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ORIGINAL PAPER

Synthesis of 5-spirocyclohexyl-2,4-dithiohydantoin derivatives: a potential anti-leishmaniasis agent

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Abstract A series of spiro-dithiohydantoins were synthesized by heating a mixture of 5-spirocyclohexyl-2,4-dithiohydantoin potassium salt and 3-chloropropanoyl chloride. These compounds were synthesized and evaluated for their activity against five *Leishmanial* strains in the promastigote stage in vitro. Seventy-two hours inoculation of a variety of products gave an IC_{100} and average IC_{50} values of 1.25 and 0.376 mg/cm^3 against all *Leishmanial* strains tested.

Keywords Dithiohydantoins · Spiro-dithiohydantoins · 1,3-Diazaspiro[4,5] decane-2,4-dithione · Leishmaniasis

Introduction

In connection with our previous research work related to aza-spiro compounds, such as 5-spirocyclohexyl-2,4-dithiohydantoin [1–7], spiro-thiopyrano[2,3-*d*]thiazolidines [8] and isothiazoles [9], we aimed at the synthesis of compounds with anti-leishmania activity based on the spiro-dithiohydantoin scaffold. It seemed of interest to us to prepare new 5-spirocyclohexyl-2,4-dithiohydantoin derivatives bearing some resemblance to the known drugs 5-ethyl-5-phenylhydantoin (Nirvanol) [10–12] and 5,5-diphenylhydantoin (Dilantin) [13]. Small heterocyclic molecules are one of the predominant types of building block in medicinal chemistry [14, 15], and among the "drug-like" heterocycles, dithiohydantoins have been widely used for constructing products with pharmaceutical applications [16]. Dithiohydantoins are sulfur analogs of hydantoins with both carbonyl groups replaced by thiocarbonyl groups. Among the known dithiohydantoins, 2-thiohydantoins are most notably known due to their wide applications as hypolipidemic, anticarcinogenic, antimutagenic, antithyroidal, and antiviral agents [17].

We now report a simple method for the preparation of 5-spiro-cyclohexyl-2,4-dithiohydantoin derivatives that can easily be scaled up in the laboratory. The reaction of 5-spirocyclohexyl-2,4-dithionhydantoin (**1**) with 1- or 2-mole equivalents of 3-chloropropanoyl chloride was examined, and the synthesized compounds were tested against five *Leishmanial* strains. In continuation [18, 19] of our efforts toward the synthesis of heterocyclic compounds, an efficient and practical preparation of 5-spirocyclohexy-2,4-dithiohydantoin derivatives **1–9** is shown.

Results and discussion

Synthesis

The direct condensation between 5-spirocyclohexyl-2,4-dithiohydantoin potassium salt **2** and 3-chloropropanoyl chloride produces 5-spirocyclohexyl-2,4-dithiohydantoin derivatives **3–9** in moderate to high yields (Scheme 1). Spirocyclohexyl-2,4-dithiohydantoin (**1**) was synthesized in high yield (96%) [1–5, 20]. The 5-spirocyclohexyl-2,4-dithiohydantoin potassium salt (**2**) was readily obtained by stirring **1** with ice-cooled KOH solution in ethanol [21, 22].

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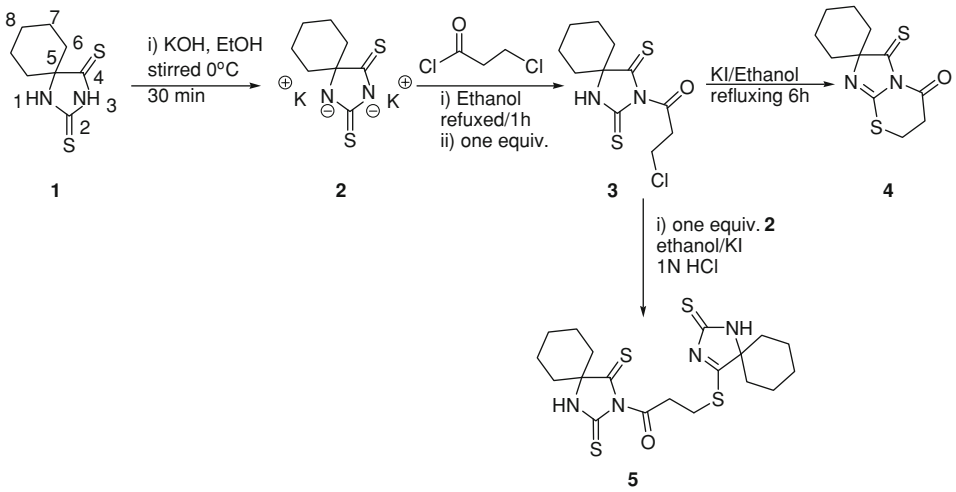
Scheme 1



Then the reaction of **2** with 3-chloropropanoyl chloride was investigated under different conditions.

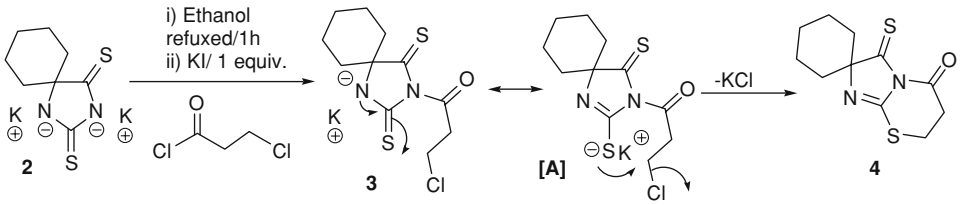
(1) When the reaction was carried out with 1-mole equivalent of 3-chloropropanoyl chloride in ethanol, 3-chloro-1-(2,4-dithioxo-1,3-diaza-spiro[4, 5]dec-3-yl)-propan-1-one (**3**) was obtained in good yield (75%). Ring closure of **3** by adding a catalytic amount of KI in refluxing ethanol produced 3′-thioxo-6′,7′-dihydro-5′*H*-spiro[cyclohexane-1,2′-imidazo[2,1-*b*][1, 3]thiazin]-5′-one **4** in excellent yield (98%). When compound **3** was reacted with 1-mole equivalent of 5-spirocyclohexyl-2,4-dithiohydantoin potassium salt (**2**) in the presence of catalytic amounts of KI, 1-(2,4-dithioxo-1,3-diaza-spiro[4, 5]dec-3yl)-3-(2-thioxo-1,3-diaza-spiro[4, 5]dec-3-en-4-ylsulfanyl)-propan-1-one (**5**) was obtained in moderate yield (49%), as depicted in Scheme 1.

The *N*-acylation and *S*-alkylation were confirmed by the fact that boiling the dithiohydantoins with dilute hydrochloric acid produced the corresponding hydantoins [10–15]. A mechanistic proposal depicting the formation of 4 including possible intermediates via ring closure of **3** undergoes competitive nucleophilic substitution reaction by the preformed sulphur anion [**A**]. The condensation type addition/substitution proceeds in a 1,4-fashion and results in an intramolecular addition on precipitation during the reaction at refluxing temperature under the action of a trace amount of ethanol in the reaction mixture or during workup on a silica gel column. A mechanistic proposal consistent with literature data [23–31] is given in Scheme 2.

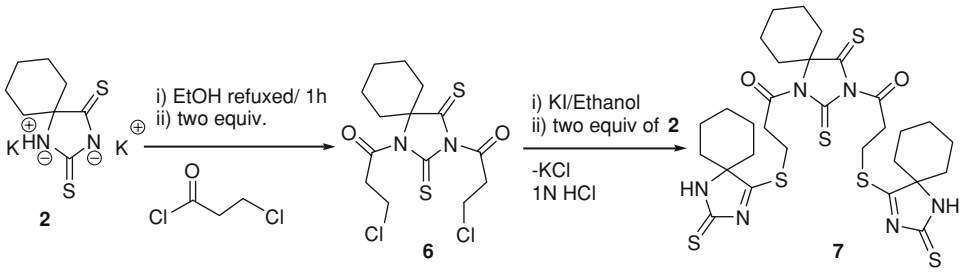
(2) Similarly, when the reaction was performed with 2-mole equivalents of 3-chloropropanoyl chloride, 3-chloro-1-[1-(3-chloro-propionyl)-2,4-dithioxo-1,3-diaza-spiro[4,5]dec-3-yl)-propan-1-one (**6**) was achieved in good yield (75%). Compound **6** was reacted further with 2-mole equivalents of 5-spirocyclohexyl-2,4-dithiohydantoin potassium salt (**2**) in the presence of catalytic amounts of KI to produce 1-{2,4-dithioxo-1-[3-(2-thioxo-1,3-diaza-spiro [4, 5]dec-3-en-4-ylsulfanyl)-propionyl]-1,3-diaza-spiro[4,5] dec-3yl)-3-(2-thioxo-1,3-diaza-spiro[4, 5]dec-3-en-4-ylsulfanyl)-propan-1-one (**7**) in 59% yield (Scheme 3). In addition, we prepared 2,4-bis{3-[2-(4-methylphenyl)sulfonyl]hydrazino-3-oxopropylthio}-1,3-diazaspiro[4,5] deca-1,3-diene (**9**) (Scheme 4). For this purpose we developed an efficient synthesis of 3-chloro-*N*′-[(4-methylphenyl)sulfonyl] propanoylhydrazide **8** by the reaction of tosyl hydrazide and 3-chlrorpropanoyl chloride in refluxing benzene for 1 h [32].

The chemical structure of **1** was established by ^{1}H and ^{13}C-NMR spectroscopies, employing the 1D and 2D (^{1}H/^{1}H and ^{1}H/^{13}C correlation spectra) NMR techniques. The ^{1}H and ^{13}C-NMR data obtained are presented in the experimental part. The ^{1}H-NMR spectra of **1**, **2**, **3**, **4**, **5**, **6**, **7**, and **9** show resonance signals at 1.5–1.75 ppm (CH_2 protons of cycloalkane residue, multiplet) and two broad signals at 8.0–14.5 ppm characteristic of NH protons. The NOEs observed for **1** exhibit an enhancement of the resonance of the alkyl protons from the cycle, when resonances at ca. δ 11.0 ppm were irradiated. This result was used to

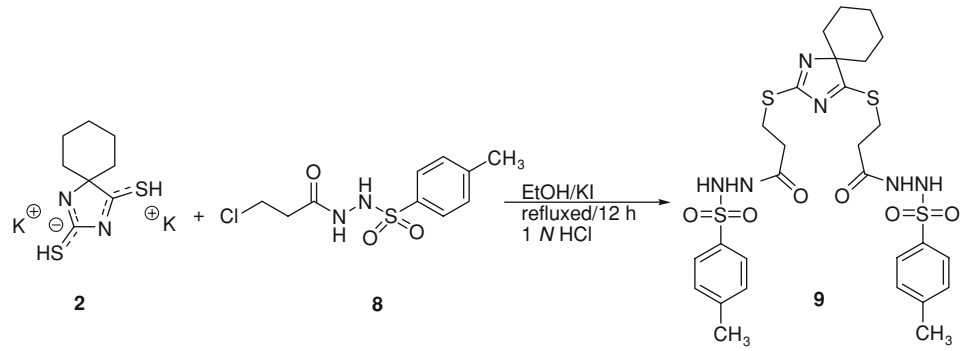
Scheme 2



Scheme 3



Scheme 4



assign the signals at ca. 11.0 ppm to H-1 and those at ca. 13.0 ppm to H-3. The ^{13}C-NMR chemical shifts assignment of the spectra was facilitated by analyses of the HMQC and HMBC spectra, which provide single and multiple bond ^{1}H/^{13}C connectivities. On the basis of the HMBC spectra, it was possible to assign the resonance peaks of carbons C-2 and C-4. Resonance peaks of C-2 and C-4 of the dithio analogues of spirodithiohydantoins **1**, **3**, **5**, **7**, and **9** appear at ca. δ 180 and 210 ppm, respectively. In the HMBC spectra of these compounds, cross peaks of C-2 with H-1 and H-3, and C-4 with H-3 were observed. The ^{13}C-NMR spectra of **1**, **3**, **5**, **7**, and **9** show an up-field shift of the resonance signals of C-4 as well as signals at 20.5 and 36.0 ppm characteristic of methylene protons of the –CH_2– group. The strong up-field shifts of the resonance signals of carbons C-4 of cycloalkane 5-spiro-(2-dithiohydantoins) **1**–**7** confirm the presence of thiocarbonyl group at C-4 position. The ^{1}H and ^{13}C-NMR data obtained confirm the structure of the compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, and **9**.

Biological studies

Evaluation of the Leishmanial activity

The different heterocyclic ring systems obtained in this investigation are known to exhibit diverse biological activities. This initiated our interest to evaluate the leishmania activity of some of these compounds. *Leishmania* spp. are intracellular parasitic hemo-flagellates that infect macrophages of the skin and viscera to produce disease in their vertebrate hosts. Three major clinical manifestations of leishmaniasis are recognized: visceral, cutaneous, and muco-cutaneous leishmaniasis [33]. The disease usually manifests itself as fever, weight loss, and hepato-splenomagaly with biochemical abnormalities of hyperglobulinemia and pancytopenia [34]. It has received increasing attention in developed countries because of the growing number of cases seen in AIDS patients [33, 35] and the occurrence of viscerotropic *L. tropica* disease among Persian Gulf War participants [33–36].

Pentavalent antimonial drugs have remained standard treatment for visceral leishmaniasis since the 1940s [36]. These drugs not only have several adverse effects [36], but drug resistance and treatment failures are becoming increasingly common, especially in immune-compromised patients who often fail to respond or relapse [35, 37, 38]. Amphotericin B and its new lipid formulations are used as second line of treatment. However, there are limitations for these drugs due to prolonged length of therapy and adverse side reactions. Thus, there is still a need for the development of new drugs [36, 39, 40]. In this article we report the anti-Leishmanial effect of *S*- and *N*- heterocyclic compounds in solution on *Leishmanial* promastigotes in vitro.

Each experiment was repeated at least three times. Concentrations of dithiohydantoins ranging from 10 to 0.07 mg/cm^3 were tested for anti-Leishmanial activity. As shown in Table 1, concentration of 1.25 mg/cm^3 was found to be leishmanicidal for all the *Leishmanial* strains tested, whereas 50% parasites of all strains were found to be dead at an average value of 0.376 mg/cm^3 after 72 h of

Table 1 In vitro activity of 5-spiro-cyclohexyl-2,4-dithiohydantoin derivatives **1–7**, **9** and 3-chloro-*N*′-[(4-methylphenyl)sulfonyl] propanoylhydrazide (**8**) on various *Leishmanial* strains in promastigote stage in vitro

Compounds no.	*L. major* IC_{50} and IC_{100} (mg cm^{-3})	*L. major Egypt* IC_{50} and IC_{100} (mg cm^{-3})	*L. tropica* IC_{50} and IC_{100} (mg cm^{-3})	*Lmex mex* IC_{50} and IC_{100} (mg cm^{-3})	*L. donovani* IC_{50} and IC_{100} (mg cm^{-3})
1	0.58 ± 0.01/0.36 ± 0.01	0.63 ± 0.01/0.36 ± 0.01	0.62 ± 0.01/0.32 ± 0.01	0.48 ± 0.01/0.33 ± 0.01	0.43 ± 0.01/0.38 ± 0.01
2	0.52 ± 0.01/0.32 ± 0.01	0.55 ± 0.01/0.37 ± 0.01	0.52 ± 0.01/0.26 ± 0.01	0.44 ± 0.01/0.16 ± 0.01	0.45 ± 0.01/0.36 ± 0.01
3	0.57 ± 0.01/0.33 ± 0.01	0.65 ± 0.01/0.36 ± 0.01	0.58 ± 0.01/0.35 ± 0.01	0.61 ± 0.01/0.37 ± 0.01	0.62 ± 0.01/0.36 ± 0.01
4	0.68 ± 0.01/0.35 ± 0.01	0.66 ± 0.01/0.33 ± 0.01	0.36 ± 0.01/0.16 ± 0.01	0.23 ± 0.01/0.12 ± 0.01	0.52 ± 0.01/0.35 ± 0.01
5	0.78 ± 0.01/0.31 ± 0.01	0.79 ± 0.01/0.37 ± 0.01	0.65 ± 0.01/0.39 ± 0.01	0.55 ± 0.01/0.31 ± 0.01	0.46 ± 0.01/0.32 ± 0.01
6	0.33 ± 0.01/0.16 ± 0.01	0.44 ± 0.01/0.15 ± 0.01	0.36 ± 0.01/0.22 ± 0.01	0.24 ± 0.01/0.13 ± 0.01	0.22 ± 0.01/0.17 ± 0.01
7	0.88 ± 0.01/0.55 ± 0.01	0.92 ± 0.01/0.66 ± 0.01	0.69 ± 0.01/0.36 ± 0.01	0.66 ± 0.01/0.33 ± 0.01	0.63 ± 0.01/0.36 ± 0.01
8	0.72 ± 0.01/0.39 ± 0.01	0.73 ± 0.01/0.35 ± 0.01	0.55 ± 0.01/0.33 ± 0.01	0.61 ± 0.01/0.37 ± 0.01	0.63 ± 0.01/0.37 ± 0.01
9	0.58 ± 0.01/0.36 ± 0.01	0.89 ± 0.01/0.55 ± 0.01	0.95 ± 0.01/0.66 ± 0.01	0.89 ± 0.01/0.46 ± 0.01	0.90 ± 0.01/0.60 ± 0.01

Each experiment was repeated at least three times

IC_{100}, concentration, which induced 100% mortality

IC_{50}, concentration, which induced 50% mortality

treatment. All five strains of *Leishmania* that were tested were found to be sensitive to the spirocyclohexylhydantoins **1–7** and **9**. No major differences in the degree of susceptibility of parasites occurred during the study except for *L. mex mex*, perhaps due to its very high multiplication rate. A careful analysis of data suggests a susceptibility order of *L. major* (Pak. Isolates) A > *L. major* > *L. donovani* > *L. tropica* > *L. mex mex*. A concentration of 1.25 mg/cm^3 was leishmanicidal, whereas 0.25 mg/cm^3 and below was leishmaniastatic. Although these concentrations are not low, it has to be taken into consideration that due to solubility issues, the anti-leishmanial components may be present in very low concentration [40]. It was interesting to find that a methanol solution of the same dithiohydantoins stock is 100% active at ~200 μg/cm^3 (unpublished data). Dithiohydantoins **1–7** and **9** are rich in sulfur containing active principles mainly in the form of cysteine derivatives, as *S*-alkyl cysteine sulfoxides, which decompose into a variety of thiosulfinates and polysulfides by the action of the enzyme allinase on extraction. Decomposed products are volatile and present in the oils of onion and garlic [41, 42]. They possess antidiabetic, antibiotic, hypocholesterolemic, fibrinolytic, and various other biological actions. In addition to free sulfoxides in onion, there are nonvolatile sulfur-containing peptides and proteins, which possess various activities and thus rendering it an important source of therapeutic agents [41, 42]. Further investigations regarding the principles of the anti-leishmanial activity of dithiohydantoins **1–7** and **9** merit consideration. Regarding mammalian cell toxicity, the compounds **7**, **8**, and **9** were, in general, less toxic than the other analogues.

Conclusions

In summary, we have described a novel synthetic method for the synthesis of 5-spirocyclohexyl-2,4-dithiohydantoins derivatives, which were tested for in vitro activity against *Leishmania* parasites. The most active compounds to emerge from this study were **5**, **7**, **8**, and **9**. They were active against free and intracellular forms of *T. cruzi* with SI higher than compounds **1–3**. The results obtained offer new possibilities for further improvements concerning the anti-parasitic activity of other closely related derivatives of these series of compounds. A more complete structure activity relationship is currently being pursued in our laboratory.

Experimental

Reactions were carried out without precautions to exclude light, atmospheric oxygen, and moisture, unless otherwise

stated. Melting points were determined on a Reichert apparatus. Elemental analyses were carried out with a Carlo Erba 1106 microanalyzer at the University of Murcia, Spain, and the microanalysis unit at Cairo University, Egypt; results agreed favorably with calculated values. IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer with Nujol mulls between polyethylene sheets. NMR spectra were recorded on a Bruker AC 200, Advance 300, or a Varian Unity 300 spectrometer at room temperature unless otherwise stated. Chemical shifts were referenced to TMS [^{1}H and ^{13}C (^{1}H) and H_3PO_4 (^{31}P)]. The NMR probe temperature was calibrated using ethylene glycol ^{1}H NMR standard methods. Chromatographic separations were carried out by TLC on silica gel (70–230 mesh).

*1,3-Diazaspiro[4, 5]decane-2,4-dithione (**1**)*

A mixture of 9.8 g cyclohexanone (0.1 mol), 5.1 g sodium cyanide (0.1 mol), 5.5 g ammonium chloride (0.1 mol), and 7.6 g carbon disulfide (0.1 mol) in 100 cm^3 ethanol was heated in a water bath at 100 °C for 12 h. After cooling to room temperature and evaporation of the solvent, a solid product was formed and was purified by recrystallization from ethyl alcohol [1, 2] to give 17.8 g (89% yield) as needless yellow crystals, mp 270–272 °C dec. (lit. [1–6] mp 267 °C, lit. [26] mp 269 °C).

1,3-Diazaspiro[4, 5]decane-2,4-dithione potassium salt (**2**, $C_8H_{10}N_2S_2K_2$)

A mixture of 1.104 g 5-spirocyclohexyl-2,4-dithiohydantoin (**1**) (0.04 mol) and 0.112 g of KOH (0.02 mol) in 25 cm^3 ethanol was stirred at 0 °C for 30 min. The solid residue was collected by filtration, washed with dry ethanol and dried under reduced pressure to isolate 1.1 g (99% yield) white powder, mp > 300 °C dec. IR (Nujol): $\bar{\nu}$ = (C=S) 1,117, (C=N and C=C) 1,536, 1,525 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 1.54–1.75 (m, 10H, cyclohexyl) ppm; ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 212.9 (C4), 180.9 (C2), 78.0 (C5), 36.6 (C6, C10), 26.3 (C8), 20.7 (C7, C9) ppm.

3-(3-Chloropropanoyl)-1,3-diazaspiro[4.5]decane-2, 4-dithione (**3**, $C_{11}H_{15}N_2OS_2Cl$)

A mixture of 2.77 g 5-spirocyclohexyl-2,4dithiohydantoin potassium salt (**2**) (0.01 mol) and 1 cm^3 3-chloropropanoyl chloride (0.01 mol) in 25 cm^3 ethanol was stirred at 0 °C for 30 min, then refluxed for 15 min and then stirred at RT again overnight. The formed KCl salts were removed by filtration. The filtrate was diluted with *Et$_2$O*, washed with saturated aqueous solutions of Na_2CO_3, NH_4Cl, brine, and dried over Na_2SO_4. After removal of the solvent a solid product was obtained, which was purified by chromatography (silica gel, *n*-hexane/ether 1:1) to give 2.2 g (75% yield) yellow solid. TLC R_f = 0.145 [*n*-hexane/ether 1:1], mp 202–205 °C dec.; IR (Nujol): $\bar{\nu}$ = (C=S) 1,115, (C=O) 1,741, (NH) 3,213 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 8.96 (s, 1H, NH), 3.75 (t, J = 6.5 Hz, 2H, –CH$_2$), 2.76 (t, J = 6.5 Hz, 2H, –CH$_2$), 1.54–1.75 (m, 10H, cyclohexyl) ppm; ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 210.8 (C4), 181.2 (C2), 178.4 (C=O), 79.5 (C5), 38.7 (CH$_2$–Cl), 37.57 (C6, C10), 36.3 (–CH$_2$–), 26.5 (C8), 20.5 (C7, C9) ppm.

3′-Thioxo-6′,7′-dihydro-5′H-spiro[cyclohexane-1, 2′-imidazo[2,1-b][1, 3]thiazin]-5′-one (**4**, $C_{11}H_{14}N_2OS_2$)

Method (*A*). To a suspension of 1.16 g 3-(3-chloropropanoyl)-1,3-diazaspiro[4.5]decane-2,4-dithione **3** (0.004 mol) in 10 cm^3 ethanolic KOH (0.8 *M*) was added 0.002 g of KI in 25 cm^3 dry ethanol, and then this was heated under reflux for 12 h. The reaction mixture was cooled and acidified by the addition of two drops of 0.1 N hydrochloric acid 37% in an ice bath. The formed KCl salts were removed by filtration. The filtrate was diluted with *Et$_2$O*, washed with saturated aqueous solutions of Na_2CO_3, NH_4Cl, and brine, and dried over Na_2SO_4. After removal of the solvent, a solid product was obtained, which was purified by chromatography (silica gel, *n*-hexane/ether 1:3) to give 1.0 g (98% yield) yellow powder. TLC R_f = 0.95 [*n*-hexane/ether 1:3], mp 242–245 °C dec. IR (Nujol): $\bar{\nu}$ = (C=S) 1,115, (C=O) 1,741, (NH) 3,213 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 4.15 (t, J = 6.4 Hz, 2H, –CH$_2$), 3.75 (t, J = 6.4 Hz, 2H, -CH$_2$), 1.54–1.75 (m, 10H, cyclohexyl) ppm; ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 210.2 (C=S), 177.2 (C=O), 163 (C=N), 79.1 (C5), 36.3 (C6, C10), 35.6 (–CH$_2$), 24.9 (–CH$_2$), 27.7 (C8), 20.0 (C7, C9) ppm.

Method (*B*). To a mixture of 2.765 g 5-spirocyclohexyl-2,4dithiohydantoin potassium salt (**2**) (0.01 mol), 1 cm^3 of 3-chloropropanoyl chloride (0.01 mol) was added 0.002 g KI in 25 cm^3 dry ethanol and then heated under reflux for 12 h. The obtained solid was collected by filtration, washed with 50 cm^3 boiling water followed by 25 cm^3 boiling ethanol, and recrystallized from DMF/ethanol to give yellow crystals of **4** in similar yield.

3-{3-[(2-Thioxo-1,3-diazaspiro[4.5]dec-3-en-4-yl)thio]propanoyl}-1,3-diazaspiro[4.5]decane-2, 4-dithione (**5**, $C_{19}H_{26}N_4OS_4$)

A mixture of 2.77 g 5-spirocyclohexyl-2,4dithiohydantoin potassium salt (**2**) (0.01 mole), 2.9 g of 3-(3-chloropropanoyl)-1,3-diazaspiro[4.5]decane-2,4-dithione **3** (0.01 mole), and 0.002 g of KI was heated under reflux in 25 cm^3 DMF for 12 h. The reaction mixture was cooled in an ice bath and then acidified by the addition of 1 N hydrochloric acid. The obtained solid was collected by filtration, washed with 25 cm^3 boiling water, and recrystallized from DMF/ethanol to give 2.25 g (49% yield) yellow powder, mp 258-260 °C dec. IR (Nujol): $\bar{\nu}$ = (C=S) 1,116, (C=N) 1,540, (C=O) 1,717, (NH) 3,219 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 10.16 (s, 1H, NH), 9.96

(s, 1H, NH), 3.15 (t, $J = 6.3$ Hz, 2H, –CH_2–S), 2.85 (t, $J = 6.3$ Hz, 2H, –CH_2–), 1.54–1.75 (m, 20H, cyclohexyl) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 210$ (C=S), 183.2 (C=S), 187 (C=S), 177.2 (C=O), 163.7 (C=N), 80.7 (C5), 60.5 (C5′), 37.1 (C6, C10), 33.4 (–$\underline{C}H_2$), 29.4 (C6′, C10′), 23.9 (–$\underline{C}H_2$), 27.7 (C8), 27.1 (C8′), 19.5 (C7, C9), 19.2 (C7′, C9′) ppm.

1,3-Bis(3-chloropropanoyl)-1,3-diazaspiro[4,5] decane-2,4-dithione (**6**, $C_{11}H_{15}N_2OS_2Cl$)

A mixture of 2.77 g 5-spirocyclohexyl-2,4dithiohydantoin potassium salt (**2**) (0.01 mol) and 2 cm^3 3-chloropropanoyl chloride (0.02 mol) was heated under reflux in 25 cm^3 dry DMF for 12 h. The reaction mixture was cooled to room temperature, and the obtained solid was collected by filtration and then washed with 50 cm^3 boiling water. The solid was purified by chromatography (silica gel, *n*-hexane/ether 1:1) to give 2.2 g (75% yields) as yellowish solid. TLC $R_f = 1.96$ [*n*-hexane/ether 1:1], mp 218–220 °C dec. IR (Nujol): $\bar{\nu} =$ (C=S) 1,118.5, (C=O) 1,746 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.75$ (t, $J = 6.5$ Hz, 4H, –CH_2), 2.76 (t, $J = 6.5$ Hz, 4H, –CH_2), 1.54–1.75 (m, 10H, cyclohexyl) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 210.8$ (C4), 181.2 (C2), 178.4 (C=O)$_2$, 79.5 (C5), 38.7 ($\underline{C}H_2$–Cl)$_2$, 37.57 (C6, C10), 36.3 (–$\underline{C}H_2$–)$_2$, 26.5 (C8), 20.5 (C7, C9) ppm.

1,3-Bis {3-[(2-thioxo-1,3-diazaspiro[4.5]dec-3-en-4-yl) thio]propanoyl}-1,3-diazaspiro[4,5]decane-2, 4-dithione (**7**, $C_{30}H_{40}N_6O_2S_6$)

A mixture of 1.10 g 5-spirocyclohexyl-2,4-ithiohydantoin potassium salt **2** (0.004 mol), 1.16 g of **6** (0.004 mol), and 0.002 g of KI was refluxed in 25 cm^3 DMF for 12 h. The reaction mixture was cooled in an ice bath and then acidified by the addition of 1 N hydrochloric acid. The obtained solid was collected by filtration, washed with 25 cm^3 boiling water, and recrystallized from DMF/ethanol to give 1.7 g (59% yield) yellow crystals, mp 290–292 °C dec. IR (Nujol): $\bar{\nu}$ = (C=S) 1,116, 1,115.8, (C=N) 1,541, (C=O) 1,654, 1,638, 1,718, 1,762, (NH) 3,218, 3,444, (CH_2) 2,855, 2,926 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 10.16$ (s, 2H, NH), 3.15 (t, $J = 6.3$ Hz, 4H, –CH_2–S), 2.85 (t, $J = 6.3$ Hz, 4H, –CH_2–), 1.21–1.75 (m, 30H, cyclohexyl) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 210$ (C=S), 187 (C=S)$_2$, 180 (C=S), 177.2 (C=O)$_2$, 163.7 (C=N)$_2$, 78.5 (C5), 60.5 (C5′)$_2$, 34.4 (C6, C10), 33.4 (–$\underline{C}H_2$)$_2$, 29.4 (C6′, C10′)$_2$, 23.9 (–$\underline{C}H_2$)$_2$, 27.7 (C8), 27.1 (C8′)$_2$, 19.5 (C7, C9), 19.2 (C7′, C9′)$_2$ ppm.

3-Chloro-N′-[(4-methylphenyl)sulfonyl] propanoylhydrazide (**8**, $C_{10}H_{13}N_2O_3SCl$)

To a solution of 4.65 g *p*-tosyl hydrazide (0.025 mol) in 50 cm^3 dry benzene was added 3.34 g of 3-chloropropanoyl chloride (4.77 cm^3, 0.025 mol), and this was heated under reflux for 1 h. After cooling to room temperature, the formed crystals were collected by filtration, washed with benzene/*n*-hexane, and recrystallized from ethanol to produce 6.5 g (93% yield) white crystals, mp 175–177 °C dec. IR (Nujol): $\bar{\nu}$ = (S=O) 1,159, (C=O and C=C) 1,678, 1,698, (NH–NH) 2,853, 2,953, 3,050, 3,300 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 10.13$–10.12 (d, $J = 3.3$ Hz, 1H, NH), 9.83–9.82 (d, $J = 3.3$ Hz, 1H, NH), 7.68 (d, $J = 8.1$ Hz, 2H, Ar–H), 7.34 (d, $J = 8.3$ Hz, 2H, Ar–H), 3.62 (t, $J = 6.3$ Hz, 2H, –CH_2), 2.46 (t, $J = 6.3$ Hz, 2H, –CH_2), 2.36 (s, 3H, –CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 170.5$ (C=O), 140.0, 143.1, 137.0, 130.1, 128.0, 39.9 ($\underline{C}H_2$–Cl), 37.5, 22.5 (CH_3) ppm.

2,4-Bis{3-[2-(4-methylphenyl)sulfonyl]hydrazino-3-oxopropylthio}-1,3-diazaspiro [4.5]deca-1,3-diene (**9**, $C_{28}H_{36}N_6O_6S_4$)

A mixture of 1.10 g 5-spirocyclohexyl-2,4-dithiohydantoin potassium salt (**2**) (0.004 mol), 1.10 g of 3-chloro-*N*′-[(4-methylphenyl)sulfonyl]propanoylhydrazide (**8**) (0.004 mol), and 0.002 g KI in 25 cm^3 DMF was heated under reflux for 12 h. The reaction mixture was cooled in an ice bath and then acidified by the addition of 1 N hydrochloric acid. The obtained solid was collected by filtration, washed with 25 cm^3 boiling water, and recrystallized from DMF/ethanol to give 1.5 g (55% yield) of **9** as a yellow powder, mp > 300 °C dec. IR (Nujol):$\bar{\nu}$ = (C=S) 1,116, 1,116, (S=O) 1,159, (C=O) 1,678, (C=C) 1,698, (NH–NH) 3,050, 3,178, 3,301 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 10.11$ (d, $J = 3.3$ Hz, 2H, NH), 9.81 (d, $J = 3.3$ Hz, 2H, NH), 7.85 (d, $J = 8.1$ Hz, 4H, Ar–Ho), 7.35 (d, $J = 8.3$ Hz, 4H, Ar–Hm), 3.64 (t, $J = 6.3$ Hz, 4H, –CH_2), 2.49 (t, $J = 6.3$ Hz, 4H, –CH_2), 2.36 (s, 6H, two –CH_3), 1.54–1.75 (m, 10H, cyclohexyl) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 178.2$ (C=O)$_2$, 165.0 (C=N), 164 (C=N), 141.9 (Cipso)$_2$, 136.9 (Cipso)$_2$, 132.1 (CH-ortho)$_2$, 126.9 (CH-meta)$_2$, 60.5 (C5), 38.9 (–$\underline{C}H_2$)$_2$, 30.4 (C6, C10), 24.9 (–$\underline{C}H_2$)$_2$, 28.7 (C8), 20.5 (C7, C9), 22.5 (CH_3)$_2$ ppm.

Biological testing

Parasite culture

All the promastigote cultures of both the reference and local Egypt *leishmanial* strains were maintained in blood agar-based bi-phasic Evan's modified Tobie's medium supplemented with RPMI-1640 with 25 m*M* TES at 25 °C. *Leishmanial* strains in promastigote stage that were used include *L. major* (JISH118), *L. major* (MHOMyPKy88y-DESTO), *L. tropica* (K27), *L. infantum* (LEM3437), *L. mex mex*. (LV4), and *L. donovani* (H43).

Viability test

Parasites in the promastigote stage were transferred from Evan's modified Tobie's modified to RPMI-1640 supplemented with 5% fetal bovine serum, 1% sterile human urine, and buffered with 25 m*M* TES, pH 7.2 (complete medium). They were grown in bulk at 25 °C. They were centrifuged at 2,500 rev./min for 10 min, and early log phase promastigotes were collected. The parasites were washed twice with RPMI (without FBS or urine) and re-suspended in the complete medium to achieve a final concentration of 106 parasites/cm^3. In order to get the 100% and IC_{50} mortality concentration, serial dilutions of heterocycles **1–9** were performed in 96-well micro-titer plates. Subsequently, 10^3 promastigotes in 100 µcm^3 of culture medium were added to each well, and the plate was incubated at 25 °C for 72 h. Negative controls (culture without heterocycles) were on the same plate. At the end of the incubation time, the plate was shaken mechanically over a plate shaker, and parasites were counted with the help of a hemocytometer. Dose-dependent viability curves were obtained.

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