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Short communication

Design, synthesis and biological evaluation of 3-[4-(7-chloro-quinolin-4-yl)-piperazin-1-yl]-propionic acid hydrazones as antiprotozoal agents

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ABSTRACT

N-Acylhydrazones derived from 7-chloro-4-piperazin-1-yl-quinoline were synthesized and biologically evaluated for blood-stage of *Plasmodium falciparum* and *Entamoeba histolytica* trophozoites. *N*-Acylhydrazone **F12** was found to inhibit the *P. falciparum* growth as well as its life cycle with good selectivity, which was achieved by inhibiting hematin formation. Compound **F24** showed better IC_{50} value than the amoebicidal drug metronidazole.

1. Introduction

Entamoeba histolytica and *Plasmodium falciparum* represent major threats to the human health worldwide, with over one billion clinical cases and between four million deaths annually [1]. The protozoan parasites have now become resistant to some of the antiprotozoal medicaments, thereby pressurizing the control measures in place to treat patients infected with malaria and amoebiasis. This scenario has necessitated the search for novel drugs to contribute to the global chemotherapeutic regimens [2,3].

Quinolines are useful source for drug development of antiprotozoal agents [4–6]. The most notable is chloroquine, which is not only an antimalarial, but also a broad-spectrum antiparasitic agent, including antiamoebic activity [7]. Indeed, a large number of naturally and synthetically-derived quinolines are antiprotozoal agents, such as iodoquinol, mefloquine, and amodiaquine [8–10].

In our previous works, chalcones and hydrazones derived from chloroquinoline exhibited antiamoebic activity of higher potency than the parent quinoline [11,12]. However, no substantial antimalarial activities were observed. The *N*-acylhydrazones have reactivity as Michael-acceptors and are classically employed as warheads during drug design [13]. The attachment of *N*-acylhydrazones has been employed both in the design of ligands for further complexation with transition metals [14] and during the processes of hit-to-lead conversion [15]. *N*-Acylhydrazone is a well-known group of antiparasitic agents, which includes antimalarial and antiamoebic properties [16]. Besides, the synthetic redesign of antiparasitics in *N*-acylhydrazones is a cost-efficient and timely manner for developing chemical libraries [17].

In view of these functional properties, it was envisaged to conjugate aminoquinoline with *N*-acylhydrazone. However, recent literature survey revealed that the most likely way of optimizing antimalarial quinolines is by using a chemical linker between the quinoline ring and a second pharmacophoric group [18,19]. In the present study piperazine was used as a linker between the aminoquinoline and *N*-acylhydrazone because of the potent

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antimalarial activities observed for trioxaquines [20]. Considering this perspective, we designed, synthesized and pharmacologically evaluated a chemical library of *N*-acylhydrazones (**F1**–**F33**) derived from 7-chloro-4-piperazin-1-yl-quinoline.

2. Results and discussion

2.1. Chemistry

The synthesis of *N*-acylhydrazones (**F1**–**F33**) was accomplished in four steps as outlined in Scheme 1. The nucleophilic substitution of 4,7-dichloroquinoline (1) with piperazine afforded 7-chloro-4-piperazin-1-yl-quinoline (2). Synthesized quinoline (2) was then reacted under neat conditions with methyl acrylate, forming 3-[4-(7-chloro-quinolin-4-yl)piperazin-1-yl]propionic acid methyl ester (3). The reaction of compound (3) with hydrazine hydrate furnished hydrazide (4) which was further reacted with commercially available aldehydes to give *N*-acylhydrazones (**F1**–**F33**).

2.2. Biology

2.2.1. Antiamoebic activity

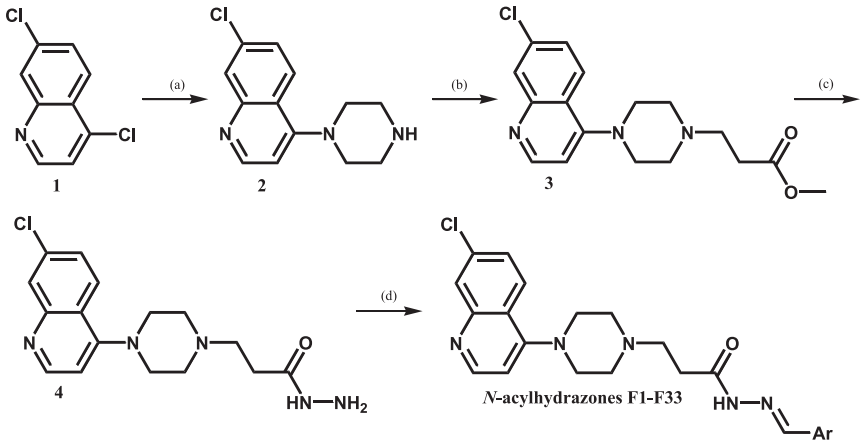
All the desired compounds were screened for antiamoebic activity against HM1:IMSS strain of *E. histolytica* and metronidazole (Mtz) was used as reference amoebicidal drug. Table 1 displays the antiamoebic activity of *N*-acylhydrazone (**F1**–**F20**). The IC_{50} of compound **F1** was 6.6 ± 0.2 μM, which was fourfold higher than the IC_{50} observed for Mtz (1.5 ± 0.1 μM). Incorporation of an alkyl substituent at *para*-position decreased the antiamoebic activity in following order: methyl (**F2**) > ethyl (**F3**) > isopropyl groups (F4). Replacing a phenyl by 2-pyridinyl (**F18**) was deleterious for activity. Electron-donating substituents attached at *para*-position, such as *N*,*N*-dimethylamino (**F5**), hydroxy (**F12**, **F13**) and alkoxy (**F14**, **F15**, **F16**, **F20**) produced weaker amoebicidal than the compound having phenyl group (**F1**). Attaching a chloro atom at *ortho* (**F9**) and *meta* (**F10**) produced active amoebicidal compounds, albeit less potent than the observed for the compound incorporating phenyl group (**F1**). However, attaching a chloro at *para*-position (**F11**) was deleterious for the amoebicidal activity. Furthermore, it was observed that a trifluoromethyl group when attached to the *ortho*-position (**F19**) led to a compound as potent as the compound bearing phenyl group (**F1**) in inhibiting the *E. histolytica* growth. Interestingly, the 4-nitro compound (**F8**) is a potent amoebicidal agent (IC_{50} = 0.95 ± 0.11 μM) without affecting mouse splenocyte viability (IC_{50} = 12.1 ± 2.4 μM). The compounds containing a nitro group at *ortho* (**F6**) and *meta* (**F7**) were less active than compound (**F8**).

The screening of *N*-acylhydrazones **F21**–**F33** (Table 2) revealed two other promising antiamoebic compounds **F24** and **F33**. Compound (**F24**) (IC_{50} = 0.13 ± 0.02 μM) was more potent against *E. histolytica* than metronidazole. Indeed, compound (**F24**) was the most potent antiamoebic agent among the studied compounds. It has low cytotoxicity to mouse splenocytes (IC_{50} = 15.8 ± 2.0 μM).

Table 1
Antiparasitic activity of *N*-acylhydrazones **F1**–**F20**.

Cpd	Ar	*P. falciparum* inhibition[a,c]	IC_{50} ± SD (μM)		
			P. falciparum[b,c]	*E. histolytica*[d]	Splenocytes[e]
4	–	74.1	41.7 ± 2.5	–	>100
F1	Ph	93.3	0.2 ± 0.05	6.6 ± 0.2	0.3 ± 0.2
F2	4-CH_3Ph	81.4	–	8.1 ± 0.15	–
F3	4-C_2H_5Ph	97.8	–	11.1 ± 0.11	–
F4	4-^{i}PrPh	97.5	1.7 ± 0.31	17.1 ± 0.15	7.9 ± 1.5
F5	4-$(CH_3)_2$NPh	98.0	1.3 ± 0.45	15.2 ± 0.2	>100
F6	2-NO_2Ph	73.1	–	11.3 ± 0.12	–
F7	3-NO_2Ph	19.9	–	12.6 ± 0.22	–
F8	4-NO_2Ph	38.4	–	0.95 ± 0.11	12.1 ± 2.4
F9	2-ClPh	97.9	0.78 ± 0.05	12.3 ± 0.13	10.8 ± 0.46
F10	3-ClPh	97.3	–	7.7 ± 0.23	>100
F11	4-ClPh	98.4	1.4 ± 0.3	87.2 ± 0.2	0.2 ± 0.04
F12	2-OHPh	98.9	0.33 ± 0.095	25.6 ± 0.15	11.5 ± 0.4
F13	4-OHPh	80.6	–	>100	–
F14	2-OC_2H_5Ph	28.1	–	>100	–
F15	4-OCH_3Ph	43.0	–	19.7 ± 0.32	–
F16	4-OC_2H_5Ph	14.9	–	12.0 ± 0.24	–
F17	4-O^{i}PrPh	98.0	0.8 ± 0.5	5.3 ± 0.22	3.6 ± 2.2
F18	2-Py	0	–	>100	–
F19	2-CF_3Ph	59.6	–	8.1 ± 0.31	–
F20	4-$(OEt)_2$CHPh	71.7	–	>100	–
Mtz	–	–	–	1.5 ± 0.1	5.4 ± 1.6
Mfq	–		0.04 ± 0.01	–	9.5 ± 0.46

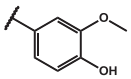
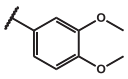
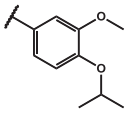
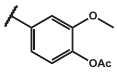
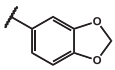
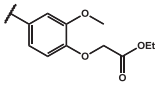
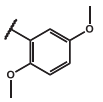
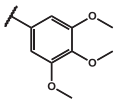
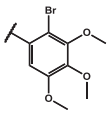
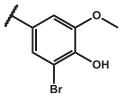
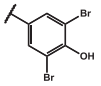
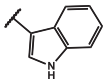
[a] % Of parasite inhibition using the concentration of 1.0 μg/mL for each compound.
[b] IC_{50}s were calculated using at least five compound concentrations.
[c] Determined 24 h after incubation of W2 strain *P. falciparum* (erythrocytic stage) with the respective compounds.
[d] Determined 72 h after incubation of HM1:IMSS strain trophozoites with the compounds. IC_{50} was calculated from at least five concentrations using concentrations in triplicate.
[e] IC_{50} values for mouse splenocytes after 24 h of incubation in the presence of the compounds. SD means standard deviation. Mtz is metronidazole; Mfq is mefloquine.



Reagents and conditions: (a) Piperazine, EtOH, reflux, 12 h (b) Methyl acrylate, r.t., 12 h (c) Hydrazine hydrate, EtOH, Reflux, 12 h (d) Aldehydes, EtOH, r.t., 12 h.

Scheme 1. Synthesis of *N*-acylhydrazones (**F1**–**F33**).

Table 2
Antiparasitic activity of *N*-acylhydrazones (**F21**–**F33**).

Cpd	Ar	*P. falciparum* % Inhibition[a,c]	IC_{50} ± SD (μM)		
			P. falciparum[b,c]	*E. histolytica*[d]	Splenocytes[e]
F21		99.1	2.1 ± 0.53	21.3 ± 0.31	9.4 ± 1.5
F22		98.4	–	11.6 ± 0.32	–
F23		98.6	4.5 ± 1.2	15.5 ± 0.15	8.8 ± 1.9
F24		0	–	0.13 ± 0.02	15.8 ± 2.0
F25		27.8	–	>100	–
F26		71.7	–	>100	–
F27		37.4	–	8.2 ± 0.2	
F28		47.4	–	12.4 ± 0.35	–
F29		42.9	–	3.7 ± 0.31	9.6 ± 0.21
F30		41.5	–	20.5 ± 0.32	–
F31		28.0	–	6.6 ± 0.35	–
F32		81.5	3.3 ± 0.63	9.6 ± 0.25	5.7 ± 1.2
F33		10.9	–	1.1 ± 0.10	8.2 ± 1.0
Mtz	–	–	–	1.5 ± 0.1	–
Mfq	–		0.04 ± 0.01	–	9.5 ± 0.46

[a] % Of parasite inhibition using the concentration of 1.0 μg/mL for each compound.
[b] IC_{50}s were calculated using at least five compound concentrations.
[c] Determined 24 h after incubation of W2 strain *P. falciparum* (erythrocytic stage) with the respective compounds.
[d] Determined 72 h after incubation of HM1:IMSS strain trophozoites with the compounds. IC_{50} was calculated from at least five concentrations using concentrations in triplicate.
[e] IC_{50} values for mouse splenocytes after 24 h of incubation in the presence of the compounds. SD means standard deviation. Mtz is metronidazole; Mfq is mefloquine.

Therefore, compound (**F24**) has a selectivity index (splenocyte/*E. histolytica*) of 121. Compound (**F33**), with IC_{50} value (1.1 ± 0.1 μM) for *E. histolytica*, had comparable ameobicidal activity to that of metronidazole. However, compound (**F33**) displayed selectivity index of 7.5, which is relatively low. All these *N*-acylhydrazone (**F1**–**F33**) showed IC_{50} value in the range (0.95 ± 0.11 μM-87.2 ± 0.02 μM). In terms of IC_{50} value, it was concluded that among the *N*-acylhydrazone (**F1**–**F20**) compound (**F8**) exhibited the most

promising antiamoebic activity (IC_{50} = 0.95 ± 0.11 μM), whereas the remaining compounds showed higher IC_{50} value than Mtz and therefore, were considered of little interest (Table 1). Further, screening of *N*-acylhydrazones (**F21**–**F33**) for amoebicidal activity revealed that two compounds, (**F24**, IC_{50} = 0.13 ± 0.02 μM) and (**F33**, IC_{50} = 1.1 ± 0.1 μM) exhibited promising antiamoebic activity. The compound (**F24**) was approximately tenfold more potent than Mtz in this assay. In comparison to our previous investigated sulfonamides and thiosemicarbazones [21,22], *N*-acylhydrazones were found more potent against *E. histolytica*.

2.2.2. Antimalarial activity

All the target compounds were screened for antimalarial activity against W2 strain of *P. falciparum* which is chloroquine resistant, therefore, mefloquine (Mfq) was used as reference inhibitory drug. The IC_{50} values of chloroquine and mefloquine against *P. falciparum* cells are (0.15 ± 0.3 μM) and (0.04 ± 0.01 μM) respectively. Toxicity to mammalian cells (IC_{50}) was determined by using mouse splenocytes (Tables 1 and 2). Compound (**F9**) having a chloro group at *ortho*-position was found to exhibit submicromolar IC_{50} value (0.78 ± 0.05 μM). Replacing this chloro group by a hydroxyl group subsequently yielded a two-fold more potent compound (**F12**, IC_{50} 0.33 ± 0.09 μM). Since the incorporation of a chloro group (**F9**) or hydroxyl group (**F12**) in *ortho* of the phenyl ring exerted significant inhibitory activity, it was concluded that polar substituents at *ortho* position are favorable for antimalarial activity. Although (**F9**) and (**F12**) exhibited promising antimalarial activity yet these were less potent than the reference drug mefloquine (Mfq). These compounds do not show toxicity against mouse splenocytes at concentrations of up to 10 μM. Moreover, uninfected erythrocytes exposed to (**F9**) or (**F12**) at 25 μM showed no hemolytic action. At concentration of 1.0 μM, the *N*-acylhydrazone (**F12**) was able to efficiently inhibit the parasite differentiation into schizont forms showing that this compound affects the parasite development in the blood-stage. Thus the data suggest that the compounds (**F9**) and (**F12**) impair erythrocytic life cycle of *P. falciparum*. However, the notable advantage is that they did not overtly affect the viability of host cells. The introduction of a nitro (compounds **F6**–**F8**) or alkoxy groups (compounds **F14**–**F17**) on the phenyl ring proved to be detrimental to antimalarial activity (Table 1). However, attaching a nitro group in *ortho* position (**F6**) maintained some level of antimalarial activity (73.1% inhibition). Compounds with trifluoromethyl (**F19**) or ethoxy (**F14**) groups at *ortho*-position and methoxy (**F15**) or ethoxy (**F16**) groups attached to the *para*-position were less potent than (**F12**). Likewise, a 2-pyridinyl ring (**F18**) was not beneficial, being inactive.

The effect of substitution (**F21**–**F33**) on the phenyl ring was analyzed. The 3-methoxyl-4-hydroxyl derivative (**F21**, 99.1% inhibition) is more active against *P. falciparum* than 4-hydroxyl derivative (**F13**, 80.6% inhibition). The same was observed for the 3,4-dimethoxy (**F22**) and 3-methoxy-4-isopropyl (**F23**) derivatives. When 3-methoxy-4-*O*-acetyl (**F24**) or 1,3-benzodioxole (**F25**) is attached, no antimalarial activity was observed. Regarding the methoxyl group, optimal antimalarial activity was observed for the 3,4-dimethoxy compound (**F22**), whereas the 4-methoxy (**F15**), 2,5-dimethoxy (**F26**), and 3,4,5-trimethoxy (**F28**) derivatives were weaker antimalarial agents. Accordingly, attaching bromo group did not improve the activity as compounds (**F29**), (**F30**) and (**F31**) were inactive. In comparison to (**F1**), which inhibited 93.3% of parasite growth, indolyl derivative (**F32**) also exhibited antimalarial activity (IC_{50} = 3.3 ± 0.63 μM) but without enough selectivity (IC_{50} = 5.7 ± 1.2 μM to splenocytes). Though ferrocenyl derivatives are well-known antimalarial agents [23], yet the ferrocenyl derivative (**F33**) (1.0 μg/mL) was unable to inhibit the *P. falciparum* growth. *N*-Acylhydrazones (**F4**, **F5**, **F9**, **F12**, **F21**, **F23**, **F32**) exhibited antimalarial activities for the blood-stage parasite. It was found that none of the compounds caused lysis in uninfected erythrocytes when tested for hemolytic properties at concentration of 25 μM (Fig. 1). Ideally, an antimalarial compound should inhibit the intra-erythrocytic life cycle of parasite. Thus we examined the ability of the potent antimalarial compound (**F12**) to inhibit intra-erythrocytic life cycle of W2 strain of *P. falciparum*, which was synchronized to ring stage and then incubated in erythrocytes. The distribution of rings, schizonts and trophozoites in erythrocytes was determined by microscopy. At the beginning, the parasitemia in untreated-infected erythrocytes is composed of immature trophozoites (ring stage). After 24 h of incubation, most ring-stage cells maturate to trophozoites and at 48 h of incubation, a significant number of schizonts were observed. Compound (**F12**) at 0.5 μM substantially reduced the number of schizonts, while at 1.0 μM, no schizonts were observed in the cell culture (Fig. 2). The mode of action of *N*-acylhydrazones is structurally similar to quinoline-based antimalarials, which are well-known to inhibit the β-hematin formation [24]. Hence, the ability of selected compounds to inhibit the formation of β-hematin was evaluated in vitro (Table 3). Interestingly, a trend showing an increase in in vitro antimalarial activity with the increase in β-hematin formation could be observed (Fig. 3). The β-hematin formation assay showed that the potent antimalarial compounds **F4**, **F5**, **F9** and **F12** were the best inhibitors, while compound **F21** & **F32** were moderate inhibitors, and compounds **4** and **F23** were least inhibitor as compared to chloroquine, which showed 87.1% inhibition at the concentration 2.5 mM.

3. Conclusion

An analysis of the antiplasmodial and antiamoebic properties for the compounds (**F1**–**F33**) revealed a potency enhancement of antiparasitic activity when compared to the hydrazide (**4**). We were able to identify compounds (**F12**) and (**F24**) as the most potent and

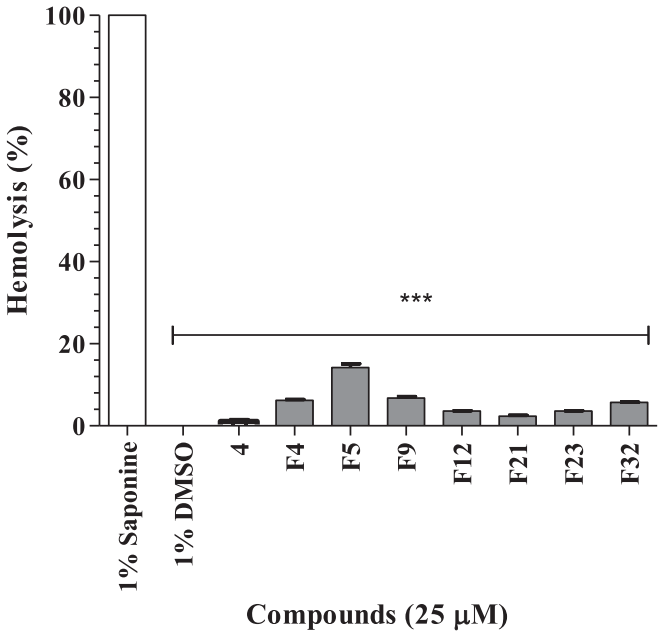


Fig. 1. Hemolytic assay for the *N*-acylhydrazones. Activity was assayed in uninfected human erythrocytes (type O^+) after 1 h of incubation with compounds (25 μM) in triplicate. $^{***}p < 0.0001$ (one-way ANOVA followed by Tukey's test).

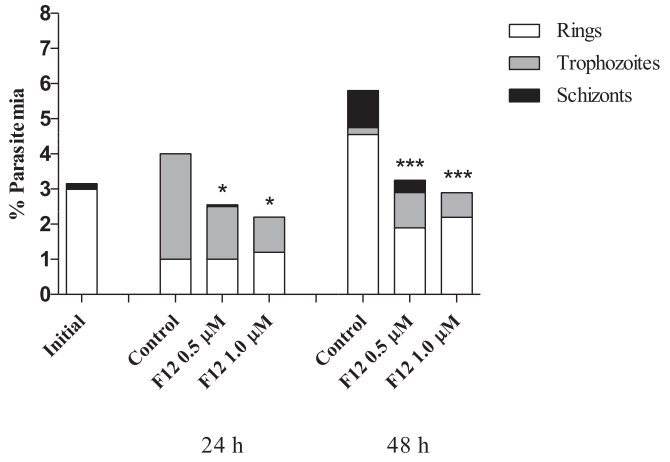


Fig. 2. Effects of *N*-acylhydrazone (**F12**) in the cell-cycle development of blood-stage *P. falciparum*. Erythrocytes were infected with W2 strain *P. falciparum* and incubated with compound **F12** (at 0.5 or 1.0 μM) or solvent (control). Each parasite forms were analyzed by confocal microscopy in different times. Assay represents two replicates per plate. Significance in reference to % of parasitemia: $^{*}p < 0.05$; $^{***}p < 0.001$ (one-way ANOVA).

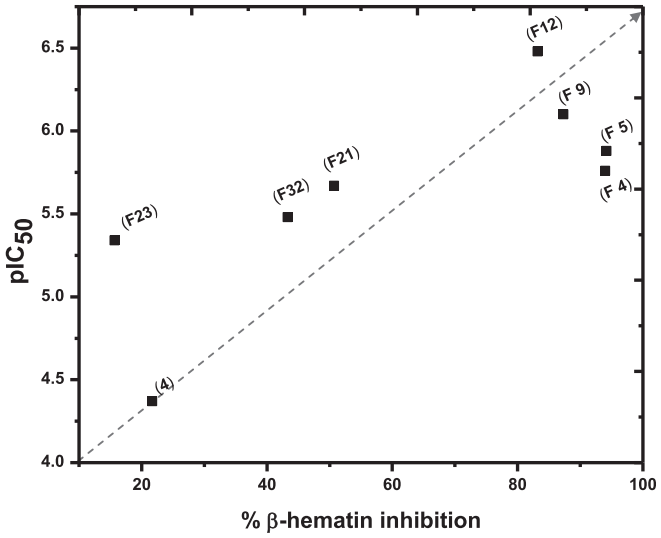


Fig. 3. Comparison between the % β-hematin inhibition and pIC_{50} values for *N*-acylhydrazones. % β-Hematin inhibition was determined by using 2.5 mM compound concentration. pIC_{50} was calculated as ($-\log IC_{50}$) by using IC_{50} values determined against *P. falciparum* W2 strain. Dot diagonal line is for visualization only and does not represent statistical analysis.

selective antimalarial and antiamoebal agents of this series, respectively. Specifically, (**F12**) inhibited the parasite growth as well as its life cycle with good potency and selectivity, in addition to inhibiting the β-hematin formation. Against *E. histolytica*, compound (**F24**) exhibited higher potency than metronidazole, while displayed lower cytotoxicity to mouse splenocytes. In overall, this work has confirmed that *N*-acylhydrazones are antiparasitic agents, and also suggested that converting antiparasitic agents in *N*-acylhydrazone derivatives is an appealing synthetic tool for the antiparasitic drug discovery.

4. Experimental protocol

The melting points were recorded on Buchi melting point apparatus Model No. M-650 and are uncorrected. Elemental analyses were performed on Elementar Vario analyzer and the results are within ±0.3% of the theoretical values. IR spectra (KBr) were acquired at Bruker FT-IR spectrophotometer. ^{1}H and ^{13}C NMR were recorded on a Bruker Spectrospin DPX 300 MHz and Bruker Spectrospin DPX 75 MHz spectrometer respectively, using $CDCl_3$ as a solvent and trimethylsilane (TMS) as the internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; t, triplet; 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.

Table 3
Inhibition of β-hematin formation.

Cpd	Substituent (Ar)	% Of hematin inhibition[a]
4	–	21.7
F4	4-^{i}PrPh	94.0
F5	4-$(CH_3)_2$NPh	94.2
F9	2-ClPh	87.3
F12	2-OHPh	83.2
F21	4-$C(OEt)_2$Ph	50.7
F23	3-OCH_3–4-O^{i}PrPh	15.7
F32	3-Indole	43.3
Cqn	–	87.1

[a] Percentage of inhibition hematin formation using the concentration of 2.5 mM for each compound. Values determined 24 h after incubation.

4.1. Synthesis of 7-chloro-4-(piperazin-1-yl)quinoline (*2*)

Piperazine (30.4 g, 353.4 mmol) was added to a mixture of 4,7-dichloro-quinoline **1** (10 g, 50.4 mmol) in ethanol (150 mL). The reaction mixture was refluxed and stirred for 12 h. The solvent was removed *in vacuo* and the resulting solid mixture was dissolved in dichloromethane (200 mL) and washed with saturated sodium bicarbonate solution until the complete removal of piperazine in the organic layer (TLC). The combined organic phases were dried over anhydrous Na_2SO_4, concentrated and then purified by recrystallization in 30% dichloromethane/hexane, (yield: 9.7 g 77%) of compound **2**. Mp: 118–120 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.72$ (d, 1H, $J = 5.1$ Hz), 8.06 (dd, 1H, $J = 2.1$ and 9 Hz), 7.97 (t, 1H, $J = 9$ Hz), 7.42 (dd, 1H, $J = 2.1$ and 9.0 Hz), 6.83 (d, 1H, $J = 5.1$ Hz), 3.33 (s, 1H), 3.14–3.21 (m, 8H); IR (KBr) $\nu_{max} = 3342$, 3024, 1579, 823 cm^{-1}; FAB-MS (*m*/*z*): $[M + 1]^{+}$, 248; Anal. calcd for $C_{13}H_{14}N_3Cl$: C 63.03, H 5.70, N 16.96%; found: C 63.23, H 5.61, N 17.02%.

4.2. Synthesis of methyl 3-[4-(7-chloro-4-quinolyl)piperazin-1-yl] propanoate (*3*)

7-Chloro-4-(piperazin-1-yl)quinoline **2** (1 g, 4.0 mmol) and methyl acrylate (8 mL) were stirred under neat conditions for 12 h at room temperature. The reaction mixture was concentrated *in vacuo* gave a crude solid, which was purified by recrystallization in 20% (DCM/hexane), (yield: 1.1 g, 85%) of compound **3**. Mp: 101 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.71$ (d, 1H, $J = 5.1$ Hz), 8.03 (d, 1H, $J = 2.1$ Hz), 7.93 (d, 1H, $J = 9$ Hz), 7.40 (dd, 1H, $J = 2.1$ and 9.0 Hz), 6.82 (d, 1H, $J = 5.1$ Hz), 3.71 (s, 3H), 3.23 (t, 4H, $J = 4.8$ Hz), 2.83 (t, 2H, $J = 7.2$ Hz), 2.75 (t, 4H, $J = 4.8$ Hz), 2.58 (t, 2H, $J = 7.2$ Hz); IR (KBr) $\nu_{max} = 3435$, 2881, 2827, 1668, 1626, 1545 cm^{-1}. FAB-MS (*m*/*z*): 333.4 $[M + 1]^{+}$, 100%; Anal. calcd for $C_{17}H_{20}N_3O_2Cl$: C 61.17, H 6.04; N 12.59%; found: C 61.38, H 6.29, N 12.51%.

4.3. Synthesis of 3-[4-(7-chloro-4-quinolyl)piperazin-1-yl] propanehydrazide (*4*)

Hydrazine hydrate (7.3 mL, 149.7 mmol) was added drop wise to a solution of 3-(4-(7-chloroquinolin-4-yl) piperazin-1-yl) propionate

3 (5 g, 14.9 mmol) in ethanol (50 mL). The reaction mixture was kept under vigorous stirring and refluxed for 12 h. The mixture was cooled to room temperature, concentrated *in vacuo*, and the resulting product was recrystallized in ethanol (yield: 4.1 g, 82%) yellow crystals. Mp: 155–156 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.9 (s, 1H), 8.74 (d, 1H, J = 4.8 Hz), 8.05 (d, 1H, J = 2.1 Hz), 7.93 (d, 1H, J = 9 Hz), 7.45–7.41 (dd, 1H, J = 2.1 and 9.0 Hz), 6.85 (d, 1H, J = 4.8 Hz), 3.9 (bs, 2H), 3.28 (t, 4H, J = 4.8 Hz), 2.79–2.75 (m, 6H), 2.50 (t, 2H, J = 6 Hz); IR (KBr) ν_{max} = 3320, 3249, 3021, 2902, 2811, 1669, 1626, 1545 cm^{-1}; FAB-MS (*m*/*z*): 333 (M^+, 100%); Anal. calcd for $C_{16}H_{20}N_5OCl$: C 57.57, H 6.04, N 20.98 found: C 57.58, H 6.29, N 20.81%.

4.4. General procedure for the preparation of compounds (**F1**–**F33**)

A mixture of 3-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)propanehydrazide **4** (1 mmol) and the respective aldehyde (1 mmol) in ethanol was stirred at room temperature for 12 h. The resulting precipitate, filtered and recrystallized in ethanol yielded 60–86% of *N*-acylhydrazones.

4.4.1. N-[(E)-Benzylideneamino]-3-[4-(7-chloro-4-quinolyl) piperazin-1-yl]propanamide (**F1**)

Colorless solid (yield: 78%); mp: 110 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.4 (s, 1H), 8.79 (s, 1H), 8.6 (d, 1H, J = 4.8 Hz), 8.02 (d, 1H, J = 1.8 Hz), 7.95 (d, 1H, J = 9 Hz), 7.75 (m, 2H), 7.65 (d, 1H, J = 3.6 Hz), 7.47–7.40 (m, 3H), 6.81 (d, 1H, J = 4.8 Hz), 3.25 (t, 4H, J = 4.8 Hz), 3.05–2.84 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 182.4, 174.3, 156.8, 151.7, 147.8, 143.6, 134.7, 133.5, 130.1, 128.6, 126.9, 125.9, 125.8, 121.7, 108.8, 53.3, 52.7, 51.9, 30.3; IR (KBr) ν_{max} = 3363, 3067, 2921, 2852, 1655, 1604,yield 1572, 1545, 1336 cm^{-1}; FAB-MS (*m*/*z*): 422.14 $[M + 1]^+$, 100%; Anal. calcd for $C_{23}H_{24}N_5OCl$: C 65.47, H, 5.73, N 16.60%; found: C 65.51, H 5.79, N 16.41%.

4.4.2. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(p-tolyl) methyleneamino]propanamide (**F2**)

Colorless solid (yield: 69%); mp: 153–154 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.4 (s, 1H), 8.79 (s, 1H), 8.03 (d, 1H, J = 2.1 Hz), 7.94 (d, 1H, J = 9 Hz), 7.7 (s, 1H), 7.55 (d, 2H, J = 8.1 Hz), 7.42–7.39 (dd, 1H, J = 1.8 and 8.8 Hz), 7.22 (d, 2H, J = 8.1 Hz), 6.81 (d, 1H, J = 5.1 Hz), 3.24 (t, 4H, J = 6.6 Hz), 2.98–2.84 (m, 8H), 2.17 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 183.4, 175.3, 155.8, 157.7, 148.8, 147.6, 136.7, 131.5, 130.1, 127.6, 126.9, 125.9, 125.8, 121.7, 108.8, 54.9, 53.3, 52.7, 31.3; IR (KBr) ν_{max} = 3435, 3152, 2980, 2881, 1665, 1604, 1566, 1332, 1134 cm^{-1}; FAB-MS (*m*/*z*): 436.08 $[M + 1]^+$, 82%; Anal. calcd for $C_{24}H_{26}N_5OCl$: C 66.12, H 6.01, N 16.06%; found: C 66.23, H 6.07, N 16.31%.

4.4.3. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-ethylphenyl)methyleneamino]propanamide (**F3**)

Colorless solid (yield: 74%); mp: 115–117 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.4 (s, 1H), 8.79 (s, 1H), 8.6 (d, 1H, J = 4.8 Hz), 8.02 (d, 1H, J = 1.8 Hz), 7.95 (d, 1H, J = 9 Hz), 7.75 (m, 2H), 7.65 (d, 1H, J = 3.6 Hz), 7.47–7.40 (m, 2H), 6.81 (d, 1H, J = 4.8 Hz), 3.25 (t, 4H, J = 4.8 Hz), 3.05–2.84 (m, 8H), 1.34 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.6, 168.4, 157.0, 151.9, 150.1, 146.9, 143.9, 134.8, 131.2, 128.7, 128.3, 127.1, 126.1, 125.2, 121.9, 108.9, 53.5, 52.9, 52.1, 30.5, 28.8, 15.3; IR (KBr) ν_{max} = 3247, 3056, 2971, 2823, 1655, 1606, 1571, 1543, 1374, 1133 cm^{-1}; FAB-MS (*m*/*z*): 450.11 $[M + 1]^+$, 100%; Anal. calcd for $C_{25}H_{28}N_5OCl$: C 66.73, H 6.2, N 15.56, found: C 66.54, H 6.21, N 15.45%.

4.4.4. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-isopropylphenyl)methyleneamino]propanamide (**F4**)

Yellow solid (yield: 77%); mp: 149 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.4 (s, 1H), 8.94 (s, 1H), 8.03 (d, 1H, J = 2.1 Hz), 7.95 (d, 1H, J = 9 Hz), 7.64 (s, 1H), 7.58 (d, 2H, J = 8.1 Hz), 7.42–7.39 (dd, 1H, J = 2.1 and 9 Hz), 7.28 (d, 2H, J = 8.1 Hz), 6.81 (d, 1H, J = 5.1 Hz), 3.25 (t, 4H, J = 3.9 Hz), 3.08–2.84 (m, 9H), 1.26 (d, 6H, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.7, 168.4, 157.0, 151.8, 150.1, 144.0, 134.9, 131.4, 128.7, 127.2, 126.9, 126.1, 125.2, 121.9, 108.9, 53.6, 52.8, 52.1, 34.1, 30.5, 23.8; IR (KBr) ν_{max} = 3465, 3053, 2958, 2816, 1677, 1604, 1564, 1378, 1359, 1197 cm^{-1}; FAB-MS (*m*/*z*): 464.21 $[M + 1]^+$, 100%; 449.21 $[M - CH_3]^+$, 21%; Anal. calcd for $C_{26}H_{30}N_5OCl$: C 67.30, H 6.52, N 15.09%, found: C 67.42, H 6.67, N 15.21%.

4.4.5. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-N,N-dimethylaminophenyl)methyleneamino]propanamide (**F5**)

Yellow solid (yield: 69%); mp: 176 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.2 (s, 1H), 8.7 (d, 1H, J = 4.8 Hz), 8.66 (s, 1H), 8.02 (d, 1H, J = 1.8 Hz), 7.95 (d, 1H, J = 9 Hz), 7.53 (d, 2H, J = 7.5 Hz), 7.42–7.39 (dd, 1H, J = 2.1 and 9 Hz), 6.81 (d, 1H, J = 4.8 Hz), 6.7 (d, 2H, J = 8 Hz), 3.25 (t, 4H, J = 4.8 Hz), 3.02 (s, 6H), 2.98–2.84 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.0, 168.0, 157.0, 151.9, 150.0, 144.4, 134.8, 129.2, 128.4, 126.0, 125.2, 121.9, 121.3, 111.8, 108.9, 53.6, 52.8, 52.1, 40.1, 30.5; IR (KBr) ν_{max} = 2231, 3068, 2892, 2824, 1651, 1596, 1574, 1545, 1364, 1130 cm^{-1}; FAB-MS (*m*/*z*): 464.2 (M^+, 100%); 465.1 $[M + 1]^+$, 77%; Anal. calcd for $C_{25}H_{29}N_6OCl$: C 64.57, H 6.29, N 18.07, found: C 64.52, H 6.37, N 18.19%.

4.4.6. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(2-nitrophenyl)methyleneamino]propanamide (**F6**)

Colorless solid (yield: 58%); mp: 217–219 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.2 (s, 1H), 9.4 (s, 1H), 8.77 (d, 1H, J = 4.8 Hz), 8.3 (s, 1H), 8.28 (d, 1H, J = 7.8 Hz), 8.08–8.01 (m, 2H), 7.95 (d, 1H, J = 8.7 Hz), 7.43 (t, 2H, J = 9.6 Hz), 6.94 (d, 1H, J = 5.1 Hz), 3.26 (t, 4H, J = 4.8 Hz), 3.04–2.88 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.3, 164.8, 155.2, 151.7, 145.7, 143.9, 137.8, 132.6, 127.3, 127.0, 126.4, 125.0, 122.9, 120.7, 108.0, 56.2, 51.8, 50.9, 17.5; IR (KBr) ν_{max} = 3376, 3071, 2926, 2810, 1682, 1574, 1514, 1350, 1426, 1350, 1132 cm^{-1}; FAB-MS (*m*/*z*): 467.05 $[M + 1]^+$, 100%; Anal. calcd for $C_{23}H_{23}N_6O_3Cl$: C 59.16, H 4.97, N 18.00, found: C 59.14, H 4.76, N 18.27%.

4.4.7. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(3-nitrophenyl)methyleneamino]propanamide (**F7**)

Yellow solid (yield: 76%); mp: 232–234 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.03 (s, 1H), 8.71 (s, 1H), 8.51 (d, 1H, J = 4.8 Hz), 8.18 (s, 1H), 8.02–7.94 (m, 4H), 7.61–7.56 (m, 1H), 7.44–7.42 (m, 1H), 6.86 (d, 1H, J = 5.1 Hz), 3.26 (m, 4H), 3.02–2.95 (m, 8H); ^{13}C NMR (75 MHz, CDC): δ = 174.3, 164.8, 155.2, 151.7, 145.7, 143.9, 137.8, 132.6, 127.3, 127.0, 126.4, 125.0, 122.9, 120.7, 108.0, 56.2, 51.8, 50.9; IR (KBr) ν_{max} = 3254, 3063, 2951, 2824, 1667, 1606, 1568, 1527, 1376, 1140 cm^{-1}; FAB-MS (*m*/*z*): 466.15 $[M + 1]^+$ 100%; Anal. calcd for $C_{23}H_{23}N_6O_3Cl$: C 59.16, H 4.97, N 18.00, found: C 59.21, H 4.79, N 18.18%.

4.4.8. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-nitrophenyl)methyleneamino]propanamide (**F8**)

Yellow solid (yield: 55%); mp: 210–211 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.4 (s, 1H), 8.79 (s, 1H), 8.6 (d, 1H, J = 4.8 Hz), 8.02 (d, 1H, J = 1.8 Hz), 7.95 (d, 1H, J = 9 Hz), 7.75 (m, 2H), 7.65 (d, 1H, J = 3.6 Hz), 7.47–7.40 (m, 3H), 6.81 (d, 1H, J = 4.8 Hz), 3.25 (t, 4H, J = 4.8 Hz), 3.05–2.84 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.3, 167.8, 155.9, 150.7, 148.7, 146.9, 139.8, 133.6, 127.3, 127.0, 126.4, 125.0, 122.9, 120.7, 108.0, 56.2, 51.8, 50.9, 17.5; IR (KBr) ν_{max} = 3173, 3075, 2922, 2853, 1667, 1579, 1518, 1495, 1337, 1141 cm^{-1}; FAB-MS

(*m*/*z*): 466.15 $[M + 1]^+$, 100%; Anal. calcd for $C_{23}H_{23}N_6O_3Cl$: C 59.16, H 4.97, N,18.00, found: C 59.21, H 4.82, N 18.07%.

*4.4.9. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(2-chlorophenyl)methyleneamino]propanamide (**F9**)*

Colorless solid, (yield: 59%); mp: 178–179 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.57 (s, 1H), 9.23 (s, 1H), 8.7 (d, 1H, J = 5.1 Hz), 8.13–8.03 (m, 2H), 7.94 (d, 1H, J = 9 Hz), 7.76–7.7 (m, 2H), 7.51–7.31 (m, 3H), 6.81 (d, 1H, J = 4.8 Hz), 3.26 (t, 4H, J = 6.9 Hz), 3.05–2.86 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.9, 167.7, 156.8, 154.4, 150.4, 145.4, 143.2, 136.3, 135.9, 132.1, 128.8, 126.5, 126.1, 125.5, 125.2, 121.7, 111.9, 54.5, 53.9, 51.1, 30.5; IR (KBr) ν_{max} = 3363, 3067, 2921, 2852, 1655, 1604, 1572, 1545, 1336, 1133 cm^{-1}; FAB-MS (*m*/*z*): 455.13 (M^+, 100%); Anal. calcd for $C_{23}H_{23}N_5OCl_2$: C 60.53, H 5.08, N 15.35, found: C 60.28, H 5.17, N 15.29%.

*4.4.10. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(3-chlorophenyl)methyleneamino]propanamide (**F10**)*

Colorless solid, (yield: 63%); mp: 197–198 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.57 (s, 1H), 9.23 (s, 1H), 8.7 (d, 1H, J = 5.1 Hz), 8.13–8.03 (m, 2H), 7.94 (d, 1H, J = 9 Hz), 7.76–7.7 (m, 2H), 7.51–7.31 (m, 3H), 6.81 (d, 1H, J = 4.8 Hz), 3.26 (t, 4H, J = 6.9 Hz), 3.05–2.86 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.9, 168.7, 156.9, 151.9, 150.1, 146.3, 142.2, 135.5, 134.9, 130.1, 128.8, 126.5, 126.1, 125.5, 125.2, 121.7, 108.9, 53.5, 52.9, 52.1, 30.5; IR (KBr) ν_{max} = 3181, 3075, 2959, 2826, 1665, 1601, 1575, 1495, 1382, 1140 cm^{-1}; FAB-MS (*m*/*z*): 455.13 (M^+, 100%), 456.33 $[M + 1]^+$, 72%; Anal. calcd for $C_{23}H_{23}N_5OCl_2$: C 60.53, H 5.08, N 15.35, found: C 60.74, H 5.29, N 15.52%.

*4.4.11. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-chlorophenyl)methyleneamino]propanamide (**F11**)*

Colorless solid (yield: 87%); mp: 155–157 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.46 (s, 1H). 8.97 (s, 1H), 8.7 (d, 1H, J = 5.1 Hz), 8.13 (s, 1H), 7.93 (d, 1H, J = 9 Hz), 7.6 (d, 2H, J = 8.4 Hz), 7.47–7.43 (dd, 1H, J = 2.1 Hz and 9 Hz), 7.38 (d, 2H, J = 8.4 Hz), 6.81 (d, 1H, J = 5.1 Hz), 3.24 (t, 4H, J = 4.2 Hz), 3.06–2.85 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.1, 167.7, 156.8, 151.8, 149.8, 142.0, 135.2, 134.5, 132.8, 128.7, 127.9, 125.8, 125.3, 121.7, 108.9, 53.1, 52.7, 51.9, 30.2; IR (KBr) ν_{max} = 3419, 3038, 2955, 2836, 1670, 1595, 1565, 1422, 1136 cm^{-1}; FAB-MS (*m*/*z*): 455.03 (M^+, 100%), 456.53 $[M + 1]^+$, 85%; Anal. calcd for $C_{23}H_{23}N_5OCl_2$: C 60.53, H 5.08, N 15.35, found: C 60.88, H 5.12, N 15.29%.

*4.4.12. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-2-(hydroxyphenyl)methyleneamino]propanamide (**F12**)*

Colorless solid (yield: 66%); mp: 203–205 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.56 (s, 1H), 11.07 (s, 1H), 8.77 (d, 1H, J = 4.8 Hz), 8.34 (s, 1H), 8.08–8.01 (m, 2H), 7.95 (d, 1H, J = 8.7 Hz), 7.57–7.48 (m, 2H), 7.43 (m, 2H), 6.94 (d, 1H, J = 5.1 Hz), 3.26 (t, 4H, J = 4.8 Hz), 3.04–2.88 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.3, 164.8, 155.2, 151.7, 145.7, 143.9, 137.8, 132.6, 127.3, 127.0, 126.4, 125.0, 122.9, 120.7, 108.0, 56.2, 51.8, 50.9, 17.5; IR (KBr) ν_{max} = 3363, 3067, 2921, 2852, 1655, 1604, 1572, 1545, 1336, 1133 cm^{-1}; FAB-MS (*m*/*z*): 438.1 $[M + 1]^+$, 100%; Anal. calcd for $C_{23}H_{24}N_5O_2Cl$: C 63.08, H 5.52, N 15.99, found: C 63.24, H 5.66, N 15.71%.

*4.4.13. 3-[4-(7-Chloro-4-quinolyl) piperazin-1-yl]-N-[(E)-(4-hydroxyphenyl)methyleneamino]propanamide (**F13**)*

Colorless solid (yield: 84%); mp: 162–164 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.67 (s, 1H), 11.34 (s, 1H), 8.75 (d, 1H, J = 4.8 Hz), 8.69 (d, 1H, J = 4.5 Hz), 8.58 (s, 1H), 7.96 (m, 1H), 7.64 (s, 1H), 7.53 (d, 2H, J = 8.1 Hz), 7.28 (d, 2H, J = 8.1 Hz), 6.84 (d, 1H, J = 5.1 Hz), 3.33–3.26 (m, 4H), 3.03–2.86 (m, 8H), ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.7, 168.4, 157.0, 151.8, 150.1, 144.0, 134.9, 131.4, 128.7, 127.2, 126.9, 126.1, 125.2, 121.9, 108.9, 53.6, 52.8, 52.1, 34.1, 30.5, 23.8; IR (KBr) ν_{max} = 3358, 3227, 3067, 2886, 2822, 1649, 1604, 1574, 1512, 1368, 1133 cm^{-1}; FAB-MS (*m*/*z*): 438.26 $[M + 1]^+$, 100%; Anal. calcd For $C_{23}H_{24}N_5O_2Cl$: C 63.08, H 5.52, N 15.99, found: C 63.15, H 5.34, N 15.82%.

*4.4.14. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(2-ethoxyphenyl)methyleneamino]propanamide (**F14**)*

Colorless solid (yield: 62%); mp: 183–185 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.4 (s, 1H), 8.94 (s, 1H), 8.03 (d, 1H, J = 2.1 Hz), 7.95 (d, 1H, J = 9 Hz), 7.64 (s, 1H), 7.58 (d, 2H, J = 8.1 Hz), 7.42–7.39 (dd, 1H, J = 2.1 and 9 Hz), 7.28 (d, 2H, J = 8.1 Hz), 6.81 (d, 1H, J = 5.1 Hz), 3.25 (t, 4H, J = 3.9 Hz), 3.08–2.84 (m, 8H), 1.46 (t, 3H, J = 6.9 Hz), 1.32 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.7, 168.4, 157.0, 151.8, 150.1, 144.0, 134.9, 131.4, 128.7, 127.2, 126.9, 126.1, 125.2, 121.9, 108.9, 53.6, 52.8, 52.1, 34.1, 30.5, 23.8; IR (KBr) ν_{max} = 3203, 3059, 2977, 2879, 1649, 1602, 1574, 1551, 1359, 1250, 1127 cm^{-1}; FAB-MS (*m*/*z*): 465.19 $[M + 1]^+$, 100%; Anal. calcd for $C_{25}H_{28}N_5O_2Cl$: C 64.44, H 6.06, N 15.03, found: C 64.38, H 6.19, N 15.33%.

*4.4.15. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-anisyl) methyleneamino]propanamide (**F15**)*

Colorless solid (yield: 81%); mp: 126–128 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.34 (s, 1H), 8.87 (s, 1H), 8.72 (m, 1H), 8.02 (s, 1H), 7.94 (d, 1H, J = 9 Hz), 7.6 (d, 2H, J = 8.7 Hz), 7.4 (dd, 1H, J = 1.8 and 9 Hz), 6.93 (d, 2H, J = 8.4 Hz), 6.89 (d, 1H, J = 4.5 Hz), 3.84 (s, 3H), 3.24 (t, 4H, J = 4.8 Hz), 3.04–2.89 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.6, 168.4, 161.2, 157.0, 151.8, 150.0, 143.7, 134.8, 129.2, 128.6, 126.1, 125.2, 121.9, 111.2, 108.9, 55.3, 53.6, 52.9, 52.0, 30.4; IR (KBr) ν_{max} = 3137, 3001, 2886, 2833, 1665, 1602, 1566, 1509, 1303, 1234, 1135 cm^{-1}; FAB-MS (*m*/*z*): 452.09 $[M + 1]^+$, 100%; Anal. calcd for $C_{24}H_{26}N_5O_2Cl$: C 63.78, H 5.80, N 15.50, found: C 63.73, H 5.61, N 15.45%.

*4.4.16. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-ethoxyphenyl)methyleneamino]propanamide (**F16**)*

Colorless solid (yield: 67%); mp: 186–187 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.35 (s, 1H), 8.79 (s, 1H), 8.7 (d, 1H, J = 5.1 Hz), 8.03 (s, 1H), 7.94 (d, 1H, J = 9 Hz), 7.58 (d, 2H, J = 8.7 Hz), 7.43–7.39 (dd, 1H, J = 2.1 and 9 Hz), 6.92 (d, 2H, J = 8.4 Hz), 6.81 (d, 1H, J = 4.8 Hz), 4.05 (q, 2H, J = 6.9 Hz), 3.33 (t, 4H, J = 5.1 Hz), 3.06–2.89 (m, 8H), 1.43 (t, 3H, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 168.3, 160.7, 157.0, 151.8, 150.1, 143.8, 134.8, 129.7, 128.6, 126.1, 125.2, 121.9, 114.7, 108.9, 63.6, 53.6, 52.9, 52.1, 30.4, 14.7; IR (KBr) ν_{max} = 3201, 3060, 2944, 2887, 1653, 1605, 1568, 1509, 1369, 1252, 1131 cm^{-1}; FAB-MS (*m*/*z*): 466.08 $[M + 1]^+$, 100%; Anal. calcd for $C_{25}H_{28}N_5O_2Cl$: C 64.44, H 6.06, N 15.03, found: C 64.48, H 6.16, N 15.07%.

*4.4.17. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-isopropoxyphenyl)methyleneamino]propanamide (**F17**)*

Colorless solid (yield: 73%); mp: 167–168 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.32 (s, 1H), 8.7 (d, 1H, J = 4.8 Hz), 8.68 (s, 1H), 8.02 (d, 1H, J = 1.8 Hz), 7.94 (d, 1H, J = 9 Hz), 7.67 (d, 2H, J = 8.4 Hz), 7.41 (dd, 1H, J = 2.1 and 9 Hz), 6.90 (d, 2H, J = 8.4 Hz), 6.81 (d, 1H, J = 5.1 Hz), 4.63–4.55 (m, 1H), 3.33 (t, 4H, J = 4.8 Hz), 3.03–2.85 (m, 8H), 1.35 (d, 6H, J = 6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.3, 168.2, 159.7, 157.0, 151.9, 150.1, 143.5, 134.8, 129.3, 128.6, 126.1, 125.2, 121.9, 115.8, 108.9, 70.0, 53.5, 52.9, 52.1, 30.5, 21.9; IR (KBr) ν_{max} = 3244, 3081, 2976, 2887, 1655, 1605, 1563, 1507, 1373, 1250, 1127 cm^{-1}; FAB-MS (*m*/*z*): 480.1 $[M + 1]^+$, 100%; Anal. calcd for $C_{26}H_{30}N_5O_2Cl$: C 65.06, H 6.30, N 14.59, found: C 65.18, H 6.26, N 14.57%.

*4.4.18. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-2-pyridylmethyleneamino]propanamide (**F18**)*

Colorless solid (yield: 76%); mp: 115–116 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.91 (s, 1H), 8.9 (s, 1H), 8.75 (d, 1H, J = 4.8 Hz), 8.59 (d, 1H, J = 4.8 Hz), 8.05 (d, 1H, J = 10.8 Hz), 7.95 (d, 1H, J = 8.7 Hz), 7.87 (s, 1H), 7.74 (t, 1H, J = 7.8 Hz), 7.43–7.45 (m, 1H), 7.32–7.30 (m, 1H), 6.9 (d, 1H, J = 5.1 Hz), 3.34 (t, 4H, J = 5.1 Hz), 3.07 (t, 2H, J = 6.6 Hz), 2.99–2.84 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 168.8, 156.9, 152.9, 151.9, 149.5, 147.6, 144.0, 136.5, 134.9, 128.7, 126.1, 125.2, 124.2, 121.2, 120.2, 108.9, 52.9, 52.5, 52.0, 30.4; IR (KBr) ν_{max} = 3467, 3040, 2970, 2829, 1687, 1604, 1579, 1378, 1215 cm^{-1}; FAB-MS (m/z): 422.16 [M + 1]$^+$, 100%; Anal. calcd for $C_{22}H_{23}N_6OCl$: C 62.48, H 5.48, N 19.87, found: C 62.58, H 5.46, N 19.77%.

*4.4.19. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-[2-(trifluoromethyl)phenyl]methyleneamino]propanamide (**F19**)*

Yellow solid (yield: 85%); mp: 187–189 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.2 (s, 1H), 9.0 (s, 1H), 8.79 (d, 1H, J = 5.1 Hz), 8.43 (s, 1H), 8.37 (d, 1H, J = 7.8), 8.07 (d, 1H, J = 1.8 Hz), 7.93 (d, 1H, J = 4.8 Hz), 7.68 (t, 2H, J = 8.7 Hz), 7.48–7.40 (m, 2H), 3.25 (t, 4H, J = 4.8 Hz), 3.04 (t, 2H, J = 6.9 Hz), 2.83 (t, 4H, J = 4.8 Hz), 2.65 (t, 2H, J = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 182.4, 174.3, 156.8, 151.7, 147.8, 143.6, 134.7, 133.5, 130.1, 128.6, 126.9, 125.9, 125.8, 121.7, 108.8, 53.3, 52.7, 51.9, 30.3; IR (KBr) ν_{max} = 3395, 3068, 2950, 2821, 1682, 1606, 1570, 1376, 1193, 1131 cm^{-1}; FAB-MS (m/z): 489.15 [M + 1]$^+$, 100%; Anal. calcd for $C_{24}H_{23}F_3N_5OCl$: C 58.84, H 4.73, N 14.29, found: C 58.65, H 4.76, N 14.35%.

*4.4.20. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-4-(diethoxymethyl)phenyl]methyleneamino]propanamide (**F20**)*

Colorless solid (yield: 65%); mp: 164–166 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.5 (s, 1H), 9.11 (s, 1H), 8.7 (d, 1H, J = 1.8 Hz), 8.13 (d, 1H, J = 1.8 Hz); 7.94 (d, 1H, J = 9 Hz), 7.7 (s, 1H), 7.66 (d, 2H, J = 8.1 Hz), 7.52 (d, 2H, J = 8.1 Hz), 6.8 (d, 1H, J = 4.8 Hz), 3.59 (q, 4H, J = 6.9 Hz), 3.24 (t, 4H, J = 5.1 Hz), 3.05–2.84 (m, 8H), 1.24 (t, 6H, J = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.1, 168.1, 157.4, 156.9, 156.4, 151.9, 150.1, 143.6, 139.7, 134.8, 131.4, 128.8, 126.0, 125.2, 122.2, 120.8, 112.1, 108.9, 63.9, 53.5, 52.9, 52.1, 30.5, 14.8; IR (KBr) ν_{max} = 3360, 3055, 2970, 2879, 1664, 1606, 1576, 1336, 1135 cm^{-1}; FAB-MS (m/z): 524.4 [M + 1]$^+$, 100%; Anal. calcd for $C_{28}H_{34}N_5O_3Cl$: C 64.17, H 6.54, N 13.36, found: C 64.18, H 6.76, N 13.27%.

*4.4.21. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-hydroxy-3-methoxyphenyl)methyleneamino]propanamide (**F21**)*

Colorless solid (yield: 61%); mp: 125–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.4 (s, 1H), 8.94 (s, 1H), 8.03 (d, 1H, J = 2.1 Hz), 7.95 (d, 1H, J = 9 Hz), 7.64 (s, 1H), 7.58 (d, 2H, J = 8.1 Hz), 7.42–7.39 (dd, 1H, J = 2.1 and 9 Hz), 7.28 (d, 2H, J = 8.1 Hz), 6.81 (d, 1H, J = 5.1 Hz), 3.85 (s, 3H), 3.25 (t, 4H, J = 3.9 Hz), 3.08–2.84 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 168.8, 156.9, 152.9, 151.9, 149.5, 147.6, 144.0, 136.5, 134.9, 128.7, 126.1, 125.2, 124.2, 121.2, 120.2, 108.9, 52.9, 52.5, 52.0, 30.4; IR (KBr) ν_{max} = 3396, 3378, 3045, 2947, 2832, 1664, 1597, 1571, 1545, 1376, 1276, 1123 cm^{-1}; FAB-MS (m/z): 468.27 [M + 1]$^+$, 100%; Anal. calcd for $C_{24}H_{26}N_5O_3Cl$: C, 61.60; H, 5.60; N, 14.97%; found: C, 61.48; H, 5.76; N, 14.74%.

*4.4.22. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(3,4-dimethoxyphenyl)methyleneamino]propanamide (**F22**)*

Colorless solid (yield: 65%); mp: 180–182 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.35 (s, 1H), 8.90 (s, 1H), 8.7 (d, 1H, J = 5.1 Hz), 8.03 (s, 1H), 7.93 (d, 1H, J = 9 Hz), 7.7 (s, 1H), 7.42–7.39 (dd, 1H, J = 1.8 Hz and 8 Hz), 7.1 (d, 1H, J = 8.4 Hz), 6.88 (d, 1H, J = 8.4 Hz), 6.82 (d, 1H, J = 5.4 Hz), 3.94 (s, 3H), 3.92 (s, 3H), 3.34 (t, 4H, J = 5.1 Hz), 3.08–2.95 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.7, 167.1, 155.7, 150.8, 149.4, 148.0, 142.2, 133.3, 127.3, 126.3, 124.7, 124.4, 120.7, 120.2, 110.0, 107.9, 107.2, 54.7, 52.5, 51.6, 50.9, 29.2; IR (KBr) ν_{max} = 3228, 3062, 2958, 2936, 1655, 1599, 1575, 1551, 1333, 1270, 1238, 1133 cm^{-1}; FAB-MS (m/z): 482.21 [M + 1]$^+$, 100%; Anal. calcd for $C_{25}H_{28}N_5O_3Cl$: C 62.30, H 5.86, N 14.53, found: C 62.48, H 5.72, N 14.47%.

*4.4.23. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(4-isopropoxy-3-methoxyphenyl)methyleneamino]propanamide (**F23**)*

Colorless solid (yield: 78%); mp: 134–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.34 (s, 1H), 8.7 (d, 1H, J = 4.8 Hz), 8.53 (s, 1H), 8.02 (d, 1H, J = 1.8 Hz), 7.93 (d, 1H, J = 9 Hz), 7.65 (s, 1H), 7.41 (dd, 1H, J = 1.8 and 9 Hz), 7.11 (d, 1H, J = 8.1 Hz), 6.9 (d, 1H, J = 8.3 Hz), 6.81 (d, 1H, J = 4.8 Hz), 4.62–4.57 (m, 1H), 3.91 (s, 3H), 3.24 (t, 4H, J = 5.1 Hz), 3.04–2.85 (m, 8H), 1.4 (d, 6H, J = 6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.4, 168.4, 156.9, 151.8, 150.4, 150.0, 149.5, 148.1, 144.0, 134.9, 128.7, 126.1, 124.9, 122.5, 121.5, 114.5, 109.0, 71.3, 56.0, 53.5, 52.9, 52.1, 30.5, 21.9; IR (KBr) ν_{max} = 3199, 3060, 2994, 2822, 1651, 1600, 1571, 1549, 1331, 1271, 1237, 1132 cm^{-1}; FAB-MS (m/z): 510.12 [M + 1]$^+$, 100%; Anal. calcd for $C_{27}H_{32}N_5O_3Cl$: C 63.58, H 6.32, N 13.73; found: C 63.43, H 6.36, N 13.72%.

*4.4.24. 4-[(E)-[3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]propanoylhydrazono]methyl]-2-methoxyphenyl]acetate (**F24**)*

Colorless solid (yield: 83%); mp: 184–185 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.47 (s, 1H), 8.87 (s, 1H), 8.70 (d, 1H, J = 4.8 Hz), 8.09 (s, 1H), 7.93 (d, 1H, J = 9 Hz), 7.71 (s, 1H), 7.43–7.39 (dd, 1H, J = 2.1 and 6.6 Hz), 7.13–7.06 (m, 2H), 6.81 (d, 1H, J = 5.1 Hz), 3.88 (s, 3H), 3.24 (t, 4H, J = 4.8 Hz), 3.06–2.83 (m, 8H), 2.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.1, 168.1, 157.4, 156.9, 156.4, 151.9, 150.1, 143.6, 139.7, 134.8, 131.4, 128.8, 126.0, 125.2, 122.2, 120.8, 112.1, 108.9, 63.9, 53.5, 52.9, 52.1, 30.5, 14.8; IR (KBr) ν_{max} = 3244, 3072, 2945, 2815, 1752, 1660, 1604, 1576, 1552, 1371, 1278, 1128 cm^{-1}; FAB-MS (m/z): 525.11 [M + 1]$^+$, 100%; Anal. calcd for $C_{27}H_{29}N_4O_5Cl$: C 61.77, H 5.57, N 10.67%; found: C 61.78, H 5.76, N 10.72%

*4.4.25. N-[(E)-1,3-Benzodioxol-5-ylmethyleneamino]-3-[4-(7-chloro-4-quinolyl) piperazin-1-yl]propanamide (**F25**)*

Colorless solid (yield: 86%); mp: 210–211 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.34 (s, 1H), 8.94 (s, 1H), 8.7 (d, 1H, J = 4.8 Hz), 8.03 (s, 1H), 7.94 (d, 1H, J = 9 Hz), 7.67 (s, 1H), 7.42–7.39 (dd, 1H, J = 1.8 and 8.7 Hz), 7.01 (d, 1H, J = 8.4 Hz), 6.89–6.78 (m, 2H), 6.02 (s, 2H), 3.25 (t, 4H, J = 4.8 Hz), 3.02–2.84 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.6, 168.8, 156.9, 151.9, 150.1, 143.0, 141.4, 134.8, 132.6, 128.8, 126.1, 125.2, 123.2, 121.9, 120.4, 109.9, 109.0, 55.9, 53.5, 52.9, 52.1, 30.6, 20.6; IR (KBr) ν_{max} = 3186, 3007, 2941, 2823, 1661, 1607, 1574, 1334, 1258, 1142 cm^{-1}; FAB-MS (m/z): 466.16 [M + 1]$^+$, 100%; Anal. calcd for $C_{24}H_{24}N_5O_3Cl$: C 61.87, H 5.19, N 15.03, found: C 61.81, H 5.26, N 15.17%.

*4.4.26. Ethyl-2-[4-[(E)-[3-[4-(7-chloro-4-quinolyl)piperazin-1-yl]propanoylhydrazono]methyl]-2-methoxyphenoxy]acetate (**F26**)*

Yellow solid (yield: 74%); mp: 137–138 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.41 (s, 1H), 8.81 (s, 1H), 8.70 (d, 1H, J = 4.8 Hz), 8.04 (s, 1H), 7.96–7.92 (dd, 1H, J = 1.5 and 9 Hz), 7.68 (s, 1H), 7.43–7.39 (dd, 1H, J = 2.1 and 9 Hz), 7.13–7.09 (dd, 1H, J = 1.8 and 8.1 Hz), 6.82–6.75 (m, 2H), 4.78 (s, 2H), 4.25 (q, 2H, J = 7.2 Hz), 3.94 (s, 3H), 3.24 (q, 4H, J = 5.1 Hz), 3.04–2.87 (m, 8H), 1.28 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 168.8, 156.9, 152.9, 151.9, 149.5, 147.6, 144.0, 136.5, 134.9, 128.7, 126.1, 125.2, 124.2, 121.2, 120.2, 108.9, 52.9, 52.5, 52.0, 30.4; IR (KBr) ν_{max} = 3244, 3072, 2945, 2815, 1752, 1660, 1604, 1576, 1552, 1371, 1278, 1128 cm^{-1}; FAB-MS (m/z): 553.1 [M + 1]$^+$, 100%; Anal. calcd for $C_{28}H_{32}N_5O_5Cl$: C 60.70, H 5.82, N 12.64, found: C 60.58, H 5.76, N 12.77%.

*4.4.27. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-(2,5-dimethoxyphenyl)methyleneamino]propanamide (**F27**)*

Colorless solid (yield: 76%); mp: 158–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.54 (s, 1H), 8.93 (s, 1H), 8.7 (d, 1H, J = 5.1 Hz), 8.04 (dd, 1H, J = 1.8 and 12 Hz), 7.94 (d, 1H, J = 9 Hz), 7.43–7.38 (m, 2H), 6.95–6.81 (m, 3H), 3.82 (s, 3H), 3.8 (s, 3H), 3.24 (t, 4H, J = 5.1 Hz), 3.06–2.83 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 168.8, 156.9, 152.9, 151.9, 149.5, 147.6, 144.0, 136.5, 134.9, 128.7, 126.1, 125.2, 124.2, 121.2, 120.2, 108.9, 52.9, 52.5, 52.0, 30.4; IR (KBr) ν_{max} = 3126, 3049, 2943, 2899, 1677, 1600, 1572, 1336, 1263, 1244, 1137 cm^{-1}; FAB-MS (m/z): 482.1 [M + 1]$^+$, 100%; Anal. calcd for $C_{25}H_{28}N_5O_3Cl$: C 62.30, H 5.86, N 14.53, found: C 62.48, H 5.71, N 14.47%.

*4.4.28. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-(E)-3,4,5-(trimethoxyphenyl)methyleneamino]propanamide (**F28**)*

Yellow solid (yield: 62%); mp: 86 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 11.33 (s, 1H), 9.35 (s, 1H), 8.68 (d, 1H, J = 4.8 Hz), 8.06 (s, 1H), 7.94–7.9 (dd, 1H, J = 2.4 and 8.7 Hz), 7.7 (s, 1H), 7.41–7.38 (dd, 1H, J = 1.8 and 9 Hz), 6.86 (s, 1H), 6.79 (d, 1H, J = 4.8 Hz), 3.87 (s, 9H), 3.24 (m, 4H), 3.05–2.84 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 168.6, 156.9, 153.5, 151.8, 150.0, 143.8, 140.2, 134.9, 128.7, 126.1, 125.1, 121.8, 108.9, 104.3, 60.9, 56.2, 52.9, 52.0, 30.5. IR (KBr) ν_{max} = 3393, 3054, 2934, 2832, 1648, 1608, 1572, 1358, 1230, 1120 cm^{-1}; FAB-MS (m/z): 512.2 [M + 1]$^+$, 29%; Anal. calcd for $C_{26}H_{30}N_5O_4Cl$: C 60.99, H 5.91, N 13.68, found: C 60.86, H 5.76, N 13.72%.

*4.4.29. N-[(E)-(2-Bromo-3,4,5-trimethoxyphenyl) methyleneamino]-3-[4-(7-chloro-4-quinolyl)piperazin-1-yl] propanamide (**F29**)*

Yellow solid (yield: 65%); mp: 170 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.3 (s, 1H), 8.76 (d, 1H, J = 5.1 Hz), 8.4 (s, 1H), 8.18 (s, 1H), 8.07–8.03 (dd, 1H, J = 2.1 and 11 Hz), 7.93 (m 1H), 7.47–7.43 (dd, 1H, J = 2.1 and 11 Hz), 6.87 (d, 1H, J = 5.1 Hz), 3.87 (s, 3H), 3.91 (s, 6H), 3.34 (m, 4H), 3.04–2.84 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 168.8, 156.9, 152.9, 151.9, 149.5, 147.6, 144.0, 136.5, 134.9, 128.7, 126.1, 125.2, 124.2, 121.2, 120.2, 108.9, 52.9, 52.5, 52.0, 30.4; IR (KBr) ν_{max} = 3363, 3067, 2921, 2852, 1655, 1604, 1572, 1545, 1336, 1133 cm^{-1}; FAB-MS (m/z): 590.12 [M + 1]$^+$, 100%; Anal. calcd for $C_{26}H_{29}BrN_5O_4Cl$: C 52.85, H 4.95, N 11.85, found: C 52.68, H 4.86, N 11.79%.

*4.4.30. N-[(E)-(3-Bromo-4-hydroxy-5-methoxyphenyl) methyleneamino]-3-[4-(7-chloro-4-quinolyl)piperazin-1-yl] propanamide (**F30**)*

Colorless solid (yield: 70%); mp: 184 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.4 (s, 1H), 11.2 (s, 1H), 8.7–8.68 (m, 1H), 8.04–7.88 (m, 3H), 7.57–7.51 (m, 1H), 7.4 (s, 1H), 7.27 (d, 1H, J = 9 Hz), 6.99 (t, 1H, J = 6.6 Hz), 3.88 (s, 3H), 3.18 (s, 4H), 2.86–2.73 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 182.4, 174.3, 156.8, 151.7, 147.8, 143.6, 134.7, 133.5, 130.1, 128.6, 126.9, 125.9, 125.8, 121.7, 108.8, 53.3, 52.7, 51.9, 30.3; IR (KBr) ν_{max} = 3363, 3067, 2921, 2852, 1655, 1604, 1572, 1545, 1336, 1133 cm^{-1}; FAB-MS (m/z): 545.3 (M$^+$, 100%); Anal. calcd for $C_{24}H_{25}BrN_5O_3Cl$: C 52.71, H 4.61, N 12.81%, found: C 52.68, H 4.46, N 12.75%.

*4.4.31. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-(E)-(3,5-dibromo-4-hydroxyphenyl)methyleneamino]propanamide (**F31**)*

Yellow solid (yield: 81%); mp: 220 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.5 (s, 1H), 11.33 (s, 1H), 8.69 (d, 1H, J = 4.8 Hz), 8.04–7.99 (m, 2H), 7.86 (s, 2H), 7.82 (s, 1H), 7.55 (d, 1H, J = 8.7 Hz), 7.01 (d, 1H, J = 4.5 Hz), 3.23 (s, 4H), 2.91 (s, 4H), 2.81 (s, 2H), 2.51 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 182.4, 174.3, 156.8, 151.7, 147.8, 143.6, 134.7, 133.5, 130.1, 128.6, 126.9, 125.9, 125.8, 121.7, 108.8, 53.3, 52.7, 51.9, 30.3; IR (KBr) ν_{max} = 3363, 3067, 2921, 2852, 1655, 1604, 1572, 1545, 1336, 1133 cm^{-1}; FAB-MS (m/z): 594.02 [M + 1]$^+$, 100%; Anal. calcd for $C_{23}H_{22}Br_2N_5O_2Cl$: C 46.37, H 3.72, N 11.76, found: C 46.48, H 3.76, N 11.67%.

*4.4.32. 3-[4-(7-Chloro-4-quinolyl)piperazin-1-yl]-N-[(E)-1H-indole-3-ylmethylene-amino]propanamide (**F32**)*

Yellow solid (yield: 78%); mp: 221–223 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.53 (s, 1H), 10.97 (s, 1H), 8.69 (s, 1H), 8.25–8.17 (m, 2H), 8.04–7.98 (m, 2H), 7.77 (s, 1H), 7.57–7.44 (m, 2H), 7.20–7.13 (m, 2H), 6.98 (s, 1H), 3.2 (s, 4H), 2.93–2.77 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 184.4, 175.5, 157.8, 153.7, 148.8, 144.6, 138.7, 134.5, 132.1, 124.6, 123.9, 122.9, 122.8, 121.7, 108.8, 53.3, 52.7, 51.9, 31.1; IR (KBr) ν_{max} = 3482, 3027, 2934, 1640, 1608, 1572, 1376, 1133 cm^{-1}. FAB-MS (m/z): 461.28 [M + 1]$^+$, 100%; Anal. calcd for $C_{25}H_{25}N_6OCl$: C 65.14, H 5.47, N 18.23 found: C 65.18, H 5.47, N 18.27%.

*4.4.33. (E)-N-(Ferrocenyl)-3-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)propanehydrazide (**F33**)*

Red solid (yield: 71%); mp: 121–123 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.004 (s, 1H), 9.03 (s, 1H), 8.7 (d, 1H, J = 5.1 Hz), 8.06 (s, 1H), 7.5 (d, 1H, J = 8.1 Hz), 7.64 (s, 1H), 7.43 (t, 1H, J = 9.9 Hz), 7.82 (d, 1H, J = 5.1 Hz), 4.59 (s, 1H), 4.39 (s, 3H), 4.19 (s, 5H), 3.26 (s, 4H), 2.86–2.85 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.1, 168.1, 157.4, 156.9, 156.4, 151.9, 150.1, 143.6, 139.7, 134.8, 131.4, 128.8, 126.0, 125.2, 122.2, 120.8, 112.1, 108.9, 63.9, 53.5, 52.9, 52.1, 30.5, 14.8; IR (KBr) ν_{max} = 3456, 3183, 2956, 2890, 1672, 1602, 1579, 1327, 1217 cm^{-1}; FAB-MS (m/z): 515 (M$^+$, 100%); Anal. calcd for $C_{27}H_{29}N_5O_2ClFe$: C 64.44, H 6.06, N 15.03, found: C 64.71, H 6.29, N 13.43%.

5. Pharmacological evaluation

5.1. Toxicity to mouse splenocytes

BALB/c mouse splenocytes were placed into a 96-well plate at a cell density of 5 × 10^6 cells/well in RPMI-1640 medium supplemented with 10% of FCS and 50 μg mL^{-1} of gentamycin. Each compound was tested in five concentrations in triplicate. To each well, an aliquot of test inhibitor suspended in DMSO was added, in addition to wells only containing solvent (untreated cells). Plate was incubated for 24 h at 37 °C and 5% CO_2. After incubation, [^{3}H]-thymidine was added to each well, and the plate was returned to the incubator. The plate was then transferred to a beta-radiation counter, and the percent of [^{3}H]-thymidine was determined. Cell viability was measured as the percent of [^{3}H]-thymidine incorporation for treated cells in comparison to untreated cells [25].

5.2. Antiamoebic activity

The compounds were tested for antiamoebic activity against the HM1:IMSS strain of *E. histolytica* by microdilution method [26]. Trophozoites were cultured in Diamond TYIS-33 growth medium [27]. The test compounds (1 mg) were dissolved in DMSO (40 mL, level at which no inhibition of amoeba occurs). The stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/mL. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (costar). Each plate included metronidazole as standard amoebicidal drug, negative control (culture medium plus amoeba) and a blank (culture medium only). All the compounds were used in triplicate concentrations. Compounds were added to the respective wells and the plate was sealed, gassed for 10 min with nitrogen and then incubated at 37 °C for 72 h. After incubation, the amoeba growth was checked under microscope. The culture medium was removed by inverting the

plate and shaking gently. Plate was then immediately washed with NaCl (0.9%) at 37 °C, dried at room temperature and the cells were fixed with methanol and once dried, stained with aqueous eosin (0.5%) for 15 min. The optical density of the resulting solution in each well was determined at 490 nm in a microplate reader. The % of inhibition of parasite growth was calculated from the optical densities of the control and test compounds. A non-linear regression analysis was used to determine the best fitting line from which the IC_{50} values were estimated. Each compound was tested at least in two independent experiments.

5.3. Antimalarial activity

It was performed using the [^{3}H]-hypoxanthine incorporation assay, as previously described [28]. W2 strain *P. falciparum* was maintained in continuous culture of human erythrocytes (blood group O^+) using the RPMI-1640 medium supplemented with 10% of human plasma. Parasites grown at 1–2% parasitemia and 2.5% hematocrit were distributed into 96-wells culture plate and incubated with the compounds (previously diluted with 4% DMSO and culture medium). After 24 h of incubation, [^{3}H]-hypoxanthine was added, the plate incubated again and parasites were harvested using a cell harvester to quantify the [^{3}H]-hypoxanthine incorporation in a β-radiation counter. Inhibition of parasite growth was evaluated by comparison with [^{3}H]-hypoxanthine uptake in treated versus untreated parasite after 24 h of incubation. Each compound was initially tested at concentration of 1 μg/mL in triplicate and the IC_{50} values were calculated in triplicate concentration using at least five concentrations. Mefloquine (Mfq) was used as a standard drug. Yet to confirm the susceptibility of *P. falciparum*, parasites were first synchronized to ring stage and incubated for 48 h with 0.5 or 1.0 μM *N*-acylhydrazone **F12** at 37 °C. Thin blood films were prepared after 24 h or 48 h of incubation, stained with fast panoptic (Laborclin, Pinhais, Brazil) and examined by light microscopy. Parasitemia were determined and at least 1000 erythrocytes were counted per slide.

5.4. Hemolysis assay

The hemolytic activity of the compounds was assayed in fresh human erythrocytes (type O^+). Cells were washed three times in phosphate buffered saline and 100 μL of this suspension (1% hematocrit) was distributed into each well to a 96-well plate. After, 100 μL of each compound (*N*-acylhydrazone), previously dissolved in phosphate buffered saline, were added in triplicate to the plate and incubated for 1 h. Saponin (Sigma–Aldrich, USA) was used as hemolytic drug at 1% v/v. After incubation, samples were centrifuged (1500 rpm for 10 min) and 100 μL of each supernatant was transferred to another microtiter plate. Released hemoglobin was monitored by measuring the absorbance at 540 nm in a spectrophotometer. The percentage of hemolysis was calculated as previously described [29].

5.5. Inhibition of β-hematin formation

50 μL of a freshly prepared solution of hemin (0.5 mg/mL) dissolved in 0.2 M NaOH was mixed to a 75 μL of 3 M sodium acetate, 25 μL of 17.4 M acetic acid and 50 μL of the tested compounds (*N*-acylhydrazones). All *N*-acylhydrazones were tested at 2.5 mM. After 24 h of incubation at 37 °C, the resulting solution was spun for 15 min at 3500 rpm, the supernatant was discarded and the pellet was washed with 200 μL of DMSO. This step was repeated once and washed with water, the pellet was dissolved in 0.1 M NaOH (150 μL). Chloroquine (Cqn) was included in each experiment as positive control. The absorption was determined at 405 nm using a spectrophotometer [30]. Results are expressed as percentage of inhibition of hematin formation in comparison to negative control (without compound). Experiments were carried out at least twice using compound concentrations in triplicate.

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