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4-Aminoquinoline-Schiff base Hybrid: Rational Drug Design Approach for Overcoming Chloroquine Resistance in Malaria

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Abstract: Antimalarials based on the 4-aminoquinoline-schiff base hybrid coupled with oxalamide functionality as linker are designed and synthesized. The molecules were evaluated for their antiplasmodial activity against choroquine-resistant (CQ-R) K1 and choroquine-sensitive (CQ-S) 3D7 of *Plasmodium falciparum* strains. Some of the novel compounds were found to be more potent than Chloroquine *in vitro* against CQ-R strain. Furthermore several molecules also showed promising *β*-hematin inhibitory activity.

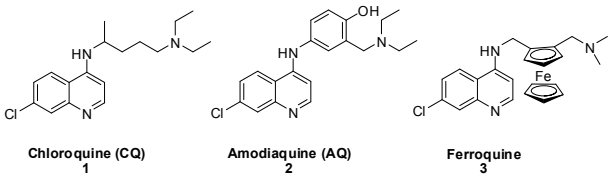
Keywords: Antimalarial, 4-Aminoquinoline, Drug resistance, Oxalamide, Schiff base

Introduction

After serving for decades as an efficient and affordable antimalarial, Chloroquine (CQ) is nearing towards the end of its effective life in medical shelves. Also effective vaccines against malaria are not available till now. The World Health Organization has set a target to develop vaccine against malaria with 80% efficacy by 2025 but according to Fauci 'we are not even close to that level of efficacy'.[1]

Plasmodium species, the causative agents of malaria, are responsible for up to 660 000 deaths each year besides very high levels of morbidity, mostly in children under five years of age. 68% of deaths due to malaria globally occur in the 10 highest-burden countries.[2] The disease has devastating impact on humans with considerable economic burden as well. Among the five major species of the malaria parasite *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* (recently discovered) that infect the humans, *P. falciparum* is the most fatal one.[3] By the end of 2011, artemisinin-based combination therapies (ACTs) had been adopted as national policy for first-line treatment where *P. falciparum* is endemic. Because in the present scenario ACTs

is the only class of efficient antimalarial therapy that can effectively eliminate multi-drug resistant malaria infections, the discovery of artemisinin resistance in parasites in western Cambodia has been viewed as a potential disaster by the international malaria community.[4] To overcome the challenges of multi-drug resistance in *P. falciparum*, many approaches currently being adopted contain optimization of treatment with available drugs including developing analogues of the existing drugs. Moreover, due to economic issues, such compounds need to be relatively inexpensive, stable and should be able to act on multiple stages of the malaria parasite. A number of clinically useful 4-aminoquinoline based antimalarial agents have been reported (Figure 1) as CQ (aminoquinolines) is known to inhibit the formation of hemozoin which makes it the target of choice for the development of new antimalarial chemotype.[5,6] Significant activity of several CQ analogues against CQ-R *P. falciparum* strains suggests that resistance could be compound-specific and not related to changes in the structure of the drug target. The structure activity relationship studies on 4-aminoquinoline antimalarials reveal that most of the structural changes of the 7-chloroquinoline ring in the CQ reduce the activity, whereas alterations of the CQ side chain appear to be a promising approach.[7-10]



Chloroquine (CQ) 1 | Amodiaquine (AQ) 2 | Ferroquine 3

Figure 1. Structures of some 4-aminoquinoline derivatives with antimalarial activity

A clue for an optimum side chain option can be taken from the contemporary trend of drug development with the molecule having two or more than two different chemical entities instead of a single pharmacophore that provides structural features to interact with numerous biological targets simultaneously within the molecule. Recently hybridization approach has been successfully explored to overcome resistance issue against CQ-R strains of *P. falciparum.*[11-14]

Schiff bases, the condensation products of aldehydes and amines are important class of biologically active molecules which are a good option to manipulate the side chain of CQ based analogues.[15,16] They could also act as valuable ligands whose biological activity has been shown to increase on complexation (Figure 2). Recently the schiff bases of N_4O_2 complex are developed as novel antimalarial drug complexes that inhibit the aggregation of hemozoin.[17] Furthermore, introduction of the oxalamide functionality is based on its greater stability towards enzyme degradation and to retain H-bonding ability (Figure 2). Oxalamide substructures based molecules show various biological activities.[18,19] The functionality shows a tendency to form two-dimensional hydrogen-bonding β-networks and its dual-binding mode of action has been confirmed through X-ray diffraction and molecular modeling studies.[20]

In our earlier attempt to introduce the oxalamide linker as flexible template in aminoquinoline, we successfully demonstrated the significant antimalarial activity of compounds having oxalamide functionality in side chain of aminoquinoline.[21] Here, as a part of our continuing efforts in malaria chemotherapy,[22-27] we have analyzed a series of 4-aminoquinoline-schiff base hybrids with oxalamide functionality as a linker that are predicted to be relevant for antimalarial activity against CQ-R parasites (**8-29**) (Table 1).

Synthesis

Proposed prototypes were prepared *via* relatively straightforward synthetic approach using inexpensive starting materials. The route

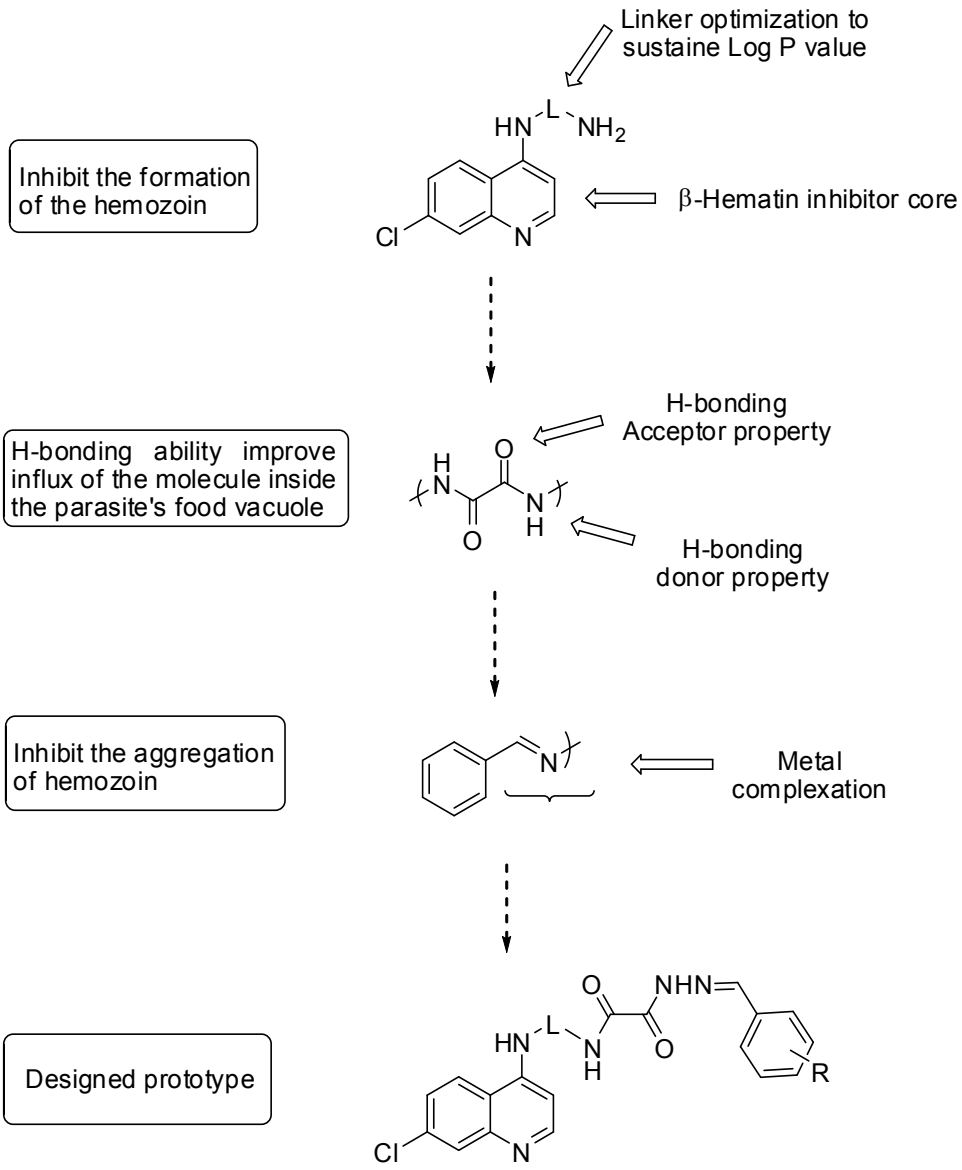
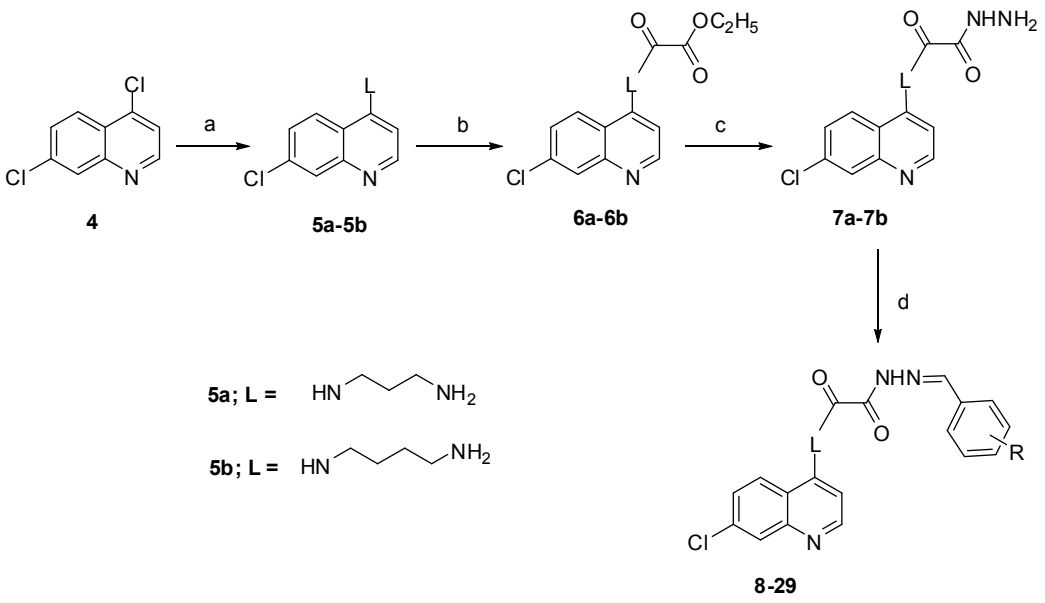


Figure 2. Designing of the desired prototype

used to synthesize representative compounds **8-29** of the series is depicted in Scheme 1. Compound **5a** and **5b** were synthesized *via* nucleophilic aromatic substitution reaction between the appropriate alkanediamine (propane-1,3-diamine, butane-1,4-diamine) and 4,7-dichloroquinoline. The diamines **5a** and **5b**, thus obtained on reaction with ethyl-2-chloro-2-oxoacetate give the corresponding oxoacetate **6a** and **6b** in quantitative yields. Further the reaction of **6a** and **6b** with hydrazine hydrate provides the corresponding hydrazinyl oxoacetamide **7a** and **7b** in promising yields. Finally, these hydrazinyl oxoacetamides on condensation with various aldehydes led to the formation of desired prototypes (Table 1) in good to excellent yields. Simple recrystallization with ethanol-hexane was sufficient for spectroscopic analysis of representative compounds of the series without any further purification. All the synthesized compounds were characterized by spectroscopic methods viz. NMR (^{1}H), mass



Scheme 1. Reagents and Conditions: (a) different diamines, neat, 80-120 °C. (b) ethyl chlorooxoacetate, DCM, rt. (c) hydrazine hydrate 80%, EtOH, rt. (d) different aldehydes, EtOH, HCl, rt.

and IR.

Results and discussion

All the synthesized compounds (**8-29**) of the series were screened for their *in vitro* antimalarial activity against the CQ-R K1 and the CQ-S 3D7 strains of *P. falciparum* using a standardized inexpensive assay based on Malaria SYBR Green I nucleic acid staining dye based fluorescence (MSF) assay. Molecules were also screened for their cytotoxicity towards VERO cell line. All the active compounds were screened for *in vitro* β-hematin inhibition too. *In vitro* antiplasmodial activity for compounds **8**–**29** against the CQ-R K1 and CQ-S 3D7 *P. falciparum* strains as indicated by their IC_{50} values, are shown in Table 1.

A systematic approach to investigate the potential antimalarial candidate was undertaken and study was planned *via* exploring the size/ property of linker and condensing substituted aromatic aldehydes, consequently establishing the structure activity relationship against CQ-R KI strain. The results reported here confirm the importance of side chain length for activity against *P. falciparum*. In general, *N*-butyl 4-aminoquinoline analogues showed activity with significant specificity (entry 8-24), however shortening the carbon chain (*N*-propyl 4-aminoquinoline) results in the loss of activity (entry 25-29) and hence the series was not explored further. After finding 4 carbon chain length as the promising linker, further pharmacomodulations consisted of introducing diversity on the C-4/C-2 position (R) of the schiff base's phenyl ring. Methyl substitution at *p*-position (entry 9) was found to enhance activity and improve selectivity as compared to their unsubstituted analogue (entry 8) and CQ against for CQ-R K1 strain. Further replacing all hydrogens of methyl group (entry 9) with fluorines by substituting it with trifluoromethyl increased activity by ~5 folds (entry 18). However, the replacement of methyl group by more lipophilic substituents (R = Et, Pr, ^{i}Pr) seems to be deleterious for activity (entry 10-

Table 1: *In vitro* antimalarial activity of compounds against 3D7 and K1 strains of *P. falciparum* and their cytotoxicity against VERO cell line.

Compd No.	Linker & R	*In vitro* antimalarial activity IC_{50} ng/mL[a]		Cytotoxicity CC_{50} ng/mL[b]	SI[c]	RI[d]
		K1	3D7			
8	1,4-Diamino butane Phenyl	>500	54.87	370	6.74	-[e]
9	1,4-Diamino butane 4-Methylphenyl	109.84	41.76	11,150	267.00	2.63
10	1,4-Diamino butane 4-Ethylphenyl	215.25	38.99	5,100	130.80	5.52
11	1,4-Diamino butane 4-Propylphenyl	243.2	44.69	5,340	119.48	5.44
12	1,4-Diamino butane 4-Isopropylphenyl	224.42	16.03	1,500	93.57	14
13	1,4-Diamino butane 4-*tert* butylphenyl	152.27	25.09	5,590	222.79	6.1
14	1,4-Diamino butane 2-Fluorophenyl	>500	20.74	5,450	262.77	-
15	1,4-Diamino butane 4-Fluorophenyl	ND[f]	NA[g]	11,260	ND	-
16	1,4-Diamino butane 4-Chlorophenyl	ND	NA	ND	ND	-
17	1,4-Diamino butane 4-Bromophenyl	360.72	160.67	36,750	228.73	2.24
18	1,4-Diamino butane 4-Trifluoromethylphenyl	20.70	58.39	5,730	98.13	0.35
19	1,4-Diamino butane 4-Methoxyphenyl	242.75	14.76	940	63.68	16.45
20	1,4-Diamino butane 3,4,5-Trimethoxyphenyl	394.36	82.90	19,630	236.79	4.76
21	1,4-Diamino butane 4-Thiomethylphenyl	176.92	18.91	5,860	309.88	9.36
22	1,4-Diamino butane 4-Nitrophenyl	271.03	26.75	7,250	57.30	10.13
23	1,4-Diamino butane 2-Fluoro-3-Pyridyl	ND	NA	ND	ND	-
24	1,4-Diamino butane 3-Indolyl	>500	37.47	42,000	1120.81	-
25	1,3-Diamino propane Phenyl	>500	37.08	51,590	1391.32	-
26	1,3-Diamino propane 4-Isopropylphenyl	ND	154.48	2,870	18.57	-
27	1,3-Diamino propane 2-Fluorophenyl	ND	NA	ND	ND	-
28	1,3-Diamino propane 4-Methoxyphenyl	356.93	37.72	5,290	140.24	9.46
29	1,3-Diamino propane 4-Thiomethylphenyl	ND	NA	ND	ND	-
	CQ	148.5	2.45	22,008	8983.0	60.61

[a] IC_{50} (ng/mL): concentration corresponding to 50% growth inhibition of the parasite. [b] CC_{50} (ng/mL): concentration corresponding to 50% growth inhibition of the cell line. [c] Selectivity index (SI): (IC_{50} values of cytotoxic activity/IC_{50} values of antimalarial activity). [d] Resistance index (IC_{50} – K1/IC_{50} – 3D7). [e] Not Applicable. [f] Not Done. [g] Not Active.

12), although *tert*-butyl at C-4 showed IC_{50} comparable with CQ against CQ-R K1 strain (entry 13). Introduction of halogens (F, Br, Cl) at 4 or 2 position led to inactive compounds (entry 14-17). Substitution with methoxy group at *p*-position decreased the activity (entry 19) and the analogue, in which methoxy group is introduced at 3, 4 and 5 position, was found to be even less potent (entry 20). Replacing the methoxy group with thiomethyl enhanced the activity as compared to the standard drug (entry 21). However, substitution with polar nitro group at C-4 was found only to be moderately active (entry 20). Further 2-fluoro-3-pyridyl and 3-indolyl substituted schiff bases-aminoquinoline hybrid were not found to be active (entry 23-24). In CQ-S 3D7 *P. falciparum* strain screened compounds were moderately active as compared to the control drug (CQ) with IC_{50} ranging from 14.76 to 160.67 ng mL^{-1}. The cytotoxicity of all the synthesized molecules (**8-29**) was carried out using Vero cell line (C1008; Monkey kidney fibroblast) following the method of Mosmann (1983) with certain modifications. Selectivity Index was calculated as SI = $CC_{50}/IC_{50.}$ Selectivity index of synthesized derivatives ranged from 57.30 to 1391.

To find out the mechanism of inhibition, all the active molecules were also evaluated for *β*-hematin inhibitory activity using the *β*-hematin inhibitory assay (BHIA) as previously described. The 50% inhibitory concentration (IC_{50}) was determined using non-linear regression analysis dose response curves. Compounds **10, 17, 19, 21, 25** and **26** (IC_{50} = 3.65, 3.29, 3.47, 3.72, 3.73 and 2.89 µg/mL respectively) showed a dose dependent inhibition in the BHIA and better potency than the CQ (IC_{50} = 3.80 µg/mL) in inhibiting hemozoin formation (Table 2).

All the synthesized compounds were also evaluated for their drug likeliness according to the Lipinski rule of five, and the results are summarized in Table 3. Molecular properties of synthesized compounds were calculated by Molinspiration software, and almost all the synthesized compounds were found to satisfy the criteria for drug likeliness (Table 3).

3. Conclusion

The results reported here demonstrate that antimalarials based on the 4-aminoquinoline-schiff base hybrid possess promising *in vitro* antiplasmodial activity against CQ-R K1 strain of *P. falciparum* and are comparable with CQ. Out of the synthesized 21 derivatives in the present series, most of the compounds were

Table 2: *β*-Hematin inhibitory activity of synthesized molecules

Compound No.	IC_{50} [a] (µg/mL)	Compound No.	IC_{50} (µg/mL)	Compound No.	IC_{50} (µg/mL)
8	4.84	**14**	14.4	**22**	5.34
9	12.3	**17**	3.29	**24**	10.6
10	3.65	**18**	4.36	**25**	3.73
11	4.99	**19**	3.47	**26**	2.89
12	4.10	**20**	5.30	**28**	4.04
13	7.33	**21**	3.72	**CQ**	3.80

[a] The IC_{50} represents the concentration of compound that inhibit β-hematin formation by 50%.

Table 3: Molinspiration calculation[a] of molecular properties for the Lipinski rule

Compound	nViol	MW	miLog P	nON	nOHNH	natoms	Nrotb
Acceptable Range	≤1	≤500	≤5	≤10	≤5	---	---
8	0	423.9	3.406	7	3	30.0	9
9	0	437.9	3.855	7	3	31.0	9
10	0	451.958	4.321	7	3	32.0	10
11	0	465.985	4.711	7	3	33	11
12	0	465.985	4.919	7	3	33	10
13	1	480.012	5.113	7	3	34	10
14	0	441.894	3.522	7	3	31	9
15	0	441.894	3.57	7	3	31	9
16	0	458.349	4.084	7	3	31	9
17	1	502.8	4.216	7	3	31	9
18	0	491.901	4.302	7	3	34	10
19	0	453.93	3.463	8	3	32	10
20	1	513.982	3.038	10	3	36	12
21	0	469.998	3.84	7	3	32	10
22	0	468.901	3.365	10	3	33	10
23	0	442.882	2.623	8	3	31	9
24	0	462.941	3.557	8	4	33	9
25	0	409.877	3.136	7	3	29	8
26	0	451.958	4.648	7	3	32	9
27	0	427.867	3.252	7	3	30	8
28	0	439.903	3.193	8	3	31	9
29	0	455.971	3.569	7	3	31	9

[a] Values calculated using online software www.molinspiration.com
nViol, no. of violations; MW, molecular weight; miLog P, molinspiration predicted Log P; nON, no. of hydrogen bond acceptors; nOHNH, no. of hydrogen bond donors; natoms, no. of atoms; nrotb, no. of rotatable bond.

found active with IC_{50} in the range of 20.70 - 394.36 ng/mL against CQ-R K1 strain of *P. falciparum* parasites along with acceptable selectivity. Moreover *β*-hematin inhibitory activity results showed promising *β*-hematin inhibitory activity. On the basis of biological evaluation and SAR study of the series, 4-aminoquinoline schiff base hybrids seem to be potential antimalarial candidates and should be explored further in future.

Experimental

General Information

NMR spectra were recorded on 300 MHz spectrometers in DMSO-d_6. Chemical shifts (δ) are reported in parts per million (ppm) for ^{1}H NMR spectra. Coupling constants *J* are reported in hertz (Hz). Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad resonance. The reaction progress was regularly monitored by thin-layer chromatography (TLC) on pre coated silica gel plates. All compounds were characterized by ^{1}H NMR, IR and Mass.

General procedure for the synthesis of compounds 5 (a-b) and 6 (a-b)

Synthesis of corresponding N^1-(7-chloroquinolin-4-yl)alkane-1,n-diamine (**5a**

and **5b**) and ethyl 2-(n-(7-chloroquinolin-4-ylamino)alkylamino)-2-oxoacetate (**6a** and **6b**) were achieved by our previously reported protocol [21].

General procedure for the synthesis of compound 7a-7b

A solution of corresponding oxoacetate **6** (1equiv) and hydrazine hydrate 80% (5 equiv) in ethanol (2 mL per 0.1 mmol of **6**) was allowed to stir at room temperature. After completion (monitored by TLC) water was added in to the reaction mixture and the insoluble product was filtered off and air dried to get corresponding hydrazinyl oxoacetamide (**7a-7b**) as tan color solid.

Typical procedure for synthesis of substituted (*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) alkyl)-2-oxo-2-(2-(substitutedbenzylidene) hydrazinyl)acetamide:

Corresponding hydrazinyl oxoacetamide **7** (1 equiv) and different aldehydes (1 equiv) in ethanol (2 mL per 0.1 mmol of **7**) with HCl (0.1 equiv) was stirred at room temperature. After completion of reaction (monitored by TLC) water was added and the insoluble product was filtered off, air dried and recrystalized with ethanol-hexane to get series of representative compounds (**8-29**).

(*E*)-2-(2-benzylidenehydrazinyl)-*N*-(4-(7-chloroquinolin-4-ylamino)butyl)-2-oxoacetamide (8): Yellow solid; yield: 82%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.61-1.65 (m, 4H), 3.24-3.26 (m, 2H), 3.41-3.43 (m, 2H), 6.71 (d, 1H, *J* = 6.3 Hz), 7.45-7.47 (m, 2H), 7.60-7.64 (m, 1H), 7.67-7.68 (m, 1H), 7.88 (brs, 1H), 8.43-8.47 (m, 3H), 8.56 (brs, 1H), 9.00 (t, 1H, *J* = 6.0 Hz), 12.09 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.5, 25.6, 37.9, 41.9, 98.1, 115.8, 122.1, 124.6, 125.0, 126.8, 128.3, 130.0, 133.4, 135.4, 142.5, 146.1, 150.2, 152.5, 156.0, 159.2. IR (KBr): 1651, 3013, 3455 cm^{-1}. MS(ES$^+$): m/z = 424.2 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-(4-methylbenzylidene) hydrazinyl)-2-oxoacetamide (9): White solid; yield: 85%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.63-1.68 (m, 4H), 2.34 (s, 3H), 3.23-3.25 (m, 4H), 6.50 (d, 1H, *J* = 5.5 Hz), 7.27 (d, 2H, *J* = 8.0 Hz), 7.77-7.79 (m, 1H), 8.28 (d, 1H, *J* = 9.1 Hz), 8.39 (d, 1H, *J* = 5.1 Hz), 8.52 (brs, 1H), 9.01 (t, 1H, *J* = 5.9 Hz), 12.05 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 20.5, 24.6, 25.8, 38.0, 41.5, 98.1, 116.8, 123.6, 126.4, 126.8, 128.9, 130.7, 133.1, 139.9, 147.9, 149.9, 150.2, 150.8, 155.9, 159.2. IR (KBr): 1687, 3028, 3441 cm^{-1}. MS(ES$^+$): m/z = 438.2 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-(4-ethylbenzylidene)hydrazinyl)-2-oxoacetamide (10): White solid; yield: 89%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.19 (t, 3H, *J* = 7.5 Hz), 1.64-1.65 (m, 4H), 2.65 (q, 2H, *J* = 7.5 Hz), 3.25-3.27 (m, 2H), 3.30-3.32 (m, 2H), 6.50 (d, 1H, *J* = 5.2 Hz), 7.29-7.31 (m, 2H), 7.42 (brs, 1H), 7.45-7.47 (m, 1H), 7.61 (d, 1H, *J* = 7.5 Hz), 7.77-7.78 (m, 1H), 8.28 (d, 1H, *J* = 9.05 Hz), 8.39 (d, 1H, *J* = 5.22 Hz), 8.53 (brs, 1H), 9.01 (t, 1H, *J* = 5.91 Hz), 12.05 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.7, 24.6, 25.8, 27.6, 38.0, 41.5, 98.1, 116.8, 123.6, 126.4, 126.9, 127.7, 130.9, 133.1, 146.1, 147.9, 149.9, 150.2, 155.9, 159.2. IR (KBr): 1654, 3021, 3413 cm^{-1}. MS(ES$^+$): m/z = 452.3 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-oxo-2-(2-(4-propylbenzylidene) hydrazinyl)acetamide (11): White solid; yield: 87%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 0.89 (t, 3H, *J* = 7.3 Hz), 1.56-1.60 (m, 2H), 1.64-1.69 (m, 4H), 2.59 (t, 2H, *J* = 7.7 Hz), 3.24-3.27 (m, 2H), 3.45-3.56 (m, 2H), 6.89 (d, 1H, *J* = 7.2 Hz), 7.26-7.29 (m, 2H), 7.59 (d, 2H, *J* = 8.2 Hz), 7.75-7.78 (m, 1H), 8.03 (brs, 1H), 8.52-8.54 (m, 1H), 8.63 (d, 1H, *J* = 9.2 Hz), 9.01

(t, 1H, *J* = 6.0 Hz), 9.50 (brs, 1H), 12.04 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.0, 23.3, 24.4, 25.5, 36.6, 37.9, 42.2, 98.0, 114.9, 118.5, 125.4, 126.1, 126.8, 128.3, 130.9, 137.3, 138.1, 142.1, 144.5, 150.3, 154.7, 155.9, 159.2. IR (KBr): 1653, 3015, 3439 cm^{-1}. MS(ES$^+$): m/z = 466.3 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-(4-isopropylbenzylidene) hydrazinyl)-2-oxoacetamide (12): Off white solid; yield: 79%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.20-1.22 (m, 6H), 1.62-1.68 (m, 4H), 2.89-2.96 (m, 1H), 3.24-3.28 (m, 4H), 6.49 (d, 1H, *J* = 5.5 Hz), 7.32-7.34 (m, 2H), 7.43-7.45 (m, 1H), 7.60 (d, 2H, *J* = 8.2 Hz), 7.78 (br s), 8.28 (d, 1H, *J* = 9.1 Hz), 8.39 (d, 1H, *J* = 5.3 Hz), 8.53 (brs), 8.98 (t, 1H, *J* = 5.9 Hz), 12.03 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.1, 24.6, 25.8, 32.8, 38.0, 41.5, 98.1, 116.9, 123.6, 126.3, 126.6, 126.9, 131.1, 133.0, 148.2, 149.7, 150.2, 150.6, 151.0, 155.9, 159.2. IR (KBr): 1646, 3026, 3443 cm^{-1}. MS(ES$^+$): m/z = 466.3 [M$^+$ + 1]

(*E*)-2-(2-(4-tert-butylbenzylidene) hydrazinyl)-*N*-(4-(7-chloroquinolin-4-ylamino)butyl)-2-oxoacetamide (13): Tan solid; yield: 93%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.29 (s, 9H), 1.64-1.70 (m, 4H), 3.24-3.28 (m, 2H), 3.54-3.56 (m, 2H), 6.88 (d, 1H, *J* = 6.6 Hz), 7.47-7.49 (m, 2H), 7.60-7.62 (m, 2H), 7.77 (brs, 1H), 8.02-8.03 (m, 1H), 8.52-8.53 (m, 1H), 8.63 (d, 1H, *J* = 9.4 Hz), 8.99 (t, 1H, *J* = 5.7 Hz), 9.46 (brs, 1H), 12.02 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.5, 30.4, 34.1, 37.9, 42.2, 98.0, 115.0, 118.7, 125.2, 125.3, 126.1, 126.7, 130.7, 137.2, 138.3, 142.4, 150.1, 152.9, 154.7, 155.9, 159.3. IR (KBr): 1623, 3011, 3419 cm^{-1}. MS(ES$^+$): m/z = 480.3 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-(2-fluorobenzylidene) hydrazinyl)-2-oxoacetamide (14): Off white solid; yield: 85%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.62-1.66 (m, 4H), 3.24-3.31 (m, 4H), 6.49 (d, 1H, *J* = 5.6 Hz), 7.27-7.31 (m, 2H), 7.37-7.39 (m, 1H), 7.43-7.46 (m, 1H), 7.50-7.52 (m, 1H), 7.78 (brs, 1H), 7.89-7.93 (m, 1H), 8.27 (d, 1H, *J* = 9.1 Hz), 8.82 (brs, 1H), 9.04 (t, 1H, *J* = 5.7 Hz), 12.32 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.5, 37.9, 42.2, 98.1, 115.5, 115.8, 116.8, 123.5, 124.6, 126.0, 126.9, 132.8, 133.2, 143.1, 149.3, 151.2, 154.5, 156.0, 159.3, 162.1. IR (KBr): 1621, 3015, 3447 cm^{-1}. MS(ES$^+$): m/z = 441.3 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-(4-fluorobenzylidene) hydrazinyl)-2-oxoacetamide (15): Off white solid; yield: 81%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.63-1.69 (m, 4H), 3.23-3.26 (m, 2H), 3.53-3.58 (m, 2H), 6.89 (d, 1H, *J* = 7.3 Hz), 7.29 (t, 2H, *J* = 8.9 Hz), 7.73-.76 (m, 1H), 7.78 (brs, 1H), 8.04-8.05 (m, 1H), 8.53 (d, 1H, *J* = 7.1 Hz), 8.56 (s, 1H), 8.66 (d, 1H, *J* = 9.0 Hz), 9.01 (t, 1H, *J* = 6.0 Hz), 9.55 (brs, 1H), 12.10 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.5, 37.9, 42.2, 98.0, 114.8, 118.6, 125.2, 126.1, 128.5, 132.4, 134.5, 137.2, 138.1, 142.4, 149.0, 154.7, 156.0, 159.1. IR (KBr): 1659, 3017, 3428 cm^{-1}. MS(ES$^+$): m/z = 442.2 [M$^+$ + 1]

(*E*)-2-(2-(4-chlorobenzylidene)hydrazinyl)-*N*-(4-(7-chloroquinolin-4-ylamino)butyl)-2-oxoacetamide (16): White solid; yield: 79%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.63-1.68 (m, 4H), 3.24-3.27 (m, 2H), 3.53-3.55 (m, 2H), 6.88 (d, 1H, *J* = 6.6 Hz), 7.51-7.53 (m, 2H), 7.70 (d, 1H, *J* = 8.4 Hz), 7.75-7.77 (m, 2H), 8.01-8.03 (m, 1H), 8.52-8.55 (m, 1H), 9.04 (t, 1H, *J* = 6.1 Hz), 9.36 (brs, 1H), 12.17 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.5, 37.9, 42.2, 98.0, 114.9, 118.6, 125.3, 126.1, 128.5, 132.3, 134.5, 137.2, 138.2, 142.3, 148.9, 154.7, 156.1, 159.1. IR (KBr): 1662, 3018, 3423 cm^{-1}. MS(ES$^+$): m/z = 458.2 [M$^+$ +

1]

(*E*)-2-(2-(4-bromobenzylidene)hydrazinyl)-*N*-(4-(7-chloroquinolin-4-ylamino)butyl)-2-oxoacetamide (17): Tan solid; yield: 83%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.63-1.69 (m, 4H), 3.24-3.27 (m, 2H), 3.54-3.56 (m, 2H), 6.89 (d, 1H, *J* = 6.8 Hz), 7.62-7.67 (m, 4H), 7.77 (brs, 1H), 8.02-8.03 (m, 1H), 8.52-8.54 (m, 1H), 8.63 (d, 1H, *J* = 9.2 Hz), 9.04 (t, 1H, *J* = 6.1Hz), 9.49 (brs, 1H), 12.18 (brs, 1H); ^{13}C NMR (DMSO-d_6 + TFA, 100 MHz) δ 24.6, 25.7, 38.1, 42.5, 98.2, 115.1, 119.0, 125.3, 126.5, 128.8, 130.8, 131.6, 131.9, 137.8, 138.2, 142.5, 149.2, 155.1, 156.3, 159.4. IR (KBr): 1617, 3024, 3445 cm^{-1}. MS(ES$^+$): m/z = 502.3 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-oxo-2-(2-(4-(trifluoromethyl) benzylidene)hydrazinyl)acetamide (18): Tan solid; yield: 87%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.64-1.69 (m, 4H), 3.25-3.28 (m, 2H), 3.53-3.56 (m, 2H), 6.89 (d, 1H, *J* = 7.1 Hz), 7.76 (br s), 7.81-7.83 (m, 2H), 7.89-7.91 (m, 2H), 8.53 (d, 1H, *J* = 7.1 Hz), 8.60-8.62 (m, 1H), 8.64 (br s), 9.07 (t, 1H, *J* = 6.0 Hz), 9.46 (t, 1H, *J* = 5.7 Hz), 12.31 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.5, 37.9, 42.2, 98.0, 114.9, 118.6, 122.1, 124.8, 125.2, 126.1, 127.4, 129.4, 129.7, 137.2, 138.2, 142.3, 148.5, 154.7, 156.2, 159.0. IR (KBr): 1629, 3020, 3432 cm^{-1}. MS(ES$^+$): m/z = 492.3 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-(4-methoxybenzylidene) hydrazinyl)-2-oxoacetamide (19): Off white solid; yield: 79%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.62-1.67 (m, 4H), 3.24-3.26 (m, 2H), 3.27-3.29 (m, 2H), 3.80 (s, 3H), 6.49 (d, 1H, *J* = 5.6 Hz), 7.01 (d, 2H, *J* = 8.8 Hz), 7.35-7.37 (m, 1H), 7.43-7.46 (m, 1H), 7.63 (d, 1H, *J* = 8.8 Hz), 7.77 (brs, 1H), 8.27 (d, 1H, *J* = 8.9 Hz), 8.39 (d, 1H, *J* = 5.4 Hz), 8.49 (brs, 1H), 8.97 (t, 1H, *J* = 6.0 Hz), 11.96 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.6, 25.8, 38.0, 41.5, 54.8, 98.1, 113.8, 116.9, 123.5, 123.6, 125.9, 126.6, 128.5, 133.0, 148.2, 149.7, 150.0, 151.0, 155.8, 159.3, 160.6. IR (KBr): 1643, 3019, 3436 cm^{-1}. MS(ES$^+$): m/z = 454.2 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino)butyl)-2-oxo-2-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl) acetamide (20): Tan solid; yield: 84%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.64-1.69 (m, 4H), 3.24-3.26 (m, 2H), 3.54-3.56 (m, 2H), 3.70 (s, 3H), 3.83 (s, 6H), 6.89 (d, 1H, *J* = 7.2 Hz), 6.97 (s, 2H), 7.75-7.78 (m, 1H), 8.02-8.03 (m, 1H), 8.46 (s, 1H), 8.53 (d, 1H, *J* = 7.1 Hz), 8.64 (d, 1H, *J* = 9.2 Hz), 8.97 (t, 1H, J = 5.9 Hz), 9.48 (brs, 1H), 12.06 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.5, 37.9, 42.2, 55.5, 59.6, 98.0, 104.1, 115.0, 118.6, 125.4, 126.1, 128.8, 137.3, 138.2, 139.1, 142.3, 150.3, 152.7, 154.7, 155.9, 159.3. IR (KBr): 1654, 3022, 3427 cm^{-1}. MS(ES$^+$): m/z = 514.3 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-(4-(methylthio)benzylidene) hydrazinyl)-2-oxoacetamide (21): Yellow solid; yield: 88%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.65-1.69 (m, 2H), 2.51 (s, 3H), 3.23-3.27 (m, 2H), 3.43-3.49 (m, 2H), 6.81 (d, 1H, *J* = 6.2 Hz), 7.23-7.28 (m, 2H), 7.54 (d, 2H, *J* = 8.1 Hz), 7.71-7.76 (m, 1H), 8.01 (brs, 1H), 8.49-8.53 (m, 1H), 8.58 (d, 1H, J = 9.0 Hz), 9.09 (t, 1H, *J* = 5.9 Hz), 12.03 (brs, 1H); ^{13}C NMR (DMSO-d_6 + TFA, 100 MHz) δ 13.7, 26.6, 36.0, 37.4, 40.4, 98.2, 114.9, 118.6, 125.1, 126.4, 127.3, 128.2, 129.8, 137.6, 141.2, 142.5, 149.9, 155.0, 155.8, 159.4, 160.4. IR (KBr): 1644, 3027, 3453 cm^{-1}. MS(ES$^+$): m/z = 470.3[M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-(4-nitrobenzylidene)hydrazinyl)-2-oxoacetamide (22): Yellow solid; yield: 81%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.64-1.66 (m, 4H), 3.25-3.26 (m, 2H), 3.47-

3.49 (m, 2H), 6.57 (d, 1H, *J* = 6.8 Hz), 7.48-7.51 (m, 1H), 7.69-7.71 (m, 1H), 7.81 (brs, 1H), 7.94-7.96 (m, 2H), 8.29-8.31 (m, 3H), 8.67 (brs, 1H), 9.05-9.08 (m, 1H), 12.40 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.5, 37.9, 42.2, 98.0, 115.0, 118.6, 125.4, 126.1, 128.6, 132.3, 134.5, 137.3, 138.1, 142.3, 149.0, 154.6, 156.3, 159. IR (KBr): 1655, 3020, 3433 cm^{-1}. MS(ES$^+$): m/z = 469.2 [M$^+$ + 1]

(*E*)-*N*-(4-(7-chloroquinolin-4-ylamino) butyl)-2-(2-((2-fluoropyridin-3-yl) methylene)hydrazinyl)-2-oxoacetamide (23): Off white solid; yield: 73%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.63-1.69 (m, 4H), 3.23-3.27 (m, 2H), 3.53-3.56 (m, 2H), 6.89 (d, 1H, *J* = 7.1 Hz), 7.44-7.48 (m, 1H), 7.74-7.78 (m, 1H), 8.04 (brs, 1H), 8.31-8.33 (m, 1H), 8.35-8.39 (m, 1H), 8.64 (d, 1H, *J* = 9.1 Hz), 8.74 (brs, 1H), 9.07 (t, 1H, *J* = 6.1 Hz), 9.50-9.52 (m, 1H), 12.44 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.5, 37.9, 42.2, 98.0, 114.9, 116.3, 118.6, 122.3, 125.4, 126.1, 137.0, 137.3, 138.2, 142.0, 142.3, 148.7, 154.7, 156.2, 158.9, 161.5. IR (KBr): 1626, 3019, 3430 cm^{-1}. MS(ES$^+$): m/z = 443.2[M$^+$ + 1]

(*E*)-2-(2-benzylidenehydrazinyl)-*N*-(3-(7-chloroquinolin-4-ylamino)propyl)-2-oxoacetamide (25): white solid; yield: 89%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.86-1.93 (m, 2H), 3.26-3.29 (m, 4H), 6.48 (d, 1H, *J* = 5.5 Hz), 7.29-7.32 (m, 1H), 7.44-7.46 (m, 3H), 7.70 (m, 1H), 7.78 (brs, 1H), 8.24 (d, 1H, *J* = 9.0 Hz), 8.39 (d, 1H, *J* = 5.2 Hz), 8.57 (brs, 1H), 9.09 (t, 1H, *J* = 5.9 Hz), 12.12 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.6, 36.2, 98.1, 115.7, 122.2, 124.6, 125.1, 126.8, 128.3, 129.9, 133.4, 135.4, 142.4, 146.2, 150.1, 152.5, 156.1, 159.4. IR (KBr): 1619, 3023, 3432 cm^{-1}. MS(ES$^+$): m/z = 410.3 [M$^+$ + 1]

(*E*)-*N*-(3-(7-chloroquinolin-4-ylamino) propyl)-2-(2-(4-isopropylbenzylidene) hydrazinyl)-2-oxoacetamide (26): White solid; yield: 82%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.20-1.22 (m, 6H), 1.85-1.92 (m, 2H), 2.88-2.95 (m, 1H), 3.29-3.32 (m, 4H), 6.48 (d, 1H, *J* = 5.2 Hz), 7.33 (d, 3H, *J* = 8.2 Hz), 7.44-7.47 (m, 1H), 7.61 (d, 2H, *J* = 8.2 Hz), 7.78 (brs, 1H), 8.24 (d, 1H, *J* = 8.9 Hz), 8.53 (brs, 1H), 9.09 (t, 1H, *J* = 6.0 Hz), 12.07 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.1, 26.7, 32.9, 36.2, 98.1, 123.4, 123.5, 126.3, 126.9, 131.1, 132.8, 148.6, 149.4, 150.2, 150.7, 151.4, 155.9, 159.4. IR (KBr): 1618, 3017, 3417 cm^{-1}. MS(ES$^+$): m/z = 452.2 [M$^+$ + 1]

(*E*)-*N*-(3-(7-chloroquinolin-4-ylamino) propyl)-2-(2-(2-fluorobenzylidene) hydrazinyl)-2-oxoacetamide (27): White solid; yield: 86%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.85-1.92 (m, 2H), 3.29-3.32 (m, 2H), 6.48 (d, 1H, *J* = 5.4 Hz), 7.27-7.29 (m, 1H), 7.35-7.37 (m, 1H), 7.44-7.47 (m, 1H), 7.61-7.63 (m, 2H), 7.77 (brs, 1H), 8.24 (d, 1H, *J* = 9.0 Hz), 8.39 (d, 1H, *J* = 5.3 Hz), 8.83 (brs, 1H), 9.12 (t, 1H, *J* = 6.1 Hz), 12.34 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.7, 36.2, 98.1, 115.6, 115.8, 116.9, 123.5, 124.5, 126.0, 127.0, 132.8, 133.2, 143.1, 149.4, 151.3, 154.5, 156.1, 159.2, 162.2. IR (KBr): 1647, 3021, 3429 cm^{-1}. MS(ES$^+$): m/z = 428.2 [M$^+$ + 1]

(*E*)-*N*-(3-(7-chloroquinolin-4-ylamino) propyl)-2-(2-(4-methoxybenzylidene) hydrazinyl)-2-oxoacetamide (28): White solid; yield: 81%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.85-1.91 (m, 2H), 3.26-3.28 (m, 2H), 3.77-3.41 (m, 2H), 3.81 (s, 3H), 6.49 (d, 1H, *J* = 5.5 Hz), 7.33-7.35 (m, 3H), 7.45-7.48 (m, 1H), 7.68 (d, 2H, *J* = 8.3 Hz), 7.82 (brs, 1H), 8.23 (d, 1H, *J* = 8.9 Hz), 8.53 (brs, 1H), 9.06 (t, 1H, *J* = 6.1 Hz), 12.09 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.7, 36.3, 54.9, 98.1, 114.1, 116.9, 123.3, 123.6, 126.0, 126.6, 128.4, 132.9, 148.2, 149.7, 150.0, 150.9, 155.8, 159.1, 160.5. IR (KBr): 1653, 3024, 3427 cm^{-1}. MS(ES$^+$): m/z = 440.2[M$^+$ + 1]

(*E*)-*N*-(3-(7-chloroquinolin-4-ylamino) propyl)-2-(2-(4-(methylthio)benzylidene) hydrazinyl)-2-oxoacetamide (29): Yellow solid; yield: 74%; mp >250 ˚C. ^{1}H (300 MHz, DMSO-d_6): δ = 1.86-1.92 (m, 2H), 2.53 (s, 3H), 3.25-3.27 (m, 2H), 3.37-3.42 (m, 2H), 6.48 (d, 1H, *J* = 5.1 Hz), 7.31-7.33 (m, 3H), 7.43-7.46 (m, 1H), 7.62 (d, 2H, *J* = 8.5 Hz), 8.24 (d, 1H, *J* = 8.9 Hz), 8.51 (brs, 1H), 9.07 (t, 1H, *J* = 6.4 Hz), 12.07 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.5, 26.6, 36.2, 98.2, 115.0, 118.6, 125.0, 126.6, 127.3, 128.2, 129.7, 137.7, 141.2, 142.6, 150.0, 155.0, 155.7, 159.4, 160.2. IR (KBr): 1637, 3018, 3456 cm^{-1}. MS(ES$^+$): m/z = 456.2[M$^+$ + 1]

Bioevaluation methods

In vitro antimalarial assay

The *in vitro* antiplasmodial SAR (structure activity relationship) initially involved evaluation of analogues against the CQ-R K1 and the CQ-S 3D7 strains (MRA-159, MR4, ATCC Manassas Virginia) of *P. falciparum* using a standardized inexpensive assay based on SYBER Green I nucleic acid staining dye based fluorescence (MSF) assay.[28] The stock (5mg/mL) solution was prepared in DMSO and test dilutions were prepared in culture medium (RPMI-1640-FBS). CQ was used as reference drug. The compounds were tested in 96 well plates (in duplicate wells). 1.0% parasitized cell suspension containing 0.8% parasitaemia was used. The plates were incubated at 37 ºC in CO_2 incubator in an atmosphere of 5% CO_2 and air mixture. After 72 h 100 µL of lysis buffer containing 1x concentration of SYBR Green-I (Invitrogen) was added to each well and incubated for another one hour at 37 ºC. The plates were examined at 485 ± 20 nm of excitation and 530 ± 20 nm of emission for relative fluorescence units (RFUs) per well using the fluorescence plate reader (FLUO star, BMG lab technologies). Data was transferred into a graphic programme (MS-EXCEL) and IC_{50} values were obtained by Logit regression analysis using pre-programmed MS-Excel spreadsheet.

In vitro assay for evaluation of cytotoxic activity

Cytotoxicity of the compounds was carried out using Vero cell line (C1008; Monkey kidney fibroblast) following the method of Mosmann (1983) with certain modifications. The cells were incubated with compound dilutions for 72 h and MTT was used as reagent for the detection of cytotoxicity.[29] 50% cytotoxic concentration (CC_{50}) was determined using nonlinear regression analysis using pre-programmed Excel spreadsheet. Selectivity Index was calculated as SI = $CC_{50}/IC_{50.}$

In vitro assay for evaluation of β-hematin inhibition

Inhibition of *in vitro β*-hematin formation was analysed by using the method reported by Pandey et al. 1999 with some modifications.[30] Male Swiss albino mice, weighing 15-20 g were inoculated with 1×10^5 *P. yoelii* infected RBCs. Blood of infected animal at 50% parasitaemia was collected by cardiac puncture in 2.0% citrate buffer and centrifuged at 5000 rpm for 10 min at 4 ˚C. The plasma was used in assay of *β*-hematin formation. The assay mixture contained 100 mM sodium acetate buffer (pH 5.1), 50 mL plasma, 100 mM hemin as the substrate and 1-20 mg compound/drug in a total reaction volume of 1.0 mL. The control tubes contained all reagents except compound. The reaction mixture in triplicate was incubated at 37 ˚C for 16 h in a rotary shaker. The reaction was stopped by centrifugation at 10,000 rpm for 10 min at 30 ˚C. The pellet was suspended in 100 mM Trise HCl buffer (pH 7.4) containing 2.5% SDS. The pellet obtained after centrifugation was washed thrice with distilled water (TDW) to remove free heme attached to *β*-hematin. The

pellet was solubilized in 50 mL of 2 N NaOH and volume was made up to 1.0 mL with TDW. Absorbance was measured at 400 nm. The 50% inhibitory concentration (IC_{50}) was determined using non-linear regression analysis of dose response curves.

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