Organic LETTERS

Letter

pubs.acs.org/OrgLett

Leonuketal, a Spiroketal Diterpenoid from *Leonurus japonicus*

Liang Xiong,[†,‡] Qin-Mei Zhou,[†,‡] Yike Zou,[§] Ming-Hua Chen,[⊥] Li Guo,[†,‡] Guan-Ying Hu,[†,‡] Zhao-Hua Liu,[∥] and Cheng Peng*[,†,‡]

[†]School of Pharmacy and [‡]State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

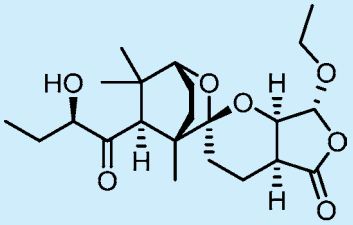
[§]Department of Chemistry, University of Pennsylvania, 231 South 34 Street, Philadelphia, Pennsylvania 19104-6323, United States

[⊥]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

[∥]Chengdu No. 1 Pharmaceutical Co. Ltd., Chengdu 610031, China

Supporting Information

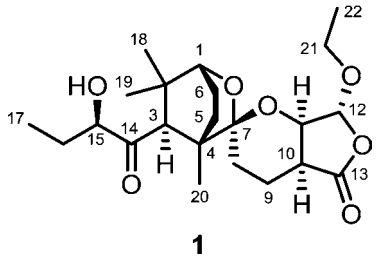
ABSTRACT: An architecturally complex spiroketal diterpenoid, leonuketal (**1**), was isolated from the aerial parts of the plant *Leonurus japonicus*. This compound possessed an unprecedented tetracyclic skeleton that comprised a bridged spiroketal moiety fused with a ketal-γ-lactone unit. The structure and absolute configuration were determined by spectroscopic analyses, a modified Mosher's method, and ECD (electronic circular dichroism) calculations. Leonuketal (**1**) showed significant vasorelaxant activity against KCl-induced contraction of rat aorta, with the EC_{50} value of 2.32 μM.



Leonuketal

In Chinese Pharmacopoeia, the aerial parts of *Leonurus japonicus* are the only certificated plant sources for "Yi Mu Cao", a common traditional Chinese medicine that is often used for the treatment of various gynecological blood disorders such as menstrual disturbance, dysmenorrhea, amenorrhea, blood stasis, and postpartum hemorrhage.[1] Chemical investigations on *Leonurus* genus (Labiatae) resulted in the discovery of a large variety of diterpene metabolites, yet with the common structural features representing the furanolabdane class.[2] This diterpenoid class displayed interesting bioactivities, including anticholinesterase inhibitory, PAF receptor antagonistic, cortical cultured neurons protective, anti-inflammatory, and estrogen sulfotransferase inhibitory properties.[3]

As part of our ongoing investigation of bioactive secondary metabolites from *L. japonicus*,[2e,4] a diterpenoid with an unprecedented tetracyclic skeleton, namely leonuketal (**1**) (Figure 1), was discovered. The highlighted structural feature includes a bridged spiroketal moiety fused with a ketal-γ-lactone ring. We herein report the isolation, structure elucidation, bioactivity, and the postulated biogenetic pathway of **1**.



1

Figure 1. Structure of leonuketal (**1**).

(−)-Leonuketal (**1**) was obtained as a colorless gum with the molecular formula ($C_{22}H_{34}O_7$) determined by HRESIMS (*m*/*z* 433.2194 $[M + Na]^+$, calcd 433.2202), which indicated six degrees of unsaturation. The IR spectrum showed characteristic absorption bands for hydroxyl (3362 cm^{-1}) and carbonyl (1787 and 1726 cm^{-1}) functionalities. To provide more detailed structural information, the ^1H NMR spectrum (Table 1) displayed typical signals that corresponded to three tertiary methyl groups (δ_H 0.75, 0.81, and 1.24), two primary methyl groups (δ_H 0.98 and 1.20), one oxymethylene group (δ_H 3.69 and 3.81), four oxymethines (δ_H 3.46, 3.96, 4.48, and 5.31), and several aliphatic methylenes and methines between δ_H 1.30 and 2.95 ppm. ^{13}C NMR data (Table 1) in combination with DEPT and HSQC spectra (Figures S7−S9 in the Supporting Information) resolved 22 carbon signals that corresponded to the protonated units (5 × CH_3, 6 × CH_2, and 6 × CH) in addition to five quaternary carbons which were one keto-carbonyl (δ_C 213.8), one carboxylic-carbonyl (δ_C 177.3), one double-oxygenated carbon (δ_C 100.7), and two all-carbon quaternary centers (δ_C 37.3 and 39.6). The above functionalities accounted for two out of the six degrees of unsaturation, and the remaining four degrees suggested **1** to be tetracyclic.

To further establish the bond connections, we began with ring A (*vide infra*). Referring to the 2D NMR spectra (Figures S9−S11 in the Supporting Information), HMBC correlations from both H_3-18 and H_3-19 to C-1, C-2, and C-3; from H_3-20 to C-3, C-4, and C-5; and from H-3 to C-1, C-2, C-4, and C-5, together with $^1H-^1H$ COSY correlations of H-1/H_2-6/H_2-5

Received: November 9, 2015
Published: December 4, 2015

DOI: 10.1021/acs.orglett.5b03227
Org. Lett. 2015, 17, 6238−6241

Table 1. ^{1}H and ^{13}C NMR Data of 1 in Me_2CO-d_6[a]

no.	δ_H	δ_C
1	3.46 dd (3.0, 2.4)	78.4
2		37.3
3	2.94 d (2.4)	52.8
4		39.6
5a	2.39 ddd (13.2, 10.8, 8.4)	20.7
5b	1.56 dddd (13.2, 10.8, 2.4, 2.4)	
6a	1.87 m	23.2
6b	1.81 m	
7		100.7
8a	1.79 ddd (13.2, 4.8, 2.4)	26.3
8b	1.34 ddd (13.2, 13.2, 4.8)	
9a	2.24 dddd (13.8, 13.2, 6.0, 4.8)	16.4
9b	2.00 dddd (13.8, 4.8, 2.4, 2.4)	
10	2.78 ddd (6.0, 4.2, 2.4)	37.5
11	4.48 d (4.2)	73.2
12	5.31 s	105.9
13		177.3
14		213.8
15	3.96 m	80.1
16a	1.90 m	27.2
16b	1.40 m	
17	0.98 t (7.2)	10.2
18	0.81 s	23.7
19	1.24 s	30.1
20	0.75 s	19.1
21a	3.81 dq (9.6, 7.2)	65.6
21b	3.69 dq (9.6, 7.2)	
22	1.20 t (7.2)	15.3

[a]NMR data (δ) were measured at 600 MHz for ^{1}H and at 150 MHz for ^{13}C. The assignments were based on DEPT, $^{1}H-^{1}H$ COSY, HSQC, and HMBC experiments.

(Figure 2), indicated the presence of a six-membered carbocycle (A) with geminal dimethyl groups at C-2 and one

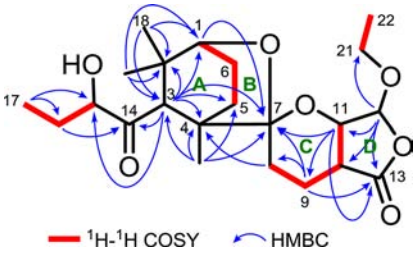


Figure 2. Key $^{1}H-^{1}H$ COSY and HMBC correlations of **1**.

methyl substituent at C-4. The existence of a 2-hydroxybutanoyl side chain was determined by the HMBC correlations, from H_3-17 to C-15 and C-16, and from H_2-16 to C-14 (δ_C 213.8), together with the $^{1}H-^{1}H$ COSY correlations of H-15/H_2-16/H_3-17.

This side chain was proven to be attached to C-3 of ring A by the HMBC correlations from H-3 to C-14 and C-15. Moving forward to elucidate the structure of rings B and C, the HMBC correlations, from H-1, H-3, H_2-5, H_2-8, H_2-9, H-11, and H_3-20 to the spiro carbon C-7; from H_2-5, H_2-6, and H_2-8 to C-4; and from H_2-8, H-10, and H-11 to C-9, in conjunction with the COSY signals of two isolated proton spin systems of H-1/H_2-6/H_2-5 and H_2-8/H_2-9/H-10/H-11 together indicated that (1) the two oxygen atoms on C-7 were attached to C-1 and C-11 respectively and (2) the two quaternary centers (C-4 and C-7) were adjacent. Rings A, B, and C therefore united a *bridged spiroketal* moiety. For the remaining part of the molecule, the low field chemical shifts for H-12 (δ_H 5.31) and C-12 (δ_C 105.9) in addition to the COSY correlation of H-21a (δ_H 3.81)/H-21b (δ_H 3.69)/H-22 (δ_H 1.20) suggested the presence of an ethyoxyl substitution.[5] Further elucidation from HMBC correlations from H_2-9 and H-11 to C-13 (the ester carbonyl, δ_C 177.3); from H-12 to C-10, C-11, C-13, and C-21; and from H_2-21 to C-12 established an ethyoxyl substituted γ-lactone unit (ring D) which was fused with ring C at the C-10 and C-11 positions. The planar structure of **1** was therefore completed.

The relative configuration of the polycyclic part was deduced from analysis of the NOESY spectrum and homonuclear coupling constants ($^{3}J_{H,H}$). NOESY correlations of H_3-18 with H-5a and H-6b indicated that the "CH_2-5−CH_2-6" bridge and H_3-18 were on the same orientation, while the NOESY correlation of H_3-19 with H-3 but with neither H_2-5 nor H_2-6 indicated that H_3-19 and H-3 were on the opposite orientation (Figure 3). The important NOESY correlation of H-8a with H-3 established the relative configuration of spiro carbon C-7.

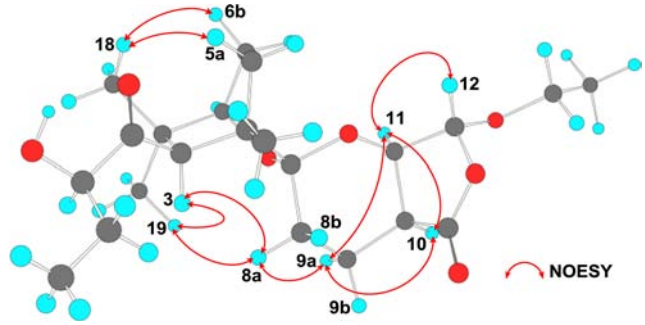


Figure 3. Key NOESY correlations of **1** (based on the computed lowest energy confirmation).

In turn, to relate the stereochemistry of C-10 and C-11 to C-7, detailed J coupling analysis ($J_{H\text{-}8a,9a}$ = 4.8 Hz, $J_{H\text{-}8a,9b}$ = 2.4 Hz, $J_{H\text{-}8b,9a}$ = 13.2 Hz, $J_{H\text{-}8b,9b}$ = 4.8 Hz, $J_{H\text{-}9a,10}$ = 6.0 Hz, $J_{H\text{-}9b,10}$ = 2.4 Hz, and $J_{H\text{-}10,11}$ = 4.2 Hz) was carried out to assign the bond orientations for the protons in the chairlike ring C. These ^{3}J values indicated that H-8a, H-9b, and H-10 were located in the equatorial positions while H-8b, H-9a, and H-11 were in the axial positions. Confirmed by the NOESY correlations of H-9a with H-8a; of H-10 with both H-9a and H-9b; of H-11 with H-9a; and of H_3-19 with H-8a and H-9a, the spatial proximity of H_3-19, H-8a, H-9a, H-10, and H-11 was revealed. The *cis* fusion of the C/D ring was verified based on the NOESY correlation of H-10 and H-11 together with the small coupling constant of $^{3}J_{H\text{-}10,11}$ (4.2 Hz).[6] The relative configuration of C-12 was assigned based on the coupling constant of $^{3}J_{H\text{-}11,12}$ (≈ 0 Hz) which suggested that the dihedral angle between H-11 and H-12 was about 90°,[7] and this orientation requires a *trans* relationship of H-11 with H-12. This assignment was confirmed by the NOESY correlation of H-12 with H-11 and no NOESY correlation of H-12 with H-10.

However, the relative configuration of C-15 to the remaining stereogenic centers could not be determined by NOESY experiment due to the bond rotations of the side chain. Nevertheless, a modified Mosher's method[8] was applied to determine the *absolute configuration* of C-15 directly. Esterification of **1** with (*R*)-(−)- and (*S*)-(+)-α-methoxy-

DOI: 10.1021/acs.orglett.5b03227
Org. Lett. 2015, 17, 6238−6241

phenylacetic acid (MPA) afforded the corresponding Mosher's ester derivatives **1a** [**1**-(*R*)-MPA] and **1b** [**1**-(*S*)-MPA]. The ^{1}H NMR data for these two diastereomers (Table S1 in the Supporting Information) were assigned on the basis of ^{1}H−^{1}H COSY experiments. By applying the MPA determination rule based on the $\Delta\delta$ values[8] (Figure 4), the absolute configuration of C-15 in **1** was therefore determined as *R*.

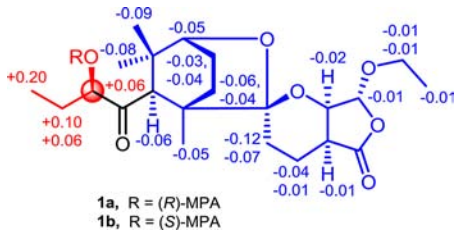


Figure 4. $\Delta\delta_H$ values ($\delta_R - \delta_S$, in ppm) for **1a** and **1b**.

To determine the absolute configuration of the polycyclic part, ECD calculations, applying the time-dependent density functional theory (TDDFT) method,[6,9] were performed. According to the above-mentioned stereochemical analysis, the absolute configuration was either 1*R*,3*S*,4*S*,7*S*,10*S*,11*R*,12*R*,15*R* (**1A**) or 1*S*,3*R*,4*R*,7*R*,10*R*,11*S*,12*S*,15*R* (**1B**), which were modeled respectively and used for the ECD calculations (Figure 5). As a result, both **1A** and **1B** showed a similar

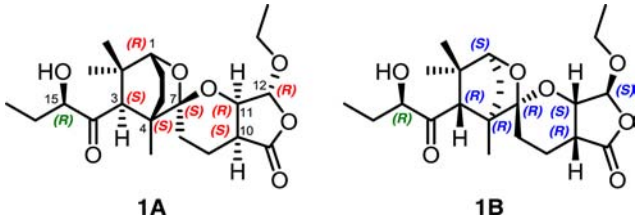


Figure 5. Proposed absolute configurations of **1** (**1A** and **1B**) in ECD calculation.

negative Cotton effect around 290 nm (Figure 6) but opposite Cotton effect around 225 nm (positive in **1A** and negative in **1B**). By comparing the 200 to 260 nm region of these three curves, the experimental ECD spectrum of **1** matched with the calculated ECD spectrum of **1A**, and the absolute configuration

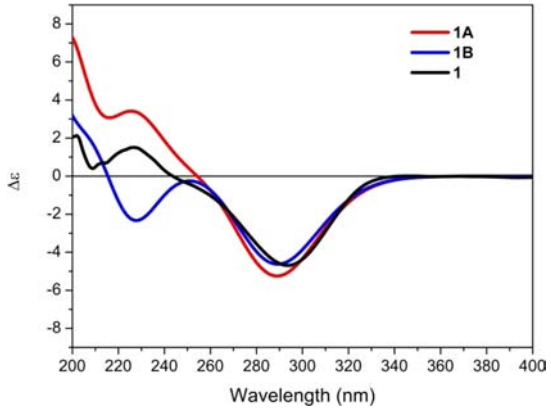
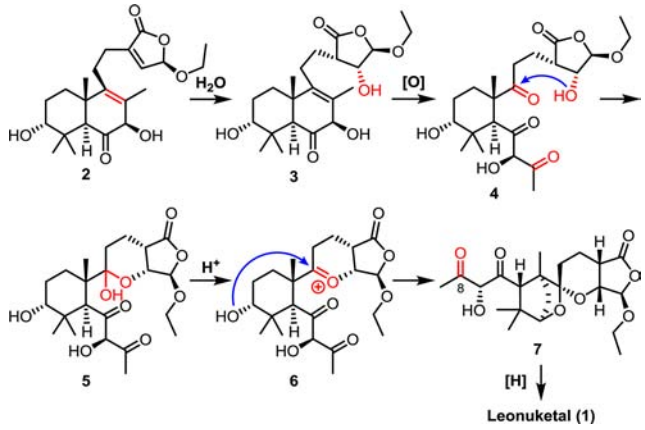


Figure 6. Experimental ECD spectrum of **1** (black) and the calculated ECD spectra of **1A** (red) and **1B** (blue).

of leonuketal A (**1**) was assigned as 1*R*,3*S*,4*S*,7*S*,10*S*,11*R*,12*R*,15*R*.

With the structure of **1** elucidated, we next postulated the biosynthetic pathway (Scheme 1). Starting from a possible

Scheme 1. Postulated Biogenetic Pathway of 1



common biosynthetic intermediate **2** that resembles sibiricinone B,[2a] Michael addition from water provided **3** which would then undergo oxidative cleavage to give triketone **4**. A subsequent cyclization led to hemiketal **5**, and the following spiroketalization via the oxocarbenium intermediate **6** afforded **7** with the tetracyclic skeleton established. Finally, the C-8 carbonyl of **7** could be reduced to yield **1**.

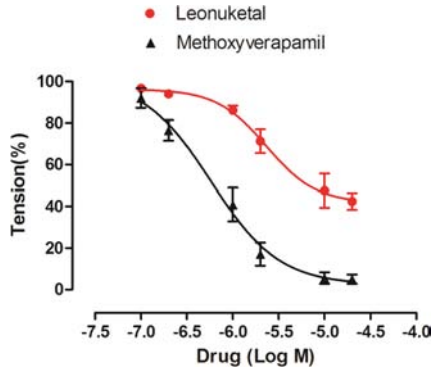


Figure 7. Concentration−response curves of leonuketal (**1**) and methoxyverapamil.

Recently, several types of diterpenoids such as kaurane-type, labdane-type, pimarane-type, and abietane-type have been found to exhibit pronounced cardiovascular effects, which hold promise for the development of cardiovascular therapeutic agents.[10] Among these, a large number of diterpenoids exhibited vasorelaxant properties against KCl-induced contraction, such as marrubenol, marrubiin, (12*R*)-12-hydroxycascarillone, *ent*-18-hydroxytrachyloban-3β-ol, *ent*-18-hydroxyisopimara-7,15-diene-3β-ol, grandifolia B, and isograndifoliol.[11] Based on this information, leonuketal (**1**) was then evaluated for the vasorelaxant activity on the aorta of Sprague−Dawley rats.[11d] The compound exhibited significant vasorelaxant effects on aortic segments with an EC_{50} value of 2.32 μM but was less potent than the positive control, methoxyverapamil (EC_{50} = 0.58 μM), a well-known cardiovascular drug with pronounced vasorelaxant activity against KCl-induced contraction.[12] Inter-

DOI: 10.1021/acs.orglett.5b03227
Org. Lett. 2015, 17, 6238−6241

estingly, leonuketal (**1**) inhibited the aortic contraction evoked by a high-level KCl solution in an obvious dose-dependent manner (Figure 7). Since Ca^{2+} plays a central role in the regulation of vascular tension and high-level K^+-induced contractions are linked to the opening of Ca^{2+} voltage-sensitive channels,[13] the vasorelaxant activity of **1** may be associated with their ability to block extracellular Ca^{2+} influx. In addition, an *in vitro* cytotoxic activity assay indicated that leonuketal (**1**) had no effect against four kinds of human cancer cell lines (A549, MCF-7, HepG-2, and MDA-MB-231) at a concentration of 50 μM.

In summary, the intriguing structure of leonuketal (**1**) as well as the disclosed bioactivity suggested it as a valuable target for further studies in related areas. A more detailed study on *L. japonicus* is ongoing in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03227.

Experimental procedures, as well as physical-chemical properties, experimental and calculated ECD spectra, 1D and 2D NMR, HRMS, and IR spectra for **1**, and ^{1}H NMR and ^{1}H−^{1}H COSY spectra for **1**-(*R*)-MPA and **1**-(*S*)-MPA (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: pengchengchengdu@126.com; pengchengchengdu@hotmail.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the National Natural Sciences Foundation of China (NNSFC, Grant No. 81303209), China Postdoctoral Science Foundation Funded Project (CPSF, Grant No. 2014M562291), and Sichuan Science and Technology Basic Research Program (Grant No. 2012JYZ005) is acknowledged.

■ REFERENCES

(1) Peng, C. *Chinese Genuine Medicinal Materials*; Chinese Press of Traditional Chinese Medicine: Beijing, 2011; pp 4187−4188.

(2) (a) Boalino, D. M.; McLean, S.; Reynolds, W. F.; Tinto, W. F. *J. Nat. Prod.* **2004**, *67*, 714−717. (b) Agnihotri, V. K.; Elsohly, H. N.; Smillie, T. J.; Khan, I. A.; Walker, L. A. *Planta Med.* **2008**, *74*, 1288−1290. (c) Shang, X.; Pan, H.; Wang, X.; He, H.; Li, M. *J. Ethnopharmacol.* **2014**, *152*, 14−32. (d) Zhou, Q.; Peng, C.; Liu, J.; Guo, L.; Xiong, L. *J. Chin. Med. Mater.* **2014**, *37*, 1691−1696. (e) Xiong, L.; Zhou, Q. M.; Peng, C.; Xie, X. F.; Liu, L. S.; Guo, L.; He, Y. C.; Yang, L.; Liu, Z. H. *Fitoterapia* **2015**, *100*, 1−6. (f) Huang, Z.; Zhu, Z. X.; Li, Y. T.; Pang, D. R.; Zheng, J.; Zhang, Q.; Zhao, Y. F.; Ferreira, D.; Zjawiony, J. K.; Tu, P. F.; Li, J. *J. Nat. Prod.* **2015**, *78*, 2276−2285.

(3) (a) Lee, C. M.; Jiang, L. M.; Shang, H. S.; Hon, P. M.; He, Y.; Wong, H. N. C. *Br. J. Pharmacol.* **1991**, *103*, 1719−1724. (b) Moon, H.-I. *Phytother. Res.* **2010**, *24*, 1256−1259. (c) Hung, T. M.; Luan, T. C.; Vinh, B. T.; Cuong, T. D.; Min, B. S. *Phytother. Res.* **2011**, *25*, 611−614. (d) Khan, S.; Shehzad, O.; Jin, H. G.; Woo, E. R.; Kang, S. S.; Baek, S. W.; Kim, J.; Kim, Y. S. *J. Nat. Prod.* **2012**, *75*, 67−71. (e) Narukawa, Y.; Niimura, A.; Noguchi, H.; Tamura, H.; Kiuchi, F. *J. Nat. Med.* **2014**, *68*, 125−131.

(4) Peng, F.; Xiong, L.; Zhao, X. M. *Molecules* **2013**, *18*, 13904−13909.

(5) (a) Fukuyama, Y.; Sato, T.; Miura, I.; Asakawa, Y. *Phytochemistry* **1985**, *24*, 1521−1524. (b) Zhang, Y.; Wang, J.; Wei, D.; Wang, X.; Luo, J.; Luo, J.; Kong, L. *Phytochemistry* **2010**, *71*, 2199−2204.

(6) Woo, J. K.; Kim, C. K.; Kim, S. H.; Kim, H.; Oh, D. C.; Oh, K. B.; Shin, J. *Org. Lett.* **2014**, *16*, 2826−2829.

(7) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870−2871.

(8) Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17−117.

(9) (a) Zhang, F.; Wang, J. S.; Gu, Y. C.; Kong, L. Y. *J. Nat. Prod.* **2012**, *75*, 538−546. (b) Oh, J.; Bowling, J. J.; Zou, Y.; Chittiboyina, A. G.; Doerksen, R. J.; Ferreira, D.; Leininger, T. D.; Hamann, M. T. *Biochim. Biophys. Acta, Gen. Subj.* **2013**, *1830*, 4229−4234. (c) Belofsky, G.; Kolaczkowski, M.; Adams, E.; Schreiber, J.; Eisenberg, V.; Coleman, C. M.; Zou, Y.; Ferreira, D. *J. Nat. Prod.* **2013**, *76*, 915−925. (d) Yang, F.; Zou, Y.; Wang, R. P.; Hamann, M. T.; Zhang, H. J.; Jiao, W. H.; Han, B. N.; Song, S. J.; Lin, H. W. *Mar. Drugs* **2014**, *12*, 4399−4416. (e) He, Y. C.; Zou, Y.; Peng, C.; Liu, J. L.; He, C. J.; Guo, L.; Xie, X. F.; Xiong, L. *Fitoterapia* **2015**, *100*, 7−10.

(10) Tirapelli, C. R.; Ambrosio, S. R.; da Costa, F. B.; de Oliveira, A. M. *Recent Pat. Cardiovasc. Drug Discovery* **2008**, *3*, 1−8.

(11) (a) El Bardai, S.; Morel, N.; Wibo, M.; Fabre, N.; Llabres, G.; Lyoussi, B.; Quetin-Leclercq, J. *Planta Med.* **2003**, *69*, 75−77. (b) Guerrero, M. F.; Puebla, P.; Carrón, R.; Martín, M. L.; San Román, L. *J. Ethnopharmacol.* **2004**, *94*, 185−189. (c) Baccelli, C.; Block, S.; Van Holle, B.; Schanck, A.; Chapon, D.; Tinant, B.; Meervelt, L. V.; Morel, N.; Quetin-Leclercq, J. *Planta Med.* **2005**, *71*, 1036−1039. (d) Kang, J.; Li, L.; Wang, D.; Wang, H.; Liu, C.; Li, B.; Yan, Y.; Fang, L.; Du, G.; Chen, R. *Phytochemistry* **2015**, *116*, 337−348.

(12) Amobi, N.; Guillebaud, J.; Smith, I. C. *Eur. J. Pharmacol.* **2010**, *627*, 285−294.

(13) (a) Karaki, H.; Weiss, G. B. *J. Pharmacol. Exp. Ther.* **1979**, *211*, 86−92. (b) Karaki, H.; Weiss, G. B. *Gastroenterology* **1984**, *87*, 960−970.

DOI: 10.1021/acs.orglett.5b03227
Org. Lett. 2015, 17, 6238−6241