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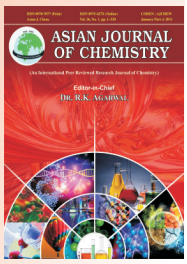

Synthesis, Characterization, Antiamoebic Activity and Toxicity of Ferrocenyl Chalcones

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Two series of ferrocenyl chalcones were synthesized and evaluated for *in vitro* antiamoebic activity against HM1:IMSS strain of *Entamoeba histolytica*. The results showed that compounds of series B having ferrocene ring adjacent to carbonyl linkage were generally more active than compounds of series A. Compounds having unsubstituted phenyl ring, methoxy substitution, thiomethyl group, chloro group and nitro group, exhibited better antiamoebic activity than the reference drug metronidazole. The toxicological studies of these compounds on human kidney epithelial cell line showed that all compounds were non-toxic. The compound 1-ferrocenyl-3-(4-nitrophenyl)-2-propen-1-one (**18**) was found most active and least toxic among all compounds.

Keywords: Ferrocenyl chalcones, Antiamoebic activity, Toxicity profile.

INTRODUCTION

Amoebiasis is the most aggressive protozoal disease and considered to be the second or third leading cause of death amongst the parasitic diseases[1]. *Entamoeba histolytica*, a protozoan parasite, is the causative agent of amoebiasis and amoebic dysentery. Though ubiquitous in distribution, this parasite is more prevalent in tropical and subtropical regions[2]. Metronidazole is known to be highly effective amoebicide and is considered to be the drug of choice for the treatment of amoebiasis, but this drug has been shown to be mutagenic in a microbiological system and carcinogenic to rodents[3-5]. Repeated treatment of *Entamoeba histolytica* infection with commonly used antiamoebic drugs results in not only increasing the toxicity potential but also leads to the development of clinical resistance. Therefore, new effective agents with less toxicity against amoebiasis are urgently required. Since the discovery of ferrocene[6,7] its derivatives have been immensely used for their potential applications in diverse fields such as homogeneous catalysis[8], organic synthesis, supramolecular chemistry[9], biosensors[10], medicinal chemistry[11-13] and material science[14]. It is now well established that ferrocene functionalized organic compounds often exhibit unexpected biological activity owing to different membrane permeation properties and anomalous metabolism[15]. Moreover, the stability and non toxicity of the ferrocenyl moiety is of particular interest rendering such drugs compatible with other treatment[12,16]. In this sense, the integration of one or more ferrocene units into a heterocyclic molecule has long been recognized as an attractive way to endow a novel molecule functionality[17]. Many ferrocenyl compounds display interesting cytotoxic, anti-tumor, antimalarial, antifungal and DNA-cleaving activity[18-20]. Recently, some new ferrocenyl-substituted heterocyclic compounds have been reported as potential pharmaceuticals[21-26]. Recently ferrocenyl chalcones demonstrated antiplasmodial[27] and nematocidal activity[28], but there is no report in literature on their *in vitro* antiamoebic activity. In view of these observations and as a part of our ongoing program devoted to the synthesis of diverse heterocyclic compounds as antiamoebic agents[29], we report herein synthesis, characterization *in vitro* antiamoebic activity and toxicity of ferrocenyl chalcones.

EXPERIMENTAL

All the chemicals were purchased from Aldrich Chemical Company (USA). Precoated aluminum sheets (silica gel 60 F254, 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 spectrophotometer 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.

ESI-MS was recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer.

General procedure for the preparation of ferrocenyl chalcones (1-18): The substituted ketone (3 mmol) and KOH (0.2 g) were dissolved in ethanol (5 mL) in a round bottomed flask and stirred at room temperature (25 ºC) for 10 min. An ethanolic solution of the substituted aromatic aldehyde (3 mmol, 5 mL) was added drop wise and the mixture was stirred at room temperature. The progress of the reaction was monitored by TLC on silica gel sheets. The reaction was stopped by neutralizing the stirred solution with 2 M HCl. In most of the cases the product was obtained as a dark red precipitate after neutralization. It was then removed by filtration, washed with water. In the absence of a precipitate on neutralization, the solution was extracted with ethyl acetate (20 mL × 3). The organic layer was dried over anhydrous sodium sulphate and removed by evaporation under reduced pressure to give a liquid residue. The latter was passed through a column of silica gel (230-400 mesh) and eluted with THF-hexane (1:4) to yield pure compound. All the synthesized compounds were well characterized by spectroscopic methods such as IR, NMR, Mass and elemental analysis and their spectral characteristics were found to be in good general agreement with those found in literature[30].

3-Ferrocenyl-1-phenyl-2-propen-1-one (1): Yield 80 %; m.p.: 140-145 °C; deep red solid; Anal. calc. for $C_{19}H_{16}FeO$: C 72.17, H 5.10 %. Found: C 72.12; H 5.06 %. IR (KBr, ν_{max}, cm^{-1}): 3031 (Ar-H), 2935 (C-H), 1655 (C=O), 1568 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.92-7.88 (m, 2H, Ar-H), 7.81 (d, 1H, *J* = 15.2 Hz, H_β), 7.57-7.15 (m, 4H, Ar-H, H_α), 4.60 (s, 2H, ferrocene), 4.48 (s, 2H, ferrocene), 4.18 (s, 5H, ferrocene); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.88 (C=O), 146.92 (C-β), (138.63, 132.44, 128.59, 128.40, aromatic), 122.17 (C-α). ESI-MS *m/z*: [M^+ + 1] 316.17.

3-Ferrocenyl-1-*p*-tolyl-2-propen-1-one (2): Yield 73 %; m.p.: 130 °C; deep red solid; Anal. calc. for $C_{20}H_{18}FeO$: C 72.75, H 5.49 %. Found: C 72.71; H 5.44 %. IR (KBr, ν_{max}, cm^{-1}): 3037 (Ar-H), 2933 (C-H), 1640 (C=O), 1562 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.89-7.83 (m, 2H, Ar-H), 7.76 (d, 1H, *J* = 15.3 Hz, H_β), 7.56-7.21 (m, 3H, Ar-H, H_α), 4.61 (s, 2H, ferrocene), 4.46 (s, 2H, ferrocene), 4.17(s, 5H, ferrocene), 2.42 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.82 (C=O), 146.48 (C-β), (138.67, 133.77, 128.59, 128.46, aromatic), 121.62 (C-α), 21.58 (CH_3). ESI-MS *m/z*: [M^+ + 1] 331.20.

3-Ferrocenyl-1-(2,5-dimethoxyphenyl)-2-propen-1-one (3): Yield 75 %; m.p.: 122 °C; deep red solid; Anal. calc. for $C_{21}H_{20}FeO_3$: C 67.04, H 5.36. Found: C 67.07, H 5.33 % IR (KBr, ν_{max}, cm^{-1}): 3041 (Ar-H), 2934 (C-H), 1646 (C=O), 1571 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.86 (s, 1H, Ar-H), 7.79 (d, 1H, *J* = 15.2 Hz, H_β), 7.61-7.38 (m, 2H, Ar-H), 7.34 (d, 1H, *J* = 15.2 Hz, H_α), 4.59 (s, 2H, ferrocene), 4.48 (s, 2H, ferrocene), 4.19 (s, 5H, ferrocene), 3.92 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.18 (C=O), 146.56 (C-β), (135.61, 134.79, 128.02, 127.56, aromatic), 122.89 (C-α), 56.65 (OCH_3). ESI-MS *m/z*: [M^+ + 1] 376.23.

3-Ferrocenyl-1-(4-methoxyphenyl)-2-propen-1-one (4): Yield 74 %; m.p.: 134 °C; deep red solid; Anal. calc. for $C_{20}H_{18}FeO_2$: C 69.39, H 5.24 %. Found: C 69.32, H 5.22 % IR (KBr, ν_{max}, cm^{-1}): 3027 (Ar-H), 2916 (C-H), 1642 (C=O), 1576 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.83-7.79 (m, 2H, Ar-H), 7.78 (d, 1H, *J* = 15.2 Hz, H_β), 7.69-7.53 (m, 2H, Ar-H,), 7.33 (d, *J* = 15.2 Hz, H_α) 4.62 (s, 2H, ferrocene), 4.44 (s, 2H, ferrocene), 4.16 (s, 5H, ferrocene), 3.82 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.29 (C=O), 145.56 (C-β), (138.07, 135.99, 131.92, 128.63, aromatic), 121.53 (C-α), 56.83 (OCH_3). ESI-MS *m/z*: [M^+ + 1] 347.00.

3-Ferrocenyl-1-(3,4,5-trimethoxyphenyl)-2-propen-1-one (5): Yield 78 %; m.p.: 123 °C; deep red solid; Anal. calc. for $C_{22}H_{22}FeO_4$: C 65.04, H 5.46 %. Found: C, 65.06, H 5.42 % IR (KBr, ν_{max}, cm^{-1}): 3054 (Ar-H), 2930 (C-H), 1650 (C=O), 1573 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.78 (s, 1H, Ar-H), 7.76 (d, 1H, *J* = 16 Hz, H_β), 7.69 (s, 1H, Ar-H), 7.36 (d, 1H, *J* = 16 Hz, H_α), 4.61 (s, 2H, ferrocene), 4.46 (s, 2H, ferrocene), 4.18 (s, 5H, ferrocene), 3.83 (s, 6H, OCH_3), 3.81 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.49 (C=O), 146.46 (C-β), (138.07, 132.70, 129.02, 128.51, aromatic), 121.98 (C-α), 60.98, 56.67 (OCH_3). ESI-MS *m/z*: [M^+ + 1] 406.25.

3-Ferrocenyl-1-(4-chlorophenyl)-2-propen-1-one (6): Yield 78 %; m.p.: 123 °C; deep red solid; Anal. calc. for $C_{19}H_{15}ClFeO$: C 65.09, H 4.31 %. Found: C, 65.04; H 4.28 % IR (KBr, ν_{max}, cm^{-1}): 3069 (Ar-H), 2930 (C-H), 1645 (C=O), 1586 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm) 7.82-7.78 (m, 2H, Ar-H), 7.75 (d, 1H, *J* = 15.2 Hz, H_β), 7.62-7.29 (m, 3H, Ar-H, H_α), 4.58 (s, 2H, ferrocene), 4.49 (s, 2H, ferrocene), 4.16 (s, 5H, ferrocene); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.23 (C=O), 145.81 (C-β), (137.73, 132.02, 128.57, 127.56, aromatic), 120.58 (C-α). ESI-MS *m/z*: [M^+ + 1] 351.6.

3-Ferrocenyl-1-(3,4-dimethylphenyl)-2-propen-1-one (7): Yield 74 %; m.p.: 120 °C; deep red solid; Anal. calc. for $C_{21}H_{20}FeO$: C 73.27, H, 5.86 %. Found: C 73.24, H 5.83 %. IR (KBr, ν_{max}, cm^{-1}): 3067 (Ar-H), 2949 (C-H), 1652 (C=O), 1566 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.83-7.77 (m, 2H, Ar-H), 7.66 (d, 1H, *J* = 15.6 Hz, H_β), 7.49-7.27 (m, 2H, Ar-H, H_α), 4.59 (s, 2H, ferrocene), 4.47 (s, 2H, ferrocene), 4.19 (s, 5H, ferrocene), 2.24 (s, 3H, CH_3), 2.16 (s,1H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.79 (C=O), 144.58 (C-β), (135.56, 132.42, 128.92, 127.63, aromatic), 121.69 (C-α). 21.45 (methyl). ESI-MS *m/z*: [M^+ +1] 345.23.

3-Ferrocenyl-1-(4-methylsulfanyl phenyl)-2-propen-1-one (8): Yield 74 %; m.p.: 140 °C; deep red solid; Anal. calc. for $C_{21}H_{20}FeOS$: C 66.31, H 5.01 %. Found: C 66.28, H 5.03 %. IR (KBr, ν_{max}, cm^{-1}): 3068 (Ar-H), 2950 (C-H), 1655 (C=O), 1564 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.86-7.84 (m, 2H, Ar-H), 7.78 (d, 1H, *J* = 15.2 Hz, H_β), 7.58 -7.43 (m, 2H, Ar-H), 7.38 (d, 1H, *J* = 15.3 Hz, H_α), 4.62 (s, 2H, ferrocene), 4.47 (s, 2H, ferrocene), 4.18 (s, 5H, ferrocene), 2.42 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ (ppm): 189.87 (C=O), 145.56 (C-β), (137.51, 133.72, 128.99, 127.43, aromatic), 122.67 (C-α). 16.7 (SCH_3). ESI-MS *m/z*: [M^+ + 1] 376.29.

3-Ferrocenyl-1-(4-nitrophenyl)-2-propen-1-one (9): Yield 71 %; m.p.: 140 °C; deep red solid; Anal. calc. for $C_{19}H_{15}FeNO_3$: C 63.18, H 4.19, N 3.85 %. Found: C 63.15, H 4.14 N 3.82 %. IR (KBr, ν_{max}, cm^{-1}): 3067 (Ar-H), 2938 (C-H), 1647 (C=O), 1578 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm) 8.12-8.06 (m, 2H, Ar-H), 7.76 (d, 1H, *J* = 15.6 Hz, H_β), 7.64 -7.22 (m, 3H, Ar-H, H_α), 4.61 (s, 2H, ferrocene), 4.48 (s, 2H, ferrocene), 4.19 (s, 5H, ferrocene); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.88 (C=O), 146.47 (C-β), (138.60, 132.72, 128.53,127.54, aromatic), 122.73 (C-α). ESI-MS *m/z*: [M^+ + 1] 362.17.

1-Ferrocenyl-3-phenyl-2-propen-1-one (10): Yield 70 %; m.p.: 122 °C; deep red solid; Anal. calc. for $C_{19}H_{16}FeO$: C 72.17, H 5.10 %. Found: C 72.14; H 5.07 %. IR (KBr, ν_{max}, cm^{-1}): 3033 (Ar-H), 2938 (C-H), 1645 (C=O), 1580 (C=C); ^1H NMR ($CDCl_3$) δ (ppm): 7.89 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.75 (d, 1H, *J* = 15.2 Hz, H_β), 7.65-7.16 (m, 4H, Ar-H, H_α), 4.92 (s, 2H, ferrocene), 4.59 (s, 2H, ferrocene), 4.22 (s, 5H, ferrocene); ^{13}C NMR ($CDCl_3$) δ (ppm): 193.04 (C=O), 144.41 (C-β), (136.07, 134.70, 130.02, 128.51, aromatic), 122.52 (C-a). ESI-MS *m/z*: [M^+ +1] 316.1.

1-Ferrocenyl-3-*p*-tolyl-2-propen-1-one (11): Yield 74 %; m.p.: 164 °C; deep red solid; Anal. calc. for $C_{20}H_{18}FeO$: C 72.75, H 5.49 %. Found: C 72.72; H 5.42 %. IR (KBr, ν_{max}, cm^{-1}): 3036 (Ar-H), 2931 (C-H), 1641 (C=O), 1582 (C=C); ^1H NMR ($CDCl_3$) δ (ppm): 7.88-7.81 (m, 2H, Ar-H), 7.73 (d, 1H, *J* = 15.6 Hz, H_β), 7.57-7.24 (m, 3H, Ar-H, H_α), 4.95 (s, 2H, ferrocene), 4.61 (s, 2H, ferrocene), 4.24 (s, 5H, ferrocene), 2.41 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 192.83 (C=O), 144.68 (C-β), (135.07, 131.70, 139.02, 128.51, aromatic), 122.89 (C-α), 21.51 (CH_3). ESI-MS *m/z*: [M^+ + 1] 331.00

1-Ferrocenyl-3-(2,5-dimethoxyphenyl)-2-propen-1-one (12): Yield 80 %; deep red liquid; Anal. calc. for $C_{21}H_{20}FeO_3$: C 67.04, H 5.36. Found: C 67.06, H 5.32 % IR (KBr, ν_{max}, cm^{-1}): 3044 (Ar-H), 2931 (C-H), 1651 (C=O), 1580 (C=C); ^1H NMR ($CDCl_3$) δ (ppm): 7.81 (s, 1H, Ar-H), 7.76 (d, 1H, *J* = 16 Hz, H_β), 7.59-7.42 (m, 2H, Ar-H), 7.36 (d, 1H, *J* = 16 Hz, H_α), 4.93 (s, 2H, ferrocene), 4.57 (s, 2H, ferrocene), 4.22 (s, 5H, ferrocene), 3.91 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 192.18 (C=O), 143.65 (C-β), (135.61, 132.70, 130.02, 128.51, aromatic), 122.23 (C-α), 55.65 (OCH_3). ESI-MS *m/z*: [M^+ +1] 376.21.

1-Ferrocenyl-3-(4-methoxyphenyl)-2-propen-1-one (13): Yield 72 %; m.p.: 121 °C; deep red solid; Anal. calc. for $C_{20}H_{18}FeO_2$: C 69.39, H 5.24 %. Found: C 69.35, H 5.26 % IR (KBr, ν_{max}, cm^{-1}): 3028 (Ar-H), 2916 (C-H), 1643 (C=O), 1574 (C=C); ^1H NMR ($CDCl_3$) δ (ppm): 7.88 -7.83 (m, 2H, Ar-H), 7.81 (d, 1H, *J* = 15.6 Hz, H_β), 7.68-7.54 (m, 2H, Ar-H,), 7.37 (d, *J* = 15.6 Hz, H_α) 4.92 (s, 2H, ferrocene), 4.58 (s, 2H, ferrocene), 4.24 (s, 5H, ferrocene), 3.84 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 192.27 (C=O), 144.44 (C-β), (135.07, 131.99, 129.92, 128.63, aromatic), 122.93 (C-α), 56.23 (OCH_3). ESI-MS *m/z*: [M^+ + 1] 347.20

1-Ferrocenyl-3-(3,4,5-trimethoxyphenyl)-2-propen-1-one (14): Yield 78 %; deep red liquid; Anal. calc. for $C_{22}H_{22}FeO_4$: C 65.04, H 5.46 %. Found: C, 65.06, H 5.42 % IR (KBr, ν_{max}, cm^{-1}): 3051 (Ar-H), 2930 (C-H), 1650 (C=O), 1565 (C=C); ^1H NMR ($CDCl_3$) δ (ppm): 7.79 (s, 1H, Ar-H), 7.74 (d, 1H, *J* = 15.2 Hz, H_β), 7.69 (s, 1H, Ar-H), 7.38 (d, 1H, *J* = 15 Hz, H_α), 4.96 (s, 2H, ferrocene), 4.58 (s, 2H, ferrocene), 4.22 (s, 5H, ferrocene), 3.85 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 192.43 (C=O), 143.49 (C-β), (135.07, 130.70, 139.02, 128.51, aromatic), 122.42 (C-α), 60.91, 56.54 (OCH_3). ESI-MS *m/z*: [M^+ + 1] 406.21

1-Ferrocenyl-3-(4-chlorophenyl)-2-propen-1-one (15): Yield 74 %; m.p.: 140 °C; deep red solid; Anal. calc. for $C_{19}H_{15}OClFe$: C 65.09, H 4.31 %. Found: C, 65.06; H 4.26 % IR (KBr, ν_{max}, cm^{-1}): 3068 (Ar-H), 2932 (C-H), 1642 (C=O), 1578 (C=C); ^1H NMR ($CDCl_3$) δ (ppm) 7.81-7.74 (m, 2H, Ar-H), 7.68 (d, 1H, *J* = 15.6 Hz, H_β), 7.51-7.26 (m, 3H, Ar-H, H_α), 4.95 (s, 2H, ferrocene), 4.61 (s, 2H, ferrocene), 4.24 (s, 5H, ferrocene); ^{13}C NMR ($CDCl_3$) δ (ppm): 192.23 (C=O), 145.14 (C-β), (135.70, 130.02, 129.53,128.51, aromatic), 122.52 (C-α). ESI-MS *m/z*: [M^+ + 1] 351.62.

1-Ferrocenyl-3-(3,4-dimethylphenyl)-2-propen-1-one (16): Yield 72 %; m.p.: 138 °C; deep red solid; Anal. calc. for $C_{21}H_{20}FeO$: C 73.27, H, 5.86 %. Found: C 73.23, H 5.87 %. IR (KBr, ν_{max}, cm^{-1}): 3068 (Ar-H), 2949 (C-H), 1650 (C=O), 1577 (C=C); ^1H NMR ($CDCl_3$) δ (ppm): 7.81-7.76 (m, 2H, Ar-H), 7.73 (d, 1H, *J* = 15 Hz, H_β), 7.58-7.29 (m, 2H, Ar-H, H_α), 4.92 (s, 2H, ferrocene), 4.58 (s, 2H, ferrocene), 4.23 (s, 5H, ferrocene), 2.24 (s, 3H, CH_3), 2.16 (s,1H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 193.49 (C=O), 144.78 (C_β), (135.51, 132.72, 128.92, 128.63, Aromatic), 122.09 (C_α). 22.45 (methyl). ESI-MS *m/z*: [M^+ + 1] 345.21.

1-Ferrocenyl-3-(4-methylsulfanyl phenyl)-2-propen-1-one (17): Yield 72 %; m.p.: 157 °C; deep red solid; Anal. calc. for $C_{21}H_{20}FeOS$: C 66.31, H 5.01 %. Found: C 66.28, H 5.03 %. IR (KBr, ν_{max}, cm^{-1}): 3068 (Ar-H), 2949 (C-H), 1685 (C=O), 1587 (C=C); ^1H NMR ($CDCl_3$) δ (ppm): 7.83-7.79 (m, 2H, Ar-H), 7.74 (d, 1H, *J* = 15.6 Hz, H_β), 7.56-7.43 (m, 2H, Ar-H), 7.33 (d, 1H, *J* = 15.6 Hz, H_α), 4.94 (s, 2H, ferrocene), 4.59 (s, 2H, ferrocene), 4.21 (s, 5H, ferrocene), 2.42 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ (ppm): 192.89 (C=O), 143.87 (C-β), (135.51, 132.72, 128.92, 128.63, aromatic), 122.14 (C-α). 16.9 (SCH_3). ESI-MS *m/z*: [M^+ + 1] 376.20.

1-Ferrocenyl-3-(4-nitrophenyl)-2-propen-1-one (18): Yield 74 %; m.p.: 145 °C; deep red solid; Anal. calc. for $C_{19}H_{15}FeNO_3$: C 63.18, H 4.19, N 3.88 %. Found: C 63.21, H 4.15 N 3.85 %. IR (KBr, ν_{max}, cm^{-1}): 3068 (Ar-H), 2935 (C-H), 1648 (C=O), 1579 (C=C); ^1H NMR ($CDCl_3$) δ (ppm) 8.13-8.05 (m, 2H, Ar-H), 7.74 (d, 1H, *J* = 15.2 Hz, H_β), 7.61-7.17 (m, 3H, Ar-H, H_α), 4.93 (s, 2H, ferrocene), 4.49 (s, 2H, ferrocene), 4.21 (s, 5H, ferrocene); ^{13}C NMR ($CDCl_3$) δ (ppm): 193.04 (C=O), 145.91 (C-β), (135.70, 130.02, 129.53,128.51, aromatic), 122.72 (C-α). ESI-MS *m/z*: [M^+ + 1] 362.10.

***in vitro* Antiamoebic assay:** All compounds **1-18** were screened *in vitro* for antiamoebic activity against HM1:IMSS strain of *E. histolytica* by microdilution method[31]. *E. histolytica* trophozoites were cultured in wells of 96-well microtiter plate by using diamond TYIS-33 growth medium[32]. The test compounds (1 mg) were dissolved in DMSO (40 mL, level at which no inhibition of amoeba occurs)[33,34]. The stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/mL. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (costar). Each test includes metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) and a blank (culture medium only). All the experiments were carried out in triplicate at each concentration level and repeated thrice. The amoeba suspension was prepared from a confluent culture by pouring off the medium at 37 °C and adding 5 mL of fresh medium, chilling the culture tube on ice to detach the organisms from the side of the flask. The number of amoeba/mL was estimated with a haemocytometer, using trypan blue exclusion to confirm the viability. The suspension was diluted to 10^5 organism/mL by adding fresh medium and 170 mL of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 mL). An inoculum of

1.7×10^4 organisms/well was chosen so that confluent, but not excessive growth, took place in control wells. Plates were sealed and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h. After incubation, the growth of amoeba in the plate was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed with sodium chloride solution (0.9 %) at 37 °C. This procedure was completed quickly and the plate was not allowed to cool in order to prevent the detachment of amoebae. The plate was allowed to dry at room temperature and the amoebae were fixed with methanol and when dried, stained with (0.5 %) aqueous eosin for 15 min. The stained plate was washed once with tap water, then twice with distilled water and allowed to dry. A 200 mL portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best fitting line from which the IC_{50} value was found. The IC_{50} values in mM are reported in Tables 1 and 2.

MTT toxicity assay: For the toxicity assay, transformed human kidney epithelial (Graham) cells were continuously maintained in culture at 37 °C in 5 % CO_2. The MTT (3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide, USB) cellular viability assay was used to determine the toxicity profile of the compound[35]. The trypsinized cell suspension was adjusted to 0.5 million cells/mL and plated out with the various compounds. After 44 h of incubation, 2 mM MTT was added to the plates and incubated for a further 4 h. DMSO was then added to stop the reaction and dissolved the formazan crystals. The absorbance was taken at 540 nm and references wavelength of 690 nm and the percentage cellular viability calculated with appropriated controls taken in account. The means ± S.D. value of IC_{50} values in Tables 1 and 2 are from three independent experiments.

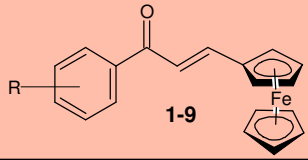
RESULTS AND DISCUSSION

Synthesis: The ferrocenyl chalcones **1-18** were synthesized according to literature procedure[27] by condensing ferrocenecarboxaldehyde/2-acetyl ferrocene/ with different substituted aromatic ketones/aldehydes in ethanolic potassium hydroxide to yield the corresponding ferrocenyl chalcones (**Scheme-I**).

Characteristic IR bands provide significant indications for the formation of the compounds**1-18**. The absence of a band at or around 2690-2660 cm^{-1} due to the aldehydic proton and the appearance of characteristic bands at 1685-1640 and 1587-1562 cm^{-1} due to α,β-unsaturated carbonyl group and ν(C=C), respectively, suggested the condensation of ferrocenecarboxaldehyde/2-acetyl ferrocene/ with different substituted aromatic ketones/aldehydes. The structures of all compounds **1-18** were further confirmed by ^1H NMR spectra. The appearance of doublets in the region of δ7.33-7.38 ppm for H_α and δ7.66-7.81 ppm for H_β with coupling constant in the range (15-16 Hz) showed that they are trans isomers. The rest of protons were appeared in the expected region. Additional support for the structure of all these compounds was obtained from ^{13}C NMR. A characteristic signal for the ferrocenyl chalcones (C=O) was appeared in the range of δ188.82-193.49 ppm. The signals at δ121.53-122.93 and 135.07-138.60 ppm revealed the presence of α, β-unsaturated keto function in all the compounds[1-18].

Antiamoebic activities: Preliminary experiments were carried out to determine the *in vitro* antiamoebic activity of all the compounds **1-18** by microdilution method using HM1: IMSS strain of *E. histolytica*. The results are summarized in Tables 1 and 2. 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 1.80 μM in our experiments. The results showed that ferrocenyl chalcones of Series B, having ferrocenyl group adjacent to carbonyl linkage were generally more

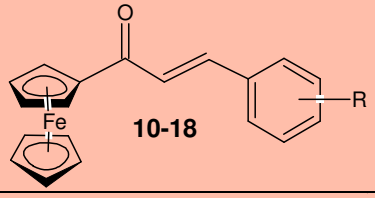
TABLE-1
FERROCENYL CHALCONES (**1-9**, **SERIES A**) THEIR ANTIAMOEBIC ACTIVITY AGAINST HM1:IMSS STRAIN OF *Entamoeba histolytica* AND TOXICITY PROFILE



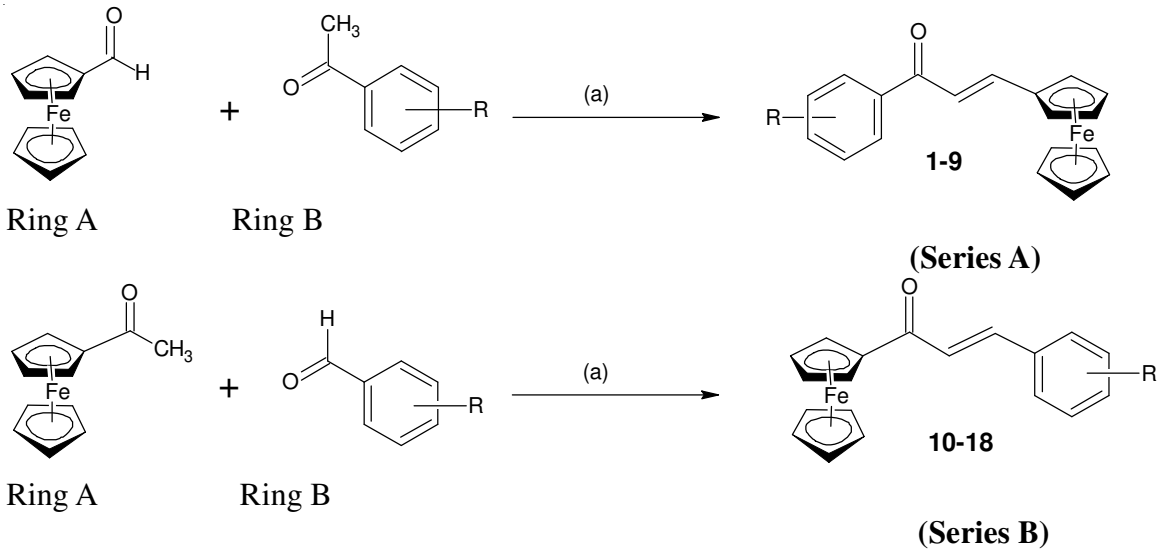
1-9

S. No.	R	Antiamoebic activity		Toxicity profile	
		IC_{50} (μM)	S.D. (±)	IC_{50} (μM)	Safety Index (S.I.)
1	H	**0.89**	0.17	>90	>101.1
2	4-Me	**2.78**	0.33	>90	>32.3
3	2,5-DiOMe	**1.76**	0.24	>90	>51.1
4	4-OMe	**1.09**	0.23	>90	>82.5
5	3,4,5-TriOMe	**1.79**	0.57	>90	>50.2
6	4-Cl	**0.51**	0.10	>90	>176.4
7	3,4-DiMe	**2.91**	0.15	>90	>30.92
8	4-SMe	**1.68**	0.27	>90	>53.5
9	4-NO_2	**0.67**	0.11	>90	>134.3

TABLE-2
FERROCENYL CHALCONES (**10-18, SERIES B**) THEIR ANTIAMOEBIC ACTIVITY AGAINST HM1:IMSS STRAIN OF *Entamoeba histolytica* AND TOXICITY PROFILE



S. No.	R	Antiamoebic activity		Toxicity profile	
		IC_{50} (μM)	S.D. (±)	IC_{50} (μM)	Safety Index (S.I.)
10	H	**0.62**	0.2	>90	>191.4
11	4-Me	**2.40**	0.45	>90	>37.5
12	2,5-DiOMe	**1.16**	0.39	>90	>77.6
13	4-OMe	**0.92**	0.41	>90	>97.8
14	3,4,5-TriOMe	**0.58**	0.12	>90	>155.1
15	4-Cl	**0.47**	0.11	>90	>191.4
16	3,4-DiMe	**2.31**	0.10	>90	>38.96
17	4-SMe	**1.57**	0.51	>90	>57.3
18	4-NO_2	**0.42**	0.15	>90	>214
19	Metronidazole	**1.80**	0.39	>100	>55.55



a = KOH, C_2H_5OH, rt , where R = Groups of different aromatic aldehydes and ketones

Compounds	Ring A	Ring B	Compound	Ring A	Ring B
1	Ph	Fc[a]	10	Fc	Ph
2	4-Me-Ph	Fc	11	Fc	4-Me-Ph
3	2,5-DiOMe-Ph	Fc	12	Fc	2,5-DiOMe-Ph
4	4-OMe-Ph	Fc	13	Fc	4-OMe-Ph
5	3,4,5-TriOMe-Ph	Fc	14	Fc	3,4,5-TriOMe-Ph
6	4-Cl-Ph	Fc	15	Fc	4-Cl-Ph
7	3,4-DiMe-Ph	Fc	16	Fc	3,4-DiMe-Ph
8	4-SMe-Ph	Fc	17	Fc	4-SMe-Ph
9	4-NO_2-Ph	Fc	18	Fc	4-NO_2-Ph

[a]Ferrocene (Fc)

Scheme-I: Synthesis of ferrocenyl chalcones (**1-18**)

active than ferrocenyl chalcones of Series A, that have ferrocene as ring A (the other ring being kept the same). These compounds (series B) had polarized carbonyl linkages, lower lipophilicities and ferrocene rings that were less readily oxidized. This is due to the fact that location of ferrocene influenced the ease of Fe^{2+} in ferrocene and the polarity of the carbonyl linkage. These parameters were found to influence the antiamoebic activity. It is evident that unsubstituted phenyl

ring found quite active in both series. Substitution of the phenyl ring with methyl group (**2**, **11**) and 3,4-dimethyl group (**7**, **16**) did not affect the activity. Thiomethyl group (**8**, **17**) mono-(**4**, **13**), Di-(**3**, **12**) and trisubstitution (**5**, **14**) of the phenyl ring with methoxy group increased the activity. Compounds (**6**, **9**, **15** and **18**) having chloro group or nitro group at *para* position of phenyl ring, exhibited an exceptionally drastic increase in the activity having IC_{50} 0.42-0.67 mM. Among all compounds studied, 1-ferrocenyl-3-(4-nitrophenyl)-2-propen-1-one (**18**) was found most active and least toxic. Therefore out of eighteen compounds screened *in vitro* for antiamoebic activity, fourteen compounds (**1**, **3-6**, **8**, **9**, **10**, **12-15**, **17** and **18**) were found more active than the reference drug metronidazole.

Toxicity profile: To ensure that the ferrocenyl chalcones were non toxic to human cells, compounds **1-18** were tested against a human kidney epithelial cell line. None of the compound inhibited cell growth at a concentration of 90 mM (Tables 1 and 2). To investigate the selectivity of the compounds, the "safety index" (SI) was calculated and defined as the toxicity IC_{50}/protozoal IC_{50}, where toxicity IC_{50} is defined as the concentration of compound that kills 50 % of the human (kidney epithelial) cell line and protozoal IC_{50} is the concentration that kills 50 % of amoeba protozoa. This allows to estimate which compound might be efficacious or toxic against human cells and potentially *in vivo*. The numerical results for each compound are given in Tables 1 and 2.

Conclusion

Two series of ferrocenyl chalcones **1-18** were synthesized by a base catalyzed Claisen-Schmidt condensation (**Scheme-I**) between ferrocenecarboxaldehyde and an appropriately substituted aromatic ketone (for Series A) and 2-acetyl ferrocene with an appropriately substituted aromatic aldehyde (for Series B). The *in vitro* antiamoebic activity was examined using HM1:IMSS strain of *E. histolytica* and results showed that compounds of series B in which ferrocene ring was adjacent to carbonyl linkage were generally more active than compounds of series A. It is due to the fact that the compounds of series B had generally lower lipophilicities, more polar carbonyl bonds and the ferrocene rings in which Fe^{2+} was more resistant to electron loss (oxidation). Out of the 18 compounds studied, 14 compounds having unsubstituted phenyl ring, methoxy substitution, thiomethyl group, chloro group and nitro group, exhibited higher antiamoebic activity than the reference drug metronidazole (IC_{50} = 1.80 mM). The MTT assay revealed that all the compounds were non-toxic to human kidney epithelial (Graham) cells. Among all compounds studied, 1-ferrocenyl-3-(4-nitrophenyl)-2-propen-1-one (**18**) was found most active and least toxic. These results identified that ferrocenyl chalcones are new leads in antiamoebic chemotherapy. The study suggests the beneficial potential of these leads that need to be further explored in order to discover and develop better and yet safer therapeutic agents for amoebiasis.

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