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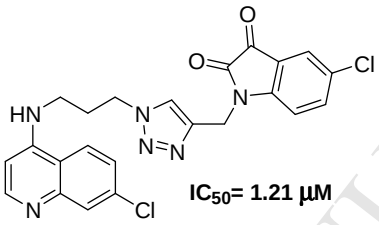

Fifteen 7-chloroquinoline-isatin conjugates have been synthesized and evaluated for their antiplasmodial profile. The compound **8h** with an optimum combination of longer alkyl chain length and chloro substitutent at C-5 position of isatin ring displayed the best activity among the test compounds.

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Abstract: We describe the synthesis and antimalarial activities of 1*H*-1,2,3-triazole tethered 7-chloroquinoline-isatin hybrids. Activity against cultured parasites was dependent on the C-5 substituent of the isatin ring as well as the alkyl chain length between the isatin and 7-chloroquinoline moieties. Compound **8h,** with an optimum alkyl chain length (n=3) and a chloro substitutent at the C-5 position of the isatin ring, displayed the best activity among the test compounds, with IC_{50} value of 1.21 μM against cultured W2-strain *Plasmodium falciparum*.

Key Words: 7-Chloroquinoline-Isatin conjugates- 1*H*-1,2,3-triazole - antimalarial evaluation- structure-activity relationship

1. Introduction

With approximately 500 million infections and 2.5 million deaths annually [1], malaria remains one of the most serious health problems of the developing world. The causative agent of the most lethal form of malaria, *Plasmodium falciparum,* has developed resistance to multiple drugs including chloroquine (CQ), previously the first line drug to treat malaria in most endemic countries [2]. CQ induces heme accumulation in the parasite digestive vacuole, leading to parasite death, possibly through degradation of parasite membranes [3]. CQ resistance is now very common, and largely due to mutations in the *P. falciparum* CQ resistance transporter (pfcrt) gene, which encodes the protein believed to mediate efflux of the drug from the digestive vacuole of the parasite, leading to sub-optimal drug concentrations [4]. The parasite has also developed resistance to other antimalarials, including cycloguanil and pyrimethamine, which are specific inhibitors of *P. falciparum* dihydrofolate reductase (PfDHFR) [5]. The most important newer drugs to treat malaria are artemisinin and its semi-synthetic derivatives, which offer rapid and potent antimalarial activity. These drugs are best used in combination with other agents as components of artemisinin-based combination therapy (ACT) [6]. However, the first cases of clinical resistance to artemisinins were reported recently from the Thai–Cambodian border, with

delayed clearance of parasites after treatment with ACTs, providing strong impetus for the development of new antimalarials [7].

The approaches currently being adopted to overcome the challenges of multi-drug resistance in *P. falciparum* include combination therapy, developing analogues of the existing drugs as well as drug resistance reversers [8]. In addition, molecular hybridization, which involves the rational design of new chemical entities by covalent fusion of two or more drugs, active compounds and/or pharmacophoric units with complimentary activities and multiple pharmacological targets, is an attractive strategy. Different and/or dual modes of action of hybrid components may optimize antimalarial efficacy and reduce undesired side effects [9]. This strategy has resulted in the development of trioxane-aminoquinoline [10], artemisinin-quinine [11] and ferrocene-CQ chimeras [12] with improved antimalarial activity compared to the parent drugs.

Isatin (1*H*-Indole-2,3-dione) is a promising new class of heterocyclic molecules with many promising activity profiles and good tolerance in humans [13,14]. The 2-oxoindole derivatives SU-5416 (semaxanib) and SU-11248 (sunitinib) reportedly have tyrosine kinase inhibitory and anti-angiogenic properties [15]. Schiff and Mannich bases of isatin are reported to exhibit broad antimicrobial properties, including antiviral [16], anti-mycobacterial [17], antifungal, and antibacterial activities [18]. A recent report by our group described the synthesis of 3-methylene-substituted indolinones and their antimalarial activities. Indolinones containing a Leu-*i*-amyl recognition moiety were found to be modest inhibitors of the *P. falciparum* cysteine protease falcipain-2, and displayed *in vitro* activity against the chloroquine-resistant *P. falciparum* W2 strain in the low micromolar range [19].

Recent disclosures from our laboratory have described the utilization of molecular hybridization towards the synthesis of β-lactam-7-chloroquinoline conjugates and evaluation of their antimalarial profiles. Activity against cultured *P. falciparum* was observed to be dependent on the *N*-substituent of the β-lactam ring as well as the presence of bis-triazole at the C-3 position. The observed activity profiles were further corroborated *via* docking simulations performed using the ligand fit module [20]. With our interest in the synthesis of novel molecular frameworks utilizing hybridization protocols [21], we sought to synthesize 1*H*-1,2,3-triazole-tethered-7-chloroquinoline-isatin conjugates with or without a well modulated alkyl chain, as depicted in **Figure 1,** and to evaluate their antimalarial activity. The triazole functionality was introduced because of its favourable properties, including

moderate dipole character, hydrogen bonding capability, rigidity and stability under *in vivo* conditions, which are evidently responsible for its diverse biological activities [22].

2. Result and Discussions

2.1 Synthetic chemistry

The synthetic protocol utilized for the synthesis of desired hybrids involved well established Cu-mediated click chemistry of *N*-propargylated isatins with varied substitutions and 7-chloroquinoline based azides with or without a spacer consisting of a well modulated alkyl chain length. The precursor, *N*-propargylated isatins **1** were synthesized by treating a solution of corresponding isatin in DMF with sodium hydride (NaH), resulting in the formation of intensely purple coloured anion that was subsequently reacted with propargyl bromide [23]. 4-Azido-7-chloroquinoline **2,** another precursor, was prepared by applying the method described by de Souza et al. [24], whereby 4,7-dichloroquinoline was reacted with 2 equiv of NaN_3 in anhydrous DMF at 65^0C for 6 h. The desired hybrids **3** were prepared *via* room temperature stirring of **1** with **2** in the presence of copper sulphate and sodium ascorbate in a C_2H_5OH:H_2O (90:10) mixture (**Scheme 1**).

(2-Azido-ethyl/propyl)-(7-chloro-quinolin-4-yl)-amine **7,** required for the synthesis of the desired hybrids, was prepared by initially reacting 4,7-dichloroquinoline with ethanolamine or 1-propanol amine at 120^0C for 12 h, followed by mesylation with methane sulphonyl chloride at 0^0C for 2-3h [25]. The mesylated product **6** was treated with sodium azide at 120^0C in dry DMF for 12h, resulting in the isolation of desired precursors **7** as depicted in **Scheme 2**. The targeted bifunctional hybrids **8a-j** were synthesized by room temperature stirring of precursors **7** and propargylated isatins **2** in a C_2H_5OH:H_2O (9:1) mixture in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate for 7 h. The progress of reactions was monitored using TLC. The reactions resulted in the isolation of crude products which were recrystallized using (9:1) $CHCl_3$:MeOH mixture.

The structure of the hybrids **3** and **8** were assigned on the basis of spectral data and analytical evidence. For example, **8c**, showed a molecular ion peak $[M+H]^+$ at 460.2432, along with characteristic peaks in ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum exhibited characteristic peaks at δ 3.71, 4.57 and 4.85 corresponding to methylene protons, a singlet at δ 7.77 corresponding to 1H of a triazole ring proton, along with the characterstic 7-chloroquinoline ring protons. The appearance of the requisite number of carbons in the ^{13}C

NMR spectrum along with the two characteristic peaks at δ 159.6 and 182.4 assigned to isatin ring carbonyls further corroborated the assigned structure.

2.2 Methods for assessment of antimalarial activity of test compounds

The W2 strain of *P. falciparum* was cultured in RPMI-1640 medium with 10% human serum, following standard methods, and parasites were synchronized with 5% D-sorbitol [26]. Beginning at the ring stage, microwell cultures were 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 medium was removed, and cultures were incubated for 48 h with 1% formaldehyde in PBS, pH 7.4, at room temperature. Fixed parasites were then transferred to 0.1% Triton X-100 in PBS containing 1nM 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 [27].

2.3 In vitro antiplasmodial activity

The test compounds were evaluated for their antimalarial profiles against the CQ resistant W2 strain of *P. falciparum*. The hybrids **3a-3e** were inactive at tested concentrations, while **8a-8j,** with varied alkyl chain length between the 7-chloroquinoline and isatin groups, showed better antiplasmodial activity (**Table 1**). Although the test compounds were not as active as standard drugs *viz.* chloroquine (CQ) and artemisinin (ART), an interesting structure-activity relationship (SAR) has been observed. Analysis of **Table 1** reveals the effects of the substituent on the C-5 position of isatin as well as the alkyl chain length on antimalarial activity. Comparing the bifunctional hybrids **8a-8e** (n=2), **8c,** with a chloro-substituent at the C-5 position of the isatin ring, displayed the best IC_{50} value (1.37 μM), while the introduction of a strong electron withdrawing fluoro substituent decreased activity, as evidenced by **8b**. The C-5 unsubstituted hybrid **8a** showed the least activity of this series against *P. falciparum*, with an IC_{50} of 3.07 μM. A similar comparison of series **8f-8j** (n=3) showed a similar trend, with **8h** (R=Cl) the most potent among the test compounds, with an IC_{50} of 1.21 μM. The C-5 unsubstituted hybrid **8f** again proved to be least active in the series in inhibiting plasmodial growth, with an IC_{50} of 3.85 μM, emphasizing the role of the

substituent at the C-5 position of the isatin ring. Thus, antimalarial efficacy showed improvement with increase in chain length, except in hybrids with unsubstituted isatin (**8a** and **8f)**.

In conclusion, we report the synthesis of 1*H*-1,2,3-triazole tethered isatin-7-chloroquinoline hybrids along with evaluation of their antiplasmodial activity. The activity profiles showed dependence on the substituents at the C-5 position of isatin as well as the length of the alkyl chain. Compound **8h,** with an optimum combination of longer alkyl chain length (n=3) and a chloro substitutent at the C-5 position of the isatin ring, displayed the best activity among the test compounds. Further work on the diversification of these conjugates *via* elaboration of alkyl chain length and introduction of halogen functionalities on the isatin ring is underway.

3. Experimental Section

Melting points were determined by open capillary using a Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 spectrophotometer. ^{1}H NMR spectra were recorded in deuterochloroform and dimethylsulfoxide-d_6 with a Jeol 300 (300 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 300 (75 MHz) and BRUKER AVANCE II (400 MHz) spectrometers in dimethylsulfoxide using TMS as internal standard. High resolution mass spectra were recorded on Bruker-micrOTOF-Q II spectrometer.

3.1 Typical procedure for the synthesis of isatin-7-Chloroquinoline conjugates (**3 or 8**): To the stirred solution of **1** (1mmol) and **2** or **7** (2mmol) in 20 mL ethanol-water (10:1) was added in succession copper sulphate (0.055 mmol) and sodium ascorbate (0.143 mmol) at room temperature. On completion of the reaction, as monitored by TLC, water (15 mL) was added to the reaction mixture and extracted with chloroform (2x50 mL). Combined oragnic layers were dried over anhydrous sodium sulphate and concentrated under reduce pressure to result in a crude product which was recrystallized using chloroform:methanol (9:1) mixture.

3.1.1 1-[1-(7-Chloro-quinolin-4-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione (**3a**): Red Solid; Yield: 85%; m.p. 240^{0}C (decomp.). IR (KBr) ν_{max}: 1741, 1609 cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 5.16 (s, 2H, -N-CH_2-); 7.16 (t, *J*=7.2Hz, 1H, ArH, H^7); 7.28 (d, *J*=7.8Hz, 1H, H^9); 7.60 (d, *J*=7.2Hz, 1H, H^6); 7.67 (t, *J*=7.8Hz, 1H, H^8); 7.79 (m, 1H, H^2+ H^3); 8.00 (d, *J*=9.0Hz, 1H, H^4); 8.27 (s, 1H, H^5); 8.90 (s, 1H, triazole H); 9.12 (d, *J*=4.5Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 35.2, 111.1, 111.2, 112.5, 116.6, 118.0, 120.3, 124.8, 125.3, 125.6, 128.5, 129.2, 135.4, 140.1, 142.3, 146.7, 149.8, 152.1, 158.9, 181.8. HRMS Calculated for $C_{20}H_{12}N_5O_2$ $[M + H]^+$ 390.7946 found 390.7941.

3.1.2 1-[1-(7-Chloro-quinolin-4-yl)-1H-[1,2,3]triazol-4-ylmethyl]-5-fluoro-1H-indole-2,3-dione (**3b**): Red Solid; Yield: 80%; m.p. 220^{0}C (decomp.). IR (KBr) ν_{max}: 1740, 1614 cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 5.16 (s, 2H, -N-CH_2-); 7.30 (dd, *J*=3.9, 8.4Hz, 1H, ArH, H^7); 7.50-7.59 (m, 2H, H^3+H^6); 7.77 (d, *J*=2.1Hz, 1H, H^9); 7.79 (d, *J*=4.5Hz, 1H, H^2); 8.00 (d, *J*=9.0Hz, 1H, H^4); 8.27 (d, *J*=2.1Hz, H^5); 8.89 (s, 1H, triazole H); 9.12 (d, *J*=4.5Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 35.0, 111.3, 111.7, 112.6, 116.8, 118.7, 120.0, 124.2, 125.4, 125.9, 128.1, 129.0, 135.4, 140.2, 142.5, 146.3, 149.4, 152.3, 158.0, 182.5. HRMS Calculated for $C_{20}H_{11}ClFN_5O_2$ $[M + H]^+$ 408.7850 found 408.7857.

3.1.3 5-Chloro-1-[1-(7-chloro-quinolin-4-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione (**3c**): Red Solid; Yield: 68%; m.p. 198^{0}C (decomp.). IR (KBr) ν_{max}: 1737, 1616 cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 5.10 (s, 2H, -N-CH_2-); 7.20 (d, *J*=8.4Hz, 1H, ArH, H^7); 7.42-7.51 (m, 2H, H^3+H^6); 7.72 (d, *J*=2.1Hz, 1H, H^9); 7.83 (d, *J*=5.1Hz, 1H, H^2); 8.01 (d, *J*=9.0Hz, 1H, H^4); 8.28 (d, *J*=2.1Hz, H^5); 8.90 (s, 1H, triazole H); 9.10 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 35.7, 111.6, 111.9, 112.4, 116.2, 118.8, 120.1, 124.8, 125.2, 125.4, 128.3, 129.1, 135.7, 140.0, 142.8, 146.7, 149.1, 152.7, 157.7, 181.8. HRMS Calculated for $C_{20}H_{11}Cl_2N_5O_2$ $[M + H]^+$ 425.2396 found 425.2390.

3.1.4 5-Bromo-1-[1-(7-chloro-quinolin-4-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione (**3d**): Red Solid; Yield: 71%; m.p. 205^{0}C (decomp.). IR (KBr) ν_{max}: 1732, 1611 cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 5.09 (s, 2H, -N-CH_2-); 7.19 (d, *J*=8.4Hz,1H, ArH, H^7); 7.39-7.48 (m, 2H, H^3+H^6); 7.68 (d, *J*=2.1Hz, 1H, H^9); 7.78 (d, *J*=5.1Hz, 1H, H^2); 7.99 (d, *J*=9.0Hz, 1H, H^4); 8.33 (d, *J*=2.1Hz, H^5); 8.95 (s, 1H, triazole H); 9.05 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 34.8, 111.1, 111.7,

112.5, 116.5, 118.4, 120.9, 124.3, 125.2, 125.6, 128.0, 129.7, 135.8, 140.1, 142.9, 146.2, 149.7, 152.2, 158.1, 182.3. HRMS Calculated for $C_{20}H_{11}BrClN_5O_2$ $[M + H]^+$ 469.6906 found 469.6901.

3.1.5 1-[1-(7-Chloro-quinolin-4-yl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-1H-indole-2,3-dione (**3e**): Red Solid; Yield: 80%; m.p. 189^0C (decomp.). IR (KBr) ν_{max}: 1740, 1614 cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 2.29 (s, 3H, -CH_3); 5.02 (s, 2H, -N-CH_2-); 7.24 (d 1H, *J*=8.4Hz, ArH, H^7); 7.48-7.57 (m, 2H, H^3+H^6); 7.74 (d, *J*=2.1Hz, 1H, H^9); 7.79 (d, *J*=4.5Hz, 1H, H^2); 8.10 (d, *J*=9.0Hz, 1H, H^4); 8.26 (d, *J*=2.1Hz, H^5); 8.90 (s, 1H, triazole H); 9.04 (d, *J*=4.5Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 21.8, 35.1, 111.4, 111.8, 112.3, 116.5, 118.7, 120.2, 124.1, 125.3, 125.5, 128.9, 129.6, 135.2, 140.0, 142.7, 146.8, 149.6, 152.1, 158.3, 182.1. HRMS Calculated for $C_{21}H_{14}ClN_5O_2$ $[M + H]^+$ 404.8212 found 404.8219.

3.1.6 1-{1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**8a**): Red Solid; Yield: 78%; m.p. 163^0C (decomp.). IR (KBr) ν_{max}: 3328 (N-H), 1737 (C=O), 1610 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 3.79-3.80 (m, 2H, -N-CH_2-); 4.64-4.66 (m, 2H, -N-CH_2-); 4.95 (s, 2H, -N-CH_2-); 6.36 (d, *J*=5.1Hz, 1H, H^2); 7.09 (t, *J*=7.5Hz, 1H, ArH); 7.26-7.32 (m, 3H, ArH+ H^4+NH exchangeable with D_2O); 7.47-7.55 (m, 2H, ArH); 7.77 (s, 1H, H^5); 7.91 (s, 1H, triazole H); 8.01 (d, *J*=6.9Hz, 1H, H^3); 8.33 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 35.2, 42.6, 48.5, 98.0, 110.8, 112.3, 117.5, 118.0, 123.4, 123.7, 124.3, 124.5, 126.6, 133.1, 141.4, 146.5, 148.8, 149.9, 151.3, 157.2, 159.7, 182.2. HRMS Calculated for $C_{22}H_{17}ClN_6O_2$ $[M + H]^+$ 424.7909 found 424.7902.

3.1.7 1-{1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-5-fluoro-1H-indole-2,3-dione (**8b**): Red Solid; Yield: 62%; m.p. 170^0C (decomp.). IR (KBr) ν_{max}: 3355 (N-H), 1732 (C=O), 1616 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 3.71-3.73 (m, 2H, -N-CH_2-); 4.57-4.59 (m, 2H, -N-CH_2-); 4.83 (s, 2H, -N-CH_2-); 6.21 (d, *J*=5.4Hz, 1H, H^2); 7.01 (dd, 1H, *J*=8.4, 1.8Hz, 1H, ArH, H^7); 7.10 (s, NH exchangeable with D_2O); 7.15 (d, *J*=8.4Hz, 1H, ArH, H^6); 7.21 (d, *J*=9.0Hz, 1H, H^4); 7.61 (d, *J*=1.8Hz, 1H, ArH, H^9); 7.71 (s, 1H, H^5); 7.85 (s, 1H, triazole H); 7.91 (d, *J*=9.0Hz, 1H, H^3); 8.21 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 100MHz): δ ppm = 35.4, 42.5, 48.4, 98.8, 111.1, 112.8, 117.4, 118.8, 123.0, 123.3, 124.6, 124.9, 126.5, 133.9, 141.1, 146.6, 147.8, 149.5,

150.6, 157.9, 159.7, 182.4. HRMS Calculated for $C_{22}H_{16}ClFN_6O_2$ $[M + H]^+$ 443.7893 found 443.7899.

3.1.8 5-Chloro-1-{1-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**8c**): Red Solid; Yield: 71%; m.p. 177^{0}C (decomp.). IR (KBr) ν_{max}: 3342 (N-H), 1732 (C=O), 1616 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 3.70-3.72 (m, 2H, -N-CH_2-); 4.55-4.59 (m, 2H, -N-CH_2-); 4.85 (s, 2H, -N-CH_2-); 6.22 (d, *J*=5.4Hz, 1H, H^2); 7.00 (d, 1H, *J*=8.4Hz, 1H, ArH, H^7); 7.10 (s, NH exchangeable with D_2O); 7.21 (d, *J*=8.7Hz, 1H, H^4); 7.35 (d, *J*=8.4Hz, 1H, ArH, H^6); 7.40 (d, *J*=1.8Hz, 1H, ArH, H^9); 7.68 (s, 1H, H^5); 7.77 (s, 1H, triazole H); 7.91 (d, *J*=8.7Hz, 1H, H^3); 8.21 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 100MHz): δ ppm = 34.9, 42.1, 48.0, 98.5, 111.5, 112.4, 117.1, 118.3, 123.8, 123.9, 124.0, 124.2, 126.9, 133.7, 141.1, 146.2, 148.2, 149.9, 151.2, 157.6, 159.6, 182.4. HRMS Calculated for $C_{22}H_{16}Cl_2N_6O_2$ $[M + H]^+$ 460.2439 found 460.2432.

3.1.9 5-Bromo-1-{1-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**8d**): Red Solid; Yield: 60%; m.p. 185^{0}C (decomp.). IR (KBr) ν_{max}: 3370 (N-H), 1739(C=O), 1609 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 3.71-3.73 (m, 2H, -N-CH_2-); 4.58-4.60 (m, 2H, -N-CH_2-); 4.82 (s, 2H, -N-CH_2-); 6.20 (d, *J*=5.4Hz, 1H, H^2); 7.01 (dd, 1H, *J*=8.4,1.8Hz, 1H, ArH, H^7); 7.09 (s, NH exchangeable with D_2O); 7.22 (d, *J*=8.7Hz, 1H, H^4); 7.37 (d, *J*=8.4Hz, 1H, ArH, H^6); 7.41 (d, *J*=1.8Hz, 1H, ArH, H^9); 7.70 (s, 1H, H^5); 7.79 (s, 1H, triazole H); 7.93 (d, *J*=8.7Hz, 1H, H^3); 8.22 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 35.4, 42.7, 48.4, 98.5, 111.1, 112.1, 117.1, 118.9, 123.2, 123.5, 124.3, 124.7, 126.1, 133.4, 141.2, 146.4, 148.7, 149.8, 151.5, 157.8, 159.6, 182.8. HRMS Calculated for $C_{22}H_{16}ClBrN_6O_2$ $[M + H]^+$ 504.6949 found 504.6945.

3.1.10 1-{1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-5-methyl-1H-indole-2,3-dione (8e): Red Solid; Yield: 60%; m.p. 169^{0}C (decomp.). IR (KBr) ν_{max}: 3327 (N-H), 1739 (C=O), 1609 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 2.31 (s, 3H, -CH_3); 3.71-3.74 (m, 2H, -N-CH_2-); 4.57-4.59 (m, 2H, -N-CH_2-); 4.82 (s, 2H, -N-CH_2-); 6.21 (d, *J*=5.4Hz, 1H, H^2); 7.01 (d, 1H, *J*=8.4Hz, 1H, ArH, H^7); 7.09 (s, NH exchangeable with D_2O); 7.22 (d, *J*=8.7Hz, 1H, H^4); 7.35-7.38 (d, *J*=8.4Hz, 1H, ArH, H^6); 7.41 (d, *J*=1.8Hz, 1H, ArH, H^9); 7.70 (s, 1H, H^5); 7.79 (s, 1H, triazole H); 7.92-7.95 (d, *J*=8.7Hz, 1H, H^3); 8.22 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 22.2, 34.8, 42.2, 48.0, 98.3, 111.2, 112.5, 117.5, 118.2, 123.2, 123.6, 124.4, 124.8, 126.7, 133.5,

141.2, 146.1, 147.9, 149.4, 151.2, 158.1, 159.7, 181.8. HRMS Calculated for $C_{23}H_{19}ClN_6O_2$ $[M + H]^+$ 439.8254 found 439.8259.

3.1.11 1-{1-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**8f**): Red Solid; Yield: 71%; m.p. 171^{0}C (decomp.). IR (KBr) ν_{max}: 3364 (N-H), 1740 (C=O), 1611 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 2.23-2.27 (m, 2H, -CH_2-); 3.78-3.80 (m, 2H, -N-CH_2-); 4.63-4.65 (m, 2H, -N-CH_2-); 4.89 (s, 2H, -N-CH_2-); 6.36 (d, *J*=5.1Hz, 1H, H^2); 7.10 (t, 1H, *J*=7.2Hz, 1H, ArH); 7.27-7.34 (m, 3H, ArH+H^4+NH exchangeable with D_2O); 7.50-7.58 (m, 2H, ArH); 7.79 (s, 1H, H^5); 7.98 (s, 1H, triazole H); 8.18 (d, *J*=6.9 Hz, 1H, H^3); 8.40 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 30.1, 36.0, 42.9, 48.8, 98.3, 111.2, 112.7, 117.5, 118.5, 123.2, 123.9, 124.4, 124.6, 126.4, 133.2, 141.6, 146.8, 148.3, 149.5, 151.6, 157.4, 159.9, 182.9. HRMS Calculated for $C_{23}H_{19}ClN_6O_2$ $[M + H]^+$ 447.8890 found 447.8895.

3.1.12 1-{1-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-1H-[1,2,3]triazol-4-ylmethyl}-5-fluoro-1H-indole-2,3-dione (**8g**): Red Solid; Yield: 69%; m.p. 150-151^{0}C. IR (KBr) ν_{max}: 3351 (N-H), 1738 (C=O), 1617 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 2.24-2.28 (m, 2H, -CH_2-); 3.74-3.76 (m, 2H, -N-CH_2-); 4.58-4.60 (m, 2H, -N-CH_2-); 4.91 (s, 2H, -N-CH_2-); 6.23 (d, *J*=5.1Hz, 1H, H^2); 7.06 (dd, 1H, *J*=8.4, 1.8Hz, 1H, ArH, H^7); 7.11 (s, NH exchangeable with D_2O); 7.17 (d, *J*=8.4Hz, 1H, ArH, H^6); 7.25 (d, *J*=9.0Hz, 1H, H^4); 7.63 (d, *J*=1.8Hz, 1H, ArH, H^9); 7.72 (s, 1H, H^5); 7.86 (s, 1H, triazole H); 7.94 (d, *J*=9.0Hz, 1H, H^3); 8.22 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 29.7, 35.0, 42.2, 48.1, 98.7, 111.7, 113.4, 117.1, 118.9, 123.1, 123.6, 124.2, 124.5, 126.6, 133.7, 141.4, 146.8, 147.8, 149.7, 150.1, 158.5, 159.5, 181.6. HRMS Calculated for $C_{23}H_{18}ClFN_6O_2$ $[M + H]^+$ 465.8794 found 465.8788.

3.1.13 5-Chloro-1-{1-[3-(7-chloro-quinolin-4-ylamino)-propyl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**8h**): Red Solid; Yield: 68%; m.p. 153-154^{0}C. IR (KBr) ν_{max}: 3372 (N-H), 1741 (C=O), 1607 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 2.20-2.22 (m, 2H, -CH_2-); 3.32-3.36 (m, 2H, -N-CH_2-); 4.45-4.50 (m, 2H, -N-CH_2-); 4.97 (s, 2H, -N-CH_2-); 6.30 (d, *J*=5.4Hz, 1H, H^2); 7.10 (d, 1H, *J*=8.4Hz, 1H, ArH, H^7); 7.15 (s, NH exchangeable with D_2O); 7.27 (d, *J*=8.7Hz, 1H, H^4); 7.40 (d, *J*=8.4Hz, 1H, ArH, H^6); 7.48 (d, *J*=1.8Hz, 1H, ArH, H^9); 7.72 (s, 1H, H^5); 7.82 (s, 1H, triazole H); 7.96 (d, *J*=8.7Hz, 1H, H^3); 8.28 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 31.0, 35.9, 42.7, 48.3, 98.8, 111.9, 112.8, 117.4, 118.4, 123.3, 123.7, 124.1, 124.6, 126.8, 133.5, 141.4, 146.4,

148.5, 150.2, 151.6, 157.9, 159.8, 182.8. HRMS Calculated for $C_{23}H_{18}Cl_2N_6O_2$ $[M + H]^+$ 482.3340 found 483.3344.

3.1.14 5-Bromo-1-{1-[3-(7-chloro-quinolin-4-ylamino)-propyl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**8i**): Red Solid; Yield: 72%; m.p. 178^{0}C (decomp.). IR (KBr) ν_{max}: 3359 (N-H), 1737 (C=O), 1607 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 2.21-2.25 (m, 2H, -CH_2-); 3.76-3.78 (m, 2H, -N-CH_2-); 4.60-4.62 (m, 2H, -N-CH_2-); 4.96 (s, 2H, -N-CH_2-); 6.29 (d, *J*=5.4Hz, 1H, H^2); 7.04 (d, 1H, *J*=8.4Hz, 1H, ArH, H^7); 7.13 (s, NH exchangeable with D_2O); 7.25 (d, *J*=8.7Hz, 1H, H^4); 7.38 (d, *J*=8.4Hz, 1H, ArH, H^6); 7.45 (d, *J*=1.8Hz, 1H, ArH, H^9); 7.71 (s, 1H, H^5); 7.83 (s, 1H, triazole H); 7.99 (d, *J*=8.7Hz, 1H, H^3); 8.24 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 31.1, 35.5, 42.5, 48.7, 98.6, 111.1, 112.5, 117.2, 118.6, 123.4, 123.7, 124.2, 124.9, 127.0, 133.9, 141.4, 146.8, 148.6, 150.1, 151.2, 157.9, 160.3, 183.2. HRMS Calculated for $C_{23}H_{18}BrClN_6O_2$ $[M + H]^+$ 526.7850 found 526.7858.

3.1.15 1-{1-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-1H-[1,2,3]triazol-4-ylmethyl}-5-methyl-1H-indole-2,3-dione (**8j**): Red Solid; Yield: 65%; m.p. 168^{0}C (decomp.). IR (KBr) ν_{max}: 3348 (N-H), 1732 (C=O), 1614 (C=O) cm^{-1}; ^{1}H NMR (300 MHz $CDCl_3$+DMSO-d_6): δ 2.23 (s, 3H, -CH_3); 2.28-2.32 (m, 2H, -CH_2-); 3.71-3.73 (m, 2H, -N-CH_2-); 4.56-4.58 (m, 2H, -N-CH_2-); 4.80 (s, 2H, -N-CH_2-); 6.24 (d, *J*=5.4Hz, 1H, H^2); 7.01 (d, 1H, *J*=8.4Hz, 1H, ArH, H^7); 7.11 (s, NH exchangeable with D_2O); 7.23 (d, *J*=9.0Hz, 1H, H^4); 7.37-7.41 (d, *J*=8.4Hz, 1H, ArH, H^6); 7.44 (d, *J*=1.8Hz, 1H, ArH, H^9); 7.78 (s, 1H, H^5); 7.90 (s, 1H, triazole H); 8.01-8.05 (d, *J*=9.0Hz, 1H, H^3); 8.32 (d, *J*=5.1Hz, 1H, H^1); ^{13}C NMR (DMSO-d_6, 75 MHz): δ ppm = 23.6, 31.4, 35.6, 42.3, 48.3, 98.6, 111.6, 112.7, 116.9, 118.5, 123.5, 123.8, 124.1, 124.6, 127.4, 134.2, 141.8, 146.5, 148.4, 150.8, 152.6, 158.8, 160.4, 182.0. HRMS Calculated for $C_{24}H_{21}ClN_6O_2$ $[M + H]^+$ 460.9155 found 460.9150.

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CAPTIONS:

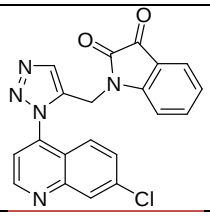
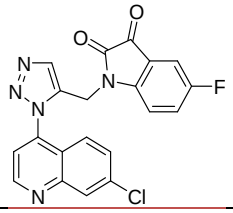
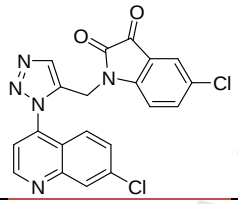
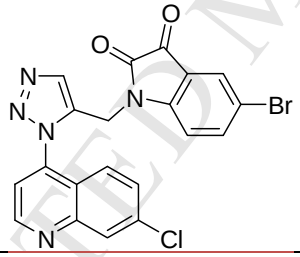
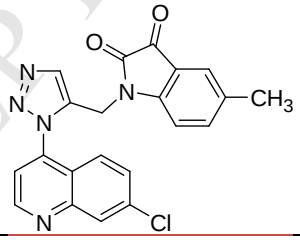
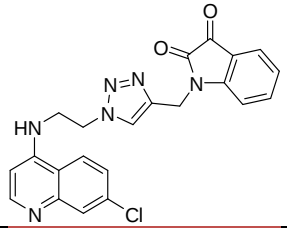
Table 1. Antimalarial activity of tested compounds

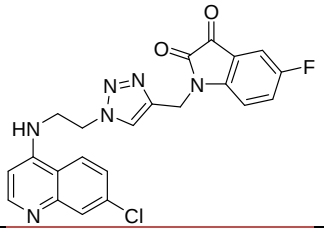
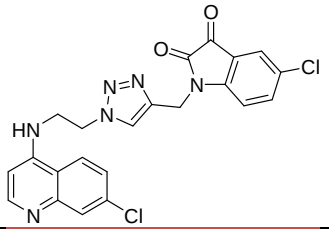
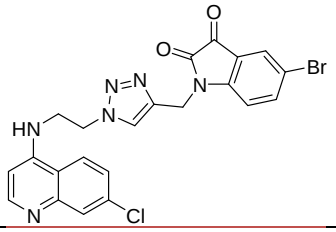
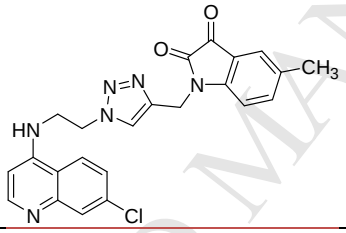
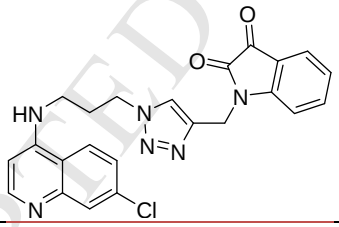
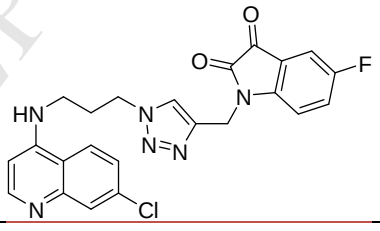
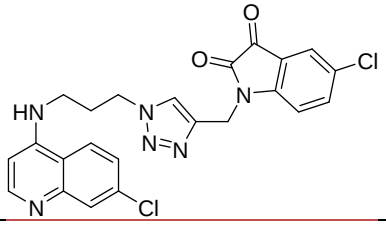
Figure 1: General structure of lead compound and target hybrid compounds

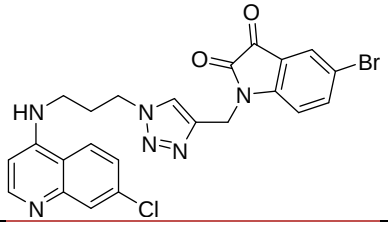
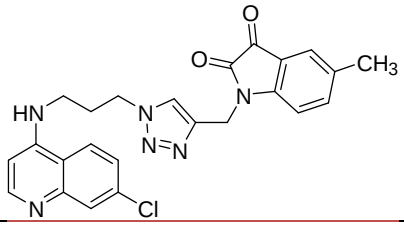
Scheme 1. Synthesis of 7-chloroquinoline-isatin-conjugates **3a**-**3e**

Scheme 2. Synthesis of 7-chloroquinoline-isatin-conjugates **8a**-**8j**

Table 1. Antimalarial activity results of tested compounds

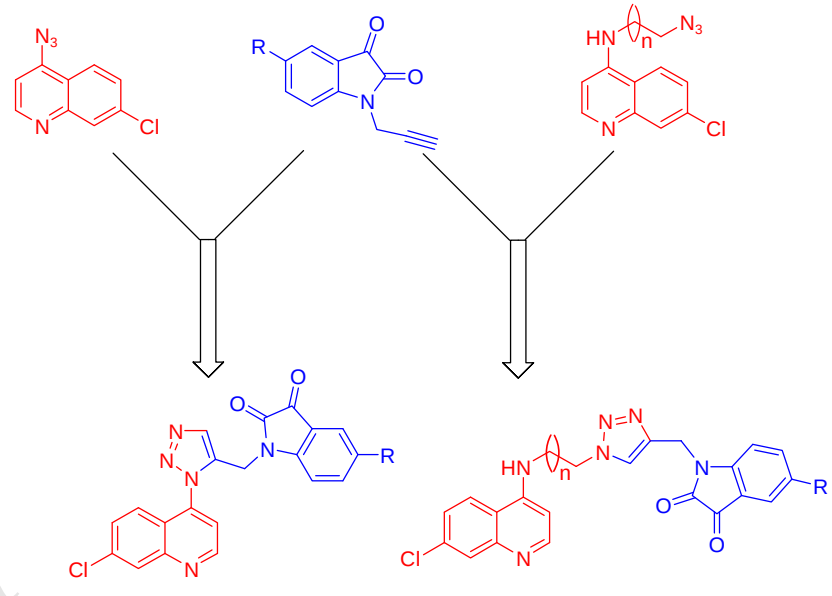
Compound	Structure	W2 (CQ-R)[a] IC_{50} (μM)
3a		>5
3b		>5
3c		>5
3d		>5
3e		>5
8a		3.07

8b		2.30
8c		1.37
8d		1.73
8e		1.63
8f		3.85
8g		1.35
8h		1.21

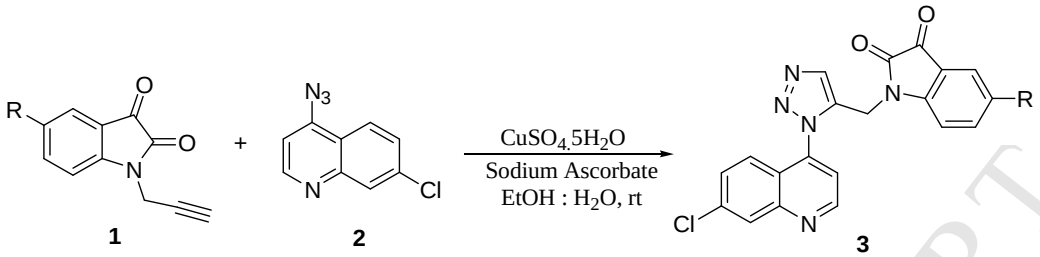
8i		1.66
8j		1.30
Chloroquine(CQ)		0.099
Artemisinin (ART)		0.014

[a] CQ-R: Chloroquine resistant

Figure 1. General structure of lead compound and target hybrid compounds

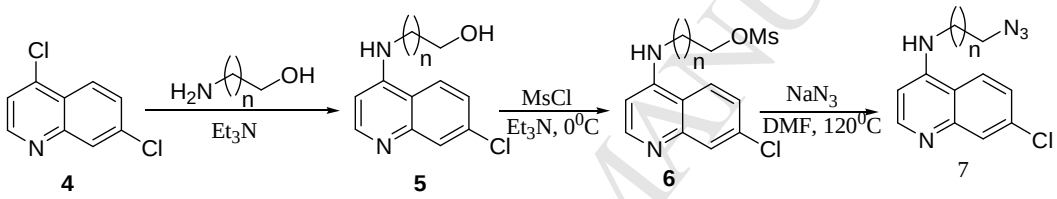


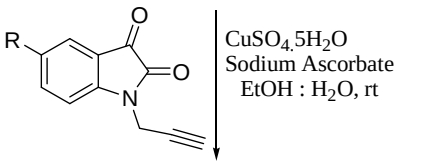
Scheme 1.Synthesis of 7-Chloroquinoline-isatin-conjugates 3a-3e



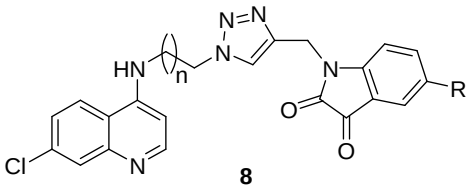
3a, R = H
3b, R = F
3c, R = Cl
3d, R = Br
3e, R = CH_3

Scheme 2. Synthesis of 7-Chloroquinoline-isatin-conjugates 8a-8j





8a, R = H, n = 1
8b, R = F, n = 1
8c, R = Cl, n = 1
8d, R = Br, n = 1
8e, R = CH_3, n = 1
8f, R = H, n = 2
8g, R = F, n = 2
8h, R = Cl, n = 2
8i, R = Br, n = 2
8j, R = CH_3, n = 2



- Synthesis of 7-chloroquinoline-isatin hybrids *via* azide-alkyne cycloaddition.
- Antiplasmodial activity of synthesized hybrids was evaluated against W2 strain.
- Activity dependence on the C-5 substituent of isatin ring and the length of alkyl chain.

Azide-alkyne cycloaddition *en route* to 1*H*-1,2,3-Triazole-tethered 7-chloroquinoline-isatin chimeras: Synthesis and antimalarial evaluation

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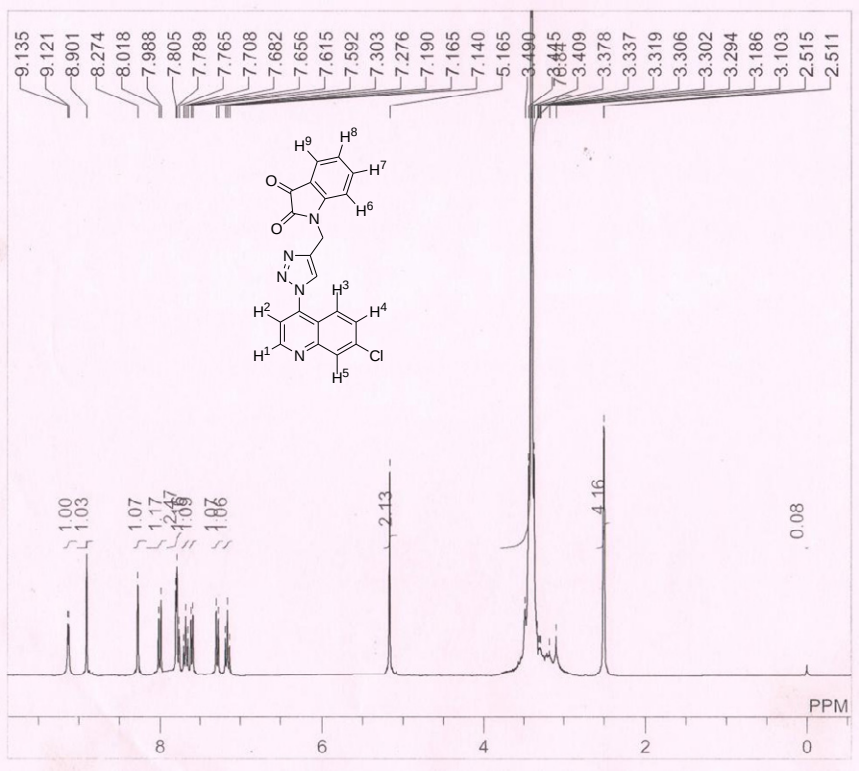
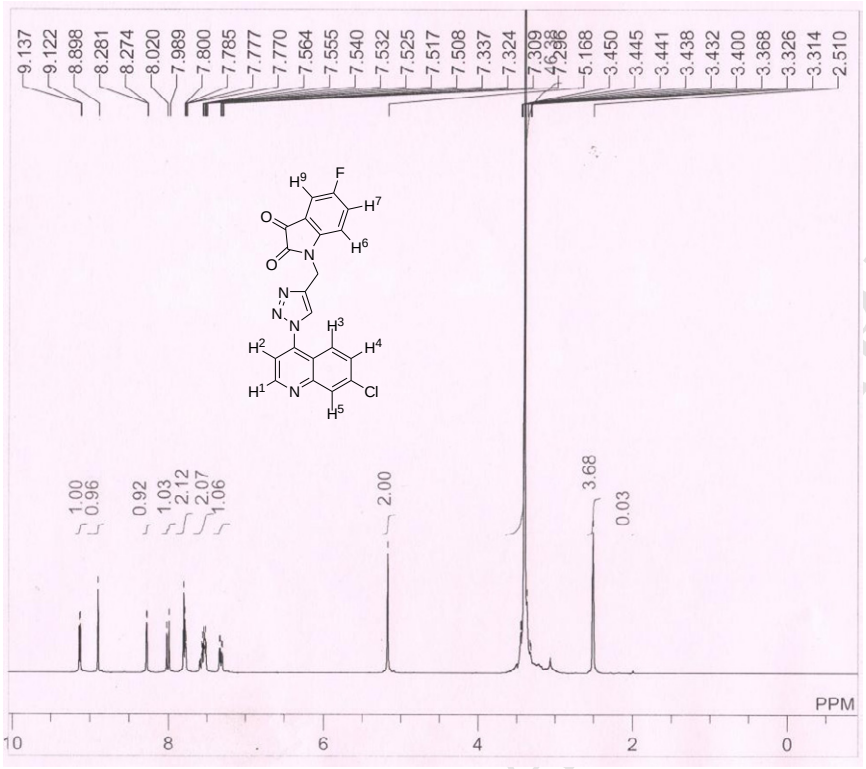
^{1}H and ^{13}C NMR Spectrum of Representative Compounds:

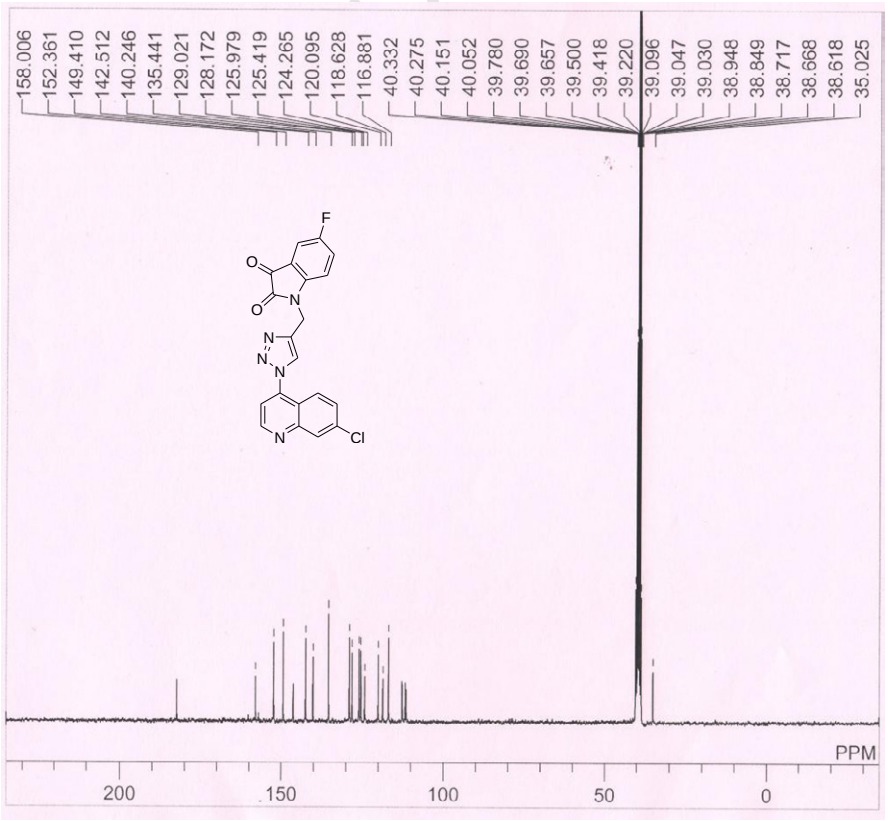
^{1}H Spectrum of **3a** :



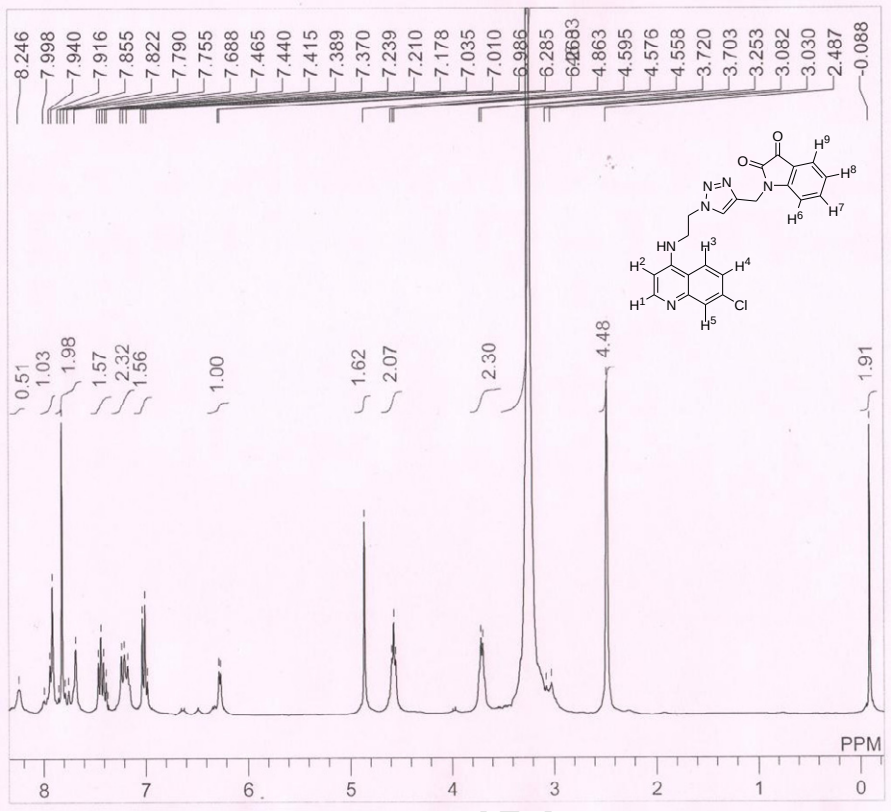
^{1}H Spectrum of **3b**:



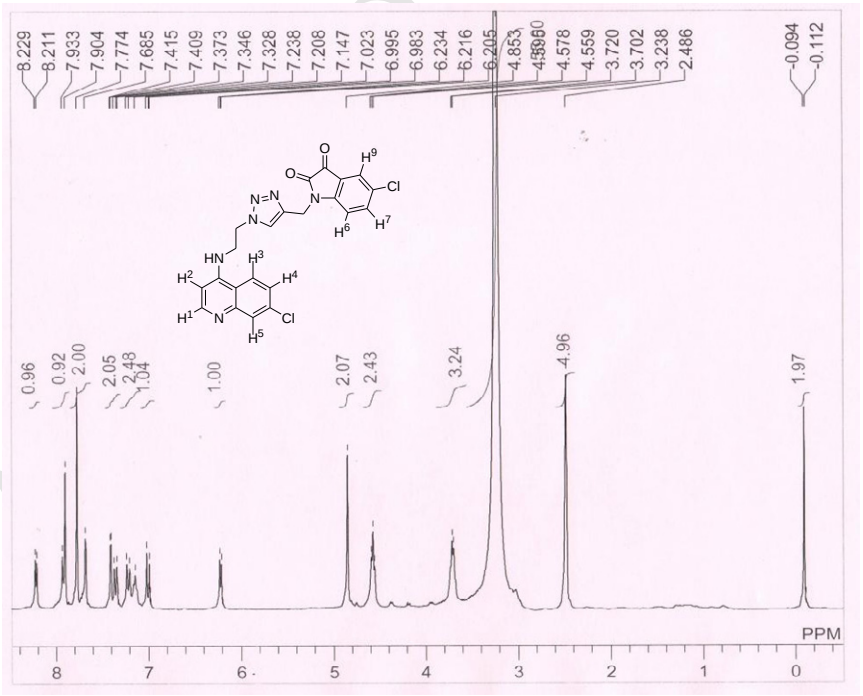
^{13}C Spectrum of **3b**:



^{1}H Spectrum of **8a**:



^{1}H Spectrum of **8c**:



^{13}C Spectrum of **8c**: