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Fortunoids A–C, Three Sesquiterpenoid Dimers with Different Carbon Skeletons from *Chloranthus fortunei*

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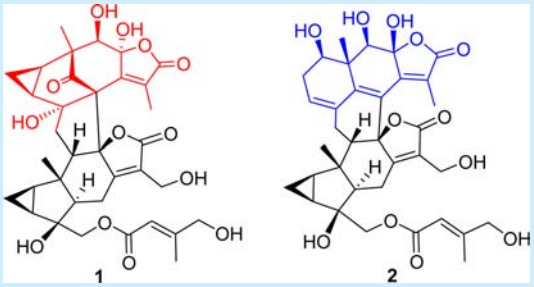
Supporting Information

ABSTRACT: Three dimeric sesquiterpenoids (**1**–**3**), fortunoid A (**1**) possessing a new carbon skeleton of rearranged lindenane dimer and fortunoids B (**2**) and C (**3**) representing the first example of the dimers of a lindenane and a eudesmane sesquiterpene, were isolated from *Chloranthus fortunei*. Their structures with absolute configurations were established by spectroscopic data and electric circular dichroism analysis. Their biosynthetic origins were also proposed. Compounds **1** and **2** showed moderate antimalarial activities.



The plants in the *Chloranthus* genus (Chloranthaceae) are widely distributed in the tropical and temperate zones of Asia.[1] There are 13 species and five varieties native to China,[2] of which many have long been used in Chinese folk medicine.[2,3] Sesquiterpenes, especially the sesquiterpenoid dimers, are the major and characteristic metabolites of this plant genus,[4] which exhibit a wide spectrum of bioactivities, e.g., HIV-1 integrase inhibition, inhibition on the delayed rectifier K^+ current, and antifungal, antitumor, and antimalarial activities.[5] *Chloranthus fortunei*, a perennial herbaceous plant, is widely distributed in the shady and moist environment of southern China.[2] Previous chemical investigations on this plant led to the isolation of a number of eudesmane and lindenane sesquiterpenoids and lindenane sesquiterpenoid dimers.[5d,6] Continuing our effort to explore the structurally interesting and bioactive components from the plants of *Chloranthus* genus,[4c,5c,d,7] three dimeric sesquiterpenoids, fortunoids A–C (**1**–**3**, Chart 1), were further isolated from *C. fortunei*. Compound **1** possessed a new carbon skeleton of rearranged lindenane dimer, and compounds **2** and **3** represented the first example of heterodimeric frameworks of a lindenane and an eudesmane sesquiterpenoid. Herein, the isolation, structure elucidation, biosynthetic origin, and antimalarial tests of these compounds are discussed.

Compound **1**, named fortunoid A, was isolated as a white, amorphous powder. It had a molecular formula of $C_{35}H_{40}O_{13}$ as determined by the sodiated HRESI(+)MS ion at m/z 691.2360 $[M + Na]^+$ (calcd 691.2367), requiring 16 double bond equivalents (DBEs). Analysis of the NMR data (Table S1) revealed the existence of two α,β-unsaturated-γ-lactone groups, a keto group (δ_C 208.1), and a γ-formylsenecioate residue. In addition, three methyls, six methylenes (two oxygenated), seven methines (one oxygenated), four oxygenated tertiary carbons, and three quaternary carbons were also distinguished with the aid of DEPT experiments. These functionalities accounted for nine out of the 16 DBEs, indicating seven additional rings in the structure of **1**. The aforementioned data suggested that **1** was likely a lindenane-type sesquiterpenoid dimer. However, an in depth NMR data analysis revealed big differences between compound **1** and the common lindenane-type sesquiterpenoid dimers regarding the chemical shifts and the linkages of some key protons and/or carbons in the component I.[4]

Analysis of the $^1H-^1H$ COSY spectrum showed the existence of two 1,2-disubstuted cyclopropane moieties as drawn with bold bonds (Figure 1), indicative of two lindenane-type sesquiterpenoid components I and II. In part I (in red), an oxygenated quaternary carbon at δ_C 104.7 was assigned to C-8 by the HMBC cross-peak of H-9/C-8 (Figure 1), involving the linkage between C-8 and C-12 via an oxygen atom to form an α,β-unsaturated γ-lactone. Although there was no direct evidence for the C-5–C-6–C-7 linkage, a bicyclo[3.3.1]nonane framework of the B and C rings in component I was established by the HMBC networks of CH_3-14/C-1, C-5 (δ_C 208.1), C-9, and C-10, H-3/C-4 and C-6, and H-9/C-7 and C-8, as well as biogenetic reasoning. It indicated that component I represented a rearranged lindenane-type framework as compared to the normal ones with a five-membered B ring. The HMBC cross-peaks of H_3-13/C-7, C-11, and C-12 (δ_C 174.2) attached Me-

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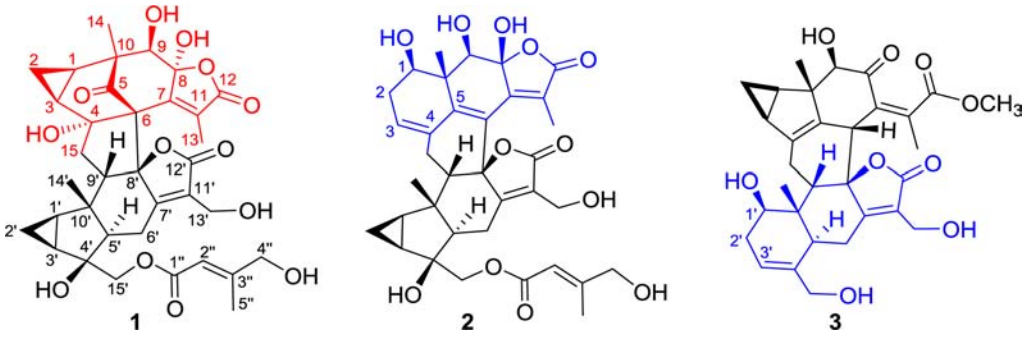
Chart 1



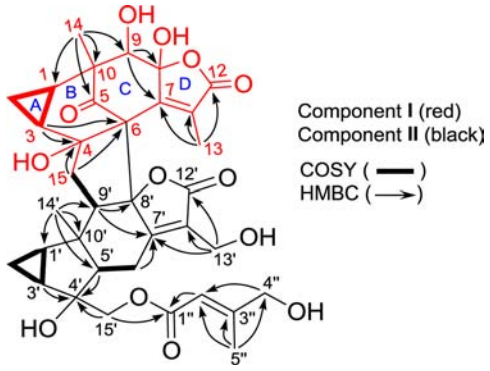


Figure 1. Key HMBC and $^1H-^1H$ COSY correlations of **1**.

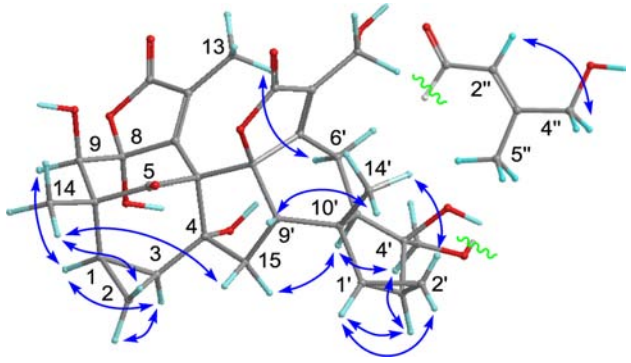


Figure 2. Key ROESY correlations of **1**.

13 at C-11 and verified the presence of the D ring of an α,β-unsaturated-γ-lactone. The component II (in black) was assembled to be a lindenane-type sesquiterpenoid motif by the HMBC data (Figure 1) in which a hydroxy group was attached to C-4′ (δ_C 79.3) via the cross-peaks of H-3′ and H-5′/C-4′, and the γ-formylsenecioate residue was attached to C-15′ by the cross-peak of H_2-15′/C-1″. The deshielded C-8′ (δ_C 102.0) and the C-12′ ester carbonyl at δ_C 173.2 suggested the presence of an α,β-unsaturated-γ-lactone. The COSY correlation of H_2-15/H-9′ and the key HMBC interactions of H-15/C-4 and C-6 connected components I and II via the bridge of C-4−C-15−C-9′. The linkage between two "loose ends" of the quaternary C-6 and C-8′ was the only possibility to satisfy the requirement for the remaining one DBE, which was consistent with their chemical shifts and biogenetic consideration. The planar structure of **1** was hence established as a new carbon skeleton.

In the ROESY spectrum (Figure 2), the cross-peaks of CH_3-14/H-2β and H-15, H-1/H-3 and H-9, and H-3/H-2α allowed the random assignment of an α-orientation for H-1, H-3, H-9, and 4-OH and a β-direction for CH_3-14, indicating that the C ring in component I took a chair conformation. Consequently, the chemical shifts of C-8 and the spatially related carbons of **1** were consistent with those of chloramultiol B and henriol A bearing an 8α-OH,[8] suggesting that the 8-OH of **1** was also α-oriented and adopted the axial bond of the chair-conformed C ring to satisfy the stereo requirement, which was supported by the correlation of CH_3-13/H-6′. Similarly, the ROESY cross-peaks of H-1′/H-2′α and H-3′, H-3′/H_2-15′, and H-5′/ H_2-15′ revealed that they were α-oriented. Accordingly, the correlations of CH_3-14′/H-2′β and H-9′/CH_3-14′ indicated that CH_3-14′ and H-9′ were β-configured. The $\Delta^{2''}$ double bond was assigned *E*-geometry by the correlation of H_2-4″/H-2″. The correlations of CH_3-13/H-6′α indicated that compound **1** was a typical endo cycloaddition product, and the C-8′−O and C-5−C-6 bonds were β-oriented, which was supported by the ROESY correlation of CH-15/H-5′.

Compound **2** possessed a molecular formula of $C_{35}H_{40}O_{12}$, as deduced by the HRESI(+)MS ion at m/z 675.2407 $[M + Na]^+$ (calcd 675.2417). Consideration of the NMR data (Table S1) and the occurrence of dimeric sesquiterpenoids in this plant suggested that **2** was also a sesquiterpenoid dimer.[4] Two partial structural components I and II were then constructed by analysis of NMR data. A five membered α,β-unsaturated lactone was identified by the chemical shifts of C-8 (δ_C 106.9) and C-12 (δ_C 174.8) as well as the HMBC cross-peaks (Figure 3) of H-9/C-8 and C-7 and CH_3-13/C-7, C-11, and C-12. The HMBC cross-peaks of CH_3-14/C-1 (δ_C 70.9), C-5, C-9 (δ_C 70.2), and C-10 attached C-1, C-5, C-9, and C-14 to C-10 and assigned two hydroxy groups at C-1 and C-9, respectively. The rest of the multiple HMBC cross-peaks as depicted finally allowed the construction of a eudesmatrien-12,8-olide for the

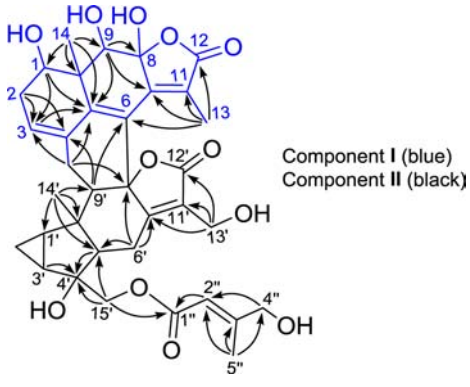


Figure 3. Selected HMBC correlations of **2**.

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component I. In the component II, four severely shielded coupling protons (δ_H 1.90, 0.64, 1.21, and 1.51) showed the existence of a 1,2-disubstituted cyclopropane motif, indicative of a lindenane-type sesquiterpenoid. Two hydroxy groups were then located at C-4 and C-13, respectively, by the HMBC cross-peaks of H-3′ and H-5′/C-4′ (δ_C 78.3) and H_2-13′ (δ_H 4.29, and 4.24)/C-7′, C-11′ and C-12′. The cross-peak from H_2-15′ to C-1″ attached the γ-formylsenecioate moiety at C-15′. The linkage of parts I and II was achieved by the key HMBC interactions of H_2-15/C-8′, C-3, and C-5 and H-9′/C-4 and C-6. The planar structure of **2** was hence established as an unprecedented heterodimer of a eudesmane and a lindenane sesquiterpene.

The relative configuration of **2** was deduced by the ROESY data (Figure S2) in which the correlations of CH_3-14/H-2β, H-1/H-2α, and H-1/H-9 allowed the random assignment of CH_3-14, 1-OH, and 9-OH as β-configured; the correlations of H-1′/H-2′α, H-1′/H-3′, and H-3′/H-5′ then suggested they were α-oriented. Accordingly, the ROESY correlations of H-2′β/CH_3-14′, CH_3-14′/H_2-6′β, and CH_3-14′/H-9′ revealed that CH_3-14′ and H-9′ were β-configured. The cross-peak of H-3′/H-15′ indicated a β-direction for 4′-OH. An *E*-geometry for the $\Delta^{2''}$double bond was assigned by the key ROESY correlation of H_2-4″/H-2″. The correlation of CH_3-13/H_2-13′ suggested a β-configuration for 8-OH, which was confirmed by the pyridine-induced chemical shift of H_3-14 ($\Delta\delta = \delta_{CDCl_3} - \delta_{C_5D_5N=} - 0.74$)[9] (Figures S12 and S13). Finally, the correlations of H-15α/H-3 and H-1′ indicated that the two flanks of the dimeric framework of **2** were highly bent and spatially close.

Compound **3** had a molecular formula $C_{31}H_{36}O_9$ as assigned by the HRESI(+)MS ion at *m*/*z* 575.2250 $[M + Na]^+$ (calcd 575.2257). The NMR data (Table S1) analysis suggested it was also a heterodimer of a lindenane (I) and an eudesmane (II) sesquiterpenoid and had the same component I as in sarcandrolide J,[10] which was verified by the HMBC data (Figure S3A). The 1D and 2D NMR data analysis showed that the component II was an eudesmadien-12,8-olide. In particular, in the HMBC (Figure S3A), the correlation networks of H_2-6′/C-7′, C-8′, and C-10′, H_2-13′ (δ_H 4.29, and 4.26)/C-7′, C-11′, and C-12′, CH_3-14′/C-1′ (δ_C 70.8), C-5′, C-9′, and C-10′, and H_2-15′ (δ_H 3.82)/C-3′, C-4′, and C-5′ verified the motif of eudesmadien-12,8-olide and located three hydroxy groups at C-1′, C-13′, and C-15′, respectively. Finally, components I and II were assembled by the key HMBCs of H_2-15/C-4 and C-3 and H-9′/C-4, C-6, and C-8′ as well as the $^1H-^1H$ COSY correlation (Figure S3A) of H_2-15/H-9′. In the ROESY spectrum (Figure S3B), the correlations of H-1/H-2α, H-3/H-2α, H-1/H-9, H-9/H-5′, H-5′/H-1′, and H-1′/H-2′α arbitrarily assigned those protons as α-oriented. Subsequently, the cross-peaks of H-2β/CH_3-14, CH_3-14/H-6, H-2′β/CH_3-14′, CH_3-14′/H-9′, and CH_3-14′/H-6′β showed they were in a β-configuration. The $\Delta^{7(11)}$ double bond was assigned a *Z*-geometry by comparing the corresponding NMR data with those of henriols C and D.[8b]

The absolute configurations of compounds **1**−**3** were established by electric circular dichroism (ECD) analysis[11] and biosynthetic consideration.[12] For example, in the ECD spectrum of **1** (Figure 4A), the first positive Cotton effect (Δε +21.7 at λ_{max} 256 nm) and the second negative Cotton effect (Δε −46.5 at λ_{max} 221 nm) were observed, indicating a positive chirality for **1**, which was corresponding with the clockwise transition dipole arrangement of two chromophores (Figure 4B).[4c] The absolute configuration of **1** was thus established as depicted. The tendency of ECD curves of compounds **2** and **3** resembled that of **1** in the range of 210−260 nm, where the first positive Cotton effects (Δε +7.3 at λ_{max} 253 nm for **2**; Δε +3.0 at λ_{max} 253 nm for**3**) and the second negative Cotton effects (Δε −37.1 at λ_{max} 217 nm for **2**; Δε −7.1 at λ_{max} 210 nm for **3**) (Figure S4) were detected, which allowed the establishment of the absolute configurations of **2** and **3** as shown. The absolute configurations assigned for compounds **1**−**3** are consistent with the biosynthesis consideration for this compound class.[12]

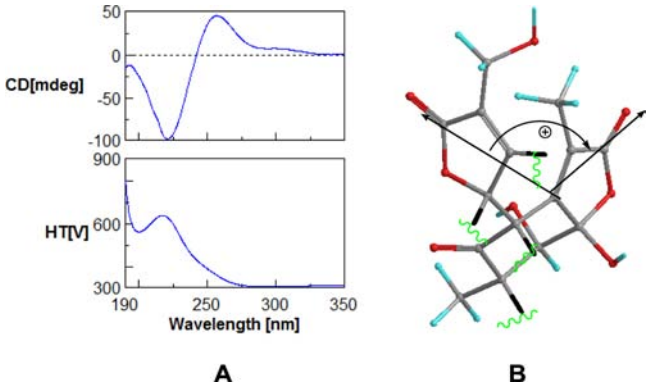
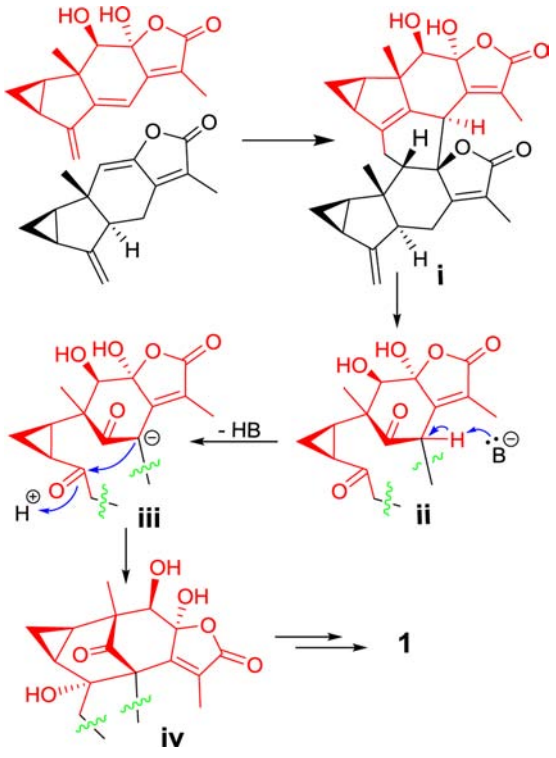


Figure 4. CD spectrum of **1** (in MeOH). The stereoview of **1**: arrows denote the electric transition dipole of the chromophores.

A possible biosynthetic pathway for **1** was proposed (Scheme 1). An intermolecular Diels−Alder cycloaddition of two lindenane sesquiterpenoids likely catalyzed by an unidentified [4 + 2] cyclase[13] would give a dimeric intermediate **i**. The oxidative cleavage of the Δ^4 double bond of **i** would produce a diketo intermediate **ii**, which was then transformed into a key intermediate **iv** (via a carbanion **iii**) by a nucleophilic addition

Scheme 1. Hypothetical Biosynthetic Pathway of 1



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to furnish the bicyclo[3.3.1]nonane core in the component **I** (red). The intermediate **iv** was finally converted to fortunoid A (**1**) by a cascade of oxidation and acylation. Similarly, for compounds **2** and **3**, an intermolecular DA cycloaddition of a lindenane and a eudesmane sesquiterpene would construct the heterodimeric cores as the key step, which were finally transformed into compounds **2** and **3**, respectively, by a series of biosynthetic modifications (Schemes S1 and S2).

Compounds **1**−**3** were tested for antimalarial activities against *P. falciparum* strain Dd2 (chloroquine-resistant) using an SYBR-Green assay with artemisinin as the positive control (IC_{50} 4.0 ± 4.2 nM) as described previously.[14] Compounds **1** and **2** showed antimalarial activities with IC_{50} values of 10.2 ± 0.37 and 0.5 ± 0.01 μM, respectively, while compound **3** was inactive.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00066.

Experimental section, selected ROESY correlations of **2**, and selected HMBC, ^{1}H−^{1}H COSY and ROESY correlations of **3**; ^{1}H and ^{13}C NMR data; hypothetical biosynthetic pathways and CD spectra for **2** and **3**; and raw spectroscopic data including IR, MS, and NMR spectra for compounds **1**−**3** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Ohwi, J. *Revised and Enlarged Flora of Japan*, new ed.; Shibundo: Tokyo, 1975; pp 461−462.

(2) Chen, Y. Q.; Cheng, D. Z.; Wu, G. F.; Cheng, P. S.; Zhu, P. Z. *Zhongguo Zhiwu Zhi*; Science Press: Beijing, 1982; Vol. *20*, pp 80−96.

(3) C. P. Committee. *Pharmacopoeia of People's Republic of China*, 2005 ed.; Chemical Industry Press, 2005; p 154.

(4) (a) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1995**, *39*, 121−125. (b) Saito, Y.; Ichihara, M.; Okamoto, Y.; Gong, X.; Kuroda, C.; Tori, M. *Tetrahedron* **2014**, *70*, 2621−2628. (c) Yang, S. P.; Gao, Z. B.; Wang, F. D.; Liao, S. G.; Chen, H. D.; Zhang, C. R.; Hu, G. Y.; Yue, J. M. *Org. Lett.* **2007**, *9*, 903−906.

(5) (a) Cao, C. M.; Peng, Y.; Xu, L. J.; Wang, Y. J.; Yang, J. S.; Xiao, P. G. *Chem. Pharm. Bull.* **2009**, *57*, 743−746. (b) Xu, Y. J.; Tang, C. P.; Ke, C. Q.; Zhang, J. B.; Weiss, H. C.; Gesing, E. R.; Ye, Y. *J. Nat. Prod.* **2007**, *70*, 1987−1990. (c) Yang, S. P.; Gao, Z. B.; Wu, Y.; Hu, G. Y.; Yue, J. M. *Tetrahedron* **2008**, *64*, 2027−2034. (d) Zhou, B.; Wu, Y.; Dalal, S.; Merino, E. F.; Liu, Q. F.; Xu, C. H.; Yuan, T.; Ding, J.; Kingston, D. G. I.; Cassera, M. B.; Yue, J. M. *J. Nat. Prod.* **2016**, DOI: 10.1021/acs.jnatprod.6b00744.

(6) (a) Wang, X. C.; Qun, W. W.; Han, L. J.; Hong, H. L. *Zhongguo Tianran Yaowu* **2008**, *6*, 404−407. (b) Wang, X. C.; Wang, L. L.; Ouyang, X. W.; Ma, S. P.; Liu, J. H.; Hu, L. H. *Helv. Chim. Acta* **2009**, *92*, 313−320. (c) Wang, X. C.; Zhang, Y. N.; Wang, L. L.; Ma, S. P.; Liu, J. H.; Hu, L. H. *J. Nat. Prod.* **2008**, *71*, 674−677.

(7) (a) Yuan, T.; Zhang, C. R.; Yang, S. P.; Yin, S.; Wu, W. B.; Dong, L.; Yue, J. M. *J. Nat. Prod.* **2008**, *71*, 2021−2025. (b) Yuan, T.; Zhu, R. X.; Yang, S. P.; Zhang, H.; Zhang, C. R.; Yue, J. M. *Org. Lett.* **2012**, *14*, 3198−3201. (c) Zhang, S.; Yang, S. P.; Yuan, T.; Lin, B. D.; Wu, Y.; Yue, J. M. *Tetrahedron Lett.* **2010**, *51*, 764−766.

(8) (a) Ran, X. H.; Chen, C. X.; Wei, G.; Hao, X. J.; Liu, H. Y. *J. Nat. Prod.* **2010**, *73*, 972−975. (b) Li, C. J.; Zhang, D. M.; Luo, Y. M.; Yu, S. S.; Li, Y.; Lu, Y. *Phytochemistry* **2008**, *69*, 2867−2874.

(9) Demarco, P. V.; Farkas, E.; Doddrell, D.; Mylari, B. L.; Wenkert, E. *J. Am. Chem. Soc.* **1968**, *90*, 5480−5486.

(10) Ni, G.; Zhang, H.; Liu, H. C.; Yang, S. P.; Geng, M. Y.; Yue, J. M. *Tetrahedron* **2013**, *69*, 564−569.

(11) (a) Harada, N.; Nakanishi, K.; Tatsuoka, S. *J. Am. Chem. Soc.* **1969**, *91*, 5896−5898. (b) Gawronski, J. K.; VanOeveren, A.; VanderDeen, H.; Leung, C. W.; Feringa, B. L. *J. Org. Chem.* **1996**, *61*, 1513−1515. (c) Ying, B. *Chin. J. Org. Chem.* **1987**, *3*, 165−173.

(12) Kawabata, J.; Mizutani, J. *Phytochemistry* **1992**, *31*, 1293−1296.

(13) Jeon, B. S.; Wang, S. A.; Ruszczycky, M. W.; Liu, H. W. *Chem. Rev.* **2016**, DOI: 10.1021/acs.chemrev.6b00578.

(14) (a) Liu, J.; He, X. F.; Wang, G. H.; Merino, E. F.; Yang, S. P.; Zhu, R. X.; Gan, L. S.; Zhang, H.; Cassera, M. B.; Wang, H. Y.; Kingston, D. G. I.; Yue, J.-M. *J. Org. Chem.* **2014**, *79*, 599−607. (b) Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 1803−1806.

DOI: 10.1021/acs.orglett.7b00066
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