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Synthesis, Characterization and Biological Evaluation of Novel 1-N-Substituted Thiocarbomoyl-3-ferrocenyl-2-pyrazoline Derivatives

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Some novel 1-*N*-substituted thiocarbomoyl-3-ferrocenyl-2-pyrazoline derivatives were synthesized and evaluated for *in vitro* antiamoebic activity against HM1:IMSS strain of *Entamoeba histolytica*. The results showed that most of the compounds exhibited promising activity (IC_{50} values in the range of 0.050-1.683 μM) than the reference drug metronidazole (IC_{50} = 1.78 μM). Active compounds were further screened for cytotoxicity against human embryonic kidney-293 (HEK-293) normal cell lines to ensure their toxic effect and the results revealed that active compounds were least toxic in the concentration range of 2.5-50 μM for 48 h and 2.5-25 μM for 72 h. At 100 μM for 48 h and at 50 μM for 72 h only four compounds **2c**, **2h**, **2k** and **2l** showed maximum viability and least cytotoxicity, respectively, concluding that all the screened compounds were least cytotoxic against human embryonic kidney-293 (HEK-293) normal cell lines in the concentration range of 2.5-50 and 2.5-25 μM.

Keywords: Novel ferrocenyl pyrazolines, *Entamoeba histolytica*, Cytotoxicity.

INTRODUCTION

Amoebiasis, also known as entamoebiasis is an infection caused by *Entamoeba histolytica*. Symptoms may include abdominal pain, mild diarrhoea, bloody diarrhoea or severe colitis with tissue death [1]. Presently, more than 50 million people are affected by this disease, causing 100,000 fatalities worldwide annually [2]. This infection also causes abscess in other body organs *viz.* liver [2] and brain [3].

Nitroimidazole such as metronidazole is the highly recommended drug for the treatment of anaerobic protozoan infections [4], but clinical resistance to nitroimidazole based drugs has been observed [5] and metronidazole also has been found carcinogenic to rodents [6]. Therefore, new therapeutic agents with least toxicity are urgently required.

Ferrocenyl derivatives with marked biologically activities have long been targets of synthetic investigations [7-10]. It has been found that the introduction of a ferrocenyl moiety into a drug molecule has been recognized as a useful approach for the development of more effective therapeutic agents [11-14].

Moreover, pyrazoles and their reduced forms, pyrazolines are an important class of nitrogen containing heterocyclic compounds which play an important role in the diverse biological activities such as antifungal [15], anticonvulsant [16], anticancer [17], antibacterial [18], antitumor [19], antimicrobial [20-24], antiamoebic [25,26], antidepressant [27,28] and anti-inflammatory [29-34].

In view of these observations and as a part of our ongoing program devoted to the synthesis of biologically active heterocycles [35-38], we report herein *in vitro* antiamoebic activity and toxicity of novel 1-*N*-substituted thiocarbomoyl-3-ferrocenyl-2-pyrazoline derivatives.

EXPERIMENTAL

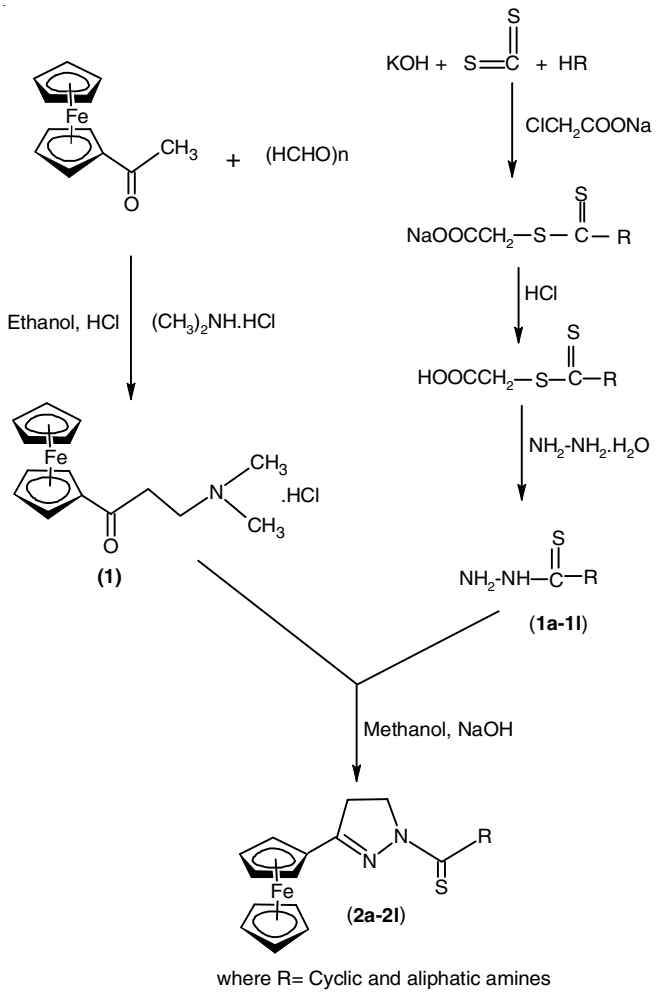
All the chemicals were purchased from Aldrich Chemical Company (USA). Precoated aluminum sheets (silica gel 60 F_{254}, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. Elemental analyses were performed on Heraeus Vario EL III analyzer. The results were within ± 0.3 % of the theoretical values. Melting points were determined on MEL-TEMP capillary melting point apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer model 1600 FT-IR RX1 spectro-

photometer as KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE 400 spectrometer using $CDCl_3$ or DMSO-d_6 as solvent with TMS as internal standard. Chemical shift values are given in ppm. ESI-MS was recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer.

General procedure for the preparation of Mannich base (1): A mixture of acetyl ferrocene (0.06 mol), paraformaldehyde (0.12 mol), dimethylamine hydrochloride (0.12 mol), absolute ethanol (150 mL) and 0.5 mL of conc. hydrochloric acid was refluxed for 3 h under nitrogen atmosphere. The resulting solution was allowed to cool and an additional 0.12 mol of paraformaldehyde was added. The mixture was then refluxed for another 3 h and allowed to cool overnight. On cooling in an ice salt bath, the red solution deposited brown crystals, which were collected and recrystallized from a minimum quantity of isopropyl alcohol to give 9.0 g (47 %) of orange yellow salt (Mannich base **1**) [39].

General procedure for the synthesis of compounds (1a-1l) thiosemicarbazones: All the *N*-4-substituted thiosemicarbazones **(1a-1l)** were prepared according to literature procedure [40], using a two-step synthetic route.

Synthesis of 1-*N*-substituted thiocarbamoyl-3-ferrocenyl-2-pyrazolines (2a-2l): To a mixture of ethanol (8 mL) acetic acid (5 mL) and water (2 mL), appropriate thiosemicarbazide (1.2 mmol) and ferrocenyl Mannich base (1 mmol) was added and refluxed under nitrogen for 10-20 h. The solvent was removed from the reaction mixture under reduced pressure. Water was added and the aqueous phase was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated (**Scheme-I**). The crude dark red residue was purified by column chromatography on silica gel (2 % ethyl acetate in chloroform) to afford the pure compound.



Scheme-I: General synthesis of 1-*N*-substituted thiocarbomoyl-3-ferrocenyl-2-pyrazoline derivatives **(2a-2l)**

3-Ferrocenyl-2-pyrazoline-1-thiocarboxamide (2a): Yield 30 %; m.p.: 160-162 °C; orange solid. Anal. calc. for $C_{14}H_{15}N_3SFe$: C 53.67, H 4.79, N 11.41 %. Found: C 53.39, H 4.82, N 11.27 %. IR (KBr, ν_{max}, cm^{-1}): 3355 (N–H), 2926 (C-H), 1545 (C=N), 1447 (C=C), 1282 (C–N), 1077 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 8.64 (s, 2H, NH_2), 4.66 (s, 1H, ferrocene), 4.52 (s, 1H, ferrocene), 4.43 (t, 2H, *J* = 9.7 Hz, H-5 pyrazoline), 4.39 (s, 2H, ferrocene), 4.06 (s, 5H, ferrocene), 3.29 (t, 2H, *J* = 9.7 Hz, H-4, pyrazoline); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.2 (C=S pyrimidine), 154.9, 68.6, 42.2 (pyrazoline) 76.0 (*ipso*-C_5H_4), 69.8, 69.6 (*meta*-C_5H_4), 68.9, 68.7 (C_5H_5), 67.7, 66.2 (*ortho*-C_5H_4), ESI-MS *m/z*: [M^++1] 314.11.

3-Ferrocenyl-2-pyrazoline-1-(N,N-diethyl)thiocarboxamide (2b): Yield 28 %; m.p.: 154-157 °C; yellow powder. Anal. calc. for. $C_{18}H_{23}N_3SFe$: C 58.53, H 5.23, N 11.38 %: Found: C 58.81, H 6.37, N 11.27 %. IR ν_{max} cm^{-1}: 2968 (C-H), 1541 (C=N), 1445 (C=C), 1225 (C–N), 1081 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.65 (s, 1H, ferrocene), 4.53 (s, 1H, ferrocene), 4.46 (t, 2H, *J* = 9.5 Hz, H-5 pyrazoline), 4.36 (s, 2H, ferrocene), 4.08 (s, 5H, ferrocene), 3.28 (t, 2H, *J* = 9.6 Hz, H-4, pyrazoline), 3.72 (q, 4H, *J* = 6.4 Hz, 2 × CH_2), 1.68 (t, 6H, *J* = 7.4 Hz, 2 × CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.0 (C=S), 155.8, 69.1, 42.2 (pyrazoline) 74.9 (*ipso*-C_5H_4), 69.8, 69.5 (*meta*-C_5H_4), 68.6, 68.32 (C_5H_5), 67.2, 66.4 (*ortho*-C_5H_4), 45.6 (2 × CH_2), 13.7 (2 × CH_3); ESI-MS *m/z*: [M^++1] 370.

3-Ferrocenyl-4,5-dihydro-1H-pyrazol-1-yl)(pyrrolidin-1-yl)methanethione (2c): Yield 38 %; m.p.: 197-199 °C; orange powder. Anal. calc. for $C_{18}H_{21}N_3S$ Fe: C 58.85, H 5.72, N 11.44 %. Found: C 58.62, H 5.81; N 11.24 %. IR (KBr, ν_{max}, cm^{-1}): 2930 (C-H), 1542 (C=N), 1437 (C=C), 1153 (C–N), 1072 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.68 (s, 1H, ferrocene), 4.54 (s, 1H, ferrocene), 4.46 (t, 2H, *J* = 8.7 Hz, H-5 pyrazoline), 4.41 (s, 2H, ferrocene), 4.07 (s, 5H, ferrocene), 3.30 (t, 2H, *J* = 9.3 Hz, H-4 pyrazoline), 2.68-3.11 (m, 4H, -CH_2 pyrrolidine), 1.79-1.89 (m, 4H, -CH_2 pyrrolidine); ^{13}C NMR (DMSO-d_6) δ (ppm): 176.8 (C=S), 155.7, 68.4, 41.6 (pyrazoline) 75.6 (*ipso*-C_5H_4), 69.9, 69.8 (*meta*-C_5H_4), 69.2, 69.0 (C_5H_5), 67.4, 66.8 (*ortho*-C_5H_4), 45.2 (2 × CH_2 pyrrolidine), 31.2 (2 × CH_2 pyrrolidine). ESI-MS *m/z*: [M^++1] 368.02.

3-Ferrocenyl-4,5-dihydro-1H-pyrazol-1-yl)(N-cyclopentyl)carbothioamide (2d): Yield 26 %; m.p.: 148-150 °C; red powder. Anal. calc. for. $C_{19}H_{23}N_3SFe$: C 59.84, H 6.03, N 11.02 % Found: C 59.59, H 6.17, N 11.32 %. IR (KBr, ν_{max}, cm^{-1}): 3335 (N-H), 2931 (C-H), 1566 (C=N), 1457 (C=C), 1173 (C–N), 1074 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 8.68 (s, 1H, N-H), 4.69 (s, 1H, ferrocene), 4.55 (s, 1H, ferrocene),

4.42 (t, 2H, *J* = 9.7 Hz, H-5 pyrazoline), 4.39 (s, 2H, ferrocene), 4.04 (s, 5H, ferrocene), 3.61 (m, 1H, -CH), 3.30 (t, 2H, *J* = 9.3 Hz, H-4, pyrazoline), 1.54-2.03 (m, 8H, $-CH_2$); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.9 (C=S), 155.6, 68.9, 42.0 (pyrazoline) 75.8 (*ipso*-C_5H_4), 69.7, 69.5 (*meta*-C_5H_4), 68.2, 68.1 (C_5H_5), 67.0, 66.3 (*ortho*-C_5H_4), 50.3 (CH), 31.2 (2 × CH_2), 24.5 (2 × CH_2). ESI-MS *m/z*: [M^++1] 380.22.

3-Ferrocenyl-2-pyrazoline-1-(N,N-dipropyl)thiocarboxamide (2e): Yield: 27 %; m.p.: 174-175 °C; red powder. Anal. calc. for $C_{20}H_{27}N_3SFe$: C 60.45, H 6.80, N 10.57 % Found: C 60.72, H 6.45, N 10.26 %. IR (KBr, ν_{max}, cm^{-1}): 2958 (C-H), 1540 (C=N), 1445 (C=C), 1226 (C–N), 1080 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.68 (s, 1H, ferrocene), 4.54 (s, 1H, ferrocene), 4.45 (t, 2H, *J* = 8.8 Hz, H-5 pyrazoline), 4.32 (s, 2H, ferrocene), 4.05 (s, 5H, ferrocene), 3.67 (t, 4H, *J* = 5.3 Hz, 2 × CH_2), 3.28 (t, 2H, *J* = 10.7 Hz, H-4, pyrazoline), 1.69-1.95 (m, 4H, 2 × CH_2), 1.05 (t, 6H, *J* = 6.6 Hz, 2 × CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 176.9 (C=S), 156.2, 69.1, 41.9 (pyrazoline) 74.3 (*ipso*-C_5H_4), 69.8, 69.6 (*meta*-C_5H_4), 68.5, 68.3 (C_5H_5), 67.7, 66.4 (*ortho*-C_5H_4), 52.6 (2 × CH_2), 32.8 (2 × CH_2), 13.7 (2 × CH_3); ESI-MS *m/z*: [M^++1] 398.

3-Ferrocenyl-2-pyrazoline-1-(N-propyl)thiocarboxamide (2f): Yield 27 %; m.p.: 176-177 °C; orange solid. Anal. calc. for. $C_{17}H_{21}N_3SFe$: C 57.46, H 5.91, N 11.83 % Found: C 57.22, H 5.61, N 11.95 %. IR (KBr, ν_{max}, cm^{-1}): 3345 (N–H), 2998 (C-H), 1555 (C=N), 1446 (C=C), 1226 (C–N), 1075 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 8.06 (s, 2H, NH_2), 4.66 (s, 1H, ferrocene), 4.53 (s, 1H, ferrocene), 4.42 (t, 2H, *J* = 9.7 Hz, H-5 pyrazoline), 4.38 (s, 2H, ferrocene), 4.02 (s, 5H, ferrocene), 3.29 (t, 2H, *J* = 9.7 Hz, H-4, pyrazoline), 3.16 (m, 2H, CH_2), 1.62-1.74 (m, 2H, CH_2), 1.02 (t, 3H, *J* = 6.15 Hz, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ (ppm): 174.5 (C=S), 155.7, 47.9, 38.2 (pyrazoline) 77.2 (*ipso*-C_5H_4), 69.7, 69.5 (*meta*-C_5H_4), 68.2, 68.1 (C_5H_5), 67.5, 67.3 (*ortho*-C_5H_4), 48.8, 23.9 (CH_2), 12.8 (CH_3); ESI-MS *m/z*: [M^++1] 356.

3-Ferrocenyl-2-pyrazoline-1-(N-butyl)thiocarboxamide (2g): Yield 36 %; m.p.: 179-180 °C; yellow powder, Anal. calc. for. $C_{18}H_{23}N_3SFe$: C 58.53, H 6.23, N 11.38 % Found: C 58.76, H 6.49, N 11.12 %. IR (KBr, ν_{max}, cm^{-1}): 3348 (N–H), 2968 (C-H), 1557 (C=N), 1446 (C=C), 1223 (C–N), 1076 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 8.15 (s, 1H, NH), 4.67 (s, 1H, ferrocene), 4.55 (s, 1H, ferrocene), 4.43 (t, 2H, *J* = 8.7 Hz, H-5 pyrazoline), 4.39 (s, 2H, ferrocene), 4.08 (s, 5H, ferrocene), 3.30 (t, 2H, *J* = 9.6 Hz, H-4, pyrazoline), 3.26 (t, 2H, *J* = 6.7 Hz, CH_2), 1.74-1.82 (m, 2H, CH_2), 1.43-1.65 (m, 2H, CH_2), 1.03 (t, 3H, *J* = 5.4 Hz, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ (ppm): 176.8 (C=S), 156.3, 69.2, 41.5 (pyrazoline) 74.2 (*ipso*-C_5H_4), 69.6, 69.3 (*meta*-C_5H_4), 68.2, 68.0 (C_5H_5), 67.3, 66.5 (*ortho*-C_5H_4), 48.6, 33.4, 22.6, (CH_2), 13.7 (CH_3); ESI-MS *m/z*: [M^++1] 370.

3-Ferrocenyl-4,5-dihydro-1H-pyrazol-1-yl)(4-methylpiperidin-1-yl)methanethione (2h): Yield 32 %; m.p.: 193-194 °C; yellow powder. Anal. calc. for $C_{20}H_{25}N_3SFe$: C 60.75, H 6.32, N 10.63 %. Found: C 60.61, H 6.67, N 10.39 %. IR (KBr, ν_{max}, cm^{-1}): 2934 (C-H), 1555 (C=N), 1463 (C=C), 1241 (C–N), 1075 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.66 (s, 1H, ferrocene), 4.52 (s, 1H, ferrocene), 4.47 (t, 2H, *J* = 10.7 Hz, H-5 pyrazoline), 4.42 (s, 2H, ferrocene), 4.05 (s, 5H, ferrocene), 3.46-3.35 (m, 4H, $-NCH_2$), 3.24 (t, 2H, *J* = 10.4 Hz, H-4 pyrazoline), 1.81-1.73 (m, 4H, $-CH_2$ piperidine), 1.49-1.34 (m, 1H, -CH, piperidine), 1.22 (s, 3H, $-CH_3$ piperidine); ^{13}C NMR (DMSO-d_6) δ (ppm): 176.0 (C=S), 156.3, 68.5, 41.5 (pyrazoline) 76.2 (*ipso*-C_5H_4), 69.8, 69.6 (*meta*-C_5H_4), 69.1, 68.4 (C_5H_5), 67.8, 66.5 (*ortho*-C_5H_4), 46.7 (2 × CH_2 piperidine), 30.9 (2 × CH_2 piperidine), 45.2 (CH), 18.9 ($-CH_3$). ESI-MS *m/z*: [M^++1] 396.25.

3-Ferrocenyl-4,5-dihydro-1H-pyrazol-1-yl)(N-cyclohexyl)carbothioamide (2i): Yield 28 %; m.p.: 203-205 °C; orange solid. Anal. calc. for $C_{20}H_{25}N_3SFe$: C 60.75, H 6.32; N 10.63 %. Found: C 60.55, H 6.41, N 10.50 %. IR (KBr, ν_{max}, cm^{-1}): 3328 (N-H), 2954 (C-H), 1568 (C=N),1455 (C=C), 1233 (C–N), 1076 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 8.57 (s, 1H, N-H), 4.67 (s, 1H, ferrocene), 4.54 (s, 1H, ferrocene), 4.43 (t, 2H, *J* = 9.7 Hz, H-5 pyrazoline), 4.41 (s, 2H, ferrocene), 4.03 (s, 5H, ferrocene), 3.72-3.61 (m, 1H, -CH), 3.29 (t, 2H, 8.6 Hz, H-4 pyrazoline), 1.78-2.24 (m, 10H, $-CH_2$); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.1 (C=S), 155.2, 69.1, 41.2 (pyrazoline) 76.4 (*ipso*-C_5H_4), 69.8, 697 (*meta*-C_5H_4), 68.3, 68.0 (C_5H_5), 67.4, 67.1 (*ortho*-C_5H_4), 53.2 (CH), 31.5, 26.3, 22.5 ($-CH_2$). ESI-MS *m/z*: [M^++1] 396.0.

3-Ferrocenyl-2-pyrazoline-1-(N-butyl methyl)thiocarboxamide (2j): Yield 27 %; m.p.: 166-168 °C; purple powder; Anal. calc. for $C_{19}H_{25}N_3SFe$: C 59.53, H 6.52, N 10.96 %. Found: C 59.82, H 6.22, N 10.76 %. IR (KBr, ν_{max}, cm^{-1}): 2954 (C-H), 1545 (C=N), 1436 (C=C), 1224 (C–N), 1084 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.68 (s, 1H, ferrocene), 4.53 (s, 1H, ferrocene), 4.41 (t, 2H, *J* = 9.3 Hz, H-5 pyrazoline), 4.37 (t, 2H, *J* = 7.9 Hz, CH_2), 4.32 (s, 2H, ferrocene), 4.04 (s, 5H, ferrocene), 3.42 (t, 2H, *J* = 9.4 Hz, CH_2), 3.28 (t, 2H, *J* = 9.8 Hz, H-4, pyrazoline), 1.65-1.84 (m, 2H, CH_2), 1.53 (s, 3H, CH_3), 1.22-1.35 (m, 2H, CH_2), 1.02 (t, 3H, *J* = 6.5 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.5 (C=S), 156.5, 69.2, 41.2 (pyrazoline) 74.2 (*ipso*-C_5H_4), 69.9, 69.7 (*meta*-C_5H_4), 68.6, 68.4 (C_5H_5), 67.6, 67.2 (*ortho*-C_5H_4), 46.3 (CH_2), 32.8 (CH_3), 31.6 (CH_2), 23.1 (CH_2), 13.3 (CH_3); ESI-MS *m/z*: [M^++1] 384.

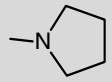
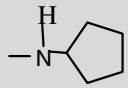
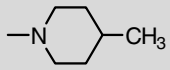
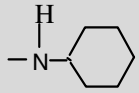
3-Ferrocenyl-2-pyrazoline-1-(N-isopropyl)thiocarboxamide (2k): Yield 22 %; m.p.: 192-194 °C; Yellow solid. Anal. calc. for. $C_{17}H_{21}N_3SFe$: C 57.47, H 5.91, N 11.82 % Found: C 57.42, H 5.93, N 11.85 %. IR (KBr, ν_{max}, cm^{-1}): 3345 (N–H), 2993 (C-H), 1558 (C=N), 1447 (C=C), 1229 (C–N), 1075 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 8.08 (s, 2H, NH_2), 4.67 (s, 1H, ferrocene), 4.50-4.56 (m, 1H, CH), 4.58 (s, 1H, ferrocene), 4.41 (t, 2H, *J* = 9.7 Hz, H-5 pyrazoline), 4.38 (s, 2H, ferrocene), 4.04 (s, 5H, ferrocene), 3.27 (t, 2H, *J* = 9.7 Hz, H-4, pyrazoline), 1.32 (t, 6H, *J* = 6.15 Hz, $-2CH_3$); ^{13}C NMR (DMSO-d_6) δ (ppm): 174.2 (C=S), 155.8, 47.3, 38.4 (pyrazoline) 77.1 (*ipso*-C_5H_4), 69.4, 69.2 (*meta*-C_5H_4), 68.3 68.1 (C_5H_5), 67.5, 67.3 (*ortho*-C_5H_4), 38.1 (CH), 14.8 ($2CH_3$); ESI-MS *m/z*: [M^++1] 356.

3-Ferrocenyl-2-pyrazoline-1-(N-isobutyl)thiocarboxamide (2l): Yield 29 %; m.p.: 122-124 °C; yellow powder, Anal. calc. for. $C_{18}H_{23}N_3SFe$: C 57.56, H 6.25, N 11.33 % Found: C 57.59, H 6.21, N 11.28 %. IR (KBr, ν_{max}, cm^{-1}): 3342 (N–H), 2968 (C-H), 1556 (C=N), 1444 (C=C), 1229 (C–N), 1072 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 8.17 (s, 1H, NH), 4.66 (s, 1H, ferrocene), 4.52 (s, 1H, ferrocene), 4.41 (t, 2H, *J* = 8.7

Hz, H-5 pyrazoline), 4.32 (s, 2H, ferrocene), 4.05 (s, 5H, ferrocene), 3.55 (t, 2H, *J* = 6.08, CH_2), 3.38 (t, 2H, *J* = 9.6 Hz, H-4, pyrazoline), 3.26 (t, 2H, *J* = 6.7 Hz, CH_2), 1.92-2.02 (m, 1H, CH, J-= 7.26), 1.05 (t, 6H, *J* = 6.08 Hz, -CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 176.5 (C=S), 157.2, 69.5, 41.8 (pyrazoline) 74.4 (*ipso*-C_5H_4), 69.5, 69.1 (*meta*-C_5H_4), 68.4, 68.2 (C_5H_5), 67.4, 66.2 (*ortho*-C_5H_4), 47.8, (CH_2), 38.9 (CH), 14.6 ($2CH_3$); ESI-MS *m/z*: [M^++1] 369.

***in vitro* Antiamoebic assay:** The newly synthesized compounds **2a-2l** were tested for *in vitro* antiamoebic activity against HM1:IMSS strain of *E. histolytica* by microdilution method according to reported procedure [36]. The IC_{50} values in μM are reported in Table-1.

TABLE-1
ANTIAMOEBIC ACTIVITY OF THE COMPOUNDS **(2a-2l)** AGAINST HM1:IMSS STRAIN OF *Entamoeba histolytica*

S. No.	R	Antiamoebic activity	
		IC_{50} (μm)	S.D.[a] (±)
2a	$-NH_2$	8.289	1.09
2b	$-N(C_2H_5)_2$	19.643	0.98
2c		**1.675**	0.34
2d		**0.504**	0.23
2e	$-N(C_3H_7)_2$	7.746	0.53
2f	$-NH-(CH_2)_2CH_3$	9.153	0.96
2g	$-NH-(CH_2)_3CH_3$	2.980	0.39
2h		**1.130**	0.65
2i		**0.128**	0.41
2j	$-N-C_4H_9(CH_3)$	**1.360**	0.77
2k	$-NH-CH(CH_3)_2$	**0.050**	1.07
2l	$-NH-CH_2CH(CH_3)_2$	**1.683**	0.82
	Metronidazole	1.780	0.19

[a]Standard Deviation. The compounds with bold font IC_{50} values are more active than metronidazole.

MTT assay protocol: The human embryonic kidney-293 (HEK-293) cells were obtained from NCCS (Pune, India). The cells were cultured in RPMI (Sigma) with 10 % foetal bovine serum and 1 % penicillin-streptomycin-neomycin. The effect of compounds **2c**, **2d**, **2h**-**2l** and the standard drug metronidazole on cell proliferation was measured by using an MTT-based assay [41]. Briefly, the cells (5,000/well) were incubated in triplicate in a 96-well plate in the presence of various concentrations of compounds **2c**, **2d**, **2h**-**2l** as well as metronidazole or vehicle (DMSO) alone in a final volume of 100 μL at 37 °C and 5 % CO_2 in and humidified atmosphere chamber for 48 h and 72 h. At the end of this time period, 20 μL of 3-(4,5-dimethylthiozole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mg/mL in PBS) was added to each well and the cells were incubated at 37 °C in a humidified atmosphere chamber for 4 h. After 4 h, the supernatant was removed from each well. The coloured formazan crystal produced from MTT was dissolved in 100 μL of DMSO and then the absorbance (A) value was measured at 570 nm by a multi-scanner auto reader. The following formula was used for the calculation of the percentage of cell viability (CV):

$$CV\ (\%) = \frac{\text{Absorbance of experimental samples}}{\text{Absorbance of the control}} \times 100$$

RESULTS AND DISCUSSION

The chemical structures of all the compounds **(2a-2l)** were characterized by IR, ^{1}H NMR, ^{13}C NMR and ESI-MS studies. Assignment of selected characteristic IR bands provides significant indications for the formation of the cyclized ferrocenyl pyrazoline analogues (**2a**-**2l**). All the compounds showed intense bands in the region 1084-1072 cm^{-1} due to the (C=S) stretch of the thiocarboxamide group. All compounds showed (C=N) stretch at 1568-1540 cm^{-1} because of the ring closure. In addition, the absorption at 1241-1153 cm^{-1} was attributed to the (C-N) stretch vibrations, which also confirms the formation of desired pyrazoline ring in all the compounds. The compounds (**2a, 2d, 2f, 2g, 2k** and **2l**) showed additional sharp band in the region 3348-3328 cm^{-1} due to the NH stretch. The structures of all compounds (**2a**-**2l**) were further supported by ^{1}H NMR spectra. The pyrazolines protons at C-4 and C-5 carbons appeared as triplets at δ 3.24-3.30 and δ 4.41-4.46 ppm, respectively. The NH protons of thiocarboxamide group of the compounds (**2a, 2d, 2f, 2g, 2k** and **2l**) showed a singlet at δ 8.04-8.68 ppm. The protons belonging to the ferrocenyl group and the other aliphatic groups were observed with the expected chemical shifts and integral values. The structures of all these compounds were further confirmed by ^{13}C NMR spectra. The C-4 and C-5 carbons of the pyrazoline ring resonate at δ 41.2-42.0 and 68.0-69.2 ppm, respectively. All the compounds showed at signal at 154.9-156.5 ppm attributed to azomethine carbon of pyrazoline ring. All compounds displayed a signal at 176.0-178.2 ppm assigned to thiocarboxamide carbon (C=S). The carbons at ferrocenyl group and 1-N substituted groups resonate at their usual positions.

Antiamoebic activities: Preliminary experiments were carried out to determine *in vitro* antiamoebic activity of all the compounds (**2a**-**2l**) by micro dilution method using HM1:IMSS strain of *E. histolytica*. The results are summarized in Table-1. The data are present in terms of percent growth inhibition relative to untreated controls and plotted as probit values as a function of drug concentration. The antiamoebic effect was compared with the most widely used antiamoebic medication metronidazole which had a 50 % inhibitory concentration (IC_{50}) of 1.78 μM in our experiments. The results showed that most of the compounds (**2c**, **2d** and **2h**-**2l**) exhibited promising activity than he reference drug metronidazole. All the pyrazoline derivatives substituted with cyclic amines (**2c**, **2d**, **2h** and **2i**) showed good activity (IC_{50} values in the range of 0.504-1.675 μM), whereas, among pyrazoline derivatives substituted with aliphatic amines only compounds (**2j**, **2k** and **2l**), exhibited increased activity (IC_{50} values in the range of 0.050-1.68 μM). Alkyl substituted pyrazolines (**2a**, **2b** and **2e**-**2g**) had no effect on the activity. Compound **2k** was emerged as most active compound with the IC_{50} value 0.050 μM among all pyrazoline derivatives. Therefore, out of

12 compounds screened for *in vitro* antiamoebic activity, 7 compounds (**2c**, **2d** and **2h**-**2l**) were found more active than the reference drug metronidazole, suggesting the bene-ficial potential of these leads that need to be further explored in order to discover and develop better and yet safer therapeutic agents for amoebiasis.

Cytotoxicity: In the present study, only active compounds (**2c, 2d** and **2h**-**2l**) were chosen for their cytotoxicity against human embryonic kidney-293 (HEK-293) normal cell lines to ensure their toxic effect. A confluent population of HEK-293 cells was treated with increasing concentrations of compounds and the number of viable cells was measured after 48 h and 72 h by MTT cell viability assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly coloured blue formazan product which, after solubilization, can be measured spectrophotometrically. This assay usually shows high correlation with number of live cells and cell proliferation. The concentration range for all the compounds (**2c, 2d**, **2h**-**2l** and MNZ) is mentioned in Fig. 1, which illustrates that all the active compounds and metronidazole were least-cytotoxic in the concentration range of 2.5-50 µM for 48 h and 2.5-25 µM for 72 h. At 100 µM for 48 h and 50 µM for 72 h, only four compounds, **2c**, **2h**, **2k** and **2l** showed maximum viability and least cytotoxicity, respectively. Therefore, it can be concluded that the cytotoxicity of all the compounds (**2c, 2d** and **2h**-**2l**) was found to be concentration-dependent and all the screened compounds were least-cytotoxic against the human embryonic kidney-293 (HEK-293) normal cell lines in the concentration range of 2.5-50 µM and 2.5-25 µM.

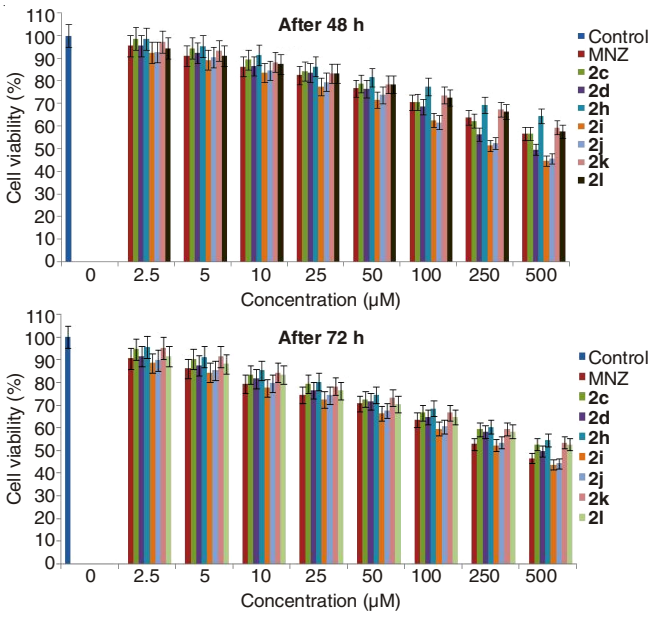


Fig. 1. Assessment of viability of HEK293 Normal cells in response to different compounds. Cells were plated in triplicates for 48 h and 72 h and treated with the compounds. Cells treated with DMSO are used as the control. MTT was added after completion of stipulated time intervals and processed. Absorbance was taken at 570 nm. Results were plotted taking control (DMSO) as 100 %

Conclusion

A new series of novel 1-*N*-substituted thiocarbomoyl-3-ferrocenyl-2-pyrazoline derivatives (**2a-2l**) were synthesized by the reaction of β-dimethylaminopropionyl ferrocene hydrochloride **(1)** with *N*-4-substituted thiosemicarbazides **(1a-1l)**. *in vitro* Antiamoebic activity was examined using HM1:IMSS strain of *E. histolytica* and results showed that most of the compounds exhibited higher antiamoebic activity (IC_{50} = 0.050-1.680 µM) than the reference drug metronidazole (IC_{50}=1.78 µM). The MTT assay revealed that active compounds and metronidazole were least toxic in the concentration range of 2.5-50 µM for 48 h and 2.5-25 µM for 72 h. At 100 µM for 48 h and at 50 µM for 72 h only four compounds **2c**, **2h**, **2k** and **2l** showed maximum viability and least cytotoxicity, respectively, concluding that all the screened compounds were least cytotoxic against human embryonic kidney-293 (HEK-293) normal cell lines in the concentration range of 2.5-50 and 2.5-25 µM. These results identified that these pyrazoline derivatives are new leads in antiamoebic chemotherapy. This study suggests that the preliminary activity of these compounds may further be explored for the development of new targets for amoebiasis.

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