Experimental Parasitology 163 (2016) 38–45

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Design, synthesis and antimalarial screening of some hybrid 4-aminoquinoline-triazine derivatives against *pf*-DHFR-TS

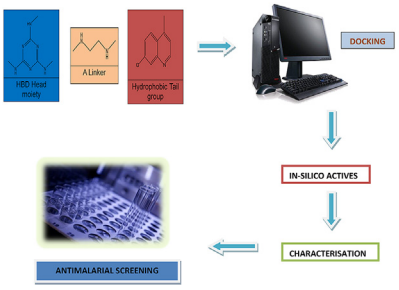
Supriya Sahu [a, *], Surajit Kumar Ghosh [a], Junmoni Kalita [a], Mayurakhi Dutta [b], Hans Raj Bhat [c]

[a] Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam 786004, India
[b] Department of Pharmacy, Assam University, Silchar, Assam 788011, India
[c] Department of Pharmaceutical Sciences, Sam Higginbottom Institute of Agriculture, Technology & Science, Deemed University, Allahabad 211007 India

HIGHLIGHTS

- Compound S1 showed encouraging IC_{50} value against 3D7 strain of *Plasmodium falciparum.*
- Presence of primary, secondary and tertiary amine in S1 made it most active.
- S1 showed favourable interaction with Met55, Phe58 and Leu164.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form
15 January 2016
Accepted 20 January 2016
Available online 25 January 2016

Keywords:
Antimalarial
Docking
Hybridisation
Triazine
Aminoquinoline

ABSTRACT

Existing antifolate antimalarial drugs have shown resistance due to the mutations at some amino acid positions of *Plasmodium falciparum* DHFR-TS. In the present study, to overcome this resistance, a new series of hybrid 4-aminoquinoline-triazine derivatives were designed and docked into the active site of *Pf*-DHFR-TS (PDB i.d. 1J3K) using validated CDOCKER protocol. Binding energy was calculated by applying CHARMm forcefield. Binding energy and the pattern of interaction of the docked compounds were analysed. Fifteen compounds were selected for synthesis based on their binding energy values and docking poses. Synthesized compounds were characterised by FTIR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and were screened for antimalarial activity against 3D7 strain of *Plasmodium falciparum.*

1. Introduction

Malaria, the most threatening parasitic infection in human has been a real concern for centuries. 300–500 million new clinical cases are reported by the World Health Organization (WHO) every year, resulting in annual deaths of about one million people. According to the World Malaria Report 2014, there are 104 countries and territories in total where malaria is presently considered endemic. Globally, an estimated 3.4 billion people are at risk of malaria. WHO estimates that there are 198 million cases of malaria leading to 584,000 deaths globally. Most cases (80%) and deaths (90%) occurred in Africa and most deaths (77%) were in children

* Corresponding author.
E-mail address: supsjrt@gmail.com (S. Sahu).

http://dx.doi.org/10.1016/j.exppara.2016.01.010
0014-4894/

below 5 years of age (World Health Organi, 2014).

For several decades, the most wonderful drug for the treatment of malaria was chloroquine (Fidock et al., 2004). *Chloroquine (CQ)* was introduced in 1944–1945 and because of its cheapness, non-toxicity and activity against all strains of malaria parasite, soon it became the mainstay of therapy and prevention. The main classes of active antimalarials are 4-aminoquinolines, aryl-alcohols including quinoline alcohols, antifolates that inhibit the synthesis of parasitic pyrimidines, artemisinin and its semisynthetic and synthetic analogs which cause parasite death due to oxidative stress produced by breakage of the endoperoxide ring present therein (Robert et al., 2001). Amongst the currently used clinical antimalarial drugs, the antifolates have the best defined molecular targets: enzymes dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), functioning in the folate metabolic pathway (Hyde, 2005). Folate metabolism is important for the viability of malaria parasites. These two pathways have been targeted in both treatment and prophylaxis of the disease. The most widely used antifolate antimalarial drugs include pyrimethamine (PYR), proguanil, sulfadoxine (SDX) and dapsone, which have long provided chemotherapy at an affordable price to the poorer nations (Sibley et al., 2001).

4-aminoquinoline, the nucleus of CQ and triazine which is the nucleus of clociguanil when joined together with a linker meets the entire structural requirement such as the presence of a hydrophobic tail and hydrogen bond donor head group respectively for inhibition of *pf*-DHFR-TS (Legesse and Prasad, 2011). Recently, conjugates of 4-aminoquinoline and 1,3,5-triazine have been widely studied as novel *pf*-DHFR-TS inhibitors (Bhat et al., 2013a; Kumar et al., 2008; Kumar et al., 2011; Manohar et al., 2010; Sharma et al., 2012). As a part of our ongoing research work to develop hybrid antimalarial molecules, we have designed a new series of hybrid 4-aminoquinolne-triazine derivatives. Based on the *in-silico* results, some selected molecules were tested for antimalarial activity against 3D7 strain of *Plasmodium falciparum.*

2. Materials and methods

2.1. Receptor preparation and docking study

The crystal structure of quadruple mutant *Pf*-DHFR-TS complex was obtained from Protein Data Bank. In the protein workspace of Accelrys Discovery Studio Version 2.5, water molecules, co-crystallized ligand WR99210 were removed and cofactors NADPH, dUMP were retained. *Pf*-DHFR-TS consist of four chains A, chain B, chain C and chain D out of which chain C & chain D are of TS domain and chain A & chain B are of DHFR domain. Since the prototype WR99210 was bound to chain A, so only chain A of the protein was used in the present work. This refined protein was simulated in the workspace by applying CHARMm forcefield and finally binding site was defined as sphere (28.0015, 5.89121, 59.8342, 16.1) around the active site of chain A.

A virtual library of hybrid 4-aminoquinolne-triazine was designed (Table 2) by joining both the nuclei with ethylenediamine and substituting the chlorines of cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) with different amines for improved H-bonding with target protein.

Docking was done using CDOCKER of Accelrys Discovery Studio version 2.5. The centre of co-crystallized ligand WR99210 was selected as the binding site for all calculations. The protocol was validated by calculating RMSD between five docked poses of WR99210 and ligand's X-ray docking pose. The X-ray pose of WR99210 was taken as reference. Docking is considered to be successful if the RMSD value is less than 2 Å (Guosheng et al., 2003). Ligands prepared were docked at the active site of the prepared protein and finally binding energy of the ligand–protein complex was calculated by using 'Calculate Binding Energy' protocol which uses CHARMm implicit solvent model.

Table 1
Heavy Atom RMSD to WR99210 X-ray pose.

Pose	RMSD (Å)
1	0.9770
2	0.9711
3	1.5187
4	1.3163
5	1.1196
WR99210	0.0000

2.2. Chemistry

All the chemicals and solvents used for synthesis, recrystallization and analysis were of AR grade and used without further purification. Melting point of the synthesized compounds was determined by Melting Point apparatus (BUCHI Melting Point M–560) at 10 °C/min temperature gradient. The UV-Spectra (λ_{max}) of the synthesized compounds were recorded on Shimadzu, UV-1800, UV-VIS spectrophotometer instrument. The FTIR spectra of the synthesized compounds were recorded on Bruker ALPHA FTIR spectrometer. Infrared spectra of compounds showed absorption bands which are characteristic of the anticipated structure of the synthesized compounds. The ^{1}H NMR spectra of the synthesized compounds were recorded in DMSO at 300 MHz by Bruker Avance DPX 300 NMR spectrometer and ^{13}C NMR was also recorded in DMSO at 100 MHz by Bruker Avance DPX 100 NMR spectrometer. The mass spectra of the synthesized compounds were recorded on ZQ-4000 equipped with an Electrospray Ionizer as an ionization method.

The synthesis of the intermediates and the final compounds was achieved by the protocol shown in Scheme 1. Compound 3 was synthesized by substituting the chlorine in fourth position of 4,7-dicholoroquinoline ring by ethylenediamine under neat condition. First and second chlorine of cyanuric chloride was substituted by different amines at 0–5 °C and at room temperature respectively. Final compounds were obtained by nucleophilic substitution of disubstituted cyanuric chloride with compound 3 at 100–120 °C.

2.2.1. Synthesis of N-(2-aminoethyl)-7-chloroquinolin-4-amine

The compound was prepared by adding ethylenediamine to melted 4,7-dichloroquinoline. This was first heated at 80 °C for 1 h and then refluxed at 120–130 °C for 2–3 h. The reaction mixture was then cooled to room temperature over a long period of time. It was then dissolved in dichloromethane and cold water was added to it. Brine solution was also added. The mixture was stirred with a glass rod and kept overnight without disturbance for settling the solid product in between the two solvent layers (colour of the solution changes from yellow to dark pink over night).The solid product was separated out by filtration. It was then dried in hot air oven at 45 °C. The dried powder was then dissolved in n-butanol and filtered. The filtrate was poured in a petridish and n-butanol was evaporated. After complete evaporation of the solvent, pale yellow crystals appeared.

2.2.2. General procedure for synthesis of mono amino-substituted 1,3,5-triazine derivatives

Cyanuric chloride was dissolved in quantity sufficient diethyl ether. It was then cooled to 0–5 °C. To this cold solution, ammonia was added and stirred for 3 h till the solution turned milky. It was then filtered and the filtrate was kept for solvent evaporation.