New amides containing selenium as potent leishmanicidal agents targeting trypanothione reductase

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Abstract

Two new series of twenty-eight selenocyanate and diselenide derivatives containing amide moiety were designed, synthesized and evaluated for their leishmanicidal activity against *Leishmania infantum* axenic amastigotes, and selectivity was assessed in human THP-1 cells. Eleven compounds exhibited excellent leishmanicidal activity with EC_{50} values lower than the reference drug miltefosine (EC_{50} = 2.84 µM). In addition, for six of them the selectivity index ranged from 9 to > 1442, greater than both references used. The most potent and selective compounds were **2h**, **2k** and **2m** that displayed EC_{50} values of 0.52, 1.19 and 0.50 µM and a high selectivity index (SI) when tested against THP-1 monocytic cells (SI = >1442, > 672 and >1100, respectively). These derivatives showed an efficacy similar to that of the reference drugs but much better SI. They also showed very interesting activity values against infected macrophages. Trypanothione reductase (TryR) activity and intracellular thiol level measurement assays were performed for the three best compounds in an attempt to elucidate their mechanism of action. Even though the new analogues exhibited comparable or better inhibitory activities than reference TryR inhibitors more studies are necessary to confirm this target. To sum up, our findings suggest that the three presented compounds could constitute lead leishmanicidal drug candidates.

Keywords: amides, diselenide, selenocyanate, thiols, trypanothione reductase

INTRODUCTION

Leishmaniasis, caused by protozoan parasites from the *Leishmania* genus, is one of the most devastating neglected tropical diseases*(1)*. These parasites belong to the *Trypanosomatidae* family and include more than 21 species with capacity of infecting humans(2). The clinical manifestations are classified into at least four major forms, including cutaneous, diffused cutaneous, muco-cutaneous and visceral leishmaniasis(3).

Actually, available treatments for leishmaniasis include pentavalent antimonials as first-line drugs, and amphotericin B, pentamidine, paromomycin, and miltefosine as second-line drugs(4). However, most of these drugs exhibit high toxicity, easily induce resistance, are responsible for severe side effects, and show increased incidence of treatment failure(5). Furthermore, there is currently no effective vaccines against this disease(6). These facts clearly emphasize the urgent need for the development of novel chemotherapies against leishmaniasis.

Currently, several investigations have been conducted to find alternative treatments for leishmaniasis and the exploitation of selenium derivatives is a viable approach. In this context, from the literature we noted the importance of selenium for the survival of these parasites acting through immune response modulation(7), alone or in combination therapy with amphotericin B(8) or glucantime(9). Moreover, selenium can interfere with redox-system enzymes such as selenophosphate synthetases(10), ascorbate peroxidases(11) or the well-known parasitic trypanothione reductase(12) or superoxide dismutase(13). Furthermore, computational analyses conducted over the genomes of several *Leishmania* species have revealed the presence of at least three

selenoproteins: Sel K, Sel T and a kinetoplastida specific Sel Tryp, which evidence the dependence of the parasites for this trace element(14).

In addition, our research group has published several articles which demonstrate that different selenium containing frameworks such as methylseleno, selenocyanate, diselenide or selenosulfonamide, incorporated in different organic structures showed potent leishmanicidal activity (12, 15-18).

Among these organoselenium compounds, selenocyanate and diselenide motifs are fascinating because of the promising results in terms of activity and selectivity(19, 20).

Prompted by the previous reported results regarding the activity of 4-aminophenylselenocyanate and bis(4-aminophenyl)diselenide we have focused on these nuclei to synthesize the compounds reported in this work. Herein we describe the synthesis and characterization of two new series containing 4-aminophenylselenocyanate and bis(4-aminophenyl)diselenide entities derivatized using an amide group as linkage. The amide based ligand has been selected for the current study due to its hydrophilic character with the aim to increase water solubility of the new compounds, improve their physico-chemical properties and for its ability to generate prodrugs that may increase drug concentration in the desired targets(21, 22). These amides have been decorated with varied substituents in order to obtain synthetic species with progressive degree of complexity in terms of electronic and steric properties or 3D structural characteristics. Among these scaffolds, different substituted phenyl rings along with carbo- and hetero-cycles with probed leishmanicidal properties have been explored(19, 20, 23). Herein we compare the effects of the following fragments: cinnamyl(24), naphtyl(25), isoxazolyl(26) and

adamantyl(27). In addition, the incorporation of an adamantyl moiety into several molecules results in compounds with relatively high lipophilicity which, in turn, can modify the biological availability of these molecules. The structures of the reported compounds are summarized in Figure 1.

(Figure 1)

Enzymes like trypanothione reductase (TryR)(28) have emerged among the most relevant druggable targets in *Leishmania* and other Trypanosomatids because of their highly relevant role in the maintenance of the parasite's redox homeostasis. The enzyme fulfills most of the more relevant requirements to be an interesting drug target since it is essential for parasite survival, it is druggable and because of its absence in the host, were is replaced by glutathione reductase(29). Trypanothion reductase acts catalyzing the reduction of trypanothione disulfide to trypanothione based on the ability of sulfur to act as donor or an acceptor of electrons. In this sense, several sulfides have demonstrated the capacity of killing leishmanial parasites binding this enzyme by competing with the substrate(30). Due to the chemical analogy between sulfur and selenium we decided to explore the ability of this molecules inhibiting TryR. Moreover, previous studies of our research group demonstrated the interesting inhibitory activity of seleno-compounds, including selenocyanates and diselenides, on TryR enzyme(23).

On the bases of the above mentioned, the leishmanicidal activity of the 28 resulting compounds was determined against the amastigote form of *L. infantum* and the cytotoxicity of these newly synthesized molecules was also assessed on one different complementary human cell line (THP-1) in order to select those compounds with high selectivity. Moreover, leishmanicidal activity

of the most active compounds was evaluated in infected macrophages. Finally, in order to elucidate the underlying molecular mechanisms, the inhibitory activity against trypanothione reductase (TryR) and their effect on intracellular thiol concentration was determined.

RESULTS

Chemistry

The target compounds **1a-1n** and **2a-2n** were synthesized in accordance with the procedures outlined in Figure 2.

(Figure 2)

The intermediates 4-aminophenylselenocyanate (**0A**) and bis-(4-aminophenyl)diselenide (**0B**) were achieved as previously reported(31). The treatment of 4-aminophenylselenocyanate with the corresponding acyl chlorides in a molar ratio 1:1 in chloroform at room temperature during 12-24h yielded derivatives **1a-1n**. The products were isolated from reaction solutions by filtration and purified by recrystallization from methanol or a mixture of methanol/water.

The structures of the final compounds were confirmed by infrared (IR), nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) as well as mass spectrometry and elemental analysis. Aromatic signals appear at δ_H 6.72-8.15 ppm depending on the nature of substituent attached. ^{13}C NMR spectra show the typical C=O signal between δ_C 156.8-176.7 ppm (see supplementary material).

The synthesis of the target compounds **2a-2c**, **2e-2j**, **2l** and **2n** was carried out by reaction of the corresponding selenocyanates, dissolved in ethanol, and sodium borohydride at room temperature. The reaction mixture was evaporated under reduced pressure and the final residue was extracted with

dichloromethane. The target compounds were purified by recrystallization. Analogues **2d**, **2k** and **2m** were obtained by reaction between bis-(4-aminophenyl)diselenide and the corresponding acyl choride in chloroform. The reaction yields are moderated ranging from 46-68%. Formation of diselenide was confirmed by the disappearance of the SeCN peak at a wavenumber around 2147-2226 cm^{-1} in infrared spectroscopy.

Biological evaluation

(i) Activity in amastigotes and cytotoxic activity in human cells. The newly synthesized compounds along with miltefosine and edelfosine (used as positive controls) were tested *in vitro* against cultured *L. infantum* amastigotes following a previously described method(19). Axenic amastigotes have been chosen because of their high similarity with the parasite stage that develops infections in humans. Parent compounds 4-aminophenylselenocyanate (**0A**) and the corresponding Bis(4-aminophenyl)diselenide (**0B**) were also investigated in order to compare their biological response. Additionally, and to assess their selectivity, these compounds were tested against a human monocytic leukemia cell line (THP-1). The results shown in Table 1 reveal that eleven compounds (**1a**, **1d**, **1h**, **1i**, **1j**, **1k**, **2h**, **2i**, **2j**, **2k** and **2m**) exhibit potent antileishmanial activity with EC_{50} values similar or lower than those of miltefosine (EC_{50} = 2.84 µM) used as reference. Compared to edelfosine, the other reference drug used in this study, **1h**, **2h**, **2i**, **2j**, and **2m** also fulfil this standard. Regarding the difference of activity between the selenocyanate and the diselenide analogs, globally considered, it is difficult to infer a general trend that either increases or reduces the activity. In order to get insight into the structure activity relationship, it has been found that the diselenide compounds derived from *p*-substituted

phenyl ring **2a-2g** and **2n** were less potent than the others (Table 1). This effect is less marked in the corresponding selenocyanates. Compounds containing heteroaryl rings on "R", *i.e*. **1h**, **1i**, **1j**, **2h**, **2i** and **2j** displayed potent activities with EC_{50} values between 0.52 and 1.96 µM. On the other hand, introduction of rigid and bulky moieties such as adamantyl (**1k**, **2k**) or naphtyl (**1m**, **2m**) reflected different behaviors. While the adamantyl motif increased the leishmanicidal activity both in selenocyanates and diselenides, the naphtyl entity was effective only with diselenide function. Compared with the chosen parent compound selenocyanate (EC_{50} = 9.29 µM), twelve of the fourteen compounds synthesized were more potent, whereas four of the diselenide derivatives were comparable to their corresponding parent molecule.

In terms of selectivity, compounds **1d**, **1h**, **1i**, **2h**, **2k** and **2m** showed SI values that ranged from 9 to > 25, greater than those of the reference drugs edelfosine and miltefosine (SI = 6 and 7, respectively). Being **2h**, **2k** and **2m** the most promising derivatives (Figure 3), they were further tested against THP-1 cells at higher concentrations in order to determine their real EC_{50} values. Interestingly, derivatives **2h** (EC_{50} = 0.52 µM, SI > 1442), **2k** (EC_{50} = 1.19 µM, SI > 672.3) and **2m** (EC_{50} = 0.50 µM, SI > 1100) are between 112 and 240-fold less toxic than edelfosine while showing similar or even better leishmanicidal activity.

(Table 1)

(Figure 3)

(ii) Leishmanicidal activity in infected macrophages. After this first screening and taking into account activity and selectivity data, compounds **2h**, **2k** and **2m** were selected for the analysis of their leishmanicidal activity on infected macrophages. Again, edelfosine was used as reference drug. The ED_{50}

for each compound was calculated as mean of three independent experiments and summarized in Table 2. These compounds reduced the parasite load of the cells comparing with the non-treated infected cells (Figure 4), exhibiting ED_{50} values of 2.20, 3.77 and 1.03 μM respectively, with similar or better effectiveness than that obtained for edelfosine (ED_{50} = 3.10 μM). Taking into account that selectivity is one of the most relevant characteristics to be achieved in new leishmanicidal drugs, the excellent SI values obtained for the three selected compounds are highly remarkable.

(Figure 4)

(Table 2)

(iii) Inhibition of *L. infantum* trypanothione reductase activity. As last topic, these derivatives have been considered as promising leads for a preliminary study of their mechanism of action. Initially, we focused on the alteration of the redox system.

In an effort to assess the mechanism by which **2h**, **2k** and **2m** inhibit *Leishmania* growth, we evaluated their activity against TryR, an essential enzyme involved in parasite detoxification of reactive oxygen species(32). Trypanothione reductase is an essential enzyme in trypanosomatids (*T. brucei, T. cruzi*, and *Leishmania* species) that has a unique role in the trypanothione-based redox metabolism and oxidant defense. Lack of this enzyme in the mammalian hosts is one of the reasons that turned it very attractive as drug-target(29).

The well stablished TryR inhibitor mepacrine was used as positive control. (Table 3).

(Table 3)

The results from the *in vitro* assays revealed that compounds **2h** and **2k** exhibit an inhibitory capacity against TryR comparable to that of the reference drug. Moreover, **2m** derivative is 3-fold more active than mepacrine. In spite of these results, numerous TryR inhibitors have been found to have only a modest effect on parasite growth. A partial explanation was provided by Krieger *et al.* who produced conditioned TryR knockout in *T. brucei*(33). They have shown that the redox metabolism of the parasite was affected only when TryR was titrated down to less than 5% of normal(34).

(iv) Redox system alteration. To further confirm the alteration of the parasite redox system by these molecules, intracellular thiol levels was determined inside the parasites by flow cytometry. As shown in Figure 5, treatment of the amastigotes for 1 hour with the three selected molecules triplicates the levels of intracellular thiols.

(Figure 5)

(v) ADME and Lipinski properties. Finally, considering that most of the new drug candidates fail in clinical trials due to reduced ADME properties, we predicted the ADME parameters for lead compounds (**2h**, **2k** and **2m**) using the PreADMET software. The values obtained were compared with the Lipinski rule of five although it is well-known that pharmacologically active compounds do not necessarily fulfill all rules. Molecular weight (MW), number of hydrogen donors (DHB), number of hydrogen acceptors (AHB), octanol/water partition coefficient (log P), apparent Caco-2 cell permeability (PCaco), and percent of human intestinal absorption (%HIA) are presented in Table 4(35, 36).

(Table 4)

The three compounds violate just two of the Lipinksi's rule of five, similar to what occurs with the most clinically relevant reference drug (miltefosine). Oral bioavailability according to VEBER and to EGAN was good for all compounds too. Moreover, all of the compounds passed the three PAINS filters, which may help to detect compounds that are unspecific assay-interfering compounds. We also used PreADMET (http://preadmet.bmdrc.org/preadmet/index.php) web-application for a rapid prediction of ADME/Tox data. Caco-2 permeability and Human Intestinal Absorption (HIA) are interesting indicators of drug absorbance. The predicted percentages of intestinal absorption are quite good for all of the compounds, with values over 97%. Otherwise, the three compounds presented middle permeability values in Caco-2 cells ranging from 38 to 53.

DISCUSSION

Selenium compounds are used as potential treatments for multiples diseases such as cancer(37) Alzheimer(38) or cardiovascular diseases(39) among others.

In addition, selenium derivatives have emerged as promising compounds against leishmaniasis and other trypanosomiasis. Previous studies of our research group demonstrated the interesting leishmanicidal activity of selenocyanate and diselenide derivatives. Moreover, several selenium containing compounds have demonstrated its trypanocidal activity against other trypanosomiasis like Chagas(40-42) or African trypanosomiasis(7, 43) The twenty-eight new compounds presented in this work contain the 4-aminopheylselenocyanate (**1a-n**) or the bis(4-aminophenyl)diselenide (**2a-n**) moieties based on the promising leishmanicidal activity reported for them.

These cores have been joined by a linker fragment, which involves a new amide bond, to a different substituted phenyl rings, heterocycles, and bulky carbocycles such as adamantyl or naphtyl. Overall, if we compared with the parent compound **0A**, thirteen of the fourteen (with the exception of **1e**) selenocyanates exhibited similar or better leishmanicidal activity than **0A**. If we focused in diselenides only four derivatives (**2h**, **2i**, **2j** and **2m**) showed comparable activity to the parent **0B** in amastigotes form. However, it is remarkable the excellent SI for diselenides, especially for **2h**, **2k** and **2m**. These data helped to select these three compounds as the best candidates for further studies. Compounds **2k** and **2m** share a bulky carbocyclic substituent (adamantyl and naphtyl, respectively) in their structure, while **2h** possess a heteroaryl monocyclic entity. The studies in infected macrophages revealed that all of them exhibited the same (**2k**) or even better (**2h, 2m**) ED_{50} values than edelfosine. Regarding their possible mechanism of action and taking into account that selenium compounds used to alter the redox system(44), intracellular thiol levels were evaluated in treated parasites. Similarly, their inhibitory activity against *L. infantum* trypanothione reductase (TryR) was determined. Trypanothion reductase emerged as an interesting target for its critical role in the trypanosomatidae family parasite's redox system control and because humans lack this enzyme(29, 45). Strong correlation between intracellular thiols disturbance, TryR inhibition and antiparasitic activity was observed for the three compounds, which may be considered as an evidence of their possible mechanism of action. Parasites, in response to an oxidative stress, seem to induce *the novo* synthesis of trypanothion and that could be the reason why the concentrations of thiols rise up. Moreover, presented

299 compounds improved the results of activity against TryR obtained so far among
300 previous selenocompounds of our research group(23). The calculated ADME
301 parameters for compounds **2h**, **2k** and **2m** predicted a good bioavailability, with
302 low Lipinski's rule violations, similar or even better (**2h**) solubility (LogP), and
303 high intestinal absorption prediction values. It is well-known that current
304 available drugs for leishmania treatment are not fully effective in all cases,
305 having only parenteral use and only miltefosine is the oral drug approved.
306 However, the appearance of resistance led to change its use from monotherapy
307 to combination therapy(46). For these reasons, the incorporation of new
308 strategies or compounds that can improve the oral bioavailability is an urgent
309 need(47).
310 In this context, the compounds described herein are promising candidates.
311 Moreover, they are also easily affordable and economical in their synthesis.
312 Further *in vivo* toxicity and efficiency studies will be carried out with these
313 promising molecules in order to confirm their potent and selective
314 antileishmanial activity. A graphical summary of the discussion drawn from this
315 work is depicted in Figure 6.

316 **(Figure 6)**

317 **MATERIAL AND METHODS**

318 **Chemistry**

319 Melting points (mp) were determined with a Mettler FP82 + FP80 apparatus
320 (Greifensee, Switzerland). Proton (^{1}H), carbon (^{13}C) and selenium (^{77}Se) NMR
321 spectra were recorded on a Bruker 400 Avance Neo spectrometer
322 (Rheinstetten, Germany) using DMSO-d_6 as solvent. IR spectra were recorded
323 on a Thermo Nicolet FTIR Nexus spectrophotometer using KBr pellets for solid

samples or NaCl plates for liquid compounds. Elemental analysis was performed on a LECO CHN-900 Elemental Analyzer. Purity of all final compounds was 95 % or higher. TLC assays were carried out in Alugram SIL G7UV254 sheets (Macherey-Nagel, Düren, Germany). Chemicals were purchased from E. Merck (Darmstadt, Germany), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Quimica, S.A. (Alcobendas, Madrid, Spain) and Acros Organics (Janssen Pharmaceuticalaan, Geel, Belgium).

General procedure of synthesis for compounds 1a-n

Compounds **1a-n** were obtained by reaction between 4-aminophenylselenocyanate and the corresponding acyl chloride, in a 1:1 molar ratio and in dry chloroform (50 mL) as solvent. The mixture was stirred at room temperature during 12-24 h. A precipitate was formed that was filtered and recrystallized from methanol or a mixture of methanol/water.

(Figure 7)

***N*-(4-selenocyanatophenyl)benzamide (1a).** From benzoyl chloride. Recrystallized from methanol. White powder; mp: 169-170 ºC. Yield: 56 %. IR (KBr) cm^{-1}: 3342 (N-H); 2154 (CN); 1663 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.52 – 7.59 (m, 3H, B, H_3+H_4+H_5), 7.72 (d, 2H, A, J_{2-3} = J_{5-6} = 8.4 Hz, H_2+H_6), 7.89 (d, 2H, A, H_3+H_5), 7.97 (d, 2H, B, H_2+H_6), 10.46 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.8 (1C, SeCN), 117.5 (1C, A, C_1), 122.0 (2C, A, C_3+C_5), 128.2 (2C, B, C_2+C_6), 128.9 (2C, B, C_3+C_5), 132.3 (2C, B, C_1+C_4), 135.1 (2C, A, C_2+C_6), 141.0 (1C, A, C_4), 166.3 (1C, C=O). MS (*m/z* % abundance): 302 (55; $M^{+}\cdot$), 105 (98), 77 (100). Elemental Analysis for $C_{14}H_{10}N_2OSe$, Calculated/Found (%): C: 55.81/55.47; H: 3.32/3.69; N: 9.30/9.21.

Antimicrobial Agents and Chemotherapy

4-chloro-*N*-(4-selenocyanatophenyl)benzamide (1b). From 4-chlorobenzoyl chloride. Recrystallized from methanol. White powder; mp: 226-227 ºC. Yield: 62 %. IR (KBr) cm^{-1}: 3322 (N-H); 2151 (CN); 1674 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.63 (d, 2H, A, $J_{2-3} = J_{5-6} = 8.3$ Hz, H_2+H_6), 7.72 (d, 2H, B, $J_{2-3} = J_{5-6} = 8.5$ Hz, H_3+H_5), 7.87 (d, 2H, B, H_2+H_6), 8.00 (d, 2H, A, H_3+H_5), 10.51 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.8 (1C, SeCN), 117.7 (1C, A, C_1), 122.1 (2C, A, C_3+C_5), 129.0 (2C, B, C_3+C_5), 130.2 (2C, B, C_2+C_6), 133.7 (1C, B, C_1), 135.0 (2C, A, C_2+C_6), 137.1 (1C, B, C_4), 140.8 (1C, A, C_4), 165.2 (1C, C=O). MS (*m/z* % abundance): 336 (20; $M^{+}\cdot$), 139 (100). Elemental Analysis for $C_{14}H_9ClN_2OSe$, Calculated/Found (%): C: 50.07/50.36; H: 2.68/3.05; N: 8.34/8.48.

4-methoxy-*N*-(4-selenocyanatophenyl)benzamide (1c). From 4-methoxybenzoyl chloride. Recrystallized from methanol. Green powder; mp: 167-168 ºC. Yield: 52 %. IR (KBr) cm^{-1}: 3394 (N-H); 2963 (C-H); 2164 (CN); 1662 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.85 (s, 3H, OCH_3), 7.08 (d, 2H, B, $J_{2-3} = J_{5-6} = 8.7$ Hz, H_3+H_5), 7.71 (d, 2H, B, $J_{2-3} = J_{5-6} = 8.6$ Hz, H_3+H_5), 7.88 (d, 2H, B, H_2+H_6), 7.98 (d, 2H, A, H_3+H_5), 10.30 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.9 (OCH_3), 105.8 (1C, SeCN), 114.1 (2C, B, C_3+C_5), 117.1 (1C, A, C_1), 122.0 (2C, A, C_3+C_5), 127.0 (1C, B, C_1), 130.2 (2C, B, C_2+C_6), 135.1 (2C, A, C_2+C_6), 141.2 (1C, A, C_4), 162.6 (1C, B, C_4), 165.6 (1C, C=O). MS (*m/z* % abundance): 332 (25; $M^{+}\cdot$), 135 (100), 77 (45). Elemental Analysis for $C_{15}H_{12}N_2O_2Se$, Calculated/Found (%): C: 54.38/54.49; H: 3.62/3.88; N: 8.46/8.32.

4-cyano-*N*-(4-selenocyanatophenyl)benzamide (1d). From 4-cyanobenzoyl chloride. Recrystallized from methanol. Yellow cotton-like powder; mp: 218-219

ºC. Yield: 63 %. IR (KBr) cm^{-1}: 3303 (N-H); 2226 (CN); 2157 (Se-CN); 1674 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.74 (d, 2H, A, J_{2-3} = J_{5-6} = 8.5 Hz, H_2+H_6), 7.87 (d, 2H, A, H_3+H_5), 8.04 (d, 2H, B, J_{2-3} = J_{5-6} = 8.2 Hz, H_2+H_6), 8.12 (d, 2H, B, H_3+H_5), 10.68 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.8 (1C, SeCN), 114.5 (1C, B, C_4), 118.1 (1C, CN), 118.7 (1C, A, C_1), 122.1 (2C, A, C_3+C_5), 129.1 (2C, B, C_2+C_6), 133.0 (2C, B, C_3+C_5), 135.0 (2C, A, C_2+C_6), 139.0 (1C, B, C_1), 140.5 (1C, A, C_4), 164.9 (1C, C=O). MS (*m/z* % abundance): 327 (20; M^{+}·), 130 (100), 102 (40). Elemental Analysis for $C_{15}H_9N_3OSe$, Calculated/Found (%): C: 55.24/54.82; H: 2.76/2.79; N: 12.88/12.44.

4-nitro-*N*-(4-selenocyanatophenyl)benzamide (1e). From 4-nitrobenzoyl chloride. Recrystallized from methanol/water (1:1). Green powder; mp: 235-236 ºC. Yield: 54 %. IR (KBr) cm^{-1}: 3304 (N-H); 2154 (CN); 1676 (C=O); 1350 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.74 (d, 2H, A, J_{2-3} = J_{5-6} = 8.6 Hz, H_2+H_6), 7.87 (d, 2H, A, H_3+H_5), 8.20 (d, 2H, B, J_{2-3} = J_{5-6} = 8.6 Hz, H_3+H_5), 8.39 (d, 2H, B, H_2+H_6), 10.77 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 106.2 (1C, SeCN), 118.7 (1C, A, C_1), 122.5 (2C, A, C_3+C_5), 124.5 (2C, B, C_3+C_5), 130.2 (2C, B, C_2+C_6), 135.5 (2C, A, C_2+C_6), 140.9 (1C, B, C_1), 141.0 (1C, A, C_4), 150.1 (1C, B, C_4), 165.1 (1C, C=O). MS (*m/z* % abundance): 347 (10; M^{+}·), 188 (100), 127 (50), 105 (32). 77 (25). Elemental Analysis for $C_{14}H_9N_3O_2Se$, Calculated/Found (%): C. 48.55/48.50; H: 2.60/2.66; N: 12.13/12.23.

4-trifluoromethyl-*N*-(4-selenocyanatophenyl)benzamide (1f). From 4-trifluoromethylbenzoyl chloride. Recrystallized from methanol/water (1:1). Green powder; mp: 214-215 ºC. Yield: 51 %. IR (KBr) cm^{-1}: 3324 (N-H); 2154 (CN); 1675 (C=O); 1176-1339 (CF_3). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.73 (d, 2H, B, J_{2-3} = J_{5-6} = 7.1 Hz, H_3+H_5), 7.87 (d, 2H, B, H_2+H_6), 7.93 (d, 2H, A, J_{2-3} = J_{5-6} =

7.8 Hz, H_3+H_5), 8.15 (d, 2H, A, H_2+H_6), 10.68 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 106.2 (1C, SeCN), 118.4 (1C, A, C_1), 122.5 (2C, A, C_3+C_5), 124.7 (q, $^1J_{C\text{-}F}$ = 272.6 Hz, 1C, CF_3), 126.3 (q, $^3J_{C\text{-}F}$ = 3.6 Hz, 2C, B, C_3+C_5), 129.7 (2C, B, C_2+C_6), 132.5 (q, $^2J_{C\text{-}F}$ = 31.9 Hz, 1C, B, C_4), 135.5 (2C, A, C_2+C_6), 139.2 (1C, B, C_4), 141.0 (1C, A, C_1), 165.5 (1C, C=O). MS (*m/z* % abundance): 370 (22; M^+·). 173 (100). Elemental Analysis for $C_{15}H_9F_3N_2OSe$, Calculated/Found (%): C: 48.78/48.91; H: 2.43/2.27; N: 7.58/7.62.

4-methyl-*N*-(4-selenocyanatophenyl)benzamide (1g). From 4-methylbenzoyl chloride. Recrystallized from methanol. Blue powder; mp: 235-236 ºC. Yield: 43 %. IR (KBr) cm^{-1}: 3334 (N-H); 2913 (C-H); 2155 (CN); 1662 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.40 (s, 3H, CH_3), 7.35 (d, 2H, B, $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 8.3 Hz, H_3+H_5), 7.70 (d, 2H, A, $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 8.7 Hz, H_3+H_5), 7.88 (d, 2H, B, H_2+H_6), 7.90 (d, 2H, A, H_2+H_6), 10.37 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.9 (1C, CH_3), 106.2 (1C, SeCN), 117.7 (1C, A, C_1), 122.4 (2C, A, C_3+C_5), 128.7 (2C, B, C_2+C_6), 129.8 (2C, B, C_3+C_5), 132.5 (1C, B, C_1), 135.5 (2C, A, C_2+C_6), 141.6 (1C, A, C_4), 142.8 (1C, B, C_4), 166.5 (1C, C=O). MS (*m/z* % abundance): 316 (25; M^+·), 119 (100), 91 (32), 65 (20). Elemental Analysis for $C_{15}H_{12}N_2OSe$, Calculated/Found (%): C: 57.14/56.77; H: 3.80/3.98; N: 8.88/8.67.

***N*-(4-selenocyanatophenyl)furan-2-carboxamide (1h).** From furoyl chloride. Recrystallized from methanol. Blue powder; mp: 146-147 ºC. Yield: 38 %. IR (KBr) cm^{-1}: 3309 (N-H); 2148 (CN); 1676 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.72 (dd, 1H, B, $J_{2\text{-}3}$= 3.5 Hz, $J_{3\text{-}4}$= 1.7 Hz, H_3), 7.38 (d, 1H, B, H_2), 7.70 (d, 2H, A, $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 8.7 Hz, H_2+H_6), 7.86 (d, 2H, A, H_3+H_5), 7.97 (d, 1H, B, H_4), 10.42 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.8 (1C, SeCN), 112.7 (1C, B, C_3), 115.8 (1C, B, C_2), 117.6 (1C, A, C_1), 122.0 (2C, A, C_3+C_5), 135.1

(2C, A, C_2+C_6), 140.4 (1C, A, C_4), 146.5 (1C, B, C_4), 147.6 (1C, B, C_1), 156.8 (1C, C=O). MS (*m/z* % abundance): 292 (48; M^+·), 95 (100). Elemental Analysis for $C_{12}H_8N_2O_2Se$, Calculated/Found (%): C: 49.48/49.40; H: 2.75/3.10; N: 9.62/9.34.

***N*-(4-selenocyanatophenyl)thiophene-2-carboxamide (1i).** From 2-thiophenecarbonyl chloride. Recrystallized from methanol. Blue powder; mp: 205-206 ºC. Yield: 56 %. IR (KBr) cm^{-1}: 3347 (N-H); 2147 (CN); 1657 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.25 (t, 1H, B, $J_{2\text{-}3}$ = $J_{3\text{-}4}$ = 4.3 Hz, H_3), 7.72 (d, 2H, A, $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 8.6 Hz, H_2+H_6), 7.83 (d, 2H, A, H_3+H_5) 7.89 (d, 1H, B, H_4), 8.05 (d, 1H, B, H_2), 10.42 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.8 (1C, SeCN), 117.6 (1C, A, C_1), 122.0 (2C, A, C_3+C_5), 128.6 (1C, B, C_4), 130.0 (1C, B, C_3), 132.8 (1C, B, C_2), 135.1 (2C, A, C_2+C_6), 140.0 (1C, A, C_4), 140.6 (1C, B, C_1), 160.6 (1C, C=O). MS (*m/z* % abundance): 308 (40; M^+·), 111 (100). Elemental Analysis for $C_{12}H_8N_2OSSe$, Calculated/Found (%): C: 46.90/46.73; H: 2.60/2.67; N: 9.12/9.26.

***N*-(4-selenocyanatophenyl)isoxazole-5-carboxamide (1j).** From isoxazole-5-carbonyl chloride. Recrystallized from methanol/water (1:1). White powder; mp: 185-186 ºC. Yield: 52 %. IR (KBr) cm^{-1}: 3297 (N-H); 2156 (CN); 1675 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.29 (d, 1H, B, $J_{2\text{-}3}$ = 1.6 Hz, H_2), 7.76 (d, 2H, A, $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 8.7 Hz, H_2+H_6), 7.86 (d, 2H, A, H_3+H_5), 8.84 (d, 1H, B, H_3), 10.94 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.7 (1C, SeCN), 107.6 (1C, B, C_2), 118.9 (1C, A, C_1), 122.4 (2C, A, C_3+C_5), 135.0 (2C, A, C_2+C_6), 139.5 (1C, A, C_4), 152.4 (1C, B, C_3), 154.7 (1C, B, C_1), 162.7 (1C, C=O). MS (*m/z* % abundance): 293 (45; M^+·), 96 (100). Elemental Analysis for $C_{11}H_7N_3O_2Se$, Calculated/Found (%): C: 45.20/44.97; H: 2.40/2.62; N:14.38/14.33.

449 ***N*-(4-selenocyanatophenyl)adamantamide (1k).** From adamantanoyl chloride.
450 Recrystallized from methanol/water (1:1). Yellow powder; mp: 180-181 ºC.
451 Yield: 72 %. IR (KBr) cm^{-1}: 3306 (N-H); 2899 (C-H); 2147 (CN); 1661 (C=O). ^{1}H
452 NMR (400 MHz, DMSO-d_6) δ: 1.71 (s, 6H, B, $2H_6$+$2H_8$+$2H_{10}$), 1.92 (s, 6H, B,
453 $2H_2$+$2H_3$+$2H_5$), 2.03 (s, 3H, B, H_4+H_7+H_9), 7.64 (d, 2H, A, $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 8.6 Hz,
454 H_2+H_6), 7.76 (d, 2H, A, H_3+H_5), 9.32 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-
455 d_6) δ: 28.1 (3C, B, C_4+C_7+C_9), 36.4 (3C, B, C_6+C_8+C_{10}), 38.6 (3C, B,
456 C_2+C_3+C_5), 41.6 (1C, B, C_1), 105.7 (1C, SeCN), 116.7 (1C, A, C_1), 121.8 (2C,
457 A, C_3+C_5), 134.9 (2C, A, C_2+C_6), 141.2 (1C, A, C_4), 176.7 (1C, C=O). MS (*m/z*
458 % abundance): 360 (22; $M^{+}\cdot$). 135 (100). Elemental Analysis for $C_{18}H_{20}N_2OSe$,
459 Calculated/Found (%): C: 60.17/60.16; H: 5.57/5.75; N: 7.79/7.70.
460 ***N*-(4-selenocyanatophenyl)cinnamamide (1l).** From cinnamoyl chloride.
461 Recrystallized from methanol/water (1:1). Blue powder; mp: 186-187 ºC. Yield:
462 67 %. IR (KBr) cm^{-1}: 3306 (N-H); 2899 (C-H); 2147 (CN); 1661 (C=O). ^{1}H NMR
463 (400 MHz, DMSO-d_6) δ: 6.86 (d, 1H, B, $J_{a\text{-}b}$ = 15.7 Hz, C**H$_a$**=CHPh), 7.36 – 7.50
464 (m, 3H, B, H_3+H_4+H_5), 7.59 – 7.67 (m, 3H, B, H_2+H_6, CH=C**H$_b$**Ph), 7.73 (d, 2H,
465 A, $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 8.4 Hz, H_2+H_6), 7.82 (d, 2H, A, H_3+H_5), 10.45 (s, 1H, NH). ^{13}C
466 NMR (100 MHz, DMSO-d_6) δ: 105.8 (1C, SeCN), 117.0 (1C, A, C_1), 121.0 (2C,
467 A, C_3+C_5), 122.4 (1C, **C$_a$H**=CH), 128.2 (2C, B, C_3+C_5), 129.5 (2C, B, C_2+C_6),
468 130.4 (1C, B, C_4), 135.1 (1C, B, C_1), 135.3 (2C, A, C_2+C_6), 141.0 (1C, A, C_4),
469 141.3 (1C, CH=**C$_b$H**), 164.3 (1C, C=O). MS (*m/z* % abundance): 328 (22; $M^{+}\cdot$),
470 131 (100), 103 (75), 77 (40). Elemental Analysis for $C_{16}H_{12}N_2O_2Se$,
471 Calculated/Found (%): C: 58.71/58.40; H: 3.67/4.06; N: 8.56/8.56.
472 ***N*-(4-selenocyanatophenyl)-1-naphthamide (1m).** From 1-naphthoyl
473 chloride. Recrystallized from methanol/water (1:1). Green cotton-like powder;

mp: 216-217 ºC. Yield: 56 %. IR (KBr) cm^{-1}:3310 (N-H); 2145 (CN); 1668 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.56 – 7.66 (m, 3H, B), 7.75 (d, 2H, A, $J_{2\text{-}3} = J_{5\text{-}6} = 8.5$ Hz, H_2+H_6), 7.78 (d, 1H, B, $J = 7.0$ Hz,), 7.91 (d, 2H, A, H_3+H_5), 8.00 – 8.06 (m, 1H, B), 8.10 (d, 1H, B, $J = 8.2$ Hz), 8.16 – 8.22 (m, 1H, B), 10.81 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 105.8 (1C, SeCN), 117.5 (1C, A, C_1), 121.6 (2C, A, C_3+C_5), 125.5 (2C, B), 126.1 (1C, B), 126.9 (1C, B), 127.6 (1C, B), 128.9 (1C, B), 130.1 (1C, B), 130.9 (1C, B), 133.6 (1C, B), 134.8 (1C, B), 135.3 (2C, A, C_2+C_6), 141.1 (1C, A, C_4), 168.0 (1C, C=O). MS (*m/z* % abundance): 352 (20; $M^{+}\cdot$), 155 (100), 127 (98), 77 (10). Elemental Analysis for $C_{18}H_{12}N_2OSe$, Calculated/Found (%): C: 61.54/61.15; H: 3.42/3.64; N: 7.97/8.00.

4-methylthio-*N*-(4-selenocyanatophenyl)benzamide (1n). From 4-methylthiobenzoyl chloride. Recrystallized from methanol. White powder; mp: 202-203 ºC. Yield: 68 %. IR (KBr) cm^{-1}: 3391 (N-H); 2913 (C-H); 2162 (CN); 1662 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.55 (s, 3H, SCH_3), 7.40 (d, 2H, B, $J_{2\text{-}3} = J_{5\text{-}6} = 8.3$ Hz, H_3+H_5), 7.70 (d, 2H, A, $J_{2\text{-}3} = J_{5\text{-}6} = 8.6$ Hz, H_2+H_6), 7.88 (d, 2H, A, H_3+H_5), 7.94 (d, 2H, B, H_2+H_6), 10.38 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.6 (1C, SCH_3), 105.8 (1C, SeCN), 117.3 (1C, A, C_1), 122.0 (2C, A, C_3+C_5), 125.4 (2C, B, C_3+C_5), 128.7 (2C, B, C_2+C_6), 130.8 (1C, B, C_1), 135.1 (2C, A, C_2+C_6), 141.0 (1C, A, C_4), 144.0 (1C, B, C_4), 165.6 (1C, C=O). MS (*m/z* % abundance): 348 (7; $M^{+}\cdot$), 151 (100), 77 (10). Elemental Analysis for $C_{15}H_{12}N_2OSSe$, Calculated/Found (%): C: 49.19/48.80; H: 3.66/3.73; N: 7.66/7.52.

General procedure of synthesis for compounds 2a-2c, **2e-2j**, **2l** and **2n.**

To a solution of the corresponding selenocyanate (Compounds **1a-1c**, **1e-1j**, **1l** and **1n)** in ethanol (50 mL) was added $NaBH_4$ (0.25 eq). The resulting mixture was stirred at room temperature during 2 h. Upon completion the solvent was removed under vacuum and the residue was treated with water (2 x 50 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. The solid residue was purified by recrystallization from ethanol.

General procedure of synthesis for compounds 2d, **2k** and **2m.**

To a solution of bis(4-aminophenyl)diselenide in dry chloroform (50 mL) the corresponding acyl chloride was added (1:2 molar ratio), and the mixture was kept at room temperature for 12-24 h. Compounds precipitated after the addition of water. After filtration, the compounds were washed with diethyl ether for purification.

***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bisbenzamide (2a).** From *N*-(4-selenocyanatophenyl)benzamide. Yellow powder; mp: 255-256 ºC. Yield: 62 %. IR (KBr) cm^{-1}: 3341 (N-H); 1665 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.52 (d, 4H, A+A', $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 8.7 Hz, $2H_2$+$2H_6$), 7.55 – 7.63 (m, 6H, B+B', $2H_3$+$2H_4$+$2H_5$), 7.77 (d, 4H, A+A', $2H_3$+$2H_5$), 7.94 (d, 4H, B+B', $J_{2\text{-}3}$ = $J_{5\text{-}6}$ = 7.1 Hz, $2H_2$+$2H_6$), 10.45 (s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.3 (4C, A+A', $2C_3$+$2C_5$), 124.4 (2C, A+A', $2C_1$), 127.8 (4C, B+B', $2C_2$+$2C_6$), 128.6 (4C, B+B', $2C_3$+$2C_5$), 131.9 (2C, B+B', $2C_4$), 132.9 (4C, A+A', $2C_2$+$2C_6$), 134.7 (2C, B+B', $2C_1$), 139.5 (2C, A+A', $2C_4$), 165.9 (2C, 2C=O). MS (*m/z* % abundance): 302 (5), 155 (55), 105 (100), 77 (80). Elemental Analysis for $C_{26}H_{20}N_2O_2Se_2$, Calculated/Found (%): C: 56.73/56.48; H: 3.64/4.03; N: 5.09/4.83

***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bis(4-chlorobenzamide) (2b).** δ: From 4-chloro-*N*-(4-selenocyanatophenyl)benzamide. Green powder; mp: 252-253 ºC. Yield: 63 %. IR (KBr) cm^{-1}: 3307 (N-H); 1646 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.56 – 7.67 (m, 8H, A+A', $2H_2$+$2H_6$, B+B', $2H_3$+$2H_5$), 7.77 (d, 4H, A+A', J_{2-3} = J_{5-6} = 8.6 Hz, $2H_3$+$2H_5$), 7.99 (d, 4H, B+B', J_{2-3} = J_{5-6} = 8.5 Hz, $2H_2$+$2H_6$) 10.45 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.6 (4C, A+A', $2C_3$+$2C_5$), 124.9 (2C, A+A', $2C_1$), 129.0 (4C, B+B', $2C_3$+$2C_5$), 130.1 (4C, B+B', $2C_2$+$2C_6$), 133.3 (4C, A+A', $2C_2$+$2C_6$), 133.9 (2C, B+B', $2C_1$), 137.0 (2C, B+B', $2C_4$), 139.7 (2C, A+A', $2C_4$), 165.0 (2C, 2C=O). MS (*m/z* % abundance): 344 (7), 231 (15), 172 (52), 139 (100), 93 (42), 77 (33), 65 (40). Elemental Analysis for $C_{26}H_{18}Cl_2N_2O_2Se_2$, Calculated/Found (%): C: 50.40/50.71; H: 2.91/3.14; N: 4.52/4.61.

***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bis(4-methoxybenzamide) (2c).** From 4-methoxy-*N*-(4-selenocyanatophenyl)benzamide. Yellow powder; mp: 261-262 ºC. Yield: 61 %. IR (KBr) cm^{-1}: 3350 (N-H); 2961 (C-H); 1652 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.84 (s, 6H, $2OCH_3$), 7.05 (d, 4H, B+B', J_{2-3} = J_{5-6} = 8.9 Hz, $2H_3$+$2H_5$), 7.59 (d, 4H, A+A', J_{2-3} = J_{5-6} = 8.7 Hz, $2H_2$+$2H_6$), 7.82 (d, 4H, A+A', $2H_3$+$2H_5$), 8.02 (d, 4H, B+B', $2H_2$+$2H_6$), 10.38 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.9 (2C, $2OCH_3$), 114.1 (4C, B+B', $2C_3$+$2C_5$), 121.6 (4C, A+A', $2C_3$+$2C_5$), 124.4 (2C, A, C_1), 127.1 (2C, B+B', C_1), 130.3 (4C, B+B', $2C_2$+$2C_6$), 133.4 (4C, A+A', $2C_2$+$2C_6$), 140.3 (2C, A+A', $2C_4$), 162.5 (2C, B+B', $2C_4$), 165.5 (2C, 2C=O). MS (*m/z* % abundance): 378 (5), 211 (15), 172 (76), 105 (90), 77 (100). Elemental Analysis for $C_{28}H_{24}N_2O_4Se_2$, Calculated/Found (%): C: 55.08/55.23; H: 3.93/3.86; N: 4.59/4.48.

546 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bis(4-cyanobenzamide) (2d).**
547 From 4-cyano-*N*-(4-selenocyanatophenyl)benzamide. Yellow powder; mp: >
548 300 ºC. Yield: 65 %. IR (KBr) cm^{-1}: 3258 (N-H); 2237 (CN); 1661 (C=O). ^{1}H
549 NMR (400 MHz, DMSO-d_6) δ: 7.64 (d, 4H, A+A', $J_{2\text{-}3} = J_{5\text{-}6}$ = 7.3 Hz, $2H_2$+$2H_6$),
550 7.77 (d, 4H, A+A', $2H_3$+$2H_5$), 8.04 (d, 4H, B+B', $J_{2\text{-}3} = J_{5\text{-}6}$ = 6.6 Hz, $2H_2$+$2H_6$),
551 8.10 (d, 4H, B+B', $2H_3$+$2H_5$), 10.61 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-
552 d_6) δ: 114.4 (2C, B+B', $2C_4$), 118.7 (2C, 2CN), 121.6 (4C, A+A', $2C_3$+$2C_5$),
553 125.2 (2C, A+A', $2C_1$), 129.1 (4C, B+B', $2C_2$+$2C_6$), 132.9 (4C, B+B', $2C_3$+$2C_5$),
554 133.3 (4C, A+A', $2C_2$+$2C_6$), 139.2 (2C, B+B', $2C_1$), 139.4 (2C, A+A', $2C_4$), 164.7
555 (2C, 2C=O). MS (*m/z* % abundance): 342 (10), 262 (5), 172 (75), 131 (75), 93
556 (100), 77 (90), 55 (85). Elemental Analysis for $C_{28}H_{18}N_4O_2Se_2$,
557 Calculated/Found (%): C: 56.00/55.62; H: 3.00/2.80; N: 9.33/9.22.
558 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bis4-nitrobenzamide (2e).** From
559 4-nitro-*N*-(4-selenocyanatophenyl)benzamide. Yellow powder; mp: 247-246 ºC.
560 Yield: 68 %. IR (KBr) cm^{-1}: 3333 (N-H); 1648 (C=O); 1350 (NO). ^{1}H NMR (400
561 MHz, DMSO-d_6) δ: 7.65 (d, 4H, A+A', $J_{2\text{-}3} = J_{5\text{-}6}$ = 8.7 Hz, $2H_2$+$2H_6$), 7.78 (d, 4H,
562 A+A', $2H_3$+$2H_5$), 8.18 (d, 4H, B+B', $J_{2\text{-}3} = J_{5\text{-}6}$ = 8.8 Hz, $2H_2$+$2H_6$), 8.37 (d, 4H,
563 B+B', $2H_3$+$2H_5$), 10.70 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.1
564 (4C, A+A', $2C_3$+$2C_5$), 123.5 (4C, B+B', $2C_3$+$2C_5$), 124.8 (2C, A+A', $2C_1$), 129.2
565 (4C, B+B', $2C_2$+$2C_6$), 132.7 (4C, A+A', $2C_2$+$2C_6$), 138.9 (2C, B+B', $2C_1$), 140.3
566 (2C, A+A', $2C_4$), 149.1 (2C, B+B', $2C_4$), 163.9 (2C, 2C=O). MS (*m/z* %
567 abundance): 342 (5), 172 (90), 131 (100), 55 (88). Elemental Analysis for
568 $C_{26}H_{18}N_4O_6Se_2$, Calculated/Found (%): C:47.85/47.41; H: 2.79/3.18; N:
569 8.58/8.48.

570 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bis-4-trifluoromethylbenzamide**
571 **(2f).** From 4-trifluoromethyl-*N*-(4-selenocyanatophenyl)benzamide. Yellow
572 powder; mp: 247-248 ºC. Yield: 64 %. IR (KBr) cm^{-1}: 3317 (N-H); 1647 (C=O);
573 1176-1339 (CF_3). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.66 (d, 4H, A+A', $J_{2\text{-}3} = J_{5\text{-}6}$
574 = 8.5 Hz, $2H_2+2H_6$), 7.78 (d, 4H, A+A', $2H_3+2H_5$), 7.93 (d, 4H, B+B', $J_{2\text{-}3} = J_{5\text{-}6} =$
575 8.2 Hz, $2H_3+2H_5$), 8.15 (d, 4H, B+B', $2H_2+2H_6$), 10.59 (s, 2H, 2NH). ^{13}C NMR
576 (100 MHz, DMSO-d_6) δ: 121.3 (4C, A+A', $2C_3+2C_5$), 123.9 (q, 2C, $^1J_{C\text{-}F} = 272.5$
577 Hz, $2CF_3$) 124.8 (2C, A+A', $2C_1$), 125.4 (q, 4C, $^3J_{C\text{-}F} = 3.6$ Hz, B+B', $2C_3+2C_5$),
578 128.7 (4C, B+B', $2C_2+2C_6$), 131.5 (q, 2C, $^2J_{C\text{-}F} = 32.0$ Hz, B+B', C_4), 132.9 (4C,
579 A+A', $2C_2+2C_6$), 138.6 (2C, A+A', $2C_4$), 139.1 (2C, B+B', $2C_1$), 164.6 (2C,
580 2C=O). MS (*m/z* % abundance): 370 (10), 173 (90), 145 (50), 121 (100).
581 Elemental Analysis for $C_{28}H_{18}F_6N_2O_2Se_2$, Calculated/Found (%): C:
582 48.98/48.41; H: 2.62/2.92; N: 4.08/4.15.
583 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bis-4-methylbenzamide (2g).**
584 From 4-methyl-*N*-(4-selenocyanatophenyl)benzamide. Yellow powder; mp: 266-
585 267 ºC. Yield: 59 %. IR (KBr) cm^{-1}: 3366 (N-H); 2915 (C-H); 1654 (C=O). ^1H
586 NMR (400 MHz, DMSO-d_6) δ: 2.39 (s, 6H, $2CH_3$), 7.35 (d, 4H, B+B', $J_{2\text{-}3} = J_{5\text{-}6} =$
587 8.1 Hz, $2H_3+2H_5$), 7.60 (d, 4H, A+A', $J_{2\text{-}3} = J_{5\text{-}6} = 8.7$ Hz, $2H_2+2H_6$), 7.78 (d, 4H,
588 A+A', $2H_3+2H_5$), 7.87 (d, 4H, B+B', $2H_2+2H_6$), 10.30 (s, 2H, 2NH). ^{13}C NMR
589 (100 MHz, DMSO-d_6) δ: 21.1 (2C, $2CH_3$), 121.1 (4C, A+A', $2C_3+2C_5$), 124.2
590 (2C, A+A', $2C_1$), 127.8 (4C, B+B', $2C_2+2C_6$), 129.1 (4C, B+B', $2C_3+2C_5$), 132.0
591 (2C, B+B', $2C_1$), 133.0 (4C, A+A', $2C_2+2C_6$), 139.7 (2C, A+A', $2C_4$), 141.9 (2C,
592 B+B', $2C_4$), 165.6 (2C, 2C=O). MS (*m/z* % abundance): 370 (5), 173 (100), 133
593 (80), 57 (90). Elemental Analysis for $C_{28}H_{24}O_2N_2Se_2.HCl$, Calculated/Found
594 (%): C: 54.60/54.23; H: 4.10/4.22; N: 4.55/4.46.

595 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bisfuran-2-carboxamide (2h).**
596 From *N*-(4-selenocyanatophenyl)furan-2-carboxamide. Yellow powder; mp: 222-
597 223 ºC. Yield: 61 %. IR (KBr) cm^{-1}: 3352 (N-H); 1660 (C=O). ^{1}H NMR (400 MHz,
598 DMSO-d_6) δ: 6.71 (dd, 2H, B+B', J_{2-3}= 3.5 Hz, J_{3-4}= 1.7 Hz, $2H_3$), 7.35 (d, 2H,
599 B+B', $2H_2$), 7.60 (d, 4H, A+A', J_{2-3} = J_{5-6} = 8.7 Hz, $2H_2$+$2H_6$), 7.74 (d, 4H, A+A',
600 $2H_3$+$2H_5$), 7.94 (d, 2H, B+B', $2H_4$), 10.32 (s, 2H, 2NH). ^{13}C NMR (100 MHz,
601 DMSO-d_6) δ: 112.1 (2C, B+B', $2C_3$), 114.9 (2C, B+B', $2C_2$), 121.0 (4C, A+A',
602 $2C_3$+$2C_5$), 124.3 (2C, A+A', $2C_1$), 132.7 (4C, A+A', $2C_2$+$2C_6$), 138.7 (2C, A+A',
603 $2C_4$), 145.8 (2C, B+B', $2C_4$), 147.2 (2C, B+B', $2C_1$), 156.1 (2C, 2C=O). MS (*m/z*
604 % abundance): 370 (5), 173 (100), 145 (70). Elemental Analysis for
605 $C_{22}H_{16}N_2O_4Se_2$, Calculated/Found (%): C: 49.81/49.71; H: 3.02/3.37; N:
606 5.28/5.21.
607 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bisthiophene-2-carboxamide (2i).**
608 From *N*-(4-selenocyanatophenyl)thiophene-2-carboxamide. Yellow powder; mp:
609 167-168 ºC. Yield: 57 %. IR (KBr) cm^{-1}: 3354 (N-H); 1666 (C=O). ^{1}H NMR (400
610 MHz, DMSO-d_6) δ: 7.23 (dd, 2H, B+B', J_{2-3}= 4.7 Hz, J_{3-4}= 3.9 Hz, $2H_3$), 7.61 (d,
611 4H, A+A', J_{2-3} = J_{5-6} = 8.4 Hz, $2H_2$+$2H_6$), 7.74 (d, 4H, A+A', $2H_3$+$2H_5$), 7.87 (d,
612 2H, B+B', $2H_2$), 8.09 (d, 2H, B+B', $2H_4$), 10.42 (s, 2H, 2NH). ^{13}C NMR (100
613 MHz, DMSO-d_6) δ: 121.6 (4C, A+A', $2C_3$+$2C_5$), 124.8 (2C, A+A', $2C_1$), 128.6
614 (2C, B+B', $2C_3$), 129.9 (2C, B+B', $2C_4$), 132.6 (2C, B+B', $2C_2$), 133.3 (4C, A+A',
615 $2C_2$+$2C_6$), 139.5 (2C, A+A', $2C_4$), 140.2 (2C, B+B', $2C_1$), 160.4 (2C, 2C=O). MS
616 (*m/z* % abundance): 342 (5), 264 (20), 184 (100). Elemental Analysis for
617 $C_{22}H_{16}N_2O_2S_2Se_2$.2HCl, Calculated/Found (%): C: 41.57/41.56; H: 2.83/2.86; N:
618 4.40/4.47.

619 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bisisoxazole-5-carboxamide (2j).**
620 From *N*-(4-selenocyanatophenyl)isoxazole-5-carboxamide. Yellow powder; mp:
621 204-205 ºC. Yield: 64 %. IR (KBr) cm^{-1}: 3347 (N-H); 1662 (C=O). ^{1}H NMR (400
622 MHz, DMSO-d_6) δ: 7.31 (d, 2H, B+B', $J_{2\text{-}3}$ =1.7 Hz, H_2), 7.65 (d, 4H, A+A', $J_{2\text{-}3}$ =
623 $J_{5\text{-}6}$ = 7.6 Hz, $2H_2$+$2H_6$), 7.77 (d, 4H, A+A', $2H_3$+$2H_5$), 8.82 (d, 2H, B+B', H_3),
624 10.93 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 107.5 (2C, B+B', $2C_2$),
625 121.9 (4C, A+A', $2C_3$+$2C_5$), 125.9 (2C, A+A', $2C_1$), 133.1 (4C, A+A', $2C_2$+$2C_6$),
626 138.5 (2C, A+A', $2C_4$), 152.3 (2C, B+B', $2C_3$), 154.6 (2C, B+B', $2C_1$), 162.8 (2C,
627 2C=O). MS (*m/z* % abundance): 264 (5), 184 (50), 93 (100), 66 (45). Elemental
628 Analysis for $C_{20}H_{14}N_4O_4Se_2$.2HCl, Calculated/Found (%): C: 39.66/39.90; H:
629 2.64/2.67; N: 9.25/9.26.
630 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))Bisadamantamide (2k).** From *N*-
631 (4-selenocyanatophenyl)adamantamide. Yellow powder; mp: 230-231 ºC. Yield:
632 54 %. IR (KBr) cm^{-1}: 3386 (N-H); 2915 (C-H); 1665 (C=O). ^{1}H NMR (400 MHz,
633 DMSO-d_6) δ: 1.69 (s, 12H, B+B', $4H_4$+$4H_6$+$4H_{10}$), 1.89 (s, 12H, B+B',
634 $4H_2$+$4H_8$+$4H_9$), 2.01 (s, 6H, B+B', $2H_3$+$2H_5$+$2H_7$), 7.50 (d, 4H, A+A', $J_{2\text{-}3}$ = $J_{5\text{-}6}$ =
635 8.8 Hz, $2H_2$+$2H_6$), 7.6 (d, 4H, A+A', $2H_3$+$2H_5$), 9.28 (s, 2H, 2NH). ^{13}C NMR (100
636 MHz, DMSO-d_6) δ: 28.1 (6C, B+B', $2C_4$+$2C_7$+$2C_9$), 36.4 (6C, B+B',
637 $2C_6$+$2C_8$+$2C_{10}$), 38.6 (6C, B+B', $2C_2$+$2C_3$+$2C_5$), 41.5 (2C, B+B', $2C_1$), 121.3
638 (4C, A+A', $2C_3$+$2C_5$), 124.1 (2C, A+A', C_1), 133.4 (4C, A+A', $2C_2$+$2C_6$), 140.2
639 (2C, A+A', $2C_4$), 176.6 (2C, 2C=O). MS (*m/z* % abundance): 344 (6), 184 (100),
640 165 (95), 93 (90), 65 (65). Elemental Analysis for $C_{34}H_{40}N_2O_2Se_2$,
641 Calculated/Found (%): C: 61.26/61.35; H: 6.05/5.95; N: 4.20/4.23.
642 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bis(3-phenylacrylamide) (2l).** δ:
643 From *N*-(4-selenocyanatophenyl)cinnamamide. Yellow powder; mp: 234-235

ºC. Yield: 67 %. IR (KBr) cm^{-1}: 3307 (N-H); 1662 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.98 (d, 2H, B+B', J_{a-b} = 15.7 Hz, C**H$_a$**=CHPh), 7.37 – 7.49 (m, 6H, B+B', H_3+H_4+H_5), 7.54 – 7.67 (m, 10H, A+A'+B+B', H_2+H_6+CH=C**H$_b$**Ph), 7.75 (d, J_{2-3} = J_{5-6} = 7.3 Hz, 4H, A+A', 2H_3+2H_5), 10.68 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.5 (4C, A+A', 2C_3+2C_5), 122.7 (2C, 2**C$_a$H**=CH), 124.3 (2C, A+A', 2C_1), 128.2 (4C, B+B', 2C_3+2C_5), 129.5 (4C, B+B', 2C_2+2C_6), 130.3 (2C, B+B', 2C_4), 133.6 (4C, A+A', 2C_2+2C_6), 135.2 (2C, B+B', 2C_1), 139.1 (2C, A+A', 2C_4), 140.7 (2C, 2CH=**C$_b$H**), 164.2 (2C, 2C=O). MS (*m/z* % abundance): 538 (5), 278 (30), 108 (100), 77 (98). Elemental Analysis for $C_{30}H_{24}N_2O_2Se_2$.HCl, Calculated/Found (%): C: 56.25/56.55; H: 3.90/4.05; N: 4.37/4.93.

***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bisnaphthamide (2m).** From *N*-(4-selenocyanatophenyl)-1-naphthamide. Yellow powder; mp: 243-247 ºC. Yield: 54 %. IR (KBr) cm^{-1}: 3392 (N-H); 2915 (C-H); 1665 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.57 – 7.65 (m, 6H, B+B'), 7.67 (d, 4H, A+A', J_{2-3} = J_{5-6} = 8.6 Hz, 2H_2+2H_6), 7.75 – 7.79 (m, 2H, B+B'), 7.83 (d, 4H, A+A', 2H_3+2H_5), 7.99 – 8.06 (m, 2H, B+B'), 8.08 (d, 2H, B+B', *J* = 8.2 Hz), 8.16 – 8.25 (m, 2H, B+B'), 10.74 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 121.1 (4C, A+A', 2C_3+2C_5), 124.8 (2C, A+A', 2C_1), 125.5 (2C, B+B'), 125.6 (2C, B+B'), 126.0 (2C, B+B'), 126.9 (2C, B+B'), 127.5 (2C, B+B'), 128.8 (2C, B+B'), 130.1 (2C, B+B'), 130.7 (2C, B+B'), 133.6 (4C, A+A', 2C_2+2C_6), 133.6 (2C, B+B'), 135.0 (2C, B+B'), 140.1 (2C, A+A', 2C_4), 167.9 (2C, 2C=O). MS (*m/z* % abundance): 344 (5), 184 (75), 172 (92), 127 (95), 57 (100). Elemental Analysis for $C_{34}H_{24}N_2O_2Se_2$, Calculated/Found (%): C: 62.78/62.86; H: 3.72/3.59; N: 4.31/4.52.

668 ***N,N'*-(4,4'-diselanediylbis(4,1-phenylene))bis-4-methylthiobenzamide (2n).**
669 From 4-methylthio-*N*-(4-selenocyanatophenyl)benzamide. Yellow powder; mp:
670 289-290 ºC. Yield: 46 %. IR (KBr) cm^{-1}: 3355 (N-H); 2930 (C-H); 1648 (C=O).
671 ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.55 (s, 6H, $2CH_3$), 7.39 (d, 4H, B+B', $J_{2-3} = J_{5-6} = 8.5$ Hz, H_3+H_5), 7.61 (d, 4H, A+A', $J_{2-3} = J_{5-6} = 8.7$ Hz, $2H_2+2H_6$), 7.77 (d, 4H,
673 A+A', $2H_3+H_5$), 7.91 (d, 4H, B+B', $2H_2+2H_6$), 10.30 (s, 2H, 2NH). ^{13}C NMR (100
674 MHz, DMSO-d_6) δ: 14.6 (2C, $2SCH_3$), 121.5 (2C, A+A', $2C_1$), 122.0 (4C, A+A',
675 $2C_3+2C_5$), 125.4 (4C, B+B', $2C_3+2C_5$), 128.7 (4C, B+B', $2C_2+2C_6$), 130.8 (2C,
676 B+B', $2C_1$), 135.1 (4C, A+A', $2C_2+2C_6$), 141.0 (2C, A+A', $2C_4$), 144.0 (2C, B+B',
677 $2C_4$), 165.6 (2C, 2C=O). MS (*m/z* % abundance): 292 (35), 107 (42), 77 (100).
678 Elemental Analysis for $C_{28}H_{24}N_2O_2S_2Se_2.2HCl$, Calculated/Found (%): C:
679 47.12/46.91; H: 3.65/3.75; N: 3.92/3.90.

680 **Biological evaluation**

681 **(i) Cells and Culture conditions.** *L. infantum* axenic amastigotes were grown
682 in M199 medium (Invitrogen, Leiden, Netherlands) supplemented with 10% heat
683 inactivated FBS, 1g/L β-alanine, 100mg/L L-asparagine, 200mg/L sacarose, 50
684 mg/L sodium pyruvate, 320 mg/L malic acid, 40mg/L fumaric acid, 70mg/L
685 succinic acid, 200mg/L α-ketoglutaric acid, 300 mg/L citric acid, 1.1 g/L sodium
686 bicarbonate, 5 g/L MES, 0.4 mg/L hemin, 10 mg/L gentamicin, pH 5.4 at 37ºC
687 and 5% CO_2. THP-1 cells were kindly provided by Dr. Michel /Université Nice
688 Sophia Antipolis, Nice, France) and were grown in RMPI-1640 medium (Gibco,
689 Lieden, Netherlands) supplemented with 10% heat inactivated FBS, 5%
690 penicillin/streptomycin, 1nM HEPES, 2mM glutamine and 1mM sodium
691 pyruvate, pH 7.2 at 37ºC and 5% CO_2 atmosphere(20).

692 **(ii) Leishmanicidal activity and cytotoxicity *in vitro* assays.** Drug treatment
693 of amastigotes and THP-1 cells was performed during the logarithmic growth
694 phase at a concentration of $2x10^6$ parasites/ml and $4x10^5$ cells/mL, respectively,
695 at 37ºC and 5% CO_2 for 24 h. Compounds were tested at five different
696 concentrations between 1.56 and 25 µM. In some cases, compounds
697 concentrations needed to be decrease until 0.2 µM. The number and
698 percentage of living parasites/cells was figured out by flow cytometry by the
699 propidium iodide (PI) exclusion method(48). After the selection of the parasite
700 population based on their forward scatter (FSC) and side scatter (SSC) values,
701 live and dead parasites cells were identified by their permeability to PI. This is a
702 conservative procedure that may underestimate LC_{50} values as parasites that
703 became fragmented as a consequence of cell death are excluded from the
704 analysis. To minimize the presence of fragmented parasites drug treatment
705 never exceeded 24 h. The selectivity index (SI) was defined as the ratio of the
706 EC_{50} values of compounds against THP-1 cells relative to those obtained
707 against *L. infantum* axenic amastigotes.
708 **(iii) Leishmania infection assay.** 120.000 cells/mL THP-1 cells were seeded
709 in 24 multidishes plates (Nunc, Roskilde, Denmark) and differentiated to
710 macrophages in 1 mL of RPMI-1640 medium containing 10 ng/mL phorbol 12-
711 myristate 13-acetate (PMA) (Sigma-Aldrich, St. Louis, MO, USA). After 24 h,
712 medium was removed and 1.2 x 10^6 *Leishmania infantum* eGFP-amastigotes in
713 1 mL of THP-1 medium were added to half of the plate wells. 4 h later, all
714 medium with non-infecting amastigotes was removed, washed 3 times with 1X
715 phosphate buffered saline (PBS) and replaced with the corresponding treatment
716 dissolved in 1 mL THP-1 medium. 48 h after the treatment, medium was

removed; Cells were washed 3 times with 1X PBS and detached with TrypLE™ Express (Invitrogen, Leiden, Netherlands) following manufacturer's protocol. Infection quantization was measured by flow cytometry(49).

(iv) Trypanothione reductase assay. The oxidorreductase activity against trypanothione reductase enzyme was determined following the method described by Toro et al.(50) Reaction was done at room temperature in 250 µL of HEPES pH 8.0 (40 mM), containing EDTA (1 mM), NADPH (150 µM), $NADP^+$ (30 µM), DTNB (25µM), T[S]2 (1 µM), glycerol (0.02%), DMSO (1.5%) and recombinant Li-TryR (7 nM). For IC_{50} values determination, the enzyme was pre-incubated with the compounds (concentrations ranging from 75 µM to 0.29 µM) for 10 min prior the addition of T[S]2 and NADPH. Enzyme activity was monitored by the increase in absorbance at 412 nm for 1 h at 26°C in a VERSAmax microplate reader (Molecular Devices, California, USA). All the assays were conducted in triplicate in at least three independent experiments. Data were analyzed using a non-linear regression model with the Grafit6 software (Erithacus, Horley, Surrey, UK). Mepacrine was used as positive control (same concentrations).

(v) Measurement of intracellular thiols. The disturbance of the intracellular thiol levels is an evidence of an oxidative stress. For its measurement, 0.5 mL/plate amastigotes at a concentration of $1x10^6$/mL were seeded in 6 multidishes plate and treated with DMSO (5 µL) as negative control, Menadione (25 µM) as positive control and compounds (25 µM). Amastigotes were incubated at 37ºC and 5% CO_2 for 1 hour. 30 minutes after the treatment CMFDA (5-chloromethylfluorescein diacetate) was added (20 µM) and

incubated 30 minutes. After this time, the parasite pellet was washed with PBS 1x and intracellular thiol content was measured by flow cytometry.

(vi) ADME and Lipinski properties. Absorption, distribution, metabolism, and excretion (ADME) properties were calculated using FAF filters. In brief, substructure searches within ligands using previous knowledge of PAINs (non-specific assay confounding compounds), reactive groups, solubility, Lipinski, Veber, and EGAN rules, among others, provide the filters for chemical compounds to flag possible non-desirable properties. Absorption properties were also calculated using PreADMET program (http://preadmet.bmdrc.org/preadmet.index.php).

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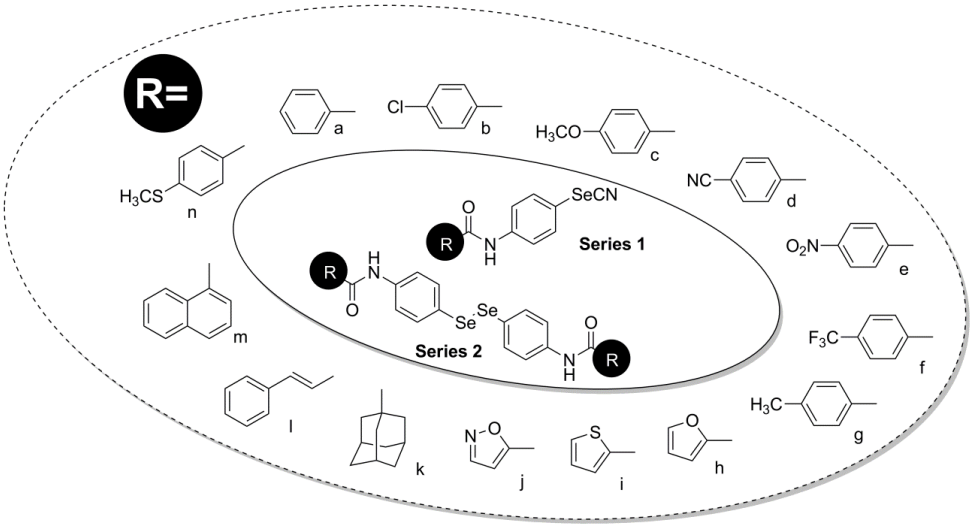
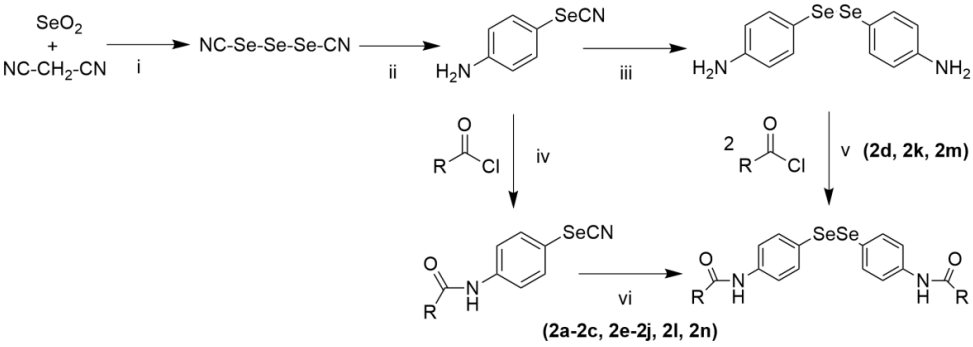


948

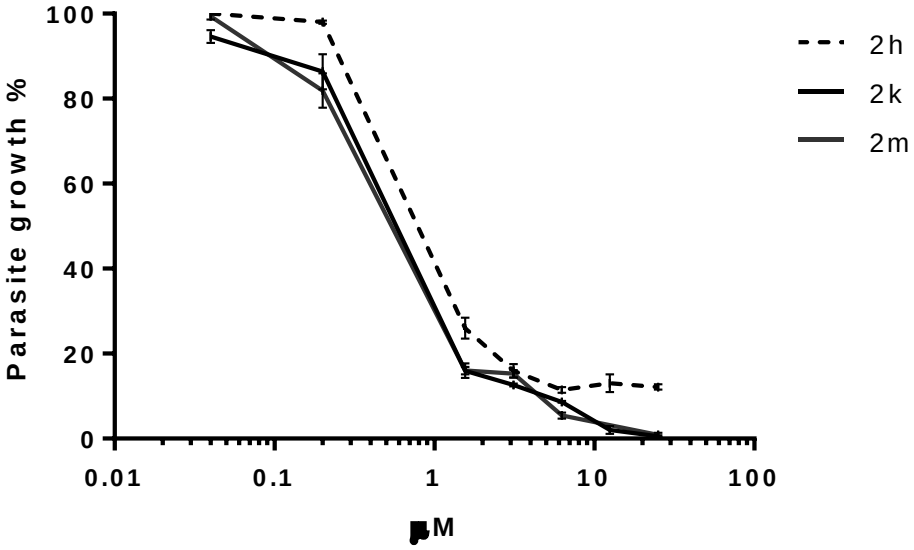
949 **Figure 1**: General structure of the 28 designed novel amides. Series 1
950 containing 4-aminophenylselenocianate (**0A**) or series 2 containing bis-
951 4(aminophenyl)diselenide (**0B**) as nucleus.



952

953 **Figure 2**: Synthetic route of the 28 compounds. Reagents and conditions: (i)
954 DMSO, 0.5 h, r.t.; (ii) Aniline, 1 h, r.t.; (iii) $NaBH_4$, ethanol, 2 h, r.t.; (iv) 1. Acid
955 chloride acid, chloroform, 12-24 h, r.t.; (v) 2. Acid chloride acid, chloroform, 12-
956 24 h, r.t.; (vi) BH_4Na, ethanol, 2 h, r.t.

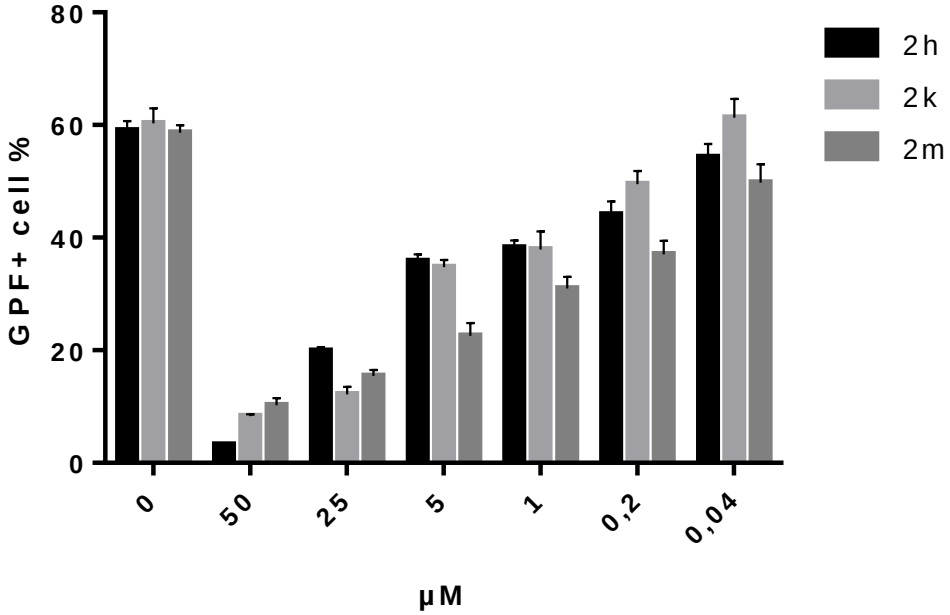
Antimicrobial Agents and Chemotherapy



957

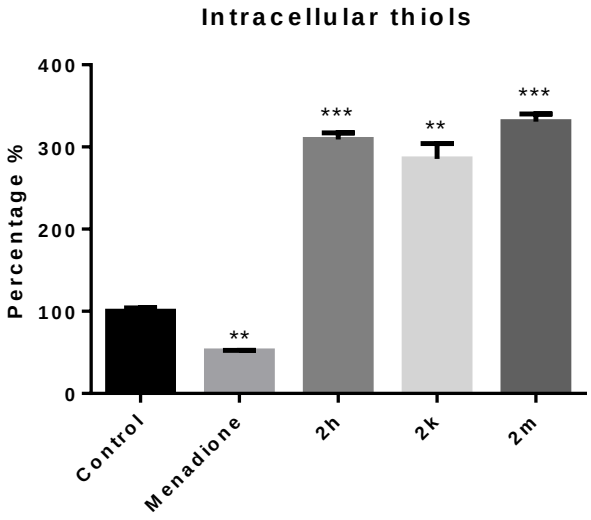
958 **Figure 3. 2h**, **2k** and **2m** compounds *in vitro* activity against *Leishmania*

959 *infantum* amastigotes.



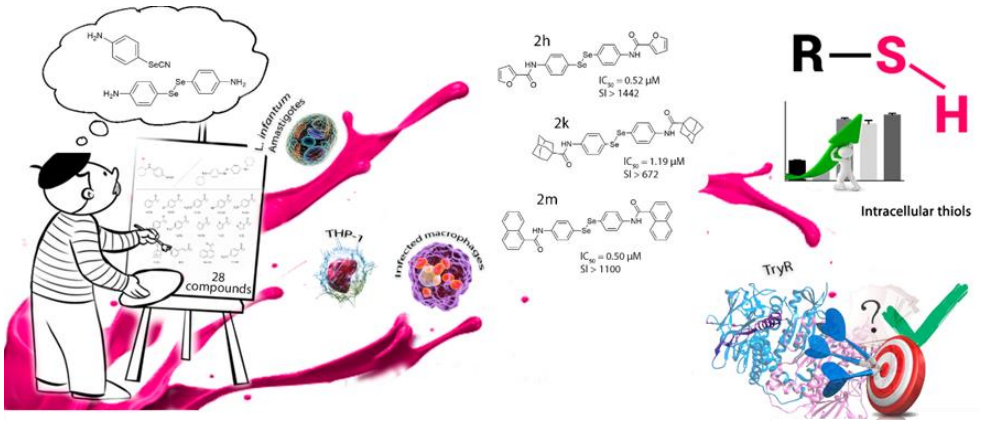
960

961 **Figure 4.** GFP+ cell % after treatments with compounds 2h, 2k and 2m for
962 different doses after 48 h.



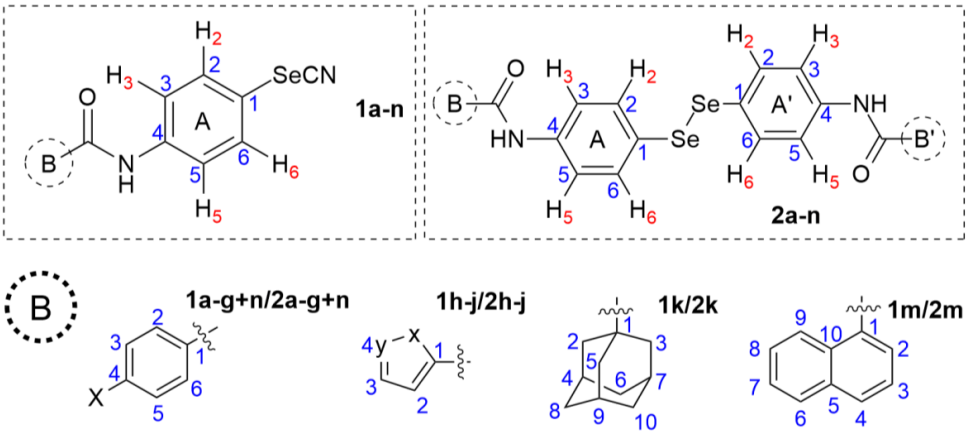
963

964 **Figure 5**: Levels of intracellular thiols after 1 h treatment with Menadione
965 (positive control), and compounds **2h**, **2k** and **2m** at 25 µM. Results are
966 expressed as a mead ± SEM of three independent experiments. **p < 0.01, ***p
967 < 0.001 with respect the control (DMSO).



968

969 **Figure 6:** Schematic illustration of the synthesis, leishmanicidal activity and
970 mechanism of action for the selenocompounds described.



971

972 **Figure 7**. General structures and nomenclature used to assign the position of

973 hydrogen and carbon in the compounds presented herein.

974

975 **Table 1.** EC_{50} ± SEM (μM) values for the compounds on amastigotes and
976 cytotoxic activity on THP-1 cells, after 24 h treatment.

Ref	Amastigote	THP-1	SI[a]	Ref	Amastigote	THP-1	SI[a]
1a	2.67 ± 0.25	6.01 ± 0.80	2.24	**2a**	14.4 ± 1.22	> 25	> 1.74
1b	6.39 ± 0.74	10.40± 1.00	1.62	**2b**	4.53 ± 0.49	> 25	> 5.52
1c	3.20 ± 0.71	4.29 ± 0.30	1.34	**2c**	10.3 ± 1.07	>25	> 2.52
1d	2.30 ± 0.57	> 25	> 10.87	**2d**	> 12.5	> 25	-
1e	11.44 ± 1.32	> 25	> 2.18	**2e**	20.1 ± 3.34	> 25	> 1.25
1f	5.68 ± 0.86	> 25	> 4.40	**2f**	> 25	>25	-
1g	4.29 ± 2.02	6.74 ± 1.69	1.57	**2g**	> 25	> 25	-
1h	0.95 ± 0.05	8.60 ± 2.80	9.05	**2h**	0.52 ± 0.04	> 750	> 1442
1i	1.96 ± 0.35	18.39 ± 1.50	9.36	**2i**	0.69 ± 0.15	7.17 ± 0.53	10.40
1j	1.76 ± 0.12	8.97 ± 0.01	5.08	**2j**	0.64 ± 0.12	7.41 ± 0.74	11.66
1k	1.21 ± 0.11	4.40 ± 1.70	3.62	**2k**	1.19 ± 0.22	> 800	> 672.3
1l	4.82 ± 0.17	6.97 ± 1.57	1.45	**2l**	3.03 ± 0.21	> 25	> 8.25
1m	5.61 ± 0.40	13.41 ± 1.18	2.39	**2m**	0.50 ± 0.10	> 550	> 1100
1n	9.39 ± 0.66	18.02 ± 1.34	1.92	**2n**	> 12.5	> 25	-
0A[b]	9.29 ± 1.16	> 50	> 5.38	**0B**[b]	0.65 ± 0.02	20.40 ± 2.80	24
Edelfosine	0.82 ± 0.13	4.96 ± 0.16	6.0	**Miltefosine**	2.84 ± 0.10	18.50 ± 0.60	7.0

977

978 [a]Selectivity index (SI) is the ratio of EC_{50} values of compounds against THP-1
979 cells relative to those against *L. infantum* amastigotes. [b]Biological data for
980 parent compounds.

981

Antimicrobial Agents and Chemotherapy

982

983 **Table 2.** ED_{50} ± SEM (μM) values for the compounds in intracellular

984 amastigotes

Compound	ED_{50}	Macrophages	SI
2h	2.20 ± 0.80	> 50	> 22.72
2k	3.77 ± 0.60	> 50	> 13.26
2m	1.03 ± 0.16	> 50	> 75.75
Edelfosine	3.10 ± 0.10	n.d.	n.d.

985

986

987 **Table 3.** IC_{50} ± SEM (μM) values for the selected compounds against TryR.

Compound	$IC_{50(TryR)}$ (μM)
2h	14.00 ± 0.90
2k	18.30 ± 0.10
2m	5.53 ± 0.01
Mepacrine	16.99 ± 1.18

988

989

990 **Table 4.** Theoretical ADME properties for lead compounds.

Compd.	cLogP	MW	*n*-OHNH donors	*n*-ON acceptor	Lipinski's violations	Absorption	
						HIA (%)	PCaco-2 (nm/s)
2h	1.45	515	2	6	2	97.80	38.91
2k	6.53	668	2	4	2	98.04	46.71
2m	5.02	652	2	4	2	98.41	53.55
Miltefosine	5.67	509	0	7	2	98.96	21.74
Edelfosine	4.34	407	1	5	0	98.43	39.38

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993