CHAPTER 3

Facile synthesis of antimalarial 1,2-disubstituted 4-quinolones from 1,3-bisaryl-monothio-1,3-diketones

3.1 Introduction

Quinolones and their derivatives have contributed substantially for the evolution of antimicrobial agents. The development of antibiotic quinolone was begin in 1962 with discovery of nalidixic acid, (Lesher et al., 1962) which was used to treat urinary tract infection. The progress of the development of new quinolones to treat bacterial infection was preceded slowly over next few years. Later, many fluoroquinolones (cinoxacin, rosoxacin, flumequine) were synthesized and studied their structure-activity relationships with antimicrobial activity and the result reveals the 4-oxo and 3-carboxyl in the quinolones are the active functional group linked to antimicrobial activity. Subsequently, many research groups discovered large number of 4-quinolones and modified their structure with broad-spectrum activity and were classified as first, second, third and fourth generation quinolones. The first generations quinolones (e.g. nalidixic acid) are effectively used inhibit gram negative bacteria. Whereas the second generation quinolones (e.g. norfloxacin, ciprofloxacin) has fluorine atom in the central ring system and hence exhibit therapeutic property against the gram negative strains such as S. aureus, pseudomonas and B. anthracis. The third (e.g. Levofloxacin) and fourth (e.g. trovafloxacin) generation quinolones extend their bactericidal activity to gram positive bacteria and other anaerobes. They have shown their chemotherapeutic property by selective inhibiting of bacterial DNA synthesis. These quinolones selectively target the topoisomerase II enzyme (DNA gyrase), which is necessary to repair the single strand
DNA during replication and maintains the chromosome in supercoiled state. Hence binding of quinolones to the target (DNA gyrase) leads to the interference of bacterial DNA replication or transcription. (Drlica and Malik, 2003) Later, gram-positive and gram-negative bacteria developed resistance to these quinolones either by developing efflux mechanism or decreased outer membrane permeability. (Yoshida et al., 1994)

Further, a newer class of fourth generation fluoroquinolones introduced to the market, which have expanded antimicrobial spectrum with large bioavailability. Hence the high serum drug concentration in tissue and fluid make them useful particularly to treat infection caused by pneumonia. The fluoroquinolones relatively safe with minimum side effect at lower concentration of drug, but the newer drug need twice or more doses per day to retains their activity. The higher concentration of the fluoroquinolone drugs leads to adverse effects such as vomiting, nausea, diarrhea, headache, dizziness and by considering these side effects, the drugs are not recommended for patients younger than 18 years or lactating or pregnant women.

Fig. 1 Types of fluoroquinolone drugs

Therefore United States introduced the potent fluoroquinolones drugs to the market such as Levofloxacin, Sparfloxacin, Grepafloxacin, Trovafloxacin, Gatifloxacin and
Moxifloxacin (Fig. 1). These available newer quinolone antibiotics have improved pharmacokinetic property compared to older quinolones. These drugs are absorbed almost completely with in short time from the gastrointestinal tract; hence the serum concentration either through oral or intravenous administration is very high. In addition to these many diversely substituted 4-quinolones have been extensively investigated as antitumour, (Nakamura et al., 2005) antiviral, (Santos et al., 2009) antidiabetic, (Edmont et al., 2000) antitrypanosomal agents (Wube et al., 2011) and HIV-1 integrase inhibitors. (Moyer et al., 1992)

3.2 Anti-malarial agents

Malaria is devastating disease caused by the infection of *Plasmodium* family of protozoan parasites. Almost, half of the world population is at health risk due to malaria infection. In 2012 alone, there have been approximated 6,27,000 deaths occur and particularly children have five year age are regularly infected by *Plasmodium falciparum*. (Murray et al., 2012) The Cinchona bark infusions was the first effective remedy used to cure malaria and was used for centuries. Later, quinine was isolated from the bark of Cinchona and identified as first antimalarial agent and the total synthesis of quinine was achieved by several groups but in economically none of the methods superior over the isolation protocol from natural source. Therefore, German chemists in twentieth century developed method for the synthesis of several quinine based antimalarial drugs, among them chloroquine, mefloquine and primaquine emerged as most successful agents to eradicate malaria. But the malarial parasites have developed a resistance against these frequently used drugs. (F Garcia-Bustos and Gamo, 2013) Hence, artemisinin-based combination therapy (ACT) has been deployed as a front-line treatment and shows rapid action against
Plasmodium falciparum malaria. However, parasites have already started developing resistance to ACT in Southeast Asia. (Dondorp et al., 2011) In view of this, the development of new drugs is an urgent necessity to control and eradicate malaria.

3.3 Synthesis of 4-quinolone: A literature survey

In early days, 4-quinolones are synthesized using classical method called Conrad-Limpach reaction (Scheme 1). This method involves the condensation of aniline (1) with acetoacetic ester (2). The amine group attack the reactive keto group of acetoacetic ester at room temperature gave Schiff base (3) in high yield, followed by cyclization at 250 °C lead to the formation of substituted 4-quinolone (keto form) (4) in low yield (30%) as final product with less predominant 4-hydroxyquinoline (enol form) (5). Later, Limpach improved the yield from 30 to 95% by using mineral oil in the cyclization step. (“Conrad–Limpach synthesis,” 2016)

![Scheme 1](image)

The high temperature, low substrate scope and use of strong acid make the less use of traditional method for the synthesis of 4-quinolones. Recently many reports demonstrated the importance of various enaminones in metal catalyzed cyclization reaction for the synthesis of 4-quinolones. These enaminones are prepared form conjugate addition of primary amines to \(\alpha,\beta\)-ynones (\(\alpha,\beta\)-ynones are obtained by Sonogoshsira coupling of commercially available 2-halo benzoyl chlorides and terminal alkynes). The 1,2-
disubstitutes-4-quinolones (9) was synthesized using through Cu catalysed heterocyclization of functionalized enaminoines (Scheme 2i). (Bernini et al., 2009) The reaction tolerates wide variety of functional group on the enaminoine (1-(2-halophenyl)-2-en-3-amin-1-ones) (8) prepared using aromatic primary amine (7) and \( \alpha,\beta \)-ynones (6). A catalyst free approach (Scheme 2ii) was developed for the synthesis of 4-quinolones (14), which involve the domino amination and Michael addition reaction of 11 with aromatic primary amines (12). (Iaroshenko et al., 2013) Further \( N \)-alkyl substituted-4-quinolones (15) are also prepared using same reaction sequence from alkyl amines (13). (Shao et al., 2012)

\[
\begin{align*}
\text{X} &= \text{Cl, Br}; R^1 &= \text{C}_6\text{H}_5, p-\text{Me-C}_6\text{H}_4, p-\text{OMe-C}_6\text{H}_4, p-\text{Cl-C}_6\text{H}_4, p-\text{CF}_2\text{C}_6\text{H}_4, p-\text{CN-C}_6\text{H}_4, m-\text{OMe-C}_6\text{H}_4, n-\text{Bu, t-Bu, Cy}; R^2 &= \text{C}_6\text{H}_5, p-\text{OMe-C}_6\text{H}_4, p-\text{Cl-C}_6\text{H}_4.
\end{align*}
\]

\[
\begin{align*}
\text{R}^2 &= \text{NH}_2, X = \text{F} \\
\text{Li}_2\text{CO}_3, \text{DMA} \\
160 \degree \text{C}, 24-30 \text{h}
\end{align*}
\]

\[
\begin{align*}
\text{X} &= \text{Cl, Br}; R^1 &= \text{C}_6\text{H}_5, p-\text{Me-C}_6\text{H}_4, p-\text{OMe-C}_6\text{H}_4, p-\text{Cl-C}_6\text{H}_4, p-\text{CF}_2\text{C}_6\text{H}_4, p-\text{CN-C}_6\text{H}_4, m-\text{OMe-C}_6\text{H}_4, n-\text{Bu, t-Bu, Cy}; R^2 &= \text{C}_6\text{H}_5, p-\text{OMe-C}_6\text{H}_4, p-\text{Cl-C}_6\text{H}_4, 3,4-\text{(OMe)}_2\text{C}_6\text{H}_3, \text{CH}_2\text{-C}_6\text{H}_5
\end{align*}
\]

\[
\begin{align*}
\text{R}^2 &= \text{NH}_2, X = \text{Cl, Br} \\
\text{K}_3\text{PO}_4, \text{3H}_2\text{O} \\
\text{DMSO, 140} \degree \text{C}
\end{align*}
\]

\[
\begin{align*}
\text{R} &= \text{H, 4-Cl, 4-Cl-5-F}; R^1 &= \text{C}_6\text{H}_5, p-\text{Et-C}_6\text{H}_4, p-\text{OMe-C}_6\text{H}_4, p-\text{Cl-C}_6\text{H}_4, p-\text{F-C}_6\text{H}_4; R^2 &= \text{n-Bu, \text{i-Pr, Me(CH}_2)_7, Me(CH}_2)_11, CH}_2\text{-CH=CH}_2
\end{align*}
\]

Scheme 2
Another convenient method which demonstrate the palladium catalyzed tandem amination reaction (Scheme 3) for the synthesis of 4-quinolone (18) in excellent yield from o-haloaryl acetylenic ketones (16) and primary amines (17). (Zhao and Xu, 2010)

\[
\text{Scheme 3}
\]

The microwave irradiation of acylated-2-\(^\text{a}\)-aminoacetophenones (19) generates 2-substituted-4-quinolones (20) in a short reaction time at 120 °C (Scheme 4). (Ding et al., 2006)

\[
\text{Scheme 4}
\]

Recently Buchwald–Hartwig (Fei et al., 2012) coupling was utilized for the preparation of 1,2-disubstituted-4-quinolones from easily accessible and cost effective starting materials such as chalcones (21) and aromatic or benzyl amines (22). The palladium catalyst used in this method plays a dual role in presence of base, namely coupling and catalytic dehydrogenation to afford wide range of 4-quinolones (23) in excellent yield (Scheme 5).
Scheme 5

The carboamination reaction was postulated as (i.e formation of C-C and C-N bond simultaneously across unsaturated C-C bonds) an efficient tool for the synthesis of many nitrogen containing heterocycles. For instance, N-phenylisatoic anhydride (24) undergo carboamination reaction with alkynes (25) in presence of nickel catalyst and gives 1-phenyl-2,3-disubstituted 4-quinolones (26, Scheme 6). (Yoshino et al., 2009)

Scheme 6

Another convenient two step strategy such as amidation reaction of o-halo acetophenones (27) with phenyl amides (28) followed by base mediated Camps cyclization of obtained intermediate 29 leads to the formation of 2,3-disubstituted-4-quinolones (30) in excellent yield (Scheme 7). (Jones et al., 2007)
3.4 Results and Discussion

3.4.1 Facile synthesis of 1,2-disubstituted 4-quinolones from β-enaminones

The facile approach used for the synthesis of 1,2-disubstituted-4-quinolones monothiodiketone involve two step procedures (Scheme 8). The first step is condensation reaction of various monothiodiketone (31) with primary aromatic amines (32) in ethanol using catalytic amount of trifluoroacetic acid. In the second step, obtained β-enaminones (33) are cyclized using palladium catalyst in presence of Cs₂CO₃ as base in DMF at reflux temperature to afford 4-quinolones (34).

![Scheme 8](image_url)

**Scheme 8** Approach to the synthesis of 1,2-disubstituted 4-quinolones

The enaminone (33a) was selected as a model substrate in which R¹ is Ph and R² is p-OMePh for the formation of 4-quinolone (34a) under different reaction conditions with DMF as a solvent of choice. Thus 33a in presence of Pd₂(dba)₃ as catalyst and K₂CO₃ as a base afforded 1,2-diphenyl-4-quinolone (34a) in 10% yield (Table 1, entry 1). However, use of ligands PPh₃ and PCy₃ along with the same catalyst and base gave quinolone (34a) in 38 and 32% yield, respectively (Table 1, entries 2 and 3). In a parallel study, the yield of (34a) was increased to 46% in presence of Pd₂(dba)₃ catalyst, PPh₃ ligand and Cs₂CO₃ base (Table 1, entry 4). Further the yield of 34a was dramatically increased to 83% in presence of catalyst Pd(OAc)₂ and base Cs₂CO₃ but without the use of ligand (Table 1, entry 5). Use of ligand and change of base reduced the yield of 34a (Table 1, entries 6-8). A reaction was conducted in the absence of Pd(OAc)₂ to assess the
role of palladium catalyst in the reaction. It took longer time with only 40% yield of 34a (Table 1, entry 9). After optimizing reaction conditions the generality and scope of the reaction was examined for the synthesis of other 4-quinolones from respective enamiones as shown in Table 2. In all cases, the yields were obtained in range of 70–85%, suggesting that the substituents have little or no effect on the cyclization reaction. The mechanism of the cyclization is discussed in scheme 9.

### Table 1 Optimization of reaction conditions for the cyclization of 1,2-disubstituted 4-quinolones

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Ligand</th>
<th>Base</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd2 (dba)3</td>
<td>-</td>
<td>K2CO3</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Pd2 (dba)3</td>
<td>PH3</td>
<td>K2CO3</td>
<td>10</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>Pd2 (dba)3</td>
<td>PCy3</td>
<td>K2CO3</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>Pd2 (dba)3</td>
<td>PH3</td>
<td>Cs2CO3</td>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>Pd2(OAc)2</td>
<td>-</td>
<td>Cs2CO3</td>
<td>5</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>Pd2(OAc)2</td>
<td>-</td>
<td>K2CO3</td>
<td>8</td>
<td>65</td>
</tr>
<tr>
<td>7</td>
<td>Pd2(OAc)2</td>
<td>PH3</td>
<td>Cs2CO3</td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td>8</td>
<td>Pd2(OAc)2</td>
<td>PCy3</td>
<td>Cs2CO3</td>
<td>6</td>
<td>56</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>Cs2CO3</td>
<td>24</td>
<td>40</td>
</tr>
</tbody>
</table>

### 3.4.2 Antimalarial activity and cytotoxicity

The antimalarial activity of the synthesized 1,2-disubstituted-4-quinolones (Table 2, 34a-o) were evaluated using chloroquine drug sensitive *P. falciparum* strain (3D7) at different concentration ranging from 0.25 μM to 100 μM. The SYBR green assay was utilized for the measurement of inhibition of parasite growth. (Smilkstein et al., 2004) The result
obtained (Table 2) was reveals that majority of synthesized 4-quinolones showed good antimalarial activity. Particularly, seven compounds such as $34d$, $34f$, $34i$, $34h$, $34l$, $34m$ and $34n$ showed IC$_{50}$ less than 2 μM. Hence these potent compounds were further evaluated for inhibition of chloroquine-resistant 7G8 and W2 strains and chloroquine-sensitive D6 strain. All the seven compounds are effectively inhibit both the drug resistant and sensitive parasites (Table 3).

**Table 2** Synthesis of 1,2-disubstituted 4-quinolones and their antimalarial activity$^a$

<table>
<thead>
<tr>
<th>Entry</th>
<th>$R^1$</th>
<th>$R^2$</th>
<th>Product</th>
<th>Yield (%)</th>
<th>IC$_{50}$ μM ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>$p$-OMe-Ph</td>
<td>34a</td>
<td>83</td>
<td>$3.92 ± 0.22$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$m$-OMe-Ph</td>
<td>34b</td>
<td>79</td>
<td>$2.18 ± 0.30$</td>
</tr>
<tr>
<td>2</td>
<td>$p$-Me-Ph</td>
<td>$p$-OMe-Ph</td>
<td>34c</td>
<td>81</td>
<td>$5.15 ± 1.71$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p$-F-Ph</td>
<td>34d</td>
<td>72</td>
<td>$0.82 ± 0.11$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$m$-Br-Ph</td>
<td>34e</td>
<td>76</td>
<td>$6.21 ± 0.33$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-naphthyl</td>
<td>34f</td>
<td>80</td>
<td>$1.53 ± 0.16$</td>
</tr>
<tr>
<td>3</td>
<td>$p$-OMe-Ph</td>
<td>Ph</td>
<td>34g</td>
<td>83</td>
<td>$16.61 ± 3.20$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$m$-OMe-Ph</td>
<td>34h</td>
<td>82</td>
<td>$1.25 ± 0.32$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p$-OMe-Ph</td>
<td>34i</td>
<td>85</td>
<td>$1.91 ± 0.16$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$o$-Br-Ph</td>
<td>34j</td>
<td>76</td>
<td>$2.23 ± 0.34$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p$-F-Ph</td>
<td>34k</td>
<td>70</td>
<td>$5.61 ± 0.84$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-naphthyl</td>
<td>34l</td>
<td>83</td>
<td>$1.53 ± 0.49$</td>
</tr>
<tr>
<td>4</td>
<td>$p$-$N$-(CH$_3$)$_2$Ph</td>
<td>$p$-Cl-CH$_2$-Ph</td>
<td>34m</td>
<td>82</td>
<td>$0.75 ± 0.15$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p,m$-(OMe)$_2$</td>
<td>34n</td>
<td>79</td>
<td>$1.26 ± 0.20$</td>
</tr>
<tr>
<td>5</td>
<td>Thiophenyl</td>
<td>$p$-OMe-Ph</td>
<td>34o</td>
<td>81</td>
<td>$71.86 ± 5.37$</td>
</tr>
</tbody>
</table>

$^a$Against the *P. falciparum* 3D7 strain. $^b$34a and 34b, $p$-Cl; 34m and 34n, $m$-Cl.
Furthermore, the cytotoxicity assay of all the compounds (34a-o) was performed on human lung cancer (A549) and kidney (HEK 293) cells using MTS assay. The majority of compounds, except two (34f and 34i) exhibit minimum cytotoxicity (Table 4). To develop an efficient drug against blood stage of parasite, it should not cause lysis of healthy cells. Hence the effect of all synthesized compounds on red blood cells at different concentration between 2.5 µM to 40 µM was tested. Interestingly, none of the synthesized compounds caused lysis of red blood cells at <20 µM. All these result demonstrate that 1,2-disubstituted-4-quinolones synthesized using facile approach are selectively inhibit the malaria parasites.

**Table 3** Antimalarial activity of 1,2-disubstituted 4-quinolones against drug-sensitive and drug resistant parasites

<table>
<thead>
<tr>
<th>Entry</th>
<th>Parasite growth inhibition (IC$_{50}$ µM ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3D7</td>
</tr>
<tr>
<td>34d</td>
<td>0.82 ± 0.11</td>
</tr>
<tr>
<td>34f</td>
<td>1.50 ± 0.16</td>
</tr>
<tr>
<td>34i</td>
<td>1.90 ± 0.16</td>
</tr>
<tr>
<td>34h</td>
<td>1.20 ± 0.32</td>
</tr>
<tr>
<td>34l</td>
<td>1.50 ± 0.49</td>
</tr>
<tr>
<td>34m</td>
<td>0.75 ± 0.15</td>
</tr>
<tr>
<td>34n</td>
<td>1.26 ± 0.20</td>
</tr>
<tr>
<td>chloroquine</td>
<td>0.13 ± 0.06</td>
</tr>
<tr>
<td>mefloquine</td>
<td>0.04 ± 0.02</td>
</tr>
</tbody>
</table>
Table 4 Cytotoxicity assay of 1,2-disubstituted 4-quinolones against human cell lines

<table>
<thead>
<tr>
<th>Entry</th>
<th>IC$_{50}$ µM ± SD$^a$</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A549 cells</td>
<td>HEK 293 cells</td>
<td></td>
</tr>
<tr>
<td>34a</td>
<td>63.6 ± 2.5</td>
<td>56.2 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>34b</td>
<td>80.4 ± 4.1</td>
<td>79.8 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>34c</td>
<td>60.6 ± 3.1</td>
<td>56.6 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>34d</td>
<td>81.0 ± 4.2</td>
<td>70.6 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>34e</td>
<td>89.6 ± 2.6</td>
<td>61.0 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>34f</td>
<td>45.0 ± 2.4</td>
<td>33.2 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>34g</td>
<td>67.3 ± 2.4</td>
<td>59.1 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>34h</td>
<td>63.6 ± 3.5</td>
<td>71.3 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>34i</td>
<td>68.1 ± 4.4</td>
<td>23.4 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>34j</td>
<td>61.2 ± 2.3</td>
<td>48.1 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>34k</td>
<td>83.1 ± 5.5</td>
<td>75.0 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>34l</td>
<td>45.0 ± 2.1</td>
<td>60.0 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>34m</td>
<td>62.8 ± 3.4</td>
<td>56.6 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>34n</td>
<td>88.5 ± 5.2</td>
<td>72.7 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>34o</td>
<td>82.9 ± 2.3</td>
<td>70.6 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The absorbances were measured using a micro plate reader at 72 h.
3.5 Conclusion

In conclusion, we have established a novel approach for the synthesis of 1,2-disubstituted 4-quinolones from 1,3-bisarylmonothio-1,3-diketone and arylamine substrates in two simple steps. In the first step, the substrates were condensed using TFA as a catalyst to form enaminones. In the second step, after evaporating the solvent of the first reaction, the enaminones were cyclized using the Pd(OAc)$_2$ catalyst and the Cs$_2$CO$_3$ base. Several of the synthesized 4-quinolones showed an excellent activity against both chloroquine-sensitive and chloroquine-resistant parasites and exhibited minimal cytotoxicity.

3.6 Experimental section

3.6.1 General procedure for the synthesis of β-enaminones (33)

In a typical experiment, to the solutions of 1-(2-haloaryl)-3-(het)arylmonothiodiketones (31, 3.1 mmol) and aryl amines (32, 3.1 mmol) in EtOH (10 mL), trifluoroacetic acid (0.5 mmol) was added and heated at 80 °C while stirring for 3-4 h. The formation of enaminones was monitored by TLC. Then the solvent was removed under reduced pressure, and the residual material was dissolved in ethyl acetate (20 mL) and water (10 mL) and thoroughly mixed. The ethyl acetate layer was washed with brine (10 mL), dried over anhydrous Na$_2$SO$_4$ and concentrated to obtain the products 33, which used for next step without purification.
3.6.2 General procedure for the synthesis of 1,2-diaryl-4-quinolones 34 (a-o)

In a typical experiment, to an oven-dried, nitrogen-purged flask containing the compound 33 (0.8 mmol) and Cs\(_2\)CO\(_3\) (1.6 mmol) in DMF (3.0 mL), Pd(OAc)\(_2\) (0.02 mmol) was added. The reaction mixture was stirred at 90-100 °C for 4 h and monitored by TLC. Upon completion, the reaction mixture was filtered through a pad of celite, residue was extracted with EtOAc (3 × 10 mL), washed with brine, water and dried over anhydrous Na\(_2\)SO\(_4\). The solvent was evaporated to give the crude product, was purified by column chromatography on silica gel (EtOAc–Hexane, 6:4) to give desired product 34.

3.6.3 Assessment of antimalarial activity and cytotoxicity

**Antimalarial activity:** *P. falciparum* parasites were cultured according to the method of Trager and Jensen. (Trager and Jensen, 1976) Briefly, 3D7, D6, W2 and 7G8 parasite strains were cultured in RPMI 1640 medium (Gibco) supplemented with 25 mM HEPES, 29 mM sodium bicarbonate, 0.005% hypoxanthine, *p*-aminobenzoic acid (2 mg per liter), gentamycin sulfate (50 mg per liter) and 5% AlbuMAX II (Invitrogen) using fresh O-positive human red blood cells at 2% hematocrit. The cultures were maintained at 37 °C under 5% O\(_2\), 5% CO, and 90% N atmosphere. The antimalarial activity of quinolone compounds was determined by a fluorometric method using SYBR Green I. Briefly, stock solutions of quinolones were prepared in DMSO (10 mM) and were diluted to 400 µM with DMSO. The solutions were then 1:2 serially diluted with complete culture medium to give concentrations ranging from 0.5 to 200 µM; the final concentration of DMSO was less than 0.1%. 100 µL of each testing solution was mixed with 100 µL of 0.2% parasitized red blood cells in the ring stage at 2% hematocrit in complete medium and dispensed into 96-well plates. Untreated- and DMSO (0.1%) vehicle-treated parasites
were used as controls. The plates were incubated at 37 °C for 72 h and the experiment was performed in triplicate. To each well, 100 µL of lysis buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 0.008% saponin, 0.08% Triton X-100) containing 0.2 µL mL⁻¹ of SYBR Green I (Life Technology) was added. The plates were wrapped with an aluminum foil and incubated in the dark at room temperature for 1 h. The fluorescence intensity was measured using a multi-well plate fluorescence reader at an excitation and emission wavelengths of 485 and 535 nm, respectively. The absorption values were expressed as relative fluorescence units. The IC₅₀ values (the effective concentrations that inhibit parasite growth by 50%) of three independent experiments were plotted using the nonlinear regression (Sigmoidal dose response) equation (GraphPad Prism, version 4.01, GraphPad Software, La Jolla, CA).

**Cytotoxicity assay:** The toxicity of quinolones against human cells was assessed by the MTS assay using human embryonic kidney cells (HEK 293 cell line) and human adenocarcinoma epithelial cells (A549 cell line). (Barltrop et al., 1991) Briefly, 5 × 10³ cells per well in 100 µL of DMEM medium supplemented with 10% bovine fetal serum were plated in 96-well plates. After 24 h, the stock solutions of quinolones in DMSO that were diluted to obtain concentrations ranging from 0.5 µM to 200 µM in 100 µl complete medium were added to each well and incubated at 37 °C for 72 h. The wells treated with DMSO (1%) alone were used as vehicle controls. To each well was added 20 µl of the MTS/PMS reagent (Promega, Madison, WI), incubated for 3 h at 37 °C and the absorbance was measured at 490 nm. The assay was performed in triplicate and the values from three independent experiments were used to calculate the IC₅₀ values (GraphPad Prism, version 4.01).
3.7 Characterization data of synthesized compounds

**7-Chloro-1-(4-methoxyphenyl)-2-phenylquinolin-4(1H)-one (34a).**

Obtained as white solid from 33a; m.p. 222-224 °C; IR (KBr) cm⁻¹: 3048, 1631, 1586, 1474, 1370, 1309, 878; ¹H NMR (400MHz, CDCl₃) δ 8.42 (d, J=8.8 Hz, 1H, ArH), 7.04-7.33 (m, 8H, ArH), 6.85-6.92 (m, 3H, ArH), 6.42 (s, 1H, CH), 3.80 (s, 3H, OMe); ¹³C NMR (100 MHz, CDCl₃) δ 177.3, 165.5, 159.6, 154.9, 143.6, 138.3, 135.4, 131.1, 130.7, 129.0, 128.7, 128.0, 127.9, 124.5, 117.7, 114.8, 112.9, 55.5; MS m/z 362 [M+H]+; Anal. Calcd for C₂₂H₁₆ClNO₂: C 73.03, H 4.46, N 3.87. Found: C 73.06, H 4.52, N 3.94.

**7-Chloro-1-(3-methoxyphenyl)-2-phenylquinolin-4(1H)-one (34b).**

Obtained as yellow solid from 33b; m.p. 114-116 °C; IR (KBr) cm⁻¹: 3056, 1673, 1547, 1498, 1398, 1278, 1247, 1121, 876; ¹H NMR (400 MHz, CDCl₃) δ 8.42 (d, J= 9.2 Hz, 1H, ArH), 7.05-7.33 (m, 5H, ArH), 6.78-7.05 (m, 6H, ArH), 6.42 (s, 1H, CH), 3.73 (s, 1H, OMe); ¹³C NMR (100MHz, CDCl₃) δ 178.0, 160.5, 159.6, 153.9, 142.6, 140.3, 131.9, 130.5, 130.2, 128.1, 126.2, 126.0, 123.7, 122.3, 118.0, 115.6, 114.6, 113.4, 112.7, 55.5; MS m/z 362 [M+H]+; Anal. Calcd for C₂₂H₁₆ClNO₂: C 73.03, H 4.46, N 3.87. Found: C 73.10, H 4.55, N 3.95.

**1-(4-Methoxyphenyl)-2-(4-methylphenyl)quinolin-4(1H)-one (34c).**

Obtained as brown solid from 33c; m.p. 216-218 °C; IR (KBr) cm⁻¹: 3078, 1614, 1603, 1458, 1402, 1318, 1275, 1063, 834; ¹H NMR (400 MHz, CDCl₃) δ 8.50 (d, 1H, J=7.2 Hz, ArH), 7.48-
7.34 (m, 2H, ArH), 7.06-6.85 (m, 9H, ArH), 6.41 (s, 1H, CH), 3.80 (s, 3H, OMe), 2.27 (s, 3H, ArMe); \(^{13}\)C NMR (100MHz, CDCl\(_3\)) \(\delta\) 177.9, 159.4, 154.5, 142.9, 138.4, 132.9, 131.9, 131.7, 130.9, 129.0, 128.6, 126.2, 126.1, 123.6, 118.1, 114.6, 112.5, 55.4, 21.2; MS m/z 342 \([\text{M+H}\]^+\); Anal. Calcd for C\(_{23}\)H\(_{19}\)NO\(_2\): C 80.92, H 5.61, N 4.10. Found: C 80.96, H 5.68, N 4.17.

1-(4-Fluorophenyl)-2-(4-methylphenyl)quinolin-4(1\(H\))-one (34d).

Obtained as white solid from 33d; m.p. 234-336 °C; IR (KBr) cm\(^{-1}\): 1618, 1594, 1562, 1488, 1421, 1341, 1172, 1068, 789; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.51 (d, \(J=8.0\) Hz, 1H, ArH), 7.50-7.37 (m, 2H, ArH), 7.16-7.02 (m, 8H, ArH), 6.87 (d, \(J=8.4\) Hz, 1H, ArH), 6.42 (s, 1H, CH), 2.28 (s, 3H, ArMe); \(^{13}\)C NMR (100MHz, CDCl\(_3\)) \(\delta\) 178.5, 160.0, 158.8, 144.2, 139.9, 134.0, 132.9, 132.1, 131.4, 130.1, 129.7, 127.4, 127.1, 124.5, 119.0, 115.9, 113.5, 23.01; MS m/z 330 \([\text{M+H}\]^+\); Anal. Calcd for C\(_{22}\)H\(_{16}\)FNO: C 80.23, H 4.90, N 4.25. Found: C 80.29, H 4.96, N 4.31.

1-(3-Bromophenyl)-2-(4-methylphenyl)quinolin-4(1\(H\))-one (34e).

Obtained as white solid from 33e; m.p. 208-210 °C; IR (KBr) cm\(^{-1}\): 1631, 1587, 1492, 1463, 1394, 1261, 1123, 767; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.50 (d, \(J=8.0\) Hz, ArH), 7.51-7.48 (t, 2H, \(J=7.6\) Hz, ArH), 7.51-7.48 (t, 2H, \(J=7.6\) Hz, ArH), 7.27-7.23 (t, 1H, \(J=8.4\) Hz, ArH), 7.12-6.87 (m, 6H, ArH), 6.41 (s, 1H, CH), 2.28 (s, 3H, ArMe); \(^{13}\)C NMR (100MHz, CDCl\(_3\)) \(\delta\) 177.9, 153.8, 142.3, 140.5, 138.9, 133.2, 132.4, 132.2, 132.0, 130.7, 128.9, 128.8, 128.8, 126.4, 125.9, 123.9, 122.8, 117.7, 112.8, 21.2; MS m/z 390,

2-(4-Methylphenyl)-1-(naphthalen-1-yl)quinolin-4(1H)-one (34f).

Obtained as brown solid from 33f; m.p. 236-238 °C; IR (KBr) cm⁻¹: 1623, 1607, 1534, 1481, 1436, 1279,1236, 1191, 1031, 842; ¹H NMR (400MHz, CDCl₃) δ 8.54 (d, J= 8.0 Hz, 1H, ArH), 7.87-6.86 (m, 14H, ArH), 6.48 (s, 1H), 2.17 (s, 3H, Me); ¹³C NMR (100MHz, CDCl₃) δ 177.9, 159.4, 154.0, 149.1, 142.7, 136.6, 133.1, 132.5, 131.7, 130.5, 129.6, 129.1, 128.0, 127.9, 127.8, 127.3, 127.0, 126.2, 126.1, 123.7, 118.2, 113.4, 122.7, 21.4; MS m/z 362 [M+H]+; Anal. Calcd for C_{26}H_{19}NO: C 86.40, H 5.30, N 3.88. Found: 86.48, H 5.36, N 3.93.

2-(4-Methoxyphenyl)-1-phenylquinolin-4(1H)-one (34g).

Obtained as white solid from 33g; m.p. 208-210 °C; IR (KBr) cm⁻¹: 3065, 1628, 1614, 1521, 1484, 1402, 1248, 1186, 1025, 841; ¹H NMR (400 MHz, CDCl₃) δ 8.43 (d, J= 8.0 Hz, 1H, ArH), 7.30-7.45 (m, 3H, ArH), 6.67-7.19 (m, 9H, ArH), 6.53 (s, 1H, CH), 3.83 (s, 3H, OMe); ¹³C NMR (100 MHz, CDCl₃) δ 175.8, 164.0, 157.8, 144.4, 141.2, 135.9, 133.2, 128.9, 128.3, 126.7, 126.4, 125.6, 125.5, 122.1, 115.5, 112.3, 110.5, 53.6; MS m/z 328 [M+H]+; Anal. Calcd for C_{26}H_{17}NO₂: C 80.71, H 5.23, N 4.28. Found: C 80.76, H 5.32, N 4.35.

1-(3-Methoxyphenyl)-2-(4-methoxyphenyl)quinolin-4(1H)-one (34h).

Obtained as white solid from 33h; m.p. 214-216 °C; IR (KBr) cm⁻¹: 3054, 1630, 1598, 1521, 1474, 1412, 1348, 1175, 1048,
838; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.50 (d, $J$=7.6 Hz, 1H, ArH), 7.49-7.11 (m, 5H, ArH), 6.98-6.67 (m, 6H, ArH), 6.42 (s, 1H, CH), 3.76 (s, 3H, OMe), 3.73 (s, 3H, OMe);
$^{13}$C NMR (100 MHz, CDCl$_3$) δ 177.9, 160.4, 159.6, 153.8, 142.5, 140.2, 131.8, 130.4, 130.2, 128.0, 126.2, 125.9, 123.7, 122.2, 118.1, 115.6, 114.6, 113.3, 112.6, 55.5, 55.2; MS m/z 358 [M+H]$^+$; Anal. Calcd for C$_{23}$H$_{19}$NO$_3$; C 77.29, H 5.36, N 3.92. Found: C 77.31, H 5.39, N 3.98.

1,2-Bis(4-methoxyphenyl)quinolin-4(1H)-one (34i).

Obtained as yellow solid from 33i; m.p. 222-224 °C; IR (KBr) cm$^{-1}$: 3068, 1628, 1614, 1532, 1464, 1436, 1261, 1163, 1087, 774; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.50 (d, $J$= 7.60 Hz, 1H, ArH), 7.48-7.33 (m, 2H, ArH), 7.10-6.71 (m, 9H, ArH), 6.41 (s, 1H, CH), 3.81 (s, 3H, OMe), 3.75 (s, 3H, OMe); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 177.9, 159.4, 159.3, 154.2, 142.9, 131.8, 131.7, 130.8, 130.5, 128.1, 126.1, 125.9, 123.5, 118.1, 114.6, 113.3, 112.5, 55.2, 55.1; MS m/z 358 [M+H]; Anal. Calcd for C$_{23}$H$_{19}$NO$_3$; C 77.29, H 5.36, N 3.92. Found: C 77.31, H 5.39, N 3.98.

1-(2-Bromophenyl)-2-(4-methoxyphenyl)quinolin-4(1H)-one (34j).

Obtained as white solid from 33j; m.p. 212-214 °C; IR (KBr) cm$^{-1}$: 3059, 1631, 1627, 1591, 1482, 1423, 1268, 1157, 1120, 846; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.51 (d, $J$=7.6 Hz, 1H, ArH), 7.61 (d, $J$= 8.0 Hz, 1H, ArH), 7.48 (t, $J$= 7.6 Hz, 1H, ArH), 7.38 (q, 2H, $J$= 8.5 Hz, ArH), 7.21-7.30 (m, 4H, ArH), 6.45 (s, 1H, CH), 3.75 (s, 3H, OMe); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 178.2, 159.9, 153.6, 141.5, 138.4, 134.0, 132.3, 132.1, 130.7, 130.3, 128.5, 127.4, 126.4, 125.9, 124.5, 123.9, 117.3, 113.2, 112.9,
55.2; MS m/z 406, 408 [M+H]; Anal. Calcd for C_{22}H_{16}BrNO_{2}: C 65.04, H 3.97, N 3.45. Found: C 65.10, H 3.99, N 3.53.

1-(4-Fluorophenyl)-2-(4-methoxyphenyl)quinolin-4(1H)-one (34k).

Obtained as white solid from 33k; m.p. 228-230 °C; IR (KBr) cm\(^{-1}\): 3063, 1622, 1583, 1511, 1486, 1417, 1326, 1161, 1018, 845; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.50 (d, \(J=7.6\) Hz, 1H, ArH), 7.48-7.34 (m, 2H, ArH), 7.16-7.03 (m, 5H, ArH), 6.71-6.95 (m, 4H, ArH), 6.42 (s, 1H, CH), 3.76 (s, 3H, OMe); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 178.9, 159.6, 154.3, 143.0, 142.6, 131.9, 131.7, 130.8, 128.2, 126.3, 123.8, 118.1, 117.7, 116.8, 116.6, 113.4, 113.3, 55.2; MS m/z 346 [M+H]; Anal. Calcd for C_{22}H_{16}FNO_{2}: C 76.51, H 4.67, N 4.06. Found: C 76.58, H 4.73, N 4.14.

2-(4-Methoxyphenyl)-1-(naphthalen-1-yl)quinolin-4(1H)-one (34l).

Obtained as yellow solid from 33l; m.p. 242-244 °C; IR (KBr) cm\(^{-1}\): 3084, 1631, 1624, 1573, 1563, 1426, 1356, 1287, 778; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.52 (d, \(J=7.6\) Hz, 1H, ArH), 7.87-7.85 (d, \(J=8.4\) Hz, 2H, ArH), 7.77-7.24 (m, 7H, ArH), 7.15-7.13 (d, \(J=8.8\) Hz, 2H, ArH), 6.89-6.87 (d, \(J=8.4\) Hz, 1H, ArH), 6.64-6.61 (d, \(J=8.8\) Hz, 2H, ArH), 6.47 (s, 1H), 3.66 (s, 3H, OMe); \(^{13}\)C NMR (100MHz, CDCl\(_3\)) \(\delta\) 177.9, 159.5, 154.1, 148.8, 142.8, 136.7, 133.1, 132.6, 131.8, 130.5, 129.7, 129.1, 128.0, 127.9, 127.8, 127.3, 127.1, 126.2, 126.0, 123.7, 118.1, 113.3, 112.7, 55.1; MS m/z 378 [M+H]; Anal. Calcd for C_{26}H_{19}NO_{2}: C 82.74, H 5.07, N 3.71. Found: C 82.79, H 5.12, N 3.78.
6-chloro-1-(4-chlorobenzyl)-2-(4-(dimethylamio)phenyl)quinolin-4(1H)-one (34m).

Obtained as white solid from 33m; m.p. 208-210 °C; IR (KBr) cm⁻¹: 3052, 1676, 1552, 1486, 1385, 1285, 1254, 1126, 868; ¹H NMR (400 MHz, CDCl₃) δ 8.46 (s, 1H, ArH), 7.42 (t, J=7.6 Hz, 1H, ArH), 7.17-7.30 (m, 5H, ArH), 6.95 (d, J=8.4 Hz, 2H, ArH), 6.64 (d, J=8.0 Hz, 2H, ArH), 6.37 (s, 1H, ArH), 5.31 (s, 2H, ArCH₂), 2.99 (s, 6H, NMe₂); ¹³C NMR (100MHz, CDCl₃) δ 176.5, 156.3, 151.1, 139.5, 135.0, 133.5, 132.3, 129.9, 129.3, 129.2, 128.3, 126.9, 126.1, 122.1, 119.0, 113.3, 111.6, 51.9, 40.1; MS m/z 423 [M+H]; Anal. Calcd for C₂₄H₂₀Cl₂N₂O: C 68.09, H 4.76, N 6.62. Found: C 68.16, H 4.82, N 6.73.

6-Chloro-1-(3,4-dimethoxyphenyl)-2-(4-(dimethylamio)phenyl)quinolin-4(1H)-one (34n).

Obtained as yellow solid from 33n; m.p. 214-216 °C; IR (KBr) cm⁻¹: 3042, 1656, 1565, 1485, 1385, 1282, 1252, 1135, 882; ¹H NMR (400 MHz, CDCl₃) δ 8.43 (s, 1H, ArH), 7.37 (d, J= 8.8 Hz, 1H, Ar-H), 6.78-7.04 (m, 5H, Ar-H), 6.61 (s, 1H, Ar-H), 6.43-6.48 (m, 3H, Ar-H), 3.90 (s, 3H, OMe), 3.75 (s, 3H, OMe), 2.92 (s, 6H, NMe₂); ¹³C NMR (100MHz, CDCl₃) δ 176.7, 154.3, 150.2, 149.6, 149.0, 141.4, 132.0, 131.7, 130.1, 129.7, 126.1, 125.4, 122.7, 122.2, 119.9, 112.8, 112.5, 111.0, 110.8, 56.1, 55.9, 40.0; MS m/z 435 [M+H]; Anal. Calcd for C₂₅H₂₃ClN₂O₃: C 69.04, H 5.33, N 6.44. Found: C 69.09, H 5.39, N 6.49.
1-(4-Methoxyphenyl)-2-(thiophen-2-yl)quinolin-4(1H)-one (34o).

Obtained as white solid from 33o; m.p. 196-198 °C; IR (KBr) cm⁻¹: 2930, 1589, 1572, 1428, 1235, 1124; ¹H NMR (400MHz, CDCl₃) δ 8.47 (d, J=8.0 Hz, 1H, ArH), 7.49-7.17 (m, 5H, ArH), 6.99-6.87 (m, 5H, ArH), 6.67 (s, 1H), 3.87 (s, 3H, OMe); ¹³C NMR (100 MHz, CDCl₃) δ 178.0, 159.2, 155.0, 141.2, 135.0, 133.0, 132.5, 132.0, 132.0, 130.0, 129.5, 128.0, 126.0, 123.5, 122.5, 114.0, 110.5, 56.5; MS m/z 334 [M+H]; Anal. Calcd for C₂₀H₁₅NO₂S: C 72.05, H 4.53, N 4.20. Found: C 72.13, H 4.58, N 4.25.
3.8 References


$^1$H and $^{13}$C spectra of compound 34a
Mass spectrum of compound 34a

IR spectrum of compound 34a
$^1$H and $^{13}$C spectra of compound 34d
Mass spectrum of compound 34d
$^1$H and $^{13}$C spectra of compound 34g
Mass spectrum of compound $34g$