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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antileishmanial activity and QSAR study of (1,3,4-thiadiazol-2-ylthio) acetamides derived from 5-nitrofuran

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Abstract A novel series of (1,3,4-thiadiazol-2-ylthio)acetamides derived from 5-nitrofuran were synthesized and evaluated against extracellular promastigotes of *Leishmania major*. The most potent anti-promastigote compounds were also evaluated in vitro against intracellular amastigotes. All compounds showed better activity than standard drug Glucantime. The most potent com pounds against the promastigotes were found to be *N*-(3,4-dimethoxyphenethyl)-2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio) acetamide (**5q**) and 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-*N*-propylacetamide (**5r**) with IC_{50} values less than 20 μM. Although, the cytotoxic evaluation of target compounds against mouse peritoneal macrophages demonstrated that these series of compounds have cytotoxicity at concentrations higher than 50 μM, but most of them exhibited antileishmanial activity at non-cytotoxic concentrations. QSAR study indicated that 2D-autocorrelation and topological descriptors are influential parameters in the antileishmanial activity.

Keywords Antileishmanial activity · 1,3,4-Thiadiazole · 5-Nitrofuran · QSAR study

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Introduction

Leishmaniasis is defined as a cluster of vector-borne diseases with diverse clinical manifestations, caused by the obligate intracellular protozoan parasite of the genus *Leishmania* (Sundar and Rai, 2002). Its manifestations includes three broad groups of disorders: visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis. These manifestations are caused by a total of about 21 leishmanial species, which are transmitted by around 30 species of phlebotomine sandflies (Desjeux, 1996; Bates, 2007; Rando *et al.*, 2008; Chappuis *et al.*, 2007). This disease is endemic in some tropical areas of the world and in underdeveloped countries, with an estimated 1.5–2 million cases per year in 88 countries (Reithinger *et al.*, 2007).

Chemotherapy of patients with leishmaniasis is still a serious problem as the treatment options are very limited. Pentavalent antimonial compounds were widely used as primary therapy for more than 50 years. However, resistance to these agents has been developed increasingly (Ouellette *et al.*, 2004). Antimonial agents may also cause acute pancreatitis and cardiac arrhythmia. Although, amphotericin B, pentamidine, and miltefosine have been discovered as effective antileishmanial drugs, but all these

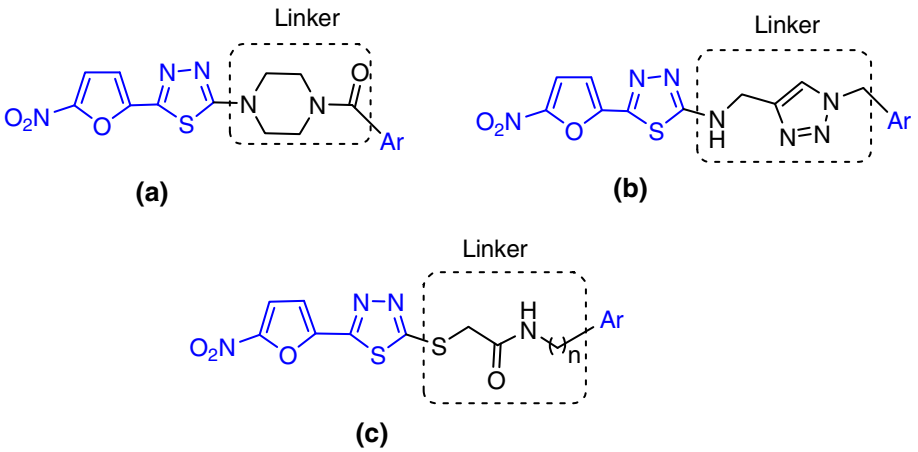


Fig. 1 Common pharmacophoric structure of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole, which is characterized by an aryl ring attached by a linker to the 2-position of 1,3,4-thiadiazole nucleus (structures **a** and **b**). 5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazole structure bearing 2-mercaptoacetamide linker (structures **c**) was designed as new antileishmanial agent

drugs suffer also from serious side effects associated with them. Major drawbacks associated with amphotericin B are life-threatening first-dose anaphylaxis, nephrotoxicity, and hypokalemia effect. Pentamidine is orally inactive and may show renal, hepatic, and pancreatic toxicity along with hypotension and dysglycemia (Kumar *et al.*, 2010). Miltefosine, originally developed as anticancer drug, is the first orally active antileishmanial agent, which have good efficacy against both visceral as well as cutaneous leishmaniasis but suffers from low therapeutic index, teratogenicity in animals, extremely long half-life (6–8 days), and relative low efficacy in HIV co-infected patients (Sindermann *et al.*, 2004). Overall, current chemotherapy against leishmaniasis is usually unsatisfactory due to some limitations including high toxicity and many adverse effects, undesirable route of administration, and unaffordable cost. These limitations lead patients withdrawing from treatment and emergence of resistant strains. Therefore, many efforts have been made to develop potential new leishmanicidal therapeutics to overcome these problems (Fuertes *et al.*, 2008; Santos *et al.*, 2008; Kedzierski *et al.*, 2009).

The most promising synthetic compounds with interesting antiparasitic activities are 5-nitroimidazoles and 5-nitrofurans which can serve as scaffolds for optimization in the field of antileishmanial chemotherapy. Recently, several compounds from 5-nitrofuran-2-yl and 5-nitroimidazolyl-1,3,4-thiadiazole series that exhibited significant antileishmanial activity have been reported by our group (Poorrajab *et al.*, 2009; Foroumadi *et al.*, 2007; Behrouzi-Fardmoghadam *et al.*, 2008; Tahghighi *et al.*, 2011, 2012). Our studies reveal the presence in all of these molecules of one common pharmacophoric portion, which is characterized by an aryl ring attached by a linker to the 2-position of 1,3,4-thiadiazole structure (Structures **a** and **b**, Fig. 1). Furthermore, the bioresponses and physicochemical properties of the molecules depends on the type of aryl ring and its linker. For example, aroylpiperazine-derived thiadiazoles (Structures **a**) bearing amide linker exhibited good antileishmanial activity against *Leishmania major* (Poorrajab *et al.*, 2009; Behrouzi-Fardmoghadam *et al.*, 2008). Therefore, it seems that the aryl pendent group with an amide linker is the most adaptable functionality for chemical change in these compounds. Accordingly, we have developed new antileishmanial agents derived from 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole structure bearing 2-mercaptoacetamide linker (Structures **c**), and displaying in vitro activity against promastigotes and amastigotes of *Leishmania major*. Thus, we report here, synthesis, antileishmanial activity, and QSAR study of 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamides **5a**–**r**.

Materials and methods

Chemistry

All starting materials, chemical reagents, and solvents used in this study were purchased from Merck AG Chemical. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide dicks). The NMR spectra were recorded on a Varian unity 500 spectrometer, and the chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. The mass spectra were run on an Agilent 6410. Merck silica gel 60 F254 plates were used for analytical TLC.

*General procedure for the synthesis of 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamides (**5a**–**r**)*

To a mixture of 5-(5-nitro-2-furyl)-1,3,4-thiadiazole-2-thiol (**3**) (1 mmol) and appropriate 2-chloroacetamide **4a**–**r** (1.1 mmol) in EtOH (8 mL), KOH solution (1.1 mmol in 2 mL H_2O) was added dropwise, and the mixture was

stirred at room temperature overnight. Then water was added, the separated solid was filtered off, washed with water, and crystallized from EtOH, to give compounds **5a–r**.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-phenylacetamide (**5a**)* Yield: 76 %; m.p. 185–187 °C; IR (KBr, cm^{-1}): 3273, 1678, 1541, 1349; ^1H NMR: 10.41 (s, 1H, NH), 7.9 (d, 1H, $J = 4$ Hz, furan), 7.62 (d, 1H, $J = 4$ Hz, furan), 7.44 (m, 2H, phenyl), 7.32 (d, 2H, $J = 8$ Hz, phenyl), 7.07 (t, 1H, $J = 8$ Hz, phenyl), 4.41 (s, 2H, $–CH_2–$); ^{13}C NMR ($CDCl_3$): 38.58 (CH_2), 114.62 (C-4, furan), 114.66 (C-3, furan), 119.19 (C-2 and C-6, phenyl), 123.71 (C-4, phenyl), 128.89 (C-3 and C-5, phenyl), 138.68 (C-1, phenyl), 145.75 (C-2, furan), 152.20 (C-5, furan), 156.25 (C-5, thiadiazole), 165.02 (C-2, thiadiazole), 167.52 (C=O). Anal. Calcd for $C_{14}H_{10}N_4O_4S_2$: C, 46.4; H, 2.78; N, 15.46. Found: C, 46.72; H, 2.50; N, 15.13.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(2-chlorophenyl)acetamide (**5b**)* Yield: 71 %; m.p. 179–181 °C; IR (KBr, cm^{-1}): 3288, 1681, 1523, 1364; ^1H NMR: 10.01 (s, 1H, NH), 7.91 (d, 1H, $J = 3.6$ Hz, furan), 7.74 (d, 1H, $J = 6.8$ Hz, phenyl), 7.64 (d, 1H, $J = 3.6$ Hz, furan), 7.51 (d, 1H, $J = 6.8$ Hz, phenyl), 7.34 (m, 1H, phenyl), 7.21 (m, 1H, phenyl), 4.41 (s, 2H, $–CH_2$); ^{13}C NMR ($CDCl_3$): 38.03 (CH_2), 114.61 (C-3 and C-4, furan), 125.77 (C-6, phenyl), 126.20 (C-4, phenyl), 126.61 (C-5, phenyl), 127.54 (C-3, phenyl), 129.57 (C-2, phenyl), 134.42 (C-1, phenyl), 145.72 (C-2, furan), 152.25 (C-5, furan), 156.32 (C-5, thiadiazole), 165.74 (C-2, thiadiazole), 167.36 (C=O). Anal. Calcd for $C_{14}H_9ClN_4O_4S_2$: C, 42.37; H, 2.29; N, 14.12. Found: C, 42.05; H, 2.54; N, 14.38.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(3-chlorophenyl)acetamide (**5c**)* Yield: 52 %; m.p. 169–171 °C; IR (KBr, cm^{-1}): 3265, 1676, 1540, 1365; ^1H NMR: 10.61 (s, 1H, NH), 7.90 (d, 1H, $J = 3.6$ Hz, furan), 7.80 (s, 1H, phenyl), 7.62 (d, 1H, $J = 3.6$ Hz, furan), 7.28–7.33 (m, 3H, phenyl), 4.42 (s, 2H, $–CH_2–$); MS (*m*/*z*, %): 396 (M^+, 8), 395 (10), 368 (34), 353 (7), 339 (23), 332 (20), 313 (46), 299 (19), 285 (23), 270 (43), 264 (69), 255 (23), 250 (23), 243 (75), 236 (53), 229 (100), 222 (23), 210 (42), 203 (34), 194 (23), 177 (100), 146 (100), 134 (60), 121 (82), 105 (7), 91 (10), 77 (10), 65 (6). Anal. Calcd for $C_{14}H_9ClN_4O_4S_2$: C, 42.37; H, 2.29; N, 14.12. Found: C, 42.35, H, 2.11; N, 13.91.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(4-chlorophenyl)acetamide (**5d**)* Yield: 62 %; m.p. 225–229 °C; IR (KBr, cm^{-1}): 3262, 1673, 1534, 1349; ^1H NMR: 10.58 (s, 1H, NH), 7.89 (d, 1H, $J = 3.6$ Hz, furan), 7.62 (d, 1H, $J = 3.6$ Hz, furan), 7.61 (d, 2H, $J = 8$ Hz, phenyl), 7.38 (d, 2H, $J = 8$ Hz, phenyl), 4.41 (s, 2H, $–CH_2–$); ^{13}C NMR ($CDCl_3$): 38.46 (CH_2), 114.59 (C-3 and C-4, furan), 120.70 (C-2 and C-6, phenyl), 127.22 (C-4, phenyl), 128.77 (C-3 and C-5, phenyl), 137.59 (C-1, phenyl), 145.70 (C-2, furan), 152.21 (C-5, furan), 156.24 (C-5, thiadiazole), 165.18 (C-2, thiadiazole), 167.34 (C=O). MS (*m*/*z*, %): 396 (M^+, 24), 270 (12), 243 (36), 210 (16), 166 (56), 127 (52), 84 (68), 66 (80). Anal. Calcd for $C_{14}H_9ClN_4O_4S_2$: C, 42.37; H, 2.29; N, 14.12. Found: C, 42.48; H, 2.62; N, 13.83.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(4-bromophenyl)acetamide (**5e**)* Yield: 60 %; m.p. 233–237 °C; IR (KBr, cm^{-1}): 3260, 1673, 1530,1364; ^1H NMR: 10.6 (s, 1H, NH), 7.89 (d, 1H, $J = 3.6$ Hz, furan), 7.62 (d, 1H, $J = 3.6$ Hz, furan), 7.56 (d, 2H, $J = 8.4$ Hz, phenyl), 7.5 (d, 2H, $J = 8.4$ Hz, phenyl), 4.41 (s, 2H, $–CH_2–$); MS (*m*/*z*, %): 441 [$(M+2)^+$, 12], 439 (18), 430 (9), 424 (12), 355 (15), 327 (15), 313 (7), 290 (23), 279 (19), 257 (68), 242 (27), 229 (69), 213 (34), 200 (38), 178 (100), 161 (61), 149 (73), 137 (27), 123 (46), 111 (50), 97 (69), 83 (65), 69 (88), 57 (73), 43 (68). Anal. Calcd for $C_{14}H_9BrN_4O_4S_2$: C, 38.11; H, 2.06; N, 12.7. Found: C, 37.92; H, 1.94; N, 12.89.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(4-methoxyphenyl)acetamide (**5f**)* Yield: 63 %; m.p. 214–215 °C; IR (KBr, cm^{-1}): 3284, 1675, 1537, 1351; ^1H NMR: 10.25 (s, 1H, NH), 7.89 (d, 1H, $J = 3.6$ Hz, furan), 7.61 (d, 1H, $J = 3.6$ Hz, furan), 7.49 (d, 2H, $J = 8.4$ Hz, phenyl), 6.89 (d, 2H, $J = 8.4$ Hz, phenyl), 4.37 (s, 2H, $–CH_2–$), 3.72 (s, 3H, $–CH_3$); MS (*m*/*z*, %): 392 (M^+, 30), 368 (7), 318 (8), 270 (100), 243 (42), 210 (11), 194 (7), 178 (8), 166 (38), 154 (7), 136 (10), 123 (96), 108 (23), 95 (11), 82 (8), 43 (7). Anal. Calcd for $C_{15}H_{12}N_4O_5S_2$: C, 45.91; H, 3.08; N, 14.28. Found: C, 45.57; H, 3.36; N, 14.15.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(4-(trifluoromethyl)phenyl)acetamide (**5g**)* Yield: 61 %; m.p. 211–212.5 °C; IR (KBr, cm^{-1}): 3273, 1678, 1535, 1359; ^1H NMR: 10.68 (s, 1H, NH), 7.93 (m, 2H, phenyl), 7.9 (d, 1H, $J = 3.6$ Hz, furan), 7.71 (m, 2H, phenyl), 7.62 (d, 1H, $J = 3.6$ Hz, furan), 4.46 (s, 2H, $–CH_2–$); ^{13}C NMR ($CDCl_3$): 38.50 (CH_2), 115.08 (C-3 and C-4, furan), 118.19 (C-4, phenyl), 119.56 (C-2 and C-6, phenyl), 123.97 (CF_3), 126.68 (C-3 and C-5, phenyl), 142.65 (C-1, phenyl), 146.16 (C-2, furan), 152.68 (C-5, furan), 156.76 (C-5, thiadiazole), 166.21 (C-2, thiadiazole), 167.72 (C=O). MS (*m*/*z*, %): 483 (M^+, 8), 430 (6), 377 (16), 356 (76), 337 (16), 270 (100), 243 (40), 218 (36), 161 (48), 145 (76), 82 (84). Anal. Calcd for $C_{15}H_9F_3N_4O_4S_2$: C, 41.86; H, 2.11; N, 13.02. Found: C, 42.91 H, 1.95; N, 12.83.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(3-nitrophenyl)acetamide (**5h**)* Yield: 70 %; m.p. 90–95 °C; IR (KBr, cm^{-1}): 3264, 1690, 1526, 1346; ^1H NMR: 10.96 (s, 1H, NH), 8.63 (s, 1H, phenyl), 7.93 (d, 1H, $J = 3.6$ Hz, furan), 7.89 (m, 2H, phenyl), 7.63 (d, 1H, $J = 3.6$ Hz, furan), 7.61 (m, 1H, phenyl), 4.46 (s, 2H, $–CH_2–$); ^{13}C NMR ($CDCl_3$): 38.41 (CH_2), 113.25 (C-4, furan), 114.65 (C-3, furan), 118.25 (C-2 and C-4, phenyl), 125.15 (C-6, phenyl), 130.41 (C-5, phenyl), 139.77 (C-1, phenyl), 145.73 (C-2, furan), 148.01 (C-3, phenyl), 152.25 (C-5, furan), 156.34 (C-5, thiadiazole), 165.93 (C-2, thiadiazole), 167.24 (C=O). MS (*m/z*, %): 407 (M^+, 8), 333 (20), 270 (100), 243 (28), 210 (8), 166 (24), 138 (28), 122 (20), 82 (44), 64 (48). Anal. Calcd for $C_{14}H_9N_5O_6S_2$: C, 41.28; H, 2.23; N, 17.19. Found: C, 41.51; H, 2.44; N, 16.9.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(2,5-dichlorophenyl)acetamide (**5i**)* Yield: 82 %; m.p. 200–202 °C; IR (KBr, cm^{-1}): 3245, 1663, 1535, 1348; ^1H NMR: 10.12 (s, 1H, NH), 7.92 (s, 1H, phenyl), 7.91 (d, 1H, $J = 3.6$ Hz, furan), 7.63 (d, 1H, $J = 3.6$ Hz, furan), 7.56 (d, 1H, $J = 9$ Hz, phenyl), 7.28 (d, 1H, $J = 9$ Hz, phenyl), 4.5 (s, 2H, $–CH_2–$); ^{13}C NMR ($CDCl_3$): 38.95 (CH_2), 113.75 (C-4, furan), 115.08 (C-3, furan), 118.19 (C-6, phenyl), 119.56 (C-4, phenyl), 123.97 (C-3, phenyl), 126. 68 (C-5, phenyl), 142.65 (C-1, phenyl), 146.15 (C-2, furan), 152.68 (C-5, furan), 156.77 (C-5, thiadiazole), 166.21 (C-2, thiadiazole), 167.71 (C=O). Anal. Calcd for $C_{14}H_8Cl_2N_4O_4S_2$: C, 38.99; H, 1.87; N, 12.99. Found: C, 39.29; H, 1.73; N, 12.75.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(2-fluoro-4-nitrophenyl)acetamide (**5j**)* Yield: 60 %; m.p. 154–156 °C; IR (KBr, cm^{-1}): 3242, 1694, 1538, 1350; ^1H NMR: 10.71 (s, 1H, NH), 8.99 (m, 1H, phenyl), 8.06 (d, 1H, $J = 8.4$ Hz, phenyl), 7.91 (d, 1H, $J = 3.6$ Hz, furan), 7.64 (d, 1H, $J = 8.4$ Hz, phenyl), 7.61 (d, 1H, $J = 3.6$ Hz, furan), 4.43 (s, 2H, $–CH_2–$); ^{13}C NMR ($CDCl_3$): 38.58 (CH_2), 114.62 (C-4, furan), 114.65 (C-3, furan), 117.93 (C-3, phenyl), 119.19 (C-5, phenyl), 123.70 (C-6, phenyl), 128.88 (C-1, phenyl), 138.68 (C-4, phenyl), 145.74 (C-2, furan), 152.25 (C-5, furan), 156.25 (C-2, phenyl), 165.02 (C-2, thiadiazole), 167.52 (C=O). MS (*m/z*, %): 425 (M^+, 14.7), 405 (43), 270 (100), 243 (39), 166 (44), 139 (27), 110 (33), 82 (48), 64 (25). Anal. Calcd for $C_{14}H_8FN_5O_6S_2$: C, 39.53; H, 1.9; N, 16.46. Found: C, 39.39; H, 1.64; N, 16.75.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(5-methylisoxazol-3-yl)acetamide (**5k**)* Yield: 75 %; m.p. 212–214 °C; IR (KBr, cm^{-1}): 3240, 1702, 1531, 1348; ^1H NMR: 11.4 (s, 1H, NH), 7.89 (d, 1H, $J = 3.6$ Hz, furan), 7.62 (d, 1H, $J = 3.6$ Hz, furan), 6.61 (s, 1H, isoxazole), 4.41 (s, 2H, $–CH_2–$), 2.3 (s, 3H, $–CH_3$); MS (*m/z*, %): 367 (M^+, 23), 313 (7), 270 (26), 258 (7), 243 (100), 229 (21), 210 (19), 194 (7), 166 (34), 153 (8), 139 (15), 125 (34), 111 (7), 99 (23), 82 (15), 69 (7), 43 (10). Anal. Calcd for $C_{12}H_9N_5O_5S_2$: C, 39.23; H, 2.47; N, 19.06. Found: C, 38.99; H, 2.61; N, 18.83.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-(benzo(d)thiazol-2-yl)acetamide (**5l**)* Yield: 51 %; m.p. 210–212 °C; IR (KBr, cm^{-1}): 3343, 1682, 1545, 1348; ^1H NMR: 7.97 (m, 1H, benzothiazole), 7.88 (d, 1H, $J = 3.6$ Hz, furan), 7.77 (d, 1H, $J = 7.6$ Hz, benzothiazole), 7.6 (d, 1H, $J = 3.6$ Hz, furan), 7.45 (m, 1H, benzothiazole), 7.32 (t, 1H, $J = 7.6$ Hz, benzothiazole), 4.55 (s, 2H, $–CH_2–$); MS (*m/z*, %): 419 (M^+, 15), 386 (15), 376 (23), 368 (65), 355 (23), 341 (19), 327 (27), 313 (40), 299 (23), 286 (100), 271 (38), 257 (57), 247 (23), 236 (73), 221 (23), 211 (23), 198 (23), 178 (27), 167 (25), 158 (19), 149 (54), 137 (30), 127 (38), 119 (23), 111 (46), 97 (71), 83 (61), 69 (77), 57 (77), 43 (69). Anal. Calcd for $C_{15}H_9N_5O_4S_3$: C, 42.95; H, 2.16; N, 16.7. Found: C, 43.21; H, 1.98; N, 16.49.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-benzylacetamide (**5m**)* Yield: 72 %; m.p. 190–192 °C; IR (KBr, cm^{-1}): 3278, 1661, 1530, 1349; ^1H NMR: 8.85 (t, 1H, $J = 5.2$ Hz, NH), 7.9 (d, 1H, $J = 3.6$ Hz, furan), 7.62 (d, 1H, $J = 3.6$ Hz, furan), 7.30–7.27 (m, 5H, phenyl), 4.32 (d, 2H, $J = 5.2$ Hz, $NCH_2–$), 4.22 (s, 2H, $SCH_2–$); ^{13}C NMR ($CDCl_3$): 37.57 (CH_2S), 42.68 (CH_2Ph), 114.49 (C-4, furan), 114.67 (C-3, furan), 126.92 (C-4, phenyl), 127.31 (C-2 and C-6, phenyl), 128.31 (C-3 and C-5, phenyl), 138.85 (C-1, phenyl), 145.79 (C-2, furan), 152.25 (C-5, furan), 156.17 (C-5, thiadiazole), 166.21 (C-2, thiadiazole), 167.68 (C=O). MS (*m/z*, %): 376 (M^+, 8), 243 (16), 229 (10), 179 (44), 181 (100), 146 (58), 123 (42), 125 (100), 110 (20), 90 (40), 82 (42), 64 (16). Anal. Calcd for $C_{15}H_{12}N_4O_4S_2$: C, 47.86; H, 3.21; N, 14.88. Found: C, 47.57; H, 3.39; N, 15.13.

*N-(4-Chlorobenzyl)-2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamide (**5n**)* Yield: 90 %; m.p. 180–181 °C; IR (KBr, cm^{-1}): 3278, 1661, 1530, 1350; ^1H NMR: 8.87 (t, 1H, $J = 5$ Hz, NH), 7.91 (d, 1H, $J = 3.6$ Hz, furan), 7.62 (d, 1H, $J = 3.6$ Hz, furan), 7.35 (d, 2H, $J = 7.6$ Hz, phenyl), 7.29 (d, 2H, $J = 7.6$ Hz, phenyl), 4.31 (d, 2H, $J = 5$ Hz, $NCH_2–$), 4.22 (s, 2H, $SCH_2–$); MS (*m/z*, %): 412 [$(M+2)^+$, 10], 409 (M^+, 7), 376 (11), 368 (8), 313 (8), 299 (9), 281 (77), 250 (46), 239 (15), 210 (10), 166 (8), 147 (100), 119 (35), 104 (19), 91 (61), 83 (15), 71 (15), 57 (19), 43 (15). Anal. Calcd for $C_{15}H_{11}ClN_4O_4S_2$: C, 43.85; H, 2.7; N, 13.64. Found: C, 44.02; H, 2.89; N, 13.85.

*N-(4-Methoxybenzyl)-2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamide (**5o**)* Yield: 57 %; m.p. 168–170 °C; IR (KBr, cm^{-1}): 3241, 1639, 1544, 1350; ^{1}H NMR: 8.77 (br s, 1H, NH), 7.91 (d, 1H, $J = 3.6$ Hz, furan), 7.62 (d, 1H, $J = 3.6$ Hz, furan), 7.18 (d, 2H, $J = 8.2$ Hz, phenyl), 6.85 (d, 2H, $J = 8.2$ Hz, phenyl), 4.24 (d, 2H, $J = 5.6$ Hz, NCH_2–), 4.19 (s, 2H, SCH_2–), 3.7 (s, 3H, $–CH_3$); ^{13}C NMR ($CDCl_3$): 37.56 (CH_2S), 42.12 (CH_2N), 55.01 (CH_3O), 113.65 (C-3 and C-5, phenyl), 114.46 (C-4, furan), 114.64 (C-3, furan), 128.71 (C-2 and C-6, phenyl), 130.73 (C-1, phenyl), 145.76 (C-2, furan), 152.23 (C-5, furan), 156.12 (C-5, thiadiazole), 158.27 (C-4, phenyl), 165.97 (C-2, thiadiazole), 167.70 (C=O). MS (*m/z*, %): 406 (M^+, 5), 329 (7), 299 (8), 177 (98), 156 (15), 146 (90), 135 (62), 121 (100), 82 (30), 64 (8). Anal. Calcd for $C_{16}H_{14}N_4O_5S_2$: C, 47.28; H, 3.47; N, 13.78. Found: C, 47.63; H, 3.24; N, 14.11.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-((furan-3-yl)methyl)acetamide (**5p**)* Yield: 80 %; m.p. 178–180 °C; IR (KBr, cm^{-1}): 3287, 1652, 1534, 1344; ^{1}H NMR: 8.85 (t, 1H, $J = 6$ Hz, –NH–), 7.91 (d, 1H, $J = 4$ Hz, furan), 7.62 (d, 1H, $J = 4$ Hz, furan), 7.20–7.32 (m, 3H, furan), 4.32 (d, 2H, $J = 6$ Hz, NCH_2–), 4.23 (s, 2H, SCH_2–); ^{13}C NMR ($CDCl_3$): 37.57 (CH_2S), 42.68 (CH_2N), 114.49 (C-4, 5-nitrofuran), 114.67 (C-3, 5-nitrofuran), 126.91 (C-3, furan), 127.31 (C-4, furan), 128.31 (C-2, furan), 138.84 (C-5, furan), 145.79 (C-2, 5-nitrofuran), 152.25 (C-5, 5-nitrofuran), 156.17 (C-5, thiadiazole), 166.21 (C-2, thiadiazole), 167.68 (C=O). MS (*m/z*, %): 366 (M^+, 3), 243 (41), 229 (17), 156 (57), 147 (100), 126 (21), 118 (100), 104 (98), 91 (100), 82 (98), 65 (80). Anal. Calcd for $C_{13}H_{10}N_4O_5S_2$: C, 42.62; H, 2.75; N, 15.29. Found: C, 42.69; H, 3.05; N, 15.04.

*N-(3,4-Dimethoxyphenethyl)-2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamide (**5q**)* Yield: 55 %; m.p. 160–162.5 °C; IR (KBr, cm^{-1}): 3321, 1653, 1542, 1345; ^{1}H NMR: 8.38 (t, 1H, $J = 5.6$ Hz, –NH–), 7.91 (d, 1H, $J = 3.2$ Hz, furan), 7.61 (d, 1H, $J = 3.2$ Hz, furan), 6.83 (d, 1H, $J = 7.6$ Hz, phenyl), 6.81 (s, 1H, phenyl), 6.7 (d, 1H, $J = 7.6$ Hz, phenyl), 4.13 (s, 2H, SCH_2–), 3.73 (s, 3H, $–CH_3$), 3.69 (s, 3H, $–CH_3$), 3.1 (m, 2H, NCH_2–), 2.66 (t, 2H, $J = 5.6$ Hz, $–CH_2$–); ^{13}C NMR ($CDCl_3$): 34.45 (CH_2Ph), 37.56 (CH_2S), 40.81 (CH_2N), 55.36 (CH_3O), 55.44 (CH_3O), 111.78 (C-2, phenyl), 112.44 (C-5, phenyl), 114.48 (C-4, furan), 114.67 (C-3, furan), 120.43 (C-6, phenyl), 131.59 (C-1, phenyl), 142.68 (C-4, phenyl), 145.80 (C-2, furan), 147.21 (C-3, phenyl), 152.23 (C-5, furan), 156.09 (C-5, thiadiazole), 166.04 (C-2, thiadiazole), 167.80 (C=O). MS (*m/z*, %): 450 (M^+, 33), 270 (19), 243 (4), 164 (100), 151 (94), 107 (25), 82 (17), 65 (16). Anal. Calcd for $C_{18}H_{18}N_4O_6S_2$: C, 47.99; H, 4.03; N, 12.44. Found: C, 47.67; H, 3.98; N, 12.40.

*2-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-N-propylacetamide (**5r**)* Yield: 62 %; m.p. 168–168.5 °C; IR (KBr, cm^{-1}): 3308, 1648, 1552, 1349; ^{1}H NMR: 8.3 (t, 1H, $J = 5.6$ Hz, –NH–), 7.9 (d, 1H, $J = 3.2$ Hz, furan), 7.62 (d, 1H, $J = 3.2$ Hz, furan), 4.14 (s, 2H, SCH_2–), 3.1 (m, 2H, NCH_2–), 1.43 (m, 2H, $–CH_2$–), 0.85 (t, 3H, $J = 6.8$ Hz, $–CH_3$); ^{13}C NMR ($CDCl_3$): 11.35 (CH_3), 22.20 ($–CH_2$–), 37.49 (CH_2S), 40.88 (CH_2N), 114.54 (C-4, furan), 114.66 (C-3, furan), 145.78 (C-2, furan), 152.26 (C-5, furan), 156.12 (C-5, thiadiazole), 165.95 (C-2, thiadiazole), 167.82 (C=O). MS (*m/z*, %): 328 (M^+, 8), 270 (15), 243 (100), 166 (17), 123 (15), 97 (44), 69 (96), 56 (78). Anal. Calcd for $C_{11}H_{12}N_4O_4S_2$: C, 40.23; H, 3.68; N, 17.06. Found: C, 39.99; H, 3.49; N, 17.33.

Biological activity

Parasite and culture

The strain of *Leishmania major* used in this study was the vaccine strain (MRHO/IR/75/ER), obtained from Pasteur Institute, Tehran (Iran) (Nazarian *et al.*, 2010). The infectivity of the parasites was maintained by regular passage in susceptible BALB/c mice. The promastigotes were grown in blood agar cultures at 25 °C. The stationary phase of promastigotes was washed with phosphate buffered saline, and re-cultured in RPMI 1640 medium (Sigma) at 2×10^6 cells/mL density, supplemented with 10 % of heat-inactivated fetal bovine serum glutamine (Sigma), pH ~7.2, 100 U/mL penicillin (Sigma), and 100 μg/mL streptomycin (Sigma).

Antileishmanial activity against promastigotes form of L. major

The antileishmanial screening of compounds **5a**–**r** was performed using MTT assay (Dutta *et al.*, 2005). It should be noted that at first, the growth curve of the *L. major* strain was determined daily under light microscope and counting in a Neubauer's chamber. Then parasites (2×10^6/mL) in the logarithmic phase were incubated with a serial concentration of test compounds for 24 h at 25 °C. To determine 50 % inhibitory concentrations (IC_{50}), the tetrazolium bromide salt (MTT) assay was used. Briefly, promastigotes from early log phase of growth were seeded in a 96-well plastic cell culture trays, containing serial dilution of compound, and phenol red-free RPMI 1640 medium, supplemented with 10 % of FBS, 2 mM glutamine, pH ~7.2, and antibiotics, in a volume of 200 μL. After 24 h of incubation at 25 °C, the media was renewed with 200 μL/well of MTT (0.5 mg/mL), and the

plates were further incubated for 4 h at 37 °C. The plates were centrifuged (2000 rpm × 5 min); the pellets were dissolved in 200 µL of DMSO. The samples were read using an ELISA plate reader at a wavelength of 492 nm.

Antileishmanial activity against amastigotes form of L. major

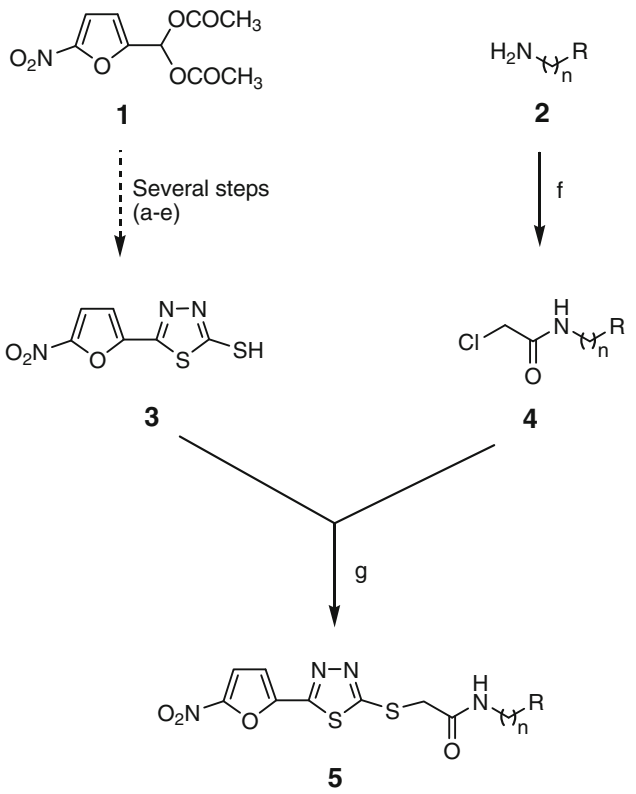
Mouse peritoneal macrophages were plated in RPMI 1640, supplemented with 10 % of heat-inactivated fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin (Sigma) ,and 100 µg/mL streptomycin. The macrophages were placed on sterile glass cover slips in 24-well plates (1×10^6/well). After 1 h, non-adherent cells were removed by washing with RPMI, the stationary phase promastigotes in RPMI were added (2×10^6 parasites/well, three parasites/macrophage) to the macrophage monolayer, and the plates were kept at 37 °C in a CO_2 incubator for 2 h. Extracellular parasites were removed by washing, and a new media containing IC_{50} concentration of the drug was added. Two sets of experiments were carried out for each drug at 24 h. Following these procedures, cells were fixed with methanol, stained with Giemsa stain (Sigma), and the number of intracellular parasites per infected macrophage was counted (100 cells were examined/well) (Tanaka *et al.*, 2007).

Toxicity against macrophages

The toxicity of compounds **5a–f**, **5h**, and **5m–r** were assessed against mouse peritoneal macrophages, plated in 96-well plates at 2×10^5 cells/well. After cell adherence, the medium was removed and replaced by the media containing different concentrations of each compounds. The plates were incubated for 24 h at 37 °C in a humidified incubator with 5 % CO_2. Cell viability was determined by MTT colorimetric assay (Kiderlen and Kaye, 1990). Two independent experiments in triplicate were performed for determination of toxicity of each compound. The CC_{50} (cytotoxic concentration for 50 % inhibition) was calculated by linear regression analysis.

QSAR study

In order to evaluate the effects of the structural parameters of the investigated derivatives on their antileishmanial activities, quantitative-structure activity relationship (QSAR) analysis with different types of molecular descriptors was performed. Several physicochemical descriptors such as hydrophobicity, topological indices, electronic parameters, and steric factors are usually used in QSAR studies in order to find the effects of different structural properties on the biological activity of



Scheme 1 Synthesis of 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio) acetamides **5**. *Reagents and conditions*: *a* thiosemicarbazide, EtOH, HCl, reflux, 1.5 h; *b* $NH_4Fe(SO_4)_2$. $12H_2O$, H_2O, reflux, 25 h; *c* $NaNO_2$, HCl, Cu, 0 °C, rt, 3 h; *d* thiourea, EtOH, reflux, 1.5 h; *e* HCl; *f* 2-chloroacetyl chloride, DMF; *g* KOH, EtOH, rt

compound of interest. Biological activity data (IC_{50}) was calibrated to their logarithmic values (pIC_{50}) in micromoles.

Since the calculated values of some electronic descriptors depend on the three-dimensional molecular geometry, the optimum 3D-geometry of the molecules was obtained by HyperChem software (Hypercube Inc, USA), using force field molecular mechanics-2 (MM2) minimization until the root mean square (RMS) gradient value becomes smaller than 0.1 kcal/mol. Minimized molecules were subjected to re-optimization via Austin model-1 (AM1) method (Khoshneviszadeh *et al.*, 2012) until the RMS gradient attained a value smaller than 0.0001 kcal/mol.

The resulting structures were used to calculate constitutional, functional, geometrical, and topological descriptors by Dragon software. Meanwhile some electronic descriptors such as frontier molecular orbital (HOMO, LUMO), dipole moment, and partial charges were calculated by the HyperChem software. For each set of descriptors, the best multilinear regression equations were obtained by the stepwise selection methods of multiple linear regression (MLR) subroutine of SPSS software (Mehdipour *et al.*, 2007).

Results and discussion

Chemistry

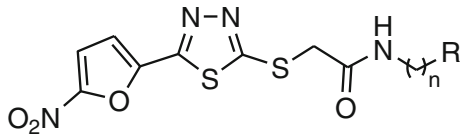
Synthesis of 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio) acetamides **5a–r** was achieved with an efficient synthetic route outlined in Scheme 1. The intermediate 5-(5-nitro-2-furyl)-1,3,4-thiadiazole-2-thiol (**3**) was prepared from 5-nitrofurfurylidine diacetate (**1**), according to the previously described method (Poorrajab *et al.*, 2009). Briefly, compound **1** was converted to thiosemicarbazone derivative and subsequently cyclized to corresponding 2-amino-1,3,4-thiadiazole by using $NH_4Fe(SO_4)_2.12\ H_2O$ in refluxing water. The 2-amino-1,3,4-thiadiazole derivative reacted with $NaNO_2$ and HCl in the presence of Cu powder. The resulting chlorinated derivative was then used in a S_N reaction with thiourea to provide the intermediate 5-(5-nitro-2-furyl)-1,3,4-thiadiazole-2-thiol (**3**). On the other hand, the 2-chloroacetamide derivatives **4a–r** were obtained from the reaction of the appropriate aliphatic or aromatic amines with chloroacetylchloride in DMF (Tahghighi *et al.*, 2011; Mohammadhosseini *et al.*, 2009). Reaction of compound **3** with 2-chloroacetamides **4a–r** gave the corresponding 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio) acetamides **5a–r** in high yield.

The structures of compounds **5a–r** were determined appropriately by IR, ^{1}H NMR, and mass spectrometry. In the IR spectrum, the title compounds showed a peak in the region of 1702–1648 cm^{-1} attributed to C=O, and absorptions in the region of 1552–1520 and 1365–1344 cm^{-1} due to the NO_2 group. The ^{1}H NMR spectroscopy revealed that the NH proton of amidic group in *N*-aryl compounds **5a–j** was located at downfield of 10.01–10.96 ppm. In the case of compounds **5m–r** containing CH_2–NH, the amidic proton was observed in the range of 8.30–8.87 as a triplet signal. The H-4 of furan ring appeared at lower field (7.88–7.93 ppm) as a doublet with coupling constant of 3.2–4.0 Hz. The doublet peak of H-3 proton of furan ring appeared at the range of 7.60–7.64 ppm with same coupling constants. The CH_2 signal of acetamide moiety was observed at 4.13–4.50 ppm. The mass spectral data of compound **5a–r** provided further evidence to their structures. In each case, the molecular ion peak was observed at the expected *m/z* value.

Biological activity

Primarily, the 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio) acetamide derivatives **5a–r** were tested against the promastigote form of the *L. major* strain in vitro using MTT assay (Table 1). The IC_{50} values of the test derivatives indicated that all derivatives exhibited better activity than Glucantime as reference drug. The most potent compounds against the promastigote form of *L. major* were found to be

Table 1 In vitro antileishmanial activity (IC_{50}, μM) of compounds **5a–r** against promastigote form of *L. major* and cytotoxic activity (CC_{50}, μM) of selected compounds against mouse peritoneal macrophages



Compounds	*R*	*n*	IC_{50} (μM)[a]	CC_{50} (μM)[b]
5a	Ph	0	46.7 ± 1.72	86.9
5b	2-Cl-Ph	0	53.8 ± 0.86	79.48
5c	3-Cl-Ph	0	40.6 ± 2.31	74.29
5d	4-Cl-Ph	0	36.4 ± 2.66	90.27
5e	4-Br-Ph	0	38.1 ± 1.03	76.71
5f	4-MeO-Ph	0	59.9 ± 1.56	64.36
5g	4-CF_3-Ph	0	122.5	–
5h	3-NO_2-Ph	0	46.7 ± 2.58	83.93
5i	2,5-di-Cl-Ph	0	178	–
5j	2-F-4-NO_2-Ph	0	559	–
5k	5-Me-isoxazol-3-yl	0	114	–
5l	Benzothiazol-2-yl	0	109.4	–
5m	Ph	1	66.4 ± 0.7	59.51
5n	4-Cl-Ph	1	54.4 ± 0.5	70.58
5o	4-MeO-Ph	1	44.6 ± 0.93	91.35
5p	Furan-3-yl	1	56.9 ± 0.71	53.55
5q	3,4-di-MeO-Ph	2	19.1 ± 1.81	55.95
5r	Me	2	19.5 ± 0.79	59.72

[a] The IC_{50} of Glucantime was 68.5 mM

[b] Cytotoxic concentration for 50 % inhibition

5q and **5r** with IC_{50} values 19.1 and 19.5 μM, respectively. By comparing the IC_{50} values of *N*-phenyl derivative **5a** with those of *N*-(3- or 4-halophenyl) analogs **5c–e**, it is revealed that halogen substituent slightly increased the anti-promastigote activity. In contrast, the introduction of 4-methoxyphenyl and 4-(trifluoromethyl)phenyl groups decreased the antileishmanial activity. Moreover, disubstituted-phenyl derivatives (**5i** and **5j**) showed less potent activity. The activity of 3-nitrophenyl compound **5h** was similar to that of *N*-phenyl derivative **5a**. Replacement of *N*-phenyl with *N*-isoxazolyl or *N*-benzothiazolyl also diminished the activity (compounds **5k** and **5l** vs. **5a**). The comparison of IC_{50} values of **5a** and **5m**, demonstrated that the introduction of *N*-benzyl instead of *N*-phenyl could not improve the inhibitory activity against promastigotes form of *L. major*. However, 4-methoxybenzyl derivative **5o** exhibited better activity (IC_{50} = 44.6 μM) than 4-methoxyphenyl counterpart **5f** (IC_{50} = 59.9 μM). Surprisingly, 3,4-dimethoxyphenethyl analog **5q** showed the highest activity. This finding showed that the distance between the aryl ring and

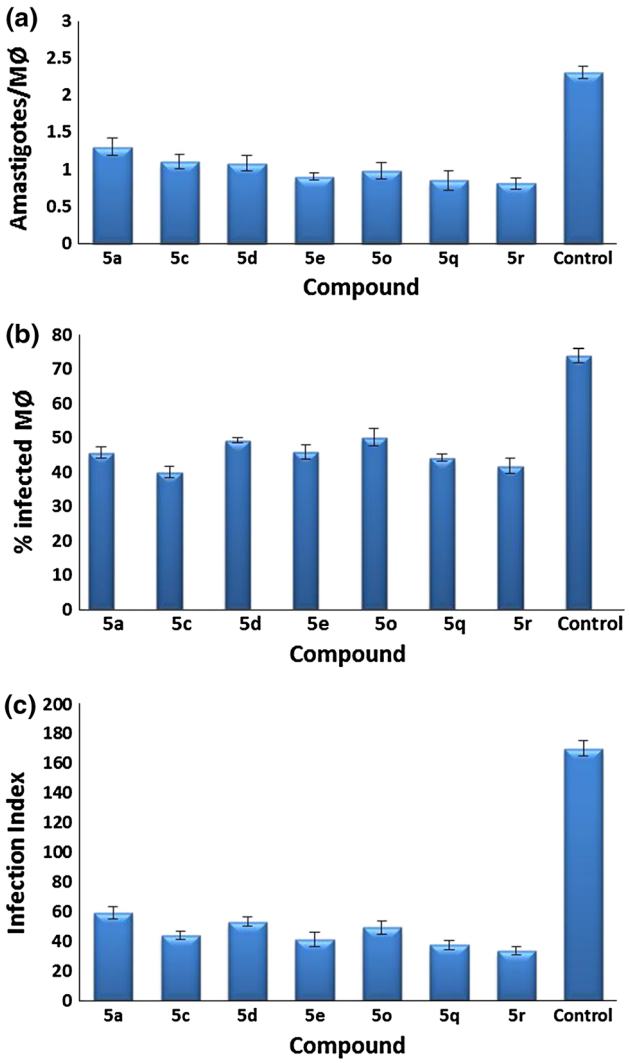


Fig. 2 In vitro activity of selected compounds against amastigotes. **a** The mean number of amastigotes per macrophage after treatment with selected compounds for 24 h. **b** The percentage of infected macrophages after treatment. **c** Infectivity index of macrophages cultured 24 h in presence of selected compound. The infectivity index was determined by multiplying the percentage of macrophages that had at least one intracellular parasite by the average number of intracellular parasite per infected macrophage (100 cells were examined/well). The infectivity index for Glucantime was 98.7

nitrogen of acetamide residue could be increased. Aside from compound **5q**, *N*-propyl derivative **5r** without any aryl ring on pendent residue, was among the most potent compounds. Thus, the aromatic ring is not required for intrinsic antileishmanial activity of these series of compounds.

Compounds **5a**, **5c–e**, **5o**, **5q**, and **5r** with IC_{50} values less than 50 μM against promastigotes were assessed against the amastigote form of *L. major*. As seen in Fig. 2, all tested compounds significantly decreased the number of intracellular amastigotes per macrophage, percentage of macrophage infectivity, and infectivity index (Fig. 2a, b, c) in comparison with control group.

Table 2 Correlation coefficient (R^2) matrix for some of descriptors used in this study

	MAXDP	MATS8e	S2k	pIC_{50} experimental
MAXDP	1	0.056	0.353	−0.713
MATS8e		1	−0.131	−0.464
S2k			1	0.184
pIC_{50} experimental				1

The cytotoxic effect of selected compounds **5a–f**, **5h**, and **5m–r** against mouse peritoneal macrophages were also evaluated using MTT assay (Table 1). The data in Table 1 revealed that compounds **5a**, **5c–e**, **5o**, **5q** and **5r** possessing IC_{50} values less than 50 μM against promastigotes had toxicity for macrophages at concentrations higher than 50 μM. Compounds **5d** and **5o** showed lower level of toxicity against macrophages ($CC_{50} > 90$ μM). In the cases of **5m** and **5p**, the inhibitory activity against macrophages was higher than inhibitory activity against promastigotes.

QSAR study

In our QSAR study, the best equations were obtained from multiple linear regressions (MLR). The correlation coefficient (R^2), standard error of regression (SE), correlation coefficient for cross-validation significance (Q^2), root mean square error (RMS), and significant level (p value) were employed to judge the validity of regression equation. As colinearity degrades the performances of the MLR-based QSAR equation, at the first, correlation analysis was performed to detect the colinear descriptors (Miri *et al.*, 2007). Thus, the correlation of descriptors with each other and with activity data was examined, and among the colinear descriptors, one of them that represented the highest correlation with activity was retained and the rest were omitted. The resulted correlation matrix is represented in Table 2 for the remaining descriptors.

Multiple regression analysis of the data gave several regression models of which the following equation was found to be the most significant model (Eq. 1):

$$\begin{aligned} pIC_{50} &= 8.780(\pm 0.742) - 1.187(\pm 0.156)\,\mathrm{MAXDP} \\ &\quad - 1.373(\pm 0.400)\,\mathrm{MATS8e} + 0.202(\pm 0.052)\,\mathrm{S2k} \\ N &= 18\; R^2 = 0.851\;\mathrm{RMScv} = 0.169 \\ \mathrm{SE} &= 0.147\; Q^2 = 0.772\; F = 26.611 \end{aligned} \quad (1)$$

In this equation, the values in the parenthesis represent the standard deviation of the coefficients.

As seen in the equation, it has high statistical quality, which can explain and predict 92.9 and 87 % of the

Table 3 Data of the selected descriptors used in this study and predicted values of pIC_{50}

Compound	MAXDP	MATS8e	S2k	pIC_{50} experimental	pIC_{50} predicted
5a	4.872	0.262	7.661	4.33	4.30
5b	4.965	0.331	8.058	4.26	4.12
5c	4.94	0.232	8.058	4.39	4.39
5d	4.924	0.153	8.058	4.44	4.37
5e	4.945	0.193	8.188	4.42	4.21
5f	4.995	0.157	8.51	4.22	4.26
5g	4.931	0.346	8.393	3.91	3.90
5h	4.983	0.049	8.456	4.33	4.67
5i	5.059	0.387	8.456	3.75	3.96
5j	5.779	0.309	8.625	3.25	3.25
5k	4.834	0.35	7.317	3.94	4.06
5l	5.115	0.275	7.903	3.96	3.83
5m	4.876	0.252	8.339	4.17	4.36
5n	4.917	0.363	8.731	4.26	4.38
5o	4.974	0.331	9.193	4.35	4.32
5p	4.756	0.362	7.81	4.24	4.18
5q	5.072	0.203	10.748	4.72	4.68
5r	4.461	0.232	7.636	4.71	4.70

variances in the antileishmanial activity. This model contains maximum electrotopological positive variation (MAXDP), Moran autocorrelation of lag8 weighted by Sanderson electronegativity (MATS8e), 2-path Kier alpha-modified shape index (S2k). The values of the descriptors used by above equation and the predicted $-\log IC_{50}$ (pIC_{50}) are listed in Table 3.

The parameter of MAXDP describes compound electrophilicity, which relates to the types of polar groups in the molecules. S2k mainly characterizes molecular shape. MATS8e is autocorrelation of Topological Structure. The 2D-autocorrelation descriptors explain how the values of certain functions, at intervals equal to the *lag*, are correlated. The 2D autocorrelation descriptors represent the topological structure of the compounds, but are more complex in nature when compared to the classical topological descriptors.

In order to confirm our results, we have predicted the antileishmanial activity of substituted 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio) acetamide derivatives against *L. major* using Eq. 1. The comparison of observed and predicted values (Table 3) demonstrated that they are close to each other evidenced by the low residual activity values. Further, it is supported by the plot of pIC_{50} observed vs. pIC_{50} predicted (Fig. 3).

In particular, to assess the predictive power of QSAR models, the correlation coefficient between the predicted and observed activities of compounds from an external test

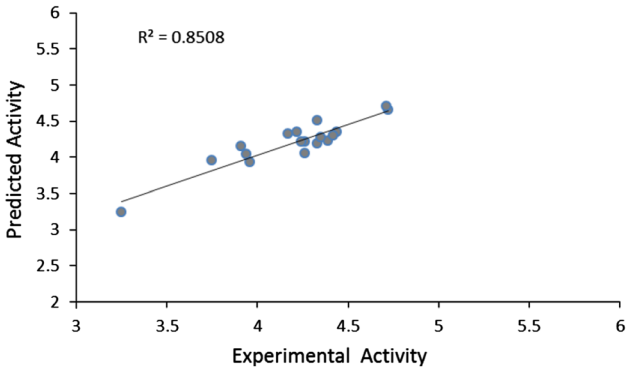


Fig. 3 Plot of cross-validated predicted values of activity by MLR against the experimental values

(R^2), the correlation coefficients for regressions through the origin (predicted versus observed activities, or observed versus predicted activities, i.e., R_0^2 or R'^2_0, respectively), and the slope of the regression lines through the origin (K and K', respectively) were calculated. A QSAR model is considered to be predictive, if all of the following conditions are satisfied: (i) $Q^2 > 0.5$, (ii) $R^2 > 0.6$, (iii) R_0^2 or R'^2_0 is close to R^2, and (iv) $0.85 \leq K \leq 1.15$ or $0.85 \leq K' \leq 1.15$ (Golbraikh and Tropsha, 2002; Tropsha *et al.*, 2003).

The proposed QSAR model in this study has all conditions to be considered as a valid model:

$$R^2 = 0.851 > 0.6;\ Q^2 = 0.772 > 0.5;\ R_0^2 = 0.88;\ R'^2_0 = 0.87;\ K = 0.95 \text{ and } K' = 0.92.$$

In overall, the Eq. 1 shows that spatial and topological descriptors play an important role in leishmaniacidal activity of these compounds. In a molecule, Moran's and Geary's spatial autocorrelation analysis tests, the value of an atomic property at one atom in the molecular structure is independent of the values of the property at neighboring atoms. If dependence exists, the property is said to exhibit spatial autocorrelation. The autocorrelation vectors represent the degree of similarity between molecules.

Conclusions

In conclusion, we have synthesized a series of 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio) acetamides and evaluated them for their antileishmanial activity against promastigotes of *L. major*. The results indicated that the anti-promastigote activity of title compounds is highly dependent on the type of substitutes positioned on the nitrogen of acetamide. The most potent compounds against the promastigotes were found to be *N*-(3,4-dimethoxyphenethyl)-2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)acetamide (**5q**)

and 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylthio)-*N*-propylacetamide (**5r**). These compounds showed also significant activity against intracellular amastigotes. Quantitative analysis of structure and anti-promastigote activity relationships of the series demonstrated that 2D-autocorrelation and topological descriptors play important roles in antileishmanial activity of these compounds. The obtained QSAR model can be used for prediction of the antileishmanial activity of further novel derivatives of this prototype.

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