In vitro evaluation of the antimalarial activity of a designed novel quinuclidine derivative

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A simple Schiff base, *N*-(pyridine-4-yl-methylene) quinuclidine-3-amine was synthesized from 3-amino-quinuclidine and 4-pyridine carboxaldehyde. The physico-chemical properties of the synthesized compound were studied. Molecular docking study was carried out and the quinuclidine derivative was evaluated for its *in vitro* antimalarial activity against chloroquine-sensitive *Plasmodium falciparum* strain. Although higher dose of synthesized compound was required for antimalarial activity (EC_{50} = 13.125 μg/ml) in comparison to chloroquine (EC_{50} 5.144 μg/ml), the correlation coefficient confirmed good fit of the data. Furthermore, the result of the molecular docking provided insights into the ligand–protein interactions responsible for the inhibitory potency.

Keywords: Antimalarial, docking, HPLC, *Plasmodium falcipurum*, quinuclidine, mass, NMR.

MALARIA, a disease affecting large populations, has proved to be an obstacle in the cultural and socio-economic progress of society in the tropical and subtropical world. It is widespread in all parts of India except in areas, which are not favourable for transmission and multiplication of malaria parasites[1–6]. Quinine, the oldest known antimalarial, led to the discovery of chloroquine, the most widely used antimalarial drug for almost more than half a century. However, chloroquine has lost its importance as an antimalarial due to widespread drug resistance[7,8]. Quinolines, however, are still considered as important lead structures and several researchers have been working on synthesis of their analogues[9]. Quinolines interact and form a non-covalent complex with heme and also inhibit the synthesis of hemozoin. On the other hand, quinuclidine(1-azabicyclo [2.2.2]octane), synthesized by Loffler and Stieze in 1909, is a structural component of some biomolecules, including quinine[10,11]. Although development of a new drug candidate against a specific disease is a difficult task, with the help of informatics-driven chemistry, it is now fairly easy. However, *in silico* prediction of biological activity is not always reflected in the biological system.

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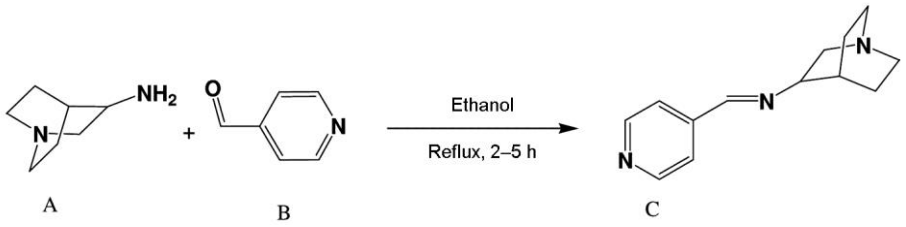
In view of the resistance developed by malarial parasites and considering the biological activity of quinuclidine moiety-bearing biomolecules, a simple Schiff base carrying the quinuclidine structural component was designed[12]. Accordingly, *N*-(pyridine-4-yl-methylene) quinuclidine-3-amine (C) was synthesized (Scheme 1) from 3-aminoquinuclidine (A) and 4-pyridine carboxaldehyde (B). On the basis of the results of *in silico* antimalarial activity study, *in vitro* study was carried out for antimalarial activity of this Schiff base. The results of this study are promising and would help in the development of novel antimalarial drugs.

All the reactions were carried out in borosilicate glassware with proper precautions. The solvents and reagents were synthesis-grade chemicals procured commercially and used as such without further tests and purification. Chemical structures have been drawn in ChemDraw version 7.0.1. Melting point (mp) of the synthesized compound was determined using a melting point apparatus (BUCHI, M-560) by open capillary method. The FT-IR spectrum of the synthesized Schiff base was obtained using FT-IR spectrometer (2000-Perkin-Elmer) in the region 4000–400 cm^{-1}. The NMR spectra (both ^{1}H and ^{13}C) of the Schiff base prepared from 3-amino quinuclidine and pyridine-4-aldehyde were recorded with a FT-NMR (BRUKER, AVANCE-DPX-300 MHz) spectrometer taking the samples in $CDCl_3$ using TMS as internal standard. The chemical shifts are reported in parts per million (ppm) relative to $CHCl_3$ (δ 7.26) for ^{1}H NMR and relative to the central $CDCl_3$ resonance (δ 77) for ^{13}C NMR. The mass spectrum was recorded using a mass spectrometer WATERS Micro-mass ZQ 4000-ESI Probe.

Qualitative HPLC analysis was carried out and the chromatogram of the compound was obtained with the mobile phase prepared with methanol and water (90 : 10). The test solution for HPLC analysis was prepared by dissolving the test sample in the mobile phase. The solution was freshly prepared and analysis was carried out using C18 column; the peaks were detected at 254 nm.

In a typical reaction, 0.01 mol of each of 3-amino-quinuclidine hydrochloride (A) was first warmed with 10% sodium bicarbonate solution to remove acid. It was then extracted in ethyl acetate and the extract dried over anhydrous sodium sulphate for about half an hour. Ethyl acetate was then removed under reduced pressure and the dried extract was taken with equimolar amount of 4-pyridine carboxaldehyde (B) in 20 ml ethanol and the mixture was refluxed for about 5 h on a water bath. The progress of reactions and purity of synthesized compounds were checked on silica gel-G TLC plates using solvent combination of different polarity. The reaction mixture was then cooled and diluted with water, whereby *N*-(pyridine-4-yl-methylene) quinuclidine-3-amine (C) precipitated out. It was then filtered and the product thus formed was recrystallized from ethanol to get 54.14% yield[12,13].



Scheme 1. Synthesis of *N*-(pyridine-4-yl-methylene) quinuclidine-3-amine (C).

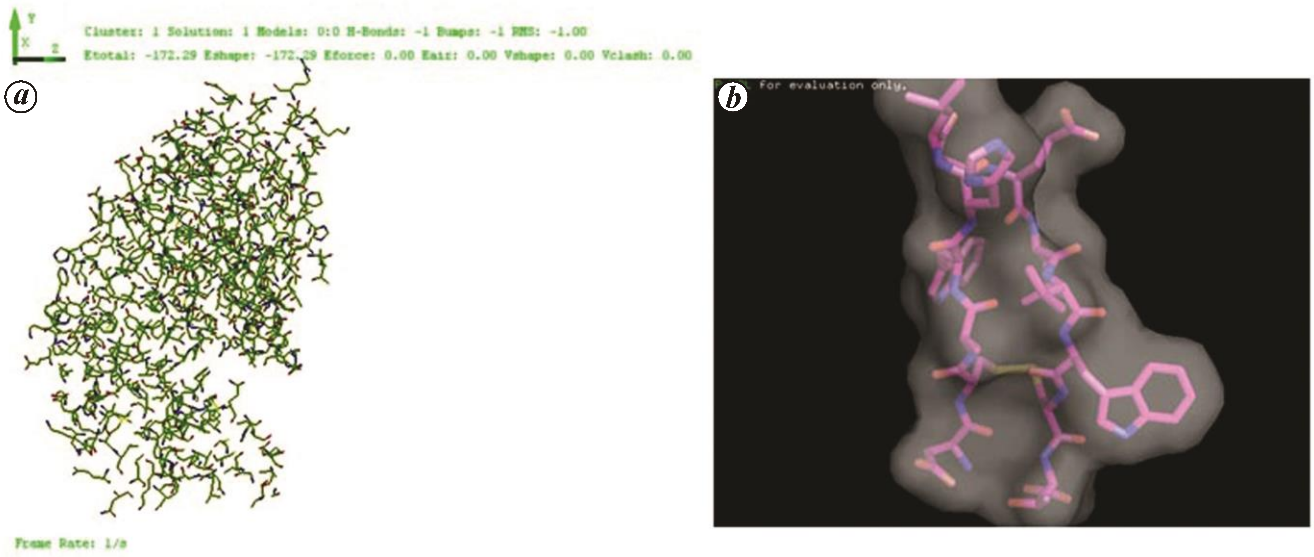


Figure 1 *a*, *b*. Result of docking study in 2D and 3D formats.

Melting point was found to be 78.5°C. The structure was determined from its characteristic ^{1}H, ^{13}C NMR, IR, mass spectral and microanalysis data.

The synthesized compound was evaluated for *in vitro* antimalarial activity against a chloroquine sensitive 3D7 strain of *Plasmodium falciparum*. Giemsa-stained slide method was adopted for the study and chloroquine was used as reference standard[14].

Continuous cultures of chloroquine-sensitive 3D7 strain of *P. falciparum* were maintained *in vitro* in O (Rh +ve) human red blood cells in RPMI-1640 medium supplemented with HEPES, D-glucose, sodium bicarbonate, gentamycin and 10% human AB (Rh +ve) serum according to standard protocol. Incubation was done at 37°C and 5% CO_2 level in a modular incubator. After 40 h of incubation, smears were prepared from each well, stained with 3% Giemsa and scanned under light microscope to ascertain percentage dead rings and tropozoites by examining a minimum of 100 asexual parasites[14]. Test results were compared with the standard results shown by chlroquine at IC_{50} dose. Each test compound was assayed in two replicates and counted against 100 asexual parasites (% dead ring + trophozoites) per replicate. The *in vitro* antimalarial activity data were analysed using the program HN-NonLin (version 1.1)[15–18].

The receptor was downloaded from RCSB Protein Data Bank (PDB) and made free from the ligand using Ligandscout (version 3.0). The energy of the PDB structure was minimized using Argus Lab (version 4.0.1). The water molecules were removed from the structure and the hydrogen atoms were merged.

Derivatives of ligand molecules were generated using Argus Lab. Two-dimensional structure was converted to 3D structure using the module Chem 3-D ultra 8.0 of ChemOffice 2004. Swiss PDB viewer was used to convert structures to compatible PDB format, and docking study was carried out using Hex (version 6.3)[19,20].

The scheme for the synthesis of the compound was exogitated. According to the route of the scheme, the Schiff base *N*-(pyridine-4-yl-methylene) quinuclidine-3-amine (C) was obtained by refluxing equimolar quantities of 3-aminoquinuclidine (A) and 4-pyridine carboxaldehyde (B) in ethanol in a water bath for 2–5 h. The Schiff base thus formed was precipitated on cooling. It was then filtered, purified by recrystallization[13] and its characteristic spectral data were recorded. Spectral analysis of the synthesized compound (C) confirmed the structure, which was then compared with the predicted structure[21].

Spectral data of Schiff base are as follows: (i) ^{1}H NMR (d ppm) ($CDCl_3$): 8.53 (d, J = 6 Hz, 2H-aromatic

–N=C*H*–), 7.54 (d, J = 6 Hz, 2H-aromatic =CH–C*H*=), 7.5 (s, 1H-aldemine–C*H*=), 3.58–3.66 (m, 4H-quinuclidine-N–CH_2–), 3.32–3.33 (d, J = 2Hz, 2H-quinuclidine–CH_2–), 1.68 (m, 2H-quinuclidine bridge head ≡C*H*), 1.41 (m, 4H, quinuclidine–CH_2–CH_2–N=); (ii) ^{13}C NMR ((d ppm) ($CDCl_3$)): 151.13, 150.40, 148.59, 121.53, 95.55, 52.56, 48.49, 47.36, 46.79; (iii) FT-IR (ν cm^{-1}) (MeOH): 2949 (CH-stretch), 1655 (C=N stretch), 1413–1452 (CH-bending); (iv) Mass (m/z): 215 (M^+); (v) Microanalysis: molecular formula $C_{13}H_{17}N_3$; required, C: 72.51, H: 7.9, N: 19.52; found, C: 72.50, H: 7.61, N: 19.33.

After synthesis, docking study was performed between *N*-(pyridine-4-yl-methylene) quinuclidine-3-amine (C) and antimalarial target plasmepsin II using docking software Hex (version 5.0). The docking study exhibited high binding affinity with *E* value of –172.29 kcal mol^{-1} (Figure 1 *a* and *b*).

The *in vitro* antimalarial activity study of synthesized compound exhibited EC_{50} of 13.125 µg/ml and correlation coefficient (R^2) 0.9818, whereas chloroquine exhibited EC_{50} of 5.144 µg/ml and $R^2 = 0.9570$. The correlation coefficient indicated good fit of the regression to the data obtained from the study and the polar nature of the compound would have an influence on binding with the receptor, resulting in better antimalarial activity. The results of the anti-malarial activity of the compound under discussion support those of the docking study.

Complete list of physical data, spectral values, viz. ^{1}H NMR, ^{13}C NMR, FTIR, mass recorded spectra. HPLC spectra of the synthesized Schiff base have been given as supporting information (see online).

Based on docking experiment, the designed Schiff base was found to exhibit antimalarial activity and the corelation coefficient confirmed good fit of the data. The antimalarial activity study may be carried out with chloroquine-resistant *P. falciparum*. The data generated in this study may be utilized for the synthesis and both *in vitro* and *in vivo* antimalarial activity studies of its analogues. The results of this study would open new avenues for further research.

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