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Posted Date: 21 December 2023

doi: 10.20944/preprints202312.1664.v1

Keywords: leishmaniasis; selenide; 2,5-dihydrooxazole; indole; chromone; coumarin; β -naphthol; 1,3,4-oxadiazole; imidazo[1,2-a]pyridine; imidazo[2,1-b]thiazole



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Article

Selenium-Containing (Hetero)Aryl Hybrids as Potential Antileishmanial Drug Candidates: *In vitro* Screening Against *L. amazonensis*

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Abstract: Leishmaniasis remains a significant global health concern, with current treatments relying on outdated drugs associated with high toxicity, lengthy administration, elevated costs, and drug resistance. Consequently, the urgent need for safer and more effective therapeutic options in leishmaniasis treatment persists. Previous research has highlighted selenium compounds as promising candidates for innovative leishmaniasis therapy. In light of this, a library of 10 selenium-containing diverse compounds was designed and evaluated in this study. These compounds included selenium substituted indole, coumarin, chromone, oxadiazole, imidazo[1,2-*a*]pyridine, Imidazo[2,1-*b*]thiazole and oxazole, among others. These compounds were subjected to screening against *L. amazonensis* promastigotes and intracellular amastigotes, and their cytotoxicity was assessed in peritoneal macrophages, NIH/3T3 and J774A.1 cells. Among the tested compounds, **MRK-106** and **MRK-108** displayed the highest potency against *L. amazonensis* promastigotes with reduced cytotoxicity. Notably, MRK-106 and MRK-108 exhibited IC₅₀ values of 3.97 μM, 4.23 μM, respectively, and most of the tested compounds showed low cytotoxicity in host cells (CC₅₀ > 200 μM). Also, compounds **MRK-107** and **MRK-113** showed activity against intracellular amastigotes (IC₅₀ 18.31 and 15.93 μM and SI 12.55 and 10.92, respectively). In conclusion, the identified selenium-containing compounds hold potential structures as antileishmanial drug candidates to be further explored in subsequent studies. These findings represent a significant step towards the development of safer and more effective therapies for leishmaniasis, addressing the pressing need for novel and improved treatments.

Keywords: leishmaniasis; organoselenide; 2,5-dihydrooxazole; indole; chromone; coumarin; β-naphthol; 1,3,4-oxadiazole; imidazo[1,2-*a*]pyridine; imidazo[2,1-*b*]thiazole

1. Introduction

Leishmaniasis encompasses a diverse spectrum of diseases, each presenting a wide array of clinical manifestations. These clinical conditions are attributed to various species of the kinetoplastid parasite *Leishmania*, affecting both humans and other mammals dwelling in tropical and subtropical regions worldwide [1,2]. According to the World Health Organization (WHO), today, more than 1 billion people live in areas endemic for leishmaniasis and are at risk of infection. An estimated 30 000 new cases of VL and more than 1 million new cases of CL occur annually, with an ongoing burden of 12 million people harboring active infections [3]. The transmission of this pathology is facilitated by blood-feeding female sandflies, with the parasites undergoing a complex life cycle involving two distinct forms: extracellular flagellated promastigotes within the vector and intracellular non-flagellated amastigotes residing within mononuclear phagocytes in the mammalian host [4].

Historically, pentavalent antimony stood as the primary treatment for leishmaniasis. However, it comes burdened with concerns of cardiotoxicity, cirrhosis, pancreatic toxicity, and the risk of resistance development [5]. Consequently, amphotericin B (including lipid formulations) emerged as a secondary option. The repertoire of drugs repurposed for leishmaniasis treatment includes amphotericin B, miltefosine, paromomycin, and pentamidine [6]. Miltefosine has also found its place in treating both VL and CL, offering the benefits of oral administration, high efficacy, and a short treatment course. Nonetheless, its usage is hampered by teratogenicity and the potential for drug resistance [6–8]. Hence, there is an immediate need to discover new therapeutic approaches and drug compounds to combat these life-threatening diseases.

Selenium compounds are gaining remarkable prominence in medicinal chemistry, representing a burgeoning frontier in the search for novel antiparasitic agents [9,10]. The strategic integration of selenium (Se) atoms into organic frameworks offers a highly auspicious avenue for the creation of enhanced, disease-specific compounds. Organoselenium compounds are renowned for their diverse pharmacological properties [9–20]. Furthermore, the biocompatibility, minimal toxicity, and chemical versatility of selenium have spurred the development of a diverse range of Se-based pharmaceuticals. Research has demonstrated that the incorporation of selenium atoms into small molecules significantly amplifies their bioactivity [21]. In broad terms, organoselenium compounds exhibit medical applications that encompass cancer treatment, managing infections, inflammation, addressing Alzheimer's disease and depression, as well as providing antioxidant benefits [21]. Intriguingly, selenium has also exhibited favorable effects in combatting parasitic diseases, including but not limited to malaria, African trypanosomiasis, Chagas Disease and intestinal parasites [22]. This highlights the pivotal role of selenium compounds in the pursuit of effective treatments against this parasitic disease [22–24]. Various Se-containing compounds have shown antimicrobial properties. Additionally, studies indicate that Se supplementation can reduce parasite burden and ameliorate symptoms associated with *Leishmania* spp. and other trypanosomiasis [22]. Moreover, recent findings have highlighted the *in vitro* leishmanicidal potential of newly synthesized compounds featuring selenium within their structures.

Similarly, heterocyclic compounds hold a pivotal role in organic chemistry, primarily due to their ubiquitous presence in pharmaceuticals, natural substances, and various chemicals integral to our daily lives [25–30]. These compounds are characterized by the presence of one or more heteroatoms within cyclic structures, with or without aromatic properties. Oxygen, nitrogen, phosphorus, and sulfur rank among the most frequently incorporated heteroatoms in a majority of heterocyclic compounds [32]. Around, 80% of major commercially available synthetic drugs contain at least one heterocyclic scaffold, with a broad spectrum of pharmacological potential, encompassing applications such as antitumor, anti-inflammatory, and especially important for several active compounds against microorganisms [31]. In the past few decades, the quest for safer and more efficacious drugs to combat leishmaniasis has spurred a multitude of research initiatives. Researchers worldwide have dedicated extensive efforts to synthesize a wide array of antileishmanial agents, distinguished by their incorporation of diverse heterocyclic moieties [33]. Among these moieties, thiazoles, pyrazoles, pyrimidines, chromanones, and imidazoles stand out, each offering a unique chemical scaffold that has garnered attention in the development of potential antileishmanial

therapeutics. [31–33]. Considering the biological importance of heteroarenes and the wide spectrum of therapeutic properties of organoselenium compounds, molecular hybridization of these structures demonstrates promising biological properties.

Thus, in line with our continuous interest in discovering and developing new sustainable, efficient methodologies for biologically relevant organoselenides and their biological evaluation [17,19,34–38], this article aims to thoroughly examine the synthetic approaches used to create novel chemical compounds for combating leishmaniasis. Herein we report the *in vitro* investigation of antileishmanial potential of selenium substituted (hetero)aryl hybrids (indole, coumarin, chromone, oxadiazole, imidazo[1,2-*a*]pyridine, Imidazo[2,1-*b*]thiazole and oxazole, among others) seeking to assess their efficacy and potential for treating this parasitic disease, with a focus on understanding the relationships between compound structures and their biological activity. Our goal is to contribute to the broader understanding of innovative drug development for addressing this widespread tropical disease.

2. Materials and Methods

2.1. Synthesis of selenium substituted (hetero)aryl hybrids:

For the current study, we selected several nitrogen and oxygen containing heterocycles. Some of privileged naturally occurring heterocycles have also been selected, for example, indole, coumarin and flavanone. Besides, oxadiazole, imidazo[1,2-*a*]pyridine, Imidazo[2,1-*b*]thiazole and oxazole were also been selected. These heterocyclic compounds have been utilized to access structurally diverse selenium substituted (hetero)aryl hybrids **MRK101-108**, **111**, **113** (Figure 1) using our previously established sustainable routes. [34–40].

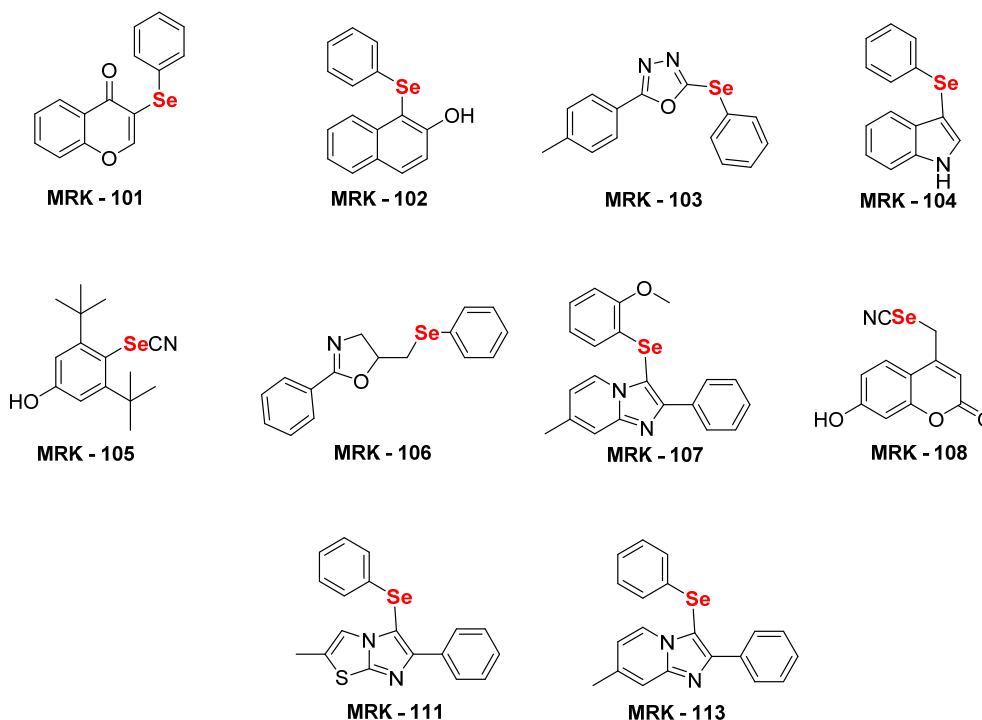


Figure 1. Chemical structure of selenium substituted (hetero)aryl hybrids [34–40], used in this study.

2.2. Mice and parasites

Female BALB/c mice, aged 6-8 weeks with an average weight of 30 g, were obtained from the Central Animal Facility of the Federal University of Mato Grosso do Sul (UFMS, Campo Grande-MS, Brazil). Animals were housed in individually ventilated cages (IVCs) within a ventilated rack system (Alesco) under specific pathogen-free (SPF) conditions. The environment maintained a temperature

of $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, a 12-hour light/dark cycle, and ad libitum access to Nuvital food and water. All procedures were approved by the Ethics Committee on Animal Experiments (CEUA) of UFMS (protocol number 1.041/2019) and adhered to relevant guidelines for animal welfare and research.

Leishmania (Leishmania) amazonensis (IFLA/BR/1967/PH8 strain) were maintained as promastigotes at 26°C in Schneider's medium (Sigma-Aldrich) supplemented with 20% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich), 10,000 U/mL penicillin, and 10 mg/mL streptomycin (Sigma-Aldrich) for a maximum of 20 serial passages. Parasites in the exponential growth phase were used for the in vitro antipromastigote assay, while those in the stationary phase were used for the antiamastigote assay. Intracellular amastigote forms were obtained by infecting murine peritoneal macrophages with promastigotes.

2.3. Anti-promastigotes assays:

Selenium-compounds MRK-101 to 113 were evaluated for their antipromastigote activity against *Leishmania (Leishmania) amazonensis* in five replicates. Serial dilutions ranging from 100, 50, 25 and $12.5 \mu\text{M}$ were prepared in supplemented Schneider's Insect Medium (Sigma-Aldrich). The microplates were then seeded with *L. amazonensis* promastigotes (1×10^5 parasites/well) and incubated at 26°C for 48 hours in a Biochemical Oxygen Demand (BOD) incubator (Cienlab, Brazil). Cell viability was assessed using the resazurin (Sigma-Aldrich) assay. A solution of resazurin (Sigma-Aldrich, USA) at $0.2 \text{ mg} \cdot \text{mL}^{-1}$ was added to each well, and after 4 h of incubation at 26°C , the absorbances at 570 nm and 600 nm were acquired and the viability calculation for all wells was performed based on the formula and instructions provided by the AlamarBlue® manufacturer's website [41]. Cell viability was evaluated from optical density and compared to untreated cells. Cells incubated with amphotericin B (1.25, 2.5, 5 and $10 \mu\text{M}$) served as reference antipromastigote drug, while DMSO in Schneider's Insect Medium (Sigma-Aldrich) was used as the negative control. The half-maximal inhibitory concentration (IC_{50}) values for each compound were calculated using a nonlinear dose-response regression curve generated Prism 5 (GraphPad software, Boston, MA). To compare variances, ANOVA with Tukey's posttest was used, supplemented by the parametric t test, with a 95% confidence interval.

2.4. Peritoneal macrophages

Following animal euthanasia, murine peritoneal macrophages were harvested. Ten milliliters of cold RPMI 1640 medium (Sigma-Aldrich) supplemented with 10,000 U/mL penicillin and 10 mg/mL streptomycin (Sigma-Aldrich) was injected into the peritoneal cavity. The abdominal region was gently massaged to facilitate macrophage release. The peritoneal fluid was then aspirated and transferred to an ice-cold erlenmeyer flask to prevent cell adherence. Cell quantification was performed using a Neubauer's chamber after trypan blue staining (Sigma-Aldrich) for viability assessment.

2.5. Treatment of infected macrophages

Murine peritoneal macrophages (1×10^6 cells/well) were seeded in three replicates onto 24-well plates containing round glass coverslips (13 mm) pre-coated with 10% FBS in RPMI 1640 medium (Sigma-Aldrich). Plates were incubated at 37°C with 5% CO_2 for 1 hour to allow adhesion, confirmed by microscopy. Following two washes with PBS (Sigma-Aldrich), adherent macrophages were infected with stationary-phase *L. amazonensis* promastigotes (4×10^6 parasites/mL) and incubated at $35^{\circ}\text{C}/5\% \text{CO}_2$. After 4 hours, free parasites were removed by two PBS washes, and infected cells were treated for 24 hours with the compounds at concentrations 6.25, 12.5, 25 and $50 \mu\text{M}$. Amphotericin B (Sigma-Aldrich; 1.25, 2.5, 5 and $10 \mu\text{M}$) served as reference drug, and untreated cells were used as a negative control. Supernatants were removed after treatment, and cells were washed twice with PBS, fixed with Bouin's solution (Sigma-Aldrich), and stained with Giemsa (Sigma-Aldrich) diluted 1:10 in distilled water. Coverslips were dehydrated through an acetone/xylene gradient (100%, 70%, 50%, 30%, 100% each with Sigma-Aldrich) and mounted for microscopic visualization. The total number

of intracellular amastigotes was counted in 200 cells per coverslip in three replicates using an optical microscope. The half-maximal inhibitory concentration (IC_{50}) values were calculated for each compound using a nonlinear regression curve in GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA).

2.6. Cytotoxicity assays:

Murine peritoneal macrophages (1×10^6 cells/well), NIH/3T3 cells (ATCC CRL-1658, mouse fibroblasts lineage) and J774A.1 (ATCC, mouse macrophages lineage) were cultured in 96-well plates (1×10^5 cells/well) in RPMI 1640 medium (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (Cultilab, Brazil), 10 IU.mL⁻¹ of penicillin, and 100 µg.mL⁻¹ of streptomycin (Sigma-Aldrich, USA) and adhered overnight at 37°C and 5% CO₂. The medium was replaced by fresh RPMI medium with different concentrations of the compounds ranging from 200 to 3.12 µM and incubated for 48 h. Cells treated with amphotericin B (50.0–0.78 µM) served as the antileishmanial drug reference. Dimethyl sulfoxide (DMSO, Sigma-Aldrich) at the concentration required to solubilize the highest test sample concentration was used as control and did not affect cell viability control, as well as fibroblasts cultured with medium alone were used as a life/viability control. Cell viability was assessed using the colorimetric resazurin method [41]. Then, 5 µl of 0.2 mg.mL⁻¹ resazurin solution (Sigma-Aldrich, USA) was added to each well, incubated for 4 h 37°C and 5% CO₂. Absorbances were acquired at 570 and 600 nm and the viability calculation were performed as described in section 2.3. The half-maximal cytotoxic concentration (CC₅₀) for each compound was calculated from a sigmoidal regression of the dose-response curve generated using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). In order to compare variances, ANOVA with Tukey's posttest was used, supplemented by the parametric *t* test, with a 95% confidence interval.

3. Results

3.1. Anti-promastigotes and anti-amastigotes assays:

The antileishmanial activity of the synthesized selenium compounds were evaluated by culturing promastigote forms of *L. amazonensis* in the presence of different concentrations of each molecule. Control wells containing only the culture medium (C) and amphotericin B (ANFB), a reference drug for antileishmanial activity, were also prepared. After 48 h of incubation, the percentage of promastigote form's viability was accessed by the resazurin colorimetric method and calculated comparing to control wells. The antileishmanial activity of the selenium compounds were also assessed in intracellular amastigote forms inside infected murine peritoneal macrophages and the representative results obtained for five of the ten compounds tested are illustrated in Figure 2.

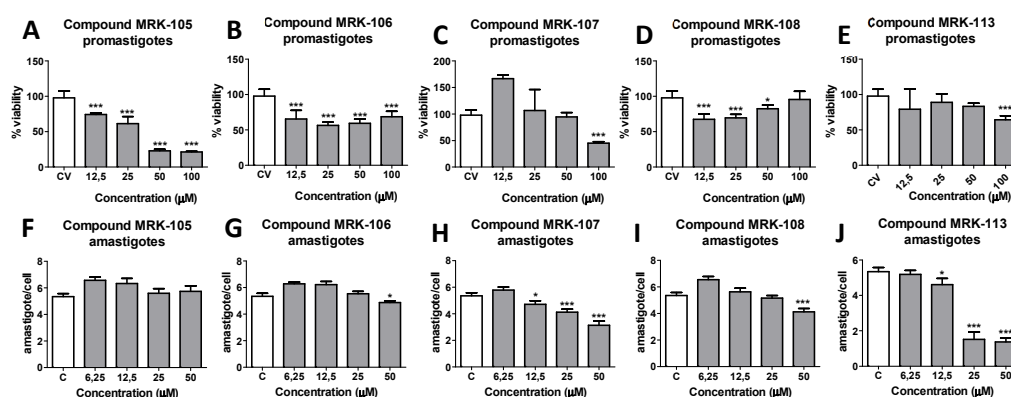


Figure 2. Antileishmanial activity of synthesized selenium compounds MRK-105, 106, 107, 108 and 113 against promastigote (A, B, C, D and E upper graphs) and intracellular amastigotes (F, G, H, I and J lower graphs) of *L. amazonensis*. * and *** means $p < 0,01$ and $p < 0,001$, respectively (ANOVA and Tukey's post test).

The half-maximal inhibitory concentration (IC₅₀) of each compound was obtained by nonlinear regression and the results are shown in Table 1. The IC₅₀ of tested compounds ranged from 3.96 to 40.98 μ M for promastigote forms, and from 15.93 to >50 μ M for amastigote forms. Amphotericin B, a reference drug for its anti-leishmanial activity, was also evaluated under the used experimental conditions, obtaining an IC₅₀ of 9.40 μ M for promastigotes and 0.97 μ M for intracellular amastigote forms.

Table 1. Antileishmanial activity of selenium compounds.

Compound	CC50 (μ M)	IC50 (μ M)		IC50 (μ M)	
	peritoneal	<i>L. amazonensis</i>	SI ^a	<i>L. amazonensis</i>	SI ^b
	macrophages	promastigotes		amastigotes	
101	> 200	30.46	6.57	> 50	ind
102	> 200	15.15	13.20	> 50	ind
103	> 200	15.48	12.92	> 50	ind
104	> 200	16.17	12.37	> 50	ind
105	> 200	12.17	16.43	> 50	ind
106	> 200	3.95	50.53	> 50	ind
107	> 200	27.37	7.31	18.31	10.92
108	> 200	4.22	47.31	> 50	ind
111	> 200	17.55	11.40	> 50	ind
113	> 200	40.98	4.88	15.93	12.55
ANFB	25.15	9.40	2.67	0.97	25.92

The antileishmanial activity of selenium-compounds was evaluated against promastigote and amastigotes forms of *L. amazonensis* after 48 and 24 hours, respectively. The cytotoxicity was performed in BALB/c peritoneal macrophages. Selectivity index (SI) was calculated as CC50/IC50 values for promastigote and amastigote forms, respectively. Abbreviations: CC50= half-maximal cytotoxic concentration; IC50 = half-maximal inhibitory concentration; SI = selectivity index. ^aSI, CC50 on murine peritoneal macrophages / IC50 on promastigotes. ^bSI, IC50 on murine peritoneal macrophages / IC50 on amastigotes. ANFB = amphotericin B used as reference drug for *L. amazonensis*. Ind = means indetermined results.

The highest activity against promastigote forms of *L. amazonensis* was observed for compound **MRK-106**, which contains a 4,5-dihydrooxazole ring in its structure and showed an IC₅₀ of 3.96 μ M. Compound **MRK-108**, a selenocyanate linked to a coumarin ring, had an IC₅₀ of 4.23 μ M, the second highest potency observed against the parasite. Following the decreasing order of anti-leishmania activity observed, the following compounds showed similar activity, between 10 and 20 μ M: **MRK-105**, a phenol selenocyanate, showed an IC₅₀ of 12.17 μ M; **MRK-102**, a beta-naphthol, showed an IC₅₀ of 15.15 μ M; compound **MRK-103**, which has a 1,3,4-oxadiazole ring, with an IC₅₀ of 15.48 μ M; **MRK-104**, which has an indolic nucleus, showed an IC₅₀ of 16.17 μ M, and compound **MRK-111**, an imidazothiazole, with an IC₅₀ of 17.55 μ M. The least active compounds had IC₅₀s above 20 μ M: **MRK-107**, an imidazopyridine, with an IC₅₀ of 27.37 μ M; **MRK-101**, a chromone, with an IC₅₀ of 30.46 μ M and **MRK-113**, also an imidazopyridine, with an IC₅₀ of 40.98 μ M.

It is interesting to note that the activity of the compounds against promastigote forms did not necessarily reflect the results of the anti-amastigote intracellular activity experiments: the compounds **MRK-106** and **MRK-108** were not active in macrophages infected with *L. amazonensis*, with IC₅₀ values estimated at >50 μ M, the highest concentration tested. On the other hand, the compounds **MRK-107** and **MRK-113**, which were poorly active against promastigote forms, showed the best results against intracellular amastigote forms: 18.31 and 15.93 μ M, respectively. As most of tested

compounds did not showed activity against intracellular amastigotes, their SI were considered indetermined (as indicated as *ind* in Table 1).

3.2. Cytotoxicity and selectivity index:

The selectivity index (SI) is defined as the ratio of the CC₅₀ obtained for host cells to the IC₅₀ obtained against the parasite cells and helps identify the compounds that exhibit a high degree of specificity in targeting the pathogen of interest while sparing host cells [42,43]. To determine the selectivity index of the selenium compounds, their cytotoxicity on murine peritoneal macrophages, NIH/3T3 murine fibroblast cell line and J774A.1 murine macrophages cell line was also evaluated, at concentrations ranging from 200 to 3.12 μ M. After 48 h, viability was assessed using the resazurin colorimetric methodology, where cells incubated only with culture medium were considered 100% viable. The representative results obtained for five of the ten compounds tested are illustrated in Figure 2. The CC₅₀ was obtained by non-linear regression and the results for CC₅₀ for peritoneal macrophages are also shown in Table 1. CC₅₀ and SI results for NIH/3T3 and J774A.1 cells for each compound are shown in Supplemental Material.

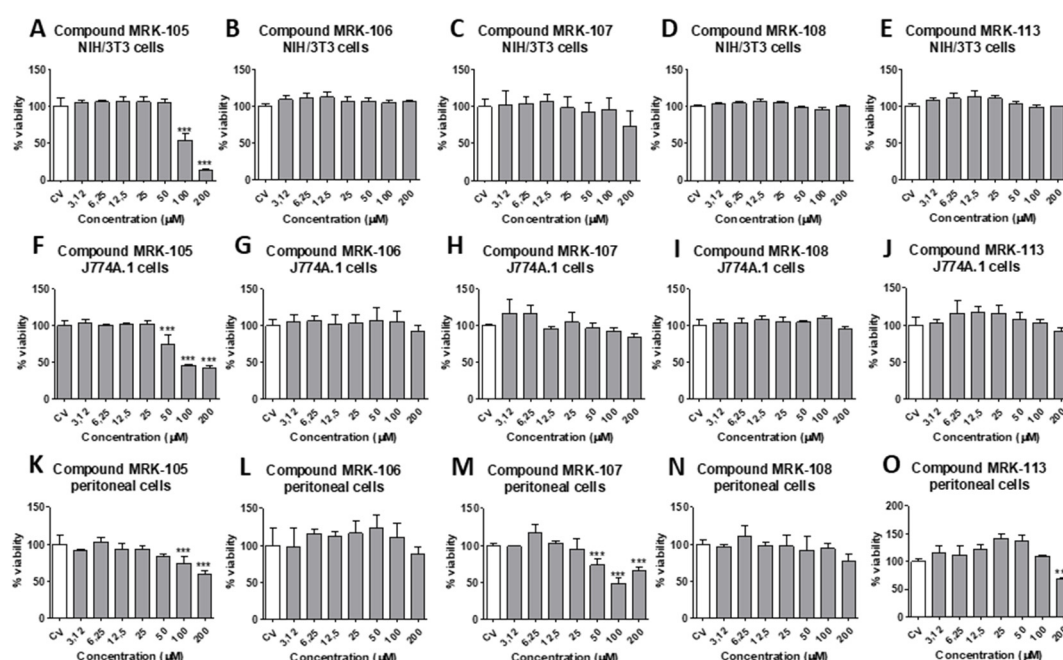


Figure 3. Cytotoxicity of synthesized selenium compounds MRK-105, 106, 107, 108 and 113 against NIH/3T3 fibroblasts (A, B, C, D and E upper graphs), J774A.1 macrophages (F, G, H, I and J middle graphs) and murine peritoneal macrophages (K, L, M, N and O lower graphs). * and *** means $p < 0.01$ and $p < 0.001$, respectively (ANOVA and Tukey's post test).

All the tested selenium compounds showed a CC₅₀ above 200 μ M in peritoneal macrophages. Some compounds showed higher cytotoxicity in other cell lines: compound **MRK-105**, whose CC₅₀ was 87.85 μ M in NIH/3T3 cells and 60.16 μ M in J774A.1 cells, and compound 104 with CC₅₀ of 126.7 μ M in J774A.1 cells. Thus, the most active compounds against *L. amazonensis* promastigotes were those compounds with the higher SI, compounds **MRK-106** and **MRK-108**, with SI above 50.53 and 47.31, respectively. However, considering the cytotoxicity in peritoneal macrophages and the activity against intracellular amastigotes of *L. amazonensis*, the most active compounds were **MRK-113** and **MRK-107**, with SI values of 12.55 and 10.92, respectively.

4. Discussion

Leishmaniasis, a neglected tropical disease, continues to pose as a significant challenge for drug discovery and development. Current antileishmanial drugs have issues such as high toxicity,

resistance development, and the necessity for hospitalization, rendering them poor adhesion [3,4]. While there have been notable strides made through combination therapy approaches, which have reduced treatment duration and cost, a critical gap persists — the urgent need for new active drugs. In the search for novel drugs, emerging evidence highlights a link between selenium and parasites, notably trypanosomatids. Certain parasites have been found to both express selenoproteins and metabolize selenium. This underscores the potential significance of selenium as a promising element for the development of new agents against leishmaniasis. Here, we demonstrated that using sustainable routes, synthesized (hetero)aryl hybrids selenium-compounds displayed anti-promastigote activity *in vitro* and a promising selectivity index, with **MRK-106**, **MRK-107**, **MRK-108** and **MRK-113** being the most potent and selective for antileishmanial activity.

MRK-106 contains a 4,5-dihydrooxazole ring (oxazoline), with substituents containing benzene rings in positions 2 and 5, showed an IC_{50} of 3.96 μM for promastigotes and a selectivity index of 50.53. Secondary metabolites featuring oxazole, oxazoline, and isoxazoline ring structures exhibit a broad distribution across marine and terrestrial organisms [44,45]. Among various heterocyclic compounds, isoxazoles and their analogs hold great significance due to their wide-ranging biological activities. This makes them pivotal structures in medicinal chemistry. Isoxazole derivatives with structural variations exhibit diverse medicinal properties, contributing significantly to the development of novel, highly effective, and less toxic bioactive drugs. These compounds are notable for their diverse and substantial biological activities, encompassing anti-tumor, antibacterial, anti-viral, anti-malarial, and immunosuppressive properties, including antileishmanial activity [44]. Moraski and colleagues undertook the synthesis of multiple compounds with oxazoline and oxazole motifs and tested for their inhibitory potential against *Mycobacterium tuberculosis*, showing promising results [45,46]. More recently, a series of compounds containing β -carboline-oxazoline were tested against promastigote and amastigote forms of *L. amazonensis*, and some of them were found to be active against the parasite [47]. Among them, compounds 8d and 8i were considered the most potent against promastigote forms, showing IC_{50} of 14.7 and 23 μM , and SI of 6.6 e 1.3, respectively. Our compound **MRK-106** also have oxazoline ring in its structure, together with Se atom and two benzene rings, which resulted in a lower IC_{50} and higher SI, compared to the β -carboline-oxazoline molecules. Another compound containing an azol is **MRK-103**, which has a 1,3,4-oxadiazole ring. Despite several examples of the use of different azol moieties in active antileishmanial compounds [44], **MRK-105** showed an IC_{50} of 15.48 μM and SI of 12.92, more than three times less selective than **MRK-108**. It is also interesting to note that, although a higher IC_{50} was obtained than for amphotericin B under the experimental conditions used, the selectivity index for **MRK-106** (50.53) is more than five times higher than that obtained for this drug (9.40) for promastigote forms. Amphotericin B is an important second-choice drug in the treatment of leishmaniasis, but it is also known for its toxic side effects and low therapeutic index [3,4]. Thus, the SI obtained for **MRK-106** is also important data for future studies of anti-leishmanial activity.

Compound **MRK-108**, a selenocyanate linked to a coumarin ring, showed an IC_{50} of 4.23 μM and an SI of 47.31. The potential for functionalization and distinctive attributes renders coumarin a privileged scaffold in the field of medicinal chemistry [48]. While coumarins are predominantly found as secondary metabolites in plants, bacteria, and fungi, numerous synthetic methods have been documented for their production. This bicyclic heterocycle, composed of a benzene ring fused with a pyrone ring, exhibits the capability to engage with diverse biological targets [48]. The pyrone ring facilitates hydrogen bonding with multiple amino acid residues, while the aromatic segment can establish hydrophobic interactions. Consequently, this versatility results in a wide array of biological properties, encompassing antioxidant, anticoagulant, anticancer, antiviral, antitrypanosomal, anticholinesterase, and antileishmanial activities. Coumarin derivatives were identified as promising structures in the search for new anti-leishmania agents in a recent review [49]. Also, the authors pointed out that the presence of electron withdrawing groups increase the antileishmanial effect. Besides, selenocyanates displayed potency against *Leishmania infantum* promastigotes [24]. In case of compound **MRK-108**, selenium atom is linked to nitrile (strongly electron-withdrawing group) [50,51] and on other side to methyl-2H-pyran-2-one of coumarin, and this may contribute to the

activity observed for **MRK-108**. The authors also highlight the work of Huang and colleagues with quinolines derivatives containing selenium as promising antileishmanial candidates [49,51]. The SI obtained for **MRK-108** (47.31) was also almost three times higher than that of amphotericin B, which could be a promising characteristic for the therapeutic use of this molecule, or a series of derivative compounds for further structure activity relationship studies.

MRK-107 and **MRK-113** showed low activity against *L. amazonensis* promastigotes (27,37 and 40,98 μ M, respectively), and it is interesting to observe that both are imidazopyridine compounds, with a phenyl-selenyl substituent in carbon 3. But the presence of a methoxy group for **MRK-107** enhanced its activity almost 1,5x compared to **MRK-113**, without methoxy, an electron-donating group. This should contribute to the antileishmanial activity and, like other compounds studied here, further structure-related activity should be carried out to address the ligand and/or the essential radicals responsible for the observed activity. Despite the results obtained for promastigote cells, both **MRK-107** and **MRK-113** were the most active compounds against intracellular amastigotes, with IC₅₀ of 18.31 and 15.93 μ M and SI 12.55 and 10.92, respectively. The imidazopyridine scaffold has gained significant importance for designing synthetic analogs targeting a range of therapeutic disorders, including cancer, diabetes, infections, inflammation, and CNS conditions. This heterocyclic system serves as a crucial pharmacophore motif, expanding medicinal chemistry tools. Additionally, imidazopyridines find use in combating helminthic, coccidial, and fungal infections, illustrating their multifaceted role in drug development [53]. Recently, imidazopyridine derivatives are reported to have potential for anti-trypanosomiasis drug discovery. Fersing et al. designed and synthesized novel 3-nitroimidazo[1,2-a]pyridine derivatives. By introducing a heteroatom bridge between the aryl group and the imidazopyridine, they obtained the desired derivatives from 8-bromo-6-chloro-3-nitro-2-(phenylsulfonylmethyl)imidazo[1,2-a]pyridine. These compounds were tested in vitro against *L. donovani* and *L. infantum* strains, alongside reference drugs like pentamidine, fexinidazole, miltefosine, and amphotericin B [54]. Such structural features can serve as a basis for the design and synthesis of new series of imidazopyridines containing selenium.

Various chemical structures containing selenium have been investigated against *Leishmania* parasites, including diselenide, selenourea, methylseleno and selenocyanate components [22]. Both **MRK-105** and **MRK-108** contain selenocyanate radical, but their structures are quite different: **MRK-105** is a phenol selenocyanate, whilst **MRK-108** is selenocyanate linked to a coumarin, as described above. Also, **MRK-8** showed higher activity against *L. amazonensis* promastigotes (4.23 μ M) than **MRK-105** (12.17 μ M), despite its poor activity against intracellular amastigotes. Regarding selectivity index, **MRK-105** was the only compound which cytotoxicity in NIH/3T3 fibroblasts was lower than 200 μ M (87.85 μ M), resulting in a low selectivity index: 7.22. Also, **MRK-104** and **MRK-105** showed higher cytotoxicity in J774A.1 cells: 126.7 and 60.16 μ M, respectively. **MRK-8**, by its turn, exhibited a SI of 47.31. These results highlight the promising structure of selenocyanate associated to coumarin ring in the search of novel antileishmanial drugs.

Based on the recommendations of the World Health Organization (WHO) Special Programme for Tropical Disease Research (TDR), some authors classify the compounds tested for *L. donovani* or *L. infantum* as active for an IC₅₀ in amastigotes in macrophages of 1-2 μ g/ml, moderately active for IC₅₀ between 1.0 and 6.0 μ M, and inactive for IC₅₀ >6.0 μ M, with a desirable SI >10 or even >20 [42,55,56]. This is an important step regarding antileishmanial potential, as intracellular amastigotes are the parasite forms found in mammalian host. However, testing new compounds against promastigote forms of *Leishmania* is still an important step in the search for their biological activity, as recently reviewed considering natural and synthetic compounds with antileishmanial activity [57,58]. So, further evaluation of these selenium compounds in other *Leishmania* species or even modifications on their structures should be carried out to outline their promising antileishmanial activity.

5. Conclusions

In conclusion, a series of selenium substituted (hetero)aryl hybrids were screened against *L. amazonensis* promastigotes and intracellular amastigotes. These hybrid compounds included

selenium substituted indole, coumarin, chromone, oxadiazole, imidazo[1,2-*a*]pyridine, Imidazo[2,1-*b*]thiazole and oxazole, among others. The biological evaluation indicate that these hybrids are promising structures against this parasite. Furthermore, their cytotoxicity was also assessed in murine peritoneal macrophages, NIH/3T3 and J774A.1 cells, with good selectivity. Compounds containing oxazoline rings, coumarin derivatives and imidazopyridine rings showed the best parameters in the evaluation of antileishmanial activity and selectivity used. These compounds may serve as a basis for the synthesis of structural derivatives and thus develop more detailed structure activity studies. These data in this study represent an important step towards the search for new antileishmanial drugs to be further explored.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, E.B.P.; S.S.; J.R. and T.B.R.; methodology, M.H.F.; A.R.N.; M.S.A.B.; C.B.V.; A.L.S.; T.E.A.F. and C.C.P.A.; software, M.H.F.; A.R.N.; M.S.A.B.; C.B.V.; A.L.S. and C.C.P.A.; validation, M.H.F.; A.R.N.; M.S.A.B.; C.B.V.; A.L.S. and C.C.P.A.; formal analysis, M.H.F.; A.R.N.; M.S.A.B.; C.B.V.; A.L.S. and C.C.P.A.; investigation, A.L.B.; S.S.; J.R. and T.B.R.; resources, A.L.B.; S.S.; J.R. and T.B.R.; data curation, M.H.F.; A.R.N.; M.S.A.B.; C.B.V.; A.L.S.; T.E.A.F. and C.C.P.A.; writing—original draft preparation, S.S.; J.R. and T.B.R.; writing—review and editing, S.S.; J.R. and T.B.R.; visualization, J.R. and T.B.R.; supervision, S.S.; J.R. and T.B.R.; project administration, S.S.; J.R. and T.B.R.; funding acquisition, S.S.; J.R. and T.B.R.. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001) and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT-MS). SS and JR are grateful to CNPq (315399/2020-1, 309975/2022-0, 422645/2021-4, 403210/2021-6, 404172/2023-7, and 405655/2023-1). S.S. would also like to acknowledge FAPEG (04/2023; EQU2023101000020).

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We gratefully acknowledge CAPES (001), CNPq, UFMS, UFG, FUNDECT MS and INCT-Catalise/CNPq/FAPESC for the support offered in this research.

Conflicts of Interest: The authors declare no conflict of interest.

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