Synthesis and evaluation of chirally defined side chain variants of 7-chloro-4-aminoquinoline to overcome drug resistance in malaria chemotherapy.

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Running Title: Side chain modified 4-aminoquinoline antimalarials

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ABSTRACT

A novel 4-aminoquinoline derivative ((*S*)-7-chloro-*N*-(4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-yl)-quinolin-4-amine triphosphate) exhibiting curative activity against chloroquine resistant malaria parasite has been identified for preclinical development as a blood schizonticidal agent. The lead molecule selected after detailed SAR studies, has good solid state properties, and has promising activity against *in vitro* and *in vivo* experimental malaria models. The *in vitro* ADME parameters have indicated favourable drug like profile.

INTRODUCTION

Malaria is a major infectious disease affecting mainly tropical and subtropical areas. Of the five species of *Plasmodia* which are responsible for human malaria, *P. falciparum* causes the most severe form of the disease (1). There were an estimated 207 million malaria cases in 2012 and an estimated 627 000 deaths of which 90% of malaria deaths occur in sub-Saharan Africa, and 77% occur in children under five years of the age (2). For several decades chloroquine (CQ), a 4-aminoquinoline, has been the frontline drug in malaria chemotherapy because of its therapeutic efficacy, ease of use and low cost (3). However due to emergence of resistant *P. falciparum* and *P. vivax* strains against commonly used drugs such as CQ, amodiaquine and artemisinin (Figure 1) there is urgent need to develop new chemical entities with a goal to overcome parasite resistance.

Aminoquinolines such as chloroquine had been the most important antimalarials for more than four decades in view of their efficacy against all species of human malaria and their safety profile. Emergence of resistance to this class of compounds during 1980's created a genuine health crisis in the developing world. Studies on elucidation of mechanism of resistance and general trend emerging from the SAR-studies revealed that chloroquine resistance does not involve any change to the target of this class of drugs but involves compound specific efflux mechanism (4). Based on this premise a number of research groups have developed short chain analogues of 4-aminoquinoline, which are active against CQ-resistant strains of *P.falciparum.* However, these derivatives undergo biotransformation (dealkylation) significantly affecting lipid solubility of the drug and decreasing the biological efficacy (5). In view of this background information it was surmised that a focussed library of

small molecules based on 4-aminoquinoline scaffold with suitable functionalities would result in molecules with improved antiplasmodial activity against CQ resistant parasite. Therefore, modifications employing side chain refinement and conformational rigidity were considered. The seminal finding of our study is that a new chemical entity having significant activity against CQ resistant parasites has been identified and the results are discussed in the present manuscript.

Stocks *et al.* synthesized and evaluated a series of short chain CQ derivatives, by replacement of diethyl amino function with more metabolically stable side chain (*tert*-butyl) as well as heterocyclic ring (piperidyl, pyrrolidino, morpholino) modifications, that led to a substantial increase in the antimalarial activity against CQ-resistant parasite strains (6). Madrid *et al.* have replaced diethylamino functionality with one propyl group as constant and replacing the other ethyl group with bulky or aromatic ring and the results indicated that some of these analogs are active against multi drug resistant strains (7).Some annals suggest that 4-aminoquinoline analogs of altered side chain such as *N*'-(7-Chloro-quinolin-4-yl)-*N*,*N*-diethyl-propane-1,3- diamine show potential leads for the development of new drugs (8). Ryckebusch *et al.* evaluated new series (1,4-bis(3-aminopropyl) piperazine derivatives) against the chloroquine resistant strains of *P. falciparum*. Compounds displayed moderate to good activity when quinoline and/or aryl moieties were attached to the above mentioned linker. In this series, compounds containing piperazine moiety were found to be active against CQ resistant strains of *P. falciparum* (9-11).

Based on these annotations, earlier from this laboratory Solomon *et al.* explored different modifications at the pendant nitrogen of the CQ lateral side chain that led to compounds with improved activity, particularly against CQ-resistant strains (12). By taking into account above facts, more recently from our laboratory Sinha *et al.*developed a generic methodology for the synthesis of chiral chloroquine and its analogues. The key feature of this methodology is that it enables the use of amino acids to generate 4-aminoquinolines with chirally defined substituted side-chain and also to address the role of hydrophobic substitution at the chiral center. It was inferred from the antiplasmodial activity data that analogues containing *N*-methylpiperazine at the terminal part of side chain showed excellent *in vitro* activity with reference to CQ against the resistant strain. These authors also examined the chain length variation by homologation of selected α-amino acids to get the corresponding β^3-and γ-amino acids (13). Most of the derivatives displayed excellent *in vitro* antiplasmodial activity, and a few compounds in the in vivo studies showed 100% parasitaemia suppression on day 4 (14).

The objective of the present study is to synthesize a new series of 4-aminoquinoline derivatives with reduced side chain amide bond with a view to increase *in vivo* stability, and also, to synthesize compounds with different substitutions at the chiral centre. These compounds have been evaluated for antiplasmodial activity against chloroquine sensitive (CQ-S) and chloroquine resistant (CQ-R) strains and the results are reported in the following paragraphs.

MATERIALS AND METHODS

Chemistry

Melting points (mp) were taken in open capillaries on Complab melting point apparatus and are uncorrected. The ^{1}H NMR (200, 300MHz) and ^{13}C NMR (50, 75 MHz) spectra were recorded in $CDCl_3$, on DPX-200 and 300 Bruker FT-NMR spectrometers. All chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), br s (broad singlet) and m (multiplet). Coupling constants are given in hertz. Mass Spectra (ESI-MS), high resolution mass spectra HRMS **(**ESI-HRMS) were recorded on Jeol (Japan)/SX-102, and Agilent 6520 Q-TOF (ESI-HRMS) spectrometers respectively. Analytical thin-layer chromatography (TLC) was carried out on Merck's pre-coated silica-gel plates 60 F_{254} and spots were visualized by irradiation with UV light (254 nm). Iodine was used as developing agent and/or by spraying with Dragendorff's reagent. Column chromatographic purification was performed over neutral alumina and silica gel (silica gel 60-120, 100-200 and 230-400 mesh) using a gradient solvent system (n-hexane/EtOAc, DCM/Hexane or chloroform/methanol as the eluent unless otherwise specified). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) and Spectrochem Pvt. Ltd (India) and were used without further purification**.** Analytes were eluted using isocratic mobile phase of methanol and 0.05% TFA in water (60:40) and detected at 254 nm. The purities of compounds submitted for biological evaluation were >98% as determined by HPLC. Yields are not optimized.

General Procedure for the Synthesis of 2a-j

Amino acids namely glycine, phenylalanine, tryptophan and methionine (1.0 equiv.) were dissolved in dioxane-water mixture (1:1) and the reaction mixture was stirred at room temperature. Addition of 2N NaOH to this reaction mixture dissolves the reactant thereby

affording a miscible solution. The reaction mixture was then cooled to 0°C and stirred for 15 min. Finally, $(Boc)_2O$ (1.1 equiv.) was added and the reaction mixture was allowed to stir at 0 °C for 10 min. The ice bath was then removed and the temperature of the reaction was allowed to rise to the room temperature. On completion of the reaction, reaction mixture was concentrated under reduced pressure. The aqueous layer was acidified with citric acid (pH 2-3) and extracted with EtOAc and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The yields were quantitative.

Amino acids namely alanine, valine, leucine, and isoleucine (1.0 equiv) in a 2N aqueous NaOH solution (17 mL) was cooled in an ice bath to 0 °C. Under vigorous stirring benzyl chloroformate (1.1 equiv) and a 2N aqueous NaOH solution were simultaneously added within 2 min. The mixture was stirred for 20 min at room temperature and extracted with diethyl ether. The aqueous layer was separated and acidified with conc. hydrochloric acid to a pH of 2-3. The resulting emulsion was extracted with EtOAc. The organic phases were combined, washed with brine, and dried with Na_2SO_4. The yields were quantitative.

2-(*Tert*-butoxycarbonylamino) acetic acid (2a)

The compound was obtained as a white solid in quantitative yield. m. p. 86-88°C; ^{1}H NMR ($CDCl_3$, 300 MHz) : δ 1.46 (s, 9H, C (CH_3)$_3$, 3.96 (s, 2H, CH_2 COOH); ESI-MS: (*m/z*) 176 $(M+H)^+$.

(*S*)-2-(Benzyloxycarbonylamino) propanoic acid (2b)

The compound was obtained as a white solid in quantitative yield. m. p. 46-48°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (d, J = 6.7 Hz, 3H, CH CH_3), 4.44 (br s, 1H, $CHCH_3$), 5.15(s, 2H, $CH_2C_6H_5$), 7.37 (m, 5H, C_6H_5); ESI-MS: (*m/z*) 224 $(M+H)^+$.

(*S*)-2-(Benzyloxycarbonylamino)-3-methylbutanoic acid (2c)

The compound was obtained as a gummy substance in quantitative yield. ^{1} H NMR (300 MHz, $CDCl_3$): δ 0.92-1.01 (m, 6H, CH(CH_3)$_2$, 2.31-2.22 (m, 1H, $CH(CH_3)_2$, 4.43 (brs, 1H, $CHCH(CH_3)_2$), 5.12 (s, 2H, $CH_2C_6H_5$), 7.35 (s, 5H, C_6H_5); ESI-MS: (*m/z*) 252 $(M+H)^+$.

(*S*)-2-(Benzyloxycarbonylamino)-4-methylpentanoic acid (2d)

The compound was obtained as a gummy substance in quantitative yield.^{1}H NMR (300 MHz, $CDCl_3$) : δ 0.98 (d, J = 6.5 Hz, 6H, CH (CH_3)$_2$, 1.55-1.61 (m, 1H, CH(CH_3)$_2$, 1.70-1.75 (m,

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2H, $CH_2CH(CH_3)_2$, 4.43 (brs, 1H, NC*H*), 5.14 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 266 $(M+H)^+$.

(*R*)-2-(Benzyloxycarbonylamino)-4-methylpentanoic acid (2e)

The compound was obtained as a gummy substance in quantitative yield.^{1}H NMR (300 MHz, $CDCl_3$) : δ 0.98 (d, J = 6.5 Hz, 6H, CH $(CH_3)_2$, 1.55-1.61 (m, 1H, $CH(CH_3)_2$, 1.70-1.75 (m, 2H, $CH_2CH(CH_3)_2$, 4.43 (brs, 1H, NC*H*), 5.14 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 266 $(M+H)^+$.

2-(Benzyloxycarbonylamino)-4-methylpentanoic acid (2f)

The compound was obtained as a gummy substance in quantitative yield.^{1}H NMR (300 MHz, $CDCl_3$) : δ 0.98 (d, J = 6.5 Hz, 6H, CH $(CH_3)_2$, 1.55-1.61 (m, 1H, $CH(CH_3)_2$, 1.70-1.75 (m, 2H, $CH_2CH(CH_3)_2$, 4.43 (brs, 1H, NC*H*), 5.14 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 266 $(M+H^+)$.

(*2S*,*3S*)-2-(Benzyloxycarbonylamino)-3-methylpentanoic acid (2g)

The compound was obtained as a gummy substance in quantitative yield.^{1}H-NMR (300 MHz, $CDCl_3$) : δ 0.93-1.08 (m, 6H, $CHCH_3CH_2CH_3$), 1.15-1.45 (m, 2H, CH_2CH_3), 1.45-1.54 (m, 1H, $CHCH_3$), 4.42 (brs,NHC*H*), 5.28(d, J = 8.3Hz, N*H*CH), 5.14 (s, 2H, $CH_2C_6H_5$),7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 266 $(M+H)^+$.

(*S*)-2-(*Tert*-butoxycarbonylamino)-4-(methylthio)butanoic acid (2h)

The compound was obtained as a gummy substance in quantitative yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.46 (s, 9H, C $(CH_3)_3$, δ 1.97-2.05 (m, 2H, $CHCH_2$), 2.13 (s, 3H, SCH_3), 2.51-2.60 (m, 2H, CH_2 SCH_3); ESI-MS: (*m/z*) 250.3 $(M+H)^+$.

(*S*)-2-(*Tert*-butoxycarbonylamino)-3-phenylpropanoic acid (2i)

The compound was obtained as a white solid in quantitative yield. m.p 85-87°C;^{1}H NMR ($CDCl_3$, 300 MHz):δ 1.41(s, 9H, $C(CH_3)_3$, 2.73-3.04 (m, 2H, $CH_2C_6H_5$), 4.07 (br s, 1H, NC*H*), 7.17-7.27 (m,5H, C_6H_5); ESI-MS: (*m/z*) 266.5 $(M+H)^+$.

(*S*)-2-(*Tert*-butoxycarbonylamino)-3-(1H-indol-3-yl)propanoic acid (2j)

The compound was obtained as a white solid in quantitative yield. m.p 134-136 °C; ^{1}H NMR ($CDCl_3$, 300 MHz):δ 1.42 (s, 9H, $C(CH_3)_3$, 3.21-3.31 (m, 2H, CH_2-Ind), 4.64 (br s, 1H,

NC*H*), 5.06 (br s, 1H, N*H*Boc), 7.02 (s, 1H, Ind-2*H*), 7.08 -7.21 (m, 2H, Ind-5, 6-*H*), 7.33 (d, *J* = 7.5 Hz, 2H, Ind-4*H*), 7.59 (d, *J* =7.8 Hz, 2H, Ind-7*H*), 8.11(br s, 1H, Ind-N*H*).

2.8.3. General Procedure for the Synthesis of 3a-j

A solution of **2a-j** (1.0 equiv.) in dry THF, was cooled to -15°C, and after 10 min, *N*-methyl morpholine (NMM) (1.2 equiv.) and isobutylchloroformate (IBCF) (1.2 equiv.) were added with stirring. After 15 min, a solution of $NaBH_4$ (2 equiv.) in water (10 mL) was added to this reaction mixture. The reaction mixture was stirred for 1h. After completion of the reaction (as monitored by TLC), water (100 mL) added to quench the reaction. Solvent was evaporated under reduced pressure. The oily residue was taken in EtOAc, organic layer was washed with 5% $NaHCO_3$, and finally with brine. The organic layer was dried over anhydrous Na_2SO_4, evaporated under reduced pressure. The products obtained were purified by the silica gel column chromatography, using a mixture of EtOAc and hexane as eluent to afford **3a-j**.

***Tert*-butyl 2-hydroxyethylcarbamate (3a)**

The compound was obtained as a gummy substance in quantitative yield.^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H, C (CH_3)$_3$), 3.30 (d, *J* = 4.8 Hz, 2H, CH_2CH_2OH), 3.72 (d, *J* = 3.5 Hz, 2H, CH_2CH_2OH), 4.96 (s, 1H, CH_2CH_2OH).

(*S*)-Benzyl 1-hydroxypropan-2-ylcarbamate (3b)

The compound was obtained as a white solid in quantitative yield. m.p 134-136°C;^{1}HNMR (300MHz, $CDCl_3$): δ 1.17 (d, *J*= 6.7 Hz, 3H, CH(CH_3), 3.53-3.68 (m, 2H, $CHCH_2OH$), 3.80-3.85 (m, 1H, $CHCH_2OH$), 4.88 (brs, 1H, $CHCH_2OH$), 5.10 (s, 2H, $CH_2C_6H_5$),7.35 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 209.9 $(M+H)^+$.

(*S*)-Benzyl 1-hydroxy-3-methylbutan-2-ylcarbamate (3c)

The compound was obtained as a white solid in quantitative yield. m.p 114-116°C; IR (KBr) 3360, 2975, 2881, 1688, 1523, 1456, 1226, 1057, 738 cm^{-1};^{1}HNMR (300 MHz, $CDCl_3$): δ 0.94-0.99 (2d, *J* = 6.7 Hz, 6H, CH(CH_3)$_2$), 1.84-1.89 (m, 1H, $CH(CH_3)_2$, 3.52-3.66 (m, 2H, $CHCH_2OH$), 3.68-3.75 (m, 1H, $CHCH_2OH$), 4.91 (brs, 1H,$CHCH_2OH$), 5.13 (s, 2H, $CH_2C_6H_5$), 7.37 (m, 5H, C_6H_5); ESI-MS: (*m/z*) 260.2 $(M+Na)^+$.

(*S*)-Benzyl 1-hydroxy-4-methylpentan-2-ylcarbamate (3d)

The compound was obtained as a gummy substance in quantitative yield; IR (neat) 3523, 2925, 1713, 1517, 1466, 1251, 1106 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.98 (d, J = 6.5 Hz, 6H, CH (CH_3)$_2$, 1.61-1.55 (m, 2H, CH_2 CH (CH_3)$_2$, 1.75-1.70 (m, 1H, CH (CH_3)$_2$, 4.43 (brs, 1H, NC*H*), 5.14 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 251.9 $(M+Na)^+$.

(*R*)-Benzyl 1-hydroxy-4-methylpentan-2-ylcarbamate (3e)

The compound was obtained as a gummy substance in quantitative yield; IR (neat) 3523, 2925, 1713, 1517, 1466, 1251, 1106 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.98 (d, J = 6.5 Hz, 6H, CH (CH_3)$_2$, 1.61-1.55 (m, 2H, CH_2 CH (CH_3)$_2$, 1.75-1.70 (m, 1H, CH (CH_3)$_2$, 4.43 (brs, 1H, NC*H*), 5.14 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, $CH_2C_6H_5$). ESI-MS: (*m/z*) 260.2 $(M+Na)^+$.

Benzyl 1-hydroxy-4-methylpentan-2-ylcarbamate (3f)

The compound was obtained as a gummy substance in quantitative yield; IR (neat) 3523, 2925, 1713, 1517, 1466, 1251, 1106 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.98 (d, J = 6.5 Hz, 6H, CH (CH_3)$_2$, 1.61-1.55 (m, 2H, CH_2 CH (CH_3)$_2$, 1.75-1.70 (m, 1H, CH (CH_3)$_2$, 4.43 (brs, 1H, NC*H*), 5.14 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, $CH_2C_6H_5$). ESI-MS: (*m/z*) 260.2 $(M+Na)^+$.

Benzyl (*2S,3S*)-1-hydroxy-3-methylpentan-2-ylcarbamate (3g)

The compound was obtained as a gummy substance in quantitative yield;^1H NMR ($CDCl_3$, 300 MHz): δ 0.88-0.97 (m, 6H, $CHCH_3CH_2CH_3$), 1.12-1.21 (m, 2H, CH_2CH_3), 1.44-1.50 (m, 1H, $CHCH_3$), 3.60-3.77 (m, 3H,$CHCH_2OH$), 4.94 (brs, CH_2OH), 5.66 (brs, N*H*CH), 5.14 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 252.0 $(M+H)^+$.

(*S*)-*Tert*-butyl 1-hydroxy-4-(methylthio)butan-2-ylcarbamate (3h)

The compound was obtained as a gummy substance in quantitative yield;^1H NMR ($CDCl_3$, 300 MHz) :δ 1.46 (s, 9H, C (CH_3)$_3$, δ 1.78-1.85 (m, 2H, $CHCH_2CH_2SCH_3$), 2.13 (s, 3H, SCH_3), 2.53-2.61 (m, 2H,$CH_2CH_2SCH_3$), 3.61-3.78 (m, 3H, $NCHCH_2OH$); ESI-MS: (*m/z*) 236.4 $(M+H)^+$.

(*S*)-*Tert*-butyl 1-hydroxy-3-phenylpropan-2-ylcarbamate (3i)

The compound was obtained as a white solid in quantitative yield; m. p 95-97 °C; 3356, 2980, 2925, 1684, 1526, 1456, 1316, 1269, 1168, 1007, 885, 775, ^1H NMR (300 MHz, $CDCl_3$) : δ 1.42 (s, 9H, C (CH_3)$_3$), 2.84 (d, J = 7.0 Hz, 2H ,CH_2 C_6H_5), 3.49-3.70 (m, 2H, CH_2OH), 3.87 (brs, 1H, NC*H*), 4.69 (t, J = 7.2 Hz, 1H, CH_2OH), 7.20-7.30 (m, 5H, C_6H_5).

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(*S*)-*Tert*-butyl 1-hydroxy-3-(1H-indol-3-yl)propan-2-ylcarbamate (3j)

The compound was obtained as a white solid in quantitative yield; m. p 118-120 °C; ^{1}H NMR (300 MHz ,$CDCl_3$): δ 1.42 (s, 9H, C (CH_3)$_3$, 2.99 (d, J = 6.72Hz,CH_2-Indole), 3.57-3.72 (m, 2H, CH_2OH), 3.98 (brs, 1H, NC*H*), 4.79 (brs, 1H, CH_2O*H*), 7.05 (s, 1H, Ind-2*H*), 7.10-7.22 (m, 2H, Ind-5, 6-*H*), 7.36 (d, J = 7.9 Hz, 2H, Ind-4*H*), 7.65 (d, J = 7.6 Hz, 2H, Ind-7*H*), 8.09 (br s, 1H, Ind-N*H*).

2.8.4. General Procedure for the Synthesis of 4a-j

To a suspension of **3a-j** (1.0 equiv.) in anhydrous THF under nitrogen atmosphere was added triethylamine (3.0 equiv.). The mixture was cooled to below 0°C. Methanesulfonyl chloride (3.0 equiv.) was added slowly, keeping the temperature below 5°C, and the reaction mixture was stirred in an ice bath for 45 min. After completion of the reaction as monitored by the TLC the reaction mixture was diluted with saturated $NaHCO_3$ solution, the reaction mixture was extracted with DCM. The combined organic extracts dried over Na_2SO_4 and concentrated under reduced pressure to afford **4a-j**. These intermediates were used without further purification.

2-(*Tert*-butoxycarbonylamino)ethyl methanesulfonate (4a)

The compound was obtained as a yellow gummy substance in quantitative yield;^{1}H NMR ($CDCI_3$, 300 MHz) : δ 1.46 (s, 9H, C (CH_3)$_3$, 3.05 (s, 3H, SO_2CH_3), 3.49 (d, J = 5.4 Hz, 2H, $CH_2CH_2OSO_3$), 4.30 (t, J = 9.9 Hz, 2H, $CH_2CH_2OSO_3$); ESI-MS: (*m/z*) 240 $(M+H)^+$.

(*S*)-2-(Benzyloxycarbonylamino) propyl methanesulfonate (4b)

The compound was obtained as a white solid in quantitative yield; m.p 84-86°C; ^{1}HNMR (300MHz, $CDCl_3$) : δ 1.28 (d, J = 6.6 Hz, 3H, CH (CH_3), 2.99 (s, 3H, SO_2CH_3), 4.01-4.26 (m, 3H, $CHCH_2OSO_2CH_3$), 4.89 (brs, 1H, N*H*CO), 5.12 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 309 $(M+Na)^+$.

(S)-2-(benzyloxycarbonylamino)-3-methylbutyl methanesulfonate (4c)

The compound was obtained as a white solid in quantitative yield; m.p 62-64°C;^{1}H NMR (300MHz, $CDCl_3$) :δ 0.89-0.97 (2d, J = 6.6, Hz, 6H, $CH(CH_3)_2$, 1.68-1.77 (m, 1H, $CH(CH_3)_2$, 2.96 (s, 3H, SO_2CH_3), 3.57-3.68 (m, 1H, NC*H*), 4.28 (d, J = 4.1Hz,2H, $SOCH_2$),

4.92 (br s,1H, N*H*), 5.12 (s, 2H, C$H_2C_6H_5$), 7.35 (s, 5H, C_6H_5); ESI-MS: (*m/z*) 338.9 $(M+Na)^+$.

(*S*)-2-(Benzyloxycarbonylamino)-4-methylpentyl methanesulfonate (4d)

The compound was obtained as a yellow solid in quantitative yield; m.p 55-57°C;^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.95 (d, J = 3.9 Hz, 6H, CH (CH_3)$_2$, 1.51-1.59 (m, 2H, CH_2 CH (CH_3)$_2$, 1.60-1.70 (m, 1H, CH(CH_3)$_2$, 2.96 (s, 3H, SO_2CH_3), 3.91-3.99 (m, 1H, NC*H*CH_2OSO$_3$), 4.01-4.47 (m, 2H, CHCH_2OSO$_3$), 5.11 (s, 2H, C$H_2C_6H_5$), 5.44 (brs, 1H, N*H*CO), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*)352.1 $(M+Na)^+$.

(*R*)-2-(Benzyloxycarbonylamino)-4-methylpentyl methanesulfonate (4e)

The compound was obtained as a yellow solid in quantitative yield; m.p 55-57°C;^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.95 (d, J = 3.9 Hz, 6H, CH (CH_3)$_2$, 1.51-1.59 (m, 2H, CH_2 CH (CH_3)$_2$), 1.60-1.70 (m, 1H, CH(CH_3)$_2$), 2.96 (s, 3H, SO_2CH_3), 3.91-3.99 (m, 1H, NC*H*CH_2OSO$_3$), 4.01-4.47 (m, 2H, CHCH_2OSO$_3$), 5.11 (s, 2H, C$H_2C_6H_5$), 5.44 (brs, 1H, N*H*CO), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*)352.1 $(M+Na)^+$.

2-(Benzyloxycarbonylamino)-4-methylpentyl methanesulfonate (4f)

The compound was obtained as a yellow solid in quantitative yield; m.p 55-57°C;^{1}H NMR ($CDCl_3$, 300 MHz) : δ 0.95 (d, J = 3.9 Hz, 6H, CH (CH_3)$_2$), 1.51-1.59 (m, 2H, CH_2 CH (CH_3)$_2$), 1.60-1.70 (m, 1H, CH(CH_3)$_2$), 2.96 (s, 3H, SO_2CH_3), 3.91-3.99 (m, 1H, NC*H*CH_2OSO$_3$), 4.01-4.47 (m, 2H, CHCH_2OSO$_3$), 5.11 (s, 2H, C$H_2C_6H_5$), 5.44 (brs, 1H, N*H*CO), 7.37 (s, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*)352.1 $(M+Na)^+$.

(*2S*, *3S*)-2-(Benzyloxycarbonylamino)-3-methylpentyl methanesulfonate (4g)

The compound was obtained as a yellow solid in quantitative yield; m.p 55-57°C; ^{1}H NMR ($CDCl_3$, 300 MHz) : δ 0.87-0.95 (m, 6H, CHCH_3CH$_2$CH_3), 1.07-1.19 (m, 2H, CH_2CH$_3$), 1.31-1.50 (m, 1H, C*H*CH$_3$), 2.90 (s, 3H, SO_2CH_3), 3.65-3.73 (m, 1H, C*H*CH$_2$OSO$_3$), 4.08-4.46 (m, 2H, CHCH_2OSO$_3$),5.24 (s, 2H, C$H_2C_6H_5$), 5.39 (br s, 1H, N*H*CO),7.38 (s, 5H, $CH_2C_6H_5$).

(*S*)-2-(*Tert*-butoxycarbonylamino)-4-(methylthio)butyl methanesulfonate (4h)

The compound was obtained as a gummy substance in quantitative yield;^{1}H NMR ($CDCl_3$, 300 MHz) :δ 1.43 (s, 9H, C (CH_3)$_3$), 1.81-2.01 (m, 2H, CHCH_2CH$_2$SCH$_3$), 2.17 (s, 3H,

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SCH_3), 2.53-2.61 (m, 2H,CH_2CH_2SCH_3), 3.67 (s, 3H, SO$_2$CH_3), 3.82-3.95 (m, 1H, NC*H*), 4.27 (d, *J* = 4.0Hz,2H, SOCH_2); ESI-MS: (*m/z*) 313.5 $(M+H)^+$.

(*S*)-2-(*Tert*-butoxycarbonylamino)-3-phenylpropyl methanesulfonate (4i)

The compound was obtained as a yellow gummy substance in quantitative yield;^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.43 (s, 9H, C (CH_3)$_3$), 2.84-2.97 (m, 2H, CH_2C$_6$H$_5$), 3.03 (s, 3H, SO$_2$CH_3), 3.85 (brs, 1H, NC*H*), 4.12-4.27 (m, 2H, SOCH_2), 4.71 (brs, N*H*), 7.22-7.33 (m, 5H, C$_6$*H*$_5$).

(*S*)-2-(*Tert*-butoxycarbonylamino)-3-(1H-indol-3-yl)propyl methanesulfonate (4j)

The compound was obtained as a yellow gummy substance in quantitative yield;^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, C(CH_3)$_3$), 2.98 (s, 3H, SO$_2$CH_3), 2.72-2.86 (m, CH_2-Indole), 3.01-3.13 (m, 2H, SOCH_2), 4.28 (br s, 1H, NC*H*), 5.15(br s,1H,N*H*Boc), 7.05 (s, 1H, Ind-2*H*), 7.10-7.22 (m, 2H, Ind-5, 6-*H*), 7.36 (d, *J* = 7.9 Hz, 2H, Ind-4*H*), 7.65 (d, *J* = 7.6 Hz, 2H, Ind-7*H*), 8.17 (br s, 1H, Ind-N*H*); ESI-MS: (*m/z*) 369.0 $(M+H)^+$.

2.8.5. General Procedure for the Synthesis of 5a-j

To a suspension of **4a-j** (1.0 equiv.) in acetonitrile under nitrogen atmosphere was added triethylamine (2.0 equiv.) and *N*-methyl piperazine (4.0 equiv.). The reaction mixture was stirred for 40hrs at room temperature. After completion of the reaction (as monitored by TLC), the solvent was evaporated under reduced pressure and the residue was dissolved in DCM, the organic layer was washed with 10% citric acid, finally the aqueous layer was basified with $NaHCO_3$ and extracted with DCM, and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The products obtained were used for the next step without further purification.

***Tert*-butyl 2-(4-methylpiperazin-1-yl) ethylcarbamate (5a)**

The compound was obtained as a gummy substance in 79% yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.46 (s, 9H, C(CH_3)$_3$), 2.29 (s, 3H, NCH_3), 2.45-2.48 (m, 10H, CH_2cycN(CH_2CH_2)$_2$NCH_3), 3.23 (d, *J* = 4.8 Hz, 2H, CH_2CH_2cycN(CH_2CH_2)$_2$NCH_3).

(*S*)-Benzyl 1-(4-methylpiperazin-1-yl) propan-2-ylcarbamate (5b)

The compound was obtained as a gummy substance in 89 % yield; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.19-1.21 (d, *J* = 6.3 Hz,3H, CH(CH_3)), 2.30 (s, 3H, NCH_3), 2.35-2.58 (m, 10H,

CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.75-3.78 (m, 1H, CH_3CHCH$_2$), 5.11 (s, 2H, CH_2C$_6$H$_5$),7.32-7.35 (m, 5H, CH$_2$C$_6$$H_5$); ESI-MS: (*m/z*) 292.3 (M+H)$^+$.

(S)-benzyl3-methyl-1-(4-methylpiperazin-1-yl) butan-2-ylcarbamate (5c)

The compound was obtained as a gummy substance in 83% yield. ^{1}H NMR (300 MHz, CDCl$_3$) : δ 0.87-0.94 (2d, J = 6.8 Hz, 6H, CH(CH_3)$_2$), 2.27 (s, 3H, NCH_3), 2.32-2.61 (m, 11H, CH(CH$_3$)CH_2cycN(CH_2CH_2)$_2$NCH_3), 3.60-3.80 (m, 1H, NHCH), 5.13 (s, 2H, CH_2C$_6$H$_5$), 7.37 (s, 5H, C$_6$$H_5$); ESI-MS: (*m/z*)320.2 (M+H)$^+$.

(*S*)-Benzyl 4-methyl-1-(4-methylpiperazin-1-yl) pentan-2-ylcarbamate (5d)

The compound was obtained as a white solid in 89% yield. ^{1}H NMR (300 MHz, CDCl$_3$) : δ 0.91 (d, J = 6.5 Hz, 6H, CH(CH_3)$_2$), 1.35-1.28 (m, 2H, CH_2CH(CH_3)$_2$), 1.73-1.64 (m, 1H, CH(CH_3)$_2$), 2.28 (s, 3H, NCH_3), 2.59-2.30 (m, 10H, CH_2cycN(CH_2CH_2)$_2$ NCH$_3$), 3.81-3.76 (m, 1H, NHCH), 4.70 (br s, 1H, NHCO), 5.11 (s, 2H, CH_2C$_6$H$_5$), 7.36-7.30 (m, 5H, CH$_2$C$_6$$H_5$); ESI-MS: (*m/z*)334.2 (M+H)$^+$.

(*R*)-Benzyl 4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-ylcarbamate (5e)

The compound was obtained as a white solid in 89% yield. ^{1}H NMR (300 MHz, CDCl$_3$) : δ 0.91 (d, J = 6.5 Hz, 6H, CH(CH_3)$_2$), 1.35-1.28 (m, 2H, CH_2CH(CH_3)$_2$), 1.73-1.64 (m, 1H, CH(CH_3)$_2$), 2.28 (s, 3H, NCH_3), 2.59-2.30 (m, 10H, CH_2cycN(CH_2CH_2)$_2$ NCH$_3$), 3.81-3.76 (m, 1H, NHCH), 4.70 (br s, 1H, NHCO), 5.11 (s, 2H, CH_2C$_6$H$_5$), 7.36-7.30 (m, 5H, CH$_2$C$_6$$H_5$); ESI-MS: (*m/z*)334.2 (M+H)$^+$.

Benzyl 4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-ylcarbamate (5f)

The compound was obtained as a white solid in 89% yield. ^{1}H NMR (300 MHz, CDCl$_3$) : δ 0.91 (d, J = 6.5 Hz, 6H, CH(CH_3)$_2$), 1.35-1.28 (m, 2H, CH_2CH(CH_3)$_2$), 1.73-1.64 (m, 1H, CH(CH_3)$_2$), 2.28 (s , 3H, NCH_3), 2.59-2.30 (m, 10H, CH_2cycN(CH_2CH_2)$_2$ NCH$_3$), 3.81-3.76 (m, 1H, NHCH), 4.70 (br s, 1H, NHCO), 5.11 (s, 2H, CH_2C$_6$H$_5$), 7.36-7.30 (m, 5H, CH$_2$C$_6$$H_5$); ESI-MS: (*m/z*)334.2 (M+H)$^+$.

Benzyl (*2S*, *3S*)-3-methyl-1-(4-methylpiperazin-1-yl) pentan-2-ylcarbamate (5g)

The compound was obtained as a gummy substance in 89% yield. ^{1}H NMR (CDCl$_3$, 300 MHz) : δ 0.87-1.05 (m, 6H, CHCH_3CH$_2$CH_3), 1.08-1.18 (m, 2H, CH_2CH$_3$), 1.40-1.48 (m, 1H, CHCH$_3$), 2.27 (s, 3H, NCH_3), 2.33-2.61 (m, 10H, CH_2cycN(CH_2CH_2)$_2$NCH$_3$), 3.63-3.77 (m,

1H, NHC*H*), 4.90 (br s, 1H, N*H*CH) 5.13 (s, 2H, C$H_2C_6H_5$), 7.32-7.38 (m, 5H, $CH_2C_6H_5$); ESI-MS: (*m/z*) 334.2 $(M+H)^+$.

(*S*)-*tert*-butyl 1-(4-methylpiperazin-1-yl)-4-(methylthio) butan-2-ylcarbamate (5h) The compound was obtained as a off-white solid in 85% yield. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.97-2.05 (m, 2H, CHCH_2), 2.13 (s, 3H, SCH_3), 2.25 (s, 3H, NCH_3), 2.39-2.67 (m, 12H, CH_2SCH$_3$CHCH_2cycN(CH_2CH_2)$_2$NCH$_3$), 3.81-3.88 (m, 1H, NHC*H*); ESI-MS: (*m/z*) 318.0 $(M+H)^+$.

(*S*)-*Tert*-butyl 1-(4-methylpiperazin-1-yl)-3-phenylpropan-2-ylcarbamate (5i)

The compound was obtained as a off-white solid in 86 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.42 (s, 9H, C(CH_3)$_3$), 2.28 (s, 3H, NCH_3), 2.30-2.61 (m, 10H, CH_2cycN(CH_2CH_2)$_2$NCH3), 2.85 (d, J = 5.8 Hz, 2H, C$H_2C_6H_5$), 3.91-4.21 (m, 1H, NC*H*), 4.59 (br s, 1H, N*H*), 7.26-7.30 (m, 5H, C_6H_5); ESI-MS: (*m/z*) 334.2 $(M+H)^+$.

(*S*)-*Tert*-butyl 1-(1*H*-indol-3-yl)-3-(4-methylpiperazin-1-yl) propan-2-ylcarbamate (5j)

The compound was obtained as a off-white solid in 86 % yield. ^{1}H NMR (300 MHz ,$CDCl_3$): δ 1.43 (s, 9H, C $(CH_3)_3$), 2.27 (s, 3H, NCH_3), 2.29-2.44 (m, 12H, Ind-CH_2CH_2cycN$(CH_2CH_2)_2$, 4.02 (br s, 1H, NCH), 4.66 (br s, 1H, NHBoc), 7.02 (s, 1H, Ind-2H), 7.08-7.20 (m, 2H, Ind-5, 6-H), 7.37 (d, J = 7.8 Hz, 2H, Ind-4H), 7.66 (d, J = 7.3 Hz, 2H, Ind-7H), 8.09 (brs, 1H, NH-Ind); ESI-MS: (*m/z*) 373.2 $(M+H)^+$.

2.8.6. General Procedure for the Synthesis of 6a-j

To a suspension of **5a, 5h, 5i** and **5j** in methanol (1.0 equiv. in 10.0 ml) were added 20% HCl/dioxane and stirred for 1 h at room temperature. After completion of the reaction, the solvent was evaporated under reduced pressure. The product was purified by trituration with diethyl ether. The hydrochloride salt was basified with the triethylamine and the products obtained were used for the next step without further purification.

To a solution of **5b, 5c, 5d, 5e, 5f,** and **5g**, in MeOH (1.0 equiv. in 10.0.ml) 10 % (w/w) Pd/C was added, and flushed two times with hydrogen gas, and the reaction mixture was agitated at room temperature for 2 h under hydrogen gas, at 30 Psi. After complete deblocking of the protecting group (as monitored by the TLC) the reaction mixture was filtered through Celite and concentrated under reduced pressure to afford the product as a

gummy residue. The products obtained were used for the next step without further purification.

2-(4-methylpiperazin-1-yl)-ethanamine (6a)

The compound was obtained as a gummy substance in quantitative yield.

(*S*)-1-(4-methylpiperazin-1-yl) propan-2-amine (6b)

The compound was obtained as a gummy substance in quantitative yield.

(*S*)-3-methyl-1-(4-methylpiperazin-1-yl)butan-2-amine (6c)

The compound was obtained as a gummy substance in quantitative yield.

(*S*)-4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-amine (6d)

The compound was obtained as a gummy substance in quantitative yield.

(*R*)-4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-amine (6e)

The compound was obtained as a gummy substance in quantitative yield.

4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-amine (6f)

The compound was obtained as a gummy substance in quantitative yield.

(*2S*,*3S*)-3-methyl-1-(4-methylpiperazin-1-yl)pentan-2-amine (6g)

The compound was obtained as a gummy substance in quantitative yield.

(*S*)-1-(4-methylpiperazin-1-yl)-4-(methylthio) butan-2-amine (6h)

The compound was obtained as a gummy substance in quantitative yield.

(*S*)-1-(4-methylpiperazin-1-yl)-3-phenylpropan-2-amine (6i)

The compound was obtained as a gummy substance in quantitative yield.

(*S*)-1-(1*H*-indol-3-yl)-3-(4-methylpiperazin-1-yl) propan-2-amine (6j)

The compound was obtained as a gummy substance in quantitative yield.

2.8.7. General Procedure for the Synthesis of 7a-r

The free amines **6a-j** were (2.0 equiv.) heated with 4,7-dichloroquinoline (1.0 equiv.) in presence of phenol. After completion of the reaction (as monitored by the TLC) the reaction mixture was dissolved in chloroform. The organic layer was washed with 10% aqueous NaOH solution and finally with brine. The organic layer was dried over Na_2SO_4, and the solvent was concentrated under reduced pressure. The crude products obtained were purified by column chromatography by using methanol-chloroform-triethylamine as eluent.

7-Chloro-*N*-(2-(4-methylpiperazin-1-yl) ethyl) quinolin-4-amine (7a)

The compound was obtained as a white solid in 78% yield; m.p 104-106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 2.34 (s, 3H, NC*H*$_3$), 2.52-2.60 (m, 8H, CH_2cycN(C*H*$_2$C*H*$_2$)$_2$NCH$_3$), 2.79-2.83 (t, J = 6.2 Hz, C*H*$_2$cycN(CH$_2$CH$_2$)$_2$NCH$_3$), 3.34 (s, 2H, C*H*$_2$CH$_2$cycN(CH$_2$CH$_2$)$_2$NCH$_3$), 6.07 (br s, N*H*), 6.39 (d, J = 5.4 Hz, 1H, Ar-*H* quinoline), 7.39-7.43 (dd, J = 2.1, 8.9 Hz, 1H, Ar-*H* quinoline), 7.70 (d, J = 8.9 Hz, 1H, Ar-*H* quinoline), 7.98 (d, J = 2.0 Hz, 1H, Ar-*H* quinoline), 8.53 (d, J = 5.3 Hz, 1H, Ar-*H* quinoline); ^{13}CNMR ($CDCl_3$, 50 MHz) :δ 45.9, 52.5, 55.3, 99.2, 117.2, 121.1, 125.4, 128.4, 135.0, 148.6, 150.0, 151.6; HRMS calculated for [$C_{20}H_{27}ClN_4$+H] 305.1528, found 305.1521; ESI-MS: (*m/z*) 305.1 $(M+H)^+$.

***N*-(2-(4-methylpiperazin-1-yl)ethyl)-7-(trifluoromethyl)quinolin-4-amine (7b)**

The compound was obtained as a white solid in 78% yield; m.p 72-74°C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 2.34 (s, 3H, NC*H*$_3$), 2.52 (brs, 4H, CH_2cycN(C*H*$_2$)$_2$), 2.61 (brs, 4H, CH_2cycN(C*H*$_2$)$_2$NCH$_3$), 2.83 (t, J = 5.8 Hz, 2H, C*H*$_2$cycN(CH$_2$CH$_2$)$_2$NCH$_3$), 3.35 (d, J = 4.6 Hz, 2H, C*H*$_2$CH$_2$cycN(CH$_2$CH$_2$)$_2$NCH$_3$), 6.09 (brs, N*H*), 6.48 (d, J = 6.8 Hz, 1H, Ar-*H* quinoline), 7.64 (d, J = 8.5 Hz, 1H, Ar-*H* quinoline), 7.88 (d, J = 8.5 Hz, 1H, Ar-*H* quinoline), 8.29 (s, 1H, Ar-*H* quinoline), 8.63 (d, J = 5.0 Hz, 1H, Ar-*H* quinoline); ^{1}H NMR (50 MHz, $CDCl_3$) : δ 38.9, 45.9, 52.5, 55.1, 100.1, 120.6, 121.0, 126.7, 127.4, 130.4, 131.1, 147.5, 149.6, 152.2; HRMS calculated for $[C_{20}H_{21}F_3N_4+H]^+$ 339.1791, found 339.1788; ESI-MS: (*m/z*) 339.1 $(M+H)^+$.

(*S*)-7-Chloro-*N*-(1-(4-methylpiperazin-1-yl) propan-2-yl) quinolin-4-amine (7c)

The compound was obtained as a white solid in 70% yield; m.p 96-98°C;^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.31 (d, J = 6.0 Hz, 3H, CH (C*H*$_3$), 2.26 (s, 3H, NC*H*$_3$), 2.42-2.67 (m, 10H, CHC*H*$_2$cycN (C*H*$_2$C*H*$_2$)$_2$NCH$_3$), 3.66-3.74 (m, 1H, C*H*CH$_3$CH$_2$), 5.99 (br s, 1H, N*H*CH), 6.44 (d, J = 5.2 Hz, 1H, Ar-*H* quinoline), 7.37-7.40- (dd, J = 1.9, 8.9 Hz,1H, Ar-*H*

quinoline), 7.72 (d, J = 8.9 Hz, 1H, Ar-*H* quinoline), 7.96 (d, J = 1.8 Hz, 1H, Ar-*H* quinoline), 8.52 (d, J = 5.2 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR ($CDCl_3$, 50 MHz) : δ 18.8, 45.9, 50.0, 55.1, 62.7, 99.6, 117.9, 121.2, 125.3, 128.6, 134.8, 149.1, 149.8, 151.9; HRMS calculated for $[C_{17}H_{23}ClN_4+H]^+$319.1684, found 319.1694; ESI-MS: (*m/z*)319.2 $(M+H)^+$.

(*S*)-*N*-(1-(4-methylpiperazin-1-yl)propan-2-yl)-7-(trifluoromethyl)quinolin-4-amine (7d)

The compound was obtained as a white solid in 66% yield; m.p 88-90°C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.33 (d, J = 6 Hz, 3H, CH (CH_3)), 2.26 (s, 3H, NCH_3), 2.42-2.75 (m, 10H, CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.67-3.79 (m, 1H, CHCH$_3$CH$_2$), 6.08 (brs, 1H, N*H*CH), 6.53 (d, J = 5.3 Hz, 1H ,Ar-*H* quinoline), 7.61 (d, J = 8.6 Hz, 1H, Ar-*H* quinoline), 7.89 (d, J = 8.5 Hz, 1H, Ar-*H* quinoline), 8.27 (s, 1H, Ar-*H* quinoline), 8.61 (d, J = 5.1 Hz, 1H, Ar-*H* quinoline);^{13}C NMR ($CDCl_3$, 75 MHz) : δ 18.7, 45.1, 45.9, 52.9, 55.1, 62.7, 100.6, 120.1, 121.0, 121.2, 122.2, 125.8, 127.6, 130.5, 131.0, 147.7, 149.6, 152.2; HRMS calculated for $[C_{18}H_{23}F_3N_4+H]^+$ 353.1948; found 353.1946; ESI-MS: (*m/z*) 353.2 $(M+H)^+$.

(*S*)-7-Chloro-*N*-(3-methyl-1-(4-methylpiperazin-1-yl) butan-2-yl) quinolin-4- amine (7e)

The compound was obtained as a white solid in 78% yield; m.p 95-97°C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 0.96-1.06 (2d, J = 6.8 Hz, 6H, CH (CH_3)$_2$), 2.24 (s, 3H, NCH_3), 2.31-2.70 (m, 11H, CH(CH$_3$)CH_2cycN(CH_2CH_2)$_2$NCH_3), 3.53-3.59 (m, 1H, NHC*H*) 5.99 (br s, 1H, N*H*CH), 6.43 (d, J = 5.3 Hz, 1H, Ar-*H* quinoline), 7.38-7.42 (dd, J = 2.0, 8.9 Hz, 1H, Ar-*H* quinoline), 7.76 (d, J = 8.8 Hz, 1H, Ar-*H* quinoline), 7.97 (d, J = 1.9 Hz, 1H, Ar-*H* quinoline), 8.52 (d, J = 5.3 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR ($CDCl_3$, 50 MHz) : δ 17.3, 18.3, 29.6, 45.9 , 53.1, 54.3, 55.1 , 56.7, 99.4, 117.8, 121.1, 125.2, 128.7, 134.85, 150.2, 151.9; HRMS calculated for $[C_{19}H_{27}ClN_4+H]^+$ 347.1997, found 347.2003; ESI-MS: (*m/z*) 347.2 $(M+H)^+$.

(*S*)-*N*-(3-methyl-1-(4-methylpiperazin-1-yl) butan-2-yl)-7-(trifluoromethyl) quinolin-4-amine (7f)

The compound was obtained as a white -off solid in 78% yield; m.p 87-89°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97-1.07 (2d, J = 6.8 Hz, 6H, CH (CH_3)$_2$), 2.25-2.29 (m, 1H, CH(CH_3)$_2$), 2.34 (s, 3H, NCH_3), 2.56-2.82 (m, 10H, CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.54-3.64 (m, 1H, NHC*H*), 6.0 (br s, 1H, N*H*CH), 6.54 (d, J = 5.4 Hz, 1H, Ar-*H* quinoline), 7.64 (d, J = 8.3 Hz, 1H, Ar-*H* quinoline), 8.05 (d, J = 8.7 Hz, 1H, Ar-*H* quinoline), 8.29 (s, 1H, Ar-*H* quinoline), 8.60 (d, J = 5.5 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR ($CDCl_3$, 50 MHz) : δ 17.3, 18.4, 28.4, 29.2, 29.6, 45.8, 53.0, 54.4, 55.1, 56.7, 100.0, 120.1, 120.9, 121.1, 122.6, 125.3, 127.6, 130.7,

131.0, 147.8, 150.0, 152.2; HRMS calculated for $[C_{20}H_{27}F_3N_4+H]^+$ 381.2161, found 381.2278. ESI-MS: (*m/z*) 381.2 $(M+H)^+$.

(*S*)-7-Chloro-*N*-(4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-yl)quinolin-4-amine (7g)

The compound was obtained as a white solid in 72% yield; m. p.103-105 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ0.96 (d, J = 6.0 Hz, 3H, CH(CH_3), 1.06 (d, J = 6.2 Hz, 3H, CH(CH_3), 1.44-1.53 (m, 2H, CH_2 CH$(CH_3)_2$), 1.67-1.83 (m, 1H, CH(CH$_3)_2$), 2.27 (s, 3H, NCH_3), 2.63-2.41 (m, 10H, CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.68 (d, J = 5.7 Hz,1H, NCH), 5.71 (d, J = 4.1 Hz, 1H, NH), 6.44 (d, J = 5.3 Hz, Ar-H quinoline), 7.37-7.41 (dd, J = 2.0, 8.8 Hz, 1H, Ar-H quinoline), 7.51 (d, J = 8.9 Hz,1H,Ar-H quinoline), 7.97 (dd, J = 2.0 Hz,1H,Ar-H quinoline), 8.53 (d, J = 5.3 Hz, 1H,Ar-H quinoline); ^{13}C NMR ($CDCl_3$, 50 MHz) : δ 22.5, 23.3, 42.6, 45.9, 53.3, 55.2, 61.2, 99.2, 117.7, 121.1, 125.3, 128.7, 134.8, 149.2, 149.8, 151.9; HRMS calculated for $[C_{20}H_{29}ClN_4+H]^+$361.2154 found 361.2151; ESI-MS: (*m/z*) 361.2 $(M+H)^+$.

(*R*)-7-Chloro-*N*-(4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-yl)quinolin-4-amine (7h)

The compound was obtained as a white solid in 72% yield; m. p. 103-105°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, J = 6.15 Hz, 3H, CH(CH_3), 1.06 (d, J = 6.18 Hz, 3H, CH(CH_3)), 1.44-1.51 (m, 2H, CH_2 CH$(CH_3)_2$), 1.66-1.75 (m, 1H, CH(CH$_3)_2$), 2.25 (s, 3H, NCH_3), 2.40-2.61 (m, 10H ,CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.66 (d, J = 5.8 Hz, 1H, NCH), 5.70 (d, J = 4.1 Hz, 1H, NH), 6.43 (d, J = 5.3Hz, Ar-Hquinoline),7.36-7.39 (dd, J = 1.9, 8.9 Hz, 1H, Ar-H quinoline), 7.69 (d, J = 8.9 Hz, 1H,Ar-H quinoline), 7.95 (dd, J = 1.8 Hz, 1H, Ar-H quinoline), 8.52 (d, J = 5.2 Hz, 1H, Ar-H quinoline); ^{13} CNMR ($CDCl_3$, 50 MHz) : δ 22.4, 23.3, 24.9, 42.6, 45.9, 53.3, 55.2, 61.2, 99.2, 117.7, 121.1, 125.2, 128.7, 134.7, 149.2, 149.7, 151.9;HRMS calculated for $[C_{20}H_{29}ClN_4+H]^+$ 361.2154 found 361.2175; ESI-MS: (*m/z*) 361.2 $(M+H)^+$.

7-Chloro-*N*-(4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-yl)quinolin-4-amine (7i)

The compound was obtained as a white solid in 72% yield; m.p.103-105 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ0.96 (d, J = 6.0 Hz, 3H, CH(CH_3)), 1.06 (d, J = 6.2 Hz, 3H, CH(CH_3)), 1.44-1.53 (m, 2H, CH_2 CH$(CH_3)_2$), 1. 67-1.83. (m, 1H, CH(CH$_3)_2$), 2.27 (s, 3H, NCH_3), 2.63-2.41 (m, 10H, CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.68 (d, J = 5.7 Hz,1H, NCH), 5.71 (d, J = 4.1 Hz, 1H, NH), 6.44 (d, J = 5.3 Hz, Ar-H quinoline), 7.37-7.41 (dd, J = 2.0, 8.8 Hz, 1H, Ar-H quinoline), 7.51 (d, J = 8.9 Hz,1H,Ar-H quinoline), 7.97 (dd, J = 2.0 Hz,1H,Ar-H quinoline), 8.53 (d, J = 5.3 Hz, 1H,Ar-H quinoline); ^{13}C NMR ($CDCl_3$, 50 MHz) : δ 22.5, 23.3, 42.6, 45.9, 53.3, 55.2, 61.2, 99.2, 117.7, 121.1, 125.3, 128.7, 134.8, 149.2, 149.8, 151.9; HRMS calculated for $[C_{20}H_{29}ClN_4+H]^+$361.2154 found 361.2151; ESI-MS: (*m/z*) 361.2 $(M+H)^+$.

(*S*)-*N*-(4-methyl-1-(4-methylpiperazin-1-yl)pentan-2-yl)-7-(trifluoromethyl)quinolin-4-amine (7j)

The compound was obtained as a white solid in 72% yield; m. p. 96-98°C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 0.97 (d, J = 6.2 Hz, 3H, CH(CH_3)), 1.07 (d, J = 6.1 Hz, 3H, CH(CH_3)), 1.50-1.52 (m, 2H, CH_2 CH (CH_3)$_2$), 1.69-1.74 (m, 1H, CH (CH_3)$_2$), 2.27 (s, 3H, NCH_3), 2.43-2.65 (m, 10H ,CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.70 (d, J = 5.3 Hz, 1H, NHCH), 5.81 (br s, 1H, NH), 6.53 (d, J = 5.19 Hz, Ar-H quinoline), 7.36-7.39 (dd, J = 1.9, 8.9 Hz, 1H, Ar-H quinoline), 7.89 (d, J = 8.4 Hz, 1H, Ar-H quinoline), 8.28 (s, 1H, Ar-H quinoline), 8.63 (d, J = 5.1 Hz, 1H, Ar-H quinoline);^{13}C NMR (50 MHz, $CDCl_3$) : δ 22.4, 23.3, 24.9, 42.4, 45.9, 53.3, 55.2, 61.2, 100.2, 120.0, 120.9, 127.6, 130.4, 131.1, 147.8, 149.5, 152.2; HRMS calculated for $[C_{21}H_{29}F_3N_4+H]^+$ 395.2417, found 395.2410. ESI-MS: (*m/z*) 395.2 $(M+H)^+$.

7-Chloro-*N*-(*2S*,*3S*)-3-methyl-1-(4-methylpiperazin-1-yl)pentan-2-yl)quinolin-4-amine (7k)

The compound obtained as a white solid in 66% yield; m. p 101-103°C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 0.92 (d, J = 6.7 Hz, 3H, CHCH_3), 1.02 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.39-1.48 (m, 2H, CH_3CH_2CHCH_3), 1.50-1.71 (m, 1H, CH_3CH_2CHCH_3), 2.26 (s, 3H, NCH_3), 2.74-2.42 (m, 10H, CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.68-3.63 (m,1H, NHCH), 5.92 (br s,1H, NHCH), 6.45 (d, J = 5.6 Hz, 1H, Ar-H quinoline), 7.42-7.45 (t, J = 6.9 1H, Ar-H quinoline), 7.88 (d, J = 1.7 Hz, 1H, Ar-H quinoline), 8.03 (d,. J = 1.7 Hz, 1H, Ar-H quinoline), 8.50 (d. J = 5.4 Hz, 1H, Ar-H quinoline); ^{13}C NMR ($CDCl_3$, 50 MHz) : δ 12.3, 14.0, 25.9, 35.8, 45.7, 55.0, 56.0, 99.2, 117.7, 121.5, 125.4, 127.9, 135.2, 148.3, 150.5, 151.1; HRMS calculated for $[C_{20}H_{29}ClN_4+H]^+$ 361.2154 found 361.2172; ESI-MS: (*m/z*) 361.2 $(M+H)^+$.

***N*-(*2S*, *3S*)-3-methyl-1-(4-methylpiperazin-1-yl)pentan-2-yl)-7 (trifluoromethyl) quinolin-4-amine (7l)**

The compound was obtained as a pale yellow solid in 60% yield; m.p 97-99 °C; ^{1}H NMR (300MHz, $CDCl_3$): δ 0.95 (d, J = 6.7 Hz, 3H, CHCH_3), 1.05 (t, J = 7.2 Hz, 3H, CH_2CH_3), 1.33-1.44 (m, 2H, CH_3CH_2CHCH_3), 1.51-1.60 (m, 1H, CH_3CH_2CHCH_3), 2.31 (s, 3H, NCH_3), 2.50-2.80 (m, 10H, CHCH_2cycN(CH_2CH_2)$_2$NCH_3), 3.70 (d, J = 4.5 Hz, 1H, NHCH), 5.94 (br s, 1H, NHCH), 6.52 (d, J = 5.2 Hz, 1H, Ar-H quinoline), 7.64 (d, J = 7.9 Hz, 1H, Ar-H quinoline), 7.99 (dd, J = 8.2 Hz, 1H, Ar-H quinoline), 8.29 (s,1H, Ar-H quinoline), 8.62 (d. J = 5.3 Hz, 1H, Ar-H quinoline); ^{13}C NMR (50 MHz, $CDCl_3$) : 12.3, 13.9, 25.9, 35.6, 45.7, 53.0, 55.0, 56.0, 100.3, 120.1, 121.1, 122.6, 125.3, 129.3, 130.7, 131.0, 147.6, 149. 9, 152.0; HRMS calculated for $[C_{21}H_{29}F_3N_4+H]^+$ 395.2417, found 395.2410. ESI-MS: (*m/z*) 395.2 $(M+H)^+$.

(*S*)-7-Chloro-*N*-(1-(4-methylpiperazin-1-yl)-4-(methylthio)butan-2-yl)quinolin-4-amine (7m)

The compound was obtained as a off-white solid in 65% yield; m.p 92-94°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.97-2.05 (m, 2H, CHCH_2), 2.12 (s, 3H, SCH_3), 2.25 (s, 3H, NCH_3), 2.37-2.66 (m, 12H, CH_2SCH$_3$CHCH_2cycN(CH_2CH_2)$_2$NCH$_3$), 3.80-3.87 (m, 1H, NHC*H*), 5.77 (d, *J* = 1H, 3.78 Hz, N*H*), 6.53 (d, J = 4.05 Hz, 1H, Ar-*H* quinoline), 7.76 (d , *J* = 6.9 Hz, 1H, Ar-*H* quinoline), 7.71 (d, *J* = 6.7 Hz, 1H, Ar-*H* quinoline), 7.97 (d, *J* = 1.17 Hz, 1H, Ar-*H* quinoline), 8.53 (d, *J* = 4.0 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.8, 30.2, 32.1, 42.4, 45.4, 45.9, 50.7, 54.5, 55.0, 59.8. 99.0, 117.4, 121.5, 125.5, 128.4, 128.5, 135.1, 149.1, 151.7, 151.8; HRMS calculated for $[C_{19}H_{27}ClN_4S+H]^+$ 379.1718, found 379.1716; ESI-MS: (*m/z*) 379.2 $(M+H)^+$.

(*S*)-*N*-(1-(4-methylpiperazin-1-yl)-4-(methylthio)butan-2-yl)-7-(trifluoromethyl)quinolin-4-amine (7n)

The compound was obtained as a white solid in 65% yield; m.p 89-91°C; H NMR (300 MHz, $CDCl_3$): δ 1.97-2.09 (m, 2H, CHCH_2), 2.13 (s, 3H, SCH_3), 2.27 (s, 3H, NCH_3), 2.44-2.73 (m, 12H, CH_2SCH$_3$CHCH_2cycN(CH_2CH_2)$_2$NCH$_3$), 3.57-3.62 (m, 1H, NHC*H*), 5.90 (d, *J* = 3.7 Hz, 1H, N*H*), 6.63 (d, *J* = 4.0 Hz, 1H, Ar-*H* quinoline), 7.60-7.63 (dd, *J* = 1.2, 6.5 Hz, 1H, Ar-*H* quinoline), 7.91 (d, *J* = 6.5 Hz, 1H, Ar-*H* quinoline), 7.97 (s, 1H, Ar-*H* quinoline), 8.62 (d, *J* = 4.02 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR (50 MHz, $CDCl_3$): δ15.9, 30.7, 32.1, 42.4, 45.4, 45.9, 51.7, 54.5, 55.4, 59.8. 99.0, 117.4, 121.5, 125.5, 128.4, 128.5, 135.1, 149.1, 151.7, 151.8; HRMS calculated for $[C_{20}H_{27}F_3N_4S+H]^+$ 413.1981, found 413.1993; ESI-MS: (*m/z*) 413.2 $(M+H)^+$.

(*S*)-7-Chloro-*N*-(1-(4-methylpiperazin-1-yl)-3-phenylpropan-2-yl)quinolin-4-amine (7o)

The compound was obtained as a yellowish white solid in 63% yield; m.p 98-100 °C ^{1}H NMR (300 MHz, $CDCl_3$) : δ 2.24 (s, 3H, NCH_3), 2.40-2.79 (m, 10H, CH_2cycN(CH_2CH_2)$_2$NCH$_3$), 2.92-3.13 (m, CH_2C$_6$H$_5$), 3.83-3.90 (m, 1H, NC*H*), 5.72 (d, *J* = 4.4Hz, 1H, N*H*CH), 6.50 (d, *J* = 5.3 Hz, 1H, Ar-*H* quinoline), 7.16-7.29 (m, 5H, CH$_2$C$_6$*H*$_5$), 7.36-7.40 (dd, *J* = 2.0, 8.9 Hz, 1H, Ar-*H* quinoline), 7.66 (d, *J* = 8.9 Hz, 1H, Ar-*H* quinoline), 7.97 (d, *J* = 2.0 Hz, 1H, Ar-*H* quinoline), 8.54 (d, *J* = 5.1 Hz, 1H, Ar-*H* quinoline); ^{13}CNMR ($CDCl_3$, 50 MHz) :δ 38.1, 45.8, 55.1, 59.8, 99.4, 117.7, 121.7, 125.5, 126.7, 128.5, 129.6, 135.0, 137.0, 138.3, 148.9, 149.6, 151.7; HRMS calculated for $[C_{23}H_{27}ClN_4+H]^+$ 394.1997 found 395.1995; ESI-MS: (*m/z*) 395.4

(*S*)-*N*-(1-(4-methylpiperazin-1-yl)-3-phenylpropan-2-yl)-7-(trifluoromethyl) quinolin-4-amine (7p)

The compound was obtained as a yellowish white solid in 65% yield; m.p 91-93°C; ^{1}H NMR (300MHz, $CDCl_3$): δ2.24 (s, 3H, NCH_3), 2.40-2.66 (m, 10H, CH_2cycN(CH_2CH_2)$_2$NCH_3), 2.92-3.14 (m, CH_2C$_6$H$_5$), 3.92 (s, 1H, NC*H*), 5.83 (brs, 1H, N*H*CH), 6.58 (d, *J* = 5.1 Hz, 1H, Ar-*H* quinoline), 7.19-7.30 (m, 5H, CH$_2$C$_6$*H*$_5$), 7.60 (d, *J* = 8.3 Hz, 1H, Ar-*H* quinoline), 7.84 (d, *J* = 8.4 Hz, 1H, Ar-*H* quinoline), 8.28 (s, 1H, Ar-*H* quinoline), 8.63 (d, *J* = 5.2 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR ($CDCl_3$, 50 MHz) :δ 38.0, 45.9, 50.3, 52.9, 55.1, 59.8, 100.4, 120.2, 121.03, 126.7, 127.6, 128.5, 129.6, 130.5, 131.2, 136.9, 147.8, 149.2, 152.2;HRMS calculated for [$C_{24}H_{27}F_3N_4$+H]$^+$ 429.2261, found 429.2272; ESI-MS: (*m/z*) 429.2 (M+H)$^+$.

(*S*)-*N*-(1-(1*H*-indol-3-yl)-3-(4-methylpiperazin-1-yl)propan-2-yl)-7-chloroquinolin-4-amine (7q)

The compound was obtained as a pale yellow solid in 60% yield; m.p 102-104 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, NCH_3), 2.35-2.69 (m, 10H, CH_2cycN(CH_2CH_2)$_2$NCH_3), 3.16-3.30 (m, CH_2-Indole), 4.05 (d, *J* = 4.7 Hz, 1H, NHC*H*), 5.75 (br s, 1H, N*H*CH), 6.56 (d, *J* = 5.3 Hz, 1H, Ar-*H* quinoline), 7.00 (d, *J* = 2.0 Hz, 1H, Ind-2*H*), 7.09-7.23 (m, 2H, Ind-5, 6-*H*), 7.33-7.36 (m, 3H, Ind-4*H* and Ar-*H* quinoline), 7.61 (d, *J* = 2.9 Hz, 2H, Ind-7*H*), 8.00 (d, *J* = 1.9 Hz, 1H, Ar-*H* quinoline), 8.21 (d, *J* = 9.3 Hz, 1H, Ar-*H* quinoline),8.54 (d, *J* = 5.4Hz, 1H, Ar-*H* quinoline); ^{13}C NMR ($CDCl_3$, 50MHz) : δ 29.6, 45.9, 55.1, 60.0, 99.4, 110.7, 111.3, 117.6, 118.8, 119.6, 121.3, 122.1, 123.1, 125.3, 128.0, 128.5, 134.9, 136.2, 149.1, 149.6, 151.8; HRMS calculated for [$C_{25}H_{28}ClN_5$+H]$^+$ 434.9763, found 434. 9743; ESI-MS: (*m/z*) 434.3 (M+H)$^+$.

(*S*)-*N*-(1-(1*H*-indol-3-yl)-3-(4-methylpiperazin-1-yl)propan-2-yl)-7-(trifluoromethyl)quinolin-4-amine (7r)

The compound was obtained as a pale yellow solid in 60% yield; m.p 96-98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 2.26 (s, 3H, NCH_3), 2.41-2.65 (m, 10H, CH_2cycN(CH_2CH_2)$_2$NCH_3), 3.20-3.25 (m, 2H, CH_2-Indole), 4.04 (d, *J* = 4.0 Hz, 1H, NHC*H*), 5.79 (d, *J* = 3.7 Hz, N*H*CH), 6.63 (d, *J* = 5.3 Hz, 1H, Ar-*H* quinoline), 7.09 (d, *J* = 2.0 Hz, 1H, Ind-2*H*), 7.10-7.23 (m, 2H, Ind-5, 6-*H*), 7.54 (d, *J* = 1.9 Hz,1H, Ar-*H* quinoline), 7.60 (d, *J* = 6.1 Hz, Ind-4*H*), 7.73 (d, *J* = 2.9 Hz, 1H, Ind-7*H*), 8.18 (d, *J* = 6.4 Hz, 1H, Ar-*H* quinoline), 8.27 (s, 1H, Ar-*H* quinoline),8.61 (d, *J* = 5.3Hz, 1H, Ar-*H* quinoline); ^{13}C NMR (50 MHz, $CDCl_3$): δ 29.6, 45.7, 45.8, 49.8, 53.0, 54.9, 55.1, 60.0, 61.1, 110.4, 110.6, 111.3, 118.7, 119.6, 121.0, 121.1, 122.1,122.6, 123.5, 125.3, 127.4, 127.9, 130.7, 131.0, 136.2, 147.7, 149.4, 152.2; HRMS calculated for [$C_{26}H_{28}F_3N_5$+H]$^+$ 468.2370, found 434. 2368; ESI-MS: (*m/z*) 468.1 (M+H)$^+$.

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598 **2.8.8. General Procedure for the synthesis of 8a-b**

599 Starting from glycine and α-phenylalanine, compounds **8a-b** were prepared by the procedure
600 described for (**2b**).

601 **2-(benzyloxycarbonylamino)acetic acid (8a)**

602 The compound was obtained as a white solid in quantitative yield; m.p 119-120°C; ^{1}H-NMR
603 (300 MHz, $CDCl_3$): δ 3.67 (s, 2H, CH_2), 5.07 (s, 2H, CH_2), 7.35 (s, 5H, $OCH_2C_6H_5$); ESI-
604 MS: (*m/z*) 210 $(M+H)^+$.

605 **(*S*)-2-(benzyloxycarbonylamino)-3-phenylpropanoic acid (8b)**

606 The compound was obtained as a white solid in quantitative yield; m.p 100-102°C; ^{1}H NMR
607 (300 MHz, $CDCl_3$): δ 2.80 (d, J = 7.0 Hz, 2H, CH_2Ph), 4.50 (m, 1H, NHC*H*), 4.95 (s, 1H,
608 N*H*CH), 5.09 (s, 2H, OCH_2Ph), 7.13-7.28 (m, 10H, Ar*H*).

609 **2.8.9. General procedure for the synthesis of 9a-b**

610 To a stirred solution of *N*-Cbz protected amino acids **8a-b** (1.0 equiv.) in dry THF at - 15°C
611 under nitrogen atmosphere were added successively isobutylchloroformate (IBCF) (1.1
612 equiv.), and *N*-methylmorpholine (NMM) (1.1 equiv.).The mixture was stirred for 15 min,
613 and then treated drop wise with an ethereal solution of excess CH_2N_2. The yellow solution
614 was allowed to warm to room temperature and stirring was continued until there was no N-
615 protected amino acid remaining (TLC control). The reaction mixture was concentrated under
616 reduced pressure, and the residue was taken up in EtOAc. The organic phase was washed
617 successively with aqueous $NaHCO_3$ solution and brine. The organic layer was dried over
618 Na_2SO_4 and concentrated under reduced pressure. The products were purified by the silica gel
619 column chromatography (EtOAc/hexane) to obtain the diazoketones **9a-b**.

620 **Benzyl 3-diazo-2-oxopropylcarbamate (9a)**

621 The compound was obtained as a white off solid in 80% yield; m.p 68-69°C; ^{1}H NMR (300
622 MHz, $CDCl_3$) δ 3.97 (d, J = 4.8 Hz, NHCH_2), 5.12 (s, 2H, CH_2Ph), 5.37 (br s, 1H, C*H*N_2),
623 5.54 (brs, 1H, NH), 7.35 (5 H, m, C_6H_5).

624 **(*S*)-benzyl 4-diazo-3-oxo-1-phenylbutan-2-ylcarbamate (9b)**

625 The compound was obtained as a yellow solid in 80% yield; m.p 80-81°C; ^{1}H NMR (300
626 MHz, $CDCl_3$): δ 3.03 (d, 2H, J = 6.8 Hz, CH_2Ph); 4.45-4.49 (m, 1H, C*H*NH), 5.21 (s, 2H,
627 OCH_2Ph), 5.31 (s, 1H, C*H*N_2), 5.35 (1H, br, NH), 7.18-7.42 (m, 10H, ArH).

628 **2.8.10. General procedure for the synthesis of 10a-b**

629 To a solution of the diazo ketone **9a-b** (1.0 equiv.) in MeOH at -25 °C under N_2 with the
630 exclusion of light was treated with a solution of silver benzoate (0.11 equiv.) in Et_3N (2.9

equiv.).The reaction mixture was allowed to warm to room temperature within 3.0 h in the dark and then concentrated under reduced pressure. The oily residue dissolved in EtOAc and washed with brine solution. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by the silica gel column chromatography.

Methyl 3-(benzyloxycarbonylamino) propanoate (10a)

The compound was obtained as a yellow solid in 75% yield; m.p 80-81°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ2.41-2.51 (m, 2H, CH_2COOCH_3), 3.43-3.51 (m, 2H, NHCH_2), 3.65 (s, 3H, OCH_3), 5.09 (s, 2H, CH_2Ph), 5.30 (1H, br, NH), 7.35 (m, 5H, ArH).

(*S*)-Methyl 3-(benzyloxycarbonylamino)-4-phenylbutanoate (10b)

The compound was obtained as a gummy substance in 75% yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.49-2.53 (m, 2H, CH_2COOCH_3), 2.80-2.99 (m, 2H, CH_2Ph), 3.67 (s, 3H, OCH_3), 4.17-4.24 (m, 1H, NHC*H*), 5.07 (s, 2H, OCH_2Ph), 5.29 (d, J = 7.3 Hz, NH), 7.15-7.35 (m, 10H, ArH); ESI-MS: (*m/z*) 328.3 $(M+H)^+$.

2.8.11. General procedure for the synthesis of 11a-b

MeOH was added drop wise over a period of 20 min to a mixture of ester of N- protected amino acid (10a-b, 1.0 equiv.) and $NaBH_4$ (2 equiv.) in THF at 50-55 °C. The mixture was stirred for 10-25 min, and then water was added to the reaction mixture. Organic solvent was concentrated under reduced pressure. The residue was taken in EtOAc and extracted with brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by the silica gel column chromatography using hexane:EtOAc as eluent.

Benzyl 3-hydroxypropylcarbamate (11a)

The compound was obtained as a gummy substance in 75% yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47-1.59 (m, 2H, $CH_2CH_2CH_2OH$),2.87-3.31 (m,$CH_2CH_2CH_2OH$), 3.47-3.61 (m, 3H, $CH_2CH_2CH_2OH$), 3.94 (brs, 1H, NHC*H*), 5.09 (s, 2H, O-CH_2Ph), 7.35 (s, 5H, Ar-*H*).

(*S*)-benzyl 4-hydroxy-1-phenylbutan-2-ylcarbamate (11b)

The compound was obtained as a gummy substance in 80% yield. ^{1}H NMR (300MHz, $CDCl_3$): δ 1.80-1.85 (m, 2H, CH_2CH_2OH),2.82-2.87 (m,$CH_2C_6H_5$), 3.65 (s, 1H, CH_2CH_2OH),3.94 (br s, 1H, NHC*H*), 4.72-4.75 (m, 2H, CH_2CH_2OH), 5.07 (s, 2H, O-CH_2Ph), 7.15-7.35 (m, 10H, Ar-*H*); ESI-MS: (*m/z*) 300 $(M+Na)^+$.

2.8.12. General procedure for the synthesis of 12a-b

Compounds **12a-b** were prepared by similar method described for **4a**.

3-(Benzyloxycarbonylamino)propyl methanesulfonate (12a)

The compound was obtained as a gummy substance in quantitative yield. ^{1}H NMR (300MHz, $CDCl_3$): δ 1.65-1.79 (m, 2H, $CH_2CH_2CH_2OSO_2CH_3$), 2.87-3.07 (m,$CH_2CH_2CH_2OSO_2CH_3$), 3.15 (s, 3H, OSO_2CH_3), 3.91(br s, 1H, NHC*H*), 4.07 (t, J = 4.7 Hz,2H,$CH_2CH_2CH_2OSO_2CH_3$), 5.07 (s, 2H, O-CH_2Ph), 7.35 (s, 5H, Ar-*H*).

(*S*)-3-(Benzyloxycarbonylamino)-4-phenylbutyl methanesulfonate (12b)

The compound was obtained as a gummy substance in quantitative yield. ^{1}H NMR (300MHz, $CDCl_3$): δ 1.69-1.80 (m, 2H, $CH_2CH_2OSO_2CH_3$),2.88-2.98 (m,$CH_2C_6H_5$), 3.15 (s, 3H, OSO_2CH_3), 3.96 (br s, 1H, NHC*H*), 4.26 (t, J = 4.5 Hz, CH_2CH_2 OSO_2CH_3), 5.08 (s, 2H, O-CH_2Ph), 7.15-7.35 (m, 10H, Ar-*H*); ESI-MS: (*m/z*) 400 $(M+Na)^+$.

2.8.13. General procedure for the synthesis of 13a-b

Compounds **12a-b** were prepared by similar method described for **5a**

Benzyl 3-(4-methylpiperazin-1-yl)propylcarbamate (13a)

The compound was obtained as a gummy substance in quantitative yield; ^{1}H NMR (300MHz, $CDCl_3$): δ 1.95 (s, 2H, CH_2CH_2cycN(CH_2CH_2)$_2$NCH_3), 2.41 (s, 3H, NCH_3), 2.61-2.67 (m, 10H, CH_2CH_2cycN(CH_2CH_2)$_2$NCH_3), 3.35-3.42 (m, 2H, NHCH_2).

(*S*)-Benzyl 4-(4-methylpiperazin-1-yl)-1-phenylbutan-2-ylcarbamate (13b)

The compound was obtained as a gummy substance in 73% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ1.67-1.79 (m, 2H, CH_2CH_2cycN(CH_2CH_2)$_2$NCH_3), 2.26 (s, 3H, NCH_3), 2.32-2.49 (m, 10H, CH_2CH_2cycN (CH_2CH_2)$_2$NCH_3), 2.76-3.09 (m,$CH_2C_6H_5$), 3.94 (br s, 1H, NHC*H*), 6.0 (brs, 1H, N*H*CH), 7.15-7.35 (m, 10H, Ar-*H*); ESI-MS: (*m/z*) 382.3 $(M+H)^+$.

2.8.14. General procedure for the synthesis of 14a-b

Compounds **15a-c** were prepared by similar method described for **6a**

3-(4-methylpiperazin-1-yl) propan-1-amine (14a)

The compound was obtained as a gummy substance and used without further purification.

(*S*)-4-(4-methylpiperazin-1-yl)-1-phenylbutan-2-amine (14b)

The compound was obtained as a gummy substance and used without further purification.

2.8.15. General procedure for the synthesis of 15a-c

The final compounds **15a-c** were prepared by similar method described for **7a-r**.

7-Chloro-N-(3-(4-methylpiperazin-1-yl)propyl)quinolin-4-amine (15a)

The compound obtained as off-white solid in 75 % yield. m.p 108-110°C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.97 (s, 2H, CH_2CH_2cycN(CH_2CH_2)$_2$NCH_3), 2.41 (s, 3H, NCH_3), 2.62-2.68 (m, 10H, CH_2CH_2cycN(CH_2CH_2)$_2$NCH_3), 3.37-3.43 (m, 2H, NHCH_2), 6.34 (d, J = 5.4 Hz, 1H, Ar-*H* quinoline), 7.33-7.37 (dd, J = 2.0, 8.8 Hz, 1H, Ar-*H* quinoline), 7.91 (d, J = 8.9 Hz, 1H, Ar-*H* quinoline), 7.97 (d, J = 1.9 Hz 1H, Ar-*H* quinoline), 8.51 (d, J = 5.2 Hz, 1H,

Ar-*H* quinoline); ^{13}C NMR (50 MHz, $CDCl_3$) : δ 23.4, 44.3, 46.2, 53.5, 55.2, 58.6, 98.4,117.4, 122.4, 124.6, 128.3, 134.7, 148.8, 150.6, 151.8; HRMS calculated for $[C_{17}H_{23}ClN_4+H]^+$ 319.1684, found 319.1683; ESI-MS: (*m/z*) 319.3 $(M+H)^+$.

***N*-(3-(4-methylpiperazin-1-yl) propyl)-7-(trifluoromethyl)quinolin-4-amine (15b)**

The compound obtained as off-white solid in 70 % yield. m.p 76-78°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.95-2.00 (m, 2H, CH_2CH_2cycN(CH_2CH_2)$_2$NCH_3), 2.41 (s, 3H, NCH_3), 2.63-2.68 (m, 10H, CH_2CH_2cycN(CH_2CH_2)$_2$NCH_3), 3.38-3.42 (m, 2H, NHCH_2), 6.41 (d, J = 4.05 Hz, 1H, Ar-*H* quinoline), 7.53-7.56 (dd, J = 1.2, 6.5 Hz, 1H, Ar-*H*quinoline), 8.07 (d, J = 6.5 Hz, 1H, Ar-*H* quinoline), 8.25 (s, 1H, Ar-*H* quinoline), 8.59 (d, J = 4.0 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.3, 44.4, 46.3, 53.5, 55.2, 58.6, 99.4, 119.3, 119.3, 120.7, 122.2, 125.9, 127.4, 127.5, 130.4, 130.9, 131.3, 147.6, 150.4, 152.4; HRMS calculated for $[C_{18}H_{23}F_3N_4+H]^+$ 353.1948, found 353.1951; ESI-MS: (*m/z*) 353.2 $(M+H)^+$.

(*S*)-7-Chloro-*N*-(4-(4-methylpiperazin-1-yl)-1-phenylbutan-2-yl)quinolin-4-amine (15c)

The compound was obtained as a gummy substance in 45 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.67-1.89 (m, 2H, CH_2CH_2cycN (CH_2CH_2)$_2$NCH_3), 2.28 (s, 3H, NCH_3), 2.36-2.47 (m, 10H, CH_2CH_2cycN (CH_2CH_2)$_2$NCH_3), 2.76-3.09 (m,$CH_2C_6H_5$), 3.91 (br s, 1H, NH*CH*), 6.40 (d, J = 4.2 Hz, 1H, Ar-*H* quinoline), 7.11-7.27 (m, 5H, $CH_2C_6H_5$), 7.76 (d, J = 6.9 Hz, 1H, Ar-*H* quinoline), 7.88 (d, J = 8.7 Hz, 1H, Ar-*H* quinoline), 7.95 (d, J = 6.5 Hz, 1H, Ar-*H* quinoline), 8.35 (d, J = 4.0 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 37.9, 40.8, 45.0, 51.7, 55.1, 58.9, 97.6, 117.7, 122.0, 124.6, 125.9, 127.6, 128.6, 128.5, 128.2, 134.6, 138.2, 146.4, 149.0, 149.6; HRMS calculated for $[C_{17}H_{23}ClN_4+H]$ 409.2154, found 409.2151; ESI-MS: (*m/z*) 409.3 $(M+H)^+$.

Biological methods

***In vitro* antimalarial assay**

The compounds were evaluated for antimalarial activity against 3D7 (CQ-sensitive) and K1 (CQ-resistant) strains of *P. falciparum* using Malaria SYBR Green I nucleic acid staining dye based fluorescence (MSF) assay as mentioned by Singh *et al* (22).The stock (5 mg/mL) solution was prepared in DMSO and test dilutions were prepared in culture medium (RPMI-1640-FBS). The final concentration of DMSO in *Plasmodium* cultures was< 1%. Chloroquine diphosphate was used as a reference drug.

Test technique: 50mL of culture medium was dispensed in 96 well plate followed by addition of 50mL of highest concentration of test compounds (in duplicate wells) in row B. Subsequent two-fold serial dilutions were prepared and finally 50mL of 1.0% parasitized cell

suspension containing 0.8% parasitaemia was added to each well except 4 wells in row'A' received non parasitized erythrocyte suspension. The plates were incubated at 37 °C in CO_2 incubator in an atmosphere of 5% CO_2 and air mixture and 72 h later 100 µL of lysis buffer containing 2x concentration of SYBR Green-I (in Nitrogen) was added to each well and incubated for 1 h at 37 °C. The plates were examined at 485±20 nm of excitation and 530±20 nm of emission for relative fluorescence units (RFUs) per well using the fluorescence plate reader (FLX800, BIOTEK).

Statistical analysis: Data was transferred into a graphic programme (EXCEL) and IC_{50} values were obtained by Logit regression analysis of dose response curves using preprogrammed Excel spreadsheet.

***In vitro* assay for evaluation of cytotoxic activity**

Cytotoxicity of the compounds was carried out using VERO cell line (C1008; Monkey kidney fibroblast) following the method as mentioned in M. Sinha *et al* (26).The cells were incubated with compound-dilutions for 72 h and MTT was used as reagent for detection of cytotoxicity. 50% cytotoxic concentration (CC_{50}) was determined using nonlinear regression analysis of dose response curves using pre-programmed Excel spreadsheet. Selectivity Index (SI) was calculated as $SI = CC_{50}/IC_{50}$

***In vivo* antimalarial assay**

The *in vivo* drug response was evaluated in Swiss mice infected with *P. yoelii* (N-67 strain) which is innately resistant to CQ (23).In addition selected compounds were also tested on multidrug resistance *P. yoelii* and *P. vinckei*. The mice (22±2 g) were inoculated with $1x10^6$ parasitized RBC on day 0 and treatment was administered to a group of five mice from day 0-6, once daily. The aqueous suspensions of compounds were prepared with 0.5%v/v Tween 80. The efficacy of test compounds was evaluated at 100 mg/kg/day and the required daily dose was administered in 0.5mL volume via oral route. Parasitemia levels were recorded from thin blood smears at regular intervals of four days throughout the period of experiment. The mean value determined for a group of five mice was used to calculate the percent suppression of parasitemia with respect to the untreated control group. Mice treated with CQ served as reference controls. Whereas, in the case of arteether, the drug was dissolved in neutralized ground nut oil and administered via intramuscular route.

Determination of hematin-4-aminoquinoline derivatives association constant

Hematin and amino quinoline association constants for the compounds synthesized in the present study were determined by spectrophotometric titration procedure in aqueous DMSO

764 at pH 7.5 (24). In this assay condition, hematin is strictly in monomeric state and
765 interpretation of results is not complicated by need to consider hematin disaggregation
766 process. Association constant calculated in this technique is a good reflection of the
767 interaction that would occur in the acidic food vacuole. The pH 7.5 improves the stability of
768 hematin solutions and quality of data.

769 ***In vitro* Inhibition of *β*-hematin formation assay**

770 The ability of the 4-aminoquinoline derivatives to inhibit *β*-Hematin polymerization was
771 induced by 1-oleoyl-rac-glycerol using UV spectrophotometer and measurements were
772 carried out at 405 nm (25).The triplicate values obtained from the assay are expressed as
773 percent inhibition relative to hemozoin formation in a drug free control. The 50% inhibitory
774 concentration (IC_{50}) values for the compounds were obtained from the sigmoidal dose
775 response curves using non-linear regression curve fitting analyses with Graph Pad Prism 30
776 v.3.00 software (26). Each IC_{50} value is the result of at least three separate experiments
777 performed in duplicate.

778 **Molecular Docking Method**

779 ***Database Preparation***

780 Compounds were used to perform docking study against heme. To do a comparison study, we
781 used chloroquine drug for docking. A database was prepared by using SYBYL-X 1.3 (Tripos
782 Inc, St. Louis, MO, USA) modeling package (27).All compounds were drawn through sketch
783 module in sybyl. Structures were minimized further by adding Gasteiger-Huckel charges
784 along with distance-dependent dielectric and the Powell conjugate gradient algorithms with a
785 convergence criterion of 0.001 kcal/mol. All structures were put into a database and finally
786 aligned with each other by way of 'Fit Atom' method.

787 ***Protomol-Based Docking Study***

788 The molecular docking studies of synthesized compounds were performed using the Surflex-
789 Dock module with standard protocols in SYBYL-X 1.3.We have extracted heme molecule
790 from a protein (XX) which was collected from a protein database bank(PDB ID). Hydrogen
791 atoms were added to heme structure to get a correct configuration. Charges were also added
792 to it using Gasteiger-Huckel charge and energy-minimizations were performed with standard
793 protocol using the Tripos force field with same Gasteiger-Huckel charges along with
794 distance-dependent dielectric and the Powell conjugate gradient algorithms with a
795 convergence criterion of 0.001 kcal/mol. After that, automatic protomol generation method

was utilized to create a grid over heme. Molecular docking was performed by placing the molecules including reference into the grid with reasonable scoring function to score the ligands and protomol guided docking. Finally, the protomol-based method and empirically derived scoring function (e.g. Total score, crash score, polar etc.) were used to calculate the binding affinities.

Experimental Procedures for the pharmacokinetic studies of 7g and 16 (phosphate salt of 7g)

***In vitro* Pharmacokinetics of 7g**

Simulated gastric fluid (SGF) was prepared by dissolving 234 mg of NaCl and 37.2 mg of KCl in 100 mL triple distilled water and pH was adjusted to 1.2 with concentrated HCl. Simulated intestinal fluid (SIF) was prepared by dissolving 680 mg of KH_2PO_4 and 90 mg of NaOH in 100 mL triple distilled water and pH was adjusted to 6.8 with orthophosphoric acid. SGF/SIF (2 mL) was taken in a test tube and pre-incubated in shaking water bath for 10-15 min at 37 ± 2°C. 5 µL of **7g** stock solution (100µg/mL) was spiked in preincubated SGF/SIF to produce a concentration of 250 ng/ml and immediately subjected to incubation. 50 µl of the incubation mixture was sampled at 0, 15, 30, 60, 90, 120 min, diluted 5 times with acetonitrile and analyzed by LC-MS/MS. Metabolic stability of **7g** was performed in duplicate using glass tubes. To each tube, 460 µL of phosphate buffer (0.1 M, pH 7.4) and 12.5 µL of microsomal protein (20.0 mg/mL) were added and incubated for at 37±0.2°C for 5 min. Then, 2.5 µL of test compound (1mM) was added. For positive control testosterone was used as the test compound. Reaction was initiated by addition of 25µL of NADPH (24 mM) and incubated for 0, 5, 10, 15, 30, 45 and 60 min. In negative control, NADPH was replaced by 25 µL of phosphate buffer. Reaction was stopped by addition of 450 µL ice-cold acetonitrile to 50 µL reaction mixture collected at predefined time intervals, followed by centrifugation for 15 min at 10,000 rpm. 100µL of supernatant was directly analyzed by LC-MS/MS. For the assessment of plasma stability the blank rat plasma (2 mL) in a test tube was pre-incubated in shaking water bath for 10 min at 37 ± 2°C. 2 µl of **7g** stock solution (100 µg/mL) was spiked to preincubated plasma to produce a concentration of 100 ng/mL and immediately subjected to incubation. 50 µl of the plasma was sampled at 0, 5, 10, 15, 30, 60, 90 and 120 min. Plasma proteins were precipitated by addition of 400µL acetonitrile at predefined time intervals, followed by centrifugation for 15 min at 10,000 rpm. 100µL of supernatant was directly analyzed by LC-MS/MS.

828 ***In vivo* Pharmacokinetic study:**

829 *Invivo* pharmacokinetic study was performed in male *SpragueDawley* rats (n=4). Intravenous
830 formulation of **7g** was prepared by dissolving accurately weighed quantity of **7g** (20 mg) in 2
831 mL of DMSO followed by addition of 400 µL of ethanol and 800 µL of glycerol and
832 vortexing for 2 min. Volume was then made up to 4 ml with TDW followed by vortexing for
833 2 min. For its oral formulation, accurately weighed quantity of **7g** (30 mg) was transferred to
834 mortar and triturated with a pestle using 200 µL of Tween 20. Volume was then made up to 4
835 ml with 0.25% CMC Suspension. The intravenous and oral formulations of its phosphate salt
836 form **(16)** were prepared by dissolving in distilled water. Blood samples were collected from
837 the retroorbital plexus of rats under light ether anesthesia into microfuge tubes containing
838 heparin as an anti-coagulant at 0.083, 0.25, 0.5, 1, 2, 3, 5, 7, 9, 24, 30 and 48 hours post-
839 dosing for intravenous study, while 0.25, 0.5, 1, 2, 3, 5, 7, 9, 24, 30 and 48 hours post-dosing
840 for oral study. Plasma was harvested by centrifuging the blood at 13000 rpm for 10 min on
841 Sigma 1-15 K (Frankfurt, Germany) and stored frozen at -70 ± 10°C until bioanalysis. Each
842 plasma sample (100 µl) was processed using protein precipitation method using 200 µl
843 acetonitrile containing piracetam as internal standard (I.S.) as protein precipitant, and 10 µl of
844 the supernatant was injected for LC-MS/MS.

845 **RESULTS AND DISCUSSION**

846 **Chemistry.** The target compounds envisaged in the present study having the general
847 structure shown in figure 2 were obtained by strategies using *α*-amino acids and *β*-amino
848 acids as shown in schemes depicted in figures 3 and 4.

849 **Synthesis of compounds 7a-r:** Synthesis of **7a-r** involves following steps starting from the
850 preparation of the Boc and/or Cbz protected *α*-amino acids. Compounds **1a-j** were converted
851 to the corresponding Boc and/or Cbz derivatives **2a-j** in quantitative yields (14, 15). The
852 Boc/Cbz protected amino acids were reduced to the corresponding alcohols by mixed
853 anhydride protocol (16). Boc/Cbz amino alcohols were subjected to mesylation to afford **4a-j**
854 in good yields (16). The mesylated products were treated with *N*-methylpiperazine under
855 nitrogen atmosphere to get **5a-j** (17). Boc deprotection was done by using 20% HCl/Dioxane
856 at room temperature in quantitative yields and Cbz group was removed by using 10% Pd/C
857 catalyst to afford free amines **6a-j**. (14, 16) Compounds **6a-j** thus obtained were fused to 4,7-
858 Dichloroquinoline or 4-chloro-7-(trifluoromethyl)quinoline in the presence of phenol to
859 obtain compounds **7a-r** (18) (Figure 3).

Synthesis of compounds 15a-c: Synthesis of **15a-c** involves following steps starting from the preparation of the *N*-Cbz protected amino acids (scheme-2). Amino acids **1a & 1i** were converted to the corresponding *N*-Cbz derivatives **8a-b** in quantitative yields (19). *N*-Cbz protected amino acids were converted into the corresponding *β*-amino esters by Arndt-Eistert reaction. The reaction involves two steps in the first step Cbz-amino acids were converted into their corresponding diazoketones **9a-b** by mixed anhydride reaction and treating the mixed anhydride intermediate with diazomethane in THF at -15°C. Diazoketones were purified by the silica gel column chromatography and characterized by ^{1}H NMR. Purified diazoketone derivatives were converted into the corresponding *β*-amino esters via Wolf rearrangement in the presence of silver benzoate to get **10a-c** (20).The *β*-amino esters were converted to *β*-amino alcohols **11a-b** (21), subsequently alcohols were transformed to mesylates **12a-b** followed by replacing the mesyl group with *N*-methyl piperazine to obtain compounds **13a-b** (17). The N-Cbz protecting group was deprotected by using Pd/C to give amines **14a-b** (16) The amines so obtained were fused with the 4,7-Dichloroquinoline or 4-chloro-7-(trifluoromethyl)quinoline in the presence of phenol resulting in compounds **15a-c** (18). The details of the synthetic steps and the reaction conditions are depicted in figure 4 (scheme 2).

Preparation of Phosphate salt of compound 7g (16)

A cold solution of 400 mg of the compound **7g** (> 99% pure) in methanol, was added to a methanolic solution of phosphoric acid made from 85% phosphoric acid (3.0 equiv). After complete conversion of free base as seen from TLC, isopropyl alcohol was added to the reaction mixture and the phosphate salt separated as oil. The alcohol layer was decanted; acetone was added to the oil and triturated /stirred until it solidified. The mixture was then filtered and the phosphate salt thus obtained was washed with acetone and quickly placed in a vacuum desiccator. Further, it was inferred by differential scanning calorimetry (DSC) (figure 5), that no freebase (**7g**) was present in the phosphate salt (**16**). The yield was quantitative. m. p 195-198°C.

***In vitro* antiplasmodial activity**

The synthesized compounds **7a-r** and **15a-c** were screened against the 3D7 (CQ-S) and K1 (CQ-R) strains of *P. falciparum in vitro* for antiplasmodial activity and the results are presented in table 1. Most of the compounds displayed excellent antiplasmodial activity in the nanomolar range. As expected reduction of the amide bond has led to a substantial increase in

antiplasmodial activity against both the strains. Our data (table 1) suggest that compounds derived from glycine, leucine and phenylalnine i.e **7a-b**, **7g, 7j** and **7o-p** displayed similar antiplasmodial activity when chloro substituent was replaced with a trifluoromethyl group against both the strains. Whereas some compounds exhibited moderate to substantial difference in the antiplasmodial activity.

Among the 21 compounds tested (**7a-r & 15a-c**), fourteen compounds namely **7a-c, 7e, 7g-k,7o-q, 15a & 15c** exhibited potent antiplasmodial activity in the range of 3.27 to 25.1 nM against CQ-S strain and IC_{50} 9.79 to 167.4 nM range against CQ-R strain. Whereas, seven compounds**7d, 7f, 7l-n, 7r** & **15b** displayed antiplasmodial activity against CQ-S strain in the range of IC_{50} 81.22 to 723 nM and one compound **7r** showed comparable activity against CQ-R with IC_{50} 259 nM, two compounds**7d** & **15b** exhibited IC_{50} 548 and 585 nM, four compounds **7f** & **7l-n** demonstrated no detectable antiplasmodial activity against K1 strain at the highest concentration tested (IC_{50}>1000 nM). On the other hand, compounds having a *iso*-butyl group (**7g-j**) and benzyl group (**7o-p & 15c**) at the chiral centre were found to be the most active in this series. Moreover, the enantiomeric pairs of **7g, 7h** and racemic compound **7i** did not show any difference in the activities against both 3D7 and K1 strains. There was more than a 1.7-fold increase in the activity against CQ-S for compound-**7a** (3.27 nM) when compared to the CQ (5.46 nM). In fact, compounds namely **7g-7j**, **7p**, **15a** and **15c** exhibited almost 20 to 28 fold increases in the activity against the CQ resistant strain with IC_{50} values of 13.57, 11.16, 11.79, 12.13, 9.97 and 11.52 nM when compared to the chloroquine. In this study we have also explored the effect of the chain length variation by homologation of selected α-amino acids to get the corresponding β^3amino acids. Increase in the chain length showed positive effects on the antiplasmodial activities against both 3D7 and K1 strain of *P. falciparum.* Compounds derived from glycine (**15a**) (IC_{50} 9.79 nM) enhanced ten folds the activity when compared to the CQ in the case of resistant strain. Whereas, in the case of phenylalanine (**15c**) (IC_{50} 11.52 nM) the activity was increased four folds with respect to the CQ.

***In vitro* cytotoxicity**

The cytotoxicity of all the synthesized molecules was determined against VERO cell line using MTT assay (table 1). Our target compounds showed selectivity index ranging from 83.20 to 37,281. Compounds namely **7a, 7g,** and **15c** exhibited excellent selectivity indices 37, 281, 22,727 and 9,852 respectively. Whereas, compounds **7e, 7k,** and **7p,** displayed

selectivity indices 8,538, 7,026, and 8,024 respectively when compared to the chloroquine. Thus these compounds demonstrated the promising safety profile.

Biophysical studies

It is well established that the mode of action of 4-aminoquinoline based antimalarial compounds such as chloroquine is by interaction with the heme leading to inhibition of the hemozoin formation. The association constant (logK) for the drug-ferriprotoporphyrin (FP) ring provides valuable information about the antimalarial activity of the synthesized molecules. Another biophysical study which involves the *in-vitro* inhibition of hemozoin formation provides the possible mode of action of the synthesized compounds. This involves inhibition of *in-vitro* polymerization of hematin to *β*-hematin. The term *β*-hematin is used for chemically or *in vitro* synthesized hemozoin pigments, while the term hemozoin is used for the biosynthetic malaria pigment. The inhibition of the *β*-hematin formation takes place due to the blockage of the growing face of the crystal by the capping effect by majority of the CQ-like compounds.

A) Association constant for hematin and-4-aminoquinoline derivatives (log K)

Heme interaction has remained the unequivocal target for antimalarial activity of 4-aminoquinoline class of compounds. Accordingly, the association constant (log K) of this interaction is calculated by titrating the hematin with different concentrations of compounds in 40% DMSO solution and log K values obtained are in the range of 4.23-6.37. There is a linear correlation between the induced hypochromic effect and the concentration of the compounds. Among all the compounds reported in the present study compounds **7c, 7e, 7g-h, 7k, 7o, 7q, 15a** and **15c** have shown very strong binding to hematin. This result is concurrent with the generally accepted mechanism of action of this class of compounds.

B) Inhibition of *β*-hematin formation assay

The results presented in table-1indicated that these derivatives inhibited *β*-hematin formation in a concentration dependent manner. The IC_{50} values calculated for all the compounds were in the range of 0.14-0.27 mM. Most of the synthesized compounds were good inhibitors of *β*-hematin formation; some of them have shown moderate antimalarial activity against CQ-S and CQ-R strains of *P. falciparum.* The most potent inhibitors were compounds **7a, 7c, 7e, 7g-7k, 7o-7q, 15a** and **15c** respectively in the hemozoin inhibition assay. These results are consistent with observed antiplasmodial activity.

955 ***In vitro* efficacy of compound 7g**

956 From the data presented in table 1 it may be inferred that, enantiomeric pair **7g, 7h** and
957 racemic compound **7i** displayed excellent antiplasmodial activity against CQ-R strain.
958 Additionally we have also evaluated the *in vitro* parasite killing efficacy against CQ-S and
959 CQ-R strains. In the case of sensitive parasite, after 12h and 24h time, when compared to CQ,
960 a marginal difference has been observed, whereas in the case of resistant parasite, the lead
961 molecule **7g** rapidly kills the parasite which is a major prerequisite for antimalarial drug like
962 candidate. It has displayed excellent IC_{90} against K1 strain. The results are shown in table 2
963 and figure 6.

964 ***In vivo* antimalarial activity**

965 On the basis of *in vitro* potency and structural diversity, compounds **7a-b, 7g-j, 7o-p, 15a** and
966 **15c** were tested for *in vivo* activity against chloroquine resistant *P. yoelii* (N-67 strain) in
967 Albino mice of Swiss strain. Initially, the *in vivo* activity of selected molecules were
968 determined through oral route at the dose of 100 mg/kg administered once daily for four or
969 seven consecutive days post-infection and monitored for parasitaemia reduction, and survival
970 of mice until day 28 post-infection. Parasitemia reductions for multi-dose regimens are
971 reported in table 3. All compounds were administered as hydrochloride salts whereas only
972 compound **7g** was administered as hydrochloride salt and phosphate salt (16).

973 Compound **7a** showed 100 % parasitaemia suppression on day 4 at a dose of 100 and 50
974 mg/kg for 4 days with survival rate 83 and 66% respectively up to day 28 and none of the
975 animals were cured. While, compound **7b** displayed 100% parasitaemia suppression on day 4
976 at a dose of 100 mg/kg for 4 days, at 50 mg/kg the same compound displayed 99.9 %
977 parasitaemia suppression, all the mice survived up to day 28, but none of them were cured.
978 Enantiomeric pair compounds namely **7g, 7h** and racemic compound **7i** showed 100 %
979 parasitaemia suppression at the dose of 100, 50 and 25 mg/kg, all mice survived as well as
980 cured up to day 28. Whereas, at the dose of 10mg/kg, racemic compound (**7i**) displayed 100%
981 survival and curative effect. Enantiomeric pair (**7g** and **7h**) exhibited 100% survival rate and
982 80% and 60% curative effect respectively. Trifluoro substituted compound (**7j**) showed 100%
983 parasitaemia suppression on day 4 at the dose of 100, 25 mg/kg, all mice survived and cured
984 at the same dose level. However, the same compound at 12.5 mg/kg exhibited 100%
985 parasitaemia suppression on day 4, four out of five mice survived and two out of five mice
986 are cured. These results are encouraging when compared with the standard drug CQ, which

showed 90% curative effect at dose of 100 mg/kg but there was no curative effect observed at dose of 25 mg/kg. Compound **7o** also administered at multiple dose regimen 100 and 50 mg/kg, at these two doses this compound has shown 100% parasitaemia suppression on day 4. At 100mg/kg five out of six mice were survived and four out of six mice were cured, while at 50 mg/kg none of the mice survived up to day 28. While, compound **7p,** exhibited 100% inhibition of parasitemia on day 4 at multiple doses, 100, 50, 25 mg/kg for four consecutive days. Nevertheless, at 100mg/kg, all mice survived and cured, at 50 mg/kg, three out of five mice survived, two mice were cured, and at 25 mg/kg none of the mice survived. Compound **15a,** inhibited 100% parasitaemia on day 4, all mice survived up to day 28 and all mice were cured, while at 50 mg/kg dose, there was 99.9% suppression of parasitaemia, showed 60% survival rate, and none of the mice were cured. Compound **15c** at the dose of 100mg/kg showed 100% parasitaemia suppression on day 4, all mice survived up to day 28 and all mice were cured.

Compound **16,** which is a phosphate salt of compound **7g** when administered orally at 25, 12.5, 10, 6.25 and 5 mg/kg doses showed curative effect at 25.0 and 12.5 and 10.0 mg/kg dose range. At lower doses although there was 100% suppression of parasitaemia but there was no curative effect. The effective curative dose of compound **16** is 10.0 mg/kg. It may be inferred from the above mentioned data that compound **16** is nearly two times more active than its corresponding free base compound **7g** possibly because of the improved GI absorption. This is consistent with the pharmacokinetic data discussed below.

Dose response studies were done for enantiomeric pairs **7g, 7h** and racemic compound **7i** against chloroquine resistant strain *P.yoelii* (N-67), and the results are shown in table 3. There was no difference observed in the *in vivo* activity of (*S*), (*R*) isomers and racemic compounds. Therefore, compound **7g** was chosen for pre-clinical studies, considering the easy availability of L-amino acid and statutory requirement of chirally defined center as against racemate in the drug discovery chain. The results of the dose response studies are shown in table 4.

In order to evaluate broad spectrum of activity of the identified molecule **7g** *in vivo* activity against MDR strain was carried out. Based on the *in vivo* potency against *P. yoelii* (N-67) strain, compounds **7g-7i,** were further selected for *in vivo* antimalarial activity against *P. yoelii* multi drug resistant strain in Albino mice of *Swiss* model (Table 5). Initially compound **7g** which is an (*S*) enantiomer, was screened at multiple doses via oral route in the form of

hydrochloride salt with 100, 50, 25 mg/kg x 7 days respectively. This compound has suppressed 100% parasitaemia on day 4 at all the doses, all mice were survived and cured at 100, 50 mg/kg, while at the dose 25 mg/kg three out of five mice were cured. Similar results were obtained with the R- enantiomer **7h** and the racemic compound **7i.** In addition to this, the broad spectrum of activity of **7g** was evaluated against *P. vinckei* which is very close to human model. The results are shown in tables 5 and 6.

Screening against *Plasmodium cynomolgi*-Rhesus monkey model

Dose response studies with compound **7g** against simian model showed that 10mg/kg x 3 dose regimen is curative against *P. cynomolgi* in monkeys. Four animals treated with initial parasitemia at 8000-15000/mm^3 showed parasite clearance within 48 hours and no recrudescence was recorded during 70 day post –treatment observation period. Treatment at 5 mg/kg x 3 dose in two monkeys showed parasite clearance in 72 hours. While one of the monkeys showed recrudescence on day 13, the other was cured. Chloroquine at 10mg/kg x 3 dose regimen is also curative in this model.

Chromatographic conditions for checking chiral purity of 7g, 7h

The lead molecule **7g** identified in the present study has a chiral center therefore it is important to establish chiral purity before proceeding further with pre-clinical investigations. Towards this objective HPLC method has been successfully developed: HPLC separation of compound **7g** and its entatiomer **7h** was achieved on a Lux 5µ cellulose-1 [250 x 4.60mm, 5µm] chiral column (Phenomenex); at 25±3°C utilizing mobile phase consisting of a mixture of hexane, isopropanol, and methanol (95:4.5:0.5); with addition of triethylamine (TEA) (0.8%) flow rate being 2.0 mL/min with detection wavelength being 254 nm. Injections were given in triplicate for each isomer and the racemate. The HPLC profiles of enantiomers vis-à-vis racemate clearly suggest that compound **7g** and **7h** are chirally pure and there is no cross contamination of the enantiomers (figure 7).

Pharmacokinetic studies

***In vitro* stability studies**

The *in vitro* pharmacokinetics data are shown below in table-7. To evaluate the stability of the compound **7g** in different conditions encountered after oral administration, the *in vitro* simulated gastric fluid (SGF), simulated intestinal fluid (SIF), metabolic and plasma stability

studies were performed. The results of these studies indicate that the candidate molecule is stable in the GI tract before getting absorbed. The compound was found to be more than 97% stable in both acidic (SGF) and basic (SIF) conditions up to 2 hrs. *In vitro* metabolic stability was performed in rat liver microsomes to assess the contribution of liver towards the total clearance of the compound from the body. Testosterone was used as a positive control to assess the activity of the microsomes. The half-life of the testosterone was in agreement with the literature reports. The half-life of **7g** was found to be 154.54 min. The compound was found to have low clearance. The plasma stability of **7g** was found to be 96.13% after 2hrs. (Table 7)

***In vivo* Pharmacokinetics**

The *in vivo* pharmacokinetic studies were performed using plasma as the matrix. This decision was taken after preliminary studies in plasma as well as blood as bioanalytical matrix. The biological levels were comparable and we could study complete pharmacokinetic profile in plasma. The whole blood partitioning (erythrocyte uptake studies), plasma and blood stability studies using blood as matrix originating from healthy as well as infected mice were also performed to check suitability of plasma as matrix (data not given). The other reason for choosing plasma was getting much cleaner and reproducible results for pharmacokinetic studies as we could eliminate interfering matrix. The analytical method used for the analysis was sensitive enough to detect the systemic levels upto 2 days of exposure, which suggests the usefulness of plasma as matrix. The mean plasma concentration-time profile of **7g** is as shown in the figure 8. Upon oral administration, it got rapidly absorbed and showed double peak phenomenon in their plasma concentration time profiles (figure 8). The peak plasma concentrations of the **7g** are 20.36±13.92 and 22.3±9.18 ng/mL at 0.44±0.38 and 3.67±0.58 h respectively. This behaviour may be due to solubility constraints of the compounds or absorption from multiples sites and enterohepatic re-circulation. The oral bioavailability (%F) of **7g** is found to be 25.30%. The discrepancy in the bioavailability data could be because of the poor solubility of **7g**. Therefore it was considered appropriate to prepare the phosphate salt of **7g** (**16**) and as expected the pharmacokinetic parameters were significantly improved when compared to the free base (**7g**). The mean plasma concentration-time profile of **16** is shown in the figure 8. Upon oral administration, the salt form rapidly got absorbed and showed double peak phenomenon in their plasma concentration time profiles (figure 8) similar to its free base. In the case of salt form (**16**) the peak plasma concentrations are 104.9±5.46 and 47.5±1.83 at 0.25 and 4 h respectively, indicating improved absorption

from the intestine. The oral bioavailability (%F) of **7g** is found to be 64.47% when the salt form **(16)** was administered. This indicates the salt form has better pharmacokinetic properties than the free base.

The pharmacological safety, toxicity, detailed pharmacokinetics (including multiple dose pharmacokinetics, tissue distribution and excretion study) and toxicokinetic studies are in progress. *In vitro* hERG assay results indicate that the compound 16 (phosphate salt of compound 7g) does not bind to hERG ion channel up to 10 µM. However, we observed the binding at the highest tested concentration i.e. 33 µM. E-4031, a known hERG ligand was used as a positive control in the assay method. These studies are part of our next manuscript which addresses mainly regulatory considerations and IND enabling endeavour.

Molecular docking studies

The ability of the compound **7g** to form a complex with Fe(III)FPIX was investigated by molecular modelling studies. A database was prepared by using SYBYL-X 1.3 (Tripos Inc, St. Louis, MO, USA) modeling package. Compound **7g** were drawn through sketch module in Sybyl. Structures were minimized further by adding Gasteiger-Huckel charges along with distance-dependent dielectric and the Powell conjugate gradient algorithms with a convergence criterion of 0.001kcal/mol. All structures were put into a database and finally aligned with each other by way of 'Fit Atom' method. The obtained docking values are reported in table 8. As shown in the figure 9, chloroquine binds with the carboxylic acid of porphyrin ring via single hydrogen bonding with C score value 4, whereas, compound **7g** interacts with another free carboxylic group of heme with the C score value 5. The docking results complement those of biophysical data confirming that mechanism of antiplasmodial activity is through heme binding.

***In silico* properties of compound 7g**

In order to evaluate drug-likeness of the compound we have used Schrodinger/QikProp/ software, and it is apparent from the data mentioned in table 9 that the selected compound doesn't violate the limitations. Molecular weight, solvent accessible surface area, rotatable bonds, H-bond donors and acceptors are within the range. More importantly it doesn't violate the lipinski rule of 5(not more than 5 hydrogen bond donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds), not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms), a molecular mass less than 500 daltons, an octanol-water partition

coefficient log *P* not greater than 5). Compound **7g** comply with all parameters (Table 9). The *in silico* evaluation confirmed that the compound **7g** had "drug-like" properties.

CONCLUSION

In conclusion, we have synthesized a novel series of chirally pure 4-aminoquinoline derivatives by using amino acids as building blocks for the side chain modifications. These analogs have displayed excellent *in vitro* as well as *in vivo* antimalarial activities against *P. falciparum* with oral efficacy in a *P. yoelli* mouse model against chloroquine resistant parasites. Based on the detailed anti-parasitic tests, speed of parasitic reduction upon administration of the compound (*in vitro*) and propensity of the parasites to recrudesce following administration (measured over 28 days) and finally efficacy validation of the compound in *P. falciparum* simian monkey model compound **16** has been identified as pre-clinical candidate molecule. Furthermore, *in vitro*, and *in vivo* ADME assays carried out on compound **16** have shown that compound **16** has excellent physicochemical properties, acceptable pharmacokinetic profile and moderate metabolic clearance in rat liver microsomes. Overall, compound **16** has "drug-like" properties. These results are in consonance with the *in silico* ADME predictions and MMV's criteria for selection of antimalarial compound. Currently the compound is under toxicological and regulatory pharmacological evaluations.

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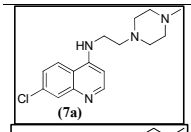
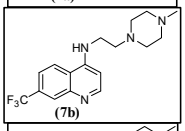
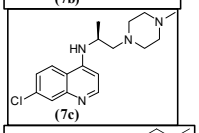
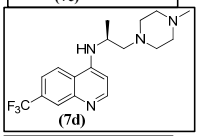
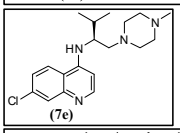
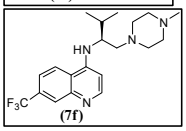
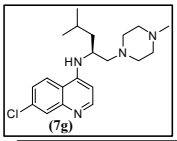
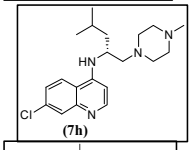
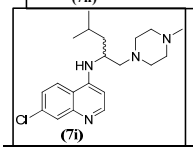
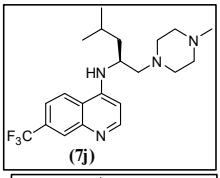
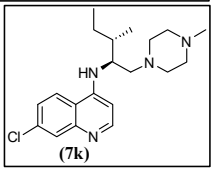
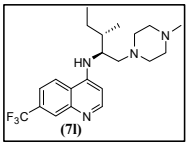
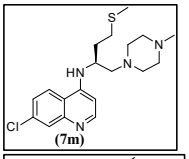
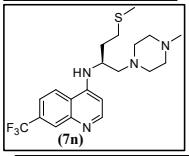
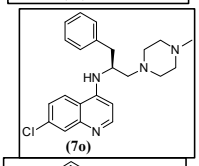
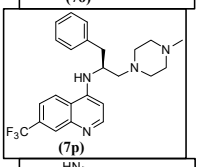
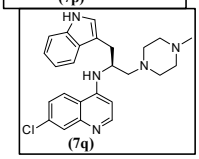
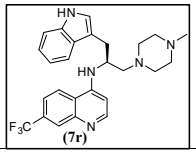
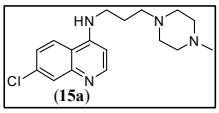
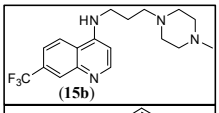
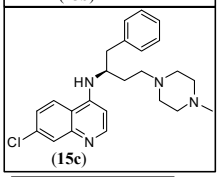
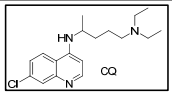
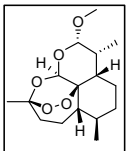
1236

1237 **Tables**

1238 **Table 1 Biological and biophysical data of the synthesized compounds**

Compound No Structure	IC_{50}(nM)[a] 3D7	IC_{50}(nM)[a] K1	SI[b]	Log K[c]	IC_{50}[d]
 (7a)	3.27	65.75	37,281	6.02±0.02	0.15±0.03
 (7b)	25.1	26.11	4013	4.96±0.04	0.22±0.01
 (7c)	16.88	122.7	5565	5.26±0.02	0.16±0.3
 (7d)	92.15	585.85	2075	4.87±0.02	0.19±0.02
 (7e)	15.18	167.4	8538	5.06±0.11	0.17±0.03
 (7f)	700	>1000	164.95	4.93±0.1	0.28±0.02
 (7g)	8.8	13.57	22,727	5.29±0.02	0.15±0.04
 (7h)	10.9	11.16	3769	5.43±0.11	0.17±0.03
 (7i)	8.36	11.79	1941	5.73±0.04	0.14±0.11

 (7j)	11.36	12.13	2820	4.63±0.02	0.15±0.13
 (7k)	14.69	84.18	7026	5.66±0.1	0.16±0.04
 (7l)	627	>1000	83.20	4.23±0.2	0.27±0.1
 (7m)	516	>1000	282	6.37±0.3	0.23±0.03
 (7n)	723	>1000	238	4.63±0.01	0.27±0.03
 (7o)	10.27	39.39	2169	5.70±0.02	0.16±0.03
 (7p)	5.3	11.42	8024	4.89±0.12	0.14±0.03
 (7q)	16.88	122.7	971	5.89±0.2	0.17±0.03
 (7r)	88.86	259.26	233	4.46±0.23	0.18±0.06

 (15a)	7.87	9.79	4922	5.62±0.02	0.15±0.06
 (15b)	81.22	548.63	2226	4.93±0.05	0.24±0.06
 (15c)	5.03	11.52	9852	5.32±0.03	0.14±0.05
 CQ	5.46	255±65	8983	5.52±0.02	0.17±0.22
 Artemether	1.37	1.32±0.11	N.D	N.D	N.D

[a] IC_{50} (nM) : Minimum concentration of compound inducing 50% parasitic cells.

[b] Selectivity index (SI): (IC_{50} for cytotoxicity to vero cells /IC_{50} for antimalarial activity).

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

[d]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).

N.D stands for not done

1250

1251 **Table 2. *In vitro* efficacy of compound 7g**

Compound	3D7 (nM)	K1 (nM)
7g	IC_{50} = 6.7-11.1	11.8 - 20.4
	IC_{90} =23.4-52.9	24.3 - 47.8
	CC_{50} = > 200µM	
CQ	IC_{50} = 3.9 - 7.0	220 – 279
	IC_{90} = 11.8-22.3	477 – 675
	CC_{50} = 125 µM	

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1253

1254 **Table 3. *In vivo* antimalarial activity against CQ resistant (N-67) in Albino mice of Swiss**

1255 **model.**

Cmpd No	Dose (p.o.)	% of suppression on day 4	Survival[a]	Cure[b]
7a	100 mg/kg x 7 days	100	5/6	0/6
	50 mg/kg x 7 days	100	4/6	0/6
7b	100 mg/kg x 7 days	100	5/5	0/5
	50 mg/kg x 4 days	99.9	5/5	0/5
7g	100 mg/kg x 7 days	100	5/5	5/5
	50 mg/kg x 7 days	100	5/5	5/5
	25 mg/kg x 7 days	100	5/5	5/5
	25 mg/kg x 4 days	100	5/5	5/5
	10 mg/kg x 4 days	100	5/5	4/5

	100 mg/kg x 7 days	100	5/5	5/5
7h	50 mg/kg x 7 days	100	5/5	5/5
	25 mg/kg x 7 days	100	5/5	5/5
	10 mg/kg x 4 days	100	5/5	3/5
	50 mg/kg x 7 days	100	5/5	5/5
7i	25 mg/kg x 7 days	100	5/5	5/5
	10 mg/kg x 4 days	100	5/5	5/5
	100 mg/kg x 4 days	100	5/5	5/5
7j	25 mg/kg x 4 days	100	5/5	5/5
	12.5 mg/kg x 4 days	100	4/5	2/5
	100 mg/kg x 7 days	100	5/6	4/6
7o	50 mg/kg x 7 days	100	0/5	0/5
	100 mg/kg x 4 days	100	5/5	5/5
7p	50 mg/kg x 4 days	100	3/5	2/5
	25 mg/kg x 4 days	100	0/5	0/5
	100 mg/kg x 7 days	100	5/5	5/5
15a	50 mg/kg x 7 days	99.9	3/5	0/5
15c	100 mg/kg x 7 days	100	5/5	5/5
	25 mg/kg x 4 days	100	5/5	5/5
	10 mg/kg x 4 days	100	8/8	8/8
16	12.5 mg/kg x 4 days	100	5/5	5/5
	6.25 mg/kg x 4 days	100	5/5	2/5

	5 mg/kg x 4 days	100	6/8	0/8
CQ	20 mg/kg 4 days	99.0	5/5	0/5
Arteether	5 mg/kg x 4 days (i.m.)*	100	5/5	5/5

[a] *Number of mice that survived till day 28 post-infection/total mice in the group.*

[b] *Number of mice without parasitaemia (cured) till day 28 post-infection.*

* *Route of administration Intramuscular.*

Table 4. Dose response studies of enantiomeric pair (7g, 7h) and racemic compound (7i) against *P.yoelii* (N-67); chloroquine resistant strain

Compound	Dose x 4 days (oral route)	Day 4 supression	No. of cured/ No. of treated
7g	100 mg/kg x 4 days	100	5/5
	50 mg/kg x 4 days	100	5/5
(*S*) isomer	25 mg/kg x 4 days	100	5/5
	10 mg/kg x 4 days	100	4/5
7h	100 mg/kg x 4 days	100	5/5
	50 mg/kg x 4 days	100	5/5
(*R*) isomer	25 mg/kg x 4 days	100	5/5
	10 mg/kg x 4 days	100	3/5
7i	100 mg/kg x 4 days	100	5/5
	50 mg/kg x 4 days	100	5/5
(Racemic)	25 mg/kg x 4 days	100	5/5
	10 mg/kg x 4 days	100	5/5

Antimicrobial Agents and Chemotherapy

Table 5. *In vivo* antimalarial activity *P.yoelii* MDR strain in Albino mice of Swiss model.

Cmpd No	Dose	% of suppression on day 4	Survival[a]	Cured[b]
7g	100 mg/kg x 7 days	100	5/5	5/5
	50 mg/kg x 7 days	100	5/5	5/5
	25 mg/kg x 7 days	100	3/5	3/5
	25 mg/kg x 4 days	99.9	0/5	0/5
7h	50 mg/kg x 7 days	100	5/5	5/5
7i	50 mg/kg x 7 days	100	3/5	3/5
CQ	20 mg/kg x 7 days	99.3	3/5	0/5

[a] *Number of mice that survived till day 28 postinfection/total mice in the group.*

[b] *Number of mice without parasitaemia (cured) till day 28 postinfection.*

Table 6. *In vivo* antimalarial activity against different rodent malaria models (compound 7g)

Compound 7g (*S*) isomer		
Parasite	**Drug Sensitivity**	**Total Curative Dose (mg/kg)**
P. yoelii	CQ resistant	25 x 4 days
P. yoelii	MDR	50 x 4 days
P. vinckei	CQ resistant	25 x 4 days

1275 **Table.7 *In vitro* pharmacokinetic parameters of 7g. (N=3)**

Parameters	Compound 7g
Simulated gastric fluid stability (% remaining, 2hr)	96.03±2.29%
Simulated intestinal fluid stability (% remaining, 2hr)	97.72±1.64%
Plasma stability (% remaining, 2hr)	96.14±3.15%
Metabolic stability, Half Life ($t_{1/2}$, min)	154.54±15.67

1276

1277 **Table 8**. Docking scores of compound **7g** and **CQ**

Cmpd	Total score	Crash score	polar	D score	PMF score	G score	Chem score	C score	Global score
7g	1.31	-0.87	1.36	161.25	-35.35	-87.17	-19.64	5	5
CQ	1.78	-0.79	1.47	130.29	-19.22	-70.26	-20.59	4	3

1278

1279 **Table 9.**Schrodinger/QikProp/ predictions **(CQ, AQ-13 and compound 7g)**

Principal Descriptors	**Chloroquine**	**AQ-13**	**Cmpd 7g**	**Range of 95% of Drugs**	
				Min	**Max**
Molecular Weight	319.876	291.823	360.929	130	725
Total SASA	649.001	603.348	667.701	300	1000
No. of rotatable bonds	8	7	6	0	15
H-bond donors	1	1	1	0	6
H-bond acceptors	4	4	6	2	20
QP log P for octanol/water	4.508	3.674	3.578	-2	6.5
QP log K hsa Serum Protein Binding	0.604	0.341	0.469	-1.5	1.5

Lipinski Rule of 5 Violations	0	0	0	(maximum is 4)
% Human Oral Absorption in GI (+-20%)	100	100	93	(<25% is poor)
Qual. Model for Human Oral Absorption	High	High	High	(>80% is high)

Figures

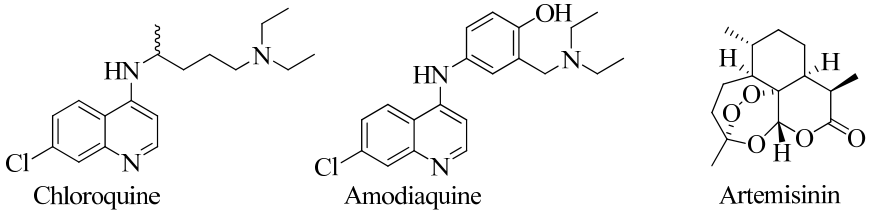


Figure 1. Antimalarial drugs

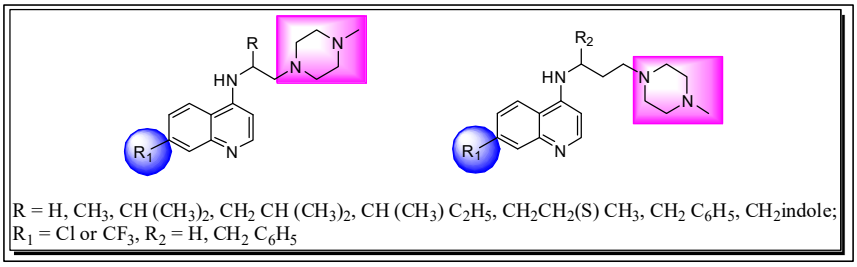
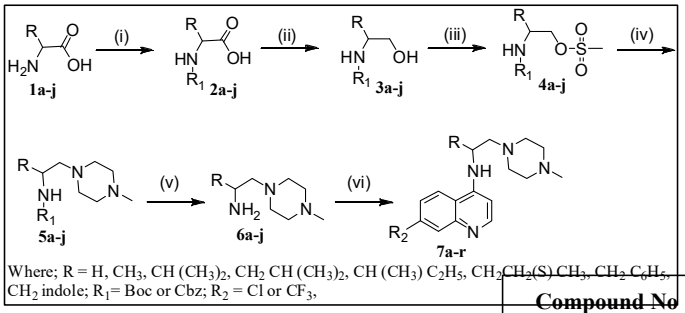


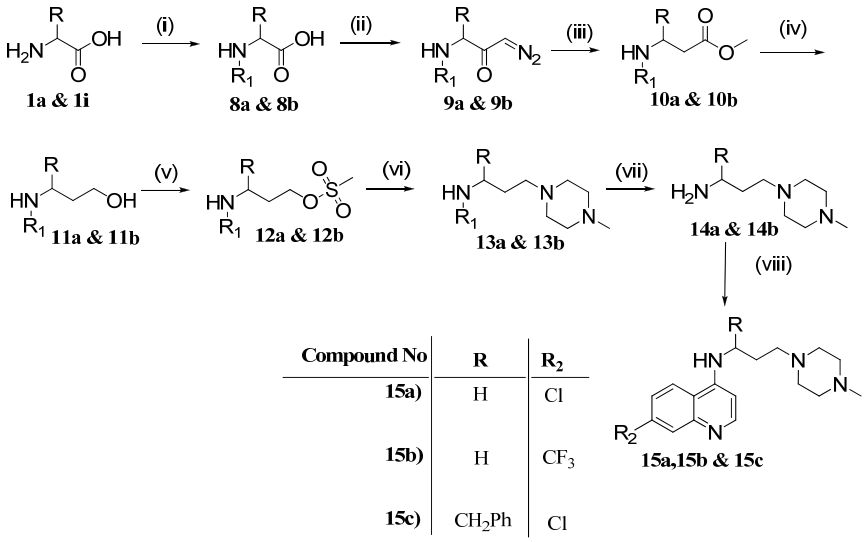
Figure 2- *General structure of compounds synthesized.*



Where; R = H, CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)C_2H_5$, $CH_2CH_2(S)CH_3$, $CH_2C_6H_5$, CH_2 indole; R_1= Boc or Cbz; R_2 = Cl or CF_3,

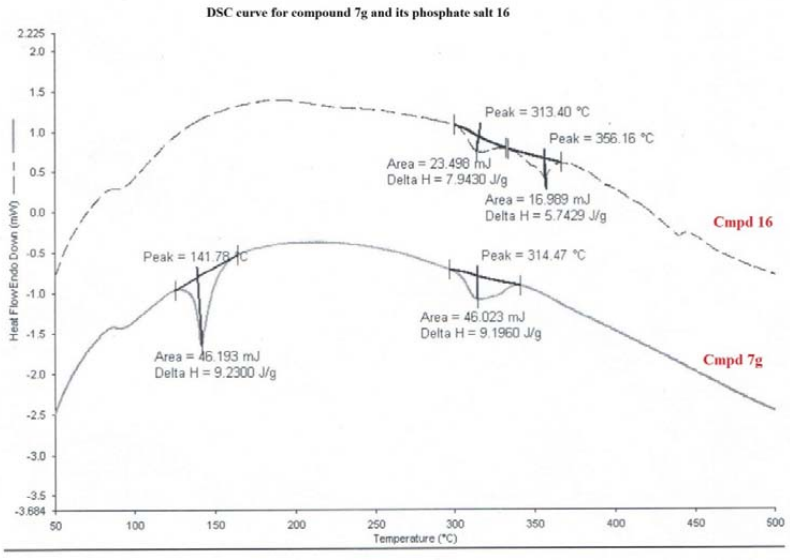
Compound No	R	R_2
7a)	H	Cl
7b)	H	CF_3
7c)	CH_3	Cl
7d)	CH_3	CF_3
7e)	$CH(CH_3)_2$	Cl
7f)	$CH(CH_3)_2$	CF_3
7g)	$CH_2CH(CH_3)_2$	Cl
7h)	$CH_2CH(CH_3)_2$	Cl
7i)	$CH_2CH(CH_3)_2$	Cl
7j)	$CH_2CH(CH_3)_2$	CF_3
7k)	$CH(CH_3)C_2H_5$	Cl
7l)	$CH(CH_3)C_2H_5$	CF_3
7m)	$CH_2CH_2SCH_3$	Cl
7n)	$CH_2CH_2SCH_3$	CF_3
7o)	CH_2PH	Cl
7p)	CH_2PH	CF_3
7q)	CH_2-indolyl	Cl
7r)	CH_2-indolyl	CF_3

Figure 3. (Scheme 1) Synthesis of compounds (7a-r); Reagents and Conditions: (i) $(Boc)_2O$, NaOH/Dioxane, 0°C, 2-3 h, and/or Benzylchloroformate, NaOH, 0°C 2-3 h; (ii) NMM, IBCF, $NaBH_4$, Dry THF, -15°C, 1h;(iii) Triethyl amine, Methane sulphonyl chloride, THF, 45 min;(iv) N-Methylpiperazine, acetonitrile, N_2, 48 h;(v) 20% HCl/dioxane and/or H_2/Pd, Methanol, 1h;(vi) 4,7-dichloroquinoline, and/or 4-chloro-7-(trifluoromethyl)quinoline amines (**6a–j**), Phenol, 140-155°C, 4-6 h.



Compound No	R	R_2
15a)	H	Cl
15b)	H	CF_3
15c)	CH_2Ph	Cl

Figure 4. (Scheme 2) Synthesis of compounds (15a-c); Reagents and Conditions: (i) Benzylchloroformate, NaOH, 0°C 2-3 h; (ii) NMM, IBCF, CH_2N_2, Dry THF, -15°C, 1h; (iii) Silver benzoate, Methanol, 70°C 1h; (iv) Methanol, $NaBH_4$, THF, 55-60°C, 45 min; (v) Triethylamine, Methanesulphonylchloride,THF,45 min; (vi) *N*-Methylpiperazine, Acetonitrile, N_2, 2days; (vii) H_2/Pd/C, Methanol, 1h;(vii) 4,7-dichloroquinoline, and/or 4-chloro-7-(trifluoromethyl)quinoline, amines (**14a–14b**), Phenol, 140-155°C, 4-6 h.



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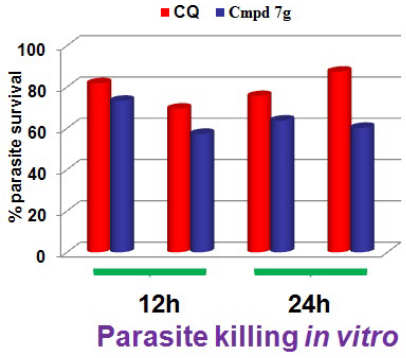
1335 **Figure5.** DSC curve for compounds **7g** and its phosphate salt **16**



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1337 **Figure6.** In vitro efficacy of compound **7g** compared to **CQ**

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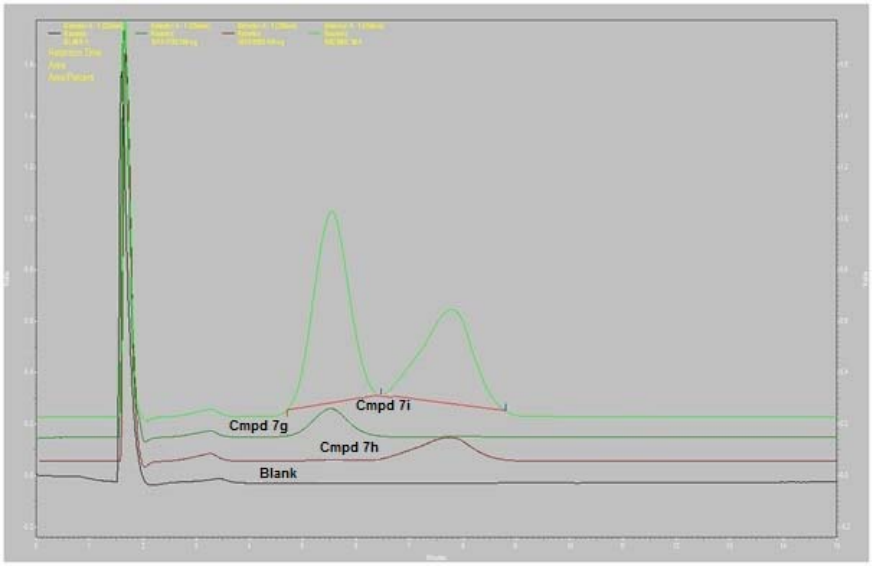


Figure 7.HPLC spectrum of enantiomers **7g, 7h** and racemic **7i** compounds.

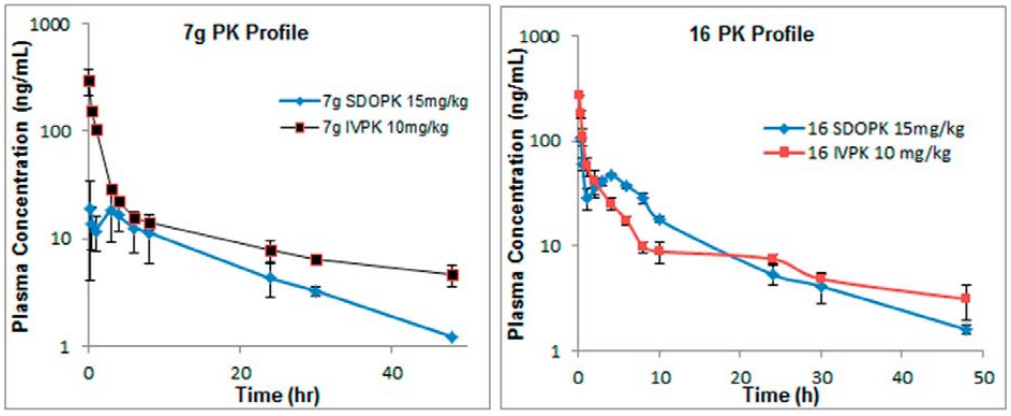
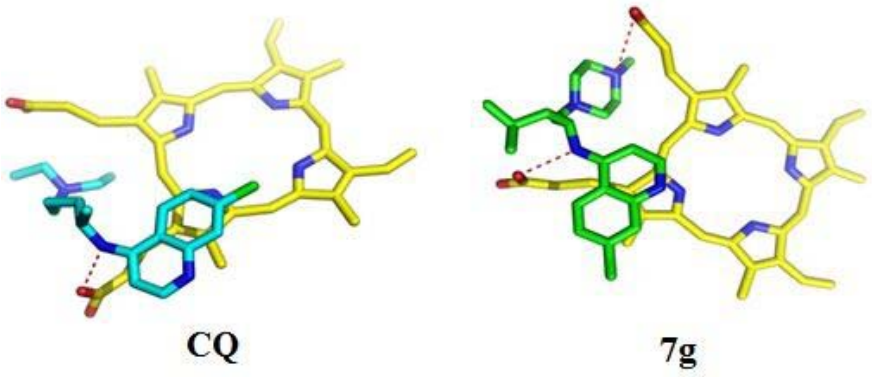


Figure 8. Intravenous (10 mg/kg) and oral (15 mg/kg) pharmacokinetic profiles of 7g and 16 in male SD rats. (n=4)



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1346 **Figure 9**. *Molecular docking studies with heme (compounds **CQ** and **7g**)*