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Amalgamating Isatin/Indole/Nitro-imidazole with 7-chloroquinolines *via* azide-alkyne cycloaddition: Synthesis, anti-plasmodial and cytotoxic evaluation

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Abstract: The present paper describes the synthesis, anti-plasmodial and cytotoxic evaluation of 7-chloroquinoline-based conjugates with isatins/indoles/ nitroimidazoles, obtained *via* Cu-promoted 1,3-dipolar cycloadditions. On contemplating SAR of the synthesized series, the inclusion of indole and nitroimidazole-core improved the anti-plasmodial activities while the isatin seemed to have a lesser effect. The conjugate with a nitroimidazole-core and hexyl chain length as a spacer between the two pharmacophores was found to be most potent among the synthesized series and displayed an IC_{50} of 0.12 μM and a selectivity index of 1748.

Keywords: 7-chloroquinoline-based Conjugates, Azide-Alkyne Cyclo-addition, Antiplasmodial activity, Cytotoxicity

Introduction

Malaria is one of the most devastating and deadliest diseases caused by parasite *Plasmodium falciparum* and transmitted *via* the bite of a female mosquito, anopheles in humans. Even after a decade of progressive efforts, the disease remains most prevalent and pernicious. According to World Malaria Report 2018, an estimated 219 million malaria cases were witnessed with 4,35,000 deaths in 2017 with an increase in 3.5 million cases compared to the previous year in African countries (1). *P. falciparum*, is responsible for more than 90% of malaria-related deaths annually despite the accessibility of an arsenal of drugs (2). In the fight against malaria, the development of 4-aminoquinoline (CQ) core had a deep impact because of its rapid onset of action, good tolerability, limited toxicity and low cost (3). However, the emergence of CQ-resistance in the 1960s paved the way for the development of Artemisinin Combination Therapy (ACT), worldwide (4,5). Unfortunately, the development of resistance to ACTs in Southeast Asia along with the lack of effective vaccine has called for urgent efforts to enrich the chemical libraries with new scaffolds with potent anti-plasmodial activities and low incidence of resistance (6).

Literary survey on 4-aminoquinolines suggested the fact that the CQ-resistance is compound specific and a large number of 4-aminoquinoline derivatives developed *via* synthetic modification of CQ-side chain have shown improved anti-plasmodial activities against drug resistant strains (7,8). 4-aminoquinoline-hybridization involving conjugation of various pharmacophores with 4-aminoquinoline is one of the emerging strategy for the development of new molecular frameworks with promising anti-plasmodial potential (9,10).

Indole, a five-membered pyrrole ring fused with benzene ring, is considered as promising drug candidate due to diverse biological properties that include anti-inflammatory (11), anti-HIV (12), anti-tubercular (13), anti-malarial (14), anti-convulsant (15), anti-diabetic (16), anti-microbial (17), anti-cancer (18), anti-oxidant (19), and anti-fungal agents (20). Currently, an indole-based compound, NITD609 (**I**) has been proposed as a promising candidate against Falciparum malaria and is currently in Phase II clinical trial (21). Aspidocarpine (**II**), isolated from trunk bark of *Aspidosperma vargasii*, is an indole based plant alkaloid with significant antiplasmodial efficacy against CQ-resistant K1 strain of *P. falciparum* (22). Thakur *et al* has recently disclosed the promising anti-plasmodial activities of a series of N^1-alkylated-3-β-C-glycoconjugate-oxopropylidene oxindoles (**III**) against 3D7 and K1 strains of *P. falciparum* (23). Recently we have disclosed the synthesis and antiplasmodial activities of isatin-7-chloroquinoline and 3-hydroxy-oxindole-7-chloroquinoline conjugates. Both have shown good activities against CQ-resistant W2 strain, while 3-hydroxy-oxindole based conjugate (**IV**) proved to

have better anti-plasmodial activities than isatin-analogues (24). Kondaparla *et al* synthesized a series of 4-aminoquinoline-imidazole derivatives (**V**) by utilizing Van Leusen multi-component approach and assessed for their antiplasmodial activity against CQ-sensitive (3D7) and CQ-resistant (K1) strains. Some of the potent compounds displayed comparable efficacy against K1 strain of *P. falciparum* along high selectivity index (**Figure 1**) (25). Few representatives of isatin/indole based molecules with significant antiplasmodial activities are depicted in **Figure 1**.

Thus, in continuation of our pursuit for synthesizing and evaluating new molecular conjugates with promising anti-plasmodial potential (26), the present work describes the synthesis of 7-chloroquinolines linked with different heterocyclic scaffolds *viz.* isatins, nitroimidazole, and indoles *via* Cu-prompted azide-alkyne cycloaddition reaction along with their anti-plasmodial evaluation against CQ-R W2 strain of *P. falciparum* as well as cytotoxic studies on mammalian Vero Kidney cells.

Experimental Section

Materials and Methods

Instrumentation: ^{1}H NMR spectra were recorded in deuterochloroform and dimethylsulfoxide-d_6 with a Jeol 400 (400 MHz) and Bruker 500 (500 MHz) spectrometer using TMS as an internal standard. Chemical shift values are expressed as parts per million downfield from TMS and J values are in hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. ^{13}C NMR spectra were recorded on Jeol 400 (100MHz) and Bruker 500 (125 MHz) spectrometer in dimethylsulfoxide using TMS as internal standard. High resolution mass spectra were recorded on Bruker-micrOTOF-Q II spectrometer.

General procedure for the preparation of 7-Chloroquinoline-Isatin/Indole/nitroimidazole conjugates (7-**9**): To the well stirred solution of **3** (1 mmol) and propargylated isatin/indole/nitroimidazole **4-6** (1 mmol) in 20 mL EtOH:H_2O (9:1) mixture was added in succession copper sulphate (0.055 mmol) and sodium ascorbate (0.143 mmol). The reaction mixture was stirred for 7-8 h at room temperature. After completion of the reaction, as monitored by TLC, water and ethyl acetate were added in the reaction mixture and organic layer was extracted and combined, dried over sodium sulphate and concentrated under reduced pressure to result in the isolation of the crude

product. The pure product was obtained *via* recrystallization using chloroform: methanol (90:10) mixture.

Biological evaluations

Methods for assessment of anti-malarial activity of test compounds

The W2 strain of *P. falciparum* was cultured at 37°C in human red blood cells at 2% hematocrit in medium RPMI-1640 supplemented with 0.5% Albumax®, 100 uM hypoxanthine, 2 μM L-glutamine and 25 μM HEPES pH 7.4 under atmosphere of 3% O_2, 5% CO_2 balance nitrogen. The parasites were synchronized with 5% D-sorbitol at ring stage (27) and incubated with different concentrations of compounds for 48 h. The compounds were added from DMSO stocks; the maximum concentration of DMSO used was 0.1 %. Controls without inhibitors included 0.1% DMSO. After 48 h, when control cultures had progressed to new rings, the culture was fixed for 48 h by adding equal volume of 2% formaldehyde in PBS, pH 7.4, at room temperature. Fixed parasites were then transferred to 0.1% Triton X-100 in PBS containing 100 μM ammonium chloride and 1 nM YOYO-1 dye (Molecular Probes). Parasitemia was determined from dot plots (forward scatter *vs.* fluorescence) acquired on a FACSort flow cytometer using Cell Quest software (Beckton Dickinson). IC_{50} values for growth inhibition were determined from plots of percent control parasitemia over inhibitor concentration using the Prism 3.0 program, (GraphPad Software), with data from duplicate experiments fitted by non linear regression (28).

Method for assessment of *in vitro* cytotoxicity of test compounds

Cell viability was determined using Vero cells (ATCC, Sigma,Germany) grown in RPMI medium (Gibco, USA), supplemented with 10% decomplemented fetal calf serum, under a 5% CO_2 atmosphere. Cells were seeded in 96-well plates at a density of 2104 cells per well in 160 mL medium and incubated overnight at 37°C to allow cells to adhere. Compounds (dissolved in DMSO) were freshly diluted to appropriate concentrations in RPMI, so as to allow addition of 20 mL volumes of the diluted compounds to the cells that resulted in final compound concentrations ranging from 100 μg/mL to 0.78 μg/mL. The maximum final concentration of DMSO was 1% (v/v) and nocytotoxic effect of DMSO was observed at this concentration. After 24 h incubation at 37°C, 20 mL of 1 μg/mL resazurin (Sigma, Germany) was added to each well and the cells were incubated for an additional 3 hours at 37°C. Fluorescence was measured in a Polarstar Omega fluorometer using appropriate filters (540 nm excitation and 590 nm emission wave length).

Percentage survival was determined by dividing fluorescence values obtained in the compound containing wells by values obtained for control wells containing cells incubated with a dilution series of DMSO and multiplying this value by 100. SDS (20%) was included as a positive control. Cytotoxic evaluation was completed twice, with each compound tested induplicate. The IC_{50} is defined as the lowest concentration of compound tested at which exactly 50% cell viability was observed and was calculated using a non-linear regression curve using Graphpad Prism 5. The SI values were determined as a function of IC_{50}/MIC_{99}.

Result and Discussions

Chemistry

The synthesis of desired conjugates involved an initial preparation of precursor **3** obtained by reacting 4,7-dichloroquinoline **1** with an excess of different diamino alkanes at 120°C for 10-12 h to yield intermediate 4-amino-7-chloroquinolines **2**. The coupling of **2** with azido acetic acid using HOBt-EDC afforded 2-azido-*N*-(2-((7-chloroquinolin-4-yl)amino)alkyl)acetamide **3** (**Scheme 1**).

Another precursor *viz.* *N*-propargylated isatins/indole (**4**/**5**) was prepared *via* base assisted propargylation of isatin/indole with propargyl bromide in the presence of sodium hydride in DMF for 2-3 h at 60°C. Similar base-promoted (K_2CO_3) propargylation of 2-methyl-4/5-nitroimidazole yielded *N*-propargylated-2-methyl-4-nitroimidazole **6** as shown in **Scheme 2** (29).

Cu-promoted azide-alkyne cycloaddition reaction of **3** with *N*-propargylated isatin/indole/nitroimidazole (**4/5/6**) in EtOH:H_2O mixture for 7 h afforded 1*H*-1,2,3-triazole linked 4-aminoquinoline-isatin/indole/imidazole conjugates **7-9** (**Scheme 3**).

The structures to the conjugates were assigned on the basis of spectral data and analytical evidence. The compound **9b,** for example, showed a molecular ion peak M^+ at 483.1545 in its High Resolution Mass Spectrum (HRMS) while its ^1H NMR spectrum showed the presence of a singlet at δ 2.37 due to the presence of methyl group, two singlets at δ 5.08 and 5.33 due to presence of two methylenes, and characteristic singlets at δ 8.13 and 8.32 corresponding to a triazole and 4-nitroimidazole ring protons. Further, the appearance of characteristic signal at δ 165.8 due to carbonyl carbon along with the required number of carbons in its ^{13}C NMR spectrum confirmed the assigned structure.

In vitro anti-malarial and cytotoxic evaluation

The synthesized compounds were assessed for their anti-plasmodial activities against CQ-resistant W2 strain of *P. falciparum* and results are enlisted in **Table 1**. As evident, most of the synthesized compounds have shown activities in the sub-micromolar concentration and exhibit an interesting Structure Activity Relationship (SAR). Among 7-chloroquinoline-isatin conjugates **7a-c**, the compounds displayed activities in the range of 0.33-1.25 μM. The conjugates with shorter alkyl chain length **7a** and **7c**, having ethyl and butyl as spacer, exhibited better anti-plasmodial activities compared to the ones with longer chain lengths *i.e.* **7d** (hexyl) and **7e** (octyl); exception being **7b** (propyl) which exhibited lowest anti-plasmodial activity among the series. SAR analysis among 7-chloroquinoline-indole conjugates **8a-e** revealed reversal in the anti-plasmodial data with conjugates exhibiting good activities at longer alkyl chain length as evident by **8d** (hexyl) and **8e** (octyl) with IC_{50s} of 0.18 and 0.36 μM, respectively. The inclusion of nitroimidazole on the side chain of 7-chloroquinoline-core substantially enhanced the activity of the synthesized conjugates as shown by **9b**, **9d** and **9e**. SAR analysis of this series revealed that the observed activities were independent upon the length of alkyl chain used as spacer and the activities are in the range of 0.12-0.26 μM. A generalized SAR of the synthesized conjugates is depicted in **Figure 2**.

In order to confirm if the observed activities of the synthesized conjugates were because of their inherent anti-plasmodial activities, the cytotoxicity of most promising compounds *viz*. **8d**, **9b**, **9d**, **9e** was determined on mammalian Vero Kidney cells (**Table 2**). As evident, the promising conjugates were proved to be non-cytotoxic against mammalian Vero cells.

Conclusion

A series of 7-chloroquinoline-based conjugates with isatins/indoles/ nitroimidazoles were synthesized *via* Cu-promoted azide-alkyne cycloaddition reactions. The anti-plasmodial activities of the synthesized conjugates on the CQ-resistant W2 strain of *P. falciparum* indicated modest to good activities. Structure-Activity Relationship studies revealed the dependence of anti-plasmodial activities on alkyl chain length among quinoline-isatins and quinoline-indoles while no such role was noticed among quinoline-indoles. The most potent and non-cytotoxic conjugate **9d** with an optimum combination of a hexyl chain length as spacer along with a nitroimidazole pharmacophore proved to be most potent and non-cytotoxic and displayed an IC_{50} value of 0.12 μM along with a selectivity index of 1748.

Conflicts of interest

There are no conflicts to declare.

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Data Availability Statement

The details of characterisation of compounds **7a-7e, 8a-8e** and **9a-9e**, along with scanned ^{1}H and ^{13}C spectra of **7c**, **8c**, **8d**, **8e and 9b** are given in supplementary file.

CAPTIONS:

Figure 1: Structures of some Isatin/Indole based antiplasmodials

Figure 2: Generalized SAR of the synthesized conjugates

Scheme 1. Synthesis of 2-azido-*N*-(2-((7-chloroquinolin-4-yl)amino)alkyl)acetamide **3**

Scheme 2. Synthesis of precursor's *viz.* *N*-propargylated isatins/indole (**4**/**5**) and *N*-propargylated-2-methyl-4-nitroimidazole **6**

Scheme 3. Synthesis of desired conjugates *viz.* 1*H*-1,2,3-triazole linked 4-aminoquinoline based isatin/indole/imidazole conjugates **7-9**

Table 1. Anti-malarial activities of synthesized compounds against CQ-R W2 strains of *P. falciparum*

Table 2. Cytotoxicity and Selectivity index of potent conjugates

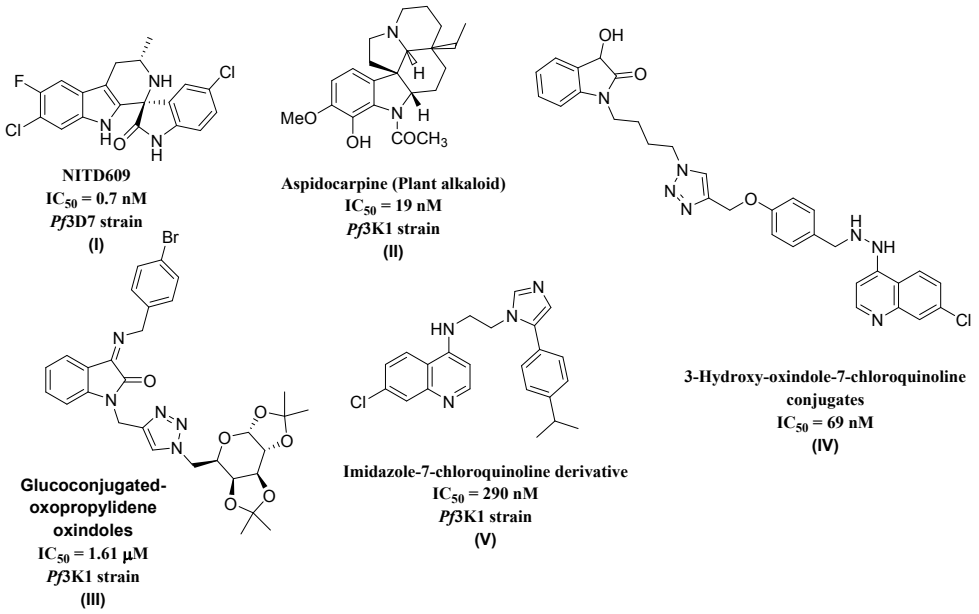


Figure 1: Structures of some Isatin/Indole based antiplasmodials

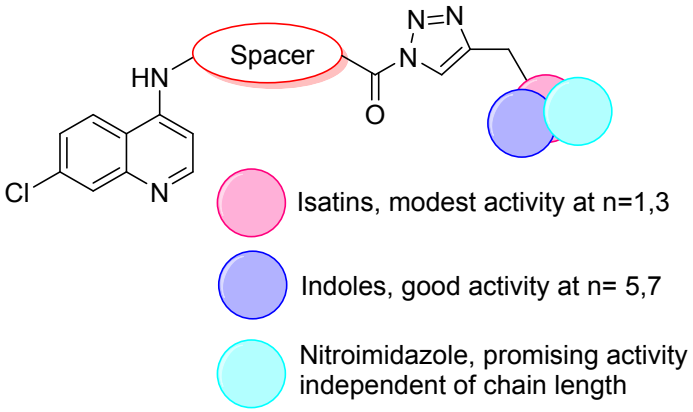
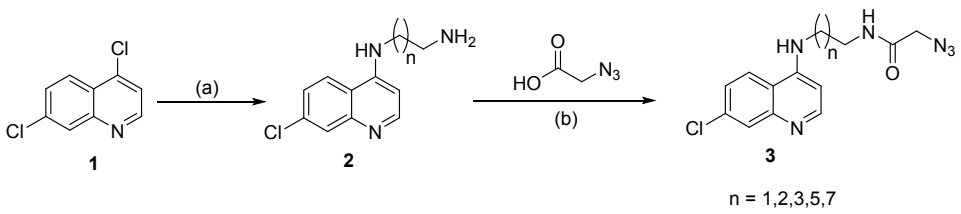
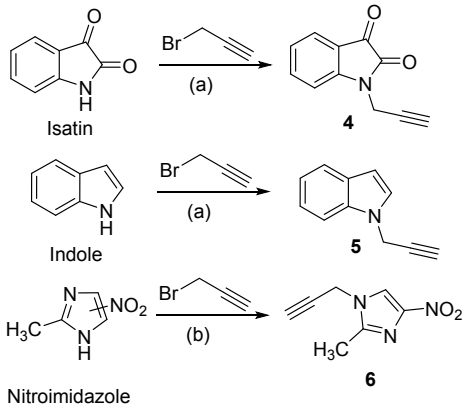


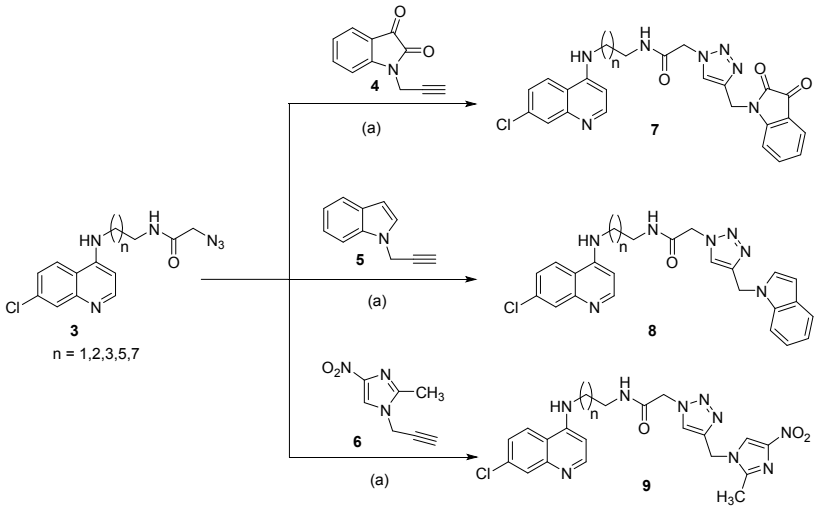
Figure 2: Generalized SAR of the synthesized conjugates



Scheme 1: Reagents and conditions: (a) Diamines, 120 °C, 10-12 h; (b) HOBt, EDC, Et_3N, Dry DCM, rt, 10 h



Scheme 2: Reagents and conditions: (a) NaH, Dry DMF, rt-60°C, 2-3 h (b) K_2CO_3, Dry DMF, rt



Scheme 3: Reagents and conditions: (a) $CuSO_4$, Sodium Ascorbate, EtOH:H_2O, rt, 7-8 h

Table 1: Anti-malarial activities of synthesized compounds against CQ-R W2 strains of *P. falciparum*

Compound	n	IC_{50} (μM)±SD
7a	1	0.33± 0.00
7b	2	1.25± 0.03
7c	3	0.39± 0.01
7d	5	1.09± 0.08
7e	7	1.12± 0.06

8a	1	1.24± 0.12
8b	2	1.20± 0.07
8c	3	0.29± 0.13
8d	5	0.18± 0.04
8e	7	0.36± 0.01
9a	1	ND
9b	2	0.18± 0.02
9c	3	ND
9d	5	0.12± 0.01
9e	7	0.26± 0.01
CQ		0.07± 0.00

Table 2: Cytotoxicity and Selectivity index of potent conjugates

Compound	**IC_{50} (μM) (Cytotoxicity)**	**IC_{50} (μM) (W2 strain *P. falciparum*)**	**SI**
8d	260.5	0.18	1447.2
9b	141.5	0.18	786.11
9d	209.8	0.12	1748.33
9e	151.3	0.26	581.92