Organic LETTERS

Letter

pubs.acs.org/OrgLett

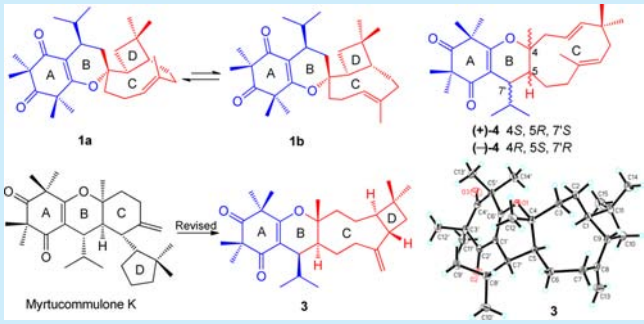
Meroterpenoids with New Skeletons from *Myrtus communis* and Structure Revision of Myrtucommulone K

Chao Liu,[†,‡] Song Ang,[†,‡] Xiao-Jun Huang,[‡] Hai-Yan Tian,[†] Yuan-Yuan Deng,[†] Dong-Mei Zhang,[†] Ying Wang,*[,†,‡] Wen-Cai Ye,*[,†,‡] and Lei Wang*[,†,‡]

[†]Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy and [‡]JNU-HKUST Joint Laboratory for Neuroscience & Innovative Drug Research, Jinan University, Guangzhou 510632, People's Republic of China

Supporting Information

ABSTRACT: Five sesquiterpene-based meroterpenoids with three kinds of new skeletons [**1**, **2**, **3**, (+)-**4**, and (−)-**4**] were isolated from the leaves of *Myrtus communis*. Compound **1** featured a new carbon skeleton with an unprecedented octahydrospiro[bicyclo[7.2.0]undecane-2,2′-chromene] tetracyclic ring system, which possessed two preferred conformations detected by variable-temperature NMR spectroscopy experiments. In addition, the structure of reported myrtucommulone K was revised to be compound **3**. The plausible biosynthetic pathways of these meroterpenoids and their cytotoxicities are discussed.



Myrtus communis L. (Myrtaceae) is an evergreen sclerophyll shrub that is widely distributed in the Mediterranean region and has been traditionally used in folk medicine as an antiseptic, disinfectant, and hypoglycemic agent.[1,2] Pharmacological studies revealed that the extracts of this plant possessed antitumor, antibacterial, anti-inflammatory, and antioxidant activities.[3] Previous phytochemical investigations of *M. communis* have led to the isolation of some monoterpenoids, triterpenoids, flavonoids, and phloroglucinol derivatives.[4−7] As a part of our search for structurally unique and biologically active constituents from Myrtaceae plants,[8,9] five new sesquiterpene-based meroterpenoids with three kinds of unusual skeletons [**1**, **2**, **3**, (+)-**4**, and (−)-**4**] (Figure 1) were isolated from the leaves of the title plant. Compound **1** featured a new carbon skeleton with an unprecedented octahydrospiro-[bicyclo[7.2.0]undecane-2,2′-chromene] tetracyclic ring system. Interestingly, **1** existed as two NMR-distinguishable conformational isomers which could be detected and analyzed by variable-temperature NMR spectroscopy experiments and quantum chemical calculations. In addition, the structure of reported myrtucommulone K[6] was revised to be compound **3**. Compounds (+)-**4** and (−)-**4** were a pair of enantiomers. In this paper, we report the isolation, structural elucidation, and cytotoxic activities of **1**−**4**. A plausible biogenetic pathway for the new compounds is also proposed.

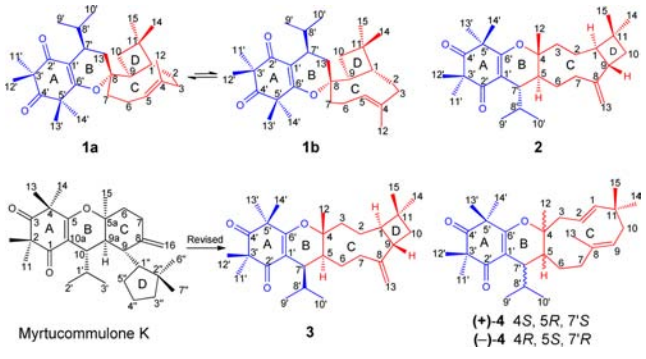


Figure 1. Chemical structures of **1**−**4**.

Compound **1** was obtained as colorless blocks. The HRESIMS of **1** showed a quasimolecular ion peak at *m*/*z* 441.3364 $[M + H]^+$ (calcd for $C_{29}H_{45}O_3$ 441.3363), consistent with the molecular formula $C_{29}H_{44}O_3$. The UV spectrum revealed the absorption maximum at 267 nm. The IR spectrum displayed the characteristic absorptions for conjugated carbonyl (1654 cm^{-1}) and olefinic bond (1601 cm^{-1}). The crystals suitable for X-ray diffraction were grown in $CHCl_3$/MeOH solution. The final refinement of the Cu Kα data resulted in a small Flack parameter of −0.14(11), allowing the assignment of the complete structure and absolute configuration of **1** (Figure 2).

It is very interesting that the ^1H and ^{13}C NMR spectra of **1** exhibited broad signals and incomplete signals at room temperature (Figure 3). These abnormal NMR signals prevented the assessment of purity and structural assignment of **1**. To investigate the structure of **1** in solution, a variable-temperature NMR spectroscopy experiment was carried out. When the ^1H NMR spectra of **1** were recorded from 298 K to 308, 318, and 328 K in DMSO-d_6 (Figure 3), the two broad peaks in olefinic bond regions (δ_H 5.04−5.14 and 5.25−5.38)

Received: June 22, 2016
Published: July 29, 2016

DOI: 10.1021/acs.orglett.6b01817
Org. Lett. 2016, 18, 4004−4007

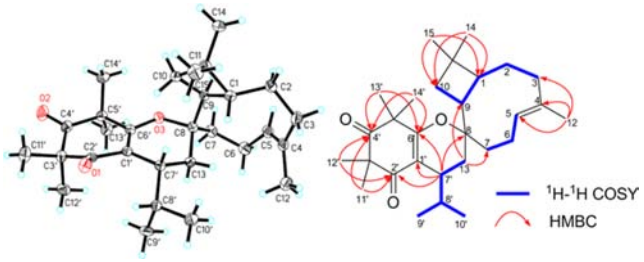


Figure 2. X-ray ORTEP drawing and key 2D NMR correlations of **1**.

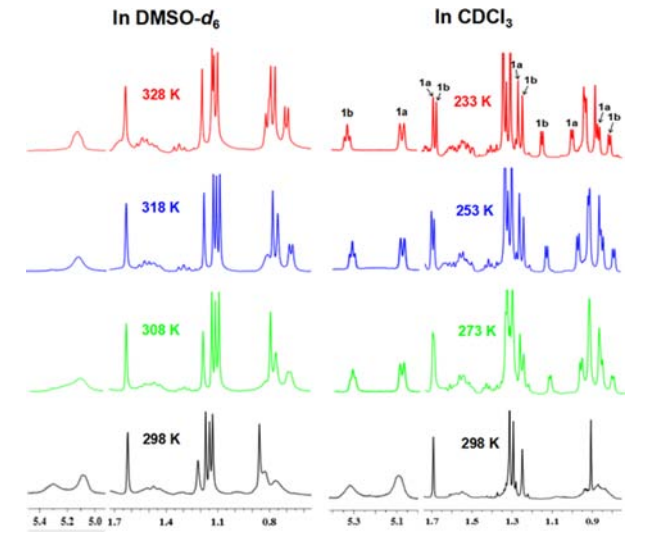


Figure 3. ^{1}H NMR spectra of **1** in DMSO-d_6 and $CDCl_3$ at different temperatures.

coalesced, but the other signals were still indistinguishable. On the other hand, when the recorded temperature was lower than 273 K (in $CDCl_3$), the less broadening and well-separated multiple signals were observed. At 233 K, the distinguishable ^{1}H and ^{13}C NMR spectra of **1** were recorded, which displayed two sets of ^{1}H NMR signals in a ratio of approximately 5:4 (**1a**/**1b**, Figure 3). The above results and the presence of a caryophyllene moiety suggested that **1** existed as a mixture of two conformers (**1a** and **1b**) in solution.[10] A comprehensive analysis of the ^{1}H−^{1}H COSY, HSQC, HMBC (Figure 2), and NOESY spectra recorded at 233 K (see the Supporting Information) allowed the assignment of NMR data of **1a** and **1b** as shown in Table S1.

In order to reveal the two major equilibrating conformers (**1a** and **1b**) in solution, the quantum chemical calculation method using the Gaussian09 package was applied. Owing to the flexibility of a nine-membered ring, two conformations of **1** were found to be the lowest energy ones. The two conformations $\beta\beta$ (**1a**) and $\beta\alpha$ (**1b**) were named according to the relative orientations of C-13 and C-12 of β-caryophyllene unit in **1**.[10] The torsion $C_6-C_5-C_4-C_{12}$ values for $\beta\beta$ and $\beta\alpha$ were 18.51° and −15.67°, respectively (Figure 4). The calculated conformational free energy difference (ΔG_{cal} = 0.127 kcal/mol) between $\beta\beta$ and $\beta\alpha$ conformations was consistent with their 5:4 ratio found experimentally in the equilibrium. In addition, the NOESY correlations between H-5 and H-9/H-6α/H-2α, H-12 and H-6β in **1a** as well as between H-5 and H-1/H-6β/H-3β, H-12 and H-6α in **1b** supported the calculated conformations (see the Supporting Information). The X-ray crystallographic structure also indicated that the

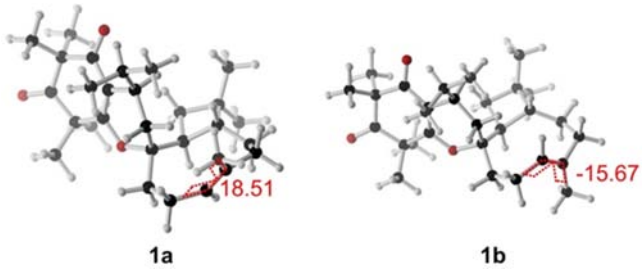


Figure 4. Two conformations [(**1a**, $\beta\beta$) and (**1b**, $\beta\alpha$)] with the lowest energy, calculated at the PWPB95-D3(BJ)/def2-QZVP level in Gaussian09.

most stable conformation $\beta\beta$ (**1a**) is the preferable one in the solid state (Figure 2).

The molecular formula of compound **2** was established as $C_{29}H_{44}O_3$ on the basis of a quasi-molecular ion at m/z 441.3361 $[M + H]^+$ in its HRESIMS spectrum (calcd for $C_{29}H_{45}O_3$ 441.3363). With the aid of ^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY experiments, the NMR signals of **2** could be assigned as shown in Table S2. A comparison of the NMR data of **2** with those of the known compound myrtucommulone L[6] suggested the presence of an isobutyl syncarpic acid unit (**a**). The spin coupling systems (H-3 to H-10 and H-7 to H-9′/H-10′) established by the ^{1}H−^{1}H COSY spectrum as well as the HMBC correlations between H-14/H-15 and C-1/C-10, between H-13 and C-7/C-9, and between H-12 and C-3/C-5 indicated the presence of a caryophyllene unit (**b**) (Figure 5).[11]

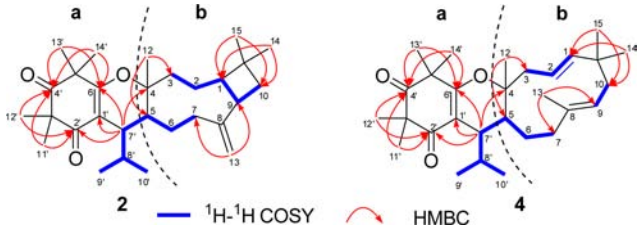


Figure 5. Key ^{1}H−^{1}H COSY and HMBC correlations of **2** and **4**.

Furthermore, the linkage of parts **a** and **b** through the C-5−C-7′ bond was confirmed by the HMBC correlations between H-7′ and C-2′/C-6′/C-4. According to the molecular formula information, the oxygen atom leftover was deduced to bridge C-4 (δ_C 83.7) and C-6′ (δ_C 171.3) to form a dihydropyran ring (ring B).

In the NOESY spectrum, the correlations between H-7′ and H-12, between H-5 and H-9′/H-10′/H-1, between H-1 and H-14, as well as between H-9 and H-15 established the relative configuration of **2** (Figure 6). The complete structure and stereochemistry of **2** were further confirmed by an X-ray diffraction analysis. The final refinement of the Cu Kα data resulted in a small Flack parameter of −0.07(18), allowing an unambiguous assignment of the absolute configurations of **2** as 1*R*,4*R*,5*S*,9*S*,7′*S* (Figure 7).

The HRESIMS data of **3** (m/z 441.3363 $[M + H]^+$; calcd for $C_{29}H_{45}O_3$: 441.3363) suggested that **3** has the same molecular formula $C_{29}H_{44}O_3$ as **2**. Detailed examination of 1D and 2D NMR spectra of **3** and comparison with those of **2** revealed they have the same planar structure. The NOESY correlations between H-5 and H-7′/H-1 as well as between H-12 and H-8′/H-9′ indicated that the isopropyl group at C-7′ should be β-orientated in **3** (Figure 6), which was further confirmed by X-

DOI: 10.1021/acs.orglett.6b01817
Org. Lett. 2016, 18, 4004−4007

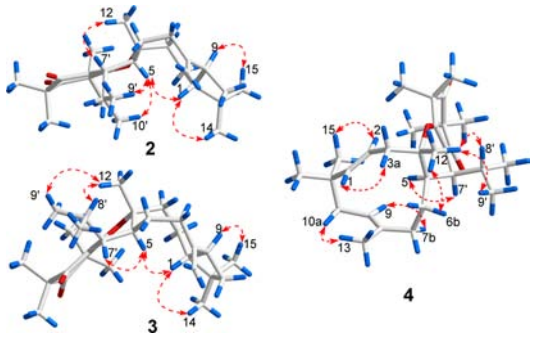


Figure 6. Key NOESY correlations of **2**–**4**.

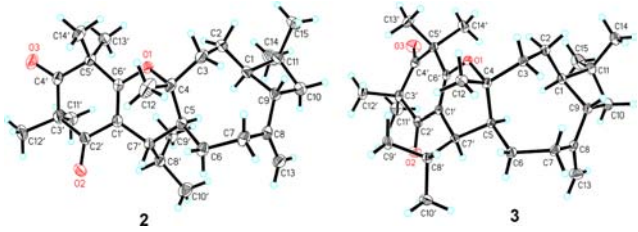


Figure 7. X-ray ORTEP drawings of **2** and **3**.

ray crystallographic analysis. The final refinement in the Cu Kα data resulted in a small Flack parameter of 0.04(16). Thus, the absolute configuration of **3** was determined as 1*R*,4*R*,5*S*,9*S*,7′*R* (Figure 7).

Recently, Cottiglia et al. reported the isolation and structural elucidation of myrtucommulone K (Figure 1) from *M. communis*.[6] The structure of myrtucommulone K was established mainly through its MS and NMR data but no X-ray analysis. Since the MS, ^{1}H NMR, and ^{13}C NMR data of myrtucommulone K are exactly identical to those of **3** (Table S3), the structure proposed for myrtucommulone K should be revised to compound **3**.

The molecular formula of compound **4** was determined to be $C_{29}H_{44}O_3$ by its HRESIMS data (*m*/*z* 441.3367 [M + H]$^+$; calcd for $C_{29}H_{45}O_3$ 441.3363). Comparison of the NMR data of **4** (Table S2) with those of **2** and **3** suggested that they had the same substructure of an isobutyl syncarpic acid unit (part **a**) (Figure 5). The ^{1}H−^{1}H COSY spectrum showed the presence of three spin systems (H-1 to H-3, H-7 to H-9′/H-10′, and H-9 to H-10) (Figure 5). In addition, the HMBC correlations between H-14/H-15 and C-1/C-10, between H-12 and C-3/C-5, as well as between H-13 and C-7/C-9 allowed the construction of a sesquiterpenoid unit (humulene, part **b**), which was further confirmed by comparison of the ^{1}H and ^{13}C NMR data of **4** with those of guajadial B.[12] Moreover, the HMBC correlations between H-7′ and C-4/C-1′/C-2′/C-6′ indicated the connection of parts **a** and **b** via C-5−C-7′ bond. Finally, the closure mode of dihydropyran ring connecting the two fragments (**a** and **b**) could be deduced on the basis of the molecular formula information and the downfield chemical shift of C-4 (δ_C 85.4).

The relative configuration of **4** was established on the basis of the NOESY data and proton coupling constants. The NOE correlations between H-2 and H-15 as well as between H-1 and H-3a suggested the presence of *E*-geometry in 1,2-double bond, which was in good agreement with the large coupling constant between H-1 and H-2 (J = 15.8 Hz). The *E*-geometry in $\Delta^{8,9}$ was also deduced by NOE correlations between H-9 and H-7b as well as between H-13 and H-10a. Moreover, the NOE correlations between H-7′ and H-5, between H-12 and H-8′/H-9′/H-6b, as well as the small coupling constants of H-7′ (J = 4.9, 4.3 Hz) indicated the relative stereochemistry of **4** as shown in Figure 6.[12] Although there were three chiral centers in **4**, the optical activity and Cotton effects were undetectable, which suggested that **4** may be a racemate. This was also supported by chiral HPLC analysis, in which two distinct chromatographic peaks appeared with a ratio of 1:1 (see the Supporting Information). To determine the absolute configurations of (+)-**4** and (−)-**4**, a comparison between the experimental and calculated circular dichroism (CD) spectra using the time-dependent DFT method of each enantiomer was performed. The measured CD spectrum of (+)-**4** showed positive Cotton effects at 319.7 ($\Delta\varepsilon$ + 5.0) and 210.1 ($\Delta\varepsilon$ + 10.3) nm as well as negative one at 272.1 ($\Delta\varepsilon$ −7.2) nm, which were consistent with those of the calculated CD spectrum for 4*S*, 5*R*, 7′*S* isomer. Meanwhile, the CD spectrum of (−)-**4** displayed opposite Cotton effects at the same wavelengths, consistent with those of 4*R*, 5*S*, 7′*R* isomer (Figure 8). Hence, the absolute configurations of (+)-**4** and (−)-**4** were elucidated as 4*S*,5*R*,7′*S* and 4*R*,5*S*,7′*R*, respectively.

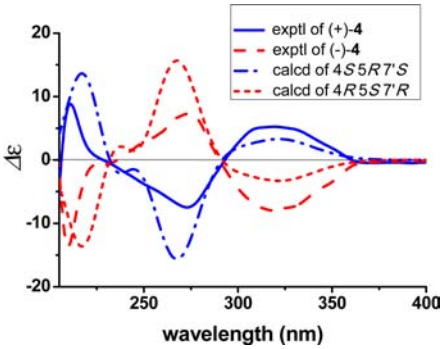
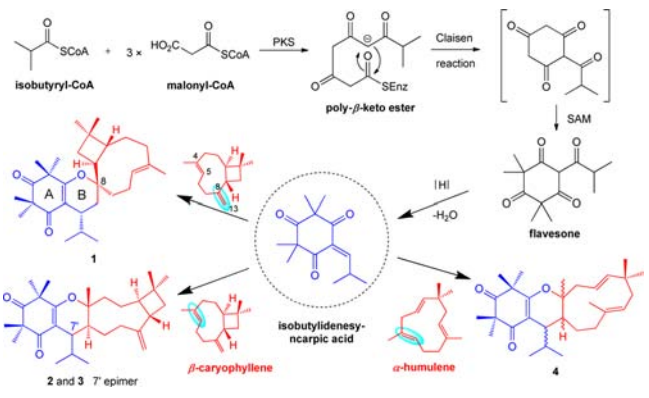


Figure 8. Calculated and experimental CD spectra of (+)-**4** and (−)-**4**.

On the basis of the key terpene precursors which were previously detected in the title plant,[13] a plausible biosynthetic pathway for compounds **1**–**4** is proposed as shown in Scheme 1. First, the isobutyryl-CoA was successively condensed with three molecules of malonyl-CoA by a polyketide synthase (PKS) to form the poly-β-keto ester. Then, cyclization and methylation could lead to the formation of intermediate flavesone, which had been previously isolated from the same plant.[14] The intermediate flavesone could be hydrogenated and

Scheme 1. Hypothetical Biogenetic Pathway of 1−4



DOI: 10.1021/acs.orglett.6b01817
Org. Lett. 2016, 18, 4004−4007

dehydrated to generate isobutylidenesyncarpic acid.[15] As a conjugated diene, isobutylidenesyncarpic acid could couple with different olefinic bond of sesquiterpene precursors such as β-caryophyllene[13] and α-humulene[13] through a hetero-Diels–Alder reaction to form **1**–**4**.

To date, about 19 caryophyllene-based meroterpenoids had been isolated by several groups from plants and fungi.[11,16] Although there are some differences in their skeletal types, all of the reported caryophyllene-based meroterpenoids possessed the same coupling positions through the endocyclic double bond (C-4 and C-5) of the caryophyllene moiety. Compound **1** is the first meroterpenoid with an unusual coupling pattern, in which the exocyclic double bond (C-8 and C-13) rather than endocyclic double bond of the caryophyllene unit is involved in the construction of dihydropyran ring (ring B) and spiro carbon (C-8) (Scheme 1). This unusual coupling pattern of **1** led to the formation of unprecedented octahydrospiro-[bicyclo[7.2.0]undecane-2,2′-chromene] tetracyclic system.

The inhibitory effects of compounds **1**–**4** on the viability of HepG2 and MDA-MB-231 cells were determined by the MTT assay. The results showed that compound **1** displayed a growth inhibitory effects against HepG2 and MDA-MB-231 cells with IC_{50} values of 4.39 ± 0.84 and 19.92 ± 4.64 μM. Compound **2** showed moderate inhibitory activity with IC_{50} values of 40.70 ± 5.19 and 40.00 ± 4.21 μM, respectively, while compounds **3** and (±)-**4** were found to be nearly inactive against these cancer cells (IC_{50} > 50 μM).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01817.

Detailed description of the experimental procedure; detailed NMR assignments of **1**; UV, IR, MS, and NMR spectra for **1**-**4**; chemical calculation details for **1** and **4** (PDF)

X-ray data for **1**(CIF)

X-ray data for **2**(CIF)

X-ray data for **3**(CIF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: wangying_cpu@163.com.
*E-mail: chyewc@gmail.com.
*E-mail: cpuwanglei@126.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the Program for the National Natural Science Foundation of China (Nos. U1401225 and 81573307), the Ministry of Science and Technology of China (Nos. 2013BAI11B05, 2013DFM30080, and 2012ZX09103201-056), and the Guangdong Natural Science Foundation for Distinguished Young Scholars (No. 2015A030306022). This work was also supported by the high-performance computing platform of Jinan University.

■ REFERENCES

(1) Leonti, M.; Casu, L.; Sanna, F.; Bonsignore, L. *J. Ethnopharmacol.* **2009**, *121*, 255–267.
(2) Alipour, G.; Dashti, S.; Hosseinzadeh, H. *Phytother. Res.* **2014**, *28*, 1125–1136.
(3) (a) Tretiakova, I.; Blaesius, D.; Maxia, L.; Wesselborg, S.; Osthoff, K. S.; Cinatl, J., Jr; Michaelis, M.; Werz, O. *Apoptosis* **2008**, *13*, 119–131. (b) Shahidi Bonjar, G. H. *Fitoterapia* **2004**, *75*, 231–235. (c) Feisst, C.; Frank, L.; Appendino, G.; Werz, O. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 389–396.
(4) Choudhary, I.; Khan, N.; Ahmad, M.; Yousuf, S.; Fun, H. K.; Soomro, S.; Asif, M.; Mesaik, M. A.; Shaheen, F. *Org. Lett.* **2013**, *15*, 1862–1865.
(5) Rotstein, A.; Lifshitz, A.; Kashman, Y. *Antimicrob. Agents Chemother.* **1974**, *6*, 539–542.
(6) Cottiglia, F.; Casu, L.; Leonti, M.; Caboni, P.; Floris, C.; Busonera, B.; Farci, P.; Ouhtit, A.; Sanna, G. *J. Nat. Prod.* **2012**, *75*, 225–229.
(7) Appendino, G.; Bianchi, F.; Minassi, A.; Sterner, O.; Ballero, M.; Gibbons, S. *J. Nat. Prod.* **2002**, *65*, 334–338.
(8) (a) Jian, Y. Q.; Huang, X. J.; Zhang, D. M.; Jiang, R. W.; Chen, M. F.; Zhao, B. X.; Wang, Y.; Ye, W. C. *Chem. - Eur. J.* **2015**, *21*, 9022–9027. (b) Shao, M.; Wang, Y.; Jian, Y. Q.; Huang, X. J.; Zhang, D. M.; Tang, Q. F.; Jiang, R. W.; Sun, X. G.; Lv, Z. P.; Zhang, X. Q.; Ye, W. C. *Org. Lett.* **2012**, *14*, 5262–5265. (c) Shao, M.; Wang, Y.; Liu, Z.; Zhang, D. M.; Cao, H. H.; Jiang, R. W.; Fan, C. L.; Zhang, X. Q.; Chen, H. R.; Yao, X. S.; Ye, W. C. *Org. Lett.* **2010**, *12*, 5040–5043.
(9) 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.
(10) Shirahama, H.; Osawa, E.; Chhabra, B. R.; Shimokawa, T.; Yokono, T.; Kanaiwa, T.; Amiya, T.; Matsumoto, T. *Tetrahedron Lett.* **1981**, *22*, 1527–1528.
(11) Yang, X. L.; Hsieh, K. L.; Liu, J. K. *Org. Lett.* **2007**, *9*, 5135–5138.
(12) Gao, Y.; Wang, G. Q.; Wei, K.; Hai, P.; Wang, F.; Liu, J. K. *Org. Lett.* **2012**, *14*, 5936–5939.
(13) Berka-Zougali, B. B.; Ferhat, M. A.; Hassani, A.; Chemat, F.; Allaf, K. S. *Int. J. Mol. Sci.* **2012**, *13*, 4673–4695.
(14) Petretto, G. L.; Maldini, M.; Addis, R.; Chessa, M.; Foddai, M.; Rourke, J. P.; Pintore, G. *Food Chem.* **2016**, *197*, 124–131.
(15) Wu, L.; Luo, J.; Zhang, Y.; Zhu, M.; Wang, X.; Luo, J.; Yang, M.; Yu, B.; Yao, H.; Dai, Y.; Guo, Q.; Chen, Y.; Sun, H.; Kong, L. *Tetrahedron Lett.* **2015**, *56*, 229–232.
(16) (a) Yang, X. W.; Li, Y. P.; Su, J.; Ma, W. G.; Xu, G. *Org. Lett.* **2016**, *18*, 1876–1879. (b) Fu, H. Z.; Luo, Y. M.; Li, C. J.; Yang, J. Z.; Zhang, D. M. *Org. Lett.* **2010**, *12*, 656–659. (c) Li, C. J.; Ma, J.; Sun, H.; Zhang, D.; Zhang, D. M. *Org. Lett.* **2016**, *18*, 168–171. (d) Qi, Q. Y.; Ren, J. W.; Sun, L. W.; He, L. W.; Bao, L.; Yue, W.; Sun, Q. M.; Yao, Y. J.; Yin, W. B.; Liu, H. W. *Org. Lett.* **2015**, *17*, 3098–3101. (e) Spence, J. T. J.; George, J. H. *Org. Lett.* **2011**, *13*, 5318–5321.

DOI: 10.1021/acs.orglett.6b01817
Org. Lett. 2016, 18, 4004–4007