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Pre-clinical evidences of the antileishmanial effects of diselenides and selenocyanates

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ABSTRACT

A series of thirty-one selenocompounds covering a wide chemical space was assessed for *in vitro* leishmanicidal activities against *Leishmania infantum* amastigotes. The cytotoxicity of those compounds was also evaluated on human THP-1 cells. Interestingly most tested derivatives were active in the low micromolar range and seven of them (**A.I.3**, **A.I.7**, **B.I.1**, **B.I.2**, **C.I.7** **C.I.8** and **C.II.8**) stood out for selectivity indexes higher than the ones exhibited by reference compounds mitelfosine and edelfosine. These leader compounds were evaluated against infected macrophages and their trypanothione reductase (TryR) inhibition potency was measured to further approach the mechanism by which they caused their action. Among them diselenide tested structures were pointed out for their ability to reduce infection rates. Three of the leader compounds inhibited TryR effectively, therefore this enzyme may be implicated in the mechanism of action by which these compounds cause their leishmanicidal effect.

Leishmaniasis is one tropical fatal disease caused by different species of protozoa belonging to the genus *Leishmania*. It is transmitted to humans by the bite of female phlebotomine and it is endemic in 98 countries. In spite of the existance of around 53 species of *Leishmania,* only 20 are capable of infecting humans[1].

Leishmaniasis can present three main clinical forms: cutaneous, which is the most common and causes skin sores; visceral or kala-azar, which is the most serious form and affects several internal organs (spleen, liver and bone marrow) and mucocutaneous, which destroys the mucous membranes of nose, mouth and throat[2–4]. Drugs including pentavalent antimonial derivatives such as Pentostam® and Glucantime® or more recently amphotericin B, miltefosine, paramomycin, sitamaquine and edelfosine have several complications such as toxicity, side effects, low efficacy, drug resistances and high costs, among others[5–9]. These reasons reinforce the urgent need of new treatments and new targets that must be explored for treating this illness[10,11].

In the search of new drugs growing evidence suggests a connection between selenium (Se) and parasites, particularly trypanosomatids. In fact, some parasites have been proven to express selenoproteins and metabolize Se[12,13]. This indicates the potential importance of Se as a privileged chemical element to design new leishmanicidal agents. In this context, in the past years, our research group has reported a set of organoselenium compounds with this activity [14–21]. Different chemical frameworks have been explored such as selenocyanate, diselenide, selenourea and methylseleno moieties. Among these series new antikinetoplastid derivatives bis(4-aminophenyl)diselenide and 4-aminophenylselenocyanate exhibited interesting properties as antiparasitic agents[18]. With these observations in mind, we decided to modulate bis (4-aminophenyl)diselenide and 4-aminophenylselenocyanate with different derivatizations (Fig. 1) with the aim to improve pharmacological properties obtaining maximal 3D structural enrichment as well as providing us with information on the effects of electrostatic modulation. Acylselenoureas (**Series A**) have been included due to their recently described presence in structures with potent leishmanicidal activity [20].

The presence of amide groups in **Series C** as a result of the reaction of the parent compounds with anhydrides provided us with a carboxylic residue that enabled us to explore the formation of imides (**Series B**). The synthetic pathway followed to yield those derivatives is described in Scheme 1.

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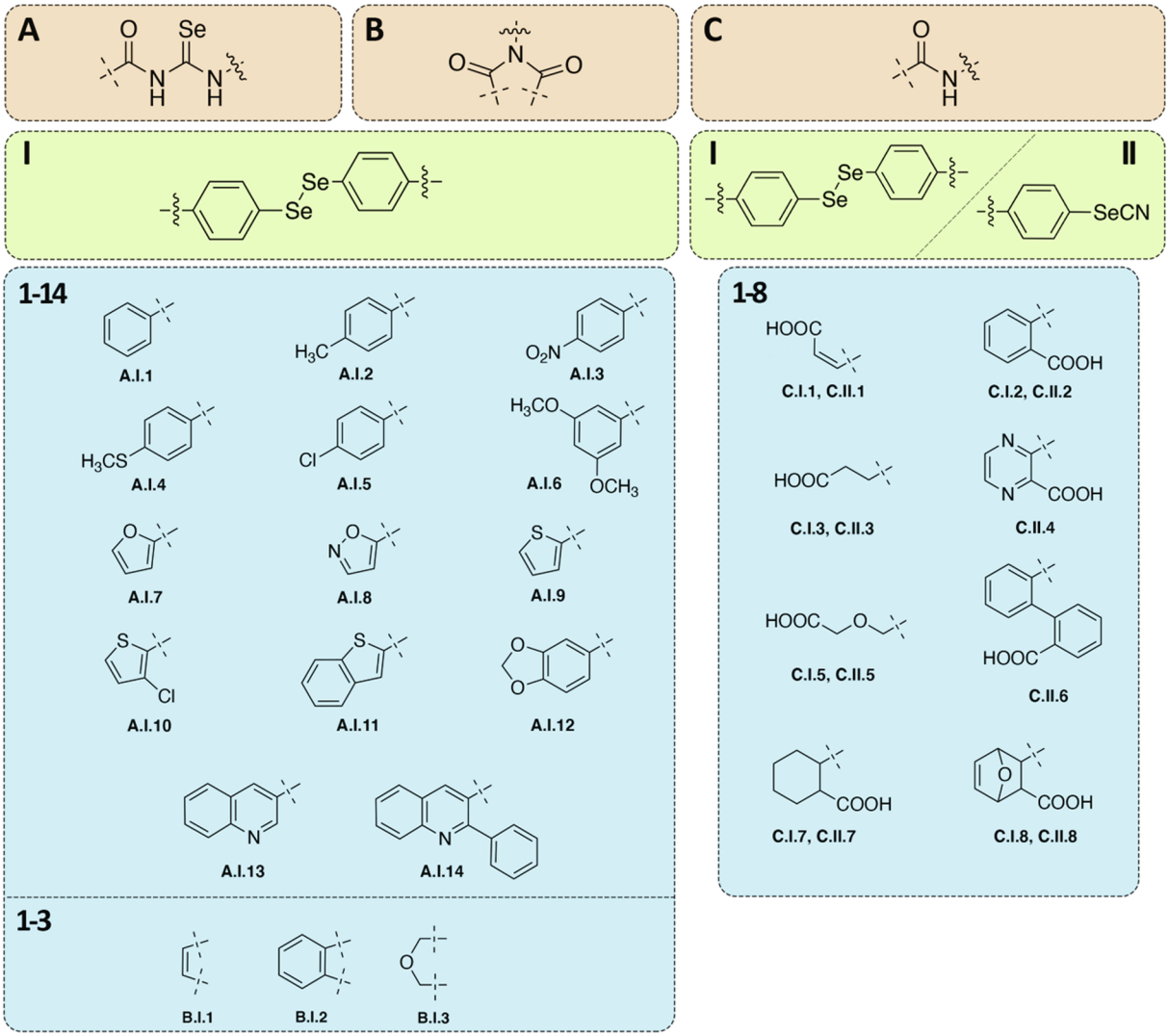
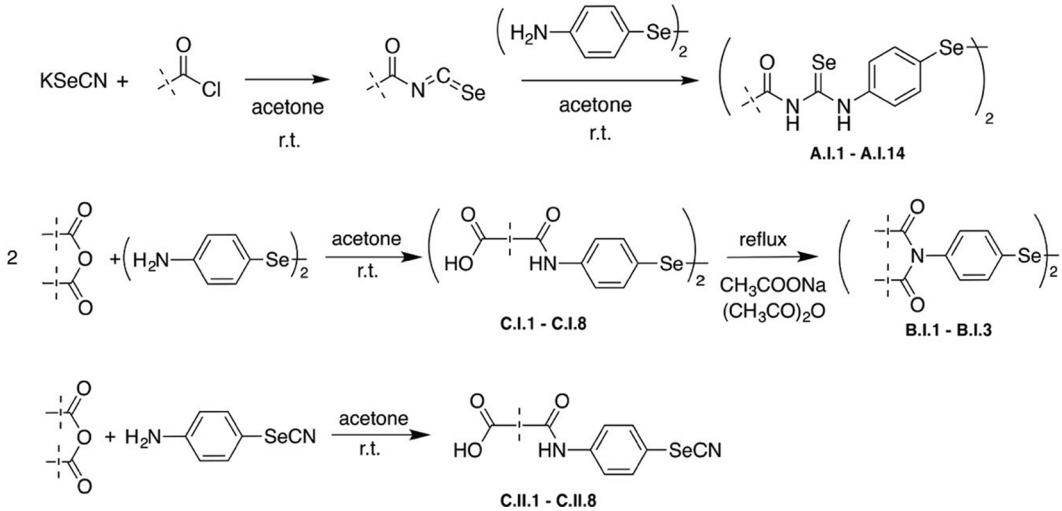


Fig. 1. Structures for the Se-containing derivatives tested in this work.



Scheme 1. General procedure of synthesis.

Among the potential targets for the treatment of leishmaniasis we focused on the redox regulatory systems. Parasites are highly sensitive to reactive oxygen species (ROS), which are produced under conditions of oxidative stress. In this sense, all *Trypanosomatids*, including *Leishmania*, present diverse regulatory pathways to protect them from the oxidative damage. Trypanothione Reductase (TryR) is one of the

most relevant and studied enzymes that plays an important role in the survival of the parasite, due to its implication in the redox system. This enzyme catalyzes the reduction of trypanothione disulfide to trypanothione. Due to its absence in the human host, TryR is considered a promising target for the development of new drugs that improve the efficacy, potency and safety of the reference drugs such as miltefosine and edelfosine[14,16,22]. On the basis of the chemical analogy of sulfur and selenium, we decided to explore diselenide and selenocyanate derivatives to generate new TryR inhibitors.

In this work, we assess the antileishmanial effect of a library of thirty-one organoseleno compounds (Fig. 1) against *Leishmania infantum* axenic amastigotes as well as the cytotoxicity against THP-1 cells in order to evaluate their selectivity. Furthermore, the most active compounds were evaluated in *L. infantum*-infected macrophages and have been tested to study the potential role played by trypanothione reductase as putative target.

The synthesis of this second-generation derivatives followed two different synthetic pathways. Briefly, the formation of acylselenoureas (**Series A**) involved acylisoselenocyanates as intermediates that were then reacted with bis(4-aminopheny)diselenide following a synthetic pathway previously described by our research group[23]. On the other hand, derivatives containing amide acids (**Series C**) or imides (**Series B**) were formed by the reaction of symmetric anhydrides with either bis (4-aminophenydiselenide) to yield compounds coded **C.I.** or 4-aminophenyselenocyanate for derivatives coded **C.II** [24]. The structure of these derivatives was determined by microanalysis, ^{1}H and ^{13}C NMR spectroscopy, infrared spectroscopy and mass spectrometry and met the required purity to be tested in order to assess their biological activity.

The leishmanicidal activity of thirty-one synthesized Se-containing compounds was analyzed against *Leishmania Infantum* axenic amastigotes at 24 h treatment time. This form of the parasite was selected as amastigotes are responsible for clinical manifestations in humans. Miltefosine and edelfosine were used as reference drugs. Three independent experiments were performed according to a previously described procedure[14] and results are expressed as IC_{50} values in Table 1. Selectivity is a compulsory property for any antileishmanial drug as toxicity in human cells is a non-desired effect in drug development. In order to asses this factor, cytotoxicity obtained after 24 h of exposure to the compounds in THP-1 cells was tested according to previously described procedure[14]. Selectivity index (SI) was calculated as the ratio of IC_{50} value obtained for THP-1 cells and IC_{50} values obtained for *L. infantum* amastigotes.

Data obtained revealed potent bioactivity for seven of the analyzed compounds (**A.I.3**, **A.I.7**, **A.I.8**, **B.I.1**, **B.I.2**, **C.I.7** and **C.I.8**) which met the threshold stated by the reference drug miltefosine (IC_{50} = 2.84 μM) in terms of potency. Derivatives **A.I.7** and **A.I.8** even exceeded the edelfosine standard for this assay (IC_{50} = 0.82 μM). Interestingly, only diselenide derivatives matched or surpassed the potency obtained for the reference drugs. The results found for compounds **A.I.7** and **A.I.8** were comparable to or lower than those obtained for bis(4-aminophenyl)diselenide (IC_{50} = 0.65 μM), common fragment for diselenide derivatives[18]. If we make the corresponding comparison of selenocyante derivatives with their parent drug 4-aminophenylselenocyanate (IC_{50} = 9.29 μM)[18], compound **C.II.8** stood out for the significant antikinetoplastid activity (see Table 2).

Low toxicitiy is required for a good antileishmanial drug, consequently IC_{50} values obtained for THP-1 and SI have to carefully be observed in order to satisfy this condition. Compounds **A.I.3**, **A.I.7**, **B.I.1**, **B.I.2**, **C.I.7**, **C.I.8** and **C.II.8** outperformed miltefosine and edelfosine (SI = 6.51 and 6.01 respectively) in terms of selectivity (Table 1).

aking into consideration both potency and selectivity, seven derivatives (**A.I.3**, **A.I.7**, **B.I.1**, **B.I.2**, **C.I.7**, **C.I.8** and **C.II.8**) were selected to be assayed in amastigote-infected THP-1 cells. Compound **A.I.3** presented reproducibility issues under the conditions used for this assay and therefore had to be discarded. All tested compounds reduced infection significantly at 3 μM concentration after 48 h treatment time as shown in Fig. 2. Compounds **A.I.7**, **B.I.2** and **C.I.8** reduced infection rates to values under the ones obtained for the treatment of the reference drug edelfosine. Five of the tested compounds correspond to diselenide derivatives, all of them exhibited infection reduction potential in the range of the one found for edelfosine. On the other hand, selenocyanate derivative **C.II.8** did not meet this standard, again this fact pointed to diselenide moiety over selenocyanate in general terms of potential as leishmanicidal structures.

To further determine the potential mechanism of action of the six leader compounds we assessed their ability to inhibit TryR. Six different concentrations ranging from 75 to 0.1 μM were screened. Mepacrine was used as a positive control due to its proven TryR inhibition properties. Three out of the six tested compounds (**A.I.7**, **B.I.1** and **C.II.8**) exceeded inhibition potency of mepacrine[25], compounds **B.I.1** and **C.II.8** were 30-fold more active than the positive control. The remaining tested compounds **B.I.2**, **C.I.7** and **C.I.8** showed IC_{50} values in the same order of magnitude as the positive control mepacrine. When compounds were ranked following their IC_{50} for this inhibition assay

Table 1
IC_{50} values for compounds on amastigotes and cytotoxic activity on THP-1 cell line.

Code	IC_{50} (mean ± SEM), μM		SI
	Amastigotes	THP-1	
A.I.1	> 25	> 25	n.d.
A.I.2	15.03 ± 0.78	> 25	> 1.66
A.I.3	2.27 ± 0.91	> 25	> 11.01
A.I.4	13.48 ± 2.21	> 25	> 1.85
A.I.5	> 25	> 25	n.d
A.I.6	9.22 ± 0.95	> 25	> 2.77
A.I.7	0.67 ± 0.15	7.44 ± 1.47	11.19
A.I.8	0.35 ± 0.06	1.07 ± 0.07	3.10
A.I.9	4.55 ± 0.71	22.16	4.87
A.I.10	5.57 ± 0.80	> 25	> 4.48
A.I.11	14.6 ± 0.90	> 25	> 1.71
A.I.12	9.98 ± 0.75	13.45 ± 1.26	1.35
A.I.13	3.08 ± 0.43	12.77 ± 1.50	4.15
A.I.14	6.18 ± 0.19	8.50 ± 0.40	1.34
B.I.1	1.54 ± 0.25	15.23 ± 2.28	9.91
B.I.2	2.07 ± 0.21	22.97 ± 1.16	11.08
B.I.3	10.40 ± 0.21	8.53 ± 1.36	0.82
C.I.1	> 25	> 25	n.d.
C.I.2	4.57 ± 0.99	> 25	> 5.47
C.I.3	7.9 ± 0.23	> 25	> 3.16
C.I.5	12.10 ± 1.72	> 25	> 2.06
C.I.7	2.03 ± 0.36	> 25	> 12.31
C.I.8	1.31 ± 0.16	> 25	> 19.13
C.II.1	> 25	> 25	n.d.
C.II.2	> 25	> 25	n.d.
C.II.3	11.33 ± 0.84	> 25	> 2.20
C.II.4	> 25	> 25	n.d.
C.II.5	> 25	> 25	n.d.
C.II.6	10.66 ± 0.51	> 25	> 2.34
C.II.7	10.61 ± 1.00	> 25	> 2.35
C.II.8	3.22 ± 0.46	21.53 ± 0.63	7.76
miltefosine	2.84 ± 0.10	18.5 ± 0.60	6.51
edelfosine	0.82 ± 0.13	4.96 ± 0.16	6.01

Table 2
IC_{50} for the selected compounds against TryR inhibition.

Code	IC_{50} (mean ± SEM), μM
A.I.7	9.22 ± 1.50
B.I.1	0.23 ± 0.01
B.I.2	21.81 ± 2.55
C.I.7	18.51 ± 0.16
C.I.8	25.00 ± 0.69
C.II.8	0.54 ± 0.13
mepacrine	16.99 ± 1.18

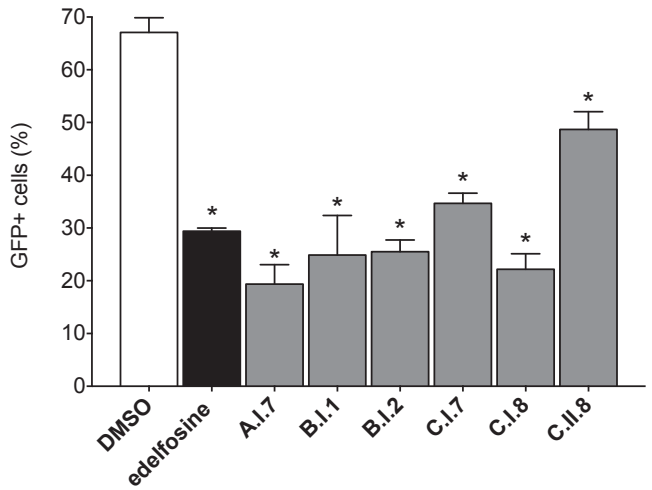


Fig. 2. Infection rates of THP-1 cells by amastigotes with 3 μM dose at 48 h treatment, autofluorescence correction applied. Aggregate results (mean ± SEM; n = 3) expressed as fold induction relative to control cells treated with DMSO. *$p < 0.05$.

and compared with the ranking obtained for the previously mentioned experiments disparities can be observed and therefore other mechanisms may be implicated in the potent leishmanicidal activity.

In conclusion, the present study reports the evaluation as potential novel class of antileishmanial agents in the promastigote and amastigote models. Moreover, their *in vitro* toxicity was assessed by the study of their cytotoxicity against THP-1 cells. The thirty-one compounds evaluated contain either bis(4-aminophenyl)diselenide (Subseries I) or 4-aminophenylselenocyanate (Subseries II) in their structure in an effort to optimize their properties as leishmanicidal agents.

In general terms diselenide derivatives were found to be more potent and selective than selenocyanate-containing structures . Seven diselenides (**A.I.3**, **A.I.7**, **A.I.8**, **B.I.1**, **B.I.2**, **C.I.7** and **C.I.8**) matched the potency of miltefosine (IC_{50} = 2.84 μM) against *L. infantum* amastigotes and derivatives **A.I.7** and **A.I.8** even surpassed the 0,65 μM IC_{50} mark obtained for edelfosine while no selenocyanate derivative fulfilled this goal.

When compared with their parent compound bis(4-aminophenyl) diselenide (IC_{50} = 0.65 μM), the previously mentioned diselenide derivatives matched or improved the activity against amastigotes. If we compare the most potent selenocyanate **C.II.8** with its parent compound 4-aminophenylselenocyanate (IC_{50} = 9.29 μM) a significant improvement in potency can be observed.

Moreover, seven of the tested derivatives (**A.I.3**, **A.I.7**, **B.I.1**, **B.I.2**, **C.I.7**, **C.I.8** and **C.II.8**) exhibited selectivity indexes higher than both reference drugs. These promising results encouraged further studies in infected macrophages with the derivatives that fulfilled this last criterion. Compounds **A.I.7**, **B.I.2** and **C.I.8** were more potent against intracellular amastigotes than edelfosine under the same conditions.

In order to get further information on the molecular mechanism implicated, the inhibition of TryR was determined. All tested compounds inhibited TryR, **C.I.7** and **C.II.8** potency was 30-fold higher than the one recorded for mepacrine. No clear correlation could be stablished when ranking derivatives according to potency and selectivity against their inhibitory potential which suggests the existence of different targets in this family of compounds.

The series of compounds described here show promising *in vitro* results as well as accomplished our goal of exploring whether the modifications made were beneficial over the parent compounds. This goal has been met for the diselenide derivatives but hardly for the selenocyanate-containing structures.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127371.

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