ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

SCHOOL OF PHARMACY

Synthesis, Antimalarial and Antileishmanial Evaluation of Some Quinazoline Derivatives

By

Yihenew Simegniew Birhan

January 2012

Synthesis, Antimalarial and Antileishmanial Evaluation of Some Quinazoline Derivatives

A Thesis Submitted to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medicinal Chemistry.

By

Yihenew Simegniew Birhan

Under the supervision of

Prof. Adnan A. Bekhit, Ph.D.

January 2012

Acknowledgment

It is my pleasure to express my deepest sense of gratitude, sincere appreciation and profound regards to my advisor, **Prof. Adnan A. Bekhit** for his scholastic guidance, constant encouragement and providing most of the chemicals for my thesis work.

I am very thankful to Dr. Ariaya Hymete for his commitment and dedication in following up, correcting and providing constructive feedback on my work and write up; Prof. Wondimagegn Mamo for his considerable support in running and interpreting the ^{1}H NMR data; Dr. Asfaw Debela for letting me use facilities at the Ethiopian Health and Nutritional Research Institute (EHNRI) laboratory to determine melting points of target compounds; Professor Yalemtsehay Mekonnen for permitting me to carry out the antimalarial activity test at the Bio-medical laboratory in Department of Biology, Faculty of Science, AAU.

I am so grateful to Debre Markos University for the sponsorship; Addis Ababa University for financially supporting this thesis work. The Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University is also acknowledged for helping in the elemental microanalysis, *in vitro* antileishmanial activity test and providing some chemicals.

My special appreciation also goes to Taddese W/Giorgis, Eshetu Assefa, Abdu Tuha, Addisu Asfaw, Girma Worku and Roza Gebre for their special support and affection!

Table of Contents

Acknowledgment i

List of Acronyms and Abbreviations x

Abstract xii

1. Introduction 1

1.1. Background on Malaria 1

1.1.1. Malaria 1

1.1.2. Global Burden of Malaria 2

1.1.3. Malaria in Ethiopia 2

1.1.4. Who is at high risk of Malaria? 3

1.1.5.1. Preventive Methods of Malaria 3

1.1.5.2.1. Quinine and Related Drugs 4

1.1.5.2.2. Antifolates and Antibiotic Antimalarials 5

1.1.5.2.3. Artemisinins and Artemisinin Combination Therapies (ACTs) 6

1.1.6. Drug Candidates for the Treatment of Malaria 7

1.1.7. Antimalarial Drug Resistance 8

1.1.7.1. Resistance to 'Older' Drugs 8

1.1.7.2. The Emergence of Artemisinin Resistance 8

1.1.8. Monitoring Antimalarial Drug Resistance 9

1.2. Background on Leishmaniasis 9

1.2.1. Leishmaniasis 9

1.2.2. Clinical Types of Leishmaniasis 10

1.2.2.1. Cutaneous Leishmaniasis (CL) 10

1.2.2.2. Visceral Leishmaniasis (VL) 11
1.2.2.3. Mucocutaneous Leishmaniasis (MCL) 11
1.2.3. Global Burden of Leishmaniasis 11
1.2.4. Leishmaniasis in Ethiopia 11
1.2.5. Who is at High Risk of Leishmaniasis? 12
1.2.6. Prevention and Treatment Strategies of Leishmaniasis 12
1.2.6.1. Prevention Methods of Leishmaniasis 12
1.2.6.2. Existing Drugs for the Treatment of Leishmaniasis 13
1.2.7. Other Drugs with Promising Antileishmanial Activities 15
1.2.8. Development of Resistance to Antileishmanial Drugs 16
1.3. Quinazolines 16
1.3.1. Chemistry of Quinazolines 16
1.3.1.1. Reported Methods of Synthesis 16
1.3.2. Biological Activities of Quinazolines 19
1.3.2.1. Quinazolines as Antimalarial Agents 19
1.3.2.2. Quinazolines as Antileishmanial Agents 22
2. Objectives 25
2.1 General objectives 25
2.2 Specific objectives 25
3. Materials and Methods 26
3.1. Materials 26
3.1.1. Chemicals and Reagents 26
3.1.2. Instruments and Apparatuses 26
3.1.3. Reference Drugs 27

3.2. Methods 27
3.2.1. Chemistry 27
3.2.1.1. Synthesis of Target Compounds 27
3.2.1.1.1. Synthesis of 2-methyl-3,1-benzoxazin-4-one (II) 28
3.2.1.1.2. Synthesis of 3-aryl-2-methyl-4(3H)-quinazolinones (III a-c) 28
3.2.1.1.3. Synthesis of 3-aryl-2-(substituted styryl)-4(3H)-quinazolinones. 29
3.2.1.2. Preparation of Stock and Working Concentrations 31
3.2.2. Biological Assays 31
3.2.2.1. Test animals and Strains 31
3.2.2.2. Culture Medium and Conditions 32
3.2.2.3. *In vivo* Antimalarial Activity 32
3.2.2.5. *In Vivo* Acute Toxicity Test 33
3.2.3. Statistical Analysis 34
4. Result and Discussion 35
4.1. Chemistry of The Synthesized Compounds 35
4.1.1. Synthesis of Target Compounds 35
4.1.1.1. Proposed Mechanism of Synthesis of 2-methyl-3,1-benzoxazin-4-one 35
4.1.1.2. Proposed Mechanism of Synthesis of 2-methyl-3-arylquinazolin-4-ones 36
4.1.1.3. Proposed Mechanism of Synthesis of 2-(substituted styryl)-3-arylquinazolin-4-ones. 36
4.1.2. Physical Constants of Target Compounds 39
4.1.3. Elemental Microanalysis of the Synthesized Compounds 40
4.1.4. Spectroscopic Analysis of the Synthesized Compounds 40
4.1.4.1. (*E*)-2-(4-chlorostyryl)-3-phenylquinazolin-4(3*H*)-one (IVa) 41

4.1.4.2. (*E*)-2-(4-chlorostyryl)-3-*p*-tolylquinazolin-4(3*H*)-one (IVb) 45

4.1.4.3. (*E*)-2-(4-nitrostyryl)-3-*p*-tolylquinazolin-4(3*H*)-one (Va)..... 50

4.1.4.4. (*E*)-2-(4-nitrostyryl)-3-*o*-tolylquinazolin-4(3*H*)-one (Vb) 53

4.1.4.5. 4-{(1E)-2-[-3,4-dihydro-3-(2-methylphenyl)-4-oxoquinazoline-2-yl)] vinyl}-2-methoxyphenyl acetate (VIb)..... 56

4.1.4.6. (*E*)-2-(4-hydroxystyryl)-3-phenylquinazolin-4(3*H*)-one (VIIa)..... 59

4.1.4.7. (*E*)-2-(4-hydroxy-3-methoxystyryl)-3-*o*-tolylquinazolin-4(*3H*)-one (VIIb) 62

4.2. Biological Activity Testing Results 65

4.2.1. *In vivo* Antimalarial Activity of the Synthesized Compounds 65

4.2.2. *In vitro* Antileishmanial Activity of the Synthesized Compounds 67

4.2.3. Oral Acute Toxicity Result 70

5. Conclusions..... 71

6. Recommendations for Further Work 72

References..... 73

List of Figures

Figure 1: Life cycle of malaria parasite. 1

Figure 2: Quinidine and quinidine related antimalarial drugs. 4

Figure 3: Dihydrofolate reductase inhibitors and antibiotic antimalarials..... 6

Figure 4: Artemesinin and artemesinin derivative antimalarials. 7

Figure 5: Life cycle of Leishmania parasite. 10

Figure 6: Currently used antileishmanial drugs 13

Figure 7: Febrifugine analogues displaying good antiplasmodial activities..... 19

Figure 8: 6-substituted-2,4-diaminiquinazolines as antimalarial agents..... 20

Figure 9: 4-aniloquinazolines as antimalarial agents..... 21

Figure 10: Thiourea analogues of 4-anolioquinazolines with antimalarial activities. 21

Figure 11: Some 2-(substituted styryl)-3-arylquinazolinones with antimalarial activities..... 22

Figure 12: 2,3-disubstituted-4(*3H*)-quinazolinones with antileishmanial activities..... 23

Figure 13: 2,4-diaminoquinazolines with potent antileishmanial activities. 23

Figure 14: Some Indolo[2,1-b]quinazolinones with antileishmanial activities. 23

Figure 15: Some styryl-4(*3H*)-quinazolinones with antileishmanial activities. 24

Figure 16: IR spectrum of compound IVa in Nujol. 42

Figure 17: ^{1}H NMR spectrum of compound IVa in $CDCl_3$ 43

Figure 18: IR spectrum of compound IVb in Nujol..... 46

Figure 19: ^{1}H NMR spectrum of compound IVb in $CDCl_3$..... 47

Figure 20: ^{13}C NMR spectrum of compound IVb in $CDCl_3$..... 48

Figure 21: IR spectrum of compound Va in Nujol. 51

Figure 22: ^{1}H NMR spectrum of compound Va in $CDCl_3$. 52

Figure 23: IR spectrum of compound Vb in Nujol. 54

Figure 24: ^{1}H NMR spectrum of compound Vb in $CDCl_3$. 55

Figure 25: IR spectrum of compound VIb in Nujol. 57

Figure 26: ^{1}H NMR spectrum of compound VIb in $CDCl_3$. 58

Figure 27: IR spectrum of compound VIIa in Nujol 60

Figure 28: ^{1}H NMR spectrum of compound VIIa in Acetone-d6. 61

Figure 29: IR spectrum of compound VIIb in Nujol. 63

Figure 30: ^{1}H NMR spectrum of compound VIIb in $CDCl_3$. 64

Figure 31: Reduction of resazurin into resorufin by metabolically active cells 68

List of Schemes

Scheme 1: One-step synthesis of fused quinazolinones. 16

Scheme 2: Synthesis of quinazolinones by one-pot condensation reaction. 17

Scheme 3: Synthesis of quinazolinones by copper catalyzed tandom reaction. 17

Scheme 4: Synthesis of quinazolinones by microwave-assisted thermal decomposition. 18

Scheme 5: Synthesis of quinazolines by cyclization of *N*-acetylanthranilic acid. 19

Scheme 6: Synthesis of 2-methyl-3,1-benzoxazin-4-one (II) from anthranilic acid. 28

Scheme 7: Synthesis of 3-aryl-2-methyl-4(*3H*)-quinazolinones. 28

Scheme 8: Synthesis of target compounds (IVa,b) 29

Scheme 9: Synthesis of target compounds (Va,b) 30

Scheme 10: Synthesis of acetylated compounds (VIa,b) 30

Scheme 11: Synthesis of target compounds (VIIa,b) 31

Scheme 12: Mechanism of synthesis of acetoanthranil (II). 35

Scheme 13: Proposed mechanism of synthesis of IIIa-c. 36

Scheme 14: Proposed mechanism of synthesis of target compounds. 37

Scheme 15: Proposed mechanism of synthesis of VIa,b. 38

Scheme 16: Mechanism of hydrolysis of target compounds (VIa,b). 39

List of Tables

Table 1: Physical constants and percent yield of the synthesized compounds. 39

Table 2: Results of elemental microanalysis for the synthesized compounds. 40

Table 3: Antimalarial activities of the synthesized compounds 66

Table 4: Antipromastigote activity (IC_{50}) of the synthesized compounds..... 69

List of Acronyms and Abbreviations

ACTs	Artemisinin Combination Therapies
AQ	Amodiaquine
ART	Artemether
AS	Artesunate
CL	Cutaneous Leishmaniasis
CQ	Chloroquine
DHA	Dihydroartemisinin
DHFR	Dihydrofolate reductase
DMF	Dimethyl formamide
DMSO	Dimethylsulfoxide
HIFCS	Heat-inactivated Fetal Calf Serum
IPT	Intermittent Preventive Treatment
ITN	Insecticide-treated Nets
LUM	Lumefantrine
mmol	Millimole
MQ	Mefloquine
NMR	Nuclear Magnetic Resonance
ppm	parts per million
PQ	Piperaquine
PRQ	Primaquine
PYR	Pyronaridine
RMPI	Roswell Park Memorial Institute
Sb^v	Pentavalent Antimonials

SP	Sulfadoxine-Pyrimethamine
SSG	Sodium stibogluconate
VL	Visceral Leishmaniasis

Abstract

Malaria and leishmaniasis are neglected tropical parasitic diseases affecting billons of people around the globe. Owing to their promising antimalarial and antileishmanial activities, seven novel 2-(substitutedstyryl)-3-aryl-4(*3H*)-quinazolinones were synthesized in good yields (65.2-86.4%) by using cyclization and condensation reactions. Structures for the synthesized compounds were determined using elemental microanalysis, IR, ^{1}H NMR and ^{13}C NMR (for compound **IVb**). The *in vivo* antimalarial and the *in vitro* antileishmanial activities of the synthesized compounds were evaluated using mice infected with *P. berghi* ANKA strain and *L. donovani* strain, respectively.

The target compounds showed poor antimalarial activities with percent suppression of 29.10-44.39% which was not significantly different from the negative control group ($P>0.05$). All the synthesized compounds displayed superior antileishmanial activities (IC_{50} values, 0.0128-3.1085 µg/ml) as compared to the standard drug miltefosine (IC_{50} = 3.1911 µg/ml). (*E*)-2-(4-chlorostyryl)-3-*p*-tolyl-4(*3H*)-quinazolinone (**IVb)** is the compound with promising antileishmanial activities (IC_{50} = 0.0128 µg/ml) which is approximately 4 and 250 times more active than the standard drugs amphotericin B deoxycholate (IC_{50} = 0.0460 µg/ml) and miltefosine (IC_{50} = 3.1911 µg/ml), respectively.

Key words: Quinazolines, Antimalarial activities, Antileishmanial activities.

1. Introduction

1.1. Background on Malaria

1.1.1. Malaria

Malaria is, a neglected tropical parasitic disease. It is caused by a *Plasmodian* protozoan species which evolved with time differentiating into four distinct species; *P. vivax, P. malarae, P. ovale* and *P. falciparum* that are specific to man [1]. It is transmitted by the bite of a female *Anopheles* mosquito through injection of sporozoites at the time of blood suck. Following hepatic and erythrocytic stages of their life cycle (Fig. 1) in the host, unidentified malaria toxin is released on rupture of schizont-erythrocytes resulting in cytokine response, which leads to clinical manifestations of the typical malaria including high fever, chills, prostration and anemia. The pathogenicity of the parasite results because of its rapid rate of asexual reproduction in the host and its ability to sequester in small blood vessels [2].

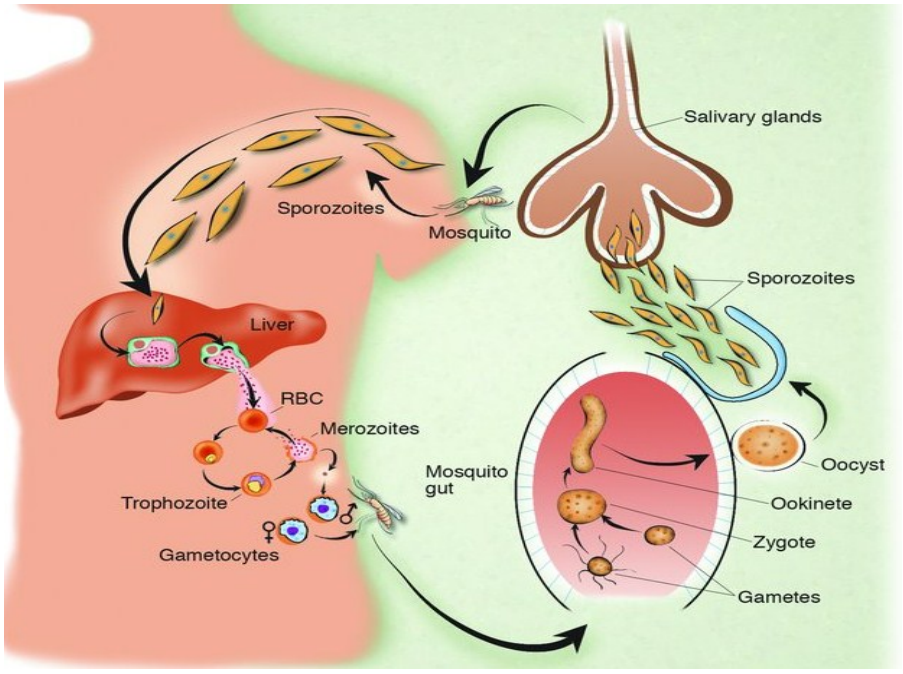


Figure 1: Life cycle of malaria parasite [3].

1.1.2. Global Burden of Malaria

In 2009 there were an estimated 225 million cases of malaria (5^{th}-95^{th} centiles, 169-294 million) worldwide down from an estimated 244 million cases in 2005. The global number of cases was estimated to have decreased since 2005 due to the impact of malaria control, especially in European region (86%) followed by the region of America (42%). The vast majority of cases in 2009 (78%) were in the African Region, followed by the South-East Asia (15%) and Eastern Mediterranean Regions (5%). The global number of malaria deaths is estimated to have decreased from 985 000 in 2000 to 781 000 in 2009. The largest percentage decreases were seen in the Region of the Americas (48%). It is estimated that 91% of deaths in 2009 were in the African Region, followed by the South East Asia (6%) and Eastern Mediterranean Regions (2%). About 85% of deaths globally were in children under 5 years of age [4].

1.1.3. Malaria in Ethiopia

Approximately 75% of Ethiopia's landmass is endemic for malaria. It usually occurs at altitudes < 2,000 meters above sea level. Recent reports indicated that epidemics have expanded to areas up to 2400 meters above sea level [5]. Additionally, malaria in Ethiopia is characterized by widespread epidemics occurring every 5-8 years; with the most recent one between 2003 and 2005 [6]. In 2009, 3 million suspected malaria cases were seen and nearly 2.3 million (77%) were tested. The number malaria cases decreased from an annual average of 3 million during 2000-2005 to 1.75 million cases in 2009 (41% decline). In the same period the malaria admissions decreased from an average of 44 000 to 30 102 in 2009 (33% decline). Inpatient malaria deaths fell by 43% in all age groups and by 60% in children under 5 years. This may be attributed to the high Insecticide-treated Nets (ITNs) coverage (66% in 2007), use of artemesinin combination therapies (ACTs) and increased funding (US $195 million in 2009) [4].

1.1.4. Who is at high risk of Malaria?

Children, pregnant women, HIV patients and travelers to sub-Saharan African countries are at increased risk for severe malaria, if infected with *P. falciparum* [7]. In Africa, more than 90% of the cases and between 1.5 and 3 million deaths occur in children under 5 years of age [8]. In 2007, 54.7 and 70.5 million pregnant women were at risk of *P. falciparum* and *P. vivax* malaria respectively. In recent years, approximately 1000-1600 episodes of malaria have been diagnosed each year in Americans after return from travel, 5 to 10% meet the criteria for severe malaria [9].

1.1.5. Prevention and Treatment Strategies of Malaria

1.1.5.1. Preventive Methods of Malaria

Vector control by the rapid deployment of ITNs for all individuals at risk of malaria is now recommended by WHO. ITNs coverage exceeds 50-60% of the populations in Equatorial Guinea, Ethiopia, Rwanda, Zambia, and Zanzibar, consequently mortality among children from such infection falls by 20-25% [10]. Sulfadoxine/pyrimethamine (SP) and chloroquine (CQ) are the drugs of choice for the prophylactic treatment of malaria. The former is used for pregnants and infants who are not taking sulfa drugs and the later for the prophylactic treatment of *vivax* malaria [11].

In addition, there is a growing interest to develop a multistage antimalarial vaccine that can avoid vaccine failure caused by polymorphic variation in parasite populations. RTS,S/AS01E is a promising vaccine candidate under Phase III clinical trial in seven malaria endemic African countries [12].

1.1.5.2. Existing Antimalarial Drugs

1.1.5.2.1. Quinine and Related Drugs

When used correctly, chemoprophylaxis appears to be highly effective in preventing malaria. Quinine (**1**), quinidine (**2**), CQ (**3**), primaquine (PRQ) (**4**), amodiaquine (AQ) (**5**), mefloquine (MQ) (**6**), piperaquine (PQ) (**7**), pyronaridine (PYR) (**8**), and the amino alcohol lumefantrine (LUM) (**9**) are structurally related antimalarial drugs.

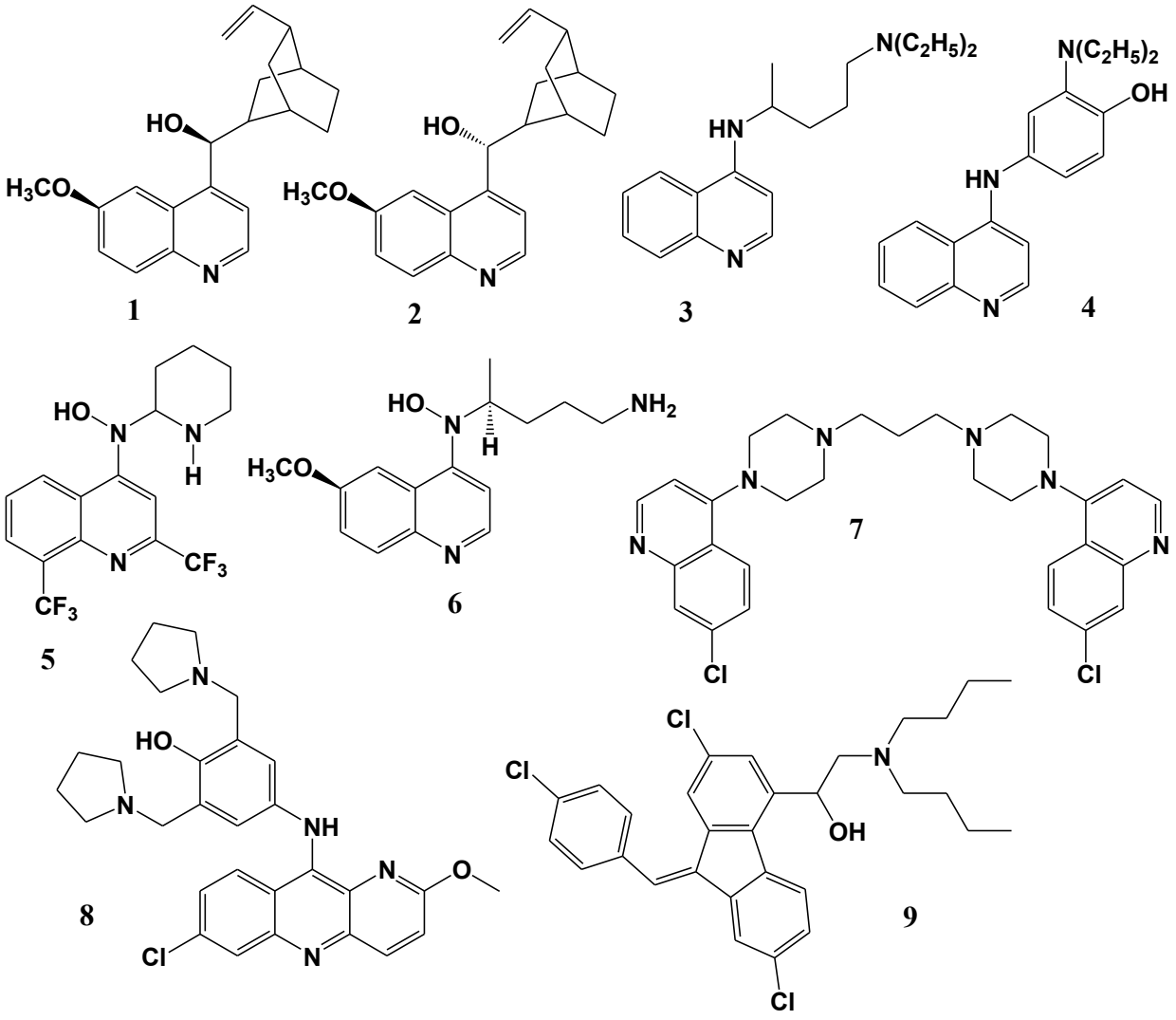


Figure 2: Quinidine and quinidine related antimalarial drugs.

Intravenous quinidine is still the drug of choice for treating severe malaria in African children [13]. In addition, quinine and mefloquine are the approved therapies for treatment of uncomplicated *falciparum* malaria, including travelers to areas with drug resistant malaria [14]. Primaquine is the only registered drug used to prevent relapse. Amiodaquine (infants and children) and mefloquine (pregnant) are among the drugs used for intermittent preventive treatment (IPTs) [15].

1.1.5.2.2. Antifolates and Antibiotic Antimalarials

Proguanil (**10**), chlorproguanil (**11**), pyrimethamine (**12**), and trimethoprim (**13**) and sulfa drugs: Dapsones (**14**), sulfamethoxazole (**15**), sulfadoxine (**16**) are drugs which are shown to have antimalarial activity by inhibiting dihydrofolate reductase (DHFR) enzyme [16].

These drugs have been used in combination (SP, Sulfamethoxazole/trimethoprim, atovaquone (**17**)-proguanil) so as to produce synergistic effect, reduce the emergence of drug resistance [17].

The antibiotic drugs; tetracycline (**18**) doxycycline (**19**) and clindamycin (**20**) are very potent antimalarials and are used for both treatment and prophylaxis in combination with other antimalarials like quinine [18]. Doxycycline is at least as safe as mefloquine for IPT during pregnancy, especially for those who do not tolerate mefloquine or travel to areas with resistance to mefloquine [19].

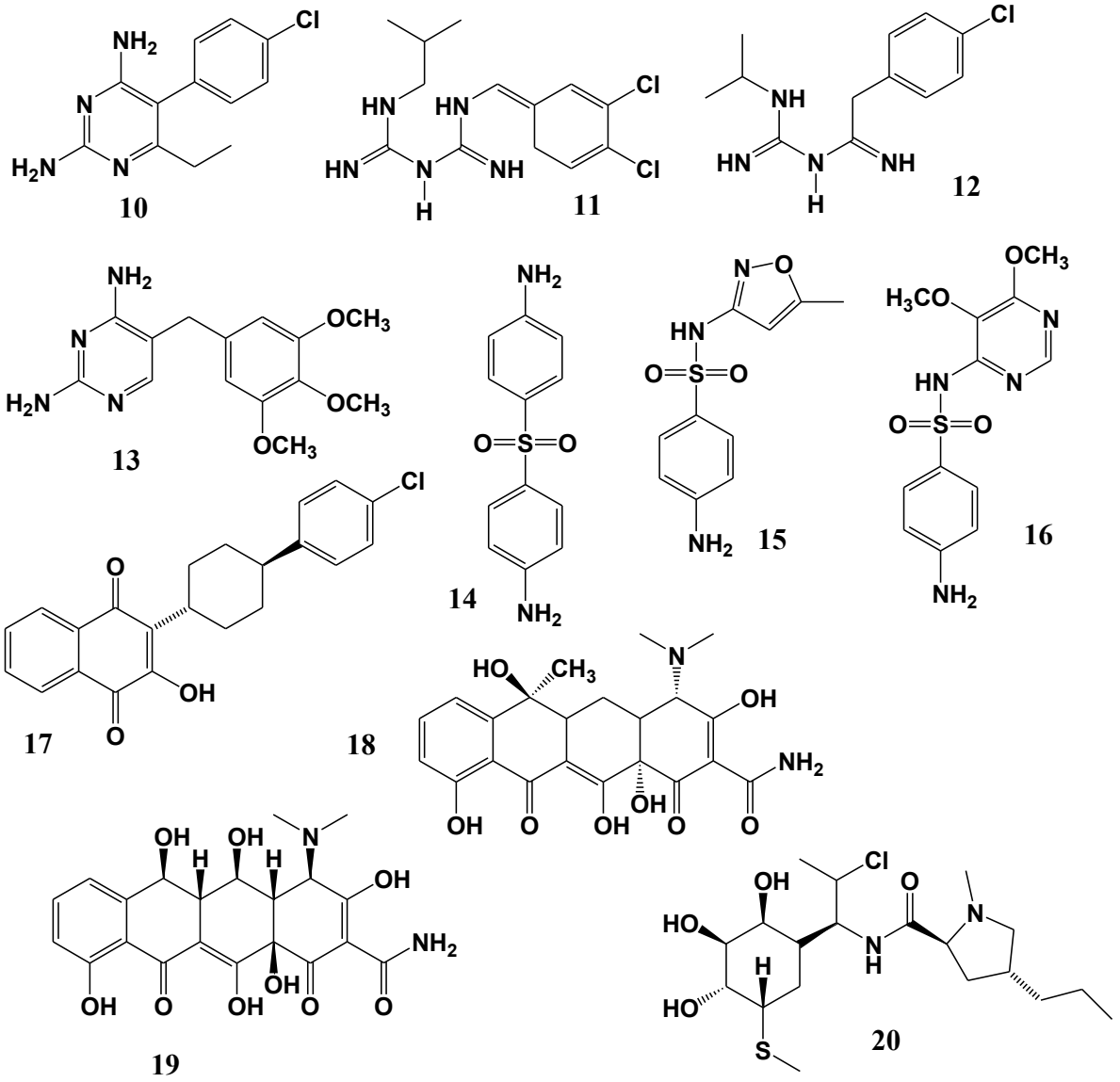
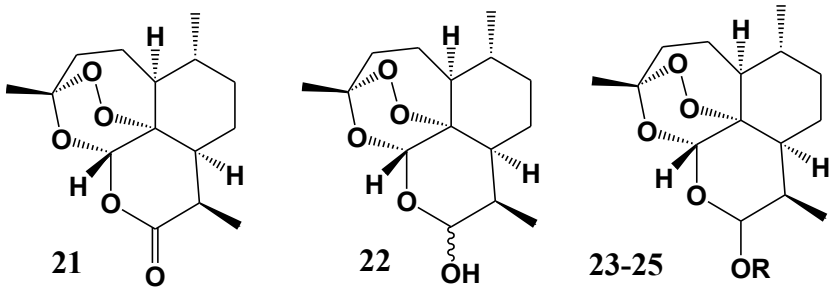


Figure 3: Dihydrofolate reductase inhibitors and antibiotic antimalarials.

1.1.5.2.3. Artemisinins and Artemisinin Combination Therapies (ACTs)

Artemisinin (**21**) dihydroartemisinin (DHA) (**22**), artemether (ART) (**23**), artether (**24**) and artesunate (AS) (**25**) are the most important new class of drugs with excellent safety profiles [20]. Intravenous and intramuscular artesunate and artemether have been highly efficacious for the treatment of severe malaria, with less side effects and better activity than quinine [21].



23: R = CH_3 ; 24: R = C_2H_5 ; 25: R = $COCH_2CH_2COOH$

Figure 4: Artemesinin and artemesinin derivative antimalarials.

ACTs are recommended by WHO as first-line treatment of uncomplicated *falciparum* malaria in all areas where malaria is endemic [22]. They are preferably formulated in fixed-dose combinations; AS-MQ, AS-AQ, ART-LUM and AS-SP, to improve adherence, acceptability, and prevent one drug being taken without its partner [23]. In addition, DHA-PQ is a new artemesinin containing fixed-dose combination treatment that has proved to be well tolerated and effective against *P. falciparum* in south East Asia [24]. DHA-PQ and ART-LUM were both highly effective in resolving uncomplicated *P. vivax* malaria, reducing anaemia and decreasing the number of gametocytes [25].

Artesunate- pyronaridine (AS-PYR) has recently completed several large-scale Phase III clinical trial and initial results demonstrate excellent efficacy. It was also reported to be effective in treating *P. vivax* malaria with similar cure rates and faster parasite and fever clearance times than chloroquine [26]. Recently, there have been signs that the efficacy of ACTs and artesunate monotherapy have declined in western Cambodia [27].

1.1.6. Drug Candidates for the Treatment of Malaria

Tafenoquine, a new 8-aminoquioline under Phase III clinical development, is found to be effective in preventing *P. falciparum* and *P. vivax* malaria as well as relapse of *P. vivax*

associated with latent hypnozoites in the liver [28]. In addition, isoquine, 4-pyridone, AQ 13 and CDRI 97/98 are in Phase I; artemisone, ferroquine, fosmidomycin-clindamycin, SAR 97276, methylene blue-AQ and tinidazole are in Phase II; eurartesim, pyramax, azithromycin-CQ and arterolane-PQ are in Phase III clinical development [29]. More recently, OZ 439 has successfully completed Phase I clinical trials, and Phase IIa trials in patients with malaria are underway [30].

1.1.7. Antimalarial Drug Resistance

1.1.7.1. Resistance to 'Older' Drugs

Chloroquine had been the drug of choice for treating *P. falciparum* malaria for more than 50 years. However, its use as a prophylactic drug and as a malaria treatment is now limited because of the selection and spread of CQ-resistant *P. falciparum* strains throughout malaria endemic areas [31]. In addition, resistant strains evolved to SP , quinine, piperaquine, mefloquine, lumefantrine and atovaquone [32, 33]. Consequently, the mortality and morbidity from malaria has increased in the past two decades. Thus, artemisinins and ACTs are recommended as a first-line treatment for *falciparum* malaria in almost all regions where malaria is endemic [22].

1.1.7.2. The Emergence of Artemisinin Resistance

Although artemisinins are potent and rapidly acting antimalarial drugs, their widespread use for treating patients with *P. falciparum* malaria raises the question of emerging drug resistance [27]. Research findings revealed that treatment failures to AS-AQ in patients with mixed infections (*P. falciparum* and *P. vivax*), observed in Papua, Indonesia [34]. Similarly, treatment failure to ART-LUM has been observed in Asia and Africa, due to *pfmdr1* selection and *pfmdr1* polymorphism in some of its residues respectively [35]. Recent data indicated that artemisinin resistance has already emerged along the Thai-Cambodian border [36]. The clinical failures of ACTs are

largely limited to the Thai-Cambodian border, where as efficacy with AS-MQ along the western borders of Thailand remains high, and high sensitivity is found in southeastern Bangladesh, an area where until very recently ACTs have not been extensively used. Thus, artemisinin resistance does not seem to be a widespread epidemiologic phenomenon yet [37].

1.1.8. Monitoring Antimalarial Drug Resistance

The history of drug resistance to the 'old' drugs, and the potential for resistance to evolve to ACTs, demonstrates that up-to-date information on efficacy of antimalarials is crucial to provide early warning of the development of resistance. A collaborative effort between the Global Malaria Program of the WHO and the World Wide Antimalarial Resistance Network (WWARN) has recently been launched. The goal of this cooperative effort is to design a system that will improve the overall quality of the data collected, analyze the collated data effectively and produce analyses that are accessible, informative and useful to all those involved in antimalarial drug deployment and use. The useful life of ACTs and other drugs that are in development must be prolonged, and the data sharing effort can certainly play an important role [38].

1.2. Background on Leishmaniasis

1.2.1. Leishmaniasis

Leishmanisis (kala-azar) is, a neglected tropical disease resulting from infection of macrophages by obligate intracellular parasites of the genus *Leishmania* [39]. It is transmitted by about 30 species of *Phlebotomine* sand flies. Infection starts following ingestion of *Leishmania* amastigotes from an infected host (Fig. 5). Thus, transformed into infective promastigotes, and infect another host upon insect bite [40, 41]. Phagocytosis by mononuclear phagocytic cells of the host (reservoir), and transformation to dividing amastigotes (lysozymes resistant) will occur

within 24 h. Eventually, the host cells lyse, releasing the free parasites which spread to new cells and tissues of different organs (spleen, liver and bone marrow) causing lesions and tissue destruction [42].

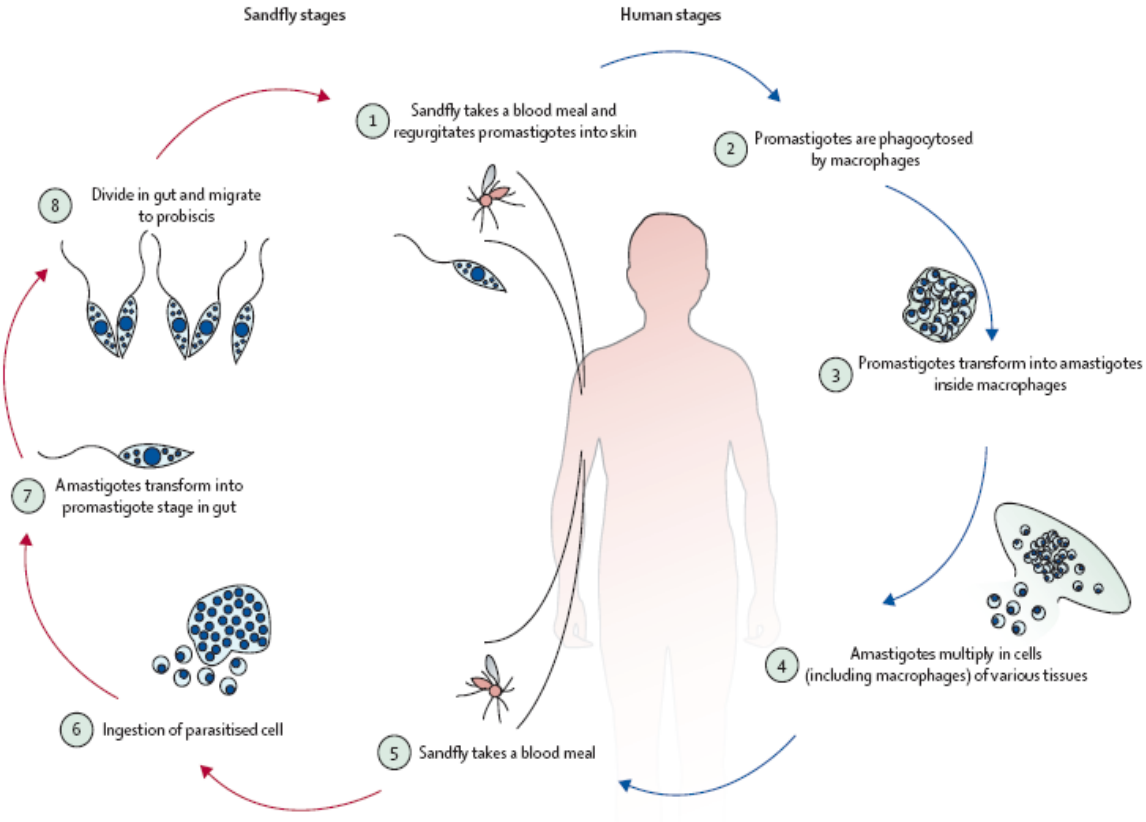


Figure 5: Life cycle of Leishmania parasite [43].

1.2.2. Clinical Types of Leishmaniasis

1.2.2.1. Cutaneous Leishmaniasis (CL)

It is the most common form of leishmaniasis with a varied spectrum of clinical manifestations which range from small cutaneous nodules to gross mucosal tissue destructions [43]. It is caused by *L. tropica, L. major, L.aethiopica, L. amazonensis* and *L. mexicana*, and produces skin ulcers on the exposed parts of the body such as the face, arms and legs, causing serious disability and leaving the patient permanently scarred. It can be either localized or diffused form [44].

1.2.2.2. Visceral Leishmaniasis (VL)

It is the most severe form, in which the parasites have migrated to vital organs. It is a severe, debilitating disease, characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and anemia. Patients gradually become ill over a period of a few months, and nearly always die if untreated. It is caused by *L. donovani, L. infantum and L. chagasi* [45].

1.2.2.3. Mucocutaneous Leishmaniasis (MCL)

It is caused by *L. aethiopica* and *L. brasiliensis* complex. It can lead to extensive and disfiguring destruction of mucous membranes of the nose, mouth, throat cavities and even cartilages [46].

1.2.3. Global Burden of Leishmaniasis

Leishmaniasis is a public health problem in at least 88 countries with an estimated 350 million people at risk. The estimated global prevalence of all forms of the disease is 12 million, with about 1.5-2 million new cases of CL and 70 000 deaths each year; 500 000 new cases of VL and 59 000 deaths occur each year [47]. CL is endemic in more than 70 countries worldwide, and 90% of cases occur in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria [48]. VL occurs in 65 countries; the majority (90%) of cases occur in Bangladesh, India, Nepal, Sudan, and Brazil [49]. The number of cases is increasing globally at an alarming rate and cases of *Leishmania*/HIV co-infection have recently increased [50].

1.2.4. Leishmaniasis in Ethiopia

Both CL and VL are endemic and growing health problems in Ethiopia. VL is mainly caused by *L. donovani* and its annual incidence ranges from 4500-5000. It is endemic in lowlands below 1500 m above sea level. Because of mass population movements, *Leishmania*/ HIV co-infection,

malnutrition and treatment failures [51], VL has appeared in areas previously free of the problem [52]. In 2005 an outbreak of VL in Libo Kemkem, South Gondar (>1800 m above sea level), claimed the lives of hundreds that were clinically misdiagnosed as chronic malaria [53].

In Ethiopia, CL is principally caused by *L. aethiopica* and rarely by *L. tropica* and *L. major*. It is found mainly at high and mid-altitudes ranging from 1400-2700 m above sea level [54]. In 2005, there was CL (skin lesion) outbreak in Silti Woreda, southern Ethiopia [55]. According to recent findings, there is an isolated zoonotic foci of CL in South-eastern Addis Ababa, with *P. longipes* as the likely vector and *Heterohyrax brucei* as the natural reservoir host [56].

1.2.5. Who is at High Risk of Leishmaniasis?

HIV/AIDS patients are special group of people highly vulnerable to leishmaniasis. In endemic regions, up to 10% of AIDS patients present with VL as an opportunistic infection, and in Northwest Ethiopia, up to 30% of VL cases are HIV co-infected [57]. With expanding travel activities, VL increasingly occurs in non-endemic areas and affects immunocompetent individuals with no other risk factors. An increase in imported *Leishmania* cases into Europe has also been attributed to an increase in international travel and the migration of immigrants and refugees from endemic regions [58].

1.2.6. Prevention and Treatment Strategies of Leishmaniasis

1.2.6.1. Prevention Methods of Leishmaniasis

Vectors and reservoirs are targeted in the prevention of leishmaniasis. But control of reservoir host and vector is difficult due to high coast, operational difficulties and frequent relapses in the host [59]. A considerable progress has been made in the last decade as far as vaccines are

concerned, but very few vaccine candidates have progressed beyond the experimental stage. As such there is no vaccine against any form of human leishmaniasis [60, 61].

1.2.6.2. Existing Drugs for the Treatment of Leishmaniasis

Pentavalent Antimonials (Sb^V)

The control of *Leishmania* infections primarily rely on the pentavalent antimonials (sodium stibogluconate (SSG) (**26**) and meglumine antimoniate (**27**)). Currently they are the mainstream treatment for VL. Cure rates of 95% or higher have been consistently obtained with a standard 30 days regimen of Sb^V in the Sudan [62], and still in use in Indian patients [63].

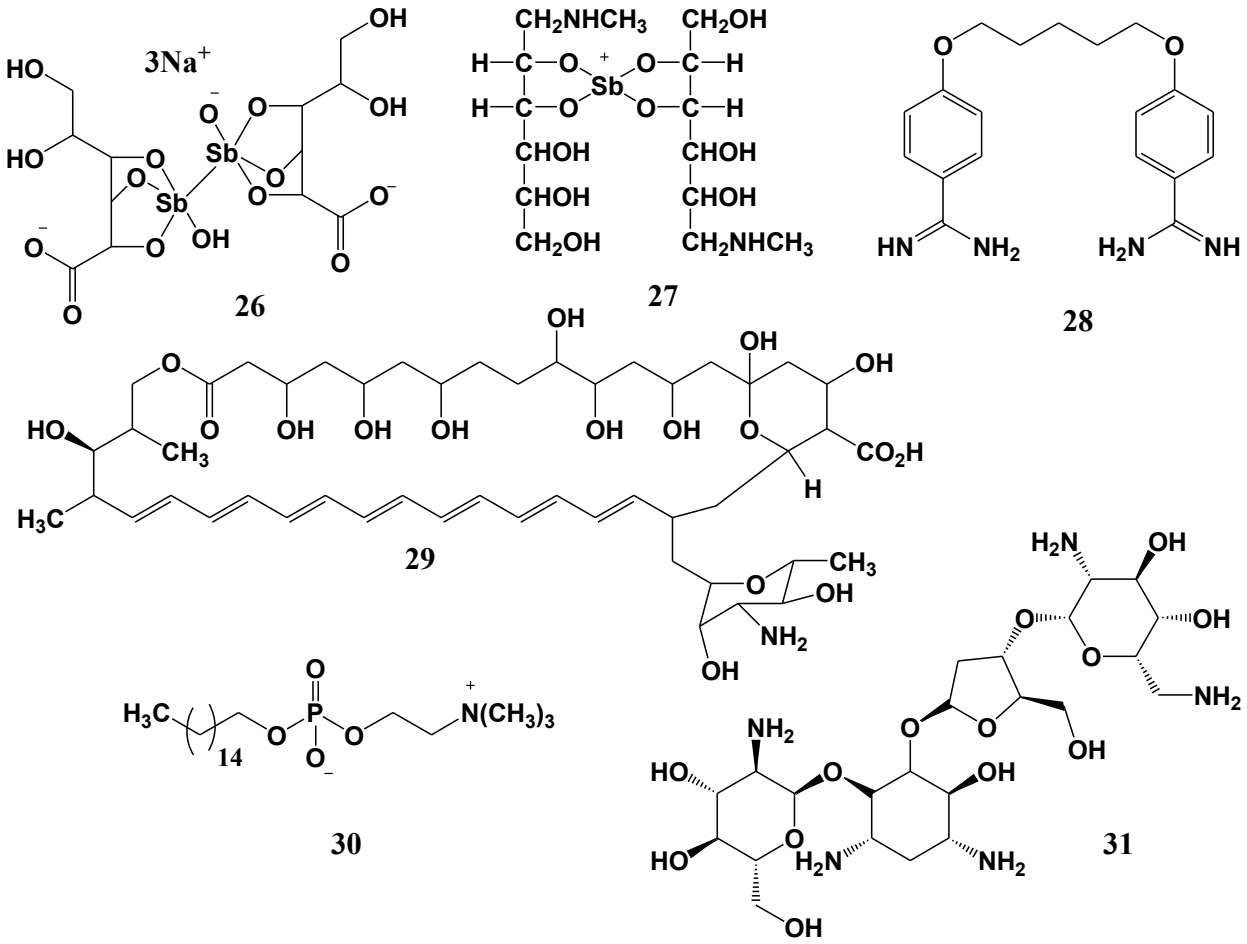


Figure 6: Currently used antileishmanial drugs

Pentamidine

Since the 1940s, pentamidine (**28**) has been successfully used against different forms of leishmaniasis. Later on, it was abandoned as first-line treatment for VL, due to the development of resistance in India and the availability of other drugs with less side effects. It is currently used as secondary prophylaxis in monthly or biweekly administration in HIV/VL patients [64].

Amphotericin

Amphotericin (**29**) is a second line drug being used in the treatment of VL cases that do not respond to Sb^V and in patients clinically resistant to pentamidine [65]. In addition to the unilamellar liposomal formulation (AmBisome®), lipid complex (Abelcet®), colloidal dispersion (Amphocil™), and amphotericin B encapsulated microspheres have been prepared and showed better activity with diminished side effects and lower cost. Nanoparticles of amphotericin B had significantly greater efficacy with a favourable safety profile [66].

Miltefosine

Miltefosine (**30**) is the first oral treatment for VL and CL including Sb^V resistant infections [67]. It was registered in India for the treatment of VL in 2002. It is also safe for the treatment of HIV/VL co-infection [68]. Miltefosine exhibited teratogenic potential, and therefore should not be administered to pregnant women [69].

Paromomycin

Both VL and CL can be treated with paromomycin (**31**). It was registered in 2007 for the treatment of VL in India [70]. But poor oral absorption has led to the development of parenteral and topical formulations for the VL and CL respectively [71]. Paromomycin ion pairing enhances the topical permeability. It can be a better therapeutic alternative for CL than meglumine antimonate [72].

Combination Therapy

Combination chemotherapy has improved prospects for decreasing the emergence of drug resistance, increasing activity, reducing required doses and thereby toxic side effects. In a recent study, WR 279,396, a topical formulation containing 15% paromomycin and 0.5% gentamicin was evaluated in a Phase II trial in Tunisia and France. The treatment for 20 days was safe and effective against CL caused by *L. Major* [73]. Preliminary results indicate that, AmBisome-Paromomycin is the most-cost effective combination among Miltefosine-Paromomycin and AmBisome-Miltefosine. But all these combinations were superior in activity compared to the standard treatments **[**74**]**. No combination has yet been used in treatment programs, except Paromomycin/SSG [75].

1.2.7. Other Drugs with Promising Antileishmanial Activities

Sitamaquine is a promising oral treatment for VL in Africa. A 28-day course of treatment was efficacious and well tolerated in 61 Kenyan patients infected by *L. donovani*, with the tested dose of 2.0 mg/kg/day. It is now in Phase IIb clinical trial [76]. The HIV protease inhibitors, nelfinavir, lopinavir [77], Ac-Leu-Val-Phenylalaninal and Saquinavir mesylate [78] showed to have promising antileishmanial activities and may be useful in combating *Leishmania*/HIV co-infection. The anticancer drugs monastrol [79], tamoxifen [80], perifosine [81] demonstrated a pronounced antileshmanial activity. In addition, pyrazinamide [82], nitazoxanide [83], furazolidone [84], azithromycin [85], and nimodipine [86] are drugs with promising antileishmanial activities that should be considered for further study and preclinical development.

1.2.8. Development of Resistance to Antileishmanial Drugs

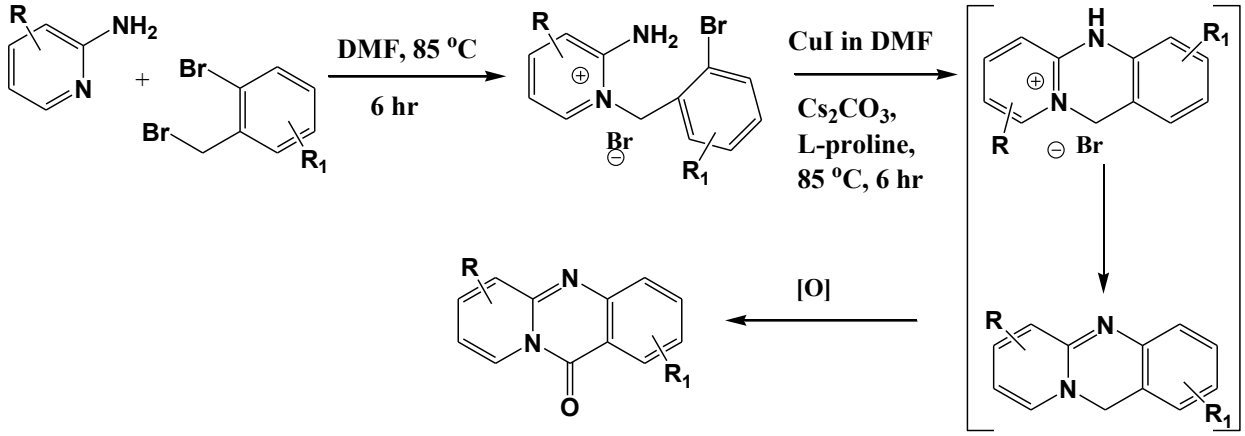
Sb^V have been used for the treatment of *Leishmania* infections. Unfortunately, in many parts of the world, the parasite has become resistant to Sb^V [87]. Treatment failure to SSG is observed in Eastern Sudan [88] and in Tigray, Northern Ethiopia [89]. Recent reports show that pentamidine also developed resistance as well as difficulties in treating HIV patients [90]. Resistance to miltefosine was shown to relatively easily develop *in vitro* through a single point mutation. Its long terminal half-life has the potential to increase the risk of development of resistant strains, especially if it is used in incomplete courses and if relapses are not thoroughly retreated [91].

1.3. Quinazolines

1.3.1. Chemistry of Quinazolines

1.3.1.1. Reported Methods of Synthesis

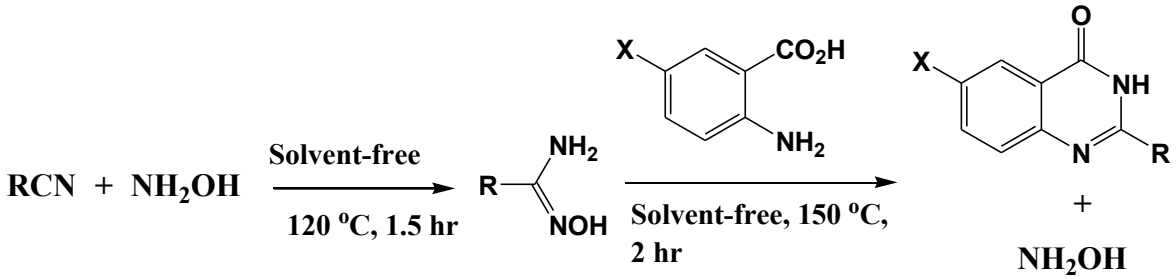
A number of synthetic methods of quinazolinones have been developed, owing to their broad pharmacological and synthetic importance. Recently, a convenient and inexpensive one-step synthesis of linear and angular fused quinazolinones has been developed.



Scheme 1: One-step synthesis of fused quinazolinones.

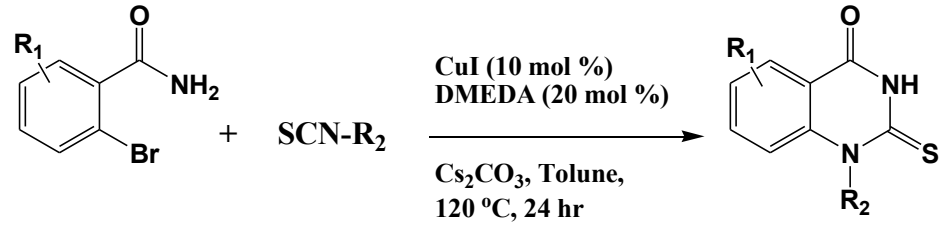
The method uses amino heterocycles and *o*-bromobenzyl/naphthyl bromides as reactants, CuI as catalyst, Cs_2CO_3 as base, L-proline as ligand, and DMF as solvent. It proceeds *via* nucleophilic aromatic substitution of the *N*-heteroaromatic cationic intermediate followed by *in situ* aerial oxidation at the benzylic position to the quinazolinone scaffold [92].

A novel and one-pot synthesis of 2-aryl/alkyl-4(3*H*)-quinazolinones is described. The *in situ* prepared amidoximes from the reaction between nitriles and hydroxylamine are condensed with anthranilic acids under solvent and catalyst-free conditions to produce the target compounds in excellent yields [93].



Scheme 2: Synthesis of quinazolinones by one-pot condensation reaction.

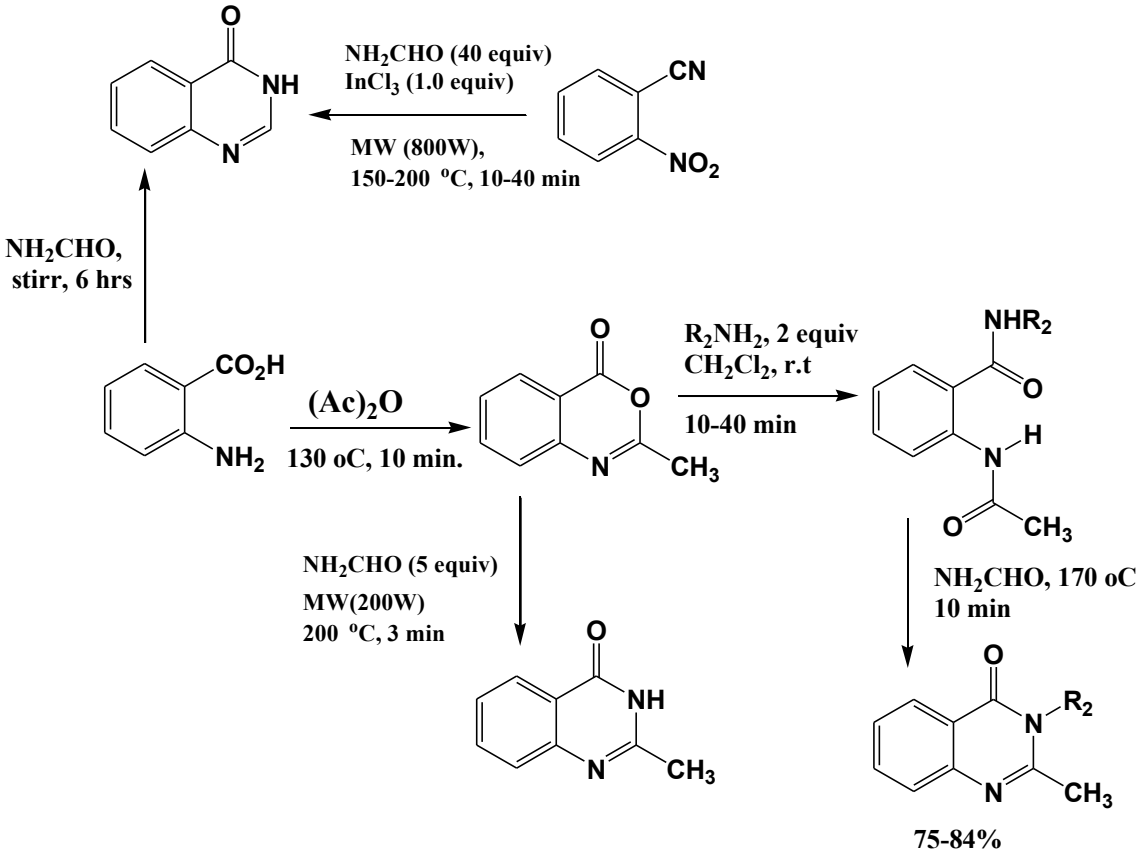
Copper-catalyzed tandem reaction of *o*-bromobenzamides and isothiocyanates is an efficient and practical route for the synthesis of 2-thioxo-2,3-dihydroquinazolin-4(1*H*)-ones. The optimal condition involved: CuI as precatalyst, Cs_2CO_3 as base, N,N′-dimethylethane-1,2-diamine (DMEDA) as ligand, and toluene as solvent, with reaction temperature at 120 °C [94].



Scheme 3: Synthesis of quinazolinones by copper catalyzed tandom reaction.

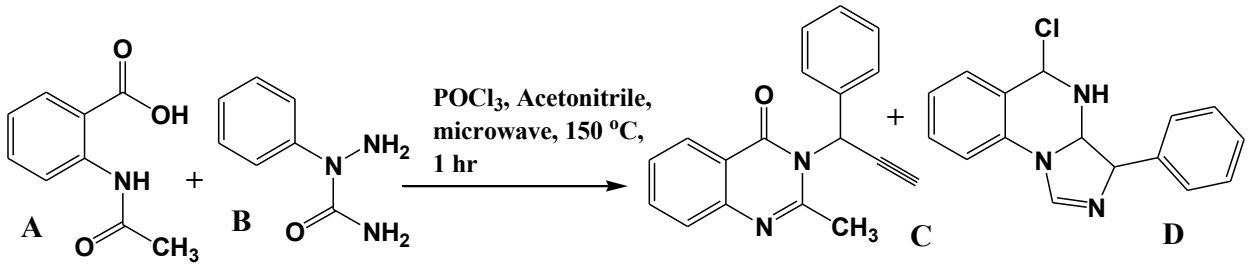
Rapid and efficient generation of CO and NH_3 in the reaction mixture *via* microwave-assisted thermal decomposition of formamide (used as a sole ammonia synthon) may represent a significant improvement over existing methods for synthesis of quinazoline derivatives [95].

Treatment of 2-nitrobenzonitrile with formamide in the presence of $InCl_3$, heated for 10-40 min at various temperatures (150-200 ^oC) resulted in the expected quinazolinones with 90% yield [96]. In addition, unsubstituted, 2-substituted and 2,3-disubstituted quinazoline-4-ones can be synthesized using anthranilic acid and formamide as a starting material, scheme 4 [97-98].



Scheme 4: Synthesis of quinazolinones by microwave-assisted thermal decomposition.

Cyclization of *N*-acylanthranilic acid (**A**) with 2-aminoacetamide (**B**) in the presence of $POCl_3$ under microwave irradiation gives a 1:1 mixture of **C** and **D** as shown in scheme 5. PCl_3 afforded exclusively compound **C** in reasonable yield [99].



Scheme 5: Synthesis of quinazolines by cyclization of *N*-acetylanthranilic acid.

1.3.2. Biological Activities of Quinazolines

The quinazolin-4(3*H*)-one nucleus, a basic unit found in various naturally occurring bioactive alkaloids, have continued to attract a widespread interest, due to their diverse pharmacological activities: Parkinson's disease [100], Alzheimer's disease [101], antiinflammatory [102], antitumor [103], CNS depressant [104], antimicrobial [105], antifungal [106], immunosuppressive [107], antihypertensive [108], anticonvulsant [109], antidepressant [110], antioxidant [111], antimalarial [112], antileishmanial activities [113], etc.

1.3.2.1. Quinazolines as Antimalarial Agents

The antimalarial activity of febrifugine **(33)**, a quinazolinone alkaloid isolated from *Dichroa febrifuga,* paved the way for the synthesis of its analogues and other bioactive compounds that contain a quinazolinone moiety as a scaffold.

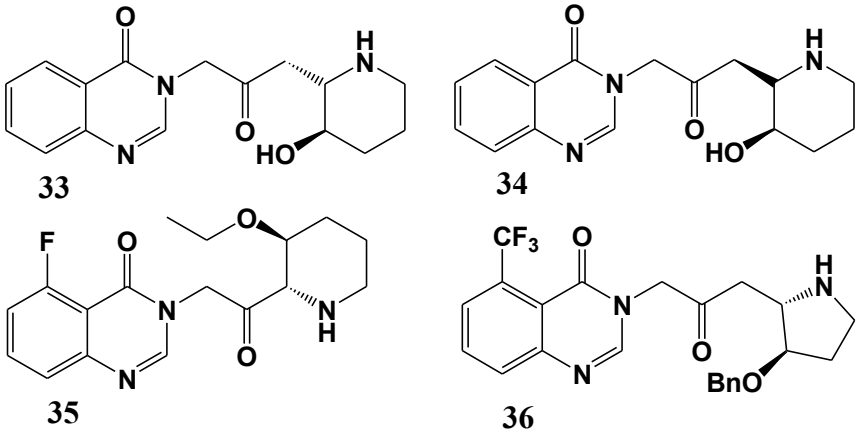


Figure 7: Febrifugine analogues displaying good antiplasmodial activities.

Several febrifugine analogs were synthesized by introducing extra nitrogen atoms, one or two electron-withdrawing group(s) or a bulky group in the quinazolinone ring [114]. Among these, isofebrifugine (**34**) (IC_{50} = 0.33 nM), 3-[2-(3-Ethoxypiperidin-2-yl)-2-oxoethyl]-5-fluoro-4(*3H*)-quinazolinone (**35**) (IC_{50} = 0.55 nM) and (2*R*,3*S*)-3-[3-(3-Benzyloxy-pyrrolidin-2-yl)-2-oxopropyl]-5-trifluoromethyl-4(*3H*)-quinazolinone (**36**) (IC_{50} = 0.19 ng/ml) have shown ten times superior activity than febrifugine and CQ. These compounds are 100 times less toxic than febrifugine. A planar aromatic ring, a 1″-amino group, and a C-2′, C-3″ *O*-functionality is crucial for their excellent antimalarial activities [115].

Several 6-substituted-2,4-diaminoquinazolines were synthesized and tested against *P. falciparum*. The 2,4-diamino-5-chloro-6-[*N*-(2,5-dimethoxybenzyl)]amino]quinazoline (**37)** and compound (**38**) are found to be active compounds with IC_{50} values of 9 nM and 33 nM respectively. Their activity was potentiated by the dihydropteroate inhibitor dapsone, an indication that this compound is a DHFR inhibitor [116].

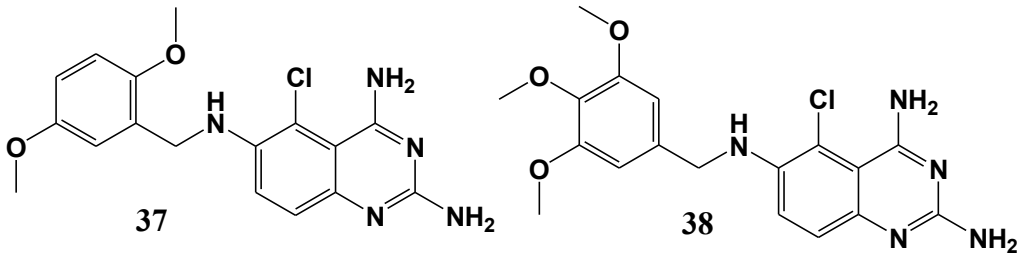


Figure 8: 6-substituted-2,4-diaminiquinazolines as antimalarial agents.

A wide range of 4-aniloquinazolines displayed potential antiplasmodial activities. Compounds (**39-43)** displayed a promising antimalarial activity with IC_{50} values 0.95, 2.2, 1.3, 1.8 and 0.4 μM respectively. Those compounds having *meta*-trifluoromethyl group in the aniline moiety, nitro group at 6-positions and tosylmethyl group at 2-position of the quinazoline scaffold, are shown to have good solubility and superior antiplsmodial activities [112].

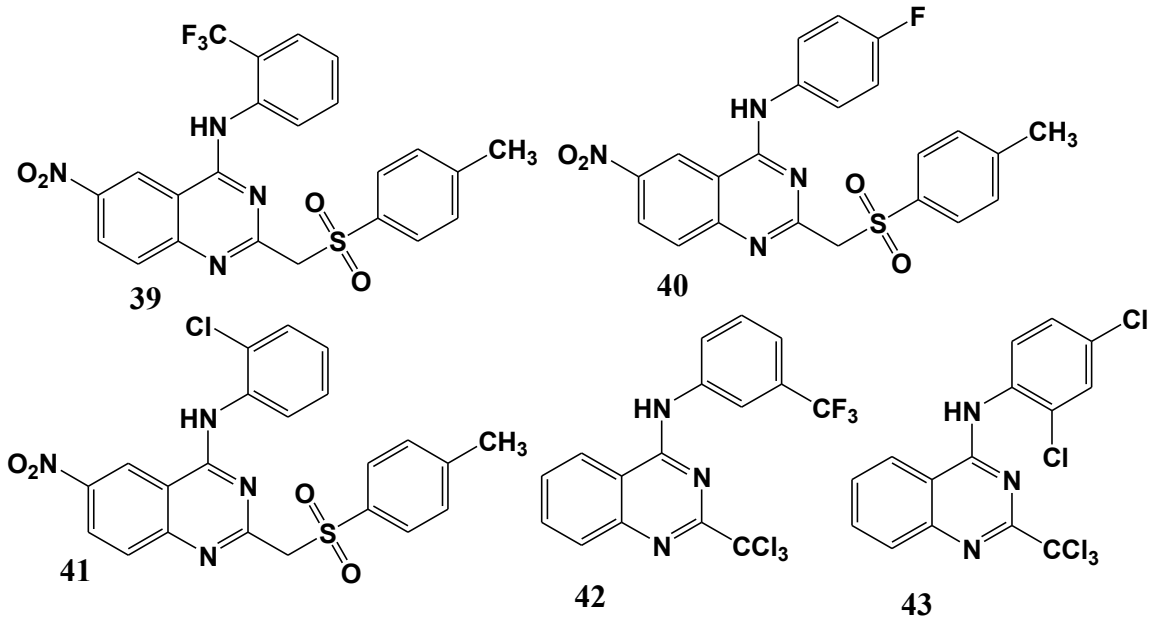


Figure 9: 4-Aniloquinazolines as antimalarial agents.

Thiourea analogues of 4-aniloquinazolines (**44-46**) showed promising *in vivo* antimalarial activity against multidrug resistant *P. yoelii nigeriensis* with 100, 85.71 and 77.19 % inhibition respectively.

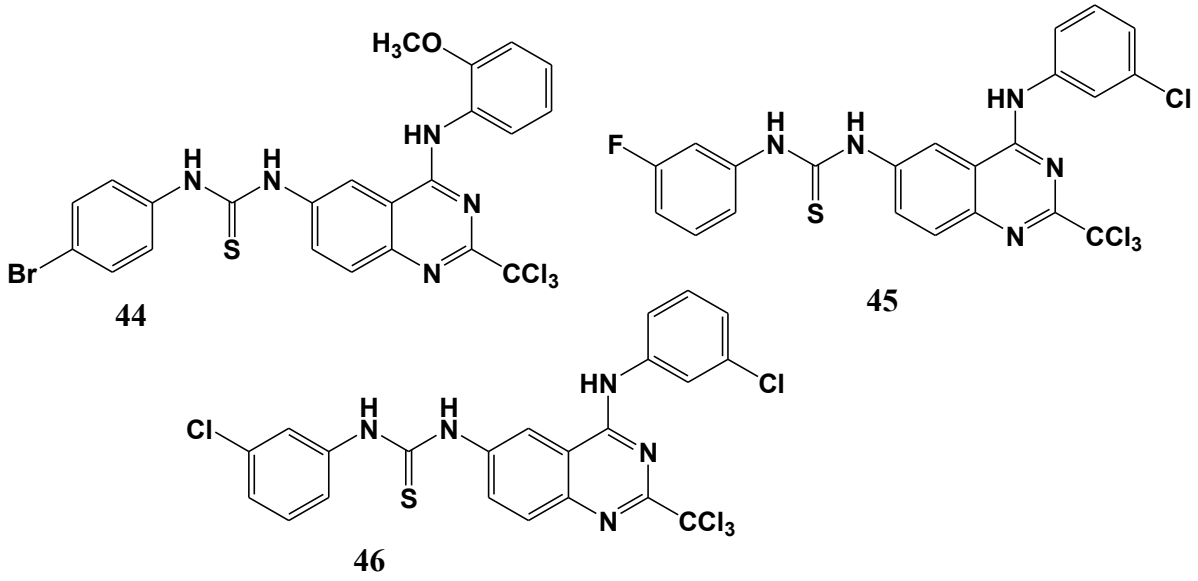


Figure 10: Thiourea analogues of 4-anolioquinazolines with antimalarial activities.

Substitution at 4-position of the phenyl ring of the aniline-part resulted in loss of antimalarial activity. In contrast, the presence of 3-Cl-phenyl or 4-Br-phenyl in the arylthiourea entity were better suited to show the antimalarial activity [117].

Several 2-(substituted styryl)-3-aryl-4(*3H*)-quinazolinones were synthesized and tested for their antimalarial activities. Compounds 47, 48 and 49 displayed promising antimalarial activities with % suppression of 81.10, 77.25 and 73.54 respectively [118, 119].

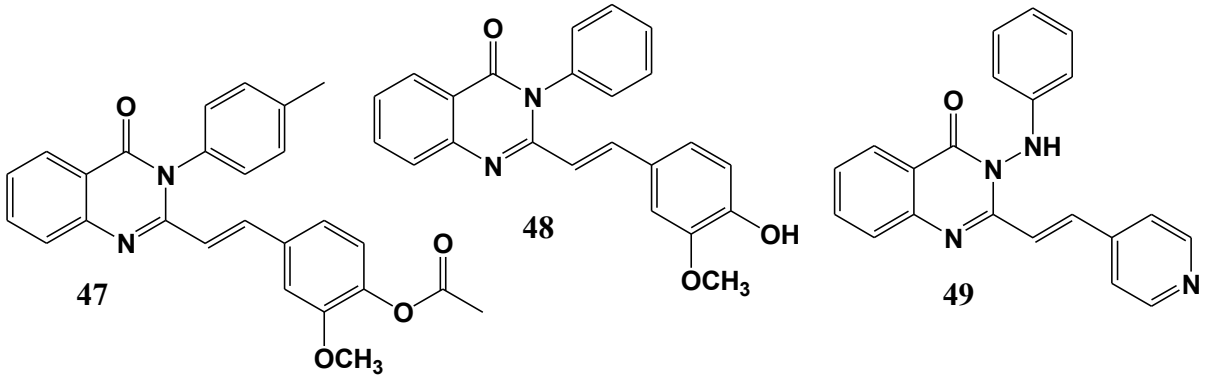


Figure 11: Some 2-styryl-4(*3H*)-quinazolinones with antimalarial activities.

1.3.2.2. Quinazolines as Antileishmanial Agents

A series of 2,3-disubstituted-4(3*H)*-quinazolinones were synthesized and tested for their antileishmanial activities. Among the tested compounds, **50** and **51** showed moderate activity. 3-benzyl-2-phenylquinazolin-4(3*H*)-one (**52**) was found to be more potent (IC_{50} = 48 μM), and is therefore anticipated to be a better new leishmanicidal candidate.

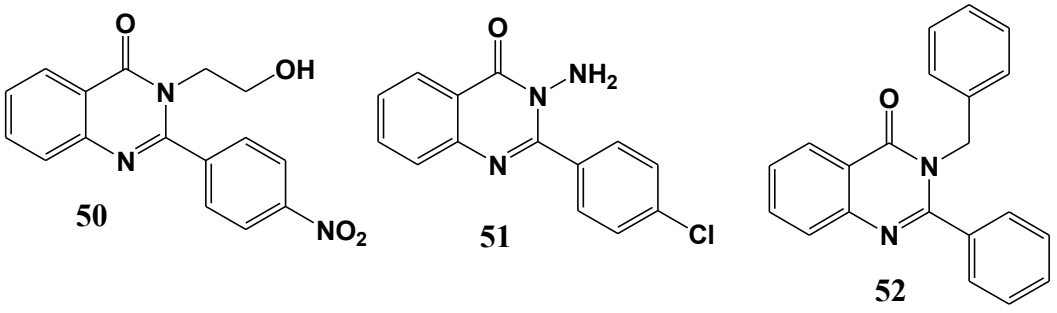


Figure 12: 2,3-Disubstituted-4(*3H*)-quinazolinones with antileishmanial activities.

The SAR of the substituents is important. The chloro substituent on the phenyl group at C-2 position, and the alkyl group at the C-3 position increase the activity, as observed in compounds **50** and **51**, respectively. A profound effect in compound **52** was observed due to the presence of the benzylic moiety at the C-3 position [113].

Among the 2,4-diaminoquinazoline derivatives synthesized as DHFR inhibitors, compound **53** and **54** showed remarkable antileishmanial activities with IC_{50} values of 12 and 91 pM, respectively and favorable therapeutic index [120].

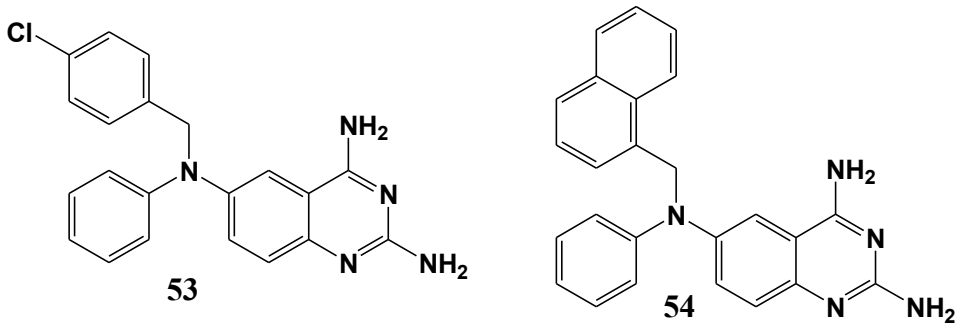


Figure 13: 2,4-Diaminoquinazolines with potent antileishmanial activities.

Several indolo[2,1-*b*]quinazoline-6,12-dione (tryptanthrin) derivatives exhibited remarkable activity at concentrations below 100 nM when tested against *L. donovani* amastigotes *in vitro*.

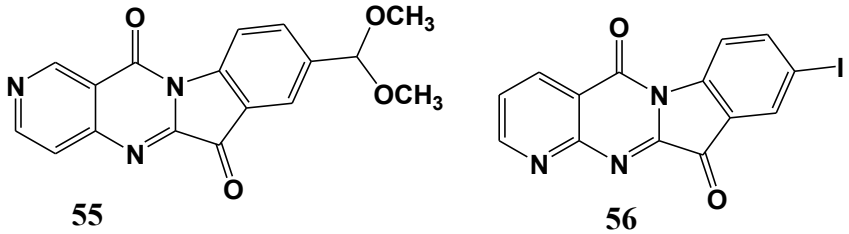
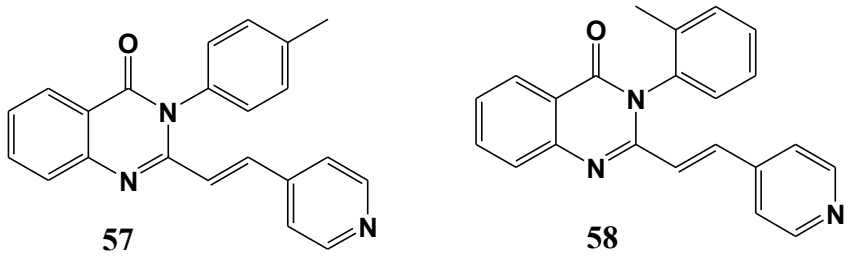


Figure 14: Some Indolo[2,1-b]quinazolinones with antileishmanial activities.

Among the indolo[2,1-*b*]quinazoline-6,12-diones, compounds **55** and **56** showed a pronounced antileishmanial activity with IC_{50} values of 16nM [121].

Among 2-(substituted styryl)-3-aryl-4(*3H*)-quinazolinones synthesized and tested for their antileishmanial activities, compounds **48** (IC_{50} =4.12 ng/ml), **57** (IC_{50} = 5.30 ng/ml) and **58** (IC_{50} = 1.10ng/ml) displayed superior antileishmanial activities than the standard drugs miltefosine and amphotericine B deoxycholate [119].



57 **58**

Figure 15: Some 2-styryl-4(*3H*)-quinazolinones with antileishmanial activities.

2. Objectives

2.1 General objectives

- To synthesis and evaluate the antimalarial and antileishmanial activities of some quinazoline derivatives.

2.2 Specific objectives

- To synthesize some quinazoline derivatives by applying different chemical reactions.
- To characterize the chemical structure of the target compounds using some physical and spectroscopic techniques.
- To test *in vivo* antimalarial activities of the synthesized compounds on infected mice relative to standard reference drug-chloroquine phosphate.
- To perform *in vitro* antileishmanial test of the synthesized compounds relative to the standard drugs.
- To perform acute toxicity test for the most active synthesized compounds.

3. Materials and Methods

3.1. Materials

3.1.1. Chemicals and Reagents

Anthranilic acid, acetic anhydride, aniline, *p*-toluidine, *o*-toluidine, acetone, dimethylsulfoxide (DMSO) and Tween 80 were obtained from BDH England. Anthranilic acid and vaniline were donated by Drug Discovery Centre, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University, Egypt. Anhydrous zinc chloride, *p*-chlorobenzaldehyde, *p*-nitrobenzaldehyde were donated by department of chemistry, Faculty of science, AAU. *p*-hydroxybenzaldehyde was donated by Ethiopian Health and Nutritional Research Institute (EHNRI). Chloroform and absolute ethanol (Research-Lab Fine Chem. Industries, India), resazurin sodium salt (Sigma Chem. Co., St.Louis, USA), anhydrous petroleum ether, distilled water, Iodine and Geimsa stain (Linear chemicals Ltd), RMPI 1640, HCl and KOH were used in the study.

3.1.2. Instruments and Apparatuses

Melting points were determined in open capillaries using electro-thermal 9100 melting point apparatus at Ethiopian Health and Nutritional Research Institute (EHNRI) and are uncorrected. IR spectra were recorded on a SHIMADZU 8400SP FT-IR spectrophotometer at EPHARM. ^{1}H NMR spectral data were performed on Bruker Avance DMX400 FT-NMR spectrometer using tetramethyl silane (TMS) as internal standard, at the Department of Chemistry, Faculty of Science, AAU, Ethiopia. Elemental microanalyses were done on Perkin Elmer 2400 elemental analyzer at Micro analytical unit, Faculty of science, Cairo University, Egypt. Silica gel TLC plates of 0.25 mm thickness were used for chromatographic analysis and spots were visualized using iodine vapor.

The following equipments were used; polystyrene tissue culture flasks (Corning), 96-well chambered plates, micropipettes (Pipetman ultra), multi-channel pipettes (Hamilton), haemocytometer (Improved Double Neubauer type), eppendroff tube, light microscope (normal and inverted types) (Motic & Olympus), class II biosafety cabinet (Labconco), autoclave (Pristage), analytical balance (Model: AB204-S), microwell plate reader (PerkinElmer), carbon dioxide (CO_2) incubator (Thermo Electro), centrifuge (Hettich/ Thermo Electro), vortex mixer (Model: whirl VIB2), pipette tips (Oxford), syringes (20 and 25 gauges) and aluminum foil were used.

3.1.3. Reference Drugs

For the *in vivo* antimalarial activity, chloroquine phosphate (EPHARM, Addis Ababa, Ethiopia) was used as a reference drug. Miltefosine/hexadecylphosphocholine (AG Scientific, San Diego, CA, USA) and amphotericin B deoxyhcholate (Fungizone®, ER Squibb, UK) were employed as reference drugs in the *in vitro* antileishmanial activity testing of the synthesized compounds.

3.2. Methods

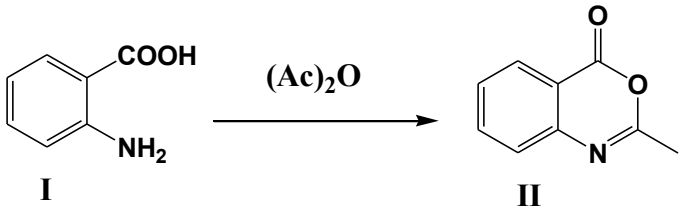
3.2.1. Chemistry

3.2.1.1. Synthesis of Target Compounds

The synthesis of target compounds (3-aryl-2-(substitutedstyryl)-4(*3H*)-quinazolinones) was achieved using cyclization, condensation and hydrolysis reactions. It involved the synthesis of acetanthranil (2-methyl-3,1-benzoxazin-4-one (**II**)) and 3-aryl-2-methyl-4(*3H*)-quinazolinones (**IIIa-c**) as intermediates. The details of each reactions and reaction conditions are mentioned in the following sections.

3.2.1.1.1. Synthesis of 2-methyl-3,1-benzoxazin-4-one (II)

A solution of anthranillic acid (**I**) (10 g, 0.073 mol) in acetic anhydride (25 ml) was heated under reflux for 1 hr. The precipitate formed after cooling was filtered and the excess acetic anhydride was washed with anhydrous petroleum ether, where up on a solid mass is obtained. This solid mass (**II**), without purification, was used for subsequent reaction (scheme 6)[122].

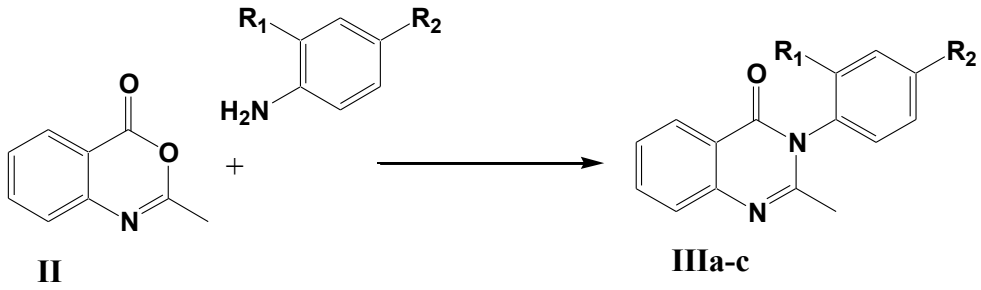


I **II**

Scheme 6: Synthesis of 2-methyl-3,1-benzoxazin-4-one (**II**) from anthranilic acid.

3.2.1.1.2. Synthesis of 3-aryl-2-methyl-4(*3H*)-quinazolinones (III a-c)

A mixture of 2-methyl-3,1-benzoxazin-4-one (**II**) (3 g, 0.017 mol) and equimolar amounts of aromatic amines (aniline, *p*-toludine, and *o*-toludine respectively) was heated under reflux at 190^{o}C for 5 hrs. The dark sticky mass formed was cooled and recrystallized from ethanol **(IIIa-c)** (scheme 7) [123].



II **IIIa-c**

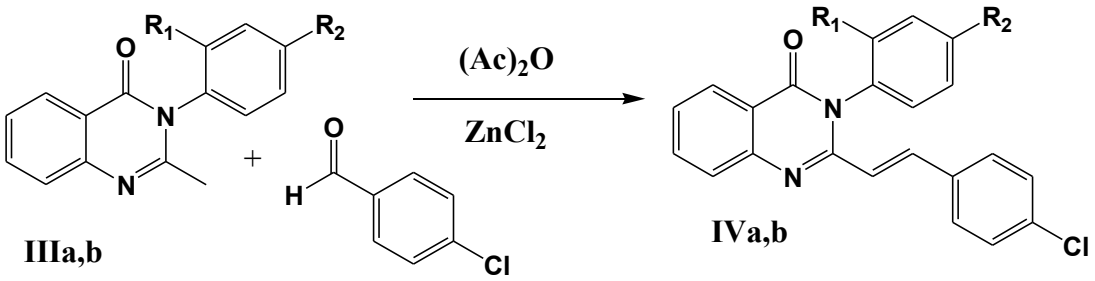
IIIa: R_1 = H, R_2 = H ; IIIb: R_1 = H, R_2 = CH_3; IIIc: R_1 = CH_3, R_2 = H

Scheme 7: Synthesis of 3-aryl-2-methyl-4(*3H*)-quinazolinones

3.2.1.1.3. Synthesis of 3-aryl-2-(substituted styryl)-4(*3H*)-quinazolinones.

Synthesis of 3-aryl-2-(4-chlorostyryl)-4(3H)-quinazolinones (IVa,b)

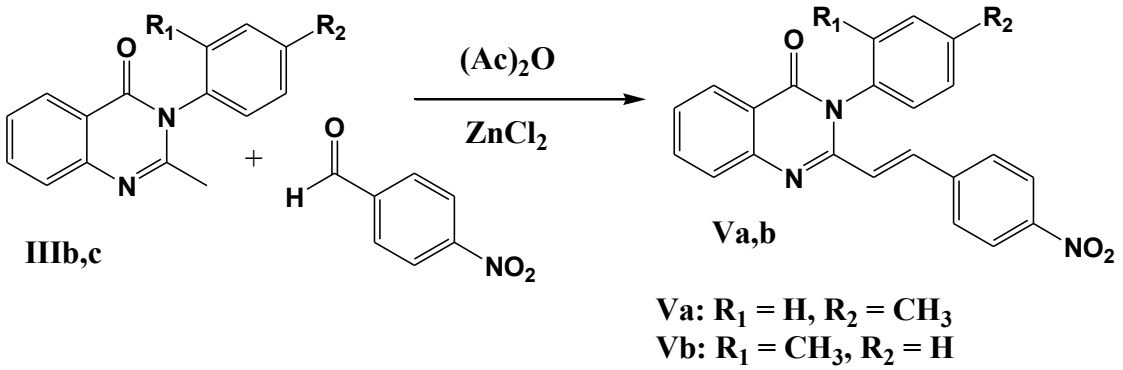
To a solution of **IIIa** or **IIIb** (0.5 g each) in acetic anhydride (10 ml), an equimolar amount of *p*-chlorobenzaldehyde was added and 10 mg of anhydrous zinc chloride as a catalyst. The reaction mixture was heated under reflux for 8 hrs, cooled and poured into ice-cooled water. The solid products formed **(IVa,b)** were filtered, dried and recrystallized from chloroform/ethanol [2:1] (scheme 8) [124].



Scheme 8: Synthesis of target compounds (**IVa,b**)

Synthesis of 3-aryl-2-(4-nitrostyryl)-4(3H)-quinazolinones (Va,b)

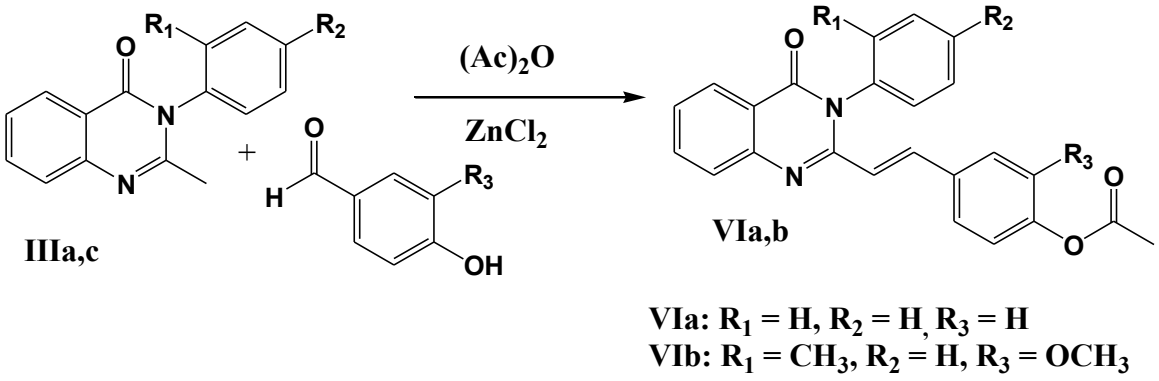
Similarly, to a solution of **IIIb** or **IIIc** (0.5 g each) in acetic anhydride (10 ml), an equimolar amount of *p*-nitrobenzaldehyde was added and 10 mg of anhydrous zinc chloride as a catalyst. The reaction mixture was heated under reflux for 8 hrs, cooled and poured into ice-cooled water. The solid products formed **(Va,b)** were filtered, dried and recrystallized from chloroform/ethanol [2:1] (scheme 9) [124].



Scheme 9: Synthesis of target compounds (**Va,b**)

Synthesis of 3-aryl-2-(4-acetylatedstyryl)-4(3H)-quinazolinones (VIa,b)

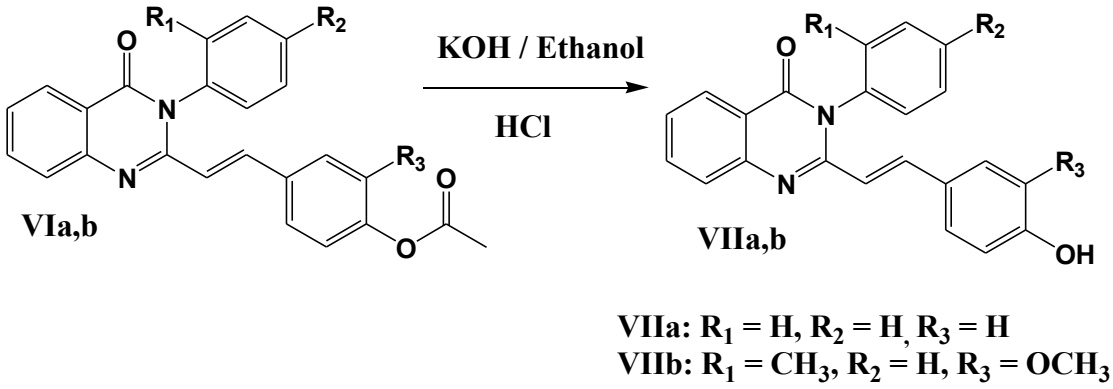
These target compounds (**VIa,b)** were synthesized in a similar fashion as the above compounds (**IVa,b** and **Va,b**) except for the acetylation of the hydroxyl groups of *p*-hydroxybenzaldehyde and vanillin due to the presence of acetic anhydride in the reaction mixture. The synthesized compounds were filtered, dried and recrystallized from ethanol (scheme 10) [124].



Scheme 10: Synthesis of acetylated compounds (**VIa,b**)

Synthesis of 3-aryl-2-(4-hydroxylstyryl)-4(3H)-quinazolinones (VIIa,b)

Subsequent treatment of **VIa, b** with 0.1 M alcoholic KOH (5ml) in the presence of ethanol followed by 0.1M HCl (6ml). The corresponding 4-hydroxyl containing compounds (**VIIa,b**) were precipitated, filtered, dried and recrystallized from ethanol (scheme 11).



Scheme 11: Synthesis of target compounds (**VIIa,b**)

3.2.1.2. Preparation of Stock and Working Concentrations

Stock solution of 10 mg/ml of the synthesized compounds were prepared by dissolving 10 mg of each in DMSO. Then stocks were diluted using complete RPMI to obtain aliquots of 10 μg/ml. Then, three-fold serial dilution with complete RPMI gave the final six working concentrations (10, 3.33, 1.11. 0.37. 0.12, 0.04 μg/ml) of each of the synthesized compounds. Amphotericin B deoxycholate and miltefosine, which were used as a positive control for comparison of the antileishmanial activities of the test compounds, were also made in three-fold serial dilutions. All the prepared drugs were stored at -20°C and retrieved only during use [125].

3.2.2. Biological Assays

3.2.2.1. Experimental animals and Strains

Swiss albino male mice of weight 20-32 g and age 6-8 weeks, *P.berghei* ANKA strain (used to infect the mice for a four-day suppressive test), were obtained from Bio-medical laboratory, Department of Biology, Faculty of Science, AAU. *L. donovani* isolate used in this study was obtained from Alexandria University, Egypt.

3.2.2.2. Culture Medium and Conditions

RPMI-1640 (Gibco, Invitrogen Co., UK), 10% heat-inactivated fetal calf serum (HIFCS), 1% penicilline-streptomycin and 1% L-glutamine all from Sigma Chem. Co., St. Louis, USA were supplied to make complete culture mediums. The *L. donovani* isolate was grown first on Novy-MacNeal-Nicolle (NNN) medium and then in tissue-cultured flasks containing RPMI 1640 medium supplemented with 10% HIFCS and 1% 100 IU penicillin/ml-100 µg/ml streptomycin solution at 22^{o}C for promastigotes.

3.2.2.3. *In vivo* Antimalarial activity

A four-day suppressive standard test was used to evaluate the *in vivo* antimalarial activities of the synthesized compounds using Swiss albino mice [126]. The mice were housed in standard cages and maintained on standard pellated diet and water for 7 days, thus, acclimatized to the laboratory conditions. Blood from a donor mouse with parasitemia level of approximately 20-30% (i.e. 20-30% of *P. berghei* ANKA strain) parasitized erythrocytes was used to infect mice. The infected blood was collected using a syringe containing trisodium citrate and diluted in physiological saline to 10^7 parasitized erythrocytes per ml.

Each mouse was given 0.2 ml (about $2x10^7$ parasites) of the inoculum intra-peritonealy on day zero, which is expected to produce a steadily rising infection [127]. After 2 hrs, the infected mice were weighed and randomly divided into nine groups of five mice per cage. Group **1** (negative control) received the vehicle containing a solution of 7% Tween 80, 3% ethanol in distilled water. Group **2** (positive control) was given 25 mg/kg/day (0.04846 mmol/kg/day) of chloroquine phosphate.

Group **3-9** were treated with equimolar amounts of the synthesized compounds through oral route for four consecutive days [128]. 24 hrs 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 four fields of approximately 100 erythrocytes per field. The efficacies of compounds were finally assessed by comparison of blood parasitemia and mouse survival time in treated and untreated mice [129].

3.2.2.4. *In Vitro* Antileishmanial Activity

In a 96-well microtitre plate, 100 µl of each of the seven threefold serial dilutions of synthesized compounds were added in triplicate wells. Then 100 µl of suspension of parasites (3.0×10^6 promastigotes/ml of *L. donovani*) were added in duplicate. The third well was left with only the target compounds of each dilution. The contents of the plates were then maintained in humidified atmosphere at 22^oC under 5% CO_2.

After 68 hrs of incubation, 10 µl of fluorochrome resazurin solution (12.5 mg dissolved in 100 ml of distilled water) was added into each well, the fluorescence intensity was measured after a total incubation period of 72 hrs using Victor3 Multilabel Counter (Perkin-Elmer), at an excitation wavelength of 530 nm and emission wavelength of 590 nm [130]. The IC_{50} values were evaluated from sigmoidal dose-response curves using Graph pad prism 5.0 software (Graph Pad Software, Inc., San Diego, CA).

3.2.2.5. *In Vivo* Acute Toxicity Test

The oral acute toxicity of compound **IVb** that exhibited promising antileishmanial activity was investigated using male Swiss albino mice (approximately 20 g each) following reported methods [131]. The experimental animals were divided into six groups and fasted over night. Group **1-5** received compound **IVb** suspended in a vehicle containing 1% gum acacia, in doses of 10, 50, 100, 200 and 300 mg/kg, respectively. The sixth group received vehicle containing 1%

gum acacia (served as a control group) at a maximum dose of 1 ml/100g of body weight by oral route. The mice were observed closely for 24 hrs with special attention to the first 4 hrs. Acute toxicity signs (sedation, lacrimation, hair erection, blinking, urination, muscle weakness, death, etc) were checked in the test mice.

3.2.3. Statistical Analysis

Results of the study were expressed as mean $\pm$ 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 2007. All data was analyzed at 95% confidence interval (P=0.05). The % parasitemia and % suppression of the synthesized compounds were calculated using the following formulae [132].

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

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

The IC_{50} values for *in vitro* promastigotes assay of target compounds were evaluated from sigmoidal dose-response curves using computer software Graph pad prism 5.0.

4. Result and Discussion

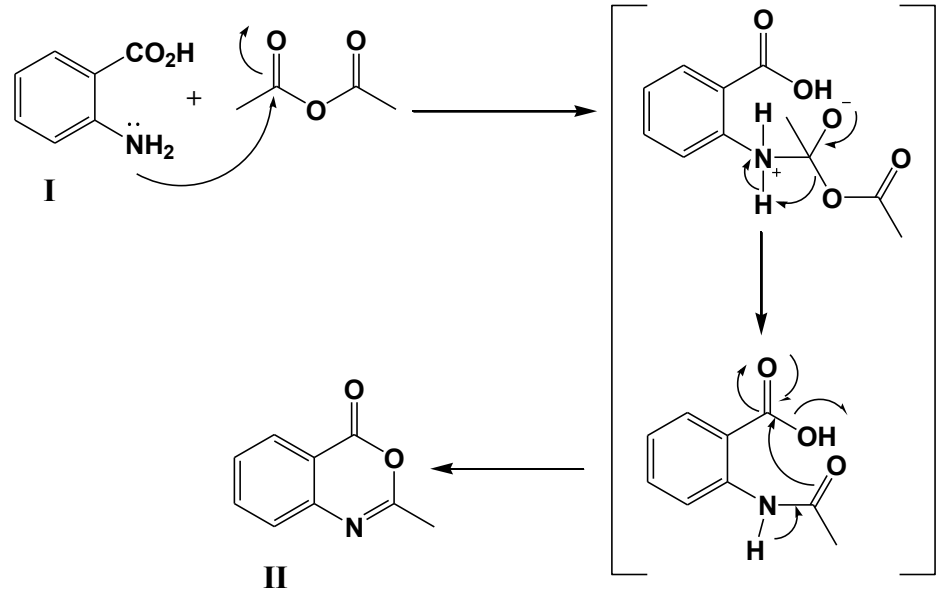
4.1. Chemistry of The Synthesized Compounds

4.1.1. Synthesis of Target Compounds

Synthesis of the target compounds involved the formation of **II** and **IIIa-c** as intermediates. It was accomplished using nucleophilic reaction (**II**), nucleophilic with ring opening and closing (**IIIa-c**), condensation reaction (**IV, V,** and **VI**) and hydrolysis reactions (**VII**). The proposed mechanism of reactions for the intermediates and target compounds is discussed below:

4.1.1.1. Proposed Mechanism of Synthesis of 2-methyl-3,1-benzoxazin-4-one (II)

The proposed mechanism for synthesis of 2-methyl-3,1-benzoxazin-4-one (**II**) involved nucleophilic attack of the strong electron-donating group (NH_2) of the anthranilic acid (**I**) with the electron deficient carbonyl group of the acetic anhydride, as shown in scheme 12.

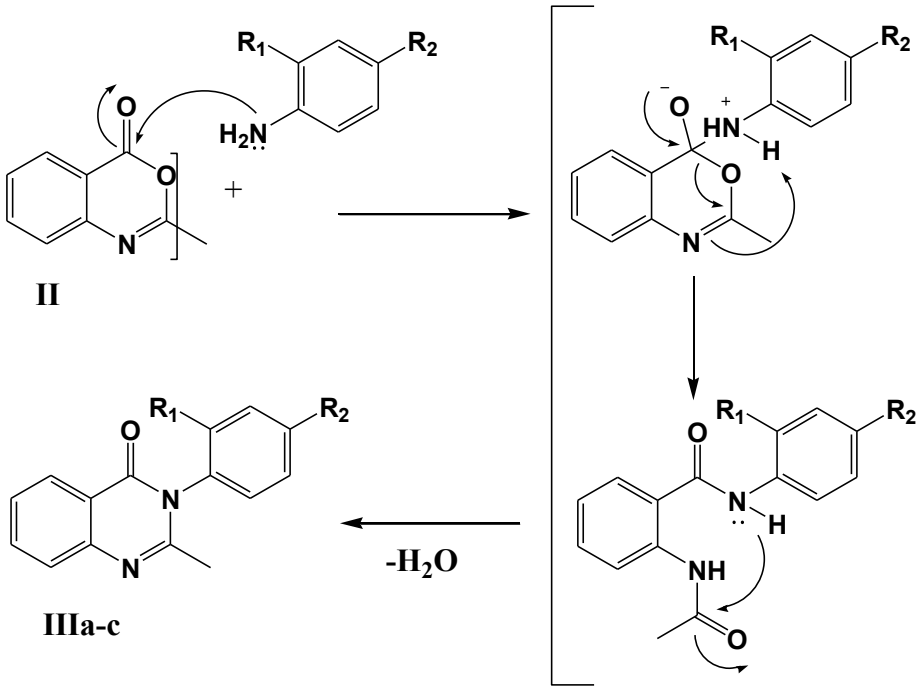


Scheme 12: Mechanism of synthesis of 2-methyl-3,1-benzoxazin-4-one (**II**).

This resulted in opening and restoration of the carbonyl group (C=O) of the acetic anhydride and there by cleavage of its C-O-C bond. Removal of a proton from the -NH- group and formation of C=N bond, trigger the formation of C-O-C bond and removal of water molecules.

4.1.1.2. Proposed Mechanism of Synthesis of 3-aryl-2-methylquinazolin-4-ones (IIIa-c)

The NH_2 group of aryl amines attacks the electron deficient carbonyl group of 2-methyl-3,1-benzoxazin-4-one (**II**) consequently the ring opened as the carbonyl group restored and amide bond formed. The -NH- group of the amide further attacks the C=O bond formed, resulting in the formation of **IIIa-c** with liberation of water molecules (scheme 13) [123].

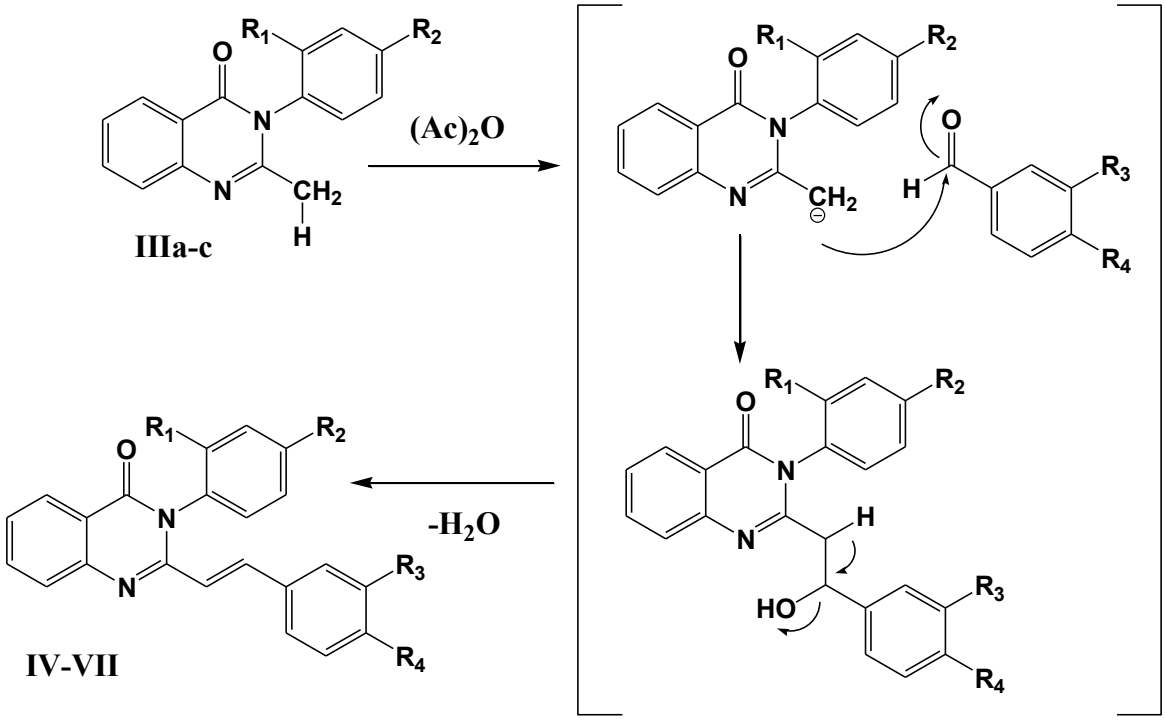


Scheme 13: Proposed mechanism of synthesis of **IIIa-c**

4.1.1.3. Proposed Mechanism of Synthesis of 3-aryl-2-(substitutedstyryl)-quinazolin-4-ones.

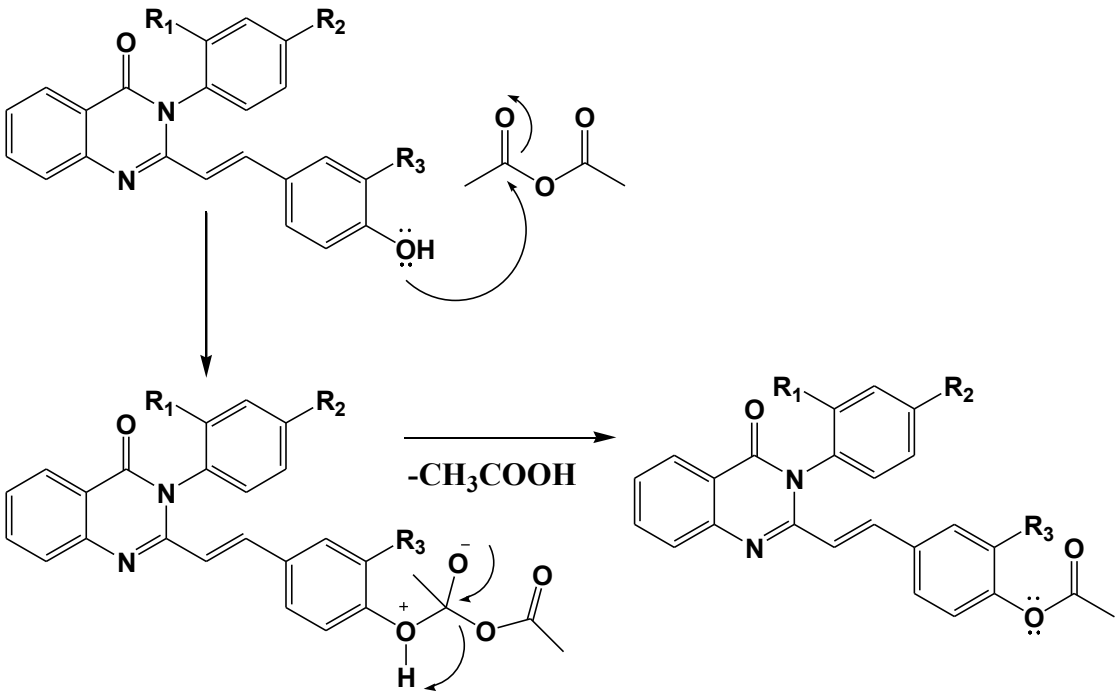
As the protons of the 2-methyl group of **IIIa-c** are acidic (stabilized by resonance with the neighboring C=N group), they can be abstracted by reactive solvents like acetic anhydride and a

strong nucleophile is formed. Thus, this strong nucleophile attacks the carbonyl group of the corresponding aldehydes, resulting in the formation of the target compounds with libration of water molecules. This reaction is catalyzed by anhydrous zinc chloride, (scheme 14). This is a common reaction that is responsible for the formation substituted styryl groups in all the synthesized compounds (**IV, V, VI** and **VII** series).



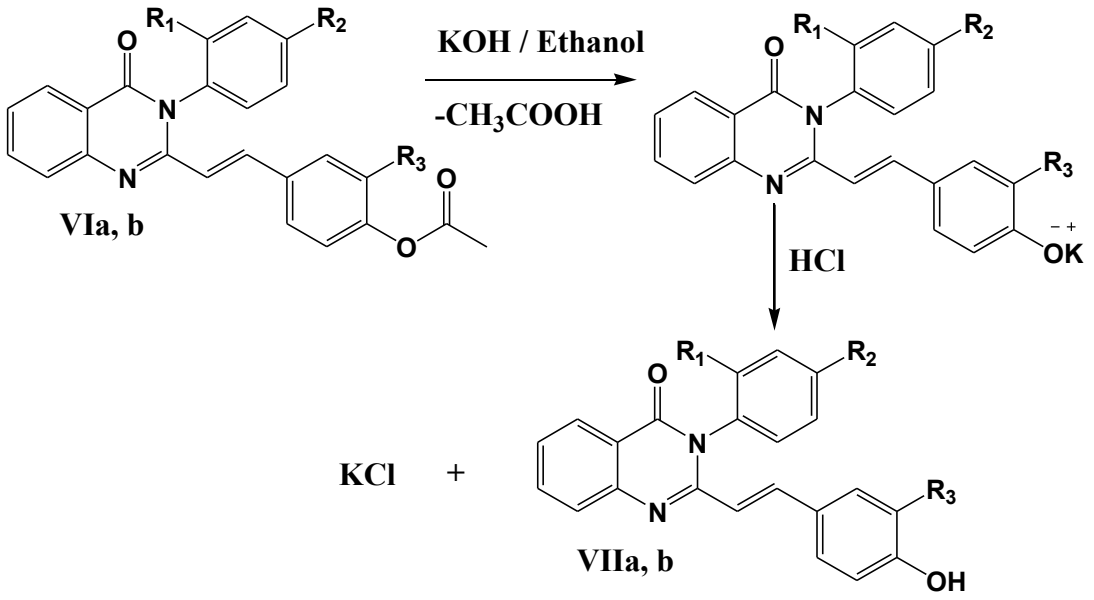
Scheme 14: Proposed mechanism of synthesis of target compounds

In reactions of **IIIb,c** with *p*-hydroxylbenzaldehyde and vanillin, the condensation reaction mentioned above was accompanied by acetylation of the hydroxyl group by the same mechanism as mentioned in 4.1.1.1. The solvent used (acetic anhydride), can participate in the reaction as it possess two nucleophilic carbonyl groups. Compound **VIa** and **VIb** are the outcomes of this phenomenon (scheme 15). Both the characteristic styryl group and acetylated styryl moiety appeared in these compounds.



Scheme 15: Proposed mechanism of synthesis of **VIa,b**.

The acetylated part of compounds **VIa, b** was hydrolyzed by the treatment of 0.1 M KOH, ethanol and acidification with 0.1 M HCl. It involved formation of salts of the target compounds giving a characteristic pink color. Up on the addition of HCl, hydroxyl derivative products (**VIIa,b**) and KCl were formed (scheme 16).



Scheme 16: Mechanism of hydrolysis of target compounds (**VIa,b**).

4.1.2. Physical Constants of Target Compounds

Physical constants like melting point, molecular weight, R_f values and percent yield of the target compounds were determined and data are shown in the Table 1.

Table 1: Physical constants and percent yield of the synthesized compounds.

Test compounds	*Molecular formula*	*Molecular weight (gram/mol)*	*% yield*	*Melting point (^oC)*	*R_f values*
IVa	$C_{22}H_{17}ClN_2O$	360.85	68.3	201-203	0.520
IVb	$C_{23}H_{19}ClN_2O$	374.87	65.2	189-191	0.577
Va	$C_{23}H_{18}N_3O_3$	384.41	74.8	214-216	0.422
Vb	$C_{23}H_{18}N_3O_3$	384.41	76.2	235-237	0.642
VIb	$C_{26}H_{24}N_2O_4$	428.49	86.4	151-153	0.781
VIIa	$C_{22}H_{18}N_2O_2$	342.40	80.3	298-300	0.524
VIIb	$C_{24}H_{22}N_2O_3$	386.45	82.2	196-198	0.711

The target compounds are synthesized in a good yield, which ranges from 65.2-86.4% and all the seven compounds have a percent yield > 65%. All the synthesized compounds were readily soluble in DMSO and chloroform except compound **VIIa** which is readily soluble in acetone. The chemical structure of these compounds was further verified based on the data obtained from elemental microanalysis, IR, ^{1}H NMR and ^{13}C NMR in the case of compound **IVb**.

4.1.3. Elemental Microanalysis of the Synthesized Compounds

Elemental microanalyses were performed on Perkin Elmer 2400 elemental analyzer and were found within the range of $\pm$ 0.4% of the theoretical values, Table 2.

Table 2: Results of elemental microanalysis for the synthesized compounds.

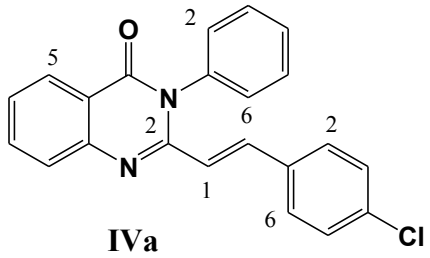
Test compounds	*Estimated Value*				*Found Value*			
	C	***H***	***N***	***Cl***	***C***	***H***	***N***	***Cl***
IVa	73.23	4.75	7.76	9.83	***73.64***	***4.92***	***7.54***	***10.22***
IVb	73.69	5.12	7.47	9.46	***73.98***	***5.38***	***7.21***	***9.35***
Va	71.86	4.72	10.93	-	***72.12***	***4.35***	***11.10***	**-**
Vb	71.86	4.72	10.93	-	***71.68***	***4.93***	***11.24***	**-**
VIb	72.88	5.65	6.54	-	***73.11***	***5.89***	***6.42***	**-**
VIIa	77.17	5.23	8.18	-	***76.86***	***5.02***	***8.38***	**-**
VIIb	74.59	5.74	7.23	-	***74.28***	***5.96***	***7.56***	**-**

4.1.4. Spectroscopic Analysis of the Synthesized Compounds

Infrared (IR) spectra of all the synthesized compounds were recorded on a SHIMADZU 8400SP FT-IR spectrophotometer using nujol mull technique. ^{1}H NMR spectrum was recorded on a

Bruker Avance DMX400-FT-NMR spectrometer and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (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 of the synthesized compounds are given below.

4.1.4.1. (*E*)-2-(4-chlorostyryl)-3-phenylquinazolin-4(3*H*)-one (IVa)



IVa

IR (Nujol) (cm^{-1}): 1682 (C=O), 1597 (C=N) and 1224 (C-Cl). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.33 (*d*, 1H, J = 15.49Hz, vinyl-C_2 H), 7.23 (*d*, 2H, J = 8.53Hz, 4-chlorophenyl $C_{3,5}$ H), 7.28 (*d*, 2H, J = 8.47Hz, 4-chlorophenyl $C_{2,6}$ H), 7.34 (*d*, 2H, J = 6.82Hz, phenyl $C_{2,6}$ H), 7.45-7.49 (*m*, 1H, quina-C_6 H), 7.58-7.63 (*m*, 3H, phenyl $C_{3,4,5}$ H), 7.75-7.79 (*m*, 2H, quina-$C_{7,8}$ H), 7.91 (*d*, 1H, J = 15.47Hz, vinyl-C_1 H), 8.29 (*d*, 1H, J = 7.95Hz, quina-C_5). Anal. calcd. for $C_{22}H_{17}ClN_2O$: C, 73.23; H, 4.75; Cl, 9.83; N, 7.76. Found: **C, 73.64; H, 4.92; Cl, 10.22; N, 7.54.**

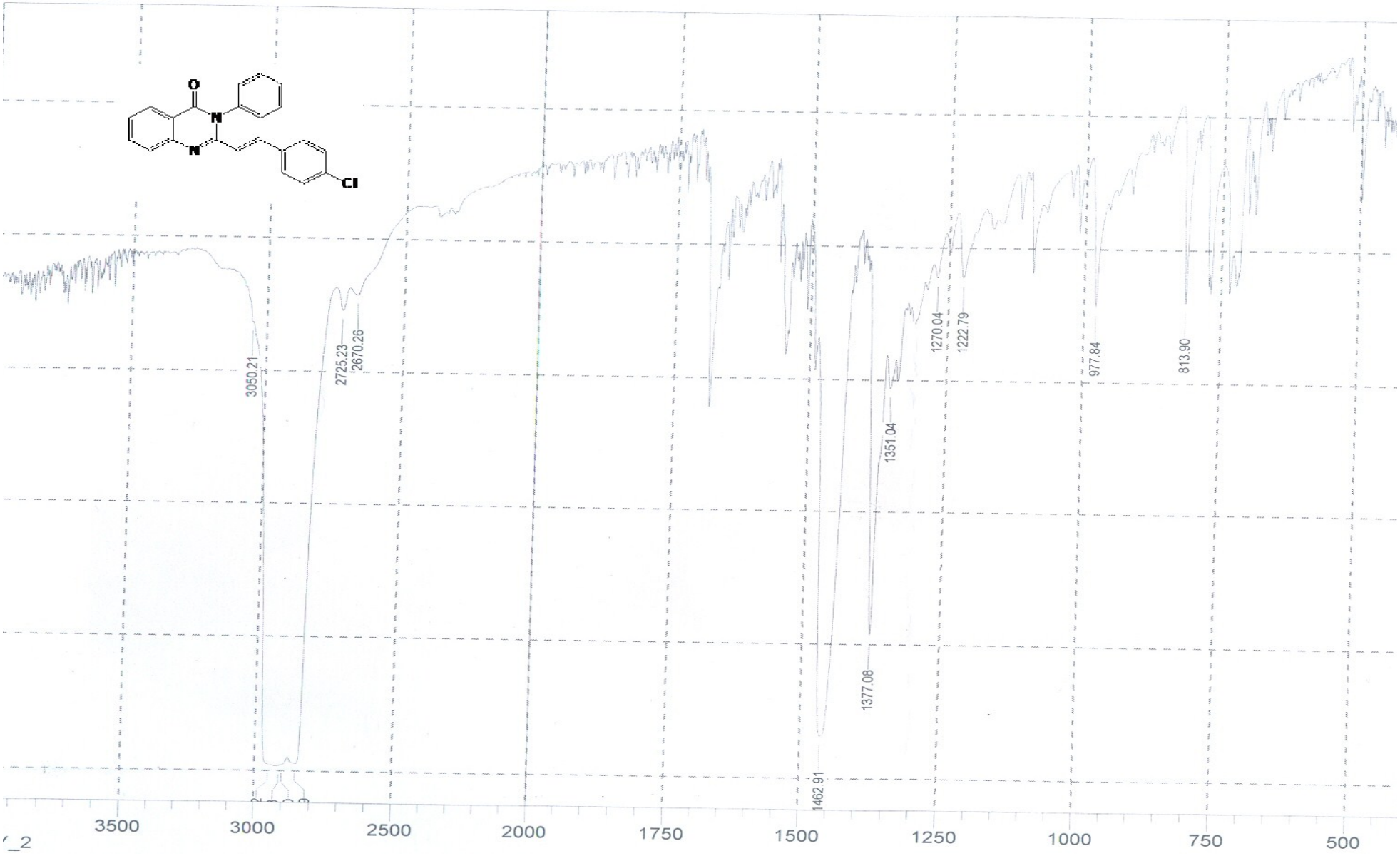


Figure 16: IR spectrum of compound **IVa** in Nujol.

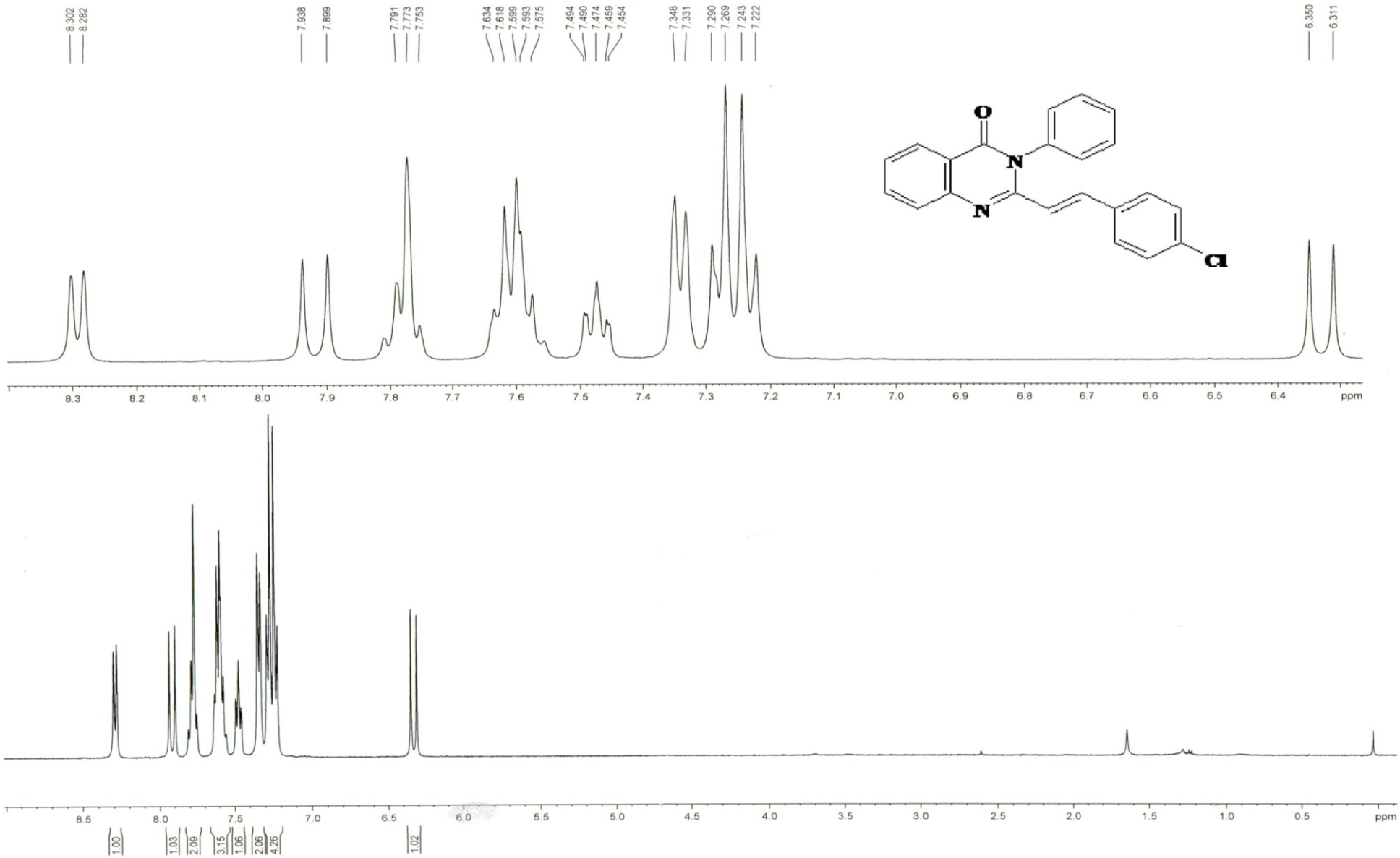


Figure 17: ^{1}H NMR spectrum of compound **IVa** in $CDCl_3$

The IR spectrum of compound **IVa** (Fig. 16), showed a strong characteristic band at 1682 cm^{-1} due to the carbonyl group of the quinazolinone moiety. Band for C=N stretch was observed at 1597 cm^{-1}. The band at 1224 cm^{-1} was attributed to the presence of C-Cl stretching.

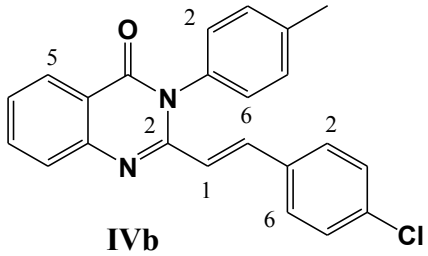
The ^{1}H NMR spectrum of compound **IVa** (Fig. 17), showed two doublets at 6.33 and 7.91 ppm with the same coupling constant, J = 15.47 Hz, attributed to vinylic $C_{2,1}$ protons respectively. These are the characteristic peaks of the target compounds formed, due to the condensation reaction between 3-arylquinazolinones (**IIIa-c**) and aromatic aldehydes [133]. It can be inferred from their coupling constants that an (*E*) configuration exists [134]. Two doublets at 7.23-7.28 ppm and another doublet peak at 7.34 ppm are due to the four protons of 4-chlorophenyl of the styryl moiety and 3-phenyl group (they integrate for six protons). There was also a peak which was integrated for three protons at 7.58-7.63 ppm, due to the three multiplet protons of 3-phenyl group. These are the characterstic peaks for the 3-phenyl substituent of quinazolinones.

In addition, the characteistic peaks of the 4(*3H*)-quinazolinone protons were obseved: multiplet peak (due to coupling with neighbouring protons) at 7.45-7.49 ppm, C_6 H; multiplet (one multiplet and one doublet) at 7.75-7.79 ppm, $C_{7,8}$ H; *dd* (due to *meta*-coupling) at 8.29 ppm, C_5 H and this was the most deshielded proton in the synthesized compounds. This might be due to the participation of the proton in the formation of a hydrogen bond with the carbonyl oxygen. Peaks of these protons with their splitting patterns are maintained in all the synthesized compounds.

The multiplicity/ splitting patterns of these peaks was the same in all the synthesized compounds regardless of the type of substituents at 2 and 3-position of the quinazolinone scaffold. These peaks may have different chemical shifts depending on the electronic effects (inductive, resonance or both) of substituents at 2 and 3-positions of the quinazolinoe moeity.

Taking this in to account, discussions regarding these peaks in the coming sections was excluded and due attention was given to the characteristic peaks of the substituents at 2 and 3- position of the quinazolinone scaffold.

4.1.4.2. (*E*)-2-(4-chlorostyryl)-3-*p*-tolylquinazolin-4(3*H*)-one (IVb)



IVb

IR (Nujol) (cm^{-1}): 1682 (C=O), 1597 (C=N) and 1224 (C-Cl). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.5 (*s*, 3H, *p*-tolyl CH_3), 6.42 (*d*, 1H, J = 15.70Hz, vinyl-C_2 H), 7.21 (*d*, 2H, 4-chlorophenyl $C_{3,5}$ H), 7.26-7.32 (*m*, 4H, *p*-tolyl $C_{2,3,5,6}$ H), 7.40 (*d*, 2H, 4-chlorophenyl $C_{2,6}$ H), 7.47-7.51 (*m*, 1H, quina-C_6 H), 7.79-7.83 (*m*, 2H, quina-$C_{7,8}$ H), 7.93 (*d*, 1H, vinyl-C_1 H), 8.32 (*d*, 1H, quina-C_5 H). ^{13}C NMR (DMSO-d_6) δ (ppm): 21.33 (1C, *p*-tolyl CH_3), 121.05 (1C, vinyl C_2), 121.29 (2C, *p*-tolyl $C_{2,6}$), 126.92 (1C, quina-C_{4a}), 127.18 (2C, 4-chlorophenyl $C_{3,5}$), 127.65 (2C, 4-chlorophenyl $C_{2,6}$), 129.07 (1C, quina-C_6), 129.55 (1C, quina-C_8), 129.66 (2C, *p*-tolyl $C_{3,5}$), 130.65 (1C, quina-C_5), 134.22 (1C, *p*-tolyl C_1), 134.67 (1C, *p*-tolyl C_4), 135.26 (1C, quina-C_7), 137.88 (1C, 4-chlorophenyl C_1), 139.18 (1C, vinyl C_1), 147.76 (1C, quina-C_{8a}), 151.86 (1C, quina-C_2), 161.81 (1C, quina-C_4, C=O). Anal. calcd. for $C_{23}H_{19}ClN_2O$: C, 73.69; H, 5.12; Cl, 9.46; N, 7.47. Found: **C, 73.98; H, 5.38; Cl, 9.35; N, 7.21.**

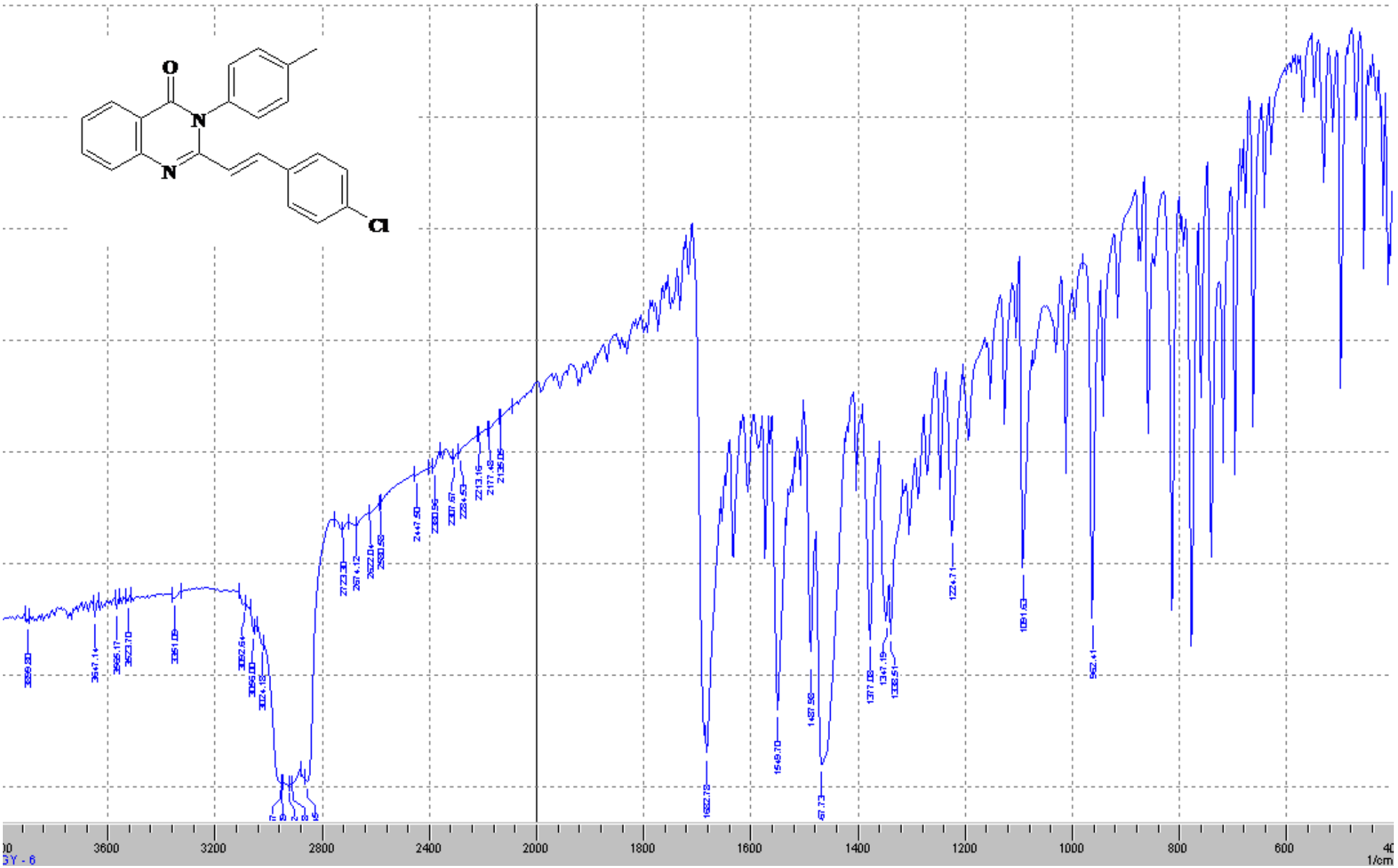


Figure 18: IR spectrum of compound **IVb** in Nujol

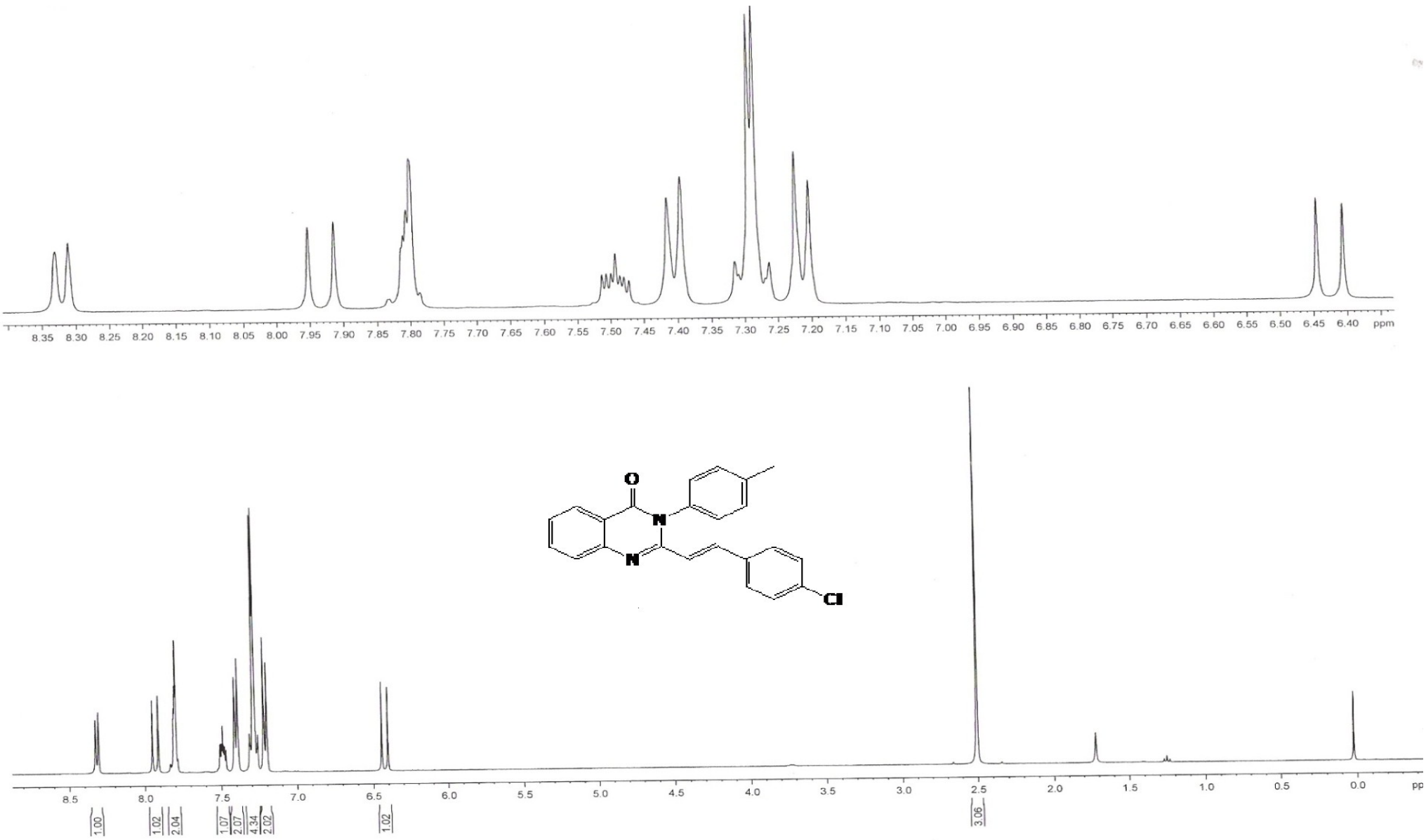


Figure 19: ^{1}H NMR spectrum of compound **IVb** in $CDCl_3$.

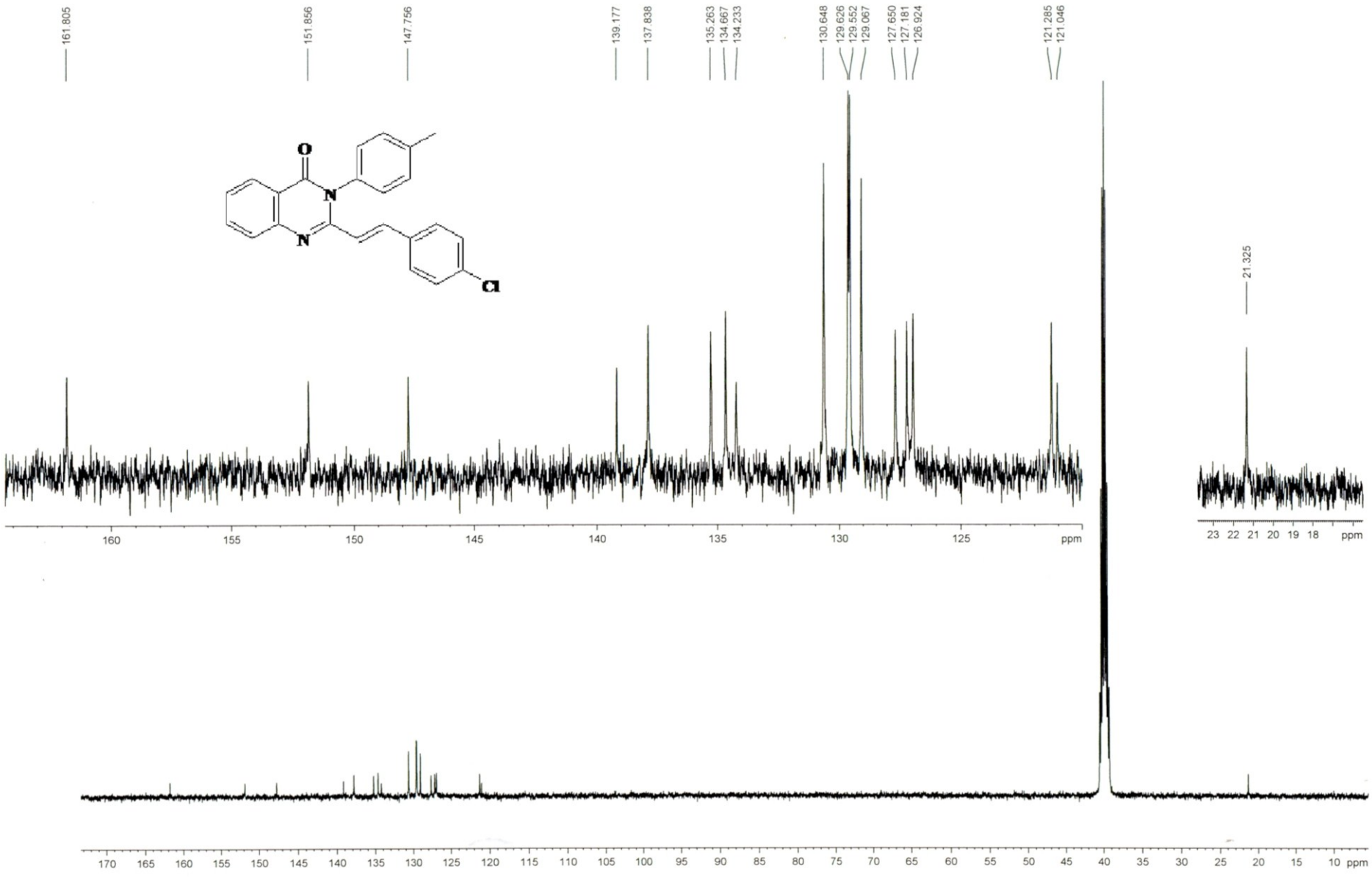


Figure 20: ^{13}C NMR spectrum of compound **IVb** in DMSO-d_6.

IR spectrum of compound **IVb** (Fig. 18) showed strong band at 1682 cm^{-1} for the carbonyl group of the quinazolinone, and the band at 1597 cm^{-1} was due to C=N stretch. The C-Cl stretch was observed around 1224 cm^{-1}.

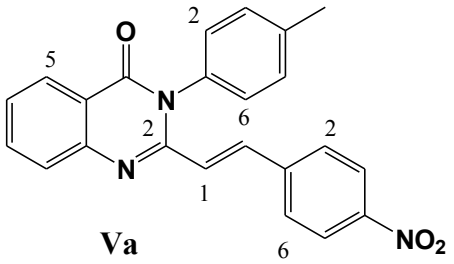
^{1}H NMR spectrum of compound **IVb** (Fig. 19) showed two doublets at 6.42 and 7.93 ppm attributed to vinylic protons. They had the same coupling constant, J = 15.53Hz confirming the existance in (*E*) configuration [134]. In addition two doublets at 7.21 and 7.40 ppm are assigned for the four protons of 4-chlorophenyl moiety. A multiplet peak at 7.26-7.32 ppm attributed to *p*-tolyl protons. The remaining peaks dipicted in Fig. 16, are the characteristic peaks of the quinazolinone scaffold.

^{13}C NMR of **IVb** (Fig. 20) exhibited two peaks at 121.05 ppm and 139.18 ppm attributed to the two vinyl $C_{2,1}$ respectively. The peaks at 121.29 ppm and 129.66 ppm were attributed to the four carbons of *p*-tolyl $C_{2,6,3,5}$ respectively. These carbons appaeared as two peaks because *p*-tolyl $C_{2,6}$ and *p*-tolyl $C_{3,5}$ are equivalent carbon atoms. On the other hand $C_{3,5}$ and $C_{2,6}$ of 4-chlorophenyl group appeared at 127.18 ppm and 127.65 ppm respectively. As this group possessed two group of equivalent carbons, only two peaks appeared. More interestingly peaks for *p*-tolyl CH_3 group, *p*-tolyl $C_{1,4}$ and 4-chlorophenyl C_1 obseved at 21.33 ppm, 134.22 ppm, 134.67 ppm, 137.88 ppm respectively. All the forementioned carbon peaks are characteristic for this compound (**IVb**) and these peaks confirm the formation of the target compound.

In addition to this, the characteristic peaks of the quinazolinone scaffold were obseved at 126.92 ppm, 129.07 ppm, 129.55 ppm, 130.47 ppm, 135.26 ppm, 147.76 ppm, 151.86 ppm, and 161.81 ppm for quinazolinone $C_{4a,6,8,5,7,8a,2,4}$ respectively. These peaks were the same for all the target compounds though they had different pattern of substitution at 2- and 3- position of the quinazolinone scaffold.

As the synthesized compounds are closely related in structure, their ^{13}C NMR spectrum resembled each other. Because ^{13}C NMR consider the type of carbon atoms present in the target compounds. **IVa/VIIa**, **IVb/Va** will have a relatively similar ^{13}C NMR data as they possess the same type of substituents at 2- and 3-position of the quinazolione scaffold regardless of the Cl or NO_2 substituents at 4-position of the styryl moiety. Similarly compounds **Vb**, **VIb** and **VIIb** are expected to have similar carbon peaks as far as 3-*o*-tolyl substituent is concerned. Based on this assumption the ^{13}C NMR of the remaining compounds was not included in this discussion.

4.1.4.3. (*E*)-2-(4-nitrostyryl)-3-*p*-tolylquinazolin-4(3*H*)-one (Va)



Va

IR (Nujol) (cm^{-1}): 1684 (C=O), 1593 (C=N), 1556 and 1377 (NO_2). ^1H NMR ($CDCl_3$) δ (ppm): 2.5 (*s*, 3H, *p*-tolyl CH_3), 6.56 (*d*, 1H, J = 15.52Hz, vinyl-C_2 H), 7.21(*d*, 2H, J = 8.19Hz, *p*-tolyl $C_{3,5}$ H), 7.41 (*d*, 2H, J = 7.97Hz, *p*-tolyl $C_{2,6}$ H), 7.46-7.53 (*m*, 3H, 4-nitrophenyl $C_{2,6}$ and quina-C_6), 7.77-7.81 (*m*, 2H, quina-$C_{7,8}$), 8.00 (*d*, 1H, J = 15.52Hz, vinyl-C_1), 8.19 (*d*, 2H, J = 8.74Hz, 4-nitrophenyl $C_{3,5}$), 8.30 (*d*, 1H, J = 8.01Hz, quia-C_5).

Anal. calcd. for $C_{23}H_{18}N_3O_3$: C, 71.86; H, 4.72; N, 10.93. Found: **C, 72.12; H, 4.35; N, 11.10**

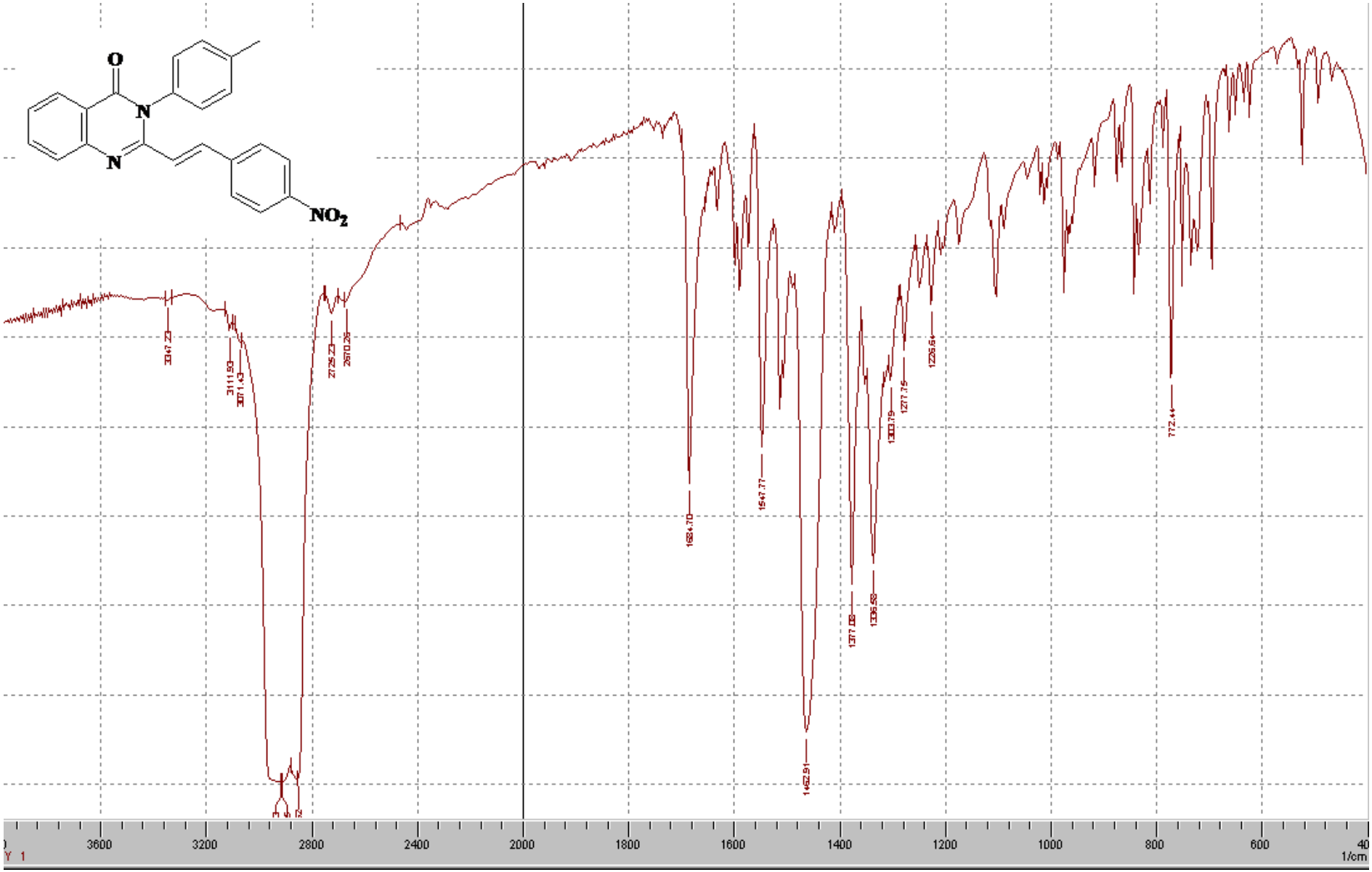


Figure 21: IR spectrum of compound **Va** in Nujol.

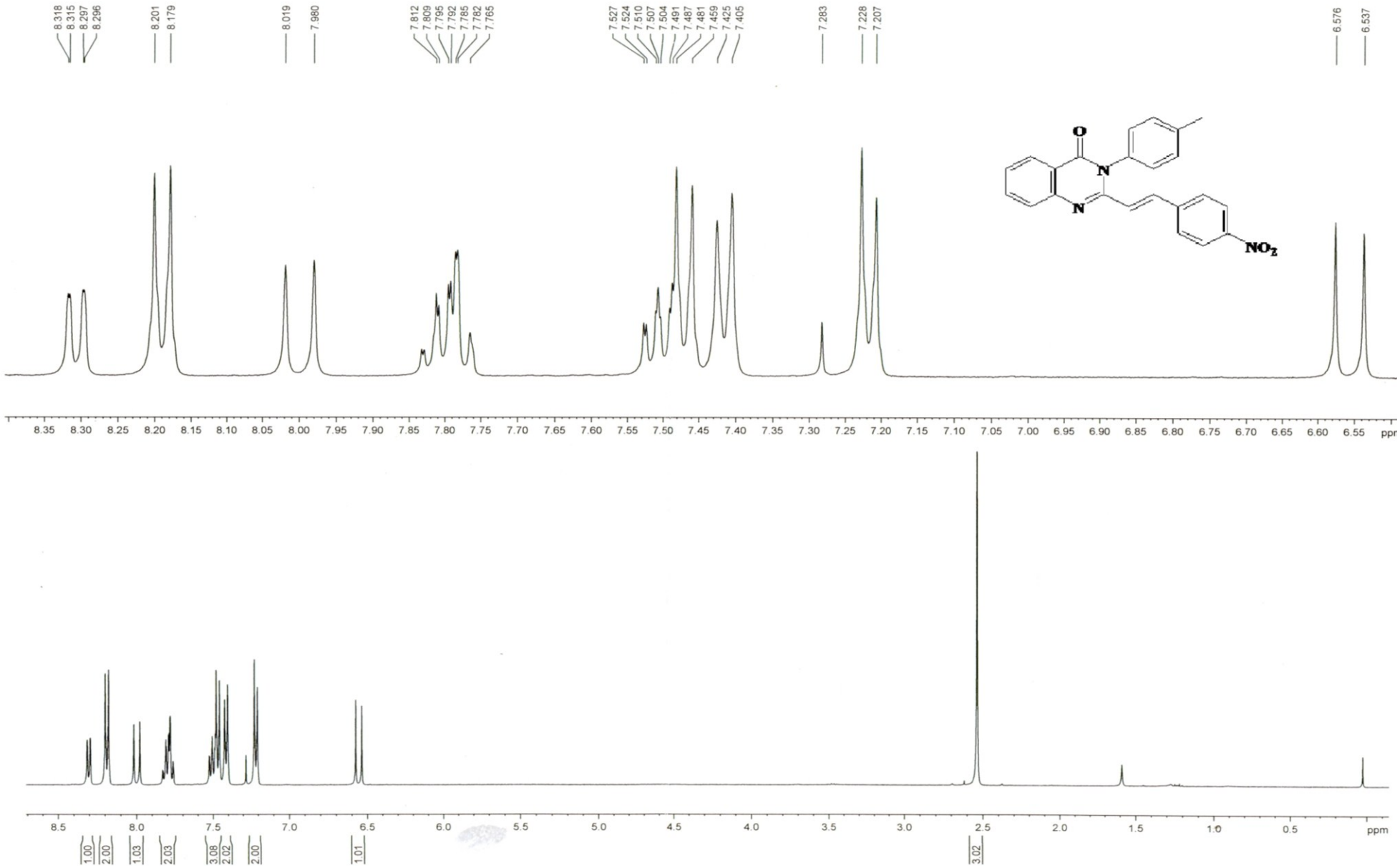
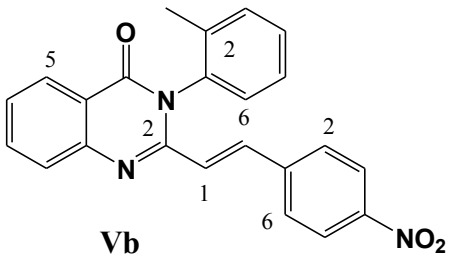


Figure 22: ^{1}H NMR spectrum of compound **Va** in $CDCl_3$.

The IR spectrum of compound **Va** (Fig. 21), showed a strong characteristic band at 1684 cm^{-1} due to the carbonyl group of the quinazolinone moiety. Band for C=N stretch was observed at 1593 cm^{-1}. The bands at 1377 cm^{-1} and 1556 cm^{-1} were attributed to the asymmetric and symmetric stretches of NO_2 respectively.

^1H NMR spectrum of compound **Va** (Fig. 22), showed a singlet peak at 2.55 ppm for *p*-tolyl CH_3 protons; two doublets at 6.55 and 7.80 ppm attributed to vinylic protons. They have the same coupling constant, J = 15.52 Hz confirming their existence in (*E*) configuration [134]; four doublets at 7.21, 7.41, 7.46-7.53 and 8.19 ppm, attributed to the protons of *p*-tolyl $C_{3,5,2,6}$ and 4-nitrophenyl ($C_{2,6,3,5}$) respectively. The protons nearest to the 4-nitro group appeared at 8.19 ppm due to their strong electron withdrawing effect.

4.1.4.4. (*E*)-2-(4-nitrostyryl)-3-*o*-tolylquinazolin-4(3*H*)-one (Vb)



Vb

IR (Nujol) (cm^{-1}): 1682 (C=O), 1593 (C=N), 1556 and 1377 (NO_2). ^1H NMR ($CDCl_3$ δ (ppm): 2.17 (*s*, 3H, *o*-tolyl CH_3), 6.47 (*d*, 1H, J = 15.66 Hz, vinyl-C_2 H), 7.25 (*d*, 1H, J= 7.91Hz, *o*-tolyl C_3 H), 7.44-7.46 (*m*, 3H, 4-nitrophenyl $C_{2,6}$ and *o*-tolyl C_6 H), 7.47-7.58 (*m*, 3H, *o*-tolyl $C_{4,5}$ and quina-C_6 H), 7.82-7.89 (*m*, 2H, quina-$C_{7,8}$ H), 8.05 (*d*, 1H, J = 15.56Hz, vinyl-C_1 H), 8.19 (*d*, 2H, J = 8.73Hz, 4-nitrophenyl $C_{4,6}$ H), 8.36 (*d*, 1H, J = 8.25Hz, quina-C_5 H). Anal. calcd. for $C_{23}H_{18}N_3O_3$: C, 71.86; H, 4.72; N, 10.93. Found: **C, 71.68; H, 4.93; N, 11.24**

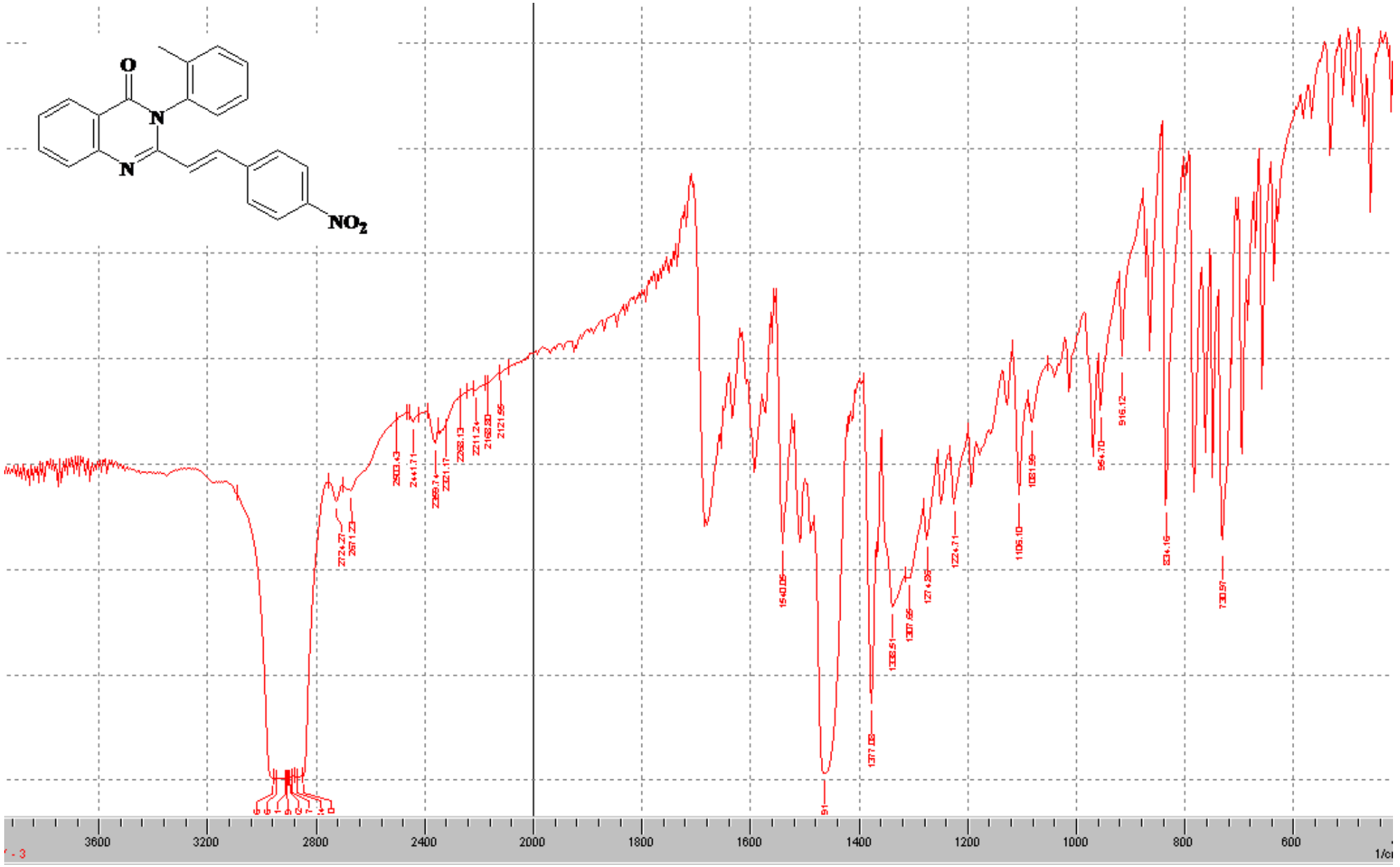


Figure 23: IR spectrum of compound **Vb** in Nujol.

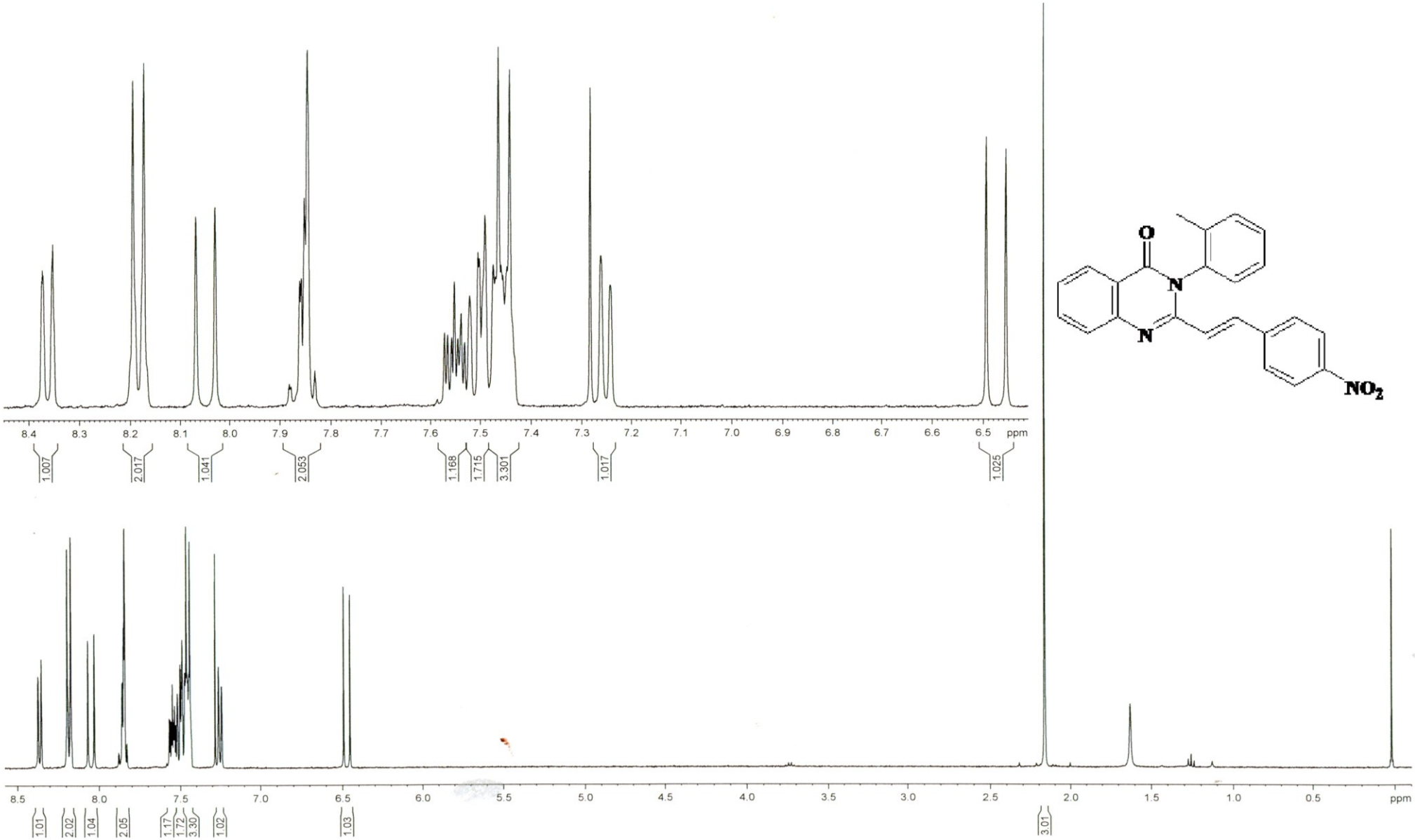
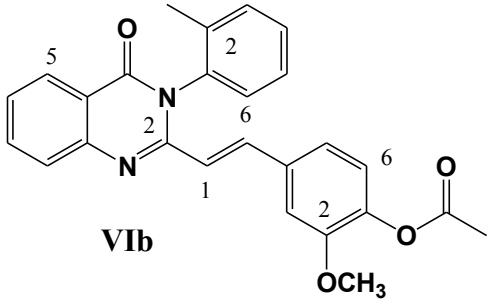


Figure 24: ^{1}H NMR spectrum of compound **Vb** in $CDCl_3$.

IR spectrum of compound **Vb** (Fig. 23), showed a strong characteristic band at 1682 cm^{-1} due to the carbonyl group of the quinazolinone moiety. Band for C=N stretch was observed at 1593 cm^{-1}. The bands at 1377 cm^{-1} and 1556 cm^{-1} attributed to the asymmetric and symmetric stretches of NO_2 respectively. ^1H NMR spectrum of compound **Vb** (Fig. 24), displayed a singlet peak at 2.17 ppm for *o*-tolyl CH_3 protons; two doublets at 6.47 and 8.05 ppm with the same coupling constant, J = 15.60 Hz, attributed to vinylic protons with (*E*) configuration [134]. Three doublets at 7.25, 7.44-7.46 and 8.19 ppm attributed to *o*-tolyl C_3 and 4-nitrophenyl $C_{2,6,3,5}$ protons respectively. The peaks for *o*-tolyl $C_{4,5,6}$ protons appeared between 7.42-7.55ppm overlapping with the quinazolinone C_6 proton. As expected, the protons near to the NO_2 group appeared at 8.19ppm because of their strong electron withdrawing effects.

4.1.4.5. 4-{(1E)-2-[-3,4-dihydro-3-(2-methylphenyl)-4-oxoquinazoline-2-yl)] vinyl}-2-methoxyphenyl acetate (VIb).



VIb

IR (Nujol) (cm^{-1}): 1761 (C=O), 1682 (C=O), 1634 (C=N), 1260 and 1149 (C-O-C). ^1H NMR ($CDCl_3$) δ (ppm): 2.15 (*s*, 3H, phenylacetate CH_3), 2.33 (*s*, 3H, *o*-tolyl CH_3), 3.80 (*s*, 3H, methoxy -O-CH_3), 6.27 (*d*, 1H, J = 15.44Hz, vinyl-C_2 H), 6.88-6.93 (*m*, 2H, 2-methoxyphenyl $C_{3,5}$ H), 6.98 (*d*, 1H, J = 8.12Hz, 2-methoxyphenyl C_6 H), 7.24 (*d*, 1H, J = 7.52Hz, *o*-tolyl C_3 H), 7.42-7.53 (*m*, 4H, *o*-tolyl $C_{4,5,6}$ H and quina-C_6 H), 7.82-7.83 (*m*, 2H, quina-$C_{7,8}$ H), 7.96 (*d*, 1H, J = 15.48Hz, vinyl-C_1 H), 8.34 (*d*, 1H, J = 7.88Hz, quina-C_5 H).

Anal. calcd. for $C_{26}H_{24}N_2O_4$: C, 72.88; H, 5.65; N, 6.54. Found: **C, 73.11; H, 5.89; N, 6.42**

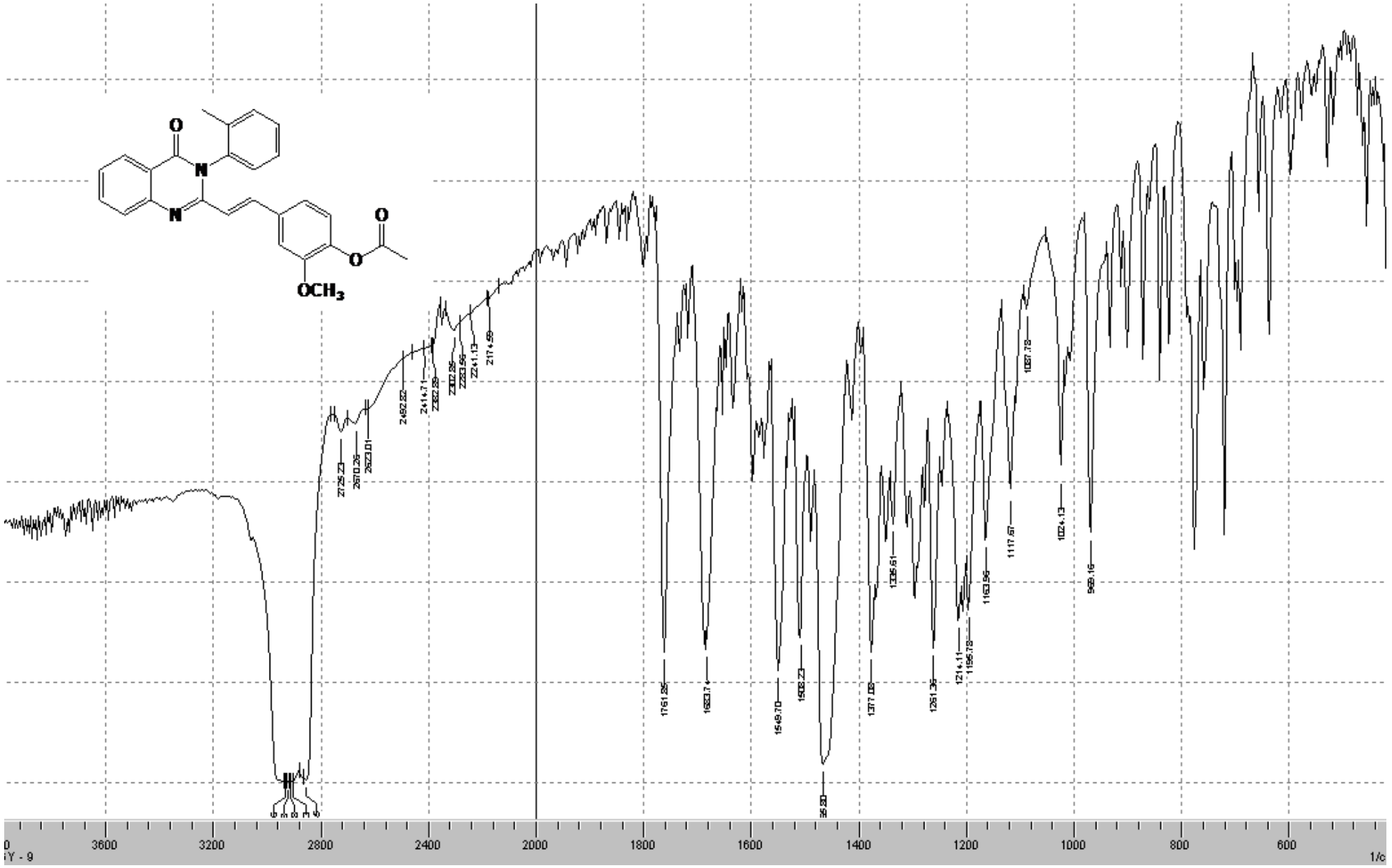


Figure 25: IR spectrum of compound **VIb** in Nujol.

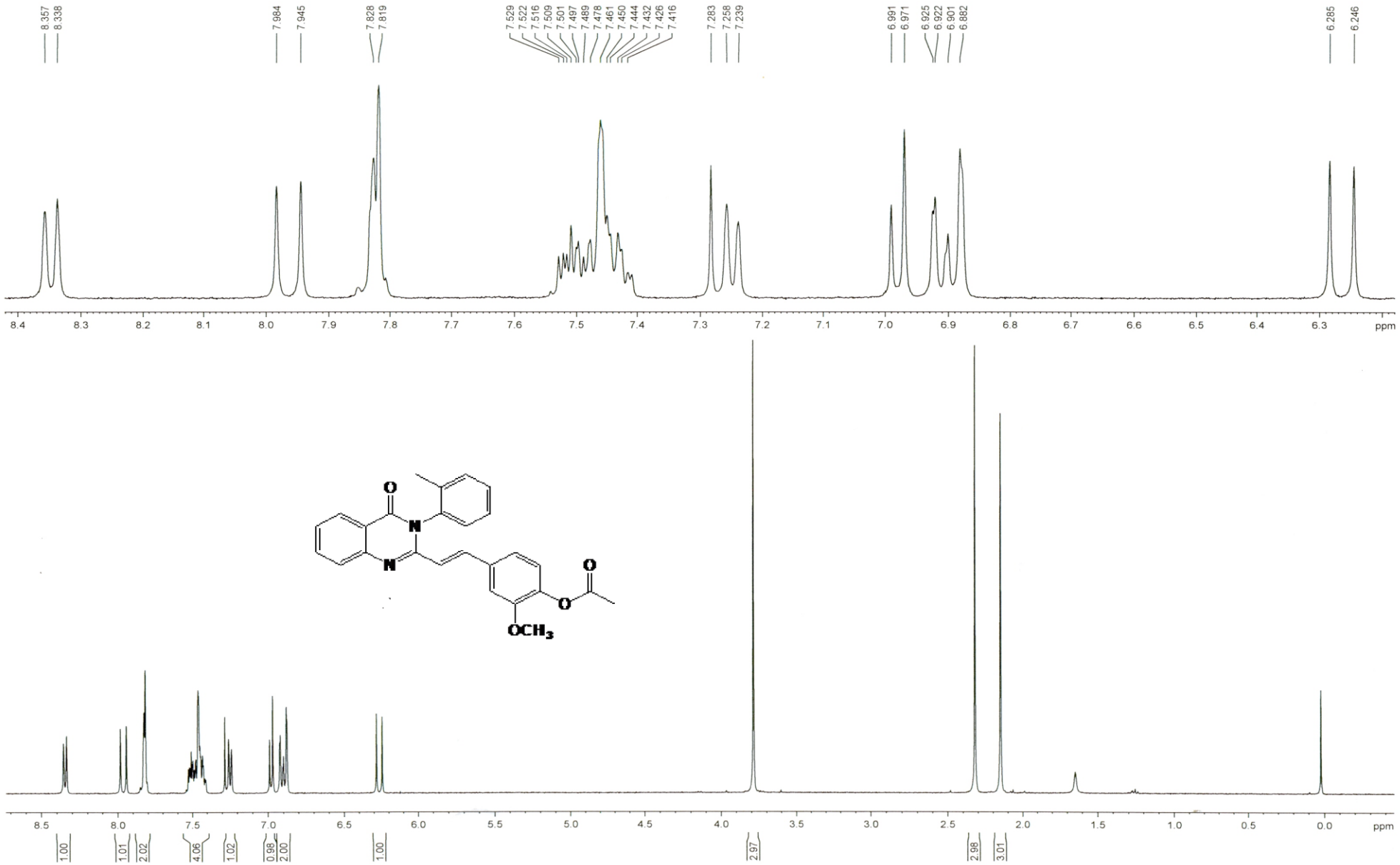
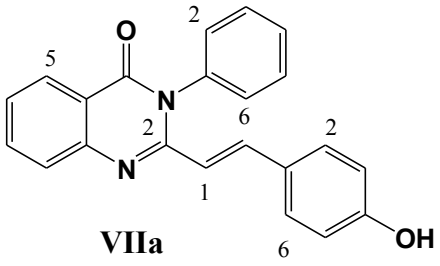


Figure 26: ^{1}H NMR spectrum of compound **VIb** in $CDCl_3$.

IR spectrum of compound **VIb** (Fig. 25), showed strong characteristic bands at 1761 and 1683 cm^{-1} due to the carbonyl group of the 3-methoxyphenylacetate and quinazolinone moiety respectively. The amidic carbonyl group appeared at lower frequency than the ester one. Band for C=N stretch was observed at 1634 cm^{-1}. The bands at 1117 and 1214 cm^{-1} were attributed to the asymmetric and symmetric stretches of C-O-C respectively. ^{1}H NMR spectrum of compound **VIb** (Fig. 26), showed singlet peaks at 2.15, 2.33, and 3.80 ppm for phenyl acetate CH_3, *o*-tolyl CH_3, and methoxy -O-CH_3 protons respectively; two doublets at 6.27 and 7.96 ppm with the same coupling constant, J = 15.455Hz attributed to vinylic protons with (*E*) configuration [134]. A multiplet peak at 6.88-6.93 ppm and a doublet peak 6.98 ppm were attributed to 2-methoxyphenyl $C_{3,5,6}$ protons. The proton of *o*-tolyl C_3 appeared as a doublet peak at 7.24 ppm and a multiplet peak for the remaining *o*-tolyl $C_{4,5,6}$ protons appeared at 7.42-7.53 ppm.

4.1.4.6. (*E*)-2-(4-hydroxystyryl)-3-phenylquinazolin-4(3*H*)-one (VIIa)



VIIa

IR (Nujol) (cm^{-1}): 3290 (OH), 1652 (C=O) and 1604 (C=N). ^{1}H NMR (Acetone-d_6) δ (ppm): 6.24 (*d*, 1H, J = 15.39Hz, vinyl-C_2 H), 6.80 (*d*, 2H, J = 8.64Hz, 4-hydroxyphenyl $C_{3,5}$ H), 7.24 (*d*, 2H, J = 8.62Hz, 4-hydroxyphenyl $C_{2,6}$ H), 7.46-7.50 (*m*, 3H, phenyl $C_{3,4,5}$ H), 7.60-7.66 (*m*, 3H, quina-C_6, phenyl $C_{2,6}$ H), 7.73 (*d*,1H, J = 8.07Hz, quina-C_8 H), 7.81-7.85 (*m*, 1H, quina-C_7 H), 7.92 (*d*, 1H, J= 15.43Hz, vinyl-C_1 H), 8.02 (*s*, 1H, 4-hydroxyphenyl -OH), 8.17 (*d*, 1H, J = 9.18Hz, quina-C_5 H). Anal. calcd. for $C_{22}H_{18}N_2O_2$: C, 7.17; H, 5.23; N, 8.18. Found: **C, 76.86; H, 5.02; N, 8.38**

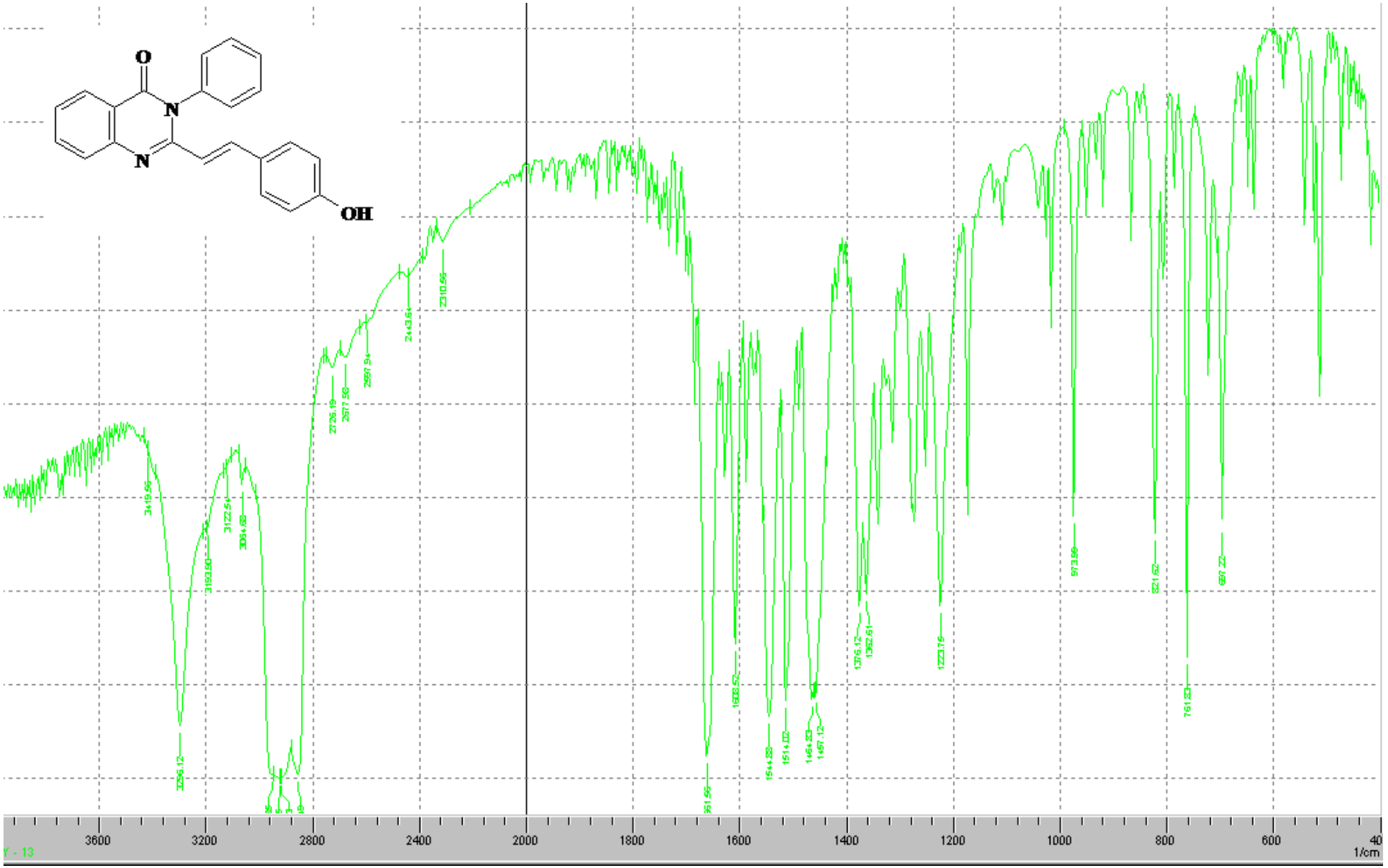


Figure 27: IR spectrum of compound **VIIa** in Nujol.

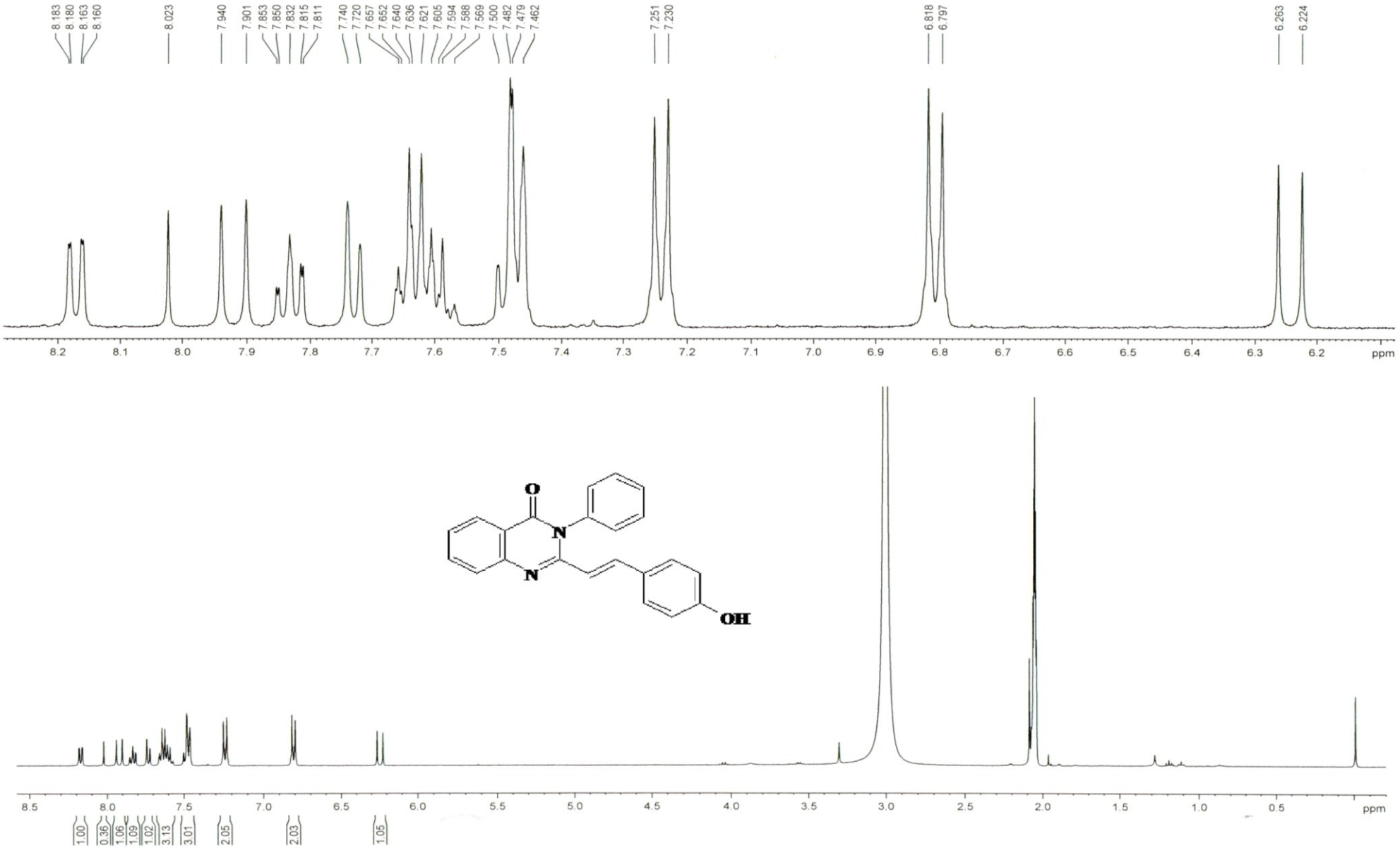
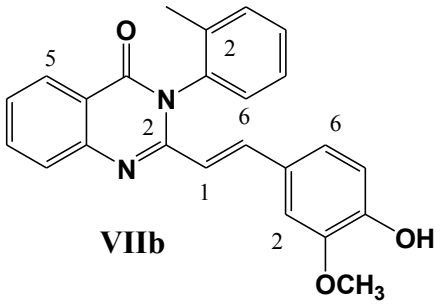


Figure 28: ^{1}H NMR spectrum of compound **VIIa** in Acetone-d6.

IR spectrum of compound **VIIa** (Fig. 27), showed a strong characteristic band at 1652 cm^{-1} due to the carbonyl group of the quinazolinone and it appeared at lower frequency as it is amidic. Band for C=N stretch was observed at 1604 cm^{-1}. The broad band around 3290 cm^{-1} belonged to the phenolic OH. ^1H NMR spectrum of compound **VIIa** (Fig. 28) showed two doublets at 6.24 and 7.92 ppm with the same coupling constant, J = 15.36 Hz that were attributed to vinylic protons with (*E*) configuration [134]. Two doublets at 6.80 and 7.24 ppm were observed belong to 4-hydroxphenyl $C_{3,5,2,6}$ protons respectively. In addition, the singlet peak at 8.02 ppm was attributed to the phenolic proton. The remaining characteristic peaks for the 3-phenyl group were observed between 7.46-7.50 ppm (phenyl $C_{3,4,5}$) and 7.60-7.66 ppm (phenyl $C_{2,6}$ overlapped with quinazolinone C_6 proton).

4.1.4.7. (*E*)-2-(4-hydroxy-3-methoxystyryl)-3-*o*-tolylquinazolin-4(*3H*)-one (VIIb)



VIIb

IR (Nujol) (cm^{-1}): 3400 (OH), 1683 (C=O), 1634 (C=N), 1211and 1148 (C-O-C). ^1H NMR ($CDCl_3$ δ (ppm): 2.15 (*s*, 3H, *o*-tolyl CH_3), 3.80 (*s*, 3H, 4-hydroxy-2-methoxyphenyl -O-CH_3), 6.10 (*s*, 1H, 4-hydroxy-2-methoxyphenyl -OH), 6.27 (*d*, 1H, J = 15.44Hz, vinyl-C_2 H), 6.88-6.93 (*m*, 2H, 4-hydroxy-2-methoxyphenyl $C_{3,5}$ H), 6.98 (*d*, 1H, J = 8.12Hz, 4-hydroxy-2-methoxyphenyl C_6 H), 7.24 (*d*, 1H, J = 7.52Hz, *o*-tolyl C_3 H), 7.42-7.53 (*m*, 4H, *o*-tolyl $C_{4,5,6}$ H and quina-C_6 H), 7.82 (*m*, 2H, quina-$C_{7,8}$ H), 7.96 (*d*, 1H, J = 15.48Hz, vinyl-C_1 H), 8.34 (*d*, 1H, J = 7.884Hz, quina-C_5H).

Anal. calcd. for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.23. Found: **C, 74.28; H, 5.96; N, 7.56**

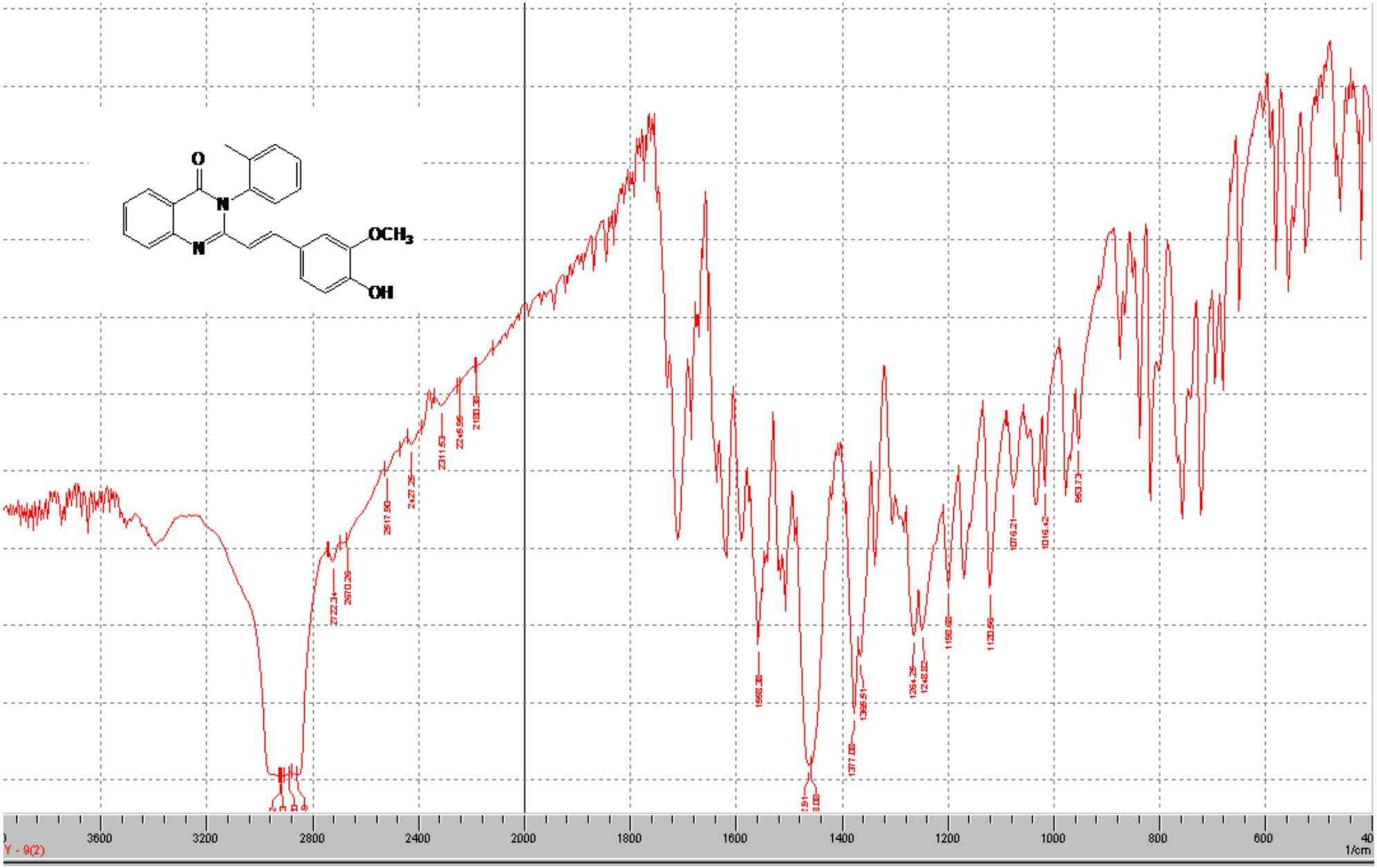


Figure 29: IR spectrum of compound **VIIb** in Nujol.

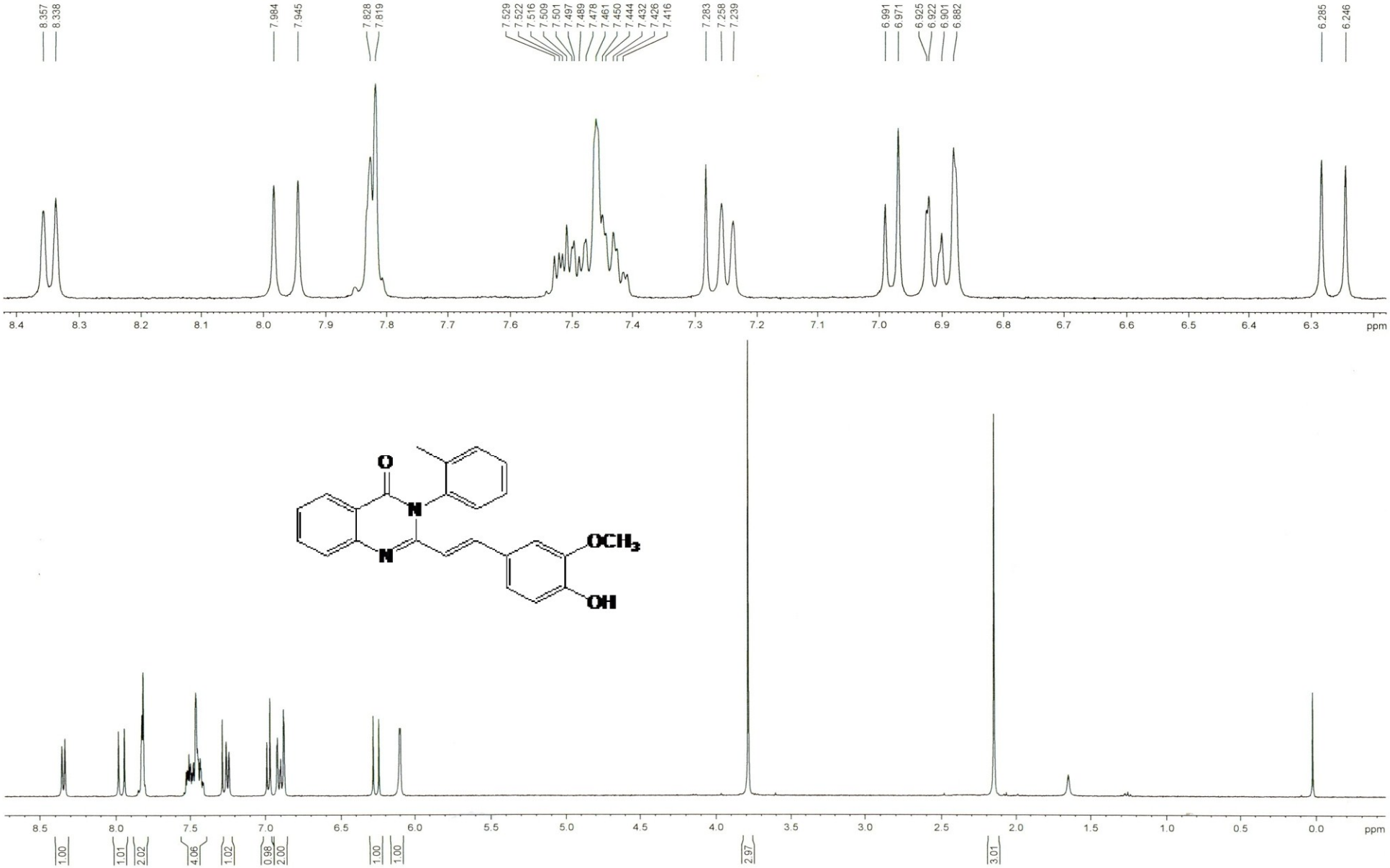


Figure 30: ^{1}H NMR spectrum of compound **VIIb** in $CDCl_3$.

The IR spectrum of compound **VIb** (Fig. 29), showed that a strong characteristic band at 1683 cm^{-1} due to the carbonyl group of the quinazolinone. Band for C=N stretch is observed at 1634 cm^{-1}. This compound lacked the ester C=O band. The bands at 1211 and 1117 cm^{-1} attributed to the symmetric and asymmetric stretches of C-O-C respectively. The band for the phenolic OH group is depicted at 3400 cm^{-1}.

^{1}H NMR spectrum of compound **VIb** (Fig. 30), showed singlet peaks at 2.15, 3.80 and 6.10 ppm for *o*-tolyl -CH_3, 4-hydroxy-2-methoxy -CH_3, and 4-hydroxy-2-methoxy -OH protons respectively. Two doublets at 6.27 and 7.96 ppm with the same coupling constant, J = 15.46 Hz were attributed to vinylic protons with (*E*) configuration [134]. A multiplet peak at 6.88-6.93 ppm and a doublet peak 6.98 ppm was attributed to 4-hydroxy-2-methoxyphenyl $C_{2,5,6}$, protons respectively. The proton of *o*-tolyl C_3 appeared as a doublet peak at 7.24 ppm and a multiplet peak for the remaining *o*-tolyl $C_{4,5,6}$ protons appeared at 7.42-7.53 ppm. The latter contains an overlapped peak of quinazolinone C_6.

4.2. Biological Activity Testing Results

4.2.1. *In vivo* Antimalarial Activity of the Synthesized Compounds

The standard four day suppressive test was used to evaluate the antimalarial activities of the synthesized compounds on *P. berghei* infected mice. Chloroquine phosphate (25 mg/kg/day or 48.46 μmol/kg/day) was use as a positive control and a vehicle containing 7% tween 80, 3% absolute ethanol in distilled water as a negative control. Equimolar amounts of the synthesized compounds were administered through the oral route after dissolving in a vehicle containing 7% tween 80, 3% absolute ethanol in distilled water. The percent suppression, percent parasitaemia, and mean survival time of the mice

treated with the synthesized compounds were compared against the control groups, as shown in Table 3.

Table 3: Data for antimalarial activities testing of the synthesized compounds (48.46 μmol/kg)*

Test compound	*Dose (mg/kg)*	*% Parasitemia*	*% Suppression*	*Mean survival time (Days)*
IVa	17.49	27.14 ± 0.91	44.38	8.3 ± 0.68
IVb	18.17	28.45 ± 0.78	41.70	7.8 ± 0.87
Va	18.63	34.60 ± 1.05	29.10	6.9 ± 0.91
Vb	18.63	29.73 ± 1.11	39.10	7.1 ± 0.99
VIb	20.76	27.14 ± 0.43	44.39	8.3 ± 0.55
VIIa	16.59	30.29 ± 0.48	37.93	7.6 ± 0.47
VIIb	18.73	29.71 ± 0.45	39.12	6.2 ± 0.43
NC**	-	48.80 ± 0.66	0.0	6.3 ± 0.86
CQ	25	0.0	100	ND

*: Values are Mean ± SD, P<0.05, NC**: Negative Control, CQ: Chloroquine phosphate

In this study, the positive control (chloroquine phosphate) suppressed parasitemia of mice infected with *P. berghei* to a non-detectable level (100%) and this result was in line with previous reports [115]. Generally all the synthesized and tested compounds displayed percent suppression lower than 50% and it is significantly lower than that of the positive control-chloroquine phosphate. Among the synthesized and tested compounds, **VIb** and **IVa** demonstrated the highest percent suppression of 44.39% and 44.38%, respectively. The mean parasitemia level of the mice treated with **VIb** (27.14 ±

0.43) and **IVa** (27.14 ± 0.91) was found to be about half that of the negative control (48.80 ± 0.66). This showed that compounds **VIb** and **IVa** reduced the parasite load. Relatively moderate antimalarial activities were observed for compounds **IVb**, **Vb** and **VIIb** with percent suppression of 41.70, 39.10 and 39.12%, respectively. Among all the tested compounds, **Va** displayed the least antimalarial activity with 29.10% suppression and 34.60 ± 1.05 percent parasitemia.

Though the target compounds contain either a strong electron withdrawing substituents (essential for strong hydrophobic interaction with the back bone of the receptor) or hydroxyl group (essential for the formation of hydrogen bonding with the back bone of the receptor) at the 4-position of the styryl moiety, they demonstrated poor antimalarial activities (< 50%) as compared to chloroquine phosphate.

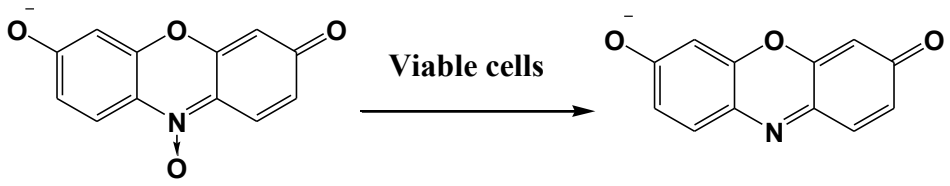
4.2.2. *In vitro* Antileishmanial Activity of the Synthesized Compounds

The antipromastigote activities of the synthesized compounds and the standard antileishmanial drugs (amphotericin B and miltefosine) were evaluated using the clinical isolate of *L.donovani* strain. Throughout the assay, the highest concentration of DMSO was adjusted not to exceed 1.0% of the synthesized compound preparation, a concentration that was reported to show no growth inhibitory effect.

The IC_{50} of the synthesized and reference drugs were evaluated from fluorescence characteristic of AlamarBlue® (resazurin). The AlamarBlue® reduction assay is considered to be better than others as it is easy, rapid, sensitive and coast effective for continuous monitoring of cell cultures and helps to accurately and quantitatively measure

proliferation of *Leishmania*. It is soluble, stable in culture medium, non-toxic to cells and does not affect the secretary abilities of cells [135].

The test works as a cell viability and proliferation indicator through the conversion of resazurin (which is the active ingredient of AlamarBlue®) to resorufin. Resazurin, a non-fluorescent indicator dye, is converted to highly pink fluorescent resorufin *via* reduction reactions of metabolically active cells (Fig. 31). The amount of fluorescence produced is proportional to the number of living cells [136, 137].



Resazurin, non-fluorescent Resorufin, highly fluorescent

Figure 31: Reduction of resazurin into resorufin by metabolically active cells

The antileishmanial activities of the synthesized compounds were evaluated following the method described in the experimental section. The results obtained were analyzed and IC_{50} values were calculated using Graph pad prism software (Table 4).

Table 4: Antipromastigote activity (IC_{50}) of the synthesized compounds*.

Test compounds	*IC_{50} values (µg/ml)*	*IC_{50} values (ng/ml)*
IVa	0.3014	301.4
IVb	**0.0128**	**12.8**
Va	0.10845	108.45
Vb	2.7017	2701.7
VIb	0.1086	108.6
VIIa	1.6472	1647.2
VIIb	3.1085	3108.5
Miltefosine	3.1911	3191.1
Amphotericin B	0.0460	46.0

*IC_{50}: effective concentration required to achieve 50% growth inhibition in µg/ml.

In this study the quinazolinone derivatives synthesized and tested for their antileishmanial activities were shown to have a good antileishmanial activity which was in line with previous reports [120, 121]. All the tested compounds exhibited better antileishmanial activity than the standard drug miltefosine as shown in Table 4. Among them, compound **IVb** was found to have a promising antileishmanial activity with an IC_{50} value of 0.0128 µg/ml which was 250 times superior than miltefosine (3.1911 µg/ml).

In addition, compound **Va**, **VIb** and **IVa** had a better antileishmanial activity with IC_{50} values of 0.108451 µg/ml, 0.10861 µg/ml and 0.3014 µg/ml respectively. Compound **Va** and **VIb** were 30 times more active than miltefosine while compound **IVa** was 10 times more active. Enhanced antileishmanial activities were observed for compounds **Vb** and **VIIa** with IC_{50} values of 2.7017 µg/ml and 1.6472 µg/ml respectively. Compound **VIIb**

(IC_{50} = 3.1085 µg/ml) possessed comparable antileishmanial activity with miltefosine (IC_{50} = 3.1911 µg/ml). Promising antipromastigote activity was observed for 2-(4-chlorostyryl)-3-*p*-tolyl-4(*3H*)-quinazolinone (**IVb**) with an IC_{50} value 0.0128 µg/ml. This compound exhibited superior antileishmanial activity to that of the standard drugs miltefosine (3.1911 µg/ml) and amphotericin B deoxycholate (0.0461 µg/ml). It was 4 times as active as amphotericin B deoxycholate and 250 times more active than that of miltefosine.

4.2.3. Oral Acute Toxicity Results

The oral acute toxicity of compound **IVb** was investigated by the procedure described in section 3.2.2.5. The results showed that it is devoid of any inherent acute toxicities.

5. Conclusions

Some 3-aryl-2-(substitutedstyryl)-4(*3H*)-quinazolinone derivatives were synthesized and tested for their antileishamanial and antimalarial activities. All the synthesized compounds displayed potential antileishmanial activities as compared to the standard drug miltefosine. In addition compound **IVb** showed pronounced antileishmanial activities as compared to miltefosine and amphotericin B deoxycholate. Thus, 2,3-disubstituted-4(3*H*)-quinazolinines containing an aromatic substitution at 3-position and substituted styryl moiety at 2-position represent a fruitful matrix for the development of antileishmanal agents.

6. Recommendations for Further Work

1. Synthesize 2,3-disubstituted-4(*3H*)-quinazolinone derivatives and evaluate for antimalarial and antileishmanial activity.
2. The antimalarial activity testing was done in a single dose. Multiple dose testing regimens need to be conducted for the antimalarial activities of these compounds.
3. The mechanisms of action of most active compounds observed on both biological studies need to be determined.
4. The antileishmanial activity screening was conducted on *L. donovani* isolate. The same work should be carried out on large variety of *Leishmania* strains.
5. *In vivo* studies should be conducted for the tested compounds as they have potent *in vitro* antileishmanial activities.

References

1. Greenwood B and Mutabingwa T (2002). Malaria in 2002. *Nature*, **415**, 670-672.
2. Mons B, Janse CJ, Boorsma EG and Van Der Kaay HJ (1985). Synchronized erythrocytic schizogony and gametocytogenesis of *Plasmodium berghei in vivo* and *in vitro***.** *Parasitology*, **91:** 423-430
3. White NJ (2004). Antimalarial drug resistance. *J. Clin. Invest.,* **113**: 1084-1092.
4. WHO (2010). The world malaria report from WHO global malaria program. World Health Organization, Geneva.
5. FMOH (Federal Ministry of Health of Ethiopia) (2007): *Proceedings of the Annual Review Meeting on Malaria Prevention and Control* Addis Ababa, Ethiopia.
6. Guthmann JP, Bonnet M, Ahoua L, Dantoine F, Balkan S, van Herp M, Tamrat A, Legros D, Brown V and Checchi F (2007). Death rates from malaria epidemics, Burundi and Ethiopia. *Emerg. Infect. Dis.*, **13**:140-143.
7. Bellanger AP, Faucher JF, Robedat P, Schmitt A, Millon L and Hoen B (2011). Malaria outbreak in French troops returning from Côte d' Ivoire. *Scand. J. Infect. Dis.,* **43**: 230-233.
8. WHO (2003). *African Malaria Report*. Geneva: World Health Organization. WHO/CDS/MAL/2003.1093. http://rbm.who.int/publications).].
9. Dellicour S, Tatem AJ, Guerra CA, Snow RW and Kuile FO (2010). Quantifying the number of pregnancies at risk of malaria in 2007: A Demographic Study. *PLoS Med.,* **7**: e1000221
10. Bhattarai A, Ali AS, Kachur SP, Martensson A, Abbas AK, Khatib R, Al-mafazy A, Ramsan M, Rotllant G, Gerstenmaier JF, Molteni F, Abdulla S, Montgomery SM,

Kaneko A and Bjorkman A (2007). Impact of artemisinin based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Med.*, **4**: e309.

11. WHO. WHO Expert Consultation on cotrimoxazole prophylaxis in HIV infection. 2006. http://www.who.int/hiv/pub/meetingreports/ctx/en/(accessed Feb 16, 2011).
12. Olotu A, Lusingu J, Leach A, Lievens M, Vekemans J, Msham S, Lang T, Gould J, Dubois MC, Jongert E, Vansadia P and Carter T (2011). Efficacy of RTS,S/AS01E malaria vaccine and exploratory analysis on anti-circumsporozoite antibody titres and protection in children aged 5-17 months in Kenya and Tanzania: a randomised controlled trial. *Lancet Infect. Dis.*, **11**: 102-109.
13. Day N and Dondorp AM (2007). The management of patients with severe malaria. *Am. J. Trop. Med. Hyg.*, **77**: 29-35.
14. Chen LH, Wilson ME and Schlagenhauf P (2007). Controversies and misconceptions in malaria chemoprophylaxis for travelers. JAMA, **297**: 2251-63.
15. Massaga JJ, Kitua AY, Lemnge MM, Akida JA, Malle LN, Ronn AN, Theander TG and Bygbjerg IC (2003). Effect of intermittent treatment with amodiaquine on anaemia and malarial fevers in infants in Tanzania: a randomised placebo-controlled trial. *Lancet*, **361**: 1853-1860.
16. Kalra BS, Chawla S, Gupta P and Valecha N (2006). Screening of antimalarial drugs. *Indian J. Pharmacol.*, **38**: 5-12.
17. Watkins WM (1997). The efficacy of antifolate antimalarial combinations in Africa: a predictive model based on pharmacodynamic and pharmacokinetic analyses. *Parasitol. Today,* **13**: 459-464.

18. Schlitzer M (2008). Antimalarial drugs-What is in use and what is in the pipeline. *Arch. Pharm.*, **341**:149-163.

19. Hellgren U and Rambo L (2010). Alternatives for malaria prophylaxis during the first trimester of pregnancy: our personal view. *J. Travel Med.*, **17**:130-132.

20. Carrara VI, Sirilak S, Thonglairuam J**,** Rojanawatsirivet C, Proux S, Gilbos V, Al Brockman A, Ashley EA, McGready R, Krudsood S, Leemingsawat S, Looareesuwan S, Singhasivanon P, White N and Nosten F (2006). Deployment of early diagnosis and mefloquine-artesunate treatment of *falciparum* malaria in Thailand: the Tak Malaria Initiative. *PLoS Med.*, **3**: e183.

21. Dondorp A, Nosten F and Stepniewska K (2005). Artesunate versus quinine for the treatment of severe *falciparum* malaria: a randomized trial. *Lancet*, **366**: 717-25.

22. Smithuis F, Kyaw MK, Phe O, Aye KH, Htet L, Barends M, Lindegardh N, Singtoroj T, Ashley E, Lwin S, Stepniewska K and White NJ (2006). Efficacy and effectiveness of dihydroartemisinin-piperaquine versus artesunate-mefloquine in falciparum malaria: an open-label randomized comparison. *Lancet,* **367:** 2075-85.

23. Alker AP, Lim P, Sem R, Shah NK, Yi P, Bouth DM, Tsuyuoka R, Maguire JD, Fandeur T, Ariey F, Wongsrichanalai C and Meshnick SR (2007). *Pfmdr1* and in vivo resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. *Am. J. Trop. Med. Hyg.*, **76**: 641-647.

24. Nosten F and White NJ (2007). Artemisinin-based combination treatment of *falciparum* malaria. *Am. J. Trop. Med. Hyg.*, **77**:181-92.

25. Ratcliff A, Siswantoro H, Kenangalem E, Maristela R, Wuwung RM, Laihad F, Ebsworth EP, Anstey NM, Tjitra E and Price RN (2007). Two fixed-dose artemisinin

combinations for drug-resistant *falciparum* and *vivax* malaria in Papua, Indonesia: an open-label randomised comparison. *Lancet*, **369**:757-765.

26. Ramharter M, Kurth F and Scherier AC (2008). Fixed-dose pyronaridine-artesunate combination for treatment of uncomplicated *falciparum* malaria in paediatric patients in Gabon. *J. Infect. Dis.*, **198**: 911-919.
27. Denis MB, Tsuyuoka R, Poravuth Y, Narann TS, Seila S, Lim C, Incardona S, Lim P, Sem R, Socheat D, Chiristophel EM and Ringwald P (2006). Surveillance of the efficacy of artesunate and mefloquine combination for the treatment of uncomplicated falciparum malaria in Cambodia. *Trop. Med. Int. Health*, **11**:1360-1366.
28. Crockett M and Kain KC (2007). Tafenoquine: a promising new antimalarial agent. *Expert. Opin. Investig. Drugs*, **16**: 705-715.
29. Olliaro, P., Wells, T.N., (2009). The global portfolio of new antimalarial medicines under development. *Clin. Pharmacol. Ther.*, **85**:584-595.
30. Franklin BS, Ishizaka ST, Lamphier M, Gusovsky F, Hansen H, Rose J, Zheng W, Ataíde MA, de Oliveira RB, Golenbock DT and Gazzinelli RT (2011). Therapeutical targeting of nucleic acid-sensing Toll-like receptors prevents experimental cerebral malaria. *Proc. Natl. Acad. Sci. USA,* **108:** 3689-3694.
31. Rijken MJ, Boel ME, Russell B, Imwong M, Leimanis ML, Phyo AP, Muehlenbachs A, Lindegardh N, McGready R, Rénia L, Snounou G, Singhasivanon P and Nosten F (2011). Chloroquine resistant *vivax* malaria in a pregnant woman on the western border of Thailand. *Malaria J.*, **10**:113
32. White NJ (2008). How antimalarial drug resistance affect post-treatment prophylaxis. *Malaria J.*, **7**: 9

33. Musset L, Bouchaud O, Matheron S, Massias L and Le Bras J (2006). Clinical atovaquone-proguanil resistance of *Plasmodium falciparum* associated with cytochrome b codon 268 mutations. *Microbes Infect.*, **8**: 2599-2604.

34. Hasugian AR, Purba HLE, Kenangalem E, Wuwung RM, Ebsworth EP, Maristela R, Penttinen PMP, Laihad F, Anstey NM, Tjitra E and Price RN (2007). Dihydroartemisinin-piperaquine versus artesunate-amodiaquine: superior efficacy and post-treatment prophylaxis against multidrug-resistant *P. falciparum* and *P.vivax* malaria. *Clin. Infect. Dis.,* **44**: 1067-1074.

35. Dokomajilar C, Nsobya SL, Greenhouse B, Rosenthal PJ and Dorsey G (2006). Selection of *Plasmodium falciparum pfmdr1* alleles following therapy with artemether-lumefantrine in an area of Uganda where malaria is highly endemic. *Antimicrob. Agents Chemother.*, **50**: 1893-1895.

36. Vijaykadga S, Rojanawatsirivej C, Cholpol S, Phoungduen D, Nakavej A and Wongsrichanalai C (2006). *In vivo* sensitivity monitoring of mefloquine monotherapy and artesunate- mefloquine combinations for the treatment of uncomplicated *falciparum* malaria in Thailand in 2003. *Trop. Med. Int. Health*, **11**: 211-219.

37. Noedl H, Socheat D and Satimai W (2009). Artemisinin-resistant malaria in Asia. *N. Engl. J. Med.*, **361**: 540-541.

38. Sibley CH, Guerin PJ and Ringwald P (2010). Monitoring antimalarial resistance: launching a cooperative effort. *Trends parasitol.,* **26**: 221-224.

39. Renslo AR and McKerrow JH (2006). Drug discovery and development for neglected parasitic diseases. *Nat. Chem. Biol.,* **2**: 701-710.

40. McConville MJ, Souza D, Saunders E, Likic VA and Thomas N (2007). Living in a phagolysosome; metabolism of *Leishmania* amastigotes. *Trends Parasitol.*, **23**: 368-375.

41. Sharma U and Singh S (2008). Insect vectors of *Leishmania*: distribution, physiology and their control. *J. Vector Borne Dis.,* **45**: 255-272.

42. Kamhawi S (2006). Phlebotomine sand flies and *Leishmania* parasites: friends or foes? *Trends Parasitol.,* **22**:439-445.

43. Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B and Brooker S (2007). Cutaneous leishmaniasis. *Lancet Infect. Dis.,* **7**: 581-596.

44. Minodier P and Parola P (2007). Cutaneous leishmaniasis treatment. *Travel Med. Infect. Dis.,* **5**: 150-158.

45. Desjeux P (2004). Leishmaniasis: current situation and new perspectives. *Comp. Immunol. Microbiol. Infect. Dis.*, **27**: 305-318.

46. Amato VS, Nicodemo AC, Amato JG, Boulos M and Neto VA (2000). Mucocutaneous leishmaniasis associated with HIV infection treated successfully with liposomal amphotericin B(AmBisome). *J. Antimicrob. Chemother.,* **46:** 341-342.

47. Manandhar KD, Yadav TP, Prajapati VK, Kumar S, Rai M, Dube A, Srivastava ON and Sundar S (2008). Antileishmanial activity of nano-amphotericin B deoxycholate. *J. Antimicrob. Chemother.,* **62**: 376-380.

48. Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B and Brooker S (2007). Cutaneous leishmaniasis. *Lancet Infect. Dis.*, **7**: 581-596.

49. Maltezou HC (2010). Drug resistance in visceral leishmaniasis. *J. Biomed. Biotechnol.,* doi:**10**.1155/2010/617521.

50. Nascimento ET, Moura MLN, Queiroz JW, Barroso AW, Araujo AF, Rego EF, Wilson ME, Pearson RD and Jeronimo SM (2011). The emergence of concurrent HIV-1/AIDS and visceral leishmaniasis in Northeast Brazil. *Trans. R. Soc. Trop. Med. Hyg.,* **105**: 298-300.

51. Maciel BLL, Lacerda HG, Queiroz JW, Galvão J, Pontes NN, Dimenstein R, McGowan SE, Pedrosa LFC and Jerônimo SMB (2008). Association of nutritional status with the response to infection with *Leishmania chagasi*. *Am. J. Trop. Med. Hyg.*, **79**: 591-598.

52. Gelanew T, Cruz I, Kuhls K, Alvar J, Canavate C, Hailu A and Schonian G (2011). Multilocus microsatellite typing revealed high genetic variability of *Leishmania donovani* strains isolated during and after a Kala-azar epidemic in Libo Kemkem district, Northwest Ethiopia. *Microbes Infect.*, **13**: 595-601.

53. Herrero M, Orfanos G, Argaw D, Mulugeta A, Aparicio P, Parreno F, Bernal O, Rubens D, Pedraza J, Lima MA, Flevaud L, Palma PP, Bashaye S, Alvar J and Bern C (2009). Natural history of visceral leishmaniasis outbreak in highland Ethiopia. *Am. J. Trop. Med. Hyg.*, **81**: 373-377.

54. Hailu A, Di Muccio T, Abebe T, Hunegnaw M, Kager PA and Gramiccia M (2006). Isolation of *Leishmania tropica* from an Ethiopian cutaneous leishmaniasis patient. *Trans. R. Soc. Trop. Med. Hyg.,* **100**: 53-58.

55. Negera E, Gadisa E, Yamuah L, Engers H, Hussein J, Kuru T, Hailu A, Gedamu L and Aseffa A (2008). Outbreak of cutaneous leishmaniasis in Silti woreda, Ethiopia: risk factor assessment and causative agent identification. *Trans. R. Soc. Trop. Med. Hyg.*, **102**: 883-890.

56. Lemma W, Erenso G, Gadisa E, Balkew M, Gebre-Michael T and Hailu A (2009). A zoonotic focus of cutaneous leishmaniasis in Addis Ababa, Ethiopia. *Parasit Vectors,* **2**:60 doi: 10.1186/1756-3305-2-60.

57. Alvar J, Aparicio P, Aseffa A, Den Boer M, Canavate C, Dedet JP, Gradoni L, Horst RT, Lopez-Velez R and Moreno J (2008). The relationship between leishmaniasis and AIDS: the second 10 years. *Clin. Microbiol. Rev.,* **21**: 334-359.

58. Herremans T, Pinelli E, Casparie M, Nozari N, Roelfsema J and Kortbeek L (2010). Increase of imported leishmaniasis in the Netherlands: a twelve-year overview (1996-2007). *Int. Health*, **2**: 42-46.

59. Thakur CP and Kumar K (1992). Post kala-azar dermal leishmaniasis: a neglected aspect of kala-azar control programmes. *Ann. Trop. Med. Parasitol.,* **86**: 355-359.

60. Kaur J, Kaur T and Kaur S (2011). Studies on the protective efficacy and immunogenicity of Hsp70 and Hsp83 based vaccine formulations in *Leishmania donovani* infected BALB/c mice. *Acta Tropica*, **119**: 50-56.

61. Abdian N, Gholami E, Zahedifard F, Safaee N and Rafati S (2011). Evaluation of DNA/DNA and prime-boost vaccination using LPG3 against *Leishmania major* infection in susceptible BALB/c mice and its antigenic properties in human leishmaniasis. *Experimental Parasitol.,* **127**: 627-636.

62. Ritmeijer K and Davidson RN (2003). Field research in humanitarian medical programmes. Medecins sans frontieres interventions against kala-azar in the Sudan, 1989-2003. *Trans. R. Soc. Trop. Med. Hyg.*, **97**: 609-613.

63. Sundar S (2001). Drug resistance in Indian visceral leishmaniasis. *Trop. Med. Int. Health,* **6**: 849-54.

64. Olliaro PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen J-A and Sundar S (2005). Treatment options for visceral leishmaniasis: a systematic review of clinical studies done in India, 1980-2004. *Lancet Infect. Dis.*, **5**: 763-774.

65. Ordonez-Gutierrez L, Espada-Fernandez R, Dea-Ayuela MA, Torrado JJ, Bolas-Fernandez F and Alunda JM (2007). *In vitro* effect of new formulations of amphotericin B on amastigote and promastigote forms of *Leishmania infantum*. *Int. J. Antimicrob. Agents.*, **30**: 325-329.

66. Veerareddy PR, Vobalaboina V and Ali N (2009). Antileishmanial activity, pharmacokinetics and tissue distribution studies of mannose-grafted amphotericin B lipid nanospheres. *J. Drug Target*, **17**:140-147.

67. Berman J, Bryceson AD, Croft S, Engel J, Gutteridge W, Karbwang J, Sindermann H, Joto S, Sundar S and Urbina JA (2006). Miltefosine: issues to be addressed in the future. *Trans. R. Soc. Trop. Med. Hyg.*, **S100**: S41-S44.

68. den Boer ML, Alvar J, Davidson RN, Ritmeijer K and Balasegaram M (2009). Developments in the treatment of visceral leishmaniasis**.** *Expert Opin. Emerging Drugs,* **14**: 395-410.

69. Sundar S and Olliaro PL (2007). Miltefosine in the treatment of leishmaniasis: clinical evidence for informed clinical risk management. *Ther. Clin. Risk Manag.,* **3**: 733-740.

70. Croft SL, Yardley V and Kendrick H (2002). Drug sensitivity of *Leishmania* species: some unresolved problems. *Trans. R. Soc. Trop. Med. Hyg.,* **96**: 127-129.

71. Sundar S, Jha TK, Thakur CP, Sinha PK and Bhattacharya SK (2007). Injectable paromomycin for visceral leishmaniasis in India. *N. Engl. J. Med.*, **356**: 2571-2581.

72. Nogueira IRL, Carneiro G, Yoshida MI, de Oliveira RB and Ferreira LAM (2011). Preparation, characterization, and topical delivery of paromomycin ion pairing. *Drug Dev. Ind. Pharm.*, **37**: 1083-1089.

73. Ben Salah A, Buffet PA, Morizot G, Ben Massoud N, Zaatour A, Ben Alaya N, Hamida NBH, El Ahmadi Z, Downs MT, Smith PL, Dellagi K and Grogl M (2009). WR279,396, a third generation aminoglycoside ointment for the treatment of *Leishmania major* cutaneous leishmaniasis: a phase 2, randomized, double blind, placebo-controlled study. *PLoS Negl. Trop. Dis.,* **3**: e432.

74. Sundar S, Sinha PK, Rai M, Verma DK, Nawin K, Alam S, Chakravarty J, Vaillant M, Verma N, Pandey K, Kumari P, Lal CS, Arora R, Sharma B, Ellis S, Strub-Wourgaft N, Balasegaram M, Olliaro P, Das P and Modabber F (2011). Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. *Lancet,* **377**: 477-486.

75. Melaku Y, Collin SM, Keus K, Gatluak F, Ritmeijer K and Davidson RN (2007). Treatment of kala-azar in southern Sudan using a 17-day regimen of sodium stibogluconate combined with paromomycin: a retrospective comparison with 30-day sodium stibogluconate monotherapy. *Am. J. Trop. Med. Hyg.,* **77**: 89-94.

76. Lopez-Martín C, Perez-Victoria JM, Carvalho L, Castanys S and Gamarro F (2008). Sitamaquine sensitivity in *Leishmania* species is not mediated by drug accumulation in acidocalcisomes. *Antimicrobial Agents Chemother.,* **52**: 4030-4036.

77. Kumar P, Lodge R, Trudel N, Ouellet M, Ouellette M and Tremblay MJ (2010). Nelfinavir, an HIV-1 protease inhibitor, induces oxidative stress-mediated, caspase-independent apoptosis in *Leishmania* amastigotes. *PLoS Negl. Trop. Dis.,* **4**: e642.

78. Valdivieso E, Rangel A, Moreno J, Saugar JM, Cañavate C, Alvar J and Dagger F (2010). Effects of HIV aspartyl-proteinase inhibitors on *Leishmania sp*. *Experimental Parasitol.,* **126**: 557-563.

79. Kaur J, Sundar S and Singh N (2010). Molecular docking, structure-activity relationship and biological evaluation of the anticancer drug monastrol as a pteridine reductase inhibitor in a clinical isolate of *Leishmania donovani*. *J. Antimicrob. Chemother.,* **65**: 1742-1748.

80. Miguel DC, Zauli-Nascimento RC, Yokoyama-Yasunaka JKU, Katz S, Barbieri CL and Uliana SRB (2009). Tamoxifen as a potential antileishmanial agent: efficacy in the treatment of *Leishmania braziliensis* and *Leishmania chagasi* infections. *J. Antimicrob. Chemother.,* **63**: 365-368.

81. Cabrera-Serra MG, Lorenzo-Morales J, Romero M, Valladares B and Piñero JE (2007). *In vitro* activity of perifosine: a novel alkylphospholipid against the promastigote stage of *Leishmania* species. *Parasitol. Res.*, **100**: 1155-1157.

82. Mendez S, Traslavina R, Hinchman M, Huang L, Green P, Cynamon MH and Welch JT (2009). The antituberculosis drug pyrazinamide affects the course of cutaneous leishmaniasis *in vivo* and increases activation of macrophages and dendritic cells. *Antimicrobial Agents Chemother.*, **53**: 5114-5121.

83. Zhang R, Shang L, Jin H, Ma C, Wu Y, Liu Q, Xia Z, Wei F, Zhu X-Q and Gao H (2010). *In vitro* and *in vivo* antileishmanial efficacy of nitazoxanide against *Leishmania donovani*. *Parasitol. Res.,***107**: 475-479.

84. Reimão JQ, Taniwaki NN and Tempone AG (2010). Furazolidone is a selective *in vitro* candidate against *Leishmania (L.) chagasi*: an ultrastructural study. *Parasitol. Res.*, **106**: 1465-1469.

85. Oliveira-Silva F, Morais-Teixeira E and Rabello A (2008). Antileishmanial activity of azithromycin against *Leishmania* (*Leishmania*) *amazonensis*, *Leishmania* (*Viannia*) *braziliensis*, and *Leishmania* (*Leishmania*) *chagasi*. *Am. J. Trop. Med. Hyg.,* **78**: 745-749.

86. Tempone AG, Taniwaki NN and Reimão JQ (2009). Antileishmanial activity and ultrastructural alterations of *Leishmania (L.) chagasi* treated with the calcium channel blocker nimodipine. *Parasitol. Res.*, **105**: 499-505.

87. Ait-Oudhia K, Gazanion E, Oury B, Vergnes B and Sereno D (2011). The fitness of antimony-resistant *Leishmania* parasites: lessons from the field. *Trends Parasitol.,* **27**: 141-142.

88. Adam GK, Abdulla MA, Ahmed AA and Adam I (2009). Maternal and perinatal outcomes of visceral leishmaniasis (kala-azar) treated with sodium stibogluconate in eastern Sudan. *Int. J. Gynecol. Obstet.,* **107**: 208-210.

89. Dorlo TPC and Kager PA (2010) Comment on: Cutaneous and mucocutaneous leishmaniasis in Tigray, northern Ethiopia: clinical aspects and therapeutic concerns. *Trans. R. Soc. Trop. Med. Hyg.,* **104**: 84-85.

90. Bray PG, Barrett MP, Ward SA and Koning HP (2003). Pentamidine uptake and resistance in pathogenic protozoa: past, present and future. *Trends Parasitol.,* **19**: 232-239.

91. Seifert K, Perez-Victoria FJ, Stettler M, Sanchez-Canete MP, Castanys S, Gamarro F and Croft SL (2007). Inactivation of the miltefosine transporter, LdMT, causes miltefosine resistance that is conferred to the amastigote stage of *Leishmania donovani* and persists *in vivo. Int. J. Antimicrob. Agents*, **30**: 229-235.

92. Maity A, Mondal S, Paira R, Hazra A, Naskar S, Sahu KB, Saha P, Banerjee S and Mondal NB (2011). A novel approach for the one-pot synthesis of linear and angular fused quinazolinones. *Tetrahedron Lett.,* **52**: 3033-3037.

93. Adib M, Ansari S, Mohammadi A and Bijanzadeh HR (2010). A novel, one-pot, solvent and catalyst-free synthesis of 2-aryl/alkyl-4(3*H*)-quinazolinones. *Tetrahedron Lett.,* **51**: 30-32.

94. Wang F, Zhao P and Xi C (2011). Copper-catalyzed one-pot synthesis of 2-thioxo-2,3-Dihydroquinazolin-4(1*H*)-ones from ortho-bromobenzamides and isothiocyanates. *Tetrahedron Lett.,* **52**: 231-235.

95. Nouira I, Kostakis IK, Dubouilh C, Chosson E, Iannelli M and Besson T (2008). Decomposition of formamide assisted by microwaves, a tool for synthesis of nitrogen-containing heterocycles. *Tetrahedron Lett.,* **49:** 7033-7036.

96. Loidreau Y and Besson T (2011). Microwave-assisted thermal decomposition of formamide: a tool for coupling a pyrimidine ring with an aromatic partner. *Tetrahedron*, **67**: 4852-4857.

97. Nouira I, Kostakis IK, Dubouilh C, Chosson E, Iannelli M and Besson T (2008). Decomposition of formamide assisted by microwaves, a tool for synthesis of nitrogen-containing heterocycles. *Tetrahedron Lett.,* **49**: 7033-7036.

98. Ye C, You J, Li XF, You R, Weng Y, Li J and Wang Y (2010). Design, synthesis and anticoccidial activity of a series of 3-(2-(2-methoxyphenyl)-2-oxoethyl) quinazolinone derivatives. *Pestic. Biochem. Physiol.*, **97**: 194-198.

99. Li G, Kakarla R, Gerritz SW, Pendri A and Ma B (2009). A facile one-step synthesis of 5-chloro-imidazo[1,5-*a*]quinazoline by microwave irradiation. *Tetrahedron Lett.,* **50**: 6048-6052.

100. Laddha SS and Bhatnagar SP (2009). A new therapeutic approach in Parkinson's disease: Some novel quinazoline derivatives as dual selective phosphodiesterase 1 inhibitors and anti-inflammatory agents. *Bioorg. Med. Chem.*, **17**: 6796-6802.

101. Fischer C, Shah S, Hughes BL, Nikov GN, Crispino JL, Middleton RE, Szewczak AA, Munoz B and Shearman MS (2011). Quinazolinones as γ-secretase modulators. *Bioorg. Med. Chem. Lett.*, **21**: 773-776.

102. Manivannan E and Chaturvedi SC (2011). Analogue-based design, synthesis and molecular docking analysis of 2,3-diarylquinazolinones as non-ulcerogenic anti-inflammatory agents. *Bioorg. Med. Chem.*, **19**: 4520-4528.

103. Rhee H-K, Yoo JH, Lee E, Kwon YJ, Seo H-R, Lee Y-S and Choo H-YP (2011). Synthesis and cytotoxicity of 2-phenylquinazolin-4(3*H*)-one derivatives. *Eur. J. Med. Chem.*, **46**: 3900-3909.

104. Kashaw SK, Kashaw V, Mishra P, Jain NK and Stables JP (2009). Synthesis, anticonvulsant and CNS depressant activity of some new bioactive1-(4-substituted-

phenyl)-3-(4-oxo-2-phenyl/ethyl-4*H*-quinazolin-3-yl)-urea. *Eur. J. Med. Chem.*, **44**: 4335-4343.

105. Jagani CL, Sojitra NA, Vanparia SF, Patel TS, Dixit RB and Dixit BC (2011). Microwave promoted synthesis and antimicrobial activity of 3-thiazole substituted 2-styryl-4(3H)-quinazolinone derivatives. *J. Saudi Chem. Soc.*, doi:**10**.1016/j.jscs.2011.02.001

106. Shivananda MK and Holla BS (2011). Antifungal activity studies of some quinazolinone derivatives. *J. Chem. Pharm. Res.,* **3**: 83-86

107. Kokuryo Y, Nakatani T, Kakinuma M, Kabaki M, Kawata K, Kugimiya A, Kawada K, Matsumoto M, Suzuki R and Ohtani M (2000). New γ-fluoromethotrexates modified in the pteridine ring: synthesis and in vitro immunosuppressive activity. *Eur. J. Med. Chem.*, **35**: 529-534.

108. Abou-Seri SM, Abouzid K and El Ella DAA, (2011). Molecular modeling study and synthesis of quinazolinone-arylpiperazine derivatives as α_1-adrenoreceptor antagonists. *Eur. J. Med. Chem.*, **46**: 647-658.

109. Aly MM, Mohamed YA, El-Bayouki KAM, Basyouni W and Abbas SY (2010). Synthesis of some new 4(3*H*)-quinazolinone-2-carboxaldehyde thiosemicarbazones and their metal complexes and a study on their anticonvulsant, analgesic, cytotoxic and antimicrobial activities - Part-1. *Eur. J. Med. Chem.*, **45**: 3365-3373.

110. Napier SE, Letourneau JJ, Ansari N, Auld DS, Baker J, Best S, Campbell-Wan L, Chan R, Craighead M, Desai H, Ho K-K, MacSweeney C, Milne R, Morphy JR, Neagu I, Ohlmeyer MHJ, Pick J, Presland J, Riviello C, Zanetakos HA, Zhao J and Webb ML (2011). Synthesis and SAR studies of novel 2-(6-aminomethylaryl-2-

aryl-4-oxo-quinazolin-3(4*H*)-*yl*) acetamide vasopressin V_{1b} receptor antagonists. *Bioorg. Med. Chem. Lett.*, **21**: 3813-3817.

111. Kumar A, Sharma P, Kumari P and Kalal BL (2011). Exploration of antimicrobial and antioxidant potential of newly synthesized 2,3-disubstituted quinazoline-4(3*H*)-ones. *Bioorg. Med. Chem. Lett.*, **21**: 4353-4357.

112. Kabri Y, Azas N, Dumetre A, Hutter S, Laget M, Verhaeghe P, Gellis A and Vanelle P (2010). Original quinazoline derivatives displaying antiplasmodial properties. *Eur. J. Med. Chem.*, **45**: 616-622.

113. Arfan M, Khan R, Khan MA, Anjum S, Choudhary MI and Ahmad M (2010). Synthesis and antileishmanial and antimicrobial activities of some 2,3-disubstituted 3*H*-quinazolin-4-ones. *J. Enzyme. Inhib. Med. Chem.*, **25**: 451-558.

114. Kikuchi H, Yamamoto K, Horoiwa S, Hirai S, Kasahara R, Hariguchi N, Matsumoto M and Oshima Y (2006). Exploration of a new type antimalarial compounds based on febrifugine. *J. Med. Chem.*, **49**: 4698-4706.

115. Zhu S, Zhang Q, Gudise C, Wei L, Smith E and Zeng, Y (2009). Synthesis and biological evaluation of febrifugine analogues as potential antimalarial agents. *Bioorg. Med. Chem.,* **17**: 4496-4502.

116. Verhaeghe P, Azas N, Gasquet M, Hutter S, Ducros C, Laget M, Rault S, Rathelot P and Vanelle P (2008). Synthesis and antiplasmodial activity of new 4-aryl-2-trichloromethylquinazolines. *Bioorg. Med. Chem. Lett.,* **18**: 396-401.

117. Mishra A, Srivastava K, Tripathi R, Puri SK and Batra S (2009). Search for new pharmacophores for antimalarial activity. Part III: Synthesis and bioevaluation of new 6-thioureido-4-anilinoquinazolines. *Eur. J. Med. Chem.*, **44**: 4404-4412.

118. Hussien M (2009). Synthesis and Biological Evaluation of Some 4(3H)-quinazolinones as Anti-malarial Agents. Master Thesis AAU, pp: 77-78.

119. Tuji G (2010). Synthesis and Biological of Some Styryl-4(3H)-quinazolinones Derivatives as Antimalarial and Antileishmanial Agents. Master Thesis AAU, pp: 50-56.

120. Berman JD, King M and Edwards N (1989). Antileishmanial activities of 2, 4-diaminoquinazoline putative dihydrofolate reductase inhibitors. *Antimicrobial Agents Chemother.*, **33**: 1860-1863.

121. Bhattacharjee AK, Skanchy DJ, Jennings B, Hudson TH, Brendle JJ and Werbovetz KA (2002). Analysis of stereoelectronic properties, mechanism of action and pharmacophore of synthetic indolo[2,1-*b*]quinazoline-6,12-dione derivatives in relation to antileishmanial activity using quantum chemical, cyclic voltammetry and 3-D-QSAR CATALYST procedures. *Bioorg. Med. Chem.,* **10**: 1979-1989.

122. Kumar A, Sharma S, Bajaj K, Sharma S, Panwar H, Singh T and Srivastava VK (2003). Some new 2,3,6-trisubstituted quinazolinones as potent anti-inflammatory, analgesic and COX-II inhibitors. *Bioorg. Med. Chem.,* **11**: 5293-5299.

123. Errede LA (1976). Acylanthranils 1. The pathway of quinazolone formation in the reaction of acylanthranils with anilines. *J. Org. Chem.,* **41**: 1763-1765.

124. Raffa D, Edler MC, 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 a new 3-heterocyclo substituted 2-styrylquinazolinones. *Eur. J. Med. Chem.*, **39**: 299-304.

125. Lara O, Raquel E, Mar´ıa A, Juan J, Francisco B and Jos´e M (2007). *In vitro* effect of new formulations of amphotericin B on amastigote and promastigote forms of *Leishmania infantum. Int. J. Antimicrob. Agents*, **30**: 325-329.

126. Peters W, Portus JH and Robinson BL (1975). The four-day suppressive *in vivo* antimalarial test. *Ann. Trop. Med. Parasitol.,* **69**: 155-171.

127. Ishih A, Miyase T and Terada M (2003). Comparison of antimalarial activity of the alkaloidal fraction of *Hydrangea macrophylla* var. *Otaksa* leaves with the hot-water extract in ICR mice infected with *Plasmodium yoelii* 17 XL. *Phytother. Res.*, **17**: 633-639.

128. Dominguez JN, Leon C, Rodrigues J, Neira GD, Gut J and Rosenthal PJ (2009). Synthesis of chlorovinyl sulfones as structural analogs of chalcones and their antiplasmodial activities. *Eur. J. Med. Chem.,* **44**: 1457-1462.

129. Trager W and Jensen JB (1976). Human malaria parasites in continuous culture. *Science*, **193**: 673-675.

130. Tariku Y, Hymete A, Hailu A and Rohloff J (2010). Essential-oil composition, antileishmanial and toxicity study of *Artemisia abyssinica* and *Satureja punctata ssp. punctata* from Ethiopia. *Chem. Biodivers*, **7:** 1013-1016.

131. Lorke D (1983). A new approach to practical acute toxicity test. *Arch. Toxicol.*, **54**: 275-286.

132. Gessler MC, Tanner M, Chollet J, Nkunya MHH and Heinrick M (1995). Tanzanian medicinal plants used traditionally for the treatment of malaria: *In vivo* antimalarial and *in vitro* cytotoxic activities. *Phytother. Res.*, **9**: 504-508.

133. Jatav V, Mishra P, Kashaw S and Stables JP (2008). CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3*H*)-ones. *Eur. J. Med. Chem.,* **43**: 1945-1954.

134. Williams DH and Fleming I (1989). *Spectroscopic Methods in Organic Chemistry*. 4th ed. revised, McGraw-Hill Book Company (UK) Limited, Table 3.27

135. Nakayama G R, Caton M C, Nova M P and Parandoosh Z (1997). Assessment of the Alamar blue assay for cellular growth and viability *in vitro*. *J. Immunol. Methods*, **204**: 205-208.

136. Al- Nasiry S, Geusens N, Hanssens M, Luyten C and Pijnenborg R (2007). The use of Alamar blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells. *Hum. Reprod.,* **25**: 1-6.

137. Shimony O and Jaffe CL (2006). Rapid fluorescent assay for screening drugs on *Leishmania* amastigotes. *J. Microbiol. Methods,* **75**: 196-200.