

1 **NOVEL UREA, THIOUREA AND SELENOUREA DERIVATIVES**
2 **OF DISELENIDES: SYNTHESIS AND LEISHMANICIDAL**
3 **ACTIVITY**

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25 **Abstract**

26 A novel series of thirty-one *N*-substituted urea, thiourea and selenourea
27 derivatives containing diphenyldiselenide entity were synthesized, fully
28 characterized by spectroscopic and analytical methods, and screened for their
29 *in vitro* leishmanicidal activities. The cytotoxic activity of these derivatives was
30 tested against *Leishmania infantum* axenic amastigotes, and selectivity was
31 assessed in human THP-1 cells. Thirteen of the synthesized compounds
32 showed a significant antileishmanial activity with EC₅₀ values lower than the
33 reference drug miltefosine (EC₅₀ = 2.84 μM). In addition, the derivatives **9**, **11**,
34 **42** and **47** with EC₅₀ between 1.1 and 1.95 μM also displayed an excellent
35 selectivity (SI ranged from 12.4 to 22.7) and were also tested against infected
36 macrophages. Compound **11**, a derivative with a cyclohexyl chain, exhibited the
37 highest activity against intracellular amastigotes with EC₅₀ values similar to
38 those observed for the standard drug edelfosine. SAR analyses revealed that
39 *N*-aliphatic substitution in urea and selenourea is recommended for the
40 leishmanicidal activity of these analogs. Preliminary studies of the mechanism
41 of action for the hit compounds was carried out by measuring their ability to
42 inhibit trypanothione reductase (TryR). Even though the obtained results
43 suggest that this enzyme is not the target for most of these derivatives, their
44 comparable activity with the standards and lack of toxicity in THP-1 cells
45 highlight the potential of these compounds to be optimized for leishmaniasis
46 treatment.

47

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

49 Leishmaniasis comprises a group of mammalian diseases caused by
50 diphasic protozoans of the genus *Leishmania spp.* It is endemic in 98 countries
51 and approximately 15 million of new cases are diagnosed every year, leading to
52 high rates of morbidity and mortality. *Leishmania spp* presents three different
53 clinical manifestations: cutaneous, mucocutaneous and visceral. Among these
54 forms, cutaneous is the most common whereas visceral is the most severe form
55 (1-2). Treatment options are limited and far from being satisfactory. Most of
56 available front-line agents were developed 50 years ago and include
57 chemotherapeutic drugs such as injectable pentavalent antimonials and sodium
58 stibogluconate and meglumine antimoniate. Second-line treatment relies in
59 highly toxic drugs such as amphotericin B or pentamidine. In this context, the
60 development of more effective and less toxic drugs represents an urgent need
61 (3). In this regard, miltefosine, an alkylphosphocholine drug, and the
62 aminoglycoside antibiotic paromomycin have proven to be effective drugs for
63 the treatment of leishmaniasis. Newly developed liposomal amphotericin B is a
64 preferred treatment in developing countries because it efficiently targets
65 *Leishmania spp* parasites with low toxic side effects. Moreover, promising
66 combination therapies are under intense investigation (4-5).

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

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

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

104 Based in previous studies, herein we present the synthesis and leishmanicidal
105 activity against the amastigote form of *L. infantum* of thirty-one new derivatives
106 related to Figure 1. The cytotoxicity of these newly synthesized molecules was
107 also assessed on one different complementary human cell line (THP-1) in order
108 to select those compounds with high selectivity. Moreover, leishmanicidal
109 activity of the most active compounds was evaluated in infected macrophages.
110 Finally, in order to elucidate the underlying molecular mechanisms, the
111 inhibitory activity against trypanothione reductase (TryR) was determined.

112

113 MATERIALS AND METHODS

114 **Chemistry.** Melting points were determined with a Mettler FP82+FP80
115 apparatus (Greifense, Switzerland) and are not corrected. The ^1H NMR and ^{13}C
116 NMR spectra were recorded on a Bruker 400 UltrashieldTM and Bruker Avance
117 Neo spectrometers (Rheinstetten, Germany) using TMS as the internal
118 standard. The IR spectra were obtained on a Thermo Nicolet FT-IR Nexus
119 spectrophotometer with KBr pellets. Mass spectrometry was carried out on a
120 MS-DIP, system MSD/DS 5973N (G2577A) Agilent. Elemental microanalyses
121 were carried out on vacuum-dried samples using a LECO CHN-900 Elemental
122 Analyzer. Silica gel 60 (0.040–0.063 mm) 1.09385.2500 (Merck KGaA, 64271
123 Darmstadt, Germany) was used for Column Chromatography and Alugram[®] SIL

124 G/UV₂₅₄ (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG. Postfach 101352,
125 D-52313 Düren, Germany) was used for Thin Layer Chromatography.
126 Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau
127 (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac,
128 Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Madrid, Spain),
129 Acros Organics (Janssen Pharmaceuticaaan 3a, 2440 Geel, België) and
130 Lancaster (Bischheim-Strasbourg, France).

131 **4,4'-Diaminodiphenyldiselenide.** The synthesis of this compound has
132 been previously described by Plano *et al.* [11].

133 **General procedure for the synthesis of ureas 1-11.** To a solution of
134 4,4'-diaminodiphenyldiselenide (1.17 mmol) in dioxane (25 mL) the
135 corresponding isocyanate was added (2.34 mmol, 1:2 molar ratio), and the
136 mixture was kept at room temperature from 24 h to 120 h. The solvent was
137 removed under vacuum by rotatory evaporation and the residue was treated
138 with ethyl ether (50 mL) and washed with water (100 mL).

139 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis(1-phenylurea) (1).** From
140 phenyl isocyanate after 24 h gave **1** as a yellow powder. Yield: 58%; mp
141 277–278 °C; IR ν_{\max} (KBr): 3294 (N–H), 1637 (C=O) cm^{-1} ; ¹H NMR (400 MHz,
142 DMSO-*d*₆, δ): 6.98 (t, 2H, $J_{4-3} = J_{4-5} = 7.0$ Hz, B+B', H₄), 7.28 (t, 4H, $J_{3-2} = J_{5-6} =$
143 8.0 Hz, B+B', H₃+H₅), 7.43–7.46 (m, 8H, A+A'+B+B', H₂+H₆), 7.52 (d, 4H, $J_{3-2} =$
144 $J_{5-6} = 8.5$ Hz, A+A', H₃+H₅), 8.74 (bs, 2H, 2NH), 8.85 (bs, 2H, 2NH); ¹³C NMR
145 (100 MHz, DMSO-*d*₆, δ): 118.7 (A+A', C₃+C₅), 119.4 (B+B', C₂+C₆), 122.5
146 (A+A', C₁), 130 (B+B', C₃+C₄+C₅), 134.1 (A+A', C₂+C₆), 140.0 (A+A', C₄), 140.7
147 (B+B', C₁), 152.8 (C=O); MS (m/z % abundance): 368 (59), 191 (100), 135 (24),

148 57 (43); Anal. Calcd for $C_{26}H_{22}N_4O_2Se_2$ (%): C: 53.8, H: 3.8, N: 9.6. Found: C:
149 54.1, H: 4.1, N: 9.1.

150 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-nitrophenyl)urea] (2).**

151 From 4-nitrophenyl isocyanate after 72 h gave **2** as a yellow powder. Yield:
152 66%; mp 245–247 °C; IR ν_{max} (KBr): 3363 (N–H), 1614 (C=O) cm^{-1} ; 1H NMR
153 (400 MHz, DMSO- d_6 , δ): 7.47 (d, 4H, $J_{2-3} = J_{6-5} = 8.5$ Hz, A+A', H₂+H₆), 7.56 (d,
154 4H, $J_{3-2} = J_{5-6} = 8.5$ Hz, A+A', H₃+ H₅), 7.69 (d, 4H, $J_{2-3} = J_{6-5} = 9.1$ Hz, B+B',
155 H₂+H₆), 8.19 (d, 4H, $J_{3-2} = J_{5-6} = 9.1$ Hz, B+B', H₃+ H₅), 9.10 (bs, 2H, 2NH), 9.50
156 (bs, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO- d_6 , δ): 118.3 (B+B', C₂+C₆), 120.5
157 (A+A', C₃+C₅), 122.8 (B+B', C₃+C₅), 126.1 (A+A', C₁), 133.3 (A+A', C₂+C₆),
158 139.7 (A+A', C₄), 142.0 (B+B', C₄), 147.1 (B+B', C₁), 152.2 (C=O); MS (m/z %
159 abundance): 588 (29), 368 (15), 99 (46), 83 (50), 57 (100); Anal. Calcd for
160 $C_{26}H_{20}N_6O_6Se_2$ (%): C: 46.6, H: 3.0, N: 12.5. Found: C: 46.5, H: 3.1, N: 12.6.

161 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-methylphenyl)urea]**

162 **(3).** From 4-methylphenyl isocyanate after 120 h gave **3** as a yellow powder.
163 Yield: 28%; mp 283–284 °C; IR ν_{max} (KBr): 3315 (N–H), 1643 (C=O) cm^{-1} ; 1H
164 NMR (400 MHz, DMSO- d_6 , δ): 2.24 (s, 6H, 2CH₃), 7.09 (d, 4H, $J_{3-2} = J_{5-6} = 8.1$
165 Hz, B+B', H₃+ H₅), 7.33 (d, 4H, $J_{2-3} = J_{6-5} = 8.1$ Hz, B+B', H₂+H₆), 7.43 (d, 4H, J_{2-3}
166 $= J_{6-5} = 8.5$ Hz, A+A', H₂+H₆), 7.51 (d, 4H, $J_{3-2} = J_{5-6} = 8.5$ Hz, A+A', H₃+ H₅),
167 8.61 (s, 2H, 2NH), 8.79 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO- d_6 , δ): 32.0
168 (CH₃), 118.1 (B+B', C₂+C₆), 118.8 (A+A', C₃+C₅), 122.4 (A+A', C₁), 127.2 (B+B',
169 C₃+C₅), 130.7 (A+A', C₂+C₆), 132.6 (B+B', C₁), 137.1 (B+B', C₄), 140.8 (A+A',
170 C₄), 154.2 (C=O); MS (m/z % abundance): 240 (24), 107 (80), 83 (58), 57 (100);
171 Anal. Calcd for $C_{28}H_{26}N_4O_2Se_2 \cdot 1/2 H_2O$ (%): C: 54.4, H: 4.2, N: 9.0. Found: C:
172 54.6, H: 4.3, N: 8.8.

173 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-chlorophenyl)urea]**
174 **(4).** From 4-chlorophenyl isocyanate after 48 h gave **4** as a yellow powder.
175 Yield: 59%; mp>300 °C; IR ν_{\max} (KBr): 3289 (N-H), 1635 (C=O) cm^{-1} , ^1H NMR
176 (400 MHz, DMSO- d_6 , δ): 7.33 (d, 4H, $J_{2-3} = J_{6-5} = 8.3$ Hz, A+A', H₂+H₆), 7.45-
177 7.54 (m, 12H, B+B', H₂+H₃+H₅+H₆, A+A', H₃+H₅), 8.86 (bs, 4H, 4NH); ^{13}C NMR
178 (100 MHz, DMSO- d_6 , δ): 119.5 (A+A', C₃+C₅), 120.3 (B+B', C₂+C₆), 122.7
179 (A+A', C₁), 126.0 (B+B', C₄), 129.1 (B+B', C₃+C₅), 134.1 (A+A', C₂+C₆), 139.0
180 (B+B', C₁), 140.5 (A+A', C₄), 152.7 (C=O); MS (m/z % abundance): 338 (29),
181 143 (85), 87 (54), 57 (100); Anal. Calcd for C₂₆H₂₀Cl₂N₄O₂Se₂ (%): C: 48.1, H:
182 3.1, N: 8.6. Found: C: 48.0, H: 3.1, N: 8.4.

183 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-cyanophenyl)urea]**
184 **(5).** From 4-cyanophenyl isocyanate after 96 h gave **5** as a yellow powder.
185 Yield: 70%; mp 185–186 °C; IR ν_{\max} (KBr): 3367 (N-H), 2221 (CN), 1689 (C=O)
186 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 , δ): 7.46 (d, 4H, $J_{2-3} = J_{6-5} = 7.5$ Hz, A+A',
187 H₂+H₆), 7.55 (d, 4H, $J_{3-2} = J_{5-6} = 7.5$ Hz, A+A', H₃+H₅), 7.64 (d, 4H, $J_{3-2} = J_{5-6} =$
188 8.0 Hz, B+B', H₃+H₅), 7.73 (d, 4H, $J_{2-3} = J_{6-5} = 8.0$ Hz, B+B', H₂+H₆), 9.02 (s, 2H,
189 2NH), 9.24 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO- d_6 , δ): 103.9 (B+B', C₄),
190 118.6 (CN), 119.7 (B+B', C₂+C₆), 123.2 (A+A', C₁), 133.8 (A+A', C₂+C₆), 134.0
191 (B+B', C₃+C₅), 140.1 (A+A', C₄), 144.5 (B+B', C₁), 152.4 (C=O); MS (m/z %
192 abundance): 156 (27), 92 (21), 83 (28), 71 (45), 57 (100); Anal. Calcd for
193 C₂₈H₂₀N₆O₂Se₂ (%): C: 53.3, H: 3.2, N: 13.3. Found: C: 53.1, H: 3.5, N: 13.2.

194 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-methoxyphenyl)urea]**
195 **(6).** From 4-methoxyphenyl isocyanate after 72 h gave **6** as a yellow powder.
196 Yield: 65%; mp 274–275 °C; IR ν_{\max} (KBr): 3288 (N-H), 1644 (C=O) cm^{-1} ; ^1H
197 NMR (400 MHz, DMSO- d_6 , δ): 3.74 (s, 6H, 2OCH₃), 6.56 (d, 2H, $J_{3-2} = 7.5$ Hz,

198 B+B', H₃), 6.94 (d, 2H, $J_{5-6} = 7.5$ Hz, B+B', H₅), 7.19 (s, 4H, B+B', H₂+H₆), 7.44
199 (d, 4H, $J_{2-3} = J_{6-5} = 6.9$ Hz, A+A', H₂+H₆), 7.53 (d, 4H, A+A', H₃+H₅), 8.73 (s, 2H,
200 2NH), 8.82 (s, 2H, 2NH); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 55.4 (CH₃), 111.1
201 (B+B', C₃+C₅), 119.4 (B+B', C₂+C₆), 122.6 (A+A', C₃+C₅), 130.0 (A+A', C₁),
202 134.1 (A+A', C₂+C₆), 140.6 (B+B', C₁), 141.2 (A+A', C₄), 152.7 (C=O), 160.2
203 (B+B', C₄); Anal. Calcd for C₂₈H₂₆N₄O₄Se₂ (%): C: 52.5, H: 4.0, N: 8.7. Found:
204 C: 52.3, H: 3.9, N: 8.5.

205 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis(1-benzylurea) (7)**. From
206 benzyl isocyanate after 96 h gave **7** as a yellow powder. Yield: 43%; mp
207 213–215 °C; IR ν_{\max} (KBr): 3335 (N–H), 1647 (C=O) cm⁻¹; ¹H NMR (400 MHz,
208 DMSO-*d*₆, δ): 4.31 (d, 4H, $J_{\text{CH}_2\text{-NH}} = 5.6$ Hz, 2CH₂), 6.68 (t, 2H, $J_{\text{NH-CH}_2} = 5.6$ Hz,
209 NH-CH₂), 7.24–7.34 (m, 10H, B+B', H₂+H₃+H₄+H₅+H₆), 7.39 (d, 4H, $J_{3-2} = J_{5-6} =$
210 8.6 Hz, A+A', H₃+H₅), 7.45 (d, 4H, $J_{2-3} = J_{6-5} = 8.4$ Hz, A+A', H₂+H₆), 8.74 (s, 2H,
211 2NH-C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 43 (CH₂), 118.8 (A+A', C₃+C₅),
212 121.7 (A+A', C₁), 127.2 (B+B', C₄), 127.6 (B+B', C₂+C₆), 128.8 (B+B', C₃+C₅),
213 134.3 (A+A', C₂+C₆), 140.7 (B+B', C₁), 141.5 (A+A', C₄), 155.5 (C=O); Anal.
214 Calcd for C₂₈H₂₆N₄O₂Se₂.1/2 H₂O (%): C: 54.5, H: 4.4, N: 9.1. Found: C: 54.6,
215 H: 4.5, N: 9.3.

216 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis[1-(4-methoxybenzyl)urea]**
217 **(8)**. From 4-methoxybenzyl isocyanate after 48 h gave **8** as a yellow powder.
218 Yield: 31%; mp 222–224 °C; IR ν_{\max} (KBr): 3305 (N–H), 1630 (C=O) cm⁻¹; ¹H
219 NMR (400 MHz, DMSO-*d*₆, δ): 3.73 (s, 6H, 2OCH₃), 4.23 (d, 4H, $J_{\text{CH}_2\text{-NH}} = 5.5$
220 Hz, 2CH₂), 6.60 (t, 2H, $J_{\text{NH-CH}_2} = 5.5$ Hz, 2NH-CH₂), 6.90 (d, 4H, $J_{2-3} = J_{6-5} = 8.5$
221 Hz, B+B', H₂+H₆), 7.23 (d, 4H, $J_{3-2} = J_{5-6} = 8.5$ Hz, B+B', H₃+H₅), 7.39 (d, 4H, J_{2-3}
222 = $J_{6-5} = 8.5$ Hz, A+A', H₂+H₆), 7.45 (d, 4H, $J_{3-2} = J_{5-6} = 8.5$ Hz, A+A', H₃+H₅),

223 8.69 (s, 2H, 2NH-C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 42.9 (CH₂), 56.0
224 (CH₃), 114.4 (B+B', C₃+C₅), 119.0 (A+A', C₃+C₅), 122.1 (A+A', C₁), 129.2 (B+B',
225 C₂+C₆), 133.6 (B+B', C₁), 134.2 (A+A', C₂+C₆), 142.3 (A+A', C₄), 158.5 (C=O),
226 159.4 (B+B', C₄); MS (m/z % abundance): 368 (7), 215 (26), 83 (44), 71 (53), 57
227 (100); Anal. Calcd for C₃₀H₃₀N₄O₄Se₂·1/2 H₂O (%): C: 53.2, H: 4.4, N: 8.3.
228 Found: C: 53.1, H: 4.4, N: 8.2.

229 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis[1-(*n*-butyl)urea] (9).** From
230 butyl isocyanate after 72 h gave **9** as a yellow powder. Yield: 42%; mp 250–251
231 °C; IR ν_{\max} (KBr): 3309 (N–H), 2958–2864 (C–H), 1630 (C=O) cm⁻¹; ¹H NMR
232 (400 MHz, DMSO-*d*₆, δ): 0.89 (t, 6H, $J_{\text{CH}_3\text{-CH}_2} = 7.2$ Hz, 2CH₃), 1.25–1.45 (m,
233 8H, 2(-CH₂-CH₂-CH₃)), 2.96–3.17 (m, 4H, 2(-NH-CH₂)), 6.18 (t, 2H, $J_{\text{NH}_2\text{-CH}_2} =$
234 5.3 Hz, 2NH-CH₂), 7.30–7.48 (m, 8H, A+A', H₂+H₃+H₅+H₆), 8.57 (s, 2H,
235 2NH-C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 14.2 (C₄), 19.1 (C₃), 32.5 (C₂),
236 39.0 (C₁), 119.1 (A+A', C₃+C₅), 122.8 (A+A', C₁), 134.7 (A+A', C₂+C₆), 142.5
237 (A+A', C₄), 156.1 (C=O); MS (m/z % abundance): 588 (12), 211 (100), 183 (26),
238 91 (34), 43 (26); Anal. Calcd for C₂₂H₃₀N₄O₂Se₂·H₂O (%): C: 47.3, H: 5.7, N:
239 10.0. Found: C: 47.4, H: 5.5, N: 9.9.

240 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis[1-(*n*-hexyl)urea] (10).**
241 From hexyl isocyanate after 72 h gave **10** as a yellow powder. Yield: 25%; mp
242 180–181 °C; IR ν_{\max} (KBr): 3313 (N–H), 2956–2856 (C–H), 1627 (C=O) cm⁻¹;
243 ¹H NMR (400 MHz, DMSO-*d*₆, δ): 0.87 (bs, 6H, 2CH₃), 1.27 (bs, 12H, 2(-
244 (CH₂)₂-(CH₂)₃-CH₃)), 1.41 (bs, 4H, 2(-CH₂-CH₂-(CH₂)₃-CH₃)), 3.06 (bs, 4H,
245 2(-CH₂-(CH₂)₄-CH₃)), 6.17 (bs, 2H, 2NH-CH₂), 7.35 (d, 4H, $J_{2-3} = J_{6-5} = 8.0$ Hz,
246 A+A', H₂+H₆), 7.43 (d, 4H, $J_{3-2} = J_{5-6} = 8.0$ Hz, A+A', H₃+H₅), 8.57 (bs, 2H,
247 2NH-C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 14.4 (C₆), 22.5 (C₅), 26.5 (C₃),

248 30.1 (C₂), 31.5 (C₁+C₄), 118.7 (A+A', C₃+C₅), 121.4 (A+A', C₁), 134.4 (A+A',
249 C₂+C₆), 141.7 (A+A', C₄), 155.4 (C=O); MS (m/z % abundance): 172 (25), 149
250 (56), 123 (100), 91 (55), 56 (74), Anal. Calcd for C₂₆H₃₈N₄O₂Se₂·1/2 H₂O (%):
251 C: 51.6, H: 6.2, N: 9.2. Found: C: 51.6, H: 6.0, N: 9.1.

252 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-cyclohexylurea] (11).**

253 From cyclohexyl isocyanate after 120 h gave **11** as a yellow powder. Yield:
254 59%; mp 255–257 °C; IR ν_{\max} (KBr): 3306 (N–H), 2927–2850 (C–H), 1645
255 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆, δ): 1.15–1.30 (m, 12H, B+B',
256 2H₃+2H₄+2H₅), 1.53–1.58 (m, 2H, B+B', H₁), 1.65 (d, 4H, $J_{2-3} = J_{2-1} = 13.0$ Hz,
257 B+B', 2H₂), 1.79 (d, 4H, $J_{6-1} = J_{6-5} = 14.1$ Hz, B+B', 2H₆), 6.13 (d, 2H, $J_{\text{NH-CH}} =$
258 7.8 Hz, 2NH–CH), 7.34 (d, 4H, $J_{2-3} = J_{6-5} = 8.6$ Hz, A+A', H₂+H₆), 7.43 (d, 4H,
259 A+A', H₃+H₅), 8.46 (s, 2H, 2NH–C₆H₄); ¹³C NMR (100 MHz, DMSO-d₆, δ)
260 24.8+25.7 (B+B', C₃+C₅), 33.3+33.8 (B+B', C₂+C₄+C₆), 48.0 (B+B', C₁), 118.6
261 (A+A', C₃+C₅), 121.5 (A+A', C₁), 127.7 (A+A', C₂+C₆), 134.4 (A+A', C₄), 154.6
262 (C=O); MS (m/z % abundance): 368 (34), 224 (71), 191 (76), 56 (100), 41 (27);
263 Anal. Calcd for C₂₆H₃₄N₄O₂Se₂ (%): C: 52.7, H: 5.7, N: 9.4. Found: C: 52.3, H:
264 5.5, N: 9.8.

265 **General procedure for the synthesis of thioureas 12–22.** To a solution
266 of diselenide (1.17 mmol) in dioxane (25 mL) the corresponding isothiocyanate
267 (2.34 mmol, 1:2 molar ratio) was added and the mixture was kept at room
268 temperature from 48 h to 144 h. The solvent was removed under vacuum by
269 rotatory evaporation and the residue was treated with ethyl ether (50 mL) and
270 washed with water (100 mL).

271 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis(1-phenylthiourea) (12).**

272 From phenyl isothiocyanate after 144 h gave **12** as a yellow powder. Yield:

273 45%; mp 142–143 °C; IR ν_{\max} (KBr): 3193 (N–H), 1588 (C=S) cm^{-1} ; ^1H NMR
274 (400 MHz, DMSO- d_6 , δ): 7.12–7.15 (m, 2H, B+B', H₄), 7.33 (t, 4H, $J_{3-2} = J_{5-6} =$
275 7.5 Hz, B+B', H₃+H₅), 7.46–7.49 (m, 8H, A+A', B+B', H₂+H₆), 7.59 (d, 4H, $J_{3-2} =$
276 $J_{5-6} = 7.5$ Hz, A+A', H₃+H₅), 9.88 (bs, 4H, 4NH); ^{13}C NMR (100 MHz, DMSO- d_6 ,
277 δ): 124.1 (A+A', C₁), 124.5 (A+A', C₃+C₅), 125.0 (B+B', C₂+C₆), 125.4 (B+B',
278 C₄), 128.9 (B+B', C₃+C₅), 132.5 (A+A', C₂+C₆), 139.8 (A+A', C₄), 140.2 (B+B',
279 C₁), 179.9 (C=S); MS (m/z % abundance): 428 (33), 386 (34), 214 (69), 172
280 (86), 135 (100), 93 (74), 80 (35); Anal. Calcd for C₂₆H₂₂N₄S₂Se₂ (%): C: 50.9, H:
281 3.6, N: 9.1. Found: C: 50.6, H: 3.8, N: 8.7.

282 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-nitrophenyl)thiourea]**
283 **(13)**. From 4-nitrophenyl isothiocyanate after 48 h gave **13** as a yellow powder.
284 Yield: 58%; mp 175–176 °C; IR ν_{\max} (KBr): 3345 (N–H), 1570 (C=S) cm^{-1} ; ^1H
285 NMR (400 MHz, DMSO- d_6 , δ): 7.49 (bs, 4H, A+A', H₂+H₆), 7.62 (bs, 4H, A+A',
286 H₃+H₅), 7.81 (bs, 4H, B+B', H₂+H₆), 8.20 (bs, 4H, B+B', H₃+H₅), 10.41 (bs, 4H,
287 4NH); ^{13}C NMR (100 MHz, DMSO- d_6 , δ): 122.2 (A+A', C₁), 124.9 (B+B', C₃+C₅),
288 126.2 (B+B', C₂+C₆), 132.4 (A+A', C₃+C₅), 139.5 (A+A', C₂+C₆), 142.9 (A+A',
289 C₄), 146.6 (B+B', C₁+C₄), 179.7 (C=S); MS (m/z % abundance): 426 (5), 386
290 (13), 344 (15), 180 (100), 172 (53), 150 (26), 134 (34), 90 (25); Anal. Calcd for
291 C₂₆H₂₀N₆O₄S₂Se₂·H₂O (%): C: 43.3, H: 2.8, N: 11.6. Found: C: 43.6, H: 2.9, N:
292 11.3.

293 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-**
294 **methylphenyl)thiourea] (14)**. From 4-methylphenyl isothiocyanate after 96 h
295 gave **14** as a yellow powder. Yield: 63%; mp 151–153 °C; IR ν_{\max} (KBr): 3203
296 (N–H), 1583 (C=S) cm^{-1} ; ^1H RMN (400 MHz, DMSO- d_6 , δ): 2.28 (s, 6H, 2CH₃),
297 7.14 (d, 4H, $J_{3-2} = J_{5-6} = 8.0$ Hz, B+B', H₃+H₅), 7.32 (d, 4H, B+B', H₂+H₆), 7.48

298 (d, 4H, $J_{2-3} = J_{6-5} = 8.2$ Hz, A+A', H₂+H₆), 7.58 (d, 4H, A+A', H₃+H₅), 9.81 (bs,
299 4H, 4NH); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 21.3 (CH₃), 123.0 (A+A', C₁),
300 125.2 (A+A', C₃+C₅), 127.9 (B+B', C₂+C₆), 129.0 (B+B', C₃+C₅), 131.5 (A+A',
301 C₂+C₆), 133.2 (B+B', C₁), 137.1 (B+B', C₄), 140.3 (A+A', C₄), 179.8 (C=S); MS
302 (m/z % abundance): 428 (33), 386 (37), 214 (65), 172 (100), 149 (86), 106 (90),
303 91 (52); Anal. Calcd for C₂₈H₂₆N₄S₂Se₂ (%): C: 52.5, H: 4.1, N: 8.7. Found: C:
304 52.1, H: 4.3, N: 8.4.

305 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-**
306 **chlorophenyl)thiourea] (15).** From 4-chlorophenyl isothiocyanate after 48 h
307 gave **15** as a yellow powder. Yield: 68%; mp 170–171 °C; IR ν_{\max} (KBr): 3210
308 (N–H), 1583 (C=S) cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆, δ): 7.44 (d, 4H, $J_{2-3} = J_{6-5} = 8.8$ Hz, A+A', H₂+H₆), 7.55 (dd, 8H, $J_{3-2} = J_{5-6} = 8.8$ Hz, A+A', B+B', H₃+H₅),
309 7.65 (d, 4H, B+B', H₂+H₆), 10.01 (bs, 4H, 4NH); ¹³C NMR (100 MHz, DMSO-*d*₆,
310 δ): 124.6 (A+A', C₃+C₅), 125.6 (B+B', C₂+C₆), 125.7 (A+A', C₁), 128.8 (B+B',
311 C₄), 128.8 (B+B', C₃+C₅), 132.5 (A+A', C₂+C₆), 138.8 (B+B', C₁), 139.9 (A+A',
312 C₄), 180.0 (C=S); MS (m/z % abundance): 428 (14), 386 (25), 214 (28), 169
313 (100), 127 (54), 111 (23); Anal. Calcd for C₂₆H₂₀Cl₂N₄S₂Se₂ (%): C: 45.8, H: 2.9,
314 N: 8.2. Found: C: 45.5, H: 3.0, N: 7.9.

316 ***N,N'*-(diselanediyldibenzene-4,1-diyl)bis[1-(4-**
317 **cyanophenyl)thiourea] (16).** From 4-cyanophenyl isothiocyanate after 144 h
318 gave **16** as a yellow powder. Yield: 44%; mp 123–124 °C; IR ν_{\max} (KBr): 3166
319 (N–H), 2224 (CN), 1584 (C=S) cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆, δ): 7.48 (d,
320 4H, $J_{2-3} = J_{6-5} = 8.4$ Hz, A+A', H₂+H₆), 7.62 (d, 4H, A+A', H₃+H₅), 7.75 (d, 4H, J_{3-5}
321 = $J_{5-6} = 8.8$ Hz, B+B', H₃+H₅), 7.78 (d, 4H, B+B', H₂+H₆), 10.25 (bs, 2H, 2NH),
322 10.28 (bs, 2H, 2NH); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 104.9 (CN), 114.7

323 (B+B', C₄), 124.2 (A+A', C₁), 124.7 (A+A', C₃+C₅), 125.3 (B+B', C₂+C₆), 129.8
324 (A+A', C₂+C₆), 132.6 (B+B', C₃+C₅), 139.0 (A+A', C₄), 144.2 (B+B', C₁), 179.8
325 (C=S); MS (m/z % abundance): 428 (6), 386 (22), 344 (23), 172 (96), 160 (100),
326 118 (30), 80 (20); Anal. Calcd for C₂₈H₂₀N₆S₂Se₂.1/2 H₂O (%): C: 50.1, H: 3.0,
327 N: 12.5. Found: C: 49.8, H: 3.3, N: 12.4.

328 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis[1-(4-**
329 **methoxyphenyl)thiourea] (17).** From 4-methoxyphenyl isothiocyanate after 72
330 h gave **17** as a yellow powder. Yield: 65%; mp 142–144 °C; IR ν_{\max} (KBr): ¹H
331 NMR (400 MHz, DMSO-*d*₆, δ): 3.75 (s, 6H, 2OCH₃), 6.91 (d, 4H, $J_{2-3} = J_{6-5} = 8.9$
332 Hz, B+B', H₂+H₆), 7.32 (d, 4H, B+B', H₃+H₅), 7.48 (d, 4H, $J_{3-2} = J_{5-6} = 8.6$ Hz,
333 A+A', H₃+H₅), 7.58 (d, 4H, A+A', H₂+H₆), 9.72 (bs, 4H, 4NH); ¹³C NMR (100
334 MHz, DMSO-*d*₆, δ): 56.0 (CH₃), 114.2 (B+B', C₃+C₅), 115.3 (A+A', C₃+C₅),
335 126.0 (B+B', C₁), 125.4 (A+A', C₁), 132.1 (B+B', C₂+C₆), 133.6 (A+A', C₂+C₆),
336 139.7 (A+A', C₄), 159.2 (B+B', C₄), 180.1 (C=S); MS (m/z % abundance): 428
337 (21), 386 (32), 213 (53), 172 (100), 166 (84), 150 (54), 108 (57), 80 (41); Anal.
338 Calcd for C₂₈H₂₆N₄O₂S₂Se₂.1/2 H₂O (%): C: 48.7, H: 4.1, N: 8.1. Found: C:
339 49.1, H 3.9, N: 7.8.

340 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis(1-benzylthiourea) (18).**
341 From benzyl isothiocyanate after 144 h gave **18** as a yellow powder. Yield:
342 71%; mp 146–147 °C; IR ν_{\max} (KBr): 3238 (N–H), 1533 (C=S) cm⁻¹; ¹H NMR
343 (400 MHz, DMSO-*d*₆, δ): 4.77 (d, $J_{\text{CH}_2\text{-NH}} = 5.3$ Hz, 4H, 2CH₂), 7.22–7.28 (m,
344 2H, B+B', H₄), 7.32–7.38 (m, 8H, B+B', H₂+H₆, H₃+H₅), 7.45 (d, 4H, $J_{2-3} = J_{6-5} =$
345 8.6 Hz, A+A', H₂+H₆), 7.57 (d, 4H, A+A', H₃+H₅), 8.27 (s, 2H, 2NH–CH₂), 9.71
346 (s, 2H, 2NH–C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 47.6 (CH₂), 124.1 (A+A',
347 C₁), 125.1 (A+A', C₃+C₅), 127.4 (B+B', C₄), 127.9 (B+B', C₂+C₆), 128.8 (B+B',

348 C₃+C₅), 132.7 (A+A', C₂+C₆), 139.3 (B+B', C₁), 140.0 (A+A', C₄), 181.1 (C=S);
349 MS (m/z % abundance): 368 (42), 191 (100), 172 (67), 57 (54); Anal. Calcd for
350 C₂₈H₂₆N₄S₂Se₂·1/2 H₂O (%): C: 51.8, H: 4.2, N: 8.6. Found: C: 51.9, H: 4.6, N:
351 8.7.

352 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis[1-(4-**
353 **methoxybenzyl)thiourea] (19).** From 4-methoxybenzyl isothiocyanate after
354 120 h gave **19** as a yellow powder. Yield: 49%; mp 174–175 °C; IR ν_{\max} (KBr):
355 3220 (N–H), 1583 (C=S) cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆, δ): 3.73 (s, 6H,
356 2OCH₃), 4.64 (bs, 4H, 2CH₂), 6.90 (d, 4H, $J_{3-2} = J_{5-6} = 8.8$ Hz, B+B', H₃+H₅),
357 7.27 (d, 4H, B+B', H₂+H₆), 7.44 (d, 4H, $J_{2-3} = J_{6-5} = 7.8$ Hz, A+A', H₂+H₆), 7.56
358 (d, 4H, A+A', H₃+H₅), 8.20 (bs, 2H, 2NH–CH₂), 9.66 (bs, 2H, 2NH–C₆H₄); ¹³C
359 NMR (100 MHz, DMSO-*d*₆, δ): 53.9 (CH₂), 57.1 (CH₃), 112.8 (B+B', C₃+C₅),
360 115.0 (A+A', C₁), 123.2 (A+A', C₃+C₅), 124.1 (B+B', C₁), 128.8 (B+B', C₂+C₆),
361 132.6 (A+A', C₂+C₆), 140.2 (A+A', C₄), 159.1 (B+B', C₄), 180.7 (C=S); MS (m/z
362 % abundance): 428 (55), 214 (100), 172 (44), 136 (96), 121 (82), 106 (36);
363 Anal. Calcd for C₃₀H₃₀N₄O₂S₂Se₂·1/2 H₂O (%): C: 50.7, H: 4.2, N: 7.9. Found:
364 C: 50.4, H: 4.2, N: 7.7.

365 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis[1-(*n*-butyl)thiourea] (20).**
366 From 4-methoxybenzyl isothiocyanate after 120 h gave **20** as a yellow powder.
367 Yield: 49%; mp 174–175 °C; IR ν_{\max} (KBr): 3220 (N–H), 1583 (C=S) cm⁻¹; ¹H
368 NMR (400 MHz, DMSO-*d*₆, δ): 3.73 (s, 6H, 2OCH₃), 4.64 (bs, 4H, 2CH₂), 6.90
369 (d, 4H, $J_{3-2} = J_{5-6} = 8.8$ Hz, B+B', H₃+H₅), 7.27 (d, 4H, B+B', H₂+H₆), 7.44 (d, 4H,
370 $J_{2-3} = J_{6-5} = 7.8$ Hz, A+A', H₂+H₆), 7.56 (d, 4H, A+A', H₃+H₅), 8.20 (bs, 2H,
371 2NH–CH₂), 9.66 (bs, 2H, 2NH–C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 14.2
372 (CH₃), 20.1 (CH₂), 30.1 (CH₂), 44.0 (CH₂), 123.7 (A+A', C₁), 124.7 (A+A',

373 C₃+C₅), 132.7 (A+A', C₂+C₆), 140.2 (A+A', C₄), 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 C₃₀H₃₀N₄O₂S₂Se₂ · 1/2 H₂O (%): C: 50.7, H: 4.2, N: 7.9. Found: C:
376 50.4, H: 4.2, N: 7.7.

377 ***N,N'*-(diselanediyl)dibenzene-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 °C; IR ν_{\max} (KBr): 3223 (N–H), 2925–2854 (C–H), 1540 (C=S) cm⁻¹;
380 ¹H NMR (400 MHz, DMSO-*d*₆, δ): 0.87 (bs, 6H, 2CH₃), 1.28 (bs, 12H, 2(-
381 (CH₂)₂-(CH₂)₃-CH₃)), 1.51 (bs, 4H, 2(-CH₂-CH₂-(CH₂)₃-CH₃)), 3.44 (bs, 4H,
382 2(-CH₂-(CH₂)₄-CH₃)), 7.42 (d, 4H, $J_{2,3} = J_{6,5} = 8.4$ Hz, A+A', H₂+H₆), 7.55 (d,
383 4H, A+A', H₃+H₅), 7.85 (bs, 2H, 2NH-CH₂), 9.55 (bs, 2H, 2NH-C₆H₄); ¹³C NMR
384 (100 MHz, DMSO-*d*₆, δ): 14.4 (CH₃), 22.5 (CH₂), 26.6 (CH₂), 28.8 (CH₂), 31.5
385 (CH₂), 44.3 (CH₂), 123.7 (A+A', C₁), 132.7 (A+A', C₂+C₃+C₅+C₆), 140.2 (A+A',
386 C₄), 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 C₂₆H₃₈N₄S₂Se₂ · 1/2 H₂O (%): C: 49.0, H: 6.0, N: 8.8. Found: C: 48.9, H: 6.0, N:
389 8.8.

390 ***N,N'*-(diselanediyl)dibenzene-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 °C; IR ν_{\max} (KBr): 3321 (N–H), 2926–2851 (C–H),
393 1587 (C=S) cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆, δ): 1.10–1.35 (m, 12H, B+B',
394 2H₃+2H₄+2H₅), 1.56 (bs, 2H, B+B', 2H₁), 1.62 (bs, 4H, B+B', 2H₂) 1.80 (bs, 4H,
395 B+B', 2H₆), 7.47 (d, 4H, $J_{3,2} = J_{5,6} = 8.6$ Hz, A+A', H₃+H₅), 7.54 (d, 4H, A+A',
396 H₃+H₅), 7.81 (d, 2H, $J_{\text{NH-CH}} = 8.1$ Hz, 2NH-CH), 9.49 (s, 2H, 2NH-C₆H₄); ¹³C
397 NMR (100 MHz, DMSO-*d*₆, δ) 25 (B+B', C₃+C₅), 26 (B+B', C₄), 32 (B+B',

398 C₂+C₆), 53 (B+B', C₁), 124 (A+A', C₁), 131 (A+A', C₂+C₃+C₅+C₆), 140 (A+A',
399 C₄), 179 (C=S); MS (m/z % abundance): 368 (12), 214 (7), 191 (24), 83 (21), 56
400 (100), 41 (33); Anal. Calcd for C₂₆H₃₄N₄S₂Se₂·1/2 H₂O (%): 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 °C 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 ν_{\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 Na₂SO₄. After
414 filtration and evaporation of the solvent, **24** was acquired as a white powder.
415 Yield: 66%; IR ν_{\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 °C for 8 h–12 h. The mixture was diluted with CH₂Cl₂
419 (50 mL), and filters through celite. Then the filtrate was washed with saturated
420 NaHCO₃ (4 x 30 mL) and brine (2x20 mL), and was dried over anhydrous
421 Na₂SO₄. After filtration and evaporation of the solvent, **25** was acquired as a
422 white powder. Yield: 69%; IR ν_{\max} (KBr): 3258 (N-H), 1670 (C=O) cm⁻¹.

423 **N-(4-cyanophenyl)formamide (26).** To a mixture of 4-aminobenzonitrile
424 (10 mmol), formic acid (30 mmol) and zinc dust pre-treated with HCl (1 mmol)
425 was added and stirred at 70 °C for 8 h–12 h. The mixture was diluted with
426 CH₂Cl₂ (50 mL), and filters through celite. Then the filtrate was washed with
427 saturated NaHCO₃ (3 x 30 mL) and brine (3 x 30 mL), and was dried over
428 anhydrous Na₂SO₄. After filtration and evaporation of the solvent, **26** was
429 acquired as a white powder. Yield: 82%; IR ν_{\max} (KBr): 3357 (N–H), 2216 (CN),
430 1637 (C=O) cm⁻¹.

431 **N-(4-methoxyphenyl)formamide (27).** To a mixture of 4-methoxyaniline
432 (11.25 mmol) and HCOOH (33.75 mmol), PEG-400 (16 g) was added. The
433 mixture was stirred at room temperature for 24 h and after completion was
434 diluted with water (50 mL) and extracted with ethyl acetate (5 x 15 mL). Then
435 the organic layer was dried over anhydrous Na₂SO₄ and concentrated. The
436 residue was subjected to column chromatography ethyl (acetate/ hexane 70/30)
437 to obtain the pure 27 as a white powder. Yield: 68.6%; IR ν_{\max} (KBr): 3245
438 (N–H), 1656 (C=O) cm⁻¹.

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

445 **N-hexylformamide (29).** To a stirred solution of hexan-1-amine (15
446 mmol) was added dropwise ethyl formate (12.10 mmol). The reaction mixture
447 was stirred at 150 °C 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 ν_{\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 °C 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 ν_{\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 ν_{\max} (KBr): 2978–2873 (C-H), 2114 (N=C=Se)
470 cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ): 6.92 (t, 2H, $J_{2-3} = J_{6-5} = 6.3$ Hz, H_2+H_6),
471 7.28 (t, 1H, H_4), 7.62 (d, 2H, $J_{3-2} = J_{5-6} = 8.4$ Hz, H_3+H_5).

472 **4-Methylphenylisoselenocyanate (32).** From *N*-(4-
473 methylphenyl)formamide **24** gave **32** as a brown syrup: Yield 7.48%; IR ν_{\max}
474 (KBr): 2921–2866 (C–H), 2153 (N=C=Se) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 ,
475 δ): 2.34 (s, 3H, CH₃), 7.21 (d, 2H, $J_{2-3} = J_{6-5} = 7.9$ Hz, H₂+H₆), 7.26 (d, 2H,
476 H₃+H₅).

477 **4-Methoxyphenylisoselenocyanate (33).** From *N*-(4-
478 methoxyphenyl)formamide **27** gave **33** as an orange syrup: Yield 6.79%; IR ν_{\max}
479 (KBr): 2944–2740 (C–H), 2121 (N=C=Se) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 ,
480 δ): 3.80 (s, 3H, OCH₃), 7.02 (d, 2H, $J_{3-2} = J_{5-6} = 8.0$ Hz, H₃+H₅), 7.45 (d, 2H,
481 H₂+H₆).

482 **Benzylisoselenocyanate (34).** From *N*-benzylformamide gave **34** as a
483 brown syrup: Yield 11.75 %; IR ν_{\max} (KBr): 2958–2871 (C–H), 2143 (N=C=Se)
484 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 , δ): 5.15 (s, 2H, CH₂), 7.41–7.54 (m, 5H,
485 H₂+H₃+H₄+H₅+H₆).

486 **General procedure for the synthesis of isoselenocyanates 35-39.** To
487 a refluxing mixture of formamide (7.2 mmol) and *N,N*-diethyletamine (30.5
488 mmol) in dry dichloromethane (25 mL) was added dropwise a solution of
489 triphosgene (3.85 mmol) in dry dichloromethane (5 mL), under N₂ atmosphere,
490 over a period of 45 min. After the addition, the resulting mixture was refluxed
491 for 2.5 h and then black selenium powder (14.4 mmol) was added and refluxed
492 for 12 h in the darkness. After filtered, solvents were removed under vacuum
493 and the residue was washed with dichloromethane (30 mL). Column
494 chromatography using ethyl acetate/hexane (70/30) as eluent afforded
495 isoselenocyanate.

496 **4-Chlorophenyl isoselenocyanate (35).** From *N*-(4-
497 chlorophenyl)formamide **25** gave **35** as a brown syrup: Yield 55.76%; IR ν_{\max}
498 (KBr): 2924–2854 (C–H), 2151 (N=C=Se) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 ,
499 δ): 7.02 (d, 2H, $J_{3-2} = J_{5-6} = 7.6$ Hz, H₃+H₅), 7.49 (d, 2H, H₂+H₆).

500 **4-Cyanophenyl isoselenocyanate (36).** From *N*-(4-
501 cyanophenyl)formamide **26** gave **36** as a brown syrup: Yield 85 %; IR ν_{\max}
502 (KBr): 2927–2856 (C–H), 2225 (CN), 2146 (N=C=Se) cm^{-1} ; ^1H NMR (400 MHz,
503 DMSO- d_6 , δ): 7.77 (d, 2H, $J_{3-2} = J_{5-6} = 8.4$ Hz, H₃+H₅), 7.79 (d, 2H, H₂+H₆).

504 **Butyl isoselenocyanate (37).** From *N*-butylformamide **28** gave **37** as a
505 dark syrup: Yield 43.5%; IR ν_{\max} (KBr): 2923–2876 (C–H), 2144 (N=C=Se) cm^{-1} ;
506 ^1H NMR (400 MHz, DMSO- d_6 , δ): 0.86–1.11 (m, 3H, CH₃), 1.21–1.32 (m, 4H,
507 CH₂-CH₂-CH₃), 1.35 (m, 2H, CH₂-NCSe).

508 **Hexyl isoselenocyanate (38).** From *N*-hexylformamide **29** gave **38** as a
509 brown syrup: Yield 62%; IR ν_{\max} (KBr): 2931–2861 (C–H), 2144 (N=C=Se) cm^{-1} ;
510 ^1H NMR (400 MHz, DMSO- d_6 , δ): 0.85–0.91 (m, 3H, CH₃), 1.05 (t, 2H, $J = 6.99$
511 Hz, (CH₂)₂-(CH₂)₂-CH₂-CH₃), 1.18–1.24 (m, 4H, (CH₂)₂-(CH₂)₂-CH₂-CH₃),
512 1.28–1.35 (m, 2H, CH₂-CH₂-(CH₂)₃-CH₃), 1.41–1.49 (m, 2H, CH₂-NCSe).

513 **Cyclohexylisoselenocyanate (39).** From *N*-cyclohexylformamide **30**
514 gave **39** as a dark syrup: Yield 92%; IR ν_{\max} (KBr): 2933–2883 (C–H), 2137
515 (N=C=Se) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 , δ): 1.07 (s, H, H₁), 1.28–1.37 (m,
516 2H, H₄), 1.56–1.62 (m, 4H, H₃+H₅), 1.85–1.90 (m, 4H, H₂+H₆).

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

521 darkness for 145 h. To afford the desired selenourea we proceed accordingly
522 two different work-up: i) Work-up method A: After stirring the precipitate was
523 filtered off, washed with dichloromethane (100 mL) and dried in order to obtain
524 the selenoureas **41** and **44**; ii) Work-up method B: After stirring the solvent was
525 evaporated to yield the solid product, which was washed with dichloromethane
526 (100 mL) and dried in order to obtain the selenoureas **40**, **42-43** and **45-48**.

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

529 ***N,N'*-(Diselanediyldibenzene-4,1-diyl)bis(1-phenylselenourea) (40).**

530 From phenyl isoselenocyanate **31**. The salt formation with hydrochloric ether
531 gave **40** as a yellow powder. Yield: 2.6%; mp 189–191 °C; IR ν_{\max} (KBr): 3427
532 (N–H), 1582 (C=Se) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 , δ): 6.43 (d, 4H, $J_{2-3} =$
533 $J_{6-5} = 7.2$ Hz, B+B', H₂+H₆), 6.63 (d, 4H, $J_{3-2} = J_{5-6} = 8.8$ Hz, A+A', H₃+H₅), 6.81
534 (t, 2H, $J_{4-3} = J_{4-5} = 7.3$ Hz, B+B', H₄), 7.20 (t, 4H, $J_{3-2} = J_{5-6} = 8.7$ Hz, B+B',
535 H₃+H₅), 7.54 (d, 4H, A+A', H₂+H₆), 9.36 (s, 2H, 2NH–C₆H₄), 9.56 (s, 2H,
536 2NH–C₆H₄Se); ^{13}C NMR (100 MHz, DMSO- d_6 , δ): 119.2 (A+A', C₁), 116.4
537 (A+A', C₃+C₅), 117.9 (B+B', C₂+C₆), 122.0 (B+B', C₄), 129.3 (B+B', C₃+C₅),
538 132.8 (A+A', C₂+C₆), 144.3 (A+A', C₄), 146.1 (B+B', C₁), 179.4 (C=Se); Anal.
539 Calcd for C₂₆H₂₂N₄Se₄·3HCl (%): C, 37.4, H, 3.2, N, 6.7. Found: C, 37.3, H, 3.0,
540 N, 6.7.

541 ***N,N'*-(Diselanediyldibenzene-4,1-diyl)bis[1-(4-**
542 **methoxyphenyl)selenourea] (41).** From 4-methoxyphenyl isoselenocyanate **32**.
543 The salt formation with hydrochloric ether gave **41** as a yellow powder. Yield:
544 7.5%; mp 212–213 °C; IR ν_{\max} (KBr): 3161 (N–H), 1577 (C=Se) cm^{-1} ; ^1H NMR
545 (400 MHz, DMSO- d_6 , δ): 2.73 (s, 6H, 2CH₃), 7.30 (d, 4H, $J_{2-3} = J_{6-5} = 8.8$ Hz,

546 B+B', H₂+H₆), 7.47 (d, 4H, $J_{3-2} = J_{5-6} = 8.6$ Hz, A+A', H₃+H₅), 7.54 (d, 4H, $J_{3-2} =$
547 $J_{5-6} = 8.8$ Hz, B+B', H₃+H₅), 7.66 (d, 4H, $J_{2-3} = J_{6-2} = 8.6$ Hz, A+A', H₂+H₆), 8.20
548 (s, 2H, 2NH-C₆H₄CH₃), 9.66 (s, 2H, 2NH-C₆H₄Se); ¹³C NMR (100 MHz,
549 DMSO-*d*₆, δ): 20.1 (CH₃), 104.3 (B+B', C₂+C₆), 118.8 (A+A', C₃+C₅), 123.3
550 (A+A', C₁), 129.0 (B+B', C₃+C₅), 134.2 (B+B', C₄), 140.7 (A+A', C₂+C₆), 162.5
551 (B+B', C₁), 173.0 (A+A', C₄), 181.8 (C=Se); MS (m/z % abundance): 222 (99),
552 197 (36), 91 (100), 65 (35); Anal. Calcd for C₂₈H₂₆N₄Se₄.3HCl (%): C, 39.8, H,
553 3.4, N, 6.6. Found: C, 39.5, H, 3.2, N, 6.6.

554 ***N,N'*-(diselanediyl)dibenzene-4,1-diyl)bis[1-(4-**
555 **chlorophenyl)selenourea] (42).** From 4-chlorophenyl isoselenocyanate **35**.
556 The salt formation with hydrochloric ether gave **42** as a yellow powder. Yield:
557 4.75%; IR ν_{\max} (KBr): 3367 (N-H), 1625 (C=Se) cm⁻¹; ¹H NMR (400 MHz,
558 DMSO-*d*₆, δ): 7.06–7.11 (m, 4H, A+A', H₃+H₅), 7.20–7.25 (m, 4H, B+B', H₂+H₆),
559 7.29 (d, 4H, $J_{2-3} = J_{6-5} = 8.6$ Hz, A+A', H₂+H₆), 7.45 (d, 4H, $J_{3-2} = J_{5-6} = 8.8$ Hz,
560 B+B', H₃+H₅), 9.33 (s, 2H, 2NH-C₆H₄CN), 9.59 (s, 2H, 2NH-C₆H₄Se); ¹³C NMR
561 (100 MHz, DMSO-*d*₆, δ): 116.3 (A+A', C₃+C₅), 119.1 (A+A', C₁), 121.3 (B+B',
562 C₂+C₆), 128.0 (B+B', C₄), 130.2 (B+B', C₃+C₅), 132.5 (A+A', C₂+C₆), 142.4
563 (B+B', C₁), 144.1 (A+A', C₄), 180.0 (C=Se); Anal. Calcd for
564 C₂₆H₂₀Cl₂N₄Se₄.4HCl (%): C, 33.9, H, 2.6, N, 6.1. Found: C, 34.1, H, 2.2, N, 5.7.

565 ***N,N'*-(Diselanediyl)dibenzene-4,1-diyl)bis[1-(4-**
566 **cyanophenyl)selenourea] (43).** From 4-cyanophenyl isoselenocyanate **36**.
567 The salt formation with hydrochloric ether gave **43** as a yellow powder. Yield:
568 7.3%; mp 165–167 °C; IR ν_{\max} (KBr): 3077 (N-H), 2223 (CN), 1607 (C=Se) cm⁻¹;
569 ¹H NMR (400 MHz, DMSO-*d*₆, δ): 6.94–7.06 (m, 4H, A+A', H₃+H₅), 7.12–7.33
570 (m, 4H, B+B', H₂+H₆), 7.35–7.53 (m, 4H, A+A', H₂+H₆), 7.56–7.74 (m, 4H, B+B',

571 H₃+H₅), 10.19 (s, 4H, 4NH); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 103.8 (B+B', C₄),
572 116.4 (A+A', C₃+C₅), 118.1 (A+A', C₁), 119.3 (CN), 120.4 (B+B', C₂+C₆), 132.2
573 (A+A', C₂+C₆), 133.0 (B+B', C₃+C₅), 144.1 (A+A', C₄), 149.2 (B+B', C₁), 180.0
574 (C=Se); MS (m/z % abundance): 446 (20), 243 (47), 189 (100), 95 (46), 56 (39
575); Anal. Calcd for C₂₈H₂₀N₆Se₄.4HCl (%): C, 44.4, H, 2.6, N, 11.1. Found: C,
576 44.5, H, 2.6, N, 11.3.

577 ***N,N'*-(Diselanediyldibenzene-4,1-diyl)bis[1-(4-**
578 **methoxyphenyl)selenourea] (44).** From 4-methoxyphenyl isoselenocyanate
579 **33.** The salt formation with hydrochloric ether gave **44** as a orange powder.
580 Yield: 8.5%; mp 201–202 °C; IR ν_{\max} (KBr): 3310 (N–H), 1553 (C=Se) cm⁻¹; ¹H
581 NMR (400 MHz, DMSO-*d*₆, δ): 3.74 (s, 6H, 2OCH₃), 6.90 (d, 4H, $J_{2-3} = J_{6-5} = 8.4$
582 Hz, B+B', H₂+H₆), 7.28 (d, 4H, $J_{3-2} = J_{5-6} = 7.7$ Hz, A+A', H₃+H₅), 7.43 (d, 4H,
583 B+B', H₃+H₅), 7.58 (d, 4H, A+A', H₂+H₆), 10.19 (bs, 4H, 4NH); ¹³C NMR (100
584 MHz, DMSO-*d*₆, δ): 55.2 (OCH₃), 113.7 (B+B', C₃+C₅), 115.1 (A+A', C₃+C₅),
585 115.5 (B+B', C₂+C₆), 116.0 (A+A', C₁), 126.2 (A+A', C₂+C₆), 133.1 (B+B', C₁),
586 147.8 (A+A', C₄), 157.4 (B+B', C₄), 179.2 (C=Se); MS (m/z % abundance): 254
587 (29), 213 (100), 197 (64), 108 (49), 63 (31); Anal. Calcd for
588 C₂₈H₂₆N₄O₂Se₄.2HCl (%): C, 33.9, H, 2.6, N, 6.1. Found: C, 34.1, H, 2.2, N, 5.7.

589 **1,1'-(4,4'-Diselanediylbis(4,1-phenylene))bis(3-benzylselenourea)**
590 **(45).** From benzyl isoselenocyanate **34.** Yellow powder. Yield: 11.75%; mp
591 145–146 °C; IR ν_{\max} (KBr): 3120 (N–H), 1570 (C=Se) cm⁻¹; ¹H NMR (400 MHz,
592 DMSO-*d*₆, δ): 4.85 (bs, 4H, 2CH₂), 7.27 (bs, 4H, B+B', H₂+H₆), 7.34 (bs, 10H,
593 A+A', H₂+H₆, B+B', H₃+H₄+H₅), 7.61 (bs, 4H, A+A', H₃+H₅), 8.66 (bs, 2H,
594 2NH–CH₂), 10.07 (bs, 2H, 2NH–C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 50.6
595 (CH₂), 125.3 (A+A', C₃+C₅), 126.6 (A+A', C₁), 127.5 (B+B', C₄), 127.9 (B+B',

596 C₂+C₆), 128.7 (B+B', C₃+C₅), 132.7 (A+A', C₂+C₆), 139.0 (B+B', C₁), 139.3
597 (A+A', C₄), 180.3 (C=Se); MS (m/z % abundance): 197 (10), 107 (27), 91 (100),
598 65 (19); Anal. Calcd for C₂₈H₂₆N₄Se₄ (%): C, 45.7, H, 3.5, N, 7.6. Found: C,
599 45.6, H, 3.6, N, 7.4.

600 **1,1'-(4,4'-Diselanediylobis(4,1-phenylene))bis(3-butylselenourea) (46).**

601 From butyl isoselenocyanate **37**. Yellow powder. Yield: 15.7%; mp 114–116 °C;
602 IR ν_{\max} (KBr): 3257 (N-H), 2957–2865 (C-H), 1620 (C=Se) cm⁻¹; ¹H NMR (400
603 MHz, DMSO-*d*₆, δ): 0.90 (t, 6H, $J_{\text{CH}_3\text{-CH}_2} = 6.8$ Hz, 2CH₃), 1.30-1.34 (m, 6H, B, -
604 $\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_3$), 1.53–1.56 (m, 6H, B' - $\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_3$), 7.33 (d, 4H,
605 $J_{3-2} = J_{5-6} = 8.1$ Hz, A+A', H₃+H₅), 7.60 (d, 4H, A+A', H₂+H₆), 8.27 (bs, 2H,
606 2NH-CH₂), 9.92 (bs, 2H, 2NH-C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 14.2
607 (CH₃), 20.0 (CH₂), 31.0 (CH₂), 47.0 (CH₂), 124.8 (A+A', C₁), 132.9 (A+A',
608 C₂+C₃+C₅+C₆), 139.6 (A+A', C₄), 179.2 (C=Se); Anal. Calcd for
609 C₂₂H₃₀N₄Se₄.H₂O (%): C, 38.6, H, 4.7, N, 8.2. Found: C, 38.3, H, 4.3, N, 8.0.

610 **1,1'-(4,4'-Diselanediylobis(4,1-phenylene))bis(3-hexylselenourea) (47).**

611 From hexyl isoselenocyanate **38**. Yellow powder. Yield: 12.2%; mp 115–117 °C;
612 IR ν_{\max} (KBr): 3195 (N-H), 2923–2854 (C-H), 1542 (C=Se) cm⁻¹; ¹H NMR (400
613 MHz, DMSO-*d*₆, δ): 0.88 (bs, 6H, 2CH₃), 1.28 (bs, 12H, 2(-CH₂)₂-(CH₂)₃-CH₃),
614 1.55 (bs, 4H, 2(-CH₂-CH₂-(CH₂)₃-CH₃), 3.53 (bs, 4H, 2(-CH₂-(CH₂)₄-CH₃),
615 7.33 (bs, 4H, A+A', H₃+H₅), 7.59 (bs, 4H, A+A', H₂+H₆), 8.23 (bs, 2H,
616 2NH-CH₂), 9.88 (s, 2H, 2NH-C₆H₄); ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 14.4
617 (CH₃), 22.5 (CH₂), 26.5 (CH₂), 28.8 (CH₂), 31.4 (CH₂), 47.3 (CH₂), 124.8 (A+A',
618 C₃+C₅), 126.2 (A+A', C₁), 133.0 (A+A', C₂+C₆), 139.6 (A+A', C₄), 179.2 (C=Se);
619 MS (m/z % abundance): 368 (5), 191 (23), 69 (8), 57 (21), 43 (100); Anal.

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

622 **1,1'-(4,4'-Diselanediybis(4,1-phenylene))bis(3-ciclohexylselenourea)**

623 **(48)**. From cyclohexylisoselenocyanate **39**. Yellow powder. Yield: 21%; mp
624 175–180 °C; IR ν_{max} (KBr): 3398 (N–H), 2968–2931 (C–H), 1655 (C=Se) cm^{-1} ;
625 1H NMR (400 MHz, DMSO- d_6 , δ): 1.09–1.33 (m, 12H, B+B', 2H₃+2H₄+2H₅);
626 1.59–1.61 (m, 2H, B+B', H₁), 1.62–2.04 (m, 8H, B+B', 2H₂+2H₆), 7.18 (s, 4H,
627 A+A', H₂+H₆), 7.53 (s, 4H, A+A', H₃+H₅), 8.69 (s, 2H, 2NH–CH), 10.47 (s, 2H,
628 2MH–C₆H₄); ^{13}C NMR (100 MHz, DMSO- d_6 , δ) 12.2 (C_{cy}), 33.0 (C_{cy}), 53.7 (C_{cy}),
629 66.3 (C_{cy}), 115.0 (A+A', C₁), 133.5 (A+A', C₂+C₆), 138.2 (A+A', C₃+C₅), 142.1
630 (A+A', C₄), 182.8 (C=Se); MS (m/z % abundance): 368 (44), 191 (100), 163
631 (54), 135 (45), 84 (59), 56 (66), 41 (87); Anal. Calcd for $C_{26}H_{34}N_4Se_4 \cdot H_2O$ (%):
632 C, 42.4, H, 4.9, N, 7.6. Found: C, 42.3, H, 4.7, N, 7.7.

633 **Biological evaluation. (i) Cells and culture conditions.** *L. infantum*
634 axenic amastigotes were grown in M199 (Invitrogen, Leiden, The Netherlands)
635 medium supplemented with 10% heat inactivated FCS, 1 g/L β -alanine, 100
636 mg/L *L*-asparagine, 200 mg/L saccharose, 50 mg/L sodium pyruvate, 320 mg/L
637 malic acid, 40 mg/L fumaric acid, 70 mg/L succinic acid, 200 mg/L α -ketoglutaric
638 acid, 300 mg/L citric acid, 1.1 g/L sodium bicarbonate, 5 g/L MES, 0.4 mg/L
639 hemin, 10 mg/L gentamicin pH 5.4 at 37 °C. THP-1 cells were kindly provided by
640 Dr. Michel (Université Nice Sophia Antipolis, Nice, France) and were grown in
641 RPMI-1640 medium (Gibco, Leiden, The Netherlands) supplemented with 10%
642 heat inactivated FCS, antibiotics, 1 mM HEPES, 2 mM glutamine and 1mM
643 sodium pyruvate, pH 7.2 at 37 °C and 5% CO₂.

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 °C or 1×10^6 parasites/mL at 37 °C 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 °C and 5% CO₂ 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 26° 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

669 in a VERSAmax microplate reader (Molecular Devices, California, USA). All the
670 assays were conducted in triplicate in at least three independent experiments.
671 Data were analyzed using a non-linear regression model with the Grafit6
672 software (Erithacus, Horley, Surrey, UK).

673 RESULTS

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

683 To obtain the planned selenoureas, the synthesis of the corresponding
684 isoselenocyanates (**31–39**), that were prepared in two steps, was necessary
685 (Figure 3). The first step involved formylation of amines to yield formamides
686 **23–30** followed by the treatment with phosgene (**31–34**) (27) or triphosgene
687 (**35–39**) (28) and selenium powder in the presence of triethylamine under reflux.
688 Compounds were purified by silica gel column chromatography using *n*-
689 hexane/ethyl acetate as eluent. The IR spectra of the isoselenocyanates are
690 quite informative about the presence of the isoselenocyanate functional group
691 ($-\text{NCSe}$). The stretching frequency was observed at 2115–2224 cm^{-1} .

692 Formamides **23–30** were prepared through different methods depending
693 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, ^1H 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

719 compounds revealed selenoyl group band at lower values, ranging from 1542 to
720 1655 cm^{-1} .

721 In ^1H NMR spectra, the characteristic singlets for N-H protons located
722 between C=X and phenyl moieties are more shielded and appear at downfield
723 shifted as singlet in a relatively wide range of 8.20 to 10.47 ppm. The typical
724 differences for aliphatic amino groups were also noted. Thus, for example, in
725 case of ureas **8–10** the signals of NHCH_2 protons are observed between 6.17
726 and 6.60 as singlets or triplets. The aromatic rings provide their signals between
727 6.90 and 7.93 ppm.

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

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

744 derived from monocytes (THP-1). EC₅₀ values obtained are summarized in
745 Table 1. The selectivity index (SI) was defined as the ratio of the EC₅₀ 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 EC₅₀ values
750 lower than miltefosine (EC₅₀ = 2.84 μM) and one of them (**40**) being more
751 effective than the standard drug edelfosine (EC₅₀ = 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**, EC₅₀ = 3.1 μM and
756 compound **40**, EC₅₀ = 0.74 μM versus **12**, EC₅₀ = 11.23 μM or compound **10**,
757 EC₅₀ = 2.03 μM and **47**, EC₅₀ = 1.95 μM versus compound **21**, EC₅₀ = 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, EC₅₀ = 12.09 μM or compound **13**, 4-NO₂, EC₅₀ =
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 leishmanicidal activity in selenoureas, while in ureas and thioureas it is
771 responsible for a significant increase. With regards to the introduction of alkyl
772 side chains, this modification confers 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** EC₅₀ = 5.69 μM versus the
779 corresponding cyclic **22** EC₅₀ = 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''*-(diselanediy)benzene-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 ED₅₀ for each compound was calculated and summarized in
795 Table 2. These compounds reduced the parasite load of the cells, exhibiting
796 ED₅₀ values of 21.5, 3.4, 14.4 and > 25 μM respectively. Among them,
797 compound **11**, with ED₅₀ = 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 EC₅₀ values obtained are gathered in **Table 3**.

809 According to the results, compound **47** potentially inhibits TryR presenting
810 an EC₅₀ 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

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

825 **DISCUSSION**

826 The present report describes the synthesis of 31 new *N*-functionalized
827 urea, thiourea and selenourea derivatives from 4,4'-diaminodiphenyldiselenide
828 along with their *in vitro* antileishmanial activity against amastigote forms of *L.*
829 *infantum*. In order to explore the selectivity, THP-1 cells were used. Fifteen
830 derivatives exhibited EC₅₀ values < 3 μM, showing thirteen of them higher
831 activity than the reference drug miltefosine, in some cases by more than 3.8
832 times. Our results demonstrate that the incorporation of urea and selenourea
833 into the central scaffold improves the leishmanicidal activity, mainly with
834 aliphatic chains.

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

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

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

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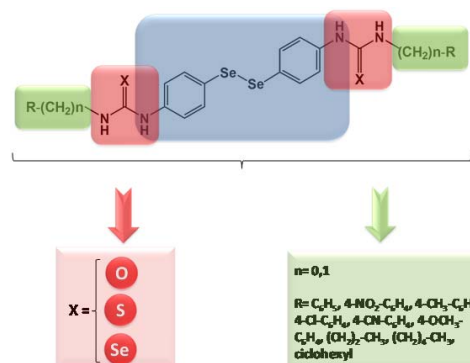
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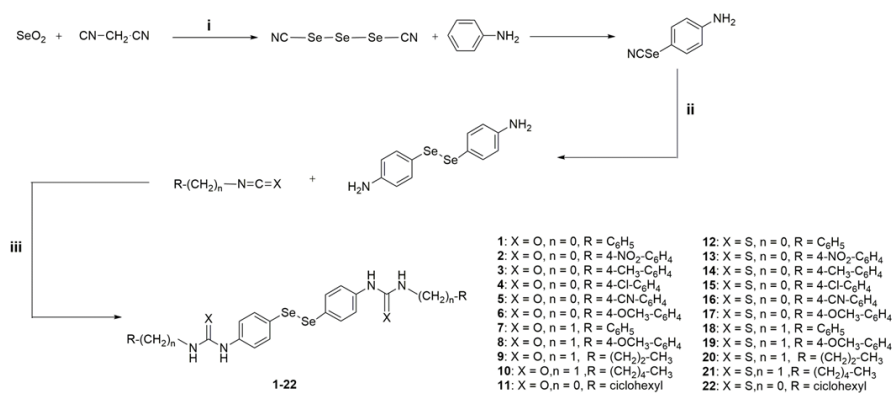
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Figure 1. Design and general structure for the proposed compounds.

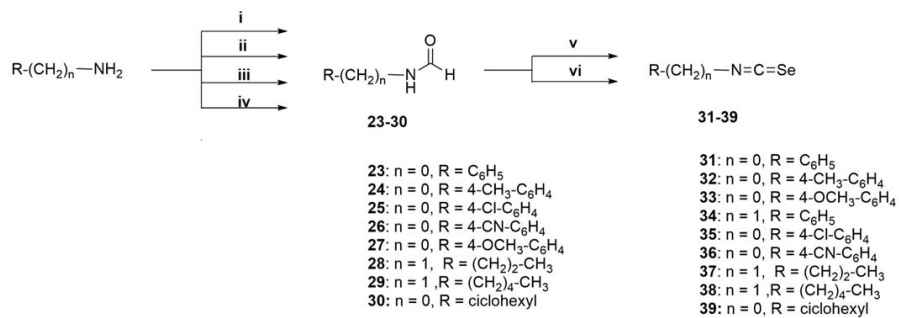


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1025 **Figure 2. General procedure of synthesis for compounds 1–22.** Reagents

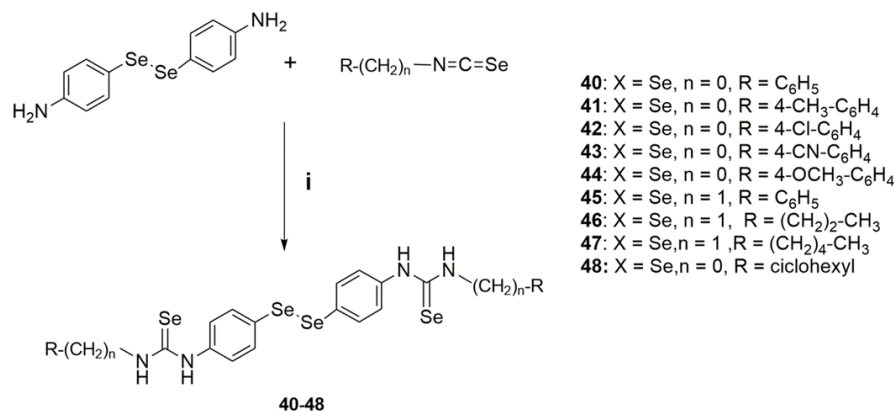
1026 and conditions: (i) DMSO, 15 min, r.t.; (ii) NaBH₄, absolute ethanol, 2 h, r.t, N₂;

1027 (iii) Dioxane (dry), 24–120 h, r.t, dark, N₂.



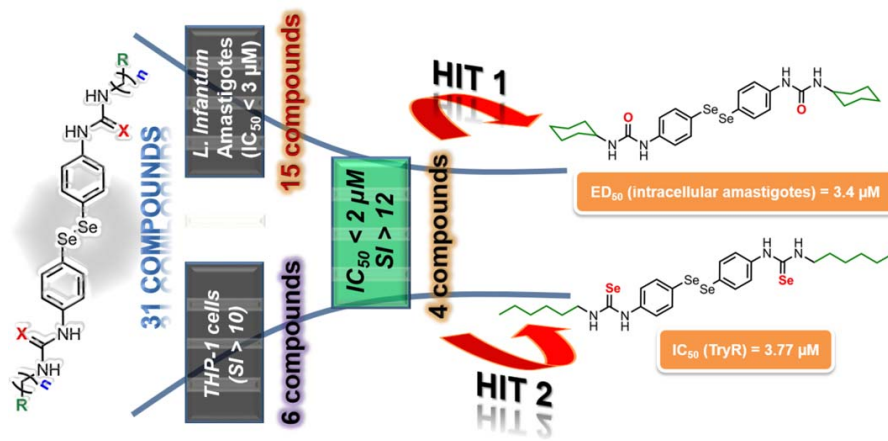
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1029 **Figure 3. General procedure of synthesis for compounds 23–39.** Reagents
 1030 and conditions: (i) HCOOC₂H₅, 12 h, reflux; (ii) HCOOH, Zn (10%), 12 h, 70 °C
 1031 (iii) HCOOH, PEG-400, r.t; (iv) HCO₂NH₄/ CH₃CN, 8–15 h, reflux; (v) Et₃N,
 1032 phosgene/toluene, 2.5 h, reflux, Se, 12 h reflux; (vi) Et₃N, triphosgene/DCM, 30
 1033 min, 0 °C, 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, N₂.



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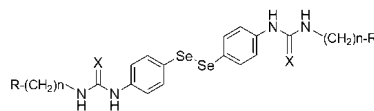
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Figure 5. Schematic illustration of conclusions.

1041 **Table 1.** EC₅₀ ± 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 ₆ H ₅	3.1 ± 0.25	11.1 ± 1.99	3.58
2	O	0	4-NO ₂ -C ₆ H ₄	4.1 ± 0.23	4.9 ± 0.18	1.2
3	O	0	4-CH ₃ -C ₆ H ₄	12.56 ± 0.77	> 25	>1.99
4	O	0	4-Cl-C ₆ H ₄	> 25	> 25	-
5	O	0	4-CN-C ₆ H ₄	2.74 ± 0.11	3.45 ± 0.2	1.26
6	O	0	4-OCH ₃ -C ₆ H ₄	5.75 ± 2.47	3.12 ± 0.2	0.54
7	O	1	C ₆ H ₅	1.54 ± 0.04	1.16 ± 0.43	0.75
8	O	1	4-OCH ₃ -C ₆ H 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 ₆ H ₅	11.23 ± 0.5	> 25	> 2.21
13	S	0	4-NO ₂ -C ₆ H ₄	17.7 ± 0.18	> 25	> 1.41
14	S	0	4-CH ₃ -C ₆ H ₄	4.55 ± 0.21	> 25	> 5.5
15	S	0	4-Cl-C ₆ H ₄	2.93 ± 0.09	> 25	> 8.52
16	S	0	4-CN-C ₆ H ₄	12.09 ± 0.57	> 25	> 2.07
17	S	0	4-OCH ₃ -C ₆ H ₄	3.2 ± 0.06	> 25	> 7.81
18	S	1	C ₆ H ₅	2.99 ± 0.06	> 25	> 9.05
19	S	1	4-OCH ₃ -C ₆ H 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 ₆ H ₅	0.74 ± 0.05	2.97 ± 0.04	4.01
41	Se	0	4-CH ₃ -C ₆ H ₄	1.12 ± 0.04	8.98 ± 0.28	8.02
42	Se	0	4-Cl-C ₆ H ₄	1.59 ± 0.28	19.84 ± 0.56	12.36
43	Se	0	4-CN-C ₆ H ₄	11.7 ± 1.01	19.46 ± 0.89	1.66
44	Se	0	4-OCH ₃ -C ₆ H ₄	2.41 ± 0.15	13.23 ± 0.21	5.49
45	Se	1	C ₆ H 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

1044 ^aSelectivity index (SI) is the ratio of EC₅₀ values of compounds against THP-1
1045 cells relative to those against *L. infantum* amastigotes.

1046

1047 **Table 2.** ED₅₀ ± 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

1049

1050 **Table 3.** EC₅₀ ± 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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