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Original Article

SYNTHESIS, ANTIPLASMODIAL AND ADMET STUDIES OF 4-METHYLAMINO-2-PHENYLQUINOLINE ANALOGS

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ABSTRACT

Objective: Synthesis, antiplasmodial and absorption, distribution, metabolism, excretion and toxicity (ADMET) studies of 4-methylamino-2-phenylquinoline analogs.

Methods: The synthesis of 4-methylamino-2-phenylquinoline analogs 7(a-j) by reacting substituted 4-(chloromethyl)-2-phenylquinoline 6(a-c) with secondary amines to explore their antimalarial property against *P. falciparum* RKL-2 strain and *in silico* absorption, distribution, metabolism, excretion and toxicity (ADMET) properties using ACD/I-Lab 2.0. The synthesized structures were confirmed by IR, NMR and Mass spectral analysis.

Results: The results revealed that at 100 µg/ml, compounds 7a, 7d and 7i were found to be potent with percentage inhibition of 88.0±1.1, 79.1±1.1, 90.2±0.1, respectively. The compounds 7b, 7e, 7f and 7h were moderately active with 59.9±1.2, 48.5±2.0, 35.2±1.1 and 52.0±0.3 and the remaining compounds 7c, 7g and 7j exhibited mild activity 32.2±1.2, 36.8±3.0 and 28.7±2.0. The absorption, distribution, metabolism, excretion and toxicity (ADMET) studies of title compounds were analyzed and found to be obeying the Lipinski rule of five and are non-toxic.

Conclusion: The C_4 of quinoline ring with morpholine 7a, piperidine 7d and imidazole 7i substitutions were promising enough to be taken as lead molecules in the drug discovery of new antimalarial. The *in silico* absorption, distribution, metabolism, excretion and toxicity (ADMET) studies of the molecules were found to be obeying the Lipinski rule of five good drug likeliness.

Keywords: Antimalarial, Chloroquine, RKL-2 strain, Pharmacokinetics, Pfitzinger reaction, *Plasmodium falciparum*

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INTRODUCTION

Malaria is a serious public health issue in certain regions of Southeast Asia, South America and Africa. Most of the malaria disease and deaths are mainly caused by the parasite *Plasmodium falciparum* [1]. The four species, *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale* are responsible for the mass of human malaria cases globally, and recently another species, *Plasmodium knowlesi* infecting humans has been documented [2]. The *Plasmodium* parasites known to give human malaria infections, *P. falciparum* is the most prevalent and deadly [3], even though the others still result in serious illness. Numbers of drugs are available to treat malaria, yet *P. falciparum* has got resistance to almost all of them. Over the years the chloroquine has remained the drug of choice for malaria chemotherapy because of its effectiveness, less toxic and affordability. The spread of resistance towards chloroquine and other available drugs by *P. falciparum* has become a major health concern in the tropical and sub-tropical regions of the world [4]. The chloroquine resistance of *P. falciparum* is associated with mutations in the *P. falciparum* chloroquine resistance transporter (pfcrt) gene; this alters the transport and accumulation of the drug in the digestive vacuole of the parasite [5, 6].

Even the Artemisinin class of drugs are now showing reduced efficacy [7], because of these factors, there is a continuing need of new antimalarial treatments to combat the disease. Therefore, development of novel, affordable and effective antimalarial drugs, is the better approach to avoid the problem faced by the present class of drugs. Keeping the interest of finding a potential lead, we tried to synthesize molecules with a quinoline ring as the core scaffold because of its proven ability as important heterocycles [8, 9], and building the structure at the C4 position with methylamines.

Quinoline is an important class of heterocycles found in many of the natural products [10, 11]. Quinoline compounds such as quinine, chloroquine and mefloquine have been used in the treatment of malaria [12-15] and also medicinal structures such as ciprofloxacin, norfloxacin and pefloxacin [16] are developed as potent antibiotics. In addition to this, quinoline derivatives are also used as an anti-arrhythmic and antiulcer agents such as quinidine [9, 17] and rebamipide [18], respectively. Furthermore, the quinoline-4-carboxylic acids and their analogs have shown interesting antiviral, analgesic, antitumor and antitubercular activities [19-22].

The quinoline antimalarial drugs act during the blood stages of the parasite's life cycle and some of them target the hepatic stages as well [23, 24]. The 4-aminoquinoline based heteroaryl piperazine is being significantly more potent against the chloroquine-resistant strains of *P. falciparum* [25]. The C-2 position is substituted by metabolically stable bulky alkyl group in 2-tert-butylprimaquine showing excellent antimalarial activity [26]. The replacement of diethyl amino function group in chloroquine with a metabolically stable heterocyclic ring (piperidyl, pyrrolidine and morpholine) in the short chain analogs leads to increase in activity [27]. Currently, antimalarial chemotherapy has been hindered by the low sensitivity of the parasite to most of the antimalarial drugs available in the market [19]. Hence, having the potential of many medicinal properties, the quinoline ring has excited us to design newer quinolines with appropriate pharmacophore in the core structure, which can be taken up as a possible lead in antimalarial treatment [28]. In continuation of our work on quinoline nucleus [29-32] to achieve this, we considered quinoline-4-carboxylic acid as key intermediate which follows many functional transformations to fix amines in 4th position on the quinoline moiety to check the potentiality of the compound on their antimalarial efficacy.

MATERIALS AND METHODS

Commercially available chemicals are used in the synthesis of compounds 7(a-j). The compounds were purified by column chromatography using silica gel 100-200 mesh with occasional monitoring by pre-coated aluminum TLC plates procured from Merck. Melting points were recorded by the open capillary method and are uncorrected by Raga Melting Point Apparatus. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 400 MHz and 100 MHz, Bruker spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were recorded on the LCMS agilent mass spectrometer. HPLC analysis of the synthesized molecules was performed to check the purity on a giant 1100 series, using a PDA detector at 254 nm using zobrax column RX-C8, 250 X 4.6 mm, 5 µm, flow rate 1.0 ml/min.

Chemicals and reagents

Isatin, Ethyl alcohol, Methyl alcohol, Acetophenone, Ethyl acetate, n-Hexane, Dichloromethane, Thionyl Chloride, $NaCO_3$, $NaHCO_3$, K_2CO_3 were procured from Sigma-Aldrich and the RKL-2 Strain, RPMI-1640 media, D-Glucose, Human serum, Giesma Stain, all the chemicals reagents and biological ingredients were procured from HIMEDIA.

General procedure

In view of the above fact, we synthesized 2-phenylquinoline-4-carboxylic acids (cinchonic acid) 3a-c by reacting substituted acetophenone 1 with isatin 2(a-c) in the presence of 33% aqueous KOH and ethanol under reflux condition. The obtained acid 3(a-c) was further esterified by using methanol and a catalytic amount of conc. H_2SO_4. The ester 4a-c was reduced to alcohol using $NaBH_4$ in the presence of methanol, the obtained alcohols 5(a-c) were further reacted with $SOCl_2$ to yield 4-(chloromethyl)-2-phenylquinolines 6(a-c). Nucleophilic substitution at C4 with secondary amines was achieved in the presence of DMF and K_2CO_3 to yield title compounds 7(a-j). The structures of all the newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, FTIR and mass spectral analysis.

I) Synthesis of 2-phenyl quinoline-4-carboxylic acid 3(a-c)

Isatin 2(a-c) (0.01 mol) and ethanol (10 v) were taken in a round bottom flask, to this 33% aq. KOH was added dropwise at 0-5 °C followed by addition of acetophenone 1 (0.01 mol). The reaction was refluxed at 75 °C for 8 hr. After completion, the reaction mixture was neutralized with dil. HCl. The precipitate thus formed was filtered, washed with ethyl acetate to remove impurities and dried.

II) Synthesis of methyl-2-phenyl quinoline-4-carboxylate 4(a-c)

2-phenyl quinoline-4-carboxylic acid 3(a-c) (0.01 mol) was taken in methanol (10 v) in a round bottom flask, to this two drops of Conc. H_2SO_4 was added. The reaction was refluxed at 75 °C for 8 hr. After completion, the reaction mixture was poured into ice-cold water, the precipitate formed was thus filtered and dried.

III) Synthesis of 2-phenyl quinoline-4-yl-methanol 5(a-c)

Methyl-2-phenyl quinoline-4-carboxylate 4(a-c) (0.01 mol), methanol (10 v) was taken in a round bottom flask, to this sodium boro hydrate (0.04 mol) was added at 0-5 °C and kept for at ambient temperature stirring at 25-30 °C for 3 hr. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice-cold water; a precipitate formed was thus filtered and dried.

IV) Synthesis of 4-(chloromethyl)-2-phenylquinolines 6(a-c)

The compound 2-phenyl quinoline-4-yl-methanol 5(a-c) (0.01 mol) was taken in DCM (10 v) in a round bottom flask, to this thionyl chloride (0.04 mol) was added at 0 °C and kept for stirring at 25-30 °C for 3 h. After completion of the reaction, the reaction mixture was neutralized with sodium bicarbonate solution and extracted with DCM; the DCM was evaporated to get a solid mass.

V) Synthesis of 4-Methylamino-2-phenylquinoline 7(a-j)

The compound 4-(chloromethyl)-2-phenylquinolines 6(a-c) (0.01 mol) was taken in DMF (10 v) in a round bottom flask, to this K_2CO_3 (0.02 mol) was added followed by addition of substituted amine (0.01 mol). The reaction was kept for stirring at 25-30 °C for 2 h. The progress of the reaction was monitored by TLC. After the complete reaction mixture was diluted with water and extracted with ethyl acetate, the organic layer was concentrated and dried. Purification of the synthesized compounds was achieved by column chromatography using n-hexane: ethyl acetate gradient as mobile phase.

Spectral data

4-(morpholinomethyl)-2-phenylquinoline (7a)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.28-8.16 (m, 4H), 7.92 (s, 1H), 7.74 (t, *J* = 4 Hz, 1H), 7.60-7.47 (m, 4H), 3.99 (s, 2H), 3.756 (t, *J* = 2 Hz, 4H), 2.586 (t, *J* = 2 Hz, 4H) ppm. ^{13}C NMR (100MHz, $CDCl_3$) δ= 156.9, 148.5, 143.9, 139.6, 130.2, 129.2, 128.7, 127.5, 126.7, 126.0, 123.9, 119.2, 66.9, 60.3, 53.9 ppm. FTIR: 3064.3, 2957.3, 1596.7, 1116.5 cm^{-1}. MS: m/z =305 (M+1). m. p.:65-68 °C.

4-((4-methylpiperazin-1-yl) methyl)-2-phenylquinoline (7b)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.23(d, *J*= 8Hz, 1H), 8.19-8.15 (m, 3H), 7.914(s, 1H), 7.71(t, *J* = 8 Hz, 1H), 7.55-7.50(m, 3H), 7.47(t, *J* = 7.2 Hz, 1H), 3.98(s, 2H), 2.55(m, 8H), 2.30 (s, 3H) ppm. ^{13}C NMR (100MHz, $CDCl_3$) δ= 157.7, 147.6, 144.4, 139.9, 132.3, 131.1, 130.4, 129.4, 128.1, 127.9, 123.9, 120.4, 60.5, 55.7, 54.0, 46.6 ppm. FTIR: 3059.0, 2963.0, 1596.7, 1097.3 cm^{-1}. MS: m/z =318 (M+1). m. p.:76-80 °C.

N-ethyl-N-((2-phenylquinolin-4-yl) methyl) ethenamine (7c)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.23(d, *J*= 1.2 Hz, 1H), 8.18(m, 3H), 8.02 (s, 1H), 7.69 (t, *J*= 7.8 Hz, 1H), 7.54-7.49 (m, 3H), 7.45 (t, *J*= 8.8 Hz, 1H), 4.04 (s, 2H), 2.63 (q, *J*= 7.2 Hz, 4H), 1.10(t, *J*= 7.2 Hz, 6H) ppm. ^{13}C NMR (100MHz, $CDCl_3$) δ= 157.0, 148.3, 146.5, 139.9, 130.1, 129.1, 128.7, 127.5, 126.5, 125.8, 123.6, 118.8, 54.9, 47.4, 11.8 ppm. FTIR: 3053.7, 2959.2, 1596.7, 1114.6 cm^{-1}. MS: m/z = 291.2 (M+1). m. p.:83-86 °C.

2-phenyl-4-(piperidin-1-ylmethyl) quinoline (7d)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.25 (d, *J*= 8.4 Hz, 1H), 8.19-8.16 (m, 3H), 7.93 (s, 1H), 7.69 (t, *J*= 7.6 Hz, 1H), 7.54-7.49 (m, 3H), 7.45 (t, *J*= 7.6 Hz, 1H), 3.93 (s, 2H), 2.51 (s, 4H), 1.61 (p, *J*= 6 Hz, 4H). ^{13}C NMR (100MHz, $CDCl_3$) δ= 156.8, 148.8, 145.1, 139.8, 130.1, 129.1, 128.7, 127.5, 126.6, 125.8, 123.9, 118.9, 60.4, 54.9, 26.0, 24.2 ppm. FTIR: 3059.5, 2935.1, 1597.7, 1115.6 cm^{-1}. MS: m/z = 303.4(M+1). m. p.:58-62 °C.

6-chloro-4-(morpholinomethyl)-2-phenylquinoline (7e)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.22 (d, *J*= 7.0 Hz, 1H), 8.15-8.10 (m, 3H), 7.92 (s,1H), 7.66 (d, *J*= 4.0 Hz, 1H), 7.55-7.48 (m, 3H), 3.93 (s, 2H), 3.76 (t, *J*= 2 Hz, 4H), 2.573 (t, *J*= 2 Hz, 4H)ppm. ^{13}C NMR (100MHz, $CDCl_3$) δ= 157.1, 147.0, 143.2, 139.2, 131.8, 131.7, 130.4, 129.5, 128.8, 127.4, 127.3, 123.2, 119.9, 66.9, 60.4, 53.8 ppm. FTIR: 3069.1, 2937.0, 1596.7, 1347.0, 1140.6, 771.3 cm^{-1}. MS: m/z = 339 (M+1). m. p.:80-83 °C.

6-chloro-4-((4-methylpiperazin-1-yl) methyl)-2-phenyl-quinoline (7f)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.21 (d, *J*= 7 Hz, 1H), 8.13-8.08 (m, 3H), 7.92 (s, 1H), 7.64 (d, *J*= 4.0 Hz, 1H), 7.59-7.47 (m, 3H), 3.93 (s, 2H), 2.61-2.50 (m, 8H), 2.31 (s, 3H) ppm.^{13}C NMR (100MHz, $CDCl_3$) δ= 157.7, 147.6, 144.4, 139.9, 132.3, 131.1, 130.4, 129.4, 128.1, 123.9, 120.4, 60.5, 55.7, 54.0, 46.6 ppm. FTIR: 3073.9, 2962.7, 1591.9, 1356.0, 1061.4, 827.5 cm^{-1}. MS: m/z = 352.8 (M+1). m. p.: 82-85 °C.

N-((6-chloro-2-phenylquinolin-4-yl) methyl)-N-ethylethan-amine (7g)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.25 (d, *J*= 2.4 Hz, 1H), 8.18-8.15 (m, 2H), 8.10 (d, *J*= 9.2 Hz, 1H), 8.03 (s, 1H), 7.63 (d, *J*= 9.2 Hz, 1H), 7.55-7.50(m, 1H), 7.46(t, *J*= 7.2 Hz, 1H) ppm. ^{13}C NMR (100MHz, $CDCl_3$) δ=159.9, 146.5, 145.5, 139.1, 131.4, 129.7, 129.1, 128.5, 127.1, 122.6, 119.1, 54.6, 47.1, 11.5 ppm. FTIR: 3063.3, 2966.9, 1597.7, 1346.0, 1071.2, 822.4 cm^{-1}. MS: m/z =325.1 (M+1). m. p.: 66-68 °C.

6-chloro-2-phenyl-4-(piperidin-1-ylmethyl) quinoline (7h)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.27-8.09 (m, 4H), 7.95 (s, 1H), 7.67-7.47 (m, 4H), 3.88 (s, 2H), 2.50 (t, 4H, *J*= 2 Hz), 1.64 (p, *J*= 3 Hz, 6H)

ppm. ^{13}C NMR (100MHz, $CDCl_3$) δ= 139.4, 131.7, 130.0, 129.4, 128.8, 127.5, 123.2, 119.7, 60.4, 54.9, 26.0, 24.2 ppm. FTIR: 2916.1, 2841.6, 1591.5, 1162.9, 817.1 cm^{-1}. MS: m/z =337.86 (M+1). m. p.: 85-88 °C.

4-((1H-imidazol-1-yl) methyl)-6-chloro-2-phenylquinoline (7i)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.17 (d,*J*= 8.8 Hz, 1H), 8.02-7.99(m, 2H), 7.87 (d, *J*= 2.4 Hz, 1H), 7.71 (d, *J*= 9.2 Hz, 1H), 7.67 (s, 1H), 7.50-7.43 (m, 3H), 7.23 (t, *J*= 1.2Hz, 1H), 7.02 (t, *J*= 1.2 Hz, 1H), 5.63 (s, 2H) ppm.^{13}C NMR (100MHz, $CDCl_3$)δ= 146.0, 141.6, 137.9, 132.1, 131.6, 130.2, 129.3, 128.3, 126.7, 120.8, 116.6, 46.7 ppm. FTIR: 3106.7, 2999.0, 1602.5, 1085.7, 834.5 cm^{-1}. MS: m/z =320.7 (M+1). m. p.: 105-108 °C.

8-fluoro-4-(morpholinomethyl)-2-phenylquinoline (7j)

^{1}H NMR (400MHz, $CDCl_3$) δ= 8.27 (d, *J*= 8 Hz, 2H), 8.18 (s, 1H), 8.124 (t, *J*= 4 Hz, 1H), 7.67-7.45 (m, 5H), 3.99 (s, 2H), 3.57 (t, *J*= 4 Hz, 4H), 2.49 (t, *J*= 2 Hz, 4H) ppm. ^{13}C NMR (100MHz, $CDCl_3$) δ= 159.9, 157.5, 156.8, 144.05, 139.1, 129.6, 128.8, 128.2, 127.6, 125.7, 119.9, 119.6, 113.5, 113.4, 66.9, 60.5, 53.872 ppm. FTIR: 2950, 1600, 748. MS: m/z = 321.4 (M+1). m. p.: 95-98 °C.

***In vitro* antimalarial activity**

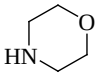
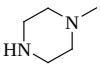
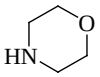
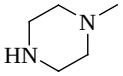
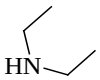
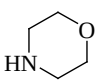
Assay protocol

All the synthesized compounds 7a-j were screened for *in vitro* antimalarial assay, carried out by adopting the protocol in a 96 well microtiter plate using a microassay protocol of Rieckmann and co-workers with minor modifications [33, 34]. The culture of *P. falciparum* RKL-2 strain was maintained in medium RPMI 1640 supplemented with 25 mmol HEPES, 0.23% sodium bicarbonate, 1% D-glucose and 10% heat inactivated human serum [35]. The asynchronous parasites of *P. falciparum* were synchronized with 5% D-sorbitol treatment to obtain ring stage parasitized cells [36]. Preliminary ring stage *parasitamia* of 0.8 to 1.5% at 3% *haematocrit* in a total volume of 200 µl of medium RPMI-1640 was determined by sample staining to evaluate the percent *parasitamia* (rings) and uniformly maintained with 50% RBCs [37]. A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and following dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at eight-fold dilutions) ranging between 0.125 µg/ml to 100 µg/ml in duplicate well containing parasitized cell. The culture plates were incubated at 37 °C, after 36 to 40 h. incubation, from each well thin blood smears, were prepared and stained with Giesma stain [38] and were microscopically observed to record maturation of ring stage parasites into the *trophozoites* and *schizonts* in the presence of different concentrations of the test samples. The test concentration which inhibited the complete maturation into *schizonts* was recorded as the percentage inhibition concentrations. Chloroquine was used as the reference drug. The mean number of rings, trophozoites and *schizonts* recorded per 100 parasites from the duplicate wells after incubation for 38 hr. The percent maturation inhibition with respect to the control group is shown in table 1; all the data were made to triplicate to calculate the statistical analysis and to get the mean of the results.

ADME-toxicity prediction

The molecular descriptors of synthesized compounds 7(a-j) are optimized using QSAR properties. The SAR activity of these compounds is significantly helping to understand the pharmacokinetics to derive physicochemical properties and predict biological activity such as absorption, distribution, metabolism, excretion and toxicity (ADMET). The Admet SAR [39] helps to evaluate biologically active molecules and eliminate the biologically poor active lead molecules which contain undesirable functional groups based on Lipinski rule. The statistical calculation for lead molecules includes surface area, geometry and fingerprint properties which help to understand biological important end points. Aqueous solubility (PlogS), blood-brain barrier penetration (QPlogBB), intestinal absorption (logHIA) [40], hepatotoxicity, Caco-2 cell permeability (QPPCaco) also helps to understand drug metabolism for top docking lead molecules [41]. Further, to predict the toxicity of lead molecules with intraperitoneal, oral, intravenous and subcutaneous toxic effects of blood, cardiovascular system, gastrointestinal, kidney, liver, and lungs to calculate sensitivity, specificity and area under the curves (AUC) that will predict the linearity of compounds.

Table 1: Physicochemical data of the synthesized compounds (7a-j)

S. No.	R	R_1	R_2	Yield %	Melting point °C
7a	H	H		72	65-68
7b	H	H		68	76-80
7c	H	H		81	83-86
7d	H	H		78	58-62
7e	Cl	H		68	80-83
7f	Cl	H		58	82-85
7g	Cl	H		70	66-68
7h	Cl	H		63	85-88
7i	Cl	H		55	105-108
7j	H	F		69	95-98

RESULTS AND DISCUSSION

Since the quinoline ring system is acknowledged, it possesses complex iron properties [42], we envisioned to combine quinolines with different secondary amines to obtain potent antimalarial [43]. Our structural design efforts were mainly dictated by the hypothesis of anti *P. falciparum* activity might be directly related to the ability of both to bind intracellular iron and to form redox-active iron complexes that generate cytotoxic ligand centered radical species. To authorize our assumption, we exploited the effect on the antimalarial potency of morpholine, piperidine, imidazole and diethylamine derivatives. Even though the exact mode of action of quinoline derivatives has not been fully clarified, it is generally accepted that quinoline derivatives readily enters the parasite *P. falciparum* along with the pH gradient and complexes [44, 45].

In the present work majority of compounds displayed antimalarial activity against *P. falciparum*, among them the compounds 7a, 7d and 7i were found to be potent with percentage inhibition 88.0±1.1, 79.1±1.1, 90.2±0.1 with 100 µg/ml, respectively. The compounds 7b, 7e, 7f and 7h were found to be moderately active with percentage inhibition 59.9±1.2, 48.5±2.0, 35.2±1.1 and 52.0±0.3 and the remaining compounds 7c, 7g and 7j were showed mild activity 32.2±1.2, 36.8±3.0 and 28.7±2.0 with 100 µg/ml.

Table 2: *In vitro* antimalarial activity of synthesized compounds against chloroquine sensitive strain of *Plasmodium falciparum*

Sample no.	% inhibition (*µg/ml*) mean±SD, n = 3							
	100	**50**	**10**	**2**	**1**	**0.5**	**0.25**	**0.125**
7a	88.0±1.1	85.1±1.0	68.5±2.0	42.3±1.5	29.7±2.2	39.2±0.6	12.3±1.0	9.2±2.0
7b	59.9±1.2	59.8±2.0	57.2±1.5	32.5±1.8	22.5±0.2	8.7±2.0	2.2±1.3	0.5±1.5
7c	32.2±1.2	31.0±0.6	26.2±1.5	18.5±2.0	11.0±0.2	12.0±2.0	1.9±1.3	0.8±1.5
7d	79.1±1.1	67.9±2.0	74.5±1.3	70.2±0.6	52.1±0.5	62.5±2.0	21.0±1.5	10.2±1.5
7e	48.5±2.0	40.0±1.2	41.2±1.1	34.8±3.2	12.2±6.4	3.0±0.3	2.2±1.3	4.1±0.1
7f	52.0±1.3	54.2±2.0	43.0±0.2	35.2±1.1	28.5±2.0	32.8±1.8	4.8±1.3	1.8±0.3
7g	36.8±3.0	28.5±0.5	30.5±2.0	12.8±0.8	22.2±1.5	5.0±2.0	1.5±1.3	0.8±1.2
7h	52.0±0.3	38.2±2.0	51.0±3.2	42.8±1.2	32.0±2.0	15.0±1.5	5.8±0.5	3.0±1.2
7i	90.2±0.1	81.5±0.5	79.1±1.2	62.2±2.0	51.1±0.5	34.0±0.6	29.0±1.1	18.1±1.5
7j	28.7±2.0	18.2±1.5	11.2±1.6	15.6±0.5	9.8±2.1	2.4±6.2	0.5±0.5	0.5±1.1
Chloroquine (std.)	100	100	98.2	97.1	98.4	89.8	88.4	91.2

µg/ml = microgram per milli liter

The structure-activity study (SAR) revealed that the activity is mainly due to the substitution of side chain with the heterocyclic ring. The basic nature of the side chain is essential for accumulation of drugs within the acidic food vacuole of the parasite [46]. The presence of a secondary nitrogen atom in the side chain reduces the ability to cross the blood-brain barrier and therefore the corresponding neurotoxicity [47]. The compound 7a, 7d and 7i having morpholine, piperidine and imidazole ring respectively, as side chain substitution at 4th position, showed promising activity. Access of the second amine on the quinoline at the 4th position is a must for good potency. The replacement of a hydrogen atom from fluorine and chlorine can alter the pKa, dipole moments, the chemical reactivity and the stability of neighboring functional groups [48] and exerts a minor steric demand at receptor sites due to greater size than hydrogen and the inductive effect of the fluorine decrease the susceptibility of adjacent groups to attack by P450 enzymes [49]. Hence, the incorporation of fluorine group at 8th position in 7j decreases the activity. The compounds 7c and 7g having diethylamine as side chain substitution showed mild activity with percentage inhibition 32.2±1.2 and 36.8±3.0, respectively. According to the result, the open chain substitution decreases the activity compared to heterocyclic compound. Inhibitory activities of synthesized compound against *P. falciparum*.

Table 3: ADME and pharmacological parameters prediction for the ligands 4-Methylamino-2-phenylquinoline 7(a-j) Derivatives using ADME SAR toolbox

ligand	PlogBB[a]	log$_{HIA}$[c]	PCaco[b]	logpGI (substrate)[d]	logpGI (non-inhibitor)[e]	PlogS[f]	Log Papp[g]
7a	0.9873	0.9953	0.5000	0.5468	0.5830	-2.5159	0.9123
7b	0.9745	0.9948	0.6371	0.8060	0.6031	-2.6139	1.1067
7c	0.9684	0.1000	0.7427	0.6342	0.7958	-3.1751	1.5569
7d	0.9822	0.9929	0.5635	0.5759	0.6951	-2.9561	0.8840
7e	0.9832	0.9966	0.5161	0.5408	0.6949	-3.5430	0.7778
7f	0.9599	0.9955	0.6360	0.8013	0.6617	-3.3748	0.9157
7g	0.9563	1.0000	0.7209	0.6232	0.8007	-4.1146	1.4550
7h	0.9735	0.9939	0.5602	0.5721	0.8109	-3.9212	0.7234
7i	0.9788	0.9938	0.6064	0.6304	0.7474	-4.6190	1.1613
7j	0.9872	0.9966	0.5000	0.5625	0.7884	-3.2512	0.7722
Indomethacin	0.9381	0.9509	0.5857	0.6360	0.9313	-4.6825	0.6287
Camptothecin	0.6345	0.8410	0.5555	0.6039	0.7852	-3.0369	1.1839
Tetracycline	0.9841	0.8006	0.7439	0.7910	0.8025	-3.0575	0.7655
Tretinoin	0.9311	0.9925	0.7603	0.6144	0.8912	-3.0895	1.7734
Levostatin	0.9287	0.9452	0.5484	0.7861	0.7046	-5.9475	0.8127
Metronidazole	0.9297	0.9805	0.5365	0.5141	0.8954	-1.3229	0.8033

[a]Predicted BB = blood/brain barrier partition coefficient (1-high penetration, 2-medium penetration and 3-Low penetration). [b]Predicted Caco-2 cell permeability in nm/s (acceptable range: -1 is poor, 1is great). [c]Predicted HIA = Human intestinal absorption in nm/s (acceptable range: 0 poor,>1 great). [d]Predicted PGI = P-glycoprotein substrate in nm/s (acceptable range of-5 is poor, 1 is great). [e]Predicted PGI = P-glycoprotein inhibitor in nm/s (accepted range: 0 to 1), [f]Predicted S = aqueous solubility, (Concern value is 0-2 highly soluble). [g]Predicted PP = probability of Caco-2 cell permeability in cm/s (Concern value is-1 to 1).

The intestinal absorption (log_{HIA}) and Caco-2 cell permeability (PCaco-2) within the range of-2 poor absorption and+2 more absorption the compounds are more permeable in the intestine and helps for good transport of metabolic drug compounds. It was noticed that the reference molecules enhance the bioavailability properties that leads to less toxic effects against the target protein. The functional groups of compounds such as Br, Cl, F, Me,-O-CH_2-O-had more logP values with a partition coefficient of drug has greatest retention within the human intestine and also has string involvement in drug metabolism. The logPGI (substrate) and non-inhibitors have drug interaction within tissue that transforms xenobiotics of vigorous reduction of drug absorption and released more bile (liver) and urine (kidney).

The reference range of-5 (poor) to+1 (good) and substrate inhibitor from 0 to 1 in which the reference and test compounds 7(a-j) show good activity with human intestinal absorption and metabolism. The aqueous solubility of compounds lies with a range of 0 (poor) to 2 (good) showed that all the molecules had good solubility and $logP_{app}$ stated that the compounds had good permeability on lipid absorption and metabolism. While the reference compounds and all test compounds came within acceptable range, the overall results predicted that test compounds have a good drug-like, lead like and fragment like properties.

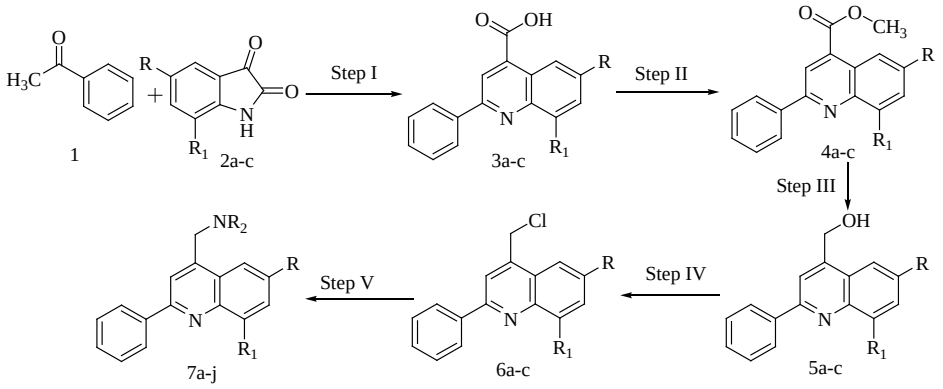
Table 4: LD_{50} and probability of health effects of 4-Methylamino-2-phenylquinoline 7(a-j) derivatives using ACD/I-Lab 2.0

ADME-TOX Parameters	Intraperitoneal a	Oral a	Intravenous a	Subcutaneous a	Blood effect b	Cardiovascular system effect b	Gastro intestnal effect b	Kidney effect b	Liver effect b	Lung effect b
7a	290(0.46)	640(0.44)	50(0.6)	370(0.44)	0.82	0.77	0.93	0.68	0.11	0.43
7b	160(0.51)	410(0.46)	38(0.44)	380(0.44)	0.71	0.87	0.93	0.38	0.15	0.83
7c	200(0.55)	430(0.56)	55(0.57)	350(0.71)	0.86	0.89	0.98	0.62	0.35	0.91
7d	190(0.44)	400(0.38)	37(0.64)	350(0.44)	0.91	0.9	0.91	0.51	0.13	0.83
7e	420(0.48)	520(0.4)	50(0.59)	370(0.42)	0.84	0.75	0.9	0.77	0.11	0.45
7f	210(0.48)	330(0.43)	43(0.54)	380(0.44)	0.75	0.88	0.95	0.44	0.13	0.78
7g	210(0.51)	380(0.53)	54(0.59)	340(0.63)	0.88	0.85	0.99	0.84	0.35	0.86
7h	250(0.39)	380(0.39)	36(0.64)	330(0.4)	0.92	0.87	0.91	0.62	0.29	0.79
7i	570(0.41)	1200(0.39)	71(0.38)	630(0.45)	0.71	0.9	0.71	0.34	0.12	0.58
7j	550(0.44)	460(0.42)	57(0.47)	540(0.33)	0.86	0.83	0.72	0.72	0.13	0.45

[a]Estimated LD_{50}-mouse value in mg/kg after intraperitoneal, oral, intravenous and subcutaneous administration, [b]Estimated probability of blood, gastrointestinal system, kidney, liver and lung effect at a therapeutic dose range of compounds. The drugs with a moderate effect on reliability index (>0.5). The drugs with borderline effect on reliability index (>0.3,<0.5).

The toxicity of the 4-(morpholino methyl)-2-phenylquinoline 7(a-j) Derivatives was predicted based on lethal dosages and functional ranges in different tissues. The mouse LD_{50} and probability of health effects were predicted using ACD/I-Lab 2.0 (guest).

The LD_{50} of potential compounds detect the increasing potential of acute toxicity when administered through oral, intraperitoneal, intravenous and subcutaneous on mouse models. The comparative analysis of reference compounds with test compounds on oral, subcutaneous, intraperitoneal and intravenous is low when compared with reference molecules. The toxicity results suggest that the compounds 7(a-j) have less toxic effect in internal tissues and no side effect when observed in the tested dosages. Further, the toxicity was tested with different organs for adverse effects on organs and their systems (blood, cardiovascular system, gastrointestinal system, kidneys, liver and lungs) and found to be within the therapeutic dose range. The probability of health effects revealed that (4-(morpholino methyl)-2-phenylquinoline 7(a-j) derivatives have less toxic effect on blood, cardiovascular, gastrointestinal, kidney, liver and lung respectively.



Scheme: **Synthesis of 4-Methylamino-2-phenylquinoline derivatives 7a-j**

CONCLUSION

The series of 4-(morpholino methyl)-2-phenylquinoline 7(a-j) derivatives were synthesized and screened for antimalarial activity. The compounds 7a, 7e and 7i were shown promising activity against chloroquine sensitive RKL 2 strain, these were identified as potential molecules in the treatment of malaria caused by *P. falciparum.* The active once will be further tested for chloroquine-resistant strains. The elucidation of the mechanism of action of the lead compounds is under investigation. The compounds can be taken up as lead molecules in the generation of new antimalarial as well as in detail testing against all plasmodium species needs to be carried out on top hits. The *in silico* Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) studies of the molecules were analyzed and found to be obeying the Lipinski rule of five good drug likeliness.

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CONFLICT OF INTERESTS

Declared none

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