Received: 23 November 2017 | Revised: 10 December 2017 | Accepted: 12 December 2017

DOI: 10.1002/aoc.4261

FULL PAPER

WILEY Applied Organometallic Chemistry

Novel Pyrimidine-based Ferrocenyl substituted Organometallic Compounds: Synthesis, Characterization and Biological Evaluation

Humaira Parveen[1] | Meshari A. Alsharif[1] | Mohammed I. Alahmdi[1] | Sayeed Mukhtar[1] | Amir Azam[2]

[1]Department of Chemistry, Faculty of Science, University of Tabuk, Tabuk 71491, Kingdom of Saudi Arabia

[2]Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India

Correspondence
Humaira Parveen, Department of Chemistry, Faculty of Science, University of Tabuk, Tabuk-71491, Kingdom of Saudi Arabia.
Email: h.nabi@ut.edu.sa

Funding information
Deanship of Scientific Research (DSR), University of Tabuk, Kingdom of Saudi Arabia, Grant/Award Number: S-0117-1438

Some novel pyrimidine-based ferrocenyl substituted organometallic compounds were synthesized via multistep reactions, well characterized by different spectroscopic techniques and elemental analyses and evaluated for *in vitro* antiprotozoal susceptibility against HM1: IMSS strain of *Entamoeba histolytica*. The results of antiprotozoal susceptibility unveiled these compounds, as new leads in protozoal chemotherapy as most of the organometallics displayed an exceptionally higher antiamoebic activity (IC_{50} = 0.055 μM - 0.815 μM) than the reference drug metronidazole which gave IC_{50} (50% inhibitory concentration) value 1.781 μM in our experiments, concluding that newly synthesized organometallic compounds have potential to be employed as effective antiamoebic agents and these organometallics can be very useful for further optimization work on amoebic chemotherapy.

KEYWORDS
antiprotozoal assay, ferrocenyl unit, HM1: IMSS strain of *Entamoeba histolytica*, new leads in amoebic chemotherapy, novel pyrimidine-based organometallics

1 | INTRODUCTION

The discovery of ferrocene and ferrocene-linked organometallic compounds have made marked influence on the growth of bioorganometallic chemistry[1–3] as these organometallics have been well established their pharmaceutical potential in drug discovery, for example, ferroquine discovered as antimalarial drug and it is in phase II clinical trials,[4] ferrocifen and hydroxy ferrocifen are discovered as anticancer drugs,[5,6] also ferrocenyl conjugates of antiestrogen tamoxifen[7] and antiandrogen nilutamide[8] have been reported as more potent, curative agents than the core drug.[9]

The unique properties of ferrocene such as low toxicity to mammals, high stability, formal oxidation potential, lipophilicity, electron donating entity and iron redox activity have justified it an outstanding pharmacophore for drug design.[9]

Amoebic dysentery (amoebiasis) is caused by an anaerobic protozoan parasite *Entamoeba histolytica*. This eukaryotic parasite penetrates into the intestinal mucosa by the ingestion of food or water carrying the cysts of *Entamoeba histolytica* and causes amoebic colitis. *Entamoeba histolytica* is also the causative agent of amoebic liver abscess.[10–12] It has been reported that fifty million cases of amoebic dysentery and one lakh deaths occur annually, due to the infectious diseases caused by this protozoan parasite (*E. histolytica*).[13]

Metronidazole (an antiprotozoal drug) is extensively used for the treatment of amoebic dysentery but it has been reported to clinical resistance to anaerobic protozoans,[14] mutagenic in microbiological system and

carcinogenic in rodents[15–17] and it has been also associated with adverse side effects such as sensory neuropathies, toxicity with ataxia, metallic taste, vomiting, diarrhea, vertigo, seizures and encephalopathy.[18,19] Moreover, in some cases, failure of treatment with metronidazole has also been reported.[10,20,21] Therefore, the search of new effective antiprotozoal agents is urgently needed to develop potent yet safer therapeutic agents for amoebiasis.

Furthermore, pyrimidines are ubiquitous, being an essential part of nucleic acids, involve in several biological processes and are main constituents of many drugs such as Zidovudine (anti-HIV), Lamivudine (anti HIV-AIDS, Hepatitis B) and many of its derivatives display widespread pharmacological properties such as anti-cancer,[22] antiviral,[23] antimalarial,[24] diuretic,[25] anti HIV-1,[26,27] antitumor[28] and antiplasmodial activities.[29] Moreover, extensive literature survey on nucleobase-derived compounds in modern medicinal chemistry also unveiled the importance of ferrocenyl nucleobases and half-sandwich nucleobase derivatives.[30–32]

In the recent decades, morpholine scaffold has emerged as an outstanding pharmacophore in medicinal chemistry and a number of clinically approved drugs[33] such as linezolid, aprepitant, and gefitinib[34,35] possesses morpholine unit in their molecules. Morpholine derivatives also have been reported as novel scaffold for new drug discovery.[36] Also, a number of reports have been suggested the potential of morpholine derivatives as effective anticancer agents.[37–43] Beside this, morpholine containing compounds also have been reported to exhibit diverse biological properties[44–49] such as γ-secretase inhibitors,[50] antioxidant[51] and anti-inflammatory.[52] According to mechanism of action of morpholine derivatives it is clear, that these derivatives prevent the biosynthesis of sterol by blocking two successive enzymatic processes: (1) inhibiting the biotransformation of lanosterol into zymosterol by blocking the enzyme C-14 sterol reductase and; (2) inhibiting the synthesis of ergosterol from the biotransformation of fecosterol into episterol by blocking the enzyme C-8 sterol isomerase.[53,54] The advantage of synthesizing morpholine derivatives resides in the fact that morpholine derivatives provide chlorhydrates that are soluble in water for pharmacological assays.[55]

In view of above observations and in continuation of our ongoing research based on to explore biologically active organometallic/heterocyclic compounds,[56,57] we report herein synthesis and characterization of some novel pyrimidine-based ferrocenyl substituted organometallic compounds containing morpholine unit, and evaluation of their in vitro antiprotozoal studies against HM1: IMSS strain of *Entamoeba histolytica*.

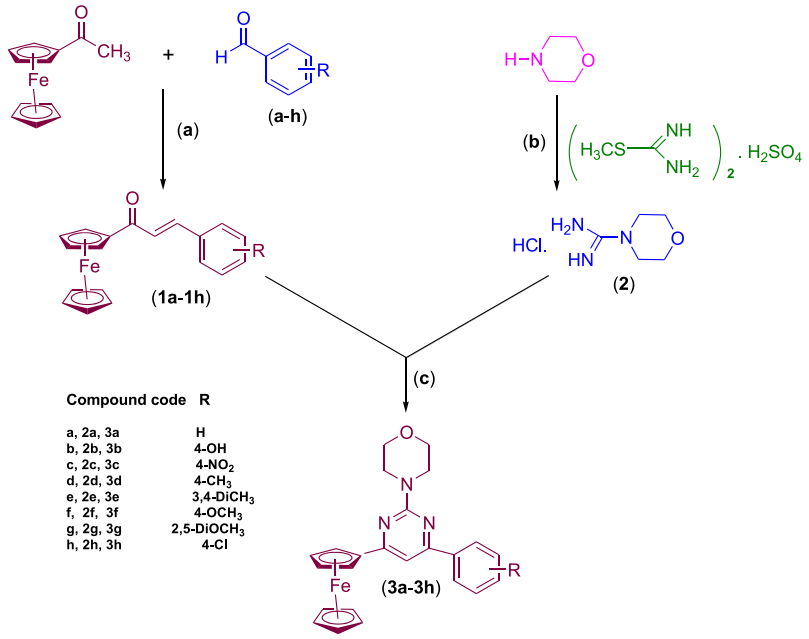
2 | RESULTS AND DISCUSSION

2.1 | Synthesis and characterization

The synthesis of novel pyrimidine-based ferrocenyl substituted organometallics (**3a-3h**) was carried out as illustrated in Scheme 1. The ferrocenyl chalcones (**1a-1h**) were synthesized according to the reported method[58] by the Claisen–Schmidt condensation of acetyl ferrocene with substituted aromatic aldehydes in the presence of potassium hydroxide and absolute ethanol. Morpholin-4-carboxamidine hydrochloride (**2**) was prepared according to reported procedure[59] by refluxing morpholine with *S*-methyl isothiourea sulfate in water. The cyclization of ferrocenyl chalcones (**1a-1h**) with morpholin-4-carboxamidine hydrochloride (**2**) in the presence of sodium isopropoxide led to the formation of corresponding pyrimidine-based ferrocenyl substituted organometallic compounds (**3a-3h).** The structures of all compounds were established by different spectroscopic techniques such as IR, ^{1}H-NMR and ^{13}C NMR and Mass spectrometry. The purity of compounds was confirmed by elemental analysis which was found in accordance with ± 0.3%.

The data of selected characteristic IR bands provide significant indications of the formation of the pyrimidine-based organometallics (**3a-3h)**. All compounds showed sharp bands in the region 1571–1577 cm^{-1} due to C=N stretch which confirmed the formation of pyrimidine ring. In addition, the absorption bands in the region 1251–1258 cm^{-1} were assigned to the C–N stretch vibrations, which also confirm the formation of desired pyrimidine ring in all organometallics. The structures of compounds were further confirmed by ^{1}H-NMR and ^{13}C NMR. The appearance of a singlet in the range of δ 7.32-7.38 was attributed to the C-H proton of pyrimidine ring in all organometallics (**3a-3h**). The chemical shift values for aromatic protons, ferrocene ring protons and morpholine ring protons resonated at their usual position, and the data are given in the experimental section.

Further evidence for the formation of pyrimidine-based organometallics was obtained from their ^{13}C NMR spectra. A signal in the range of 166.2–166.9 and 162.5–162.8 was attributed to C=N and C=C respectively in all organometallics (**3a-3h)**. The characteristic signal for C-H of pyrimidine ring in all organometallics has appeared in the range of 104.2-104.6, which clearly favored the formation of pyrimidine nucleus in all compounds (**3a-3h**). Other signals attributed to phenyl, ferrocenyl and morpholine moieties, resonated at their usual positions, and their values are given in the experimental section.



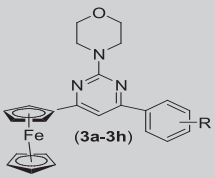
SCHEME 1 General synthesis of pyrimidine-based ferrocenyl substituted organometallics (**3a-3h**)

2.2 | Pharmacology (In vitro antiamoebic activity)

All novel organometallics (**3a-3h)** were evaluated in vitro antiamoebic activity against HM1: IMSS strain of *Entamoeba histolytica* by microdilution method.[60] All experiments were performed in triplicate at each concentration level and repeated three times. The results of the antiprotozoal assay are given in Table 1.

The data have been given in terms of percent growth inhibition relative to untreated controls and plotted as probit values as a function of drug concentration.

TABLE 1 Novel pyrimidine-based ferrocenyl substituted organometallics (**3a-3h**), their antiamoebic activity against HM1: IMSS strain of *Entamoeba histolytica*



S. No.	R	Antiamoebic activity	
		IC_{50} (μM)	S. D.[a] (±)
3a	H	**0.815**	±0.011049
3b	4-OH	**0.142**	±0.010578
3c	4-NO_2	3.780	±0.003505
3d	4-CH_3	4.541	±0.002976
3e	3,4-DiCH_3	**0.697**	±0.002659
3f	4-OCH_3	**0.055**	±0.004103
3g	2,5-Di OCH_3	**0.251**	±0.004257
3h	4-Cl	7.717	±0.001923
Metronidazole		1.781	±0.003151

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

Metronidazole was used as a reference drug to compare antiamoebic effect which gave IC_{50} (50% inhibitory concentration) 1.781 μM in our experiments.

The results showed that in pyrimidine-based organometallics when the R group was a phenyl ring the morpholine-linked compound (**3a**) showed IC_{50} 0.815 μM. Substitution of the phenyl ring with hydroxyl group (**3b**), dimethyl group (**3e**), monomethoxy (**3f**) and dimethoxy (**3g**) enhanced the antiamoebic activity, as all these compounds exhibited IC_{50} in the range of 0.055 μM-0.815 μM, which is higher than the standard drug metronidazole. However, nitro group (3c), methyl group (3d) and chloro group (**3h**) did not affect the activity. In general, compound (**3f**) emerged as the most active antiamoebic organometallic with IC_{50} 0.055 μM with respect to standard drug metronidazole (IC_{50} 1.781 μM).

The results of antiamoebic activity identified these organometallics as useful leads in amoebic chemotherapy.

3 | CONCLUSION

A series of some novel pyrimidine-based ferrocenyl substituted organometallic compounds were synthesized as new leads in antiamoebic chemotherapy. The structures of all organometallics (**3a**-3h) were characterized by different analytical techniques and elemental analyses. The in vitro antiamoebic activity was performed by microdilution method against HM1: IMSS strain of *Entamoeba histolytica*.

The results of antiamoebic activity revealed that most of the organometallics exhibited exceptionally higher IC_{50} values ranging from 0.055 μM to 0.815 μM, than the reference drug Metronidazole (IC_{50} = 1.781 μM). 4-(4-methoxyphenyl)-6-ferrocenyl-2-morpholin-1-yl-pyrimidine (**3f**) was emerged as most potent antiamoebic agent with the IC_{50} = 0.055 μM. The results of antiamoebic activity concluded that these novel organometallic compounds have potential to be employed as effective antiamoebic agents and can be very useful for further optimization work on amoebic chemotherapy.

4 | EXPERIMENTAL

Acetylferrocene, substituted aromatic aldehydes, morpholine, other chemicals, and reagents were purchased from Sigma Aldrich, St. Louis, MO, USA). Thin-layer chromatography (TLC) was performed on precoated aluminum sheets (silica gel 60 F_{254}, Merck, Darmstadt, Germany) 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 Thomas Hoover capillary melting 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 $CDCl_3$ as a solvent with tetramethylsilane (TMS) as an internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; m, multiplet. Chemical shift (δ) values are given in ppm. ESI-MS was recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer.

4.1 | General procedure for the synthesis of ferrocenyl chalcones (1a-1h)

Ferrocenyl chalcones (**1a-1 h)** were synthesized according to reported method[58] and their data has been found in agreement with the reported ones.[58] A mixture of acetyl ferrocene (6 mmol) and potassium hydroxide (7.14 mmol) was dissolved in absolute ethanol (50 ml) and stirred for 15 minutes at room temperature, then a solution of substituted aromatic aldehydes (prepared in absolute ethanol) was added drop wise to the reaction mixture and was further stirred at room temperature. The reaction was monitored by thin layer chromatography on precoated silica gel sheets. After completion the reaction mixture was neutralized by 2 M hydrochloric acid (HCl), leading to the formation of a deep red precipitate which was filtered and first washed with water alone several times then with cold ethanol-water mixture, dried under reduced pressure to obtain pure respective ferrocenyl chalcone.

4.2 | General procedure for the synthesis of morpholine-4-carboxamidine hydrochloride (2)

Morpholine-4-carboxamidine hydrochloride (**2**) was synthesized according to reported procedure[59] by refluxing morpholine with *S*-methyl isothiourea sulfate in water.

4.3 | General procedure for the synthesis of pyrimidine-based ferrocenyl substituted organometallics (3a-3h)

To a solution of morpholine-4-carboxamidine hydrochloride (6.10 mmol) in isopropanol (50 ml), sodium metal (6.71 mmol) was added. The reaction mixture was refluxed for two hours, then substituted ferrocenyl chalcone **(1a-1h)** (6.10 mmol) was added to the reacion mixture and further refluxed for eight hours. Progress of

reaction was checked by TLC, after completion of the reaction, solvent was removed under reduced pressure, then water was added to the reaction mixture and the aq. layer was extracted with chloroform then washed with 10% NaCl solution. The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The crude product was crystallized from CH_3OH or C_2H_5OH to yield, respective pure organometallic compound.

4.3.1 | 4-Phenyl-2-morpholin-1-yl-6-ferrocenyl pyrimidine (3a)

Yield: 68%; m.p: 212 °C; Maroon solid. Anal. calc. for $C_{24}H_{23}FeN_3O$: C 67.78, H 5.45, N 9.88 % . Found: C 67.72, H 5.47, N 9.79 %. IR ν_{max} (cm^{-1}): 3048 (Ar–H), 2924 (C-H), 1577 (C=N), 1472 (C=C), 1251 (C–N), 1120 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.62-7.85 (m, 5H, Ar-H), 7.33 (s, 1H, Pyrimidine), 4.78 (s, 2H, ferrocene), 4.51 (s, 2H, ferrocene), 4.20 (s, 5H, ferrocene), 3.79 (t, 4H, J=4.5 Hz, morpholine), 2.85 (t, 4H, J=4.5 Hz, morpholine); ^{13}C NMR ($CDCl_3$) δ(ppm): 166.2 (C=N pyrimidine), 162.8 (C=C pyrimidine), 162.5 (N=C-N pyrimidine), 145.5- 118.2 (Ar-C), 104.2 (C-H pyrimidine), 66.5 (C-O-C, morpholine), 44.2 (C-N-C, morpholine), ESI-MS m/z: [M^++1] 425.31.

4.3.2 | 4-(4-hydroxyphenyl)-6-ferrocenyl-2-morpholin-1-yl-pyrimidine (3b)

Yield: 71%; m.p: 168 °C; Dark red solid. Anal. calc. for $C_{24}H_{23}FeN_3O_2$: C 65.38, H 5.22, N 9.59 % . Found: C 65.32, H 5.24, N 9.51 %. IR ν_{max} (cm^{-1}): 3046 (Ar–H), 2929 (C-H), 1572 (C=N), 1469 (C=C), 1256 (C–N), 1118 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.12 (d, 2H, H-2,6 phenyl, J = 8.55 Hz), 7.05 (d, 2H, H-3,5 phenyl, J = 8.55 Hz), 7.36 (s, 1H, Pyrimidine), 4.78 (s, 2H, ferrocene), 4.68 (s, 1H, OH), 4.52 (s, 2H, ferrocene), 4.18 (s, 5H, ferrocene), 3.77 (t, 4H, J = 4.6 Hz, morpholine), 2.86 (t, 4H, J = 4.6 Hz, morpholine); ^{13}C NMR ($CDCl_3$) δ(ppm): 166.9 (C=N pyrimidine), 162.5 (C=C pyrimidine), 162.1 (N=C-N pyrimidine), 145.2- 120.4 (Ar-C), 104.6 (C-H pyrimidine), 66.3 (C-O-C, morpholine), 44.2 (C-N-C, morpholine). ESI-MS m/z: [M^++1] 442.11.

4.3.3 | 4-(4-nitrophenyl)-6-ferrocenyl-2-morpholin-1-yl-pyrimidine (3c)

Yield: 69%; m.p: 195 °C; Reddish brown solid. Anal. calc. for $C_{24}H_{22}FeN_4O_3$: C 61.32, H 4.72, N 11.94 %. Found: C 61.35, H 4.75, N 11.88 %. IR ν_{max} (cm^{-1}): 3048 (Ar–H), 2926 (C-H), 1577 (C=N), 1466 (C=C), 1254 (C–N), 1120 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.26 (d, 2H, H-2,6 phenyl, J = 8.65 Hz), 7.18 (d, 2H, H-3,5 phenyl, J = 8.65 Hz), 7.38 (s, 1H, Pyrimidine), 4.76 (s, 2H, ferrocene), 4.55 (s, 2H, ferrocene), 4.21 (s, 5H, ferrocene), 3.79 (t, 4H, J = 4.6 Hz, morpholine), 2.88 (t, 4H, J = 4.6 Hz, morpholine); ^{13}C NMR ($CDCl_3$) δ(ppm): 166.5 (C=N pyrimidine), 162.8 (C=C pyrimidine), 162.2 (N=C-N pyrimidine), 145.6- 111.9 (Ar-C), 104.6 (C-H pyrimidine), 66.9 (C-O-C, morpholine), 44.5 (C-N-C, morpholine). ESI-MS m/z: [M^++1] 471.3.

4.3.4 | 4-Ferrocenyl-2-morpholin-1-yl-6-p-tolyl-pyrimidine (3d)

Yield: 72%; m.p: 251 °C; Reddish brown solid. Anal. calc. for $C_{25}H_{25}FeN_3O$: C 68.85, H 6.02, N 9.28 % . Found: C 68.81, H 6.05, N 9.25 %. IR ν_{max} (cm^{-1}): 3047 (Ar–H), 2929 (C-H), 1571 (C=N), 1469 (C=C), 1255 (C–N), 1116 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.36 (s, 1H, Pyrimidine), 7.06 (d, 2H, H-2,6 Phenyl, J = 8.85 Hz), 6.99 (d, 2H, H-3,5 phenyl, J = 8.85 Hz), 4.79 (s, 2H, ferrocene), 4.55 (s, 2H, ferrocene), 4.19 (s, 5H, ferrocene), 3.76 (t, 4H, J = 4.5 Hz, morpholine), 2.88 (t, 4H, J = 4.5 Hz, morpholine), 2.35 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$) δ(ppm): 166.2 (C=N pyrimidine), 162.6 (C=C pyrimidine), 162.1 (N=C-N pyrimidine), 145.1- 112.7 (Ar-C), 104.2 (C-H pyrimidine), 66.2 (C-O-C, morpholine), 44.5 (C-N-C, morpholine), 22.4 (CH_3), ESI-MS m/z: [M^++1] 440.34.

4.3.5 | 4-(3,4-Dimethylphenyl)-6-ferrocenyl-2-morpholin-1-yl-pyrimidine (3e)

Yield: 66%; m.p: 295 °C; dark red solid. Anal. calc. for $C_{26}H_{27}FeN_3O$: C 69.41, H 6.28, N 8.96 %. Found: C 69.49, H 6.21, N 8.95 %. IR ν_{max} (cm^{-1}): 3049 (Ar–H), 2926 (C-H), 1577 (C=N), 1466 (C=C), 1258 (C–N), 1115 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.09-7.14 (m, 2H, phenyl), 7.03 (s, 1H, phenyl), 7.32 (s, 1H, Pyrimidine), 4.76 (s, 2H, ferrocene), 4.52 (s, 2H, ferrocene), 4.21 (s, 5H, ferrocene), 3.78 (t, 4H, J = 4.5 Hz, morpholine), 2.86 (t, 4H, J = 4.5 Hz, morpholine), 2.22 (s, 3H, -CH_3), 2.16 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$) δ(ppm): 166.2 (C=N pyrimidine), 162.8 (C=C pyrimidine), 162.5 (N=C-N pyrimidine), 145.1- 118.7 (Ar-C), 104.4 (C-H pyrimidine), 66.5 (C-O-C, morpholine), 44.6 (C-N-C, morpholine), 22.4 (2×CH_3), ESI-MS m/z: [M^++1] 454.37.

4.3.6 | 4-(4-methoxyphenyl)-6-ferrocenyl-2-morpholin-1-yl-pyrimidine (3f)

Yield: 71%; m.p: 168 °C; dark red solid. Anal. calc. for $C_{25}H_{25}FeN_3O_2$: C 65.91, H 5.54, N 9.26 % . Found: C

65.95, H 5.58, N 9.29 %. IR ν_{max} (cm^{-1}): 3048 (Ar–H), 2929 (C-H), 1572 (C=N), 1468 (C=C), 1256 (C–N), 1120 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.02 (d, 2H, H-2,6 phenyl, J = 8.65 Hz), 6.94 (d, 2H, H-3,5 phenyl, J = 8.65 Hz), 7.32 (s, 1H, Pyrimidine), 4.78 (s, 2H, ferrocene), 4.55 (s, 2H, ferrocene), 4.22 (s, 5H, ferrocene), 3.85 (s, 3H, OCH_3), 3.77 (t, 4H, J = 4.6 Hz, morpholine), 2.88 (t, 4H, J = 4.6 Hz, morpholine); ^{13}C NMR ($CDCl_3$) δ(ppm): 166.4 (C=N pyrimidine), 162.8 (C=C pyrimidine), 162.5 (N=C-N pyrimidine), 145.1- 116.4 (Ar-C), 104.2 (C-H pyrimidine), 66.5 (C-O-C, morpholine), 44.4 (C-N-C, morpholine), 55.7 (-OCH_3); ESI-MS m/z: [M^+ +1] 456.34.

4.3.7 | 4-(2,5-dimethoxyphenyl)-6-ferrocenyl-2-morpholin-1-yl-pyrimidine (3g)

Yield: 76%; m.p: 241 °C; Reddish solid. Anal. calc. for $C_{26}H_{27}FeN_3O_3$: C 64.35, H 5.62, N 8.66 % . Found: C 64.32, H 5.65, N 8.62 %. IR ν_{max} (cm^{-1}): 3045 (Ar–H), 2922 (C-H), 1577 (C=N), 1467 (C=C), 1255 (C–N), 1120 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 6.92 (s, 1H, phenyl), 6.88-6.84 (m, 2H, phenyl), 7.32 (s, 1H, Pyrimidine), 4.79 (s, 2H, ferrocene), 4.56 (s, 2H, ferrocene), 4.18 (s, 5H, ferrocene), 3.90 (s, 3H, OCH_3), 3.79 (t, 4H, J = 4.6 Hz, morpholine), 3.76 (s, 3H, OCH_3), 2.87 (t, 4H, J = 4.6 Hz, morpholine); ^{13}C NMR ($CDCl_3$) δ(ppm): 166.5 (C=N pyrimidine), 162.8 (C=C pyrimidine), 162.4 (N=C-N pyrimidine), 145.2- 118.4 (Ar-C), 104.6 (C-H pyrimidine), 66.5 (C-O-C, morpholine), 44.2 (C-N-C, morpholine), 56.8, 55.2 (2×-OCH_3); ESI-MS m/z: [M^+ +1] 486.37.

4.3.8 | 4-(4-Chlorophenyl)-6-ferrocenyl-2-morpholin-1-yl-pyrimidine (3h)

Yield: 62%; m.p: 310 °C; Reddish solid. Anal. calc. for $C_{24}H_{22}ClFeN_3O$: C 62.72, H 4.82, N 9.14 % . Found: C 62.75, H 4.85, N 9.18 %. IR ν_{max} (cm^{-1}): 3043 (Ar–H), 2929 (C-H), 1576 (C=N), 1468 (C=C), 1257 (C–N), 1119 (C-O-C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.31 (d, 2H, H-2,6 phenyl, J = 8.85 Hz), 7.25 (d, 2H, H-3,5 phenyl, J = 8.85 Hz), 7.38 (s, 1H, Pyrimidine), 4.78 (s, 2H, ferrocene), 4.56 (s, 2H, ferrocene), 4.22 (s, 5H, ferrocene), 3.78 (t, 4H, J = 4.6 Hz, morpholine), 2.88 (t, 4H, J = 4.6 Hz, morpholine); ^{13}C NMR ($CDCl_3$) δ(ppm): 166.8 (C=N pyrimidine), 162.5 (C=C pyrimidine), 162.2 (N=C-N pyrimidine), 145.1- 118.4 (Ar-C), 104.2 (C-H pyrimidine), 66.6 (C-O-C, morpholine), 44.2 (C-N-C, morpholine). ESI-MS m/z: [M^++1] 460.76.

4.4 | In vitro antiamoebic assay

All novel organometallic compounds (**3a-3 h)** were evaluated *in vitro* antiamoebic activity against HM1: IMSS strain of *Entamoeba histolytica* by microdilution method.[60] The culture of *Entamoeba histolytica* trophozoites was prepared in the wells of 96-well microtiter plate by Diamond TYIS-33 growth medium.[61] The organometallics (1 mg) were dissolved in dimethyl sulfoxide 40 μL, level at which no inhibition of amoeba occurs].[62,63] The stock solutions of the organometallics were prepared freshly at a concentration of 1 mg/ml two-fold serial dilutions were made in the wells of a 96-well microtiter plate (costar). Each test included metronidazole as a standard drug, control wells (culture medium plus amoebae) and a blank (culture medium only). All the experiments were performed in triplicate at each concentration level and repeated three times. 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 remove the amoebae 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 amoebae/mL by adding fresh medium and 170 μL of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 μL). An inoculum of 1.7×10^4 amoebae/well was chosen so that confluent, but not excessive growth, took place in control wells. Plates were sealed and gassed for 10 minutes with nitrogen before incubation at 37 °C for 72 hours. 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 0.9% NaCl solution at 37 °C. This procedure has been performed quickly and the plate was not allowed to cool to avoid detachment of amoebae. After this step, the plate was allowed to dry at room temperature and the amoebae were fixed with CH_3OH and dried then stained with 0.5% aq. eosin for 15 minutes. The stained plate was first washed with tap water, then washed twice with distilled water and was allowed to dry. A 200 μL 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 percentage of 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 μM are reported in Table 1.

ACKNOWLEDGEMENTS

The authors would like to thank, the Deanship of Scientific Research, University of Tabuk, Tabuk, Saudi Arabia for the financial support for this research work via Grant No. S-0117-1438.

ORCID

Humaira Parveen http://orcid.org/0000-0001-8365-0014

REFERENCES

[1] (a) T. J. Kealy, P. L. Pauson, *Nature* **1951**, *168*, 1039. (b) G. Jaouen (Ed), *Bioorganometallics: Biomolecules, Labeling, Medicine*, Wiley-VCH, New York **2006**. (c) L. V. Snegur, A. A. Simenel, A. N. Rodionov, V. I. Boev, *Russ. Chem. Bull. Int. Ed.* **2014**, *63*, 26.

[2] D. R. van Stavern, N. Metzler-Nolte, *Chem. Rev.* **2004**, *104*, 5931.

[3] N. Metzler-Nolte, M. Salmain, in Ferrocenes: Ligands, Materials and Biomolecules, (Ed: P. Stepnicka), Wiley-VCH, Chichester **2008** (Chapter 13).

[4] C. Biot, D. Taramelli, I. Forfar-Bares, L. A. Maciejewski, M. Boyce, G. Nowogrocki, J. S. Brocard, N. Basilico, P. Olliaro, T. J. Egan, *Mol. Pharmaceutics* **2005**, *2*, 185.

[5] E. A. Hillard, A. Vessières, L. Thouin, G. Jaouen, C. Amatore, *Angew. Chem. Int. Ed.* **2006**, *45*, 285.

[6] D. Hamels, P. Dansette, E. A. Hillard, S. Top, P. Pigeon, G. Jaouen, D. Mansuy, *Angew. Chem. Int. Ed.* **2009**, *48*, 9124.

[7] E. Hillard, A. Vessières, L. Thouin, G. Jaouen, C. Amatore, *Angew. Chem. Int. Ed.* **2006**, *45*, 285.

[8] O. Payen, S. Top, A. Vessières, E. Brulé, M. A. Plamont, M. J. McGlinchey, H. Müller-Bunz, G. Jaouen, *J. Med. Chem.* **2008**, *51*, 1791.

[9] S. Top, C. Thibaudeau, A. Vessières, E. Brulé, F. Le Bideau, J. M. Joerger, M. A. Plamont, S. Samreth, A. Edgar, J. Marrot, P. Herson, G. Jaouen, *Organometallics* **2009**, *28*, 1414.

[10] S. L. Stanley Jr., *Lancet* **2003**, *361*, 1025.

[11] E. Oku, K. Nomura, T. Nakamura, S. Morishige, R. Seki, R. Imamura, M. Hashiguchi, K. Osaki, S. Mizuno, K. Nagafuji, T. Okamura, *Kansenshogaku Zasshi* **2012**, *86*, 773.

[12] W. A. Petri, R. Haque, *Handb. Clin. Neurol.* **2013**, *114*, 147.

[13] K. S. Ralston, W. A. Petri, *Essays Biochem.* **2011**, *91*, 193.

[14] S. M. Townson, H. Laqua, P. F. Boreman, P. Upcrofit, J. A. Upcroft, *Trans. R. Soc. Trop. Med. Hyd.* **1922**, *86*, 521.

[15] M. C. Conde-Bonfil, C. De la M ora-Zerpa, *Salud Publica Mex.* **1992**, *34*, 335.

[16] R. Cedillo-Rivera, A. Tapia-Contreras, J. Torres, O. Munoz, *Arch. Med. Res.* **1997**, *28*, S295.

[17] K. Kapoor, M. Chandra, D. Nag, J. K. Paliwal, R. C. Gupta, R. C. Saxena, *Int. J. Clin. Pharmacol. Res.* **1999**, *19*, 83.

[18] I. S. Adagu, D. Nolder, D. C. Warhurts, J. F. Rossignol, *J. Antimicrob. Chemother.* **2002**, *49*, 103.

[19] G. Tarun-Ziouni, A. Ozdemir, K. Guven, *Arch. Pharm. (Weinheim)* **2005**, *338*, 96.

[20] F. J. Roe, R. Perez, *Hum. Pathol.* **1970**, *1*, 351.

[21] J. C. Samuelson, A. Burke, J. M. Courval, *Antimicrob. Agents Chemother.* **1992**, *36*, 2392.

[22] J. Jadhav, A. Juvekar, R. Kurane, S. Khanapure, R. Salunkhe, G. Rashinkar, *Eur. J. Med. Chem.* **2013**, *65*, 232.

[23] L. M. Beauchamp, B. L. Serling, J. E. Kelsey, K. K. Biron, P. Collins, J. Selway, J.- C. Lin, H. I. Schaeffer, *J. Med. Chem.* **1988**, *31*, 144.

[24] T. Ishihara, Y. Okada, M. Kurobushi, T. Shinozaki, T. Ando, *Chem. Lett.* **1988**, *5*, 819.

[25] A. Monge, V. Martiner-Merino, C. Sammartin, M. C. Ochoa, E. Fernandez-Alvarez, *Arzneim. Forsch.* **1990**, *40*, 1349.

[26] T. Miyasaka, H. Tanaka, M. Baba, H. Hayakawa, R. T. Walker, J. Balzarini, E. De Clercq, *J. Med. Chem.* **1989**, *32*, 2507.

[27] H. Tanaka, H. Takashima, M. Ubasawa, K. Sekiya, I. Nitta, M. Baba, S. Shigeta, R. T. Walker, E. De Clercq, T. Miyasaka, *J. Med. Chem.* **1992**, *35*, 337.

[28] Y. Guo, S.-Q. Wang, Z.-Q. Ding, J. Zhou, B.-F. Ruan, *J. Organomet. Chem.* **2017**, *851*, 150.

[29] R. Chopra, C. de Kock, P. Smith, K. Chibale, K. Singh, *Eur. J. Med. Chem.* **2015**, *100*, 1.

[30] K. Kowalski, *Coord. Chem. Rev.* **2016**, *317*, 132.

[31] K. Kowalski, Ł. Szczupak, S. Saloman, D. Steverding, A. Jabłoński, V. Vrček, A. Hildebrandt, H. Lang, A. Rybarczyk-Pirek, *Chem Plus Chem.* **2017**, *82*, 303.

[32] J. Skiba, C. Schmidt, P. Lippmann, P. Ensslen, H.-A. Wagenknecht, R. Czerwieniec, F. Brandl, I. Ott, T. Bernaś, B. Krawczyk, D. Szczukocki, K. Kowalski, *Eur. J. Inorg. Chem.* **2017**, *297*.

[33] M. Andrs, J. Korabecny, D. Jun, Z. Hodny, J. Bartek, K. Kuca, *J. Med. Chem.* **2015**, *58*, 41.

[34] Z. Yu, G. Shi, Q. Sun, H. Jin, Y. Teng, K. Tao, et al., *Eur. J. Med. Chem.* **2009**, *44*, 4726.

[35] T. V. Abramova, P. A. Bakharev, S. V. Vasilyeva, V. N. Silnikov, *Tetrahedron Lett.* **2004**, *45*, 4361.

[36] M. L. Leathen, B. R. Rosen, J. P. Wolfe, *J. Org. Chem.* **2009**, *74*, 5107.

[37] A. D. Tereshchenko, J. S. Myronchuk, L. D. Leitchenko, I. V. Knysh, G. O. Tokmakova, O. O. Litsis, A. Tolmachev, K. Liubchak, P. Mykhailiuk, *Tetrahedron* **2017**, *73*, 750.

[38] H. G. R. Kumar, K. R. Asha, H. N. K. Kumar, L. S. Inamdar, G. M. Advi Rao, *Nucleotides Nucleic Acids* **2015**, *34*, 525.

[39] M. A. Ibrahim, S. M. Abou-Seri, M. M. Hanna, M. M. Abdalla, N. A. El Sayed, *Eur. J. Med. Chem.* **2015**, *99*, 1.

[40] M. Taha, S. A. A. Shah, M. Afifi, M. Zulkeflee, S. Sultan, A. Wadood, F. Rahim, N. H. Ismail, *Chin. Chem. Lett.* **2017**, *28*, 607.

[41] P. Diao, Q. Li, M. Hu, Y. Ma, W. You, K. H. Hong, P. Liang, *Eur. J. Med. Chem.* **2017**, *134*, 110.

[42] R. S. Kumar, M. Moydeen, S. S. Al-Deyab, A. Manilal, A. Idhayadhulla, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 66.

[43] M. Sankarganesh, J. Rajesh, G. G. Vinoth Kumar, M. Vadivel, L. Mitu, R. Senthil Kumar, J. Dhaveethu Raja, J. Saudi Chem. *Soc.* **2017**, https://doi.org/10.1016/j.jscs.2017.08.007.

[44] A. Ahmadi, M. Khalili, R. Hajikhani, N. Safari, B. Nahri-Niknafs, *Med. Chem. Res.* **2012**, *21*, 3532.

[45] A. M. Ismaiel, L. M. Gad, S. A. Ghareib, F. H. Bamanie, M. A. Moustafa, *Med. Chem. Res.* **2011**, *20*, 381.

[46] N. K. Gasparyan, G. A. Gevorgyan, R. G. Paronikyan, A. E. Tumadzhyan, G. A. Panosyan, *Pharm. Chem. J.* **2005**, *39*, 361.

[47] V. O. Kozminykh, A. O. Belyaev, E. N. Kozminykh, R. R. Makhmudov, T. F. Odegova, *Pharm. Chem. J.* **2004**, *38*, 431.

[48] C. H. Lee, M. Jiang, M. Cowart, G. Gfesser, R. Perner, K. H. Kim, Y. G. Gu, M. Williams, M. F. Jarvis, E. A. Kowaluk, A. O. Stewart, S. S. Bhagwat, *J. Med. Chem.* **2001**, *44*, 2133.

[49] Vainilavicius, *IL Farmaco* **2003**, *58*, 323.

[50] Z. Zhao, D. A. Pissarnitski, H. T. B. Josien, T. A. Bara, J. W. Clader, H. Li, M. D. McBriar, M. Rajagopalan, R. Xu, G. Terracina, L. Hyde, L. Song, L. Zhang, E. M. Parker, R. Osterman, A. V. Buevich, *Eur. J. Med. Chem.* **2016**, *124*, 36.

[51] E. M. Ladopoulou, A. N. Matralis, A. Nikitakis, A. P. Kourounakis, *Bioorg. Med. Chem.* **2015**, *23*, 7015.

[52] A. Rathore, R. Sudhakar, M. J. Ahsan, A. Ali, N. Subbarao, S. S. Jadav, S. Umar, M. S. Yar, *Bioorg. Chem.* **2017**, *70*, 107.

[53] A. Kerkenaar, *vol. 1. Prous Science Publ*, **1987** 523.

[54] A. Polak, *Ann. N. Y. Acad. Sci.* **1988**, *554*, 221.

[55] R. G. M. P. Pinto, B. V. Silva, A. C. Pinto, **2013**. In: 35th Annual Meeting of the Brazilian Chemical Society.

[56] H. Parveen, S. Mukhtar, A. Azam, *J. Heterocyclic Chem.* **2016**, *53*, 473.

[57] H. Parveen, R. A. S. Alatawi, N. H. El Sayed, S. Hasan, S. Mukhtar, A. U. Khan, *Arabian J. Chem.* **2017**, *10*, 1098.

[58] X. Wu, P. Wilairat, *M. L. Go. Biorg. Med.Chem. Lett.* **2002**, *12*, 2299.

[59] J. M. Andrews, N. Anand, A. R. Todd, A. Topham, *J. Chem. Soc.* **1949**, *2490*.

[60] C. W. Wright, M. J. O'Neill, J. D. Phillipson, D. C. Warhurst, *Antimicrob. Agents Chemother.* **1988**, *32*, 1725.

[61] L. S. Diamond, D. R. Harlow, C. C. R. Cunnick, *Trans. Soc. Trop. Med. Hyg.* **1978**, *72*, 431.

[62] F. D. Gillin, D. S. Reiner, M. Suffness, *Antimicrob. Agents Chemother.* **1982**, *22*, 342.

[63] A. T. Keene, A. Harris, J. D. Phillipson, D. C. Warhurst, *Planta Med.* **1986**, *52*, 278.

How to cite this article: Parveen H, Alsharif MA, Alahmdi MI, Mukhtar S, Azam A. Novel Pyrimidine-based Ferrocenyl substituted Organometallic Compounds: Synthesis, Characterization and Biological Evaluation. *Appl Organometal Chem*. 2018;e4261. https://doi.org/10.1002/aoc.4261