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Synthesis and biological evaluation of a new class of 4-aminoquinoline–rhodanine hybrid as potent anti-infective agents

Kuldeep Chauhan [a], Moni Sharma [a], Juhi Saxena [b], Shiv Vardan Singh [c], Priyanka Trivedi [d], Kumkum Srivastava [b], Sunil K. Puri [b], J.K. Saxena [c], Vinita Chaturvedi [d], Prem. M.S. Chauhan [a,*]

[a] Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, Lucknow 226001, India
[b] Division of Parasitology, CSIR-Central Drug Research Institute, Lucknow 226001, India
[c] Division of Biochemistry, CSIR-Central Drug Research Institute, Lucknow 226001, India
[d] Drug Target Discovery and Development Division, CSIR-Central Drug Research Institute, Lucknow 226001, India

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ABSTRACT

Synthesis of novel 4-aminoquinoline–rhodanine hybrid using inexpensive starting materials *via* easy to operate methodology, and their biological activity is reported. All the compounds were screened for their *in vitro* antimalarial activity against chloroquine-resistant (K1) and chloroquine-sensitive (3D7) strains of *Plasmodium falciparum,* and their cytotoxicity toward VERO cell line. Compounds **9**, **19**, **21** and **23** exhibited excellent antimalarial activity with IC_{50} value ranging from 13.2 to 45.5 nM against chloroquine-resistant (K1) strain. Biochemical studies revealed that inhibition of hemozoin formation is the primary mechanism of action of these analogs for their antimalarial activity. Additionally, some derivatives (**14**, **18** and **26**) of this series also exhibited the antimycobacterial activity against $H_{37}Rv$ strain of *Mycobacterium tuberculosis* with MIC value of 6.25 μM.

1. Introduction

Despite the enormous progress in medicinal chemistry, infectious diseases remain a biggest threat to society and have provided new challenges to researchers worldwide [1]. Among these, malaria and tuberculosis (TB) are the two lethal infectious diseases in the world [2–4]. *Plasmodium falciparum* is the most virulent species among the five major species of malarial parasites and it accounts for more than 95% of malarial cases related to morbidity and mortality [5]. There were an estimated 655000 malaria deaths in 2010, of which 91% were in Africa. Approximately 86% of malaria deaths worldwide were of children under the age of 5 years [6]. In conjunction with malaria, tuberculosis (TB) is a chronic infectious disease and one of the major AIDS-associated infections. According to the first World Health Organization (WHO) report on global TB care and control to include estimates of the burden of TB disease among children, with approximate of 490,000 cases and 64,000 deaths each year [7]. Current therapies for malaria and tuberculosis are unsatisfactory and the development of drug resistance for the common antimalarials [8–11] (aminoquinoline, artemisinin, artemether and antifolates) and antitubercular [12] (rifampicin and isoniazid) have stimulated the research efforts in related enterprise globally. As a consequence, the pipeline of potential new drugs has expanded, but humans have suffered from the burden of malarial and TB infections for thousands of years [13–17]. After the several decades of drug development, quinoline pharmacophore has a long and successful history as antimalarials [18] and antitubercular [19] and its derivatives also exhibited various biological activities such as anti-HIV, antifungal, antibacterial and antileishmanial [20–22]. Among the quinoline derivatives, 4-amino-7-chloro quinoline class of therapeutics remain a front line drug and the selection of this pharmacophore is based on its excellent clinical efficacy, low toxicity, cheap synthesis and ease of administration [23]. Quinoline-based compounds disrupt the conversion of toxic heme into hemozoin (crystal of ferriprotoporphyrin IX (FP–Fe (III)). Hemozoin is essential for FP–Fe (III) detoxification in the parasite and it is the main target of quinoline antimalarials that can modulate immune and inflammation responses [24]. Quinoline-based hybrids such as aminoquinoline–isatin [25], aminoquinoline–clotrimazole [26], aminoquinoline–triazine [27], aminoquinoline–pyrimidine [28,29], quinoline-thiazolidin-4-one [30], aminoquinoline–ferrocene based hydrazones [31] and more

* Corresponding author. Tel.: +91 522 2612411; fax: +91 522 2623405.
E-mail addresses: prem_chauhan_2000@yahoo.com, premsc58@hotmail.com (Prem.M.S. Chauhan).

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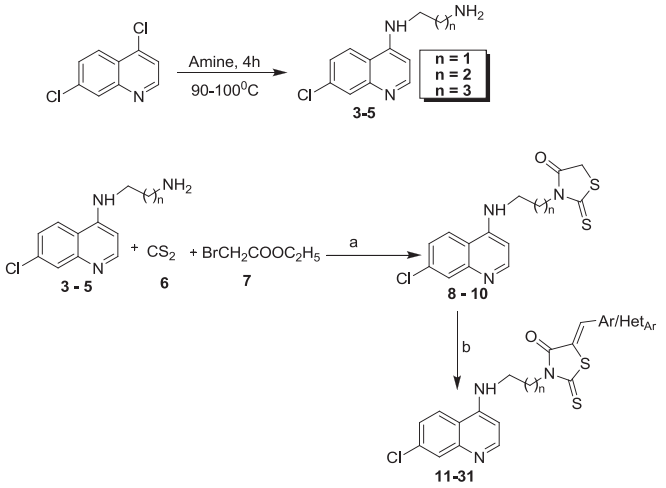
recently, 4-aminoquinolines-3-(2,2,2-trifluoroethyl)-γ-hydroxy-γ-lactam [32], enone and chalcone-chloroquine hybrid analogs [33] and 15-membered azalide-4-aminoquinoline [34] were reported in the literature as anti-infective agents. (Fig. 1)

On the other hand, rhodanine scaffold is a powerful device for medicinal chemist and have a broad substrate scope for the synthesis of various heterocyclic moieties with wide range of pharmacological activities such as antimalarial [35], antimicrobial [36–38], antiviral, anti-diabetic [39], and anticonvulsant effects [40] (Fig. 1). This scaffold showing important therapeutic targets against *Plasmodium* fatty acid synthesis pathway (FAS-II) found in the parasites which is distinct from type I fatty acid synthesis pathway (FAS-I) present in the human host [41]. Therefore in continuation of our ongoing anti-infective research programme [42–45], herein, we envisaged that combining two intrinsically pharmacologically active scaffolds 4-aminoquinoline and rhodanine based on molecular hybridization approach [46]. The newly synthesized hybrids exhibited not only antimalarial activity but also showed good activity against $H_{37}Rv$ strain of *Mycobacterium tuberculosis*.



Scheme 1. Synthesis of 4-aminoquinoline–rhodanine hybrids. Reagent and Conditions (a) Acetonitrile, rt, 3–5 h, (b) Acetic acid, Ammonium acetate, Aromatic/ Hetro-aromatic aldehydes, 90 °C, 4–6 h

2. Results and discussion

2.1. Chemistry

Malaria and tuberculosis severely influence the health and economies of the poorest countries, therefore low-cost of antimalarials and antitubercular drugs are equally important alongside efficacy and safety. Herein, we report an economical methodology for the synthesis of compounds (**8**–**31**) which is depicted in Scheme 1. Commercially available 4, 7-dichloroquinoline was condensed with various diamines to afford the 4-aminoquinoline derivatives (**3**–**5**). Further 4-aminoquinoline, carbon disulphide (**6**) and ethyl-bromoacetate (**7**) provided cyclized intermediate 3-((7-chloroquinolin-4-ylamino) alkyl)-2-thioxothiazolidin-4-one (**8**–**10**) *via* addition–elimination reaction in one operation at room temperature. (*Z*)-5-arylidene-3-((7-chloroquinolin-4-ylamino) alkyl)-2-thioxothiazolidin-4-one (**11**–**31**) were obtained by Knoevenagel condensation with appropriate aldehydes [47,48]. All the compounds were well characterized by Mass, IR, NMR and elemental analysis.

2.2. Biological assay

All the 4-aminoquinoline–rhodanine hybrids (**8**–**31**) were evaluated for their *in vitro* antimalarial efficacy against CQ-resistant (K1) and CQ-sensitive (3D7) strains of *P. falciparum*, using malaria SYBER Green I nucleic acid staining dye based fluorescence (MSF) assay, and also screened against $H_{37}Rv$ strain of *M. tuberculosis*, which carried out with a recommended protocol. The antimalarial activity, β-hematin inhibition assay and antitubercular activity results are presented in Tables 1–3. Among the 24 compounds, compound **23** was found to be the most active against the chloroquine-resistant (K1) strain with IC_{50} value of 13.2 nM, which is almost 20-fold more potent than chloroquine (IC_{50} = 254.05 nM). Eight compounds of this series (**9**, **10**, **16**, **19**–**22** and **31**) exhibited IC_{50} values ranging from 42.2 to 59.3 nM, showed more potency than chloroquine-resistant (K1) strain and eleven compounds (**8**, **11**, **12**, **14**, **15**, **17** and **24**–**28**) found to be comparable (IC_{50} ranging from 66.2 to 153.3 nM) with the standard. Structure activity relationship (SAR) may be attributed to the factors like length

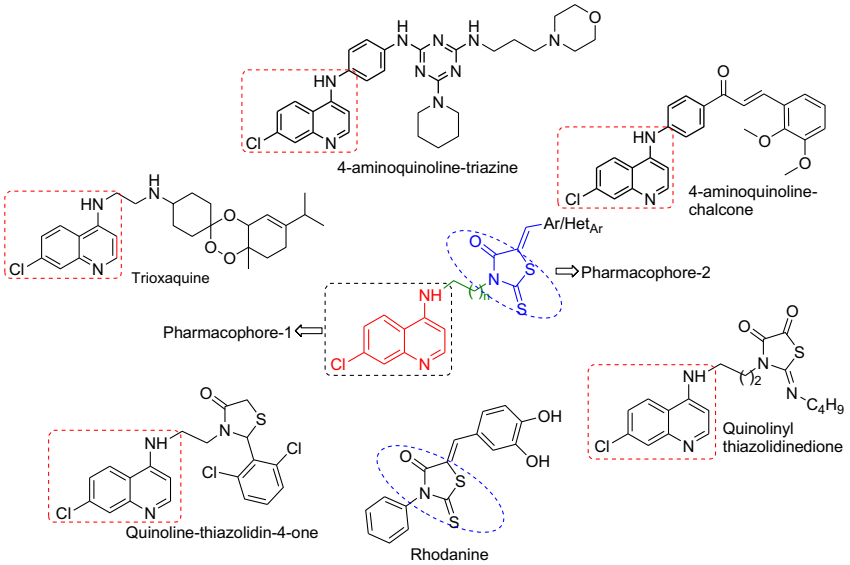
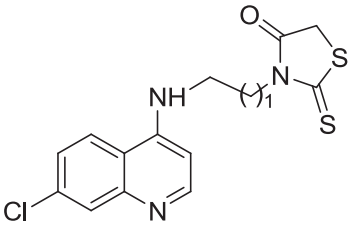
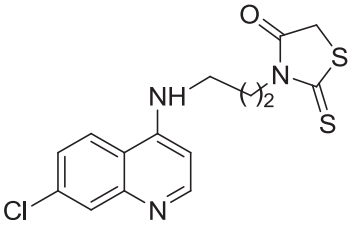
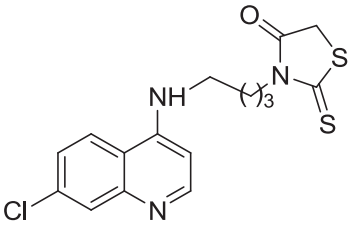
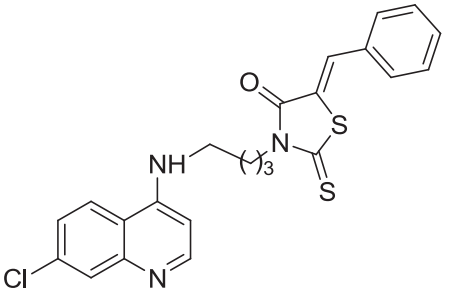
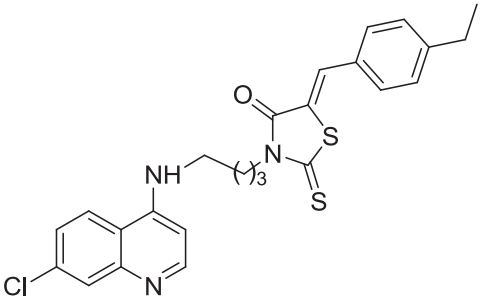
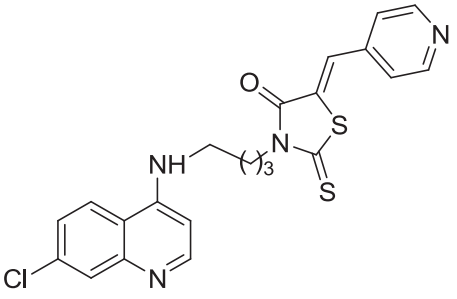


Fig. 1. Designing of 4-aminoquinoline–rhodanine hybrid based on 4-aminoquinoline and rhodanine scaffolds showing antimalarial and antitubercular activity.

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

Compd.	Structure	[a]IC_{50} (nM) 3D7 K1		[b]SI	[c]Log *P*
8		181.0	80.1	112.54	2.82
9		200.1	45.1	88.30	3.09
10		241.6	58.2	75.44	3.37
11		43.5	153	113.53	4.80
12		54.5	143.9	102.86	5.72
13		93.5	308.4	60.39	3.51

(*continued on next page*)

Table 1 (*continued*)

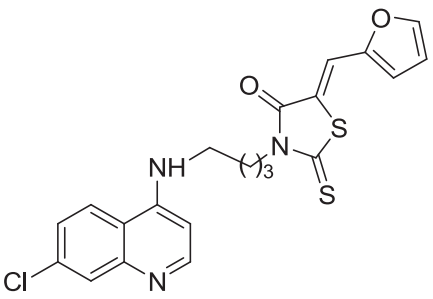
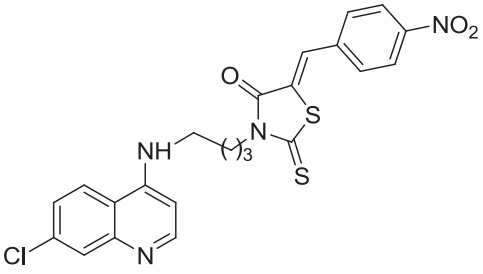
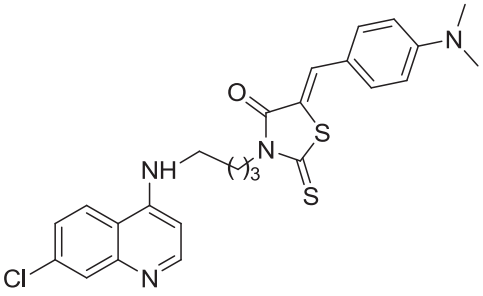
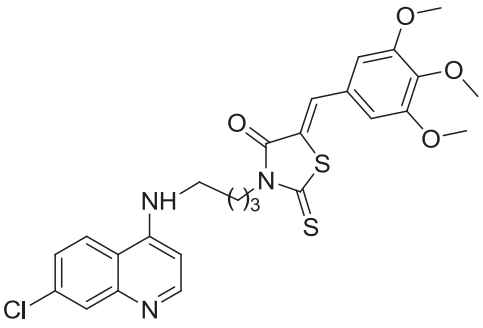
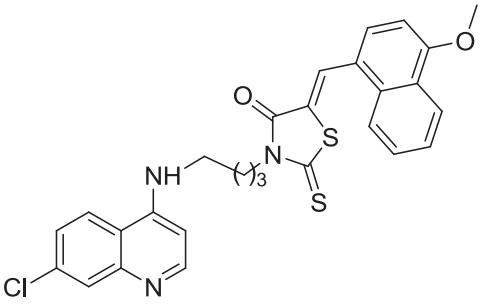
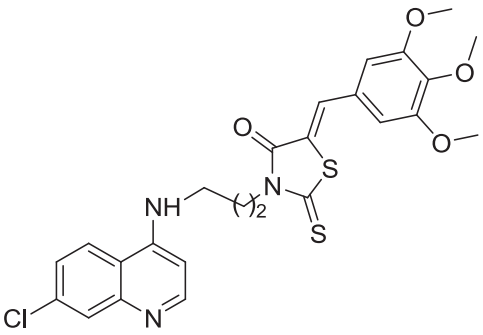
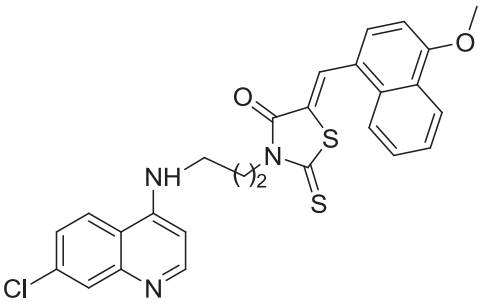
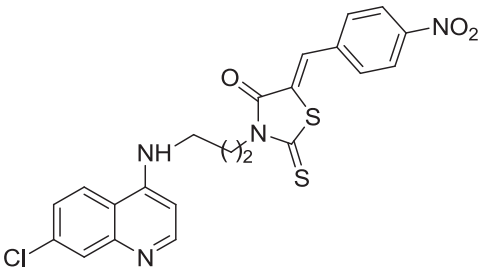
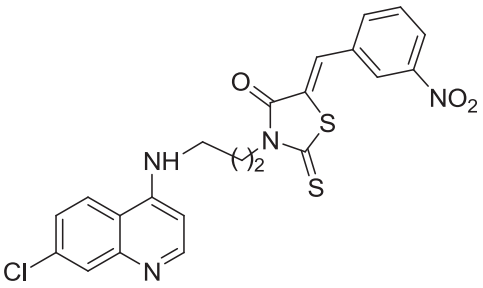
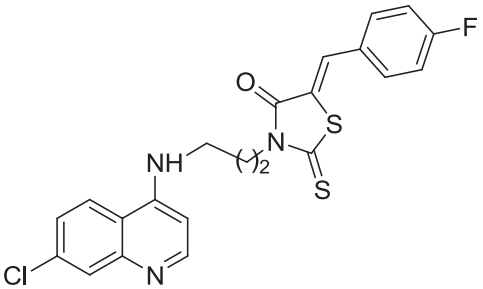
Compd.	Structure	[a]IC_{50} (nM) 3D7 K1		[b]SI	[c]Log *P*
14		35.0	151.4	92.72	4.06
15		40.0	88.8	3720.88	4.76
16		31.0	54.6	422.41	4.61
17		43.1	70.1	110.08	4.43
18		279.9	284.2	356.27	5.97

Table 1 (*continued*)

Compd.	Structure	[a]IC_{50} (nM) 3D7 K1		[b]SI	[c]Log *P*
19		56.0	42.2	193.92	4.16
20		90.7	59.3	64.55	5.70
21		23.5	45.5	180.38	4.49
22		96.0	50.4	106.40	4.47
23		93.9	13.2	2083.58	4.70

(*continued on next page*)

Table 1 (*continued*)

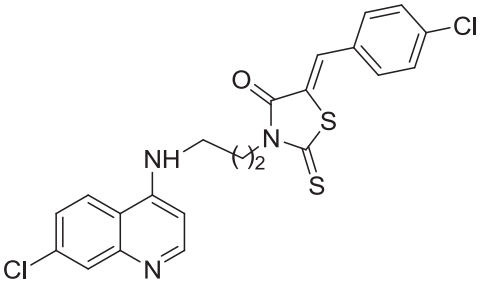
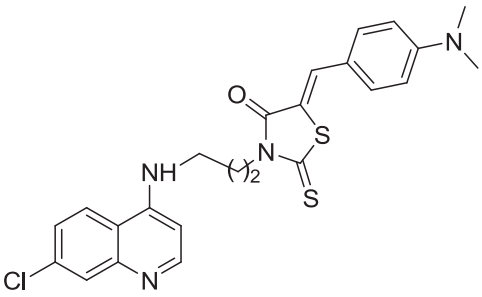
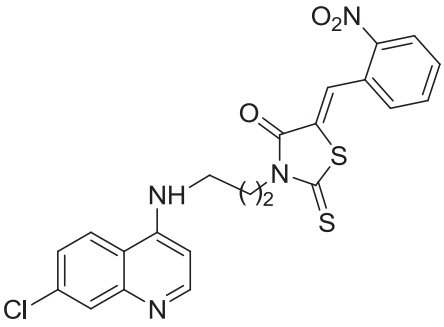
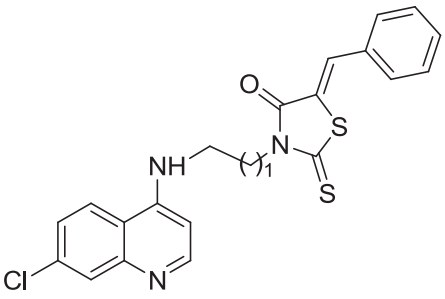
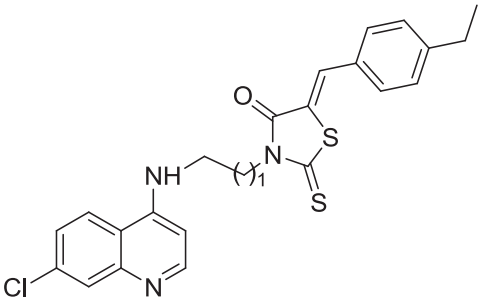
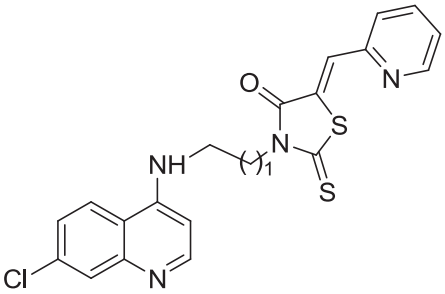
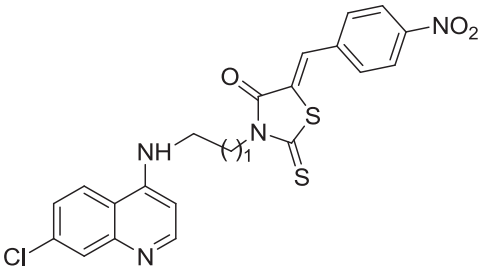
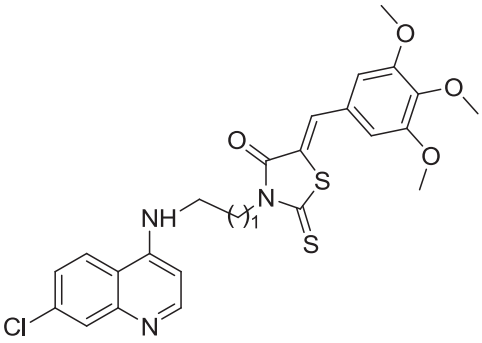
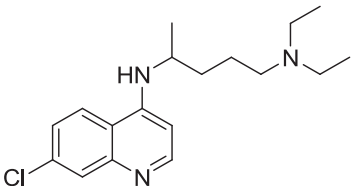
Compd.	Structure	[a]IC_{50} (nM) 3D7 K1		[b]SI	[c]Log *P*
24		76.5	94.2	442.83	5.20
25		49.3	66.2	4195.05	3.51
26		38.2	83.8	639.19	4.06
27		58.7	104.1	29.16	4.76
28		42.7	95.7	17.51	4.61

Table 1 (*continued*)

Compd.	Structure	[a]IC_{50} (nM) 3D7 K1		[b]SI	[c]Log *P*
29		>1174	>1104	ND	3.09
30		869.9	840.3	56.19	4.22
31		34.8	59.3	88.79	3.89
CQ		5.40	254.05	8983.00	5.00

[a] IC_{50} (nM): concentration corresponding to 50% growth inhibition of the parasite.
[b] Selectivity index (SI): (IC_{50} values of cytotoxic activity/IC_{50} values of antimalarial activity).
[c] Log *P* values calculated using on line software www.molinspiration.com.

Table 2
β-hematin inhibitory activity of molecules.[a]

Compound no.	IC_{50} (μg/mL)	Compound no.	IC_{50} (μg/mL)
8	4.90 ± 0.31[b]	**21**	3.23 ± 0.12
9	8.16 ± 0.12	**22**	3.93 ± 0.12
10	6.21 ± 0.12	**23**	4.30 ± 0.10
11	4.03 ± 0.07	**24**	3.26 ± 0.17
12	6.21 ± 0.12	**25**	6.35 ± 0.13
13	6.30 ± 0.10	**26**	3.30 ± 0.13
14	2.78 ± 0.16	**27**	8.21 ± 0.42
15	17.33 ± 1.25	**28**	6.73 ± 0.22
16	4.23 ± 0.12	**29**	16.48 ± 0.97
17	3.93 ± 0.17	**30**	12.75 ± 1.39
18	4.05 ± 0.13	**31**	7.31 ± 0.68
19	3.33 ± 0.15	**CQ**	4.30 ± 0.18
20	2.65 ± 0.13	–	–

[a] The IC_{50} represents the concentration of compound that inhibit β-hematin formation by50%.
[b] Standard deviation.

of the linker and the substituents on the arylidene moiety concurrent with rhodanine. The more potent activity against the chloroquine-resistant (K1) strain underscores the importance of three carbon linker between the quinoline and rhodanine scaffold.

Table 3
In vitro antitubercular activity of active compounds against *M. tuberculosis* H_{37}Rv.

Compounds	MIC (μM)	Cytotoxicity [IC_{50}]	
		VERO	MBMDMϕ[a]
10	12.5	ND	ND
14	6.25	>100	70.71
17	12.5	ND	ND
18	6.25	>100	>100
26	6.25	>100	70.71
Isoniazid	0.217	>100	>100
Rifampicin	0.243	>100	>100

[a] Mouse bone marrow derived macrophages.

Compound **23** was the most active of the series having *p*-F substituent at the phenyl ring while incorporation at *p*-NO_2 (**21**), *p*-$N(CH_3)_2$ (**25**) and *p*-Cl (**24**) reduced the antimalarial activity IC_{50} = 45.5 nM, IC_{50} = 66.2 nM and IC_{50} = 94.2 nM. On the other hand compound **21** was found to be the best active against chloroquine sensitive (3D7) strain, with IC_{50} value 23.5 nM. Compound **15**, which contained electron withdrawing substituent (*p*-nitro) showed moderate antimalarial activity (IC_{50} = 40.0 nM) and with propyl linker (**21**) led to increase in antimalarial potency (IC_{50} = 23.5 nM), however, ethyl linker (compound **30**) caused a major loss of activity (IC_{50} = 869.9 nM). Our SAR studies indicated that the electron withdrawing substituent plays a crucial role in the activity. In case of trimethoxy substituted derivatives **17**, **19** and **31** with butyl linker demonstrated IC_{50} values 43.1, 56.0 and 59.3 nM respectively. Compound **16** with butyl linker and *N,N*-dimethyl showed IC_{50} value 31.0 nM and with propyl linker as in compound **25** decrease in antimalarial potency (IC_{50} = 49.3 nM). Furthermore, all the compounds were also tested for their cytotoxicity toward VERO cell line. Among all derivatives, compounds **15**, **23** and **25** exhibited very high selectivity index (SI); 3720.88, 2083.58, 4195.05 respectively without any toxicity.

To understand the interaction with free heme is envisaged to the mode of action of these antimalarials, the β-hematin inhibitory (BHIA) assay of all the molecules were carried out. Interestingly, twelve compounds **11**, **14**, **16**–**24** and **26** showed better activity with IC_{50} values in the range of 2.65 ± 0.13–4.30 ± 0.10 μg/mL than CQ (4.30 ± 0.18 μg/mL). This study suggests that an interaction with free heme is necessary for antimalarial activity, although previously it has been observed that activities measured in the BHIA assay do not always match the parasite growth assay [49].

Furthermore the antitubercular activity of the synthesized analogs against *M. tuberculosis* $H_{37}Rv$ were also performed and among the tested analogs, butyl linker carrying compound **14** with furyl in place of arylidene and compound **18** with 4-methoxy arylidene substituent exhibited MIC values 6.25 μM. However, propyl linker did not affect the antitubercular activity of compound **26** with *o*-nitro substituent (MIC = 6.25 μM). The compounds containing ethyl linker did not exhibit activity against *M. tuberculosis*.

3. Conclusion

In conclusion, the synthesis of new 4-aminoquinoline–rhodanine derivatives successfully designed through molecular hybridization approach, exhibited potent *in-vitro* antimalarial and antitubercular activities with low toxicity. The most potent compounds in this series, **23** exhibited nanomolar activity against resistant strain of *P. falciparum* with the high selectivity (IC_{50} = 13.2 nM, SI = 2083.58). Compounds **14**, **18** and **26** displayed good antitubercular activity against $H_{37}Rv$ strain of *Mycobacterium tuberculosis* (MIC = 6.25 μM). In addition, twelve compounds were found to be most efficient *in vitro* β-hematin inhibition in comparison to CQ. Plausible SAR could be derived from the substitution pattern of the arylidene moiety and chain length of the linker. Therefore fluoro, trimethoxy and nitro were all found to be suitable substituents in order to obtain good antimalarial and anti-TB properties. We believe that the observed results could be useful in guiding future global efforts to discover new compounds of 4-aminoquinoline which might be serve as a valuable prototype with improved potency.

4. Experimental methods

4.1. General information

Commercially available reagents and solvents were used without further purification. Thin-layer chromatography (TLC) was carried out with silica gel plates (silica gel 60 F254), that were visualized by exposure to ultraviolet light. IR spectra were recorded on an FTIR spectrophotometer Shimadzu 8201 PC and are reported in terms of frequency of absorption (cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Supercon Magnet Avance DRX-300 or DPX 200 FT spectrometers using TMS as an internal reference and the samples were dissolved in suitable deuterated solvents (Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (J) in Hz). Electro Spray Ionisation Mass spectra (ESI-MS) were recorded by micromass quattro II instrument. HR-DART MS were recorded on JEOL, JMS T100LC Accu TOF. Purity of all tested compounds was ascertained on the basis of their elemental analysis and was carried out on Carlo-Erba-1108 instrument. Column chromatography purifications were performed in flash using 60–120 or 100–200 mesh silica gel. The melting points were recorded on an electrically heated melting point apparatus and are uncorrected.

Representative procedure for Synthesis of intermediate compound:

4.2. Synthesis of 3-(2-(7-chloroquinolin-4-ylamino) ethyl)-2-thioxothiazolidin-4-one (**8**)

A mixture of N^1-(7-chloroquinolin-4-yl) ethane-1, 2-diamine (1.20 g, 5.43 mmol), carbon disulphide (0.33 mL, 5.47 mmol) and ethylbromoacetate (0.30 mL, 2.69 mmol) were taken in acetonitrile (5 mL) and stirred magnetically at room temperature till the reaction mixture gets solidified (observed on TLC). The acetonitrile was evaporated under reduced pressure and crude product purified through column chromatography (60–120 mesh) using methanol-chloroform as eluent to afford the desired products. Brown solid in 78% Yield, mp 155–157 °C; IR (KBr): ν 3431, 3020, 1723, 1216, 766 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ: 8.50 (s, 1H), 7.99 (s, 1H), 7.94 (s, 1H), 7.85 (d, 1H, J = 9.2 Hz), 6.65 (d, 1H, J = 6.7 Hz), 4.60 (s, 2H), 4.12 (s, 2H), 3.83 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$ + TFA): δ: 202.4, 175.9, 155.9, 142.9, 141.2, 138.1, 129.1, 123.0, 119.9, 116.7, 115.0, 112.9, 98.2, 42.7, 35.5, 29.6; ESI-MS (*m/z* %): 338.0 (M^++H); HRMS: calc.: 338.0183 (MH^+); Found: 338.0184 (MH^+); Calcd. for $C_{14}H_{12}ClN_3OS_2$: C, 49.77; H, 3.58; N, 12.44. Found: C, 49.73; H, 3.52; N, 12.42.

4.3. Synthesis of 3-(3-(7-chloroquinolin-4-ylamino) propyl)-2-thioxothiazolidin-4-one (**9**)

Compound **9** was prepared by the reaction of N^1-(7-chloroquinolin-4-yl) propane-1, 2-diamine (1.20 g, 5.10 mmol), carbon disulfide (0.30 mL, 4.97 mmol) and ethylbromoacetate (0.28 mL, 2.51 mmol), Brown solid in 76% Yield, mp 148–150 °C; IR (KBr): ν 3440, 3020, 1729, 1215, 761 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ: 8.43 (d, 1H, J = 6.0 Hz), 8.06 (d, 1H, J = 9.0 Hz), 7.94 (s, 1H), 7.68 (d, 1H, J = 8.9 Hz), 6.68 (d, 1H, J = 7.0 Hz), 4.21 (t, 2H, J = 5.9 Hz), 4.13 (s, 2H), 3.58 (d, 2H, J = 8.9 Hz), 2.19 (t, 2H, J = 5.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$ + TFA): δ: 194.0, 175.0, 155.7, 142.7, 140.7, 138.4, 128.5, 128.0, 123.3, 120.0, 117.3, 115.2, 113.5, 98.0, 41.4, 40.7, 35.5, 25.3; ESI-MS (*m/z* %): 352.0 (M^++H); HRMS: calc.: 352.0340 (MH^+); Found: 352.0337 (MH^+); Calcd. for $C_{15}H_{14}ClN_3OS_2$: C, 51.20; H, 4.01; N, 11.94. Found: C, 51.18; H, 3.96; N, 11.90.

4.4. Synthesis of 3-(4-(7-chloroquinolin-4-ylamino) butyl)-2-thioxothiazolidin-4-one (**10**)

Compound **10** was prepared by the reaction of N^1-(7-chloroquinolin-4-yl) butane-1, 2-diamine (1.20 g, 4.81 mmol), carbon disulfide (0.29 mL, 4.80 mmol) and ethylbromoacetate (0.27 mL, 2.42 mmol), Brown solid in 80% Yield, mp 158–160 °C; IR (KBr): ν 3440, 3020, 1729, 1215, 761 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ: 8.30

(s, 1H), 8.07 (d, 1H, J = 8.9 Hz), 7.84 (s, 1H), 7.52 (d, 1H, J = 8.7 Hz), 6.62 (s, 1H, J = 6.7 Hz), 4.06–4.03 (m, 4H), 3.61 (s, 2H), 1.84 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$ + TFA): δ: 201.4, 175.1, 155.8, 142.3, 140.7, 138.2, 128.4, 123.5, 120.8, 119.7, 117.0, 113.2, 98.2, 43.6, 35.4, 29.7, 24.8, 24.0; ESI-MS (m/z %): 366.0 (M^++H); HRMS: calc.: 366.0496 (MH^+); Found: 366.0489 (MH^+); Calcd. for $C_{16}H_{16}ClN_3OS_2$: C, 52.52; H, 4.41; N, 11.48. Found: C, 52.49; H, 4.40; N, 11.42.

4.5. Representative procedure for synthesis of compound (**11**)

A mixture of 3-(4-(7-chloroquinolin-4-ylamino) butyl)-2-thioxothiazolidin-4-one (0.50 g, 1.37 mmol), benzaldehyde (0.14 mL, 1.39 mmol) and ammonium acetate (0.16 g, 2.05 mmol) were taken in acetic acid and stirred with magnetic stirrer. The reaction mixture was refluxed for an appropriate time and the progress of reaction was monitored with TLC. After completion of the reaction, acetic acid was removed under vacuum and residue was washed with water then purified through column chromatography.

The following compounds (**12**–**31**) were organized using a similar procedure which described for compound **11**, followed the corresponding compounds **8**, **9** and **10** with suitable aldehyde.

4.6. (Z)-5-Benzylidene-3-(4-(7-chloroquinolin-4-ylamino) butyl)-2-thioxothiazolidin-4-one (**11**)

Yellow solid in 74% Yield; mp 220–222 °C; IR (KBr): ν 3433, 3022, 1635, 1217, 770 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ: 8.55 (d, 2H, J = 7.0 Hz), 7.89 (s, 1H), 7.77 (d, 2H, J = 4.7 Hz), 7.62–7.53 (m, 5H), 6.93 (d, 1H, J = 7.2 Hz), 4.11 (s, 2H), 3.56 (s, 2H), 1.80–1.78 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6 + TFA): δ: 193.2, 170.0, 156.0, 142.3, 141.3, 138.0, 135.8, 132.6, 131.8, 129.5, 128.7, 123.80, 122.1, 120.1, 119.6, 112.5, 43.8, 30.6, 30.1, 27.5, 24.9, 18.6; ESI-MS (m/z %) : 454.0 (M^++H); HRMS: calc.: 454.0809 (MH^+); Found: 454.0809 (MH^+); Calcd. for $C_{23}H_{20}ClN_3OS_2$: C, 60.85; H, 4.44; N, 9.26. Found: C, 60.81; H, 4.39; N, 9.23.

4.7. (Z)-3-(4-(7-Chloroquinolin-4-ylamino) butyl)-5-(4-ethylbenzylidene)-2-thioxothiazolidin-4-one (**12**)

Yellow solid in 78% Yield, mp 170–172 °C; IR (KBr): ν 3430, 3024, 1628, 1210, 779 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ: 8.37 (d, 1H, J = 5.1 Hz), 8.24 (d, 1H, J = 9.0 Hz), 7.74 (s, 2H), 7.54 (d, 1H, J = 8.0 Hz), 7.43–7.37 (m, 2H), 6.86 (s, 1H), 6.61 (s, 1H), 6.46 (d, 1H, J = 5.3 Hz), 4.23 (t, 2H, J = 6.3 Hz), 2.69–2.64 (m, 2H), 1.70–1.60 (m, 6H), 1.21–1.16 (m, 3H); ^{13}C NMR (50 MHz, DMSO-d_6 + TFA): δ: 193.5, 167.0, 151.3, 147.6, 139.1, 130.8, 128.9, 128.0, 124.8, 121.0, 117.9, 112.2, 44.0, 34.3, 30.3, 28.1, 20.9, 18.7; ESI-MS (m/z %): 482.0 (M^++H); HRMS: calc.: 482.1122 (MH^+); Found: 482.1129 (MH^+); Calcd. for $C_{25}H_{24}ClN_3OS_2$: C, 62.29; H, 5.02; N, 8.72. Found: C, 62.25; H, 5.00; N, 8.71.

4.8. (Z)-3-(4-(7-Chloroquinolin-4-ylamino) butyl)-5-(pyridin-4-ylmethylene) thioxothiazolidin-4-one (**13**)

Yellow solid in 76% Yield, mp 235–237 °C; IR (KBr): ν 3434, 3022, 1630, 1214, 769 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ: 8.60 (d, 2H, J = 8.2 Hz), 8.37 (d, 1H, J = 5.3 Hz), 8.26 (d, 1H, J = 8.6 Hz), 7.80 (s, 1H), 7.53 (d, 2H, J = 8.0 Hz),7.32 (d, 2H, J = 5.8 Hz), 6.44 (d, 1H, J = 5.2 Hz), 4.18 (t, 2H, J = 6.1 Hz), 3.53 (s, 2H), 1.78–1.74 (m, 4H); ESI-MS (m/z %) : 455.0 (M^++H); HRMS: calc.: 455.0762 (MH^+); Found: 455.0776 (MH^+); Calcd. for $C_{22}H_{19}ClN_4OS_2$: C, 58.07; H, 4.21; N, 12.31. Found: C, 58.02; H, 4.18; N, 12.28.

4.9. (Z)-3-(4-(7-Chloroquinolin-4-ylamino) butyl)-5-(furan-2-ylmethylene)-2thioxothiazolidin-4-one (**14**)

Yellow solid in 82% Yield, mp 165–167 °C; IR (KBr): ν 3431, 3023, 1625, 1212, 768 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ: 8.39 (d, J = 5.3 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.76 (s, 1H), 7.62 (s, 1H), 7.45 (d, J = 8.9 Hz, 1H), 7.30 (s, 1H), 7.22 (s, 1H), 6.79 (s, 1H), 6.47(d, J = 5.4 Hz, 1H), 4.10–4.05 (m, 2H), 1.79–1.68 (m, 6H); ^{13}C NMR (50 MHz, DMSO-d_6): δ: 194.2, 166.7, 151.7, 150.0, 149.5, 148.9, 133.4, 127.3, 124.0, 120.4, 119.0, 118.8, 117.4, 114.0, 98.6, 43.9, 41.9, 25.0, 24.3. ESI-MS (m/z %) : 444.0 (M^++H); HRMS: calc.: 444.0602 (MH^+); Found: 444.0624 (MH^+); Calcd. for $C_{21}H_{18}ClN_3O_2S_2$: C, 56.81; H, 4.09; N, 9.46. Found: C, 56.78; H, 4.10; N, 9.43.

4.10. (Z)-3-(4-(7-chloroquinolin-4-ylamino) butyl)-5-(4-nitrobenzylidene)-2-thioxothiazolidin-4-one (**15**)

Yellow solid in 78% Yield, mp 234–236 °C; IR (KBr): ν 3433, 3004, 1630, 1217, 772 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ: 8.41 (d, 1H, J = 5.9 Hz), 8.37 (d, 2H, J = 8.3 Hz), 8.29 (d, 1H, J = 8.7 Hz), 7.90 (d, 2H, J = 5.6 Hz), 7.71 (d, 2H, J = 8.3 Hz), 7.50 (d, 1H, J = 9.0 Hz), 6.54 (d, 1H, J = 5.2 Hz), 4.24 (t, 2H, J = 6.2 Hz), 4.11–4.01 (m, 2H), 2.31–2.28 (m, 4H); ESI-MS (m/z %) : 499.0 (M^++H); HRMS: calc.: 499.0660 (MH^+); Found: 499.0679 (MH^+); Calcd. for $C_{23}H_{19}ClN_4O_3S_2$: C, 55.36; H, 3.84; N, 11.23. Found: C, 55.32; H, 3.85; N, 11.20.

4.11. (Z)-3-(4-(7-Chloroquinolin-4-ylamino) butyl)-5-(4-(dimethylamino) benzylidene)-2-thioxothiazolidin-4-one (**16**)

Brown solid in 86% Yield, mp 195–197 °C; IR (KBr): ν 3429, 3021, 1628, 1221, 770 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ: 8.53 (d, 1H, J = 5.3 Hz), 7.94 (s, 1H), 7.75 (d, 1H, J = 8.9 Hz), 7.67 (s, 1H), 7.41 (d, 3H, J = 8.8 Hz), 6.74 (d, 2H, J = 8.9 Hz), 6.43 (d, 1H, J = 5.3 Hz), 4.25–4.20 (m, 2H), 3.42 (s, 6H), 1.85–1.83 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 193.4, 167.8, 156.1, 152.6, 143.5, 135.2, 133.9, 127.5, 126.1, 121.5, 120.7, 119.7, 117.7, 112.9, 110.1, 99.2, 44.3, 43.5, 25.6, 24.8; ESI-MS (m/z %) : 497.0 (M^++H); HRMS: calc.: 497.1231 (MH^+); Found: 497.1289 (MH^+); Calcd. for $C_{25}H_{25}ClN_4OS_2$: C, 60.41; H, 5.07; N, 11.27. Found: C, 60.38; H, 5.05; N, 11.24.

4.12. (Z)-3-(4-(7-Chloroquinolin-4-ylamino) butyl)-2-thioxo-5-(3,4,5-trimethoxybenzylidene) thiazolidin-4-one (**17**)

Yellow solid in 76% Yield, mp 248–250 °C; IR (KBr): ν 3430, 3023, 1639, 1220, 770 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ: 8.39 (s, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.74 (s, 1H), 7.45 (d, J = 8.8 Hz, 1H), 7.30 (s, 1H), 6.92 (s, 2H), 6.48 (d, J = 5.3 Hz, 1H), 4.10 (s, 2H), 3.85 (s, 9H) 1.81–1.70 (m, 6H); ^{13}C NMR (50 MHz, DMSO-d_6): δ: 193.2, 166.9, 153.2, 151.7, 150.0, 148.9, 139.9, 133.4, 128.4, 127.3, 124.0, 121.0, 117.4, 108.1, 98.6, 60.2, 56.0, 44.0, 41.9, 24.9, 24.2; ESI-MS (m/z %): 544.0 (M^++H); HRMS: calc.: 544.1126 (MH^+); Found: 544.1154 (MH^+); Calcd. for $C_{26}H_{26}ClN_3O_4S_2$: C, 57.40; H, 4.82; N, 7.72. Found: C, 57.37; H, 4.80; N, 7.74.

4.13. (Z)-3-(4-(7-Chloroquinolin-4-ylamino) butyl)-5-((4-methoxynaphthalen-1-yl) methylene) 2-thioxothiazolidin-4-one (**18**)

Yellow solid in 74% Yield, mp 242–244 °C, IR (KBr): ν 3432, 3032, 1635, 1218, 773 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ: 8.39 (s, 2H), 8.27–8.23 (m, 2H), 8.19 (d, J = 8.5 Hz, 1H), 7.75 (s, 1H), 7.71–7.60 (m, 3H), 7.44 (d, J = 8.9 Hz, 1H), 7.32 (s, 1H), 7.20 (d, J = 8.3 Hz, 1H), 6.49 (d, J = 5.5 Hz, 1H), 4.12–4.10 (m, 2H), 4.06 (s, 3H), 1.82–1.71 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 194.3, 167.1, 158.1, 152.2, 150.6,

149.4, 133.9, 132.8, 129.8, 128.8, 127.8, 126.8, 125.4, 124.6, 123.5, 122.8, 117.9, 105.3, 99.1, 56.6, 44.5, 25.6, 24.8; ESI-MS (*m/z* %) : 534.0 (M^++H); HRMS: calc.: 534.1071 (MH^+); Found: 534.1094 (MH^+); Calcd. for $C_{28}H_{24}ClN_3O_2S_2$: C, 62.97; H, 4.53; N, 7.87. Found: C, 62.93; H, 4.50; N, 7.78.

*4.14. (Z)-3-(4-(7-Chloroquinolin-4-ylamino) propyl)-2-thioxo-5-(3,4,5-trimethoxybenzylidene) thiazolidin-4-one (**19**)*

Yellow solid in 82% Yield, mp 168–170 °C; IR (KBr): *ν* 3433, 3000, 1638, 1211, 768 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ*: 8.53 (d, 1H, *J* = 5.3 Hz), 7.93 (s, 1H), 7.82 (d, 1H, *J* = 8.9 Hz), 7.65 (s, 1H), 7.39–7.36 (m, 1H), 6.69 (s, 2H), 6.41 (d, 1H, *J* = 5.3 Hz), 5.71 (s, 1H), 4.34 (t, 2H, *J* = 6.2 Hz), 3.93 (s, 9H), 3.41–3.39 (m, 2H), 2.20–2.16 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 193.4, 168.2, 153.6, 151.7, 149.5, 148.8, 140.9, 135.0, 134.4, 128.4, 125.4, 121.3, 121.1, 117.2, 108.0, 98.7, 61.1, 56.2, 41.9, 39.6, 25.5; ESI-MS (*m/z* %) : 530.0 (M^++H); HRMS: calc.: 530.0970 (MH^+); Found: 530.0994 (MH^+); Calcd. for $C_{25}H_{24}ClN_3O_4S_2$: C, 56.65; H, 4.56; N, 7.93. Found: C, 56.61; H, 4.54; N, 7.90.

*4.15. (Z)-3-(3-(7-Chloroquinolin-4-ylamino) propyl)-5-((4-methoxynaphthalen-1-yl) methylene)-2-thioxothiazolidin-4-one (**20**)*

Yellow solid in 74% Yield, mp 235–237 °C; IR (KBr): *ν* 3442, 3022, 1638, 1217, 769 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ*: 8.54 (s, 1H), 8.39 (d, 1H, *J* = 7.8 Hz), 8.02 (s, 1H), 7.97 (d, 1H, *J* = 8.6 Hz), 7.63 (d, 2H, *J* = 8.0 Hz), 7.44 (d, 1H, *J* = 8.2 Hz), 7.32 (s, 1H), 7.09 (d, 1H, *J* = 5.0 Hz), 6.96 (d, 1H, *J* = 8.1 Hz), 6.66 (d, 1H, *J* = 8.0 Hz), 6.45 (s, 1H), 5.04 (s, 3H), 4.97–4.93 (m, 4H), 4.11 (s, 2H); ESI-MS (*m/z* %) : 520.0 (M^++H); HRMS: calc.: 520.0915 (MH^+); Found: 520.0959 (MH^+); Calcd. for $C_{27}H_{22}ClN_3O_2S_2$: C, 62.36; H, 4.26; N, 8.08. Found: C, 62.35; H, 4.22; N, 8.04.

*4.16. (Z)-3-(3-(7-Chloroquinolin-4-ylamino) propyl)-5-(4-nitrobenzylidene)-2 thioxothiazolidin-4-ones (**21**)*

Yellow solid in 70% Yield, mp 225–227 °C; IR (KBr): *ν* 3433, 3020, 1635, 1199, 781 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ*: 8.41 (d, 1H, *J* = 5.3 Hz), 8.35 (d, 2H, *J* = 8.6 Hz), 8.23 (d, 1H, *J* = 8.9 Hz), 7.85 (s, 1H), 7.82 (d, 2H, *J* = 5.1 Hz), 7.74 (s, 1H), 7.43 (d, 1H, *J* = 8.7 Hz), 6.48 (d, 1H, *J* = 5.3 Hz), 4.22–4.20 (m, 2H), 2.12–2.08 (m, 2H), 1.41–1.33 (m, 2H); ESI-MS (*m/z* %) : 485.0 (M^++H); HRMS: calc.: 485.0503 (MH^+); Found: 485.0542 (MH^+); Calcd. for $C_{22}H_{17}ClN_4O_3S_2$: C, 54.48; H, 3.53; N, 11.55. Found: C, 54.45; H, 3.50; N, 11.53.

*4.17. (Z)-3-(3-(7-Chloroquinolin-4-ylamino) propyl)-5-(3-nitrobenzylidene)-2 thioxothiazolidin-4-ones (**22**)*

Yellow solid in 76% Yield, mp 176–178 °C; IR (KBr): *ν* 3435, 3019, 1627, 1207, 768 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ*: 8.40 (d, 2H, *J* = 9.4 Hz), 8.32 (d, 1H, *J* = 7.8 Hz), 8.20 (d, 1H, *J* = 8.8 Hz), 7.98 (d, 1H, *J* = 7.7 Hz), 7.84–7.78 (m, 2H), 7.71 (s, 1H), 7.40 (d, 1H, *J* = 9.2 Hz), 6.46 (d, 1H, *J* = 5.4 Hz), 4.22–4.18 (m, 2H), 3.16–3.14 (m, 2H) 2.12–2.08 (m, 2H); ESI-MS (*m/z* %) : 485.0 (M^++H); Calcd. for $C_{22}H_{17}ClN_4O_3S_2$: C, 54.48; H, 3.53; N, 11.55. Found: C, 54.45; H, 3.51; N, 11.54.

*4.18. (Z)-3-(3-(7-Chloroquinolin-4-ylamino) propyl)-5-(4-fluorobenzylidene)-2-thioxothiazolidin-4-one (**23**)*

Yellow solid in 74% Yield, mp 224–226 °C; IR (KBr): *ν* 3395, 3021, 1635, 1216, 784 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ*: 8.43 (d, 2H, *J* = 5.3 Hz), 8.28 (d, 1H, *J* = 9.0 Hz), 7.79–7.77 (m, 2H), 7.64 (s, 2H), 7.44–7.42 (m, 1H), 7.33 (s, 1H), 6.51 (d, 1H, *J* = 5.3 Hz), 4.26 (t, 2H, *J* = 6.5 Hz), 2.33–2.16 (m, 4H); ESI-MS (*m/z* %) : 458.0 (M^++H); HRMS: calc.: 458.0558 (MH^+); Found: 485.0564 (MH^+); Calcd. for $C_{22}H_{17}ClFN_3OS_2$: C, 57.70; H, 3.74; N, 9.18. Found: C, 57.68; H, 3.75; N, 9.15.

*4.19. (Z)-5-(4-Chlorobenzylidene)-3-(3-(7-chloroquinolin-4-ylamino) propyl)-2-thioxothiazolidin-4-one (**24**)*

Yellow solid in 82% Yield, mp 230–232 °C; IR (KBr): *ν* 3432, 3016, 1627, 1218, 772 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ*: 8.40 (d, 2H, *J* = 5.4 Hz), 8.23 (d, 1H, *J* = 9.0 Hz), 7.75–7.73 (m, 2H), 7.62 (s, 2H), 7.43–7.41 (m, 1H), 7.31 (s, 1H), 6.48 (d, 1H, *J* = 5.3 Hz), 4.21 (t, 2H, *J* = 6.5 Hz), 2.30–2.06 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$ + TFA): *δ*: 193.0, 169.4, 155.7, 142.8, 141.2, 138.2, 134.0, 132.0, 131.7, 129.9, 128.9, 122.9, 120.0, 116.5, 108.9, 98.1, 41.3, 40.4, 30.8, 29.7; ESI-MS (*m/z* %) : 474.0 (M^++H); HRMS: calc.: 474.0263 (MH^+); Found: 474.0284 (MH^+); Calcd. for $C_{22}H_{17}Cl_2N_3OS_2$: C, 55.70; H, 3.61; N, 8.86. Found: C, 55.68; H, 3.60; N, 8.82.

*4.20. (Z)-3-(3-(7-chloroquinolin-4-ylamino) propyl)-5-(4-(dimethylamino) benzylidene)-2-thioxothiazolidin-4-one (**25**)*

Yellow solid in 74% Yield, mp 174–176 °C; IR (KBr): *ν* 3435, 2980, 1635, 1207, 769 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): *δ*: 8.72 (d, 2H, *J* = 5.3 Hz), 8.14 (d, 1H, *J* = 8.8 Hz), 7.92 (s, 1H), 7.64 (d, 2H, *J* = 8.6 Hz), 7.53 (s, 1H), 6.97 (d, 1H, *J* = 8.8 Hz), 6.89 (d, 1H, *J* = 8.1 Hz), 6.64 (d, 1H, *J* = 5.2 Hz), 4.55–4.51 (m, 2H), 3.62 (s, 6H), 2.28–2.22 (m, 4H); ESI-MS (*m/z* %) : 483.0 (M^++H); HRMS: calc.: 483.1075 (MH^+); Found: 483.1096 (MH^+); Calcd. for $C_{24}H_{23}ClN_4OS_2$: C, 59.67; H, 4.80; N, 11.60. Found: C, 59.64; H, 4.78; N, 11.58.

*4.21. (Z)-3-(3-(7-Chloroquinolin-4-ylamino) propyl)-5-(2-nitrobenzylidene)-2thioxothiazolidin-4-one (**26**)*

Yellow solid in 72% Yield, mp 166–168 °C; IR (KBr): *ν* 3506, 3021, 1661, 1217, 772 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ*: 8.42 (d, 2H, *J* = 9.2 Hz), 8.26 (d, 1H, *J* = 7.4 Hz), 8.21 (m, 2H), 8.02 (d, 1H, *J* = 7.1 Hz), 7.84 (d, 1H, *J* = 7.1 Hz), 7.73 (s, 1H), 7.42 (d, 1H, *J* = 9.2 Hz), 6.44 (d, 1H, *J* = 5.8 Hz), 3.66–3.54 (m, 2H), 2.12–2.08 (m, 4H); ESI-MS (*m/z* %) : 485.0 (M^++H); Calcd. for $C_{22}H_{17}ClN_4O_3S_2$: C, 54.48; H, 3.53; N, 11.55. Found: C, 54.45; H, 3.51; N, 11.54.

*4.22. (Z)-5-Benzylidene-3-(2-(7-chloroquinolin-4-ylamino) ethyl)-2-thioxothiazolidin-4-one (**27**)*

Yellow solid in 80% Yield, mp 208–210 °C; IR (KBr): *ν* 3434, 3041, 1635, 1207, 768 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ*: 8.53 (d, 2H, *J* = 7.2 Hz), 7.87 (s, 1H), 7.74 (d, 2H, *J* = 5.1 Hz), 7.63–7.52 (m, 5H), 6.83 (d, 1H, *J* = 7.4 Hz), 3.50 (m, 2H), 2.18 (m, 2H); ESI-MS (*m/z* %) : 426.0 (M^++H); HRMS: calc.: 426.0496 (MH^+); Found: 426.0496 (MH^+); Calcd. for $C_{21}H_{16}ClN_3OS_2$: C, 59.21; H, 3.79; N, 9.86. Found: C, 59.20; H, 3.75; N, 9.84.

*4.23. (Z)-3-(2-(7-Chloroquinolin-4-ylamino) ethyl)-5-(4-ethylbenzylidene)-2-thioxothiazolidin-4-one (**28**)*

Yellow solid in 72% Yield, mp 174–176 °C; IR (KBr): *ν* 3430, 3028, 1631, 1212, 780 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): *δ*: 8.45 (d, 1H, *J* = 5.3 Hz), 8.10 (d, 1H, *J* = 9.0 Hz), 7.79 (d, 2H, *J* = 5.3 Hz), 7.58 (d, 3H, *J* = 7.9 Hz), 7.42 (d, 2H, *J* = 8.3 Hz), 6.71 (d, 1H, *J* = 3.6 Hz), 4.30 (s, 2H), 3.69 (s, 2H), 2.71–2.63 (m, 3H), 1.23 (m, 2H); ESI-MS (*m/z* %) : 454.0 (M^++H); HRMS: calc.: 454.0809 (MH^+); Found: 454.0818 (MH^+); Calcd. for $C_{23}H_{20}ClN_3OS_2$: C, 60.85; H, 4.44; N, 9.26. Found: C, 60.81; H, 4.38; N, 9.22.

*4.24. (Z)-3-(2-(7-chloroquinolin-4-ylamino) ethyl)-5-(pyridin-2-ylmethylene)-2-thioxothiazol idin-4-one (**29**)*

Yellow solid in 76% Yield, mp 232–234 °C; IR (KBr): ν 3438, 3022, 2140, 1638, 1207, 770 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ: 10.1 (d, 1H, $J = 7.1$ Hz), 8.76 (s, 1H), 8.44 (d, 1H, $J = 9.1$ Hz), 8.22–8.13 (m, 2H), 7.78–7.73 (m, 2H), 7.50–7.46 (m, 1H), 7.33–7.29 (m, 2H), 1.35–1.22 (m, 4H); ESI-MS (m/z %) : 427.0 (M^++H); HRMS: calc.: 427.0449 (MH^+); Found: 427.0437 (MH^+); Calcd. for $C_{20}H_{15}ClN_4OS_2$: C, 56.26; H, 3.54; N, 13.12. Found: C, 56.25; H, 3.52; N, 13.10.

*4.25. (Z)-3-(2-(7-chloroquinolin-4-ylamino) ethyl)-5-(4-nitrobenzylidene)-2-thioxothiazolidin-4-one (**30**)*

Yellow solid in 86% Yield, mp 235–237 °C; IR (KBr): ν 3428, 3000, 1638, 1207, 768 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ: 8.44 (d, 2H, $J = 8.7$ Hz), 8.35 (d, 2H, $J = 8.7$ Hz), 7.97 (d, 2H, $J = 8.5$ Hz), 7.72–7.68 (m, 2H), 7.33 (d, 2H, $J = 8.5$ Hz), 4.26 (s, 2H, $J = 6.5$ Hz), 1.71–1.61 (m, 2H); ESI-MS (m/z %) : 471.0 (M^++H); Calcd. for $C_{21}H_{15}ClN_4O_3S_2$: C, 53.56; H, 3.21; N, 11.90. Found: C, 53.53; H, 3.18; N, 11.86.

*4.26. (Z)-3-(2-(7-chloroquinolin-4-ylamino) ethyl)-2-thioxo-5-(3,4,5-trimethoxybenzylidene) thiazolidin-4-one (**31**)*

Yellow solid in 78% Yield, mp 260–262 °C; IR (KBr): ν 3431, 3035, 1633, 1218, 778 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ: δ 8.72 (s, 1H), 8.61 (d, $J = 5.91$ Hz, 1H), 8.24 (d, $J = 9.18$ Hz, 1H), 7.74 (s, 1H), 7.70 (d, $J = 8.97$ Hz, 1H), 6.95 (s, 3H), 3.85 (s, 9H), 3.75 (m, 4H); ^{13}C NMR (50 MHz, DMSO-d_6) :δ: 191.2, 167.1, 153.5, 146.6, 140.0, 136.3, 133.2, 128.3, 126.0, 124.7, 122.4, 121.2, 116.4, 108.1, 98.3, 60.2, 56.0, 42.3; ESI-MS (m/z %) : 516.0 (M^++H); HRMS: calc.: 516.0813 (MH^+); Found: 516.0828 (MH^+); Calcd. for $C_{24}H_{22}ClN_3O_4S_2$: C, 55.86; H, 4.30; N, 8.14. Found: C, 55.78; H, 4.28; N, 8.11.

4.27. Bioevaluation methods

4.27.1. In vitro assay for evaluation of antimalarial activity

The compounds were evaluated for antimalarial activity against both 3D7 (CQ-sensitive) and K1 (CQ-resistant) strains of *P. falciparum* using Malaria SYBER Green I nucleic acid staining dye based fluorescence (MSF) assay as mentioned by Singh et al. (2011) [50]. The stock (5 mg/ml) solution was prepared in DMSO and test dilutions were prepared in culture medium (RPMI-1640-FBS). Chloroquine was used as reference drug. The compounds were tested in 96well plate (in duplicate wells). 1.0% parasitized cell suspension containing 0.8% parasitemia was used. The plates were incubated at 37 °C in CO_2 incubator in an atmosphere of 5% CO_2 and air mixture and 72 h later 100 μL of lysis buffer containing 2× concentration of SYBR Green-I (Invitrogen) was added to each well and incubated for 1 h at 37 °C. 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 (EXCEL) and IC_{50} values were obtained by Logit regression analysis using pre-programmed Excel spreadsheet.

4.27.2. 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 as mentioned by Sashidhara et al. (2012) [51]. The cells were incubated with compound dilutions for 72 h and MTT was used as reagent for the detection of cytotoxicity. 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}.

4.27.3. In vitro assay for evaluation of β-hematin inhibition

Inhibition of *in vitro* β-hematin formation was analyzed by using the method of Pandey et al. (1999) with some modifications [52]. Male swiss albino mice, weighing 15-20 g were inoculated with 1×10^5 *Plasmodium yoelii* infected RBCs. Blood of infected animal at 50% parasitemia 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 μL plasma, 100 μM hemin as the substrate and 1–20 μg 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 Tris–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 μL 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 (IC50) was determined using nonlinear regression analysis of dose response curves.

4.27.4. In vitro assay for evaluation of antitubercular activity

Evaluation of antitubercular activity against *M. tuberculosis* H37Rv was carried out with a recommended protocol [53] using Middlebrook (MB) 7H10 agar medium. A 100 μL of serial two fold dilutions of the stock (1.0 mg/mL in DMSO, Dimethyl Sulphoxide) of test compounds and standard antitubercular drug {isoniazid (INH)} were incorporated in the medium (final volume, 2 mL/tube) supplemented with OADC (oleic acid, albumin fraction IV, dextrose and catalase). Compounds/drug containing tubes were kept in slanting position till the medium solidified. Culture of *M. tuberculosis* H37Rv grown on Lowenstein-Jensen (L-J) was harvested in N-saline containing 0.05% Tween-80. The culture was agitated with glass beads to make a single cell suspension. A working inoculum (2×10^7 cfu/mL; 10μL/tube) of mycobacterium was spread on the surface of the medium and the tubes were kept at 37 °C for 4 weeks for the appearance of colonies. Tubes containing no drug served as control. The minimum concentration of the drug (INH)/compounds that completely inhibited the growth of mycobacterium was recorded as Minimum Inhibitory Concentration (MIC) with respect to the used inoculum.

4.27.5. In vitro assay for evaluation of cytotoxic activity

4.27.5.1. Evaluation against VERO cells. Cell line was procured from laboratory animal division of CDRI. The cell suspension was plated in 96-well tissue culture plates at a density of 20,000 cells per well (in 100 μL) in minimal essential medium (MEM) with antibiotics + 10% fetal bovine serum (FBS). The monolayers were then incubated overnight at 37 °C and 5% CO_2 for allowing adherence of cells. Compounds of different concentrations were added in MEM + 10% FBS. As a positive control, a known toxic compound was used. DMSO was used as negative control. After 24 h incubation, 20 μL of MTS solution (tetrazolium compound, Owen's reagent) was added to each well and incubated for 2 h at 37 °C, 5% CO_2. Reading was taken at 490 nm using a plate reader. Absorbance shown by DMSO containing wells is taken as 100% survivors [54]. A compound is considered toxic if it causes 50% inhibition at concentration 10 fold higher than its MIC.

4.27.5.2. Evaluation against mouse bone marrow derived macrophages (MBMDMQs). Mouse was euthenized by exposure to CO_2 and the femur bones were dissected out. The bones were trimmed

at each end, and the marrow was flushed out (using 26-gauge needle) with 5 mL of Dulbecco's minimal essential medium (DMEM) supplemented with 10% FBS, 15% L-929 fibroblast conditioned supernatant (prepared as described below), and non essential amino acids. Cells were washed twice and plated in 96-well tissue culture plates at a concentration of 10^5 cells per well (100 μL) in supplemented DMEM. The monolayer's were then incubated at 37 °C in 5% CO_2 with the medium change every 3rd day. Macrophages were used 5 days later. Different concentrations of compounds were added in antibiotic free, FBS supplemented, DMEM and incubated at 37 °C in 5% CO_2. After 48 h, 20 μL of MTS solution was added to each well and incubated for 2 h at 37 °C in 5% CO_2. Reading was taken at 490 nm using a plate reader. Absorbance shown by DMSO containing wells is taken as 100% survivors [55]. A compound is considered non-toxic if it causes 50% inhibition at concentration $\geq$ 10 fold higher than its MIC.

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