JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Abietane-Type Diterpenoid Amides with Highly Potent and Selective Activity against *Leishmania donovani* and *Trypanosoma cruzi*

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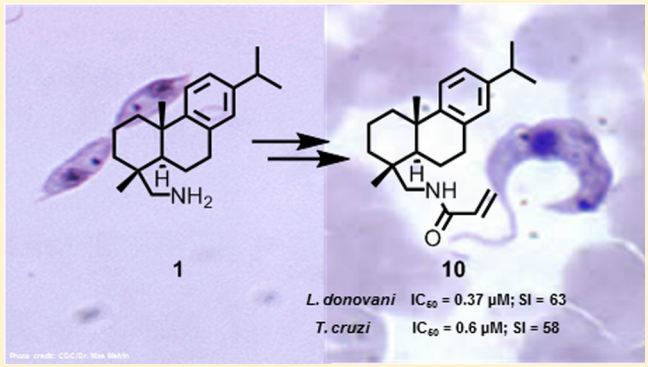
Supporting Information

ABSTRACT: Dehydroabietylamine (**1**) was used as a starting material to synthesize a small library of dehydroabietyl amides by simple and facile methods, and their activities against two disease-causing trypanosomatids, namely, *Leishmania donovani* and *Trypanosoma cruzi,* were assayed. The most potent compound, **10**, an amide of dehydroabietylamine and acrylic acid, was found to be highly potent against these parasites, displaying an IC_{50} value of 0.37 μM against *L. donovani* axenic amastigotes and an outstanding selectivity index of 63. Moreover, compound **10** fully inhibited the growth of intracellular amastigotes in *Leishmania donovani*-infected human macrophages with a low IC_{50} value of 0.06 μM. This compound was also highly effective against *T. cruzi* amastigotes residing in L6 cells with an IC_{50} value of 0.6 μM and high selectivity index of 58, being 3.5 times more potent than the reference compound benznidazole. The potent activity of this compound and its relatively low cytotoxicity make it attractive for further development in pursuit of better drugs for patients suffering from leishmaniasis and Chagas disease.



Leishmaniasis is a common tropical disease caused by trypanosomatids that are transmitted by the bite of several sandfly species.[1] The most common clinical forms of leishmaniasis are cutaneous, mucocutaneous, and visceral leishmaniasis.[1] Cutaneous leishmaniasis is the mildest form, causing ulcers at the site of the insect bite that normally self-heal with significant scarring. Mucocutaneous leishmaniasis is a debilitating form that causes mutilation of the membranes in the mouth, nose, and throat. This form does not self-heal and responds poorly to treatment. Visceral leishmaniasis, the most severe and life-threatning form, is caused by *L. donovani*. It affects vital human organs causing fever, weight loss, and anemia and is generally life-threatening if not diagnosed and treated. Leishmaniasis occurs mostly in poor developing countries of Asia, South America, and Africa and can be associated with HIV infection.[1] Current therapeutic drugs against leishmaniasis are pentavalent antimonials (sodium stibogluconate and meglumine antimoniate),[2] amphotericin B and its lipid-based formulations,[3] pentamidine isethionate,[4] sitamaquine,[5] paromomycin, and miltefosine.[6] However, there are several limitations and drawbacks for most of these drugs such as high cost, toxicity, difficult route of administration, lack of efficacy in endemic areas, and parasite resistance.[7]

Chagas disease is another parasitic disease caused by a trypanosomatid, namely, *Trypanosoma cruzi*, and like leishmaniasis it infects millions of people worldwide.[8] Chagas disease is also known as American trypanosomiasis.[8] It is a two-stage disease with an initial acute phase bearing mild symptoms that include fever, headache, enlarged lymph glands, and characteristic swelling of the eyelid close to the site of the bite of the insect. During the chronic phase symptoms such as enlargement of the ventricles of the heart and enlarged esophagus or colon will develop.[8] Chemotherapy for Chagas disease includes mainly the drugs benznidazole and nifurtimox.[7a] These drugs can be administered either separately or simultaneously, and

Received: November 2, 2015

ACS Publications © XXXX American Chemical Society and American Society of Pharmacognosy

DOI: 10.1021/acs.jnatprod.5b00990
J. Nat. Prod. XXXX, XXX, XXX−XXX

treatments can cure up to 80% of the infections in the acute phase. However, as with leishmaniasis, drug efficacy is a problem: the efficacy of the drugs decreases dramatically when treating the chronic phase of the disease, curing only 5−20% of the patients. Thus, there is an urgent need for more effective and safer drugs for the treatment of these diseases.

Plant- or marine-derived natural compounds, such as various alkaloids, terpenoids, flavonoids, and quinones, are promising sources of antileishmanial agents.[9] One example is represented by the abietane-type diterpenoids present in plants.[10] These compounds exhibit a wide range of biological activities including anti-inflammatory, antibacterial, antifungal, and antimalarial properties.[10] To date, only a few naturally occurring and semisynthetic abietane-type diterpenoids have shown promising trypanocidal activity such as those isolated from *Plectranthus barbatus* Andrews,[11] *Dracocephalum komarovii* Lipsky,[12] *Salvia cilicica* Boiss,[13] and *Juniperus procera* Hochst. ex Endl. berries,[14] a quinone derivative of dehydroabietic acid,[15] 12-methoxycarnosic acid,[16] and a few others.[17]

Figure 1 depicts compounds that were tested against intracellular forms of *L. donovani* and *T. cruzi* and their

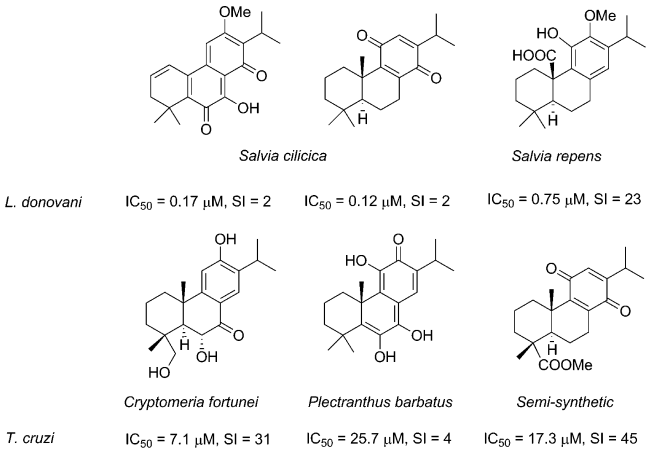


Figure 1. Naturally occurring and semisynthetic abietane-type diterpenoids with activity (IC_{50} values, μM) against intracellular forms of *L. donovani* and *T. cruzi*. SI = selectivity index.

respective potency and selectivity. The diterpenoids from *Salvia cilicica*[13] showed potent activity against *L. donovani* but limiting toxicity, whereas 12-methoxycarnosic acid from *Salvia repens*[16] displayed better selectivity. The abietane isolated from *Cryptomeria fortunei* Hooibr. ex Otto & A.Dietr.[17a,e] and the quinone derivative of dehydroabietic acid[15] were active against *T. cruzi* with high selectivity indices. More recently, dehydroabietylamine (**1**) and (*N*-dehydroabietyl)benzamide (**13**) (Scheme 1) have been reported as potent antimalarial agents.[18] In continuation of previous work with bioactive terpenoid derivatives,[19] we herein report the preparation of a small library of abietane-type diterpenoid amides using commercially available dehydroabietylamine (**1**) as a starting material and the evaluation of their activity against *L. donovani* and *T. cruzi*.

RESULTS AND DISCUSSION

Commercially available dehydroabietylamine (**1**) was used as a starting material to produce amides **2**−**13** by two simple methods with slightly varying reaction conditions. Compounds **2** and **3** were synthesized by reacting diterpenoid **1** with various carboxylic acids using 1,3-dicyclohexylcarbodiimide (DCC) as a coupling reagent, and compounds **4**−**12** were synthesized by reacting **1** with various acyl chlorides or anhydrides in the presence of a basic catalyst. The reaction conditions were as follows: propiolyl amide (**2**) and pyrrole-2-carboxylic acid amide (**3**) were prepared by treating a mixture of **1**, the corresponding carboxylic acid, and *N*,*N*-diisopropylethylamine (DIPEA) in CH_2Cl_2 with DCC at ice-bath temperature. Cinnamoyl amide **4** and *p*-nitrocinnamoyl amide **5** were obtained after reaction of the corresponding acyl chlorides with amine **1** in the presence of 4-dimethylaminopyridine (DMAP) in pyridine. Maleic acid amide **6** was prepared from maleic anhydride and amine **1**. Compounds **7**−**9** (amides of butyric acid (**7**), phthalic acid (**8**), and acetic acid (**9**)) were prepared by reacting the corresponding acyl chloride (**7**) or anhydride (**8**, **9**) with amine **1** in pyridine in the presence of DMAP and triethylamine (TEA). The amide of acrylic acid **10** was prepared from the corresponding acyl chloride and **1** in CH_2Cl_2. Amides of 2,3-pyrazinedicarboxylic acid **11**, succinic acid **12**, and benzoic acid **13** were prepared by stirring a mixture of the corresponding anhydride (**11**, **12**) or acyl chloride (**13**) and TEA in CH_2Cl_2 for 1−24 h at room temperature.

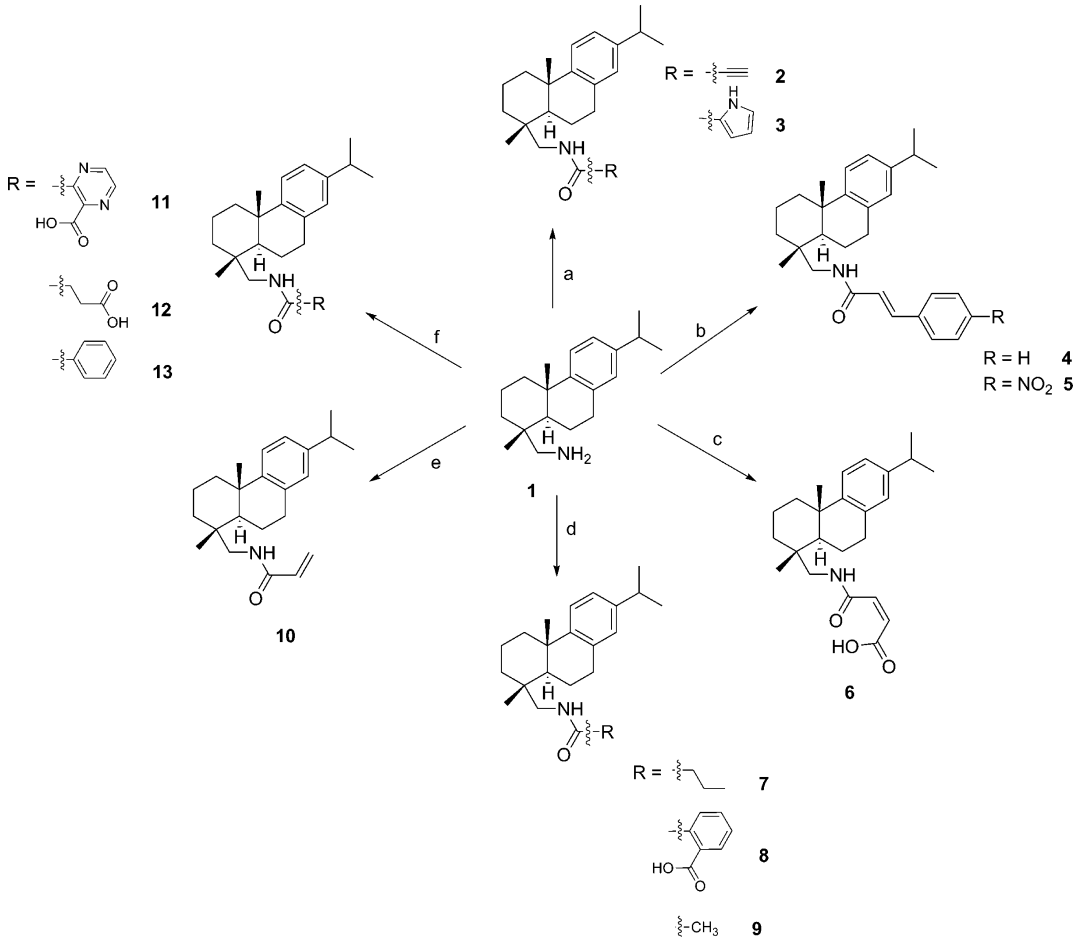
Initial screening against *L. donovani* axenic amastigotes was performed at 50 μM. Compounds showing significant growth inhibition (≥70%) at this concentration were assayed at 15 μM (Table 1). If significant activity was still observed at the lower concentration, the 50% growth inhibition values (IC_{50}) on the axenic amastigotes and human monocyte cell line THP-1, i.e., toxicity (TOX-IC_{50}), were determined. Selectivity indices (SI) were then calculated (SI = TOX-IC_{50}/IC_{50} axenic amastigotes). Finally, the best derivatives were assayed for activity against intracellular amastigotes using infected THP-1 macrophages. The highest compound concentration showing no toxicity on THP-1 cells was chosen for use in the latter assay.

At the highest compound concentration tested, 50 μM, most of the dehydroabietyl amide derivatives displayed excellent antileishmanial activity (Table 1). Dehydroabietylamine (**1**) showed moderate inhibition of parasite growth (68.7%), while derivatives containing large aromatic groups, **4**, **5**, and **11**, had only slight inhibitory activity against axenic amastigotes (42.4%, 42.7%, and 9.4%, respectively). In addition, compound **11** was poorly soluble in DMSO. The phthalic acid derivative **8** and benzoic acid derivative **13** performed well at 50 μM (98.5% and 95.7%, respectively), but activities were clearly diminished at 15 μM (34.9% and 56.3%, respectively). Poor to moderate activities may be explained by steric hindrance caused by large, rigid, and planar aromatic groups. Derivatives possessing smaller side chains displayed excellent activities at 50 μM and good to excellent activities even at 15 μM. Interestingly, succinic acid derivative **12** with a saturated carboxylic side chain displayed clearly poorer activity (55.0%) when compared to maleic acid derivative **6** (90.7%) with an unsaturated side chain. The butyric acid derivative **7** displayed moderate activity (73.2%). Compounds **2**, **3**, **6**, **9**, and **10** were the most potent and showed excellent activities (>90%) at 15 μM. Overall, all the compounds displayed either micro- or submicromolar IC_{50} values against *L. donovani* axenic amastigotes that ranged from 0.37 to 5.1 μM (Table 1). The most promising was compound **10**, which is an amide built from acrylic acid.

Only compound **2** was found to be highly toxic, whereas the remaining compounds all showed mild or low toxicity with TOX-IC_{50} values ranging from 23.2 to 145.2 μM. SI values of

DOI: 10.1021/acs.jnatprod.5b00990
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 1. [a]



[a]Reagents and conditions: (a) propiolic acid (for **2**) or pyrrole-2-carboxylic acid (for **3**), DMAP, DCC, DIPEA, CH_2Cl_2, rt, overnight; (b) cinnamoyl chloride (for **4**) or 4-nitrocinnamoyl chloride (for **5**), DMAP, pyridine, 40 °C, overnight; (c) maleic anhydride, TEA, pyridine, rt, 2.5 h; (d) butanoyl chloride (for **7**) or phthalic anhydride (for **8**) or acetic anhydride (for **9**), DMAP, TEA, pyridine, 1−2 h; (e) acryloyl chloride, DMAP, TEA, CH_2Cl_2, rt, 1 h; (f) 2,3-pyrazinedicarboxylic acid anhydride (for **11**), succinic anhydride (for **12**), or benzoyl chloride (for **13**), TEA, CH_2Cl_2, rt, 1−24 h.

Table 1. Antileishmanial Activity of Dehydroabietylamine (1) and Its Derivatives (2−13) against Axenic Amastigote Forms[a]

	% inhibition ± SE[b] (*L. donovani*)					
compound	50 μM	15 μM	IC_{50} ± SE (μM)[b] (*L. donovani*)	TOX-IC_{50} ± SE (μM)[b] (THP1)	SI[c]	% *L. donovani* inhibition ± SE iMΦ (at 5 μM)[b]
1	68.7 ± 1.4					
2	99.8 ± 0.1	98.1 ± 0.3	4.1 ± 0.20	<3.0	<1	
3	99.1 ± 0.2	90.6 ± 0.3	3.2 ± 0.30	33.3 ± 1.5	10	
4	42.4 ± 0.9					
5	42.7 ± 1.3					
6	98.8 ± 0.1	90.7 ± 0.3	1.9 ± 0.09	145.2 ± 4.3	76	34.7 ± 8.0
7	96.6 ± 0.1	73.2 ± 1.0	5.1 ± 0.22	56.3 ± 10.3	11	
8	98.5 ± 0.1	34.9 ± 1.5				
9	98.5 ± 0.1	96.0 ± 0.5	2.4 ± 0.10	33.3 ± 1.5	14	
10	99.8 ± 0.1	99.6 ± 0.1	0.37 ± 0.02	23.2 ± 3.1	63	94.7 ± 2.2[d]
11	9.4 ± 3.7					
12	98.1 ± 0.2	55.0 ± 1.6				
13	95.7 ± 0.1	56.3 ± 2.0				

[a]Amphotericin B was included as a positive control on each plate and gave >90% inhibition of parasite growth at 1 μM. [b]Average inhibition of triplicates from at least two independent experiments. [c]SI = TOX-IC_{50}/IC_{50} axenic amastigotes. [d]IC_{50} value for compound **10** on the infected macrophages was determined as 0.06 μM (n = 2).

20 or higher are considered "hit activity criteria for protozoa" when screening for new bioactive compounds.[20] In fact, compounds **10** and **6** exhibited outstanding SI values of 63 and 76, respectively (Table 1). It was found also that despite

DOI: 10.1021/acs.jnatprod.5b00990
J. Nat. Prod. XXXX, XXX, XXX−XXX

the potent activity of the set of compounds on hand against *L. donovani* axenic amastigotes, only compounds **6** and **10** were able to effectively kill the parasites inside human macrophages, at 5 μM (Table 1). These two compounds possess the necessary physicochemical properties that allow them to efficiently cross the cell membranes and kill the parasites residing inside the host cells, reflecting their potential to act at the relevant stages responsible for the disease. Determination of the IC_{50} value for compound **10** on the infected macrophages (Table 1) showed that this compound is 6-fold more potent against infected macrophages than axenic amastigotes.

The most promising compounds in the set were then tested for activity against *T. cruzi* amastigotes in L6 cells (Table 2).

Table 2. Activity of Dehydroabietylamine (1) and Compounds 6, 9, and 10 against *T. cruzi* Amastigotes in L6 Cells (Rat Myoblasts)

compound	IC_{50} ± SE (μM) (*T. cruzi*)	IC_{50} ± SE (μM) (L6)	SI[a]
1	7.4 ± 1.2	6.5 ± 0.4	1
6	9.5 ± 5.4	33.5 ± 15.5	4
9	3.7 ± 1.2	24.3 ± 11.2	6
10	0.6 ± 0.5	36.7 ± 12.3	58
benznidazole	2.1 ± 0.1		
podophyllotoxin		0.02 ± 0.01	

[a]SI = IC_{50} (L6)/IC_{50} (*T. cruzi*), n = 4.

Dehydroabietylamine (**1**) was also tested for comparison. According to expectations, it was found that compounds **6**, **9**, and **10** were also active against *T. cruzi* amastigotes, with IC_{50} values ranging from 0.6 to 9.5 μM. Their toxicity was again evaluated against L6 cells, and the SI values were less promising (<10) with the exception of that of compound **10**, which again proved to be the best compound, 3.5 times more potent than the reference compound, benznidazole, displaying an IC_{50} value of 0.6 μM and an exceptional SI value of 58. Overall, chemical modifications of the parent compound **1** proved successful, resulting in improved activity against both protozoan parasites and with reduced cytotoxicity.

The potent activity of compound **10** against *L. donovani*-infected macrophages and *T. cruzi* intracellular amastigotes combined with its low cytotoxicity makes it an excellent candidate for further development of new compounds for the treatment of leishmaniasis and Chagas disease. This compound combines the ability to target parasites residing inside infected cells with low IC_{50} values and excellent selectivity and is readily available through facile synthetic methods. When compared to the abietane-type diterpenoids with reported activity against *L. donovani* and *T. cruzi* (Figure 1), some of the compounds in the present set display a much more promising combination of potency and selectivity. To the best of our knowledge, compound **10** is the most potent and selective abietane-type diterpenoid against these protozoan parasites reported so far. Further studies are currently under way to identify its mode of action. The present work highlights clearly the value of abietane-type diterpenoids in discovering novel therapeutic options for patients suffering from protozoal infections caused by trypanosomatids.

EXPERIMENTAL SECTION

General Experimental Procedures. Commercially available reagents were used without further purification. Dehydroabietylamine (TCI D1588, **1**) was obtained from Tokyo Chemical Industry TCI Europe (Zwijndrecht, Belgium). All of the solvents were HPLC grade. Anhydrous solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reactions in anhydrous solvents were performed in oven-dried glassware under an inert atmosphere of anhydrous argon or nitrogen. Thin-aluminum-layer chromatography (TLC) was performed on E. Merck (Darmstadt, Germany) silica gel 60 backed plates, with visualization accomplished by UV illumination and staining with 5% H_2SO_4 in MeOH. Melting points were obtained with a Sanyo Gallenkamp (Moriguchi, Osaka, Japan) apparatus without correction. The Fourier transform infrared (FTIR) spectra were recorded on a Nicolet iS50 FT-IR (Waltham, MA, USA) using built-in diamond ATR. The ^1H and ^{13}C NMR spectra were measured on a Bruker Avance III 500 MHz NMR spectrometer (Billerica, MA, USA). ^1H and ^{13}C NMR spectra were recorded in solution in $CDCl_3$. Chemical shifts (δ) are given as parts per million (ppm) relative to the NMR solvent signals ($CDCl_3$ 7.26 and 77.00 ppm for ^1H and ^{13}C NMR, respectively). HRMS were measured to determine purity of all tested compounds and were measured on a Waters Acquity UPLC system (Waters, Milford, MA, USA) equipped with a Synapt G2 HDMS mass spectrometer (Waters). Optical rotations were determined with an Autopol IV automatic polarimeter (Rudolf Research Analytical, Hackettstown, NJ, USA) using a 1-mL capacity cell with a 50-mm path length. $[\alpha]_D^{25}$ values are given in (deg·mL)/(g·dm).

***N*-Dehydroabietylpropynamide (2).** Dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), propiolic acid (0.49 g, 7.0 mmol), DIPEA (0.68 g, 5.3 mmol), and DMAP (21 mg, 0.18 mmol) were dissolved in CH_2Cl_2 (20 mL). To the stirred solution at ice-bath temperature was slowly added DCC (1.1 g, 5.3 mmol) in small portions. The reaction mixture was slowly allowed to reach room temperature, and it was stirred overnight. The resulting mixture was cooled again with an ice bath, and another portion of propiolic acid (0.25 g, 3.5 mmol), DCC (0.55 g, 2.6 mmol), and DIPEA (0.34 g, 2.6 mmol) were added. The reaction mixture was allowed to reach room temperature, and it was stirred for 3 h. The formed urea byproduct was filtered, and the filtrate was evaporated. The solid residue was dissolved in ethyl acetate (50 mL) and washed with 1 M HCl (2 × 50 mL). The remaining and precipitated urea was removed by filtration. The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 50 mL) and dried with anhydrous Na_2SO_4, and solvents were evaporated to give amide **2** as a brown solid in 67% yield: mp 98 °C; $[\alpha]_D^{25}$ −30.7 (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3266, 3202, 2927, 2857, 2106, 1705, 1620, 1549, 1451, 1273, 823, 697, 674 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.16 (1H, d, J = 1.6 Hz), 6.99 (1H, d, J = 1.6 Hz), 6.89 (1H, s), 5.95 (1H, s), 3.30 (1H, dd, J = 13.8, 6.6 Hz), 3.11 (1H, dd, J = 13.8, 6.7 Hz), 2.82−2.89 (1H, m), 2.77 (1H, s), 2.28 (2H, d, J = 12.7 Hz), 1.68−1.75 (2H, m), 1.32−1.45 (4H, m), 1.29 (3H, s), 1.21 (3H, s), 1.21 (3H, s), 0.95 (3H, s), 0.89 (1H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.3, 146.9, 145.7, 134.7, 126.9, 124.1, 123.9, 77.4, 73.4, 50.0, 45.2, 38.2, 37.5, 37.4, 36.1, 33.4, 32.6, 30.5, 26.50, 25.2, 23.9, 18.8, 18.8, 18.7; HRMS $[M + Na]^+$ m/z 360.2305 (calcd for $C_{23}H_{30}NONa$ 360.2303).

***N*-(Dehydroabietyl)pyrrole-2-carboxamide (3).** Dehydroabietylamine (**1**) (0.50 g, 1.8 mmol), pyrrole-2-carboxylic acid (0.20 g, 1.8 mmol), DMAP (0.010 g, 0.090 mmol), and DIPEA (0.45 g, 3.5 mmol) were dissolved in CH_2Cl_2 (20 mL). To this mixture was slowly added DCC (0.72 g, 3.5 mmol) in small portions at ice-bath temperature. The reaction mixture was allowed to reach room temperature, and it was stirred overnight. The formed urea was filtered, and the filtrate was washed with 0.5 M HCl (30 mL) and 1 M HCl (30 mL). The remaining and precipitated urea was filtered, and the filtrate was washed with a saturated aqueous solution of $NaHCO_3$ (30 mL). The organic layer was dried with anhydrous Na_2SO_4, and solvents were evaporated. The crude product was purified with SiO_2 column chromatography (hexane−EtOAc, 1:3) and recrystallized from a refluxing hexane−EtOAc (1:1, 40 mL) mixture to give the amide **3** in 30% yield: mp 181 °C (dec); $[\alpha]_D^{25}$ −2.4 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3248, 2929, 1606, 1561, 1408, 1328, 1131, 822, 735, 609 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), δ 9.71 (1H, s), 7.17 (1H, d, J = 8.2 Hz),

DOI: 10.1021/acs.jnatprod.5b00990
J. Nat. Prod. XXXX, XXX, XXX−XXX

6.99 (1H, d, J = 7.9 Hz), 6.90 (2H, d, J = 11.6 Hz), 6.51 (1H, s), 6.21 (1H, s), 5.89 (1H, s), 3.40 (1H, dd, J = 6.7 Hz), 3.29 (1H, dd, J = 6.8 Hz), 2.90−2.95 (1H, m), 2.81−2.85 (2H, m), 2.28 (1H, d), 1.96−1.99 (2H, m), 1.67−1.80 (3H, m), 1.49 (2H, d, J = 11.9 Hz), 1.31−1.41 (2H, m), 1.23 (3H, s), 1.23 (3H, s), 1.22 (3H, s), 0.99 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.2, 147.0, 145.6, 134.7, 126.9, 125.9, 124.2, 123.8, 121.4, 109.6, 108.3, 49.7, 45.6, 38.3, 37.7, 37.5, 36.3, 33.4, 30.4, 25.4, 23.9, 19.1, 18.7, 18.6; HRMS $[M + Na]^+$ m/z 401.2570 (calcd for $C_{25}H_{34}N_2ONa$ 401.2569).

(*E*)-*N*-(Dehydroabietyl)-3-phenylprop-2-enamide (4). A mixture of dehydroabietylamine (**1**) (5.0 g, 18 mmol), cinnamoyl chloride (4.4 g, 0.030 mol), and DMAP (3.2 g, 26 mmol) in pyridine (100 mL) was stirred at 40 °C for 24 h. Solvents were evaporated, the solid residue was dissolved in toluene (400 mL), and the resulting solution was washed with 5% HCl (3 × 200 mL), water (150 mL), a saturated aqueous solution of $NaHCO_3$ (2 × 150 mL), and water (150 mL). The organic layer was dried with anhydrous Na_2SO_4, and the filtrate was evaporated. The obtained product was dried in a vacuum oven at 40 °C overnight to give the amide **4** as a white solid (5.05 g, 70%): mp 72 °C; $[\alpha]_D^{25}$ −8.2 (c 1.0, $CHCl_3$); IR (neat) ν_{max} 3287, 2924, 2865, 1655, 1617, 1547, 1449, 1334, 1217, 976, 821, 763, 680, 486 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.62 (1H, d, J = 15.6 Hz), 7.49 (2H, dd, J = 7.9, 1.8 Hz), 7.30−7.40 (3H, m), 7.17 (1H, d, J = 8.2 Hz), 6.99 (1H, dd, J = 8.1, 1.5 Hz), 6.36 (1H, d, J = 15.4 Hz), 6.90 (1H, s), 5.56 (1H, t, J = 5.9 Hz), 3.25 (1H, dd, J = 13.3, 6.7 Hz), 3.37 (1H, dd, J = 13.7, 6.4 Hz), 2.77−2.98 (3H, m), 2.30 (1H, d, J = 12.8 Hz), 1.90−1.97 (1H, m), 1.65−1.85 (3H, m), 1.44−1.50 (1H, m), 1.27−1.44 (2H, m), 1.19−1.25 (9H, m), 1.03 (1H, t, J = 7.3 Hz), 0.99 (1H, s), 0.83−0.92 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.0. 147.1, 145.6, 141.0, 134.8, 134.8, 129.5, 129.0, 128.7, 128.2, 127.7, 126.9, 125.2, 124.1, 123.8, 120.8, 49.8, 45.2, 38.3, 37.6, 37.4, 36.2, 33.4, 30.1, 25.2, 23.9, 23.9, 18.9, 18.8, 18.6; HRMS m/z $[M + Na]^+$ 438.2775 (calcd for $C_{29}H_{37}NONa$ 438.2773).

(*E*)-*N*-(Dehydroabietyl)-3-(4-nitrophenyl)prop-2-enamide (5). A mixture of 4-nitrocinnamic acid (10.0 g, 51.8 mmol), thionyl chloride (38.0 mL, 524 mmol), and a drop of *N*,*N*-dimethylformamide was stirred at 40 °C for 21 h. Solvents were evaporated, and the resulting solids were dissolved in chloroform (100 mL). Chloroform was evaporated, and the treatment was repeated three times to remove residual thionyl chloride. The crude product was obtained as a yellow solid (14.4 g) and used in the next reaction step without further purification. A mixture of dehydroabietylamine (**1**) (5.0 g, 18 mmol), 4-nitrocinnamoyl chloride (4.1 g, 19 mmol), and DMAP (2.4 g, 20 mmol) in pyridine (100 mL) was stirred at 40 °C for 24 h. The precipitate formed was filtered off, and the filtrate was evaporated. The residue was dissolved in toluene (400 mL) and washed with 5% HCl (3 × 200 mL), water (150 mL), a saturated aqueous solution of $NaHCO_3$ (2 × 150 mL), and water (150 mL). The organic layer was dried with anhydrous Na_2SO_4 and evaporated. The crude product (5.7 g) was purified with SiO_2 column chromatography (hexane−EtOAc, 1:2). The product was dried in a vacuum oven at 60 °C overnight to yield the amide **5** as a yellow solid (3.4 g, 42%): mp 102−107 °C; $[\alpha]_D^{25}$ −16.6 (c 1.0, $CHCl_3$); IR (neat) ν_{max} 3294, 2929, 1658, 1622, 1517, 1340, 1215, 978, 843, 822, 719, 630, 482 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.21 (2H, d, J = 8.8 Hz), 7.66 (1H, d, J = 15.6 Hz), 7.62 (2H, d, J = 8.8 Hz), 7.18 (1H, d, J = 8.0 Hz), 7.00 (1H, dd, J = 8.2, 1.7 Hz), 6.90 (1H, d, J = 1.2 Hz), 6.10 (1H, d, J = 15.6 Hz), 5.71 (1H, t, J = 6.3 Hz), 3.23 (1H, dd, J = 13.8, 6.7 Hz), 3.42 (1H, dd, J = 13.9, 6.4 Hz), 2.95 (1H, dd, J = 17.3, 6.3 Hz), 2.77−2.89 (2H, m), 2.31 (1H, d, J = 13.0 Hz), 1.88−1.97 (1H, m), 1.58−1.85 (1H, m), 1.47 (2H, dd, J = 12.5, 2.4 Hz), 1.40 (1H, dd, J = 13.1 Hz, 3.7 Hz), 1.27−1.43 (3H, m), 1.19−1.26 (9H, m), 1.03 (1H, t, J = 7.3 Hz), 0.99 (1H, s), 0.70−0.91 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.8, 148.1, 147.1, 145.8, 141.1, 138.5, 134.7, 128.3, 126.9, 125.0, 124.1, 124.1, 123.9, 49.9, 46.2, 45.1, 38.3, 37.7, 37.4, 36.2, 33.4, 30.1, 25.2, 24.0, 23.9, 19.0, 18.9, 18.8, 18.6; HRMS m/z $[M + Na]^+$ 483.2634 (calcd for $C_{29}H_{36}N_2O_3Na$ 483.2624).

(*Z*)-*N*-(Dehydroabietyl)-3-carboxyprop-2-enamide (6). A mixture of dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), maleic anhydride (0.34 g, 3.5 mmol), and triethylamine (1.0 mL, 7.2 mmol) in pyridine (15 mL) was stirred at room temperature for 2.5 h. Pyridine was evaporated, and the residual solids were dissolved in CH_2Cl_2 (50 mL). The resulting solution was washed with 5% HCl (3 × 50 mL) and water (3 × 50 mL), dried with anhydrous Na_2SO_4, and evaporated to give the amide **6** as a pale yellow solid (0.95 g, 71%): mp 208 °C; $[\alpha]_D^{25}$ +42.2 (c 1.0, $CHCl_3$); IR (neat) ν_{max} 3266, 2929, 1705, 1692, 1524, 1497, 1279, 1215, 1038, 883, 626, 593, 407 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.73−7.61 (1H, m), 7.15 (1H, d, J = 8.4 Hz), 6.98 (1H, dd, J = 8.4, 1.5 Hz), 6.89 (1H, d, J = 1.1 Hz), 6.43 (1H, d, J = 12.8 Hz), 6.23 (1H, d, J = 12.8 Hz), 3.43 (1H, dd, J = 13.7, 7.0 Hz), 3.16 (1H, dd, J = 13.7, 6.2 Hz), 2.97−2.75 (3H, m), 2.29 (1H, d, J = 12.8 Hz), 1.97−1.86 (1H, m), 1.83−1.63 (3H, m), 1.50−1.27 (4H, m), 1.22 (3H, s), 1.21 (3H, s), 1.20 (3H, s), 0.98 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.6, 166.0, 146.9, 145.7, 135.9, 134.6, 132.0, 127.0, 124.1, 50.8, 45.1, 38.0, 37.5, 36.1, 33.4, 30.0, 25.4, 24.0, 19.1, 18.8, 18.5; HRMS m/z $[M + H]^+$ 384.2540 (calcd for $C_{24}H_{34}NO_3$ 384.2539).

***N*-(Dehydroabietyl)butanamide (7).** A mixture of dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), butanoyl chloride (0.36 mL, 3.5 mmol), triethylamine (0.50 mL, 3.6 mmol), and DMAP (0.39 g, 3.5 mmol) in pyridine (20 mL) was stirred at room temperature for 1 h. Pyridine was evaporated, and the residual solids were dissolved in CH_2Cl_2 (50 mL). The resulting solution was washed with 5% HCl (3 × 50 mL) and water (3 × 50 mL), dried with anhydrous Na_2SO_4, and evaporated to give the amide **7** as a yellow solid (0.68 g, 55%): mp 70 °C; $[\alpha]_D^{25}$ +24.6 (c 1.0, $CHCl_3$); IR (neat) ν_{max} 3304, 2958, 2926, 2868, 1642, 1547, 1456, 1381, 1208, 820, 629 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.17 (1H, d, J = 8.2 Hz), 7.00 (1H, dd, J = 8.2, 7.7 Hz), 6.90 (1H, d, J = 1.3 Hz), 5.46 (1H, t, J = 5.9 Hz), 3.20 (1H, dd, J = 13.7, 6.3 Hz), 3.13 (1H, dd, J = 13.7, 6.7 Hz), 2.96−2.76 (3H, m), 2.32−2.24 (1H, m), 2.13 (2H, td, J = 7.5, 3.6 Hz), 1.93−1.85 (1H, m), 1.82−1.59 (6H, m), 1.44−1.38 (2H, m), 1.38−1.33 (1H, m), 1.26−1.19 (11H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.1, 147.1, 145.6, 126.9, 124.2, 123.9, 49.7, 45.4, 39.0, 38.3, 37.4, 37.3, 36.2, 33.4, 30.2, 25.3, 24.0, 19.3, 18.9, 18.7, 18.6, 13.8; HRMS m/z $[M + Na]^+$ 378.2773 (calcd for $C_{24}H_{37}NONa$ 378.2773).

2-(Dehydroabietylcarbamoyl)benzoic acid (8). A mixture of dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), phthalic anhydride (0.52 g, 3.5 mmol), triethylamine (0.50 mL, 3.6 mmol), and DMAP (0.39 g, 3.5 mmol) in pyridine (15 mL) was stirred at room temperature for 2 h. Pyridine was evaporated, and the residual solids were dissolved in CH_2Cl_2 (50 mL). The resulting solution was washed with 5% HCl (3 × 50 mL) and water (3 × 50 mL), dried with anhydrous Na_2SO_4, and evaporated to give the amide **8** as a pale yellow solid (0.73 g, 48%): mp 81 °C; $[\alpha]_D^{25}$ +6.0 (c 1.0, $CHCl_3$); IR (neat) ν_{max} 2925, 1736, 1676, 1534, 1454, 1296, 1167, 1107, 820, 695, 631, 437 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.87 (1H, d, J = 7.5 Hz), 7.47−7.39 (1H, m), 7.39−7.33 (2H, m), 7.15 (1H, d, J = 8.3 Hz), 6.98 (1H, d, J = 8.3, 1.8 Hz), 6.88 (1H, d, J = 1.2 Hz), 6.77−6.67 (1H, m), 3.31 (1H, dd, J = 13.9, 6.7 Hz), 3.18 (1H, dd, J = 13.9, 5.8 Hz), 2.95−2.70 (3H, m), 2.26 (1H, d, J = 12.3 Hz), 1.92−1.80 (1H, m), 1.78−1.65 (3H, m), 1.65−1.57 (1H, m), 1.47−1.38 (2H, m), 1.38−1.25 (3H, m), 1.22 (3H, s), 1.20 (3H, s), 1.09 (3H, s), 0.94 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.0, 169.8, 147.0, 145.5, 137.3, 134.7, 132.0, 129.8, 129.6, 127.9, 126.9, 124.2, 123.8, 50.8, 45.9, 38.2, 37.5, 37.4, 36.0, 33.3, 30.2, 25.4, 24.0, 18.9, 18.5, 18.5; HRMS m/z $[M + H]^+$ 434.2696 (calcd for $C_{25}H_{39}NO_3$ 434.2695).

***N*-(Dehydroabietyl)acetamide (9).** A mixture of dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), acetic anhydride (0.33 mL, 3.5 mmol), triethylamine (1.0 mL, 7.2 mmol), and DMAP (0.39 g, 3.5 mmol) in pyridine (15 mL) was stirred at room temperature for 2 h. Pyridine was evaporated, and the residual solids were dissolved in CH_2Cl_2 (50 mL). The resulting solution was washed with 5% HCl (3 × 50 mL) and water (3 × 50 mL), dried with anhydrous Na_2SO_4, and evaporated to give the amide **9** as a pale yellow solid (0.77 g, 67%): mp 58−64 °C; $[\alpha]_D^{25}$ +30.4 (c 1.0, $CHCl_3$); IR (neat) ν_{max} 3295, 2925, 2865, 1647, 1554, 1440, 1374, 1287, 820, 599 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.17 (1H, d, J = 8.2 Hz), 7.00 (1H, dd, J = 8.2, 3.3 Hz), 6.90 (1H, d, J = 1.2 Hz), 5.57−5.42 (1H, m), 3.23 (1H, dd, J = 13.8, 6.4 Hz), 3.08 (1H, dd, J = 13.7, 6.6 Hz), 2.97−2.77 (2H, m), 2.33−2.24 (1H, m),

DOI: 10.1021/acs.jnatprod.5b00990
J. Nat. Prod. XXXX, XXX, XXX−XXX

1.96 (3H, s), 1.92−1.86 (1H, m), 1.80−1.63 (3H, m), 1.46−1.33 (3H, m), 1.23 (3H, s), 1.22 (3H, s), 1.21 (3H, s), 0.93 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.1, 147.1, 145.6, 134.8, 126.9, 124.1, 123.8, 49.7, 45.1, 38.3, 37.2, 36.1, 33.4, 30.1, 25.3, 24.0, 23.5, 18.9, 18.8, 18.5; HRMS m/z $[M + Na]^+$ 350.2461 (calcd for $C_{22}H_{33}NONa$ 350.2460).

***N*-(Dehydroabietyl)prop-2-enamide (10).** A mixture of dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), acryloyl chloride (0.28 mL, 3.5 mmol), triethylamine (1.0 mL, 7.2 mmol), and DMAP (0.39 g, 3.5 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 1 h. The reaction mixture was washed with 5% HCl (3 × 50 mL) and water (3 × 50 mL), dried with anhydrous Na_2SO_4, and evaporated to give the amide **10** as a pale yellow solid (0.76 g, 64%): mp 60 °C; $[\alpha]_D^{25}$ +21.8 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3287, 2926, 2866, 1656, 1624, 1547, 1456, 1406, 1234, 986, 953, 821, 708, 630 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.18 (1H, d, *J* = 8.2 Hz), 7.00 (1H, dd, *J* = 8.2, 1.8 Hz), 6.90 (1H, s), 6.28 (1H, dd, *J* = 16.9, 1.5 Hz), 6.06 (1H, dd, *J* = 16.9, 10.3 Hz), 5.63 (1H, dd, *J* = 10.3, 1.4 Hz), 5.59 (1H, t, *J* = 6.0 Hz), 3.32 (1H, dd, *J* = 13.5, 6.6 Hz), 3.19 (1H, dd, *J* = 13.8, 6.7 Hz), 2.97−2.77 (3H, m), 2.32−2.24 (1H, m), 1.94−1.86 (1H, m), 1.82−1.63 (4H, m), 1.44 (2H, dd, *J* = 12.3, 2.2 Hz), 1.41−1.24 (2H, m), 1.23 (3H, s), 1.22 (6H, s), 0.96 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.6, 147.1, 145.7, 134.8, 130.8, 126.9, 126.7, 124.2, 123.9, 123.8, 49.6, 45.1, 38.2, 37.5, 37.4, 36.1, 33.4, 30.2, 25.3, 24.0, 24.0, 18.9, 18.8, 18.5; HRMS m/z $[M + H]^+$ 360.2642 (calcd for $C_{23}H_{34}NO$ 360.2640).

3-(*N*-Dehydroabietylcarbamoyl)pyrazine-2-carboxylic acid (11). A mixture of dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), 2,3-pyrazinedicarboxylic acid anhydride (0.53 g, 3.5 mmol), and triethylamine (1.0 mL, 7.2 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 1 h. The reaction mixture was washed with 5% HCl (2 × 50 mL) and water (3 × 50 mL), dried with anhydrous Na_2SO_4, and evaporated to give the amide **11** as a yellow solid (1.34 g, 88%): mp 157 °C; $[\alpha]_D^{25}$ +4.0 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3369, 2925, 1739, 1637, 1537, 1448, 1267, 1177, 1109, 820, 695, 632, 443 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.98 (1H, dd, *J* = 2.2 Hz), 8.67 (1H, dd, *J* = 2.2 Hz), 8.59−8.52 (1H, m), 7.16 (1H, d, *J* = 8.2 Hz), 6.99 (1H, dd, *J* = 8.3, 8.1 Hz), 6.90 (1H, d, *J* = 1.4 Hz), 3.57 (1H, dd, *J* = 13.7, 7.0 Hz), 3.33 (1H, dd, *J* = 13.7, 6.5 Hz), 3.00−2.76 (4H, m), 2.30 (1H, dd, *J* = 12.9 Hz), 2.01−1.91 (1H, m), 1.86−1.66 (4H, m), 1.55 (1H, d, *J* = 12.9 Hz), 1.47 (1H, dd, *J* = 12.7, 1.9 Hz), 1.38 (1H, td, *J* = 13.3 Hz, 3.9 Hz), 1.23 (3H, s), 1.22 (3H, s), 1.20 (3H, s), 1.04 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.6, 164.0, 147.2, 146.8, 145.9, 145.8, 143.6, 141.8, 134.6, 127.0, 124.2, 124.0, 50.7, 45.7, 38.1, 37.9, 37.6, 36.3, 33.4, 30.3, 25.5, 23.9, 23.9, 19.1, 18.9, 18.5; HRMS m/z $[M + H]^+$ 436.2599 (calcd for $C_{26}H_{34}N_3O_3$ 436.2600).

***N*-(Dehydroabietyl)-3-carboxypropanamide (12).** A mixture of dehydroabietylamine (**1**) (1.0 g, 3.5 mmol), succinic anhydride (0.42 g, 4.2 mmol), and triethylamine (4.0 mL, 28.7 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature overnight. The reaction mixture was washed with 5% HCl (3 × 100 mL) and water (2 × 100 mL). The organic layer was dried with anhydrous Na_2SO_4 and evaporated to give the amide **12** as a pale yellow solid (1.2 g, 89%): mp 65−70 °C; $[\alpha]_D^{25}$ +20.8 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3320, 2925, 2867, 1713, 1647, 1550, 1380, 1171, 821, 626, 478 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.16 (1H, d, *J* = 8.2 Hz), 6.99 (1H, dd, *J* = 8.2, 1.8 Hz), 6.89 (1H, d, *J* = 1.3 Hz), 5.99 (1H, t, *J* = 6.4 Hz), 3.22−3.09 (2H, m), 2.95−2.75 (4H, m), 2.68−2.57 (2H, m), 2.46 (2H, t, *J* = 2.5 Hz), 2.28 (1H, t, *J* = 12.7 Hz), 1.90−1.81 (1H, m), 1.81−1.60 (4H, m), 1.45−1.32 (4H, m), 1.22 (3H, s), 1.21 (3H, s), 1.20 (3H, s), 0.92 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.6, 172.6, 147.1, 145.7, 134.7, 126.9, 124.2, 123.9, 50.1, 45.4, 38.3, 37.4, 37.3, 36.1, 33.4, 31.0, 30.1, 25.3, 24.0, 18.9, 18.6, 18.6; HRMS m/z $[M + H]^+$ 386.2693 (calcd for $C_{24}H_{36}NO_3$ 386.2695).

(*N*-Dehydroabietyl)benzamide (13). A mixture of dehydroabietylamine (**1**) (3.00 g, 10.5 mmol), benzoyl chloride (1.62 g, 11.6 mmol), and TEA (2.36 g, 23.1 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed with 5% HCl (2 × 50 mL) and water (100 mL), dried with anhydrous Na_2SO_4, and evaporated. The crude product was purified with SiO_2 column chromatography on silica gel (1:4 → 1:2 hexane−EtOAc) to yield the amide **13** as an off-white solid (3.1 g, 76%): mp 145 °C; $[\alpha]_D^{25}$ +4.0 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3351, 2926, 1637, 1536, 1293, 823, 698, 639 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.73 (2H, dd, *J* = 8.1, 1.5 Hz), 7.44 (3H, m), 7.17 (1H, d, *J* = 8.4 Hz), 6.99 (1H, dd, *J* = 8.1, 1.8 Hz,), 6.89 (1H, d, *J* = 1.5 Hz), 6.12 (1H, bt), 3.39 (2H, m), 2.86 (3H, m), 2.31 (1H, d, *J* = 13.2 Hz), 1.23 (3H, s), 1.22 (6H, d, *J* = 6.0 Hz), 1.02 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.6, 147.0, 145.6, 134.9, 134.7, 131.4, 128.6, 126.9, 126.8, 124.2, 123.7, 50.3, 45.8, 45.8, 38.3, 37.7, 37.6, 36.4, 33.4, 30.4, 25.4, 23.9, 23.9, 19.1, 18.9, 18.8, 18.6; HRMS m/z $[M + H]^+$ 390.2794 (calcd for $C_{27}H_{36}NO$ 390.2797). Spectroscopic data were consistent with literature values.[21]

Antileishmanial Activity. *L. donovani* (MHOM/SD/1962/1S-Cl 2d) was used in all bioassays. Screening of the compounds for leishmanicidal activity using axenic amastigotes was carried out as previously described[22] using the AlamarBlue (AbD Serotec, Oxford, UK) viability assay similar to that reported for leishmanial promastigotes. Axenic amastigotes were grown at 37 °C in a 5% CO_2 incubator in complete RPMI 1640 containing 20% fetal calf serum (FCS), pH 5.5.[23] Compounds to be assayed were diluted in the complete amastigote medium containing 1% DMSO to twice the final concentration used in the assays and were aliquoted in triplicate (125 μL/well) into 96-well flat-bottom plates (Nunc, Roskilde, Denmark). Initial screening was carried out at 50 μM. IC_{50} values were determined for the most active compounds using serial 2-fold dilutions of the test compounds from 50 to 0.4 μM. Amastigotes (5.0×10^5 cells/mL; 125 μL/well) were added to each well and incubated for 24 h at 37 °C in a 5% CO_2 incubator. The AlamarBlue viability indicator was added (25 μL/well), and the plates were incubated for an additional 24 h, at which time the fluorescence (λ_{ex} = 544 nm; λ_{em} = 590 nm) was measured in a microplate reader (Fluoroskan Ascent FL, Finland). Complete medium both with and without DMSO was used as negative controls (0% inhibition of amastigote growth). Amphotericin B (Sigma-Aldrich, St. Louis, MO, USA), a drug used to treat visceral leishmaniasis, was included as a positive control on each plate and gave >90% inhibition of parasite growth at 1 μM. Toxicity was measured using the AlamarBlue viability indicator on human leukemia monocyte cells (THP-1, 6.4×10^4 cells/well) that were differentiated with retinoic acid as previously described.[24] IC_{50} values were determined using serial 2-fold dilutions of the test compounds in triplicate from 500 to 0.25 μM. Inhibition of intracellular amastigote growth in infected THP-1 cells (1.0×10^5 cells/well) was carried out using transgenic Ld:pSSU-int/LUC promastigotes that express luciferase.[25] Amphotericin B (1 μM) was included as a positive control on each plate. Complete medium both with and without DMSO was used as negative controls. Calculation of the IC_{50}'s and statistical analysis were carried out using GraphPad Prism version 6.0b (GraphPad Software, Inc., San Diego, CA, USA).

Activity against *Trypanosoma cruzi*. Rat skeletal myoblasts (L6 cells) were seeded in 96-well microtiter plates at 2000 cells/well in 100 μL of RPMI 1640 medium with 10% FCS and 2 mM L-glutamine. After 24 h, the medium was removed and replaced by fresh medium (100 μL per well) containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 with the β-galactosidase (Lac Z) gene.[26] After 48 h, the medium was removed from the wells and replaced by 100 μL of fresh medium with or without a serial drug dilution of seven 3-fold dilution steps covering a range from 90 to 0.123 μg/mL. After 96 h of incubation, the plates were inspected under an inverted microscope to ensure growth of the controls and sterility. Then, the substrate CPRG/Nonidet (50 μL) was added to all wells. A color reaction developed within 2−6 h and could be read photometrically at 540 nm. Data were transferred into the graphic program Softmax Pro (Molecular Devices), to caculate IC_{50} values. Benznidazole was the reference drug used.

Cytotoxicity against L6 Cells. Assays were performed in 96-well microtiter plates, with each well containing 100 μL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% FCS, and 4×10^4 L6 cells (a cell line derived from rat skeletal myoblasts). Serial drug dilutions of seven 3-fold dilution steps covering a range from 90 to 0.123 μg/mL were prepared. After 72 h of incubation, the

DOI: 10.1021/acs.jnatprod.5b00990
J. Nat. Prod. XXXX, XXX, XXX−XXX

plates were inspected under an inverted microscope to ensure growth of the controls and sterile conditions. A 10 μL amount of AlamarBlue solution was then added to each well, and the plates were incubated for another 2 h. Then, the plates were read with a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the microplate reader software Softmax Pro. Podophyllotoxin was the reference drug used.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00990.

NMR spectra for compounds **2**−**12** (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to Darin Al Ramahi and Yukho Sok-sar for their excellent technical assistance. This study was supported by the Finnish Funding Agency for Technology and Innovation (project 40375/11), the Foundation for Research of Natural Resources in Finland, Marjatta ja Eino Kollin Säätiö, the European Commission (contract no. EU-KBBE-227239-ForestSpeCs), the Academy of Finland (projects 252308, 256515, 264020, and 265481), and the COST Actions CM-0801 (New Drugs for Neglected Diseases) and CM1307 (Targeted Chemotherapy towards Diseases cause by Endoparasites). C.L.J. holds the Michael and Penny Feiwel Chair of Dermatology.

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DOI: 10.1021/acs.jnatprod.5b00990
J. Nat. Prod. XXXX, XXX, XXX−XXX