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SYNTHESIS FROM NORAMBREINOLIDE, STRUCTURE, AND ANTIMICROBIAL ACTIVITY OF DIHOMODRIMANE SESQUITERPENOIDS WITH AZINE, HYDRAZIDE, AND DIHYDRAZIDE FRAGMENTS

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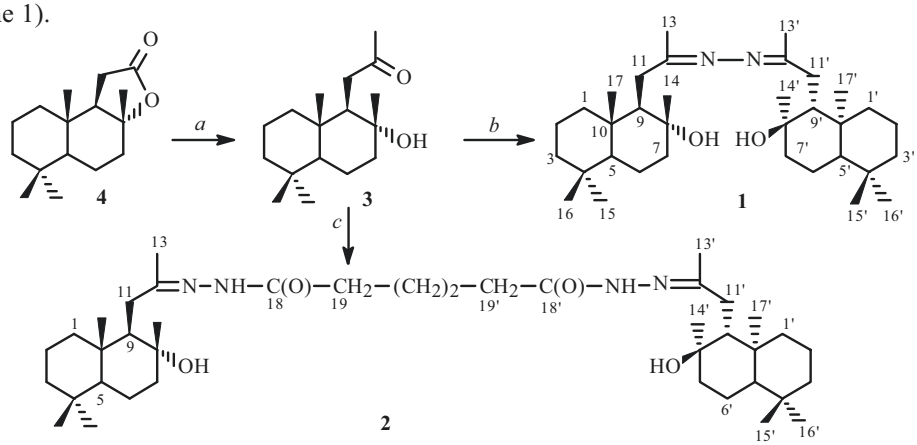
12,12′-Azino-di-8α-hydroxy-11-dihomodrimane, N,N′*-di-(8α-hydroxy-11-dihomodrim-12-ylidene)-adipic acid dihydrazide,* N,N′*-di-(*$\Delta^{8,13}$*-bicyclohomofarnesenoyl)-hydrazine, and hydrazides of* $\Delta^{8,9}$*- and* $\Delta^{8,13}$*-bicyclohomofarnesenoic acids were synthesized.*

Keywords: dihomodrimane sesquiterpenoids, norambreinolide, azines, hydrazides, bicyclohomofarnesenoic acid, synthesis.

New dihomodrimanes containing pharmacophoric azine and hydrazide fragments were synthesized in order to discover new biologically active compounds and to continue our studies on the synthesis of *N*-containing drimane sesquiterpenoids [1–5]. Several azines and hydrazides are known to possess high and varied biological activity [6, 7], including antituberculosis and antibacterial. We supposed that adding these fragments to the drimane scaffold could increase its biological potential.

The starting material for synthesizing azine **1**, dihydrazide **2**, and 11-dihomodriman-8α-ol-12-one (**3**) was prepared from norambreinolide **4**, as described previously by us [8]. Reaction of **3** with hydrazine hydrate in MeOH formed **1**. Refluxing **3** with adipic acid dihydrazide in MeOH gave **2**, in which two ketones were condensed through the carbonyls to adipic acid dihydrazide (Scheme 1).



a. CH_3Li, Et_2O, 20°C, 15 min, 65%; *b*. $N_2H_4{\cdot}H_2O$, CH_3OH, Δ, 10 h, 80%; *c*. $NH_2NHCO(CH_2)_4CONHNH_2$, CH_3OH, 20°C, 48 h, Δ, 20 h, 31%

Scheme 1

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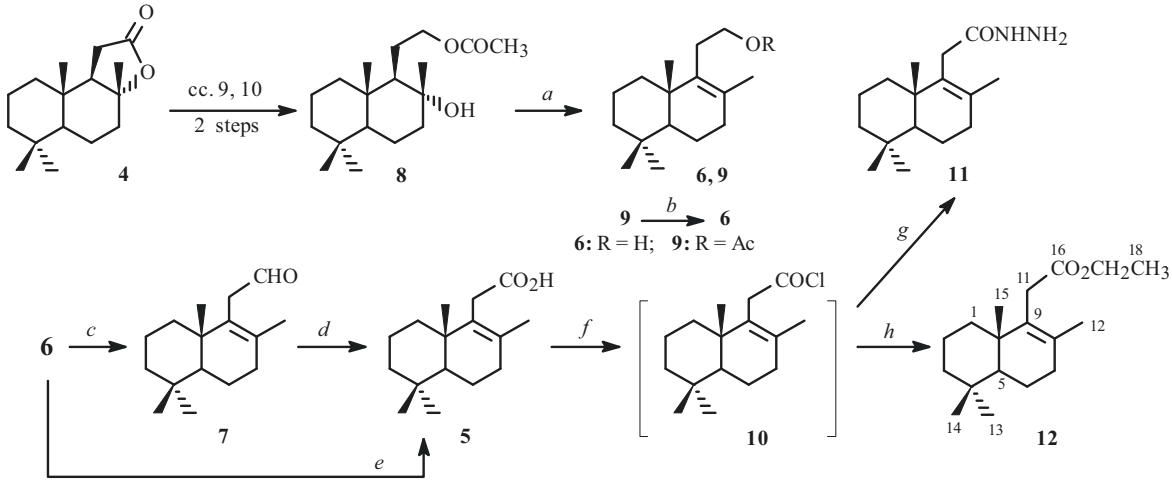
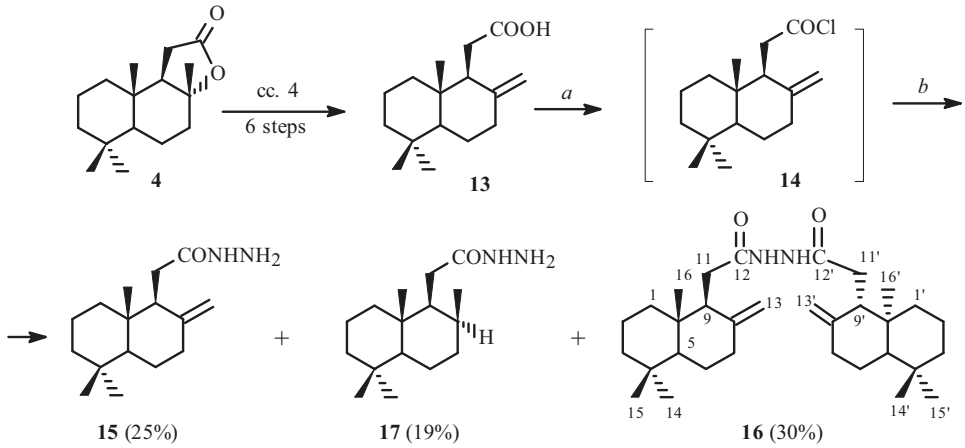
Norambreinolide (**4**) yielded (in six steps) in 62% overall yield $\Delta^{8,9}$-bicyclohomofarnesenoic acid (**5**). The key steps in this synthesis were oxidation of $\Delta^{8,9}$-bicyclohomofarnesen-12-ol (**6**) by P_2O_5 and DMSO into aldehyde **7** and subsequent oxidation of this aldehyde by $NaClO_2$ into acid **5**. Direct oxidation of **6** by Jones reagent gave acid **5** in only 42% yield. Alcohol **6** was produced via dehydration of sclaradiol monoacetate **8** [9, 10] by $MeSO_3SiMe_3$ (trimethylsilylmethanesulfonate) in MeCN to form acetate **9** that was subsequently hydrolyzed. Reaction of acid **5** with $(COCl)_2$ produced *in situ* acid chloride **10**, reaction of which with $N_2H_4 \cdot H_2O$ in CH_2Cl_2 afforded hydrazide **11**. The main product from the reaction in EtOH was ethyl $\Delta^{8,9}$-bicyclohomofarnesenoate (**12**) in 55% yield (Scheme 2).



a. $MeSO_3SiMe_3$, CH_3CN, 20°C, 5 h, 72%; *b*. KOH, MeOH, 20°C, 2 h, 99%; *c*. DMSO, P_2O_5, CH_2Cl_2, 0°C, 10 min, 20°C, 45 min; Et_3N, 0°C, 10 min, 20°C, 45 min, 95 %; *d*. $NaClO_2$, $NaH_2PO_4 \cdot 2H_2O$, 2-Me-2-butene, *t*-BuOH, 20°C, 2 h, 94 %; *e*. Jones reagent, $(CH_3)_2CO$, 0°C, 48 h, 42 %; *f*. $(COCl)_2$, C_6H_6, 20°C, 1 h, Δ, 1 h; *g*. $N_2H_4 \cdot H_2O$, CH_2Cl_2, 20°C, 2 h, Δ, 7 h, 65%; *h*. $N_2H_4 \cdot H_2O$, C_2H_5OH, 20°C, 7 h, 55%

Scheme 2

Norambreinolide **4** was also used to synthesize $\Delta^{8,13}$-bicyclohomofarnesenoic acid (**13**) in 60% overall yield as before [4]. Reaction of its acid chloride **14** with hydrazine hydrate in CH_2Cl_2 gave its hydrazide **15** and *N*,*N*′-di-($\Delta^{8,13}$-bicyclohomofarnesenoyl)-hydrazine (**16**) and hydrazide **17** with a reduced double bond. These compounds were isolated pure by liquid chromatography (Scheme 3).



a. $(COCl)_2$, C_6H_6, 20°C, 1 h, Δ, 1 h; *b*. $N_2H_4 \cdot H_2O$, CH_2Cl_2, 20°C, 10 h, Δ, 10 h

Scheme 3

PMR, ^{13}C NMR, ^{15}N NMR, and IR spectroscopic data and mass spectral analysis including high-resolution in addition to an X-ray crystal structure analysis for the initially prepared **1**, **2**, **5**–**7**, **11**, **12**, and **15**–**17** agreed with the formulas given in the schemes. ^1H–^1H NOESY experiments demonstrated that the C-8 and C-10 methyls coupled so that C-8 of hydrazide **17** was assigned the *S*-configuration. PMR, ^{13}C NMR, and ^{15}N NMR spectra of **1** in $CDCl_3$ were consistent with two isomers in a 2:1 ratio. Analogously, PMR and ^{13}C NMR spectra of dihydrazide **2** in $CDCl_3$ showed that it also existed as two isomers in an approximately 1:1 ratio. Special studies are required to establish their structures.

TABLE 1. Crystal Data and Structure Refinement for **1**

Empirical formula	$C_{34}H_{60}N_2O_2$
Formula weight	528.84
Crystal system	Monoclinic
Space group	$P2_1$
a, Å	15.1323(7)
b, Å	6.1227(3)
c, Å	17.6171(8)
β, deg.	95.567(4)
V, Å^3	1624.53(13)
Z	2
ρ (calcd), g/cm^3	1.081
μ, mm^{-1}	0.499
F(000)	588
Crystal size, mm	$0.35 \times 0.08 \times 0.02$
θ-range, deg.	7.358–133.19
Limiting indices	$-18 \le h \le 18$; $-7 \le k \le 7$; $-20 \le l \le 20$
Reflections collected/unique (R_{int})	19919/5473 (0.0967)
Number of refined parameters	356
GOOF	1.039
R- factor ($I > 2\sigma(I)$)	$R_1 = 0.0569$ ($wR_2 = 0.1252$)
R-factor (whole dataset)	$R_1 = 0.0759$ ($wR_2 = 0.1362$)
$\Delta\rho_{max}$, $\Delta\rho_{min}$, *e* Å^{-3}	0.14, –0.16

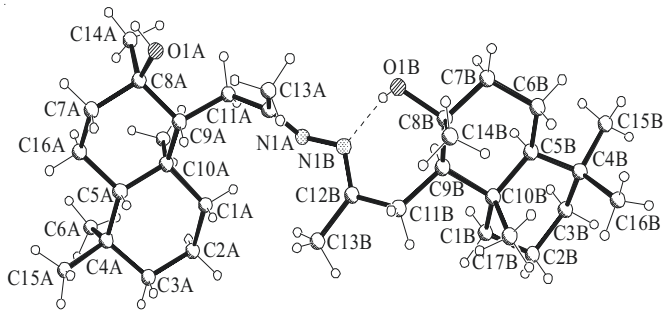


Fig. 1. Molecular structure of **1**.

An X-ray crystal structure analysis of **1** found that the molecule had the *trans*-configuration in the single crystal. Figure 1 shows the molecular structure of the asymmetric unit. It consisted of two chemically equivalent but crystallographically independent parts designated A and B and joined by the N1A–N1B bond of 1.417(5) Å.

An intermolecular H-bond with O1A–H as the proton donor and O1B–H as the proton acceptor formed in the crystal. However, O1B–H acted as a donor in forming an intramolecular H-bond with N1B. The molecules were combined into infinite chains as a result of these interactions.

Compounds **1**, **2**, **11**, **15**, **16**, and **17** were tested *in vitro* for antifungal and antibacterial activity against pure cultures of five fungal species (*Aspergillus flavus*, *Fusarium*, *Penicillium chrysogenum*, *P. frequentans*, *Alternaria alternata*) and Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Bacillus* sp.) bacteria. Compound **2** exhibited significant antifungal activity with a characteristic minimum inhibiting concentration (MIC) of 0.50 μg/mL, which was comparable with the activity of the known antifungal agent caspofungin that was used as the reference drug (MIC = 0.25 μg/mL), and moderate antibacterial activity (MIC = 24 μg/mL) compared with the reference antibiotic kanamycin (MIC = 3 μg/mL).

Thus, new dihomodrimane sesquiterpenoids containing azine, hydrazide, and dihydrazide fragments were synthesized. It was found that *N*,*N*′-di-(8α-hydroxy-11-dihomodrim-12-ylidene)-adipic acid dihydrazide (**2**) possessed significant antifungal and antibacterial activity.

EXPERIMENTAL

Melting points were measured on a Boetius apparatus; $[\alpha]_D$ values, in $CHCl_3$ on a JASCO P-2000 polarimeter. IR spectra were recorded on a PerkinElmer Spectrum 100 FTIR spectrophotometer. PMR, ^{13}C NMR, and ^{15}N NMR spectra were recorded in $CDCl_3$ on a Bruker Avance III 400 spectrometer (400, 100, and 40 MHz). Chemical shifts were given on the δ-scale in ppm relative to $CD(H)Cl_3$ resonances as internal standards (resonances of δ 7.24 and 77.00 ppm, respectively) in PMR and ^{13}C NMR spectra and relative to $MeNO_2$ external standard in ^{15}N NMR spectra. Resonances in ^{13}C NMR spectra were assigned using ^1H–^{13}C DEPT, ^1H–^1H COSY-45, ^1H–^{13}C HMQC, ^1H–^{13}C HMBC, and ^1H–^1H NOESY experiments; in ^{15}N NMR spectra, ^1H–^{15}N HMBC and ^1H–^{15}N HMQC experiments. The course of reactions was monitored by TLC on Silufol plates with detection by I_2 vapor. Products were isolated using column chromatography over Fluka-60 silica gel and a Gilson HPLC with a refraction detector. The product compositions were determined and mass spectra were recorded on an Agilent 7890A chromatograph with an MSD 5975C VL quadrupole MS detector and an HP-5ms capillary column (30 m × 0.25 μm). The vaporizer temperature was 250°C; ionization potential 70 eV. Analysis conditions: T_1 = 70°C (2 min), 5°C/min to 200°C, 20°C/min to 300°C, T_2 = 300°C (5 min). The He flow rate was 1 mL/min. High-resolution mass spectra (HR-EI-MS) were recorded on an AEI MS 902 spectrometer (EI 70 eV). Et_2O and EtOAc extracts were dried over anhydrous $MgSO_4$. Solvents were distilled from extracts at reduced pressure. Elemental analyses of all compounds agreed with those calculated.

Preparation of 12,12′-Azino-di-8α-hydroxy-11-dihomodrimane (1). A solution of **3** (100 mg, 0.37 mmol) in MeOH (3 mL) was treated with $N_2H_4 \cdot H_2O$ (0.2 mL, 0.206 g, 4.11 mmol), stirred, and refluxed for 10 h. The MeOH was removed at reduced pressure. The residue was treated with H_2O (5 mL) and EtOAc (30 mL) and stirred. The organic layer was separated. The aqueous layer was extracted with EtOAc (10 mL). The combined EtOAc extracts were washed with H_2O (3 × 5 mL) and dried. The EtOAc was distilled. The crystalline solid was recrystallized from MeCN to afford **1** (80 mg, 80%), mp 168–169°C (MeCN), $[\alpha]_D^{26}$ –151.1° (*c* 0.53, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3381, 3222 (OH), 1636, 1619 (C=N). **Main isomer.** ^1H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80 (6H, s, CH_3-16, 16′), 0.82 (6H, s, CH_3-17, 17′), 0.87 (6H, s, CH_3-15, 15′), 0.95* (2H, m, H-5α, 5′α), 1.19 (6H, s, CH_3-14, 14′), 1.86* (2H, m, H-9α, 9′α), 1.93 (6H, s, CH_3-13, 13′), 2.31 (2H, dd, J = 16.7, 6.4, H-11a, 11′a), 2.50 (2H, dd, J = 16.7, 2.3, H-11b, 11′b), 3.77 (2H, br.s, 2 × (OH)). (*overlapped resonances). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 15.49 (C-17, 17′), 18.47 (C-2, 2′), 18.71 (C-13, 13′), 20.44 (C-6, 6′), 21.44 (C-16, 16′), 24.13 (C-14, 14′), 33.35 (C-4, 4′), 33.38 (C-15, 15′), 34.54 (C-11, 11′), 38.81 (C-10, 10′), 39.63 (C-1, 1′), 41.84 (C-3, 3′), 44.24 (C-7, 7′), 56.11 (C-5, 5′), 57.19 (C-9, 9′), 73.12 (C-8, 8′), 168.54 (C-12, 12′). ^{15}N NMR spectrum (40 MHz, $CDCl_3$, δ, ppm): 336, 329 (2 × (C=N)). **Minor isomer.** ^1H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.77 (6H, s, CH_3-17, 17′), 0.80 (6H, s, CH_3-16, 16′), 0,87 (6H, s, CH_3-15, 15′), 0.95* (2H, m, H-5α, 5′α), 1.27 (6H, s, CH_3-14, 14′), 1.80* (2H, m, H-11a, 11′a), 2.09 (6H, s, CH_3-13, 13′), 2.70* (2H, m, H-11b, 11′b), 5.48 (2H, br.s, 2 × (OH)). ^{15}N NMR spectrum (40 MHz, $CDCl_3$, δ, ppm): 338, 331 (2 × (C=N)). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 495 (M^+ – 34 (2 OH), 7), 405 (4), 355 (4), 315 (9), 281 (41), 246 (52), 207 (100), 191 (82), 175 (27), 137 (29), 121 (40), 109 (71), 95 (48), 81 (47), 69 (59), 55 (48), 43 (68). HR-EI-MS: found 529.4709. $C_{34}H_{60}N_2O_2$. Calcd 528.8602.

Preparation of *N*,*N*′-Di-(8α-hydroxy-11-dihomodrim-12-ylidene)-adipic Acid Dihydrazide (2). Adipic acid dihydrazide (32 mg, 0.18 mmol) was dissolved with heating in MeOH (16 mL). The solution was stirred, cooled to room temperature, treated with ketone **3** (100 mg, 0.37 mmol), stirred at 20°C for 48 h, and refluxed for 20 h. The MeOH was removed at reduced pressure. The residue was dried over P_2O_5 in a vacuum desiccator, treated with $CHCl_3$ (5 mL), and stirred for 1 h. The insoluble precipitate (15 mg) was filtered off and rinsed with $CHCl_3$. The filtrates were evaporated. The residue was dried over P_2O_5 to afford a product (136 mg) that was recrystallized from hexane. The crystalline precipitate was filtered off and rinsed with hexane to afford a mixture (80 mg) of the reaction product and starting ketone **3**. The hexane filtrate was evaporated to give unreacted **3** (37 mg). Target compound **2** was separated from its mixture (80 mg) with **3** by column chromatography over silica gel (4 g). Ketone **3** (9 mg) was eluted by $CHCl_3$; dihydrazide **2** (31%; considering unreacted **3**, the yield of dihydrazide **2** was 57%), by $CHCl_3$–MeOH (24:1). **Dihydrazide 2.** White crystals, mp 101–102°C (hexane), $[\alpha]_D^{26}$ –60.6° (*c* 1.35, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3400, 3227, 2923, 1662, 1534, 1454, 1387, 1250, 1067, 938. ^{15}N NMR spectrum (40 MHz, $CDCl_3$, δ, ppm): 169 (NH), 299, 301, 302, 305 (C=N). **Main isomer.** ^1H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80 (6H, s, CH_3-15, 15′), 0.83 (6H, s, CH_3-17, 17′), 0.84 (6H, s, CH_3-16, 16′), 1.22, 1.25 (6H, s, CH_3-14, 14′), 2.11, 2.13 (6H, s, CH_3-13, 13′), 3.0 (2H, br.s, 2 × (OH)), 8.27, 8.37 (2H, br.s, 2 × NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 15.20, 15.26 (C-17, 17′), 18.43, 18.50 (C-6, 6′), 20.36 (C-2, 2′), 21.39, 21.51 (C-16, 16′), 23.99, 24.16 (C-14, 14′), 24.05, 24.49 (C-20, 20′), 26.11, 26.19 (C-13, 13′), 28.29 (C-11, 11′), 32.04, 32.67 (C-19, 19′), 33.30 (C-4, 4′),

33.41 (C-15, 15′), 38.70, 38.76 (C-10, 10′), 39.72, 39.87 (C-1, 1′), 41.72, 41.80 (C-3, 3′), 43.96, 44.11 (C-7, 7′), 56.24 (C-5, 5′), 57.22, 57.27 (C-9, 9′), 74.96, 75.29 (C-8, 8′), 156.61, 157.85 (C-12, 12′), 175.07, 175.26 (C-18, 18′). **Minor isomer.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.86 (6H, s, CH_3-15, 15′), 0.88 (6H, s, CH_3-16, 16′), 0.89 (6H, s, CH_3-17, 17′), 1.21 (6H, s, CH_3-14, 14′), 1.85, 1.89 (6H, s, CH_3-13, 13′), 3.0 (2H, br.s, 2 × (OH)), 11.44, 11.46 (2H, br.s, 2 × NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 15.43, 15.48 (C-17, 17′), 15.65, 16.07 (C-13, 13′), 18.22, 18.26 (C-6, 6′), 20.36 (C-2, 2′), 21.43, 21.47 (C-16, 16′), 24.05, 24.49 (C-20, 20′), 24.24 (C-14, 14′), 32.04, 32.67 (C-19, 19′), 33.20 (C-4, 4′), 33.28 (C-15, 15′), 34.59, 34.70 (C-11, 11′), 38.80, 38.87 (C-10, 10′), 40.03, 40.20 (C-1, 1′), 41.45, 41.51 (C-3, 3′), 44.36, 44.50 (C-7, 7′), 55.84, 55.90 (C-5, 5′), 56.85, 56.91 (C-9, 9′), 73.55, 73.97 (C-8, 8′), 154.68, 155.61 (C-12, 12′), 169.70, 170.23 (C-18, 18′). HR-EI-MS: found 671.5471. $C_{40}H_{70}N_4O_4$. Calcd 671.0174.

Preparation of 12-Acetoxy-$\Delta^{8,9}$-bicyclohomofarnesen-12-ol (9). A solution of sclaradiol monoacetate (**8**, 2 g, 6.75 mmol) [9, 10] in anhydrous MeCN (18 mL) was treated with Me_3SiSO_3Me (5.3 mL, 5.78 g, 34.3 mmol), stirred for 5 h at 20°C, diluted with H_2O (100 mL) and Et_2O (300 mL), and stirred. The aqueous layer was separated. The Et_2O layer was washed with $NaHCO_3$ solution (3 × 30 mL) and H_2O (3 × 30 mL) and dried. The Et_2O was evaporated to afford a mixture of the $\Delta^{8,9}$, $\Delta^{7,8}$, and $\Delta^{8,13}$ isomers (1.6 g, 85%) in an 85:11:2 ratio (from PMR spectra and GC-MS analysis). The mixture (1 g) of isomers was chromatographed over a column of silica gel (100 g). Elution by petroleum ether–Et_2O (98.5:1.5) eluted the isomer (0.8 g) with the double bond in the C_8–C_9 position (**9**). PMR and ^{13}C NMR and IR spectroscopic data and mass spectral analysis of this compound agreed fully with those in the literature [11].

Preparation of $\Delta^{8,9}$-Bicyclohomofarnesen-12-ol (6). Acetate **9** (0.46 g, 16.5 mmol) was treated with a solution of KOH (0.46 g, 82.0 mmol) in MeOH (14 mL) and stirred for 2 h at 20°C. The MeOH was distilled at reduced pressure. The residue was treated with H_2O (25 mL) and Et_2O (50 mL) and stirred. The aqueous layer was extracted with Et_2O (3 × 15 mL). The combined Et_2O extracts were washed with H_2O (5 × 10 mL) and dried. The Et_2O was evaporated to afford **6** (386 mg, 99%). $C_{16}H_{28}O$, $[\alpha]_D^{16}$ +95.2° (*c* 1.39, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3306, 1036, 1019 (OH). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83 (3H, s, CH_3-15), 0.88 (3H, s, CH_3-14), 0.95 (3H, s, CH_3-16), 1.09 (1H, dd, J = 12.6, 6.1, H-5α), 1.61 (3H, s, CH_3-13), 1.96 (1H, dd, J = 17.7, 6.5, H-7a), 2.05 (1H, td, J = 17.7, 10.9, 7.0, H-7b), 2.24 (1H, td, J = 13.3, 9.7, 6.2, H-11a), 2.39 (1H, td, J = 13.3, 9.8, 6.9, H-11b), 3.59 (1H, td, J = 16.5, 9.7, 6.2, H-12a), 3.62 (1H, td, J = 16.5, 9.8, 6.9, H-12b). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 19.00 (C-2), 19.02 (C-6), 19.94 (C-13), 20.09 (C-16), 21.70 (C-15), 31.50 (C-11), 33.32 (C-14), 33.34 (C-4), 33.66 (C-7), 37.15 (C-1), 38.68 (C-10), 41.73 (C-3), 51.69 (C-5), 62.64 (C-12), 128.56 (C-8), 136.19 (C-9). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 236 (M^+, 40), 221 (63), 203 (26), 191 (97), 177 (42), 163 (34), 151 (41), 135 (43), 121 (85), 107 (88), 95 (100), 79 (61), 69 (55), 55 (54), 41 (69).

Preparation of $\Delta^{8,9}$-Bicyclohomofarnesen-12-al (7). A solution of **6** (0.24 g, 1.01 mmol) in CH_2Cl_2 (10 mL) was stirred, cooled in an ice bath, and treated with anhydrous DMSO (0.6 mL, 0.66 g, 8.45 mmol) and P_2O_5 (0.97 g, 6.83 mmol). After 10 min, the flask was removed from the ice bath. The solution was stirred for another 45 min at room temperature, treated with Et_3N (0.72 mL, 0.52 g, 5.14 mmol) with cooling in ice, stirred in the ice bath for 10 min and at room temperature for 45 min, treated dropwise with H_2O (3 mL) and HCl (5%, 3 mL) with cooling in the ice bath, and extracted with CH_2Cl_2 (3 × 25 mL). The extract was washed with H_2O (2 × 8 mL) and dried. The solvent was evaporated to afford **7** (0.23 g, 95% according to GC-MS analysis). $C_{16}H_{26}O$, $[\alpha]_D^{16}$ +138.6° (*c* 1.40, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1723 (CHO). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.84 (3H, s, CH_3-15), 0.90 (3H, s, CH_3-14), 0.94 (3H, s, CH_3-16), 1.55 (3H, s, CH_3-13), 1.19* (1H, m, H-5α), 3.05, 3.11 (each 1H, AB system, J = 17.1, H-11a, 11b), 9.53 (1H, t, J = 2.3, H-12). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 18.89 (C-2, 6), 19.77 (C-16), 19.93 (C-13), 21.58 (C-15), 33.19 (C-14), 33.29 (C-4), 33.86 (C-7), 37.22 (C-1), 38.46 (C-10), 41.49 (C-3), 43.18 (C-11), 51.70 (C-5), 131.25 (C-8), 132.15 (C-9), 201.43 (C-12). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 234 (M^+, 17), 219 (32), 201 (43), 190 (95), 175 (100), 163 (23), 149 (50), 133 (21), 123 (43), 105 (60), 91 (55), 79 (36), 69 (39), 55 (35), 41 (47).

Preparation of $\Delta^{8,9}$-Bicyclohomofarnesenoic Acid (5). A solution of **7** (0.23 g, 0.98 mmol) in *t*-BuOH (23 mL) and 2-methyl-2-butene (5 mL) was stirred, treated over 10 min with a solution of $NaClO_2$ (1.13 g, 12.5 mmol) and $NaH_2PO_4 \cdot 2H_2O$ (1.15 g, 7.37 mmol) in H_2O (10 mL), and stirred for 2 h at 20°C. The solvents were evaporated at reduced pressure. The residue was treated with H_2O (25 mL), acidified with HCl (10%), and extracted with Et_2O (3 × 50 mL). The Et_2O extract was washed with H_2O (3 × 15 mL) and dried. The Et_2O was evaporated to afford **5** (0.24 g, 97% according to GC-MS analysis). $C_{16}H_{26}O_2$, $[\alpha]_D^{17}$ +70.2° (*c* 1.44, $CHCl_3$). IR spectrum (ν, cm^{-1}): 2670, 1703 (COOH). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83 (3H, s, CH_3-15), 0.89 (3H, s, CH_3-14), 0.93 (3H, s, CH_3-16), 1.21 (1H, dd, J = 12.6, 1.90, H-5α), 1.59 (3H, s, CH_3-13), 2.02 (1H, dd, J = 18.6, 6.2, H-7a), 2.13 (1H, dd, J = 18.6, 11.3, H-7b), 3.01 (1H, d, J = 17.3, H-11a), 3.14 (1H, d, J = 17.3, H-11b), 9.58 (1H, br.s, OH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 18.86 (C-2), 18.89 (C-6), 19.73 (C-16),

20.10 (C-13), 21.61 (C-15), 32.94 (C-11), 33.13 (C-14), 33.27 (C-4), 33.56 (C-7), 36.26 (C-1), 38.58 (C-10), 41.48 (C-3), 51.38 (C-5), 130.78 (C-8), 133.42 (C-9), 178.85 (C-12). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 250 (M^+, 35), 235 (66), 190 (100), 175 (96), 165 (79), 153 (27), 139 (67), 119 (77), 105 (77), 91 (72), 79 (46), 69 (42), 55 (39), 41 (66).

Preparation of $\Delta^{8,9}$-Bicyclohomofarnesenoic Acid Hydrazide (11). A solution of **5** (100 mg, 0.40 mmol) in anhydrous benzene (2 mL) was treated with a solution of $(COCl)_2$ (0.4 mL, 0.58 g, 4.58 mmol) in benzene (1 mL), stirred at 20°C for 1 h and refluxed for 1 h. The benzene and excess of $(COCl)_2$ were evaporated at reduced pressure. The residue was treated with CH_2Cl_2 (2 mL) and $N_2H_4{\cdot}H_2O$ (1 mL), stirred for 2 h at 20°C, refluxed for 7 h, and diluted with CH_2Cl_2 (2 mL). The excess of $N_2H_4{\cdot}H_2O$ was separated. The organic layer was washed with H_2O (3 × 1 mL) and dried. The solvent was evaporated to afford a product (89 mg) that was chromatographed over a column of silica gel (2.7 g) ($CHCl_3$–MeOH, 49:1) to afford **11** (76 mg, 65%). Hydrazide **11**, $C_{16}H_{28}N_2O$, $[\alpha]_D^{20}$ +22.1° (*c* 1.3, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3425, 3291 (NH_2), 1655, 1626 (CONH). ^1H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83 (3H, s, CH_3-15), 0.90 (3H, s, CH_3-14), 0.94 (3H, s, CH_3-16), 0.95*–1.80* (8H, m), 1.14 (1H, dd, J = 21.0, 12.7, H-5α), 1.59 (3H, s, CH_3-13), 2.07 (2H, m, H-7a, 7b), 2.94 (1H, d, J = 17.4, H-11a), 3.09 (1H, d, J = 17.4, H-11b), 3.86 (2H, br.s, NH_2), 7.03 (1H, br.s, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 18.74 (C-2), 18.77 (C-6), 19.84 (C-16), 20.15 (C-13), 21.58 (C-15), 33.19 (C-14), 33.32 (C-4), 33.50 (C-7), 34.37 (C-11), 36.01 (C-1), 38.80 (C-10), 41.47 (C-3), 51.87 (C-5), 131.68 (C-7), 134.73 (C-8), 172.16 (C-12). ^{15}N NMR spectrum (40 MHz, $CDCl_3$, δ, ppm): 127 (NH). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 264 (M^+, 23), 249 (31), 232 (38), 190 (100), 175 (81), 163 (50), 149 (35), 135 (31), 121 (50), 105 (59), 91 (52), 69 (41), 55 (39), 41 (47).

Preparation of Ethyl $\Delta^{8,9}$-Bicyclohomofarnesenoate (12). A solution of **5** (100 mg, 0.40 mmol) in anhydrous benzene (2 mL) was treated with a solution of $(COCl)_2$ (0.4 mL, 0.58 g, 4.58 mmol) in benzene (1 mL), stirred at 20°C for 1 h, and refluxed for 1 h. The benzene and excess of $(COCl)_2$ were evaporated at reduced pressure. The residue was treated with anhydrous EtOH (2 mL) and $NH_2NH_2{\cdot}H_2O$ (1 mL, 1.03 g, 20.5 mmol), stirred at 20°C for 7 h, diluted with H_2O (10 mL), and extracted with EtOAc (3 × 15 mL). The extract was washed with H_2O (3 × 5 mL) and dried. The EtOAc was evaporated to afford a product (97 mg) that was chromatographed over a column of silica gel (2.9 g). Ethyl ester **12** (60 mg, 55%) was eluted by petroleum ether–Et_2O (19:1). $C_{18}H_{30}O_2$, $[\alpha]_D^{17}$ +79.8° (*c* 1.41, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1738, 1154 (CO_2Et). ^1H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83 (3H, s, CH_3-15), 0.89 (3H, s, CH_3-14), 0.93 (3H, s, CH_3-16), 1.25 (3H, t, J = 7.3, CH_3-18), 1.57 (3H, s, CH_3-13), 2.95 (1H, d, J = 16.8, H-11a), 3.07 (1H, d, J = 16.8, H-11b), 4.12 (2H, m, 2 × H-17). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 14.24 (C-18), 18.93 (C-2), 18.94 (C-6), 19.74 (C-16), 20.07 (C-13), 21.63 (C-15), 33.13 (C-11), 33.17 (C-14), 33.27 (C-4), 33.61 (C-7), 36.28 (C-1), 38.54 (C-10), 41.54 (C-3), 51.39 (C-5), 60.30 (C-17), 130.14 (C-8), 133.92 (C-9), 172.96 (C-12). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 278 (M^+, 18), 263 (25), 217 (10), 205 (14), 190 (100), 175 (91), 163 (28), 147 (16), 133 (22), 119 (42), 105 (46), 91 (34), 79 (21), 55 (21), 41 (27).

Reaction of $\Delta^{8,13}$-Bicyclohomofarnesenoic Acid (13) with $N_2H_4{\cdot}H_2O$. A solution of **13** (100 mg, 0.40 mmol) in anhydrous benzene (2 mL) was treated with a solution of $(COCl)_2$ (0.4 mL, 0.58 g, 4.58 mmol) in benzene (1 mL), stirred for 1 h at 20°C, and refluxed for 1 h. The benzene and excess of $(COCl)_2$ were evaporated at reduced pressure. The residue was treated with CH_2Cl_2 (2 mL) and $N_2H_4{\cdot}H_2O$ (1 mL), stirred for 10 h at room temperature, refluxed for 10 h, and diluted with CH_2Cl_2 (2 mL). The excess of $N_2H_4{\cdot}H_2O$ was separated. The organic layer was washed with H_2O (3 × 1 mL) and dried. The solvent was evaporated to afford crude product (107 mg) that was chromatographed over a column of silica gel (3.2 g). A product (30 mg, 30%) that was recrystallized from MeOH to afford disubstituted hydrazine **16** (25 mg) was eluted by $CHCl_3$. Then, a mixture (46 mg, 43%) of hydrazides **15** and **17** (1.5:1 ratio, PMR data and GC-MS analysis) was eluted ($CHCl_3$–MeOH, 99:1). The mixture (15 mg) of **15** and **17** was separated using HPLC (semi-preparative μBondapak™ C18 column, 10 μm, 125 Å, MeOH–H_2O, 9:1, 0.38 psi, flow rate 1 mL/min) to afford **15** (5.2 mg, 4.9%) and **17** (5 mg, 4.7%).

$\Delta^{8,13}$-Bicyclohomofarnesenoic Acid Hydrazide (15). $C_{16}H_{28}N_2O$, $[\alpha]_D^{17}$ –15.8° (*c* 0.31, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3294, 3198 (NH_2), 1641, 1616 (CONH). ^1H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69 (3H, s, CH_3-16), 0.81 (3H, s, CH_3-15), 0.89 (3H, s, CH_3-14), 1.2* (1H, m, H-5α), 2.10 (1H, dt, J = 12.9, 5.2, H-7a), 2.22 (1H, dd, J = 15.9, 10.8, H-11a), 2.37* (1H, m, H-7b), 2.39* (1H, m, H-11b), 3.88 (2H, br.s, NH_2), 4.47 (1H, br.s, H-13a), 4.79 (1H, br.s, H-13b), 7.04 (1H, br.s, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 14.52 (C-16), 19.27 (C-2), 21.70 (C-15), 24.06 (C-6), 30.44 (C-11), 33.54 (C-4), 33.56 (C-14), 37.67 (C-7), 38.91 (C-1), 39.19 (C-10), 41.96 (C-3), 52.09 (C-9), 55.12 (C-5), 106.48 (C-13), 149.19 (C-8), 174.01 (C-12). ^{15}N NMR spectrum (40 MHz, $CDCl_3$, δ, ppm): 128 (NH). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 264 (M^+, 12), 249 (94), 233 (48), 217 (28), 190 (95), 175 (47), 163 (24), 137 (64), 129 (25), 123 (52), 121 (72), 119 (49), 113 (84), 109 (65), 95 (84), 91 (87), 81 (89), 69 (83), 55 (72), 41 (100).

Bicyclohomofarnesenoic Acid Hydrazide (17). $C_{16}H_{30}N_2O$. $[\alpha]_D^{17}$ +6.40° (*c* 0.30, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3287, 3198 (NH_2), 1645, 1616 (CONH). ^1H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.81 (3H, s, CH_3-15), 0.84 (3H,

s, CH_3-16), 0.86 (3H, s, CH_3-14), 0.94 (3H, d, J = 7.1, CH_3-13), 0.98* (1H, m, H-5α), 1.60* (2H, m, H-7a, 7b), 1.82* (2H, m, H-8α, 9α), 2.01 (1H, dd, J = 14.2, 9.7, H-11a), 2.32 (1H, dd, J = 14.2, 3.9, H-11b), 3.90 (2H, br.s, NH_2), 6.70 (1H, br.s, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 15.88 (C-13), 16.54 (C-16), 17.40 (C-6), 18.38 (C-2), 21.54 (C-15), 31.27 (C-8), 32.72 (C-11), 33.34 (C-4), 33.45 (C-14), 34.44 (C-7), 38.13 (C-10), 39.39 (C-1), 42.04 (C-3), 50.41 (C-9), 56.45 (C-5), 174.51 (C-12). ^{15}N NMR spectrum (40 MHz, $CDCl_3$, δ, ppm): 128 (NH). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 266 (M^+, 0.9), 264 (2), 251 (6), 249 (6), 235 (7), 217 (7), 193 (13), 177 (9), 163 (3), 149 (4), 137 (22), 123 (33), 109 (33), 95 (33), 81 (36), 74 (100), 69 (34), 55 (35), 41 (36).

***N,N′*-Di-($\Delta^{8,13}$-bicyclohomofarnesenoyl)-hydrazine (16).** White crystals, mp 273–274°C (MeOH), $[\alpha]_D^{16}$ –3.83° (*c* 0.12, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3440, 3258 (NH), 1645, 1616 (CONH). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.70 (6H, s, CH_3-16, 16′), 0.81 (6H, s, CH_3-15, 15′), 0.88 (6H, s, CH_3-14, 14′), 1.21* (2H, m, H-5α, 5′α), 2.40* (2H, m, H-9, 9′), 2.40* (4H, m, H-11a, 11′a, 11b, 11′b), 4.51 (2H, br.s, H-13a, 13′a), 4.80 (2H, br.s, H-13b, 13′b), 8.40 (2H, br.s, 2NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 14.54 (C-16), 19.30 (C-2), 21.72 (C-15), 24.08 (C-6), 30.32 (C-11), 33.34 (C-4), 33.56 (C-14), 37.66 (C-7), 38.98 (C-1), 39.25 (C-10), 42.03 (C-3), 52.00 (C-9), 55.16 (C-5), 106.75 (C-13), 148.92 (C-8), 169.52 (C-12). ^{15}N NMR spectrum (40 MHz, $CDCl_3$, δ, ppm): 134 (NH). HR-EI-MS: found 497.4108. $C_{32}H_{52}N_2O_2$. Calcd 496.7750.

X-ray Crystal Structure Experiment. A dataset for a crystal of **1** was collected at room temperature (293 K) on an Oxford Diffraction Super Nova diffractometer using a Nova Cu Kα microfocusing X-ray tube. The dataset was processed using the CrysAlis PRO program (England, 2012). The structure was solved by direct methods using the SHELX-S program [12] and refined by anisotropic full-matrix least squares methods for non-hydrogen atoms using SHELXL-2013 [12]. Positions of H atoms on O atoms were determined in difference Fourier syntheses and refined with the corresponding geometric H-bond parameters. Positions of other H atoms were calculated geometrically and refined using a rigid-body model with $U_H = 1.2\ U_{eq}$ or $1.5\ U_{eq}$ (U_{eq} correspond to the C atoms).

Table 1 presents the crystal data and refinement parameters.

Positional and thermal parameters for atoms in the structure of **1** were deposited in the Cambridge Crystallographic Data Centre (No. CCDC 1453861; deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Biological Activity. Antifungal and antibacterial activities of **1**, **2**, **11**, and **15**–**17** were studied using *in vitro* diffusion tests on standard nutrient agar in Petri dishes. Solutions of samples of concentrations 0.5%, 1%, and 2% were prepared by dissolving the appropriate amounts of test compounds in fixed volumes of DMSO. Microorganism suspensions were prepared using sequential dilutions. A symmetric inhibition zone formed as an ellipse after incubation for 48 h at 37°C. The minimum inhibiting concentrations (MICs) were determined in units of μg/mL. Results were observed visually using an Olympus SZY 160 microscope (Japan) and photography.

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REFERENCES

1. K. I. Kuchkova, A. N. Arycu, and P. F. Vlad, *Chem. Nat. Compd.*, **45**, 367 (2009).
2. K. I. Kuchkova, A. N. Aryku, P. F. Vlad, C. Deleanu, and A. Nicolescu, *Chem. Nat. Compd.*, **46**, 539 (2010).
3. K. I. Kuchkova, A. N. Aryku, A. N. Barba, P. F. Vlad, Ya. Lipkovskii, Yu. A. Simonov, and V. Kh. Kravtsov, *Chem. Nat. Compd.*, **47**, 223 (2011).
4. K. Kuchkova, A. Aricu, E. Secara, A. Barba, P. Vlad, N. Ungur, C. Tuchilus, S. Shova, Gh. Zbancioc, and I. I. Mangalagiu, *Med. Chem. Res.*, **23**, 1559 (2014).
5. K. I. Kuchkova, A. N. Arycu, E. S. Secara, A. N. Barba, P. F. Vlad, F. Z. Makaev, E. Melnic, and V. Kh. Kravtsov, *Chem. Nat. Compd.*, **51**, 684 (2015).
6. B. F. Garifullin, I. Yu. Strobykina, G. G. Mordovskoi, V. F. Mironov, and V. E. Kataev, *Chem. Nat. Compd.,* **47**, 55 (2011).

7. R. M. Mohareb, K. A. El-Sharkawy, M. M. Hussein, and H. M. El-Sehrawi, *J. Pharm. Sci. Res*., **2**, 185 (2010).
8. K. I. Kuchkova, Yu. M. Chumakov, Yu. A. Simonov, G. Bocelli, A. A. Panasenko, and P. F. Vlad, *Synthesis*, 1045 (1997).
9. E. Demole and H. Wuest, *Helv. Chim. Acta*, **50**, 1314 (1967).
10. P. F. Vlad, G. A. Dragalina, and M. N. Coltsa, *J. Gen. Chem. USSR*, **47**, 943 (1977) [Engl. Transl.].
11. V. Kulcitki, N. Ungur, M. Gavagnin, M. Carbone, and G. Cimino, *Tetrahedron: Asymmetry*, **15**, 423 (2004).
12. G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Crystallogr.*, **64**, 112 (2008).