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 areas where leishmaniasis is endemic, 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 2 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), 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 show promising antileishmanial activity, but despite 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, and diselenide) onto organic scaffolds can be considered a promising rational design to achieve potent and selective cytotoxic compounds (11). Several reports have shown vast and miscellaneous types of structures applying this approach, resulting in very promising antitumoral compounds in preclinical models (12, 13). Recently, our research group has been using this rational design in order to obtain new derivatives with potent and selective antileishmanial 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 heteroaryl selenocyanates and diselenides as potent antileishmanial agents. 

Most promising lead molecule for leishmanicidal drug design.
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 *Leishmania infantum* and *Leishmania major*, and selectivity indexes higher than those observed for the reference drugs miltefosine, edelfosine, and paromomycin. Additionally, some of them induced nitric oxide production and alterations in gene expression profiling related to proliferation (PCNA), treatment resistance (ABC-transporter and *H*9251-/-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 registered as nifurtimox, and derivatives of the benzodioxol core (36) have been selected. In addition, substituted five-membered heterocyclic rings such as isoxazol (37) and thiophenyl (38) or pirrol (39) have been tested as leishmanicidal agents. Furthermore, related to heterocycles derivatives, some fused aryl azo and triazo molecules have been described (34, 40). Finally, some carbocycles, such as ones with an adamantane ring (41) or anthraquinone structure (42, 43), have been described.

Among the potential molecular targets for the treatment of leishmaniasis, 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 in the search for drugs against members of this family. TryR catalyzes the reduction of trypanothione disulfide to trypanothione (44). Therefore, during recent years a great number of inhibitors of this key enzyme have been reported (45–47). Based on the chemical analogy of sulfur and selenium, we decided to explore the relevance of this trace element to generate new TryR inhibitors.

In summary, as a continuation of an ongoing program aiming to find new structural leaders with potential leishmanicidal activity, we have constructed a new class of seleno derivatives. 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 23 new Se compounds (Fig. 1) and their leishmanicidal activities against the amastigote form of *L. infantum*. In parallel, the cytotoxicities of these newly synthesized molecules were assessed. Moreover, the leishmanicidal activities of the most active compounds were evaluated in *L. infantum*-infected macrophages. Finally, in order to elucidate a preliminary mechanism of action, their inhibitory activities against trypanothione reductase were determined.

**MATERIALS AND METHODS**

**Chemistry.** Melting points (mp) were determined with a Mettler FP82+FP80 apparatus (Greifensee, Switzerland) and are not corrected. The 1H nuclear magnetic resonance (NMR) and 13C NMR spectra were recorded on a Bruker 400 Ultrashield spectrometer (Rheinstetten, Germany) using TMS (tetramethylsilane) as the internal standard. The infrared (IR) spectra were obtained on a Thermo Nicolet FT-IR Nexus spec-
trophotroph with KBr pellets. Mass spectrometry was carried out on an ICP-MS (inductively coupled plasma mass spectrometer), Agilent system MSD/DS 5973N (G2577A). Elemental microanalyses were carried out on vacuum-dried samples using a LECO CHN-900 elemental analyzer. Silica gel 60 (0.040 to 0.063 mm) 1.09385.2500 (Merck KGaA, Darmstadt, Germany) was used for column chromatography, and Alugram SIL G/VUV34 (layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was used for thin-layer chromatography (TLC). Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Alcobendas, Madrid, Spain), Acros Organics (Janssen Pharmaceuticalca Jaa, Geel, Belgium) and Lancaster (Bischheim-Strasbourg, France).

General procedure for the synthesis of compounds 1a to 1o. The synthesis of compounds 1a to 1o was carried out according to the procedure described in the literature (48–50), with a few modifications. Briefly, KsCN (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 to 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 with a high degree of purity.

**Quinolin-8-yl)methyl selenocyanate (1a).** Compound 1a was from 8-bromomethylquinoline and potassium selenocyanate. The compound was washed with ethyl ether (2 × 50 ml). Brown solid. Yield: 71.5%. mp: 49.5 to 50.5°C. IR (KBr) cm⁻¹: 2,138 (s, C≡N); 1,593 (f, C=C).1H NMR (400 MHz, DMSO-d₆): 6.89 (s, 2H, CH₂-Se); 7.58 to 7.63 (m, 2H, H₂ + H₃); 7.88 (m, 1H, H₇); 7.97 (dd, 1H, H₂, J = 8.1 Hz, I = 1.6 Hz); 8.44 (dd, 1H, J₅,J₆ = 8.4 Hz, J₄,₅ = 2.2 Hz); 8.94 (dd, 1H, H₂, J = 4.3 Hz, J₄,₅ = 2.2 Hz).13C NMR (100 MHz, DMSO-d₆): 6.95 (CH₂-Se); 106.1 (CN); 113.8 (C₄); 115.1 (C₃); 130.6 (C₈); 136.0 (C₉); 142.1 (C₅); 149.4 (C₆). MS (m/z [percent abundance]): 204 (12); 193 (58); 126 (100); 75 (19); 50 (15). Elemental analysis for C₁₁H₈N₂Se, calculated/found (percent): C, 53.44/53.52; H, 3.33/3.49; N, 11.33/11.06.

**Quinolin-2,3-diylmethanediyl bisselenocyanate (1d).** Compound 1d was from 2-chloromethylquinoline and potassium selenocyanate. The compound was washed with ethyl ether (2 × 50 ml). Yellow solid. Yield: 28.1%. mp: 197 to 199°C. IR (KBr) cm⁻¹: 2,150 (m, C≡N); 1,709 (s, C=O).1H NMR (400 MHz, DMSO-d₆): 6.33 (s, 3H, OCH₃); 7.37 to 7.42 (m, 3H, H₃ + H₂ + H₁); 7.70 (d, 1H, H₄, J = 7.8 Hz); 7.70 (d, 1H, H₄, J = 7.8 Hz).13C NMR (100 MHz, DMSO-d₆): 29.5 (CH₂-Se); 106.1 (CN); 129.9 (C₆); 132.2 (C₅, C₆); 141.3 (C₈, C₉); 151.0 (C₇, C₈). MS (m/z [abundance]): 262 (100); 235 (44); 156 (72); 129 (72); 76 (20). Elemental analysis for C₁₂H₁₀N₄O₂Se, calculated/found (percent): C, 39.34/39.16; H, 2.18/2.17; N, 15.30/15.06.

**6,7-Dimethoxy-2-oxo-2H-chromen-4-yl)methyl selenocyanate (1c).** Compound 1c was from 6,7-dimethoxy-4-bromomethyl-2H-chromen-2-one and potassium selenocyanate. The compound was washed with ethyl ether (2 × 50 ml). Yellow solid. Yield: 28.1%. mp: 197 to 199°C. IR (KBr) cm⁻¹: 2,150 (m, C≡N); 1,709 (s, C=O).1H NMR (400 MHz, DMSO-d₆): 6.33 (s, 3H, OCH₃); 7.37 to 7.42 (m, 3H, H₃ + H₂ + H₁); 7.70 (d, 1H, H₄, J = 7.8 Hz); 7.70 (d, 1H, H₄, J = 7.8 Hz).13C NMR (100 MHz, DMSO-d₆): 29.5 (CH₂-Se); 106.1 (CN); 129.9 (C₆); 132.2 (C₅, C₆); 141.3 (C₈, C₉); 151.0 (C₇, C₈). MS (m/z [abundance]): 262 (100); 235 (44); 156 (72); 129 (72); 76 (20). Elemental analysis for C₁₂H₁₀N₄O₂Se, calculated/found (percent): C, 39.34/39.16; H, 2.18/2.17; N, 15.30/15.06.
analysis for C\textsubscript{16}H\textsubscript{12}BrN\textsubscript{3}Se, calculated/found (percent); C, 25.62/25.44; H, 1.42/1.38; N, 4.98/4.59.

(2-Chlorothiophen-5-yl)methyl selenocyanate (1j). Compound 1j was from 5-bromo-2-chlorothiophenol and potassium selenocyanate.

The brown oil obtained after washing with water was extracted with ethyl ether (3 × 50 ml). The organic layer was washed with water (3 × 50 ml) and dried with Na\textsubscript{2}SO\textsubscript{4}. The ethyl ether was removed under vacuum, and the brown solid was obtained. Yield: 91.0%. mp: 154 to 156°C. IR (KBr) cm\textsuperscript{-1}: 3427, 2953, 2846, 1698, 1578, 1480, 1460, 1380, 1236, 977, 764. 1\textsuperscript{H} NMR (400 MHz, DMSO-\text{d}\textsubscript{6}) \&: 7.61 (t, 1H, H1, J\textsubscript{1,2} = 7.6 Hz); 7.66 (t, 1H, H2, J\textsubscript{2,3} = 7.6 Hz); 8.02 (d, 1H, H3, J\textsubscript{3,4} = 7.6 Hz); 8.12 (d, 1H, H4, J\textsubscript{4,5} = 7.5 Hz). 1\textsuperscript{3}C NMR (100 MHz, DMSO-\text{d}\textsubscript{6}) \&: 105.3 (CN); 112.3 (C8); 119.8 (C4); 120.1 (C2); 121.5 (C7); 136.1 (C4); 147.0 (C6); MS (m/z [abundance]): 292 (100); 258 (7); 234 (5); 190 (5); 166 (4); 142 (4); 118 (4); 94 (3); 70 (3); 56 (2); 42 (2); 30 (2); 18 (2); 15 (1); 13 (1); 11 (1); 9 (1); 7 (1). Elemental analysis for C\textsubscript{16}H\textsubscript{12}BrN\textsubscript{3}Se, calculated/found (percent); C, 30.36/30.46; H, 1.23/1.23; N, 4.16/4.16.

(3,4-Dimethoxyphenyl)dimethyl selenocyanate (1k). Compound 1k was from 1-[(4-(bromomethyl)phenyl)-1H-pyrrole with potassium selenocyanate.

The brown oil obtained after washing with water was extracted with dichloroethane. The resultant solid was washed with ethyl ether (3 × 20 ml) and dried over Na\textsubscript{2}SO\textsubscript{4}. The dichloroethane was removed under vacuum, and the residue was recrystallized from ethanol to give an orange solid. Yield: 99.0%. mp: 154 to 156°C. IR (KBr) cm\textsuperscript{-1}: 3434, 2965, 2856, 1693, 1578, 1460, 1380, 1236, 969, 752. 1\textsuperscript{H} NMR (400 MHz, DMSO-\text{d}\textsubscript{6}) \&: 11.09 (s, C6-Se); 114.9 (C3); 127.5 (C4); 136.1 (C9, C10); 146.9 (C4); 183.4 (CO); MS (m/z [abundance]): 178 (4); 164 (2); 150 (2); 136 (2); 122 (2); 108 (2); 94 (2); 80 (1); 66 (1); 52 (1); 38 (1); 24 (1); 10 (1); 8 (1). Elemental analysis for C\textsubscript{16}H\textsubscript{12}BrN\textsubscript{3}Se, calculated/found (percent); C, 30.93/30.95; H, 1.22/1.22; N, 4.16/4.16.

General procedure for the synthesis of compounds 2a to 2g. The appropriate selenocyanate derivative (3 mmol) was added in absolute ethanol (20 ml), and Na\textsubscript{2}BH\textsubscript{4} (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'-Diselenodimethylidenedi)diquinoline (2a). Compound 2a was obtained from (quinolin-8-yl)methyl selenocyanate (1a) and sodium borohydride. The resultant solid was washed with ethyl ether (3 × 50 ml) and recrystallized from ethanol to give an orange solid. Yield: 60.3%. mp: 103 to 104°C. IR (KBr) cm\textsuperscript{-1}: 3424 (C\textsubscript{6}H\textsubscript{4}C\textsubscript{6}H\textsubscript{4})-Hz; 1553 (C\textsubscript{6}H\textsubscript{4}C\textsubscript{6}H\textsubscript{4}). 1\textsuperscript{H} NMR (400 MHz, DMSO-\text{d}\textsubscript{6}) \&: 4.58 (s, 4H, CH\textsubscript{2}-CH\textsubscript{2}); 7.43 to 7.48 (m, 4H, H1, H2, H3, H4). 1\textsuperscript{3}C NMR (100 MHz, DMSO-\text{d}\textsubscript{6}) \&: 42.1 (CH\textsubscript{2}); 129.8 (C1); 128.8 (C2); 132.9 (C3); 146.1 (C4); MS (m/z [abundance]): 160 (100); 128 (7), 106 (6), 94 (5), 82 (4), 70 (3), 58 (2), 46 (2), 34 (2), 22 (2), 10 (2), 8 (2), 6 (2). Elemental analysis for C\textsubscript{16}H\textsubscript{12}N\textsubscript{2}Se\textsubscript{2}, calculated/found (percent); C, 47.30/47.30; H, 2.50/2.50; N, 6.30/6.30.
Compound 3 was from (1,3-dioxo-1,3-dihydro-2H-imidazol-4-yl)methyl selenocyanate (1o) (0.75 mmol) and sodium borohydride. A white solid was obtained after washing with water (3 x 50 ml) and dried with anhydrous Na2SO4. The yellow powder was removed under vacuum, and a yellow white powder was obtained. Yield: 29%. mp: 49 to 50°C. IR (KBr) cm⁻¹: 3,099 (w, C-H); 7.22 (s, Se-Sc). 1H NMR (400 MHz, DMSO-d₆) δ: 4.04 (s, 4H, CH₂-Se, CH₂-Se); 7.00 (d, 2H, H₃, H₄ = 5.6 Hz); 7.58 (2H, H₃, H₄ = 5.6 Hz). 13C NMR (100 MHz, DMSO-d₆) δ: 26.5 (CH-Se); 111.1 (C₆, C₇); 128.4 (C₈, C₉); 130.6 (C₆, C₇); 140.0 (C₅, C₆). MS (m/z [percent abundance]): 510 (M⁺, 3); 175 (100); 199 (69); 45 (8). Elemental analysis for C₂₂H₂Br₂N₂O₂Se₄ calculated (found, percent): C, 38.06/38.40; H, 2.77/2.74; N, 19.61/19.39.

2H,1H-benzotriazolo[1,1-c][1,2-dione] (2f). Compound 2f was from (2-bromothiophene-3-yl)methyl selenocyanate (1i) (0.75 mmol) and sodium borohydride. A yellow powder was obtained. Yield: 29%. mp: 49 to 50°C. IR (KBr) cm⁻¹: 3,099 (w, C-H); 7.22 (s, Se-Se). 1H NMR (400 MHz, DMSO-d₆) δ: 6.19 (s, 4H, N-CH₃-Se, N-CH₃-Se); 7.44 (t, 2H, H₃, H₄ = 8.2 Hz); 7.86-7.89 (m, 4H, H₃, H₄, H₅, H₆). 13C NMR (100 MHz, DMSO-d₆) δ: 31.4 (CH₂-Se); 127.6 (C₆, C₇, C₈, C₉, C₁₀, C₁₁); 133.2 (C₁₂, C₁₃); 134.1 (C₁₄, C₁₅, C₁₆, C₁₇); 153.5 (C₁₈, C₁₉, C₂₀, C₂₁); 148.0 (C₂₂, C₂₃); 183.2 (CO). MS (m/z [percent abundance]): 317 (100); 149 (13); 96 (22); 69 (17); 51 (8). Elemental analysis for C₃₀H₂₂Br₂N₂O₂Se₄ calculated (found, percent): C, 38.00/38.40; H, 2.77/2.74; N, 19.61/19.39.

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3.4-(Diselenodiyldimethanediyl)bis[2-bromophene] (2d). Compound 2d was from (2-bromophene-3-yl)methyl selenocyanate (11) and sodium borohydride. The mixture was extracted with ethyl ether (3 x 50 ml). The organic phase was washed with water (3 x 50 ml) and dried with anhydrous Na₂SO₄. The yellow powder was obtained. Yield: 29%. mp: 49 to 50°C. IR (KBr) cm⁻¹: 3,099 (w, C-H); 722 (s, Se-Se). 1H NMR (400 MHz, DMSO-d₆) δ: 4.04 (s, 4H, CH₂-Se, CH₂-Se); 7.00 (d, 2H, H₃, H₄ = 5.6 Hz); 7.58 (2H, H₃, H₄ = 5.6 Hz). 13C NMR (100 MHz, DMSO-d₆) δ: 26.5 (CH-Se); 111.1 (C₆, C₇); 128.4 (C₈, C₉); 130.6 (C₆, C₇); 140.0 (C₅, C₆). MS (m/z [percent abundance]): 510 (M⁺, 3); 175 (100); 199 (69); 45 (8). Elemental analysis for C₁₀H₈Br₂S₂Se₂ calculated (found, percent): C, 38.06/38.40; H, 1.58/1.52.

1.1-(Diselenodiyldimethanediyl)bis[1H-benzotriazole] (2e). Compound 2e was from 1H-benzotriazol-1-ylmethyl selenocyanate (11) and sodium borohydride. The mixture was extracted with dichloromethane (3 x 50 ml). The organic phase was washed with water (3 x 50 ml) and dried with anhydrous Na₂SO₄. The dichloromethane was removed under vacuum, and a white powder was obtained. Yield: 25%. mp: 197 to 199°C. IR (KBr) cm⁻¹: 744 (s, Se-Se). 1H NMR (400 MHz, DMSO-d₆) δ: 6.19 (s, 4H, N-CH₃-Se, N-CH₃-Se); 7.44 (t, 2H, H₃, H₄ = 8.2 Hz); 7.86-7.89 (m, 4H, H₃, H₄, H₅, H₆). 13C NMR (100 MHz, DMSO-d₆) δ: 43.6 (CH₂-Se); 112.2 (C₆, C₇, C₈, C₉); 120.2 (C₁₀); 125.3 (C₁₁); 128.3 (C₁₂); 133.0 (C₁₃); 146.0 (C₁₄). MS (m/z [percent abundance]): 132 (86); 77 (100). Elemental analysis for C₁₄H₁₂N₂Se₂O₂ calculated (found, percent): C, 45.19/45.25; H, 2.51/2.70; N, 5.86/5.64.

2.2-(Diselenodiyldimethanediyl)bis[1H-isooindole-1,3(2H)-dione] (2g). Compound 2g was from (1,3-dioxo-1,3-dihydro-2H-isooindole-2-yl)methyl selenocyanate and sodium cyanoethoxide. A white solid was obtained. Yield: 28%. mp: 162 to 164°C. IR (KBr) cm⁻¹: 1,774 and 1,715 (C=O) cm⁻¹; 1,715 and 1,715 (C=O) cm⁻¹; 1,774 and 1,715 (C=O) cm⁻¹; 1,715 and 1,715 (C=O) cm⁻¹. 1H NMR (400 MHz, DMSO-d₆) δ: 4.21 (s, 4H, N-CH₃-Se, N-CH₃-Se); 7.70 (d, 2H, H₃, H₄ = 8.1 Hz); 7.86-7.89 (m, 4H, H₃, H₄, H₅, H₆); 7.95 (s, 2H, H₃, H₄); 8.06 (d, 2H, HI, H₄, J₄ = 8.1 Hz); 8.09 to 8.11 (m, 4H, H₃, H₄, H₅, H₆). 13C NMR (100 MHz, DMSO-d₆) δ: 31.4 (CH₂-Se); 127.6 (C₆, C₇, C₈, C₉, C₁₀, C₁₁); 133.2 (C₁₂, C₁₃); 134.1 (C₁₄, C₁₅, C₁₆, C₁₇); 153.5 (C₁₈, C₁₉, C₂₀, C₂₁); 132.3 (C₁₄); 135.6 (C₁₅); 167.3 (CO). MS (m/z [percent abundance]): 478 (M⁺, 2); 160 (100). Elemental analysis for C₃₄H₁₈N₂O₂Se₄ calculated (found, percent): C, 45.19/45.25; H, 2.51/2.70; N, 5.86/5.64.

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3.-(Diselenodiyldimethanediyl)bis[2-bromophene] (2d). Compound 2d was from (2-bromophene-3-yl)methyl selenocyanate (11) and sodium borohydride. The mixture was extracted with ethyl ether (3 x 50 ml). The organic phase was washed with water (3 x 50 ml) and dried with anhydrous Na₂SO₄. The ethyl ether was removed under vacuum, and a yellow powder was obtained. Yield: 29%. mp: 49 to 50°C. IR (KBr) cm⁻¹: 3,099 (w, C-H); 722 (s, Se-Se). 1H NMR (400 MHz, DMSO-d₆) δ: 4.04 (s, 4H, CH₂-Se, CH₂-Se); 7.00 (d, 2H, H₃, H₄ = 5.6 Hz); 7.58 (2H, H₃, H₄ = 5.6 Hz). 13C NMR (100 MHz, DMSO-d₆) δ: 26.5 (CH-Se); 111.1 (C₆, C₇); 128.4 (C₈, C₉); 130.6 (C₆, C₇); 140.0 (C₅, C₆). MS (m/z [percent abundance]): 510 (M⁺, 3); 175 (100); 199 (69); 45 (8). Elemental analysis for C₁₀H₈Br₂S₂Se₂ calculated (found, percent): C, 38.06/38.40; H, 1.58/1.52.
Heteroaryl Selenocyanates as Leishmanicidal Agents

(Compounds 1a to 1o) were obtained in variable yields (27 to 92%) by reaction between the commercially available haloalkyl carbo or heterocyclic reactives with potassium selenocyanate in the molar ratio 1.1 in acetone under reflux over 3 to 4 h (24). The subsequent reduction of compounds 1a to 1o with sodium borohydride or sodium cyanoborohydride in ethanol afforded derivatives 2a to f and 2g (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 reaction conditions (temperature, solvents, or molar ratio) resulted in decomposition of the starting materials by rupture of the bond between the 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 ($\text{NaBH}_3\text{CN}$) to reduce elemental selenide in DMF ($\text{NaBH}_4$ or NaBH$_3$CN) was confirmed by the disappearance of the signal corresponding to the Se-Se group and lacked the CN band, confirming 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$_2$CN.

All of the compounds prepared during the course of these investigations are stable. 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 (Fig. 2).

The IR spectra of compounds 1a to 1o illustrate sharp peaks around 2,138 to 2,155 cm$^{-1}$ due to a CN group. Derivatives 2a to 2g showed multiple bands in the range of 710 to 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 in the supplemental material.

**Biological evaluation. (i) Activity in amastigotes and cytotoxic activity in human cells.** Compounds 1a to 1o, 2a to 2g, and 3 were tested for their antiprotozoal activity against amastigotes of the pathogenic *Leishmania infantum* 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 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 “gold 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 50% effective dose (ED$_{50}$) values. In addition, for a compound to be a candidate for antileishmanial drug, it is required to have both high leishmanicidal activity and low cytotoxicity.

**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 miltefosine and edelfosine are included in all cases for comparison. Biological data evidenced that most of the screened compounds (15 out of 23) showed high bioactivity (ED$_{50}$ $\leq$ 2.53 $\mu$M) against *L. infantum*, being more potent than miltefosine (ED$_{50}$ = 2.84 $\mu$M). In addition, under our experimental conditions, seven compounds (1d, 1e, 1h, 1m, 1n, 2e, and 2f) displayed *in vitro* potency comparable to or higher than that of edelfosine (ED$_{50}$ = 0.82 $\mu$M).

Different authors have claimed that compounds having SI values greater than 20 can be considered ideal candidates for further development as leishmanicidal drugs (58). However, in this study and with rigorous criteria, we have considered the SI threshold of >25 for further analysis of their activity in amastigote-infected THP-1 cells. This requirement is satisfied by compounds 1h, 2d, 2e, and 2f considering them as the lead ones in this series due to their excellent biological behavior.

**(ii) Leishmanicidal activity in infected macrophages.** According to their activity and selectivity, four compounds (1h, 2d,
TABLE 1 ED₅₀ values for compounds on amastigotes and cytotoxic activity in the THP-1 cell line

<table>
<thead>
<tr>
<th>Compound</th>
<th>R (substituted group)</th>
<th>ED₅₀ (mean ± SEM), μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amastigotes</td>
<td>THP-1</td>
</tr>
<tr>
<td>la</td>
<td>Quinol-8-yl</td>
<td>4.49 ± 0.21</td>
</tr>
<tr>
<td>lb</td>
<td>Quinol-2-yl</td>
<td>1.76 ± 0.04</td>
</tr>
<tr>
<td>lc</td>
<td>Acridin-9-yl</td>
<td>7.40 ± 0.60</td>
</tr>
<tr>
<td>ld</td>
<td>Quinoxalin-2,3-diylnmethanediyl</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>le</td>
<td>6,7-Dimethoxy-2-oxo-2H-chromen-4-yl</td>
<td>0.82 ± 0.07</td>
</tr>
<tr>
<td>lf</td>
<td>5-Nitrofur-2-yl</td>
<td>1.99 ± 0.23</td>
</tr>
<tr>
<td>lg</td>
<td>6-Bromo-1,3-benzodioxol-5-yl</td>
<td>10.10 ± 1.81</td>
</tr>
<tr>
<td>lh</td>
<td>3,3-Dimethylisoxazol-4-yl</td>
<td>0.73 ± 0.10</td>
</tr>
<tr>
<td>li</td>
<td>2-Bromothien-3-yl</td>
<td>2.86 ± 0.29</td>
</tr>
<tr>
<td>lj</td>
<td>5-Chlorothien-2-yl</td>
<td>1.85 ± 0.33</td>
</tr>
<tr>
<td>lk</td>
<td>N-Phenylpyrrol-4-yl</td>
<td>8.87 ± 1.32</td>
</tr>
<tr>
<td>ll</td>
<td>Benzotriazol-1-yl</td>
<td>1.11 ± 0.21</td>
</tr>
<tr>
<td>lm</td>
<td>2-Adamant-1-yl-2-oxoethyl</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td>ln</td>
<td>9,10-Dioxo-9,10-dihydroanthracen-2-yl</td>
<td>0.74 ± 0.18</td>
</tr>
<tr>
<td>lo</td>
<td>Phthalimidyl</td>
<td>2.53 ± 0.32</td>
</tr>
<tr>
<td>la</td>
<td>Quinol-8-yl</td>
<td>2.05 ± 0.24</td>
</tr>
<tr>
<td>lb</td>
<td>Acridin-9-yl</td>
<td>5.46 ± 0.01</td>
</tr>
<tr>
<td>lc</td>
<td>6-Bromo-1,3-benzodioxol-5-yl</td>
<td>3.99 ± 0.62</td>
</tr>
<tr>
<td>ld</td>
<td>2-Bromothien-3-yl</td>
<td>1.20 ± 0.03</td>
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<tr>
<td>le</td>
<td>Benzotriazol-1-yl</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>lf</td>
<td>9,10-Dioxo-9,10-dihydroanthracen-2-yl</td>
<td>0.68 ± 0.30</td>
</tr>
<tr>
<td>lg</td>
<td>Phthalimidyl</td>
<td>1.35 ± 0.17</td>
</tr>
<tr>
<td>lh</td>
<td>Phthalimidyl</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Edelfosine</td>
<td></td>
<td>0.82 ± 0.13</td>
</tr>
<tr>
<td>Miltefosine</td>
<td></td>
<td>2.84 ± 0.10</td>
</tr>
</tbody>
</table>

*a Selectivity index (SI) is the ratio of ED₅₀ values of compounds against THP-1 cells relative to their corresponding ED₅₀ against L. infantum amastigotes.

2e, and 2f) were advanced for testing leishmanicidal activity in amastigote-infected THP-1 cells. Compound 2f (ED₅₀ = 0.68 μM; SI = 36.8) was not further tested due to the reproducibility issues showed by this derivative pertaining to its lack of solubility under the assay conditions. The ED₅₀ values for the other selected derivatives (1h, 2d, and 2e) were calculated and summarized in Table 2. The potency of the analogues was compared with that of edelfosine, a current antileishmanial agent (ED₅₀ = 2.84 μM; SI = 23.2, 14.0, and 14.4 μM, respectively, with the members of diselenide family being the most potent compounds.

(iii) Inhibition of L. infantum trypanothione reductase activity. In an attempt to investigate a possible mechanism of action, the ability to inhibit the trypanothione reductase activity for the most active compounds was 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 a vehicle. The 50% inhibitory concentrations (IC₅₀) obtained are shown in Table 3.

The compounds 1h and 2d were able to inhibit TryR with IC₅₀ values of 0.46 and 6.85 μM, respectively. It is remarkable 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, which 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 promising antileishmanial lead candidates because they show a strong inhibitory activity against axenic amastigotes (IC₅₀ = 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 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 one violation (edelfosine). It has been well established that optimal lipophi-
DISCUSSION

There are many available antileishmanial agents, but the drug of choice is still awaited because of 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 2 decades, several interesting drug targets have been proposed, including many proteins and enzymes that differ from mammalian counterparts which can interfere with the redox system. Among the promising targets that the scientific community considers for the design of useful therapies, enzymes (trypanothione reductase, proteinases, superoxide dismutase, dihydrofolate reductase, metacaspase, topoisomerase, kinases, sirtuins, etc.) are one of the classes with the most representation. In this context, we notice that selenium plays an important role in medicinal chemistry, particularly in 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 seleno derivatives presented in this work, there seems to exist a tendency suggesting that analogues with the diselenide unit were more active than those with the selenocyanate moiety (1a versus 2a, 1c versus 2b, 1g versus 2c, 1i versus 2d, 1l versus 2e, and 1o versus 2g) against *L. infantum* amastigotes. Regarding the selectivity index, the addition of the diselenide scaffold clearly improved the selectivity, for example, in compounds 1g versus 2c, 1i versus 2d, 1l versus 2e, and 1n versus 2f. In general, it was observed that tricyclic nitrogemated 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 (ED₅₀ of <2.5 μM and SI of >25), four derivatives, one selenocyanate, 1h, and two diselenides (2d and 2e) were selected for further studies. Despite the fact that compound 2f fulfilled these criteria, it could not be tested due to solubility problems. When we performed intracellular form tests, these derivatives did not improve the activity compared to that of edelfosine. However, their lack of toxicity against THP-1 cells (Table 1) represents a remarkable advantage over the reference drug.

We hypothesized that TryR inhibition may be related with the leishmanicidal activity observed for seleno derivatives. 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 is this enzyme 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 were shown 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, and isozazole) and their *in vitro* leishmanicidal activities against *L. infantum* amastigotes along with their cytotoxicities in THP-1 cells. Fifteen such compounds exhibited better potency against axenic amastigotes than the standard drug miltefosine. Based on their antiparasitic activities and low toxicity in THP-1 cells, compounds 1h, 2d, 2e, and 2f were identified as the best candidates for further studies with 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. infantum* TryR was determined. A clear correlation between enzyme inhibition and antiparasitic activity was observed for compounds 1h and 2d, which may be considered 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 (absorption, distribution, metabolism, and excretion) parameters calculated for derivatives 1h and 2e predict a good bioavailability. *In silico* ADME profiling and drug score results along with *in vitro* leishmanicidal activity, cytotoxicity, and TryR inhibitory activity make 1h a promising drug candidate for the development of a new leishmanicidal drug.

### Table 4 Theoretical ADME properties for lead compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molinspiration calculations</th>
<th>Osiris calculations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MW</td>
<td>miLogP</td>
</tr>
<tr>
<td>1h</td>
<td>215.1</td>
<td>1.2</td>
</tr>
<tr>
<td>2d</td>
<td>510.0</td>
<td>5.8</td>
</tr>
<tr>
<td>2e</td>
<td>422.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Edelfosine</td>
<td>523.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*MW, molecular weight; miLogP, octanol-water partition coefficient; TPSA, topological polar surface area; nON, hydrogen bond acceptors; nOHNH, hydrogen bond donors; NV, number of violations; Vol, volume.*
lead compound for the development of more potent antiparasitic drugs. A graphical summary of the conclusions drawn from this work is depicted in Fig. 3.

Moreover, to the best of our knowledge, this is 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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28. Carole Gopinath


