N,N-Bis(trifluoromethylquinolin-4-yl)diamino Alkanes: Synthesis and Antimalarial Activity

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Abstract: A series of N,N-*bis*(trifluoromethylquinolin-4-yl)- and N,N-*bis*[2,8-bis(trifluoromethyl)quinolin-4-yl] diamino alkane and piperazine derivatives were synthesised by employing a simple and rapid displacement reaction of the 4-chloro group on the 2-trifluoromethyl- and 2,8-*bis*(trifluoromethyl)-quinoline by diaminoalkane or piperazine groups. Results of *in vitro* antimalarial activity evaluations of these compounds against the chloroquine-sensitive (D10) and chloroquine-resistant (K1) strains of *Plasmodium falciparum* indicate that compounds with trifluoromethyl groups in both the 2 and 8 positions coupled with diaminoalkyl bridging chains of 2 to 6 carbon atoms exhibit a slightly higher activity than compound with only a trifluoromethyl group at position 2, and those with a piperazine bridge These compounds exhibit higher activity in the chloroquine-resistant than in the chloroquine-sensitive strains of the Plasmodium. Comparative studies indicate that the compounds are more selective in their cytotoxicity against the parasite cells. Except for compounds containing a piperazine bridge, this new series of compounds interact with ferriprotoporphyrin IX to more or less the same extent.

Key Words: N,N-*Bis*(Trifluoromethylquinolin-4-yl)diamino alkanes, N,N-*bis*[2,8-bis(trifluoromethyl)quinolin-4-yl]diamino alkanes, antimalarial activity, synthesis, Ferriprotoporphyrin IX, cytotoxicity.

INTRODUCTION

If present in the human body, the growing malaria organism normally needs haemoglobin as a source of intracellular supply of iron for its metabolic requirements. This haemoglobin undergoes catabolism in the acidic digestive vacuoles, and during this process a deposition of a malaria pigment called haemozoin takes place [1]. Trapped within the haemozoin is the parasite's endogenous antimalarial agent called ferriprotoporphyrin IX (FP) in the form of haem [2], which in an aggregated form serves as a receptor for the concentration of antimalarial drugs [3]. Malaria parasites attempt to prevent the accumulation of FP within the food vacuoles by converting it to the haemozoin, an insoluble inert substance. The main criterion for selection or design of a compound which exhibit antimalarial activity is that it must be able to form a strong complex with FP through interaction of the π-electron cloud of the aromatic ring with that of the haem molecule, and thus inhibit the formation of haemazoin [4]. In addition, the compound must have the capacity to accumulate to pharmacologically relevant concentrations in the food vacuoles, as the site of drug action [5]. It is during the accumulation in the food vacuoles that the tertiary amino group in the side chain and the heterocyclic nitrogen atom in the quinoline ring become fully protonated [6]. This then assists the accumulation of more drug molecules in the food vacuoles. The increased concentrations of the FP-drug complex may then cause hemolysis of the parasite by a colloidal-osmotic mechanism [7]. Both free FP and its complex with chloroquine also inhibit the proteases which are essential for the degradation of haemoglobin and the growth of the parasites [8]. Thus both the structural and thermodynamic factors of the drug molecule must be such that they allow for its increased cellular accumulation and a favourable co-ordination to FP [9,10] so that the formation of haemazoin is prevented.

Although used in the chemotherapy of malaria for a number of years, the quinoline-type compounds are still the mainstay of research on compounds exhibiting antimalarial activity. Most these compounds contain at least one nitrogen atom at the 4-position either attached directly to the quinoline ring or as part of the various substituents attached to this position. The activity of such a drug molecules is more likely to be related to the nature and geometry of these types of substituents as well as the nature and position of other functional groups on the quinoline ring, rather than to an overall property of the molecule [11]. Molecules exhibiting high biological activity are those that are more electron-rich in the quinoline portion of the molecule due to a stronger resonance effect [9] and which contain a basic side chain with characteristics that will assists in the accumulation of the drug in the acid food vacuoles [5,13]. The stronger resonance effect resulting in conjugation in the molecule is efficient for the quinoline ring systems that are substituted at C-4 with aminoalkyl groups. The stronger the conjugation effect in the

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molecule, the more the increase in the stability of the drug-haem complex.

The aminoalkyl side-chain at position 4 of the quinoline ring is also a requirement for strong antiplasmodial activity by probably assisting in drug accumulation in the acidic digestive vacuoles [14]. The number of carbon atoms between the two nitrogen atoms in the alkyldiamino side chain is a major determinant of activity against the chloroquine- resistant *P. falciparum* [15]. For the aminoquinolines, biological activity is more robust to changes in the basic 4-amino side chain, with activity being approximately equally maintained with between a 2 and 5 carbon atom chain, but decreases if this chain is shortened or lengthened beyond these points [16], while branching is tolerated. An aliphatic tertiary nitrogen atom is a major determinant of activity in chloroquine-resistance [17]. This has been shown by the fact that the replacement of the piperidine moiety by alkyl chain or a pyrimidino ring leads to a loss of activity of the compound [18]. The weak basic property of these compounds resides solely with the piperidine nitrogen (pKa 9,6) because the alkyl derivatives do not have the ability to concentrate in the parasite food vacuoles [19]. A moderate increase in lipophilicity around the nitrogen atom is quite beneficial for antimalarial potency [20]. Quinoline compounds containing large nonpolar substituents such as a N-*tert* butyl or cyclohexyl group show substantially reduced antimalarial activity, while those with alkyl groups that have a 3 carbon backbone with no greater than one branch give optimum antimalarial activity [21]. Changes in the length of the aminoalkyl side chain have little influence on activity against the chloroquine-sensitive strains but profound influence against the chloroquine-resistant strains of *P. falciparum* [14,21], suggesting that whatever the mechanism is contributing to chloroquine resistance, it is extremely structure specific [22]. This is evidenced by the fact that compounds with a shortened side chain at the 4-amino group, while are structurally related to chloroquine, manage to overcome chloroquine-resistance to a greater extent than chloroquine itself. When an alkyl chain length exceeds the permissible linear dimension, energetically less favourable folding of the chain may occur, leading to a decrease in activity [11]. Toxicity appears to increase with greater chain length. In addition, the nature and position of the substituents on the quinoline ring may affect the shape, electronegativity and lipophilicity of the compounds, characteristics which are known to have a tremendous influence on the antimalarial activity [23]. The trifluoromethyl group has aroused considerable interest in the chemotherapy of malaria since the discovery of the most effective but non-phototoxic, 2,8-*bis*(trifluoromethyl)-α-(2-piperidyl)-4-quinolinemethanol (mefloquine) [24] and the phenanthrenemethanol, halofantrine [25] both of which are effective against the chloroquine-resistant strain of *P. falciparum* and have become the first-choice drugs for antimalarial prophylaxis [26]. Being a highly electronegative group, the triflouromethyl group attached to the quinoline ring appears to make mefloquine to have greater access than chloroquine to identical receptors, thus interacting strongly both with FP and certain phospholipids [27, 28].

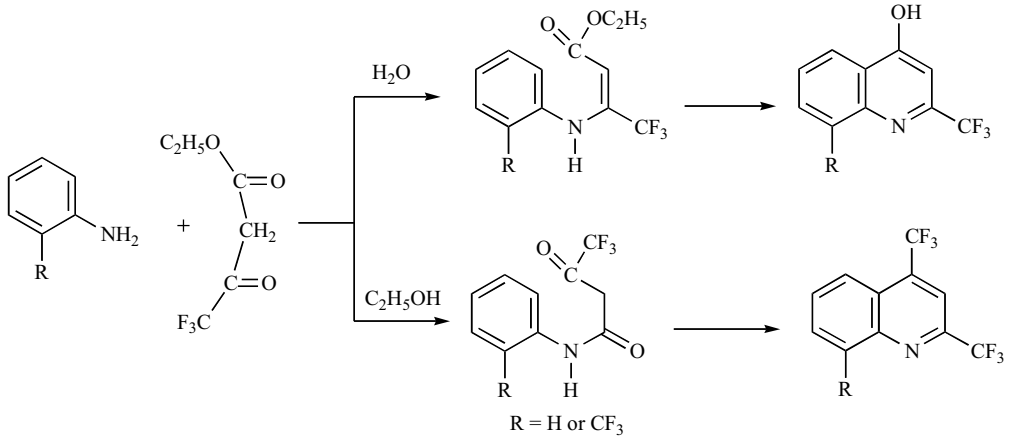
The main objective of this study is to synthesise and evaluate the activity of a series of N,N-*Bis*(trifluoro-methylquinolin-4-yl)diamino alkane derivatives as potential alternatives to the currently available antimalarial agents for possible use against both the chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. The rationale for this investigation is also to assessment the effect of the presence of one or two trifluoromethyl groups attached to the quinoline ring, the length and shape of the diaminoalkyl chain linking the two quinoline rings at position 4 on antimalarial activity.

CHEMISTRY

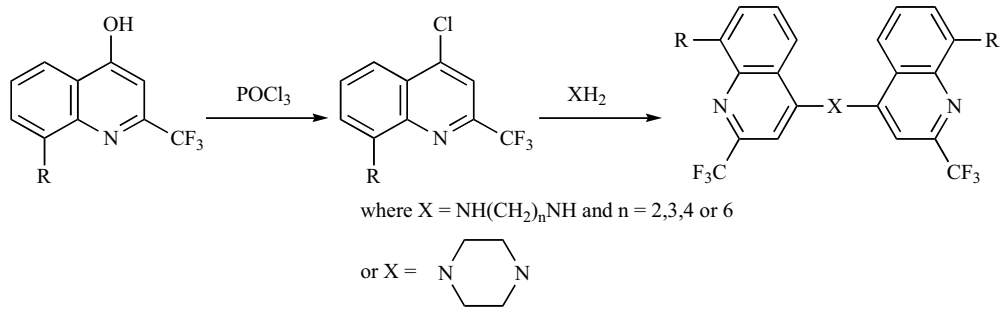
The 4-chloro-2- trifluoromethyl- and 2,8-bis(trifluoromethyl)quinolines used as starting materials were prepared in the previous study [29] according to the following schemes.

ANTIMALARIAL ACTIVITY

The antimalarial activity of the bisquinolines was assessed against the chloroquine-sensitive strain (D10) and chloroquine-resistant strain (K1) of *Plasmodium falciparum*. Continuous *in vitro* cultures of the asexual erythrocyte stages of these strains were maintained using a modified method of [30]. Quantitative assessment of the antiplasmodial activity *in vitro* was determined using the parasite lactate dehydrogenase assay procedures [31]. The parasite lactate dehydro-



Scheme 1. Synthesis of 4-Quinolinol and its by-product, 2-Quinolinol.



Scheme 2. Synthesis of N,N-Bis(trifluoromethylquinolin-4 yl)- and N,N-bis{bis(trifluioromethyl)quinolin-4 yl}-diamino alkanes.

genase (pLDH) activity differs from the host LDH activity in its use of the 3-acetyl pyridine adenine dinucleotide (APAD) [32] as a coenzyme. This difference has afforded a useful assay procedure both for diagnostic and screening purposes for Plasmodium infections. The sensitivity of the assay can be used to detect a level of 0,02% parasitemia of *P. falciparum grown in vitro*. It has been shown that chloroquine interacts specifically with pLDH but not with erythrocyte LDH [32]. The difference could be due to the fact that pLDH is not subject to ternary substrate inhibition by pyruvate and NAD^+, which is a common property for LDH molecules from other sources [33]. This difference raises the possibility that inhibitor molecules could be designed that would specifically target the parasite enzyme. Spectroscopic changes associated with the interaction of the compounds with ferriprotoporphyrin IX was performed following a modified known procedure [34, 35].

Determination of the Cytotoxicity of the Compounds

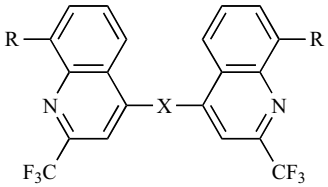
In order to determine whether the synthesized compounds are selective in their action against the plasmodial cells in the presence of the host cells, a selectivity index (SI) can be determined. This index is based on the ratio of the IC_{50} values obtained against the normal host cells (cytotoxicity) and the IC_{50} values obtained against the plasmodial cells. The measurement of the surviving and/or proliferating mammalian cells (cytotoxicity assay) after treatment with these compounds is the rapid colorimetric assay procedure based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) [36,37]. Mammalian cell-line, Chinese Hamster Ovarian (CHO) cells were treated with those compounds exhibiting more active against the chloroquine-resistant strains of *P. falciparum* were subjected to the MTT assay procedure. The tetrazolium ring of the MTT used to measure cell growth and chemosensitivity is cleaved in the active mitochondria, so that the reaction can occur only in living cells. When incubated with these cells, the pale yellow MTT substrate produces a dark blue formazan product [36]. This forms the basis of this colorimetric assay.

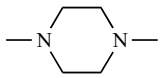
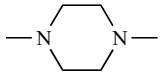
RESULTS AND DISCUSSION

A series of ten N,N-*bis*(trifluoromethylquinolin-4-yl)- and N,N-*bis*[2,8-*bis*(trifluoromethyl)quinolin-4-yl]diamino alkanes and piperazines were successfully synthesised by employing a simple but rapid displacement reaction of the 4-chloro group of 2-trifluoromethyl- and 2,8-*bis*(trifluoromethyl)quinolines by diaminoalkane or piperazino groups. Evaluation of the *in vitro* antimalarial activity of these compounds was conducted against the chloroquine-sensitive (D10) and chloroquine-resistant (K1) strains of *Plasmodium falciparum* following the procedure of the lactate dehydrogenase activity and the results are reflected on Table (**1**). The results indicate that against the chloroquine-sensitive strains, the compounds exhibit moderate activity when compared to their activity against the chloroquine-resistant strains of *P. falciparum*. However, when compared to mefloquine under identical experimental conditions, the compounds are shown to exhibit much lower antimalarial activity. While the activity of the majority of these compounds is essentially of the same order of magnitude, compounds containing the trifluoromethyl groups at both positions 2 and 8 and the diaminoalkyl bridging chains of 2 to 6 carbon atoms are consistently more active than those with a single trifluoromethyl group at position 2 of the quinoline ring. Compound **2** is the most active against the chloroquine-sensitive and chloroquine-resistant strain of *P. falciparum* with IC_{50} values of 500 and 100 ng/ml respectively. These results are comparable to those of mefloquine under identical experimental conditions. Compounds **4**, **6** and **8** with bridging diaminoalkyl chain of three to six carbon atoms and trifluoromethyl groups at positions 2 and 8 are equally more active than those in which the quinoline ring contains a single trifluoromethyl group at position 2. It was shown earlier that quinoline compounds with a trifluoromethyl group at position 8 only show initial inhibition followed by stimulation of cell growth in a dose-dependant manner [38]. Compounds **9** and **10** with the piperazino linking bridge exhibit reduced antimalarial activity than those compounds with a diaminoalky bridging chain against the chloroquine-sensitive strains, although compound **10** with trifluoromethyl groups at positions 2 and 8 show comparable activity to those with diaminoalky bridging chains.

Changes in the absorbance maxima of the Soret band of the UV spectrum is sometimes used as a measure of interaction of the drug with FP through the formation of π–π complexes with the induced-shift pattern, upfield or downfield, being different for each drug molecule, leading to different complex structure [39]. Spectrophotometric titrations of FP with these newly synthesised compounds indicate identical patterns in the downward shift of the absorbance maxima of the Soret band of the FP from 350 to 327 nm, with apparent slight weakening of the bands at 280 and 300 nm as shown in Fig. (**1**). While these finding cannot be used to draw conclusions on the structure-activity relationships of these new compounds, compounds **9** and **10**, both containing a piperaz-

Table I. The *In Vitro* IC_{50} Values (μg/ml)of the N,N-bisquinolin-4 yl Derivatives



Compound R	X	Chloroquine-sensitive	Chloroquine-resistant
1	-H -HN$(CH_2)_2$NH-	6.0	ND
2	-CF_3 -HN$(CH_2)_2$NH-	0.5	0.10
3	-H -HN$(CH_2)_3$NH-	13.0	> 100
4	-CF_3 -HN$(CH_2)_3$NH-	6.0	1.20
5	-H -HN$(CH_2)_4$NH-	4.0	35.3
6	-CF_3 -HN$(CH_2)_4$NH-	2.1	1.50
7	-H -HN$(CH_2)_6$NH-	3.8	> 100
8	-CF_3 -HN$(CH_2)_6$NH-	3.3	1.60
9	-H	10.7	> 100
10	-CF_3	12.0	1.02
Mefloquine			0.145
Chloroquine		0.,085	113.0

ino bridge instead of the diaminoalkyl chain, show very little effect on the absorbance of the FP. It may be inferred from this behaviour that the low or lack of activity of these two

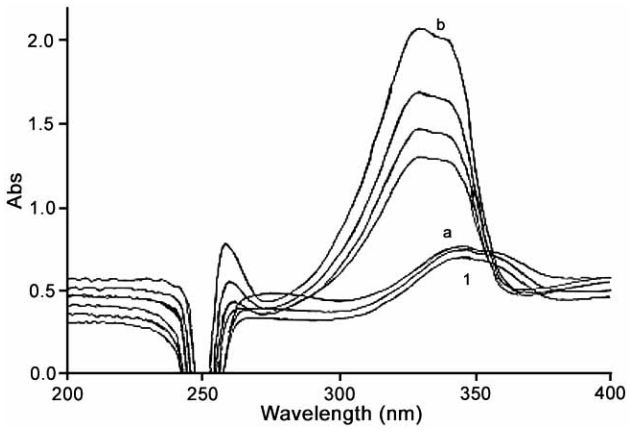


Fig. (1). Spectrophotometric titration of Ferriprotoporphyrin IX (1.3 μM) with increasing concentrations of compound **2**. In each case 1 is a curve for Ferriprotopophyrin IX alone, and *a* to *b* represent the concentrations of compound **2** added. At *a* the concentration of compound **2** is 0.822 μM, while at *b* is 1.37 μM. A reference cell was simultaneously titrated with approximately equal amounts of compound **2** and the absorbance values corrected for dilution.

piperazine compounds on the chloroquine-sensitive strains of *Plasmodium falciparum* is due to their inability to associate with and form complexes with FP. This could be ascribed to either addition of micromolar concentration of the bisquinolines unable to induce aggregation with FP or a reflection of the lack of capacity of these compounds to associate and complex with FP, and therefore inhibit the formation of haematin [40].

A quinoline ring is a complexing group with FP, while the aminoalkyl side chain assists in drug accumulation *via* the pH trapping [14]. The amino group at 4-position of the quinoline ring plays an important role in activity through interaction with the propionate side chains of FP [10]. It would appear that sufficiently large changes in diaminoalkyl side chain are responsible for overcoming chloroquine-resistance without having to change to the 4-amino-7-haloquinoline template responsible for FP complexation and inhibition of β-haematin formation [14]. It is for this reason that changes in the length of the diaminoalkyl briding chain have little influence on activity against chloroquine-sensitive strains of *P. falciparum* but profound influence on activity against the chloroquine-resistant strains of the parasite. There is also a speculation that the quinoline N-1 atom and the heteroatom (N or O) of the heteroaryl group in most of the active 2-heteroarylquinolines are favourably positioned to form specific hydrogen-bonded complexes with a biologi-

cal receptor [12]. A strong conjugation effect of the 4-amino group with the quinoline would increase the stability of such complex by increasing electron density at the quinoline nitrogen atom. The lack of the activity against the chloroquine-sensitive strain of the Plasmodium may be due to the absence of primary amino groups on the piperazine bridge to assist in the accumulation of these compounds in the acid food vacuoles. One of the bisquinolines synthesised earlier [41], the (±) trans-N^1,N^2-*bis*(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine, possesses superior *in vitro* and *in vivo* activity against the chloroquine susceptible and some moderately chloroquine-resistant clones of *P. falciparum.* The presence of the imino hydrogen atoms in these compounds as compared to the newly synthesised compounds in this study, could account for the activity of these compounds. The two enantiomers of this bisquinoline, the S,S and R,R have higher levels of inhibition of haem polymerisation than both chloroquine and mefloquine *in vitro* [42]. These isomers differ in their abilities to inhibit chloroquine resistant parasite growth, indicating that transport factors affecting the compound's accumulation in the parasite food vacuole may play a role. In-spite of the high potency of the S,S enantiomer of bisquinoline, *trans*-N^1,N^2-*bis*(7-chloroquinolin-4-yl)cyclohexane-1,-2-diamine, toxicological liabilities, particularly phototoxicity and the attendant photocarcinogenicity precluded its further development [42, 43]. However, these types of bisquinolines have potent activity against the chloroquine-resistant strains of *P. falciparum.* There is an indication that the optimal chain length for the linker group between the two quinoline rings is 6 carbons, and that linking through the 8-position is better than through the 6 - position and that a chlorine substituent is not necessary [3]. Bisquinolines with alkyl bridges of three, four or twelve carbon atoms are inactive, while those with bridges of five and nine carbon atoms are active, with a methyl substituent in the bridge improving the antimalarial activity [44].

The results of the *in vitro* cytotoxicity determination against the mammalian cell-line, Chinese Hamster Ovarian (CHO) cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) are represented in Table (**2**). The good selectivity index shown by these new compounds in the preliminary colorimetric cytotoxicity studies indicate that these compounds do not affect the living and proliferating mammalian cells, as only live cells will actively cleave MTT, while dead cells are almost completely negative even immediately after complement-mediated lysis [36].

CONCLUSION

On the basis of the IC_{50} values obtained by subjecting the new compounds to the chloroquine-sensitive and chloroquine-resistant strains *P.falciparum* it can be concluded that the two trifluoromethyl groups on the quinoline ring system are essential for *in vitro* antimalarial activity particularly against the chloroquine-resistant strains of *P. falciparum*. The type and size or length of the bridging groups between the two quinoline rings have an influence on the activity of this series of compounds. Bridging chains containing primary amino groups seem to be more active than those compounds which lack imino hydrogen atoms. This is particularly noticeable in compounds used against the chloroquine-sensitive than chloroquine-resistant strains of the *Plasmodium falciparum*. Except for compound **2**, the moderately low IC_{50} values obtained for these newly synthesised and screened compounds against both the chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum in vitro* suggest that the compounds are not sufficiently potent enough to warrant further investigation. Unfortunately the results of the interaction of these new compounds with FP cannot be used to assess the structure-activity relationships since identical shift patterns are observed in the UV spectra. The new compounds are highly selective in their action against the plasmodial cells and no serious cytotoxicity is observed against the Chinese Hamster Ovarian cell lines in the preliminary investigation using MTT.

EXPERIMENTAL SECTION

Synthesis

Melting points were determined in open capillary tubes on a Büchi B-545 apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer. ^{1}H NMR spectra were taken on a GEMINI 2000BB spectrometer. Chemical shifts are reported in δ (p.p.m) relative to internal trimethylsilane in DMSO-d_6. ^{13}C NMR spectra were recorded on the GEMINI 2000BB spectrometer at 75 MHz. Electron ionisation mass spectra as m/z (% relative intensity) values were carried out on a Turbomass GC-Mass spectrometer. TLC was carried out on pre-

Table 2. The *In Vitro* IC_{50} Values (μg/ml) of the N,N-Bisquinolin-4 yl Derivatives on the Chloroquine-Resistant Strain K1 of *Plasmodium falciparum* and CHO Cell-Line

Compound	K1: IC_{50}	CHO: IC_{50}	SI
4	1.20	50.82	42
6	1.50	41.49	28
8	1.60	>100	>62.5
10	1.00	>100	>100
Chloroquine	113.00		
Emetine		0.09	

Selectivity index (SI) = cytotoxicity IC_{50}/Antiplasmodial IC_{50}.

coated silica gel $60F_{254}$ analytical plates and the resulting chromatograms visualised under UV light (254 nm).

General Methods for the Synthesis of the trifluoromethylquinolin-4yl Diamino- Alkanes

2-Trifluoromethyl- or 2,8-bis(trifluoromethyl)-4-chloroquinoline (0.01 mole), diaminoalkane (including piperazine) (0.005 mole) and triethylamine (0.01 mole) were refluxed in 2-hydroxyethanol (20 ml) for 4 hours under a slight positive nitrogen pressure. The mixture was cooled and shaken with ethyl acetate (10 ml) and water (10 ml). The solid that formed was filtered and washed with ethyl acetate and water and dried.

N,N-bis(2-trifluoromethylquinolin-4-yl)diamino ethane (1): Yield 27%. Recrystallisation solvent DMF, M.p. 286 °C (Found: C, 58.6; H, 3.7; N, 12.3. $C_{22}H_{16}N_4F_6$ requires C, 58.7; H, 3.6; N, 12.4). ^{1}H N.M.R. δ 2.7, t, CH_2; 3.3, t, CH_2; 3.9, bs, NH; 7.8, t , H 6; 7.9, t, H 7; 8.0, s, H 3; 8.2, d, J 6 Hz, H 5; 8.4, d, J 6 Hz, H 8. ^{13}C N.M.R. δ: 46.1, CH_2; 109.5, C 3; 119.0, C 4a; 121.9, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.8, C 5; 126.0, C 6; 129.6, C 8; 130.4, C 7; 143.2, C 8a; 147.3, q, $^2J_{CF}$ 32 Hz, C 2; 152.2, C 4. E.i. mass spectrum *m/z*: 450 (M, 12%), 226 (72), 225 (100), 197 (15), 128 (7). ν_{max} (KBr): 3298s, 1581s, 1356s, 1295s, 1180s, 1128s.

N,N-bis[2,8-bis(trifluoromethyl)quinolin-4-yl]diamino ethane (2): Yield 33%. Recrystallisation solvent, ethanol. M.p. 222 °C. (Found: C, 50.8; H, 2.9; N, 9.6. $C_{24}H_{14}N_4F_{12}$ requires C, 49.2; H, 2.4; N, 9.6). ^{1}H N.M.R. δ: 2.6, t, CH_2; 2.8, t, CH_2; 3.5, bs, NH; 3.8, bs, NH; 7.0, t, H 6; 8.1, s, H 3; 8.3, d, J 6 Hz, H 5; 8.5, d, J 6 Hz, H 5. ^{13}C N.M.R. δ: 43.6, CH_2; 94.3, CH_2; 117.4, C 3; 118.5, C 4a; 119.5, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.6, q, $^2J_{CF}$ 32 Hz, C 8); 125.9, bq, $^1J_{CF}$ 213 Hz, 8-CF_3; 126.1, C 7; 129.1, C 6; 133.1, C 5; 144.0, C 8a; 148.2, q, $^2J_{CF}$ 32 Hz, C 2); 152.5, C 4. E.i. mass spectrum *m/z*: 586 (M, 54%), 567 (27), 293 (100), 273 (25), 224 (33). ν_{max} (KBr): 3368m, 1594s, 1443m, 1313s, 1136s.

N,N-bis(2-trifluoromethylquinolin-4-yl)diamino propane (3): Yield 17%. Recrystallisation solvent methanol. M.p. 180 °C. (Found: C, 58.2; H, 4.0; N, 11.4. $C_{23}H_{18}N_4F_6$ requires C, 59.5; H, 3.9; N, 12.1). ^{1}H N.M.R. δ: 2.1, q, CH_2; 3.4, q, CH_2; 3.9, q, CH_2; 4.4, bs, NH; 7.7, t, H 6; 7.8, t, H 7; 8.0, s, H 3; 8.2, d, J 6 Hz, H 5; 8.3, d, J 6 Hz, H 8. ^{13}C N.M.R. δ: 46.2, CH_2; 62.8, CH_2; 108.9, C 3; 117.6, C 4a; 119.3, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.8, C 5; 125.9, C 6; 129.5, C 8; 130.3, C 7; 144.3, C 8a; 146.5, q, $^2J_{CF}$ 32 Hz, C 2); 152.1, C 4. E.i. mass spectrum *m/z*: 464 (M, 69%), 252 (18), 239 (100), 213 (63). ν_{max} (KBr): 3445w, 1580s, 1305m, 1132s, 1014w.

N,N-bis[2,8-bis(trifluoromethyl)quinolin-4-yl]diamino propane (4): Yield 34%. Recrystallisation solvent, DMF. M.p. 240 °C. (Found C, 50.9; H, 2.8; N, 9.2. $C_{25}H_{16}N_4F_{12}$ requires C, 50.0; H, 2.7; N, 9.3). ^{1}H N.M.R. δ: 1.8, t, CH_2; 2.5, t, CH_2; 3.1, t, CH_2; 3.3, bs, NH; 7.1, t, H 6; 8.2, s, H 3; 8.3, d, J 6 Hz, H 7; 8.5, d, J 6 Hz, H 5. ^{13}C N.M.R. δ: 46.6, CH_2; 62.7, CH_2; 110.2, C 3; 118.6, C 4a; 119.5, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.4, q, $^2J_{CF}$ 32 Hz, C 8; 125.9, bq, $^1J_{CF}$ 213 Hz, 8-CF_3; 126.3, C 7; 129.6, C 6; 133.1, C 5; 143.9, C 8a; 146.5, q, $^2J_{CF}$ 32 Hz, C 2; 152.4, C 4. E.i. mass spectrum *m/z:* 600 (M, 82%), 343 (61), 319 (21), 307 (66), 293 (43), 280 (100), 273 (20), 265 (60), 224 (21), 196 (33), 176 (30), 57 (20), 45 (65). ν_{max} (KBr): 3467m, 2924w, 1595s, 1312s, 1151s, 952m.

N,N-bis(2-trifluoromethylquinolin-4-yl)diamino butane (5): Yield 31%. Recrystallisation solvent, DMF. M.p. 219 °C. (Found C, 59.8; H, 4.6; N, 11.1. $C_{24}H_{20}N_4F_6$ requires C, 60.2; H, 4.2; N, 11.7). ^{1}H N.M.R. δ: 3.3, bs, NH; 3.6, d, J 4,6 Hz, CH_2; 3.9, m, CH_2; 7.1, t, H 6; 7.4, t, H 7; 7.8, s, H 3; 8.3, d, J 6 Hz, H 7; 8.5, d, J 6 Hz, H 5. ^{13}C N.M.R. δ: 26.8, CH_2; 44.8, CH_2; 109.7, C 3; 118.6, C 4a; 119.5, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.9, C 5; 127.8, C 6; 130.4, C 7; 132.6, C 8; 143.8, C 8a; 147.1, q, $^2J_{CF}$ 32 Hz, C 2; 152.3, C 4. E.i. mass spectrum *m/z*: 478 (M, 53%), 266 (48), 239 (90), 237 (49), 225 (100), 213 (85), 205 (25). ν_{max} (KBr): 3386s, 1652s, 1407s, 1001m, 831m.

N,N-bis[2,8-bis(trifluoromethyl)quinolin-4-yl]diamino butane (6): Yield 22%. Recrystallisation solvent, DMF. M.p. 242 °C. (Found: 51.0; 3.2; N, 8.1. $C_{26}H_{18}N_4F_{12}$ requires C, 50.8; H, 3.0; N, 9.1). ^{1}H N.M.R δ (acetone): 3.0, bs, NH; 3.6, m, CH_2; 6.9, t, H 6; 8.1, s, H 3; 8.4, d, J 6 Hz, H 7; 8.6, d, J 6 Hz, H 5. ^{13}C N.M.R. δ: 26.9, CH_2; 44.1, CH_2; 110.3, C 3; 119.4, C 4a; 122.9, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.3, q, $^2J_{CF}$, 32 Hz, C 8; 125.7, bq, $^1J_{CF}$ 213 Hz, 8-CF_3; 126.4, C 7; 129.6, C 6; 133.4, C 5; 144.8, C 8a; 146.1, q, $^2J_{CF}$ 32 Hz, C 2; 153.9, C 4. E.i. mass spectrum *m/z* : 614 (M, 19%), 573 (23), 382 (24), 334 (37), 314 (24), 307 (43), 293 (54), 281 (49), 267 (27), 224 (24), 97 (24), 69 (33), 57 (69), 44 (100), 28 (54). ν_{max} (KBr): 2942w, 1592m, 1312s, 1138s, 950m, 764m.

N,N-bis(2-trifluoromethylquinolin-4-yl)diamino hexane (7): Yield 32%. Recrystallisation solvent, DMF. M.p. 145 °C. (Found: C, 61.7, H, 5.0; N, 10.9. $C_{26}H_{24}N_4F_6$ requires C, 61.6; H, 4.8; N, 11.1). ^{1}H N.M.R. δ 1.4, bs, NH; 1.7, bs, NH; 3.4, m, CH_2; 6.9, t, H 6; 7.5, t, H 7; 7.7, s, H 3; 7.9, d, J 6 Hz, H 5; 8.3 d, J 6 Hz, H 8. ^{13}C N.M.R. δ: 26.4, CH_2; 30.8, CH_2; 42.5, CH_2; 118.9, C 3; 122.0, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 123.2, C 5; 125.9, C 4a; 129.6, C 6; 130.4, C 7; 133.5, C 8; 144.3, C 8a; 148.0, q, $^2J_{CF}$ 32 Hz, C 2; 152.1, C 4. E.i. mass spectrum *m/z* : 506 (M, 17%), 486 (18), 281 (62), 239 (100), 205 (22), 44 (16). ν_{max} (KBr): 3449m, 2931m, 1586s, 1289m, 1128s, 937m.

N,N-bis[2,8-bis(trifluoromethyl)quinolin-4-yl]diamino hex-ane (8): Yield 29%. Recrystallisation solvent, DMF. M.p. 145 °C. (Found: C, 52.5; H, 3.6; N, 8.5. $C_{28}H_{22}N_4F_{12}$ requires C, 52.3; H, 3.5; N, 8.7). ^{1}H N.M.R.δ: 1.3, t, CH_2; 1.4, t, CH_2; 3.8, t, CH_2; 3.9, t, CH_2; 4.5, bs, NH; 7.2, t, H 6; 7.7, s, H 3; 8.0, d, J 6 Hz, H 7; 8.8, d, J 6 Hz, H 5. ^{13}C N.M.R. δ: 26.1, CH_2; 42.4, CH_2; 65.2, CH_2; 110.2, C 3; 118.5, C 4a; 119.4, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.3, q, $^2J_{CF}$ 32 Hz, C 8; 125.2, bq, $^1J_{CF}$ 213 Hz, 8-CF_3; 128.8, C 7; 130.2, C 6; 133.4, C 5; 143.9, C 8a; 147.9, q, $^2J_{CF}$ 32 Hz, C 2; 152.4, C 4. E.i. mass spectrum *m/z* : 642 (M, 18%), 362 (16), 349 (46), 307 (100), 293 (77), 273 (23), 224 (30), 44 (44). ν_{max} (KBr): 3423w, 2938m, 1593s, 1446w, 1173s.

N,N-bis(2-trifluoromethylquinolin-4-yl) piperazine (9): Yield 44%. Recrystallisation solvent, methanol. M.p. 310 °C. (Found: C, 60.0; H, 3.8; N, 11.7. $C_{24}H_{18}N_4F_6$ requires C, 60.5; H, 3.8; N, 11.8). ^{1}H N.M.R. δ: 3.2, t, CH_2; 3.3, t, CH_2; 7.1, t, H 6; 7.2, t, H 7; 7.4, s, H 3; 7.9, d, J 6 Hz, H 5; 8.1, d, J 6 Hz, H 8. ^{13}C N.M.R. δ: 25.7, CH_2; 42.5, CH_2; 65.3, CH_2;

109.7, C 3; 118.4, C 4a; 120.2, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.5, C 5; 125.7, C 6; 128.0, C 8; 132.8, C 7; 142.8, C 8a; 146.4, q, $^2J_{CF}$ 32 Hz, C 2; 151.4, C 4. E.i. mass spectrum *m/z*: 476 (M, 67%), 251 (100), 224 (44), 89 (18), 45 (34). ν_{max} (KBr): 3386w, 2934w, 1583s, 1419s, 1303m, 1242m, 1111s.

N,N-bis[2,8-bis(trifluoromethyl)quinolin-4-yl]piperazine (10): Yield 30%. Recrystallisation solvent, DMF. M.p. 350 °C (Found: C, 51.5; H, 2.6; N, 9.4. $C_{26}H_{16}N_4F_{12}$ requires C, 51.0; H, 2.6; N, 9.2). ^{1}H N.M.R. δ: 3.1, t, CH_2; 3.3, t, CH_2; 7.1, t, H 6; 7.8, s, H 3; 8.2, d, J 6 Hz, H 7; 8.5, d, J 6 Hz, H 5. ^{13}C N.M.R. δ: 26.8, CH_2; 43.0, CH_2; 65.5, CH_2; 109.7, C 3; 118.3, C 4a; 119.5, bq, $^1J_{CF}$ 213 Hz, 2-CF_3; 124.3, q, $^2J_{CF}$ 32 Hz, C 8; 125.6, bq, $^1J_{CF}$ 213 Hz, 8-CF_3; 126.8, C 7; 129.5, C 6; 132.9, C 5; 144.0, C 8a; 147.8, q, $^2J_{CF}$ 32 Hz, C 2; 152.5, C 4. E.i. mass spectrum *m/z*: 612 (M, 52%), 319 (100), 292 (38), 223 (42), 44 (22). ν_{max} (KBr): 3423w, 2938m, 1593s, 1314s, 1137s, 765m.

ASSESSMENT OF ANTIMALARIAL ACTIVITY AND CYTOTOXICITY

Preparation of Compound Suspension

The test compounds were dissolved in 10% methanol to give a 2 mg/ml stock solution. All the compounds formed a milky suspension in this solvent system and were tested as such. Chloroquine (CQ) was used as the reference drug in all experiments. The stock solutions of the compounds were stored at – 20 °C until use. A full dose response was performed with a starting concentration of 100 μg/ml, which was serially diluted 2-fold in complete culture medium (RPMI 1640 containing 25 mmol/*l* HEPES buffer, 20 μg/ml gentamycin, 27 mmol $NaHCO_3$, and 10% normal type A human serum) to give 10 concentrations ranging from 0.01 to 100 μg/ml. Chloroquine was tested at a starting concentration of 1000 ng/ml using the same dilution technique. The highest concentration of solvents to which the parasites were exposed to had no measurable effect on the parasite viability.

Preparation of the Parasites

The parasites inocula used in the experiments consisted of isolates of the chloroquine-sensitive (D10) and chloroquine-resistant (K1) strains of *P. falciparum* obtained from Groote Schuur Hospital, Cape Town, South Africa. These were used when the tropozoite stage parasitaemia was adjusted to at least 2 % with normal type A human red blood cells (2% hematocrit). The parasite culture was suspended in complete tissue culture medium (RPMI 1640 containing 25 mmol/*l* HEPES buffer, 20 μg/ml gentamycin, 27 mmol $NaHCO_3$) and 10% normal type A human serum. 25 μ*l* of the drug suspension to contain from 0,01 to 100 μg/ml of the drugs were added in triplicate at 0.2 ml*l*/well into 96-well flat-bottom microtitre plates, leaving one row for positive control (no drug containing parasitised red blood cells) and one row for background control (no parasites but only red blood cells). Incubation was carried out at 37 °C in a humidified atmosphere of 6% CO_2, 3 % O_2 and 91% N_2 for 48 hours.

Harvesting (the Parasite Lactate Dehydrogenase Assay)

After the incubation period, the parasite Lactate dehydrogenase (pLDH) was determined. 10 μl from each well of the suspended culture was transferred into another 96 well, flat bottom microtiter plate that contained 100 μg of the Malstat reagent. The plates were left in the dark for 0.5 – 1.0 hour. At the end of this period, the reduction of APAD to APADH was followed kinetically. To each well 25 μ*l* of a 20:1 mixture of nitroblue tetrazoluim (NBT) and phenazine ethosulphate (PES) was added and the reduction of the tetrazolium to the blue formazan salt was followed for 10 minutes at 650 nm. Finally the blue formazan product was evaluated after addition of 30 μ*l* of $2NH_2SO_4$ by end-point analysis at 650 nm using the Anthos Labotec. HT2 model 1.06 (Anthos Labotec Instruments, Salzburg, Austria. The 50% inhibitory concentration (IC_{50}) values were obtained using a non-linear dose-response curve fitting analyses *via* GraphPad Prism v 4.0 software.

Interaction of the Compounds with Ferriprotoporphyrin IX

Stock solutions were prepared by dissolving between 6 to 8 mg of accurately weighed Ferriprotoporphyrin IX (Sigma-Aldrich Chemie, Steinheim, Germany) in 10 ml AR grade DMSO (Sigma-Aldrich Chemie). These stock solutions were stored in the dark. Aqueous-DMSO (40% v/v) solutions of FP were prepared daily by mixing 20 μ*l* of the FP stock solution with 4 ml DMSO and 1 ml 0. 2 M HEPES buffer (pH 7,4) and making up to 10 ml with deionised water. Solutions of the compounds of interest were prepared by dissolving them in 0.02M HEPES and 40% DMSO to obtain final concentrations of about 2 mM. The ferriprotoporphyrin IX - compounds interactions were monitored by spectrophotometric titration of both sample and reference solutions in a thermostated cell holders using a Cary 100 Conc UV/VIS Spectrophotometer (Varian Australia (Pty) Ltd., Mulgrave Victoria, Australia) at 25 °C and measuring the absorbance of the Soret band at 230 nm. A reference cell was titrated simultaneously with the compounds. The compounds seem to give similar reaction with Ferriprotoporphyrin IX.

Determination of the Cytotoxicity of the Compounds

The mammalian cell line, Chinese hamster Ovarian (CHO) obtained from the Department of Medicine, University of Cape Town were grown in RPMI 1640 supplemented with 50 μM 2-mercaptoethanol and 5 – 10% foetal bovine serum in a 6% CO_2 atmosphere, were used for the study. Stock solutions of compounds exhibiting highest activity against the chloroquine-resistant strains of *P. falciparum,* compounds **4**, **6**, **8** and **10,** were prepared to a 2 mg/ml in 10% methanol and stored at –20 °C until use. All the compounds formed milky suspensions. The initial concentration of Emetine (Sigma-Aldrich Chemie, Steinheim, Germany) used as a standard, was 100 μg/ml and was serially diluted in complete medium to 10-fold dilutions to give 6 concentrations ranging from 0.001 to 100 μg/ml. Similarly, the test compounds were diluted from the stock solutions to form concentrations from 0.01 to 100 μg/ml. The highest concentration of the solvent to which the cells were exposed to did not have a measurable effect on the cell viability.

Colorimetric Assay

3-(4,5-dimethilthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich Chemie, Germany) was dis-

solved in phosphate-buffered saline at 5 mg/ml and filtered to sterilise and remove a small amount of insoluble residue present to form a stock solution. At regular intervals this stock solution (10 µl per 100 µl) was added to all the wells of the 96-well flat bottom trays, each of which contains 1 x 10^6 cells to which 25 µl of the drug suspensions varying in concentrations from 0.01 to 100 µg/ml was added. One row contained the cells and the Emetine standard ranging in concentration from 0.01 to 100 µg/ml. The plates were incubated at 37 ^oC for 4 hours. Acid-isopropanol (100 µl of 0.04 N HCl is isopropanol) was added to all the wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at room temperature, the plates were read at 650 nm using the Anthos Labotec.HT2 model 1.06 Microelisa reader linked to a computer with GraphPad Prism v.4 software. The 50% inhibitory concentration (IC_{50}) values were obtained from the full dose-response curves using a non-linear dose response curve fitting analysis. The plates were normally read within 1 hour of adding the isopropanol.

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