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Leptosperols A and B, Two Cinnamoylphloroglucinol–Sesquiterpenoid Hybrids from *Leptospermum scoparium*: Structural Elucidation and Biomimetic Synthesis

Ji-Hong Gu,[‖] Wen-Jing Wang,[‖] Jun-Zi Chen, Jun-Shan Liu, Ni-Ping Li, Min-Jing Cheng, Li-Jun Hu, Chuang-Chuang Li,* Wen-Cai Ye,* and Lei Wang*

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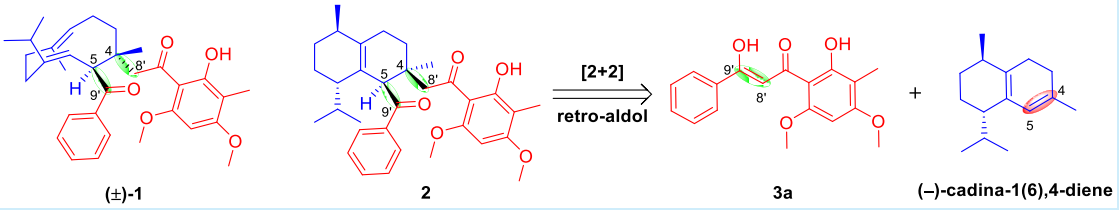

ABSTRACT: Leptosperols A and B (**1** and **2**), two cinnamoylphloroglucinol–sesquiterpenoid hybrids featuring unprecedented 1-benzyl-2-(2-phenylethyl) cyclodecane and 2-benzyl-3-phenylethyl decahydronaphthalene backbones, along with their biosynthetic precursor (**3**), were isolated from *Leptospermum scoparium*. Compounds **1** and **2** represent the first example of phloroglucinol derivatives biogenetically constructed by a De Mayo reaction. The biomimetic synthesis of leptosperol B (**2**) was achieved using the proposed biosynthetic pathway. In addition, compounds **1** and **2** showed significant anti-inflammatory effects in zebrafish acute inflammatory models.

Leptospermum scoparium (Myrtaceae), which is best known as the source of manuka honey, is widely distributed in New Zealand and has been traditionally used as herbal medicine in the treatment of dysentery and urinary and skin disease.[1] Previous phytochemical studies on *L. scoparium* had led to the isolation of some terpenes, *β*-triketones, and phloroglucinol derivatives.[2] In recent years, Myrtaceous phloroglucinols with various backbones and bioactivities have attracted much attention from chemists and pharmacologists.[3] To date, more than 500 Myrtaceous phloroglucinols have been reported. Biogenetically, these natural products are constructed from different building blocks (terpenes, *β*-triketones, and phloroglucinols) via Michael addiction,[3a] Diels–Alder cycloaddition,[4] electrophilic addition,[5] radical reaction,[6] or [3 + 2] cycloaddition.[7]

In our continuing studies on structurally unique and bioactive natural products from Myrtaceae plants,[6−8] two cinnamoylphloroglucinol–sesquiterpenoid hybrids, leptosperols A and B, with unprecedented 1-benzyl-2-(2-phenylethyl) cyclodecane (**1**) and 2-benzyl-3-phenylethyl decahydronaphthalene (**2**) skeletons, along with their biosynthetic precursor (**3**), were isolated from the leaves of *L. scoparium*. Compounds (+)-**1** and (−)-**1** were a pair of enantiomers. Interestingly, these compounds represent the first example of phloroglucinol derivatives biogenetically constructed by a De Mayo reaction ([2 + 2] cycloaddition followed by retro-aldol condensation),[9] resulting in a sesquiterpenoid unit "inserted" into the cinnamoylphloroglucinol moiety with formation of two new bonds and C8′–C9′ bond cleavage (Figure 1). To provide experimental support for the proposed biosynthetic pathway, the biomimetic synthesis of **2** was achieved using a visible light mediated De Mayo reaction approach. The absolute configuration of each compound was determined by X-ray diffraction and chemical calculations. Moreover, compounds **1** and **2** displayed anti-inflammatory effects on zebrafish acute inflammatory models. Herein, the structural elucidation, anti-inflammatory activities, and putative biogenetic pathways of **1** and **2** as well as biomimetic synthesis of **2** are reported.

Compound **1** was isolated as colorless crystals (MeOH). The molecular formula of **1** ($C_{33}H_{42}O_5$) was determined by its HRESIMS data (*m*/*z* 519.3107 $[M + H]^+$, calcd for $C_{33}H_{43}O_5$ 519.3105), indicating the presence of 13 degrees of unsaturation. The UV spectrum of **1** displayed absorption maxima at 239 and 294 nm. The IR spectrum indicated the presence of a conjugated carbonyl group (1677 cm^{-1}) and aromatic ring (1618, 1458 cm^{-1}). The ^1H NMR spectrum of **1** showed signals for a hydrogen-bonded phenolic hydroxyl, a monosubstituted benzene ring, a pentasubstituted benzene

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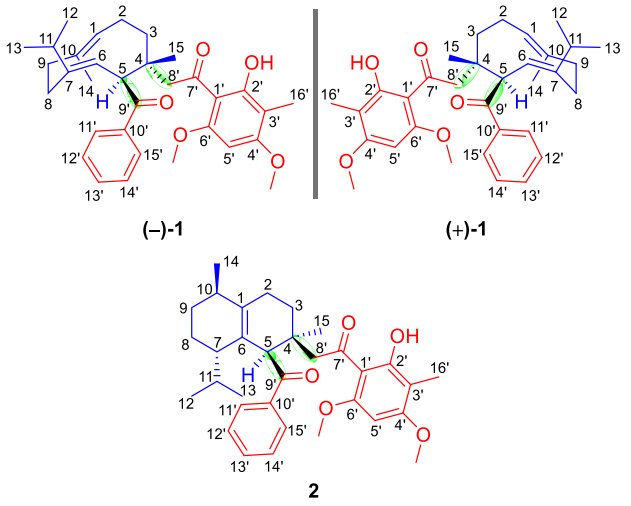

Figure 1. Chemical structures of **1** and **2**.

ring, two olefinic protons, two methoxy groups, and five methyl groups. The ^{13}C NMR and DEPT spectra of **1** displayed 33 carbon signals including those for two ketocarbonyls, two benzene rings, two olefinic bonds, and 15 aliphatic carbons. The aforementioned spectroscopic data suggested that **1** could be a phloroglucinol–sesquiterpenoid adduct.

Further 2D NMR analysis enabled the construction of the structure of **1** (Figure 2). The spin systems from H-1 to H-3,

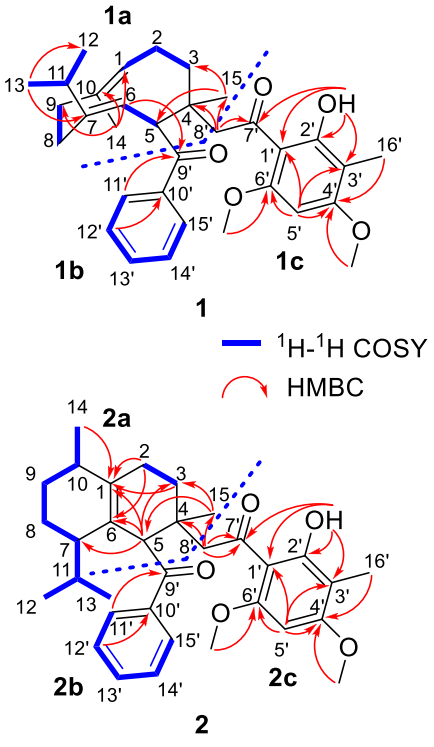

Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1** and **2**.

from H-5 to H-6, from H-8 to H-9, and from H-11 to H-12/H-13 were deduced by their ^{1}H–^{1}H COSY correlations. The HMBC correlations between H-6 and C-8/C-11, between H-13 and C-7/C-11/C-12, between H-14 and C-1/C-9/C-10, and between H-15 and C-3/C-4/C-5 indicated the presence of a germacrene C unit (**1a**).[10] In addition, the ^{1}H–^{1}H COSY correlations from H-11′ to H-15′ and the HMBC correlations between H-6 and C-9′ revealed that a benzoyl unit (**1b**) was substituted at the C-5 position of **1a**. The remaining resonances assignable to three methyls (including two oxygenated ones), six aromatic carbons, and a carbonyl were in good agreement with those of 2-hydroxy-4,6-dimethoxy-3-methylacetophenone,[11] indicating the presence of an acylphloroglucinol moiety (**1c**). Furthermore, the HMBC cross peaks between H-8′ and C-4/C-15/C-7′ indicated that **1a** and **1c** were connected via the remaining unassigned CH_2 unit (CH_2-8′).

The relative stereochemistry of **1** could be elucidated by a NOESY experiment. The NOE correlations between H-1 and H-9 and between H-6 and H-11 indicated that the double bonds (C-6═C-7 and C-1═C-10) were both in *E* configuration. In addition, NOE correlations between H-5 and H-8′ suggested that H-5 and CH_2-8′ were cofacial, which was further confirmed by a single-crystal X-ray diffraction experiment (Figure 3).

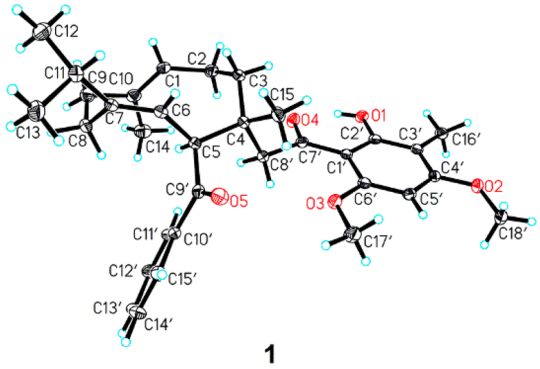

Figure 3. X-ray ORTEP drawing of **1**.

Although there were two chiral centers in **1**, the space group $P\bar{1}$ of the X-ray data and the lack of optical activity suggested that **1** could be a racemate. Separation of **1** by chiral HPLC yielded a pair of enantiomers with a ratio of 1:1 (see the Supporting Information). The absolute configuration of each enantiomer was determined by electronic circular dichroism (ECD) calculation using the time-dependent DFT method. The experimental ECD spectrum of (+)-**1** displayed positive Cotton effects at 216 and 297 nm and negative ones at 210 and 251 nm, which were similar to the calculated ECD data for 4*S*,5*R* isomer. In addition, the experimental ECD spectrum of (−)-**1** showed similar Cotton effects as those of 4*R*,5*S* isomer (Figure 4). As a result, the absolute configurations of (+)-**1** and (−)-**1** were determined as shown in Figure 1.

The HRESIMS data of **2** (*m*/*z* 519.3104 $[M + H]^+$, calcd for $C_{33}H_{43}O_5$ 519.3105) indicated that the molecular formula of **2** is the same as **1**. On the basis of the detailed examination of the NMR data, **2** was suggested to have the same benzoyl (**2b**) and acylphloroglucinol (**2c**) moieties as **1**. Interpretation of the ^{1}H–^{1}H COSY spectrum suggested the presence of two isolated spin systems in the remaining part (Figure 2). The HMBC correlations between H-2 and C-1/C-4/C-6, between H-5 and C-1/C-3/C-6/C-7, between H-3/H-14 and C-1, and between H-15 and C-3/C-4/C-5 indicated the presence of a cadina-1,4(6)-diene unit (**2a**) with a 6/6 bicyclic system.[2a] Additionally, the HMBC correlations between H-5 and C-9′ and between H-8′ and C-3/C-5/C-15/C-7′ indicated that **2a** was

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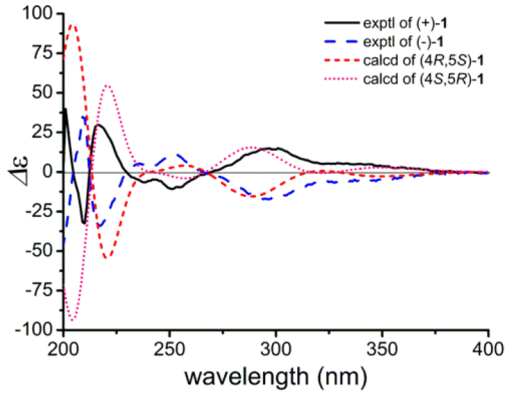


Figure 4. Experimental and calculated ECD spectra of (+)-**1** and (−)-**1**.

connected to **2c** through C-4−C-8′ bond and to **2b** via C-5−C-9′ bond (Figure 2).

In the NOESY spectrum, cross peaks between H_3-15 and H-2α/H-5, between H-5 and H-11/H_3-13, and between H_3-14 and H-2β established β configuration of CH_3-14 and H-7, as well as α configuration of CH_3-15 and H-5 (Figure 5). The

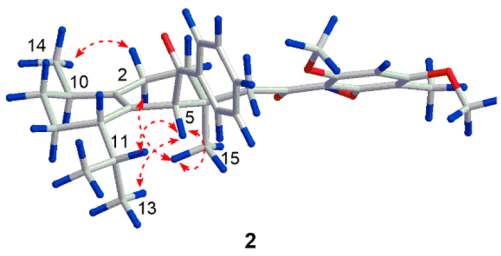


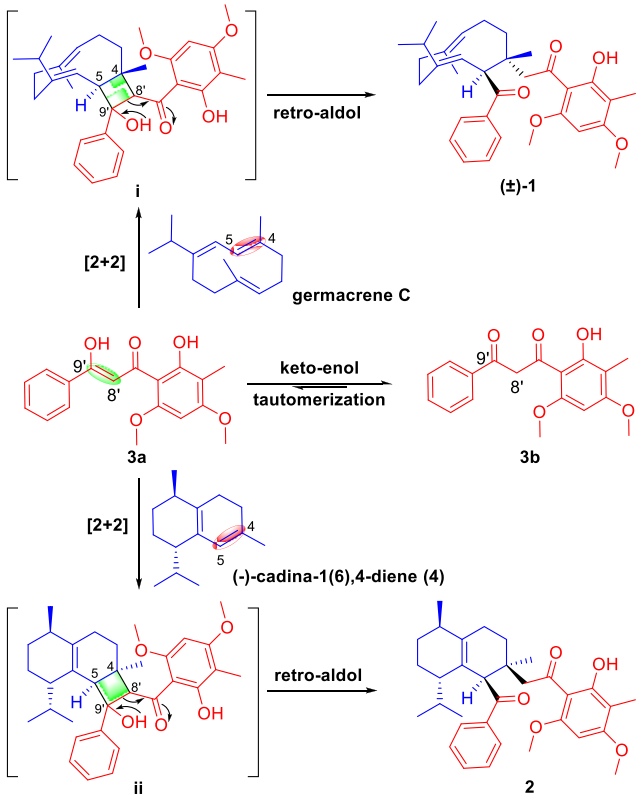
Figure 5. Key NOESY correlations of **2**.

absolute configuration of **2** was finally identified by comparison of the experimental ECD spectrum with the calculated ones. The ECD data of **2** showed positive Cotton effects at 238 and 292 nm, which were consistent with the calculated curves of 4*S*,5*R*,7*S*,10*R*-**2** (Figure S2). Thus, the absolute configuration of **2** was determined.

Based on the terpene precursors that were previously reported in *L. scoparium*,[2a,c] a plausible biosynthetic pathway for compounds **1** and **2** is proposed as shown in Scheme 1. In the present study, 2′,β-dihydroxy-3′-methyl-4′,6′-dimethoxychalcone (**3a**) and its keto tautomer (**3b**) were found to be major components in the leaves of *L. scoparium* (see the Supporting Information). The C8′═C9′ double bond of **3a** could couple with the olefin bond of different sesquiterpenoids [germacrene C or (−)-cadina-1(6),4-diene] via a [2 + 2] photocycloaddition to generate intermediate **i** or **ii** with a β-acylcyclobutanol unit. Subsequently, a retro-aldol reaction of the formed intermediates **i** or **ii** could lead to C8′−C9′ cleavage and produce compounds **1** and **2**. Notably, the intermediates **i** and **ii** are unstable due to the presence of the β-hydroxyketone moiety that could induce a retro-aldol process.[9,12] Thus, **1** and **2** were proposed to be formed by De Mayo reaction.

To confirm that leptosperols A and B are natural products, an UPLC-MS analysis was performed. The ion peaks corresponding to **1** and **2** could be found in the crud extract of the fresh leaves of *L. scoparium*, which indicated the natural

Scheme 1. Plausible Biosynthetic Pathways of 1 and 2



existence of leptosperols A and B (see the Supporting Information).

As an important class of natural products, Myrtaceous phloroglucinols possessed various skeletons and different biosynthetic pathways.[3a,4−7] However, leptosperols A and B represent the first examples of phloroglucinol derivatives that are biogenetically constructed by De Mayo reaction. To validate the proposed biosynthetic pathways and further confirm the structure of **2**, a biomimetic synthesis of **2** was carried out. Although the volatile (−)-cadina-1(6),4-diene (**4**) has been detected in the title plant by GC-MS analysis,[2a] it was hardly able to be isolated directly due to its high volatility. Thus, a synthetic route of **4** from commercially available (−)-menthone was performed (Scheme 2). Formylation of (−)-menthone with LDA and 2,2,2-trifluoroethyl formate,[13] followed by treatment with methyl vinyl ketone (MVK) and Et_3N, gave **6**.[14] The diketone **6** underwent a chemoselective Wittig reaction to provide compound **7** in 76% yield.[15] Subsequently, treatment of **7** with KHMDS and Comins' reagent afforded **8** and its regioisomer (**8a**) in 90% combined yield.[16] Finally, Suzuki coupling of **8** with pinacol vinylboronate using $Pd(PPh_3)_4$ as a catalyst furnished **9**, which underwent the ring closing metathesis reaction using Grubbs' II as catalyst to give the desired sesquiterpenoid (**4**) in 53% overall yield.[15]

With (−)-cadina-1(6),4-diene (**4**) in hand, the synthesis of leptosperol B (**2**) was conducted by De Mayo reaction (Scheme 3). Irradiating the mixture of **3a**, **4**, and the photocatalyst (**10**) in dry DCM using a blue LED light (455 nm) under N_2 at room temperature for 12 h produced **2** in 16% yield.[17] The physicochemical data of synthetic **2** (Supporting Information) were identical to that of leptosperol B (**2**). During the isolation and synthesis process, the

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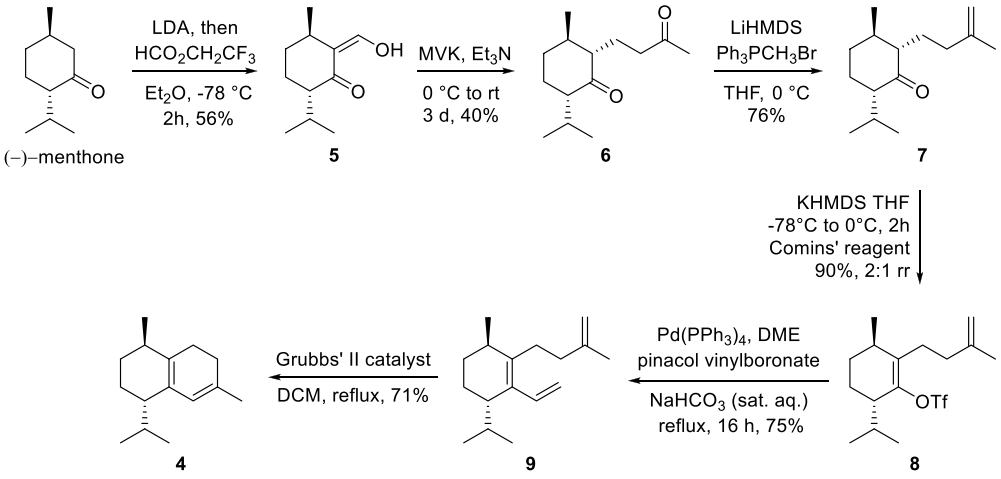
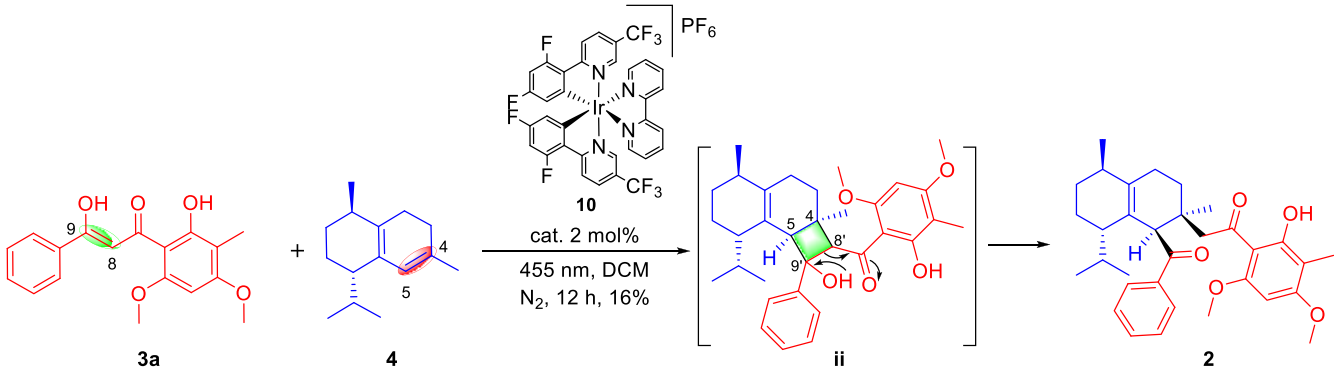
Scheme 2. Synthesis of (−)-Cadina-1(6),4-diene (4)



Scheme 3. Biomimetic Synthesis of 2



regioisomers of **1** and **2** were not isolated. The observed regioselectivity could be explained by the stability of radicals in their intermediate[17] (Supporting Information). Additionally, a synthetic byproduct was identified (Figure S9), but it was not found in the crude extract of the plant. Previous studies revealed that the De Mayo reaction could occur spontaneously under light mediated conditions (UV and visible light).[9,17] However, it remains to be an interesting question whether this step is enzyme- or nonenzyme-catalyzed in the plant.

Compounds **1** and **2** were evaluated for their anti-inflammatory effects *in vivo*. As a result, both **1** and **2** (at 2.5 μM) significantly reduced the neutrophil number (green fluorescence) in inflammatory sites in zebrafish acute inflammatory models, which were induced by $CuSO_4$, tail injury, or LPS (see the Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c00109.

Experimental details, UV, IR, MS, and NMR spectra for compounds **1**−**3**, and ECD calculations for **1**−**2** (PDF)

Accession Codes

CCDC 1963827, 1965781, and 1984690 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

■ AUTHOR INFORMATION

Corresponding Authors

Chuang-Chuang Li − *Department of Chemistry, Shenzhen Grubbs Institute, Southern University of Science and Technology, Shenzhen 518055, P. R. China;* orcid.org/0000-0003-4344-0498; Email: ccli@sustech.edu.cn

Wen-Cai Ye − *Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China;* orcid.org/0000-0002-2810-1001; Email: chywc@aliyun.com

Lei Wang − *Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China;* orcid.org/0000-0001-9242-1109; Email: cpuwanglei@126.com

Authors

Ji-Hong Gu − *Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China*

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Wen-Jing Wang − *Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China*

Jun-Zi Chen − *Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China*

Jun-Shan Liu − *School of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, People's Republic of China*

Ni-Ping Li − *Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China*

Min-Jing Cheng − *Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China; Department of Chemistry, Shenzhen Grubbs Institute, Southern University of Science and Technology, Shenzhen 518055, P. R. China*

Li-Jun Hu − *Institute of Traditional Chinese Medicine & Natural Products and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c00109

Author Contributions

[‖]J.-H.G. and W.-J.W. contributed equally.

Notes

The authors declare no competing financial interest.

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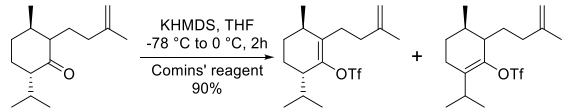

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