# NOVEL ADAMANTANE-CHLOROQUINOLIN CONJUGATES TO OVERCOME *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE

**MOFENGE OPUTE YVETTE**

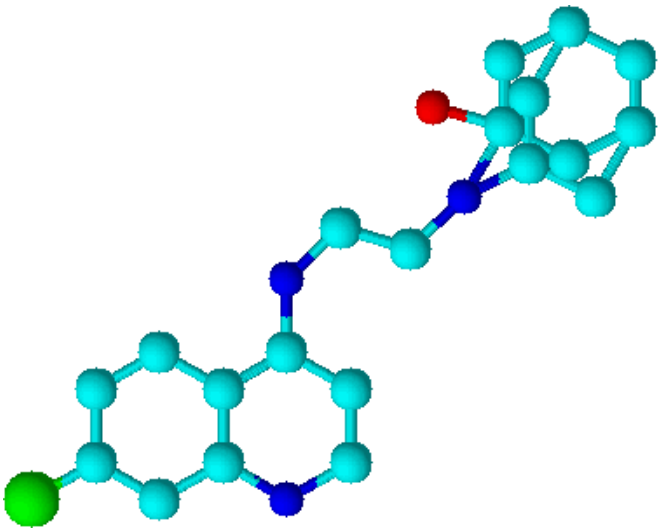
A dissertation submitted in partial fulfilment of the requirements for the degree *Magister Scientiae* in the Department of Pharmaceutical Chemistry, School of Pharmacy**,** University of the Western Cape.

Supervisor: Prof. Jacques Joubert

Co-supervisor: Prof. Sarel Malan



October 2017



**UNIVESRSITY OF THE WESTERN CAPE**

**FALCULTY OF SCIENCE**

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By

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**3106217**

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# DECLARATION

I declare that this research titled *"Novel Adamantane-chloroquinolin conjugates to overcome Plasmodium falciparum chloroquine resistance"* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

**Opute Yvette Mofenge** **2017**

**----------**

**Prof Jacques Joubert** **2017**



**----------**

*This study is firstly dedicated to God Almighty, then to my late beloved grandpa Joseph Bruno Opute.*

# ACKNOWLEDGEMENTS

The completion of my masters has been made possible by the supervision, assistance, guidance and prayer of many people to whom I extend my sincere gratitude and hereby gratefully acknowledged a few.

- God Almighty for granting me life till now to pursue my dreams

- My supervisors Prof. Jacques Joubert and Prof. Sarel F Malan for their patience, understanding, financial assistance and constructive contributions towards this study while giving me the opportunity to build myself. Thank you Profs, your interest in this study was a great motivation and significantly appreciated.

.

- Ms A. Ramplin, Mr. Y Kippie, Dr. E. Antunes for their invaluable technical advice and assistance making the findings of this study possible and meaningful.

- Prof Peter Smith and associates at UCT Pharmacology Department for their support in performing the biological assays. I am most indebted to you Dale Taylor for your patience and reliable results. Thank you

- My foyer crew Heather, Ireen, Locarno and Yemi for making this journey enjoyable especially in the low times. The outings were great. Love you guys.

- My fellow Drug Design and Discovery research group colleagues especially Frank, Ayo and Dr Sharma for all the invaluable assistance in the lab. Those were difficult days but you guys made it doable.

- The NRF and UWC for financial support

- The School of Pharmacy (UWC) for provision of research facilities and technical support.

- The Gwanfogbe family for the continuous push for me to reach my ultimate potential. Thank you for the constant encouragement and support in all facets of my life. I love you infinitely. The Nguatem family for their support, care and talking me to believe in God. Thank you for all the prayers and time devoted to proofread my drafts. I thank God for introducing you all into my life. I appreciate all your sacrifices

- To my family, I cannot say thank you enough. I am forever indebted to your love, care and sacrifices. I am grateful for everything.

# KEY WORDS

Novel adamantane-chloroquinolin conjugates to overcome plasmodium falciparum chloroquine resistance

**Mofenge Opute Yvette**

Malaria

Antimalarials

Aminoquinolines (AQ)

Chloroquine (CQ)

Drug resistance

Chloroquine resistance (CQR)

*Plasmodium falciparum*

*Plasmodium falciparum* chloroquine resistance transporter (*Pf*CQRT)

Resistance reversal agents

Reversed chloroquine (RCQ) molecules

Polycyclic cage compounds

Adamantane

# ABBREVIATIONS

| | |
|---|---|
| % | Percentage |
| µg | Microgram |
| µl | Micro litre |
| $^{13}C$ | Carbon 13 |
| $^{1}H$ | Proton |
| AcOH | Acetic acid |
| ACQ | Aminoquinoline |
| ACTs | Artemisinin-based combination therapies |
| AD-CQ | Adamantane-chloroquinolin conjugates |
| aq. | Aqueous |
| ATR | Attenuated total reflectance |
| bs | Broad singlet |
| CDC | Centre for Disease Control and Prevention |
| CHO | Chinese Hamster Ovarian |
| CQ | Chloroquine |
| CQR | Chloroquine resistance |
| $CQ^{R}$ | Chloroquine resistant parasite strain |
| $CQ^{S}$ | Chloroquine sensitive parasite strain |
| d | Doublet |
| DCE | Dichloroethane |
| DCM | Dichloromethane |
| DCQ | Dichloroquinoline |
| dd | Doublet(s) of doublet |
| DDT | Dichloro-diphenyltrichoroethane |
| DHA | Dihydroartemisinin |
| DHFR | Dihydrofolate reductase |
| DHPS | Dihydropteroate synthase |
| DMSO | Dimethylsulfoxide |
| DV | Digestive vacuole |
| FPIX | Ferriprotoporphyrin IX |
| FT-IR | Fourier transmission infrared radiation |
| g | Grams |
| G6PD | Glucose-6-Phosphate Dehydrogenase |
| GTS | Global Technical strategy |
| $H_2O_2$ | Hydrogen peroxide |
| HCl | Hydrochloric acid |
| HEPES | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| Hz | Hertz |
| $IC_{50}$ | 50 % inhibitory concentration |
| IR | Infrared spectroscopy |
| $LiAlH_4$ | Lithium aluminum hydride |
| M | Molar |
| m | Multiplet |

| | |
|---|---|
| m-CPBA | m-Chloroperbenzoic acid |
| MeOH | Methanol |
| mg | Milligram |
| MHz | Megahertz |
| ml | Millilitre |
| mM | Millimolar |
| mmol | Millimole |
| MMV | Medicines for Malaria Venture |
| mp | Melting point |
| MRC | Medical Research Council |
| MS | Mass spectrometry |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tertrazoliumbromide |
| MW | Microwave |
| NaBH(OAc)3 | Sodium triacetoxyborohydride |
| $NaHCO_3$ | Sodium hydrogen carbonate |
| NaOH | Sodium hydroxide |
| ND | Not determined |
| NGOs | Non-governmental organisations |
| $NH_4OH.H_2O$ | Ammonia water |
| nM | Nanomolar |
| NMDA | *N*-methyl-D-aspartate |
| NMR | Nuclear magnetic resonance |
| -OH | Hydroxyl group |
| PCU | Pentacycloundecylamine |
| PDC | Pyridinium dichromate |
| *Pf*CQRT | *Plasmodium falciparum* chloroquine resistance transporter |
| pLDH | parasite lactate dehydrogenase |
| ppm | Parts per million |
| PQ | Primaquine |
| psi | pound-force per square inch |
| QN | Quinine |
| RA | Reversal agent |
| RBCs | Red blood cells |
| RCQ | Reversed chloroquine |
| RI | Resistance index |
| Ro5 | Rule of five |
| RPMI | Roswell Park Mermorial Institute |
| rt. | Room temperature |
| s | Singlet |
| SAR | Structure-activity relationship |
| SD | Standard deviation |
| SI | Selective index |
| SQ | Single Quad |
| t | Triplet |
| THF | Tetrahyrofuran |
| TLC | Thin layer chromatography |
| TMS | Tetramethylsilane |

| | | | |
|---|---|---|---|
| UCT | University of Cape Town | WHO | World Health Organisation |
| USA | United States of America | X 2 | Twice |
| UV | Ultra violent | X 3 | Trice |
| W | Watt | δ | delta shift |

# ABSTRACT

Malaria poses devastating health and socioeconomic outcomes on global health especially among pregnant women and children below the age of 5 in endemic areas. This is exacerbated by *Plasmodium falciparum* resistance to available antimalarial drugs, especially chloroquine (CQ), which was the drug of choice for many years against the blood stage of malaria. CQ resistance is mostly associated with mutations in the putative transporter protein known as the *P. falciparum* chloroquine resistance transporter (*Pf*CQRT) localized in the parasitic digestive vacuole (DV) membrane. It enhances CQ efflux out of the DV and reduces its accumulation at its site of action. Consequently there is need for continued research for a more effective therapeutic drug to solve the problem of CQ resistance. The use of reversed CQ compounds which involves the hybridization of a CQ-like nucleus to a reversal agent (RA) is a feasible strategy of identifying innovative antimalarial compounds to overcome *P. falciparum* CQ resistance. Prior research has shown that the channel blocking ability of polycyclic cage compounds, such as pentacycloundecylamines (PCU), aids in CQ resistance reversal. In continuation of this research, the structurally related polycyclic adamantane moiety with inherent channel blocking ability was identified as a potential RA for this study. The aim of this study was to synthesize novel adamantane-chloroquinolin (AD-CQ) conjugates as potentially improved 'reversed chloroquine' compounds to overcome *P. falciparum* CQ resistance.

The AD-CQ conjugates consisted of the CQ-like 4-aminoquinoline pharamacophore conjugated to the adamantane moiety (as the reversal agent) *via* an alkyl linker. Firstly, the CQ-like nucleus was conjugated to alkyl linkers of different chain lengths by amination reaction using microwave irradiation to generate the various aminoquinoline (ACQ) intermediates. These compounds were then used for synthesis of the novel AD-CQ conjugates in series 1 and 2. The aza-adamantanols of series 1 were synthesized from the conjugation of an adamantane diketone and appropriate ACQ intermediates *via* reductive amination followed by transannular cyclization. The adamantane diketone was synthesized from 2-adamantanone *via* a 3 step process. The imine-adamantanes of series 2 were synthesized from the direct conjugation between 2-adamantone and ACQ intermediates in a 1:1 ratio. A total of eight novel AD-CQ conjugates were synthesized containing the bulky adamantane moiety which made the incorporation of a tertiary amine possible. The compounds were structurally confirmed by NMR, IR and MS. *In vitro* MTT and pLDH

assays were used to evaluate the cytotoxicity and antimalarial activity of the novel conjugates against $CQ^S$ and $CQ^R$ strains NF54 and K1 of the *P. falciparum* parasite.

All the novel AD-CQ compounds were non-toxic (CHO IC50 = 37860 – 279420 nM) and some of the conjugates exhibited potent antimalarial activity *in vitro*, superior to CQ against the $CQ^R$ strain and thus overcoming *P. falciparum* CQ resistance. Compound **5**, **6** and **9** were highly active compounds on both $CQ^S$ and $CQ^R$ strains ($IC_{50}$< 100 nM). Compound **5** showed the lowest resistance index (RI = 2.11) with good activity against the $CQ^R$ strain ($IC_{50}$ = 98.92 nM) and was identified as the most promising novel AD-CQ conjugate. Its ability to retain activity in the $CQ^R$ strain was 18-fold better than that of CQ. The adamantane moiety, especially in the aza-adamantanols, was shown to be a significant *P. falciparum* CQ resistance reversal agent compared to the previously used structurally related PCU moiety. Hence, the hybridization of a CQ-like nucleus to an adamantane moiety results in reversed CQ molecules with improved antimalarial activity that could overcome *P. falciparum* CQ resistance.

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# CHAPTER 1

# INTRODUCTION

## 1.1. INTRODUCTION

Malaria is a life-threatening parasitic infectious disease caused by the *Plasmodium* parasite and the different species responsible for infection in humans are *P. falciparum, P. vivax, P. malariae, P. ovale* and the most recently discovered *P. knowlesi*. Of these five species *P. falciparum* is the most common and lethal species (Greenwood *et al.*, 2005; Carter & Mendis, 2002; Liu *et al.*, 2010), associated with most deaths especially in Africa (Maguene *et al*., 2015; Kaur *et al*., 2009; Baird, 2005; WHO, 2017c).

Malaria infection is a major global health problem that accounts for millions of morbidity and mortality cases with increase in health cost annually (Yearick *et al*., 2008; Kelly *et al.*, 2007). Although substantial progress has been made to control and manage the disease in the past decades of research, about 50 % of the world's populations are still at risk of being infected with the malaria parasite. In the 212 million clinical cases encountered globally in 2015, 429,000 deaths were registered (WHO, 2016a). Moreover, the burden of malaria is felt mostly in low to middle income countries of sub-Saharan Africa. In addition, the poorest, marginalized communities and young children below the age of 5 and pregnant women are the most vulnerable (Bray *et al.*, 2005; Breman, Alilio and Mills, 2004; Bloland, 2001). It also places economic burden on the malaria endemic countries, as it impedes economic growth which affects livelihood and settlement patterns (Sachs & Malaney, 2002; Gallus & Sachs, 2001).

Chloroquine (CQ), (figure 1.1) was a cost effective antimalarial drug discovered in the 1940s. Additionally, it was readily available, well tolerated with a rapid onset of action and a relatively good safety profile (Greenwood*et al.*, 2005; Ridley, 2002; Wellems & Plowe, 2001). These qualities made CQ a 'wonder drug' that was used extensively worldwide as the mainstay drug for the prophylaxis and treatment of blood stage malaria for many decades since its discovery (Bruce-Chwatt, 1954). CQ exerts its antimalarial activity by inhibiting the formation of inert haemozoin leading to build up of toxic free haem in the parasitic food vacuole. Nonetheless, the emergence of *P. falciparum* CQ resistance in 1957 in the Thai-Cambodian border area and subsequent increasing wide spread of the resistant strains of the

parasite have rendered CQ ineffective and its use as an antimalarial drug restricted (Joubert *et al*., 2014; Sunduru *et al.*, 2009; Trape *et al*., 1998). This has put all the excellent qualities of CQ to waste as the problem is not with CQ itself but with the advancement of the parasite.

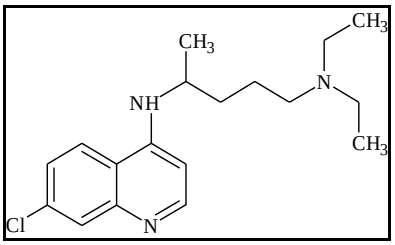



**Figure 1.1:** CQ structure

*P. falciparum* resistance to CQ is due to mutations in *Plasmodium falciparum* chloroquine resistance transporter (*Pf*CQRT) protein, a putative transporter in the parasitic vacuole membrane. This transporter promotes CQ efflux out of the parasitic vacuole thus decreasing CQ accumulation at its site of action (Fidock *et al.*, 2000). Furthermore, *P. falciparum* is not resistant to CQ only but to almost all available antimalarial drugs even the newer artemisinin-based combination therapies (ACTs) thus limiting the number of affordable efficacious drugs available to fight the disease. The greatest consequences of these limitations of fewer drugs and high cost have been the complication and delay of malaria treatment, control and eradication strategies especially in sub-Saharan Africa (Ridley *et al*., 1996). Thus there is indeed a dire need for the discovery of new antimalarial drugs to expand the chemotherapy agents' portfolio before it is too late. This study seeks to overcome *P. falciparum* CQ resistance using adamantane-chloroquinolin conjugates as potential reversed CQ agents. This could go a long way to restore the usefulness of CQ derivatives as antimalarial drugs.



## 1.2. RATIONALE OFTHE STUDY

As already stated, malaria poses health threat to 50 % of the world's population as the most common strain *P. falciparum*, in addition to being lethal has become resistant to every antimalarial drug available especially CQ. Thus more people mainly in sub-Saharan Africa are at risk of being infected with the malaria parasite each year with increased malaria morbidity and mortality. This is due to the availability of a limited number and range of antimalarial drugs, which greatly hinders the management and control of malaria.

The control of malaria infection is multifaceted including vector control, chemotherapy and policy strengthening (Crater & Mendis, 2002). Currently, the limitation of vector control,

inadequate surveillance of policies as well as the delay of an effective vaccine makes chemotherapy the mainstay strategy, to control, cure and possibly eliminate malaria (Aguiar *et al.*, 2012). Thus, it is required that new drugs be developed against all the stages of the complex life cycle of the parasite. However, the interest of this study is focused on continued research efforts to modify existing successful pipe-line drugs, especially the modification of existing blood stage antimalarial agent scaffolds, in order to produce improved novel antimalarial agents. CQ has been used as a lead scaffold and modified as described below to synthesize novel AD-CQ conjugates to overcome *P. falciparum* CQ resistance.

### 1.2.1. 4-Aminoquinoline Pharmacophore

Resistance to CQ has made its use restricted in malaria infection and has led to the rise of artemisinin-based combination therapy (ACT) as first line treatment in *P. falciparum* infections. However, the availability of ACT is limited and resistance to artemisinin have also emerged (Noedl *et al.*, 2008; Dondrop *et al.*, 2009). Also, the effectiveness of some alternatives like mefloquine and quinine has reduced significantly due to cross resistance. These drugs mentioned above do not match the low cost, quick onset of action, safety profile, availability and simple structure of CQ (Mushtaque & Shahjahan, 2015; WHO, 2014; Peyton, 2012; Saenz *et al*., 2012; van Schalkwyk & Egan, 2006; Wellems & Plowe, 2001, Ridley *et al.*, 1996; Whitty *et al*., 2008; Olliaro, Taylor & Rigal, 2001).



In this project, the 4-aminoquinoline pharmacophore (CQ-like nucleus) was used as lead scaffold to synthesize novel adamantane-chloroquinolin conjugates in order to modulate and enhance the favourable qualities of CQ. Moreover, the 4-aminoquinoline pharmacophore (7-chloro-4-aminoquinoline-based antimalarial drugs) is still an attractive scaffold of interest because its mechanism of action and mechanism of resistance are independent of each other and resistance to it developed slowly over a long period of time (Omodeo-Sale *et al*., 2009; Egan *et al*., 2000). Structure-activity relationship (SAR) on the 4-aminoquinoline pharmacophore has shown it to posssesses antimalarial activity,especially the inhibition of beta-hematin formation (haemozoin) and enhanced accumulation of the drug at the targeted site (O'Neill, 1998; Muraleedharan & Avery, 2007). New analogues with the 4-aminoquinoline core as reversed CQ molecules have been shown to have enhanced antimalarial activity *in vitro* compared to CQ against CQ resistant ($CQ^R$) *P. falciparum* strains as well CQ sensitive ($CQ^S$) strains (Joubert *et al*., 2014; Pandey *et al.*, 2013; Sunduru *et al*., 2009).

### 1.2.2. Reversed Chloroquine Molecule Strategy

Despite the controversies on the mechanism of CQ resistance, it is agreed that CQ resistance occurs due to decreased CQ access to target free haem as a result of reduced drug accumulation in the parasitic digestive vacuole (Saenz *et al*., 2012; Kelly *et al*., 2007; Wellems & Plowe, 2001). This has shown to be strongly associated to point mutations of *Plasmodium falciparum* chloroquine resistance transporter (*Pf*CQRT) protein in the vacuole of the parasite. This is a transporter which promotes the efflux of the drug out of the vacuole, the site of CQ action (Burgess *et al*., 2010; Van Schalkwyk & Egan, 2006; Djimdé *et al*., 2004).

Fortunately, CQ resistance can be reversed using reversal agents and/or chemosensitiers. These are molecules that reverse resistance to CQ by inhibiting the efflux mechanism or making the parasite sensitive to CQ again. The interest in research on agents to reverse CQ resistance started in 1987, when Krogstad and co-workers discovered that CQ resistance was due to increased efflux rate of the drug out of the food vacuole (Krogstad *et al.*, 1987). This explains the low drug accumulation and why several compounds, such as calcium channel blockers like verapamil (Martin *et al.*, 1987), tricyclic antidepressants like imipramine (Adam *et al.*, 2004; Bhattacharjee, Kyle and Vennerstrom, 2001) and antihistamines like chlorpheniramine (Basco & Bras, 1994; Brasco & Bras, 1991), have slowed the rate of this efflux and increased CQ accumulation in the vacuole (Kelly *et al.*, 2007). A similar result was obtained by Martin, Oduola and Milhous (1987), when they investigated verapamil and found it reversed CQ resistance in resistant *P. falciparum* strains with no effect on CQ sensitive strains at the same concentration.

The findings of these studies, though ground breaking, had the limitation of the poly-pharmacy approach of CQ and chemosensitizers, which is costly and inconvenient. To address this problem, Burgess and colleagues (2006) introduced the new attractive strategy of reversing CQ resistance *via* the hybridization of a reversal agent (RA) to the CQ pharmacophore (7-chloro-4-aminoquinoline nucleus) in the development of a single antimalarial molecule. This was termed a 'reversed CQ (RCQ) compound' (**figure1.2**) with greater advantage of increasing the accumulation of the compound in the vacuole.

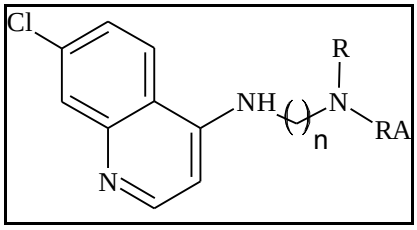



**Figure 1.2:** Generalized structure of a RCQ compound

Many other researchers have demonstrated the feasibility of this strategy by the investigation of different RA design strategies and have produced potent novel RCQ compounds (Joubert *et al*., 2014; Van Schalkwyk & Egan, 2006; Andrews *et al*., 2009).

The hybridization of the RAs to the side chain of 7-chloro-4-aminoquinoline nucleus produced compounds with the excellent antimalarial properties as CQ and this is attributed to the 7-chloro-4-aminoquinoline scaffold. The 7-chloro-4-aminoquinoline is responsible for the antimalarial activity of the compounds by facilitating haem target binding. This hybridized reversed CQ molecule is generally a potential improved dual function compound (Kumar *et al.*, 2011) and it is the interest of this study. This hybridization is advantageous to the patient, as it produces a single chemical entity with dual activity and decreases the concentration of each compound required, when compared to the polypharmacy approach. The result of this is decreased cost, increased efficacy, adherence and reduced toxicity (Joubert *et al*., 2014; Peyton, 2012). These molecules are known to also have enhanced activity against resistant strains of *P. falciparum* as well as improved metabolic stability which prevents cross resistance.



### 1.2.3. Polycyclic Cage Compounds

Polycyclic amines like NGP1-01 (**figure 2.13**), a prototype of pentacycloundecylamine (PCU) has shown significant inherent voltage-gated calcium channels and *N*-methyl-D-aspartate channels blocking activities. It has been investigated and found to act as a chemosensitizer to CQ and further investigation led to the discovery of aza-derivatives of PCUs with better resistance reversal activity when hybridized to a CQ-like nucleus (Joubert *et al.*, 2016; Joubert *et al.*, 2014). Based on these findings, adamantane which is structurally related to the PCU of NGP1-01 as a polycyclic cage compound with inherent ability to block *N*-methyl-D-aspartate (NMDA) channels (Kademani *et al.*, 2014; Parsons & Gilling, 2007; Danysz *et al.*, 1997) was chosen to be explored as a resistance reversal agent in this study. Furthermore, to the best of our knowledge this is the first time adamantane the smallest diamondoid is been investigated as a RA conjugated to the CQ-like nucleus.

The interest to explore adamantane is also encouraged by the following: The known RAs used for other RCQ compounds like calcium channel blockers, antihistamines and antidepressants have high cardiac and central neuronal system side effects and cross-resistance have also been observed with these classes of molecules (Mushtaque & Shahjahan, 2015; Zishiri *et al*., 2011, Kelly *et al.*, 2007). Adamantane on the other hand have not been associated with these effects. The adamantane moiety also facilitates the formation of a tertiary amine with the *N*-alkyl amino side chain of the proposed structures which is necessary for antimalarial activity *via* its protonation (Kelly *et al.*, 2007). Furthermore, the diamondoid and bulky nature of the adamantane moiety could also confer metabolic stability to the terminal tertiary amine from *N*-dealkylation as in the case of CQ (Geldenhuys *et al*., 2005). This could then lead to prevention of pruritus side effect commonly associated with CQ administration as well as cross resistance with CQ (Aghahowa *et al*., 2010).

There are controversies as to the appropriate length of linker neccessary for optimum activity between the RA and the CQ-like nucleus. While some studies show that the optimum length is between 2-3 carbons (Joubert *et al.*, 2014), others say it can also be more than a 10 carbon chain linker (Ridley *et al.*, 1996; Hocart *et al.*, 2011). This implies the linker length has a role to play in the antimalarial activity of the compounds. Thus, the effect of the linker length was also evaluated in this study by varying the carbon chain length.



## 1.3. AIM AND OBJECTIVES OF STUDY

The main aim of this study is to synthesize two series of novel adamantane-chloroquinolin (AD-CQ) conjugates (**figure 1.3**) as potentially improved reversed CQ compounds to overcome CQ resistance by *Plasmodium falciparum* and to investigate their antimalarial activity. To obtain this aim, the following objectives were set:

- Design model compounds that fulfil the requirements of potential reversed CQ agents.
- Synthesis, purification and structural elucidation of the *N*-(7-chloroquinolin-4-yl)alkanes-1-n-diamines of different lengths (aminoquinoline (ACQ) intermediates, compounds 1 - 4).
- Synthesis of the adamantane diketone moiety through a 3 step synthesis process.

- Synthesis of the AD-CQ conjugates of series 1 (aza-adamantanols, compounds 5 - 8) (**figure 1.3**).
- Synthesis of AD-CQ conjugates of series 2 (imine-adamantanes, compounds 9 – 12)(**figure 1.3**)
- Structure elucidation of the synthesized series of compounds using NMR, MS and IR.
- Screening of the synthesized compounds for cytotoxicity.
- Evaluation of the compounds for antimalarial activity and resistance reversal activity

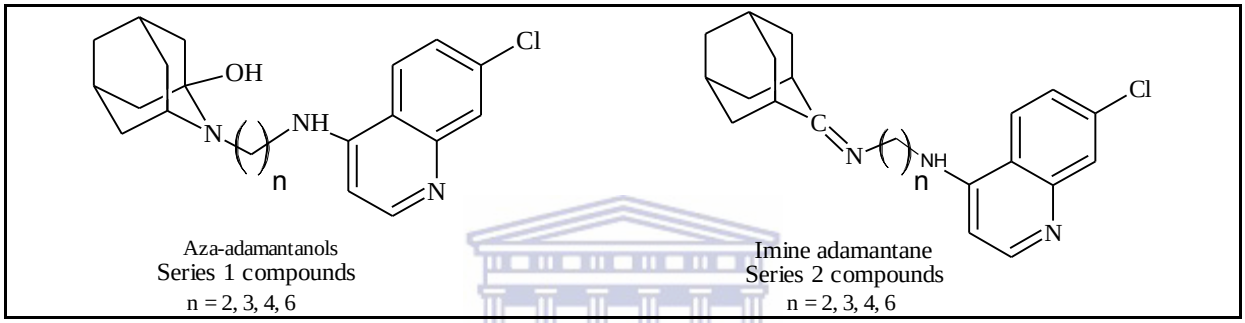



**Figure 1.3:** Representative structures of the two adamantane-chloroquinolin conjugates series.



## 1.4 HYPOTHESIS

CQ reversed molecule is a viable strategy to reverse CQ resistance in *P. falciparum* CQ resistant strains. Adamantane derivatives are known to have inherent channel blocking activity (Joubert *et al.*, 2011; Geldenhuys *et al.*, 2007). However it is not known whether such inherent channel blocking activity can inhibit the efflux pumps associated with malaria resistance. In this study, the series of synthesized novel adamantane-chloroquinolin compounds are proposed to exhibit the following properties:

- The 4-aminoquinoline pharmacophore (CQ-like portions) of the molecule will confer antimalarial activity to the molecule by binding to the target haem.
- The channel blocking properties of the adamantane may enable these molecules to block the efflux pump associated with CQ resistance and enhance accumulation of the drug, thus improving its use in malaria chemotherapy. It is also expected that its lipophillic nature will enhance permeability into the food vacuole.

- The presence of the tertiary amine will increase the basicity of the proposed novel structures. This will increase the degree of protonation of the novel molecules thus enabling greater accumulation of the drug in the food vacuole where it is proposed to act.

- The aza-adamantane moiety is bulky enough for steric hindrance, which will prevent metabolism of the tertiary amine required for antimalarial activity. It will also prevent cross resistance with CQ as well as avoid formation of the metabolite involved in psoriases which is a major reason for non-adherence.

## 1.5 RESEARCH QUESTIONS

Malaria poses a global health problem which is exacerbated by the increasing spread of *Plasmodium falciparum* resistance to CQ. Due to *P. falciparum* resistance to almost all antimalarial agents especially to CQ, this study seeks to develop improved novel antimalarial alternatives for blood stage malaria. The good qualities of CQ encouraged the optimization of the already successful 4-aminoquinoline pharmacophore in this study to develop improved novel antimalarial alternatives for blood stage malaria parasite.

The main question posed in this research is, can adamantane with inherent channel blocking ability act as a *P. falciparum* CQ resistance reversal agent to produce improved RCQ compounds? Other questions include:

1. How would the series of novel adamantane-chloroquinolin conjugates be designed and synthesized?

2. What is the toxic effect of this novel AD-CQ conjugates on non-parasitic cells?

3. Do these novel compounds demonstrate any antimalarial activity?

4. Do these novel compounds exhibit any ability to reverse CQ resistance?

5. What is the reversal effect of the use of adamantane moiety as CQ resistance reversal agent compared to the pentacycloundecylamine moiety previously used?

6. What is the appropriate chain length for optimal activity of the linker between the 4-aminoquinoline pharmacophore and the reversal agent?

7. How would the presence or absence of a hydroxyl (-OH) substitution on the compounds affect the activity?

8. What is the effect of the pKa value of the terminal tertiary amine on the antimalarial activity of the compounds?

## 1.6 CHAPTERS OUTLINE

This study is divided into five chapters. Chapter 1 provides the context in which the research is set, the importance of the research, the aim and objectives of the study, the hypothesis and research questions. It also includes the chapter outline and conclusion.

Chapter two is the literature review. This chapter provides a background of malaria and available blood stage antimalarial drugs especially CQ. It also focuses on *P. falciparum* resistance, the development of reversed CQ agents as well as the importance of the polycyclic cage moieties in producing improved reversed CQ molecules.

Chapter three present the synthesis of the novel compounds which highlights all the experimental work carried out in this study to produce the novel adamantane-chloroquinolin (AD-CQ) conjugates, including the intermediates required. These comprise what was done and used, giving detailed synthetic procedures for the designed series of compounds as well as challenges that were encountered. Two test series of the novel AD-CQ conjugates of four compounds each were synthesised by conjugating the adamantane moiety to a CQ-like nucleus.

Chapter four deals with the biological assays done and results obtained. The cytotoxicity profiles of the compounds were investigated. It also explains how the synthesized compounds were evaluated for antimalarial activity on both $CQ^S$ and $CQ^R$ strains and the results used to investigate their resistance reversal ability against $CQ^R$ isolates of *P. falciparum.*

Chapter five gives an overall conclusion of the study and recommendations for future studies.

## 1.7 CONCLUSION

Resistance to antimalarial drugs especially CQ is a major setback in malaria chemotherapy for the prophylaxis and treatment of blood stage malaria. The hybridization of adamantane as reversal agent to a 4-aminoquinoline nucleus *via* an alkyl amino linker of appropriate length

may produce a potentially improved reversed CQ molecule. It is expected that these novel synthesized compounds will have improved antimalarial activity compared to CQ because of their potential ability to accumulate in the food vacuole of chloroquine resistant strains by blocking the CQ efflux system mediated by the *Pf*CQRT. This ability may make the resistant strains sensitive to the CQ nucleus again. Being designed with proposed reverse CQ agents in mind, the compounds may also have improved pharmacokinetic properties as a result of the lipophillic nature of adamantane. These molecules will potentially lead to improved chemotherapy to overcome the malaria disease, resulting in decreased morbidity and mortality associated with malaria, especially in Africa. In addition, these hybrid-reversed CQ molecules are expected to reduce the burden of cocktail administration of CQ and a reversal agent as proposed in other studies in that it will decrease cost, increase efficacy and adherence, and reduce toxicity by decreasing the concentration of each compound required.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 INTRODUCTION

This chapter reviews some literature on malaria. It provides a background of malaria and the available classes of blood stage acting antimalarials especially chloroquine (CQ). It looks at *P. falciparum* resistance to CQ, the development of reversed CQ agents as well as the importance of the polycyclic cage moieties as reversal agents in producing improved reversed CQ molecules. The aim of this chapter was to understand the vulnerability of the parasite, the basic mechanisms by which CQ antimalarial drugs act and how resistance to CQ developed. This enables the optimisation of CQ, so as to design new chemical therapeutic derivatives, rather than focusing on discovering new entities with new mechanism of action, to save time and cost.

The 4-aminoquinolines and their derivatives especially CQ have been the most significant antimalarial agents synthesized and used for the treatment and prevention of malaria. However, the increasing emergence and widespread multidrug resistant *P. falciparum* strains, has put chemotherapy (the mainstay of malaria management and control) under constraint, thus raising health concerns. Therefore, there is a need for the continued search for improved synthetic alternatives. This research is aimed at making a contribution by developing an improved alternative to CQ to address the problem of CQ resistance.



## 2.2 MALARIA BACKGROUND

### 2.2.1 History of malaria

Malaria is an ancient disease that dates centuries, following the awareness of the characteristic poor health, malarial (mash) fevers and enlarged spleens seen in people living in marshy places. For many years, the idea that malaria was air borne was generally accepted, thus the name *mal'aria* meaning 'spoiled air' in Italian (Reiter, 2000). With the discovery of bacteria in 1676, and the incrimination of microorganisms as causes of infectious diseases followed by the development of the germ theory of infection by Louis Pasteur and Robert Koch in 1878-1879, the search for the cause of malaria intensified. However, scientific

understanding of the malaria disease was possible only after the discovery of the plasmodium parasites in the blood of malaria patients by Charles Louis Alphonse Laveran in 1880 and the discovery of the plasmodium sexual stage in infected birds by William MacCallum (Bruce-Chwatt, 1988; Sullivan, 2002).

Furthermore, the identification of mosquitoes as the malaria vector was first shown for avian malaria by Ronald Ross in 1897 following his elucidation of the whole transmission cycle of the avian malaria parasite *P. relictum* in culicine mosquitoes (Meshnick & Dobson, 2001). With further studies by the Italian malariologists Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava between 1898 and 1900, mosquitoes were confirmed to also transmit malaria to humans (Bruce-Chwatt, 1988; Cox, 2010).

Today, malaria is known as a devastating mosquito-borne infectious disease of humans and other animals, caused by the parasitic protozoan *Plasmodium*. It is transmitted principally by the female Anopheles mosquitoes during feeding on a blood meal. In some rare but possible cases malaria is transmitted from human to human *via* transfusion of malaria infected blood (Ekwunife *et al*., 2011; Owusu-Ofor, Gadzo and Bates, 2016; WHO, 2016a). Also, malaria can be 'congenital', transmitted from an infected mother to foetus before or during delivery (Solomon, Okere and Daminabo, 2014).



Five species of Plasmodium are known to affect humans and these include *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. Of all these species, *P. falciparum* is the deadliest species associated with most deaths (Maguene *et al*., 2015; Burgess *et al*., 2010; Yearick *et al*., 2008; Kaur *et al*., 2009). *P. falciparum* is different from the other species known to affect humans in that it has the ability to adhere to the endothelium of blood vessels during the blood stage of the infection and limit blood supply to different organs. *P. vivax* and *P. ovale* infections make eradication of malaria difficult because they have the ability to remain dormant in the liver for months as hypnozoites without any symptoms and cause relapses after malaria treatment or years of initial infection. *P. malariae* is unique in that it is able to persist asymptomatic for years in the blood stage while *P. knowlesi* is a zoonotic infection. *P. knowlesi* is an infection of monkeys transmitted to humans by the mosquito vector.

*P. vivax* although less researched is highly disabling and malaria infection by it, is common in tropical areas outside Africa as a result of the absence of the Duffy blood group antigen

receptor necessary for *P. vivax* invasion of the red blood cells (RBCs) expressed in most Africans. Also, *P. vivax* can develop within the mosquito at a lower temperature than that required for *P. falciparum* thus can survive in cold temperatures (Greenwood *et al.*, 2008). Baird (2013) concurs with Anstey and colleagues (2009) to argue that *P. vivax* is an equally life threatening Plasmodium species associated with as many debilitating clinical cases as *P. falciparum.* However, *P. falciparum* is of greater importance in this study because it is the most prevalent malaria parasite on the African continent where more deaths occur. Also, it is associated with severe malaria and responsible for most malaria-related deaths globally. Additionally, it is the most implicated parasite in antimalarial resistance making the control of the disease a challenge (Trampuz *et al*., 2003; WHO, 2016a; MMV, 2017; WHO, 2016b).

### 2.2.2 Life cycle of malaria

Malaria infection is caused by the parasitic protozoan of the genus *Plasmodium* which has a complex life cycle completed in three stages. These include the exo-erythrocytic stage (in the liver), the intra-erythrocytic stage (in blood) and the sporogonic stage (in the mosquito) as shown in **figure 2.1**. It involves two hosts - the female *Anopheles* mosquito (vector) and humans or animals (secondary host) (Foley & Tilley, 1998; Hay *et al*., 2010; Kumar, Kumari and Pandey, 2014).



The life cycle of Plasmodium illustrated in **figure 2.1** begins with the inoculation of sporozoites, the motile infective form of the parasite by the female Anopheles mosquito into the blood stream of the human host during a blood meal. The sporozoites target and access the liver rapidly through the blood stream with the help of the circumsporozoite protein and invade the hepatocytes (liver cells) (**A**). This begins the asymptomatic liver stage of the infection (Sinnis *et al.*, 1994). The parasite stays in the liver cells for 5 to 16 days where it replicates asexually *via* tissue schizogony to produce thousands of merozoites contained in schizonts (mother cell). *P. vivax* and *P. ovale* hypnozoites attack this stage and remain dormant in the liver cells (White, 2011).

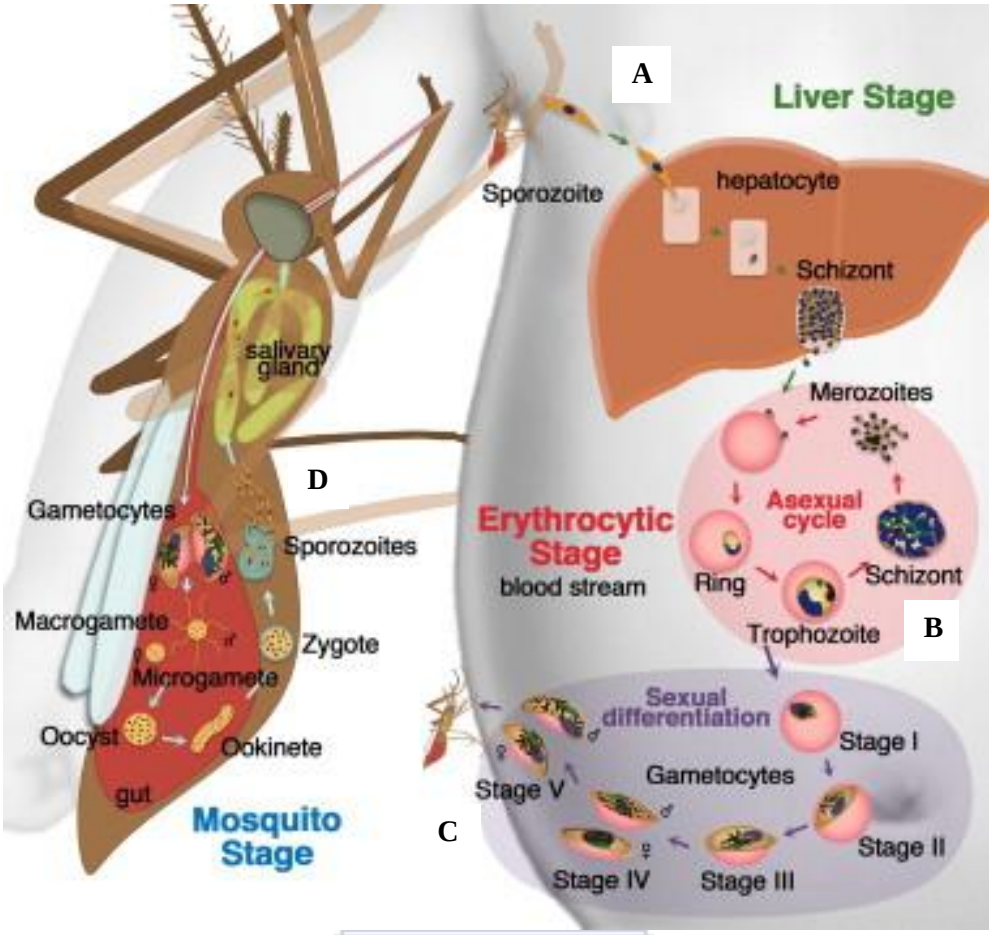





**Figure 2.1:** The life cycle of Plasmodium. (Adapted from: Baimote, Wanner and Le Roch, 2013)

A rupture of the liver cells as the schizonts burst, releases thousands of merozoites which flood into the blood stream and infect red blood cells (RBCs) (Sturm *et al.*, 2006). This begins the blood stage of the malaria infection cycle. The merozoites in RBCs start another series of rapid asexual proliferations known as blood schizogony producing 8 to 32 new schizonts, each containing merozoites. These merozoites differentiate through different forms from ring forms to trophozoites to schizonts as shown in **figure 2.1 (B)**. The infected RBCs rupture as the schizonts burst and many merozoites are again released into the blood stream to start a new infective cycle infecting new RBCs. The release of merozoites in blood and infection of new RBCs is an almost indefinitely repeated process taking place every 24 hours (*P. knowlesi*), 48 hours (*P. falciparum*, *P. vivax* and *P. ovale*) and 72 hours (*P. malariae*). This increases the number of parasites in the blood (Greenwood *et al.*, 2008) and is responsible for the symptomatic phase of malaria infections. In the course of disease progression, some trophozoites differentiate sexually from immature gametocytes to mature male and female gametes that circulate in the peripheral blood awaiting ingestion by a female

anopheles mosquito for further development (**C**) (Cooke, Mohandas and Coppel, 2001; Cowman, Berry and Baum 2012; Miller, Good and Milon, 1994). Targeting of the blood stage is therefore most critical for the control of clinical cases of the disease and associated morbidity and mortality.

The circulating gametes are fertilized in the gut of the mosquito once they are ingested during another blood meal on the infected person by the female anopheles mosquito as illustrated in **figure 2.1** (**D**). This is the asymptomatic mosquito stage. In the mosquito gut, fertilization occurs between the gametes to form a motile zygote called an ookinete. The zygote then develops into new sporozoites *via* sporogony which migrate to the mosquito salivary glands in preparation for inoculation during the next blood meal starting the cycle over again (Cowman, Berry and Baum 2012). Development of drugs that target this stage are important to prevent transmission.

### 2.2.3 Epidemiology

Malaria still remains a devastating infectious parasitic disease of great public health impact worldwide with 50 % of the world's population (3.2 billion people) at risk of being infected with the malaria parasite annually. According to the World Malaria Report (2017a) released by the World Health Organisation (WHO), estimates of 212 million malaria cases and 429 000 deaths were observed globally in 2015 only. This statistic estimate a 21 % drop in malaria new cases and 29 % decline in mortality rates registered worldwide when analysed from 2010 to 2015, resulting in a total of 6.8 million deaths by malaria averted. Despite this success registered in lowered disease burden, malaria still remains endemic in more than 91 countries (WHO, 2017a; WHO, 2017c). This means more than 40 % of the people in these countries are infected with malaria.

This is a cause for concern because a greater number of malaria deaths are not reported as they are handled at home with complementary medicines and strategies to notify and record these deaths are nowhere to be found. This was investigated by Murray and colleagues (Murray *et al.*, 2012) and indicated that counting all the undiagnosed, untreated and unreported malaria cases doubles the reported mortality rate (Talisuna, Bloland and D'Alessandro 2004; Breman, 2001). Nevertheless, malaria is preventable and curable when diagnosed and treated on time using effective antimalarial drugs. Although malaria is also common in the tropical and subtropical areas of Asia and Latin America, its main impact is

felt in Sub-Saharan Africa where almost 92 % of the global mortality burden occurs (WHO, 2016a; Kaur, *et al*., 2009; Greenwood & Mutabingwa, 2002).

In 1954, Africa was identified as the most malaria plagued continent and this was attributed to the late introduction of residual insecticides as the modern vector control strategy used in other areas at that time like USA in 1951 (Bruce-Chwatt, 1954). Recent data by WHO (2016a) revealed that increased control efforts implemented using insecticides bed-nets and surveillance are dramatically reducing the malaria burden in many places and especially in children below 5 years and pregnant women in Africa (Breman, Alilio and Mills, 2004). However, Africa still remains the most plagued malaria WHO region. From the above it can be seen that the intensity of malaria transmission in Africa is determined by epidemiological factors related to the vector, parasite, human host and environmental conditions.

There are more than 400 different species of *Anophelines* mosquito vectors that transmit the malaria parasite but only about 40 are malaria vectors of major importance known as "dominant vector species (DVS)" by malaria experts. DVS are capable of significant malaria transmission (Hay *et al.*, 2010; Sinka *et al.*, 2012). Each species of these *Anopheles* mosquitoes has a preferred aquatic habitat such as small, shallow collections of fresh water, puddles or hoof prints, which favours transmission. These habitats are abundant during the rainy season in tropical countries (Autino *et al.*, 2012). Transmission is more intense in places where the mosquito lifespan is longer for complete development of parasites and their preference of feeding on humans rather than other animals. The long lifespan and strong human-biting habit of the African vector species contributes to the reasons why nearly 90% of the world's malaria cases are in Africa (Coluzzi, 1999). These vector species include the common *Anopheles gambiae* complex: *An. gambiae, An. arabiensis, An. merus, An. melas* and *Anopheles funestus, An. moucheti and An. nili* (Sinka *et al.*, 2010).*Anopheles gambiae* is a very efficient vector in malaria transmission because, they are long-lived, breed readily, occur in high densities in the tropical climates and also, they prefer to feed on humans rather than other animals. These species are exclusively found in sub-Saharan Africa (Gallup & Sachs, 2001). Their effective transmission rate which is the number of sporozoite-positive mosquito bites per person per year varies between 1 in part of Latin America and Southeast Asia to about 300 in some parts of tropical Africa. This is measured using entomological inoculation rate (EIR) (Birley & Charlewood, 1987). *Anopheles funestus* is also a major vector in transmitting *P. falciparum* in Africa despite being sadly neglected, with most of the

research focusing on members of the *An. gambiae* complex (Charlewood *et al.*, 1995; Coetzee & Fontenille, 2004).

Human immunity or correctly put tolerance is also significant in malaria transmission. In areas of intense or stable malaria transmission where a healthy human is exposed to a fairly constant rate of malaria inoculation (from mosquito bites) all year round throughout their whole lives, morbidity and mortality during early childhood is very significant. The most vulnerable group of people affected by malaria are children below the age of 5 and pregnant women due to immature and compromised immune system (Luxemburger *et al.*, 1997; Carter & Mendis, 2002). However, for those who survive early childhood, some partial immunity against malaria is developed (Baird, 1995; Baird *et al.*, 1991). This partial immunity does not provide complete protection but reduces the risk of severe disease from malaria infection in adulthood by making the infection asymptomatic. On the other hand, in areas with low, erratic or focal transmission, full immunity is not achieved and symptomatic severe malaria cases may occur at all ages. This is known as unstable malaria. In addition, this immunity gained is lost if the individual is exposed to a long period of no reinfection such as in relocation from an endemic area to a malaria free area (Thomson, 1933). The above explain why children and pregnant women with low immunity in African countries with intense malaria transmission are more vulnerable to severe attack than adults whereas in low transmission areas, all age groups are at equal risk.



Furthermore, natural climatic conditions such as heavy rainfall patterns, temperature and relative humidity and altitude also greatly affect malaria transmission. This is because they affect the number and survival of mosquitoes (Carter & Mendis, 2002). In areas of unstable malaria like in South Africa (SA), transmission is mostly seasonal with the peak season during or just after the rainy season in many places. Malaria peaks in SA from September to May. In addition, human activities such as agriculture, deforestation, urbanisation, increase in international travel and influx of people with low immunity into intense malaria areas also affect malaria transmission and can cause a malaria epidemic (Autino *et al.*, 2012; Keiser *et al.*, 2004). Malaria epidemics or emergency is a threat for all, even in non-malarious areas due to global warming and seasonal change patterns threatening the world today. Thus malaria does not solely belong to the tropical countries, as it can occur anywhere when climate and other conditions favour transmission (Snow, 2015; Parham *et al.*, 2011)

The socioeconomic impact of malaria has been investigated by Gallup and Sachs (2001) and Sachs and Malaney (2002), and they found that malaria is a disease commonly associated to poverty and decreased economic growth with a causal relationship from both ends. At a global level, malaria incidence is concentrated in the world's poorest countries especially in sub-Saharan Africa. Although one cannot categorically say malaria causes poverty or poverty cause malaria, it is hard not to see the link between poverty and malaria. Malaria's impact on economic growth is huge. For this reason, malaria ridden countries cannot monitor and control the disease such that there is high malaria incidence in these countries. This eventually leads to high cost, disease burden and absences from work which leads to depression in economic growth and limits the countries' ability to monitor and control the disease. Thus it maintains a vicious cycle of disease and poverty (Breman, 2001).

### 2.2.4 Pathology of malaria and clinical manifestation

The pathology of malaria illness arises as a result of the processes that take place in the asexual blood stage. These processes involve the rupture of infected red blood cells to release parasitic waste and many merozoites parasites into the blood stream. These infect new red blood cells causing RBCs degradation by plamepsins and falcipains enzymes (aspartic acid proteases) (Egan *et al.*, 2000; Goldberg *et al.*, 1990). The proteolysis of the infected RBCs results in cell debris and toxins, which when released into the blood stream stimulate macrophages and other host cells to produce cytokines and other soluble factors responsible for an abnormal immune response and the clinical symptoms of malaria (CDC, 2010). Also, lysis of erythrocytes and hepatocytes directly affect blood counts and the liver. The involvement of blood makes malaria a potential multisystem disease because all organs of the body are in contact with blood. The clinical symptoms are manifested as either uncomplicated (typical) or severe malaria (Sunduru *et al*., 2009).

The typical symptoms of malaria are flu-like in nature with regular periodic occurrence of sudden coldness accompanied by shivering and then fever and sweating know as paroxysm. This occurs every four days (quartan fever) as in *P. falciparum* and *P. malariae* infections and every three days (tertian fever) in *P. vivax* and *P. ovale* infections (Carter & Mendis, 2002). Other symptoms include headaches and nausea, fatigue, body aches, and spleen enlargement if left untreated (MMV, 2015). Symptoms of malaria can recur after varying symptom-free periods following relapse, reinfection or recrudescence. Relapse occurs when symptoms reappear after parasite elimination from the blood by malaria treatment, due to

persistent parasites as hypnozoites in the liver cells and usually occurs between 8 - 24 weeks (Nadjm & Behrens, 2012). Recrudescence is the return of symptoms after a symptom-free period caused by parasites that survived in the blood as a result of inadequate or ineffective treatment (White, 2011; Baird & Rieckmann 2003). Reinfection means the parasite that caused the past infection was eliminated from the body but a new parasite was introduced. Reinfection cannot readily be distinguished from recrudescence, although recurrence of infection within two weeks of treatment from the initial infection is typically attributed to treatment failure (Tran *et al.,* 2012; WHO, 2015)

If not treated, uncomplicated malaria infections become severe leading to other life threatening conditions and are mostly associated with the *P. falciparum* parasite. In severe malaria, parasite proliferation in the erythrocyte causes structural, biochemical and mechanical modifications which increase the rigidity and adhesiveness of the parasitized RBCs. This increased adhesiveness causes the infected RBCs to continuously and simultaneously bind to vascular endothelium (cytoadherence) and to non-infected erythrocytes (rosettling). This binding can cause the accumulation of parasitized cells in the small vessels and locally sited capillaries, resulting in vascular occlusion and inflammation. This results in decreased blood supply thus limiting oxygen supply to vital organs causing dysfunction of the organs. Some serious complications associated with severe malaria include respiratory distress, glomerulonephritis (kidney inflammation), acute renal failure, hypoglycaemia, severe anaemia, cerebral malaria, metabolic acidosis and pulmonary oedema (WHO, 2015; White, 2004; Wilairatana *et al.,* 2002). Cerebral malaria and respiratory distress are the major causes of death by *P. falciparum* especially in infants as it leads to convulsions, coma and death if not attended to immediately (Chen, Schlichtherle and Wahlgren, 2000; Foley & Tilley, 1998; Wiesner *et al.,* 2003; Miller, Good and Milon, 1994).

The malaria parasite has been able to survive from ancient times until now *via* the development of many mechanisms through mutations to avoid natural as well as host induced immune responses. The most notorious survival strategy of the parasite is its ability to undergo almost unlimited antigenic variations, changing the antigens on the surface red blood cells such that it is not recognized. This provides for temporary parasitisation and often causes death of their host in the end (Chen, Schilchtherle & Wahlgren 2000).

### 2.2.5 Control of malaria

To decrease and eliminate host morbidity and mortality, malaria must be controlled. The control of malaria infection involves the integrated efforts of vector control, chemotherapy and policies. Currently, the limitation of vector control as well as the delay to get an effective vaccine and ineffective policies surveillance makes chemotherapy the mainstay strategy to control and eliminate malaria as per WHO Global Technical Strategy (GTS) for Malaria 2016-2030 (WHO, 2017b; Aguiar *et al.*, 2012). Presently, the fight against malaria is steered towards malaria elimination which is defined as the reduction to zero new malaria cases of a particular human malaria parasite within a defined geographical area as a result of deliberate activities. This will require integrated efforts to prevent transmission re-establishment. Once this is achieved permanently worldwide, then malaria can be eradicated for a malaria free world (WHO, 2017b).

Malaria had once occurred widely in the temperate areas of Western Europe and the USA. However, it was successfully eradicated through the vector control campaign with DDT (dichloro-diphenyl-trichoroethane) insecticide between the late 1940s and early 1950s (Zucker, 1996). The success of this campaign led to the creation of the Global Malaria Eradication in 1955 by the World Health Organisation. This programme which depended on CQ for both treatment and prevention and DDT for vector control was relatively successful only in areas with relative low transmission rate and developed economy (Carter & Mendis, 2002; Tanner & Savigny, 2008). Nonetheless, the goal of 'Global malaria eradication' was never achieved in most parts of Africa, and was officially abandoned in 1972 because of the lost political will due to war and unrest at the time, emergence of CQ resistant parasites and DDT insecticide resistant anopheles mosquitoes (Brito, 2001; Bruce-Chwatt, 1988; Greenwood *et al*., 2008).

With the 'crushed hope' of achieving global malaria eradication, the new strategy of 'Malaria control' was then introduced to reduce malaria incidence, prevalence or mortality (disease burden) to a level where it is not a public health problem anymore (Mendis *et al.*, 2009; WHO, 2017b). From the lessons of past failure, it was postulated that strengthening of health systems, infrastructure development and poverty reduction, especially in Africa, was required in addition to chemotherapy and vector control in order to effectively and efficiently control malaria (Crater & Mendis, 2002).

The introduction of the new strategy was followed by a global gloom period in the fight against malaria with increased malaria burden (Tanner *et al.*, 2015). The DDT resistant vectors led to the focus of malaria control and elimination being shifted and greatly dependent on chemotherapy (Aguiar *et al.*, 2012). However, there existed too few antimalarial drugs which made them vulnerable to emergence of resistance. Moreover, malaria at the time was known as a disease of the poor and this made investment into antimalarial drugs to be seen as a market failure by pharmaceutical industries and thus was not pursued. The public sectors such as government did not have all the innovative methods and finances to invest in malaria research (Mendis *et al.*, 2009). These limitations called for a partnership between the private and public as well as military sectors to combine research efforts and to finance international malaria programmes to fight this common enemy that threatened the world (Brito, 2001; Rieckmann & Sweeney, 2012).

Presently this has been looked into after the Global Malaria Control Strategy adoption of 1992 (WHO, 1993) and there are many new partnerships between governmental, academic, non-governmental organisations (NGOs) and pharmaceutical companies such as WHO's Medicines for Malaria Ventures (MMV). MMV was created in 1999 for the discovery, development and delivery of new antimalarials in collaboration with the public and the private sectors and many other funding organisations. These organisations include the World Bank's Roll Back Malaria (RBM) program, Bill and Melinda Gates Foundation's Malaria Vaccine Initiative and Multilateral Initiatives on Malaria and other national initiatives. The success of such partnership can be seen in South Africa (SA) in the collaboration of SA Medical Research Council (MRC) and UCT with Bill and Melinda Gates foundation to develop new medicines, vaccines and other biotechnologies against HIV/AIDS, TB and Malaria (Haldar & Philips, 2013).

Currently, no antimalarial vaccine exist commercially, except the RTS,S/AS01 vaccine based on the *P. falciparum* circumsporozoite protein which is in clinical trials. It is a pre-blood stage vaccine with promising results (Bejon *et al.*, 2008; Schwartz *et al.*, 2012). Also being tested are vaccines targeting the anti-merozoite surface protein of blood stages (McCa-rthy *et al.*, 2011) and the blockade of transmission (Herrera, Corradin & Arévalo-Herrera, 2007; Gregory *et al.*, 2012). With the adaptation by WHO that RTS,S be rolled out in 3 sub-Saharan countries as pilot projects and the funding for it already secured, the vaccinations are expected to start in 2018. However, vaccine development is still a challenge and a success story is yet to be told.

The history of malaria gives researchers the advantage to understand the disease and how it has evolved overtime as well as the prospects of understanding the gaps in malaria research which is one of the aims of this chapter. This section has shown that from a statistical point of view, malaria is a devastating parasitic infectious disease worldwide as 50 % of the world's population is at risk of transmission. Since all the clinical manifestations of malaria infection are now known to be from the blood stage, targeting the infection at this stage will decrease disease morbidity and mortality especially in sub-Saharan Africa. This research aims at synthesizing adamantane-chloroquinolin conjugates as reversed CQ molecules to overcome *P. falciparum* CQ resistance as a strategy to add to the antimalarial armoury to control and eventually eradicate the disease.

## 2.3 SCHIZONTICIDES: BLOOD STAGE ACTING ANTIMALARIAL AGENTS

The plasmodium parasite responsible for malaria pathology is most susceptible to drug attack at the blood stage. Antimalarials acting on the asexual erythrocytic *Plasmodium* parasites are known as blood schizonticides. Blood schizonticides are used for clinical prophylaxis (prevention of clinical symptoms by acting on the asexual parasite in blood); clinical cure which entails relief of immediate flu-like symptoms of an attack without necessarily eliminating infection and radical cure which is complete elimination of blood and tissue parasite from the body (Olliaro, 2001). For decades, malaria chemotherapy has been sustained by a handful of drugs, each with their own pharmacological limitations, of which parasite resistance has been the most significant (Biagini *et al.*, 2003). The classes of drugs commonly used with blood schizonticidal activity include antifolates (e.g. pyrimethamine, proguanil); artemisinin and related derivatives (artesunate, artemether, arteether) and the quinolines (e.g. CQ, mefloquine, quinine, amodiaquine) (Olliaro, 2001; Shaik, 2010; von Seidlein & Greenwood, 2003; Jana & Paliwal, 2007; Saifi *et al.*, 2013). These are presented in **figure 2.2**.

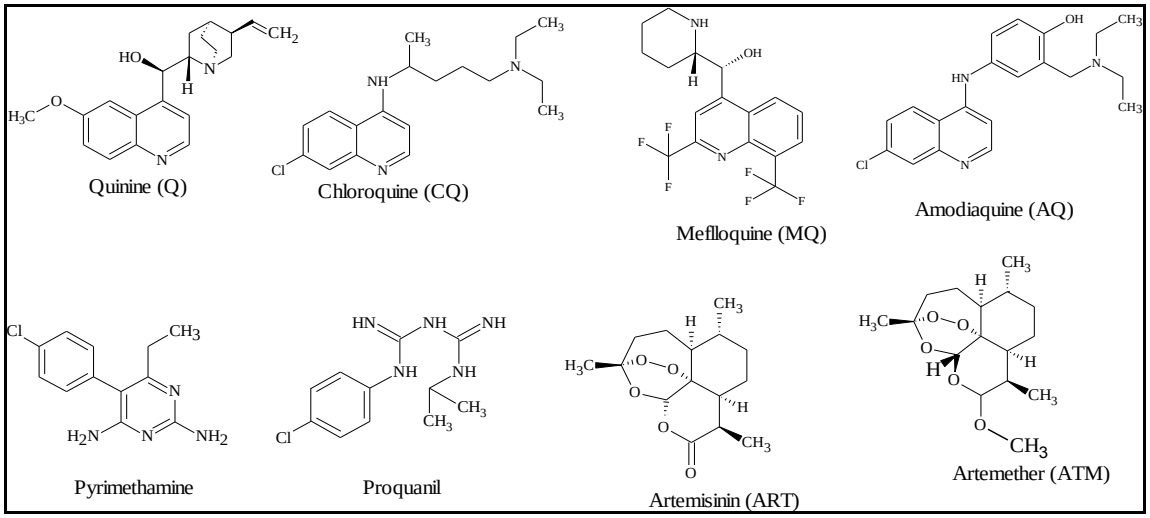



**Figure 2.2:** Structures of some blood stage Schizonticides

### 2.3.1 Antifolates

Antifolate antimalarials are drugs that inhibit the parasitic folate pathway enzymes: dihydrofolate reductase (DHFR) by pyrimethamine, cycloguanil and dihydropteroate synthase (DHPS) by sulfadoxine and other sulfa drugs in the production of parasitic folate (**figure 2.3)**. These enzymes are involved in the biosynthesis of parasitic nucleic acids and some amino acids necessary for parasitic growth (Saifi *et al.*, 2013). These drugs have been used successfully for the treatment of malaria but the rapid emergence of drug resistance through point mutations in the genes coding for DHFR and DHPS enzymes, have restricted their use (Biagini *et al.*, 2003; Sibley *et al.*, 2001).

The first antifolate drug produced was proguanil marketed as Paludrine® in 1945. Further work on it by Carrington and co-workers (1951) showed progunail to be a prodrug as its antimalarial activity was attributed to one of its metabolites, cycloquanil, an active form of the drug. It later became the template for pyrimethamine synthesis by Falco and colleagues (1951). DHPS inhibitors had unacceptable toxicity profiles and low efficacy when used alone in antimalarial therapy, but showed good synergistic effects with DHFR inhibitors. Thus, they were used in combination therapy. The most widely used combination was sulfadoxine and pyrimethamine (SP) marketed as Fansidar® (Nazila 2006; Saifi *et al.*, 2013). Despite the combination of DHFR and DHPS inhibitors, it soon faced resistance and was short lived.

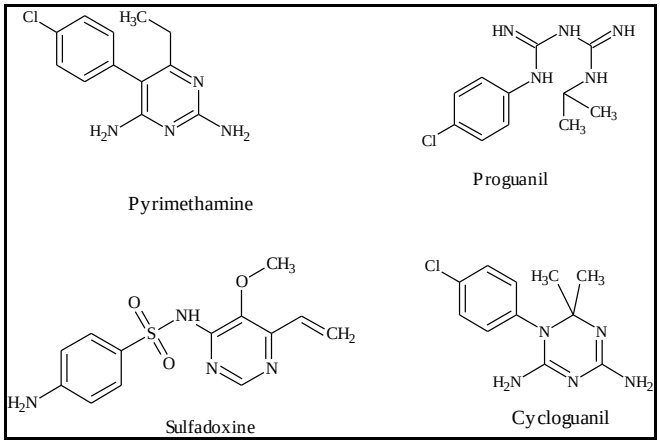



**Figure 2.3:** Antifolates antimalarial drugs

### 2.3.2 Artemisinin and derivatives

Artemisinin (ART) discovered by Chinese scientists from sweet wormwood *Arteminsia annua* in 1972, is a sesquiterpene lactone with an endoperoxide bridge essential for antimalarial activity. The discovery of artemisinin rescued the antimalarial chemotherapy as all the other antimalarial classes available at the time were faced with resistance (White, 2008).

The antimalarial activity of artemisinin and derivatives is by interaction with haem *via* the endoperoxide bridge to form oxygen free radicals. These free radicals alkylate protein and damage parasitic micro-organelles and membranes (Meshnick, Taylor and Kamchonwongpaisan, 1996). The endoperoxides also inhibit parasitic polymerization of degraded haemoglobin to haemozoin.

The natural structure of artemisinin served as a template for the development of semi-synthetic derivatives like artemether and artesunate in 1987 as well as dihydroartemisinin (DHA), an active metabolite of artemisinin in 1992. The derivatives were modified to improve water and oil solubility to ease formulation into different dosage forms (Krishna, Uhlemann and Haynes, 2004; Biagini *et al.*, 2003). The use of artemisinin and derivatives in monotherapy had as a major drawback, susceptibility to an increased rate of malaria infection recrudescence due to their short half-lives and short-course of treatment (Meshnick, Taylor and Kamchonwongpaisan 1996). Consequently, artemisinin-based combination therapies (ACTs) were introduced in an attempt to reduce recrudescence with monotherapy and to slow down potential resistance. Artemisinin-based combination therapies (ACT) were declared by the WHO as the first line treatment for *P. falciparum* malaria infection in 2004 (WHO, 2006; Schoepflin *et al.*, 2010). In addition to other drawbacks, ART and its semi-synthetic

derivatives are commercially limited because it is a natural product thus requires extraction which is costly.

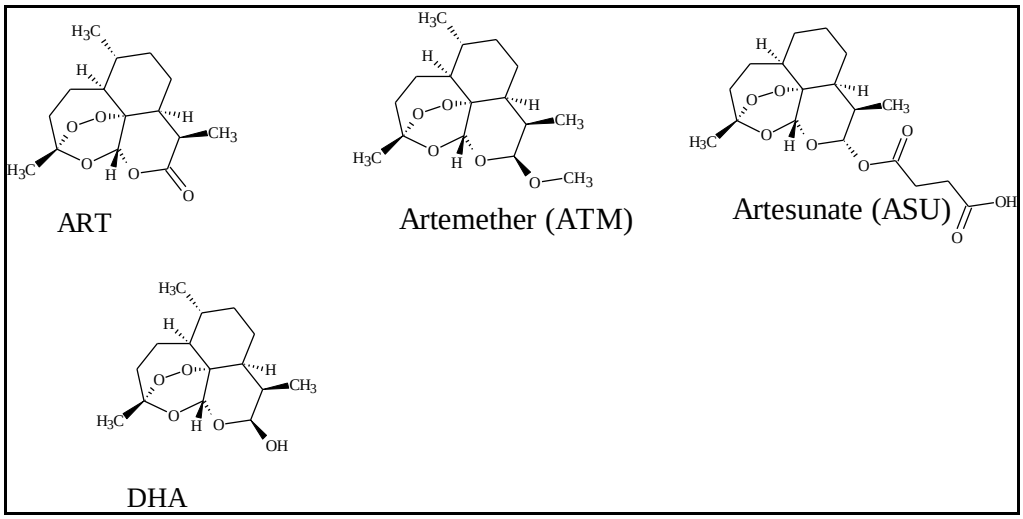



**Figure 2.4:** Artemisinin and semi-synthetic derivatives

Further work on artemisinin led to the discovery of other synthetic antimalarial drugs like pyronaridine in 1973 and lumefantrine (benflumetol) in 1976, to expatiate on the ACTs development. Synergistic effects between lumefantrine and artemether resulted in a new combination in China in 1992. This new combination drug was registered in 1999 in Switzerland as Coartem® and added to the WHO Essential List in 2001 (Premji, 2009). Another combination drug artemisinin and piperaquine was tested and DHA/piperaquine phosphate was registered and produced in Vietnam in 1997 as Artekin®. Artemisinin and derivatives have also shown to have activities against other parasites, viruses and cancers (Krishna *et al.*, 2008). However, the emergence of *Plasmodium falciparum* resistance to artemisinin threatens the usable life span of ACTs and the armoury of antimalarial chemotherapy.

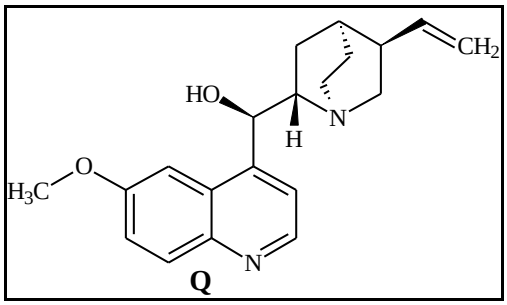


### 2.3.3 Aminoquinolines and derivatives

Quinine (Q) is a natural alkaloid extracted from the bark of cinchona tree and was the first antimalarial agent to be used as standard for intermittent fever worldwide. Quinine has a weak gametocidal activity against *P. vivax* and *P. malariae* and is still used in the treatment of acute cases of severe *P. falciparum* infection. Quinine provided the scaffold for discovery and synthetic aminoquinolines development.

Further research in search of new antimalarial drugs to address the disaster caused by malaria in World War I spearheaded the exploration and discovery of new synthetic antimalarials.

This was due to the fact that world supply of quinine was cut off by the Japanese and quinine extraction from Chinchona bark was expensive and difficult and it also causes severe cardiac side effects (Brito, 2001).




**Q**

**Figure 2.5:** Structure of Quinine

In 1856, Henry attempted to synthesise QN but failed. However, in his failure, he succeeded in synthesizing the first synthetic water resistant textile dye. As dyes became useful to microbiologists and commonly used to stain and identify pathogens that were difficult to see under the microscope, methylene blue (**MB**) was discovered to be particularly effective in staining malaria parasites and found to have antimalarial activity.

Using MB as a prototype, scientists from Bayer a once German dye company turned pharmaceutical company, began to develop new synthetic antimalarials and discovered Pamaquine (**PM**) (plasmoquine) in 1925. **PM**, the first 8-aminoquinoline antimalarial drug was also capable of preventing relapses in *vivax* and *ovale* malaria. Nevertheless, it is no longer used clinically as per WHO recommendation because it is less efficacious than it is toxic (WHO, 2010; Peters, 1999)

In1932, they developed mepacrine (**MP**) (Atabrine®) which was effective against falciparum malaria. One of the biggest problems with this compound was its discolouration of the urine, skin and eyes. Although this was used for some time to monitor patient compliance, it greatly limited the use of mepacrine (Coatney, 1963).

Research for new antimalarial drugs continued and resochin was developed in 1934. Although it had potent antimalarial activity, reports from clinical trials showed it was too toxic and thus abandoned. In 1936, a derivative of resochin was developed known as sontochin (**NQ**) (nivaquine) which seemed less toxic. However, due to the greater loss of soldiers to malaria in the Pacific War of the 1940s, the Vietnam War, World War II and others in malaria zones than to the war itself, more efforts were made by Australian and American military to find the best antimalarial drugs to fight the disease (Brabin, 2014;

Rieckmann & Sweeney, 2012). Compounds previously synthesised were re-assessed and resochin was among them. The 'wonder properties' of resochin renamed **CQ** were seen and appreciated only in 1946 and was then designated the drug of choice for malaria treatment (Talisuna, Bloland and D'Alessandro 2004; Khan & Chand, 2015; Coatney, 1963).

With the success story of CQ, many other compounds previously made were reinvestigated against malaria and Camoquin (amodiaquine AQ) another 4-aminoquinoline was discovered. Amodiaquine is an orally active 4-aminoquinoline with antimalarial and anti-inflammatory properties. It is structurally similar to CQ but is superior in activity against CQ resistant *P. falciparum* strains. However, it has been associated with agranulocytosis and severe fatal acute hepatitis which limits its use in chemoprophylaxis (O'Neill *et al.*, 2003).

In the search for effective clinical derivatives of **PM**, primaquine (**PQ**) was discovered. **PQ** is the only approved drug commercially available for attacking the tissue hypnozoites reservoir of infection of *P. vivax* and *P.ovale* (Baird & Rieckmann, 2003; Greenwood, 1995). It is the prototype used for the design and synthesis of anti-hypnozoite derivatives for radical cure. Primaqiune is an all stages antimalarial drug: It kills the early and late stage tissue parasites in the liver as well as sterilizes the gametocytes of the blood stage of all species, thus stops disease transmission from host to vector (Davanco *et al.*, 2014). Although **PQ** exerts schizontocidal activity (Vale, Moreira and Gomes, 2009), it is less effective on *P. falciparum*. It interferes with ubiquinone function as an electron carrier in the respiratory chain of the parasite, thus disrupting its metabolic processes in the mitochondria (Hill *et al.*, 2006; Schlesinger, Krogstad and Herwaldt, 1988). Works of Alving and colleagues (1955) demonstrated that there is a synergy effect between **PQ** and blood schizonticides like CQ or quinine since it showed decreased efficacy when not co-administered with CQ (Baird & Rieckmann, 2003). However, metabolites of PQ cause serious side effects such as haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD) deficient patients and necessitate a pre-screening before the use of PQ. This limits the use of PQ only as a last resort (Fernando, Rodrigo and Rajapakse, 2011; Alving *et al.*, 1960). Therefore, PQ needs a better alternative that is safer, well tolerated by all, require no special diagnostics and with a short course of treatment.

Tafenoquine (**TQ**) a promising PQ analogue developed by the US Army and GSK (Elmes *et al.*, 2008; Wells, Burrows and Baird, 2010) shows better antimalarial activity against all three stages of malaria parasite life cycle compared to primaquine. Nonetheless, it still has the

potential side effect of haemolysis in G6PD deficient patients. Thus, **TQ** will have the same limitations as primaquine.

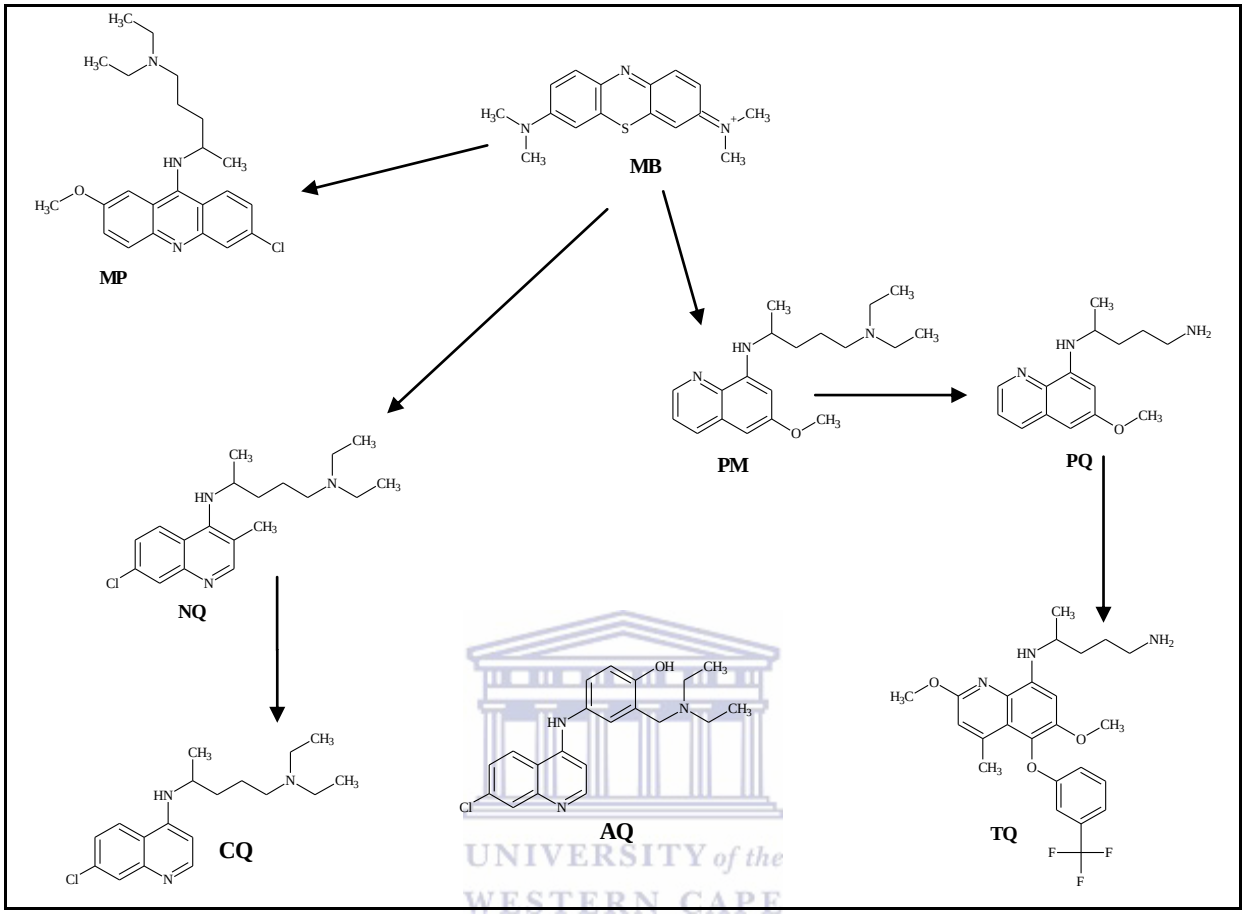



**Figure 2.6:** Aminoquinoline based antimalarial drugs and their analogues

## 2.4 CHLOROQUINE: BACKGROUND AND PROPOSED MECHANISMS OF ACTION

CQ is a diprotic weak base synthetic analogue of the natural quinoline quinine. This is illustrated in **figure 2.7**. Since its recognition as a potent and safe antimalarial in the 1940s, CQ has been used for many years as the mainstay malaria chemotherapy agent for both prophylaxis and treatment of the blood stage malaria (Bruce-Chwatt, 1981). This was because of its effectiveness and simple regimen, affordability, quick onset of action and readily accessibility in hyper-endemic areas, as well as a low toxicity profile that permitted its use in pregnant women and children who are the most vulnerable victims of the disease (Hawley *et al.*, 1998; Burgess *et al.*, 2010; Mushtaque & Shahjahan, 2015; Omodeo-Sale *et al.*, 2009; Wellems & Plowe, 2001). CQ is also useful in rheumatoid arthritis therapy as a disease modifying agent to decrease the symptoms.

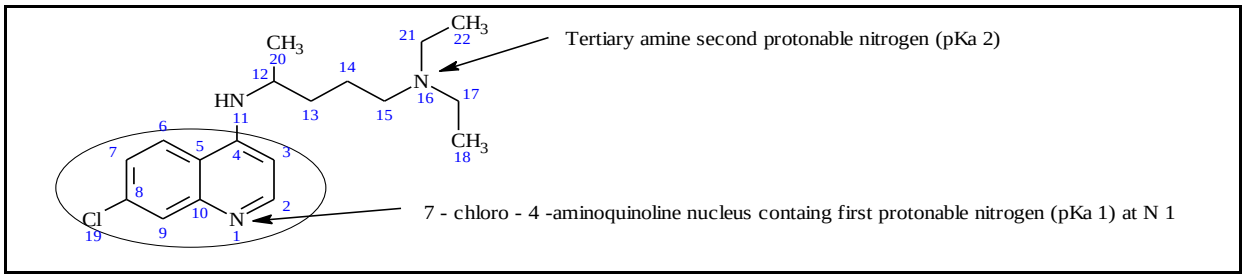



**Figure 2.7:** Structure of CQ showing protonable nitrogens

The site of action for CQ and other 4-aminoquinoline drugs to exert their antimalarial activity is the acidic parasitic digestive vacuole (DV) of pH 5.2 – 5.8 (Egan, Ross and Adams, 1994). The mechanism of action of CQ can be broadly explained by the two major theories of drug accumulation in the acidic DV by ion trapping and inhibition of haem polymerization **(**Kaschula *et al.*, 2002).

### 2.4.1 pH (Ion) trapping in parasitic DV (weak base) theory

At physiological pH (pH = 7.4), CQ is uncharged and very membrane permeable thus diffuses into the acidic DV (pH = 5.5) where it becomes double- ionized because of its dibasic nature (pKa1 = 8.1 and pKa2 = 10.2) (**figure 2.7**) (Martin *et al*., 2009; Olliaro, 2001). The ionized form is DV membrane impermeable hence it is trapped in and accumulates in the acidic food vacuoles depicted in **figure 2.8** (Homewood *et al.*, 1972). Ample evidence have demonstrated that the difference of pH gradient between the DV and external red blood cells is important for the accumulation of CQ and its derivatives in the DV (O'Neil *et al.*, 2011; Foley & Tilley, 1998; Martiney, Cerami and Slater, 1995; Geary *et al.*, 1990). Hence, an increase in vacuolar pH will reduce the proton gradient responsible for drug concentration and thus lead to decreased drug uptake and accumulation. The pH trapping aids in ensuring that the compound is concentrated at the site of action and is essential for drug activity (Hawley *et al.*, 1998).

Based on this theory, the adamantane-chloroquinolin conjugates in this study were designed with a tertiary amine on the side chain to have pKa close to that of CQ to ensure protonation in the DV.

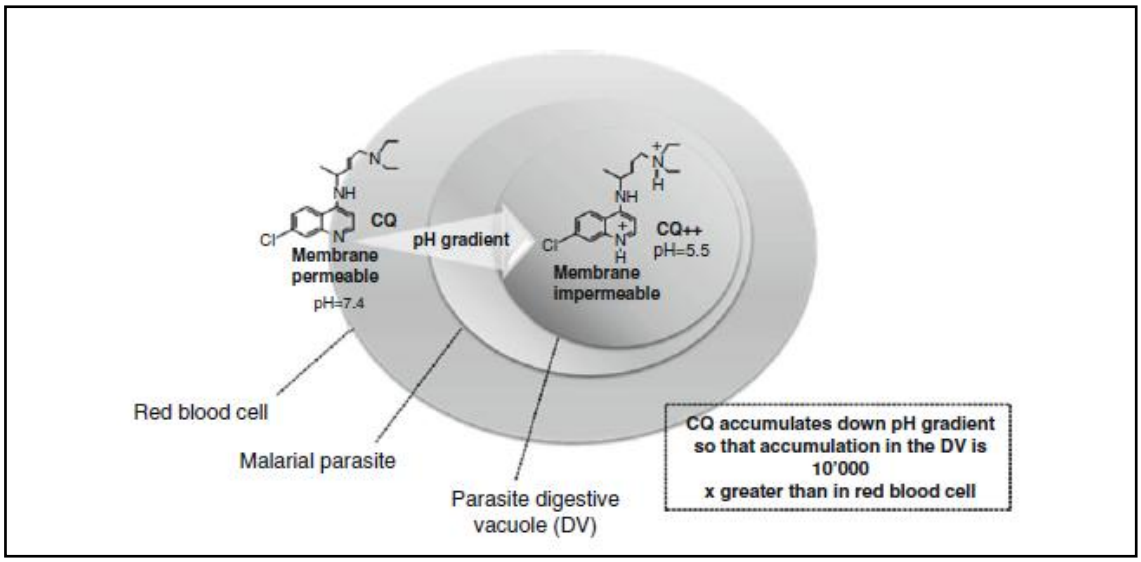



**Figure 2.8:** Diffusion of CQ and ion trapping due to pH gradient. (Adapted from: O'Neil *et al.*, 2011)

### 2.4.2 Haem polymerization to haemozoin inhibition theory

Once trapped, the concentration of CQ increases in the DV, the site of haemoglobin degradation. The trapped drug then react/complex with haematin (ferriprotoporphyrin IX (FPIX) from degraded red blood cells and inhibits parasitic haematin crystallization and detoxification to non-toxic insoluble haemozoin (Hz) crystals (Bray *et al.*, 1998; Slater & Cerami, 1992; Pagola *et al.*, 2000). The diagram in **figure 2.9** represents the process of haemozoin inhibition.



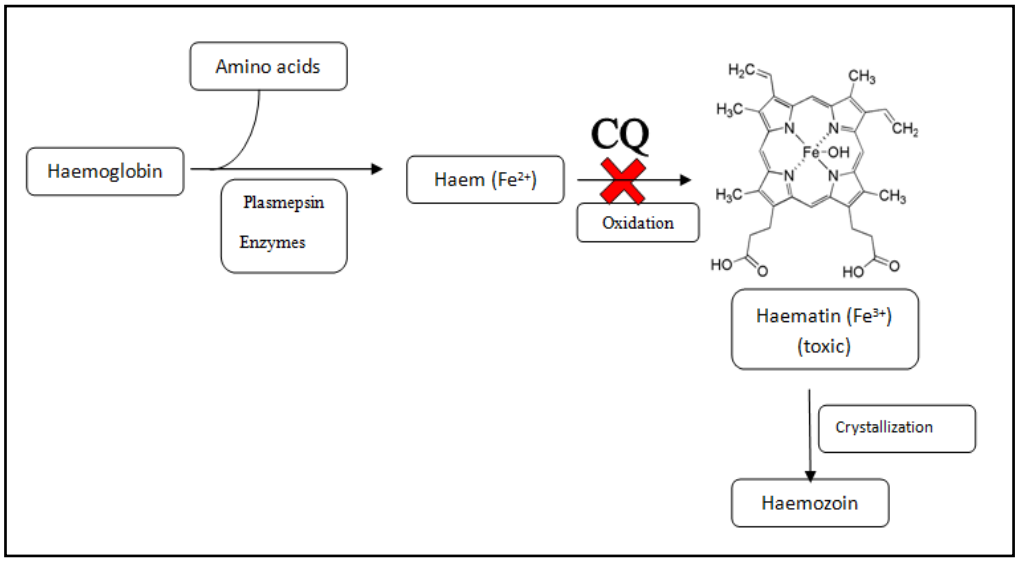



**Figure 2.9:** CQ inhibition of toxic haematin detoxification to harmless haemozoin

Intra-erythrocytic *P. falciparum* degradation of haemoglobin provides amino acids used for parasitic growth and also releases free haem which is rapidly oxidized to haematin toxic to the parasite. However, this toxic by-product is detoxified in the DV into haemozoin or malaria pigment crystals which are non-toxic to the plasmodium parasites but toxic to humans (Mushtaque & Shahjahan, 2015; Dorn *et al.*, 1995).

The CQ-FPIX complex inhibits the detoxification *via* crystallization and the accumulation of significant concentrations of toxic FPIX and complex adductsis ultimately responsible for killing the parasite (Foley & Tilley, 1998; Combrinck *et al*., 2013; Omodeo-Sale *et al*., 2009; Sullivan *et al*., 1998).

These mechanisms of actions above are attributed to the 7-chloro-4-aminoquinoline nucleus and basic amino side chain shown in **figure 2.10**. The 4-aminoquinoline pharmacophore is said to be essential for the complexation of CQ to FPIX resulting in inhibition of haemozoin formation and parasite growth while the basic amino side chain aids in trapping high concentrations of the drug in the acidic DV of the parasite (Egan *et al*., 2000; Glans *et al*., 2012; Yearick*et al*., 2008; Muraleedharan& Avery, 2007; Cheruku *et al.*, 2003).

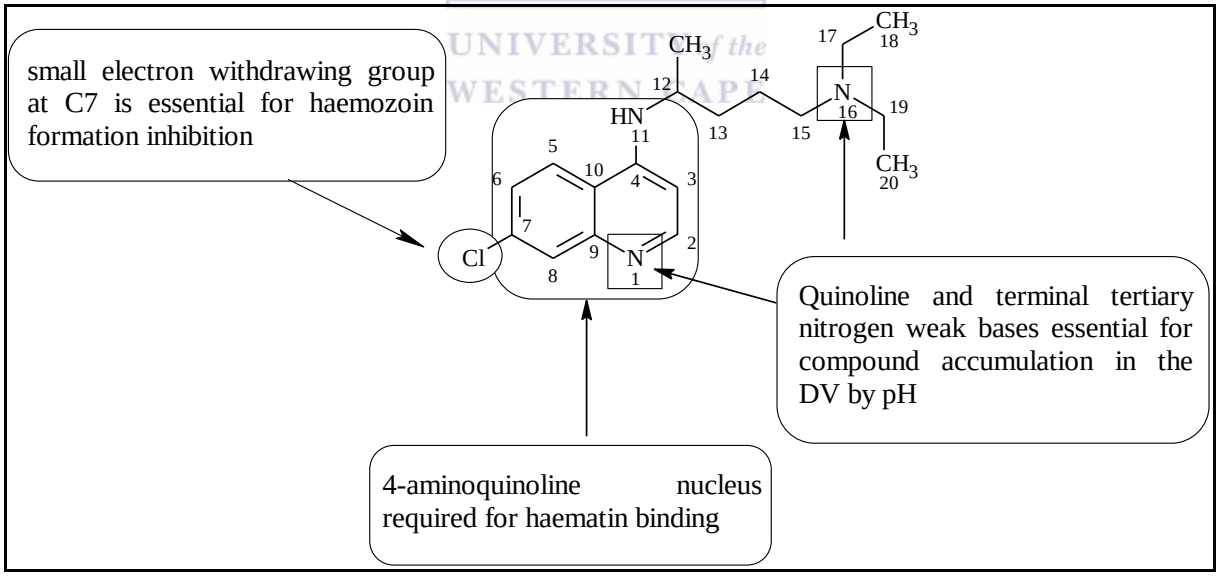



**Figure 2.10:** Structure activity relationship (SAR) of CQ

### 2.4.3 Inhibition of Glutathione and hydrogen peroxide mediated haem decomposition theory

Although it is generally accepted that CQ exerts its antimalarial activity by inhibiting the polymerization of haemozoin in the DV of the parasite (Egan *et al.*, 2000), it has been questioned and debated if this is the only means by which CQ is effective against the

plasmodia parasite. Other different mechanisms have been proposed (Sullivan, 2002), but the inhibition of both hydrogen peroxide-mediated decomposition of haem and cytosolic glutathione-mediated degradation of haem would be mentioned in this study. This is because these two routes have been proposed and investigated as defence mechanisms of detoxification by the parasite (Opsenica *et al*., 2013). Ferriprotoporphyrin IX is a pro-oxidant and catalyses reactive oxygen species production. This is because in addition to sequestering of the toxic haem by-product into insoluble haemozoin, ferrous haem is oxidized to ferric haem with the release of oxygen and superoxide for the production of hydrogen peroxide ($H_2O_2$) and other oxygen radicals (Loria *et al.*, 1999). This causes oxidative stress in the DV. Ginsburg and co-workers, (1998) also proposed that not all ferriprotoporphyrin is converted to haemozoin thus the remainder exits the DV to the cytosol where it is decomposed by glutathione.

These proposed mechanisms cannot be discredited by the findings of Egan and colleagues (2000); they rather strengthen each other as their mechanisms seem to be connected. Thus in addition to inhibition of haemozoin formation, the inhibition to both hydrogen peroxide and glutathione degradation of toxic haem are additional mechanisms to the mechanism of action of CQ to bring about parasite death. Although the rate of one can be more than the others, none can stand alone.



Additionally, despite resistance to CQ, novel drugs with CQ-like nuclei and proposed CQ mechanism of action are still in quest because the mechanism of action and mechanism of resistance of CQ are independent of each other (Omodeo-Sale *et al*., 2009; Egan *et al*., 2000).

## 2.5 *P. FALCIPARUM* CHLOROQUINE RESISTANCE

Despite the effective mechanism of action of CQ, *P. falciparum* with time has and continues to develop resistance to it and almost all other available antimalarial drugs, even the newest artemisinin-based combinations. This thus makes optimal treatment, control and ultimately elimination of malaria difficult (Saenz *et al*., 2012). Parasite resistance has been defined as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject". This takes into consideration that "the drug must gain access to the parasite or the infected red blood cells for the duration of the time necessary for

the normal action of the drug" (Bruce-Chwatt *et al.*, 1986;WHO, 2010;WHO, 2015;WHO, 2016c).

The first case of CQ resistant *P. falciparum* was observed in the Cambodia-Thailand border of Southeast Asia in late 1957, and it spread steadily through South America (Venezuela and Colombia) and India in the 1960s and 1970s. It was only in 1978 that Africa also joined the queue, when resistant *P. falciparum* strains were detected in Kenya and Tanzania. However, within a decade almost the entire continent was swept with resistance (Wellems & Plowe, 2001).

The frequent malaria treatment failures observed with CQ during this period led to the abandonment of CQ as a first line drug and ultimately a stop to its use in malaria infections. Thailand was the first country to replace CQ as a first-line drug in 1973 followed by several others in Asia and South America. Nonetheless, CQ was still useful in Africa until 1988 when KwaZulu-Natal in South Africa replaced CQ with Sulfodoxine-Pyrimethamine (SP) as a first line treatment for malaria (Bredenkamp *et al.*, 2001). Notwithstanding, the first country to change their national drug treatment policy as per WHO requirements was Malawi in 1993, followed by Kenya, South Africa and Botswana four years later (Bloland *et al.*, 1998; Talisuna, Bloland and D'Alessandro, 2004). This was the decline of the success story of CQ usefulness in malaria infections. It has been established that *P. falciparum* CQ resistance is as a result of decreased accumulation of CQ in the DV, for which there are many proposed mechanisms pointing to either increased efflux or reduced intake of drug (Van Schalkwyk & Egan, 2006;Wellems & Plowe, 2001). Consequently, there is decreased access of CQ to free heme for complexing and thus ineffectiveness of CQ. Many propositions have been made as to the mechanism of limited CQ accumulation in the DV of *P. falciparum* parasites, of which the major ones considered in this study are follows.



### 2.5.1 Increase CQ efflux rate out of parasitic DV theory

CQ resistance is based on reduced CQ accumulation in the DV of resistant parasites (Verdier *et al.*, 1985). This theory was confirmed by Krogstad and colleagues (1987) and shown to be due to CQ efflux from the DV. They demonstrated that CQ is efflux from $CQ^R$ parasites at a rate 40-50 times faster than in $CQ^S$ parasites despite the same rate of accumulation. This finding suggested a simultaneous expulsion of CQ out of the DV by an energy dependent process. This process was mediated by the ATP-fuelled multidrug efflux pump, *p*-glycoprotein (*p*GP) which is capable to expel a number of different drugs (Sharom, 2011).

Bray and co-workers in 1992 also investigated this phenomenon and discovered that both $CQ^S$ and $CQ^R$ parasites of *P. falciparum* have equal rates of CQ efflux. However, this new finding did not contradict the efflux pump theory of Krogstad and colleagues (1987), but suggested that more than the efflux of CQ out of parasitic DV is responsible for CQ resistance. This made room for further research into the difference in the $CQ^S$ and $CQ^R$ strains of *P. falciparum*. Consequently, mutation in the gene coding for *Pf*CQRT found in the $CQ^R$ strains was implicated in *P. falciparum* CQ resistance.

### 2.5.2 *Pf*CQRT mutations theory

CQ resistant *P. falciparum* accumulate less CQ compared to CQ sensitive parasites. The major explanation for the decreased CQ accumulation in the DV of resistant strains is attributed to multiple point mutations in the transmembrane protein *Plasmodium falciparum* CQ resistance transporter (*Pf*CQRT) localized in the parasitic DV membrane (Fidock *et al.*, 2000; Fitch, 2004; Djimdé *et al.*, 2004; Pulcini *et al.*, 2015). This putative drug/metabolite transporter recognizes and expels CQ out of the DV, its site of action (Bray *et al*., 2005; Burgess *et al*., 2010). The inhibition of this transporter is thus imperative.



## 2.6 REVERSED CHLOROQUINE (RCQ) MOLECULES

The search for new antimalarial agents is significant in the fight against plasmodium parasite resistance to today's drugs in order to increase the chemotherapy armoury. The strategies commonly used for new drug discovery are combination therapy, the use of resistance reversal agents (chemosensitizers), natural products, repurposing of drugs originally developed against other diseases, structural modification of existing antimalarial drugs and search of drugs with new parasitic targets and mechanism of action (Muregi & Ishih, 2010; Grimberg & Mehlotra, 2011). Chemosensitizers or reversal agents (RAs) are molecules that reverse resistance to a drug and a RA generally inhibits the efflux mechanism (Peyton, 2012). The aim of this study was to structurally modify the existing ineffective drug CQ by conjugating its pharmacophore with a potential resistance RA to form a dual functioned compound.

The emergency of CQ resistance led to increased morbidity and mortality (Trape *et al.*, 1998). This fuelled researchers to study the disease and parasite to understand them better. It was discovered that CQ resistance is due to decreased CQ accumulation in the DV thus

decrease access to the target haem-detoxification process (Bray *et al.*, 1998). The decreased CQ concentration is as a result of efflux of the drug by *Pf*CQRT and not as a change in the degradation process of haemoglobin or structure of free haem the target of CQ. Hence, targeting the inhibition of haem degradation is still a viable strategy as the unchanged process of haem degradation makes the parasite vulnerable to 4-aminoquinoline-based drugs at the right concentration. The discovery of compounds that enhance the accumulation of CQ in the DV hence, restore CQ sensitivity in $CQ^R$ strains when given together with CQ was ground breaking in the fight against *P. falciparum* CQ resistance. Some of these compounds which include verapamil (VP), despiramine and chloropheniramine (**figure 2.11)** are structurally different and belong to different classes of drugs such as: calcium channel blockers, antidepressants and antihistamines (Peyton, 2012; van Schalkwyk & Egan, 2006; Martin *et al*., 1987). They have been demonstrated to bind directly to the *Pf*CQRT in the DV membrane inhibiting the *Pf*CQRT CQ efflux system (Bray *et al*., 2005). Nonetheless, due to high doses often required for the polypharmacy administration of the chemosensitizers and CQ, and associated toxic effects, studies were turned towards the use of a more attractive hybrid-drug approach (Burgess *et al*., 2006; Joubert *et al*., 2014; Kelly *et al*., 2007; Peyton, 2012 ).



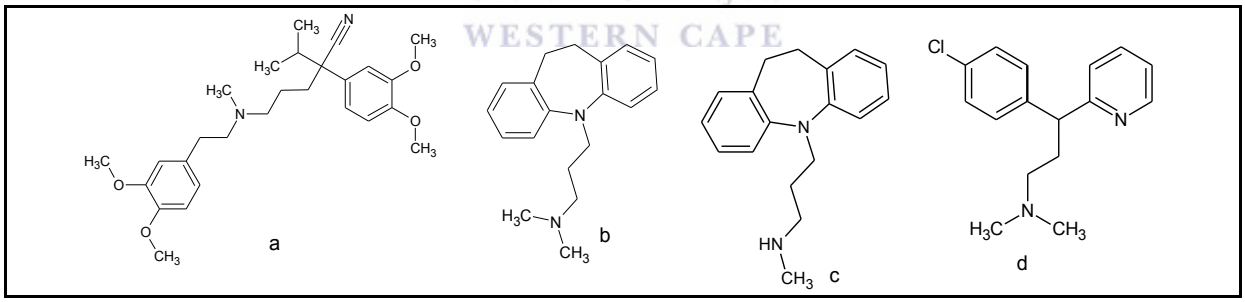



**Figure 2.11:** Some reported CQ reverser compounds. a. Verapamil b. Imipramine c. Desimipramine d. Chlorpheniramine

Drug hybridization is the conjugation of two pharmacophores to produce a single hybrid molecule with dual functionality or targets by rational drug design. This hydrid agent can then be further optimised. The conjugation of a 4-aminoquinoline pharmacophore (CQ-nucleus) to a resistance reversal agent using an alkyl linker is an innovative strategy to reverse CQ resistance in *P. falciparum* resistant strains (Joubert *et al*., 2014; Sunduru *et al*., 2009; Deane *et al*., 2014; Muregi & Ishih, 2010). This strategy was first demonstrated by Burgess and colleagues (2006) using imipramine as the reversal agent and they called the resultant class of aminoquinoline-hybrids, "reversed chloroquine (RCQ) molecules" (**figure**

**2.12)**. They carried out further studies to demonstrate the feasibility of this approach and synthesised many optimised compounds which showed good antimalarial activity and low toxicity compared to CQ. The antimalarial activity of compounds linking any reversal-agent-like moieties to a 4-aminoquinoline (RCQ compounds) is by inhibition of haemozoin formation in a similar way as CQ (Andrews *et al.*, 2009; Burgess *et al.*, 2010; Peyton, 2012). All RCQ compounds share common features, such as bulky hydrophobic heterocyclic rings of the RA and 4-aminoquinoline (CQ like nucleus) linked to one another by an alkyl side chain linker with a tertiary amine as a bond acceptor site (Sharma, Tiwari and Parate, 2015).

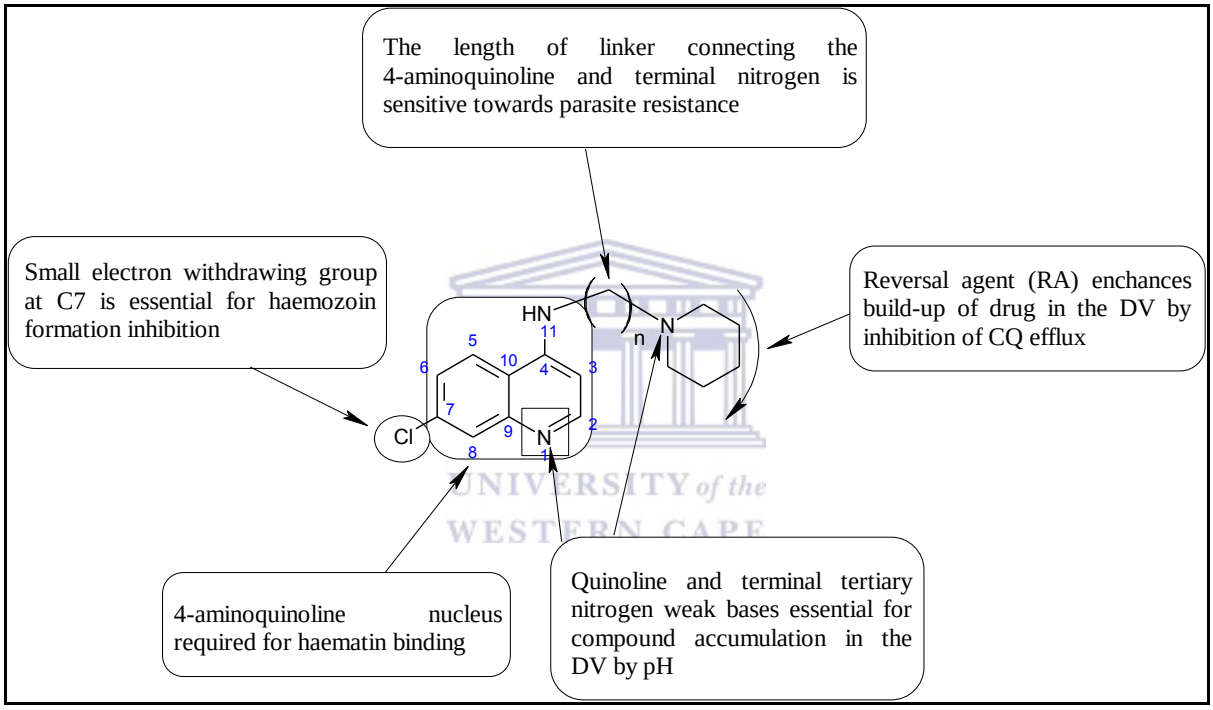



**Figure 2.12:** Representative structure and structure activity relationship of RCQ compounds

## 2.7 POLYCYCLIC CAGE MOLECULES

Recently the field of drug design and discovery has witnessed a shift of interest in developing drugs with multi-functions by targeting different mechanisms of disease etiologies (Youdim & Buccafusco, 2005; Van der Schyf & Geldenhuys, 2009). This is made possible in some instances by the use of polycyclic compounds. The synthesis of pentacyclo [5.4.0.0$^{2,6}$.0$^{3}$.0$^{5,9}$]undecane-8,11-dione, commonly known as Cookson's diketone, in 1958 (Cookson *et al.*, 1958) stimulated the interest for research on saturated polycyclic hydrocarbon cage compounds by medicinal chemists. This interest was later fuelled by the

discovery of the antiviral activity of the adamantane derivative - amantadine by Davies and co-workers in 1964 (Davies *et al*., 1964), as well as its subsequently observed antiparkison activity. The antiparkison activity was discovered following an unanticipated symptomatic improvement when the drug was administered to patients with Parkinson disease for treatment of influenza (Oliver & Malan, 2007; Schwab *et al*., 1969). Since then many polycyclic compounds have been synthesized and evaluated, especially adamantane derivatives, such as amantadine, memantine and pentacycloundecylamines (PCU). These compounds have been ascertained to have diverse pharmacological activities including antiviral activity against influenza, anti-parkinson activity (Joubert *et al*., 2012; Malan *et al*., 2003) and significant calcium and sodium channels antagonism (Malan *et al*., 2000).

Polycyclic amines like NGP1-01 an oxa-prototype of PCU, has been shown to possess significant inherent channel blocking activity (Kadernani *et al.*, 2014). NGP1-01 has been investigated further by Joubert and co-workers (2014) and shown to act as a chemosensitizer to CQ when given together at increasing concentrations. NGP1-01 has no intrinsic antimalarial activity but when co-administered with CQ, increases the activity of CQ against the $CQ^R$ parasite. It reverses the *P. falciparum* resistance to CQ by 40% and 52% at 1 µM and 10 µM respectively. This polypharmacy approach shows that for a higher reversal of CQ resistance, a higher concentration of the chemosensitizer (NGP1-01) is required (**Table 4.4**) (Joubert *et al.*, 2014). To address the poly-pharmacy approach, NGP1-01 was conjugated to a CQ-like nucleus, producing novel PCU-CQ compounds which are aza-derivatives of PCUs (**PCU-CQ 1 - 3**). These compounds, especially PCU-CQ 1, had good resistance reversal ability on the $CQ^R$ strain. From further investigation on PCUs, the aza-derivatives (**aza-PCU1** and **aza-PCU2**) were identified to have significant voltage-gated calcium channel blocking activities and this was found to have a direct correlation with the compounds capacity to act as modulating agents to CQ resistance. This implied that the inherent channel blocking activities of these polycyclic compounds enabled better resistance reversal activity (Joubert *et al.*, 2016; Joubert *et al.*, 2014).

Based on this finding, adamantane (**AD**), a polycyclic compound with inherent channel blocking ability like NGP1-01 (Kademani *et al.*, 2014; Danysz *et al.*, 1997) was investigated in this study for its potential to reverse CQ resistance. To the best of our knowledge this is the first time adamantane, the smallest diamondoid has been explored as a RA attached to a CQ-like nucleus, even though its derivatives have been explored as antimalarial agents (**S1 – S5**) by Solaja and colleagues (Solaja *et al.*, 2008).

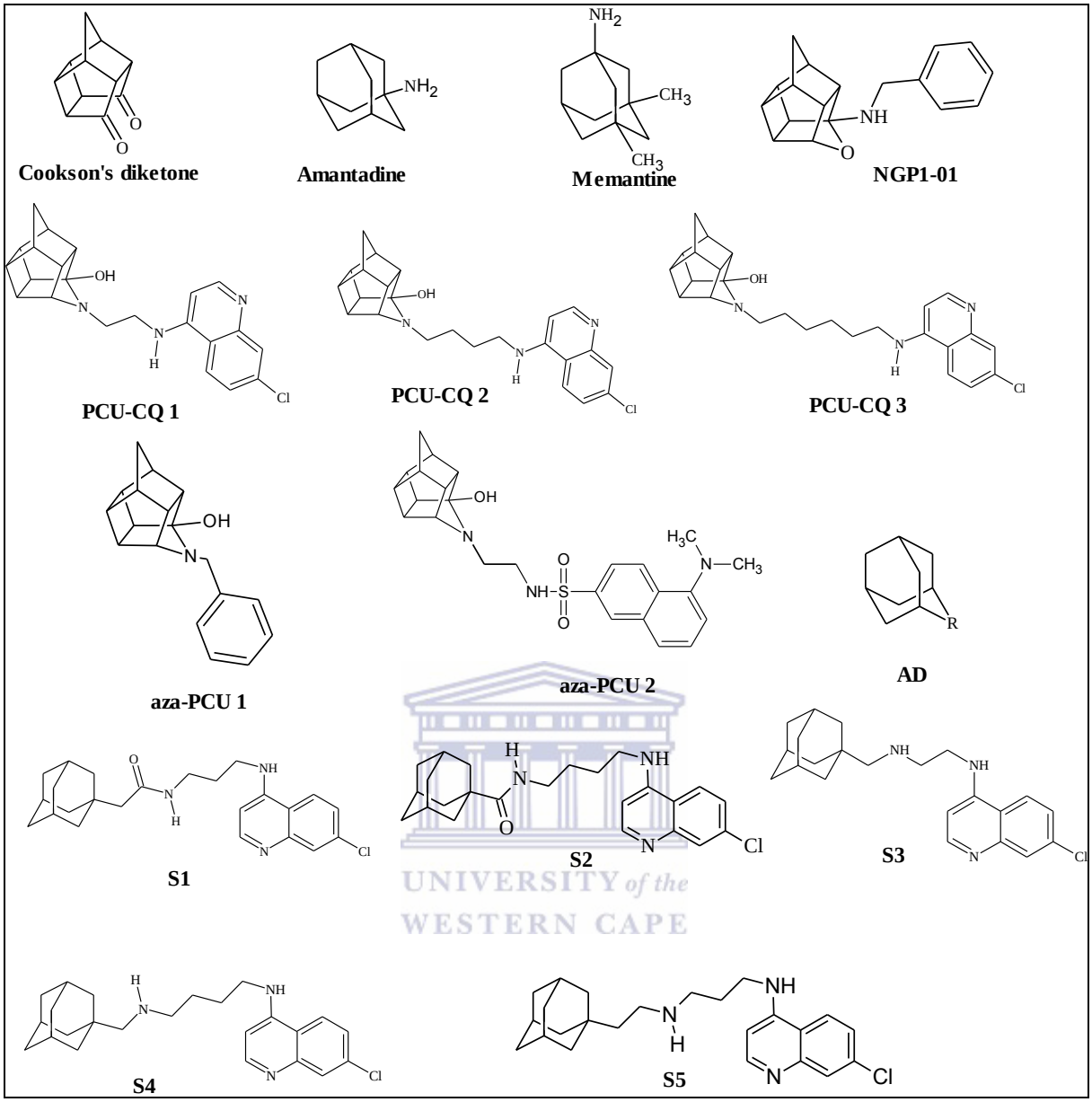



**Figure 2.13:** Polycyclic cage compounds and their derivatives with antimalarial activity

Adamantane is highly lipophilic in nature, thus the conjugation of the adamantane moiety to several privileged molecules can favourably modify and improve their pharmacokinetics and pharmacodynamics properties (Brookes *et al*., 1992) and enhances the movement of the privileged compounds across biological membranes (Zah *et al*., 2003). Also, their unique properties of high density, moderate strain energy and great stability convey metabolic stability to the conjugated compounds (Joubert *et al*., 2012; Geldenhuys *et al*., 2005).

Adamantane and its derivatives are known *N*-methyl-D-asparate (NMDA) receptor channel blockers which have also been proven to modulate voltage-gated calcium channels (Parsons,

Danysz and Quack, 1999; Malan *et al.,* 2000; Joubert *et al.,* 2011). The inherent channel blocking ability of these moieties, suggest that the adamantane moiety may block the *Pf*CQRT and inhibit the efflux of CQ-like antimalarials and render them useful as a reversal agents to overcome plasmodia CQ resistance (Joubert *et al*., 2014). The adamantane moiety facilitates the formation of a tertiary amine with the *N*-alkyl amino side chain of the proposed structures which is necessary for antimalarial activity *via* its protonation (Joubert *et al.*, 2016; Kelly *et al.*, 2007).

## 2.8 CONCLUSION

Many strategies have been developed and used to fight malaria, yet it remains a devastating parasitic disease with major health and socioeconomic impact worldwide despite decades of research. The 4-aminoquinolines and their derivatives, especially CQ, have been the most significant antimalarial agents synthesized and used for the treatment and prevention of malaria. However, the increasing emergence and alarmingly widespread multidrug resistant *P. falciparum* strains, especially to CQ, have put chemotherapy which is the mainstay of malaria management and control under constraint. This is intensified by the fact that very few antimalarial drugs are available.



History has taught us that *P. falciparum* resistance is ever evolving and continual research must thus be undertaken in search for new suitable antimalarial drugs to expand the available range to withstand and overcome the problem of CQ resistance. Although there is a shift towards genomic exploration for a better understanding of the disease to discover new interventions, it is agreed that this has to be balanced with research that deals with immediate priorities like optimal implementation and protection of existing treatment and control tools. Thus the optimization of CQ *via* the viable strategy of 'reversed CQ molecules'. The hybridization of adamantane as a reversal agent to a CQ-like nucleus with a linker of appropriate carbon chain length could produce a potential improved reversed CQ molecule and add to the number of antimalarial agents.

# CHAPTER 3

# SYNTHETIC PROCEDURES

## 3.1. INTRODUCTION

This chapter highlights all the experimental work carried out in this study to synthesize the novel adamantane-chloroquinolin (AD-CQ) conjugates including the required intermediates. Detailed synthetic procedures for the designed series of compounds as well as challenges that were encountered are reported herein. Two series of novel AD-CQ conjugates of four compounds each were synthesized by conjugating an adamantane moiety to a CQ-like nucleus via different tethered linkers. The reactions used included amination and reductive amination. The compounds were characterized by nuclear magnetic resonance (NMR), mass spectrometry (MS) and infrared (IR) spectroscopy.

## 3.2. STANDARD EXPERIMENTAL PROCEDURES

### 3.2.1. Materials

Unless otherwise specified, all reagents and solvents used were purchased from Sigma-Aldrich and other commercial suppliers and used without further purification. Some solvents were dried by simple distillation technique.



### 3.2.2. Instrumentation for product characterization

**Nuclear magnetic resonance spectroscopy (NMR):** Proton ($^{1}H$) and Carbon ($^{13}C$) NMR spectra were obtained using a Bruker Avance IIIHD Nanobay spectrometer equipped with a 5 mm BBO probe at a resonance frequency of 400 MHz and 100 MHz respectively. All chemical shifts (δ) were reported in parts per million (ppm) relative to the signal of the internal standard Tetramethylsilane (TMS; δ = 0) added to an appropriate deuterated solvent of methanol, chloroform or DMSO. The following abbreviations are used to describe the multiplicity of the respective signals: s - singlet, bs - broad singlet, ds – doublet of singlet, d - doublet, dd - doublet of doublet, t - triplet and m - multiplet. Relevant spectra of compounds are included in the annexure.

**Mass spectroscopy (MS):** The MS spectra of samples were recorded on a Perkin Elmer Flexar Single Quad (SQ) 300 mass spectrometer. The samples were dissolved in methanol, filtered through a 0.2 micron filter and diluted with 50 % (aq.) methanol before injecting 100

µl of the 50 ppm solution *via* the UHPLC auto-sampler into the SQ 300 MS. Relevant spectra are included in the annexure.

**Infrared spectroscopy (IR):** The IR spectra were recorded on a Perkin Elmer Spectrum 400 FT-IR (Fourier-transform infrared) spectrometer attached to a computer for analyzing data. Approximately 0.1 mg of the sample to be analyzed was placed on the fitted diamond attenuated total reflectance (ATR) attachment. Pressure was then applied on the ATR and the spectra was generated and obtained on the computer. Relevant spectra are included in the annexure.

**Melting point (mp) determination:** Melting points for the solid form of the compounds were determined using a Lasec SMP-10 melting point apparatus and capillary tubes. The melting points were uncorrected

**Microwave (MW) reactor:** Synthetic procedures that involved microwave irradiation were performed utilizing a CEM Discover® SP focused closed vessel reactor. This method drastically shortened reaction times as reactions were completed in minutes as opposed to hours and days for conventional methods. MW synthesis reactions are reproducible because of the highly and accurately controlled temperature system that makes heating uniform in the reaction vessel. The reaction yields are generally higher making it an ideal method for medicinal chemists as it enables the optimisation of reaction conditions (Koopmans *et al.*, 2006).



### 3.2.3. Chromatographic techniques

**Thin layer chromatography (TLC):** Analytical TLC was performed on 0.20 mm thick aluminum silica gel sheets (TLC Silica gel 60 F245 Merck KGaA). The mobile phases for this technique were prepared on a volume-to-volume basis in the ratio 10:1 of methanol/ammonia. Visualization was achieved using UV light (254 nm and 366 nm), and/or iodine vapour. This was used mainly to monitor reaction progress.

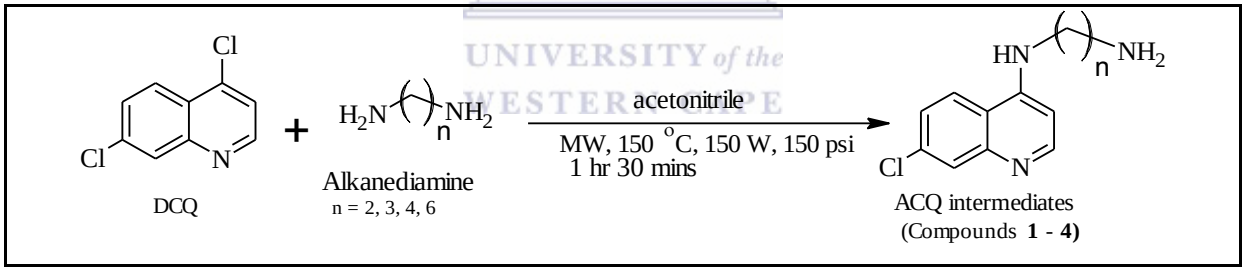
**Column chromatography:** Product mixtures were purified using a standard glass columns varying in size. The stationary phase used was silica gel (0.063 - 0.200 mm/70 - 230 mesh ASTM, Macherey-Nagel, Duren, Germany) with methanol/ammonia in the ratio 10:1 as mobile phase.

## 3.3. GENERAL SYNTHETIC PROCEDURES

### 3.3.1. Synthesis of *N*-(7-chloroquinolin-4-yl)alkane-1-n-diamines (ACQ intermediates; compounds 1- 4)

The synthesis of the appropriate *N*-(7-chloroquinolin-4-yl)alkane-1-n-diamines (**ACQ**-intermediates) involved the amination of 4,7-dichloroquinoline with excess of the appropriate alkanediamine employing microwave (MW) irradiation as represented in **scheme 3.1**. A mixture of 4,7-dichloroquinoline (1.00 g, 5.05 mmol) and the appropriate alkanediamine was dissolved in acetonitrile (4 ml) and irradiated at 150 ˚C, 150 W and 150 psi for 1 hour 30 minutes. The cooled reaction mixture was then basified with 5 % $NaHCO_3$ (30 ml) and extracted with dichloromethane (DCM) (20 ml x 3). The combined organic fractions were collected and washed with water and then with brine to wash off the excess alkanediamine. The washed organic solution was then evaporated *in vacuo* to generate a pale yellow crude residue of *N*-(7-chloroquinolin-4-yl)alkane-1-n-diamines (compounds **1– 4**) (Joubert *et al.*, 2014; Sunduru *et al.*, 2009; Natarajan *et al.*, 2008). The residue was used without further purification or further purified by column chromatography rendering the product as an off-white solid.

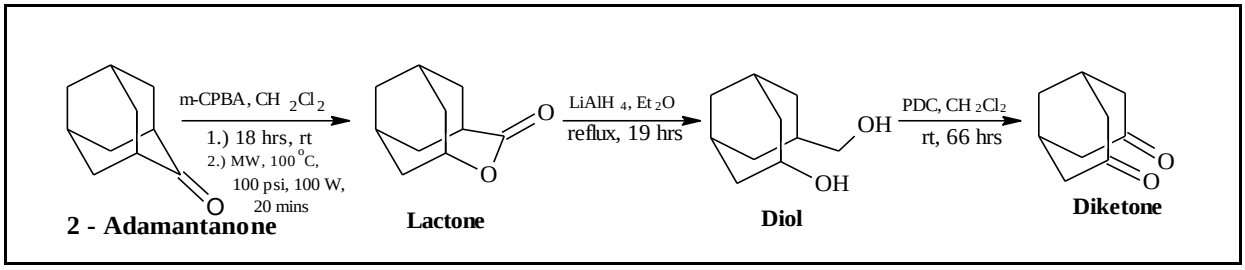





**Scheme 3.1:** Schematic representation of ACQ synthesis

### 3.3.2. Synthesis of the aza-adamantanol derivatives (compounds 5 – 8, Series 1)

Two series of the novel adamantane-chloroquinolin (AD-CQ) reversed CQ conjugates were synthesized. Series 1 consisted of the aza-adamantanols (compounds **5 - 8**). Prior to their synthesis, an adamantane diketone (bicyclo[3.3.1]nonane-3,7-dione) intermediate was required to be synthesized. This was required to provide a tertiary amine needed in the AD-CQ derivatives. The diketone was synthesized according to a modified method from the published methods of Zalikowski, Gilbert and Borden (1980) and Banister and co-workers (2011). This reaction involved a three-step synthetic route starting with the commercially available 2-adamantone. The schematic representation is shown in **scheme 3.2**.

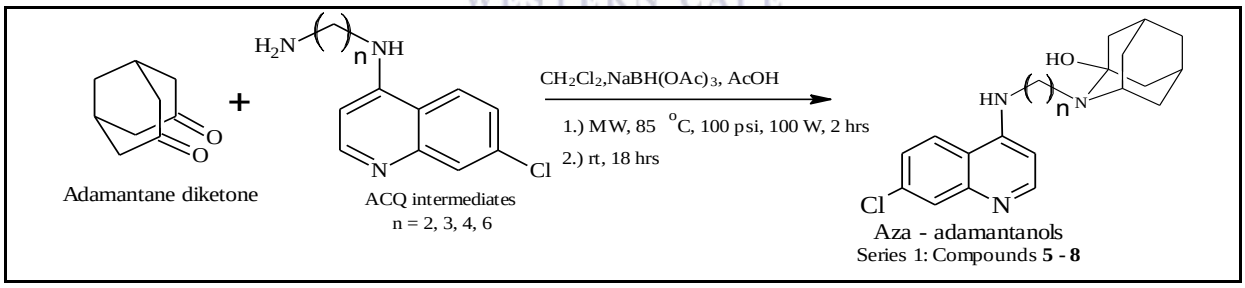



**Scheme 3.2:** A schematic representation of the adamantane diketone synthesis

The 2-adamantanone was subjected to a Baeyer-Villiger oxidation reaction with m-chloroperbenzoic acid (m-CPBA) to generate a lactone (4-oxatricyclo[4.3.1.1$^{3,8}$]undecan-5-one) using conventional and MW methods 1 and 2. The lactone was reduced with lithium aluminium hydride ($LiAlH_4$) to a diol [7-(hydroxymethyl)bicyclo[3.3.1]nonan-3-ol] which was then oxidized with pyridinium dichromate to yield the adamantane diketone (bicyclo[3.3.1]nonane-3,7-dione).

The adamantane diketone was then used in the synthesis of the aza-adamantanol derivatives (**5 – 8**) *via* the two methods presented in **scheme 3.3**. The diketone was conjugated to the appropriate *N*-(7-chloroquinolin-4-yl) alkanediamines (compounds **1 – 4**) in a 1:1.25 mmol ratio, by reductive amination and transannular cyclization using sodium triacetoxylborohydride ($NaBH(OAc)_3$) (1.60 mmol) to generate the aza-adamantanols.






**Scheme 3.3:** Synthetic route for the aza-adamantanols of series 1 (compounds **5** – **8**)

Some compounds of series 1 were synthesized by a modified method from the microwave-assisted method (1 in **scheme 3.3**) used by Joubert and colleagues (2013) and the conventional method (2 in **scheme 3.3**) of Banitster and co-workers (2011).

In the microwave-assisted method, an equimolar mixture of the diketone and the appropriate *N*-(7-chloroquinolin-4-yl)alkanediamine was dissolved in ethanol (5 ml) in a closed microwave vessel and irradiated at 85 ˚C, 100 W, 100 psi for 10 minutes to dissolve all solids. The reaction mixture was then transferred to a round bottom flask where ethanol (10 ml) and acetic acid (150 µl) was added. The mixture was then treated with sodium

triacetoxyborohydride (1.3 mmol) and allowed to stir for 2 hours at room temperature, where after it was concentrated *in vacuo*. Water (15 ml) was added to the concentrate to dissolve the mixture and this was stirred further with the addition of sodium hydrogen carbonate until the evolution of the $CO_2$ gas ceased. Then an excess of the sodium hydrogen carbonate (2 g) was added. The aqueous sodium hydrogen carbonate layer was extracted with DCM (20 ml x3) and the combined organic fractions was washed with water (25 ml) and concentrated *in vacuo* to obtain the crude yellowish solid of aza-adamantanol, compound **7**.

In the conventional method of Banitster *et al.*, (2011), a mixture of diketone (1 mmol) and *N*-(7-chloroquinolin-4-yl)alkanediamines (1 mmol) was dissolved in 1,2-dichloroethane (DCE) with glacial acetic acid (75 µl) and stirred for 10 minutes until all solids were dissolved. The mixture was then treated with sodium triacetoxyborohydride (1.3 mmol) and further stirred for 18 hours at room temperature. The reaction mixture was quenched with sodium hydroxide (3 M, 1 ml), stirred for a further 10 minutes and then partitioned between sodium hydroxide (0.5 M) and dichloromethane (DCM) in the ratio 6:5 to wash off any acetic acid left. The DCM layer was collected, the aqueous phase extracted with more DCM (25 ml x 2) and the combined organic layers washed with brine and concentrated *in vacuo* to get compound **6**. The drawback with this method is the lengthy reaction time.



These two methods were worked into a modified method which makes use of the short reaction time of the MW and a neat work-up. In the modified method, an equimolar mixture of the adamantane diketone (1 mmol) and *N*-(7-chloroquinolin-4-yl)alkanediamine (1 mmol) was dissolved in 1,2-dichloroethane (DCE; 4.5 ml). Glacial acetic acid (AcOH; 75 µl) was added to the mixture to activate the carbonyl group. The mixture was transferred to a sealed microwave vessel (25 ml capacity) and irradiated at 85 ˚C, 100 W and 100 psi for 10 minutes to dissolve all the solids. The reaction mixture was transferred into a round bottom flask, treated with sodium triacetoxyborohydride and stirred at room temperature for 2 hours. The reaction was monitored with TLC using methanol/ammonia in the ratio 10:1 as mobile phase. After 2 hours, the reaction was quenched with sodium hydroxide (NaOH; 3 M, 1 ml) and stirred for 10 minutes. The reaction was then partitioned between sodium hydroxide solution and DCM in the ratio 6:5 and the DCM layer was collected. The aqueous NaOH layer was extracted with more DCM (20 ml x 2). The combined organic fractions were washed with brine and concentrated *in vacuo* to a yellowish product of compound **5** and **8**. A summary of the three methods is presented on **table 3.1**, as used to synthesize the different compounds of series 1.

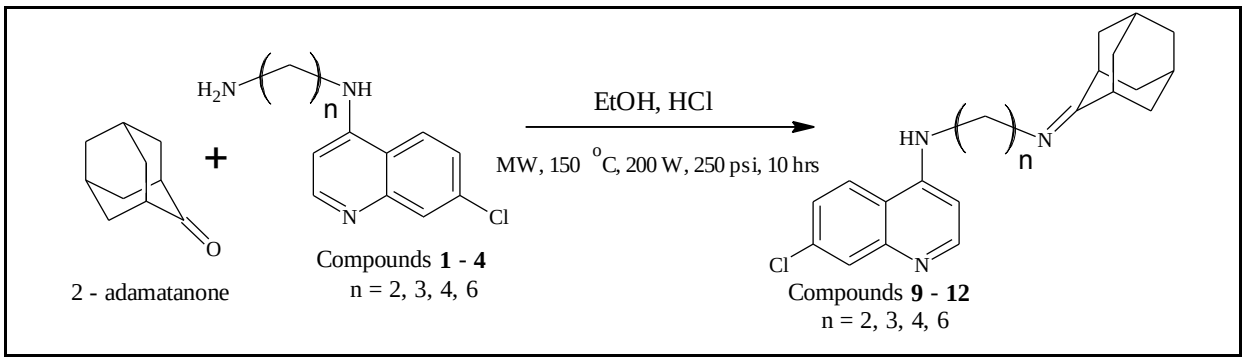
**Table 3.1:** Summary of series 1 aza-adamantanols synthetic methods

| Method used | Reaction time | % yield | Comments |
|---|---|---|---|
| **Conventional method by Banitster and co-workers (2011)** | 18 hours | Cpd.**6**: 50 | Lengthy reaction time |
| **MW assisted method by Joubert and colleagues (2013)** | 2 hours | Cpd.**7**: 24 | Much compound was lost during the workup. Also reaction should be monitored frequently to prevent breakdown of compounds. |
| **Modified method (combining both methods above)** | 2 hours | Cpd.**5**: 38<br>Cpd.**8**: 41 | Short time, reasonable yields, but introduction of unknown impurities were observed |



### 3.3.3. Synthesis of imine-adamantane derivatives (compounds **9 – 12,** Series 2**)**

Series 2 of the novel AD-CQ conjugates consist of compounds **9 – 12**. They were synthesized through the direct conjugation of commercially available 2-adamantanone to the appropriate *N*-(7-chloroquinolin-4-yl)alkanediamines as illustrated in **scheme 3.4**.



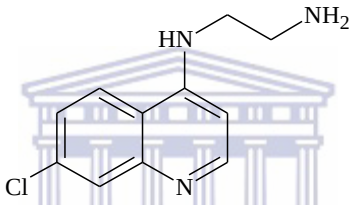

**Scheme 3.4:** Synthetic route of series 2 imine-adamantanes

An equimolar mixture of 2-adamantanone and the appropriate *N*-(7-chloroquinolin-4-yl)alkanediamine was dissolved in ethanol (10 ml) in a closed microwave vessel. The mixture

was made acidic by the addition of two drops of hydrochloric acid (HCl) to pH 5 to ensure nucleophilic attack at the carbonyl carbon. The mixture was irradiated at 150 ˚C, 200 W and 250 psi at 2 hour intervals for 9 – 10 hours in the presence of 4 Å molecular sieves to remove the water byproduct formed. The reaction was monitored with TLC using methanol/ammonia (10:1) as mobile phase. The reaction mixture was filtered through Celite® and the filtrate concentrated *in vacuo* without any further work-up to yield an off-white solid powder of imine-adamantanes (compounds **9 – 12)**. The compounds were characterized and confirmed with NMR MS and IR.

## 3.4. SYNTHESIS OF INDIVIDUAL COMPOUNDS

### 3.4.1. *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine (Compound **1)**

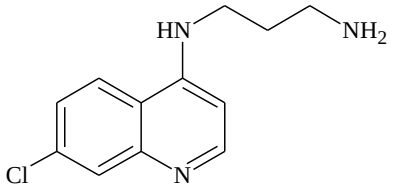





**Synthesis:** Dichloroquinoline (DCQ; 1.0247 g, 5.17 mmol) and ethane-1,2-diamine (1.73 ml, 25.87 mmol) dissolved in acetonitrile (4 ml) was microwave irradiated in a closed vessel at 150 ˚C, 150 W and 150 psi at 30 minute intervals for 1 hour. The reaction was monitored with TLC using methanol/ammonia (10:1) as mobile phase. The reaction mixture was basified with 5 % $NaHCO_{3\,(aq)}$ (30 ml) and extracted with DCM (20 ml x 2). The organic layers were collected, combined and washed with water (20 ml) and then with brine (15 ml). The workup washed off the excess ethane-1,2-diamine from the organic layer which was concentrated under reduced pressure to yield *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine, compound **1** as a pale yellow solid. This solid was further purified by column chromatography using methanol/ammonia (10:1) as mobile phase producing an off-white powder (Yield: 0.6762 g, 59 %) (Fortuin, 2014; Natarajan *et al.*, 2008).

**Physical data:** $C_{11}H_{12}ClN_3$; **$^1$H-NMR** (400 MHz, DMSO-$d_6$) δ: 8.47 – 8.45 (d, 1H, *J* = 6.40 Hz), 8.30 – 8.28 (d, 1 H, *J* = 9.2 Hz), 7.82 – 7.82 (ds, 1 H, *J* = 2.4 Hz), 7.52 – 7.48 (dd, 1 H, *J* = 9.2, 2.4 Hz), 6.58 – 6.57 (d, 1 H, *J* = 5.6 Hz), 3.55 – 3.52 (t, 2 H, *J* = 12.0, 6.4 Hz), 3.11 – 3.08 (t, 2 H, *J* = 12.4, 6.4Hz); **MS** (ESI-MS) m/z: 222.11 $[M+H]^+$

### 3.4.2. *N*-(7-chloroquinolin-4-yl) propane-1,3-diamine (Compound **2**)

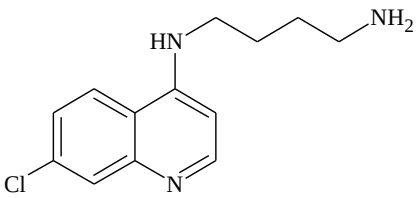



**Synthesis:** Dichloroquinoline (1.0030 g, 5.06 mmol) and propane-1,3-diamine (2.11 ml, 25.24 mmol) dissolved in acetonitrile (4 ml) was microwave irradiated in a closed vessel at 150 ˚C, 150 W and 150 psi at 30 minute intervals for 1 hour. The reaction was monitored with TLC using methanol/ammonia (10:1) as mobile phase. The reaction mixture was basified with 5 % $NaHCO_{3\,(aq)}$ (30 ml) and extracted with DCM (20 ml x 2). The organic layers were collected, combined and washed with water (20 ml) and then with brine (15 ml). The workup washed off the excess propane-1,3-diamine from the organic layer which was concentrated under reduced pressure to yield *N*-(7-chloroquinolin-4-yl)propane-1,3-diamine, compound **2** as a pale yellow solid. This solid was further purified by column chromatography using methanol/ammonia (10:1) as mobile phase producing an off-white powder (Yield: 0.9947 g, 83%) (Fortuin, 2014; Natarajan *et al.*, 2008).



**Physical data:** $C_{12}H_{14}ClN_3$; **$^1$H-NMR** (400 MHz, DMSO-$d_6$) δ: 8.83 – 8.81 (d, 1 H, *J* = 9.2 Hz), 8.59 – 8.57 (d, 1 H, *J* = 7.2 Hz), 8.12 – 8.11 (ds, 1 H, *J* = 2.4 Hz), 7.77 – 7.74 (dd, 1 H, *J* = 9.2, 2.4Hz), 6.94 – 6.92 (d, 1 H, *J* = 7.2 Hz), 3.67 – 3.63 (m, 2 H), 2.96 – 2.91 (m, 2 H), 2.04 – 1.97 (m, 2H); **MS** (ESI-MS) m/z: 236.00 $[M+H]^+$.

### 3.4.3. *N*-(7-chloroquinolin-4-yl) butane-1,4-diamine (compound **3**)

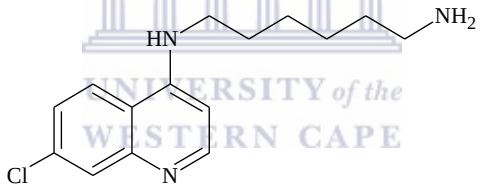



**Synthesis:** Dichloroquinoline (1.0022 g, 5.06 mmol) and butane-1,4-diamine (2.60 ml, 25.86 mmol) dissolved in acetonitrile (4 ml) was microwave irradiated in a closed vessel

microwave reactor at 150 ˚C, 150 W and 150 psi at 30 minutes interval for 1 hour. The reaction was monitored with TLC using methanol/ammonia (10:1) as mobile phase. The reaction mixture was basified with 5 % $NaHCO_{3aq}$ (30 ml) and extracted with DCM (20 ml x 2). The combined organic layers were collected and washed with water (20 ml) and then brine (15 ml). The workup washed off the excess butane-1,4-diamine from the organic layer which was concentrated under reduced pressure to yield *N*-(7-chloroquinolin-4-yl)butane-1, 4-diamine, compound **3** as a pale yellow oily solid. This was further purified by column chromatography using methanol/ammonia (10:1) as mobile phase producing a yellowish waxy solid (Yield: 0.7178 g, 56%) (Fortuin, 2014).

**Physical data:** $C_{13}H_{16}ClN_3$; **$^1$H-NMR** (400 MHz, MeOD) δ: 8.17 – 8.15 (d, 1 H, *J* = 5.6 Hz), 7.92 – 7.90 (d, 1 H, *J* = 8.8 Hz), 7.60 – 7.59 (ds, 1 H, *J* = 2.0 Hz), 7.21 – 7.18 (dd, 1 H, *J* = 8.8, 2.4 Hz), 6.30 – 6.29 (d, 1 H, *J* = 6.0 Hz), 3.21 – 3.17 (t, 2 H, *J* = 15.2, 7.6 Hz), 2.67 – 2.63 (t, 2 H, *J* = 14.8, 7.2 Hz), 1.66 – 1.48 (m, 4 H); **MS** (ESI-MS) m/z: 250.10 $[M+H]^+$.

### 3.4.4. *N*-(7-chloroquinolin-4-yl) hexane-1,6-diamine (Compound **4)**

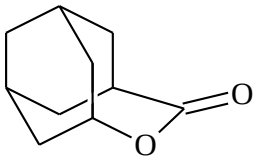





**Synthesis:** Dichloroquinoline (1.0050 g, 5.07 mmol) and hexane-1,6-diamine (3.31 ml, 25.35 mmol) dissolved in acetonitrile (4 ml) was microwave irradiated in a closed vessel at 150 ˚C, 150 W and 150 psi at 30 minutes interval for 1 hour. The reaction was monitored with TLC using methanol/ammonia (10:1) as mobile phase. The reaction mixture was basified with 5 % $NaHCO_{3aq}$ (30 ml) and extracted with DCM (20 ml x 2). The organic layers were collected, combined and washed with water (20 ml)) and then with brine (15 ml). The workup washed off the excess hexane-1,6-diamine from the organic layer which was concentrated under reduced pressure to yield *N*-(7-chloroquinolin-4-yl)hexane-1,6-diamine, compound **4** as a pale yellow waxy solid. This solid was further purified by column chromatography using methanol/ammonia (10:1) as mobile phase producing an off-white powder (Yield: 0.9817g, 69 %) (Fortuin, 2014; Natarajan *et al.*, 2008).

**Physical data:** $C_{15}H_{20}ClN_3$; **$^1$H-NMR** (400 MHz, MeOD) δ (ppm): 8.48 – 8.45 (d, 1 H, *J* = 9.2 Hz), 8.40 – 8.38 (d, 1 H, *J* = 7.2 Hz), 7.88 – 7.88 (ds, 1 H, *J* = 2.0 Hz), 7.70 – 7.68 (dd, 1 H, *J* = 9.2, 2.4 Hz), 6.90 (d, 1 H, *J* = 7.2 Hz), 3.62 (t, 2 H, *J* = 15.2, 7.2 Hz), 2.95 (t, 2 H, *J* = 15.6, 7.6 Hz), 1.88 – 1.81 (m, 2 H), 1.75 – 1.67 (m, 2 H), 1.52 – 1.48 (m, 4 H); **MS** (ESI-MS) m/z:. 278.06 $[M+H]^+$.

### 3.4.5. 4-Oxatricyclo[4.3.1.1$^{3,8}$]undecan-5-one (Lactone)



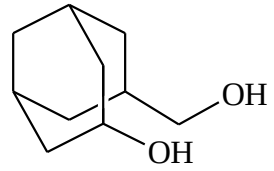

**Synthesis:** 2-Adamantanone (5.0070 g, 33.33mmol) dissolved in DCM (50 ml) was treated with m-CPBA (5.8486 g, 33.89 mmol) and stirred at room temperature (rt) for 18 hours while the reaction was monitored with TLC using MeOH/$NH_4OH$ (10:1) as mobile phase. A white suspension was formed, which was filtered and the filtrate poured into a separating funnel and washed with aqueous sodium hydroxide (NaOH; 1 M, 100 ml) and the DCM layer was collected. The aqueous phase was extracted with more DCM (25 ml x 2) and the combined organic fractions were collected and concentrated *in vacuo*. The residue was allowed to dry in the fume cupboard to yield a white solid of 4-oxatricyclo[4.3.1.1$^{3,8}$]undecan-5-one (Lactone) (Yield: 5.3727 g, 97 %). The structure of the product was elucidated by NMR and confirmed to be similar as per literature (Banister *et al.*, 2011).

This reaction is also possible through the utilization of microwave irradiation (method 2 in **scheme 3.2**) and is much quicker. 2-Adamantanone (0.1759 g, 1.17 mmol) dissolved in DCM (15 ml) was treated with m-CPBA (0.2040 g, 1.18 mmol). The mixture was microwave irradiated at 100 ˚C, 100 W and 100 psi for 20 minutes at a 10 minute interval. The reaction was monitored by TLC using methanol/ammonia in the ratio 10:1 as mobile phase. The reaction mixture was washed with NaOH (1 M; 20 ml) and the DCM layer was collected. The aqueous phase was extracted with DCM (15 ml x 2). The combined organic layers were collected and concentrated *in vacuo* to yield a white solid of lactone (Yield: 0.1911 g, 98.11 %). This microwave synthesis saves considerable time and is very convenient for small scale synthesis. However, the 20 minutes seem to be too long as other minor peaks of impurities

are seen in the $^{1}$H-NMR (spectrum 42). These could be attributed to energetic breakdown of compounds forming unknown degradation products.

**Physical data:** $C_{10}H_{14}O_2$; **mp**: 281 – 290 ˚C (Lit.: 285 – 287; Zalikowski *et al.*, 1980); **$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ (Spectrum 6): 4.50 -4.6(m, 1 H), 3.08 – 3.05(t, 1 H, *J* = 12.00, 4.00 Hz), 2.10 – 1.73(m, 12 H).

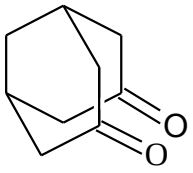
### 3.4.6. 7-(Hydroxymethyl)bicyclo[3.3.1]nonan-3-ol (Diol)




**Synthesis:** Oxatricyclo[4.3.1.1$^{3,8}$]undecan-5-one (4.8653 g, 29.27 mmol) was dissolved in a gray suspension of lithium aluminum hydride ($LiAlH_4$, 1.5220 g, 40.10 mmol) in diethyl ether (50 ml). The reaction was refluxed overnight, cooled and quenched with a mixture of THF and water (1:1, 2 ml), NaOH solution (4 M, 1 ml) and water (3 ml) to deactivate active hydride. This sequence is known as the Fieser work up, necessary to prevent the formation of an emulsion from aluminum hydroxide by converting the aluminate salts to alumina which can easily to rinsed and filtered. There was gas evolution and formation of a white suspension on addition of the water. The suspension was slowly transferred into a separating-funnel (500 ml capacity) containing water (150 ml). The denser aqueous phase was collected first and filtered to collect the alumina salt residue, which was washed with hot THF. The aqueous filtrate was extracted with diethyl ether (25 ml x 2) and was combined with the synthetic diethyl ether fraction and the hot THF. The combined organic fractions were concentrated *in vacuo* to yield 7-(Hydroxymethyl)bicyclo[3.3.1]nonan-3-ol (diol) (Yield: 2.65 g, 54.41 %) as a white crystalline solid. The structure of the product was confirmed by NMR and was found to be similar as reported in the literature (Banister *et al.*, 2011).

**Physical data:** $C_{10}H_{18}O_2$;**mp**: 169 – 173 (Lit.: 164-168, 170 – 174) (Banister et al., 2011; Zalikowski, Gilbert and Borden, 1980); **$^{1}$H-NMR** (400 MHz, MeOD) δ (Spectrum 7): 4.083 (m, 1 H), 3.331 – 3.326 (d, 2 H, overlapping with MeOD signal, *J* = 6.00 Hz), 2.082 (m, 2 H), 1.977 – 1.584 (m, 11 H), 1.195 – 1.161 (d, 1 H, *J* = 13.60 Hz).

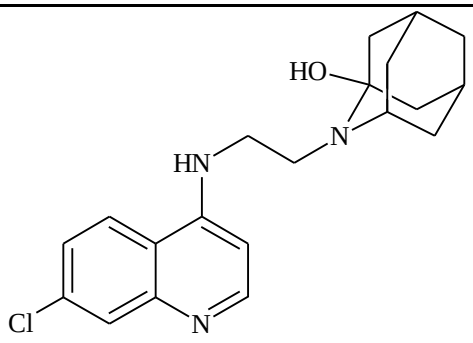
**3.4.7. Bicyclo[3.3.1]nonane-3,7-dione(**Adamantane diketone**)**




**Synthesis:** The diol (0.3903 g, 2.29 mmol) was added to a suspension of pyridinium dichromate (PDC; 4.5829 g, 12.18mmol) in diethyl ether (30 ml). The mixture was stirred at rt for three days and the reaction was monitored by TLC using methanol/ammonia (10:1) until completion. The reaction mixture was diluted with more diethyl ether (50 ml) and mixed with Celite® to absorb the fine traces of the PDC and it was filtered by vacuum filtration to remove the PDC particles. The diethyl ether filtrate was collected and concentrated under pressure to yield bicyclo[3.3.1]nonane-3,7-dione (adamantane diketone) (Yield: 0.2793 g, 80.05 %) as a brownish solid with physical properties that match that found in the literature (Banister *et al.*, 2011; Toşa, 2009).

**Physical data:** $C_9H_{12}O_2$; **mp**: 210˚C (initial droplet appears) 257 ˚C (final melting) (Lit.: 249 – 255; Banister *et al.*, 2011) **$^1$H-NMR** (400 MHz, $CDCl_3$) δ (Spectrum 8) 2.866 (s, 2 H), 2.613 – 2.560 (dd, 4 H, *J* = 15.60, 5.60 Hz), 2.434 – 2.395 (d, 4 H, *J* = 15.60), 2.203 (m, 2H).

**3.4.8. 2-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-2-azatricyclo[3.3.1.1$^{3,7}$]decan-1-ol (**Compound **5)**



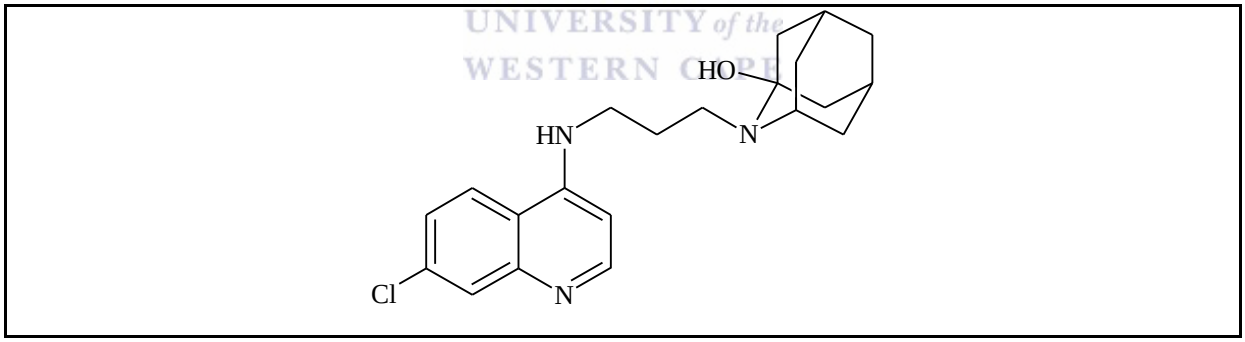

**Synthesis**: The *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**1**; 0.1663 g, 0.75 mmol) and diketone (0.0978 g, 0.64 mmol) were dissolved in 1,2-dichloroethane (3.5 ml). Glacial acetic acid (75 µl) was added as a proton donor to the mixture and stirred for 10 minutes to dissolve all solids. The reaction mixture was then treated with sodium triacetoxyborohydride

($NaBH(OAc)_3$) (0.2756 g, 1.30 mmol) and stirred at rt for 18 hours and subsequently partitioned between $NaOH_{aq}$ (0.5 M) and dichloromethane (DCM) in the ratio of 6:5 respectively. The denser DCM layer was collected. The NaOH layer was further extracted with more DCM (15 ml x 3) and the combined DCM layers was washed with brine (25 ml). The combined DCM layer was then concentrated under reduced pressure to yield compound **5** (Yield: 85.6 mg, 38.40 %) as an off-white solid.

**Physical data:** $C_{20}H_{24}ClN_3O$; **$^1$H-NMR** (400 MHz, MeOD) δ (ppm) (Spectrum 9): 8.36 – 8.34 (d, 1 H, *J* = 5.6 Hz), 8.05 – 8.03 (d, 1 H, *J* = 9.2 Hz), 7.78 – 7.77 (ds, 1 H, *J* = 2.4 Hz), 7.42 – 7.39 (dd, 1 H, *J* = 9.2, 2.0 Hz), 6.56 – 6.54 (d, 1 H, *J* = 6.0 Hz), 3.37 – 3.34 (t, 2 H, *J* = 13.2, 6.4 Hz), 3.12 – 3.09 (t, 2 H, *J* = 13.2, 6.4 Hz), 2.20 (m, 2 H), 2.01 – 1.95 (m, 4 H), 1,80 – 1.75 (d, 2 H, *J* = 16.4 Hz), 1.71 – 1.68 (d, 2 H, *J* = 12.4 Hz), 1.55 – 1.52 (d, 2 H, *J* = 12.0 Hz), 1.29 (bs, 1 H); **$^{13}$C-NMR** (100 MHz) δ:153.4, 150.5, 147.4, 137.6, 126.9, 124.7, 118.4, 99.874, 94.4, 57.7, 50.8, 44.8, 36.6, 34.5, 32.0, 30.6; **MS** (ESI-MS) m/z (Spectrum11): 358.26 $[M + H]^+$, 360.36 $[M + H]^+ + 2$; **IR** (ATR, $cm^{-1}$) Vmax: 3261, 2926, 1574, 1408, 1012, 922, 802, 763.



### 3.4.9. 2-{3-[(7-Chloroquinolin-4-yl)amino]propyl}-2-azatricyclo[3.3.1.1$^{3,7}$]decan-1-ol (Compound **6**)



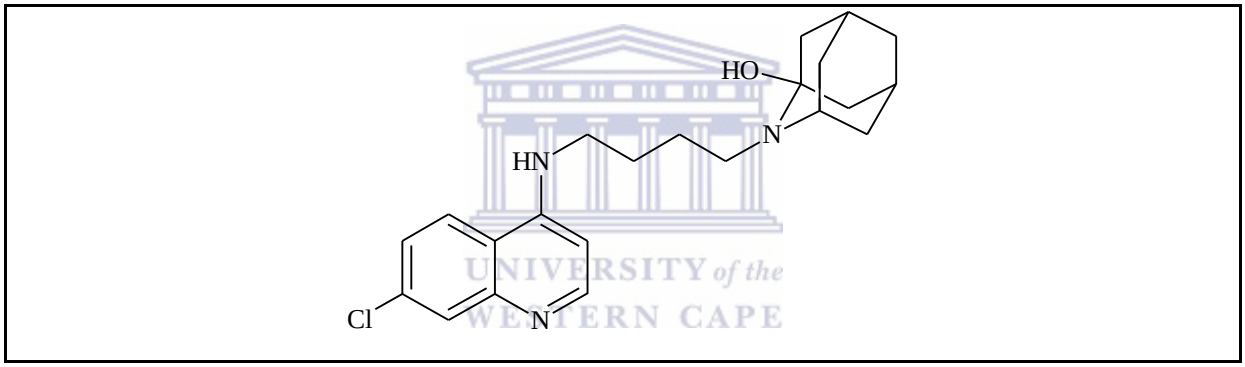

**Synthesis:** The *N*-(7-chloroquinolin-4-yl)propane-1,3-diamine **2** (0.2895 g, 1.23 mmol) and diketone (0.1565 g, 1.03 mmol) were dissolved in 1,2-dichloroethane (3.5 ml). Glacial acetic acid (75 µl) was added as a proton donor to the mixture and stirred for 10 minutes to dissolve all solids. The reaction mixture was then treated with sodium triacetoxyborohydride ($NaBH(OAc)_3$) (0.3444 g, 1.62 mmol) and stirred at rt for 18 hours and subsequently partitioned between $NaOH_{aq}$(0.5 M) and dichloromethane (DCM) in the ratio 6:5 respectively. The denser DCM layer was collected. The NaOH layer was further extracted with more DCM (15 ml x 3) and the combined organic fractions was washed with brine (25

ml). The combined DCM layer was then concentrated under reduced pressure to yield compound **6** (Yield: 183.1 mg, 47.9 %) as an off-white solid.

**Physical data:** $C_{21}H_{26}ClN_3O$; **$^1$H-NMR** (400 MHz, MeOD) δ (Spectrum 13): 8.36 – 8.34 (d, 1 H, *J* = 5.6 Hz), 8.11 – 8.09 (d, 1 H, *J* = 8.8 Hz), 7.78 – 7.77 (ds, 1 H, *J* = 2.4 Hz), 7.41 – 7.38 (dd, 1 H, *J* = 11.2, 9.2, 2.4 Hz), 6.57 – 6.55 (d,1 H, *J* = 5.6 Hz), 3.47 – 3.43 (t, 2 H, *J* = 13.6, 6.8, Hz), 2.95 – 2.92 (t, 2 H, *J* = 13.6, 6.8Hz), 2.18 (m, 2 H), 1.99 – 1.97 (d, 4 H, *J* = 12.4 Hz), 1.89 – 1.86 (m, 3 H), 1.78 – 1.73 (d, 2 H, *J* = 17.6 Hz), 1.65 – 1.62 (d, 2 H, *J* = 11.6 Hz), 1.52 – 1.49 (d, 2 H, J = 12.8 Hz); **$^{13}$C-NMR** (100 MHz, MeOD) δ: 150.73, 147.93,134.77, 126.42, 124.54, 122.35, 117.13, 98.04, 81.59, 77.02, 53.13, 47.33, 41.29, 36.99, 35.33, 32.50, 31.30 26.61; **MS** (ESI-MS) m/z: 372.16 $[M + H]^+$, 374.18 $[M + H]^+ + 2$; **IR** (ATR, $cm^{-1}$) Vmax: 3192, 2916, 1591, 1454, 1217, 803, 762.

### 3.4.10. 2-{4-[(7-Chloroquinolin-4-yl)amino]butyl}-2-azatricyclo[3.3.1.1$^{3,7}$]decan-1-ol

**(**Compound **7)**

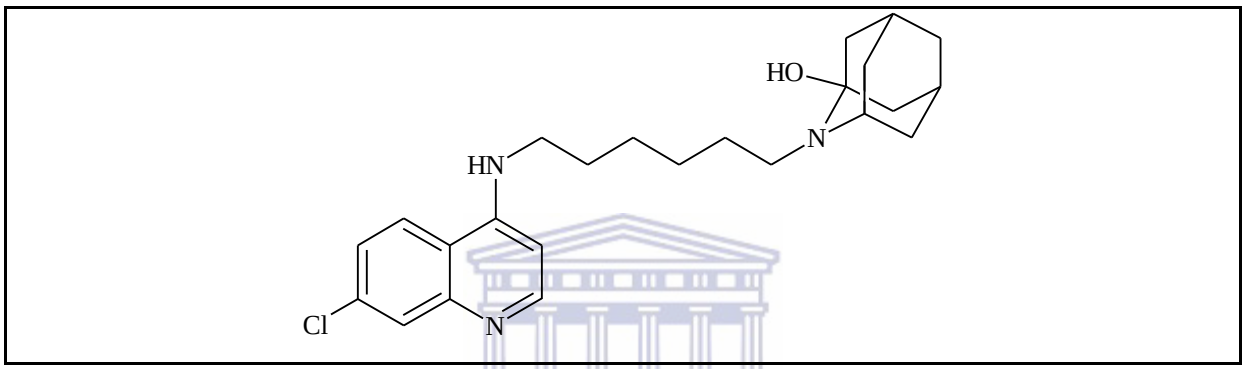




**Synthesis:** The *N*-(7-chloroquinolin-4-yl)butane-1,4-diamine **3** (0.1146 g, 0.46 mmol) and diketone (0.0611 g, 0.40 mmol) were dissolved in 1,2-dichloroethane (4.5 ml). Glacial acetic acid (75 µl) was added to the mixture and microwave irradiated at 85 ˚C, 100 W, and 100 psi for 10 minutes to dissolve all the solids. The reaction mixture was then treated with sodium triacetoxyborohydride ($NaBH(OAc)_3$ (0.1361 g, 0.64 mmol) and stirred at room temperature for 2 hrs. The reaction was then quenched with sodium hydroxide (3 M, 2 ml), stirred for 10 minutes and subsequently partitioned between $NaOH_{(aq)}$ (0.5 M) and dichloromethane (DCM) in the ratio 6:5 respectively. The denser DCM layer was collected. The NaOH layer was further extracted with more DCM (15 ml x3) and the combined organic fractions was washed with brine (25 ml). The combined DCM layer was then concentrated under reduced pressure to yield compound **7** as a yellow waxy solid. The mixture was further purified by column chromatography using methanol/ammonia (10:1) as mobile phase (Yield: 36.5mg, 23.56 %).

**Physical data:** $C_{22}H_{28}ClN_3O$; **$^1$H-NMR** (400 MHz, MeOD) δ (Spectrum 17): 8.34 (d, 1 H, *J* = 6.0 Hz), 8.11 – 8.09 (d, 1 H, *J* = 9.2 Hz), 7.76 (ds, 1 H, J = 4.4 Hz), 7.40 – 7.37 (dd, 1 H, *J* = 11.6, 9.2, 2.0 Hz), 6.52 – 6.51 (d, 1 H, *J* = 5.2 Hz), 3.38 – 3.37 (m, 2 H) 2.84 – 2.80 (m, 2 H), 2.64 (t, 2 H, *J* =14.8, 7.2 Hz), 2.38 – 1.46 (m, 14 H); **$^{13}$C-NMR** (100 MHz, MeOD) δ: 152.5, 147.9, 146.2, 138.8, 127.6, 125.9, 124.2, 99.5, 82.6, 51.2, 49.5, 46.0, 43.9, 41.8, 36.8, 33.3, 31.3, 24.2; **MS** (ESI-MS) m/z: 386.26 $[M + H]^+$, 388.25 $[M + H]^+ + 2$; **IR** (ATR, $cm^{-1}$) Vmax; 3297, 2924, 2852, 1581, 1413,1163, 919, 801, 762.

### 3.4.11. 2-{6-[(7-Chloroquinolin-4-yl)amino]hexyl}-2-azatricyclo[3.3.1.1$^{3,7}$]decan-1-ol (Compound **8)**

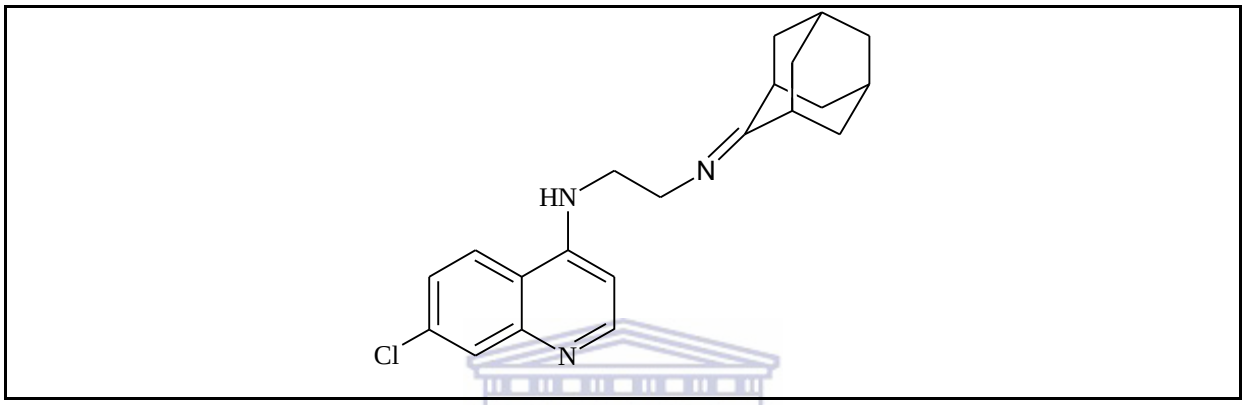



**Synthesis:** The *N*-(7-chloroquinolin-4-yl)hexane-1,6-diamine **4** (0.1040 g, 0.37 mmol) and diketone (0.0456 g, 0.30mmol) were dissolved in ethanol (5 ml). The reaction mixture was microwave irradiated at 80 ˚C, 100 W, 100 psi for 10 minutes to dissolve all solids. More ethanol (10 ml) was added to the reaction mixture and transferred to a round bottom flask. Acetic acid (150 µl) was added to the mixture as a proton donor. The mixture was then treated with sodium triacetoxyborohydride ($NaBH(OAc)_3$ (0.0850 g, 0.40 mmol) and stirred for 4 hours at room temperature. The reaction mixture was concentrated *in vacuo* and water (20 ml) was added to the residue. The resultant suspension was stirred with the addition of sodium bicarbonate in small portion until the complete evolution of gas and then an excess sodium bicarbonate solid was added to the mixture. This was to neutralize any excess acetic acid from the product. The mixture was then extracted with DCM (20 ml x 4). The combined organic layers was washed with water (25 ml x 2) and concentrated *in vacuo* and a yellow microcrystalline solid was obtained. This was precipitated with ethyl acetate to give compound **8** (Yield: 51.2 mg, 41.28 %).

**Physical data:** $C_{24}H_{32}ClN_3O$; **$^1$H-NMR** (400 MHz, $CDCl_3$) δ (Spectrum 21): 8.34 – 8.33 (d, 1 H, *J* = 5.6 Hz), 8.11 – 8.09 (d, 1 H, *J* = 8.8 Hz), 7.77 – 7.76 (ds, 1 H, *J* = 2.4 Hz), 7.40 – 7.37 (dd, 1 H,

*J* = 11.6, 9.6, 2.8 Hz), 6.51 – 6.50 (d, 1 H, *J* = 5.2 Hz), 3.37 – 3.35 (m, 2 H), 2.98 (m, 2 H), 2.37 (m, 2H), 2.26 – 2.19 (m, 4H), 2.07 (m, 8 H); **$^{13}$C-NMR** (100 MHz, MeOD) δ: 152.7, 152.4, 149.7, 136.3, 127.6, 125.9, 124.3, 118.8, 99.7, 95.5, 55.3, 46.0, 42.8, 40.4, 36.6, 34.5, 31.9, 31.2, 29,7, 28.2, 27.3; **MS** (ESI-MS) m/z: 414.32 $[M + H]^+$, 416.31 $[M + H]^+ + 2$; **IR** (ATR, $cm^{-1}$) Vmax; 3343, 2921, 1577, 1450, 1035, 975, 850, 768.

### 3.4.12. 2-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-2-tricyclo[3.3.1.1$^{3,7}$]dec-2-ylideneamine (Compound **9**)



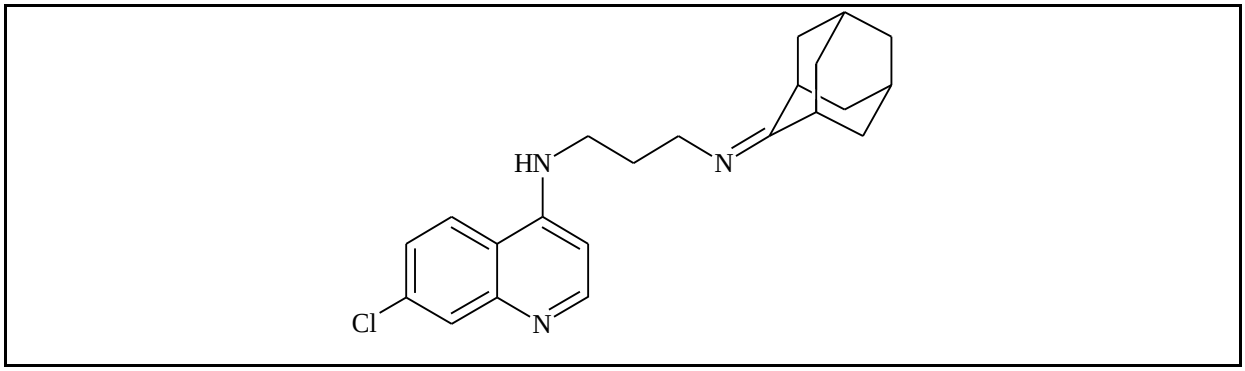

**Synthesis:** The *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine**, 1** (0.0217 g, 0.098 mmol) was added to commercially available 2-admantantone (0.0188 g, 0.125 mmol) dissolved in ethanol (10 ml) in a microwave vessel. The mixture was made acidic by the addition of two drops of hydrochloric acid (HCl) to pH 5 to ensure nucleophilic attack at the carbonyl carbon. The mixture was microwave irradiated at 150 ˚C, 200 W and 250 psi at 2 hour intervals for 10 hrs in the presence of molecular sieves. The reaction was monitored by TLC using methanol/ammonia in the ratio 10:1 as mobile phase. The reaction mixture was then filtered through Celite® and the filtrate concentrated under reduced pressure without any further work up to yield compound **9** (Yield: 0.0171 g, 49.4 %) as an off-white solid powder.



**Physical data**: $C_{21}H_{24}ClN_3$; **$^1$H-NMR** (400 MHz, DMSO-$d_6$) δ (Spectrum 25): 8.84 – 8.81 (d, 1 H, *J* = 9.2 Hz), 8.65 – 8.63 (d, 1 H, *J* = 7.2 Hz), 8.12 (s, 1 H ), 7.80 – 7.78 (d, 1 H, *J* = 9.2 Hz), 7.02 – 7.00 (d, 1 H, *J* = 7.2 Hz), 3.85 (m, 2 H), 3.16 (m, 2 H), 2.38 (m, 3 H), 2.05 – 1.87 (m, 17 H); **$^{13}$C-NMR** (100 MHz, MeOD) δ (Spectrum 26): 175.8, 158.0, 144.5, 141.3, 140.1, 129.0, 126.5, 120.4, 117.3, 100.4, 42.0, 38.9, 38.44, 37.8, 34.7, 34.3, 28.6; **MS** (ESI-MS) m/z: 354.29 $[M + H]^+$, 356.23 $[M + H]^+ + 2$; **IR** (ATR, $cm^{-1}$) Vmax: 3416 (N-H), 2897(C-H), 1573 (C=N), 1408, 1013, 903, 802, 763.

.

### 3.4.13. 2-{3-[(7-Chloroquinolin-4-yl)amino]propyl}-2-tricyclo[3.3.1.1$^{3,7}$]dec-2-ylideneamine (Compound **10)**

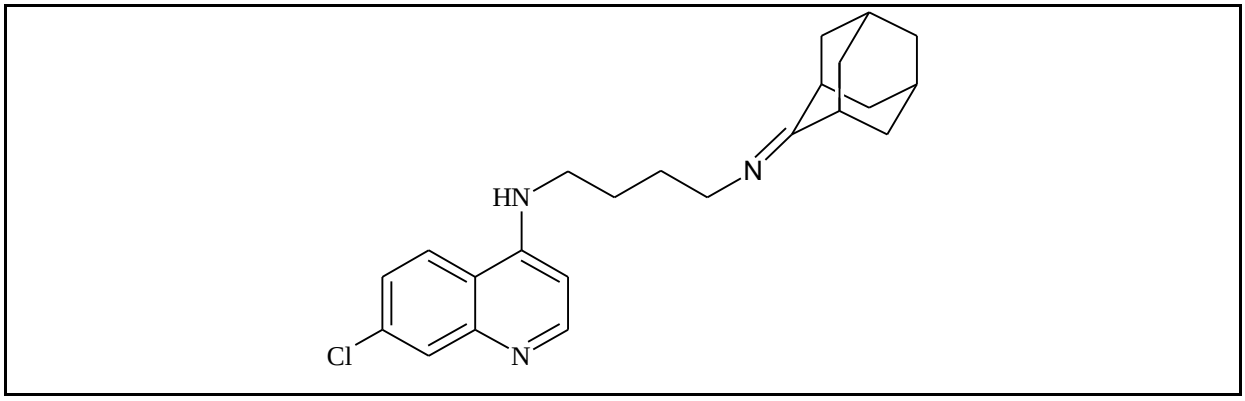



**Synthesis:** The *N*-(7-chloroquinolin-4-yl)propane-1,3-diamine **3** (0.3138 g, 1.33mmol) was added to commercial 2-admantantone (0.1996 g, 1.33mmol) dissolved in ethanol (10 ml) in a microwave vessel. The mixture was made acidic by the addition of two drops of hydrochloric acid (HCl) to pH 5 to ensure nucleophilic attack at the carbonyl carbon. The mixture was microwave irradiated at 150 ˚C, 200 W and 250 psi at 2 hour intervals for 10 hours in the presence of molecular sieves. The reaction was monitored by TLC using methanol/ammonia in the ratio 10:1 as mobile phase. The reaction mixture was then filtered through Celite and the filtrate was concentrated under reduced pressure without any further work up to yield compound **10** (Yield: 0.3007 g, 61.4 %) as an off-white solid.



**Physical data:** $C_{22}H_{26}ClN_3$. **$^1$H-NMR**(400 MHz, DMSO) δ (Spectrum 29): 8.42 – 8.40 (d, 1H, *J* = 5.6 Hz), 8.36 – 8.34 (d, 1 H, *J* = 8.8 Hz), 7.79 – 7.79 (ds, 1 H, *J* = 2.4 Hz), 7.46 – 7.44 (dd, 1 H, *J* = 11.2, 9.2, 2.4 Hz), 6.53 – 6.52 (d, 1 H, *J* = 5.6 Hz), 3.41 – 3.38 (t, 2 H, J = 13.6, 6.8 Hz), 2.92 – 2.89 (t, 2 H, *J* = 14.4, 7.2 Hz), 2.38 (m, 2 H) 2.04 – 1.86 (m, 16 H); **$^{13}$C-NMR** (100 MHz, MeOD) δ: 170.01, 153.02, 151.65, 148.69, 137.03, 126.92, 126.51, 124.48, 118.62, 99.79, 48.43, 40.94, 40.39, 38.75, 37.16, 28.91, 27.59; **MS** (ESI-MS) m/z: 368.13 [M + H], 370.18 $[M + H]^+$+ 2**; IR** (ATR, $cm^{-1}$) Vmax: 3244, 2863, 1591, 1454, 1217, 803, 762.

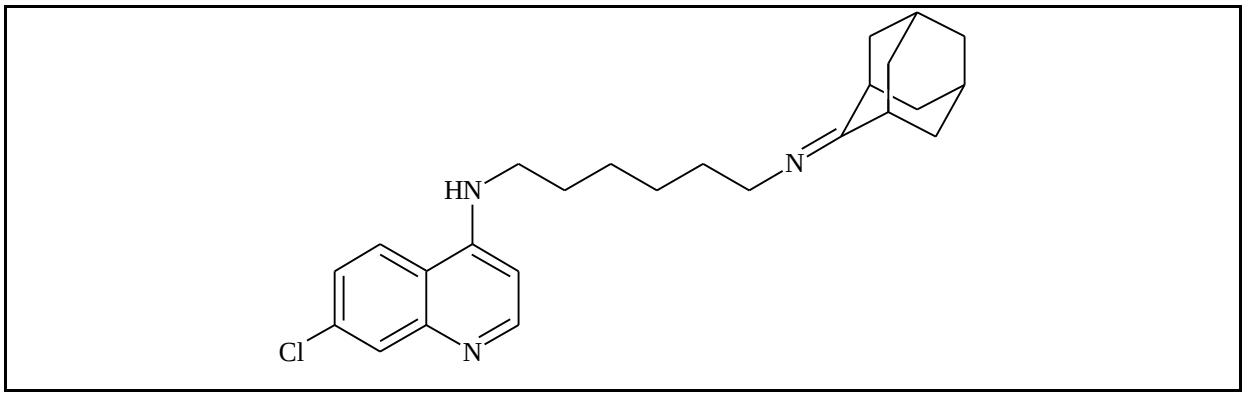
### 3.4.14. 2-{4-[(7-Chloroquinolin-4-yl)amino]butyl}-2-tricyclo[3.3.1.1$^{3,7}$]dec-2-ylideneamine (Compound **11)**




**Synthesis:** The *N*-(7-chloroquinolin-4-yl)butane-1,4-diamine, **3** (0.1806 g, 0.72 mmol) was added to commercial 2-admantantone (0.1040 g, 0.70 mmol) dissolved in ethanol (10 ml) in a microwave vessel. The mixture was made acidic by the addition of two drops of hydrochloric acid (HCl) to pH 5 to ensure nucleophilic attack at the carbonyl carbon. The mixture was microwave irradiated at 150 ˚C, 200 W and 250 psi at 2 hour intervals for 10 hours in the presence of molecular sieves. The reaction during this duration was monitored by TLC using methanol/ammonia in the ratio 10:1 as mobile phase. The reaction mixture was then filtered through Celite® and the filtrate concentrated under reduced pressure without any further work up to yield compound **11 (**Yield: 0.1573 g, 59.49 %) as a waxy solid.

**Physical data:** $C_{23}H_{28}ClN_3$; **$^1$H-NMR** (400 MHz, MeOD) δ (Spectrum 33): 8.39 – 8.38 (d, 1 H, *J* = 6.0 Hz), 8.34 – 8.36 (d, 1 H, *J* = 4.0 Hz), 7.83 – 7.82 (ds, 1 H, *J* = 1.6 Hz), 7.57 – 7.54 (dd, 1 H, *J* =10.8, 8.8, 2.0 Hz), 6.81 – 6.79 (d, 1H, *J* = 6.8 Hz), 3.61 – 3.58 (t, 2 H, *J* = 13.2, 6.4 Hz), 3.05 – 3.02 (t, 2 H, *J* = 14.4, 6.8 Hz), 2.46 (m, 2 H), 2.16 – 2.13– 1.81 (m, 14 H), 1.68 – 1.58 (m, 1 H); **$^{13}$C-NMR** (100 MHz, MeOD) δ: 178.14, 156.10, 146.15, 142.50, 139.50, 127.90, 125.76, 122.25, 117.24, 99.85, 48.38, 47.40, 43.89, 40.34, 37.13, 28.85, 26.12, 26.03; **MS** (ESI-MS) m/z: 382.32 $[M + H]^+$, 384.34 $[M + H]^+$+ 2; **IR** (ATR, $cm^{-1}$) Vmax: 3228, 2904, 1611, 1451, 1056, 874, 764.

### 3.4.15.2-{6-[(7-Chloroquinolin-4-yl)amino]hexyl}-2-tricyclo[3.3.1.1$^{3,7}$]dec-2-ylideneamine(Compound **12)**




**Synthesis:** The *N*-(7-chloroquinolin-4-yl)hexane-1,6-diamine, **4** (0.0551 g, 0.22 mmol) was added to commercial 2-admantantone (0.0305 g, 0.20 mmol) dissolved in ethanol (10 ml) in a microwave vessel. The mixture was made acidic by the addition of two drops of hydrochloric acid (HCl) to pH 5 to ensure nucleophilic attack at the carbonyl carbon. The mixture was then microwave irradiated at 150 ˚C, 200 W and 250 psi at 2 hour intervals for 10 hours in the presence of molecular sieves. The reaction was monitored by TLC using methanol/ammonia in the ratio 10:1 as mobile phase. The reaction mixture was then filtered through Celite and the filtrate concentrated under reduced pressure without any further work up to yield compound **12 (**Yield: 0.0437 g, 52.5 %) as an off-white solid.



**Physical data:** $C_{25}H_{32}ClN_3$; **$^1$H-NMR** (400 MHz, MeOD) δ (Spectrum 37): 8.48 – 8.46 (d, 1 H, *J* = 8.0 Hz), 8.40 – 8.38 (d, 1 H, *J* = 7.2 Hz), 7.89 – 7.88 (ds, 1 H, *J* = 2.0 Hz), 7.71 – 7.68 (dd, 1 H, *J* = 11.2, 9.2, 2.4 Hz), 6.91 – 6.89 (d, 1 H, *J* = 7.2 Hz), 3.64 – 3.60 (t, 2 H, *J* = 14.8, 6.8 Hz), 2.97 – 2.95 (t, 2 H, J = 15.2, 7.6 Hz), 2.47 (m, 2 H), 2.17 – 1.50 (m, 12 H); **$^{13}$C-NMR** (100 MHz, MeOD) δ: 173.54, 157.56, 143.77, 140.92, 140.07, 128.65, 126.13, 120.32, 116.93, 99.73, 48.43, 44.74, 40.62, 40.38, 38.38, 37.13, 34.77, 34.26, 28.91, 28.58, 27.47, 27.14; **MS** (ESI-MS) m/z: 410.34 $[M + H]^+$, 412.32 $[M + H]^+$+ 2; **IR** (ATR, $cm^{-1}$) Vmax: 3333, 2913, 1615, 1448, 1056, 873, 735.

## 3.5. STRUCTURE ELUCIDATION

The ACQ-intermediates (compound **1**– **4**), other intermediates and the adamantane diketone were confirmed with $^1$H-NMR and MS and compared with literature. In confirming the novel synthesized AD-CQ derivatives, the 2-adamantanone and its intermediates were analyzed and used to extrapolate and assign their characteristic peaks to the AD-CQ derivatives. In the proton NMR, the 4-aminoquinoline moiety showed five aromatic proton peaks down-field (9

– 6 ppm) in the characteristic d, d, ds, dd and d pattern (Natarajan *et al.*, 2008; Fortuin, 2014). The protons of the differently tethered linkers appeared around 3.5 – 2 ppm and the adamantane cage protons were seen up-field at around 2 – 1 ppm. In the $^{13}$C-NMR, the cage carbons were still found up-field while the linkersare around 60 – 40 ppm and the aromatic carbons were seen down-field. The molecular ion masses of the compounds from the MS spectra were similar to the calculated masses confirming the compounds. Characteristic functional moieties such as the hydroxyl group in aza-adamantanol series and the C=N bond in imine-adamantane series as well C-H, C=C, N-H bonds were identified. On the IR spectra, the hydroxyl group was observed at 3450 -3200 $cm^{-1}$and the imine bond at 1650 – 1550 $cm^{-1}$. The structures of the novel compounds were confirmed with analysis from NMR, MS and IR.

## 3.6. CONCLUSION

A total of 12 test compounds presented in **figure 3.1** were synthesized successfully. They consisted of four aminoquinoline intermediates (compounds **1- 4**) and eight novel adamantane-chloroquinolin (AD-CQ) conjugates (compounds **5 – 12**). These conjugates contained the bulky adamantane moiety which made the synthesis of a tertiary amine possible.



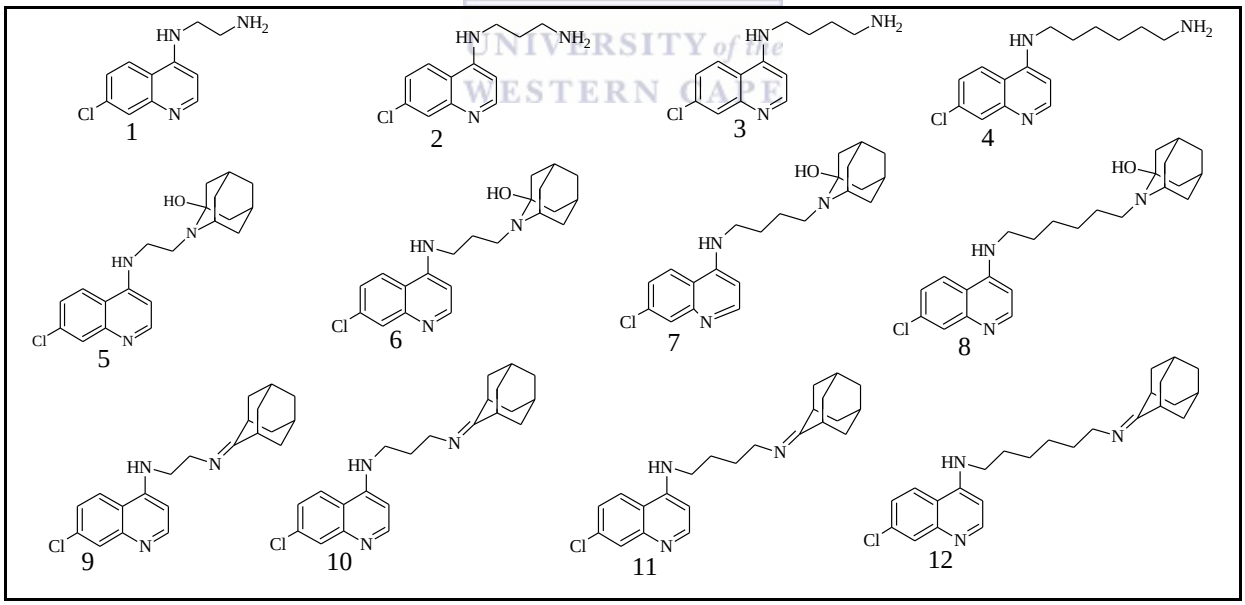



**Figure 3.1**: Structures of synthesized compounds used for biological evaluations

The percentage yield of the final products ranged from 23.56 % to 83.40 %. The low to moderate yields observed may be due to the formation of by-products during synthesis and compound lost during purification. However, optimization of the various synthetic techniques and purification procedures used in this study as well as further purification of the remaining

fractions of product mixture after column chromatography could improve yields. The compounds were characterized and confirmed using NMR, MS and IR spectroscopy.

In order to meet all the objectives of this study, the 12 test compounds (compounds **1 - 12**) were subjected to *in vitro* cytotoxicity (MTT) and parasite lactate dehydrogenase (pLDH) biological assays. These assays and the results obtained are discussed in chapter 4.

# CHAPTER 4

# BIOLOGICAL EVALUATION AND RESULTS

## 4.1. INTRODUCTION

This chapter focuses on the *in vitro* bioassays carried out in this study. It explains the investigation of the cytotoxicity profiles of the novel compounds against a non-parasitic cell line. Furthermore, it includes the results of the synthesized compounds (adamantane-chloroquinolin (**AD-CQ**) conjugates and aminoquinoline (**ACQ**) intermediates) evaluated in *in vitro* assays for their antimalarial activity on both $CQ^S$ (NF54) and $CQ^R$ (K1) strains of *P. falciparum* using the parasitic lactate dehydrogenase (pLDH) assay. In addition, these assays provide data on the ability of the compounds to overcome resistance in the $CQ^R$ isolates of *P. falciparum* (K1). The results and discussion of the biological assays carried out on the test compounds are herein presented in relation to the research questions asked in chapter 1.



## 4.2. BIOLOGICAL ASSAYS

*In vitro* cytotoxicity studies were conducted on the novel test compounds against a Chinese hamster ovarian (CHO) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tertrazoliumbromide (MTT) colorimetric assay. The MTT assay assesses the growth and survival of the CHO cell line used (Mosmann, 1983) based on viable cells' ability to reduce the yellow MTT into water-insoluble purple-blue formazan mediated by dehydrogenase enzymes of endoplasmic recticulum and mitochondria (Twentyman & Luscombe, 1987; *Fotakis* & Timbrell, 2006). Thereafter, the antimalarial activity of the AD-CQ compounds was quantitatively determined against both *P. falciparum* $CQ^S$ (NF54) and $CQ^R$ (K1) strains using a modified parasite lactate dehydrogenase (pLDH) assay first described by Makler and Hinrichs (1993). This is based on the measurement of inhibition of pLDH activity by the compounds. From the dose-inhibition

measurements, the 50 % inhibitory concentration ($IC_{50}$) values of pLDH activity for the test compounds were calculated and compared with CQ (Penna-Coutinho *et al.*, 2011).

## 4.3. MATERIALS AND METHODS

### 4.3.1. MTT Assay Procedure

Stock solutions of the test compounds were dissolved in 100 % dimethylsulfoxide (DMSO), to a concentration of 20 mg/ml and stored at -20 ˚C. To enhance solubility, the stock solutions were sonicated and test compounds that did not fully go into solution were tested in suspension. All further dilutions were freshly prepared in complete culture medium on the day of the experiment and tested in triplicate.

Initially, the stock solution was thawed and starting from a 100 µg/ml concentration, it was serially diluted in 10-fold dilutions to give 6 different concentrations in complete medium and the lowest concentration was 0.001µg/ml. The reference drug emetine was also serially diluted with the same dilution technique as above. Lastly, the 50 % inhibitory concentrations of cell line growth were obtained using non-linear dose-response curve fitting analysis with GraphPad Prism v.4 software from full dose-response curves.

### 4.3.2. Cells and *P. Falciparum* Parasite Cultures

To quantitatively determine the effect of the synthesized compounds on biological systems, two strains of *P. falciparum* were selected to be used in this study; NF54 and K1, the *P. falciparum* $CQ^S$ and $CQ^R$ strains respectively. The parasite strains were cultured continuously according to a modified method of Trager and Jensen (1976) in normal type A human erythrocytes of 2 % haematocrit. The parasite culture was maintained in a complete tissue culture medium of RPMI 1640 supplemented with 25 mM HEPES buffer, 20 µg/ml gentamicin, 27 mM sodium hydrogen carbonate and 10 % normal type A human serum (Trager & Jensen, 1976; Tan-ariya *et al.*, 1997). This was incubated at 37 ˚C in an atmosphere of 3 % $O_2$, 6 % $CO_2$ and 91 % $N_2$.

### 4.3.3. Parasite Lactate Dehydrogenase (pLDH) Assay Procedure

Prior to the experiment, the parasitemia level of the culture-derived parasitized erythrocytes was adjusted to 2 % suspension by the addition of a normal type A human erythrocytes. The stock solutions of the novel test compounds were prepared and stored at -20 ˚C. All further dilutions were freshly prepared in complete culture medium on the day of the experiment and tested in triplicate.

On the day of the experiment, the frozen stock parasite suspension was thawed at room temperature and 0.2 ml of parasite suspension was collected and dispensed into a well of a 96-well microtitre plate in triplicate for each compound concentration. Subsequently, an aliquot of the frozen stock synthesized test compounds were thawed and freshly diluted to the desired final test concentration with complete medium. Starting from a concentration of 1000 ng/ml, the test compounds were serially diluted 2-fold to 2 ng/ml concentrations in complete medium. A total of 10 concentrations were achieved. Each dilution was distributed in the 96-well microtiter plates with parasite suspension in triplicate and then incubated at 37 ºC for 72 hours. The same dilutions technique was done on the reference drug CQ. Finally, the cultures in each well were re-suspended carefully and aliquots were removed and spectrophotometerically analyzed at 650 nm for pLDH activity. Then, the $IC_{50}$ values were obtained by the non-linear dose-response curve fitting analysis using GraphPad Prism v.4.0 for windows. The antimalarial activity of the test compounds were expressed as $IC_{50}$ values (mean ± SD) which is inversely proportional to the potency of the test compounds.



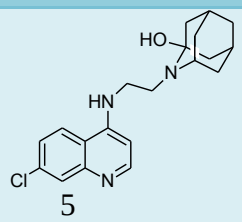
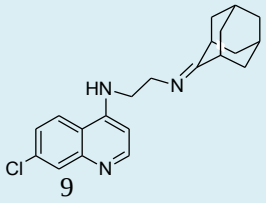
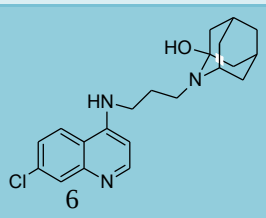
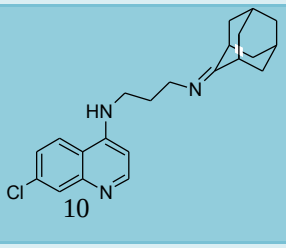
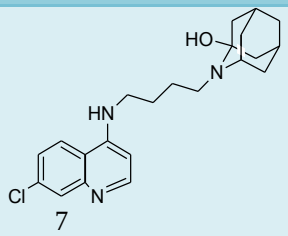
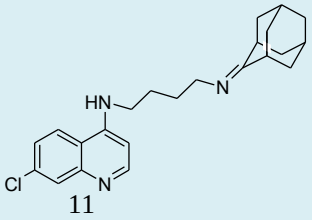
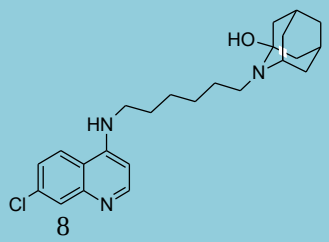
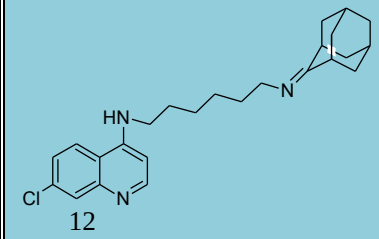
## 4.4. RESULTS AND DISCUSSION

### 4.4.1. Cytotoxicity Study

One of the aims of this study was to establish the toxicity of these conjugates, thus they were subjected to cytotoxicity evaluation using the Chinese Hamster Ovarian cell line (CHO). The results are presented in **table 4.1**. It can be seen from the result that all the AD-CQ conjugates of both series, have very high CHO $IC_{50}$ values ($IC_{50}$: 37860 – 279420 nM). The implication of these high values is that the

novel conjugates have very low toxicity towards non-parasitic cells and as such are much safer when compared to the cytotoxic reference drug emetine ($IC_{50}$ = 61 nM).

**Table 4.1:** Cytotoxicity assay $IC_{50}$ values of the novel AD-CQ conjugates

| Series 1 aza-adamantanols | CHO $IC_{50}$ (nM) | Series2 imine-adamantanes | CHO $IC_{50}$(nM) |
|---|---|---|---|
|  5 | 279420 |  9 | 98500 |
|  6 | 45190 |  10 | 66390 |
|  7 | 37860 |  11 | 57080 |
|  8 | 80760 |  12 | 103750 |
| Emetine | 61 | | |

CHO = Chinese Hamster Ovarian.

### 4.4.2. Antimalarial Activity

The main aim of this study was to synthesize novel AD-CQ conjugates and have them overcome *P. falciparum* CQ resistance. However, these conjugates are also expected to fundamentally possess antimalarial activity because of the

incorporation of the 4-aminoquinoline moiety of CQ (Peyton, 2012). The antimalarial activities of the conjugates were defined as follows: $IC_{50}$ > 1000 nM is inactive; $IC_{50}$ < 1000 nM is active and $IC_{50}$ < 100 nM is highly active. From the results presented in **table 4.2**, both series of novel synthesized AD-CQ conjugates (compounds **5 - 12**) displayed good antimalarial activity in the low nanomolar range ($IC_{50}$ = <5 – 112.69 nM) comparable to the reference drug CQ ($IC_{50}$ = 7.8 nM) against the $CQ^S$ NF54 strain. Among all the AD-CQ conjugates, compound **10**, showed every good activity ($IC_{50}$ = < 5 nM) even better than the reference CQ on the $CQ^S$ NF54 strain followed by compound **6**, **7** and **5** ($IC_{50}$ NF54 = 22.32, 33.94 and 46.94 nM). The work of Makler and Hinrichs (1993) has shown that there is a linear correlation between parasitemia levels and pLDH activity. Hence, the $IC_{50}$ values are inversely proportional to the potency of the test compounds, which implies the lower the $IC_{50}$ value the more potent the compound.

Focusing on the $CQ^R$ strain K1, seven of the novel conjugates **(5 – 11**) were active antimalarial compounds ($IC_{50}$ = 93 nM – 784 nM) and compound **12** was considered inactive with an $IC_{50}$ value greater than 1000 nM ($IC_{50}$ = 1580.28 nM). Three of the compounds **5**, **6** and **9** were highly active ($IC_{50}$ = 98.92, 96.80 and 93.81 nM). These results showed that the novel AD-CQ conjugates exhibit marked antimalarial activity. Furthermore, the selectivity index (SI), defined as the ratio of the $IC_{50}$ on the CHO cell line to the $IC_{50}$ on $CQ^R$ K1 was calculated. This estimates the potential of the novel conjugates to selectively inhibit *P. falciparum* growth. A very low SI (below 25) indicates the possibility that the antimalarial activity determined is due to cytotoxicity rather than antimalarial activity against the parasite. In the same line the higher the SI (greater than 25) the more selective the conjugates towards *P. falciparum* (Valdés *et al.*, 2010; Soh & Benoit-Vical, 2007). As shown in **table 4.2**, all the novel conjugates show greater selectivity towards the resistant parasite strains K1 with high SI (SI = 73 – 2825). This implies the activity of the novel conjugates recorded is because of antimalarial activity.

Between the novel compounds, the series 1-aza-adamantanols (**5 – 8**) showed better activity with lower $IC_{50}$ values against $CQ^R$ strain compared to the series 2-

imine-adamantanes (**9 – 12**). Evaluating the two series structurally in relation to their activity, it was established that the presence of the hydroxyl group (-OH) may play a role in the improved reversed CQ activity of the aza-adamantanols (series 1). This implies that the hydroxyl group could help or play a part in the binding of the compounds to the *Pf*CQRT and aid in blocking this channel. This can be investigated further by investigating the mechanism of action of these conjugates. It is worth mentioning that although the imine-adamantane compounds showed significant activity against the CQ sensitive strain, they had markedly reduced activity against the CQ resistant strain. This loss of activity may be directly linked to the lower pKa values observed or other structural features making the imine-adamantane moiety a weaker RA.

**Table 4.2:** Antimalarial activity of novel adamantane-chloroquinolin conjugates

| Compounds | NF 54 $IC_{50}$ (nM) | K1 $IC_{50}$ (nM) | SI |
|---|---|---|---|
| 5 | 46.94 | 98.92 | 2825 |
| 6 | 22.32 | 96.80 | 467 |
| 7 | 33.94 | 198.22 | 191 |
| 8 | 112.57 | 283.59 | 285 |
| 9 | 26.28 | 93.81 | 1050 |
| 10 | < 5 | 191.62 | 346 |
| 11 | 108.39 | 783.89 | 73 |
| 12 | 112.69 | 1580.28 | 104 |
| CQ | 7.8 | 300.00 | ND |

Selectivity Index = $IC_{50}$ CHO/$IC_{50}$ K1. ND = not determined

After confirming that these conjugates have antimalarial activity, it was further evaluated if they retained enough activity in the $CQ^R$ strain to overcome *P. falciparum* CQ resistance. The ability of the conjugates to overcome the problem of *P. falciparum* CQ resistance was elucidated from the antimalarial activity of the conjugates against the $CQ^S$ and $CQ^R$ strains. From the antimalarial activity data it was possible to get the ratio of the $IC_{50}$ of the $CQ^R$ strain K1 to that of the $CQ^S$ strain NF54. This ratio is called the resistance index (RI) shown in **table 4.3**.

RI gives a degree of how resistant the parasite is to a particular compound by reducing its activity on the resistant strain such that a higher concentration of the drug compound is required to inhibit the growth of 50 % of the parasite in the resistant strain than in the sensitive strain. The higher the K1 $IC_{50}$, the higher the RI and the higher the level of resistance (Nzila & Mwai, 2010).

As shown in **table 4.3**, all the novel conjugates have smaller RI, ranging from 2.11 to > 16, compared to CQ with a RI of 38.46. This implies that the novel compounds exhibit the ability to retain their activity in *P. falciparum* CQ resistant strain thus can overcome the problem of *P. falciparum* CQ resistance.

**Table 4.3:** Resistance reversal activity of the novel AD-CQ conjugates

| Compounds | NF 54 $IC_{50}$ (nM) | K1 $IC_{50}$ (nM) | RI | Ratio to CQ RI* |
|---|---|---|---|---|
| **5** | 46.94 | 98.92 | 2.11 | 18.2 |
| **6** | 22.32 | 96.80 | 4.34 | 8.9 |
| **7** | 33.94 | 198.22 | 5.84 | 6.6 |
| **8** | 112.57 | 283.59 | 3.81 | 10.1 |
| **9** | 26.28 | 93.81 | 3.56 | 10.8 |
| **10** | < 5 | 191.62 | >16 | 2.4 |
| **11** | 108.39 | 783,89 | 7.23 | 5.3 |
| **12** | 112.69 | 1580.28 | 14.02 | 2.7 |
| **CQ** | 7.8 | 300.00 | 38.46 | 1 |

Resistance Index = $IC_{50}$ K1/$IC_{50}$ NF54. *Ratio to CQ RI = RI of CQ/RI of compound

Between the two series, the aza-adamantanols of series 1 are better at retaining their activity in the resistant strain than the imine-adamantane series. This implies that the aza- adamantanols are promising reversed CQ compounds. Although the ACQ intermediates (compounds **1 – 4**) with a primary terminal amine were also tested, they did not have significant activity against the $CQ^S$ strain at

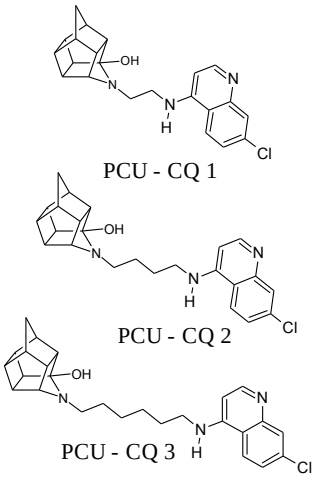
concentrations of less than 10 µM and were thus not tested further. This result confirmed that the terminal tertiary amine is essential for antimalarial activity especially in the $CQ^R$ strain to increase the drug accumulation in the acidic digestive vacuole (DV) (Egan *et al.*, 2000). Evaluation of the ACQ intermediates offered information about the effect of adamantane as a reversal agent as well as some preliminary SAR of the AD-CQ. The result showed that the addition of the adamantane in the novel compounds greatly decreased the concentration required for 50 % inhibition of the parasite. Hence the ability of the novel compounds to retain their activity in the $CQ^R$ strain and overcome the problem of *P. falciparum* CQ resistance is attributed to the adamantane moiety. This has the implication that the adamantane moiety is a resistance reversal agent in *P. falciparum* CQ resistant strain.

Prior work in our research group, have shown NGP1-01 may act as a good reversal agent. The pentacycloundecylamine (PCU) of NGP1-01 was used to develop aza PCU-CQ derivatives (**PCU-CQ 1 - 3)** which are good reversed CQ compounds (Fortuin, 2014; Joubert *et al.*, 2014). This is demonstrated by their resistance index compared to CQ, presented in **table 4.4**. The same assay methods as described in this study were used to evaluate the PCU-CQ compounds. Thus, in this study we were also interested to evaluate the reversal effect of the adamantane moiety as RA compared to the PCU moiety. This was possible by comparing the RI of the AD-CQ conjugates with that of the PCU-CQ compounds. The PCU-CQ compounds have RIs ranging from 7 – 22 compared to CQ while the RI of the novel AD-CQ conjugates range from 2 to 16. However, for easy and clear comparison of the resistance reversal activity of the PCU and adamantane moieties, the RI of the novel AD-CQ conjugates and the PCU-CQ compounds were modified by calculating the ratio of each RI of the various compounds compared to CQ. This made comparison between the compounds meaningful. The ratio revealed that **PCU-CQ 1** (a 2C linker) was 3.29 times better at retaining activity than CQ whereas compound **5** (also with a 2C linker) of the novel AD-CQ conjugates is 18 times better at retaining activity than CQ in the $CQ^R$ strain. From **figure 4.1**, it can be clearly seen that compound **5** overcomes the problem of *P. falciparum* CQ resistance by 18-fold compared to CQ. Therefore, the aza-

adamantanol moiety as used in this study produced more potent reversed CQ compounds than the PCU moiety.

**Table 4.4:** PCU-CQ compounds with PCU of NGP1-01as reversal agent RI and corresponding structures

| Previous study compounds | RI | Ratio to CQ RI* |
|---|---|---|
| PCU-CQ1 | 7 | 3.29 |
| PCU-CQ2 | 22 | 1.05 |
| PCU-CQ3 | 14 | 1.60 |
| CQ | 23 | 1 |




Resistance Index = IC50 K1/IC50 NF54. *Ratio to CQ RI = RI of CQ/RI of compound

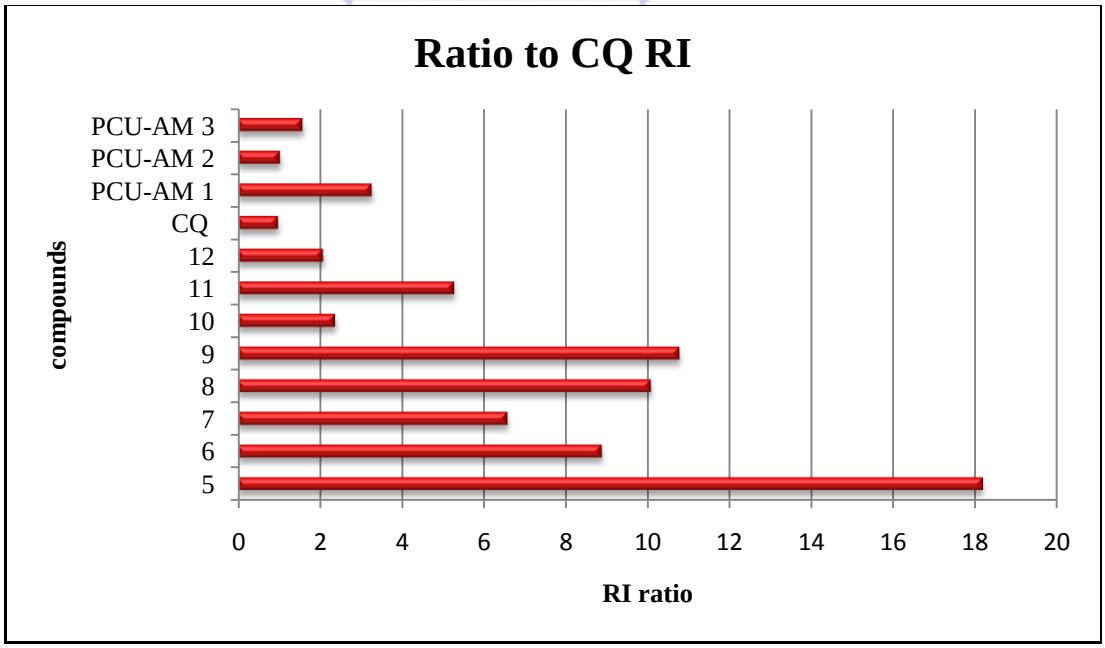



**Figure 4.1:** Graphic display of the potency of the compounds in reversing CQ resistance.

The result presented in **table 4.2** shows that, the $IC_{50}$ values of the test compounds increases as the chain length of the alkyl linker increases. This trend is significant in the resistant strain K1 and concurs with literature that chain length changes has little influence on $CQ^S$ NF54 activity but a profound influence on the $CQ^R$ strain

and thus resistance (De, *et al.*, 1996). This can be explained in that as the chain length increases the degree of flexibility of the compounds in the *Pf*CQRT protein increases and may adopt an unfavourable conformation whereas the short chain adopts a more favourable conformation and thus improves the efficacy of the compounds. From both series and putting all data together, it can be concluded that the conjugates with alkyl linkers of 2 - 3 carbons show optimum activity for both $CQ^S$ NF54 and $CQ^R$ K1. This answers the research question "What is the appropriate chain length for optimal activity of the linker between 4-ACQ pharmacophore and the RA?" asked in chapter 1.

From the data shown in **table 4.5** below, the series 1-aza-adamantanols have log P values of less than 5 (Log P = 3.1 – 4.59) in the range of CQ (log P = 4.69) compared to the large values of the series 2-imine-adamantane (Log P = 6.34 – 7.93). Furthermore, all the novel compounds have hydrogen bond donors and acceptors less than five and ten respectively with molecular mass below 500 Daltons. The conjugates of series 1 obey all the Lipinski rule of five (Ro5) (Lipinski *et al.*, 1997). This implies that compounds of this series possess desired drug-like properties of solubility and permeability necessary for orally effective drug candidate development. Therefore, the activity of the novel compounds is mainly due to the reversal ability of the adamantane moiety *via* inhibition of the *Pf*CQRT efflux pump and little to do with the lipophllic nature of the adamantane RA. The imine adamantanes failed to obey all the RoF with the log P values which can affect their solubility. The table below represents a summary of all the results.

**Table 4.5:** *In vitro* antimalarial activity $IC_{50}$ values of the novel adamantane-chloroquinolin conjugates and reference compounds

| Compounds | NF 54 $IC_{50}$ (nM) | K1 $IC_{50}$ (nM) | CHO $IC_{50}$ (nM) | RI | SI | Log P | $pKa_2$ |
|---|---|---|---|---|---|---|---|
| **5** | 46.94 | 98.92 | 279420 | 2.11 | 2825 | 3.11 | 8.3 |
| **6** | 22.32 | 96.80 | 45190 | 4.34 | 467 | 3.34 | 8.8 |
| **7** | 33.94 | 198.22 | 37860 | 5.84 | 191 | 3.72 | 9.1 |
| **8** | 112.57 | 283.59 | 80760 | 3.81 | 285 | 4.59 | 9.2 |
| **9** | 26.28 | 93.81 | 98500 | 3.56 | 1050 | 6.34 | 6.3 |
| **10** | < 5 | 191.62 | 66390 | >16 | 346 | 6.67 | 6.7 |
| **11** | 108.39 | 783,89 | 57080 | 7.23 | 73 | 7.00 | 7.1 |
| **12** | 112.69 | 1580.28 | 103750 | 14.02 | 104 | 7.93 | 8.9 |
| **CQ** | 7.8 | 300.00 | ND | 38.46 | ND | 4.69 | 10.2 |
| **Emetine** | ND | ND | 61 | ND | ND | ND | ND |

Resistance Index = IC50 K1/IC50 NF54. Selectivity Index = IC50 CHO/IC50 K1. ND = not determined. CHO = Chinese Hamster Ovarian. Log P values calculated using ACD Chemsketch. The pKa calculated using the ACE and JChem acidity and basicity calculator, available at http://epoch.uky.edu/ace/public/pka.jsp)c

According to Kaschula and co-workers (2002), pKa of terminal nitrogen ranges from 7.65 to 10.2 in amino derivatives. This results in 97 % of drug in the DV due to pH trapping. Based on this it was expected that conjugates with a pKa value closer to that of CQ would have better antimalarial activity. Thus, the pKa values of the compounds were calculated and are presented in **table 4.5** to get a picture on the degree of protonation for drug accumulation in the DV. From the calculated pKa values, all the compounds have a quinolyl nitrogen pKa value of 7.3 and terminal side chain nitrogen of varied pKa values. This may lead to

different degrees of drug accumulation because of the differences in 2nd protonation. It was thus expected that the antimalarial activity will also increase in each series as pKa values increases. Although this was true between the two series in that the more active series 1- aza-adamantanols showed higher pKa values (closer to CQ, pKa = 10.2), it was not true within each series as carbon chain length increases.

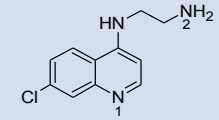
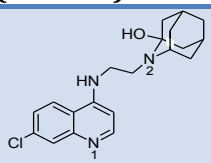
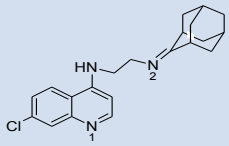
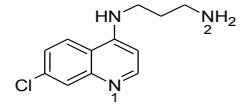
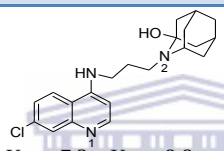
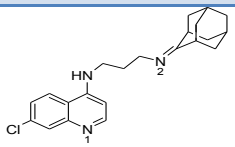
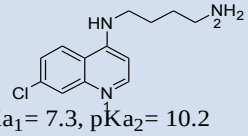
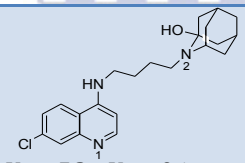
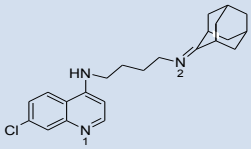
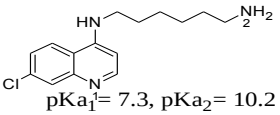
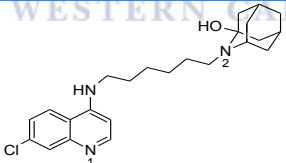
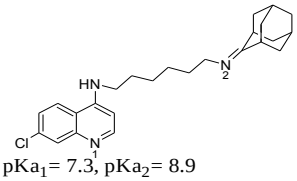
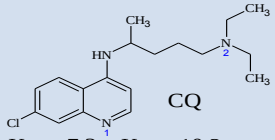
| ACQ intermediates | Aza-adamantanols (Series 1) | Imine-adamantane (Series 2) |
|---|---|---|
|  $pKa_1$ = 7.3, $pKa_2$= 9.6 |  $pKa_1$= 7.3, $pKa_2$= 8.3 |  $pKa_1$= 7.3, $pKa_2$= 6.3 |
|  $pKa_1$= 7.3, $pKa_2$= 9.6 |  $pKa_1$= 7.3, $pKa_2$= 8.8 |  $pKa_1$= 7.3, $pKa_2$= 6.7 |
|  $pKa_1$= 7.3, $pKa_2$= 10.2 |  $pKa_1$= 7.3, $pKa_2$= 9.1 |  $pKa_1$= 7.3, $pKa_2$= 7.1 |
|  $pKa_1$= 7.3, $pKa_2$= 10.2 |  $pKa_1$= 7.3, $pKa_2$= 9.2 |  $pKa_1$= 7.3, $pKa_2$= 8.9 |
|  CQ $pKa_1$= 7.3, $pKa_2$= 10.2 | | |

**Figure 4.2:** Calculated pKa values of the novel compounds.

The pKa calculated using the ACE and JChem acidity and basicity calculator, available at http://epoch.uky.edu/ace/public/pka.jsp)c

From **figure 4.2** above, the ACQ intermediates (**1 – 4**) with terminal primary amines were expected to have good activity against the $CQ^S$ strain when considering pKa values alone, however they did not have significant activity when tested ($IC_{50}$ $CQ^S$ > 1 µM). This concurs with literature (Egan *et al.*, 2000) in that

the terminal tertiary amine is essential for antimalarial activity especially in the $CQ^R$ strain. It supports the argument that although drug accumulation via pH trapping is possible by the second protonation of the tertiary amine, increases in pKa cannot be considered alone for increased activity as this series of compounds had higher pKa values closer to that of CQ (pKa = 10.2). Thus, a primary terminal amine decreases antimalarial activity.

## 4.5. CONCLUSION

The novel compounds exhibited potent antimalarial activity *in vitro* comparable to CQ on the $CQ^S$ NF54 strain and superior to CQ against the $CQ^R$ KI strain and overcame *P. falciparum* CQ resistance. Although compound **5** was not the compound with the best activity on both $CQ^S$ and $CQ^R$ strains, it had the lowest RI (RI = 2.11) and good activity ($IC_{50}$ = 98.92 nM) towards the resistant parasite strain K1. Compound **5** which showed an 18-fold enhancement at retaining its activity against the *P. falciparum* $CQ^R$ strain K1 compared to CQ is thus a promising candidate to substitute CQ in *P. falciparum* resistant malaria. The adamantane moiety, especially in the aza-adamantanols, was shown to be a significant *P. falciparum* CQ resistance reversal agent. Hence, the hybridization of a CQ-like nucleus to an adamantane moiety resulted in adamantane-chloroquinolin conjugates with improved antimalarial activity that overcomes the problem of *P. falciparum* CQ resistance.

# CHAPTER 5

# SUMMARY AND CONCLUSION

## 5.1. INTRODUCTION

From the review of literature, malaria remains a significant parasitic infection. This is because of the devastating impact it has on global health and the socioeconomic status of endemic areas especially in sub-Saharan Africa (Sachs & Malaney, 2002; Gallup & Sachs, 2001). Half of the worlds' population are at risk of malaria parasite infection. Although efforts are being made to fight the disease, there are still thousands of malaria-related deaths annually and the most vulnerable predominantly include pregnant women and children below the age of 5 (WHO, 2016a). The burden of malaria is being aggravated by the emergence of *Plasmodium falciparum* resistance to the few antimalarial drugs available, in particular CQ (Sidhu, Verdier-Pinard and Fidock, 2002). CQ had been the drug of choice for the prophylaxis and treatment of malaria infection. However, *P. falciparum* resistance to it has restricted its use in malaria therapy and put to waste its good qualities of cost-effectiveness, safety, affordability and availability (Ridley, 2002). CQ resistance is mainly as a result of low drug concentration in the parasitic food vacuole due to its efflux by the mutated *Plasmodium falciparum* CQ resistance transporter (*Pf*CQRT) (Pulcini *et al.*, 2015; Sidhu, Verdier-Pinard and Fidock, 2002; Fidock *et al.*, 2000). Hence, the inhibition of this efflux transporter of CQ could overcome the prevailing issue of *P. falciparum* resistance to CQ-like drugs. In an attempt to solve the problem, some compounds known as reversal agents (RA) were found to reverse/inhibit the effect of *Pf*CQRT (Deane, *et al*., 2014). This led to the attractive strategy of making "reversed CQ" (RCQ) compounds which involved the hybridization of the CQ-like 4-aminoquinoline pharmacophore to a reversal agent *via* an alkyl linker (Burgess *et al.*, 2006, Andrews *et al.*, 2009). Thus, this study sought to synthesize a series of novel adamantane-chloroquinolin (AD-CQ) conjugates as potentially improved reversed CQ agents. These compounds are intended to overcome the CQ resistance by

*Plasmodium falciparum* and could add to the antimalarial armoury to control malaria.

## 5.2. SYNTHESIS

The AD-CQ conjugates (compounds **5** - **12**) consisted of the CQ-like 4-aminoquinoline pharmacophore conjugated to adamantane (as the reversal agent) *via* an alkyl linker. Their synthesis involved multiple intermediary steps which posed many challenges in this study. The idea in designing the novel compounds was to explore adamantane as a RA, whilst incorporate a terminal tertiary amine as well as a hydroxyl group in the conjugates. The starting material 2-adamantanol enabled all these features. Firstly, the various aminoquinoline (ACQ) intermediates were synthesized by the amination of 4, 7-dichloroquinoline with different chain length diaminoalkane linkers using microwave irradiation. These compounds were used for synthesis of the novel AD-CQ conjugates of series 1 and 2.

The compounds in series 1 (compounds **5** - **8**) had the hydroxyl group. They were synthesized by the conjugation of the adamantane diketone and appropriate ACQ intermediates *via* reductive amination followed by transannular cyclization (Joubert *et al.*, 2014). The adamantane diketone was synthesized from the 2-adamantanone *via* a 3 step process. The compounds in series 2 (compounds **9** – **12**) were synthesized from the direct conjugation between 2-adamantone and ACQ intermediates in a 1:1 ratio.

As presented in **figure 3.1**, a total of eight novel AD-CQ conjugates (compounds **5** – **12**) were successfully synthesized with reasonable yields. The compounds were purified with column chromatography using methanol/ammonia as mobile phase in a 10:1 ratio. NMR and IR were used to characterize significant signals and the MS confirmed the presence of the desired conjugates by their molecular masses. Characterization of the compounds was possible by making inference to the characteristic signals observed for each specific compound using the signals of 2-adamatnatone and its intermediates as reference for the adamantane part of the conjugates.

## 5.3. BIOLOGICAL EVALUATIONS

The cytotoxicity profile of the synthesized novel AD-CQ conjugates were investigated using the MTT assay and all compounds were found to be non-toxic on the Chinese Hamster Ovarian cell line. Their antimalarial activity was then investigated against both CQ sensitive ($CQ^S$) NF54 and resistant ($CQ^R$) K1 strains using the parasitic lactate dehydrogenase assay (Makler *et al.*, 1993). The results obtained are presented in **table 4.5** of the preceding chapter.

All the compounds of both series demonstrated *in vitro* antimalarial activity comparable to CQ against the $CQ^S$ strain and superior to CQ against the $CQ^R$ strains. The compounds also had lower resistance index (RI) of 2 to 16, which gave the degree of magnitude by which the activity of the compounds were retained in the $CQ^R$ strain compared to CQ (RI = 38). In general the compounds in series 1 had better activity. The degree by which *P. falciparum* CQ resistance was overcome in the resistant strain was calculated as the ratio to CQ RI and ranged from 2 to 18-folds. This result illustrated that compound **5** overcame *P. falciparum* CQ resistance in the resistant strain K1 by 18-fold compared to CQ. Thus it stood out as a promising lead compound for further optimization to substitute CQ in *P. falciparum* malaria infections. The adamantane moiety, especially in the compounds of series **1**, was shown to be a significant *P. falciparum* CQ resistance reversal agent compared to the previously used structurally related PCU moiety (Joubert *et al*,. 2014).



## 5.4. CONCLUSION

Resistance to antimalarial drugs especially CQ is a major setback in the use of chemotherapy in the control of malaria. This thus created the need to find and develop new improved antimalarial agents.

This study has shown that the hybridization of adamantane moieties to a CQ-like nucleus *via* alkyl linkers of 2-3 carbon chain lengths results in improve reversed CQ compounds with significant *in vitro* antimalarial activity that overcome *P. falciparum* CQ resistance. From all the observations and analysis made, this study has deduced that the antimalarial activity of the novel compounds is dependent on

a number of hypothesized factors that need to be assessed collectively and not individually. These include:

- The 4-aminoquinoline pharmacophore is still a viable class for antimalarial compounds.
- The incorporation of the tertiary amine group is essential for significant activity especially in the $CQ^R$ strain as it increases drug accumulation in the acidic DV
- The use of the adamantane moiety as a RA produced reversed CQ compounds with enhanced antimalarial activity compared to CQ in the *P. falciparum* CQ resistant strain.
- The length of the alkyl linker between the CQ-like nucleus and the adamantane RA should be between 2-3 carbons for optimum ability to overcome resistance.

From **table 4.5**, it was observed that compound **5** had the lowest RI and good activity towards the resistant parasite strain K1 even though it was not the compound with the best activity on both $CQ^S$ and $CQ^R$ strains. It retained its activity in the $CQ^R$ strain by 18-fold compared to CQ and was thus identified as a promising candidate to substitute CQ in *P. falciparum* resistant malaria. However, its clinical use and safety in humans still needs to be proven. Therefore, the next step will be to carry out further *in vitro* and *in vivo* biological and mechanistic studies to elaborate on the molecular mechanism (s) involved in parasite-killing and reversal of the *Pf*CQRT efflux effect. Also, the role of the hydroxyl group on the activity of the aza AD-CQ conjugates should be investigated further to build on the SARs of the compounds. Also, further work on compound 10 with NF54 IC50 value lower than CQ is recommended.

# REFERENCES


1. Aghahowa, S.E., Obianwu, H.O., Isah, A.O. and Arhewoh, I.M. (2010). Chloroquine-induced pruritus. *Indian Journal of Pharmaceutical Sciences*, *72*(3), pp.283.
2. Aguiar, A.C.C., de Meneses Santos, R., Figueiredo, F.J.B., Cortopassi, W.A., Pimentel, A.S., França, T.C.C., Meneghetti, M.R. and Krettli, A.U. (2012). Antimalarial activity and mechanisms of action of two novel 4-aminoquinolines against chloroquine-resistant parasites. *Public Library of Science* (*PLoS) One*, *7*(5), pp.e37259.
3. Alving, A.S., Arnold, J., Hockwald, R.S., Clayman, C.B., Dern, E., Beutler, E. and Flanagan, C.L. (1955). Potentiation of the Curative Action of Primaquine in vivax Malaria by Quinine and Chloroquine. *Journal of Laboratory and Clinical Medicine*, *46*(2), pp.301-306.
4. Alving, A.S., Johnson, C.F., Tarlov, A.R., Brewer, G.J., Kellermeyer, R.W. and Carson, P.E. (1960). Mitigation of the haemolytic effect of primaquine and enhancement of its action against exoerythrocytic forms of the Chesson strain of Plasmodium vivax by intermittent regimens of drug administration: a preliminary report. *Bulletin of the World Health Organization*, *22*(6), pp.621.
5. Andrews, S., Burgess, S.J., Skaalrud, D., Kelly, J.X. and Peyton, D.H. (2009). Reversal agent and linker variants of reversed chloroquines: activities against Plasmodium falciparum. *Journal of Medicinal Chemistry*, *53*(2), pp.916-919.
6. Anstey, N.M., Russell, B., Yeo, T.W. and Price, R.N. (2009). The pathophysiology of vivax malaria. *Trends in Parasitology*, *25*(5), pp.220-227.
7. Autino, B., Noris, A., Russo, R. and Castelli, F. (2012). Epidemiology of malaria in endemic areas. *Mediterranean Journal of Hematology and Infectious Diseases*, *4*(1), pp.e2012060.

8. Baird, J.K. (1995). Host age as a determinant of naturally acquired immunity to Plasmodium falciparum. *Parasitology Today*, *11*(3), pp.105-111.
9. Baird, J.K. (2005). Effectiveness of antimalarial drugs. *New England Journal of Medicine*, *352*(15), pp.1565-1577.
10. Baird, J.K. (2013). Evidence and implications of mortality associated with acute Plasmodium vivax malaria. *Clinical Microbiology Reviews*, *26*(1), pp.36-57.
11. Baird, J.K. and Rieckmann, K.H. (2003). Can primaquine therapy for vivax malaria be improved? *Trends in Parasitology*, *19*(3), pp.115-120.
12. Baird, J.K., Jones, T.R., Danudirgo, E.W., Annis, B.A., Bangs, M.J., Basri, P.H. and Masbar, S. (1991). Age-dependent acquired protection against Plasmodium falciparum in people having two years exposure to hyper endemic malaria. *The American Journal of Tropical Medicine and Hygiene*, *45*(1), pp.65-76.
13. Banister, S.D., Yoo, D.T., Chua, S.W., Cui, J., Mach, R.H. and Kassiou, M. (2011). N-Arylalkyl-2-azaadamantanes as cage-expanded polycarbocyclic sigma (σ) receptor ligands. *Bioorganic &Medicinal Chemistry Letters*, *21*(18), pp.5289-5292.
14. Bejon, P., Lusingu, J., Olotu, A., Leach, A., Lievens, M., Vekemans, J., Mshamu, S., Lang, T., Gould, J., Dubois, M.C. and Demoitié, M.A.(2008). Efficacy of RTS, S/AS01E vaccine against malaria in children 5 to 17 months of age. *New England Journal of Medicine*, *359*(24), pp.2521-2532.
15. Biagini, G.A., O'Neill, P.M., Nzila, A., Ward, S.A. and Bray, P.G. (2003). Antimalarial chemotherapy: young guns or back to the future? *Trends in Parasitology*, *19*(11), pp.479-487.
16. Biamonte, M.A., Wanner, J. and Le Roch, K.G. (2013). Recent advances in malaria drug discovery. *Bioorganic &Medicinal Chemistry Letters*, *23*(10), pp.2829-2843.
17. Birley, M. and Charlewood, J.D. (1987). Sporozoite rate and malaria prevalence. *Parasitology Today*, *3*(8), pp.231-232.

18. Bloland, P.B.(2001). Drug resistance in malaria. World Health Organization.
19. Bloland, P.B., Kazembe, P.N., Oloo, A.J., Himonga, B., Barat, L.M. and Ruebush, T.K. (1998). Chloroquine in Africa: critical assessment and recommendations for monitoring and evaluating chloroquine therapy efficacy in sub-Saharan Africa. *Tropical Medicine & International Health*, *3*(7), pp.543-552.
20. Brabin, B.J. (2014). Malaria's contribution to World War One–the unexpected adversary. *Malaria Journal*, *13*(1), pp.497.
21. Bray, P.G., Howells, R.E., Ritchie, G.Y. and Ward, S.A. (1992). Rapid chloroquine efflux phenotype in both chloroquine-sensitive and chloroquine-resistant Plasmodium falciparum: a correlation of chloroquine sensitivity with energy-dependent drug accumulation. *Biochemical Pharmacology*, *44*(7), pp.1317-1324.
22. Bray, P.G., Martin, R.E., Tilley, L., Ward, S.A., Kirk, K. and Fidock, D.A. (2005). Defining the role of *Pf*CRT in Plasmodium falciparum chloroquine resistance. *Molecular Microbiology*, *56*(2), pp.323-333.
23. Bray, P.G., Mungthin, M., Ridley, R.G. and Ward, S.A. (1998). Access to hematin: the basis of chloroquine resistance. *Molecular Pharmacology*, *54*(1), pp.170-179.
24. Bredenkamp, B.L., Sharp, B.L., Mthembu, S.D., Durrheim, D.N. and Barnes, K.I. (2001). Failure of sulphadoxine-pyrimethamine in treating Plasmodium falciparum malaria in KwaZulu-Natal. *South African Medical Journal (Suid-Afrikaanse tydskrif vir geneeskunde)*, *91*(11), pp.970-972.
25. Breman, J.G. (2001). The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *The American Journal of Tropical Medicine and Hygiene*, *64*(1 suppl), pp.1-11.
26. Breman, J.G., Alilio, M.S. and Mills, A. (2004). 'Conquering the intolerable burden of malaria: what's new, what's needed: a summary'. *The American Journal of Tropical Medicine and Hygiene*, *71*(2 suppl), pp.1-15.

27. Brito, I. (2001). 'Eradicating malaria: high hopes or a tangible goal?' *Health Policy at Harvard*, *2*, pp.61-66.
28. Brookes, K. B., Hickmott, P. W., Jutle, K. K. and Schreyer, C. A. (1992) Introduction of pharmacophoric groups into polycyclic systems. Part 4. Aziridine, oxiran and tertiary b-hyddroethylamine derivatives of adamantine. *South African Journal of Chemistry*. 45, pp. 8-11
29. Bruce-Chwatt, L.J. (1954). Problems of malaria control in tropical Africa. *British Medical Journal*, *1*(4855), pp.169.
30. Bruce-Chwatt, L.J. (1981). *Chemotherapy of malaria* 27, 2nd edition, Geneva, Switzerland; WHO.
31. Bruce-Chwatt, L.J. (1988). History of malaria from prehistory to eradication. *Malaria Principles and Practice of Malariology*, Edited by W. H Wernsdorfer and I McGregor. Vol. 1. Edinburgh: Churchill Livingstone, *1*, pp.1-59.
32. Bruce-Chwatt, L.J., Black, R. H., Canfield, C. J., Clyde, D. F., Peters, W., Wernsdorfer, W. H. And World Health Organization (1986). Chemotherapy of Malaria.World Health Organization. Available at http://www.who.int/iris/handle/10665/38605.
33. Burgess, S.J., Kelly, J.X., Shomloo, S., Wittlin, S., Brun, R., Liebmann, K. and Peyton, D.H. (2010). Synthesis, Structure− Activity Relationship, and Mode-of-Action Studies of Antimalarial Reversed Chloroquine Compounds. *Journal of Medicinal Chemistry*, *53*(17), pp.6477-6489.
34. Burgess, S.J., Selzer, A., Kelly, J.X., Smilkstein, M.J., Riscoe, M.K. and Peyton, D.H. (2006). A chloroquine-like molecule designed to reverse resistance in Plasmodium falciparum. *Journal of Medicinal Chemistry*, *49*(18), pp.5623-5625.
35. Carrington, H.C., Crowther, A.F., Davey, D.G., Levi, A.A. and Rose, F.L. (1951). A metabolite of 'Paludrine' with high antimalarial activity. *Nature*, *168*(4286), pp.1080-1080.
36. Carter, R. and Mendis, K.N. (2002). Evolutionary and historical aspects of the burden of malaria. *Clinical Microbiology Reviews*, *15*(4), pp.564-594.

37. CDC. (2010). Malaria. Center for Disease Control and Prevention. [Online] Available at: http://www.cdc.gov/malaria/about/disease.html [Accessed 25 Apr. 2015]
38. Charlwood, J.D., Smith, T., Kihonda, J., Heiz, B., Billingsley, P.F. and Takken, W. (1995). Density independent feeding success of malaria vectors (Diptera: Culicidae) in Tanzania. *Bulletin of Entomological Research*, *85*(01), pp.29-35.
39. Chen, Q., Schlichtherle, M. and Wahlgren, M. (2000). Molecular aspects of severe malaria. *Clinical Microbiology Reviews*, 13(3), pp. 439-450.
40. Cheruku, S.R., Maiti, S., Dorn, A., Scorneaux, B., Bhattacharjee, A.K., Ellis, W.Y. and Vennerstrom, J.L. (2003). Carbon Isosteres of the 4-Aminopyridine Substructure of Chloroquine: Effects on pKa, Hematin Binding, Inhibition of Hemozoin Formation, and Parasite Growth. *Journal of Medicinal Chemistry*, *46*(14), pp.3166-3169.
41. Coatney, G.R. (1963). Pitfalls in a discovery: the chronicle of chloroquine. *American Journal of Tropical Medicine and Hygiene*, *12*(121), p.8.
42. Coetzee, M. and Fontenille, D. (2004). Advances in the study of Anopheles funestus, a major vector of malaria in Africa. *Insect Biochemistry and Molecular Biology*, *34*(7), pp.599-605.
43. Coluzzi, M. (1999). The clay feet of the malaria giant and its African roots: hypotheses and inferences about origin, spread and control of Plasmodium falciparum. *Parassitologia*, *41*(1-3), pp.277-283.
44. Combrinck, J., Mabotha, T., Ncokazi, K., Ambele, M., Taylor, D., Smith, P., Hoppe, H. and Egan, T. (2013). Insights into the role of heme in the mechanism of action of antimalarials. *American Chemical Society (ACS) Chemical Biology*, 8(1), pp. 133-137.
45. Cooke, B.M., Mohandas, N. and Coppel, R.L. (2001). The malaria-infected red blood cell: structural and functional changes. *Advances in Parasitology*, *50*, pp.1-86.

46. Cookson, R. C., Grundwell, W. and Hudec, W. (1958). Synthesis off cage-like molecules by irradiation of Diels-Alder adducts. *Indian Journal of Chemistry*, pp. 1003-1004
47. Cowman, A.F., Berry, D. and Baum, J. (2012). The cellular and molecular basis for malaria parasite invasion of the human red blood cell. *Journal of Cell Biology*, *198*(6), pp.961-971.
48. Cox, F.E. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites & Vectors*, *3*(1), pp.5.
49. Danysz, W., Parsons, C.G., Kornhuber, J., Schmidt, W.J. and Quack, G. (1997). Aminoadamantanes as NMDA receptor antagonists and antiparkinsonian agents -preclinical studies. *Neuroscience & Biobehavioral Reviews*, *21*(4), pp.455-468.
50. Davanço, M.G., Aguiar, A.C.C., dos Santos, L.A., Padilha, E.C., Campos, M.L., de Andrade, C.R., da Fonseca, L.M., dos Santos, J.L., Chin, C.M., Krettli, A.U. and Peccinini, R.G. (2014). Evaluation of antimalarial activity and toxicity of a new primaquine prodrug. *Public Library of Science (PloS) one*, *9*(8), pp.e105217.
51. Davies, W.L., Grunert, R.R., Haff, R.F., McGahen, J.W., Neumayer, E.M., Paulshock, M., Watts, J.C., Wood, T.R., Hermann, E.C. and Hoffmann, C.E. (1964). Antiviral activity of 1-adamantanamine (amantadine). *Science*, 144(3620), pp.862-863.
52. De, D., Krogstad, F.M., Cogswell, F.B. and Krogstad, D.J. (1996). Aminoquinolines that circumvent resistance in Plasmodium falciparum in vitro. *The American journal of Tropical Medicine and Hygiene*, 55(6), pp.579-583.
53. Deane, K. J., Summers, R. L., Lehane, A. M., Martin, R. E. and Barrow, R. A. (2014). Chlorpheniramine analogues reverse chloroquine resistance in P. falciparum by inhibiting PfCRT. *ACS Medicinal Chemistry Letters*, 5(5), pp. 576-581.
54. Djimdé, A.A., Dolo, A., Ouattara, A., Diakité, S., Plowe, C.V. and Doumbo, O.K. (2004). Molecular diagnosis of resistance to antimalarial

drugs during epidemics and in war zones. *Journal of Infectious Diseases*, *190*(4), pp.853-855.

55. Dondorp, A.M., Nosten, F., Yi, P., Das, D., Phyo, A.P., Tarning, J., Lwin, K.M., Ariey, F., Hanpithakpong, W., Lee, S.J. and Ringwald, P. (2009).Artemisinin resistance in Plasmodium falciparum malaria. *New England Journal of Medicine*, *361*(5), pp.455-467
56. Dorn, A., Stoffel, R., Matile, H., Bubendorf, A. and Ridley, R.G. (1995). Malarial haemozoin/beta-haematin supports haem polymerization in the absence of protein. *Nature*, *374*(6519), pp.269.
57. Egan, T., Ross, D. and Adams, P. (1994). Quinoline anti-malarial drugs inhibit spontaneous formation of β-haematin (malaria pigment). Federation of European Biochemical Societies (*FEBS) Letters*, 352(1), pp.54-57.
58. Egan, T.J., Hunter, R., Kaschula, C.H., Marques, H.M., Misplon, A. and Walden, J. (2000). Structure-function relationships in aminoquinolines: effect of amino and chloro groups on quinoline-hematin complex formation, inhibition of β-hematin formation, and antiplasmodial activity. *Journal of Medicinal Chemistry*, *43*(2), pp.283-291.
59. Ekwunife, C. A., Ozumba, N. A., Eneanya, C. andNandwaorgu, O. C. (2011). Malaria infection among blood donors in Onitsha urban, Southeast Nigeria. *Sierra Leone Journal of Biomedical Researcch,* 3(1), pp. 21-26.
60. Elmes, N.J., Nasveld, P.E., Kitchener, S.J., Kocisko, D.A. and Edstein, M.D. (2008). The efficacy and tolerability of three different regimens of tafenoquine versus primaquine for post-exposure prophylaxis of Plasmodium vivax malaria in the Southwest Pacific. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *102*(11), pp.1095-1101.
61. Falco, E.A., Goodwin, L.G., Hitchings, G.H., Rollo, I.M. and Russell, P.B. (1951). 2, 4-diaminopyrimidines - a new series of antimalarials. *British Journal of Pharmacology and Chemotherapy*, *6*(2), pp.185-200.
62. Fernando, D., Rodrigo, C. and Rajapakse, S. (2011). Primaquine in vivax malaria: an update and review on management issues. *Malaria Journal*, *10*(1), pp.351.

63. Fidock, D.A., Nomura, T., Talley, A.K., Cooper, R.A., Dzekunov, S.M., Ferdig, M.T., Ursos, L.M., Naudé, B., Deitsch, K.W., Su, X.Z. and Wootton, J.C. (2000). Mutations in the *P. falciparum* digestive vacuole transmembrane protein *Pf*CRT and evidence for their role in chloroquine resistance. *Molecular cell*, *6*(4), pp.861-871.
64. Fitch, C.D. (2004). Ferriprotoporphyrin IX, phospholipids, and the antimalarial actions of quinoline drugs. *Life sciences*, *74*(16), pp.1957-1972.
65. Foley, M. and Tilley, L. (1998). Quinoline antimalarials: Mechanism of action and resistance and prospects for new agents. *Pharmacology and Therapeutics*, 79(1), pp. 55-87.
66. Fortuin, E. E. (2014). Novel aminoquinoline-polycyclic hybrid molecules as potential antimalarial agents. A dissertation submitted in partial fulfillment of the Requirements of University of the Western Cape for the Degree of Magister Scientiae. Bellville: University of the Western Cape. Available at http://hdl.handle.net/11394/4463 [Accessed 25 September 2015]
67. Fotakis, G. and Timbrell, J.A. (2006). In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicology Letters*, *160*(2), pp.171-177.
68. Gallup, J.L. and Sachs, J.D. (2001). The economic burden of malaria. *The American Journal of Tropical Medicine and Hygiene*, *64*(1 suppl), pp.85-96.
69. Geary, T.G., Divo, A.D., Jensen, J.B., Zangwill, M. and Ginsburg, H. (1990). Kinetic modeling of the response of Plasmodium falciparum to chloroquine and its experimental testing in vitro: implications for mechanism of action of and resistance to the drug. *Biochemical Pharmacology*, *40*(4), pp.685-691.
70. Geldenhuys, W.J., Malan, S.F., Bloomquist, J.R. and Van der Schyf, C.J. (2007). Structure–activity relationships of pentacycloundecylamines at the

*N*-methyl-d-aspartate receptor. *Bioorganic & Medicinal Chemistry*, *15*(3), pp.1525-1532.

71. Geldenhuys, W.J., Malan, S.F., Bloomquist, J.R., Marchand, A.P. and Van der Schyf, C.J. (2005). Pharmacology and structure-activity relationships of bioactive polycyclic cage compounds: A focus on pentacycloundecane derivatives. *Medicinal Research Reviews*, *25*(1), pp. 21-48.
72. Ginsburg, H., Famin, O., Zhang, J. and Krugliak, M. (1998). Inhibition of glutathione-dependent degradation of heme by chloroquine and amodiaquine as a possible basis for their antimalarial mode of action. *Biochemical Pharmacology*, 56(10), pp.1305-1313.
73. Glans, L., Ehnbom, A., de Kock, C., Martínez, A., Estrada, J., Smith, P.J., Haukka, M., Sánchez-Delgado, R.A. and Nordlander, E.(2012). Ruthenium (II) arene complexes with chelating chloroquine analogue ligands: synthesis, characterization and in vitro antimalarial activity. *Dalton Transactions*, *41*(9), pp.2764-2773.
74. Goldberg, D.E., Slater, A.F., Cerami, A. and Henderson, G.B. (1990). Hemoglobin degradation in the malaria parasite Plasmodium falciparum: an ordered process in a unique organelle. *Proceedings of the National Academy of Sciences*, *87*(8), pp.2931-2935.
75. Greenwood, B. and Mutabingwa, T. (2002).Malaria in 2002.*Nature*, *415*(6872), pp.670-672.
76. Greenwood, B.M., Bojang, K., Whitty, C.J. and Targett, G.A. (2005). *Malaria Lancet*, 365, pp.1487− 1498
77. Greenwood, B.M., Fidock, D.A., Kyle, D.E., Kappe, S.H., Alonso, P.L., Collins, F.H. and Duffy, P.E. (2008). Malaria: progress, perils, and prospects for eradication. *The Journal of Clinical Investigation*, *118*(4), pp.1266-1276.
78. Greenwood, D. (1995). Conflicts of interest: the genesis of synthetic antimalarial agents in peace and war. *Journal of Antimicrobial Chemotherapy*, *36*(5), pp.857-872.
79. Gregory, J.A., Li, F., Tomosada, L.M., Cox, C.J., Topol, A.B., Vinetz, J.M. and Mayfield, S.(2012). Algae-produced Pfs25 elicits antibodies that

inhibit malaria transmission. *Public Library of Science(PLOS) one*, *7*(5), p.e37179.

80. Grimberg, B.T. and Mehlotra, R.K. (2011). Expanding the Antimalarial Drug Arsenal Now, But How? *Pharmaceuticals*, *4*(5), pp.681-712.
81. Haldar, K. & Philips M. (2013). Malaria targets and drugs for all stages. *PLOS*: *Medicine Pathogens Neglected Tropical Disease*. Speaking medicine community blog.
82. Hawley, S.R., Bray, P.G., Mungthin, M., Atkinson, J.D., O'Neill, P.M. and Ward, S.A. (1998). Relationship between antimalarial drug activity, accumulation, and inhibition of heme polymerization in Plasmodium falciparum *in vitro*. *Antimicrobial Agents and Chemotherapy*, *42*(3), pp.682-686.
83. Hay, S., Sinka, M., Okara, R., Kabaria, C., Mbithi, P., Tago, C., Benz, D., Gething, P., Howes, R., Patil, A., Temperley, W., Bangs, M., Chareonviriyaphap, T., Elyazar, I., Harbach, R., Hemingway, J., Manguin, S., Mbogo, C., Rubio-Palis, Y. and Godfray, H. (2010). Developing global maps of the dominant anopheles vectors of human malaria. *Public Library of Science Medicine (PLoS Med)*, 7(2), p.e1000209.
84. Herrera, S., Corradin, G. and Arévalo-Herrera, M.(2007). 'An update on the search for a Plasmodium vivax vaccine'. *Trends in Parasitology*, *23*(3), pp.122-128.
85. Hill, D.R., Baird, J.K., Parise, M.E., Lewis, L.S., Ryan, E.T. and Magill, A.J. (2006). Primaquine: report from CDC expert meeting on malaria chemoprophylaxis I. *The American Journal of Tropical Medicine and Hygiene*, *75*(3), pp.402-415.
86. Hocart, S.J., Liu, H., Deng, H., De, D., Krogstad, F.M. and Krogstad, D.J. (2011). 4-aminoquinolines active against chloroquine-resistant Plasmodium falciparum: basis of antiparasite activity and quantitative structure-activity relationship analyses. *Antimicrobial Agents and Chemotherapy*, *55*(5), pp.2233-2244.

87. Homewood, C.A., Warhurst, D.C., Peters, W. and Baggaley, V.C. (1972). Lysosomes, pH and the anti-malarial action of chloroquine. *Nature*, *235*, pp.50-52.

88. Jana, S. and Paliwal, J. (2007). Novel molecular targets for antimalarial chemotherapy. *International Journal of Antimicrobial Agents*, *30*(1), pp.4-10.

89. Joubert, J., Fortuin, E.E., Taylor, D., Smith, P.J. and Malan, S.F. (2014). Pentacycloundecylamines and conjugates thereof as chemosensitizers and reversed chloroquine agents. *Bioorganic & Medicinal Chemistry Letters*, *24*(23), pp.5516-5519.

90. Joubert, J., Geldenhuys, W., Van der Schyf, C., Oliver, D., Kruger, H., Govender, T. and Malan, S. (2012). Polycyclic cage structures as lipophilic scaffolds for neuro-active drugs. *ChemMedChem*, 7(3), pp.375-384.

91. Joubert, J., Kapp, E., Taylor, D., Smith, P.J. and Malan, S.F. (2016). Polycyclic amines as chloroquine resistance modulating agents in Plasmodium falciparum. *Bioorganic & Medicinal Chemistry Letters*, *26*(4), pp.1151-1155.

92. Joubert, J., Sharma, R., Onani, M. and Malan, S.F., 2013. Microwave-assisted methods for the synthesis of pentacyclo [5.4. 0.0 2, 6. 0 3, 10. 0 5, 9] undecylamines. *Tetrahedron Letters*, *54*(50), pp.6923-6927.

93. Joubert, J., van Dyk, S., Green, I.R. and Malan, S.F.(2011). Synthesis and evaluation of fluorescent heterocyclic aminoadamantanes as multifunctional neuroprotective agents. *Bioorganic & Medicinal Chemistry*, *19*(13), pp.3935-3944.

94. Kadernani, Y.E., Zindo, F.T., Kapp, E., Malan, S.F. and Joubert, J. (2014). Adamantane amine derivatives as dual acting NMDA receptor and voltage-gated calcium channel inhibitors for neuroprotection. *Medicinal Chemistry Communications (MedChemComm)*, *5*(11), pp.1678-1684.

95. Kaschula, C.H., Egan, T.J., Hunter, R., Basilico, N., Parapini, S., Taramelli, D., Pasini, E. and Monti, D. (2002). Structure activity

relationships in 4-aminoquinoline antiplasmodials. The role of the group at the 7-position. *Journal of Medicinal Chemistry, 45*(16), pp.3531-3539.

96. Kaur, K., Jain, M., Kaur, T. and Jain, R. (2009).Antimalarials from nature. *Bioorganic & Medicinal Chemistry, 17*(9), pp.3229-3256.
97. Keiser, J., Utzinger, J., De Castro, M.C., Smith, T.A., Tanner, M. and Singer, B.H. (2004). Urbanization in sub-Saharan Africa and implication for malaria control. *The American Journal of Tropical Medicine and Hygiene, 71*(2 suppl), pp.118-127.
98. Kelly, J.X., Smilkstein, M.J., Cooper, R.A., Lane, K.D., Johnson, R.A., Janowsky, A., Dodean, R.A., Hinrichs, D.J., Winter, R. and Riscoe, M. (2007). Design, synthesis, and evaluation of 10-*N*-substituted acridones as novel chemosensitizers in *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy*, *51*(11), pp.4133-4140.
99. Khan, N. and Chand, A. (2015). Antimalarial metabolization: what have we learnt so far? *World*, *1*(1), pp.002-010.
100. Koopmans, C., Iannelli, M., Kerep, P., Klink, M., Schmitz, S., Sinnwell, S. and Ritter, H. (2006). Microwave-assisted polymer chemistry: Heck-reaction, transesterification, Baeyer–Villiger oxidation, oxazoline polymerization, acrylamides, and porous materials. *Tetrahedron*, *62*(19), pp.4709-4714.
101. Krishna, S., Bustamante, L., Haynes, R.K. and Staines, H.M.(2008). Artemisinins: their growing importance in medicine. *Trends in Pharmacological Sciences*, *29*(10), pp.520-527.
102. Krishna, S., Uhlemann, A.C. and Haynes, R.K.(2004). Artemisinins: mechanisms of action and potential for resistance. *Drug Resistance Updates*, *7*(4), pp.233-244.
103. Krogstad, D.J., Gluzman, I.Y., Kyle, D.E., Oduola, A.M., Martin, S.K., Milhous, W.K. and Schlesinger, P.H. (1987). Efflux of chloroquine from Plasmodium falciparum: mechanism of chloroquine resistance. *Science*, *238*(4831), pp.1283-1286.
104. Kumar, A., Srivastava, K., Kumar, S.R., Siddiqi, M.I., Puri, S.K., Sexana, J.K. and Chauhan, P.M.(2011). 4-Anilinoquinoline triazines: A

novel class of hybrid antimalarial agents. *European Journal of Medicinal Chemistry*, *46*(2), pp.676-690.

105. Kumar, S., Kumari, R. and Pandey, R. (2014). New insight-guided approaches to detect, cure, prevent and elimate malaria. *Protoplasma, 252(3), pp. 717-753.*

106. Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J.(1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced: Drug Delivery Reviews*, 23(1-3), pp.3-25.

107. Liu, W., Li, Y., Learn, G.H., Rudicell, R.S., Robertson, J.D., Keele, B.F., Ndjango, J.B.N., Sanz, C.M., Morgan, D.B., Locatelli, S. and Gonder, M.K. (2010). Origin of the human malaria parasite Plasmodium falciparum in gorillas. *Nature*, *467*(7314), p.420.

108. Loria, P., Miller, S., Foley, M. and Tilley, L. (1999). Inhibition of the peroxidative degradation of haem as the basis of action of chloroquine and other quinoline antimalarials. *Biochemical Journal*, *339*(2), pp.363-370.

109. Luxemburger, C., Ricci, F., Nosten, F., Raimond, D., Bathet, S. and White, N.J.(1997). The epidemiology of severe malaria in an area of low transmission in Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *91*(3), pp.256-262.

110. Maguene, G.M., Lekana-Douki, J.B., Mouray, E., Bousquet, T., Grellier, P., Pellegrini, S., Ndouo, F.S.T., Lebibi, J. and Pélinski, L. (2015). Synthesis and in vitro antiplasmodial activity of ferrocenyl aminoquinoline derivatives. *European Journal of Medicinal Chemistry*, *90*, pp.519-525.

111. Makler, M.T. and Hinrichs, D.J.(1993). Measurement of the lactate dehydrogenase activity of Plasmodium falciparum as an assessment of parasitemia. *The American Journal of Tropical Medicine and Hygiene*, *48*(2), pp.205-210.

112. Makler, M.T., Ries, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbins, B.L. and Hinrichs, D.J.(1993). Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. *The American Journal of Tropical Medicine and Hygiene*, *48*(6), pp.739-741.

113. Malan, S.F. Van der Walt, J.J. and Van der Schyf, C.J. (2000). Structure-activity relationships of polycyclic aromatic amines with calcium channel blocking activity. *Archiv der Pharmazie*, 333(1), pp. 10-16.

114. Malan, S.F., Dyason, K., Wagenaar, B., Van der Walt, J.J. and Van der Schyf, C.J. (2003). The structure and ion channel activity of 6-Benzylamino-3-hydroxyhexacyclo[6.5.0.03,7.04,12.05,10.09,13]tridecane. *Archiv der Pharmazie*, 336(2), pp. 127-133.

115. Martin, R. E., Marchetti, R. V., Cowan, A. I., Howitt, S. M., Broer, S. and Kirk, K. (2009). Chloroquine transport *via* the malaria parasite's chloroquines resistance transporter. Sciences, 325, pp. 1680-1682.

116. Martin, S.K., Oduola, A.M. and Milhous, W.K. (1987).Reversal of chloroquine resistance in Plasmodium falciparum by verapamil. *Science*, *235*, pp.899-902.

117. Martiney, J.A., Cerami, A. and Slater, A.F. (1995). Verapamil reversal of chloroquine resistance in the malaria parasite Plasmodium falciparum is specific for resistant parasites and independent of the weak base effect. *Journal of Biological Chemistry*, *270*(38), pp.22393-22398.

118. McCarthy, J.S., Marjason, J., Elliott, S., Fahey, P., Bang, G., Malkin, E., Tierney, E., Aked-Hurditch, H., Adda, C., Cross, N. and Richards, J.S. (2011). 'A phase 1 trial of MSP2-C1, a blood-stage malaria vaccine containing 2 isoforms of MSP2 formulated with Montanide® ISA 72. *PLoS One*, *6*(9), p.e24413.

119. Medicine for Malaria Venture (2017). Five species, viewed 22 February 2017 http://www.mmv.org/malaria-medicines/five-species

120. Medicine for Malaria Venture. (2015). Definitions and symptoms. [Online] Available from http://www.mmv.org/malaria-medicines/definitions-and-symptoms. [Accessed: 21/04/2015]

121. Mendis, K., Rietveld, A., Warsame, M., Bosman, A., Greenwood, B. and Wernsdorfer, W.H. (2009). 'From malaria control to eradication: The WHO perspective'. *Tropical Medicine & International Health*, *14*(7), pp.802-809.

122. Meshnick, S.R. and Dobson, M.J. (2001). The history of antimalarial drugs. In *Antimalarial Chemotherapy*, pp. 15-25. Humana Press.

123. Meshnick, S.R., Taylor, T.E. and Kamchonwongpaisan, S. (1996). Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiological Reviews*, *60*(2), pp.301-315.

124. Miller, L.H., Good, M.F. and Milon, G. (1994). Malaria pathogenesis. *Science*, *264*(5167), pp.1878-1883.

125. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*(1-2), pp.55-63.

126. Muraleedharan, K.M. and Avery, M.A. (2007). Advances in the discovery of new antimalarials. *Comprehensive Medicinal Chemistry II*, *7*, pp.765-814.

127. Muregi, F.W. and Ishih, A. (2010). Next-generation antimalarial drugs: hybrid molecules as a new strategy in drug design. *Drug Development Research*, *71*(1), pp.20-32.

128. Murray, C.J., Rosenfeld, L.C., Lim, S.S., Andrews, K.G., Foreman, K.J., Haring, D., Fullman, N., Naghavi, M., Lozano, R. and Lopez, A.D. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *The Lancet*, *379*(9814), pp.413-431.

129. Mushtaque, M. and Shahjahan. (2015). Reemergence of chloroquine (CQ) analogs as multi-targeting antimalarial agents: A review. *European Journal of Medicinal Chemistry*, 90, pp. 280-295.

130. Nadjm, B. and Behrens, R.H. (2012). Malaria: An update for physicians. *Infectious Disease Clinics of North America*, *26*(2), pp.243-259.

131. Natarajan, J.K., Alumasa, J.N., Yearick, K., Ekoue-Kovi, K.A., Casabianca, L.B., de Dios, A.C., Wolf, C. and Roepe, P.D. (2008). 4-*N*-, 4-*S*-, and 4-*O*-chloroquine analogues: influence of side chain length and quinolyl nitrogen pKa on activity against chloroquine resistant malaria. *Journal of Medicinal Chemistry*, *51*(12), pp.3466-3479.

132. Noedl, H., Se, Y., Schaecher, K., Smith, B.L., Socheat, D. and Fukuda, M.M. (2008). Evidence of artemisinin-resistant malaria in Western Cambodia. *New England Journal of Medicine*, *359*(24), pp.2619-2620.

133. Nzila, A. (2006). The past, present and future of antifolates in the treatment of *Plasmodium falciparum* infection. *Journal of Antimicrobial Chemotherapy*, *57*(6), pp.1043-1054.

134. Nzila, A. and Mwai, L. (2010). *In vitro* selection of *Plasmodium falciparum* drug-resistant parasite lines. *Journal of Antimicrobial Chemotherapy*, *65*(3), pp.390-398.

135. O'Neill, P.M., Barton, V.E., Ward, S.A. and Chadwick, J. (2011). 4-Aminoquinolines: chloroquine, amodiaquine and next-generation analogues. *In Treatment and Prevention of Malaria* (pp. 19-44). Springer Basel.

136. Oliver, D. and Malan, S. (2007). Medicinal chemistry of polycyclic cage compounds in drug discovery research. *Medicinal Chemistry Research*, 17(2-7), pp.137-151.

137. Olliaro, P. (2001). Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacology & Therapeutics*, *89*(2), pp.207-219.

138. Olliaro, P., Taylor, W.R. and Rigal, J. (2001). 'Controlling malaria: challenges and solutions'. *Tropical Medicine & International Health*, *6*(11), pp.922-927.

139. Omodeo-Sale, F., Cortelezzi, L., Basilico, N., Casagrande, M., Sparatore, A. and Taramelli, D.(2009). Novel antimalarial aminoquinolines: heme binding and effects on normal or Plasmodium falciparum-parasitized human erythrocytes. *Antimicrobial Agents and Chemotherapy*, *53*(10), pp.4339-4344.

140. O'Neill, P.M., Bray, P.G., Hawley, S.R., Ward, S.A. and Park, B.K. (1998). '4-Aminoquinolines—Past, present, and future; A chemical perspective'. *Pharmacology & Therapeutics*, *77*(1), pp.29-58.

141. O'Neill, P.M., Mukhtar, A., Stocks, P.A., Randle, L.E., Hindley, S., Ward, S.A., Storr, R.C., Bickley, J.F., O'Neil, I.A., Maggs, J.L. and Hughes, R.H. (2003). 'Isoquine and related amodiaquine analogues: a new

generation of improved 4-aminoquinoline antimalarials'. *Journal of Medicinal Chemistry*, *46*(23), pp.4933-4945.

142. Opsenica, I., Tot, M., Gomba, L., Nuss, J., Sciotti, R., Bavari, S., Burnett, J. and Šolaja, B. (2013). 4-Amino-7-chloroquinolines: Probing Ligand Efficiency Provides Botulinum Neurotoxin Serotype A Light Chain Inhibitors with Significant Antiprotozoal Activity. *Journal of Medicinal Chemistry*, 56(14), pp.5860-5871.
143. Owusu-Ofori, A., Gadzo, D. and Bates, I. (2016). Transfusion-transmitted malaria: donor prevalence of parasitaemia and a survey of healthcare workers knowledge and practices in a district hospital in Ghana. *Malaria Journal*, *15*(1), pp.234.
144. Pagola, S., Stephens, P.W., Bohle, D.S., Kosar, A.D. and Madsen, S.K. (2000). The structure of malaria pigment β-haematin. *Nature*, *404*(6775), pp.307-310.
145. Pandey, S., Agarwal, P., Srivastava, K., RajaKumar, S., Puri, S.K., Verma, P., Saxena, J.K., Sharma, A., Lal, J. and Chauhan, P.M. (2013). Synthesis and bioevaluation of novel 4-aminoquinoline-tetrazole derivatives as potent antimalarial agents. *European Journal of Medicinal Chemistry*, *66*, pp.69-81.
146. Parham, P.E., Christiansen-Jucht, C., Pople, D. and Michael, E. (2011). Understanding and modeling the impact of climate change on infectious diseases—progress and future challenges. *Climate Change Socioeconomic Effects*.
147. Parsons, C.G. and Gilling, K.(2007). Memantine as an example of a fast, voltage-dependent, open channel *N*-methyl-*D*-aspartate receptor blocker. *Patch-Clamp Methods and Protocols*, pp.15-36
148. Parsons, C.G., Danysz, W. and Quack, G. (1999). Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist - a review of preclinical data. *Neuropharmacology*, 38(6), pp. 735-776.
149. Penna-Coutinho, J., Cortopassi, W.A., Oliveira, A.A., França, T.C.C. and Krettli, A.U. (2011). Antimalarial activity of potential inhibitors of

Plasmodium falciparum lactate dehydrogenase enzyme selected by docking studies. *Public Library of Science(PloS) one*, *6*(7), pp.e21237.

150. Peters, W. (1999). The evolution of tafenoquine—antimalarial for a new millennium? *Journal of the Royal Society of Medicine*, *92*(7), pp.345-352.
151. Peyton H. David.(2012). Reversed chloroquine molecules as a strategy to overcome resistance in malaria. *Current Topics in Medicinal Chemistry*, *12*(5), pp.400-407.
152. Premji, Z.G. (2009). Coartem®: the journey to the clinic. *Malaria Journal*, *8*(1), p.S3.
153. Pulcini, S., Staines, H.M., Lee, A.H., Shafik, S.H., Bouyer, G., Moore, C.M., Daley, D.A., Hoke, M.J., Altenhofen, L.M., Painter, H.J. and Mu, J. (2015). Mutations in the *Plasmodium falciparum* chloroquine resistance transporter, *Pf*CRT, enlarge the parasite's food vacuole and alter drug sensitivities. *Scientific Reports*, *5*, pp.srep14552.
154. Reiter, P. (2000). From Shakespeare to Defoe: malaria in England in the Little Ice Age. *Emerging Infectious Diseases*, *6*(1), pp.1.
155. Reyburn, H. (2010). New WHO guidelines for the treatment of malaria. *British Medical Journal*, *340*, pp.c2637.
156. Ridley, R.G.(2002). Medical need, scientific opportunity and the drive for antimalarial drugs.*Nature*, *415*(6872), pp.686-693.
157. Ridley, R.G., Hofheinz, W., Matile, H., Jaquet, C., Dorn, A., Masciadri, R., Jolidon, S., Richter, W.F., Guenzi, A., Girometta, M.A. and Urwyler, H. (1996). 4-aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy*, *40*(8), pp.1846-1854.
158. Rieckmann, K.H. and Sweeney, A.W. (2012). Army malaria institute: Its evolution and achievements. First decade: 1965-1975. *Journal of Military and Veterans Health*, *20*(2), pp.17.
159. Sachs, J. and Malaney, P. (2002). The economic and social burden of malaria. *Nature*, *415*(6872), pp.680-685.

160. Sáenz, F.E., Mutka, T., Udenze, K., Oduola, A.M. and Kyle, D.E. (2012). Novel 4-aminoquinoline analogs highly active against the blood and sexual stages of Plasmodium *in vivo* and *in vitro*. *Antimicrobial Agents and Chemotherapy*, *56*(9), pp.4685-4692.

161. Saifi, M.A., Beg, T., Harrath, A.H., Altayalan, F.S.H. and Al Quraishy, S. (2013). Antimalarial drugs: Mode of action and status of resistance. *African Journal of Pharmacy and Pharmacology*, *7*(5), pp.148-156.

162. Schlesinger, P.H., Krogstad, D.J. and Herwaldt, B.L. (1988). Antimalarial agents: mechanisms of action. *Antimicrobial Agents and Chemotherapy*, *32*(6), pp.793.

163. Schoepflin, S., Lin, E., Kiniboro, B., DaRe, J.T., Mehlotra, R.K., Zimmerman, P.A., Mueller, I. and Felger, I. (2010). Treatment with coartem (artemether-lumefantrine) in Papua New Guinea. *The American journal of tropical medicine and hygiene*, *82*(4), pp.529-534.

164. Schwab, R. S., England, A. C Jr., Poskanzer, D. C., Young, R. R. (1969). Amantadine in the treatment of Parkinson's disease. *Journal of the American Medical Association*, 208(7), pp. 1168-117

165. Schwartz, L., Brown, G.V., Genton, B. and Moorthy, V.S. (2012). A review of malaria vaccine clinical projects based on the WHO rainbow table. *Malaria Journal*, *11*(1), pp.11.

166. Shaik, A. (2010). Evaluation of antihistamines for in vitro antimalarial activity against P. falciparum. A full thesis submitted in fulfillment of the requirements for the degree of Magister Pharmaceuticiae in the Faculty of Natural Science, School of Pharmacy, University of the Western Cape.

167. Sharma, R., Tiwari, A. and Parate, A. (2015). Synthesis of new chloroquine derivatives as antimalarial agents. *Pharmaceutical Chemistry Journal*, *49*(8), pp.537-542.

168. Sharom, F.J. (2011). The *p*-glycoprotein multidrug transporter. *Essays in Biochemistry*, *50*, pp.161-178.

169. Sibley, C.H., Hyde, J.E., Sims, P.F., Plowe, C.V., Kublin, J.G., Mberu, E.K., Cowman, A.F., Winstanley, P.A., Watkins, W.M. and Nzila, A.M.

(2001). Pyrimethamine–sulfadoxine resistance in Plasmodium falciparum: what next? *Trends in Parasitology*, *17*(12), pp.570-571.

170. Sidhu, A.B.S., Verdier-Pinard, D. and Fidock, D.A. (2002). Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by pfcrt mutations. *Science*, *298*(5591), pp.210-213.

171. Sinka, M.E., Bangs, M.J., Manguin, S., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W. and Okara, R.M. (2010). The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites & Vectors*, *3*(1), pp.117.

172. Sinka, M.E., Bangs, M.J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H. and Gething, P.W.(2012). A global map of dominant malaria vectors. *Parasites &Vectors*, *5*(1), p.69.

173. Sinnis, P., Clavijo, P., Fenyö, D., Chait, B.T., Cerami, C. and Nussenzweig, V. (1994). Structural and functional properties of region II-plus of the malaria circumsporozoite protein. *Journal of Experimental Medicine*, *180*(1), pp.297-306.

174. Slater, A.F.G. and Cerami, A. (1992). Inhibition by chloroquine of a novel haem polymerase enzyme activity in malaria trophozoites. *Nature*, *355*(6356), pp.167.

175. Snow, R.W. (2015). Global malaria eradication and the importance of *Plasmodium falciparum* epidemiology in Africa. *BioMed Central Medicine*, 13(1), pp.23.

176. Soh, P.N. and Benoit-Vical, F. (2007). Are West African plants a source of future antimalarial drugs? *Journal of Ethnopharmacology*, *114*(2), pp.130-140.

177. Šolaja, B.A., Opsenica, D., Smith, K.S., Milhous, W.K., Terzić, N., Opsenica, I., Burnett, J.C., Nuss, J., Gussio, R. and Bavari, S. (2008). Novel 4-aminoquinolines active against chloroquine-resistant and sensitive *P. falciparum* strains that also inhibit botulinum serotype A. *Journal of Medicinal Chemistry*, *51*(15), pp.4388-4391

178. Solomon, L., Okere, H. and Daminabo, V. (2014). Understanding human malaria: Further Review on the literature, pathogenesis and disease control. *Rep Opinion*, *6*, pp.55-63.

179. Sturm, A., Amino, R., Van de Sand, C., Regen, T., Retzlaff, S., Rennenberg, A., Krueger, A., Pollok, J.M., Menard, R. and Heussler, V.T. (2006). Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. *Science*, *313*(5791), pp.1287-1290.

180. Sullivan, D. J., Matile, H., Ridley, R. G. and Goldberg, D. E. (1998). A common mechanism for blockade of heme polymerization by antimalarial quinolines. *Journal of Biological Chemistry*, 273(47), pp. 31003-31107.

181. Sullivan, D.J. (2002). Theories on malarial pigment formation and quinoline action. *International Journal for Parasitology*, *32*(13), pp.1645-1653.

182. Sunduru, N., Sharma, M., Srivastava, K., Rajakumar, S., Puri, S.K., Saxena, J.K. and Chauhan, P.M. (2009). Synthesis of oxalamide and triazine derivatives as a novel class of hybrid 4-aminoquinoline with potent antiplasmodial activity. *Bioorganic & Medicinal Chemistry*, *17*(17), pp.6451-6462.



183. Talisuna, A.O., Bloland, P. and d'Alessandro, U. (2004). History, dynamics, and public health importance of malaria parasite resistance. *Clinical Microbiology Reviews*, *17*(1), pp.235-254.

184. Tan-ariya, P., Na-Bangchang, K., Ubalee, R., Boutes, S., Riengchainam, S. and Karbwang, J. (1997). In vitro blood schizonticidal activity of sera containing mefloquine-quinine against *Plasmodium falciparum*. *Tropical Medicine & International Health*, *2*(2), pp.159-164.

185. Tanner, M. and Savigny, D.D. (2008). Malaria eradication back on the table. *Bulletin of the World Health Organization*, *86*(2), pp.82-82.

186. Tanner, M., Greenwood, B., Whitty, C.J., Ansah, E. K., Price, R. N., Dondorp, A. M., von Seidlein, L., Baird, J. K., Beeson, J. G., Fowkes, F. J. and Hemingway, J., Marsh, K. and Osier, F. (2015). Malaria eradication and elimination: views on how to translate a vision into reality. BioMed Central Medicine, 13(1), pp. 167

187. Thomson, J.G. (1933). Immunity in malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *26*(6), pp.483IN1499-498514.

188. Toşa, N.I. (2009). Contributions to the Synthesis and Structural Analysis of Some Bicyclic Compounds and to the Fabrication of 3D Metallic Micro-and Nanostructures by Laser Induced Photochemistry (Doctoral dissertation).

189. Trager, W. and Jensen J. B. (1976). Human malaria parasites in continuous culture. *Science*, 193(4254), pp.673-675.

190. Trampuz, A., Jereb, M., Muzlovic, I. and Prabhu, R.M. (2003). Clinical review: Severe malaria. *Critical Care*, 7(4), pp.315.

191. Tran, T.M., Samal, B., Kirkness, E. and Crompton, P.D. (2012). Systems immunology of human malaria. *Trends in Parasitology*, *28*(6), pp.248-257.

192. Trape, J.F., Pison, G., Preziosi, M.P., Enel, C., du Loû, A.D., Delaunay, V., Samb, B., Lagarde, E., Molez, J.F. and Simondon, F. (1998).Impact of chloroquine resistance on malaria mortality. *Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie*, *321*(8), pp.689-697.

193. Twentyman, P.R. and Luscombe, M. (1987). A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. *British Journal of Cancer*, *56*(3), pp.279.

194. Valdés, A.F.C., Martínez, J.M., Lizama, R.S., Gaitén, Y.G., Rodríguez, D.A. and Payrol, J.A. (2010). *In vitro* antimalarial activity and cytotoxicity of some selected Cuban medicinal plants. *Revista do Instituto de Medicina Tropical de Sao Paulo*, *52*(4), pp.197-201.

195. Vale, N., Moreira, R. and Gomes, P. (2009). Primaquine revisited six decades after its discovery. *European Journal of Medicinal Chemistry*, *44*(3), pp.937-953.

196. Van der Schyf, C.J. and Geldenhuys, W.J. (2009). Polycyclic compounds: Ideal drug scaffolds for the design of multiple mechanism drugs? *Neurotherapeutics*, *6*(1), pp.175-186.

197. Van Schalkwyk, D.A. and Egan, T.J. (2006). Quinoline-resistance reversing agents for the malaria parasite *Plasmodium falciparum*. *Drug Resistance Updates*, *9*(4), pp.211-226.

198. Verdure, F., Le Bras, J., Clavier, F., Hatin, I. and Blayo, M.C. (1985). Chloroquine uptake by Plasmodium falciparum-infected human erythrocytes during in vitro culture and its relationship to chloroquine resistance. *Antimicrobial Agents and Chemotherapy*, *27*(4), pp.561-564.

199. Von Seidlein, L. and Greenwood, B. M. (2003). Mass administration of antimalarial drugs. *Trends in Parasitology*, 19(10), pp. 452--460

200. Wellems, T.E. and Plowe, C.V. (2001). Chloroquine-resistant malaria. *Journal of Infectious Diseases*, *184*(6), pp.770-776.

201. Wells, T.N., Burrows, J.N. and Baird, J.K. (2010). Targeting the hypnozoite reservoir of *Plasmodium vivax*: the hidden obstacle to malaria elimination. *Trends in Parasitology*, *26*(3), pp.145-151.

202. White, N.J. (2008). Qinghaosu (artemisinin): the price of success. *Science*, *320*(5874), pp.330-334.

203. White, N.J. (2011). Determinants of relapse periodicity in Plasmodium vivax malaria. *Malaria Journal*, *10*(1), pp.297.

204. White, N.J. 2004. Antimalarial drug resistance. *Journal of Clinical Investigation*, *113*(8), p.1084.

205. Whitty, C.J., Chandler, C., Ansah, E., Leslie, T. and Staedke, S.G. (2008). Deployment of ACT antimalarials for treatment of malaria: challenges and opportunities. *Malaria Journal*, *7*(1), pp.S7.

206. Wiesner, J., Ortmann, R., Jomaa, H. and Schlitzer, M. (2003). New antimalarial drugs. *Angewandte Chemie International Edition*, *42*(43), pp.5274-5293.

207. Wilairatana, P., Krudsood, S., Treeprasertsuk, S., Chalermrut, K. and Looareesuwan, S. (2002). The future outlook of antimalarial drugs and recent work on the treatment of malaria. *Archives of Medical Research*, *33*(4), pp.416-421.

208. World Health Organization. (2010). Global report on antimalarial drug efficacy and drug resistance: 2000 - 2010

209. World Health Organization. (2016a). World Malaria Report 2015. World Health Organization.

210. World Health Organization. (2016b). Malaria fact sheet 2011. *World Health Organization*

211. World Health Organization. (2016c). WHO Malaria Terminology

212. World Health Organization. (2017a). World Malaria Report 2016. Geneva, Switzerland. World Health Organization.

213. World Health Organization. (2017b). Global Technical Strategy for Malaria 2016-2030. Geneva: World Health Organization. Available at http://www.who.int/malaria/areas/global_technica_strategy/draft_strategy/en/.

214. World Health Organization. (2017c). Malaria Factsheets *World Health Organization*. Available at [http://www.who.int/mediacentre/factsheets/fs094/en/]viewed 14/09.2017

215. World Health Organization (1999). World Health Report 1999: Making a difference. *World Health Organization*.

216. World Health Organization. (2006). Guidelines for the treatment of malaria. 2006. *Geneva: World Health Organization Google Scholar*.

217. World Health Organization. (2014). World Malaria Report 2013. Geneva: *World Health Organization*. *Fecha de consulta*, *23*, p.238.

218. World Health Organization. (2015). Guidelines for the treatment of malaria. 3rd ed. World Health Organization.

219. World Health Organization. (1993). A Global Strategy for Malaria Control. WHO, Geneva. Available at http://whqlibdoc.who.int/publications/9241561610.pdf

220. Yearick, K., Ekoue-Kovi, K., Iwaniuk, D.P., Natarajan, J.K., Alumasa, J., de Dios, A.C., Roepe, P.D. and Wolf, C. (2008).Overcoming drug resistance to heme-targeted antimalarials by systematic side chain variation of 7-chloro-4-aminoquinolines. *Journal of Medicinal Chemistry*, *51*(7), pp.1995-1998.

221. Youdim, M.B. and Buccafusco, J.J. (2005). Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends in Pharmacological Sciences*, *26*(1), pp.27-35.

222. Zah, J., Terre'Blanche, G., Erasmus, E. and Malan, S.F. (2003). Physicochemical prediction of a brain–blood distribution profile in polycyclic amines. *Bioorganic & Medicinal Chemistry*, *11*(17), pp.3569-3578.

223. Zalikowski, J.A., Gilbert, K.E. and Borden, W.T. (1980). Oxidation of 7-(hydroxymethyl) bicyclo [3.3. 1] nonan-3-ol. Convenient synthesis of bicyclo [3.3. 1] nonane-3, 7-dione. *The Journal of Organic Chemistry*, *45*(2), pp.346-347.

224. Zishiri, V.K., Hunter, R., Smith, P.J., Taylor, D., Summers, R., Kirk, K., Martin, R.E. and Egan, T.J. (2011). A series of structurally simple chloroquine chemosensitizing dibemethin derivatives that inhibit chloroquine transport by *Pf*CRT. *European Journal of Medicinal Chemistry*, *46*(5), pp.1729-1742.

225. Zucker, J.R. (1996). 'Changing patterns of autochthonous malaria transmission in the United States: a review of recent outbreaks'. *Emerging Infectious Diseases*, *2*(1), p.37.

# ANNEXURE

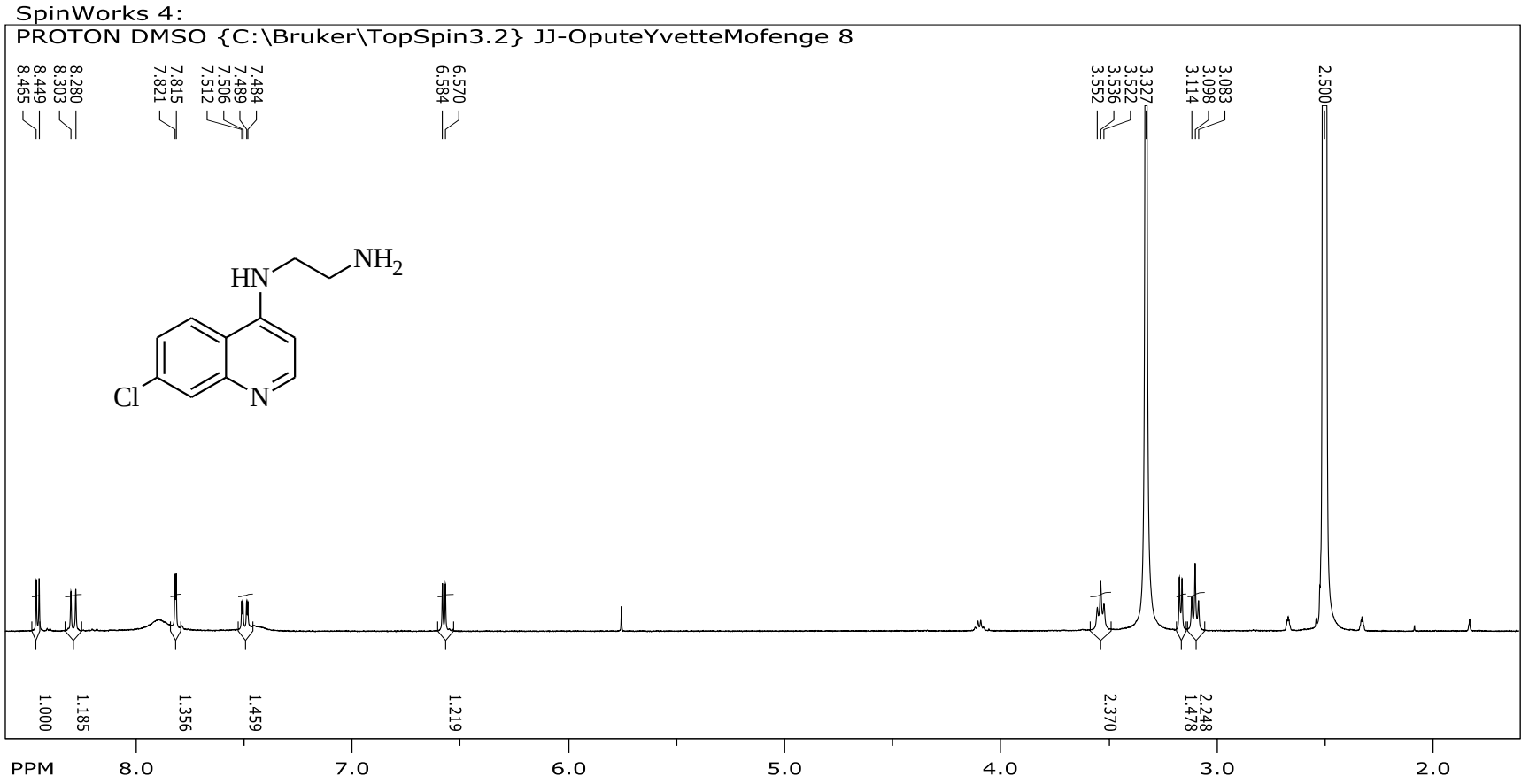
# SPECTRAL DATA:

## NUCLEAR MAGNETIC RESONANCE

## INFRARED AND MASS SPECTROSCOPY

## SPECTRA

**<u>SPECTRUM 1:</u> Compound 1 $^{1}$H-NMR**

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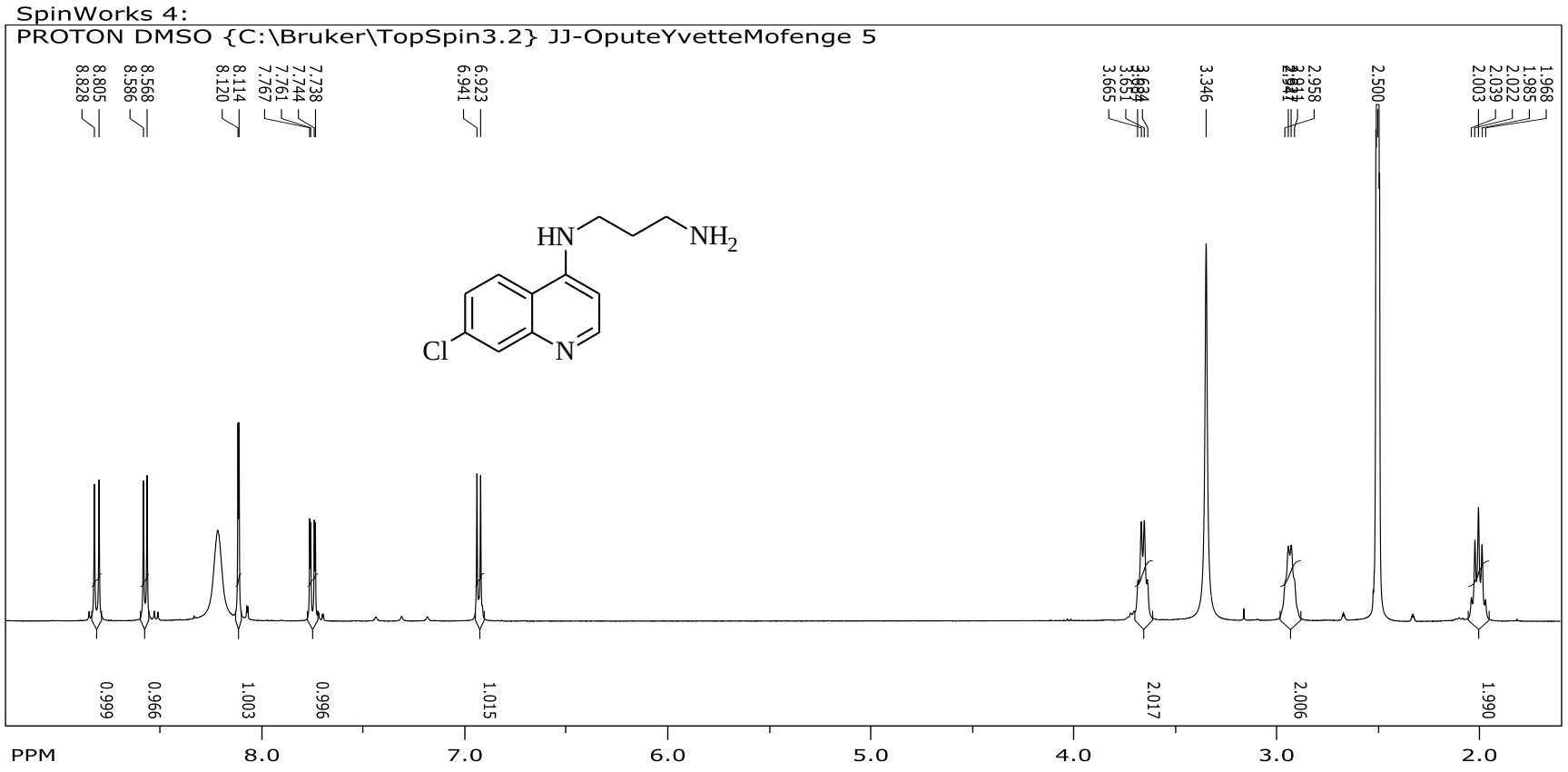
**SPECTRUM 2: Compound 2 1H-NMR**

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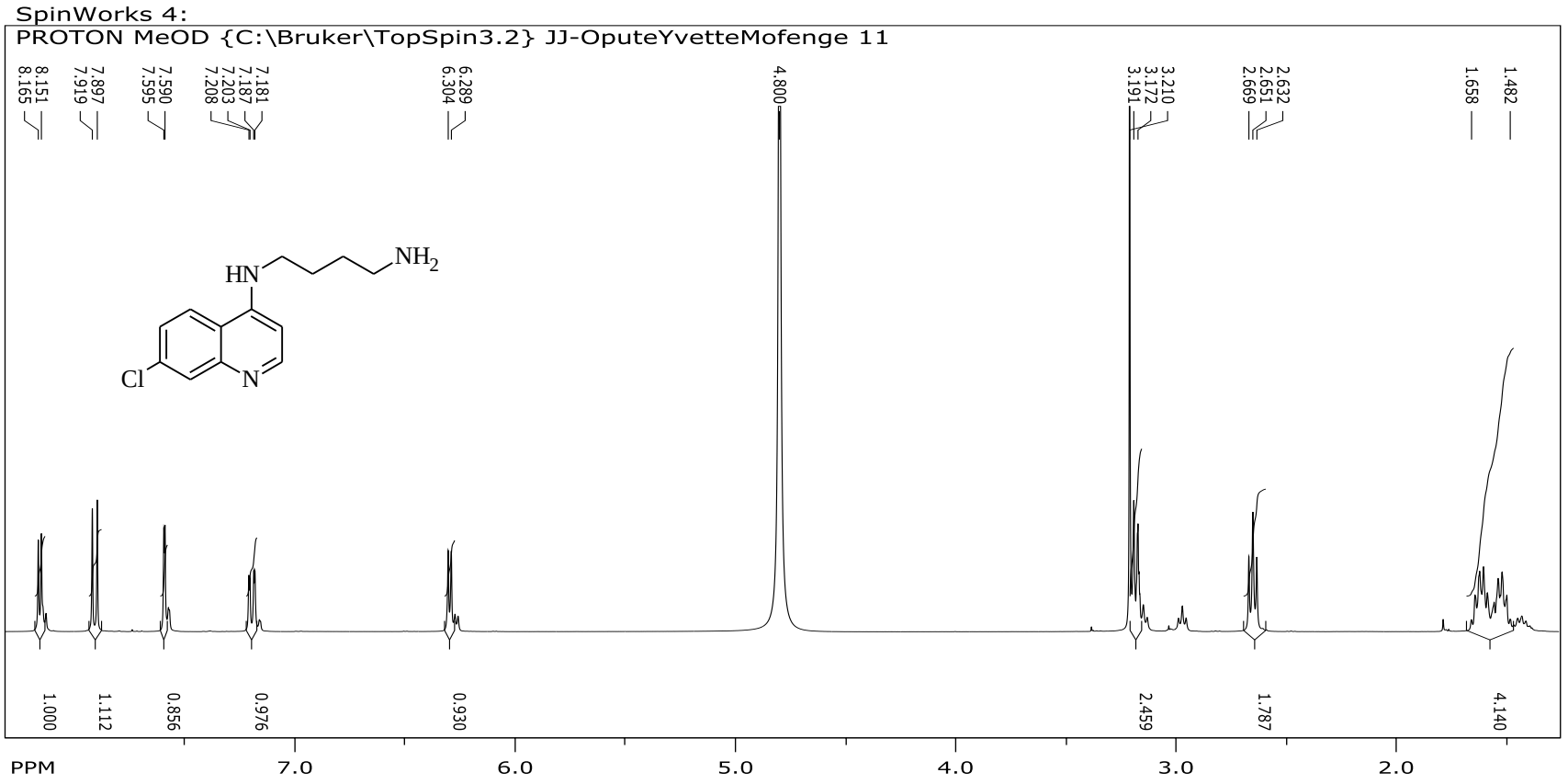


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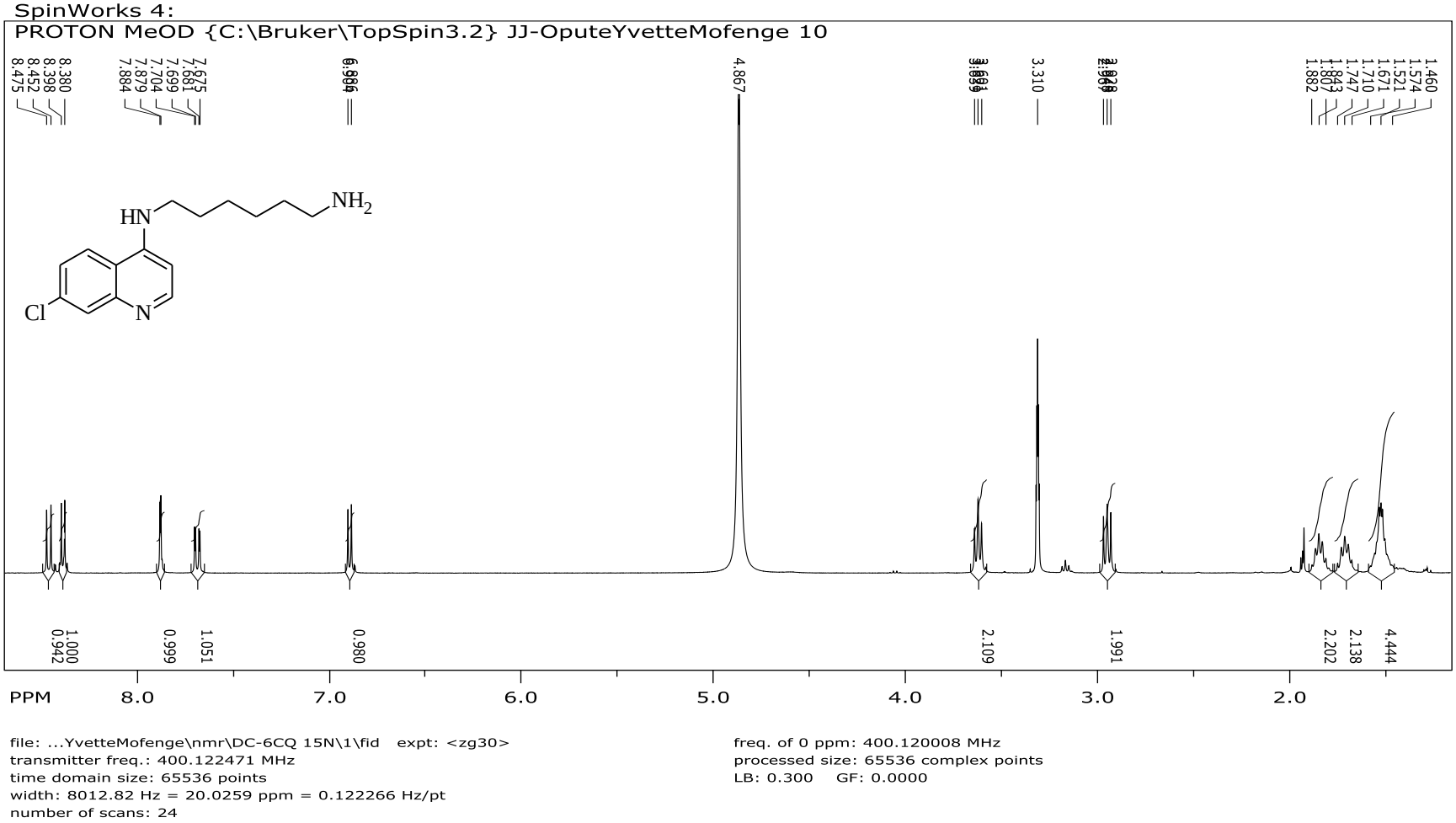


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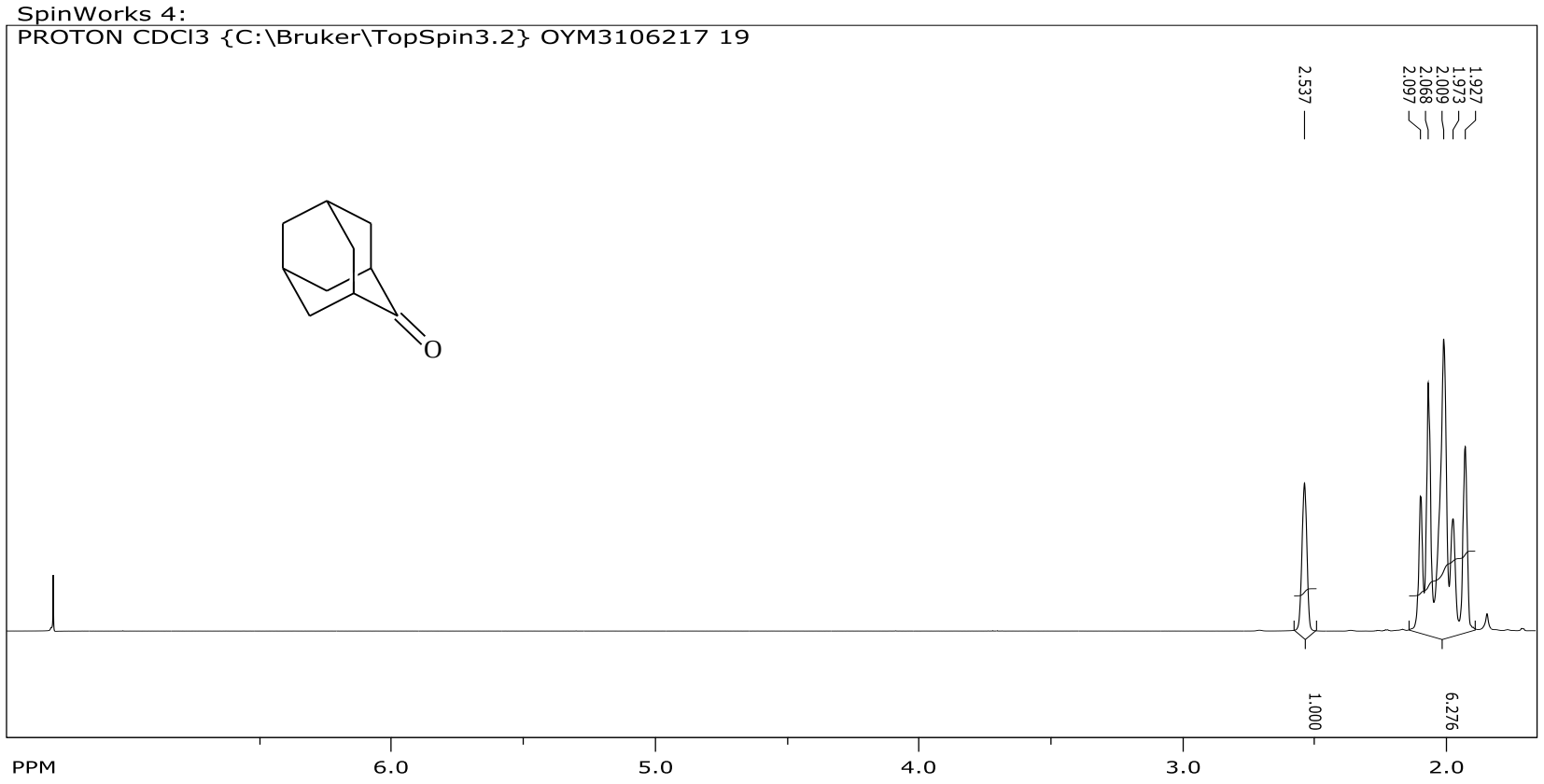
**<u>SPECTRUM 5</u>: 2-Adamantanone 1H-NMR**

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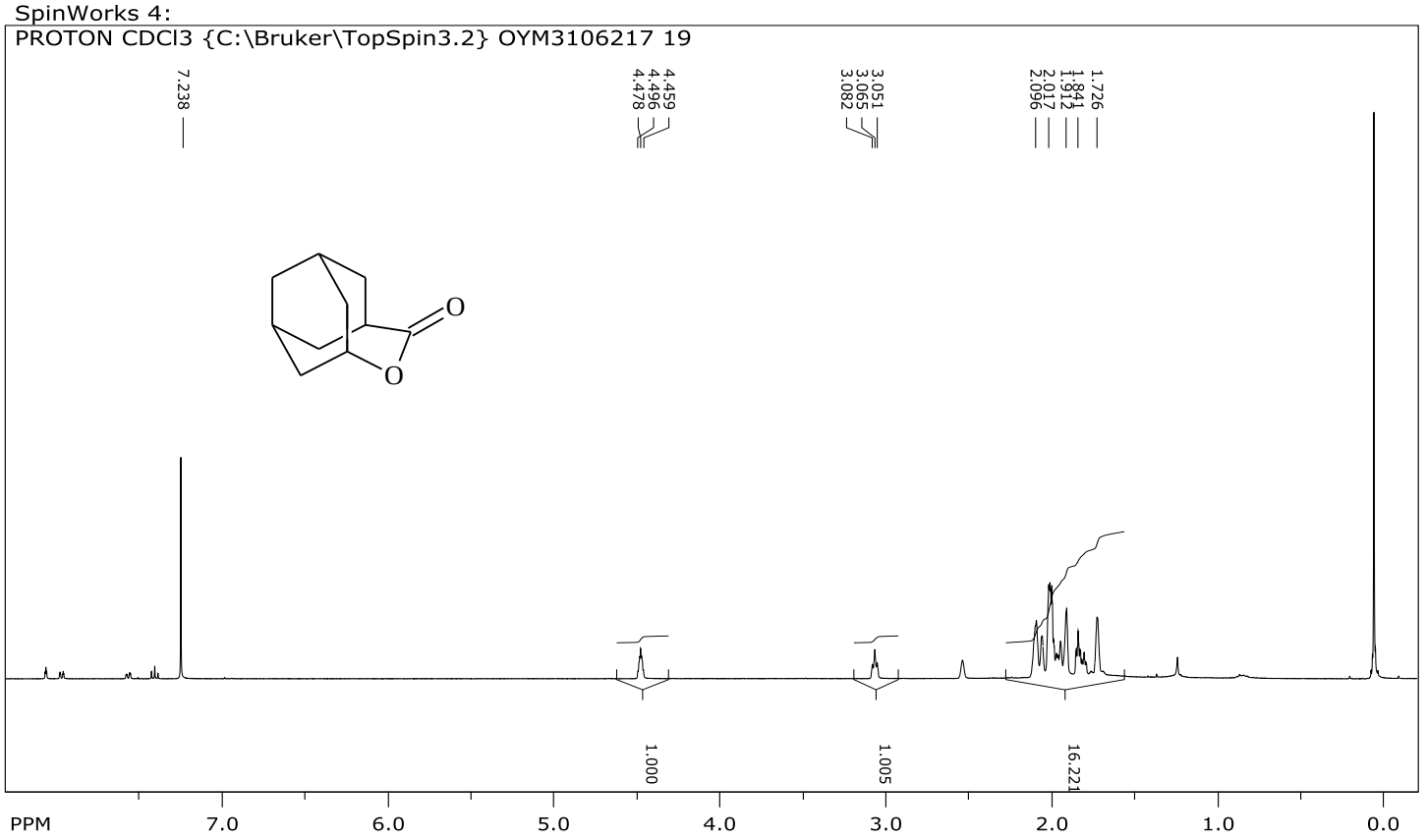
**<u>SPECTRUM 6</u>: Lactone 1H-NMR**

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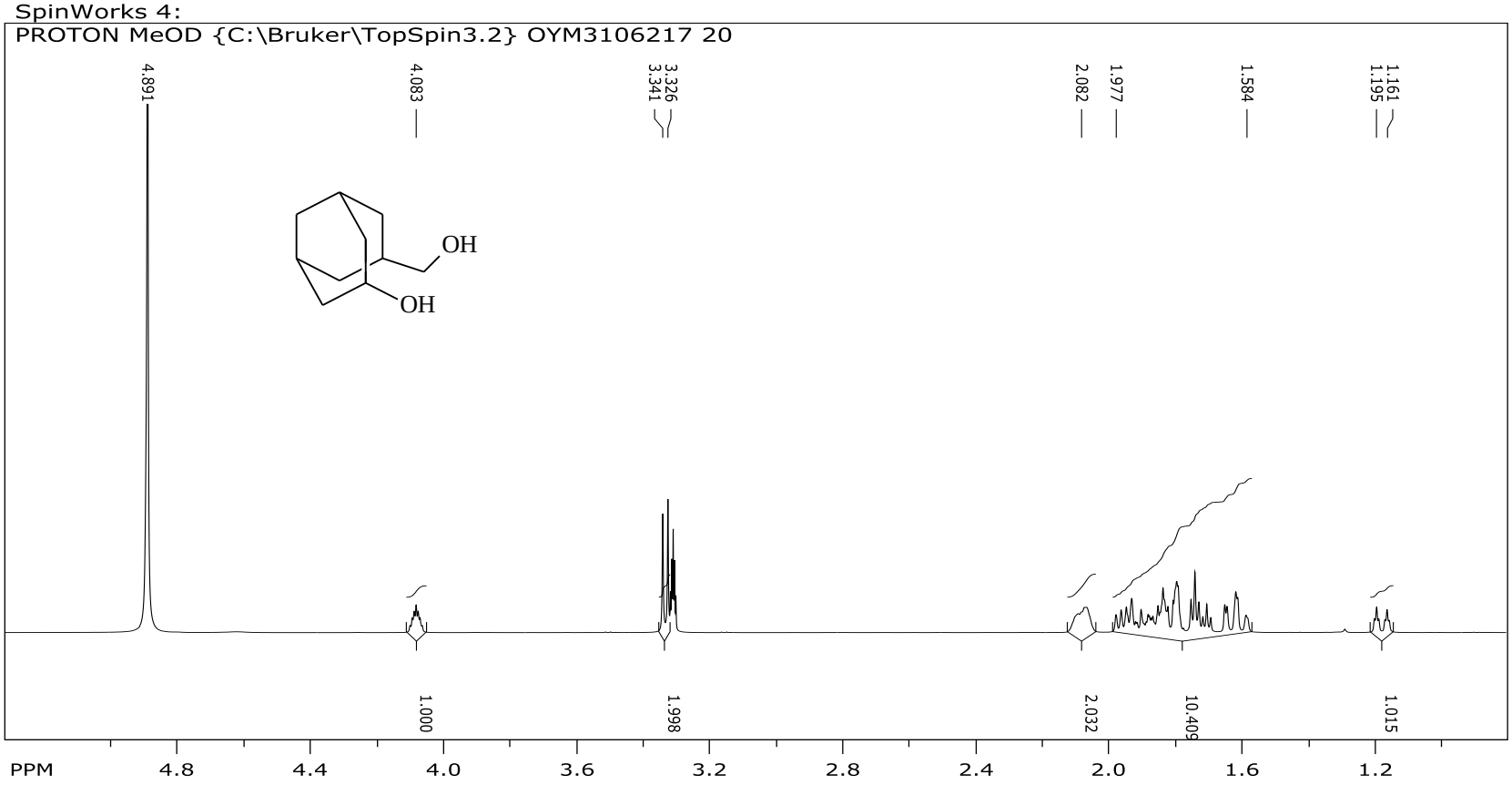
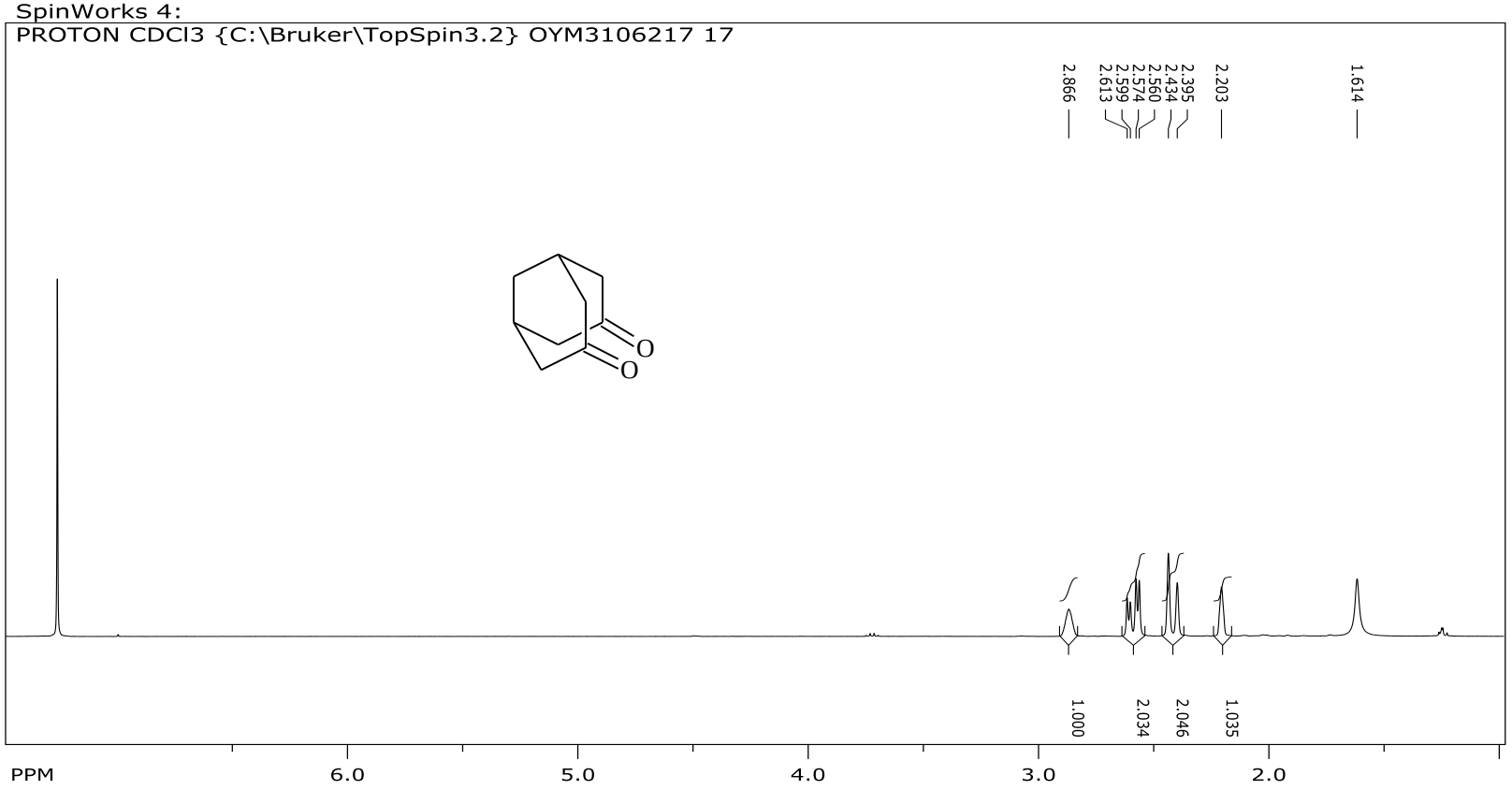
**SPECTRUM 7: Diol 1H-NMR**



**SPECTRUM 8: Adamantane Diketone 1H-NMR**

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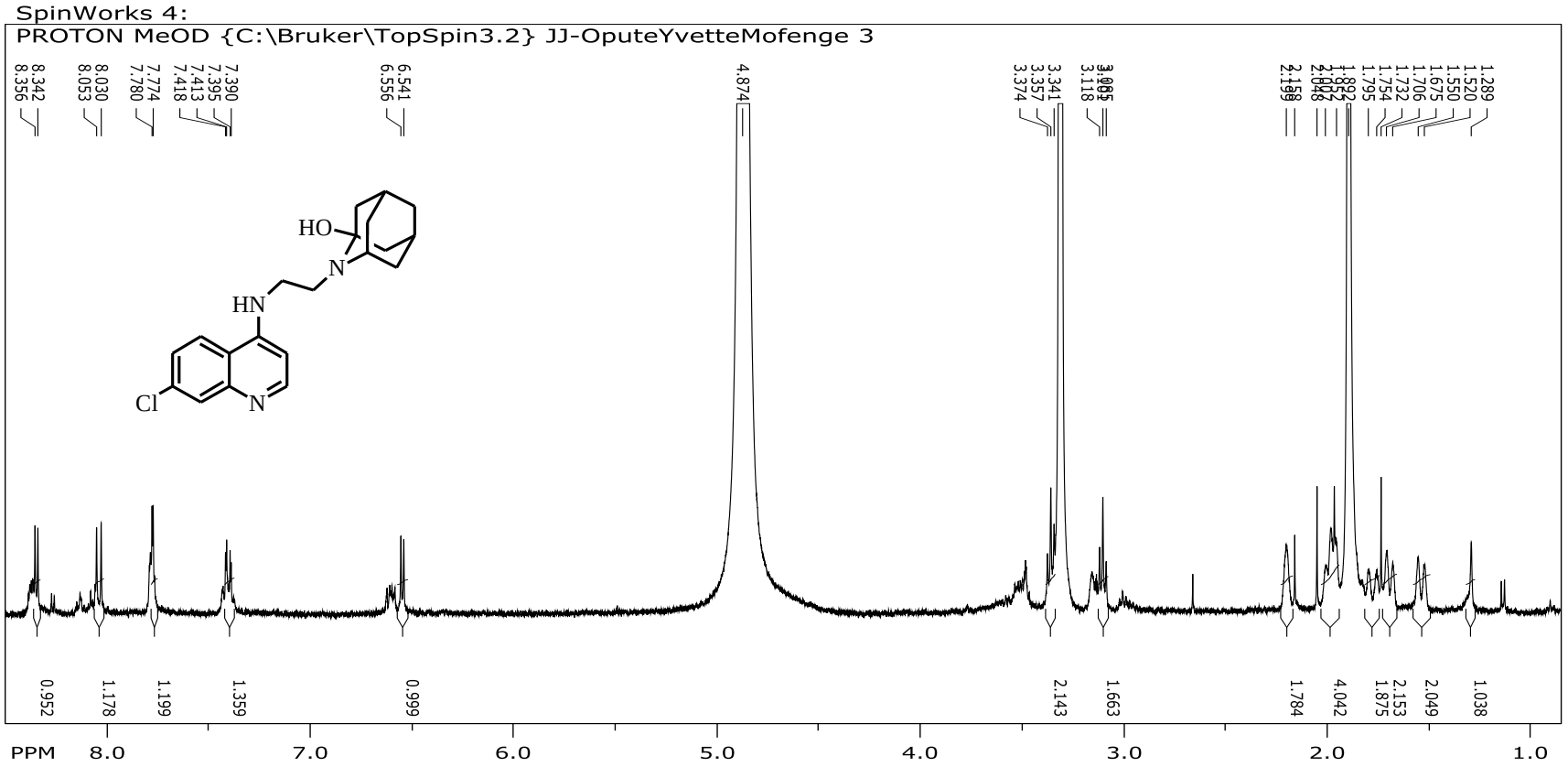
**SPECTRUM 9: Compound 5 1H-NMR**

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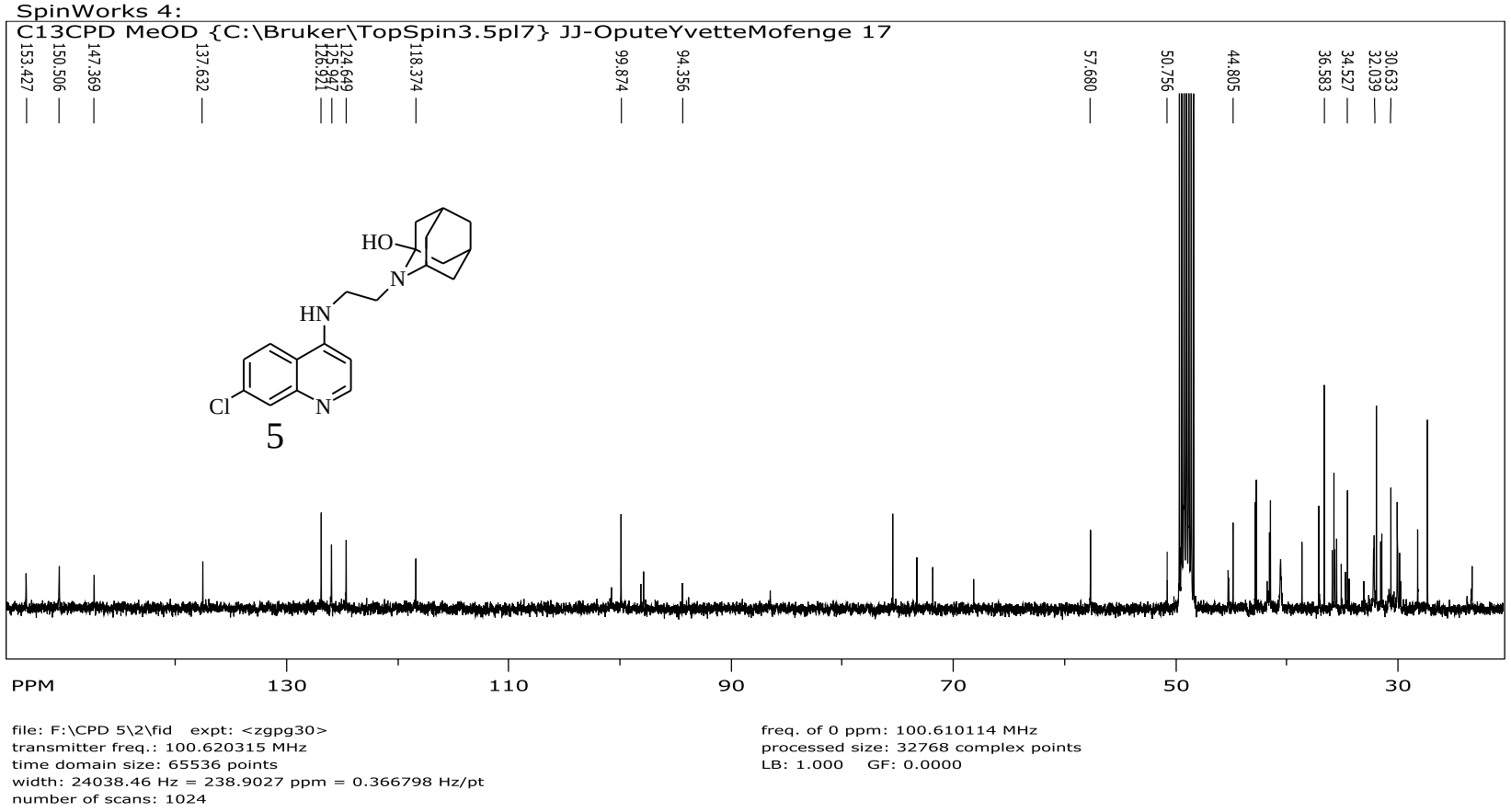
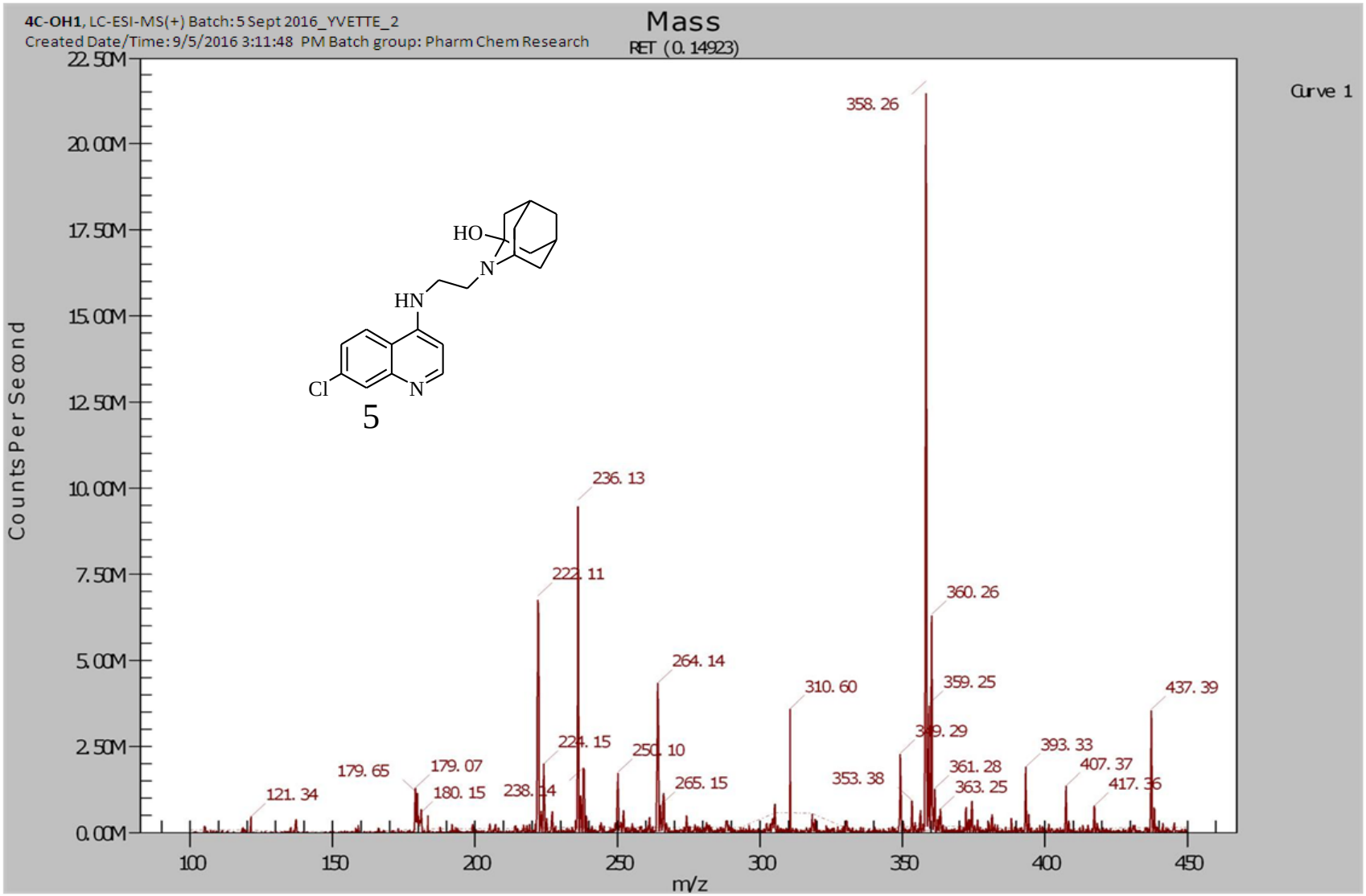
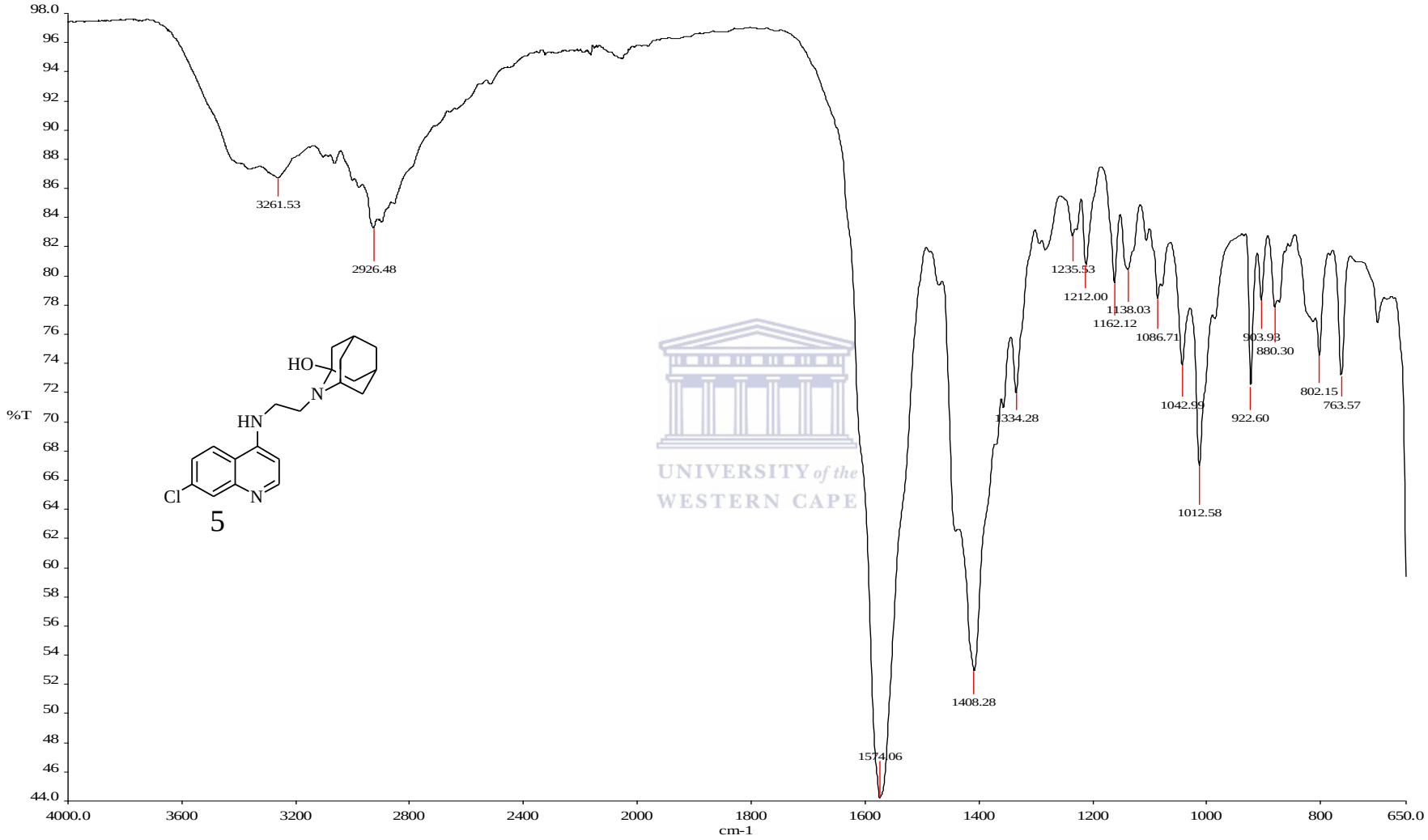
**SPECTRUM 10: Compound 5 13C-NMR**



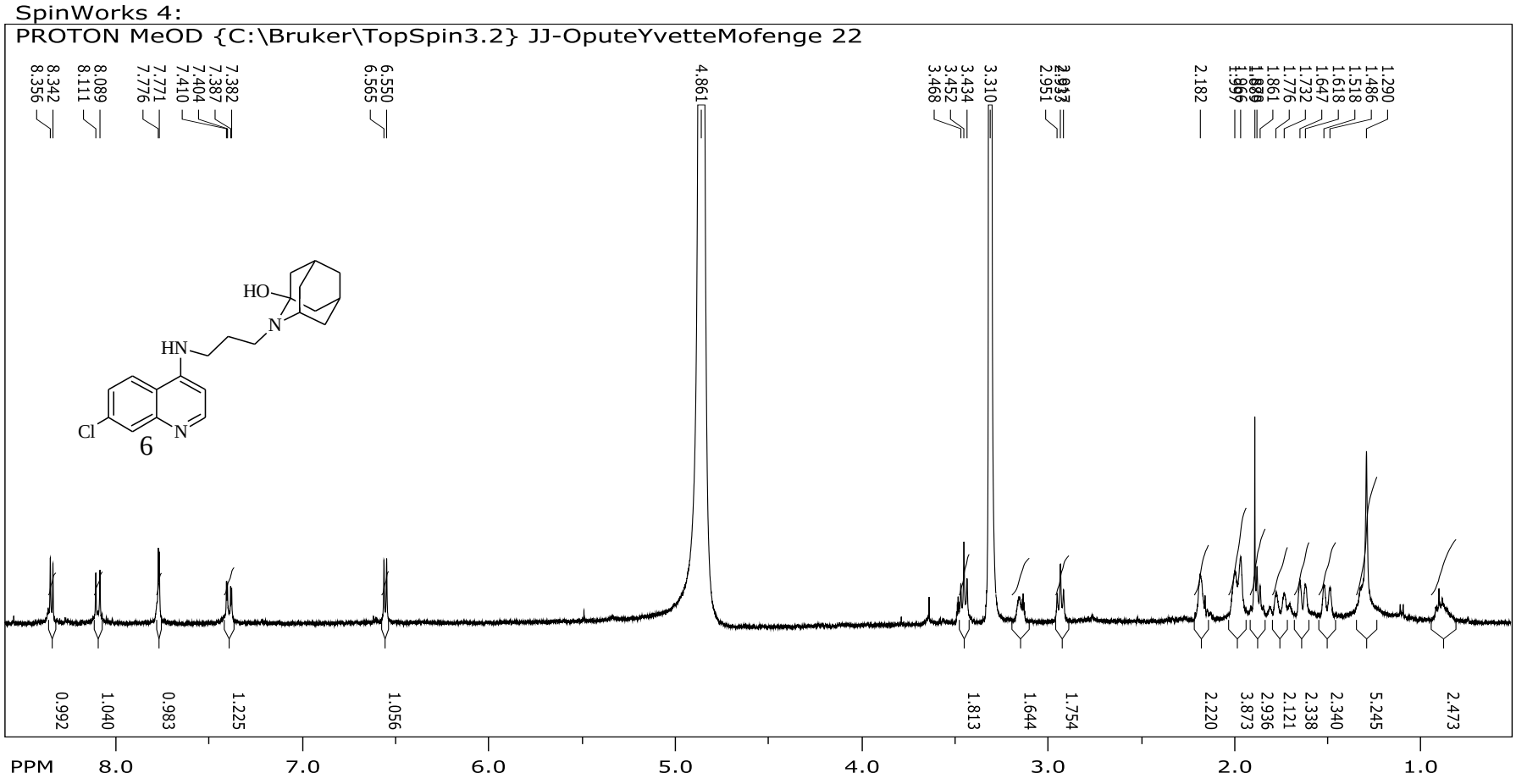
**SPECTRUM 11: Compound 5 MS**



**SPECTRUM 12: Compound 5 IR**



**SPECTRUM 13: Compound 6 1H-NMR**

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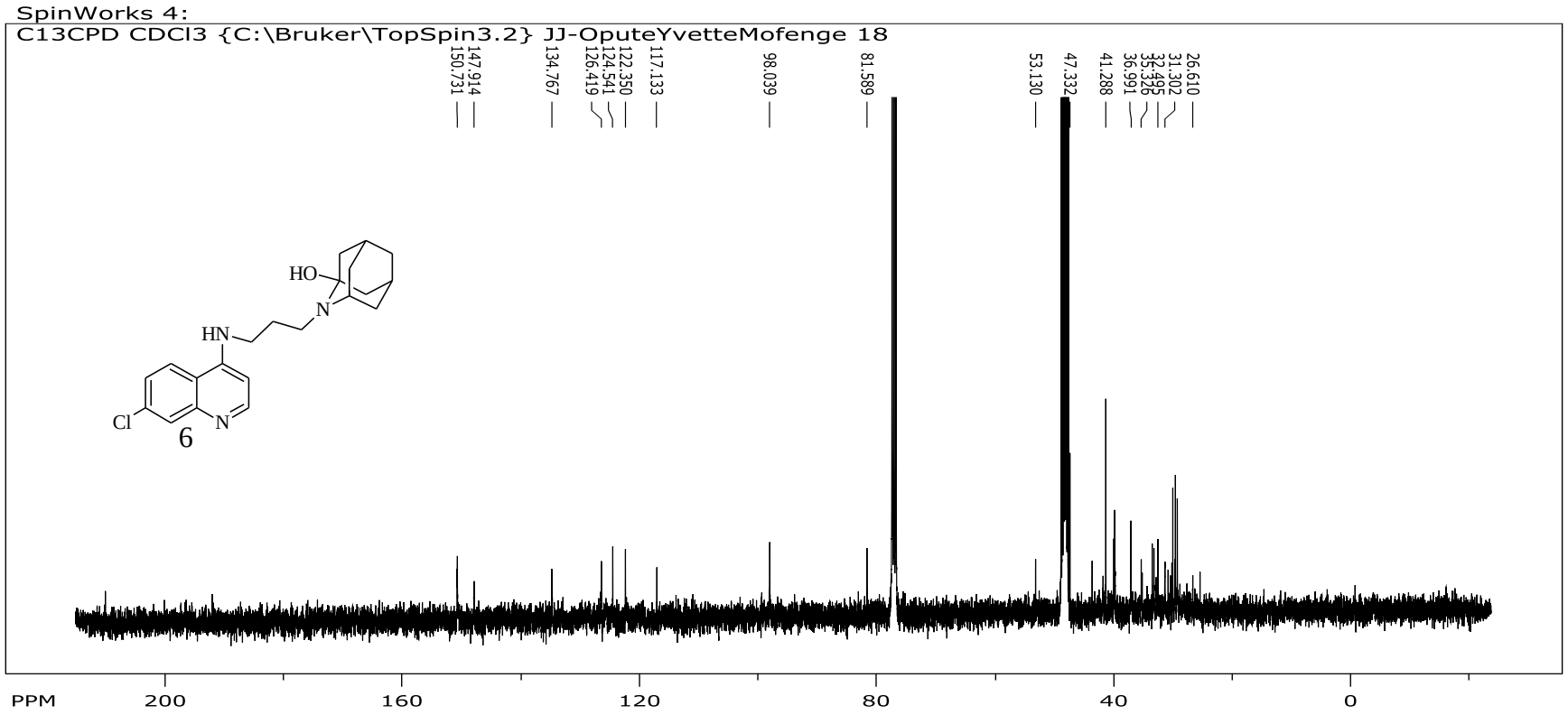
**SPECTRUM 14: Compound 6 13C-NMR**

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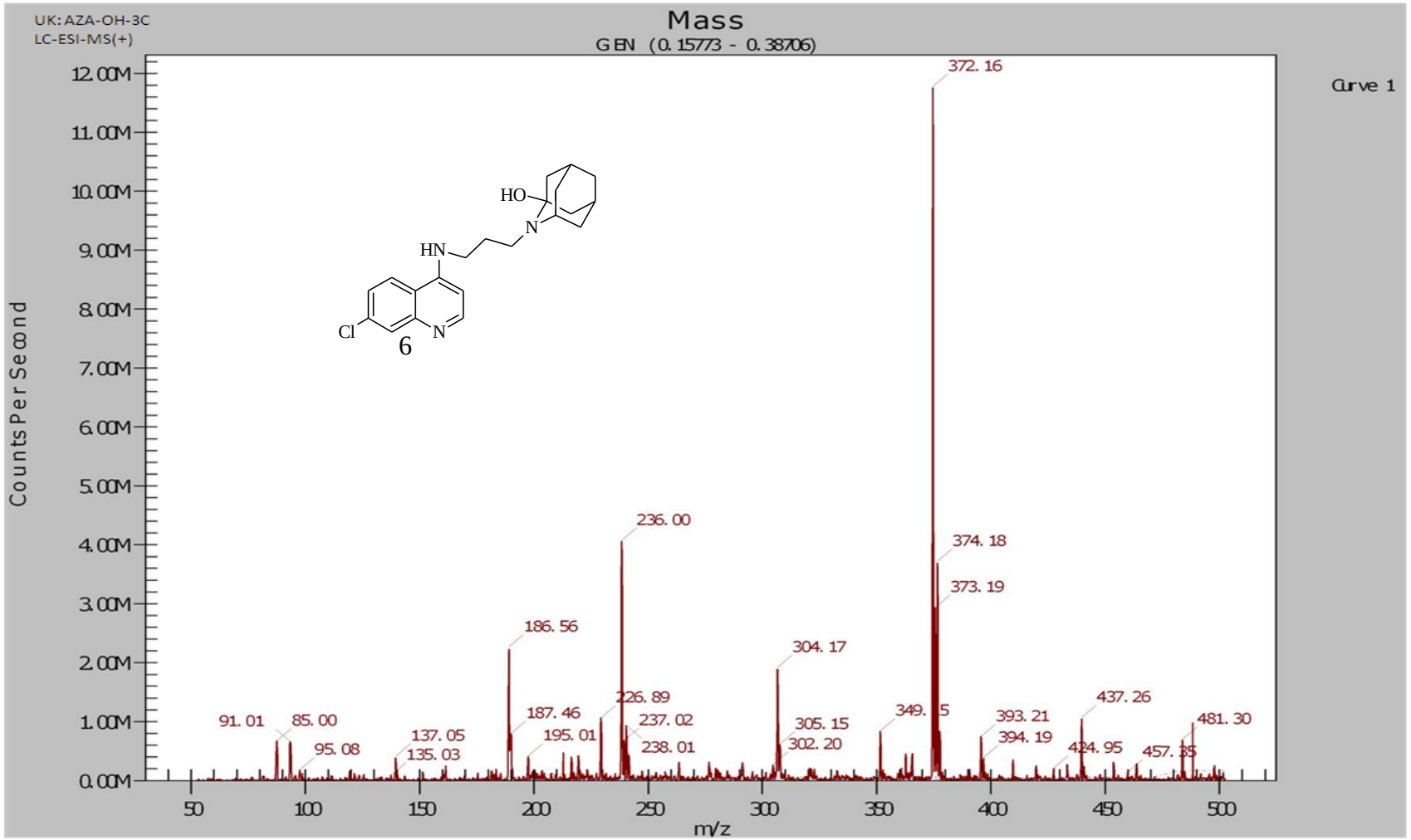
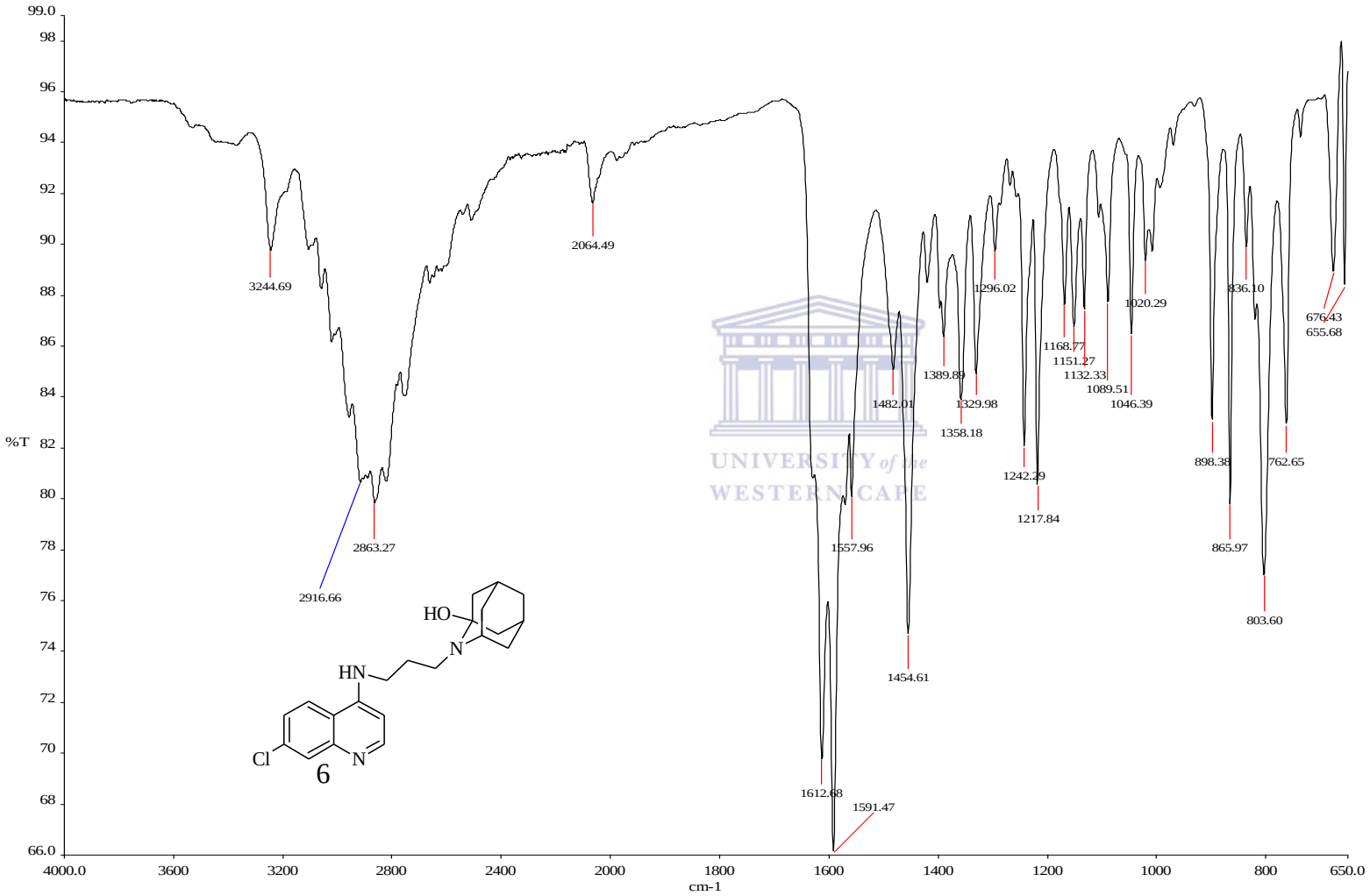
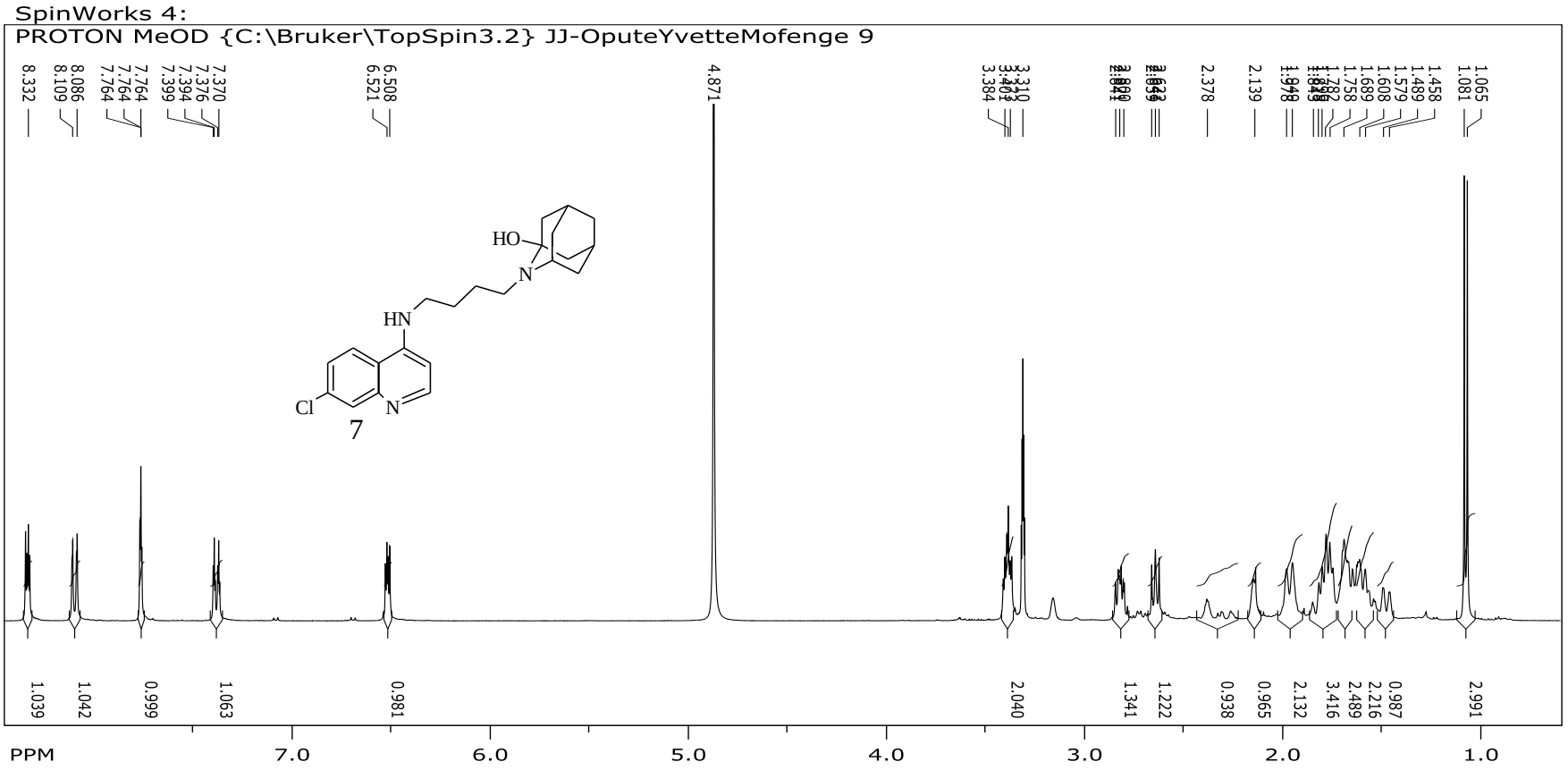
**SPECTRUM 15: Compound 6 MS**



**SPECTRUM 16: Compound 6 IR**



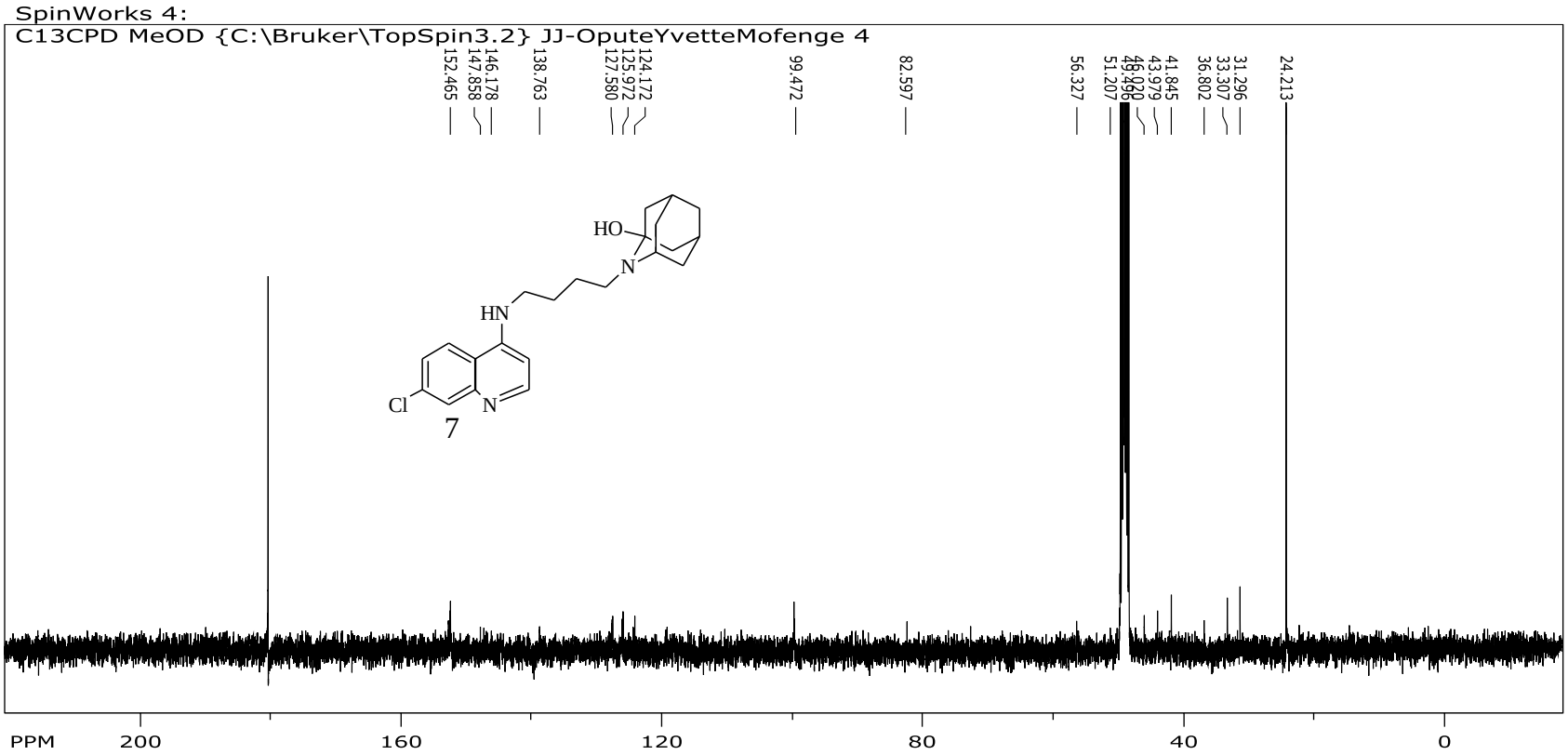
**SPECTRUM 17: Compound 7 1H-NMR**



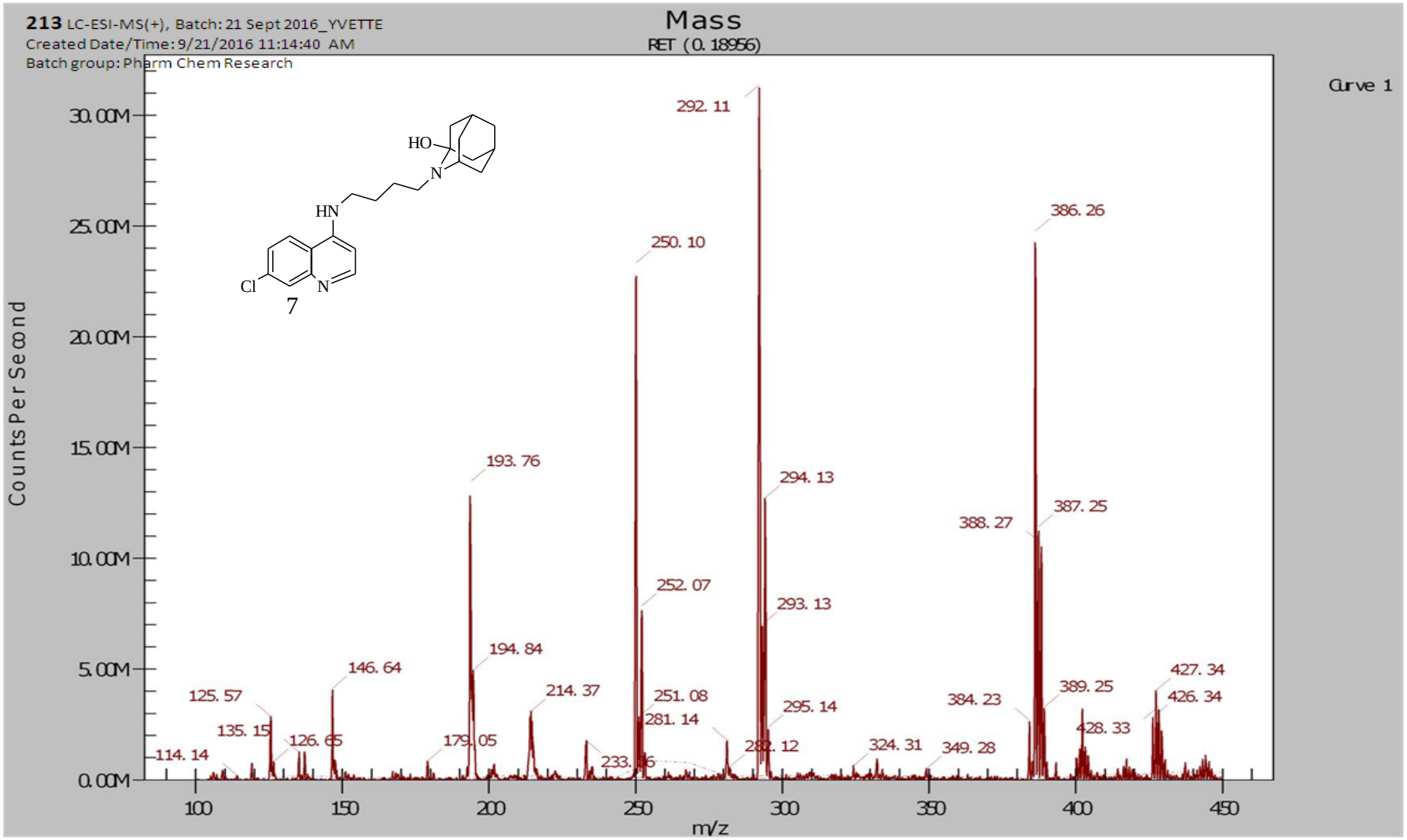

file: ...vetteMofenge\nmr\4C-OH 14 EA\1\fid expt: <zg30>
transmitter freq.: 400.122471 MHz
time domain size: 65536 points
width: 8012.82 Hz = 20.0259 ppm = 0.122266 Hz/pt
number of scans: 24

freq. of 0 ppm: 400.120008 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

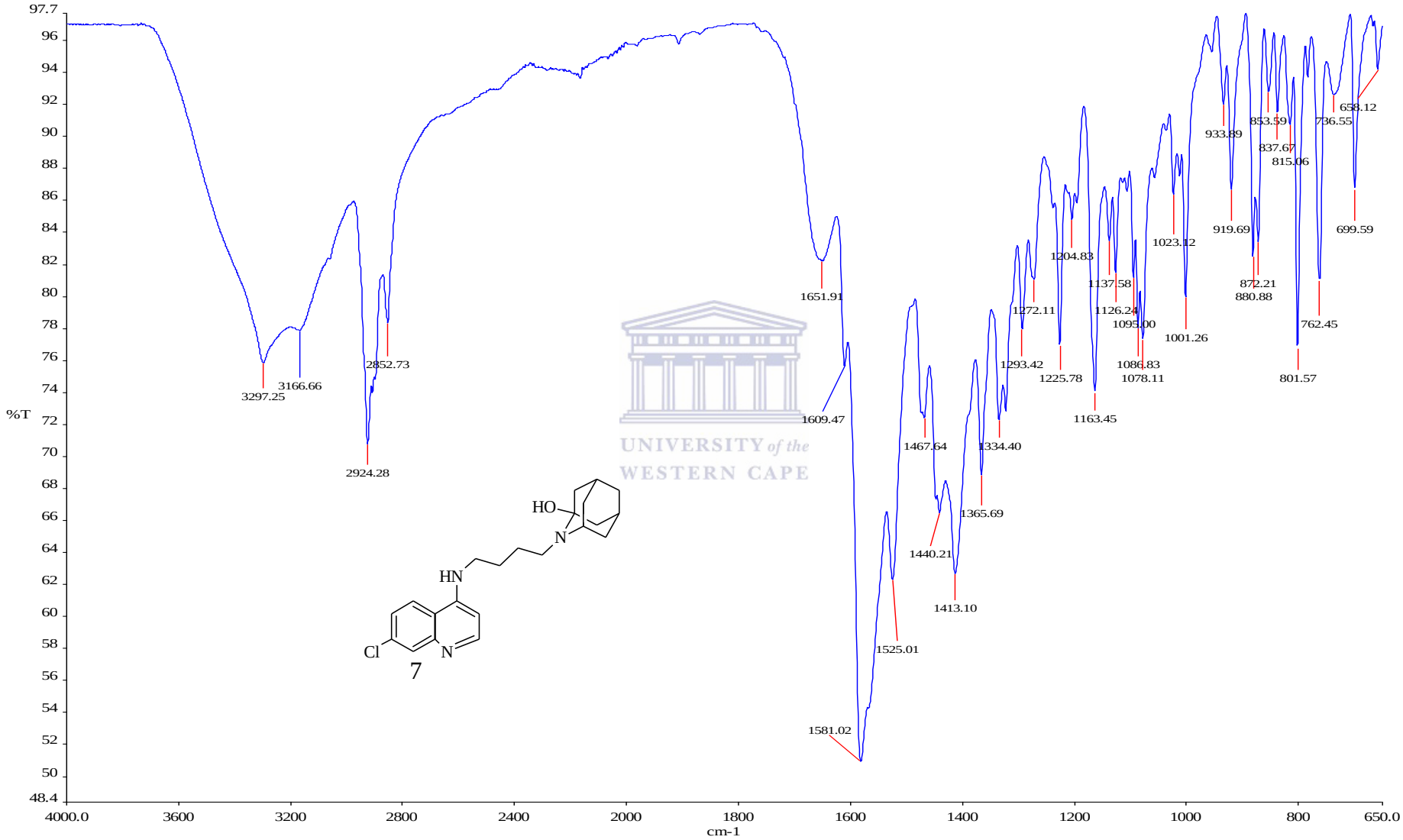
**SPECTRUM 18: Compound 7 13C NMR**



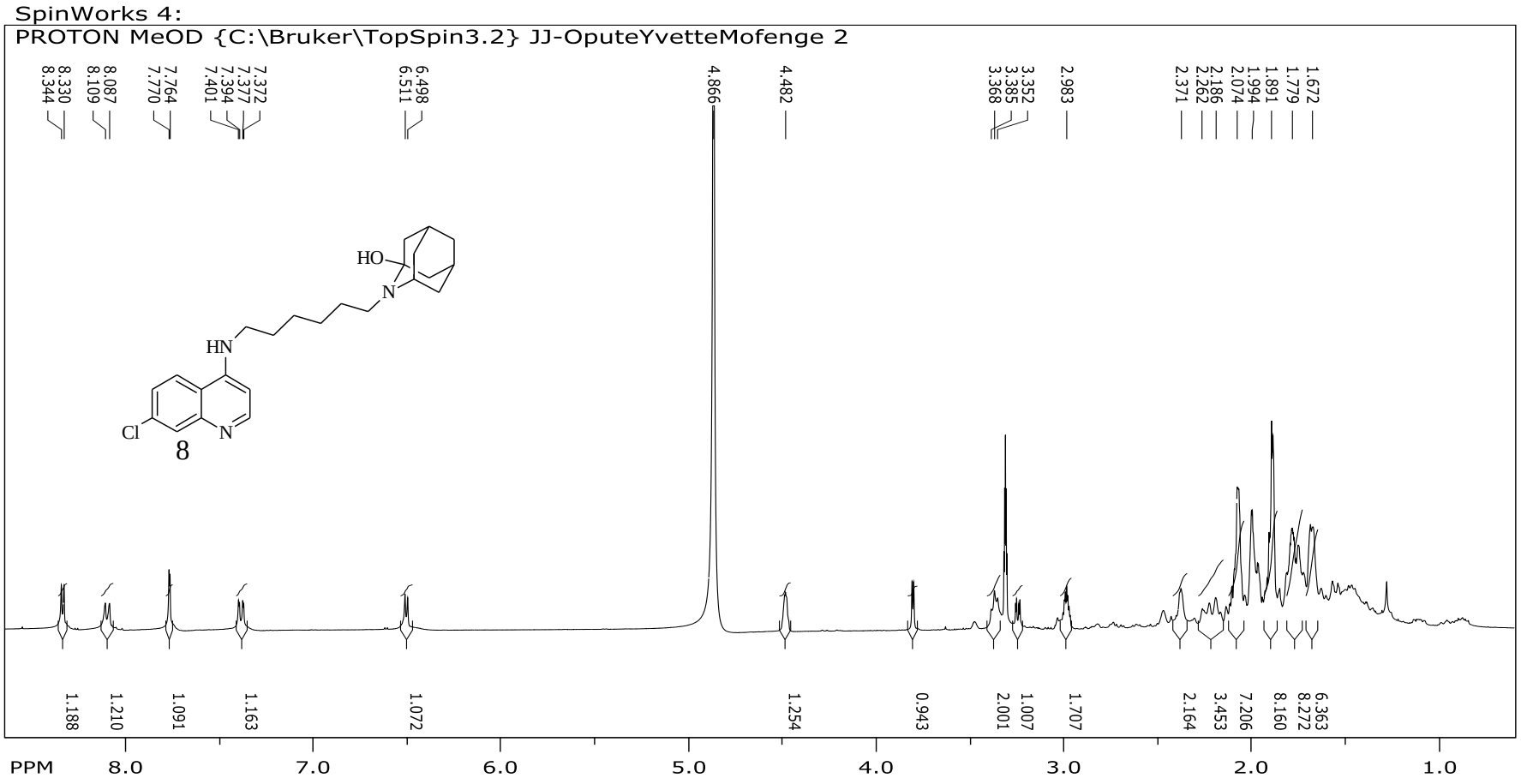
**SPECTRUM 19: Compound 7 MS**



**SPECTRUM 20: Compound 7 IR**



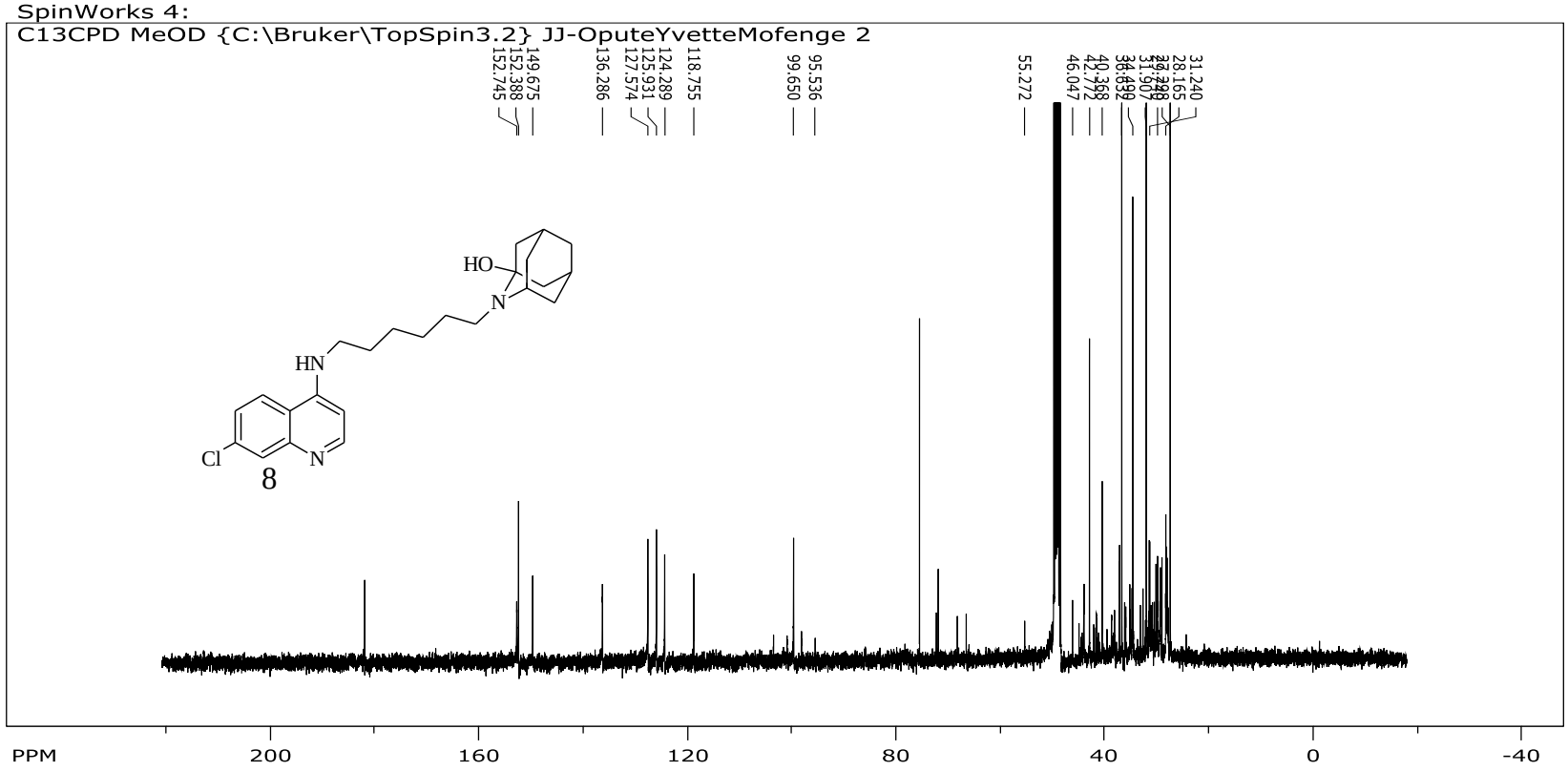
**SPECTRUM 21: Compound 8 PROTON NMR**



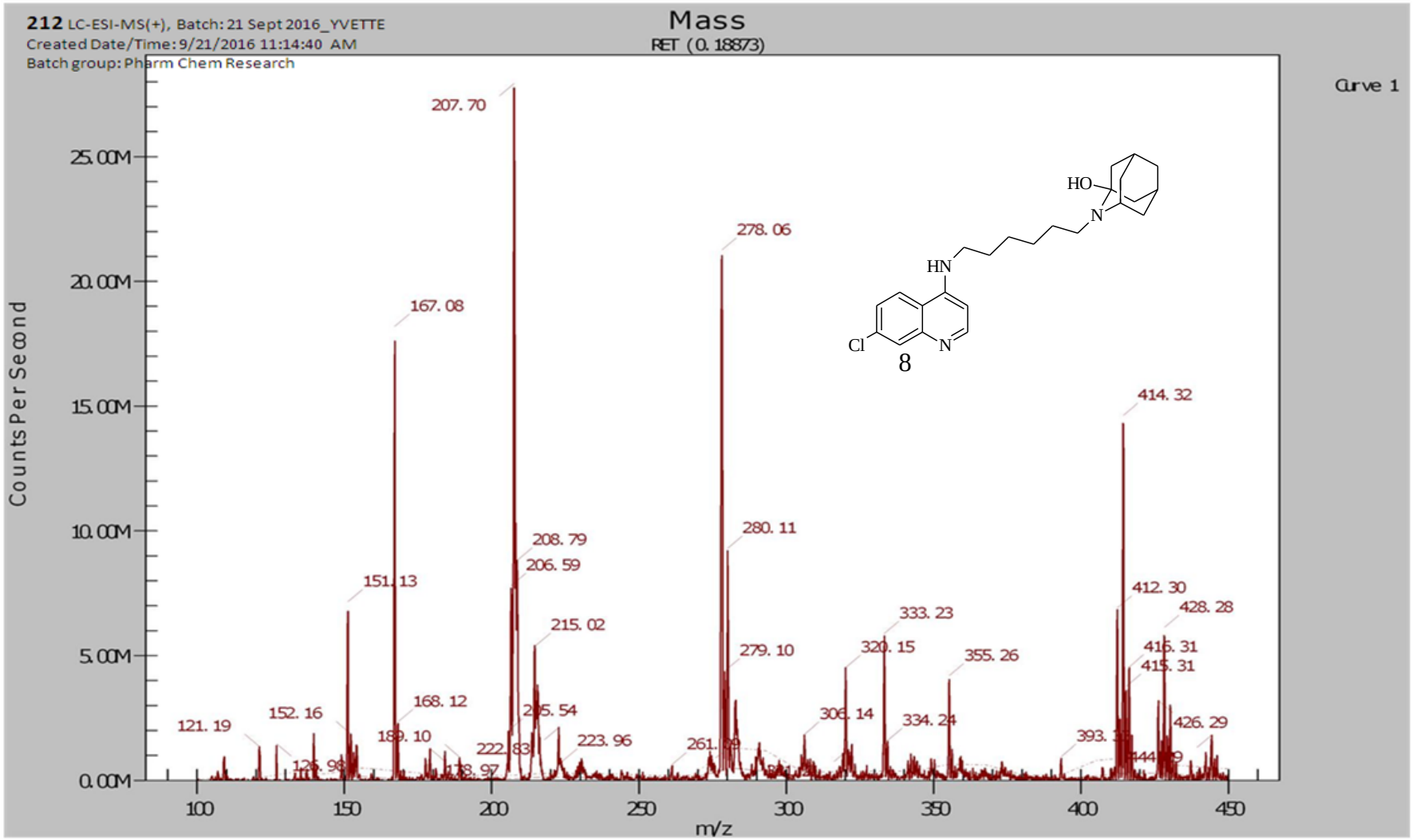

file: G:\6C-OHA\2\fid expt: <zg30>
transmitter freq.: 400.122471 MHz
time domain size: 65536 points
width: 8012.82 Hz = 20.0259 ppm = 0.122266 Hz/pt
number of scans: 24

freq. of 0 ppm: 400.120008 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

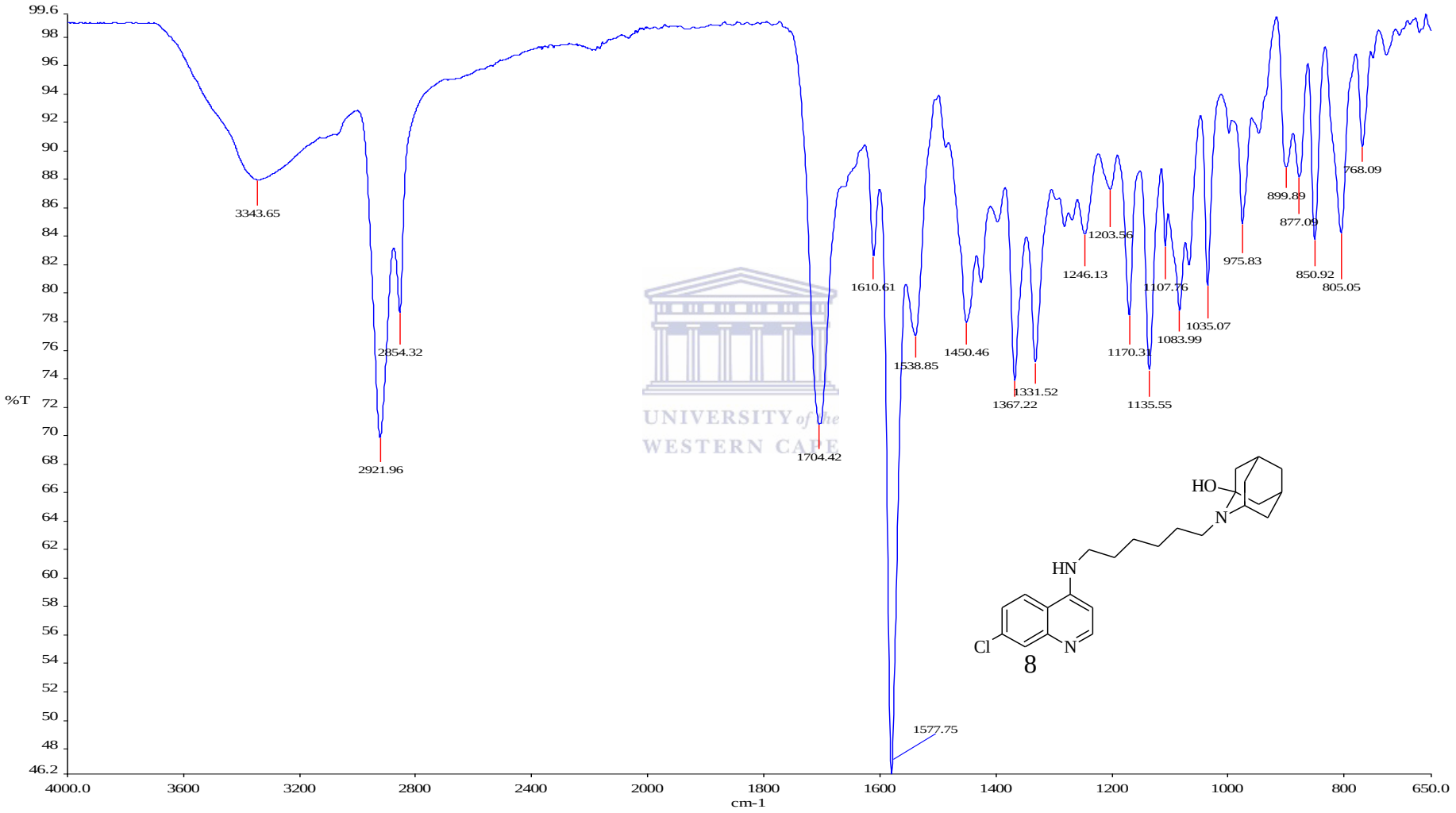
**SPECTRUM 22: Compound 8 13C-NMR**



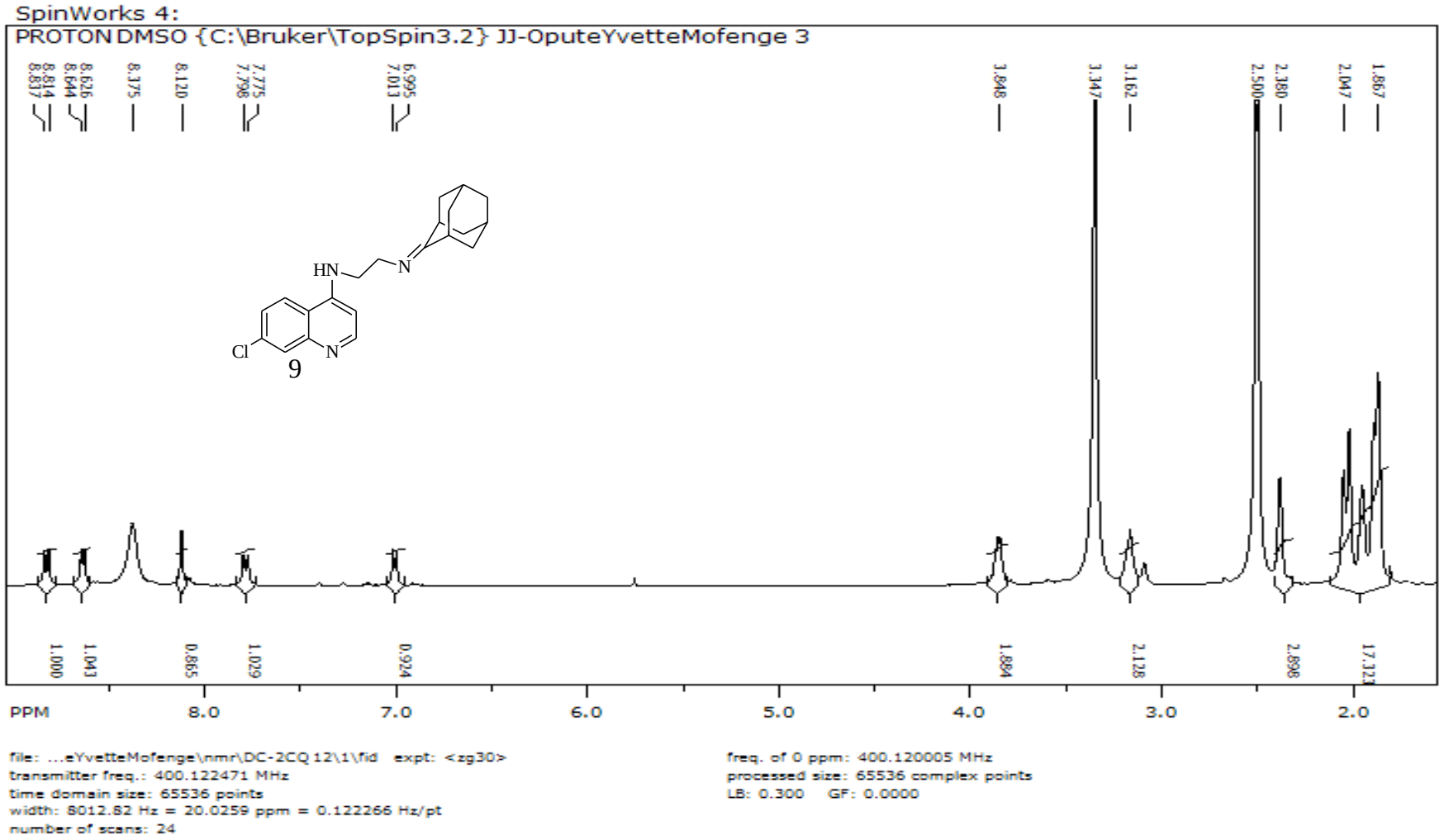
**SPECTRUM 23: Compound 8 MS**



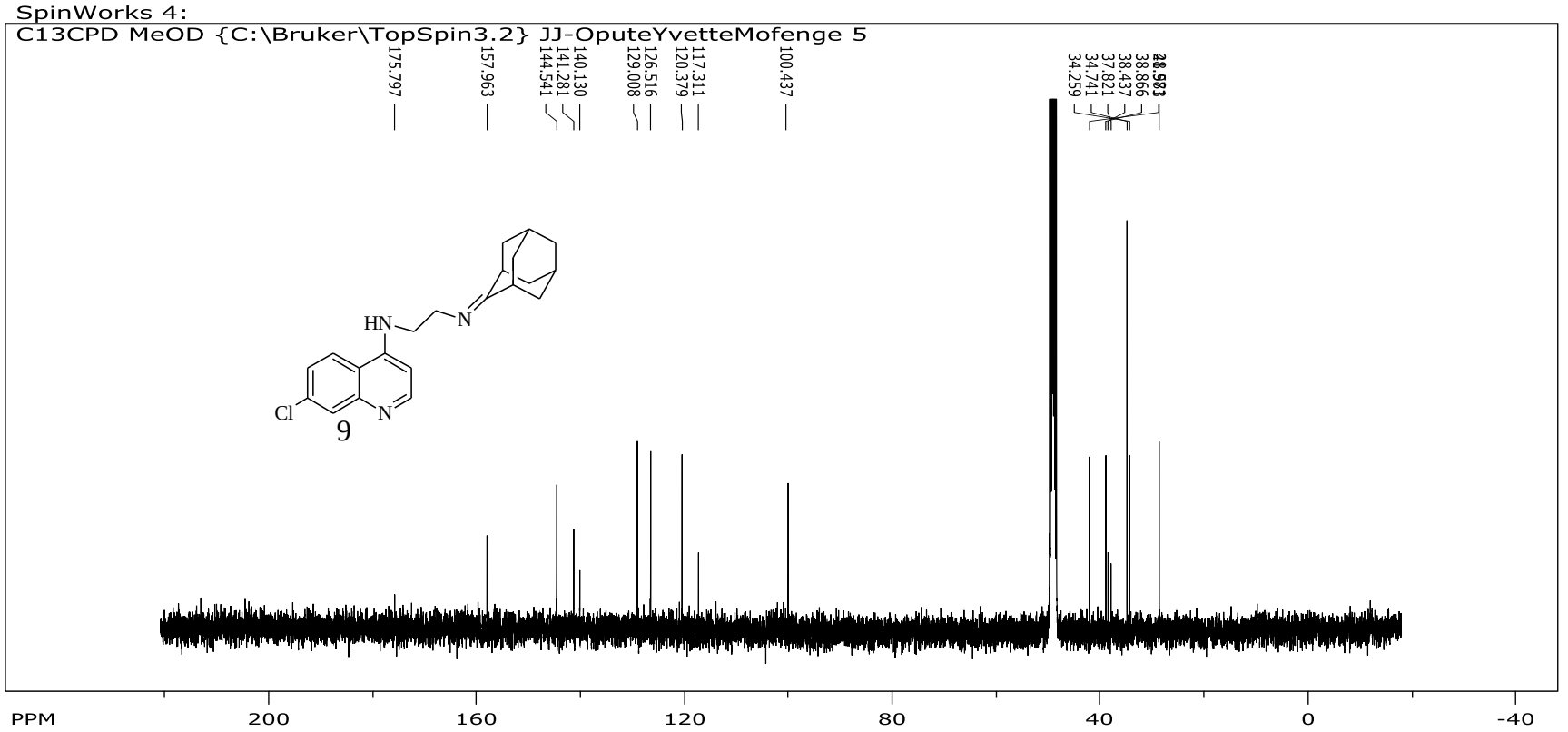
**SPECTRUM 24: Compound 8 IR**



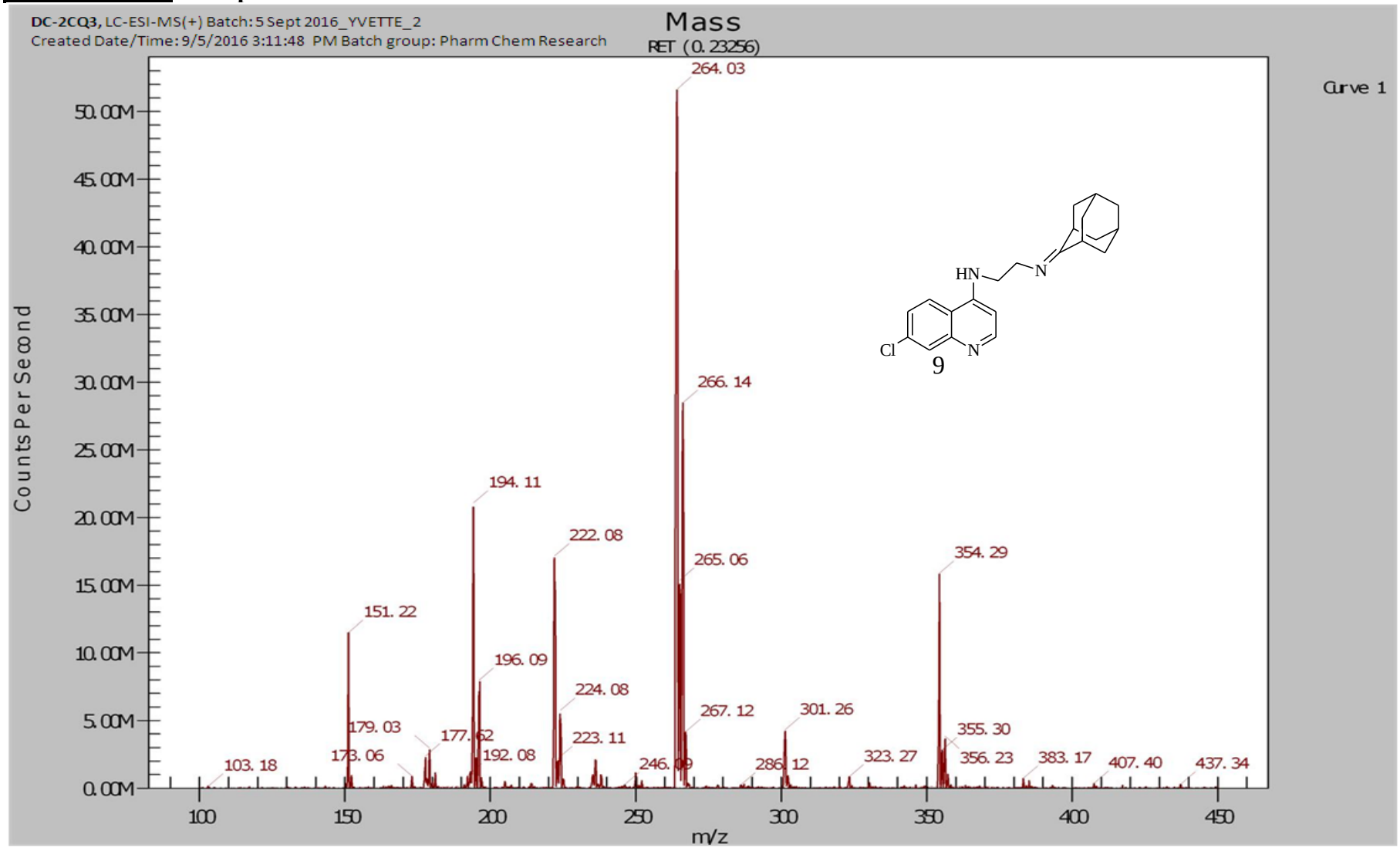
**<u>SPECTRUM 25</u>: Compound 9 PROTON NMR**



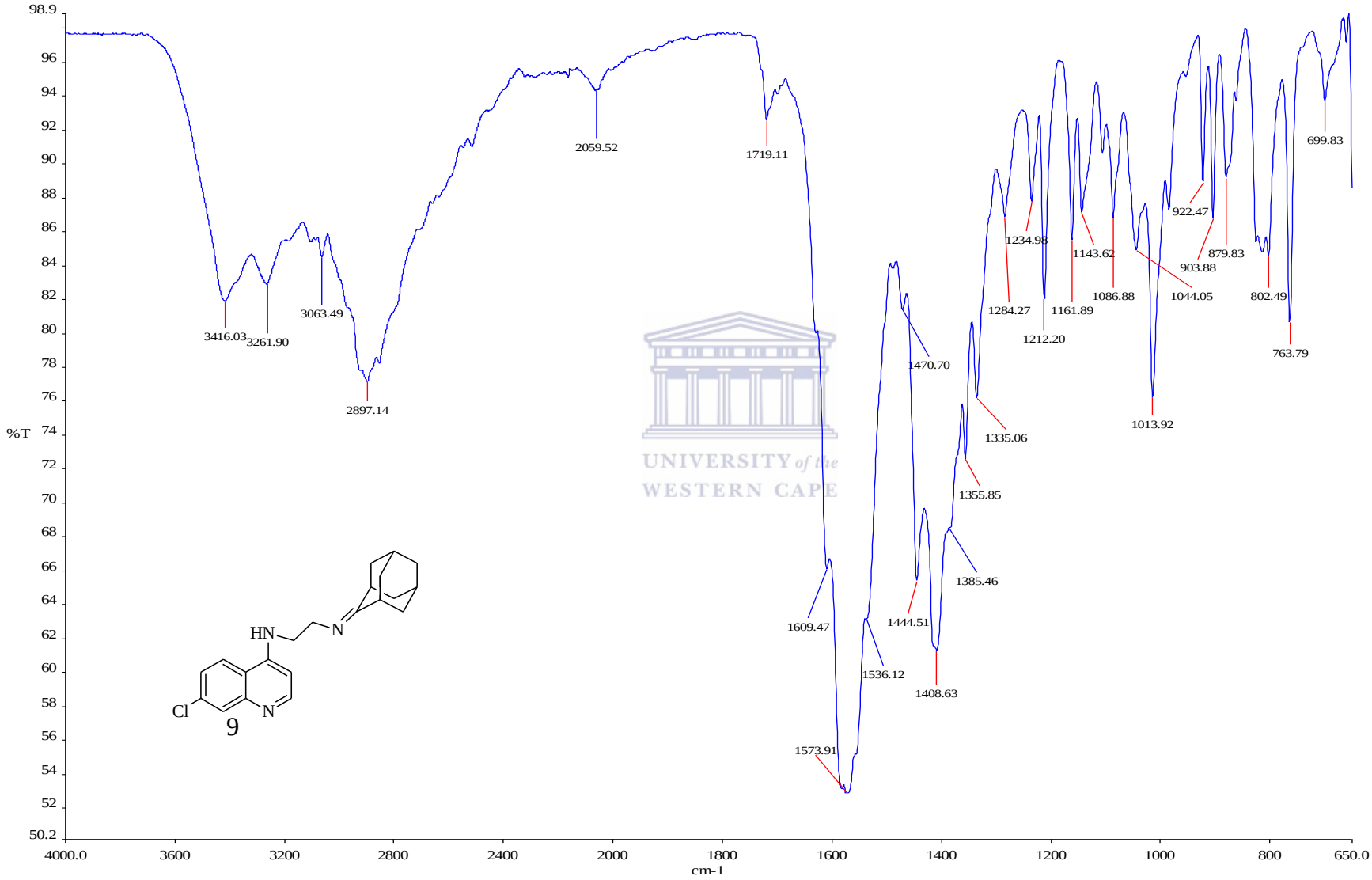
**SPECTRUM 26: Compound 9 13C NMR**



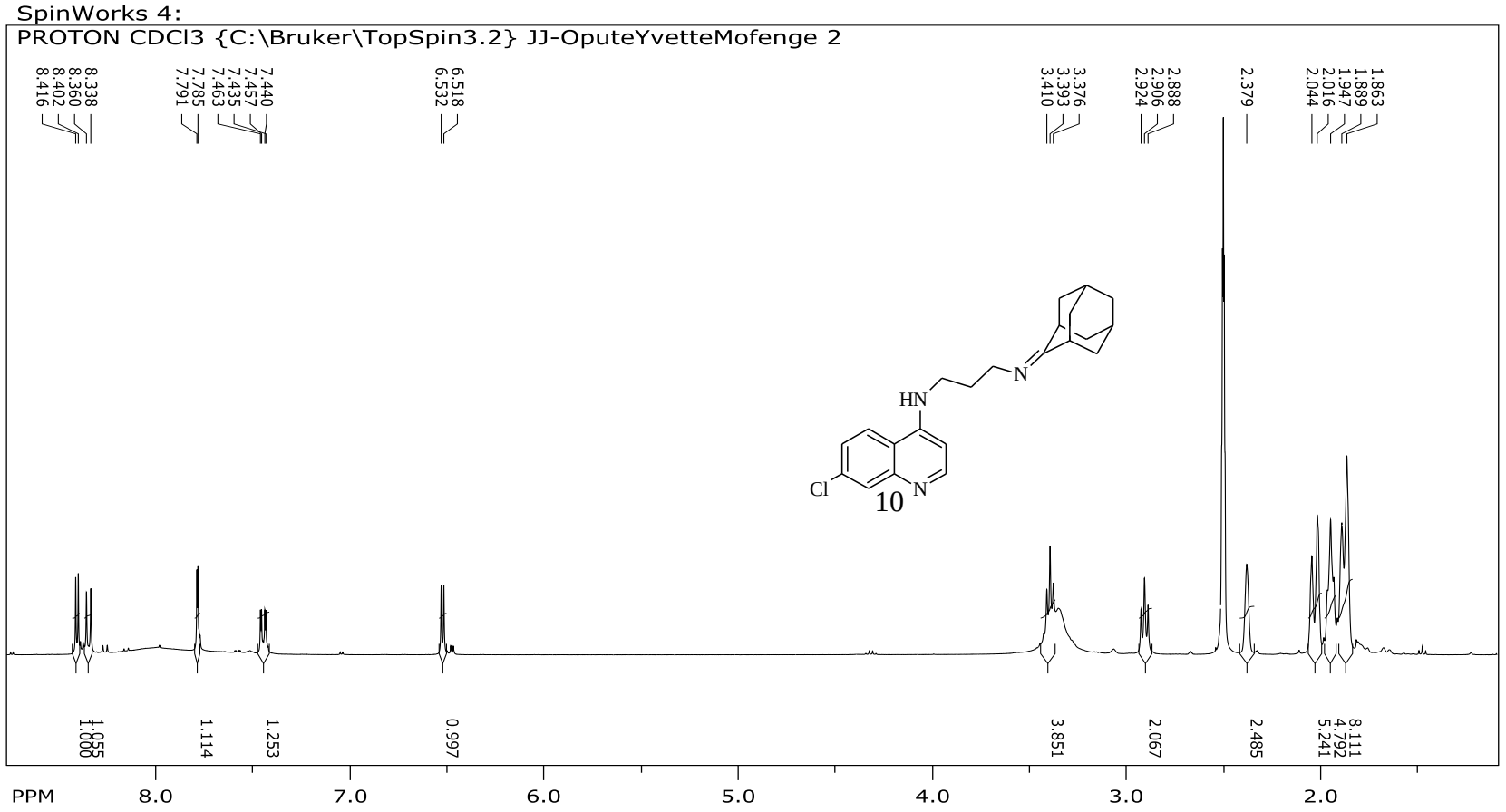
**SPECTRUM 27: Compound 9 MS**



**SPECTRUM 28: Compound 9IR**



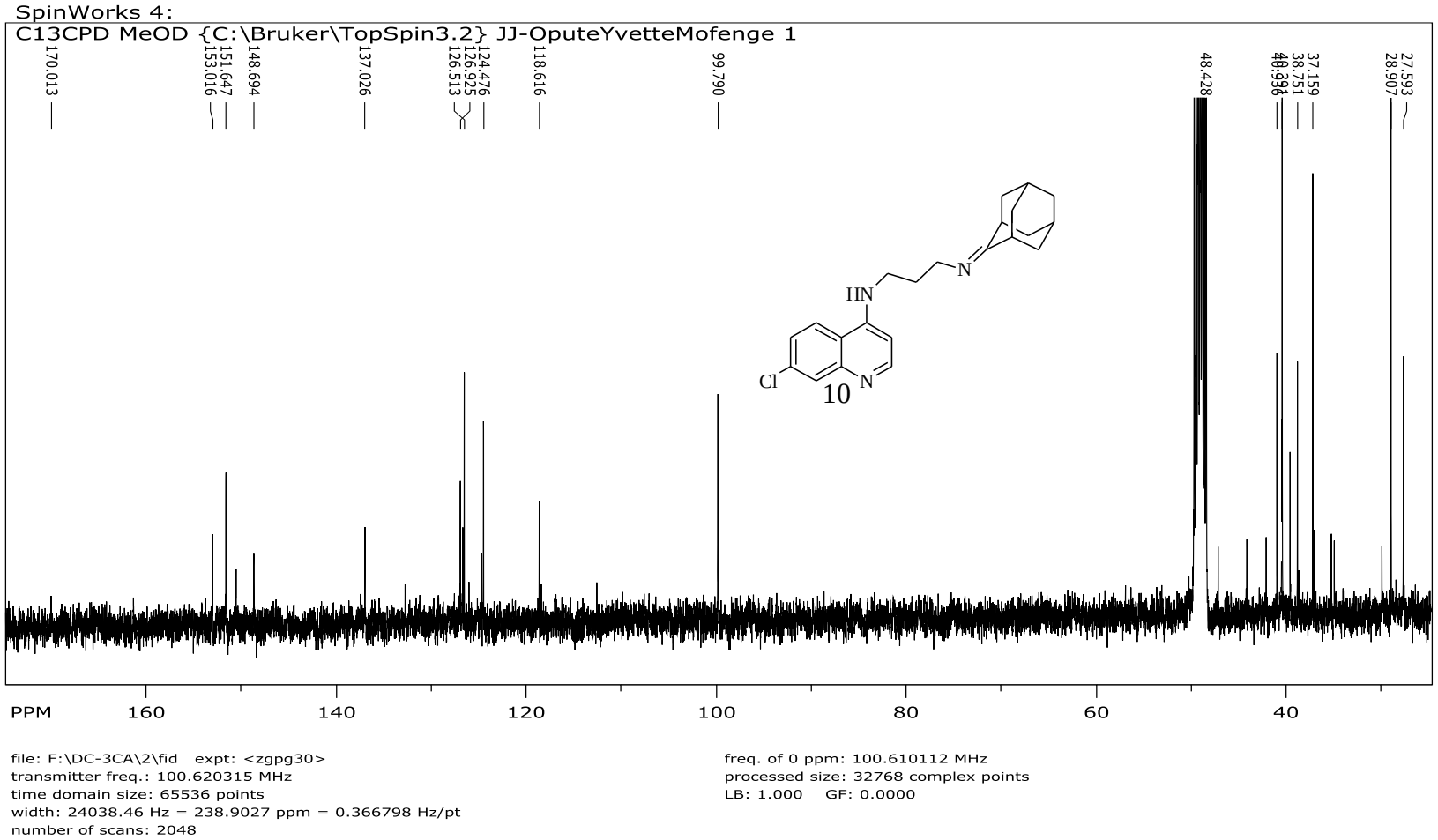
**SPECTRUM 29: Compound 10 1H-NMR**



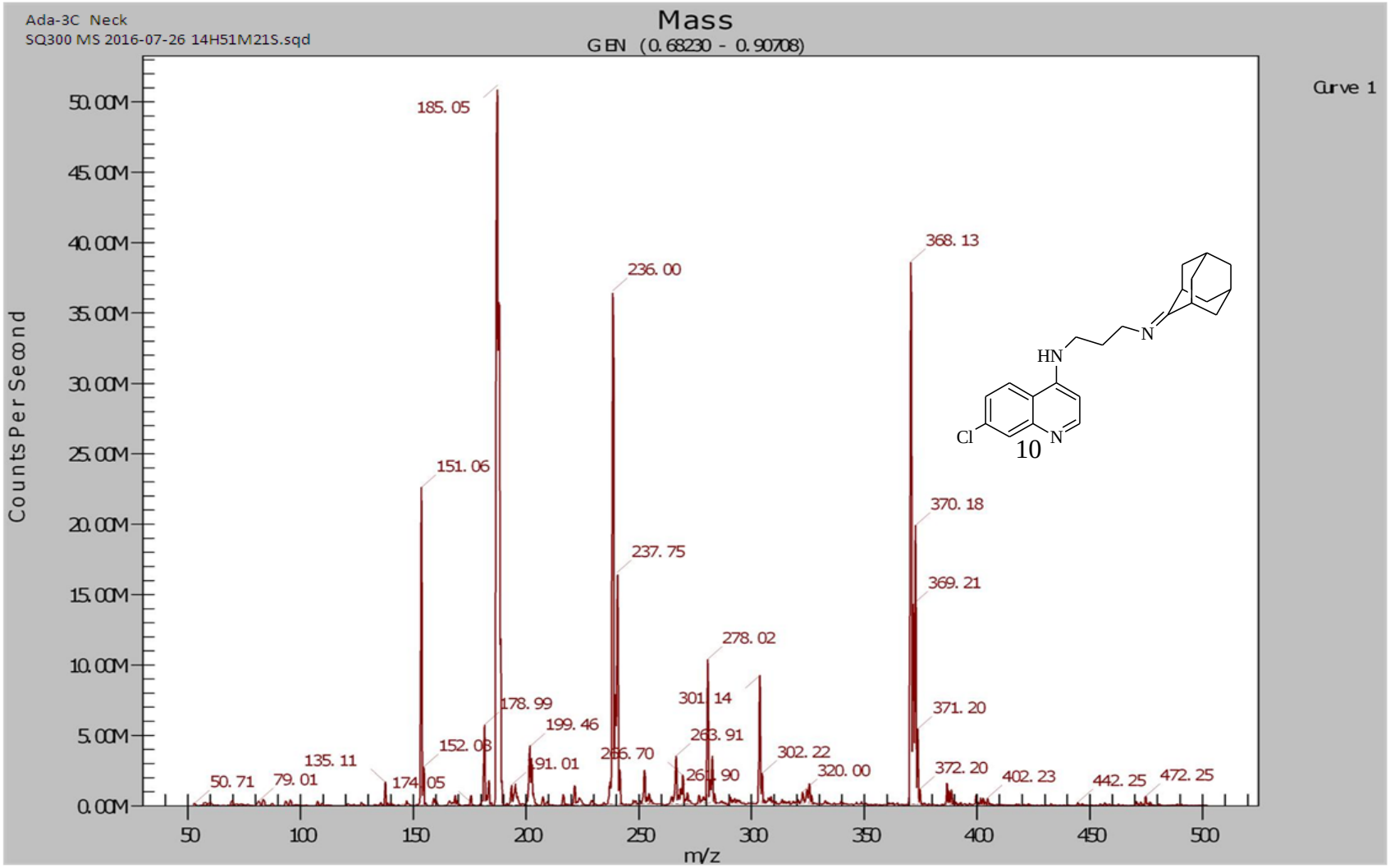

file: ...eYvetteMofenge\nmr\DC-3CQ LD\1\fid expt: <zg30>
transmitter freq.: 400.122471 MHz
time domain size: 65536 points
width: 8012.82 Hz = 20.0259 ppm = 0.122266 Hz/pt
number of scans: 24

freq. of 0 ppm: 400.121905 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

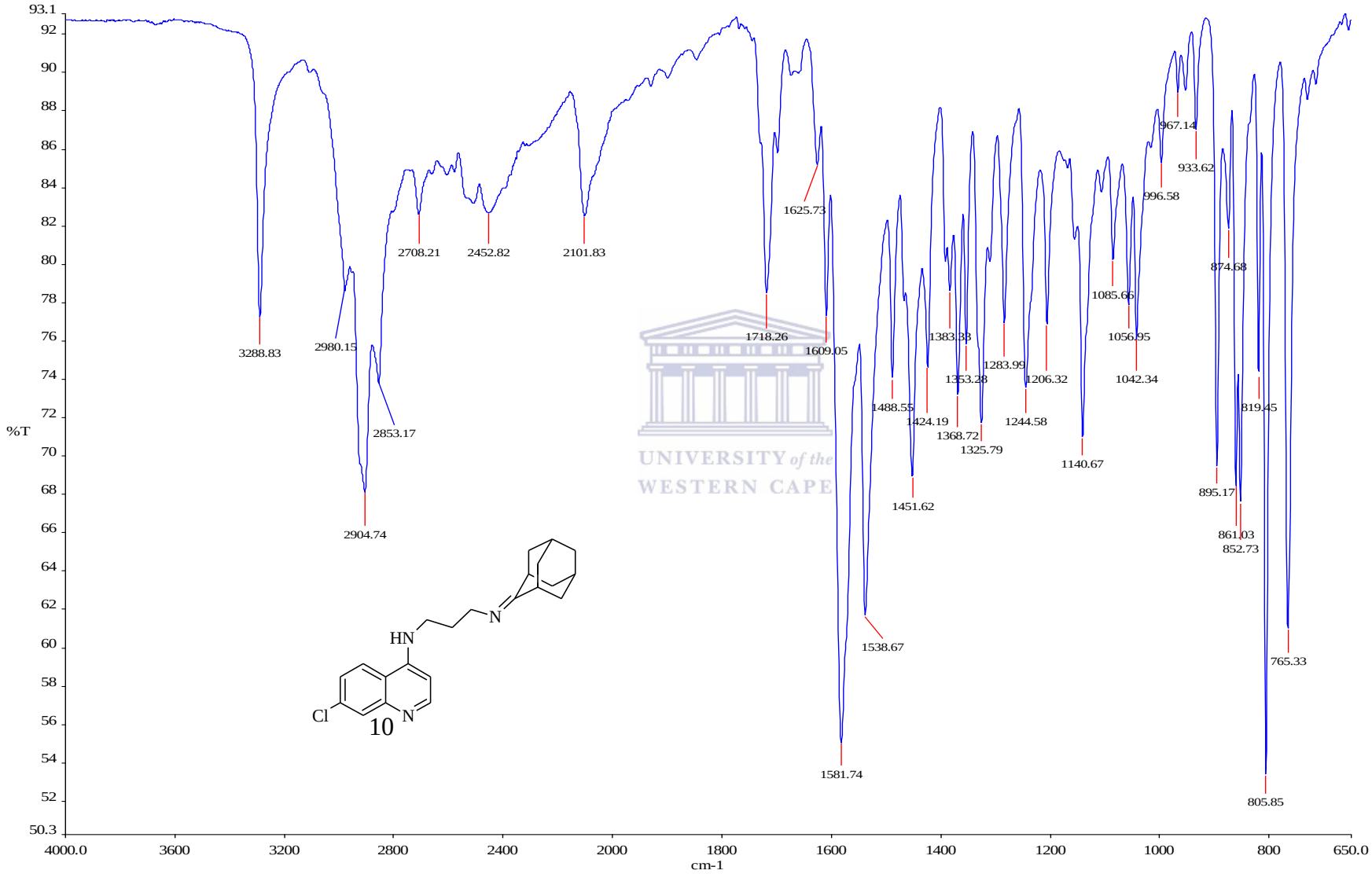
**<u>SPECTRUM 30</u>: Compound 10 13C-NMR**



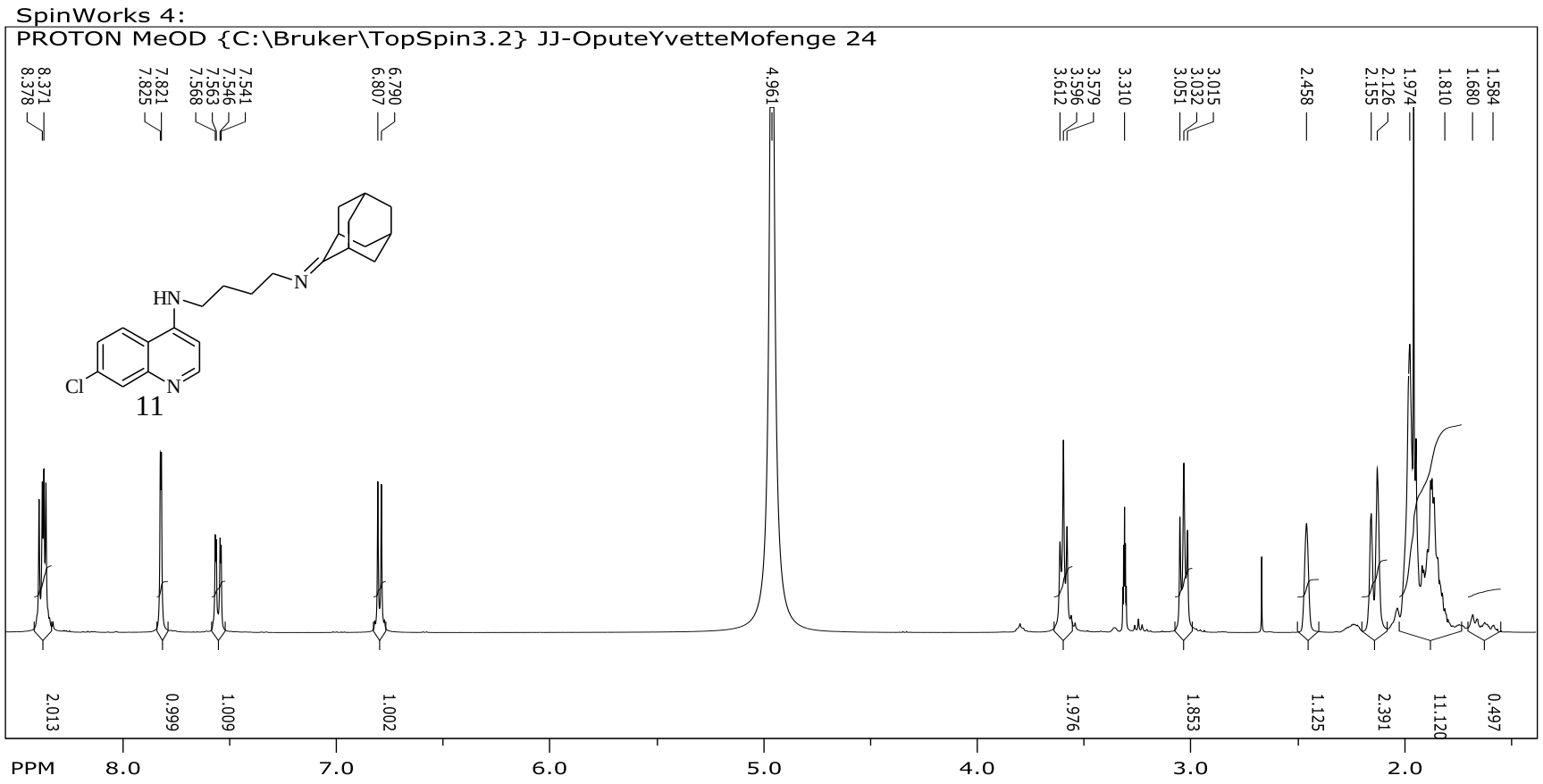
**SPECTRUM 31: Compound 10 MS**



**SPECTRUM 32: Compound 10 IR**



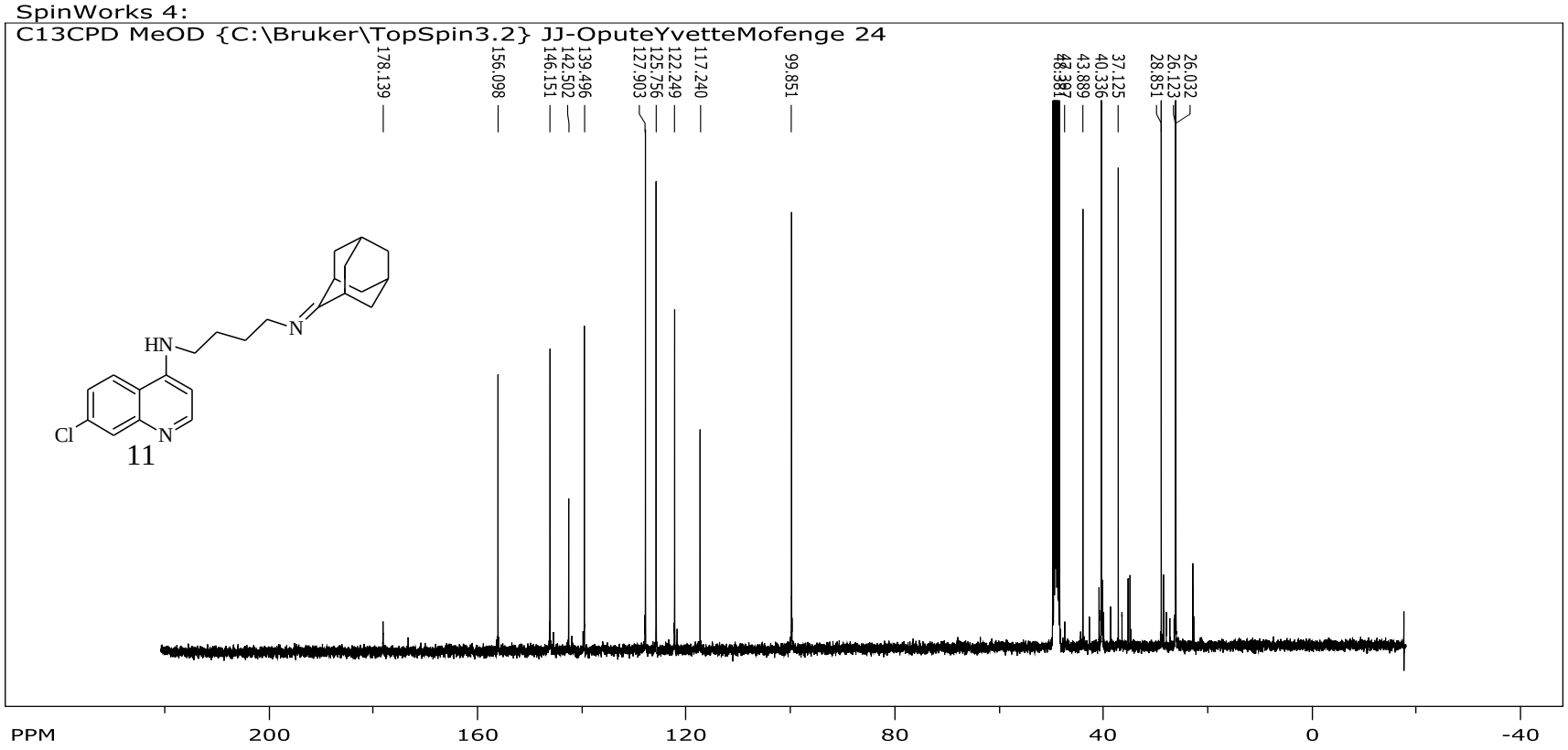
**SPECTRUM 33: Compound 11 1H-NMR**




file: F:\DC-4CA\1\fid expt: <zg30>
transmitter freq.: 400.122471 MHz
time domain size: 65536 points
width: 8012.82 Hz = 20.0259 ppm = 0.122266 Hz/pt
number of scans: 24

freq. of 0 ppm: 400.120008 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

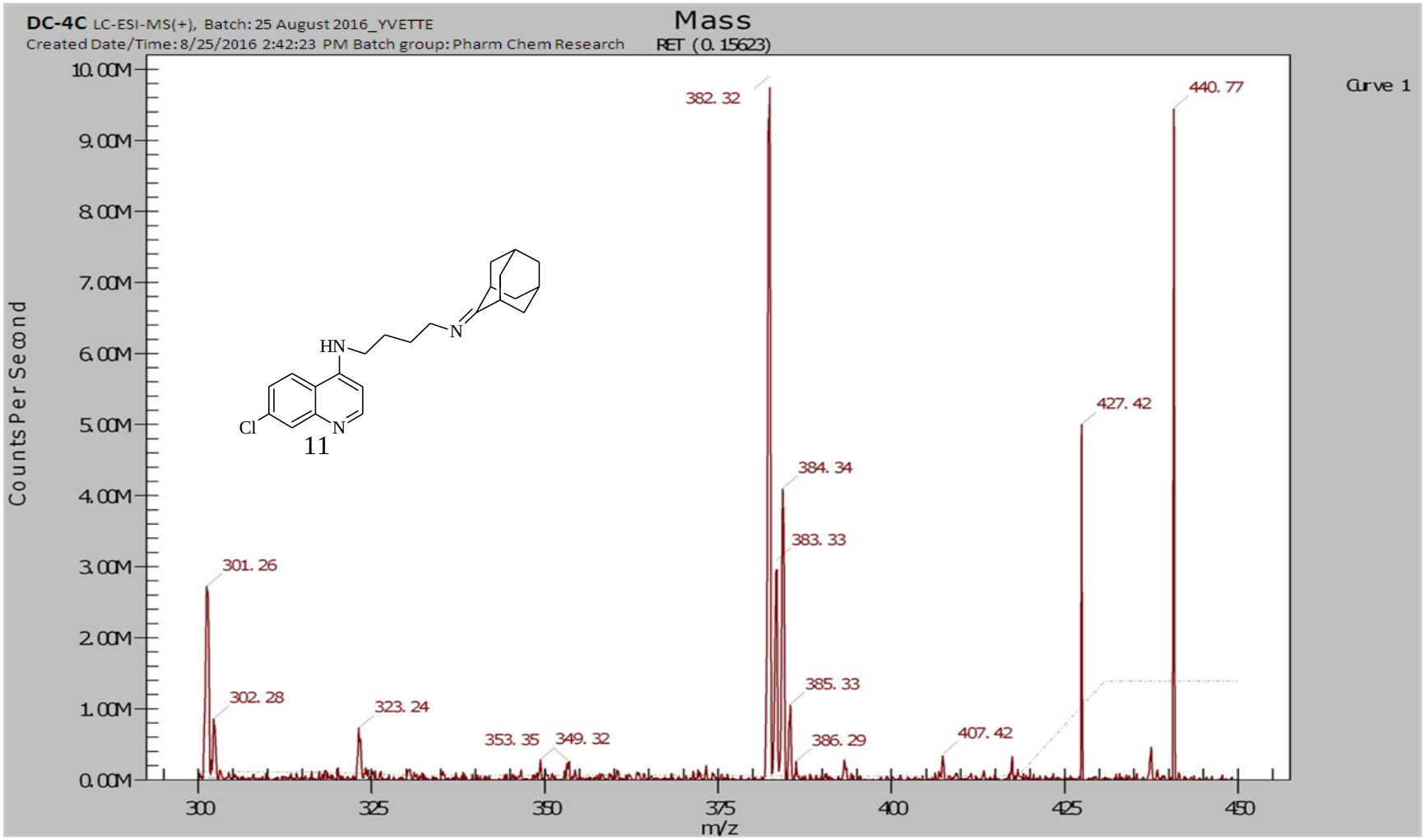
**SPECTRUM 34: Compound 11 13C-NMR**



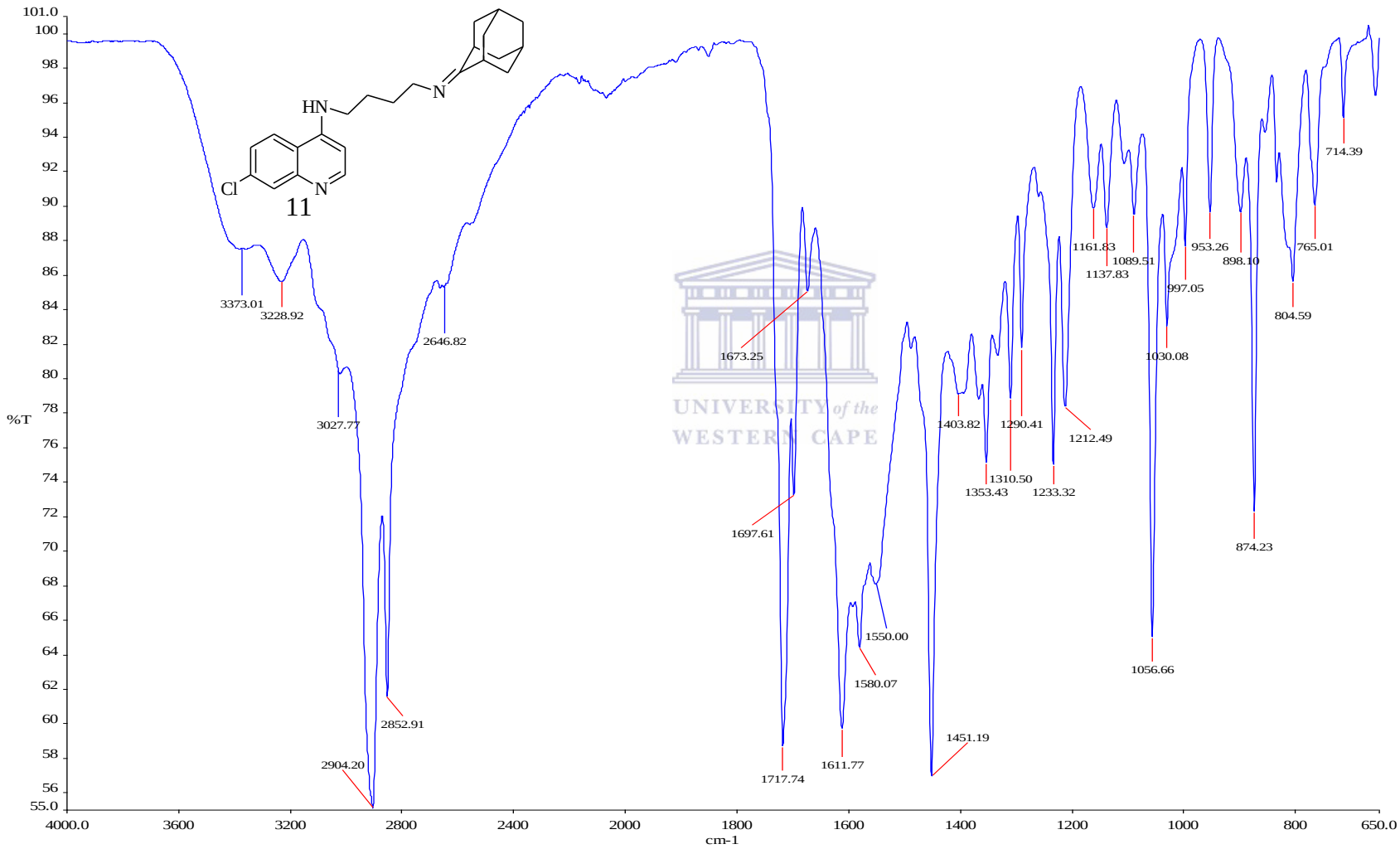

file: F:\DC-4CA\2\fid expt: <zgpg30>
transmitter freq.: 100.620315 MHz
time domain size: 65536 points
width: 24038.46 Hz = 238.9027 ppm = 0.366798 Hz/pt
number of scans: 2048

freq. of 0 ppm: 100.610117 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000

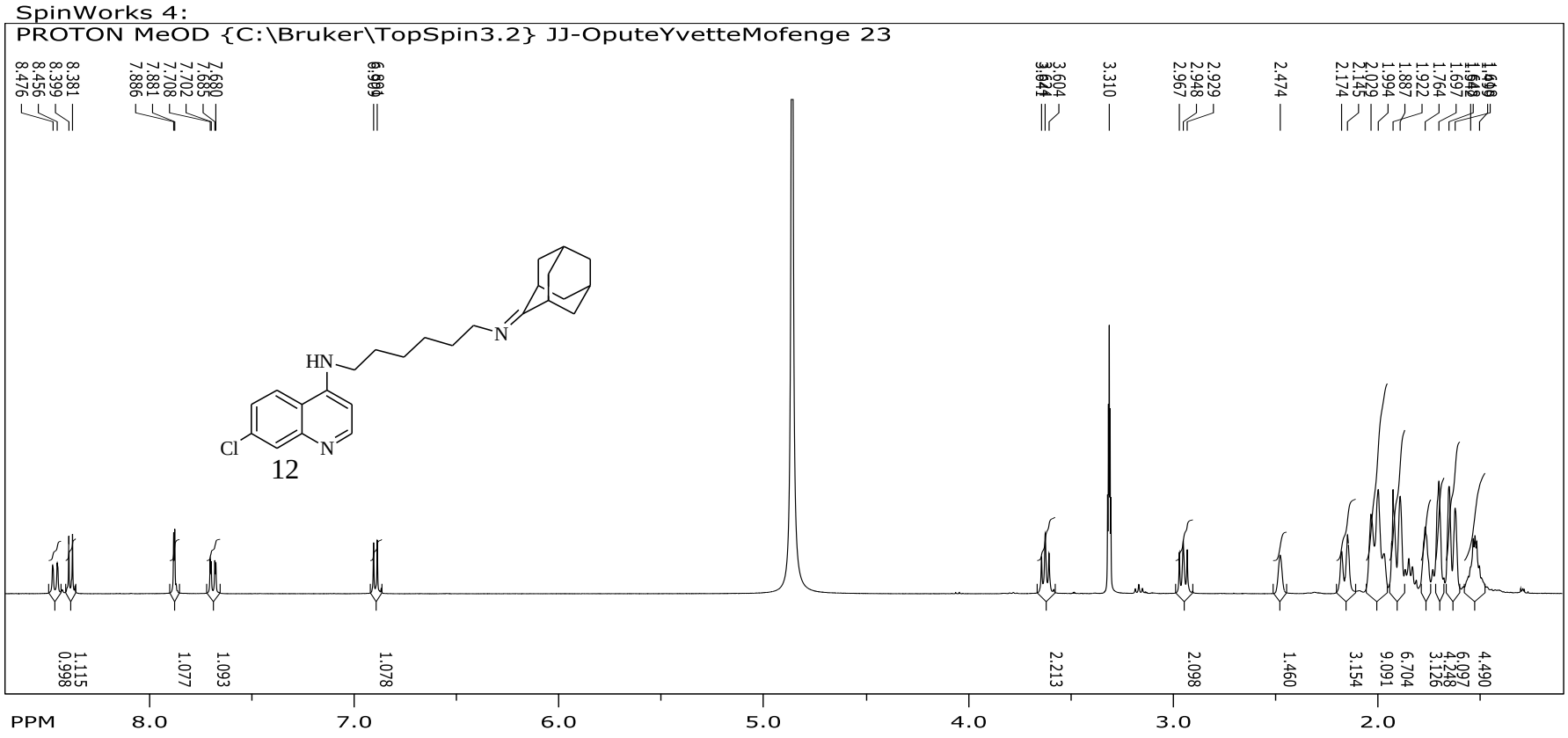
**SPECTRUM 35: Compound 11 MS**



**SPECTRUM 36: Compound 11 IR**



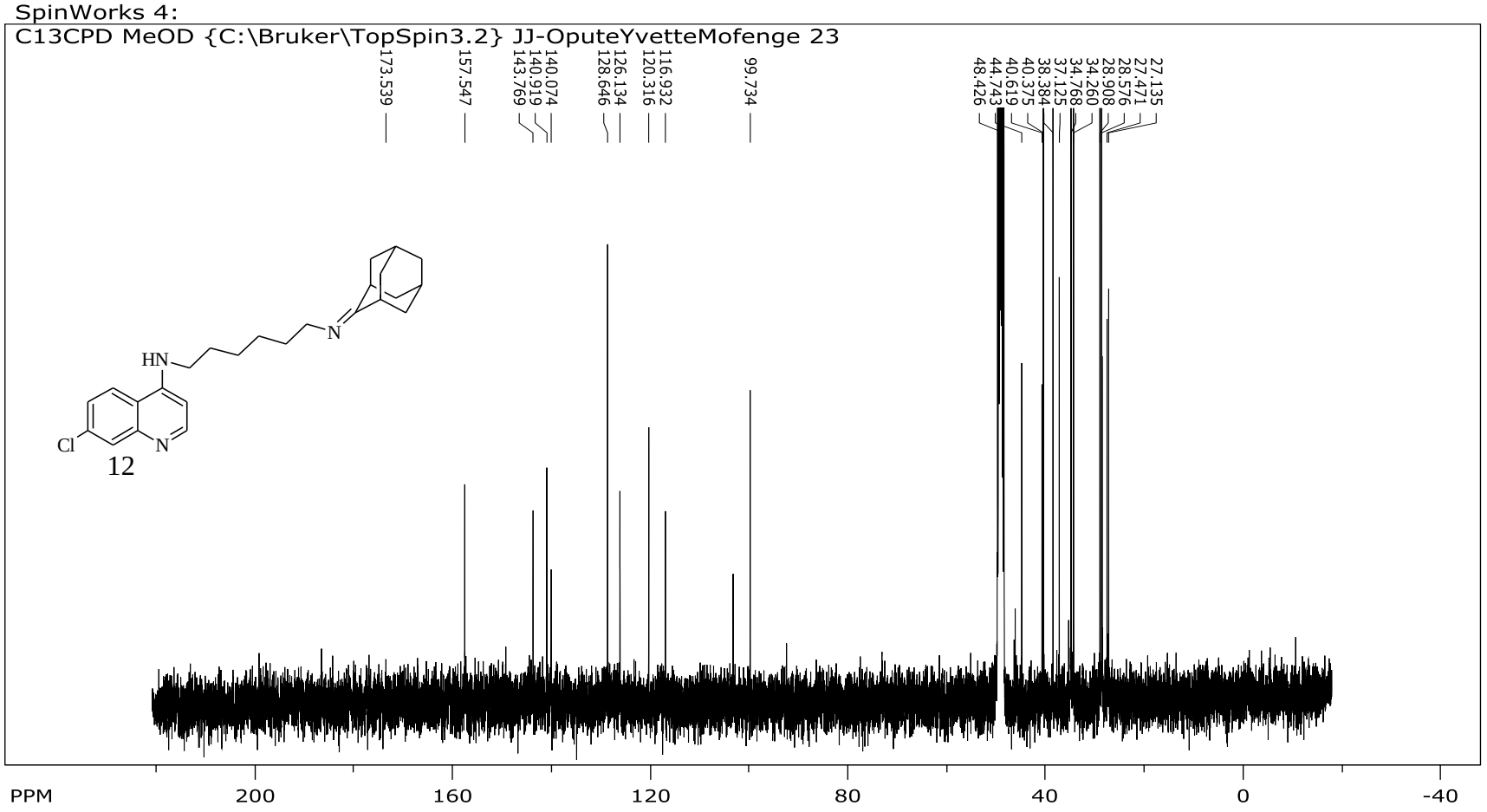
**SPECTRUM 37: Compound 12 1H-NMR**




file: F:\DC-6CA\1\fid expt: <zg30>
transmitter freq.: 400.122471 MHz
time domain size: 65536 points
width: 8012.82 Hz = 20.0259 ppm = 0.122266 Hz/pt
number of scans: 24

freq. of 0 ppm: 400.120008 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000

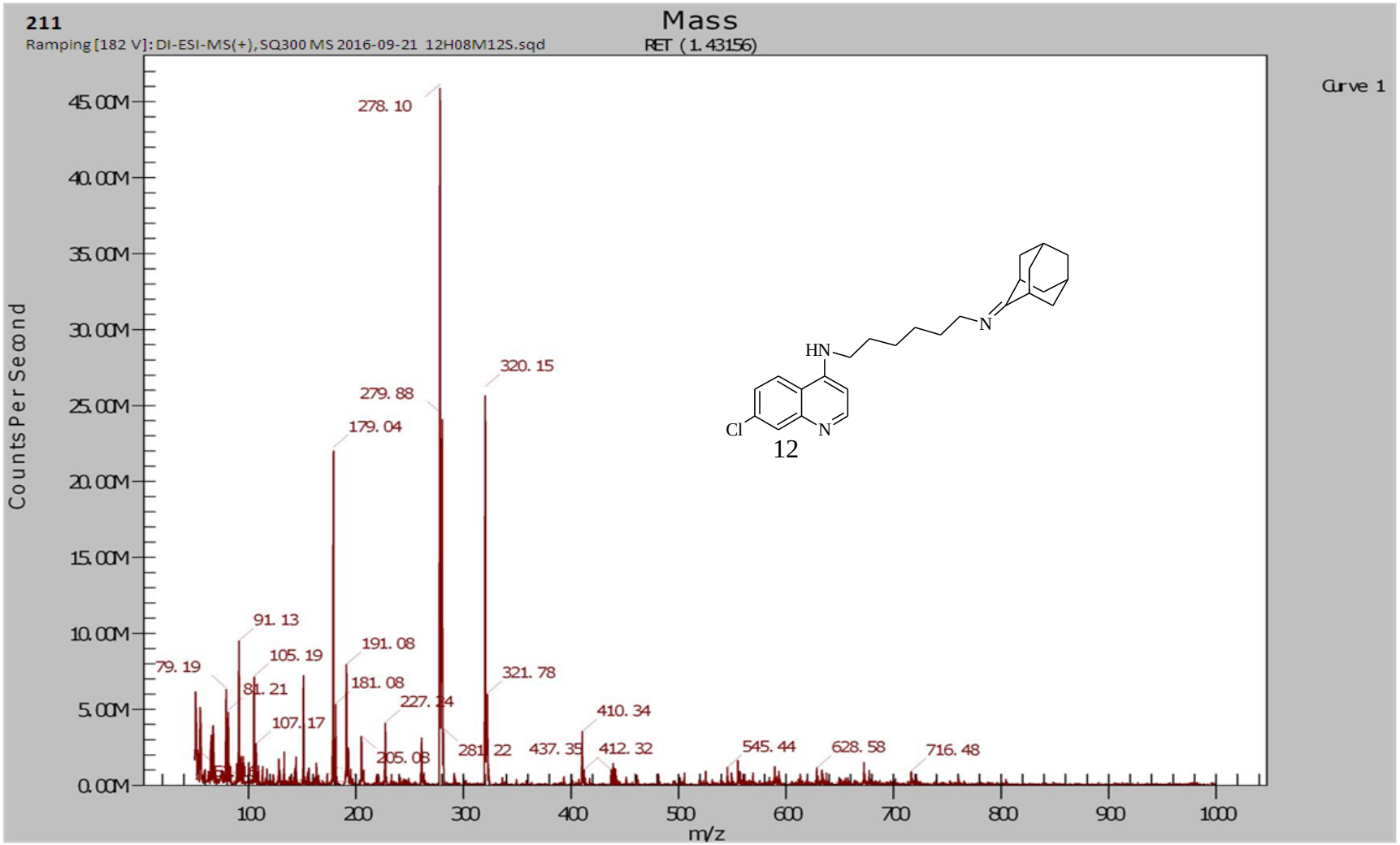
**SPECTRUM 38: Compound 12 13C-NMR**



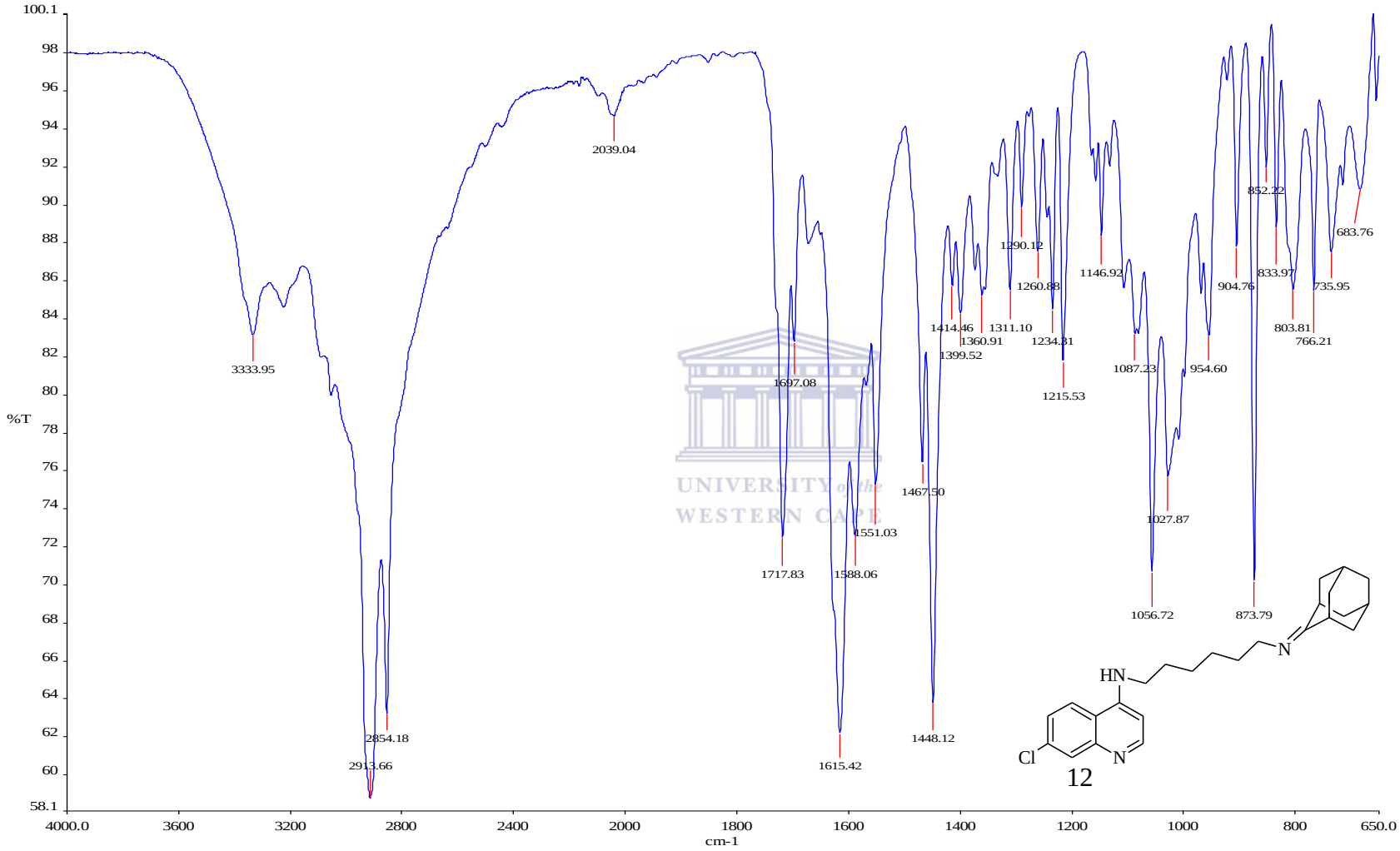

file: F:\DC-6CA\2\fid expt: <zgpg30>
transmitter freq.: 100.620315 MHz
time domain size: 65536 points
width: 24038.46 Hz = 238.9027 ppm = 0.366798 Hz/pt
number of scans: 2048

freq. of 0 ppm: 100.610113 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000

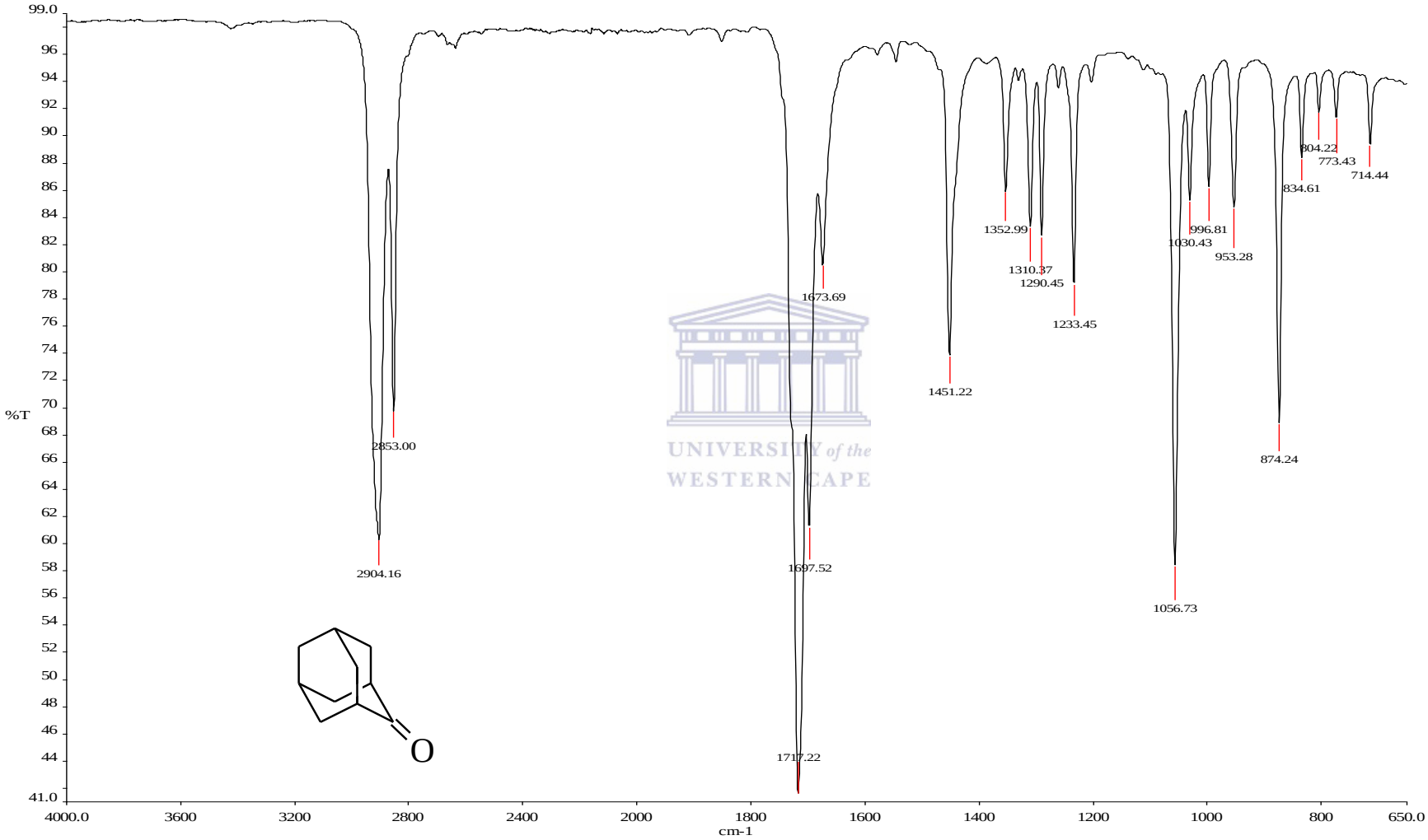
**SPECTRUM 39: Compound 12 MS**



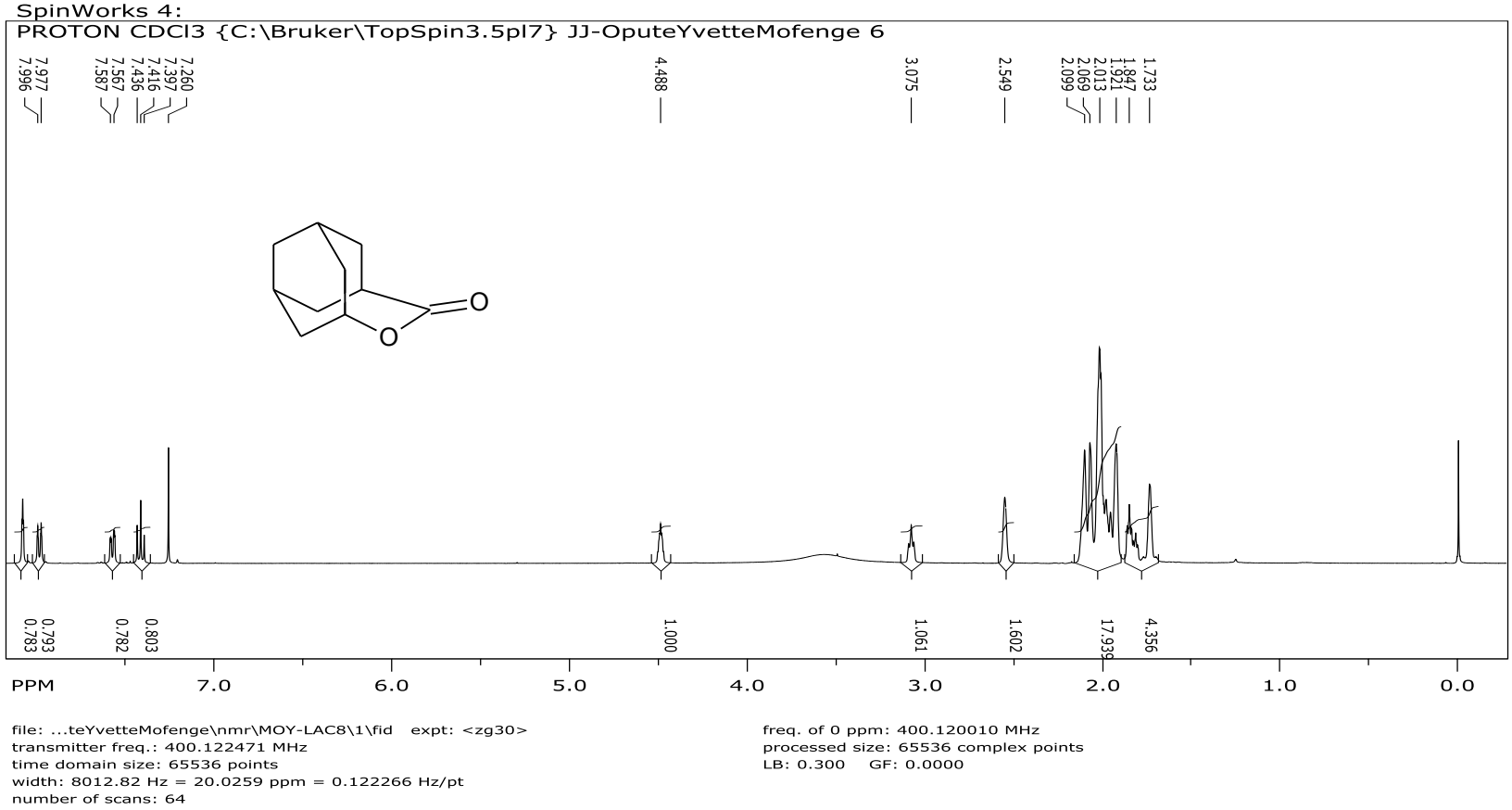
**SPECTRUM 40: Compound 12 IR**



**SPECTRUM 41: ADAMANTANONE IR**



**<u>SPECTRUM 42</u>: Proton NMR for Lactone by MW synthesis**




file: ...teYvetteMofenge\nmr\MOY-LAC8\1\fid expt: <zg30>
transmitter freq.: 400.122471 MHz
time domain size: 65536 points
width: 8012.82 Hz = 20.0259 ppm = 0.122266 Hz/pt
number of scans: 64

freq. of 0 ppm: 400.120010 MHz
processed size: 65536 complex points
LB: 0.300 GF: 0.0000