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New Lupanes from *Alstonia scholaris* Reducing Uric Acid Level

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ABSTRACT

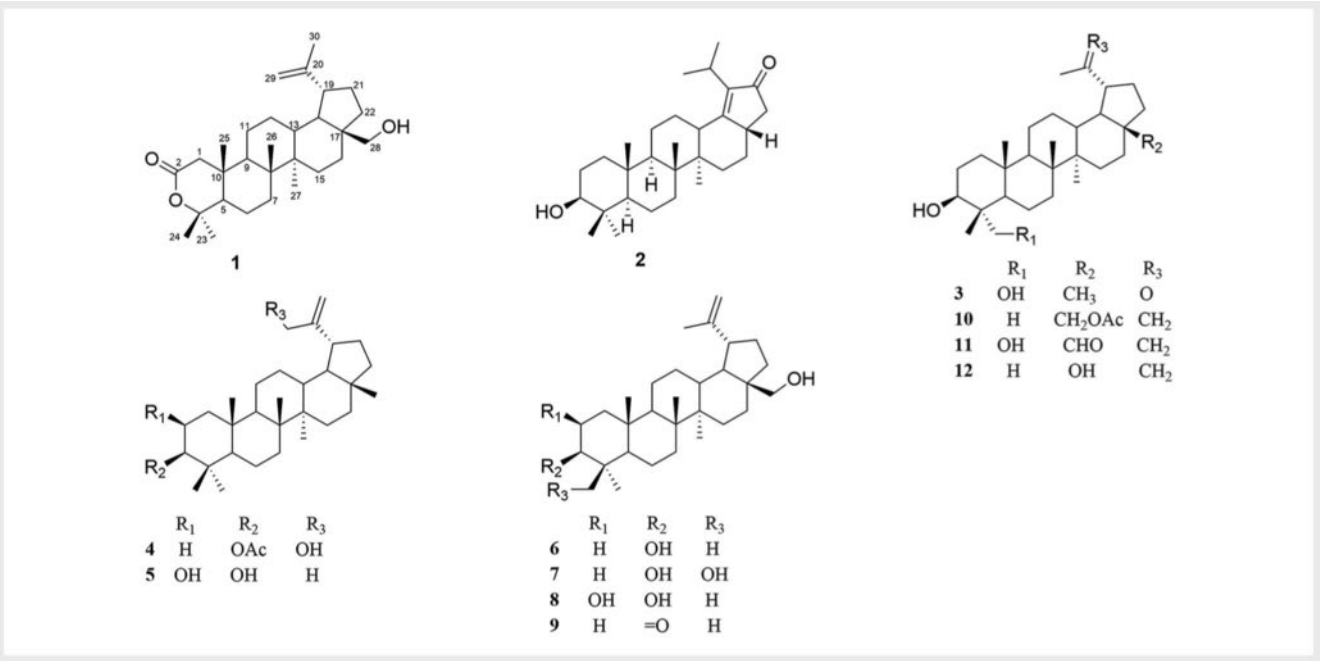
Twelve lupanes including three new compounds named alstoscholarilups A–C (**1–3**) were isolated from the leaves of *Alstonia scholaris*. Their structures were elucidated by spectroscopic analysis and ECD calculation. Structurally, compound **1** with a rare A ring-seco skeleton formed lactone and degraded C-3, while **2** with a 28-*nor* and **3** with a 29-*nor*-lupane skeleton supported the phytochemical diversity and novelty of the plant. Pharmacologically, compounds **4, 7**, and **10** reduced the serum uric acid (UA) levels of mice significantly.

Introduction

Hyperuricemia (HUA) is closely related with gout [1, 2], nephropathy [3, 4], and cardiovascular risk [5]. Lacking the functional uricase, hominoids are the only mammals that cannot degrade urate [6]. In the clinic, HUA is commonly classified into three types: the urate overproduction type, the urate underexcretion type, and the combined type [7]. Therefore, the effective clinical approach for HUA management is to reduce the uric acid (UA) level by increasing the excretion of UA and decreasing the synthesis of UA with anti-hyperuricemic drugs. However, many of the reported drugs for hyperuricemia are either expensive or have serious side effects [8]. Then, natural products may be a promising resource for drug discovery and development.

Pentacyclic triterpenoids, an important class of secondary metabolites derived from plants, exhibit a wide range of pharmacological activities. Four pentacyclic triterpenoids were launched as drugs in China, including oleanolic acid, glycyrrhizic acid, asiaticoside, and carbenoxolone [9]. Betulin (3-lup-20(29)-ene-3β,28-diol), structurally belonging to pentacyclic lupane triterpenoids [10], inhibited xanthine oxidase significantly [11, 12] and is a major compound of *Alstonia scholaris*. Previous studies of *A. scholaris* reported some structurally diverse and bioactive alkaloids [13–20] and triterpenoids [21–24], and then, reducing the UA levels in lupanes, structurally related to betulin, encouraged our further investigation into it. As a result, 12 lupanes, including three new compounds named alstoscholarilups A–C (**1–3**) and nine known analogues (**4–12**), were isolated from *Alstonia scholaris* (L.) R. Br. (Apocynaceae) (▶ **Fig. 1**). Compounds **1, 4, 7, 8, 10**, and **11** exhibited better tendencies of reducing UA levels in monosodium urate (MSU)-induced human renal tubular epithelial cell (HK-2) model at a concentration of 5 μM and were chosen for further evaluation in mice. The results exhibited that compounds **4, 7**, and **10** at 5 mg/kg decreased the serum UA levels, which showed significant anti-hyperuricemic effects *in vivo*.

* These authors contributed equally to this work.



▶ **Fig. 1** Lupanes **1**–**12** from *A. scholaris*.

Results and Discussion

The molecular formula of compound **1** was determined to be $C_{29}H_{46}O_3$ based on the HRESIMS at *m/z* 443.35397 $[M + H]^+$ (calcd for $C_{29}H_{47}O_3$, 443.35197), with 7 degrees of unsaturation. The ^1H NMR spectral data (▶ **Table 1**) presented two olefinic protons, two oxygenated protons, and six characteristic singlet methyls of lupanes. With the aid of HSQC and DEPT experiments, the ^{13}C NMR (▶ **Table 1**) displayed 29 carbons, comprising of 6 methyls, 11 methylenes (including 1 exocyclic olefinic and 1 oxygenated carbons), 5 methines, and 7 quaternary carbons (including 1 ketone and 1 olefinic carbon). The above spectral data suggested that **1** was similar with jughopenoid B [25]. According to the key HMBC correlations (▶ **Fig. 2**) of δ_H 3.78, 3.35 (2H, H-28) with δ_C 29.1 (C-16), 47.76 (C-17), and 33.9 (C-22), and of δ_H 1.61 (1H, H-18) with δ_C 60.5 (C-28), an oxygenated methylene in **1** was deduced.

Biogenetically, β-orientation was positioned for Me-25 [25], and then, the NOE correlations (▶ **Fig. 3**) of δ_H 0.98 (H_3-25) with δ_H 1.34 (H_3-24), 1.06 (H_3-26) and δ_H 2.66 (Ha-1), of δ_H 1.06 (H_3-26) with δ_H 1.65 (H-13) and 3.78 (Ha-28), and of δ_H 3.78 (Ha-28) with δ_H 2.40 (H-19) positioned all of these protons as β-orientation. The NOE correlations of δ_H 1.81 (Hb-1) with δ_H 1.42 (H_3-23) and 1.40 (H-9) and of δ_H 1.00 (H_3-27) with δ_H 1.42 (H-5), 1.40 (H-9) and 1.61 (H-18) placed these protons at α-orientation. Comparing the experimental CD spectrum with the calculated ECD curves (▶ **Fig. 4a**), the absolute configurations of **1** were 5*R*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*, 17*S*, 18*R*, and 19*R*.

Alstoscholarilup A (**1**) could be tracked back to betulin (**6**), which yields 2β,3β,28-lup-20(29)-en-triol (**8**) by a hydroxylation reaction. Further oxidative cleavage reaction at C-2/3 of 2β,3β,28-lup-20(29)-en-triol could yield the intermediate A. Intermediate B might be derived from intermediate A by a decarboxylation reaction. Finally, alstoscholarilup A (**1**) might be afforded from intermediate B via a hydroxylation reaction and following an esterification reaction (▶ **Fig. 5**).

Compound **2** was obtained as a white powder. Its molecular formula was determined to be $C_{29}H_{46}O_2$ by the HRESIMS at *m/z* 427.35663 $[M + H]^+$ (calcd for $C_{29}H_{47}O_2$, 427.35706). The ^1H NMR spectral data (▶ **Table 1**) displayed five singlet methyls, two doublet methyls, and one oxygenated proton. The ^{13}C NMR and DEPT spectral data displayed 29 carbons, comprising seven methyls, nine methylenes, six methines (including one oxygenated carbon), and seven quaternary carbons (including one ketone and two olefinic carbons) (▶ **Table 1**). All these data suggested that the **2** might be a *nor*-lupane derivative similar to betulinic acid [26]. In its HMBC spectrum, the presence of a ketone at C-21 and two olefinic quaternary carbons were deduced by the correlations of δ_H 2.42 (Ha-22) and 3.19 (H-20) with δ_C 211.5 (C-21) and of δ_H 3.19 (H-20) with δ_C 177.8 (C-18) and 143.1 (C-19). The absence of a carboxyl and an olefinic methylene were deduced by the correlations of δ_H 2.42 (Ha-22) and 1.98 (Ha-16) with the methine δ_C 41.9 (C-17) and the ^1H-^1H COSY correlations of δ_H 3.19 (1H, H-20) with δ_H 1.15 (3H, H-29) and 1.18 (3H, H-30) (▶ **Fig. 2**). The NOE correlations of δ_H 1.38 (H-9) with δ_H 0.943 (H_3-27) and 0.76 (H-5) and of δ_H 0.96 (H_3-23) with δ_H 3.16 (H-3) and 0.76 (H-5) positioned them at α-orientation and 3β-OH, and of δ_H 2.77 (H-13) with δ_H 2.43 (H-17) placed 17β-H. Another partial relative configuration of **2** was supported to be the same as **1** based on its NOE correlations (▶ **Fig. 3**). Furthermore, the absolute configuration of **2** was elucidated to be 3*S*, 5*R*, 8*R*, 9*R*, 10*R*, 13*S*, 14*R*, and 17*S* by the ECD calculation (▶ **Fig. 4b**).

Compound **3** possessed a molecular formula of $C_{29}H_{48}O_3$ by the positive HRESIMS ion peak at *m/z* 445.36822 $[M + H]^+$ (calcd

▶ **Table 1** ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds **1–3** (δ in ppm).

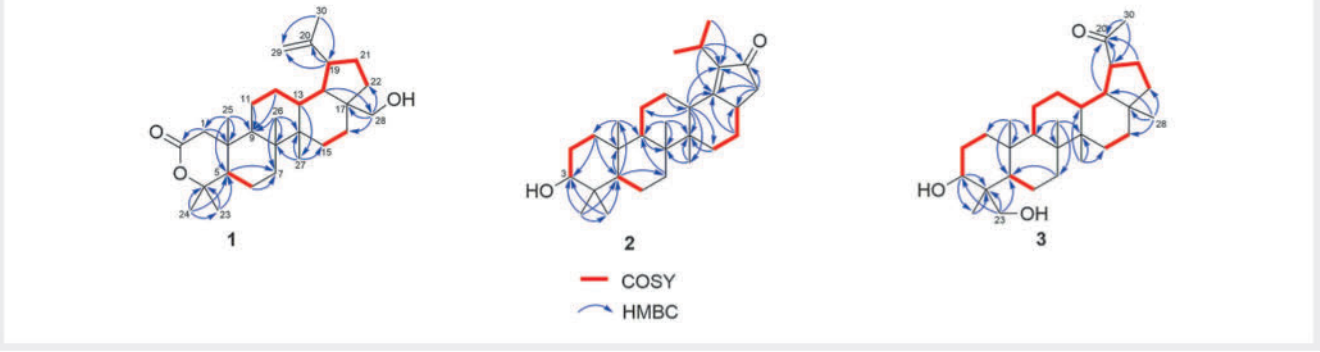
NO.	1[a]		2[b]		3[a]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	Ha: 2.66, d (16.7)	46.6, t	Ha:1.79, m	38.8, t	Ha: 1.66, m	38.4, t
	Hb: 1.81, d (16.7)		Hb:1.03, m		Hb: 0.88, m	
2		170.9, s	1.62 (2H), m	26.6, t	1.60 (2H), m	26.9, t
3			3.16, dd (11.5, 5.12)	78.1, d	3.62, t-like (8.3)	76.6, d
4		85.8, s		41.0, s		41.9, s
5	1.42, m	52.4, d	0.76, m	55.4, d	0.83, m	49.9, d
6	1.33 (2H), m	20.9, t	Ha:1.57, m	18.0, t	Ha: 1.41, m	18.4, t
			Hb:1.44, overlap		Hb: 1.32, m	
7	1.48 (2H), m	32.7, t	Ha:1.54, m	34.8, t	Ha:1.49, m	33.9, t
			Hb:1.44, overlap		Hb:1.36, m	
8		40.7, s		37.0, s		40.7, s
9	1.40, m	47.77, d	1.38, dd (12.4, 3.5)	51.3, d	1.29, m	50.3, d
10		36.4, s		38.6, s		37.1, s
11	1.43 (2H), m	29.7, t	Ha:1.64, m	21.0, t	Ha: 1.44, m	20.9, t
			Hb:1.34, m		Hb: 1.27, m	
12	Ha: 1.70, m	24.9, t	Ha:1.79, m	31.2, t	1.03 (2H), m	27.2, t
	Hb: 1.07, m		Hb:1.42, m			
13	1.65, m	37.2, d	2.77, dd (12.9, 3.2)	47.2, d	1.57, m	37.0, d
14		42.8, s		45.2, s		42.7, s
15	Ha: 1.73, m	27.0, t	Ha:2.01, m	27.6, t	1.03 (2H), m	27.3, t
	Hb: 1.08, m		Hb:1.91, m			
16	Ha: 1.98, m	29.1, t	Ha:1.98, m	31.8, t	Ha: 1.50, m	34.9, t
	Hb: 1.23, m		Hb:1.18, m		Hb: 1.42, m	
17		47.76, s	2.43, m	41.9, d		43.1, s
18	1.61, m	48.6, d		177.8, s	1.81, m	49.7, d
19	2.40, m	47.77, d		143.1, s	2.60, m	52.6, d
20		150.2, s	3.19, m	24.8, d		213.3, s
21	1.47 (2H), m	21.1, t		211.5, s	Ha: 2.05, m	27.7, t
					Hb: 1.47, m	
22	Ha: 1.88, m	33.9, t	Ha:2.42, dd (16.2, 1.7)	41.4, t	Ha: 1.46, m	39.8, t
	Hb: 1.05, m		Hb:1.82, brd (16.2)		Hb: 1.35, m	
23	1.42, s	32.6, q	0.96, s	27.2, q	Ha: 3.71, d (10.4)	71.9, t
					Hb: 3.41, d (10.4)	
24	1.34, s	24.5, q	0.77, s	14.8, q	0.86, s	11.3, q
25	0.98, s	16.7, q	0.938, s	16.0, q	0.87, s	16.4, q
26	1.06, s	15.5, q	1.17, s	15.9, q	1.01, s	15.9, q
27	1.00, s	14.7, q	0.943, s	15.3, q	0.96, s	14.5, q

continued

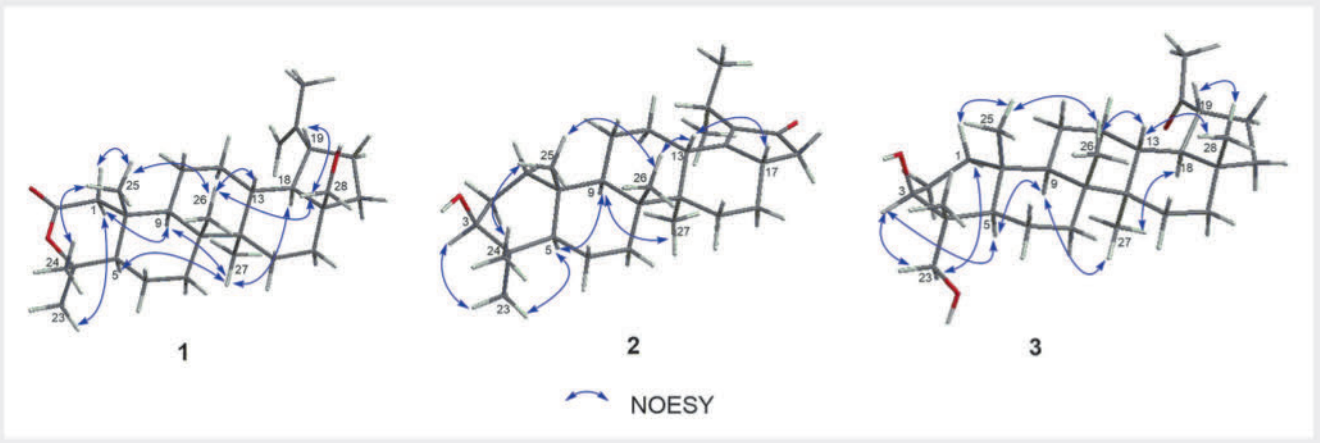
▶ **Table 1** *Continued*

NO.	1[a]		2[b]		3[a]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
28	Ha:3.78, d (10.0)	60.5, t			0.77, s	18.0, q
	Hb: 3.35, d (10.0)					
29	Ha: 4.69, brs	110.0, t	1.15, d (6.6)	19.2, q		
	Hb: 4.60, brs					
30	1.69, s	19.1, q	1.18, d (7.0)	19.1, q	2.15, s	29.3, q

[a] recorded in $CDCl_3$; [b] recorded in MeOD

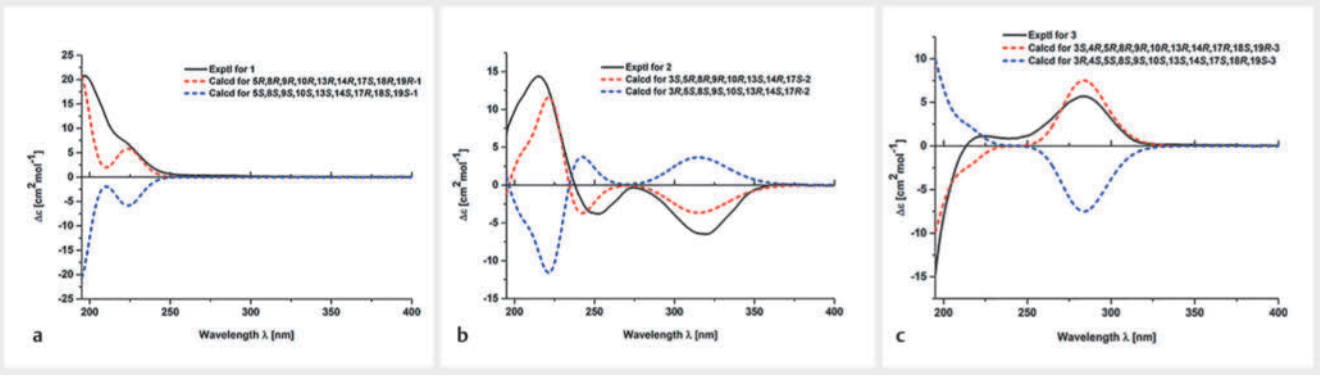


▶ **Fig. 2** Key HMBC and ^{1}H-^{1}H COSY correlations of **1**–**3**.

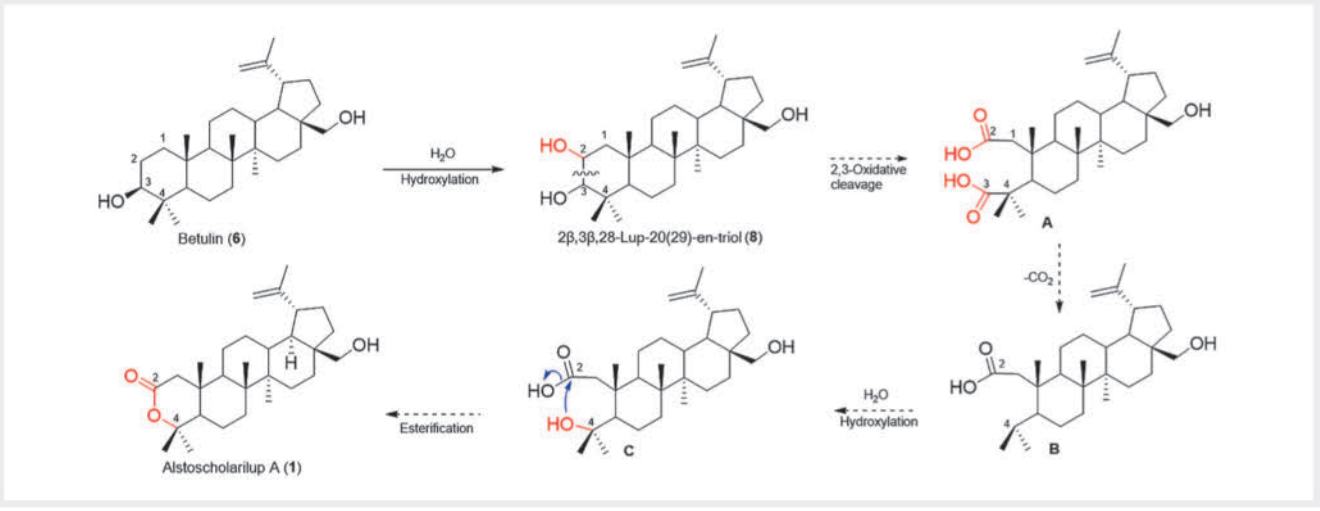


▶ **Fig. 3** Key NOE correlations of **1**–**3**.

for $C_{29}H_{49}O_3$, 445.36762), with 6 degrees of unsaturation. Six methyl singlets and three oxygenated protons were observed in its ^{1}H NMR spectrum. The ^{13}C NMR and DEPT spectral data (▶ **Table 1**) suggested that **3** was also a *nor*-lupane derivative similar to messagenin [15], except for a downfield oxygenated methylene in **3**. The HMBC correlations of δ_H 3.71, 3.41 (2H, H-23) with δ_C 76.6 (C-3), 41.9 (C-4) and 49.9 (C-5), and of δ_H 0.77 (3H, H-28) with δ_C 34.9 (C-16), 43.1 (C-17), 49.7 (C-18) and 39.8 (C-22) supported an oxygenated C-23 (▶ **Fig. 2**). The NOE correlations of δ_H 3.62 (H-3) with δ_H 0.83 (H-5) and 3.71 (Ha-23), and of δ_H 0.83 (H-5) with δ_H 1.29 (H-9), indicated them at α-orientation (▶ **Fig. 3**). Another partial relative configuration of **3** was supported to be the



▶ **Fig. 4** Experimental ECD spectra together with the calculated ECD spectra of **1** (**a**), **2** (**b**), and **3** (**c**) at the B3LYP/6-311G (d, p) level with the PCM in MeOH.



▶ **Fig. 5** Plausible Pathway for the Biogenesis of **1**.

same as **1** by its NOESY spectrum data. Furthermore, its absolute configuration was elucidated to be 3*S*, 4*R*, 5*R*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*, 17*R*, 18*S*, and 19*R* by same way (▶ **Fig. 4c**).

Nine known compounds were identified as 3*β*-acetoxylup-20(29)-en-30-ol (**4**) [27], 2*β*,3*β*-dihydroxylup-20(29)-ene (**5**) [28], betulin (**6**) [29], ilekudinol C (**7**) [30], 2*β*,3*β*,28-lup-20(29)-en-triol (**8**) [31], betulone (**9**) [32], 28-*O*-acetylbetulin (**10**) [29], swinniol (**11**) [33], and 28-norlup-20(29)-ene-3*β*,17*β*-diol (**12**) [34] by comparison with the NMR spectrum data with the literature.

To investigate the effect of reducing the UA levels of all the lupanes isolated from *A. scholaris in vitro*, we first exposed the human renal tubular epithelial cells (HK-2) to all the lupanes (5 μM) for 24 h. The viability of the HK-2 cells was a little different compared with the control upon treatment with all the lupanes ($p > 0.05$, ▶ **Table 2**). Thus, test articles at 5 μM were chosen for further determination. We established a standard model of hyperuricemia *in vitro*: HK-2 was induced by MSU treatment. The UA level in cell supernatants after treatment with MSU alone was increased significantly compared with that in the control group ($p < 0.01$, ▶ **Table 3**). Of note, compared with the model group, compound **4** significantly reduced the serum UA level, while compounds **1, 2, 7, 8, 10**, and **11** showed decreasing tendency of UA levels. Unfortunately, because of the limited quality of **2**, it was not enough to carry out evaluation *in vivo*. Therefore, the other six bioactive lupanes were selected for further investigation.

We further investigated the effects of six active compounds on the serum UA using the potassium oxonate (PO)-induced model in mice. As shown in ▶ **Fig. 6**, the serum UA levels of mice in the model group significantly increased after PO administration ($p < 0.01$). However, the serum UA levels in the positive control group (Ben, $p < 0.01$) and compounds group at 5 or 2.5 mg/kg were decreased. Of note, compounds **4, 7**, and **10** at 5 mg/kg dose on lowering UA were more obvious ($p < 0.05$), which reduced the UA levels significantly *in vivo*.

Twelve lupane-triterpenoids including three new compounds (**1–3**) were obtained from the leaves of *A. scholaris*. In comparison with common lupanes, **1** possessed a rare A ring-*seco* skeleton to form lactone with degraded C-3, while **2** was a 28-*nor* and **3** was a 29-*nor*-lupane derivative, which indicated the phytochemical diversity and novelty of the plant. Further pharmacological investi-

▶ **Table 2** The cell viability of all compounds in HK-2 cells.

Group	UA in supernatant (mg/L)
Control	100.3 ± 4.3
1	98.2 ± 1.4
2	97.1 ± 0.9
3	96.2 ± 0.8
4	96.4 ± 0.5
5	98.3 ± 1.5
6	98.9 ± 1.3
7	98.5 ± 2.7
8	94.5 ± 2.8
9	87.4 ± 8.9
10	97.2 ± 1.7
11	97.6 ± 1.1
12	98.1 ± 2.0

Data represent the cell viability of compounds on HK-2 cells using a 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide assay. Experiments were performed in triplicate (n = 3). All compounds had no inhibitory effect on HK-2 cells ($p > 0.05$).

▶ **Table 3** Lupanes reduced UA levels *in vitro*.

Group	UA in supernatant (mg/L)
Control	15.3 ± 0.2
Model	51.1 ± 1.6$^{\Delta\Delta}$
Benzbromarone	60.1 ± 1.5*
1	57.1 ± 1.7
2	63.8 ± 4.4
3	52.7 ± 0.3
4	61.6 ± 3.5*
5	45.2 ± 2.0
6	48.2 ± 4.9
7	56.3 ± 1.6
8	57.4 ± 2.0
9	55.7 ± 1.5
10	64.5 ± 5.0
11	65.1 ± 4.9
12	53.8 ± 0.3

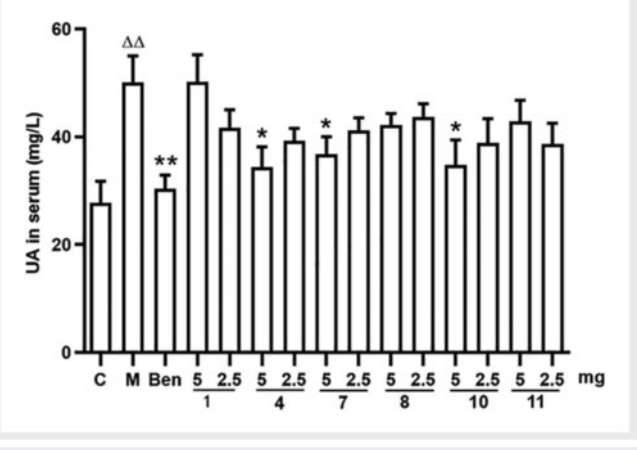
Data exhibited the level of extracellular UA in the MSU-induced HK-2 cell model. Experiments were performed in triplicate (n = 3). Lupane concentration is 5 μM, and Ben is 5 μg/mL. Statistics: $\Delta\Delta$ $p < 0.01$ vs. control; */** $p < 0.05/0.01$ vs. model.

gation showed compounds **4, 7**, and **10** reducing serum UA levels significantly *in vivo*, which also supported the traditional use of *A. scholaris* as an anti-hyperuricemic medicine. The primary structure–activity relationship (SAR) of the lupanes assumed that the hydroxymethyl groups or aldehyde group of **4, 7**, and **10** seem to be essential in reducing UA levels, but more bioactive lupanes are needed for further SAR analysis.

Material and Methods

General experimental procedures

The Autopol VI was used to record optical rotations. The Shimadzu spectrometer was used to record UV spectra. The Agilent serial Applied Photophysics was used to obtain the CD spectra. KBr pellets were used to obtain the IR spectra on a NICOLET iS10 infrared spectrophotometer. NMR spectra were recorded on a Bruker AVANCE NEO 400 MHz spectrometer, and chemical shifts (δ) were expressed in ppm with reference to the solvent signals. An Agilent 1290 UPLC/6545 Q-TOF mass spectrometer was used for HRESIMS analyses. Column chromatography (CC) was used for many separate materials, such as C-18 silica gel (40–60 μm), silica gel (200–300 mesh), and Sephadex LH-20 (Amersham Pharmacia, Sweden). Thin-layer chromatography (TLC) was performed on silica gel plates (GF254 silica gel plates). Compounds were purified by an Agilent 1260 liquid chromatograph semi-preparative HPLC (equipped with an Agilent Zorbax SBC18 column 250 mm × 9.4 mm, i. d., 5 μm; flow rate: 2 mL/min).



▶ **Fig. 6** The effect of reducing the UA levels of six lupanes in PO-induced mice. C, control; M, model; Ben, benzbromarone. $\Delta\Delta$ $p < 0.01$ vs. C; */** $p < 0.05/0.01$ vs. M. Ben (10 mg/kg) was used as the positive control.

Plant material

The voucher specimen of *A. scholaris* (Luo 20 130 601) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences.

Extraction and isolation

The air-dried leaves of *A. scholaris* (10 kg) were powdered and, then, refluxed with EtOH (90%, 3 h × 4) and evaporated to afford an EtOH extract. Afterward, 0.3% aqueous HCl solution was used to dissolve the crude extract. Then, 300 g of the undissolved residue was selected and fractionated by using silica gel column chromatography (CC) to generate six portions (Fr. A–F) with petroleum ether/acetone (1 : 0–0 : 1) as solvents. Among them, Fr.C.1–Fr.C.19 were generated from Fr.C (31.0 g) by using a C-18 column and MeOH/H_2O (40 : 60–100 : 0) as solvents. Silica gel CC was used to separate Fr.C.8 (2.0 g) washed with petroleum ether/acetone (1 : 0–0 : 1) to provide Fr.C.8.1–Fr.C.8.7. Fr.C.8.6 (97.4 mg) was purified by using semi-preparative HPLC (MeCN/H_2O, 87%) to afford compound **12** (5.2 mg, t_R = 14.2 min). Using petroleum ether/acetone (1 : 0–0 : 1) as the mobile phase, Fr.C.9 (733.7 mg) was separated by silica gel CC and further purified by semi-preparative HPLC carried with 97% MeCN/H_2O to yield compound **9** (36.8 mg, t_R = 11.5 min) and compound **3** (13.5 mg, t_R = 17.7 min). Sephadex LH-20 with MeOH was used to separate Fr.C.13 (910.0 mg) and further purified by semi-preparative HPLC with 99% MeCN/H_2O to afford compound **10** (15.6 mg, t_R = 33.0 min). A recrystallization method was used to obtain and purify compound **4** (87.3 mg) from Fr.C.14 (305.2 mg) in MeOH. Compound **5** (24.0 mg, t_R = 31.3 min) was purified from Fr.C.16 (186.5 mg) via Sephadex LH-20 by MeOH and further purified by semi-preparative HPLC by 98% MeCN/H_2O.

Fr.D (35.0 g) was cut into seven fractions (Fr.D.1–Fr.D.8) by using C-18 CC and MeOH/H_2O (30 : 70–100 : 0) as solvents. Fr.D.6.1–Fr.D.6.3 were generated from Fr.D.6 (3.0 g) by using Sephadex LH-20 with MeOH. Fr.D.6.2 (1.4 g) was fractionated by using silica gel CC and petroleum ether/acetone (1 : 0–0 : 1) as solvents to generate Fr.D.6.2.1–Fr.D.6.2.10. Fr.D.6.2.9 (70.3 mg) was carried out on semi-preparative HPLC washed with 87% MeCN/H_2O to produce compound **1** (10.0 mg, t_R = 12.9 min), and compound **2** (3.5 mg, t_R = 18.9 min) was purified from Fr.D.6.2.10 (81.7 mg) in the same way. Compound **6** (2.5 g) was obtained and purified from Fr.D.8 (6.3 g) in MeOH with the recrystallization method.

Seven fractions (Fr.F.1–Fr.F.7) were yielded from Fr.F (43.2 g) by a C-18 column and MeOH/H_2O (30 : 70–100 : 0) as solvents. Fr. F.6 (451.8 mg) was fractionated by using silica gel CC to generate Fr.F.6.1–Fr.F.6.7 with petroleum ether/acetone (1 : 0–0 : 1) as solvents. Compound **8** (10.5 mg) was recrystallized from Fr.F.6.1 (68.3 mg, MeOH). Fr.F.6.2 (85.0 mg) was carried out on semi-preparative HPLC washed with 93% MeCN/H_2O to produce compound **11** (9.5 mg, t_R = 13.7 min). Compound **7** (10.7 mg) was obtained and purified from Fr.F.6.6 (64.9 mg) in MeOH with the recrystallization method.

Alstoscholarilup A (***1***)*:* white powder (MeOH); $[\alpha]_D^{23}$ + 70.5 (*c* 0.06, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 259 (+ 0.43), 197 (+ 20.78); IR (KBr) ν_{max} 3435, 2940, 2869, 1711, 1454, 1377, 1294, 1113, 1027; HRESIMS *m/z*: 443.35397 $[M + H]^+$ (calcd for $C_{29}H_{47}O_3$, 443.35197); ^1H and ^{13}C NMR spectral data, see ▸ **Table 1**.

Alstoscholarilup B (***2***)*:* white powder (MeOH); $[\alpha]_D^{23}$ − 67.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 243 (3.49), 195 (3.11); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 365 (+ 0.08), 319 (− 6.46), 274 (− 0.33), 252 (− 3.80), 215 (+ 14.37), 195 (+ 6.77); IR (KBr) ν_{max} 3429, 2942, 2869, 1696, 1611, 1453, 1380, 1028; HRESIMS *m/z*: 427.35663 $[M + H]^+$ (calcd for $C_{29}H_{47}O_2$, 427.35706); ^1H and ^{13}C NMR spectral data, see ▸ **Table 1**.

Alstoscholarilup C (***3***)*:* white powder (MeOH); $[\alpha]_D^{23}$ + 10.2 (*c* 0.07, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 328 (+ 0.14), 284 (+ 5.69), 243 (+ 0.86), 225 (+ 1.12), 195 (− 14.67); IR (KBr) ν_{max} 3400, 2939, 2867, 1702, 1455, 1383, 1046; HRESIMS *m/z*: 445.36822 $[M + H]^+$ (calcd for $C_{29}H_{49}O_3$, 445.36762); ^1H and ^{13}C NMR spectral data, see ▸ **Table 1**.

Animals

ICR male mice weighing approximately 22–24 g were purchased from Kunming Medical University (License number SCXK 2020-0004). In the SPF-grade laboratory, all mice were housed in a room maintained at 24 ± 1 °C and 40–70% relative humidity with a 12 h light–dark cycle (license number SYXK 2018–0005). Food and water were provided *ad libitum*. The Institutional Animal Care and Use Committee of the Kunming Institute of Botany, Chinese Academy of Sciences, approved our experiments (approved code: Kib202107007), and the date of approval was July 16th, 2021. In accordance with the international guidelines of animal experiments and internationally accepted ethical principles for laboratory animal use and care, the animal studies were performed.

Chemicals

PO and MSU were obtained from Sigma-Aldrich (St. Louis, MO, USA). The concentrations of UA were determined by biochemical kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Assay of the Level of UA in MSU-Induced Cell Model

According to the methods described previously in the literature [35], we harvested and diluted HK-2 cells (purchased from Shanghai Meixuan Biotechnology Co. (Shanghai, China)) into 1×10^5 cells/mL using Dulbecco's modified Eagle's medium (DMEM) complete medium. Then HK-2 cells at 200 µL/well were plated in 96-well plates for one night. Culture medium at 200 µL was added into control wells. An equal amount of culture medium with 5 µM tested lupanes was added into other wells. Subsequently, MSU (8 mg/dL) was supplemented into wells after 24 h of incubation. The supernatant and broken cells were collected for the quantification of UA via biochemical kits. Benzbromarone (Ben, 5 µg/mL) was used as the positive medicine. MTT assay was used to evaluate the effects of tested lupanes on HK-2 cell viability at the same concentration.

Assay of Reducing UA Level–PO-Induced Hyperuricemic Mice Model

According to the methods described previously in the literature [22], an experiment was conducted with the PO-induced hyperuricemic mice model to evaluate the effect of lupane triterpenoids against hyperuricemia. Ten mice were first selected randomly arranged for the control group. For the hyperuricemia model mice, mice were administered intragastrically with PO for five consecutive days at a dose of 300 mg/kg, and an equal volume of distilled water was given to the control group. On day 6, 140 modeling mice were randomly divided into 14 groups with 10 mice in each group. The model group mice were treated with

0.5% DMSO. Positive control mice were intragastrically administrated with Ben at 10 mg/kg. The lupane-testing groups were administered intraperitoneally with compounds **1, 4, 7, 8, 10**, and **11** at 5 and 2.5 mg/kg, respectively. Tested lupanes were intraperitoneally injected respectively at 30 min after PO was intragastrically administrated (300 mg/kg). Meanwhile, the control group and positive group were administered with the corresponding 0.5% DMSO and Ben. Blood samples were collected from the eyeballs at 1.5 h after administration; serum UA levels were measured by using the commercial reagent immediately.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, Inc.). Data from the experiment are expressed as the mean ± standard error of the mean (SE). The two-tailed Student's test was used to determine statistical significance with ($\Delta\Delta$) $p < 0.01$, (**) $p < 0.01$, or (*) $p < 0.05$ denoted as significance values in all analyses.

Supporting Information

Computational data of compounds **1–3**; NMR, HRESIMS, ORD, CD, and IR of compounds **1–5**; the physical and spectroscopic data of the known compounds are available as Supporting Information.

Contributors' Statement

Conception and design of the work: X. D. Luo; data collection: B. Y. Hu, Y. L. Zhao, Y. Xu, X. N. Wang; analysis and interpretation of the data: X. D. Luo, B. Y. Hu, Y. L. Zhao, Y. Xu, X. N. Wang; statistical analysis: Y. Xu, X. N. Wang; drafting the manuscript: X. D. Luo, B. Y. Hu, Y. L. Zhao, Y. Xu, X. N. Wang; critical revision of the manuscript: X. D. Luo, B. Y. Hu, Y. L. Zhao.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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