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Novel heteroaryl selenocyanates and diselenides as potent antileishmanial agents

Ylenia Baquedano[a,b,1], Verónica Alcolea[a,b,1], Miguel Ángel Toro[c], Killian Jesús Gutiérrez[c], Paul Nguewa[b], María Font[a,b], Esther Moreno[a,b], Socorro Espuelas[b], Antonio Jiménez-Ruiz[c], Juan Antonio Palop[a,b], Daniel Plano[a,b], Carmen Sanmartín[a,b,*]

[a]*Departamento de Química Orgánica y Farmacéutica,* [b]*Instituto de Salud Tropical, University of Navarra (ISTUN), Irunlarrea, 1. E-31008 Pamplona, Spain*

[c]*Departamento de Bioquímica y Biología Molecular, Universidad de Alcalá, Carretera Madrid-Barcelona km 33,600. E-28871, Alcalá de Henares, Madrid, Spain*

[1]These authors have contributed equally to this manuscript.

* 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

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Abstract

A serie of new selenocyanates and diselenides bearing interesting bioactive scaffolds (quinoline, quinoxaline, acridine, chromene, furane, isosazole…) were synthesized and their *in vitro* leishmanicidal activity against *L. infantum* amastigotes along with their cytotoxicity in human THP-1 cells were determined. Interestingly, most tested compounds were active in the low micromolar range and led us to identify four lead compounds (**1h**, **2d**, **2e** and **2f**) with ED_{50} values ranging from 0.45 to 1.27 µM and selectivity indexes > 25 for all of them, much higher than those observed for the reference drugs. These active derivatives were evaluated against infected macrophages and, in order to gain a preliminary knowledge about their possible mechanism of action, the inhibition of trypanothione reductase (TryR) was measured. Among these novel structures, compounds **1h** (3,5-dimethyl-4-isoxazolyl selenocyanate) and **2d** [3,3'-(diselenodiyldimethanediyl)bis(2-bromothiophene)] exhibited good association between TryR inhibitory activity and antileishmanial potency pointing **1h**, for its excellent theoretical ADME properties, as the most promising lead molecule for leishmancidal drug design.

Keywords: Diselenide, selenocyanate, leishmanicidal, trypanothione reductase

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Introduction

Leishmaniasis is an infectious poverty-associated disease caused by protozoan parasites of the genus *Leishmania*. In fact, this term includes a wide spectrum of vector-borne diseases with great epidemiological and clinical diversity. Even though exact statistical data are lacking (1, 2), within the 350 million people that live in endemic areas, approximately 12 million people get infected per year. There are three major clinical types: cutaneous (CL), mucocutaneous (MCL), and visceral leishmaniasis (VL; also known as kala-azar) which differ in their immunopathologies and degrees of morbidity and mortality (3). Among the different manifestations, VL is the most severe form with nearly 200,000 to 400,000 new cases, causing more than 20,000 deaths per year. Left untreated, it is usually fatal within two years.

The efficacy of the different drugs available seems to vary according to the *Leishmania* species and the current chemotherapy is far from being satisfactory. Furthermore, they present several problems, including toxicity, many adverse side-effects and high costs. The most relevant problem is related to the fact that many of these drugs were developed many years ago, and currently, there are resistant strains (4).

Since their discovery in the 1940s, the toxic pentavalent antimony [Sb(V)] compounds have been the mainstay of treatment against all forms of leishmaniasis through parenteral administration and their efficacy is progressively decreasing owing to the development of resistance (5). For this reason, in the last decades several drugs, such as amphotericin B and miltefosine (6),

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paromomycin and pentamidine (7), sitamaquine (8) and edelfosine (9), have been used in the treatment of leishmaniasis. Nevertheless, their high cost and therapeutic complications limit their use. Nowadays, several other drugs based on natural products have shown promising antileishmanial activity but, despite the significant progress, an ideal drug is still awaited (10).

The development of new antiparasitic drugs has not been much of a priority for the pharmaceutical industry because many of the parasitic diseases occur in poor countries where the populations cannot afford to pay a high price for the drugs. Thus, although important initiatives such as the Drugs for Neglected Diseases Initiative (DNDi) are attracting more interest in these neglected pathologies, an investment in drug development against parasitic diseases is needed.

The incorporation of different functionalities bearing the Se atom (i.e. methylseleno, selenocyanate, diselenide...) onto organic scaffolds can be considered a promising rational design to achieve potent and selective cytotoxic compounds (11). Several reports have shown a vast and miscellaneous types of structures applying this approach, resulting in very promising antitumoral compounds in pre-clinical models (12, 13). Recently, our research group has been using this rational design in order to obtain new derivatives with potent and selective antileishmanicidal activity. Continuing with these efforts, herein we have designed novel Se compounds which gather two different chemical entities: the selenium entity on its selenocyanate and diselenide forms; and different carbo- and hetero-cyclic entities with proven leishmanicidal activity. Below in this section, a brief description with several reported data that supports the selection for each of these sub-units can be found.

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During the last years, various reports have shown that an increase in plasma selenium levels has been recognized as a new defensive strategy against *Leishmania* infection (14, 15). The choice of the chemical form for the selenium derivatives can modulate the level of this element on the basis of several metabolic routes (16). The mechanism of action for selenium is unknown though some enzymatic pathways such as mitochondrial peroxiredoxins (17), selenophosphate synthetases (18) or ascorbate peroxidases (19) could be implicated. On the other hand, the incorporation of selenium into novel nanomaterials has demonstrated effectiveness in the treatment of leishmaniasis (20). We have reported (21-24) new selenium compounds with potent *in vitro* antiparasitic activity against *L. infantum* and *L. mayor*, and selectivity indexes higher than those observed for the reference drugs miltefosine, edelfosine or paromomicyn. Additionally, some of them induced nitric oxide production and alterations in gene expression profiling related to proliferation (PCNA), treatment resistance (ABC-transporter and α-tubulin) and virulence (QDPR) (23). Among the various antileishmanial scaffolds containing selenium earlier reported by us, selenocyanate and diselenide showed promising activity against *Leishmania* parasites (24).

We have payed special attention to quinoline, which constitutes the central nucleus of sitamaquine (25, 26), acridine (27, 28), quinoxaline (29-31) and coumarins (32, 33). On the other hand, nitrofuran compounds (34, 35), the most relevant registrated as nifurtimox, and derivatives of the benzodioxol core (36) have been selected. Besides, substituted five-membered heterocyclic rings such as isoxazol (37) and thiophenyl (38) or pirrol (39) have been tested as leishmanicidal agents. Finally, related to heterocycles derivatives, some fused

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aryl azo and triazo molecules have been described (34, 40). Furthermore, some carbocycles, such as adamantane ring (41) and anthraquinone structure (42, 43). Among the potential molecular targets for the treatment of leihshmaniasis, trypanothione reductase (TryR) is considered an ideal enzyme since it is involved in the unique thiol-based metabolism observed in the trypanosomatidae family and is a validated target for the search of antitrypanosomatidae drugs. TryR catalyzes the reduction of trypanothione disulfide to trypanothione (44). Therefore, during the last years a great number of inhibitors of this key enzyme have been reported (45-47). Based on the chemical analogy of sulphur and selenium, we decided to explore the relevance of this trace element to generate new TryR inhibitors.

In summary, and as a continuation of an ongoing program aiming to find new structural leaders with potential leishmanicidal activity, we have constructed a new class of selenoderivatives. They were designed by incorporating selenocyanate or diselenide moieties onto other bioactive carbo or heterocycles selected on the basis of the above mentioned findings. In this work, we present the synthesis of twenty-three new Se compounds **(Figure 1)** and their leishmanicidal activity against the amastigote form of *L. infantum*. In parallel, the cytotoxicity of these newly synthesized molecules was assessed. Moreover, leishmanicidal activity of the most active compounds was evaluated in *L. infantum*-infected macrophages. Finally, in order to elucidate a preliminary mechanism of action, their inhibitory activity against trypanothione reductase was determined.

MATERIALS AND METHODS

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Chemistry. Melting points (mp) 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™ spectrometer (Rheinstetten, Germany) using TMS as the internal standard. The IR spectra were obtained on a Thermo Nicolet FT-IR Nexus spectrophotometer with KBr pellets. Mass spectrometry was carried out on a MS-DIP, system MSD/DS 5973N (G2577A) Agilent. Elemental microanalyses were carried out on vacuum-dried samples using a LECO CHN-900 Elemental Analyzer. Silica gel 60 (0.040−0.063 mm) 1.09385.2500 (Merck KGaA, 64271 Darmstadt, Germany) was used for Column Chromatography and Alugram® SIL G/UV_{254} (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG. Postfach 101352, D-52313 Düren, Germany) was used for Thin Layer Chromatography. Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Madrid, Spain), Acros Organics (Janssen Pharmaceuticalaan 3a, 2440 Geel, België) and Lancaster (Bischheim-Strasbourg, France).

General procedure for the synthesis of compounds 1a−o. The synthesis of compounds **1a−o** was carried out according to the procedure described in the literature (48-50) with few modifications. Briefly, KSeCN (4 mmol) was added to a solution of the appropriate halyl derivative (4 mmol) in acetone (50 mL) and the mixture was heated under reflux for 2−4 h. The resulting precipitate (KBr) was filtered off. The filtrate was evaporated under vacuum and the residue was treated with water (2×50 mL) and dried. The target compounds were obtained in high purity.

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(Quinolin-8-yl)methyl selenocyanate (1a). From 8-bromomethylquinoline and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). Brown solid. Yield: 71.5%; mp: 49.5−50.5 ºC. IR (KBr) cm^{-1}: 2138 (s, C≡N); 1593 (f, C=N).^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.89 (s, 2H, CH_2-Se); 7.58−7.63 (m, 2H, H_6 + H_7); 7.88 (m, 1H, H_3); 7.97 (dd, 1H, H_5, J_{5-6} = 8.1 Hz, J_{5-7}=1.6 Hz); 8.44 (dd, 1H, H_4, J_{4-3} = 8.4 Hz, J_{4-2} = 2.2 Hz); 8.94 (dd, 1H, H_2, J_{2-3} = 4.3 Hz, J_{2-4} = 2.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.5 (CH_2-Se); 106.1 (CN); 123.6 (C_3); 127.3 (C_7); 129.5 (C_5, C_6); 131.7 (C_8); 136.0 (C_9); 138.1 (C_4); 146.9 (C_2); 151.4 (C_{10}). MS (*m/z*, % abundance): 222 (58); 158 (35); 142 (100); 130 (13); 115 (18); 89 (8); 63 (6). Elemental Analysis for $C_{11}H_8N_2Se$, Calcd/Found (%): C: 53.44/53.35; H: 3.33/3.49; N: 11.33/11.06.

(Quinolin-2-yl)methyl selenocyanate (1b). From 2-chloromethylquinoline hydrochloride and potassium selenocyanate. In first time, the quinoline hydrochloride was treated with an aqueous solution of NaOH (1N) in water/methanol (40:20) during 15 minutes in order to obtain 2-chloromethylquinoline. The white powder obtained was washed with water (4×25 mL) and dried. The compound was washed with ethyl ether (2×50 mL). Brown solid. Yield: 27.5%; mp: 83−84 ºC. IR (KBr) cm^{-1}: 2142 (m, C≡N); 1591 (m, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.73 (s, 4H, $2CH_2$); 7.62 (t, 2H, H_6 + H_7, J_{6-5} = J_{7-8} = 11.0 Hz); 7.79 (d, 1H, H_3, J_{3-4} = 9.4 Hz); 7.98 (dd, 2H, H_5 + H_8, J_{5-6} = J_{8-7} = 11.0 Hz, J_{5-7} = J_{8-6} = 9.4 Hz); 8.41 (d, 1H, H_4, J_{4-3} = 9.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 35.8 (CH_2-Se); 105.9 (CN); 122.6 (C_3); 127.3 (C_5, C_7); 129.0 (C_6, C_9); 131.7 (C_8); 138.2 (C_4); 147.8 (C_{10}); 158.0 (C_2). MS (*m/z*, % abundance): 248 (25); 142 (100); 115 (35); 89 (8); 63 (5); 51 (4). Elemental Analysis for $C_{11}H_8N_2Se$, Calcd/Found (%): C: 53.44/53.52; H: 3.33/3.62; N: 11.33/11.10.

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Acridin-9-ylmethyl selenocyanate (1c). From 9-(bromomethyl)acridine and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). Yellow solid. Yield: 92.3%; mp: 137−138 ºC. IR (KBr) cm^{-1}: 2145 (m, C≡N); 1625 (d, C=N).^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.47 (s, 2H, CH_2-Se); 7.69 (t, 2H, H_2 + H_7, $J_{2-1} = J_{7-8}$ = 9.3 Hz); 7.87 (t, 2H, H_3 + H_6, $J_{3-4} = J_{6-5}$ = 9.1 Hz); 8.19 (d, 2H, H_4 + H_5, $J_{4-3} = J_{5-6}$ = 9.1 Hz); 8.58 (d, 2H, H_1 + H_8, $J_{1-2} = J_{8-7}$ = 9.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 24.4 (CH_2-Se); 104.0 (CN); 124.9 (C_{12}, C_{14}); 125.3 (C_1, C_{11}); 127.0 (C_2, C_{10}); 130.7 (C_4, C_8); 132.2 (C_3, C_9); 142.1 (C_{13}); 149.4 (C_5, C_7). MS (*m/z*, % abundance): 204 (12); 193 (100); 177 (5); 165 (9); 87 (7); 63 (4). Elemental Analysis for $C_{15}H_{10}N_2Se$, Calcd/Found (%): C: 60.60/60.74; H: 3.36/3.36; N: 9.42/9.13.

Quinoxalin-2,3-diyldimethanediyl bisselenocyanate (1d). From 2,3-bis(bromomethyl)quinoxaline and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). Brown solid. Yield: 46.5%; mp: 153−154 ºC. IR (KBr) cm^{-1}: 2153 (s, C≡N); 1608 (m, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.91 (s, 4H, CH_2-Se); 7.87−7.89 (m, 2H, H_6 + H_7); 8.06−8.08 (m, 2H, H_5 + H_8). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 32.3 (CH_2-Se); 105.0 (CN); 129.9 (C_6, C_9); 132.2 (C_7, C_8); 141.3 (C_5, C_{10}); 151.0 (C_2, C_3). MS (*m/z*, % abundance): 262 (100); 235 (44); 156 (72); 129 (27); 102 (21); 76 (20). Elemental Analysis for $C_{12}H_8N_4Se_2$, Calcd/Found (%): C: 39.34/39.16; H: 2.18/2.17; N: 15.30/15.06.

(6,7-Dimethoxy-2-oxo-2*H*-chromen-4-yl)methyl selenocyanate (1e). From 6,7-dimethoxy-4-bromomethyl-2*H*-chromen-2-one and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). Yellow solid. Yield: 28.1%; mp: 197−199 ºC. IR (KBr) cm^{-1}: 2150 (m, C≡N); 1709 (s, C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.83 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 4.42 (s, 2H,

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CH_2-Se); 6.33 (s, 1H, CH-CO); 7.10 (s, 1H, H_5); 7.43 (s, 1H, H_8).^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.5 (CH_2-Se); 56.3 (OCH_3); 58.7 (OCH_3); 100.1 (C_9); 102.7 (CN); 107.8 (C_6); 110.0 (C_3); 114.6 (C_5); 147.2 (C_{10}); 150.3 (C_7); 152.2 (C_8); 154.1 (C_4); 161.5 (CO). MS (*m/z*, % abundance): 325 (59); 219 (73); 191 (100); 163 (12); 147 (25); 119 (7); 69 (8). Elemental Analysis for $C_{13}H_{11}NO_4Se$, Calcd/Found (%): C: 48.15/48.02; H: 3.40/3.40; N: 4.32/4.20.

(5-Nitrofuran-2-yl)methyl selenocyanate (1f). From 5-nitro-2-bromomethylfurane and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). Yellow solid. Yield: 42%; mp: 87-88 ºC. IR (KBr) cm^{-1}: 2152 (m, C≡N).^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.45 (s, 2H, CH_2-Se); 6.80 (d, 1H, H_3, $J_{3\text{-}4}$ = 7.8 Hz); 7.70 (d, 1H, H_4, $J_{4\text{-}3}$ = 7.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) 23.0 (CH_2-Se); 101.1 (CN); 109.4 (C_3); 111.2 (C_4); 150.0 (C_5); 156.3 (C_2). MS (*m/z*, % abundance): 126 (100); 113 (85). Elemental Analysis for $C_6H_4N_2O_3Se$, Calcd/Found (%): C: 31.17/31.28; H: 1.73/2.02; N: 12.12/11.72.

(6-Bromo-1,3-benzodioxol-5-yl)methyl selenocyanate (1g). From 5-bromo-6-(bromomethyl)-1,3-benzodioxole and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). White solid. Yield: 78.6%; mp: 110−111 ºC. IR (KBr) cm^{-1}: 2150 (s, C≡N); 1033 (s, C-Br).^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.36 (s, 2H, CH_2-Se); 6.10 (s, 2H, O-CH_2-O); 7.11 (s. 1H, H_6); 7.27 (s, 1H, H_3).^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.3 (CH_2-Se); 103.5 (O-CH_2-O); 105.9 (CN); 111.3 (C_6); 113.8 (C_4); 115.1 (C_3); 130.6 (C_5); 148.2 (C_2); 149.4 (C_1). MS (*m/z*, % abundance): 213 (100); 157 (7); 75 (19); 50 (15). Elemental Analysis for $C_9H_6BrNO_2Se$, Calcd/Found (%): C: 33.86/34.02; H: 1.88/2.05; N: 4.39/4.13.

3,5-Dimethyl-4-isoxazolyl selenocyanate (1h). From 4-chloromethyl-3,5-dimethylisoxazole and potassium selenocyanate. The brown oil obtained after

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washed with water was extracted with dichloromethane (3×50 mL). The organic layer was dried with Na_2SO_4. The dichloromethane was removed under vacuum and the residue was treated with ethyl ether (3×25 mL) and a clear brown powder was obtained. Yield: 21.3%; mp: 69−70 ºC. IR (KBr) cm^{-1}: 2143 (s, C≡N); 1628 (s, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.23 (s, 3H, CH_3-C_5); 2.40 (s, 3H, CH_3-C_3); 4.19 (s, 2H, CH_2-Se). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 10.6 (C_3-CH_3); 11.8 (C_5-CH_3); 21.4 (-CH_2-Se-); 105.9 (C_4); 112.3 (CN); 159.9 (C_3); 168.2 (C_5). MS (*m/z*, % abundance): 156 (4); 110 (100); 68 (89); 52 (5); 43 (45). Elemental Analysis for $C_7H_8N_2OSe$, Calcd/Found (%): C: 39.08/39.14; H: 3.75/3.88; N: 13.02/12.89.

(2-Bromothiophene-3-yl)methyl selenocyanate (1i). From 2-bromo-3-bromomethylthiophene and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). Brown solid. Yield: 88%; mp: 53−55 ºC. IR (KBr) cm^{-1}: 2148 (m, C≡N).^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.27 (s, 2H, CH_2-Se); 7.09 (d, 1H, H_2, $J_{1\text{-}2}$ = 5.7 Hz); 7.63 (d, 1H, H_1, $J_{2\text{-}1}$ = 5.7 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 26.5 (CH_2); 105.3 (CN); 112.5 (C_2); 128.4 (C_5); 129.7 (C_4); 138.3 (C_3). MS (*m/z*, % abundance): 281 ($M^{+\cdot}$, 3); 175 (100). Elemental Analysis for $C_6H_4BrNSSe$, Calcd/Found (%): C: 25.62/25.44; H: 1.42/1.38; N: 4.98/4.59.

(2-Chlorothiophene-5-yl)methyl selenocyanate (1j). From 5-bromo-2-chloro-methylthiophene and potassium selenocyanate. The brown oil obtained after washed with water was extracted with ethyl ether (3×50 mL). The organic layer was washed with water (3×50 mL) and dried with Na_2SO_4. The ethyl ether was removed under vacuum and a brown solid was obtained. Brown solid. Yield: 60%; mp: 37−39 ºC. IR (KBr) cm^{-1}: 2149 (m, C≡N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.52 (s, 2H, CH_2-Se); 6.98 (d, 1H, H_3, $J_{3\text{-}4}$ = 7.8 Hz); 7.00 (d, 1H, H_4, $J_{4\text{-}3}$ =

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7.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 28.6 (CH_2); 106.5 (CN); 127.5 (C_4); 128.4 (C_3); 129.3 (C_2); 141.4 (C_5). MS (*m/z*, % abundance): 131 (100); 95 (7); 87 (5); 69 (5); 45 (5). Elemental Analysis for $C_6H_4ClNSSe$, Calcd/Found (%): C: 30.44/30.04; H: 1.69/1.82; N: 5.92/5.80.

4-(1*H*-Pyrrol-1-yl)benzyl selenocyanate (1k). From 1-[4-(bromomethyl)phenyl]-1*H*-pyrrole and potassium selenocyanate. The solid obtained after the treatment with water was solved in THF and the insoluble fraction was rejected. The THF was removed under vacum and the residue was washed with water (3×25 mL) and with hexane (3×25 mL). Brown solid. Yield: 73.3%; mp: 154−155 ºC. IR (KBr) cm^{-1}: 2145 (m, C≡N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.35 (s, 2H, CH_2-Se); 6.27 (t, 2H, H_3 + H_4, J_{3-2} = J_{4-5} = 8.5 Hz); 7.38 (t, 2H, H_2 + H_5, J_{2-3} = J_{5-4} = 8.5 Hz); 7.45 (d, 2H, $H_{3'}$ + $H_{5'}$, $J_{3'-2'}$ = $J_{5'-6'}$ = 8.5 Hz); 7.58 (d, 2H, $H_{2'}$ + $H_{6'}$, $J_{2'-3'}$ = $J_{6'-5'}$ = 8.5 Hz. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 33.1(-CH_2-Se-); 105.8 (CN); 111.5 (C_3, C_4); 119.8 (C_2, C_5); 120.1 ($C_{2'}$, $C_{6'}$); 131.1 ($C_{3'}$, $C_{5'}$); 136.1 ($C_{4'}$); 140.2 ($C_{1'}$). MS (*m/z*, % abundance): 262 (M+1$^+$, 3); 156 (100); 128 (17); 89 (8); 78 (4). Elemental Analysis for $C_{12}H_{10}N_2Se$, Calcd/Found (%): C: 55.18/55.59; H: 3.86/4.01; N: 10.73/10.07.

1*H*-Benzotriazol-1-ylmethyl selenocyanate (1l). From 1-chloromethyl-1*H*-benzotriazole and potassium selenocyanate. The orange oil obtained after washed with water was extracted with dichloromethane (3×50 mL). The organic phase was washed with water (3×20 mL) and dried over Na_2SO_4. The dichloromethane was removed under vacuum and the residue was recrystallized from ethanol to give an orange solid. Yield: 19.7%; mp: 158−160 ºC. IR (KBr) cm^{-1}: 2151 (m, C≡N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.51 (s, 2H, CH_2-Se); 7.49 (t, 1H, H_2, J_{2-1} = 7.5 Hz, J_{2-3} = 7.6 Hz); 7.66 (t, 1H, H_3, J_{3-4} = 7.5 Hz, J_{3-2} = 7.6 Hz);

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8.02 (d, 1H, H_1, J_{1-2} = 7.5 Hz); 8.12 (d, 1H, H_4, J_{4-3} = 7.5 Hz).^{13}C NMR (100 MHz, DMSO-d_6) δ: 45.4 (CH_2); 105.3 (CN); 112.3 (C_8); 120.4 (C_5); 125.6 (C_6); 128.8 (C_7); 132.9 (C_9); 146.41 (C_4). MS (*m/z*, % abundance): 132 (62); 77 (100). Elemental Analysis for $C_8H_6N_4Se$, Calcd/Found (%): C: 40.51/40.47; H: 2.53/2.75; N: 23.63/23.95.

2-Adamant-1-yl-2-oxoethyl selenocyanate (1m). From 1-adamant-1-yl-2-bromoethanone and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). Yellow solid. Yield: 77%; mp: 108−110 ºC. IR (KBr) cm^{-1}: 2149 (s, C≡N); 1678 (s, C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.63−1.67 (m, 6H, C$\underline{H}_2$-CH); 1.80−1.81 (m, 6H, C$\underline{H}_2$-C-CO); 1.99 (s, 3H, CH); 4.63 (s, 2H, C$\underline{H}_2$-Se). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.1 (CH_2-Se); 29.0 (C$\underline{H}$); 36.7 (C$\underline{H}_2$-CH); 38.4 (C$\underline{H}_2$-C-CO); 47.1 (C-CO); 104.5 (CN); 210.2 (C=O). MS (*m/z*, % abundance): 163 (4); 135 (100); 107 (8); 93 (17); 79 (18); 67 (7). Elemental Analysis for $C_{13}H_{17}NOSe$, Calcd/Found (%): C: 55.31/55.38; H: 6.03/6.19; N: 4.96/4.70.

(9,10-Dioxo-9,10-dihydroanthracen-2-yl)methyl selenocyanate (1n). From 2-chloromethylanthraquinone and potassium selenocyanate. The compound was washed with ethyl ether (2×50 mL). Yellow solid. Yield: 75.3%; mp: 168−169 ºC. IR (KBr) cm^{-1}: 2145 (m, C≡N); 1674 (s, C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.51 (s, 2H, CH_2-Se); 7.90 (dd, 1H, H_3, J_{3-4} = 8.1 Hz, J_{3-1} = 2.5 Hz); 7.93−7.96 (m, 2H, H_1 + H_4); 8.22−8.25 (m, 4H, H_5 + H_6 + H_7 + H_8).^{13}C NMR (100 MHz, DMSO-d_6) δ: 33.3 (CH_2-Se); 106.5 (CN); 128.5 (C_1, C_4, C_8, C_{11}); 133.2 (C_3); 134.1 (C_9, C_{10}); 135.0 (C_5, C_7, C_{12}, C_{14}); 146.9 (C_2); 183.4 (CO). MS (*m/z*, % abundance): 256 (9); 221 (100); 207 (4); 165 (22); 139 (5); 76 (4); 63 (4). Elemental Analysis for $C_{16}H_9NO_2Se$, Calcd/Found (%): C: 58.89/58.74; H: 2.76/2.86; N: 4.29/4.25.

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(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl selenocyanate (1o). From *N*-bromomethylphthalimide and potassium selenocyanate. The oil obtained after washed with water was extracted with dichloromethane (3×50 mL). The organic phase was washed with water (3×20 mL) and dried over Na_2SO_4. The dichloromethane was removed under vacuum and the residue was recrystallized from ethanol to give a white-pink solid. Yield: 50.4%; mp: 162−164 ºC. IR (KBr) cm^{-1}: 2155 (m, C≡N); 1778 (CO); 1718 (CO).^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.28 (s, 2H, CH_2); 7.89−7.98 (m, 4H, H_3 + H_4 + H_5 + H_6).^{13}C NMR (100 MHz, DMSO-d_6) δ: 35.0 (CH_2); 105.2 (CN); 124.5 (C_4); 132.2 (C_3); 136.0 (C_5); 166.8 (C_2). MS (*m/z*, % abundance): 160 (100); 104 (22). Elemental Analysis for $C_{10}H_6N_2O_2Se$, Calcd/Found (%): C: 45.29/44.93; H: 2.26/2.49; N: 10.57/10.37.

General procedure for the synthesis of compounds 2a−g. The appropriate selenocyanate derivative (3 mmol) was solved in absolute ethanol (40 mL) and $NaBH_4$ (6.2 mmol) was added in small portions with caution to the solution. For the obtention of compound **2g**, $NaBH_3CN$ (6.2 mmol) was used. The mixture was stirred at room temperature for 2 h. The solvents were removed under vacuum by rotary evaporation and the residue was treated with water (50 mL) and purified in order to obtain the target compounds.

8,8'-(Diselenodiyldimethanediyl)diquinoline (2a). From (quinolin-8-yl)methyl selenocyanate (**1a**) and sodium borohydride. The resultant solid was washed with ethyl ether (3×50 mL) and recystallized from ethanol to give a yellow solid. Yield: 60.3%; mp: 103−104 ºC. IR (KBr) cm^{-1}: 1591 (s, C=C); 790 (s, Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.58 (s, 4H, 2 CH_2-Se); 7.43−7.48 (m, 4H, H_6 + H_7, $H_{6'}$ + $H_{7'}$); 7.57 (dd, 2H, H_3, $H_{3'}$, J_{3-4} = 8.1 Hz, J_{3-2} = 4.6 Hz); 7.89 (dd, 2H, H_5, $H_{5'}$, J_{5-6} = 8.1 Hz, J_{5-7} = 2.5 Hz); 8.38 (dd, 2H, H_4, $H_{4'}$, J_{4-3} = 8.1 Hz, J_{4-2} = 2.5 Hz); 8.97

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(dd, 2H, H_2, $H_{2'}$, J_{2-3}= 4.6 Hz, J_{2-4} = 2.5 Hz).^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.2 (CH_2-Se); 122.5 (C_3, $C_{3'}$); 127.1 (C_7, $C_{7'}$); 128.0 (C_5, C_6, $C_{5'}$, $C_{6'}$); 130.7 (C_8, $C_{8'}$); 137.9 (C_9, $C_{9'}$); 138.4 (C_4, $C_{4'}$); 146.2 (C_2, $C_{2'}$); 150.1 (C_{10}, $C_{10'}$). MS (*m/z*, % abundance): 442 ($M^{+\cdot}$, 5); 222 (75); 142 (100); 130 (10); 115 (17); 89 (7); 63 (5). Elemental Analysis for $C_{20}H_{16}N_2Se_2$, Calcd/Found (%): C: 54.30/54.80; H: 3.62/4.00; N: 6.33/6.10.

9,9'-(Diselenodiyldimethanediyl)diacridine (2b). From acridin-9-ylmethyl selenocyanate (**1c**) and sodium borohydride. The resultant solid was washed with ethyl ether (3×50 mL) and recystallized from ethanol to give an orange solid. Yield: 45%; mp: 108−109 ºC. IR (KBr) cm^{-1}: 1552 (s, C=C); 752 (s, Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.22 (s, 4H, CH_2-Se, $CH_{2'}$-Se); 7.63 (t, 4H, H_2 + H_7, J_{2-3} = J_{7-6} = 9.0 Hz); 7.84 (t, 4H, H_3 + H_6, J_{3-2} = J_{6-7} = 9.0 Hz); 8.14 (d, 4H, H_4 + H_5, J_{4-3} = J_{5-6} = 9.0 Hz); 8.41 (d, 4H, H_1 + H_8, J_{1-2} = J_{8-7} = 9.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 26.3 (CH_2-Se); 123.1 (C_{12}, C_{14}, $C_{12'}$, $C_{14'}$); 125.0 (C_1, C_{11}, $C_{1'}$, $C_{11'}$); 128.6 (C_2, C_{10}, $C_{2'}$, $C_{10'}$); 130.0 (C_4, C_8, $C_{4'}$, $C_{8'}$); 133.4 (C_3, C_9, $C_{3'}$, $C_{9'}$); 141.8 (C_{13}, $C_{13'}$); 149.9 (C_5, C_7, $C_{5'}$, $C_{7'}$). MS (*m/z*, % abundance): 204 (100); 165 (47); 63 (6). Elemental Analysis for $C_{28}H_{20}N_2Se_2$, Calcd/Found (%): C: 61.99/61.62; H: 3.69/4.03; N: 5.16/5.12.

5,5'-(Diselenodiyldimethanediyl)bis(6-bromo-1,3-benzodioxole) (2c). From (6-bromo-1,3-benzodioxol-5-yl)methyl selenocyanate (**1g**) and sodium borohydride. The resultant solid was washed with ethyl ether (3×50 mL) and recystallized from ethanol to give a yellow solid. Yield: 70.3%; mp: 90−91 ºC. IR (KBr) cm^{-1}: 766 (s, Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.03 (s, 4H, CH_2-Se, $CH_{2'}$-Se); 6.00 (s, 4H, O-CH_2-O, O-$CH_{2'}$-O); 6.77 (s, 2H, H_6, $H_{6'}$); 7.01 (s, 2H, H_3, $H_{3'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 33.3 (CH_2-Se); 103.1 (O-CH_2-O); 111.6

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(C_6, $C_{6'}$); 113.7 ($C_{4'}$, $C_{4'}$); 115.2 (C_3, $C_{3'}$); 132.9 (C_5, $C_{5'}$); 147.0 (C_2, $C_{2'}$); 148.8 (C_1, $C_{1'}$). MS (*m/z*, % abundance): 213 (100); 157 (9); 135 (9); 75 (10); 50 (5). Elemental Analysis for $C_{16}H_{12}Br_2O_4Se_2$, Calcd/Found (%): C: 32.76/32.99; H: 2.04/2.02.

3,3'-(Diselenodiyldimethanediyl)bis(2-bromothiophene) (2d). From (2-bromothiophene-3-yl)methyl selenocyanate (**1i**) and sodium borohydride. The mixture was extracted with ethyl ether (3×50 mL). The organic phase was washed with water (3×50 mL) and dried with anhydrous Na_2SO_4. The ethyl ether was removed under vacuum and a yellow powder was obtained. Yield: 29%; mp: 49−50 ºC. IR (KBr) cm^{-1}: 3099 (w, C-H); 722 (s, Se-Se). ^1H NMR (400 MHz, DMSO-d_6) δ: 4.04 (s, 4H, CH_2-Se, $CH_{2'}$-Se); 7.00 (d, 2H, H_2 + $H_{2'}$, J_{1-2} = 5.6 Hz); 7.58 (d, 2H, H_1 + $H_{1'}$, J_{2-1} = 5.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.5 (CH_2-Se); 111.1 (C_2, $C_{2'}$); 128.4 ($C_{4'}$, $C_{4'}$); 130.6 (C_5, $C_{5'}$); 140.0 (C_3, $C_{3'}$). MS (*m/z*, % abundance): 510 ($M^{+\cdot}$, 3); 175 (100); 96 (19); 69 (9); 45 (8). Elemental Analysis for $C_{10}H_8Br_2S_2Se_2$, Calcd/Found (%): C: 23.55/23.49; H: 1.58/1.52.

1,1'-(Diselenodiyldimethanediyl)bis(1*H*-benzotriazole) (2e). From 1*H*-benzotriazol-1-ylmethyl selenocyanate (**1l**) and sodium borohydride. The mixture was extracted with dichloromethane (3×50 mL). The organic phase was washed with water (3×50 mL) and dried with anhydrous Na_2SO_4. The dichloromethane was removed under vacuum and a white powder was obtained. Yield: 25%; mp: 197−199 ºC. IR (KBr) cm^{-1}: 744 (s, Se-Se). ^1H NMR (400 MHz, DMSO-d_6) δ: 6.19 (s, 4H, N-CH_2-Se, N-$CH_{2'}$-Se); 7.44 (t, 2H, H_3 + $H_{3'}$, J_{3-2} = 8.2 Hz, J_{3-4} = 8.2 Hz); 7.57 (t, 2H, H_2 + $H_{2'}$, J_{2-1} = 8.2 Hz, J_{2-3} = 8.2 Hz); 7.87 (d, 2H, H_4 + $H_{4'}$, J_{3-4} = 8.2 Hz); 8.07 (d, 2H, H_1 + $H_{1'}$, J_{1-2} = 8.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 43.6 (CH_2-Se); 112.2 (C_8, C_{14}, $C_{12'}$, $C_{14'}$); 120.2 (C_5,); 125.3 (C_6); 128.3 (C_7); 133.0

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399 (C_9); 146 (C_4). MS (*m/z*, % abundance): 132 (86); 77 (100). Elemental Analysis
400 for $C_{14}H_{12}N_6Se_2 \cdot H_2O$, Calcd/Found (%): C: 38.80/38.40; H: 2.77/2.74; N:
401 19.61/19.39.
402 **2,2'-(Diselenodiyldimethanediyl)di(9,10-dihydroanthracene-9,10-dione) (2f).**
403 From (9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl selenocyanate (**1n**) and
404 sodium borohydride. The resultant solid was washed with ethyl ether (3×50 mL)
405 and recystallized from ethanol to give a yellow solid. Yield: 52%; mp: 186−187
406 ºC. IR (KBr) cm^{-1}: 1666 (CO); 710 (m, Se-Se). ^1H NMR (400 MHz, DMSO-d_6) δ:
407 4.21 (s, 4H, CH_2-Se, $CH_{2'}$-Se); 7.70 (d, 2H, H_3, $H_{3'}$, $J_{3\text{-}4}$ = 8.1 Hz); 7.86−7.89 (m,
408 4H, H_5 + H_8, $H_{5'}$ + $H_{8'}$); 7.95 (s, 2H, H_1, $H_{1'}$); 8.06 (d, 2H, H_4, $H_{4'}$, $J_{4\text{-}3}$ = 8.1 Hz);
409 8.09−8.11 (m, 4H, H_6 + H_7, $H_{6'}$ + $H_{7'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 31.4
410 (CH_2-Se); 127.6 (C_1, C_4, C_8, C_{11}, $C_{1'}$, $C_{4'}$, $C_{8'}$, $C_{11'}$); 133.2 (C_3, $C_{3'}$); 134.1 (C_9, C_{10},
411 $C_{9'}$, $C_{10'}$); 135.3 (C_5, C_7, C_{12}, C_{14}, $C_{5'}$, $C_{7'}$, $C_{12'}$, $C_{14'}$); 148.0 (C_2, $C_{2'}$); 183.2 (CO).
412 MS (*m/z*, % abundance): 177 (100); 149 (13); 96 (22); 69 (17); 51 (8). Elemental
413 Analysis for $C_{30}H_{18}O_4Se_2 \cdot ½H_2O$, Calcd/Found (%): C: 59.11/59.12; H: 3.12/3.44.
414 **2,2'-(Diselenodiyldimethanediyl)bis(1*H*-isoindole-1,3(2*H*)-dione) (2g).** From
415 (1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl selenocyanate and sodium
416 cyaneborohydride. A white solid was obtained. Yield: 28%; mp: 162−164 ºC. IR
417 (KBr) cm^{-1}: 1774 and 1715 (vs, C=O); 719 (m, Se-Se). ^1H NMR (400 MHz,
418 DMSO-d_6) δ: 5.07 (s, 4H, N-CH_2-Se, N-$CH_{2'}$-Se); 7.86 (s, 8H, H_3 + $H_{3'}$ + H_4 + $H_{4'}$
419 + H_5 + $H_{5'}$ + H_6 + $H_{6'}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.0 (CH_2-Se); 124.2
420 (C_4); 132.3 (C_3); 135.6 (C_5); 167.3 (CO). MS (*m/z*, % abundance): 478 ($M^{+\cdot}$, 2);
421 160 (100). Elemental Analysis for $C_{18}H_{12}N_2O_4Se_2$, Calcd/Found (%): C:
422 45.19/45.25; H: 2.51/2.70; N: 5.86/5.64.

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2,2'-(Selenodiyldimethanediyl)bis(1*H*-isoindole-1,3(2*H*)-dione) (3). From (1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl selenocyanate (**1o**) (0.75 mmol) and sodium borohydride (0.75 mmol). Yield: 47%; mp: 230−232ºC. IR (KBr) cm^{-1}: 1772 and 1718 (vs, C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.13 (s, 4H, N-CH_2-Se, N-$CH_{2'}$-Se); 7.87−7.89 (m, 8H, H_3 + $H_{3'}$ + H_4 + $H_{4'}$ + H_5 + $H_{5'}$ + H_6 + $H_{6'}$).^{13}C NMR (100 MHz, DMSO-d_6) δ: 31.5 (CH_2-Se); 124.2 (C_4); 132.5 (C_3); 135.6 (C_5); 167.8 (CO). MS (*m/z*, % abundance): 400 ($M+1^+$, 2); 160 (100). Elemental Analysis for $C_{18}H_{12}N_2O_4Se$, Calcd/Found (%): C: 54.13/54.13; H: 3.00/2.79; N: 7.02/7.07.

Biological evaluation. (i) Cells and culture conditions. *L. infantum* promastigotes (MCAN/ES/ 89/IPZ229/1/89) were grown in RPMI-1640 medium (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS), antibiotics, and 25 mm HEPES (pH 7.2) at 26°C.

L. infantum axenic amastigotes were obtained by incubation of $4.5x10^6$ late logarithmic promastigotes in 5 mL of M199 medium (Invitrogen, Leiden, The Netherlands) supplemented with 10% heat-inactivated FCS, 1 gL^{-1} β-alanine, 100 mgL^{-1} L-asparagine, 200 mgL^{-1} sucrose, 50 mgL^{-1} sodium pyruvate, 320 mgL^{-1} malic acid, 40 mgL^{-1} fumaric acid, 70 mgL^{-1} succinic acid, 200 mgL^{-1} α ketoglutaric acid, 300 mgL^{-1} citric acid, 1.1 gL^{-1} sodium bicarbonate, 5 gL^{-1} MES, 0.4 mgL^{-1} hemin, and 10 mgL^{-1} gentamicine, pH 5.4 at 37 °C. After 48 hours of incubation, all parasites had a rounded morphology without flagellum and divided during several weeks under the described conditions.

THP-1 cells 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.

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(ii) Leishmanicidal activity and cytotoxicity assays. Drug treatment of amastigotes was performed during the logarithmic growth phase at a concentration of 2×10^6 parasites/mL at 26 ºC or 1×10^6 parasites/mL at 37 ºC for 24 h, respectively. Drug treatment of THP-1 cells was performed during the logarithmic growth phase at a concentration of 4×10^5 cells/mL at 37 ºC and 5% CO_2 for 24 h. The percentage of living cells was evaluated by flow cytometry by the propidium iodide (PI) exclusion method (51). Drug concentrations ranged from 0.2 µM to 25 µM.

(iii) Leishmania infection assay. Human THP-1 monocytic cells were seeded at 1.2 x 10^5 cells/mL in 24 multidishes plates (Nunc, Roskilde, Denmark) and differentiated to macrophages for 24 h 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 1.2×10^6 *Leishmania* amastigotes in 1 mL of THP-1 medium were added to each well. 4 h later all medium with non-infective 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 h 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 quantization was measured by flow cytometry. Drug concentrations ranged from 0.2 µM to 25 µM.

(iv) Trypanothione reductase assay. Oxidoreductase activity was determined according to the method described by Toro *et al.* (52). Briefly, reactions were carried out at 26ºC 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%

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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-lineal regression model with the Grafit6 software (Erithacus, Horley, Surrey, UK).

Drug-likeness parameters. The drug-likeness and drug score values along with the TPSA values and the properties described in the Lipinski's Rule of Five [molecular weight (MW) ≤500 Da, log P ≤ 5, H-bond donors (HBD) ≤5 and H-bond acceptors (HBA) ≤10] were calculated using the online available Osiris (53) and Molinspiration property calculation programs (54), respectively. Topological polar surface area was used to calculate the percentage of absorption (%ABS) according to the equation: %ABS = 109 − [0.345 × TPSA] (55).

RESULTS

Chemistry. The synthetic approaches adopted to obtain the target compounds are depicted in Figure 2. The selenocyanate derivatives (compounds **1a−o**) were obtained in variable yields (27–92%) by reaction between the commercially available haloalkyl carbo or heterocyclic reactives with potassium selenocyanate in a molar ratio 1.1 in acetone under reflux during 3−4 h (24). The subsequent reduction of compounds **1a−o** with sodium borohydride or sodium cyanoborohydride in ethanol afforded derivatives **2a−f** and **2g**, respectively (24) in yields ranging from 25 to 70%. Unfortunately, for some selenocyanates (**1a**, **1d**, **1e**, **1f**, **1h**, **1i**, **1k** and **1m**) several difficulties were found and this procedure failed to afford the expected products. This prompted us to seek alternative routes to prepare the corresponding diselenides. Surprisingly, modification of the

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reaction conditions (temperature, solvents or molar ratio) resulted in decomposition of the starting materials by rupture of the bond between heterocycle and methylene. This hypothesis was confirmed by the disappearance of the signal corresponding to the methylene group in ^{1}H NMR. Additionally, an undesired mixture of side compounds was identified in TLC. The alternative strategy employing 100% hydrazine hydrate and sodium hydroxide in DMF to reduce elemental selenium and generate sodium diselenide followed by reaction with the corresponding haloalkyl reactives (56) did not allow the synthesis of the corresponding diselenides. These results can be explained by the greater steric hindrance so as the quick degradation of the starting materials in the reduction process.

Finally, and contrary to our expectations, the reduction of **1o** with $NaBH_4$ in ethanol yielded compound **3**, an unexpected compound instead of the corresponding diselenide that was obtained by reaction with $NaBH_3CN$.

All of the compounds prepared during the course of these investigations are stable and their purity was assessed by TLC and elemental analyses and their structures were identified from spectroscopic data. IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elementary analysis methods were used for structure elucidation **(Figure 2)**.

The IR spectra of compounds **1a−o** illustrate sharp peaks around 2138−2155 cm^{-1} due to CN group. Derivatives **2a−g** showed multiple bands in the range 710−790 cm^{-1} attributable to the Se-Se group and lacked the CN band, confirming the reduction. In NMR all the signals were fully consistent with proposed structures. Copies of the registered ^{1}H- and ^{13}C-NMR spectra for the selected compounds (**1h**, **2d** and **2e**) can be found as supplementary material.

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Biological evaluation. (i) Activity in amastigotes and cytotoxic activity in human cells. Compounds **1a−o**, **2a−g** and **3** were tested for their antiprotozoal activity against the pathogenic *Leishmania infantum* amastigotes using miltefosine and edelfosine as standard drugs according to a previously described procedure (57). Although most of the studies on the *in vitro* biological activity of new compounds against *Leishmania spp*. are performed on promastigote forms, this assay must be considered as preliminary because this stage of parasite is significantly more susceptible to drug-induced effects than the amastigote form. Moreover, promastigotes are not the developed forms of the parasite in vertebrate hosts so the evaluations made with them are merely indicative of the potential leishmanicidal activity of the compounds tested. Accordingly, because amastigotes are responsible for all clinical manifestations in humans, the intracellular amastigote model has been cited as the golden standard for *in vitro Leishmania* drug discovery research. Taking this into account, all the analyses were carried out in the amastigote form with a minimum of three independent experiments and the results are expressed as ED_{50} values. In addition, for a compound to be a candidate for antileishmanial drug, it is required both high leishmanicidal activity and low cytotoxicity. Cytotoxicity on THP-1 cell line was evaluated for all compounds in order to identify drugs with low toxicity in human cells and as a prelude to selecting drugs for *in vitro* assay on the relevant clinical *Leishmania* amastigote stage. The selectivity index (SI) of the compounds is expressed by the ratio between cytotoxicity (ED_{50} value on THP-1 cells) and activity (ED_{50} value on *L. infantum* amastigotes).

Table 1 shows the ED_{50} values obtained after 24 h of exposure to the compounds in *L. infantum* axenic amastigotes. Values for the reference drugs

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548 miltefosine and edelfosine are included in all cases for comparison. Biological
549 data evidenced that most of the screened compounds (fifteen out of twenty-three)
550 showed high bioactivity (ED_{50} ≤ 2.53 μM) against *L. infantum*, being more potent
551 than miltefosine (ED_{50} = 2.84 μM). In addition, under our experimental conditions
552 seven compounds (**1d**, **1e**, **1h**, **1m**, **1n**, **2e** and **2f**) displayed comparable or
553 higher *in vitro* potency than edelfosine (ED_{50} = 0.82 μM).
554 Different authors have claimed that compounds having SI values greater than 20
555 can be considered ideal candidates for further development as leishmanicidal
556 drugs (58). However, in this study and with rigorous criteria, we have considered
557 the SI threshold > 25 to further analyse their activity in amastigote-infected THP-
558 1 cells. This requirement is satisfied by compounds **1h**, **2d**, **2e** and **2f** considering
559 them as the lead ones in this series due to their excellent biological behaviour.
560 **(ii) Leishmanicidal activity in infected macrophages.** According to their
561 activity and selectivity, four compounds (**1h**, **2d**, **2e** and **2f**) were advanced for
562 testing leishmanicidal activity in amastigote-infected THP-1 cells. Compound **2f**
563 (ED50 = 0.68 μM, SI = 36.8) was not further tested due to the reproducibility
564 issues showed by this derivative pertaining to its lack of solubility in the assay
565 conditions. The ED_{50} values for the other selected derivatives (**1h**, **2d** and **2e**)
566 were calculated and summarized in **Table 2**. The potency of the analogues was
567 compared with edelfosine, a current antileishmanial agent (ED_{50} = 3.1 ± 0.1 μM).
568 These compounds reduced the parasite load of the cells exhibiting ED_{50} values
569 of 23.2, 14.0 and 14.4 μM respectively, the members of diselenide family being
570 the most potent compounds.
571 **(iii) Inhibition of *L. infantum* trypanothione reductase activity.** In an attempt
572 to investigate a possible mechanism of action, the ability to inhibit the

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trypanothione reductase activity for the most active compounds were first screened at six different concentrations between 0.1 and 75 μM. Mepacrine, a well known TryR inhibitor, was used as positive control (59) and DMSO as vehicle. The IC_{50} values obtained are gathered in **Table 3**.

The compounds **1h** and **2d** were able to inhibit TryR with IC_{50} values of 0.46 and 6.85 μM, respectively. It is remarkably, that derivative **1h** was 37-fold more active than the positive control. **1h** and **2d** exhibited good association between TryR inhibitory activity and antileishmanial potency (intracellular forms of the parasite). The results for **2e**, that did not show inhibitory activity, suggest an alternative mechanism of action for its potent leishmanicidal activity. Compound **1h**, as well as compound **2d**, can be considered as promising antileishmanial lead candidates because they show a strong inhibitory activity against axenic amastigotes (IC_{50} values of 0.73 and 1.27 μM), excellent SI (29.9 and 25.8) and a marked inhibitory activity against TryR.

Drug-likeness properties. Employing the Molinspiration (54) and Osiris (53) software the selected compounds (**1h**, **2d** and **2e**) were subjected to the Lipinski's rule of five analyses (drug-likeness), which helps to predict and explain the biological behavior of small molecules. This preliminary analysis allows prediction of the physicochemical properties related to their absorption and bioavailability. We have found that the derivatives **1h** and **2e** show no violations of Lipinski's Rule of Five **(Table 4)**. Among leishmanicidal drugs available on the market, only miltefosine does not violate Lipinski's Rule of Five; all other drugs have, at least, 1 violation (edelfosine). It has been well established that optimal lipophilicity range along with low logP (< 5) and low topological polar surface area (TPSA) are the major driving forces that lead to good absorption, including

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intestinal absorption, bioavailability, Caco-2 permeability, and blood-brain barrier penetration. Molecules with TPSA < 140 $Å^2$ are indicative of excellent bioavailability (60). According to the theoretical study carried out by De Toledo *et al.* (61), the TPSA of most leishmanicidal drugs currently on the market is higher than this limit, which probably restricts their absorption and bioavailability. The logP and TPSA values for compounds **1h**, **2d** and **2e** range from 0.75 to 4.68 and 0 to 61.44 respectively, suggesting that these compounds are potentially able to cross cell membranes in a permeation process, which could explain the ability to reach the amastigotes inside the phagolysosome.

The drug score uses and relates other molecular parameters, such as drug-likeness, logP, molecular weight, and toxicity risks, being considered a convenient value that may be used to judge the overall potential of a compound to become a drug. A value of 0.5 or more is indicative of a promising lead for future development of a safe and efficient drug (62). Compound **1h** possess the maximum drug-score value (0.5) for the selected compounds whereas edelfosine presented a drug score value 0.3.

DISCUSSION

There are many available antileishmanial agents, but the drug of choice is still awaited by several limitations of current drugs such as high cost, poor compliance, drug resistance, low efficacy and poor safety. The high prevalence and severity of this illness justify the urgency for the discovery of new drugs. In the last two decades, several “interesting drug targets” have been proposed including many proteins and enzymes that differ from mammalian counterpart which can interfere with the redox system. Among the promising targets that scientific community considers for the design of useful therapies, enzymes

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(trypanothione reductase, proteinases, superoxide dismutase, dihydrofolate reductase, metacaspase, topoisomerase, kinases, sirtuins …) are one of the most representatives. In this context, we notice that selenium plays an important role in medicinal chemistry particularly as antioxidant, antitumoral, chemopreventive or antiparasitic agents. Thus, we have described here the synthesis and leishmanicidal activity of novel selenocyanate and diselenide compounds.

For the novel selenoderivatives presented in this work it seems to exist a tendency suggesting that analogues with the diselenide unit were more active than those with the selenocyanate moiety (**1a** vs **2a**, **1c** vs **2b**, **1g** vs **2c**, **1i** vs **2d**, **1l** vs **2e** and **1o** vs **2g**) against *L. infantum* amastigotes. Regarding the selectivity index, the addition of the diselenide scaffold clearly improved the selectivity, for example in compounds **1g**/**2c**, **1i**/**2d**, **1l**/**2e, 1n**/**2f**. In general, it was observed that tricyclic nitrogenated rings such as acridine (**1c** and **2b**) are detrimental to the biological activity and selectivity compared with bicyclic nitrogenated rings (**1b**, **1d**, **1l**, **1o**, **2e** and **2g**). Furthermore, no regular order of decrease or increase in the activity among the rest derivatives can be concluded.

Taking into account the results related to activity and selectivity and considering our exigent criteria for both parameters (ED_{50} < 2.5 μM and SI > 25), four derivatives, one selenocyanate, **1h**, and two diselenides (**2d** and **2e**) were selected for further studies. Despite compound **2f** fullfiled these criteria, it could not be tested due to solubility problems. Then, when we performed intracellular form tests these derivatives did not improve the activity compared to edelfosine. However, their lack of toxicity against THP-1 cells **(Table 1)** represents a remarkable advantage over the reference drug.

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We hypothesized that TryR inhibition may be related with the leishmanicidal activity observed for selenoderivatives. The results for *in vitro* assays revealed that compounds **1h** and **2d** exhibited a good correlation between leishmanicidal activity and TryR inhibition confirming our previous proposal. On the other hand, **2e** did not show inhibitory activity, suggesting that not only this enzyme is involved in its potent leishmanicidal activity, but also other mechanisms can be implicated.

Finally, *in silico* prediction studies were performed in order to determine the drug-like properties for the lead compounds. Considering these properties, derivatives **1h** and **2e** showed to meet the Lipinski's Rule of Five, indicating favorable properties for drug development. The *in silico* toxicity profile, drug-likeness, and drug-score (0.5) data for compound **1h** make it a promising leader for future development of safer and more efficient leishmanicidal drugs.

In conclusion, the present study reports the synthesis of new selenocyanates and diselenides bearing interesting bioactive scaffolds (quinoline, quinoxaline, acridine, chromene, furane, isosazole...) and their *in vitro* leishmanicidal activity against *L. infantum* amastigotes along with their cytotoxicity in THP-1 cells. Fifteen of such compounds exhibited better potency against axenic amastigotes than the standard drug miltefosine. Based on its antiparasitic activity and low toxicity in THP-1 cells, compounds **1h**, **2d**, **2e** and **2f** were identified as the best candidates for further studies in infected macrophages. Although their potency against intracellular amastigotes is lower than that observed for the reference drug, these compounds combined a potent leishmanicidal activity with excellent selectivity index (>25) resulting in promising therapeutic utility. In order to get further insight into their putative mechanism of action, their activity against *L.*

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infantum TryR was determined. A clear correlation between enzyme inhibition and antiparasitic activity was observed for compounds **1h** and **2d**, which may be considered as an evidence for one of their many possible mechanisms of action. No correlation was detected for **2e,** which suggest the existence of different targets in this family of compounds. The ADME parameters calculated for derivatives **1h** and **2e** predict a good bioavailability. *In silico* ADME profiling and drug score results along with *in vitro* leishmanicidal, cytotoxicity and TryR inhibitory activity make **1h** a promising lead compound for the development of more potent antiparasitary drugs. A graphical resume of the conclusions drawn from this work is depicted in **Figure 3**.

Moreover, to the best of our knowledge, this is in itself the first study that reports new selenoderivatives as leishmanicidal and TryR inhibitors and opens new possibilities in the field of neglected diseases.

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Figure 1. General structure of new pharmacophoric Se compounds.

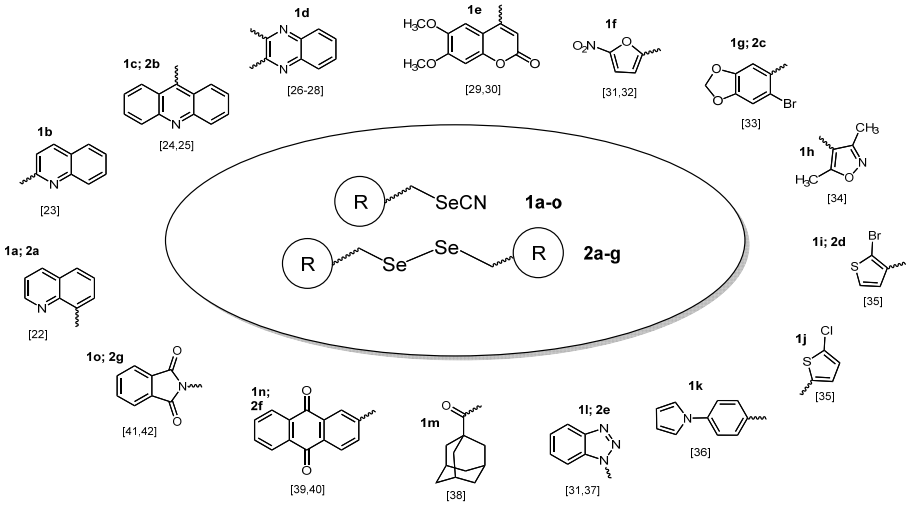

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Figure 2. Synthesis of compounds **1a−o**, **2a−g** and **3**.

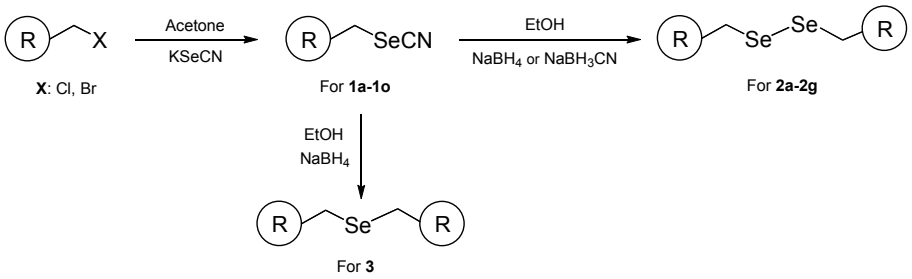
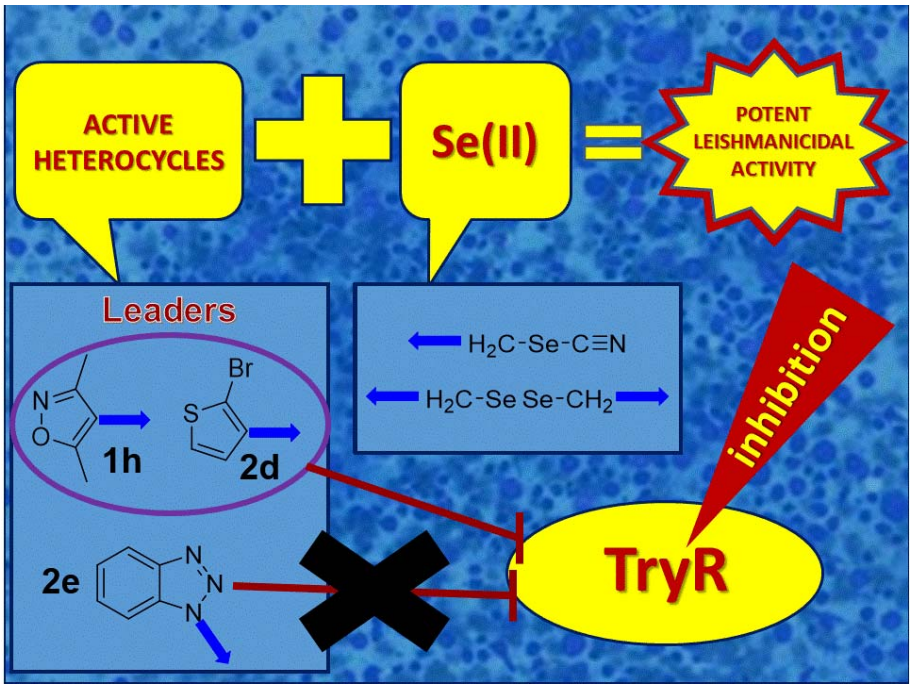


Figure 3. Schematic ilustration of conclusions.



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

Compound	R	Amastigote	THP-1	SI[a]
1a	Quinol-8-yl	4.49 ± 0.21	14.48 ± 0.37	3.2
1b	Quinol-2-yl	1.76 ± 0.04	14.91± 0.92	8.5
1c	Acridin-9-yl	7.40 ± 0.60	15.01 ± 0.68	2.0
1d	Quinoxalin-2,3-diylmethanediyl	0.69 ± 0.06	13.83 ± 1.59	20.0
1e	6,7-Dimethoxy-2-oxo-2*H*-chromen-4-yl	0.82 ± 0.07	15.95 ± 1.54	19.4
1f	5-Nitrofur-2-yl	1.99 ± 0.23	3.95 ± 0.29	2.0
1g	6-Bromo-1,3-benzodioxol-5-yl	10.10 ± 1.81	15.26 ± 1.19	1.5
1h	3,5-Dimethylisoxazol-4-yl	0.73 ± 0.10	21.82 ± 2.40	29.9
1i	2-Bromothien-3-yl	2.86 ± 0.29	19.54 ± 0.52	6.8
1j	5-Chlorothien-2-yl	1.85 ± 0.33	21.02 ± 0.52	11.4
1k	*N*-Phenylpyrrol-4-yl	8.87 ± 1.32	23.74 ± 0.48	2.7
1l	Benzotriazol-1-yl	1.11 ± 0.21	22.00 ± 1.20	19.8
1m	2-Adamant-1-yl-2-oxoethyl	0.83 ± 0.03	19.68 ± 1.98	23.7
1n	9,10-Dioxo-9,10-dihydroanthracen-2-yl	0.74 ± 0.18	6.05 ± 1.19	8.2
1o	Phthalimidyl	2.53 ± 0.32	22.50 ± 1.63	8. 9
2a	Quinol-8-yl	2.05 ± 0.24	8.60 ± 1.10	5.6
2b	Acridin-9-yl	5.46 ± 0.01	3.76 ± 0.07	< 1
2c	6-Bromo-1,3-benzodioxol-5-yl	3.99 ± 0.62	>25	>6.3
2d	2-Bromothien-3-yl	1.20 ± 0.03	30.9 ± 0.02	25.8

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2e	Benzotriazol-1-yl	0.45 ± 0.03	> 25	> 55.5
2f	9,10-Dioxo-9,10-dihydroanthracen-2-yl	0.68 ± 0.30	>25	36.8
2g	Phthalimidyl	1.35 ± 0.17	9.26 ± 0.31	6.8
3	Phthalimidyl	> 25	> 25	-
Edelfosine	-------	0.82 ± 0.13	4.96 ± 0.16	6.0
Miltefosine	-------	2.84 ± 0.10	18.50 ± 0.60	7.0

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

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

Compound	ED_{50}
1h	23.2 ± 4.3
2d	14.0 ± 2.1
2e	14.4 ± 2.6
Edelfosine	3.1 ± 0.1

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

Compound	IC_{50}
1h	0.46 ± 0.01
2d	6.85 ± 0.49
2e	> 75
Mepacrine	16.99 ± 1.18

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

Compd.	Molinspiration calculations							Osiris calculations	
	MW	miLogP	TPSA	nON	nOHNH	NV	VOL	Drug-likeness	Drug score
1h	215.1	1.2	49.8	3	0	0	151.7	-6.8	0.5
2d	510.0	5.8	0.00	0	0	2	253.2	-7.5	0.1
2e	422.2	3.6	61.4	6	0	0	278.1	-8.0	0.1
Edelfosine	523.7	0.7	77.1	7	0	1	550.9	-58.2	0.3

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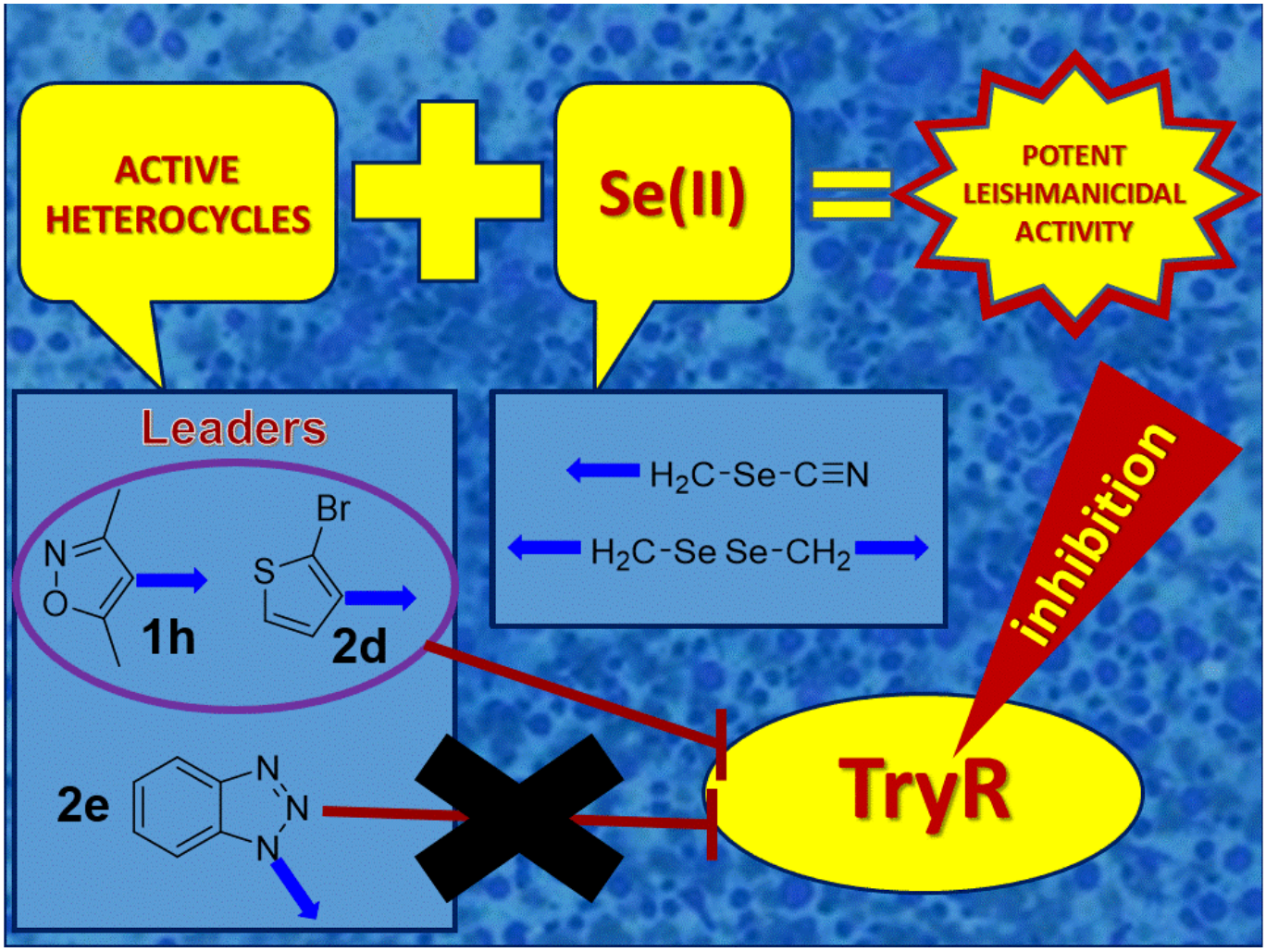

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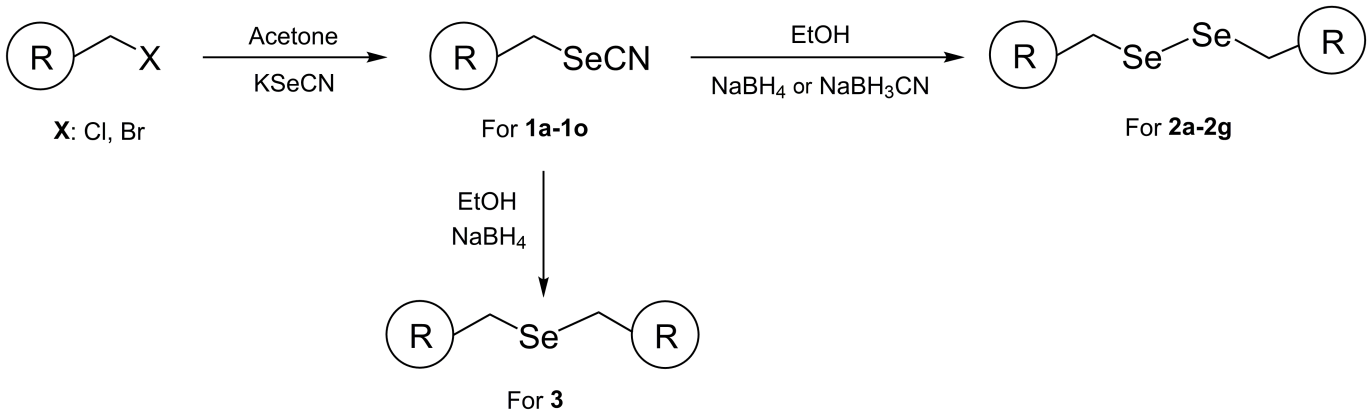

X: Cl, Br

For **1a-1o**

For **2a-2g**

For **3**

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