Cytotoxic Isoprenylated Flavonoids from the Roots of *Sophora flavescens*

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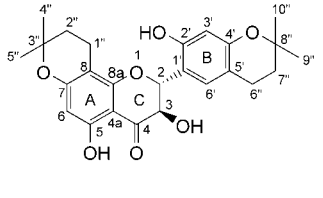
Three new flavonoids, which are isoprenylated by fused 2,2-dimethyl-3,4-dihydro-2*H*-pyran moieties, were isolated from the roots of *Sophora flavescens* and named flavenochromanes A – C (**1** – **3**). Their structures were elucidated by spectroscopic methods, including 2D-NMR techniques. Flavenochromane C (**3**) showed strong cytotoxic activity against A549 (lung carcinoma), 1A9 (ovarian carcinoma), KB (epidermoid carcinoma of the nasopharynx), and KB-Vin (drug-resistant variant KB) cell lines with IC_{50} values ≤ 1.7 μM, and significant activity against the MCF-7 (breast adenocarcinoma) cell line with an IC_{50} value of 3.6 μM. Flavenochromane B (**2**) displayed slightly lower inhibitory effects (IC_{50} 3.2 – 6.9 μM) as compared with **3**.

Introduction. – The dried roots of *Sophora flavescens* AIT. (Leguminosae) are commonly used as the traditional Chinese medicine 'Kushen' for the treatment of skin and gynaecological diseases, such as eczema, dermatitis, and colpitis [1][2]. A series of isoprenylated or lavandulylated flavonoids have been isolated from this plant [3 – 10]. Some of these compounds exhibited significant antibacterial activity against *Gram*-positive bacteria [3] and weak antiviral activity against herpes simplex virus types I and II [4], as well as cytotoxic activity against human myeloid leukemia HL-60 cells [5][6] and potent inhibitory activity against cGMP phosphodiesterase 5 [7]. In the course of our continued screening for cytotoxic phenolic compounds from *Sophora* medicinal plants, the Et_2O-soluble fraction from an EtOH extract of the roots of *S. flavescens* was subjected to repeated column chromatography to afford three new flavonoids, which are isoprenylated by fused 2,2-dimethyl-3,4-dihydro-2*H*-pyran moieties, and are named flavenochromanes A – C (**1** – **3**)[1]).



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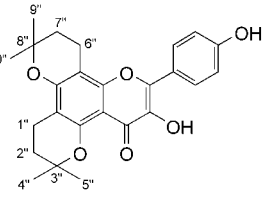

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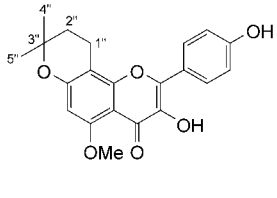

3[1)]

[1]) Arbitrary numbering, derived from the trivial flavonoid numbering; for systematic names, see *Exper. Part*.

This paper reports the isolation and structure elucidation of the new compounds **1**–**3**, as well as their evaluation against a panel of human tumor cell lines, including human lung carcinoma (A549), ovarian carcinoma (1A9), breast adenocarcinoma (MCF-7), epidermoid carcinoma of the nasopharynx (KB), and its drug-resistant variant (KB-Vin).

Results and Discussion. – The Et_2O-soluble fraction of the EtOH extract was subjected to repeated column chromatography to give three new isoprenylated flavonoids, flavenochromanes A–C (**1**–**3**).

Flavenochromane A (**1**) was obtained as white amorphous optically active powder. The quasi-molecular ion $[M+Na]^+$ was detected by HR-ESI-MS at m/z 463.1725, consistent with the formula $C_{25}H_{28}O_7$. Compound **1** gave a positive reaction with the $FeCl_3$ reagent, indicative of a phenol moiety. The IR spectrum showed absorption bands characteristic of an OH group (3420 cm^{-1}), a conjugated C=O group (1640 cm^{-1}), and an aromatic ring (1578 and 1511 cm^{-1}). The UV spectrum was consistent with that of a hydroxyflavanone with maxima at 342 (sh) and 296 nm [11].

The ^{1}H-NMR spectrum of **1** showed the presence of two 2,2-dimethyl-3,4-dihydro-2*H*-pyran moieties fused to rings A and B of a flavonoid, respectively, with signals at δ 2.60 (t, J = 6.8 Hz, 2 H), 1.83 (t, J = 6.8 Hz, 2 H), and 1.34 (s, 6 H), and δ 2.72 (t, J = 6.7 Hz, 2 H), 1.80 (t, J = 6.7 Hz, 2 H), and 1.31 (s, 6 H) [12] (*Table 1*). This hypothesis was further supported by the fragment ions at m/z 221 ($[A_1+H]^+$) and 220 (A_1^+, $B_1^{+\bullet}$) in the EI-MS due to the *retro-Diels–Alder* cleavage of the hydroxyflavanone C ring [11]. A fragment ion at m/z 220 ($B_1^{+\bullet}$) indicated that ring B also contained an OH substituent in addition to the fused 2,2-dimethyl-3,4-dihydro-2*H*-pyran moiety. Signals at δ 12.09 (d, J = 2.4 Hz, 1 H), 8.31 (s, 1 H), and 4.64 (t, J = 3.8 Hz, 1 H) in the ^{1}H-NMR spectrum, which disappeared on D_2O exchange and did not correlate to C-signals in the HMQC spectrum, could

Table 1. *^{1}H-NMR Data of* **1**–**3**. δ in ppm, J in Hz. Arbitrary numbering[1]).

	1[a])	**2**[b])	**3**[c])
H–C(2)	5.44 (d, J = 11.6)	–	–
H–C(3)	4.87 (d, J = 11.6)	–	–
OH–C(3)	4.64 (t, J = 3.8)	7.76 (br. s)	8.70 (br. s)
OH–C(5)	12.09 (d, J = 2.4)	–	–
MeO–C(5)	–	–	3.81 (s)
H–C(6)	5.82 (s)	–	6.33 (s)
H–C(2′)	–	8.14 (d, J = 8.9)	8.04 (d, J = 8.8)
OH–C(2′)	8.31 (s)	–	–
H–C(3′)	6.32 (s)	7.02 (d, J = 8.9)	6.94 (d, J = 8.8)
OH–C(4′)	–	8.84 (br. s)	9.97 (br. s)
H–C(5′)	–	7.02 (d, J = 8.9)	6.94 (d, J = 8.8)
H–C(6′)	7.19 (s)	8.14 (d, J = 8.9)	8.04 (d, J = 8.8)
CH_2(1″)	2.60 (t, J = 6.8)	2.63 (t, J = 6.8)	2.87 (t, J = 6.6)
CH_2(2″)	1.83 (t, J = 6.8)	1.82 (t, J = 6.8)	1.88 (t, J = 6.6)
Me(4″)	1.34 (s)	1.37 (s)	1.35 (s)
Me(5″)	1.34 (s)	1.37 (s)	1.35 (s)
CH_2(6″)	2.72 (t, J = 6.7)	2.97 (t, J = 6.8)	–
CH_2(7″)	1.80 (t, J = 6.7)	1.95 (t, J = 6.8)	–
Me(9″)	1.31 (s)	1.41 (s)	–
Me(10″)	1.31 (s)	1.41 (s)	–

[a]) In (D_6)acetone at 500 MHz. [b]) In (D_6)acetone at 400 MHz. [c]) In (D_6)DMSO at 400 MHz.

Table 2. *^{13}C-NMR Data of* **1**–**3**. δ in ppm. Arbitrary numbering[1]).

	1[a])	**2**[b])	**3**[c])		**1**[a])	**2**[b])	**3**[c])
C(2)	79.6	142.2	141.8	C(5′)	113.5	116.8	115.5
C(3)	72.8	138.3	137.3	C(6′)	130.9	130.0	128.6
C(4)	199.4	172.5	171.1	C(1″)	16.7	18.0	16.0
C(4a)	104.9	106.3	105.7	C(2″)	32.7	32.7	31.1
C(5)	162.3	153.7	158.1	C(3″)	77.5	75.9	75.9
C(6)	97.1	106.7	96.2	C(4″)	27.3	27.3	26.3
C(7)	164.3	157.5	157.8	C(5″)	27.2	27.3	26.3
C(8)	103.1	101.1	100.9	C(6″)	22.8	17.6	–
C(8a)	162.3	155.4	154.9	C(7″)	34.0	32.8	–
C(1′)	116.7	124.6	122.2	C(8″)	75.3	77.0	–
C(2′)	156.5	130.0	128.6	C(9″)	27.6	27.3	–
C(3′)	105.0	116.8	115.5	C(10″)	27.4	27.3	–
C(4′)	156.5	159.8	158.5	*Me*O–C(5)			55.9

[a]) In (D_6)acetone at 125 MHz. [b]) In (D_6)acetone at 100 MHz. [c]) In (D_6)DMSO at 100 MHz.

be assigned to three OH protons. The ^{1}H-NMR spectrum also showed signals due to three isolated aromatic protons (δ 7.19 (*s*, 1 H), 6.32 (*s*, 1 H), and 5.82 (*s*, 1 H)), and two coupled hydroxyflavanone methine protons H–C(2) (δ 5.44 (*d*, $J = 11.6$ Hz, 1 H)) and H–C(3) (δ 4.87 (*d*, $J = 11.6$ Hz, 1 H)). The ^{13}C-NMR spectrum of **1** contained signals for a C=O (δ 199.4), and the hydroxyflavanone C(2) (δ 79.6) and C(3) (δ 72.8) (*Table 2*).

The fusion site of the 2,2-dimethyl-3,4-dihydro-2*H*-pyran moiety at ring A was established at C(7) and C(8) by an HMBC experiment[1]). The methylene protons at δ 1.83 (CH_2(2″)) and 2.60 (CH_2(1″)) correlated with C(8) (δ 103.1), and the latter methylene protons also coupled with C(7) (δ 164.3). In addition, the chelated OH at δ 12.09 (OH–C(5)) and the isolated aromatic proton at δ 5.82 (H–C(6)) both correlated with C(5) (δ 162.3). The fusion site of the dihydropyran moiety at ring B, *i.e.*, at C(4′) and C(5′), was also deduced by correlations of CH_2(7″) (δ 1.80) with C(5′) (δ 113.5), of CH_2(6″) (δ 2.72) with C(5′) (δ 113.5), C(4′) (δ 156.5), and C(6′) (δ 130.9), and of H–C(6′) (δ 7.19) with C(2) (δ 79.6), C(4′) (δ 156.5), and C(2′) (δ 156.5). A cross-peak between the OH at δ 8.31 and C(1′) (δ 116.7) indicated that the OH group of ring B was at C(2′), which was also supported by the absence of a cross-peak between the aromatic proton at δ 7.19 (H–C(6′)) and C(3′) (δ 105.0), which bore a proton appearing at δ 6.32 (H–C(3′)) (*Fig. 1*). With the aid of HMBC and HMQC experiments, all ^{1}H- and ^{13}C-NMR signals were fully assigned.

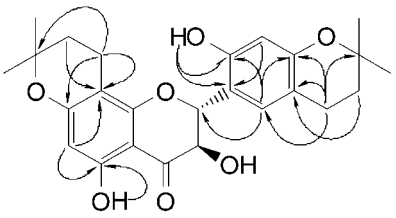

Fig. 1. *Selected HMBC correlations of* **1**

The absolute configuration at C(2) of **1** was determined as (*R*) from the CD spectrum, which showed a positive *Cotton* effect at 336 nm and a negative one at 302 nm [3][13]. Subsequently, the absolute configuration at C(3) was determined as (*R*) from the coupling constant between H–C(2) and H–C(3) ($J = 11.6$ Hz) [14]. Thus, **1** was characterized as (2*R*,3*R*)-2-(3,4-dihydro-7-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-yl)-2,3,9,10-tetrahydro-3,5-dihydroxy-8,8-dimethyl-4*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-4-one.

Flavenochromane B (**2**) was obtained as yellow tabular crystals. It had a molecular formula of $C_{25}H_{26}O_6$ established by HR-ESI-MS ($[M + Na]^+$, *m*/*z* 445.1630). Its IR

spectrum showed absorptions characteristic of an OH group (3200 cm^{-1}), a conjugated C=O group (1644 cm^{-1}), and an aromatic ring (1604, 1558, 1512, and 1484 cm^{-1}). The UV spectrum exhibited maximum absorptions at 367, 330 (sh), 304 (sh), 272, and 257 (sh) nm, indicating a hydroxyflavone skeleton [11]. Based on further spectroscopic data, **2** was identified as 3,4,7,8-tetrahydro-11-hydroxy-10-(4-hydroxyphenyl)-2,2,6,6-tetramethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-12-one.

The absence of the signals due to H–C(2) and H–C(3) in the ^{1}H-NMR spectrum of **2**, as well as the appearance of two additional δ(C) of oxygenated olefinic C-atoms (δ 142.2 and 138.3) and an upfield-shifted C=O signal (δ 172.5) in the ^{13}C-NMR spectrum indicated the presence of a hydroxyflavone skeleton [14]. The ^{1}H-NMR spectrum also exhibited signals for two 2,2-dimethyl-3,4-dihydro-2*H*-pyran moieties (δ 2.97 (*t*, *J* = 6.8 Hz, 2 H), 1.95 (*t*, *J* = 6.8 Hz, 2 H), and 1.41 (*s*, 6 H), and 2.63 (*t*, *J* = 6.8 Hz, 2 H), 1.82 (*t*, *J* = 6.8 Hz, 2 H), and 1.37 (*s*, 6 H)), and two OH groups (δ 8.84 (br. *s*, 1 H) and 7.76 (br. *s*, 1 H)) (*Table 1*). The remaining four aromatic protons (δ 8.14 (*d*, *J* = 8.9 Hz, 2 H) and 7.02 (*d*, *J* = 8.9 Hz, 2 H)) formed an A_2B_2 system, indicating that the two 2,2-dimethyl-3,4-dihydro-2*H*-pyran moieties were both located at ring A, and that C(4′) of ring B was substituted by an OH group. These assignments were further supported by the presence of a fragment ion at *m*/*z* 121 ($B_1^{+\cdot}$) in the EI-MS due to the *retro-Diels–Alder* cleavage of the hydroxyflavone C ring [11].

The fusion sites of the two 2,2-dimethyl-3,4-dihydro-2*H*-pyran moieties at the hydroxyflavone skeleton were determined unambiguously to be C(5) and C(6), and C(7) and C(8) of ring A, with the O-atoms connected to C(5) and C(7) based on the following HMBC cross-peak correlations CH_2(2″) (δ 1.82)/C(6) (δ 106.7), CH_2(1″) (δ 2.63)/C(6) (δ 106.7), C(5) (δ 153.7), and C(7) (δ 157.5), CH_2(7″) (δ 1.95)/C(8) (δ 101.1), CH_2(6″) (δ 2.97)/C(8) (δ 101.1), C(7) (δ 157.5), and C(8a) (δ 155.4) (*Fig. 2*).

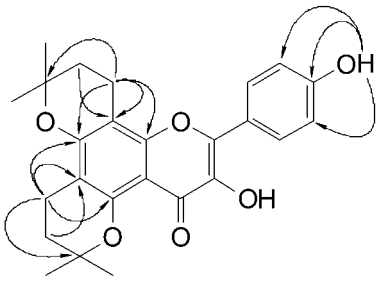

Fig. 2. *Selected HMBC correlations of* **2**

Flavenochromane C (**3**) was obtained as yellow needles. The HR-ESI-MS analysis ($[M+Na]^+$, *m*/*z* 391.1158) afforded the molecular formula $C_{21}H_{20}O_6$. The IR and UV spectra of **3** were similar to those of **2**. Comparison of the ^{1}H-NMR data of **3** with those of **2** revealed that the chemical-shift values and splitting patterns of **3** agreed well with those of **2**, with a few exceptions. Detailed analysis of further spectral data allowed us to establish the structure of 9,10-dihydro-3-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8,8-dimethyl-4*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-4-one for **3**.

The signals of one 2,2-dimethyl-3,4-dihydro-2*H*-pyran moiety of **2** were replaced by signals for a MeO group (δ 3.81 (*s*, 3 H)) and an isolated aromatic proton (δ 6.33 (*s*, 1 H)) in **3** (*Table 1*). The ^{13}C-NMR data of **3** were also identical to those of **2**, except for the appearance of an additional MeO signal at δ 55.9 instead of the δ(C) of a 2,2-dimethyl-3,4-dihydro-2*H*-pyran moiety in **2** (*Table 2*). Therefore, it could be inferred that **3** contained only one 2,2-dimethyl-3,4-dihydro-2*H*-pyran moiety and a MeO group at ring A rather than the two dihydropyran moieties in **2**. This was confirmed by the enhancement of the isolated-aromatic-proton signal at δ 6.33 in the difference NOE spectrum upon saturation of the MeO signal at δ 3.81.

In the ^{13}C-NMR spectrum of **3**, the noticeable upfield shift of C(6) ($\Delta\delta$ (C) = – 10.5 ppm) as compared to **2** and the downfield shift of C(5) ($\Delta\delta$ (C) = + 4.4 ppm) suggested that the MeO group of **3** was possibly attached

to C(5), which was also supported by the correlations of the isolated aromatic proton at δ 6.33 (H–C(6)) with C(7) (δ 157.8), C(8) (δ 100.9), and C(4a) (δ 105.7), and the MeO protons at δ 3.81 with C(5) (δ 158.1) in the HMBC spectrum. In addition, the CH_2(2″) signal at δ 1.88 correlated with C(8) (δ 100.9), and the CH_2(1″) signal at δ 2.87 coupled with C(7) (δ 157.8), C(8a) (δ 154.9), and C(8) (δ 100.9), indicating that the 2,2-dimethyl-3,4-dihydro-2*H*-pyran moiety was located at C(7) and C(8) (*Fig. 3*).

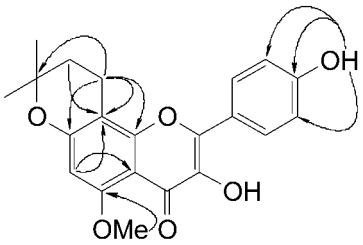

Fig. 3. *Selected HMBC correlations of* **3**

The three isolated flavenochromanes A–C (**1**–**3**) were tested *in vitro* against a panel of human tumor cell lines, and the results are summarized in *Table 3*. The cytotoxicity spectra were broad, with slightly higher potency against A549 (lung carcinoma) than the other four tumor cell lines. Interestingly, the compounds showed comparable potency against the KB (epidermoid carcinoma of the nasopharynx) cell line and its drug-resistant variant (KB-Vin). Of the compounds tested, flavenochromane C (**3**) was the most potent, and exhibited strong cytotoxic activities against A549, 1A9 (ovarian carcinoma), KB, and KB-Vin cells with IC_{50} values of 1.0, 1.2, 1.3, and 1.7 μM, respectively, and significant activity against MCF-7 (breast adenocarcinoma) with an IC_{50} value of 3.6 μM. The positive control, etoposide, showed corresponding IC_{50} values of 1.0, 0.3, 1.4, 14.5, and 2.0 μM. With IC_{50} values of 3.2–6.9 μM, flavenochromane B (**2**) was slightly less potent than **3** against the human tumor cell-line panel, while flavenochromane A (**1**) was not active (IC_{50} 13.9–16.1 μM).

Table 3. *Cytotoxicities of* **1**–**3** *against Human Tumor Cell Lines.* IC_{50} in μM.

	A549	1A9	MCF-7	KB	KB-Vin
1	13.9	16.1	15.4	15.6	15.5
2	3.2	5.0	4.9	6.9	6.5
3	1.0	1.2	3.6	1.3	1.7
Etoposide	1.0	0.3	2.0	1.4	14.5

Thus, hydroxyflavones **2** and **3**, which have two hydrophobic groups (2,2-dimethyl-3,4-dihydro-2*H*-pyran, MeO) at ring A, a hydrophilic group (4-OH group) at ring B, and a C(2)=C(3) in ring C, were significantly more potent than hydroxyflavanone **1**, which bears both hydrophobic and hydrophilic groups at ring A and ring B simultaneously, and a saturated C(2)–C(3) bond in ring C. This limited data set indicated that the hydroxyflavone skeleton and the distribution of hydrophobic and hydrophilic groups at different rings might be important to the cytotoxic activity. In addition, comparison of the structure and cytotoxic effect of **3** with those of **2** revealed that the presence of a MeO group at C(5) in **3** possibly led to enhanced cytotoxicity.

However, further investigation is necessary to fully elucidate the structural determinants for cytotoxic activity.

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Experimental Part

General. Column chromatography (CC): silica gel *H*, 200–300 mesh (*Qingdao,* China), *Diaion HP-20* resin (*Mitsubishi*, Japan). TLC: precoated silica-gel plates GF_{254}, 10–40 μm (*Yantai*, China); detection by UV light and visualization by staining with aq. 10% (*v*/*v*) H_2SO_4 spraying soln. followed by heating. Melting points (m.p.): *XT-4* micro-melting-point apparatus; uncorrected. Optical rotations: *Jasco P-1020* digital polarimeter. UV Spectra: *Shimadzu UV-260* UV/VIS recording spectrophotometer; λ_{max} (logε) in nm. CD Spectra: *Jasco J-715* spectropolarimeter; λ ([ϑ]) in nm. IR Spectra: *Avatar 360-FT-IR* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-400* or *-500* spectrometers; ^1H at 400 and 500 MHz, resp., ^{13}C at 100 and 125 MHz, resp.; (D_6)acetone or (D_6)DMSO solns. at r.t. with $SiMe_4$ as internal standard; δ in ppm, *J* in Hz. EI-MS: *Hewlett-Packard 5989A* mass spectrometer; in *m*/*z* (rel. %). HR-ESI-MS: *AB-QSTAR-Pulsar* mass spectrometer.

Plant Material. The roots of *Sophora flavescens* AIT. (Leguminosae) were purchased from *Huayu Materia Medica Co., Ltd.*, Shanghai, in February 2001. A voucher specimen (KS-SH-0102) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.

Extraction and Isolation. The pulverized roots of *S. flavescens* (14 kg) were extracted 4 × with aq. 1% (*v*/*v*) H_2SO_4 soln. to obtain the total alkaloids (250 g). The residues were then air-dried and extracted with 95% EtOH (6 × 15 l) at r.t. The EtOH extract (1440 g) was suspended in H_2O (2.5 l) and partitioned with Et_2O (6 × 3 l). The Et_2O-soluble fraction (195 g) was subjected to CC (silica gel, petroleum ether (60–90°), petroleum ether/AcOEt 50:1, 20:1, 15:1, 10:1, 5:1, 3:1, 1:1, and AcOEt): *Fractions 1–9*. *Fr. 6* (4.90 g) was subjected to CC (silica gel, petroleum ether/$CHCl_3$/Me_2CO 10:10:1): **1** (4 mg). *Fr. 7* (10.40 g) was subjected to CC (*Diaion HP-20*, MeOH/H_2O gradient); the 90% MeOH fraction (1.24 g) was then subjected to CC (silica gel; petroleum ether/$CHCl_3$/Me_2CO 10:10:1): **2** (11 mg). *Fr. 8* (15.77 g) was subjected to CC (*Diaion HP-20*, H_2O/MeOH 30:70): **3** (16 mg).

Flavenochromane A (= *(2*R,*3*R*)-2-(3,4-Dihydro-7-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-yl)-2,3,9,10-tetrahydro-3,5-dihydroxy-8,8-dimethyl-4*H,*8*H*-benzo[1,2*-b:*3,4*-b′*]dipyran-4-one*; **1**): White amorphous powder. $[\alpha]_D^{21} = +70.3$ (*c* = 0.20, MeOH). UV (MeOH): 342 (sh, 3.42), 296 (4.28), 230 (sh, 4.31), 209 (4.56). CD (MeOH): 336 (+7493), 302 (−24112), 279 (+4322), 259 (+4618), 229 (+23414), 211 (−26285). IR (KBr): 3420, 2976, 2934, 2855, 1640, 1578, 1511, 1455, 1384, 1369, 1296, 1279, 1156, 1116, 1093, 882, 822, 767. ^1H- and ^{13}C-NMR ((D_6)acetone): *Tables 1* and *2*. EI-MS: 441 (10), 440 (33, M^+), 422 (10), 227 (31), 221 (56), 220 (10), 218 (65), 192 (14), 191 (37), 165 (76), 163 (37), 149 (35), 148 (100), 105 (92), 77 (53), 69 (34), 57 (36), 55 (39), 43 (37). HR-ESI-MS: 463.1725 ($[M+Na]^+$, $C_{25}H_{28}NaO_7^+$; calc. 463.1733).

Flavenochromane B (= *3,4,7,8-Tetrahydro-11-hydroxy-10-(4-hydroxyphenyl)-2,2,6,6-tetramethyl-2*H*,6*H*,12*H*-benzo[1,2*-b:*3,4*-b′:*5,6*-b″*]tripyran-12-one*; **2**): Yellow tabular crystals (petroleum ether/Me_2CO 10:1). M.p. 294–296°. UV (MeOH): 367 (4.40), 330 (sh, 4.26), 304 (sh, 4.15), 272 (4.47), 257 (sh, 4.39), 209 (4.60). IR (KBr): 3200, 2975, 2932, 2849, 1644, 1604, 1558, 1512, 1484, 1425, 1370, 1282, 1277, 1216, 1178, 1118, 1079, 948, 915, 838, 795. ^1H- and ^{13}C-NMR ((D_6)acetone): *Tables 1* and *2*. EI-MS: 423 (28), 422 (100, M^+), 407 (2), 379 (14), 368 (15), 367 (67), 366 (72), 351 (11), 323 (12), 312 (18), 311 (99), 310 (98), 309 (24), 282 (15), 121 (30), 43 (12), 41 (11). HR-ESI-MS: 445.1630 ($[M+Na]^+$, $C_{25}H_{26}NaO_6^+$; calc. 445.1627).

Flavenochromane C (= *9,10-Dihydro-3-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8,8-dimethyl-4*H*,8*H*-benzo[1,2*-b:*3,4*-b′*]dipyran-4-one*; **3**): Yellow needles (EtOH/H_2O 1:2). M.p. 268–270°. UV (MeOH): 420 (sh, 3.15), 360 (4.40), 306 (4.11), 268 (4.41), 207 (4.52). IR (KBr): 3330, 2975, 2932, 1652, 1614, 1590, 1562, 1515, 1444, 1419, 1406, 1373, 1327, 1310, 1275, 1220, 1196, 1177, 1160, 1120, 1087, 1027, 1007, 942, 840, 822. ^1H- and ^{13}C-NMR ((D_6)DMSO): *Tables 1* and *2*. EI-MS: 369 (25), 368 (100, M^+), 367 (11), 350 (13), 322 (24), 314 (15), 313 (77), 311 (12), 294 (13), 284 (21), 283 (24), 267 (16), 255 (22), 179 (22), 156 (12), 121 (57), 93 (15), 65 (14), 55 (11), 41 (10). HR-ESI-MS: 391.1163 ($[M+Na]^+$, $C_{21}H_{20}NaO_6^+$; calc. 391.1158).

Cytotoxicity Assay. Drug stock solns. were prepared in DMSO and stored at $-70°$. Upon dilution into culture medium, the final DMSO concentration was $\leq 1\%$ (*v*/*v*) DMSO, a concentration without effect on cell replication. The human tumor cell-line panel consisted of lung carcinoma (A549), ovarian carcinoma (1A9), breast adenocarcinoma (MCF-7), epidermoid carcinoma of the nasopharynx (KB), and its subclone (KB-Vin). Cell culture and other procedures were the same as those reported previously [15].

All stock cultures were grown in T-25 flasks containing 4 ml of RPMI-1640 medium supplemented with 25 mM HEPES, 0.2% (*w*/*v*) $NaHCO_3$, 10% (*v*/*v*) fetal bovine serum, and 100 μg/ml of kanamycin at 37° in a humidified atmosphere containing 5% CO_2. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 2500–10000 cells per well. Initial seeding densities varied among the cell lines to ensure a final absorbance reading in control (untreated) cultures in the range 1–2.5 A_{560} units. Tumor cells were incubated at 37° for 72 h in the presence of various concentrations of drugs from DMSO-diluted stock solns. Cultures were monitored briefly at daily intervals by microscopic examination. After 3 days, attached cells were fixed with ice-cold 10% (*w*/*v*) CCl_3COOH soln. and then stained with 0.4% (*w*/*v*) sulforhodamine B (SRB) (*Sigma Chemical Co.*, St. Louis, MO). The absorbance at 562 nm was measured with an automated microculture plate reader (*Molecular Devices*, Menlo Park, CA) after solubilizing the bound dye. The IC_{50} values, the drug concentration resulting in 50% growth inhibition, were interpolated from dose-response data. Each test was performed $3\times$, and the s.e.m. of IC_{50} values varied no more than 5% (*Table 3*).

REFERENCES

[1] The State Pharmacopoeia Commission of the People's Republic of China, 'Pharmacopoeia of the People's Republic of China', Vol. I, Chemical Industry Press, Beijing, 2000, p. 197.
[2] Jiangsu New Medical College, 'Dictionary of Chinese Traditional Medicine', Shanghai Science and Technology Press, Shanghai, 1977, p. 1283.
[3] M. Kuroyanagi, T. Arakawa, Y. Hirayama, T. Hayashi, *J. Nat. Prod.* **1999**, *62*, 1595.
[4] E. R. Woo, J. H. Kwak, H. J. Kim, H. Park, *J. Nat. Prod.* **1998**, *61*, 1552.
[5] T. H. Kang, S. J. Jeong, W. G. Ko, N. Y. Kim, B. H. Lee, M. Inagaki, T. Miyamoto, R. Higuchi, Y. C. Kim, *J. Nat. Prod.* **2000**, *63*, 680.
[6] W. G. Ko, T. H. Kang, N. Y. Kim, S. J. Lee, Y. C. Kim, G. I. Ko, S. Y. Ryu, B. H. Lee, *Toxicol. in Vitro* **2000**, *14*, 429.
[7] H. J. Shin, H. J. Kim, J. H. Kwak, H. O. Chun, J. H. Kim, H. Park, D. H. Kim, Y. S. Lee, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2313.
[8] L. J. Wu, T. Miyase, A. Ueno, M. Kuroyanagi, T. Noro, S. Fukushima, *Yakugaku Zasshi* **1985**, *105*, 736.
[9] M. Komatsu, T. Tomimori, K. Hatayama, N. Mikuriya, *Yakugaku Zasshi* **1970**, *90*, 463.
[10] S. S. Kang, J. S. Kim, K. H. Son, H. W. Chang, H. P. Kim, *Fitoterapia* **2000**, *71*, 511.
[11] The Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Science, 'Handbook of the Systematic Identification of Flavonoids', Science Press, Beijing, 1981, p. 399, 519, 697, 703.
[12] Y. Hano, N. Itoh, A. Hanaoka, T. Nomura, *Heterocycles* **1995**, *41*, 2313.
[13] W. Gaffield, *Tetrahedron* **1970**, *26*, 4093.
[14] R. S. Xu. 'The Chemistry of Natural Products', Science Press, Beijing, 1993, p. 592, 593.
[15] H. H. Cheng, H. K. Wang, J. Ito, K. F. Bastow, Y. Tachibana, Y. Nakanishi, Z. Xu, T. Y. Luo, K. H. Lee, *J. Nat. Prod.* **2001**, *64*, 915.

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