Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 2157–2165
Bioorganic & Medicinal Chemistry

Design and synthesis of new antimalarial agents from 4-aminoquinoline

V. Raja Solomon,[a] Sunil K. Puri,[b] Kumkum Srivastava[b] and S. B. Katti[a,*]

[a]*Division of Medicinal and Process Chemistry, Central Drug Research Institute, Lucknow 226 001, India*
[b]*Division of Parasitology, Central Drug Research Institute, Lucknow 226 001, India*

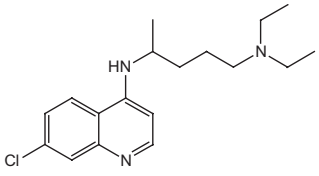
Received 8 December 2004; revised 28 December 2004; accepted 29 December 2004
Available online 21 January 2005

Abstract—This study describes the synthesis of new 4-aminoquinoline derivatives and evaluation of their activity against a chloroquine sensitive strain of *P. falciparum* in vitro and chloroquine resistant N-67 strain of *P. yoelii* in vivo. All the analogues were found to form strong complex with hematin and inhibit the β-hematin formation in vitro. These results suggest that these compounds act on heme polymerization target.

1. Introduction

Malaria remains one of the most widespread diseases in the world, affecting mainly the population living in tropical and subtropical areas. Approximately 40% of the world population lives in malaria endemic areas. Every year, 300–500 million people suffer from acute malaria and 0.5–2.5 million die from this disease.[1] Over the years chloroquine (CQ) has remained the drug of choice for malaria chemotherapy because it is highly effective, less toxic and cheap drug.[2] During the past two decades emergence of drug resistance has severely limited the choice of available antimalarial drugs. The biochemical studies on CQ and closely related 4-aminoquinoline antimalarial compounds suggested that accumulation of the chemotherapeutic agents in the parasite vacuole is critical for their antimalarial activity.[3] However, there are conflicting reports in the literature on the significance of vacuolar accumulation ratio (VAR) and cellular accumulation ratio (CAR) in terms of antimalarial activity.[3d] Studies on the mechanism underlying the drug resistance have indicated that the resistance is consequence of decreased accumulation of the drug as a result of enhanced efflux, reduced uptake or a combination of both.[4] This class of compounds enter the food vacuole and inhibit the parasite growth by forming complex with hematin (Fe(III)FPIX) (π–π interaction) thereby inhibiting the haemozoin formation.[5] Accumulation of hematin is detrimental to the parasite survival.

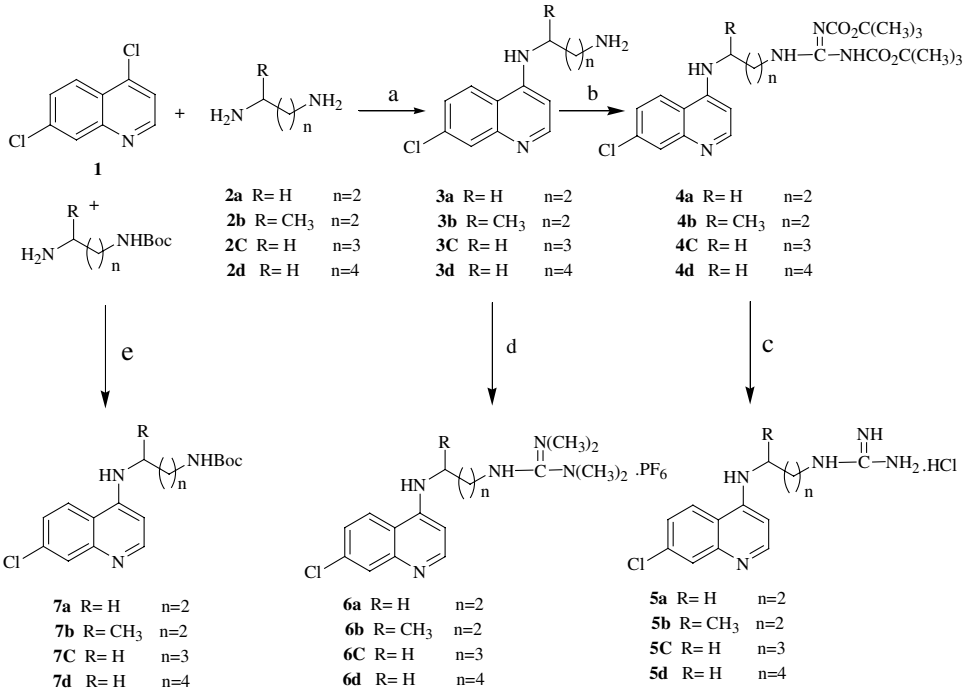
Recent reports have shown that close analogues of CQ and its derivatives are active against CQ-resistant parasite strains,[6] strongly suggesting that the resistance mechanism does not involve any change to the target of this class of drug but involves a compound specific resistance. Based on this premise a number of groups have developed short-chain analogues of quinoline derivatives, which are significantly more potent than CQ against a CQ-resistant strain of *P. falciparum* in in vitro studies.[7] These findings have given impetus to the antimalarial drug discovery programme further augmenting the realization that rational choice of inputs based on known antimalarial scaffold could lead to molecules with desirable activity profile. Encouraged by these results we designed new compounds by selectively modifying the pendant amino group (Scheme 1) with a view to facilitate, (i) their accumulation in the food vacuole, and (ii) achieve better interaction with hematin leading to improved antimalarial activity. The results are described in this communication.



Chloroquine (CQ)

Keywords: 4-Aminoquinoline; Antimalarial; Heam binding.
* Corresponding author. Tel.: +91 0522 2220586; fax: +91 0522 223405; e-mail: setu_katti@yahoo.com

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.12.051



Scheme 1. Reagents and conditions: (a) 80 °C for 1 h, 120–130 °C for 6–8 h; (b) 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudurea, THF, 60 °C; (c) 20% dioxane/HCl, rt, 1 h; (d) HBTU, dry CH_3CN, rt, 8 h; (e) 80 °C for 1 h, 120–130 °C for 6–8 h.

2. Chemistry

4-Aminoquinoline (**3a**–**d**) were prepared by aromatic nucleophilic substitution on 4,7-dichloroquinoline **1** with excess of diaminoalkane in neat conditions with simple standard workup procedure in excellent yields. Here we performed nucleophilic substitution in neat amine to avoid the use of phenol as a solvent, which is prone to polymerization.[8] The guanidine derivatives (**5a**–**d**) were prepared by refluxing 4-aminoquinoline with guanylating reagents like 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudurea in 2% aqueous THF. The Boc-protected derivatives (**4a**–**d**) thus obtained were deblocked with 20% HCl/dioxane. Tetramethylguanidine derivatives (**6a**–**d**) were synthesized in a single step, by the reaction of 4-aminoquinoline with HBTU (2-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate in dry acetonitrile solvent at room temperature stirring. These compounds were obtained as PF_6 salt. This was confirmed by ^{31}P NMR studies wherein, a septet at −159.73 to −194.70 ($J = 704$) corresponding to the PF_6 was observed in all these compounds (**6a**–**d**). Mono Boc-protected 4-aminoquinoline were synthesized by aromatic nucleophilic substitution of 4,7-dichloroquinoline with excess of mono Boc-protected diaminoalkane. The compounds reported in this study have been thoroughly characterized by elemental analysis and spectral data.

3. Result and discussion

A recent study by Ridley et al.[6a] has shown that 4-aminoquinoline analogues with altered chain length exhibit antimalarial activity against CQ-resistant strains of *P. falciparum*. Despite improved in vitro activity these compounds were less active than CQ in the in vivo model. This was attributed to rapid N-dealkylation in the biological milieu. On the basis of these results we surmised that appropriate modification of the side chain amino group could give compounds with improved antimalarial activity. Accordingly, *tert*-butoxycarboxyl (Boc), guanyl and tetramethylguanyl moieties were introduced at the side chain amino group of the 4-aminoquinoline. All compounds (Table 1) were tested for in vitro antimalarial activity against the CQ sensitive NF-54 strain of *P. falciparum*. The activity data (Table 1) clearly suggest that compounds with four carbon chain in the side chain (**4d**, **5d**, **6d**, **7d**) are more active than the compounds with three (**4c**, **5c**, **6c**, **7c**) or two (**4a**, **4b**, **5a**, **5b**, **6a**, **6b**, **7a**, **7b**) carbon chain. Furthermore the data also suggest that the compounds having Boc protecting group (**7a**–**d**) were more active than corresponding amino compounds (**3a**–**d**). This is because under assay conditions Boc derivatives of 4-aminoquinoline (**7a**–**d**) and 4-aminoquinoline guanidine (**4a**–**d**) do not undergo protonation and this protecting group is hydrophobic. As a result they have proclivity to diffuse freely and rapidly across the biological membrane and are transformed to mono or diprotonated derivatives within the parasite food vacuole. Once they are protonated they are less permeable, and are trapped in the food vacuole resulting in increased drug accumulation at the target site. It has been shown previously that accumulation of the compound at the target site viz parasite food vacuole has a direct bearing on the antimalarial activity.[3d] The level of accumulation depends on the pK_a of side chain nitrogen.[5d] Therefore, we hypothesized that a systematic change in the pK_a of the side chain amino group could provide insight into the role of amino nitrogen with respect to pK_a,

Table 1. Biological and biophysical data of the compounds

C. No.	MIC (μM)	Log *P*	pK_{a1}	pK_{a2}	VAR	CAR	Log *K*[a]	IC_{50}[b]
3a	9.02	1.63	7.85	10.00	62882.12	2012.23	5.99 ± 0.01	0.23 ± 0.08
4a	1.08	3.59	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
5a	6.66	1.33	8.00	13.83	63095.70	2019.06	5.56 ± 0.02	0.35 ± 0.02
6a	21.50	2.15	8.53	16.28	63095.73	2019.06	5.70 ± 0.04	1.00 ± 0.05
7a	6.21	3.18	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
3b	4.24	2.17	8.03	10.00	62900.20	2012.81	6.02 ± 0.05	0.46 ± 0.15
4b	1.05	4.12	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
5b	31.83	1.86	8.18	13.83	63095.71	2019.06	6.32 ± 0.01	0.49 ± 0.03
6b	20.84	2.69	8.71	16.32	63095.73	2019.06	7.26 ± 0.02	1.23 ± 0.02
7b	2.98	3.72	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
3c	8.49	1.99	8.08	10.15	62984.20	2015.49	6.24 ± 0.03	0.50 ± 0.06
4c	1.05	3.94	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
5c	6.36	1.68	8.23	14.00	63095.72	2019.06	6.04 ± 0.01	0.69 ± 0.05
6c	104.20	2.52	8.23	16.10	63095.73	2019.06	6.48 ± 0.02	0.86 ± 0.06
7c	5.96	3.54	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
3d	8.00	2.50	8.76	10.15	62979.32	2015.34	6.45 ± 0.02	0.51 ± 0.11
4d	1.02	4.45	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
5d	6.09	2.19	8.76	14.00	63095.72	2019.06	7.14 ± 0.03	0.62 ± 0.02
6d	20.25	3.02	8.76	16.42	63095.73	2019.06	7.65 ± 0.04	1.19 ± 0.07
7d	1.42	4.05	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
CQ	0.39	4.72	8.41	10.27	63002.78	2016.08	5.52 ± 0.02	0.17 ± 0.02

[a] 1:1 complex formation in 40% aqueous DMSO, 20 mM HEPES buffer, pH 7.5 at 25 °C (data are expressed as means ± SD from at least three different experiments in duplicate).

[b] 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 means ± SD from at least three different experiments in duplicate).

[c] The values not determined.

drug accumulation viz-a-viz antimalarial activity. Towards this objective compounds having guanyl (**5a**–**d**) and tetramethylguanyl (**6a**–**d**) derivatives were synthesized. It may be appropriate to mention here that guanidine group is a privileged pharmacophore found in many biologically active compounds.[9] Guanidine itself, the imine of urea has strongly basic character (pK_a of guanidinium ion approximately 13.5). Due to their strongly basic character, guanidines are fully protonated under physiological conditions. The positive charge thus imposed on the molecules forms the basis for specific interaction between ligand and receptor or enzyme and substrate, mediated by hydrogen bonds or electrostatic interactions.[10] In this particular case it was expected that compounds with guanyl (**5a**–**d**) and tetramethylguanyl (**6a**–**d**) moieties would form tighter association complex with hematin leading to improved antimalarial activity. Since such a modification would alter pK_a of the side chain nitrogen in turn effecting the accumulation of the compound at the target site. Therefore we have calculated the log *P*, pK_{a1} and pK_{a2} values of these compounds using Pallas.[11] The data presented in Table 1 clearly showed that introduction of guanyl (**5a**–**d**) and tetramethylguanyl (**6a**–**d**) moieties increased the pK_a values. Although in some cases there is no direct correlation between drug accumulation and antimalarial activity, VAR and CAR could be considered while designing the compounds. Accordingly with the help of pK_a values we have calculated the VAR and CAR. As expected introduction of guanyl moiety has increased the pK_a values as compared to CQ while there is marginal increase in the VAR and CAR values. Therefore it was expected that guanylated compounds to accumulate at higher concentration within the parasite food vacuole. However the antimalarial activity of these compounds (**5a**–**d**, **6a**–**d**) is much lower than CQ suggesting that the pK_a values must be in the range of 8.41 (pK_{a1}) and 10.27 (pK_{a2}) in order to exhibit maximum activity.

The ability of the 4-aminoquinoline guanidine (**5a**–**d**) and tetramethylguanidine (**6a**–**d**) derivatives to form association complex with Fe(III)FPIX was investigated by UV spectrophotometer. The results are shown in Table 1. Boc-protected derivatives of 4-aminoquinoline guanidine (**4a**–**d**) and 4-aminoaquinoline (**7a**–**d**) will transform (according to weak base effect) to corresponding free amino compounds. Therefore, no attempt was made for the determination of association constant and inhibition of β-hematin formation for these compounds. The data shows that all the compounds bind or interact with Fe(III)FPIX and form a complex, in the range of 5.56–7.65. Because of marginally higher accumulations and strongly basic character of these compounds there is a tight binding to Fe(III)FPIX. These results explain that the principle interaction might be involving π–π stacking interaction of the quinoline ring with the porphyrin ring system. The second weak electrostatic interaction is of the positively charged centre of guanidine and tetramethylguanidine derivatives with carboxyl groups of hematin. All the 4-aminoquinoline guanidine (**5a**–**d**) and tetramethylguanidine (**6a**–**d**) derivatives inhibited β-hematin formation (Table 1) in a concentration-dependent manner. Data suggest that this class of compound binds to hematin monomer or hematin μ-oxo dimers. This binding inhibits hematin polymerization by shifting hematin dimerization

Table 2. In vivo antimalarial activity against CQ resistant N-67 strain of *Plasmodium yoelii* in Swice mice

C. No.	% Suppression on day 4[a]	Mean survival time (MST in days) ± SE[b]
4d	76.08	15.80 ± 1.24
5d	88.98	14.80 ± 1.77
6d	73.92	14.67 ± 1.76
5c	56.72	16.00 ± 1.92
CQ	100.00	All animals survived
Control	—	13.20 ± 1.07

[a] Percent suppression = $[(C-T)/C] \times 100$; where C = parasitaemia in control group, and T = parasitaemia in treated group.
[b] MST calculated for the mice which died during 28-day observation period and the mice which survived beyond 28 days are excluded.

equilibrium to the μ-oxo dimer, thus reducing the availability of monomeric hematin for incorporation into hemozoin. Although none of the new derivatives were more potent than CQ the study has provided substantial evidence to highlight the importance of pK_a, drug accumulation and formation of association complex with β-hematin on the overall antimalarial activity of this class of compounds.

The most effective analogues (**4d**, **5d**, **6d**, **5c**) were selected for in vivo bio evaluation against CQ-resistant N-67 strain of *P. yoelii* in Swiss mice at 30.0 mg/kg by intraperitoneal route (Table 2). The in vivo activity exhibited by these compounds is much less than CQ. It is possible to improve the in vivo activity by fine tuning the modifications at the side chain amino group of the 4-aminoquinoline moiety.

4. Conclusion

In summary, the synthesis of a new series of 4-aminoquinoline derivatives has been described. These derivatives have exhibited promising antimalarial activity against CQ sensitive strain of NF-54 in in vitro and CQ resistant N-67 strain of *P. yoelii* in vivo. The order of activity appears to be Boc-protected (**4a–d**) compounds are more active than the guanylated compounds. The present biophysical studies have suggested that this class of compounds form association complex with hematin and thereby inhibit the hemozoin formation. The pK_a of second nitrogen in the range of 10.27 is critical for activity. Either increase or decrease in the pK_a beyond this limit has adverse effect on the antimalarial activity.

5. Experimental

Melting points (mp) were taken in open capillaries on Complab melting point apparatus and are uncorrected. Elemental analysis was performed on a Perkin–Elmer 2400 C,H,N analyzer and values were within the acceptable limits of the calculated values. Infrared (IR) spectra were recorded on an FT-IR Perkin–Elmer spectrometer. The ^{1}H spectra were recorded on a DPX-200/DRX-300 MHz Bruker FT-NMR spectrometer using $CDCl_3$, CD_3OD, CD_3CN and DMSO-d_6 as solvent. The chemical shifts were reported as parts per million (δ ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). The progress of the reaction was monitored on readymade 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. Chromatographic purification was performed over silica gel (100–200 mesh). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt. Ltd (India) and were used without further purification.

6. Biological and physicochemical studies

6.1. In vitro antimalarial efficacy test

The in vitro antimalarial assay was carried out in 96 well-microtitre plates. The cultures of *P. falciparum* NF 54 strain are routinely maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, the initial ring stage parasitaemia of 0.8–1.5% at 3% haematocrit in a total volume of 200 μL of medium RPMI-1640 was uniformly maintained. The test compound in 20 μL volume concentrations ranging between 0.5 and 50 μg/mL in duplicate well were incubated with parasitized cell preparation at 37 °C in a candle jar. After 36–40 h incubation, the blood smears from each well prepared and stained with giemsa stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in the presence of different concentrations. The test concentrations which inhibited the complete maturation into schizonts were recorded as the minimum inhibitory concentration (MIC). CQ was used as the standard reference drug.

6.2. In vivo antimalarial efficacy test

The in vivo efficacy of test compounds was evaluated against CQ resistant N-67 strain of *P. yoelii* in Swiss mice model at 30.0 mg/kg/day. The mice (22 ± 2 g) were inoculated with 1×10^6 parasitized RBC on day 0 and treatment was administered to a group of five mice from day 0 to 3, once daily. The aqueous suspension of compounds were prepared with a few drops of Tween 80. The required drug dose was administered in 0.2 mL volume via intraperitoneal route. Parasitaemia level were recorded from thin blood smears between days 4 and 28.[12] The mean value determined for a group of 5 mice was used to calculate the percent suppression of parasitaemia with respect to the untreated control group. Mice treated with CQ served as positive controls.

6.3. Determination of log P and pK_a values

The log P and pK_a values of 4-aminoquinoline guanidine (**5a–d**) and tetramethylguanidine (**6a–d**) derivatives were

calculated by Pallas.[11] In the case of pK_a two values were obtained. These data presented in Table 1 show pK_a of quinoline N (the acid dissociation constant of the quinolinuim cation and referred to below as pK_{a1}) and pK_a of tertiary amino group in the lateral chain (the acid dissociation constant of the tertiary ammonium group in the lateral side chain is referred to pK_{a2}). With the help of these values we predicted theoretical vacuolar accumulation ratio (VAR) by the following equation.[3d]

$$\mathrm{VAR} = \frac{1 + 10^{(pK_{a1} - pHv)} + 10^{(pK_{a1} + pK_{a2} - 2pHv)}}{1 + 10^{(pK_{a1} - pHo)} + 10^{(pK_{a1} + pK_{a2} - 2pHo)}}$$

This equation proceeds from a derivation of the Henderson–Hasselbach equation, where pHv = pH inside the vacuole (assumed to be pH 5.0) pHo = pH externally (assumed to be pH 7.4).

Making the assumptions that the parasites acid vacuole occupies 3.2% of its total cell volume, corresponding cellular drug accumulation ratio's (CARs) for each cell type were then calculated using the following equation.[3d]

$$\mathrm{CAR} = \mathrm{VAR} \times \text{Fractional cell volume occupied by acid vacuoles}$$

6.4. Determination of hematin-4-aminoquinoline guanidine and tetramethylguanidine derivatives association constant

Association constant for hematin-4-aminoquinoline guanidine (**5a**–**b**) and tetramethylguanidine (**6a**–**d**) derivatives complex formation were determined by spectrometric titration procedure in aqueous dimethylsulfoxide (DMSO) at pH 7.5.[13] The major advantage of this titration method is that, in this condition Fe(III)PPIX is strictly in monomeric state and interpretation of results is not complicated by the need to consider Fe(III)PPIX disaggregation process. Association constant measured in this method is a good reflection of the interaction would occur in the acidic food vacuole. Utilizing a pH of 7.5, rather than more acidic conditions, improves the stability of Fe(III)PPIX solutions and quality of data.

6.5. In vitro inhibition of β-hematin polymerization

The ability of the 4-aminoquinoline guanidine (**5a**–**d**) and tetramethylguanidine (**6a**–**d**) derivatives to inhibit β-hematin polymerization was induced by parasite lysate using UV spectrophotometer and measurements were carried out at 405 nm.[14] The values obtained from the assay were expressed as percent inhibition relative to hemozoin formation in a drug-free control. The values of triplicate assays were plotted semi-logarithmically on GraphPad Prism 3.5 and the IC_{50} values (mM) calculated graphically ±SD (standard deviation).

6.6. General synthetic procedure for N^1-(7-chloroquinolin-4-yl)diaminoalkane (3a–d)

A mixture of 4,7-dichloroquinoline **1** (2.5 g, 12.5 mmol) and 1,2-diaminoalkane (25 mmol) was heated slowly from room temp 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 temp 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.

6.6.1. N^1-(7-Chloroquinolin-4-yl)ethane-1,2-diamine (3a). This compound was obtained as a yellowish white solid in 85% yield. Mp 131–132 °C; IR (KBr) 3327.2 cm^{-1}; 1583.2 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.09–3.15 (m, 4H, C*H*$_2$), 3.27 (br s, 2H, N*H*$_2$), 5.82 (br s, 1H, N*H*), 6.38–6.41 (d, J = 5.34 Hz, 1H, 3*H* quinoline), 7.32–7.37 (dd, J = 8.96, 1.72 Hz, 1H, 6*H* quinoline), 7.73–7.77 (d, J = 8.92 Hz, 1H, 5*H* quinoline), 7.94–7.95 (d, J = 1.92 Hz, 1H, 8*H* quinoline), 8.49–8.52 (d, J = 5.34 Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 222 $[M+H]^+$; Anal. Calcd for $C_{11}H_{12}ClN_3$: C, 59.60; H, 5.46; N, 18.95; found: C, 60.01; H, 5.56; N, 19.00.

6.6.2. N^2-(7-Chloroquinolin-4-yl)-propane-1,2-diamine (3b). This compound was obtained as a yellowish white solid in 82% yield. mp 126–127 °C; IR (KBr) 3342.3 cm^{-1}; 1581.9 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.23–1.26 (d, J = 6.31 Hz, 3H, C*H*$_3$), 1.59 (br s, 2H, N*H*$_2$), 2.94–3.04 (m, 2H, C*H*$_2$), 3.25–3.36 (m, 1H, CH$_3$–C*H*–), 5.62 (br s, 1H, N*H*), 6.36–6.39 (d, J = 5.38 Hz, 1H, 3*H* quinoline), 7.32–7.38 (dd, J = 8.92, 2.02 Hz, 1H, 6*H* quinoline), 7.71–7.76 (d, J = 8.94 Hz, 1H, 5*H* quinoline), 7.94–7.95 (d, J = 2.1 Hz, 1H, 8*H* quinoline), 8.49–8.52 (d, J = 5.36 Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 236 $[M+H]^+$; Anal. Calcd for $C_{12}H_{14}ClN_3$: C, 61.15; H, 5.99; N, 17.83; found: C, 61.20; H, 5.98; N, 17.80.

6.6.3. N^1-(7-Chloroquinolin-4-yl)-propane-1,3-diamine (3c). This compound was obtained as a yellowish white solid in 88% yield. mp 96–98 °C; IR (KBr) 3328.7 cm^{-1}; 2236.7 cm^{-1}; 1587.5 cm^{-1}; 1216.4 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.90–1.93 (m, 2H, C*H*$_2$), 2.76 (br s, 2H, N*H*$_2$), 2.99–3.05 (m, 2H, C*H*$_2$), 3.32–3.40 (m, 2H, C*H*$_2$), 6.29–6.32 (d, J = 5.30 Hz, 1H, 3*H* quinoline), 7.26–7.30 (dd, J = 8.78, 1.96 Hz, 1H, 6*H* quinoline), 7.38 (br s, 1H, N*H*), 7.68–7.73 (d, J = 8.88 Hz, 1H, 5*H* quinoline), 7.91–7.92 (d, J = 2.02 Hz, 1H, 8*H* quinoline), 8.46–8.48 (d, J = 5.29 Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 236 $[M+H]^+$; Anal. Calcd for $C_{12}H_{14}ClN_3$: C, 61.15; H, 5.99; N, 17.83; found: C, 61.15; H, 6.00; N, 17.91.

6.6.4. N^1-(7-Chloroquinolin-4-yl)-butane-1,4-diamine (3d). This compound was obtained as a yellowish white solid in 76% yield. mp 122–124 °C; IR (KBr) 3329.2 cm^{-1}; 2235.9 cm^{-1}; 1587.4 cm^{-1}; 1218.4 cm^{-1};

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.57–1.61 (m, 2H, CH_2), 1.64–1.68 (m, 2H, CH_2), 2.73 (br s, 2H, NH_2), 2.78–2.85 (m, 2H, CH_2–NH$_2$), 3.25–3.34 (m, 2H, –NH–CH_2), 6.00 (br s, 1H, N*H*), 6.35–6.38 (d, J = 5.42 Hz, 1H, 3*H* quinoline), 7.30–7.35 (dd, J = 8.95, 2.12 Hz, 1H, 6*H* quinoline), 7.72–7.76 (d, J = 8.96 Hz, 1H, 5*H* quinoline), 7.93–7.94 (d, J = 2.08 Hz, 1H, 8*H* quinoline), 8.48–8.51 (d, J = 5.39 Hz, 1H, 2*H* quinoline); FAB-MS *m*/*z* 250 [M+H]$^+$; Anal. Calcd for $C_{13}H_{16}ClN_3$: C, 65.52; H, 6.46; N, 16.83; found: C, 65.95; H, 6.38; N, 16.72.

6.7. General synthetic procedure for *N*,*N*^{1}-bis-(*tert*-butoxycarbonyl)-*N*^{11}-[(7-chloroquinolin-4-ylamino)-alkyl]-guanidine (4a-d)

1,3-Bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudurea (4.6 mmol) and 4-aminoquinoline (3 mmol) (**3a–d**) were heated at 60 °C for 6 h in 2% aq. THF. Solvent was removed, and the residue was treated with 5% aq. $NaHCO_3$, followed by extraction with dichloromethane (2×). After brine wash, the crude product was chromatography over silica gel using chloroform-methanol.

6.7.1. *N*,*N*^{1}-Bis-(*tert*-butoxycarbonyl)-*N*^{11}-[2-(7-chloroquinolin-4-ylamino)-ethyl]-guanidine (4a). This compound was obtained as white solid in 65% yield. mp 143–145 °C; R_f 0.62; IR (KBr) 3324.4 cm^{-1}; 2980.2 cm^{-1}; 1723.7 cm^{-1}; 1582.6 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.49 (s, 9H, –C–NH–COO–C(CH_3)$_3$), 1.61 (s, 9H, –C=N–COO–C(CH_3)$_3$), 1.89 (br s, 1H, N*H*), 3.42–3.58 (m, 2H, CH_2), 3.81–4.01 (m, 2H, CH_2), 6.29–6.31 (d, J = 5.34 Hz, 1H, 3*H* quinoline), 7.16 (br s, 1H, N*H*), 7.32–7.37 (dd, J = 8.96, 2.01 Hz, 1H, 6*H* quinoline), 7.84–7.88 (d, J = 8.94 Hz, 1H, 5*H* quinoline), 7.93–7.94 (d, J = 2.08 Hz, 1H, 8*H* quinoline), 8.48–8.51 (d, J = 5.38 Hz, 1H, 2*H* quinoline), 8.91 (br s, 1H, N*H*); FAB-MS *m*/*z* 464 [M+H]$^+$; Anal. Calcd for $C_{22}H_{30}ClN_5O_4$: C, 56.95; H, 6.52; N, 15.09; found: C, 57.02; H, 6.48; N, 15.12.

6.7.2. *N*,*N*^{1}-Bis-(*tert*-butoxycarbonyl)-*N*^{11}-[2-(7-chloroquinolin-4-ylamino)-propyl]-guanidine (4b). This compound was obtained as white solid in 65% yield. Mp 116–117 °C; R_f 0.72; IR (KBr) 3325.3 cm^{-1}; 2980.5 cm^{-1}; 1724.5 cm^{-1}; 1615.3 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.32–1.35 (d, J = 6.2 Hz, 3H, CH_3), 1.50 (s, 9H, –C–NH–COO–C(CH_3)$_3$), 1.51 (s, 9H, –C=N–COO–C(CH_3)$_3$), 1.74 (br s, 1H, N*H*), 2.62–2.64 (m, 2H, CH_2), 3.43–3.46 (m, 1H, CH$_3$–C*H*–), 6.43–6.45 (d, J = 5.34 Hz, 1H, 3*H* quinoline), 6.66 (br s, 1H, N*H*), 7.27–7.32 (dd, J = 8.96, 1.98 Hz, 1H, 6*H* quinoline), 7.77–7.82 (d, J = 9.02 Hz, 1H, 5*H* quinoline), 7.92–7.93 (d, J = 1.92 Hz, 1H, 8*H* quinoline), 8.62–8.65 (d, J = 5.40 Hz, 1H, 2*H* quinoline), 8.93 (br s, 1H, N*H*); FAB-MS *m*/*z* 478 [M+H]$^+$; Anal. Calcd for $C_{23}H_{32}ClN_5O_4$: C, 57.79; H, 6.75; N, 14.65; found: C, 57.82; H, 6.78; N, 14.56.

6.7.3. *N*,*N*^{1}-Bis-(*tert*-butoxycarbonyl)-*N*^{11}-[3-(7-chloroquinolin-4-ylamino)-propyl]-guanidine (4c). This compound was obtained as white solid in 63% yield. Mp 115–116 °C; R_f 0.70; IR (KBr) 3324.3 cm^{-1}; 2979.8 cm^{-1}; 1722.5 cm^{-1}; 1578.9 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.52 (s, 9H, –C–NH–COO–C(CH_3)$_3$), 1.54 (s, 9H, –C=N–COO–C(CH_3)$_3$), 1.69–1.73 (m, 2H, CH_2), 1.81 (br s, 1H, N*H*), 3.41–3.46 (m, 4H, CH_2), 6.40–6.42 (d, J = 5.54 Hz, 1H, 3*H* quinoline), 6.94 (br s, 1H, N*H*), 7.31–7.36 (dd, J = 8.90, 2.10 Hz, 1H, 6*H* quinoline), 7.77–7.82 (d, J = 9.02 Hz, 1H, 5*H* quinoline), 7.93–7.94 (d, J = 2.12 Hz, 1H, 8*H* quinoline), 8.47–8.50 (d, J = 5.41 Hz, 1H, 2*H* quinoline), 8.60 (br s, 1H, N*H*); FAB-MS *m*/*z* 478 [M+H]$^+$; Anal. Calcd for $C_{23}H_{32}ClN_5O_4$: C, 57.79; H, 6.75; N, 14.65; found: C, 57.82; H, 6.78; N, 14.68.

6.7.4. *N*,*N*^{1}-Bis-(*tert*-butoxycarbonyl)-*N*^{11}-[4-(7-chloroquinolin-4-ylamino)-butyl]-guanidine (4d). This compound was obtained as white solid in 60% yield. Mp 121–122 °C; R_f 0.72; IR (KBr) 3328.3 cm^{-1}; 2981.7 cm^{-1}; 1721.1 cm^{-1}; 1417.6 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.43 (s, 9H, –C–NH–COO–C(CH_3)$_3$), 1.50 (s, 9H, –C=N–COO–C(CH_3)$_3$), 1.52–1.64 (m, 4H, CH_2), 1.83 (br s, 1H, N*H*), 3.38–3.41 (m, 2H, CH_2), 3.49–3.52 (m, 2H, CH_2), 5.73 (br s, 1H, N*H*), 6.39–6.41 (d, J = 5.36 Hz, 1H, 3*H* quinoline), 7.28–7.35 (dd, J = 8.98, 1.92 Hz, 1H, 6*H* quinoline), 7.79–7.84 (d, J = 8.95 Hz, 1H, 5*H* quinoline), 7.94–7.95 (d, J = 1.89 Hz, 1H, 8*H* quinoline), 8.50–8.53 (d, J = 5.28 Hz, 1H, 2*H* quinoline), 8.60 (br s, 1H, N*H*); FAB-MS *m*/*z* 478 [M+H]$^+$; Anal. Calcd for $C_{24}H_{34}ClN_5O_4$: C, 58.59; H, 6.97; N, 14.23; found: C, 58.62; H, 7.02; N, 14.34.

6.8. General synthetic procedure for *N*-[(7-chloroquinolin-4-ylamino)-alkyl]-guanidine hydrochloride (5a–d)

N,*N*^{1}-Bis-(*tert*-butoxycarbonyl)-*N*^{11}-[(7-chloro-quinolin-4-ylamino)-alkyl]-guanidine (**4a–d**) were treated with 20% HCl/dioxane solution and kept for 1 h. The solvent was evaporated under reduced pressure and the residue was scraped with ether. Finally the ethereal layer was evaporated to get the product.

6.8.1. *N*-[2-(7-Chloroquinolin-4-ylamino)-ethyl]-guanidine hydrochloride (5a). This compound was obtained as white solid in 55% yield. Mp 143–144 °C; R_f 0.32; ^{1}H NMR (200 MHz, $CDCl_3$+CD_3OD): δ 2.36 (br s, 2H, NH_2), 2.51 (br s, 1H, N*H*), 3.36–3.42 (m, 2H, CH_2), 3.75–3.96 (m, 2H, CH_2), 4.26 (br s, 1H, N*H*), 6.59–6.61 (d, J = 5.32 Hz, 1H, 3*H* quinoline), 7.02 (br s, 1H, N*H*), 7.27–7.32 (dd, J = 8.98, 2.02 Hz, 1H, 6*H* quinoline), 7.74–7.78 (d, J = 9.02 Hz, 1H, 5*H* quinoline), 7.83–7.84 (d, J = 2.04 Hz, 1H, 8*H* quinoline), 8.38–8.41 (d, J = 5.34 Hz, 1H, 2*H* quinoline); FAB-MS *m*/*z* 264 [M+H]$^+$; Anal. Calcd for $C_{12}H_{15}Cl_2N_5$: C, 48.01; H, 5.04; N, 23.33; found: C, 48.06; H, 5.41; N, 23.45.

6.8.2. *N*-[2-(7-Chloroquinolin-4-ylamino)-propyl]-guanidine hydrochloride (5b). This compound was obtained as white solid in 58% yield. Mp 222–223 °C; R_f 0.28; IR (KBr) 3426.3 cm^{-1}; 2928.3 cm^{-1}; 2372.5 cm^{-1}; 1809.6 cm^{-1}; 1662.0 cm^{-1}; 1589.6. cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$+CD_3OD): δ 1.30–133 (d, J = 6.38 Hz, 3H, CH_3), 2.38 (br s, 2H, NH_2), 2.49 (br s, 1H, N*H*), 2.58–2.59 (m, 2H, CH_2), 3.63–3.66 (m,

1H, –C*H*–CH_3), 4.25 (br s, 1H, N*H*), 6.89–6.91 (d, $J = 5.64$ Hz, 1H, 3*H* quinoline), 7.73–7.80 (dd, $J = 9.16$, 2.02 Hz, 1H, 6*H* quinoline), 7.89 (br s, 1H, N*H*), 8.05–8.55 (d, $J = 9.02$ Hz, 1H, 5*H* quinoline), 8.56–8.57 (d, $J = 1.98$ Hz, 1H, 8*H* quinoline), 9.29–9.31 (d, $J = 5.64$ Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 278 $[M+H]^+$; Anal. Calcd for $C_{13}H_{17}Cl_2N_5$: C, 49.69; H, 5.45; N, 22.29; found: C, 49.72; H, 5.48; N, 22.45.

6.8.3. *N*-[3-(7-Chloroquinolin-4-ylamino)-propyl]-guanidine hydrochloride (5c). This compound was obtained as white solid in 58% yield. mp 242–244 °C; R_f 0.28; IR (KBr) 3429.5 cm^{-1}; 3022.5 cm^{-1}; 2118.4 cm^{-1}; 1637.5 cm^{-1}; 1217.1 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+CD_3OD): δ 1.68–1.74 (m, 2H, CH_2), 2.39 (br s, 2H, NH_2), 2.52 (br s, 1H, N*H*), 3.21–3.44 (m, 4H, CH_2), 4.33 (br s, 1H, N*H*), 5.68 (br s, 1H, N*H*), 6.38–6.41 (d, $J = 5.36$ Hz, 1H, 3*H* quinoline), 7.32–7.36 (dd, $J = 8.98$, 1.96 Hz, 1H, 6*H* quinoline), 7.79–7.80 (d, $J = 1.98$ Hz, 1H, 5*H* quinoline), 8.12–8.16 (d, $J = 8.96$ Hz, 1H, 8*H* quinoline), 8.38–8.41 (d, $J = 5.38$ Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 288 $[M+H]^+$; Anal. Calcd for $C_{13}H_{17}Cl_2N_5$: C, 46.69; H, 5.45; N, 22.29; found: C, 47.04; H, 5.57; N, 22.35.

6.8.4. *N*-[4-(7-Chloroquinolin-4-ylamino)-butyl]-guanidine hydrochloride (5d). This compound was obtained as white solid in 54% yield. Mp 109–110 °C; R_f 0.29; IR (KBr) 3428.5 cm^{-1}; 3024.5 cm^{-1}; 2116.4 cm^{-1}; 1635.5 cm^{-1}; 1214.1 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+CD_3OD): δ 1.69–1.85 (m, 4H, CH_2), 1.91 (br s, 2H, NH_2), 2.76 (br s, 1H, N*H*), 3.17–3.29 (m, 4H, CH_2), 4.53 (br s, 1H, C=N*H*), 5.65 (br s, 1H, N*H*), 6.38–6.41 (d, $J = 5.12$ Hz, 1H, 3*H* quinoline), 7.30–7.35 (dd, $J = 9.02$, 2.02 Hz, 1H, 6*H* quinoline), 7.69–7.74 (d, $J = 8.98$ Hz, 1H, 5*H* quinoline), 7.93–7.94 (d, $J = 1.98$ Hz, 1H, 8*H* quinoline), 8.44–8.47 (d, $J = 5.16$ Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 292 $[M+H]^+$; Anal. Calcd for $C_{14}H_{19}Cl_2N_5$: C, 51.23; H, 5.83; N, 21.34; found: C, 51.33; H, 5.97; N, 21.35.

6.9. General synthetic procedure for *N*-[(7-chloroquinolin-4-ylamino)-alkyl]-tertramethylguanidine hexafluorophosphate (6a–d)

HBTU (2-1H-benzotriazol-1-yl)-1,1,3,3-tertamethyluronium hexaflurophosphate (4.6 mmol) and 4-aminoquinoline derivatives (**3a–d**) (3 mmol) were stirred at room temp for 8 h in dry acetonitrile. Solvent was removed, and the residue was treated with 5% aq $NaHCO_3$, followed by extraction with dichloromethane (2×). After brine wash, the crude product was chromatography over LH-20 using chloroform–methanol.

6.9.1. *N*-[2-(7-Chloroquinolin-4-ylamino)-ethyl]-*N*′,*N*′,*N*″,*N*″-tetramethylguanidine hexafluorophosphate (6a). This compound was obtained as gummy matter in 52% yield. IR (neat) 3391.1 cm^{-1}; 2926.8 cm^{-1}; 2370.9 cm^{-1}; 2144.1 cm^{-1}; 1580.3 cm^{-1}; 1425.1 cm^{-1}; 1221.4 cm^{-1}; 1137.3 cm^{-1}; 1080.7 cm^{-1}; ^1H NMR (300 MHz, CD_3CN) δ 2.24 (s, 6H, N(CH_3)$_2$), 2.86 (s, 6H, N(CH_3)$_2$), 3.49–3.57 (m, 4H, CH_2), 5.95 (br s, 1H, N*H*), 6.06 (br s, 1H, N*H*), 6.56–6.58 (d, $J = 5.40$ Hz, 1H, 3*H* quinoline), 7.45–7.49 (dd, $J = 9.0$, 2.1 Hz, 1H, 6*H* quinoline), 7.89–7.90 (d, $J = 2.1$ Hz, 1H, 8*H* quinoline), 7.95–7.98 (d, $J = 9.00$ Hz, 1H, 5*H* quinoline), 8.49–8.51 (d, $J = 5.40$ Hz, 1H, 2*H* quinoline); ^{32}P NMR (120 MHz, CD_3CN) −159.7 to −194.7 (sept, $J = 704.0$ Hz, 1P, PF_6); FAB-MS *m/z* 320 $[M+H]^+$; Anal. Calcd for $C_{16}H_{23}ClF_6N_5P$: C, 41.26; H, 4.98; N, 15.04 Found: C, 41.60; H, 5.02; N, 15.20.

6.9.2. *N*-[2-(7-Chloroquinolin-4-ylamino)-propyl]-*N*′,*N*′,*N*″,*N*″-tetramethylguanidine hexafluorophosphate (6b). This compound was obtained as gummy matter in 58% yield. ^1H NMR (200 MHz, CD_3CN) δ 1.27–1.30 (d, $J = 6.34$ Hz, 3H, CH_3), 2.24 (s, 6H, N(CH_3)$_2$), 2.89 (s, 6H, N(CH_3)$_2$), 2.96–2.99 (m, 2H, CH_2), 3.03–3.10 (m, 1H, –C*H*–CH_3), 5.98 (br s, H, N*H*), 6.06 (br s, 1H, N*H*), 6.78–6.81 (d, $J = 5.34$ Hz, 1H, 3*H* quinoline), 7.30–7.35 (dd, $J = 9.02$, 2.12 Hz, 1H, 6*H* quinoline), 7.89–7.90 (d, $J = 2.02$ Hz, 1H, 8*H* quinoline), 7.96–7.99 (d, $J = 9.00$ Hz, 1H, 5*H* quinoline), 8.61–8.64 (d, $J = 5.36$ Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 334 $[M+H]^+$; Anal. Calcd for $C_{17}H_{25}ClF_6N_5P$: C, 42.55; H, 5.25; N, 14.60; found: C, 42.86; H, 5.29; N, 14.98.

6.9.3. *N*-[3-(7-Chloroquinolin-4-ylamino)-propyl]-*N*′,*N*′,*N*″,*N*″-tetramethylguanidine hexafluorophosphate (6c). This compound was obtained as gummy matter in 55% yield. ^1H NMR (200 MHz, CD_3CN) δ 1.90–1.93 (m, 2H, CH_2), 2.31 (s, 6H, N(CH_3)$_2$), 2.64–2.67 (m, 2H, CH_2), 2.91 (s, 6H, N(CH_3)$_2$), 2.96–2.99 (m, 2H, CH_2), 5.92 (br s, 1H, N*H*), 6.15 (br s, 1H, N*H*), 6.27–6.29 (d, $J = 5.60$ Hz, 1H, 3*H* quinoline), 7.32–7.36 (dd, $J = 9.0$, 1.98 Hz, 1H, 6*H* quinoline), 7.92–7.93 (d, $J = 1.96$ Hz, 1H, 8*H* quinoline), 8.00–8.40 (d, $J = 8.96$ Hz, 1H, 5*H* quinoline), 8.48–8.51 (d, $J = 5.58$ Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 334 $[M+H]^+$; Anal. Calcd for $C_{17}H_{25}ClF_6N_5P$: C, 42.55; H, 5.25; N, 14.60; found: C, 42.75; H, 5.32; N, 14.56.

6.9.4. *N*-[4-(7-Chloroquinolin-4-ylamino)-butyl]-*N*′,*N*′,*N*″,*N*″-tetramethylguanidine hexafluorophosphate (6d). This compound was obtained as gummy matter in 55% yield. ^1H NMR (200 MHz, CD_3CN) δ 1.41–1.60 (m, 4H, CH_2), 2.46 (s, 6H, N(CH_3)$_2$), 2.94 (s, 6H, N(CH_3)$_2$), 3.14–3.36 (m, 4H, CH_2), 5.66 (br s, 1H, N*H*), 6.10 (br s, 1H, N*H*), 6.34–6.37 (d, $J = 5.52$ Hz, 1H, 3*H* quinoline), 7.36–7.42 (dd, $J = 8.96$, 2.02 Hz, 1H, 6*H* quinoline), 7.92–7.93 (d, $J = 1.96$ Hz, 1H, 8*H* quinoline), 8.00–8.40 (d, $J = 8.96$ Hz, 1H, 5*H* quinoline), 8.46–8.48 (d, $J = 5.37$ Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 350 $[M+H]^+$; Anal. Calcd for $C_{18}H_{27}ClF_6N_5P$: C, 43.78; H, 5.51; N, 14.18; found: C, 43.96; H, 5.84; N, 14.22.

6.10. General synthetic procedure for [(7-chloroquinolin-4-ylamino)-alkyl]-carbamic acid *tert*-butyl ester (7a–d)

A mixture of 4,7-dichloroquinoline **1** (2.5 g, 12.5 mmol) and monoprotected 1,2-diaminoalkane (25 mmol) were heated slowly from room temp 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 temp and taken up in dichloromethane. The organic layer was successively washed with 5% aq $NaHCO_3$ followed by water and then finally with brine. The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure to get a final product.

6.10.1. [2-(7-Chloroquinolin-4-ylamino)-ethyl]-carbamic acid *tert*-butyl ester (7a). This compound was obtained as white solid in 75% yield. Mp 98–100 °C; R_f 0.57; IR (KBr) 3313.2 cm^{-1}; 1582.6 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.44 (s, 9H, –NH–COO–C(CH_3)$_3$), 3.29–3.39 (m, 2H, CH_2), 3.62–3.74 (m, 2H, CH_2), 4.95 (br s, 1H, N*H*), 5.61 (br s, 1H, N*H*), 6.38–6.41 (d, J = 5.34 Hz, 1H, 3*H* quinoline), 7.32–7.37 (dd, J = 8.96, 1.94 Hz, 1H, 6*H* quinoline), 7.73–7.77 (d, J = 8.92 Hz, 1H, 5*H* quinoline), 7.94–7.95 (d, J = 1.96 Hz, 1H, 8*H* quinoline), 8.59–8.62 (d, J = 5.34 Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 322 $[M+H]^+$; Anal. Calcd for $C_{16}H_{20}ClN_3O_2$: C, 59.72; H, 6.26; N, 13.06; found: C, 60.04; H, 6.41; N, 13.45.

6.10.2. [2-(7-Chloroquinolin-4-ylamino)-propyl]-carbamic acid *tert*-butyl ester (7b). This compound was obtained as white solid in 68% yield. Mp 209–210 °C; R_f 0.54; IR (KBr) 3320.9 cm^{-1}; 2981.3 cm^{-1}; 1705.8 cm^{-1}; 1579.3 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.32–1.35 (d, J = 6.64 Hz, 3H, CH_3), 1.44 (s, 9H, –NH–COO–C(CH_3)$_3$), 2.43–2.72 (m, 2H, CH_2), 3.42–3.47 (m, 1H, CH_3–C*H*–), 5.42 (br s, 1H, N*H*), 6.36–6.39 (d, J = 5.42 Hz, 1H, 3*H* quinoline), 7.32–7.38 (dd, J = 8.94, 1.86 Hz, 1H, 6*H* quinoline), 7.72–7.77 (d, J = 9.02 Hz, 1H, 5*H* quinoline), 7.97–7.98 (d, J = 1.92 Hz, 1H, 8*H* quinoline), 8.22–8.25 (d, J = 5.46 Hz, 1H, 2*H* quinoline), 8.42 (br s, 1H, N*H*); FAB-MS *m/z* 336 $[M+H]^+$; Anal. Calcd for $C_{17}H_{22}ClN_3O_2$: C, 60.80; H, 6.60; N, 12.51; found: C, 60.96; H, 7.02; N, 12.75.

6.10.3. [3-(7-Chloroquinolin-4-ylamino)-propyl]-carbamic acid *tert*-butyl ester (7c). This compound was obtained as white solid in 70% yield. Mp 137–138 °C; R_f 0.52; IR (KBr) 3378.9 cm^{-1}; 2236.7 cm^{-1}; 1705.4 cm^{-1}; 1216.4 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.44 (s, 9H, –NH–COO–C(CH_3)$_3$), 1.61–1.64 (m, 2H, CH_2), 3.16–3.21 (m, 4H, CH_2), 3.26 (br s, 1H, N*H*), 4.91 (br s, 1H, N*H*), 6.30–6.33 (d, J = 5.64 Hz, 1H, 3*H* quinoline), 7.34–7.39 (dd, J = 8.94, 1.86 Hz, 1H, 6*H* quinoline), 7.63–7.68 (d, J = 9.00 Hz, 1H, 5*H* quinoline), 7.99–8.00 (d, J = 2.02 Hz, 1H, 8*H* quinoline), 8.21–8.24 (d, J = 5.62 Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 336 $[M+H]^+$; Anal. Calcd for $C_{17}H_{22}ClN_3O_2$: C, 60.80; H, 6.60; N, 12.51; found: C, 60.92; H, 6.72; N, 12.55.

6.10.4. [4-(7-Chloroquinolin-4-ylamino)-butyl]-carbamic acid *tert*-butyl ester (7d). This compound was obtained as white solid in 68% yield. Mp 144–146 °C; R_f 0.55; IR (KBr) 3313.2 cm^{-1}; 1582.6 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.44 (s, 9H, –NH–COO–C(CH_3)$_3$), 1.51–1.53 (m, 2H, CH_2), 1.54–1.56 (m, 2H, CH_2), 3.12–3.17 (m, 2H, CH_2–NH–), 3.31–3.35 (m, 2H, –NH–CH_2–), 4.65 (br s, 1H, N*H*), 5.64 (br s, 1H, N*H*), 6.38–6.41 (d, J = 5.09 Hz, 1H, 3*H* quinoline), 7.32–7.37 (dd, J = 8.91, 1.84 Hz, 1H, 6*H* quinoline), 7.71–7.75 (d, J = 8.94 Hz, 1H, 5*H* quinoline), 7.95–7.96 (d, J = 1.86 Hz, 1H, 8*H* quinoline), 8.45–8.48 (d, J = 5.37 Hz, 1H, 2*H* quinoline); FAB-MS *m/z* 350 $[M+H]^+$; Anal. Calcd for $C_{18}H_{24}ClN_3O_2$: C, 61.79; H, 6.91; N, 12.01; found: C, 61.84; H, 7.02; N, 12.31.

Acknowledgements

The authors thank the Director, CDRI for the support and the SAIF for the spectral data. One of the authors V.R.S. thanks the CSIR, New Delhi for Senior Research Fellowship. CDRI communication no. 6691.

References and notes

1. Wiesner, J.; Ortmann, R.; Schlitzer, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5274.
2. Winstanely, P. A.; Breckenridge, A. M. *Ann. Trop. Med. Parasitol.* **1987**, *81*, 619.
3. (a) Homewood, C. A.; Warhurst, D. C.; Peters, W.; Baggaley, V. C. *Nature* **1972**, *235*, 50; (b) Krogstad, D. J.; Schlesinger, P. H. *Biochem. Pharmacol.* **1986**, *35*, 547; (c) Schlesinger, P. H.; Krogstad, D. J.; Herwaldt, B. L. *Antimicrob. Agents Chemother.* **1988**, *32*, 793; (d) Hawley, S. R.; Bray, P. G.; O'Neill, P. M.; Park, B. K.; Ward, S. A. *Biochem. Pharmacol.* **1996**, *52*, 723; (e) Hawley, S. R.; Bray, P. G.; Mungthin, M.; Atkinson, J. D.; O'Neill, P. M.; Ward, S. A. *Antimicrob. Agents Chemother.* **1998**, *42*, 682.
4. (a) Bray, P. G.; Howells, R. E.; Ward, S. A. *Biochem. Pharmacol.* **1992**, *43*, 1219; (b) Bray, P. G.; Howells, R. E.; Ritchie, G. Y.; Ward, S. A. *Biochem. Pharmacol.* **1992**, *44*, 1317.
5. (a) Warhurst, D. C. *Biochem. Pharmacol.* **1981**, *30*, 3323; (b) Goldberg, D. E.; Slater, A. F. G.; Cerami, A.; Henderson, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2931; (c) Ridley, R. G. *J. Pharm. Pharmacol.* **1997**, *49*(2), 43; (d) Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Waldon, J. C. *J. Med. Chem.* **2000**, *43*, 283; (e) Kaschula, C. H.; Egan, T. J.; Hunter, R.; Basilico, N.; Parapani, S.; Tarameli, D.; Pasini, E.; Monti, D. *J. Med. Chem.* **2002**, *45*, 3531; (f) Ridley, R. G. *Nature* **2002**, *415*, 686.
6. (a) Ridley, R. G.; Hofheinz, Z.; Matile, H.; Jaquet, C.; Dorn, A.; Masciadri, R.; Jolidon, S.; Richter, W. F.; Guenzi, A.; Girometta, M. A.; Urwyler, H.; Thaithong, S.; Peters, W. *Antimicrob. Agents Chemother.* **1996**, *40*, 1846; (b) De, D.; Krogstad, F. M.; Byers, L. D.; Krogstad, D. J. *J. Med. Chem.* **1998**, *41*, 4918; (c) Biot, C.; Glorian, G.; Maciejewski, L. A.; Brocard, J. S. *J. Med. Chem.* **1997**, *40*, 3715.
7. Stocks, P. A.; Raynes, K. J.; Bray, P. G.; Park, B. K.; O'Neill, P. M.; Ward, S. A. *J. Med. Chem.* **2002**, *45*, 4975.
8. Surrey, A. R.; Cutler, R. A. *J. Am. Chem. Soc.* **1951**, *73*, 2623.
9. Greenhill, J. V.; Lue, P. Amidines and Guanidines in Medicinal Chemistry. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier Sciences: Amsterdam, The Netherlands, 1993; Vol. 30, pp 203.
10. *Guanidines: Historical, Biological, Biochemical and Clinical Aspects of the Naturally Occurring Guanido Compounds*;

Mori, H. M., Cohen, B. D., Lowenthal, A., Eds.; Plenum: New York.
11. Pallas 2.0, Compu-Drug Chemistry Ltd. San Francisco, CA.
12. Puri, S. K.; Singh, N. *Expl. Parasit.* **2000**, *94*, 8.
13. (a) Egan, T. J.; Mavuso, W. W.; Ross, D. C.; Marques, H. M. *J. Inorg. Biochem.* **1997**, *68*, 137; (b) Egan, T. J.; Marques, H. M. *Coord. Chem. Rev.* **1999**, *190*, 493.
14. Tripathi, A. K.; Khan, S. I.; Walker, L. A.; Tekwani, B. L. *Anal. Chem.* **2004**, *325*, 85.