NOVEL UREA, THIOUREA AND SELENOUREA DERIVATIVES OF DISELENIDES: SYNTHESIS AND LEISHMANICIDAL ACTIVITY

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Abstract

A novel series of thirty-one *N*-substituted urea, thiourea and selenourea derivatives containing diphenyldiselenide entity were synthesized, fully characterized by spectroscopic and analytical methods, and screened for their *in vitro* leishmanicidal activities. The cytotoxic activity of these derivatives was tested against *Leishmania infantum* axenic amastigotes, and selectivity was assessed in human THP-1 cells. Thirteen of the synthesized compounds showed a significant antileishmanial activity with EC_{50} values lower than the reference drug miltefosine (EC_{50} = 2.84 μM). In addition, the derivatives **9**, **11**, **42** and **47** with EC_{50} between 1.1 and 1.95 μM also displayed an excellent selectivity (SI ranged from 12.4 to 22.7) and were also tested against infected macrophages. Compound **11**, a derivative with a cyclohexyl chain, exhibited the highest activity against intracellular amastigotes with EC_{50} values similar to those observed for the standard drug edelfosine. SAR analyses revealed that *N*-aliphatic substitution in urea and selenourea is recommended for the leishmanicidal activity of these analogs. Preliminary studies of the mechanism of action for the hit compounds was carried out by measuring their ability to inhibit trypanothione reductase (TryR). Even though the obtained results suggest that this enzyme is not the target for most of these derivatives, their comparable activity with the standards and lack of toxicity in THP-1 cells highlight the potential of these compounds to be optimized for leishmaniasis treatment.

Keywords: selenium, selenourea, thiourea, trypanothione reductase, urea

Leishmaniasis comprises a group of mammalian diseases caused by diphasic protozoans of the genus *Leishmania spp*. It is endemic in 98 countries and approximately 15 million of new cases are diagnosed every year, leading to high rates of morbidity and mortality. *Leishmania spp* presents three different clinical manifestations: cutaneous, mucocutaneous and visceral. Among these forms, cutaneous is the most common whereas visceral is the most severe form (1-2). Treatment options are limited and far from being satisfactory. Most of available front-line agents were developed 50 years ago and include chemotherapeutic drugs such as injectable pentavalent antimonials and sodium stibogluconate and meglumine antimoniate. Second-line treatment relies in highly toxic drugs such as amphotericin B or pentamidine. In this context, the development of more effective and less toxic drugs represents an urgent need (3). In this regard, miltefosine, an alkylphosphocholine drug, and the aminoglycoside antibiotic paromomycin have proven to be effective drugs for the treatment of leishmaniasis. Newly developed liposomal amphotericin B is a preferred treatment in developing countries because it efficiently targets *Leishmania spp* parasites with low toxic side effects. Moreover, promising combination therapies are under intense investigation (4-5).

The trace element selenium is a micronutrient element with broad functions in biological systems. Selenium derivatives have been recognized by antioxidant, cancer preventing, and antiviral activities. Selenoproteins interfere with kinetoplastid biochemistry and have anti-parasite activities (6). Similarly, increased selenium concentration in plasma has been proposed as a new defensive strategy against *Leishmania spp* infection (7). In recent years, our research group and others have been engaged in the design, synthesis and

biological evaluation of new selenium compounds with potent *in vitro* antitrypanosomatic activity (8), mainly against *L. infantum*. Our data revealed that some of these compounds possess a potent activity with higher selectivity than the reference drugs miltefosine and edelfosine. Additionally, leishmanicidal activity in infected macrophages (THP-1 cells) was comparable to edelfosine (9-16). Among the different selenium entities tested, 4,4'-diaminodiphenyldiselenide showed one of the most promising leishmanicidal activities. This derivative contains as essential pharmacophore the diselenide group within the framework of molecular symmetry that, in our opinion, appears as a key factor for leishmanicidal activity. Herein, we designed several modifications on the side chain of the diselenide core in order to develop compounds with improved leishmanicidal activity and ADMET properties. For this purpose, the hit 4,4'-diaminodiphenyldiselenide was modulated by two strategies: i) the amine group was derivatized to urea, thiourea and selenourea in order to adjust polarity, solubility and ability to interact and form hydrogen bonds; ii) introduction of various aromatic systems or a cyclic or linear aliphatic chain of variable length and flexibility into the pendent amino groups of the ureidic function. Urea moiety is commonly found in various potent leishmanicidal compounds (17-18). On the other hand, the thiourea scaffold has been described for treating parasitic disorders by itself (19-20) or combined with metals (21). Finally, we further expanded the scope of the reaction to the synthesis of selenoureas in order to assess the importance of the number of selenium atoms in the leishmanicidal activity. Regarding the modulation in the pendent amino groups, various substituents were introduced to the terminal phenyl ring with the purpose of exploring their influence on activity by regulating

the electronic and steric features (22). Moreover, both cyclic and acyclic aliphatic chains have been validated as attractive scaffolds for the development of new leishmanicidal drugs, given the structural analogy with leishmanicidal derivatives containing aminoalkylchains previously reported in the literature (23-24). Figure 1 shows the general structure of the new designed compounds.

Based in previous studies, herein we present the synthesis and leishmanicidal activity against the amastigote form of *L. infantum* of thirty-one new derivatives related to Figure 1. 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) was determined.

MATERIALS AND METHODS

Chemistry. Melting points were determined with a Mettler FP82+FP80 apparatus (Greifense, Switzerland) and are not corrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 Ultrashield™ and Bruker Avance Neo spectrometers (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. (MontcadaiReixac, Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Madrid, Spain), Acros Organics (Janssen Pharmaceuticalaan 3a, 2440 Geel, België) and Lancaster (Bischheim-Strasbourg, France).

4,4'-Diaminodiphenyldiselenide. The synthesis of this compound has been previously described by Plano *et al***.** [11]**.**

General procedure for the synthesis of ureas 1-11. To a solution of 4,4'-diaminodiphenyldiselenide (1.17 mmol) in dioxane (25 mL) the corresponding isocyanate was added (2.34 mmol, 1:2 molar ratio), and the mixture was kept at room temperature from 24 h to 120 h. The solvent was removed under vacuum by rotatory evaporation and the residue was treated with ethyl ether (50 mL) and washed with water (100 mL).

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis(1-phenylurea) (1).** From phenyl isocyanate after 24 h gave **1** as a yellow powder. Yield: 58%; mp 277−278 ºC; IR υ_{max} (KBr): 3294 (N−H), 1637 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 6.98 (t, 2H, $J_{4-3} = J_{4-5} = 7.0$ Hz, B+B', H_4), 7.28 (t, 4H, $J_{3-2} = J_{5-6} = 8.0$ Hz, B+B', H_3+H_5), 7.43-7.46 (m, 8H, A+A'+B+B', H_2+H_6), 7.52 (d, 4H, $J_{3-2} = J_{5-6} = 8.5$ Hz, A+A', H_3+H_5), 8.74 (bs, 2H, 2NH), 8.85 (bs, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 118.7 (A+A', C_3+C_5), 119.4 (B+B', C_2+C_6), 122.5 (A+A', C_1), 130 (B+B', C_3+C_4+C_5), 134.1 (A+A', C_2+C_6), 140.0 (A+A', C_4), 140.7 (B+B', C_1), 152.8 (C=O); MS (m/z % abundance): 368 (59), 191 (100), 135 (24),

57 (43); Anal. Calcd for $C_{26}H_{22}N_4O_2Se_2$ (%): C: 53.8, H: 3.8, N: 9.6. Found: C: 54.1, H: 4.1, N: 9.1.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-nitrophenyl)urea] (2).** From 4-nitrophenyl isocyanate after 72 h gave **2** as a yellow powder. Yield: 66%; mp 245−247 ºC; IR υ_{max} (KBr): 3363 (N−H), 1614 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 7.47 (d, 4H, $J_{2\text{-}3} = J_{6\text{-}5} = 8.5$ Hz, A+A', H_2+H_6), 7.56 (d, 4H, $J_{3\text{-}2} = J_{5\text{-}6} = 8.5$ Hz, A+A', H_3+ H_5), 7.69 (d, 4H, $J_{2\text{-}3} = J_{6\text{-}5} = 9.1$ Hz, B+B', H_2+H_6), 8.19 (d, 4H, $J_{3\text{-}2} = J_{5\text{-}6} = 9.1$ Hz, B+B', H_3+ H_5), 9.10 (bs, 2H, 2NH), 9.50 (bs, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 118.3 (B+B', C_2+C_6), 120.5 (A+A', C_3+C_5), 122.8 (B+B', C_3+C_5), 126.1 (A+A', C_1), 133.3 (A+A', C_2+C_6), 139.7 (A+A', C_4), 142.0 (B+B', C_4), 147.1 (B+B', C_1), 152.2 (C=O); MS (m/z % abundance): 588 (29), 368 (15), 99 (46), 83 (50), 57 (100); Anal. Calcd for $C_{26}H_{20}N_6O_6Se_2$ (%): C: 46.6, H: 3.0, N: 12.5. Found: C: 46.5, H: 3.1, N: 12.6.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-methylphenyl)urea] (3).** From 4-methylphenyl isocyanate after 120 h gave **3** as a yellow powder. Yield: 28%; mp 283−284 ºC; IR υ_{max} (KBr): 3315 (N−H), 1643 (C=O) cm^{-1};^1H NMR (400 MHz, DMSO-d_6, δ): 2.24 (s, 6H, $2CH_3$), 7.09 (d, 4H, $J_{3\text{-}2} = J_{5\text{-}6} = 8.1$ Hz, B+B', H_3+ H_5), 7.33 (d, 4H, $J_{2\text{-}3} = J_{6\text{-}5} = 8.1$ Hz, B+B', H_2+H_6), 7.43 (d, 4H, $J_{2\text{-}3} = J_{6\text{-}5} = 8.5$ Hz, A+A', H_2+H_6), 7.51 (d, 4H, $J_{3\text{-}2} = J_{5\text{-}6} = 8.5$ Hz, A+A', H_3+ H_5), 8.61 (s, 2H, 2NH), 8.79 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 32.0 (CH_3), 118.1 (B+B', C_2+C_6), 118.8 (A+A', C_3+C_5), 122.4 (A+A', C_1), 127.2 (B+B', C_3+C_5), 130.7 (A+A', C_2+C_6), 132.6 (B+B', C_1), 137.1 (B+B', C_4), 140.8 (A+A', C_4), 154.2 (C=O); MS (m/z % abundance): 240 (24), 107 (80), 83 (58), 57 (100); Anal. Calcd for $C_{28}H_{26}N_4O_2Se_2$ .1/2 H_2O (%): C: 54.4, H: 4.2, N: 9.0. Found: C: 54.6, H: 4.3, N: 8.8.

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***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-chlorophenyl)urea]**

(4). From 4-chlorophenyl isocyanate after 48 h gave **4** as a yellow powder. Yield: 59%; mp>300 ºC; IR υ_{max} (KBr): 3289 (N−H), 1635 (C=O) cm^{-1}, ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.33 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.3 Hz, A+A', H_2+H_6), 7.45-7.54 (m, 12H, B+B', H_2+H_3+H_5+H_6, A+A', H_3+H_5), 8.86 (bs, 4H, 4NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 119.5 (A+A', C_3+C_5), 120.3 (B+B', C_2+C_6), 122.7 (A+A', C_1), 126.0 (B+B', C_4), 129.1 (B+B', C_3+C_5), 134.1 (A+A', C_2+C_6), 139.0 (B+B', C_1), 140.5 (A+A', C_4), 152.7 (C=O); MS (m/z % abundance): 338 (29), 143 (85), 87 (54), 57 (100); Anal. Calcd for $C_{26}H_{20}Cl_2N_4O_2Se_2$ (%): C: 48.1, H: 3.1, N: 8.6. Found: C: 48.0, H: 3.1, N: 8.4.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-cyanophenyl)urea]**

(5). From 4-cyanophenyl isocyanate after 96 h gave **5** as a yellow powder. Yield: 70%; mp 185−186 ºC; IR υ_{max} (KBr): 3367 (N−H), 2221 (CN), 1689 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.46 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 7.5 Hz, A+A', H_2+H_6), 7.55 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 7.5 Hz, A+A', H_3+ H_5), 7.64 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.0 Hz, B+B', H_3+H_5), 7.73 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.0 Hz, B+B', H_2+H_6), 9.02 (s, 2H, 2NH), 9.24 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 103.9 (B+B', C_4), 118.6 (CN), 119.7 (B+B', C_2+C_6), 123.2 (A+A', C_1), 133.8 (A+A', C_2+C_6), 134.0 (B+B', C_3+C_5), 140.1 (A+A', C_4), 144.5 (B+B', C_1), 152.4 (C=O); MS (m/z % abundance): 156 (27), 92 (21), 83 (28), 71 (45), 57 (100); Anal. Calcd for $C_{28}H_{20}N_6O_2Se_2$ (%): C: 53.3, H: 3.2, N: 13.3. Found: C: 53.1, H: 3.5, N: 13.2.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-methoxyphenyl)urea]**

(6). From 4-methoxyphenyl isocyanate after 72 h gave **6** as a yellow powder. Yield: 65%; mp 274−275 ºC; IR υ_{max} (KBr): 3288 (N−H), 1644 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.74 (s, 6H, 2OCH_3), 6.56 (d, 2H, $J_{3\text{-}2}$ = 7.5 Hz,

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B+B', H_3), 6.94 (d, 2H, $J_{5\text{-}6'}$ = 7.5 Hz, B+B', H_5), 7.19 (s, 4H, B+B', H_2+H_6), 7.44 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 6.9 Hz, A+A', H_2+H_6), 7.53 (d, 4H, A+A', H_3+H_5), 8.73 (s, 2H, 2NH), 8.82 (s, 2H, 2NH);^{13}C NMR (100 MHz, DMSO-d_6, δ): 55.4 (CH_3), 111.1 (B+B', C_3+C_5), 119.4 (B+B', C_2+C_6), 122.6 (A+A', C_3+C_5), 130.0 (A+A', C_1), 134.1 (A+A', C_2+C_6), 140.6 (B+B', C_1), 141.2 (A+A', C_4), 152.7 (C=O), 160.2 (B+B', C_4); Anal. Calcd for $C_{28}H_{26}N_4O_4Se_2$ (%): C: 52.5, H: 4.0, N: 8.7. Found: C: 52.3, H: 3.9, N: 8.5.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis(1-benzylurea) (7).** From benzyl isocyanate after 96 h gave **7** as a yellow powder. Yield: 43%; mp 213−215 ºC; IR υ_{max} (KBr): 3335 (N−H), 1647 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.31 (d, 4H, $J_{CH2\text{-}NH}$ = 5.6 Hz, 2CH_2), 6.68 (t, 2H, $J_{NH\text{-}CH2}$ = 5.6 Hz, NH-C$\underline{H_2}$), 7.24−7.34 (m, 10H, B+B', H_2+H_3+H_4+H_5+H_6), 7.39 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.6 Hz, A+A', H_3+H_5), 7.45 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.4 Hz, A+A', H_2+H_6), 8.74 (s, 2H, 2N$\underline{H}$-C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 43 (CH_2), 118.8 (A+A', C_3+C_5), 121.7 (A+A', C_1), 127.2 (B+B', C_4), 127.6 (B+B', C_2+C_6), 128.8 (B+B', C_3+C_5), 134.3 (A+A', C_2+C_6), 140.7 (B+B', C_1), 141.5 (A+A', C_4), 155.5 (C=O); Anal. Calcd for $C_{28}H_{26}N_4O_2Se_2$ .1/2 H_2O (%): C: 54.5, H: 4.4, N: 9.1. Found: C: 54.6, H: 4.5, N: 9.3.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-methoxybenzyl)urea] (8).** From 4-methoxybenzyl isocyanate after 48 h gave **8** as a yellow powder. Yield: 31%; mp 222−224 ºC; IR υ_{max} (KBr): 3305 (N−H), 1630 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.73 (s, 6H, 2OCH_3), 4.23 (d, 4H, $J_{CH2\text{-}NH}$ = 5.5 Hz, 2C$\underline{H_2}$), 6.60 (t, 2H, $J_{NH\text{-}CH2}$ = 5.5 Hz, 2N$\underline{H}$−CH_2), 6.90 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.5 Hz, B+B', H_2+H_6), 7.23 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.5 Hz, B+B', H_3+H_5), 7.39 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.5 Hz, A+A', H_2+H_6), 7.45 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.5 Hz, A+A', H_3+ H_5),

8.69 (s, 2H, 2NH−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 42.9 (CH_2), 56.0 (CH_3), 114.4 (B+B', C_3+C_5), 119.0 (A+A', C_3+C_5), 122.1 (A+A', C_1), 129.2 (B+B', C_2+C_6), 133.6 (B+B', C_1), 134.2 (A+A', C_2+C_6), 142.3 (A+A', C_4), 158.5 (C=O), 159.4 (B+B', C_4); MS (m/z % abundance): 368 (7), 215 (26), 83 (44), 71 (53), 57 (100); Anal. Calcd for $C_{30}H_{30}N_4O_4Se_2$.1/2 H_2O (%): C: 53.2, H: 4.4, N: 8.3. Found: C: 53.1, H: 4.4, N: 8.2.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(n-butyl)urea] (9).** From butyl isocyanate after 72 h gave **9** as a yellow powder. Yield: 42%; mp 250−251 ºC; IR υ_{max} (KBr): 3309 (N−H), 2958−2864 (C−H), 1630 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 0.89 (t, 6H, $J_{CH3-CH2}$ = 7.2 Hz, $2CH_3$), 1.25−1.45 (m, 8H, 2(-CH_2−CH_2−CH_3)), 2.96−3.17 (m, 4H, 2(-NH−CH_2), 6.18 (t, 2H, $J_{NH2-CH2}$ = 5.3 Hz, 2NH−CH_2), 7.30−7.48 (m, 8H, A+A', H_2+H_3+H_5+H_6), 8.57 (s, 2H, 2NH−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 14.2 (C_4), 19.1 (C_3), 32.5 (C_2), 39.0 (C_1), 119.1 (A+A', C_3+C_5), 122.8 (A+A', C_1), 134.7 (A+A', C_2+C_6), 142.5 (A+A', C_4), 156.1 (C=O); MS (m/z % abundance): 588 (12), 211 (100), 183 (26), 91 (34), 43 (26); Anal. Calcd for $C_{22}H_{30}N_4O_2Se_2.H_2O$ (%): C: 47.3, H: 5.7, N: 10.0. Found: C: 47.4, H: 5.5, N: 9.9.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(n-hexyl)urea] (10).** From hexyl isocyanate after 72 h gave **10** as a yellow powder. Yield: 25%; mp 180−181 ºC; IR υ_{max} (KBr): 3313 (N−H), 2956−2856 (C−H), 1627 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 0.87 (bs, 6H, $2CH_3$), 1.27 (bs, 12H, 2(-$(CH_2)_2$−$(CH_2)_3$−CH_3)), 1.41 (bs, 4H, 2(-CH_2−CH_2−$(CH_2)_3$−CH_3)), 3.06 (bs, 4H, 2(-CH_2−$(CH_2)_4$−CH_3)), 6.17 (bs, 2H, 2NH−CH_2), 7.35 (d, 4H, J_{2-3} = J_{6-5} = 8.0 Hz, A+A', H_2+H_6), 7.43 (d, 4H, J_{3-2} = J_{5-6} = 8.0 Hz, A+A', H_3+H_5), 8.57 (bs, 2H, 2NH−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 14.4 (C_6), 22.5 (C_5), 26.5 (C_3),

30.1 (C_2), 31.5 (C_1+C_4), 118.7 (A+A', C_3+C_5), 121.4 (A+A', C_1), 134.4 (A+A', C_2+C_6), 141.7 (A+A', C_4), 155.4 (C=O); MS (m/z % abundance): 172 (25), 149 (56), 123 (100), 91 (55), 56 (74), Anal. Calcd for $C_{26}H_{38}N_4O_2Se_2$.1/2 H_2O (%): C: 51.6, H: 6.2, N: 9.2. Found: C: 51.6, H: 6.0, N: 9.1.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-cyclohexylurea] (11).** From cyclohexyl isocyanate after 120 h gave **11** as a yellow powder. Yield: 59%; mp 255−257 ºC; IR υ_{max} (KBr): 3306 (N−H), 2927−2850 (C−H), 1645 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 1.15−1.30 (m, 12H, B+B', 2H_3+2H_4+2H_5), 1.53−1.58 (m, 2H, B+B', H_1), 1.65 (d, 4H, $J_{2\text{-}3}$ = $J_{2\text{-}1}$ = 13.0 Hz, B+B', 2H_2), 1.79 (d, 4H, $J_{6\text{-}1}$ = $J_{6\text{-}5}$ = 14.1 Hz, B+B', 2H_6), 6.13 (d, 2H, $J_{NH\text{-}CH}$ = 7.8 Hz, 2N<u>H</u>−CH), 7.34 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.6 Hz, A+A', H_2+H_6), 7.43 (d, 4H, A+A', H_3+H_5), 8.46 (s, 2H, 2N<u>H</u>−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ) 24.8+25.7 (B+B', C_3+C_5), 33.3+33.8 (B+B', C_2+C_4+C_6), 48.0 (B+B', C_1), 118.6 (A+A', C_3+C_5), 121.5 (A+A', C_1), 127.7 (A+A', C_2+C_6), 134.4 (A+A', C_4), 154.6 (C=O); MS (m/z % abundance): 368 (34), 224 (71), 191 (76), 56 (100), 41 (27); Anal. Calcd for $C_{26}H_{34}N_4O_2Se_2$ (%): C: 52.7, H: 5.7, N: 9.4. Found: C: 52.3, H: 5.5, N: 9.8.

General procedure for the synthesis of thioureas 12-22. To a solution of diselenide (1.17 mmol) in dioxane (25 mL) the corresponding isothiocyanate (2.34 mmol, 1:2 molar ratio) was added and the mixture was kept at room temperature from 48 h to 144 h. The solvent was removed under vacuum by rotatory evaporation and the residue was treated with ethyl ether (50 mL) and washed with water (100 mL).

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis(1-phenylthiourea) (12).** From phenyl isothiocyanate after 144 h gave **12** as a yellow powder. Yield:

45%; mp 142−143 ºC; IR υ_{max} (KBr): 3193 (N−H), 1588 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.12-7.15 (m, 2H, B+B', H_4), 7.33 (t, 4H, J_{3-2} = J_{5-6} = 7.5 Hz, B+B', H_3+H_5), 7.46-7.49 (m, 8H, A+A', B+B', H_2+H_6), 7.59 (d, 4H, J_{3-2} = J_{5-6} = 7.5 Hz, A+A', H_3+H_5), 9.88 (bs, 4H, 4NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 124.1 (A+A', C_1), 124.5 (A+A', C_3+C_5), 125.0 (B+B', C_2+C_6), 125.4 (B+B', C_4), 128.9 (B+B', C_3+C_5), 132.5 (A+A', C_2+C_6), 139.8 (A+A', C_4), 140.2 (B+B', C_1), 179.9 (C=S); MS (m/z % abundance): 428 (33), 386 (34), 214 (69), 172 (86), 135 (100), 93 (74), 80 (35); Anal. Calcd for $C_{26}H_{22}N_4S_2Se_2$ (%): C: 50.9, H: 3.6, N: 9.1. Found: C: 50.6, H: 3.8, N: 8.7.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-nitrophenyl)thiourea] (13).** From 4-nitrophenyl isothiocyanate after 48 h gave **13** as a yellow powder. Yield: 58%; mp 175−176 ºC; IR υ_{max} (KBr): 3345 (N−H), 1570 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.49 (bs, 4H, A+A', H_2+H_6), 7.62 (bs, 4H, A+A', H_3+H_5), 7.81 (bs, 4H, B+B', H_2+H_6), 8.20 (bs, 4H, B+B', H_3+H_5), 10.41 (bs, 4H, 4NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 122.2 (A+A', C_1), 124.9 (B+B', C_3+C_5), 126.2 (B+B', C_2+C_6), 132.4 (A+A', C_3+C_5), 139.5 (A+A', C_2+C_6), 142.9 (A+A', C_4), 146.6 (B+B', C_1+C_4), 179.7 (C=S); MS (m/z % abundance): 426 (5), 386 (13), 344 (15), 180 (100), 172 (53), 150 (26), 134 (34), 90 (25); Anal. Calcd for $C_{26}H_{20}N_6O_4S_2Se_2 .H_2O$ (%): C: 43.3, H: 2.8, N: 11.6. Found: C: 43.6, H: 2.9, N: 11.3.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-methylphenyl)thiourea] (14).** From 4-methylphenyl isothiocyanate after 96 h gave **14** as a yellow powder. Yield: 63%; mp 151−153 ºC; IR υ_{max} (KBr): 3203 (N−H), 1583 (C=S) cm^{-1}; ^{1}H RMN (400 MHz, DMSO-d_6, δ): 2.28 (s, 6H, $2CH_3$), 7.14 (d, 4H, J_{3-2} = J_{5-6} = 8.0 Hz, B+B', H_3+H_5), 7.32 (d, 4H, B+B', H_2+H_6), 7.48

(d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.2 Hz, A+A', H_2+H_6), 7.58 (d, 4H, A+A', H_3+H_5), 9.81 (bs, 4H, 4NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 21.3 (CH_3), 123.0 (A+A', C_1), 125.2 (A+A', C_3+C_5), 127.9 (B+B', C_2+C_6), 129.0 (B+B', C_3+C_5), 131.5 (A+A', C_2+C_6), 133.2 (B+B', C_1), 137.1 (B+B', C_4), 140.3 (A+A', C_4), 179.8 (C=S); MS (m/z % abundance): 428 (33), 386 (37), 214 (65), 172 (100), 149 (86), 106 (90), 91 (52); Anal. Calcd for $C_{28}H_{26}N_4S_2Se_2$ (%): C: 52.5, H: 4.1, N: 8.7. Found: C: 52.1, H: 4.3, N: 8.4.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-chlorophenyl)thiourea] (15).** From 4-chlorophenyl isothiocyanate after 48 h gave **15** as a yellow powder. Yield: 68%; mp 170−171 ºC; IR υ_{max} (KBr): 3210 (N−H), 1583 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.44 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.8 Hz, A+A', H_2+H_6), 7.55 (dd, 8H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.8 Hz, A+A', B+B', H_3+H_5), 7.65 (d, 4H, B+B', H_2+H_6), 10.01 (bs, 4H, 4NH);^{13}C NMR (100 MHz, DMSO-d_6, δ): 124.6 (A+A', C_3+C_5), 125.6 (B+B', C_2+C_6), 125.7 (A+A', C_1), 128.8 (B+B', C_4), 128.8 (B+B', C_3+C_5), 132.5 (A+A', C_2+C_6), 138.8 (B+B', C_1), 139.9 (A+A', C_4), 180.0 (C=S); MS (m/z % abundance): 428 (14), 386 (25), 214 (28), 169 (100), 127 (54), 111 (23); Anal. Calcd for $C_{26}H_{20}Cl_2N_4S_2Se_2$ (%): C: 45.8, H: 2.9, N: 8.2. Found: C: 45.5, H: 3.0, N: 7.9.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-cyanophenyl)thiourea] (16).** From 4-cyanophenyl isothiocyanate after 144 h gave **16** as a yellow powder. Yield: 44%; mp 123−124 ºC; IR υ_{max} (KBr): 3166 (N−H), 2224 (CN), 1584 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.48 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.4 Hz, A+A', H_2+H_6), 7.62 (d, 4H, A+A', H_3+H_5), 7.75 (d, 4H, $J_{3\text{-}5}$ = $J_{5\text{-}6}$ = 8.8 Hz, B+B', H_3+H_5), 7.78 (d, 4H, B+B', H_2+H_6), 10.25 (bs, 2H, 2NH), 10.28 (bs, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 104.9 (CN), 114.7

(B+B', C_4), 124.2 (A+A', C_1), 124.7 (A+A', C_3+C_5), 125.3 (B+B', C_2+C_6), 129.8 (A+A', C_2+C_6), 132.6 (B+B', C_3+C_5), 139.0 (A+A', C_4), 144.2 (B+B', C_1), 179.8 (C=S); MS (m/z % abundance): 428 (6), 386 (22), 344 (23), 172 (96), 160 (100), 118 (30), 80 (20); Anal. Calcd for $C_{28}H_{20}N_6S_2Se_2$.1/2 H_2O (%): C: 50.1, H: 3.0, N: 12.5. Found: C: 49.8, H: 3.3, N: 12.4.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-methoxyphenyl)thiourea] (17).** From 4-methoxyphenyl isothiocyanate after 72 h gave **17** as a yellow powder. Yield: 65%; mp 142−144 ºC; IR υ_{max} (KBr): ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.75 (s, 6H, 2OCH_3), 6.91 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.9 Hz, B+B', H_2+H_6), 7.32 (d, 4H, B+B', H_3+H_5), 7.48 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.6 Hz, A+A', H_3+H_5), 7.58 (d, 4H, A+A', H_2+H_6), 9.72 (bs, 4H, 4NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 56.0 (CH_3), 114.2 (B+B', C_3+C_5), 115.3 (A+A', C_3+C_5), 126.0 (B+B', C_1), 125.4 (A+A', C_1), 132.1 (B+B', C_2+C_6), 133.6 (A+A', C_2+C_6), 139.7 (A+A', C_4), 159.2 (B+B', C_4), 180.1 (C=S); MS (m/z % abundance): 428 (21), 386 (32), 213 (53), 172 (100), 166 (84), 150 (54), 108 (57), 80 (41); Anal. Calcd for $C_{28}H_{26}N_4O_2S_2Se_2$ .1/2 H_2O (%): C: 48.7, H: 4.1, N: 8.1. Found: C: 49.1, H 3.9, N: 7.8.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis(1-benzylthiourea) (18).** From benzyl isothiocyanate after 144 h gave **18** as a yellow powder. Yield: 71%; mp 146−147 ºC; IR υ_{max} (KBr): 3238 (N−H), 1533 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.77 (d, $J_{CH2\text{-}NH}$ = 5.3 Hz, 4H, 2CH_2), 7.22−7.28 (m, 2H, B+B', H_4), 7.32-7.38 (m, 8H, B+B', H_2+H_6, H_3+H_5), 7.45 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.6 Hz, A+A', H_2+H_6), 7.57 (d, 4H, A+A', H_3+H_5), 8.27 (s, 2H, 2N<u>H</u>−CH_2), 9.71 (s, 2H, 2N<u>H</u>−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 47.6 (CH_2), 124.1 (A+A', C_1), 125.1 (A+A', C_3+C_5), 127.4 (B+B', C_4), 127.9 (B+B', C_2+C_6), 128.8 (B+B',

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C_3+C_5), 132.7 (A+A', C_2+C_6), 139.3 (B+B', C_1), 140.0 (A+A', C_4), 181.1 (C=S); MS (m/z % abundance): 368 (42), 191 (100), 172 (67), 57 (54); Anal. Calcd for $C_{28}H_{26}N_4S_2Se_2$ .1/2 H_2O (%): C: 51.8, H: 4.2, N: 8.6. Found: C: 51.9, H: 4.6, N: 8.7.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-methoxybenzyl)thiourea] (19).** From 4-methoxybenzyl isothiocyanate after 120 h gave **19** as a yellow powder. Yield: 49%; mp 174−175 ºC; IR υ_{max} (KBr): 3220 (N−H), 1583 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.73 (s, 6H, $2OCH_3$), 4.64 (bs, 4H, $2CH_2$), 6.90 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.8 Hz, B+B', H_3+H_5), 7.27 (d, 4H, B+B', H_2+H_6,), 7.44 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 7.8 Hz, A+A', H_2+H_6), 7.56 (d, 4H, A+A', H_3+H_5), 8.20 (bs, 2H, 2N<u>H</u>−CH_2), 9.66 (bs, 2H, 2N<u>H</u>−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 53.9 (CH_2), 57.1 (CH_3), 112.8 (B+B', C_3+C_5), 115.0 (A+A', C_1), 123.2 (A+A', C_3+C_5), 124.1 (B+B', C_1), 128.8 (B+B', C_2+C_6), 132.6 (A+A', C_2+C_6), 140.2 (A+A', C_4), 159.1 (B+B', C_4), 180.7 (C=S); MS (m/z % abundance): 428 (55), 214 (100), 172 (44), 136 (96), 121 (82), 106 (36); Anal. Calcd for $C_{30}H_{30}N_4O_2S_2Se_2$ .1/2 H_2O (%): C: 50.7, H: 4.2, N: 7.9. Found: C: 50.4, H: 4.2, N: 7.7.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(n-butyl)thiourea] (20).** From 4-methoxybenzyl isothiocyanate after 120 h gave **20** as a yellow powder. Yield: 49%; mp 174−175 ºC; IR υ_{max} (KBr): 3220 (N−H), 1583 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.73 (s, 6H, $2OCH_3$), 4.64 (bs, 4H, $2CH_2$), 6.90 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.8 Hz, B+B', H_3+H_5), 7.27 (d, 4H, B+B', H_2+H_6,), 7.44 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 7.8 Hz, A+A', H_2+H_6), 7.56 (d, 4H, A+A', H_3+H_5), 8.20 (bs, 2H, 2N<u>H</u>−CH_2), 9.66 (bs, 2H, 2N<u>H</u>−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 14.2 (CH_3), 20.1 (CH_2), 30.1 (CH_2), 44.0 (CH_2), 123.7 (A+A', C_1), 124.7 (A+A',

373 C_3+C_5), 132.7 (A+A', C_2+C_6), 140.2 (A+A', C_4), 180.6 (C=S); MS (m/z %
374 abundance): 428 (55), 214 (100), 172 (44), 136 (96), 121 (82), 106 (36); Anal.
375 Calcd for $C_{30}H_{30}N_4O_2S_2Se_2$ .1/2 H_2O (%): C: 50.7, H: 4.2, N: 7.9. Found: C:
376 50.4, H: 4.2, N: 7.7.

377 ***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(n-hexyl)thiourea] (21).**
378 From hexyl isothiocyanate after 120 h gave **21** as a yellow powder. Yield: 65%;
379 mp 132−134 ºC; IR υ_{max} (KBr): 3223 (N−H), 2925−2854 (C−H), 1540 (C=S) cm^{-1};
380 ^{1}H NMR (400 MHz, DMSO-d_6, δ): 0.87 (bs, 6H, $2CH_3$), 1.28 (bs, 12H, 2(-
381 $(CH_2)_2$−(CH_2)$_3$−CH_3)), 1.51 (bs, 4H, 2(-CH_2−CH_2−$(CH_2)_3$−CH_3)), 3.44 (bs, 4H,
382 2(-CH_2−$(CH_2)_4$−CH_3)), 7.42 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.4 Hz, A+A', H_2+H_6), 7.55 (d,
383 4H, A+A', H_3+H_5), 7.85 (bs, 2H, 2NH−CH_2), 9.55 (bs, 2H, 2NH−C_6H_4); ^{13}C NMR
384 (100 MHz, DMSO-d_6, δ): 14.4 (CH_3), 22.5 (CH_2), 26.6 (CH_2), 28.8 (CH_2), 31.5
385 (CH_2), 44.3 (CH_2), 123.7 (A+A', C_1), 132.7 (A+A', C_2+C_3+C_5+C_6), 140.2 (A+A',
386 C_4), 180.6 (C=S); MS (m/z % abundance): 428 (14), 386 (6), 214 (32), 172 (45),
387 135 (73), 115 (92), 72 (47), 57 (56), 43 (100); Anal. Calcd for
388 $C_{26}H_{38}N_4S_2Se_2$.1/2 H_2O (%): C: 49.0, H: 6.0, N: 8.8. Found: C: 48.9, H: 6.0, N:
389 8.8.

390 ***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-cyclohexylthiourea]**
391 **(22).** From cyclohexylisothiocyanate after 96 h gave **22** as a yellow powder.
392 Yield: 62%; mp 143−144 ºC; IR υ_{max} (KBr): 3321 (N−H), 2926−2851 (C−H),
393 1587 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.10−1.35 (m, 12H, B+B',
394 $2H_3$+$2H_4$+$2H_5$)), 1.56 (bs, 2H, B+B', $2H_1$), 1.62 (bs, 4H, B+B', $2H_2$) 1.80 (bs, 4H,
395 B+B', $2H_6$), 7.47 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.6 Hz, A+A', H_3+H_5), 7.54 (d, 4H, A+A',
396 H_3+H_5), 7.81 (d, 2H, $J_{NH\text{-}CH}$ = 8.1 Hz, 2NH−CH), 9.49 (s, 2H, 2NH−C_6H_4); ^{13}C
397 NMR (100 MHz, DMSO-d_6, δ) 25 (B+B', C_3+C_5), 26 (B+B', C_4), 32 (B+B',

398 C_2+C_6), 53 (B+B', C_1), 124 (A+A', C_1), 131 (A+A', C_2+C_3+C_5+C_6), 140 (A+A',
399 C_4), 179 (C=S); MS (m/z % abundance): 368 (12), 214 (7), 191 (24), 83 (21), 56
400 (100), 41 (33); Anal. Calcd for $C_{26}H_{34}N_4S_2Se_2$ .1/2 H_2O (%): C: 49.4, H: 5.2, N:
401 8.8. Found: C: 49.2, H: 5.3, N: 8.5.

402 **Preparation of formamides 23-30.**

403 ***N*-phenylformamide (23).** To a stirred solution of aniline (9.2 mmol) was
404 added dropwise ethyl formate (9.6 mmol). The reaction mixture was stirred at
405 150 ºC for 12 h. The reaction mixture was cooled to room temperature and the
406 precipitate was collected by filtration, dried and washed with ethyl ether (100
407 mL) to give **23** as a white powder. Yield: 77%; IR υ_{max} (KBr): 3364 (N−H), 1634
408 (C=O) cm^{-1}.

409 ***N*-(4-methylphenyl)formamide (24).** A mixture of 4-methylaniline (5
410 mmol) and anhydrous ammonium formate (7.5 mmol) in dry acetonitrile (15 mL)
411 was heated at 100°C for 24 h. Acetonitrile was removed under reduced
412 pressure. The residue was diluted with ethyl acetate (25 mL) and washed with
413 water (4 x 15 mL). The organic layer was dried over anhydrous Na_2SO_4. After
414 filtration and evaporation of the solvent, **24** was acquired as a white powder.
415 Yield: 66%; IR υ_{max} (KBr): 3117 (N−H), 1637 (C=O) cm^{-1}.

416 ***N*-(4-chlorophenyl)formamide (25).** To a mixture of 4-chloroaniline (10
417 mmol), formic acid (30 mmol) and zinc dust pre-treated with HCl (1 mmol) was
418 added and stirred at 70 ºC for 8 h−12 h. The mixture was diluted with CH_2Cl_2
419 (50 mL), and filters through celite. Then the filtrate was washed with saturated
420 $NaHCO_3$ (4 x 30 mL) and brine (2x20 mL), and was dried over anhydrous
421 Na_2SO_4. After filtration and evaporation of the solvent, **25** was acquired as a
422 white powder. Yield: 69%; IR υ_{max} (KBr): 3258 (N−H), 1670 (C=O) cm^{-1}.

***N*-(4-cyanophenyl)formamide (26).** To a mixture of 4-aminobenzonitrile (10 mmol), formic acid (30 mmol) and zinc dust pre-treated with HCl (1 mmol) was added and stirred at 70 ºC for 8 h−12 h. The mixture was diluted with CH_2Cl_2 (50 mL), and filters through celite. Then the filtrate was washed with saturated $NaHCO_3$ (3 x 30 mL) and brine (3 x 30 mL), and was dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, **26** was acquired as a white powder. Yield: 82%; IR υ_{max} (KBr): 3357 (N−H), 2216 (CN), 1637 (C=O) cm^{-1}.

***N*-(4-methoxyphenyl)formamide (27).** To a mixture of 4-methoxyaniline (11.25 mmol) and HCOOH (33.75 mmol), PEG-400 (16 g) was added. The mixture was stirred at room temperature for 24 h and after completion was diluted with water (50 mL) and extracted with ethyl acetate (5 x 15 mL). Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was subjected to column chromatography ethyl (acetate/ hexane 70/30) to obtain the pure 27 as a white powder. Yield: 68.6%; IR υ_{max} (KBr): 3245 (N−H), 1656 (C=O) cm^{-1}.

***N*-butylformamide (28).** To a stirred solution of butan-1-amine (25 mmol) was added dropwise ethyl formate (20.16 mmol). The reaction mixture was stirred at 150 ºC for 12 h. The reaction mixture was cooled to room temperature and the precipitate was collected by filtration, dried and washed with ethyl ether (100 mL) to give **28** as a white powder. Yield: 74.7%; IR υ_{max} (KBr): 3291 (N−H), 2960−2869 (C−H), 1665 (C=O) cm^{-1}.

***N*-hexylformamide (29).** To a stirred solution of hexan-1-amine (15 mmol) was added dropwise ethyl formate (12.10 mmol). The reaction mixture was stirred at 150 ºC for 12 h. The reaction mixture was cooled to room

448 temperature and the precipitate was collected by filtration, dried and washed
449 with ethyl ether (100 mL) to give **29** as a white powder. Yield: 92%; IR υ_{max}
450 (KBr): 3361 (N−H), 2978−2868 (C−H), 1630 (C=O) cm^{-1}.

451 ***N*-ciclohexylformamide (30).** To a mixture of cyclohexylamine (25
452 mmol), formic acid (75 mmol) and zinc dust pre-treated with HCl (5 mmol) was
453 added and stirred at 70 ºC for 8 h−12 h. The mixture was diluted with CH_2Cl_2
454 (50 mL), and filters through celite. Then the filtrate was washed with saturated
455 $NaHCO_3$ (4 x 25 mL) and brine (2 x 25 mL), and was dried over anhydrous
456 Na_2SO_4. After filtration and evaporation of the solvent, **30** was acquired as a
457 white powder. Yield: 16%; IR υ_{max} (KBr): 3412 (N−H), 2933−2858 (C−H), 1661
458 (C=O) cm^{-1}.

459 **General procedure for the synthesis of isoselenocyanates 31-34.** To
460 a mixture of formamide (6.29 mmol) and *N*,*N*-diethyletanamine (26.8 mmol) in
461 dry toluene (50 mL) was added dropwise a solution of phosgene (3.35 mmol) in
462 dry toluene (10 mL), under N_2 atmosphere, on ice over a period of 30 min. Then
463 black selenium powder (12.58 mmol) was added and the resulting mixture was
464 refluxed for 24 h in the darkness. After filtered, solvents were removed under
465 vacuum and the residue was washed with dichloromethane (30 mL). Column
466 chromatography using ethyl acetate/hexane (70/30) as eluent afforded
467 isoselenocyanate.

468 **Phenylisoselenocyanate (31).** From *N*-phenylformamide **23** gave **31** as
469 a brown syrup: Yield 2.66%; IR υ_{max} (KBr): 2978−2873 (C−H), 2114 (N=C=Se)
470 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 6.92 (t, 2H, $J_{2\text{-}3} = J_{6\text{-}5} = 6.3$ Hz, H_2+H_6),
471 7.28 (t, 1H, H_4), 7.62 (d, 2H, $J_{3\text{-}2} = J_{5\text{-}6} = 8.4$ Hz, H_3+H_5).

4-Methylphenylisoselenocyanate (32). From *N*-(4-methylphenyl)formamide **24** gave **32** as a brown syrup: Yield 7.48%; IR υ_{max} (KBr): 2921−2866 (C−H), 2153 (N=C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 2.34 (s, 3H, CH_3), 7.21 (d, 2H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 7.9 Hz, H_2+H_6), 7.26 (d, 2H, H_3+H_5).

4-Methoxyphenylisoselenocyanate (33). From *N*-(4-methoxyphenyl)formamide **27** gave **3**3 as an orange syrup: Yield 6.79%; IR υ_{max} (KBr): 2944−2740 (C−H), 2121 (N=C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 3.80 (s, 3H, OCH_3), 7.02 (d, 2H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.0 Hz, H_3+H_5), 7.45 (d, 2H, H_2+H_6).

Benzylisoselenocyanate (34). From *N*-benzylformamide gave **34** as a brown syrup: Yield 11.75 %; IR υ_{max} (KBr): 2958−2871 (C−H), 2143 (N=C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 5.15 (s, 2H, CH_2), 7.41−7.54 (m, 5H, H_2+H_3+H_4+H_5+H_6).

General procedure for the synthesis of isoselenocyanates 35-39. To a refluxing mixture of formamide (7.2 mmol) and *N*,*N*-diethyletanamine (30.5 mmol) in dry dichloromethane (25 mL) was added dropwise a solution of triphosgene (3.85 mmol) in dry dichloromethane (5 mL), under N_2 atmosphere, over a period of 45 min. After the addition, the resulting mixture was refluxed for 2.5 h and then black selenium powder (14.4 mmol) was added and refluxed for 12 h in the darkness. After filtered, solvents were removed under vacuum and the residue was washed with dichloromethane (30 mL). Column chromatography using ethyl acetate/hexane (70/30) as eluent afforded isoselenocyanate.

4-Chlorophenyl isoselenocyanate (35). From *N*-(4-chlorophenyl)formamide **25** gave **35** as a brown syrup: Yield 55.76%; IR υ_{max} (KBr): 2924−2854 (C−H), 2151 (N=C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 7.02 (d, 2H, $J_{3\text{-}2} = J_{5\text{-}6} = 7.6$ Hz, H_3+H_5), 7.49 (d, 2H, H_2+H_6).

4-Cyanophenyl isoselenocyanate (36). From *N*-(4-cyanophenyl)formamide **26** gave **36** as a brown syrup: Yield 85 %; IR υ_{max} (KBr): 2927−2856 (C−H), 2225 (CN), 2146 (N=C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 7.77 (d, 2H, $J_{3\text{-}2} = J_{5\text{-}6} = 8.4$ Hz, H_3+H_5), 7.79 (d, 2H, H_2+H_6).

Butyl isoselenocyanate (37). From *N*-butylformamide **28** gave **37** as a dark syrup: Yield 43.5%; IR υ_{max} (KBr): 2923−2876 (C−H), 2144 (N=C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 0.86−1.11 (m, 3H, CH_3), 1.21−1.32 (m, 4H, $\underline{CH_2}$−$\underline{CH_2}$−CH_3), 1.35 (m, 2H, $\underline{CH_2}$-NCSe).

Hexyl isoselenocyanate (38). From *N*-hexylformamide **29** gave **38** as a brown syrup: Yield 62%; IR υ_{max} (KBr): 2931−2861 (C−H), 2144 (N=C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 0.85−0.91 (m, 3H, CH_3), 1.05 (t, 2H, J = 6.99 Hz, $(CH_2)_2$−$(CH_2)_2$−$\underline{CH_2}$−CH_3), 1.18−1.24 (m, 4H, $(CH_2)_2$−$\underline{(CH_2)_2}$−CH_2−CH_3), 1.28−1.35 (m, 2H,CH_2−$\underline{CH_2}$− $(CH_2)_3$−CH_3), 1.41−1.49 (m, 2H, $\underline{CH_2}$-NCSe).

Cyclohexylisoselenocyanate (39). From *N*-ciclohexylformamide **30** gave **39** as a dark syrup: Yield 92%; IR υ_{max} (KBr): 2933−2883 (C−H), 2137 (N=C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 1.07 (s, H, H_1), 1.28-1.37 (m, 2H, H_4), 1.56−1.62 (m, 4H, H_3+H_5), 1.85−1.90 (m, 4H, H_2+H_6).

General procedure for the synthesis of selenoureas 40-48. To a solution of the corresponding isoselenocyanate (2.33 mmol) in dry dioxane (40 mL), at room temperature under nitrogen atmosphere, was added a diselenide solution (1.17 mmol) in dry dioxane (10 mL). The reaction was kept in the

darkness for 145 h. To afford the desired selenourea we proceed accordingly two different work-up: i) Work-up method A: After stirring the precipitate was filtered off, washed with dichloromethane (100 mL) and dried in order to obtain the selenoureas **41** and **44**; ii) Work-up method B: After stirring the solvent was evaporated to yield the solid product, which was washed with dichloromethane (100 mL) and dried in order to obtain the selenoureas **40**, **42**-**43** and **45**-**48**.

Optimal purification method for compounds **40**-**44** was the formation of the corresponding salts by reaction with hydrochloric acid in ethyl ether.

***N´,N´´´*-(Diselanediyldibenzene-4,1-diyl)bis(1-phenylselenourea) (40).** From phenyl isoselenocyanate **31**. The salt formation with hydrochloric ether gave **40** as a yellow powder. Yield: 2.6%; mp 189−191 ºC; IR υ_{max} (KBr): 3427 (N−H), 1582 (C=Se) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 6.43 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 7.2 Hz, B+B', H_2+H_6), 6.63 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.8 Hz, A+A', H_3+H_5), 6.81 (t, 2H, $J_{4\text{-}3}$ = $J_{4\text{-}5}$ = 7.3 Hz, B+B', H_4), 7.20 (t, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.7 Hz, B+B', H_3+H_5), 7.54 (d, 4H, A+A', H_2+H_6), 9.36 (s, 2H, 2N<u>H</u>−C_6H_4), 9.56 (s, 2H, 2N<u>H</u>−C_6H_4Se); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 119.2 (A+A', C_1), 116.4 (A+A', C_3+C_5), 117.9 (B+B', C_2+C_6), 122.0 (B+B', C_4), 129.3 (B+B', C_3+C_5), 132.8 (A+A', C_2+C_6), 144.3 (A+A', C_4), 146.1 (B+B', C_1), 179.4 (C=Se); Anal. Calcd for $C_{26}H_{22}N_4Se_4$.3HCl (%): C, 37.4 H, 3.2, N, 6.7. Found: C, 37.3, H, 3.0, N, 6.7.

***N´,N´´´*-(Diselanediyldibenzene-4,1-diyl)bis[1-(4-methylphenyl)selenourea] (41).** From 4-methylphenyl isoselenocyanate **32**. The salt formation with hydrochloric ether gave **41** as a yellow powder. Yield: 7.5%; mp 212−213 ºC; IR υ_{max} (KBr): 3161 (N−H), 1577 (C=Se) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.73 (s, 6H, 2CH_3), 7.30 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.8 Hz,

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B+B', H_2+H_6), 7.47 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.6 Hz, A+A', H_3+H_5), 7.54 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.8 Hz, B+B', H_3+H_5), 7.66 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}2}$ = 8.6 Hz, A+A', H_2+H_6), 8.20 (s, 2H, 2N<u>H</u>−$C_6H_4CH_3$), 9.66 (s, 2H, 2N<u>H</u>−C_6H_4Se); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 20.1 (CH_3), 104.3 (B+B', C_2+C_6), 118.8 (A+A', C_3+C_5), 123.3 (A+A', C_1), 129.0 (B+B', C_3+C_5), 134.2 (B+B', C_4), 140.7 (A+A', C_2+C_6), 162.5 (B+B', C_1), 173.0 (A+A', C_4), 181.8 (C=Se); MS (m/z % abundance): 222 (99), 197 (36), 91 (100), 65 (35); Anal. Calcd for $C_{28}H_{26}N_4Se_4$.3HCl (%): C, 39.8, H, 3.4, N, 6.6. Found: C, 39.5, H, 3.2, N, 6.6.

***N´,N´´´*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-chlorophenyl)selenourea] (42).** From 4-chlorophenyl isoselenocyanate **35**. The salt formation with hydrochloric ether gave **42** as a yellow powder. Yield: 4.75%; IR υ_{max} (KBr): 3367 (N−H), 1625 (C=Se) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.06−7.11 (m, 4H, A+A', H_3+H_5), 7.20−7.25 (m, 4H, B+B', H_2+H_6), 7.29 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.6 Hz, A+A', H_2+H_6), 7.45 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 8.8 Hz, B+B', H_3+H_5), 9.33 (s, 2H, 2N<u>H</u>−C_6H_4CN), 9.59 (s, 2H, 2N<u>H</u>−C_6H_4Se); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 116.3 (A+A', C_3+C_5), 119.1 (A+A', C_1), 121.3 (B+B', C_2+C_6), 128.0 (B+B', C_4), 130.2 (B+B', C_3+C_5), 132.5 (A+A', C_2+C_6), 142.4 (B+B', C_1), 144.1 (A+A', C_4), 180.0 (C=Se); Anal. Calcd for $C_{26}H_{20}Cl_2N_4Se_4$.4HCl (%): C, 33.9, H, 2.6, N, 6.1. Found: C, 34.1, H, 2.2, N, 5.7.

***N´,N´´´*-(Diselanediyldibenzene-4,1-diyl)bis[1-(4-cyanophenyl)selenourea] (43).** From 4-cyanophenyl isoselenocyanate **36**. The salt formation with hydrochloric ether gave **43** as a yellow powder. Yield: 7.3%; mp 165−167 ºC; IR υ_{max} (KBr): 3077 (N−H), 2223 (CN), 1607 (C=Se) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 6.94-7.06 (m, 4H, A+A', H_3+H_5), 7.12-7.33 (m, 4H, B+B', H_2+H_6), 7.35-7.53 (m, 4H, A+A', H_2+H_6), 7.56-7.74 (m, 4H, B+B',

H_3+H_5), 10.19 (s, 4H, 4NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 103.8 (B+B', C_4), 116.4 (A+A', C_3+C_5), 118.1 (A+A', C_1), 119.3 (CN), 120.4 (B+B', C_2+C_6), 132.2 (A+A', C_2+C_6), 133.0 (B+B', C_3+C_5), 144.1 (A+A', C_4), 149.2 (B+B', C_1), 180.0 (C=Se); MS (m/z % abundance): 446 (20), 243 (47), 189 (100), 95 (46), 56 (39); Anal. Calcd for $C_{28}H_{20}N_6Se_4$.4HCl (%): C, 44.4, H, 2.6, N, 11.1. Found: C, 44.5, H, 2.6, N, 11.3.

***N´,N´´´*-(Diselanediyldibenzene-4,1-diyl)bis[1-(4-methoxyphenyl)selenourea] (44).** From 4-methoxyphenyl isoselenocyanate **33**. The salt formation with hydrochloric ether gave **44** as a orange powder. Yield: 8.5%; mp 201−202 ºC; IR υ_{max} (KBr): 3310 (N−H), 1553 (C=Se) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.74 (s, 6H, 2OCH_3), 6.90 (d, 4H, $J_{2\text{-}3}$ = $J_{6\text{-}5}$ = 8.4 Hz, B+B', H_2+H_6), 7.28 (d, 4H, $J_{3\text{-}2}$ = $J_{5\text{-}6}$ = 7.7 Hz, A+A', H_3+H_5), 7.43 (d, 4H, B+B', H_3+H_5), 7.58 (d, 4H, A+A', H_2+H_6), 10.19 (bs, 4H, 4NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 55.2 (OCH_3), 113.7 (B+B', C_3+C_5), 115.1 (A+A', C_3+C_5), 115.5 (B+B', C_2+C_6), 116.0 (A+A', C_1), 126.2 (A+A', C_2+C_6), 133.1 (B+B', C_1), 147.8 (A+A', C_4), 157.4 (B+B', C_4), 179.2 (C=Se); MS (m/z % abundance): 254 (29), 213 (100), 197 (64), 108 (49), 63 (31); Anal. Calcd for $C_{28}H_{26}N_4O_2Se_4$.2HCl (%): C, 33.9, H, 2.6, N, 6.1. Found: C, 34.1, H, 2.2, N, 5.7.

1,1'-(4,4'-Diselanediylbis(4,1-phenylene))bis(3-benzylselenourea) (45). From benzyl isoselenocyanate **34**. Yellow powder. Yield: 11.75%; mp 145−146 ºC; IR υ_{max} (KBr): 3120 (N−H), 1570 (C=Se) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.85 (bs, 4H, 2CH_2), 7.27 (bs, 4H, B+B', H_2+H_6), 7.34 (bs, 10H, A+A', H_2+H_6, B+B', H_3+H_4+H_5), 7.61 (bs, 4H, A+A', H_3+H_5), 8.66 (bs, 2H, 2N<u>H</u>−CH_2), 10.07 (bs, 2H, 2N<u>H</u>-C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 50.6 (CH_2), 125.3 (A+A', C_3+C_5), 126.6 (A+A', C_1), 127.5 (B+B', C_4), 127.9 (B+B',

C_2+C_6), 128.7 (B+B', C_3+C_5), 132.7 (A+A', C_2+C_6), 139.0 (B+B', C_1), 139.3 (A+A', C_4), 180.3 (C=Se); MS (m/z % abundance): 197 (10), 107 (27), 91 (100), 65 (19); Anal. Calcd for $C_{28}H_{26}N_4Se_4$ (%): C, 45.7, H, 3.5, N, 7.6. Found: C, 45.6, H, 3.6, N, 7.4.

1,1'-(4,4'-Diselanediylbis(4,1-phenylene))bis(3-butylselenourea) (46). From butyl isoselenocyanate **37**. Yellow powder. Yield: 15.7%; mp 114−116 ºC; IR υ_{max} (KBr): 3257 (N−H), 2957−2865 (C−H), 1620 (C=Se) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 0.90 (t, 6H, $J_{CH3-CH2}$ = 6.8 Hz, $2CH_3$), 1.30-1.34 (m, 6H, B, -CH_2-CH_2−CH_2−CH_3), 1.53−1.56 (m, 6H, B' -CH_2-CH_2−CH_2−CH_3), 7.33 (d, 4H, J_{3-2} = J_{5-6} = 8.1 Hz, A+A', H_3+H_5), 7.60 (d, 4H, A+A', H_2+H_6), 8.27 (bs, 2H, 2NH−CH_2), 9.92 (bs, 2H, 2NH−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 14.2 (CH_3), 20.0 (CH_2), 31.0 (CH_2), 47.0 (CH_2), 124.8 (A+A', C_1), 132.9 (A+A', C_2+C_3+C_5+C_6), 139.6 (A+A', C_4), 179.2 (C=Se); Anal. Calcd for $C_{22}H_{30}N_4Se_4.H_2O$ (%): C, 38.6, H, 4.7, N, 8.2. Found: C, 38.3, H, 4.3, N, 8.0.

1,1'-(4,4'-Diselanediylbis(4,1-phenylene))bis(3-hexylselenourea) (47). From hexyl isoselenocyanate **38**. Yellow powder. Yield: 12.2%; mp 115−117 ºC; IR υ_{max} (KBr): 3195 (N−H), 2923−2854 (C−H), 1542 (C=Se) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ): 0.88 (bs, 6H, $2CH_3$), 1.28 (bs, 12H, $2(-CH_2)_2-(CH_2)_3-CH_3$), 1.55 (bs, 4H, $2(-CH_2-CH_2-(CH_2)_3-CH_3$), 3.53 (bs, 4H, $2(-CH_2-(CH_2)_4-CH_3$), 7.33 (bs, 4H, A+A', H_3+H_5), 7.59 (bs, 4H, A+A', H_2+H_6), 8.23 (bs, 2H, 2NH−CH_2), 9.88 (s, 2H, 2NH−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 14.4 (CH_3), 22.5 (CH_2), 26.5 (CH_2), 28.8 (CH_2), 31.4 (CH_2), 47.3 (CH_2), 124.8 (A+A', C_3+C_5), 126.2 (A+A', C_1), 133.0 (A+A', C_2+C_6), 139.6 (A+A', C_4), 179.2 (C=Se); MS (m/z % abundance): 368 (5), 191 (23), 69 (8), 57 (21), 43 (100); Anal.

Calcd for $C_{26}H_{38}N_4Se_4.H_2O$ (%): C, 42.2, H, 5.4, N, 7.6. Found: C, 42.1, H, 5.1, N, 7.5.

1,1'-(4,4'-Diselanediylbis(4,1-phenylene))bis(3-ciclohexylselenourea) (48). From cyclohexylisoselenocyanate **39**. Yellow powder. Yield: 21%; mp 175−180 ºC; IR υ_{max} (KBr): 3398 (N−H), 2968−2931 (C−H), 1655 (C=Se) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ): 1.09−1.33 (m, 12H, B+B', $2H_3$+$2H_4$+$2H_5$); 1.59−1.61 (m, 2H, B+B', H_1), 1.62-2.04 (m, 8H, B+B', $2H_2$+$2H_6$), 7.18 (s, 4H, A+A', H_2+H_6), 7.53 (s, 4H, A+A', H_3+H_5), 8.69 (s, 2H, 2NH−CH), 10.47 (s, 2H, 2*NH*−C_6H_4); ^{13}C NMR (100 MHz, DMSO-d_6, δ) 12.2 (C_{cy}), 33.0 (C_{cy}), 53.7 (C_{cy}), 66.3 (C_{cy}), 115.0 (A+A', C_1), 133.5 (A+A', C_2+C_6), 138.2 (A+A', C_3+C_5), 142.1 (A+A', C_4), 182.8 (C=Se); MS (m/z % abundance): 368 (44), 191 (100), 163 (54), 135 (45), 84 (59), 56 (66), 41 (87); Anal. Calcd for $C_{26}H_{34}N_4Se_4.H_2O$ (%): C, 42.4, H, 4.9, N, 7.6. Found: C, 42.3, H, 4.7, N, 7.7.

Biological evaluation. (i) 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 ºC.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 1mM sodium pyruvate, pH 7.2 at 37 ºC and 5% CO_2.

644 **(ii) Leishmanicidal activity and cytotoxicity assays.** Drug treatment of
645 amastigotes was performed during the logarithmic growth phase at a
646 concentration of 2×10^6 parasites/mL at 26 ºC or 1×10^6 parasites/mL at 37 ºC for
647 24 h, respectively. Drug treatment of Jurkat and THP-1 cells was performed
648 during the logarithmic growth phase at a concentration of 4×10^5 cells/mL at 37
649 ºC and 5% CO_2 for 24 h. The percentage of living cells was evaluated by flow
650 cytometry by the propidium iodide (PI) exclusion method (25).

651 **(iii) Leishmania infection assay.** THP-**1** cells were seeded at 120,000
652 cells/mL in 24 multidishes plates (Nunc, Roskilde, Denmark) and differentiated
653 to macrophages for 24 hours in 1mL of RPMI-1640 medium containing 10
654 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich, St. Louis, MO,
655 USA). Medium culture was removed and 1.2×10^6 *Leishmania* amastigotes in
656 1mL of THP-1 medium were added to each well. 4 hours later all medium with
657 non-infecting amastigotes was removed, washed 3 times with 1X phosphate
658 buffered saline (1X PBS) and replaced with new THP-1 medium and
659 corresponding treatment. After 48 hours treatment, medium was removed; THP-
660 1 cells were washed 3 times with 1X PBS and detached with TrypLE™ Express
661 (Invitrogen, Leiden, The Netherlands) according to the manufacturer's
662 indications. Infection was evaluated by flow cytometry.

663 **(iv) Trypanothione reductase assay.** Oxidoreductase activity was
664 determined according to the method described by Toro *et al.* (26). Briefly,
665 reactions were carried out at 26º C in 250 µL of 40 mM pH 8.0 HEPES buffer
666 containing 1 mM EDTA, 150 µM NADPH, 30 µM NADP+, 25 µM DTNB, 1 µM
667 T[S]2, 0.02% glycerol, 1.5% DMSO and 7 nM of recombinant Li-TryR. Enzyme
668 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-lineal regression model with the Grafit6 software (Erithacus, Horley, Surrey, UK).

RESULTS

Chemistry. The synthesis of the compounds described here was carried out according to Figures 2−4. 4,4'-diaminodiphenyldiselenide (Figure 2) was used as starting material to prepare the target compounds. This compound was synthesized in good yield and purity as previously described by our group (12). Compounds **1−22** were synthesized according to Figure 2. Diselenide and commercial available isocyanate or isothiocyanate were mixed in dioxane at a molar ratio 1:2, respectively, at room temperature for 24−120 hours. After removing the solvent, the residue was treated with ethyl ether and washed with water. The compounds were obtained in yields ranging from 25 to 71%.

To obtain the planned selenoureas, the synthesis of the corresponding isoselenocyanates (**31−39**), that were prepared in two steps, was necessary (Figure 3). The first step involved formylation of amines to yield formamides **23−30** followed by the treatment with phosgene (**31−34**) (27) or triphosgene (**35−39**) (28) and selenium powder in the presence of triethylamine under reflux. Compounds were purified by silica gel column chromatography using *n*-hexane/ethyl acetate as eluent. The IR spectra of the isoselenocyanates are quite informative about the presence of the isoselenocyanate functional group (–NCSe). The stretching frequency was observed at 2115−2224 cm^{-1}.

Formamides **23−30** were prepared through different methods depending on the type of primary amine (Figure 3). Ethyl formate was used for compounds

694 **23**, **28** and **29**; formic acid in the presence of zinc dust (29) for derivatives **25**,
695 **26** and **30** or in presence of PEG−400 for **27** (30). Derivative **24** was prepared
696 with ammonium formate in acetonitrile (31). After isolation of the product,
697 formamides were afforded in moderate to good overall yields 16−92 %.

698 Reaction of isoselenocyanates with 4,4'-diaminodiphenyldiselenide in a
699 molar ratio 2:1 respectively, under nitrogen atmosphere, in dried dioxane and in
700 darkness generated selenoureas **40−48**, (Figure 4). However, isolation of
701 selenoureas from the crude reaction mixture was highly tedious and
702 contaminations from different impurities remained with the desired derivatives,
703 thus diminishing final yields. Some of them (**41** and **44**) precipitated and were
704 collected by filtration and the other ones (**40**, **42**, **43**, **45**, **46**, **47** and **48**) were
705 obtained after the solvent was concentrated to dryness. In both cases the
706 residue was washed with different solvents or solvent mixtures (ethyl ether,
707 hexane, ethanol...) generating the target compounds for derivatives **45−48**,
708 exclusively. Optimal purification method for compounds **40−44** was the
709 formation of the corresponding salts by reaction with hydrochloric acid in ethyl
710 ether.

711 The structures and purity of final compounds as well as all intermediates
712 were confirmed by spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR), MS and
713 elemental analyses.

714 IR spectra of urea, thiourea and selenourea compounds revealed
715 characteristic strong intensity bands between 3427 and 3120 cm^{-1} as a broad
716 signal due to the presence of hydrogen bonding for the introduction of four N-H
717 groups. Just above 3000 cm^{-1} Ar−H stretch was evident and carbonyl group for
718 ureas appeared as an intense band about 1644 cm^{-1}. IR spectra of selenourea

compounds revealed selenoyl group band at lower values, ranging from 1542 to 1655 cm^{-1}.

In ^{1}H NMR spectra, the characteristic singlets for N-H protons located between C=X and phenyl moieties are more shielded and appear at downfield shifted as singlet in a relatively wide range of 8.20 to 10.47 ppm. The typical differences for aliphatic amino groups were also noted. Thus, for example, in case of ureas **8−10** the signals of $NHCH_2$ protons are observed between 6.17 and 6.60 as singlets or triplets. The aromatic rings provide their signals between 6.90 and 7.93 ppm.

In ^{13}C NMR, maximum downfield carbon is the carbon attached to selenium, appearing in the range of 179−183 ppm, whereas carbonyl carbon appears at 152−156 ppm. Aromatic carbons provide their signals between 160 and 114 ppm. As a representative example of related structures, the close range of ^{13}C NMR shifts of C=S (179) for derivative **22** and C=Se (183) for selenourea derivative **48** indicates their chemical similarity compared with the C=O (156) of urea derivative **11**. Most of the compounds proved to be unstable towards the harsh conditions of MS and therefore the nominal mass was not observed.

Biological evaluation. (i) *In vitro* antileishmanial activity and cytotoxicity. The synthesized diselenides (**1−22** and **40−48**) were initially tested against *L. infantum* axenic amastigotes according to a previously described procedure [9]. All the analyses were carried out with a minimum of three independent experiments. In these assays miltefosine and edelfosine were used as reference drugs. EC_{50} values are collected in Table 1. In order to assess their selectivity, these compounds were tested against leukemia cells

744 derived from monocytes (THP-1). EC_{50} values obtained are summarized in
745 Table 1. The selectivity index (SI) was defined as the ratio of the EC_{50} values of
746 compounds against THP-1 cells relative to those obtained against *L. infantum*
747 axenic amastigotes.

748 The newly synthesized compounds displayed high activity, thirteen of
749 them (**5**, **7**, **9**, **10**, **11**, **20**, **22**, **40**, **41**, **42**, **44**, **47** and **48**) showing EC_{50} values
750 lower than miltefosine (EC_{50} = 2.84 μM) and one of them (**40**) being more
751 effective than the standard drug edelfosine (EC_{50} = 0.82 μM). In light of the
752 results, the following structural considerations could be made. Regarding
753 derivatization of the amine group and, as a general trend, the ureas **1−11** and
754 selenoureas **40−48,** considered as a whole, have better leishmanicidal activity
755 than the corresponding thiourea analogues (compound **1**, EC_{50} = 3.1 μM and
756 compound **40**, EC_{50} = 0.74 μM versus **12**, EC_{50} = 11.23 μM or compound **10**,
757 EC_{50} = 2.03 μM and **47**, EC_{50} = 1.95 μM versus compound **21**, EC_{50} = 5.69 μM).
758 Regarding the relevance of the presence of additional selenium atoms,
759 comparison of compounds **43** and **46** with analogues **5** and **9**, where the
760 selenium was replaced by oxygen, revealed higher activity in the oxygen
761 containing molecules, particularly in the case of the urea analogue. This fact
762 revealed that the introduction of two additional atoms of selenium is not crucial
763 for the activity.

764 Inspection of the data in Table 1 shows that within thiourea compounds,
765 introduction of electron-withdrawing substituents in *para* position decreases the
766 activity (compound **16**, 4-CN, EC_{50} = 12.09 μM or compound **13**, 4-NO_2, EC_{50} =
767 17.7 μM). The elongation effect of the methylene group as spacer between the
768 aromatic ring and the functional derivatization in compounds **1**, **12** and **40** (n =

769 0) and **7**, **18** and **45** (n =1) was also evaluated. Thus, this spacer causes a drop
770 in the leishmancidal activity in selenoureas, while in ureas and thioreas it is
771 responsible for a significant increase. With regards to the introduction of alkyl
772 side chains, this modification confreres a marked leishmanicidal increase in the
773 three series of compounds (**9**, **10**, **11**, **20**, **21**, **22**, **46**, **47** and **48**), seven of them
774 being more active than miltefosine. This phenomenon can indicate that the
775 activity correlates with an increase in the lipophilicity of the compounds.
776 Lipophilic compounds are more permeable to cellular membranes, which could
777 justify this higher *in vitro* activity. In addition, cyclization of the aliphatic chain
778 improved the activity for thioureas (compound **21** EC_{50} = 5.69 μM versus the
779 corresponding cyclic **22** EC_{50} = 2.71 μM).

780 In terms of selectivity, compounds **8**, **9**, **10** and **11** for ureas, **15**, **17–20**
781 and **22** for thioureas and **41**, **42**, **45** and **47** for selenoureas show SI values in
782 the range of 6.1–22.76, comparable or better than reference drugs. These
783 compounds also displayed the best inhibitory activity in the cultured amastigote
784 model for each series. The most selective was *N',N'''*-(diselanediylbenzene-4,1-
785 diyl)bis[1-(*n*-butyl)urea] (**9**), with SI > 22.7, followed by derivatives **11** (SI >
786 15.2), **47** (SI > 12.82) and **42** (SI > 12.36). Particularly, compound **9** was found
787 to be 3.8 and 3.2 times more selective than edelfosine (SI > 22.7 versus SI = 6)
788 and miltefosine (SI > 22.7 versus SI = 7) respectively. These results confirm a
789 low toxicity for these diselenide compounds.

790 **(ii) Leishmanicidal activity in infected macrophages.** After the first
791 screening and considering their activity and selectivity, four derivatives (**9**, **11**,
792 **42** and **47**) were selected and further tested for their leishmanicidal activity on
793 infected THP-1 macrophages. Again, edelfosine was used as comparative

794 reference. The ED_{50} for each compound was calculated and summarized in
795 Table 2. These compounds reduced the parasite load of the cells, exhibiting
796 ED_{50} values of 21.5, 3.4, 14.4 and > 25 μM respectively. Among them,
797 compound **11**, with ED_{50} = 3.4 μM, presented a similar effectiveness to the
798 reference drug.

799 **(iii) Inhibition of *L. infantum* trypanothione reductase activity.** Going
800 one step further, we investigated whether the most active compounds could act
801 as trypanothione reductase (TryR) inhibitors. Given the essential role of TryR in
802 the antioxidant defences of trypanosomatids, this enzyme has become one of
803 the main exploited targets in *Leishmania spp* (32-34). Different inhibitors have
804 been described in literature, although so far none of them proceeded to the
805 further step of drug development (35). With this purpose, hit compounds were
806 screened at six different concentrations between 0.1 and 75 μM. Mepacrine, a
807 well-known TryR inhibitor, was used as positive control (36) and DMSO as
808 vehicle. The EC_{50} values obtained are gathered in **Table 3**.

809 According to the results, compound **47** potently inhibits TryR presenting
810 an EC_{50} value of 3.77 μM. Noteworthy, this derivative was 4.5-fold more active
811 than the positive control. This inhibitory effect is also accompanied by a good
812 leishmanicidal activity in axenic amastigotes, which suggests that inhibition of
813 TryR could be involved in the mechanism of action of this molecule. Its low
814 activity against intracellular amastigotes suggests that this compound might not
815 enter into the parasitophorous vacuole or, alternatively, the compound could be
816 altered inside it before entering the parasites.

817 Compound **11**, which demonstrated to be the most potent against
818 infected macrophages, shows mild inhibitory activity towards TryR, which

indicates that this enzyme is not its main target. The other two compounds, **9** and **42**, evinced mild leishmanicidal effect on infected macrophages and on TryR activity. In general, the inhibitory effect of these compounds over TryR is not strong enough to support the notion that TryR may be their main target inside the cell. Consequently, additional studies are necessary to elucidate the mechanism of action of the compounds presented herein.

DISCUSSION

The present report describes the synthesis of 31 new *N*-functionalized urea, thiourea and selenourea derivatives from 4,4'-diaminodiphenyldiselenide along with their *in vitro* antileishmanial activity against amastigote forms of *L. infantum*. In order to explore the selectivity, THP-1 cells were used. Fifteen derivatives exhibited EC_{50} values < 3 μM, showing thirteen of them higher activity than the reference drug miltefosine, in some cases by more than 3.8 times. Our results demonstrate that the incorporation of urea and selenourea into the central scaffold improves the leishmanicidal activity, mainly with aliphatic chains.

Four compounds (**9**, **11**, **42** and **47**) showing high activity and selectivity, were tested for their activity in infected macrophages and for their ability to inhibit trypanothione reductase, a potential therapeutic target for the treatment of leishmaniasis. Compound **11** showed antiparasitic activity comparable to edelfosine. On the other hand, compound **47** showed activity against the targeted enzyme while the rest of the derivatives do not follow this apparent trend since they are mild inhibitors of TryR. These results indicate that different mechanisms must be involved on the leishmanicidal activity exerted by these hit

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compounds. A graphical summary of the conclusions drawn from this work is depicted in Figure 5.

Our results provide a basis for further scaffold optimization and structure-based drug design aimed towards the identification and develop of more active, safe and cost-effective antileishmanial agents.

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1021

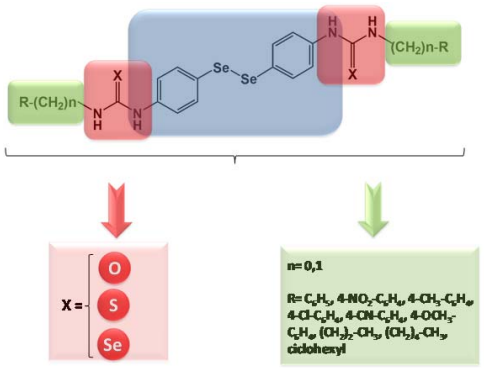

Figure 1. Design and general structure for the proposed compounds.

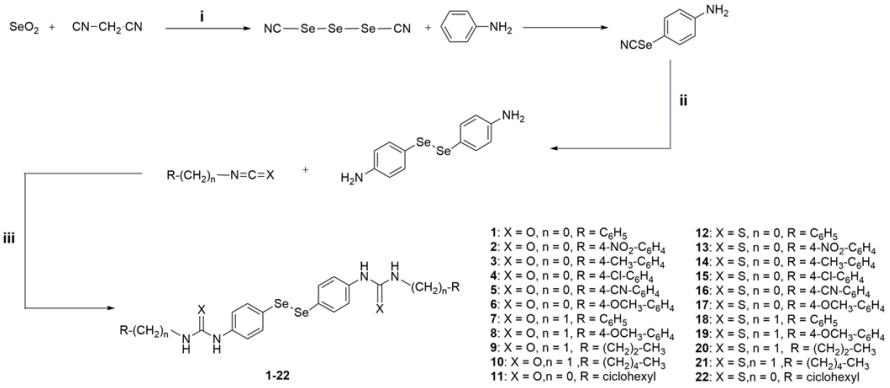

Figure 2. General procedure of synthesis for compounds 1−22. Reagents and conditions: (i) DMSO, 15 min, r.t; (ii) $NaBH_4$, absolute ethanol, 2 h, r.t, N_2; (iii) Dioxane (dry), 24−120 h, r.t, dark, N_2.

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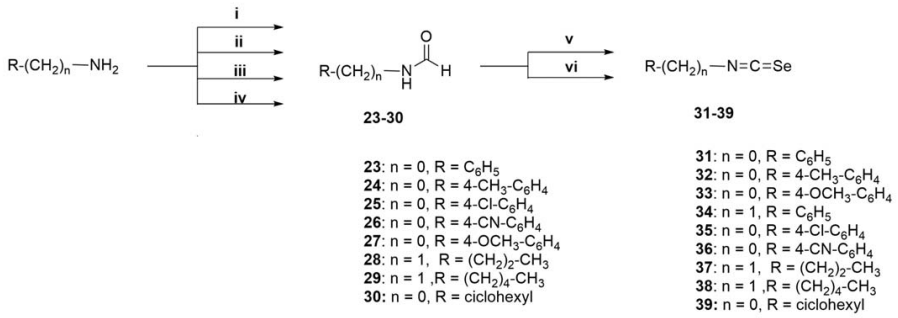

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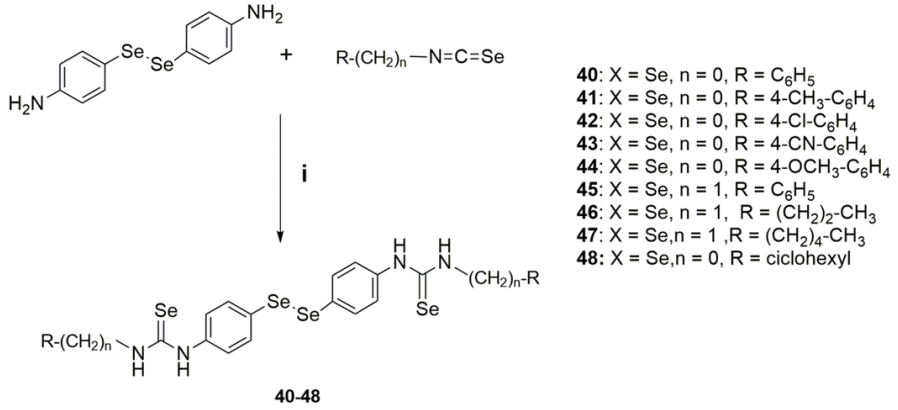
1029 **Figure 3. General procedure of synthesis for compounds 23−39.** Reagents

1030 and conditions: (i) $HCOOC_2H_5$, 12 h, reflux; (ii) HCOOH, Zn (10%), 12 h, 70 ºC

1031 (iii) HCOOH, PEG−400, r.t; (iv) HCO_2NH_4/ CH_3CN, 8−15 h, reflux; (v) Et_3N,

1032 phosgene/toluene, 2.5 h, reflux, Se, 12 h reflux; (vi) Et_3N, triphosgene/DCM, 30

1033 min, 0 ºC, Se, 24h reflux.



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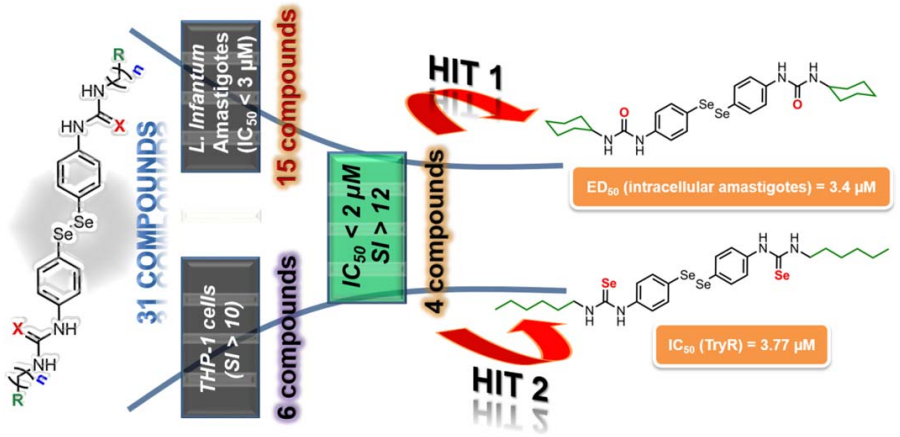
1035 **Figure 4. General procedure of synthesis for compounds 40−48.** Reagents

1036 and conditions: (i) Dioxane (dry), 24−120 h, r.t, dark, N_2.



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1038 **Figure 5.** Schematic illustration of conclusions.

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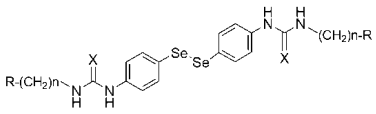
1041 **Table 1**. EC_{50} ± SEM (μM) values for the compounds on amastigotes and
1042 cytotoxic activity in THP-1 cell lines.

1043 

Comp.	X	n	R	Amastigote	THP-1	SI[a]
1	O	0	C_6H_5	3.1 ± 0.25	11.1 ± 1.99	3.58
2	O	0	4−NO_2−C_6H_4	4.1 ± 0.23	4.9 ± 0.18	1.2
3	O	0	4−CH_3−C_6H_4	12.56 ± 0.77	> 25	>1.99
4	O	0	4−Cl−C_6H_4	> 25	> 25	-
5	O	0	4−CN−C_6H_4	2.74 ± 0.11	3.45 ± 0.2	1.26
6	O	0	4−OCH_3−C_6H_4	5.75 ± 2.47	3.12 ± 0.2	0.54
7	O	1	C_6H_5	1.54 ± 0.04	1.16 ± 0.43	0.75
8	O	1	4−OCH_3−C_6H_4	4.1 ± 0.29	> 25	> 6.10
9	O	1	propyl	1.1 ± 0.2	> 25	>22.7
10	O	1	pentyl	2.03 ± 0.2	> 25	> 12.3
11	O	0	ciclohexyl	1.68 ± 0.02	> 25	>15.2
12	S	0	C_6H_5	11.23 ± 0.5	> 25	> 2.21
13	S	0	4−NO_2−C_6H_4	17.7 ± 0.18	> 25	> 1.41
14	S	0	4−CH_3−C_6H_4	4.55 ± 0.21	> 25	> 5.5
15	S	0	4−Cl−C_6H_4	2.93 ± 0.09	> 25	> 8.52
16	S	0	4−CN−C_6H_4	12.09 ± 0.57	> 25	> 2.07
17	S	0	4−OCH_3−C_6H_4	3.2 ± 0.06	> 25	> 7.81
18	S	1	C_6H_5	2.99 ± 0.06	> 25	> 9.05
19	S	1	4−OCH_3−C_6H_4	3.2 ± 0.15	> 25	> 7.81

20	S	1	propyl	2.36 ± 0.44	> 25	> 10.59
21	S	1	pentyl	5.69 ± 0.02	>25	> 4.39
22	S	0	ciclohexyl	2.71 ± 0.23	>25	> 9.22
40	Se	0	C_6H_5	0.74 ± 0.05	2.97 ± 0.04	4.01
41	Se	0	4−CH_3−C_6H_4	1.12 ± 0.04	8.98 ± 0.28	8.02
42	Se	0	4−Cl−C_6H_4	1.59 ± 0.28	19.84 ± 0.56	12.36
43	Se	0	4−CN−C_6H_4	11.7 ± 1.01	19.46 ± 0.89	1.66
44	Se	0	4−OCH_3−C_6H_4	2.41 ± 0.15	13.23 ± 0.21	5.49
45	Se	1	C_6H_5	3.28 ± 0.10	> 25	> 7.62
46	Se	1	propyl	4.39 ± 0.36	10.88 ± 0.73	2.47
47	Se	1	pentyl	1.95 ± 0.78	> 25	>12.82
48	Se	0	cyclohexyl	1.36 ± 0.27	3.74 ± 0.31	2.75
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 EC_{50} values of compounds against THP-1 cells relative to those against *L. infantum* amastigotes.

1047 **Table 2.** ED_{50} ± SEM (μM) values for the compounds in intracellular
1048 amastigotes.

Compound	ED_{50}
9	21.5 ± 4.3
11	3.4 ± 0.1
42	14.4 ± 2.6
47	> 25
Edelfosine	3.1 ± 0.1

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1050 **Table 3.** EC_{50} ± SEM (μM) values for the selected compounds against TryR
1051 inhibition.

Compound	EC_{50}
9	37.46 ± 5.16
11	33.85 ± 4.49
42	24.35 ± 1.49
47	3.77 ± 0.58
Mepacrine	16.99 ± 1.18

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