0.88 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.74 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 439 (M$^+$ + 1). Anal. (C$_{29}$H$_{26}$O$_4$·$^1/_2$H$_2$O) C, H, O.

2-(Phenanthren-9′-yl)-6,8,8-triethyldesmosdumotin B (33). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.06 (1H, chelated-O*H*), 8.86−8.72 (m, 2H, 4′- and 5′-*H*), 8.04−7.94 (m, 2H, 1′- and 8′-*H*), 7.92−7.62 (m, 5H, 2′-, 3′-, 6′-, 7′-, and 10′-*H*), 6.89 (s, 1H, 3-*H*), 2.49 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.24−2.11 (m, 2H, 8-CH_2CH$_3$), 1.98−1.83 (m, 2H, 8-CH_2CH$_3$), 1.07 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.75 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 439 (M$^+$ + 1). Anal. (C$_{29}$H$_{26}$O$_4$·$^1/_2$H$_2$O) C, H, O.

2-(Biphen-4′-yl)-6,8,8-triethyldesmosdumotin B (34). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.08 (1H, chelated-O*H*), 7.88 (d, 2H, J = 7.8 Hz, 2′- and 6′-*H*), 7.78 (d, 2H, J = 7.8 Hz), 7.64 (d, 2H, J = 8.2 Hz), 7.55−7.43 (m, 3H, 8′-, 9′-, and 10′-*H*), 6.95 (s, 1H, 3-*H*), 2.46 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.36−2.21 (m, 2H, 8-CH_2CH$_3$), 2.10−1.96 (m, 2H, 8-CH_2CH$_3$), 1.05 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.69 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). Anal. (C$_{27}$H$_{26}$O$_4$) C, H, O.

2-(4′-Methylnaphthalen-1′-yl)-6,8,8-trimethyldesmosdumotin B (35). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.12 (s, 1H, chelated-O*H*), 8.16−8.12 (m, 1H, 5′-*H*), 8.00−7.96 (m, 1H, naphthyl-*H*), 7.68−7.57 (m, 3H, naphthyl-*H*), 7.45 (d, 1H, J = 7.3 Hz, naphthyl-*H*), 6.79 (s, 1H, 3-*H*), 2.80 (s, 3H, 4′-CH_3), 1.90 (s, 3H, 6-CH_3), 1.53 (s, 6H, 8-CH_3 × 2). Anal. (C$_{23}$H$_{20}$O$_4$) C, H, O.

2-(2′,3′-Dihydrobenzo[*d*][1′,4′]dioxin-6′-yl)-6,8,8-trimethyldesmosdumotin B (36). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.22 (s, 1H, chelated-O*H*), 7.34−7.30 (m, 2H, Ar-*H*), 7.03−7.00 (m, 1H, Ar-*H*), 6.77 (s, 1H, 3-*H*), 4.40−4.30 (m, 4H, -OCH_2CH_2O-), 1.86 (s, 3H, 6-CH_3), 1.56 (s, 6H, 8-CH_3 × 2). MS (ESI$^+$) m/z: 355 (M$^+$ + 1). Anal. (C$_{20}$H$_{18}$O$_6$·$^1/_2$H$_2$O) C, H, O.

2-(Benzo[*b*]thiophen-3′-yl)-6,8,8-trimethyldesmosdumotin B (37). ^{1}H NMR (400 MHz, CDCl$_3$): δ 13.08 (s, 1H, chelated-O*H*), 8.11 (d, 1H, J = 8.1 Hz, 4′- or 7′-*H*), 8.07 (s, 1H, 2′-*H*), 7.98 (d, 1H, J = 8.1 Hz, 4′- or 7′-*H*), 7.60−7.48 (m, 2H, 5′- and 6′-*H*), 6.92 (s, 1H, 3-*H*), 1.90 (s, 3H, 6-CH_3), 1.60 (s, 6H, 8-CH_3 × 2). MS (ESI$^+$) m/z: 351 (M$^+$ − 1). Anal. (C$_{20}$H$_{16}$O$_4$S·$^1/_4$H$_2$O) C, H, O.

6,8,8-Triethyl-7-methoxy-2-(naphthalen-1′-yl)-4*H*-chromene-4,5(8*H*)-dione (40a). **40a** was prepared according to the previously reported procedure.[1b] ^{1}H NMR (300 MHz, CCDCl$_3$): δ 8.04 (d, 1H, J = 8.2 Hz, Ar-*H*), 8.00−7.92 (m, 2H, Ar-*H*), 7.67−7.54 (m, 4H, Ar-*H*), 6.72 (s, 1H, 3-*H*), 4.00 (s, 3H, OCH_3), 2.60 (q, 2H, J = 7.3 Hz, 6-CH_2CH$_3$), 2.16−2.00 (m, 2H, 8-CH_2CH$_3$), 2.24−1.89 (m, 2H, 8-CH_2CH$_3$), 1.18 (t, 3H, J = 7.3 Hz, 6-CH$_2$CH_3), 0.76 (t, 6H, J = 7.3 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z: 403 (M$^+$ + 1). Anal. (C$_{26}$H$_{26}$O$_4$) C, H, O.

2-(Naphthalen-1-yl)-4-thioxo-6,8,8-triethyldesmosdumotin B (41). Lawesson's reagent (48 mg, 0.12 mmol) was added to a solution of **3** (40 mg, 0.10 mmol) in toluene (1.5 mL). After the mixture was refluxed for 6.5 h, the volatile solvent was removed in vacuo. The residue was purified by SiO$_2$ column chromatography (EtOAc/hexane, gradient) to obtain **41** (20 mg, 50%) and **42** (3 mg, 7%) along with starting material (8 mg, 20%). ^{1}H NMR (300 MHz, CDCl$_3$): δ 13.50 (1H, chelated-O*H*), 8.10 (d, 1H, J = 7.9 Hz, 5′-*H*), 8.02−7.95 (m, 2H, 3′- and 8′-*H*), 7.73 (dd, 1H, J = 7.2 and 1.1 Hz, 2′-*H*), 7.66−7.60 (m, 3H, 4′-, 6′-, and 7′-*H*), 7.62 (s, 1H, 3-*H*), 2.52 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.31−2.07 (m, 2H, 8-CH_2CH$_3$), 2.01−1.86 (m, 2H, 8-CH_2CH$_3$), 1.07 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.74 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z 405 (M$^+$ + 1). Anal. (C$_{25}$H$_{24}$O$_3$S) C, H, O.

4,7-Dithioxo-2-(naphthalen-1-yl)-6,8,8-triethyldesmosdumotin B (42). ^{1}H NMR (300 MHz, CDCl$_3$): δ 14.30 (1H, chelated-O*H*), 8.11 (d, 1H, J = 8.2 Hz, 5′-*H*), 8.03−7.94 (m, 2H, 3′- and 8′-*H*), 7.76 (dd, 1H, J = 7.4 and 1.3 Hz, 2′-*H*), 7.67−7.57 (m, 3H, 4′-, 6′-, and 7′-*H*), 7.62 (s, 1H, 3-*H*), 2.99 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.58−2.42 (m, 2H, 8-CH_2CH$_3$), 2.30−2.16 (m, 2H, 8-CH_2CH$_3$), 1.10 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.64 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z 421 (M$^+$ + 1). Anal. (C$_{25}$H$_{24}$O$_2$S$_2$·H$_2$O) C, H, O.

3-Bromo-6,8,8-triethyl-7-methoxy-2-(naphthalen-1′-yl)-4*H*-chromene-4,5(8*H*)-dione (43). PhI(OAc)$_2$ (280 mg, 0.87 mmol) was suspended in anhydrous CH$_2$Cl$_2$ (1.5 mL) under argon at room temperature. Bu$_4$NBr (281 mg, 0.87 mmol) was added, and the mixture was stirred for 30 min. **40a** (64 mg, 0.16 mmol) in anhydrous CH$_2$Cl$_2$ (1.0 mL) was added, and the mixture was stirred at room temperature for 4 days. The reaction was quenched with saturated aqueous NH$_4$Cl and extracted with CH$_2$Cl$_2$. The organic layer was dried over Na$_2$SO$_4$, and the solvent was evaporated in vacuo. The residue was purified by SiO$_2$ column chromatography (EtOAc/hexane, gradient) to obtain **43** (22 mg, 29%). ^{1}H NMR (300 MHz, CCDCl$_3$): δ 8.07 (dd, 1H, J = 2.1 and 7.4 Hz, 5′-*H*), 8.01−7.96 (m, 1H, Ar-*H*), 7.68−7.52 (m, 5H, Ar-*H*), 3.99 (s, 3H, OCH_3), 2.61 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.10−1.95 (m, 2H, 8-CH_2CH$_3$), 1.90−1.78 (m, 2H, 8-CH_2CH$_3$), 1.17 (t, 3H, J = 7.3 Hz, 6-CH$_2$CH_3), 0.73 (br s, 6H, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z 481 and 483 (M$^+$ + 1). Anal. (C$_{26}$H$_{25}$ BrO$_4$) C, H, O.

3-Bromo-2-(naphthalen-1′-yl)-6,8,8-triethyldesmosdumotin B (44). To a solution of **43** (18 mg, 0.038 mmol) in anhydrous CH$_2$Cl$_2$ (1.0 mL), BBr3 (0.1 mL, 0.1 mmol, 1.0 M solution in CH$_2$Cl$_2$) was added at −78 °C under N$_2$. The mixture was stirred overnight at −78 to 0 °C. The reaction mixture was quenched with water and extracted with CH$_2$Cl$_2$. The organic phase was dried over Na$_2$SO$_4$, and concentrated.

The residue was purified by SiO_2 column chromatography (EtOAc/hexane, gradient) to obtain **44** (13 mg, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.63 (1H, chelated-O*H*), 8.14−8.08 (m, 1H, 5′-*H*), 8.03−7.98 (m, 1H, 8′-*H*), 7.71−7.52 (m, 5H, 2′-, 3′-, 4′-, 6′-, and 7′-*H*), 2.49 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.20−2.07 (m, 2H, 8-CH_2CH$_3$), 1.87−1.72 (m, 2H, 8-CH_2CH$_3$), 1.07 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.74 (t, 6H, J = 7.4 Hz, 8-CH$_2$CH_3 × 2). MS (ESI$^+$) m/z 467 and 469 ($M^+ + 1$). Anal. ($C_{22}H_{23}BrO_4$) C, H, O.

2,3-Dihydro-2-(naphthalen-1′-yl)-6,8,8-triethyldesmosdumotin B (48). Compound **39** (R = Et, Ar = naphthyl,[1b] 58 mg, 0.14 mmol) was dissolved in HOAc (2.0 mL). Then 45% HI (1.6 mL) was added to the mixture, which was refluxed for 20 h. The reaction mixture was cooled to room temperature, and the solvent was removed in vacuo. Water was added to the residue. The whole mixture was neutralized to pH 7 with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by SiO_2 column chromatography (EtOAc/hexane, gradient) to obtain **48** (20 mg, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.64 (1H, chelated-O*H*), 8.01−7.92 (m, 3H, naphthyl-*H*), 7.64−7.52 (m, 4H, naphthyl-*H*), 6.37 (dd, 1H, J = 12.3 and 4.1 Hz, 2-*H*), 3.19 (dd, 1H, J = 12.3 and 17.3 Hz, 3-H_{ax}), 3.14 (dd, 1H, J = 4.1 and 17.3 Hz, 3-H_{eq}), 2.40 (q, 2H, J = 7.4 Hz, 6-CH_2CH$_3$), 2.12−1.98 (m, 2H, 8-CH_2CH$_3$), 1.90−1.66 (m, 2H, 8-CH_2CH$_3$), 1.00 (t, 3H, J = 7.4 Hz, 6-CH$_2$CH_3), 0.77 (t, 3H, J = 7.4 Hz, 8-CH$_2$CH_3), 0.50 (t, 3H, J = 7.4 Hz, 8-CH$_2$CH_3). MS (ESI$^+$) m/z 389 ($M^+ - 1$). Anal. ($C_{25}H_{26}O_4$) C, H, O

5-Methoxybenzothiophene-3-carboxaldehyde (55). To a solution of **53** (122 mg, 0.74 mmol) in anhydrous CH_2Cl_2 (1.5 mL), $SnCl_4$ (1.5 mL, 1.5 mmol, 1.0 M solution in CH_2Cl_2) was added dropwise at 0 °C under inert gas. Subsequently, dichloromethyl methyl ether (0.1 mL, 1.11 mmol) was added dropwise at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was quenched with saturated $NaHCO_3$ at 0 °C and stirred for 2 h at room temperature. After extraction with CH_2Cl_2, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on SiO_2 with EtOAc−hexane gradient to give **55** (107 mg, 0.56 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.71 (1H, s, C*H*O), 8.39 (d, 1H, J = 5.6 Hz, 3-*H*), 7.97 (d, 1H, J = 9.0 Hz, 7-*H*), 7.65 (d, 1H, J = 5.6 Hz, 2-*H*), 7.04 (d, 1H, J = 9.0 Hz, 6-*H*) 3.96 (s, 3H, OCH_3). MS (ESI$^+$) m/z 193 ($M^+ + 1$).

5-Methylbenzothiophene-3-carboxaldehyde (56). The title compound was prepared in 82% yield following the same procedure as **55** starting from **54** (119.5 g, 0.81 mmol), $SnCl_4$ (2.4 mL, 2.4 mmol, 1.0 M solution in CH_2Cl_2), and dichloromethyl methyl ether (0.11 mL, 1.21 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.13 (1H, s, C*H*O), 8.51−8.48 (m, 1H, 4-*H*), 8.32 (s, 1H, 2-*H*), 7.86 (d, 1H, J = 8.2 Hz, 7-*H*), 7.30 (dd, 1H, J = 8.2 and 1.6 Hz, 6-*H*) 2.5 (s, 3H, CH_3). MS (ESI$^+$) m/z 177 ($M^+ + 1$).

Antiproliferative Activity Assay. All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500−7500 cells per well, with compounds added from DMSO stock solutions and then successively diluted into medium. The highest concentration of DMSO in the cultures (0.5% v/v) was without effect on cell replication under the culture conditions used. After 3 days in culture, attached cells were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B. The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The mean GI_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions and is the average from at least three independent and similar determinations. All values presented in Table 2 are statistically significant, and standard deviations are shown in the Supporting Information. For the verapamil reversal experiments, cells were co-treated with verapamil (1 μg/mL). Control experiments showed this concentration had no effect on the replication of KB-VIN cells. The following human tumor cell lines were used in the assay: A549 (lung carcinoma), MCF-7 (breast cancer), HCT-8 (colon adenocarcinoma), PC-3 (prostate cancer), KB (nasopharyngeal carcinoma), and KB-VIN (vincristine-resistant KB subline). All cell lines were obtained from the Lineberger Cancer Center (UNC-CH) or from ATCC (Manassas, VA) except KB-VIN, which was a generous gift of Professor Y.-C. Cheng, Yale University, CT. Cells were cultured in RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin.

Tubulin Assays. Tubulin assembly was measured by turbidimetry at 350 nm as described previously.[15] Assay mixtures contained 1.0 mg/mL (10 μM) tubulin, and varying compound concentrations and were preincubated 15 min at 30 °C without guanosine 5′-triphosphate (GTP). The samples were placed on ice, and 0.4 mM GTP was added. Reaction mixtures were transferred to 0 °C cuvettes, and turbidity development was followed for 20 min at 30 °C following a rapid temperature jump. Compound concentrations that inhibited 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.[16] Incubation of 1.0 μM tubulin with 5.0 μM [^{3}H]colchicine and 5.0 μM inhibitor was for 10 min at 37 °C, when about 40−60% of maximum colchicine binding occurs in control samples.

Immunofluorescence Staining of Tubulin. A549 tumor cells were maintained in four-well chamber slides (Lab-Tech) for 12 h prior to treatment with DMSO, 0.1 nM compound **21**, 100 nM colchicine, 1.5 nM pacritaxel, or 500 nM doxorubicin for 24 h at 37 °C. Cells were fixed with 4% paraformaldehyde in phospate buffered saline (PBS), permeabilized with 0.5% Triton X-100 in PBS, and then tubulin was immunostained with monoclonal antibody to α-tubulin (B5-1-2, Sigma) followed by fluorescein 5-isothiocyanate (FITC) conjugated secondary antibody. Nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI).[17] Fluorescence labeled tubulin and nuclei were observed using a Zeiss Axioplan fluorescence microscope, and images were captured by a XL16 Excel cooled digital camera controlled by the Dage Exponent software (Dage-MTI). Final images were prepared using Adobe Photoshop.

Pharmacophore Analysis. Pharmacophore analysis was conducted using Molecular Operating Environment (MOE) software (version 2009.10, Chemical Computing Group, Inc.). First, low-energy 3D conformations were calculated for compounds **3**, **21**, and **26**, as well as two known antitubulin compounds, colchicine and combretastatin-A4 (CA-4). The pharmacophore was determined by aligning the 3D structures of colchicine and CA-4. This resulting pharmacophore was later used to search the best-fit 3D confirmations of compounds **3**, **21**, and **26**.

■ ASSOCIATED CONTENT

Supporting Information. Elemental analysis data for compounds **5**−**36** and HPLC analysis results for **46**−**53**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*For K.N.-G.: phone, 919-843-5209; fax, 919-966-3893; e-mail, goto@email.unc.edu. For K.F.B.: phone, 919-966-7633; fax, 919-966-3893; e-mail, Ken_Bastow@unc.edu. For K.-H.L.: phone, 919-962-0066; fax, 919-966-3893; e-mail, khlee@unc.edu.

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■ ABBREVIATIONS USED

CS, collateral sensitivity; CA-4, combretastatin A-4; VERAP, verapamil; CSI, colchicine site inhibitior; MOE, Molecular Operating Environment

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