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

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## Abstract

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, fifteen novel analogs (**7**–**15** and **21**–**26**) were synthesized, and evaluated for cytotoxity 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 cytotoxity against A549, A431, 1A9, and HCT-8 with $ED_{50}$ values of 1.0, 1.2, 0.9, and 1.3 μg/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 fivefold 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 thirteen 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**) has a distinctive chalcone skeleton with an unusual non-aromatic A ring possessing a *gem*-dimethyl

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group on C-3′ and 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 $IC_{50}$ values of 4.0 and 3.5 (μg/mL, respectively.[10] In addition, it was more active against drug-resistant KB-VIN cells than against the parent KB (epidermoid nasopharyngeal carcinoma) cell line. Thus, **1** is a promising new 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 two- to three-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 synthesis[11] 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 eq. of ethyl or propyl iodide in the presence of sodium methoxide provided the corresponding trialkylacetophenones (**32** and **33**, respectively). Similarly, treatment of **30**[12] with 1 mol eq. of ethyl iodide afforded **31**. Selective methylation of the resulting trialkylacetophenones, **31**–**33**, with an excess of $TMSCHN_2$ 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 $ED_{50}$ values (μg/mL) are listed in Table 1.

Compound **1** showed activity against A549, 1A9, HCT-8 and HUVEC with $ED_{50}$ 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 ($ED_{50}$ 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 $ED_{50}$ values of 0.9–2.3 μg/mL. In addition, **26** was not affected by multidrug resistant expressing P-glycoprotein, since it showed similar and potent cell growth inhibition of KB and

KB-VIN cell replication ($ED_{50}$ 0.9 μg/mL). The strong activity of **26** could be predicted since it combines the structural features of 4-bromo **2** and 3′,3′,5′-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 $ED_{50}$ 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 $ED_{50}$ values of 4.6, 3.3 and 2.3 μg/mL, respectively. These three analogs also inhibited the growth of A431 cells with $ED_{50}$ 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 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 two- to five-fold ratio of KB/KB-VIN selectivity (see **8**–**14**). However, the insertion of 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 compounds were also evaluated in a standard anti-angiogenesis assay using HUVEC. All tested compounds, except **20**, showed potent activity with $ED_{50}$ values less than 4.0 μg/mL. Compound **14** showed the highest potency with an $ED_{50}$ 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 non-specific. However, analogs **16**–**18**, in which the phenyl B-ring was replaced with 5-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 $ED_{50}$ value of 2.4 μg/mL against HUVEC compared to $ED_{50}$ 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) 5-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 cytotoxity 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 ($^1$H NMR) spectra were measured on a 300MHz 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 1N HCl, 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.

## 4-Fluoro desmosdumotin C (7)

Yellow prisms, mp 109–110 °C ($CH_2Cl_2$-hexane). $^1$H-NMR ($CDCl_3$): δ 19.18 (s, 1H, chelated-O*H*), 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, OC$H_3$), 2.00 (s, 3H, 5′-C$H_3$), 1.38 (s, 6H, 3′-C$H_3$×2). MS *m*/*z* 331 ($M^+$+1). Anal. ($C_{19}H_{19}FO_4$) C, H, O.

## 4-Methyl desmosdumotin C (8)

Yellow prisms, mp 110–111 °C ($CH_2Cl_2$-hexane). $^1$HNMR (300 MHz, $CDCl_3$): δ = 19.32 and 18.92 (3:1, each s, 1H, chelated-O*H*), 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, OC$H_3$), 2.00 and 1.96 (3:1, each s, 3H, 5′-C$H_3$), 1.47 and 1.38 (1:3, each s, 6H, 3′-C$H_3$×2). MS *m*/*z* 327 ($M^+$+1). Anal. ($C_{20}H_{22}O_4$) C, H, O.

## 2-Methyl desmosdumotin C (9)

Yellow prisms, mp 93–94 °C ($CH_2Cl_2$-hexane). $^1$H NMR (300 MHz, $CDCl_3$): δ = 19.32 and 18.62 (4:1, each s, 1H, chelated-O*H*), 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, OC$H_3$), 2.63 (s, 3H, Ar-C$H_3$), 2.15 and 2.01 (4:1, each s, 3H, 5′-C$H_3$), 1.52 (s, 6H, 3′-*CH*$_3$×2). MS *m*/*z* 327 ($M^+$+1). Anal. ($C_{20}H_{22}O_4$) C, H, O.

## 4-Ethyl desmosdumotin C (10)

Yellow prisms, mp 90–91 °C ($CH_2Cl_2$-hexane). $^1$H NMR (300 MHz, $CDCl_3$): δ 19.19 and 18.80 (2:1, each s, 1H, chelated-O*H*), 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, $OCH_3$), 2.68 (q, 2H, *J* = 7.7 Hz, Ar-*CH*$_2$CH$_3$), 2.00 and 1.96 (2:1. each s, 3H, 5′-C$H_3$), 1.47 and 1.38 (1:2, each s, 6H, 3′-C$H_3$×2), 1.25 (t, 3H, *J* = 7.7 Hz, Ar-CH$_2$C$H_3$). MS *m*/*z* 339 ($M^+$−1). Anal. ($C_{21}H_{24}O$) C, H, O.

### 2,6-Dimethyl desmosdumotin C (11)

Yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): δ 19.14 and 18.80 (2:1, each s, 1H, chelated-O*H*), 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, OC$H_3$), 2.44 (s, 6H, Ar-2,6-C$H_3$), 2.00 and 1.92 (2:1, each s, 3H, 5′-C$H_3$), 1.48 and 1.35 (1:2, each s, 6H, 3′-*C*$H_3$×2). MS *m*/*z* 341 ($M^+$+1). Anal. ($C_{21}H_{24}O_4$) C, H, O.

### 3,5-Dimethyl desmosdumotin C (12)

Yellow prisms, mp 105–106 °C ($CH_2Cl_2$-hexane). $^1$H NMR (300 MHz, $CDC1_3$): δ 19.20 (s, 1H, chelated-O*H*), 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, OC$H_3$), 2.34 (s, 6H, Ar-3,5-C$H_3$), 2.00 (s, 3H, 5′-C$H_3$), 1.39 (s, 6H, 3′-*C*$H_3$×2). MS *m*/*z* 341 ($M^+$+1). Anal. ($C_{21}H_{24}O_4$) C, H, O.

### 2,4,6-Trimethyl desmosdumotin C (13)

Yellow prisms, mp 87–88 °C (AcOEt-hexane). $^1$H NMR (300 MHz, $CDC1_3$): δ 19.17 and 18.48 (3:1, each s, 1H, chelated-O*H*), 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, OC$H_3$), 2.43 and 2.41 (3:1, each s, 6H, Ar-2,6-C$H_3$), 2.30 and 2.29 (1:3, each s, 3H, Ar-4-C$H_3$), 2.00 and 1.92 (3:1. each s, 3H, 5′-C$H_3$), 1.47 and 1.35 (1:3, each s, 6H, 3′-C$H_3$×2). MS *m*/*z* 355 ($M^+$+1). Anal. ($C_{22}H_{26}O_4 \cdot 1/8H_2O$) C, H.

### 3-Vinyl desmosdumotin C (14)

Yellow prisms, mp 76–77 °C ($CH_2Cl_2$-hexane). $^1$H NMR (300 MHz, $CDC1_3$): δ 19.19 and 18.79 (3:1, each s, 1H, chelated-O*H*), 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=$CH_2$), 5.81 (d, 1H, *J* = 17.4 Hz, Ar-CH=$CH_2$), 5.31 (d, 1H, *J* = 10.8 Hz, Ar-CH=$CH_2$), 3.96 and 3.89 (3:1, each s, 3H, OC$H_3$), 2.00 and 1.96 (3:1, each s, 3H, 5′-C$H_3$), 1.48 and 1.39 (1:3, each s, 6H, *3*′-C$H_3$×2). MS *m*/*z* 359 ($M^+$+1). Anal. ($C_{21}H_{22}O_4$) C, H, O.

### 2-[1-Hydroxy-3-(naphthalen-1-yl)allyidene]-5-methoxy-4,6,6-trimethylcyclohex-4-ene-1,3-dione (15)

Yellow prisms, mp 128–129 °C ($CH_2Cl_2$-hexane). $^1$H NMR (300 MHz, $CDCl_3$): δ 19.2 (s, 1H, chelated-O*H*), 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=$CH_2$), 5.81 (d, 1H, *J* = 17.4 Hz, Ar-CH=C$H_2$), 5.31 (d, 1H, *J* = 10.8 Hz, Ar-CH=$CH_2$), 3.96 and 3.89 (3:1, each s, 3H, OC$H_3$), 2.00 and 1.96 (3:1, each s, 3H, 5′-C$H_3$), 1.48 and 1.39 (1:3, each s, 6H, 3′-C$H_3$×2). MS *m*/*z* 363 ($M^+$+1). Anal. ($C_{23}H_{22}O_4$) C, H, O.

### 3′,3′-Dimethyl-5′-ethyl desmosdumotin C (21)

75% Yield. Yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): δ 19.37 and 18.90 (2:1, each s, 1H, chelated-O*H*), 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, OC$H_3$), 2.65 and 2.62 (2:1, each q, *J* = 7.4 Hz, 2H, 5′-C$H_2$CH$_3$), 1.62 and 1.53 (1:2, each s, 6H, 3′-C$H_3$×2), 1.32 and 1.29 (2:1, each t, *J* = 7.4 Hz, 3H, *5*′-$CH_2$C$H_3$). MS *m*/*z* 325 ($M^+$−1). Anal. ($C_{20}H_{22}O_4$) C, H, O.

### 3′,3′,5′-Triethyl desmosdumotin C (22)

57% Yield. Yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): δ 19.30 and 18.89 (3:2, each s, 1H, chelated-O*H*), 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, $OCH_3$), 2.60 and 2.56 (3:2, each q, 2H, *J* = 7.4 Hz, 5′-C*H*$_2$$CH_3$), 2.04-1.76 (m, 4H, 3′-C*H*$_2$$CH_3$×2), 1.20 and 1.16 (3:2, each t, 3H, *J* = 7.4 Hz, 5′-$CH_2$C*H*$_3$), 0.71 and 0.70 (2:3, each t, 3H, *J* = 7.4 Hz, 3′-$CH_2$C*H*$_3$×2). MS *m*/*z* 355 ($M^+$+1). Anal. ($C_{22}H_{26}O_4$) C, H, O.

### 4-Methyl-3′,3′,5′-triethyl desmosdumotin C (23)

63% Yield. Yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): δ 19.30 and 18.91 (3:2, each s, 1H, chelated-O*H*), 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, OC*H*$_3$), 2.60 and 2.56 (3:2, each q, 2H, *J* = 7.4 Hz, 5′-C*H*$_2$$CH_3$), 2.38 and 2.35 (3:2, each s, 3H, Ar-4-C*H*$_3$), 2.07-1.90 and 1.90-1.72 (3:2, each m, 4H, 3′-C*H*$_2$$CH_3$×2), 1.30-1.21 (m, 3H, 5′-$CH_2$C*H*$_3$), 0.70 (t, 6H, *J* = 7.4 Hz, 3′-$CH_2$C*H*$_3$×2). MS *m*/*z* 367 ($M^+$−1). Anal. ($C_{22}H_{24}O_4$) C, H.

### 4-Methyl-3′,3′,5′-triethyl desmosdumotin C (24)

61% Yield. Yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): δ 19.30 and 18.93 (2:1, each s, 1H, chelated-O*H*), 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, OC*H*$_3$), 2.55-2.50 (m, 4H, C*H*$_2$$CH_3$×2), 2.08-1.72 (m, 4H, C*H*$_2$$CH_3$×2), 1.33-1.10 (m, 6H, C*H*$_2$$CH_3$×2), 0.80-0.64 (m, 6H, C*H*$_2$$CH_3$×2). MS *m*/*z* 381 ($M^+$−1). Anal. ($C_{24}H_{30}O_4$) C, H, O.

### 3′,3′,5′-Tripropyl desmosdumotin C (25)

76% Yield. Yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): δ 19.29 and 18.90 (2:1, each s, 1H, chelated-O*H*), 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, OC*H*$_3$), 2.56-2.42 (m, 2H, 5′-C*H*$_2$Et), 2.00-1.65 (m, 4H, 3′-C*H*$_2$Et×2), 1.65-1.48 (m, 2H, 3′-$CH_2$C*H*$_2$$CH_3$), 1.16-0.96 (m, 7H, 5′-$CH_2$C*H*$_2$$CH_3$×2 and 3′-$CH_2CH_2$C*H*$_3$), 0.90-0.77 (m, 6H, 3′-$CH_2CH_2$C*H*$_3$×2). MS *m*/*z* 395 ($M^+$−1). Anal. ($C_{25}H_{32}O_4$) C, H, O.

### 4-Bromo-3′,3′,5′-tripropyl desmosdumotin C (26)

38% Yield. Yellow prisms, mp 114–115 °C. $^1$H NMR (300 MHz, $CDCl_3$): δ 19.28 and 18.81 (2:1, each s, 1H, chelated-O*H*), 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, OC*H*$_3$), 2.58-2.41 (m, 2H, 5′-C*H*$_2$Et), 2.00-1.80 (m, 4H, 3′-C*H*$_2$Et×2), 1.80-1.46 (m, 2H, 3′-$CH_2$C*H*$_2$$CH_3$), 1.18-0.93 (m, 7H, 5′-$CH_2$C*H*$_2$$CH_3$×2 and 3′-$CH_2CH_2$C*H*$_3$), 0.93-0.77 (m, 6H, 3′-$CH_2CH_2$C*H*$_3$×2). MS *m*/*z* 475 and 477 ($M^+$−1). Anal. ($C_{25}H_{31}O_4Br$) 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 volatile solvent, the residue was partitioned between EtOAc and in aqueous HCl. The water phase was extracted with EtOAc (×3). The combined organic layers were dried over $Na_2SO_4$

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). $^1$H NMR (300 MHz, DMSO-$d_6$): δ 19.00 (br s, 1H, chelated-O*H*), 2.48 [s, 3H, C(O)C*H*$_3$], 2.35 (q, 2H, *J* = 7.2 Hz, C*H*$_2$CH$_3$), 1.29 (s, 6H, 4-C*H*$_3$×2), 0.95 (t, 3H, 6-CH$_2$C*H*$_3$). MS *m/z* 223 ($M^+$−1). Anal. ($C_{12}H_{16}O_4$·1/8$H_2O$) 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 in aqueous HCl, then extracted with EtOAc (×3). The combined organic layers were dried over $Na_2SO_4$ 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). $^1$H NMR (300 MHz, DMSO-$d_6$): δ 18.98 (br s, 1H, chelated-O*H*), 2.51 [s, 3H, C(O)C*H*$_3$], 2.42 (q, 2H, *J* = 7.2 Hz, 6-C*H*$_2$CH$_3$), 1.81 (q, 4H, *J* = 7.4 Hz, 4-C*H*$_2$CH$_3$×2), 0.97 (t, 3H, *J* = 7.2 Hz, 6-CH$_3$C*H*$_3$), 0.57 (t, 3H, *J* = 7.2 Hz, 4-CH$_2$C*H*$_3$×2). MS *m/z* 251 ($M^+$+1). Anal. ($C_{21}H_{22}O_4$·1/16$H_2O$) 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). $^1$H NMR (300 MHz, $CD_3OD$): δ 2.53 [s, 3H, C(O)C*H*$_3$], 2.42 (t, 2H, *J* = 7.5 Hz, 6-C*H*$_2$Et), 1.94-1.74 (m, 4H, 4-C*H*$_2$Et×2), 1.54-1.38 (m, 2H, , 6-CH$_3$C*H*$_2$CH$_3$), 1.16-0.90 (m, 4H, 4- CH$_3$C*H*$_2$CH$_3$×2), 0.94 (t, 3H, *J* = 7.4 Hz, 6-CH$_3$CH$_2$C*H*$_3$), 0.81 (t, 6H, *J* = 7.1 Hz, 6-CH$_3$CH$_2$C*H*$_3$×2). MS *m/z* 293 ($M^+$−1). Anal. ($C_{17}H_{26}O_4$) 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 $TMSCHN_2$ (2 mL, 4.0 mmol, 2 M solution in diethyl ether) was added slowly at −78 °C under argon atmosphere and the mixture was stirred for 3 h. Acetic acid was then added to destroy the excess $TMSCHN_2$. 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. $^1$H NMR (300 MHz, $CDCl_3$): δ 18.97 and 18.14 (2:1, each s, 1H, chelated-O*H*), 3.97 and 3.90 (2:1, each s, 3H, OC*H*$_3$), 2.71 and 2.61 [1:2, each s, 3H, C(O)C*H*$_3$], 2.48 and 2.43 (2:1, each q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 1.45 and 1.34 (1:2, each s, 6H, 4-C*H*$_3$), 1.16 and 1.11 (2:1, each t, 3H, 6-CH$_2$C*H*$_3$×2). MS *m/z* 237 ($M^+$−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. $^1$H NMR (300 MHz, $CDCl_3$): δ 19.00 and 18.12 (3:2, each s, 1H, chelated-O*H*), 4.02 and 3.93 (3:2, each s, 3H, OC*H*$_3$), 2.71 and 2.64 [2:3, each s, 3H, C(O)C*H*$_3$], 2.57 and 2.51 (3:2, each q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.03-1.72 (m, 4H, 4- C*H*$_2$CH$_3$×2), 1.18 and 1.12 (3:2, each t, 3H, 6-CH$_2$C*H*$_3$), 0.67 and 0.65 (2:3, each t, 6H, 4-CH$_2$C*H*$_3$×2). MS *m/z* 267 ($M^+$+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. $^1$H NMR (300 MHz, $CDCl_3$): δ 18.99 and 18.15 (3:2, each s, 1H, chelated-O*H*), 3.98 and 3.90 (3:2, each s, 3H, OC*H*$_3$), 2.67 and 2.63 [2:3, each s, 3H, C(O) C*H*$_3$], 2.53-2.40 (m, 2H, 6-C*H*$_2$Et), 1.95-1.42 (m, 6H, 6-CH$_2$C*H*$_2$CH$_3$ and 4-C*H*$_2$Et×2), 1.10-0.95 (m, 7H, 6-CH$_2$CH$_2$C*H*$_3$ and 4-CH$_2$C*H*$_2$CH$_3$×2), 0.83 and 0.80 (3:2, each t, 6H, 4-CH$_2$CH$_2$C*H*$_3$×2). MS *m/z* 307 ($M^+$−1).

### Cytotoxic Activity Assay[15]

All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtitre 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-VTN (vincristine-resistant KB subline), 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 μg/mL kanamycin.

### Angiogenesis Assay

Method is according to the NCI's Angiogenesis Resource Center protocol. HUVEC were purchased from Cambrex Bio-science. HUVEC ($1.5\times10^3$) were plated in a 96-well plate in 100 μl of EGM-2 (Clonetec *H* CC3162). After 24 h (day zero), the test compound (100 μl) was added to each well at 2× the desired concentration in EGM-2 medium. One plate was stained with 0.5% crystal violet in 20% MeOH for 10 minutes, rinsed with water, and air-dried. The remaining plates were incubated for 72 h at 37 °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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

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## Abbreviations

**HUVEC**
: human umbilical vein endothelial cell

**KB**
: vincristine-resistant

**KB-VIN**
: expressing P-glycoprotein, cell subline

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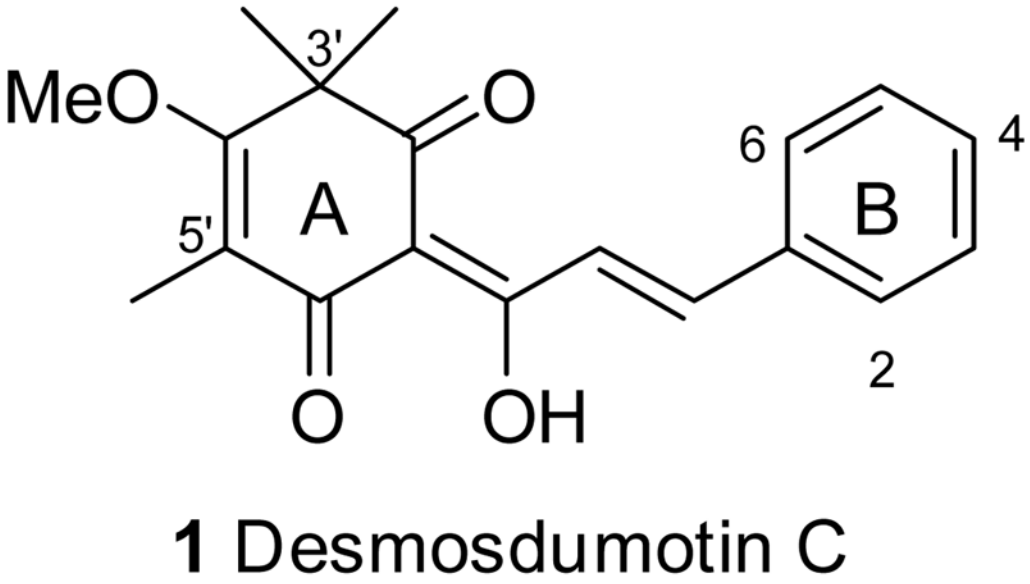
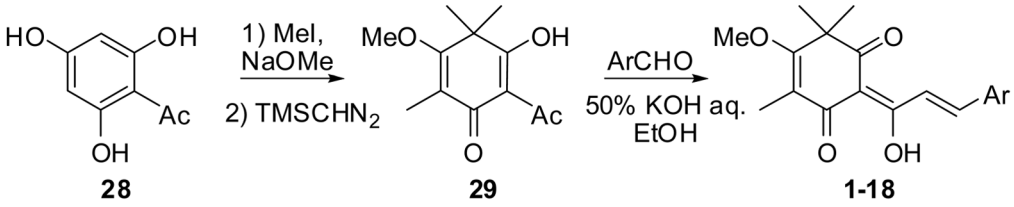
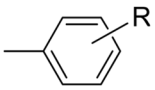
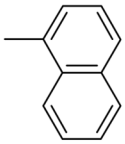
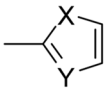


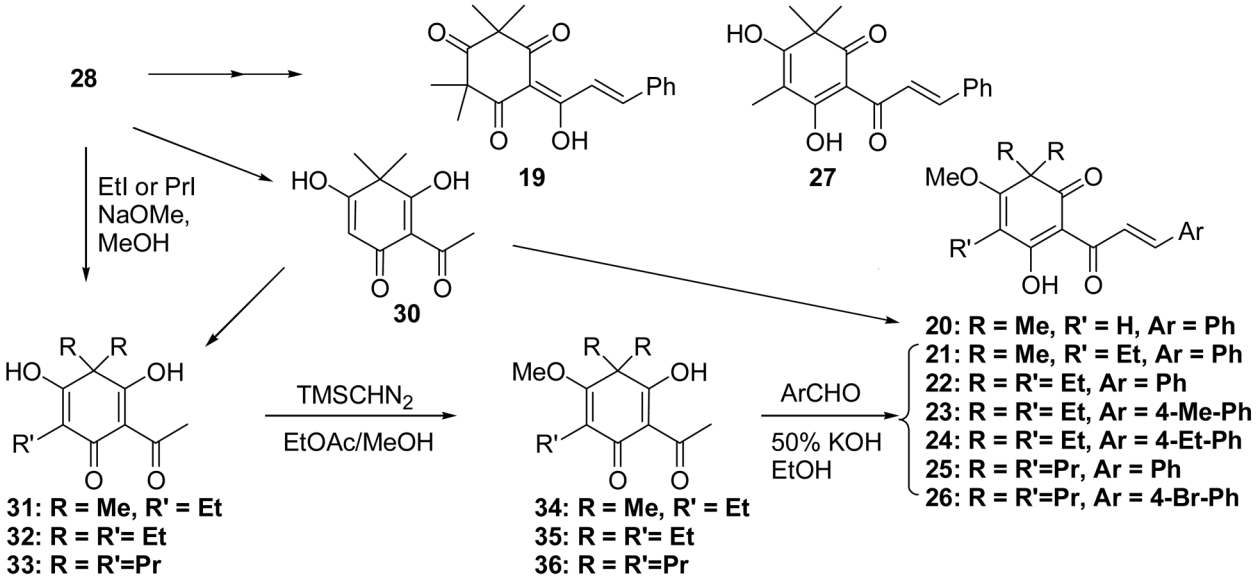

**1** Desmosdumotin C

**Figure 1.**
Structure of **1**




| Ar | R or X | | Yield (%) |
|---|---|---|---|
|  | H | **1** | 88[a,b] |
| | 4-Br | **2** | 61[b,c] |
| | 4-OMe | **3** | 82[c] |
| | 3-OMe | **4** | 66[c] |
| | 2-OMe | **5** | 89[c] |
| | 4-OH | **6** | 35[b,c] |
| | 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[b,c] |
| | X=S, Y=C | **17** | 61[b,c] |
| | X=S, Y=N | **18** | 50[b,c] |

**Scheme 1.**
Syntheses of **1**-Analogs: B-ring Modifications
[a] See reference [11].[b] Based on recovered starting material.[c] See reference [12].




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

**Table 1**

Cytotoxic Activity Data for **1**-Analogs

| Cmpd | ED$_{50}$ (μg/mL)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 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.

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