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

Marta Díaz,a,b Héctor de Lucio,c Esther Moreno,a,b,d Socorro
Espuelas,a,b,d Carlos Aydillo,a,b,d Antonio Jiménez-Ruiz,c Miguel
Ángel Toro,c Killian Jesús Gutiérrez,c Víctor Martínez-Merino,e
Alfonso Cornejo,e Juan Antonio Palop,a,b,d Carmen Sanmartín,a,b,d,*
Daniel Planoa,b,d

Departamento de Tecnología y Química Farmacéuticasa and Instituto de Salud
Tropical,b University of Navarra, Pamplona, Spain; Departamento de Biología
de Sistemas, Universidad de Alcalá, Madrid, Spain; c Instituto de
Investigaciones Sanitarias de Navarra (IdiSNA), Pamplona, Spain; d
Departamento de Química Aplicada, Universidad Pública de Navarra,
Pamplona, Spain e

* Prof. Carmen Sanmartín
Department of Organic and Pharmaceutical Chemistry
University of Navarra
Irunlarrea, 1, E-31008 Pamplona
SPAIN
+34 948 425 600 (Telephone)
+34 948 425 649 (Fax)
e-mail: sanmartin@unav.es
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/UV254 (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG. Postfach 101352, D-52313 Düren, Germany) was used for Thin Layer Chromatography. Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Madrid, Spain), Acros Organics (Janssen Pharmaceuticaal 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 °C; IR νmax (KBr): 3294 (N−H), 1637 (C=O) cm−1; 1H 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$_{5-2}$ = 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$_{5-2}$ = 8.5 Hz, A+A’, H$_3$+H$_5$), 8.74 (bs, 2H, 2NH), 8.85 (bs, 2H, 2NH); 13C 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), 6
N"N""-(diselenadiylidibenzene-4,1-diyld)bis[1-(4-nitrophenyl)urea] (2).

From 4-nitrophenyl isocyanate after 72 h gave 2 as a yellow powder. Yield: 66%; mp 245–247 °C; IR \( \nu_{\text{max}} \) (KBr): 3363 (N−H), 1614 (C=O) cm\(^{-1}\); \(^{1}\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 7.47 (d, 4H, \(J_{2-3} = J_{b-5} = 8.5\) Hz, A+A', H2+H6), 7.56 (d, 4H, \(J_{3-2} = J_{b-5} = 9.1\) Hz, B+B', H2+H6), 8.19 (d, 4H, \(J_{2-3} = J_{b-5} = 9.1\) Hz, B+B', H3+H5), 9.10 (bs, 2H, 2NH), 9.50 (bs, 2H, 2NH); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\)): 118.3 (B+B', C2+C6), 120.5 (A+A', C3+C5), 122.8 (B+B', C3+C5), 126.1 (A+A', C1), 133.3 (A+A', C2+C6), 139.7 (A+A', C4), 142.0 (B+B', C4), 147.1 (B+B', C1), 152.2 (C=O); MS (m/z % abundance): 588 (29), 368 (15), 99 (46), 83 (50), 57 (100); Anal. Calcd for C26H20N6O6Se2 (%): C: 46.6, H: 3.0, N: 12.5. Found: C: 46.5, H: 3.1, N: 12.6.

N"N""-(diselenadiylidibenzene-4,1-diyld)bis[1-(4-methylphenyl)urea] (3). From 4-methylphenyl isocyanate after 120 h gave 3 as a yellow powder. Yield: 28%; mp 283–284 °C; IR \( \nu_{\text{max}} \) (KBr): 3315 (N−H), 1643 (C=O) cm\(^{-1}\); \(^{1}\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 2.24 (s, 6H, 2CH3), 7.09 (d, 4H, \(J_{3-2} = J_{5-6} = 8.1\) Hz, B+B', H3+H5), 7.33 (d, 4H, \(J_{2-3} = J_{b-5} = 8.1\) Hz, B+B', H2+H6), 7.43 (d, 4H, J2), 7.51 (d, 4H, \(J_{3-2} = J_{5-6} = 8.5\) Hz, A+A', H3+H5), 8.61 (s, 2H, 2NH), 8.79 (s, 2H, 2NH); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\)): 32.0 (CH3), 118.1 (B+B', C2+C6), 118.8 (A+A', C3+C5), 122.4 (A+A', C1), 127.2 (B+B', C3+C5), 130.7 (A+A', C2+C6), 132.6 (B+B', C4), 137.1 (B+B', C1), 140.8 (A+A', C4), 154.2 (C=O); MS (m/z % abundance): 240 (24), 107 (80), 83 (58), 57 (100); Anal. Calcd for C28H26N4O2Se2 \(\cdot\)1/2 H2O (%): C: 54.4, H: 4.2, N: 9.0. Found: C: 54.6, H: 4.3, N: 8.8.
\(N^\prime,N^\prime\prime\prime-(\text{diselanediyldibenzene-4,1-diyl})\text{bis}[1-(4-\text{chlorophenyl})\text{urea}]\) (4). From 4-chlorophenyl isocyanate after 48 h gave 4 as a yellow powder.

Yield: 59%; mp > 300 °C; IR \(\nu_{\text{max}}\) (KBr): 3289 (N–H), 1635 (C=O) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 7.33 (d, 4H, \(J_{2,3} = J_{6,5} = 8.3\) Hz, A+A’, H2+H6), 7.45-7.54 (m, 12H, B+B’, H2+H3+H5+H6, A+A’, H3+H5), 8.86 (bs, 4H, 4NH); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\)): 119.5 (A+A’, C3+C5), 120.3 (B+B’, C2+C6), 122.7 (A+A’, C1), 126.0 (B+B’, C4), 129.1 (B+B’, C3+C5), 134.1 (A+A’, C2+C6), 139.0 (B+B’, C1), 140.5 (A+A’, C4), 152.7 (C=O); MS (m/z % abundance): 338 (29), 143 (85), 87 (54), 57 (100); Anal. Calcd for C\(_{26}\)H\(_{20}\)Cl\(_2\)N\(_4\)O\(_2\)Se\(_2\) (%): C: 48.1, H: 3.1, N: 8.6. Found: C: 48.0, H: 3.1, N: 8.4.

\(N^\prime,N^\prime\prime\prime-(\text{diselanediyldibenzene-4,1-diyl})\text{bis}[1-(4-\text{cyanophenyl})\text{urea}]\) (5). From 4-cyanophenyl isocyanate after 96 h gave 5 as a yellow powder.

Yield: 70%; mp 185–186 °C; IR \(\nu_{\text{max}}\) (KBr): 3367 (N–H), 2221 (CN), 1689 (C=O) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 7.46 (d, 4H, \(J_{2,3} = J_{6,5} = 7.5\) Hz, A+A’, H2+H6), 7.55 (d, 4H, \(J_{3,2} = J_{5,6} = 7.5\) Hz, A+A’, H3+H5), 7.73 (d, 4H, \(J_{2,3} = J_{6,5} = 8.0\) Hz, B+B’, H2+H6), 9.02 (s, 2H, 2NH), 9.24 (s, 2H, 2NH); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\)): 103.9 (B+B’, C4), 118.6 (CN), 119.7 (B+B’, C2+C6), 123.2 (A+A’, C1), 133.8 (A+A’, C2+C6), 134.0 (B+B’, C3+C5), 140.1 (A+A’, C4), 144.5 (B+B’, C1), 152.4 (C=O); MS (m/z % abundance): 156 (27), 92 (21), 83 (28), 71 (45), 57 (100); Anal. Calcd for C\(_{28}\)H\(_{20}\)Cl\(_2\)N\(_6\)O\(_2\)Se\(_2\) (%): C: 53.3, H: 3.2, N: 13.3. Found: C: 53.1, H: 3.5, N: 13.2.

\(N^\prime,N^\prime\prime\prime-(\text{diselanediyldibenzene-4,1-diyl})\text{bis}[1-(4-\text{methoxyphenyl})\text{urea}]\) (6). From 4-methoxyphenyl isocyanate after 72 h gave 6 as a yellow powder.

Yield: 65%; mp 274–275 °C; IR \(\nu_{\text{max}}\) (KBr): 3288 (N–H), 1644 (C=O) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 3.74 (s, 6H, 2OCH\(_3\)), 6.56 (d, 2H, \(J_{5,2} = 7.5\) Hz,
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 °C; IR ν max (KBr): 3335 (N−H), 1647 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 4.31 (d, 4H, JCH₂-NH = 5.6 Hz, 2CH₂), 6.68 (t, 2H, JNH-CH₂ = 5.6 Hz, NH-CH₂), 7.24–7.34 (m, 10H, B+B', H₂+H₃+H₄+H₅+H₆), 7.39 (d, 4H, J₃-2 = J₅-₆ = 8.6 Hz, A+A', H₃+H₅), 7.45 (d, 4H, J₂-₃ = J₆-₅ = 8.4 Hz, A+A', H₂+H₆), 8.74 (s, 2H, 2NH-C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ): 43 (CH₂), 118.8 (A+A', C₃+C₅), 121.7 (A+A', C₁), 127.2 (B+B', C₄), 127.6 (B+B', C₂+C₆), 128.8 (B+B', C₃+C₅), 134.3 (A+A', C₂+C₆), 140.7 (B+B', C₁), 141.5 (A+A', C₄), 155.5 (C=O); Anal. Calcd for C₂₈H₂₆N₄O₄Se₂ .1/2 H₂O (%): 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 °C; IR ν max (KBr): 3305 (N−H), 1630 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 3.73 (s, 6H, 2OCH₃), 4.23 (d, 4H, JCH₂-NH = 5.5 Hz, 2CH₂), 6.60 (t, 2H, JNH-CH₂ = 5.5 Hz, 2NH-CH₂), 6.90 (d, 4H, J₃-2 = J₅-₆ = 8.5 Hz, B+B', H₃+H₆), 7.23 (d, 4H, J₃-2 = J₅-₆ = 8.5 Hz, B+B', H₃+H₆), 7.39 (d, 4H, J₂-₃ = J₆-₅ = 8.5 Hz, A+A', H₂+H₆), 7.45 (d, 4H, J₃-2 = J₅-₆ = 8.5 Hz, A+A', H₃+H₆),
8.69 (s, 2H, 2NH−C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ): 42.9 (CH₂), 56.0 (CH₃), 114.4 (B+B’, C₃+C₅), 119.0 (A+A’, C₂+C₅), 122.1 (A+A’, C₁), 129.2 (B+B’, C₂+C₆), 133.6 (B+B’, C₁), 134.2 (A+A’, C₂+C₆), 142.3 (A+A’, C₄), 158.5 (C=O), 159.4 (B+B’, C₄); MS (m/z % abundance): 368 (7), 215 (26), 83 (44), 71 (53), 57 (100); Anal. Calcd for C₃₀H₃₀N₄O₄Se₂·1/₂ H₂O (%): 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 ʋ max (KBr): 3309 (N−H), 2958−2864 (C−H), 1630 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 0.89 (t, 6H, J(CH₃−CH₂ = 7.2 Hz, 2CH₃), 1.25−1.45 (m, 8H, 2(-CH₂−CH₂−CH₃)), 2.96−3.17 (m, 4H, 2(-NH−CH₂), 6.18 (t, 2H, J NH₂−CH₂ = 5.3 Hz, 2NH−CH₂), 7.30−7.48 (m, 8H, A+A’, H₂+H₃+H₅+H₆), 8.57 (s, 2H, 2NH−C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ): 14.2 (C₄), 19.1 (C₃), 32.5 (C₂), 39.0 (C₁), 119.1 (A+A’, C₃+C₅), 122.8 (A+A’, C₁), 134.7 (A+A’, C₂+C₆), 142.5 (A+A’, C₄), 156.1 (C=O); MS (m/z % abundance): 588 (12), 211 (100), 183 (26), 91 (34), 43 (26); Anal. Calcd for C₂₂H₃₀N₄O₂Se₂.H₂O (%): 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 ʋ max (KBr): 3313 (N−H), 2956−2856 (C−H), 1627 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 0.87 (bs, 6H, 2CH₃), 1.27 (bs, 12H, 2(-CH₂−(CH₂)₂−CH₃)), 1.41 (bs, 4H, 2(-CH₂−CH₂−(CH₂)₃−CH₃)), 3.06 (bs, 4H, 2(-CH₂−(CH₂)₄−CH₃)), 6.17 (bs, 2H, 2NH−CH₂), 7.35 (d, 4H, J₂-3 = J₆-₅ = 8.0 Hz, A+A’, H₂+H₆), 7.43 (d, 4H, J₃-₄ = J₅-₆ = 8.0 Hz, A+A’, H₃+H₅), 8.57 (bs, 2H, 2NH−C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ): 14.4 (C₆), 22.5 (C₅), 26.5 (C₃), 39.2 (C₁), 119.1 (A+A’, C₃+C₅), 122.8 (A+A’, C₁), 134.7 (A+A’, C₂+C₆), 142.5 (A+A’, C₄), 156.1 (C=O); MS (m/z % abundance): 588 (12), 211 (100), 183 (26), 91 (34), 43 (26); Anal. Calcd for C₂₂H₃₀N₄O₂Se₂.H₂O (%): C: 47.3, H: 5.7, N: 10.0. Found: C: 47.4, H: 5.5, N: 9.9.
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), 74 (67), Anal. Calcd for C$_{26}$H$_{38}$N$_4$O$_2$Se$_2$·1/2 H$_2$O (%): C: 51.6, H: 6.2, N: 9.2. Found: C: 51.6, H: 6.0, N: 9.1.


From cyclohexyl isocyanate after 120 h gave 11 as a yellow powder. Yield: 59%; mp 255–257 °C; IR $\nu$$_{max}$ (KBr): 3306 (N–H), 2927–2850 (C–H), 1645 (C=O) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$): 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, $J$_2-3 = $J$_2-1 = 13.0$ Hz, B+B', 2H$_2$), 1.79 (d, 4H, $J$_6-1 = $J$_6-5 = 14.1$ Hz, B+B', 2H$_6$), 6.13 (d, 2H, $J_{NH-CH} = 7.8$ Hz, 2NH–CH), 7.34 (d, 4H, $J$_2-3 = $J$_6-5 = 8.6$ Hz, A+A', H$_2$+H$_6$), 7.43 (d, 4H, A+A', H$_3$+H$_5$), 8.46 (s, 2H, 2NH–C$_6$H$_4$); $^{13}$C NMR (100 MHz, DMSO-d$_6$, $\delta$)

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$_{38}$N$_4$O$_2$Se$_2$ (%): 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 °C; IR \( \nu_{\text{max}} \) (KBr): 3193 (N−H), 1588 (C=S) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 7.12-7.15 (m, 2H, B+B', H4), 7.33 (t, 4H, J3-2 = J5-6 = 7.5 Hz, B+B', H3+H5), 7.46-7.49 (m, 8H, A+A', B+B', H2+H6), 7.59 (d, 4H, J5-6 = 7.5 Hz, A+A', H3+H5), 9.88 (bs, 4H, 4NH); \(^13\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\)): 124.1 (A+A', C1), 124.5 (A+A', C3+C5), 125.0 (B+B', C2+C6), 125.4 (B+B', C4), 128.9 (B+B', C1+C4), 132.5 (A+A', C2+C6), 139.8 (A+A', C4), 140.2 (B+B', C1), 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\(_4\)S\(_2\)Se\(_2\) (%): 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 °C; IR \( \nu_{\text{max}} \) (KBr): 3345 (N−H), 1570 (C=S) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 7.49 (bs, 4H, A+A', H2+H6), 7.61 (bs, 4H, A+A', H3+H5), 7.81 (bs, 4H, B+B', H2+H6), 8.20 (bs, 4H, B+B', H3+H5), 10.41 (bs, 4H, 4NH); \(^13\)C NMR (100 MHz, DMSO-\(d_6\), \(\delta\)): 122.2 (A+A', C1), 124.9 (B+B', C3+C5), 126.2 (B+B', C2+C6), 132.4 (A+A', C3+C5), 139.5 (A+A', C2+C6), 142.9 (A+A', C4), 146.6 (B+B', C1+C4), 179.7 (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\(_{20}\)N\(_6\)O\(_4\)S\(_2\)Se\(_2\)·H\(_2\)O (%): 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 °C; IR \( \nu_{\text{max}} \) (KBr): 3203 (N−H), 1583 (C=S) cm\(^{-1}\); \(^1\)H RMN (400 MHz, DMSO-\(d_6\), \(\delta\)): 2.28 (s, 6H, 2CH\(_3\)), 7.14 (d, 4H, J3-2 = J5-6 = 8.0 Hz, B+B', H3+H5), 7.31 (d, 4H, B+B', H2+H6), 7.48
(d, 4H, J_{2-3} = J_{6-5} = 8.2 Hz, A+\text{A}', H_{2}+H_{6}), 7.58 (d, 4H, A+\text{A}', H_{3}+H_{5}), 9.81 (bs, 4H, 4\text{NH}); ^{13}\text{C NMR (100 MHz, DMSO-\text{d}_{6}, \delta)}: 21.3 (\text{CH}_{3}), 123.0 (A+\text{A}', C_{1}), 125.2 (A+\text{A}', C_{3}+C_{5}), 127.9 (B+\text{B}', C_{2}+C_{6}), 129.0 (B+\text{B}', C_{3}+C_{5}), 131.5 (A+\text{A}', C_{2}+C_{6}), 133.2 (B+\text{B}', C_{1}), 137.1 (B+\text{B}', C_{4}), 140.3 (A+\text{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_{26}H_{20}Cl_{2}N_{4}S_{2}Se_{2} (%): C: 45.8, H: 2.9, N: 8.2. Found: C: 45.5, H: 3.0, N: 7.9.

\textit{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 °C; IR \nu_{\text{max}} (KBr): 3210 \text{cm}^{-1}; ^{1}\text{H NMR (400 MHz, DMSO-\text{d}_{6}, \delta)}: 7.44 (d, 4H, J_{2-3} = J_{6-5} = 8.8 Hz, A+\text{A}', H_{2}+H_{6}), 7.55 (dd, 8H, J_{3-2} = J_{5-6} = 8.8 Hz, A+\text{A}', B+\text{B}', H_{3}+H_{5}), 7.65 (d, 4H, B+\text{B}', H_{2}+H_{6}), 10.01 (bs, 4H, 4\text{NH}); ^{13}\text{C NMR (100 MHz, DMSO-\text{d}_{6}, \delta)}: 124.6 (A+\text{A}', C_{3}+C_{5}), 125.6 (B+\text{B}', C_{2}+C_{6}), 125.7 (A+\text{A}', C_{1}), 128.8 (B+\text{B}', C_{4}), 128.8 (B+\text{B}', C_{3}+C_{5}), 132.5 (A+\text{A}', C_{2}+C_{6}), 138.8 (B+\text{B}', C_{1}), 139.9 (A+\text{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_{2}N_{4}S_{2}Se_{2} (%): C: 45.8, H: 2.9, N: 7.9. Found: C: 45.5, H: 3.0, N: 7.9.

\textit{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 °C; IR \nu_{\text{max}} (KBr): 3166 \text{cm}^{-1}; ^{1}\text{H NMR (400 MHz, DMSO-\text{d}_{6}, \delta)}: 7.48 (d, 4H, J_{2-3} = J_{6-5} = 8.4 Hz, A+\text{A}', H_{2}+H_{6}), 7.62 (d, 4H, A+\text{A}', H_{3}+H_{5}), 7.75 (d, 4H, J_{3-5} = J_{5-6} = 8.8 Hz, B+\text{B}', H_{3}+H_{5}), 7.78 (d, 4H, B+\text{B}', H_{2}+H_{6}), 10.25 (bs, 2H, 2\text{NH}), 10.28 (bs, 2H, 2\text{NH}); ^{13}\text{C NMR (100 MHz, DMSO-\text{d}_{6}, \delta)}: 104.9 (CN), 114.7
(B+B', C4), 124.2 (A+A', C1), 124.7 (A+A', C3+C5), 125.3 (B+B', C2+C6), 129.8
(A+A', C2+C6), 132.6 (B+B', C3+C5), 139.0 (A+A', C3), 144.2 (B+B', C1), 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 C28H20N6S2Se2.1/2 H2O (%): C: 50.1, H: 3.0,
N: 12.5. Found: C: 49.8, H: 3.3, N: 12.4.

\[N',N''-(diselanediylidibenzene-4,1-diyl)bis[1-(4-methoxyphenyl)thiourea]\] (17). From 4-methoxyphenyl isothiocyanate after 72 h gave 17 as a yellow powder. Yield: 65%; mp 142−144 ºC; IR \( \nu_{\text{max}} \) (KBr): \( ^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \), \( \delta \)): 3.75 (s, 6H, 2OCH3), 6.91 (d, 4H, 2J = J3 = J5 = 8.9 Hz, A+A', H 2+H6), 7.32 (d, 4H, B+B', H 3+H5), 7.48 (d, 4H, A+A', H 3+H5), 7.58 (d, 4H, A+A', H 2+H6), 9.72 (bs, 4H, 4NH); 13C NMR (100 MHz, DMSO-\( d_6 \), \( \delta \)): 56.0 (CH3), 114.2 (B+B', C3+C5), 115.3 (A+A', C3+C5),
126.0 (B+B', C1), 125.4 (A+A', C1), 132.1 (B+B', C2+C6), 133.6 (A+A', C2+C6),
139.7 (A+A', C4), 159.2 (B+B', C4), 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.
Calcld for C28H20N6O2S2Se2 .1/2 H2O (%): C: 48.7, H: 4.1, N: 8.1. Found: C:
49.1, H 3.9, N: 7.8.

\[N',N''-(diselanediylidibenzene-4,1-diyl)bis(1-benzylthiourea)\] (18). From benzyl isothiocyanate after 144 h gave 18 as a yellow powder. Yield:
71%; mp 146−147 ºC; IR \( \nu_{\text{max}} \) (KBr): 3238 (N−H), 1533 (C=S) cm⁻¹; \( ^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \), \( \delta \)): 4.77 (d, 2JCH2=NH = 5.3 Hz, 4H, 2CH2), 7.22−7.28 (m, 2H, B+B', H4), 7.32-7.38 (m, 8H, B+B', H2+H6, H3+H5), 7.45 (d, 4H, 2J2 = J6 = 8.6 Hz, A+A', H 2+H6), 7.57 (d, 4H, A+A', H 3+H5), 8.27 (s, 2H, 2NH−CH2), 9.71 (s, 2H, 2NH−CH2); 13C NMR (100 MHz, DMSO-\( d_6 \), \( \delta \)): 47.6 (CH2), 124.1 (A+A', C1), 125.1 (A+A', C3+C5), 127.4 (B+B', C4), 127.9 (B+B', C2+C6), 128.8 (B+B', C5+C6), 129.8 (B+B', C4), 132.6 (B+B', C3+C5), 139.0 (A+A', C3), 144.2 (B+B', C1), 179.8 (C=S).
C₃+C₅), 132.7 (A+A', C₂+C₆), 139.3 (B+B', C₁), 140.0 (A+A', C₄), 181.1 (C=S); MS (m/z % abundance): 368 (42), 191 (100), 172 (67), 57 (54); Anal. Calcd for C₂₈H₂₆N₄S₂Se₂·½H₂O (%): 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 νmax (KBr): 3220 (N−H), 1583 (C=S) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 3.73 (s, 6H, 2OCH₃), 4.64 (bs, 4H, 2CH₂), 6.90 (d, 4H, J₃−₂ = J₅−₆ = 8.8 Hz, B+B', H₃+H₅), 7.27 (d, 4H, B+B', H₂+H₆), 7.44 (d, 4H, J₂−₃ = J₆−₅ = 7.8 Hz, A+A', H₂+H₆), 7.56 (d, 4H, A+A', H₃+H₅), 8.20 (bs, 2H, 2NH−CH₂), 9.66 (bs, 2H, 2NH−C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ): 53.9 (CH₂), 57.1 (CH₃), 112.8 (B+B', C₃+C₅), 115.0 (A+A', C₁), 123.2 (A+A', C₃+C₅), 124.1 (B+B', C₁), 128.8 (B+B', C₂+C₈), 132.6 (A+A', C₂+C₆), 140.2 (A+A', C₄), 159.1 (B+B', C₄), 180.7 (C=S); MS (m/z % abundance): 428 (55), 214 (100), 172 (44), 136 (96), 121 (82), 106 (36); Anal. Calcd for C₃₀H₃₀N₄O₂S₂Se₂·½H₂O (%): 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 νmax (KBr): 3220 (N−H), 1583 (C=S) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 3.73 (s, 6H, 2OCH₃), 4.64 (bs, 4H, 2CH₂), 6.90 (d, 4H, J₃−₂ = J₅−₆ = 8.8 Hz, B+B', H₃+H₅), 7.27 (d, 4H, B+B', H₂+H₆), 7.44 (d, 4H, J₂−₃ = J₆−₅ = 7.8 Hz, A+A', H₂+H₆), 7.56 (d, 4H, A+A', H₃+H₅), 8.20 (bs, 2H, 2NH−CH₂), 9.66 (bs, 2H, 2NH−C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ): 14.2 (CH₃), 20.1 (CH₂), 30.1 (CH₂), 44.0 (CH₂), 123.7 (A+A', C₁), 124.7 (A+A',...
$N'N''\text{-diselanediyldibenzene-4,1-diyl} \text{bis[1- (n-hexyl)thiourea]}$ (21).

From hexyl isothiocyanate after 120 h gave 21 as a yellow powder. Yield: 65%; mp 132–134 °C; IR $\nu_{\text{max}}$ (KBr): 3223 (N–H), 2925–2854 (C–H), 1540 (C=S) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$): 0.87 (bs, 6H, 2CH$_3$), 1.28 (bs, 12H, 2(-$(CH_2)_2$=CH$\equiv$CH$_2$)), 1.51 (bs, 4H, 2(-CH$_2$=CH$\equiv$CH$_2$)=(-CH$_2$)$_3$=CH$_3$)), 3.44 (bs, 4H, 2=CH$_2$=(-CH$_2$)$_3$=CH$_3$)), 7.42 (d, 4H, $J_{3-2}=J_{5-6}=8.4$ Hz, A+A', H$_2$+H$_6$), 7.55 (d, 4H, A+A', H$_3$+H$_5$), 7.85 (bs, 2H, 2NH−CH$_2$), 9.55 (bs, 2H, 2NH−C$_6$H$_4$); $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$) 14.4 (CH$_3$), 22.5 (CH$_2$), 26.6 (CH$_2$), 28.8 (CH$_2$), 31.5 (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', C$_4$), 180.6 (C=S); MS (m/z % abundance): 428 (14), 386 (6), 214 (32), 172 (45), 135 (73), 115 (92), 72 (47), 57 (56), 43 (100); Anal. Calcd for C$_{30}$H$_{30}$N$_4$O$_2$S$_2$Se$_2$.1/2 H$_2$O (%): C: 50.7, H: 4.2, N: 7.9. Found: C: 50.4, H: 4.2, N: 7.7.

$N'N''\text{-diselanediyldibenzene-4,1-diyl} \text{bis[1-cyclohexylthiourea]}$ (22).

From cyclohexyl isothiocyanate after 96 h gave 22 as a yellow powder. Yield: 62%; mp 143–144 °C; IR $\nu_{\text{max}}$ (KBr): 3321 (N–H), 2926–2851 (C–H), 1587 (C=S) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$): 1.10−1.35 (m, 12H, B+B', 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, B+B', 2H$_6$), 7.47 (d, 4H, $J_{3-2}=J_{5-6}=8.6$ Hz, A+A', H$_3$+H$_5$), 7.54 (d, 4H, A+A', H$_3$+H$_5$), 7.81 (d, 2H, J$_{NH-CH}=8.1$ Hz, 2NH−CH$_2$), 9.49 (s, 2H, 2NH−C$_6$H$_4$); $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$) 25 (B+B', C$_3$+C$_5$), 26 (B+B', C$_4$), 32 (B+B',
C$_2$+C$_6$), 53 (B+B’, C$_1$), 124 (A+A’, C$_1$), 131 (A+A’, C$_2$+C$_5$+C$_6$), 140 (A+A’, C$_4$), 179 (C=S); MS (m/z % abundance): 368 (12), 214 (7), 191 (24), 83 (21), 56 (100), 41 (33); Anal. Calcd for C$_{26}$H$_{34}$N$_4$S$_2$Se$_2$ .1/2 H$_2$O (%): C: 49.4, H: 5.2, N: 8.8. Found: C: 49.2, H: 5.3, N: 8.5.

Preparation of formamides 23-30.

**N-phenylformamide (23).** To a stirred solution of aniline (9.2 mmol) was added dropwise ethyl formate (9.6 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 23 as a white powder. Yield: 77%; IR $\nu_{\max}$ (KBr): 3364 (N−H), 1634 (C=O) cm$^{-1}$.

**N-(4-methylphenyl)formamide (24).** A mixture of 4-methylaniline (5 mmol) and anhydrous ammonium formate (7.5 mmol) in dry acetonitrile (15 mL) was heated at 100°C for 24 h. Acetonitrile was removed under reduced pressure. The residue was diluted with ethyl acetate (25 mL) and washed with water (4 x 15 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$. After filtration and evaporation of the solvent, 24 was acquired as a white powder. Yield: 66%; IR $\nu_{\max}$ (KBr): 3117 (N−H), 1637 (C=O) cm$^{-1}$.

**N-(4-chlorophenyl)formamide (25).** To a mixture of 4-chloroaniline (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$_2$Cl$_2$ (50 mL), and filters through celite. Then the filtrate was washed with saturated NaHCO$_3$ (4 x 30 mL) and brine (2x20 mL), and was dried over anhydrous Na$_2$SO$_4$. After filtration and evaporation of the solvent, 25 was acquired as a white powder. Yield: 69%; IR $\nu_{\max}$ (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 CH₂Cl₂ (50 mL), and filters through celite. Then the filtrate was washed with saturated NaHCO₃ (3 x 30 mL) and brine (3 x 30 mL), and was dried over anhydrous Na₂SO₄. After filtration and evaporation of the solvent, 26 was acquired as a white powder. Yield: 82%; IR ν 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 Na₂SO₄ 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 ν 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 ν 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 temperature and the precipitate was collected by filtration, dried and washed with ethyl ether (100 mL) to give 29 as a white powder. Yield: 82.5%; IR ν max (KBr): 3291 (N−H), 2960−2869 (C−H), 1665 (C=O) cm⁻¹.
temperature and the precipitate was collected by filtration, dried and washed
with ethyl ether (100 mL) to give 29 as a white powder. Yield: 92%; IR $\nu_{\text{max}}$
(KBr): 3361 (N–H), 2978–2868 (C–H), 1630 (C=O) cm$^{-1}$.

**N-cyclohexylformamide (30).** To a mixture of cyclohexylamine (25
mmol), formic acid (75 mmol) and zinc dust pre-treated with HCl (5 mmol) was
added and stirred at 70 ºC for 8 h–12 h. The mixture was diluted with CH$_2$Cl$_2$
(50 mL), and filters through celite. Then the filtrate was washed with saturated
NaHCO$_3$ (4 x 25 mL) and brine (2 x 25 mL), and was dried over anhydrous
Na$_2$SO$_4$. After filtration and evaporation of the solvent, 30 was acquired as a
white powder. Yield: 16%; IR $\nu_{\text{max}}$ (KBr): 3412 (N–H), 2933–2858 (C–H), 1661
(C=O) cm$^{-1}$.

**General procedure for the synthesis of isoselenocyanates 31-34.** To
a mixture of formamide (6.29 mmol) and $N,N$-diethyletanamine (26.8 mmol) in
dry toluene (50 mL) was added dropwise a solution of phosgene (3.35 mmol) in
dry toluene (10 mL), under N$_2$ atmosphere, on ice over a period of 30 min. Then
black selenium powder (12.58 mmol) was added and the resulting mixture was
refluxed for 24 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.

**Phenylisoselenocyanate (31).** From N-phenylformamide 23 gave 31 as
a brown syrup: Yield 2.66%; IR $\nu_{\text{max}}$ (KBr): 2978–2873 (C–H), 2114 (N=C=Se)
cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$): 6.92 (t, 2H, $J_{2,3} = J_{6,5} = 6.3$ Hz, H$_2$+H$_6$),
7.28 (t, 1H, H$_4$), 7.62 (d, 2H, $J_{3,2} = J_{5,6} = 8.4$ Hz, H$_3$+H$_5$).
4-Methylphenylisoselenocyanate (32). From N-(4-methylphenyl)formamide 24 gave 32 as a brown syrup: Yield 7.48%; IR \(\nu_{\text{max}}\) (KBr): 2921−2866 (C−H), 2153 (N=C=Se) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 2.34 (s, 3H, CH\(_3\)), 7.21 (d, 2H, \(J_{2,3} = J_{6,5} = 7.9\) Hz, H\(_2\)+H\(_6\)), 7.26 (d, 2H, H\(_3\)+H\(_5\)).

4-Methoxyphenylisoselenocyanate (33). From N-(4-methoxyphenyl)formamide 27 gave 33 as an orange syrup: Yield 6.79%; IR \(\nu_{\text{max}}\) (KBr): 2944−2740 (C−H), 2121 (N=C=Se) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 3.80 (s, 3H, OCH\(_3\)), 7.02 (d, 2H, \(J_{3,2} = J_{5,6} = 8.0\) 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 \(\nu_{\text{max}}\) (KBr): 2958−2871 (C−H), 2143 (N=C=Se) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \(\delta\)): 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 $\nu_{\text{max}}$ (KBr): 2924–2854 (C-H), 2151 (N=C=Se) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$): 7.02 (d, 2H, $J_{3,2} = J_{5,6} = 7.6$ 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 $\nu_{\text{max}}$ (KBr): 2927–2856 (C-H), 2225 (CN), 2146 (N=C=Se) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$): 7.77 (d, 2H, $J_{3,2} = J_{5,6} = 8.4$ 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 $\nu_{\text{max}}$ (KBr): 2923–2876 (C-H), 2144 (N=C=Se) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$): 0.86–1.11 (m, 3H, CH$_3$), 1.21–1.32 (m, 4H, CH$_2$–CH$_2$–CH$_3$), 1.35 (m, 2H, CH$_2$-NCSe).

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

Cyclohexylisoselenocyanate (39). From N-cyclohexylformamide 30 gave 39 as a dark syrup: Yield 92%; IR $\nu_{\text{max}}$ (KBr): 2933–2883 (C-H), 2137 (N=C=Se) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$): 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.

\[ N',N'''-(\text{Diselanediyldibenzen-4,1-diyl})\text{bis}(1\text{-phenylselenourea}) \] (40).

From phenyl isoselenocyanate 31. The salt formation with hydrochloric ether gave 40 as a yellow powder. Yield: 2.6%; mp 189−191 °C; IR \( \nu_{\text{max}} \) (KBr): 3427 (N−H), 1582 (C=Se) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \( \delta \)): 6.43 (d, 4H, \( J_{2-3} = J_{6-5} = 7.2 \) Hz, \( B+B' \), \( H_2+H_6 \)), 6.63 (d, 4H, \( J_{3-2} = J_{5-6} = 8.8 \) Hz, \( A+A' \), \( H_3+H_5 \)), 6.81 (t, 2H, \( J_{4-3} = J_{4-5} = 7.3 \) Hz, \( B+B' \), \( H_4 \)), 7.20 (t, 4H, \( J_{5-2} = J_{5-6} = 8.7 \) Hz, \( B+B' \), \( H_3+H_5 \)), 7.54 (d, 4H, \( A+A' \), \( H_2+H_6 \)), 9.36 (s, 2H, 2NH−C\(_6\)H\(_4\)), 9.56 (s, 2H, 2NH−C\(_6\)H\(_4\)Se); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\), \( \delta \)): 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\(_4\)Se\(_4\).3HCl (%): C, 37.4 H, 3.2, N, 6.7. Found: C, 37.3, H, 3.0, N, 6.7.

\[ N',N'''-(\text{Diselanediyldibenzen-4,1-diyl})\text{bis}[1\text{-}(4\text{-methylphenyl})\text{selenourea}] \] (41). From 4-methylphenyl isoselenocyanate 32. The salt formation with hydrochloric ether gave 41 as a yellow powder. Yield: 7.5%; mp 212−213 °C; IR \( \nu_{\text{max}} \) (KBr): 3161 (N−H), 1577 (C=Se) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), \( \delta \)): 2.73 (s, 6H, 2CH\(_3\)), 7.30 (d, 4H, \( J_{2-3} = J_{6-5} = 8.8 \) Hz,
B+B', H2+H6), 7.47 (d, 4H, J3-2 = J5-6 = 8.6 Hz, A+A', H3+H5), 7.54 (d, 4H, J3-2 = J5-6 = 8.8 Hz, A+H', H2+H6), 8.20 (s, 2H, 2NH−C6H4CH3), 9.66 (s, 2H, 2NH−C6H4Se); 13C NMR (100 MHz, DMSO-d6, δ): 20.1 (CH3), 104.3 (B+B', C2+C6), 118.8 (A+A', C3+C5), 123.3 (A+A', C1), 129.0 (B+B', C3+C5), 134.2 (B+B', C4), 140.7 (A+A', C2+C6), 162.5 (B+B', C1), 173.0 (A+A', C4), 181.8 (C=Se); MS (m/z % abundance): 222 (99), 197 (36), 91 (100), 65 (35); Anal. Calcd for C28H26N4Se4.3HCl (%): 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 νmax (KBr): 3367 (N−H), 1625 (C=Se) cm⁻¹; 1H NMR (400 MHz, DMSO-d6, δ): 7.06-7.11 (m, 4H, A+A', H3+H5), 7.20-7.25 (m, 4H, B+B', H2+H6), 7.29 (d, 4H, J2-3 = J6-5 = 8.6 Hz, A+H', H2+H6), 7.45 (d, 4H, J3-2 = J5-6 = 8.8 Hz, B+B', H3+H5), 9.33 (s, 2H, 2NH−C6H4CN), 9.59 (s, 2H, 2NH−C6H4Se); 13C NMR (100 MHz, DMSO-d6, δ): 116.3 (A+A', C3+C5), 119.1 (A+A', C1), 121.3 (B+B', C2+C6), 128.0 (B+B', C4), 130.2 (B+B', C3+C5), 132.5 (A+A', C2+C6), 142.4 (B+B', C1), 144.1 (A+A', C4), 180.0 (C=Se); Anal. Calcd for C26H20Cl2N4Se4.4HCl (%): 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 νmax (KBr): 3077 (N−H), 2223 (CN), 1607 (C=Se) cm⁻¹; 1H NMR (400 MHz, DMSO-d6, δ): 6.94-7.06 (m, 4H, A+H', H3+H5), 7.12-7.33 (m, 4H, B+B', H2+H6), 7.35-7.53 (m, 4H, A+H', H2+H6), 7.56-7.74 (m, 4H, B+B', H2+H6), 7.80-7.98 (m, 4H, A+A', H3+H5), 8.15-8.22 (m, 4H, B+B', H3+H5), 8.35-8.42 (m, 4H, A+A', H2+H6), 8.58-8.65 (m, 4H, B+B', H2+H6), 8.78-8.85 (m, 4H, A+A', H3+H5), 8.98-9.05 (m, 4H, B+B', H3+H5).
H3+H5), 10.19 (s, 4H, 4NH); 13C NMR (100 MHz, DMSO-d6, δ): 103.8 (B+B', C4), 116.4 (A+A', C3+C5), 118.1 (A+A', C1), 119.3 (CN), 120.4 (B+B', C2+C6), 132.2 (A+A', C2+C6), 133.0 (B+B', C3+C5), 144.1 (A+A', C4), 149.2 (B+B', C1), 180.0 (C=Se); MS (m/z % abundance): 446 (20), 243 (47), 189 (100), 95 (46), 56 (39); Anal. Calcd for C28H20N6Se4.4HCl (%): 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 an orange powder.

Yield: 8.5%; mp 201–202 ºC; IR ʋ max (KBr): 3310 (N−H), 1553 (C=Se) cm−1; 1H NMR (400 MHz, DMSO-d6, δ): 3.74 (s, 6H, 2OCH3), 6.90 (d, 4H, J2-3 = J6-5 = 8.4 Hz, B+B', H 2+H6), 7.28 (d, 4H, J3-2 = J5-6 = 7.7 Hz, A+A', H3+H5), 7.43 (d, 4H, B+B', H3+H5), 7.58 (d, 4H, A+A', H2+H6), 10.19 (bs, 4H, 4NH); 13C NMR (100 MHz, DMSO-d6, δ): 55.2 (OCH3), 113.7 (B+B', C3+C5), 115.1 (A+A', C3+C5), 115.5 (B+B', C2+C6), 116.0 (A+A', C1), 125.3 (A+A', C1), 126.2 (A+A', C2+C6), 133.1 (B+B', C1), 147.8 (A+A', C4), 157.4 (B+B', C4), 179.2 (C=Se); MS (m/z % abundance): 254 (29), 213 (100), 197 (64), 108 (49), 63 (31); Anal. Calcd for C28H26N4O2Se4.2HCl (%): C, 33.9, H, 2.6, N, 6.1. Found: C, 34.1, H, 2.2, N, 5.7.

**1,1’-(4,4’-Diselanediyldibenzene-4,1-diyl)bis(3-benzylselenourea)** (45). From benzyl isoselenocyanate 34. Yellow powder. Yield: 11.75%; mp 145–146 ºC; IR ʋ max (KBr): 3120 (N−H), 1570 (C=Se) cm−1; 1H NMR (400 MHz, DMSO-d6, δ): 4.85 (bs, 4H, 2CH2), 7.27 (bs, 4H, B+B', H2+H6), 7.34 (bs, 10H, A+A', H2+H6, B+B', H 3+H4+H5), 7.61 (bs, 4H, A+A', H3+H5), 8.66 (bs, 2H, 2NH−CH2), 10.07 (bs, 2H, 2NH-C6H4); 13C NMR (100 MHz, DMSO-d6, δ): 50.6 (CH2), 125.3 (A+A', C3+C5), 126.6 (A+A', C1), 127.5 (B+B', C4), 127.9 (B+B', 2NH−CH2).
1,1’-(4,4’-Diselanediylbis(4,1-phenylene))bis(3-butylselenourea) (46).

From butyl isoselenocyanate 37. Yellow powder. Yield: 15.7%; mp 114–116 °C;
IR νmax (KBr): 3257 (N−H), 2957−2865 (C−H), 1620 (C=Se) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 0.90 (t, 6H, JCH₃-CH₂ = 6.8 Hz, 2CH₃), 1.30-1.34 (m, 6H, B−CH₂−CH₂−CH₂−CH₃), 1.53−1.56 (m, 6H, B’-CH₂−CH₂−CH₂−CH₃), 7.33 (d, 4H, J₃-2 = J₅-6 = 8.1 Hz, A + A’, H₃+H₅), 7.60 (d, 4H, A + A’, H₂+H₆), 8.27 (bs, 2H, 2NH−CH₂), 9.92 (bs, 2H, 2NH−C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ): 14.2 (CH₃), 20.0 (CH₂), 31.0 (CH₂), 47.0 (CH₂), 124.8 (A + A’, C₁), 132.9 (A + A’, C₂+C₃+C₅+C₆), 139.6 (A + A’, C₄), 179.2 (C=Se); Anal. Calcd for C₂₂H₃₀N₄Se₄ (%): 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-hexylselenourea) (47).

From hexyl isoselenocyanate 38. Yellow powder. Yield: 12.2%; mp 115–117 °C;
IR νmax (KBr): 3195 (N−H), 2923−2854 (C−H), 1542 (C=Se) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 0.88 (bs, 6H, 2CH₃), 1.28 (bs, 12H, 2(-CH₂)₂−(CH₂)₃−CH₃), 1.55 (bs, 4H, 2(-CH₂−CH₂−(CH₂)₃−CH₃), 3.53 (bs, 4H, 2(-CH₂−(CH₂)₄−CH₃), 7.33 (bs, 4H, A + A’, H₃+H₅), 7.59 (bs, 4H, A + A’, H₂+H₆), 8.23 (bs, 2H, 2NH−CH₂), 9.88 (s, 2H, 2NH−C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ): 14.4 (CH₃), 22.5 (CH₂), 26.5 (CH₂), 28.8 (CH₂), 31.4 (CH₂), 47.3 (CH₂), 124.8 (A + A’, C₂+C₃+C₅+C₆), 126.2 (A + A’, C₁), 133.0 (A + A’, C₂+C₆), 139.6 (A + A’, C₄), 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_{4}Se_{4}.H_{2}O (%): 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-cyclohexylselenourea)**

(48). From cyclohexyliselenocyanate 39. Yellow powder. Yield: 21%; mp 175–180 °C; IR $\nu_{\text{max}}$ (KBr): 3398 (N−H), 2968–2931 (C−H), 1655 (C=Se) cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$): 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, 2NH−C$_6$H$_4$); $^{13}$C NMR (100 MHz, DMSO-d$_6$, $\delta$) 12.2 (C$_\text{cy}$), 33.0 (C$_\text{cy}$), 53.7 (C$_\text{cy}$), 66.3 (C$_\text{cy}$), 115.0 (A+A’, C$_1$), 133.5 (A+A’, C$_2$+C$_8$), 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$_{4}$Se$_{4}$.H$_{2}$O (%): 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 °C. THP-1 cells were kindly provided by Dr. Michel (Université Nice Sophia Antipolis, Nice, France) and were grown in RPMI-1640 medium (Gibco, Leiden, The Netherlands) supplemented with 10% heat inactivated FCS, antibiotics, 1 mM HEPES, 2 mM glutamine and 1 mM sodium pyruvate, pH 7.2 at 37 °C and 5% CO$_2$. 
(ii) Leishmanicidal activity and cytotoxicity assays. Drug treatment of amastigotes was performed during the logarithmic growth phase at a concentration of \(2 \times 10^6\) parasites/mL at 26 °C or \(1 \times 10^6\) parasites/mL at 37 °C for 24 h, respectively. Drug treatment of Jurkat and THP-1 cells was performed during the logarithmic growth phase at a concentration of \(4 \times 10^5\) cells/mL at 37 °C and 5% CO\(_2\) for 24 h. The percentage of living cells was evaluated by flow cytometry by the propidium iodide (PI) exclusion method (25).

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

(iv) Trypanothione reductase assay. Oxidoreductase activity was determined according to the method described by Toro et al. (26). Briefly, reactions were carried out at 26° C in 250 µL of 40 mM pH 8.0 HEPES buffer containing 1 mM EDTA, 150 µM NADPH, 30 µM NADP+, 25 µM DTNB, 1 µM T[S]2, 0.02% glycerol, 1.5% DMSO and 7 nM of recombinant Li-TryR. 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).

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

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

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

IR spectra of urea, thiourea and selenourea compounds revealed characteristic strong intensity bands between 3427 and 3120 cm⁻¹ as a broad signal due to the presence of hydrogen bonding for the introduction of four N-H groups. Just above 3000 cm⁻¹ Ar−H stretch was evident and carbonyl group for 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 ¹H 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 NHCH₂ 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 ¹³C 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 ¹³C 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. EC₅₀ values are collected in Table 1. In order to assess their selectivity, these compounds were tested against leukemia cells
derived from monocytes (THP-1). EC50 values obtained are summarized in Table 1. The selectivity index (SI) was defined as the ratio of the EC50 values of compounds against THP-1 cells relative to those obtained against *L. infantum* axenic amastigotes.

The newly synthesized compounds displayed high activity, thirteen of them (5, 7, 9, 10, 11, 20, 22, 40, 41, 42, 44, 47 and 48) showing EC50 values lower than miltefosine (EC50 = 2.84 µM) and one of them (40) being more effective than the standard drug edelfosine (EC50 = 0.82 µM). In light of the results, the following structural considerations could be made. Regarding derivatization of the amine group and, as a general trend, the ureas 1-11 and selenoureas 40-48, considered as a whole, have better leishmanicidal activity than the corresponding thiourea analogues (compound 1, EC50 = 3.1 µM and compound 40, EC50 = 0.74 µM versus 12, EC50 = 11.23 µM or compound 10, EC50 = 2.03 µM and 47, EC50 = 1.95 µM versus compound 21, EC50 = 5.69 µM).

Regarding the relevance of the presence of additional selenium atoms, comparison of compounds 43 and 46 with analogues 5 and 9, where the selenium was replaced by oxygen, revealed higher activity in the oxygen containing molecules, particularly in the case of the urea analogue. This fact revealed that the introduction of two additional atoms of selenium is not crucial for the activity.

Inspection of the data in Table 1 shows that within thiourea compounds, introduction of electron-withdrawing substituents in para position decreases the activity (compound 16, 4-CN, EC50 = 12.09 µM or compound 13, 4-NO2, EC50 = 17.7 µM). The elongation effect of the methylene group as spacer between the aromatic ring and the functional derivatization in compounds 1, 12 and 40 (n =
0) and 7, 18 and 45 (n = 1) was also evaluated. Thus, this spacer causes a drop in the leishmanicidal activity in selenoureas, while in ureas and thioureas it is responsible for a significant increase. With regards to the introduction of alkyl side chains, this modification confers a marked leishmanicidal increase in the three series of compounds (9, 10, 11, 20, 21, 22, 46, 47 and 48), seven of them being more active than miltefosine. This phenomenon can indicate that the activity correlates with an increase in the lipophilicity of the compounds. Lipophilic compounds are more permeable to cellular membranes, which could justify this higher in vitro activity. In addition, cyclization of the aliphatic chain improved the activity for thioureas (compound 21 EC₅₀ = 5.69 µM versus the corresponding cyclic 22 EC₅₀ = 2.71 µM).

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

(ii) Leishmanicidal activity in infected macrophages. After the first screening and considering their activity and selectivity, four derivatives (9, 11, 42 and 47) were selected and further tested for their leishmanicidal activity on infected THP-1 macrophages. Again, edelfosine was used as comparative
reference. The ED$_{50}$ for each compound was calculated and summarized in Table 2. These compounds reduced the parasite load of the cells, exhibiting ED$_{50}$ values of 21.5, 3.4, 14.4 and > 25 µM respectively. Among them, compound 11, with ED$_{50} = 3.4$ µM, presented a similar effectiveness to the reference drug.

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

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

Compound 11, which demonstrated to be the most potent against 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
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.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Foundation for Applied Medical Investigation (FIMA), University of Navarra. The authors also acknowledge the Ministerio de Educación y Ciencia, Spain (grant SAF2015-64629-C2) and Comunidad de Madrid (BIPEDD-2-CM ref. S-2010/BMD-2457) for financial support. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

FUNDING INFORMATION

This work, including the efforts of Marta Díaz, was funded by Foundation for Applied Medical Investigation (ISTUN-API-2011/02). This work, including the efforts of Antonio Jiménez-Ruiz, was funded by Ministerio de Educación y Ciencia, Spain (grant SAF2015-64629-C2) and Comunidad de Madrid (BIPEDD-2-CM ref. S-2010/BMD-2457). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
REFERENCES


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$_2$. 

1. $X = O, n = 0, R = CH_3H_2$
2. $X = O, n = 0, R = 4-NO_2C_6H_4$
3. $X = O, n = 0, R = 4-(NO_2C_6H_4)$
4. $X = O, n = 0, R = 4-ClC_6H_4$
5. $X = O, n = 0, R = 4-BrC_6H_4$
6. $X = O, n = 0, R = 4-OHcC_6H_4$
7. $X = O, n = 1, R = CH_3H_2$
8. $X = O, n = 1, R = 4-OHcC_6H_4$
9. $X = O, n = 1, R = CH_3C_6H_4$
10. $X = O, n = 1, R = 4-CH_3C_6H_4$
11. $X = O, n = 0, R = 4-ClC_6H_4$
12. $X = S, n = 0, R = CH_3H_2$
13. $X = S, n = 0, R = 4-NO_2C_6H_4$
14. $X = S, n = 0, R = 4-(NO_2C_6H_4)$
15. $X = S, n = 0, R = 4-ClC_6H_4$
16. $X = S, n = 0, R = 4-BrC_6H_4$
17. $X = S, n = 0, R = 4-OHcC_6H_4$
18. $X = S, n = 1, R = CH_3H_2$
19. $X = S, n = 1, R = 4-OHcC_6H_4$
20. $X = S, n = 1, R = CH_3C_6H_4$
21. $X = S, n = 1, R = 4-CH_3C_6H_4$
22. $X = S, n = 0, R = 4-ClC_6H_4$
Figure 3. General procedure of synthesis for compounds 23−39. Reagents and conditions: (i) HCOOC(CH₃)₂, 12 h, reflux; (ii) HCOOH, Zn (10%), 12 h, 70 ºC (iii) HCOOH, PEG−400, r.t; (iv) HCO₂NH₂/ CH₃CN, 8−15 h, reflux; (v) Et₃N, phosgene/toluene, 2.5 h, reflux, Se, 12 h reflex; (vi) Et₃N, triphosgene/DCM, 30 min, 0 ºC, Se, 24h reflux.

Figure 4. General procedure of synthesis for compounds 40−48. Reagents and conditions: (i) Dioxane (dry), 24−120 h, r.t, dark, N₂.
Figure 5. Schematic illustration of conclusions.
Table 1. EC\textsubscript{50} ± SEM (µM) values for the compounds on amastigotes and cytotoxic activity in THP-1 cell lines.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>X</th>
<th>n</th>
<th>R</th>
<th>Amastigote</th>
<th>THP-1</th>
<th>SI'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O</td>
<td>0</td>
<td>C\textsubscript{6}H\textsubscript{5}</td>
<td>3.1 ± 0.25</td>
<td>11.1 ± 1.99</td>
<td>3.58</td>
</tr>
<tr>
<td>2</td>
<td>O</td>
<td>0</td>
<td>4-NO\textsubscript{2}-C\textsubscript{6}H\textsubscript{4}</td>
<td>4.1 ± 0.23</td>
<td>4.9 ± 0.18</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>O</td>
<td>0</td>
<td>4-CH\textsubscript{3}-C\textsubscript{6}H\textsubscript{4}</td>
<td>12.56 ± 0.77</td>
<td>&gt; 25</td>
<td>&gt;1.99</td>
</tr>
<tr>
<td>4</td>
<td>O</td>
<td>0</td>
<td>4-Cl-C\textsubscript{6}H\textsubscript{4}</td>
<td>&gt; 25</td>
<td>&gt; 25</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>O</td>
<td>0</td>
<td>4-CN-C\textsubscript{6}H\textsubscript{4}</td>
<td>2.74 ± 0.11</td>
<td>3.45 ± 0.2</td>
<td>1.26</td>
</tr>
<tr>
<td>6</td>
<td>O</td>
<td>0</td>
<td>4-OCH\textsubscript{3}-C\textsubscript{6}H\textsubscript{4}</td>
<td>5.75 ± 2.47</td>
<td>3.12 ± 0.2</td>
<td>0.54</td>
</tr>
<tr>
<td>7</td>
<td>O</td>
<td>1</td>
<td>C\textsubscript{6}H\textsubscript{5}</td>
<td>1.54 ± 0.04</td>
<td>1.16 ± 0.43</td>
<td>0.75</td>
</tr>
<tr>
<td>8</td>
<td>O</td>
<td>1</td>
<td>4-OCH\textsubscript{3}-C\textsubscript{6}H\textsubscript{4}</td>
<td>4.1 ± 0.29</td>
<td>&gt; 25</td>
<td>&gt; 6.10</td>
</tr>
<tr>
<td>9</td>
<td>O</td>
<td>1</td>
<td>propyl</td>
<td>1.1 ± 0.2</td>
<td>&gt; 25</td>
<td>&gt; 22.7</td>
</tr>
<tr>
<td>10</td>
<td>O</td>
<td>1</td>
<td>pentyl</td>
<td>2.03 ± 0.2</td>
<td>&gt; 25</td>
<td>&gt; 12.3</td>
</tr>
<tr>
<td>11</td>
<td>O</td>
<td>0</td>
<td>ciclohexyl</td>
<td>1.68 ± 0.02</td>
<td>&gt; 25</td>
<td>&gt; 15.2</td>
</tr>
<tr>
<td>12</td>
<td>S</td>
<td>0</td>
<td>C\textsubscript{6}H\textsubscript{5}</td>
<td>11.23 ± 0.5</td>
<td>&gt; 25</td>
<td>&gt; 2.21</td>
</tr>
<tr>
<td>13</td>
<td>S</td>
<td>0</td>
<td>4-NO\textsubscript{2}-C\textsubscript{6}H\textsubscript{4}</td>
<td>17.7 ± 0.18</td>
<td>&gt; 25</td>
<td>&gt; 1.41</td>
</tr>
<tr>
<td>14</td>
<td>S</td>
<td>0</td>
<td>4-CH\textsubscript{3}-C\textsubscript{6}H\textsubscript{4}</td>
<td>4.55 ± 0.21</td>
<td>&gt; 25</td>
<td>&gt; 5.5</td>
</tr>
<tr>
<td>15</td>
<td>S</td>
<td>0</td>
<td>4-Cl-C\textsubscript{6}H\textsubscript{4}</td>
<td>2.93 ± 0.09</td>
<td>&gt; 25</td>
<td>&gt; 8.52</td>
</tr>
<tr>
<td>16</td>
<td>S</td>
<td>0</td>
<td>4-CN-C\textsubscript{6}H\textsubscript{4}</td>
<td>12.09 ± 0.57</td>
<td>&gt; 25</td>
<td>&gt; 2.07</td>
</tr>
<tr>
<td>17</td>
<td>S</td>
<td>0</td>
<td>4-OCH\textsubscript{3}-C\textsubscript{6}H\textsubscript{4}</td>
<td>3.2 ± 0.06</td>
<td>&gt; 25</td>
<td>&gt; 7.81</td>
</tr>
<tr>
<td>18</td>
<td>S</td>
<td>1</td>
<td>C\textsubscript{6}H\textsubscript{5}</td>
<td>2.99 ± 0.06</td>
<td>&gt; 25</td>
<td>&gt; 9.05</td>
</tr>
<tr>
<td>19</td>
<td>S</td>
<td>1</td>
<td>4-OCH\textsubscript{3}-C\textsubscript{6}H\textsubscript{4}</td>
<td>3.2 ± 0.15</td>
<td>&gt; 25</td>
<td>&gt; 7.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>S 1</td>
<td>propyl</td>
<td>2.36 ± 0.44</td>
<td>&gt; 25</td>
<td>&gt; 10.59</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>S 1</td>
<td>pentyl</td>
<td>5.69 ± 0.02</td>
<td>&gt; 25</td>
<td>&gt; 4.39</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>S 0</td>
<td>ciclohexyl</td>
<td>2.71 ± 0.23</td>
<td>&gt; 25</td>
<td>&gt; 9.22</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Se 0</td>
<td>C₆H₅</td>
<td>0.74 ± 0.05</td>
<td>2.97 ± 0.04</td>
<td>4.01</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>Se 0</td>
<td>4−CH₃−C₆H₄</td>
<td>1.12 ± 0.04</td>
<td>8.98 ± 0.28</td>
<td>8.02</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Se 0</td>
<td>4−Cl−C₆H₄</td>
<td>1.59 ± 0.28</td>
<td>19.84 ± 0.56</td>
<td>12.36</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Se 0</td>
<td>4−CN−C₆H₄</td>
<td>11.7 ± 1.01</td>
<td>19.46 ± 0.89</td>
<td>1.66</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>Se 0</td>
<td>4−OCH₃−C₆H₄</td>
<td>2.41 ± 0.15</td>
<td>13.23 ± 0.21</td>
<td>5.49</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>Se 1</td>
<td>C₆H₅</td>
<td>3.28 ± 0.10</td>
<td>&gt; 25</td>
<td>&gt; 7.62</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>Se 1</td>
<td>propyl</td>
<td>4.39 ± 0.36</td>
<td>10.88 ± 0.73</td>
<td>2.47</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Se 1</td>
<td>pentyl</td>
<td>1.95 ± 0.78</td>
<td>&gt; 25</td>
<td>&gt; 12.82</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Se 0</td>
<td>ciclohexyl</td>
<td>1.36 ± 0.27</td>
<td>3.74 ± 0.31</td>
<td>2.75</td>
<td></td>
</tr>
</tbody>
</table>

Edelfosine

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Miltefosine

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Selectivity index (SI) is the ratio of EC₅₀ values of compounds against THP-1 cells relative to those against *L. infantum* amastigotes.
Table 2. ED\textsubscript{50} ± SEM (μM) values for the compounds in intracellular amastigotes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>21.5 ± 4.3</td>
</tr>
<tr>
<td>11</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>42</td>
<td>14.4 ± 2.6</td>
</tr>
<tr>
<td>47</td>
<td>&gt; 25</td>
</tr>
<tr>
<td>Edelfosine</td>
<td>3.1 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3. EC\textsubscript{50} ± SEM (μM) values for the selected compounds against TryR inhibition.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>37.46 ± 5.16</td>
</tr>
<tr>
<td>11</td>
<td>33.85 ± 4.49</td>
</tr>
<tr>
<td>42</td>
<td>24.35 ± 1.49</td>
</tr>
<tr>
<td>47</td>
<td>3.77 ± 0.58</td>
</tr>
<tr>
<td>Mepacrine</td>
<td>16.99 ± 1.18</td>
</tr>
</tbody>
</table>
$X = \begin{cases} O \\ S \\ Se \end{cases}$

$n = 0, 1$

$R = C_6H_4, 4-NO_2C_6H_4, 4-CH_3C_6H_4, 4-ClC_6H_4, 4-CN-C_6H_4, 4-OCH_3-C_6H_4, (CH_2)_2-CH_3, (CH_2)_4-CH_3, ciclohexyl$