Med Chem Res (2016) 25:1148–1162
DOI 10.1007/s00044-016-1555-5

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

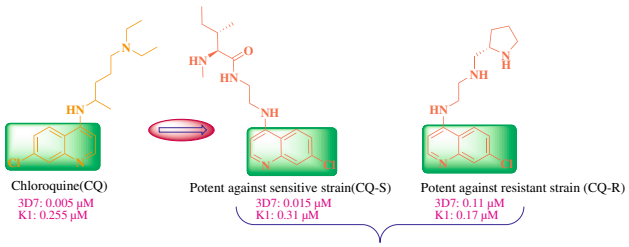
Design, synthesis, and in vitro antiplasmodial activity of 4-aminoquinolines containing modified amino acid conjugates

Kondaparla Srinivasarao[1] · Pooja Agarwal[2] · Kumkum Srivastava[2] · W. Haq[1] · Sunil K. Puri[2] · S. B. Katti[1]

Received: 16 April 2015 / Accepted: 5 March 2016 / Published online: 25 March 2016

Abstract A new series of side chain-modified 4-aminoquinolines were synthesized and screened for in vitro antiplasmodial activity against both chloroquine-sensitive (3D7) and chloroquine-resistant (K1) strains of *Plasmodium falciparum*. Among the series, compounds **30** and **31** showed significant inhibition of parasite growth against K1 strain of *P. falciparum* with IC_{50} values 0.28 and 0.31 μM, respectively, whereas compounds **34**, **35,** and **38** exhibited superior activity against K1 strain with IC_{50} values 0.18, 0.22, and 0.17 μM, respectively, as compared to 0.255 μM for chloroquine (CQ). All the compounds displayed good resistance factor between 1.54 and >34.48 as against 51.0 for CQ. All these analogues were found to form strong complex with hematin and inhibited the *β*-hematin formation in vitro, suggesting that this class of compounds act on a heme polymerization target. Overall results suggest that present series of compounds appear to be promising for further lead optimization to obtain compounds active against drug-resistant parasites.

Graphical Abstract



Keywords Antiplasmodial activity · Heme binding · Chloroquine · *Plasmodium falciparum* · *β*-Hematin · Chloroquine-sensitive strain · Chloroquine-resistant strain

Abbreviations

CQ	Chloroquine
DCC	*N*,*N*′-dicyclohexylcarbodiimide
HOBt	Hydroxybenzotriazole
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide

Introduction

Malaria remains one of the most common parasitic diseases in the developing countries. According to recent World Health Organization (WHO) report, an estimated 3.3 billion people were at risk of malaria and approximately 584,000 deaths occurred worldwide in 2013. Of these estimated deaths, 90 % occurred in sub-Saharan Africa, especially young children and pregnant women are the most affected (WHO: World Malaria Report 2014). The disease is caused by five different species (namely *P.*

✉ S. B. Katti
sb_katti@cdri.res.in;
setu_katti@yahoo.com

[1] Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, Sector-10, Jankipuram Extension, Sitapur Road, Lucknow 226031, India

[2] Parasitology Division, CSIR-Central Drug Research Institute, Sector-10, Jankipuram Extension, Sitapur Road, Lucknow 226031, India

falciparum, *vivax*, *ovale*, *malariae,* and *knowlesi*) of protozoan *Plasmodium*. Of these, *P. falciparum* is the most lethal and most prevalent in sub-Saharan Africa. Since the development of quinine (*cinchona* alkaloid) as antimalarial, drugs containing the quinoline nucleus had became a mainstay of antimalarial therapy (Kaur *et al.*, 2010; Na-Bangchang and Karbwang, 2009; Schlitzer, 2008; Vasanth *et al.*, 2014). For many years, the most effective and least expensive quinoline-based antimalarial was chloroquine (CQ) (Fig. 1) mainly because of its rapid onset of action and low toxicity (Wellems and Plowe, 2001). Unfortunately, the significance of CQ and other existing antimalarials were seriously diminished due to the emergence and widespread of drug-resistant parasites in every region where *P. falciparum* is prevalent. So, there is an urgent need for new antimalarial agents active against drug-resistant strains. In order to search a new antimalarial agent and to overcome drug resistance, several quinoline-based scaffolds were developed in the last few years (Teixeira *et al.*, 2014). Most of them contain 7-chloro-4-aminoquinoline skeleton with slight variations in the side chain length and basic nature of the pendant nitrogen atom.

It is reported that substitution of 7-chloro group of the 4-aminoquinoline nucleus with electron-donating groups (NH_2, OCH_3) or electron-withdrawing group (NO_2) reduces the antimalarial activity due to either an increase or a decrease in the pK_a of quinoline nitrogen atom. Therefore, the presence of 7-chloro group is optimal for antimalarial activity in 4-aminoquinoline class of compounds (De *et al.*, 1998). Apart from this, 4-aminopyridine moiety of the 4-aminoquinoline is crucial and essential for heme binding as well as accumulation of drug in the acidic food vacuole of the parasite (Dorn *et al.*, 1995; Pandey *et al.*, 2001) (Fig. 2). This is further supported by experimental and molecular modelling studies by Cheruku *et al.* (2003).

The importance of aminoalkyl side chain in 4-aminoquinolines was investigated, and the results suggested that analogues having shorter chain length (2–3 carbon) between the two nitrogen atoms of the side chain consistently show better activity against chloroquine-resistant strains of *P. falciparum* as compared to longer chain (4-10 carbon) analogues (Ridley *et al.*, 1996). Further, basicity of

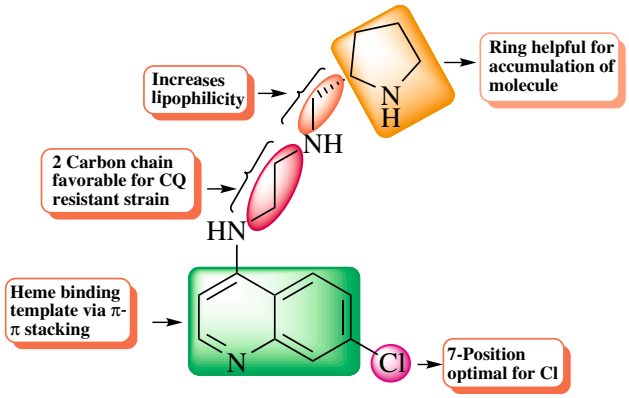
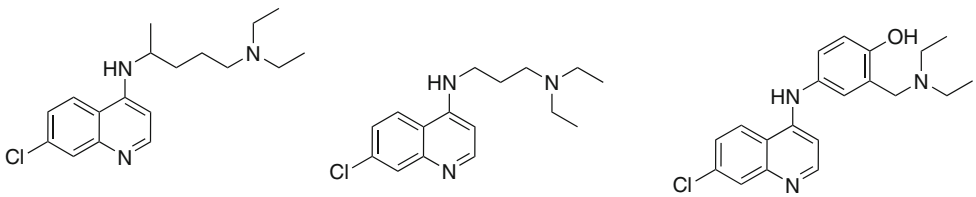


Fig. 2 SAR of the synthesized potent active molecule

the pendant nitrogen atom also plays a vital role in the antimalarial activity. Madrid and coworkers synthesized a series of AQ-13 derivatives, by replacing the diethylamino functionality of AQ-13 with one propyl group as constant and other with different substituted heterocycles (furan, thiophene, and imidazole). These analogues led to substantial increase in the antimalarial activity against 3D7 and W2 strains of *P. falciparum* (Ray *et al.*, 2010). Later, Bavari *et al.* (2007) reported shorter side chain 4-aminoquinoline analogues obtained by derivatization of pendant amino (NH_2) group with substituted cholesterol derivatives with enhanced activity against CQ-sensitive strains. On the other hand, recent studies also revealed that chloroquine (CQ) analogues having modifications at the side chain showed better antimalarial activity predominantly against CQ-resistant strains (Hocart *et al.*, 2011; Madrid *et al.*, 2004; Stocks *et al.*, 2002) as compared to the available drugs. These findings gave impetus to our antimalarial drug research by further augmenting the realization that rational choice of inputs based on known antimalarial scaffold could lead to molecules with desirable antimalarial activity profile.

Previously from our group, we have reported a novel series of 4-aminoquinoline analogues obtained by modifying the pendant amino group with amide bond (–CO–NH–) using cationic amino acids, lysine, and ornithine (Fig. 3a). These analogues showed promising in vitro

Fig. 1 Structures of some 4-aminoquinolines having antimalarial activity



Chloroquine 1 AQ-13 2 Amodiaquine 3

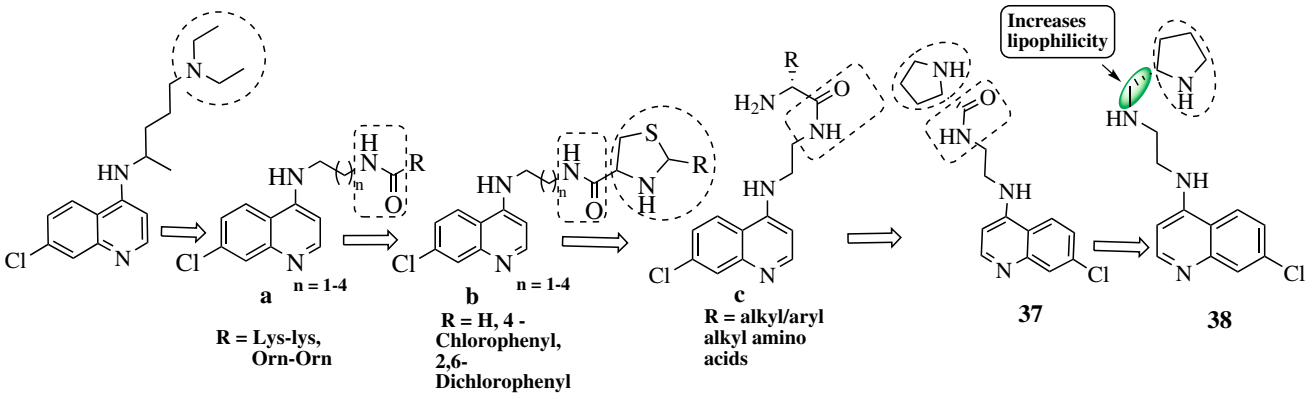


Fig. 3 Some lead molecules of 4-aminoquinoline derived antimalarials developed from this laboratory

antimalarial activity (Solomon *et al.*, 2008). Later, we introduced biologically privileged thiazolidine ring system in the side chain in order to enhance the lipophilicity (Fig. 3b). These analogues showed remarkable activity against CQ-sensitive strain (NF-54) of *P. falciparum* in vitro and CQ-resistant strain (N-67) of *P. yoelii* in vivo (Solomon *et al.*, 2013). In continuation of our efforts to develop effective antimalarial agents, recently we have synthesized a series of 4-aminoquinolines with different amino acids at the lateral side chain connected through 1,2 diamine tether (Fig. 3c). These molecules were found to have moderate activity against chloroquine-sensitive strain (3D7) and promising activity against chloroquine-resistant strain (K1). The results indicated that the hydrophobicity of the side chain and pK_a of the pendant amine group are important factors which may help the molecule to exhibit activity by involving in hydrogen bonding (Deshpande 2010). In addition to these studies, we have also investigated the quantitative structure–activity relationship (QSAR) studies on two distinct series of N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl) piperazine derivatives using DRAGON descriptors in order to rationalize the activity. The results obtained from computational study clearly suggested that amide (–CO–NH–) moiety in the pharmacophore is essential for the antimalarial activity of 4-AQ class (Deshpande *et al.*, 2009).

Encouraged by these results herein, we report a series of amide bond (–CO–NH–) containing 4-aminoquinolines with free pendant amino group (NH_2) using various amino acids (Tyr, Thr, Ser, and Pro). Further, with a view to facilitate the accumulation of molecule in the acidic food vacuole and to accomplish better interaction with hematin, we introduced methyl group at pendant amino group of 4-aminoquinoline. Finally, we reduced the amide (–CO–NH–) bond present in this series of compounds to further enhance the lipophilic nature of the lateral side chain, which could help the molecule to exhibit better in vivo activity against both strains of *P. falciparum*. The results are described in the present communication.

Materials and methods

General information

Meting points (mp) were determined on a Complab melting point apparatus and are uncorrected. The ^{1}H NMR (300 MHz) and ^{13}C NMR (50 MHz) spectra were recorded in $CDCl_3$, CD_3OD, D_2O, and DMSO-d_6, used as solvents on DPX-300 (^{1}H NMR) Bruker FT-NMR and DPX-200 (^{13}C NMR) spectrometers. Tetramethylsilane (δ 0.0 ppm) was used as an internal standard. Mass Spectra (ESI–MS), and high-resolution mass spectrometry HRMS (ESI–HRMS) were recorded on Jeol (Japan)/SX-102, Agilent 6520 Q-Tof (ESI–HRMS) spectrometer. The progress of the reaction was monitored on readymade TLC silica gel plates (Merck) using chloroform/methanol (9:1) as a solvent system. Iodine was used as developing agent or by spraying with Dragendorff's reagent. Column chromatographic purification was performed over silica gel (230–400 mesh). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK), or Spectrochem Pvt. Ltd (India) and were used without further purification.

Experimental procedure for the synthesis of intermediates (6a-6i)

(S)-2-(Tert-butoxycarbonyl(methyl)amino)-3-hydroxypropanoic acid ***(6a)*** Gummy substance (This compound was prepared by adding neat sodium hydride (10 equiv.) in portion wise over a period of 2.0 h to a cooled (0 °C) solution of (*S*)-2-(*tert*-butoxycarbonylamino)-3-hydroxypropanoic acid (1 equiv.) and iodomethane (10 equiv.) in dry THF under a stream of nitrogen. The reaction mixture was stirred at

room temperature for 24 h under nitrogen atmosphere and then diluted with ether (20 mL) and quenched with water (30 mL). The layers were separated and the aqueous layer was extracted with ether (2 × 15 mL), acidified to pH 3 with a 20 % aqueous solution of citric acid and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over Na_2SO_4 and evaporated to afford the corresponding *N*-methylated product in 90 % yield as Gummy substance.);^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 3.92–3.89$ (1H, m, C*H*NCH$_3$), 3.59–3.56 (2H, m, CHCH_2OH), 2.79 (3H, s, CHNCH_3), 1.38 (9H, s, C(CH_3)$_3$); ESI–MS (*m/z*): 220.3 $[M + H]^+$ (100).

(S)-2-(Tert-butoxycarbonyl (methyl)amino)-3-(4-methoxyphenyl)propanoic acid (***6b***) Gummy substance (The method for synthesis was the same as reported for compound **6**a. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonylamino)-3-(4-methoxyphenyl)propanoic acid. It was obtained as gummy substance in 93 % yield.); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.25–7.21$ (4H, m, CHCH$_2$C$_6$*H*$_4$OCH$_3$), 4.34–4.23 (1H, m, C*H*CH$_2$C$_6$H$_4$ OCH$_3$), 3.46 (3H, s, CHCH$_2$C$_6$H$_4$OCH_3), 3.20 (3H, s, CHNCH_3), 3.16-3.12 (2H, m, CHCH_2C$_6$H$_4$OCH$_3$), 1.38 (9H, s, C(CH_3)$_3$); ESI–MS (*m/z*): 310.2 $[M + H]^+$ (100).

(2S,3R)-2-(Tert-butoxycarbonyl(methyl)amino)-3-methoxybutanoic acid (***6c***) Gummy substance (The method for synthesis was the same as reported for compound **6**b. While the amino acid used was (2*S*,3*R*)-2-(*tert*-butoxycarbonylamino)-3-methoxybutanoic acid. It was obtained as gummy substance in 95 % yield.); ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 4.09–3.94$ (1H, m, C*H*CHOHCH$_3$), 3.74–3.70 (1H, m, CHC*H*OHCH$_3$), 3.30 (3H, s, CHNCH_3), 2.93 (3H, s, CHCHOCH_3), 1.37 (9H, s, C(CH_3)$_3$), 1.19–1.13(3H, m, CHCHOHCH_3), ESI–MS (*m/z*): 248.3 $[M + H]^+$ (100).

(S)-2-(Tert-butoxycarbonyl(methyl)amino)acetic acid (***6d***) Gummy substance (The method for synthesis was the same as reported for compound **6**c. While the amino acid used was 2-(*tert*-butoxycarbonylamino)acetic acid. It was obtained as gummy substance in 94 % yield.); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 3.93$ (2H, s, CH_2COOH), 2.79 (3H, s, CH$_2$NCH_3), 1.39 (9H, s, C(CH_3)$_3$), ESI–MS (*m/z*): 190.3 $[M + H]^+$ (100).

(S)-2-(Tert-butoxycarbonyl(methyl)amino)propanoic acid (***6e***) Gummy substance (The method for synthesis was the same as reported for compound **6**d. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonylamino)propanoic acid. It was obtained as gummy substance in 93 % yield.); ^{1}H NMR ($CDCl_3$, 300 MHz,): $\delta = 4.43–4.41$ (1H, m, CH$_3$C*H*NCH$_3$), 2.85 (3H, s, CHNCH_3), 1.42 (3H, d, $J = 5.7$ Hz, CHCH_3), 1.41 (9H, s, C(CH_3)$_3$); ESI–MS (*m/z*): 204.3 $[M + H]^+$ (100).

(S)-2-(Tert-butoxycarbonyl(methyl)amino)-3-methylbutanoic acid (***6f***) Gummy substance (The method for synthesis was the same as reported for compound **6**e. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonylamino)-3-methylbutanoic acid. It was obtained as gummy substance in 91 % yield.); ^{1}H NMR ($CDCl_3$, 300 MHz,): $\delta = 4.10$ (1H, d, $J = 10.4$ Hz, C*H*NCH$_3$), 2.87 (3H, s, CHNCH_3), 1.46 (9H, d, $J = 6.1$ Hz, C(CH_3)$_3$), 1.25 (1H, s, CHC*H*(CH$_3$)$_2$), 1.00 (3H, d, $J = 7.5$ Hz, CHCHCH_3), 0.92 (3H, d, $J = 6.6$ Hz, CHCHCH_3); ESI–MS (*m/z*): 232.3 $[M + H]^+$ (100).

(S)-2-(Tert-butoxycarbonyl(methyl)amino)-3-methylpentanoic acid (***6g***) Gummy substance (The method for synthesis was the same as reported for compound **6**f. While the amino acid used was (2*S*)-2-(*tert*-butoxycarbonylamino)-3-methylpentanoic acid. It was obtained as gummy substance in 98 % yield.); ^{1}H NMR ($CDCl_3$, 300 MHz,): $\delta = 3.97–3.93$ (1H, m, C*H*CHCH$_3$CH$_2$CH_3), 2.73 (3H, s, CHNCH_3), 2.46–2.43(1H, m, CHC*H*CH$_3$CH$_2$CH_3), 1.56–1.45 (2H, m, CHCHCH$_3$CH_2CH$_3$), 1.38 (9H, s, C(CH_3)$_3$), 1.29–0.99 (3H, m, CHCHCH_3CH$_2$CH$_3$), 0.97–0.82 (3H, m, CHCHCH$_3$CH$_2$CH_3); ESI–MS (*m/z*): 246.3 $[M + H]^+$(100).

(S)-2-(Tert-butoxycarbonyl(methyl)amino)-4-methylpentanoic acid (***6h***) Gummy substance (The method for synthesis was the same as reported for compound **6**g. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonylamino)-4-methylpentanoic acid. It was obtained as gummy substance in 95 % yield.); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 4.16–4.11$ (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 2.81 (3H, s, CHNCH_3), 1.75–1.70 (2H, m, CHCH_2CH(CH$_3$)$_2$), 1.58–1.54 (1H, m, CH$_2$C*H*(CH$_3$)$_2$), 1.46 (9H, s, C(CH_3)$_3$), 0.94 (6H, d, $J = 5.9$ Hz, CH$_2$CH(CH_3)$_2$); ESI–MS (*m/z*): 246.7 $[M + H]^+$(100).

(S)-2-(Tert-butoxycarbonyl(methyl)amino)-3-phenylpropanoic acid (***6i***) Gummy substance (The method for synthesis was the same as reported for compound **6**h. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonylamino)-3-phenylpropanoic acid. It was obtained as gummy substance in 92 % yield.); ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 7.34–7.25$ (5H, m, CHCH$_2$C$_6$*H*$_5$), 4.81–4.63 (1H, m, C*H*CH$_2$C$_6$H$_5$), 2.88 (3H, s, CHNCH_3), 2.74 (2H, d, $J = 5.5$ Hz, CHCH_2C$_6$H$_5$), 1.41 (9H, s, C(CH_3)$_3$); ESI–MS (*m/z*): 280.3 $[M + H]^+$(100).

*General procedure for the synthesis of (S)-1-Methylpyrrolidine-2-carboxylic acid (**7a**)* L-Proline (2.0 g, 17.4 mmol) was dissolved in methanol (20 mL) and 40 % aqueous formaldehyde (1.4 mL, 19.1 mmol) was added to this solution. Next, 10 % Pd/C catalyst (500 mg) was added to the reaction mixture and the resulting slurry was stirred in hydrogen overnight. The slurry was then

filtered through a Celite pad to remove the catalyst. The pad was washed with methanol and the combined filtrates were concentrated under reduced pressure. The residue was dissolved in ethanol/benzene (1:1, 100 mL) and concentrated second time to provide a solid that was re-crystallized in methanol/diethyl ether. In this way *N*-methylproline **7a** was isolated as fine needles (2.1 g, 92 % yield); mp 109–111 °C; ^{1}H NMR (D_2O, 300 MHz): δ = 3.95–3.90 (1H, m, CH_2 $(CH_2)_2CHNCH_3$), 3.24–3.15 (2H, m, CH_2 $(CH_2)_2CHNCH_3$), 2.97 (3H, s, CH_2 $(CH_2)_2$ $CHNCH_3$), 2.22–2.01 (4H, m, CH_2 $(CH_2)_2CHNCH_3$); ESI–MS (*m/z*):130.2 $[M + H]^+$(100).

*General procedure for the synthesis of N^1-(7-Chloroquinolin-4-yl) ethane-1,2-diamine (**9**)* Yellow solid (A mixture of 4,7-dichloroquinoline (1 equiv.) and 1,2-diaminoethane (5 equiv.) was heated slowly from room temperature to 80 °C over 1 h with stirring and subsequently at 120–130 °C for 6–8 h with continued stirring to drive the reaction to completion. The reaction mixture was cooled to room temperature and taken up in dichloromethane. The organic layer was successively washed with 5 % aq $NaHCO_3$ followed by water wash and then finally with brine. The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure and the residue was precipitated by the addition of 80:20 hexane–chloroform. It was obtained as yellow solid in 85 % yield.); mp 131–132 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 8.50 (1H, d, J = 5.1 Hz, H-2), 7.99–7.95 (1H, dd, J = 2.1, 8.1 Hz, H-5), 7.70 (1H, d, J = 2.1 Hz, H-8), 7.67 (1H, d, J = 9.0 Hz, H-6), 6.40 (1H, d, J = 5.4 Hz, H-3), 5.87 (1H, br s, $NHCH_2CH_2NH_2$), 4.73 (2H, br s, $NHCH_2CH_2NH_2$), 3.45–3.30 (2H, m, $NHCH_2$ CH_2NH_2), 3.16–3.10 (2H, m, $NHCH_2CH_2NH_2$); ESI–MS (*m/z*): 222.3 $[M + H]^+$(100).

General procedure for the synthesis of final compounds 13–15, 25–33, 35 and 37

(S)-2-Amino-N-(2-(7-chloroquinolin-4-ylamino) ethyl)-3-hydroxypropanamide (***13***) Gummy residue (1-hydroxybenzotriazole HOBt (1.1 equiv.) and *N*,*N*′-dicyclohexylcarbodiimide DCC (1.5 equiv.) are added successively to a suspension of (*S*)-2-(*tert*-butoxycarbonylamino)-3-hydroxypropanoic acid (1 equiv.) in 15 mL of DMF. The mixture was left under stirring for 30 min before adding *N^1*-(7-chloroquinolin-4-yl) ethane-1,2-diamine, the stirring was continued for 4–6 h at 0 °C temperature. The reaction medium is then diluted with 40 mL of ethyl acetate and then filtered, and washed with 40 mL of water saturated with NaCl. The organic phase is dried over Na_2SO_4, filtered and then evaporated. The crude product obtained is purified by Column chromatography on silica gel (230–400 mesh eluent: dichloromethane/Methanol 9:1). The fractions containing the desired compound according to TLC revealed under UV are pooled and evaporated. The quantitative yields were obtained for desired intermediates. Further Boc deprotection was done by TFA/DCM at room temperature for 4–6 h which leads to desired product in good yield. It was obtained as gummy substance in 76 % yield); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.85 (1H, d, J = 9.1 Hz, H-2), 8.56 (1H, d, J = 8.9 Hz, H-5), 8.12 (1H, d, J = 1.8 Hz, H-8), 7.73–7.71(1H, dd, J = 1.8, 8.9 Hz, H-6), 6.92 (1H, d, J = 7.0 Hz, H-3), 3.73–3.65 (1H, m, $NHCOCHCH_2OH$), 3.56 (2H, s, $COCHCH_2OH$), 3.45–3.36 (4H, m, $NH(CH_2)_2NHCO$); ^{13}C NMR (DMSO-d_6, 50 MHz,): δ = 167.2 (CO, CONH), 155.4 (C, C-4), 138.8 (C, C-2), 142.3 (CH, C-9), 137.7 (CH, C-7), 126.5 (C, C-8), 126.3 (C, C-5), 119.1 (C, C-6), 115.7 (C, C-10), 98.5 (CH, C-3), 59.9 (CH_2, CH_2OH), 54.4 (CH, CHCO), 42.1 (CH_2, $NHCH_2$), 37.2 (CH_2, CH_2NHCO); ESI–MS (*m/z*): 309.2 $[M + H]^+$ (100); HRMS (*m/z*): 309.1106 $[C14H17ClN4O_2 + H]^+$ (calcd. 309.1113).

(S)-2-Amino-N-(2-(7-chloroquinolin-4-ylamino)ethyl)-3-(4-hydroxyphenyl)propan amide (***14***) Gummy residue (The method for synthesis was the same as reported for compound 13. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonylamino)-3-(4-hydroxyphenyl)propanoic acid. It was obtained as gummy substance in 77 % yield.); ^{1}H NMR (DMSO-d_6, 300 MHz,): δ = 8.40 (1H, d, J = 6.1 Hz, H-2), 8.26 (1H, d, J = 9.0 Hz, H-5), 7.81 (1H, d, J = 8.1 Hz, H-8), 7.41–7.29 (2H, m, $CH_2C_4H_2OH(CH)_2$), 6.95–6.93 (1H, dd, J = 2.0, 8.9 Hz, H-6), 6.71 (2H, d, J = 6.3 Hz, $CH_2C_4H_2OH(CH)_2$), 6.58 (1H, d, J = 5.4 Hz, H-3), 3.86–3.83 (1H, m, $COCHCH_2C_6H_4OH$), 3.41–3.30 (4H, m, $NH(CH_2)_2NHCO$), 2.86 (2H, d, J = 2.5 Hz, $COCHCH_2C_6H_4OH$); ^{13}C NMR (DMSO-d_6, 50 MHz,): δ = 168.4 (CO, CONH), 155.9 ($1C_{aromatic}$), 142.4 (C, C-4), 138.2 (C, C-2), 137.8 (CH, C-9), 130.3 (CH, C-7), 127.7, 127.3 ($2C_{aromatic}$), 126.8 (C, C-8), 125.2 (C, C-5), 124.5 (C, C-6), 118.2 (C, C-10), 115.1, 109.8 ($2C_{aromatic}$), 98.5 (CH, C-3), 53.7 (CH, CHCO), 42.3 (CH_2, $NHCH_2$), 36.8 (CH_2, CH_2NHCO), 35.6 (CH_2, CH_2Ph); ESI–MS (*m/z*): 385.3 $[M + H]^+$ (100); HRMS (*m/z*): 385.1469 $[C20H21ClN4O_2 + H]^+$ (calcd. 385.1426).

(2S)-2-Amino-N-(2-(7-chloroquinolin-4-ylamino) ethyl)-3-hydroxybutanamide (***15***) Gummy residue (The method for synthesis was the same as reported for compound 14. While the amino acid used was (2*S*)-2-(*tert*-butoxycarbonylamino)-3-hydroxybutanoic acid. It was obtained as gummy substance in 81 % yield.); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.59 (1H, d, J = 5.6 Hz, H-2), 8.48 (1H, d, J = 9.1 Hz, H-5), 7.99 (1H, d, J = 1.8 Hz, H-8), 7.81–7.78 (1H, dd, J = 1.8, 8.9 Hz, H-6), 6.96 (1H, d, J = 5.4 Hz, H-3), 3.95 (1H, s, $COCHOHCHCH_3$),

3.89–3.85 (5H, m, $NH(CH_2)_2NHCOCH$), 1.08 (3H, d, $J = 6.2$ Hz, $COCHOHCHCH_3$); ^{13}C NMR (DMSO-d_6, 50 MHz): $\delta = 168.7$ (CO, CONH), 155.7 (C, C-4), 151.8 (C, C-2), 148.7 (CH, C-9), 134.7 (CH, C-7), 129.8 (C, C-8), 127.4 (C, C-5), 121.3 (C, C-6), 116.6 (C, C-10), 98.5 (CH, C-3), 69.4 (CH, CH_3CHOH), 65.3 (CH, $CHNH_2$), 43.3 (CH_2, $NHCH_2$), 38.4 (CH_2, CH_2NHCO), 18.3 (CH_3, $CHCH_3$); ESI–MS (*m/z*): 323.1 $[M + H]^+$ (100); HRMS (*m/z*): 323.1276 $[C_{15}H_{19}ClN_4O_2 + H]^+$ (calcd. 323.1269).

(S)-N-(2-(7-Chloroquinolin-4-ylamino) ethyl)-3-hydroxy-2-(methylamino) propan amide (***25***) Gummy residue (The method for synthesis was the same as reported for compound 15. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-hydroxypropanoic acid. It was obtained as gummy substance in 81 % yield.); ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 8.95$ (1H, d, $J = 9.1$ Hz, H-2), 8.65 (1H, d, $J = 6.6$ Hz, H-5), 8.23 (1H, d, $J = 7.8$ Hz, H-8), 7.84–7.82 (1H, dd, $J = 2.0$, 8.9 Hz, H-6), 7.01 (1H, d, $J = 6.8$ Hz, H-3), 4.06 (1H, s, $COCHNHCH_3$), 3.84–3.76 (6H, m, $NH(CH_2)_2$ $NHCOCHCH_2OH$), 3.31 (3H, s, $COCHNHCH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz,): $\delta = 165.1$ (CO, CONH), 158.0 (C, C-4), 143.9 (C, C-2), 141.1 (CH, C-9), 140.0 (CH, C-7), 128.8 (C, C-8), 126.1 (C, C-5), 120.3 (C, C-6), 117.0 (C, C-10), 99.9 (CH, C-3), 44.9 (CH, CHCO), 44.7 (CH_2, CH_2OH), 39.0 (CH_2, $NHCH_2$), 37.5 (CH_2, CH_2NHCO), 30.7 (CH_3, $NHCH_3$); ESI–MS (*m/z*): 323.1 $[M + H]^+$ (100); HRMS (*m/z*): 323.1261 $[C_{15}H_{19}ClN_4O_2 + H]^+$ (calcd 323.1269).

(S)-N-(2-(7-Chloroquinolin-4-ylamino)ethyl)-3-(4-methoxyphenyl)-2-(methylamino)propanamide (***26***) Gummy residue (The method for synthesis was the same as reported for compound 25. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-(4-methoxyphenyl)propanoic acid. It was obtained as gummy substance in 79 % yield.); ^1H NMR (CD_3OD, 300 MHz,): $\delta = 8.43$ (1H, d, $J = 7.0$ Hz, H-2); 8.33 (1H, d, $J = 9.0$ Hz, H-5), 7.90 (1H, d, $J = 1.9$ Hz, H-8), 7.72–7.70 (1H, dd, $J = 2.1$, 9.0 Hz, H-6), 7.10 (2H, d, $J = 8.4$ Hz, $CH_2(CH)_2C_4H_2$), 6.88 (2H, d, $J = 7.0$ Hz, $CH_2C_4H_2(CH)_2$), 6.71 (1H, d, $J = 5.3$ Hz, H-3), 4.07–4.03 (1H, m, $COCHCH_2C_6H_4OCH_3$), 3.62 (3H, s, $COCHCH_2$ $C_6H_4OCH_3$), 3.57–3.54 (4H, m, $NH(CH_2)_2NHCO$), 3.10–2.93 (2H, m, $COCHCH_2C_6H_4OCH_3$), 2.66 (3H, s, $NHCOCHNHCH_3$); ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 169.5$ (CO, CONH), 160.6 ($1C_{aromatic}$), 157.7 (C, C-4), 144.1 (C, C-2), 141.1 (CH, C-9), 140.0 (CH, C-7), 131.7, 128.9, ($3C_{aromatic}$), 126.8 (C, C-8), 126.5 (C, C-5), 120.3 (C, C-6), 117.0 (C, C-10), 115.3 ($2C_{aromatic}$), 100 (CH, C-3), 64.1 (CH, CHCO), 55.8 (CH_3, OCH_3), 44.8 (CH_2, $NHCH_2$), 38.7 (CH_2, CH_2NHCO), 36.8 (CH_2, CH_2Ph), 32.9 (CH_3, $NHCH_3$); ESI–MS (*m/z*): 413.2 $[M + H]^+$ (100); HRMS (*m/z*): 413.1733 $[C_{22}H_{25}ClN_4O_2 + H]^+$ (calcd 413.1739).

(2S,3R)-N-(2-(7-Chloroquinolin-4-ylamino)ethyl)-3-methoxy-2-(methylamino)butanamide (***27***) Gummy residue (The method for synthesis was the same as reported for compound 26. While the amino acid used was (2*S*,3*R*)-2-(tert-butoxycarbonyl(methyl)amino)-3-methoxybutanoic acid. It was obtained as gummy substance in 73 % yield.); ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 8.49$ (1H, d, $J = 5.6$ Hz, H-2), 8.42 (1H, d, $J = 8.8$ Hz, H-5), 7.89 (1H, d, $J = 1.7$ Hz, H-8), 7.73–7.71 (1H, dd, $J = 2.1$, 8.8 Hz, H-6), 6.69 (1H, d, $J = 6.4$ Hz, H-3), 3.53–3.38 (5H, m, $NH(CH_2)_2NHCOCHCHCH_3$), 3.17–3.10 (1H, m, $COCHCHCH_3OCH_3$), 3.02 (3H, s, $COCHCHCH_3OCH_3$), 2.29 (3H, s, $COCHNHCH_3$), 0.87 (3H, d, $J = 5.7$ Hz, $COCHCHCH_3OCH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz,): $\delta = 167.8$ (CO, CONH), 157.9 (C, C-4), 144.2 (C, C-2), 141.2 (CH, C-9), 140.0 (CH, C-7), 128.9 (C, C-8), 126.4 (C, C-5), 120.4 (C, C-6), 117.1 (C, C-10), 100.2 (CH, C-3), 76.3 (CH, $CHCH_3$), 68.2 (CH, CHCO), 57.2 (CH_3, OCH_3), 44.2 (CH_2, $NHCH_2$), 39.0 (CH_2, CH_2NHCO), 33.0 (CH_3, $NHCH_3$), 15.9 (CH_3, $CHCH_3$); ESI–MS (*m/z*): 350.1 $[M + H]^+$ (100); HRMS (*m/z*): 351.1588 $[C_{17}H_{23}ClN_4O_2 + H]^+$ (calcd 351.1582).

N-(2-(7-Chloroquinolin-4-ylamino) ethyl)-2-(methylamino)acetamide (***28***) White solid (The method for synthesis was the same as reported for compound 27. While the amino acid used was 2-(*tert*-butoxycarbonyl(methyl)amino)acetic acid. It was obtained as white solid in 89 % yield.); mp 206–208 °C; ^1H NMR (CD_3OD, 300 MHz): $\delta = 8.47$ (1H, d, $J = 5.3$ Hz, H-2), 8.43 (1H, d, $J = 8.9$ Hz, H-5), 7.89 (1H, d, $J = 1.7$ Hz, H-8), 7.72–7.69(1H, dd, $J = 1.7$, 9.0 Hz, H-6), 6.98 (1H, d, $J = 7.0$ Hz, H-3), 3.82 (2H, s, $NHCH_2CH_2NHCOCH_2$ $NHCH_3$), 3.79–3.62 (2H, m, $NHCH_2CH_2NHCOCH_2$ $NHCH_3$), 3.52–3.45(2H, m, $NHCH_2CH_2NHCOCH_2$ $NHCH_3$), 2.71 (3H, s, $COCH_2NHCH_3$); ^{13}C NMR (CD_3 OD, 50 MHz,): $\delta = 167.4$ (CO, CONH), 157.9 (C, C-4), 144.0 (C, C-2), 141.2 (CH, C-9), 131.6 (CH, C-7), 128.9 (C, C-8), 126.4 (C, C-5), 120.4 (C, C-6), 117.1 (C, C-10), 99.8 (CH, C-3), 50.7 (CH_3, $NHCH_3$), 44.3 (CH_2, CH_2CO), 38.9 (CH_2, $NHCH_2$), 33.7 (CH_2, CH_2NHCO); ESI–MS (*m/z*): 293.1 $[M + H]^+$ (100); HRMS (*m/z*): 293.1191 $[C_{14}H_{17}ClN_4O + H]^+$ (calcd 293.1164).

(S)-N-(2-(7-chloroquinolin-4-ylamino) ethyl)-2-(methylamino) propanamide (***29***) White solid (The method for synthesis was the same as reported for compound 28. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonyl(methyl)-amino)propanoic acid. It was obtained as white solid in 87 % yield.); mp 210–212 °C; ^1H NMR (CD_3OD,

300 MHz): $\delta = 8.46$ (1H, d, $J = 5.4$ Hz, H-2), 8.43 (1H, d, $J = 7.0$ Hz, H-5), 7.90 (1H, d, $J = 1.7$ Hz, H-8), 7.73–7.72 (1H, dd, $J = 1.8$, 8.8 Hz, H-6), 7.00 (1H, d, $J = 6.4$ Hz, H-3), 3.91–3.86 (1H, m, COC*H*CH_3NHCH_3), 3.84–3.76 (4H, m, NH(CH_2)$_2$NHCO), 2.64 (3H, s, COCHCH_3NHCH_3), 1.47 (3H, d, $J = 6.9$ Hz, COCH CH_3NHCH_3); ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 171.1$ (CO, CONH), 157.9 (C, C-4), 144.0 (C, C-2), 141.1 (CH, C-9), 140.0 (CH, C-7), 128.8 (C, C-8), 126.4 (C, C-5), 120.3 (C, C-6), 117.0 (C, C-10), 100.0 (CH, C-3), 68.2 (CH, $CHCH_3$), 44.2 (CH_2, $NHCH_2$), 38.9 (CH_2, CH_2 NHCO), 31.9 (CH_3, $NHCH_3$), 16.3 (CH_3, $CHCH_3$); ESI–MS (*m/z*): 307.1 $[M + H]^+$ (100); HRMS (*m/z*): 307.1324 $[C_{15}H_{19}ClN_4O + H]^+$ (calcd 307.1320).

(S)-N-(2-(7-Chloroquinolin-4-ylamino) ethyl)-3-methyl-2-(methylamino) butanamide (***30***) Gummy residue (The method for synthesis was the same as reported for compound 29. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-methylbutanoic acid. It was obtained as gummy residue in 85 % yield.); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.47$ (1H, d, $J = 5.6$ Hz, H-2), 8.17 (1H, d, $J = 8.9$ Hz, H-5), 7.78 (1H, d, $J = 2.0$ Hz, H-8), 7.49–7.46 (1H, dd, $J = 2.1$, 9.0 Hz, H-6), 6.68 (1H, d, $J = 5.5$ Hz, H-3), 3.42–3.34 (5H, m, NH(CH_2)$_2$ NHCOC*H*NHCH_3), 3.20 (3H, s, COCHNHCH_3), 2.09–1.98 (1H, m, COCHC*H*$(CH_3)_2$), 0.91–0.84 (6H, m, COCHCH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 50 MHz,): $\delta = 170.1$ (CO, CONH), 159.1 (C, C-4), 145.3 (C, C-2), 142.3 (CH, C-9), 141.2 (CH, C-7), 130.1 (C, C-8), 127.5 (C, C-5), 121.6 (C, C-6), 118.2 (C, C-10), 101.3 (CH, C-3), 69.4 (CH, NHCH), 45.5 (CH_2, $NHCH_2$), 39.9 (CH_2, CH_2-NHCO), 34.4 (CH_3, $NHCH_3$), 32.3 (CH, CH_3CHCH_3), 20.0 (CH_3, CH_3CHCH_3), 19.5 (CH_3, CH_3CHCH_3); ESI–MS (*m/z*): 335.1$[M + H]^+$(100); HRMS (*m/z*): 335.1634 $[C_{17}H_{23}ClN_4O + H]^+$ (calcd 335.1633).

(2S,3S)-N-(2-(7-Chloroquinolin-4-ylamino)ethyl)-3-methyl-2-(methylamino)pentanamide (***31***) Gummy residue (The method for synthesis was the same as reported for compound 30. While the amino acid used was (2*S*,3*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-methylpentanoic acid. It was obtained as gummy residue in 80 % yield.); ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 8.47$ (1H, d, $J = 5.8$ Hz, H-2), 8.42 (1H, d, $J = 8.8$ Hz, H-5), 7.89 (1H, d, $J = 1.7$ Hz, H-8), 7.74–7.71 (1H, dd, $J = 1.7$, 8.9 Hz, H-6), 7.00 (1H, d, $J = 6.9$ Hz, H-3), 3.79–3.71 (m, 4H, NH(CH_2)$_2$CO), 3.68 (3H, s, COCHNHCH_3), 3.57–3.53 (1H, m, C*H*CHCH_3CH_2CH_3), 1.50–1.38 (1H, m, CHC*H*CH_3CH_2 CH_3), 1.22–1.08 (2H, m, CHCHCH_3CH_2CH_3), 0.99–0.95 (3H, m, CHCHCH_3CH_2CH_3), 0.93–0.90 (3H, m, CHCHCH_3CH_2CH_3); ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 168.9$ (CO, CONH), 157.8 (C, C-4), 143.9 (C, C-2), 141.6 (CH, C-9), 140.0 (CH, C-7), 129.4 (C, C-8), 126.6 (C, C-5), 120.6 (C, C-6), 117.1 (C, C-10), 100.0 (CH, C-3), 68.3 (CH, NHCH), 44.4 (CH_2, $NHCH_2$), 39.0 (CH_2, CH_2 NHCO), 37.5 (CH, $CHCH_3$), 33.6 (CH_3, $NHCH_3$), 26.7 (CH_2, CH_2CH_3), 15.1 (CH, $CHCH_3$), 12.5 (CH_2, CH_2CH_3); ESI–MS (*m/z*): 349.2 $[M + H]^+$ (100); HRMS (*m/z*): 349.1794 $[C_{18}H_{25}ClN_4O + H]^+$ (calcd 349.1790).

(S)-N-(2-(7-Chloroquinolin-4-ylamino)ethyl)-4-methyl-2-(methylamino) pentanamide (***32***) Gummy residue (The method for synthesis was the same as reported for compound 31. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-4-methylpentanoic acid. It was obtained as gummy residue in 82 % yield.); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.67$ (1H, d, $J = 9.0$ Hz, H-2), 8.32 (1H, d, $J = 2.1$ Hz, H-5), 7.88 (1H, d, $J = 6.6$ Hz, H-8), 7.80–7.78 (1H, dd, $J = 2.0$, 8.9 Hz, H-6), 6.77 (1H, d, $J = 4.2$ Hz, H-3), 3.53–3.37 (5H, m, NH(CH_2)$_2$ NHCOC*H*NHCH_3), 2.64 (3H, s, COCHNHCH_3), 1.84–1.81 (1H, m, COCHCH_2C*H*$(CH_3)_2$), 1.65–1.62 (2H, m, COCHCH_2CH$(CH_3)_2$), 0.95 (6H, d, $J = 5.7$ Hz, COCHCH_2CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 50 MHz,): $\delta = 168.9$ (CO, CONH), 156.4 (C, C-4), 142.5 (C, C-2), 140.4 (CH, C-9), 138.7 (CH, C-7), 125.8 (C, C-8), 119.6 (C, C-5), 116.2 (C, C-6), 111.9 (C, C-10), 98.5 (CH, C-3), 67.3 (CH, NHCH), 60.7 (CH_2, $NHCH_2$), 43.1 (CH_2, $CHCH_2$), 38.9 (CH_2, CH_2NHCO), 31.6 (CH_3, $NHCH_3$), 24.9 (CH, $CHCH_3$), 23.1 (CH_3, CH_3CHCH$_3$), 22.0 (CH_3, CH_3 CHCH_3); ESI–MS (*m/z*): 349.1 $[M + H]^+$ (100); HRMS (*m/z*): 349.1795 $[C_{18}H_{25}ClN_4O + H]^+$ (calcd 349.1790).

(S)-N-(2-(7-Chloroquinolin-4-ylamino)ethyl)-2-(methylamino)-3-phenylpropa namide (***33***) Gummy residue (The method for synthesis was the same as reported for compound 32. While the amino acid used was (*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-phenylpropanoic acid. It was obtained as gummy residue in 91 % yield.); ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 8.45$ (1H, d, $J = 5.6$ Hz, H-2), 8.45 (1H, d, $J = 6.5$ Hz, H-5), 8.36 (1H, d, $J = 9.0$ Hz, H-8), 7.91–7.89 (1H, dd, $J = 2.0$, 8.9 Hz, H-6), 7.76–7.47 (5H, m, $CH_2C_6H_5$), 6.90 (1H, d, $J = 6.7$ Hz, H-3), 4.08–3.87 (1H, m, COC*H*NHCH_3), 3.53 (4H, s, NH(CH_2)$_2$NHCO), 3.22–3.08 (2H, m, COC$H_2C_6H_5$), 2.66 (3H, s, COCHNHCH_3); ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 166.9$ (CO, CONH), 153.7 (C, C-4), 150.3 (C, C-2), 149.2 (CH, C-9), 144.4 ($1C_{aromatic}$), 139.9 (CH, C-7), 139.3 (C, C-8), 138.2, 138.1 ($4C_{aromatic}$), 135.7 ($1C_{aromatic}$), 129.7 (C, C-5), 109.5 (C, C-6), 107.8 (C, C-10), 98.3 (CH, C-3), 73.4 (CH, NHCH), 46.9 (CH_2, $NHCH_2$), 42.6 (CH_2, CH_2 NHCO), 41.4 (CH_2, CH_2Ph), 40.0 (CH_3, $NHCH_3$); ESI–MS (*m/z*): 383.1 $[M + H]^+$ (100); HRMS (*m/z*): 383.1640 $[C_{21}H_{23}ClN_4O + H]^+$ (calcd 383.1633).

(S)-N-(2-(7-Chloroquinolin-4-ylamino)ethyl)-1-methylpyrrolidine-2-carboxamide (***35***) White solid (The method for

synthesis was the same as reported for compound 33. While the amino acid used was (*S*)-1-methylpyrrolidine-2-carboxylic acid. It was obtained as white solid in 91 % yield.); mp 195–197 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.44$ (1H, d, $J = 5.0$ Hz, H-2), 8.39 (1H, d, $J = 8.9$ Hz, H-5), 8.02 (1H, d, $J = 2.0$ Hz, H-8), 7.45–7.43 (1H, dd, $J = 2.1$, 8.9 Hz, H-6), 6.34 (1H, d, $J = 6.1$ Hz, H-3), 3.76–3.72 (2H, m, $NHCH_2CH_2NHCO$), 3.17–3.14 (1H, m, $NHCOCH(CH_2)_2CH_2NCH_3$), 3.12–3.02 (2H, m, $NHCH_2CH_2NHCO$), 2.43 (3H, s, $NHCO\,CH(CH_2)_2CH_2NCH_3$), 2.28–2.23 (2H, m, $NHCOCH\,(CH_2)_2CH_2NCH_3$), 1.75–1.68 (4H, m, $NHCOCH(CH_2)_2\,CH_2NCH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 178.0$ (CO, CONH), 151.4 (C, C-4), 150.4 (C, C-2), 148.5 (CH, C-9), 135.0 (CH, C-7), 127.9 (C, C-8), 125.5 (C, C-5), 122.2 (C, C-6), 117.1 (C, C-10), 98.0 (CH, C-3), 68.6, 56.6 ($2C_{pyrrole}$), 46.1 (CH_2, $NHCH_2$), 41.8 (CH_3, NCH_3), 38.3 (CH_2, CH_2NHCO), 31.1, 24.3 ($2C_{pyrrole}$); ESI–MS (*m/z*): 333.2 $[M + H]^+$ (100); HRMS (*m/z*): 333.1484 $[C_{17}H_{21}ClN_4O + H]^+$ (calcd 333.1477).

(S)-N-(2-(7-Chloroquinolin-4-ylamino) ethyl) pyrrolidine-2-carboxamide (***37***) Gummy residue (The method for synthesis was the same as reported for compound 35. While the amino acid used was (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid. It was obtained as gummy residue in 88 % yield.); ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 8.34$ (1H, d, $J = 5.6$ Hz, H-2), 8.01 (1H, d, $J = 8.8$ Hz, H-5), 7.65 (1H, d, $J = 2.0$ Hz, H-8), 7.29–7.26 (1H, dd, $J = 2.0$, 9.0 Hz, H-6), 6.44 (1H, d, $J = 5.4$ Hz, H-3), 3.64 (1H, s, $COCH(CH_2)_2CH_2NH$), 3.21 (2H, d, $J = 5.2$ Hz, $NHCH_2CH_2CO$), 2.68 (2H, s, $NHCH_2CH_2CO$), 2.03 (2H, t, $J = 1.7$ Hz, $COCH(CH_2)_2\,CH_2NH$), 1.77–1.36 (4H, m, $COCH(CH_2)_2CH_2NH$); ^{13}C NMR (DMSO-d_6, 50 MHz): $\delta = 168.9$ (CO, CONH), 156.1 (C, C-4), 143.1 (C, C-2), 138.9 (CH, C-9), 138.3 (CH, C-7), 127.1 (C, C-8), 126.7 (C, C-5), 119.2 (C, C-6), 116.0 (C, C-10), 98.9 (CH, C-3), 66.8, 59.3 ($2C_{pyrrole}$), 45.8 (CH_2, $NHCH_2$), 42.6 (CH_2, CH_2NHCO), 29.7, 24.0 ($2C_{pyrrole}$); ESI–MS (*m/z*): 319.1 $[M + H]^+$ (100); HRMS (*m/z*): 319.1327 $[C_{16}H_{19}ClN_4O + H]^+$ (calcd 319.1320).

Experimental procedure for the synthesis of final compounds 34 and 38

(S)-N^1-(2-(7-Chloroquinolin-4-ylamino)ethyl)-N^2,4-dimethylpentane-1,2-diamine (***34***) Gummy residue (Amide **32** (1.7 g, 5.34 mmol) in dry THF (15 mL) was added to a slurry of $NaBH_4$ (1 g, 27 mmol) in dry THF (15 mL) in a two-neck septum capped round-bottom flask. I_2 (3 g, 12 mmol) in dry THF (20 mL) was added under nitrogen atmosphere at 0 °C for 2.5 h. The mixture was refluxed for 6 h; cooled to 0 °C and the excess hydride was carefully destroyed with 3 N HCI (5 mL). After the gas evolution ceased, it was neutralized using 3 N NaOH (8 mL). The organic layer was separated and aqueous layer was extracted with ether (3 × 10 mL). The combined organic extracts were washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the amine product was purified by chromatography on silica gel column (hexane: ethyl acetate as 5:15). The fractions containing the desired compound according to TLC revealed under UV are pooled and evaporated. It was obtained as gummy residue in 76 % yield); ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 8.39$ (1H, d, $J = 6.5$ Hz, H-2), 7.70 (1H, d, $J = 8.8$ Hz, H-5), 7.68 (1H, d, $J = 2.0$ Hz, H-8), 7.59–7.56 (1H, dd, $J = 2.1$, 8.9 Hz, H-6), 6.58 (1H, d, $J = 6.4$ Hz, H-3), 4.02 (4H, d, $J = 6.5$ Hz, $NH(CH_2)_2$ NH), 3.85 (1H, s, $CH_2CHCH_2CH(CH_3)_2$), 3.69–3.55 (2H, m, $CH_2CHCH_2CH(CH_3)_2$), 2.67 (3H, s, $CH_2CHNHCH_3$), 1.72–1.68 (1H, m, $CH_2CHCH_2CH(CH_3)_2$), 1.55–1.51 (2H, m, $CH_2CHCH_2CH(CH_3)_2$), 0.93 (6H, d, $J = 8.7$ Hz, $CH_2CHCH_2CH(CH_3)_2$); ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 153.8$ (C, C-4), 152.8 (C, C-2), 148.9 (CH, C-9), 132.6 (CH, C-7), 128.8 (C, C-8), 124.8 (C, C-5), 121.6 (C, C-6), 117.3 (C, C-10), 98.7 (CH, C-3), 58.9 (CH, NHCH), 53.8 (CH_2, $NHCH_2CHNH$), 48.8 (CH_2, $NHCH_2$), 47.6 (CH_2, CH_2NHCH_2), 42.3 (CH_2, $CHCH_2CHNH$), 32.3 (CH_3, $NHCH_3$), 25.3 (CH, CH_3CHCH_3), 23.3 (CH_3, CH_3 $CHCH_3$), 22.1 (CH_3, CH_3CHCH_3); ESI–MS (*m/z*): 335.2 $[M + H]^+$ (100); HRMS (*m/z*): 335.1999 $[C_{18}H_{27}ClN_4 + H]^+$ (calcd 335.1997).

(S)-N^1-(7-Chloroquinolin-4-yl)-N^2-(pyrrolidin-2-yl-methyl)ethane-1,2-diamine (***38***) Gummy residue (The method for synthesis was the same as reported for compound 34. While the amide used was (*S*)-*N*-(2-(7-chloroquinolin-4-ylamino)ethyl)pyrrolidine-2-carboxamide. It was obtained as gummy substance in 73 % yield.); ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 8.64$ (1H, d, $J = 5.4$ Hz, H-2), 8.51 (1H, d, $J = 8.9$ Hz, H-5), 7.92 (1H, d, $J = 1.9$ Hz, H-8), 7.74–7.71 (1H, dd, $J = 2.0$, 8.8 Hz, H-6), 7.08 (1H, d, $J = 5.6$ Hz, H-3), 3.91–3.53 (4H, m, $NH(CH_2)_2NH$), 3.42 (1H, s, $CH_2CH(CH_2)_2CH_2NH$), 3.24–3.17 (2H, m, $CH_2CH(CH_2)_2CH_2NH$), 2.20–2.08 (2H, m, $CH_2CH(CH_2)_2CH_2NH$), 1.33–1.28 (4H, m, CH_2 $CH(CH_2)_2CH_2NH$); ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 153.3$ (C, C-4), 152.2 (C, C-2), 148.5 (CH, C-9), 133.4 (CH, C-7), 128.4 (C, C-8), 123.3 (C, C-5), 121.1 (C, C-6), 117.3 (C, C-10), 98.4 (CH, C-3), 60.2 ($1C_{pyrrole}$), 51.5 (CH_2, $NHCH_2$), 48.3 (CH_2, $NHCH_2CH_2NH$), 47.3 (CH_2, $NHCH_2CH_2NH$), 46.3, 30.6, 24.2 ($3C_{pyrrole}$); ESI–MS (*m/z*): 305.2 $[M + H]^+$ (100); HRMS (*m/z*): 305.1534 $[C_{16}H_{21}ClN_4 + H]^+$ (calcd 305.1528).

Biological assay

In vitro antimalarial assay

The compounds were evaluated for antimalarial activity against 3D7 (CQ-sensitive) and K1 (CQ-resistant) strains of *Plasmodium falciparum* using Malaria SYBR Green I nucleic acid staining dye-based fluorescence (MSF) assay as mentioned by Singh *et al.* (2011). The stock (10 mM) solution was prepared in DMSO, and test dilutions were prepared in culture medium (RPMI-1640-FBS). Chloroquine diphosphate (SIGMA) was used as reference drug. For assessment of antimalarial activity, 50 µl of culture medium was dispensed in 96-well plate followed by addition of 50 µl of highest concentration (containing less than 0.5 % of DMSO) of test compounds (in duplicate wells) in row B. Subsequent twofold serial dilutions were prepared in culture medium, and finally, 50 µl of 2.0 % parasitized cell suspension containing 0.8 % parasitaemia (Asynchronous culture containing more than 80 % ring stages) was added to each well except 4 wells in row 'A' received non-parasitized erythrocyte suspension. The plates were incubated at 37 °C in CO_2 incubator in an atmosphere of 5 % CO_2 and air mixture (Srivastava and Puri, 2009), 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 (FLX800, BIOTEK). Data were transferred into a graphic program (EXCEL), and IC_{50} values were obtained by logit regression analysis of dose–response curves using pre-programmed Excel spreadsheet. Three replicates were carried out for each compound.

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 in Sinha *et al.* (2014). The cells were incubated with compound dilutions for 72 h, MTT was used as reagent for detection of cytotoxicity, and 50 % cytotoxic concentration (CC_{50}) was determined using nonlinear regression analysis of dose–response curves using pre-programmed Excel spreadsheet. Selectivity index (SI) was calculated as

$$SI = CC_{50}/IC_{50}$$

Determination of association constant for hematin 4-aminoquinoline derivatives

Association constant for hematin 4-aminoquinoline derivatives complex formation was determined by spectrometric titration procedure in aqueous DMSO at pH-7.5 (Egan *et al.,* 1997). In this assay condition, hematin is strictly in monomeric state and interpretation of results is not complicated by the need to consider hematin disaggregation process. Association constant calculated in this technique is a good reflection of the interaction that would occur in the acidic food vacuole, and pH-7.5 improves the stability of hematin solutions and quality of the data.

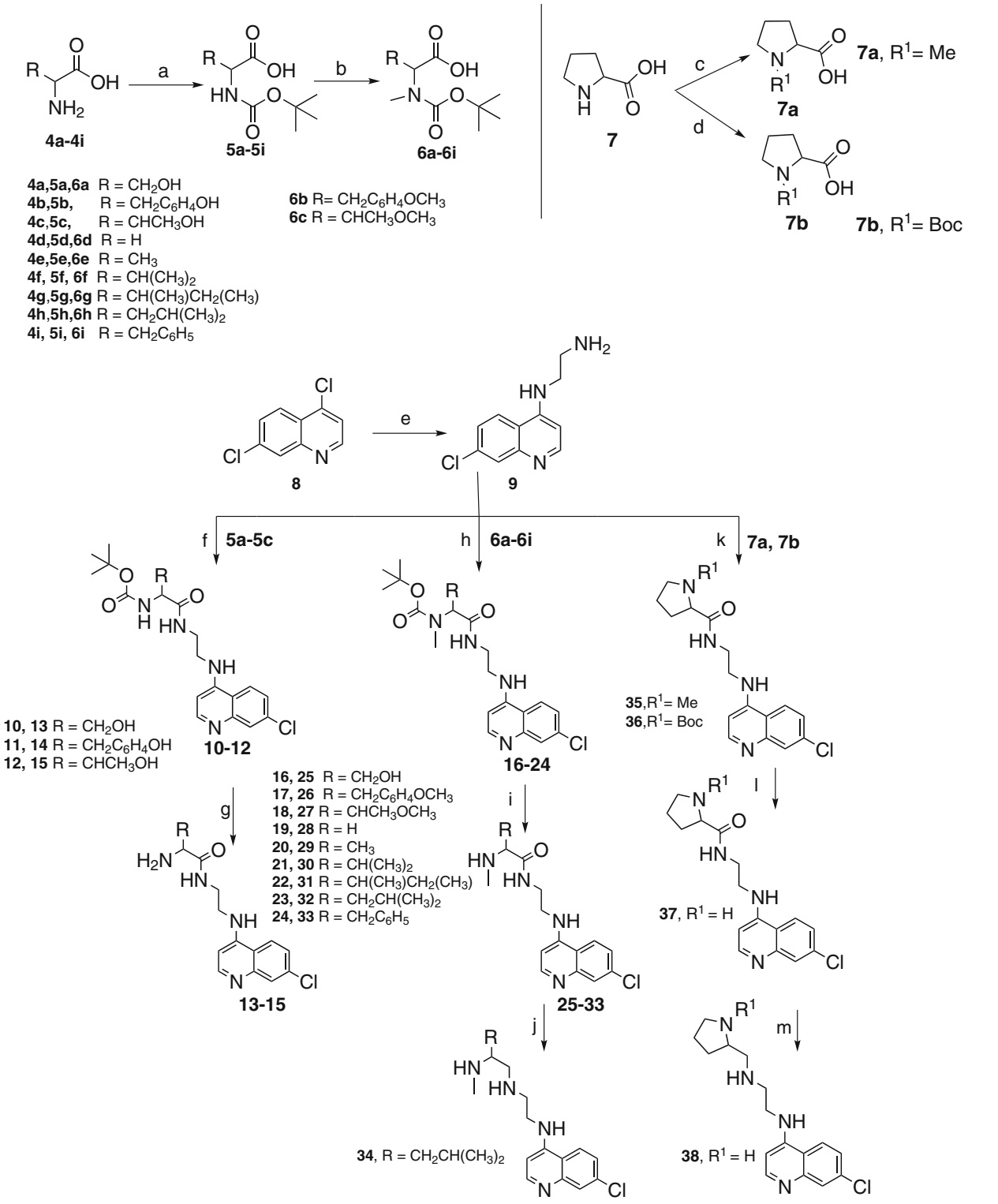
In vitro Inhibition of β-hematin formation assay

The ability of the 4-aminoquinoline derivatives to inhibit β-hematin formation was induced by 1-oleoyl-rac-glycerol. Spectroscopic measurements were done using UV spectrophotometer wave length 405 nm (Tripathi *et al.,* 2004). The IC_{50} values obtained from the assay are expressed as percent inhibition relative to β-hematin formation in a drug-free control. The 50 % inhibitory concentration values for the compounds were obtained from the sigmoidal dose–response curves using nonlinear regression curve fitting analyses with GraphPad Prism v.3.00 software (GraphPad Prism, 1999).

Results and discussion

Chemistry

Synthesis of targeted compounds **13–15** and **25–38** is outlined in scheme 1. The primary intermediates (**5a–5c**) and (**6a–6i**) were prepared by using reported protocol (Malkov *et al.*, 2009). The key intermediate N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**9**) was synthesized by reaction with 4,7-dichloroquinoline (**8**) and ethane-1,2-diamine through aromatic nucleophilic substitution in neat condition with standard workup procedure in excellent yields. Here, we performed nucleophilic substitution reaction in neat condition to avoid the use of phenol as a solvent, which is prone to polymerization (Sunduru *et al.*, 2009). The synthesis of intermediates **10–12** and **16–24** was achieved by coupling of suitably derivatized amino acids (**5a–5c**) and (**6a–6i**) with N^1-(7-chloroquinolin-4-yl) ethane-1,2-diamine (**9**) using the method reported by Sheehan and Hess (1955). Finally, deprotection of Boc group led to the desired compounds **13–15** and **25–33**. Same protocol was repeated for the synthesis of compounds **35** and **36** using **7a** and **7b**. Furthermore, compounds **34** and **38** are obtained by the peptide bond reduction of compounds **32** and **37,** respectively, using $I_2/NaBH_4$ in dry THF at 70 °C (Scheme 1) (Prasad *et al.*, 1992). Completion of the reaction was monitored by TLC using methanol–chloroform (1:9) as eluent. All the synthesized compounds were characterized by using ^{1}H NMR, ^{13}C NMR, mass spectrometry, and HRMS.



Scheme 1 Synthesis of 4-aminoquinolines-aminoacid conjugates. Reagents and Conditions**: a** $(Boc)_2O$, dioxane, water, (1:1) NaOH, 0 °C, 2 h, 97 %; **b** NaH, CH_3I, 0 °C, dryTHF, 24 h, 90–98 %; **c** 40 % aq HCHO, 10 % Pd/C, H_2 atm, overnight, methanol, 92 %; **d** $(Boc)_2O$, dioxane, water, (1:1) NaOH, 0 °C, 2 h, 84 %; **e** Amine, 80 °C, 1 h, 120–130 °C, neat, 6–8 h, 85 %; **f** DCC, HOBt, DMF, 0 °C, 4–6 h; **g** TFA/DCM(1:1), 4–6 h, r.t, 73–91 %; **h** DCC, HOBt, DMF, 0 °C, 4–6 h; **i** TFA/DCM(1:1), 4–6 h, r.t, 73–91 %; **j** $I_2/NaBH_4$, Dry THF, N_2 atm, 70 °C, 6–8 h, 76 %; **k** DCC, HOBt, DMF, 0 °C, 4–6 h, 90–91 %; **l** TFA/DCM(1:1), 4–6 h, r.t, 88 %; (m) $I_2/NaBH_4$, Dry THF, N_2 atm, 70 °C, 6–8 h, 73 %

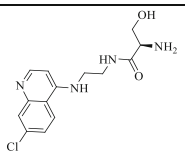
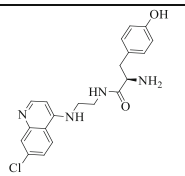
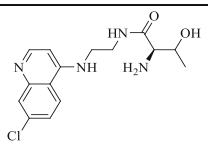
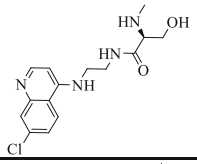
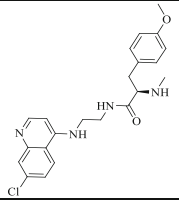
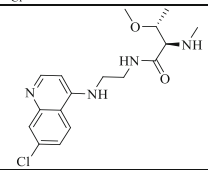
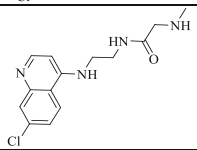
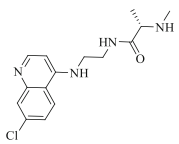
In vitro antiplasmodial activity

Initially, all the synthesized compounds **13–15**, **25–35,** and **37–38** were evaluated for in vitro antiplasmodial activity against the 3D7-chloroquine-sensitive (CQ-S) and K1-chloroquine-resistance (CQ-R) strains of *P. falciparum* using the method reported by Singh *et al.* (2011), and the IC_{50} values are presented in Table 1. The in vitro activity data (IC_{50}) revealed that derivatives having a basic nitrogen atom at the lateral side chain showed significant antimalarial activity. This suggests that the modification at the lateral side chain nitrogen atom is very well tolerated for antimalarial activity. Among the sixteen compounds tested, 6 compounds (**29–32**, **35,** and **37**) showed IC_{50} values in the range between 0.015–0.038 μM, 0.017, and 0.029 μM, respectively, and remaining ten compounds exhibited IC_{50} values ranging between 0.043 and 3.22 μM, against CQ-S strain of *P. falciparum*. Further, in case of CQ-R strain of *P. falciparum,* compounds **30**, **31**, **34**, **35,** and **38** showed IC_{50} values 0.28, 0.31, 0.18, 0.22, and 0.17 μM, respectively. Remaining compounds in the series exhibited IC_{50} values ranging between 0.55 and >1.0 μM. This difference in the activity profile can be associated with the factors such as methyl group substitution on the pendant amine group and presence of amine bond (–CH_2–NH–) in the lateral side chain obtained by reduction of amide (–CO–NH–) bond. Among the tested compounds, compounds **15** (IC_{50} = 0.058 μM (3D7)) and **37** (IC_{50} = 0.029 μM (3D7)) having pendant amino (NH_2) group showed promising activity against CQ-S strain of *P. falciparum.* These results are in consonance with the earlier activity data from our laboratory (Deshpande 2010). Further, with a view to increase the pK_a of lateral side chain which could help the molecule to show better activity against resistant strains of malaria, we introduced the methyl group at the pendant amine group resulting the compounds **27** (IC_{50} = 0.044 μM (3D7), >1.0 μM (K1)) and **35** (IC_{50} = 0.017 μM (3D7), 0.22 μM (K1)) with substantial increase in the activity against CQ-S strain of *P. falciparum*. It is important to mention here that latter compound exhibited slightly improved activity than CQ (IC_{50} = 0.255 μM (K1)) when screened against CQ-R strain of *P. falciparum*. Further, the decrease in the hydrophobicity of the side chain results the compound **29** (IC_{50} = 0.037 μM (3D7), >1.0 μM (K1)) with reduction in the activity against CQ-R strain, but showed promising activity against CQ-S strain. Additional decrease in the hydrophobicity causes the compound **28** (IC_{50} = 0.054 μM (3D7), 0.96 μM (K1)) with further reduction in the activity against both (3D7 & K1) strains of *P. falciparum*. Interestingly, when the propensity of the side chain changed to more lipophilic groups such as isopropyl (compound **30,** IC_{50} = 0.038 μM (3D7), 0.28 μM (K1)), *sec*-butyl **31** (IC_{50} = 0.015 μM (3D7), 0.31 μM (K1)), and isobutyl **32** (IC_{50} = 0.038 μM (3D7), 0.64 μM (K1)), we observed considerable enhancement in the activity against CQ-S and CQ-R strains of *P. falciparum*. Moreover, the two front runner compounds (**30** and **31**) showed comparable activity to CQ (IC_{50} = 0.255 μM (K1)) when screened against CQ-R strain of *P. falciparum*. Furthermore, the replacement of aliphatic side chain with more hydrophobic benzylic group results the compound **33** (IC_{50} = 0.058 μM (3D7), 0.55 μM (K1) with considerable decrease in the activity against both 3D7 and K1 strains of *P. falciparum*. However, the introduction of biologically privileged tyrosyl group results the compound **26** (IC_{50} = 0.052 μM (3D7)) with 2.4-fold increase in the activity against CQ-S strain as compared to its parent compound **14** (IC_{50} = 0.126 μM (3D7)). This activity may be due to difference in the pK_a of pendant amino group. Furthermore, it is reported in the literature that amide bond (–CO–NH–) is metabolically unstable in the biological milieu (Dalal *et al.,* 2012). Therefore, based on this affirmation and to enhance the metabolic stability of the molecule in the acidic food vacuole, we reduced the amide bond of some selected compounds, namely **32** (IC_{50} = 0.038 μM (3D7), 0.64 μM (K1)) and **37** (IC_{50} = 0.029 μM (3D7), > 1.0 μM (K1)) leading to reduced compounds **34** (IC_{50} = 0.043 μM (3D7), 0.18 μM (K1)) and **38** (IC_{50} = 0.11 μM (3D7), 0.17 μM (K1)), respectively. These two compounds **34** and **38** showed 3.6-fold and > 6-fold improved activity than **32** and **37**, respectively, against CQ-R strain of *P. falciparum.* The improved activities might be due to the enhanced lipophilic character of compounds **34** and **38**. So, it clearly demonstrates that lipophilicity is very important aspect for antiplasmodial activity. It may be appropriate to reveal here that resistance factor which is calculated as a ratio of IC_{50} in chloroquine-resistant (K1) vs chloroquine-sensitive (3D7) strains has been used as an index to measure chances of parasite developing resistance to a particular class of compounds. Consequently, it is believed that smaller the resistance factor, lesser the chance of developing resistance to that compound (Solomon *et al.,* 2010). Interestingly, all the compounds in the series displayed good resistance factor between 1.54 and >34.48 as against 51.0 for CQ (Table 1). Therefore, the compelling antimalarial activity exhibited by the present series of compounds appears to be promising for further lead optimization to obtain compounds active against drug-resistant parasites.

In vitro cytotoxicity

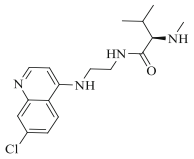
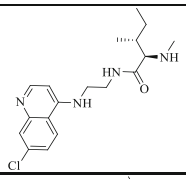
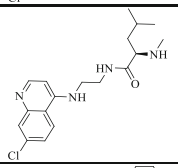
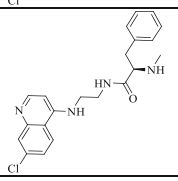
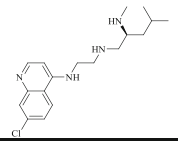
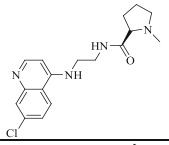
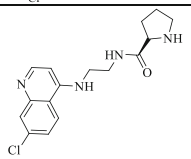
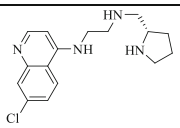
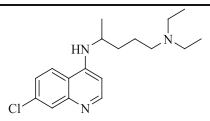
The cytotoxicity of all synthesized compounds **13–15**, **25–35,** and **37–38** was determined by MTT assay against VERO cell line (Table 1). Our target compounds showed selectivity index (SI) ranging from 12.08 to 13,242.66. Some compounds, namely **15**, **26–29**, **32,** and **33,** exhibited moderate activity against sensitive (3D7) strain with good selectivity

Table 1 Biological and Biophysical data of the synthesized compounds (**13–15),** (**25–35**) & (**37–38**)

Comp no	Structure	*In vitro* antiplasmodial activity IC_{50}(μM±SD)[a]		SI[b]	LogP[c]	Resistance factor[d]	Log K[e]	IC_{50}[f]
		3D7	K1					
13		0.244±0.021	>1.0±0.039	423.56	-0.24	>4.09	4.83±0.02	0.68±0.04
14		0.126±0.015	>1.0±0.045	698.80	1.90	>7.93	4.53±0.03	0.59±0.02
15		0.058±0.005	0.84±0.031	1472.58	0.08	14.48	4.84±0.02	0.69±0.02
25		3.22±0.041	ND	12.08	0.28	NA	4.21±0.03	0.81±0.04
26		0.052±0.004	>1.0±0.043	1120.57	2.69	>19.23	4.39±0.02	0.57±0.02
27		0.044±0.003	>1.0±0.044	4385.45	0.96	>22.72	4.79±0.03	0.66±0.02
28		0.054±0.005	0.96±0.029	>6325.37	0.65	17.77	4.63±0.02	0.70±0.02
29		0.037±0.003	>1.0±0.033	6723.24	1.14	>27.02	4.41±0.03	0.63±0.03

indices 1472.58, 1120.57, 4385.45, >6325.37, 6723.24, 5870.00, and 2607.24, respectively. Compounds **30**, **31**, **34**, **35,** and **37** have shown promising antiplasmodial activity against 3D7 as well as K1 strain and also showed better selectivity indices (SI) 4026.85, 13,242.66, 1844.65, 7746.47, and 7904.48, respectively. In general, most of the compounds of the series exhibited reasonable activity against 3D7 strain and less cytotoxic effect with fairly high selectivity index. Thus, these compounds have demonstrated the promising safe activity profile and deserve further optimization.

Table 1 continued

30		0.038±0.005	0.28±0.024	4026.84	2.02	7.36	4.71±0.02	0.35±0.02
31		0.015±0.002	0.31±0.016	13242.66	2.44	20.66	5.29±0.01	0.37±0.03
32		0.038±0.004	0.64±0.039	5870.00	2.37	16.84	4.63±0.02	0.53±0.04
33		0.058±0.003	0.55±0.043	2607.24	2.81	9.48	4.83±0.03	0.43±0.03
34		0.043±0.002	0.18±0.017	1844.65	2.97	4.18	5.32±0.01	0.19±0.02
35		0.017±0.002	0.22±0.019	7746.47	1.49	12.94	5.23±0.02	0.21±0.02
37		0.029±0.002	>1.00±0.035	7904.48	1.12	>34.48	5.18±0.02	0.64±0.03
38		0.11±0.003	0.17±0.002	631.00	1.71	1.54	4.97±0.02	0.20±0.03
CQ		0.005±0.002	0.255±0.049	8983	4.50	51.0	5.52±0.02	0.17±0.02

[a] IC_{50} (μM): Concentration corresponding to 50 % growth inhibition of the parasite

[b] Selectivity index (SI): (CC_{50} for cytotoxicity to vero cells/IC_{50}(3D7) for antiplasmodial activity)

[c] log *P* values calculated using ChemBioDraw ultra software

[d] Resistance factor (RI) = IC_{50} (K1)/IC_{50} (3D7)

[e] 1:1 (compound: Hematin) complex formation in 40 % aqueous DMSO, 20 mM HEPES buffer, pH 7.5 at 25 °C (data are expressed as mean ± SD from at least three different experiments in triplicate)

[f] The IC_{50} represents the millimolar equivalents of test compounds, relative to hemin, required to inhibit *β*-hematin formation by 50 % (data are expressed as mean ± SD from at least three different experiments in triplicate). *NA* Not applicable, *ND* Not done

In vitro inhibition of β-hematin polymerization

The mode of action of new series of 7-chloro-4-aminoquinoline derivatives **13–15**, **25–35,** and **37–38** was investigated by the method reported by Ekoue-Kovi *et al.* (2009), and the results are shown in Table 1. However, from the data (Table 1), compounds **34, 35,** and **38** formed strong complex with hematin and range of log K was found to be 4.97–5.32. Additionally, compound **25** showed weak binding effect to hematin because of its weak lipophilic character as compared to CQ and also exhibited weak antiplasmodial activity against CQ-S strain of *P. falciparum.* Among all the reported compounds in the present study, compound **34** exhibited very tight binding to hematin because of more lipophilic character and also showed significant antiplasmodial activity. These results are concurrent with the previous results from our laboratory (Solomon *et al.,*2007, 2005) as well as reported literature evidences (Egan *et al.,* 2000). The data suggest that the principle interaction may be hydrophobic, as well as electrostatic interactions between the 4-aminoquinoline and the porphyrin ring system, and plays a role in the hematin binding.

All the synthesized side chain-modified 4-aminoquinoline derivatives **13–15**, **25–35,** and **37–38** inhibited the β-hematin formation in a concentration-dependent manner (Table 1). However, most of the new series of 4-aminoquinoline derivatives were good inhibitors of β-hematin formation. It is reported that there was no linear correlation between inhibition of hemozoin formation and antiplasmodial activity (Solomon *et al.,* 2010). As reported, some of our compounds showed moderate antiplasmodial activity against CQ-S and CQ-R strains of *P. falciparum*, but exhibited better affect on inhibition of hemozoin formation (Table 1). In this present study, most potent inhibitor was compound **34** with an IC_{50} of 0.19 μM in the hemozoin inhibition assay and also showed good antiplasmodial activity in CQ-R strain.

Conclusion

The present study elaborates the synthesis and antiplasmodial activity of novel series of 4-aminoquinoline derivatives having *N*-methylated basic side chain secondary nitrogen. From the activity data of synthesized compounds, a few compounds have shown promising in vitro antiplasmodial activity, and some compounds showed superior antiplasmodial activity than CQ in the resistance strain (K1) of *P. falciparum*. The present findings are sufficient to establish that the basic nature of the side chain nitrogen is very much required to exhibit antimalarial activity of 4-aminoquinolines and opens up a new strategy for developing new antimalarial agents.

Acknowledgments One of the authors (K.S.R) thanks the CSIR, New Delhi, for Senior Research Fellowship. Authors thank the Director, CDRI, for the support, and the SAIF division for the spectral data. The CDRI Communication No is 9197.

Compliance with ethical standards

Conflicts of interest The authors have no conflict of interest to declare.

References

Burnett JC, Opsenica D, Sriraghavan K, Panchal RG et al (2007) A refined pharmacophore identifies potent 4-amino-7-chloroquinoline-based inhibitors of the botulinum neurotoxin serotype A metalloprotease. J Med Chem 50:2127–2136. doi:10.1021/jm061446e

Cheruku SR, Maiti S, Dorn A, Scorneaux B, Bhattacharjee AK, Ellis WY, Vennerstrom JL (2003) Carbon isosteres of the 4-aminopyridine substructure of chloroquine: effects on pK(a), hematin binding, inhibition of hemozoin formation, and parasite growth. J Med Chem 46:3166–3169. doi:10.1021/jm030038x

Dalal S, Ragheb DR, Klemba M (2012) Engagement of the S1, S1′ and S2′ subsites drives efficient catalysis of peptide bond hydrolysis by the M1-family aminopeptidase from Plasmodium falciparum. Mol Biochem Parasitol 183:70–77. doi:10.1016/j.molbiopara.2012.02.003

De D, Krogstad FM, Byers LD, Krogstad DJ (1998) Structure-activity relationships for antiplasmodial activity among 7-substituted 4-aminoquinolines. J Med Chem 41:4918–4926. doi:10.1021/jm980146x

Deshpande S (2010) Synthesis, biological evaluation and QSAR studies of novel antimalarial agents PhD Thesis, Jawaharlal Nehru University, New Delhi

Deshpande S, Solomon VR, Katti SB, Prabhakar YS (2009) Topological descriptors in modelling antimalarial activity: N(1)-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine as prototype. J Enzyme Inhib Med Chem 24:94–104. doi:10.1080/14756360801915377

Dorn A, Stoffel R, Matile H, Bubendorf A, Ridley RG (1995) Malarial haemozoin/beta-haematin supports haem polymerization in the absence of protein. Nature 374:269–271. doi:10.1038/374269a0

Egan TJ, Hunter R, Kaschula CH, Marques HM, Misplon A, Walden J (2000) Structure-function relationships in aminoquinolines: effect of amino and chloro groups on quinoline-hematin complex formation, inhibition of beta-hematin formation, and antiplasmodial activity. J Med Chem 43:283–291. doi:10.1021/jm990437l

Egan TJ, Mavuso WW, Ross DC, Marques HM (1997) Thermodynamic factors controlling the interaction of quinoline antimalarial drugs with ferriprotoporphyrin IX. J Inorg Biochem 68:137–145

Ekoue-Kovi K, Yearick K, Iwaniuk DP, Natarajan JK et al (2009) Synthesis and antimalarial activity of new 4-amino-7-chloroquinolyl amides, sulfonamides, ureas and thioureas. Bioorg Med Chem 17:270–283. doi:10.1016/j.bmc.2008.11.009

GraphPad Prism v3.0 (1999) GraphPad Software Inc., 10855 Sorrento Valley Rd. #203, San Diego. CA 92121

Hocart SJ, Liu H, Deng H, De D, Krogstad FM, Krogstad DJ (2011) 4-aminoquinolines active against chloroquine-resistant Plasmodium falciparum: basis of antiparasite activity and quantitative structure-activity relationship analyses. Antimicrob Agents Chemother 55:2233–2244. doi:10.1128/AAC.00675-10

Kaur K, Jain M, Reddy RP, Jain R (2010) Quinolines and structurally related heterocycles as antimalarials. Eur J Med Chem 45:3245–3264. doi:10.1016/j.ejmech.2010.04.011

Madrid PB, Wilson NT, DeRisi JL, Guy RK (2004) Parallel synthesis and antimalarial screening of a 4-aminoquinoline library. J Comb Chem 6:437–442. doi:10.1021/cc0340473

Malkov AV, Vrankova K, Cerny M, Kocovsky P (2009) On the selective N-methylation of BOC-protected amino acids The. J Org Chem 74:8425–8427. doi:10.1021/jo9016293

Na-Bangchang K, Karbwang J (2009) Current status of malaria chemotherapy and the role of pharmacology in antimalarial drug research and development. Fund Clin Pharmacol 23:387–409. doi:10.1111/j.1472-8206.2009.00709.x

Pandey AV, Bisht H, Babbarwal VK, Srivastava J, Pandey KC, Chauhan VS (2001) Mechanism of malarial haem detoxification inhibition by chloroquine. Biochem J 355:333–338

Prasad ASB, Kanth JVB, Periasamy M (1992) Convenient method for the reduction of amides, nitriles, carboxylic esters, acids and hydroboration of alkenes. Tetrahedron 48:4623–4628

Ray S, Madrid PB, Catz P, Levalley SE, Furniss MJ et al (2010) Development of a new generation of 4-aminoquinoline antimalarial compounds using predictive pharmacokinetic and toxicology models. J Med Chem 53:3685–3695. doi:10.1021/jm100057h

Ridley RG, Hofheinz W, Matile H, Jaquet C, Dorn A et al (1996) 4-aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant Plasmodium falciparum. Antimicrob Agents Chemother 40:1846–1854

Schlitzer M (2008) Antimalarial drugs—what is in use and what is in the pipeline. Arch Pharm 341:149–163. doi:10.1002/ardp.200700184

Sheehan JC, Hess GP (1955) A new method of forming peptide bonds. J Am Chem Soc 77:1067–1068. doi:10.1021/ja01609a099

Singh S, Srivastava RK, Srivastava M, Puri SK, Srivastava K (2011) In-vitro culture of Plasmodium falciparum: utility of modified (RPNI) medium for drug-sensitivity studies using SYBR Green I assay. Exp Parasitol 127:318–321. doi:10.1016/j.exppara.2010.08.007

Sinha M, Dola VR, Agarwal P, Srivastava K, Haq W, Puri SK, Katti SB (2014) Antiplasmodial activity of new 4-aminoquinoline derivatives against chloroquine resistant strain. Bioorg Med Chem 22:3573–3586. doi:10.1016/j.bmc.2014.05.024

Solomon VR, Haq W, Smilkstein M, Srivastava K, Puri SK, Katti SB (2010) 4-Aminoquinoline derived antimalarials: synthesis, antiplasmodial activity and heme polymerization inhibition studies. Eur J Med Chem 45:4990–4996. doi:10.1016/j.ejmech.2010.07.068

Solomon VR, Haq W, Smilkstein M, Srivastava K, Rajakumar S, Puri SK, Katti SB (2008) Synthesis and antimalarial activity of novel side chain modified antimalarial agents derived from 4-aminoquinoline. Med Chem 4:446–456. doi:10.2174/157340608785700207

Solomon VR, Haq W, Srivastava K, Puri SK, Katti SB (2007) Synthesis and antimalarial activity of side chain modified 4-aminoquinoline derivatives. J Med Chem 50:394–398. doi:10.1021/jm061002i

Solomon VR, Haq W, Srivastava K, Puri SK, Katti SB (2013) Design, synthesis of 4-aminoquinoline-derived thiazolidines and their antimalarial activity and heme polymerization inhibition studies. J Enzyme Inhib Med Chem 28:619–626. doi:10.3109/14756366.2012.666537

Solomon VR, Puri SK, Srivastava K, Katti SB (2005) Design and synthesis of new antimalarial agents from 4-aminoquinoline. Bioorg Med Chem 13:2157–2165. doi:10.1016/j.bmc.2004.12.051

Srivastava K, Puri S (2009) Recent Developments in Plasmodium falciparum in vitro model for Drug Discovery. Proc Natl Acad Sci, India 79:37–47

Stocks PA, Raynes KJ, Bray PG, Park BK, O'Neill PM, Ward SA (2002) Novel short chain chloroquine analogues retain activity against chloroquine resistant K1 Plasmodium falciparum. J Med Chem 45:4975–4983. doi:10.1021/jm0108707

Sunduru N, Sharma M, Srivastava K, Rajakumar S, Puri SK, Saxena JK, Chauhan PM (2009) Synthesis of oxalamide and triazine derivatives as a novel class of hybrid 4-aminoquinoline with potent antiplasmodial activity. Bioorg Med Chem 17:6451–6462. doi:10.1016/j.bmc.2009.05.075

Teixeira C, Vale N, Perez B, Gomes A, Gomes JR, Gomes P (2014) "Recycling" classical drugs for Malaria. Chem Rev 114(22):11164–11220. doi:10.1021/cr500123g

Tripathi AK, Khan SI, Walker LA, Tekwani BL (2004) Spectrophotometric determination of de novo hemozoin/beta-hematin formation in an in vitro assay. Anal Biochem 325:85–91. doi:10.1016/j.ab.2003.10.016

Vasanth RD, Srinivasarao K, Katti SB (2014) Recent developments in the side chain modified 4-aminoquinolines as antimalarial agents. Chem Biol Interface 4:206–222

Wellems TE, Plowe CV (2001) Chloroquine-resistant malaria. J Infect Dis 184:770–776. doi:10.1086/322858

World Malaria Report (WHO) (2014) http://www.who.int/malaria/publications/world_malaria_report_2014/en/