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SHORT REPORT

Inhibition of the NLRP3 inflammasome by Callinudin A: A novel 3,4-seco-labdane diterpenoid derived from *Callicarpa nudiflora*

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Abstract: A previously uncharacterized 3,4-seco-labdane diterpenoid, which is designated Callinudin A (1), along with two known diterpenoids of the same type (2, 3), was isolated from *Callicarpa nudiflora* leaves. Multiple spectroscopic techniques and literature comparisons were used to elucidate the structures of these compounds. Furthermore, these compounds were tested in vitro to assess their effects on the NLRP3 inflammasome in lipopolysaccharide (LPS)-induced THP-1 macrophages. Analysis revealed that these compounds exhibit inhibitory effects on the NLRP3 inflammasome.

Keywords: *Callicarpa nudiflora*, Callinudin A, 3,4-seco-labdane diterpenoid, NLRP3 inflammasome

Cite this article as:
Zhang et al. Inhibition of the NLRP3 inflammasome by Callinudin A: A novel 3,4-seco-labdane diterpenoid derived from *Callicarpa nudiflora*. (2026). *Records of Natural Products*, 20(4):e25123770

Received: *20 December 2025*
Revised: *01 March 2026*
Accepted: *02 March 2026*
Published: *30 March 2026*

1 Plant Source

In this research, Callicarpa nudiflora leaves were collected from Baisha County in Hainan Province, China. These leaves were identified by Professor Tian Jianping, a recognized expert at Hainan Medical University, who confirmed that the samples were dried *Callicarpa nudiflora* Hook. et Arn. (Verbenaceae). The Herbarium of Changbai Mountain Nature Reserve received a voucher specimen (ANTU 4283).

2 Previous Studies

Callicarpa nudiflora is a species that belongs to the Verbenaceae family within the *Callicarpa* genus. It is found predominantly in southern China, specifically, Hainan, Guangdong, and Guangxi provinces. This plant is recognized as a characteristic Li minority herb in Hainan and has various pharmacological benefits, including anti-inflammatory, antibacterial, and hemostatic properties. Clinically, it is used to treat various acute and chronic inflammatory conditions, as well as bleeding disorders (Liu, 2008; Yang et al., 2021). A preliminary literature review revealed that the chemical constituents that have been isolated from *C. nudiflora* include terpenoids, flavonoids, phenylethanoid glycosides, and phenylpropanoids (Ma et al., 2022; Zhang et al., 2022; Sun et al., 2018; Lin et al., 2024; Wu et al., 2025; Zhao et al., 2023; Dong et al., 2013; Dong et al., 2014; Chen et al., 2023). Diterpenoids feature complex structures and often contain multiple hydroxyl, carboxyl, and cyclic groups, which endow them with diverse pharmacological activities and excellent target selectivity. These diterpenoids are categorized into three primary types: labdane-type, primarane-type, and isopimarane-type. Among these, labdane-type diterpenoids are the predominant class of diterpenoids, with 3,4-seco-labdane-type diterpenoids being the most abundant. These compounds exhibit unique structural features in which the A ring is cleaved at the 3,4 position, thus making them distinctive and characteristic constituents of *C. nudiflora*. In our previous study on this plant, we isolated four 3,4-seco-labdane-type diterpenes (Dong et al., 2013; Dong et al., 2014).

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Table 1. NMR spectroscopic data of Callinudin A (**1**) (600 MHz for ^{1}H, and 125 MHz for ^{13}C) in $CDCl_3$

Position	δ_H (J in Hz)	δ_C
1	1.76 (m); 1.62 (m)	32.3
2	2.51 (m); 2.42 (m)	27.8
3	–	175.4
4	–	146.7
5	2.26 (m)	50.6
6	1.72 (m); 1.65 (m)	29.7
7	2.43 (m); 2.04 (m)	37.6
8	–	147.6
9	2.39 (m)	44.0
10	–	41.5
11	1.77 (m); 1.61 (m)	31.7
12	4.61 (m)	67.1
13	–	173.6
14	6.02 (s)	114.4
15	–	173.7
16	4.94 (brs)	71.1
17	4.96 (brs); 4.45 (brs)	107.2
18	4.89 (brs); 4.72 (brs)	114.0
19	1.75 (s)	23.3
20	0.73 (s)	17.6
21	4.15 (q, J = 7.5 Hz)	60.9
22	1.28 (t, J = 7.5 Hz)	14.2

3 Present Study

In the present investigation, various chromatographic techniques were effectively used to isolate a novel 3,4-seco-labdane diterpene, Callinudin A (1), along with two previously identified diterpenoid compounds (**2**, **3**) from *C. nudiflora*. In this study, the isolation, structural characterization, and analysis of the inhibitory effects of these diterpenoids on the NLRP3 inflammasome are presented.

The leaves of *C. nudiflora* were air dried, subjected to two extraction procedures with 95% ethanol, and refluxed for 4 h each. The extracts were subsequently concentrated under vacuum until the scent of ethanol disappeared. To further purify these extracts, each extract was dissolved in water and subsequently partitioned with dichloromethane. A total of 1500 g of the dichloromethane extract was subjected to coarse separation through silica gel column chromatography (SGCC). The eluent used was a gradient mixture of petroleum ether (PE) and ethyl acetate (EA), with a ratio that ranged from 100:0 to 0:100 (v/v). This method enabled the isolation of nine distinct fractions, which were labeled 1–9. Following this initial separation, fraction 6 was subjected to further separation. This separation involved another round of SGCC, for which a more refined gradient solvent system of PE and EA (40:1 to 0:1) was used. This meticulous process culminated in the collection of nine fractions, which were designated Fr.6-A to Fr.6-I. Fraction 6-D was separated by SGCC, which yielded seven subfractions (Fr. 6-D-1 to Fr. 6-D-7). These subfractions were eluted using a gradient system that consisted of PE and acetone (ACE) at a ratio that ranged from 40:1 to 0:1. For further purification, Fr. 6-D-4 underwent semipreparative HPLC at a flow rate of 2 mL/min. For the gradient elution process, a mixture of 70% CH_3OH/H_2O (0.2% HCOOH) was used for the initial 20 min, which was followed by a transition to a 64% CH_3OH/H_2O (0.2% HCOOH) mixture from 20 to 100 min. This procedure resulted in the successful isolation of compound **2** (5 mg, t_R = 65 min). Additionally, fraction 6-E was isolated via semipreparative HPLC (with a flow rate of 2 mL/min, using 73% CH_3OH/H_2O (0.2% HCOOH)), which resulted in the extraction of compound **1** (16.8 mg, t_R = 58 min). Similarly, Fr. 6-F was separated through SGCC, which resulted in eight subfractions (Fr. 6-F-1 to Fr. 6-F-8). These subfractions were eluted with a gradient of PE and EA (from 100:1 to 0:100). Fr. 6-F-5 was subsequently purified using semipreparative HPLC (2 mL/min, 78% CH_3OH/H_2O (0.2% HCOOH)), which yielded compound **3** (8.2 mg, t_R = 25 min).

Callinudin A (**1**), which is a yellow oily substance with molecular formula $C_{22}H_{32}O_5$, was confirmed by its $[M+Na]^+$ ion peak at m/z 399.2147. This value aligns perfectly with the calculated mass of $C_{22}H_{32}O_5Na$, thereby confirming the molecular identity of the compound. The data obtained from the ^{1}H and ^{13}C NMR (refer to Table 1) suggested the presence of an iso-propenyl moiety [δ_H 1.75 (3H, s), 4.72 (1H, brs), 4.89 (1H, brs); δ_C 23.3, 114.0, 146.7], an olefinic methylene group [δ_H 4.45 (1H, s), 4.96 (1H, brs), δ_C 107.2, 147.6], a methyl group attached to a quaternary carbon [δ_H 0.73 (3H, s), δc 17.6], and a carbonyl carbon in the carboxylic acid [δc 175.4]. On the basis of the distortionless enhancement by polarization transfer (DEPT) spectral information, the remaining signals were interpreted as representing one methyl group, seven methylenes, four methines, and three quaternary carbons. Thorough heteronuclear multiple bond correlation (HMBC) and ^{1}H-^{1}H correlated spectroscopy (COSY) correlations (see Figure 2) indicated that compound **1** features a 3,4-seco-labdane framework, which is structurally similar to that of a previously identified compound, Nudiflopene U (Chen et al., 2023). Although the NMR data for compound **1** closely resembled those for Nudiflopene U, there were notable differences in the chemical shift values for C-21 and C-22. Analysis of the ^{1}H-^{1}H COSY and HMBC spectra revealed that, in contrast to Nudiflopene U, compound **1** differs by the attachment of an ethyl group to the carboxyl group at the C-3 position. HMBC analysis revealed a significant correlation between H-21 and both C-22 and C-3, which indicated that the ethyl group is connected to the carboxyl group at the C-3 position of **1**. To further explore the relative configuration of **1**, the ROESY spectrum was analyzed. In particular, the observed ROESY correlations between H-5 and H-9, H-9 and H-7β, H_3-20 and H_2-11, and H_3-20 and H_3-19 suggest that the Me-20 group, the side chain at C-9, and the isopropenyl moiety at C-5 were oriented in the same α-axial direction. Additionally, the hydroxyl group at C-12 was positioned in the α direction, as supported by the correlation observed between H-9 and H-12. Upon analyzing the experimental ECD spectrum of compound **1** alongside the computed ECD spectra (Neese, 2012; Stephens & Harada, 2010), the absolute configuration

http://doi.org/10.25135/rnp.2512.3770
Rec. Nat. Prod. 2026, 20(4):e25123770

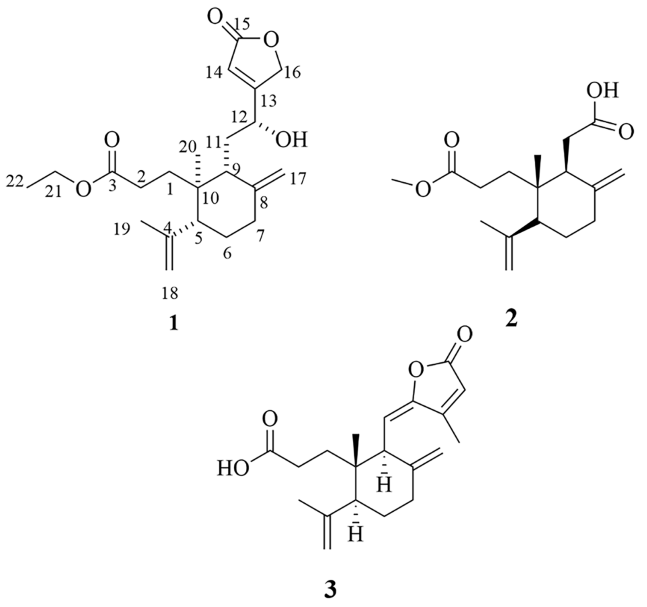

Figure 1. Structures of Callinudin A (1) and two other compounds isolated from *C. nudiflora*

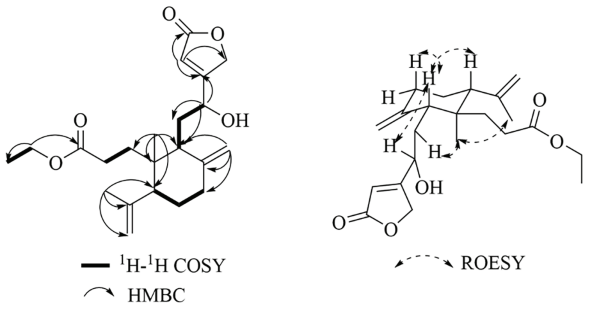

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and ROESY correlations of Callinudin A (**1**)

was determined to be significantly correlated with the calculated spectrum for 5R, 9R, 10R, and 12R (Figure 3). As a result of these analyses, the structure of 1 was confirmed, and it was identified as Callinudin A.

*Callinudin A(**1**)*: Yellow oil. (CH_3OH), $[\alpha]_{25}^{D}$ = 25 (c 0.1), UV (MeOH) λ_{max} (log ε) 205(3.06). ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR data (see Table 1); HR-ESI-MS: *m/z* 399.2147 ([M+Na]$^+$, calcd. 399.2147 for $C_{22}H_{32}O_5Na$).

Two known 3,4-seco-labdane-type diterpenoids (**2,3**) (Figure 1) were also isolated and identified as callnudoid F (**2**) (Zhang et al., 2022) and nudiflopene H (**3**) (Sun et al., 2018) through a comparison of their physical properties and spectroscopic characteristics with previously published data.

In this study, a novel labdane-type diterpenoid (**1**) and two known compounds (**2** and **3**) were isolated from the leaves of

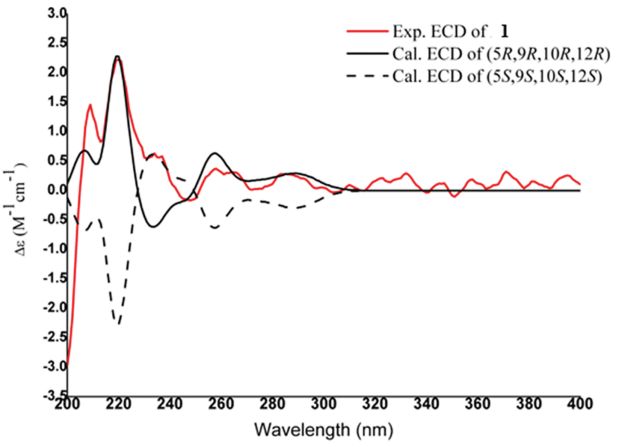

Figure 3. Calculated and experimental ECD spectra of Callinudin A (**1**)

C. nudiflora, all of which are classified as 3,4-seco-labdane-type diterpenoids. These compounds have been identified only in *C. nudiflora* and can be considered characteristic metabolites of this species. This discovery plays a crucial role in distinguishing *C. nudiflora* from other species in the *Callicarpa* genus, thereby improving our understanding of the chemotaxonomic connections among *C. nudiflora* and other *Callicarpa* varieties. Furthermore, this research not only provides novel phytochemical information concerning *C. nudiflora* but also lays the groundwork for subsequent studies in chemotaxonomy.

The NLRP3 inflammasome is among the most well-characterized inflammasomes and plays a crucial role in regulating immune and inflammatory responses. Overactivation of the NLRP3 inflammasome is frequently associated with various inflammatory diseases (Zhan et al., 2023). Considering the clinical applications of *C. nudiflora*, we investigated the inhibitory effects of **1–3** on the NLRP3 inflammasome in LPS-induced THP-1 macrophages by following established experimental procedures outlined in the literature (Wang et al., 2020). The cytotoxicity of **1–3** on THP-1 macrophages was examined (Figure 4). Furthermore, after treatment with **1** at various doses (6 and 14 μM), we examined the effects of this compound on the expression levels of NLRP3, IL-1β, and IL-18 in LPS-induced THP-1 macrophages through Western blotting. The results of Western blot analysis (Figure 5) illustrated that **1** could attenuate the LPS-induced overexpression of the NLRP3 inflammasome. The effects of compounds **2** and **3** on the levels of NLRP3, IL-1*β*, and IL-18 in cells were detected by using a reagent kit (Figure 5). The results showed that **1–3** inhibit the NLRP3 inflammasome. All the compounds exhibit anti-inflammatory activity.

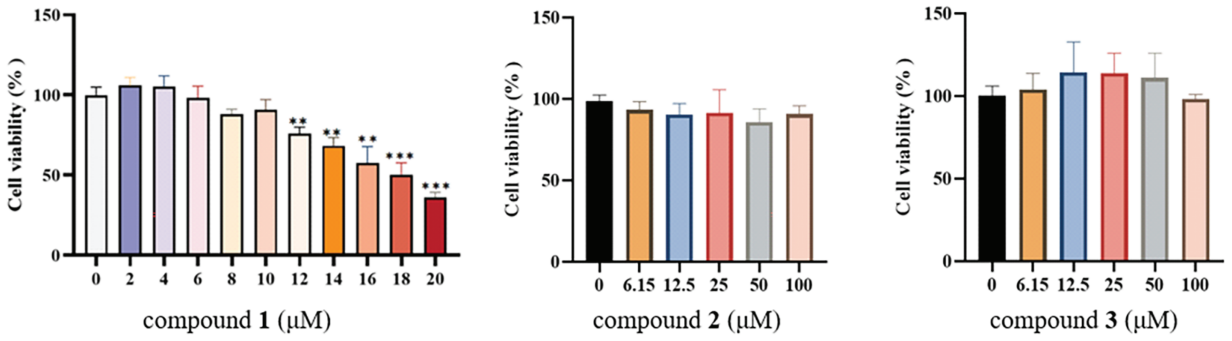

Figure 4. Cell viability of THP-1 macrophages after 24 h of exposure to compounds **1–3**, which was evaluated using a CCK-8 assay. **P < 0.01 and ***P < 0.001 vs. the control group

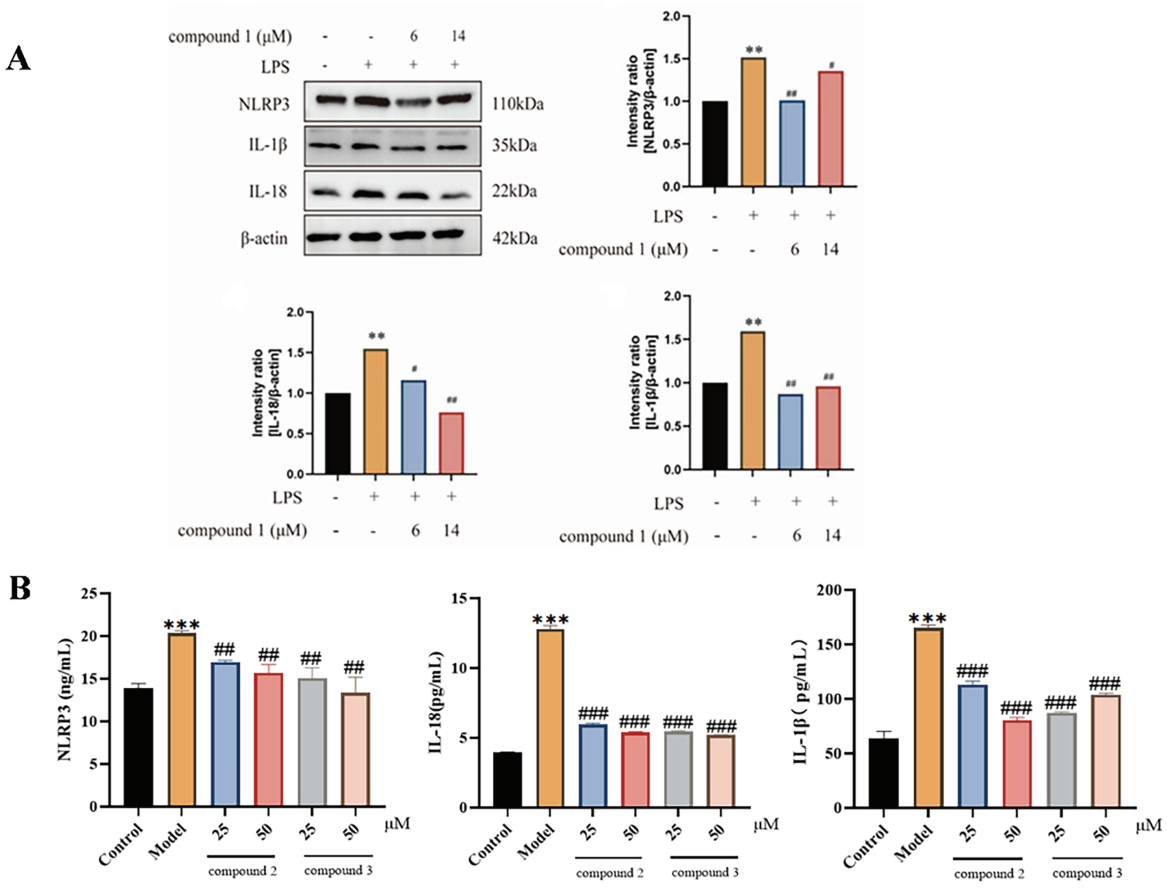

Figure 5. Effects of compounds **1–3** on the NLRP3 inflammasome in LPS-induced THP-1 macrophages. (A) Effects of compound **1** on the levels of NLRP3, IL-1*β*, and IL-18 in LPS-treated THP-1 macrophages. (B). Effects of compounds **2** and **3** on the concentrations of NLRP3, IL-1*β*, and IL-18 in these cells, which were was assessed using a reagent kit. Each data point represents the mean ± SD (n = 3 per group). (*P < 0.05, **P < 0.01, and ***P < 0.001 vs. the normal control; $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, and $^{\#\#\#}$P < 0.001 vs. the model group.)

Funding Statement

This research received funding from the Key Research and Development Program of Hainan Province, supported by the Hainan Province Science and Technology Special Fund (No. ZDYF2023SHFZ113), the Joint Program on Health Science & Technology Innovation of Hainan Province (No. WSJK2025MS208), as well as the Engineering Research Center of Tropical Medicine Innovation and Transformation of the Ministry of Education Open Project (No. TMIT2024Y05).

Author Contributions

Xiaofeng Zhang: Methodology, Investigation, Writing-original draft; Tali Mei: Investigation, Data cuation, Writing-original draft preparation; Xuemei Luo:

Investigation, Visualization, Formal analysis; Jinye Yan: Visualization, Data curation; Yong Wang: Data curation; Xiaowen He: Software; Jinping Cai: Formal analysis; Lin Dong: Conceptualization, Methodology, Funding acquisition, Writing-review & editing; Qinghu Wang: Supervision, Methodology, Resources, Writing-review & editing. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they have no competing financial interests.

Supporting Information

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

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