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Additional Information

Synthesis and Biological Studies of (+)-Liquiditerpenoic Acid A (Abietopinoic Acid) and Representative Analogues: SAR Studies

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ABSTRACT: The first semisynthesis and biological profiling of the new abietane diterpenoid (+)-liquiditerpenoid acid (abietopinoic acid) (**7**) along with several analogues are reported. The compounds were obtained from readily available methyl dehydroabietate (**8**), which was derived from (-)-abietic acid (**1**). Biological comparison was conducted according to the different functional groups leading to some basic structure-activity relationship (SAR). In particular, the ferruginol and sugiol analogues **7** and **10-16**, were characterized by the presence of an acetylated phenolic moiety, an oxidized C-7 as a carbonyl, and a different functional group at C-18 (methoxycarbonyl, carboxylic acid, and hydroxymethyl). The biological properties of these compounds were investigated against a panel of six representative human tumor solid cells (A549, HBL-100, HeLa, SW1573, T-47D, and WiDr), five leukemia cellular models (NALM-06, KOPN-8, SUP-B15, UoCB1, and BCR-ABL), and four *Leishmania* species (*L. infantum*, *L. donovani*, *L. amazonensis*, and *L. guyanensis*). A molecular docking study pointed out some targets in these *Leishmania* species. In addition, the ability of the compounds to modulate GABA_A receptors ($\alpha_1\beta_2\gamma_{2s}$), is also reported. The combined findings indicate that these abietane diterpenoids offer a source of novel bioactive molecules with promising pharmacological properties from cheap chiral-pool building blocks.

The study of the chemistry of terpenoids started nearly 200 years ago with the analysis of turpentine oil, including the investigation of the first resin acid, abietic acid (**1**, Figure 1) from pine oleoresin, that was isolated in an impure form in 1824.¹ Abietic acid (**1**) occurs in plants of the genus *Abies*² and is the first member of the abietane-type diterpenoids. This class is characterized by a tricyclic ring system and has shown a wide range of chemical diversity and biological activity, including antitumor and anti-infective properties.^{3,4} To date, new abietanes are still being discovered and are of increasing interest to scientists in multiple disciplines. For example, medicinal chemists have studied derivatives of the two readily available materials, dehydroabietic acid (**2**) and dehydroabietylamine (**3**), also called leelamine.⁴ To date, there is only one commercial drug based on abietane-type diterpenoids, namely Ecabet® (**4**, ecabet sodium). Ecabet® is used for the treatment of reflux esophagitis and peptic ulcer disease.⁵ Nevertheless, there are several on-going clinical trials on related abietanes, such as tanshinones and triptolide.⁶ For example, ferruginol (**5**), the simplest phenolic abietane diterpenoid, has recently shown antitumor activity through inhibiting non-small cell lung cancer growth by inducing caspase-associated apoptosis. In fact, intraperitoneal administration of ferruginol (**5**) significantly suppressed the growth of subcutaneous CLI-5 xenografts.⁷ Ferruginol (**5**) also exhibits antiproliferative effects in human ovarian cancer cells by inducing apoptosis and inhibiting cancer cell migration.⁸ In addition, ferruginol (**5**) has shown promising antileishmanial activity (IC₅₀ 12.2 μM) against *Leishmania donovani* promastigotes.⁹ The related C-7 oxidized sugiol (**6**, Fig. 1) displayed potent anti-inflammatory and hepatoprotective activities,¹⁰ in vitro cytotoxicity against human pancreatic (MIAPaCa-2, IC₅₀ 17.9 μM) and melanoma (MV-3, IC₅₀ 34.1 μM) tumor cellular models,¹¹ and antitumor promoting activity against EBV-EA activation.¹² Additionally, studies of sugiol (**6**) demonstrated in vivo antitumor activity in a DU145 prostate xenograft murine model. The effects of sugiol (**6**) on tumor growth inhibition are presumed to occur by inhibition of the STAT3 pathway.¹³ STAT3 is a promising molecular target for the treatment of various cancers as it is an upregulated oncogenic protein. Recently, a series of β-aminoalcohol analogues of sugiol (**6a**) showed improved biological activity against human solid tumor cell lines, in comparison to that of the parent sugiol (**6**).¹⁴

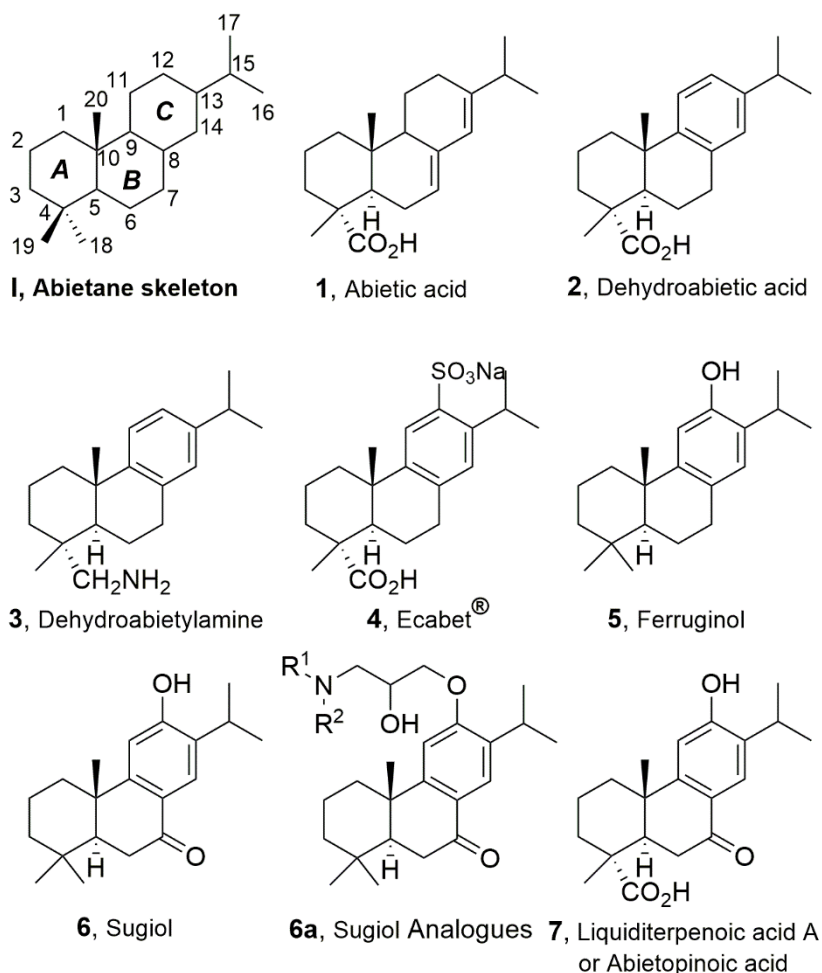


Figure 1. Abietane numbering system and some typical examples.

The promising biological activity of abietane-type diterpenoids, the isolation of a new sugiol analogue, (+)-liquiditerpenoic acid A (**7**) (abietopinoic acid) in 2014 by Hua and co-workers from the resin of *Liquidambar formosana*¹⁵ and by Kuo and co-workers from *Pinus massoniana*¹⁶ in two independent studies, and the lack of synthetic and biological studies of this molecule, prompted the synthesis and assessment of the biological properties of compound **7** and analogues with different C-18 functional groups and oxidation pattern at C-7.

Finally, it should be noted that compounds such as 12-hydroxydehydroabietic acid (**15**, Scheme 1) have recently attracted attention after being patented for protein tyrosine phosphatase 1B (PTP1B) inhibitory activity, following promising *in vitro* tests. The potential of compound **15** as drug in the prevention, delay, or treatment

of PTP1B-mediated diseases such as diabetes and obesity, or treatment of the complications thereof, thus merits further study.¹⁷

In the present study, the semisynthesis of (+)-liquiditerpenoic acid A (**7**, or abietopinoic acid) and related analogues (**9-16**) from methyl dehydroabietate (**8**), their evaluation against a cellular panel of six representative human solid tumors, five leukemia cellular lines, and four *Leishmania* species (including molecular docking analysis of potential biological targets of *Leishmania*), and their modulating activity of GABA_A receptor subtype ($\alpha_1\beta_2\gamma_{2s}$) are reported.

RESULTS AND DISCUSSION

Chemistry.

The compounds were synthesized from readily available methyl dehydroabietate (**8**) (Scheme 1), which was obtained from commercially available (–)-abietic acid (**1**), following the reported method *via* methylation and aromatization.¹⁸ The starting material contains the aromatic ring and three stereocenters (C-4, C-5, C-10), which are present in the target, (+)-liquiditerpenoic acid A (**7**, or abietopinoic acid), including the C-18 carboxylic group. The aromatic diterpenoid **7** also features a phenolic moiety at C-12 and a 7-carbonyl group, and thus, its semisynthesis can follow a synthetic approach similar to that developed to generate (–)-sugikurojin A.¹⁹ Based on the previous synthetic studies, a concise synthesis of compound **10** was performed via Friedel-Crafts acetylation (AcCl/AlCl₃) followed by Baeyer-Villiger oxidation (*m*-CPBA/TFA in DCM) to introduce the C-12 phenolic moiety affording the key intermediate **10** (Scheme 1) in 80% overall yield for the two steps. Compound **10** was used to access a series of diverse analogues, and the synthesized natural product **7**. First, oxidation (CrO₃ in HOAc) at the benzylic C-7 position afforded ketone **11**, which after deacetylation (K₂CO₃ in MeOH) resulted in sugiol analogue **14**. Compound **14** was sparingly soluble in organic solvents. Nucleophilic methyl ester cleavage of **14** (LiI in 2,4,6-collidine) afforded the (+)-liquiditerpenoic acid A (abietopinoic acid) (**7**) in 34% overall yield for the five steps. The combined analytical data [¹H and ¹³C NMR in DMSO-*d*₆ and acetone-*d*₆, and

HRMS (calcd for $C_{20}H_{27}O_4$ $[M+H]$: 331.1909; found: 331.1887)] were in complete agreement with the reported data for the natural product **7** (Scheme 1).^{15,16} It is important to highlight that there is a discrepancy in the reported specific rotation. Hua et al.¹⁵ reported $[\alpha]_D^{25} +80.3$ (*c* 0.137, MeOH), while Kuo et al.¹⁶ reported $[\alpha]_D^{25} +21.8$ (*c* 0.5, $CHCl_3$). Clearly, the difference in solvent systems and concentration may affect the outcome. Our specific rotation data of synthesized **7** ($[\alpha]_D^{25} +23.8$, *c* 0.4, MeOH) were in agreement with Kuo et al.¹⁶ However, when the optical rotation was measured in $CHCl_3$ at the same concentration reported by Kuo et al. (*c* 0.5), an $[\alpha]_D^{24} +33.0$ was obtained. It is likely that the presence of an impurity in their samples affected their data. Our data were recorded by independent investigators and the same conclusions were drawn. As shown by NMR data and elemental analysis, the purity was high.

Further functional group manipulation of intermediate **10** provided, after deacetylation with K_2CO_3 in MeOH, phenol **12**, which was subsequently treated with LiI in 2,4,6-collidine to afford compound **15**. An alternative approach led directly to 18-hydroxyferruginol (**13**) via reduction of **10** with $LiAlH_4$ in THF. Compound **13** was subsequently acetylated (Ac_2O in pyridine), oxidized at C-7 (CrO_3 in HOAc), and deacetylated (K_2CO_3 in MeOH) to afford the new compound 18-hydroxysugiol (**16**) (Scheme 1).

Table 1. Antiproliferative Activity (GI₅₀) of Liquiditerpenoic acid (**7**, Abietopinoic acid) Analogues **9-10** and **12** Against Human Solid Tumor Cells.^a

compound	Cell line (origin)					
	A549 (lung)	HBL- 100 (breast)	HeLa (cervix)	SW1573 (lung)	T-47D (breast)	WiDr (colon)
8^b	15 ± 2.6	19 ± 0.2	15 ± 3.0	22 ± 6.2	16 ± 4.7	10 ± 3.6
9	18 ± 2.3	15 ± 1.2	11 ± 1.0	11 ± 4.6	10 ± 1.5	17 ± 2.4
10	11 ± 1.8	15 ± 3.9	4.4 ± 1.1	12 ± 3.2	14 ± 2.9	16 ± 2.5
12	9.4 ± 2.6	9.7 ± 2.7	5.8 ± 0.5	11 ± 2.0	19 ± 3.8	23 ± 4.4
etoposide	1.5 ± 0.3	1.4 ± 0.1	3.3 ± 1.6	15 ± 1.5	22 ± 5.5	23 ± 3.1
cisplatin	4.9 ± 0.2	1.9 ± 0.2	1.8 ± 0.5	2.7 ± 0.4	17 ± 3.3	23 ± 4.3

^a Values are given in μM and represent the mean \pm standard deviation of at least two independent experiments.

^b Values taken from reference 22: Stadler, M. et al. *Planta Med. Int. Open* **2017**, *4*, e89-e92.

Table 2. Antiproliferative Activity (EC₅₀) of Liquiditerpenoic acid (**7**, or Abietopinoic acid) Analogues **10-11** Against Leukemia Cellular Models.^a

compound	Cell line					
	NALM-06	BCR-ABL	KOPN-8	SUP-B15	UoCB1	BJ
10	8.8	14.3	21.0	20.2	>40	N.A
11	10.7	12.2	18.5	21.7	>40	N.A
STS	1.4	0.35	0.71	0.55	2.0	>40

^a Values are given in μM , N.A.: no activity at the tested concentrations. Negative control: Vehicle; Positive control: Staurosporine (STS).

The standard anticancer drugs etoposide, cisplatin and staurosporine were used for comparison and control. Most of the compounds were active ($GI_{50} < 25 \mu\text{M}$) in the solid tumor cells with higher activity than the parent dehydroabietic acid (**2**) from previous results.²² The target molecule, (+)-liquiditerpenoic acid (**7**, or abietopinoic acid), its deoxy acid, 12-hydroxydehydroabietic acid (**15**) and the sugiol methyl ester analog (**14**) were generally inactive ($GI_{50} > 70 \mu\text{M}$) in the solid tumor cells assays (Table S2, Supporting Information). The most potent compound in general in solid tumor assays, was compound **10**, characterized by an acetate group at C12 and a methyl ester at C18. The activity of **10** (4-16 μM) is comparable to that of cisplatin (a currently approved drug) and the human topoisomerase II α catalytic inhibitor, etoposide. Compound **10** was more potent than etoposide and cisplatin for the more resistant cell lines T-47D and WiDr, indicating potential for future mechanistic studies. Compound **9** was also more potent against T-47D and WiDr cell lines than the standard of care drug, cisplatin. One clear SAR that can be deduced is that acetylation on the hydroxy group at C12 results in more potent compounds (Table S2, compounds **10** and **11** vs. **12** and **14**). In general, as found previously in similar systems,¹⁸ the order of activity in the ferruginol series with different functional groups at C18 (compounds **12**, **13**, and **15**) was ester \geq alcohol $>$ acid, which is a trend similar to that of C19 callitricis-derivative series.²²

Previous reports on the antiproliferative activity of sugiol β -amino alcohols analogues (**6a**, Figure 1),¹⁴ provided similar cytotoxicity results against A2780, WiDr and SW1573 cancer cells after 48 h of exposure using the sulforhodamine B (SRB) assay.¹⁴ An important difference in the β -amino alcohols analogues is the presence of a *gem*-dimethyl group at C4 in contrast with the C18-functionalized position in the current study. 18-Hydroxyferruginol (**13**) isolated from *Juniperus brevifolia* has also been described previously as a moderate cytotoxic agent against HeLa cells.²³

Next, these compounds were evaluated against human pre-B leukemia cellular models (NALM-06, KOPN-8, SUP-B15, UoCB1), and murine cellular model (BCR-ABL), which showed similar response to the solid tumor models, particularly for compounds **10** and **11**. Not surprisingly, compounds showed no cytotoxicity at tested concentrations in the glucocorticoid-resistant leukemia cell line (UoC-B1). The acetylated compounds **10** and

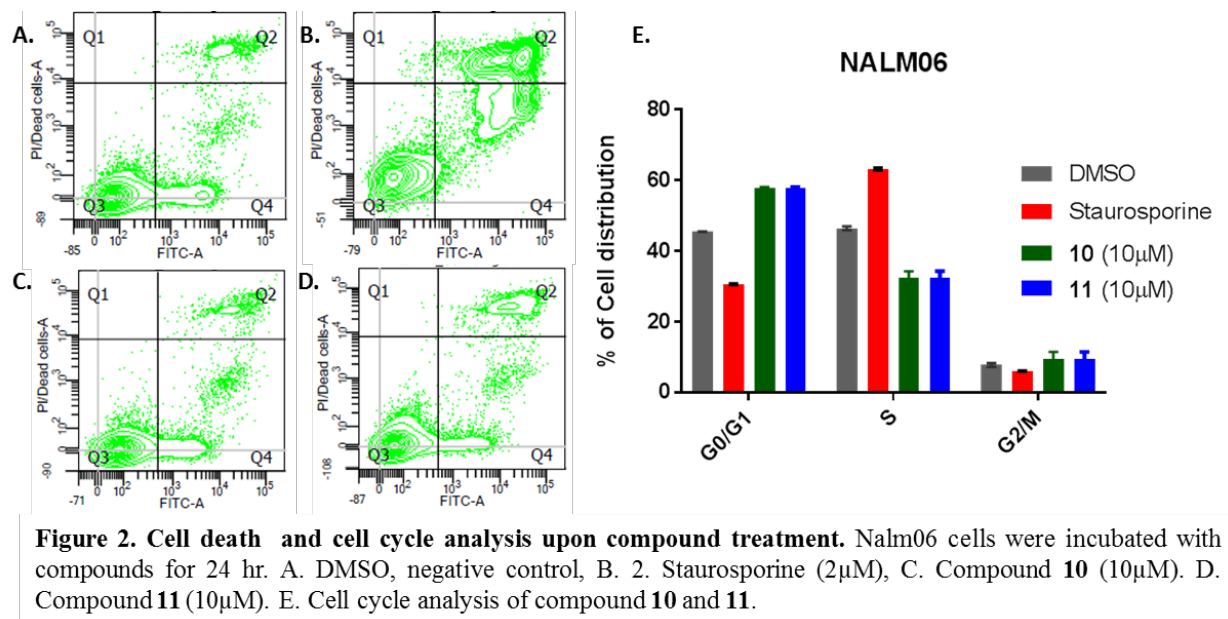
11 showed the highest potency against the NALM-06 cellular model, with EC_{50} values in the low micromolar range (8.8 and 10.7 μM , respectively). These compounds displayed no cytotoxicity against the normal fibroblast BJ cellular model at the tested concentrations, indicating a favorable therapeutic index (see Supporting Information for experimental detail).

Our results indicate that compounds **10** and **11** have superior antiproliferative properties than the corresponding free hydroxyl/acid groups as the non-acetylated sugiol analogues **7**, **14**, and **16** displayed either no or little activity in the leukemia lines tested ($EC_{50} > 40 \mu\text{M}$) (Table S1, Supporting Information), confirming that the absence of the keto group at C7 was required for anti-leukemic activity.

Interestingly, the acetyl intermediate **9** ($EC_{50} > 40 \mu\text{M}$) and compounds **13** and **16** with a hydroxy group at C18, showed poor activity in these leukemia cell lines (Table S1, Supporting Information), in contrast to the antiproliferative activity against the solid tumor cell lines. Such experimental observations illustrate the fact that different survival pathways drive solid and hematological malignancies.

To validate apoptosis (or programmed cell death) for compounds **10** and **11**, the appearance of phosphatidyl serine residues on the surface of the cell, an early event in apoptosis, was determined with Annexin V conjugated to fluorescein isothiocyanate (FITC) and propidium iodide (PI) via flow cytometry.²⁴ In addition DNA content was analyzed (by PI staining) for cell cycle progression²⁵ in Nalm06 and KOPN8 cellular models for 24 and 32 h, respectively. Representative Annexin V-FITC/PI plot images of independent experiments are shown in figure 2. For controls, DMSO (negative control) and staurosporine (2 μM ; positive control) are indicated along with compound **10** (10.0 μM) and **11** (10.0 μM) for Nalm06 (Fig. 2). Fluorescent images show Annexin V-FITC positive apoptotic cells and the PI positive necrotic cells. No significant cell death was observed at the tested concentration for compound **10** (Fig. 2), while compound **11** showed progression of cell death in a dose dependent manner (see Supporting Information). However, both compounds indicated cell arrest at G0/G1 and

G2/M as illustrated in diagram E. We hypothesized that these compounds display cytotoxicity at longer incubation times in the NALM-06 cellular model.



Compounds **10** and **11** were evaluated at higher concentrations (20 μ M) and showed a profound effect on cell death in the KOPN8 cellular model with more than 85% of cells being in the late apoptotic state (Fig. 3). Cell treated with compound **10** displayed a similar distribution pattern as staurosporine, while compound **11** induced significant cell arrest at the S phase. The combined data illustrate that compound **11** might be acting differently on these cellular models as they have different genomic backgrounds.

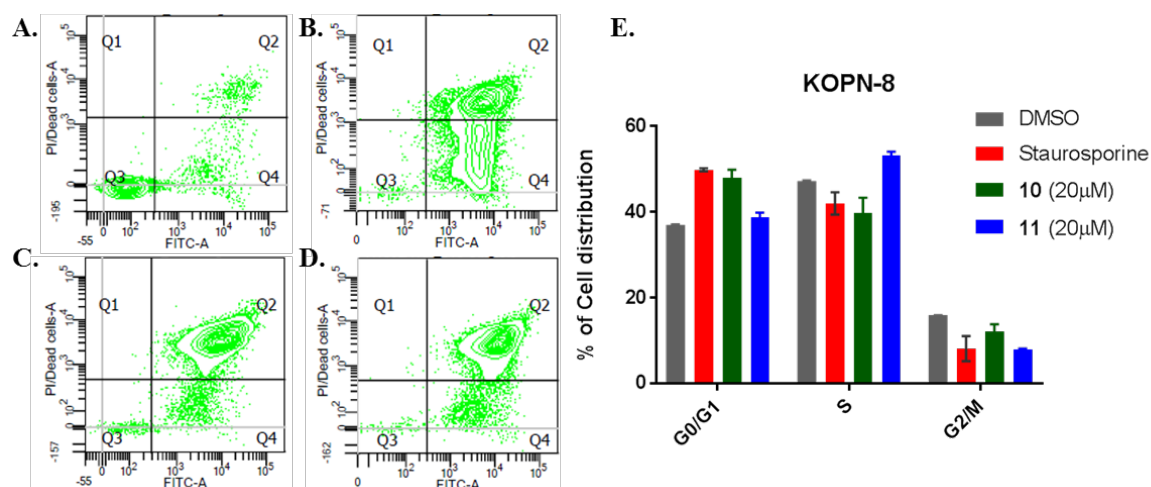


Figure 3. Cell death induced by compound treatment. KOPN-8 cells were incubated with compounds of interest for 32 hr. A. DMSO, negative control, B. 2. Staurosporine (1μM), C. Compound 10 (20μM). D. Compound 11(20μM) respectively.

GABA_A Receptor Modulating Activity.

In an experiment based on the oocyte assay, the compounds **7** and **9-16** were tested for their effects on the most abundant GABA_A receptor subtype ($\alpha 1\beta 2\gamma 2S$) by means of the two-microelectrode voltage clamp technique in *Xenopus laevis* oocytes.²⁶ All compounds were screened at concentrations of 10 and 100 μM and compared with dehydroabiatic acid (DHA) (**2**) as a positive control.²⁶ The results are summarized in Figure 4 as a bar diagram and tabulated in Table 3. Depicted is the enhancement of a GABA-evoked chloride-current (I_{GABA}), which elicits 5% of the maximum response ($\sim 5\mu M$ GABA). Compounds **12-14**, analogues of ferruginol (**5**) and sugiol (**6**), showed considerable potentiation of GABA-evoked currents at 100 μM. Interestingly, compounds **7** and **16** do not potentiate, but act as weak inhibitors. Basic SAR shows that the most potent compound is phenol **12** (I_{GABA} 378% at 100 μM) bearing a methyl ester group at C18. Replacement of either the methyl ester by a hydroxymethyl group (compound **13**) or the introduction of a keto group at C7 (compound **14**), led to less active compounds. Specifically, half of the activity (compound **13**) or two thirds (compound **14**) of the parent activity (compound **12**) was provoked doing these functional group changes. Acetylation at C12 (compounds **10** and **11**), as well as the presence of a carboxylic acid at C18 (compounds **7** and **15**), disfavoured

GABA_A potentiation. In all cases, the activity was considerably lower in comparison to the positive control, DHA (2).

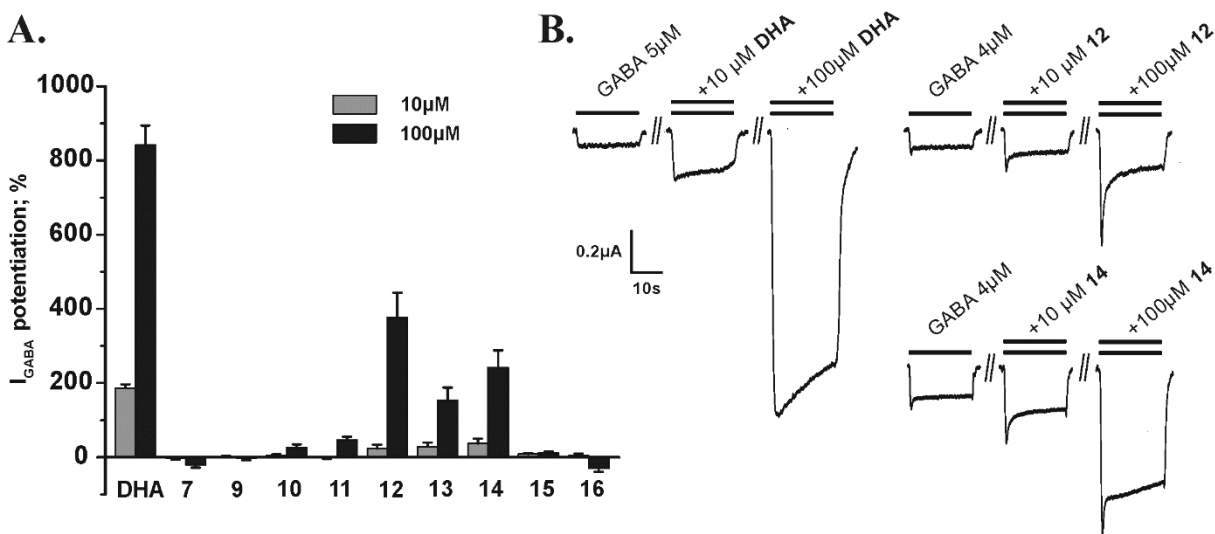


Figure 4. **A.** Potentiation of GABA-evoked currents (I_{GABA}) through $\alpha 1\beta 2\gamma 2\text{S}$ receptors by indicated compounds at 10 and 100 μM . Data represent mean \pm SEM from at least three different oocytes and two oocyte batches. **B.** Representative current traces of DHA (2), 12 and 14 in the presence of 20 s application of a GABA EC_5 (single bar) or co-application of GABA EC_5 (4-5 μM) and 10 or 100 μM compound (double bar). Currents were recorded from *Xenopus laevis* oocytes expressing $\alpha 1\beta 2\gamma 2\text{S}$ receptors voltage-clamped at -70 mV. GABA EC_5 was determined individually for every oocyte.

Table 3. Potentiation of I_{GABA} in $\alpha 1\beta 2\gamma 2S$ Receptors by Compounds **7** and **9-16**.

Compound	Conc.; μM	I_{GABA} potentiation (%)	SEM
DHA (2)	10	186.9	9.6
	100	841.3	52.9
7	10	-2.0	4.4
	100	-21.1	7.1
9	10	2.7	0.9
	100	-2.9	5.1
10	10	5.1	2.1
	100	26.3	8.9
11	10	-1.0	2.8
	100	46.7	8.3
12	10	23.7	9.6
	100	378.0	65.6
13	10	29.3	10.6
	100	154.1	34.2
14	10	37.4	12.7
	100	242.4	46.0
15	10	8.9	2.3
	100	12.1	2.9
16	10	4.5	5.8
	100	-30.3	9.5

According to the literature, DHA (**2**) induced significant receptor modulation in the oocyte assay, with a maximal potentiation of I_{GABA} of $397.5\% \pm 34.0\%$, and EC_{50} of $8.7 \mu M \pm 1.3 \mu M$. This was the first report of dehydroabiatic acid as a positive $GABA_A$ receptor modulator.²⁶ Also, dehydroabiatic acid (**2**) exhibit large-conductance voltage- and Ca^{2+} -activated K^+ (BK channels) channel-opening activities. However, the structurally related 12-hydroxydehydroabiatic acid (**15**) showed no significant activity by other authors, concluding that hydroxyl groups at C12 are detrimental for BK channel-opening activities.²⁷

Antileishmanial Activity.

Compounds **7** and **9-16** (Scheme 1) were evaluated for antileishmanial activity against four different *Leishmania* species (*L. infantum*, *L. donovani*, *L. amazonensis* and *L. guyanensis*), as well as for cytotoxicity against J774 macrophages following established procedures.²⁸ The results on extracellular forms (promastigotes) are summarized in Table 4. All the compounds were active against the different species of *Leishmania* except compound **7**. In the case of *L. donovani* no compound improved the activity of the reference drug, miltefosine. However, several compounds proved to be more potent than the reference compound for other species. For example, compounds **9-13** and **16** displayed better potency than miltefosine against *L. amazonensis*. Interestingly, compound **11** exhibited sub-micromolar IC_{50} , being 73 times more potent than the reference drug. In the case of *L. guyanensis*, compounds **10-12** showed lower IC_{50} values than miltefosine, while compounds **9**, **13** and **16** showed a similar IC_{50} value to that of miltefosine. Again, compound **11** was the most potent (13-fold more potent than miltefosine). With regard to *L. infantum*, compounds **9-11** and **13** were more potent than miltefosine, being compound **11** the most potent as well for this species. Thus, compound **11** resulted to be the most potent of all tested compounds. The toxicity of compound **11**, however, was not negligible but showed a good selectivity index (SI) in the range 4.06–27.26. Moreover, the best balance of activity-selectivity was exhibited by compound **9**, which displayed a good level of antileishmanial activity with a very valuable SI (between 8.76-52.49) associated with a reduced toxicity. Therefore, compound **9** was selected for studying its activity against amastigotes (*L. amazonensis* and *L. infantum*) (Table 5).

Table 4. IC₅₀ Leishmanicidal and Cytotoxic Effects (in μM) of C-7- and C-12-Functionalized Dehydroabietic Acid Derivatives in an in vitro Promastigote Assay.

Compound	<i>L. amazonensis</i>		<i>L. guyanensis</i>		<i>L. donovani</i>		<i>L. infantum</i>		<i>Macrophages J774</i>
	IC ₅₀ ^a ±SD	SI ^b	IC ₅₀ ±SD	SI	IC ₅₀ ±SD	SI	IC ₅₀ ±SD	SI	CC ₅₀ ^c
7	NA ^d		NA		NA		NA		219.4±15.0
9	11.6±0.6	11.2	14.2±0.4	9.1	14.8±0.9	8.8	2.5±0.6	51.8	129.6±8.9
10	3.2±0.3	8.2	5.5±0.7	4.8	8.1±0.7	3.3	1.3±0.1	20.4	26.5±2.7
11	0.65±0.03	27.4	1.3±0.1	13.6	4.4±0.5	4.1	0.7±0.2	25.4	17.8±2.8
12	3.9±0.1	8.7	5.9±1.1	5.8	9.21±0.06	3.7	5.0±0.3	6.8	34.2±3.1
13	7.7±0.8	3.0	12.5±2.2	1.9	13.9±0.1	1.7	0.7±0.1	33.4	23.4±4.6
14	ND ^e		ND		ND		ND		153.9±49.3
15	54.1±6.5	1.6	71.4±1.5	1.2	58.1±0.7	1.5	43.6±3.4	2.0	85.8±28.8
16	10.2±0.6	1.4	16.5±0.7	0.9	19.7±0.4	0.7	9.7±0.8	1.5	14.4±2.8
Miltefosine	47.7 ± 5.0	2.9	18.2 ± 0.6	7.5	0.15 ± 0.02	909	3.4 ± 0.6	40.1	136.4 ± 1.4

^a IC₅₀, concentration of the compound that produced a 50% reduction in parasites; SD: standard deviation. ^b Selectivity index, SI = CC₅₀/IC₅₀. ^c CC₅₀, concentration of the compound that produced a 50% reduction of cell viability in treated culture cells with respect to untreated ones. ^d NA, no activity. ^e ND, not determined.

Table 5. IC₅₀ Leishmanicidal and Cytotoxic Effects (in μM) of Compound **9** on in vitro Amastigote Assay on *L. amazonensis* and *L. infantum*.

Compound	<i>L. amazonensis</i>		<i>L. infantum</i>		<i>Macrophages J774</i>
	IC ₅₀ ^a ±SD	SI ^b	IC ₅₀ ±SD	SI	CC ₅₀ ^c
9	31.4±6.2	4.1	37.2±2.4	3.5	129.6±8.9

^a IC₅₀, concentration of the compound that produced a 50% reduction in parasites; SD: standard deviation.

^b Selectivity index, SI = CC₅₀/IC₅₀. ^c CC₅₀, concentration of the compound that produced a 50% reduction of cell viability in treated culture cells with respect to untreated ones.

Ferruginol (**5**) isolated from *Juniperus* berries displayed moderate antiparasitic activities (IC₅₀ 12.2 μM) against *Leishmania donovani* promastigotes, as well as anti-*Plasmodium falciparum* chloroquine-sensitive (D6; IC₅₀ 14.7 μM) and chloroquine-resistant (W2; IC₅₀ 12.2 μM) strains.⁹ The selected analogues, **7** and **9-16**, also showed important activity against *L. donovani*, with the sugiol analog **11** (IC₅₀ 4.38 μM) three times more potent than the isolated ferruginol (**5**) from *Juniperus* berries. The methyl ester of 12-hydroxydehydroabiatic acid, compound **12**, recently described as a new natural product,²⁹ has demonstrated activity against *Trypanosoma cruzi* in epimastigote and axenic amastigote forms (IC₅₀ c.a. 6.0 mM).³⁰ In our studies, compound **12** exhibited promising antileishmanial activity with IC₅₀ ranging from 3.92 to 9.21 μM and selectivity indexes (SI) from 3.72 to 8.74.

Molecular Docking for *Leishmania* spp. Targets

To propose a plausible mechanism of action of these compounds, we carried out molecular docking studies on some enzymes described as potential targets for antileishmanials (see Supplemental Information for the complete list of the analyzed enzymes and their docking results). This methodology has been used previously for the study of potential antiparasitics including *Leishmania*^{31,32} and *Trypanosoma*.³³ Docking studies suggested UDP-glucose pyrophosphorylase, dihydroorotate dehydrogenase, methionyl-tRNA synthetase, pteridine reductase, phosphomannomutase and N-myristoyltransferase as potential targets, since compounds **7-16** exhibited the highest theoretical affinity on these enzymes. Interestingly, docking scores on UDP-glucose pyrophosphorylase have relatively good correlation with *in vitro* leishmanicidal activities in three of the four parasites studied ($r^2 > 0.71$), considering compound **15** as outlier (Table 6). This latter compound has an ionizable carboxylic group which could interfere on the access of the compound to the active site due to poor membrane permeability, particularly in the case of dihydroorotate dehydrogenase which is located inside the mitochondria.³⁴

Table 6. Results of Docking Studies Carried Out on Potential Leishmanicidal Targets and Correlation with in vitro Activity (Log(1/IC₅₀) on *L. donovani*.

Compound	Log (1/IC ₅₀)	UDPGP	DHODH	MtRNAS	PtRd	S14DM	NMyrT
9	-1.1700	-76.67	-87.73	-89.10	-84.99	-78.54	-81.78
10	-0.9096	-103.75	-92.73	-97.45	-92.25	-89.16	-91.23
11	-0.6415	-105.01	-96.99	-101.41	-89.75	-83.16	-91.38
12	-0.9643	-82.91	-85.14	-90.39	-79.21	-75.03	-76.87
13	-1.1440	-79.12	-81.21	-90.12	-85.58	-72.18	-77.08
15	-1.7642	-80.21	-86.49	-97.46	-83.42	-72.76	-80.38
16	-1.2945	-79.95	-86.38	-94.69	-75.17	-73.45	-82.68
Average docking score		-87.90	-88.37	-93.86	-84.49	-78.59	-83.50
r ^{2*}		0.7335	0.5990	0.4957	0.4330	0.4172	0.3933

UDPGP= UDP-Glucose Pyrophosphorylase (PDB: 2OEG), DHODH= dihydroorotate dehydrogenase (PDB: 3MHU), MtRNAS = methionyl-tRNA synthetase (PDB: 3KFL), PtRd= Pteridine Reductase (PDB: 1E7W), S14DM= sterol 14-alpha demethylase (PDB: 3L4D), NMyrT= N-myristoyltransferase (PDB: 4A30).

*Considering compound **15** as outlier

Analysis of the interactions of compounds **9-16** in the active site of UDPGP help to explain our SAR observations. Particularly, interactions with Gly83 and Gly84 seem critical for a better binding, as the most active compounds (**10** and **11**) have an *O*-acetyl group in position 12 that interacts through hydrogen bonds with these residues, while these interactions are absent in the case of compounds that bear a hydroxyl or acetyl group in this position (Figure 5).

Table 7. Calculated Molecular Properties (Drug-likeness) by Molinspiration Online Software for Compounds **7**, and **9-16**.^{a,b}

Compound	miLog <i>P</i>	MW	n-HBA	n-HBD	TPSA	Lipinski's violation
7	4.21	330.42	4	2	74.60	0
9	5.64	356.51	3	0	43.38	1
10	5.29	372.50	4	0	52.61	1
11	4.39	386.49	5	0	69.68	0
12	5.73	330.47	3	1	46.53	1
13	5.24	302.46	2	2	40.46	1
14	4.83	344.45	4	1	63.60	0
15	5.11	316.44	3	2	57.53	1
16	4.33	316.44	3	2	57.53	0
Rule of five	not >5	<500	not >10	not >5		1 violation allowed

^a Values were calculated using Molinspiration Cheminformatics software (Molinspiration, Slovensky Grob, Slovak Republic, 2015, <http://www.molinspiration.com>)

^b P= partition coefficient; MW= Molecular weight; n-HBA= number of hydrogen bond accepting groups; n-HBD= number of hydrogen bond donating groups; TPSA= Total polar surface area.

In summary, we have demonstrated the first chemical synthesis of the abietane-type natural product (+)-liquiditerpenoic acid **A** (**7**, or abietopinoic acid). It is accessed in a short synthetic sequence of five steps in 34% overall yield from the readily available methyl dehydroabietate (**8**). Methyl dehydroabietate (**8**) is easily obtained from chiral (–)-abietic acid, a building block available for ~\$0.4 USD/gram of purity higher than 80%.³⁸ During this process, parallel routes led to a number of oxidized analogues, some either naturally occurring (**13** and **15**) or new (**16**). Their availability allowed a biological profiling of several analogues as well as of the target compound. This work definitively establishes a versatile platform for the synthesis of a number of bioactive abietanes with the possibility of accessing even further oxidized, unnatural analogues of these diterpenoids using

similar chemistry. The combined findings indicate that these abietane-diterpenoid natural product analogues offer a source of novel bioactive molecules with promising pharmacological and drug-likeness properties.

ASSOCIATED CONTENT

Supporting Information Available: Complete experimental details for the synthesis of compounds **7** and **9-16**, including copies of ^1H NMR and ^{13}C NMR spectra, and for all the biological assays. A list with the complete results of the docking studies carried out on the analyzed targets and SMILES codes is also available in the S.I. This material is available free of charge via the Internet at <http://pubs.acs.org> (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. Specifically, the study was conceived and chemical experiments were designed by MAGC. DH did the chemical synthesis. MS and SH participated in the GABA_A receptor modulating activities, while JMP, FR and RB contributed with the antiproliferative studies. MADA designed and performed the antileishmanial studies and MLM did the in silico docking work. MAGC, JMP, FR, MADA and MLM analyzed results. MAGC wrote the manuscript. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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