Design, Synthesis and Antimalarial Activity of a New Class of Iron Chelators

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Abstract: Iron is crucial for many biochemical reactions involved in the growth and multiplication of the malaria parasite *Plasmodium falciparum*. There are many reports indicating that the iron chelators have antimalarial activity *in vitro*, *in vivo* and in human studies. However, these compounds suffer from a number of serious problems such as limited membrane permeability, short half-life and require long subcutaneous infusions. To circumvent these drawbacks we have designed a new class of iron chelators, wherein EDTA is tethered to 4-aminoquinoline. Here 4-aminoquinoline scaffold is used as a carrier to penetrate biological membrane and facilitate targetting the compounds to acidic food vacuole of the parasite. This study describes the synthesis of novel iron chelators and their *in vitro* antimalarial activity against *P. falciparum* strain of NF-54. The calculated LogP values of these compounds suggest the importance of lipophilicity for the antimalarial activity. The EDTA esters are more active than the corresponding acids. The biophysical studies suggest that these compounds may inhibit the parasite growth by iron chelation mechanism.

Key Words: Iron chelator, 4-aminoquinoline, antimalarial agents.

INTRODUCTION

Malaria is one of the foremost public health problems in developing countries affecting nearly 40% of the global population [1]. Historically 4-aminoquinoline based entities, particularly chloroquine (CQ), have remained the first choice in the malaria chemotherapy [2]. The biochemical studies have suggested that these class of compounds enter the food vacuole and inhibit the parasite growth by forming a complex with hematin (Fe(III)FPIX), π-π interaction thereby inhibiting the hemozoin formation, which exerts a toxic effect on the parasite [3-5]. But over the years extensive use of CQ has led to the drug resistant *P. falciparum*, thus limiting the armoury of antimalarial drugs. In this setting, development of antimalarial agents, preferably with a different mechanism of action than the classical 4-aminoquinoline is the need of the hour.

During the life cycle of the malaria parasite the intra erythrocytic replication is highly dependent on iron [6-8]. A number of biochemical studies have indicated that deprivation of iron would adversely affect the activity of ribonucleotide reductase, cytochrome and other vital cellular functions, which in turn inhibit the growth of the malaria parasite [9-11]. On the contrary; host cells have the ability to recover growth after temporary withdrawal of iron. The proclivity of the malaria parasite for free iron suggested that selective sequestering of iron could be an attractive approach for malaria chemotherapy [12, 13]. Accordingly, deferoxamine (DFO), a siderophore used to treat patients with excess iron, and a number of chemical entities capable of selective iron chelation have been explored as antimalarial agents [14-21]. They include α-keto hydroxyl pyridones, pyridoxyl isonicotinoyl hydrazones and thiosemicarbazones (Table **1**). However, these compounds suffer from a number of serious problems including: limited membrane permeability, short half-life and require long subcutaneous infusions [22]. To circumvent these limitations we have designed a new class of EDTA based iron chelators linked to 4-aminoquinoline moiety. The designing of analogues was guided by the a *priori* knowledge that EDTA is a well-known hexadentate, with an affinity constant for iron (III) of the order of 10^{25}, would readily form complex with iron (III) [23, 24]. Further it is surmised that aminoquinoline moiety would facilitate membrane permeability [25] and accumulation [26] of the iron chelator in the parasite food vacuole. With envisaged modifications the new compounds are expected to have better antimalarial activity as compared to known iron chelators reported in the literature.

Table 1. Iron Chelating Agents that Inhibit the Growth of *P. falciparum* in Cultured Erythrocytes

Specific agents	IC_{50} (μM)*	Ref
Deferoxamine	4-35	14-16
Deferiprone	15-45	17, 18
Pyridoxal isonicotinoyl hydrazone (PIH)	30	19
Salicylaldehyde isonicotinoyl hydrazone (SIH)	18-30	20
Ethane-1,2-bis(N-1-amino-3-ethylbutyl-3-thiol) (BAT)	6-9	21

*Concentration of iron chelator that produce 50% growth inhibition after 48-72 hr of culture

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CHEMISTRY

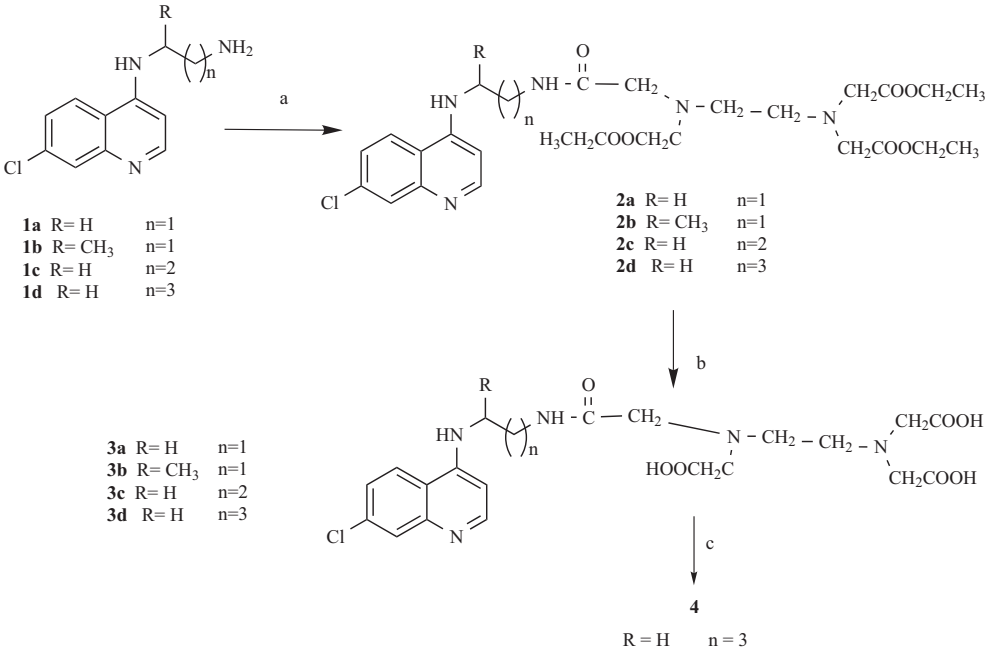
The synthesis of desired 4-aminoquinoline derivatives (**1a-1d)** was carried out by the procedure reported earlier from this laboratory [27]. The EDTA esters (**2a-2d)** were synthesized as shown in scheme **1**. Ethylenediaminetetra-acetic acid triethyl ester (Et_3EDTA) was synthesized by two-step process, in which EDTA was converted into EDTA tetraethyl ester with the help of thionyl chloride/ethanol protocol [28]. This was further selectively hydrolyzed by sodium hydroxide at pH 5.0. 4-Aminoquinoline derivatives (**1a-1d)** were coupled with Et_3EDTA using 1-Hydroxy-benzotriazolehydrate/N,N'-Dicyclohexylcarbodiimide (HOBt/DCC) in dry THF at room temperature. The compounds (**3a-3d)** were obtained by cleavage of EDTA ester derivatives (**2a-2d)** with help of lithium hydroxide (LiOH).

All the above-synthesized compounds were characterized by elemental analysis and spectral data. The ferric complex of EDTA acid **4** was prepared by treating methanolic solution of the acid **3d** with ferric chloride. The complex is insoluble in most of the organic solvents such as carbon tetrachloride and ether but is readily soluble in dimethylsulfoxide. The iron complex was characterized by IR and Mass spectral data.

The infrared spectrum of EDTA acid **3d** contains band at 3427.1 cm^{-1} (O-H str). In the yellowish white EDTA-Fe (III) complex **4**, this band disappears. Compound **3d** shows a strong band at 1723 cm^{-1}, which can be assigned to $\upsilon_{c=o}$ stretching of a carbonyl of COOH functionality. This band is shifted to 1655.1 from 1743 cm^{-1} in the case of iron complex **4**. Such a decrease in the carbonyl stretching frequency is a clear indication that metal coordination involves the carboxyl oxygen. Furthermore in the mass spectra of the complexes molecular ion peak appeared at 578 $(M+H)^+$ corresponding to the 1:1 complexation of free acid **3d** with iron.

RESULTS AND DISCUSSION

All the compounds were tested *in vitro* for antimalarial activity against the *P. falciparum* strain of NF-54. Among all the compounds tested, four compounds showed MIC (minimal inhibitory concentration) range between 3.3 to 5.2 μM. The remaining compounds have shown MIC above the 95.0 μM. Furthermore the data suggest that the compounds having EDTA ester substitution (**2a-2d**) are more active than the corresponding acid derivatives (**3a-3d**). However, the observed biological activities of the EDTA esters as against the corresponding free acids could be rationalized as follows: recent studies have shown that lipophilicity plays a role in iron chelators for antimalarial activity [29]. The LogP values presented in Table **2** (Fig. **1**) are indicative of an excellent positive correlation between the antimalarial activity and LogP (R^2 = 0.9726, P = 0.010). It clearly highlights the importance of lipophilicity in the antimalarial activity of the synthesized compounds. Compounds (**3a-3d)** have lower LogP values compared to EDTA esters (**2a-2d**). Secondly, basicity of the ring nitrogen (pKa1) and the side chain nitrogen (pKa2) has a direct bearing on the accumulation of the compounds at the target site, which is affected in both the



Reagents and conditions :
(a) ethylenediaminetetraacetic acid triethyl ester, HOBt/DCC, rt, 4h ; (b) LiOH, rt, 1h; (c) $FeCl_3$, rt, 2h.

Scheme 1.

Table 2. Biological and Biophysical Data of the Compounds

C. No	MIC (μM)	LogP	pKa1	pKa2	Log K[a]	IC_{50}[b]
2a	5.2	2.13	8.23	4.05	3.83±0.02	1.25±0.18
3a	100.8	-0.58	8.23	6.96	2.28±0.01	1.99±0.32
2b	3.4	2.67	8.41	4.05	3.76±0.03	1.87±0.04
3b	98.1	-0.04	8.41	6.96	2.26±0.01	1.93±0.02
2c	3.4	2.49	8.23	4.05	2.76±0.01	0.90±0.04
3c	98.1	-0.22	8.23	6.96	2.21±0.04	1.86±0.13
2d	3.3	3.00	8.76	4.05	2.66±0.03	1.48±0.01
3d	95.4	-0.29	8.76	6.96	2.34±0.02	1.91±0.31
4	-[c]	-[d]	-[d]	-[d]	2.22±0.01	1.89±0.06
CQ	0.4	4.72	8.41	10.27	5.52±0.02	0.17±0.02

[a]1:1 (compound : hematin) complex formation in 40% aqueous DMSO, 20 mM HEPES buffer, pH 7.5 at 25^{0}C (data are expressed as means ± SD from at least three different experiments in duplicate)

[b]The IC_{50} represents the milimolar 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]Inactive at 200 μM

[d]The values not determined

cases [30]. The decrease in pKa's in the case of esters (**2a-2d**) is much less as compared to the free acids (**3a-3d**), hence ester derivatives are more active than the corresponding free acids (Table **2**). It may be appropriate to mention here that the esters on account of being more lipophilic than the corresponding free acids have better permeability across the membrane. At the intracellular site it is likely that they are hydrolyzed by estrases liberating free acids. The free acids would then effectively sequester iron thereby suggesting that the esters are acting as prodrugs and their higher activity is due to better membrane permeability. We have observed that the size of the spacer also affects the biological activity as evident from the activity results. Compounds (**2a, 2b**) having two carbons in the side chain are less active compared to (**2c, 2d**) which have three or four carbon atoms in the side chain. These findings are in consonance with the earlier data from this laboratory [27].

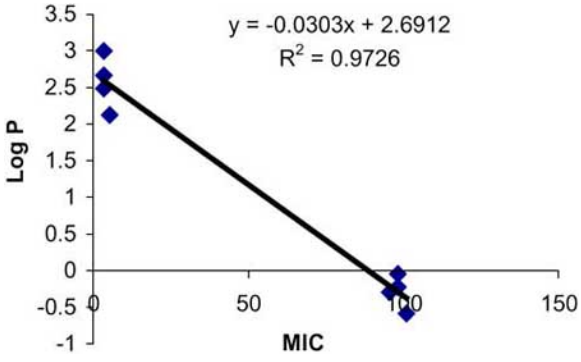


Fig. (1). Correlation between antimalarial activity and Log P.

The ability of the compounds synthesized in the present study to form association complex with hematin was investigated by UV spectrophotometer and it may be inferred from the data shown in Table **2** that all the compounds poorly interact with the hematin. Moreover the compounds (**2a-2d, 3a-3d**) inhibited the β-hematin formation albeit in a concentration dependent manner, data suggest that this class of compounds inhibit the hemozoin polymerisation very poorly. Approximately 10 fold higher concentrations of dose are required to inhibit the hemozoin polymerisation. Since these compounds contain aminoquinoline skeleton as a carrier molecule there is a possibility that they may interact with hematin leading to inhibition of hemozoin formation, which is, accepted mode of action of aminoquinoline class of compounds. Generally it is known that in order for compounds to be effective through this pathway the association constant (Log K) has to be in the range 4 to 5.5 and association constant below this range are considered to be acting through different mode of action [31, 32]. Accordingly Log K values for compounds (**2a-2d, 3a-3d**) reported in Table **2** are indicative of different mode of action other than haem binding and more likely through iron chelation.

The lower pKa values, weaker hematin association and weaker hemozoin formation inhibiting properties of this class of compounds indicate that they have different mode of action and more likely through iron chelation mechanism. If the mode of action of this class of compounds is through selective iron chelation, pre-complexation should render the compounds inactive. As a proof of concept iron complex of compound **3d** was prepared, characterized *(vide supra)* and when assayed in the *in vitro* activity the compound **4** was

inactive thereby suggesting that the mode of action of these compounds is through selective iron chelation.

CONCLUSION

In summary, the synthesis of a new series of EDTA-4-aminoquinoline conjugates has been described. These compounds have exhibited moderate antimalarial activity against the *P. falciparum* strain of NF-54 *in vitro*. The order of activity appears to be EDTA esters are more active than the corresponding acids. The present biophysical studies have suggested that this class of compounds may be inhibiting the malarial growth through iron chelation mechanism. Although the activity of the test compound is relatively weak but the present data suggest that there is scope for further improvement towards development of better antimalarial agents.

EXPERIMENTAL

General

Melting points (mp) were taken in open capillaries on Complab melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on an FT-IR Perkin-Elmer spectrometer. The ^{1}H spectra were recorded on a DPX-200 MHz Bruker FT-NMR spectrometer. The chemical shifts were reported as parts per million (δppm) tetramethyl-silane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 C,H,N analyzer and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on readymade silica gel 60 F_{254} 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.

General Synthetic Procedure for ([2-(Bis-ethoxycarbonyl-methyl-amino)-alkyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid Ethyl Ester (2a-2d)

4-Aminoquinoline derivatives (1a-1d) (2.35 mmol) was suspended in dry tetrahydrofuran. After addition of ethylenediaminetetraacetic acid triethyl ester (2.35 mmol), HOBt (2.35 mmol) and DCC (2.35 mmol) was slowly added to the reaction mixture. The reaction was allowed to proceed at room temperature for 4h. Dicyclohexylurea (DCU) was removed by filtration and filtrate was evaporated to dryness in vacuo. The residue was taken in ethyl acetate and washed with 5% $NaHCO_3$ and brine, dried on anhydrous Na_2SO_4. The crude product was chromatography over silica gel using chloroform-methanol.

([2-(Bis-ethoxycarbonylmethyl-amino)-ethyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid Ethyl Ester (2a)

This compound was obtained as gummy matter in 68% yield. R_f 0.78; IR (Neat) 3397.2 cm^{-1}; 2922.0 cm^{-1}; 1739.0 cm^{-1}; 1627.0 cm^{-1}; 1459.3 cm^{-1}; 1375.3 cm^{-1};^{1}H NMR (200 MHz, $CDCl_3$): δ 1.19-1.25 (m, 9H, -CH_2CH_3), 2.75 (s, 4H, -N-CH_2-CH_2-N-), 3.35-3.47 (m, 10H, -N-CH_2-), 3.71-3.75 (m, 2H, -NH-CH_2-), 4.04-4.14 (m, 6H, -CH_2CH$_3$), 6.28-6.30 (d, *J*=5.4 Hz, 1H, 3*H* quinoline), 6.85 (s br, 1H, N*H*), 7.31-7.35 (dd, *J*=8.9, 1.8 Hz, 1H, 6*H* quinoline), 7.86-7.90 (m, 3H, Ar-*H* quinoline), 8.92 (s br, 1H, N*H*); FAB-MS *m/z* 580 [M+H]$^+$; Anal. Calcd for $C_{27}H_{38}ClN_5O_7$: C, 55.90; H, 6.60; N, 12.07; found: C, 55.98; H, 6.64; N, 12.09.

([2-(Bis-ethoxycarbonylmethyl-amino)-propyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid Ethyl Ester (2b)

This compound was obtained as gummy matter in 65% yield. R_f 0.67; IR (Neat) 3305.5 cm^{-1}; 2982.5 cm^{-1}; 1736.7 cm^{-1}; 1650.4 cm^{-1}; 1581.3 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.15-1.18 (d, *J*=6.9 Hz, 3H, C*H*$_3$), 1.25-1.40 (m, 9H, -CH_2CH_3), 2.75 (s, 4H, -N-CH_2-CH_2-N-), 3.29-3.36 (m, 1H, -C*H*-CH$_3$), 3.42-3.47 (m, 10H, -N-CH_2-), 4.04-4.15 (m, 6H, -NH-CH_2-), 6.27-6.30 (d, *J*=5.5 Hz, 1H, 3*H* quinoline), 6.92 (s br, 1H, N*H*), 7.32-7.38 (dd, *J*=8.9, 2.1 Hz, 1H, 6*H* quinoline), 7.80-7.84 (d, *J*=8.9 Hz, 1H, 5*H* quinoline), 7.91-7.92 (d, *J*=2.0 Hz, 1H, 8*H* quinoline), 8.45-8.48 (d, *J*=5.4 Hz, 1H, 2*H* quinoline), 8.94 (s br, 1H, N*H*); FAB-MS *m/z* 595 [M+H]$^+$; Anal. Calcd for $C_{28}H_{40}ClN_5O_7$: C, 56.61; H, 6.79; N, 11.79; found: C, 56.62; H, 6.81; N, 11.74.

([3-(Bis-ethoxycarbonylmethyl-amino)-propyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid Ethyl Ester (2c)

This compound was obtained as gummy matter in 62% yield. R_f 0.78; IR (Neat) 3364.6 cm^{-1}; 2984.6 cm^{-1}; 1738.4 cm^{-1}; 1668.1 cm^{-1}; 1626.3 cm^{-1}; 1594.9 cm^{-1}; 1445.2 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.22-1.29 (m, 9H, -CH_2CH_3), 1.84-1.89 (m, 2H, CH_2), 2.78 (s, 4H, -N-CH_2-CH_2-N-), 3.37-3.43 (m, 2H, -CH_2-NH), 3.49-3.52 (m, 10H, -N-CH_2-), 4.09-4.20 (m, 6H, CH_2-CH$_3$), 6.36-6.39 (d, *J*=5.5 Hz, 1H, 3*H* quinoline), 6.89 (s br, 1H, N*H*), 7.30-7.36 (dd, *J*=8.9, 2.0 Hz, 1H, 6*H* quinoline), 7.91-8.00 (m, 2H, Ar-*H* quinoline), 8.43-8.46 (d, *J*=5.5 Hz, 1H, 2*H* quinoline), 8.93 (s br, 1H, N*H*); FAB-MS *m/z* 595 [M+H]$^+$; Anal. Calcd for $C_{28}H_{40}ClN_5O_7$: C, 56.61; H, 6.79; N, 11.79; found: C, 56.68; H, 6.84; N, 11.83.

([4-(Bis-ethoxycarbonylmethyl-amino)-butyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid Ethyl Ester (2d)

This compound was obtained as gummy matter in 60% yield. R_f 0.62; IR (Neat) 3364.cm^{-1}; 2987.6 cm^{-1}; 1736.5 cm^{-1}; 1656.2 cm^{-1}; 1612.0 cm^{-1}; 1579.6 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.18-1.28 (m, 9H, -CH_2CH_3), 1.74-1.76 (m, 4H, CH_2), 2.78 (s, 4H, -N-CH_2-CH_2-N-), 3.30-3.35 (m, 2H, -CH_2-NH), 3.51-3.67 (m, 10H, -N-CH_2-), 4.09-4.19 (m, 6H, CH_2-CH$_3$), 5.98 (s br, 1H, N*H*), 6.35-6.38 (d, *J*=5.5 Hz, 1H, 3*H* quinoline), 7.29-7.34 (dd, *J*=9.0, 2.0 Hz, 1H, 6*H* quinoline), 7.87-7.92 (m, 2H, Ar-*H* quinoline), 8.46-8.49 (d, *J*=5.4 Hz, 1H, 2*H* quinoline), 8.92 (s br, IH, N*H*); FAB-MS *m/z* 608 [M+H]$^+$; Anal. Calcd for $C_{29}H_{42}ClN_5O_7$: C, 57.28; H, 6.96; N, 11.52; found: C, 57.33; H, 6.99; N, 11.53.

General Synthetic Procedure for ([2-(Bis-carboxymethyl-amino)-alkyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethyl-carbamoyl]-methyl}-amino)-acetic Acid (3a-3d)

To the solution of **2a-2d** (2.0 mmol) in ethanol was added LiOH (6.6 mmol) dissolved in water. The reaction mixture was stirred at room temperature for 1h.The solvent was evaporated, water was added, and the solution was extracted with chloroform. The aqueous phase was acidified to pH 4.0 and extracted again with the chloroform. The combined organic phases were dried on anhydrous Na_2SO_4. The crude product was separated by chromatography on sephadex LH-20 using chloroform-methanol.

([2-(Bis-carboxymethyl-amino)-ethyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid (3a)

This compound was obtained as gummy matter in 64% yield. IR (Neat) 3429.7 cm^{-1}; 2925.3 cm^{-1}; 2925.3 cm^{-1}; 2363.5 cm^{-1}; 1667.3 cm^{-1}; 1388.5 cm^{-1}; ^{1}H NMR (200 MHz, $DMSOd_6$ + $CDCl_3$): δ 2.91 (s, 4H, -N-C*H*$_2$-C*H*$_2$-N-), 3.02 (s, 2H, -CO-C*H*$_2$-N-), 3.34 (s, 6H, -C*H*$_2$COOH), 3.42-3.46 (m, 4H, C*H*$_2$), 4.24 (s br, 1H, N*H*), 6.18-6.21 (d, *J*=6.8 Hz, 1H, Ar-*H* quinoline), 6.95-7.00 (dd, *J*=9.1, 2.0 Hz, 1H, Ar-*H* quinoline), 7.29-7.31 (d, *J*=2.0 Hz, 1H, Ar-*H* quinoline), 7.53 (s br, 1H, N*H*), 7.77-7.80 (d, *J*=6.4 Hz, 1H, Ar-*H* quinoline), 8.27-8.35 (d, *J*=9.2 Hz, 1H, Ar-*H* quinoline), 9.84 (s br, 3H, COO*H*) ; FAB-MS *m/z* 496 $[M+H]^+$; Anal.Calcd for $C_{21}H_{26}ClN_5O_7$: C, 50.86; H, 5.28; N, 14.12; found: C, 50.90; H, 5.34; N, 14.14.

([2-(Bis-carboxymethyl-amino)-propyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid (3b)

This compound was obtained as gummy matter in 60% yield. IR (Neat) 3432.6 cm^{-1}; 2930.5 cm^{-1}; 2362.9 cm^{-1}; 1655.4 cm^{-1}; 1561.3 cm^{-1}; 1516.8 cm^{-1}; 1459.8cm^{-1}; ^{1}H NMR (200 MHz, $DMSOd_6$+ $CDCl_3$): δ 1.36-1.40 (d, *J*=6.4 Hz, 3H, C*H*$_3$), 2.90 (s, 4H, -N-C*H*$_2$-C*H*$_2$-N-), 3.04 (s, 2H, -CO-C*H*$_2$-N-), 3.34-3.36 (m, 2H, C*H*$_2$-NH), 3.36 (s, 6H, -C*H*$_2$COOH), 3.37-3.39 (m, 1H, C*H*-CH$_3$), 3.76 (s br, 1H, N*H*), 6.99-7.03 (d, *J*=7.0 Hz, 1H, Ar-*H* quinoline), 7.62-7.67 (dd, *J*=8.6, 2.0 Hz, 1H, Ar-*H* quinoline), 7.86-7.87 (d, *J*=1.9 Hz, 1H, Ar-*H* quinoline), 8.02-8.06 (d, *J*=6.9 Hz, 1H, Ar-*H* quinoline), 8.36 (s br, 1H, N*H*), 8.70-8.74 (d, *J*=8.8 Hz, 1H, Ar-*H* quinoline), 9.64 (s br, 3H, COO*H*); FAB-MS *m/z* 509 $[M+H]^+$; Anal.Calcd for $C_{21}H_{26}ClN_5O_7$: C, 50.86; H, 5.28; N, 14.12; found: C, 50.90; H, 5.34; N, 14.14.

([3-(Bis-carboxymethyl-amino)-propyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid (3c)

This compound was obtained as gummy matter in 58% yield. IR (Neat) 3432.9 cm^{-1}; 2932.1 cm^{-1}; 2363.5 cm^{-1}; 1660.7 cm^{-1}; 1562.5 cm^{-1}; 1513.2 cm^{-1}; 1462.6 cm^{-1}; ^{1}H NMR (200 MHz, $DMSOd_6$ + $CDCl_3$): δ 1.91-1.95 (m, 2H, C*H*$_2$), 2.99 (s, 4H, -N-C*H*$_2$-C*H*$_2$-N-), 3.31-3.36 (m, 4H, -C*H*$_2$-NH, NH-C*H*$_2$-), 3.54 (s, 2H, -CO-C*H*$_2$-N-), 3.64 (s, 6H, CH$_2$COOH), 4.64 (s br, 1H, NH), 6.82-6.85 (d, *J*=6.4 Hz, 1H, Ar-*H* quinoline), 7.98-8.04 (dd, *J*=8.5, 1.9 Hz, 1H, Ar-*H* quinoline), 8.43-8.45 (d, *J*=1.9 Hz, 1H, Ar-*H* quinoline), 8.67 (s br, 1H, N*H*), 8.83-8.87 (d, *J*=6.5 Hz, 1H, Ar-*H* quinoline), 9.00-9.04 (d, *J*=8.6 Hz, 1H, Ar-*H* quinoline), 9.91 (s br, 3H, COO*H*); FAB-MS *m/z* 511 $[M+H]^+$; Anal.Calcd for $C_{22}H_{28}ClN_5O_7$: C, 51.82; H, 5.53; N, 13.73; found: C, 51.90; H, 5.58; N, 13.77.

([4-(Bis-carboxymethyl-amino)-butyl]-{[2-(7-chloro-quinolin-4-ylamino)-ethylcarbamoyl]-methyl}-amino)-acetic Acid (3d)

This compound was obtained as gummy matter in 55% yield. R_f 0.62; IR (KBr) 3429.8 cm^{-1}; 2927.1 cm^{-1}; 2367.1 cm^{-1}; 1723.0 cm^{-1}; 1660.9 cm^{-1}; 1585.8 cm^{-1}; 1455.7 cm^{-1}; ^{1}H NMR (200 MHz, $DMSOd_6$ + $CDCl_3$): δ 2.58-2.63 (m, 4H, C*H*$_2$), 2.92 (s, 4H, -N-C*H*$_2$-C*H*$_2$-N-), 2.94-3.19 (m, 4H, NH-C*H*$_2$, -C*H*$_2$-NH-), 3.22 (s, 2H, -CO-C*H*$_2$-N-), 3.47 (s, 6H, C*H*$_2$-COOH), 4.68 (s br, 1H, N*H*), 7.00-7.04 (d, *J*=7.0 Hz, 1H, Ar-*H* quinoline), 7.71-7.77 (dd, *J*=9.0, 1.7 Hz, 1H, Ar-*H* quinoline), 8.32-8.33 (d, *J*=1.8 Hz, 1H, Ar-*H* quinoline), 8.58-8.61 (d, *J*=6.9 Hz, 1H, Ar-*H* quinoline), 8.74 (s br, 1H, N*H*), 9.00-9.05 (d, *J*=9.1 Hz, 1H, Ar-*H* quinoline), 10.25 (s br, 3H, COO*H*); FAB-MS *m/z* 524 $[M+H]^+$; Anal.Calcd for $C_{23}H_{30}ClN_5O_7$: C, 52.72; H, 5.77; N, 13.37; found: C, 52.76; H, 5.75; N, 13.34.

Synthesis of the EDTA acid (3d) -Fe(III) Complex (4)

The ferric complex of EDTA acid was prepared by treating methanloic solution of the acid EDTA **3d** (0.20 mmol) with ferric chloride (0.20 mmol in methanol). The reaction mixture was stirred for 2h. The solvent was evaporated and the residue was triturated with ether. The ethereal layer was discarded up till to get yellowish white product in 51% yield. IR (KBr) 2925.3 cm^{-1}; 2866.5 cm^{-1}; 1655.1cm^{-1}; 1362.7 cm^{-1}; 1279.8 cm^{-1}; 1099.8 cm^{-1}; 1025.2 cm^{-1}; 768.2 cm^{-1}; FAB-MS *m/z* 578 $[M+H]^+$.

BIOLOGICAL AND BIOPHYSICAL STUDIES

Measurement of *In Vitro* Antimalarial Activity

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 25mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum [33]. 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 to 1.5% at 3% haematocrit in a total volume of 200µl of medium RPMI-1640 was uniformly maintained. The test compound in 20µl volume concentrations ranging between 0.5 - 50 µg/ml in duplicate well were incubated with parasitized cell preparation at 37 ^{0}C in a candle jar. After 36 to 40h incubation, the blood smears from each well prepared and stained with giemsa stain [34]. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations. The test concentrations, which inhibited the complete maturation into schizonts, were recorded as the MIC. CQ was used as the standard reference drug.

Determination of LogP and pKa Values

The LogP and pKa values of the compounds (**2a-d** and **3a-d**) were calculated by Pallas [35]. In case of pKa two values were obtained. These data presented in Table **2** shows that pKa of quinoline N (the acid dissociation constant of the quinolinium cation and referred to below as pKa1) and pKa of tertiary amino group in the lateral chain (the acid dissociation constant of the tertiary ammonium group in the lateral chain is referred to pKa2).

Determination of Hematin Association Constant

The complex formation of the newly synthesized compounds with hematin as reflected in the form of association constants (Log K) was determined by spectrometric titration procedure in aqueous dimethylsulfoxide (DMSO) at pH-7.5 [36, 37]. 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 [38]. Utilizing a pH of 7.5, rather than more acidic conditions, improves the stability of Fe(III)PPIX solutions and quality of data.

In Vitro Inhibition of β-Hematin Polymerization

The ability of the compounds (**2a-d, 3a-d**) to inhibit β-hematin polymerization was induced by parasite lysate using UV spectrophotometer and measurements were carried out at 405nm [39]. The values obtained from the assay are 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).

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