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Short communication

Antiprotozoal activity of chloroquinoline based chalcones

Faisal Hayat [a], Emma Moseley [b], Attar Salahuddin [a], Robyn L. Van Zyl [b], Amir Azam [a,*]

[a] Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India
[b] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of Witwatersrand, Johannesburg 2193, South Africa

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ABSTRACT

A new series of chloroquinoline based chalcones were synthesized and evaluated for *in vitro* antiamoebic and antimalarial activities. The results showed that out of fifteen compounds, four were found to be more active against the *Entamoeba histolytica*; while one compound was moderatively active compared to the standard drug metronidazole (IC_{50} = 1.46 μM). In contrast, *in vitro* antimalarial activity against the chloroquine-sensitive (3D7) strain of *P. falciparum* indicated relatively low activity when compared to controls such as chloroquine and quinine (IC_{50} = 0.0065 μM and 0.14 μM, respectively). The toxicological studies of these compounds on human breast cancer MCF-7 cell line showed that all the compounds were non-toxic at the concentration range of 1.56–50 μM.

1. Introduction

Parasitic protozoa are the causative agents of numerous diseases and affect an immense proportion of the world's population [1]. Two such protozoa are *Entamoeba histolytica* and *Plasmodium falciparum*, the causative agents of amoebiasis and malaria, respectively. Amoebiasis continues to be a major problem in developing countries partly due to a lack of adequate sanitation and health education [2]. Countries where *E. histolytica* remains endemic include India, South Africa and Mexico to name a few [2]. Invasive amoebiasis is the second most common cause of mortality due to parasitic infections worldwide [3] killing one in 30 children in Bangladesh alone [4]. It results primarily in an infection of the colon, but may also be spread via the haematogenous path to other organs, especially the liver [5] and is characterized by its high capacity to destroy host tissues, leading to potentially life-threatening diseases such as ulcerative colitis or liver abscess. Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) is the drug of choice for the treatment of amoebiasis and giardiasis [6] and is reported to cause several toxic effects such as genotoxicity, gastric mucus irritation and spermatozoid damage [7,8]. Furthermore, failures in the treatment of several intestinal protozoan parasites may result from drug resistant by parasites [9,10] A similar trend has been observed in the treatment of malaria, a haematoprotozoan parasite of the *Plasmodium* species, carried by certain types of the *Anopheles* mosquito [11]. It is one of the most prevalent diseases of the developing world, affecting over a hundred countries and resulting in an estimated 300–500 million cases annually [12]. In sub-Saharan Africa alone over 90% of reported malaria cases are attributed to the *P. falciparum* parasite [13–15]. Epidemiological models have suggested that *P. falciparum* infections outside of Africa, specifically in Southeast Asia, are expected to be 200% higher than figures originally published by the World Health Organization, with 76% of the total cases originating from India [12]. The widespread resistance of the parasite to chloroquine, the backbone of malaria chemotherapy, has highlighted the fact that the investigation of new chemotherapeutics with similar mechanisms of action and toxicity profile to that of chloroquine is highly desired. The mechanism of action of chloroquine lies in the fact that, once haemoglobin has been degraded by

Abbreviations: μg, microgram; μL, microliter; μM, micromole; mL, milliliter; mg, milligram; mmol, millimole; nm, nanometer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC_{50}, the drug concentration at which 50% growth inhibition occurs.

* Corresponding author. Tel.: +91 11 2698 1717/3254; fax: +91 11 2698 0229.
E-mail address: amir_sumbul@yahoo.co.in (A. Azam).

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plasmodial cysteine proteases, the toxic haem intermediate cannot be detoxified into haemozoin, resulting in oxidative stress and death of the parasite [16]. A therapeutic agent capable of acting in a similar manner as well as interacting favourably with other established antimalarials would be invaluable.

It is well known that chalcones and quinoline incorporating heterocycles play an important role in medicinal chemistry and possess various biological activities such as antimicrobial, anti-inflammatory, analgesic, antimalarial, anticancer, antiviral, anti-leishmanial and antitubercular [17–22]. According to the literature iodoquinol is currently used as antiamoebic drug in medical practice [23] and quinoline based hydrazones have shown antimalarial and antitubercular activity [24–26]. Many natural products bearing a quinoline nucleus such as quinidine, quinidinone, and quinine from the plant *Cinchona ledgeriana* were also found to be antiamoebic [27]. In recent years it is reported that the incorporation of a quinoline nucleus could alter the course of reaction, as well as the biological properties of the molecules [28,29].

Since chalcones and quinoline incorporating heterocycles are biologically active and as a part of our continuous efforts towards the development of more potent antiprotozoal agents, we herein report the synthesis, characterization, antiamoebic, antiplasmodial and cytotoxic properties of a new series of chloroquinoline based chalcones (**4**–**18**) in an attempt to improve upon the efficacy of current antiamoebic and antimalarial agents.

2. Chemistry

The synthesis of the chloroquinoline based chalcones (**4**–**18**) was performed in a manner as outlined in Scheme 1. The reaction of 2-chloro-3-formylquinolines (**1**–**3**) with different commercially available aromatic ketones in presence of aqueous sodium hydroxide in ethanol gave the quinolinyl chalcones. All the compounds are insoluble in water, but soluble in most of the organic solvents. Melting points were recorded on KSW melting point apparatus and are uncorrected.

3. Pharmacology

All quinolinyl chalcones (**4**–**18**) were screened *in vitro* against HM1:IMSS strain of *E. histolytica* by microdilution method [30]. All the experiments were carried out in triplicate at each concentration level and repeated thrice. Cytotoxicity of active compounds has been studied using the MTT cell viability assay on the breast cancer MCF-7 cell line. The results of antiamoebic activity and cytotoxicity are summarized in Table 1. *In vitro* antimalarial activity was carried out on the chloroquine-sensitive (3D7) strain of *P. falciparum* by use of the [^{3}H]-hypoxanthine-incorporation assay. All experiments were repeated, at least, in triplicate. To determine a possible mechanism of antimalarial action the inhibition of β-haematin formation, as well as free radical scavenging activity were determined. Drug toxicity was determined by examining the haemolytic effects of the compounds on healthy erythrocytes. Out of the **14** compounds, one lead compound was selected and combined with quinine to determine possible drug interactions. The results of the above are summarized in Table 2 and Fig. 2.

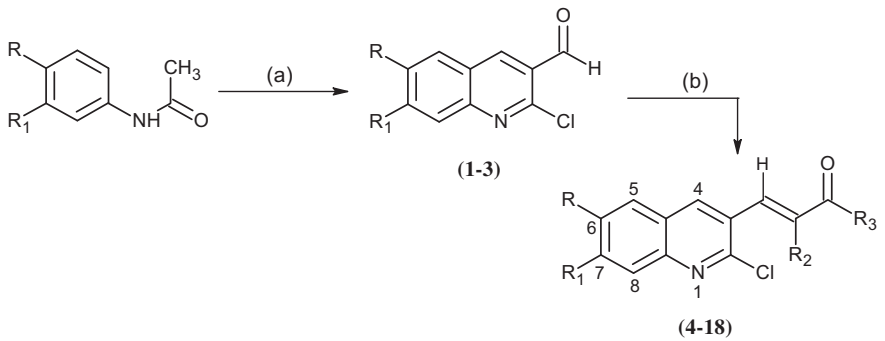
4. Results and discussion

4.1. Synthesis

The chloroquinoline based chalcones were prepared by one-step Claisen–Schmidt condensation [31] of substituted 2-chloro-3-formylquinolines with different aromatic ketones using the reaction sequence as shown in Scheme 1. For this purpose a series of 2-chloro-3-formylquinolines (**1**–**3**) were prepared from different acetanilides [32]. The required acetanilides were prepared by direct condensation of substituted anilines with acetic acid in presence of acetic anhydride [33]. The prepared 2-chloro-3-formylquinolines were condensed with different commercially available aromatic ketones in presence of aqueous sodium hydroxide (2 mL, 40%) in 50 mL ethanol to yield the quinolinyl chalcones (**4**–**18**) in good yield. All the compounds were characterized by IR, ^{1}H and ^{13}C NMR and mass spectra. The purity of the compounds was confirmed by elemental analysis and the data was found in accordance with ±0.3%.

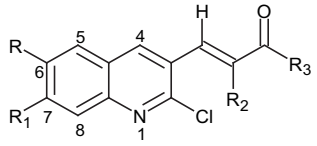
4.2. Antiamoebic activity

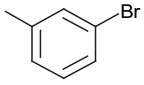
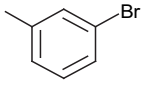
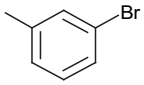
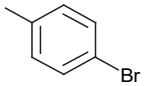
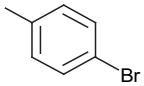
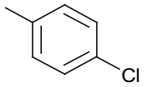
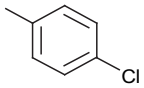
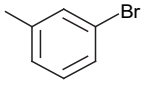
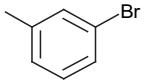
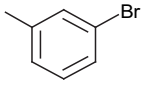
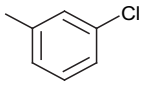
Preliminary experiments were carried out to determine the *in vitro* antiamoebic activity of all the compounds (**4**–**18**) by microdilution method using the HM1:IMSS strain of *E. histolytica*. The antiamoebic effect was compared with the most widely used antiamoebic medication, namely metronidazole which had a 50% inhibitory concentration (IC_{50}) of 1.46 μM (Table 1). SAR showed that compounds (**4**–**10**) which contained chloro and bromo groups as substituent's at **C-3** and **C-4** position of the phenyl ring; while compounds (**4**, **5**, **7** and **9**) with a methyl group as a substituent at **C-6** and **C-7** position of the chloroquinoline ring (excluding compounds **6**, **8** and **10**) showed IC_{50} value in the range of 0.05–7.53 μM. Compounds (**11**–**15**) besides containing the same substitution of chloro and bromo groups at **C-3** and **C-4** position of the phenyl and methyl group at **C-6** and **C-7** position of the chloroquinoline ring except the compound (**13**), contain a methyl group at the α-β unsaturated carbonyl position showed IC_{50} value in the range of 0.06–7.03 μM. Compounds (**16**–**18**) having the same chloroquinoline ring in their structure contain a pyrimidine ring



Scheme 1. Synthesis of chloroquinoline based chalcone (**4**–**18**). Reagent and conditions: (a) DMF/$POCl_3$, 75 °C reflux 17 h, (b) aq. NaOH, C_2H_5OH, different aromatic ketones, where **R**, **R$_1$**, **R$_2$** and **R$_3$** are the different substituted groups.

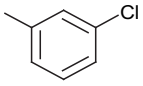
Table 1
In vitro antiamoebic activity of chloroquinoline based chalcones (**4**–**18**) against HM1:IMSS strain of *E. histolytica* and cytotoxicity profile of compounds **5**, **10**, **11**, **15**, **17** and metronidazole.



Compound	R	R_1	R_2	R_3	Antiamoebic activity		Cytotoxicity profile (MCF-7)	
					IC_{50} (μM) SD[a]		IC_{50} (μM) SD[a]	
4	H	CH_3	H		7.53	0.14	N.D.	N.D.
5	CH_3	H	H		**1.03**	0.22	>100	0.18
6	H	H	H		3.45	0.16	N.D.	N.D.
7	CH_3	H	H		4.09	0.12	N.D.	N.D.
8	H	H	H		3.38	0.23	N.D.	N.D.
9	H	CH_3	H		6.91	0.19	N.D.	N.D.
10	H	H	H		**0.05**	0.12	>100	0.14
11	H	CH_3	CH_3		**0.09**	0.22	>100	0.14
12	CH_3	H	CH_3		2.79	0.11	N.D.	N.D.
13	H	H	CH_3		3.65	0.32	N.D.	N.D.
14	H	CH_3	CH_3		7.03	0.11	N.D.	N.D.

(*continued on next page*)

Table 1 (*continued*).

Compound	R	R_1	R_2	R_3	Antiamoebic activity		Cytotoxicity profile (MCF-7)	
					IC_{50} (μM) SD[a]		IC_{50} (μM) SD[a]	
15	CH_3	H	CH_3		**0.06**	0.14	>100	0.28
16	CH_3	H	H		1.45	0.17	N.D.	N.D.
17	H	CH_3	H		**0.05**	0.10	75.7	0.16
18	H	H	H		5.30	0.22	N.D.	N.D.
				Metronidazole	**1.46**	0.17	>100	0.11

[a] Standard deviation, N.D. = Not determined.
The compounds with bold IC_{50} values are more active than metronidazole.

instead of the phenyl ring. The compounds (**16**) and (**17**) except (**18**) having the chloroquinoline ring being substituted by methyl group at **C-6** and **C-7** position showed IC_{50} value in the range of 0.05–5.30 μM. From these 15 compounds, five (**5**, **10**, **11**, **15** and **17**) were found to be more active against *.E. histolytica* than the reference drug, metronidazole (IC_{50} = 1.46 μM). Out of these five compounds, four compounds **10** (IC_{50} = 0.05 μM), **11** (IC_{50} = 0.09 μM), **15** (IC_{50} = 0.06 μM) and **17** (IC_{50} = 0.05 μM) showed excellent antiamoebic activity being 16–29 times more active than metronidazole. While compound **5** (IC_{50} = 1.03 μM) was moderately active compare to metronidazole.

4.3. Antimalarial activity

A series of chloroquinoline based chalcones (**4**–**18**) were examined for their *in vitro* antimalarial activity, their interactions with classic antimalarials, as well as their ability to inhibit the formation of β-heamatin and scavenge free radicals. The results have been summarized in Table 2. The hypoxanthine incorporation assay indicated that, although not comparable to the reference agents chloroquine and quinine (IC_{50} values = 0.0065 μM and 0.14 μM, respectively), compounds **10**, **16**, **17** and **18** (IC_{50} values = 39.17 μM, 31.54 μM, 31.31 μM and 31.98 μM, respectively) showed the most promising antimalarial activity. Compounds **16**–**18** were the only compounds to have a pyridine ring substitutions at R_3 and a hydrogen group at R_2, with either hydrogen or methyl groups at R and R_1. Compound **10**, which had the highest IC_{50} value out of the 4, has hydrogen groups at R, R_1 and R_2 with a 4-chloro phenyl ring at R_3. It appears as if the substitution of the hydrogen group, instead of a methyl group, at R_2 positively influences the activity of compounds **4** and **5** when compared to compounds **11**–**13**, all of which have a bromophenyl ring. However, when all methyl groups are replaced with hydrogen groups, as seen in compound **6**, activity diminishes. The positioning of the bromo

Table 2
The *in vitro* antimalarial and haemolytic activity of chloroquinoline based chalcones (**4**–**18**) and reference agents along with their effect on β-haematin formation.

Compound	Antimalarial activity		Haemolytic activity	Inhibition Of β-haematin formation	
	IC_{50} ± S.D. (μM)	Maximum inhibition at 100 μM (%)	% Lysis at 100 μM	IC_{50} ± S.D. (μM)	Maximum inhibition at 100 μM (%)
4	59.73 ± 1.19		1.09 ± 0.55	>100	27.50 ± 4.80
5	50.11 ± 3.85		1.68 ± 0.66	>100	14.88 ± 6.86
6	>100	61.22 ± 13.09	1.38 ± 0.67	>100	16.03 ± 4.81
7	74.84 ± 3.39		1.40 ± 0.96	>100	55.38 ± 19.1
8	44.56 ± 1.75		2.20 ± 0.54	>100	13.55 ± 2.61
9	N.D.	N.D.	N.D.	N.D.	N.D.
10	39.17 ± 2.05		1.74 ± 0.67	>100	19.49 ± 5.35
11	47.06 ± 2.76		1.04 ± 0.64	>100	10.54 ± 0.88
12	>100	51.15 ± 8.1	1.53 ± 0.34	>100	13.30 ± 5.38
13	>100	34.3 ± 8.93	2.17 ± 0.59	>100	9.35 ± 5.76
14	50.44 ± 3.22		1.44 ± 0.66	>100	5.64 ± 3.72
15	>100	8.74 ± 13.66	1.24 ± 0.78	>100	14.42 ± 4.21
16	31.54 ± 3.03		1.55 ± 1.15	>100	11.52 ± 5.20
17	31.31 ± 0.87		0.47 ± 0.77	>100	15.00 ± 0.99
18	31.98 ± 1.27		1.10 ± 1.04	>100	12.29 ± 4.35
Chloroquine	0.0065 ± 0.0002		0.72 ± 0.32	24.97 ± 5.3	
Quinine	0.14 ± 0.007		1.22 ± 0.98	65.80 ± 1.77	

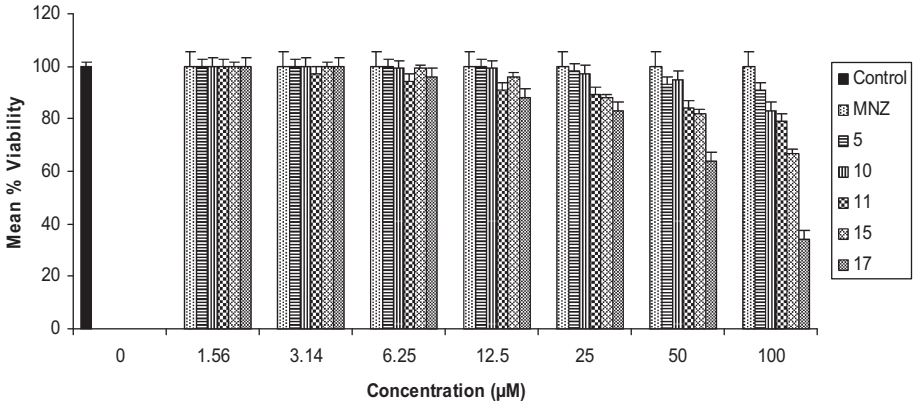


Fig. 1. Percentage of viable cells after 48 h pre-treatment of human breast cancer MCF-7 cells with compounds **5**, **10**, **11**, **15**, **17** and metronidazole (MNZ), evaluated by the MTT assay.

group on the phenyl ring appeared not to influence antimalarial activity. A study conducted against the FCB1 chloroquine-resistant strain of *P. falciparum* indicated that quinolinyl chalcones with a single chloro substitution at position 4 on the phenyl ring displayed no antimalarial activity (>200 μM). However, once di-substituted at positions 2, 4 and 2, 5, the IC_{50} values decreased to 19 μM and 48.6 μM, respectively, whilst di-substitutions at positions 3 and 4 diminished the antimalarial activity [34]. The same study also indicated that the addition of bromo, methoxy or hydrogen groups to the phenyl ring diminished antimalarial activity. The above results indicate differences in drug sensitivity between chloroquine-sensitive and -resistant strains, but also highlight the significant role that not only the various substitutions, but also the positioning of the substitutions, play. None of the compounds were capable of inhibiting the formation of β-haematin, the synthetic molecule with the same chemical structure as haemozoin, as effectively as chloroquine or quinine (IC_{50} = 24.9 μM and 65.8 μM, respectively). Compound **7** exhibited the greatest activity with 55.38% inhibition at 100 μM, however this was not well correlated with antimalarial activity. None of the compounds were capable of scavenging the free electron of DPPH• as effectively as ascorbic acid (IC_{50} = 20.10 μM), compounds **1**, **2**, **10**, **11**, **13** and **14** showed some anti-oxidant activity at 80 μM, or a ratio of drug to DPPH• of 1:1 (% scavenging activity = 7.79, 2.06,3.69, 2.27, 1.43, and 0.78%, respectively) and were comparable to the anti-oxidant activity of chloroquine and quinine (% scavenging activity = 4.06 and 2.65%, respectively). The mechanism of action of this class of compounds remains unclear, when tested against the *P. falciparum* cysteine protease, falcipain, the quinolinyl chalcones showed weak activity that was not correlated with antimalarial activity [34]. In an examination of the interaction of compound **17** and quinine, an additive interaction was noted. This is possibly due, to the different complementary mechanisms of actions of these two compounds.

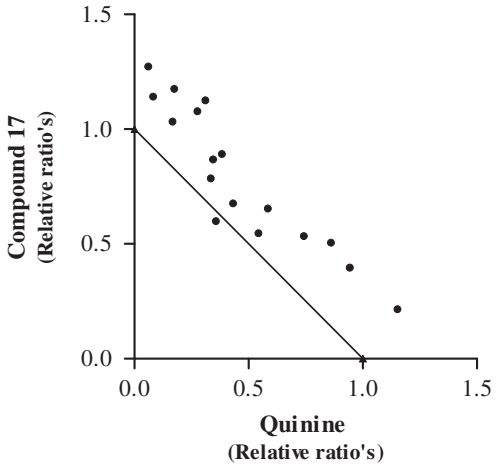


Fig. 2. Isobologram depicting the additive interaction between compound **17** and quinine.

4.4. Cytotoxicity profile

Since compounds **5**, **10**, **11**, **15** and **17** were more potent than metronidazole, they were screened for cytotoxicity against the human breast cancer MCF-7 cell line. Compounds **5**, **10**, **11** and metronidazole inhibited <20% cell growth. Whilst compound **17** inhibited cell growth in a dose dependant manner, where 50 μM inhibited 36% and 100 μM, inhibited 66% cell growth (Fig. 1). To ensure the antimalarial activity noted for these compounds were due to a direct inhibitory effect on the intra-erythrocytic parasite, all the compounds were screened against healthy red blood cells for any haemolytic effects (Table 2). None of the compounds showed any notable haemolytic effects when compared to antimalarials such as chloroquine or quinine (% haemolysis of 0.72 and 1.22%, respectively). The greatest haemolytic activity was noted for compounds **8** and **13** (% haemolysis of 2.2 and 2.17%, respectively).

5. Conclusion

The 15 chloroquinoline based chalcones (**4**–**18**) were synthesized by condensation of substituted 2-chloro-3-formylquinolines with different aromatic ketones. *In vitro* antiamoebic and antimalarial activities were determined against the HM1:IMSS strain of *E. histolytica* and 3D7 strain of *P. falciparum*, respectively. Antiamoebic activity indicated that out of the 15 compounds, 5 compounds exhibited more potent activity than the reference drug metronidazole (IC_{50} = 1.46 μM). Antimalarial activity was not as promising, none of the chloroquinoline based chalcones were capable of inhibiting parasite growth as effectively as the reference drugs chloroquine and quinine (IC_{50} values = 0.0065 μM and 0.14 μM, respectively). When combined with quinine, compound **17** interacted in a favourable additive manner. Both the inhibition of β-haematin formation and DPPH• free radical scavenging assays indicated that the antimalarial mechanisms of action of the compounds do not lie in their ability to inhibit haemozoin formation or act as anti-oxidants. The MTT assay revealed that compounds **5**, **10**, **11**, **15** and metronidazole were non-toxic, however compound **17** was found to inhibit 66% cell growth at 100 μM. Whilst, the red blood cell toxicity assay showed negligible

amounts of haemolysis when compared to that of chloroquine and quinine. The results obtained indicate that these compounds show excellent antiamoebic activity, with 16–29 times the activity of the standard treatment, metronidazole. No correlation was noted between the antiamoebic and antimalarial activity with the above compounds exhibiting only moderate antimalarial activity when compared to standard treatments. Generally the compounds proved to be non-toxic. Investigation into derivatives of this class of compounds is essential, to examine the exact nature of their antimalarial activity, as well as the effect of structural changes on antiamoebal and antiplasmodial activities.

6. Experimental protocol

All the required chemicals were purchased from Merck and Aldrich Chemical Company (USA). 2-Chloro-3-formylquinolines were synthesized by using the methods reported in literature [32]. Precoated aluminium sheets (silica gel 60 F_{254}, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. Elemental analysis was carried out on CHNS Elementar (Vario EL-III) and the results were within ±0.3% of the theoretical values. 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 Spectrospin DPX 300 MHz and Bruker Spectrospin DPX 75 MHz spectrometer respectively using $CDCl_3$ as a solvent and trimethylsilane (TMS) as an internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; m, multiplet. Chemical shift values are given in ppm. The FAB mass spectra of the compounds were recorded on JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon (6 KV, 10 mA) as the FAB gas and *m*-nitrobenzyl alcohol (NBA) was used as the matrix.

6.1. Preparation of 2-chloro-3-formylquinolines (**1**–**3**)

Substituted 2-chloro-3-formylquinolines were prepared by a reported method [32].

6.2. General procedure for the synthesis of chloroquinoline based chalcones (**4**–**18**)

A mixture of substituted 2-chloro-3-formylquinolines (0.01 mol), the respective aromatic ketones (0.01 mol), and sodium hydroxide (2 mL, 40% aqueous) in 50 mL ethanol was stirred at room temperature for 24 h. The resulting precipitate was collected by filtration, washed with water and recrystallized from ethyl acetate.

6.2.1. (E)-1-(3-Bromophenyl)-3-(2-chloro-7-methylquinoline-3yl) prop-2-en-1-one (**4**)

Yield 88% (Ethyl acetate); mp: 276 °C; Anal. calc. for $C_{19}H_{13}NOClBr$: C 59.02, H 3.39, N 3.62%; found: C 59.04, H 3.33, N 3.58%; IR ν_{max} (cm^{-1}): 1652 (C=O), 1579 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.46 (s,1H, H_4 quinoline), 8.24 (s,1H, phenyl), 8.19 (d,1H, $J = 15.6$ Hz, H_β), 7.99 (d,1H, $J = 7.5$ Hz, phenyl), 7.80–7.73 (m, 2H, H_5 H_8 quinoline), 7.56 (d,1H, $J = 15.6$ Hz, H_α), 7.46–7.39 [m, 3H, (2H phenyl, H_6 quinoline)], 2.58 (s, 3H, CH_3 quinoline); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.3 (C=O), 150.3 (C–Cl), 148.2 (C-β), 142.8, 140.4, 139.4, 135.9, 131.6, 130.4, 127.5, 127.1, 126.7 (Ar–C), 123.0 (C-α), 22.1 (CH_3 quinoline). FAB-MS (*m*/*z*): [M^++1] 387.34.

6.2.2. (E)-1-(3-Bromophenyl)-3-(2-chloro-6-methylquinolin-3-yl) prop-2-en-1-one (**5**)

Yield 74% (Ethyl acetate); mp: 142–144 °C; Anal. calc. for $C_{19}H_{13}NOClBr$: C 59.02, H 3.39, N 3.62%; found: C 59.03, H 3.32, N 3.56%. IR ν_{max} (cm^{-1}): 1662 (C=O), 1581 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.39 (s, 1H, H_4 quinoline), 8.22 (s, 1H, phenyl), 8.16 (d, 1H, $J = 15.6$ Hz, H_β), 7.98 (d, 1H, $J = 7.5$ Hz, phenyl), 7.69 (s, 1H, H_5 quinoline) 7.66–7.60 [m, 2H, (1H phenyl, H_8 quinoline)], 7.57 (d, 1H, $J = 15.6$ Hz, H_α), 7.49–7.39 [m, 2H, (1H phenyl, H_7 quinoline)], 2.55 (s, 3H, CH_3 quinoline); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.8 (C=O), 149.4 (C–Cl), 146.5 (C-β), 140.1, 137.9, 136.3, 135.6, 134.0, 132.0, 130.01, 128.1, 127.6, 126.8 (Ar–C), 122.5 (C-α), 21.6 (CH_3 quinoline); FAB-MS (*m*/*z*): [M^++1] 387.25.

6.2.3. (E)-1-(3-Bromophenyl)-3-(2-chloroquinoline-3yl) prop-2-en-1-one (**6**)

Yield 78% (Ethyl acetate); mp: 173 °C; Anal. calc. for $C_{18}H_{11}NOClBr$: C 59.95, H 3.77, N 3.50%; found: C 59.89, H 3.71, N 3.44%. IR ν_{max} (cm^{-1}): 1659 (C=O), 1583 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.41 (s, 1H, H_4 quinoline), 8.24 (s, 1H, phenyl), 8.21 (d, 1H, $J = 15.6$ Hz, H_β), 7.97 (d, 1H, $J = 7.5$ Hz, phenyl), 7.94–7.78 (m, 3H, H_5 H_7 H_8 quinoline), 7.54 (d, 1H, $J = 15.6$ Hz, H_α), 7.46–7.35 [m, 3H, (2H phenyl, H_6 quinoline)]; ^{13}C NMR ($CDCl_3$) δ (ppm): 188.9 (C=O), 148.9 (C–Cl), 145.6 (C-β), 139.0, 137.7, 134.6, 133.4, 129.6, 127.6 (Ar–C), 126.6 (C-α); FAB-MS (*m*/*z*): [M^++1] 373.11.

6.2.4. (E)-1-(4-Bromophenyl)-3-(2-chloro-6-methylquinoline-3yl) prop-2-en-1-one (**7**)

Yield 84% (Ethyl acetate); mp: 276 °C; Anal. calc. for $C_{19}H_{13}NOClBr$: C 59.02, H 3.39, N 3.62%; found: C 59.03, H 3.33, N 3.56%. IR ν_{max} (cm^{-1}): 1651 (C=O), 1578 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.41 (s, 1H, H_4 quinoline), 8.22 (d, 1H, $J = 15.6$ Hz, H_β), 8.05–7.91 [m, 3H, (2H phenyl, H_8 quinoline)], 7.64 (s,1H, H_5 quinoline), 7.63–7.52 [m, 3H, (1H phenyl, H_α, H_7 quinoline)], 2.56 (s, 3H, CH_3 quinoline); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.4 (C=O), 149.4 (C–Cl), 146.4 (C-β),139.5, 137.5, 135.5, 133.1, 128.6, 127.6 (Ar–C), 122.7 (C-α), 22.5 (CH_3 quinoline); FAB-MS (*m*/*z*): [M^++1] 387.39.

6.2.5. (E)-1-(4-Bromophenyl)-3-(2-chloroquinoline-3yl)prop-2-en-1-one (**8**)

Yield 88% (Ethyl acetate); mp: 223 °C; Anal. calc. for $C_{18}H_{11}NOClBr$: C 58.02, H 2.98, N 3.76%; found: C 58.05, H 2.94, N 3.70%. IR ν_{max} (cm^{-1}): 1651 (C=O), 1586 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.50 (s, 1H, H_4 quinoline), 8.24 (d,1H, $J = 15.6$ Hz, H_β), 8.05 (d, 1H, $J = 8.4$ Hz, H_5 quinoline), 7.95–7.77 [m, 4H, (2H phenyl, H_6 H_8 quinoline)], 7.69–7.60 [m, 3H, (2H phenyl, H_7 quinoline)], 7.55 (d, 1H, $J = 15.6$ Hz, Hα); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.86 (C=O), 150.3 (C–Cl), 147.9 (C-β), 139.9, 136.2, 131.7, 130.1, 129.4, 128.4 (Ar–C), 125.7 (C-α). FAB-MS (*m*/*z*): [M^++1] 373.44.

6.2.6. (E)-3-(2-Chloro-7-methylquinolin-3-yl)-1-(4-chlorophenyl) prop-2-en-1-one (**9**)

Yield 78% (Ethyl acetate); mp: 158 °C; Anal. calc. for $C_{19}H_{13}NOCl_2$: C 66.68, H 3.83, N 4.09%; found: C 66.63, H 3.77, N 4.11%. IR ν_{max} (cm^{-1}): 1650 (C=O), 1563 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.47 (s, 1H, H_4 quinoline), 8.19 (d, 1H, $J = 15.7$ Hz, H_β), 8.06 (d, 1H, $J = 7.6$ Hz, phenyl), 7.89 (d, 1H, $J = 7.5$ Hz, phenyl), 7.87–7.76 (m, 2H, H_5 H_8 quinoline), 7.56 (d, 1H, $J = 15.6$ Hz, H_α),7.48–7.33 [m, 3H, (2H phenyl, H_6 quinoline), 2.55 (s, 3H, CH_3 quinoline); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.9 (C=O), 149.9 (C–Cl), 147.1 (C-β), 139.2, 138.4, 136.8, 134.6, 132.2, 131.2, 129.7, 128.0, 127.6 (Ar–C), 124.6 (C-α), 21.25 (CH_3 quinoline); FAB-MS (*m*/*z*): [M^++1] 343.03.

6.2.7. (E)-1-(4-Chlorophenyl)-3-(2-chloroquinoline-3yl)prop-2-en-1-one (**10**)

Yield 86% (Ethyl acetate); mp: 167 °C; Anal. calc. for $C_{18}H_{11}NOCl_2$: C 65.87, H 3.38, N 4.27%; found: C 65.82, H 3.31, N 4.22%. IR ν_{max} (cm^{-1}): 1662 (C=O),1580 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.50 (s, 1H, H_4 quinoline), 8.24 (d, 1H, $J = 15.6$ Hz, H_β), 8.05 (d, 1H, $J = 8.2$ Hz, H_5 quinoline), 8.01–7.77 [m, 4H, (2H phenyl, H_6 H_8

quinoline), 7.65–7.60 [m, 3H, (2H phenyl, H_7 quinoline)], 7.56 (s, 1H, J = 15.6 Hz, H_α); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.6 (C=O), 150.6 (C–Cl), 147.7 (C-β), 139.4, 136.2, 135.2, 131.2, 130.0, 128.3, 127.5, 126.3 (Ar–C), 125.2 (C-α); FAB-MS (m/z): [M^++1] 329.24.

*6.2.8. (E)-1-(3-Bromophenyl)- 3-(2-chloro-7-methylquinoline-3yl)-2-methylprop-2-en-1-one (**11**)*

Yield 82% (Ethyl acetate); mp: 275 °C; Anal. calc. for $C_{20}H_{15}NOClBr$: C 59.95, H 3.77, N 3.50%; found: C 59.89, H 3.73, N 3.46%; IR ν_{max} (cm^{-1}): 1657 (C=O), 1580 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.11 (s, 1H, H_4 quinoline), 8.01 (s, 1H, phenyl), 7.79–7.74 [m, 3H, (1H phenyl, H_5 H_8 quinoline)], 7.72 (s, 1H, H_β, C**H**$_\beta$=CMe), 7.44–7.36 [m, 3H, (2H phenyl, H_6 quinoline)], 2.57 (s, 3H, CH_3 quinoline), 2.20 (s, 3H, CH_β=**CMe**); ^{13}C NMR ($CDCl_3$) δ (ppm): 196.8 (C=O), 149.2 (C–Cl), 147.2 (C-β), 141.9, 139.2, 138.7, 138.0, 137.1, 129.8, 129.7, 128.0, 127.3, 126.9 (Ar–C), 124.5 (C-α), 21.9 (CH_3 quinoline), 14.1 [CH_3 (CH_β=**CMe**)]; FAB-MS (m/z): [M^++1] 401.22.

*6.2.9. (E)-1-(3-Bromophenyl)-3-(2-chloro-6-methylquinolin-3-yl)-2-methylprop-2-en-1-one (**12**)*

Yield 84% (Ethyl acetate); mp: 181–183 °C; Anal. calc. for $C_{20}H_{15}NOClBr$: C 59.95, H 3.77, N 3.50%; found: C 59.87, H 3.71, N 3.44%; IR ν_{max} (cm^{-1}): 1645 (C=O), 1577 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.17 (s, 1H, H_4 quinoline), 8.02 (s, 1H, phenyl), 7.94 (d, 1H, J = 8.3 Hz, phenyl), 7.81 (d, 1H, J = 7.5 Hz, H_8 quinoline), 7.73 (s, 1H, H_β C**H**$_\beta$=CMe),7.63 (s, 1H, H_5 quinoline), 7.42–7.37 (m, 2H, phenyl), 7.33 (d, 1H, J = 7.8 Hz, H_7 quinoline), 2.55 (s, 3H, CH_3 quinoline), 2.19 (s, 3H, CH_3 CH_β=**CMe**); ^{13}C NMR ($CDCl_3$) δ (ppm): 196.3 (C=O), 148.0 (C–Cl), 147.2 (C-β), 141.3, 138.7, 137.6, 135.5, 133.6, 132.3, 129.1, 128.1, 127.6 (Ar–C), 126.4 (C-α), 21.3 (CH_3 quinoline), 14.5 [CH_3 (CH_β = **CMe**)]; FAB-MS (m/z): [M^++1] 401.32.

*6.2.10. (E)-1-(3-Bromophenyl)-3-(2-chloroquinoline-3yl)-2-methylprop-2-en-1-one (**13**)*

Yield 89% (Ethyl acetate); mp: 217 °C; Anal. calc. for $C_{19}H_{13}NOClBr$: C 59.02, H 3.39, N 3.62%; found: C 59.03, H 3.32, N 3.58%. IR ν_{max} (cm^{-1}): 1644 (C=O), 1579 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.17 (s, 1H, H_4 quinoline), 8.05 (s, 1H, phenyl), 7.89 (d, 1H, J = 8.4 Hz, phenyl), 7.82 (d, 1H, J = 7.8 Hz, H_8 quinoline), 7.79–7.73 (m,2H, H_5 H_6 quinoline), 7.71(s, 1H, C**H**$_\beta$ = CMe), 7.64–7.59 (1H, t, J = 7.2 Hz, H_7 quinoline), 7.42–7.33 (m, 2H, phenyl), 2.21 (s, 3H, CH_β=**CMe**); ^{13}C NMR ($CDCl_3$) δ (ppm): 196.5 (C=O), 149.5 (C–Cl), 147.3 (C-β), 139.2, 138.5, 136.1, 135.4, 132.4, 128.6, 127.5 (Ar–C), 124.2 (C-α), 14.3[CH_3 (CH_β=**CMe**)]. FAB-MS (m/z): [M^++1] 387.16.

*6.2.11. (E)-3-(2-Chloro-7-methylquinoline-3yl)-1-(3-chlorophenyl)-2-methylprop-2-en-1-one (**14**)*

Yield 84% (Ethyl acetate); mp: 236 °C; Anal. calc. for $C_{20}H_{15}NOCl_2$: C 67.43, H 4.24, N 3.93%; found: C 67.38, H 4.18, N 3.86%. IR ν_{max} (cm^{-1}): 1655 (C=O), 1576 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.12 (s, 1H, H_4 quinoline), 7.86 (s, 1H, phenyl), 7.81 (s, 1H, H_8 quinoline), 7.77–7.74 [m, 2H, (1H phenyl, H_5 quinoline)], 7.73 (s, 1H, C**H**$_\beta$=CMe), 7.47–7.33 [m, 3H, (2H phenyl, H_6 quinoline)] 2.55 (s, 3H, CH_3 quinoline), 2.20 (s, 3H, CH_β=**CMe**); ^{13}C NMR ($CDCl_3$) δ (ppm): 197.0 (C=O), 149.8 (C–Cl), 145.0 (C-β), 139.1, 137.1, 134.6, 132.1, 129.8, 127.6 (Ar–C), 124.5 (C-α), 22.0 (CH_3 quinoline), 14.2 [CH_3 (CH_β=**CMe**)]; FAB-MS (m/z): [M^++1] 357.08.

*6.2.12. (E)-3-(2-Chloro-6-methylquinolin-3-yl)-1-(3-chlorophenyl)-2-methylprop-2-en-1-one (**15**)*

Yield 88% (Ethyl acetate); mp: 178 °C Anal. calc. for $C_{20}H_{15}NOCl_2$: C 67.43, H 4.24, N 3.93%; found: C 67.39 H 4.19, N 3.88%. IR ν_{max} (cm^{-1}): 1651 (C=O), 1578 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.16 (s, 1H, H_4 quinoline), 8.07 (s, 1H, phenyl), 7.94 (d, 1H, J = 8.4 Hz, phenyl), 7.82 (d, 1H, J = 7.5 Hz, H_8 quinoline), 7.74 (s, 1H, C**H**$_\beta$=CMe), 7.63 (s, 1H, H_5 quinoline) 7.48–7.32 [m, 3H, (2H phenyl, H_7 quinoline)], 2.56 (s, 3H, CH_3 quinoline), 2.20 (s, 3H, CH_β=**CMe**); ^{13}C NMR ($CDCl_3$) δ (ppm): 196.3 (C=O), 148.7 (C–Cl), 145.9 (C-β), 138.5, 137.4, 136.3, 134.1, 133.4, 132.2, 129.1, 127.6 (Ar–C), 124.3 (C-α) 21.3, (CH_3 quinoline), 14.5 [CH_3 (CH_β=**CMe**)]; FAB-MS (m/z): [M^++1] 357.41.

*6.2.13. (E)-3-(2-Chloro-6-methylquinoline-3yl)-1-(pyridine-2yl) prop-2-en-1-one (**16**)*

Yield 78% (Ethyl acetate); mp: 188 °C; Anal. calc. for $C_{18}H_{13}N_2OCl$: C 70.02, H 4.24, N 9.07%; found: C 70.03, H 4.19, N 9.02%. IR ν_{max} (cm^{-1}): 1651 (C=O), 1560 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.78 (d, 1H, J = 4.2 Hz, pyridine), 8.58 (s,1H, H_4 quinoline), 8.46 (d, 1H, J = 15.9 Hz, H_β), 8.39 (d, 1H, J = 2.4 Hz, pyridine), 8.26 (d, 1H, J = 7.8 Hz, H_8 quinoline), 7.95–7.90 [m, 2H, (1H pyridine, H_5 quinoline)] 7.66–7.59 [m, 2H, (1H pyridine, H_7 quinoline)], 7.56 (d, 1H, J = 15.6 Hz, H_α), 2.56 (s, 3H, CH_3 quinoline); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.5 (C=O), 149.7 (C–Cl), 148.8 (C-β), 146.5, 139.1, 137.6, 135.6, 133.8, 128.0, 127.1, 126.8 (Ar–C), 124.5 (C-α), 21.5 (CH_3 quinoline). FAB-MS (m/z): [M^++1] 309.37.

*6.2.14. (E)-3-(2-Chloro-7-methylquinoline-3yl)-1-(pyridine-2yl) prop-2-en-1-one (**17**)*

Yield 88% (Ethyl acetate); mp: 235 °C; Anal. calc. for $C_{18}H_{13}N_2OCl$: C 70.02, H 4.24, N 9.07%; found: C 70.05, H 4.18, N 9.03%; IR ν_{max} (cm^{-1}): 1658 (C=O), 1578 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.76 (d, 1H, J = 4.2 Hz, pyridine), 8.63 (s,1H, H_4 quinoline), 8.40 (d, 1H, J = 15.9 Hz, H_β), 8.38 (d, 1H, J = 2.3 Hz, pyridine), 8.26 (d, 1H, J = 7.5 Hz, H_5 quinoline), 7.95–7.81 [m, 2H (1H pyridine, H_8 quinoline)], 7.67–7.57 [m, 2H (1H pyridine, H_6 quinoline)], 7.54 (d, 1H, J = 15.9 Hz, H_α), 2.56 (s, 3H, CH_3 quinoline); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.4 (C=O), 150.3 (C–Cl), 148.4 (C-β), 142.7, 140.4, 139.9, 137.6, 129.1, 127.2, 127.9, 125.3 (Ar–C), 124.2 (C-α), 22.4 (CH_3 quinoline); FAB-MS (m/z): [M^++1] 309.41.

*6.2.15. (E)-3-(2-Chloroquinoline-3yl)-1 (pyridine-2yl)prop-2-en-1-one (**18**)*

Yield 89% (Ethyl acetate); mp: 248 °C; Anal. calc. for $C_{17}H_{11}N_2OCl$: C 69.28, H 3.76, N 9.50%; found: C 69.24, H 3.72, N 9.47%. IR ν_{max} (cm^{-1}): 1653 (C=O), 1566 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.78 (d, 1H, J = 4.2 Hz, pyridine), 8.67 (s, 1H, H_4 quinoline), 8.42 (d, 1H, J = 15.9 Hz, H_β), 8.35 (d, 1H, J = 2.4 Hz, pyridine), 8.26 (d, 1H, J = 7.5 Hz, H_5 quinoline), 8.05–7.76 [m, 3H, (1H pyridine, H_6 H_8 quinoline)], 7.63–7.58 [m, 2H, (1H pyridine, H_7 quinoline)], 7.55 (d, 1H, J = 15.9 Hz, H_α), ^{13}C NMR ($CDCl_3$) δ (ppm): 188.6 (C=O), 150.9 (C–Cl), 148.1 (C-β), 139.9, 137.3, 128.2, 127.8, 125.3 (Ar–C), 124.4 (C-α); FAB-MS (m/z): [M^++1] 295.13.

6.3. In vitro antiamoebic assay

All the compounds (**4**–**18**) were screened *in vitro* for anti-amoebic activity against HM1:IMSS strain of *E. histolytica* by microdilution method [30]. *E. histolytica* trophozoites were cultured in a 96-well microtiter plate suspended in Diamond TYIS-33 growth medium [35]. The test compounds (1 mg) were dissolved in DMSO (40 μL, concentration at which no inhibition of amoeba growth occurred) [27,36]. The stock solutions (1 mg/mL) of the compounds were freshly prepared and twofold serial dilutions were made in the wells of a 96-well microtiter plate. The following controls were included in each plate: 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 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 the Trypan blue exclusion assay to confirm viability. The suspension was diluted to 10^5 organism per mL in fresh medium and 170 μL of this suspension was added to the test and control wells in the plate such that an inoculum of 1.7×10^4 organisms/well was achieved to ensure confluency but no excessive growth 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. The plate was then immediately washed with prewarmed (37 °C) 0.9% (w/v) sodium chloride solution. This procedure was completed as quickly as possible to ensure the plate did not cool, in order to prevent the detachment of amoebae. The plate was allowed to dry at room temperature and the amoebae were fixed with chilled (−20 °C) 100% methanol and when dried, stained with 0.5% aqueous eosin for 15 min. The stained plate was washed 3 times with distilled water and allowed to dry before 200 μL 0.1 N sodium hydroxide was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated taking into account the controls and then plotted against the logarithm of the compound concentration. Linear regression analysis was used to determine the best fitting line from which the IC_{50} value was found (Table 1).

6.3.1. In vitro antimalarial assay

Antimalarial activity of the compounds, against the chloroquine-sensitive (3D7) strain of *P. falciparum,* was performed using the [^{3}H]-hypoxanthine-incorporation assay [37]. The 3D7 strain was continuously maintained *in vitro* in supplemented RPMI-1640 culture media and at a haematocrit of 5%. The culture was incubated at 37 °C in a gaseous atmosphere of 5% CO_2, 3% O_2, 92% N_2 [38] and synchronized at the ring stage with 5% D-sorbitol [39] before being adjusted to a final parasitemia of 0.5% and haematocrit of 1%. This suspension (200 μL) was added to each well of the 96-well plate with the exception of some wells which received non-parasitized red blood cells. Stock solutions of the test compounds were made up in DMSO and serially diluted in a 96-well microtiter plate [37]. The microtiter plate was then incubated for 24 h. Following the incubation period, 25 μL of the radiolabeled [^{3}H]-hypoxanthine isotope (Amersham) at a concentration of 0.5 μCi/well was added to each well. The microtiter plate was then incubated for a further 24 h. The parasitic DNA was harvested onto glass fibre filter mats by use of a Titertek™ semi-automatic cell harvester. The mats were then transferred to sample bags containing scintillation fluid (Perkin–Elmer) and the β-radioactivity counted on the Wallac 1205 Betaplate scintillation counter. The counts per minute (cpm) were generated and the parasite survival rate calculated. The concentration required to inhibit parasite growth by 50% (IC_{50} value) was determined from log sigmoid dose-response curves using the Enzfitter® software. Chloroquine and quinine were used as reference agents. Each experiment was repeated, at least, in triplicate.

6.3.2. Combination study

The antagonistic, synergistic or additive effect of the compound exhibiting the most promising antimalarial activity and quinine were determined by using the tritiated hypoxanthine incorporation assay and constructing isobolograms. This involved preparing various ratios of the two drugs (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9) from which serial dilutions were prepared and from each combination, an IC_{50} value was obtained. The IC_{50} values were used to construct an isobologram from which the type of interaction could be determined [40,41]. Each experiment was repeated, at least, in triplicate.

6.4. Inhibition of β-haemozoin formation assay

To determine whether the compounds had a similar mechanism of action to that of chloroquine, the following were combined in a 96-well microtiter plate: 12.5 μL of the test compound, 12.5 μL of a 1 mg/mL haemin (Sigma) solubilised in DMSO, 25 μL H_2O and finally 50 μl of a 0.5M acetate buffer. The acetate buffer was utilized to simulate the acidic conditions (pH 4.7) of the parasitic food vacuole. The plates were then incubated for 24 h and 100 μL of the solution removed and the same volume substituted with DMSO, the plates were then centrifuged at 1500 g for 10 min. This was repeated 3 times to remove any unreacted haemin. Following this, 100 μL was removed and substituted with a 2M NaOH solution to dissolve the β-haematin crystals. The solution was diluted twofold and the absorbance read at 405 nm [42]. From the data obtained the concentration at which β-haematin formation was inhibited by 50% (IC_{50} value) was determined by use of Enzfitter® software. Classic antimalarials, such as quinine and chloroquine, were used as positive control. Each experiment was repeated, at least, in triplicate.

6.5. DPPH• assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay was used to measure the ability of the compounds to scavenge the stable free radical of DPPH• in comparison to ascorbic acid. DPPH• (100 μL) dissolved in HPLC grade methanol, was added along with 25 μL of the compounds to a 96-well microtiter plate. Controls included a drug-free DPPH• control and a background control consisting of MeOH and DMSO. The plate was then incubated in the dark at room temperature for 30 min before the absorbance read at 540 nm. The DPPH• scavenging activity of the compounds were expressed as the percentage decrease in the absorbance compared with the drug-free DPPH• control. Each experiment was triplicated.

6.6. Cytotoxicity assays

6.6.1. MTT assay

MCF-7 cells were cultured and maintained as a monolayer in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% of fetal calf serum (Sigma) and antibiotics (100 IU/mL of penicillin and 100 μg/mL of streptomycin, Sigma). All cells were cultured at 37 °C in a 100% humidity atmosphere and 5% CO_2 [43]. Exponentially growing viable cells were plated at 1.2×10^4 cells per well into 96-well plates and incubated for 48 h before the addition of the compounds/metronidazole. Stock solutions of compounds were initially dissolved in 20% (v/v) DMSO and further diluted with fresh complete medium. The growth-inhibitory effects of the compounds were measured using standard tetrazolium MTT assay. After 48 h of incubation at 37 °C, the medium was removed and 25 μL of MTT (5 mg/mL) in serum free medium was added to each well. The plates were incubated at 37 °C for 4 h. At the end of the incubation period, the medium was removed and 100 μL DMSO added to all wells. The metabolized MTT product dissolved in DMSO was quantified by reading the absorbance at 570 nm with a reference wavelength of 655 nm in an ELISA plate reader (Labsystems Multiskan RC, Helsinki, Finland). All assays were performed in triplicate. Percent viability was defined as the relative absorbance of treated versus untreated control cells.

6.6.2. Red blood cell toxicity assay

The haemolytic activities of the compounds were evaluated in comparison to the standard antimalarial agents, chloroquine and quinine as well as metronidazole [44]. A suspension of fresh human red blood cells was adjusted to a 1% haematocrit in culture media and plated together with each test compound (100 μM).This suspension was incubated for 48 h before the absorbance was read at 412 nm. The % haemolysis was calculated using a 0.5% Triton X100 solution as the 100% haemolytic control. These results were used to generate a log sigmoid dose-response curve to calculate IC_{50} values and the mean ± s.d. were calculated from at least triplicate values.

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