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Article

Cytotoxic Chalcones from *Desmodium oxyphyllum*

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Three new chalcones were isolated from *Desmodium oxyphyllum*. Their structures were elucidated by spectroscopic methods including extensive 1D- and 2D-nuclear magnetic ressonance techniques. The compounds were evaluated for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7). The results showed that the compounds exhibited weak cytotoxicity against some selected cell lines with IC_{50} values ranging from 3.6 to 8.9 μM.

Keywords: chalcones, cytotoxicity, *Desmodium oxyphyllum*

Introduction

Desmodium oxyphyllum (Leguminosae) is a sub-shrub that grows to a height between 30 cm and 1.5 m in southern China. It has been used in folk medicine to treat rheumatic pains, leucorrhea disorder in woman, snake bites, infantile malnutrition and measles, and to reduce traumatic swelling and pain.[1] Previous phytochemical studies of *D. oxyphyllum* revealed the presence of flavonols, isoflavones, and coumaronochromones.[1,2] Motivated by a search for new bioactive metabolites from local plants, our group investigated the chemical constituents of the whole plant of *D. oxyphyllum* growing in Puer Prefecture, which led to the isolation and characterization of three new chalcones (**1**-**3**), together with known isobavachromene (**4**),[3] artonin A (**5**),[4] licoflavonol (**6**),[5] leachianone G (**7**),[6] kenusanone I (**8**),[7] sophoraflavanone B (**9**),[8] and naringenin (**10**).[9] This paper deals with the isolation and structural elucidation of these compounds, and the evaluation of the cytotoxicity of **1**-**3** against human tumor cell lines human acute promyelocytic leukemia cells (NB4), human lung adenocarcinoma (A549), human neuroblastoma (SHSY5Y), human prostate (PC3), and human breast adenocarcinoma (MCF7).

Experimental

General experimental procedures

UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Bruker Tenor 27 spectrophotometer was used for scanning infrared (IR) spectroscopy with KBr pellets. 1D- and 2D- nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX-500 NMR spectrometer with tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals [$(CD_3)_2CO$]. High-resolution electrospray ionisation mass spectrometry (HRESIMS) was performed on a VG Autospec-3000 spectrometer. Semipreparative high performance liquid chromatography (HPLC) was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography (CC) was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by thin layer chromatography (TLC), and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

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Plant material

The whole plant of *D. oxyphyllum* was collected in Puer Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Dr Yuan N. from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (YNNU 2010-10-10) has been deposited in our laboratory.

Extraction and isolation

The air-dried and powdered *D. oxyphyllum* (1.5 kg) plant material was extracted four times with 80% aqueous ethanol (4 × 5 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (140 g) was decolorized by MCI. The portion of the extract soluble in 90% methanol (50 g) was chromatographed on a silica gel column eluting with a $CHCl_3$-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions (A-F). Separation of fraction C (8:2, 10.4 g) by silica gel CC, eluted with petroleum ether-acetone (9:1-1:2), yielded fractions C1-C7. Fraction C2 (8:2, 1.01 g) was subjected to silica gel CC using petroleum ether-acetone and semi-preparative HPLC (40% MeOH-H_2O) to give **1** (6.6 mg), **2** (7.3 mg), **5** (1.8 mg), **7** (2.2 mg), and **10** (5.9 mg). Fraction C3 (7:3, 0.64 g) was subjected to silica gel CC using petroleum ether-acetone and semi-preparative HPLC (35% MeOH-H_2O) to give **3** (6.1 mg), **4** (2.5 mg), **6** (1.2 mg), and **8** (8.2 mg). Fraction C4 (6:4, 0.28 g) was subjected to semi-preparative HPLC (32% MeOH-H_2O) to give **9** (1.7 mg).

Oxyphyllumchalcone A (**1**)

Pale yellow gum; UV (MeOH) λ_{max}/nm 210, 252, 362; IR (KBr) ν_{max}/cm^{-1} 3415, 3140, 3076, 2951, 2837, 1685, 1605, 1532, 1467, 1335, 1188, 1064, 892, 738; ^{1}H and ^{13}C NMR data [500 and 125 MHz, $(CD_3)_2CO$], see Table 1; positive ESIMS *m/z* 377 $[M + Na]^+$; positive HRESIMS *m/z,* calcd. for $C_{21}H_{22}O_5Na$ $[M + Na]^+$: 377.1365, found: 377.1373.

Oxyphyllumchalcone B (**2**)

Pale yellow gum; UV (MeOH) λ_{max}/nm 210, 250, 360; IR (KBr) ν_{max}/cm^{-1} 3418, 3142, 3074, 2953, 2837, 1684, 1600, 1527, 1465, 1332, 1180, 1063, 899, 736; ^{1}H and ^{13}C NMR data [500 and 125 MHz, $(CD_3)_2CO$], see Table 1; positive ESIMS *m/z* 377 $[M+Na]^+$; positive HRESIMS *m/z,* calcd. for $C_{21}H_{22}O_5Na$ $[M+Na]^+$: 377.1365, found: 377.1358.

Oxyphyllumchalcone C (**3**)

Pale yellow gum; UV (MeOH) λ_{max}/nm 210, 252, 364; IR (KBr) ν_{max}/cm^{-1} 3408, 3140, 3085, 2962, 2835, 1680, 1602, 1531, 1468, 1335, 1182, 1065, 898, 734; ^{1}H and ^{13}C NMR data (500 and 125 MHz, $(CD_3)_2CO$), see Table 1; positive ESIMS *m/z* 393 $[M+Na]^+$; positive HRESIMS *m/z,* calcd. for $C_{21}H_{22}O_6Na$ $[M+Na]^+$: 393.1314, found: 393.1322.

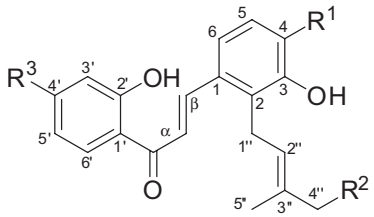
Cytotoxicity assay

Colorimetric assays were performed to evaluate cytotoxicity. Human acute promyelocytic leukemia cells (NB4), human lung adenocarcinoma (A549), human neuroblastoma (SHSY5Y), human prostate (PC3), and human breast adenocarcinoma (MCF7) tumor cell lines were purchased from the American Type Culture Collection (ATCC). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) or Dulbecco's modification of Eagle's medium (DMEM) medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO). Briefly, 100 µL of suspended adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition. In addition, suspended cells were seeded just before drug addition, with an initial density of 1×10^5 cells mL^{-1} in 100 µL of medium. Each tumor cell line was exposed to each test compound at various concentrations in triplicate for 48 h; Paclitaxel (Sigma, purity > 95%) was used as a positive control. After the incubation, MTT (100 µg) was added to each well, and the incubation was continued for 4 h at 37 °C. The cells were lysed with 100 µL of 20% SDS-50% DMF after removal of 100 µL of the medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.[10]

Results and Discussion

Whole plants of *D. oxyphyllum* were extracted with 80% aqueous ethanol. The extract was subjected repeatedly to column chromatography on silica gel, RP-18, and semi-preparative RP-HPLC separation to afford compounds **1**-**3**. The ^{1}H and ^{13}C NMR data of the compounds **1**-**3** are listed in Table 1.

Compound **1** gave a *quasi*-molecular ion by HRESIMS at *m/z* 377.1373 $[M + Na]^+$ corresponding to the molecular formula $C_{21}H_{22}O_5$. The ^{1}H and ^{13}C NMR signals of **1**



1 R^1 = OH, R^2 = H, R^3 = OMe
2 R^1 = OMe, R^2 = H, R^3 = OH
3 R^1 = OH, R^2 = OH, R^3 = OMe

Figure 1. Structures of chalcones from *D. oxyphyllum.*

(Table 1) were assigned by the DEPT, HSQC and HMBC spectra. The ^{1}H, ^{13}C, and DEPT NMR spectra showed signals for 21 carbons and 24 hydrogen atoms. The carbon signals [δ_C 126.9 s, 130.2 s, 144.0 s, 148.0 s, 113.8 d, 120.0 d, 114.2 s, 164.5 s, 102.9 d, 168.5 s, 107.6 d, 133.1 d, 143.2 d, 119.2 d], the characteristic protons signals for a 1,2,3,4-tetrasubstituted aromatic ring at δ_H 6.80 (d, 1H, *J* 8.5 Hz, Ph-H), 7.42 (d, 1H, *J* 8.5 Hz, Ph-H), and a 1,2,4-trisubstituted aromatic ring at δ_H 6.37 (d, 1H, *J* 2.2 Hz, Ph-H), 6.45 (dd, 1H, *J* 8.8 Hz, 2.2, Ph-H), and 8.08 (d, 1H, *J* 8.8 Hz, Ph-H), as well as two olefinic protons at δ_H 7.65 (d, 1H, *J* 15.1 Hz, CH) and 8.18 (d, 1H, *J* 15.1 Hz, CH), indicated the NMR pattern of a chalcone. The ^{1}H NMR spectrum (Table 1) revealed a triplet at δ_H 5.14 (t, 1H, *J* 6.8 Hz, CH), a doublet at δ_H 3.60 (d, 2H, *J* 6.8 Hz, CH_2), a singlet at δ_H 1.83 (s, 3H, CH_3), and a singlet at δ_H 1.63 (s, 3H, CH_3), which established the presence of a prenyl group. HMBC correlations (Figure 2) of H-1" (δ_H 3.60) with C-1, C-2, C-3, and those of H-2" (δ_H 5.14) with C-2, together with the ^{1}H-^{1}H COSY correlations H-1"/H-2", confirmed the prenyl group at C-2 in **1**. The connectivity of C-1' and the carbonyl carbon was established by the HMBC corrections from H-6' (δ_H 8.08) to C-1' and the carbonyl carbon, and from H-3' (δ_H 6.37) to C-1'. The HMBC correlations of three hydroxy protons at δ_H 12.51, 12.83 and 13.17, with C-4 (δ_C 148.0 s)/C-3 (δ_C 144.0 s)/C-5 (δ_C 113.0 d), C-4 (δ_C 148.0 s)/C-3 (δ_C 144.0 s)/C-1 (δ_C 126.1 s), and C-2' (δ_C 164.5 s), respectively, placed the phenolic OH groups at C-4, C-3, and C-2', respectively. A methoxy group was located at C-4' from HMBC and ROESY correlations of its signal at δ_H 3.80 with C-4' (δ_C 168.5 s), H-3' (δ_H 6.37, d, 1H, *J* 2.2 Hz, Ph-H) and H-5' (δ_H 6.45, dd, 1H, *J* 8.8 Hz, 2.2, Ph-H). Doublets at H-5, H-6, H-3', H-6' as well as a doublet of doublets at H-5' indicated no substituent at C-5, C-6, C-3', C-5' and C-6'. Thus, the structure of **1** was established as shown, and named as oxyphyllumchalcone A.

Compound **2**, obtained as a pale yellow gum, showed a *quasi*-molecular ion at *m/z* 377.1358 [M + Na]$^+$ in the HRESIMS, corresponding to the molecular formula $C_{21}H_{22}O_5$. The ^{13}C NMR and DEPT spectra of compound **2** were similar to those of **1**, except that C-4 was downfield shifted ($\Delta\delta_C$ +2.2 ppm), and C-4' was upfield shifted ($\Delta\delta_C$ −2.4 ppm). Detailed comparison of the NMR spectra of **1** and **2** showed, as major difference, the position of the methoxy group, which was placed at C-4 in **2**, as indicated by the HMBC correlation of the methoxy protons at δ_H 3.80 with C-4 (δ_C 150.2). Accordingly, the structure of oxyphyllumchalcone B (**2**) was determined as shown.

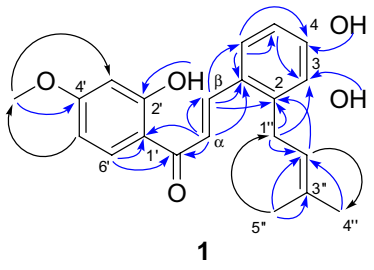


Figure 2. Selected HMBC ( ) and ROESY ( ) correlations of **1**.

Compound **3**, obtained as a pale yellow gum, had the molecular formulas $C_{21}H_{22}O_6$ as determined by positive HRESIMS at *m/z* 393.1322 [M + Na]$^+$. Comparison of ^{13}C NMR data between **3** and **1** suggested that **3** was similar to **1** except for an additional hydroxy group at C-4" in **3**. This was further confirmed by an oxygenated signal of C-4" (δ_C 68.9 t, δ_H 3.96 s, 2H, CH_2), the lack of the one singlet signal of the methyl group, and the HMBC correlations from H_2-4" to C-2", C-3", and C-5". The ROESY correlations of H-1" with H-5", and of H-2" with H-4" indicated an *E*-configuration for the C-2", C-3" double bond [Figure S17, in the Supplementary Information (SI) section].[11,12] Accordingly, the structure of oxyphyllumchalcone C (**3**) was determined as shown.

Since some previously reported flavonoids from *Desmodium* plants exhibited cytotoxicity,[13,14] we tested compounds **1**-**3** for cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) using the MTT method as reported previously.[15] Paclitaxel was used as the positive control. The results are depicted in Table 2.

Conclusions

Three new chalcones were isolated from *D. oxyphyllum*. The structures of **1-3** were elucidated by spectroscopic methods including extensive 1D- and 2D-NMR techniques. These three compounds presented a weak cytotoxic activity against tumor cell lines NB4, A549, SHSY5Y, PC3, and MCF7.

Table 1. ^{1}H and ^{13}C NMR data of compounds **1**-**3** in $(CD_3)_2CO$ (δ in ppm)

No.	**1**		**2**		**3**	
	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)
1	126.9 s	–	126.1 s	–	126.6 s	–
2	130.2 s	–	130.9 s	–	131.0 s	–
3	144.0 s	–	143.4 s	–	144.1 s	–
4	148.0 s	–	150.2 s	–	147.3 s	–
5	113.8 d	6.80 (d, 1H, *J* 8.5, Ph-H)	113.0 d	6.87 (d, 1H, *J* 8.5, Ph-H)	113.6 d	6.81 (d, 1H, *J* 8.5, Ph-H)
6	120.0 d	7.42 (d, 1H, *J* 8.5, Ph-H)	120.2 d	7.47 (d, 1H, *J* 8.5, Ph-H)	120.0 d	7.43 (d, 1H, *J* 8.5, Ph-H)
1'	114.2 s	–	114.9 s	–	114.6 s	–
2'	164.5 s	–	164.1 s	–	164.2 s	–
3'	102.9 d	6.37 (d, 1H, *J* 2.2, Ph-H)	103.9 d	6.37 (d, 1H, *J* 2.2, Ph-H)	102.7 d	6.38 (d, 1H, *J* 2.2, Ph-H)
4'	168.5 s	–	166.1 s	–	168.2 s	–
5'	107.6 d	6.45 (dd, 1H, *J* 8.8, 2.2, Ph-H)	108.7 d	6.49 (dd, 1H, *J* 8.8, 2.2, Ph-H)	107.3 d	6.48 (dd, 1H, *J* 8.8, 2.2, Ph-H)
6'	133.1 d	8.08 (d, 1H, *J* 8.8, Ph-H)	133.1 d	8.09 (d, 1H, *J* 8.8, Ph-H)	133.1 d	8.04 (d, 1H, *J* 8.8, Ph-H)
1"	25.3 t	3.60 (d, 2H, *J* 6.8, CH_2)	25.2 t	3.60 (d, 2H, *J* 6.8, CH_2)	23.2 t	3.43 (d, 2H, *J* 6.8, CH_2)
2"	124.0 d	5.14 (t, 1H, *J* 6.8, CH)	123.9 d	5.13 (t, 1H, *J* 6.8, CH)	123.2 d	5.21 (t, 1H, *J* 6.8, CH)
3"	132.0 s	–	132.0 s	–	134.5 s	–
4"	26.0 q	1.63 (s, 3H, CH_3)	25.9 q	1.68 (s, 3H, CH_3)	68.9 t	3.96 (s, 2H, CH_2)
5"	18.0 q	1.83 (s, 3H, CH_3)	17.9 q	1.85 (s, 3H, CH_3)	14.3 q	1.68 (s, 3H, CH_3)
α	119.2 d	7.65 (d, 1H, *J* 15.1, CH)	119.4 d	7.63 (d, 1H, *J* 15.1, CH)	119.1 d	7.62 (d, 1H, *J* 15.1, CH)
β	143.2 d	8.18 (d, 1H, *J* 15.1, CH)	143.0 d	8.19 (d, 1H, *J* 15.1, CH)	143.0 d	8.20 (d, 1H, *J* 15.1, CH)
C=O	192.3 s	–	192.5 s	–	192.5 s	–
4-OMe	–	–	55.9 q	3.80 (s, 3H, CH_3)	–	–
4'-OMe	56.0 q	3.80 (s, 3H, CH_3)	–	–	56.0 q	3.80 (s, 3H, CH_3)
3-OH	–	12.83 (s, 1H, OH)	–	12.88 (s, 1H, OH)	–	12.85 (s, 1H, OH)
4-OH	–	12.51 (s, 1H, OH)	–	–	–	12.50 (s, 1H, OH)
2'-OH	–	13.17 (s, 1H, OH)	–	13.45 (s, 1H, OH)	–	13.18 (s, 1H, OH)
4'-OH	–	–	–	13.66 (s, 1H, OH)	–	–

Table 2. Cytotoxicity data (IC_{50}, μM) for compounds **1**-**3** from *D. oxyphyllum*

Compound	NB4	A549	SHSY5Y	PC3	MCF7
1	> 10	8.2	5.6	> 10	6.5
2	3.6	5.8	> 10	8.7	> 10
3	8.9	7.6	> 10	8.5	5.7
Paclitaxel	0.03	0.02	0.2	0.2	0.1

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br.

Acknowledgements

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