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Synthesis, molecular modelling studies of indolyl chalcone derivatives and their antimalarial activity evaluation

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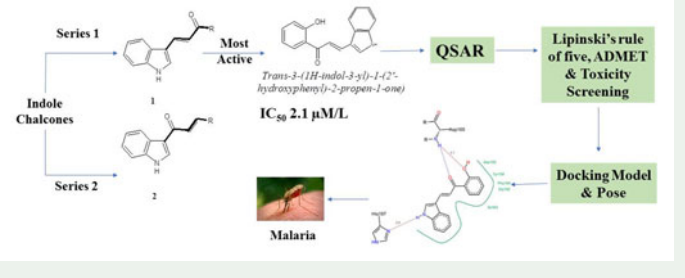
ABSTRACT

Twenty one chalcone derivatives were synthesized using Claisen-Schmidt condensation, their antimalarial activity against *Plasmodium falciparum* was determined and quantitative structure–activity relationship (QSAR) was developed. Condensation of substituted acetophenones with various aromatic aldehydes at room temperature gave chalcones in 75–96% yield. Chalcones are secondary metabolites of terrestrial plants, precursors for the biosynthesis of flavonoids and exhibit various biological activities. Antiplasmodial IC_{50} (half-maximal inhibitory concentration) activity of a compound against malaria parasites *in vitro* provides a good first screen for identifying the antimalarial potential of the compound. The most active compound was *Trans-3-(1H-indol-3-yl)-1-(2′-hydroxyphenyl)-2-propen-1-one* **(1b)** with IC_{50} of 2.1 µM/L. Molecular mechanism was explored through *in silico* docking & ADMET studies for the active compounds.



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1. Introduction

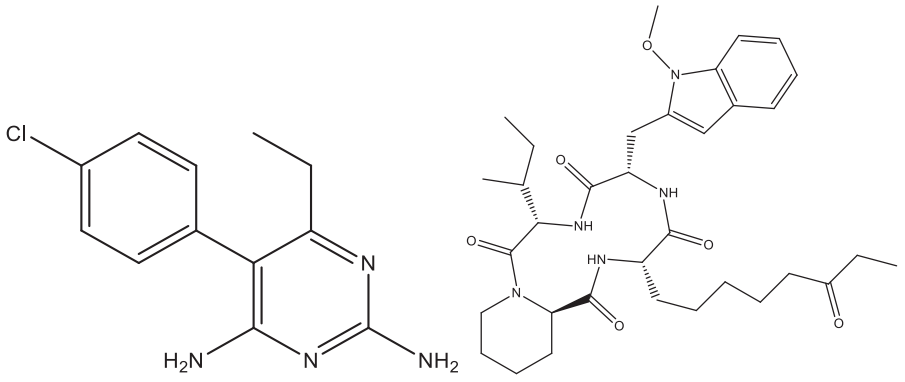
Malaria, one of the most devastating infectious diseases, affects almost half of the global population and poses a major socioeconomic hazard to humanity at large (World Malaria Report 2018). The above grim scenario is further aggravated by the persistent tendency of *Plasmodium falciparum*, the most common malaria parasite, to rapidly develop resistance against any newly introduced drug (Klayman 1985; Luo and Shen 1987). In fact some recent reports have already indicated tolerance of this parasite toward artemisinin and its derivatives (Naß and Efferth 2019), the current World Health Organization reports the guidelines for treatment of chloroquine (CQ) resistant malaria (Geneva: World Health Organization 2015).

Indole and its derivatives represent one of the most active class of compounds possessing a wide spectrum of antiparasitic activities (Butcher et al. 2000; Enserink 2000; Gardner et al. 2000; Rastelli et al. 2000; Shrivastava and Chauhan 2001; Ursos and Roepe 2002; Kumar et al. 2003a, 2003b). Some of the individual compounds having nitrogen in their ring which is responsible for the antimalarial activity are pyrimethamine (**I**) and apicidin (**II**) (Figure 1). Pyrimethamine is an antiparasitic drug used for treating *Toxoplasma gondii* and other plasmodia infections. Indole alkaloids as apicidin (**II**) have shown potent antimalarial activity against *P. falciparum*. Apicidin is known to be a potent inhibitor of HDAC (histone deacetylase), a nuclear enzyme that regulates gene transcription and the assembly of newly synthesized chromatin. Apicidin reversibly induces histone hyperacetylation, causing altered transcriptional regulation and ultimately cell death. Studies on apicidin have suggested that the indole region is a key constituent of enzyme binding and HDAC activity (Singh et al. 1996; Colletti et al. 2001). Compounds, which act on more than one target sites are more liable to be active. Keeping in view all of the above facts we have synthesized chalcone derivatives containing indole (HDAC inhibitor). Antimalarial drugs as quinine, mefloquine, have piperidine nucleus and amopyroquine, cycloquine have pyrrolidine moiety. A large number of compounds having piperidine, pyrrolidine, piperazine and morpholine moiety have shown potent antimalarial activity (Delarue et al. 2001; Ryckebusch et al. 2003; Brinner et al. 2005). These results prompted us to synthesize compounds having indole moiety. As part of our ongoing programme devoted to the synthesis of chalcone derivatives as antidiabetic (Gaur et al. 2014), antimicrobial (Gaur et al. 2015), anticancer (Gaur et al. 2015) and anti-TBagents (Gaur et al. 2015). This communication describes the synthesis of substituted indoles **1a**-**1j**, **2a**-**2k** as antimalarial agents.

In continuation of our work published in Gaur et al. 2015, in the present study, we have synthesized two different series (Figure 2) of novel indolyl chalcones **1a**-**1j** (Scheme 1) and **2a**-**2k** (Scheme 2) and evaluated their *in vitro* antimalarial activity.

2. Results and discussions

General methods for the preparation of chalcones involve Claisen-Schmidt condensation of appropriate aryl methyl ketones and aldehydes in presence of acid or base (Delarue et al. 2001; Ryckebusch et al. 2003; Brinner et al. 2005). We have prepared both series of indolyl chalcones by using afore mentioned reference conditions in



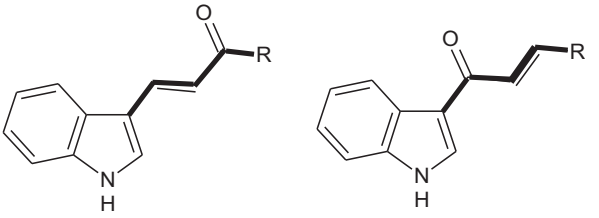
I II

Figure 1. Anti-malarial drugs having nitrogen ring: Pyrimethamine (**I**) aromatic nitrogen ring; Apicidin (**II**) indole ring.

good yields. Indolyl chalcones **1a-j** were prepared by the reaction of appropriate acetophenone **4** with indol-3-carboxaldehyde **5** in the presence of NaOH at RT (Scheme 1) (Bhagat et al. 2006; Srinivasan et al. 2009). Indolyl chalcones **2a-k** were prepared by the reaction of 3-acetylindole **6** with appropriate aldehyde **7** in the presence of $SOCl_2$ (Scheme 2) (Jeong et al. 2004; Gaur et al 2015). Acetophenones (**4d, 4e, 4h**) for the preparation of the compounds **1d**, **1e** and **1h** were prepared by etherification of p-hydroxy (**3d**), m,p-hydroxy (**3e**) and p-hydroxy (**3h**) acetophenones respectively with allyl bromide in the presence of KBr in acetone using refluxing condition.

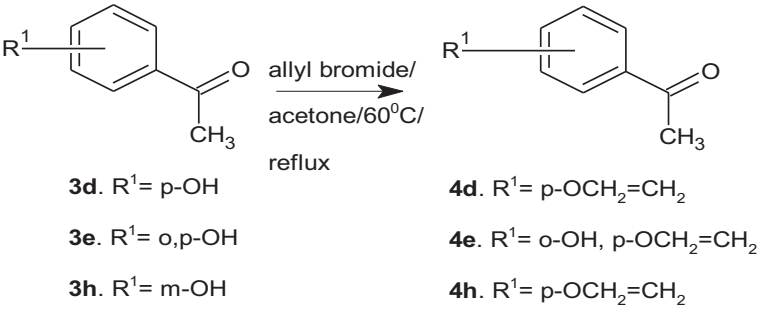
Among all the twenty one compounds tested, four compounds, **1b**, **1d, 1e, 1f** and **2k** showed MIC below 3 μM/L and **1b** was most active with IC_{50} 2.1 μM/L, while five compounds, **1a**, **1c**, **2b**, **2d**, **2e**, have shown MIC below 6 μM/L. Only the compound **2k** having thiophene ring showed MIC 2.8 μM/L. Most of the compounds of series 2 showed MIC more than 12 μM/L. All the compounds, of series 1 having electron donating group in the ring showed better activity than compounds of series 2. These results emphasize the better efficacy of series 1 compounds over series 2 compounds in antimalarial activity (Supplementary material, Table S1).

All these indolyl chalcones were assayed for their *in vitro* antimalarial activity against *P. falciparum,* NF54 strain. Varying the substituents on the phenyl ring of series 2 chalcone derivatives in which ketone group of α,β-unsaturated chalcone is near indole ring, have no significant effect on the activity, while substitution at the second position of phenyl ring of series 1 chalcone derivatives plays a crucial role in exerting antimalarial activity, in which double bond of α,β-unsaturated chalcone is near indole ring. On introducing hydroxyl group at position 2 of phenyl ring of **1b** showed good antimalarial activity with IC_{50} 2.1 μM/L. On introducing ether group with OH group activity dropped to 2.5 μM/L. On the removal of the hydroxyl group the activity dropped to 2.6 μM/L. These results emphasize the better efficacy of hydroxyl group over ether group in series 1 chalcone derivatives for antimalarial activity.



Series 1. **Series 2**

Figure 2. Indole Chalcones skeleton; Series 1 and Series 2.



3d. R^1= p-OH

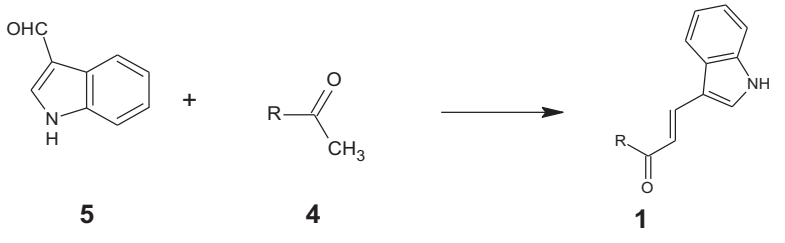
3e. R^1= o,p-OH

3h. R^1= m-OH

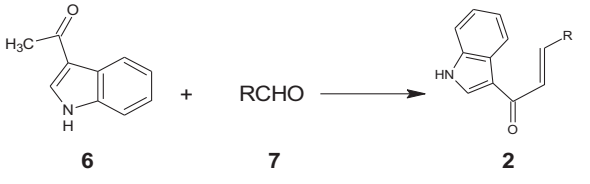
4d. R^1= p-OCH_2=CH_2

4e. R^1= o-OH, p-OCH_2=CH_2

4h. R^1= p-OCH_2=CH_2



Scheme 1. Reagents and conditions: NaOH, methanol, 1–15 h, RT.



Scheme 2. Reagents and conditions: $SOCl_2$, methanol, 1–2 h, RT.

2.1. Molecular docking study

During docking study a series of bioactive chalcone derivatives were screened for the inhibitory effects in *P. falciparum* by targeting the best characterized plasmodium cysteine protease (Falcipain-2) of papain family, consequently this family of enzymes is a potential target for antimalarial drugs. Falcipain-2 is one of the key haemoglobinases of erythrocytic parasite, playing an essential role in the degradation of human

haemoglobin to provide nutrients for the pathogen (Rarey et al. 1996). The aim of docking was to explore the binding site conformation of chalcone derivatives against falcipain-2 and to inhibit the falcipain-2 activity, the results of docking studies suggest that the compounds inhibit the activity of falcipain-2 by showing higher binding energy as indicated by docking score. In this docking study, we explore the orientation and binding energy of active chalcone derivatives towards anti-malarial target falcipain-2 (PDB: 2GHU). The binding energy of control drugs artemisinin and di-hydro artemisinin (DHA) were obtained from docking study and compared with the studied chalcone derivatives, the active derivatives were bound in the active binding site of falcipain-2 and showed higher binding energy as comparable to artemisinin and DHA. The binding energy of artemisinin and DHA were obtained by docking software was −14.9698 and −13.8422 KJ/Mol, which was lower than studied chalcone derivatives (**1a, 1b, 1c, 1d,1e**, **1f, 1g**, **1h**, **1i**, **1j**, **2a, 2b, 2c, 2d, 2e, 2f, 2g, 2h, 2i, 2j** and **2k** i.e. −23.3609, −20.1176, −18.0378, −21.1530, −19.7242, −15.6122, −23.8243, −19.5057, −18.1345, −34.7210, −23.2514, −20.1176, −22.7645, −19.929, −24.9142, −30.3430, −27.6112, −30.5480, −32.2120, − 32.2012 and −28.0341 KJ/Mol and formed hydrogen bonds with interacting residues respectively.

When we compared how the binding site pocket residues of falcipain-2 interact with chalcone derivatives, we found that the compounds **1b**, **1c**, **1d**, **1e, 1f**, **1h, 1i**, **2a**, **2b**, **2c**, and **2d** showed interaction with specific amino acids residues which were also found conserved in artemisinin and DHA docking pose respectively. Although compounds **2b**, **2e** and **1f** do not have any single conserved residue with artemisinin or DHA. During docking against falcipain-2, results showed similar binding site residues for most favourable conformations of predicted active indole chalcones as well as for DHA and artemisinin (Supplementary material, Table S2). Among the all studied compounds, **1j** showed higher binding energy with falcipain-2 and found to bind in the active pocket of protein, although the bioactive chalcone derivatives were showing higher binding energy as compared to artemisinin and DHA.

2.2. Screening through in-silico *pharmacokinetics parameters*

The chemical descriptors for the pharmacokinetic properties were calculated, to check the compliance of studied compounds with the standard range. The ADMET properties were calculated to check the failure of lead candidates, which may cause toxicity or be metabolized by body into an inactive formor one unable to cross the intestinal membranes. Results revealed that artemisinin and indole chalcones *i.e.* compound **1a–1j** and **2a–2k** follows Lipinski's rule of five for oral bioavailability and follow rule of 5 violation i.e., 0 (Supplementary material, Tables S3 and S4). The bi-plot showed two confidence ellipses of 95% and 99% for blood-brain barrier penetration and human intestinal absorption models (Supplementary material, Figure S1). The polar surface area (PSA) was showed to have inverse relationship with percent human intestinal absorption and membrane permeability. Evaluation through standard ADMET parameters showed that most of the studied compounds have low solubility level, Blood-Brain Barrier (BBB) penetration level is very high which showed that studied compounds can easily penetrate the BBB. On the other hand most of the compounds

showed False Cytochrome (CYP- 2D6) binding parameter which showed, the compounds have good inhibitory property with CYP 2D6, and compounds for hepato-toxicity showed true parameter. All the studied compounds except **2d**, **2k**, artemisinin and DHA, showed high plasma-protein binding (PPB), thus seems to be easily distributed in the body. The bi-plot showed confidence ellipses of 95% and 99% for the blood–brain barrier penetration and human intestinal absorption models (Supplementary material, Figure S1). The polar surface area (PSA) was shown to have an inverse relationship with percent human intestinal absorption and membrane permeability. All the compounds were found inside the plot, which represent good bioavailability and permeability. The toxic effect of all the studied compounds were calculated in comparison to the standard drugs (Artemisinin, DHA) (Supplementary material, Table S5). The computed toxicity results, showed that all the compounds were non-mutagenic except **1a**, **1j**, **2f** and **2k** compound. Studied compounds **1f**, **1g**, **1c**, **2b**, **2d**,**2c**, **1b**, **1d**, **1h**, and **1e** showed none skin irritancy while artemisinin, DHA, **1a**, **1i**, **1j**, **2a**, **2e, 2f, 2g, 2i, 2j** and **2k** showed mild skin irritancy. For ocular irritancy only DHA, compound **1a**, **1i**, **2b, 2d, 2g** and **2k** showed severe irritancy rest of the compounds showed either mild or none irritancy.

3. Experimental

For general experimental procedures of synthesis, extraction and isolation of indolyl chalcones, antimalarial activity, in silico and NMR spectra of the synthesized compounds (Supplementary material, Figure S2) see supplementary file Lecaille et al. 2002; Lipinski et al. 2001; Markler & Hinrich 1993; Pandey et al. 2005.

4. Conclusion

The present study suggested that the newly synthesized indole derivatives are new lead in antimalarial chemotherapy. These molecules can be very useful for further optimization work in malarial chemotherapy.

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Disclosure statement

The author(s) confirm that this article content has no conflicts of interest.

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