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Short communication

Synthesis and biological evaluation of dehydroabietic acid derivatives

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ABSTRACT

A series of C18-oxygenated derivatives of dehydroabietic acid were synthesized from commercial abietic acid and evaluated for their cytotoxic, antimycotic, and antiviral activities.

1. Introduction

Diterpene resin acids are important defense compounds of conifers against potential herbivores and pathogens [1]. The biological activity of natural abietane acids has been reviewed [2]. Antimicrobial, antiulcer and cardiovascular activities are the most representative for this class of diterpenoids. Dehydroabietic acid (DHA)(Fig. 1, R=COOH), a natural occurring diterpene resin acid, and its derivatives exhibit a broad spectrum of biological action. For example, they have shown antiulcer [3], antimicrobial [4], anxiolytic [5], antiviral [6], antitumor [7], and cytotoxic activities [8]. Recent studies have demonstrated that DHA and some derivatives are chemical modulators, particularly openers, of large-conductance calcium-activated K^+ channels (BK channels) [9]. This feature makes DHA a new scaffold in the treatment of acute stroke, epilepsy, asthma, hypertension, gastric hypermotility and psychoses. Also, DHA was reported to have properties of enhancing the inhibitory activity of anticancer drugs in cervical carcinoma cells, hepatocellular carcinoma cells, or breast cancer cells [10]. However, the cytotoxic activity of easily available derivatives of DHA have not yet been reported so far.

Continuing our research program on the synthesis of bioactive terpenoids, we were interested in confirming the results reported in a recent patent on the use of abietic acid **1** and derivatives as antitumor agents [11] (Scheme 1). As a result, we studied a series of abietic acid derivatives and found that methyl abietate **2** displayed the highest cytotoxicity against HeLa cancer cells (CC_{50} 11 μM), and showed good selectivity towards non-cancerous cells (selectivity index 13.7) [12]. Encouraged by these research results, DHA was chosen as the starting material in screening a series of derivatives for new potential bioactive compounds.

In this communication, we describe the syntheses of a number of derivatives of DHA from commercially available (−)-abietic acid (**1**) (Scheme 1) [13], and the results of preliminary evaluation of their cytotoxic, antimycotic and antiviral activities. In this study, an oxygenated moiety (such as methyl ester, alcohol, or aldehyde) was introduced into the lipophilic dehydroabietane skeleton. In this context, simple and sequential modifications were performed in the molecule of DHA (**4**). Compound **4** and nine derivatives with different functional groups at C7 and C18 were tested. All the compounds were easily obtained in good yield, by standard or reported chemical procedures. Some of the synthesized compounds have been isolated as natural products, in particular, dehydroabietinol (pomiferin A) (**5**) [14], dehydroabietinol acetate (**6**) [15], 7α-hydroxydehydroabietinol (**11**) [16] and 7-oxodehydroabietinol (**12**) [17], however, few reports have appeared on their biological activities.

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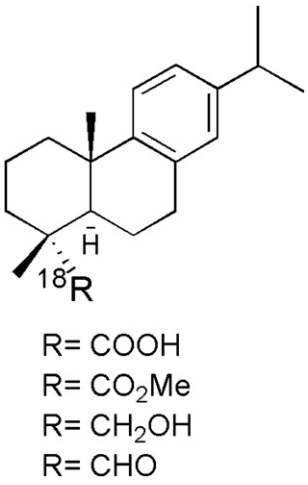


Fig. 1. Chemical structure of some tested dehydroabietanes.

2. Results and discussion

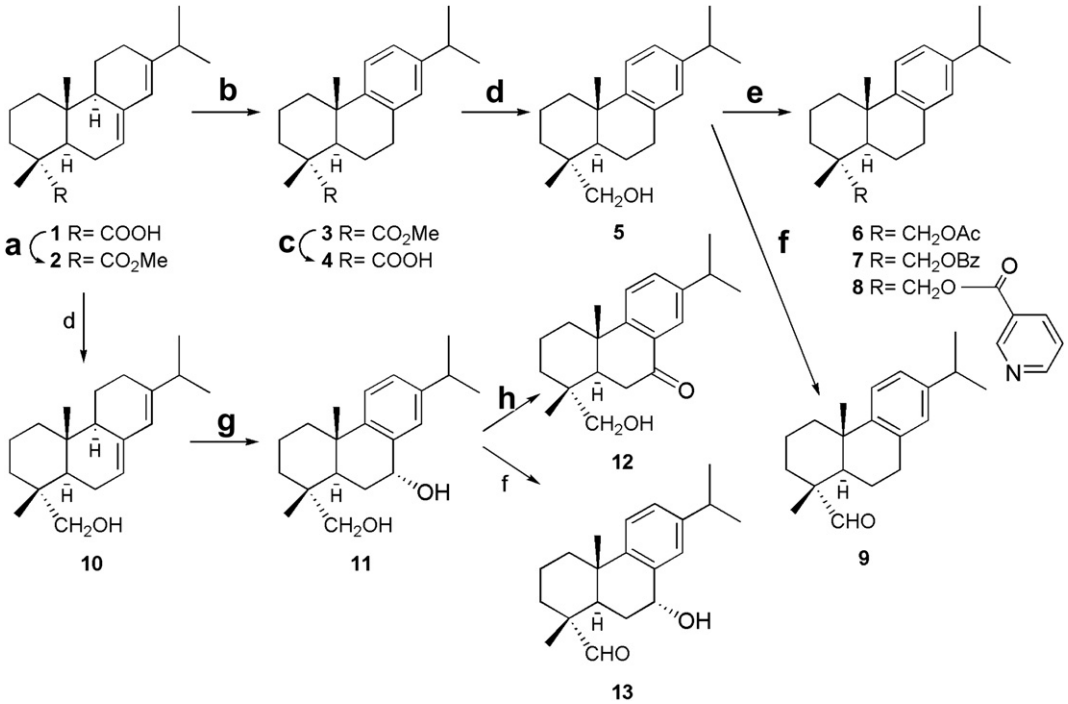
2.1. Chemistry

The synthesis of the C18-functionalized dehydroabietanes used in this work begins with the preparation of the required methyl ester **2** (methyl abietate) from commercial (−)-abietic acid, following a reported procedure (Scheme 1) [12]. Thus, abietic acid **1** was esterified by treatment with lithium hydroxide and methyl sulphate to give ester **2** in quantitative yield. With the ester **2** in hand, we carried out the reaction of aromatization to obtain the dehydroabietane skeleton (Scheme 1). The ester **2** was treated in a similar manner as pine rosin is treated to obtain disproportionated rosin [18]. Thus, neat ester **2** was heated at 250 °C in the presence of 5% Pd/C to give methyl dehydroabietate **3** in 85% yield. Then, we carried out the funtional group interconversions necessary to obtain six more derivatives. Thus, ester **3** was saponified with KOH in aqueous methanol to give dehydroabietic acid (**4**). Reduction of **3** with $LiAlH_4$ in dry tetrahydrofuran at reflux gave dehydroabietinol **5** in 90% yield. Esterification of **5** with acetyl chloride, benzoyl chloride and nicotinic chloride gave the corresponding esters **6**, **7**, and **8**, respectively. Finally, oxidation with Dess–Martin periodinane [19] of **5** afforded aldehyde **9** in 95% yield.

On the other hand, reduction of ester **2** with $LiAlH_4$ in dry tetrahydrofuran at reflux gave alcohol (abietinol) **10** in quantitative yield. Then, we reacted alcohol **10** with SeO_2 to produce the aromatization and simultaneous functionalization at C7 (Compound **11**) [20]. Finally, alcohol **11** was oxidized with MnO_2 and Dess–Martin periodinane to give the keto–alcohol **12** and the aldehyde **13**, respectively.

2.2. Biological evaluation

All compounds **1**–**13** (Scheme 1) were tested for antimycotic, cytotoxic and antiviral activity with the exception of compound **8** which was not soluble under the experimental conditions. Firstly, the compounds did not show antimycotic activity against *Candida parapsilosis, Candida krusei*, *Candida tropicalis and Candida albicans* in concentrations below 100 μg/mL (data not shown). MIC values for the two reference antifungal drugs, amphotericine B and itraconazole (Sigma, New Jersey, USA), used as positive controls, were within the established values for the AFST-EUCAST protocol. The reaction of aromatization of abietanes did not improve anti-*Candida* activity when we compare with the results of our previous report [12]. In contrast, the dehydroabietanes **4** and **11** showed anti-Aspergillus activity. The dehydroabietane **4** showed activity against *Aspergillus terreus* with MIC value of 39.7 μg/mL whereas compound **11** showed activity against *Aspergillus fumigates* and *Aspergillus niger* with MIC values of 50 and 63 μg/mL, respectively. Thus, the reaction of aromatization of abietanes improves anti-Aspergillus activity when we compare with the results of our previous report in which abietic acid **1** was not active.



Scheme 1. Reagents and conditions: a) LiOH, Me_2SO_4,DMF, 100%; b) 5% Pd/C, 250 °C, 85%; c) KOH, MeOH, H_2O, 75%; d) $LiAlH_4$, THF, 90%; e) AcCl, BzCl, nicotinic chloride, Et_3N, DCM, 75%, 70%, 80%, respectively; f) Dess–Martin periodinance, 90% for **9**,85% for **13**;g) SeO_2, TBHP, DCM, 65%; h) MnO_2, DCM, 60%.

Next, the compounds **1**–**13** (Scheme 1) were tested *in vitro* for potential antitumor and cytotoxic activities determining the concentration of the compound that induces 50% growth inhibition (IC_{50}) of the HeLa and Jurkat tumor cell lines, and the Vero no-tumor cell line (Table 1). All these compounds produced a dose-dependent inhibition on the growth of the HeLa and Jurkat tumor cell lines and Vero cell line, with R^2 (coefficient of linear regression) > 0.8. The dehydroabietane that showed the highest antitumor in vitro activity on the HeLa and Jurkat tumor lines was the dehydroabietane **5** with IC_{50} values of 13.0 ± 2.8 μg/mL and 9.7 ± 0.7 μg/mL, respectively. However, the dehydroabietane that showed the lowest cytotoxicity on non-cancerous cells (Vero cell line) and highest antitumor *in vitro* activity on the Jurkat tumor line was the dehydroabietane **6** with IC_{50} values of 95.0 ± 13.0 μg/mL and 22.0 ± 3.6 μg/mL, respectively. This means that the dehydroabietane with the highest selectivity index (SI) was dehydroabietinol acetate (**6**) (SI = 4.3). Comparing the cytotoxic activity on HeLa cells of our report on abietane derivatives [12] with these dehydroabietane derivatives, the compound with the highest selectivity was methyl abietate (**2**) (SI = 13.7), now the compound dehydroabietinol (**5**) displayed the highest cytotoxic activity to both HeLa and Jurkat cells.

In Jurkat cells, the cytotoxicity of dehydroabietinol **5** was higher than that of methyl abietate (**2**). In contrast, the abietinol **10** was more cytotoxic on Vero cells than on Jurkat cells. Specifically, according to SI value the dehydroabietane derivatives are more selective to Jurkat cells than abietane derivatives. Selectivity refers to the ability of a compound to recognize its target without interacting with other target. In this study, it is likely that the dehydroabietane derivatives recognize targets in Jurkat cells which are not present in HeLa cells. The introduction of an oxygenated functional group in position C-7 in compound **5** (compounds **11** and **12**) led to less active compounds.

Gigante and co-workers [21] evaluated the antitumoral *in vitro* activity of different catechols from abietic acid. The order of activity in the catechol series with different functional groups at position C18 was ester > acid ≥ alcohol. Our previous study on the biological activity of abietic acid derivatives were consistent with this order of activity but in these series of dehydroabietane derivatives is not possible to establish this relationship.

Table 1
Cytotoxic activity of dehydroabietane diterpenes determined by the MTT technique expressed as IC_{50} (μg/mL).[a]

Compound	Cell lines[b]				
	Vero	HeLa	Jurkat		
	IC_{50}[c]	IC_{50}	SI[d]	IC_{50}	SI[d]
1[e]	53.0 ± 5.6	15.0 ± 0.6	3.5	≥32.0	–
2[e]	49.0 ± 3.0	3.6 ± 1.0	13.7	24.0 ± 1.5	2.0
3	36.0 ± 5.8	28.0 ± 5.0	1.3	21.0 ± 3.4	1.7
4	91.0 ± 7.5	101.0 ± 10.3	0.9	28.0 ± 2.7	3.2
5	38.0 ± 6.5	13.0 ± 2.8	2.9	9.7 ± 0.7	3.9
6	95.0 ± 13.0	28.0 ± 4.7	3.4	22.0 ± 3.6	4.3
7	≥200	≥200	–	57.0 ± 11.0	3.5
8	[f]	[f]	[f]	[f]	[f]
9	32.0 ± 5.0	24.0 ± 4.4	1.3	17.0 ± 0.6	1.9
10[e]	13.0 ± 2.4	5.2 ± 0.5	2.5	15.0 ± 3.6	0.9
11	30.0 ± 5.4	20.0 ± 3.8	1.5	12.0 ± 1.0	2.5
12	27.0 ± 5.0	30.0 ± 1.2	0.9	17.0 ± 0.3	1.6
13	36.0 ± 5.5	23.0 ± 4.3	1.6	12.0 ± 1.5	3.0
Vincristine[a]	1.1 ± 0.2	0.05 ± 0.01	2.2	–	

[a] Concentration of compounds that induces 50% growth inhibition in 48 h.
[b] HeLa, human cervix epitheloid carcinoma ATCC CCL-2; Vero, *Cercopithecus aethiops* African green monkey kidney ATCC CCL-81. Jurkat, human acute T cell leukemia ATCC TIB-152.
[c] IC_{50} values are expressed as the mean ± S.E.M. of at least four dilutions by quadruplicate.
[d] SI, selectivity index is defined as VERO IC_{50} over HeLa IC_{50}.
[e] These compounds were tested in a previous report [12].
[f] This compound did not dissolved in DMSO.

Table 2
Cytotoxicity and anti-HSV-1 activity of dehydroabietane diterpenes on Vero[e] Cells determined by the end-point titration technique.

Compound	CC_{100} (μg/mL)[a]	Viral Reduction Factor[b]	Antiviral Activity (μg/mL)[c]
1[d]	>100	NA	–
2[d]	50	$10^{0.5}$	6.25
3	>50	–	–
4	>50	–	–
5	50	–	–
6	>50	10^{1}	25
7	>50	$10^{0.5}$	50
9	50	–	–
10[d]	25	10^{1}	6.25
11	25	–	–
12	25	–	–
Heparin	>100 I.U./mL	10^{2}	0.5 I.U./mL
Acyclovir	>600	10^{4}	6.0

[a] Minimal toxic dose that detached 100% of the cell monolayer.
[b] Ratio of the virus titer in the absence over virus titer in the presence of the tested compound.
[c] Maximal nontoxic dose that showed the highest viral reduction factor. N.A: no activity.
[d] These abietane activities were reported in a previous study on HeLa cells [12].
[e] The dehydroabietanes did not show activity on HeLa cells.

The antiviral activity of the dehydroabietanes against herpes simplex virus type 1 (HSV-1) was determined using a modified end-point titration technique (EPPT)(Table 2) [12]. Only the dehydroabietanes **6** and **7** reduced the HSV-1 replication with values below 100 μg/mL. The dehydroabietane that showed the highest antiviral activity was dehydroabietinol acetate (**6**). According to Vlietinck et al. [22], only the compounds with reduction factor (*Rf*) of the viral titer over 1×10^3 (*Rf*: ratio of the virus titer in the absence over virus titer in the presence of the tested compound) show relevant antiviral activity. The ester dehydroabietinol acetate (**6**) was found to be slightly active against HSV-1 over infected confluent monolayers of Vero cells with *Rf* value of 1×10^1 at a concentration of 25 μg/mL. Comparing the antiviral activity of the abietinol (**10**) [12] with dehydroabietinol (**5**), the aromatization of ring C did not improve the activity.

3. Conclusions

In conclusion, we have prepared and tested several dehydroabietanes for their antitumor, antifungal and antiviral activities *in vitro*. In general, the aromatization in ring *C* of abietic acid led to less active compounds. Also, the antitumor activity of the dehydroabietanes was more selective to Jurkat cells than HeLa cells. Dehydroabietinol acetate (**6**) presented the highest SI on Jurkat cells and it also showed the highest anti-herpes activity. These results confirm that abietane and dehydroabietane-type diterpenes do have interesting antitumor properties and encourage us to research about what targets are recognized for these diterpenes in HeLa and Jurkat cells. Furthermore, these results also encourage us to synthesize additional abietane and dehydroabietane derivatives with the aim of obtaining compounds more potent biologically.

4. Experimental

4.1. Chemistry

Optical rotations were determined using a 5-cm path-length cell, using dichloromethane as solvent (concentration expressed in g/100 mL). $[\alpha]_D$-values are given in 10^{-1} deg cm^2 g^{-1}. NMR spectra

were recorded on a 300 MHz spectrometer with tetramethylsilane as an internal standard. All spectra were recorded in $CDCl_3$ as solvent unless otherwise described. Complete assignments of ^{13}C NMR multiplicities were made on the basis of DEPT experiments. *J* values are given in Hz. Mass spectra (MS) were run by electron impact (EI) at 70 eV. Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F-254 in 0.25 mm-thick plates. Compounds on TLC plates were detected under UV light at 254 nm and visualized by immersion in a 10% sulfuric acid solution and heating on a hotplate. Purifications were performed by flash chromatography on Merck silica gel (230–400 mesh). All non-aqueous reactions were carried out in an argon atmosphere in oven-dried glassware. Commercial reagent grade solvents and chemicals were used as received unless otherwise noted. Combined organic extracts were washed with brine, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Methyl abietate (**2**) was prepared from commercial abietic acid (Aldrich) as reported by us [12]. All compounds prepared in this work exhibit spectroscopic data in agreement with the proposed structures.

4.1.1. Methyl 8,11,13-abietatrien-18-oate (methyl dehydroabietate, **3**)

To neat methyl abietate (**2**) (4.0 g, 12 mmol) 5% Pd/C (200 mg) was added and then heated to 240–250 °C for 2 h. Then, the mixture was cooled to rt and diluted with hexane-ethyl acetate 8:2 and purified by flash chromatography, using hexane-ethyl acetate (8:2) as eluent, to give methyl dehydroabietate **3** (3.3 g, 85%) as an orange oil: $[\alpha]^{20}_D + 52.0$ (*c* 1.0); ^1H NMR (300 MHz) δ 7.16 (1H, d, $J = 8.1$), 6.99 (1H, dd, $J = 8.1, 1.5$), 6.88 (1H, br s), 3.65 (3H, s), 1.27 (3H, s), 1.23 (3H, s), 1.21 (6H, s); ^{13}C NMR (75 MHz) δ_C 179.1 (s), 146.9 (s), 145.7 (s), 134.7 (s), 126.8 (d), 124.1 (d), 123.9 (d), 51.9 (d), 47.6 (s), 44.8 (d), 37.9 (t), 36.9 (s), 36.6 (t), 33.4 (d), 29.9 (t), 25.1 (q), 24.0 (q), 24.0 (q), 21.7 (t), 18.6 (t), 16.5 (q); HRMS (EI) *m/z* 314.2231 $[M]^+$, calcd for $C_{21}H_{30}O_2$: 314.2246.

4.1.2. 8,11,13-Abietatrien-18-oic acid (dehydroabietic acid, **4**)

A mixture of ester **3** (200 mg, 0.63 mmol), KOH (85%, 1.0 g, 15 mmol), H_2O (2 mL) and methanol (12 mL) was refluxed for 16 h. Then, additional KOH (85%, 400 mg, 7 mmol) was added and reflux continued for 5 h more. After this time, the reaction mixture was then cooled, poured into aqueous HCl (1.5 M, 20 mL) and extracted three times with ethyl acetate. The organic extract was dried over $MgSO_4$ and concentrated under reduced pressure to give the crude acid which was purified by chromatography eluting with hexane-ethyl acetate (3:7) to give acid **5** (142 mg, 75%) as a foam: $[\alpha]^{20}_D + 59.7$ (c 0.5); ^1H NMR (300 MHz) δ 7.16 (1H, d, $J = 8.4$), 6.99 (1H, dd, $J = 8.1, 1.5$), 6.88 (1H, br s), 2.85 (1H, m), 1.28 (3H, s), 1.21 (6H, d, $J = 6.9$); ^{13}C NMR (75 MHz) δ_C 184.5 (s), 146.7 (s), 145.7 (s), 134.7 (s), 126.9 (d), 124.1 (d), 123.8 (d), 44.6 (d), 37.9 (t), 36.8 (s), 36.7 (t), 33.4 (d), 30.0 (t), 25.1 (q), 24.0 (q), 24.0 (q), 21.7 (t), 18.5 (t), 16.2 (q); HRMS (EI) *m/z* 300.2068 $[M]^+$, calcd for $C_{20}H_{28}O_2$: 300.2089.

4.1.3. 8,11,13-Abietatrien-18-ol (dehydroabietinol, **5**)

To a solution of ester **3** (1.78 g, 5.7 mmol) in tetrahydrofuran (25 mL), $LiAlH_4$ (1.20 g, 31.5 mmol) was added in portions and the resulting mixture was refluxed for 16 h. Then, it was cooled to 0 °C and 1.2 mL of H_2O, 1.2 mL of 15% NaOH and 3.6 mL of H_2O were added sequentially and carefully. The resulting white solid was filtered off and washed with ethyl acetate. The extract was concentrated and purified by chromatography eluting with hexane-ethyl acetate (6:4) to give 1.46 g (90%) of pure alcohol **5** as a slightly yellow oil: $[\alpha]^{20}_D + 43.5$ (c 0.6); ^1H NMR (300 MHz) δ 7.17 (1H, d, $J = 8.1$), 6.98 (1H, dd, $J = 8.1, 1.5$), 6.88 (1H, br s), 3.45 (1H, d, $J = 10.8$), 3.21 (1H, d, $J = 10.8$), 2.85 (1H, m), 1.21 (6H, d, $J = 6.9$), 1.21 (3H, s), 0.87 (3H, s); ^{13}C NMR (75 MHz) δ_C 147.2 (s), 145.4 (s), 134.7 (s), 126.7 (d), 124.1 (d), 123.7 (d), 72.1 (t), 43.8 (d), 38.3 (t), 37.8 (s), 37.2 (s), 35.0 (t), 33.4 (d), 30.0 (t), 25.2 (q), 24.0 (q), 24.0 (q), 18.8 (t), 18.6 (t), 17.3 (q); HRMS (EI) *m/z* 286.2265 $[M]^+$, calcd for $C_{20}H_{30}O$: 286.2297.

4.1.4. 8,11,13-Abietatrien-18-yl acetate (dehydroabietinol acetate, **6**)

A solution of the alcohol **5** (300 mg, 1 mmol) and dimethylaminopyridine (12 mg, 0.1 mmol) in triethylamine (8 mL) at 0 °C was treated with acetyl chloride (355 μL, 5 mmol) dropwise. The resulting mixture was stirred for 16 h. Then, it was diluted with diethyl ether and washed with water and brine. The aqueous phase was extracted twice with diethyl ether and the combined organic extracts were dried and concentrated. The residue was purified by chromatography eluting with hexane-ethyl acetate (7:3) to give 258 mg (75%) of acetate **6** as a slightly yellow oil: $[\alpha]^{20}_D + 8.5$ (c 0.5); ^1H NMR (300 MHz) δ 7.18 (1H, d, $J = 8.1$), 6.99 (1H, dd, $J = 8.1, 1.5$), 6.90 (1H, br s), 3.98 (1H, d, $J = 10.8$), 3.69 (1H, d, $J = 10.8$), 2.85 (1H, m), 2.02 (3H, s), 1.22 (6H, d, $J = 6.6$), 1.21 (3H, s), 0.94 (3H, s); ^{13}C NMR (75 MHz) δ_C 171.3 (s), 147.1 (s), 145.6 (s), 134.7 (s), 126.8 (d), 124.2 (d), 123.9 (d), 72.4 (t), 44.0 (d), 38.2 (t), 37.4 (s), 36.7 (s), 35.5 (t), 33.4 (d), 30.2 (t), 25.3 (q), 24.0 (q), 24.0 (q), 21.0 (q), 18.9 (t), 18.5 (t), 17.5 (q); HRMS (EI) *m/z* 328.2422 $[M]^+$, calcd for $C_{22}H_{32}O_2$: 328.2402.

4.1.5. 8,11,13-Abietatrien-18-yl benzoate (dehydroabietinol benzoate, **7**)

A solution of the alcohol **5** (300 mg, 1 mmol) and dimethylaminopyridine (12 mg, 0.1 mmol) in triethylamine (8 mL) at 0 °C was treated with benzoyl chloride (580 μL, 5 mmol) dropwise. The resulting mixture was stirred for 16 h. Then, it was diluted with diethyl ether and washed with water and brine. The aqueous phase was extracted twice with diethyl ether and the combined organic extracts were dried and concentrated. The residue was purified by chromatography eluting with hexane-ethyl acetate (7:3) to give 286 mg (70%) of benzoate **7** as a slightly yellow oil: ^1H NMR (300 MHz) δ 8.01 (2H, m), 7.53 (1H, m), 7.41 (2H, m), 7.20 (1H, d, $J = 8.1$), 7.00 (1H, dd, $J = 8.1, 1.5$), 6.88 (1H, br s), 4.21 (1H, d, $J = 10.8$), 3.98 (1H, d, $J = 10.8$), 2.85 (1H, m), 1.25 (3H, s), 1.22 (6H, d, $J = 6.9$), 1.04 (3H, s); ^{13}C NMR (75 MHz) δ_C 166.6 (s), 146.9 (s), 145.5 (s), 134.7 (s), 132.8 (d), 130.3 (s), 129.5 × 2 (d), 128.4 × 2 (d), 126.9 (d), 124.3 (d), 123.9 (d), 73.0 (t), 44.9 (d), 38.3 (t), 37.6 (s), 37.1 (s), 35.8 (t), 33.4 (d), 30.1 (t), 25.5 (q), 23.9 (q), 23.9 (q), 19.1 (t), 18.6 (t), 17.6 (q); HRMS (EI) *m/z* 390.2537 $[M]^+$, calcd for $C_{27}H_{34}O_2$: 390.2559.

4.1.6. 8,11,13-Abietatrien-18-yl nicotinate (dehydroabietinol nicotinate, **8**)

A suspension of nicotinic acid (2.46 g, 20 mmol) in thionyl chloride (10 mL) was refluxed for 2 h, then it was cooled and concentrated under vacuum. Then, 500 mg of the resulting solid was added to a solution of the alcohol **5** (300 mg, 1 mmol) and dimethylaminopyridine (12 mg, 0.1 mmol) in triethylamine (8 mL) at 0 °C. The resulting mixture was stirred for 48 h. Then, it was diluted ethyl acetate and washed with water and brine. The aqueous phase was extracted twice with ethyl acetate and the combined organic extracts were dried and concentrated. The residue was purified by chromatography eluting with hexane-ethyl acetate (7:3) to give 328 mg (80%) of nicotinate **8** as a slightly yellow solid: ^1H NMR (300 MHz) δ 9.21 (1H, br d, $J = 2.1$), 8.75 (1H, dd, $J = 4.8, 1.5$), 8.26 (1H, ddd, $J = 7.8, 2.1, 2.1$), 7.36 (1H, dd, $J = 8.1, 5.1$), 7.19 (1H, d, $J = 8.1$), 7.00 (1H, dd, $J = 8.1, 1.8$), 6.88 (1H, br s), 4.25 (1H, d, $J = 10.8$), 4.01 (1H, d, $J = 10.8$), 2.85 (1H, m), 1.25 (3H, s), 1.22 (6H, d, $J = 6.9$), 1.04 (3H, s); ^{13}C NMR (75 MHz) δ_C 165.1 (s), 153.3 (d),

150.7 (d), 146.7 (s), 145.5 (s), 136.9 (d), 134.4 (s), 126.8 (d), 126.1 (s), 124.2 (d), 123.8 (d), 123.2 (d), 73.2 (t), 44.8 (d), 38.2 (t), 37.4 (s), 37.1 (s), 35.7 (t), 33.3 (d), 30.3 (t), 25.4 (q), 23.8 (q), 23.8 (q), 19.0 (t), 18.4 (t), 17.5 (q); HRMS (EI) *m/z* 391.2526 $[M]^+$, calcd for $C_{26}H_{33}NO_2$: 391.2511.

4.1.7. 8,11,13-Abietatrien-18-al (dehydroabietinal, **9**)

A solution of alcohol **5** (1.1 g, 3.84 mmol) in DCM (11 mL) was treated with Dess–Martin periodinane [19] (2.4 g, 5.7 mmol). After being stirred for 16 h, the mixture was diluted with diethyl ether, saturated sodium thiosulphate solution, and saturated sodium hydrogen carbonate solution. The resulting mixture was stirred vigorously for 1 h and then extracted with ethyl acetate. The combined organic extracts were washed with brine, dried and concentrated. Then, the residue was chromatographed on silica eluting with hexane-ethyl acetate (7:3) to give 1.0 g (90%) of aldehyde **9** as a slightly yellow semisolid: $[\alpha]^{20}_D + 55.0$ (c 0.63); ^{1}H NMR (300 MHz) δ 9.25 (1H, s), 7.18 (1H, d, $J = 8.4$), 6.99 (1H, dd, $J = 8.1$, 1.8), 6.89 (1H, br s), 2.85 (1H, m), 1.22 (3H, s), 1.22 (6H, d, $J = 6.9$), 1.15 (3H, s); ^{13}C NMR (75 MHz) δ_C 206.0 (d), 146.1 (s), 145.8 (s), 134.3 (s), 127.0 (d), 124.1 (d), 123.9 (d), 49.7 (s), 42.7 (d), 37.8 (t), 36.2 (s), 33.4 (d), 31.9 (t), 29.7 (t), 25.1 (q), 23.9 (q), 23.9 (q), 21.3 (t), 17.7 (t), 13.9 (q); HRMS (EI) *m/z* 284.2125 $[M]^+$, calcd for $C_{20}H_{28}O$: 284.2140.

4.1.8. Abietadien-18-ol (abietinol, **10**)

A suspension of $LiAlH_4$ (3.10 g, 82 mmol) in tetrahydrofuran (100 mL) was stirred as abietic acid (**1**) (70%, 5 g, 11.6 mmol) in tetrahydrofuran (50 mL) was added. The mixture was refluxed for 15 h, then it was cooled to 0 °C and 3.1 mL of H_2O, 3.1 mL of 15% NaOH and 9.3 mL of H_2O were added sequentially and carefully. The resulting white solid was filtered off and washed with ethyl acetate. The extract was concentrated and purified by chromatography eluting with hexane-ethyl acetate (7:3) to give 3.3 g (100%) of pure alcohol **10** as a white solid: mp 81–83 °C; $[\alpha]^{20}_D - 132.0$ (c 0.33); ^{1}H NMR (300 MHz) δ 5.75 (1H, s), 5.37 (1H, br s), 3.31 (1H, d, $J = 11.0$), 3.07 (1H, d, $J = 11.0$), 0.99 (3H, d, $J = 6.8$), 0.98 (3H, d, $J = 6.8$), 0.84 (3H, s), 0.80 (3H, s); ^{13}C NMR (75 MHz) δ_C 144.9 (s), 135.4 (s), 122.4 (d), 120.9 (d), 71.8 (t), 50.7 (d), 43.4 (d), 38.8 (t), 37.3 (s), 35.6 (t), 34.7 (d), 34.5 (s), 27.4 (t), 23.7 (t), 22.6 (t), 21.3 (q), 20.7 (q), 18.1 (t), 17.6 (q), 14.1 (q); HRMS (EI) *m/z* 288.2440 $[M]^+$, calcd for $C_{20}H_{32}O$: 288.2453.

4.1.9. 7α,18-Dihydroxyabieta-8,11,13-triene (7α-hydroxydehydroabietinol, **11**)

A suspension of selenium dioxide (3.3 g, 29.7 mmol) in DCM (40 mL) was treated with 70% tert-butyl hydroperoxide (8.8 mL, 66 mmol) and stirred for 15 min. Then, a solution of the alcohol **10** (1.9 g, 6.6 mmol) in DCM (20 mL) was added and stirring continued for 20 h. Then, the reaction was diluted with DCM and washed with brine. The aqueous phase was extracted with ethyl acetate and the combined organic extracts were dried and concentrated. The resulting residue was chromatographed on silica (8:2–4:6 hexane-EtOAc as eluant) to give 1.3 g (65%) of the aromatic diol **11** as an yellow solid: mp 85–86 °C; $[\alpha]^{20}_D + 10.1$ (c 5.7); ^{1}H NMR (300 MHz) δ 7.17 (1H, d, $J = 8.4$), 7.16 (1H, s), 7.09 (1H, dd, $J = 8.1$, 1.8), 4.68 (1H, t, $J = 2.4$), 3.47 (1H, d, $J = 11.7$), 2.90 (1H, d, $J = 11.4$), 2.86 (1H, m), 1.23 (6H, d, $J = 7.2$), 1.11 (3H, s), 0.74 (3H, s); ^{13}C NMR (75 MHz) δ_C 147.2 (s), 145.9 (s), 135.8 (s), 127.8 (d), 126.2 (d), 124.2 (d), 70.2 (t), 68.0 (d), 38.0 (t), 37.5 (s), 37.2 (s), 37.0 (d), 34.4 (t), 33.4 (d), 27.8 (t), 24.3 (q), 24.0 (q), 23.8 (q), 18.6 (t), 17.6 (q); HRMS (EI) *m/z* 302.2265 $[M]^+$, calcd for $C_{20}H_{30}O_2$: 302.2246.

4.1.10. 7-Oxoabieta-8,11,13-trien-18-ol (7-oxodehydroabietinol, **12**)

To a solution of diol **11** (115 mg, 0.38 mmol) in DCM (4 mL) activated manganese dioxide (85%, 970 mg, 9.5 mmol) was added in one portion. After being stirred for 16 h, the mixture was filtered through a pad of celite and concentrated. Then, the residue was chromatographed on silica eluting with hexane-ethyl acetate (7:3) to give 68 mg (60%) of ketone **12** as a colorless oil: $[\alpha]^{20}_D + 15.0$ (c 2.85); ^{1}H NMR (300 MHz) δ 7.80 (1H, d, $J = 2.1$), 7.36 (1H, dd, $J = 8.1$, 2.1), 7.28 (1H, d, $J = 8.1$), 3.45 (1H, d, $J = 10.8$), 3.13 (1H, d, $J = 10.8$), 2.88 (1H, sept., $J = 6.9$), 1.24 (3H, s), 1.22 (3H, d, $J = 6.9$), 1.21 (3H, d, $J = 6.9$), 0.92 (3H, s); ^{13}C NMR (75 MHz) δ_C 199.8 (s), 153.6 (s), 146.5 (s), 132.4 (d), 130.5 (s), 124.8 (d), 123.6 (d), 70.7 (t), 42.3 (d), 37.6 (s), 37.4 (t), 35.8 (t), 34.6 (t), 33.5 (d), 23.8 (q), 23.7 (q), 23.7 (q), 18.2 (t), 17.2 (q); HRMS (EI) *m/z* 300.2077 $[M]^+$, calcd for $C_{20}H_{28}O_2$: 300.2089.

4.1.11. 7α-Hydroxyabieta-8,11,13-trien-18-al (7α-hydroxydehydroabietinal, **13**)

A solution of diol **11** (275 mg, 0.91 mmol) in DCM (3 mL) was treated with Dess–Martin periodinane [19] (390 mg, 0.91 mmol). After being stirred for 4 h, the mixture was diluted with diethyl ether, saturated sodium thiosulphate solution, and saturated sodium hydrogen carbonate solution. The resulting mixture was stirred vigorously for 1 h and then diluted with ethyl acetate. The aqueous phase was extracted with ethyl acetate twice and the combined organic extracts were washed with brine, dried and concentrated. Then, the residue was chromatographed on silica eluting with hexane-ethyl acetate (7:3) to give 232 mg (85%) of aldehyde **13** as a slightly yellow oil: ^{1}H NMR (300 MHz) δ 9.28 (1H, s), 7.19 (1H, d, $J = 8.4$), 7.17 (1H, br s), 7.12 (1H, dd, $J = 8.1$, 2.1), 4.74 (1H, br d, $J = 2.7$), 2.85 (1H, m), 1.22 (6H, d, $J = 6.9$), 1.16 (3H, s), 1.14 (3H, s); ^{13}C NMR (75 MHz) δ_C 206.3 (d), 146.6 (s), 145.9 (s), 135.7 (s), 128.0 (d), 126.5 (d), 124.2 (d), 67.6 (d), 49.1 (s), 37.4 (t), 37.2 (d), 36.5 (s), 33.4 (d), 31.8 (t), 30.9 (t), 24.2 (q), 23.9 (q), 23.8 (q), 17.7 (t), 13.9 (q); HRMS (EI) *m/z* 300.2058 $[M]^+$, calcd for $C_{20}H_{28}O_2$: 300.2089.

4.2. Biological assays

Stock solutions of compounds were prepared in dimethyl sulfoxide (DMSO, Sigma) and frozen at −70 °C until required. The concentration of DMSO in biological assays was of 0.05%. Cell controls with DMSO at 0.05% were used.

4.3. Antifungal assay

The antifungal activity of dehydroabietanes was evaluated following the standard method proposed by the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing (AFST-EUCAST) [23] for fermentative yeasts. *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 6258), *C. tropicalis* (CECT 11901), *C. albicans* (ATCC 10231) were used to evaluate antifungal activity. Briefly, seven serial dilutions of the compounds were dispensed into 96-well flat-bottom microdilution plates in duplicate at final concentrations between 100 μg/mL and 2 μg/mL. Amphotericine B (Sigma Chemical Co, MO, USA) and itraconazole (Sigma Chemical Co, MO, USA) were used as positive controls at a range of 0.031–16 μg/mL. The plates were frozen at −70 °C until required. The inoculum size for microdilution plates were $0.5–2.5 \times 10^5$ CFU/mL for yeast. For the AFST-EUCAST method, the Minimal Inhibitory Concentrations (MICs) were determined after 24 h of incubation and is defined as the lowest concentration that resulted in a 90% reduction of growth. MICs results were expressed as range and geometric mean (GM) of triplicates of each compound tested three times against each of the fungi species in different assays.

4.4. Antitumor activity and cytotoxicity

The cell lines used were human cervix epitheloid carcinoma cells (HeLa cell line ATCC CCL-2), acute T cell leukemia human cells (Jurkat cell line ATCC TIB-152) and *Cercopithecus aethiops* african green monkey kidney cells (Vero cell line ATCC CCL-81). HeLa and Vero cells were grown in MEM supplemented with 10% FBS, 100 units/mL of penicillin, 100 μg/mL of streptomycin, 20 mg/mL of glutamine, 0.14% $NaHCO_3$, and 1% each of nonessential amino acids and vitamin solution. Jurkat cells was grown in RPMI-1640 (Sigma Chemical Co, St Louis, MI, USA) supplemented with 10% FBS, 100 units/mL of penicillin and 100 μg/mL of streptomycin. The cultures were maintained at 37 °C in humidified 5% CO_2 atmosphere.

The antitumor activity on HeLa and Jurkat cells, and cytotoxic activity on Vero cells have been carried out using *in vitro* assay on cell growth and tetrazolium- dye (MTT) cytotoxicity assay, according to the protocol reported by us [24], which was used with a few modifications. HeLa and Vero cell monolayers were trypsinized and washed with culture medium and then plated at 1.5×10^4 cells per well for HeLa and 1.25×10^4 cells per well for Vero cells in a 96-well flat-bottomed plate. After 24 h of incubation, each diluted compound was added to the appropriate wells and the plates were incubated for further 48 h at 37 °C in a humidified incubator with 5% CO_2. Jurkat cells that grow in suspension were plated at 3.00×10^4 cells per well and diluted compounds were added to plates and incubated for 48 h at 37 °C. Vincristine was used as positive control. The IC50 for each compound were obtained from dose–effect curves for linear regression methods and IC50 values are expressed as the mean ± S.E.M. of at least four dilutions by quadruplicate.

4.5. Antiviral assays

Herpes simplex virus type 1 (HSV-1) was obtained from the Center for Disease Control (Atlanta, GA). The virus stock was prepared from HSV-1-infected HeLa cell cultures. The antiviral activity against HSV-1 have been carried out on HeLa and Vero cells using end-point titration technique, according to the protocol reported by us [24], which was used with a few modifications. Two-fold dilutions of the compounds and viral suspension (one infection dose, 1 D.I) were mixed and incubated for 0.5 h at 37 °C before they were added on confluent monolayer cells and incubated again at 37 °C in humidified 5% CO_2 atmosphere for 36 h. Acyclovir and Heparin sodium salt were used as positive controls.

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