IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2016 Vol.:7, Issue:4

Design, Synthesis and Evaluation of Hybrids Based on Quinoline and Acridine Scaffolds as Antimalarial Agents

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Submission: 5 November 2016
Accepted: 10 November 2016
Published: 25 November 2016

Keywords: PfDHFR-TS, quinoline-acridine hybrids, docking, antimalarials

ABSTRACT

Plasmodium falciparum dihydrofolate reductase is an important target for antimalarial drugs. Despite the development of resistance against DHFR inhibitor drugs, there is still significant potential for designing new chemical entity with affordable, safe and efficacious antimalarials. A strategy that has attracted considerable attention in current medicinal chemistry is based on the conjugation of two biologically active molecules into one hybrid compound. In present study quinoline-acridine hybrids were designed and interaction of these conjugate hybrids was investigated by docking studies in the binding site of PfDHFR-TS (1J3I) enzyme using Glide v 5.6. Among the series of designed compounds five compounds with good potential were synthesized. Structural confirmation of these compounds was done by FT-IR, ^{1}H-NMR and Mass spectroscopy. The compounds were evaluated for *in vitro* antimalarial activity against resistant strain of *plasmodium falciparum* by microdilution technique. The activity of compound K3 was found to be comparable with chloroquine and better than the quinine. The above study could be very useful for further design and development of new antimalarials.

1. INTRODUCTION

Malaria is one of the most widespread disease in the world, caused by parasites of the *Plasmodium* family and transmitted by female *Anopheles* mosquitoes. According to World Health Organization (WHO) globally approx. 3.3 billion people are at risk of being infected with malaria and 1.2 billion are at high risk.[1]

Despite continuous research efforts, malaria continuous to exert the tremendous burden on the health due to development of resistance to currently available antimalarial like 4-aminoquinolines and antifolates. Therefore, there is urgent need to develop new affordable, safe, and efficacious antimalarials. Among the available ways to optimize the research for new therapeutic agents, various techniques are being adopted: for example, the use of hybrid compounds is considered an extension of the concept of combination therapy, where the coupling of two pharmacophoric groups, often covalently joined, is observed, creating a single chemical entity capable of modulating multiple targets. The use of hybrid drugs is an interesting way to discover new drugs, making it possible to circumvent the resistance of parasites, a phenomenon that appears to be composition-specific and not related to the changes in the action of the drug target. With these thoughts, various research groups have synthesized a large number of hybrid molecules by combination of chloroquine with different pharmacophores acting on different targets. The most common antimalarial agents from these studies include hybrid based on chloroquine and thiazolidinone scaffolds,[2] keto-enamine chalcone-chloroquine hybrids,[3] 4-aminoquinoline-pyrimidine hybrids.[4] Some of these hybrids have shown promising *in vitro* and *in vivo* antimalarial activity against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum.*

To overcome the resistance, the bulky bisquinolines were designed stating the hypothesis that steric hindrance would not allow drug efflux by the proteinaceous transporter. Though bisquinolines such as Ro 47-7737 and other piperaquine, hydroxypiperaquine and dichloroquinazine exhibited promising antimalarial efficacy but toxic liabilities ruled out their development as drug candidate. In addition, bis acridine derivatives with di-, tri-, and tetramine linker have been investigated for their effect on the antimalarial activity. Encouraged by these results and research towards the synthesis of novel antimalarial agents we have designed a new series of 4-aminoquinoline-acridine hybrids by using o-phenylenediamine, m-phenylenediamine and p-phenylenediamine as linkers so as to develop structurally diverse series of compounds in order to gain structural insight for improved

antimalarial activity. Thus in the present study, we report the synthesis and antimalarial activity of a new series of 4-aminoquinoline-acridine hybrids. Also, we studied the interaction of these hybrids in the binding site of *P. falciparum* dihydrofolate reductase (PfDHFR) using Glide docking studies.

2. MATERIALS AND METHODS

2.1 Computational studies

2.1.1 Docking validation

The most eloquent method to check the accuracy of docking method is to determine the closeness between the lowest energy conformer and scoring function. Glide score simulate an experimental binding mode as deciphered by X-ray crystallography. Assurance of docking process was done by analyzing the RMSD value, it is used to indicate whether correct docking pose was obtained by Glide or not. Normally RMSD of 2Å and higher precision analysis is not meaning full. Docking was done by using protein PfDHFR-TS using PDB ID IJ3I. The RMSD values among docked pose and its bound conformation for IJ3I are in range of 0.003 to 0.043, which indicates that docking was performed well for PfDHFR. After this validation, all of the fifteen PFDHFR inhibitors were docked in the binding pocket of X-ray crystallographic structure of IJ3I.

2.1.2 Ligand preparation

ChemDraw Ultra 12.0 was used to draw 2D molecular structures of designed hybrids. All these 2D structures were converted into 3D with help of Chem 3D ultra-version 8.0.3. These 3D structures were introduced into Maestro implemented in Schrödinger; energy minimization of 3D structures was done by using Ligprep v 2.4 program. Different ionization states were generated at user defined pH. ConfGen was used to generate various conformers for each ligand and minimization was done by using Impref module of Schrödinger suit by using OPLS-2005 force field to correct its bond length and bond order.

2.1.3 Protein preparation

The three-dimensional crystal structure of PfDHFR (PDB ID IJ3I) was downloaded from RCSB Protein Data Bank and prepared by protein preparation wizard. Preparation and refinement are two components of protein preparation wizards. After confirmation of

chemical accuracy, addition of hydrogens and side chain neutralization was done by using force field OPLS-2005. Only those side chains were neutralized that neither participate in salt bridge formation nor were present in contact with binding cavity. Minimization was performed until the average root mean square deviation of the non-hydrogen atoms reached 0.3 Å. In final step flip no flip model of prepared protein was obtained and this was used for grid generation.

2.1.4 Receptor Grid Generation

Receptor grid generation starts by picking and selecting co-crystallized ligand from the active site of prepared protein. Finally, the grid was generated to define the active site of protein which was visualized in form of box at the point of work space. The complete process was run by default settings. This grid file was further used to perform docking.

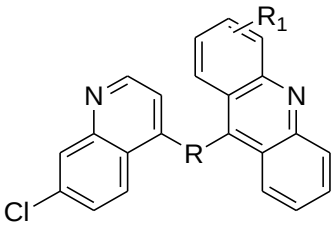
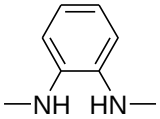
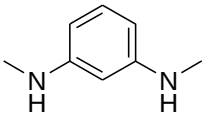
2.1.5 Docking studies

Ligand docking was done by using Glide, v 5.6. The prepared ligands and the file obtained from receptor grid generation panel were selected and all the designed hybrids of 4-aminoquinoline and acridine derivatives were docked within the binding site of PfDHFR (PDB ID IJ3I). Flexible docking was done by employing Extra Precision (XP) mode of Glide. Glide score of compounds was obtained and various interaction of ligand with protein was studied. The final energy evaluation was done with the GlideScore and a single best pose was generated as output for a particular ligand with the help of following equation.

$$GScore = a*vdW+ b*Coul + Lipo + H\ bond + Metal + Bury\ P+ RotB + Site$$

Where vdW = Vander Waal energy, Coul = Coulomb energy, Lipo = Lipophilic contact term, HBond = Hydrogen-bonding term, Metal = Metal-binding term, BuryP = Penalty for buried polar group, RotB = Penalty for freezing rotable bonds, Site = Polar interaction at active site, and the coefficient of vdW and Coul are a = 0.065, b = 0.0130. The best pose for a given ligand was determined by the Emodel score, while different compounds were ranked using Glide score.

Table 1: Structures of designed compounds along with compound code, linkers, substituents and molecular weight

General structure of hybrids	R	Compound code
	 o-phenylenediamine	K1,K4,K7,K10,K13
	 m-phenylenediamine	K2,K5,K8,K11,K14
	 p-phenylenediamine	K3,K6,K9,K12,K15

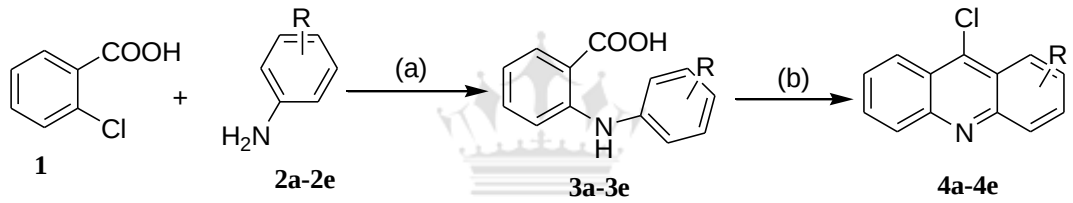
S.No	Compound code	R_1	Molecular weight	Molecular formula
1	K1	-H	446.13	$C_{28}H_{19}ClN_4$
2	K2	-H	446.13	$C_{28}H_{19}ClN_4$
3	K3	-H	446.13	$C_{28}H_{19}ClN_4$
4	K4	$-3NO_2$	491.11	$C_{28}H_{18}ClN_5O_2$
5	K5	$-3NO_2$	491.11	$C_{28}H_{18}ClN_5O_2$
6	K6	$-3NO_2$	491.11	$C_{28}H_{18}ClN_5O_2$
7	K7	$-2CH_3$, $-4\ CH_3$	474.16	$C_{30}H_{23}ClN_4$
8	K8	$-2CH_3$, $-4\ CH_3$	474.16	$C_{30}H_{23}ClN_4$
9	K9	$-2CH_3$, $-4\ CH_3$	474.16	$C_{30}H_{23}ClN_4$
10	K10	$-2OCH_3$	476.14	$C_{29}H_{21}ClN_4O$
11	K11	$-2OCH_3$	476.14	$C_{29}H_{21}ClN_4O$
12	K12	$-2OCH_3$	476.14	$C_{29}H_{21}ClN_4O$
13	K13	$-4OCH_3$	476.14	$C_{29}H_{21}ClN_4O$
14	K14	$-4OCH_3$	476.14	$C_{29}H_{21}ClN_4O$
15	K15	$-4OCH_3$	476.14	$C_{29}H_{21}ClN_4O$

2.2 Synthesis

Chemistry

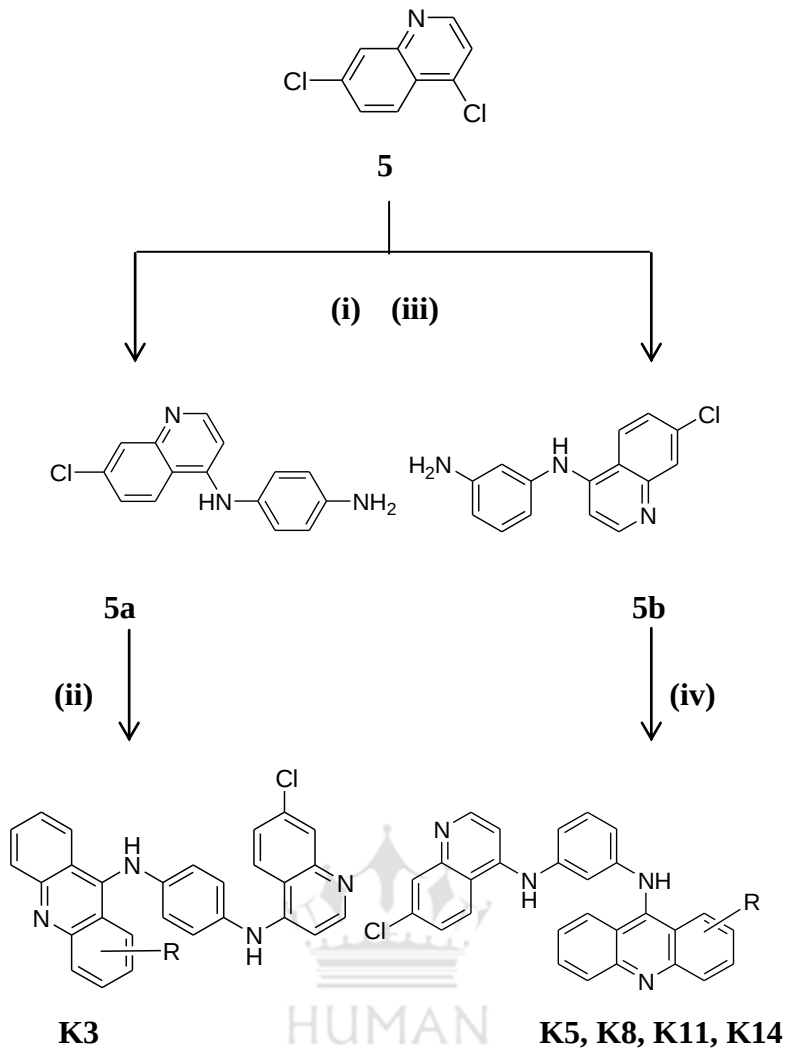
9-chloro substituted acridine derivatives were synthesized by reaction of o-chlorobenzoic acid with (substituted/unsubstituted) aniline in the presence of copper (acts as catalyst), isoamyl alcohol as a solvent in presence of dry potassium carbonate to give intermediate product named as N-phenyl anthranilic acid derivative. Cyclization of intermediate product with freshly distilled phosphorus oxychloride gives 9-chloroacridine derivatives **(Scheme 1)**.

4,7-dichloroquinoline was coupled with m-phenylenediamine and p-phenylenediamine by using catalyst p-TSA in ethanol gives the N^1-(7-chloroquinolin-4-yl)-benzene-1,4-diamine **(5a)** and N^1-(7-chloroquinolin-4-yl)-benzene-1,3-diamine **(5b)** respectively. The nucleophilic substitution of above intermediates was done with 9-chloroacridine derivatives in ethanol using p-TSA as catalyst to yield quinoline-acridine hybrids as depicted in scheme 1.



R= 2a= -H; 2b= -3NO_2; 2c= -2,4 dimethyl; 2d= -o methyl; 2e= -p methyl

Scheme 1: Synthesis of Acridine derivatives: (**a**) Cu, K_2CO_3, Heat, 8 hours (**b**) reflux, $POCl_3$, 4 hours.



Scheme 2: Synthesis of Quinoline-Acridine hybrids K3 and K5, K8, K11, K14: (**5**) 4, 7-dichloroquinoline, **(i)** ethanol, p-phenylenediamine, p-TSA, 3 hours, **(ii)** ethanol, p-TSA, 4 hours, 4b - 4e **(iii)** ethanol, m-phenylenediamine, p-TSA, 3 hours, **(iv)** ethanol, p-TSA, 4 hours and 4a.

A. Synthesis of *N*-phenyl anthranilic acid derivatives (3a-3e)

A mixture of *o*-chlorobenzoic acid (0.038 moles), aniline derivatives (0.038 moles) and copper powder (0.12 g) in 40 ml isoamyl alcohol was taken in round bottom flask. Then 6 g of dry potassium carbonate was gradually added to it and the mixture was refluxed for 7-8 hours. The excess amount of isoamyl alcohol was separated by distillation process, remaining mixture was poured into 500 ml of hot water and then it was filtered. To obtain crude precipitate acidification of filtrate was done by adding concentrated hydrochloric acid. Precipitates were filtered, washed with hot water and collected. Then these precipitates were dissolved in aqueous NaOH solution. The solution was boiled in the presence of charcoal and

filtered. Precipitates were obtained by acidifying filtrate with concentrated hydrochloric acid, and then it was filtered and washed with hot water. The final crude product was collected and recrystallized from methanol.

B. General procedure for synthesis of 9-chloroacridine derivatives (4a-4e)

N-phenyl anthranilic acid derivative (0.023 moles) and phosphorous oxychloride (0.176) were mixed in a 500 ml round bottom flask fitted with a condenser. The mixture was placed in water bath and heated for 15 minutes at 85-90^{o}C. After 15 minutes, the round bottom flask was heated on heating mantle for 3 hours at 140-150^{o}C. After cooling residue was mixed in mixture of 20 ml of chloroform, 20 ml concentrated ammonia solution and 50 g of ice. The flask was rinsed with 20-30 ml of chloroform-ammonia mixture. When no more solid remains, the layer containing chloroform was removed. The chloroform layer containing crude product was dried on 1 g of calcium chloride, filtered and the solvent was removed by evaporation. The crude product was dried at 70-80^{o}C for 10 minutes.

C. General procedure for synthesis of 4-aminoquinoline (5a and 5b)

A solution of 4,7-dichloroquinoline (1.0 equiv.) and p-phenylenediamine or m-phenylenediamine in absolute ethanol was refluxed in presence of p-TSA as a catalyst for 3 hours. During the reflux precipitation of compound occurred, precipitates were collected, washed with ethanol and dried to get desired 4-anilino derivatives named as N^1-(7-chloroquinolin-4-yl)-benzene-1,3-diamine **(5a)** and N^1-(7-chloroquinolin-4-yl)-benzene-1,4-diamine **(5b)** respectively.

D. General procedure for synthesis of quinoline-acridine hybrids (K3, K5, K8, K11, and K14)

4-aminoquinolines i.e., N^1-(7-chloroquinolin-4-yl)-benzene-1,2-diamine and N^1-(7-chloroquinolin-4-yl)-benzene-1,4-diamine were further subjected to nucleophilic substitution with 9-chloro-3-nitroacridine / 9-chloro-2, 4-dimethylacridine / 9-chloro-2-methoxyacridine / 9-chloro-4-methoxyacridine / 9-chloroacridine (equivalent moles) and refluxed for 3 hours in absolute ethanol using catalyst p-TSA to form hybrids K3, K5, K8, K11 and K14.

Thin layer chromatography was used to monitor the progress of the reactions. Infrared spectra were recorded on SHIMADZU FT/IR spectrophotometer using KBr pellets at S.G.S.I.T.S,

Indore, and values were expressed in cm^{-1}.^{1}HNMR spectra were recorded using a Bruker ADVANCE II 400 NMR spectrophotometer at IIT Delhi and values were reported in ppm downfield from TMS (Tetramethylsilane) as an internal standard. The NMR spectra were obtained in DMSO. The molecular mass of synthesized hybrid was determined by mass spectroscopy. Mass spectra were recorded using CIF Mass Facility IISER Bhopal and results were reported in terms of their m/z values. Melting points were determined by open capillary method.

N^1-(acridin-9-yl)-N^4-(7-chloroquinolin-4-yl) benzene-1, 4-diamine (K3):

Yellow ; Yield 72%; m.p. 252-258^{o}C; IR (KBr, cm^{-1}): 3200.04 (N-H, stretch), 3065.02 (C-H, stretch, aromatic), 1592.31 (C=N), 1525.76 (N-H, bend), 1445.71 (C=C, aromatic), 1217.14 (C-N), 819.78 (p-substitution), 684.76 (C-H, bend, aromatic), 566.13 (C-Cl); ^{1}H-NMR (DMSO): δ 8.616 (1H, d, quinoline), 8.06 (2H, d, acridine), 7.931 (1H, s, quinoline), 7.895 (1H, d, quinoline), 7.691 (2H, d, acridine), 7.506 (2H, m, acridine), 7.480 (2H, m, acridine), 7.125(1H, d, quinoline), 6.938 (1H, d, quinoline), 6.895 (4H, d, benzene), 3.442 (2H, s, H-N, aromatic); the molecular ion peak was not observed but m/z at 268.9 (M-$C_9H_6ClN_2$) and 131.1 (M-C_9H_7N) confirmed the structure of compound.

N^1-(7-chloroquinolin-4-yl)-N^3-(3-nitroacridin-9-yl) benzene-1, 3-diamine (K5):

Brownish black; Yield 75%; m.p. 250-256^{o}C; IR (KBr, cm^{-1}): 2985.94 (N-H, stretch), 2947.36 (C-H, stretch, aromatic), 1611 (C=N), 1582.66 (N-H, bend), 1463.07 (C=C, aromatic), 1356.02 (-NO_2), 1252.82 (C-N), 877.65 (m-substitution), 818.82 (C-H, bend, aromatic), 766.74 (C-Cl); ^{1}H-NMR (DMSO): δ 8.731 (1H, s, acridine), 8.508 (1H, d, quinoline), 8.173 (1H, d, acridine), 8.146 (1H, d, acridine), 7.970 (1H, s, quinoline), 7.902 (2H, d, acridine), 7.579 (1H, d, quinoline), 7.548 (1H, d, acridine), 7.518 (1H, m, acridine), 7.452 (1H, d, quinoline), 7.343 (1H, m, acridine), 6.773 (1H, m, benzene), 6.484 (1H, d, quinoline), 5.900 (2H, d, benzene), 5.182 (1H, s, benzene), 3.92 (2H, s, H-N, aromatic); the molecular ion peak was not observed but m/z at 410.9 (M-81) and 131.1 (M-C_9H_7N) confirms the structure of compound.

N^1-(7-chloroquinolin-4-yl)-N^3-(2,4-dimethylacridin-9-yl)benzene-1,3-diamine (K8):

Brownish black; Yield 65%; m.p. 280-283^oC; IR (KBr, cm^{-1}): 3336.99 (N-H, stretch), 2952.18 (C-H, stretch, aromatic), 2824.87 (C-H, stretch, methyl), 1609.18 (C=N), 1569.16 (N-H, bend), 1460.18 (C=C, aromatic), 1376.27 (C-H, bend, methyl), 1272.11 (C-N), 860.29 (m-substitution), 766.74 (C-H, bend, aromatic), 689.58 (C-Cl); ^{1}H-NMR (DMSO): δ 8.752 (1H, d, quinoline), 8.178 (1H, d, acridine), 7.985 (1H, s, quinoline), 7.869 (1H, d, acridine), 7.701 (1H, m, acridine), 7.595 (1H, d, quinoline), 7.454 (1H, s, acridine), 7.429 (1H, d, quinoline), 7.365 (1H, m, acridine), 7.166 (1H, s, acridine), 6.839 (1H, m, benzene), 6.485 (1H, d, quinoline), 6.387 (2H, d, benzene), 5.171 (1H, s, benzene), 4.311 (2H, s, H-N, aromatic), 2.240 (6H, s, methyl); m/z 475 [M+1].

N^1-(7-chloroquinolin-4-yl)-N^3-(2-methoxyacridin-9-yl) benzene-1, 3-diamine (K11):

Brownish black; Yield 68%; m.p. 269-273^oC; IR (KBr, cm^{-1}): 2995.58 (N-H, stretch), 2949.29 (C-H, stretch, aromatic), 1607.74 (C=N), 1569.10 (N-H, bend), 1480.43 (C=C, aromatic), 1123.58 (C-O), 1082.11 (C-N), 901.76 (C-H, bend, aromatic), 867.04 (m-substitution), 687.65 (C-Cl); ^{1}H-NMR (DMSO): δ 8.521 (1H, d, quinoline), 8.427 (1H, d, acridine), 7.912 (1H, s, quinoline), 7.869 (1H, d, acridine), 7.582 (1H, m, acridine), 7.520 (1H, d, quinoline), 7.492 (1H, d, acridine), 7.456 (1H, m, acridine), 7.346 (1H, d, quinoline), 7.297 (1H, d, acridine), 7.069 (1H, s, acridine), 6.894 (1H, m, benzene), 6.517 (1H, d, quinoline), 6.404 (2H, d, benzene), 5.106 (1H, s, benzene), 3.778 (1H, s, R-O-C-H), 3.427 (1H, s, H-N, aromatic); m/z 475 [M-1].

N^1-(7-chloroquinolin-4-yl)-N^3-(4-methoxyacridin-9-yl) benzene-1, 3-diamine (K14):

Brownish black; Yield 70%; m.p. 276-279^oC; IR (KBr, cm^{-1}): 3187.51 (N-H, stretch), 2985.94 (C-H, stretch, aromatic), 1606.77 (C=N), 1569.16 (N-H, bend), 1492.97 (C=C, aromatic), 1275.97 (C-N), 1163.13 (C-O), 867.04 (m-substitution), 820.75 (C-H, bend, aromatic), 691.51 (C-Cl); ^{1}H-NMR (DMSO): δ 8.537 (1H, d, quinoline); ^{1}H-NMR (DMSO): δ 8.537 (1H, d, quinoline), 8.439 (1H, d, acridine), 7.915 (1H, s, quinoline), 7.869 (1H, d, acridine), 7.606 (1H, d, quinoline), 7.574 (1H, m, acridine), 7.466 (1H, d, quinoline), 7.357 (1H, m, acridine), 7.252 (1H, d, acridine), 7.252 (1H, d, acridine), 7.226 (1H, m, acridine), 6.902 (1H, d, acridine), 6.886 (1H, m, benzene), 6.481 (1H, d, quinoline), 6.384 (2H, d, benzene), 5.167 (1H, s, benzene), 4.056 (2H, s, H-N, aromatic), 3.732 (1H, s, R-O-C-H); m/z 475 [M-1].

2.3 *In vitro* Antimalarial activity:

All the synthesized compounds were screened for *in vitro* antimalarial activity at Microcare laboratory & TRC, Surat, Gujarat. The *in vitro* antimalarial assay was carried out in 96 well microtiter plates according to the microassay protocol of Rieckmann and co-workers with minor modifications. All the cultures of *P. falciparum* strains were maintained in medium RPMI1640 supplemented with 25m MHEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat-inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was determined by samples, prepared in DMSO and their subsequent dilutions were prepared with culture Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and maintained with 50 % RBCs (O^+). A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium, then diluted samples were added to the test wells so as to obtain final concentrations ranging between 0.4µg/ml-100µg/ml in duplicate well containing parasite cell preparation. The culture plates were incubated at37^0C in a candle jar, after 36-40 hours of incubation; thin blood smear slides were prepared from each well and stained with JSB stain. The slides were observed under microscope to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the IC_{50}value of test compounds.

3. RESULTS AND DISCUSSION

3.1 Docking studies:

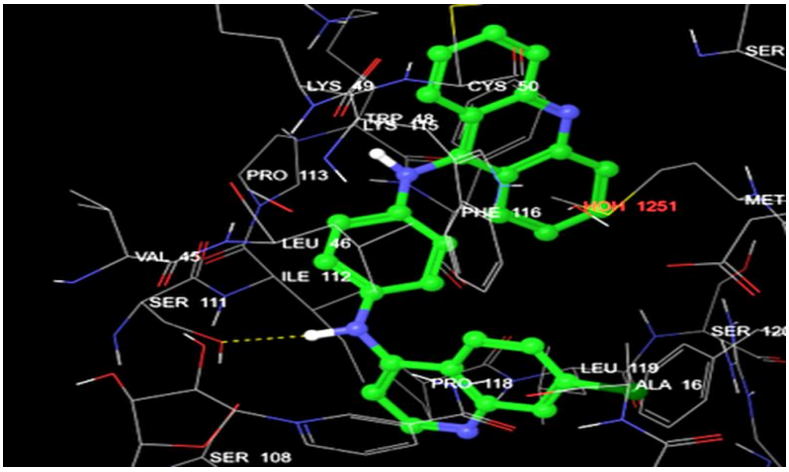
The result of the extra precision docking experiments of all the designed compounds along with standard drug is summarized in table 2. The X-ray crystallographic structure of PfDHFR-TS (PDB: 1J3I) complexed with WR99210 inhibitor, were obtained from protein data bank through internet. Inspection of quinoline-acridine hybrid in the active site of enzyme revealed hydrogen bonding and π-π interaction with the residues SER111, ILE164, GLY165, LEU164, VAL45 and ARG122.

All the designed compounds revealed molecular interaction into the active site of enzyme. The observed interaction of compound K3, K9 and K10 into the active site of PfDHFR enzyme are shown in fig 1 (a), (b) and (c). For K3 and K9 the hydrogen atom of amino group (linker) forms hydrogen bond with the oxygen atom of SER111 with bond length of 2.073 and 2.057 respectively. For K10, oxygen of methoxy group form bond with hydrogen of amino group of VAL45 and LEU46 with bond length of 2.444. In case of chloroquine, ring nitrogen form hydrogen bond with SER511, ARG510 having bond length 2.448, 2.244 and the bridge nitrogen shows hydrogen bonding with TYR 533 having bond length 2.073 respectively. The good binding interaction of compound K3 with enzyme explains highest antimalarial activity.

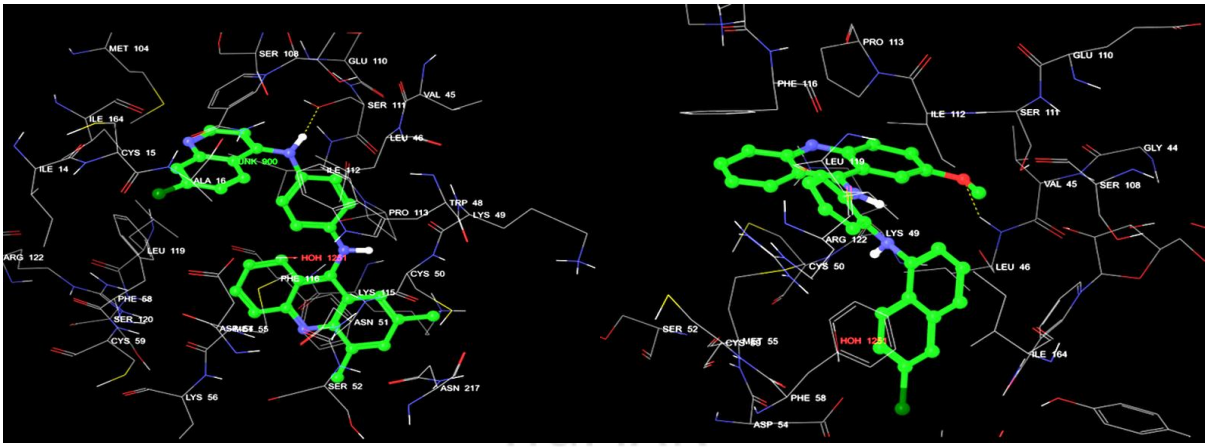
Table 2: Compound code, Glide score, Emodel energy, RMSD, amino acid interactions and IC_{50} values of compounds and standard with PfDHFR-TS.

S.No	Compound code	Glide Score	Glide EModel energy	RMSD	Interacting amino acid residues	IC_{50} value
1	K1	-10.495	-69.530	0.014	ILE164,GLY164	-
2	K2	-11.476	-74.835	0.003	SER 111	-
3	K3*	-10.533	-86.392	0.037	SER111,GLY165, ILE164	0.04
4	K4	-9.237	-105.756	0.011	GLY165,LEU164, ARG 122	-
5	K5*	-10.375	-80.971	0.043	SER111, ILE112	0.98
6	K6	-10.333	-100.632	0.018	SER111,ARG 122	-
7	K7	-10.019	-86.152	0.005	ILE164, GLY165	-
8	K8*	-9.384	-80.317	0.017	ILE164, GLY165	-
9	K9	-12.300	-94.578	0.034	SER111	1.25
10	K10	-10.635	-79.120	0.031	LEU46, VAL45	-
11	K11*	-10.192	-82.199	0.004	ILE164.GLY165	1.50
12	K12	-11.701	-91.635	0.027	SER111	-
13	K13	-10.347	-88.187	0.013	ILE164	-
14	K14*	-9.783	-90.320	0.042	ILE164,GLY165	0.72
15	K15	-11.446	-95.429	0.027	SER 111	-
16	Chloroquine	-8.743	59.672	0.009	SER511, ARG510, TYR533	0.020
17	Quinine	-	-	-	-	0.268

* Synthesized compounds



(a)



(b) (c)

Fig 1: Binding mode of compound K3, K9 and K10 into active site of PfDHFR enzyme showing hydrogen bonding interaction

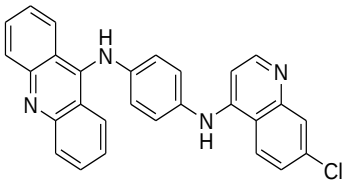
3.2 Synthesis:

Synthesis of quinoline-acridine hybrids were carried out as outlined in reaction schemes 1 and 2. Acridine derivatives **4a-4e** were synthesized by reaction of o-chlorobenzoic acid **(1)** with aniline or its derivatives i.e, 3-nitroaniline, 2, 4-dimethylaniline, p-anisidine and o-anisidine respectively (Scheme 1). Then N^1-(7-chloroquinolin-4-yl)-benzene-1,4-diamine **(5a)** and N^1-(7-chloroquinolin-4-yl)-benzene-1, 2-diamine **(5b)** were synthesized by the reaction of 4, 7-dichloroquinoline **(5)** with excess of p-phenylenediamine or m-phenylenediamine using ethanol and p-TSA for 3 hours (Scheme 2). The 4-aminoquinolines **(5a-5b)** with free amino group were refluxed with acridine derivatives **(4a-4e)** using absolute

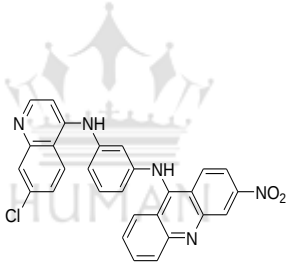
ethanol as a solvent and p-TSA as a catalyst to yield hybrids **K3, K5, K8, K11, and K14** (Scheme 2).

3.3 *In vitro* Antimalarial activity:

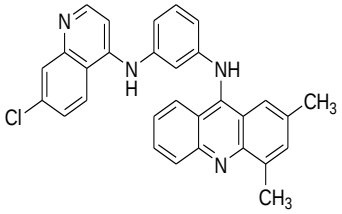
All the five synthesized hybrids were evaluated for their antimalarial activity against chloroquine resistant strain of *P. Falciparum* using chloroquine and quinine as reference drugs (Table 2). In order to get structural insight, two point variations were made in the quinoline-acridine hybrids **(K3, K5, K8, K11, and K14)**. In the structural motive of these hybrids, the aminoquinoline and acridine rings were kept common and variation was made in connecting linker (5a-5b) while another variation was introduced in acridine ring. Among these hybrids, compound K3 which possess unsubstituted acridine ring was found to be active against chloroquine sensitive strain with IC_{50} value 0.04 µg/ml. The activity of compound K3 was found to be comparable with chloroquine and better than the quinine (Table 2). While in case of other compounds having substituted acridine ring, results are comparable to the reference drugs.



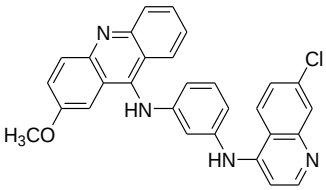
K3



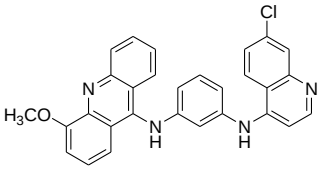
K5



K8



K11



K14

Fig: 2 Structures of synthesized compounds

4. CONCLUSION

In the present study, we have reported docking, synthesis and antimalarial activity of series of 4-aminoquinoline-acridine hybrids. XP Glide docking scores and docking poses of designed compounds and standard suggest that these compounds adopt similar binding mode with active site residue of PfDHFR-TS (1J3I) as hydrogen bond, hydrophobic and π-π stacking interactions, which help in the stabilization of drug in active site. The *in vitro* evaluation of synthesized hybrids against chloroquine sensitive strain of *P. falciparum* depicted activity in nanomolar range. The compound K3 exhibited comparable antimalarial activity with chloroquine and better activity than the quinine. Antimalarial activity of other compounds was comparable to the standard drugs. The good *in vitro* antimalarial activity exhibited by the 4-aminoquinoline-acridine hybrids and docking pattern in the *P. falciparum* DHFR described in present study reveals that in near future, they could be developed as lead for antimalarial compounds.

ACKNOWLEDGEMENT

The authors are thankful to Director, Shri G. S. Institute of Technology and Science, Indore for providing the facility to carry out the research work.

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