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Jacaranone analogs from *Senecio scandens*

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Bioassay-guided fractionation of the ethanolic extract of *Senecio scandens* led to the isolation of four new compounds **4**, **5**, **7**, and **8**, along with four known jacaranone analogs (**1**, **2**, **3**, **6**). Their structures were elucidated on the basis of spectral and chemical evidence. Compound **7** was obtained as a tautomeric mixture of α/β-epimer. The cytotoxic activities of these compounds were evaluated. Among these, compounds **5** and **8** showed potent cytotoxicities. The benzoquinone derivative, jacaranone ethyl ester (**1**), was the major cytotoxic constituent in this plant with IC_{50}s at a range of 0.5–1.0 μg/ml against various tumor cell lines. The SAR of these jacaranone analogs (**1–8**), isolated from *S. scandens*, was also discussed.

Keywords: *Senecio scandens*; Jacaranone analog; tetrahydrojacaranone; benzoquinone

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1. Introduction

Senecio scandens Buch-Ham, an annual plant distributed in the southwestern and southeastern China, is a folk medicine used for the treatment of inflammatory, bacteria infection, arthritis, and rheumatic disease. To our knowledge, no thorough phytochemical investigation on this herb has been reported in the literature. In our continuing search for new anticancer agents from Chinese folk medicines, the ethanolic extract of *S. scandens* was found to demonstrate significant cytotoxic effects. Therefore, bioassay-guided fractionation of the extract was carried out, and four new compounds, jacaranone analogs **4** and **5** and jacaranone glucosides **7** and **8**, along with four known benzoquinone derivatives, jacaranone (**2**), jacaranone ethyl ester (**1**), jacaranone methyl ester (**3**), and senecio lactone (**6**), were isolated after four jacaranone glucosides [1]. Compound **7** was isolated as an inseparable mixture due to free terminal C-1″ tautomerization in its sugar part, whose structure was confirmed unambiguously by the UV, IR, MS, and 1D- and 2D-NMR spectra. The cytotoxic activities of these compounds were tested, respectively.

2. Results and discussion

Compound **4**, pale yellow gum, exhibited the pseudomolecular ion peak $[M - H]^-$ at *m/z* 171.0 in EI-MS, and its HR-EI-MS gave the molecular formula of $C_8H_{12}O_4$. In the NMR spectra of **4,** the proton signals at δ_H 1.86–1.92 (2H, m), 2.02–2.13 (4H, m), and 2.62–2.73 (2H, m) and the carbon signals at δ_C 210.2, 37.2 ($4 \times C$), and 69.1 suggested the presence of 4-oxocyclohexanone. In addition, the proton signal at δ_H 2.60 (2H, s) and the carbon signals at δ_C 45.7 and 173.5 suggested the presence of a substituted acetic acid moiety in compound **4**. Comparison of its ^1H and ^{13}C NMR spectral data with those of **4a** and **4b** in the literature [2] revealed that

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the structure of **4** was similar to that of **4a** and **4b**, except for the absence of a methoxyl of **4a** or ethoxyl group of **4b**. Thus, **4** was identified as tetrahydrojacaranone.

Compound **5** exhibited a pseudomolecular ion peak $[M + H]^+$ at *m/z* 215 in EI-MS. The molecular formula was established as $C_{10}H_{15}O_5$ by HR-EI-MS at *m/z* 215.0914 $[M + H]^+$. In the NMR spectra of compound **5**, the proton signals at δ_H 5.98 (1H, d, $J = 10.2$ Hz) and 6.78 (1H, d, $J = 10.2$ Hz) and the carbon signals at δ_C 197.1, 129.6, 148.7, and 70.0 indicated the existence of cyclohexanone moiety. The proton signals at δ_H 1.26 (3H, t, $J = 7.2$ Hz), 2.63 (2H, s), and 4.18 (2H, q, $J = 7.2$ Hz), along with the carbon signals at δ_C 42.0, 171.7, 61.5, and 14.0, suggested the presence of an mono-substituted acetic acid ethyl ester. The proton signals at δ_H 4.00–4.03 (1H, m) and 2.56–2.87 (2H, m) and the corresponding carbon signals at δ_C 71.2 and 41.1 revealed that **5** was the H_2O adduct of jacaranone ethyl ester **1**. Comparison of its spectral data with those of **5a** [3] revealed that the methyl ester in **5a** was replaced by an ethyl ester in **5**. Consequently, the structure of **5** was elucidated as 2,3-dihydro-3-hydroxyljacaranone ethyl ester.

Compound **7**, whose molecular formula was determined as $C_{14}H_{18}O_9$ by HR-ESI-MS at *m/z* 329.0880 $[M - H]^-$, was a jacaranone glycose ester. The ^1H and ^{13}C NMR spectra (Table 1) of **7** suggested the presence of one jacaranone and one sugar moiety [4]. An important HMBC correlation between H-2′ (δ_H 4.42) and C-8 (δ_C 170.3) was observed, confirming that jacaranone was linked to the C-2′ position of the sugar. The sugar obtained from the basic hydrolysis of **7** was identical to an authentic sample of glucose by TLC and HPLC analyses. Consequently, **7** was deduced as C-2′ monoester, as shown in Figure 1. The ratio of the α/β mixture was 3:2 as determined by the ^1H NMR spectrum.

Compound **8** exhibited a pseudomolecular ion peak ($[M + Na]^+$) at *m/z* 382.1 in its ESI-MS. Its molecular formula $C_{16}H_{12}O_9$ was furnished by HR-ESI-MS spectroscopy. Except for the typical signals for jacaranone ethyl acetate moiety [4], glucose signals were observed in its ^1H and ^{13}C NMR spectra. The doublet at δ_H 4.21 (1H, d, $J = 7.6$ Hz) in the ^1H NMR spectrum was attributed to the anomeric proton of the sugar, corresponding to the β-configuration. In TLC and HPLC analyses, the sugar obtained from the acid hydrolysis of **8** was identical to an authentic sample of D-glucose. Consequently, its structure was assigned as jacaranone ethyl ester 4-*O*-glucoside (Figure 1).

Table 1. Cytotoxicity data of jacaranone ethyl ester (**1**) (IC_{50} values, μg/ml).

Tumor cell lines	**1**	Flurouracil (FU)
HCT-8	0.56	0.67
CaEa-17	0.56	0.89
A2780	0.57	0.65
HeLa	0.60	0.57
BEL-7402	0.63	0.54
KB	0.78	0.64
PC-3M	0.850	0.77

The cytotoxic activities of compounds **1**–**8** were evaluated. Among them, compound **7** exhibited moderate cytotoxicities. Compounds **5** and **8** showed potent cytotoxicities with IC_{50}s at a range of 0.699–7.660 μg/ml against five tumor cell lines (Table 2). Jacaranone ethyl ester (**1**) was significantly cytotoxic with IC_{50}s at a range of 0.5–1.0 μg/ml against various tumor cell lines (Table 1).

Compounds **1–3**, jacaranone and its methyl and ethyl esters showed significant cytotoxic activities *in vitro*. Compound **5** retained strong cytotoxic activity, while compounds **4** and **6** were inactive. This result suggested the essence of α,β-unsaturated carbonyl group in their cytotoxicities, a segment that has been previously known to be crucial to cytotoxicity in various compounds [5–8]. The inactivity of **4** is not surprising due to the absence of α,β-unsaturated carbonyl segment, and the inactivity of **6** may be arisen from its steric hindrance in comparison with **5**. This postulation was partly confirmed by reduced antitumor activity of **8**, which may also be attributed to increased steric hindrance.

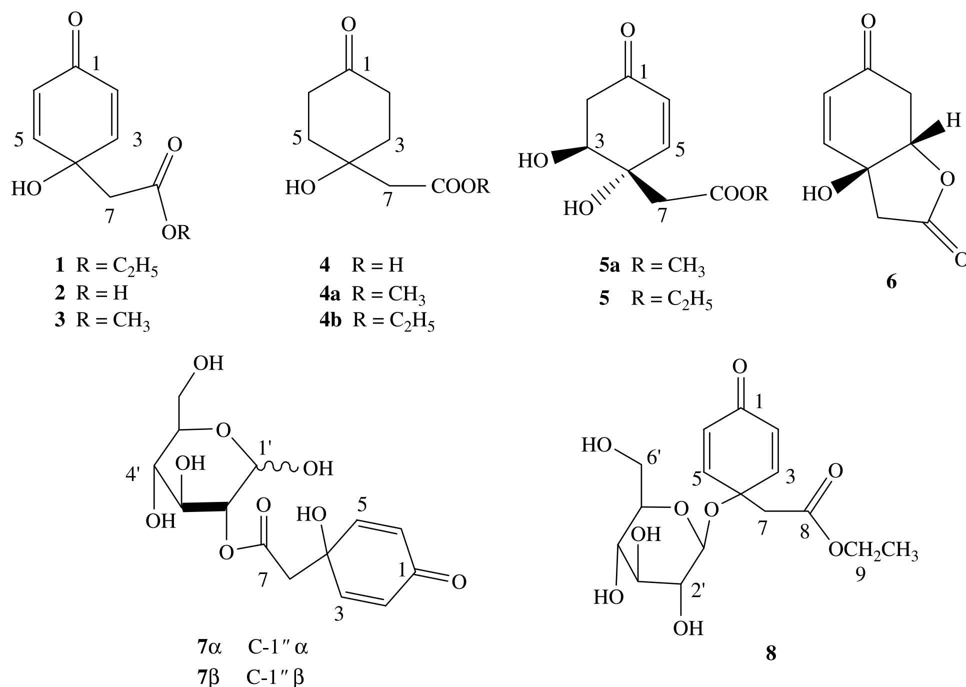

Figure 1. Structures of compounds **1**–**8**.

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The relatively low activity of jacaranone ester glucoside **7** (Table 3) may also be ascribed to steric factors. Furthermore, glycoside **8** retained potent activity, suggesting that substituents attached to the hydroxyl may be well tolerated, in comparison with the modification at the carboxyl of jacaranone moiety in compound **7**.

The major cytotoxic constituent jacaranone ethyl ester (**3**) was isolated in more than 0.075% (dry weight of plant materials); thus we were able to examine its *in vivo* activity. It displayed antitumor activity against H_{22} liver tumor-transplanted mice with 30% tumor growth inhibition at a dose of 15 mg/kg, and with 53% inhibition at 50 mg/kg. However, the dosage of 50 mg/kg led to 4/10 death of the mice indicating its high toxicity at this dose. Activities of jacaranone analogs and its glycosides *in vivo* are undergoing.

Bioassay-guided fractionation led to the isolation of jacaranone and its analogs. The activity result suggested that jacaranone ethyl

Table 2. Cytotoxicity data of jacaranone and its derivatives (IC_{50} values, μg/ml).

	A549	BEL-7402	BGC-823	HCT-8	MCF-7
2	5.37	0.58	2.73	3.64	2.91
3	5.20	0.95	6.02	4.58	8.37
4	>50	>50	>50	>50	>50
5	3.88	0.70	3.23	2.50	7.66
6	>50	>50	>50	>50	>50
7	>10	>10	>10	>10	>10
8	5.86	4.10	4.83	4.86	5.68
Flurouracil (FU)	0.18	0.54	0.69	0.67	0.57

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Table 3. ^{1}H and ^{13}C NMR spectral data for **7** in CD_3OD (500 MHz for ^{1}H and 125 MHz for ^{13}C).

	δ_H		δ_C	
No.	**7α** (major)	**7β** (minor)	**7α** (major)	**7β** (minor)
1			187.4	187.4
2	6.10 (d, 10.0)	6.10 (d, 10.0)	127.2	127.1
3	7.03 (d, 10.0)	7.0 (d, 10.0)	152.7	152.7
4			70.7	70.6
5	7.03 (d, 10.0)	7.03 (d, 10.0)	152.7	152.7
6	6.10 (d, 10.5)	6.10 (d, 10.5)	127.2	127.2
7	2.71 (s)	2.73 (s)	44.8	45.8
8			170.3	170.3
1′	5.01 (d, 4.0)	4.40 (d, 7.5)	92.8	97.0
2′	4.42 (m)	4.42 (m)	75.1	75.1
3′	3.20–4.00 (m)	3.20–4.00 (m)	73.7	73.2
4′	3.20–4.00 (m)	3.20–4.00 (m)	72.6	71.8
5′	3.20–4.00 (m)	3.20–4.00 (m)	76.9	76.8
6′a	4.10 (m)	4.10 (m)	61.5	61.7
6′b	4.32 (m)	4.32 (m)		

ester was the major cytotoxic constituent of *S. scandens*, and preliminary structure–activity relationship indicated the α,β-unsaturated carbonyl segment is responsible for the antitumor effect. In order to determine whether jacaranone ethyl ester (**1**) is the artifact of jacaranone during the process of extraction and isolation, the aerial parts of *S. scandens* was extracted with MeOH at ambient temperature. The residue was divided into petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH fractions by extraction. Both the petroleum ether and $CHCl_3$ extractions showed the presence of **1** comparing with the authentic compound on TLC. Compounds **5** and **8** were detected to exist in the EtOAc and *n*-BuOH fractions, respectively, by the HPLC method.

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XT_4-100X micromelting point apparatus (uncorrected). Optical rotations were measured on a PerkinElmer 241 digital polarimeter. IR spectra were recorded on a Nicolet-Impact 400 IR spectrometer with KBr disk. UV spectra were recorded on a HP 8453 spectrophotometer. NMR experiments were performed on MERCURY-400 and INOVA-500 or 300 spectrometers using TMS as internal standard, and the chemical shifts were given on the δ scale. Assignments were confirmed by COSY, HMBC, and HMQC experiments. Mass spectra were obtained on an Autospec-Ultima ETOF. ESI-MS measurements were carried out on an Agilent 1100 series LC/MSD Trap SL mass spectrometer.

Silica gel (60–100 and 200–300 meshes) for column chromatography and silica gel GF_{254} for TLC were obtained from Qingdao Marine Chemical Company (Qingdao, Shandong Province, China). RpC-18 (40–60 microns) silica gel was purchased from Fuji Silysica Chemical Ltd. (Kasugai, Japan). Size-exclusion chromatography was performed using Sigma Lipophilic Sephadex LH-20 (Milwaukee, WI, USA). HPLC was carried out on an Agilent 1100 series.

3.2 Plant material

The aerial parts of *S. scandens* Buch-Ham were collected from Dali, Yunnan Province of China, in October 2001. It was identified by Prof. Guangming Liu, Dali Medicinal College. The authentic sample of the plant has been deposited at the Herbarium of the

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Institute of Materia Medica, Chinese Academy of Medical Sciences (no. 817).

3.3 Extraction and isolation

The dried aerial parts (8.0 kg) of *S. scandens* were powdered and extracted with the boiling ethanol (95%, 30 L × 3, each 3 h). The combined alcoholic extracts were concentrated *in vacuo* to yield a dark brown residue (1.2 kg), which were chromatographed on silica gel (60–100 mesh, 4 kg) and eluted with petroleum ether–acetone (90:10, 13.4 L; 50:50, 13.0 L), acetone (7.4 L), and methanol (9.0 L) to provide fractions I, II, III, IV, and V. Bioactive factions I and IV were further isolated and tested for cytotoxicity. Fraction I (50 g) was chromatographed on silica gel (2 kg) eluting with petroleum ether–acetone (75:25, 5.2 L) to give eight major fractions (I-1 to I-8) based on TLC behaviors. Activity test showed that fractions I-6 to I-8 exhibited strong cytotoxicities. Fraction I-6 (0.99 g) was further subjected to a silica gel column (200–300 mesh, 90 g) eluting with petroleum ether–acetone (90:10, 8.7 L; 80:20, 5.8 L) to afford four subfractions (I-6-1 to I-6-4). Subfraction I-6-3 (0.540 g) was purified on silica gel column chromatography (200–300 mesh, 5.0 g) eluting with cyclohexane–ethyl acetate (9:1, 2.6 L) to afford jacaranone ethyl ester **1** (0.260 g). Fraction I-7 (0.200 g) was subjected to column chromatography (200–300 mesh, 5.0 g) using cyclohexane–ethyl acetate (9:1, 1.5 L) as eluent to give jacaranone **2** (30 mg). Fraction I-8 (17.0 g) was chromatographed on silica gel (60–100 mesh, 1000 g) using petroleum ether–acetone (9:1, 12.4 L) as eluent to afford 16 subfractions [I-8-1 to I-8-16]. Subfractions I-8-9, -10, -11, and -12 showed strong cytotoxicities and were further subjected to silica gel chromatography eluting with cyclohexane–ethyl acetate (9:1). Fraction I-8-9 (3.317 g) was chromatographed on silica gel (200–300 mesh, 250 g) to afford jacaranone methyl acetate **3** (0.015 g) and jacaranone ethyl acetate **1** (2.843 g). Fraction I-8-10 (1.215 g) was chromatographed on silica gel (200–300 mesh, 90 g) to give jacaranone ethyl acetate **1** (0.953 g). Fraction I-8–11 (1.324 g) was separated on silica gel column chromatography (85 g) to afford jacaranone ethyl acetate **1** (0.905 g), **4** (0.010 g), **5** (0.006 g), and senecio lactone **6** (0.040 g). Fraction I-8–12 (0.213 g) was isolated by silica gel column chromatography to give jacaranone ethyl acetate **1** (0.063 g) and senecio lactone **6** (0.034 g).

Bioactive fraction IV (353 g) was subjected to silica gel column chromatography (1.0 kg) eluting with $CHCl_3$–MeOH (95:5, 12.6 L; 90:10, 9 L; 85:15, 14.5 L; 80:20, 8.5 L; 70:30, 14.0 L; 50:50, 20 L) to afford six fractions (A1, A2, A3, A4, A5, and A6). Bioactive fraction A2 (45.0 g) was subjected to silica gel column chromatography (200–300 mesh, 2 kg) eluting with MeOH–$CHCl_3$ (30:70, 4 L) to afford 11 fractions (A2-1 to A2-11). Fraction A2-4 (1.54 g) was chromatographed on silica gel using $CHCl_3$–CH_3OH–H_2O (80:20:1, 9.0 L) as eluent to afford six subfractions. Subfraction A2-4–6 (0.885 g) was further separated with Sephadex LH-20 using MeOH as eluent and purified by HPLC on ODS (YMC-Pack ODS-A C-18, 250 × 20 mm; eluent, 20% MeOH and 0.1% CF_3COOH; flow rate, 5.0 ml/min; detection UV at 210 nm) to afford **7** (0.004 g) and **8** (0.014 g).

*3.3.1 Compound **4***

Pale yellow gum; UV (MeOH) λ_{max} (log ε) 242 (3.31) nm; IR (KBr) ν_{max} 3057, 1729 cm^{-1}; ^1H NMR (CD_3COCD_3, 300M Hz) δ_H 2.67–2.73 (2H, m, H-2a, 6a), 2.02–2.13 (4H, m, H-2e, 3e, 5e, 6e), 1.86–1.92 (2H, m, H-3a, 5a); 2.60 (2H, s, H-7); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ_C 210.2 (C-1), 37.2 (C-2, 3, 5, 6), 69.1 (C-4), 45.7 (C-7), 173.5 (C-8); EI-MS *m/z* (%) 171.1 ($[M - H]^-$, 30), 154.0 ($M - H_2O$, 90), 112.0 ($M - CH_2COOH$, 100); HR-EI-MS *m/z* 171.0663 $[M - H]^-$ (calcd for $C_8H_{11}O_4$, 171.0663).

3.3.2 Compound 5

Pale yellow gum; $[\alpha]_D^{20}$ -12.5 (c 1.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 244 (3.26) nm; IR (KBr) ν_{max} 3438, 1732, 1682 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 5.98 (1H, d, $J = 10.2$ Hz, H-6), 6.78 (1H, d, $J = 10.2$ Hz, H-5), 4.00–4.03 (1H, dd, $J = 6.9$, 3.6 Hz, H-3), 2.56–2.87 (2H, m, H-2), 2.57 (2H, s, H-7), 4.18 (2H, dd, $J = 7.2$ Hz, H-9), 1.26 (3H, t, $J = 7.2$ Hz, H-10); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 197.1 (C-1), 129.6 (C-6), 148.7 (C-5), 70.0 (C-4), 71.2 (C-3), 41.1 (C-2), 42.0 (C-7), 171.7 (C-8), 61.5 (C-9), 14.0 (C-10); EI-MS *m/z* 214 (M^+), 170, 142, 96, 82; HR-EI-MS *m/z* 215.0914 $[M + H]^+$ (calcd for $C_{10}H_{15}O_5$, 215.0915).

3.3.3 Compound 7

Pale yellow gum; UV (MeOH) λ_{max} (log ε) 231 (2.35) nm; IR (KBr) ν_{max} 3429, 1728, 1599, 1080, 1036 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 3; (−)HR-ESI-MS *m/z* 329.0878 $[M - H]^-$ (calcd for $C_{14}H_{17}O_9$, 329.0881); ESI-MS *m/z* 329.1 $[M - H]^-$.

3.3.4 Compound 8

Pale yellow gum; UV (MeOH) λ_{max} (log ε) 239 (2.73) nm; IR (KBr) ν_{max} 3394, 1732, 1672, 1082, 1034 cm^{-1}; ^1H NMR (CD_3OD, 400 M Hz) δ_H 1.17 (3H, t, $J = 7.2$ Hz, H-10a), 2.71 2H, s, H-7), 3.83 (1H, m, H-9a), 3.55 (1H, m, H-9b), 6.08 (2H, d, $J = 10.0$ Hz, H-2, 6), 7.03 (2H, d, $J = 10.0$ Hz, H-3, 5), 4.21 (1H, d, $J = 7.2$ Hz, H-1′), 4.37 (1H, dd, $J = 2.0$, 12.0 Hz, H-6′a), 4.13 (1H, dd, $J = 5.6$, 12.0 Hz, H-6′b), 3.08–3.40 (4H, m, H-2′, 3′, 4′, 5′); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 187.4 (C-1), 128.3 (C-2), 152.7 (C-3), 77.8 (C-4), 152.8 (C-5), 128.3 (C-6), 45.9 (C-7), 170.3 (C-8), 66.3 (C-9), 15.5 (C-10), 104 (C-1′), 75.0 (C-5′), 75.1 (C-3′), 68.1 (C-4′), 71.6 (C-2′), 65.0 (C-6′); ESI-MS *m/z* 382.1 $[M + Na]^+$; HR-ESI-MS *m/z* 357.1191 $[M - H]^-$ (calcd for $C_{16}H_{21}O_9$, 357.1193).

3.4 Cytotoxicity assay

We evaluated the cytotoxic activity of jacaranone and its analogs on many tumor cell lines. Fluorouracil was used as a positive control. The cells were continuously treated with the samples for 96 h. The supernatant was doffed off and 0.1 ml MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 0.4 mg/ml in RPMI 1640) was added, after each well had been carefully washed with RPMI 1640. The cell viabilities were measured with an MTT assay procedure (Tables 1 and 2) [9,10].

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