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Article

Dehydrohispanolone Derivatives Attenuate the Inflammatory Response through the Modulation of Inflammasome Activation

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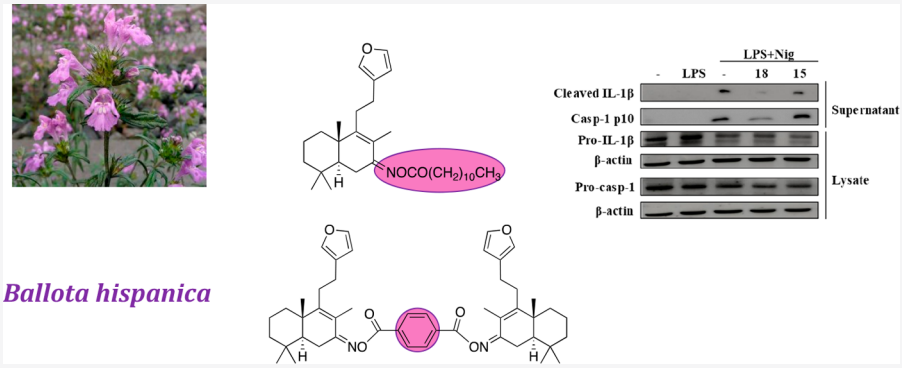

ABSTRACT: The NLRP3 inflammasome plays a critical role in inflammation-mediated human diseases and represents a promising drug target for novel anti-inflammatory therapies. Hispanolone is a labdane diterpenoid isolated from the aerial parts of *Ballota* species. This diterpenoid and some derivatives have demonstrated anti-inflammatory effects in classical inflammatory pathways. In the present study, a series of dehydrohispanolone derivatives (**1**−**19**) was synthesized, and their anti-inflammatory activities toward NLRP3 inflammasome activation were evaluated. The structures of the dehydrohispanolone analogues produced were elucidated by NMR spectroscopy and mass spectrometry. Four derivatives significantly inhibited IL-1β secretion, with **15** and **18** being the most active (IC_{50} = 18.7 and 13.8 μM, respectively). Analysis of IL-1β and caspase-1 expression revealed that the new diterpenoids **15** and **18** are selective inhibitors of the NLRP3 inflammasome, reinforcing the previously demonstrated anti-inflammatory properties of hispanolone derivatives.

Inflammation is a protective physiological response of the body triggered by microbial infections or tissue injuries, with a central role in the pathogenesis of many inflammatory conditions and autoimmunity. The inflammatory response is initiated by cellular sensing of either pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs). Soluble PRRs are able to form large intracellular multiprotein complexes known as inflammasomes, with a pivotal role in the molecular control of the inflammatory process.[1,2]

In recent years, the activation of inflammasomes, in particular the nucleotide-binding oligomerization domain (NOD)-like receptor containing pyrin domain 3 (NLRP3) inflammasome, is emerging as a critical molecular mechanism in the pathogenesis of many inflammation-associated diseases, including diabetes, acute myocardial infarction, inflammatory bowel disease, gout, and Alzheimer's disease, among others.[3−7] The assembly of this complex results in the activation of caspase-1, which promotes the cleavage of pro-IL-1β and pro-IL-18 to produce mature and functional IL-1β and IL-18.[8,9] Growing evidence substantiates inflammasome inhibition as a therapeutic option for the treatment of inflammatory diseases.[3] Additionally, neutralization of IL-1β has proven efficacious in the treatment of inflammation.[10] Nevertheless, there are no drugs available clinically that specifically target NLRP3. It is therefore of importance to develop specific NLRP3 inflammasome inhibitors (NLRP3Is) as novel anti-inflammatory therapies.

Terpenoids are a very large group of natural products with a plethora of pharmacological properties.[11−18] Recently, some terpenoids have been reported to possess inhibitory effects on the NLRP3 inflammasome pathway.[19] The genus *Ballota* is a valuable source of bioactive compounds, mainly terpenoids

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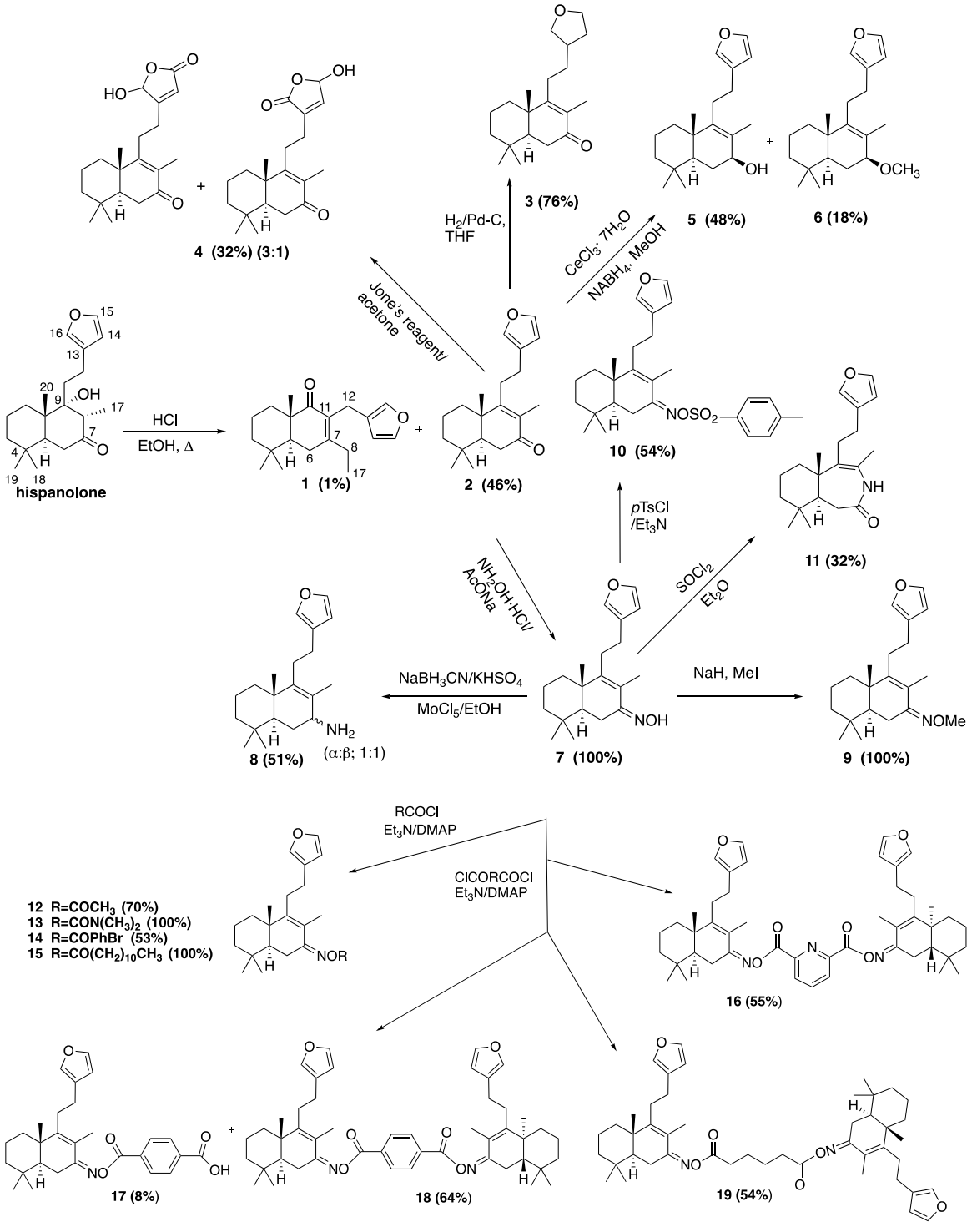
Scheme 1. Preparation of Diterpenoid Derivatives (1−19)



and flavonoids with therapeutic potential in different diseases.[20] Hispanolone is a furolabdane diterpenoid isolated from the aerial parts of species of the *Ballota* genus such as *Ballota hispanica* or *B. hirsuta*.[21,22] Previous studies have reported the anti-inflammatory, antitumor, and cardioprotective effects of hispanolone derivatives.[23−27] The anti-inflammatory potential of these compounds has been largely associated with the impairment of classical inflammatory signaling pathways. Nevertheless, the effects of these compounds on inflammasome modulation remain unexplored.

To investigate the potential of diterpenoids as NLRP3 inhibitors, a series of new dehydrohispanolone derivatives was semisynthesized. Two derivatives (**15** and **18**) were identified as potent and selective NLRP3 inhibitors.

RESULTS AND DISCUSSION

Preparation of Derivatives 1−19. Nineteen derivatives (**1−19**) related to the natural diterpenoid hispanolone were prepared in order to evaluate the role of the different functional groups and moieties in their anti-inflammatory

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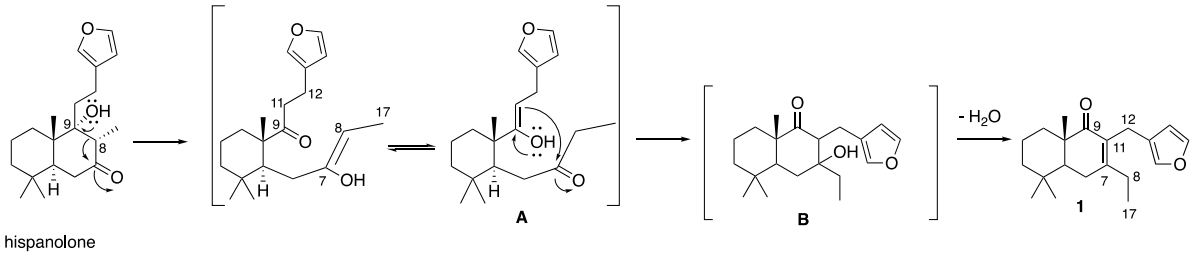
Scheme 2. Plausible Formation of Compound 1



activities (Scheme 1). Most of them were obtained from compound **2** through modifications on the carbonyl group at C-7 and the furan ring. Thus, the treatment of hispanolone with HCl in EtOH under reflux for 18 h afforded two compounds, **1** (1%) and dehydrohispanolone (**2**) (46%) (Scheme 1).[28] The major compound **2** was obtained from the dehydration of the alcohol, while compound **1** was obtained from a retro-aldol reaction followed by an intramolecular aldol condensation, as shown in Scheme 2. First, the cleavage of the C-8, C-9 bond of hispanolone takes place to give the dicarbonyl intermediate A, which undergoes an intramolecular aldol reaction to yield the hydroxy intermediate B, which dehydrates, affording compound **1** with a new diterpenoid skeleton. The hydrogenation of compound **2** with Pd−C in THF yielded the corresponding tetrahydrofuran derivative in 76% yield.

When compound **2** was treated with Jones reagent, the furan ring was oxidized and a mixture of two inseparable hydroxy-unsaturated lactones **4** in a 3:1 ratio (32%) was obtained. The reduction of the carbonyl group at C-7 in compound **2** with $NaBH_4$/MeOH in the presence of $CeCl_3{\cdot}7H_2O$ gave the corresponding hydroxy derivative **5** (48%), together with compound **6** (18%), which has a methoxy group. The β-orientation of the hydroxy group was determined on the basis of the multiplicity and the value of the ^{1}H NMR coupling constant for H-7α (δ 4.10, t, J = 8.4 Hz) and also by the NOE effect detected between H-7α and Me-18. The oxime **7** was obtained quantitatively when compound **2** was treated with hydroxylamine hydrochloride. The reduction of compound **7** with $NaBH_3CN/KHSO_4/MoCl_5$[29] in EtOH afforded a 1:1 mixture of the epimeric amino derivatives **8** (53%). The *O*-methyl oxime **9** was produced quantitatively when **7** was treated with NaH/MeI, while the *p*-toluenesulfonyl derivative **10** was obtained in a 54% yield when **9** was reacted with *p*-toluensulfonyl chloride and Et_3N. The lactam **11** was formed in low yield (32%) by Beckmann rearrangement of oxime **7** with $SOCl_2/Et_2O$.[30] The structure of compound **11** was ratified by the presence in the ^{1}H NMR spectrum of a multiplet at δ 3.23 (2H) corresponding to H-6 and the presence in the ^{13}C NMR spectrum of a quaternary carbon at δ 175.5 assignable to the lactam carbonyl. The *O*-acyloxime derivatives (**12**−**15**) were prepared by treatment of the oxime **7** with several acyl chlorides of different size, lipophilicity, and stereoelectronic properties. With the aim of preparing dimers, it was decided to react **7** with some aromatic diacyl dichlorides such as 2,6-pyridinedicarbonyl dichloride, terephthaloyl chloride, or the aliphatic adipoyl dichloride. Under the usual acylation conditions, three dimers (**16**, **18**, and **19**) were obtained, as shown in Scheme 1. When terephthaloyl chloride was used, together both the dimer **18** and the derivative **17**, having just one oxime unit, were obtained.

The diterpenoids **1**−**19** were tested as anti-inflammatory agents, with focus on the NLRP3 inflammasome.

Potential cytotoxicity was analyzed by cell viability assays with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in a mouse macrophage J774 A.1 model. As shown in Figure 1, compounds **2**, **5**, **8**, and **17** reduced the cell viability at the tested concentrations, whereas no significant cytotoxicity was observed with the remaining compounds.

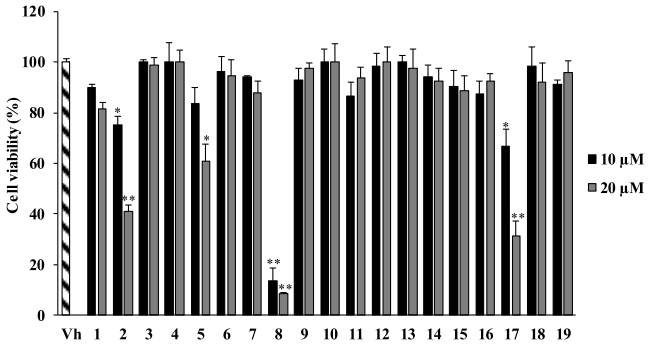


Figure 1. Cell viability after treatment with hispanolone derivatives. J774A.1 macrophages were treated with vehicle (Vh) or 10 and 20 μM of hispanolone derivatives (**1**−**19**) for 24 h. Cell viability was determined by an MTT assay. Experiments were carried out in triplicate. Results are the means ± SD of three different experiments. *p < 0.05 and **p < 0.01 with respect to cells treated with vehicle (Vh).

Two of the cytotoxic diterpenoids, **5** and **8**, have at C-7 hydrogen bond donors such as $-NH_2$ and $-OH$, respectively. The presence of a carbonyl group at C-7 together with the furan ring (**2**) produces cytotoxicity since compound **3**, having a carbonyl group at C-7 and a tetrahydrofuran ring, was not cytotoxic. The occurrence of a $-COOH$ moiety also produced low cell viability since compound **17** was found to be the only cytotoxic oxime derivative.

To test the specificity of the derivatives on NLRP3 inhibition, the ability of nontoxic diterpenoids to regulate nitric oxide (NO) production was previously evaluated. NO is a relevant pro-inflammatory mediator released upon exposure of macrophages to bacterial lipopolysaccharide (LPS). Ten derivatives exhibited discernible inhibitory effects on NO release, with a reduction of more than 50% for **1**, **3**, and **14** (Figure 2).

The inflammasome is a multiprotein complex that mediates activation of caspase-1, leading to the secretion of the proinflammatory cytokines IL-1β and IL-18. NLRP3 inflammasome activation involves a two-step process: a first signal called "priming" that induces NLRP3, pro-IL-1β, and pro-IL-18 expression, and a second signal required for full activation of the inflammasome that is triggered by diverse stimuli

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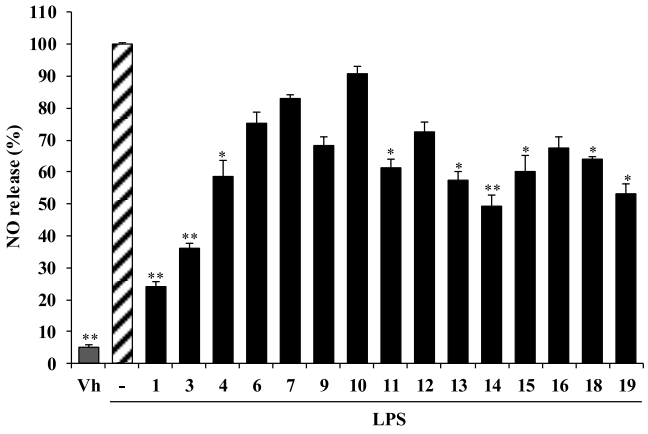

Figure 2. Effects of diterpenoids on NO production. J774A.1 macrophages were treated with vehicle (Vh), LPS (1 μg/mL), or LPS and 20 μM of nontoxic hispanolone derivatives for 24 h. The accumulation of nitrite in the culture medium was measured with the Griess reagent. Experiments were carried out in triplicate. Results are the means ± SD of three different experiments. *p < 0.05 and **p < 0.01 with respect to LPS treatment.

including nigericin, adenosine triphosphate (ATP), and monosodium urate crystals (MSU).[31] To investigate the effects of dehydrohispanolone derivatives on IL-1β secretion, LPS-primed mouse macrophages were treated with nigericin in the presence or absence of derivatives. Four derivatives, **1**, **3**, **15**, and **18**, reduced nigericin-induced IL-1β secretion in a significant manner (Figure 3).

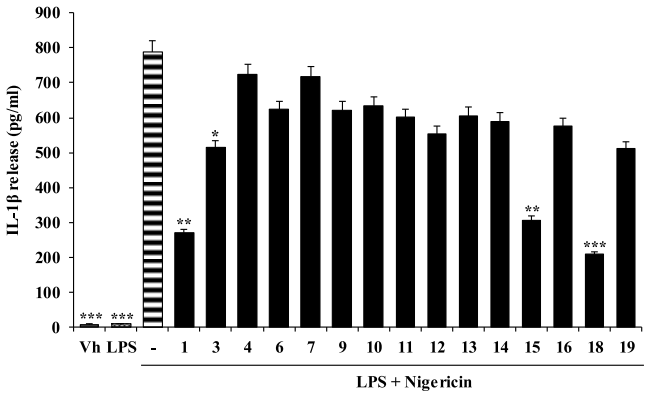

Figure 3. Inhibitory effects of dehydrohispanolone derivatives on IL-1β secretion. J774A.1 macrophages were treated with vehicle (Vh) or primed for 5 h with LPS (1 μg/mL), followed by treatment with 20 μM of hispanolone derivatives for 30 min and then nigericin (20 μM) for 45 min. Levels of IL-1β in the culture medium were measured by ELISA. Experiments were carried out in triplicate. Results are the means ± SD of three independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001 with respect to LPS + nigericin treatment.

From these results, some structure−activity relationships can be outlined. Thus, in the dimer series (compounds **16**, **18**, and **19**) the importance of the linker was evident since only compound **18**, with a terephthaloyl type linker, significantly reduced IL-1β release. Regarding the oxime derivatives **7**, **9**, **10**, **12**, **13**, **14**, and **15**, the lipophilicity seems to play a role in their activity, because compound **15**, with the highest log p (log p 9.39) of the series, showed the highest value of IL-1β release reduction. Compound **1**, with a modified furanlabdane skeleton, was active. The presence of a tetrahydrofuran ring instead of a furan ring led to a decrease in IL-1β release (compound **3**).

According to their inhibitory effects on IL-1β secretion, compounds **15** and **18** gave IC_{50} values of 18.7 and 13.8 μM, respectively. Since they exhibited an inhibitory effect on IL-1β release without affecting NO production, they were selected for further analysis. Treatment with the NLRP3 inflammasome inhibitor MCC950 was used to corroborate the involvement of NLRP3 in IL-1β activation. As expected, the levels of cleaved IL-1β increased in cells exposed to LPS and nigericin, an effect abolished by the addition of the MCC950 inflammasome inhibitor (Figure 4A).

Similarly, reduction of cleaved caspase-1 and cleaved IL-1β levels in cell culture supernatants by derivatives **18** and **15** was also observed by Western blot analysis (Figure 4B).

Compounds **15** and **18** also inhibited IL-1β secretion stimulated by other NLRP3 agonists, including ATP and MSU (Figure 5), suggesting that both compounds act as broad-spectrum inhibitors of the NLRP3 inflammasome.

Concluding Remarks. The search for new anti-inflammatory agents is challenging due to the complexity of the inflammatory process and the role of inflammation as a key component of many diseases. A significant body of evidence has emerged supporting that the NLRP3 inflammasome is critical for inflammatory responses. Indeed, dysregulation of inflammasome activation is linked to a variety of inflammatory pathologies. Therefore, NLRP3 has become a prime focus for the development of novel anti-inflammatory therapies. Diterpenoids are an interesting natural products group with marked structural and biological diversity. The reported findings herein have identified two new dehydrohispanolone oxime derivatives (**15** and **18**) as selective NLRP3 inflammasome inhibitors, reinforcing the anti-inflammatory properties of labdane diterpenoids. These derivatives deserve additional attention as potential multitargeting anti-inflammatory compounds and strongly encourage further studies, as they could serve as leads for new therapeutics against NLRP3-driven diseases.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 241 polarimeter. IR spectra were obtained using a Bruker IFS28/55 spectrophotometer. NMR spectra were recorded in $CDCl_3$ at 400, 500, or 600 MHz for ^{1}H NMR and 100 or 150 MHz for ^{13}C NMR. Chemical shifts (δ) are given in parts per million, and coupling constants (J) in hertz (Hz). ^{1}H and ^{13}C NMR spectra were referenced using the solvent signal as internal standard. HREIMS were recorded using a high-resolution magnetic trisector (EBE) mass analyzer. Analytical TLC plates used were Polygram-Sil G/UV254. Preparative TLC was carried out with Analtech silica gel GF plates (20 × 20 cm, 1000 μm) using appropriate mixtures of ethyl acetate and hexanes. All solvents and reagents were purified by standard techniques reported[32] or used as supplied from commercial sources. The hispanolone used as starting material was obtained from *Ballota hispanica* Benth. (Lamiaceae), following the procedure described in ref 33.

Preparation of Compounds 1 and 2. To 3.0 g (9.45 mol) of hispanolone in 175 mL of EtOH was added 10 mL of concentrated HCl, and the reaction mixture was heated under reflux for 18 h. Next, this was treated with 100 mL of H_2O and extracted with CH_2Cl_2 (3 × 30 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed. The residue was purified by column chromatography with hexanes/EtOAc (95:5) to yield 0.14 g (1.0%) of **1** and 1.3 g of **2** (46%) as yellow oils.

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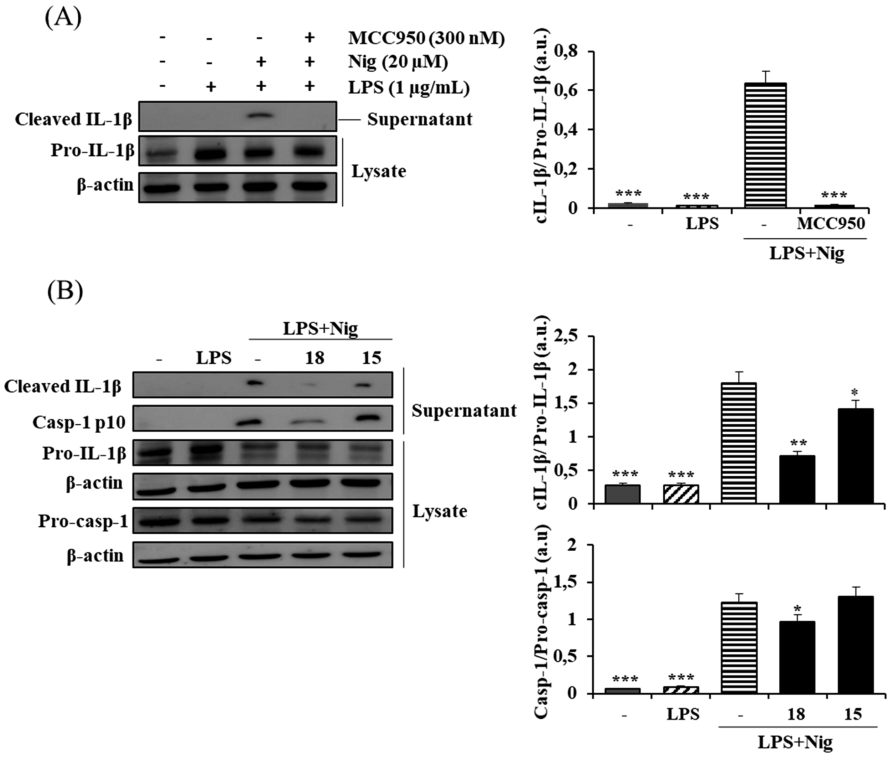

Figure 4. Dehydrohispanolone derivatives inhibit NLRP3 inflammasome activation. J774A.1 macrophages were primed with LPS (1 μg/mL) for 5 h and treated with nigericin (20 μM) for 45 min in the presence or absence of the inflammasome inhibitor MCC950 (300 nM) (A) or derivatives **18** and **15** (20 μM) (B). Supernatants and cell extracts were analyzed by inmunoblot analysis of cleaved IL-1β (cIL-1 β) (17 kDa), cleaved caspase-1 (10 kDa), pro-IL-1β (31 kDa), and pro-caspase-1 (45 kDa) expression. β-Actin was immunoblotted as a loading control. A representative experiment of three performed is shown. Bar graphs show densitometry quantification of the bands from three independent experiments. cIL-1β/Pro-IL-1β and casp1/procasp-1 ratios were calculated from data obtained by densitometry. *p < 0.05, **p < 0.01, and ***p < 0.001 with respect to LPS + nigericin treatment.

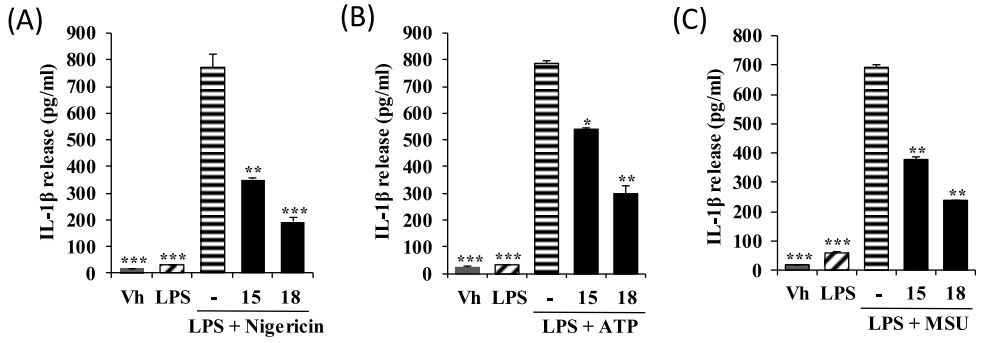

Figure 5. Diterpenoids **15** and **18** inhibit NLRP3 inflammasome activation by diverse stimuli. J774A.1 macrophages were treated with vehicle (Vh) or primed for 5 h with LPS (1 μg/mL), followed by incubation with 20 μM **15** and **18** for 30 min, and subsequent treatment with (A) nigericin (20 μM, 45 min), (B) ATP (5 mM, 45 min), and (C) MSU (100 μg/mL, 24 h). Supernatants were analyzed for IL-1β release. Results show the means ± SD of three independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001 with respect to LPS + stimuli treatment.

*(4aS,8aS)-3-Ethyl-2-(furan-2-ylmethyl)-5,5,8a-trimethyl-4a,5,6,7,8,8a-hexahydronaphthalen-1(4H)-one (**1**).* Yellow oil; $[\alpha]^{20}_{D}$ −91 (*c* 0.9, $CHCl_3$); IR (neat) ν_{max} 3150, 2970, 2850, 1665, 1505, 1465, 1305, 1160, 1030, 995, 765 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.26 (1H, bs, H-15), 7.11 (1H, bs, H-16), 6.18 (1H, s, H-14), 3.53 (1H, d, J = 14.8 Hz, H-6a), 3.25 (1H, d, J = 14.8 Hz, H-6b), 2.33 (2H, m, H-12), 1.86 (1H, dd, J = 13.7, 1.4 Hz, H-5), 1.59 (3H, m, H-2, H-1), 1.43 (1H, dd, J = 13.2, 1.4 Hz, H-3), 1.32 (1H, td, J = 13.4, 5.1 Hz, H-8), 1.19 (1H, td, J = 13.0, 5.1 Hz, H-8), 1.07 (3H, t, J = 7.7 Hz, H_3-17), 0.99 (3H, s, H_3-20), 0.98 (3H, s, H_3-19), 0.91 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.7 (C, C-9), 158.6 (C, C-11), 142.5 (CH, C-15), 139.1 (CH, C-16), 130.8 (C, C-7), 124.0 (C, C-13), 111.2 (CH, C-14), 48.4 (CH, C-5), 44.2 (C, C-10), 41.7 (CH_2, C-3), 33.7 (CH_2, C-1), 33.7 (C, C-4), 32.3 (CH_3, C-19), 28.1 (CH_2, C-6), 27.8 (CH_2, C-12), 22.2 (CH_3, C-20), 20.8 (CH_2, C-8), 18.3 (CH_2, C-2), 17.2 (CH_3, C-18), 12.4 (CH_3, C-17); EIMS m/z 300 ([M^+], 100) 285 (8), 271 (11), 267 (10), 229 (15), 149 (32), 147 (30), 81 (54); HRESIMS m/z 323.1981 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_2Na$, 323.1987).

*Compound **2**.* Yellow oil; $[\alpha]^{20}_{D}$ +40 (*c* 1.1, $CHCl_3$); IR (neat) ν_{max} 3150, 3120, 2950, 2880, 1420, 1350, 1330, 1260, 1165, 1070, 920, 810, 785 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37 (1H, t, J = 1.6 Hz, H-15), 7.27 (1H, s, H-16), 6.30 (1H, d, J = 0.8 Hz, H-14), 2.47 (5H, m, H-12, H-11, H-6a), 2.37 (1H, dd, J = 17.5, 14.4 Hz, H-6b), 1.97 (1H, d, J = 12.4 Hz, H-1a), 1.80 (3H, s, H_3-17), 1.71 (2H, m, H-5, H-2a), 1.60 (1H, m, H-2b), 1.49 (1H, dd, J = 13.3, 1.1 Hz, H-3a), 1.41 (1H, td, J = 12.9, 3.7 Hz, H-1b), 1.23 (1H, td, J = 13.5, 4.1 Hz, H-3b), 1.10 (3H, s, H_3-20), 0.92 (3H, s, H_3-19), 0.89 (3H, s, H_3-

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18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.7 (C, C-7), 166.6 (C, C-9), 142.8 (CH, C-15), 138.4 (CH, C-16), 130.1 (C, C-8), 124.3 (C, C-13), 110.4 (CH, C-14), 50.1 (CH, C-5), 41.1 (CH_2, C-3), 40.7 (C, C-10), 35.7 (CH_2, C-1), 35.0 (CH_2, C-6), 32.9 (C, C-4), 32.3 (CH_3, C-19), 30.0 (CH_2, C-11), 24.0 (CH_2, C-12), 21.1 (CH_3, C-18), 18.4 (CH_2, C-2), 17.9 (CH_3, C-20), 11.2 (CH_3, C-17); EIMS m/z 300 ([M^+], 21), 285 (12), 205 (12), 176 (54), 163 (9), 148, 135 (33), 81 (100); HRESIMS m/z 323.1983 [M + Na]$^+$ (calcd for $C_{20}H_{28}O_2Na$, 323.1987).

Preparation of Compound 3. To 44.3 mg (0.15 mmol) of compound **2** in 9 mL of dry THF was added a catalytic amount of 10% Pd/C, and the reaction mixture was hydrogenated for 13 h. After removal of the solvent, the resulting residue was purified by preparative TLC using hexanes−EtOAc (4:1) to yield 34.8 mg (76%) of compound **3** as a colorless oil: IR (neat) ν_{max} 3382, 2865, 2243, 1760, 1605, 1332, 1254, 1151, 1077, 977, 620 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.92 (1H, t, J = 8.1 Hz), 3.86 (1H, m, H-15), 3.75 (1H, dd, J = 15.7, 7.6 Hz, H-16a), 3.39 (1H, td, J = 7.9, 2,7 Hz, H-16b), 2.48 (1H, dd, J = 13.9, 3.6 Hz, H-6a), 2.34 (1H, m, H-6b), 2.19 (4H, m, H-11, H-12), 1.89 (1H, dd. J = 11.8, 2.7 Hz, H-1a), 1.74 (3H, s, H_3-17), 1.68 (2H, m, H-5, H-13), 1.53 (5H, m, H-1b, H-2, H-14), 1.36 (1H, m, H-3b), 1.21 (1H, m, H-3a), 1.07 (3H, s, H_3-20), 0.90 (3H, s, H_3-19), 0,87 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.3 (C, C-7), 167.7 (C, C-9), 130.1 (C, C-8), 73.2 (CH_2, C-16), 68.0 (CH_2, C-15), 50.4 (CH, C-5), 41.4 (CH_2, C-3), 41.0 (C, C-10), 40.4 (CH, C-13), 36.1 (CH_2, C-1), 35.3 (CH_2, C-6), 33.2 (C, C-4), 32.6 (CH_3, C-19), 32.4 (CH_2, C-14), 32.3 (CH_2, C-11), 28.6 (CH_2, C-12), 21.3 (CH_3, C-18), 18.7 (CH_2, C-2), 18.3 (CH_3, C-20), 11.5 (CH_3, C-17); EIMS m/z 289 ([M]$^+$, 100), 135 (84), 205 (51), 123 (76); HREIMS m/z 304.2462 [M]$^+$ (calcd for $C_{20}H_{32}O_2$, 304.2404).

Preparation of Compound 4. To 128 mg (0.43 mmol) of compound **2** in 9 mL of acetone was added dropwise 1 mL of Jones reagent. Next, the reaction mixture was extracted with CH_2Cl_2 (3 × 15 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed. The residue was purified by column chromatography with hexanes/EtOAc (7:3) to yield 44.9 mg (32%) of compound **4** as an inseparable mixture of isomers in a 1:3 ratio, as a yellow oil: IR (neat) ν_{max} 3385, 2948, 1753, 1643, 1458, 1338, 1182, 1135, 943, 895 cm^{-1}; EIMS m/z 332 ([M]$^+$, 14), 317 (4), 314 (6), 299 (3), 288 (8), 270 (41), 269 (25), 237 (26), 220 (15), 205 (69), 161 (28), 149 (27), 135 (99), 123 (57), 109 (33), 91 (79), 77 (41), 69 (59), 60 (100); HREIMS m/z 332.1991 [M]$^+$ (calcd for $C_{20}H_{28}O_4$, 332.1988). Major isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.05 (1H, s, H-16), 5.94 (1H, s, H-14), 4.07 (1H, bs, OH), 2.51 (5H, H-6a, H-11, H-12), 2.37 (1H, dd, J = 17.6, 14.5 Hz, H-6b), 1.92 (1H, d; J = 12.1 Hz, H-1a), 1.77 (3H, s, H_3-17), 1.71 (3H, m, H-5, H-2), 1.52 (1H, d, J = 12.1 Hz, H-3b), 1.35 (1H, m, H-1b), 1.23 (1H, dd, J = 13.8, 3.1 Hz, H-3b), 1.12 (3H, s, H_3-20), 0.93 (3H, s, H_3-19), 0.90 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.2 (C, C-7), 170.3 (C, C-15), 167.7 (C, C-13), 165.2 (C, C-9), 131.4 (C, C-8), 118.1 (CH, C-14), 98.4 (CH, C-16), 50.5 (CH, C-5), 41.4 (CH_2, C-3), 41.1 (C, C-10), 35.7 (CH_2, C-1), 36.1 (C, C-4), 35.3 (CH_2, C-1), 33.3 (CH_2, C-6), 32.6 (CH_3, C-18), 27.0 (CH_2, C-12), 26.5 (CH_2, C-11), 21.4 (CH_3, C-19), 18.7 (CH_2, C-2), 18.3 (CH_3, C-20), 11.6 (CH_3, C-17). Minor isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92 (1H, s, H-14), 6.13 (1H, s, H-15), 4.07 (1H, bs, OH), 2.50 (5H, H-6α, H-11, H-12), 2.36 (1H, dd, J = 17.6, 14.5 Hz, H-6b), 1.95 (1H, d; J = 12.7 Hz, H-1a), 1.77 (3H, s, H_3-17), 1.70 (3H, m, H-5, H-2), 1.47 (1H, d, J = 12.7 Hz, H-3b), 1.37 (1H, m, H-1b), 1.21 (1H, dd, J = 13.9, 4.3 Hz, H-3b), 1.10 (3H, s, H_3-20), 0.93 (3H, s, H_3-19), 0.90 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.7 (C, C-7), 171.2 (C, C-16), 166.1 (C, C-9), 143.5 (CH, C-14), 137.4 (C, C-13), 130.9 (C, C-8), 96.7 (CH, C-15), 50.2 (CH, C-5), 41.2 (CH_2, C-3), 41.0 (C, C-10), 35.8 (CH_2, C-1), 35.2 (CH_2, C-6), 33.1 (C, C-4), 32.5 (CH_3, C-18), 27.1(CH_2, C-11), 24.6 (CH_2, C-12), 21.3 (CH_3, C-19), 18.5 (CH_2, C-2), 18.1 (CH_3, C-20), 11.5 (CH_3, C-17).

Preparation of Compounds 5 and 6. To 40.8 mg (0.14 mmol) of compound **2** in 2 mL of MeOH were added 1 equiv (52,16 mg) of $CeCl_3{\cdot}7H_2O$ and 4 equiv of $NaBH_4$ (5.4 mg) at room temperature. The reaction mixture was stirred for 30 min until the disappearance of starting material. Next, the reaction mixture was treated with H_2O and extracted with CH_2Cl_2 (3 × 15 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography with hexanes/EtOAc (9:1) to yield 20.3 mg (48%) of compound **5** and 7.9 mg of compound **6** (18%).

Compound **5**. Colorless oil; $[\alpha]^{20}_D$ +76 (c 0.1, $CHCl_3$); IR (neat) ν_{max} 3382, 2992, 2243, 1760, 1460, 1373, 1262, 1076, 977, 873, 730 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, s, H-15), 7.24 (1H, s, H-16), 6.30 (1H, bs, H-14), 4.10 (1H, t, J = 8.4 Hz, H-7), 2.48 (2H, t, J = 8.6 Hz, H-6), 2.27 (1H, m, H-11a), 2.13 (2H, m, H-12), 1.85 (1H, d, J = 12.4 Hz, H-1a), 1.73 (3H, s, H_3-17), 1.73 (1H, m, H-11b), 1.42 (3H, m, H-5, H-3a, H-1b), 1.19 (3H, m, H-3b, H-2), 1.03 (3H, m, H_3-20), 0.90 (3H, s, H_3-19), 0.87 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.4 (C, C-9), 142.9 (CH, C-15), 138.6 (CH, C-16), 129.2 (C, C-8), 125.5 (C, C-13), 110.9 (CH, C-14), 73.1 (CH, C-7), 50.0 (CH, C-5), 41.7 (CH_2, C-3), 40.0 (C, C-10), 37.1 (CH_2, C-1), 33.2 (CH_2, C-6), 33.1 (C, C-4), 30.1 (CH_3, C-19), 29.1 (CH_2, C-11), 25.2 (CH_2, C-12), 21.8 (CH_3, C-18), 20.3 (CH_2, C-2), 19.0 (CH_3, C-20), 14.9 (CH_3, C-17); EIMS m/z 220 (100), 176 (27), 135 (26), 119 (21), 81 (81); HREIMS m/z 302.2162 [M]$^+$ (calcd for $C_{20}H_{30}O_2$, 302.2140).

Compound **6**. Colorless oil; $[\alpha]^{20}_D$ +53 (c 0.1, $CHCl_3$); IR (neat) ν_{max} 3382, 2927, 2865, 1760, 1460, 1373, 1262, 1076, 977, 873, 730 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, bs, H-15), 7.24 (1H, s, H-16), 6.29 (1H, s, H-14), 3.39 (3H, s, OMe), 3.39 (1H, m, H-7), 2.48 (2H, t, J = 8.9 Hz, H-6), 2.24 (2H, m, H-12), 1.85 (1H, d, J = 12.5 Hz, H-1a), 1.88 (2H, m, H-11), 1.74 (3H, s, H_3-17), 1.54 (5H, m, H-1b, H-2, H-3a, H-5), 1.22 (1H, m, H-3b), 0.93 (6H, s, H_3-19, H_3-20), 0.86 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.8 (C, C-9), 142.8 (CH, C-15), 138.5 (CH, C-16), 126.7 (C, C-8), 125.7 (C, C-13), 110.9 (CH, C-14), 80.0 (CH, C-7), 56.8 (CH_3, OMe), 46.1 (CH, C-5), 41.5 (CH_2, C-3), 36.4 (CH_2, C-1), 36.4 (C, C-10), 33.1 (CH_2, C-6), 29.8 (C, C-4), 29.0 (CH_3, C-19), 25.3 (CH_2, C-11), 22.7 (CH_2, C-12), 21.9 (CH_3, C-18), 19.1 (CH_2, C-2), 18.4 (CH_3, C-20), 17.7 (CH_3, C-17); EIMS m/z 221 ([M]$^+$, 100), 133 (38), 81 (46); HREIMS m/z 316.2464 [M]$^+$ (calcd for $C_{21}H_{32}O_2$, 316.2402).

Preparation of Compound 7. To 66.3 mg (0.22 mmol) of compound **2** in 16 mL of EtOH were added 3 equiv (47.0 mg) of hydroxylamine hydrochloride and 1.7 equiv of sodium acetate (30.7 mg) dissolved in 10 mL of H_2O. The reaction mixture was heated under refux for 19 h. Then, the EtOH was removed, and the aqueous solution was extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was eliminated to yield quantitatively compound **7** as an amorphous white solid: $[\alpha]^{20}_D$ −15 (c 0.3, $CHCl_3$); IR (neat) ν_{max} 3149, 3105, 2922, 1764, 1607, 1501, 1463, 1290, 1153, 1067, 934, 811 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 (1H, bs, H-15), 7.15 (1H, s, H-16), 6.20 (1H, bs, H-14), 3.08 (1H, dd, J = 13.9, 4.0 Hz, H-6a), 2.53 (2H, m, H-12), 2.44 (1H, m, H-11a), 2.37 (1H, m, H-11b), 2.11 (1H, dd, J = 13.9, 14,1 Hz, H-6b), 1.94 (1H, bd, J = 12.4 Hz, H-1a), 1.76 (3H, s, H_3-17), 1.55 (1H, m, H-2b), 1.47 (1H, bd, J = 13.4 Hz, H-3a), 1.39 (1H, dd, J = 3.8, 13.8 Hz, H-5), 1.35 (1H, m, H-1b), 1.20 (1H, td, J = 3.9, 13.4 Hz, H-3b), 0.91 (3H, s, H_3-20), 0.88 (6H, s, H_3-19, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.6 (C, C-7), 154.2 (C, C-9), 143.0 (CH, C-15), 138.7 (CH, C-16), 125.0 (C, C-13), 124.1 (C, C-8), 110.8 (CH, C-14), 48.7 (CH, C-5), 41.9 (CH_2, C-3), 39.7 (C, C-10), 36.4 (CH_2, C-1), 35.6 (C, C-4), 33.0 (CH_3, C-18), 29.4 (CH_2, C-11), 25.4 (CH_2, C-12), 21.5 (CH_3, C-19), 21.0 (CH_2, C-6), 19.0 (CH_2+CH_3, C-2, C-20), 13.2 (CH_3, C-17); EIMS m/z 284 ([M]$^+$, 24), 234 (39), 220 (100), 150 (31), 81 (34); HREIMS m/z 315.2191 [M]$^+$ (calcd for $C_{20}H_{29}O_2N$, 315.2198).

Preparation of Compound 8. To 54.7 mg (0.174 mmol) of oxime **7** in 2 mL of EtOH were added 4 equiv of $NaBH_3CN$ (43.6 mg), 3 equiv (71.1 mg) of $KHSO_4$, and 1 equiv (41.2 mg) of $MoCl_5$ in 4 mL of EtOH. The reaction mixture was heated under reflux for 40 min until the disappearance of the starting material. Then, it was

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extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed. The residue was purified by preparative TLC with hexanes/EtOAc (4:1) to yield 28 mg (51%) of compound **8** as a colorless oil: IR (neat) ν_{max} 2926, 2097, 1610, 1462, 1374, 1253, 1160, 1064, 978, 873, 778 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, bs, H-15), 7.23 (1H, s, H-16), 6.29 (1H, bs, H-14), 3.65 (1H, m, H-7, *epimer A*), 3.38 (1H, m, H-7, *epimer B*), 2.47 (2H, d, J = 8.5 Hz, H-6), 2.18 (4H, m, H-11, H-12), 1.55 (2H, m, H-1b, H-2b), 1.73 (3H, s, H_3-17), 1.60 (1H, m, H-5), 1.32 (4H, m, H-1a, H-2a, H-3), 1.03 (3H, s, H_3-20), 0.89 (3H, s, H_3-19), 0.85 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.4 (C, C-9), 142.9 (CH, C-15), 138.6 (CH, C-16), 129.2 (C, C-8), 125.5 (C, C-13), 110.9 (CH, C-14), 73.1 (CH, C-7), 50.0 (CH, C-5, *epimer A*), 50.5 (CH, C-5, *epimer B*), 41.7 (CH_2, C-3), 39.9 (C, C-10, *epimer A*), 40.0 (C, C-10, *epimer B*), 37.1 (CH_2, C-1), 33.2 (CH_2, C-6), 33.2 (C, C- 4), 30.1 (CH_3, C-19, *epimer A*), 31.0 (CH_3, C-19, *epimer B*), 29.1 (CH_2, C-11), 25.2 (CH_2, C-12, *epimer A*), 25.3 (CH_2, C-12, *epimer B*), 21.8 (CH_3, C-18), 20.3 (CH_2, C-2, *epimer A*), 20.5 (CH_2, C-2, *epimer B*), 19.0 (CH_3, C-20), 14.9 (CH_3, C-17, epimer A), 15.9 (CH_3, C-17, *epimer B*); HREIMS m/z 324.2309 $[M + Na]^+$ (calcd for $C_{20}H_{31}NONa$, 324.2303).

Preparation of Compound 9. To 25.1 mg (0,08 mmol) of oxime **7** in 5 mL of THF were added 1 equiv of NaH (1.92 mg) under an inert atmosphere. The reaction mixture was stirred for 10 min, and then 1.2 equiv (6 μL) of methyl iodide was also added and the reaction mixture was left at room temperature for 4 days. The solvent was removed, and the residue was treated with 10 mL of H_2O and extracted with Et_2O (3 × 10 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was eliminated under reduced pressure. The residue was purified by TLC with hexanes/EtOAc (95:5) to yield 26.3 mg (100%) of compound **9** as a colorless oil: $[\alpha]^{20}_D$ −20 (c 0.4, $CHCl_3$); IR (neat) ν_{max} 2926, 2856, 2091, 1737, 1504, 1380, 1160, 979, 877 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37 (1H, t, J = 1.5 Hz, H-15), 7.26 (1H, s, H-16), 6.31 (1H, d, J = 0.6 Hz, H-14), 3.92 (3H, s, OMe), 2.96 (1H, dd, J = 13.9, 4.1 Hz, H-6a), 2.51 (2H, m, H-12); 2.45 (1H, m, H-11a), 2.35 (1H, m, H-11b), 2.08 (1H, dd, J = 13.9, 14.1 Hz, H-6b), 1.93 (1H, d, J = 6.1 Hz, H-1a), 1.86 (3H, s, H_3-17), 1.62 (1H, m, H-2a), 1.54 (1H, m, H-2b), 1.45 (1H, dd, J = 12.2, 1.1 Hz, H-3a), 1.37 (2H, m, H-5, H-1b), 1.19 (1H, td, J = 13.4, 4.0 Hz, H-3b), 0.96 (3H, s, H_3-20), 0.93 (6H, s, H_3-18, H_3-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.7 (C, C-7), 153.4 (C, C-9), 143.0 (CH, C-15), 138.7 (CH, C-16), 125.1 (C, C-8), 124.4 (C, C-13), 110.9 (CH, C-14), 61.8 (CH_3, OCH_3), 48.7 (CH, C-5), 42.0 (CH_2, C-3), 39.5 (C, C-10), 36.5 (CH_2, C-1), 33.5 (C, C-4), 33.0 (CH_3, C-18), 29.5 (CH_2, C-11), 25.3 (CH_2, C-12), 21.5 (CH_3, C-19), 21.4 (CH_2, C-6), 19.0 (CH_2, C-2), 18.9 (CH_3, C-20), 12.9 (CH_3, C-17); EIMS m/z 298 ($[M]^+$, 16), 248 (97), 234 (100), 97 (37), 85 (32), 81 (53), 71 (44), 57 (64), 55 (49); HREIMS m/z 329.2357 $[M]^+$ (calcd for $C_{21}H_{31}NO_2$, 329.2328).

Preparation of Compound 10. To 40.5 mg (0.13 mmol) of compound **7** in 2 mL of CH_2Cl_2 were added 2.5 equiv of *p*-toluenesulfonyl chloride (61.28 mg), 2.5 equiv of triethylamine (45.3 μL), and a catalytic amount of DMAP dissolved in 4 mL of CH_2Cl_2. The reaction mixture was heated under reflux for 24 h and then treated with 10 mL of brine and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed. The residue was purified by preparative TLC with hexanes/EtOAc (4:1) to yield 31.8 mg (54%) of compound **10** as an oil: $[\alpha]^{20}_D$ −11 (c 0.3, $CHCl_3$); IR (neat) ν_{max} 2930, 2868, 1755, 1652, 1453, 1292, 1177, 1023, 975, 875, 731, 663 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.91 (2H, d, J = 8.0 Hz, H-2′, H-6′), 7.36 (1H, bs, H-15), 7.33 (2H, d, J = 8.0 Hz, H-3′, H-5′), 7.24 (1H, s, H-16), 6.28 (1H, bs, H-14), 2.97 (1H, dd, J = 14.6, 3.7 Hz, H-6a), 2.44 (3H, s, Me-Ar), 2.35 (4H, m, H-11, H-12), 2.20 (1H, dd, J = 14.6, 14.3 Hz, H-6b), 1.91 (1H, d, J = 12.4 Hz, H-1a), 1.76 (3H, s, H_3-17), 1.76 (1H, m, H-5), 1.63 (1H, d, J = 13.5 Hz, H-2a), 1.46 (1H, d, J = 13.4 Hz, H-1b), 1.32 (2H, m, H-3), 1.16 (1H, td, J = 13.3, 3.8 Hz, H-3a), 0.91 (9H, s, H_3-20, H_3-18, H_3-19); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 165.0 (C, C-7), 159.7 (C, C-9), 144.8 (C, C-1′), 143.1 (CH, C-15), 138.7 (CH, C-16), 133.1 (C, C-4′), 129.5 (2CH, C-3′, C-5′), 129.2 (2CH, C-2′, C-6′), 124.7 (C, C-13), 123.1 (C, C-8), 110.7 (CH, C-14), 48.4 (CH, C-5), 41.7 (CH_2, C-3), 39.8 (C, C-10), 36.1 (CH_2, C-1), 33.4 (C, C-4), 32.8 (CH_3, C-18), 29.7 (CH_2, C-11), 25.0 (CH_2, C-12), 22.3 (CH_2, C-6), 21.8 (CH_3, C-19), 21.4 (CH_2, C-2), 18.7 (CH_3, C-20), 12.9 (CH_3, C-17); HRESIMS m/z 492.2182 $[M + Na]^+$ (calcd for $C_{27}H_{35}O_4SNa$, 492.2185).

Preparation of Compound 11. To 48.5 mg (0.15 mmol) of compound **7** in 5 mL of dry dioxane at 0 °C was added 12 equiv of thionyl chloride (0.13 mL). The reaction mixture was stirred at 0 °C for 1 h and then was left stirring at room temperature overnight. Then 20 mL of water was added, and it was extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed under reduced pressure. The residue was purified by preparative TLC with hexanes/EtOAc (7:3) to yield 15 mg (32%) of compound **11** as a colorless oil: $[\alpha]^{20}_D$ −11 (c 0,2, $CHCl_3$); IR (neat) ν_{max} 3149, 2922, 1764, 1723, 1501, 1331, 1256, 1153, 1067, 957, 811 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38 (1H, s, H-15), 7.26 (1H, s, H-16), 6.32 (1H, s, H-14), 6.00 (1H, bs, NH), 3.23 (2H, m, H-6), 2.50 (4H, m, H-11, H-12), 2.00 (3H, s, H_3-17), 1.98 (1H, m, H-1a), 1.80 (1H, d, J = 12.2 Hz, H-3a), 1.70 (1H, d, J = 10.7 Hz, H-2a), 1.45 (3H, m, H-5, H-3b, H-1b), 1.15 (1H, td, J = 13.4, 3.9 Hz, H-2b), 0.95 (9H, bs, H_3-18, H_3-19, H_3-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.5 (C, C-7), 154.0 (C, C-9), 143.1 (CH, C-15), 138.8 (CH, C-16), 126.9 (C, C-8), 124.8 (C, C-13), 110.8 (CH, C-14), 58.1 (CH, C-5), 44.6 (CH_2, C-3), 41.0 (CH_2, C-1), 39.8 (C, C-10), 38.7 (CH_2, C-6), 35.1 (C, C-4), 33.8 (CH_3, C-18), 31.5 (CH_2, C-11), 25.0 (CH_2, C-12), 22.7 (CH_3, C-19), 22.6 (CH_2, C-2), 19.1 (CH_3, C-20), 17.7 (CH_3, C-17); EIMS m/z 315 ($[M]^+$, 100), 314 (73), 300 (65), 258 (32), 234 (55) 206 (32), 191 (70), 190 (79), 149 (64), 109 (36), 95 (30), 191 (70), 94 (32), 81 (91), 55 (46); HREIMS m/z 315.2163 $[M]^+$ (calcd for $C_{20}H_{29}O_2N$, 315.2198).

Preparation of Compound 12. To 25 mg (0.08 mmol) of oxime **7** in 8 mL of CH_2Cl_2 were added 1.5 equiv of acetyl chloride (8.5 μL), 3 equiv of triethylamine (33 μL), and catalytic amounts of DMAP. The reaction mixture was stirred for 5 h, and then the solvent was removed under reduced pressure. The residue was treated with 10 mL of H_2O and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed. The residue was purified by preparative TLC with hexanes−EtOAc (9:1) to yield 20.0 mg (70%) of compound **12** as an oil: $[\alpha]^{20}_D$ −43 (c 0.3, $CHCl_3$); IR (neat) ν_{max} 2953, 2859, 1766, 1610, 1579, 1441, 1367, 1204, 944, 873 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37 (1H, bs, H-15), 7.26 (1H, s, H-16), 6.31 (1H, bs, H-14), 3.01 (1H, dd, J = 14.3, 3.9 Hz, H-6a), 2.51 (3H, m, H-12, H-11a), 2.38 (1H, m, H-11b), 2.25 (1H, m, H-6b), 2.24 (3H, s, H_3-17), 1.94 (1H, m, H-1a), 1.94 (3H, s, CH_3COO−), 1.56 (2H, m, H-2), 1.47(1H, bd, J = 13.1 Hz, H-3a), 1.40 (2H, H-5, H-1b), 1.20 (1H, td, J = 13.4, 3.9 Hz, H-3b), 0.97 (3H, s, H_3-20), 0.94 (3H, s, H_3-19), 0.93 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$,125 MHz) δ 170.0 (C, -$OCOCH_3$), 164.3 (C, C-7), 158.8 (C, C-9), 143.1 (CH, C-15), 138.7 (CH, C-16), 124.1 (C, C-8), 124.0 (C, C-13), 110.8 (CH, C-14), 48.5 (CH, C-5), 41.9 (CH_2, C-3), 41.7 (CH_2, C-3), 39.8 (C, C-10), 36.2 (CH_2, C-1), 33.5 (C, C-4), 32.8 (CH_3, C-19), 29.7 (CH_3, C-11), 25.0 (CH_2, C-12), 22.5 (CH_2, C-6), 21.4 (CH_3, C-19), 20.2 (CH_3, −$OCOCH_3$), 18.8 (CH_2, C-2), 13.2 (CH_3, C-17); EIMS m/z 298 ($[M^+]$, 64), 297 (100), 284 (86), 282 (42), 234 (34), 218 (36), 202 (42) 175 (67), 81(72); HRESIMS m/z 380.2206 $[M + Na]^+$ (calcd for $C_{22}H_{31}NO_3Na$, 380.2202).

Preparation of Compound 13. To 25.0 mg (0.08 mmol) of oxime **7** in 6 mL of CH_2Cl_2 were added 1.5 equiv of *N,N*-dimethylcarbamoyl chloride (8.3 μL), 3 equiv of triethylamine (33.6 μL), and catalytic amounts of DMAP. The reaction mixture was stirred under reflux for 24 h. Then, the solvent was removed, and the residue was treated with 10 mL of H_2O and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was eliminated. The residue was purified by preparative TLC with hexanes/EtOAc (4:1) to yield 30.8 mg (100%) of compound **13** as an amorphous white solid: $[\alpha]^{20}_D$ −20 (c 0.4, $CHCl_3$); IR (neat) ν_{max} 3397, 2927, 2863, 2347, 2114,

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1727, 1444, 1379, 1152, 1019, 975, 870 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.35 (1H, t, J = 1.6 Hz, H-15), 7.25 (1H, s, H-16), 6.30 (1H, d, J = 0.8 Hz, H-14), 3.00 (1H, dd, J = 14.0, 3.9 Hz, H-6a), 2.99 (6H, s, 2×N-Me), 2.50 (3H, m, H-11a, H-12), 2.31 (1H, m, H-11b), 2.24 (1H, dd, J = 14.0, 14.1 Hz, H-6b), 1.96 (3H, s, H_3-17), 1.95 (1H, m, H-1a), 1.57 (2H, m, H-2), 1.46 (1H, d, J = 12.9 Hz, H-3a), 1.38 (2H, m, H-5, H-1b), 1.20 (1H, td, J = 13.4, 4.0 Hz, H-3b), 0.97 (3H, s, H_3-20), 0.93 (3H, s, H_3-19), 0.91 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.0 (C, $-OCON(CH_3)_2$), 157.2 (C, C-7), 155.2 (C, C-9), 142.8 (CH, C-15), 138.5 (CH, C-16), 124.7 (C, C-8), 124.1 (C, C-13), 110.6 (CH, C-14), 48.3 (CH, C-5), 41.5 (CH_2, C-3), 39.5 (C, C-10), 36.1 (CH_2, C-1), 36.1 (2×CH_3, $-OCON(CH_3)_2$), 33.2 (CH_3, C-18), 32.7 (C, C-4), 29.5 (CH_2, C-11), 24.9 (CH_2, C-12), 22.2 (CH_2, C-6), 21.3 (CH_3, C-20), 18.7 (CH_3, C-19), 18.6 (CH_2, C-2), 13.1 (CH_3, C-17); EIMS m/z 298 ([M^+], 19), 297 (56), 284 (32), 282 (24), 175 (26), 81 (50), 72 (100); HRESIMS m/z 409.2462 [M + Na]$^+$ (calcd for $C_{23}H_{34}N_2O_3Na$, 409.2467).

Preparation of Compound 14. To 25.6 mg (0.08 mmol) of oxime **7** in 6 mL of CH_2Cl_2 were added 1.5 equiv of *p*-bromobenzoyl chloride (26.6 mg), 3 equiv of triethylamine (33.5 μL), and catalytic amounts of DMAP. The reaction mixture was stirred at room temperature for 16 h, and the solvent was eliminated. Then the residue was treated with 10 mL of H_2O and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed under reduced pressure. The residue was purified by preparative TLC with hexanes/EtOAc (9:1) to yield 21.0 mg (53%) of compound **14** as an amorphous white solid: $[\alpha]^{20}_D$ −27 (c 0.3, $CHCl_3$); IR (neat) ν_{max} 3125, 2927, 2389, 2112, 1921, 1741, 1586, 1358, 1170, 972, 907, 813 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.94 (2H, d, J = 8.5 Hz, H-3′, H-7′), 7.64 (2H, d, J = 8.5 Hz, H-4′, H-6′), 7.38 (1H, s, H-15), 7.28 (1H, s, H-16), 6.32 (1H, s, H-14), 3.15 (1H, dd, J = 14.0, 3.9 Hz, H-6a), 2.54 (3H, m, H-11a, H-12), 2.40 (2H, m, H-11b, H-6b), 2.03 (3H, s, H_3-17), 1.98 (1H, d, J = 12.7 Hz, H-1a), 1.67 (2H, m, H-2), 1.47 (2H, m, H-5, H-1b), 1.41 (1H, td, J = 12.8, 3.5 Hz, H-3a), 1.23 (1H, td, J = 13.4, 3.8 Hz, H-3b), 1.02 (3H, s, H_3-20), 0.97 (3H, s, H_3-19), 0.95 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.8 (C, C-1′), 163.4 (C, C-7), 159.3 (C, C-9), 143.0 (CH, C-15), 138.5 (CH, C-16), 132.0 (CH, C-2′), 131.0 (2×CH, C-3′, C-7′), 128.5 (CH, C-4′, C-6′), 128.2 (CH, C-5′), 124.6 (C, C-13), 124.0 (C, C-8), 110.5 (CH, C-14), 48.5 (CH, C-5), 41.6 (CH_2, C-3), 39.9 (C, C-10), 36.1 (CH_2, C-1), 33.5 (CH_3, C-18), 32.9 (C, C-4), 29.8 (CH_2, C-11), 25.0 (CH_2, C-12), 22.7 (CH_2, C-6), 21.6 (CH_3, C-19), 18.8 (CH_3, C-20), 18.7 (CH_2, C-2), 13.3 (CH_3, C-17); EIMS m/z 298 ([M^+] 36), 297 (74), 285 (20), 284 (88), 282 (29), 218 (34), 202 (88), 182 (100), 175 (64), 174 (24), 156 (25), 81 (50); HRESIMS m/z 520.1460 [M + Na]$^+$ (calcd for $C_{27}H_{32}NO_3{}^{79}BrNa$, 520.1463), 522.1450 (calcd for $C_{27}H_{32}NO_3{}^{81}BrNa$, 522.1443).

Preparation of Compound 15. To 25.5 mg (0.08 mmol) of compound **7** in 6 mL of CH_2Cl_2 were added 1.5 equiv of lauroyl chloride (27.5 μL), 3 equiv of triethylamine (33.2 μL), and catalytic amounts of DMAP. The reaction mixture was stirred for 18 h at room temperature. Then, the solvent was removed, and the residue was treated with 20 mL of H_2O and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was again removed. The residue was purified by preparative TLC with hexanes/EtOAc (9:1) to yield 39.8 mg (100%) of compound **15** as an amorphous white solid: $[\alpha]^{20}_D$ −24 (c 0.3, $CHCl_3$); IR (neat) ν_{max} 2849, 2669, 1697, 1501, 1432, 1375, 1279, 1248, 1193, 1021, 954, 872 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.36 (1H, t, J = 1.6 Hz, H-15), 7.26 (1H, s, H-16), 6.30 (1H, d, J = 0.8 Hz, H-14), 3.01 (1H, dd, J = 13.9, 4.0 Hz, H-6a), 2.49 (5H, m, H-12, H-11, H-2′), 2.37 (1H, m, H-11b), 2.24 (1H, dd, J = 13.9, 14.2 Hz, H-6b), 1.95 (3H, s, H_3-17), 1.94 (1H, m, H-1a), 1.63 (4H, m, H-2, H-3′), 1.47 (1H, d, J = 13.4 Hz, H-3a), 1.39 (7H, m, H-5, H-1b, H-6′, H-5′a, H-4′), 1.25 (11H, bs, H-11′, H-10′, H-9′, H-8′, H-7′, H-5′b), 1.20 (1H, td, J = 13.4, 4.0 Hz, H-3b), 0.98 (3H, s, H_3-20), 0.94 (3H, s, H_3-19), 0.93 (3H, s, H_3-18), 0.87 (3H, t, J = 6.8 Hz, H-12′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.6 (C, $-OCO-(CH_2)_{10}CH_3$), 158.7 (C, C-7), 155.5 (C, C-9), 143.0 (CH, C-15), 138.7 (CH, C-16), 124.9 (C, C-13), 123.7 (C, C-8), 110.8 (CH, C-14), 48.6 (CH, C-5), 41.9 (CH_2, C-3), 39.7 (C, C-10), 36.3 (CH_2, C-1), 34.2 (CH_3, C-18), 33.6 (CH_2, C-10′), 32.9 (C, C-4), 32.0 (CH_2, C-2′), 29.7 (2×CH_2, C-7′, C-8′), 29.6 (CH_2, C-6′), 29.5 (2×CH_2, C-5′, C-9′), 29.4 (CH_2, C-11), 29.2 (2×CH_2, C-3′, C-4′), 25.4 (CH_2, C-12), 24.9 (CH_2, C-11′), 22.8 (CH_2, C-6), 21.5 (CH_3, C-19), 19.0 (CH_2, C-2), 18.9 (CH_3, C-20), 14.2 (CH_3, C-12′); 13.4 (CH_3, C-17); EIMS m/z 315 ([$M]^+$, 16), 298 (22), 234 (36), 220 (100), 150 (26), 81 (41); HRESIMS m/z 320.3762 [M + Na]$^+$ (calcd for $C_{32}H_{51}NO_3Na$, 320.3767).

Preparation of Compound 16. To 30.0 mg (0.09 mmol) of compound **7** in 5 mL of CH_2Cl_2 were added 2 equiv of 2,6-pyridinedicarbonyl dichloride (36.7 mg), 1.5 equiv of triethylamine (18.8 μL), and a catalytic amount of DIMAP. The reaction mixture was stirred for 24 h. Then the solvent was removed, and the residue was treated with 20 mL of H_2O and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was eliminated. The residue was purified by preparative TLC with hexanes/EtOAc (9:1) to yield 20.0 mg (55%) of compound **16** as an amorphous white solid: $[\alpha]^{20}_D$ −15 (c 0.3, $CHCl_3$); IR (neat) ν_{max} 2915, 2846, 1747, 1434, 1319, 1241, 1018, 952, 871, 763, 663 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.31 (2H, d, J = 7.9 Hz, H-3′, H-5′), 8.02 (1H, m, H-4′), 7.36 (2H, s, 2×H-15), 7.26 (2H, s, 2×H-16), 6.30 (2H, s, 2×H-14), 3.06 (2H, dd, J = 14.0, 4.3 Hz, 2×H-6a), 2.51 (4H, m, 2×H-12), 2.45 (2H, m, 2×H-11a), 2.35 (2H, m, 2×H-11b), 2.15 (2H, dd, J = 14.0, 14.2 Hz, 2×H-6b), 1.93 (2H, d, J = 12.6 Hz, 2×H-1a), 1.84 (6H, s, 2×H_3-17), 1.64 (4H, m, 2×H-2a, 2×H-5), 1.55 (2H, m, 2×H-2b), 1.45 (2H, d, J = 13.3 Hz, 2×H-3a), 1.23 (2H, m, H-1b), 1.19 (2H, td, J = 13.5, 4.0 Hz, 2×H-3b), 0.97 (6H, s, 2×H_3-20), 0.94 (12H, s, 2×H_3-19, 2×H_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.2 (2×C, C-1′+C-7′), 159.0 (2×C, 2×C-7), 154.1 (2×C, 2×C-9), 148.3 (2×C, C-2′+C-6′), 143.0 (2×CH, 2×C-15), 138.7 (3×CH, 2×C-16+C-4′), 128.2 (2×CH, C-3′+C-5′), 125.0 (2×C, C-13), 124.1 (2×C, C-18), 110.8 (2×CH, 2×C-14), 48.6 (2×CH, 2×C-5), 41.9 (2×CH_2, 2×C-3), 39.6 (2×C, 2×C-10), 36.4 (2×CH_2, 2×C-1), 33.5(2×C, 2×C-4), 33.0 (2×CH_3, 2×C-19), 29.4 (2×CH_2, 2×C-11), 25.3 (2×CH_2, 2×C-12), 21.5 (2×CH_3, 2×C-19), 20.8 (2×CH_2, 2×C-6), 19.0 (2×CH_2, 2×C-2), 18.9 (2×CH_3, 2×C-20), 13.1 (2×CH_3, 2×C-17); HRESIMS m/z 784.4305 [M + Na]$^+$ (calcd for $C_{47}H_{59}N_3O_6Na$, 784.4302).

Preparation of Compounds 17 and 18. To 47.6 mg (0.15 mmol) of compound **7** in 6 mL of CH_2Cl_2 were added 1.5 equiv of terephthaloyl chloride (46.0 mg), 3 equiv of triethylamine (63.1 μL), and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 12 h, and then the solvent was removed. The resulting residue was purified by preparative TLC to yield 2.7 mg (8%) of compound **17** and 36.4 mg (64%) of compound **18**.

Compound 17. Yellow oil; $[\alpha]^{20}_D$ −21 (c 0.3 $CHCl_3$); IR (neat) ν_{max} 3633, 2904, 2595, 1731, 1392, 1226, 1014, 798, 775, 721, 609 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.19 (2H, bs, H-3′+H-4′), 8.12 (2H, bs, H-2′+H-6′), 7.38 (1H, bs, H-15), 7.27 (1H, bs, H-16), 6.32 (1H, bs, H-14), 3.17 (1H, d, J = 14.9 Hz, H-6a), 2.54 (3H, m, H-12, H-11a), 2.40 (2H, m, H-11b, H-6b), 2.02 (3H, s, H_3-17), 1.97 (1H, d, J = 10.0 Hz, H-1a), 1.63 2H, m, H-2), 1.47 (1H, m, H-5), 1.40 (1H, td, J = 12.7, 2.8 Hz, H-3a), 1.22 (2H, m, H-1b, H-3b), 1.02 (3H, s, H_3-20), 0.97 (3H, s, H_3-19), 0.95 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.0 (C, CO_2H), 163.5 (C, NOCO), 159.6 (C, C-7), 151.6 (C, C-9), 143.1 (CH, C-15), 138.7 (CH, C-16), 135.8 (C, C-1′), 130.4 (CH, C-3′, C-5′), 129.6 (CH, C-2′, C-6′), 128.4 (C, C-4′), 124.7 (C, C-13), 124.1 (C, C-8), 110.8 (CH, C-14), 45.8 (CH, C-5), 41.6 (CH_2, C-3), 39.9 (C, C-10), 36.1 (CH_2, C-1), 33.5 (C, C-4), 32.9 (CH_3, C-18), 29.8 (CH_2, C-11), 25.0 (CH_2, C-12), 22.7 (CH_2, C-6), 21.6 (CH_3, C-19), 18.8 (CH_2, C-2), 18.7 (CH_3, C-20), 13.3 (CH_3, C-17); HRESIMS m/z 462.2283 [M − H]$^+$ (calcd for $C_{28}H_{32}NO_5$, 462.2280).

Compound 18. Amorphous white solid; $[\alpha]^{20}_D$ −28 (c 0.4, $CHCl_3$); IR (neat) ν_{max} 2911, 2846, 1743, 1569, 1234, 1068, 1014, 968, 779, 601 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.21 (4H, bs, 4×CHAr), 7.38 (2H, bs, 2×H-15), 7.28 (2H, bs, 2×H-16), 6.33 (2H, bs, 2×H-14), 3.17 (2H, dd, J = 14.1, 3.8 Hz, 2×H-6a), 2.55 (6H, m,

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2×H-12, 2×H-11a), 2.42 (4H, m, 2×H-6b, 2×H-11b), 2.04 (6H, s, 2×H-17), 1.98 (2H, d, J = 12.4 Hz, 2×H-1a), 1.64 (4H, m, 2×H-2), 1.49 (4H, m, 2×H-3a, 2×H-5), 1.42 (2H, td, J = 12.8, 3.3 Hz, 2×H-1b), 1.24 (2H, td, J = 13.3, 3.8 Hz, 2×H-3b), 1.03 (6H, s, 2×H_3-20), 0.99 (6H, s, 2×H_3-18), 0.97 (6H, s, 2×H_3-19); ^{13}C NMR ($CDCl_3$, 125 MHz,) δ 166.1 (2×C, 2×C═O), 163.4 (2×C, 2×C-7), 159.6 (2×C, 2×C-9), 143.1 (2×CH, 2×C-15), 138.7 (2×CH, 2×C-16), 133.7 (2×C, 2×CAr), 129.8 (4xCH, 4xCHAr), 124.7 (2×C, 2×C-8), 124.1 (2×C, 2×C-13), 110.8 (2×CH, 2×C-14), 48.5 (2×CH, 2×C-5), 41.6 (2×CH_2, 2×C-3), 39.9 (2×C, 2×C-10), 36.1 (2×CH_2, 2×C-1), 33.5 (2×C, 2×C-4), 32.9 (2×CH_3, 2×C-18), 29.8 (2×CH_2, 2×C-11), 25.0 (2×CH_2, 2×C-12), 22.7 (2×CH_2, 2×C-6), 21.6 (2×CH_3, 2×C-19), 18.8 (2×CH_2, 2×C-2), 18.7 (2×CH_3, 2×C-20), 13.3 (2×CH_3, 2×C-17); HRESIMS m/z 783.4344 $[M + Na]^+$ (calcd for $C_{48}H_{60}N_2O_6Na$, 783.4349).

Preparation of Compound 19. To 15.0 mg (0.04 mmol) of compound **7** in 5 mL of CH_2Cl_2 were added 0.5 equiv of adipoyl chloride (4.0 μL) and 1.5 equiv of triethylamine (10.0 μL). The reaction mixture was stirred for 72 h. Then, the solvent was removed, and the residue was treated with 5 mL of H_2O and extracted with CH_2Cl_2 (3 × 10 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed. The residue was purified by preparative TLC with hexanes/EtOAc (7:3) to yield 8.0 mg (54%) of compound **19** as an amorphous white solid: $[\alpha]^{20}_D$ −24 (c 0.3, $CHCl_3$); IR (neat) ν_{max} 2842, 2834, 1643, 1365, 1230, 1052, 987, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37 (2H, bs, 2×H-15), 7.26 (2H, bs, 2×H-16), 6.31 (2H, d, J = 0.6 Hz, 2×H-14), 2.94 (2H, dd, J = 14.2, 3.9 Hz, 2×H-6a), 2.49 (4H, m, H-2′+H-5′), 2.45 (4H, m, 2×H-11a, 2×H-12), 2.32 (2H, m, H-11b), 2.18 (2H, dd, J = 14.3, 14.2 Hz, 2×H-6b), 1.89 (2H, d, J = 9.1 Hz, 2×H-1a), 1.87 (6H, s, 2×H_3-17), 1.77 (4H, m, H-3′+H-4′), 1.58 (2H, dt, J = 13.8, 2.8 Hz, 2×H-5), 1.51 (4H, m, 2×H-2), 1.40 (2H, d, J = 13.3 Hz, 2×H-3a), 1.33 (2H, m, 2×H-1b), 1.14 (2H, td, J = 13.4, 3.9 Hz, 2×H-3b), 0.92 (6H, s, 2×H_3-20), 0.88 (6H, s, 2×H_3-18), 0.87 (6H, s, 2×H_3-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.9 (2×C, C-1′+C-6′), 164.5 (2×C, 2×C-7), 158.8 (2×C, 2×C-9), 143.1 (2×CH, 2×C-15), 138.7 (2×CH, 2×C-16), 124.8 (2×C, 2×C-13), 124.1 (2×C, 2×C-8), 110.8 (2×CH, 2×C-14), 48.5 (2×CH, 2×C-5), 41.7 (2×CH_2, C-3), 39.8 (2×C, 2×C-10), 36.2 (2×CH_2, 2×C-1), 33.5 (2×C, 2×C-4), 32.9 (2×CH_2, C-2′+C-5′), 32.8 (2×CH_3, 2×C-18), 29.8 (2×CH_2, 2×C-11), 25.0 (2×CH_2, 2×C-12), 24.5 (2×CH_2, C-3′+C-4′), 22.6 (2×CH_2, 2×C-6), 21.5 (2×CH_3, 2×C-19), 18.8 (2×CH_2, 2×C-2), 18.8 (2×CH_3, 2×C-20), 13.2 (2×CH_3, 2×C-17); HRESIMS m/z 763.4660 $[M + Na]^+$ (calcd for $C_{46}H_{64}N_2O_6Na$, 763.4662).

Cell Culture and Viability Assay. J774A.1 murine macrophage cells were purchased from American Type Cell Culture (ATCC, Manassas, VA, USA) and were cultured in Dulbecco's modified Eagle medium with 10% fetal bovine serum, 1% penicillin/streptomycin, and L-arginine (1 mM) (Life Technologies).

Cell viability was assessed by the mitochondrial-dependent reduction of the MTT (Sigma) to formazan, as described previously.[34]

Determination of NO Production. The production of NO was determined by the accumulation of nitrite in the medium, using the Griess reagent.[17]

Inflammasome Activation. For induction of NLRP3 inflammasome activation, confluent cells were plated overnight in six-well plates at a density of 10^6 cells/mL. Then, the medium was changed, and cells were primed with 1 μg/mL LPS for 5 h. Diterpenoids were added for another 30 min, and next, the cells were stimulated with 20 μM nigericin and 5 mM ATP for 45 min or 100 μg/mL MSU for 24 h (Sigma). MCC950 (300 nM) was used as reference inflammasome inhibitor (InvivoGen).

Enzyme-Linked Immunosorbent Assay (ELISA). Supernatants from cell cultures were collected, and IL-1β levels were determined by a mouse Il-1β ELISA kit (R&D Systems), according to the manufacturer's instructions.

Immunoblot Analysis. J774A.1 cells were lysed by buffer A, containing 0.5% Chaps, 10 mM Tris pH 7.5, 1 mM EGTA, 1 mM $MgCl_2$, 10% glycerol, and 5 mM β-mercaptoetanol, supplemented with phosphatase and protease inhibitor cocktails (Sigma). Protein content was assayed with the Bio-Rad protein reagent.

Cell lysates and supernatant proteins were separated by SDS-PAGE gel electrophoresis and transferred onto PVDF membranes (Millipore) and proved with anti-cleaved IL-1β and anti-pro-IL-1β, anti-cleaved caspase-1 and anti-pro-caspase-1, and anti-β-actin (Santa Cruz Biotechnology) antibodies. Blots were developed with ECL according to the manufacturer's instructions (GE Healthcare).

Statistical Analysis. All values were expressed as means ± standard deviation (SD) from at least three experiments and were analyzed by one-way ANOVA, considering a significance level of $p < 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00200.

^{1}H NMR and ^{13}C NMR spectra of compounds **1**−**19** (PDF)

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Notes

The authors declare no competing financial interest.

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