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Short communication

Synthesis, characterization, antiamoebic activity and cytotoxicity of novel series of pyrazoline derivatives bearing quinoline tail

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ABSTRACT

The cyclization of chalcones (**1a**–**1j**) with 2-(quinolin-8-yloxy) acetohydrazide (**2**) under basic condition led to the formation of new compounds, pyrazoline derivatives (**3a**–**3j**). In vitro antiamoebic activity was performed against HM1: IMSS strain of *Entamoeba histolytica*. The results showed that the compounds **3d**, **3g**, **3h**, and **3j** exhibited promising antiamoebic activity (IC_{50} = 0.05 μM, 0.31 μM, 0.06 μM, 0.29 μM) respectively than the standard drug metronidazole (IC_{50} = 1.84 μM). The toxicological studies of these compounds on human breast cancer MCF-7 cell line showed that all the compounds **3d**, **3g**, **3h**, **3j** and metronidazole were nontoxic at the concentration range of 1.56–50 μM.

1. Introduction

Human infections with *Entamoeba histolytica* may results in different clinical out comes such as asymptomatic gut colonization, amoebic colitis or extra intestinal abscess formation in various organs [1,2]. The prevalence of infection is higher than 5–10% in endemic areas [3] and sometimes as high as 55% [4]. The highest prevalence is found in developing countries in the tropics, particularly in Mexico, India, Central and South America and tropical areas of Asia and Africa. Occasionally, *E. histolytica* trophozoites penetrate the intestinal mucosa, causing amoebic colitis and spread via portal circulation to other organs, most commonly to the liver, where they induce amoebic liver abscess (ALA), the most common extra intestinal manifestation of invasive amoebiasis [5]. Majority of amoebic liver abscess patients have a single abscess of variable sizes located in the right lobe, predominantly with in segment six to eight [6]. In most cases, amoebic liver abscess can be efficiently treated by intake of metronidazole alone [7], but the recent studies have shown that this drug have several toxic effects such as genotoxicity, gastric mucus irritation, and spermatozoid damage [8,9]. Furthermore, failures in the treatment of several intestinal protozoan parasites may result from drug resistant to parasites [10,11]. Considering these problems, there is a pressing need for a new effective drug to treat these infections.

The study of pyrazolines has become of much interest on account of their diverse biological properties such as antitumor, immunosuppressive, antibacterial, anti-inflammatory, anticancer, antidiabetic, and antidepressant properties [12–18]. The recent success of pyrazole COX-2 inhibitor [19] has further highlighted the importance of these heterocycles in medicinal chemistry. A systematic investigation of this class of compounds revealed that pyrazole containing pharmacoactive agents play important role in medicinal chemistry.

The quinoline nucleus occurs in several natural compounds (cinchona alkaloids) and pharmacologically active substances, displaying a broad range of biological activity [20]. In recent years it is reported that the incorporation of quinoline nucleus could alter the course of reaction as well as the biological properties [21,22].

Prompted by the above mentioned biological properties of pyrazoline and quinoline incorporating heterocycles, we here in report the synthesis of a novel series of pyrazoline derivatives (**3a**–**3j**) bearing quinoline tail.

2. Chemistry

The synthesis of novel series of pyrazoline derivatives (**3a**–**3j**) was performed in a manner as outlined in Scheme 1. The cyclization of chalcones (**1a**–**1j**) with 2-(quinolin-8-yloxy) acetohydrazide (**2**) under basic condition (aq·NaOH 10%, 1 mL) in 50 mL absolute

Abbreviations: μg, microgram; μL, microliter; μM, micromole; mL, milliliter; mg, milligram; mmol, millimole.

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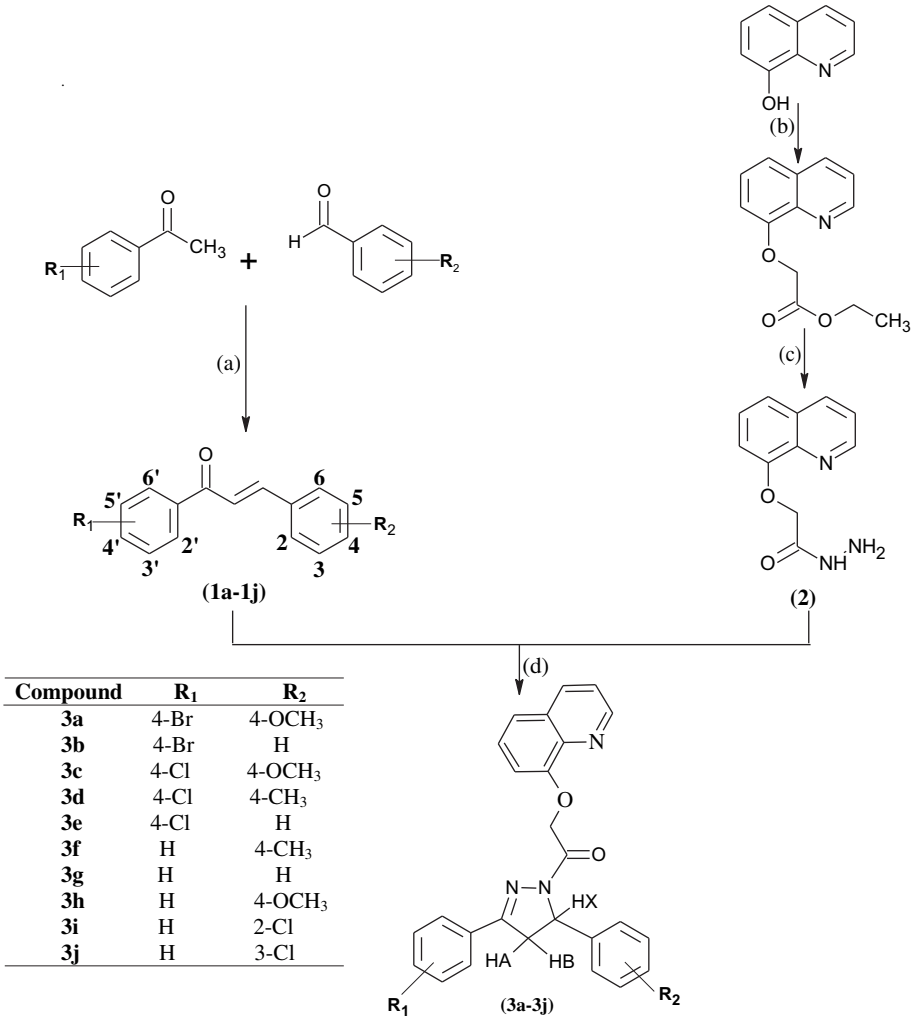


Compound	R_1	R_2
3a	4-Br	4-OCH_3
3b	4-Br	H
3c	4-Cl	4-OCH_3
3d	4-Cl	4-CH_3
3e	4-Cl	H
3f	H	4-CH_3
3g	H	H
3h	H	4-OCH_3
3i	H	2-Cl
3j	H	3-Cl

Reagent and conditions: (a) aq. NaOH, C_2H_5OH, RT (24 hrs.) (b) Ethyl chloroacetate, K_2CO_3, Acetone (c) $N_2H_4.2H_2O$ (d) NaOH, C_2H_5OH, Reflux 12-15hrs

Scheme 1. General method for the synthesis of pyrazoline derivatives (**3a**–**3j**).

ethanol led to the formation of novel series of pyrazoline derivatives. All the compounds were stable in solid state. Melting points were recorded on KSW melting point apparatus and are uncorrected (Fig. 1).

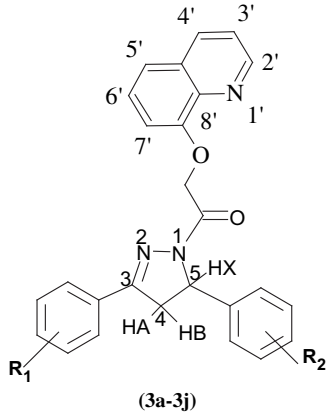


Fig. 1. Structure of pyrazoline derivatives (**3a**–**3j**).

3. Pharmacology

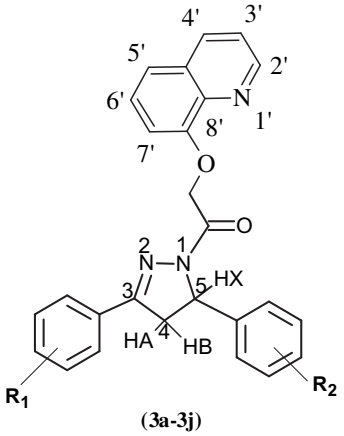
All pyrazoline derivatives (**3a**–**3j**) were screened in vitro against HM1:IMSS strain of *E. histolytica* by microdilution method [23]. All the experiments were carried out in triplicate at each concentration level and repeated thrice. Cytotoxicity of active compounds has been studied by MTT assay on human breast cancer MCF-7 cell line. The results of biological activity and cytotoxicity are summarized in Table 1 and Fig. 2.

4. Results and discussion

4.1. Synthesis

Synthesis of novel series of pyrazoline derivatives (**3a**–**3j**) is described in this study and their reaction sequence is outlined in Scheme 1. A series of chalcones (**1a**–**1j**) were prepared by base-catalyzed Claisen–Schmidt condensation of appropriate substituted acetophenones and benzaldehydes in good yield (75%–80%) [24]. The cyclization of chalcones (**1a**–**1j**) with 2-(quinolin-8-yloxy) acetohydrazide (**2**) in presence of base led to the formation

Table 1
Novel pyrazoline derivatives (**3a**–**3j**), their in vitro antiamoebic activity against HM1:IMSS strain of *E. histolytica* and cytotoxicity profile of compounds **3d**, **3g**, **3h**, **3j** and metronidazole.



(3a-3j)

Compound	R_1	R_2	Antiamoebic activity		Cytotoxicity profile	
			IC_{50} (μM)	± SD[a]	IC_{50} (μM)	± SD[a]
3a	4-Br	4-OCH_3	7.71	0.16	N.D.	N.D.
3b	4-Br	H	16.3	0.02	N.D.	N.D.
3c	4-Cl	4-OCH_3	1.68	0.04	N.D.	N.D.
3d	4-Cl	4-CH_3	**0.05**	0.02	>100	0.26
3e	4-Cl	H	0.72	0.13	N.D.	N.D.
3f	H	4-CH_3	7.50	0.09	N.D.	N.D.
3g	H	H	**0.31**	0.10	>100	0.14
3h	H	4-OCH_3	**0.06**	0.03	98.3	0.18
3i	H	2-Cl	0.71	0.08	N.D.	N.D.
3j	H	3-Cl	**0.29**	0.12	>100	0.14
Metronidazole			**1.84**	0.19	>100	0.28

The compounds with bold font IC_{50} values are more active than metronidazole.
[a] The value obtained in at least three separate assay done in triplicate, N.D. = Not done, S.D. = Standard deviation.

of novel series of pyrazoline derivatives (**3a**–**3j**). All the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies and their data are presented in the experimental section.

4.2. Antiamoebic activity

Preliminary experiments were carried out to determine the in vitro antiamoebic activity of all the compounds (**3a**–**3j**) by microdilution method using HM1: IMSS strain of *E. histolytica* and their IC_{50} values are reported in Table 1. The metronidazole was used as reference drug having IC_{50} = 1.84 μM in our experiment. The results were estimated as the percentage of growth inhibition compared with the untreated controls and plotted as probit values as a function of the drug concentration. IC_{50} and 95% confidence limits were interpolated in the corresponding dose response curve. The pyrazoline derivatives (**3a**–**3j**) showed IC_{50} values in the range 0.05–16.3 μM. In terms of structure–activity relationship, compound **3d** (IC_{50} = 0.05 μM) substituted at **C-4** position of phenyl rings with methyl and chloro groups and compound **3h** (IC_{50} = 0.06 μM) with methoxy substituted and unsubstituted phenyl rings showed excellent antiamoebic activity against the HM1: 1MSS strain of *E. histolytica*. The cyclized pyrazoline analogs **3e** (IC_{50} = 0.72 μM), **3g** (IC_{50} = 0.31 μM), **3i** (IC_{50} = 0.71 μM), and **3j** (IC_{50} = 0.29 μM) having chloro substituted and unsubstituted phenyl rings also showed good antiamoebic activity and the compound **3c** (IC_{50} = 1.68 μM) moderately active than the standard drug metronidazole. Therefore, out of ten compounds screened in vitro for antiamoebic activity, four compounds **3d**, **3g**, **3h** and **3j** were more active than the standard drug metronidazole.

4.3. Cytotoxicity profile

To examine the effect of antiamoebic compounds **3d**, **3g**, **3h**, **3j** and metronidazole on cell proliferation, we ensured their cytotoxicity on human breast cancer MCF-7 cell line. A subconfluent population of MCF-7 cells was treated with increasing concentrations of compounds and the number of viable cells was measured after 48 h by MTT cell viability assay. The concentration range for all the compounds **3d**, **3g**, **3h**, **3j** and metronidazole was 1.56–100 μM. Figure-2 depicts the compounds **3d**, **3g**, **3h**, **3j** and metronidazle exhibited >80% viability at the concentration range of 1.56–50 μM. On increasing the concentration range up to 100 μM only compound **3g** (Viability 55%) and **3h** (Viability 49%) showed moderate cytotoxicity against the human breast cancer MCF-7 cell line. The cytotoxicity IC_{50} values along with the standard deviation values of compounds **3d**, **3g**, **3h**, **3j** and metronidazle are given in Table 1.

5. Conclusion

In summary, novel pyrazoline derivatives (**3a**–**3j**) were synthesized by the cyclization of chalcones (**1a**–**1j**) with 2-(quinolin-8-yloxy) acetohydrazide (**2**) under basic condition. The in vitro antiamoebic activity was examined using HM1: IMSS strain of *E. histolytica* and results showed that out of ten compounds four

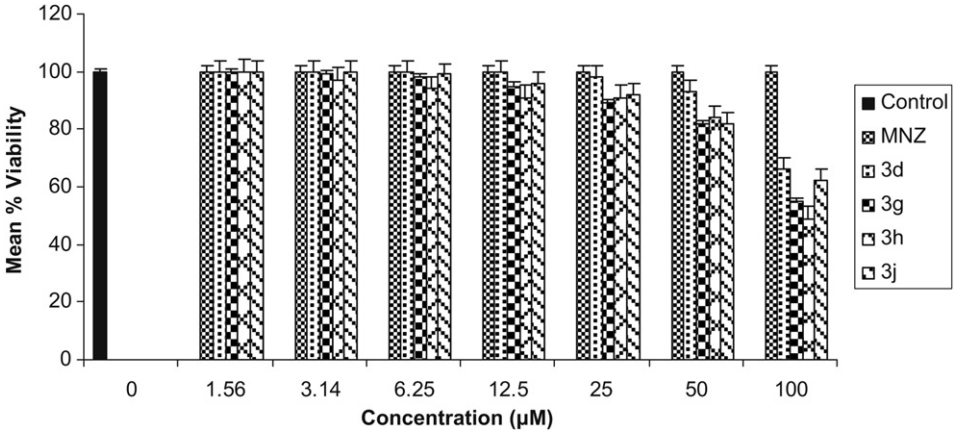


Fig. 2. Cytotoxic effect of compounds **3d**, **3g**, **3h**, **3j** and metronidazole on human breast cancer MCF-7 cells as assessed by the MTT dye reduction assay following a 48 h treatment. Data represent the arithmetic means ± S.D. of at least three independent experiments (n = 3 in triplicates).

compounds **3d**, **3g**, **3h** and **3j** exhibited higher antiamoebic activity than the reference drug metronidazole (IC_{50} = 1.84 μM). The MTT assay revealed that all the compounds **3d**, **3g**, **3h**, **3j** and metronidazole were nontoxic at the concentration range of 1.56–50 μM. On increasing the concentration range up to 100 μM, only compound **3g** and **3h** showed moderate cytotoxicity against the human breast cancer MCF-7 cell line.

6. Experimental protocol

All the required chemicals were purchased from Merck and Aldrich Chemical Company (USA). 2-(Quinolin-8-yloxy) acetohydrazide (**2**) was synthesized by using the method reported in literature [25]. Precoated aluminium sheets (silica gel 60 F254, 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-nitro benzyl alcohol (NBA) was used as the matrix.

6.1. General procedure for the synthesis of substituted chalcones (*1a–1j*)

A mixture of substituted acetophenone (0.01 mol) and substituted benzaldehyde (0.01 mol), and sodium hydroxide (1 mL, 10% aqueous) in 50 mL absolute ethanol was stirred at room temperature for 24 h. The resulting solid was filtered, washed with water, dried, and recrystallized from ethanol.

6.1.1. (2E)-1-(4-Bromophenyl)-3-(4-methoxyphenyl) propenone (*1a*)

Yield: 78%; mp: 67 °C; white crystals; Anal. calc. for $C_{16}H_{13}O_2Br$: C 60.59, H 4.13%; found: C 60.48, H 4.08%; IR ν_{max} (cm^{-1}): 3036 (Ar–H), 2933 (C–H), 1676 (C=O), 1442 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.89 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.81 (d, 1H, J = 15.6 Hz, H-β), 7.65–7.59 (m, 4H, Ar–H), 7.37 (d, 1H, J = 15.6 Hz, H-α), 6.95 (d, 2H, J = 8.7 Hz, H-3, H-5), 3.86 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.73 (C=O), 144.41 (C-β), (136.07, 134.70, 130.02, 128.51, Ar–H), 122.52 (C-α), 53.81 (OCH_3); FAB-MS (m/z): [M^+ + 1] 318.

6.1.2. (2E)-1-(4-Bromophenyl)-3-phenyl propenone (*1b*)

Yield: 74%; mp: 163 °C; white crystals; Anal. calc. for $C_{15}H_{11}OBr$: C 62.74, H 3.86%; found: C 62.63, H 3.75%; IR ν_{max} (cm^{-1}): 3056 (Ar–H), 2931 (C–H), 1656 (C=O), 1446 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.89 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.79 (d, 2H, J = 8.1 Hz, H-3′, H-5′), 7.56 (d, 1H, J = 15.6 Hz, H-β), 7.44–7.38 (m, 2H, Ar–H), 7.31(1H, J = 15.6 Hz, H-α), 7.15–6.86 (m, 3H, Ar–H); ^{13}C NMR ($CDCl_3$); δ (ppm): 189.83 (C=O), 145.41 (C-β), (136.07, 134.70, 130.02, 128.51, Ar–H), 121.52 (C-α); FAB-MS (m/z): [M^+ + 1] 288.

6.1.3. (2E)-1-(4-Chlorophenyl)-3-(4-methoxyphenyl) propenone (*1c*)

Yield: 80%; mp: 94 °C; light green crystals; Anal. calc. for $C_{16}H_{13}O_2Cl$: C 70.46, H 4.80%; found: C 70.34, H 4.68%; IR ν_{max} (cm^{-1}): 3064 (Ar–H), 2934 (C–H), 1677 (C=O), 1442 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.83 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.81 (d, 1H, J = 15.6 Hz, H-β), 7.64–7.58 (m, 4H, Ar–H), 7.37 (d, 1H, J = 15.6 Hz, H-α), 6.96 (d, 2H, J = 8.7 Hz, H-3, H-5), 3.85 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 190.18 (C=O), 144.65 (C-β), (135.61, 132.70, 130.02, 128.51, Ar–H), 122.23 (C-α), 53.65 (OCH_3); FAB-MS (m/z): [M^+ + 1] 273.

6.1.4. (2E)-1-(4-Chlorophenyl)-3-(4-methylphenyl) propenone (*1d*)

Yield: 72%; mp: 153 °C; white crystals; Anal. calc. for $C_{16}H_{13}O_2Cl$: C 74.85, H 5.10%; found: C 74.69, H 5.04%; IR ν_{max} (cm^{-1}): 3028 (Ar–H), 2916 (C–H), 1657 (C=O), 1448 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.91 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.82 (d, 1H, J = 15.6 Hz, H-β), 7.55–7.48 (m, 4H, Ar–H), 7.46 (d, 1H, J = 15.6 Hz, H-α), 7.25–7.21 (m, 2H, Ar–H) 2.39 (s,3H, CH3); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.27 (C=O), 145.44 (C-β), (139.07, 131.99, 129.92, 128.63, Ar–H), 120.53 (C-α), 21.54 (methyl); FAB-MS (m/z): [M^+ + 1] 257.

6.1.5. (2E)-1-(4-Chlorophenyl)-3-phenyl propenone (*1e*)

Yield: 78%; mp: 67 °C; white crystals. Anal. calc. for $C_{15}H_{11}OCl$: C 74.23, H 4.57%; found: C 74.12, H 4.45%; IR ν_{max} (cm^{-1}): 3051 (Ar–H), 2930 (C–H), 1683 (C=O), 1440 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.92 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.83 (d, 1H, J = 15.6 Hz, H-β), 7.79–7.53 (m, 4H, H-2, H-6, H-3′, H-5′), 7.49 (d, 1H, J = 15.6 Hz, H-α), 7.37–6.99 (m, 3H, H-3, H-4, H-5); ^{13}C NMR ($CDCl_3$) δ (ppm): 191.43 (C=O), 143.41 (C-β), (136.07, 134.70, 130.02, 128.51, Ar–H), 121.42 (C-α); FAB-MS (m/z): [M^+ + 1] 243.

6.1.6. (2E)-3-(4-Methylphenyl)-1-phenyl propenone (*1f*)

Yield: 76%; mp: 189 °C; light yellow crystals; Anal. calc. for $C_{16}H_{14}O$: C 86.45, H 6.35%; found: C 86.31, H 6.24%; IR ν_{max} (cm^{-1}): 3058 (Ar–H), 2932 (C–H), 1659 (C=O), 1447 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.02 (d, 2H, J = 6.9 Hz, H-2′, H-6′), 7.82 (d, 1H, J = 15.6 Hz, H-β), 7.60–7.49 (m, 4H, Ar–H), 7.46 (d, 1H, J = 15.6 Hz, H-α), 7.25–7.18 (m, 3H, Ar–H), 2.39 (s,3H, CH3).^{13}C NMR ($CDCl_3$) δ (ppm): 189.23 (C=O), 144.41 (C-β), (134.70, 130.02, 129.53,128.51, phenyl), 122.52 (C-α), 22.45 (methyl); FAB-MS (m/z): [M^+ + 1] 223.

6.1.7. (2E)-1,3-diphenyl propenone (*1g*)

Yield: 75%; mp: 57 °C; yellow crystals; Anal. calc. for $C_{15}H_{12}O$: C 86.51, H, 5.81%; found: C 86.38, H 5.69%; IR ν_{max} (cm^{-1}): 3064 (Ar–H), 2939 (C–H), 1658 (C=O), 1460 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.81 (m, 10H, Ar–H), 7.74 (d, 1H, J = 15 Hz, H-β), 7.53 (d, 1H, J = 15 Hz, H-α); ^{13}C NMR ($CDCl_3$) δ (ppm): 190.49 (C=O), 144.78 (C-β), (134.51, 132.72, 128.92, 127.63, Ar–H), 122.09 (C-α); FAB-MS (m/z): [M^+ + 1] 209.

6.1.8. (2E)-3-(4-Methoxyphenyl)-1-phenyl propenone (*1h*)

Yield: 78%; mp: 122 °C; light green crystals; Anal. calc. for $C_{16}H_{14}O_2$: C 80.65, H 5.92%; found: C 80.51, H 5.81%; IR ν_{max} (cm^{-1}): 3054 (Ar–H), 2938 (C–H), 1656 (C=O), 1446 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.97 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.65 (d, 1H, J = 15.6 Hz, H-β), 7.56–7.44 (m, 4H, Ar–H), 7.39 (d, 1H, J = 15.6 Hz, H-α), 7.38–7.11(m, 3H, Ar–H), 3.86 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 191.83 (C=O), 144.41 (C-β), (135.17, 134.70, 132.02, 128.59 Ar–H), 121.52 (C-α), 54.65 (OCH_3); FAB-MS (m/z): [M^+ + 1] 239.

6.1.9. (2E)-3-(2-Chlorophenyl)-1-phenyl propenone (*1i*)

Yield: 74%; mp: 133 °C; light yellow crystals; Anal. calc. for $C_{15}H_{11}OCl$: C 74.23, H 4.57%; found: C 74.17, H 4.48%; IR ν_{max} (cm^{-1}): 3060 (Ar–H), 2931 (C–H), 1662 (C=O), 1465 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.20 (d, 1H, J = 5.4 Hz, H-6′), 8.15 (d, 1H, J = 5.4 Hz, H-2′), 8.01 (d, 1H, J = 15 Hz, H-β), 7.76–7.30 (m, 8H, Ar–H, H-α); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.83 (C=O), 143.41 (C-β), (136.98, 135.43, 134.20, 130.02, 128.58, Ar–H), 123.56 (C-α); FAB-MS (m/z): [M^+ + 1] 243.

6.1.10. (2E)-3-(3-Chlorophenyl)-1-phenyl propenone (*1j*)

Yield: 98%; mp: 172 °C; light green crystals; Anal. calc. for $C_{15}H_{11}OCl$: C 74.23, H 4.57%; found: C 74.13, H 4.41%; IR ν_{max} (cm^{-1}): 3068 (Ar–H),

2913 (C–H), 1683 (C=O), 1466 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.94 (d, 2H, J = 8.3 Hz, H-2′, H-6′), 7.65 (d, 1H, J = 15.6 Hz, H-β), 7.53–7.37 (m, 5H, Ar–H), 7.36(d, 1H, J = 15.615.6 HzHz, H-α), 7.31–7.27(m, 2H, Ar–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 190.03 (C=O), 142.41 (C-β), (136.71, 134.94, 130.17, 128.66 Ar–H), 123.52 (C-α); FAB-MS (m/z): [M^+ + 1] 243.

6.2. Preparation of 2-(quinolin-8-yloxy) acetohydrazide (**2**)

2-(Quinolin-8-yloxy) acetohydrazide was prepared by a reported method [25].

6.3. General procedure for the synthesis of pyrazoline derivatives (**3a**–**3j**)

A mixture of the chalcones **1a**–**1j** (10 mmol), 2-(quinolin-8-yloxy) acetohydrazide **2** (10 mmol) and NaOH (25 mmol) was refluxed in ethanol (50 mL) for 12–15 h. The contents were reduced under *vacuo*, cooled, and poured onto crushed ice and kept for 1 h at room temperature. The resulting precipitate was collected by filtration and purified by column chromatography using hexane:ethyl acetate (7:3 v/v) as eluent to yield (**3a**–**3j**).

6.3.1. 1-(3-(4-Bromophenyl)-4,5-dihydro-5-(4-methoxyphenyl) pyrazol-1-yl)-2-(quinolin-8-yloxy) ethanone (**3a**)

Yield: 18%; mp: 104 °C; white solid; Anal. calc. for $C_{27}H_{22}N_3O_3Br$: C 62.80, H 4.29, N 8.14%; found: C 62.73, H 4.24, N 8.16%; IR ν_{max} (cm^{-1}): 3035 (Ar–H), 2928 (CH_2), 1683 (C=O), 1592 (C=N), 1446 (C=C), 1238 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.92 (d, 1H, J = 3.6 Hz, H-2′quinoline ring), 8.10 (d, 1H, J = 8.1 Hz, H-4′quinoline ring), 7.64–6.80 (m, 12H, phenyl and quinoline ring), 5.61 [dd, 1H, J = 4.5, 11.7 Hz, H_x (pyrazoline ring)], 5.40 (s, 2H, OCH_2), 3.75 [dd, 1H, J = 12, 18 Hz, H_A (pyrazoline ring)], 3.21 [dd, 1H, J = 4.5, 17.7 Hz, H_B (pyrazoline ring)], 3.67 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 165.88 (C=O), 155.75 (C-3, pyrazoline ring), (148.23, 141.25, 134.73, 132.03, 128.12,,123.53, 121.26, 109.50, quinoline and phenyl ring), 65.91 (CH_2), 55.97 (OCH_3), 55.29 (C-5, pyrazoline ring), 41.51 (C-4, pyrazoline ring); FAB-MS (m/z): [M^+ + 1] 517.

6.3.2. 1-(3-(4-Bromophenyl)-4,5-dihydro-5-phenylpyrazol-1-yl)-2-(quinolin-8-yloxy) ethanone (**3b**)

Yield: 15%; mp: 125 °C; white solid; Anal. calc. for $C_{26}H_{20}N_3O_2Br$: C 64.21, H 4.14, N 8.64%; found: C 64.16, H 4.09, N, 8.51%; IR ν_{max} (cm^{-1}): 3031 (Ar–H), 2921 (CH_2), 1681 (C=O), 1591 (C=N), 1439 (C=C), 1229 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.92 (d, 1H, J = 3.7 Hz, H-2′quinoline ring), 8.11 (d, 1H, J = 8.1 Hz, H-4′quinoline ring), 7.65–6.97 (m, 13H, phenyl and quinoline ring), 5.66 [dd, 1H, J = 4.5, 11.7 Hz, H_x (pyrazoline ring)], 5.43 (s, 2H, OCH_2), 3.81 [dd, 1H, J = 11.7, 17.7 Hz, H_A (pyrazoline ring)], 3.24 [dd, 1H, J = 4.8, 17.7 Hz, H_B (pyrazoline ring)]; ^{13}C NMR ($CDCl_3$) δ (ppm): 165.82 (C=O), 155.69 (C-3, pyrazoline), (148.25, 140.25, 133.73, 132.03, 127.18, 126.62, 123.93, 121.36, 109.58, quinoline and phenyl ring), 65.31 (CH_2), 55.32 (C-5, pyrazoline), 41.84 (C-4, pyrazoline); FAB-MS (m/z): [M^+ + 1] 487.

6.3.3. 1-(3-(4-Chlorophenyl)-4,5-dihydro-5-(4-methoxyphenyl) pyrazol-1-yl)-2-(quinolin-8-yloxy) ethanone (**3c**)

Yield: 8%; mp: 110 °C; white solid; Anal. calc. for $C_{27}H_{22}N_3O_2Cl$: C 68.71, H 4.70, N 8.90%; found: C 68.62, H 4.61, N 8.82%; IR ν_{max} (cm^{-1}): 3036 (Ar–H), 2934 (CH_2), 1677 (C=O), 1593 (C=N), 1442 (C=C), 1180 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.92 (d, 1H, J = 3.7 Hz, quinoline ring), 8.10 (d, 1H, J = 8.1 Hz, quinoline ring), 7.71–6.80 (m, 12H, phenyl and quinoline ring), 5.61 [dd, 1H, J = 4.8, 11.7 Hz, H_x (pyrazoline ring)], 5.46 (s, 2H, OCH_2), 3.78 [dd, 1H, J = 11.7, 17.4 Hz, H_A (pyrazoline ring)], 3.22 [dd, 1H, J = 4.8, 17.7 Hz, H_B(pyrazoline ring)], 3.68 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 165.98 (C=O), 155.29 (C-3, pyrazoline ring), (149.21, 141.25, 134.73, 132.03,128.28, 126.76, 123.33, 121.30,109.88, quinoline ring and phenyl ring), 65.31 (CH_2), 55.27 (OCH_3), 55.32 (C-5, pyrazoline), 41.84 (C-4, pyrazoline); FAB-MS (m/z): [M^+ + 1] 472.

6.3.4. 1-(3-(4-Chlorophenyl)-4,5-dihydro-5-p-tolylpyrazol-1-yl)-2-(quinolin-8- yloxy) ethanone (**3d**)

Yield: 12%; mp: 132 °C; white solid; Anal. calc. for $C_{27}H_{22}N_3O_2Cl$: C 71.13, H 4.86, N 9.22%; found: C 71.18, H 4.74, N 9.13%; IR ν_{max} (cm^{-1}): 3034 (Ar–H), 2927 (CH_2), 1682 (C=O), 1594 (C=N), 1440 (C=C), 1198 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.92 (d, 1H, J = 3.6 Hz, H-2′quinoline ring), 8.10 (d, 1H, J = 8.1 Hz, H-4′quinoline ring), 7.71–6.97 (m, 12H, phenyl and quinoline ring), 5.62 [dd, 1H, J = 4.5, 11.7 Hz, H_x (pyrazoline ring)], 5.42 (s, 2H, OCH_2), 3.78 [dd, 1H, J = 12, 17.7 Hz, H_A (pyrazoline ring)], 3.21 [dd, 1H, J = 4.5, 17.7 Hz, H_B (pyrazoline)], 2.29 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 165.27 (C=O), 154.29 (C-3, pyrazoline ring), (149.23, 140.25, 135.73, 132.03, 128.28, 126.76, 123.33, 121.30, 109.88, quinoline and phenyl ring), 66.31 (CH_2), 55.27 (OCH_3), 55.32 (C-5, pyrazoline), 41.51 (C-4, pyrazoline); FAB-MS (m/z): [M^+ + 1] 456.

6.3.5. 1-(3-(4-Chlorophenyl)-4,5-dihydro-5-phenylpyrazol-1-yl)-2-(quinolin-8-yloxy) ethanone (**3e**)

Yield: 14%; mp: 96 °C; white solid; Anal. calc. for $C_{26}H_{20}N_3O_2Cl$: C 70.67, H 8.02, N 9.51%; found: C 70.54, H 8.06, N 9.43%; IR ν_{max} (cm^{-1}): 3087 (Ar–H), 2924 (CH_2), 1678 (C=O), 1592 (C=N), 1432 (C=C), 1241 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.93 (d, 1H, J = 3.9 Hz, H-2′quinoline ring), 8.11 (d, 1H, J = 8.1 Hz, H-4′quinoline ring), 7.79–6.96 (m, 13H, phenyl and quinoline ring), 5.62 [dd, 1H, J = 4.5, 11.7 Hz, H_x (pyrazoline ring)], 5.43 (s, 2H, OCH_2), 3.85 [dd, 1H, J = 12, 18 Hz, H_A (pyrazoline)], 3.24 [dd, 1H, J = 4.8, 18 Hz, H_B (pyrazoline ring)]; ^{13}C NMR ($CDCl_3$) δ (ppm): 165.37 (C=O), 154.49 (C-3, pyrazoline ring), (148.23, 141.35, 135.73, 131.63, 128.28, 127.76, 123.33, 121.30, 108.88, quinoline and phenyl ring), 65.31 (CH_2), 55.35 (C-5, pyrazoline ring), 41.51 (C-4, pyrazoline ring); FAB-MS (m/z): [M^+ + 1] 442.

6.3.6. 1-(4,5-Dihydro-3-phenyl-5-p-tolylpyrazol-1-yl)-2-(quinolin-8-yloxy)ethanone (**3f**)

Yield: 12%; mp: 103 °C; white solid; Anal. calc. for $C_{27}H_{23}N_3O_2$: C 76.94, H 5.50, N 9.97%; found: C 76.81, H 5.41, N 9.91%; IR ν_{max} (cm^{-1}): 3042 (Ar–H), 2928 (CH_2), 1684 (C=O), 1595 (C=N),1434 (C=C), 1244 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.93 (d, 1H, J = 3.9 Hz, H-2′quinoline ring), 8.10 (d, 1H, J = 8.1 Hz, H-4′quinoline ring), 7.79–6.98 (m, 13H, phenyl and quinoline ring), 5.62 [dd, 1H, J = 4.5, 11.7 Hz, H_x (pyrazoline ring)], 5.44 (s, 2H, OCH_2), 3.82 [dd, 1H, J = 12, 18 Hz, H_A (pyrazoline ring)], 3.26 [dd, 1H, J = 4.8, 18 Hz, H_B (pyrazoline ring)], 2.29 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 165.42 (C=O),155.02 (C-3, pyrazoline ring), (149.28, 141.38, 133.73, 130.63, 128.98, 127.36, 123.33, 121.70, 109.88, quinoline and phenyl ring), 66.31 (CH_2), 55.33 (C-5, pyrazoline ring), 41.65 (C-4, pyrazoline ring); FAB-MS (m/z): [M^+ + 1] 422.

6.3.7. 1-(4,5-Dihydro-3,5-diphenylpyrazol-1-yl)-2-(quinolin-8-yloxy)ethanone (**3g**)

Yield: 13%; mp: 115 °C; white solid; Anal. calc. for $C_{26}H_{21}N_3O_2$: C 76.64, H 5.19, N 9.97%; found: C 76.53, H 5.14, N 9.89%; IR ν_{max} (cm^{-1}): 3034 (Ar–H), 2928 (CH_2), 1676 (C=O), 1591 (C=N), 1466 (C=C), 1123 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.92 (d, 1H, J = 3.6 Hz, H-2′quinoline ring), 8.10 (d, 1H, J = 8.1 Hz, H-4′quinoline ring), 7.79–6.99 (m, 14H, phenyl and quinoline ring), 5.66 [dd, 1H, J = 5.1, 11.7 Hz, H_x (pyrazoline ring)], 5.43 (s, 2H, OCH_2), 3.84 [dd, 1H, J = 12, 18 Hz, H_A (pyrazoline ring)], 3.28 [dd, 1H, J = 4.8, 17.7 Hz, H_B (pyrazoline ring)]; ^{13}C NMR ($CDCl_3$) δ (ppm): 168.47 (C=O), 155.89 (C-3, pyrazoline ring), (149.83, 141.35, 134.63, 131.63, 127.68, 127.76, 123.33, 120.34, 109.18, quinoline and phenyl ring), 67.31 (CH_2), 55.45 (C-5, pyrazoline ring), 41.21 (C-4, pyrazoline ring); FAB-MS (m/z): [M^+ + 1] 408.

*6.3.8. 1-(4, 5-Dihydro-5-(4-methoxyphenyl)-3-phenylpyrazol-1-yl)-2-(quinolin-8-yloxy) ethanone (**3h**)*

Yield: 8%; mp: 98 °C; white solid; Anal. calc. for $C_{27}H_{23}N_3O_3$: C, 74.12, H 5.30, N 9.60%; found: C 74.14, H 5.22, N 9.51%; IR ν_{max} (cm^{-1}): 3038 (Ar–H), 2926 (CH_2), 1674 (C=O), 1593 (C=N), 1441 (C=C), 1185 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.93 (d, 1H, J = 3.7 Hz, H-2′quinoline ring), 8.12 (d,1H, J = 8.1 Hz, H-4′quinoline ring), 7.79–7.06 (m, 13H, phenyl and quinoline ring), 5.62 [dd, 1H, J = 5.1, 11.7 Hz, H_x (pyrazoline ring)], 5.42 (s, 2H, OCH_2), 3.83 [dd, 1H, J = 12,18 Hz, H_A (pyrazoline ring)], 3.26 [dd, 1H, J = 4.8, 17.7 Hz, H_B (pyrazoline ring)]; ^{13}C NMR ($CDCl_3$) δ (ppm): 165.37 (C=O), 154.49 (C-3, pyrazoline ring), (148.23, 141.35, 135.73, 131.63, 128.28, 127.76, 123.33, 121.30, 108.88 quinoline and phenyl ring), 65.31 (CH_2), 57.80 (OCH_3), 55.35 (C-5, pyrazoline ring), 41.52 (C-4, pyrazoline ring); FAB-MS (m/z): [M^+ + 1] 438.

*6.3.9. 1-(5-(2-Chlorophenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)-2-(quinolin-8-yloxy) ethanone (**3i**)*

Yield: 12%; mp: 117 °C; white solid; Anal. calc. for $C_{26}H_{20}N_3O_2Cl$: C 70.67, H 4.56, N 9.51%; found: C 70.59, H 4.46, N 9.45%; IR ν_{max} (cm^{-1}): 3043 (Ar–H), 2927 (CH_2), 1682 (C=O), 1594 (C=N), 1440 (C=C), 1218 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.93 (d, 1H, J = 3.8 Hz, H-2′quinoline ring), 8.11 (d, 1H, J = 8.1 Hz, H-4′quinoline ring), 7.77–7.07 (m, 13H, phenyl and quinoline ring), 5.62 [dd, 1H, J = 5.1, 11.7 Hz, H_x (pyrazoline ring)], 5.49 (s, 2H, OCH_2), 3.88 [dd, 1H, J = 12, 18 Hz, H_A (pyrazoline ring)], 3.16 [dd, 1H, J = 4.8, 17.7 Hz, H_B (pyrazoline ring)]; ^{13}C NMR ($CDCl_3$) δ (ppm): 166.31 (C=O), 155.69 (C-3, pyrazoline ring), (148.63, 140.15, 134.76, 132.83, 128.88, 127.76, 123.33, 121.30, 109.43, quinoline and phenyl ring), 66.31 (CH_2), 55.25 (C-5, pyrazoline ring), 41.59 (C-4, pyrazoline ring); FAB-MS (m/z): [M^+ + 1] 442.

*6.3.10. 1-(5-(3-Chlorophenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)-2-(quinolin-8-yloxy) ethanone (**3j**)*

Yield: 16%; mp: 146 °C; white solid; Anal. calc. for $C_{26}H_{20}N_3O_2Cl$: C 70.67, H 4.56, N 9.51%; found: C 70.56, H 4.49, N 9.42%; IR ν_{max} (cm^{-1}): 3068 (Ar–H), 2913 (CH_2), 1683 (C=O), 1596 (C=N),1466 (C=C), 1239 (C–N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.94 (d, 1H, J = 3.8 Hz, H-2′quinoline ring), 8.10 (d, 1H, J = 8.1 Hz, H-4′quinoline ring), 7.79–7.01 (m, 13H, phenyl and quinoline ring), 5.62 [dd, 1H, J = 4.5, 11.7 Hz, H_x (pyrazoline ring)], 5.44 (s, 2H, OCH_2), 3.85 [dd, 1H, J = 12, 18 Hz, H_A (pyrazoline ring)], 3.25 [dd, 1H, J = 4.8, 17.7 Hz, H_B (pyrazoline ring)]; ^{13}C NMR ($CDCl_3$) δ (ppm): 165.24 (C=O), 155.69 (C-3, pyrazoline ring), (149.27, 141.34, 134.73, 132.63, 128.18, 127.76, 123.44, 121.50, 108.68, quinoline and phenyl ring), 66.31 (CH_2), 55.15 (C-5, pyrazoline ring), 41.57 (C-4, pyrazoline ring); FAB-MS (m/z): [M^+ + 1] 442.

6.4. In vitro antiamoebic assay

All the compounds (**3a**–**3j**) were screened in vitro for antiamoebic activity against HM1: IMSS strain of *E. histolytica* by microdilution method [23]. *E. histolytica* trophozoites were cultured in wells of 96-well microtiter plate by using Diamond TYIS-33 growth medium [26]. The test compounds (1 mg) were dissolved in DMSO (40 μL) at which level no inhibition of amoeba occurs [27,28]. The stock solutions of the compounds were freshly prepared 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 hemocytometer, using trypan blue exclusion to confirm the viability. The suspension was diluted to 10^5 organism/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 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 chilled methanol for 15 min 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 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 % 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.

6.5. MTT assay

The human breast cancer MCF-7 cells used in this study were purchased from National Center for Cell Science (NCCS), Pune, India. The cells were cultured and maintained as monolayer in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% of fetal calf serum (Sigma), antibiotics: 100 IU/mL of penicillin (Sigma) and 100 μg/mL of streptomycin (Sigma). All cells were cultured in T-75 corning culture flasks at 37 °C in the 100% humidity atmosphere and 5% of CO_2 [29]. Only viable cells were used in the assay. Exponentially growing cells were plated at 1.2×10^4 cells per well into 96-well plates and incubated for 48 h before the addition of drugs to achieve the maximum confluency of the cells. Stock solutions were prepared by dissolving the compounds in 20% (v/v) DMSO and further diluted with fresh complete medium to achieve 1 M concentration. 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 reagent (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 pure DMSO 100 μL was added to each well. The metabolized MTT product dissolved in DMSO was quantified by measuring the absorbance at 570 nm on an ELISA plate reader (Labsystems Multiskan RC, Helsinki, Finland) with a reference wavelength of 655 nm. All assays were performed in triplicate and repeated thrice. Percent viability was defined as the relative absorbance of treated versus untreated control cells.

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References

[1] R. Haque, C.D. Huston, M. Hughes, E. Houpt, W.A. Petri Jr., N. Engl. J. Med. 348 (2003) 1565–1573.

[2] S.L. Stanley Jr., Lancet 361 (2003) 1025–1034.
[3] J. Blessman, I.K.M. Ali, P.A.T. Nu, B.T. Dinh, T.Q.N. Viet, A.L. Van, et al., J. Clin. Microbiol. 41 (2003) 4745–4750.
[4] R. Haque, P. Duggal, I.M. Ali, M.B. Hossain, D. Mondal, R.B. Sack, et al., J. Infect. Dis. 186 (2002) 547–552.
[5] C.D. Wells, M. Arguedas, Southampt. Med. J. 97 (2004) 673–682.
[6] J. Blessmann, H.D. Binh, D.M. Hung, E. Tannich, G. Burchard, Trop. Med. Int. Health 8 (2003) 1030–1034.
[7] W.A. Petri, Parasitology 19 (2003) 523–526.
[8] F.A. el-Nahas, M.I. el-Ashmawy, Basic Clin. Pharmacol. Toxicol. 94 (2004) 226–231.
[9] V. Purohit, K.A. Basu, Chem. Res. Toxicol. 13 (2000) 673–692.
[10] P. Abboud, V. Lemee, G. Gargala, P. Brasseur, J.J. Ballet, F. Borsa-Lebas, et al., Clin. Infect. Dis. 32 (2001) 1792–1794.
[11] W. Petri, Trends Parasitol. 19 (2003) 523–526.
[12] Z. Ratković, Z.D. Juranić, T. Stanojković, D. Manojlović, R.D. Vukićević, N. Radulović, et al., Bioorg. Chem. 38 (2010) 26–32.
[13] M.S. Karthikeyan, B.S. Holla, N.S. Kumari, Eur. J. Med. Chem. 42 (2007) 30–36.
[14] B.S. Holla, P.M. Akberali, M.K. Shivananda, Farmaco 55 (2000) 256–263.
[15] E. Bansal, V.K. Srivastava, A. Kumar, Eur. J. Med. Chem. 36 (2001) 81–92.
[16] F. Manna, F. Chimenti, R. Fioravanti, A. Bolasco, D. Secci, P. Chimenti, et al., Bioorg. Med. Chem. Lett. 15 (2005) 4632–4635.
[17] A.J. Hee, K. Hye-Min, J.S. Ho, K.S. Kyu, K.K. Rok, R.S. Dal, et al., Bioorg. Med. Chem. Lett. 14 (2004) 4461–4465.
[18] Y.R. Prasad, A.L. Rao, L. Prasoona, K. Murali, P.R. Kumar, Bioorg. Med. Chem. Lett. 15 (2005) 5030–5034.
[19] M.V.R. Reddy, V.K. Billa, V.R. Pallela, M.R. Mallireddigari, R. Boominathan, J.L. Gabriel, et al., Bioorg. Med. Chem. 16 (2008) 3907–3916.
[20] P.M.S. Chauhan, S.K. Srivastava, Curr. Med. Chem. 8 (2001) 1535–1542.
[21] K.H. Chikhalia, M.J. Patel, D.B. Vashi, Arkivoc xiii (2008) 189–197.
[22] M. Azad, M.A. Munawar, H.L. Siddiqui, J. Appl. Sci. 7 (2007) 2485–2489.
[23] C.W. Wright, M.J. O'Neill, J.D. Phillipson, D.C. Warhurst, Antimicrob. Agents Chemother. 32 (1988) 1725–1729.
[24] J.N. Dominguez, C. Leon, J. Rodrigues, N.G. Dominguez, J. Gut, P.J. Rosenthal, Eur. J. Med. Chem. 44 (2009) 1457–1462.
[25] M. Ahmed, R. Sharma, D.P. Nagda, J.L. Jat, G.L. Talesara, Arkivoc xi (2006) 66–75.
[26] L.S. Diamond, D.R. Harlow, C.C.R. Cunnick, Trans. R. Soc. Trop. Med. Hyg. 72 (1978) 431–432.
[27] F.D. Gillin, D.S. Reiner, M. Suffness, Antimicrob. Agents Chemother. 22 (1982) 342–345.
[28] A.T. Keene, A. Harris, J.D. Phillipson, D.C. Warhurst, Planta Med. 52 (1986) 278–284.
[29] F. Gümüs, Ö. Algül, G. Eren, H. Eroğlu, N. Diril, S. Gür, et al., Eur. J. Med. Chem. 38 (2003) 473–480.