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Novel hybrid selenosulfonamides as potent antileishmanial agents

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ABSTRACT

Diselenide and sulfonamide derivatives have recently attracted considerable interest as leishmanicidal agents in drug discovery. In this study, a novel series of sixteen hybrid selenosulfonamides has been synthesized and screened for their *in vitro* activity against *Leishmania infantum* intracellular amastigotes and THP-1 cells. These assays revealed that most of the compounds exhibited antileishmanial activity in the low micromolar range and led us to identify three lead compounds (derivatives **2**, **7** and **14**) with IC_{50} values ranging from 0.83 to 1.47 μM and selectivity indexes (SI) over 17, much higher than those observed for the reference drugs miltefosine and edelfosine. When evaluated against intracellular amastigotes, hybrid compound **7** emerged as the most active compound (IC_{50} = 2.8 μM), showing higher activity and much less toxicity against THP-1 cells than edelfosine. These compounds could potentially serve as templates for future drug-optimization and drug-development efforts for their use as therapeutic agents in developing countries.

1. Introduction

Leishmaniasis is defined as a cluster of vector-borne diseases with diverse clinical manifestations [1] and it constitutes one of the six priority diseases in the "Tropical Diseases Research" program of the World Health Organization (WHO). Leishmaniasis [2,3] are a complex of neglected tropical diseases caused by obligate intracellular protozoan parasites from the genus *Leishmania* that are transmitted by around 30 species of the phlebotomine sandflies through the bite of females infected with the pathogen [4,5].

The life cycle of the parasite is relatively simple. Sandflies inoculate the skin with flagellated promastigotes, which are phagocytosed by local and immediately recruited host cells. Within the phagolysosomes of resident macrophages surviving promastigotes transform and replicate as amastigotes, which infect additional macrophages either locally or in distant tissues after dissemination [6]. The clinical manifestations of leishmaniasis vary depending on the pathogenic species; they encompass some groups of disorders: cutaneous, diffuse cutaneous, mucocutaneous and visceral leishmaniasis. The latter is the most severe form of the disease, and it is usually fatal if left untreated [7].

Leishmaniasis is endemic in broad tropical areas of the world including many underdeveloped countries, which makes it a major international health problem. It has high morbidity and mortality rates and is classified as an emerging and uncontrolled disease by the World Health Organization. The migration of population from endemic to non-endemic regions and tourist activities in endemic zones are contributing to the spread of the disease to new areas. Currently, about 350 million people in 88 countries around the world are at risk of infection; 12 million people worldwide are infected and 500,000 new cases of visceral leishmaniasis emerge every year, causing about 60,000 deaths [8]. At present available chemotherapy is far from satisfactory and presents several problems including toxicity, many adverse effects, high costs and development of drug resistance [9].

Two pentavalent antimonial [Sb(V)] compounds, sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®), were first introduced in the 1940s and have been used since then as first-line chemotherapeutic agents against all forms of leishmaniasis through parenteral administration.

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However, their efficacy is becoming increasingly smaller and highly dependent on *Leishmania* species and endemic regional variations, even within the same country [10]. Besides, antimonial agents may also cause acute pancreatitis and cardiac arrhythmia.

In the recent years, new potential therapies have been introduced for visceral leishmaniasis. They include an amphotericin B liposome formulation [11]; oral miltefosine [12]; a parenteral formulation of aminosidine (paromomycin) [13] and oral sitamaquine [14]. Edelfosine [15–17], another alkyl-phospholipid, has also been tested and found to display higher *in vitro* activity than miltefosine. However, all current treatments suffer from limitations derived either from their high cost, route of administration, drug resistance or extended treatment regimens and, especially, serious side effects such as nephrotoxicity, hypokalemia, hepatic and pancreatic toxicity, hypotension and dysglycemia among others [18,19]. Therefore, there is an urgent need for the development of improved treatments for leishmaniasis that are safe, inexpensive and easily available to the patients. Furthermore, the discovery of new lead compounds for this disease is a pressing concern for global health programs.

During the last few years, various reports have shown the relevance of the trace element selenium, whose increased concentration in plasma has been recognized as a new defensive strategy against *Leishmania* infection [20,21]. Selenium derivatives have antioxidant, cancer preventing, and antiviral activities and also appear to improve the immune response of hosts against various bacterial and viral species [22,23]. Recently, we reported [24,25] new selenium compounds with *in vitro* antiparasitic activity against *Leishmania infantum*. Some of them possess a powerful activity with selectivity indexes higher than the reference drugs miltefosine and edelfosine. Additionally, their leishmanicidal activity in infected macrophages (THP-1 cells) was comparable to that of edelfosine. Among others, compounds referable to formula (Fig. 1) which contains as essential pharmacophore the diselenide group within the framework of molecular symmetry that, in our opinion, appear as a critical factor for activity. In view of its remarkable performance, we considered interesting to explore the modulation by derivatization of amine groups in order to adjust polarity and solubility, facilitate cellular uptake, optimize antileishmanial activity and reduce cytotoxicity improving the level of selectivity. With this aim, we chose the sulfonamide group, because: 1. Hydrolysis of this group inside the cell could release the active moieties; 2. This functional group assists drug transport to the cell and it could improve the permeability through lipid membranes by transitory partial loss of basicity of aminic nitrogen; 3. Sulfonamide, according to literature, shows intrinsic antileishmanial activity and is an important structural core in leishmanicidal therapy [26–31]; 4. The sulfonamide group can be used as a chemical link that allows binding of other potential "active components" such as aromatic and heteroaromatic systems with demonstrated antiparasitic activity [32–34]. Concerning theses aromatic rings, we have added several different substituents in order to explore new characteristics related to volume, rigidity and lipophilicity in the new molecules. On the other hand, some heterocycles are interesting molecular scaffolds in the design of new efficient leishmanicidal compounds; it is well established that many quinoline derivatives act as antiprotozoal agents [35–38] and also that imidazo derivatives are highly active against *Leishmania infantum*, *Leishmania mexicana* and *Leishmania donovani* [39–41]. Finally, the incorporation of the thienyl moiety has been explored due to its demonstrated effectiveness in leishmanicidal therapy by itself, combined, or fused with other skeletons [42,43]. In summary, we designed a new class of diselenide derivatives by molecular combination between 4,4′-diselanediyldianiline, as the core, and other bioactive substructures trying to make use of the "Medicinal Chemical Hybridization" (MCH) as one of the approaches to design a polyvalent drug. This strategy can be carried out by joining through appropriate linkers to pharmacophores, the linkers usually are susceptible to metabolic cleavage [44–46]. From our point of view, molecular hybridization is a relatively new concept in drug design and development. It is based on the combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved physico-chemical and bioactive properties, in an attempt to obtain synergistic effects through the action of some mechanistic routes and the reduction of undesired side effects when compared to the parent compounds.

In light of these findings, and as a part of our efforts to develop new compounds for the treatment of leishmaniasis, we present the synthesis and the leishmanicidal activity of sixteen new hybrids (Fig. 2) based on the 4,4′-diselanediyldianiline with sulfonamide moiety bound to different rings. Their activity was evaluated against both axenic and, for the most interesting compounds, intracellular amastigotes. Cytotoxicity against the human cell line THP-1 was also assessed in order to exclude those molecules showing unfavorable toxicological profile from further development.

2. Results and discussion

2.1. Chemistry

We have previously described the syntheses of various diselenide derivatives by reduction of the corresponding selenocyanates with sodium borohydride in ethanol [25]. This method was employed for the preparation of 4,4′-diselanediyldianiline, which was used as template and coupled to the corresponding sulfonyl chlorides (1:2 or 1:2.5 M ratio) to give the target compounds. The reaction was carried out in dry ether under N_2 atmosphere, at room temperature during 48–72 h. This synthesis was based on a published procedure with modifications [47] following our own protocol. The compounds were obtained in yields ranging from 3.6 to 38.5% and were purified by recrystallization from ethanol/water, washed with dichloromethane, ethyl ether, or water, and one of them was purified by column chromatography. The purity of the compounds was assessed by TLC and elemental analyses and their structures were identified from spectroscopic data. IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elementary analysis methods were used for structure elucidation (Scheme 1). All the signals were fully consistent with proposed structures. In this regard we found an interesting observation in the ^{1}H NMR spectra obtained for compounds **7** and **10**, as they did not show doublets corresponding to the coupling of fluorine and hydrogen signals in the FC=CH

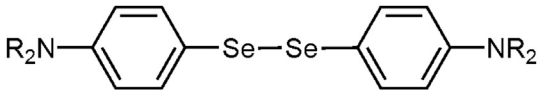

Fig. 1. Formula of 4,4′-diselanediyldianiline.

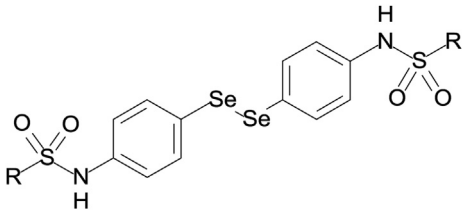

Fig. 2. General structure of hybrid compounds.