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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

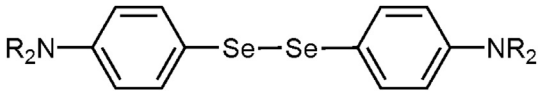


Fig. 1. Formula of 4,4′-diselanediyldianiline.

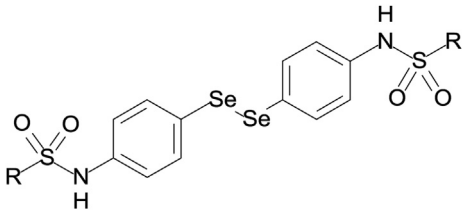
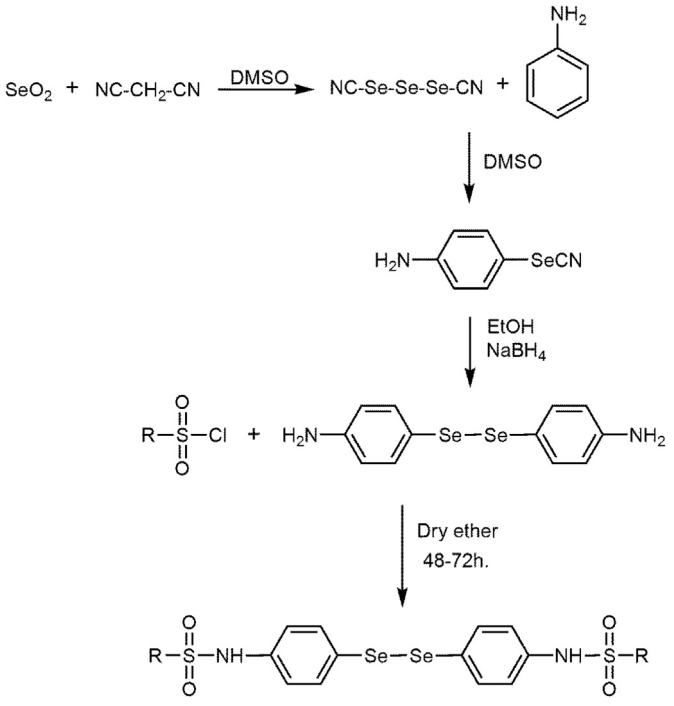


Fig. 2. General structure of hybrid compounds.



Scheme 1. Synthesis of compounds **1**–**16**.

moieties. The strategy for the preparation of sulfonamide derivatives (compounds **1**–**16**) is outlined in Scheme 1.

2.2. Biological evaluation

2.2.1. Activity in axenic amastigotes

The antileishmanial activity of the sixteen synthesized sulfonamides was analyzed against *L. infantum* axenic amastigotes using miltefosine and edelfosine as standard drugs according to a previously described procedure [48]. Analyses were carried out with a minimum of three independent experiments and results were expressed as IC_{50} values. Biological data evidenced that most of the screened compounds (twelve of them) show similar or even higher bioactivity than miltefosine (IC_{50} = 2.84 μM). From a structural point of view, introduction of different substituents on the pendent phenyl group does not modify the activity with the exception of the 4-nitro and 4-chloro substitutions which slightly decrease it. In general, both electron-donating and electron-withdrawing groups are tolerated. We also checked the influence of the introduction of other bulky aryl rings and heteroaryl moieties with known antiparasitic activity. Our results demonstrate that biphenyl (**12**) and naphthyl (**13**) derivatives are inactive whereas the 8-quinolinyl (**14**), 2-thienyl (**15**) and 2-(1-methyl-1*H*-imidazolyl) (**16**) analogs display high activity. Based on the comparison of compounds **7** and **8** (IC_{50} values of 1.47 and 3.63 μM, respectively) with analogs **10** and **11** (IC_{50} values of 1.66 and 1.17 μM) location of the atom of halogen in the *para* (**7** and **8**) or *ortho* (**10** and **11**) position does not seem to markedly influence the antileishmanial activity.

2.2.2. Cytotoxicity

Both high leishmanicidal activity and low cytotoxicity are required in a good antileishmanial drug. Accordingly, cytotoxicity against the THP-1 human cell line was evaluated and the selectivity index (SI) of the compounds was calculated as the ratio of cytotoxicity (IC_{50} value on THP-1 cells) to activity (IC_{50} value on *L. infantum* amastigotes). Compounds **2**, **3**, **6**, **7**, **10**, **11**, and **14** (SI = 18, 14, 9.5, 17, 11, 15, and 30, respectively) outperformed edelfosine and miltefosine (SI = 6 and 7 respectively) with regards to this therapeutic index (Table 1).

2.2.3. Leishmanicidal activity in infected macrophages

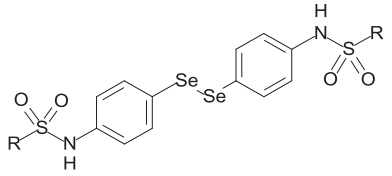
Because of their activity and selectivity, three compounds (**2**, **7** and **14**) were selected for testing their leishmanicidal activity in amastigote-infected THP-1 cells. The IC_{50} for each compound was calculated and displayed in Table 2. The potency of the analogs was compared with edelfosine, a current antileishmanial agent (IC_{50} = 3.1 ± 0.1 μM). We observed that these derivatives are active against the intracellular form of the parasite and reduce the parasite load of the cells exhibiting IC_{50} values of 4.1, 2.8 and 6.2 μM, respectively.

Because of its activity on amastigotes and infected macrophages and on its selectivity against the parasites (therapeutic index > 17), analog **7** seems to be the best compound, which makes it a suitable lead structure for the development of future antiparasitic drugs.

3. Conclusions

A series of sixteen diselenide-sulfonamide hybrids has been designed, synthesized, completely characterized and bioevaluated as a new series of *in vitro* leishmanicides whose activities were compared with the standard drugs miltefosine and edelfosine. All the synthetic derivatives, with the exception of compounds **12** and **13**, exhibited a remarkable inhibition of *L. infantum* amastigotes growth. Compounds **2**, **7** and **14** combined a very good antileishmanial activity with low toxicity against the human cell line THP-1, two properties essential for the development of new drug candidate prototypes. Regarding their structure/activity analysis, no clear-cut correlation was found but, taken together these results suggest that the sulfonamide scaffold could be a valuable linker for the parent diselenide-containing antileishmanial compounds

Table 1
IC_{50} (μM) ± SEM values for the compounds on amastigotes and cytotoxic activity in THP-1 cell line.



Compound	*R*	Amastigote	THP-1	SI[a]
1	phenyl	1.64 ± 0.05	11.9 ± 1.0	7.3
2	4-methylphenyl	1.40 ± 0.09	>25	>18
3	4-methoxyphenyl	1.30 ± 0.08	18.1 ± 0.2	14
4	4-cyanophenyl	1.76 ± 0.30	3.6 ± 0.3	2
5	4-nitrophenyl	3.74 ± 0.11	17.1 ± 0.8	4.6
6	4-trifluoromethylphenyl	2.01 ± 0.62	19.1 ± 0.8	9.5
7	4-fluorophenyl	1.47 ± 0.07	>25	>17
8	4-chlorophenyl	3.63 ± 0.12	>25	>7
9	4-bromophenyl	2.80 ± 0.29	5.2 ± 0.05	2
10	2-fluorophenyl	1.66 ± 0.25	17.8 ± 0.6	10.7
11	2-chlorophenyl	1.17 ± 0.08	17.4 ± 1.5	15
12	4-biphenyl	13.81 ± 0.32	>25	>2
13	2-naphtyl	5.65 ± 0.22	>25	>4.4
14	8-quinolinyl	0.83 ± 0.04	>25	>30
15	2-thienyl	1.54 ± 0.14	8.6 ± 1.1	5.6
16	2-(1-methyl-1*H*-imidazolyl)	1.05 ± 0.07	4.4 ± 0.02	4
Edelfosine	–	0.82 ± 0.13	4.9 ± 0.1	6
Miltefosine	–	2.84 ± 0.10	18.5 ± 0.6	7

[a] Selectivity index (SI) is the ratio of IC_{50} values of compounds against THP-1 cells relative to those against *L. infantum* amastigotes.

Table 2
IC_{50} (μM) ± SEM values for the compounds in amastigote-infected THP-1 cells.

Compound	*R*	IC_{50}
2	4-methylphenyl	4.1 ± 0.1
7	4-fluorophenyl	2.8 ± 0.1
14	8-quinolinyl	6.2 ± 0.9
Edelfosine	–	3.1 ± 0.1

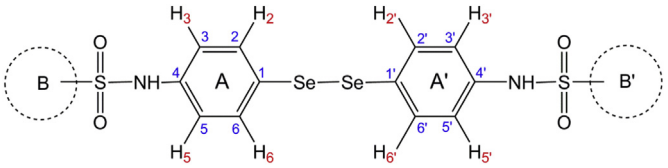


Fig. 3. Assignment for the chemical shifts in NMR spectroscopy.

resulting in hybrid derivatives that can allow synergistic effects. To sum up, the potent leishmanicidal activity, synthetic accessibility and good selectivity strongly encourage further optimization of **2**, **7** and **14** as leads to develop more potent leishmanicidal agents. Furthermore, this work contributes to validate the choice of the molecular symmetry as a useful template to design new active compounds.

4. Experimental protocols

4.1. Chemistry

Melting points were determined with a Mettler FP82 + FP80 apparatus (Greifensee, Switzerland) and are not corrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 Ultrashield™ spectrometer (Rheinstetten, Germany) using TMS as the internal standard. The IR spectra were obtained on a Thermo Nicolet FT-IR Nexus spectrophotometer with KBr pellets. Mass spectrometry was carried out on a MS-DIP, system MSD/DS 5973N (G2577A) Agilent. Elemental microanalyses were carried out on vacuum-dried samples using a LECO CHN-900 Elemental Analyzer. Silica gel 60 (0.040–0.063 mm) 1.09385.2500 (Merck KGaA, 64271 Darmstadt, Germany) was used for Column Chromatography and Alugram® SIL G/UV_{254} (Layer: 0.2 mm) (Macherey–Nagel GmbH & Co. KG. Postfach 101352, D-52313 Düren, Germany) was used for Thin Layer Chromatography. Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma–Aldrich Química, S.A. (Alcobendas, Madrid, Spain), Acros Organics (Janssen Pharmaceuticalaan 3a, 2440 Geel, België) and Lancaster (Bischheim-Strasbourg, France).

4.1.1. General procedure for the synthesis of compounds ***1–16***

To a stirred solution of compound 4,4′-diselanediyldianiline (1 mmol) solved in dry ether (50 mL), different sulfonyl chlorides (2 or 2.5 mmol) were added dropwise (when the sulfonyl chloride was solid, previously, it was solved in dry ether 10 mL). The reaction mixture was stirred at different time intervals (48–72 h) at room temperature under N_2 atmosphere (progress and completion of reaction was confirmed by TLC). Then, a precipitate was produced which was collected by filtration.

One of the following work-up methods was used:

Work-up method A: After 72 h of stirring at room temperature, the precipitate was filtered off, washed and purified in order to obtain the target compounds (**1**–**4**, **6**–**8**, **12**, **14** and **16**).

Work-up method B: Once filtered the solid, the organic layer reaction mixture was treated suitably in order to obtain derivatives **5**, **9**–**11**, **13** and **15**.

In order to assign the chemical shifts in NMR spectroscopy the following assignment has been done (Fig. 3): Central rings A and A′; External Rings B and B′. For derivative **12**, which possesses two rings in the external position have been named B1, B2, B1′ and B2′, respectively.

*4.1.1.1. N,N′-(Diselanediyldibenzene-4,1-diyl)dibenzenesulfonamide (**1**).* From 4,4′-diselanediyldianiline and benzenesulfonyl chloride. The precipitate was washed with water (150 mL), ethyl ether (4 × 25 mL) and recrystallized from ethanol:water (50:50). Yield: 26%; mp: 162–164 °C. IR (KBr) cm^{-1}: 3238 (m, N–H), 1160 (s, SO_2), 820 (m, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.02 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.6$ Hz); 7.38 (d, 4H, A + A′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.6$ Hz); 7.56 (t, 4H, B + B′, $H_3 + H_5$); 7.61 (dd, 2H, B + B′, H_4, $J_{4-3} = 7.4$ Hz, $J_{4-2} = 1.2$ Hz); 7.77 (dd, 4H, B + B′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 7.4$ Hz, $J_{2-4} = J_{6-4} = 1.2$ Hz); 10.50 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.1 (4C, A + A′, C_3, C_5); 125.6 (2C, A + A′, C_1); 127.5 (4C, B + B′, C_2, C_6); 130.2 (4C, B + B′, C_3, C_5); 133.9 (4C, A + A′, C_2, C_6); 134.2 (2C, B + B′, C_4); 138.8 (2C, A + A′, C_4); 140.1 (2C, B + B′, C_1). MS (*m*/*z*, % abundance): 136 (100). Elemental Analysis for $C_{24}H_{20}N_2O_4S_2Se_2$, Calcd/Found (%): C: 46.30/46.21; H: 3.21/3.62; N: 4.50/4.46.

*4.1.1.2. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(4-methylbenzenesulfonamide) (**2**).* From 4,4′-diselanediyldianiline and 4-methylbenzenesulfonyl chloride. The precipitate was washed with dichloromethane (20 mL) and the dichloromethane was evaporated to reduced pressure. Yield: 4%; mp: 89–90 °C. IR (KBr) cm^{-1}: 3235 (m, N–H), 1159 (s, SO_2), 812 (m, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.33 (s, 6H, $2CH_3$); 7.02 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.7$ Hz); 7.34 (d, 4H, B + B′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.2$ Hz); 7.38 (d, 4H, A + A′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.7$ Hz); 7.64 (d, 4H, B + B′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.2$ Hz); 10.43 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.8 (1C, CH_3); 121.1 (4C, A + A′, C_3, C_5); 125.4 (2C, A + A′, C_1); 127.6 (4C, B + B′, C_2, C_6); 130.6 (4C, B + B′, C_3, C_5); 134.1 (4C, A + A′, C_2, C_6); 137.3 (2C, A + A′, C_4); 138.9 (2C, B + B′, C_1); 144.3 (2C, B + B′, C_4). MS (*m*/*z*, % abundance): 184 (100). Elemental Analysis for $C_{26}H_{24}N_2O_4S_2Se_2$, Calcd/Found (%): C: 48.00/47.77; H: 3.69/4.09; N: 4.30/4.20.

*4.1.1.3. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(4-methoxybenzenesulfonamide) (**3**).* From 4,4′-diselanediyldianiline and 4-methoxybenzenesulfonyl chloride. The precipitate was washed with dichloromethane (20 mL) and the dichloromethane was evaporated under reduced pressure. Yield: 5%; mp: 78–79 °C. IR (KBr) cm^{-1}: 3251 (m, N–H), 1156 (s, SO_2), 822 (m, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.79 (s, 6H, $2OCH_3$); 7.02 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.7$ Hz); 7.05 (d, 4H, B + B′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 9.0$ Hz); 7.40 (d, 4H, A + A′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.7$ Hz); 7.70 (d, 4H, B + B′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 9.0$ Hz); 10.36 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.5 (1C, O–CH_3); 115.3 (4C, B + B′, C_3, C_5); 121.0 (4C, A + A′, C_3, C_5); 125.3 (2C, A + A′, C_1); 129.7 (4C, B1 + B1′, C_2, C_6); 131.7 (2C, B + B′, C_1); 134.1 (4C, A + A′, C_2, C_6); 139.0 (2C, A + A′, C_4); 163.4 (2C, B + B′, C_4). MS (*m*/*z*, % abundance): 172 (100). Elemental Analysis for $C_{26}H_{24}N_2O_6S_2Se_2$, Calcd/Found (%): C: 45.75/45.36; H: 3.52/3.82; N: 4.10/3.75.

*4.1.1.4. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(4-cyanobenzenesulfonamide) (**4**).* From 4,4′-diselanediyldianiline and 4-cyanobenzenesulfonyl chloride. The precipitate was washed with ethyl ether (4 × 20 mL) and dried. Yield: 27%; mp: 194–195 °C. IR (KBr) cm^{-1}: 3251 (m, N–H), 1159 (s, SO_2), 834 (m, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.03 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-}$

$_6$ = 8.5 Hz); 7.44 (d, 4H, A + A′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 8.5 Hz); 7.92 (d, 4H, B + B′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 8.4 Hz); 8.05 (d, 4H, B + B′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 8.4 Hz); 10.76 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 116.4 (2C, B + B′, C_4); 118.3 (2C, CN); 121.6 (4C, A + A′, C_3, C_5); 126.3 (2C, A + A′, C_1); 128.3 (4C, B + B′, C_2, C_6); 133.9 (4C, A + A′, C_2, C_6); 134.4 (4C, B + B′, C_3, C_5); 138.0 (2C, A + A′, C_4); 144.2 (2C, B + B′, C_1). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for $C_{26}H_{18}N_4O_4S_2Se_2 \cdot 0.5$ HCl, Calcd/Found (%): C: 45.20/45.33; H: 2.75/2.74; N: 8.11/7.90.

*4.1.1.5. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(4-nitrobenzenesulfonamide) (**5**).* From 4,4′-diselanediyldianiline and 4-nitrobezenesulfonyl chloride. The organic layer was evaporated under reduced pressure and the residue was stirred in water (50 mL) at room temperature during 24 h. The resultant solid was filtered and dried. Yield: 38%; mp: 215–216 °C. IR (KBr) cm^{-1}: 3268 (m, N–H), 1164 (s, SO_2), 835 (m, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.05 (d, 4H, A + A′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 8.7 Hz); 7.45 (d, 4H, A + A′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 8.7 Hz); 8.00 (d, 4H, B + B′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 8.9 Hz); 8.37 (d, 4H, B + B′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 8.9 Hz); 10.81 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.7 (4C, A + A′, C_3, C_5); 125.6 (2C, A + A′, C_1); 128.4 (4C, B + B′, C_3, C_5); 129.1 (4C, B + B′, C_2, C_6); 133.9 (4C, A + A′, C_2, C_6); 137.9 (2C, A + A′, C_4); 145.5 (2C, B + B′, C_1); 150.8 (2C, B + B′, C_4). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for $C_{24}H_{18}N_4O_8S_2Se_2$, Calcd/Found (%): C: 40.45/40.62; H: 2.53/2.78; N: 7.87/7.90.

*4.1.1.6. N,N′-(Diselanediyldibenzene-4,1-diyl)bis[4-(trifluoromethyl) benzenesulfonamide] (**6**).* From 4,4′-diselanediyldianiline and 4-(trifluoromethyl)benzenesulfonyl chloride. The precipitate was washed with water (150 mL) and recrystallized from ethanol:water (80:20). Yield: 28%; mp: 243–244 °C. IR (KBr) cm^{-1}: 3256 (m, N–H), 1161 (s, SO_2), 840 (m, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.04 (d, 4H, A + A′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 8.3 Hz); 7.44 (d, 4H, A + A′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 8.3 Hz); 7.96 (s, 8H, B + B′, H_2 + H_3 + H_5 + H_6); 10.72 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.5 (4C, A + A′, C_3, C_5); 122.8 (2C, CF_3); 125.5 (2C, A + A′, C_1); 126.2 (4C, B + B′, C_3, C_5); 127.5 (4C, B + B′, C_2, C_6); 128.5 (2C, B + B′, C_4); 134.1 (4C, A + A′, C_2, C_6); 138.2 (2C, A + A′, C_4); 144.1 (2C, B + B′, C_1). MS (*m*/*z*, % abundance): 172 (100). Elemental Analysis for $C_{26}H_{18}F_6N_2O_4S_2Se_2$, Calcd/Found (%): C: 41.16/41.46; H: 2.37/2.53; N: 3.69/3.48.

*4.1.1.7. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(4-fluorobenzenesulfonamide) (**7**).* From 4,4′-diselanediyldianiline and 4-fluorobenzenesulfonyl chloride. The precipitate was washed with water (150 mL) and recrystallized from ethanol:water (80:20). Yield: 13%; mp: 181–182 °C. IR (KBr) cm^{-1}: 3279 (m, N–H), 1157 (s, SO_2), 816 (w, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.03 (d, 4H, A + A′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 8.6 Hz); 7.37–7.43 (m, 8H, A + A′, H_2 + H_6, B + B′, H_3 + H_5); 7.82 (m, 4H, B + B′, H_2 + H_6); 10.52 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 116.8 (4C, B + B′, C_3, C_5); 122.1 (4C, A + A′, C_3, C_5); 125.8 (2C, A + A′, C_1); 130.7 (4C, B + B′, C_2, C_6); 133.6 (4C, A + A′, C_2, C_6); 136.5 (2C, B + B′, C_1); 138.6 (2C, A + A′, C_4); 166.5 (2C, B + B′, C_4). MS (*m*/*z*, % abundance): 172 (100). Elemental Analysis for $C_{24}H_{18}F_2N_2O_4S_2Se_2$, Calcd/Found (%): C: 43.76/43.31; H: 2.73/2.83; N: 4.25/4.21.

*4.1.1.8. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(4-chlorobenzenesulfonamide) (**8**).* From 4,4′-diselanediyldianiline and 4-chlorobenzenesulfonyl chloride. The precipitate was washed with water (150 mL) and recrystallized from ethanol:water (80:20). Yield: 7%; mp: 199–200 °C. IR (KBr) cm^{-1}: 3254 (m, N–H), 1158 (s, SO_2), 806 (w, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.02 (d, 4H, A + A′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 8.6 Hz); 7.43 (d, 4H, A + A′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 8.6 Hz); 7.63 (d, 4H, B + B′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 8.5 Hz); 7.75 (d, 4H, B + B′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 8.5 Hz); 10.57 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.8 (4C, A + A′, C_3, C_5); 125.9 (2C, A + A′, C_1); 129.5 (4C, B + B′, C_2, C_6); 130.4 (4C, B + B′, C_3, C_5); 134.1 (4C, A + A′, C_2, C_6); 138.4 (2C, B + B′, C_4); 138.6 (2C, A + A′, C_4); 138.9 (2C, B + B′, C_1). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for $C_{24}H_{18}Cl_2N_2O_4S_2Se_2$, Calcd/Found (%): C: 41.68/41.46; H: 2.60/2.68; N: 4.05/3.96.

*4.1.1.9. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(4-bromobenzenesulfonamide) (**9**).* From 4,4′-diselanediyldianiline and 4-bromobenzenesulfonyl chloride. The organic layer was evaporated under reduced pressure and the residue was recrystallized from ethanol:water (80:20).Yield: 9%; mp: 173–174 °C. IR (KBr) cm^{-1}: 3252 (m, N–H), 1160 (s, SO_2), 816 (w, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.03 (d, 4H, A + A′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 7.1 Hz); 7.43 (d, 4H, A + A′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 7.1 Hz); 7.67 (d, 4H, B + B′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 7.3 Hz); 7.78 (d, 4H, B + B′, H_2 + H_6, $J_{2-3} = J_{HF-HE}$ = 7.3 Hz); 10.36 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.6 (4C, A + A′, C_3, C_5); 126.0 (2C, A + A′, C_1); 127.9 (2C, B + B′, C_4); 129.5 (4C, B + B′, C_2, C_6); 133.3 (4C, B + B′, C_3, C_5); 134.0 (4C, A + A′, C_2, C_6); 138.4 (2C, A + A′, C_4); 139.4 (2C, B + B′, C_1). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for $C_{24}H_{18}Br_2N_2O_4S_2Se_2 \cdot 1$HCl, Calcd/Found (%): C: 34.80/35.27; H: 2.36/2.32; N: 3.26/3.42.

*4.1.1.10. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(2-fluorobenzenesulfonamide) (**10**).* From 4,4′-diselanediyldianiline and 2-fluorobenzenesulfonyl chloride. The organic layer was evaporated under reduced pressure and the residue was solved in water (50 mL). The water phase was extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried with anhydrous sodium sulfate and evaporated to dryness. The sticky residue was recrystallized from ethanol:water (80:20). Yield: 13%; mp: 137–138 °C. IR (KBr) cm^{-1}: 3237 (s, N–H), 1160 (s, SO_2), 816 (m, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.03 (d, 4H, A + A′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 7.9 Hz); 7.37–7.44 (m, 8H, A + A′, H_2 + H_6, B + B′, H_3 + H_5); 7.67–7.71 (m, 2H, B + B′, H_4); 7.82–7.87 (m, 2H, B + B′, H_6); 10.82 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 118.1 (2C, B + B′, C_3); 120.9 (4C, A + A′, C_3, C_5); 125.7 (2C, A + A′, C_1); 127.6 (2C, B + B′, C_5); 131.3 (2C, B + B′, C_1); 134.1 (4C, A + A′, C_2, C_6); 137.0 (2C, B + B′, C_6); 138.2 (2C, A + A′, C_4); 157.7 (2C, B + B′, C_4); 160.2 (2C, B + B′, C_2). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for $C_{24}H_{18}F_2N_2O_4S_2Se_2$, Calcd/Found (%): C: 43.76/44.23; H: 2.74/3.20; N: 4.25/4.23.

*4.1.1.11. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(2-chlorobenzenesulfonamide) (**11**).* From 4,4′-diselanediyldianiline and 2-chlorobenzenesulfonyl chloride. The organic layer was evaporated under reduced pressure and the residue was solved in water (50 mL). The water phase was extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried with anhydrous sodium sulfate and evaporated to dryness. The sticky residue was treated with water (50 mL) during 24 h and the resultant solid was recrystallized from ethanol:water (80:20). Yield: 14%; mp: 200–201 °C. IR (KBr) cm^{-1}: 3279 (m, N–H), 1164 (s, SO_2), 814 (w, Se–Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.01 (d, 4H, A + A′, H_3 + H_5, $J_{3-2} = J_{5-6}$ = 8.6 Hz); 7.36 (d, 4H, A + A′, H_2 + H_6, $J_{2-3} = J_{6-5}$ = 8.6 Hz); 7.53 (m, 4H, B + B′, H_4 + H_5); 7.62 (d, 2H, B + B′, H_3, J_{3-4} = 3.7 Hz); 8.05 (d, 2H, B + B′, H_6, J_{6-5} = 7.8 Hz); 10.82 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.5 (4C, A + A′, C_3, C_5); 125.4 (2C, A + A′, C_1); 128.6 (2C, B + B′, C_5); 131.5 (2C, B + B′, C_6); 132.5 (2C, B + B′, C_3); 132.8 (2C, B + B′, C_2); 134.2 (4C, A + A′, C_2, C_6); 135.7 (2C, B + B′, C_4); 137.1 (2C, A + A′, C_4); 138.2 (2C, B + B′, C_1). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for

$C_{24}H_{18}Cl_2N_2O_4S_2Se_2$, Calcd/Found (%): C: 41.68/41.61; H: 2.60/3.01; N: 4.05/3.71.

*4.1.1.12. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(biphenyl-4-sulfonamide) (**12**).* From 4,4′-diselanediyldianiline and biphenyl-4-sulfonyl chloride. The precipitate was washed with water (150 mL) and recrystallized from ethanol:water (80:20). Yield: 18%; mp: 197–198 °C. IR (KBr) cm^{-1}: 3250 (s, N–H), 1157 (s, SO_2), 806 (m, Se–Se). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.07 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.6$ Hz); 7.43 (d, 4H, A + A′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.6$ Hz); 7.47 (m, 6H, B2 + B2′, $H_3 + H_4 + H_5$); 7.69 (dd, 4H, B2 + B2′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 7.1$ Hz, $J_{2-4} = J_{6-4} = 1.5$ Hz); 7.84–7.87 (m, 8H, B1 + B1′, $H_2 + H_3 + H_5 + H_6$); 10.57 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.8 (4C, A + A′, C_3, C_5); 125.6 (2C, A + A′, C_1); 127.9 (4C, B1 + B1′, C_3, C_5); 129.0 (2C, B2 + B2′, C_4); 129.4 (4C, B1 + B1′, C_2, C_6); 129.6 (4C, B2 + B2′, C_2, C_6); 130.3 (4C, B2 + B2′, C_3, C_5); 133.7 (4C, A + A′, C_2, C_6); 134.6 (2C, B1 + B1′, C_4); 138.8 (2C, A + A′, C_4); 139.0 (2C, B1 + B1′, C_1); 145.3 (2C, B2 + B2′, C_1). MS (*m*/*z*, % abundance): 169 (100). Elemental Analysis for $C_{36}H_{28}N_2O_4S_2Se_2$, Calcd/Found (%): C: 55.81/55.35; H: 3.62/3.51; N: 3.61/3.58.

*4.1.1.13. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(naphthalene-2-sulfonamide)·0.5 hydrochloride (**13**).* From 4,4′-diselanediyldianiline and naphthalene-2-sulfonyl chloride. The organic layer was evaporated under reduced pressure and the residue was stirred in water (50 mL) at room temperature during 24 h. The resultant solid was recrystallized from ethanol:water (80:20). Yield: 36%; mp: 97–98 °C. IR (KBr) cm^{-1}: 3249 (m, N–H), 1158 (s, SO_2), 806 (m, Se–Se). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.04 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.6$ Hz); 7.33 (d, 4H, A + A′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.6$ Hz); 7.61–7.68 (m, 4H, B + B′, $H_4 + H_5$); 7.75 (dd, 2H, B + B′, H_8, $J_{8-7} = 8.0$ Hz, $J_{8-2} = 2.0$ Hz); 7.99 (d, 2H, B + B′, H_7, $J_{7-8} = 8.0$ Hz); 8.09 (dd, 4H, B + B′, $H_3 + H_6$, $J_{3-4} = J_{6-5} = 13.6$ Hz, $J_{3-5} = J_{6-4} = 8.4$ Hz); 8.45 (s, 2H, B + B′, H_2); 10.59 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.6 (4C, A + A′, C3, C5); 122.7 (2C, B + B′, C3); 125.8 (2C, A + A′, C1); 128.7 (2C, B + B′, C1); 129.8 (2C, B + B′, C6); 130.0 (2C, B + B′, C9); 130.1 (2C, B + B′, C7); 130.3 (2C, B + B′, C8); 130.5 (2C, B + B′, C4); 132.3 (2C, B + B′, C10); 133.5 (4C, A + A′, C2, C6); 134.6 (2C, B + B′, C2); 135.1 (2C, B + B′, C5); 138.7 (2C, A + A′, C4). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for $C_{32}H_{24}N_2O_4S_2Se_2 \cdot 0.5HCl$, Calcd/Found (%): C: 51.87/51.63; H: 3.37/3.77; N: 3.78/3.96.

*4.1.1.14. N,N′-(Diselanediyldibenzene-4,1-diyl)diquinoline-8-sulfonamide (**14**).* From 4,4′-diselanediyldianiline and quinoline-8-sulfonyl chloride. The precipitate was recrystallized from ethanol:water (80:20). Yield: 4%; mp: 226–228 °C. IR (KBr) cm^{-1}: 3268 (m, N–H), 1164 (s, SO_2), 835 (m, Se–Se). ^1H NMR (400 MHz, DMSO-d_6) δ: 6.95 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.5$ Hz); 7.17 (d, 4H, A + A′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.5$ Hz); 7.68–7.72 (m, 4H, B + B′, $H_6 + H_3$); 8.27 (dd, 2H, B + B′, H_5, $J_{5-6} = 8.2$ Hz, $J_{5-7} = 1.3$ Hz); 8.37 (dd, 2H, B + B′, H_7, $J_{7-6} = 7.3$ Hz, $J_{7-5} = 1.3$ Hz); 8.50 (dd, 2H, B + B′, H_4, $J_{4-3} = 8.4$ Hz, $J_{4-2} = 1.7$ Hz); 9.11 (dd, 2H, B + B′, H_2, $J_{2-3} = 4.2$ Hz, $J_{2-4} = 1.7$ Hz); 10.30 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.2 (2C, B + B′, C_3); 121.6 (4C, A + A′, C_3, C_5); 123.5 (2C, B + B′, C_8); 125.0 (2C, A + A′, C_1); 126.5 (2C, B + B′, C_7); 129.2 (2C, B + B′, C_5); 133.2 (4C, A + A′, C_2, C_6); 134.4 (2C, B + B′, C_6); 135.8 (2C, B + B′, C_4); 138.9 (2C, A + A′, C_4); 143.5 (2C, B + B′, C_9); 151.9 (2C, B + B′, C_{10}); 152.7 (2C, B + B′, C_2). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for $C_{30}H_{22}N_4O_4S_2Se_2$, Calcd/Found (%): C: 49.72/49.56; H: 3.03/3.11; N: 7.73/7.69.

*4.1.1.15. N,N′-(Diselanediyldibenzene-4,1-diyl)ditiophene-2-sulfonamide (**15**).* From 4,4′-diselanediyldianiline and thiophene-2-sulfonyl chloride. The organic layer was evaporated under reduced pressure and the residue was stirred in water (50 mL) at room temperature during 24 h. The resultant solid was chromatographed on silica gel (toluene:dioxane, 1:1). Yield: 5%; mp: 114–115 °C. IR (KBr) cm^{-1}: 3246 (m, N–H), 1154 (s, SO_2), 814 (w, Se–Se). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.08 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.6$ Hz); 7.12 (dd, 2H, B + B′, H_4, $J_{4-3} = 4.9$ Hz, $J_{4-5} = 3.8$ Hz); 7.45 (d, 4H, A + A′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.6$ Hz); 7.56 (dd, 2H, B + B′, H_5, $J_{5-4} = 3.8$ Hz, $J_{5-3} = 1.3$ Hz); 7.90 (dd, 2H, B + B′, H_3, $J_{3-4} = 4.9$ Hz, $J_{3-5} = 1.3$ Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.6 (4C, A + A′, C_3, C_5); 126.0 (2C, A + A′, C_1); 128.5 (2C, B + B′, C_5); 133.5 (4C, A + A′, C_2, C_6); 134.2 (2C, B + B′, C_4); 134.4 (2C, B + B′, C_3); 138.6 (2C, A + A′, C_4); 140.6 (2C, B + B′, C_2). MS (*m*/*z*, % abundance): 177 (100). Elemental Analysis for $C_{20}H_{16}N_2O_4S_4Se_2$, Calcd/Found (%): C: 37.85/38.02; H: 2.52/2.86; N: 4.41/4.09.

*4.1.1.16. N,N′-(Diselanediyldibenzene-4,1-diyl)bis(1-methyl-1H-imidazole-2-sulfonamide)·0.5 hydrochloride (**16**).* From 4,4′-diselanediyldianiline and 1-methyl-1*H*-imidazole-2-sulfonyl chloride. The precipitate was washed with water (150 mL) and recrystallized from ethanol:water (80:20). Yield: 8%; mp: 244–245 °C. IR (KBr) cm^{-1}: 3249 (m, N–H), 1145 (s, SO_2), 825 (m, Se–Se). ^1H NMR (400 MHz, DMSO-d_6) δ: 3.80 (s, 6H, N–CH_3); 7.02 (s, 2H, B + B′, H_4), 7.10 (d, 4H, A + A′, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.1$ Hz); 7.41 (s, 2H, B + B′, H_5); 7.47 (d, 4H, A + A′, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 8.1$ Hz); 10.99 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 35.4 (2C, N–CH_3); 121.5 (4C, A + A′, C_3, C_5); 125.8 (2C, A + A′, C_1); 127.5 (2C, B + B′, C_5); 128.7 (2C, B + B′, C_4); 133.9 (4C, A + A′, C_2, C_6); 138.2 (2C, A + A′, C_4); 142.5 (2C, B + B′, C_2). MS (*m*/*z*, % abundance): 57 (100). Elemental Analysis for $C_{20}H_{20}N_6O_4S_2Se_2 \cdot 0.5HCl$, Calcd/Found (%): C: 36.99/36.90; H: 3.15/3.51; N: 12.94/12.52.

4.2. Biological evaluation

4.2.1. Cells and culture conditions

L. infantum axenic amastigotes were grown in M199 (Invitrogen, Leiden, The Netherlands) medium supplemented with 10% heat inactivated FCS, 1 g/L β-alanine, 100 mg/L L-asparagine, 200 mg/L sacarose, 50 mg/L sodium pyruvate, 320 mg/L malic acid, 40 mg/L fumaric acid, 70 mg/L succinic acid, 200 mg/L α-ketoglutaric acid, 300 mg/L citric acid, 1.1 g/L sodium bicarbonate, 5 g/L MES, 0.4 mg/L hemin, 10 mg/L gentamicin pH 5.4 at 37 °C. THP-1 cells were kindly provided by Dr. Michel (Université Nice Sophia Antipolis, Nice, France) and were grown in RPMI-1640 medium (Gibco, Leiden, The Netherlands) supplemented with 10% heat inactivated FCS, antibiotics, 1 mM HEPES, 2 mM glutamine and 1 mM sodium pyruvate, pH 7.2 at 37 °C and 5% CO_2.

4.2.2. Leishmanicidal activity and cytotoxicity assays

Drug treatment of amastigotes was performed during the logarithmic growth phase at a concentration of 2×10^6 parasites/mL at 26 °C or 1×10^6 parasites/mL at 37 °C for 24 h, respectively. Drug treatment of THP-1 cells was performed during the logarithmic growth phase at a concentration of 4×10^5 cells/mL at 37 °C and 5% CO_2 for 24 h. The percentage of living cells was evaluated by flow cytometry by the propidium iodide (PI) exclusion method [49].

4.2.3. Leishmania infection assay

THP-1 cells were seeded at 120.000 cells/mL in 24 multidishes plates (Nunc, Roskilde, Denmark) and differentiated to macrophages for 24 h in 1 mL of RPMI-1640 medium containing 10 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma–Aldrich, St. Louis, MO, USA). Medium culture was removed and 1.2×10^6 *Leishmania* amastigotes expressing the eGFP protein were added to each well reaching a final volume of 1 mL. 4 h later all medium with non

infecting amastigotes was removed, wells were washed 3 times with phosphate buffered saline (PBS) and cells were grown in the presence of new THP-1 medium and the corresponding treatment. After 48 h of treatment, medium was removed; THP-1 cells were washed 3 times with PBS and detached with TrypLE™ Express (Invitrogen, Leiden, The Netherlands) according to the manufacturer's indications. Rates of infection were measured by flow cytometry according to established protocols [24].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.12.030.

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