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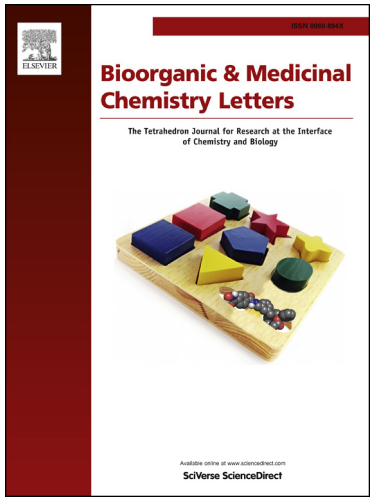

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PII: S0960-894X(15)00865-3
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.08.030
Reference: BMCL 23024

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 28 May 2015
Revised Date: 23 July 2015
Accepted Date: 11 August 2015

Please cite this article as: Parthiban, A., Muthukumaran, J., Manhas, A., Srivastava, K., Krishna, R., Surya Prakash Rao, H., Synthesis, *in vitro* and *in silico* antimalarial activity of 7-chloroquinoline and 4*H*-chromene conjugates, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.08.030

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Synthesis, *in vitro* and *in silico* antimalarial activity of 7-chloroquinoline and 4*H*-chromene conjugates

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Abstract

A new series of chloroquinoline-4*H*-chromene conjugates incorporating piperizine or azipane tethers were synthesized and their anti-malarial activity were evaluated against two *Plasmodium falciparum* strains namely 3D7 chloroquine sensitive (CQS) and K1 chloroquine resistant (CQR). Chloroquine was used as the standard and also reference for comparison. The conjugates exhibit intense UV absorption with λmax located at 342 nm (log ε = 4.0), 254 nm (log ε = 4.2), 223 nm (log ε = 4.4) which can be used to spectrometrically track the molecules even in trace amounts. Among all the synthetic compounds, two molecules namely 6-nitro and *N*-piperazine groups incorporated **7d** and 6-chloro and *N*-azapane incorporated **15b** chloroquinoline-4*H*-chromene conjugates showed significant anti-malarial activity against two strains (3D7 and K1) of *P. falciparum*. These values are lesser than the values of standard antimalarial compound. Molecular docking results suggested that these two compounds showing strong binding affinity with *P. falciparum* Lactate dehydrogenase (PfLDH) and also they occupy the co-factor position which indicated that they could be the potent inhibitors for dreadful disease malaria and specifically attack the glycolytic pathway in parasite for energy production.

Key words: Chloroquinoline-4*H*-chromene conjugates, *in vitro* antimalarial activity and *in silico* analysis.

Malaria is one of the most infectious and parasitic disease affecting about 40% of the human population, particularly those living in tropical and poorer countries.[1] According to World Health Organization (WHO) World Malaria Report 2014 out of an estimated 198 million cases nearly 584,000 malaria deaths occurred in 2013.[2] Because of the devastating effects on human population, WHO rates malaria as one of the top three of the infectious

diseases.[3] Malarial disease is caused by any one of the species of the *Plasmodium* parasite, namely *P. falciparum, P. malariae*, *P. ovale*, *P. vivax and P.knowlesi.*[4] Among all the species, *P. falciparum* is the most severe pathogenic.[5]

Treatment of malaria began in earlier days by administering decoction made from cinchona (*Cinchona rubra,* Rubiaceae) bark. Subsequently, quinine **1** was isolated from this tree and shown to have anti-malarial properties.[6] Medicinal chemists developed simpler synthetic analogues of quinine **1** like pamaquine **2**, mepacrine **3**, chloroquine **4,** amodiaquine **5** and piperaquine **6** (Fig. 1), all of which had pharmacopore of quinine, but did not have multiple stereogenic centers. Among these, chloroquine **4** is the most efficient and important drug and served humanity for over five decades.[7] Although in recent years artemisinin-based combination therapy (ACT) has been employed for treatment of most cases of malaria, owing to being inexpensive and efficacious, chloroquine **4** is still used as a first-line drug in some Central American and Caribbean countries.[8] Moreover it remains as a first-line drug for treatment of malaria caused by *P. vivax*.[9] Extensive use of chloroquine over the years lead to emergence of the drug resistance in parasite and this forced medicinal chemists to look for alternate molecules.[10] Since, artemisinin and its close analogs have the problem of low bio-availability, tedious isolation from natural sources or difficult to synthesize in industrial scale, reliance of chloroquine continues with room for further development in its structural features.[11]

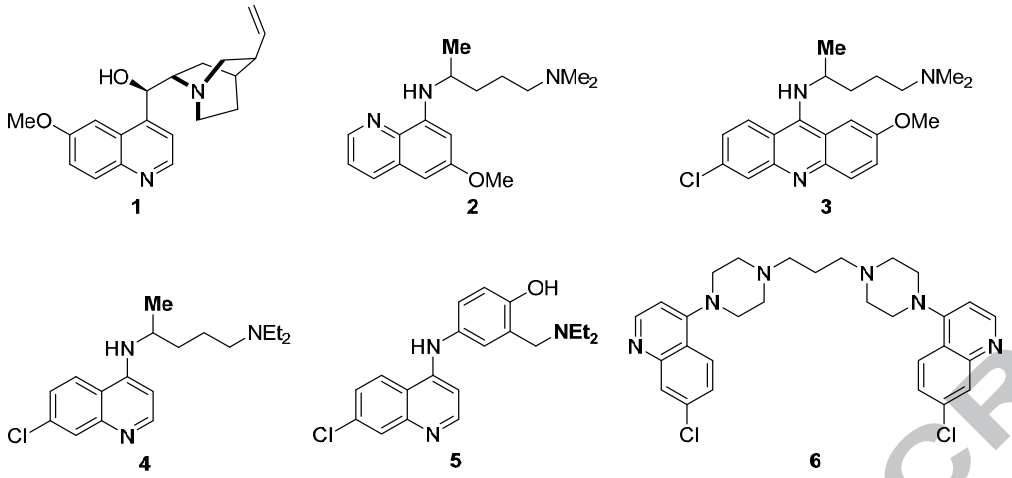


Figure 1. Structure of quinoline based antimalarial drugs. Quinine **1**, pamaquine **2**, mepacrine **3**, chloroquine **4,** amodiaquine **5** and piperaquine **6**.

One of the major issues with chloroquine and its analogues is the hydrophobic nature of the molecule, which allows it to cross the blood brain barrier (BBB). To restrict crossing of BBB it is desirable to attach a polar group to the chloroquine pharmacophore. Our experience in synthesis of 4-aryl-4*H*-chromenes[12] prompted us link 6-chloroquineand 4*H*-chromene through electron rich aromatic ring tether to generate chloroquinoline-4*H*-chromene conjugates **7** (Fig. 2). The chloroquinoline-4*H*-chromene conjugates **7** possess medicinally important rigid and nearly flat 4*H*-chromene framework with hydrogen bond acceptor C1 oxygen, hydrogen bond donor and acceptor C2-amino group and highly polarized C3 nitro group and extremely stable push-pull alkene located between C2 and C3.[13] For structural diversity there are several possibilities of installation of different substitution in the aromatic ring in the 4*H*-chromene. There are also possibilities for changes in the *N*-phenylpiperazine moiety in **7**. The 4*H*chromene-chloroquinoline conjugate **7** possess highly polarized nitroketene-*N,O*-acetal substructure which is not expected to allow **7** to cross BBB. Moreover, nitroketene-*N,O*-acetal moiety in **7** is expected to be responsible for intense UV absorption with λ_{max} at about 345 nm and 255 nm (log ε = 4.0) which should allow detection

of the sample present in trace amounts. We report, herein, synthesis of a small library of chloroquinoline-4*H*-chromene conjugates **7** and their antimalarial activity was tested against drug resistant strains of *P. falciparum*. Furthermore, we report on *in silico* studies performed with PfLDH for all synthetic compounds to delineate structure activity relationships. The experimental part of the chemistry, *in vitro* and *in silico* studies are given in supplementary section.

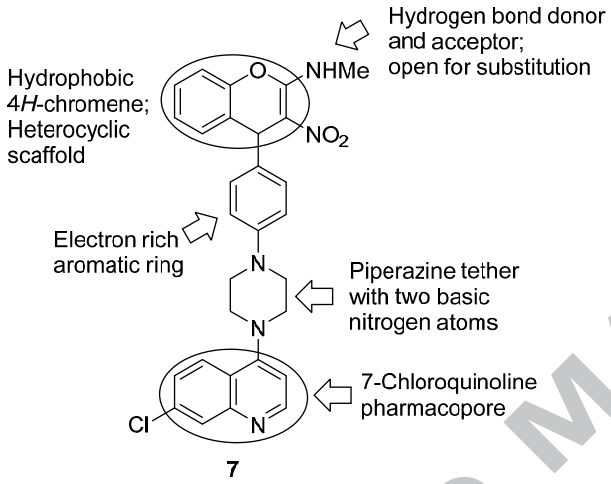
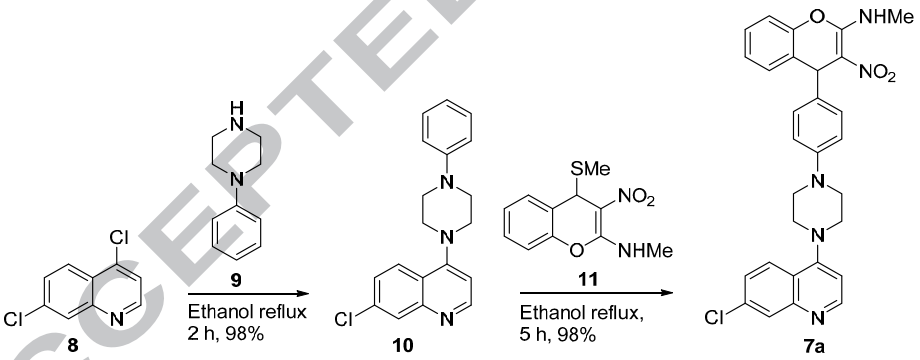


Figure 2. Structures of present target chloroquinoline-4*H*-chromene conjugates **7**.

In the first step of the two-step sequence towards **7a**, we subjected commercially available 4,7-dichloroquinoline **8** to reaction with *N*-phenylpiperizine **9** in EtOH reflux. In this reaction, regio-selective nucleophilic displacement of C(4)Cl in **8** took place readily to provide *N*-phenylpiperazine substituted chloroquinoline **10** in near quantitative yield (Scheme 1). The nucleophilic displacement of C(4)SMe in **11** took place readily on treatment with **10** in ethanol reflux to provide chloroquinoline-4-aryl-4*H*-chromene conjugate in near quantitative yield. Mechanistically, the displacement of SMe in **11** went through aromatic electrophilic substitution on electron rich and sterically less crowded C(4) of *N*-phenylpiperizine moiety in **10.**[14] The product **7a** generated as faintly yellow colored solid precipitated out of the reaction and thus no work-up and column chromatographic

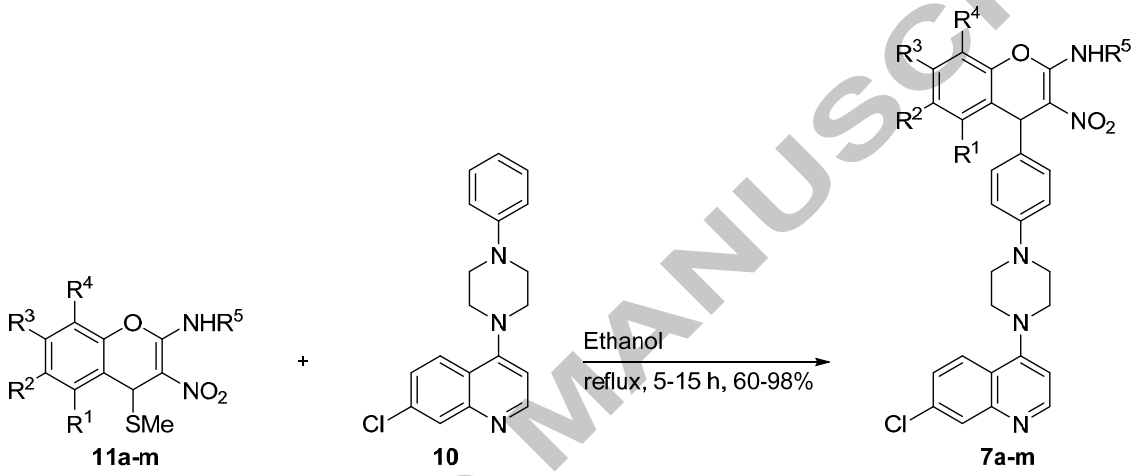
purification was necessary. The UV-Vis spectrum of **7a** displayed three intense bands with λ_{max} located at 342 nm (log ε = 4.0), 254 nm (log ε = 4.2), 223 nm (log ε = 4.4). Of the three one with λ_{max} at 223 nm was due to 6-chloroquinoline chromophore since chloroquine displayed λ_{max} at 228 nm (log ε = 3.8). The other two high intensity bands at 342 nm (log ε = 4.0) and 254 nm (log ε = 4.2) are clearly due to nitroketene-*N,O*-acetal moiety and these bands can be used to track the molecule **7a**. The ^{1}H NMR spectrum of **7a** exhibited a signal for C(4)H of 4-aryl-4*H*-chromene portion at 5.40 ppm. The doublet at 3.26 ppm for NH-Me and a broad singlet at 10.46 ppm were the other characteristic peaks for the 4-aryl-4*H*-chromene portion. Two types of CH_2 protons of the piperazinyl moiety gave rise to two triplets located at 3.33 and 3.36 ppm. Two doublets located at 8.72 and 7.96 ppm were due to C(2)H and C(8)H of quinoline moiety. The ^{13}C NMR spectrum of **7** showed four signals at δ 27.9, 40.9, 49.2 and 52.2 ppm in the aliphatic region which were assigned to N-Me, C4, two methylene carbons of piperazine ring.



Scheme 1. Synthesis of chloroquinoline 4-aryl-4*H*-chromene conjugate **7a**.

After having established conditions for the synthesis of chloroquinoline-4*H*-chromene conjugate **7a** the sequence was repeated for synthesis of twelve conjugates **7b-m** by varying substitutions in the aromatic ring and the alkyl group on C(2) nitrogen of the 4*H*-chromene

(Scheme 2). Spectral (UV-Vis, IR, ^{1}H NMR, ^{13}C NMR and DEPT) and analytical (ESI-MS HRMS) data of the chloroquinoline conjugates **7b-m** agreed well with the assigned structures. Surprisingly the reaction of **10** did not take place with 4*H*-chromene having C(2)N phenyl group. It is possible that due to electron withdrawing effect of the phenyl group makes C(2) nitrogen less electron donating for ejection of C(4)SMe group.



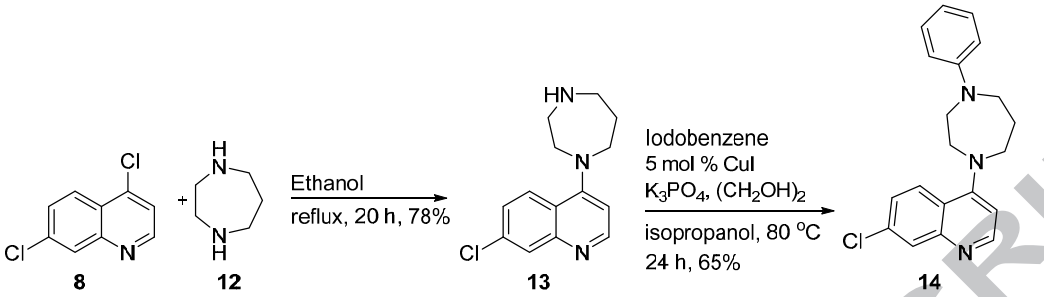
11a: $R^1 = R^2 = R^3 = R^4 = R^5 =$ Me;
11b: $R^2 =$ Cl, $R^1 = R^3 = R^4 = R^5 =$ Me;
11c: $R^2 =$ Br, $R^1 = R^3 = R^4 = R^5 =$ Me;
11d: $R^2 = NO_2$, $R^1 = R^3 = R^4 = R^5 =$ Me;
11e: $R^2 =$ OMe, $R^1 = R^3 = R^4 = R^5 =$ Me;
11f: $R^4 =$ OEt, $R^1 = R^2 = R^3 = R^5 =$ Me;
11g: $R^4 =$ OMe, $R^1 = R^2 = R^3 = R^5 =$ Me;
11h: $R^2 = R^4 =$ Cl, $R^1 = R^3 = R^5 =$ Me;
11i: $R^2 = R^4 =$ Br, $R^1 = R^3 = R^5 =$ Me;
11j: $R^1 =$ CHO, $R^3 =$ Me $R^2 = R^4 =$ Cl, $R^5 =$ Me;
11k: $R^1 = R^2 = R^3 = R^4 =$ H, $R^5 =$ Bn;
11l: $R^1 = R^2 = R^3 = R^4 =$ H; $R^5 = CH_2CH_2Ph$;
11m: $R^1 = R^2 = R^3 = R^4 =$ H, $R^5 =$ *n*-Bu.

7a: $R^1 = R^2 = R^3 = R^4 = R^5 =$ Me (5 h, 98%);
7b: $R^2 =$ Cl, $R^1 = R^3 = R^4 = R^5 =$ Me (10 h, 75%);
7c: $R^2 =$ Br, $R^1 = R^3 = R^4 = R^5 =$ Me (6 h, 98%);
7d: $R^2 = NO_2$, $R^1 = R^3 = R^4 = R^5 =$ Me (15 h, 98%);
7e: $R^2 =$ OMe, $R^1 = R^3 = R^4 = R^5 =$ Me (5 h, 70%);
7f: $R^4 =$ OEt, $R^1 = R^2 = R^3 = R^5 =$ Me (5 h, 96%);
7g: $R^4 =$ OMe, $R^1 = R^2 = R^3 = R^5 =$ Me (10 h, 82%);
7h: $R^2 = R^4 =$ Cl, $R^1 = R^3 = R^5 =$ Me (10 h, 85%);
7i: $R^2 = R^4 =$ Br, $R^1 = R^3 = R^5 =$ Me (5 h, 98%);
7j: $R^1 =$ CHO, $R^3 =$ Me, $R^2 = R^4 =$ Cl, $R^5 =$ Me (15 h, 70%);
7k: $R^1 = R^2 = R^3 = R^4 =$ H, $R^5 =$ Bn (6 h, 83%);
7l: $R^1 = R^2 = R^3 = R^4 =$H, $R^5 = CH_2CH_2Ph$ (15 h, 92%);
7m: $R^1 = R^2 = R^3 = R^4 =$ H, $R^5 =$ *n*-Bu (15 h, 60%).

Scheme 2: Synthesis of chloroquinolin-4-ylpiperazin substituted 4*H*-chromene conjugates **7a-m**.

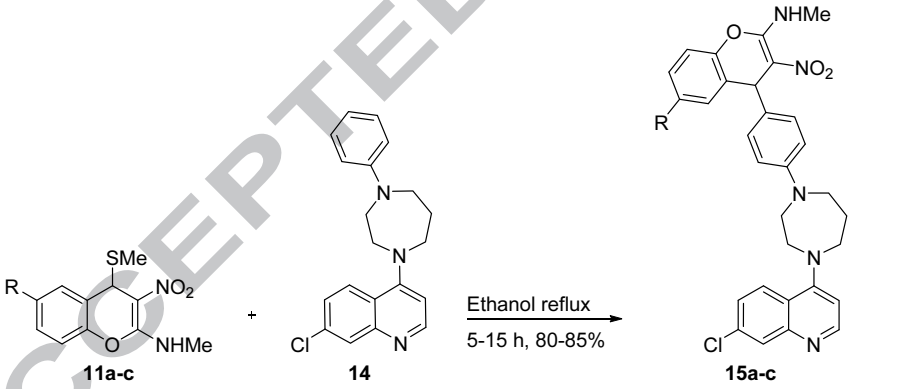
Next, our attention was focused on changing six member piperazinyl ring in **10** to seven-member 1,4-diazepane ring (homopiperazine) **14** to evaluate if the ring size and concomitant change of orientation would affect antimalarial activity. Synthesis of 4-(4-(4-(7-

chloroquinolin-4-yl)-1,4-diazepan-1-yl)phenyl)-*N*-methyl-3-nitro-4*H*-chromen-2-amine derivatives **15a-c** is given in Schemes 3 and 4.



Scheme 3. Synthesis 7-chloro-4-(4-phenyl-1,4-diazepan-1-yl)quinoline **14**.

The synthesis started with commercially available 4,7-dichloroquinoline **8** and 1,4-diazapine (homopiperazine) **12**. The nuclophilic displacement of C(4)Cl in **8** with secondary amine in **12** worked well to provide **13**. The Buchwald–Hartwigamination[15] with iodobenzene in the presence of catalytic amount of copper (I) iodide afforded *N*-phenyldiazepan substituted chloroquinoline **14** in good yield (Scheme 3).



Scheme 4. Synthesis of chloroquinoline-4*H*-chromene conjugates **15a-c**.

The reaction of 4-methylsulfanyl-4*H*-chromene derivatives **11a-c** and *N*-phenyldiazepan substituted chloroquinoline**14** furnished diazepan incorporated

chloroquinoline-4*H*-chromene conjugates **15a-c** in high yields (Scheme 4). The structures of chloroquinoline-conjugates **15a-c** were confirmed on the basis of spectral (UV-Vis, IR, ^{1}H NMR, ^{13}C NMR and DEPT) and analytical (ESI-MS HRMS) data and they compared well with the those having piperazine moiety **7a-m**.The structures of chloroquinoline-4*H*-chromene conjugates **7a-m** and **15a-c** prepared in present study along with yield of the product obtained are gathered in Fig. 3.

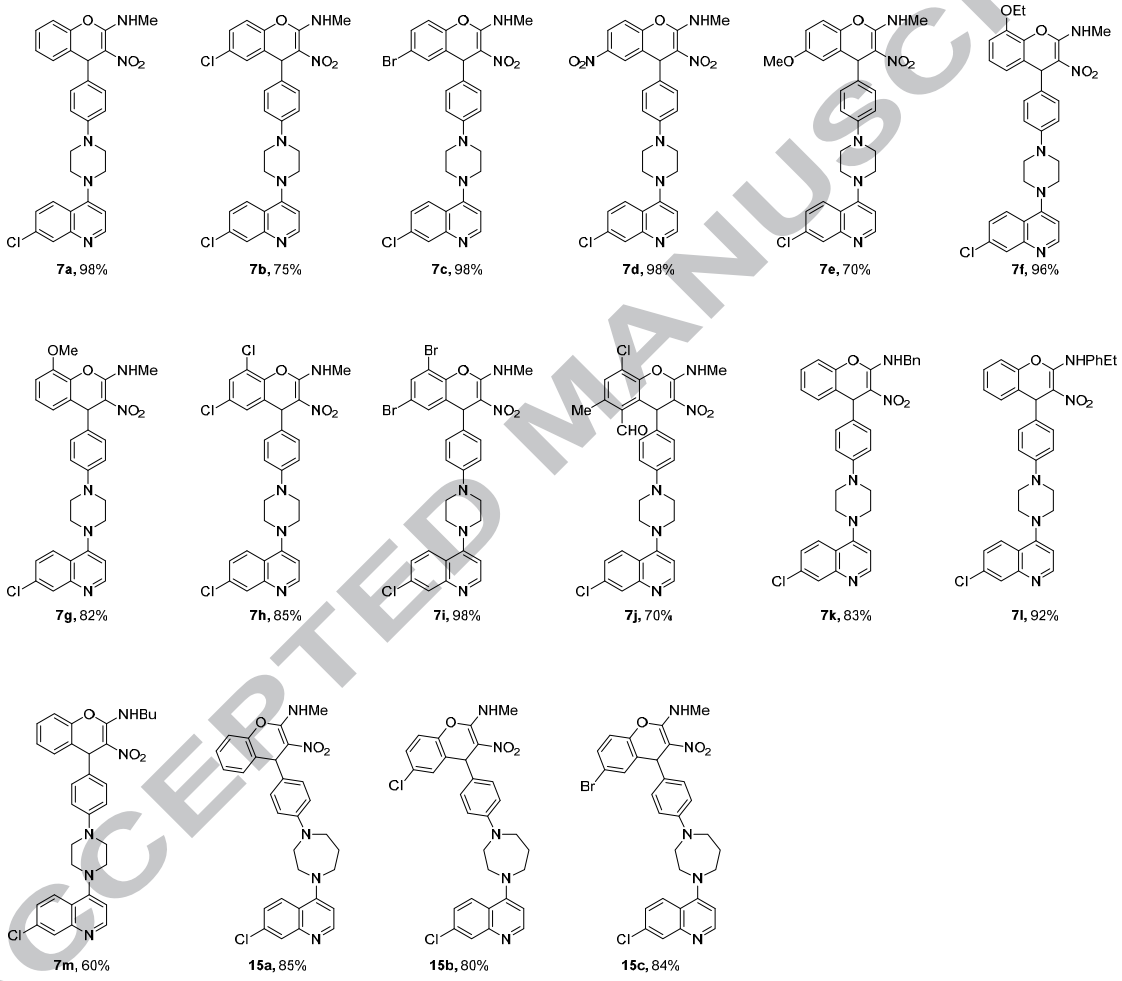


Figure 3. Library synthesis of chloroquinoline-4*H*-chromene conjugates **7a-m** and **15a-c**.

After the successful synthesis and characterization, the chloroquinoline-4*H*-chromene conjugates (**7a-m** and**15a-c**) were evaluated for their anti-malarial activity against two

pathogenic strains of *P. falciparum* namely chloroquine sensitive (CQS; 3D7) and chloroquinine resistant (CQR; K1). Chloroquine was used as the standard and reference drug for comparison. Table 1 shows 50% inhibitory concentrations (IC_{50}) against 3D7 & K1 parasite strains, 50% cytotoxic concentration (CC_{50}) against Vero cell line (C1008; monkey kidney fibroblast) and selectivity index SI (SI = CC_{50}/IC_{50}) of all the newly made chloroquinoline-4*H*-chromene conjugates (**7a-m** and **15a-c**).

Table 1. *In vitro* antimalarial activity and cytotoxicity of the chloroquinoline-4*H*-chromene conjugates **7a-m** and **15a-c** against 3D7 and K1 strains of *P. falciparum*.

Compounds	IC_{50} (μM) [3D7,CQS]	IC_{50} (μM) [K1,CQR]	CC_{50} (μM)	SI [3D7,CQS]	SI [K1, CQR]
7a	1.11	3.38	>200	>180.2	>59.2
7b	1.37	2.99	74.46	54.3	24.9
7c	1.88	1.75	88.56	47.1	50.6
7d	**0.62**	**1.78**	**31.87**	**51.4**	**17.9**
7e	2.48	4.25	81.92	33.0	19.3
7f	2.93	2.95	40.38	13.7	13.6
7g	2.47	2.40	21.84	8.8	9.1
7h	3.92	1.45	97.77	24.9	67.4
7i	>5.0	2.93	>200	~40.0	>68.2
7j	2.34	0.87	83.98	35.8	96.5
7k	>5.0	>5.0	66.45	13.3	13.3
7l	3.31	1.74	131.34	39.6	75.5
7m	3.09	3.57	106.91	34.6	29.9
15a	>5.0	>5.0	91.41	<18.3	<18.3
15b	**0.29**	**0.496**	**76.97**	**265.4**	**155.2**

15c	2.24	1.27	>200	89.3	157.5
10	2.69	2.49	178.57	66.3	2.5
14	3.98	4.47	147.93	37.1	33.1
Chloroquine	0.005±0.0009	0.254± 0.020	125	25000	492.13

CQS- chloroquine sensitive and CQR - chloroquine resistant

CC_{50}- 50% Cytotoxic concentration, SI -selectivity index (CC_{50}/ IC_{50}).

Out of thirteen conjugates (**7a-7m)** which incorporated *N*-phenylpiperazine tether, one (**7d)** with C(6)-nitro group exhibited IC_{50} value 0.62 µM against 3D7 (CQS) strain and 1.78 µM in case of K1 (CQR) strain. Interestingly, the conjugate **15b** which has seven-member 1,4-diazepane ring in the tether was most potent in the set of all sixteen compounds. It displayed significant anti-malarial activity with the IC_{50} values of 0.29 µM against 3D7 strain and 0.49 µM against K1 strain. This result indicates that bent structure and chloro-group in 4*H*-portion were responsible for enhanced activity. Although none of the newly synthesized compounds exhibited antimalarial activity better than chloroquine, they have the advantage of being polar and resistant to crossing bbb.

PfLDH is an important enzyme that involves the inter-conversion of pyruvate to lactate and *vice-versa*.[16] For the conversion of pyruvate to lactate NADH is employed as a cofactor, which in turn gets converted into NAD^+. Being an essential enzyme for glycolysis in the parasite, it is a good target for anti-malarial drugs. The amino acid sequences for human and PfLDH are very different (sequence homology: 30.7 %), hence this implies that the synthetic compounds specifically interact with glycolytic enzyme of parasite.[17] Chloroquine is known to interact with PfLDH effectively through competitive inhibition of binding of NADH at the active site.[18] We reasoned that our newly synthesized molecules which possess both chloroquine and 4*H*-chromene portions could bind to the active site more

efficiently and effectively. Hence, we have used PfLDH as a drug target for our newly synthesized compounds and chloroquine was used as a reference. Molecular docking results suggested that out of sixteen compounds screened, (evaluated independently in both *R* and *S* configuration) and the reference chloroquine (-6.61 kcal/mol), the *R* form of the conjugates **7d** and **15b** displayed good docking score (**7d** -10.91 kcal/mol and **15b** -10.96 kcal/mol) and strong binding affinity with PfLDH. The conjugates occupied the co-factor position at the binding site which indicated that they could be the competitive inhibitors. *In silico* interaction results of **7d** and **15b** with target enzyme is presented here (Fig. 4) and interactions of the remaining molecules are given in supplementary information. The ligand **7d** interacted with the target enzyme mainly through hydrogen bonding interaction and hydrophobic contacts. However, the ligand **15b** made the hydrophobic contacts with the target enzyme. The terminal oxygen atom of nitro group in **7d** formed hydrogen bond with Ile31 residue of target enzyme separated by an ideal distance of 2.94 Å. As **7d**, the following amino acid residues Gly27, Gly29, Met30, Phe52, Asp53, Ile54, Tyr85, Ala98, Gly99, Thr101 and Ile119were formed significant hydrophobic contacts with the ligand **15b**. Aforementioned interactions are essential to stabilize the ligands in the active site of PfLDH.

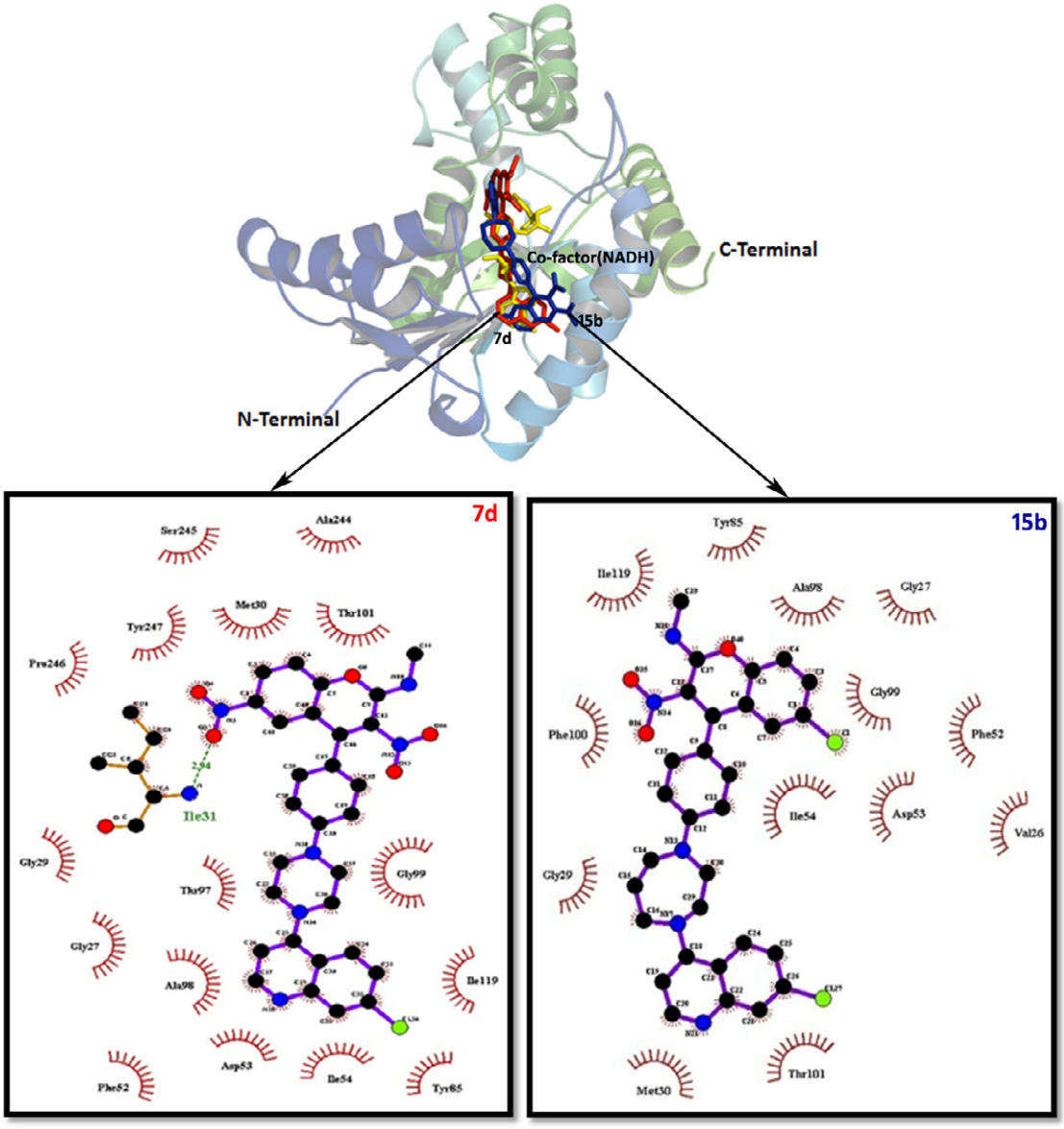


Figure 4. Orientation of 1,4-Dihydronicotinamide adenine dinucleotide (Co-factor), **7d** and **15b** with PfLDH (PDB Accession Code: 1LDG). Two-dimensional representation of PfLDH with ligand **7d** and **15b**. The three-dimensional representation of protein-ligand complex was prepared by PyMOL. The LigPlot shows the amino acid residues of target enzyme around the ligand molecule with hydrogen bond and hydrophobic contacts.

In conclusion, we have synthesized sixteen derivatives of chloroquinoline-4*H*-chromene conjugates and evaluated *in vitro* antimalarial activity against *P. falciparum* strains. Of all conjugates, 6-nitro and *N*-piperazine incorporated **7d** and 6-chloro-*N*-azapane incorporated **15b** chloroquinoline-4*H*-chromene conjugates showed good anti-malarial activity against *P. falciparum* strains (3D7 and K1) and their efficiency was almost equal to that of chloroquine. Newly prepared molecules have the added advantage of possessing polar 4*H*-chromene to make the drug candidates brain impregnable. The conjugates display strong bands at λmax 345, 254 and 223 nm which can be the used for their detection even in trace quantities. The theoretical calculations corroborated experimental results. Overall, the molecular docking results, along with experimental data, suggest that two molecules (**7d** and **15b**) are potent inhibitors for malarial parasite by attacking the parasite specific glycolytic pathway.

Acknowledgement

AP thanks Pondicherry University for fellowship. JM thanks UCIBIO (UID/Multi/04378/2013), Fundação para a Ciência e a Tecnologia (FCT) (PTDC/QUI-BIQ/117799/2010), (SFRH/BPD/97719/2013) Portugal for providing support to carry out *in silico* part of the research work. RK thank the Centre for Bioinformatics (Funded by Department of Biotechnology and Department of Information Technology, New Delhi, India), Pondicherry University, India. AP thanks Central Instrumentation Facility, Pondicherry University for providing the spectroscopic instruments to carry out the research work. We thank Open Source Drug Discovery (OSDD) program of CDRI, Lucknow for testing antimalarial activity**.** HSPR thanks the UGC for financial support under major research project (MRP) and the Special Assistance Program (UGC SAP). He also thanks the Department of Science and Technology (DST) for the Fund for Improvement of S&T Infrastructure in Higher Educational Institutions (FIST).

Graphical Abstract

Synthesis, *in vitro* and *in silico* antimalarial activity of 7-chloroquinoline and 4*H*-chromene conjugates

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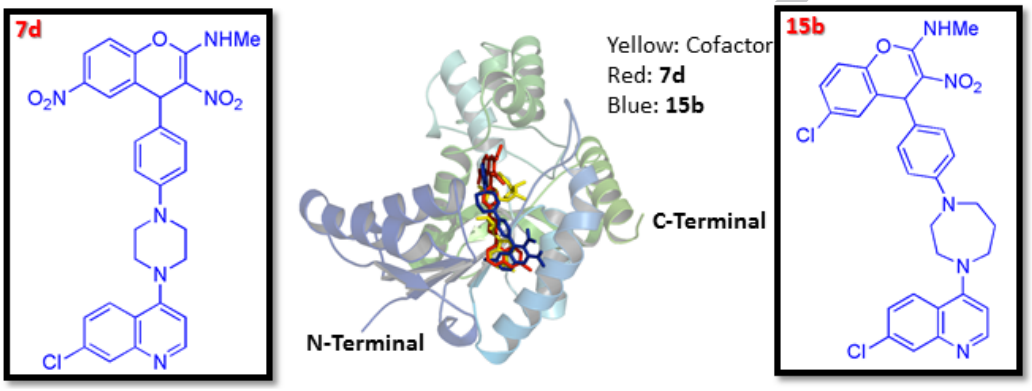

IC_{50} values: **7d**: 0.62 µM CQS strain & 1.78 µM CQR strain and **15b**: 0.29 µM CQS strain & 0.496 µM CQR strain

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