IJBPAS, December, Special Issue, 2021, 10(12): 640-653
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'
www.ijbpas.com

# Synthesis and *In Vivo* Antimalarial Investigation of Some New 3-Aryl, 2-Substituted 4 (3H)-Quinazolinone Derivatives

**ALEMU TADESSE FEROCHE**

***Corresponding Author: Mr. Alemu Tadesse Feroche**

**Received 20th Aug. 2021; Revised 24th Sept. 2021; Accepted 30th Oct. 2021; Available online 1st Dec. 2021**

https://doi.org/10.31032/IJBPAS/2021/10.12.2061

## ABSTRACT

**Background**: In this study, some new 3-aryl, 2-substituted quinazoline-4(3H)-one of derivatives was synthesized using a three-step synthetic route to evaluate the antimalarial activity. To evaluate the *in vivo* antimalarial activities a four-day suppressive standard test was used, and the acute toxicity test was investigated using male Swiss Albino mice (20g each) according to reported methods for the synthesized compounds. The *in vivo* antimalarial activity of these compounds on *P. berghei* infected mice was found to be moderate to high at oral dose 0.04846 mmol/kg /day. This dose is equal to 25 mg/kg of chloroquine phosphate which cause 100% inhibition of the parasite. It is worth mentioning that most active compounds (*E*) -3 Phenyl -2- [2-(pyridine-4-yl) vinyl]-4(3H)-quinazolinone **IVa** (64.02%, (*E*)-2- [2-(4 -Hydroxy-3-methoxystyryl)-vinyl)-3-phenyl-4(3H)-quinazolinone **IVc** (77.25%) and (*E*)-2-[2-(Pyridin-4-yl)-vinyl]-3-phenenylamine-4(3H)- quinazolinone **IVe** (73.54%) showed a dose-dependent increase in present suppression in antimalarial activities. The results of acute toxicity indicated that all test compounds proved to be non-toxic and well tolerated by the experimental animals up to 300 mg/kg in oral and 140 mg/kg in parental studies.

**Keywords: Acute toxicity; Antimalarial; *Plasmodium berghei*; Quinazolin-4(3H)-one derivative**

## Background on Malaria

Malaria is one of the most prevalent parasitic infections in the world and certainly the most detrimental. Each year, over two million people die from the disease, with the vast majority of the deaths in children under five years old in sub-Saharan Africa [1]. In addition to the over whelming death toll, over 213 million malarial attacks lead to more than 800 million days of illness in Africa annually [2].

According to the 2008 WHO reports, half of the world population was at risk of malaria, and an estimated 250 million cases led to nearly 1 million deaths in 2006. According to this report, there were an estimated 247 million cases worldwide, of which 91% were due to *P. falciparum*. The vast majority of cases (86%) were in the Africa region, followed by the south East Asia (9%) and eastern Mediterranean regions (3%). According to the World Health Organization's World Malaria Report 2009 and Global Malaria Action Plan, 3.3 billion people (half of the world population) live in areas at risk of malaria transmission in 109 countries and territories. Out of these 109 countries, 35 (30 in sub-Saharan Africa and 5 in Asia) accounts for 98% of global malaria death [3]. Ethiopia had approximately 6% of malaria cases in the Africa region in 2006. The estimated numbers of cases in the country by the years 2006 were 12,405, 000 among which 2,073,000 are children under 5 years old [4].

In humans, one or more of four species of intracellular protozoan parasites belonging to the genius P*lasmodium*; P. *falciparum, P. malarae, P. vivax* and *P. ovalae* cause malaria infection in human *p.*

*falciparum* malaria receives special attention because it is major causes of death [5].

**Current drugs in use for the treatments of malaria**

**Quinine and related agents**

Quinine, along with its dextro-isomer guanidine, has been the drugs of last resort for the treatment of malaria, especially server disease [6]. Chloroquine is a 4-aminoquioline derivative of quinine and it has been the drug of choice even through drugs resistance has dramatically reduced its usefulness. Amodiaquine is a relatively widely available compound closely related to chloroquine. Other quinine related compounds in common use include mefloquine and primaquine.

**Antifolate combination drugs**

These drugs are various combination of dihydrofolate reductase inhibitors (proguanil **7,** chlorproguanil, pyrimethamine,trimethoprim) and sulfa drugs (dapsone,sulfamethoxazole, sulfadoxine and others). When used in combination, these drugs produce a synergistic effect on the parasite and can be effective even in the presence of resistance to the individual components [7].

**Antibiotics**

Tetracycline and its derivative as such as doxycycline are very potent antimalarials and are used for both treatment and prophylaxis. In area where response to quinine has deteriorated, tetracyclines are often in used combination with quinine to improve cure rates. Clindamycinhas been used but offers only limited advantage when compared to others antimalarial drugs [8].

**Artemisinin and derivatives**

Artemisinin is a drug used to treat multi-drug resistant strains of *falciparum* malaria. The compound is isolated from the plant *Artemisia annua*. Combination therapy has a reportedly been responsible for inhibiting intensification of drug resistance and for decreased malaria transmission levels in south East Asia [9].

Several derivatives of artemisinin have been prepared. Reduction of artemisinin produces dihydroartemisinin which in turn led to the preparation of series of semi-synthetic first-generation analogues such as artemether and artether [10]. Sodium artesunate was developed as a water-soluble alternative to artemether and artether. However, sodium artesunate is unstable in aqueous solution and also has extremely short plasma half-life [11].

**Combination therapy of antimalarial drugs**

The use of two antimalarials simultaneously, especially when the antimalarials have different mechanism of action, has the potential for inhibiting the development of resistance to either of the components. The combination of drugs currently prescribed can be divided into two categories: non-artemisinin and artemisinin-based combinations.

**Antimalarial drugs resistance**

Several medicines ranging from chloroquine to currently used medicines, such as sulfadoxine pyrimethamine, mefloquine have sequentially fallen to resistance, in particular in the case of *p. falciparum* malaria, and have thus become in effective for treatment in many parts of the world. Moreover, according to research report in 2008, the now days widely used drug, artemisinin is losing its potency in Cambodia and increased efforts are required to prevents drug resistant malaria from spreading across the globe [12]. This finding was subsequently supported by a detailed study in 2009 from Western Cambodia [13].

Resistance to antimalarial drugs has been observed in *P. falciparum* and *P. vivax*. P. *falciparum* has developed resistance to nearly all antimalarial drugs in current use [14]. Therefore, there is an urgent need to discover and develop new effective and safe drugs for the treatment of this disease.

**Biological Activities of Quinazolinones**

4(3H)-quinazolinone are a frequently encountered heterocyclic in medicinal chemistry literature with applications including antibacterial and antifungal [15], anti-inflammatory and analgesic [16], antimalarial [17], antihypertensive [18] and antiviral [19].

**Quinazolinones as antimalarial agents**

Most of the commonly used antimalarial drugs are developing resistance. On contrary, newly emerging drugs which are under development are showing comparable activity whose activity towards the resistant strains is reported to be good. Among those under development are the synthetic febrifugines that demonstrate antimalarial activity against P. *falciparum,* of similar potency to that of natural product febrifugines, with high selectivity.

According to this report, quinazolinones derivatives substituted at positions 2 and 3 play pivotal role in the hypotensive and antimalarial activity. Several

bioactive natural products such as febrifugine and iso-febrifugine contain quinazolinone moieties with potential antimalarial activity. The results also suggest that basicity of both nitrogen atoms of febrifugine is crucial in conferring powerful antimalarial activity. The compound had exhibited highest *in vivo* antimalarial activity against *P. berghei,* with no serious side effect [20].

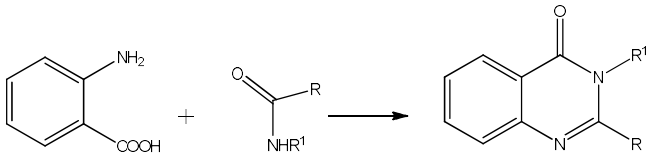
Furthermore, in our laboratory some 4(3H)-quinazolinone derivatives were synthesized and evaluated for their antimalarial activity against *P. berghei* infected mice. The result showed that 2-Methyl-3 anilino-4(3H)-quinazolinone (70.7%), (1*E*)-2-(3,4-dihydro-4-oxo-3-p-tolyl quinazoline -2-yl) vinyl-2-methoxyphenyl acetate (78.4%) and 2-(2-nitrostrostyryl)-3- anilino quinazoline-4-(3H)- one (71.6%) were the most active compounds with better percent suppression [21].

**Reported Method of Synthesis of Quinazolinone Derivatives**

4(3H)-quinazolinone derivatives can be synthesized in many ways. Some of the methods include

**a. Niementowski quinazoline synthesis**

This method is the formation of 4-oxo-3,4-dihydroquinazolines by cyclization of anthranilic acid by amides [22], scheme 1.

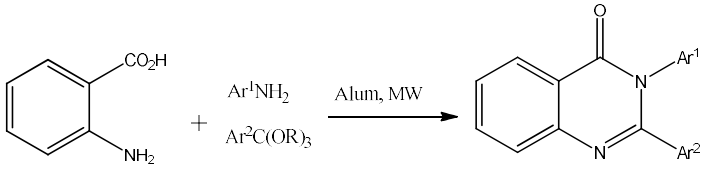



$R^1$, R = Me, Ph, Ar

**Scheme 1**: Synthesis of 2,3-disubstituted 4(3H)- quinazolinones

**b. One-pot synthesis of 2,3 disubstituted -4(3H)-quinazolinones**

A one-pot, three-component condensation of anthranilic acid with aromatic amines and ortho-esters on the surface of KAl $(SO_4)_2$.12 $H_2O$ (Alum) under microwave irradiation as a new efficient method to produce 2,3-disubstituted -4(3H) – quinazolinones in good yields. 2,3- distributed-4(3H)-quinazolinones formation can be rationalized as shown in scheme 2 [23].

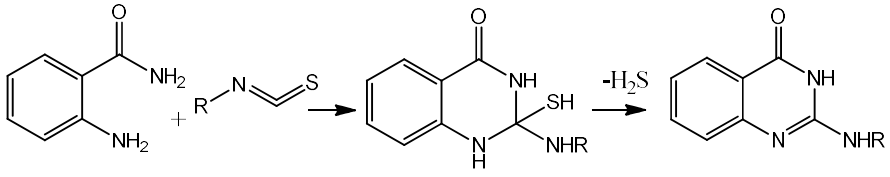



R = Me, Et, Ph

**Scheme 2: One-pot synthesis of 2,3-distributed 4(3H)-quinazolinones**

**c. One-pot synthesis of 2-(alkylamino) or 2-(arylamino) -4(3H)- quinazolinones**

A convenient one-pot preparation of 2-(alkylamino) and 2-(arylamino)-4(3H)-quinazolinones in synthetically useful yields is reported, scheme 3. The approach is based on the reaction of isothiocyanates with 2- aminobenzamide. The reaction could be either heated under reflux for 1-4.5 hours in toluene (15ml), or exposed to microwave irradiation for 3-4 minutes. The solid material was crystallized from ethanol [24].




R = Me, Et, Cyclohexyl,4-F-$C_6H_4$, 4-Me-$C_6H_4$

**Scheme 3: One pot synthesis of 2-substituted 4(3H)-quinazolinones**

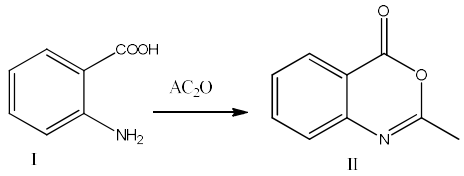
**Methods**

**Chemistry**

**Synthesis of 4(3H)-Quinazolinone Derivatives**

**a. Synthesis of 2-methyl-3,1-benzoxazin-4-one II (Acetanthranil) [**25]

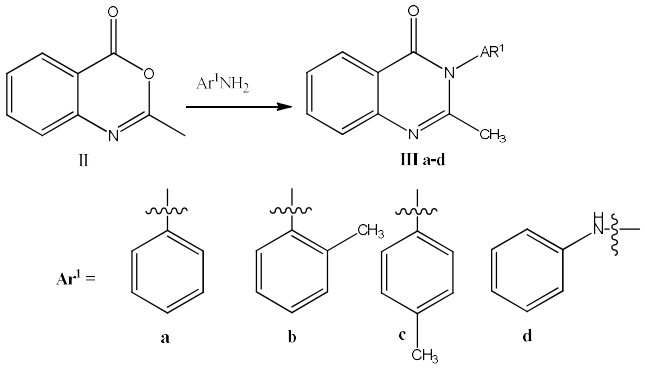
A solution of anthracitic acid **I** (20g, 0.145 moles) in acetic anhydride (23 ml) was heated under reflux for 1 hr. The excess acetic anhydride was then washed with anhydrous petroleum ether, where upon a solid mass was obtained which, without purification was suitable for the subsequent reaction, scheme 4.




**Scheme 4: Synthesis of 2-methyl-3,1-benzoxazin-4-one**

**b. Synthesis of 3-aryl-2-methyl-4(3H)-Quinazolinone; III a-c** [26] **and 3-arylamino-2-methyl-4(3H)-Quinazolinone; d** [21].

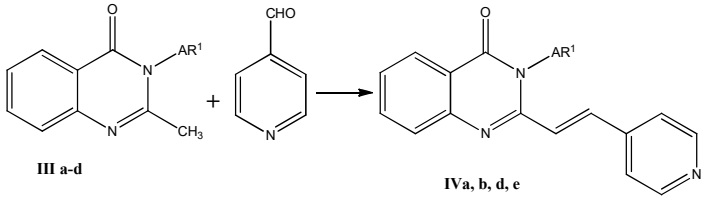
A mixture of Acetanthranil: II (1.175gm, 7.5 mmole) and equimolar amounts of the appropriate aromatic amine or phenyl hydrazine was heated under reflux at $190^0$C for 5 hrs. The dark sticky mass formed was cooled and recrystallized from ethanol, scheme 5.



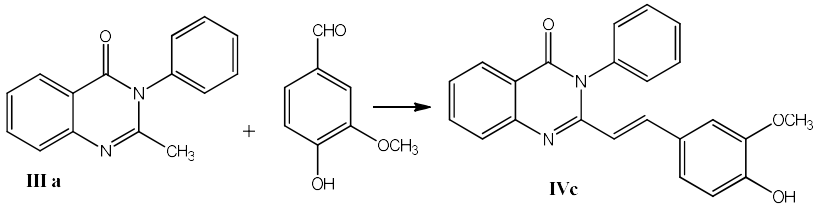

**Scheme 5: Synthesis of 3-aryl-2-methyl-4(3H)-Quinazolinone**

**c. Synthesis of 3-aryl-2-(substituted styryl)-4(3H)-Quinazolinones; IV a-f** [27].

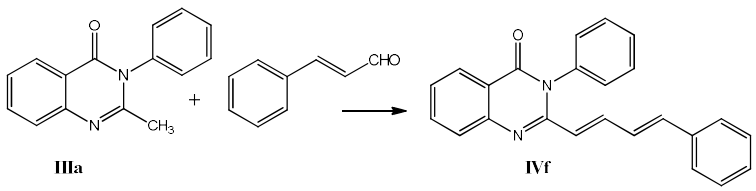
To a solution of 3-aryl-2-(substituted styryl)-4(3H)-Quinazolinones; **III a-c** (10mmole) and 3-arylamino-2-methyl-4(3H)-quinazolinones; **III d** (10 mmole) in acetic anhydride (10ml), an equimolar amount of appropriate aldehyde was added and 10mg of anhydrous zinc chloride as catalyst. The reaction mixture was heated under reflux for 10 hrs. and set aside at room temperature. The reaction mixture was poured into ice-cooled water (50ml). The separated solid product was filtered, dried and recrystallized from ethanol, scheme 6, 7 and 8.




**Scheme 6: Synthesis of (*E*)-3-aryl-2-(2-(pyridine-4-yl) vinyl)-4(3H)-quinazolinones**




**Scheme 7: Synthesis of 2-(4-hydroxy-3-methoxystyryl)-3-phenyl-4(3H)-quinazolinone**




**Scheme 8: Synthesis of (*1E, 3E*)-3- phenyl-2-(4-phenylbuta-1,3-dienyl)-4(3H)-**quinazolinone

***In vivo* antimalarial activity**

A four-day suppressive standard test was used to evaluate the in vivo antimalarial activities of the synthesized compounds [28]. *P. berghei* was maintained in the laboratory mice through infected blood transfusion. Mice were allowed to acclimatize to the laboratory environment under room temperature for a week before being used for the experiments.

A donor mouse with parasitemia level of approximately 20-30% (i.e of *P. berghei* ANKA strain) parasitized erythrocytes was used to infect mice. Infected blood from donor mouse was collected using syringe containing trisodium citrate and diluted in physiological saline to $10^7$ parasitized erythrocytes per ml.

Each experiment was given with inoculations of 0.2 ml (about $2x10^7$ parasites) intra-peritoneal on day zero, which is expected to produce a steadily rising infection. After 2 hrs., the infected mice were weighed and randomly divided in to eight groups of five mice per group per cage. Group 1 received a vehicle containing 7% Tween 80 and 3% ethanol in distilled water that served as a negative control. Group 2 that served as positive control was given 25 mg/kg/day (0.04846 mmole/kg/day) of the standard drug, chloroquine phosphate (Mwt = 515.86g/mol). Groups 3, 4, 5, 6, 7 and 8 were treated with equimolar amount of the synthesized compounds (0.04846 mmol/kg/day) that was dissolved in 7% Tween 80 and 3 ethanol through oral route [29].

The above treatment was continued over 4 days. Twenty-four hours after the last treatment ($5^{th}$ day), blood smears were prepared from the tail of all mice, air dried, fixed with absolute methanol and stained with 6% Giemsa. The parasitemia was then determined microscopically by counting 4 fields of approximately 100 erythrocytes per field [30].

The efficacies of compounds were finally assessed by comparison of blood parasitemia and mouse survival time in treated and untreated mice [31]. The 4-day standard suppressive test was repeated for the second time for all compounds at a dose level of twice the amount in the first dose (0.09692 mmol/kg/day).

***In vivo* acute toxicity test**

The oral acute toxicity of compounds **IVa, IVb, IVc, IVd, IVe and IVf** was investigated using male Swiss Albino mice (20g each) according to reported methods [32]. The mice were divided into groups of six mice each and fasted overnight. The compounds were given orally, suspended in 1% gum acacia, in dose of 10, 50, 100, 200 and 300mg/kg. After oral administration to the target compounds, the mice were observed closely during 24 hrs. with special attention to the first four hours. Additionally, the test compounds were investigated for their parenteral acute toxicity in groups of six mice per cage. The compounds or their vehicle, propylene glycol (control) was given by intraperitoneal injection in doses of 20, 40, 80, 120, 140mg/kg. The percentage survival was followed up to 7 days [33]. Acute toxicity signs like sedation, lacrimation, hair erection, blinking, urination, muscle weakness, convulsion in motor, diarrhea, sleep, coma and death were checked in the test mice.

**Statistical analysis**

The results of the study were expressed as mean ± standard deviation and statistical significance for suppressive test was determined by one-way ANOVA using Origin 6.0 software. Data on survival time, % parasitemia and % suppression was analyzed using Microsoft Office Excel 2010. All data was analyzed at 95% confidence interval (P = 0.05).

The $IC_{50}$ values for in vitro promastigote assay of target compounds were evaluated from sigmoidal

dose-response curves using computer software Graph Pad Prism 5.0.

Percentage parasitemia and percentage suppression fore antimalarial activity were calculated using the following formulae:

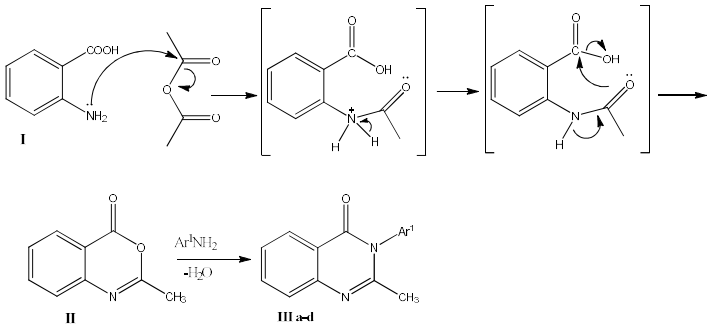
$$\% \text{ Parasitemia} = \frac{\text{Number of infected RBC}}{\text{Number of total RBC}} \times 100$$

$$\% \text{ Suppression} = \frac{\text{Parasitemia in untreated group - Parasitemia in treated group}}{\text{Parasitemia in untreated group}} \times 100$$

**Result and discussion**

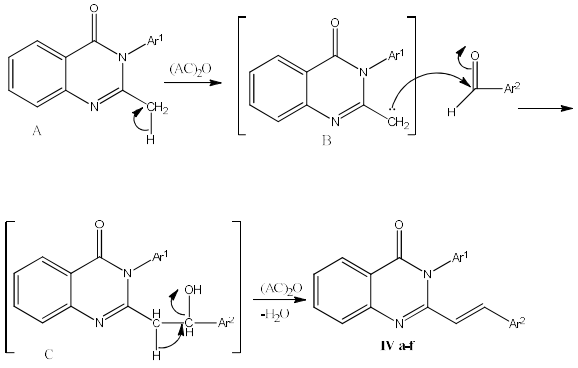
**Synthesis of the target compound**

The largest compounds were synthesized according to schemes 6, 7 and 8. The intermediates 2-methyl-3,1-benzoxazin-4-one (Acetanthranil) **II** was prepared by heating under reflex anthranilic acid with acetic anhydride, scheme 4. The mechanism of synthesis of Acetanthranil starts with acetylating amino group of anthranilic acid followed by dehydration by the effect of acetic anhydride, scheme 9.



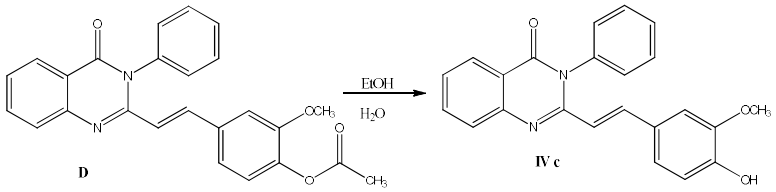
**Scheme 9: Mechanism of formation of intermediate compounds (II and III a-d)**

Condensation of acetanthranil **II** with appropriate aromatic amines or phenyl hydrazine afforded compounds **III a-d** schemes 5. Compounds **IVa-f** were synthesized by condensations of 2-methyl-3-substituted 4 (3H)-quinazolinone with acetic anhydride in the presence of anhydrous zinc chloride. Acetic anhydride will abstract hydrogen from the methyl group to form carbanion which in turn attacked the carbonyl group of the selected aldehydes to offered the hydroxyl intermediate **C**. Finally, dehydration by acetic anhydride and zinc chloride afforded the target compounds (**IV a-f**) scheme 10.



**Scheme 10: Mechanism of formation of target compounds (IVa-f)**

The crude compounds **D,** scheme 11 crystallized from reaction mixture was subjected to IR spectral analysis and showed two characteristic peaks at 1673 and 1763 $cm^{-1}$ assigned for acetyl carbonyl group and quinazolinone carbonyl group. After crystallization from aqueous ethanol (95%), the obtained $^{1}H$ NMR spectrum devoid from characteristic peak of the acetyl methyl group confirming hydrolysis of the weak phenolic ester.




**Scheme 11: Hydrolysis of weak phenolic ester to (E)-2-(4-hydroxy-3-methoxystyryl)-3-phenyl-4(3H)-quinazolinone IVc.**

Physical constants and yield percent of the synthesized compounds are listed in table 1. The progress of the reactions in the synthesis part of the work was monitored by thin-layer chromatography (TLC). The $R_f$ values for each synthesized compound were calculated, Table 1. The spots on the TLC plate were visualized using iodine vapor.

**Table 1: Physical constants and yield % of the synthesized compounds**

| Cpd No. | Mol. Formula | Mol. Wt (g/mol) | Yield (%) | M.P ($^0$C) | $R_f$ [Chloroform: Benzene (9:1)] |
|---|---|---|---|---|---|
| **IVa** | $C_{21}H_{15}N_3O$ | **325.36** | **71.2** | **250-252** | **0.718** |
| **IVb** | $C_{22}H_{17}N_3O$ | **339.39** | **78.0** | **223-225** | **0.744** |
| **IVc** | $C_{23}H_{18}N_2O_3$ | **370.40** | **63.4** | **260-262** | **0.795** |
| **IVd** | $C_{22}H_{17}N_3O$ | **339.39** | **59.5** | **224-226** | **0.690** |
| **IVe** | $C_{21}H_{16}N_4O$ | **340.38** | **67.5** | **264-266** | **0.738** |
| **IVf** | $C_{24}H_{18}N_2O$ | **350.41** | **85.0** | **228-230** | **0.643** |

The Compounds were synthesized in a good yield (59.5-85%). All the synthesized compounds were readily soluble in dimethyl sulphoxide and partially soluble in methanol and chloroform. The structures of the final compounds were verified based on data from elemental microanalysis, IR and $^1$H NMR spectral studies.

**Elemental microanalysis**

Elemental microanalyses were performed on Perkin Elmer 2400 elemental analyzer and were founds with ±0.4% of the theoretical values, Table 2.

**Table 2: Results of the elemental microanalysis**

| | % Composition | | | | | |
|---|---|---|---|---|---|---|
| **Cpd No.** | **Estimated** | | | **Found** | | |
| | **C** | **H** | **N** | **C** | **H** | **N** |
| **IVa** | **77.52** | **4.65** | **12.91** | **77.21** | **4.82** | **13.13** |
| **IVb** | **77.86** | **5.05** | **12.38** | **78.10** | **4.85** | **12.11** |
| **IVc** | **74.58** | **4.90** | **7.56** | **74.27** | **5.22** | **7.81** |
| **IVd** | **77.86** | **5.05** | **12.38** | **78.08** | **4.79** | **12.54** |
| **IVe** | **74.10** | **4.74** | **16.26** | **74.36** | **4.48** | **16.24** |
| **IVf** | **82.26** | **5.18** | **7.99** | **82.46** | **4.88** | **8.08** |

**Spectroscopic analysis of the synthesized compounds**

Infrared (IR) spectra were recorded on a SHIMADZU 8400 SP FT-IR spectrophotometer using Nujol disc technique. $^1$H NMR spectrum was recorded on Bruker Avance DMX400-FT-NMR spectrometer and the chemical shifts are given in δ (ppm) downfield from tertramethylsilane (TMS) which served as an internal standard. Splitting patterns were designed as follows: *s*: singlet; *d*: doublet; *m*: multiplet. The summarized characteristic stretching and bending IR vibration frequencies and the $^1$H NMR chemical shifts for each compound are discussed below.

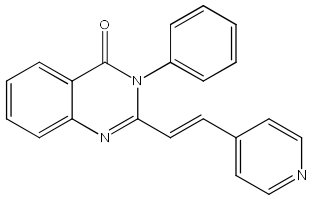


**Figure 1: (*E*)-3-phenyl-2-[2-(pyridine-4-yl) vinyl]-4(3H)-quinazolinone,IVa:**

IR (Nujol) ($cm^{-1}$): 1680 (C=O) and 1593 (C=N)
$^1$H NMR ($CDCl_3/CCl_4$) ppm: 6.55 (*d*, 1H, J = 15.53 Hz, vinyl-$C_2$ H), 7.16 (d, 2H, J = 5.29 Hz, pyridine-$C_{2,6}$ H), 7.35 (*d*, 2H, J = 6.38 Hz, phenyl-$C_{3,5}$ H), 7.55 (*t*,1H, J = 8.08 Hz, quinazolin-$C_7$ H), 7.58-7.67 (*m*, 3H, phenyl-$C_{2,4,6}$ H), 7.78-7.86 (*m*, 2H, quinazolin-$C_{6,8}$ H), 7.90 (*d*, 1H, J = 15.53 Hz, vinyl-$C_1$ H), 8.32 (*dd*,1H, $J_1$ = 0.490Hz, $J_2$ = 1.407 Hz quinazolin-$C_5$ H) and 8.57 (*d*,2H,J= 5.29 Hz, pyridine-$C_{3,5}$ H).
The IR spectrum compound **IVa** (Figure 1), showed strong characteristic band at 1680 $cm^{-1}$attributed to 4 (3H)-quinazolinone carbonyl group stretching. The peak appeared at low frequency as it is amide carbonyl group. Moreover, a medium characteristic band for C=N appeared at 1593 $cm^{-1}$.
$^1$H NMR spectrum of compound **IVa** (Figure 1), showed two doublets at 6.55 ppm and 7.90 ppm attributed to vinyl protons. They have the same coupling constant, J = 15.53 Hz confirming the existence in (*E*) configuration [34]. In addition, two doublets appeared at 7 .16 ppm and 8.57 ppm assigned to protons of pyridine protons which assure the formation of the regret's compounds.

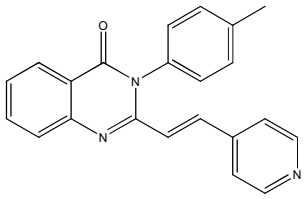


**Figure 2: (*E*)-2-[2-(pyridine-4-yl) vinyl]-3-p-tolyl-4(3H)-quinazolinone IVb:**

IR (Nujol) ($cm^{-1}$): 1682 (C=O) and 1614 (C=N)
$^1$H NMR (DMSO-$d_6$) ppm: 2.4 (*s*, 3H, p-tolyl $CH_3$), 6.60 (*d*, 1H, j = 15.70 Hz, vinyl $C_2$ H), 7.30-7.45 (*m*, 6H *p*-tolyl $C_{2,35,6}$ H and pyridine-$C_{2,6}$ H), 7.60 (t, 1H, J=7.84 Hz, quinazolin-$C_7$ H), 7.75-7.85 (*m* ,2H, vinyl-$C_1$ H and quinazolin-$C_8$ H), 7.90 (*t*, 1H, J =7.10 Hz, quinazolin-$C_6$ H), 8.15 (*dd*, 1H, $J_1$ = 0.460 Hz, $J_2$ = 1.391 Hz, quinazolin-$C_5$H) and 8.55 (*d*, 2H, j = 5.28 Hz, pyridin-$C_{3,5}$ H).
The IR spectrum of compound **IVb** (Figure 2)**,**showed strong characteristic absorption band at 1682 $cm^{-1}$ that indicated the presence of a 4(3H)-quinazolinone carbonyl group. The band appeared at low frequency as it is amidic carbonyl group. Strong absorption at 1614 $cm^{-1}$ assigned to the C=N stretch.
$^1$H NMR spectrum of compound **IVb** (Figure 2), showed two doublet peaks at 6.60 and 7.80 ppm that appeared as overlapping multiplet with quinazolin-$C_8$H attributed to vinylic protons. The coupling constant, J = 15.70 Hz confirm that the compounds is in (*E*) configuration [34]. In addition, the overlapping multiplet peaks at 7.30-7.45 ppm afford to four aromatic protons of *p*-tolyl and pyridine-$C_{2,6}$ H. Similarly, doublet peak at 8.55 ppm due to pyridine-$C_{3,5}$ protons. The presence of these peaks confirmed the formation of the target compound.

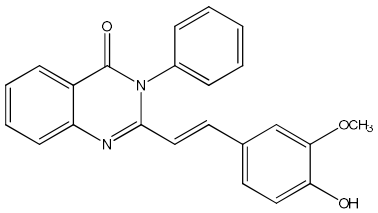


**Figure 3: (*E*)-2-(4-hydrox-3-methoxystystyryl)-3-phenyl-4(3H)-quinazolinone IVc:**

IR (Nujol) ($cm^{-1}$): 1673 (C=O);1637 (C=N); 1120 and 1206 (C-O-C)
$^1$H NMR ($CDCl_3/CCL_4$) ppm:3.8 (*s*,3H, -O-$CH_3$), 6.25 (*d*,1H, J=15.69 Hz, vinyl-$C_2$H), 6.85-7.0 (*m*,3H, 4-hydroxy-3-methoxphenyl-$C_{2,5,6}$ H), 7.35 (*d*, 2H, J=7.84 Hz, phenyl-$C_{3,5}$ H),7.50 (*t*,1H, J = 7.79 Hz, quinazolin-$C_7$ H), 7.55-7.65 (*m*,3H, phenyl-$C_{2,4,6}$ H) ,7.80 (*m*, 2H, quinazolin-$C_{6,8}$ H), 7.92 (*d*,1H, J = 15.69 Hz, vinyl-$C_1$ H), 8.30 (*dd*,1H, $J_1$= 0.510 Hz, $J_2$ =1.430 Hz quinazolin-$C_5$H).
The IR spectrum of **IVc** (Figure 3) revealed characteristic medium band at 1673 $cm^{-1}$ attributed to the quinazolinone carbonyl group. The appeared at low frequently as it is amidic carbonyl group. The

other characteristic medium band at 1637 $cm^{-1}$ assigned to C=N stretching. The bands at 1206 and 1120 $cm_{-1}$ are characteristic to ether symmetric and asymmetric stretching.

The $^{1}$HNMR spectrum of compound **IVc** (Figure 3), showed two doublet peaks at 6.25 and 7.92 ppm attributed to vinyl protons. The coupling constant of these protons (J= 15.69 Hz) confirm that the compounds is in (*E*) configuration [34]. In addition, the peaks at 6.8 -7.0 ppm (*m*,3H) afforded to aromatic protons of 4-hydroxy-3-methoxyphenyl group and the up-field singlet peak at 3.8 ppm conforms the formation of the target compound.

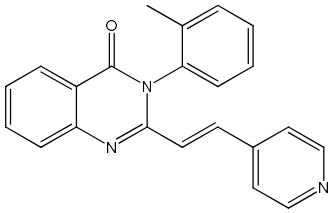


**Figure 4: (*E*)-2-[2-(Pyridin-4-yl)-vinyl]-3-o-tolyl-4(3H)-quinazolinone IVd:**

IR (Nujol) ($cm^{-1}$):1652 (C=O) and 1634 (C=N)

$^{1}$HNMR (DMSO-$d_6$) ppm:2.4 (*s*,3H, $CH_3$), 6.60 (*d*,1H, J=15.63Hz, vinyl-$C_2$ H),7.30-7.45 (*m*, 6H, tolyl-$C_{3,4,5,6}$ H and pyridin-$C_{2,6}$ H),7.60 (*t*,1H, J=7.7.3 Hz, quinazolin-$C_7$ H),7.75 -7.85 (*m*,2H, vinyl-$C_1$ H and quinazolin-$C_8$ H),7.90 (t, 1H, J=7.34 Hz, quinazolin-$C_6$ H), 8.15 (*dd*, 1H, $J_1$ =0.415 Hz, $J_2$ =1.390Hz quinazolin-$C_5$ H) and 8.55 (*d*,1H, J=5.32 Hz pyridine-$C_{3,5}$ H).

The IR spectrum of compound **IVd** (Figure 4), exhibited a characteristic absorption band at 1652 $cm^{-1}$ attributed to quinazolinone carbonyl group. The peak appeared at low frequency as it is amidic carbonyl group. The absorption band appeared at 1634 $cm^{-1}$ is due to C=N stretching.

The $^{1}$HNMR spectrum of compound **IVd** (Figure 4), displayed two doublets at 6.60 ppm and in overlapping multiplet at 7.75-7.85 ppm. The coupling constant of these protons (J=15.63 Hz) confirm that the compound is in (*E*) configuration [34]. The overlapping multiplet peaks at 7.30-7.45 ppm attributed to two protons of pyridine-$C_{2,6}$ H. The highly de-shielded peak at 8.55 ppm afforded to pyridine-$C_{3,5}$ H confirm the foundation of the trade compounds.

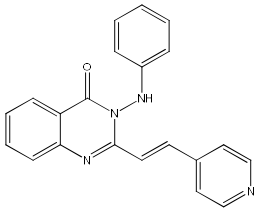


**Figure 5: (*E*)-2-[2-(pyridin-4-yl)-vinyl]-3-phenylamine-4(3H)-quinazolinone IVe:**

IR (Nujol) ($cm^{-1}$): 3250 (N-H); 1700 (C=O) and 1625 (C=N)

$^{1}$HNMR (DMSO-$d_6$) ppm: 6.70 (*d*,2H,J=8.39Hz, phenyl-$C_{2,6}$ H), 6.85 (*t*,1H, J=7.36Hz, 3-phenylamine-C4 H), 7.23 (*t*,2H, J=8.34Hz, 3-phenylamne-$C_{3,5}$ H), 7.58 (*t*,1H, J=7.61Hz, quinazolin-$C_7$ H),7.62 (*d*,2H, J=5.31Hz, pyridine-$C_{2,6}$ H), 7.67 (*d*,1H, J=15.90Hz,vinyl-$C_2$ H),7.82 (*d*,1H, j=8.27Hz, quinazolin-$C_8$ H), 7.90 (*t*,1H, J=8.30Hz, quinazolin-$C_6$ H), 8.00 (*d*,1H, J=15.90Hz, vinyl-$C_1$ H), 8.15 (*dd*,1H, $J_1$=0.450Hz, $J_2$=0.976Hz, quinazolin-$C_5$ H), 8.60 (*d*,2H, pyridine-$C_{3,5}$ H) and 9.25 (*s*,1H, 3-phenylamine N-H).

The IR spectrum of compound **IVe** (Figure 5), revealed a sharp absorption at 3250 $cm^{-1}$ which is characteristic for the presence of N-H group. Another characteristic medium band at 1700 $cm^{-1}$ assigned to the 4(3H)-quinazolinone carbonyl group. The peak appeared at high frequency as it forms five membered rigs by hydrogen bond with the N-H. In addition, the band at 1652 $cm^{-1}$ were attributed to the C=N stretching.

The $^{1}$HNMR spectrum of compound **IVe** (Figure 5), displayed two doublets at 7.67 ppm and 8.00 ppm assigned to vinylic protons. They have the same coupling constants, J= 15.90Hz confirming that the

compound is in (*E*) configuration [34]. In addition, two doublets at 7.62 & 8.60 ppm appeared due to pyridine protons which assure the formation of the target compound.

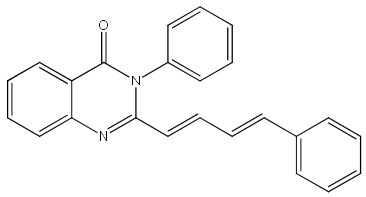


**Figure 6: (*1E,3E*)-3-phenyl-2-[4 phenylbut-1,3-dienyl]-4(3H)-quinazoline IVf:**

IR (Nujol) ($cm^{-1}$): 1679 (C=O) and 1625 (C=N)
$^1$HNMR ($CDCl_3$ /$CCL_4$) ppm: 5.95 (*d*,1H, J=14.69 Hz, butdiene-$C_4$ H), 6.75 (*t*, 1H, J = 15.43 Hz, butdiene-$C_2$ H), 6.95 (d, 1H, J= 15.43Hz, butdien-$C_1$ H), 7.25-7.35 (*m*, 5H, butadiene-$C_3$ H), quinazolin-$C_{6,8}$ H and phenyl-$C_{3,5}$ H),7.73 -7.88 (*m*,3H, quinazolin-$C_7$H and phenyl $C_{2,6}$ H), 7.40 -7.70 (*m*,6H, phenyl-$C_4$ H and but phenyl-$C_{2,3,4,5,6}$ H) and 8.3 (*dd*,1H, $J_1$ =0.897Hz, $J_2$=1.380Hz quinazolin-$C_5$H).

The IR spectrum of compound **IVf** (Figure 6), exhibited strong characteristic bands at 1679 $cm^{-1}$ for carbonyl group and the peak appeared at low frequency as it is amidic carbonyl group. Medium band at 1625 $cm^{-1}$ was assigned for C=N stretching.

In the $^1$HNMR spectrum of compound **IVf** (Figure 6), the protons of the conjugating double bonds of 2-(4-phenylbuta-1,3-dienyl) appeared at 5.95 ppm, 6.75 ppm and 6.95 ppm for carbon number 4,2 and 1 proton respectively. The coupling constants (J= 14.69 Hz and J= 15.43 Hz) of these three protons confirming that the compound is in (*E, E*) configuration. In addition, coupling constants of protons on carbon number 1 and 2 (J=15.43 Hz) show that they are adjacent protons [34].

The protons of butadiene-$C_3$H appeared as over applying multiplet at 7.25-7.35 ppm with two protons of quinazolin-$C_{6,8}$ H and phenyl-$C_{3,5}$ H. The five protons on but-phenyl showed overlapping multiplet peak with one proton of phenyl-$C_4$ H at 7.40-7.70 ppm confirming the formation of the target compound.

**Biological Activity Results**

***In vivo* antimalarial activity of synthesized compounds**

The 4-day suppressive test is a standard test commonly used for antimalarial screening, and the determination of percent suppression (inhibition) of parasitemia is most reliable parameter. The target compounds were evaluated for their *in vivo* antimalarial activity on *p. berghei,* infected mice and the result are listed in Table 3 and 4. Chloroquine phosphate was used as positive control. The target compounds were used in two doses. The initial dose was 48.46 µmol/kg/day of chloroquine phosphate and equimolar concentration of each of the synthesized compounds. The dose was doubled (96.92 µmol/kg/day) to investigate the antimalarial effect is dose-dependent or not.

When a standard antimalarial drug is used in mice infected with *p. berghei*, it suppresses parasitemia to non-detectable levels [35], which is in agreement with the effect of chloroquine phosphates in this study. Moreover, as shown in the Table 3, the percentage of parasitemia measured for all test compounds changed significantly from those in the negative control ($p<0.05$). This shows that the compounds are significantly active. This significant antimalarial activity of the target compounds was also in agreement with that of the activity of 4(3H)-quinazolinone derivates on the same strain [21].

**Table 3: Antimalarial activities of IVa-f at doses of 48.46 µmol/kg***

| Test compound | Dose (mg /kg) | % Parasitemia | % Suppression | Mean survival time (Days) |
|---|---|---|---|---|
| IVa | 15.77 | 33.67 ± 0.70 | 50.67 | 7.9 ± 0.40 |
| IVb | 16.45 | 46.00 ± 0.69 | 32.60 | 7.0 ± 0.20 |
| IVc | 17.95 | 17.75 ± 0.39 | 73.99 | 9.8 ± 0.50 |
| IVd | 16.45 | 45.50 ± 0.77 | 33.33 | 6.7 ± 0.34 |
| IVe | 16.49 | 22.75 ± 0.5 | 66.67 | 9.1 ± 1.12 |
| IVf | 16.98 | 27.67 ± 0.29 | 59.46 | 8.2 ± 0.30 |
| NC** | 1ml/100g | 68.25 ± 0.73 | 0.0 | 6.1 ± 0.17 |
| Chloroquine phosphate | 25 | 0.0 | 100 | ND |

***Values are mean ± SD, P<0.05, **NC: Negative control, ND: No death recorded over the experimental period**

In the first does, compound **IVa**, **IVc**, **IVe** and **IVf** displayed mean percent suppression of greater than 50%. On the other hand, **IVb** and **IVd** had less than 50 % mean percent suppression compared to the untreated group, Table 3.

Compound **IVc** was the most active of the tested compounds with mean percent suppression 73.99 %. The mean parasitemia level in mice treated with **IVc** (17.75 ± 0.39) was found to be approximately four times lower than in the negative control (68.25 ± 0.73) showing the compound has greatly reduced the parasite load. This highest activity was further supported by better mean survival time (9.8 ± 0.5) compared to other target compounds but less than those of positive control group that did not show any death during the experimental period, table 3.

This highest suppression effect of **IVc** can be attributed to the hydroxyl group (OH) at *Para* position of 2-styryl group that forms hydrogen bonding with the receptors site.

Compound **IVe** reveled the next better antimalarial activity with percent suppression of 66.67 that is further confirmed by the mean survival time (9.1 ± 1.12), table 3. This could be because of the presence of secondary amine group (N-H) on the 3-quinazolinone positions that increases the binding strength by hydrogen bonding. Moreover, the planner shape of the phenyl group can be considered as a good input in this regard.

The mean percent suppression of **IVb** and **IVd** was not significantly different (p=0.374) at first does showing that their activity is comparable but lower than that of **IVa**. This could be due to the tetrahedral shape of the *ortho* or *para* methyl groups in the structure of compounds, **IVb** and **IVd** that hinders the strong hydrophobic interaction with the receptor pocket.

The activity of compounds **IVf** was less than of **IVc** that could be due to the extension (increase in length) of the group at 3-qiuinazolinone positions and lack of electronegative element.

**Table 4: Antimalarial activists of IVa-f at dose of 96.92µmol/kg***

| Test compound | Dose (mg/kg) | % Parasitemia | % Suppression | Mean survival time (Days) |
|---|---|---|---|---|
| **IVa** | **31.54** | **22.67 ± 0.58** | **64.02** | **8.7 ± 1.0** |
| **IVb** | **32.90** | **37.67 ± 0.72** | **40.21** | **7.4 ± 0.57** |
| **IVc** | **35.90** | **14.33 ± 0.40** | **77.25** | **10.0 ± 1.1** |
| **IVd** | **32.90** | **43.00 ± 0.26** | **31.75** | **7.0 ± 0.5** |
| **IVe** | **32.98** | **16.67 ± 0.25** | **73.54** | **9.5 ± 0.8** |
| **IVf** | **33.96** | **27.67 ± 0.47** | **56.08** | **8.2 ± 0.5** |
| **NC**** | **1ml/100g** | **63.00 ± 0.40** | **0.0** | **6.3 ± 0.1** |

***Values are mean ± SD, p<0.05, **NC; negative control.**

When the dose was doubled compounds: **IVa, IVb, IVc and IVe showed** a dose-dependent increase in mean percent suppression, Table 4. It is interesting to note that the mean percent suppression increased 50.67% to 64.02%, 32 .60 % to 40.21 %, 73.99% to 77.25 % and 66.67% to 73.54% for **IVa, IVb, IVc and IVe** respectively. This result is also in agreement with longer mean survival time of each corresponding compound in the second dose.

On the other hand, despite the fact that compound **IVd** had some suppressing effect, the mean percentage suppression at the two doses was not significantly different at 95% confidence level (p> 0.05). This indicates that the activity of this compound is not dose dependent. The mean percent suppression of compound **IVf** at the two dose levels was significantly different conforming that there is a decrease in the activity (from 59.46 % to 56.08%). This might be due to the toxicity of the compound that may weaken the mice immune system.

The other parameter used to check the antimalarial activity of the synthesized compounds; mean survival time of the compound is in agreement with the results discussed for the change of mean percent suppression at the two doses studied. The group of mice treated with compound **IVc** showed the highest survival time (10.0 ± 1.1) and followed by compound **IVe** (9.5 ± 0.8) while those treated with compound **IVd** had the least survival time (7.0 ± 0.5) at dose levels of 0.09692 mmol/kg (96.92 µmol kg/day).

**In vivo acute toxicity test**

Oral and parental acute toxicity test was performed for all compounds **IVa-f**. The test compounds were administrated through oral route in doses of 10, 50,

100, 200 and 300 mg /kg. The results indicated that test compounds proved to be non-toxic and well tolerated by the experimental animal up to 300 mg/kg. Moreover, these compounds were tested for their toxicity through parenteral route in doses of 20, 40, 80, 120, 140 mg/kg [33]. The results revealed absence of any acute toxicity signs like lacrimation, hair erection, blinking, urination, muscle weakness, sedation and convulsion, reduction in motion, diarrhea, sleep, coma and death in both oral and parental studies for all test compounds.

**Conclusion**

Some 2,3-distributed-4(3H)-quinazolinone derivatives, **IVa-f** were synthesized to investigate their antimalarial activities. The target compounds were obtained in a good yield (59.5- 85%) by applying different chemical reactions like cyclization and condensation reaction. The chemical structure of the final compounds was verified by using elemental microanalysis, IR and $^{1}$HNMR.

The *in vivo* antimalarial activity of these compounds was tested on *p. berghei* infected mice at two doses (48.46 µmol /kg /day and 96.92 µml kg/day. Chloroquine phosphate which causes 100 % inhibition of the parasite was used as positive control. Most active compounds **IVa** (64.02%), **IVc** (77.25%) and **IVe** (73.54%) showed a dose dependent increase in antimalarial activities. Despite the fact that the tested compounds rendered a significantly longer survival time and percent suppression of up to 77.25%, none of the synthesized compounds was as active as the standards drug chloroquine phosphate.

The results of acute toxicity indicated that all test compounds (**IVa-f)** proved to be non-toxic and well tolerated by the experimental animals up to 300 mg/kg in oral and 140 mg/kg in parenteral studies. Although that some compound **IVa, IVc** and **IVe** showed moderate antimalarial activities compared to chloroquine phosphate. Meanwhile acute toxicity test showed that all test compounds have good safety margin. Therefore, the test compounds **IVa-f** would represent a fruitful matrix for the development of new class for antimalarial agents that would deserve further investigation and derivatization.

**Reference**


1. Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H. Y and Hay, S. I. (2005). The global distribution of clinical episodes of *plasmodium falciparum* malaria. *Nature*,**434**:214-17.
2. Berman, J. G., Alilio, M. S., Mills. A. (2004). Conquering the intolerable burden of malaria. *Am. J. trop. Med. Hyg*., **71**: 1-15.
3. WHO, (2009). The World Malaria Report from WHO and the Global malarial Action Plan. http://www.cdc.gov/malaria/malaria_worldwide/impact.html. (accessed on 2010-05-05).
4. WHO, (2008). The world malaria report from WHO and UNICEF. World Health Organization, Geneva.
5. Mbatchi, S. F., Mbatchi, B., Banzouzi, J. T., Bansimba, T., Nsonde, G. F., Ntandou, J., Ouamba, M., Berry, A. and Vical, B. F. (2006). *In vitro* antiplasmodial activity of 18 plants used in congo Brazzaville traditional medicine. *Journal of ethnopharmacology*, **104**: 168-174.
6. Greenwood D. (1992). The quinine connection. *J. Antimicrob. Chemoter*., **30**: 417-427.
7. Watkins, W. M. (1997). The efficacy of antifolate antimalarial combination in Africa: a predictive model based on pharmacodynamic and pharmacokinetic analysis. *Parasitology today*, **13**: 459-464.
8. Kremsner, P. G. (1994). Clindamycin in combination with chloroquine or quinine is an effective therapy for uncomplicated *plasmodium falciparum* malaria in children from Gabon. *The journal of infection disease*, **169**: 467-470.
9. Price, R.N (1996). Effect of artemisinin derivatives on malaria transmissibility. *Lancet*, **347**: 1654 -1658
10. Wellems, T. and Plowe, C.V. (2001). Chloroquine-resistant malaria. *J. Infect. Dis*., **184** 770-776.
11. Bornstik, K. (2002). Antimalarial chemotherapeutic peroxides: artemisinin, yingzhaosu A and related compounds. *Int. J. Parasitol*., **32**:166 -1667.
12. Noedl, H., Schaecher, K., Smith, B. L., Socheat, D. and Fukuda, M.M. (2008). "Evidence of artemisinin-resistant malaria in western Cambodia." *N. Engl. J. Med*., **359** (24): 2619-20.
13. Dondorp, A. M., Nosten, F., Das, D., Phyo, A. P and Tarning, J. (2009). Artemisinin-resistance in *plasmodium falciparum* malaria. *N. Engl. J. Med*., **361** (5): 455-67.

14. Nallan, L., Bauer, K. D. and Bendale, P. (2006). Protein farnesyl transferase inhibitors exhibit potent antimalarial activity. *J. Med. Chem*., **48**: 3704 -3713.
15. Desai, N.C Shihora, P.N and Moradia, D.L. (2007). Synthesis and characterization of new quinazolines as potential antimicrobial agents. *Indian J. Chem*., **46B**: 550-553.
16. Yesilada, A., Koyunologlu, S., Sagili, N., Kupeli, E., Yesilada, E., Bedir, E. and Khan, I. (2004). Synthesis anti-inflammatory and analgesic activity screening of some new 4(3H) quinazolinone derivatives. *Arch. Pharma. Med. Chem*. **337**: 96 -104.
17. Martin T. A., Wheller, A.G., Majewski, R.F. and Corrigan, J.R. (1964). *J. Med. Chem*.,**7**:812
18. Dienei, J.B., Dowalo, F., Hoeven, H.V., Bender, P. and Love, B. (1973). *J. Med Chem*.,**16**:633.
19. Alagarsamy, V., Giridhar, R., Yadav,M. R., Revathi, R., Ruckmai, K. and De Clercq, E. (2006). Anti-HIV, antibacterial, and antifungal activities of some novel 1,4-disubstitued-1,2,4-triazolo [4,3-a] quinazolin-5(4H)-ones. *Indian J. Pharm. Sci*. **68**:532-535.
20. Palle, V.R., Acharyulu, P.K., Dubey, P. V., Prasada, R. and Thatipally, S. (2008). Synthesis of new 4(3H)-quinazolinone derivatives under solvent-free conditions using PEG-400. *General Papers ARKIVOC*,**11**: 104-111.
21. Bule M.H., Haymete A., Kefale B. (2015). Synthesis and *In-Vivo* Pharmacological Evaluation of Some Novel 4(3H)-Quinazolinone Derivatives as Potential Anti-malarial Agents. *Drug Des.,***4**: 121. doi:10.4172/2169-0138.1000121.
22. Raff, D., Edler, M., Daidone, G., Maggio. B., Merickech, M., Plescia, S., Schillaci, D., Bai, R. and Hamel, E. (2004). Synthesis, cytotoxicity and inhibitory effects on tubulin polymerization of new 3-heterocyclo substituted 2-styrylquinazolinones. *Eur. J. of Med Chem*. **39**: 299-304.
23. Mohammadi, A. A., Mohammedi, M.H., and Sharifan, R. (2008). KAl $(So4)_2.12H_2O$ (Alum) catalyzed one-pot synthesis of some new 4(3H)-quinazolinones under microwave irradiations. *J. of App. Chem. Res*., **6**: 55-61.
24. Tavallaii. Z., Sabzevari, O., Bakavoli, and Rahimizadeh, M. (2007). A novel synthesis of 2-(alkylamino) and 2-(arylamino-4(3H)-quinazolinones by heterocyclization of 2-aminobenzamide with isothiocyanates or (isocyanates) under microwave irradiation. *Scien. Iranica*. **14**: 320-322.
25. Farghaly, A. M., Soliman, R., Khali, M.A. and Bekhit, A.A. (1994). Non-steroidal anti-inflammatory agents: synthesis of novel pyrazolyl-1,2-oxazolyl-, and 1,3-diazinyl derivatives of 4(3H)-quinazolinones. *Arch. Pharma*. (Weinheim), **327**: 27-35.
26. Farghaly, A.M., Chaaban, I., Khalil, M.A., Bekhit, A.A. (1990). Non-steroidal anti-inflammatory agents. III: synthesis of novel pyrazole derivatives 4(3H)-quinazolinones. *Alex. J. Pharm.Sci*.,**4**: 52-62.
27. Farghaly, A.M., Chaaban, I., Khalil, M.A. and Bekhit, A.A. (1990). Non-steroidal anti-inflammatory agents, synthesis of novel 2-pyrazolyl-4(3H)-quinazolinones. *Arch. Pharm. (Weinheim)* **323**: 833-40.
28. David, A., Philip, J., Simon, L., Reto, B. and Solomon, N. (2004). Antimalarial drug discovery: efficacy model for compound screening *in vivo* and *in vitro* protocols http://www.mmv.org/IMG/pdf/SCREENING PDF.pdf (Accessed on 15.10.10).
29. Dominguez, J. N., Leon, C., Rodrigues, J., Neira, G. D., Gut, J. and Rosenthal, P. J. (2009). Synthesis of chlorovinyl sulfones as structural analogs of chalcones and their antiplasmodial activities. *Eur. J. Med. Chem*., **44**:1457-1462.
30. Mesele, A. (2008). *In vivo* antimalarial activity of crude hydroalcoholic extracts of Melia azedarach and Hypostes triflorate in Mice Infected with *Plasmodium berghei. Master thesis AAU*, pp: 18-19.
31. Trager, W. and Jensen, J.B. (1976). Human malaria parasites in continuous culture. *Science*,**193**: 673-675.
32. OECD guideline for testing of chemicals, (2001). Acute oral toxicity up and down procedure. http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL420.pdf. (Accessed on 23.08.07).
33. Bekhit A.A. and Baraka, A.M. (2005). Novel Milrinone analogs of pyridine-3-carbonitrile

derivatives as promising cardiotonic agents. *Eur. J. Med. Chem*., **40**:1405-1413.

34. Williams, D.H. and Fleming, I. (1989). Spectrometric methods in Organic Chemistry, 4th ed. Revied, McGraw-Hill Book Company (UK) Limited. Table 3.27.
35. Kiseko, K., Hiroyuki, M., Syun-ichi, F., Ryuiichi, F., Tomotaka, K. and Seiji, M. (2000). Antimalarial activity of leaf extract of *hydrangea macrophyla*, a common Japanese plant. *Acta. Med. Okayama*, **54**: (5):227-232.