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LEISHMANICIDAL INSIGHTS FROM PINE RESINE DERIVATIVES

PERCEPÇÕES LEISHMANICIDAS A PARTIR DE DERIVADOS DA RESINA DE PINHEIRO

PERSPECTIVAS LEISHMANICIDAS A PARTIR DE DERIVADOS DE LA RESINA DE PINO

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Regis Silveira Barbosa[1], Ana Cláudia Cerqueira Oliveira[2], Mário Mateus Naves[3], Michelle de Aguiar Pimenta Julioti[4], Marília Vitória Franco da Silva[5], Carolina Ferreira Vaz[6], Laércio Couto[7], Herbert Júnior Dias[8], Lizandra Guidi Magalhães[9], Vladimir Constantino Gomes Heleno[10]

ABSTRACT

Due to the known potential of diterpenes to display biological activities, the aim of this work was to produce amide derivatives from dehydroabietic acid, naturally occurring in pine resins and evaluate them against Leishmania amazonensis. Our venture into the derivatives production led us to eight different amides which were properly identified and characterized by Nuclear Magnetic Resonance spectroscopy. Biological assays were carried out with all derivatives and the natural precursor against promastigote forms of L. amazonensis. Results show that two derivatives can be considered of better activity than the precursor against this parasite. This opens future perspectives to further explore that kind of diterpenes against Leishmania species.

Keywords: Abietanes. Dehydroabietic Acid. Structural Modification. Biological Assays. *Leishmania Amazonensis*.

[1] Master. Universidade de Franca. E-mail: Regissb11@outlook.com
Lattes: http://lattes.cnpq.br/1152058834427226
[2] Universidade de Franca. E-mail: acco1901@gmail.com
Lattes: http://lattes.cnpq.br/5655979824229654
[3] Universidade de Franca. E-mail: naves.mario02@gmail.com
[4] Master's degree of Science. Universidade de Franca. E-mail: miaguiar_pimenta@hotmail.com
Lattes: http://lattes.cnpq.br/4147148004485091
[5] Graduated in Biomedicine. Universidade de Franca. E-mail: vitoriamarilia8@gmail.com
Lattes: http://lattes.cnpq.br/9920963264358240
[6] Instituto Federal de Goiás. E-mail: carolina.vaz@estudante.ifgoiano.edu.br
Lattes: http://lattes.cnpq.br/1075044183373125 Orcid: https://orcid.org/0009-0001-4951-0103
[7] Dr. of Forest Science. Centro Brasileiro para Conservação da Natureza e Desenvolvimento Sustentável.
E-mail: laercio.couto45@gmail.com Lattes: http://lattes.cnpq.br/1437178545562509
[8] Dr. of Chemistry. Instituto Federal de Goiás. E-mail: herberthdias@ifgoiano.edu.br
Lattes: http://lattes.cnpq.br/6644735736454239 Orcid: https://orcid.org/0000-0001-6612-2295
[9] Dr, in Biomedicine. Universidade de Franca. E-mail: lizandra.magalhaes@unifran.edu.br
Lattes: http://lattes.cnpq.br/6987777227549255 Orcid: https://orcid.org/0000-0003-2959-6752
[10] Dr. of Science. Universidade de Franca. E-mail: heleno.vcg@gmail.com
Lattes: http://lattes.cnpq.br/8691255594471749 Orcid: https://orcid.org/0000-0002-4505-6978

RESUMO
Devido ao conhecido potencial dos diterpenos em apresentar atividades biológicas, o objetivo deste trabalho foi produzir derivados amídicos a partir do ácido desidroabiético, naturalmente presente em resinas de pinheiro, e avaliá-los contra Leishmania amazonensis. O desenvolvimento desses derivados resultou na obtenção de oito amidas diferentes, devidamente identificadas e caracterizadas por espectroscopia de Ressonância Magnética Nuclear. Ensaios biológicos foram realizados com todos os derivados e com o precursor natural frente às formas promastigotas de L. amazonensis. Os resultados demonstram que dois derivados apresentam atividade superior à do precursor contra esse parasito, o que abre perspectivas futuras para a exploração adicional desse tipo de diterpeno contra espécies de Leishmania.

Palavras-chave: Abietanos. Ácido Desidroabiético. Modificação Estrutural. Ensaios Biológicos. *Leishmania amazonensis*.

RESUMEN
Debido al conocido potencial de los diterpenos para presentar actividades biológicas, el objetivo de este trabajo fue producir derivados amídicos a partir del ácido deshidroabiético, presente de forma natural en las resinas de pino, y evaluarlos frente a Leishmania amazonensis. El desarrollo de estos derivados dio lugar a la obtención de ocho amidas diferentes, debidamente identificadas y caracterizadas mediante espectroscopía de Resonancia Magnética Nuclear. Se realizaron ensayos biológicos con todos los derivados y con el precursor natural frente a las formas promastigotas de L. amazonensis. Los resultados muestran que dos derivados presentan una actividad superior a la del precursor frente a este parásito, lo que abre perspectivas futuras para una mayor exploración de este tipo de diterpenos contra especies de Leishmania.

Palabras clave: Abietanos. Ácido Deshidroabiético. Modificación Estructural. Ensayos Biológicos. *Leishmania amazonensis*.

1 ABOUT NATURAL PRODCTS AND DITERPENES

Substances originating from living organisms, including plants, animals, and microorganisms, are classified as natural products (NPs). Natural products exhibit a wide diversity of chemical structures and encompass several distinct structural classes, which often confer various biological activities, such as antiparasitic, antiproliferative, and antimicrobial effects (Pertino *et al*., 2014; Soares *et al*., 2018). This diversity is of considerable scientific interest, as it contributes to the discovery and development of new drugs, supports the treatment of diseases, and enhances the therapeutic potential of existing medicines (Brusotti *et al*., 2014).

In medicine, many drugs are direct or indirect derivatives of natural products, and they are widely used in the treatment of cancer and infectious diseases. To ensure the efficacy of these medicines in clinical use, multiple regulatory and quality control measures are implemented to demonstrate their safety and effectiveness before they reach the market (Xie *et al*., 2018).

Studies on natural products can provide important insights into their biological potential and support the production of active substances from the vast diversity of plant species. Such investigations allow these compounds to be applied for the benefit of humanity by elucidating their mechanisms of action in specific living organisms. Given this remarkable potential, natural products are widely regarded as a promising source of new drugs (Soares *et al*., 2018).

Terpenoids are a large and important class of natural products widely distributed in nature. They can be subdivided into several groups, including monoterpenes, sesquiterpenes, diterpenes, and others (Reveglia *et al*., 2018). Among these subgroups, diterpenes are particularly prominent and are found in plants, fungi, bacteria, and animals, occurring in both marine and terrestrial environments (Garcia *et al*., 2007). They exhibit a wide range of biological activities, including antimicrobial, antiparasitic, anti-inflammatory, and antiviral effects, among others (Porto *et al*., 2009; Sebisubi *et al*., 2010; Jiang *et al*., 2015 [a]; Jiang *et al*., 2015 [b]).

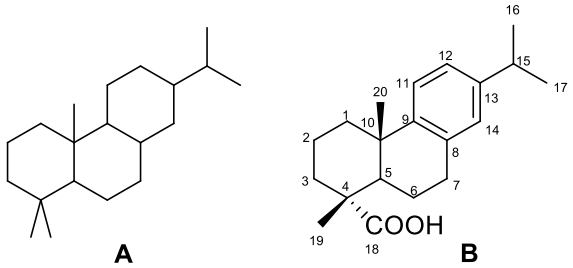
Pinus species represent an important plant group with high potential as a source of natural products. These trees are widely distributed and commonly found in countries such as China, the United States, India, and Brazil. A distinctive characteristic of *Pinus* species is the production of resin, a viscous yellow to brown liquid with a strong odor. This resin consists of a volatile fraction, known as turpentine, which contains monoterpenes and

sesquiterpenes, and a non-volatile fraction, known as pitch, which is rich in diterpenes (Sousa *et al*., 2018).

Diterpenes exhibit a wide range of chemical structures and are subdivided into several classes, including abietanes, kauranes, pimaranes, clerodanes, and labdanes, among others. The abietane class is particularly notable, as its members are commonly found in conifers of the genus *Pinus*. Abietanes possess a characteristic tricyclic skeleton and occur largely in the form of resin acids, which can account for up to 21% of the resin (Guo *et al*., 2019) (Figure 1 A).

Figure 1

A. Tricyclic skeleton of the abietan diterpenes. B. Chemical structure of dehydroabietic acid



Within this class of diterpenes, numerous resin-derived compounds have been reported, including abietic acid, isopimaric acid, dehydroabietic acid, among others (Fallarelo *et al*., 2013). One of the major components of Pinus resin is dehydroabietic acid (DI) (Figure 1B), a tricyclic abietane-type diterpene that exhibits several biological activities, including antiulcer, anti-inflammatory, and antimicrobial effects, among others. These properties indicate that dehydroabietic acid is a highly valuable starting material for the synthesis of new compounds with relevant industrial and pharmacological applications (Gouiric *et al*., 2004; Boeck *et al*., 2005; Tanaka *et al*., 2008).

Despite these findings, the antiparasitic activity of abietane diterpenes remains relatively underexplored in literature. In this context, this chapter focuses on recent advances achieved by our research group in the development of abietane-based molecules, with particular emphasis on their leishmanicidal activity. For this purpose, semi-synthetic methods have been established to obtain a series of derivatives from this class of compounds, which will be discussed below.

2 EXPERIMENTAL PROCEDURES

Several methods for the isolation of metabolites from *Pinus* resins have been reported in the literature. In the following sections of this chapter, the methodological sequence and corresponding results obtained by our research group in studies on *Pinus* oleoresins will be presented. Some *Pinus* resin sources are available from commercial suppliers. In Brazil, these resins can be obtained from the company Tecflora, which provides material from different Pinus species, such as *Pinus elliotti* from Buri, São Paulo-SP.

2.1 SEPARATION AND PURIFICATION OF OLEORESINS

The separation procedures were performed by vacuum liquid chromatography (VLC) and classical column chromatography (CCC) in glass columns, using silica gel 60 (70–230 mesh, 0.063–0.200 mm) and silica gel 60H (70–230 mesh, 0.040–0.063 mm), both Merck®, as the stationary phase. The choice of technique and column dimensions was adjusted according to the amount of sample available.

^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker® AVANCE DRX400 spectrometer operating at 400 MHz. The experiments were carried out at Department of Chemistry of the University of São Paulo - USP de Ribeirão Preto. Compound identification was supported by ultraviolet (UV) irradiation and comparative thin-layer chromatography (TLC) on Merck® aluminum plates coated with silica gel 60 GF254 (0.25 mm thick), with visualization using vanillin sulfuric acid reagent followed by heating.

High-performance liquid chromatography (HPLC) analyses were performed on a Shimadzu Prominence system (CBM-20A, LC-6AD) equipped with a manual injector, DGU-20A5 degasser, SPD-20A diode array detector (DAD), and a microcomputer running LCsolution software for data acquisition and processing. A reversed-phase C18 Shimadzu column (4.6 mm × 250 mm, 5 µm) with a 20 µL injection loop was used, with acetonitrile/water (9:1) as the mobile phase at a flow rate of 1.0 mL/min. HPLC-grade reagents from J. T. Baker® and ultrapure water from a Milli-Q® system were employed. The solvents used for isolation, purification, and as reaction media were ethyl acetate (AcOEt), hexane (Hex), dichloromethane (DCM), methanol (MeOH), and ethanol (EtOH), all of analytical grade (P.A.).

The resin was coded as RP-1. These resins consist of a white solid mass impregnated with a light-yellow oily fraction. Samples from the three *Pinus* resins were

analyzed by HPLC to identify and compare their major constituents in both the oily fraction and the solid mass. Thus, the solid masses and oily fractions are described as Resin 1 (mass): RP-1M; Resin 1 (oil): RP-1O.

The fractionation of RP-1O was done by VLC. In this case, it was carried out only with the "oil" part and the following fractions were generated, which were thus coded.

- Fraction 1 - Hex: RP-1O.
- Fraction 2 - 9:1 (Hex/EtOAc): RP-1O.
- Fraction 3 - 8:2 (Hex/EtOAc): RP-1O.
- Fraction 4 - 7:3 (Hex/EtOAc): RP-1O.
- Fraction 5 - 6:4 (Hex/EtOAc): RP-1O.
- Fraction 6 - 1:1 (Hex/EtOAc): RP-1O.CLV-6.
- Fraction 7 - 4:6 (Hex/EtOAc): RP-1O.
- Fraction 8 - 3:7 (Hex/EtOAc): RP-1O.
- Fraction 9 - EtOAc: RP-1. LVC -9.
- Fraction 10 - EtOH: RP-1O. LVC -10.

The isolation and purification of the chemical constituents from *Pinus elliottii* resin were performed starting from 50.0 g of crude material. The procedure began with a VLC separation (Pelletier *et al.*, 1986), using 240.0 g of silica gel 60 and 240.0 g of silica gel 60H as stationary phases. The column was packed under vacuum with hexane as the initial solvent, and the sample was adsorbed onto 77.6 g of silica gel 60 prior to loading. During the chromatographic run, ten fractions were collected, using an elution gradient of increasing polarity as summarized in Table 1.

Table 1

Elution scheme used in RP-1O fractionation

Solvent used	Volume collected (mL)	Fraction	Mass obtained (g)
Hex	500	RP-1O.VLC-1	0.018
Hex/EtOAc 9:1	500	RP-1O.VLC-2	0.720
Hex/EtOAc 8:2	500	RP-1O.VLC-3	0.960
Hex/EtOAc 7:3	500	RP-1O.VLC-4	41.450
Hex/EtOAc 6:4	500	RP-1O.VLC-5	0.550

Hex/EtOAc 1:1	500	RP-1O.VLC-61.430
Hex/EtOAc 4:6	500	RP-1O.VLC-72.000
Hex/EtOAc 3:7	500	RP-1O.VLC-80.990
EtOAc	500	RP-1O.VLC-90.460
EtOH	500	RP-1O.VLC-100.340

Hex: hexane; EtOAc: ethyl acetate; EtOH: ethanol

All fractions obtained from this procedure were analyzed through CTLC using as mobile phase a mixture of Hex/EtOAc (8:2). In this analysis, it was possible to observe that the fractions RP-1O.CLV-3 and RP-1O.CLV-4 had a similar chemical profile and therefore were selected to continue the isolation. The portions designated as “oils” afforded higher yields than the corresponding “mass” portions. RP-1M provided approximately 12% essential oil, whereas RP-1O gave yield of 17%.

2.2 STRUCTURAL MODIFICATIONS

2.2.1 General procedure for obtaining amides from dehydroabietic acid (DI)

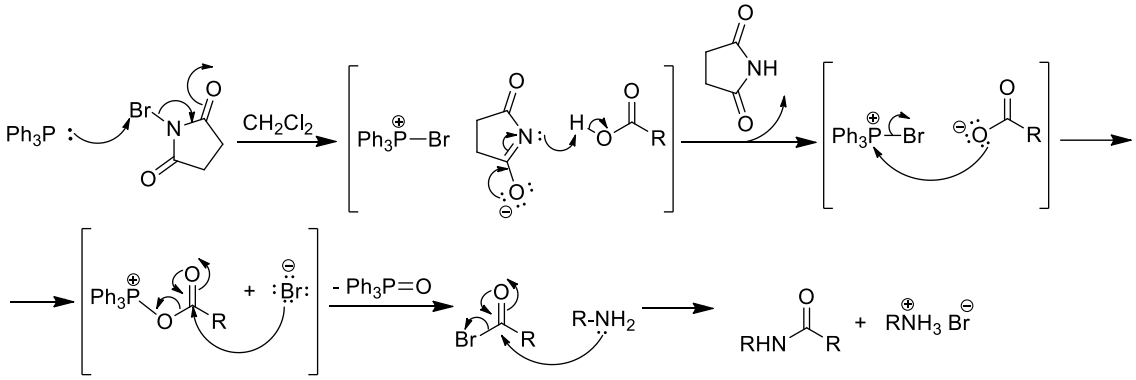
The fractionation of *Pinus* oleoresin affords dehydroabietic acid (DI) as a purified product, which is subsequently used as a substrate for the semi-synthesis of amide derivatives. A general procedure was adopted for all DI amide-forming reactions. First, dichloromethane (DCM) was predried with calcium sulfate ($CaSO_4$) using 2.0 g of $CaSO_4$ per 100 mL of DCM under reflux for 3 h. The DCM was then collected and transferred to a second drying system containing phosphorus pentoxide (P_2O_5) added in an amount sufficient for complete drying, and the mixture was further refluxed for 3 h.

Recrystallization of N-bromosuccinimide (NBS) was carried out as follows. NBS (20.0 g) and deionized water were added to a 0.5 L Erlenmeyer flask to a final volume of 290 mL, and the mixture was heated in a water bath at 70-80 °C until complete dissolution. The resulting orange solution was allowed to stand at room temperature for 24 h, during which a crystalline solid precipitated. The solid was collected by vacuum filtration, washed several times with deionized water, and dried in an oven at 120 °C for 3 h. The dry material was then transferred to a light-protected bottle and stored in a desiccator.

According to the procedure described by Bandgar and co-workers (2004), and as outlined in the reaction mechanism shown in Figure 1, amidation reactions were carried out as follows. One equivalent of DI (40.0 mg) and two equivalents of triphenylphosphine (Ph_3P, 69.9 mg) were dissolved in 2 mL of DCM, and the mixture was cooled to 0-5 °C under stirring. Subsequently, 2.5 equivalents of N-bromosuccinimide (NBS, 59.3 mg) were added, and the reaction was stirred for 15 min. After this period, 4-5 equivalents of the corresponding amine were added, while maintaining the same temperature and continuous stirring.

Figure 2

Proposed scheme for amidation reaction using dehydroabietic acid, amine, triphenylphosphine and N-bromossuccinimide (Bandgar et al., 2004).



In all cases where a greater amount of product was required, the procedures for its preparation and purification were repeated as originally described. After each reaction, the mixture was concentrated by solvent evaporation, and the crude product was stored in a freezer prior to purification by classical column chromatography. Our research group proposed in this sense a synthesis of eight DI-amide-derivatives, follow the reactions performed are described in according to Figure 3 and Table 2

Figure 3

Scheme of the amidation reaction of dehydroabietic acid

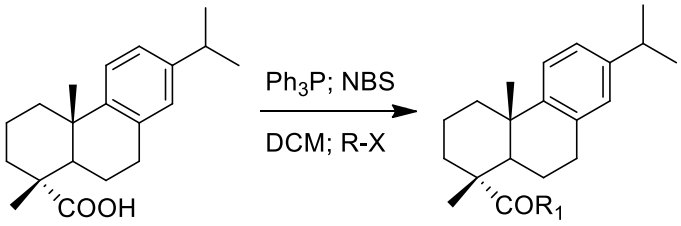


Table 2

Amidation reactions carried out with dehydroabietic acid

Reaction	Reagent (R-X)	Ramification (R_1)	Reaction time (min)
1	Cyclohexylamine	C6H11N	11
2	3-amino-1-propanol	C3H8NO	45
3	*p*-anisidine	C7H8NO	9
4	Terc-butylamine	C4H10N	25
5	Isopropylamine	C3H8N	8
6	Aniline	C6H6N	9
7	Methyl piperazine	C5H11N2	9
8	Pyrrolidine	C4H8N	8

3 RESULTS

3.1 PRODUCTS OBTAINED FROM DEHYDROABIETIC ACID.

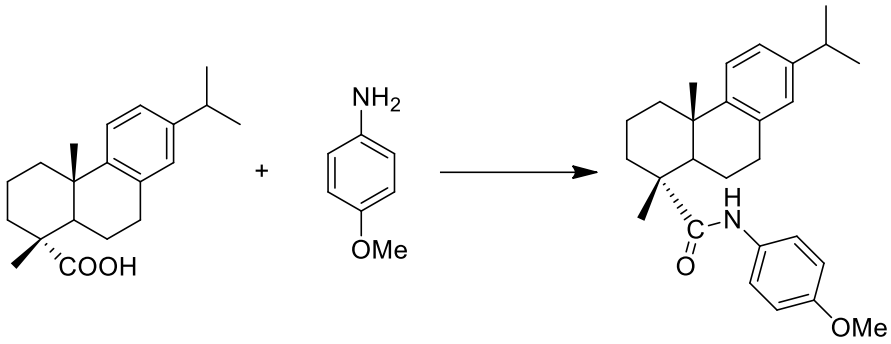
The dehydroabietic acid (DI) isolated from Pinus elliottii resin was subjected to structural modification at the hydroxyl region of the carboxylic acid group through reactions with different amines, leading to amidation. This process afforded four new amide derivatives, enabling comparative evaluation of their structural features relative to DI, which served as the starting material in each reaction. The ^{1}H NMR spectra of the products were recorded, and the signals were assigned and compared with those of the parent DI spectrum to confirm the proposed structural changes. Data for three of the obtained derivatives are presented below, with selected signals highlighted to illustrate the modifications introduced in each structure.

3.1.1 Identification of reaction product 3 (D1)

The identification of the product obtained was confirmed by ^{1}H NMR spectrum of the reaction 3 (Figure 4). Product data obtained: F.M. = $C_{27}H_{35}NO_2$ and M.M. = 405.27 g mol^{-1}.

Figure 4

Reaction of dehydroabietic acid with p-anisidine



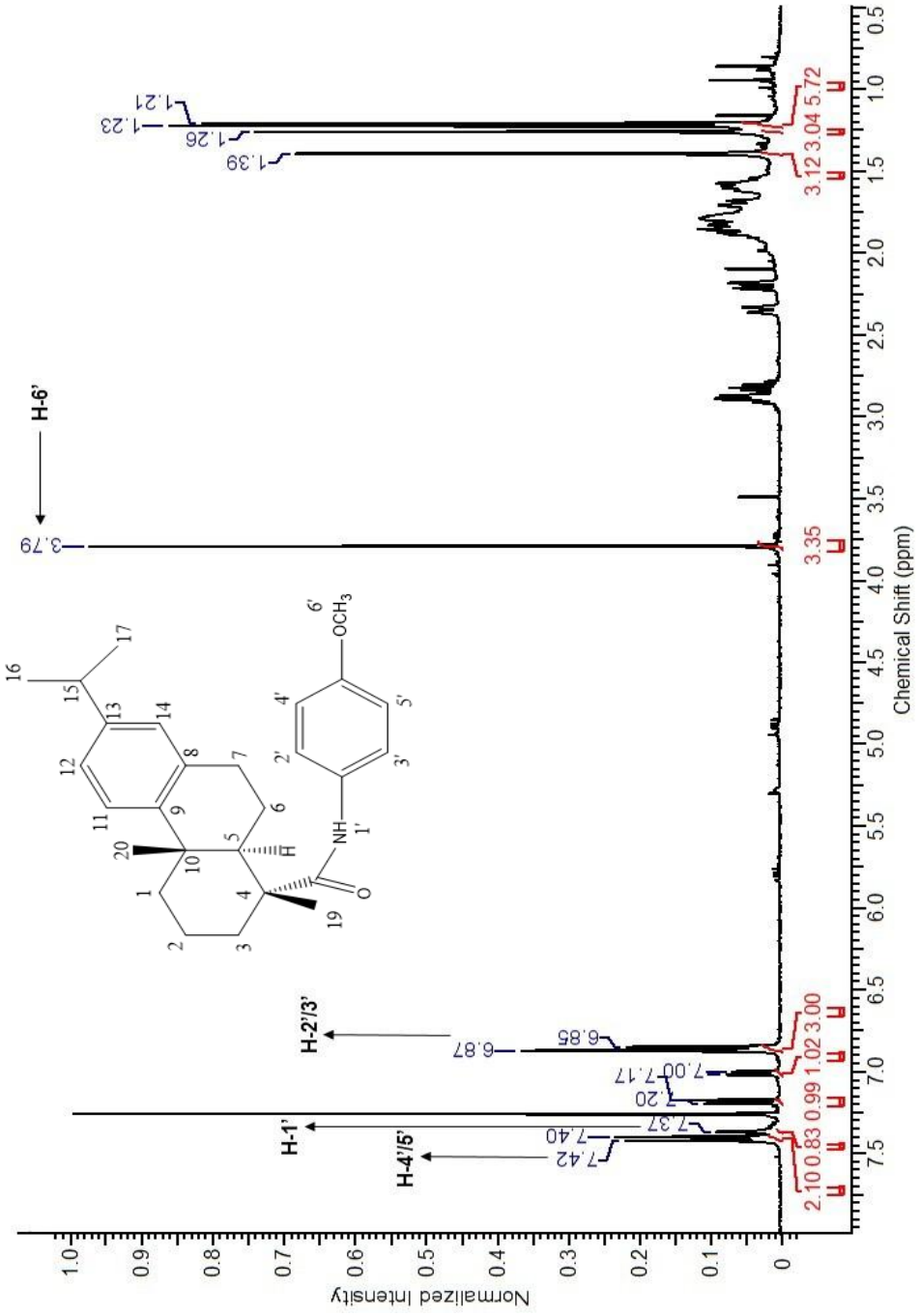
The ^{1}H NMR spectrum shown in Figure 5, in addition to the characteristic signals of DI, displays a new singlet at *δ* 3.79 ppm with an integral corresponding to 3H, attributable to the hydrogens of a methoxy group. This signal is consistent with the expected chemical shift for a methyl group bound to an ether oxygen, typically observed between *δ* 3.00 and 4.00 ppm. The signals obtained in the spectrum of Figure 4, in addition to the characteristic signs of DI, clearly show us the presence of a signal that differentiates it from the starting material, corresponding to a singlet with *δ* of 3.79 ppm and relative integral for 3H, referring to the hydrogens of the methoxy group. This signal is expected for this structure, since the methyl group bound to the ether group has its region found between *δ* 3.00 and 4.00 ppm.

A further diagnostic signal appears at *δ* 7.37 ppm with an integral for 1H, corresponding to a proton directly attached to nitrogen. This resonance agrees with the chemical shift range reported for amide NH hydrogens, usually found between *δ* 5.00 and 8.00 ppm. In addition, aromatic hydrogens in positions closer to the oxygen atom appear more deshielded, giving a signal at *δ* 7.41 ppm with an integral 2H.

A second set of aromatic protons, less deshielded and located nearer to the nitrogen atom, is observed at *δ* 6.86 ppm with an apparent integral for 3H. This higher integral value results from overlap with the H-14 signal of the parent DI, which contributes 1H to this resonance. Comparison with the spectrum of DI and the assignment of these new or shifted signals support that the amidation proceeded as expected.

Figure 5

^1H NMR spectrum of the product obtained from reaction 3, 400 MHz, $CDCl_3$

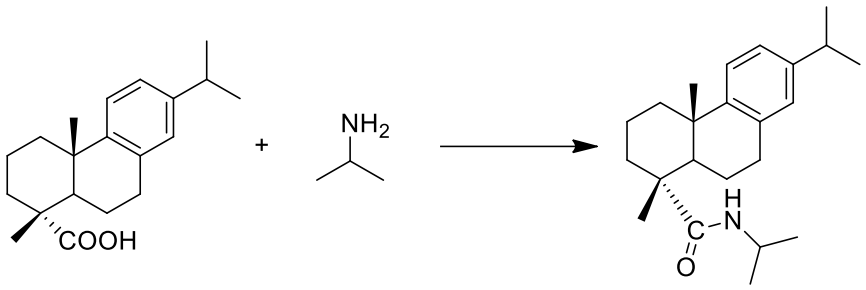


3.1.2 Identification of reaction product 5 (D3)

The identification of the product obtained was confirmed by NMR spectrum of 1H, shown in Figure 6. Product data obtained: F.M. = $C_{23}H_{35}NO$ and M.M. = 341.27 g mol $^{-1}$.

Figure 5

Reaction of dehydroabietic acid with isopropyl amine

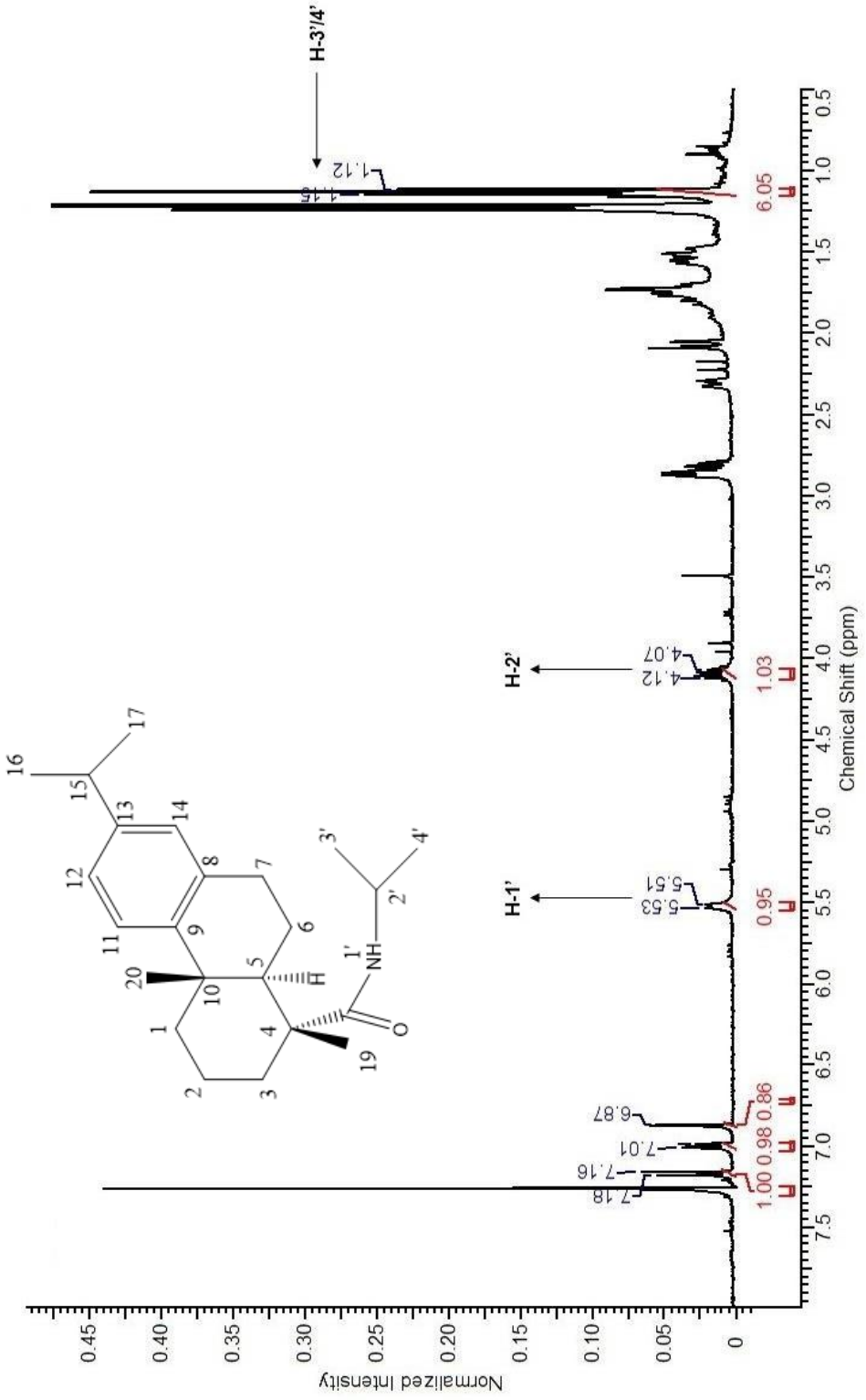


In Figure 7, the signals corresponding to the starting material DI are still observed, including H-11, H-12, and H-14, which appear more downfield due to their aromatic nature. In contrast, a new diagnostic signal (*d*) at *δ* 5.52 ppm with an integral for 1H is observed, assigned to the proton directly attached to the amide functional group. This resonance is consistent with the expected chemical shift range for amide NH protons and thus confirms the introduction of the amide function in the product

In addition to these signals, the spectrum in Figure 7 also shows a set of upfield resonances in an overlapping region at *δ* 1.13 ppm with an integral corresponding to 6H, which are attributed to the methyl protons of the product. A further signal at *δ* 4.10 ppm with an integral for 1H is assigned to the proton attached to the methine carbon of the newly formed fragment. Taken together, the presence of both the characteristic signals of the starting material and the new resonances assigned to the product confirms that the reaction proceeded satisfactorily.

Figure 7

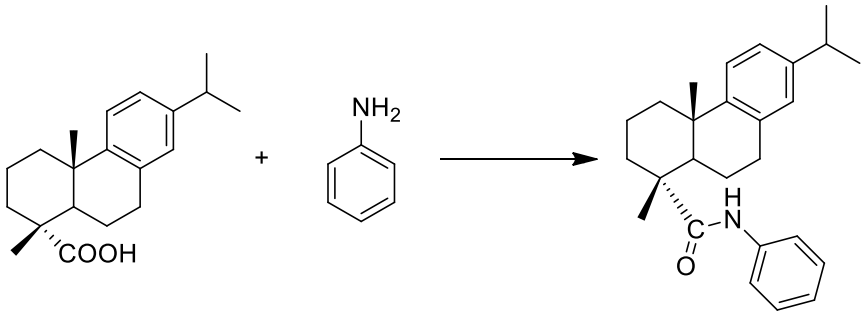
^1H NMR spectrum of the product obtained from reaction 5, 400 MHz, $CDCl_3$



The identification of product 6 obtained was confirmed by NMR spectrum of 1H, shown in figure 9. Product data obtained: F.M. = $C_{26}H_{33}NO$ and M.M. = 375.26 g mol $^{-1}$.

Figure 8

Diagram of the reaction of dehydroabietic acid with aniline



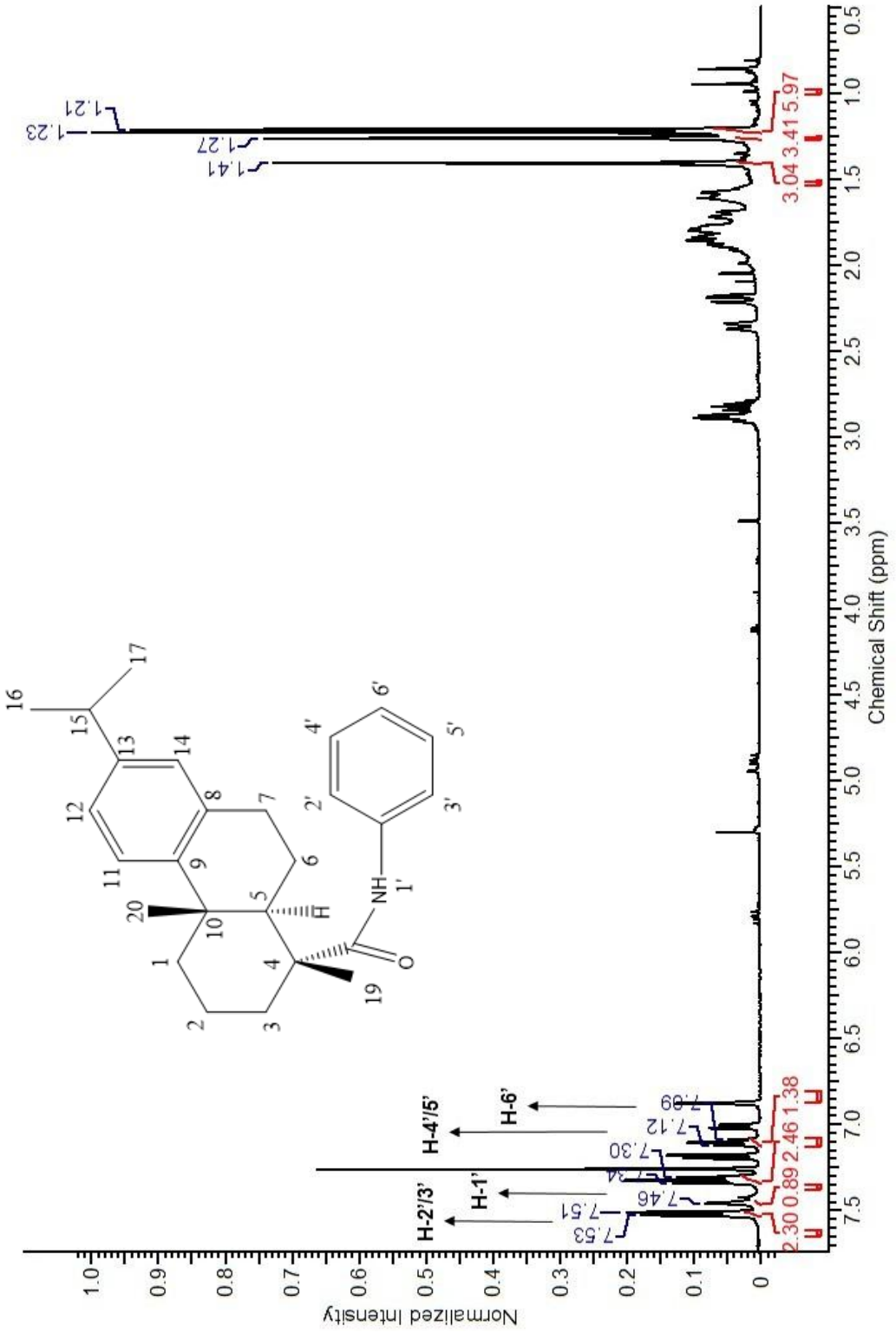
According to the spectrum shown in Figure 9, in addition to the most shielded methyl signals attributable to the starting material, several resonances distinct from those of DI are observed. A double doublet at *δ* 7.52 ppm with an integral for 2H is assigned to aromatic protons that are more deshielded and located closer to the amide functional group. These hydrogens experience a similar electronic environment and therefore appear as a single signal in the spectrum.

Another signal is observed at *δ* 7.33 ppm with an integral for 2H, corresponding to aromatic protons that are relatively less deshielded and appear as a single resonance due to their similar chemical environment. In addition, a signal at *δ* 7.11 ppm with an integral for 1H is attributed to the most shielded aromatic proton in the structure. All these aromatic proton signals fall within the expected chemical shift range for aromatic hydrogens, typically between *δ* 6.50 and 8.00 ppm, and are fully consistent with the proposed structure.

A singlet at *δ* 7.46 ppm with an integral corresponding to 1H was also observed and assigned to the proton directly bonded to nitrogen. This signal is consistent with the expected chemical shift range for amide NH protons, typically found between *δ* 5.00 and 8.00 ppm. Therefore, comparison with the spectrum of the starting material and the presence of these new diagnostic resonances indicate that the reaction proceeded as expected and that the product is distinct from DI.

Figure 9

^1H NMR spectrum of the product obtained from reaction 6, 400 MHz, $CDCl_3$



3.1.3 Evaluation of Leishmanicidal Activity

The biological activity investigated in this study was the leishmanicidal effect against promastigote forms of *Leishmania (L.) amazonensis*. The assays were carried out in collaboration with the Parasitology Laboratory of the University of Franca, Franca, São Paulo, Brazil. Initially, samples of the crude resin (RP-1), its essential oil (RP-1-EO), and the fixed fraction (RP-1-PF) obtained after essential oil extraction were evaluated. Under the experimental conditions employed, none of these samples exhibited significant leishmanicidal activity, as shown in Table 3.

Table 3

Leishmanicidal activity against promastigote forms of L. (L.) amazonensis and determination of inhibitory concentration values 50% (IC_{50}) in the 24-hour incubation period

% of inhibition of flagellar motility ± D.P.						
Substances	**50**	**25**	**12.5**	**6.25**	**3.12**	**IC_{50} (µg/mL)**
RP-1	54.59±0.59	22.97±16.81	28.91±1.52	19.05±0.95	11.62±1.14	>50
RP-1-PF	34.45±0.95	28.51±1.33	17.43±0.57	10.54±0.00	5.81±1.72	>50
RP-1-OE	21.89±0.76	15.54±2.10	8.91±1.47	0.00±0.00	0.00±0.00	>50
RP-1-FR (7:3)	88.78±0.95	84.05±0.00	69.18±1.91	58.51±1.33	50.67±1.33	3.43±0.73
Dehydroabietic acid	88.67±0.66	83.49±0.33	75.94±1.66	57.07±2.01	49.76±1.33	1.05±0.79
Other component	48.10±3.05	35.54±3.24	20.67±3.63	11.89±0.38	0.00±0.00	49.73±1.02
	0.19	**0.095**	**0.047**	**0.023**	**0.011**	
Amphotericin B	99.88±0.60	78.33±24.43	68.74±21.97	54.67±17.77	42.44±20.97	0.011±0.54

Positive Control: Amphotericin B 1 µg/mL Negative Control: Medium RPMI + 0.1% DMSO

However, after VLC of the resin, several fractions were obtained, and one of them contained the most abundant diterpenes in its composition. This fraction (RP-1-FR 7:3) was therefore considered rich in major diterpenic constituents. Evaluation of the leishmanicidal activity of this fraction yielded a notable result, revealing significant activity of the mixture against promastigote forms of the parasite (IC_{50} = 3.43 □g/mL). The activity-guided assessment then focused on identifying the component responsible for this effect by testing dehydroabietic acid (DI) and the other, not yet fully identified, constituent of the fraction. Dehydroabietic acid showed a much stronger leishmanicidal effect (IC_{50} = 1.05 □g/mL) than

the other component (IC_{50} = 49.73 □g/mL), supporting the conclusion that dehydroabietic acid is the main compound responsible for the observed activity.

These results reinforce the relevance of pursuing structural modifications of DI with the aim of obtaining derivatives that may display even more pronounced leishmanicidal activity. In this context, derivatives D1 to D4 were evaluated, and their IC_{50} values, expressed in micromolar units as appropriate for pure substances, are presented in Table 4.

Table 4

Leishmanicidal activity against promastigote forms of L. (L.) amazonensis and determination of inhibitory concentration values 50% (IC_{50}) in the 24-hour incubation period

% of inhibition of flagellar motility ± D.P.						
Substances	**50**	**25**	**12.5**	**6.25**	**3.12**	**IC50 (µM)**
Dehydroabietic acid	88.67±0.66	83.49±0.33	75.94±1.66	57.07±2.01	49.76±1.33	3.49±0.79
D1	76.35±0.19	71.35±1.52	65.13±0.38	59.45±1.14	50.40±1.72	2.76±0.41
D2	75.67±0.76	74.45±1.72	70.67±0.57	60.13±1.33	47.70±2.10	2.97±0.48
D3	72.43±0.76	63.10±1.33	55.40±1.14	48.51±1.33	45.40±0.76	6.16±0.40
D4	70.94±4.01	62.29±2.10	51.62±0.77	40.67±0.57	36.75±0.38	10.35±0.54
	0.19	**0.095**	**0.047**	**0.023**	**0.011**	
Amphotericin B	99.88±0.60	78.33±24.43	68.74±21.97	54.67±17.77	42.44±20.97	0.011±0.54

Positive Control: Amphotericin B 1 µg/mL Negative Control: RPMI medium + 0.1% DMSO

The results obtained were promising, as all derivatives exhibited activity against the promastigote forms of the parasite. In addition, two of these derivatives were even more active than the precursor, dehydroabietic acid, which was the most active component of the resin. The next step in the search for bioactive compounds will be to evaluate the cytotoxicity of the natural products and the semi-synthetic derivatives.

4 CONCLUSION AND PERSPECTIVES

This chapter provides evidence that *Pinus* oleoresin is a valuable source of abietane-type diterpenes with leishmanicidal potential, particularly dehydroabietic acid and its semi-synthetic amide derivatives. Fractionation of the crude resin enabled the identification of a diterpene-enriched fraction (RP-1-FR 7:3) with significant activity against promastigote forms of *Leishmania (L.) amazonensis*, and subsequent assays identified dehydroabietic

acid as the principal bioactive component of this mixture. These findings align with the growing body of literature indicating that terpenoids and other plant-derived metabolites represent promising scaffolds for the discovery of new antileishmanial agents, particularly in the context of neglected tropical diseases. In addition, the successful application of a simple amidation protocol underscores the value of dehydroabietic acid as a synthetic platform for structural diversification and increasing antiparasitic activity.

Leishmanicidal profile observed for DI and derivatives D1–D4 underscores the importance of continued structure–activity relationship studies aimed at improving potency and selectivity, especially through rational modifications of the abietane skeleton and systematic evaluation against both promastigote and amastigote forms. Future investigations should prioritize cytotoxicity assays, determination of selectivity index, and mechanistic studies to elucidate cellular targets and pathways involved in parasite death, thereby meeting current criteria for early-stage drug discovery in leishmaniasis. Moreover, in vivo assessments, exploration of alternative formulations, and integration with contemporary approaches such as *in silico* modeling may facilitate the progression of these derivatives from promising hits to preclinical candidates, contributing to the development of safer and more effective therapies derived from natural products.

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