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Aristolochic acid derivatives from the rhizome of *Arisolochia championii*

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ABSTRACT

Four new aristolochic acid derivatives aristchamic A (**1**), aristchamic B (**2**), aristochamic C (**3a**), aristchamic D (**3b**) and one new aristolactam aristolactam-CV (**4**), together with 10 known compounds (**5**–**14**), were isolated from the rhizomes of *Aristolochia championii*. Their structures were assigned by detailed analysis of MS and NMR spectroscopic data. All of the isolated compounds were evaluated for their cytotoxic activities against HCT-116, HepG2, BGC-823, NCI-H1650, and A2780 cell. Compound **1** exhibited significant cytotoxic activity against HCT-116, HepG2, BGC-823, and NCI-H1650, with IC_{50} values of 0.50, 7.37, 2.66, and 0.75 μM, respectively.

1. Introduction

Aristolochia championii Merr. et Chun., a member of genus *Aristolochia* from aristolochiaceae family, is an herbaceous plant mainly distributed in the southwest of China [1]. Aristolochia species were previous reported to possess abundant bioactive components, such as diverse aristolochic acids [2–5], aristolactams [6,7], alkaloids [8], and terpenoids [9,10]. With the aim of searching novel bioactive constituents from this genus, phytochemical investigations were conducted with the rhizome of *A. championii* and resulted in the isolation and structural identification of four new aristolochic acid derivatives aristchamic A (**1**), aristchamic B (**2**), aristchamic C (**3a**), aristchamic D (**3b**) and one new aristolactam aristolactam-CV (**4**), together with 10 known compounds (**5**–**14**). Their cytotoxic activities against HCT-116, HepG2, BGC-823, NCI-H1650, and A2780 cell were also evaluated.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a JASCO P-2000 polarimeter, and UV spectra with a JASCO V-650 spectrophotometer (JASCO Corporation, Tokyo, Japan). IR spectra were recorded on a Nicolet 5700 spectrometer (Thermo Electron Scientific Instruments Corp.) by an FT-IR microscope transmission method. CD spectra were measured on a JASCO-815 CD spectrometer. NMR measurements were performed on Bruker AV500-III and Bruker AV 600 IIIHD spectrometers using TMS as an internal reference (Bruker Biospin Corporation, Fallanden, Switzerland) in $CDCl_3$ and DMSO. HRESIMS were obtained using an Agilent 1100 series LC/MSD ion trap mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (GE), and ODS (50 μm, YMC, Kyoto, Japan) were used for column chromatography. Analytical thin-layer chromatography (TLC) was carried out with GF254 plates (Qingdao Marine Chemical Factory). Preparative HPLC experiments were performed on a preparation YMC-Pack ODS-column (10 μm, 250 × 20 mm; YMC, Kyoto, Japan) or a Chiralpak AD-H column (5 μm, 250 × 20 mm; Daicel Chiral Technologies Co. Ltd., Shanghai, China) equipped with Shimadzu SPD-6A UV spectrophotometric detector (Shimadzu, Kyoto, Japan) and Shimadzu LC-6AD pumping system. Spots were visualized by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

2.2. Plant material

The rhizomes of *Aristolochia championii* Merr. et Chun were purchased from the Yulin City, Guangxi Province, China, in June 2014, and were identified by associate professor Lin Ma (Institute of Materia Medica, Chinese Academy of Medical Science & Peking Union Medical College). A voucher specimen (ID-S-2566) has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Science & Peking Union Medical College, Beijing.

2.3. Extraction and isolation

Air-dried rhizomes of *Aristolochia championii* (60 kg) were extracted three times with 95% EtOH (3 × 150 L) under reflux (2 h each). The

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combined extracts were concentrated under reduced pressure to dryness. The residue (3 kg) was subjected to fractionation using silica gel eluted with petroleum ester, CH_2Cl_2, EtOAc, acetone and MeOH, successively, to give five fractions (A, B,C, D, and E, respectively). The CH_2Cl_2 fraction (230 g) was applied to a silica gel eluted gradient with $CHCl_3$/MeOH (200:1–50:1, v/v) to afford seven major fractions (B_1–B_7). Fraction B_3 (10.6 g) was chromatographed on silica gel (petroleum ester-Me_2CO 20:1–4:1, v/v) to give subfractions $B_{3\text{-}1}$–$B_{3\text{-}20}$, fraction $B_{3\text{-}5}$ was followed by Sephadex LH-20 column ($CHCl_3$:MeOH 50:50) to give five subfractions ($B_{3\text{-}5\text{-}1}$–$B_{3\text{-}5\text{-}5}$). Fraction $B_{3\text{-}5\text{-}2}$ was purified by preparative HPLC (MeOH:H_2O, 85:15) to yield **1** (12 mg), and $B_{3\text{-}5\text{-}3}$ also was purified by preparative HPLC (MeOH:H_2O, 75:25) to yield **2** (14 mg), **5** (27 mg). $B_{3\text{-}5\text{-}4}$ was chromatographed over silica gel eluted with $CHCl_3$/MeOH (100:1, v/v) to give **6** (21 mg) and **7** (12 mg). Fraction $B_{3\text{-}13}$ and $B_{3\text{-}15}$ were subjected separately to separation over Sephadex LH-20 (MeOH) to yield **8** (47 mg) from $B_{3\text{-}13}$ and **9** (14 mg) from $B_{3\text{-}17}$. Fraction B_5 (14.7 g) was subjected to a silica gel column chromatography and eluted with a step gradient of petroleum ester-Me_2CO (50:1–2:1, v/v) to yield five subfractions ($B_{5\text{-}1}$–$B_{5\text{-}5}$). Fraction $B_{5\text{-}3}$ was purified by Sephadex LH-20 column (MeOH) to yield **4** (11 mg). Fraction $B_{5\text{-}4}$ was purified by preparative HPLC (MeOH:H_2O, 80:20) to yield **10** (11 mg) and **11** (8 mg). Fraction $B_{5\text{-}4}$ was also purified by preparative HPLC (MeOH:H_2O, 75:25) to yield **12** (16 mg). Fraction B_6 (14.2 g) was chromatographed on RP-C_{18} silica gel to obtain 14 subfractions ($B_{6\text{-}1}$–$B_{6\text{-}14}$). Fraction $B_{6\text{-}10}$ was subjected to a Sephadex LH-20 column (MeOH) and purified by preparative HPLC (acetonitrile:H_2O, 60:40) to yield **3** (9 mg) and **14** (21 mg). Subsequent separation of **3** by HPLC on a semi-preparative Chiralpak AD-H column (*n*-hexane:*i*PtOH, 1:1, 2.0 mL/min) afforded **3a** (4.2 mg, $t_R = 9.0$ min) and **3b** (4.1 mg, $t_R = 15.2$ min). Fraction $B_{6\text{-}12}$ was further separated by repeated Sephadex LH-20 column chromatography to afford **13** (32 mg).

*2.3.1. Aristchamic A (**1**)*

Yellow solid; $[\alpha]_D^{20}$ − 179.8 (0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (4.01), 267 (4.58), 314 (3.92), 326 (3.79) nm; IR (KBr) ν_{max} 3360, 2955, 2920, 1720, 1611, 1553 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS (positive) *m/z* 388.1039 $[M + H]^+$ (calcd. for $C_{19}H_{18}NO_8$, 388.1027).

*2.3.2. Aristchamic B (**2**)*

Yellow solid; $[\alpha]_D^{20}$ + 23.9 (0.27, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (4.41), 258 (4.36), 311 (4.24) nm; CD ($CHCl_3$) λ_{max} (Δε) 250 (+0.28), 298 (−0.28), 341 (+0.58); IR (KBr) ν_{max} 3481, 1750, 1708, 1621 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS (positive) *m/z* 362.0868 $[M + H]^+$ (calcd. for $C_{17}H_{16}NO_8$, 362.0870).

*2.3.3. Aristchamic C (**3a**) and aristchamic D (**3b**)*

Yellow solid; UV ($CHCl_3$) λ_{max} (log ε) 242 (4.82), 262 (4.78), 284 (4.55), 325 (4.28), 395 (4.16) nm; IR (KBr) ν_{max} 3471, 1715, 1610 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS (positive) *m/z* 574.1298 $[M + Na]^+$ (calcd. for $C_{28}H_{25}NO_{11}Na$, 574.1320).

3a: Yellow solid; $[\alpha]_D^{20}$ − 21.6 (0.04, $CHCl_3$); CD ($CHCl_3$) λ_{max} (Δε) 246 (+0.44), 318 (+0.31).

3b: Yellow solid; $[\alpha]_D^{20}$ + 19.7 (0.04, $CHCl_3$); CD ($CHCl_3$) λ_{max} (Δε) 243 (−0.24), 222 (−0.16).

*2.3.4. Aristolactam-CV (**4**)*

Yellow solid; UV ($CHCl_3$) λ_{max} (log ε) 232 (4.18), 256 (4.29), 278 (4.35), 288 (4.37) nm; IR (KBr) ν_{max} 3492, 1687, 1652 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS (positive) *m/z* 296.0925 $[M + H]^+$ (calcd. for $C_{17}H_{14}NO_4$, 296.0917).

Table 1
^1H NMR and ^{13}C NMR spectral data for **1**–**4**.

	1[a]		**2**[a]		**3a**[b] **and 3b**[b]		**5**[c]	
No.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		124.6		173.4		120.7		131.3
2	7.49 (s)	109.9		76.4	7.78 (s)	121.0		119.1
3		148.1	3.20 (d, 18.7) 3.13 (d, 18.7)	40.5		144.5		148.6
4		149.1		201.0		141.2		150.4
4a		119.4		131.2		122.0		119.2
4b		130.5		138.4		132.7		125.6
5	7.38 (d, 2.3)	104.1	8.28 (d, 1.6)	96.4	8.64 (d, 1.7)	102.7	9.05 (d, 7.2)	126.5
6		159.8		166.2		161.7	o	125.4
7	6.47 (d, 2.3)	98.9	6.67 (d, 1.6)	100.7	6.88 (d, 1.7)	99.4	o	127.2
8		157.6		158.4		157.4	7.97 (d, 7.0)	129.1
8a		111.9		128.8		114.5		134.5
9	4.23 (dd, 17.6, 2.4) 2.81 (dd, 17.6, 5.4)	26.5	9.35 (s)	121.6	8.60 (s)	120.0	7.16 (s)	104.7
10	6.67 (dd, 2.4, 5.4)	78.8		149.1		142.7		135.1
10a		126.2		134.3		116.9		121.7
11		166.6				166.7		169.6
12	6.28 (d, 1.4) 6.13 (d, 1.4)	102.4					5.11 (s)	59.8
1′						126.5		
2′					7.25 (d, 1.7)	112.0		
3′						147.7		
4′						147.4		
5′					6.87 (d, 8.1)	115.5		
6′					7.08 (dd,8.1,1.7)	120.7		
7′					5.22 (d, 8.3)	77.3		
8′					4.54 (m)	77.8		
9′					3.69 (m) 3.48 (m)	60.4		

o: overlapped.
[a] Recorded at 500 MHz (^1H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$.
[b] Recorded at 600 MHz (^1H NMR) and 150 MHz (^{13}C NMR) in DMSO.
[c] Recorded at 500 MHz (^1H NMR) and 125 MHz (^{13}C NMR) in DMSO.