SUPPLEMENTARY MATERIAL

Scillapersicene: A new Homoisoflavonoid with cytotoxic activity from the bulbs of *Scilla persica* HAUSSKN

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Abstract

The phytochemical investigation of *Scilla persica* HAUSSKN bulbs led to the isolation of a novel homoisoflavonoid that named Scillapersicene (**1**) and identified as 3-(3',4'-dihydroxybenzylidene)-8-hydroxy-5,7-dimethoxychroman-4-one along with five known homoisoflavonoids **2-6**, whose structures were elucidated by using HRFAB-MS, 1D and 2D NMR spectroscopic data. The known compounds were identified as 3-(3',4'-dihydroxybenzyl)-5,8-dihydroxy-7-methoxychroman-4-one (**2**), 3,9-dihydro-autumnalin (**3**), autumnalin (**4**), 3-(3',4'-dihydroxybenzylidene)-5,8-dihydroxy-7-methoxychroman-4-one (**5**) and scillapersicone (**6**). All compounds obtained, except **2** and **4**, showed strong cytotoxic activity against AGS cell line. The toxicity on AGS cell line was measured by **1**, **3**, **5** and **6** with IC₅₀ values of 8.4, 30.5, 10.7 and 24.2 µM, respectively. In addition, the physico-chemical properties of these natural compounds were optimized by using density functional method (B3LYP) with standard 6-311+G* basis set. These natural products have low energy gaps between the first ionization potentials and HOMO. In conclusion, the low energy gap could cause reason for cytotoxic activity of Homoisoflavonoids.

Keywords: AGS; B3LYP; Cytotoxic activity; Scillapersicene; Homoisoflavonoid; *Liliaceae*; *Scilla Persica*.

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Experimental

Plant material

Fresh bulbs of *S. persica* HAUSSKN were collected in the village of Valliv from Sardasht, West Azerbaijan Province, Iran, in March 2013 at an altitude of 1700-1800 m, and were taxonomically identified by Afsaneh Kolbadi. A voucher specimen (No. 6334) was deposited in the Herbarium of the Agricultural Research Center and Natural Resources of Sari, Iran.

Extraction and isolation

The bulbs (300 g) of *S. persica* were crushed and extracted with Et₂O (3×2 L, rt for 24 h). Evaporation of solvent at reduced pressure afforded 6 g of a light brown gum. A portion (4 g) was fractionated on a CC eluted with n-hexane-EtOAc-MeOH [10:0:0, 9.5:0.5:0, 9:1:0, 8.5:1.5:0, 8:2:0, 7:3:0, 6:4:0, 5:5:0, 4:6:0, 2:8:0, 0:10:0, 0:8:2 and 0:5:5 (v/v)] to obtain fractions Fr.1-Fr.10 after pooling according to their TLC profiles. **Fr.4** (247 mg), obtained from the elution with n-hexane-EtOAc-MeOH of 8.5:1.5:0, was consequently subjected to CC on silica gel using CHCl₃ and on Sephadex LH-20 column using MeOH gave the compounds **2** (12.5 mg, with R_f = 0.74 for CHCl₃/MeOH: 18/2) and **3** (27.3 mg, with R_f = 0.69 for CHCl₃/MeOH: 15/5). **Fr.6** (261 mg), obtained from elution with n-hexane-EtOAc-MeOH of 7:3:0, was further subjected via silica gel CC, eluting with a CHCl₃/MeOH gradient (10:0 to 0:4) to give compound **4** (46.7 mg, with R_f = 0.65 for CHCl₃/MeOH: 9/1). **Fr.7** (185 mg), obtained from elution with n-hexane-EtOAc-MeOH of 6:4:0 and 5:5:0, was consequently subjected to CC on silica gel using CHCl₃ and was chromatographed on a Sephadex LH-20 column using MeOH that gave the pure compounds **5** (20.3 mg, with R_f = 0.73 for CHCl₃/MeOH: 9.5/0.5). **Fr.8** (296 mg), obtained from the elution with n-hexane-EtOAc-MeOH of 4:6:0 to 0:10:0, was submitted to CC on silica gel using CHCl₃, and consequently on Sephadex LH-20 column using MeOH to obtain the compounds **1** (34.5 mg, with R_f = 0.77 for CHCl₃/MeOH: 9/1) and **6** (43 mg, with R_f = 0.69 for CHCl₃/MeOH: 9/1). The ¹H and ¹³C NMR spectroscopic data for 3-(3',4'-dihydroxybenzyl)-5,8-dihydroxy-7-methoxychroman-4-one (**2**) (Adinolfi et al. 1987), 3,9-dihydro-autumnalin (**3**) (Silayo et al. 1999; Mutanyatta et al. 2003; Nishida et al. 2008), autumnalin (**4**) (Silayo et al. 1999), 3-(3',4'-dihydroxybenzylidene)-5,8-dihydroxy-7-methoxychroman-4-one (**5**) (Masterova et al. 1991) and scillapersicone (**6**) (Hafez Ghoran et al. 2014) have been reported previously.

MTT assay

Cell viability was measured by MTT (3-[4, 5-dimethylthiazol-2-yl] 2, 5-iphenyltetrazolium bromide) assay according to the manufacturer's instructions (Sigma-Aldrich, USA). Briefly, AGS

cells were seeded onto 96-well plates and allowed to adhere and grow overnight in 200 μ l DMEM medium. The cells were then incubated with fresh medium containing serial concentrations (0-200 μ M) of compounds 1-6 dissolved in 1% DMSO for 48 h. Dendrosmal curcumin as an anti-proliferative compound was also employed as positive control. Afterward, 20 μ l of 5 mg/ml MTT was added to each well and incubated for additional 4 h at 37 $^{\circ}$ C followed by addition of 200 μ l of DMSO (Mosmann 1983; Babaei et al. 2012). The relative cell viability was determined at 540 nm by a 96-well plate reader (Biorad-USA) and the concentration, at which cell growth was inhibited by 50% (IC₅₀), was determined by standard curve method (Milach et al. 1997; Nguyen et al. 2006). Each experiment was carried out in triplicate wells and repeated at least three times.

Computational details

The calculations were performed at DFT levels on personal computer using Gaussian 98 program (Frisch et al. 1998). The geometry of **1-6** were optimized by using density functional method (B3LYP) with standard 6-311+G* basis set (Table S3).

NMR data of Known compounds

Compound (2): Yellow-Brown powder; ¹H-NMR (DMSO-*d*₆, 500 MHz) δ 12.02 (1H, s, OH-5), 6.45 (1H, dd, *J*= 1.8, 7.9 Hz, H-6'), 6.60 (1H, d, *J*= 7.5 Hz, H-5'), 6.68 (1H, d, *J*= 1.6 Hz, H-2'), 2.91 (1H, m, H-3), 2.94 (1H, dd, *J*= 5.4, 11 Hz, H-9a), 2.54 (1H, dd, *J*= 10.6, 13.6 Hz, H-9b), 6.11 (1H, s, H-6), 4.35 (1H, dd, *J*= 4.7, 11.5 Hz, H-2a), 4.12 (1H, dd, *J*= 7.7, 12.3 Hz, H-2b), 3.80 (3H, s, OCH₃-7); ¹³C-NMR (DMSO-*d*₆, 125 MHz) δ 69.41 (C-2), 46.52 (C-3), 199.15 (C-4), 102.35 (C-4a), 157.61 (C-5), 92.97 (C-6), 156.38 (C-7), 127.00 (C-8), 148.50 (C-8a), 31.96 (C-9), 129.21 (C-1'), 116.72 (C-2'), 145.26 (C-3'), 144.25 (C-4'), 116.23 (C-5'), 120.19 (C-6'), 55.98 (OCH₃, C-7).

Compound (3): Yellowish powder; ¹H-NMR (DMSO-*d*₆, 500 MHz) δ 12.1 (1H, s, OH-5), 7.0 (each 1H, d, *J*= 9 Hz, H-2' and H-6'), 6.86 (each 1H, d, *J*= 8 Hz, H-3' and H-5'), 3.02 (1H, dd, *J*= 5.2, 13 Hz, H-9a), 2.88 (1H, m, H-3), 2.54 (1H, dd, *J*= 9.2, 13.7 Hz, H-9b), 5.88 (1H, s, H-8), 4.19 (1H, dd, *J*= 5.1, 12 Hz, H-2a), 4.0 (1H, dd, *J*= 8.7, 11.5 Hz, H-2b), 3.68 (3H, s, OCH₃-6); ¹³C-NMR (DMSO-*d*₆, 125 MHz) δ 68.59 (C-2), 46.17 (C-3), 199.05 (C-4), 101.46 (C-4a), 155.79 (C-5), 128.47 (C-6), 159.86 (C-7), 95.20 (C-8), 158.34 (C-8a), 31.58 (C-9), 129.39 (C-1'), 130.51 (C-2'), 115.7 (C-3'), 156.25 (C-4'), 115.7 (C-5'), 130.51 (C-6'), 60.38 (OCH₃, C-6).

Compound (4): Yellowish powder; $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 500 MHz) δ 12.85 (1H, s, OH-5), 7.2 (each 1H, s, H-2' and H-6'), 6.86 (each 1H, s, H-3' and H-5'), 7.71 (1H, s, H-9), 5.9 (1H, s, H-8), 5.3 (2H, d, $J=1.4$ Hz, H-2), 3.8 (3H, s, OCH_3 -6); $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 125 MHz) δ 67.22 (C-2), 125.63 (C-3), 185.84 (C-4), 102.51 (C-4a), 159.31 (C-5), 126.72 (C-6), 159.39 (C-7), 94.84 (C-8), 155.98 (C-8a), 136.98 (C-9), 129.30 (C-1'), 132.27 (C-2'), 115.60 (C-3'), 160.00 (C-4'), 115.56 (C-5'), 132.30 (C-6'), 59.81 (OCH_3 , C-6).

Compound (5): Yellow-red powder; $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 500 MHz) δ 12.34 (1H, s, OH-5), 6.78 (1H, dd, $J=1.7, 8.2$ Hz, H-6'), 6.81 (1H, d, $J=7.6$ Hz, H-5'), 6.85 (1H, d, $J=2.1$ Hz, H-2'), 7.54 (1H, s, H-9), 6.30 (1H, s, H-6), 5.28 (2H, d, $J=1.5$ Hz, H-2), 3.79 (3H, s, OCH_3 -7); $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 125 MHz) δ 67.19 (C-2), 127.26 (C-3), 185.49 (C-4), 102.35 (C-4a), 156.74 (C-5), 92.23 (C-6), 157.28 (C-7), 125.37 (C-8), 146.98 (C-8a), 137.31 (C-9), 125.29 (C-1'), 117.87 (C-2'), 145.61 (C-3'), 147.99 (C-4'), 115.75 (C-5'), 123.42 (C-6'), 56.10 (OCH_3 , C-7).

Compound (6): Yellowish powder; $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 500 MHz) δ 8.75 (each 1H, brs, OH-3' and OH-4'), 8.14 (1H, brs, OH-8), 6.48 (1H, dd, $J=2, 8.1$ Hz, H-6'), 6.52 (1H, d, $J=7.7$ Hz, H-5'), 6.65 (1H, d, $J=7.9$ Hz, H-2'), 2.61 (1H, m, H-3), 2.93 (1H, dd, $J=4.9, 13.7$ Hz, H-9a), 2.50 (1H, dd, $J=10.2, 13.7$ Hz, H-9b), 6.29 (1H, s, H-6), 4.20 (1H, dd, $J=4.2, 11.2$ Hz, H-2a), 4.02 (1H, dd, $J=8, 11.2$ Hz, H-2b), 3.82 (3H, s, OCH_3 -5), 3.74 (3H, s, OCH_3 -7); $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 125 MHz) δ 68.66 (C-2), 47.52 (C-3), 190.33 (C-4), 105.13 (C-4a), 153.48 (C-5), 90.45 (C-6), 154.00 (C-7), 128.03 (C-8), 150.62 (C-8a), 31.55 (C-9), 129.32 (C-1'), 115.65 (C-2'), 145.10 (C-3'), 143.74 (C-4'), 116.43 (C-5'), 120.00 (C-6'), 55.96 (OCH_3 , C-5), 55.96 (OCH_3 , C-7).

Figure captions:

Figure S1. Key HMBC correlations for Scillapersicene (**1**).

Figure S2. Distribution patterns of frontier molecular orbitals for the compound **1**.

Table captions:

Table S1. NMR spectroscopic data for Scillapersicene (1)

Table S2. Results of cytotoxic assay on compounds 1–6.

Table S3. The physico-chemistry properties[†] of the compounds 1-6 computed using DFT at the B3LYP/6-311+G* basis set.

(Table S1)

No.	Scillapersicene (1) ^{a,b}				
	$\delta_{\text{H}}^{\text{a}}$ (<i>J</i> in Hz)	$\delta_{\text{H}}^{\text{b}}$ (HSQC) (<i>J</i> in Hz)	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	HMBC
2	5.19, <i>d</i> (1.5)	5.31, <i>d</i> (1.2)	67.91	67.85	C3, C4, C8a, C9
3	—	—	130.03	130.43	—
4	—	—	179.13	179.00	—
4a	—	—	107.46	107.35	—
5*	—	—	154.65	154.81	—
6	6.38, <i>s</i>	6.46, <i>s</i>	91.88	91.56	C4a, C5, C7, C8
7*	—	—	153.98	152.91	—
8	—	—	128.43	128.64	—
8a	—	—	150.40	149.79	—
9	7.44, <i>s</i>	7.61, <i>s</i>	135.46	135.06	C4, C1', C5', C6'
1'	—	—	126.06	126.81	—
2'	6.8, <i>d</i> (2)	6.93, <i>d</i> (1.9)	117.84	117.17	C9, C4', C6'
3'	—	—	145.81	145.26	—
4'	—	—	147.73	146.90	—
5'	6.82, <i>d</i> (8.1)	6.95, <i>d</i> (8)	116.29	115.63	C1', C3'
6'	6.73, <i>dd</i> (2.1, 8.3)	6.8, <i>dd</i> (2, 8)	123.25	122.96	C9, C2', C4'
8-OH	8.25, <i>broad signal</i>	7.2, <i>broad signal</i>	—	—	—
3'-OH**	9.41, <i>broad signal</i>	8.57, <i>broad signal</i>	—	—	—
4'-OH**	9.41, <i>broad signal</i>	8.57, <i>broad signal</i>	—	—	—
C5-OMe	3.79, <i>s</i>	3.86, <i>s</i>	56.58	55.83	C5
C7-OMe	3.88, <i>s</i>	3.96, <i>s</i>	56.46	55.61	C7

^a NMR recorded in DMSO-*d*₆; ^b NMR recorded in CO(CD₃)₂, (500 MHz for δ_{H} , 125 MHz for δ_{C})

*, **: The assignments may be interchanged.

(Table S2)

Compounds	IC ₅₀ (μM)	
	AGS ^a cell line	Normal fibroblasts
1	8.4	125
2	120.3	125
3	30.5	100
4	74.6	100
5	10.7	120
6	24.2	115
Dendrosomal curcumin ^c	16.6	ND

^a Human gastric carcinoma (AGS cell);

^b ND: Not Detected;

^c Dendrosomal curcumin as an anti-proliferative compound was employed as positive control.

(Table S3)

	Natural Compound	HOMO	LUMO	$\Delta\epsilon$	D _M (Debye)	HF	E _F (μ)	<i>S</i>	η	ω	<i>X</i>	ΔN_{max}
B3LYP/6-311+G*	1	-5.60	-2.02	3.58	3.8302	-1222.36728	-3.81	0.895	1.79	4.05	3.81	2.13
	2	-5.75	-1.85	3.86	4.1336	-1184.29968	-3.80	0.965	1.93	3.74	3.80	1.97
	3	-6.15	-1.89	4.26	4.5331	-1109.05045	-4.02	1.065	2.13	3.79	4.02	1.89
	4	-6.04	-2.44	3.60	5.8352	-1107.82798	-4.24	0.90	1.80	4.99	4.24	2.36
	5	-5.64	-2.38	3.26	1.0976	-1183.07781	-4.01	0.815	1.63	4.47	4.01	2.46
	6	-5.25	-0.96	4.29	5.7546	-1223.62146	-3.11	1.07	2.15	2.25	3.11	1.45

[†] The parameters are units of eV.

Original Spectra of new Compound (1)

¹H-NMR spectrum (DMSO-*d*₆ 500 MHz)

¹H-NMR spectrum (expansion)

^1H -NMR spectrum (CD_3COCD_3 500 MHz)

HMBC Spectrum

HSQC Spectrum

Note : -
Inlet : Direct Ion Mode : FFB+
Spectrum Type : Normal Ion (MF-Linear)
RT : 3.58 min Scan# : 22
BP : m/z 89.0275 Int. : 1599.91
Output m/z range : 50.0000 to 500.0000 Cut Level : 0.00 %
16776256 89.0 107.0 137.1 154.1 155.1

HRFAB-MS

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