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An abietane diterpene from *Salvia cuspidata* and some new derivatives are active against *Trypanosoma cruzi*

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ABSTRACT

The Plant Kingdom is an excellent source for obtaining natural compounds with antiprotozoal activity. In the present work, we studied the effect of the diterpene 12-hydroxy-11,14-diketo-6,8,12-abietatrien-19,20-olide (HABTO) obtained from the aerial parts of *Salvia cuspidata* on *Trypanosoma cruzi* epimastigotes. This compound was found to inhibit parasite growth even at low concentrations (IC_{50} 5 μg/mL) and with low toxicity on mammalian cells. In addition, this diterpene induced an intense vacuolization within the parasites. In order to obtain analogs with greater lipophilicity, chemical modifications on the enol moiety were carried out to obtain the acetyl (AABTO), the sylil (SABTO) and the allyl (ALLABTO) derivatives. We observed that the SABTO was the most effective one on the parasites, and the effect could be attributed to a greater lipophilicity of this compound. Taking into account these data we conclude that the increase of lipophilicity by chemical modifications is an adequate strategy for improving the trypanocidal activity of this kind abietane diterpenes.

Trypanosoma cruzi is a monoflagellate parasite causative of Chagas' disease. About 6 million to 7 million people are estimated to be infected worldwide, mostly in Latin America where Chagas disease is endemic.[1,2] Over the last decades, many natural and synthetic compounds have been tested to treat this disease; however, results have not been satisfactory.[3,4] Current drugs, benznidazole (Bz) and nifurtimox (Nx), have poor efficacy, are highly toxic and have disadvantages such as the development of drug resistance, and high costs. The weak effects of Bz and Nx during the chronic phase of Chagas disease can be attributed to their relatively short half-lives, as well as their poor permeability properties, which afford them limited tissue penetration.[5] Thus, there is a need for new trypanocidal drugs having low toxicity to the host's cells. Secondary metabolites isolated from plants are one of the main sources for the development of new antiparasitic agents.[6] Some plant species from the genus *Salvia* have been used as antiparasitic remedies.[7–10] and some molecules effective against different stages of *T. cruzi* have been identified.[11–13] Among such structures, those belonging to the large terpenoid family and their derivatives appear to be attractive candidates to treat Chagaś disease. In fact, many of them have already been demonstrated to be effective on *T. cruzi*.[11–13] The Plant Kingdom is an interesting source of antiparasitic molecules, due to the abundance of different families of compounds exhibiting a lot of molecular connectivities and a well defined stereochemistry.[14–16]

On the other hand, the development of improved bioactive natural products has benefited from the use of substituents that are chemically stable, resistant to metabolic transformation, and the possibility to combine lipophilicity with polarity to modulate receptor binding or the transport across membranes.[17]

In this sense, the Functional Group Interconversion of bioactive molecules is a useful strategy to increase the bioavailability of drugs. The chemical transformation of a single functional group into a bioactive compound can affect the overall electronics properties, solubility, and steric dimensions. In addition, the overall effect of a given functional group depends upon all of the other functional groups surrounding it or attached to it.[18]

In this study, we tested the antiprotozoal activity of an abietane diterpene (HABTO) isolated from *Salvia cuspidata* (Ruiz & Pav. Subsp. *gilliesii* (Benth) J.R.I. Wood, (Lamiaceae) and some less polar derivatives prepared by chemical derivatization[19] (Supplementary data).

The search for new molecules with trypanocidal activity has been a constant concern in the fight against Chagas' disease. Natural products are a growing source of new drugs with activity against different pathogens.

In this work we evaluated the in vitro activity of a new diterpene obtained from S. cuspidata and some of its semi-synthetic

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derivatives on *T. cruzi* epimastigotes. Compound **1**, (12-hydroxy-11,14-diketo-6,8,12-abietatrien-19,20-olide) (HABTO), has a diterpene structure belonging to the abietane group (Fig. 1). As observed in the Figure 2A, this compound inhibits the growth of *T. cruzi* at very low concentrations, with an IC_{50} ~5 μg/mL (Table 2), and induces an intense vacuolization within the parasites (Fig. 3), similar to that observed with other terpenes.[11,20,21] This antiparasitic effect was similar to that observed in cultures treated with the trypanocidal drug Bz (data not shown).

It is known that *T. cruzi* is highly sensitive to compounds bearing quinone groups,[12,13,22] a feature that would account for the antiparasitic effect of HABTO observed (Fig. 1 and Table 2). However, this molecule contains other chemical groups that could be involved in the biological activity of this compound. From the structure of compound **1** it is possible to deduce that the polarity observed from the Clog*p* (−0.1591) is due to the hydroxyl group on C-12 (C ring). Several strategies can be used to modify this property to obtain a more lipophilic analogous. One of these strategies it to transform this functional group into an ester by acetylation (R^1); however, this alternative is not totally appropriate taking into account that the ester functionality can be cleaved in vivo by esterases. A second alternative is the transformation to a trialkylsilyl enol ether functional group (R^2); this type of linkage renders a functional group resistant to hydrolysis and, in turn, it is highly lipophilic. Finally, the synthesis of an allylether (R^3) constituted an interesting alternative; this kind of functionality is common in some skeletons of natural products and has proved to be resistant to hydrolytic processes occurring in living organisms. Based on these criteria, derivatives **2** (AABTO); **3** (SABTO), and **4** (ALLABTO), were prepared (Fig. 1).

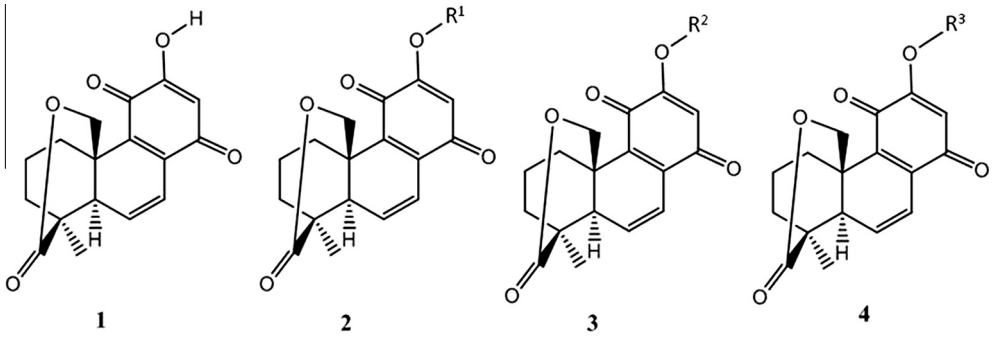

Figure 1. Structures of HABTO (1), AABTO (2), SABTO (3) and ALLABTO (4).

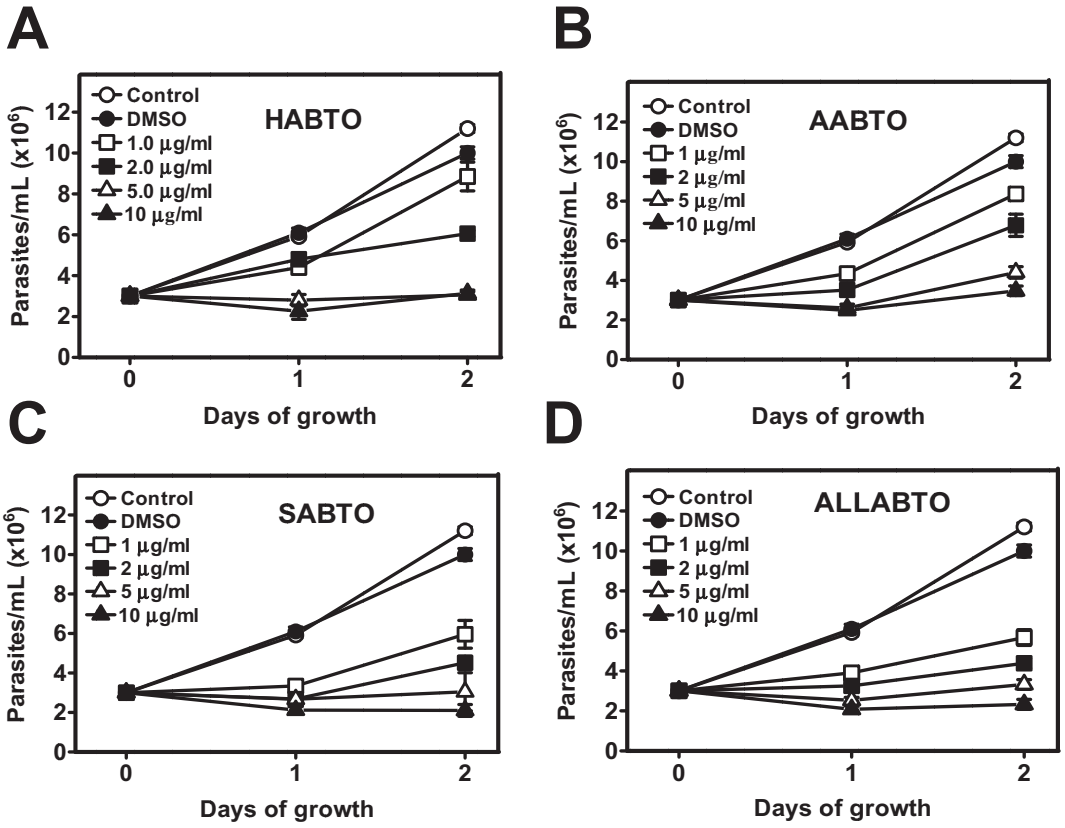

Figure 2. The effect of HABTO (A) and its derivatives AABTO (B), SABTO (C) and ALLABTO (D) on the growth of *T. cruzi* epimastigotes. Parasites were incubated in the absence (control) or in the presence of each compound at the indicated concentrations. Values represent the means ± SD of parasite concentration from four independent experiments.

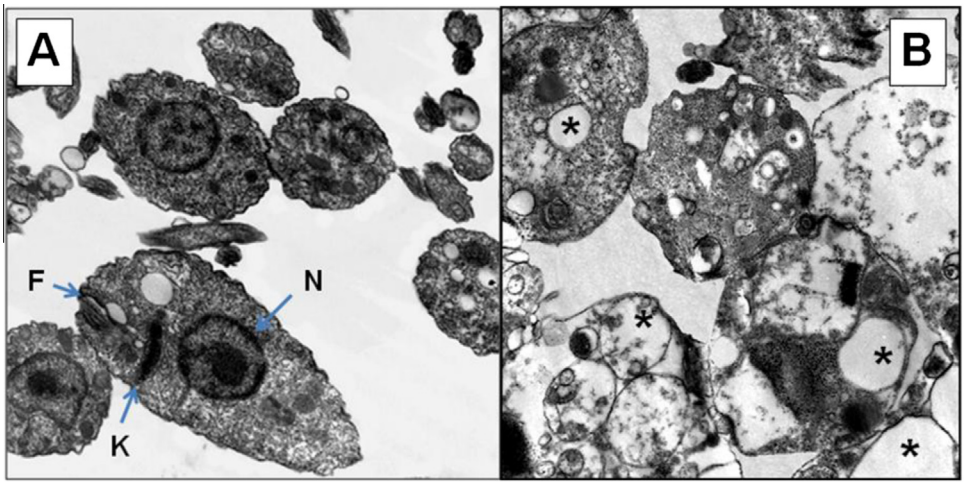

Figure 3. Ultrastructural features of *T. cruzi* epimastigotes grown for 48 h in liquid culture medium, either in the absence (A) or in the presence (B) of 5 μg/mL HABTO. N: nucleus, K: kinetoplast, F: flagellum. Asterisks: vacuolization. Magnification: ×3000 (A); ×3500 (B).

Table 1
Values of *Clogp* of the compounds

Compound	*Clogp*
1	−0.1591
2	0.9227
3	1.8240
4	1.4694

Table 2
Viability of *T. cruzi* epimastigotes after 24 h of treatment with the compounds at the indicated concentrations

Compound	HABTO	AABTO	SABTO	ALLABTO
1 μg/ml	99.2 ± 0.7	99.2 ± 0.7	85.5 ± 0.9	85.5 ± 0.9
2 μg/ml	76.4 ± 1.3	89.5 ± 0.9	49.5 ± 0.9	65.5 ± 0.9
5 μg/ml	49.7 ± 2.5	39.5 ± 2.5	29.5 ± 2.5	29.5 ± 2.5
10 μg/ml	16.3 ± 1.9	16.3 ± 1.9	15.3 ± 1.5	18.3 ± 1.5

Values represent percentages (means) of alive parasites ± SD from four independent experiments.

Table 3
Viability of Vero cells after 24 h of treatment with the compounds at the indicated concentrations

Compound	HABTO	AABTO	SABTO	ALLABTO
1 μg/ml	99.2 ± 0.5	99.8 ± 0.4	99.7 ± 0.5	99.8 ± 0.5
2 μg/ml	99.5 ± 0.7	99.7 ± 0.6	99.6 ± 0.6	99.6 ± 0.6
5 μg/ml	98.8 ± 0.9	99.2 ± 0.8	99.1 ± 0.8	99.1 ± 0.9
10 μg/ml	88.4 ± 1.4	92.4 ± 1.3	83.6 ± 1.5	85.5 ± 1.5

Values represent percentages (means) of alive cells ± SD from four independent experiments.

In the 12-acetoxyl derivative (**2**) an acyl group (R^1) was introduced by treatment of **1** with acetic anhydride and pyridine. The formation of a *O*-trimethylsilyl group in compound **1** was performed with hexamethyldisilazane/trimethylchlorosilane in pyridine to obtain the 12-trymethylsilyloxy derivative (R^2) (**3**). Finally, the alkylation of **1** with allyl bromide by means of NaH in DMF provided the 12-allyloxy derivative (R^3) (**4**). All these derivatives (**2**–**4**) are less polar than the starting material **1** (Fig. 1 and Table 1). We observed that these derivatives were also able to inhibit the growth of epimastigotes (Fig. 2B–D), being the 12-trymethylsilyloxy derivative (**3**) the most cytotoxic to the parasites (Table 2). Interestingly, all the compounds under study exhibited low toxicity to mammalian cells (Table 3).

Taken into account that *Clogp* can be considered as a measure of the hydrophobicity, the values presented in Table 1 suggest that the most lipophilic compound is the 12-trymethylsilyloxy derivative (**3**). It is well known that silyl derivatives are generally more volatile, less polar but more resistant to hydrolysis, because the O–Si bond is relatively stable. Our research group has recently reported that silylethers represent a plausible strategy to introduce lipophilicity in drugs.[23]

Results indicate that the substitution 12-trimethylsilyloxy (SABTO) enhances the compound bioactivity (IC_{50}, 2 μg/mL) probably due to the high lipophilicity that it confers to the molecule, allowing it to enter the parasites easily, and the high resistance to hydrolysis due to the lack of an enzyme in the parasites that can cleave the linkage O–Si. The latter effect is not achieved by the replacement by other groups (12-acetoxyl, 12-allyloxy) (Table 2).

Taking into account these results, we conclude that abietane could be a useful lead compound for the development of semi-synthetic drugs active against *T. cruzi*, that have low toxicity on the hosts cells.

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Supplementary data

Supplementary data (experimental procedures) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.10.082.

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