Organic LETTERS

Letter

pubs.acs.org/OrgLett

Four Matrine-Based Alkaloids with Antiviral Activities against HBV from the Seeds of *Sophora alopecuroides*

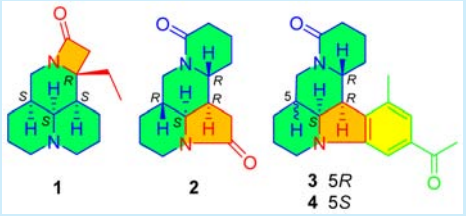
Yu-Bo Zhang,[†,‡] Xiao-Li Zhang,[†,‡] Neng-Hua Chen,[†,‡] Zhong-Nan Wu,[†,‡] Wen-Cai Ye,*[,†,‡] Yao-Lan Li,*[,†,‡] and Guo-Cai Wang*[,†,‡]

[†]Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, People's Republic of China

[‡]Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangzhou 510632, People's Republic of China

Supporting Information

ABSTRACT: Four novel matrine-based alkaloids (**1**–**4**) were isolated from the seeds of *Sophora alopecuroides*. Compounds **1** and **2** possess unprecedented 6/6/6/4 and 6/5/6/6 ring systems, respectively, while **3** and **4** are a pair of stereoisomeric matrine–acetophenone alkaloids with an unusual skeleton. Their structures were elucidated by means of spectroscopic methods and single-crystal X-ray diffraction. Hypothetical biogenetic pathways for **1**–**4** are proposed, and their antiviral activities are also discussed.



The plant *Sophora alopecuroides* L., belonging to the family Leguminosae, is widely distributed in western and central Asia, especially in northwest China.[1] Traditionally, the seeds and roots of this plant have been used as traditional Chinese medicine for the treatment of rheumatism, fever, bacterial infection, and heart disease.[2] Previous chemical investigations of *S. alopecuroides* have revealed that matrine-type alkaloids[3−7] and flavonoids[8−10] are the main chemical constituents of this plant. The matrine-type alkaloids belong to a class of quinolizidine alkaloids with unique 6/6/6/6 tetracyclic skeletons[5] that display antiviral,[11,12] antibacterial,[5] antitumor,[13] and anti-inflammatory[14] activities. Two representative matrine-type alkaloids, matrine and oxymatrine, have been used for treatment of hepatitis B, cancer, dysentery, psoriasis, and pyogenic infections of the skin in clinic.[15−17] Because of their complex stereostructure and diverse biological effects, matrine-type alkaloids have become increasingly attractive targets for both pharmacologists and synthetic chemists.

We recently reported several novel dimeric matrine-type alkaloids from the *Sophora* plant (*Sophora flavescens*).[11] In our continuing search for antiviral and structurally unique constituents of Chinese medicinal plants,[11,18] four novel matrine-based alkaloids, sophalines A–D (**1**–**4**) (Figure 1), were isolated from the ethanol extract of seeds of *S. alopecuroides*. Compound **1** is a novel matrine-type alkaloid with a rearranged 6/6/6/4 tetracyclic skeleton, while **2** represents the first normatrine-type alkaloid with a 6/5/6/6 tetracyclic system. Compounds **3** and **4** are a pair of stereoisomeric matrine–acetophenone alkaloids with an unprecedented skeleton. Herein we report the structure elucidation, hypothetical biogenetic pathways, and anti-HBV activities of **1**–**4**.

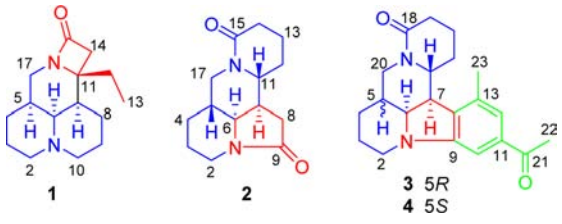


Figure 1. Chemical structures of **1**–**4**.

Air-dried and pulverized seeds (30.0 kg) of *S. alopecuroides* were extracted with 95% ethanol to obtain a crude extract (1.9 kg). A total alkaloid extract (897 g) was obtained by acidification and subsequent basicification treatments followed by separation via column chromatography over macroporous resin (silica gel, ODS, Sephadex LH-20) and preparative HPLC to yield compounds **1** (5.3 mg), **2** (12.5 mg), **3** (8.5 mg), and **4** (27.4 mg).

Sophaline A (**1**) was obtained as colorless crystals. The molecular formula was determined to be $C_{15}H_{24}N_2O$ on the basis of HR-ESI-MS data (*m*/*z* 249.1956 $[M + H]^+$, calcd for $C_{15}H_{25}N_2O$ 249.1961). Its UV spectrum exhibited an absorption maximum at 206 nm, and the IR spectrum revealed the existence of a carbonyl bond (1735 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) showed the presence of a methyl group at δ_H 0.92 (3H, t, J = 7.4 Hz) and two characteristic protons at δ_H 3.39 (1H, dd, J = 13.3, 5.7 Hz) and 2.93 (1H, t, J = 13.3 Hz). The ^{13}C NMR spectrum displayed a carbonyl carbon (δ_C 168.7), a methyl group (δ_C 7.5), a tertiary carbon connected to a heteroatom (δ_C 65.3), and a quaternary carbon connected to a heteroatom (δ_C 58.3). The ^{13}C NMR data of **1**

Received: December 15, 2016
Published: January 9, 2017

DOI: 10.1021/acs.orglett.6b03685
Org. Lett. 2017, 19, 424–427

Table 1. NMR Data for 1 and 2 (in CD_3OD, J in Hz)

	1[a]		2[b]	
no.	δ_H	δ_C	δ_H	δ_C
2α	1.74	59.1	2.83 (td, 13.3, 3.7)	41.1
2β	2.84		4.05 (dd, 13.3, 5.1)	
3a	1.62	22.0	1.77 (m)	25.7
3b	1.44		1.47 (m)	
4a	1.68	28.6	1.93 (m)	28.8
4b	1.58		1.28 (m)	
5	1.79	37.3	1.89 (m)	35.4
6	2.13 (t, 3.6)	65.3	3.25	62.4
7	1.80	45.1	2.64	37.1
8a	1.88	25.7	2.64	35.6
8b	1.62		2.23 (d, 10.4)	
9a	1.92	22.9	–	173.9
9b	1.44			
10α	1.91	59.0	–	–
10β	2.84			
11	–	58.3	3.66 (dt, 10.0, 5.8)	56.8
12a	2.53 (dq, 21.8, 7.4)	26.9	2.05 (m)	28.5
12b	1.65		1.60 (m)	
13α	0.92 (t, 7.4)	7.5	1.83 (m)	18.7
13β			1.69 (m)	
14α	2.44 (d, 14.8)	48.0	2.34 (t, 6.5)	33.3
14β	2.96 (dd, 14.8, 1.3)		2.34 (t, 6.5)	
15	–	168.7	–	173.1
17α	3.39 (dd, 13.3, 5.7)	38.6	3.41 (dd, 13.4, 5.3)	47.9
17β	2.93 (t, 13.3)		3.24 (t, 13.4)	

[a]Measured at 500 (^{1}H) and 125 (^{13}C) MHz. [b]Measured at 400 (^{1}H) and 100 (^{13}C) MHz. Overlapped signals are reported without designating multiplicity.

showed a number of similarities to those of matrine,[19] except for the signals assigned to C-11, C-12, C-13, C-14, C-15, and C-17. The main differences were the absence of a methine and a methylene that are present in matrine and the presence of a methyl at δ_C 7.5 and a quaternary carbon at δ_C 58.3 in **1**.

The ^{1}H−^{1}H COSY spectrum of **1** revealed the presence of two spin systems, as shown in Figure 2. In the HMBC

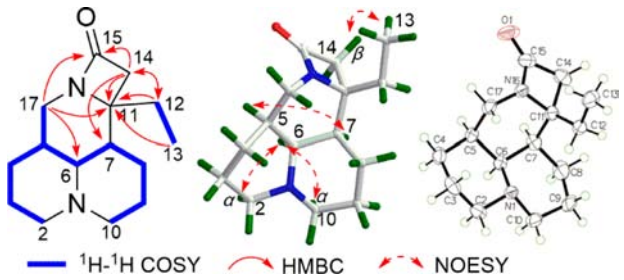


Figure 2. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations and X-ray ORTEP drawing of **1**.

spectrum, the correlations between H_2-12 and C-11/C-14, between H_3-13 and C-11, and between H_2-17 and C-6/C-11/C-15 suggested that an ethyl group is connected to C-11. Moreover, the HMBC correlations between H_2-14 and C-7/C-12, together with the deshielded quaternary carbon (δ_C 58.3) at C-11, suggested that C-14 is linked to C-11 to form a four-membered ring. In the NOESY spectrum, the correlations of H-2α/H-6/H-10α, H-5/H-7, and H_3-13/H-14β established the relative configuration of **1**. Finally, X-ray diffraction (Cu Kα) analysis of **1** resulted in a small Flack parameter of 0.1(3), allowing an explicit assignment of the absolute configurations of **1** as 5*S*, 6*S*, 7*S*, and 11*R* (Figure 2).

Sophaline B (**2**) was isolated as colorless crystals. The molecular formula of **2** was determined to be $C_{14}H_{20}N_2O_2$ by HR-ESI-MS data (m/z 249.1608 $[M + H]^+$; calcd for $C_{14}H_{21}N_2O_2$ 249.1598). The IR spectrum displayed the characteristic absorptions for carbonyl bonds (1680 and 1628 cm^{-1}). The ^{1}H NMR spectrum of **2** showed two characteristic protons at δ_H 3.66 (1H, dt, J = 10.0, 5.8 Hz) and 3.41 (1H, dd, J = 13.4, 5.3 Hz). The ^{13}C NMR spectrum displayed two carbonyls at δ_C 173.9 and 173.1 and two methines connected to heteroatoms at δ_C 62.4 and 56.8. The above data are similar to those for sophoridine,[20] except for the absence of two methylenes and the presence of another carbonyl group in **2**. The ^{1}H−^{1}H COSY spectrum of **2** revealed the presence of a spin system (C-2 to C-8/C-14/C-17) (Figure 3). In the

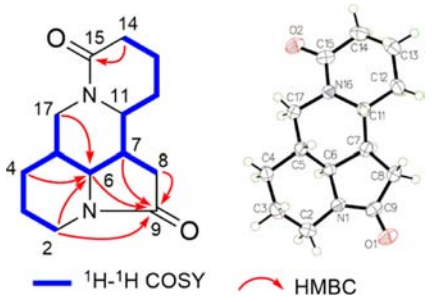


Figure 3. Key ^{1}H−^{1}H COSY and HMBC correlations and X-ray ORTEP drawing of **2**.

HMBC spectrum, the correlations between H_2-2/H_2-4/H_2-17 and C-6, between H_2-2/H-6/H-7/H_2-8 and C-9, and between H_2-14 and C-15 allowed the assignment of the planar structure of **2** (Figure 3). The relative configuration of **2** was elucidated by its ROESY data. Furthermore, the complete structure and stereochemistry of **2** were confirmed by a single-crystal X-ray diffraction experiment (Figure 3). The Cu Kα data resulted in a reasonable Flack parameter of 0.13(6), allowing an unambiguous assignment of the absolute configurations of **2** as 5*R*, 6*S*, 7*R*, and 11*R*.

Sophaline C (**3**) was isolated as colorless crystals from CH_3OH, $[\alpha]_{25}^{D}$ +10.4 (c 0.5, CH_3OH). The molecular formula of **3** was determined to be $C_{21}H_{26}N_2O_2$ on the basis of its HR-ESI-MS ion peak at m/z 339.2065 $[M + H]^+$ (calcd for $C_{21}H_{27}N_2O_2$ 339.2067). Its UV spectrum exhibited absorption maxima at 206 and 250 nm. The IR spectrum displayed characteristic absorptions for carbonyl bonds (1735 and 1628 cm^{-1}) and an aromatic ring (1567 and 1444 cm^{-1}). The ^{1}H NMR spectrum of **3** showed signals for two aromatic protons [δ_H 7.13 (1H, s) and 6.88 (1H, s)] and two methyl groups [δ_H 2.54 (3H, s) and 2.39 (3H, s)]. The ^{13}C NMR and DEPT data suggested the existence of 21 carbons, including two carbonyls (δ_C 200.6 and 173.4) and a tetrasubstituted benzene ring (δ_C 154.1, 138.8, 137.0, 135.3, 123.4, and 104.4). With the aid of 2D NMR experiments, including HSQC, ^{1}H−^{1}H COSY, and HMBC (Figure 4), the ^{1}H and ^{13}C NMR signals of **3** were assigned as shown in Table 2.

The ^{1}H−^{1}H COSY spectrum of **3** revealed the presence of a spin system (C-2 to C-17/C-20) (Figure 4). In the HMBC spectrum, the correlations between H-4b and C-20, between H-7 and C-5/C-15, between H-17 and C-18, and between H_2-20

DOI: 10.1021/acs.orglett.6b03685
Org. Lett. 2017, 19, 424−427

Table 2. NMR Data for 3 and 4 (in CD_3OD, *J* in Hz)

	3[a]		4[a]	
no.	δ_H	δ_C	δ_H	δ_C
2α	2.99 (m)	45.5	2.47 (td, 12.0, 2.5)	46.7
2β	3.81 (m)		3.74 (m)	
3a	1.62 (m)	23.8	1.85	21.5
3b	1.62 (m)		1.67	
4α	1.32 (m)	30.0	1.85	27.2
4β	1.88		1.85	
5	1.93	31.6	2.07 (m)	33.5
6	3.24 (dd, 10.5, 8.7)	68.1	3.20 (m)	69.4
7	3.52 (m)	49.5	3.16 (m)	46.2
8	–	135.3	–	136.6
9	–	154.1	–	154.5
10	6.88 (s)	104.4	6.96 (s)	104.9
11	–	138.8	–	138.7
12	7.13 (s)	123.4	7.24 (s)	123.3
13	–	137.0	–	135.3
14	4.04 (dt, 9.2, 6.0)	55.1	3.35 (m)	57.6
15a	2.25 (m)	31.1	1.82	27.4
15b	2.12 (m)		1.61 (m)	
16a	1.93	19.2	2.04 (m)	18.7
16b	1.77 (m)		1.71	
17a	2.41 (m)	33.3	2.40 (m)	33.3
17b	2.41 (m)		2.33 (m)	
18	–	173.4	–	171.9
20α	3.38 (m)	47.2	4.43 (dd, 12.8, 5.4)	43.5
20β	3.38 (m)		3.12 (t, 12.8)	
21	–	200.6	–	201.0
22	2.54 (s)	26.8	2.54 (s)	26.8
23	2.39 (s)	21.8	2.37 (s)	19.8

[a]Measured at 500 (^{1}H) and 125 (^{13}C) MHz. Overlapped signals are reported without designating multiplicity.

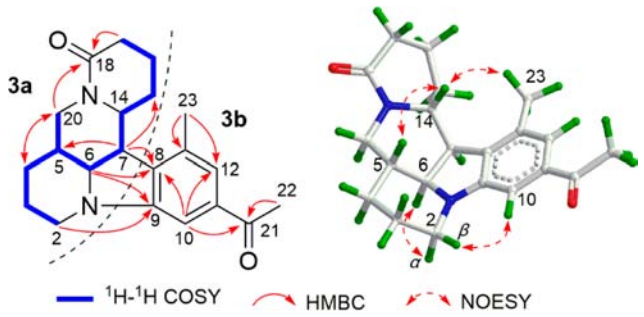


Figure 4. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations of **3**.

and C-4/C-18 allowed the establishment of the planar structure of **3a** (Figure 4). Similarly, the HMBC correlations between H-10 and C-8/C-12/C-21, between H_3-22 and C-21, and between H_3-23 and C-12/C-13 suggested the presence of the planar structure of **3b**. Moreover, the 1D NMR data of **3b** were similar to those of 3′-methylacetophenone,[21] except that the tertiary carbons at C-4 (δ_C 134.2) and C-5 (δ_C 129.2) in 3′-methylacetophenone were replaced by two quaternary carbons at δ_C 137.0 and 154.1, respectively. The HMBC correlations between H-6/H-7 and C-8 clearly defined the connection of **3a** and **3b** via the C-7−C-8 bond (Figure 4). Furthermore, a closure mode of pyrrole ring formation to connect the two units (**3a** and **3b**) was established on the basis of the deshielded quaternary carbon at δ_C 154.1 (C-9) together with the HMBC correlations between H-2α/H-6 and C-9.

The relative stereochemistry of **3** was partially deduced by the NOE correlations of H-5/H-14/H_3-23, H-2α/H-6, and H-2β/H-10 (Figure 4). Finally, the complete structure and stereochemistry of **3** were confirmed by single-crystal X-ray diffraction (Cu Kα radiation), allowing the assignment of the absolute configurations as 5*R*, 6*S*, 7*R*, and 14*R* [with a reasonable Flack parameter of 0.08(18)] (Figure 5).

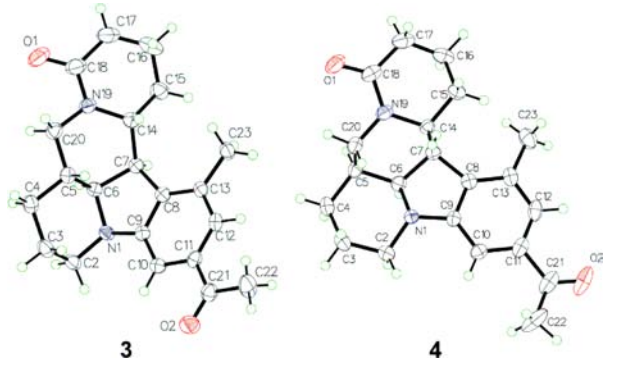


Figure 5. X-ray ORTEP drawings of **3** and **4**.

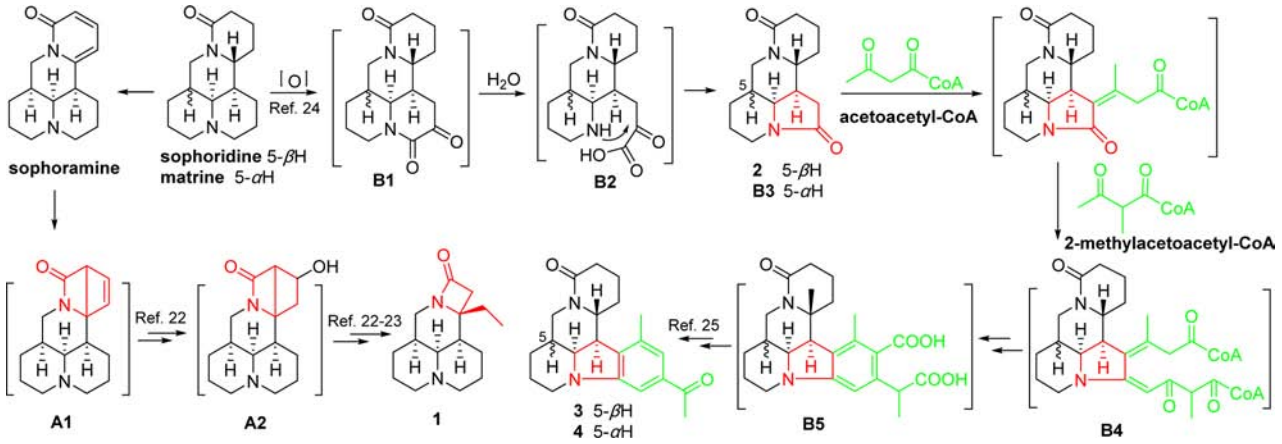
Sophaline D (**4**) exhibited the same molecular formula as **3** ($C_{21}H_{26}N_2O_2$) on the basis of its HR-ESI-MS ion peak at *m*/*z* 339.2079 $[M + H]^+$ (calcd for $C_{21}H_{27}N_2O_2$ 339.2067). The ^{1}H and ^{13}C NMR data of **4** were very similar to those of **3**, except for the signals assigned to C-3, C-4, C-5, C-7, C-14, and C-20. The differences between **3** and **4** showed a number of similarities to the differences between sophoridine[20] and matrine,[19] which are a pair of epimers at C-5. This implied that compounds **3** and **4** might also be a pair of stereoisomers at C-5. The planar structure of **4** was elucidated by interpretation of the ^{1}H−^{1}H COSY and HMBC spectra. In the NOESY spectrum of **4**, the correlations of H-2α/H-6, H-2β/H-10, H-5/H-7, H-12/H_3-22, and H-14/H-20β indicated that the orientation of H-5 is the α configuration, suggesting that the absolute configuration of C-5 in **4** is *S*. Furthermore, Cu Kα X-ray crystallographic analysis with a small Flack parameter [−0.1(2)] further confirmed the absolute configurations of **4** (Figure 5).

Plausible biosynthetic pathways for **1**−**4** are proposed as shown in Scheme 1. Compound **1** could originate from matrine.[19] First, matrine could be dehydrogenated to give sophoramine.[12] Then **A1** could be formed through an electrocyclization reaction. Subsequently, **A1** could be further hydrated to yield **A2**.[22] Finally, **1** could be formed by a retro-aldol-type C−C bond cleavage of **A2**[22] followed by a reduction reaction.[23] Compounds **3** and **4** could also be considered to be derived from sophoridine and matrine, respectively, which could be oxidized and then hydrolyzed to yield **B1** and **B2**, respectively.[24] After decarboxylation and cyclization reactions, **B2** could afford **2** and **B3**, which could be coupled with acetoacetyl-CoA and 2-methylacetoacetyl-CoA molecules to generate intermediates **B4**. Subsequently, intermediates **B4** may be cyclized and hydrolyzed to afford **B5**. Finally, **B5** could further generate **3** and **4** through decarboxylation and subsequent oxygenation reactions.[25]

Compounds **1**−**4** and matrine were tested for their antiviral activities against hepatitis B virus (HBV) in HepG2.2.15 cells. The results of the assay (see the Supporting Information) showed that compounds **2**−**4** significantly inhibited HBsAg secretion by more than 50.0% at non-cytotoxic concentrations

DOI: 10.1021/acs.orglett.6b03685
Org. Lett. 2017, 19, 424−427

Scheme 1. Hypothetical Biosynthetic Pathways for 1−4



of 0.2 or 0.4 mM, which suggests that these natural products are more potent than the positive control lamivudine (3TC) (31.5% at a concentration of 1.0 mM).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03685.

Detailed experimental procedures, NMR, HR-ESI-MS, UV, and IR spectra, X-ray crystallography data, and bioassay results for **1**−**4** (PDF)

Crystallographic data in CIF format for **1**−**4** (ZIP)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: chyewc@gmail.com.
*E-mail: tliyl@jnu.edu.cn.
*E-mail: twangguocai@jnu.edu.cn.

ORCID

Wen-Cai Ye: 0000-0002-2810-1001

Author Contributions

All authors have given approval to the final version of the article.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China (U1401225 and 81473116) and the Science and Technology Planning Project of Guangdong Province (2016B030301004).

■ REFERENCES

(1) *Flora of China*; Science Press: Beijing, 1994; Vol. *40*, p 80.
(2) Song, L. R.; Hong, X.; Ding, X. L.; Zang, Z. Y. *Dictionary of Modern Medicine*; People's Medical Publishing House: Beijing, 2001; pp 1224−1226.
(3) Atta-ur-Rahman; Choudhary, M. I.; Parvez, K.; Ahmed, A.; Akhtar, F.; Nur-e-Alam, M.; Hassan, N. M. *J. Nat. Prod.* **2000**, *63*, 190−192.
(4) Gao, H. Y.; Li, G. Y.; Wang, J. H. *Helv. Chim. Acta* **2012**, *95*, 1108−1113.
(5) Küçükboyaci, N.; Özkan, S.; Adigüzel, N.; Tosun, F. *Turk. J. Biol.* **2011**, *35*, 379−385.
(6) Yu, Y.; Ding, P.; Chen, D. *Anal. Chim. Acta* **2004**, *523*, 15−20.
(7) Liu, B.; Li, J. L.; Yuan, Y. J. *Chin. Tradit. Herb Drugs* **2001**, *32*, 293−296.
(8) Wan, C. X.; Luo, J. G.; Ren, X. P.; Kong, L. Y. *Phytochemistry* **2015**, *116*, 290−297.
(9) Kwon, J.; Basnet, S.; Lee, J. W.; Seo, E. K.; Tsevegsuren, N.; Hwang, B. Y.; Lee, D. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3314−3318.
(10) Ni, K.; Yang, L.; Wan, C.; Xia, Y.; Kong, L. *Nat. Prod. Res.* **2014**, *28*, 2195−2198.
(11) Zhang, Y. B.; Zhan, L. Q.; Li, G. Q.; Wang, F.; Wang, Y.; Li, Y. L.; Ye, W. C.; Wang, G. C. *J. Org. Chem.* **2016**, *81*, 6273−6280.
(12) Pan, Q. M.; Li, Y. H.; Hua, J.; Huang, F. P.; Wang, H. S.; Liang, D. *J. Nat. Prod.* **2015**, *78*, 1683−1688.
(13) Li, L. Q.; Li, X. L.; Wang, L.; Du, W. J.; Guo, R.; Liang, H. H.; Liu, X.; Liang, D. S.; Lu, Y. J.; Shan, H. L.; Jiang, H. C. *Cell. Physiol. Biochem.* **2012**, *30*, 631−641.
(14) Guzman, J. R.; Koo, J. S.; Goldsmith, J. R.; Muhlbauer, M.; Narula, A.; Jobin, C. *Sci. Rep.* **2013**, *3*, 1629.
(15) Zhang, J. F.; Han, F. *West. J. Tradit. Chin. Med.* **2012**, *25*, 107−110.
(16) Wang, B. E. *J. Gastroenterol. Hepatol.* **2000**, *15*, E67−E70.
(17) Yong, J.; Wu, X.; Lu, C. *Curr. Pharm. Des.* **2015**, *21*, 3673−3680.
(18) (a) Liu, C.; Ang, S.; Huang, X. J.; Tian, H. Y.; Deng, Y. Y.; Zhang, D. M.; Wang, Y.; Ye, W. C.; Wang, L. *Org. Lett.* **2016**, *18*, 4004−4007. (b) Cao, J. Q.; Huang, X. J.; Li, Y. T.; Wang, Y.; Wang, L.; Jiang, R. W.; Ye, W. C. *Org. Lett.* **2016**, *18*, 120−123. (c) Zhao, B. X.; Wang, Y.; Zhang, D. M.; Huang, X. J.; Bai, L. L.; Yan, Y.; Chen, J. M.; Lu, T. B.; Wang, Y. T.; Zhang, Q. W.; Ye, W. C. *Org. Lett.* **2012**, *14*, 3096−3099. (d) Zhao, B. X.; Wang, Y.; Zhang, D. M.; Jiang, R. W.; Wang, G. C.; Shi, J. M.; Huang, X. J.; Chen, W. M.; Che, C. T.; Ye, W. C. *Org. Lett.* **2011**, *13*, 3888−3891.
(19) Galasso, V.; Asaro, F.; Berti, F.; Pergolese, B.; Kovač, B.; Pichierri, F. *Chem. Phys.* **2006**, *330*, 457−468.
(20) Xiao, P.; Kubo, H.; Komiya, H.; Higashiyama, K.; Yan, Y. N.; Li, J. S.; Ohmiya, S. *Phytochemistry* **1999**, *50*, 189−193.
(21) Ruan, J.; Li, X.; Saidi, O.; Xiao, J. *J. Am. Chem. Soc.* **2008**, *130*, 2424−2425.
(22) Kaneko, C.; Katagiri, N.; Sato, M.; Muto, M.; Sakamoto, T.; Saikawa, S.; Naito, T.; Saito, A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1283−1288.
(23) Larsson, M.; Nguyen, B. V.; Högberg, H. E.; Hedenström, E. *Eur. J. Org. Chem.* **2001**, *2001*, 353−363.
(24) Lee, B. H.; Clothier, M. F.; Pickering, D. *J. Org. Chem.* **1997**, *62*, 7836−7840.
(25) Zhang, C.; Srivastava, P.; Ellis-Guardiola, K.; Lewis, J. C. *Tetrahedron* **2014**, *70*, 4245−4249.

DOI: 10.1021/acs.orglett.6b03685
Org. Lett. 2017, 19, 424−427