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Synthesis and Antileishmanial Activity of C7- and C12-Functionalized Dehydroabietylamine Derivatives

M. Auxiliadora Dea-Ayuela[a,b], Pablo Bilbao-Ramos[b], Francisco Bolás-Fernández[b] and Miguel A. González-Cardenete,[*c]

[a] *Departamento de Farmacia, Universidad CEU Cardenal Herrera, Avda. Seminario s/n, 46113 Moncada (Valencia), Spain*

[b] *Departamento de Parasitología, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain*

[c] *Instituto de Tecnología Química, Universitat Politècnica de València-Consejo Superior de Investigaciones Científicas, Avda. de los Naranjos s/n, 46022 Valencia, Spain*

Abstract— Abietane-type diterpenoids, either naturally occurring or synthetic, have shown a wide range of pharmacological actions, including antiprotozoal properties. In this study, we report on the antileishmanial evaluation of a series of (+)-dehydroabietylamine derivatives functionalized at C7 and/or C12. Thus, the activity *in vitro* against *Leishmania infantum*, *Leishmania donovani*, *Leishmania amazonensis* and *Leishmania guyanensis*, was studied. Most of the benzamide derivatives showed activities at low micromolar concentration against cultured promastigotes of *Leishmania* spp. (IC_{50} = 2.2-46.8 μM), without cytotoxicity on J774 macrophage cells. Compound **15**, an acetamide, was found to be the most active leishmanicidal agent, though it presented some cytotoxicity on J774 cells. Among the benzamide derivatives, compounds **8** and **10**, were also active against *L. infantum* intracellular amastigotes, being 18- and 23-fold more potent than the reference compound miltefosine, respectively. Some structure-activity relationships have been identified for the antileishmanial activity in these dehydroabietylamine derivatives.

Keywords: Leishmanicidal, abietane, diterpene, dehydroabietylamine

Introduction

Leishmaniasis is a poverty-associated disease caused by more than 20 species of protozoan parasites of the genus *Leishmania*. It is a wide spectrum of vector born diseases with great epidemiological and clinical diversity. Three major clinical forms of the disease are visceral, cutaneous, and mucocutaneous leishmaniasis, which differ in immunopathologies and degree of morbidity and mortality. Visceral leishmaniasis (VL, also known as Kala-azar) is the most severe form, fatal, if it is not treated. Moreover, VL has emerged as an important opportunistic infection associated with HIV and co-infection is becoming an increasing problem that requires urgent attention [1]. Administration of pentavalent antimonials remains as the first choice therapy, followed by amphotericin B and miltefosine. However, the growing incidence of parasitic resistance, the route of administration, long-term treatments, high toxicity and the drug cost makes the development of new drugs necessary [2].

Natural products are potential sources of new and selective agents for the treatment of a number of diseases, including those caused by parasites. To date, many compounds from plant sources have shown potential antileishmanial activity, though none of them has undergone clinical evaluation [3]. Among these, some naturally occurring abietane-type diterpenoids and semisynthetic derivatives have shown promising antiparasitic activities [4]. For example, ferruginol (**1**), isolated from *Juniperus procera*, showed good antileishmanial activity (IC_{50} = 12.2 μM) against *Leishmania donovani* promastigotes and 12-methoxycarnosic acid (**2**) (Fig. 1), isolated from *Salvia repens*, showed potent activity (IC_{50} = 0.75 μM) against axenic *L. donovani* amastigotes [5,6]. On the other hand, semisynthetic ferruginol analogues **3** (Fig. 1) displayed antileishmanial activity (IC_{50}= 22.3-35.0 μM) on intracellular amastigotes (*L. infantum* and *L. braziliensis*), being more active and less toxic than the reference drug glucantime [7]. Recently, Moreira *et al.* reported on the antileishmanial activity of some dehydroabietic acid derivatives (e.g. cyclohexyl-L alanine derivative **4**, Fig. 1)

[*] To whom correspondence should be addressed. (M.A.G.C.) Tel.: +34-96-3877800; fax: +34-96-3879444. E-mail: migoncar@itq.upv.es

and some dehydroabietylamine amides (e.g. acrylic acid derivative **5**, Fig. 1), which were able to kill intracellular *L. donovani* amastigotes in the range 0.4 to 9.0 μM [8].

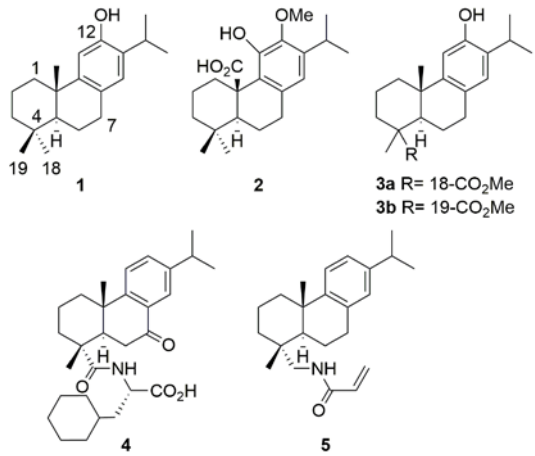


Figure 1. Examples of antileishmanial abietane diterpenoids.

The present study is a continuation of our research programs to synthesize and discover bioactive diterpenoids [9]. It includes the synthesis of several amide derivatives from commercially available (+)-dehydroabietylamine (**6**, Scheme 1) with potential antileishmanial activity. Among these derivatives, several nitroaromatic compounds were envisaged as antileishmanial prodrugs, as it is known that these compounds require activation before exerting their therapeutic effects [10]. Herein, we describe the antileishmanial activity of dehydroabietylamine derivatives (**7-15**) (Scheme 1) against *Leishmania infantum*, *L. donovani*, *L. amazonensis* and *L. guyanensis*. Our aim is to identify new antileishmanial drug leads and their structure-activity relationships on modifying the functionalization present at C7, C12 and C18.

Results and Discussion

Chemistry

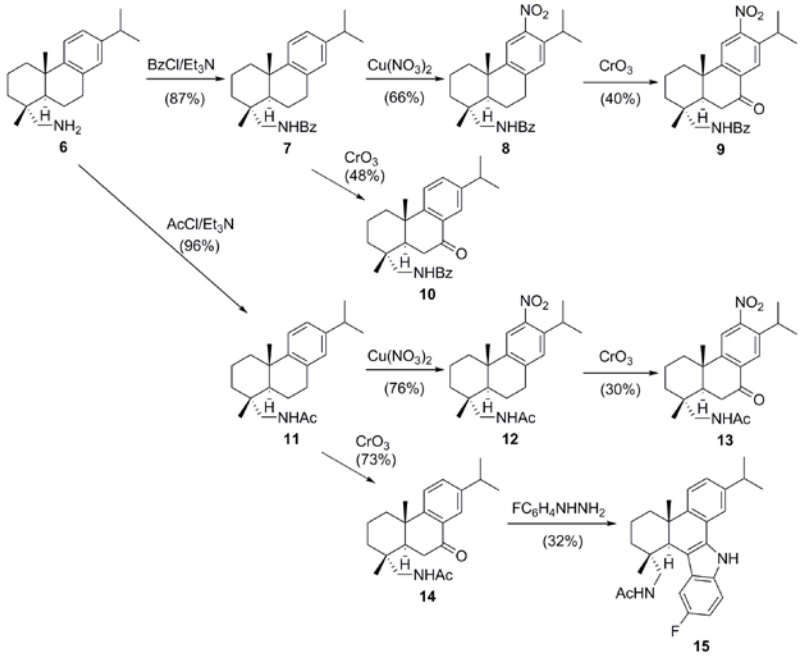
The compounds **7-15** were synthesized from (+)-dehydroabietylamine (**6**), as shown in Scheme 1. Compounds **13** and **15** are novel derivatives.

Benzamides **7**, **8** and **10** were obtained following similar conditions to those reported in the literature [11]. Thus, compound **7** (*N*-benzoyldehydroabietylamine) was synthesized by acylation of dehydroabietylamine (**6**) with benzoyl chloride in high yield (87%). Then, benzamide **7** was subjected to nitration at C-12 with acetic anhydride-cupric nitrate to give nitroderivative **8** (66% yield), while benzylic oxidation with CrO_3 gave ketone **10** in 48% yield. Similar benzylic oxidation at C-7 of nitroderivative **8** gave benzamide **9** in 40% yield. A similar synthetic sequence using acetyl chloride as acylating agent of **6** instead of benzoyl chloride, afforded the corresponding acetamides **11-14** in reasonable yields. Finally, compound **15** was obtained by Fischer indole reaction of **14** with a fluorinated phenylhydrazine [12].

Biological Evaluation

Compounds **6-15** (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 (see Experimental section). The results on extracellular forms (promastigotes) are summarized in Table 1.

As can be seen in Table 1, compounds (benzamides) **7-10** showed relevant activity without significant cytotoxicity on J774 macrophage cells (CC_{50} > 200 μM), being *L. donovani* the most sensitive species. Compound **15**, an acetamide, was found to be the most active leishmanicidal agent, though it presented some cytotoxicity on J774 cells. The remaining acetamides, compounds **11-14**, also presented cytotoxicity and were not included in further studies.



Scheme 1. Synthesis of the tested compounds **7-15**.

Table 1. IC_{50} Leishmanicidal and cytotoxic effects (in μM) of C7- and C12-functionalized dehydroabietylamine derivatives on *in vitro* promastigote assay.

Compound	*L. infantum*		*L. donovani*		*L. amazonensis*		*L. guyanensis*		*Macrophages J774*
	IC_{50}[a]±SD	SI [b]	IC_{50}±SD	SI	IC_{50}±SD	SI	IC_{50}±SD	SI	CC_{50}[c]
6	-	-	-	-	-	-	-	-	14,5± 1,6
7[d]	3,1±0,2	>64	2,2±0,2	>90	3,7±0,2	>54	20,4± 5,7	>10	>200
8	11,1±0,8	>18	3,2± 0,1	>62	20,7± 0,5	>10	94,5± 26,1	>2	>200
9	23,9±1,2	>8	5,4± 0,4	>37	12,2± 2,2	>16	38,5± 1,4	>5	>200
10	8,7±0,8	>23	4,0± 0,3	>50	4,9± 0,5	>41	8,6± 1,2	>23	>200
11	-	-	-	-	-	-	-	-	21,6± 1,6
12	-	-	-	-	-	-	-	-	13,7± 1,5
13	-	-	-	-	-	-	-	-	39,2± 7,3
14	-	-	-	-	-	-	-	-	72,3± 6,1
15	1,5±0,1	19,2	2,8± 0,4	10,3	4,0± 0,2	7,2	ND[e]		28,8± 1,9
Miltefosine	3,4±0,6	40,1	0,15± 0,02	909	47,7± 5,0	2,9	18,2± 0,6	7,5	136,4± 1,4

[a] IC_{50}, concentration of the compound that produced a 50% reduction in parasites; SD: standard deviation. [b] Selectivity index, SI = CC_{50}/IC_{50}. [c] CC_{50}, concentration of the compound that produced a 50% reduction of cell viability in treated culture cells with respect to untreated ones. [d] IC_{50} values obtained from six data points instead of eight. The two points removed because of problems solubility corresponded to the more concentrated dilutions 100 and 50 μg/mL of test compound. [e] ND, not determined.

It is interesting to note that benzamides **7-10** showed better activity against *L. amazonensis* parasites compared to the standard drug miltefosine, used as reference. They also showed better selectivity index (SI = CC_{50}/IC_{50}) in this *Leishmania* spp. In general, *L. guyanensis* was the least sensitive parasite strain to the tested compounds. Unfortunately, the tested acetamide derivatives displayed toxicity against the J774 cell line. On the contrary, the benzamide derivatives showed no cytotoxicity at 200 μM.

In the mammalian hosts *Leishmania* parasites exist as intracellular amastigotes within phagolysosomes of macrophages. Therefore, benzamides **7-10**, which are the most active compounds in the antipromastigote assay (SI from >2 to >90), could be good candidates for subsequent investigations against the clinically relevant *Leishmania* amastigote forms. Consequently, these benzamides were tested against *L. infantum* and *L. amazonensis* intracellular amastigotes (Table 2).

When the assay was carried out on *L. infantum* amastigotes, the antileishmanial activity of the tested derivatives was similar in comparison to extracellular forms. However, *L. amazonensis* amastigotes were more sensitive to these compounds. Compound **10** was the most potent for both parasite strains (IC_{50} = 2.5 and 3.0 μM, respectively).

Different authors have claimed that a compound should have a selectivity index (SI) value > 20 to be considered to possess leishmanicidal properties [13]. This requirement is satisfied by compounds **7-10** against *L. infantum* and *L. amazonensis*, except compound **9** against *L. infantum*.

Table 2. IC_{50} Leishmanicidal and cytotoxic effects (in μM) of C7- and C12-functionalized dehydroabietylamine derivatives on intracellular amastigote assay.

Compound	*L. infantum*		*L. amazonensis*		*Macrophages J774*
	IC_{50}[a] ±SD	SI[b]	IC_{50} ±SD	SI	CC_{50}[c]
7[d]	4,7±0,2	> 42	5,0±0,2	> 40	>200
8	3,3±0,2	> 61	3,5±0,2	> 57	>200
9	17,5±0,4	> 11	3,7±0,5	> 54	>200
10	2,5±0,9	> 80	3,0±0,4	> 67	>200
Miltefosine	58,1±4,4	2,3	16,5±1,9	8,2	136,4

[a] IC_{50}, concentration of the compound that produced a 50% reduction in parasites; SD: standard deviation. [b] Selectivity index, SI = CC_{50}/IC_{50}. [c] CC_{50}, concentration of the compound that produced a 50% reduction of cell viability in treated culture cells with respect to untreated ones. [d] IC_{50} values obtained from six data points instead of eight. The two points removed because of problems solubility corresponded to the more concentrated dilutions 100 and 50 μg/mL of test compound.

The resultant data indicates that the introduction of a nitro group at C-12 or a carbonyl group at C-7 increases the antileishmanial activity. In the case of *L. infantum* amastigotes both modifications on the parent molecule led to lower activity.

Conclusions

In summary, a study of antileishmanial activity of several dehydroabietylamine derivatives has been carried out *in vitro*. Particular modifications of the molecular scaffold abieta-8,11,13-triene functionalized at C-7, C-12, and C-18 have led to potent antileishmanial agents. Two benzamide derivatives, compounds **8** and **10**, were found to consistently reduce parasitimia at low micromolar concentrations. The presence of a benzamide at C-18 and a carbonyl group at C-7 in the molecule, compound **10**, resulted in the highest antileishmanial activity and selectivity index against *L. infantum* amastigotes (IC_{50} = 2.5 μM, SI > 80). Moreover, the activity of compound **10** was remarkable, as it was more potent than the reference drug miltefosine, and showed good selectivity. Thus, the abietane-type diterpenoids are promising scaffolds for the development of treatments for Leishmaniasis.

Experimental

General.

See supporting information.

Chemistry.

Materials. Compounds **7-15** have been prepared following similar conditions of reported procedures [11, 12]. All compounds prepared in this work exhibit spectroscopic data in agreement with the proposed structures. Purity of all final compounds was 95% or higher.

***N*-benzoyl-dehydroabietylamine (7).** To a solution of dehydroabietylamine (**6**) (2.0 g, 7 mmol) and triethylamine (2.0 mL, 14 mmol) in THF (35 mL) at 0 ºC, benzoyl chloride (0.9 mL, 7.7 mmol) was added dropwise. Then, it was allowed to warm to rt and stirred overnight (16 h). The resulting reaction mixture was treated with 1 M HCl (20 mL), phases separated, and the aqueous phase was extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The resulting residue was purified by flash chromatography, using DCM as eluent, to give benzamide **7** (2.44 g, 87%) as a white solid: $[\alpha]^{20}_D$ +2.7 (c 3.0, $CHCl_3$); ^{1}H NMR (300 MHz) δ 7.75-7.70 (2H, m), 7.52-7.39 (3H, m), 7.17 (1H, d, *J* = 8.5), 7.00 (1H, dd, *J* = 8.5, 1.5), 6.90 (1H, br s), 6.16 (1H, t, *J* = 6.0), 3.44 (1H, dd, *J* = 15.0, 6.0), 3.35 (1H, dd, *J* = 15.0, 6.0), 2.31 (1H, br d, *J* = 12.0), 1.25 (3H, s), 1.22 (6H, d, *J* = 6.5), 1.02 (3H, s); ^{13}C NMR (75 MHz) δ_C 167.6 (s), 147.0 (s), 145.6 (s), 134.7 (s), 131.3 (s), 128.6 x 2 (d), 126.9 (d), 126.8 x 2 (d), 124.2 (d), 123.8 (d), 50.3 (t), 45.8 (d), 38.3 (t), 37.6 (s), 37.5 (s), 36.4 (t), 33.4 (d), 30.4 (t), 25.4 (q), 23.9 (q), 23.9 (q), 19.1 (t), 18.8 (q), 18.6 (t); HRMS (ESI) *m/z* 390.2825 $[M+H]^+$, calcd for $C_{27}H_{36}NO$: 390.2797. The NMR and MS data agree with those reported in the literature [8b, 11].

***N*-benzoyl-12-nitrodehydroabietylamine (8).** Powdered cupric nitrate trihydrate (2.20 g, 9.0 mmol) was added in portions to a stirred solution of benzamide **7** (1.74 g, 4.47 mmol) in acetic anhydride (70 mL) and the blue solution was stirred at 5 ºC for 48 h. Then, the mixture was poured into water with stirring and the resulting precipitate was filtered off, washed with water and crystallized from methanol to give the nitro derivative **8** (1.28 g, 66%) as a pale yellow solid: $[\alpha]^{20}_D$ +24.2 (c 3.0, $CHCl_3$); ^{1}H NMR (300 MHz) δ 7.75-7.70 (2H, m), 7.62 (1H, s), 7.52-7.39 (3H, m), 7.06 (1H, s), 6.18 (1H, m), 3.56 (1H, dd, *J* = 15.0, 6.0), 3.20 (1H, dd, *J* = 15.0, 6.0), 2.29 (1H, br d, *J* = 12.0), 1.27-1.19 (9H, m), 1.01 (3H, m); ^{13}C NMR (75 MHz) δ_C 167.7 (s), 149.0 (s), 148.3 (s), 141.0 (s), 139.5 (s), 134.8 (s), 131.5 (d), 128.6 x 2 (d), 127.9 (d), 126.2 x 2 (d), 120.3 (d), 50.2 (t), 45.1 (d), 38.3 (t), 37.7 (s), 37.6 (s), 36.3 (t), 30.2 (t), 28.7 (d), 25.3 (q), 2 x 23.5 (q), 18.7 (t), 18.6 (q), 18.4 (t); HRMS (ESI) *m/z* 435.2739 $[M+H]^+$, calcd for $C_{27}H_{35}N_2O_3$: 435.2648. The NMR and MS data agree with those reported in the literature [11].

***N*-benzoyl-12-nitrodehydroabietylamine-7-one (9).** A solution of CrO_3 (500 mg, 5.0 mmol) in 25 mL of acetic acid was added dropwise to a solution of nitro-benzamide **8** (1.1 g, 2.50 mmol) in 10 mL of acetic acid. The mixture was stirred at room temperature for one hour and heated at 60 ºC for 17 h. Then, the mixture was poured into water and ice, and extracted with chloroform. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The resulting crude was purified by flash chromatography, using hexane-ethyl acetate (6:4) as eluent, to give ketone **9** (455 mg, 40%) as a yellowish foam: $[\alpha]^{20}_D$ +16.9 (c 1.3, $CHCl_3$); ^{1}H NMR (300 MHz) δ 8.08 (1H, s), 7.75-7.70 (2H, m), 7.59 (1H, s), 7.52-7.35 (3H, m), 6.19 (1H, m), 3.35 (1H, m), 3.25 (1H, m), 2.32 (1H, br d, *J* = 12.0), 1.30-1.19 (9H, m), 1.10 (3H, m); ^{13}C NMR (75 MHz) δ_C 197.2 (s), 167.9 (s), 154.0 (s), 153.0 (s), 140.0 (s), 134.8 (s), 133.0 (s), 131.6 (d), 128.6 x 2 (d), 127.4 (d), 126.8 x 2 (d), 119.1 (d), 49.7 (t), 44.4 (d), 37.9 (s), 37.9 (s), 37.2 (t), 36.0 (t), 35.9 (t), 28.4 (d), 23.9 (q), 2 x 23.4 (q), 18.4 (q), 17.9 (t); HRMS (ESI) *m/z* 449.2463 $[M+H]^+$, calcd for $C_{27}H_{33}N_2O_4$: 449.2440.

***N*-benzoyl-dehydroabietylamine-7-one (10).** A solution of CrO_3 (0.36 g, 3.6 mmol) in 20 mL of acetic acid was added dropwise to a solution of benzamide **7** (700 mg, 1.8 mmol) in 10 mL of acetic acid. The mixture was stirred at room temperature for one hour and heated at 60 ºC for 17 h. Then, the mixture was poured into water and ice, and extracted with chloroform. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The resulting yellowish crude oil was purified by flash chromatography, using petroleum ether-acetone (3:1) as eluent, to give ketone **10** (350 mg, 48%) as a yellowish foam: $[\alpha]^{20}_D$ -22.1 (c 2.8, $CHCl_3$); ^{1}H NMR (300 MHz) δ 7.82-7.83 (1H, m), 7.75-7.70 (2H, m), 7.49-

7.27 (5H, m), 6.40 (1H, br s), 3.52 (1H, dd, *J* = 15.0, 6.0), 3.20 (1H, dd, *J* = 15.0, 6.0), 3.00-2.72 (3H, m), 2.34 (1H, br d, *J* = 12.0), 1.28 (3H, s), 1.23-1.20 (6H, m), 1.09 (3H, s); ^{13}C NMR (75 MHz) δ_C 199.3 (s), 168.0 (s), 153.4 (s), 146.8 (s), 134.6 (s), 132.7 (d), 131.4 (d), 130.1 (s), 128.5 x 2 (d), 126.9 x 2 (d), 125.0 (d), 123.6 (d), 49.6 (t), 44.7 (d), 37.9 (s), 37.8 (s), 37.4 (t), 36.0 (t), 35.9 (t), 33.5 (d), 23.9 (q), 23.7 (q), 23.7 (q), 18.5 (q), 18.2 (t); HRMS (ESI) *m/z* 404.2634 $[M+H]^+$, calcd for $C_{27}H_{34}NO_2$: 404.2590. The NMR and MS data agree with those reported in the literature [11].

***N*-acetyl-dehydroabietylamine (11).** To a solution of dehydroabietylamine (**6**) (1.45 g, 5 mmol) and triethylamine (1.4 mL, 10 mmol) in THF (25 mL) at 0 ºC, acetyl chloride (0.4 mL, 5.5 mmol) was added dropwise. Then, it was allowed to warm to rt and stirred overnight (16 h). The resulting reaction mixture was treated with 1 M HCl (20 mL), phases separated, and the aqueous phase was extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The resulting yellowish crude oil was purified by flash chromatography, using hexane-ethyl acetate (4:6) as eluent, to give acetamide **11** (1.59 g, 96%) as a yellowish foam: $[\alpha]^{20}_D$ +18.2 (c 0.55, $CHCl_3$); ^{1}H NMR (300 MHz) δ 7.29 (1H, d, *J* = 8.5), 7.12 (1H, dd, *J* = 8.5, 1.5), 7.02 (1H, br s), 6.89 (1H, t, *J* = 6.0), 3.40 (1H, dd, *J* = 15.0, 6.0), 3.16 (1H, dd, *J* = 15.0, 6.0), 2.41 (1H, br d, *J* = 12.0), 2.08 (3H, s), 1.36 (6H, d, *J* = 6.0), 1.35 (3H, s), 1.07 (3H, s); ^{13}C NMR (75 MHz) δ_C 170.2 (s), 146.8 (s), 145.0 (s), 134.4 (s), 126.5 (d), 123.7 (d), 123.4 (d), 53.1 (s), 49.6 (t), 44.8 (d), 37.9 (t), 37.0 (s), 35.8 (t), 33.0 (d), 29.8 (t), 25.0 (q), 23.6 (q), 23.6 (q), 22.7 (q), 18.6 (t), 18.4 (q), 18.3 (t); HRMS (ESI) *m/z* 328.2621 $[M+H]^+$, calcd for $C_{22}H_{34}NO$: 328.2640. The NMR and MS data agree with those reported in the literature [14].

***N*-acetyl-12-nitrodehydroabietylamine (12).** Powdered cupric nitrate trihydrate (1.10 g, 4.55 mmol) was added in portions to a stirred solution of acetamide **11** (745 mg, 2.27 mmol) in acetic anhydride (30 mL) and the blue solution was stirred at 5 ºC for 17 h. Then, the mixture was poured into water and extracted with ethyl acetate. The organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The resulting residue was purified by chromatography eluting with hexane-ethyl acetate (4:6) to give 0.64 g (76%) of pure nitro derivative **12** as a slightly orange foam: $[\alpha]^{20}_D$ +45.3 (c 0.75, $CHCl_3$); ^{1}H NMR (300 MHz) δ 7.62 (1H, s), 7.08 (1H, s), 5.48 (1H, m), 3.48-3.30 (2H, m), 2.27 (1H, br d, *J* = 12.0), 1.97 (3H, s), 1.27-1.21 (9H, m), 0.94 (3H, s); ^{13}C NMR (75 MHz) δ_C 170.2 (s), 148.4 (s), 147.6 (s), 141.0 (s), 139.5 (s), 127.9 (d), 120.2 (d), 49.8 (t), 44.7 (d), 38.1 (t), 37.5 (s), 37.3 (s), 36.0 (t), 30.0 (t), 28.0 (d), 25.1 (q), 23.6 (q), 23.5 (q), 18.6 (q), 18.6 (t), 18.3 (q); HRMS (EI) *m/z* 373.2472 $[M+H]^+$, calcd for $C_{22}H_{33}N_2O_3$: 373.2491. The NMR and MS data agree with those reported in the literature [14].

***N*-acetyl-12-nitrodehydroabietylamine-7-one (13).** A solution of CrO_3 (600 mg, 6.0 mmol) in 28 mL of acetic acid was added dropwise to a solution of nitro-acetamide **12** (1.1 g, 3.0 mmol) in 12 mL of acetic acid. The mixture was stirred at room temperature for one hour and heated at 60 ºC for 17 h. Then, the mixture was poured into water and ice, and extracted with chloroform. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The resulting crude oil was purified by flash chromatography, using hexane-ethyl acetate (15:85) as eluent, to give ketone **13** (330 mg, 30%) as a light orange foam: $[\alpha]^{20}_D$ +27.4 (c 5.0, $CHCl_3$); ^{1}H NMR (300 MHz) δ 8.05 (1H, s), 7.63 (1H, s), 6.33 (1H, m), 3.26 (1H, m), 3.14 (2H, m), 2.34 (1H, br d, *J* = 12.0), 1.94 (3H, s), 1.32-1.25 (9H, m), 1.05 (3H, s); ^{13}C NMR (75 MHz) δ_C 197.3 (s), 170.6 (s), 154.1 (s), 152.8 (s), 139.6 (s), 132.9 (s), 127.0 (d), 119.0 (d), 48.9 (t), 43.5 (d), 37.5 (s), 37.4 (s), 37.1 (t), 35.7 (t), 35.4 (t), 28.2 (d), 23.6 (q), 23.2 (q), 23.2 (q), 23.1 (q), 18.3 (q), 17.8 (t); HRMS (EI) *m/z* 387.2245 $[M+H]^+$, calcd for $C_{22}H_{31}N_2O_4$: 387.2284.

***N*-acetyl-dehydroabietylamine-7-one (14).** A solution of CrO_3 (0.45 g, 4.5 mmol) in 20 mL of acetic acid was added dropwise to a solution of acetamide **11** (740 mg, 2.2 mmol) in 10 mL of acetic acid. The mixture was stirred at room temperature for one hour and heated at 60 ºC for 17 h. Then, the mixture was poured into water and ice, and extracted with chloroform. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The resulting yellowish crude oil was purified by flash chromatography, using hexane-ethyl acetate (4:6 to 3:7) as eluent, to give ketone **14** (565 mg, 73%) as a yellowish oil: $[\alpha]^{20}_D$ -26.7 (c 0.6, $CHCl_3$); ^{1}H NMR (300 MHz) δ 7.70 (1H, d, *J* = 2.0), 7.38 (1H, dd, *J* = 8.5, 2.0), 7.31 (1H, d, *J* = 8.5), 6.64 (1H, t, *J* = 6.0), 3.33 (1H, dd, *J* = 15.0, 6.0), 3.00 (1H, dd, *J* = 15.0, 6.0), 2.36 (1H, br d, *J* = 12.0), 1.95 (3H, s), 1.27 (3H, s), 1.19 (6H, d, *J* = 6.0), 1.04 (3H, s); ^{13}C NMR (75 MHz) δ_C 199.3 (s), 170.7 (s), 153.5 (s), 146.4 (s), 132.6 (d), 130.2 (s), 124.5 (d), 123.6 (d), 48.7 (t), 43.7 (d), 2 x 37.5(s), 37.4 (t), 35.8 (t), 35.5 (t), 33.3 (d), 2 x 23.6 (q), 23.5 (q), 23.1 (q), 18.5 (q), 18.1 (t); HRMS (ESI) *m/z* 342.2417 $[M+H]^+$, calcd for $C_{22}H_{32}NO_2$: 342.2433. The NMR and MS data agree with those reported in the literature [15].

***N*-(((1*R*,4a*S*,13c*R*)-12-fluoro-7-isopropyl-1,4a-dimethyl-2,3,4,4a,9,13c-hexahydro-1*H*-dibenzo[a,c]carbazol-1-yl)methyl)acetamide (15).** It was followed a method reported in the literature [12]. To a solution of 4-fluoroaniline (50 mmol) in 50 ml of 20% HCl was added dropwise the solution of $NaNO_2$ (3.63 g, 52.5 mmol) in 8 ml of water while cooling with an ice-water bath. The reaction mixture was stirred at 0 ºC for 1 h to give a clear solution. Then to the solution was added dropwise a solution of $SnCl_2$ (19 g, 0.1 mol) in 30 ml of 35% HCl at 0 ºC. Then, the mixture was stirred at room temperature for 1-2 h. The solid product was filtered, washed with 35% HCl for 3 times and dried in a vacuum desiccator containing phosphorous pentoxide. The product (7 g) could be used in subsequent reactions without further purification.

To a solution of ketone-acetamide (**14**) (190 mg, 0.56 mmol) in EtOH (2 mL) was added the above prepared phenylhydrazine hydrochloride (200 mg, 1.21 mmol) and 36% HCl (0.2 mL). The mixture was refluxed for 3 h, water/ice added and extracted with DCM. The combined organic extracts were washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated. The resulting brown crude oil was purified by flash chromatography, using DCM-MeOH (95:5) as eluent, to give carbazole **15** (77 mg, 32%) as a dark orange oil: $[\alpha]^{20}_D$ 0 (c 3.5, $CHCl_3$); ^{1}H NMR (300 MHz) δ 9.38 (1H, s, NH), 7.73 (1H, dd, *J* = 11.0, 2.0), 7.39 (1H, d, *J* = 1.5), 7.34 (1H, dd, *J* = 8.5, 5.0), 7.27 (1H, d, *J* = 8.5), 7.16 (1H, dd, *J* = 8.5, 1.5), 6.90 (1H, dt, *J* = 8.5, 2.0), 5.76 (1H, t, *J* = 6.0), 4.15 (1H, dd, *J* = 15.0, 6.0), 3.16 (1H, dd, *J* = 15.0, 6.0), 2.28 (1H, br d, *J* = 12.0), 1.99 (3H, s), 1.45 (3H, s), 1.30-1.25 (6H, m), 0.99 (3H, s); ^{13}C NMR (75 MHz) δ_C 170.7 (s), 157.0 (d, $J_{C,F}$ = 235), 146.7 (s), 146.7 (s), 136.9 (s), 133.9 (s), 126.7 (s), 126.2 (d), 123.9 (d, $J_{C,F}$ = 20), 123.5 (d), 119.3 (d), 111.6 (d, $J_{C,F}$ = 10), 111.0 (d, $J_{C,F}$ = 5), 109.0 (d, $J_{C,F}$ = 25), 107.7 (d, $J_{C,F}$ = 25), 53.4 (s), 51.3 (t), 48.8 (d), 39.9 (s), 37.2 (t), 36.4 (t), 33.7 (d), 23.9 (q), 23.9 (q), 23.4 (q), 21.3 (q), 19.6 (q), 17.6 (t); HRMS (ESI) *m/z* 433.2676 $[M+H]^+$, calcd for $C_{28}H_{34}FN_2O$: 433.2655.

Biology

Antileishmanial activity

Parasites and culture procedure

The following species of *Leishmania* were used: *L. donovani* (MHOM/IN/80/DD8) was purchased (ATCC, USA), an autochthonous isolate of *L. infantum* (MCAN/ES/96/BCN150) obtained from an asymptomatic dog from the Priorat region (Catalunya, Spain), kindly given by Prof. Montserrat Portús (Universidad de Barcelona); *L. amazonensis* (MHOM/Br/79/Maria) and *L. guyanensis* 141/93 were kindly provided by Prof. Alfredo Toraño (Instituto de Salud Carlos III, Madrid). Promastigotes were cultured in Schneider's Insect Medium supplemented with 10% heat-inactivated Foetal Bovine Serum (FBS) and 1000 U/L of penicillin plus 100 mg/L of streptomycin in 25 mL culture flasks at 26 °C.

Promastigote assay

The activity of functionalized dehydroabietylamine amide derivatives on *Leishmania* promastigotes was performed according to the method previously reported by us [16]. Parasites (2.5×10^5 promastigotes/well) were cultured in 96-well flat-bottom plates. Stock solutions of the tested compounds were solubilized at 100 mg/mL in DMSO. Serial dilutions 1:2 of test compounds in fresh culture medium were performed (100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/mL) in a final volume of 200 µL; the solutions then were added to the parasites suspension. After an incubation period of 48 h at 26 °C, a volume of 20 µL of 2.5 mM resazurin solution in PBS was added and the plates were incubated 3 h under the same conditions. Finally, the Relative Fluorescence Units (RFU) (535_{ex} – 590_{em} nm wavelength) was determined in a fluorimeter (Infinite 200 Tecan i-Control). Growth inhibition (%) was calculated by 100 - [(RFU treated wells – RFU signal-to-noise)/(RFU untreated – RFU signal-to-noise) x 100]. All tests were carried out in triplicate and the IC_{50} was calculated by probit analysis using SPSS 17.0 Statistics Software. Miltefosine (Sigma) was used as reference drug and all tests were carried out in triplicate.

Intracellular amastigote assay

We performed on *L. amazonensis* and *L. infantum* according to the novel fluorometric method reported by us [17]. To carry out the *in vitro* macrophage infections, $5x10^4$ cells were cultured in RPMI-1640 medium and then were infected with $5x10^5$ promastigotes. In *L. infantum* assays, the plates were incubated during 48 h at 37 °C, while in *L. amazonensis* assays, were incubated at 33 °C the first 24 h and then the temperature was increased to 37 °C for the next 24 h. After, the culture medium was removed and the cells were washed with buffered RPMI-HEPES at pH 7.4 for several times in order to eliminate the non-internalized promastigotes. One hundred microliters of fresh medium tempered at 37 °C was added to the infected cells, which then were exposed to 100 µL of test compounds in RPMI-1640 at different concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 µg/mL in a final volume 200 µL) for 48 h at 37 °C. The culture medium was removed by centrifugation at 3500 rpm for 5 min (Centrifuge 5403 Eppendorf) and a lysis solution (0.02 % sodium dodecyl sulphate in RPMI-HEPES) was added. After 20 min, the treated cells were harvested (centrifugation at 3,500 rpm for 5 min at 4° C) and the supernatants were replaced by 200 µL of fresh Schneider´s culture medium. The plates were then incubated at 26 °C for other 3 days to allow the transformation of viable amastigotes into promastigotes and proliferation. Afterwards, 20 µL/well of 2.5 mM resazurin was added and incubated for another 3 h. Finally, URF was measured and IC_{50} was estimated as described above. All tests were carried out in triplicate. Miltefosine was used as reference drug.

Cytotoxic activity

Cell culture

J774 murine macrophages were grown in RPMI-1640 medium (Sigma) supplemented with 10 % heat-inactivated FBS (30 min at 56 °C), penicillin G (100 U/mL) and streptomycin (100 µg/mL). Cell cultures were maintained in a humidified 5% CO_2/95 % air atmosphere at 37 °C. Cells in the pre-confluence phase were harvested mechanically to accomplish the assays.

Cytotoxicity Assay

The assay was carried out according our methods previously described by us [18]. J774 macrophages cell lines were seeded (5×10^4 cells/well) in 96-well flat-bottom plates with 100 µL of RPMI 1640 medium. The cells were allowed to attach for 2 h at 37 °C, 5% CO_2, and then was added 100 µL of RPMI-1640 medium with test compound at different concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/mL to the final volume) and exposed for

another 48 h. Growth controls and signal-to-noise were also included. Afterwards, a volume of 20 μL of 2.5 mM resazurin solution in PBS was added, and plates were returned to the incubator for another 3 h to evaluate cell viability. The reduction of resazurin was determined by fluorometry as in the promastigote assay. Each concentration was assayed by triplicate. Cytotoxicity effect of compounds was defined as the 50% reduction of cell viability of treated culture cells with respect to untreated culture (CC_{50}).

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