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Total synthesis and bioactivity of unique flavone desmosdumotin B and its analogs☆

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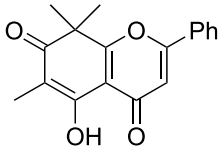
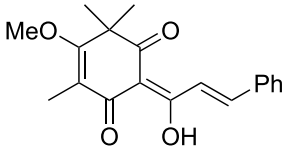
Abstract—The first total synthesis of a unique flavone natural product, desmosdumotin B (**1**), was accomplished. Furthermore, three novel flavonoids, **6**–**8**, and a novel chalcone, **9**, were synthesized. The new compounds were evaluated as in vitro inhibitors of human cancer cell growth. The synthetic **1** showed significant cytotoxic activity against a multi-drug resistant cell line (KB-VIN) with an ED_{50} value of 2.0 μg/mL compared to >40 μg/mL against the parental KB cell line. Flavone **7** displayed selective activity against 1A9 ovarian carcinoma with an ED_{50} value of 0.7 μg/mL. Selected **1**-analogs and synthetic intermediates were also screened for anti-tumor-promoting effects as inhibitors of EBV-EA activation. Among them, trihydroxyacetophenone derivatives **11** and **14** showed good activity.

Desmos (Annonaceae) spp. are used in China as folk medicines because of their antimalarial, insecticidal, antirheumatic, antispasmodic, and analgesic properties.[1] Several interesting flavonoids including chalcones with an oxygen function (OH or OMe) at the C-5 position (flavonoid numbering) and a highly substituted A-ring have been isolated from these species.[2] In particular desmosdumotin B (**1**)[3] was isolated and characterized as a unique flavonoid with an unusual A-ring structure. Desmosdumotin C (**2**)[4] is the possible biosynthetic precursor of **1**. Both compounds were isolated from the root bark of *Desmos dumosus* in 2001 and 2002, respectively.[3,4] Flavone **1**, also isolated from the stems and leaves of *Dasymaschalon trichophorum* and named dasytrichone by Liu et al. in 1992,[5] inhibited the metabolism of the carcinogen benzo[*a*]pyrene in hamster embryo cell culture, and thus was regarded as a potential cancer chemopreventive agent (see Fig. 1).



1 Desmosdumotin B



2 Desmosdumotin C

Figure 1. Structures of desmosdumotins B (**1**) and C (**2**).

We previously reported the total synthesis of desmosdumotin C (**2**) from trihydroxyacetophenone (**4**)[6] and biological evaluation of several derivatives with different terminal aromatic rings.[7] These promising results led us to synthesize desmosdumotin B (**1**). Intramolecular cyclization of **2** could give flavone **1** as well as other flavonoid compounds. Accordingly, herein, we report the syntheses of **1**, **6**, (±)-**8**, and **9** from **2**, as well as the novel flavone **7** from ceroptene (**3**)[7,8] and their cytotoxic activity data. We also describe the antitumor-promoting effects of selected compounds.

Treatment of **2** with catalytic iodine and concd H_2SO_4 in DMSO afforded desmosdumotin B (**1**) in 33% yield along with its 7-methoxy derivative **6** in 55% yield.[9,10] The resulting **6** was also converted to **1** in 68% yield by treatment with BBr_3 along with 18% of recovered

Keywords: Desmosdumotin B; Desmosdumotin C; Cytotoxicity; Anti-tumor promoter.
☆ Antitumor Agents 244 and Cancer Preventive Agents 4.
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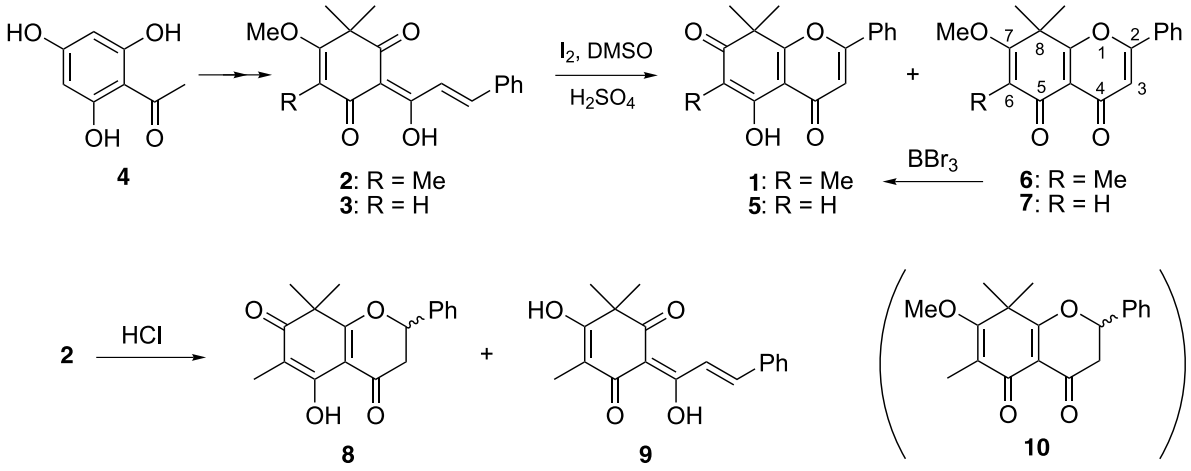

Scheme 1. Synthesis of **1** and its analogs **6**–**9**.

starting material.[11] The synthetic desmosdumotin B (**1**) has spectroscopic data identical with those of the natural product.[3] Flavone **7** was also obtained from ceroptene (**3**) in the same manner as described above. Unfortunately, demethylation of **7** to give **5** was unsuccessful using several methods. On the other hand, an intramolecular Michael reaction of **2** would provide flavanone **8** and/or the 7-methoxy derivative **10**. Although using a well-known procedure employing NaOAc gave only recovered starting material, (±)-**8** was obtained in 13% yield together with the uncyclized chalcone **9** in 40% yield by refluxing a MeOH–acetone solution of **2** with concentrated HCl (see Scheme 1).[12]

The compounds obtained were evaluated in vitro against human tumor cell replication in the following cell lines: lung carcinoma A549, ovarian carcinoma 1A9, breast cancer MCF-7, nasopharyngeal carcinoma KB, and KB-VIN, a multi-drug resistant (MDR) variant (Table 1). Synthesized desmosdumotin B (**1**) showed interesting and significant activity against the Pgp-multidrug resistant (KB-VIN) cell line with an ED_{50} value of 2.0 μg/mL, although it was inactive (ED_{50} > 40 μg/mL) against the KB cell line. Interestingly, the other two flavones, **6** and **7**, with a methoxy group at the C-7 position displayed no difference in KB and KB-VIN cell lines. Novel flavone **7** showed quite strong and selective in vitro cytotoxic activity against 1A9 ovarian carcinoma with an ED_{50} value of 0.7 μg/mL, although flavone **6** with a methyl group on the C-6 position was less active against all cell lines.

Selected synthetic **1**-analogs, **6**, **8**, **9**, and **16**,[7] and trihydroxyacetophenone derivatives, **11**–**15**,[6,7] which may be biosynthetic intermediates of the desmosdumotins, were examined in an in vitro assay for inhibition of EBV-EA (Epstein–Barr virus early antigen) activation in Raji cells (see Fig. 2). As shown in Table 2, all five hydroxyacetophenones showed greater activity than the desmosdumotins. Particularly, compounds **11** and **14**, which bear two acetoxy groups on the ring, exhibited significant inhibitory effects (94–95% inhibition of activation at 1×10^3 mol ratio/TPA, 61–62% inhibition at 500 mol ratio/TPA, and 29–31% inhibition of activation even at 100 mol ratio/TPA). Therefore, these compounds might be potential antitumor promoters, because inhibitory effect on EBV-EA activation generally correlates well with antitumor-promoting activity in vivo.[13] In vivo evaluation of **11** and further structural modifications are in progress.

Table 1. Activities of **1**-analogs against human tumor cell replication

Compound	ED_{50} (μg/mL)[a] versus cell line[b]				
	A549	1A9	MCF-7	KB	KB-VIN
1	28.0	36.0	>5 (21)	>40 (33)	2.0
2[c]	3.5	3.5	3.8	3.9	3.2
3[c]	>5 (48)	3.63	>5 (47)	4.1	2.9
6	33.5	38.0	>5 (25)	38.5	23.0
7	2.0	0.7	NA	1.8	1.8
8	13.5	8.0	>5 (44)	10.5	7.5
9	9.0	8.0	>5 (25)	10.5	6.5

[a] Cytotoxicity as ED_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay. If inhibition was observed at the highest test concentration but was less than 50%, the percentage inhibition is the values in parentheses. The values are averages from two independent determinations and variation between the replicates was not greater than 5% of any value.

[b] Human lung carcinoma (A549), human ovarian carcinoma (1A9), breast cancer (MCF-7), human epidermoid carcinoma of the nasopharynx (KB), and multi-drug resistant expressing P-glycoprotein (KB-VIN).

[c] See Ref. 7.

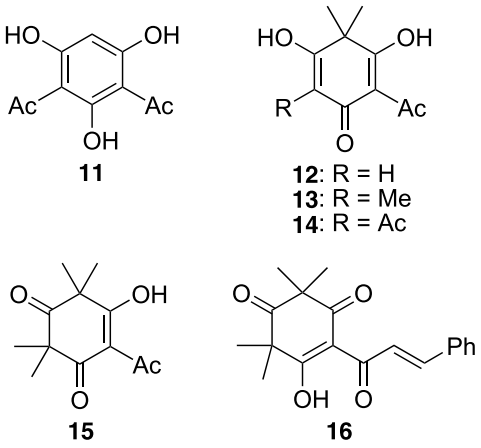

Figure 2. Structures of **11**–**16**.

Table 2. Relative ratio[a] of EBV-EA activation with respect to positive control (100%)[b]

Compound	% of control (% viability)[c] versus concentration (mol ratio/TPA)[d]			
	1000[d]	500[d]	100[d]	10[d]
1[e]	21.6 (70)[c] ± 1.1	52.8 ± 1.9	85.0 ± 2.9	100 ± 0.2
2[e]	10.1 (70) ± 0.5	41.6 ± 1.6	75.0 ± 2.1	100 ± 0.4
6	13.7 (70) ± 0.6	45.0 ± 1.7	79.1 ± 2.7	100 ± 0.3
8	15.9 (70) ± 1.0	47.6 ± 1.7	82.0 ± 2.9	100 ± 0.1
9	10.1 (70) ± 0.5	49.3 ± 1.8	76.7 ± 2.2	100 ± 0.3
11	4.0 (60) ± 0.3	38.1 ± 1.1	69.3 ± 1.9	90.6 ± 0.5
12	8.5 (60) ± 0.3	41.3 ± 1.3	72.6 ± 2.3	96.3 ± 0.4
13	7.3 (60) ± 0.8	40.5 ± 1.4	70.9 ± 2.0	95.1 ± 0.5
14	5.3 (60) ± 0.6	39.1 ± 1.3	71.4 ± 2.1	93.2 ± 0.4
15	9.0 (60) ± 0.5	44.7 ± 1.5	72.1 ± 2.2	98.3 ± 0.3
16	13.4 (70) ± 1.4	51.4 ± 1.8	77.2 ± 2.2	100 ± 0.1

[a] Values represent relative percentage to positive control value (100%). Data are expressed as means.
[b] For experimental details, see Ref. 15.
[c] Values in parentheses are viability percentages of Raji cells.
[d] TPA concentration is 20 ng/mL (32 pmol/mL).
[e] See Ref. 14.

Acknowledgments

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9. All chemicals and solvents were used as purchased. All melting points were measured on a Fisher–Johns melting point apparatus without correction. ^{1}H NMR and ^{13}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 parts per million. NMR spectra were referenced to the residual solvent peak; chemical shifts δ in ppm; apparent scalar coupling constants J in Hertz. Mass spectral data were obtained on a TRIO 1000 mass spectrometer. IR spectra were recorded on Perkin–Elmer 1320 spectrophotometer. 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}H and IR spectral analyses and MS analyses.
10. Preparation of **1** and **6**. To a solution of desmosdumotin C (**2**, 97 mg, 0.31 mmol) in anhydrous DMSO (3 mL), 0.5 v/w % of I_2 in DMSO (1 mL, 0.02 mmol of I_2) and concd H_2SO_4 (0.03 mL) were added. The reaction mixture was heated at 80 °C for 1 h. An additional identical portion of concd H_2SO_4 was added. After 1.5 h, the reaction mixture was quenched with ice-cold aqueous 10% $Na_2S_2O_3$ and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with EtOAc–hexane (1/2, v/v) and 5% MeOH in EtOAc to afford desmosdumotin B (**1**, 30 mg, 33%) and **6** (52 mg, 54%). Desmosdumotin B (**1**). Pale yellow prisms, mp: 224–224.5 °C (CH_2Cl_2–hexane) (lit. 217–218 °C).[3] IR (KBr): 1669, 1633, 1602, 1553, 1454, 1432, 1300, 1162, 873 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 13.1 (s, 1H, chelated-O*H*), 7.84–7.77 (m, 2H, Ar-2′,6′-*H*), 7.66–7.53 (m, 3H, Ar-3′,4′,5′-*H*), 6.90 (s, 1H, 3-*H*), 1.88 (s, 3H, 6-CH_3), 1.59 (s, 6H, 8-CH_3 × 2). MS *m*/*z* 297 (100) (M^++1), 282 (7) (M^++1-CH_3), 269 (3) (M^++1-CO). 7-Methoxy-6,8,8-trimethyl-2-phenyl-8*H*-chromene-4,5-dione (**6**). Pale yellow prisms, mp: 212–213 °C (EtOAc–hexane). IR (KBr): 1675, 1405, 1124 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.80–7.74 (m, 2H, Ar-2′,6′-*H*), 7.58–7.50 (m, 3H, Ar-3′,4′,5′-*H*), 6.84 (s, 1H, 3-*H*), 3.96 (s, 3H, 7-OCH_3) 1.99 (s, 3H, 6-C*H* $_3$), 1.65 (s, 6H, 8-CH_3 × 2). MS *m*/*z* 311 (M^++1).
11. Preparation of Desmosdumotin B (**1**) from **6**. To a solution of **6** (11 mg, 0.035 mmol) in 1.0 mL of anhydrous CH_2Cl_2, 0.2 mL of BBr_3 (1.0 M solution in CH_2Cl_2) was added at –78 °C. The mixture was allowed to warm to room temperature spontaneously. After stirring for 21 h, the mixture was partitioned between water and CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with EtOAc–hexane (1/2 v/v) and 5% MeOH in EtOAc to provide **1** (7 mg, 68%) and **6** (2 mg, 18%).
12. Preparation of **8** and **9**. To a solution of desmosdumotin C (**2**, 94 mg, 0.30 mmol) in anhydrous acetone (6 mL) and MeOH (3 mL), concd HCl (1 mL) was added. The reaction mixture was refluxed for 1 h. After cooling, the volatile solvents were removed in vacuo. The residue was parti-

tioned between EtOAc and water. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc–hexane (1/4–1/1, v/v) as an eluent to afford compounds **8** (12 mg, 13%) and **9** (36 mg, 40%).

(±)-7-Hydroxy-6,8,8-trimethyl-2-phenyl-2,3-dihydro-8*H*-chromene-4,5-dione (**8**). Yellow powder, mp: 159–160.5 °C (CH_2Cl_2–hexane). IR (KBr): 2978, 2930, 2359, 1669, 1622, 1559, 1413, 1290, 1150 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.62 (s, 1H, O*H*, D_2O-exchangeable), 7.52–7.38 (m, 5H, Ar-*H*), 5.58 (dd, 1H, J = 13.8, 3.8 Hz, 2-*H*), 3.10 (dd, 1H, J = 17.3, 13.8 Hz, 3-H_{ax}), 2.88 (dd, 1H, J = 17.3, 3.8 Hz, 3-H_{eq}), 1.81 (s, 3H, 5-CH_3), 1.45 (s, 3H, 8-CH_3), 1.41 (s, 3H, 8-CH_3). MS m/z 299 (M^++1).

5-Hydroxydesmosdumotin C (**9**). Yellow powder, mp: 150–151 °C (CH_2Cl_2–hexane). IR (KBr): 3264 (br), 1621, 1510, 1447, 1470, 1433, 1230, 1117 cm^{-1}. ^1H NMR ($CDCl_3$): δ 19.19 (s) and 18.71 (s) (3:1, 1H, chelated-O*H*), 8.59 (d) and 8.33 (d) (1:3, 1H, J = 15.4 Hz, trans-olefinic proton), 7.96 (d) and 7.92 (d) (1:3, 1H, J = 15.4 Hz, trans-olefinic proton), 7.74–7.62 (m, 2H, Ar-2″,6″-*H*), 7.47–7.34 (m, 3H, Ar-3″,4″,5″-*H*), 5.89 (s) and 5.36 (s) (3:1, 1H, 5-O*H*, D_2O-exchangeable), 1.93 (s) and 1.90 (s) (3:1, 3H, 4-CH_3), 1.54 (s) and 1.45 (s) (1:3, 3H, 6-$CH_3 \times 2$). MS m/z 297 (M^+–1). Compound **9** was obtained as a mixture of isomers. The major enolic isomer is shown in Scheme 1. As discussed and shown in our prior paper (Ref. 7), conceivable structures of the minor enolic isomers would have a cinnamoyl side chain with a conjugated enol at position 1 or 3.

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