








Myrrhalindenane C, a New Eudesmane Sesquiterpenoid From *Lindera Myrrha* Roots

Hoang-Dung Nguyen ^{1,2}, Huy Truong Nguyen ³, Thi-Hoai-Thu Nguyen ⁴,
Jirapast Sichaem ⁵, Huu-Hung Nguyen ^{6*}, Ngoc-Hong Nguyen ^{7*}
and Thuc-Huy Duong ^{8*}

¹Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam

²NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh city, Vietnam

³Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam

⁴Faculty of Basic Sciences, University of Medicine and Pharmacy at Ho Chi Minh City, 217 Hong Bang Street, District 5, Ho Chi Minh City 700000, Vietnam

⁵Research Unit in Natural Products Chemistry and Bioactivities, Faculty of Science and Technology, Thammasat University Lampang Campus, Lampang 52190, Thailand

⁶Faculty of Applied Technology, School of Engineering and Technology, Van Lang University, 45 Nguyen Khac Nhu, District 1, Ho Chi Minh City, Vietnam

⁷CirTech Institute, HUTECH University, 475 A Dien Bien Phu Street, Binh Thanh District, Ho Chi Minh City 700000, Vietnam

⁸Department of Chemistry, Ho Chi Minh City University of Education, Ho Chi Minh City, Vietnam

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Abstract: Phytochemical investigation of *Lindera myrrha* roots growing in Vietnam afforded a new eudesmane sesquiterpenoid, myrrhalindenane C (1), along with seven known compounds, *rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (2), 1-*O*-(4-hydroxy-2,6-dimethoxyphenoxy)-6-*O*-[*rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienyl]-β-D-glucopyranose (3), curcumin (4), demethoxycurcumin (5), bisdemethoxycurcumin (6), (1*E*,6*E*)-1,7-bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (7), and sanjoseolide (8). The structures were determined by analysis of their MS and NMR data as well as by comparison with literature values. All compounds were evaluated for antimicrobial activity against antibiotic-resistant, pathogenic bacteria *Enterococcus faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii*.

Keywords: Lauraceae, *Lindera myrrha*, myrrhalindenane C, eudesmane, antimicrobial activity. © 2022 ACG Publications. All rights reserved.

1. Introduction

The genus *Lindera*, which belongs to the Lauraceae family, has economic applications in eastern Asia and America [1]. Many *Lindera* plants are associated with ethnopharmacological claims, including treatment of urinary system diseases, inflammatory-related health hazards, antispasmodic

* Corresponding authors: E-Mail: huydt@hcmue.edu.vn (T.H. Duong); Phone: 084-919-011884; Fax: 084-919-011884; nn.hong@hutech.edu.vn (N.H. Nguyen); Phone: 084-908-644000; Fax: 084-908-644000

properties, as well as beneficial effects in gastric ulcer, cholera, and beriberi [2, 3]. Previous phytochemical investigation of *Lindera* species reported a wealth of skeletons which include mainly sesquiterpenes (lindenanes, eudesmanes, and germacranes) and aporphine alkaloids [2]. *Lindera myrrha* (Lour.) Merr. is a native species in Vietnam. Chemical data of these Vietnamese plants are scarce. The first phytochemical investigation, conducted in 1994, indicated the isolation of several newly-reported aporphine alkaloids [4]. More recently, lindermyrhrin and myrrhalindenanes A-B were further described from this bio-source [5, 6]. The current report describes the isolation and structural elucidation of one new compound named myrrhalindenane C, together with seven known compounds, *rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-penta-dienoic acid (**2**) [7], 1-*O*-(4-hydroxy-2,6-dimethoxyphenoxy)-6-*O*-[*rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoyl]- β -D-glucopyranose (**3**) [8], curcumin (**4**) [9], demethoxycurcumin (**5**) [9], bisdemethoxycurcumin (**6**) [9], (1*E*,6*E*)-1,7-bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (**7**) [10], and sanjoseolide (**8**) [11] (Figure 1). Additionally, all isolated compounds were evaluated for antimicrobial activity against the antibiotic-resistant, pathogenic bacteria *Enterococcus faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii*.

2. Materials and Methods

2.1. General Experimental Procedures

NMR spectra were recorded on a Bruker Avance III spectrometer (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) with TMS as internal standard. HRESIMS was recorded using a MicroTOF-Q mass spectrometer on an LC-Agilent 1100 LC-MSD Trap spectrometer. Thin-layer chromatography (TLC) was carried out using precoated silica gel 60 F₂₅₄ or 60 RP-18 F_{254S} (Merck). Spots were visualized by spraying with 10% H₂SO₄ solution followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Himedia).

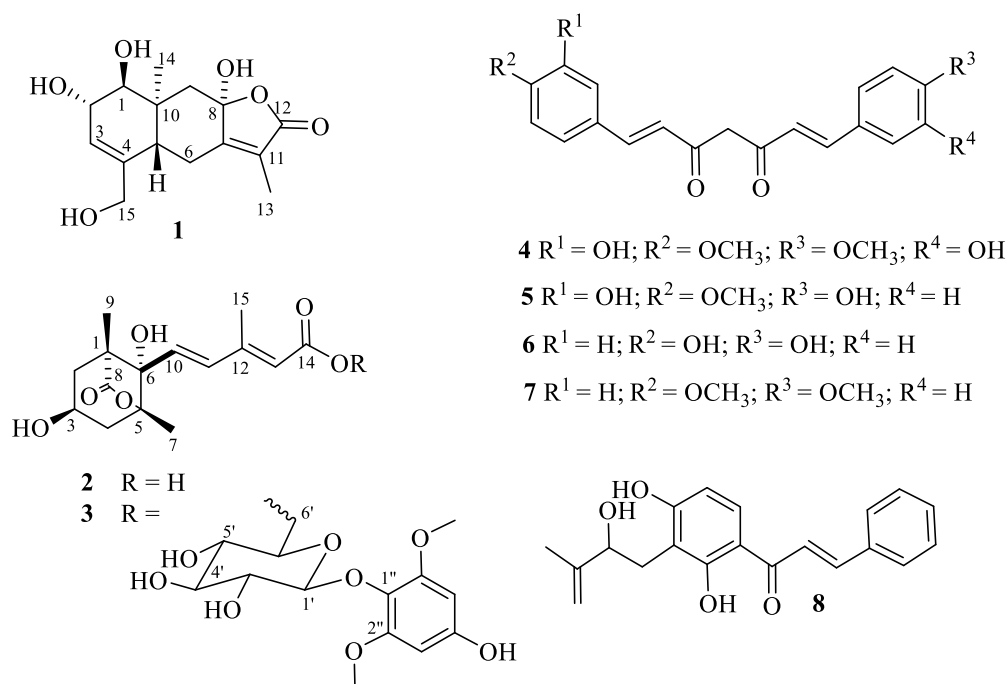
2.2. Plant Material

The roots of *L. myrrha* were collected from Cu Chi District, Ho Chi Minh City, Vietnam, in July, 2019. The botanical sample was authenticated by Dr. Le Van Minh, Research Center of Ginseng and Materia Medica, Ho Chi Minh City, Vietnam. A voucher specimen (No UP-B05) was deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh University of Education and in Institute of Tropical Biology, Vietnam Academy of Science and Technology (VNM-Dung0025).

2.3. Extraction and Isolation

Dried and ground roots of *Lindera myrrha* (5.2 kg) were extracted by maceration with EtOAc (3 \times 10 L) at ambient temperature. The filtrated solution was evaporated to dryness under reduced pressure to obtain a crude extract (145 g). The extract was applied to Sephadex LH-20 gel column chromatography (CC), eluted with MeOH to afford fractions EA1-EA5. Fraction EA5 (23.3 g) was re-chromatographed by Sephadex LH-20 gel CC to afford fractions EA5.1-4. Fraction EA5.4 (6.7 g) was applied to normal phase silica gel CC, and isocratically eluted with a solvent system of *n*-hexane-EtOAc (5:1, v/v) to afford fractions D1-D5. Fraction D5 (677 mg) was applied to a C₁₈ reversed-phase (RP) CC eluted with MeOH/H₂O (5:1, v/v) to yield subfractions D5.1- D5.3. Subsequently, subfraction D5.1 (135 mg) was purified by semi-preparative HPLC to obtain compounds **4** (2.1 mg), **5** (2.5 mg), **6** (2.3 mg), **7** (2.7 mg), and **8** (1.6 mg). Subfraction D5.3 was applied to a C₁₈-RP CC eluted with MeOH/H₂O (3:2, v/v) to obtain compounds **1** (1.9 mg), **2** (4.7 mg), and **3** (1.5 mg).

A new eudesmane sesquiterpenoid

**Figure 1.** Chemical structures of compounds **1-8**

Myrrhalindenane C (1): colorless gum. UV (MeOH), λ_{\max} (log ϵ) 207 (4.58) nm; IR ν_{\max} (neat): 3480, 1709, 1701, 1260, 1101 cm^{-1} . ^1H NMR (500 MHz, DMSO- d_6 , δ ppm, J/Hz): 7.12 (1H, s, 8-OH), 5.57 (1H, brs, H-3), 4.85 (1H, d, $J = 4.0$ Hz, 1-OH), 4.80 (1H, d, $J = 4.5$ Hz, 2-OH), 4.75 (1H, t, $J = 5.5$ Hz, 15-OH), 4.01 (1H, dd, $J = 13.5, 5.5$ Hz, H-15a), 3.90 (1H, dd, $J = 13.5, 5.5$ Hz, H-15b), 3.84 (1H, m, H-2), 3.21 (1H, d, $J = 4.0$ Hz, H-1), 2.96 (1H, d, $J = 10.0$ Hz, H-6a), 2.21 (1H, d, $J = 13.5$ Hz, H-5), 2.18 (1H, dd, $J = 10.0, 13.5$ Hz, H-6b), 1.88 (1H, d, $J = 13.5$ Hz, H-9a), 1.86 (1H, d, $J = 13.5$ Hz, H-9b), 1.73 (3H, s, H-13), 1.03 (3H, s, H-14). ^{13}C -NMR (125 MHz, DMSO- d_6 , δ ppm): 7.9 (C-13), 16.1 (C-14), 22.3 (C-6), 36.8 (C-10), 39.9 (C-5), 45.4 (C-9), 62.8 (C-15), 70.4 (C-2), 76.6 (C-1), 104.6 (C-8), 120.4 (C-11), 122.9 (C-3), 137.7 (C-4), 161.6 (C-7), 171.8 (C-12). HR-ESI-MS m/z 295.1191 $[\text{M}-\text{H}]^-$ (calcd. for $\text{C}_{15}\text{H}_{19}\text{O}_6$, 295.1182).

2.4. Semi-Preparative HPLC Method

The selected fraction was applied to preparative HPLC LC-8A Shimadzu, equipped with an SPD-20A detector, following a published method with modification [12]. Samples were injected separately. The mobile phase used (ACN) as solvent A and (H_2O) as solvent B, with an isocratic mode of 60% A and 40% for 60 min. The flow rate was 5 mL/min, and 1 mL of each sample was injected. The detection wavelength was 254 nm. A Luna C18 column (Phenomenex, 150 mm \times 10 mm. i.d., 5 μm) and a C18 guard column (Phenomenex, Torrance, CA, USA) were used.

2.5. Antimicrobial Activity Assay

Evaluation of the antibacterial activity of all the isolated compounds followed a previous procedure [13]. The agar well diffusion method was used to evaluate the antibacterial activity of the isolated compounds on antibiotic-resistant, pathogenic bacteria *Enterococcus faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii*. Three bacterial pathogens were cultured in nutrient broth at 37°C for 18 hours. The cultures were diluted with sterile 0.9% NaCl to obtain bacterial solutions of 1.5×10^8 CFU/mL. This solution with the volume of 100 μL was spread on a Mueller-Hinton agar plate. Holes with a diameter of 8 mm were punched aseptically to create wells on the surface of the Mueller-Hinton

agar. The compounds were then dissolved in DMSO. Following this, the amount of 50 μg of each compound solution was inserted into the wells. The plates were incubated at 37°C for 16-18 hours and the antibacterial activity of each compound was recorded by measuring the diameters of the inhibition zones surrounding the wells. DMSO was used as a control. The positive control, apramycin showed the inhibition zone of 21 mm, 20 mm, and 21 mm against *Enterococcus faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii*, respectively.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1**, colorless gum, had the molecular formula of $\text{C}_{15}\text{H}_{20}\text{O}_6$ based on HRESI mass data. The ^1H NMR spectrum, in accordance with the HSQC spectrum, showed the presence of four hydroxy groups, one olefin proton [δ 5.57 (1H, brs, H-3)], one oxygenated methylene [δ 4.01 (1H, dd, J = 13.5, 5.5 Hz, H-15a), 3.90 (1H, dd, J = 13.5, 5.5 Hz, H-15b)], two oxymethines [δ 3.84 (1H, m, H-2) and 3.21 (1H, d, J = 4.0 Hz, H-1)], one methine, two methyls (δ 1.73 and 1.03), and two methylenes. The ^{13}C NMR spectrum revealed one lactone carbonyl carbon, four olefinic carbons, one hemiketal carbon, two oxygenated methines, and one oxygenated methylene. At a higher magnetic field, there was the presence of one methine, two methylenes, two methyls, and a quaternary carbon. The above spectroscopic data indicated that **1** had a eudesmane-type sesquiterpene lactone [6, 14-17]. The HMBC correlations of the methyl at δ 1.73 to carbons at δ 171.8 (C-12), 161.6 (C-7), and 120.4 (C-11) and of the hemiacetal proton at δ 7.12 to carbons at δ 104.6 (C-8) and C-7, indicated the presence of the lactone ring (so-called C-ring) Figure 2. Moreover, HMBC cross-peaks of the methylenes at δ 2.96 (H-6a), 1.88 (H-9a), and 1.86 (H-9b) to both C-7 and C-8 defined the connectivity through C-6-C-9. The methyl at δ 1.03 gave HMBC correlations to C-5, C-9, and C-10, confirmed the structure of the B-ring. This group also gave a HMBC cross-peak to an oxymethine carbon at δ 76.6 (C-1), indicating the position of the 1-OH group. The oxymethylene at δ 4.01 and 3.90 gave HMBC correlations to carbons at 122.9 (C-3), 137.7 (C-4), and C-5 while the hydroxy protons at δ 4.80 had the HMBC correlations to carbons C-1 and C-3. These correlations, along with the HMBC cross-peaks of H-1 with carbons C-2, C-3, and C-5 indicated the position of 2-OH, which further confirmed the structure of the A-ring. NMR data of **1** were highly similar to those of eudebeiolide J [14], except for the replacement of the oxymethylene H₂-15 of **1** for the methyl H₃-15 of eudebeiolide J (Table S1) [14]. Altogether, the chemical structure of **1** was elucidated as shown, namely myrrhalindenane C.

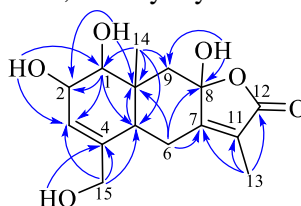


Figure 2. Key HMBC correlations of **1**

The stereochemistry of **1** was determined by J -coupling analysis, NOESY correlations, and comparison with the literature. At first, proton H-5 had a large coupling constant (J = 13.5 Hz). This indicated its axial position, which was similar to those of previously reported eudesmane sesquiterpenes [6]. Next, the coupling constant of H-1 and H-2 indicated their stereochemistry. Particularly, the null coupling constant of H-1 and H-2 indicated that both protons are in *trans* orientation. This phenomenon can be found in previously-reported eudesmanolides from *Mikania campunulata* [15] or artemisargin A isolated from *Artemisia argyi* [17]. In contrast, in the case of eudebeiolide J, the coupling constant of H-1 and H-2 was 4.0 Hz, assigning that both H-1 and H-2 were *syn*-facial. Finally, the NOESY correlation of H₃-14 and H-1 indicated an equal orientation. This methyl also gave the NOESY cross peak to H-6b, while proton H-5 had a strong NOESY correlation to H-6a, indicating the *trans* orientation of H-5 and H₃-14. Based on the abovementioned spectroscopic information, the relative stereochemistry of **1** was

defined except for the C-8 chiral center. The undefined C-8 center led to 4 possible stereoisomers of **1** (Figure S10). ECD spectrum of **1** was recorded to define the absolute stereochemistry of **1**. ECD spectra of **1** and eudbeiolide J [14] gave two similar characteristic Cotton effects at -220 and -241 nm (Figure S9), indicating that both compounds have a similar stereochemistry at C-8. Thus, the absolute configuration of **1** was proposed as 1*S*,2*R*,5*R*,8*R*,10*S*.

To the best of our knowledge, compounds **1-6** and **8** were found for the first time in the *Lindera* genus.

3.2. Antimicrobial Activity

The compounds displayed antimicrobial activity against antibiotic-resistant, pathogenic bacteria *Enterococcus faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii*. Compound **8** showed strong activity against *S. aureus* with a MIC value of 8 µg/mL. This compound showed moderate activity against *Enterococcus faecium* and *Acinetobacter baumannii* with the inhibition zones of 13 and 11 mm at the concentration of 50 µg/mL but it is inactive against the other strain. Other compounds failed to reveal any activity.

Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

ORCID

Hoang-Dung Nguyen: [0000-0002-3338-3816](https://orcid.org/0000-0002-3338-3816)

Huy Truong Nguyen: [0000-0002-7725-9876](https://orcid.org/0000-0002-7725-9876)

Thi-Hoai-Thu Nguyen: [0000-0002-3551-084X](https://orcid.org/0000-0002-3551-084X)

Jirapast Sichaem: [0000-0003-1407-496X](https://orcid.org/0000-0003-1407-496X)

Huu-Hung Nguyen: [0000-0003-0078-2340](https://orcid.org/0000-0003-0078-2340)

Ngoc-Hong Nguyen: [0000-0002-3119-1802](https://orcid.org/0000-0002-3119-1802)

Thuc-Huy Duong: [0000-0001-9891-4326](https://orcid.org/0000-0001-9891-4326)

References

- [1] M. L. Zhao, Y. Song, J. Ni, X. Yao, Y. H. Tan and Z. F. Xu (2018). Comparative chloroplast genomics and phylogenetics of nine *Lindera* species (Lauraceae), *Sci. Rep.* **8**, 8844-8855.
- [2] Y. Cao, B. Xuan, B. Peng, C. Li, X. Chai and P. Tu (2016). The genus *Lindera*: a source of structurally diverse molecules having pharmacological significance, *Phytochem. Rev.* **15**, 869-906.
- [3] H. Tanaka, K. Ichino and K. Ito (1985). A novel flavanone, linderatone, from *Lindera umbellata*. *Chem. Pharm. Bull.* **33**, 2602-2604.
- [4] B. H. Phan, E. Seguin, F. Tillequin and M. Koch (1994). Aporphine alkaloids from *Lindera myrrha*, *Phytochemistry* **35**, 1363-1365.
- [5] H. D. Nguyen, V. K. Nguyen, N. K. T. Pham, J. Sichaem, and T. H. Duong (2021). Lindermyrhrin, a novel 3,4-dihydroisocoumarin from *Lindera myrrha* roots, *Nat. Prod. Res.* **35** 1122-1126.
- [6] T. H. Duong, M. A. Beniddir, N. T. Trung, C. T. D. Phan, V. G. Vo, V. K. Nguyen, Q. L. Le, H. D. Nguyen and P. Le Pogam (2020). Atypical lindenane-type sesquiterpenes from *Lindera myrrha*, *Molecules* **25**, 1830-1839.
- [7] H. Kikuzaki, S. Kayano, N. Fukutsuka, A. Aoki, K. Kasamatsu, Y. Yamasaki, T. Mitani and N. Nakatani (2004). Absciscic acid related compounds and lignans in prunes (*Prunus domestica* L.) and their oxygen radical absorbance capacity (ORAC), *J. Agric. Food Chem.* **52**, 344-349.

- [8] A. Mimura, H. Sumioka, K. Matsunami and H. Otsuka (2010). Conjugates of an abscisic acid derivative and phenolic glucosides, and a new sesquiterpene glucoside from *Lindera strychnifolia*, *J. Nat. Med.* **64**, 153–160.
- [9] A. N. Nurfini, M. S. Reksohadiprodjo, H. Timmerman, U. A. Jenie, D. Sugiyanto and H. van der Goot (1997). Synthesis of some symmetrical curcumin derivatives and their antiinflammatory activity, *Eur. J. Med. Chem.* **32**, 321-328.
- [10] C. R. Polaquini, L. G. Morão, A. C. Nazaré, G. S. Torrezan, G. Dilarri, L. B. Cavalca, D. L. Campos, I. C. Silva, J. A. Pereira, D. J. Scheffers, C. Duque, F. R. Pavan, H. Ferreira and L. O. Regasini (2019). Antibacterial activity of 3,3'-dihydroxycurcumin (DHC) is associated with membrane perturbation, *Bioorg. Chem.* **90**, 103031.
- [11] C. V. Shaffer, S. Cai, J. Peng, A. J. Robles, R. M. Hartley, D. R. Powell, L. Du, R. H. Cichewicz and S. L. Mooberry (2016). Texas native plants yield compounds with cytotoxic activities against prostate cancer cells, *J. Nat. Prod.* **79**, 531-540.
- [12] T. H. Duong, M. A. Beniddir, G. Genta-Jouve, T. Aree, M. Chollet-Krugler, J. Boustie, S. Ferron, A. Sauvager, H. H. Nguyen, K. P. P. Nguyen, W. Chavasiri and P. Le Pogam (2018). Tsavoenones A–C: unprecedented polyketides with a 1,7-dioxadispiro[4.0.4.4]tetradecane core from the lichen *Parmotrema tsavoense*, *Org. Biomol. Chem.* **16**, 5913-5919.
- [13] C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney (1997), Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Adv. Drug Deliv. Rev.* **23**, 3-25.
- [14] H. J. Jang, S. Lee, S. J. Lee, H. J. Lim, K. Jung, Y. H. Kim, S. W. Lee and M. C. Rho (2017). Anti-inflammatory activity of eudesmane-type sesquiterpenoids from *Salvia plebeia*, *J. Nat. Prod.* **80**, 2666-2676.
- [15] M. Krautmann, E. C. de Riscalca, E. Burgueño-Tapia, Y. Mora-Pérez, C. A. N. Catalán, and P. Joseph-Nathan (2007). C-15-functionalized eudesmanolides from *Mikania campanulata*, *J. Nat. Prod.* **70**, 1173-1179.
- [16] Y. Liu and Y. Chen (2021). Eudesmane sesquiterpenoids from *Salvia plebeia*, *Rec. Nat. Prod.* **15**, 613-616.
- [17] L. Zhang, Y. M. Yan, S. X. Wang, Z. Ren, and Y. X. Cheng (2021). Three new sesquiterpenoids with cytotoxic activity from *Artemisia argyi*, *Nat. Prod. Res.* **35**, 893-899.

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