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TWO NEW CHROMANONE DERIVATIVES FROM THE ROOTS AND STEMS OF *NICOTIANA TABACUM* AND THEIR CYTOTOXICITY

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Abstract – Two new chromanone derivatives, tabchromones A and B (**1**-**2**), together with four known compunds (**3**-**6**) were isolated from the roots and stems of *Nicotiana tabacum*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR techniques. Compounds **1-6** were tested for their in vitro cytotoxicity against five human tumor (NB4, A549, SHSY5Y, PC3, and MCF7) cell lines. Compound **1** showed significant inhibitory effect against SHSY5Y cell line, with IC_{50} values of 2.8 μM, and compounds **2-4** showed moderate activity for some selected cell lines, with IC_{50} values in the range of 4.8-8.0 μM.

INTRODUCTION

Nicotiana tabacum L. is one of the most commercially valued agricultural crops in the world.[1,2] Its leaves are the most important row material for cigarette industry. In addition to being used in cigarette industry, *N. tabacum* is also used as insecticides, anesthetics, diaphoretics, sedatives, and emetic agents in Chinese folklore medicines because of its containing many useful chemical compounds.[1,3] The stems and roots of *N. tabacum* are rich in secondary metabolities and are normally used as organic fertilizer. The

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multipurpose utilization of the stems and roots of *N. tabacum* is an interesting focus, and receives more and more attentions.[4,5]

Our previous investigation of this species led to the discovery of a number of new compounds, and those compounds were found to show various bioactivaties.[6-9] With the aim of continuing efforts to utilize *N. tabacum* and identify bioactive natural products the phytochemical investigation of the roots and stems of Honghua Dajinyuan (a variety of *N. tabacum*) was carried out, and led to two new chromanone derivatives (**1**-**2**), together with four known chromone derivatives (**3**-**6**). This paper reports the isolation, structural elucidation, and their cytotoxicity.

RESULTS AND DISCUSSION

The 90% aqueous ethanol extract prepared from the roots and stems of *N. tabacum* was subjected repeatedly to column chromatography on silica gel, sephadex LH-20, RP-18 and preparative HPLC to afford two new chromanone derivatives, tabchromones A and B (**1**-**2**), together with four known compunds (**3**-**6**), The structures of compounds **1**-**6** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of the compounds **1** and **2** were listed in Table 1. The known compounds, compared with literature data, were identified as: 6-(3-hydroxy-4-methoxystyryl)-4-methoxy-2*H*-pyran-2-one (**3**),[10] pestaloficiol G (**4**),[11] takanechromone C (**5**),[12] greveichromenol (**6**).[13]

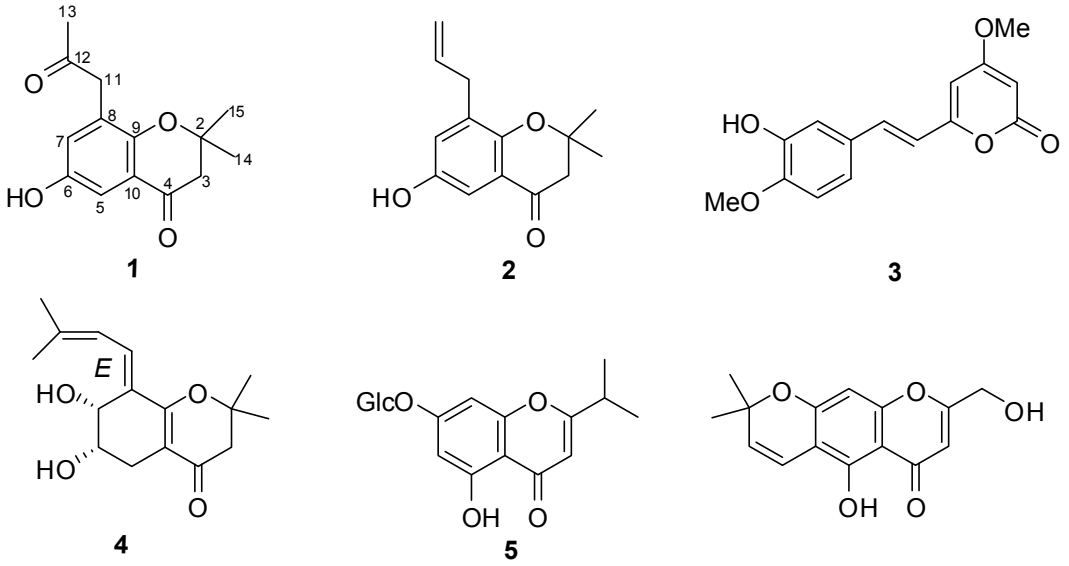

Figure 1. The chromone derivatives form N. tabacum

Compound **1** was obtained as pale yellow oil. It gives a parent ion by HR-ESIMS at m/z 247.0976 $[M-H]^-$ (calcd for 247.0976) corresponding to a molecular formula $C_{14}H_{16}O_4$, requiring seven degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **1** along with analysis of the DEPT spectra (Table 1)

displayed 14 carbon signals and 16 proton signals, respectively, corresponding to an chromanone nucleus[10] (δ_C 79.2 s, 50.3 t, 192.0 s, 108.0 d, 151.0 s, 123.7 d, 131.6 s, 152.2 s, 120.9 s, 25.9 q (2C)), an acetonyl group (-$CH_2C(O)CH_3$) (δ_C 49.8 t, 206.8 s, 30.0 q; δ_H 4.08 s, 2.65 s), and a phenolic hydroxy group (δ_H 8.43 br. s). Strong absorption bands accounting for hydroxy (3436 cm^{-1}), carbonyl group (1722, 1670 cm^{-1}) and aromatic groups (1615, 1556, 1436 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 260 and 210 nm, which confirmed the existence of the aromatic functions. The HMBC correlations (Figure 2) of H-11 (δ_H 4.08) with C-7 (δ_C 123.7), C-8 (δ_C 131.6) and C-9 (δ_C 152.2), of H-7 (δ_H 6.89) with C-11 (δ_C 49.8) indicated that the acetonyl group should be located at C-8 on the chromone ring. The phenolic hydroxy group located at C-6 was supported by the HMBC correlations of the hydroxy proton (δ_H 8.43) with C-5 (δ_C 108.0), C-6 (δ_C 151.0), and C-7 (δ_C 123.7). Thus, the structure of **1** was established as shown, and given the name as tabchromone A.

Compound **2** was also obtained as pale yellow oil, and showed quasi molecular ion at *m/z* 231.1027 $[M-H]^-$ in the HRESIMS (calcd *m/z* 231.1021), corresponding to the molecular formula of $C_{14}H_{16}O_3$. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1** in C-2～C-10, C-14 and C-15. The obvious chemical shift differences resulted from the disappearance of an acetonyl group signals, and appearance of a 2-propenyl group signals (δ_C 39.0 t, 137.9 d, 117.8 t; δ_H 3.17 m, 5.96 m, 5.03 m) in **2**. This indicated that the acetonyl group in **1** was substituted by a 2-propenyl group in **2**. The HMBC correlations of H-11 (δ_H 3.17) with C-7 (δ_C 122.2), C-8 (δ_C 133.0) and C-9 (δ_C 152.4), of H-12 (δ_H 5.96) with C-8 (δ_C 133.0), of H-7 (δ_H 6.91) with C-11 (δ_C 39.0) indicated that the 2-propenyl group should be located at C-8 of the chromone ring. Thus, the structure of **2** was established and it was named tabchromone B.

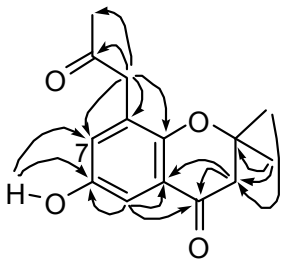

Figure 2. Key HMBC correlations ( ) of **1**

Table 1. ^1H NMR and ^{13}C NMR data of compounds **1** and **2** in $CDCl_3$

No.	**1**		**2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
2	79.2 s		79.0 s	
3	50.3 t	2.65 s	49.8 t	
4	192.0 s		191.7 s	
5	108.0 d	7.08, d, *J* = 2.4	107.8 d	7.09, d, *J* = 2.4
6	151.0 s		151.3 s	
7	123.7 d	6.89, d, *J* = 2.4	122.2 d	6.91, d, *J* = 2.4

8	131.6 s		133.0 s	
9	152.2 s		152.4 s	
10	120.9 s		119.1 s	
11	49.8 t	4.08 s	39.0 t	3.17 m
12	206.8 s		137.9 d	5.96 m
13	30.0 q	2.65 s	117.8 t	5.03 m
14,15	25.9 q		26.1 s	1.46 s
OH-6		8.43 brs		8.49 brs

Since certain of the chromone derivatives exhibit potential cytotoxicity,[14-16] the compounds **1**-**6** were tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) using the MTT method as reported previously.[17] Taxol was used as the positive control.

Table 2. The cytotoxicity data for the compounds **1**-**6**

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	8.3	5.1	2.8	8.7	>10
2	>10	4.8	7.2	>10	6.5
3	6.2	8.0	>10	7.4	>10
4	7.6	>10	8.8	>10	>10
5	>10	>10	>10	>10	>10
6	>10	>10	>10	>10	>10
Taxol	0.03	0.02	0.2	0.2	0.1

The results were shown in Table 2. Compounds **5** and **6** showed low active (IC_{50} values >10 μM) for all tested tumor cell lines. Compound **1** showed high cytotoxicity against SHSY5Y cell with IC_{50} values of 2.8 μM. Compounds **2-4** also showed moderate cytotoxicity for some selected cell line with IC_{50} valve below 10 μM.

EXPERIMENTAL

General. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10～40 μm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

Plant material. The roots and stems of Honghua Dajinyuan (a variety of *N. tabacum*) were collected in Yuxi Prefecture, Yunnan Province, People's Republic of China, in September 2010.

Extraction and Isolation. The air-dried and powdered roots and stems of *N. tabacum* (5.0 kg) were extracted four times with 90% aq. EtOH (4 ×5.0 L) at room temperature and filtered. The crude extract (298 g) was applied to silica gel (200 – 300 mesh) column chromatography, eluting with a $CHCl_3$-$(Me)_2CO$ gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. The further separation of fraction C (8:2, 11.5 g) by silica gel column chromatography, eluted with $CHCl_3$-MeOH (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures C1–C5. Fraction C1 (9:1, 1.57 g) was subjected to preparative HPLC (42% MeOH, flow rate 12 mL/min) to give **1** (10.6 mg), **2** (14.8 mg), **3** (15.2 mg), **4** (22.5 mg), and **6** (13.2 mg). The further separation of fraction E (6:4, 32.6 g) by silica gel column chromatography, and preparative HPLC (28% MeOH, flow rate 12 mL/min) gave **5** (18.2 mg).

Tabchromone A (1). Obtained as pale yellow oil; UV (MeOH) max (log ε) 210 (4.57), 260 (4.15), 358 (3.02) nm; IR (KBr) ν_{max} 3436, 2918, 2872, 1722, 1670, 1615, 1556, 1436, 1358, 1137, 946, 853 cm^{-1}; ^1H NMR and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; negative ESIMS *m/z* 247 $[M-H]^-$; negative HRESIMS *m/z* 247.0976 $[M-H]^-$ (calcd for $C_{14}H_{16}O_4$, 247.0970).

Tabchromone B (2). Obtained as pale yellow oil; UV (MeOH), λ_{max} (log ε) 210 (4.53), 254 (4.08), 355 (3.11) nm; IR (KBr) ν_{max} 3418, 2975, 2926, 1669, 1614, 1538, 1469, 1247, 1154, 943, 862 cm^{-1}; ^1H NMR and ^{13}C NMR data ($CDCl_3$, 500 MHz and 150 MHz), see Table 1; negative ESIMS *m/z* 231 $[M-H]^-$; negative HRESIMS *m/z* 231.1027 $[M-H]^-$ (calcd 231.1021 for $C_{14}H_{16}O_3$).

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