Diacetal Ditellurides as Highly Active and Selective Antiparasitic Agents toward *Leishmania amazonensis*

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Supporting Information

ABSTRACT: Leishmaniasis is a neglected tropical disease and a public health concern in at least 98 countries, affecting mainly the poorest populations. Pharmaceuticals and chemotherapies available for leishmaniasis treatment have several limitations, which clearly justify the efforts to find new potential antileishmanial drugs. In this context, antiprotozoal activities toward different *Leishmania* species have been reported for hypervalent tellurium compounds, which motivated us to investigate, for the first time, the leishmanicidal properties of some nonhypervalent diaryl ditellurides. Thus, this work describes *in vitro* activity against *Leishmania amazonensis* and the cytotoxicities of diaryl ditellurides. Ditelluride **LQ7** revealed a strong leishmanicidal activity on promastigotes and amastigotes at submicromolar levels ($IC_{50} = 0.9 \pm 0.1$ and $0.5 \pm 0.1 \mu\text{mol L}^{-1}$, respectively) and presented selectivity indexes greater than those of reference drug miltefosine. This preliminary study suggests that diaryl ditellurides may be promising scaffolds for the development of new agents for leishmaniasis treatment.

KEYWORDS: Organotellurium chemistry, ditellurides, antiparasitic agents, neglected tropical disease, *Leishmania amazonensis*

Leishmaniasis is a parasitic infection caused by more than 20 species of protozoans of the genus *Leishmania*, which is transmitted to mammals via the bite of infected female sandflies. According to the World Health Organization (WHO), leishmaniasis is endemic in at least 98 tropical and temperate countries, both developed and developing, and it is considered to be a neglected tropical disease. Furthermore, about 1.5–2 million new cases occur annually, resulting in approximately 70 000 deaths per year, thus representing a public health concern.¹ Clinical manifestations of leishmaniasis are divided into visceral, cutaneous, or mucocutaneous forms. Among these, cutaneous leishmaniasis remains the most widespread manifestation of the disease, of which one of the etiological agents in South America is *Leishmania amazonensis*.

Leishmania parasite has demonstrated a complex life cycle which alternates in two distinct stages: promastigote, which is the extracellular form found in the female sandfly vectors, and amastigote, the intracellular forms that replicates in the mammalian hosts and are responsible for the clinical symptoms. This complex biology of *Leishmania* parasite makes the development of new antileishmanial agents highly challenging.² Currently, leishmaniasis treatment drugs include the pentavalent antimonials, represented by pentostam and glucantime. Amphotericin B is recommended as a second-line therapy. Additionally, all of these drugs must be administered by parenteral route and usually exhibit variable or poor efficacy, high toxicity, and relatively long periods of administration, which hinder the continuity of treatment.³ Miltefosine, the first oral drug available for leishmaniasis treatment, is generally more effective, but some factors like renal toxicity, potential teratogenicity, and high cost limit the

access and popularization.^{4,5} Because drug-resistant *Leishmania* parasite emergence has increased significantly and conventional chemotherapies have several limitations,^{6,7} the relevance of research on new potential antileishmanial drugs is easily justified.

In this context, antiprotozoal activities of well-known electrophilic organic and inorganic hypervalent tellurium compounds (telluranes) have been reported for different *Leishmania* species (Figure 1). **RT01** was evaluated against *L. amazonensis* promastigotes and presented IC_{50} value of approximately $4 \mu\text{mol L}^{-1}$ ($2 \mu\text{g mL}^{-1}$).⁸ *In vitro* and *in vivo* activities of **RF07** were determined against *L. chagasi* amastigotes and showed inhibitory effect at submicromolar concentrations ($IC_{50} = 0.5 \mu\text{mol L}^{-1}$).⁹ *In vitro* efficacy of inorganic tellurane **AS101** against *L. donovani* promastigotes has been recently reported with IC_{50} value of $27 \mu\text{mol L}^{-1}$.¹⁰

Among the well-known classes of organotellurium compounds, the largest studied set are diaryl ditellurides (RTeTeR , $\text{R} = \text{aryl}$) because most of them are solid, almost odorless, stable to air or/and light and, consequently, easily handled, unlike their repulsive dialkyl derivatives ($\text{R} = \text{alkyl}$).¹¹ Moreover, these species are versatile because they have dual behavior as nucleophiles or electrophiles, depending on the nature of the other species involved.

Toxicity data on organotellurium compounds are still scarce in the current literature.¹² (Di)tellurides have been reported as

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Figure 1. Organic and inorganic tellurium compounds with antileishmanial activities.

highly toxic agents to the central nervous system of rodents¹³ and as potential inhibitors of squalene monooxygenases¹⁴ but also as presenting a broad range of biological applications,¹⁵ including antioxidant,¹⁶ antibacterial,¹⁷ antifungal,¹⁸ anti-cancer,¹⁹ and anti-inflammatory²⁰ properties. To the best of our knowledge, there has been no investigation about antileishmanial activities of diaryl ditellurides. Thus, we report *in vitro* leishmanicidal activity of some diaryl ditellurides (**LQ1**, **LQ6**, **LQ7**, and **LQ8**) against *Leishmania amazonensis* (promastigotes and amastigotes) and cytotoxicity on non-infected macrophages.

To evaluate the effect of substitution pattern, *ortho* (**LQ8**), *meta* (**LQ6**), and *para*-substituted (**LQ7**) diaryl ditellurides were prepared according to literature (Scheme 1).^{21,22} First,

Scheme 1. Synthesis of the Diaryl Ditellurides LQ1 and LQ6–8 from the Corresponding Brominated Precursors

bromoacetophenones were transformed into their corresponding cyclic acetals (2,2-dimethyl-1,3-dioxolanes), which was performed with ethylene glycol and *p*-toluenesulfonic acid (*p*TSA) in 69–98% isolated yields (Scheme 1).²³ Incorporation of acetal moiety was considered for two main reasons: they are compatible with basic conditions during the second step (use of organomagnesium and organolithium compounds, followed by oxidation in water)²⁴ and cyclic acetal-containing substances have been described to have promising biological activities, including antileishmanial properties.²⁵

Meta (1,2-bis(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)-ditellane, **LQ6**) and *para* (1,2-bis(4-(2-methyl-1,3-dioxolan-2-yl)phenyl)-ditellane, **LQ7**) ditellurides were obtained as almost odorless red solids and were synthesized in 67 and 59% yields, respectively, by reaction between proper brominated precursor and magnesium, followed by elemental tellurium insertion and subsequently air oxidation (Scheme 1). This approach was not efficient to *ortho*-substituted derivatives; therefore, **LQ8** was

synthesized by reaction of aryl lithium acetal (generated by bromine–lithium exchange) with elemental tellurium followed by air oxidation to give the product 1,2-bis(2-(2-methyl-1,3-dioxolan-2-yl)phenyl)-ditellane as almost odorless yellow solid in 51% yield (Scheme 1). Diphenyl ditelluride (**LQ1**) was also prepared by reaction between phenylmagnesium bromide and elemental tellurium (Scheme 1) to evaluate the influence or necessity of substituents in benzene ring in further biological activity assays. All structures were characterized by ¹H and ¹³C NMR, and the insertion of tellurium was confirmed by ¹²⁵Te NMR analyses. Spectroscopic data (¹H, ¹³C, and ¹²⁵Te NMR) of compounds are in agreement with literature²⁰ data and are available in the Supporting Information.

Subsequently, **LQ1**, **LQ6**, **LQ7**, **LQ8**, and miltefosine were assayed against *L. amazonensis* promastigotes (extracellular forms), and leishmanicidal activities were expressed as IC_{50} values, defined by the inhibitory concentration able to kill 50% of the parasites in relation to the negative control (Table 1).

Table 1. *In Vitro* Activity, Cytotoxicity, and Selectivity Index of LQ1, LQ6, LQ7, LQ8, and Miltefosine^a

entry	substance	promastigote IC_{50}^b ($\mu\text{mol L}^{-1}$)	macrophage CC_{50}^c ($\mu\text{mol L}^{-1}$)	SI ^d
1	LQ1	1.4 ± 0.3	59 ± 9	42
2	LQ6	0.9 ± 0.2	22 ± 4	25
3	LQ7	0.9 ± 0.1	177 ± 5	194
4	LQ8	8.0 ± 1.4	19 ± 1	2
5	miltefosine	20.7 ± 0.2	55 ± 2	3

^aData are expressed as the mean $\pm$ SD determined from three different experiments. ^b IC_{50} : the concentration required to give 50% inhibition. ^c CC_{50} : against macrophages after 72 h. ^dSelectivity index: CC_{50}/IC_{50} .

The cytotoxic concentration on macrophage cell line J774A1, represented by the CC_{50} index (cytotoxic concentration able to kill 50% of the cells in relation to the untreated control), was also measured to establish the selectivity of tested compounds. Furthermore, the selectivity index (SI) was determined for each tested compound as the ratio between the CC_{50} and the corresponding IC_{50} value in promastigotes (Table 1).

Biological assays evidenced that all screened diaryl ditellurides exhibited activities against *L. amazonensis* promastigotes greater than those of reference miltefosine (Table 1). The most active substances were *meta*- (**LQ6**) and *para*-substituted (**LQ7**) diaryl ditellurides with submicromolar IC_{50} values of 0.9 ± 0.2 and $0.9 \pm 0.1 \mu\text{mol L}^{-1}$ (corresponding to about $0.53 \mu\text{g mL}^{-1}$, entries 2 and 3, Table 1), respectively,

about 20-fold more active than miltefosine ($IC_{50} = 20.7 \pm 0.2 \mu\text{mol L}^{-1}$, corresponding to $8.15 \mu\text{g mL}^{-1}$, entry 5, Table 1). Diphenyl ditelluride (LQ1), the unsubstituted derivative, was slightly less active ($IC_{50} = 1.4 \pm 0.3 \mu\text{mol L}^{-1}$, entry 1, Table 1) than disubstituted analogues LQ6 and LQ7. This result may indicate that the presence of substituents on the aromatic ring did not have a pivotal role in antileishmanial effect. However, when *ortho*-substituted analogue LQ8 was evaluated against *L. amazonensis* promastigotes, it presented $IC_{50} = 8 \pm 1 \mu\text{mol L}^{-1}$ (entry 4, Table 1), which was about 8-fold less active than LQ1, LQ6 and LQ7, indicating that *ortho*-substitution pattern led to a decrease in antileishmanial activity. Despite the lower antileishmanial potential, it is worth noting that LQ8 presented activity about threefold higher than miltefosine.

Because preliminary *in vitro* evaluation demonstrated that LQ6–8 have significant inhibitory effect on *L. amazonensis* promastigotes, they were evaluated through determination of cytotoxicity on host macrophages. The most selective substance was *para*-substituted ditelluride LQ7, which presented a CC_{50} of $177 \pm 5 \mu\text{mol L}^{-1}$ and a SI of 194 (entry 2, Table 1). In other words, LQ7 was about 194-fold more selective for *L. amazonensis* protozoa than healthy reference cells. Moreover, LQ7 was much less toxic to host macrophages than the reference, exceeding SI of miltefosine by 74-fold (entry 5, Table 1).

The second most selective substance was unsubstituted derivative LQ1 with $CC_{50} = 59 \pm 9 \mu\text{mol L}^{-1}$ and SI = 42 (entry 1, Table 1), which was also less toxic to host cells than miltefosine. LQ1 presented selectivity index lower than that of LQ7 (SI = 194, entry 5, Table 1), and this result suggests that *para*-substituted pattern has a pivotal role in the cytotoxic potential of screened diaryl ditellurides. This hypothesis was confirmed when CC_{50} values of *meta*- (LQ6) and *ortho*-substituted (LQ8) analogues were determined. Despite being highly selective, LQ6 and LQ8 presented cytotoxicity ($CC_{50} = 22 \pm 5$ and $19 \pm 1 \mu\text{mol L}^{-1}$, entries 2 and 4, Table 1, respectively) higher than that of *para*- (LQ7) and unsubstituted (LQ1) analogues, which implied in lower selectivity indexes (SI = 25 and 2, Table 1). Notwithstanding the minor selectivity, LQ6 was about 10-fold more selective than miltefosine. LQ8 was the least selective substance, suggesting that the *ortho* substitution pattern has a negative influence on toxicity of diaryl ditellurides.

The effects of substituents in *ortho* position in aromatic tellurium compounds have been studied extensively since 1970.²⁶ It has been found that tellurium frequently interacts with a nearby heteroatom (O, N, S, P), producing *quasi*-cyclic systems. This phenomenon can be explained by assuming a noncovalent interaction between tellurium and a lone pair of electrons from the donor heteroatom, which interact with the antibonding orbital of tellurium atom ($\sigma^* \text{Te-R}$).²⁷ Because the oxygen atom in acetal group is capable of coordination with the tellurium in *ortho* position, an enrichment of electronic density in tellurium can be observed in LQ8. The existence of the noncovalent intramolecular interaction $O \rightarrow \text{Te}$ was clearly evidenced by ^{125}Te NMR analysis (Figure 2), where *ortho*-substituted compound LQ8 presented chemical shift ($\delta_{\text{Te}} = 369 \text{ ppm}$) lower than those of LQ7 ($\delta_{\text{Te}} = 408 \text{ ppm}$) and LQ6 ($\delta_{\text{Te}} = 426 \text{ ppm}$), *para*- and *meta*-substituted, respectively. This noncovalent interaction causes an increase in electronic density in tellurium in LQ8, justifying the greater shielding and, therefore, the lowest chemical shift when

Figure 2. ^{125}Te NMR spectra of diaryl ditellurides LQ6, LQ7, and LQ8 (126.2 MHz, PhTeTePh ($\delta = 422$), 30°C)

compared to derivatives LQ6 and LQ7, which do not have this kind of intramolecular interaction.

Leishmania parasites have been described as containing high levels of thiol-dependent enzymes, for example cathepsin B, a cysteine protease which has been found to be involved in all the life cycle stages of the parasites.²⁸ Electrophilic hypervalent organotellurium compounds have also been described as potent inhibitors of thiol-dependent cysteine protease, including cathepsins.²⁹ Furthermore, Barbiéri and coworkers described a possible relationship between the leishmanicidal effect of organotelluranes and cathepsin B inhibition based on *in vivo* studies.⁹ Ditellurides present well-known dual nucleophilic or electrophilic behavior which allows us to propose ditellurides as electrophiles in inhibition of some thiol-dependent enzymes of *L. amazonensis*. This hypothesis could also explain the decrease in activity observed for LQ8 because Te atoms in this ditelluride are richer in electrons than LQ6 and LQ7. Another point to be considered is steric hindrance intrinsic to *ortho* position.

Subsequently, the effect of the most active substance LQ7 on the intracellular amastigotes was investigated (Table 2). In

Table 2. *In Vitro* Activity, Cytotoxicity, and Selectivity Index of LQ7 and Miltefosine against Intracellular Amastigote^as

entry	substance	amastigote IC_{50} ($\mu\text{mol L}^{-1}$)	macrophage CC_{50} ($\mu\text{mol L}^{-1}$)	SI ^b
1	LQ7	0.5 ± 0.1	8.3 ± 0.2	18
2	miltefosine	1.7 ± 0.1	20.3 ± 0.5	12

^aData are expressed as the mean $\pm$ SD determined in three different experiments. ^b CC_{50}/IC_{50} .

in vitro evaluation demonstrated that LQ7 has significant inhibitory effect against amastigote infection on *L. amazonensis* at submicromolar concentration ($IC_{50} = 0.5 \pm 0.1 \mu\text{mol L}^{-1}$, Table 2, entry 1). Although the cytotoxicity of LQ7 was higher than that of the reference drug ($CC_{50} = 8.3 \pm 0.2$ and $20.3 \pm 0.5 \mu\text{mol L}^{-1}$, Table 2, entries 1 and 2, respectively), the SI value (18, entry 1, Table 2) demonstrated that this diaryl ditelluride has great potential as an antileishmanial agent.

Considering a possible hydrolysis of ketal group under the assays conditions, corresponding acetyl derivative LQ64 was prepared through the deprotection of LQ7 under acid conditions and reflux (Scheme 3, see Supporting Information), and then its cytotoxicity was evaluated (Table 3). The CC_{50} value of LQ64 ($53 \pm 2 \mu\text{mol L}^{-1}$) was lower than that observed for LQ7 ($177 \pm 5 \mu\text{mol L}^{-1}$), indicating that this compound is more toxic to healthy cells than LQ7. This result suggests that the compound LQ7 does not suffer alterations in

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