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Research Paper

Synthesis of 4-Amino substituted Quinolines and their β –hematin inhibitory activity

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Abstract: In present study, new side chain modified 4-aminoquinoline derivatives and quinoline pyrimidine hybrids were synthesized and evaluated *in vitro* against β-hematin formation. Compounds 20, 21, 22,23 have shown significant inhibitory activity against β-hematin formation.

Introduction

Malaria is one of the major problems of many tropical and subtropical countries in the world. It is estimated that with 40% of the world's population exposed to the threat of malaria, there are an estimated 500 million clinical cases per year and 2 million deaths. Malaria is caused by protozoan parasites, namely *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae,* and is transmitted to humans by female mosquitoes belonging to the genus Anopheles. Endemic maps indicates that *P. falciparum* and *P. vivax* account for 95%of malaria infections [1,2]. There are a several classes of antimalarial drugs, which inhibit different stages of the malaria parasite. (chloroquine, mefloquine and amodiaquine, cycloguanil, pyrimethamine), but as the parasites rapidly develop permanent resistance against the different subclasses, there is a great urge to develop new and effective drugs [3]. CQ has been the mainstay of malaria therapy for decades because of its efficacy, safety and low cost until the emergence and spread of CQ-resistance. Pyrimethamine-sulfadoxine (fansidar) was another good therapeutic option after CQ but rendered ineffective in most of malaria endemic regions due to spread of resistance [4, 5]. Currently, natural endoperoxide artemisinin and its semi-synthetic derivatives (artemether, arteether and artesunate) are the most potent and fast acting antimalarials effective against resistant strains of *P. falciparum* [6-10].

Despite the emergence of resistance to heme-targeted antimalarials mainly quinoline-based drugs, drug target heme is still being exploited extensively for designing new antimalarial agents because

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biochemical drug target heme cannot either be mutated or expressed by the parasite [11-12]. During the intra-erythrocytic stage of life cycle, parasite invades the red blood cells of human host for feeding the hemoglobin which is a major source of nutrition for its growth and development. In the acidic food vacuole of the parasite, a toxic by-product free heme is generated as a result of hemoglobin degradation into amino acids by enzymes aspartic, cysteine, and metallo proteases [13,14]. As a defence mechanism for its survival, parasite converts the free heme into non-toxic inert crystalline pigment called hemozoin [15]. CQ and other aminoquinoline drugs disrupt the conversion of toxic heme into hemozoin, consequently substantial accumulation of toxic heme lead to the death of the parasite [16,17]. Despite the persistent heavy drug pressure of CQ for several decades, the delayed emergence of resistance to CQ is considered due to the complexity of digestive vacuole environment and the immutable nature of heme target [18]. Multiple point mutations in *P. falciparum* chloroquine resistance transporter protein (pfcrt) conferred resistance to CQ characterized by the substantially reduced accumulation of CQ level in food vacuole. Interaction of CQ with pfcrt induces resistance very slowly to *P. falciparum* owing to the complexity in amino acid substitutions in pfcrt [19-20]. The comprehensive structure-activity relationship studies on CQeHematin binding have been explored to identify the optimal structural requirements for designing the new CQ-based antimalarial agents. It was established that the 7-chloro-4-aminoquinoline is critical for the antimalarial activity and basicity of the side chain nitrogen is also equally important for accumulation of drug within acidic food vacuole of the parasite [21,22]. Modification in the side chain of CQ led to the new CQ analogues with improved activity effective against CQ-resistant strain. Altering the chain length of CQ had the little effect on CQ-sensitive strain but produced the remarkable effect on the CQ-resistant strain suggesting that CQ-resistance is compound specific [23-25]. In addition to the side chain modified new CQ analogues; several functionalized 4-anilinoquinolines were identified as potential antimalarials. Amodiaquine is still clinically useful for low degree CQ-resistant parasites but it has some serious side effects like heptatotoxicity and agranulocytosis [26, 27]. To prevent the formation of toxic metabolite, amodiaquine quinoneimine, a direct regioisomer of amodiaquine, isoquine was developed having potent in vitro activity against CQ-resistant parasites, however the unacceptable high first pass metabolism of isoquine to dealkylated metabolites did not allow further drug development process [28]. Modification of isoquine guided the discovery of drug candidate N-tert-butyl isoquine [29]. In addition, 40-Fluoro-N-tert-butylamodiaquine was identified as a back-up compound for N-tertbutyl isoquine based on potent activity against CQ-sensitive and CQ-resistant parasites [30]. Thus, these findings manifest considerable scope for developing new antimalarial with quinoline nucleus. Along with improvement of efficacy, preventing and delaying the emergence of drug resistance is an essential goal of antimalarial drug development. To combat the increasingly becoming resistant parasite, hybrid drug approach is gaining attention as a viable option for effective long term strategy of antimalarial chemotherapy [31]. Hybrid drug approach involves the incorporation of two drug pharmacophore in one single molecule with attention of dual drug action. Given the unique pharmacological effect of quinoline based antimalarials targeting the heme, quinoline moiety has been the integral component of designing hybrid antimalarials. It was

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exemplified by several potential antimalarials that include trioxaferroquines [32], trioxaquines, artemisinin-quinine hybrid 4-aminoquinoline based tetraoxanes [33], clotrimazole-based 4-aminoquinolines [34], ferrocene-chloroquine analogues [35] inhibitors of glutathione reductase conjugated to a 4-aminoquinoline and 4-aminoquinolines based on natural product scaffold isatin [36]. In addition, a chloroquine reversal agent hybrid of CQ like moiety and imipramine was identified as potential antimalarial agent [37]. More recently, the dual function acridones as new antimalarial chemotype were discovered that combined the heme targeting character of acridones, together with a chemosensitizing component that counteracts resistance to quinoline antimalarial drugs [38]. The dihydrofolate reductase (DHFR) is one of the well-defined and successfully exploited targets in malarial chemotherapy. Pyrimethamine and cycloguanil, the two important therapeutic drugs commonly employed for the prophylaxis and treatment of malaria target the DHFR. However, in the recent years, rapid spread of antifolate resistant *P.falciparum* seriously compromised the clinical utilities of these drugs and consequently necessitates the need to search for new potent antifolate antimalarials [8,39,40]. Toward this goal, additional pyrimethamine and cycloguanil analogues were identified as potential inhibitors of resistant DHFR [41]. Apart from this, structurally similar to cycloguanil, triazines have been reported to possess promising antimalarial activity [42]. We envisaged that combining two intrinsically active antimalarial moiety 4-aminoquinoline and pyrimidine would lead to develop more potent antimalarials. The haemozoin pigment is formed by polymerization of the haem that is released following haemoglobin digestion [1]. The enzyme haem polymerase, which catalyses the formation of haemozoin from free haem, and chloroquine was reported to inhibit haem polymerization *in vitro* [1]. Several quinoline antimalarial drugs have been reported to inhibit haem polymerization. We have synthesised 4-aminoquinolino pyrimidines and tested for β –hematin inhibitory activities. Compounds 20, 21, 22,23 have shown better activity than the reference drug chloroquine (IC_{50} 3.95 µg/ml)

Chemistry

Synthesis of 2-chloro 4-aminopyrimidine derivatives-

The synthesis of targeted compounds 3-11 is shown in scheme-1. The compound (1) was reacted with amine (2) which afforded the nucleophilic substitution in presence of DIPEA as base and ethanol as solvent to obtain the final targeted compounds **(3-10).**

General procedure for the synthesis of compounds 2-10 –

To a stirred solution of 2, 4, dichloropyrimidine (1) (1 mmol) in ethanol (6 ml) , DIPEA (1.2 mmol) was added, after this respected amine (2 mmol) was added drop-wise and then this reaction mixture was allowed to be stirred at room temperature for 24 hrs. The solvent was removed under reduced pressure and the resultant residue was dissolved in dichloromethane (30 ml) and this organic layer was washed with water, dried over anhyd.Na_2SO_4 the solution was evaporated to dryness and the crude material was purified by flash chromatography (Scheme-1).

Synthesis of 4-aminoquinoline pyrimidine derivatives –

Here commercially available 4,7-Dichloroquinoline (11) was refluxed

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overnight with commercially available piperazine (12) using ethanol as the solvent to yield compound(13). The solvent was removed under reduced pressure and the resultant residue was dissolved in dichloromethane (30 ml) and this organic layer was washed with water, dried over anhyd.Na_2SO_4 the solution was evaporated to dryness and the crude material was purified by flash chromatography (Scheme-2a).

In scheme 2b step (a) Compound (13) obtained from scheme 2a was further reacted with compound (14) at room temperature using DIPEA (15) as base and ethanol as solvent to yield a couple of compounds (16). In step (b) compounds (16) were reacted with different amines using dioxane as solvent and K_2CO_3 as base in a sealed tube at 120 ^oC for 10 hours to obtain the desired compounds (17-23). The solvent was removed under reduced pressure and the resultant residue was dissolved in dichloromethane (30 ml) and this organic layer was washed with water, dried over anhyd.Na_2SO_4 the solution was evaporated to dryness and the crude material was purified by flash chromatography (Scheme-2b).

Materials and Methods

All the reactions were monitored by thin layer chromatography over silica gel-G and basic alumina coated TLC plates. The spot on TLC plates were developed by iodine vapours, potassium permagnate spray or Dragendroff spray as the developing agents.The melting points were recorded on an electrically heated melting point apparatus.IR spectra were recorded on Beckman Aculab-10, Perkin Elmer 881 and FTIR 8210 PC, Shimadzu spectrophotometers either on KBr discs or in neat and values are expressed in cm^{-1}.Nuclear Magnetic Resonance (NMR) spectra were recorded on either Bruker Avance DRX-300 MHz or Bruker DRX 200 FT spectrometers using TMS as an internal reference. Chemical shifts (δ in ppm) were reported relative to solvent peak ($CHCl_3$ in $CDCl_3$ at 7.23 ppm and DMSO in DMSO-d_6 at 2.49 ppm) or TMS. Signals were designated as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet.FAB mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer using Argon/ Xenon (6 KV, 10 mA) as the FAB gas. EI mass spectra were recorded on JEOL JMS-D-300 spectrometer with the ionization potential of 70 eV and ES mass on Quantro-II, micro mass. Silica gel (60-120mesh) for column chromatography and silica gel (230-400 mesh) for flash column chromatography were used. Room temperature mentioned range between 22-30˚C unless stated otherwise. Anhydrous solvents were prepared as per general procedure mentioned in text book of practical organic chemistry by A.I. Vogel. Common solvents for general use were purchased from E. Merck, Qualigens, Ranbaxy and S. D. Fine Chemicals. Reagents were purchased from Sigma, Aldrich and Across Chemicals.

Characterisation of synthesized compounds:- 2 – 10

N-tert-butyl-2-chloropyrimidin-4-amine (2) –

Yield: 85% ,mp: 203-205^{0}C,FT-IR ν (cm^{-1}) : 3281, 2363, 1594, 1346, 1217, 996, 772. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 7.98 (d, j=5.1Hz, 1H), 6.29 (d, j=4.8Hz, 1H) , 5.23 (s, 1H), 1.46 (s, 9H). Elemental analysis Calcd. for $C_8H_{12}ClN_3$: C 51.76, H 6.51, N 22.63; Found: C 51.96, H 6.31, N 22.43.

2-chloro-4-(4-methylpiperazin-1-yl)pyrimidine (3)-

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Yield: 85% ,mp: 170-175^{0}C, FT-IR ν (cm^{-1}) : 3505, 3432, 1593, 1268, 1155, 984, 772. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.05 (d, j=6.3Hz, 1H), 6.40 (d, j=6.3Hz, 1H), 3.69 (s, 4H), 2.50 (t, j=5.1Hz, 4H), 2.35 (s, 3H). Elemental analysis Calcd. for $C_9H_{13}ClN_4$: C 50.83, H 6.61, N 26.24; Found: C 50.72, H 6.72, N 26.05.

2-chloro-*N*-(4-methoxybenzyl) pyrimidin-4-amine (4)

Yield: 80% , mp: 179-180^{0}C FT-IR ν (cm^{-1}) : 3491, 3440, 3022, 1637, 1216, 768, 671. ^{1}H NMR (300MHz, DMSO) : δppm 8.39 (s, 2H), 7.44 (d, j=8.7Hz, 2H), 6.97 (d, j=8.4Hz, 2H), 3.92 (s, 2H), 3.76 (s, 3H). Elemental analysis Calcd. for $C_{12}H_{12}ClN_3O$: C 57.72, H 4.84, N 16.83; Found: C 57.42, H 4.64, N 16.63.

N-butyl-2-chloropyrimidin-4-amine (5)

Yield: 80% ,mp: 180-182^{0}C, FT-IR ν (cm^{-1}) : ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.03 (s, 1H), 6.27 (d, j=6Hz, 1H), 3.32 (s, 2H), 1.66-1.57 (m, 2H), 1.48-1.39 (m, 2H), 0.99-0.95 (m, 3H). Elemental analysis Calcd. for $C_8H_{12}ClN_3$: C 51.76, H 6.51, N 22.63; Found: C 51.42, H 6.74, N 22.43.

2-chloro-*N*-(4-methylbenzyl) pyrimidine-4-amine (6)

Yield: 79% ,mp: 200-202^{0}C, FT-IR ν (cm^{-1}) : 3432, 2362, 1601, 1350, 1219, 771, 673. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.03 (d,j=5.4Hz, 1H), 7.20 (d, j=3Hz, 4H), 6.25 (d, j=6Hz, 1H), 4.51 (s, 2H), 2.36 s, 3H). Elemental analysis Calcd. for $C_{12}H_{12}ClN_3$: C 61.67, H 5.18, N 17.98; Found: C 61.42, H 5.34, N 17.63.

2-chloro-N-(2-chlorobenzyl)pyrimidine-4-amine (7)

Yield: 89%, mp: 190-192^{0}C, FT-IR ν (cm^{-1}) : 3434, 3264, 2362, 1600, 1500, 1347, 1188, 1046, 976, 756, 674. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.04 (d, j=4.5Hz, 1H), 7.41-7.28 (m,4H), 6.27 (d, j=4.67Hz, 1H). 4.51 (s, 2H) Elemental analysis Calcd. for $C_{11}H_9Cl_2N_3$: C 51.99, H 3.57, N 16.54; Found: C 51.52, H 3.34, N 16.34.

2-chloro-4-(4-ethylpiperazin-1-yl)pyrimidine (8)

Yield: 82% ,mp: 200-202^{0}C FT-IR ν (cm^{-1}) : 3435, 3344, 2971, 2820, 1590, 1536, 1357, 1154, 981, 807, 759, 698. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.04 (d, j=6Hz, 1H), 6.40 (d, j=6Hz, 1H), 3.68 (s, 4H), 2.55-2.43 (m, 6H), 1.14 (t, j=7.2Hz, 3H). Elemental analysis Calcd. for $C_{10}H_{15}ClN_4$: C 52.98, H 6.67, N 24.71; Found: C 52.52, H 6.34, N 24.34.

2-chloro-*N*-(3,4-dichlorobenzyl)pyrimidin-4-amine (9)

Yield: 83%,mp: 180-182^{0}C, FT-IR ν (cm^{-1}) : 3275, 2928, 1625, 1596, 1500, 1343, 1186, 971, 904, 823, 680. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.06 (d, j=5.7Hz, 1H), 7.46-7.43 (m, 2H), 7.19-7.17 (m, 1H), 6.26 (d, j=5.7Hz, 1H), 4.57 (d, j=4.5Hz, 2H). Elemental analysis Calcd. for $C_{11}H_8Cl_3N_3$: C 45.79, H 2.79, N 14.56; Found: C 45.52, H2.34, N 14.34.

4-(2-chloropyrimidin-4-yl)morpholine (10)

Yield: 85%, mp: 188-190^{0}C FT-IR ν (cm^{-1}) : 3858, 3364, 2922, 2854, 2361, 1590,1475, 1235, 1113,774. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 7.99 (d, j=6Hz, 1H), 5.89 (d, j=6Hz, 1H), 3.79-3.75 (m, 4H), 3.58-3.55 (m, 4H). **Elemental analysis** Calcd. for $C_8H_{10}ClN_3O$: C 48.13, H 5.05, N 21.05; Found: C 48.52, H5.34, N 21.34.

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Synthesis of 4-aminoquinoline pyrimidine derivatives –

General procedure for the synthesis of compounds (13)

Here commercially available 4,7-Dichloroquinoline (1 mmol) (11) was refluxed overnight with commercially available piperazine (3 mmol) (12) using ethanol as the solvent to yield compound (13). The solvent was removed under reduced pressure and the resultant residue was dissolved in dichloromethane (30 ml) and this organic layer was washed with water, dried over anhyd.Na_2SO_4 the solution was evaporated to dryness and the crude material was purified by flash chromatography yielded (13). Yield 85%

General procedure for the synthesis of compounds (16-23)

In scheme 2b step (a) Compound (1 mmol) (13) obtained from scheme 2a was further reacted with compound(1.2 mmol) (14) at room temperature using DIPEA (15) as base and ethanol as solvent to yield compounds (16).Yield 75% .
The compounds (1 mmol) (16) were reacted with different amines (2 mmol) using dioxane as solvent and K_2CO_3 (1.5 mmol) as base in a sealed tube at 120 ^oC for 10 hours to obtain the desired compounds (17-23). The solvent was removed under reduced pressure and the resultant residue was dissolved in dichloromethane (30 ml) and this organic layer was washed with water, dried over anhyd.Na_2SO_4 the solution was evaporated to dryness and the crude material was purified by flash chromatography.

Characterisation of synthesized compound :- 16 – 23(Scheme 2b)

7-chloro-4-(4-(2-chloropyrimidin-4-yl)piperazin-1-yl)quinoline (16)-
Yield: 88%,mp: 200-202^{0}C, FT-IR ν (cm^{-1}) : 3497, 3338, 3241, 2900, 2365, 1583, 1351, 1231, 1157, 975, 809. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.76 (d, j=5.1Hz, 1H), 8.17-8.14 (m, 2H), 8.02 (d, j=9Hz, 1H), 7.53 (dd, j_1=9Hz, j_2=2.1Hz, 2H), 6.8 (d, j=5.7Hz, 1H), 6.5 (d, j=5.7Hz, 1H), 3.98 (s, 4H), 3.31 (t, j=5.1Hz, 4H), Elemental analysis Calcd. for $C_{17}H_{15}Cl_2N_5$: C 56.68, H 4.20, N 19.44; Found: C 56.58, H 4.10, N 19.14.

7-chloro-4-(4-(2-chloro-6-methyl pyrimidin -4-yl)piperazin-1-yl) quinoline (17) –

Yield: 78%,mp: 210-212^{0}C FT-IR ν (cm^{-1}) : 3457, 2900, 2365, 1594, 1494, 1426, 1236, 770. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.76 (d, j=5.1Hz, 1H), 8.10 (d, j=2.1Hz, 1H), 8.01 (d, j=8.97, 1H), 7.51 (dd, j_1=9Hz,j_2=2.1Hz, 1H), 6.8 (d, j=5.7Hz, 1H), 6.3 (s, 1H),3.95 (s, 4H), 3.32 (t, j=5.1Hz, 4H), 2.40 (s, 3H). Elemental analysis Calcd. for $C_{18}H_{17}Cl_2N_5$: C 57.76, H 4.58, N 18.71; Found: C 57.46, H 4.38, N 18.41

4-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-*N,N*,6-trimethylpyrimidin-2-amine (18) –

Yield: 88%,mp: 220-222^{0}C, FT-IR ν (cm^{-1}) : 3457, 2900, 2360,1600, 1494, 1426, 1236, 770 ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.73 (d, j=5.1Hz, 1H), 8.06-7.97(m, 2H), 7.46 (dd, j_1=9.0Hz, j_2=1.8Hz, 1H), 6.84 (d, j=4.8Hz, 1H), 5.90 (d, j=6Hz, 1H), 3.86 (s, 4H), 3.27 (t, j=4.5Hz, 4H), 3.15 (s, 6H). Elemental analysis Calcd. for $C_{20}H_{23}ClN_6$: C 62.74, H 6.05, N 21.95; Found: C 62.44, H 6.25, N 21.62.

4-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-*N,N*-dimethylpyrimidin-2-amine (19) –

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Yield: 75%, mp: 225-228^{0}C FT-IR ν (cm^{-1}) : 2923, 2852, 2365, 1571, 1396, 1191, 992, 962. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.77 (d, j=5.1Hz, 1H), 8.02-8.09(m, 3H), 7.46 (dd, j_1=9.0Hz, j_2=1.8Hz, 1H), 6.84 (d, j=4.8Hz, 1H), 5.90 (d, j=6Hz, 1H), 3.87 (s, 4H), 3.27 (t, j=4.5Hz, 4H), 3.15 (s, 6H). Elemental analysis Calcd. for $C_{19}H_{21}ClN_6$: C 61.87, H 5.74, N 22.78; Found C 61.66, H 5.44, N 22.58.

4-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-*N*-(4-methoxybenzyl)-6-methylpyrimidin-2-amine (20) –

Yield: 78%,mp: 190-194^{0}C, FT-IR ν (cm^{-1}) : 3456, 3393, 2363, 1573, 1432,1245, 770. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.76 (d, j=4.5Hz, 1H), 8.08 (s, 1H), 8.02 (d, j=9Hz, 1H), 7.49 (d, j=7.8Hz,1H), 7.30 (d, j=7.8Hz,2H), 6.88 (brs, 3H), 5.87 (s, 1H), 5.18 (brs, 1H), 4.56 (d, j=5.1Hz, 2H) 3.87 (bs, 4H),3.80 (s, 3H), 3.25 (s, 4H), 2.26 (s, 3H). Elemental analysis Calcd. for $C_{26}H_{27}ClN_6O$: C 65.74, H 5.73, N 17.69; Found C 65.50, H 5.62, N 17.46.

4-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-*N*-(4-methylbenzyl)pyrimidin-2-amine (21) –

Yield: 85% mp: 198-200^{0}C, FT-IR ν (cm^{-1}) : 3456,2900, 3393, 2363, 1573, 1432,1245, 770,^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.72 (d, j=5.1Hz, 1H), 8.05 (m,2H), 7.45 (dd, j_1=9Hz, j_2=1.8Hz, 4H), 6.83 (d, j=4.8Hz, 2H), 5.82 (s.1H), 3.83 (bs, 6H), 3.24 (s, 4H), 2.25-2.50 (m, 6H), Elemental analysis Calcd. for $C_{26}H_{27}ClN_6$: C 68.04, H 5.93, N 18.31; Found C 68.24, H 5.63, N 18.10

7-chloro-4-(4-(2-(4-ethylpiperazin-1-yl)pyrimidin-4-yl)piperazin-1-yl)quinoline (22) –

Yield: 80%, mp: 190-194^{0}C, FT-IR ν (cm^{-1}) : 3456, 3393, 2900,2363, 1573, 1432,1245, 770^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.71 (d, j=4.8Hz, 1H), 8.04-7.95 (m, 3H), 7.44 (dd, j_1=8,.7Hz,j_2=1.8Hz, 1H), 6.83 (d, j=5.1Hz, 1H), 5.92 (d, j=6Hz, 1H), 3.82-3.79 (m, 8H), 3.25 (t, j=4.5Hz, 4H), 2.51-2.41 (m, 6H), 1.13 (t, j=7.2Hz, 3H). Elemental analysis Calcd. for $C_{23}H_{28}ClN_7$: C 63.07, H 6.44, N 22.39; Found C 63.27, H 6.24, N 22.12.

4-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-N-(4-methoxybenzyl)pyrimidin-2-amine (23)

Yield: 85%, mp: 188-190^{0}C FT-IR ν (cm^{-1}) : 3436, 3229, 1582, 1425,1232, 770. ^{1}H NMR (300MHz, $CDCl_3$) : δppm 8.67 (d, j=4.2Hz,1H), 7.99-7.85 (m, 3H), 7.40 (dd, j_1=9Hz, j_2=1.8Hz, 1H), 7.21 (d, j=8.7Hz, 2H), 6.79-6.76 (m,3H), 5.90 (d, j=6Hz, 1H), 4.47 (d, j=5.4Hz, 2H), 3.96 (s, 4H), 3.71 (s, 3H), 3.17 (s, 4H) . Elemental analysis Calcd. for $C_{25}H_{25}ClN_6O$: C 65.14, H 5.47, N 18.23; Found C 65.34, H 5.68, N 18.03.

Result & Discussion

Result

Most of the synthesized compounds showed moderate β –hematin inhibitory activities (IC_{50} > 3.5 µg/ml).Compounds 20, 21, 22,23 have shown better than the reference drug chloroquine (IC_{50} 3.95 µg/ml) (Table 2).

Inhibition of β-hematin formation assay –

Male swiss mice, weighing 15–20 g were inoculated with 1X10^5 *P. yoelii* infected RBCs. Blood of infected animal at~50% parasitemia was collected by cardiac puncture in 2.0% citrate buffer and centrifuged at 3000 rpm for 10 min at 4^{0}C. The plasma was used in assay of β hematin

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formation. The assay mixture contained 100 mM sodium acetate buffer pH (5.1), 50 µL plasma, 100 µM hemin as the substrate and 1–10 µg compound/drug in a total volume of 1.0 mL. The control tube contained all reagents except compound. The reaction mixture in triplicate was incubated at 37°C for 16 h in a rotary shaker. The reaction was stopped by centrifugation at10,000 rpm for 10 min at 30° C. The pellet was suspended in 100 mM Tris–HCl buffer pH (7.4) containing 2.5% SDS. The pellet obtained after centrifugation was washed thrice with distilled water (TDW) to remove free hemin attached to β-hematin. The pellet was solubilized in 50 µL of 2 N NaOH and volume was made up to 1.0 mL with TDW. Absorbance was measured at 400 nm [43].

Discussion

Chloroquine (CQ), 4-aminoquinoline, has been the mainstay of malaria therapy for decades because of its efficacy, safety and low cost that has dominated the market for years. CQ is a safe and affordable drug, and it was effective before mutations led to the emergence of resistant strains in the 1960s.

Through this work we aimed at designing 4-amino substituted quinolines having antimalaraial activity comparable to chloroquine and to overcome the resistance developed against chloroquine due to mutations by developing a hybrid compound by linking two pharmcophores, with already established antimalarial activity, through a linker. Here we linked the two pharmocophores, 4-aminoquinoline nucleus and pyrimidine nucleus through a piperazine linker. We synthesized a series of compounds and their structure was confirmed by using spectroscopic techniques like Infra-red (IR) spectroscopy, Nuclear Magnetic resonance (NMR) spectroscopy. Out of 19 compounds 16-23 have shown inhibitory activity against β-hematin formation.

Conclusion

We conclude that the appropriate and suitable changes in the side chain of Chloroquine does help to overcome the resistance developed in the plasmodium species against Chloroquine. We aimed at increasing its basicity so that the drug is retained for a longer duration in the food vacuole contrary to its quick efflux in the resistant strains. Compounds having inhibitory activity against β-hematin formation can be new lead in malalia chemotherapy.

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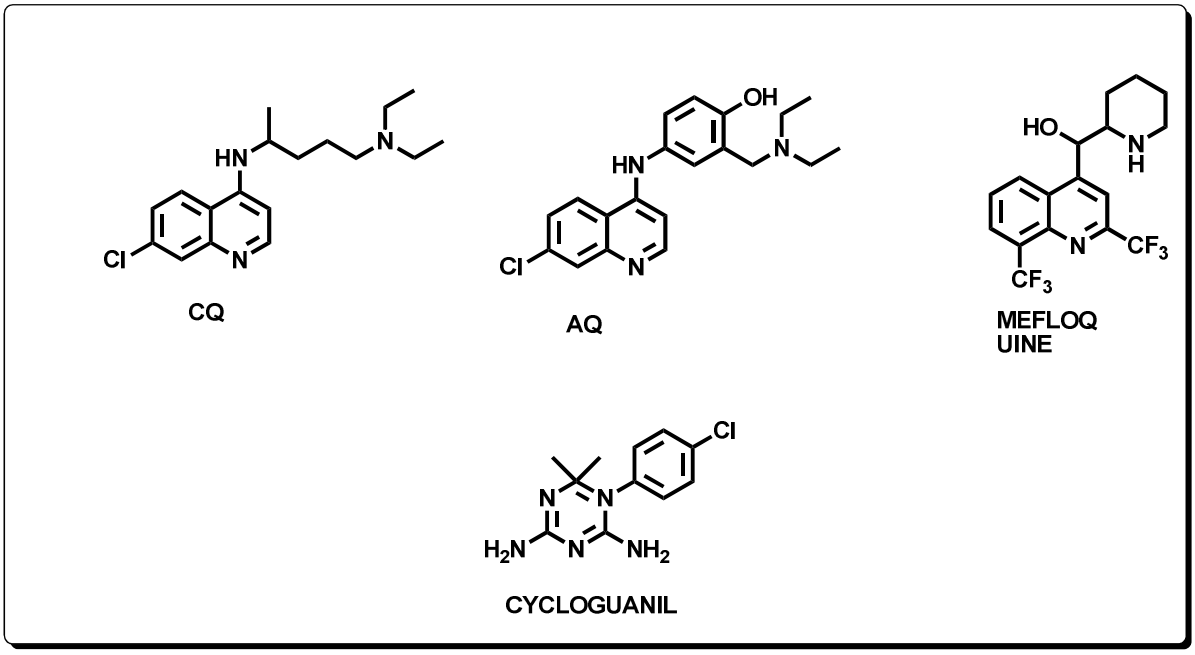
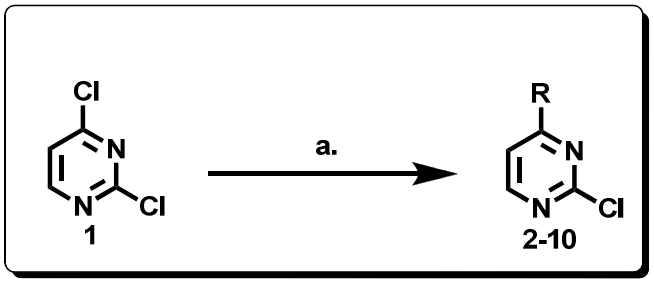


Fig 1 A few common Anti-Malarial drugs

SCHEME -1



Scheme 1

Reagents and Reaction conditions- 2,4 dichloropyrimidine (1 mmol), DIPEA (1.2 mmol), different amines (2 mmol), EtOH, rt, 24 hrs

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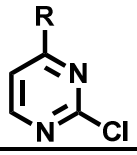
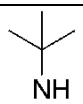
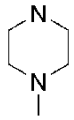
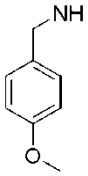
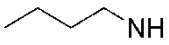
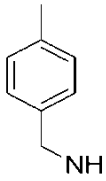
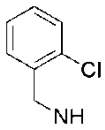
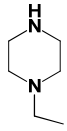
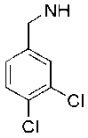
Table 1 .List of compounds of scheme 1



SNo.	*Compd. No.*	*R*
1	**2**	
2	**3**	
3	**4**	
4	**5**	
5	**6**	
6	**7**	
7	**8**	
8	**9**	
9	**10**	

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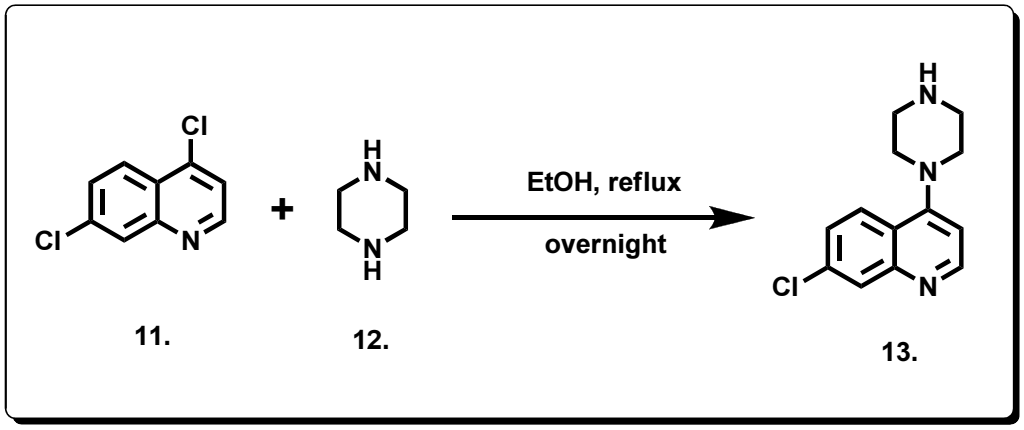
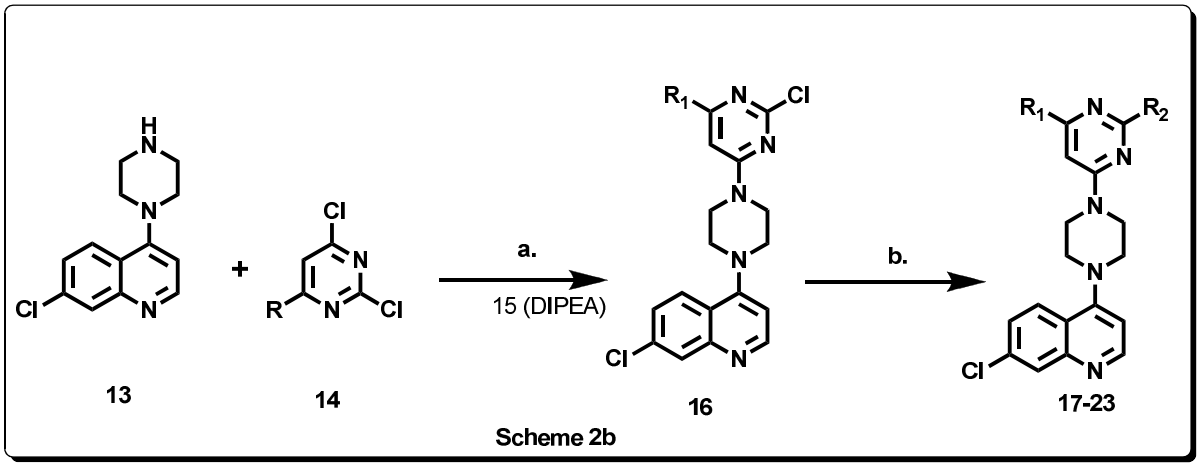


Fig 2. Scheme 2a



Reagents and conditions: (a)EtOH, 15 (DIPEA), rt (b) various amines, Dioxane, K_2CO_3, sealed tube, 120^oC, 10 hrs.

Table: 2List of compounds of scheme 2 a & 2b

Fig 3. Scheme 2b

SNo.	*Compd. No.*	R_1	R_2
10	**16**	H	Cl
11	**17**	CH_3	Cl

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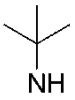
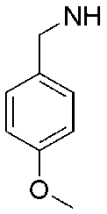
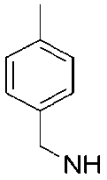
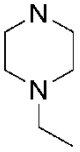
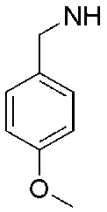
12	**18**	CH_3	
13	**19**	H	
14	**20**	CH_3	
15	**21**	CH_3	
16	**22**	H	
17	**23**	H	

Inhibition of β-hematin formation : -

Most of the synthesized compounds showed moderate β –hematin inhibitory activities (IC_{50} > 3.5 µg/ml).Compounds 20, 21, 22,23 have shown better than the reference drug chloroquine (IC_{50} 3.95 µg/ml) (Table 2).

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Table 2. *β*-hematin inhibitory activity of selected compounds

Compounds	Inhibition of β-hematin formation IC_{50}[a] (µg/mL)
16	**3.14**
17	**4.10**
18	**8.93**
19	**6.53**
20	**2.03**
21	**2.05**
22	**2.46**
23	**2.56**
Chloroquine	**3.95**

[a]The IC_{50} represents the concentration of compound that inhibit β-hematin formation by 50%. and express in µg/ml

Data are the mean of three different experiments in triplicate.

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References

[1] (a) P.M.S. Chauhan, S.K. Srivastava, Curr. Med. Chem. **2001,** 8, 1535-1542,(b) A. Kumar, S.B. Katiyar, A. Agarwal, P.M.S. Chauhan, Drugs Fut. **2003,** 28, 243-255,(c) A. Kumar, S.B. Katiyar, A. Agarwal, P.M.S. Chauhan, Curr. Med. Chem. **2003,** 10 , 1241-1253, (d) Moni Sharma, Kuldeep Chauhan, Shikha S. Chauhan, Ashok Kumar, Shiv Verdan Singh, Jitendra K. Saxena, Pooja Agarwal, Kumkum Srivastava, S. Raja Kumar, Sunil K. Puri, Priyanka Shah, M.I. Siddiqi and Prem M. S. Chauhan, Medicinal Chemistry Communications**, 2012**, 3, 71-79(e)Moni Sharma and Prem M S Chauhan, Future Medicinal Chemistry, **2012**, 4, 1335-1365,(f)Ashok Kumar, Kumkum Srivastava, S. Raja Kumar, M. I. Siddiqi, Sunil K. Puri,Jitendra K. Sexana and Prem M. S. Chauhan, European Journal of Medicinal Chemistry, **2011**, 46, 676-690, (g) Moni Sharma, Shikha Chauhan, P.M.S.Chauhan, Drug News & Perspectives, **2010**, *23,632-46.*

[2] U. Frevert, Trend Parasitol. **2004, 20,** 417-424.

[3] V. Narasimhan, A. Attaran, Malar. J. **2003**, 2, 8.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 5, 347-361

[4] M.H. Gelb, Curr. Opin. Chem. Biol. **2007,** 11 , 440-445.
[5] J. Wiesner, R. Ortmann, H. Jomaa, M. Schlitzer, Angew. Chem. Int. Ed. **2003,** 42, 5274-5293.
[6] G.A. Biagini, P.M. O'Neill, P.G. Bray, S.A. Ward, Curr. Opin. Pharmacol. **2005,** 5, 473-478.
[7] R.G. Ridley, Nature **2002,** 415, 686-693.
[8] J.E. Hyde, Parasitol. Today **1989,** 5, 252-255.
[9] P.M. O'Neill, G.H.A. Posner, J. Med. Chem. **2004**, 47,2945-2964.
[10] S. Yeung, W. Pongtavornpinyo, I.M. Hastings, A.J. Mills, N.J. White, Am. J. Trop. Med. Hyg. **2004**, 71, 179-186.
[11] N.J. White, P.L. Olliaro, Parasitol. Today **1996**, 12,399-401.
[12] P.B. Bloland, M. Ettling, S. Meek, Bull. World Health Org. **2000,** 78, 1378-1388.
[13] T.K. Mutabingwa, Acta Trop. **2005,** 95, 305-315.
[14] R. Jambou, E. Legrand, M. Niang, N. Khim, P. Lim, B. Volney, M.T. Ekala, C. Bouchier, P. Esterre, T. Fandeur, O. Mercereau-Puijalon, Lancet **2005,** 366, 1960-1963.
[15] (a) D. Payne, Parasitol. Today**1988**, 4 ,112-115
(b) L.M.B. Ursos, P.D. Roepe, Med. Res. Rev. **2002**, 22 , 465-491.
[16] M. Krugliak, J. Zhang, H. Ginsburg, Mol. Biochem. Parasitol. **2002,** 119,249-256.
[17] A. Yayon, Z.I. Cabantchik, H. Ginsburg, EMBO J. **1984,** 3,2695-2700.
[18] S. Pagola, P.W. Stephens, D.S. Bohle, A.D. Kosar, S.K. Madsen, Nature **2000,** 404,307-310.
[19] A. Leed, K. DuBay, L.M. Ursos, D. Sears, A.C. de Dios, P.D. Roepe, Biochemistry **2002**, 41, 10245-10255.
[20] A.C. de Dios, R. Tycko, L.M.B. Ursos, P.D. Roepe, J. Phys. Chem. A **2003,** 107,5821-5825.
[21] A. Dorn, S.R. Vippagunta, H. Matile, C. Jaquet, J.L. Vennerstrom, R.G. Ridley, Biochem. Pharmacol. **1998,** 55 , 727-736.
[22] M. Foley, L. Tilley, Int. J. Parasitol. **1997,** 27 231-240. (a)A.B. Sidhu, D. Verdier-Pinard, D.A. Fidock, Science **2002,** 298,210-213.
[23] H. Zhang, M. Paguio, P.D. Roepe, Biochemistry (Mosc) **2004,** 43,8290-8296.
[24] T.N. Bennett, A.D. Kosar, L.M. Ursos, S. Dzekunov, S.A.B. Singh, D.A. Fidock, P.D. Roepe, Mol. Biochem. Parasitol. **2004,** 133, 99-114.
[25] S.R. Cheruku, S. Maiti, A. Dorn, B. Scorneaux, A.K. Bhattacharjee, W.Y. Ellis, J.L. Vennerstrom, J. Med. Chem. **2003,** 46,3166-3169.
[26] T.J. Egan, R. Hunter, C.H. Kaschula, H.M. Marques, A. Misplon, J. Walden, J. Med. Chem. **2000,** 43 , 283-291.
[27] R.G. Ridley, H. Hofheinz, H. Matile, C. Jacquet, A. Dorn, R. Masciadri, S. Jolidon, W.F. Richter, A. Guenzi, M.A. Girometta, H. Urwyler, W. Huber, S. Thiathong, W. Peters, Antimicrob. Agents Chemother. **1996,** 40 , 1846-1854.
[28] P.B. Madrid, A.P. Liou, J.L. DeRisi, K. Guy, J. Med. Chem. **2006**, 49,4535-4543. (a). K. Yearick, K. Ekove-Kovi, D.P. Iwaniuk, J.K. Natarajan, J. Alumasa, A.C. de Dios, P.D. Roepe, C.W. Wolf, J. Med. Chem. **2005**, 51,1995-1998.
[29] K.A. Neftel, W. Woodtly, M. Schmid, Br. Med. J. **1986**, 292,721-723.
[30] D.E. Lind, J.A. Levi, P.C. Vincent, Br. Med. J. **1973**, 1,458-460.
[31] P.M. O'Neill, A. Mukhtar, P.A. Stocks, L.E. Randle, S. Hindley, S.A. Ward, R.C. Storr, J.F. Bickley, I.A. O'Neill, J.L. Maggs, R.H. Hughes, P.A. Winstanely, P.G. Bray, B.K. Park, J. Med. Chem. **2003,** 46 , 4933-4945.
[32] P.M. O'Neill, B.K. Park, A.E. Shone, J.L. Maggs, P. Roberts, P.A. Stocks, G.A. Biagini, P.G. Bray, P. Gibbons, N. Berry, P.A. Winstanley, A. Mukhtar, R. Bonar-law, S. Hindley, R.B. Bambal, C.B. Davis, M. Bates, T.K. Hart, S.L. Gresham, R.M. Lawrence, R.A. Brigandi, F.M. Gomez-delas-Heras, D.V. Gargallo, S.A. Ward, J. Med. Chem. **2009,** 52, 1408-1415.
[33] P.M. O'Neill, A.E. Shone, D. Tanford, G. Nixon, E. Adollahy, B.K. Park, J.L. Maggs, P. Roberts, P.A. Stocks, G. Biagini, P.G. Bray, J. Davies, N. Berry, C. Hall, K. Rimmer, P.A. Winstanley, S. Hindley, R.B. Bambal, C.B. Davis, M. Bates, S.L. Gresham, R.A. Brigandi, F.M. Gomez-delas-Heras, D.V. Gargallo, S. Parapini, L. Vivas, H. Lander, D. Taramelli, S.A. Ward, J. Med. Chem. **2009,** 52,1828-1844.
[34] J.J. Walsh, A. Bell, Curr. Pharm. Design **2009,** 15,2970-2985.
[35] F. Bellot, F. Cosledan, L. Vendier, J. Brocard, B. Meunier, A. Robert, J. Med. Chem. **2010**, 53, 4103-4109.
[36] (a) O. Dechy-Cabaret, F. Benoit-Vical, A. Robert, B. Meunier, ChemBioChem **2000,** 1,281-283.
(b) A. Robert, O. Dechy-Cabaret, J. Cazelles, B. Meunier, Acc. Chem. Res. **2000,** 35,167-174.
(c) O. Dechy-Cabaret, F. Benoit-Vical, C. Loup, A. Robert, H. Gornitzka, Bonhoure, H. Vial, J.F. Magnaval, J.P. Seguela, B. Meunier, Chemistry **2004,** 10, 1625-1636.
(d) F. Benoit-Vical, J. Lelievre, A. Berry, C. Deymier, O. Dechy-Cabaret, J. Cazelles, C. Loup, A. Robert, J.F. Magnaval, B. Meunier, Antimicrob. Agents Chemother. **2007,** 51, 1463-1472.
(e) C. Singh, H. Malik, S.K. Puri, Bioorg. Med. Chem.,**2004**, 12, 1177-1182.
[37] J.J. Walsh, D. Coughlan, N. Heneghan, C. Gaynor, A. Bell, **,** Bioorg. Med. Chem. Lett. **2007,** 17 , 3599-3602.
[38] I. Opsenica, D. Opsenica, C.A. Lanteri, L. Anova, W.K. Milhous, K.S. Smith, B.A. Salaja, J. Med. Chem. **2008,** 51, 6216-6219.
[39] S. Gemma, G. Campiani, S. Butini, B.P. Joshi, G. Kukreja, S.S. Coccone, M. Burrutti, M. Persico, V. Nacci, I. Fiorini, E. Novellino, D. Taramerlli, N. Banilico, S. Parapini, V. Yardley, S. Croft, S.K. Maerk, M. Rottman, R. Brun, M. Coletta, S.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 5, 347-361

Marini, G. Guiso, S. Caccia, C. Fattorusso, J. Med. Chem. **2009,** 52, 502-513.

[40] (a) D. Dive, C. Biot, Chem. Med. Chem. **2008,** 3, 383--391;
(b) M.A. Blackie, P. Beagley, S. L Croft, H. Kendrick, J.R. Moss, K. Chibale, Bioorg. Med. Chem. **2007,** 15, 6510-6516.

[41] (a) E. Davioud-Charvet, S. Delarue, C. Biot, B. Schwobel, C.C. Boehme, Mussigbrodt, L. Maes, C. Sergheraert, P. Grellier, R.H. Schirmer, K.A. Becker, J. Med. Chem. **2001,** 44, 4268;
(b) W. Friebolin, B. Jannack, N.Wenzel, J. Furrer, T. Oeser, C.P. Sanchez, M. Lanzer, V. Yardley, K. Becker, E. Davioud-charvet, J. Med. Chem. **2008,** 51,1260-1277.

[42] I. Chiyanzu, C. Clarkson, P.J. Smith, J. Gut, P.J. Rosenthal, K. Chibale, Bioorg. Med. Chem. **2005,** 13, 3249-3261.

[43] Pandey, A. V.; Singh, N.; Tekwani, B. L.; Puri, S. K.; Chauhan, V. S. *J. Pharm. Biomed. Anal*. **1999**, *20*, 203-7, (a) M. Sharma, K. Chauhan, S.S Chauhan, A. Kumar, S.V. Singh, J.K. Saxena, Pooja Agarwal, Kumkum Srivastava, S. Raja Kumar[c], Sunil K. Puri, Priyanka Shah, M.I. Siddiqi and Prem M. S. Chauhan, Medicinal Chemistry Communications**, 2012**, 3, 71-79.