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SHORT COMMUNICATION

Active sesquiterpene lactones against *Leishmania amazonensis* and *Leishmania braziliensis*

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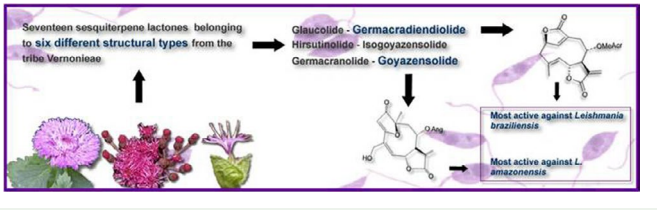
ABSTRACT

Seventeen sesquiterpene lactones (SLs) isolated from five species of the tribe Vernonieae were evaluated for their *in vitro* activity against promastigotes of *Leishmania amazonensis* and *Leishmania braziliensis*. Additionally, a quantitative structure activity relationship has been made, since all these natural compounds were found to have potent to mild antileishmanial properties. The most active compounds against *L. braziliensis* were **16** and **17** (IC_{50} values 1.45 and 1.34 μM, respectively), followed by compound **15** with IC_{50} value of 1.60 μM against *L. amazonensis*. The three glaucolide-type SLs (**4**–**6**) were the least active against both parasites. The computational study allowed us to establish that lipophilicity and polarisability play an important role in the antiparasitic activity. This is the first report of the known germacradiendiolides **16** and **17** from *Elephantopus mollis*. The activity data of the compounds **1**–**17** assayed against *Leishmania* parasites are reported here for the first time.



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1. Introduction

Leishmaniasis is a parasitic disease caused by protozoan species of the genus *Leishmania* of which over 20 are known to be pathogenic to humans. Parasites are inoculated by the bite of infected female phlebotomine sandflies. According to recent WHO reports, 98 countries are affected, comprehending 12 million infected people. The lack of effective antiprotozoal

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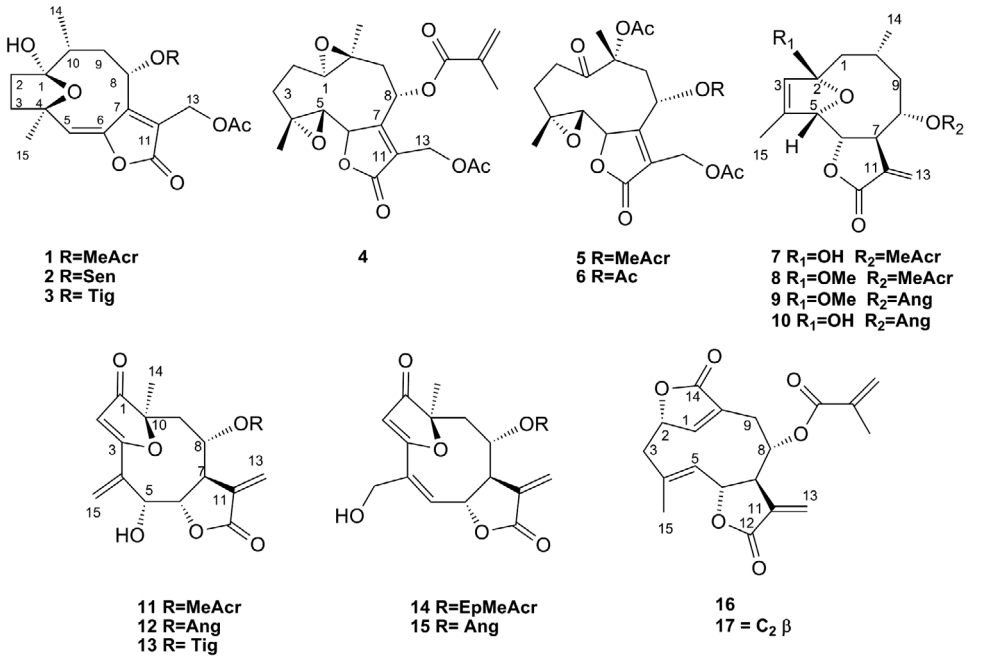

Figure 1. Structures of sesquiterpene lactones **1**–**17**.

drugs has caused a renewed interest in the study of medicinal plants as sources of new chemotherapeutic compounds with better activities and fewer side effects. Many people, who live in endemic areas, rely on traditional medicines for treatment. Over 100 plants have been reported to be active against various forms of leishmanial parasites. Therefore, the search of novel, effective and safe drugs for the treatment of the disease has been a priority for health researchers (WHO 2015). In this work, we isolated a series of highly oxygenated sesquiterpene lactones (SLs), characteristic of the tribe Vernonieae. These natural compounds belong to six structural types: hirsutinolides **1**–**3**, glaucolides **4**–**6**, germacranolides **7**–**10**, isogoyazensolides **11**–**13**, goyazensolide **14** and **15** and germacradiendiolides **16** and **17** (Figure 1). Hirsutinolides (**1**–**3**) and glaucolides (**4**–**6**) carry a C(7)=C(11) endocyclic bond, a C(13)–OAc group and an ester chain at C(8). Compounds **7**–**10**, **11**–**15** possess furanone and exomethylidene-γ-lactone rings in their structures. Compounds **16** and **17** are characteristics metabolites from the subtribe Elephantopodinae and carry two-γ-lactone rings. In the present article, we evaluate and report the leishmanicidal effects of SLs on promastigotes of *Leishmania amazonensis* and *Leishmania braziliensis*. In addition, a quantitative structure activity relationship study of the selected SLs from the New World Asteraceae was carried out.

2. Results and discussion

2.1. Promastigotes inhibition by SLs

Natural products have been systematically studied against *Leishmania* parasites and demonstrated a promising alternative due to their antileishmanial potential, not only obtained from plants, such as the rich source of SLs, *Tithonia diversifolia* (de Toledo et al. 2014), but

also from marine organisms, such as the *Laurencia dendroidea*, a rich sesquiterpene red alga (da Silva Machado et al. 2011) and recently from the brown propolis prepared by *Apis mellifera* (Santana et al. 2014). In the present study, all the SLs tested showed moderate to high antiparasitic activity for *L. amazonensis* and *L. braziliensis*, being the 65% of the products more effective for the control of *L. braziliensis*.

The IC_{50} values of SLs **1**–**17** on the promastigote forms are displayed in Table S1. As can be seen, the best leishmanicidal effects were exerted by compounds **16** and **17**, mainly against *L. braziliensis* (IC_{50} values of 1.45 and 1.34 μM, respectively), followed by compound **15** (IC_{50} value of 1.60 μM) against *L. amazonensis*. Compounds **8**, **10**, **11** and **12** display a high activity against both strains with IC_{50} values between 2.80 and 8.30 μM. Compounds **1**–**3**, **13** and **14** showed significant activities with IC_{50} values in the range of 5.70–14.1 μM towards both strains. Finally, the three glaucolide-type SLs tested (**4**–**6**) were the least active compounds against both promastigote forms (IC_{50} range values 23.4–47.8 μM). The results showed that compounds **16** and **17** were about two times more potent than the **15** (IC_{50} value of 2.94 μM) and **8** (IC_{50} value of 2.76 μM) towards *L. braziliensis*. This significant activity may be related to the presence of two lactone rings in their structures. It is well known that molecules possessing at least one lactone ring function can act as antiparasitic (Schmidt et al. 2009). Furthermore, compound **15** was about six and four times more potent than **14** with IC_{50} values of 12.5 μM for *L. amazonensis* and 10.4 μM for *L. braziliensis,* respectively. The difference may be due to the presence of the angelate ester side chain at C(8).

Much of the biological activities displayed by the SLs are attributed to the exomethylidene-γ-lactone ring which may react with nucleophiles, especially sulfhydryl groups of proteins, by a Michael-type addition reaction (Kupchan et al. 1971). Although the possibility of reacting with sulfhydryl groups in different environments may increase with steric flexibility, compounds **4**–**6** with a flexible macrocycle skeleton and a γ-lactone ring with a C(13) allylic acetate function, showed the weakest leishmanicidal activity. Differences in molecular conformation may affect both steric accessibility of Michael addition sites and lipophilicity (Beekman et al. 1997). While the presence of the exomethylidene-γ-lactone moiety is often considered essential is not enough for the leishmanicidal activity. Therefore, we conclude that the activity depends on a combination of structural features of the compound, including the type of the germacranolide skeleton and the functional groups.

Isodeoxyelephantopin (**16**) and deoxyelephantopin (**17**) have been isolated previously (Zhang et al. 1986), but this is the first report from *Elephantopus mollis.*

2.2. Computational analysis

Results obtained from the quantitative structure–activity study showed that descriptors related to the partition coefficient (LogP) and polarisability (bpol) best correlated with the experimental activities. LogP is an indicator of the lipophilic character of a molecule, and it has a strong influence on ADME (absorption, distribution, metabolism and excretion) properties of drugs. For this study, CLogP (based on the fragmental method developed by Leo & Hansch 1979) and ALogP (based on atomic contributions) were used. Both descriptors provide values of the same property and only differ from one to another on the algorithms used to calculate LogP (Medić-Šarić et al. 2004). Figures S1a and S1b show correlations between LogP values and the IC_{50} obtained for *L. amazonensis* and *L. braziliensis*. As can be seen, CLogP and ALogP correlated with the experimental activity through a parabolic function

with R^2 values greater than 0.77, resulting better predictive models for *L. amazonensis* than *L. braziliensis* according to the R^2 values (Table S2).

The quadratic relationship between LogP and the biological activity indicates that there is an optimum range of LogP (all around a value of **2**) beyond which, increasing or decreasing hydrophobicity causes a loss in the activity. This fact can be explained as a poor absorption or permeation on the biological system due to inadequate range of hydrophobicity, which suggests that an optimun balance between hydrophilic and lipophilic properties is most convenient. The most active compounds against the two strains (**8**, **10**, **12**, **15**–**17**) showed LogP values between **1** and **3**, suggesting that this would be the range of hydrophobicity in which SLs best display their antiprotozoal effects.

It is also possible to notice that the less active compounds (**4**–**6**) against both parasite strains are in turn the most 'hydrophilic' (LogP values <1) according to the data obtained for CLogP and ALogP (Figures S1a and S1b, respectively).

These findings are in agreement with previous reports in which the contribution of LogP to the *in vitro* antiprotozoal activity was studied (Daunes & D'Silva 2001). LogP values of compounds showed a correlation predominantly parabolic with the antiparasitic activity showing an optimum range of LogP values. Then, the activity is controlled to some degree by their ability to enter cells.

Regarding the atomic polarisability of the molecules (Figure S1c), compounds **16** and **17** with bpol values of 34.9 and 32.5 cm^3, respectively, proved to be the most active against *L. braziliensis* with IC_{50} values <3.30 μM. Compound **15** was the most active against *L. amazonensis* with a bpol value of 34.8 cm^3. As can be seen in Figure S1, the quadratic function showed the best correlation between the antiparasitic activity and the bpol values calculated for the SLs with R^2 values of 0.83 and 0.80 for *L. amazonensis* and *L. braziliensis,* respectively (Table S2). It is noteworthy that there is a short range of polarisability values (30–40 cm^3) in which the compounds display their best antiparasitic activity. The quadratic relationship for bpol is in agreement with the above mentioned for LogP considering that both descriptors (LogP and bpol) are closely related as follows: the partition coefficient is a function of the solvation energy which is made up of a number of components where the most important are the electrostatic and polarisation energies, the latter being a linear function of polarisability (Lewis 1989). On the other hand, polarisability plays an important role not only on compounds' penetration through the cellular membrane but also on the ligand–receptor recognition where the Van der Waals interactions are involved. From the above mentioned, it is possible to conclude that for the studied SLs, both closely related properties (lipophilicity and polarisability) need optimum values inside a range and they have a strong influence on the antiparasitic activity.

3. Conclusions

Our study, focus on the detection of plant secondary metabolites with leishmanicidal activity, has been done using the promastigote form of the parasite because it is easier to maintain under *in vitro* conditions. Since the promastigote is not the infective form of the parasite in vertebrate hosts, evaluations done with promastigotes have only an indicative value of the possible leishmanicidal activity of the SLs tested. However, our results of structure–activity relationship reported herein on the series of the 17 SLs can be useful in the design of new

antiparasitic drugs with the structural, topological, geometric and/or electronic requirements associated with such activity.

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