

## Antitumor Agents 260. New Desmosdumotin B Analogues with Improved In Vitro Anticancer Activity

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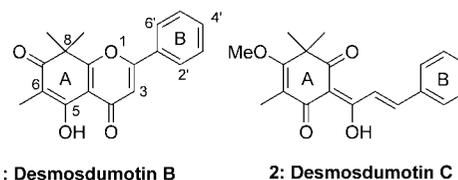
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Sixteen analogues (**3–16**, **33**, and **48**) of the unique flavonoid desmosdumotin B (**1**) were prepared and evaluated as in vitro inhibitors of the human KB cancer cell line and its MDR subclone, KB-VIN. 6,8,8-Triethyl analogues **10–13** showed enhanced KB-VIN selectivity. In particular, 4'-alkyl derivatives **11** (4'-Me) and **12** (4'-Et) showed significant ED<sub>50</sub> values of 0.03 and 0.025 μg/mL, respectively, against KB-VIN with selectivities of >460- and 320-fold compared with that of KB. This report is the first to describe compounds showing such high activity against MDR cells versus non-MDR cells. The unique activity of **1**-analogues is likely MDR-mediated because cotreatment with verapamil, a P-gp inhibitor, partially reversed the selective toxicity of both **1** and **10**. Interestingly, only **1**-analogues with a naphthalene B-ring (**8** and **14**) showed significant cytotoxic activity against KB and other cancer cell lines. Thus, **1**-analogues might be a new class of potent drug candidates, especially as **11** and **12** express direct selective action against tumors expressing MDR.

### Introduction

Chemotherapy is a useful treatment for cancer. However, its usefulness is often obstructed by the intrinsic or acquired resistance of cancer cells to anticancer drugs.<sup>1,2</sup> Resistance to one drug often implies simultaneous resistance to structurally and mechanistically diverse anticancer drugs, called multidrug resistance (MDR).<sup>3–7</sup> This efflux phenotype is mediated in part by the overexpression of plasma membrane transporters, including P-glycoprotein (P-gp, MDR1 or ABCB1)<sup>8</sup> or the multidrug resistance-associated protein (MRP1 or ABCC1),<sup>9–11</sup> which both belong to the superfamily of ATP-binding-cassette (ABC) transporters.<sup>12</sup> The emergence of MDR pumps anticancer drugs out of the cell utilizing the energy of ATP hydrolysis and thus results in reducing intracellular drug concentrations below cytotoxic levels. As a result, tumor cells overexpressing MDR show resistance to most of the currently used antitumor drugs. The development of agents targeted toward MDR1 or MRP1 is greatly needed in order to improve anticancer chemotherapeutic strategies.<sup>13–17</sup> MDR1/P-gp is the best characterized factor of the efflux system and most important mediator of MDR. Therefore, the major pharmacological approaches to overcome MDR have been focused on exploring the reversal of P-gp mediated MDR by inhibiting the function and suppressing the expression of MDR.<sup>13–16</sup> Numerous compounds have been found and examined as MDR modulators that inhibit P-gp function and are classified into first, second, and third generation chemosensitizers. Many second and third generation chemosensitizers, some of which are currently in clinical trials, are more potent and less toxic than first generation compounds like verapamil or cyclosporin A, yet they still suffer from adverse



**Figure 1.** Structures of **1** and **2**.

effects, poor solubility, and undesirable changes in pharmacokinetics present with many marketed antitumor drugs.

Among many MDR modulator candidates, flavonoids are compounds capable of modulating P-gp, hMRP1 transport, and ATPase activities.<sup>18–20</sup> We recently found that the flavone desmosdumotin B (DesB, **1**)<sup>21</sup> and its possible biosynthetic intermediate chalcone, desmosdumotin C (DesC, **2**) (Figure 1),<sup>22</sup> showed higher activity against the P-gp-expressing multidrug-resistant KB-VIN cell line (vincristine-resistant KB) than its parental nonresistant KB tumor cell (human epidermoid carcinoma of the nasopharynx). Interestingly, DesB (**1**) strongly inhibited the growth of KB-VIN cells with an ED<sub>50</sub> value of 2.0 μg/mL,<sup>23</sup> while it showed no activity against the other tested tumor cell lines, including the parental KB cell line (ED<sub>50</sub> > 40 μg/mL). The amazing selective activity against only MDR cells, with a >20-fold selectivity for KB-VIN versus KB, implies that desmosdumotins act via unusual mechanisms that differ from those of classical MDR modulators, which are inhibitors of the efflux pump.

We have previously accomplished the total synthesis of both **1**<sup>23</sup> and **2**,<sup>24</sup> and the published routes have wide-ranging application for future analogue syntheses. Focused modification of **2** and the bioactivity of the resulting analogues have also been reported.<sup>25,26</sup> We have continued the syntheses of **1**-analogues and evaluation of in vitro anticancer activity, mainly focused against non-MDR (KB) and MDR (KB-VIN) cell lines. Herein, we report the syntheses and bioactivity of novel **1**-analogues as well as observed structure–activity relationships (SAR) in detail.

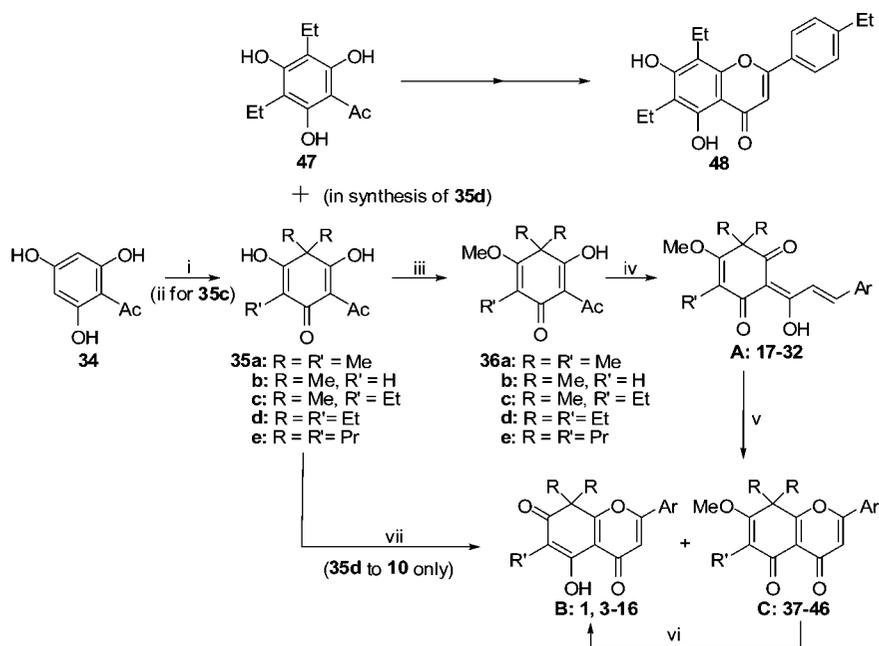
**Chemistry.** Fourteen **1**-analogues, **3–16**, were synthesized from the related **2**-analogues through intramolecular cyclization

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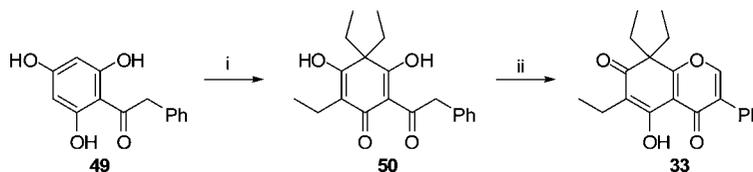
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## Scheme 1. Syntheses of 1-Analogues



R	R'	Ar	A	B	C	R	R'	Ar	A	B	C
Me	Me	Ph	2	1	37	Me	Et	Ph	25	9	39
		4-Me-Ph	17	3	-	Et	Et	Ph	26	10	40
		2-Me-Ph	18	4	-			4-Me-Ph	27	11	41
		2,6-diMe-Ph	19	-	-			4-Et-Ph	28	12	42
		3,5-diMe-Ph	20	5	-			2-Me-Ph	29	13	43
		2,4,6-triMe-Ph	21	6	-			Naphthalen-1-yl	30	14	44
		4-Et-Ph	22	7	-			Phenanthren-9-yl	31	15	45
		Naphthalen-1-yl	23	8	-	Pr	Pr	Ph	32	16	46
Me	H	Ph	24	-	38						

<sup>a</sup> Reaction conditions: (i) RI (3 mol equiv), NaOMe (3 mol equiv), MeOH, reflux. (R = Me for **35a**, R = Et for **35d**, R = Pr for **35e**). (ii) (1)  $\text{BF}_3 \cdot \text{OEt}_2$ ,  $\text{HOAc}/\text{Ac}_2\text{O}$ , (2) MeI, NaOMe, MeOH, reflux, (3) 85%  $\text{H}_2\text{SO}_4$ , heat, (4) EtI (2 mol equiv), NaOMe (1 mol equiv), MeOH, reflux. (iii)  $\text{TMSCHN}_2$ ,  $-78$  to  $-40^\circ\text{C}$ . (iv)  $\text{ArCHO}$ , 50% KOH, EtOH, rt, 1–3 days. (v)  $\text{I}_2$ , DMSO, conc  $\text{H}_2\text{SO}_4$ , see Tables 1 and 2. (vi)  $\text{BBr}_3$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$  to rt. (vii) (1)  $\text{PhCHO}$ , 50% KOH, EtOH, microwave,  $90^\circ\text{C}$ , 20 min; (2)  $\text{I}_2$ , DMSO, microwave,  $190^\circ\text{C}$ , 5 min.

Scheme 2. Synthesis of isoflavone **33**<sup>a</sup>

<sup>a</sup> (i) EtI (3 mol equiv), NaOMe (3 mol equiv), MeOH, reflux, 50%. (ii)  $\text{BF}_3 \cdot \text{OEt}_2$ ,  $\text{PCl}_5$ , DMF, 68%.

and demethylation transformations as shown in Scheme 1. Three 2-analogues, **29–31**, were newly synthesized from 2,4,6-trihydroxyacetophenone (**34**) according to the methodology in our prior report,<sup>24</sup> and 13 2-analogues, **17–28**, and **32**, were prepared previously.<sup>25,26</sup> As shown in Table 1, compounds **17**, **18**, **20–23**, and **26** were converted directly to the related 1-analogues, **3–8** and **10**, by heating at  $80–95^\circ\text{C}$  with catalytic iodine and 1–2% conc  $\text{H}_2\text{SO}_4$  in DMSO for several hours in moderate yields. However, certain compounds (e.g., **19**, **21**, and **26**) gave unsatisfying low yield of products, even employing a higher temperature and longer reaction time. Bulkiness in the aryl ring (2',6'-dimethylphenyl for **19** and **21**) or at the C-8 position (*gem*-diethyl groups for **26**) might hinder the reaction. Alternatively, a two-step conversion involving cyclization followed by demethylation was carried out to improve the yield (Table 2). At this point, a shorter reaction time (less than 1.5 h) was used for the cyclization step to avoid demethylation and

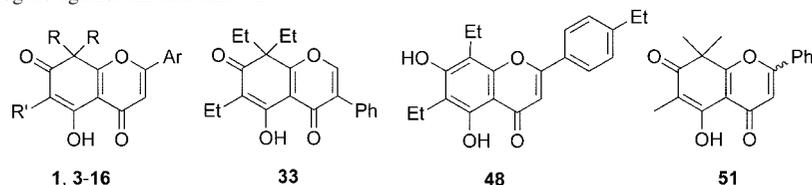
**Table 1.** Conditions of Cyclization Step (Method A: Direct Conversion)

starting material (mmol)	1–2% conc $\text{H}_2\text{SO}_4$ in DMSO (mL)	temp ( $^\circ\text{C}$ )	time (h)	product (% yield)
<b>17</b> (0.15)	1.5	90	5	<b>3</b> (52)
<b>18</b> (0.16)	1.8	90	6	<b>4</b> (48)
<b>20</b> (0.17)	2.0	80	5	<b>5</b> (44)
<b>21</b> (0.34)	2.3	80–100	24	<b>6</b> (10)
<b>22</b> (0.12)	1.2	95	6	<b>7</b> (51)
<b>23</b> (0.13)	1.8	90	6	<b>8</b> (45)
<b>26</b> (0.24)	2.3	90–100	7	<b>10</b> (10)

decomposition. For example, compound **26** was heated at  $95^\circ\text{C}$  for 1.5 h in the presence of catalytic iodine and 1–2% conc  $\text{H}_2\text{SO}_4$  in DMSO to obtain methylated **40** in 68% yield along with **10** in 19% yield. Demethylation of **40** was performed by using 3 mol equiv of  $\text{BBr}_3$  in  $\text{CH}_2\text{Cl}_2$  to afford **10** in 82% yield (79% overall yield for two steps). This improved method was

**Table 2.** Conditions of Cyclization Step (Method B: Two-Step Conversion)

starting material (mmol)	1–2% conc H <sub>2</sub> SO <sub>4</sub> in DMSO (mL)	temp (°C)	time (min)	products in step 1 (% yield)		product in step 2 <sup>a</sup> (% yield)	overall % yield in two steps
<b>25</b> (0.28)	1.8	95	60	<b>9</b> (41)	<b>39</b> (34)	<b>9</b> (74)	66
<b>26</b> (0.22)	1.6	95	90	<b>10</b> (19)	<b>40</b> (68)	<b>10</b> (82)	79
<b>27</b> (0.19)	1.7	95	50	<b>11</b> (8)	<b>41</b> (72)	<b>11</b> (76)	62
<b>28</b> (0.12)	1.2	95	60	w/o separation		<b>12</b> (–)	64
<b>29</b> (0.31)	1.7	90	90	w/o separation		<b>13</b> (–)	66
<b>30</b> (0.11)	1.8	90	60	w/o separation		<b>14</b> (–)	82
<b>31</b> (0.08)	1.7	90	60	w/o separation		<b>15</b> (–)	71
<b>32</b> (0.34)	1.6	95	90	<b>16</b> (2)	<b>46</b> (75)	<b>16</b> (90)	76

<sup>a</sup> BBr<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> rt overnight from **39**–**46**.**Table 3.** Activities of 1-Analogues against KB and KB-VIN

compound	R			ED <sub>50</sub> (μg/mL) <sup>a</sup>		selectivity KB/KB-VIN
	R	R'	Ar	KB	KB-VIN	
<b>1</b>	Me	Me	Ph	>40 (33)	2.0	20
<b>3</b>			4-Me-Ph	22.54	1.09	20.7
<b>4</b>			2-Me-Ph	37.28	1.75	21.3
<b>5</b>			3,5-diMe-Ph	53.05	2.91	18.2
<b>6</b>			2,4,6-triMe-Ph	NA <sup>b</sup>	5.82	>3.4
<b>7</b>			4-Et-Ph	20.0	1.54	13.0
<b>8</b>			naphthalen-1-yl	0.51	0.16	3.2
<b>9</b>		Et	Ph	>20 (12)	0.6	>33
<b>10</b>	Et	Et	Ph	>80	0.36	222
<b>11</b>			4-Me-Ph	13.8	0.03	460
<b>12</b>			4-Et-Ph	8.0	0.025	320
<b>13</b>			2-Me-Ph	18.0	0.1	60
<b>14</b>			naphthalen-1-yl	0.6	0.3	2.0
<b>15</b>			phenanthren-9-yl	5.0	4.3	1.2
<b>16</b>	Pr	Pr	Ph	4.0	0.63	6.4
<b>33</b>				16.0	3.5	4.6
<b>48</b>				>20 (18)	5.0	>4
<b>51</b>				10.5	7.5	1.4
<b>VIN<sup>c</sup></b>				0.015	7.0	0.002

<sup>a</sup> Cytotoxicity as ED<sub>50</sub> 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. <sup>b</sup> Test compound (20 μg/mL) did not reach 50% inhibition. <sup>c</sup> Vincristine.

applied to the remaining 2-analogues, **25**–**32**, to give the desired compounds **9**–**16** in 62–82% overall yields in two steps through **39**–**46**.

However, as our synthetic focus was on 1-analogues, we also designed a synthetic pathway that avoided the corresponding 2-analogues as intermediates. Aldol condensation of **35d** with phenylaldehyde in the presence of 50% aq KOH proceeded only under microwave conditions (90 °C, 20 min). The resulting aldol adduct was treated without purification with iodine in DMSO, again using microwave technology, to provide **10** in 35% overall yield. The overall yields using the two synthetic pathways (with or without the 2-analogue intermediate) were almost identical. However, the direct pathway from **35d** to **10** had fewer reaction steps and required less time. Thus, we have developed an alternative synthetic pathway to 1-analogues using microwave technology.

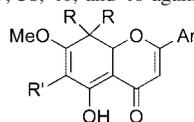
Three additional compounds (isoflavone **33**, flavone **48**, and flavanone **51**) were screened for cytotoxic activity in our SAR study. During ethylation of **34** to give **35d**, 3,5-diethylacetophenone (**47**) was produced as a side product. Compound **47** was converted to the related flavone **48** by the same reaction sequence indicated above. In addition, triethylation of 2,4,6-trihydroxy phenylacetophenone (**49**) was successfully achieved using the methods described above to afford **50** in moderate

yield. Treatment of **50** with *N,N*-dimethyl(chloromethylene)-ammonium chloride, generated by DMF and PCl<sub>5</sub>, in the presence of BF<sub>3</sub>·OEt<sub>2</sub>,<sup>27</sup> produced the desired isoflavone **33** in good yield. The last compound (**51**) investigated in our study was reported in our prior papers.<sup>23</sup>

## Results and Discussion

All synthesized 1-analogues were evaluated in vitro against two human tumor cell lines, the KB-VIN cell line, which is an MDR P-gp-expressing cloned subline stepwise selected using vincristine, and its parental non-MDR KB cell line. Selected 1-analogues were also evaluated in vitro against four additional human tumor cell lines, A549 (human lung carcinoma), MCF-7 (breast cancer), HCT-8 (colon adenocarcinoma), and PC-3 (prostate cancer).

The cytotoxic activity data for **1** and its analogues **3**–**16**, **33**, **48**, and **51** are listed in Table 3. Because the KB-VIN selectivity of **1** was higher than that of **2**, we hypothesized that analogues with the flavone skeleton would show enhanced inhibition of KB-VIN (MDR) cell growth with less activity against KB (non-MDR) cells than the related chalcone analogues. In fact, most 1-analogues (**3**–**5**, **7**, and **9**–**13**) did show remarkably enhanced inhibition of the growth of KB-VIN cells

**Table 4.** Activities of **37**, **38**, **40**, and **46** against KB and KB-VIN

compound				ED <sub>50</sub> (μg/mL)		selectivity KB/KB-VIN
	R	R'	Ar	KB	KB-VIN	
<b>37</b>	Me	Me	Ph	38.5	23.0	1.7
<b>38</b>	Me	H	Ph	1.8	1.8	1.0
<b>40</b>	Et	Et	Ph	20.0	15.5	1.3
<b>46</b>	Pr	Pr	Ph	8.5	7.0	1.2

**Table 5.** Data for Selected of 1-Analogues against Various Cancer Cell Lines

compd	Ed <sub>50</sub> (μg/mL)			
	A549	MCF-7	HCT-8	PC-3
<b>1</b>	>20(16)	>20(23)	NA	NA
<b>8</b>	0.6	0.6	0.4	0.5
<b>10</b>	NA	>20(38)	NA	NA
<b>11</b>	16.0	>20(38)	15.0	18.0
<b>14</b>	0.6	0.2	0.4	0.4
<b>15</b>	8.0	4.0	5.6	6.0
<b>33</b>	18.0	10.0	12.0	>20(32)
VIN	0.004	0.010	0.020	0.010

but were not significantly cytotoxic against KB cells. These **1**-analogues had KB-VIN selectivities of 6.4–460 (Table 3), while tested C-7 methylated analogues (**37**, **38**, **40**, and **46**) were not selective (Table 4). Important pharmacophores for the enhanced KB-VIN activity were a nonaromatic trialkyl A-ring, 2,3-double bond, and monophenyl B-ring, as discussed below.

Monoalkylated B-ring analogues **3**, **4**, and **7**, as well as 3,5-dimethyl analogue **5**, showed almost the same activity and selectivity as the nonsubstituted parent compound (**1**). However, the 2,4,6-trimethyl B-ring analogue **6** displayed decreased activity against KB-VIN cells. This finding could imply that the loss of rotational flexibility around the B–C ring axis caused by 2,6-disubstitution might adversely affect the cytotoxic activity against MDR cells.<sup>28</sup> In addition, analogues **8** and **14**, in which the phenyl B-ring was replaced with a naphthyl ring, strongly inhibited KB-VIN cell growth with ED<sub>50</sub> values of 0.16 and 0.3 μg/mL, although they also inhibited the parental KB cells with ED<sub>50</sub> values of 0.5 and 0.6 μg/mL, respectively. This result is interesting because **1**-analogues without a naphthyl ring did not show strong cytotoxic activity against KB cells.

Among the A-ring analogues, changing the substituents on the A-ring caused dramatic effects on KB-VIN selectivity. Replacing the C-6 methyl group (**1**) with an ethyl group (**9**) resulted in increased cytotoxic activity against KB-VIN (ED<sub>50</sub> 0.6 versus 2.0 μg/mL) and, correspondingly, in enhanced KB-VIN selectivity (>33 versus 20). Furthermore, 6,8,8-triethyl-DesB **10**, in which all three A-ring methyl groups have been replaced with ethyl groups, possessed even greater activity against KB-VIN cells (ED<sub>50</sub> 0.36 μg/mL) without activity against the KB cell line, resulting in greater than 222-fold selectivity for the KB-VIN cell line. However, extending the substituent size to three carbons (6,8,8-tripropyl **16**) led to enhanced cytotoxic activity against both cell lines, MDR and non-MDR, with decreased KB-VIN selectivity of only 6.4. Although, as discussed above, B-ring alkylation of trimethyl substituted **1** had no or little effect on activity or selectivity, B-ring alkylation of triethyl-DesB (**10**) did have significant effects. Adding a 2'-Me (**13**) increased potency against both cell lines. More notably, introduction of either a methyl (**11**) or

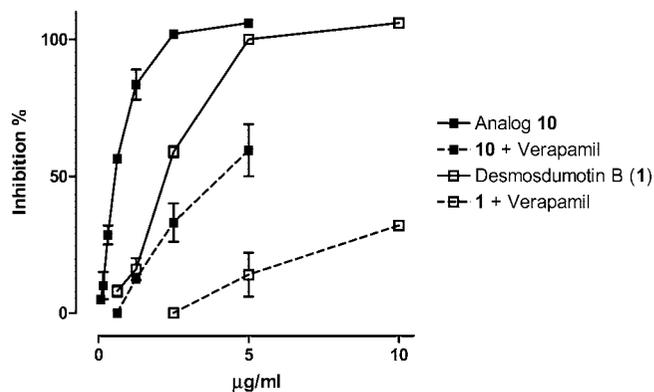
ethyl (**12**) at C-4' greatly potentiated the inhibitory activity against KB-VIN cells, resulting in remarkably enhanced ED<sub>50</sub> values of 0.03 and 0.025 μg/mL, respectively. Because these two compounds did not significantly inhibit the parent KB cell growth, the KB-VIN selectivities for 4'-methyl **11** and 4'-ethyl **12** were 460 and 320, respectively.

Comparison of **12** and **48** showed that an aromatized A-ring in the latter disturbed both activity and KB-VIN selectivity or, alternatively, that 6,8,8-trialkyl A-ring substitution was required for selectivity. Moreover, a C-2,3 double bond in the B-ring was also necessary because the flavanone **51** showed decreased activity against and no selectivity for KB-VIN compared to the related flavone **1**. Isoflavonoid analogue **33**, which has the B-ring connected to the C-3 position, also showed only 4.6-fold KB-VIN selectivity. In addition, as shown in Table 4, putting a methoxy group at the C-7 position (enol ether) generally resulted in no inhibitory activity against KB-VIN or KB cells and no KB-VIN selectivity. The one exception was **38**, which showed potent activity against both KB and KB-VIN cell lines (ED<sub>50</sub> 1.8 μg/mL). This compound is unsubstituted at the C-6 position.

As discussed above, most **1**-analogues tested possessed unique activity against KB-VIN cells with less or comparable activity against the parent tumor cell line. The following key SAR observations were found regarding the KB-VIN selectivity: (1) A flavone skeleton, as opposed to chalcone or flavanone, was important for the selectivity. (2) A 6,8,8-trialkyl nonaromatic A-ring resulted in better selectivity than a 6,8-dialkyl aromatic ring. (3) The rank order of selectivity for **1**-analogues decreased in the following order: 6,8,8-triethyl > 6-ethyl-8,8-dimethyl > 6,8,8-trimethyl > 6,8,8-tripropyl. (4) A 4'-alkyl group enhanced the selectivity in triethyl desB analogues and a methyl group was better than an ethyl group.

Meanwhile, selected analogues (parent compound **1**, analogues **10** and **11**, which showed high selectivity for MDR (KB-VIN) versus non-MDR (KB) cells, naphthyl B-ring analogues **8** and **14**, which displayed potent cytotoxic activity against both KB and KB-VIN cell lines, and isoflavonoid **33**) were tested against additional cancer cell lines (Table 5). As was expected, analogues **8** and **14** showed significant cell growth inhibition against all tested cell lines, A549, MCF-7, HCT-8, and PC-3, with ED<sub>50</sub> values of 0.2–0.6 μg/mL. In contrast, no cytotoxic activity was observed with **1**, **10**, and **11** as well as **33**. The lack of cytotoxic activity against these cell lines suggested that P-gp might be necessary for the activity of these **1**-analogues.

To examine whether functional P-gp was required for the unique activity of **1**-analogues against KB-VIN cells, experiments testing the effect of cotreatment with verapamil, a first generation P-gp inhibitor, were conducted. The results of this preliminary approach shown in Figure 2 clearly show that verapamil significantly reduced the selective toxicity of compounds **1** and **10**, suggesting that for these two agents, P-gp activity was indeed required. Advanced mechanistic investigations are now in progress, but it is worthwhile noting that the hyperactivity of **1** and **10–12** is not general for MDR-cell lines but is restricted to only a few (P. C. Chiang, K. H. Lee, and K. F. Bastow, unpublished observation). One possible explanation is that P-gp microheterogeneity is important in determining hypersensitivity. More than 50 single-nucleotide polymorphisms (SNP) are known, some of which are silent but which can still radically affect P-gp conformation (any can potentially alter drug interaction).<sup>29,30</sup> Efforts to test the importance of P-gp-microheterogeneity for the unprecedented high collateral sensitivity of KB-VIN and other MDR cell-lines to **11** are underway, and mechanistic work will be reported in due course.



**Figure 2.** Reversal effects of **1** and **10** in KB-VIN cells by verapamil cotreatment.

In summary, 6,8,8-triethyl-4'-methyl analogue **11** possessed the most significant and unique activity in this study. It inhibited KB-VIN cell growth with an  $ED_{50}$  value of  $0.03 \mu\text{g/mL}$ , had no toxicity against the other tested cancer cells, including KB, and showed 460-fold KB-VIN selectivity. This surprising sensitivity of a MDR versus non-MDR cell line has not been reported since the U.S. National Cancer Institute (NCI) 60 cell line anticancer drug screen was developed in the late 1980s.<sup>31</sup> Currently, a thiosemicarbazone derivative, NSC73306,<sup>32</sup> is undergoing advanced preclinical testing by NCI as a drug lead for targeting MDR tumor cell populations. It was selectively toxic to P-gp-expressing tumor cells; however, the sensitivity of KB-VIN (MDR)/KB-3-1 (non-MDR) was only 7.8-fold. The proposed mechanisms of action for NSC73306 do not involve direct interaction with P-gp but are P-gp-dependent and P-gp-specific. In our study, because **1**-analogues showed amazingly sensitive and potent cytotoxic activity against MDR cells with no inhibitory activity against non-MDR cells, these compounds seem to require the existence of P-gp for activity and might not act as normal MDR modulators, which inhibit the drug efflux pump and thereby reverse MDR via chemosensitization activity. Thus, although the mechanism of action of **1**-analogues is not yet clear, it might be unique and different from that of NSC73306. Triethyl-DesB analogues **10**–**12** could provide a new class of promising antitumor drug candidates able to overcome the current serious MDR problem. In addition, naphthyl analogues **8** and **14** showed significant cytotoxic activity against both MDR and non-MDR cell lines and would be good antitumor drug candidates that act regardless of the presence of P-gp.

## Experimental Section

All chemicals and solvents were used as purchased. All melting points were measured on a Fisher-Johns melting point apparatus without correction.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Varian Gemini 2000 (300 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  $\delta$  in ppm, apparent scalar coupling constants  $J$  in Hz. Mass spectroscopic data were obtained on a TRIO 1000 mass spectrometer. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). All target compounds were characterized by  $^1\text{H}$  NMR, MS, and elemental analyses.

**General Synthetic Procedures for 1-Analogues. Method A (Direct Conversion).** Compounds **17**, **18**, **20**–**23**, and **26** were dissolved separately in 1–2% conc  $\text{H}_2\text{SO}_4$  in DMSO, and then  $\text{I}_2$  (0.1 mol equiv) was added and the reaction mixture heated for several hours (see below). The reaction mixture was quenched with

ice-cold aqueous 10%  $\text{Na}_2\text{S}_2\text{O}_3$  and extracted with EtOAc. The extract was washed with brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated in vacuo. The residue was chromatographed on silica gel eluting with EtOAc–hexane (1:4 to 1:2, v/v) to afford analogues **3**–**8** and **10**.

**Method B (Two-Step Conversion).** Compounds **25**–**32** were dissolved separately in 1% conc  $\text{H}_2\text{SO}_4$  in DMSO, and then  $\text{I}_2$  (0.1 mol equiv) was added and the reaction mixture heated at  $90$ – $95^\circ\text{C}$  for 1 h. The reaction mixture was treated in the same manner as described above to afford 7-methoxy substituted analogues **39**–**46**, along with a small amount of **9**–**16**. Compounds **39**–**46** were dissolved separately in anhydrous  $\text{CH}_2\text{Cl}_2$ , and the mixture cooled to  $0^\circ\text{C}$ .  $\text{BBr}_3$  (3 mol equiv, 1.0 M solution in  $\text{CH}_2\text{Cl}_2$ ) was added to the solution, which was warmed to rt spontaneously and stirred overnight. After addition of water, the reaction mixture was extracted three times with  $\text{CH}_2\text{Cl}_2$ . The extracts were combined, washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated in vacuo. The residue was chromatographed on silica gel eluting with EtOAc–hexane (1:4) to obtain analogues **9**–**16**.

**4'-Methyl desmosdumotin B (3).** Yellow prisms, mp  $201$ – $202^\circ\text{C}$  ( $\text{CH}_2\text{Cl}_2$ –hexane).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.16 (s, 1H, chelated-OH), 7.70 (d, 2H,  $J = 8.2$  Hz, 2'- and 6'-H), 7.36 (d, 2H,  $J = 8.2$  Hz, 3'- and 5'-H), 6.86 (s, 1H, 3-H), 2.46 (s, 3H, 4'- $\text{CH}_3$ ), 1.88 (s, 3H, 6- $\text{CH}_3$ ), 1.58 (s, 6H, 8- $\text{CH}_3 \times 2$ ). MS  $m/z$ : 311 ( $M^+ + 1$ ). Anal. ( $\text{C}_{19}\text{H}_{18}\text{O}_4 \cdot \frac{1}{4}\text{H}_2\text{O}$ ) C, H, O.

**2'-Methyl desmosdumotin B (4).** Yellow prisms, mp  $146$ – $147^\circ\text{C}$  ( $\text{CH}_2\text{Cl}_2$ –hexane).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.10 (s, 1H, chelated-OH), 7.51–7.44 (m, 2H, Ar-H  $\times 2$ ), 7.40–7.31 (m, 2H, Ar-H  $\times 2$ ), 6.61 (s, 1H, 3-H), 2.47 (s, 3H, 4'- $\text{CH}_3$ ), 1.88 (s, 3H, 6- $\text{CH}_3$ ), 1.52 (s, 6H, 8- $\text{CH}_3 \times 2$ ). MS  $m/z$ : 311 ( $M^+ + 1$ ). Anal. ( $\text{C}_{19}\text{H}_{18}\text{O}_4 \cdot \frac{1}{8}\text{H}_2\text{O}$ ) C, H, O.

**3',5'-Dimethyl desmosdumotin B (5).** Colorless prisms, mp  $112$ – $113^\circ\text{C}$  ( $\text{CH}_2\text{Cl}_2$ –hexane).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.16 (s, 1H, chelated-OH), 7.38 (s, 2H, 2'- and 6'-H), 7.23 (s, 1H, 4'-H), 6.85 (s, 1H, 3-H), 2.42 (s, 6H, 3'- and 5'- $\text{CH}_3$ ), 1.88 (s, 3H, 6- $\text{CH}_3$ ), 1.59 (s, 6H, 8- $\text{CH}_3 \times 2$ ). MS  $m/z$ : 325 ( $M^+ + 1$ ). Anal. ( $\text{C}_{20}\text{H}_{20}\text{O}_4 \cdot \frac{1}{8}\text{H}_2\text{O}$ ) C, H, O.

**2',4',6'-Trimethyl desmosdumotin B (6).** Colorless prisms, mp  $196$ – $197^\circ\text{C}$  ( $\text{CH}_2\text{Cl}_2$ –hexane).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.08 (s, 1H, chelated-OH), 6.99 (s, 2H, 3'- and 5'-H), 6.44 (s, 1H, 3-H), 2.36 (s, 3H, 4'- $\text{CH}_3$ ), 2.22 (s, 6H, 2'- and 6'- $\text{CH}_3$ ), 1.88 (s, 3H, 6- $\text{CH}_3$ ), 1.47 (s, 6H, 8- $\text{CH}_3 \times 2$ ). MS  $m/z$ : 339 ( $M^+ + 1$ ). Anal. ( $\text{C}_{21}\text{H}_{22}\text{O}_4 \cdot \frac{1}{4}\text{H}_2\text{O}$ ) C, H, O.

**4'-Ethyl desmosdumotin B (7).** Colorless prisms, mp  $203$ – $204^\circ\text{C}$  ( $\text{CH}_2\text{Cl}_2$ –hexane).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.16 (s, 1H, chelated-OH), 7.72 (d, 2H,  $J = 8.2$  Hz, 2'- and 6'-H), 7.38 (d, 2H,  $J = 8.2$  Hz, 3'- and 5'-H), 6.36 (s, 1H, 3-H), 2.75 (q, 2H,  $J = 7.4$  Hz, 4'- $\text{CH}_2\text{CH}_3$ ), 1.88 (s, 3H, 6- $\text{CH}_3$ ), 1.58 (s, 6H, 8- $\text{CH}_3 \times 2$ ), 1.29 (t, 3H,  $J = 7.4$  Hz, 4'- $\text{CH}_2\text{CH}_3$ ). MS  $m/z$ : 323 ( $M^+ - 1$ ). Anal. ( $\text{C}_{20}\text{H}_{20}\text{O}_4 \cdot \frac{1}{8}\text{H}_2\text{O}$ ) C, H, O.

**2-(Naphthalen-1-yl)desmosdumotin B (8).** Colorless prisms, mp  $164$ – $165^\circ\text{C}$  ( $\text{CH}_2\text{Cl}_2$ –hexane).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.10 (s, 1H, chelated-OH), 8.87 (d, 1H,  $J = 8.0$  Hz, Ar-H), 8.02–7.94 (m, 2H, Ar-H), 7.71–7.67 (m, 1H, Ar-H), 7.65–7.58 (m, 3H, Ar-H), 6.82 (s, 1H, 3-H), 1.91 (s, 3H, 6- $\text{CH}_3$ ), 1.54 (s, 6H, 8- $\text{CH}_3 \times 2$ ). MS  $m/z$ : 347 ( $M^+ + 1$ ). Anal. ( $\text{C}_{22}\text{H}_{18}\text{O}_4 \cdot \frac{1}{8}\text{H}_2\text{O}$ ) C, H, O.

**6,8,8-Triethyl-7-methoxy desmosdumotin B (40).** Colorless prisms, mp  $131$ – $132^\circ\text{C}$  ( $\text{CH}_2\text{Cl}_2$ –hexane).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.78–7.72 (m, 2H, Ar-H), 7.58–7.50 (m, 3H, Ar-H), 6.84 (s, 1H, 3-H), 4.01 (s, 3H, 7- $\text{OCH}_3$ ), 2.58 (q, 2H,  $J = 7.4$  Hz, 6- $\text{CH}_2\text{CH}_3$ ), 2.26–2.01 (m, 4H, 8- $\text{CH}_2\text{CH}_3 \times 2$ ), 1.15 (t, 3H,  $J = 7.4$  Hz, 6- $\text{CH}_2\text{CH}_3$ ), 0.71 (t, 6H,  $J = 7.4$  Hz, 8- $\text{CH}_2\text{CH}_3 \times 2$ ). MS  $m/z$ : 353 ( $M^+ + 1$ ). Anal. ( $\text{C}_{22}\text{H}_{24}\text{O}_4 \cdot \frac{1}{8}\text{H}_2\text{O}$ ) C, H, O.

**6,8,8-Tripropyl-7-methoxy desmosdumotin B (46).** Colorless prisms, mp  $124$ – $125^\circ\text{C}$  ( $\text{CH}_2\text{Cl}_2$ –hexane).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.78–7.72 (m, 2H, Ar-H), 7.59–7.51 (m, 3H, Ar-H), 6.83 (s, 1H, 3-H), 3.99 (s, 3H, 7- $\text{OCH}_3$ ), 2.54–2.45 (m, 2H, 6- $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 2.16–1.92 (m, 4H, 8- $\text{CH}_2\text{CH}_2\text{CH}_3 \times 2$ ), 1.60–1.46 (m, 2H, 6- $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 1.21–1.04 (m, 2H, 8- $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 1.02–0.86 (m, 2H, 8- $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 0.98 (t, 3H,  $J = 7.4$  Hz,

6-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.83 (t, 6H, *J* = 7.4 Hz, 8-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub> × 2). MS *m/z*: 395 (M<sup>+</sup>). Anal. (C<sub>25</sub>H<sub>30</sub>O<sub>4</sub>·<sup>1</sup>/<sub>4</sub>H<sub>2</sub>O) C, H, O.

**6-Ethyl-8,8-dimethyl desmosdumotin B (9)**. Method B. 66% two-step overall yield. Pale-yellow prisms, mp 212–213 °C (CH<sub>2</sub>Cl<sub>2</sub>–hexane). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.03 (1H, chelated-OH), 7.84–7.78 (m, 2H, 2'- and 6'-H), 7.64–7.53 (m, 3H, 3', 4'- and 5'-H), 6.90 (s, 1H, 3-H), 2.44 (q, 2H, *J* = 7.3 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 1.59 (s, 6H, 8-CH<sub>3</sub> × 2), 1.04 (t, 3H, *J* = 7.3 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>). MS *m/z*: 310 (M<sup>+</sup> - 1). Anal. (C<sub>19</sub>H<sub>18</sub>O<sub>4</sub>) C, H, O.

**6,8,8-Triethyl-2-methyl desmosdumotin B (10)**. Method A. 10% yield. Pale-yellow prisms, mp 204–205 °C (CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.06 (1H, chelated-OH), 7.84–7.77 (m, 2H, 2'- and 6'-H), 7.66–7.54 (m, 3H, 3', 4'- and 5'-H), 6.91 (s, 1H, 3-H), 2.46 (q, 2H, *J* = 7.3 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 2.34–2.20 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 2.08–1.94 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.05 (t, 3H, *J* = 7.3 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 0.68 (t, 6H, *J* = 7.3 Hz, 8-CH<sub>2</sub>CH<sub>3</sub> × 2). MS *m/z*: 339 (M<sup>+</sup> + 1). Anal. (C<sub>21</sub>H<sub>22</sub>O<sub>4</sub>·<sup>1</sup>/<sub>4</sub>H<sub>2</sub>O) C, H, O.

**6,8,8-Triethyl-4'-methyl desmosdumotin B (11)**. Method B. 55% two-step overall yield. Pale-yellow prisms, mp 182–183 °C (CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.16 (1H, chelated-OH), 7.70 (d, 2H, *J* = 8.1 Hz, 2'- and 6'-H), 7.37 (d, 2H, *J* = 8.1 Hz, 3'- and 5'-H), 6.88 (s, 1H, 3-H), 2.47 (s, 3H, 4'-CH<sub>3</sub>), 2.36–2.18 (m, 2H, 6-CH<sub>2</sub>CH<sub>3</sub>), 2.12–1.94 (m, 4H, 8-CH<sub>2</sub>CH<sub>3</sub> × 2), 1.04 (t, 3H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 0.67 (t, 6H, *J* = 7.4 Hz, 8-CH<sub>2</sub>CH<sub>3</sub> × 2). MS *m/z*: 351 (M<sup>+</sup> - 1). Anal. (C<sub>22</sub>H<sub>24</sub>O<sub>4</sub>) C, H, O.

**6,8,8-Triethyl-4'-ethyl desmosdumotin B (12)**. Method B. 66% two-step overall yield. Colorless prisms, mp 159–160 °C (CH<sub>2</sub>Cl<sub>2</sub>–hexane). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.13 (1H, chelated-OH), 7.72 (d, 2H, *J* = 8.2 Hz, 2'- and 6'-H), 7.39 (d, 2H, *J* = 8.2 Hz, 3'- and 5'-H), 6.88 (s, 1H, 3-H), 2.75 (q, 4H, *J* = 7.4 Hz, 4'-CH<sub>2</sub>CH<sub>3</sub>), 2.45 (q, 4H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 2.36–2.18 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 2.08–1.94 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.29 (t, 3H, *J* = 7.4 Hz, 4'-CH<sub>2</sub>CH<sub>3</sub>), 1.04 (t, 3H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 0.67 (t, 6H, *J* = 7.4 Hz, 8-CH<sub>2</sub>CH<sub>3</sub> × 2). MS *m/z*: 367 (M<sup>+</sup> + 1). Anal. (C<sub>23</sub>H<sub>26</sub>O<sub>4</sub>) C, H, O.

**6,8,8-Triethyl-2'-methyl desmosdumotin B (13)**. Method B. 64% two-step overall yield. Pale-yellow prisms, mp 146–147 °C (CH<sub>2</sub>Cl<sub>2</sub>–hexane). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.08 (1H, chelated-OH), 7.52–7.42 (m, 2H, Ar-H), 7.40–7.32 (m, Ar-H), 6.62 (s, 1H, 3-H), 2.46 (s, 3H, 2'-CH<sub>3</sub>), 2.52–2.42 (m, 2H, 6-CH<sub>2</sub>CH<sub>3</sub>), 2.26–2.13 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.98–1.84 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.04 (t, 3H, *J* = 7.3 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 0.67 (t, 6H, *J* = 7.4 Hz, 8-CH<sub>2</sub>CH<sub>3</sub> × 2). MS *m/z*: 351 (M<sup>+</sup> - 1). Anal. (C<sub>22</sub>H<sub>24</sub>O<sub>4</sub>·<sup>1</sup>/<sub>2</sub>H<sub>2</sub>O) C, H, O.

**2-(Naphthalen-1'-yl)-6,8,8-triethyl desmosdumotin B (14)**. Colorless prisms, mp 185–186 °C (EtOAc–hexane). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.07 (1H, chelated-OH), 8.09 (d, 1H, *J* = 7.9 Hz, Ar-H), 8.02–7.91 (m, 2H, Ar-H), 7.69 (dd, 1H, *J* = 7.2 and 1.3 Hz, Ar-H), 7.66–7.58 (m, 3H, Ar-H), 6.83 (s, 1H, 3-H), 2.49 (q, 2H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 2.26–2.12 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.98–1.84 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.07 (t, 3H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 0.73 (t, 6H, *J* = 7.4 Hz, 8-CH<sub>2</sub>CH<sub>3</sub> × 2). Anal. (C<sub>25</sub>H<sub>24</sub>O<sub>4</sub>) C, H, O.

**6,8,8-Triethyl-2-(phenanthren-9'-yl) desmosdumotin B (15)**. Colorless prisms, mp 184–185 °C (EtOAc–hexane). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.07 (1H, chelated-OH), 8.84–8.72 (m, 2H, Ar-H), 8.04–7.94 (m, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 7.92–7.62 (m, 5H, Ar-H), 6.89 (s, 1H, 3-H), 2.49 (q, 2H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 2.24–2.12 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.97–1.83 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.07 (t, 3H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 0.75 (t, 6H, *J* = 7.4 Hz, 8-CH<sub>2</sub>CH<sub>3</sub> × 2). Anal. (C<sub>26</sub>H<sub>26</sub>O<sub>4</sub>·<sup>1</sup>/<sub>2</sub>H<sub>2</sub>O) C, H, O.

**6,8,8-Tripropyl desmosdumotin B (16)**. Method B. 75% two-step overall yield. Pale-yellow prisms, mp 115–116 °C (CH<sub>2</sub>Cl<sub>2</sub>–hexane). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.04 (1H, chelated-OH), 7.82–7.78 (m, 2H, 2'- and 6'-H), 7.64–7.54 (m, 3H, 3', 4' and 5'-H), 6.90 (s, 1H, 3-H), 2.44–2.36 (m, 2H, 6-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.26–2.14 (m, 2H, 8-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.36–2.18 (m, 2H, 8-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.54–1.41 (m, 2H, 6-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.21–1.04 (m, 2H, 8-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.01–0.84 (m, 2H, 8-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.95

(t, 3H, *J* = 7.3 Hz, 6-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.79 (t, 6H, *J* = 7.8 Hz, 8-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub> × 2). MS *m/z*: 379 (M<sup>+</sup> - 1). Anal. (C<sub>24</sub>H<sub>26</sub>O<sub>4</sub>) C, H, O.

**6,8,8-Triethyl-5-hydroxy-3-phenyl-4H-chromene-4,7(8H)-dione (33)**. Colorless prisms, mp 146–147 °C (EtOAc–hexane). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 13.09 (1H, chelated-OH), 8.08 (s, 1H, 2-H), 7.56–7.44 (m, 5H, Ar-H), 2.46 (q, 2H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 2.25–2.14 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 2.00–1.89 (m, 2H, 8-CH<sub>2</sub>CH<sub>3</sub>), 1.04 (t, 3H, *J* = 7.4 Hz, 6-CH<sub>2</sub>CH<sub>3</sub>), 0.67 (t, 6H, *J* = 7.4 Hz, 8-CH<sub>2</sub>CH<sub>3</sub> × 2). Anal. (C<sub>21</sub>H<sub>22</sub>O<sub>4</sub>·<sup>1</sup>/<sub>4</sub>H<sub>2</sub>O) C, H, O.

**6,8-Diethyl-2-(4-ethylphenyl)-5,7-dihydroxychromen-4-one (48)**. Pale-yellow powder, mp 263–264 °C (CH<sub>2</sub>Cl<sub>2</sub>–MeOH). <sup>1</sup>H NMR (300 MHz, 10% CD<sub>3</sub>OD in CDCl<sub>3</sub>) δ: 7.83 (d, 2H, *J* = 8.2 Hz, 2'- and 6'-H), 7.36 (d, 2H, *J* = 8.2 Hz, 3'- and 5'-H), 6.66 (s, 1H, 3-H), 2.91 (q, 2H, *J* = 7.3 Hz, 4'-CH<sub>2</sub>CH<sub>3</sub>), 2.80–2.66 (m, 4H, 6- and 8-CH<sub>2</sub>CH<sub>3</sub>), 1.35–1.22 (m, 6H, 6- and 8-CH<sub>2</sub>CH<sub>3</sub>), 1.18 (t, 3H, *J* = 7.3 Hz, 4'-CH<sub>2</sub>CH<sub>3</sub>). MS *m/z*: 338 (M<sup>+</sup>). Anal. (C<sub>21</sub>H<sub>22</sub>O<sub>4</sub>) C, H, O.

**Cytotoxic 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-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<sub>50</sub> 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. For the verapamil reversal experiments, cells were cotreated 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 (human 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 Lineberger Cancer Center (UNC-CH) or from ATCC (Rockville, MD), except KB-VIN which was a generous gift of Professor Y.-C. Cheng, Yale University. 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.

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**Supporting Information Available:** Elemental analysis data for compounds **3–16**, **33**, **40**, **46**, and **48**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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