Med Chem Res
DOI 10.1007/s00044-016-1644-5

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimalarial activity evaluation and docking studies of some novel tetraoxaquines

Mukesh Kumar Kumawat[1,2] · Pratap Parida[3] · Dipak Chetia[1]

Received: 19 June 2015 / Accepted: 4 July 2016

Abstract Tetraoxaquines, molecular hybrids of 1,2,4,5-tetraoxane and 4-aminoquinoline, were designed via molecular docking analysis against Falcipain-2. Among the studied compounds, 11 top scoring analogues showing low binding energy were further selected for synthesis and evaluated for their in vitro antimalarial activity. In inhibitory assay, five compounds showed significant activity against chloroquine-resistant strain of *P. falciparum*-RKL-9 with IC_{50} values of 3.906, 3.942, 4.272, 3.906, 4.814 µg/ml.

Keywords Antimalarial activity · Tetraoxaquine · *P. falciparum* · Molecular docking studies · Falcipain-2

Introduction

Malaria is a global infectious disease responsible for major public health problem in tropical and subtropical regions of the world. It accounted for the death of nearly half million people against the 207 million cases of infection in 2013 (WHO, 2013). The African subcontinent has the highest burden of the disease, for instance, more than 75 % malaria-related deaths are reported in pregnant women and children under the age of five (www.rbm.who.int). *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi* are the causative organisms of malaria. These parasites are transmitted from an infected person to the healthy human by female mosquitoes. Among them, infection due to *P. falciparum* (*Pf*) accounts for majority of morbidity (i.e., >95 %) and mortality cases.

Malaria is a curable disease if treated in the earlier stage of infection. However, the resistant strains of malaria has seriously jeopardized the clinical efficacy of currently used drugs. This has made the urgent necessity for the discovery and development of novel drugs that would be effective against the resistant strains of malarial parasites (Kumawat et al., 2011; Casteel, 1997).

Quinoline-based drugs such as chloroquine and its derivatives are known to affect the parasite metabolism and interfering with its survival by suppressing the polymerization of toxic haem into an insoluble and non-toxic pigment, hemozoin. It results in lysis of cell. Compounds belonging to the family of 1,2,4,5-tetraoxane show excellent antimalarial activity via reaction with haem (or free Fe (II)) to generate cytotoxic radicals for its antimalarial activity (O'Neill et al., 2010). Therefore, it has been postulated that the development of hybrid conjugates comprising of quinoline and tetraoxane (Tetraoxaquines) into single scaffold could result into new class of antimalarials with considerable efficacy than the molecules used alone. More recently, Opsenica et al. (2008) synthesized such hybrid molecules which showed excellent in vitro and in vivo activity against both chloroquine-sensitive and chloroquine-resistant strains of *Pf*. This observation supports the rational for the design and synthesis of tetraoxaquines as a new class of antimalarial drugs.

The present study was undertaken to design and synthesize hybrid conjugates of 4-aminoquinoline and 1,2,4,5-tetraoxane (Tetraoxaquines) via covalent linker (Scheme 1).

✉ Mukesh Kumar Kumawat
phmukesh@gmail.com

[1] Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004 Assam, India

[2] Anand College of Pharmacy, Keetham, Agra 282006 Uttar Pradesh, India

[3] Regional Medical Research Centre, ICMR (NE Region), Post Bag No. 105, Dibrugarh, Assam, India

Published online: 21 July 2016

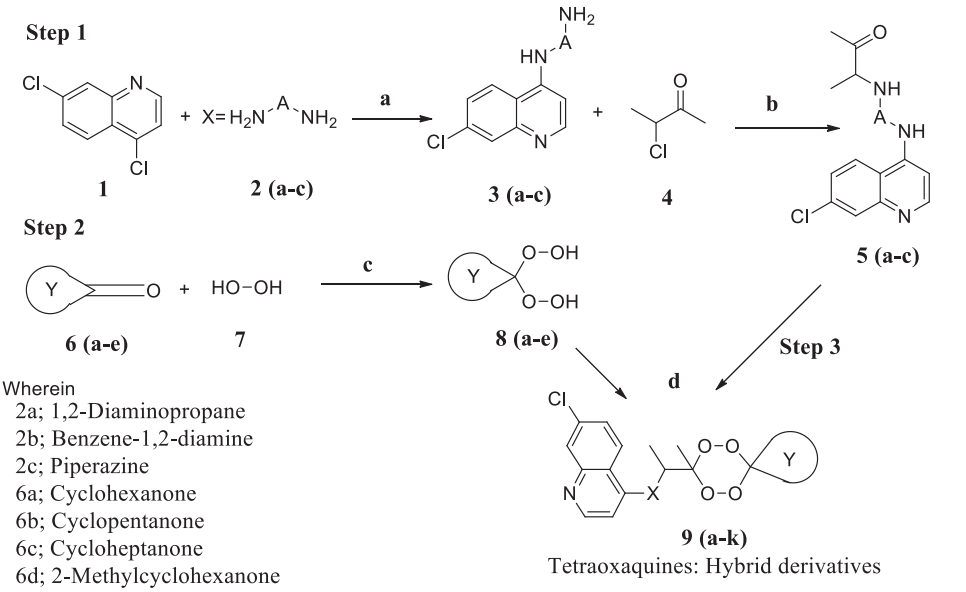
These compounds were subsequently tested against CQ-resistant strain (RKL-9) of *Pf*. Docking study was also performed with these inhibitors against falcipain-2.

Materials & Methods

Chemistry

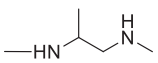
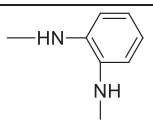
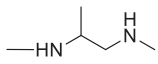
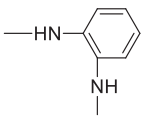
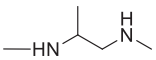
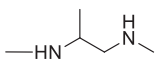
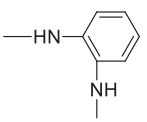
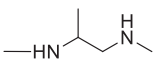
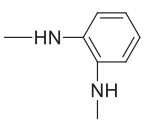
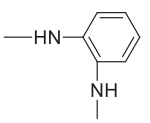
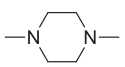
Structural investigation

All the Chemicals were procured either from Sigma-Aldrich Corporation, USA or Merck Specialties Pvt. Ltd., Mumbai and were used without further purification. The TLC plates coated with silica gel-G was utilized for monitoring the completion of the reaction using various solvent combinations. The spots were detected with iodine vapours and observed under UV-light. The melting point of the intermediates, as well as the target compounds were determined by open capillary method using Veego-MPI melting point apparatus and are uncorrected. The UV-Visible spectra of the synthesized compounds were recorded on *Shimadzu UV*-1800 UV-visible spectrophotometer. The Infrared spectra were recorded on FTIR *Perkin-Elmer* spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a *Bruker Avance-II* 400



Scheme 1 *Reagents and conditions*: **a** Reflux for 8–10 h; **b** Reflux for 8–10 h; **c** CH_2Cl_2/CH_3CN mixture (1:3 v/v), Conc^{n} HCl, Stirring for 2 h at rt; **d** CH_3CN, CH_2Cl_2, Conc^{n} H_2SO_4, stirring at 0–10 °C

Wherein
2a; 1,2-Diaminopropane
2b; Benzene-1,2-diamine
2c; Piperazine
6a; Cyclohexanone
6b; Cyclopentanone
6c; Cycloheptanone
6d; 2-Methylcyclohexanone
6e; Acetone

S. No.	Compound Code	X	Y	S. No.	Compound Code	X	Y
1	9a		R1= -CH_3 R_2 = -CH_3	7	9g		
2	9b			8	9h		
3	9c						
4	9d			9	9i		
5	9e			10	9j		
6	9f		R1= -CH_3 R_2 = -CH_3	11	9k		R1= -CH_3 R_2 = -CH_3

NMR spectrometer using either DMSO-d_6 or $CDCl_3$ as solvent with tetramethylsilane as an internal standard. Mass spectra were obtained on a Waters Q-TOF MICROMASS LC mass spectrometer. Elemental analysis (CHN & O) were carried out on Eager Xperience Elemental analyzer (Coates, 2000; Pasto et al., 1992; Mathieson, 1965; Silverstein and Webster, 1963).

General Procedure

Synthesis of 4-substituted-7-chloroquinolines (**3a**–**3b**) A mixture of 4,7-dichloroquinoline (**1**) (12.6 mmol) and diamine, different in each case, (**2a–2c**) (56.8 mmol) was heated slowly with stirring at elevated temperature (80 °C) for 1 h. The mixture was then heated at 120–130 °C for 6–8 h with stirring to drive the reaction to completion. The reaction mixture was cooled to room temperature and taken up in dichloromethane with subsequent washing with 1 M sodium hydroxide and brine solution to afford an aqueous layer, an organic layer and a particulate precipitate. The resulting precipitate was filtered-out and dried to obtain the corresponding product.

Synthesis of Intermediate 2-butanone derivatives (**5a–5c**) A mixture of 4-substituted-7-chloroquinolines (**3a**–**3c**) (12.6 mmol) and 3-chlorobutan-2-one (4) (56.8 mmol) in dry acetone was heated slowly with stirring and temperature was raised to 80 °C for 1 h, subsequently at 120–130 °C for 6–8 h. Then the reaction mixture was cooled to room temperature and taken up in dichloromethane followed by washing with 1 M sodium hydroxide and brine solution to afford an aqueous layer, an organic layer, and a particulate precipitate. The resulting precipitate was filtered-out and dried to obtain the corresponding product.

Synthesis of dihydroproxides (**8a–8e**) Cyclic or alicyclic ketone (**6a**–**6e**) (1 ml, 10 mmol) was taken in a mixture of CH_2Cl_2/CH_3CN (20 ml, 1:3 v/v) at room temperature followed by addition of 30 % H_2O_2 (10.4 ml, 0.1 mol) and concentrated HCl (3 ml). The resulting mixture was further stirred for 2 h at room temperature and then quenched with saturated $NaHCO_3$ and CH_2Cl_2. The organic layer thus obtained was separated out, and the water layer was filtered to collect the precipitate and dried to obtain the desired product

Synthesis of targeted tetraoxaquines (**9a**–**9k**) The 2-butanone derivative (**5a–5c**) (2.3 mmol) prepared was added to a cooled solution (ice bath) of dihydroperoxide (**8a–8e**) (2.3 mmol) in CH_2Cl_2. The resultant mixture was stirred for 30 min followed by the dropwise addition of 20 ml of cooled H_2SO_4/CH_3CN mixture (1:10 v/v). After an additional 50 min of stirring, the mixture was quenched with saturated $NaHCO_3$ and CH_2Cl_2. The organic layer was separated out and the precipitate in the water layer was collected by filtration to furnish the target compounds.

N-(1-aminopropan-2-yl)-7-chloroquinolin-4-amine (**3a**) Odourless yellow solid; soluble in ethanol, chloroform; melting range 105–107 °C; %yield 87; R_f value 0.75 (acetone: ethanol: 2:1); Spectroscopic analysis: λ_{max} (in $CHCl_3$) 255.06 nm; FTIR spectrum (ν_{max}, in cm^{-1}, Film) 3501.81, 3435.96 (sym. and asymm. N–H stretching, $–NH_2$), 3222.98 (N–H stretching, >NH), 3100.50–3061.27 (aromatic C–H stretching), 3016.10–2823.23 (C–H stretching, $>CH_2$ and $–CH_3$), 2024.98–1534.00 (N–H bending), 1517.60–1425.72 (C=C–C, aromatic ring stretching), 1396.47–1132.19 (C–N stretching), 1238.63–960.92 (aromatic C–H in-plane bend), 1084.98 (C–Cl stretching, Ar–Cl), 907.53–676.06 (aromatic C–H out-of-plane bend).

N^1-(7-chloroquinolin-4-yl)benzene-1,2-diamine (**3b**) Odourless blackish brown solid; soluble in dichloromethane; melting range 150–152 °C; %yield 89; R_f value 0.51 (Acetone: Pet. ether: 1:2); Spectroscopic analysis: λ_{max} (in $CHCl_3$) 240.51 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, Film) 3565.24, 3461.94 (sym. & asymm. N–H stretching, $–NH_2$), 3374.62 (N–H stretching, >NH), 3034.39 (aromatic C–H stretching), 2926.08 (aliphatic C–H stretching), 1915.54-1567.15 (N–H bending), 1531.00-1421.82 (C=C-C, aromatic ring stretching), 1368.59-1277.77 (C-N stretching), 1237.15–913.02 (aromatic C–H in-plane bend), 1080.16 (C–Cl stretching, Ar–Cl), 875.15–592.35 (aromatic C-H out-of-plane bend).

7-chloro-4-piperazine-1-ylquinoline (**3c**) A mixture of 4,7-dichloroquinoline (**1**) (0.505 mol), piperazine (**2c**) (1.5 mol) and potassium iodide (0.12 mol) in isopropyl alcohol (20 ml) was refluxed for 10 h, and cooled to room temperature. The precipitated solid was filtered, washed with isopropyl alcohol and evaporated in vacuo. The crude product, thus obtained was diluted further with dichloromethane (20 ml) and washed twice with water. The pH of dichloromethane layer was adjusted using conc. HCl. The separated water layer was made alkaline with 10 % NaOH solution and extracted again with dichloromethane. The organic layer was evaporated to dryness to get 7-chloro-4-piperazine-1-ylquinoline. Odourless off-white solid; soluble in acetone, dichloromethane; melting range 161–162 °C; %Yield 50; R_f value 0.76 (dichloromethane: ethanol: 1:1); Spectroscopic analysis: λ_{max} (in DMSO) 321.52 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3250.01 (N–H stretching, >NH piprazinyl), 3035.30 (aromatic C–H stretching, quinolyl), 2925.41–2830.10 (C–H stretching, $>CH_2$ piprazinyl), 1918.46–1496.85 (N–H bending, >NH piprazinyl), 1452.82–1419.76 (C=C–C, aromatic ring stretching, quinolyl), 1373.06–1281.11 (C–N stretching), 1250.50–922.34 (aromatic C–H in-plane bend), 1067.64 (C–Cl stretching,

quinolyl–Cl), 867.05–618.50 (aromatic C–H out-of-plane bend, quinolyl).

3-(2-(7-chloroquinolin-4-ylamino)propylamino)butan-2-one (**5a**) Reddish brown solid with characteristic odour; soluble in dichloromethane, chloroform; melting range 251–252 °C; %Yield 95.78; R_f value 0.72 (acetone: carbon tetrachloride: 1:2); Spectroscopic analysis: λ_{max} (in $CHCl_3$) 252.53 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3565.11, 3219.34 (N–H stretching, >NH), 3063.37 (aromatic C–H stretching), 2923.94–2856.30 (C–H stretching, $>CH_2$ and $–CH_3$), 1742.19–1645.48 (C=O stretching), 1918.13–1532.77 (N–H bending), 1518.54–1425.51 (C=C–C, aromatic ring stretching), 1368.29–1140.06 (C–N stretching), 1238.63–960.92 (aromatic C–H in-plane bend), 1085.31 (C–Cl stretching, Ar–Cl), 845.69–602.69 (aromatic C–H out-of-plane bend); ^{1}H NMR (400 MHz, $CHCl_3$) δ 1.01–1.03 (d, 3H, $J = 8$Hz, $–CH_3$), 1.05–1.07 (d, 3H, $J = 8$Hz, $–CH_3$), 2.50 (s, 1H, >NH), 3.58 (s, 3H, $–CH_3$), 2.02–2.31 (m, 1H, $–CH_2$), 3.87–3.89 (d, 1H, $J = 8$Hz, >C–H), 3.92–3.94 (d, 1H, $J = 8$Hz, >C–H), 4.44 (s, 1H, >NH), 6.09–6.10 (d, 1H, $J = 4$Hz, quinolinyl-H), 7.27–7.29 (d, 1H, $J = 8$Hz, quinolinyl-H), 7.56–7.58 (d, 1H, $J = 8$Hz, quinolinyl-H), 7.71–7.74 (d, 1H, $J = 12$Hz, quinolinyl-H), 7.89 (s, 1H, quinolinyl-H).

3-(2-(7-chloroquinolin-4-ylamino)phenylamino)butan-2-one (**5b**) Odourless blackish solid; soluble in dichloromethane; melting range112–113 °C; %Yield 72.69; R_f value 0.71 (Pet. ether: acetone: 1: 2); Spectroscopic analysis: λ_{max} (in $CHCl_3$) 240.51 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3565.76, 3451.26 (sym. and asymm. N–H stretching, $–NH_2$), 3373.43 (N–H stretching, >NH), 3105.25 (aromatic C–H stretching), 2927.29 (aliphatic C–H stretching), 1916.62–1566.15 (N–H bending), 1743.95–1706.32(>C=O stretching) 1531.70–1425.58 (C=C–C, aromatic ring stretching), 1368.53–1318.67 (C–N stretching), 1211.26–904.97 (aromatic C–H in-plane bend), 1088.47 (C–Cl stretching, Ar–Cl), 849.91–581.16 (aromatic C–H out-of-plane bend); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.11–2.33 (m, 1H, >C–H), 2.49–2.50 (d, 3H, $J = 4$Hz, $–CH_3$), 2.99 (s, 3H, $–CH_3$), 4.49 (bs, 2H, 2×>NH), 6.19–6.21(d, 1H, $J = 8$Hz, Ar–H), 6.61–6.65 (t, 1H, $J = 16$Hz, Ar–H), 6.83–6.85 (d, 1H, $J = 8$Hz, Ar–H), 7.02–7.07 (m, 1H, Ar–H), 7.40–7.46 (m, 1H, quinolinyl-H), 7.09–7.10 (d, 1H, $J = 4$Hz, quinolinyl-H), 8.02 (s, 1H, quinolinyl-H), 8.28–8.29 (d, 1H, $J = 4$Hz, quinolinyl-H), 8.53–8.55 (d, 1H, $J = 8$Hz, quinolinyl-H).

3-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)butan-2-one (**5c**) Odourless off-white solid; soluble in acetone, dichloromethane; melting range 140–142 °C; %Yield 51.18; R_f value 0.77 (Pet. ether: acetone: 1:1); Spectroscopic analysis: λ_{max} (in DMSO) 325.48 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3247.73-3061.40 (aromatic C–H stretching, quinolyl), 2960.36-2858.89 (C–H stretching, $>CH_2$, alkyl and piprazinyl), 1730.72 (>C=O stretching), 1608.58–1424.47 (C=C–C, aromatic ring stretching, quinolyl), 1367.37–908.15 (aromatic C–H in-plane bend, quinolyl), 1015.32 (C–Cl stretching, quinolyl–Cl), 873.44–619.00 (aromatic C–H out-of-plane bend, quinolyl).

1,1-dihydroperoxycyclohexane (**8a**) White solid characteristic odour; soluble in Pet. Ether; melting range 63 °C; %Yield 30.8; R_f value 0.61 (Ethanol: acetone: 1:1); Spectroscopic analysis: λ_{max} (in DMSO) 275 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3419.09–3387.32 (–OH stretching, dihydroperoxide), 2938.52–2855.97 (C–H stretching, cyclohexyl), 1551.89 (–OH bending, dihydroperoxide), 1447.21–1342.74 (C–H bending, cyclohexyl), 1272.68–1158.23 (C–C–O symmetrical stretching), 946.13–910.15 (C–C–O asymmetrical stretching), 868.21–823.88 (peroxide, C–O–O stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.40–1.42 (t, 2H, $J = 8$Hz, cyclohexyl–H), 1.46–1.48 (t, 2H, $J = 8$Hz, cyclohexyl–H), 1.49–1.75 (m, 2H, cyclohexyl–H), 1.79–1.85 (m, 2H, cyclohexyl–H), 2.28 (s, 2H, 2× –OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.07 (cyclohexane $–CH_3–$), 24.36 (cyclohexane–CH_2–), 25.04 (cyclohexane–CH_2–), 26.47 (cyclohexane–CH_2–), 29.38 (cyclohexane–CH_2–), 108.02 (cyclohexane–C–O–); Mass Spectrum (TOF MS, *m/z*) Calculated 148.07, Observed 142.2 (100 %), 137.1 (38.06 %), 173(52.92 %).

1,1-dihydroperoxycyclopentane (**8b**) Odourless off-white solid; soluble in dichloromethane; melting range 80–82 °C; %Yield 23; R_f value 0.71(chloroform: dichloromethane: 3:1); Spectroscopic analysis: λ_{max} (in $CHCl_3$) 243.04 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3417.72 (–OH stretching, dihydroperoxide), 2949.97–2873.68 (C–H stretching, cyclopentyl), 1454.31–1395.70 (C–H bending, cyclopentyl), 1161.99–1073.47 (C–C–O symmetrical stretching), 977.25–952.31 (C–C–O asymmetrical stretching), 827.85 (peroxide, C–O–O– stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.79–1.81 (t, 2H, $J = 8$Hz, cyclopentane–CH_2–), 1.83–1.86 (t, 2H, $J = 12$Hz, cyclopentane–CH_2–), 1.87–1.89 (t, 2H, $J = 8$Hz, cyclopentane–CH_2–), 1.90–1.98 (m, 2H, cyclopentane–CH_2–), 2.06 (s, 2H, 2× –OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.65 (cyclopentane–CH_2–), 24.11 (cyclopentane–CH_2–), 32.67 (cyclopentane–CH_2–), 37.68 (cyclopentane–CH_2–), 119.90 (cyclopentane–C–O–); Mass Spectrum (TOF MS, *m/z*) Calculated 134.06; Observed 341.1 (100 %), 101.1(63.35 %), 441.2 (56.04 %), 241.1(32.91 %).

1,1-dihydroperoxycycloheptane (**8c**) White solid with characteristic in odour; soluble in Chloroform; melting range 90–92 °C; %Yield 47.5; R_f value 0.65 (n-Butanol:

Benzene: 1:1); Spectroscopic analysis: λ_{max} (in $CHCl_3$) 249.05 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3797.13 (–OH stretching, dihydroperoxide), 2924.03–2851.74 (C–H stretching, cycloheptyl), 1454.56–1348.56 (C–H bending, cycloheptyl), 1168.70–1017.26 (C–C–O symmetrical stretching), 991.80–895.47 (C–C–O asymmetrical stretching), 791.90 (peroxide, C–O–O- stretching).

1,1-dihydroperoxy-2-methylcyclohexane (**8d**) Pale yellow liquid with characteristic odour; soluble in Dichloromethane, Chloroform; Boiling range 95–96 °C; %Yield 28.12; R_f value 0.72 (Pet ether: chloroform: 1:1); Spectroscopic analysis: λ_{max} (in $CHCl_3$) 242.00 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3565.12 (–OH stretching, dihydroperoxide), 2935.66–2865.04 (C–H stretching, methylcyclohexyl), 1540.24–1513.22 (–OH bending, dihydroperoxide), 1454.81–1376.00 (C–H bending, cyclohexyl), 1234.67–1089.90 (C–C–O symmetrical stretching), 990.33–935.39 (C–C–O asymmetrical stretching), 841.58 (peroxide, C–O–O– stretching).

2,2-dihydroperoxypropane (**8e**) White solid with a characteristic odour; soluble in ethanol, dichloromethane, DMSO; melting range 95 °C; %Yield 70; R_f value 0.64 (Pet. ether: ether: 4:1); Spectroscopic analysis: λ_{max} (in ethanol) 268.40 nm; FTIR Spectrum (film, in cm^{-1}) 3374.77 (–OH stretching, dihydroperoxide), 3006.71–2946.00 (C–H stretching, gem dimethyl group), 1633.47–1549.83 (–OH bending, dihydroperoxide), 1456.45–1360.31 (C–H bending, gem dimethyl group), 1273.60–1174.54 (C–C–O symmetrical stretching), 996.75–841.08 (C–C–O asymmetrical stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38(s, 6H, 2× $–CH_3$), 1.54(s, 1H, –OH), 1.69(s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.96 ($–CH_3$), 21.06($–CH_3$), 106.68(aliphatic C–C); Mass Spectrum (TOF MS, *m/z*) Calculated 108.04; Observed 173 (100 %), 247.1(87.43 %), 99(48.37 %), 141(33.53 %).

N2-(7-chloroquinolin-4-yl)-N1-(1-(3,6,6-trimethyl-1,2,4,5-tetraoxan-3-yl)ethyl)propane-1,2-diamine (**9a**) Odourless pink solid; soluble in dichloromethane, DMSO; melting range 258–260 °C; %Yield 22; R_f value 0.90 (Pet. ether: methanol: 1: 1); Spectroscopic analysis: λ_{max} (in DMSO) 339.56 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3247.25 (N–H stretching, >NH), 3111.60 (aromatic C–H stretching), 2922.43–2853.03 (aliphatic C–H stretching), 1730.33–1554.45 (N–H bending), 1453.33–1371.06 (C=C–C, aromatic ring stretching), 1213.62 (C–N stretching), 1087.09 (aromatic C–H in-plane bend), 1049.08 (C–Cl stretching, Ar–Cl), 896.76 (C–C-O stretching), 868.95 (aromatic C–H out-of-plane bend), 813.19–766.68 (peroxide, C–O–O stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83–0.84 (d, 6H, J = 4Hz, 2× CH_3), 1.22 (d, 6H, J = 4Hz, 2× CH_3), 1.49 (bs, 3H, $–CH_3$), 1.54–2.01 (m, 2H, >C–H), 2.05–2.38 (m, 1H, >C–H), 2.50 (s, 1H, >N–H), 3.01–3.18 (m, 1H, >C–H), 3.62 (bs, 1H, >N–H), 7.56–7.67 (d, 1H, J = 8Hz, quinolinyl-H), 7.90 (s, 1H, quinolinyl-H), 8.19–8.20 (d, 1H, J = 4Hz, quinolinyl-H), 8.36–8.38 (d, 1H, J = 8Hz, quinolinyl-H), 8.46–8.50 (d, 1H, J = 16Hz, quinolinyl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.48 (1×C, $–CH_3$), 22.07 (1×C, $–CH_3$), 24.85 (1×C, $–CH_3$), 28.68 (2×C, $–CH_3$) 28.98 (1×C, >C–H), 31.27 (1×C, $>CH_2$), 38.93 (1×C, >C–H), 39.14, 39.34, 39.55, 39.76 (2×C, tetraoxane), 39.97, 40.18, 78.45, 78.78, 79.11 (9×C, quinolinyl); Mass Spectrum (TOF MS, *m/z*) Calculated 395.88, Observed 342.2 (100 %), 378.3 (34.83 %), 330.2 (29.70 %), 396.89 (11.34 %) $[M+H]^+$; Elemental Analysis for $C_{22}H_{30}ClN_3O_4$; Calculated C, 57.64; H, 6.62; N 10.61; O, 16.17 Observed C, 57.373; H, 6.329; N, 10.933; O, 16.490.

N2-(7-chloroquinolin-4-yl)-N1-(1-(3-methyl-1,2,4,5-tetraoxaspiro[5.5] undecan-3-yl)ethyl)propane-1,2-diamine (**9b**) Blackish brown solid with characteristic odour; soluble in dichloromethane, DMSO; melting range 245–247 °C; % Yield 11.12; R_f value 0.73 (Pet. ether: acetone: 3:1); Spectroscopic analysis: λ_{max} (in DMSO) 341.77 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3254.20 (N–H stretching, >NH), 3109.22 (aromatic C–H stretching), 2924.53–2853.14 (aliphatic C–H stretching), 1710.60–1577.31 (N–H bending), 1451.97–1335.61 (C=C–C, aromatic ring stretching), 1238.85–1212.91 (C–N stretching), 1082.01 (aromatic C–H in-plane bend), 1048.66 (C–Cl stretching, Ar–Cl), 897.70 (C–C–O stretching), 871.10 (aromatic C–H out-of-plane bend), 809.33–764.82 (peroxide, C–O–O stretching); ^{1}HNMR (400 MHz, DMSO-d_6) δ 1.23–1.29 (d, 6H, J = 24Hz, 2× CH_3), 1.38–1.42 (t, 6H, J = 16Hz, 3×$>CH_2$, cyclohexyl), 1.51 (bs, 3H, $–CH_3$), 1.70–1.76 (t, 4H, J = 24Hz, 2×$>CH_2$, cyclohexyl), 2.20–2.28 (d, 2H, J = 32Hz, >C–H), 2.50 (s, 1H, >N–H), 2.67–2.89 (m, 1H, >C–H), 3.08–3.82 (m, 1H, >C–H), 3.99 (s, 1H, >N–H), 7.00–7.02 (d, 1H, J = 8Hz, quinolinyl-H), 7.57–7.62 (m, 1H, quinolinyl-H), 7.68–7.90 (m, 1H, quinolinyl-H), 8.34 (s, 1H, quinolinyl–H), 8.46–8.51 (d, 1H, J = 20Hz, quinolinyl–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.06 (1×C, $–CH_3$), 24.39 (1×C, $–CH_3$), 24.85, 24.93 (2×C, 2×$>CH_2$, cyclohexyl), 25.09 (1×C, $–CH_3$), 27.76, 27.83, 28.69 (3×C, 3×$>CH_2$, cyclohexyl), 33.33 (1×C, >C–H), 33.48 (1×C, >C–H), 33.61 (1×C, $>CH_2$), 38.83, 39.04 (2×C, tetraoxane), 39.25, 39.46, 39.67, 39.88, 40.8, 63.47, 162, 174, 176 (9×C, quinolinyl); Mass Spectrum (TOF MS, *m/z*) Calculated 435.19, Observed 342.1 (100 %), 344.1 (27.13 %), 412.1 (18.88 %); Elemental Analysis for $C_{19}H_{26}ClN_3O_4$; Calculated C, 60.61; H, 6.94; N 9.64; O, 14.68; Observed C, 60.577; H, 6.942; N, 9.269; O, 14.841.

N2-(7-chloroquinolin-4-yl)-N1-(1-(8-methyl-6,7,9,10-tetraoxaspiro[4.5]decan-8-yl)ethyl)propane-1,2-diamine (**9c**)

Odourless brownish solid; soluble in dichloromethane, DMSO; melting range 270–271 °C; %Yield 13; R_f value 0.90 (Pet. ether: methanol: 1:1); Spectroscopic analysis: λ_{max} (in DMSO) 341.46 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film): 3255.64 (N–H stretching, >NH), 3114.47 (aromatic C–H stretching), 2923.16–2853.42 (aliphatic C–H stretching), 1729.34–1611.90 (N–H bending), 1453.28–1373.58 (C=C–C, aromatic ring stretching), 1210.50–1164.69 (C–N stretching), 1092.67 (aromatic C–H in-plane bend), 1022.68 (C–Cl stretching, Ar–Cl), 900.12 (C–C–O stretching), 868.86 (aromatic C–H out-of-plane bend), 813.23-764.15 (peroxide, C–O–O- stretching).

N2-(7-chloroquinolin-4-yl)-N1-(1-(3-methyl-1,2,4,5-tetraoxaspiro[5.6]dodecan-3-yl)ethyl)propane-1,2-diamine (**9d**) Odourless blackish brown solid; soluble in DMSO; melting range 265–266 °C; %Yield 13.6; R_f value 0.83 (Pet. ether: methanol: 1:1); Spectroscopic analysis: λ_{max} (in DMSO) 335.76 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3246.65 (N–H stretching, >NH), 3106.87 (aromatic C–H stretching), 2923.15–2853.33 (aliphatic C–H stretching), 1731.61–1545.44 (N–H bending), 1452.40–1369.82 (C=C–C, aromatic ring stretching), 1240.41–1160.56 (C–N stretching), 1084.70 (aromatic C–H in-plane bend), 1018.65 (C–Cl stretching, Ar–Cl), 896.90 (C–C–O stretching), 871.17 (aromatic C–H out–of-plane bend), 806.82–765.03 (peroxide, C–O–O– stretching).

N2-(7-chloroquinolin-4-yl)-N1-(1-(3,7-dimethyl-1,2,4,5-tetraoxaspiro[5.5]undecan-3-yl)ethyl)propane-1,2-diamine (**9e**) Odourless dark brown solid; soluble in dichloromethane, DMSO; melting range 260–262 °C; %Yield 08.98; R_f value 0.89 (Pet. ether: Methanol: 1: 1); Spectroscopic analysis: λ_{max} (in DMSO) 340.19 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3230.87 (N–H stretching, >NH), 3060.40 (aromatic C–H stretching), 2920.67–2851.15 (aliphatic C–H stretching), 1991.73–1517.05 (N–H bending), 1477.87–1340.47 (C=C–C, aromatic ring stretching), 1214.62 (C–N stretching), 1087.18 (aromatic C–H in-plane bend), 1042.15 (C–Cl stretching, Ar–Cl), 896.27 (C–C–O stretching), 868.05 (aromatic C–H out-of-plane bend), 809.08–765.95 (peroxide, C–O–O- stretching); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.25-0.32 (t, 3H, J = 28Hz, $-CH_3$, methylcyclohexyl), 0.98–1.23 (d, 3H, J = 100Hz, 2×-CH_3), 1.49 (s, 3H, $-CH_3$), 1.65–1.93 (m, 4H, 3×>CH_2, methylcyclohexyl), 2.03–2.09 (t, 4H, J = 24Hz, 2×>CH_2, methylcyclohexyl), 2.27 (s, 1H, >N–H), 2.49–2.61 (t, 2H, J = 48Hz, >CH_2), 2.75–2.99 (m, 1H, >C–H), 3.60 (s, 1H, >N–H), 4.23–4.42 (m, 1H, >C–H), 5.95–5.96 (d, 1H, J = 4Hz, quinolinyl-H), 6.30–6.54 (m, 1H, quinolinyl-H), 7.23–7.82 (m, 1H, quinolinyl–H), 7.96–8.17 (m, 1H, quinolinyl–H), 8.44 (s, 1H, quinolinyl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.1 (2×C, $-CH_3$), 38.91 (1×C, $-CH_3$, methylcyclohexyl), 39.12 (2×C, 2×>CH_2, methylcyclohexyl), 39.33 (1×C, $-CH_3$), 39.54, 39.75 (3×C, 3×>CH_2, methylcyclohexyl), 39.95 (1×C, >C–H), 40.16 (1×C, >C–H), (1×C, >CH_2), 78.44, 78.76 (2×C, tetraoxane), 79.09, 143.01 (quinolinyl-C).

(7-chloroquinolin-4-yl)-N2-(1-(3,6,6-trimethyl-1,2,4,5-tetraoxan-3-yl)ethyl)benzene-1,2-diamine (**9f**) Odourless brownish red solid; soluble in methanol; melting range 179–180 °C; %yield 10.56; R_f value 0.87 (acetone: methanol: 1:4); spectroscopic analysis: λ_{max} (in methanol) 323.15 nm; FTIR spectrum (ν_{max}, in cm^{-1}, film) 3222.09 (N–H stretching, >NH), 3060.81 (aromatic C–H stretching), 2920.32–2851.80 (aliphatic –C–H stretching), 1917.89–1581.53 (N–H bending), 1533.04–1451.67 (C=C–C, aromatic ring stretching), 1370.82 (C–N stretching), 1207.90–1027.49 (aromatic C–H in-plane bend), 1093.02 (C–Cl stretching, Ar–Cl), 873.94–571.28 (aromatic C–H out–of-plane bend), 815.97 (C–C–O stretching), 757.39–720.21 (peroxide, C–O–O– stretching).

N1-(7-chloroquinolin-4-yl)-N2-(1-(8-methyl-6,7,9,10-tetraoxaspiro[4.5]decan-8-yl)ethyl)benzene-1,2-diamine (**9g**) Odourless black solid; soluble in Dichloromethane; melting range 275–277 °C; %Yield 15.23; R_f value 0.88 (Pet. ether: methanol: 1:4); Spectroscopic analysis: λ_{max} (in dichloromethane) 288.81 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3223.23 (N–H stretching, >NH), 3061.54 (aromatic C–H stretching), 2922.23–2852.55 (aliphatic –C–H stretching), 1917.56–1517.18 (N–H bending), 1447.00 (C=C–C, aromatic ring stretching), 1368.72–1332.57 (C–N stretching), 1207.44–1091.81 (aromatic C–H in-plane bend), 1028.05 (C–Cl stretching, Ar–Cl), 906.14–500.00 (aromatic C–H out-of-plane bend), 817.47 (C–C–O stretching), 759.43 (peroxide, C–O–O– stretching); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (s, 3H, $-CH_3$), 1.53–1.58 (d, 3H, J = 20Hz, $-CH_3$), 1.82–196 (m, 4H, 2×>CH_2, cyclopentyl), 2.24–2.51 (m, 4H, 2×>CH_2, cyclopentyl), 2.69–3.04 (m, 1H, >C–H), 3.55 (s, 2H, 2×>N–H), 6.34–6.35 (d, 2H, J = 4Hz, Ar–H), 6.43–7.14 (m, 2H, Ar–H), 7.54–7.60 (d, 1H, J = 24Hz, quinolinyl-H), 7.86–8.58 (m, 2H, quinolinyl-H), 8.70 (s, 1H, quinolinyl-H), 8.89–8.93 (d, 1H, J = 16Hz, quinolinyl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.03 (2×C, $-CH_3$), 38.98 (2×C, 2×>CH_2, cyclopentyl), 39.19 (2×C, 2×>CH_2, cyclopentyl), 39.40 (1×C, >C–H), 39.60, 39.81(2×C, tetraoxane), 40.02, 40.23 (4×C, Ar), 77.79, 78.12, 78.45 (9×C, quinolinyl); Mass Spectrum (TOF MS, *m/z*) Calculated 455.93 Observed 270.2 (100 %), 272.2 (24.02 %), 410.3 (7.08 %); Elemental Analysis for $C_{24}H_{26}ClN_3O_4$; Calculated C, 63.22; H, 5.75; N 9.22; O, 14.04 Observed C, 62.928; H, 5.994; N, 08.925; O, 14.143.

N1-(7-chloroquinolin-4-yl)-N2-(1-(3-methyl-1,2,4,5-tetraoxaspiro[5.5]undecan-3-yl)ethyl)benzene-1,2-diamine (**9h**) Black solid with characteristic in odour; soluble in ethanol,

dichloromethane; %Yield 08.08; R_f value 0.86 (Pet. ether: methanol: 1:4); Spectroscopic analysis: λ_{max} (in dichloromethane) 228.57 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3235.48 (N–H stretching, >NH), 3062.80 (aromatic C–H stretching), 2925.57–2857.58 (aliphatic –C–H stretching), 1707.19–1541.02 (N–H bending), 1455.56 (C=C–C, aromatic ring stretching), 1394.14 (C–N stretching), 1167.25–1097.28 (aromatic C–H in-plane bend), 1042.33 (C–Cl stretching, Ar–Cl), 960.80–580.83 (aromatic C–H out-of-plane bend), 819.77 (C–C–O stretching), 736.52–695.67 (peroxide, C–O–O– stretching).

N1-(7-chloroquinolin-4-yl)-N2-(1-(3-methyl-1,2,4,5-tetraoxaspiro[5.6]dodecan-3-yl)ethyl)benzene-1,2-diamine (**9i**) Odourless black solid; soluble in methanol; melting range 280-282 °C; %Yield 07.78; R_f value 0.88 (Pet. ether: methanol: 1:1); Spectroscopic analysis: λ_{max} (in DMSO) 341.46 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3221.07 (N–H stretching, >NH), 3061.02 (aromatic C–H stretching), 2919.95–2851.39 (aliphatic –C–H stretching), 1917.59–1516.78 (N–H bending), 1451.38 (C=C–C, aromatic ring stretching), 1368.82 (C–N stretching), 1200.85 (aromatic C–H in-plane bend), 1024.68 (C–Cl stretching, Ar–Cl), 908.69–572.72 (aromatic C–H out-of-plane bend), 813.79 (C–C–O stretching), 754.81 (peroxide, C–O–O– stretching).

N1-(7-chloroquinolin-4-yl)-N2-(1-(3,7-dimethyl-1,2,4,5-tetraoxaspiro[5.5]undecan-3-yl)ethyl)benzene-1,2-diamine (**9j**) Blackish brown semisolid with characteristic odour; soluble in ethanol, dichloromethane; %Yield 06.96; R_f value 0.77 (acetonitrile: ethanol: 1:1); Spectroscopic analysis: λ_{max} (in $CHCl_3$) 239.56 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3224.09 (N–H stretching, >NH), 3063.32 (aromatic C–H stretching), 2927.32–2860.35 (aliphatic –C–H stretching), 1706.98–1540.20 (N–H bending), 1454.88–1414.69 (C=C–C, aromatic ring stretching), 1375.30 (C–N stretching), 1169.57 (aromatic C–H in-plane bend), 1089.94–1043.16 (C–Cl stretching, Ar–Cl), 928.68–582.08 (aromatic C–H out-of-plane bend), 822.13 (C–C–O stretching), 756.76 (peroxide, C–O–O– stretching).

7-chloro-4-(4-(1-(3,6,6-trimethyl-1,2,4,5-tetraoxan-3-yl) ethyl)piperazin-1-yl)quinoline (**9k**) Black semisolid with characteristic odour; soluble in dichloromethane, chloroform; %Yield 05.54; R_f value 0.91 (Pet. ether: methanol: 1:4); Spectroscopic analysis: λ_{max}(in $CHCl_3$) 251.27 nm; FTIR Spectrum (ν_{max}, in cm^{-1}, film) 3060.00 (aromatic C–H stretching), 2921.16–2852.83 (C–H stretching, $>CH_2$, alkyl and piprazinyl), 1867.16–1458.33 (C=C–C, aromatic ring stretching, quinolyl), 1373.86–876.70 (aromatic C–H in-plane bend, quinolyl), 1038.55 (C–Cl stretching, quinolyl–Cl), 917.60-578.47 (aromatic C–H out-of-plane bend, quinolyl), 876.70 (C–C–O stretching), 722.73–696.00 (peroxide, C–O–O– stretching); ^{1}HNMR (400 MHz, $CHCl_3$) δ 0.85-0.91 (m, 3H, $–CH_3$), 1.27 (s, 6H, 2×$–CH_3$), 2.96 (s, 3H, $–CH_3$), 2.00–2.10 (m, 1H, >C–H), 2.33–2.37 (t, 4H, $J = 16$Hz, 2×$>CH_2$, piperazinyl–H), 2.91–2.94 (t, 2H, $J = 12$Hz, 2×$>CH_2$, piperazinyl–H), 7.00–7.18 (m, 1H, quinolinyl-H), 7.28 (s, 1H, quinolinyl-H), 7.48–7.52 (t, 1H, $J = 16$Hz, quinolinyl-H), 7.59–7.61 (d, 1H, $J = 8$Hz, quinolinyl-H), 7.81–7.83 (t, 1H, $J = 8$Hz, quinolinyl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.12 (1×C, $–CH_3$), 22.69 (1×C, $–CH_3$), 24.76 27.09 (2×C, $–CH_3$), 29.27, 29.36, 29.70 (1×C, >CH), 31.93, 33.84 (4×C, piperazine), 76.70 (1×C, tetraoxane), 77.02 (1×C, tetraoxane), 77.34 (1×C, quinolinyl), 128.11 (2×C, quinolinyl), 130.67 (4×C, quinolinyl).

Antimalarial Activity Evaluation All the synthesized compounds were evaluated for in vitro antimalarial activity against chloroquine-resistant strain (RKL-9) of *Pf* using 96 well-microtitre plates. The laboratory adapted strain of *Pf* was routinely cultured at 37 °C temperature and 5 % CO_2 environment in RPMI-1640 medium supplemented with 25 mM HEPES, 1 % D-glucose, 0.23 % sodium bicarbonate and 10 % heat inactivated human serum. For antimalarial activity testing, the asynchronous parasites of *Pf* were synchronized to obtain only the ring stage parasitized cells by 5 % D-sorbitol treatment. For carrying out the assay, the initial ring stage parasitaemia of 0.8–1.5 % at 3 % haematocrit in a total volume of 100 ml of medium RPMI-1640 was uniformly maintained. A stock solution of 1 mg/ml was prepared by dissolving the test compounds in DMSO and subsequent dilutions were made with the culture medium. Hundred microlitres of the test compounds at 100 µg/ml concentrations in triplicate wells was incubated with parasitized cell preparation at 37 °C and 5 % CO_2 in a CO_2 incubator. After an incubation period of 36–40 h, the blood smears were prepared from each well and stained with 3 % Giemsa stain. The slides were microscopically observed and the per cent dead rings and schizonts were scored against 200 asexual parasites with respect to the control group. Chloroquine was used as the standard reference drug (Trager and Jensen, 1976).

Molecular Docking Studies The three dimensional (3D) crystal structure of Falcipain-2 (PDB code 3BPF) was retrieved from the protein data bank (PDB) (www.rcsb.org/pdb). The native auto inducer and all water molecules were removed. The CHARMm force field (FF) was used to add atom types and hydrogens in the proteins. 3D structures of all synthesized compounds were constructed and energy minimized using the Discovery Studio 2.5/Builder module. Docking studies were performed using the CDOCKER module of Discovery Studio 2.5. CDOCKER is a grid-based molecular docking method, where the receptor is held

rigid while the ligands are allowed to flex during the refinement. The CHARMm FF was used as an energy grid FF for docking and scoring function calculations. Random ligand conformations were generated from the initial structure through high temperature molecular dynamics, followed by random rotations which were further refined by grid-based (GRID 1) simulated annealing and a final grid-based minimization. Of the 10 best poses, one (conformation) having highest docking score (−CDOCKER energy) was used for the binding energy calculations and further analysis. The higher negative value of CDOCKER energy represents more favourable binding of the complex. This means that ligands with high docking scores are able to fit snugly in the active site pocket with the minimal steric clashes. CDOCKER score (−CDOCKER Energy) includes internal ligand strain energy and receptor–ligand interaction energy, and is used to sort the different conformations of each input ligand (Oliveira et al., 2013; Liu et al., 2012).

Results and Discussion

Chemistry

The synthesis of target tetraoxaquine hybrids was achieved in three step reactions as shown in scheme 1.

The step 1 corresponds to the synthesis of 2-butanone derivatives (**5a–5c**), which was afforded by the reaction between 4-substituted-7-chloroquinolines (**3a–3c**) and 3-chlorobutan-2-one (**4**). The template, i.e., compound **3a–c** was synthesized by reacting 4,7-dichloroquinoline (**1**) with corresponding diamines **2a–c**.

The corresponding cyclic or alicyclic ketone (**6a–6e**) was allowed to react with hydrogen peroxide (**7**) to afford resultant dihydroperoxides (**8a–8e**), which was then conjugated with derivatives of step 1 (**5**) to furnish target compounds (**9a–9k**).

The structures of the synthesized compounds were confirmed with the aid of various spectroscopic techniques. FTIR spectra showed the stretching in the frequency range between 3200–3400 cm^{-1} attributable to N–H. The presence of stretching band amid 3100–3000 cm^{-1} corresponds to aromatic C–H. The other stretching bands in range of 2800–2950 cm^{-1} were attributable to aliphatic –C–H, while 1250–900 cm^{-1} confirmed the presence of C-C-O stretching. The peroxide, C–O–O– of compounds showed stretching at 900–750 cm^{-1}. The ^{1}H NMR spectra of the compounds showed the characteristic singlet, doublet, triplet or multiplet at δ (ppm). More specifically the 0.50–2.50 was attributable to aliphatic –C–H, a singlet at 2.50–3.50 caused by N–H. A doublet, triplet or multiple at 3.00–3.70 showed the presence of tetraoxane –C–H. Moreover, a singlet, doublet, triplet or multiple at δ 6.72–8.26 are due to Ar–H or quinolinyl-H. The analytical and spectral data of the compounds were found in compliance with the structure of the synthesized compounds.

Biological activity

In vitro antimalarial activity

All the eleven synthesized compounds were tested for in vitro antimalarial activity against the chloroquine-resistant strain RKL-9 (Rourkela, Orissa (India)) of *Pf*.

Among the tested compounds, compounds **9a**, **9b**, **9g**, **9e** and **9k** showed activity against chloroquine-resistant *Pf*

Table 1 In vitro antimalarial activity profile of the synthesized compounds against chloroquine-resistant *Pf* strain RKL-9

S. No.	Compound code	MIC (μM)	MIC (μg/ml)	IC_{50} (μg/ml)	IC_{90} (μg/ml)
1	**9a**	78.94	31.25	3.906	4.498
2	**9b**	71.81	31.25	3.942	24.982
3	**9c**	296.26	125	4.686	101.230
4	**9d**	ND	ND	4.573	167.736
5	**9e**	138.90	62.5	4.272	52.761
6	**9f**	1163.06	500	5.629	96.637
7	**9g**	68.69	31.25	3.906	4.166
8	**9h**	1063.92	500	4.886	145.485
9	**9i**	258.80	125	5.024	94.339
10	**9j**	ND	ND	4.271	153.491
11	**9k**	153.23	62.5	4.814	46.129
12	**CQ**	78.32	25	0.393	1.218

ND = MIC was not determined

MIC, IC_{50} and IC_{90} values were means of three independent experiments

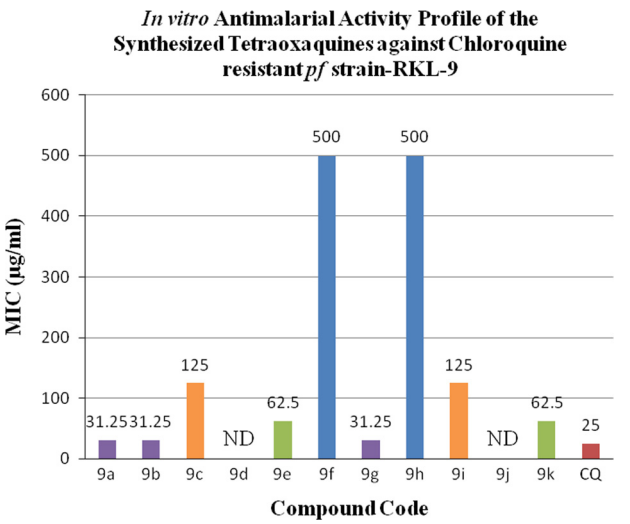


Fig. 1 Comparison in MIC against CQ-resistant *Pf* strain RKL-9, where ND is not determined

strain RKL-9 with MIC 31.25, 31.25, 31.25, 62.50, 62.50 µg/ml and IC_{50} values 3.906, 3.942, 3.906, 4.272, 4.814 µg/ml, respectively. In comparison test, CQ showed prominent inhibition with MIC 25 µg/ml and IC_{50} 0.393 µg/ml (Table 1 and Fig. 1).

The designed hybrid compounds exhibited significant activity against CQ-resistant strain. Compounds containing spiro-cycloheptane substitution on tetraoxane moiety of tetraoxaquine showed less antimalarial activity as compared to other congeners having dimethyl, spiro-cyclohexane, spiro-cyclopentane. It has been observed that nature of linkers (1,2-diaminopropane, o-phenylenediamine and piperazine) have its unique role on the pharmacological activity. Particularly, tetraoxaquines with 1,2-diaminopropane and o-phenylenediamine as linkers exhibit prominent activity than compounds having piperazine linker.

Molecular Docking Studies

In order to gain insight into the key structural requirements and the basis of the distinct activity profile of the test compounds in *Pf* parasite, molecular docking study was carried out. The docking studies of the target compounds were performed into the binding pocket of Falcipain-2 (PDB code 3BPF). The results and docked conformations of the ligands in the active site are illustrated in Table 2 and Fig. 2 and 3. The results showed that the targeted molecules were snugly fitted into the active site with considerable and diverse CDOCKER energy (−1.9658 to −17.3049) along with the formation of hydrogen bonds and hydrophobic interactions.

The results indicate that most promising inhibitor, i.e., compound **9b** showed very low binding energy (−15.3656) against falcipain-2; other compounds, e.g., **9f** also showed very low binding energy (−17.3049) with less antimalarial activity. The formation of two hydrogen bonds with Glu14 and Asn173 through the involvement of 1,2,4,5-tetraoxane nucleus and 7-chloroquinoline nucleus, respectively, was noted. The stability of these complexes (post-docked ligand–receptor) and their low binding energies are attributed to the formation of greater intermolecular hydrogen bonding and hydrophobic interactions with the amino acid residues of falcipain-2 (Fig. 2).

The 2,3-diaminopropyl bridge used to join 4-aminoquinoline with 1,2,4,5-tetraoxane was predicted to be predominantly engaged in hydrogen bonding with

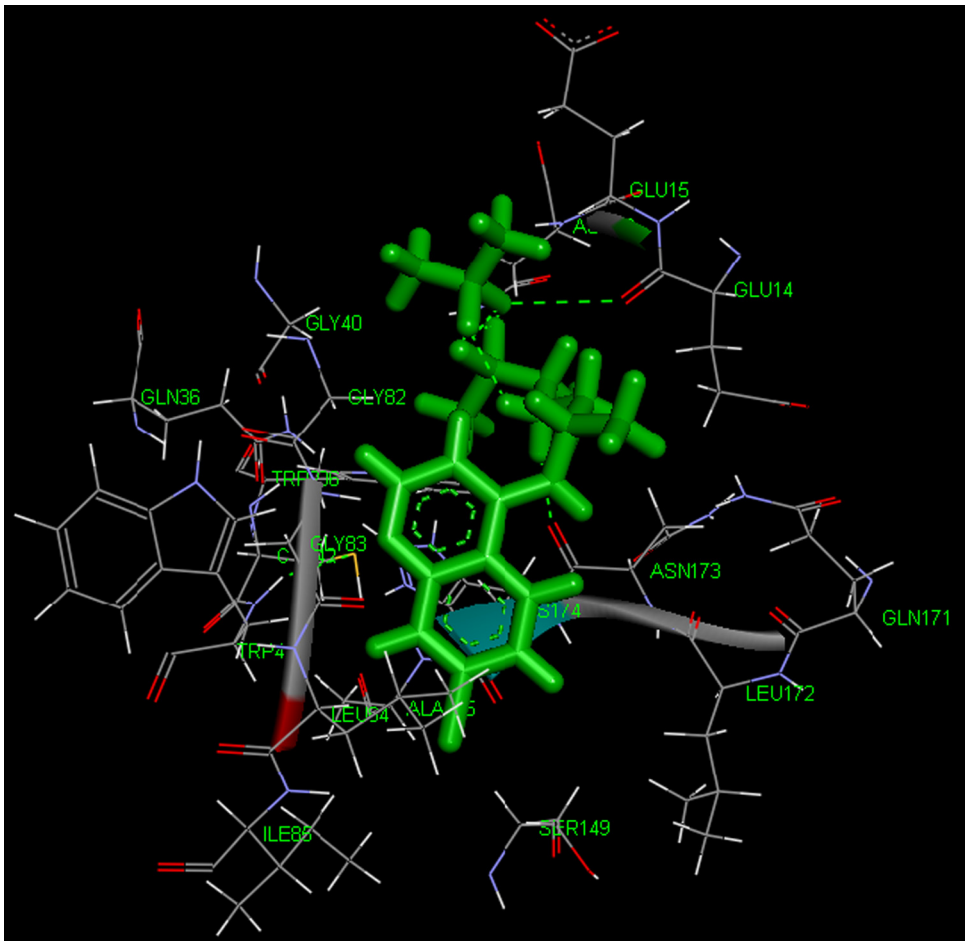


Fig. 2 Docked complex of compound **9b** in the binding pocket of FP-2

Table 2 Molecular docking interactions

S. No.	Compound code	CDOCKER energy (kcal/mol)	CDOCKER interaction energy (kcal/mol)	Hydrogen bonds	Hydrophobic bonds
1	**9a**	−1.9658	−44.6887	Glu14, Asn173	Glu15, Asn16, Gln36, Gly40, Cys42, Gly82, Gly83, Leu84, Ile85, Trp43, Ser149, Gln171, Leu172, Asn173, His174, Ala175, Trp206
2	**9b**	−15.3656	−42.1005	Glu14, Asn173	Glu15, Asn16, Gln36, Gly40, Cys42, Gly82, Gly83, Leu84, Ile85, Trp43, Ser149, Gln171, Leu172, Asn173, His174, Ala175, Trp206
3	**9c**	−15.0352	−47.1908	Glu14, Asn173	Glu15, Asn16, Gln36, Gly40, Gly82, Gly83, Leu84, Ile85, Trp43, Ser149, Leu172, Asn173, His174, Ala175, Trp206
4	**9d**	−10.8618	−47.0260	Glu14, Asn173	Glu15, Asn16, Gln36, Gly40, Cys42, Gly82, Gly83, Ile85, Trp43, Ser149, Gln171, Leu172, Asn173, Ala175, Trp206
5	**9e**	−11.0352	−47.1908	Glu14, Gly83, Asn173,	Glu15, Asn16, Gln36, Lys37, Asn38, Cys39, Gly40, Cys43, Cys80, Asn81, Gly82, Gly83, Leu84, Ile85, Ser149, Val150, Val152, Gln171, Leu172, Asn173, His174, Ala175, Trp206, Asp234
6	**9f**	−9.0973	−48.2636	Glu14, Gly83, ASN173	Glu15, Asn16, Val152, Asn173, Val150, Leu172, Gln171, Ile85, Leu84, Gly83, Gly82, Ser41, Asn81, Cys80, Cys39, Lys37, Asn38, Trp206, Asp234, Ser149, Asn173, His174, Ala175
7	**9g**	−17.3049	−52.7430	Glu14, Gly83, ASN173	Glu15, Asn16, Val152, Asn173, Val150, Leu172, Gln171, Ile85, Leu84, Gly83, Gly82, Ser41, Asn81, Cys80, Cys39, Lys37, Asn38, Trp206, Asp234, Ser149, Asn173, His174, Ala175
8	**9h**	−17.3049	−52.7430	Glu14, Gly83, ASN173	Glu15, Asn16, Val152, Asn173, Val150, Leu172, Gln171, Ile85, Leu84, Gly83, Gly82, Ser41, Asn81, Cys80, Cys39, Lys37, Asn38, Trp206, Asp234, Ser149, Asn173, His174, Ala175
9	**9i**	−2.7859	−41.3373	Glu14, Gly83, Asn173	Lys37, Asn38, Gln36, Cys39, Cys80, Ser41, Gly40, Cys42, Ssn81, Gly82, Trp43, Leu84, Ile85, Glu15, Asn16, Trp206, Val152, Gln171, Asn173, Leu172, His174, Ala175, Asp234
10	**9j**	−12.6214	−41.4067	Glu14, Gly83, ASN173	Glu15, Asn16, Val152, Asn173, Val150, Leu172, Gln171, Ile85, Leu84, Gly83, Gly82, Ser41, Asn81, Cys80, Cys39, Lys37, Asn38, Trp206, Asp234, Ser149, Asn173, His174, Ala175
11	**9k**	−5.7859	−47.3373	Glu14, Gly83	Asn16, Val152, Asn173, Val150, Gln171, Ile85, Leu84, Gly83, Gly82, Ser41, Cys39, Lys37, Asn38, Trp206, Asp234, Ser149, Asn173, Ala175

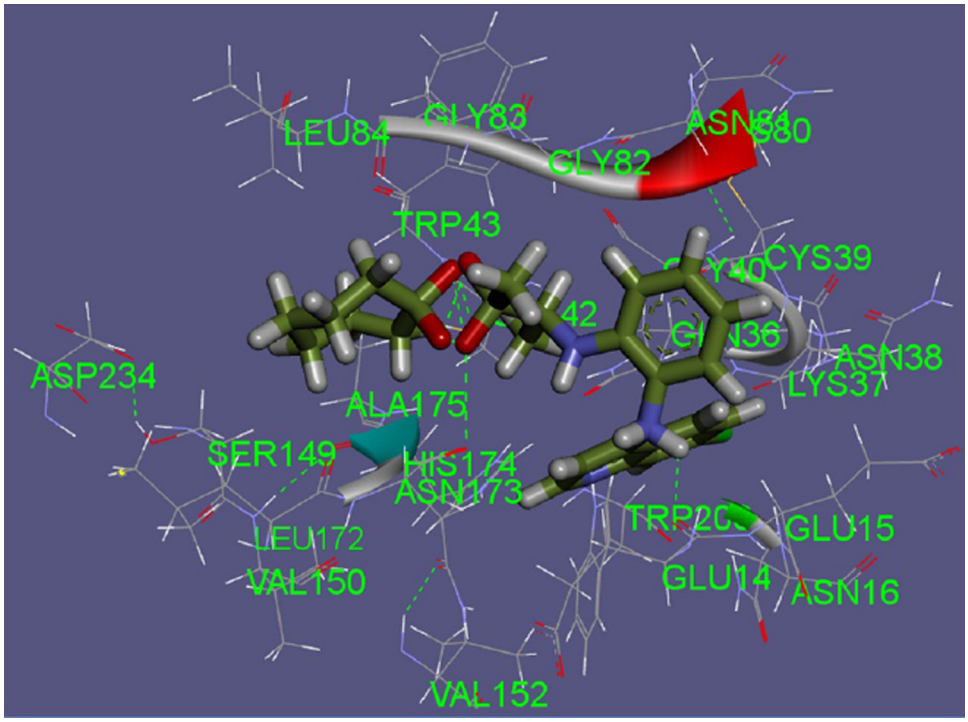


Fig. 3 Docked complex of compound **9h** in the binding pocket of FP-2

key amino acids, i.e.,Glu14 and Asn173. The hydrophobic interactions through the participation of 1,2,4,5-tetraoxane, the cyclohexyl and its amine linkage with Glu15, Asn16, Gln36, Gly40, Cys42, Gly82, Gly83, Leu84, Ile85, Trp43, Ser149, Gln171, Leu172, Asn173, His174, Ala175, Trp206 suggest the role of hydrophobic interactions in biological activity. Compound **9b** showed formation of two hydrogen bonds with Glu14 and Asn173 through oxygen atom of 1,2,4,5-tetraoxane and nitrogen of amino group of side chain modified 7-chloroquinoline. Compounds **9a**, **9c** and **9d** showed similar fashion of interactions like **9b**. In the case of compounds **9e**, **9f**, **9g**, **9h** (Fig. 3), **9i**, **9j** and **9k**, where a diaminophenyl and piperazinyl group are present at the positions of the diaminopropyl revealed a diverse pattern of interaction with the binding site. The Hydrogen bonding with Glu14, Gly83, ASN173, as well as hydrophobic interaction with Glu15, Asn16, Val152, Asn173, Val150, Leu172, Gln171, Ile85, Leu84, Gly83, Gly82, Ser41, Asn81, Cys80, Cys39, Lys37, Asn38, Trp206, Asp234, Ser149, Asn173, His174, Ala175 were observed for rest of the compounds.

The results support H-bonding interaction is the main predictor for the activity of the ligands (Table 2).

Conclusion

The synthesis and antimalarial activity of a series of tetraoxaquine hybrids have been reported in this paper. The in vitro evaluation against chloroquine-resistant strain of *Pf* RKL-9 showed activity in the designed compounds. The promising antimalarial activity exhibited by the novel tetraoxaquine hybrids reported in the present study emphasizes their potential for further development as antimalarial drugs.

Acknowledgments The authors are thankful to Ipca Laboratories Ltd., Indore, India for providing gift sample of 4,7-dichloroquinoline. The authors are also thankful to S.A.I.F., Punjab University, Chandigarh, India for carrying out spectroscopic analysis of the synthesized compounds.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Casteel DA (1997), Antimalarial agents. In: Abraham DJ (ed) Burger's medicinal chemistry and drug discovery, Vol-5 – Therapeutic agents, 5th edn., Wiley Interscience, New York, pp 3–75

Coates J (2000), Interpretation of infrared spectra, a practical approach. In: Meyers RA (ed) Encyclopaedia of analytical chemistry. John Wiley & Sons Ltd, Chichester, pp 10815–10837

Kumawat MK, Singh UP, Singh B, Prakash A, Chetia D (2011) Synthesis and antimalarial activity evaluation of 3-(3-(7-chloroquinolin-4-ylamino)propyl)-1,3-thiazinan-4-one derivatives. Arab J Chem doi:10.1016/j.arabjc.2011.07.007, in Press

Liu Y, Lu WQ, Cui KQ, Luo W, Wang J, Guo C (2012) Synthesis and biological activities of novel artemisinin derivatives as cysteine protease falcipain-2 inhibitors. Arch Pharm Res 35(9):1525–1531

Mathieson DW (1965) Interpretation of Organic Spectra. Academic Press, New York

O'Neill PM, Amewu RK, Nixon GL, Garah FB, Mungthin M, Chadwick J, Shone AE, Vivas L, Lander H, Barton V, Muangnoicharoen S, Bray PG, Davies J, Park BK, Wittlin S, Brun R, Preschel M, Zhang K, Ward SA (2010) Identification of a 1,2,4,5-tetraoxane antimalarial drug-development candidate (RKA182) with superior properties to the semisynthetic artemisinins. Angew Chem Int Ed 49:5693–5697

Oliveira R, Newton AS, Guedes RC, Miranda D, Amewu RK, Srivastava A, Gut J, Rosenthal PJ, O'Neill PM, Ward SA, Lopes F, Moreira R (2013) An endoperoxide-based hybrid approach to deliver falcipain inhibitors inside malaria parasites. Chem Med Chem 8:1528–1536

Opsenica I, Opsenica D, Lanteri CA, Anova L, Milhous WK, Smith KS, Olaja BAS (2008) New chimeric antimalarials with 4-aminoquinoline moiety linked to a tetraoxane skeleton. J Med Chem 51:6216–6219

Pasto DJ, Johnson CR, Miller MJ (1992) Experiments and Techniques in Organic Chemistry. Prentice Hall, NJ, USA

Silverstein RM, Webster FX (1963) Spectrometric Identification of organic compounds. John Wiley and Sons Ltd, New York

Trager W, Jensen JB (1976) Human Malaria Parasites in Continuous Culture. Science 193:673–675

WHO, (2013). World Malaria Report. WHO Global Malaria Programme. www.who.int/malaria. Accessed 10 June 2014