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Hyperjapones A–E, Terpenoid Polymethylated Acylphloroglucinols from *Hypericum japonicum*

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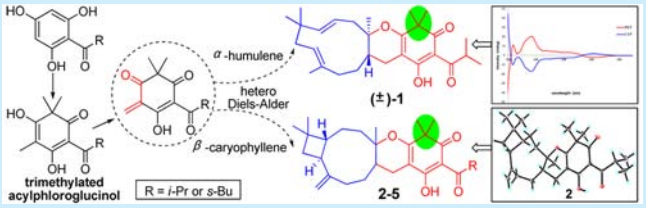
Supporting Information

ABSTRACT: Hyperjapones A–E (**1**–**5**), novel terpenoid polymethylated acylphloroglucinols (TPAPs) with unusual architectures, were characterized from *Hypericum japonicum*. Their structures and absolute configurations were determined by comprehensive spectroscopic data and X-ray diffractions. Compound **1** was obtained as a racemic mixture and was separated by a column coated with cellulose tris(4-methylbenzoate) after attempts with various chiral materials. Compounds **1**, **2**, and **4** exhibited moderate antitumor activities *in vitro*.



Our team has long been committed to the investigation of polycyclic polyprenylated acylphloroglucinols (PPAP),[1] owing to their fascinating chemical structures and intriguing biological activities.[2] To date, this special class of complex natural products has been isolated only from the plants of family Guttiferae (mainly from the genera *Hypericum* and *Garcinia*). Previous phytochemical studies have led to the isolation of a series of PPAPs with diverse structures, of which the majority are bicyclic polyprenylated acylphloroglucinols featuring a bicyclo[3.3.1]nonane-2,4,9-trione core and adamantane-type PPAPs with tricyclo[3.3.1.1]decane or tricyclo[4.3.1.1]undecane cores.[1,3]

However, no such types of metabolites were found in our recent search for acylphloroglucinol derivatives from *Hypericum japonicum* Thunb., a traditional Chinese medicinal plant used for the treatment of hepatitis and "dampness-heat" disease.[4] Instead, another type of acylphloroglucinols derivatives, terpenoid polymethylated acylphloroglucinols (TPAPs) including hyperjapones A–E (**1**–**5**, Figure 1) possessing unusual carbon skeletons, were characterized. Biogenetically, these compounds could be derived by condensation of a trimethylated acylphloroglucinol core and a sesquiterpenoid unit rather than decoration of acylphloroglucinol with several prenyl units for the PPAPs.[2a] Although meroterpenoids (such as psidial A) with a similar hybrid pattern have been previously isolated from the plants of family Myrtaceae and several fungi,[5] the highly functionized acylphloroglucinol cores of TPAPs are distinct from those of the reported meroterpenoids. Dimethylation of C-5 breaks up the aromatic feature of the phloroglucinol in TPAPs, accompanied by the formation of an enol-β-triketone system. Furthermore, it is the first time the hybridization of a sesquiterpenoid unit with a trimethylated acylphloroglucinol in *Hypericum* species has been reported, which expands plant resources for diverse meroterpenoids. Compound **1**, possessing a 11/6/6 fused ring system, was obtained as a racemic mixture and was separated by a column coated with cellulose tris(4-methylbenzoate) after attempts with various chiral materials. Compounds **2**–**5**, containing a caryophyllane-type sesquiterpenoid moiety in their molecules, were two pairs of diastereoisomers. In the bioassay, compounds **1**, **2**, and **4** exhibited moderate cytotoxic activities *in vitro*, and compound **4** was found to inhibit Hsp90.

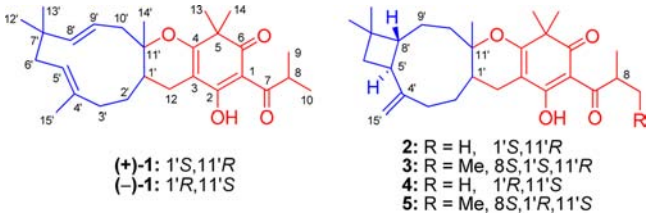


Figure 1. Structures of compounds **1**–**5**.

Hyperjapone A (**1**) was obtained as colorless block crystals. Its molecular formula $C_{28}H_{40}O_4$ was established by its ^{13}C NMR and HRESIMS data (m/z 441.2999, $[M + H]^+$). The UV (242, 293, and 320 nm) and IR (3435, 1660, and 1627 cm^{-1}) spectra indicated the presence of an enolic 1,3-diketone system.[6] The unusual downfield active proton at δ_H 19.17 (OH-2) in the ^1H NMR spectrum recorded in $CDCl_3$ (Figure S7, Supporting Information), together with a shielded olefinic carbon at δ_C 104.9 (C-1) and three carbonyls at δ_C 189.3 (C-2), 196.6 (C-6), and 207.8 (C-7) in the ^{13}C NMR spectrum (Table 1), suggested the presence of an enol-β-triketone

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Table 1. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR Spectroscopic Data for 1 and 2 in Acetone-d_6

	1		2	
no.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	104.9, C		104.9, C	
2	189.3, C		189.6, C	
3	102.9, C		102.7, C	
4	173.7, C		173.5, C	
5	48.8, C		48.9, C	
6	196.6, C		196.6, C	
7	207.8, C		207.8, C	
8	35.7, CH	3.96, sept (6.8)	35.7, CH	3.95, sept (6.8)
9	19.2, CH_3	1.08, d (6.8)	19.2, CH_3	1.08, d (6.8)
10	19.3, CH_3	1.09, d (6.8)	19.3, CH_3	1.09, d (6.8)
12	22.3, CH_2	2.77, brd (11.8)	25.2, CH_2	2.36, dd (16.5, 5.0)
		1.82, m		1.91, m
13	24.3, CH_3	1.34, s	25.3, CH_3	1.26, s
14	25.1, CH_3	1.28, s	24.2, CH_3	1.31, s
1′	35.5, CH	1.83, overlap	34.5, CH	2.05, m
2′	30.2, CH_2	1.41, brt (12.8)	33.7, CH_2	1.77, m
		1.20, m		1.57, m
3′	38.2, CH_2	2.10, dd (12.8, 7.6)	35.8, CH_2	2.47, m
		1.89, t (12.8)		2.18, m
4′	137.3, C		152.8, C	
5′	123.7, CH	5.10, brd (12.4)	42.7, CH	2.49, m
6′	42.1, CH_2	2.23, t (12.4)	36.9, CH_2	1.71, t (10.5)
		1.74, dd (12.4, 4.1)		1.59, dd (10.5, 7.7)
7′	38.7, C		34.1, C	
8′	143.7, CH	5.22, d (15.8)	53.8, CH	1.94, m
9′	120.6, CH	5.03, dd (15.8, 10.8)	23.3, CH_2	1.78, overlap
				1.47, m
10′	42.5, CH_2	2.55, brd (14.3)	37.8, CH_2	2.23, m
		2.45, dd (14.3, 11.2)		1.94, overlap
11′	85.8, C		85.3, C	
12′	24.1, CH_3	1.02, s	22.3, CH_3	0.99, s
13′	30.3, CH_3	1.03, s	30.3, CH_3	0.96, s
14′	20.2, CH_3	1.15, s	21.1, CH_3	1.19, s
15′	17.2, CH_3	1.64, s	110.6, CH_2	4.90, brs
				4.89, brs

system.[6] This deduction was further evidenced by the correlations of OH-2 with C-1, C-2, and C-7 in the HMBC spectrum, which also indicated the formation of a *pseudo* six-membered heterocyclic ring due to strong intramolecular hydrogen bonding between the active hydrogen and O-7 (Figure 2).[7]

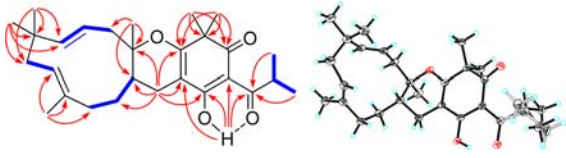

Figure 2. HMBC and $^{1}H-^{1}H$ COSY correlations, and X-ray crystallographic structure of **1**.

In the HMBC spectrum, the correlations of a gem-dimethyl at δ_H 1.34 (Me-13) and 1.28 (Me-14) with three quaternary carbons at δ_C 48.8 (C-5), 173.7 (C-4), and C-6 suggested the linkage of C-4/C-5/C-6. Furthermore, the correlations from H_2-12 (δ_H 2.77 and 1.82) to δ_C 102.9 (C-3), C-2, and C-4 indicated the connection of C-2/C-3/C-4. An isopropyl linked to C-7 was deduced by the correlations of both δ_H 1.08 (Me-9) and 1.09 (Me-10) with δ_C 35.7 (C-8) and C-7 (Figure 2). These fragments, combined with the established enol-β-triketone system, constructed a trimethylated acylphloroglucinol moiety of **1** (the red part in Figure 1).

Besides the aforementioned 13 carbon signals in the ^{13}C and DEPT NMR spectra of **1**, the remaining 15 resonances assignable to three quaternary carbons (δ_C 137.3, C-4′; 38.7, C-7′; and 85.8, C-11′), four methines (including three olefinic ones), four methylenes, and four methyls indicated a humulane-type sesquiterpenoid moiety (the blue part in Figure 1). This assumption was further confirmed by the correlations of H-1′/H_2-2′/H_2-3′, H-5′/H_2-6′, and H-8′/H-9′/H_2-10′ in the $^{1}H-^{1}H$ COSY plot, together with the HMBC correlations from both δ_H 1.02 (Me-12′) and δ_H 1.03 (Me-13′) to δ_C 42.1 (C-6′), C-7′, and 143.7 (C-8′); from δ_H 1.15 (Me-14′) to δ_C 35.5 (C-1′), 42.5 (C-10′), and C-11′; and from δ_H 1.64 (Me-15′) to δ_C 38.2 (C-3′), 123.7 (C-5′), and C-4′ (Figure 2).

The linkage of C-12/C-1′ was deduced by the correlations from H_2-12 to C-1′, C-2′ (δ_C 30.2), and C-11′, which combined the acylphloroglucinol and sesquiterpenoid moieties. The formation of the dihydropyran ring was indicated by the indices of hydrogen deficiency along with the downfield chemical shifts of C-4 (δ_C 173.7) and C-11′ (δ_C 85.8).

In the ROESY spectrum, the cross peak between Me-14′ and H-2′ and between H-1′ and H_2-10′ suggested that the sesquiterpenoid moiety was *trans*-fused with the dihydropyran ring. Furthermore, the correlation between Me-15′ and δ_H 2.23 (H-6′), in combination with the coupling constant of H-9′ (d, J = 15.8), defined the *E*-configuration of C-4′/C-5′ and C-8′/C-9′ double bonds. Interestingly, the crystal structure of **1** (CCDC 1430903) was demonstrated to be racemic by the space group $P2_1/n$ and the absence of any CD maximum. After attempts of various chiral materials (Table S3, Supporting Information), a CHIRALCEL OJ-RH column (cellulose tris(4-methylbenzoate) coated on 5 μm silica gel) was suitable to separate this pair of strongly lipophilic enantiomers. The subsequent chiral HPLC resolution of **1** afforded the anticipated enantiomers (+)-**1** and (−)-**1**, whose CD curves were completely reversed (Figure 3). Finally, the generally matched CD curves of (+)-**1** with **2** and **3** and of (−)-**1** with **4**

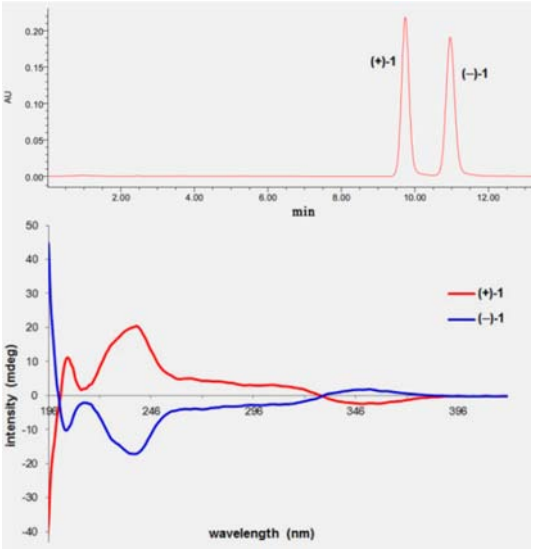

Figure 3. Chiral HPLC chromatogram of (±)-**1** and their CD spectra.

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and **5** (Figures 3 and 6) assigned 1′*S*,11′*R* for (+)-**1** and 1′*R*,11′*S* for (−)-**1**, despite the fact that the CD profile of **2**−**5** was slightly affected by the newly formed stereogenic centers (C-5′ and C-8′) far away from the phloroglucinol chromophore.

Hyperjapone B (**2**) was obtained as colorless crystals with a molecular formula $C_{28}H_{40}O_4$, as accommodated collectively by its HRESIMS and NMR spectral data. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were very similar to those of hyperjapone A. However, one methyl (δ_C 17.2, Me-15′) and three olefinic methines (δ_C 120.6, C-9′; 123.7, C-5′; and 143.7, C-8′) in **1** were replaced by two upfield methines at δ_C 42.7 and 53.8, one methylene at δ_C 23.3, and one terminal olefinic carbon at δ_C 110.6 in **2**, which implied further cyclization between C-5′ and C-8′ to form a caryophyllane-type sesquiterpenoid moiety in **2**. This deduction was subsequently confirmed by the correlations of δ_H 4.90, 4.89 (2H, H-15′) with δ_C 35.8 (C-3′) and 42.7 (C-5′) in the HMBC spectrum, coupled with the proton spin coupling system H_2-6′/H-5′/H-8′/H_2-9′. The remaining partial structure of **2** was determined to be the same as that of **1** by detailed analysis of 2D NMR spectroscopic data (Figure 4).

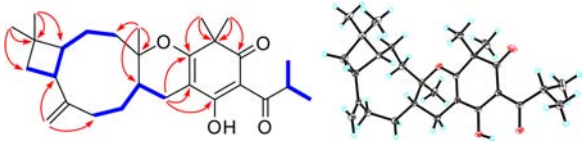

Figure 4. HMBC and ^{1}H−^{1}H COSY correlations, and X-ray crystallographic structure of **2**.

The overlapped signals in the upfield ^{1}H NMR spectrum precluded the definition of the relative configuration of **2**. Therefore, the final refinement on the Cu Kα data of the crystal of **2** (CCDC 1430904) [the Flack parameter is 0.15(16), and the Hooft parameter is 0.09(7) for 1741 Bijvoet pairs] allowed an unambiguous assignment of the absolute configuration as 1′*S*,5′*S*,8′*R*,11′*R* (Figure 4).[8]

Hyperjapone C (**3**) was assigned the molecular formula $C_{29}H_{42}O_4$ from its ^{13}C NMR (Table S1, Supporting Information) and HRESIMS data (*m*/*z* 455.3158, [M + H]$^+$), 14 mass units more than that of **2**. Detailed analysis of their 1D and 2D NMR data suggested that the isopropyl group in **2** was replaced by a *sec*-butyl group in **3**, as evidenced by the HMBC correlations from Me-9 (δ_H 1.08) to C-7 (δ_C 207.1) and C-8 (δ_C 42.1) and from Me-11 (δ_H 0.86) to C-8 and C-10 (δ_C 27.3). The absolute configuration of **3** (CCDC 1430905) was also determined as 8*S*,1′*S*,5′*S*,8′*R*,11′*R* by a single-crystal X-ray diffraction study (Figure 5).

Hyperjapones D (**4**) and E (**5**) shared the same planar structures as **2** and **3**, respectively, as deduced by the detailed analysis of their HRESIMS and NMR spectroscopic data. However, the experimental CD curves of **4** and **5** were almost

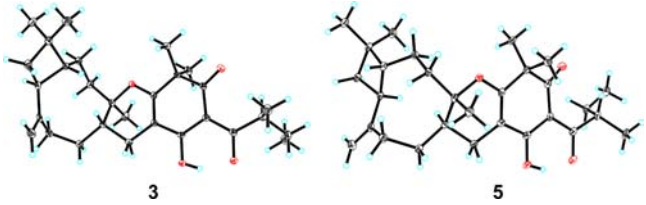

Figure 5. X-ray crystallographic structures of **3** and **5**.

the reverse of those for **2** and **3** (Figure 6), implying the chiral differences near the chromophore of these compounds. Finally,

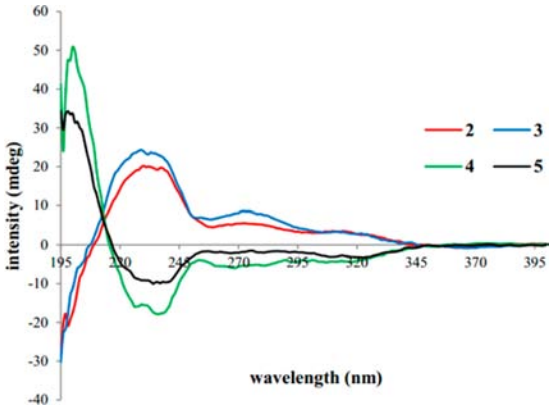

Figure 6. Experimental CD spectra of **2**−**5**.

the X-ray diffraction experiment of **5** (CCDC 1430906) confirmed the absolute configuration of 1′*R*,11′*S* for **5**, which were opposite to those of **2** and **3**. Furthermore, the well matched CD curves of **4** and **5** defined 1′*R*,11′*S* for **5**.

Biosynthetically, compounds **1**−**5** could be derived from a "mixed" biosynthetic pathway (Scheme 1). Methylation of the acylphloroglucinol core affords trimethylated acylphloroglucinols.[2b,9] Then, dehydrogenation of the intermediates may form an α,β-unsaturated ketone moiety, which may further cyclize with α-humulene or β-caryophyllene to form (±)-**1** and **2**−**5**,[10] respectively, by a key hetero-Diels−Alder machanism.[11] For the normal PPAPs from other *Hypericum* species (Scheme 1), prenylation of the acylphloroglucinols affords monocyclic polyprenylated acylphloroglucinols (MPAP), which may be further cyclized to PPAP type metabolites with diverse carbon skeletons.[2a,3] It is an interesting phenomenon that compounds **1**−**5** undergo a different biogenetic pathway from those of PPAPs obtained from other *Hypericum* species, which deserve further study.

The inhibitory activities of the isolates against HSP90 and the six human tumor cell lines AGS, Hela, HepG2, HCT116, MDA-MB-468, and PANC-1 were examined. Compound **1** exhibited moderate cytotoxic activity against Hela and HepG2 with IC_{50} values of 7.9 and 13.2 μM, respectively, while compounds **2** and **4** showed activity against AGS (IC_{50} 14.8 and 12.3 μM). In addition, compound **4** was found to inhibit Hsp90 with an IC_{50} value of 21.3 μM.

In conclusion, five TPAPs were characterized in this study to possess two unusual carbon skeletons. Their architectures were totally different from the normal PPAP type metabolites from *Hypericum* plants as well as the filicinic acid derivatives from this plant.[6] Although different kinds of hybrid natural products have been reported,[2b,5] it is the first report of these TPAP type metabolites from *Hypericum* species. In addition, we had predicted that the existence of abundant double bonds and carbonyl groups in the structures allowed more acylphloroglucinol derivatives with novel scaffolds via [4 + 2] cycloadditions in *Hypericum* plants.[1e] The characterization of **1**−**5** can be seen as another example of complex acylphloroglucinol derivatives resulting from such a cycloaddition, which further confirmed our prediction. Our finding also presents challenging natural products for organic synthesis and also

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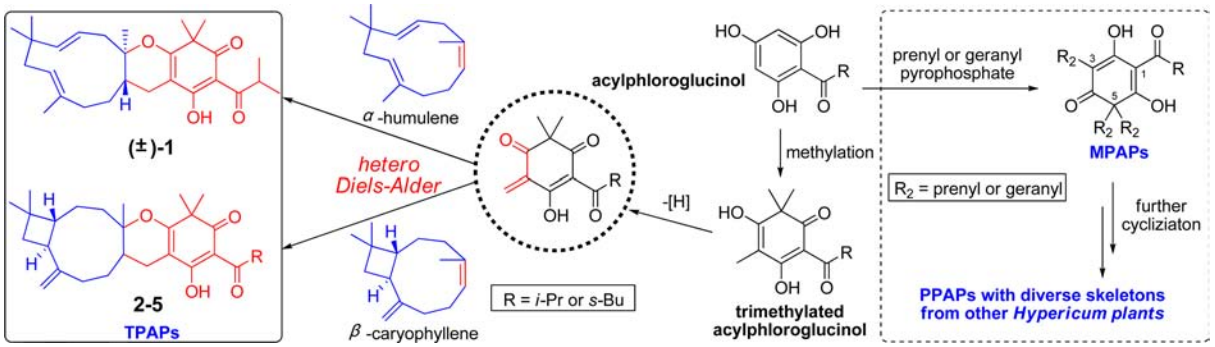
Scheme 1. Putative Biosynthetic Pathways to 1−5 from *H. japonicum* and PPAPs from Other *Hypericum* Plants



might provide a clue for the separation of extremely lipophilic racemic compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00650.

Details of isolation and biological experimental procedures, original MS and NMR spectra (PDF)
Crystallographic data for **1** (CIF)
Crystallographic data for **2** (CIF)
Crystallographic data for **3** (CIF)
Crystallographic data for **5** (CIF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Xu, G.; Kan, W. L. T.; Zhou, Y.; Song, J. Z.; Han, Q. B.; Qiao, C. F.; Cho, C. H.; Rudd, J. A.; Lin, G.; Xu, H. X. *J. Nat. Prod.* **2010**, *73*, 104−108. (b) Yang, X. W.; Deng, X.; Liu, X.; Wu, C. Y.; Li, X. N.; Wu, B.; Luo, H. R.; Li, Y.; Xu, H. X.; Zhao, Q. S.; Xu, G. *Chem. Commun.* **2012**, *48*, 5998−6000. (c) Yang, X. W.; Ding, Y.; Zhang, J. J.; Liu, X.; Yang, L. X.; Li, X. N.; Ferreira, D.; Walker, L. A.; Xu, G. *Org. Lett.* **2014**, *16*, 2434−2437. (d) Zhang, J. J.; Yang, J.; Liao, Y.; Yang, X. W.; Ma, J. Z.; Xiao, Q. L.; Yang, L. X.; Xu, G. *Org. Lett.* **2014**, *16*, 4912−4915. (e) Liao, Y.; Liu, X.; Yang, J.; Lao, Y. Z.; Yang, X. W.; Li, X. N.; Zhang, J. J.; Ding, Z. J.; Xu, H. X.; Xu, G. *Org. Lett.* **2015**, *17*, 1172−1175.

(2) (a) Ciochina, R.; Grossman, R. B. *Chem. Rev.* **2006**, *106*, 3963−3986. (b) Singh, I. P.; Bharate, S. B. *Nat. Prod. Rep.* **2006**, *23*, 558−591. (c) Shimizu, Y.; Shi, S.-L.; Usuda, H.; Kanai, M.; Shibasaki, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 1103−1106. (d) Biber, N.; Mows, K.; Plietker, B. *Nat. Chem.* **2011**, *3*, 938−942. (e) Richard, J.-A.; Pouwer, R. H.; Chen, D. Y.-K. *Angew. Chem., Int. Ed.* **2012**, *51*, 4536−4561. (f) Sparling, B. A.; Moebius, D. C.; Shair, M. D. *J. Am. Chem. Soc.* **2013**, *135*, 644−647. (g) Grenning, A. J.; Boyce, J. H.; Porco, J. A., Jr. *J. Am. Chem. Soc.* **2014**, *136*, 11799−11804.

(3) Yang, X. W.; Li, M. M.; Liu, X.; Ferreira, D.; Ding, Y.; Zhang, J. J.; Liao, Y.; Qin, H. B.; Xu, G. *J. Nat. Prod.* **2015**, *78*, 885−895.

(4) Li, Y. H. In *Flora of China*; Wu, Z. Y., Ed.; Science Press: Beijing, 1990; Vol. *50*, pp 47−48.

(5) (a) Yang, X. L.; Hsieh, K. L.; Liu, J. K. *Org. Lett.* **2007**, *9*, 5135−5138. (b) Lawrence, A. L.; Adlington, R. M.; Baldwin, J. E.; Lee, V.; Kershaw, J. A.; Thompson, A. L. *Org. Lett.* **2010**, *12*, 1676−1679. (c) Spence, J. T. J.; George, J. H. *Org. Lett.* **2011**, *13*, 5318−5321. (d) 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.

(6) (a) Ishiguro, K.; Yamaki, M.; Kashihara, M.; Takagi, S.; Isoi, K. *Planta Med.* **1990**, *56*, 274−276. (b) Ishiguro, K.; Nagata, S.; Fukumoto, H.; Yamaki, M.; Isoi, K. *Phytochemistry* **1994**, *35*, 469−471.

(7) Yu, Q.; Ravu, R. R.; Xu, Q. M.; Ganji, S.; Jacob, M. R.; Khan, S. I.; Yu, B. Y.; Li, X. C. *J. Nat. Prod.* **2015**, *78*, 2748−2753.

(8) (a) Flack, H. D. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *39*, 876−881. (b) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2008**, *41*, 96−103.

(9) Hu, L. H.; Khoo, C.-W.; Vittal, J. J.; Sim, K.-Y. *Phytochemistry* **2000**, *53*, 705−709.

(10) Although the putative precursors were not isolated, the sesquiterpenoids and acylphloroglucinol derivatives with similar structures have been reported from this plant and other plants of *Hypericum*; see (a) Ishiguro, K.; Yamaki, M.; Kashihara, M.; Takagi, S. 1986, 288−290. (b) Dordevic, A.; Zlatkovic, B.; Lazarevic, J.; Palic, R. *J. Med. Plants Res.* **2011**, *5*, 5486−5492. (c) Verma, R. S.; Padalia, R. C.; Chauhan, A.; Chanotiya, C. S.; Yadav, A. *J. Essent. Oil Res.* **2012**, *24*, 501−505.

(11) Zhao, N.; Ren, X.; Ren, J.; Lü, H.; Ma, S.; Gao, R.; Li, Y.; Xu, S.; Li, L.; Yu, S. *Org. Lett.* **2015**, *17*, 3118−3121.

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