

Antitumor Agents 259. Design, Syntheses, and Structure–Activity Relationship Study of Desmosdumotin C Analogs

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Received March 6, 2007

Desmosdumotin C (**1**) and its analogs previously showed potent, selective in vitro anticancer activity. To explore structure–activity relationships of **1** and further increase potency and selectivity, 15 novel analogs (**7–15** and **21–26**) were synthesized and evaluated for cytotoxicity against several human tumor cell lines, as well as inhibition of human endothelial (HUVEC) replication. 4-Bromo-3',3',5'-tripropyl analog **26** showed significant cytotoxicity against A549, A431, 1A9, and HCT-8 with ED₅₀ values of 1.0, 1.2, 0.9, and 1.3 $\mu\text{g/mL}$, respectively. Compound **26** also strongly inhibited the growth of matched tumor cells, KB-VIN and its parent cell KB. Furthermore, analogs **13** and **21** were over 5-fold more potent against KB-VIN than KB. Bromination of ring-B and tripropyl functionalization of ring-A enhanced activity, while alkylation of ring-B promoted KB-VIN/KB selectivity. 2-Furyl analog **16** showed selective activity against HUVEC, suggesting that it may have potential as a new prototype for angiogenesis inhibition.

Introduction

Natural products are a significant source of drug candidates. An impressive number of modern drugs have been developed from natural sources, especially from plants used as traditional folk medicines.¹ Thus, drug discovery from medicinal plants plays an important role in the treatment and prevention of various human diseases, and the continuous importance of natural products in modern medicine has been highlighted in several recent reviews and reports.^{2–6} Our research interest is the discovery and development of novel anticancer drugs from natural plants. Currently, about three-quarters of anticancer drugs come from either natural products or their derivatives.⁷ Cancer is a leading cause of death worldwide accounting for 13 percent of all deaths in 2005,⁸ even though many effective and diverse cancer treatments have been approved and are used. Major problems associated with cancer chemotherapy include undesirable toxic side effects and multidrug resistance. Therefore, a pressing need to develop more effective antitumor drugs still remains.

Desmos dumosus (Roxb.) is a climber plant found in alluvial forests in southern Asia and has been used in Chinese folk medicine as an antimalarial, insecticidal, antirheumatic, antispasmodic, and analgesic agent.⁹ Recently, some novel bioactive flavonoids named desmosdumotins B and C were isolated from the plant root.^{10,11} Desmosdumotin C (**1**; Figure 1) has a distinctive chalcone skeleton with an unusual nonaromatic A ring possessing a *gem*-dimethyl group on C-3' and a methyl group on C-5'. It showed significant and selective in vitro cytotoxicity against 1A9 (ovarian cancer) and A549 (human lung carcinoma) cell lines, with IC₅₀ values of 4.0 and 3.5 $\mu\text{g/mL}$, respectively.¹⁰ In addition, it was more active against drug-resistant KB-VIN^a cells than against the parent KB (epidermoid nasopharyngeal carcinoma) cell line. Thus, **1** is a promising new

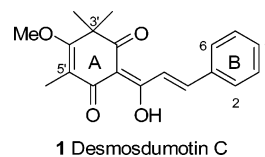


Figure 1. Structure of **1**.

lead for further new antitumor analog development. We previously achieved a simple first total synthesis of **1**¹¹ as well as some modifications of the A- and B-rings and reported the cytotoxic activity data against four tumor cell lines, 1A9, A549, KB, and KB-VIN.¹² Among the tested compounds, 4-bromodesmosdumotin C (**2**) showed 2- to 3-fold enhanced activity compared with **1**. This promising result encouraged us to continue the modification of this series to develop novel anticancer drug candidates. In this paper, we describe further modifications of the A- and B-rings as well as evaluation of newly and previously synthesized analogs against seven human tumor cell lines, A549, 1A9, KB, KB-VIN, A431 (epidermoid skin carcinoma), HCT-8 (colon adenocarcinoma), and PC-3 (prostate cancer), as well as HUVEC.

Chemistry

The simplicity of our accomplished synthesis¹¹ of **1** allows easy modification of the A-ring, by using another alkyl iodide rather than methyl iodide in the first step, and the B-ring, by using a different aromatic aldehyde from benzaldehyde in the final step (Scheme 1). First, nine B-ring modified analogs, **7–15**, were synthesized from intermediate **29** using 4-fluorophenylaldehyde, *o*- and *p*-tolualdehyde, 4-ethylbenzaldehyde, 2,6- and 3,5-dimethylbenzaldehyde, 2,4,6-trimethylbenzaldehyde, 2-vinylbenzaldehyde, and 1-naphthaldehyde under basic aldol conditions. Second, modification of the A-ring to introduce an ethyl or propyl group at the C-3 and C'-5' positions was accomplished by the following method. Treatment of trihydroxyacetophenone **28** with 3 mol equiv of ethyl or propyl iodide in the presence of sodium methoxide provided the corresponding trialkylacetophenones (**32** and **33**, respectively, Scheme 2). Similarly, treatment of **30**¹² with 1 mol equiv of ethyl iodide afforded **31**. Selective methylation of the resulting

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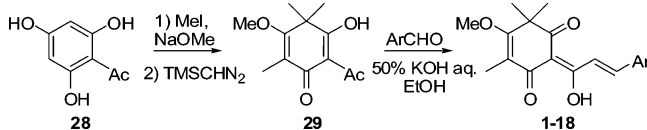
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^a Abbreviations: HUVEC, human umbilical vein endothelial cell; KB-VIN, vincristine-resistant KB, expressing P-glycoprotein, cell subline.

Scheme 1. Syntheses of 1-Analogs: B-Ring Modifications



Ar	R or X	Yield (%)	
	H	1	88 ^{ab}
	4-Br	2	61 ^{bc}
	4-OMe	3	82 ^c
	3-OMe	4	66 ^c
	2-OMe	5	89 ^c
	4-OH	6	35 ^{bc}
	4-F	7	60
	4-Me	8	98
	2-Me	9	100
	4-Et	10	88
	2,6-diMe	11	81
	3,5-diMe	12	82
	2,4,6-triMe	13	54
	2-Vinyl	14	75
	-	15	97
	X=O, Y=C	16	74 ^{bc}
	X=S, Y=C	17	61 ^{bc}
	X=S, Y=N	18	50 ^{bc}

^a See ref 11. ^b Based on recovered starting material. ^c See ref 12.

trialkylacetophenones, **31–33**, with an excess of TMSCHN₂ at low temperature, followed by aldol reaction with benzaldehyde, gave **21**, **22**, and **25**. The aldol reaction of **35** and **36** with 4-methyl, 4-ethyl, or 4-bromobenzaldehyde gave A- and B-ring analogs, **23**, **24**, and **26**, respectively. All synthesized compounds, except for **7**, **12**, and **15**, exist as a mixture of two tautomeric isomers as discussed in our prior papers.^{12,13} Compounds **1–6**, **16–19**, **20**, and **27** were synthesized previously.¹²

Results and Discussion

Together with **1** and previously synthesized analogs, all newly synthesized compounds were evaluated for in vitro anticancer activity against several human tumor cell lines, A549, A431, 1A9, HCT-8, PC-3, KB, and KB-VIN, and against HUVEC, a normal cell useful for assessing anti-angiogenic potential. The average ED₅₀ values (μg/mL) are listed in Table 1.

Compound **1** showed activity against A549, 1A9, HCT-8, and HUVEC with ED₅₀ values of 3.5, 3.5, 3.7, and 2.1 μg/mL, respectively, but was less active against A431 and PC-3 cells. Compound **1** was also 1.3-fold more active against KB-VIN cells (ED₅₀ 3.0 μg/mL) than the parent KB cell line. Except for **14**, **25**, and **26**, most **1**-analogs showed similar activity patterns, as described later.

Significantly enhanced cytotoxic activities were observed with **2**, 2-vinyl desmosdumotin C (**14**), 3',3',5'-tripropyl desmosdumotin C (**25**), and 4-bromo-3',3',5'-tripropyl desmosdumotin C (**26**). Especially, **26** displayed enhanced in vitro antitumor activity against all cell lines with ED₅₀ values of 0.9–2.3 μg/mL. In addition, **26** was not affected by multidrug resistant expressing P-glycoprotein, because it showed similar and potent cell growth inhibition of KB and KB-VIN cell replication (ED₅₀ 0.9 μg/mL). The strong activity of **26** could be predicted because it combines the structural features of 4-bromo **2** and 3',3',5'-

Table 1. Cytotoxic Activity Data for 1-Analogs

cmpd	ED ₅₀ ^a (μg/mL)							
	A549	A431	1A9	HCT-8	PC-3	KB	KB-VIN	HUVEC
1	3.5	5.1	3.5	3.7	11.1	4.0	3.0	2.1
2	1.4	9.0	1.1	7.4	17.7	1.7	1.9	3.6
3	3.0	9.2	2.8	5.6	18.2	3.6	2.9	2.5
4	2.8	4.5	2.9	2.9	14.3	3.3	2.4	1.6
5	2.4	6.6	2.5	4.1	15.6	3.3	2.8	1.7
6	3.1	14.9	2.9	14.8	NA ^b	3.8	3.8	4.0
7	3.3	4.1	NT ^c	3.6	7.2	3.7	2.7	2.0
8	4.3	5.1	NT	4.1	8.2	3.9	1.9	2.4
9	4.6	5.2	NT	4.0	9.0	4.1	1.6	2.3
10	6.1	12.0	5.3	10.5	15.1	6.9	1.9	NT
11	8.3	10.1	NT	7.8	17.1	7.1	2.1	3.0
12	4.3	4.0	NT	2.9	8.8	3.1	1.6	2.5
13	8.0	20.0	6.2	8.4	NA	NA	1.84	NT
14	2.2	2.6	NT	2.1	4.6	1.9	1.1	1.1
15	3.6	4.1	NT	2.3	9.0	2.8	1.3	1.8
16	10.0	14.7	7.9	17.5	20.6	9.9	8.8	2.4
17	4.5	10.8	4.0	8.6	18.2	5.5	3.4	2.5
18	3.5	6.0	3.5	5.1	10.8	4.3	3.0	1.6
19	>5	10.5	4.6	5.0	11.1	7.3	3.8	2.0
20	>5 (48)	7.0	3.6	3.3	14.2	4.1	2.9	NA
21	5.5	13.8	3.8	9.4	18.9	4.3	0.3	NT
22	3.4	5.4	2.1	2.8	7.7	2.8	1.2	NT
23	4.6	10.3	4.3	9.4	15.0	3.0	1.0	NT
24	3.8	9.8	3.8	8.5	15.0	3.6	2.6	NT
25	1.4	1.9	1.5	1.4	3.3	0.6	0.8	NT
26	1.0	1.2	0.9	1.3	2.3	0.9	0.9	NT
27	9.0	14.0	8.0	15.8	17.8	10.5	6.5	2.1

^a See Experimental "Cytotoxic Activity Assay" for cell line descriptions.

^b Not active: >20 μg/mL. ^c Not tested.

tripropyl **25**, which also were quite potent against A549, 1A9, KB, and KB-VIN cell lines. Notably, **25** strongly inhibited the growth of KB and KB-VIN cells with ED₅₀ values of 0.6 and 0.8 μg/mL, respectively.

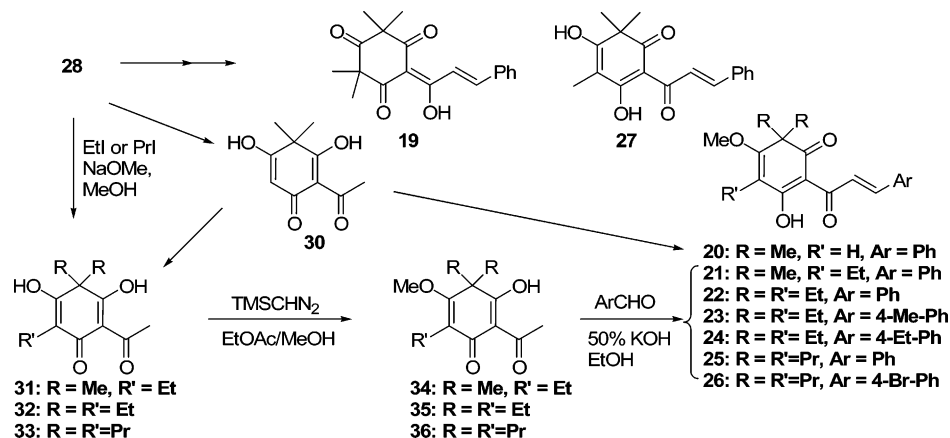
Interestingly, while most analogs, including **2**, were generally less active against PC-3 replication, three compounds, **14**, **25**, and **26**, displayed enhanced activity against PC-3 with ED₅₀ values of 4.6, 3.3, and 2.3 μg/mL, respectively. These three analogs also inhibited the growth of A431 cells with ED₅₀ values of 2.6, 1.9, and 1.2 μg/mL, respectively. Furthermore, in the PC-3, A431, and HCT-8 cell lines, bromination at C-4 (**2**) decreased cytotoxic activity relative to **1**, while fluorination (**7**) increased activity.

From a comparison of the data for **1** with that of **3–5** and **8–9**, the insertion of a methoxy or methyl group on the phenyl B-ring had either no effect on or slightly decreased activity, regardless of the substitution position. The 4-ethyl (**10**), 2,6-dimethyl (**11**), and trimethyl (**13**) derivatives also showed decreased activity against all cell lines, although **1** and the 3,5-dimethyl derivative (**12**) had comparable potencies. However, it is noteworthy that the introduction of an alkyl group on the B-ring enhanced the growth inhibition of the KB-VIN cell line resulting in a 2- to 5-fold ratio of KB/KB-VIN selectivity (see **8–14**). However, the insertion of a hetero aromatic ring rather than the phenyl ring did not affect the cytotoxic activity (see **16–18**).

For the A-ring analogs, tetramethyl analog **19**, dimethyl analog **20** (ceroptene),¹⁴ 3',3'-dimethyl-5'-ethyl analog **21**, and 4'-demethyl analog **27** were less potent against all cell lines than the parent compound **1**. However, **21** showed a remarkably high KB/KB-VIN selectivity ratio of 14.3. Among the triethyl compounds, **23** and **24** were less active than **1**, while **22**, without a substituent on the B-ring, showed slightly enhanced activity against most cell lines, except for A549 and A431.

Angiogenesis is necessary for tumor growth and metastasis; therefore, it presents another target for cancer treatment. Selected

Scheme 2. Syntheses of 1-Analogs: A/B-ring Modifications



compounds were also evaluated in a standard anti-angiogenesis assay using HUVEC. All tested compounds, except **20**, showed potent activity with ED₅₀ values less than 4.0 μg/mL. Compound **14** showed the highest potency with an ED₅₀ value of 1.1 μg/mL. However, this compound also showed strong activity against other tumor cell lines, suggesting that the activity against HUVEC may be nonspecific. However, analogs **16–18**, in which the phenyl B-ring was replaced with five-membered heteroaromatic rings, demonstrated significant activity against HUVEC with less activity against tumor cell replication. In particular, 2-furyl analog **16** possessed the highest differential, with an ED₅₀ value of 2.4 μg/mL against HUVEC compared to ED₅₀ values of 7.9–20.6 μg/mL against the other tumor cell lines. This selective activity suggests that **16** may have potential as a new prototype as an angiogenesis inhibitor.

In summary, the preliminary SAR studies led to the following observations: (1) a bromide function on the B-ring enhanced activity (**1** vs **2** and **25** vs **26**); (2) the order of potency for C-3' and -5' functionalities was 3',3',5'-tripropyl > 3',3',5'-trimethyl > 3',3',5'-triethyl > 3',3'-dimethyl-5'-ethyl > 3',3'-dimethyl > 3',3',5',5'-tetramethyl; (3) an alkyl group on the B-ring promoted KB-VIN/KB selectivity; (4) a C-4' methoxy group is required for activity; (5) five-membered hetero B-rings increased the differential angiogenesis activity. From all results, **26** showed significant promise as a new antitumor drug candidate because of its potent cytotoxicity against tumor cell lines and lack of multidrug cross resistance. Focused studies on the KB-VIN/KB selectivity of desmosdumotin series are currently ongoing, and the results will be reported elsewhere in the near future.

Experimental Section

Materials and Methods. Melting points were measured with a Fisher Johns melting apparatus without correction. Proton nuclear magnetic resonance (¹H NMR) spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. All chemical shifts are reported in ppm. Mass spectra were measured on a PE-SCIEX API 3000 instrument with turbo ion spray source or Agilent-1100, LC/MSD-Trap. Thin-layer chromatography (TLC) and preparative TLC were performed on precoated silica gel GF plates purchased from Merck, Inc. Biotage Flash or Isco Companion systems were used for medium-pressure column chromatography. Silica gel (200–400 mesh) from Aldrich, Inc., was used for column chromatography. All other chemicals were obtained from Aldrich, Inc.

General Procedures for the Aldol Reactions. A solution of acetyl compound (**29** or **34–36**) in EtOH–50% aq KOH (1:1, v/v) and an appropriate 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 CH₂Cl₂.

The extract was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was chromatographed on silica gel with CH₂Cl₂–hexane as eluent to afford the target compound, which was crystallized from CH₂Cl₂–hexane.

4-Fluoro Desmosdumotin C (7). Yellow prisms, mp 109–110 °C (CH₂Cl₂–hexane). ¹H NMR (CDCl₃): δ 19.18 (s, 1H, chelated-OH), 8.27 (d, 1H, *J* = 15.6 Hz, *trans*-olefinic proton), 7.89 (d, 1H, *J* = 15.6 Hz, *trans*-olefinic proton), 7.71–7.60 (m, 2H, Ar-2,6-*H*), 7.12–7.00 (m, 2H, Ar-3,5-*H*), 3.96 (s, 3H, OCH₃), 2.00 (s, 3H, 5'-CH₃), 1.38 (s, 6H, 3'-CH₃ × 2). MS *m/z* 331 (M⁺ + 1). Anal. (C₁₉H₁₉FO₄) C, H, O.

4-Methyl Desmosdumotin C (8). Yellow prisms, mp 110–111 °C (CH₂Cl₂–hexane). ¹H NMR (300 MHz, CDCl₃): δ 19.32 and 18.92 (3:1, each s, 1H, chelated-OH), 8.52 and 8.31 (1:3, each d, 1H, *J* = 15.7 Hz, olefin), 7.95 and 7.93 (1:3, each d, 1H, *J* = 15.7 Hz, olefin), 7.62–7.54 (m, 2H, Ar-2', 6'-*H*), 7.24–7.17 (m, 2H, Ar-3, 5-*H*), 3.96 and 3.89 (3:1, each s, 3H, OCH₃), 2.00 and 1.96 (3:1, each s, 3H, 5'-CH₃), 1.47 and 1.38 (1:3, each s, 6H, 3'-CH₃ × 2). MS *m/z* 327 (M⁺ + 1). Anal. (C₂₀H₂₂O₄) C, H, O.

2-Methyl Desmosdumotin C (9). Yellow prisms, mp 93–94 °C (CH₂Cl₂–hexane). ¹H NMR (300 MHz, CDCl₃): δ 19.32 and 18.62 (4:1, each s, 1H, chelated-OH), 8.34 (s, 2H), 8.05–7.94 (m, 1H), 7.51–7.25 (m, 3H, Ar-*H*), 4.10 and 4.03 (4:1, each s, 3H, OCH₃), 2.63 (s, 3H, Ar-CH₃), 2.15 and 2.01 (4:1, each s, 3H, 5'-CH₃), 1.52 (s, 6H, 3'-CH₃ × 2). MS *m/z* 327 (M⁺ + 1). Anal. (C₂₀H₂₂O₄) C, H, O.

4-Ethyl Desmosdumotin C (10). Yellow prisms, mp 90–91 °C (CH₂Cl₂–hexane). ¹H NMR (300 MHz, CDCl₃): δ 19.19 and 18.80 (2:1, each s, 1H, chelated-OH), 8.52 and 8.32 (1:2, each d, 1H, *J* = 15.9 Hz, olefin), 7.96 and 7.94 (1:2, each d, 1H, *J* = 15.9 Hz, olefin), 7.62 and 7.60 (1:2, each d, 2H, *J* = 7.9 Hz, Ar-2, 6-*H*), 7.23 and 7.22 (1:2, each d, 2H, *J* = 7.9 Hz, Ar-3, 5-*H*), 3.95 and 3.89 (2:1, each s, 3H, OCH₃), 2.68 (q, 2H, *J* = 7.7 Hz, Ar-CH₂-CH₃), 2.00 and 1.96 (2:1, each s, 3H, 5'-CH₃), 1.47 and 1.38 (1:2, each s, 6H, 3'-CH₃ × 2), 1.25 (t, 3H, *J* = 7.7 Hz, Ar-CH₂-CH₃). MS *m/z* 339 (M⁺ - 1). Anal. (C₂₁H₂₄O) C, H, O.

2,6-Dimethyl Desmosdumotin C (11). Yellow oil. ¹H NMR (300 MHz, CDCl₃): δ 19.14 and 18.80 (2:1, each s, 1H, chelated-OH), 8.17 (d, 1H, *J* = 15.7 Hz, olefin), 7.87 (d, 1H, *J* = 15.7 Hz, olefin), 7.20–7.01 (m, 3H, Ar-3,4,5-*H*), 3.95 and 3.88 (2:1, each s, 3H, OCH₃), 2.44 (s, 6H, Ar-2,6-CH₃), 2.00 and 1.92 (2:1, each s, 3H, 5'-CH₃), 1.48 and 1.35 (1:2, each s, 6H, 3'-CH₃ × 2). MS *m/z* 341 (M⁺ + 1). Anal. (C₂₁H₂₄O₄) C, H, O.

3,5-Dimethyl Desmosdumotin C (12). Yellow prisms, mp 105–106 °C (CH₂Cl₂–hexane). ¹H NMR (300 MHz, CDCl₃): δ 19.20 (s, 1H, chelated-OH), 8.31 (d, 1H, *J* = 15.7 Hz, olefin), 7.91 (d, 1H, *J* = 15.7 Hz, olefin), 7.30 (s, 2H, Ar-2', 6'-*H*), 7.03 (s, 1H, Ar-4-*H*), 3.96 (s, 3H, OCH₃), 2.34 (s, 6H, Ar-3,5-CH₃), 2.00 (s, 3H, 5'-CH₃), 1.39 (s, 6H, 3'-CH₃ × 2). MS *m/z* 341 (M⁺ + 1). Anal. (C₂₁H₂₄O₄) C, H, O.

2,4,6-Trimethyl Desmosdumotin C (13). Yellow prisms, mp 87–88 °C (AcOEt–hexane). ¹H NMR (300 MHz, CDCl₃): δ 19.17

and 18.48 (3:1, each s, 1H, chelated-OH), 8.21–8.09 (m, 1H, olefin), 7.96 and 7.90 (1:3, each d, 1H, $J = 15.5$ Hz, olefin), 6.92 and 6.90 (1:3, each s, 2H, Ar-3, 5-H), 3.95 and 3.88 (3:1, each s, 3H, OCH₃), 2.43 and 2.41 (3:1, each s, 6H, Ar-2,6-CH₃), 2.30 and 2.29 (1:3, each s, 3H, Ar-4-CH₃), 2.00 and 1.92 (3:1, each s, 3H, 5'-CH₃), 1.47 and 1.35 (1:3, each s, 6H, 3'-CH₃ × 2). MS m/z 355 ($M^+ + 1$). Anal. (C₂₂H₂₆O₄·1/8H₂O) C, H.

3-Vinyl Desmosdumotin C (14). Yellow prisms, mp 76–77 °C (CH₂Cl₂–hexane). ¹H NMR (300 MHz, CDCl₃): δ 19.19 and 18.79 (3:1, each s, 1H, chelated-OH), 8.54 and 8.34 (1:3, each d, 1H, $J = 15.9$ Hz, olefin), 7.96 and 7.93 (1:3, each d, 1H, $J = 15.9$ Hz, olefin), 7.67 and 7.65 (1:3, each br s, 1H, Ar-2-H), 7.60 and 7.58 (1:3, each d, 1H, $J = 7.4$ Hz, Ar-4 or 6-H), 7.47 and 7.45 (1:3, each d, 1H, $J = 7.4$ Hz, Ar-4 or 6-H), 7.37 and 7.36 (1:3, each t, 1H, Ar-5-H), 6.74 (dd, 1H, $J = 10.8$ and 17.4 Hz, Ar-CH=CH₂), 5.81 (d, 1H, $J = 17.4$ Hz, Ar-CH=CH₂), 5.31 (d, 1H, $J = 10.8$ Hz, Ar-CH=CH₂), 3.96 and 3.89 (3:1, each s, 3H, OCH₃), 2.00 and 1.96 (3:1, each s, 3H, 5'-CH₃), 1.48 and 1.39 (1:3, each s, 6H, 3'-CH₃ × 2). MS m/z 359 ($M^+ + 1$). Anal. (C₂₁H₂₂O₄) C, H, O.

2-[1-Hydroxy-3-(naphthalen-1-yl)allylidene]-5-methoxy-4,6,6-trimethylcyclohex-4-ene-1,3-dione (15). Yellow prisms, mp 128–129 °C (CH₂Cl₂–hexane). ¹H NMR (300 MHz, CDCl₃): δ 19.2 (s, 1H, chelated-OH), 8.83 (d, 1H, $J = 15.4$ Hz, olefin), 8.42 (d, 1H, $J = 15.4$ Hz, olefin), 8.28 (d, 1H, $J = 7.5$ Hz, naphthyl-2 or 8-H), 8.04 (d, 1H, $J = 7.5$ Hz, naphthyl-2 or 8-H), 7.96–7.84 (m, 2H, naphthyl-H), 7.64–7.46 (m, 3H, naphthyl-H), 7.37 and 7.36 (1:3, each t, 1H, Ar-5-H), 6.74 (dd, 1H, $J = 10.8$ and 17.4 Hz, Ar-CH=CH₂), 5.81 (d, 1H, $J = 17.4$ Hz, Ar-CH=CH₂), 5.31 (d, 1H, $J = 10.8$ Hz, Ar-CH=CH₂), 3.96 and 3.89 (3:1, each s, 3H, OCH₃), 2.00 and 1.96 (3:1, each s, 3H, 5'-CH₃), 1.48 and 1.39 (1:3, each s, 6H, 3'-CH₃ × 2). MS m/z 363 ($M^+ + 1$). Anal. (C₂₃H₂₂O₄) C, H, O.

3',3'-Dimethyl-5'-ethyl Desmosdumotin C (21). Yield 75%; yellow oil. ¹H NMR (300 MHz, CDCl₃): δ 19.37 and 18.90 (2:1, each s, 1H, chelated-OH), 8.67 and 8.47 (1:2, each d, 1H, $J = 15.9$ Hz, olefin), 8.10 and 8.07 (1:3, each d, 1H, $J = 15.9$ Hz, olefin), 7.89–7.76 (m, 2H, Ar-2,6-H), 7.59–7.48 (m, 3H, Ar-3,4,5-H), 4.12 and 4.06 (2:1, each s, 3H, OCH₃), 2.65 and 2.62 (2:1, each q, $J = 7.4$ Hz, 2H, 5'-CH₂CH₃), 1.62 and 1.53 (1:2, each s, 6H, 3'-CH₃ × 2), 1.32 and 1.29 (2:1, each t, $J = 7.4$ Hz, 3H, 5'-CH₂CH₃). MS m/z 325 ($M^+ - 1$). Anal. (C₂₀H₂₂O₄) C, H, O.

3',3',5'-Triethyl Desmosdumotin C (22). Yield 57%; yellow oil. ¹H NMR (300 MHz, CDCl₃): δ 19.30 and 18.89 (3:2, each s, 1H, chelated-OH), 8.52 and 8.44 (2:3, each d, 1H, $J = 15.3$ Hz, olefin), 7.96 and 7.93 (2:3, each d, 1H, $J = 15.3$ Hz, olefin), 7.73–7.66 (m, 2H, Ar-2,6-H), 7.43–7.36 (m, 3H, Ar-3,4,5-H), 4.03 and 3.96 (3:2, each s, 3H, OCH₃), 2.60 and 2.56 (3:2, each q, 2H, $J = 7.4$ Hz, 5'-CH₂CH₃), 2.04–1.76 (m, 4H, 3'-CH₂CH₃ × 2), 1.20 and 1.16 (3:2, each t, 3H, $J = 7.4$ Hz, 5'-CH₂CH₃), 0.71 and 0.70 (2:3, each t, 3H, $J = 7.4$ Hz, 3'-CH₂CH₃ × 2). MS m/z 355 ($M^+ + 1$). Anal. (C₂₂H₂₆O₄) C, H, O.

4-Methyl-3',3',5'-triethyl Desmosdumotin C (23). Yield 63%; yellow oil. ¹H NMR (300 MHz, CDCl₃): δ 19.30 and 18.91 (3:2, each s, 1H, chelated-OH), 8.50 and 8.41 (2:3, each d, 1H, $J = 15.3$ Hz, olefin), 7.96 and 7.93 (2:3, each d, 1H, $J = 15.3$ Hz, olefin), 7.64–7.55 (m, 2H, Ar-2,6-H), 7.25–7.14 (m, 2H, Ar-3,5-H), 4.03 and 3.95 (3:2, each s, 3H, OCH₃), 2.60 and 2.56 (3:2, each q, 2H, $J = 7.4$ Hz, 5'-CH₂CH₃), 2.38 and 2.35 (3:2, each s, 3H, Ar-4-CH₃), 2.07–1.90 and 1.90–1.72 (3:2, each m, 4H, 3'-CH₂CH₃ × 2), 1.30–1.21 (m, 3H, 5'-CH₂CH₃), 0.70 (t, 6H, $J = 7.4$ Hz, 3'-CH₂CH₃ × 2). MS m/z 367 ($M^+ - 1$). Anal. (C₂₂H₂₄O₄) C, H.

4-Methyl-3',3',5'-triethyl Desmosdumotin C (24). Yield 61%; yellow oil. ¹H NMR (300 MHz, CDCl₃): δ 19.30 and 18.93 (2:1, each s, 1H, chelated-OH), 8.50 and 8.42 (1:2, each d, 1H, $J = 15.3$ Hz, olefin), 7.97 and 7.93 (1:2, each d, 1H, $J = 15.3$ Hz, olefin), 7.68–7.56 (m, 2H, Ar-2,6-H), 7.30–7.18 (m, 2H, Ar-3,5-H), 4.03 and 3.95 (2:1, each s, 3H, OCH₃), 2.55–2.50 (m, 4H, CH₂CH₃ × 2), 2.08–1.72 (m, 4H, CH₂CH₃ × 2), 1.33–1.10 (m, 6H, CH₂CH₃ × 2), 0.80–0.64 (m, 6H, CH₂CH₃ × 2). MS m/z 381 ($M^+ - 1$). Anal. (C₂₄H₃₀O₄) C, H, O.

3',3',5'-Tripropyl Desmosdumotin C (25). Yield 76%; yellow oil. ¹H NMR (300 MHz, CDCl₃): δ 19.29 and 18.90 (2:1, each s, 1H, chelated-OH), 8.49 and 8.42 (1:2, each d, 1H, $J = 15.6$ Hz, olefin), 7.95 and 7.92 (1:2, each d, 1H, $J = 15.6$ Hz, olefin), 7.74–7.65 (m, 2H, Ar-2,6-H), 7.44–7.34 (m, 3H, Ar-3,4,5-H), 4.00 and 3.92 (2:1, each s, 3H, OCH₃), 2.56–2.42 (m, 2H, 5'-CH₂Et), 2.00–1.65 (m, 4H, 3'-CH₂Et × 2), 1.65–1.48 (m, 2H, 3'-CH₂CH₂CH₃), 1.16–0.96 (m, 7H, 5'-CH₂CH₂CH₃ × 2 and 3'-CH₂CH₂CH₃), 0.90–0.77 (m, 6H, 3'-CH₂CH₂CH₃ × 2). MS m/z 395 ($M^+ - 1$). Anal. (C₂₅H₃₂O₄) C, H, O.

4-Bromo-3',3',5'-tripropyl Desmosdumotin C (26). Yield 38%; yellow prisms, mp 114–115 °C. ¹H NMR (300 MHz, CDCl₃): δ 19.28 and 18.81 (2:1, each s, 1H, chelated-OH), 8.48 and 8.40 (1:2, each d, 1H, $J = 15.6$ Hz, olefin), 7.86 and 7.84 (1:2, each d, 1H, $J = 15.6$ Hz, olefin), 7.60–7.47 (m, 5H, Ar-H), 4.00 and 3.92 (2:1, each s, 3H, OCH₃), 2.58–2.41 (m, 2H, 5'-CH₂Et), 2.00–1.80 (m, 4H, 3'-CH₂Et × 2), 1.80–1.46 (m, 2H, 3'-CH₂CH₂CH₃), 1.18–0.93 (m, 7H, 5'-CH₂CH₂CH₃ × 2 and 3'-CH₂CH₂CH₃), 0.93–0.77 (m, 6H, 3'-CH₂CH₂CH₃ × 2). MS m/z 475 and 477 ($M^+ - 1$). Anal. (C₂₅H₃₁O₄Br) C, H, O.

Synthesis of Intermediates 31–36. 2-Acetyl-6-ethyl-3,5-dihydroxy-4,4-dimethylcyclohexa-2,5-dienone (31). A solution of 2-acetyl-3,5-dihydroxy-4,4-dimethylcyclohexa-2,5-dienone (**30**, 713 mg, 3.6 mmol) and sodium methoxide (2.0 mL, 9.3 mmol, 25% MeOH solution) in anhydrous MeOH (3 mL) containing ethyl iodide (0.3 mL, 3.8 mmol) was refluxed for 4 h. After removal of the volatile solvent, the residue was partitioned between EtOAc and 1 N aqueous HCl. The water phase was extracted with EtOAc (×3). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc–hexane (1:9 to 1:4, v/v) as an eluent to provide **31** (196 mg, 24%) along with recovered starting material. Colorless prisms, mp 153–154 °C (EtOAc–hexane). ¹H NMR (300 MHz, DMSO-*d*₆): δ 19.00 (br s, 1H, chelated-OH), 2.48 [s, 3H, C(O)-CH₃], 2.35 (q, 2H, $J = 7.2$ Hz, CH₂CH₃), 1.29 (s, 6H, 4-CH₃ × 2), 0.95 (t, 3H, 6-CH₂CH₃). MS m/z 223 ($M^+ - 1$). Anal. (C₁₂H₁₆O₄·1/8H₂O) C, H, O.

2-Acetyl-4,4,6-triethyl-3,5-dihydroxycyclohexa-2,5-dienone (32). A solution of 2,4,6-trihydroxyacetophenone (1.117 g, 6.7 mmol) and sodium methoxide (4.8 mL, 22.2 mmol, 25% MeOH solution) in anhydrous MeOH (5 mL) containing ethyl iodide (1.65 mL, 20.6 mmol) was refluxed for 7 h. The reaction mixture was cooled to 0 °C and acidified with 1 N aqueous HCl and then extracted with EtOAc (×3). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc–hexane (1:9 to 1:4, v/v) as an eluent to provide **32** (723 mg, 43%). Colorless prisms, mp 149–150 °C (EtOAc–hexane). ¹H NMR (300 MHz, DMSO-*d*₆): δ 18.98 (br s, 1H, chelated-OH), 2.51 [s, 3H, C(O)CH₃], 2.42 (q, 2H, $J = 7.2$ Hz, 6-CH₂CH₃), 1.81 (q, 4H, $J = 7.4$ Hz, 4-CH₂CH₃ × 2), 0.97 (t, 3H, $J = 7.2$ Hz, 6-CH₃CH₃), 0.57 (t, 3H, $J = 7.2$ Hz, 4-CH₂CH₃ × 2). MS m/z 251 ($M^+ + 1$). Anal. (C₂₁H₂₂O₄·1/16H₂O) C, H, O.

2-Acetyl-3,5-dihydroxy-4,4,6-tripropylcyclohexa-2,5-dienone (33). The procedure was identical to that described above except with propyl iodide instead of ethyl iodide to provide **33** (41%). Colorless prisms, mp 95–96 °C (EtOAc–hexane). ¹H NMR (300 MHz, CD₃OD): δ 2.53 [s, 3H, C(O)CH₃], 2.42 (t, 2H, $J = 7.5$ Hz, 6-CH₂Et), 1.94–1.74 (m, 4H, 4-CH₂Et × 2), 1.54–1.38 (m, 2H, 6-CH₃CH₂CH₃), 1.16–0.90 (m, 4H, 4-CH₃CH₂CH₃ × 2), 0.94 (t, 3H, $J = 7.4$ Hz, 6-CH₃CH₂CH₃), 0.81 (t, 6H, $J = 7.1$ Hz, 6-CH₃CH₂CH₃ × 2). MS m/z 293 ($M^+ - 1$). Anal. (C₁₇H₂₆O₄) C, H, O.

2-Acetyl-6-ethyl-3-hydroxy-5-methoxy-4,4-dimethylcyclohexa-2,5-dienone (34). To a solution of **31** (124 mg, 0.55 mmol) in anhydrous EtOAc–MeOH (5:1, 1.8 mL), a solution of TMSCHN₂ (2 mL, 4.0 mmol, 2 M solution in diethyl ether) was added slowly at –78 °C under an argon atmosphere, and the mixture was stirred for 3 h. Acetic acid was then added to destroy the excess TMSCHN₂. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography with EtOAc–hexane (1:9 to 1:4 v/v) as an eluent to obtain **34** (117 mg, 89%);

yellow oil. $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 18.97 and 18.14 (2:1, each s, 1H, chelated-OH), 3.97 and 3.90 (2:1, each s, 3H, OCH_3), 2.71 and 2.61 [1:2, each s, 3H, $\text{C}(\text{O})\text{CH}_3$], 2.48 and 2.43 (2:1, each q, 2H, $J = 7.4$ Hz, $6\text{-CH}_2\text{CH}_3$), 1.45 and 1.34 (1:2, each s, 6H, 4-CH_3), 1.16 and 1.11 (2:1, each t, 3H, $6\text{-CH}_2\text{CH}_3 \times 2$). MS m/z 237 ($\text{M}^+ - 1$).

2-Acetyl-4,4,6-triethyl-3-hydroxy-5-methoxycyclohexa-2,5-dienone (35). The same procedure was employed to obtain **35** (80%) from **32**; yellow oil. $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 19.00 and 18.12 (3:2, each s, 1H, chelated-OH), 4.02 and 3.93 (3:2, each s, 3H, OCH_3), 2.71 and 2.64 [2:3, each s, 3H, $\text{C}(\text{O})\text{CH}_3$], 2.57 and 2.51 (3:2, each q, 2H, $J = 7.4$ Hz, $6\text{-CH}_2\text{CH}_3$), 2.03–1.72 (m, 4H, $4\text{-CH}_2\text{CH}_3 \times 2$), 1.18 and 1.12 (3:2, each t, 3H, $6\text{-CH}_2\text{CH}_3$), 0.67 and 0.65 (2:3, each t, 6H, $4\text{-CH}_2\text{CH}_3 \times 2$). MS m/z 267 ($\text{M}^+ + 1$).

2-Acetyl-3-hydroxy-5-methoxy-4,4,6-tripropylcyclohexa-2,5-dienone (36). The same procedure was employed to obtain **36** (53%) from **33** along with recovered starting material (32%); yellow oil. $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 18.99 and 18.15 (3:2, each s, 1H, chelated-OH), 3.98 and 3.90 (3:2, each s, 3H, OCH_3), 2.67 and 2.63 [2:3, each s, 3H, $\text{C}(\text{O})\text{CH}_3$], 2.53–2.40 (m, 2H, $6\text{-CH}_2\text{-Et}$), 1.95–1.42 (m, 6H, $6\text{-CH}_2\text{CH}_2\text{CH}_3$ and $4\text{-CH}_2\text{Et} \times 2$), 1.10–0.95 (m, 7H, $6\text{-CH}_2\text{CH}_2\text{CH}_3$ and $4\text{-CH}_2\text{CH}_2\text{CH}_3 \times 2$), 0.83 and 0.80 (3:2, each t, 6H, $4\text{-CH}_2\text{CH}_2\text{CH}_3 \times 2$). MS m/z 307 ($\text{M}^+ - 1$).

Cytotoxic Activity Assay.¹⁵ 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-diluted stock. 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 ED_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent determinations that were reproducible and statistically significant. The following human tumor cell lines were used in the assay: A549 (human lung carcinoma), A431 (epidermoid skin carcinoma), 1A9 (human ovarian carcinoma), HCT-8 (colon adenocarcinoma), PC-3 (prostate cancer), KB (nasopharyngeal carcinoma), KB-VIN (vincristine-resistant KB subline), and HUVEC (human endothelial). All cell lines were obtained from the Lineberger Cancer Center (UNC-CH) or from ATCC (Rockville, MD) and were cultured in RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 $\mu\text{g}/\text{mL}$ kanamycin.

Angiogenesis Assay. The method is according to the NCI's Angiogenesis Resource Center protocol. HUVEC were purchased from Cambrex Bio-science. HUVEC (1.5×10^3) were plated in a 96-well plate in 100 μL of EGM-2 (Clonetec # CC3162). After 24 h (day zero), the test compound (100 μL) was added to each well at $2 \times$ the desired concentration in EGM-2 medium. One plate was stained with 0.5% crystal violet in 20% MeOH for 10 min, rinsed with water, and air-dried. The remaining plates were incubated for

72 h at 37 $^\circ\text{C}$. After 72 h, plates were stained with 0.5% crystal violet in 20% MeOH, rinsed with water, and air-dried. The stain was eluted with 1:1 solution of EtOH/0.1 M sodium citrate, and absorbance was measured at 540 nm with an ELISA reader.

Acknowledgment. This work was supported by a NIH Grant CA17625 from the National Cancer Institute, awarded to K.-H.L. and Grant No. 30572213 from the National Natural Science Foundation of China, awarded to J.-H.W.

Supporting Information Available: Elemental analysis data for compounds **7–15**, **21–26**, and **31–33**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JM0702534