Synthesis and biological evaluation of hydrophilic derivatives of decoquinate

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Abstract

Malaria is a mosquito borne disease that is caused by a parasitic protozoan, belonging to the genus, *Plasmodium*. Five *Plasmodium* species are known to infect humans, of which *Plasmodium falciparium* is the most virulent. Malaria poses a global health threat, with 40% of the world's population at risk of contracting the disease. In 2015 alone, 216 million people were reportedly infected with malaria, of which 438,000 died from the disease. This renders malaria the third leading cause of deaths, following tuberculosis (TB) and acquired immunodeficiency syndrome (AIDS). Sub-Saharan Africa accounts for 90% of the total malarial burden.

Tuberculosis (TB) is caused by mycobacteria, with *Mycobacterium tuberculosis* (*Mtb*) being the most important. In 2015, 9.6 million cases of TB and 1.4 million related deaths were reported, making this disease the leading cause of human mortalities. Eastern Europe and the South-East Asia regions carry the highest TB burden and account for 58% of the total TB load, while sub-Saharan Africa accounts for 28% of all reported TB cases.

The troublesome fact about these two diseases is the development and spread of pathogenic strains that are resistant towards all drugs currently being used clinically for their treatment. *P. falciparum*, for example, had developed resistance towards prominent anti-malarial drugs, like chloroquine, Fansidar and mefloquine. A *P. falciparum* strain that is resistant to artemisinin combination therapies (ACTs), which are currently used as frontline drugs for the treatment of malaria, has been reported in at least six different regions in Asia. *Mtb* strains that are resistant towards the first line TB treatment regimens, (i.e. rifampicin andisoniazid) and towards the last treatment option for TB (i.e. fluoroquinolones and injectable TB drugs, such as kanamycin, amikacin and capreomycin) have been documented worldwide.

Such growing development and spread of malaria and TB drug resistant pathogens emphasise the urgent need for identifying and developing new drugs that would help curb the spread of these diseases.

Decoquinate (DQ) is a safe and inexpensive drug that has been in use for over 30 years for the treatment of coccidiosis infections in livestock. DQ has also demonstrated potent anti-malarial activity *in vitro* against the liver, asexual blood stages and gametocytic stages of malaria parasites. Interestingly, DQ has a flexible and long alkyl chain, rendering it

highly lipophilic. This characteristic is expected to make DQ easily permeable to lipophilic layers, such as the mycolic acid wall that surrounds the *Mtb*. However, it is in fact its poor solubility that has hampered the development of DQ as a human therapeutic agent. DQ also has a metabolically susceptible ester group, which, if hydrolysed into a carboxylic acid *in vivo*, may reduce the bio-availability of DQ.

During this study, to address the poor solubility of DQ and its undesirable, metabolically susceptible ester, a total of seventy-seven derivatives of DQ were synthesised and evaluated *in vitro* for their anti-malarial activities against chloroquine sensitive (NF54) and multi-drug resistant (W2 and K1) strains of *P. falciparum*, for their anti-tubercular activities against a rifampicin sensitive strain of *Mtb* (H37RV) and for their cytotoxicities against normal human fetal lung fibroblast (Wl-38) cell lines. These results are presented in chapters 4, 5 and 6.

Chapter 4: "Straightforward conversion of decoquinate into inexpensive tractable new quinolones with significant anti-malarial activities". This chapter presents the syntheses, cytotoxicity and anti-malarial evaluations of a series of thirty-five decoquinate derivatives. These compounds were prepared by using either simple aminolysis, during which the ethyl ester in DQ was converted into an amide in the presence of the corresponding amine reagent, and/or through acylation, during which the N-1 nitrogen atom of DQ was converted into an amide, upon reacting it with an acyl chloride. All reactions occurred in a basic medium in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The most active compound among this series was a derivative, bearing an acetyl group attached to N-1 of decoquinate. This compound was found five times more active than decoquinate against the *Pf* NF54 and K1 strains, having an activity profile that was comparable to those of artemether and artesunate against these strains. The selectivity index for this compound was >6494 with respect to normal human fetal lung fibroblast (Wl-38) cell lines, indicating that this compound was not toxic.

Chapter 5: "Syntheses of new decoquinate derivatives with potent anti-mycobacterial activities". This chapter presents the syntheses of twenty-five decoquinate derivatives, their anti-tubercular activities against the H37Rv strain of *Mtb* and their cytotoxicity profiles against the WI-38 cell line. These compounds were prepared, either through *N*-alkylation at N-1 of DQ with an alkyl bromide and aminolysis, or through *N*-acylation and aminolysis. Twenty-three of these compounds showed moderate to good activities against *Mtb*, with the most active compound having an MIC_{99} of 1 μM. This compound, that contains an ethyl group attached to N-1, and an *N*-[2-(2-hydroxyethoxy)ethyl]acetamide group at C-3 of the

quinolone nucleus, had a selectivity index higher than 10, indicative of adequate selectively against *Mtb*.

Chapter 6: "New decoquinate derivatives with improved solubilities and *in vitro* antimalarial activities". This chapter reports on the syntheses and anti-malarial activities of a series of seventeen decoquinate derivatives. The compounds in this series were synthesised in a manner similar to those described in chapters 4 and 5. The biological assessments were conducted, using the same strains and cell lines, as in those previous chapters. The most active compound in this part of the study was found to be the DQ derivative, bearing a sulfonyl containing group attached to N-1 of the quinolone. It possessed a twenty-six-fold higher activity than decoquinate against *Pf* NF54, with an overall activity profile (IC_{50} ~1 nM) that was superior to those of artemether and artesunate, regardless of the *P. falciparum* strain used. This derivative showed no toxicity towards mammalian cells, as evidenced by its high selectivity index (SI) of 71428.

In summary, this study has uncovered a cost-effective anti-tubercular hit, synthesised from the non-active parent drug, decoquinate. Furthermore, this study has also led to the discovery of three new derivatives with superior anti-malarial activities *in vitro*, compared to decoquinate, artemether, artesunate and chloroquine against both chloroquine sensitive and -resistant strains of *P. falciparum*. It is anticipated that these promising compounds may qualify as potential candidates for further investigation in the search for new and effective anti-malarial and anti-tubercular drugs.

Keywords: Decoquinate, anti-malarial, anti-tubercular, decoquinate derivatives, resistance.

Opsomming

Malaria is 'n muskiet-oordraagbare siekte, wat veroorsaak word deur 'n parasiet wat deel uitmaak van die genus, *Plasmodium*. Vyf *Plasmodium*-spesies is daarvoor bekend dat hulle die mens infekteer, waarvan *Plasmodium falciparum* die mees gevaarlikste is. Malaria hou 'n globale gesondheidsbedreiging in, met 40% van die wêreld se bevolking wat die risiko dra om die siekte op te doen. In 2015 alleen, was 216,000,000 mense na bewering met malaria besmet, waarvan 438,000 daaraan gesterf het. Dit maak malaria die derde grootste oorsaak van sterftes, naas tuberkulose (TB) en verworwe immuniteitsgebreksindroom (VIGS). 90% van die totale malaria-las kom in Sub-Sahara-Afrika voor.

Tuberkulose (TB) word deur mikobakterieë veroorsaak, waaronder *Mycobacterium tuberculosis* (MTB) die belangrikste is. In 2015 is 9.6 miljoen gevalle van TB en 1,4 miljoen verwante sterftes aangemeld, wat hierdie siekte die grootste oorsaak van menslike sterftes maak. Oos-Europa en Suid-Oos-Asië dra die hoogste TB-las en maak 58% van die totale aantal gevalle uit, terwyl 28% van alle gerapporteerde TB-gevalle in Sub-Sahara-Afrika voorkom.

Die kommerwekkende feit aangaande hierdie twee siektes is die ontwikkeling en verspreiding van patogene stamme, wat bestand is teen alle middels wat tans klinies gebruik word vir die behandeling daarvan. *P. falciparum*, byvoorbeeld, het weerstand teen prominente teen-malaria-middels, soos chlorokien, Fansidar en meflokien ontwikkel. ‗n *P. falciparum*-stam, wat bestand is teen artemisinien-kombinasie-terapieë (AKTs), wat tans as die voorste linie middels vir die behandeling van malaria gebruik word, is in minstens ses verskillende streke in Asië aangemeld. MTB-stamme, wat bestand is teen die eerste linie TB-behandelingsmiddels (nl. rifampisien en isoniasied) en teen die laaste behandelingsopsies vir TB (nl. fluorokinoloon en inspuitbare TB-middels, soos kanamisien, amikasien en kapreomisien), is wêreldwyd gedokumenteer.

Hierdie stygende ontwikkeling en verspreiding van malaria en TB-middel weerstandige patogene beklemtoon die dringende behoefte aan die identifisering en ontwikkeling van nuwe middels wat sal help om die verspreiding van hierdie siektes te bekamp.

Dekoquinaat (DQ) is 'n veilige en goedkoop middel, wat al vir meer as 30 jaar vir die behandeling van koksidiose-infeksies in vee gebruik word. DQ het ook kragtige *in vitro* teen-

malaria aktiwiteite getoon teen die lewer-, ongeslagtelike bloed- en gametosiet-stadia van malaria-parasiete. Interessant genoeg, het DQ 'n buigsame en lang alkielketting, wat dit hoogs lipofiel maak. Hierdie eienskap behoort DQ volgens verwagting maklik deurlaatbaar te maak vir lipofiele lae, soos die mikoliensuurwand, wat die MTB omring. Dit is egter in werklikheid sy swak oplosbaarheid wat die ontwikkeling van DQ, as 'n menslike terapeutiese agent, tot dusver belemmer het. DQ het ook 'n metabolies vatbare estergroep, wat, indien dit na 'n karboksielsuur *in vivo* gehidroliseer word, sy bio-beskikbaarheid kan verlaag.

Tydens hierdie studie, in 'n poging om die swak oplosbaarheid van DQ en sy ongewenste, metabolies vatbare ester aan te spreek, is 'n totaal van sewe-en-sewentig derivate van DQ gesintetiseer en *in vitro* geëvalueer vir hul teen-malaria aktiwiteite teen chlorokien-sensitiewe (NF54) en multi-middel-weerstandige (W2 en K1) stamme van *P. falciparum*, vir hul anti-tuberkulêre aktiwiteite teen 'n rifampisien-sensitiewe stam van MTB (H37Rv) en vir hul sitotoksisiteit teen normale menslike fetale long fibroblaste (WL-38) sellyne. Hierdie resultate word in hoofstukke 4, 5 en 6 aangebied.

Hoofstuk 4: "Eenvoudige omskakeling van dekoquinaat in goedkoop, plooibare, nuwe kinolone met beduidende teen-malaria aktiwiteite". Hierdie hoofstuk bied die sintese, sitotoksisiteit en teen-malaria-evaluerings van 'n reeks van vyf-en-dertig dekoquinaat afgeleides aan. Hierdie verbindings is berei, deur óf gebruik te maak van eenvoudige aminolise, waartydens die etielester in DQ in die teenwoordigheid van die ooreenstemmende amienreagens in 'n amied omskep is, en/of deur asetilering, waartydens die N-1 stikstofatoom van DQ in 'n amied omskep is, deur dit met 'n asetielchloried te laat reageer. Alle reaksies het in 'n basiese medium plaasgevind in die teenwoordigheid van 1,8-diazabisiklo[5.4.0]undek-7-een (DBU). Die mees aktiewe verbinding in hierdie reeks was 'n afgeleide, wat die draer was van 'n asetielgroep, gekoppel aan N-1 van dekoquinaat. Hierdie verbinding is vyf keer meer aktief as dekoquinaat teen die *Pf* NF54 en K1-stamme bevind, met 'n aktiwiteitsprofiel wat vergelykbaar met daardie van artemeter en artesonaat teen hierdie stamme was. Die selektiwiteitsindeks vir hierdie verbinding was > 6494 met betrekking tot normale menslike fetale long fibroblaste (WL-38) sellyne, wat daarop beduidend was dat hierdie verbindings nie giftig was nie.

Hoofstuk 5: "Sintese van nuwe dekoquinaat-derivate met kragtige anti-mikobakteriële aktiwiteite". Hierdie hoofstuk bied die sintese van vyf-en-twintig dekoquinaat afgeleides, hul anti-tuberkulêre aktiwiteite teen die H37Rv-stam van MTB en hul sitotoksiese profiele teen die WI-38 sellyn aan. Hierdie verbindings is berei, hetsy deur middel van N-alkilering op N-1 van DQ met 'n alkiel-bromied en aminolise, of deur middel van N-

asetilering en aminolise. Drie-en-twintig van hierdie verbindings het matige tot goeie aktiwiteite teen MTB getoon, met die mees aktiewe verbinding wat 'n MIC_{99} van 1 μM opgelewer het. Hierdie verbinding, wat 'n etielgroep, gekoppel aan N-1 bevat het, sowel as 'n N-[2-(2-hidroksie-etoksie)etiel]asetamied-groep op C-3 van die kinolienkern, het 'n selektiwiteitsindeks van hoër as 10 opgelewer, wat beduidend was van voldoende selektiwiteit teen MTB.

Hoofstuk 6: "Nuwe dekoquinaat -derivate met verbeterde oplosbaarhede en *in vitro* teen-malaria aktiwiteite". Hierdie hoofstuk doen verslag oor die sintese en teen-malaria aktiwiteite van 'n reeks van sewentien dekoquinaat afgeleides. Die verbindings in hierdie reeks is op 'n soortgelyke wyse gesintetiseer, as daardie wat in hoofstukke IV en V beskryf word. Die biologiese evaluerings is uitgevoer deur van dieselfde stamme en sellyne gebruik te maak, as in daardie vorige hoofstukke. Die mees aktiewe verbinding in hierdie deel van die studie was _n DQ afgeleide, wat 'n sulfonielbevattende groep, gebonde aan N-1 van die kinoloon, bevat het. Dit het oor 'n ses-en-twintig keer hoër aktiwiteit as dekoquinaat teen *Pf* NF54 beskik, met 'n algehele aktiwiteitsprofiel (IC_{50} ~ 1 nm) wat beter was as dié van artemeter en artesonaat, ongeag die *P. falciparum*-stam wat gebruik is. Hierdie afgeleide het geen toksisiteit teenoor soogdierselle getoon nie, soos uit sy hoë selektiwiteitsindeks van 71428 geblyk het.

Ter opsomming het hierdie studie 'n koste-effektiewe, anti-tuberkulêre treffer ontbloot, wat vanuit die onaktiewe stammiddel, dekoquinaat, gesintetiseer is. Hierdie studie het voorts ook aanleiding gegee tot die ontdekking van drie nuwe derivate met uitstaande teen-malaria aktiwiteite *in vitro*, in vergelyking met dekoquinaat, artemeter, artesonaat en chlorokien teen beide chlorokien-sensitiewe en -weerstandige stamme van *P. falciparum*. Daar word verwag dat hierdie belowende middels as potensiële kandidate mag kwalifiseer vir verdere ondersoek in die soeke na nuwe en doeltreffende teen-malaria en anti-tuberkulêre middels.

***Sleutelwoorde**: dekoquinaat, teen-malaria, anti-tuberkulêre, dekoquinaat -derivate, weerstand.*

Preface

Decoquinate (DQ) is a quinolone based compound that is being used to prevent and treat coccidiosis in the digestive tract of farm animals. It has also been reported to have a wide safety margin, with no adverse effect noticed when given to cavlves at a dose fourty times higher than the recommended dosage.

Studies carried out in 2011 and 2012 reported for the very first time very potent *in vitro* activity of DQ against the liver and gametocyte stages of malarial parasites. Activity against the foregoing stages is very crucial for malarial eradication. However, poor aqueous solubility makes DQ an unsuitable hit against systemic malaria parasites which are mainly located in liver cells and RBCs. This project saw the need to circumvent the poor solubility associated with DQ by synthesizing polar derivatives of DQ. It was also paramount to evaluate these derivatives for anti-TB activity as the present anti-TB quinolone based drugs (FQs) are not safe and are also witnessing increased resistance mediated by efflux pumps.

This dissertation contains seven chapters; Chapter 1 contains the aims and objectives of this dissertation. Chapter 2 makes a summary on the chemotherapeutic agents used to treat malarial. Chapter 3 summarises trends in the development of some anti-malarial quinolone hits. Chapter 4 contains the syntheses, anti-malarial, and cytotoxicity activity of a series of thirty five decoquinate derivatives. Chapter 5 contains the design, syntheses, anti-TB, and cytotoxicity activity of a series of twenty five decoquinate derivatives. Chapter 6 contains the syntheses, anti-malarial, and cytotoxicity activity of a series of fifteen decoquinate derivatives. Chapter 7 contains concluding remark.

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Acronyms and abbreviations

μM	Micro molar	*Pf*ATPase6	*Plasmodium falciparum* adenosine triphosphatase 6
^{13}CNMR	Carbon nuclear magnetic resonance	*pf*CRT	*Plasmodium falciparum* chloroquine resistance transporter
^{1}HNMR	Proton nuclear magnetic resonance	*Pf*mdr1	*Plasmodium falciparum* multi-drug resistant gene
ACT	Artemisnin combination therapy	*Pf*TCTP	*Plasmodium falciparum* translationally controlled tumour protein
AQ	Amodiaquine	PND	Pyronaridine
AVQ	Atovaquone	PQ	Piperaquine
BC	Before Christ	QC	Quinacrine
CDC	Centre for disease control	QN	Quinine
cLogP	Calculated LogP	RBCs	Red blood cells
CQ	Chloroquine	RDTs	Rapid diagnostic tests
DEPT 135	Distortionless enhancement of polarization transfer using a 135 degree decoupler pulse	ROS	Reactive oxygen species

DHA	Dihydroartemisinin	SERCA	Sarco/endoplasmic reticulum Ca^{2+} ATPase
DHFR	Dihydrofolatereductase	TB	Tuberculosis
DHPS	Dihydropteroate synthase	THF	Tetrahydrofolate
DNA	Deoxyribonucleic acid	TQ	Tafenoquine
DQ	Decoquinate	US	United states
DV	Digestive vacuole	WHO	World health organization
G6PD	Glucose-6-phosphate dehydrogenase deficiency		
HRMS	High resolution mass spectrometry		
i.e.	That is		
IC_{50}	50 % inhibitory concentration		
IRS	Indoor residual spraying		
IX	Nine		
LLINs	Long lasting insecticidal nets		
MIC_{99}	99 % minimum inhibitory concentration		
MMV	Medicines for malaria venture		

Mtb	*Mycobacterium tuberculosis*
nM	Nano molar
NMR	Nuclear magnetic resonance
P	*Plasmodium*
pABA	*Para* -aminobenzoic acid

Chapter 1

Introduction and problem statement

1.1. Background

Malarial infection is the third leading cause of deaths worldwide. Over 40% of the world's total population currently inhabit endemic malaria areas, including Sub-Saharan Africa, Asia, and South and Central America. 80% of the total endemic malaria region exist in Sub-Saharan Africa alone, according to the World Health Organization (WHO, 2015). These endemic areas are further divided into chloroquine resistant, chloroquine sensitive and multi-drug resistant zones (Arsic, 2012) (Fig. 1).

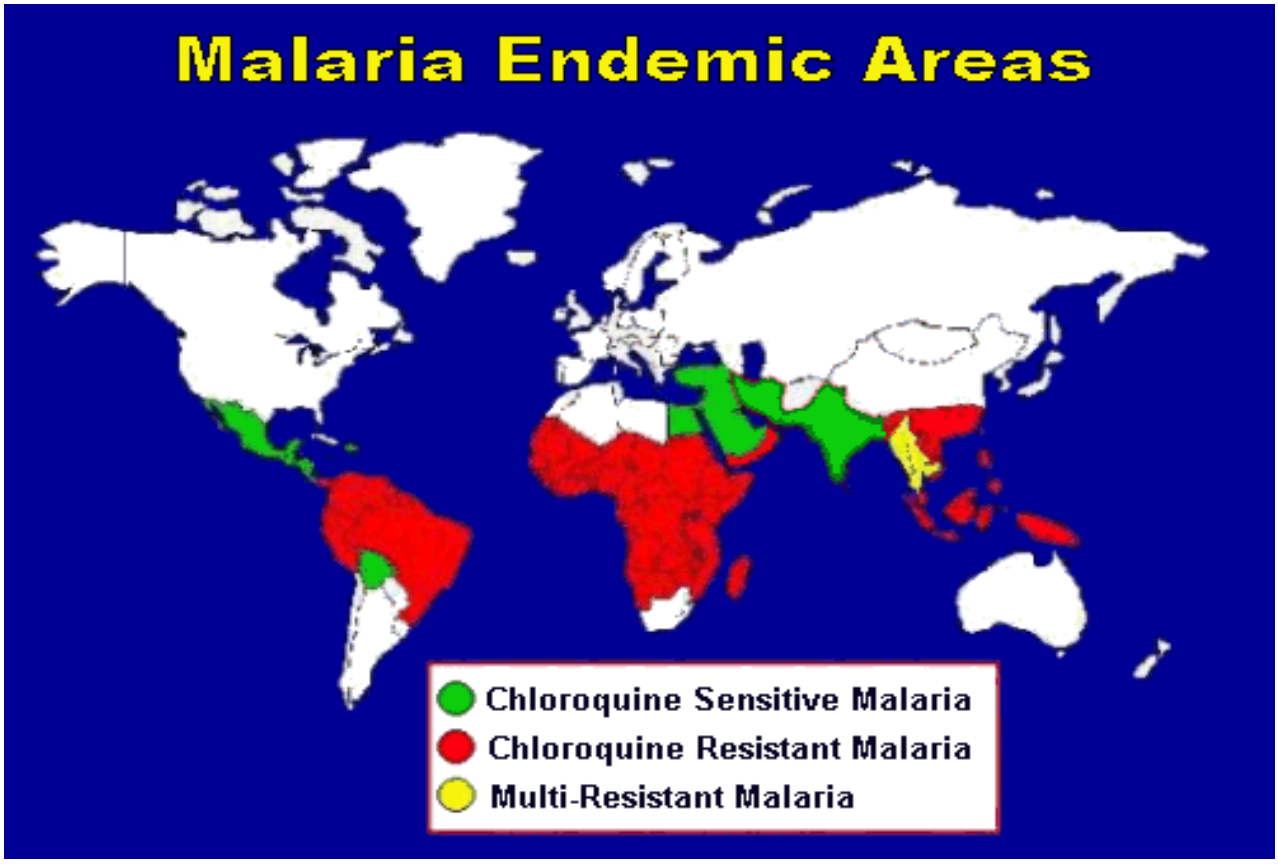


Figure 1: Graphical representation of the current demographics of endemic malaria regions worldwide (Arsic, 2012).

In 2014, 438,000 people reportedly died from the disease (WHO, 2015). Pregnant women and children under the age of five are the most vulnerable to malaria infection (Shetty, 2012), since they either have reduced immunity, or have not yet acquired any immunity against the disease (Roggelin and Cramer, 2014).

Malaria is caused by a parasitic protozoan that invades red blood cells (RBCs). This parasite, which belongs to the genus *Plasmodium* (*P*), is transmitted to humans following the bite of an infected female *Anopheles* mosquito (Kesara and Juntra, 2009). Five species of *Plasmodium*, namely *P. falciparum, P. ovale, P. vivax, P. malariae* and *P. knowlesi* cause malaria in humans. Of these species, *P. falciparum* is responsible for the most severe form of malaria and its prevalence is higher in Sub-Saharan Africa, than in any of the other endemic malaria regions (Guerra *et al*., 2010).

Hitherto, chemotherapy has remained the sole option for malaria treatment (Frevert, 2004). Quinine, an alkaloid that is present in the bark of cinchona trees, had been discovered as the first effective treatment for malaria (Meshnick and Dobson, 2001; Achan *et al*., 2011). Once its structure had been established by Rabe (Rabe, 1907), the syntheses of quinine analogues became the next focus. This has led to the discovery of the quinoline chloroquine (Fig. 2) and related drugs, such as amodiaquine and piperaquine. Other quinolines, bearing a benzylic hydroxyl group, as in the case of quinine, were also prepared, of which mefloquine was the most important (Gelb, 2007). Chloroquine turned out to be the most successful anti-malarial drug, as it was cheap, relatively safe, and remained effective for decades, before the parasite started to develop resistance against it. Structurally quite different drugs, as represented by Fansidar (a combination of sulfadoxine and pyrimethamine), were also introduced, but here also resistance has developed (Ridley, 2002).

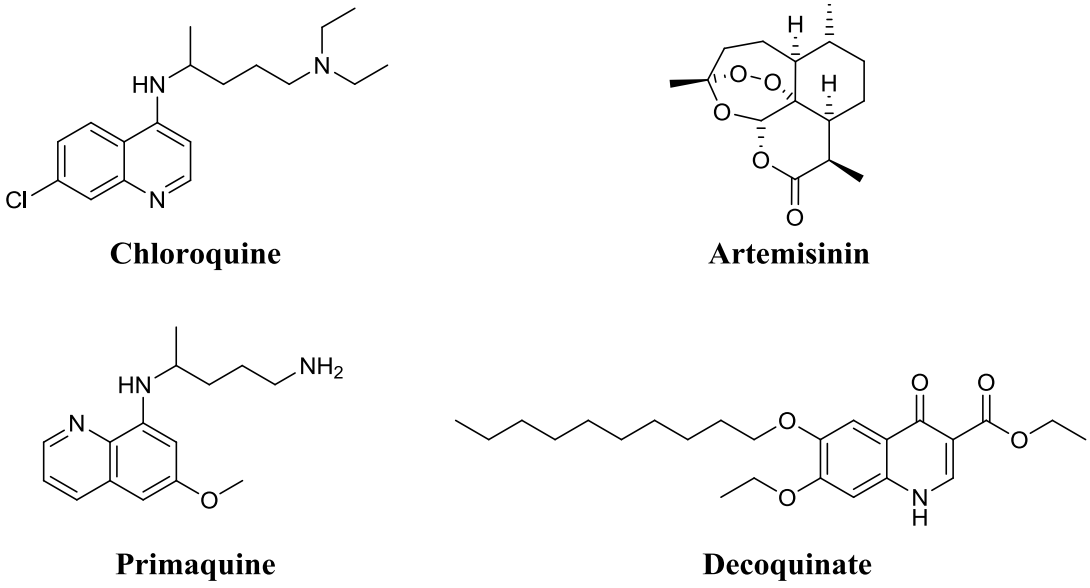


Figure 2: Chemical structures of some anti-malarial drugs in clinical use and the old anti-coccidiosis drug, decoquinate, which was the research focus of this thesis.

Artemisinin (Fig. 2) and its derivatives, referred to as artemisinins, are another important class of anti-malarial drugs. They are fast acting and potent against all resistant strains of the malaria parasite (O'Neill, 2004; Haynes *et al*., 2013). To avoid the artemisinins from suffering the same fate of other anti-malarial drugs, i.e. the development of parasite resistance, the World Health Organization (WHO) recommended the use of these drugs in combination, rather than in monotherapy. This led to the introduction and adoption of artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria worldwide. ACT combines an artemisinin derivative with a longer half-life anti-malarial drug. Despite this measure, resistance to ACTs by the malaria parasite had already been reported in South-East Asia (Phyo *et al*., 2012; Makam *et al*., 2014). Resistance to ACTs has been ascribed to an emerging resistance to artemisinins. Although the mechanism through which artemisinin resistance develops is yet to be established, single nucleotide mutation in the *K13*-propeller gene has been confirmed as one of the key factors (Mok *et al*., 2015).

The anti-malarial drugs that are discussed above are most effective against the blood stage, than any other stage of the malaria parasite's life cycle. The only clinically approved drug that

effectively kills hypnozoites (the liver stage of the *P. vivax* parasite) and that is also active against gametocytes (the transmission stage of the parasite), is primaquine (Fig. 2). However, since primaquine causes fatal haemolysis in patients with glucose-6-phosphate dehydrogenase deficiency, its use has been largely limited (Mazier, 2009).

Overall, resistance and the identified adverse side effects that are associated with currently available anti-malarial drugs, have created a driving force for the repurposing of known drugs, and for the search for new chemical entities that have novel modes of action, that are active against resistant strains and that are readily available. As an existing drug that is readily available, decoquinate (Fig. 2) was recently proven to have potent anti-malarial activities. Decoquinate has been used for a long time as an agent for the treatment of coccidiosis in poultry (Taylor and Bartram, 2012). It has gametocytocidal and schizontocidal activities, with a unique mode of action, involving the cytochrome bc_1 complex as target (da Cruz *et al*., 2012). Decoquinate also has potent activity against hypnozoites, both *in vivo* and *in vitro* (Bonamy *et al*., 2011). Decoquinate therefore meets the requirements of the Medicine for malarial venture (MMV) for a next generation of drugs that are desperately needed for the eradication of malaria (Burrows *et al.*, 2013). The requirements of a suitable drug candidate for malaria eradication are that it should be able to kill gametocytes, hypnozoites and other liver stages, thereby inhibiting transmission and relapse, as well as providing prophylaxes for the disease.

1.2. Aim of the study

Although decoquinate (DQ) shows potent anti-malarial activity, it is very lipophilic, with a calculated Log P (cLogP) value of 8.45 ±1.37 (ACD, 2014). It is also very poorly soluble in water. These qualities severely restrict its use as an anti-malarial drug and as a therapeutic agent for several other systemic infectious diseases. To address the poor solubility of DQ, this project had as its aim the synthesis of more hydrophilic (less lipophilic) derivatives through modification at the C-3 ester and/or at the N-1 of the quinolone ring. It was necessary that these more hydrophilic derivatives retain the anti-malarial activity of DQ against different stages of the malaria parasite, as well as being potent against resistant strains of the parasite. A successful outcome to this project would lead to the potential availability of an affordable and efficacious anti-malarial drug candidate.

In light of the above considerations, this study focused on the synthesis, characterisation and *in vitro* evaluation of the anti-malarial activities and cytotoxicities of the new, more hydrophilic DQ derivatives.

1.3. Primary objectives

The primary objectives of this study were:

- The synthesis of new, more hydrophilic derivatives of decoquinate.
- The characterisation of all intermediates by means of infrared (IR), mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.
- The determination of the *in vitro* anti-malarial and cytotoxicity activities of the newly synthesised decoquinate derivatives.

1.4. Secondary objective

The secondary objective of this study was:

- The determination of the *in vitro* activities of the decoquinate derivatives against *Mycobacterium tuberculosis*, the principal causative pathogen of tuberculosis.

Chapter 2
Literature review

This chapter summarises the historic discoveries that had led to our current understanding of the malaria patho-physiology and of the life cycle of the malarial parasite, the signs and symptoms of the disease, the modes of diagnoses, malaria vector control strategies, anti-malarial drugs in clinical usage, as well as a brief history of quinolones and their targets.

2.1. Historic discoveries

Malaria is a disease of antiquity. It had shown high prevalence and had been responsible for infecting humans in different geographical regions, including Egypt, Italy, India and Peru (Cox, 2010). Ancient documentaries, dating back to 400 BC, describe a disease that had evidently been a scourge to the population, with fever and enlarged spleens being mentioned as typical signs and symptoms of the disease. These documentaries also note that the disease had especially infected people living in marshy areas (Cox, 2010), which had evidently favoured the mosquito that we now know as being responsible for transmitting the disease.

Although different names were used in the different regions to refer to this disease, current knowledge of malaria pathogenesis, the vector of the disease and the life cycle of its causative agent, make it clear that such ancient documentaries were all making reference to malaria.

The word, malaria, is believed to originate from the Italian words, *mal aria*, meaning *bad air*. The Italians of the Renaissance believed that miasma evaporating from stagnant marshes had been the cause of malaria. With the discovery of bacteria and their implication in diseases and also with the coming of the germ theory of diseases in 1878 (Capanna, 2006), the idea of miasma from the stagnant wetlands being a possible cause of the diseases, had faded. In light of these new discoveries, people started searching for the possible causal agent(s) of malaria.

Malarial research had intensified, following the discovery of malaria parasites in the blood of malaria patients, by Charles Louis Alphonse Laveran in 1880. From the year 1880 onwards, several other discoveries had helped to clarify the role that the malaria parasite played in the disease and the life cycle of the parasite, as listed below (Cox, 2010; Kakkilaya, 2015):

- The discovery of mosquitos as vector for avian malaria by Ronald Ross in 1897.
- The discovery of gametocytes and sexual reproduction in crows by William MacCallum in 1897.
- The discovery of mosquitos as vector for human malaria by Battista Grassi in 1898.
- The discovery of exo-erythrocytic (liver) stages of malaria parasites in the livers of birds by William MacCallum in 1898.
- The discovery of female *Anopheles* mosquitos as the sole vector for human malaria by Battista Grassi in 1900.
- The discovery of exo-erythrocytic stages of the parasite in the livers of monkeys by Henry Shortt and Cyril Garnham in 1947.
- The discovery of exo-erythrocytic stages of *P. vivax* and *P. falciparum* in the livers of human volunteers by Henry Shortt and Cyril Garnham in 1949.
- The discovery of exo-erythrocytic stages of *P. ovale* in the livers of human volunteers by Henry Shortt and Cyril Garnham in 1954.
- The discovery of exo-erythrocytic stages of *P. malariae* in the livers of chimpanzees by Robert Bray in 1960.
- The discovery of hypnozoites by Wojciech Krotoski in 1982.

2.2. Life cycle of the malaria parasite

Malaria is a disease, caused by protozoa species, belonging to the genus *Plasmodium* and phylum *Apicomplexa*. The disease is transmitted among humans by infected female *Anopheles* mosquitos (Painter *et al*., 2011). Other human diseases being caused by protozoans include trypanosomiasis, leishmaniasis, toxoplasmosis and cryptosporidiosis (Andrews *et al*., 2014).

The protozoan that causes malaria in humans has a very complex life cycle, which involves humans as the host and female *Anopheles* mosquitoes as the vector. The life cycle comprises of three phases, i.e. schizogony, gamogony and sporogony. Gamogony and sporogony occur within the mosquito, while schizogony occurs within humans. In each phase, different stages of the parasite are produced and in each stage, the parasite has a unique structure, shape and specialised proteins. These constant changes in shape, structure and specialised proteins throughout the life cycle pose big challenges to the development of effective vaccines and drugs for use against the parasite (Floren *et al*., 2002).

2.2.1. Schizogony

The malaria parasite life cycle (Fig. 3) starts when an infected female *Anopheles* mosquito bites a human for a blood meal. During feeding, sporozoites, along with saliva are injected into the skin of the victim. Some of the injected sporozoites find their way to nearby blood vessels from where they are transported to liver cells (hepatocytes) hours later. Within the hepatocytes, the pre-erythrocytic schizogony phase begins and progresses as follows: Each of the infective sporozoites develops into an actively feeding form, called a trophozoite. The feeding trophozoite develops into a schizont, which later undergoes multiple fissions to produce ten- to thirty-thousand merozoites. Merozoites are contained in a merosome, which conceals them from Kupffer cells that would otherwise destroy the merozoites. The budding off from the liver and subsequent rupturing of the merosome releases merozoites directly into the blood stream, where they initiate erythrocytic, or RBC schizogony (Baer *et al*., 2007).

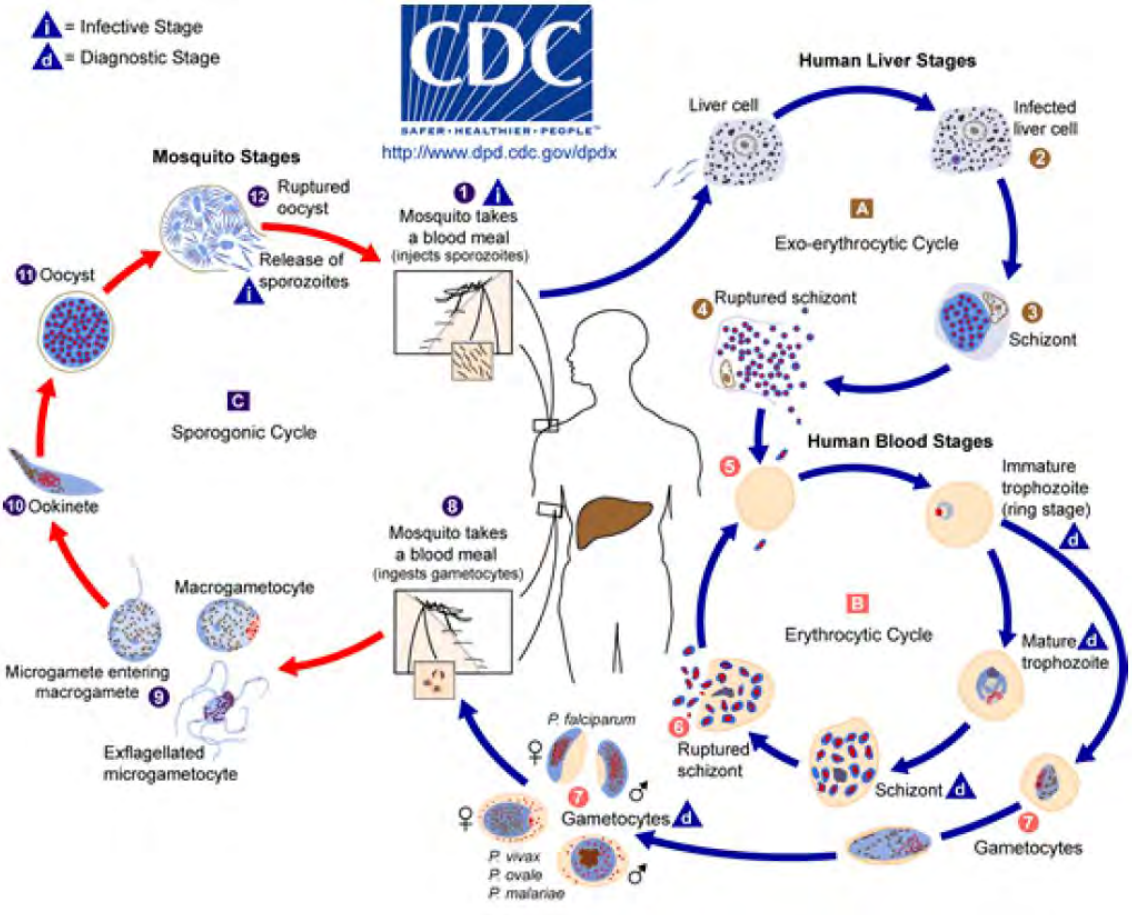


Figure 3: Graphical representation of the life cycle of the malaria parasite (CDC, 2015).

The cycle starts from human liver cells (pre-erythrocytic schizogony) and progresses into the blood (erythrocytic schizogony), and finally into the mosquitoes (gamogony/sporogony). In humans, the parasite only multiples asexually, while it undergoes both sexual and asexual multiplication in the mosquito.

In *P. vivax* and *P. ovale*, some sporozoites remain dormant in the liver for months. These dormant species are called hypnozoites. Hypnozoites later develop into schizonts, which progress as described above, causing relapse of the disease months after being bitten by an infected mosquito (Janneck *et al*., 2011).

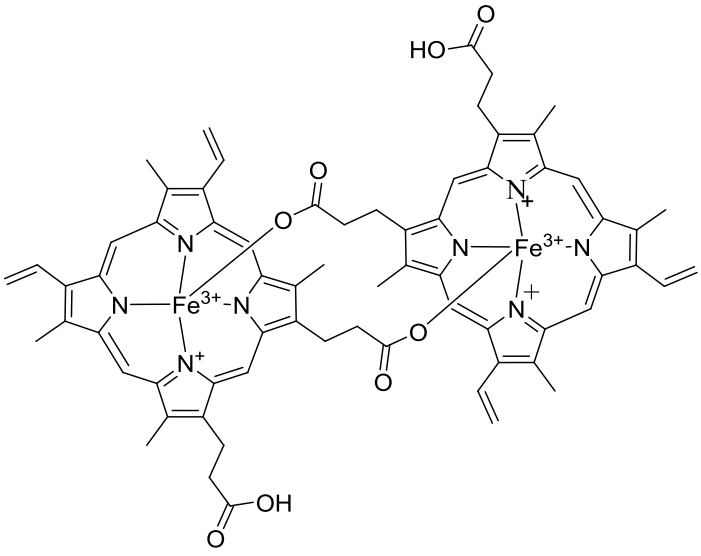
Once released into the blood stream, the merozoites quickly invade red blood cells (RBCs). The invasion is made possible through ligand-receptor interaction, where specific ligands on the surfaces of merozoites interact with specific receptors on the surfaces of RBCs. For example, the Duffy antigen on RBCs acts as a sole receptor for ligands on the merozoites of *P. vivax* (Molina *et al*., 2012), whereas ligands on the merozoites of *P. falciparum* recognise and interact with many receptors on the membranes of RBCs (Mayera *et al*., 2009).

Inside RBCs, each merozoite develops into a ring stage, which catabolises haemoglobin. The ring stage develops into a mature trophozoite, which then develops into a schizont. The schizont undergoes multiple fissions to produce ten to eighteen merozoites, which are released back into the blood stream when the infected RBCs rupture. Some of these merozoites invade new RBCs and start the erythrocytic schizogony anew, while a few develop into micro-gametocytes (male) and macro-gametocytes (female) (Wykes and Horne-Debets, 2012).

The rupturing of infected RBCs occurs at regular intervals and the concomitant fever and other symptoms, typical of malaria, are due to immune response to waste materials resulting from the catabolic activities of the parasites. This interval is 24 hours for *P. knowlesi*, 48 hours for *P. falciparum, P. vivax* and *P. ovale* and 72 hours for *P. malariae* (Greenwood *et al*., 2008).

During erythrocytic schizogony, almost 75% of the RBCs‘ cytoplasmic content, including haemoglobin, is digested to generate free amino acids. Digestion occurs within the parasite‘s food vacuole, where haemoglobin is first cleaved into haem and globin. Globin is digested further to release amino acids needed by the parasite for synthesis of its own proteins. Although free haem (Fe^{2+}- protoporphyrin IX) is toxic to the parasite, it undergoes rapid oxidation into heme-Fe (III) that spontaneously self-associates to generate haemozoin (Fig.4). Haemozoin forms beta-haematin crystals, which precipitate within the food vacuole (Cross, 2010).

As the *P. falciparum* parasite progresses from the ring stage to the schizont, it causes infected RBCs to become spherical and less flexible. It also induces the formation of sticky knobs on the surfaces of infected RBCs (Quadt *et al*., 2012). All of these cause the infected RBCs to adhere to walls of blood vessels, such as capillaries. The adherence to capillaries (sequestration) blocks blood flow to vital organs, such as the brain and kidney, leading to cerebral malaria and kidney failure, as characterised by a coma (Franke-Fayard *et al*., 2010).



Haemozoin

Figure 4: Chemical structure of haemozoin.

2.2.2. Gamogony/Sporogony

During a blood meal by an *Anopheles* mosquito on an infected human, macro-gametocytes (female) and micro-gametocytes (male) are taken up along with blood. Within the mosquito‘s midgut, each micro-gametocyte undergoes three mitotic divisions in a process called exflagellation, to produce a flagellated micro-gamete. At the same time, each macro-gametocyte develops into a macro-gamete. The flagellated micro-gamete swims to and fuses with the macro-gamete to produce a zygote. This fusion is called gamogony (Bogitsh *et al*., 2012). Within 24 hours, the diploid zygote elongates into an ookinete, which penetrates the midgut wall of the mosquito and develops into an oocyst. The oocyst grows in size and develops several encapsulated spiroblasts. Each spiroblast in the oocyst undergoes multiple fissions (sporogony) to produce thousands of sporozoites. The oocyst finally ruptures to release sporozoites, which eventually invade the mosquito‘s salivary gland, ready to be injected into humans, when the mosquito feeds again (Bogitsh *et al*., 2012).

2.3. Signs and symptoms of malaria

Signs and symptoms of malaria occur between 10 - 30 days, following infection. They are due to either inflammatory immune responses, which are triggered by the release of parasitic waste materials and antigens, as mentioned above, or because of anaemia, following the lysis of RBCs. These signs and symptoms, non-specific to malaria, can easily be confused with those of other diseases, such as influenza, or gastro-intestinal infection. They include moderate to severe shaking, chills, high fever, profuse sweating, headaches, nausea, vomiting, diarrhoea, muscle pain, convulsions, coma and bloody stools (Bogitsh *et al*., 2012; Kakkilaya, 2015).

2.4. Malaria diagnosis

Malaria diagnosis involves the identification of signs and symptoms of the disease (clinical diagnosis) and/or identifying the presence of malaria parasites, or parasitic products in blood samples (laboratory diagnosis). As signs and symptoms of malaria are non-specific, clinical diagnosis should always be accompanied by laboratory diagnosis. This is to avoid unnecessary use of anti-malarial drugs and hence delay the onset of drug resistance (WHO, 2010). There are several types of laboratory diagnoses, of which the most routinely used tests are microscopy and rapid diagnostic tests (RDTs) (WHO, 2010).

Microscopy is the gold standard for malaria diagnoses. It involves visualising a thick blood smear to identify parasites and a thin blood smear to differentiate between the parasite species. Besides identification and differentiation, microscopy also determines the parasite load in patients. The disadvantages of microscopy are the need for an energy source and a trained technician (WHO, 2010; Tangpukdee *et al*., 2009).

RDTs rely on the interaction between parasite antigen and parasite anti-bodies. They detect the presence of parasite antigens. Although RDTs give qualitative results, they are simple, fast, do not require a trained technician, and can be deployed in remote settings (WHO, 2010; Tangpukdee *et al*., 2009).

2.5. Malaria vector control

The spread of malaria can be prevented through effective vector control strategies. Malaria vector control aims at avoiding contact between *Anopheles* mosquitos and humans and hence stops malaria transmission. It also aims at reducing the life span of the vector. Among the WHO recommended malaria vector control strategies are indoor residual spraying (IRS) and the use of long lasting insecticidal nets (LLINs) (WHO, 2013).

IRS involves spraying long lasting, residual insecticides on surfaces of walls, ceilings and other potential resting places of the vector, before and after a blood meal. During resting, the vector absorbs the insecticide and eventually dies (WHO, 2013).

LLINs are insecticide-impregnated nets. They have dual working modes, i.e. the nets provide a physical barrier, which prevents mosquitos from reaching humans, while the impregnated insecticide repels, or kills the mosquitos (WHO, 2015).

2.6. Chemotherapy

Although the disease has long been known, the number of chemical agents approved for the treatment and prevention of this disease are relatively few. These agents generally belong to seven chemical classes (chemotypes), as outlined below. Agents belonging to the same chemotype are believed to have the same mode of action (Schlitzer, 2008).

2.6.1. Arylamino alcohols

Drugs belonging to this class include quinine (**1**), mefloquine (**2**), halofantrine (**3**) and lumefantrine (**4**) (Fig. 5). Quinine (**1**) is the first chemical agent used to treat malaria. It was introduced as a pure substance for the treatment of malaria in the early 19th Century (Achan *et al*., 2011). Although it has been used for such a long period of time, quinine is still effective against *P. falciparum* parasites. Widespread use of quinine stopped when chloroquine, an inexpensive and safer analogue of quinine, was introduced (Dinio *et al*., 2012). Quinine, in combination with tetracycline, is often used to treat malaria caused by quinine resistant malaria parasites. This combination is also recommended for cases where the first line treatment fails, or is unavailable (Achan *et al*., 2011). Although quinine causes hypoglycaemia and other serious side effects when administered intra-venously (Noubiap, 2014), it is still recommended for the treatment of severe malaria in high transmission areas (WHO, 2010; Dondorp *et al*., 2010; Kremsner *et al*., 2012).

Mefloquine (**2**) is a racemic mixture of two enantiomers that has a simplified structure, relative to quinine (Schlitzer *et al*., 2008). Before the advent of parasite resistance to mefloquine, it had been used as a prophylactic and a mainstay drug against chloroquine resistant *P. falciparum* (Wisedpanichkij *et al*., 2009). Presently, mefloquine is used in combination with artesunate to treat uncomplicated malaria (Bukirwa and Orton, 2012). Mefloquine use is associated with neurological and psychiatric side effects, such as insomnia and depression (Schlitzer, 2007).

The mechanism of action of arylamino alcohols is still unclear. They are active against the erythrocytic stages of malaria parasites and their most probable mode of action involves inhibition of haemozoin formation, by forming a complex with Fe^{2+}- protoporphyrin IX, which ultimately leads to a build-up of haem in the parasite‘s digestive vacuole. This complex, just like free haem, is toxic to the parasite (Haynes *et al*., 2013).

Resistance to arylamino alcohols is associated with an increased number of *P. falciparum* multi-drug resistant (*Pf*mdr1) genes (Schlitzer, 2008). This gene codes for the P-glycoprotein transporter, *Pf*mdr1, which is located on the membrane of the digestive vacuole (Preechapornkul *et al*., 2009) . How increase in *Pf*mdr1 genes causes resistance, is not yet understood.

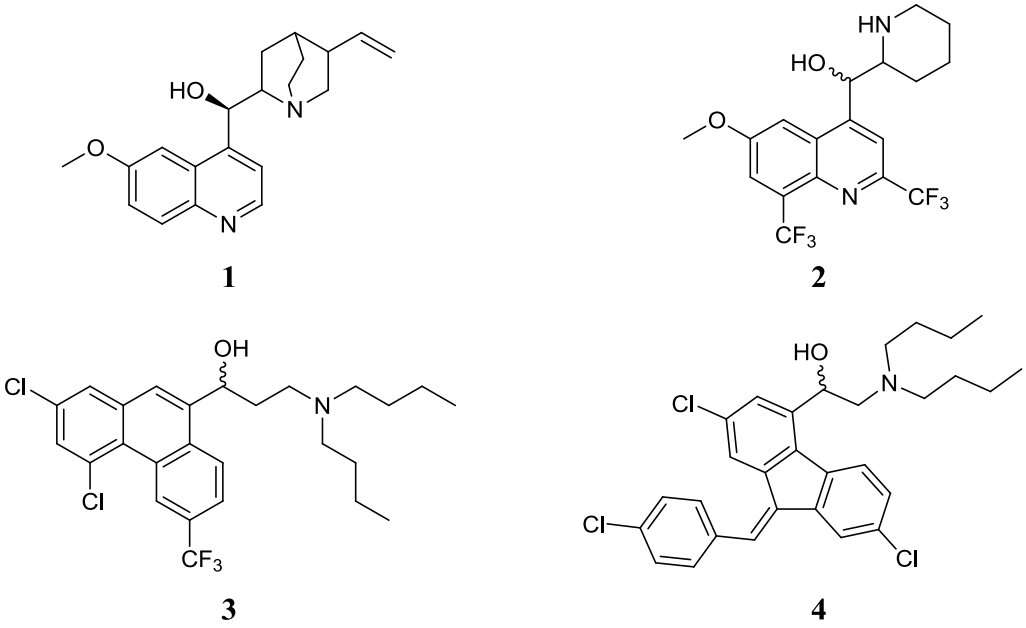
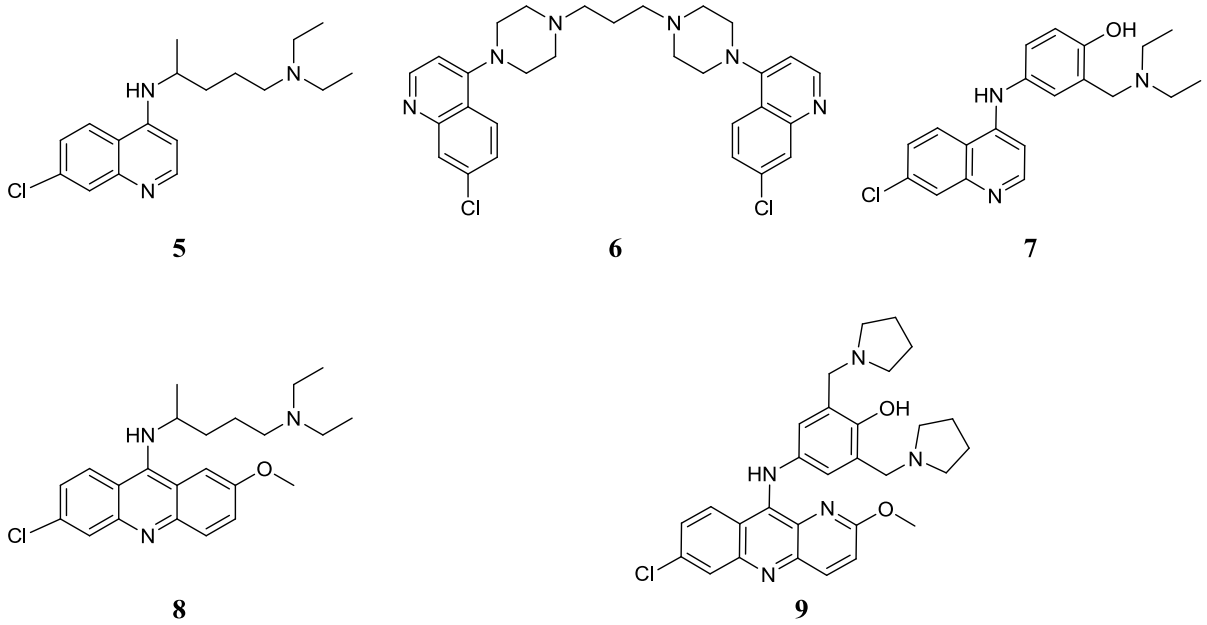


Figure 5: Chemical structures of arylamino alcohol anti-malarials: quinine (**1**), mefloquine (**2**), halofantrine (**3**) and lumefantrine (**4**).

2.6.2. 4-Aminoquinolines

Prominent in this class are chloroquine (**5**), piperaquine (**6**), amodiaquine (**7**), quinacrine (**8**) and pyronaridine (**9**) (Hobbs and Duffy, 2011).



5 **6** **7**

8 **9**

Figure 6: Chemical structures of 4-aminoquinolines: chloroquine (**5**), piperaquine (**6**), amodiaquine (**7**), quinacrine (**8**) and pyronaridine (**9**).

Chloroquine (**5**) is the most successful anti-malarial drug ever used. After its development in the 1930s, chloroquine was the first anti-malarial drug used worldwide for the treatment and prevention of malaria (Jensen and Mehlhorn, 2009). This is because chloroquine was very effective, cheap and easily administered, even in non-clinical settings (Wells and Poll, 2010). Although widespread resistance by *P. falciparum* strains currently exists towards chloroquine (Lehane *et al*., 2012), chloroquine is still effective in treating malaria caused by *P. vivax* and *P. ovale* (Jensen and Mehlhorn, 2009).

A re-introduction of chloroquine in areas where its usage had been stopped for at least 8 years (chloroquine re-cycling), seems to restore its original effectiveness against *P. falciparum* strains. This is believed to be due to the fact that the phenotype of the resistant parasites had changed into a mild, or chloroquine sensitive phenotype (Jensen and Mehlhorn, 2009; Read and Huijben, 2009; Mangera *et al*., 2012).

Piperaquine (**6**) is a bisquinoline, having two 4-aminoquinoline units linked by a bispiperazine-propylene unit. It is equipotent with chloroquine against chloroquine sensitive strains of the malaria parasite and more potent than chloroquine against chloroquine resistant parasites (Kaur *et al*., 2010). Piperaquine activity against chloroquine resistant parasites may be attributed to its larger size, which prevents it from binding to the *P. falciparum* chloroquine resistance transporter (*pf*crt), a protein that pumps chloroquine out of the digestive food vacuole of the parasite (Schlitzer, 2007).

Piperaquine evidently is a safer drug than chloroquine (Davis *et al*., 2005). A combination of piperaquine and dihydro-artemisinin is more effective than artemether-lumefantrine in Africa, while it is better tolerated by malaria patients in Asia, than artesunate-mefloquine (Zani e*t al*., 2014).

Amodiaquine (**7**) is a Mannich base, which is very potent compared to chloroquine (Obua *et al*., 2006). Despite this increased activity, its use as a treatment, or prophylactic for malaria is hampered by its hepatotoxicity and the induction of agranulocytosis (Cairns *et al*., 2010). Amodiaquine is metabolically oxidised into a reactive quinone imine metabolite (Fig. 6), which reacts with glutathione to form a conjugate. This conjugate further reacts with proteins and lipids present in cells, causing the side effects (Cairns *et al*., 2010). Despite the adverse effects associated with amodiaquine when used as a prophylactic, a fixed dose combination of artesunate-amodiaquine as combination therapy is potent and well tolerated (Schramm *et al*., 2013). This combination is adopted in many countries as the first line treatment against uncomplicated *P. falciparum* malaria (Barroso *et al*., 2015).

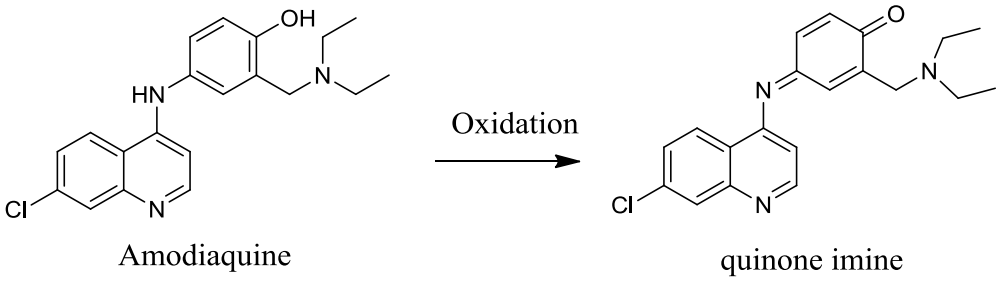


Figure 7: Oxidation of amodiaquine to its toxic quinone imine metabolite.

Quinacrine (**8**) is an acridine derivative, which was widely used to treat malaria during World War II, but was later abandoned due to the fact that it caused bone marrow suppression, psoriasis and skin colouration (Clarke *et al*., 2001). Presently, quinacrine is the second line treatment option for treating malarial patients with retinal toxicity, resulting from the use of chloroquine (González-Sixto *et al*., 2010).

Pyronaridine (**9**) is an aza analogue of quinacrine. Pyronaridine, like amodiaquine, is a Mannich base. Hence, its metabolic oxidation also produces a quinone imine metabolite. However, unlike amodiaquine, the quinone imine from pyronaridine is not reactive towards glutathione. This is due to the steric barrier provided by the two *N*-methylpyrrolidine units located *ortho* to the quinone carbonyl group of the quinone methide of pyronaridine (Biagini *et al*., 2005). Pyronaridine is active against chloroquine resistant *P. falciparum* and *P. vivax* strains in both Asia and Africa (Vivas *et al*., 2008; Price *et al*., 2010). The first report on the phase III clinical studies of fixed dose pyronaridine-artesunate (3:1) suggests that this combination has a longer duration and a more rapid onset of activity against *P. falciparum*, compared to artemether-lumefantrine, the most widely used ACT for treating uncomplicated malaria (Tshefu *et al*., 2010; Croft *et al*., 2012) .

Like many other anti-malarial drugs, several modes of action have been suggested for 4-aminoquinolines. It is worth noting that this class of anti-malarial drugs is very active against the erythrocytic stages of the malaria parasite, during which the parasite is degrading haemoglobin as a source of food. Most researchers believe that the probable mode of action of 4-aminoquinolines involves inhibition of haemozoin formation within the parasite‘s digestive vacuole. They do this by forming a complex with haem (Fe^{2+}- protoporphyrin IX), which ultimately leads to a build-up of haem in the parasite‘s digestive vacuole. This complex, just like free haem, is toxic to the parasite (Schlitzer, 2008).

Resistance has been noted for all 4-aminoquinolines, although the extent thereof varies among the drugs. With respect to chloroquine, the most prominent drug in this class, resistance is associated with changes in *Pf*crt and *Pf*mdr I, both of which are trans-membrane proteins, located in the membrane of the parasite‘s digestive vacuole. Point mutation at amino acid 76, wherein a lysine is replaced by a threonine, seems to cause mutated *Pf*crt to pump chloroquine out of the digestive vacuole (O‘Neill *et al*., 2012). Similarly to the arylamino alcohols, an over expression of *Pf*mdr I gene has also been suggested to cause chloroquine resistance, although this is still controversial (Ibraheem *et al*., 2014).

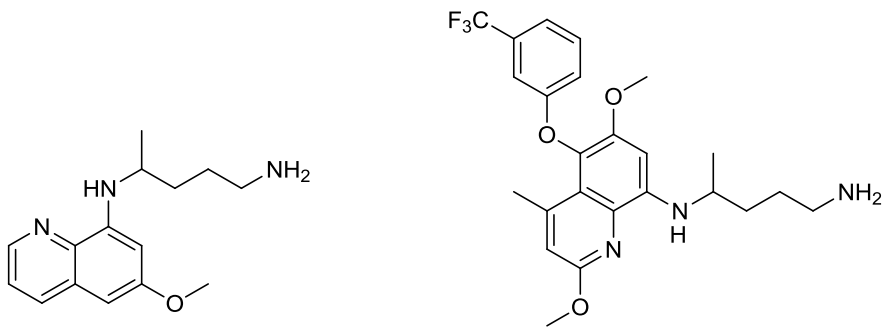
2.6.3. 8-Aminoquinolines

Compounds in this class include pamaquine, primaquine (**10**) and tafenoquine (**11**) (Howes *et al*., 2013) (Fig. 8). These compounds are not very potent against erythrocytic stages of *P. falciparum*, but they are very active against all gametocytic and hypnozoitic stages of *P. vivax* and *P. ovale*. Pamaquine is the first synthetic anti-malarial drug developed for clinical use against *P. falciparum* gametocytes and *P. vivax* hypnozoites. Pamaquine, however, induces haemolytic reactions in patients with glucose-6-phosphate dehydrogenase deficiency, which has limited its use (Sweeney *et al*., 2004; Recht *et al*., 2014).

Primaquine (**10**), the only authorised compound in this class, plays a pivotal role in the radical cure and eradication of malaria, due to its potent activity against gametocytes and hypnozoites (Kondrashin *et al*., 2014). This pamaquine successor is more active against *P. falciparum* gametocytes and *P. vivax* hypnozoites, than pamaquine. Primaquine is less toxic than pamaquine, but it also causes formation of methaemoglobin and induces haemolytic anaemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Recht *et al*., 2014). Due to its short half-life of four hours, primaquine must be taken daily for fourteen days in order to achieve complete cure of malaria caused by *P. vivax* and *P. ovale* (Recht *et al*., 2014).

The shortcomings of primaquine necessitated the search for a new primaquine analogue, with a higher therapeutic index (safety window) and better pharmacokinetic profile. This led to the discovery of tafenoquine (**11**). However, unlike primaquine, tafenoquine is active against the erythrocytic stages of *P. falciparum*, more potent against gametocytes and hypnozoites of *P. vivax* and *P. ovale* and has a longer half-life of fourteen days. Despite the foregoing merits over primaquine, tafenoquine also causes haemolytic anaemia in patients with G6PD deficiency (Recht *et al*., 2014). Tafenoquine is currently undergoing phase III clinical studies as an anti-malarial drug (GSK, 2014).

Little is known about the mode of action of the 8-aminoquinoline class of anti-malarial drugs. However, they are metabolised *in vivo* into hydroxylated and carboxylated metabolites. It is thus assumed that these metabolites are responsible for both their anti-malarial activity, as well as their toxicity. Indeed, hydroxylated metabolites ultimately lead to increased H_2O_2 and methaemoglobin levels and decreased glutathione levels in RBCs, after a series of events. These changes increase oxidative stress, which ultimately kills the parasite (Vennerstrom *et al*., 1999; Vangapandu *et al*., 2007).



10 **11**

Figure 8: Chemical structures of 8-aminoquinoline anti-malarials: primaquine (**10**) and tafenoquine (**11**).

2.6.4. Anti-folates

Anti-folates, also known as anti-metabolites, are compounds that inhibit certain key enzymes along folate bio-synthetic pathway, thereby stopping *de novo* folate synthesis and/or blocking folate salvage pathways. Folate, a co-factor, comprising of pteridine, *para*-aminobenzoic acid (pABA) and L-glutamic acid, acts as a one carbon donor in the bio-synthesis of amino acids and in the methylation of nucleic acid (Wells *et al*., 2009). Nucleic acid methylation and amino acid synthesis are essential for cell growth and differentiation (Salcedo-Sora and Ward, 2013). Anti-folates that are clinically deployed for the control and treatment of malaria are broadly classified into type I and type II anti-folates (Nzila, 2006).

Type I anti-folates include dapsone (**12**), sulphadoxine (**13**) and sulfalene. They inhibit dihydropteroate synthase (DHPS), an enzyme that catalyses the synthesis of dihydropteroate. Dihydropteroate is required for the synthesis of dihydrofolate (Nzila, 2006). Type II anti-folates include pyrimethamine (**14**), proguanil (**15**), chlorproguanil (**16**) and cycloguanil (**17**). Cycloguanil is the pro-drug of proguanil and chlorproguanil. Type II anti-folates inhibit dihydrofolatereductase (DHFR), an enzyme that converts dihydrofolate (DHF) and folates from exogenous sources into tetrahydrofolate (THF) (Nzila, 2006).

Anti-folates (Fig. 9) have never been very effective when used in mono-therapy, as the malaria parasites had easily developed resistance towards them (Peters, 1987). The most successful anti-folate to date in malaria chemotherapy has been Fansidar, a combination of sulphadoxine and pyrimethamine. Fansidar has been used instead of chloroquine in some parts of the world as a first line treatment for uncomplicated malaria. Where resistance to Fansidar has developed, it is used in

combination with artesunate as the first line treatment for uncomplicated malaria (Adeel, 2012). Other anti-folate drug combinations include dapsone-chlorproguanil (Lapdap) and cycloguanil-atovaquone (Malarone). Malarone is mainly used as a prophylactic (Hawkins *et al*., 2007). Lapdap was introduced in the late 1990s as an effective, low cost regiment for the treatment of uncomplicated malaria, following an increased resistance to Fansidar. It was, however, withdrawn in 2008, as a result of reports of haemolytic anaemia in patients with G6PD deficiency (Luzzatto, 2010).

Type I anti-folates work by competing with *para*-aminobenzoic acid for the active site of DHPS enzyme, while type II anti-folates compete with dihydrofolate for the active site of DHFR. When type I anti-folate inhibits DHPS, less dihydropteroate is produced, which culminates in less DHF being produced in the pathway. With less DHF, type II anti-folates face little, or no competition for the active site of DHFR. For this reason, their activity against the target increases. The reduced production of DHF, caused by type I anti-folates and the parallel increased activity of type II anti-folates, account for the synergistic action achieved with anti-folate combinations (Nzila, 2006). Resistance to anti-folates is due to several mutations on genes coding for DHPS and DHFR (Khatoon *et al*., 2013).

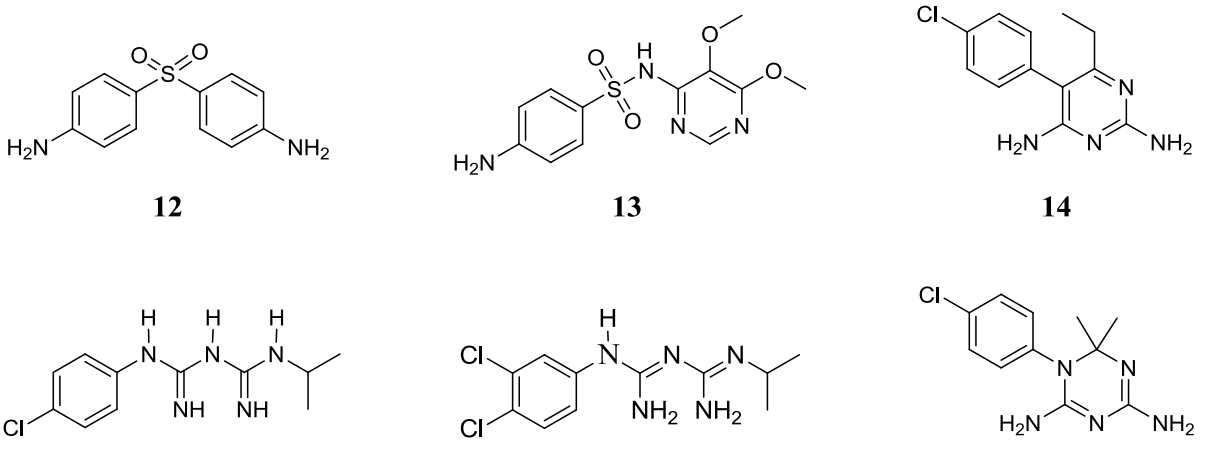
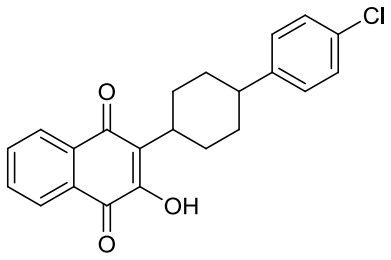


Figure 9: Chemical structures of anti-folate anti-malarial drugs: dapsone (**12**), sulphadoxine (**13**), pyrimethamine (**14**), proguanil (**15**), chlorproguanil (**16**) and cycloguanil (**17**).

2.6.5. Hydroxynapthoquinones

The only anti-malarial drug from this class (Fig. 10) in clinical use is atovaquone (**18**). Atovaquone is not very effective when used as mono-therapy and the malaria parasite easily develops resistance against it (Olliaro, 2001). As a result, the drug is always used in combination with proguanil. This combination is used for casual prophylaxis and treatment of uncomplicated *P. falciparum* malaria (Patel and Kain, 2005; Hawkins *et al*., 2007; Kimura *et al*., 2012).

Atovaquone acts by intercepting electron flow at cytochrome *bc1* complex, an enzyme located along the mitochondrial respiratory chain. The electron interception causes depolarization of parasite‘s mitochondrial membrane potential and the subsequent death of the mitochondrion (Baggish and Hill, 2002; Kessl *et al*., 2007). The demise of the mitochondrion brings an end to pyrimidine bio-synthesis, followed by inhibition of DNA synthesis and replication, which ultimately causes death of the parasite (Hyde, 2002).



18

Figure 10: Chemical structure of atovaquone.

2.6.6. Artemisinins

This class of anti-malarial drugs includes artemisinin (**19**) and its semi-synthetic derivatives, i.e. artemether (**21**), arte-ether (**22**) and artesunate (**23**) (Woodrow *et al*., 2005). Artemisinin, a sesquiterpene lactone, containing an endo-peroxide bridge, is extracted from *Artemisia annua* (qinghao), a plant native to China and to temperate zones of Europe (Liao, 2009). In China, qinghao had been used as a traditional herb for treatment of fevers and chills. However, artemisinin (qinghaosu) and its anti-malarial properties were only discovered later in 1972 as a consequence of the Chinese government policy to screen herbs in search for new anti-malarial drugs (Li, 2012).

To date, *Artemisia annua* remains the only natural source of artemisinin, which is neither soluble in water, nor in oil. To improve the pharmacokinetics properties of artemisinin, semi-

synthetic derivatives that are either oil soluble (artemether (**21**) and arte-ether (**22**)), or water soluble (artesunate (**23**)), have been synthesised (Boareto *et al*., 2012).

Unlike the other anti-malarial drugs, artemisinins (Fig. 11) are fast acting (reducing parasite burden by 10,000-fold within 48 hours), are active against all *Plasmodium* species that infect humans and they are potent even against multi-drug resistant *P. falciparum* strains (Woodrow *et al*., 2005). These properties make artemisinins the most important drugs in the fight against malaria (Shakir *et al*., 2011).

Despite their superior drug properties, the artemisinins have short half-lives of less than three hours, which leads to recrudescence in patients, when used as mono-therapy (Rehman *et al*., 2014). To prevent parasite recrudescence in patients treated with artemisinins and also to slow down the spread of artemisinin resistant parasites, WHO recommended the use of artemisinins in combination with a long life partner drug (WHO, 2006), i.e. artemisinin combination therapy.

Artemisinin combination therapies (ACTs) currently are the most important drugs in the treatment of uncomplicated malaria caused by *P. falciparum*. ACTs combine an artemisinin derivative with a longer half-life anti-malarial drug. The rationale is that the fast acting artemisinin clears a larger proportion of the parasites within its short pharmacological half-life, whilst the longer half-life partner drug then continues the clearance, as the artemisinin concentration falls to sub-therapeutic levels. Examples of ACTs in clinical use include artemether-lumefantrine, amodiaquine-artesunate, artesunate-mefloquine, artesunate-fansidar and artesunate-pyronaridine (Ding *et al*., 2011).

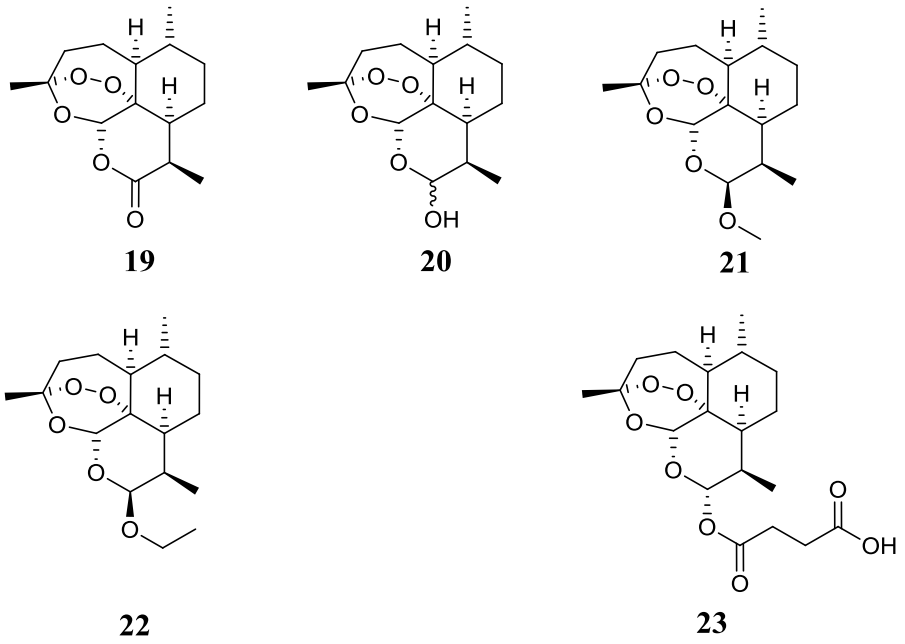
Clinically used semi-synthetic artemisinins are converted by cytochrome p-450, or through hydrolysis in the case of artesunate, into dihydro-artemisinin (DHA) (**20**), which is their active metabolite (Ho *et al*., 2014). The anti-malarial properties of artemisinins result from the presence of the endo-peroxide bridge (Woodrow *et al*., 2005). The mode of action of artemisinins is still controversial, as several modes are proposed in the literature, with no consensus between them. Although the target as such has not yet been definitively identified, several have been proposed to explain the mode of action of artemisinins (O'Neill *et al*., 2010; Ding *et al*., 2011) and they include:

- Translationally controlled tumour protein (*Pf*TCTP): this protein is probably involved in cell growth. It has been suggested that artemisinin radicals alkylate *Pf*TCTP and other proteins of the parasite, thereby interfering with the parasite's growth.

- Sarco/endo-plasmic reticulum Ca^{2+} ATPase (SERCA) enzyme: this mammalian protein regulates intra-cellular calcium levels and it is inhibited by thapsigargin, a sesquiterpene lactone. By analogy, it is assumed that artemisinins being sesquiterpene lactones, work by inhibiting *P. falciparum* adenosine triphosphatase 6 (*Pf*ATPase6), a protein orthologous to SERCA.
- Haem: it has been surmised that heme-Fe^{2+} causes cleavage of the peroxide, leading to the formation of O-radicals, which are re-arranged to form C-radicals. These radicals covalently form adducts with haem, which are toxic to the parasite.

The above proposals have their problems (Haynes *et al*., 2013). It has been demonstrated that artemisinin and its derivatives rapidly accept electrons from reduced flavin co-factors and related electron donors, for example, the reduced conjugates of flavin, i.e. adenine dinucleotide (FAD), flavine mono-nucleotide (FMN), riboflavin and others. They thereby prevent critical flavo-enzymes from donating the electrons that are required for generating reduced glutathione (GSH) and related thiols, which regulate intra-cellular redox homeostasis and other effects. At the same time, the artemisinin is irreversibly reduced into an inactive product. Overall, the artemisinins abruptly interrupt the cellular mechanisms involved in redox homeostasis, an event that results in a rapid build-up of cytototoxic reactive oxygen species (ROS) (Haynes *et al*., 2012).

Although at present, no widespread resistance towards the artemisinins yet exists, as is the case with most anti-malarial drugs, artemisinin resistance had been confirmed in Western Cambodia (where the number of treatment failure is still on the rise) and has now spread or emerged independently in other areas of South-East Asia, including Myanmar, Thailand and Southern Vietnam (Ashley *et al*., 2014). Artemisinin resistance in these areas has been ascribed to mutations in *K13*-propeller gene of *P. falciparum* (Cravoa *et al*., 2015). Although mutations akin to those observed in Asia are yet to be identified in Africa, several polymorphisms in the *K13*-propeller gene have been noted in Africa and are yet to be associated with any form of artemisinin resistance in Africa (Taylor *et al*., 2015).



19 **20** **21**

22 **23**

Figure 11: Chemical structures of artemisinin (**19**) and its semi-synthetic derivatives, dihydro-artemisinin (**20**) artemether (**21**), arte-ether (**22**) and artesunate (**23**). Of these, only dihydro-artemisinin, artemether and artesunate are currently used in clinics.

2.6.7. Antibiotics

Antibiotics that have been used for malaria prophylaxis and treatment include doxycycline (**24**) (Fig. 12), clindamycin and azithromycin. They are effective as mono-therapies against *P. falciparum* parasites, but have a slow onset of action. For this reason, they are usually combined with quinine for the treatment of uncomplicated malaria (Obonyo and Juma, 2012; Andrews *et al*., 2014). The mode of action of antibiotics, for example doxycycline, against the malarial parasite, is still unknown. Doxycycline is the cheapest and safest antibiotic anti-malarial, making it the most clinically used (CDC, 2012).

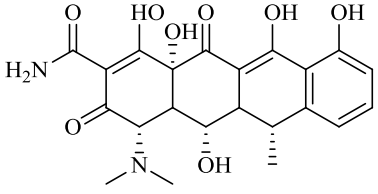


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Figure 12: Chemical structure of doxycycline.

2.6.8. Quinolones

Quinolones are synthetic compounds, containing the 4-oxo-1,4-dihydroquinoline skeleton that may be written as the tautomeric 4-hydroxyquinoline (Fig. 13). They are mostly used as antibiotics. The first anti-bacterial quinolone, nalidixic acid, was discovered as a by-product during the synthesis of chloroquine (Emmerson and Jones, 2003). Since the discovery of nalidixic acid and its anti-bacterial properties against gram negative bacteria, several structural modifications have been made to widen its spectrum of activity to also include gram positive bacteria (Cheng *et al*., 2013).

Besides possessing bactericidal properties, the quinolone scaffold is present in the structures of certain anti-cancer (Rajabalian *et al*., 2007) and anti-viral drugs (Tabarrini *et al*., 2008) and also in anti-oxidants (Greeff *et al*., 2012). The scaffold is also incorporated into compounds that display anti-malarial activity (Winter *et al*., 2008).

Reports on the anti-malarial properties of quinolones compared to reports on their anti-bacterial properties are relatively limited. Recent research involving the evaluation of anti-malarial properties of quinolones, indicates that these compounds demonstrate promising anti-malarial potential. They show very good efficacies, with 50% inhibitory concentration (IC_{50}) values of less than 10 nM, and target more than one stage in the malaria parasite‘s life cycle, including the blood, liver and gametocyte stages. They also seem to have novel modes of action, different from those of most clinically used drugs (Pidathala *et al.*, 2012).

Despite having good anti-malarial properties, there is just one quinolone based anti-malarial drug that has been patented in the US (Aymé *et al*., 2011). The second most advanced quinolone in this therapeutic area, ELQ-300, is undergoing formulation studies, due to its poor solubility in preparations required for clinical trials as an anti-malarial drug (MMV, 2013). A full literature account on the research of quinolones as anti-malarial agents is presented in Chapter 3.

2.6.8.1. Decoquinate

The first carboxyquinolone to show anti-malarial activity was ICI 56 780 (**25**). Since this compound was discovered and later abandoned, little subsequent research has been carried out to either optimise its activity, or to develop new carboxyquinolones with better anti-malarial activity (Winter *et al*., 2008). However, decoquinate (**26**), an old coccidiostat (Taylor and Bartram, 2012), is a carboxyquinolone that has recently shown to possess anti-malarial activity during a high

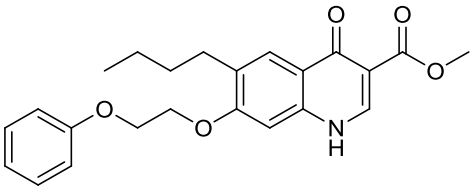
throughput screening programme conducted in search of compounds that are active against liver stage malaria parasites.

Decoquinate, like many carboxyquinolones, is prominent in veterinary medicines. It is useful in the treatment and prevention of protozoal diseases such as coccidiosis in poultry and toxoplasmosis in sheep (Taylor and Bartram, 2012).

Although malaria, like coccidiosis and toxoplasmosis, is caused by a protozoan, little interest had been shown in the past in promoting the use of decoquinate for the treatment and prophylaxis of malaria. Recently, decoquinate garnered more interest following the discovery of its picomolar activities against the liver stages of *P. falciparum* (Nam *et al*., 2011). This promoted more research that culminated in the findings that DQ has nano-molar activities against the blood and early gametocyte stages of *P. falciparum* (da Cruz *et al*., 2012). Besides its potent efficacy against different stages of the malarial parasite, it is also noteworthy that decoquinate is a very cheap compound. Indeed, with all of these attributes, decoquinate seems to have an anti-malarial potential that is superior to that of chloroquine.

DQ likely has low bio-availability. This is because decoquinate, like most hits discovered *via* high throughput screening (HTS) (Keseru and Makara, 2006), suffers from high lipophilicity and poor aqueous solubility (0.06 µg/L in purified water and <0.01 µg/L in buffered water pH 4–9) (Wang *et al*., 2013). This may in part be due to the lipophilic n-decyl chain attached to oxygen at position 6 of the quinolone ring. It also has a metabolically labile ester functional group, which, if hydrolysed into carboxylic acid, might reduce the ability of decoquinate to cross biological membranes.

Thus, this research project is aimed at improving the anti-malarial activity and aqueous solubility of DQ and at replacing the metabolically susceptible ester of DQ through the syntheses of new derivatives, using simple and cost-effective chemical transformations.



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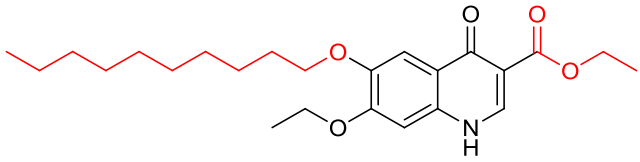

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Figure 13: Chemical structures of promising quinolone anti-malarials: ICI 56 780 (**25**) and decoquinate (**26**).

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Chapter 3

Recent progress in the development of anti-malarial quinolones

This chapter contains a review article published in Malaria journal. It presents anti-malarial quinolone hits and their activities against malaria parasites.

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MALARIA JOURNAL

REVIEW Open Access

Recent progress in the development of anti-malarial quinolones

Richard M Beteck[1], Frans J Smit[2], Richard K Haynes[2] and David D N'Da[2*]

Abstract

Available anti-malarial tools have over the ten-year period prior to 2012 dramatically reduced the number of fatalities due to malaria from one million to less than six-hundred and thirty thousand. Although fewer people now die from malaria, emerging resistance to the first-line anti-malarial drugs, namely artemisinins in combination with quinolines and arylmethanols, necessitates the urgent development of new anti-malarial drugs to curb the disease. The quinolones are a promising class of compounds, with some demonstrating potent *in vitro* activity against the malaria parasite. This review summarizes the progress made in the development of potential anti-malarial quinolones since 2008. The efficacy of these compounds against both asexual blood stages and other stages of the malaria parasite, the nature of putative targets, and a comparison of these properties with anti-malarial drugs currently in clinical use, are discussed.

Keywords: Endochin, Quinolone, Decoquinate

Background

Malaria represents a significant global health threat, with 40% of the world's population being at risk of contracting this disease. During 2012, nearly six-hundred and thirty thousand people died from the disease [1], with pregnant women and children under the age of five being the most vulnerable to infection [2]. By far the most (around 90%) deaths occur in sub-tropical and tropical Africa south of the Sahara (representing 564,300 of the total 627,000 deaths reported in 2012) [1], indicative of the endemic proportions that malaria has reached in this region.

Malaria arises from the invasion of red blood cells (RBCs) by a protozoan of the genus, *Plasmodium* [3]. Five species of the *Plasmodium* genus, i.e. *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax, Plasmodium malariae,* and *Plasmodium knowlesi* cause human malaria. Of these species, *P. falciparum* is responsible for the most severe form of malaria [4]. The malaria parasite is transmitted to humans following the bite of an infected female *Anopheles* mosquito. The parasite has a complex life cycle, involving the vector and a vertebrate host. Figure 1 illustrates the malaria parasite life cycle that progresses through three different phases, with each phase comprising its own different stages.

The liver phase (A): following the bite of an infected *Anopheles* mosquito, sporozoites (1) (infectious stage) are introduced into the bloodstream of the victim (host), from where they migrate to the liver. In the liver, each sporozoite develops into a tissue schizont (3). In *P. ovale* and *P. vivax*, the sporozoites develop into hypnozoites (2), the dormant form responsible for the relapse of the disease, months after the initial infection.

The blood phase (B): when the tissue schizont (3) ruptures in the liver, merozoites are released into the bloodstream, where they invade the RBCs. Within the RBCs, each merozoite transforms into a trophozoite (6) and later into a blood schizont (7), which multiplies asexually, giving rise to 16–32 merozoites (4). When the infected RBCs rupture, merozoites are released into the bloodstream to further invade more RBCs and hence continue asexual multiplication. The clinical manifestations of the disease (e.g. fever and chills) appear during this phase. Some of these merozoites develop into gametocytes (8).

The mosquito phase (C): when a mosquito feeds on an infected person, it ingests gametocytes with the blood. The gametocytes undergo asexual reproduction within the mosquito's mid-gut, producing thousands of sporozoites (1),

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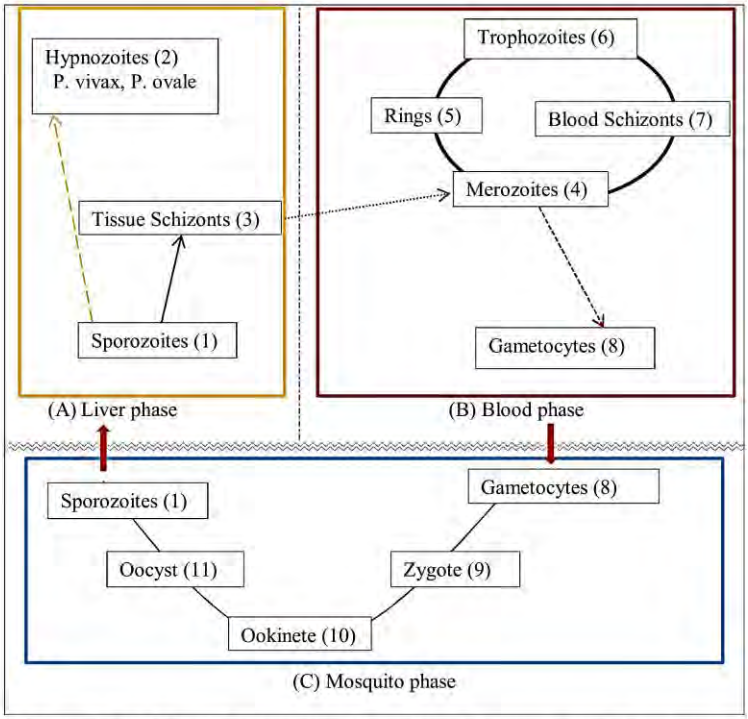


Figure 1 Schematic life cycle of malaria parasite. (A) Liver phase, **(B)** Blood phase, **(C)** Mosquito phase. The cycle progresses from **(A)** to **(B)**, and then to **(C)**.

which then migrate into the salivary glands of the mosquito, from where they are injected into humans during a blood meal.

Hitherto, chemotherapy has remained the sole option for malaria treatment [5]. Quinine, an alkaloid present in the bark of Cinchona trees, was the first effective treatment for malaria [6]. Once the structure of quinine had been established by Rabe *et al.* [7], the syntheses of quinine analogues became the next focus. This led to the discovery of the quinoline chloroquine (CQ) (Figure 1) and related compounds, such as amodiaquine and piperaquine. Other quinolines bearing a benzylic hydroxyl group as in the case of quinine were also prepared, the most important of which was mefloquine [8]. CQ turned out to be the most successful drug: it was cheap, relatively safe and was used for decades, before the parasite developed resistance to the drug. Structurally quite different drugs, as represented by Fansidar (a combination of sulphadoxine and pyrimethamine), have since been introduced, but unfortunately efficacy has also been impeded by the development of resistance [9].

Artemisinin and its derivatives (**2**, **3**–**6**, Figure 2), referred to as the 'artemisinins', are another class of antimalarial drugs that are fast acting and potent against all resistant strains of the malaria parasite [10]. In an attempt to protect the artemisinins against the development of parasite resistance, the World Health Organization (WHO) recommended the use of these drugs in combination with other drugs, rather than in monotherapy. This led to the adoption of artemisinin based combination therapy (ACT) for the treatment of uncomplicated malaria in endemic countries. ACT combines an artemisinin derivative with a longer half-life anti-malarial drug. The rationale is that the fast acting artemisinin clears a larger proportion of the parasites within its short pharmacological half-life, whilst the longer half-life partner drug then continues the clearance as the artemisinin concentration falls to subtherapeutic levels. In spite of this, tolerance to ACTs by the parasite has been reported in South-East Asia [11,12], which is indicative of emerging resistance to the artemisinins. With the recent identification of genetic markers of the resistant phenotype and the

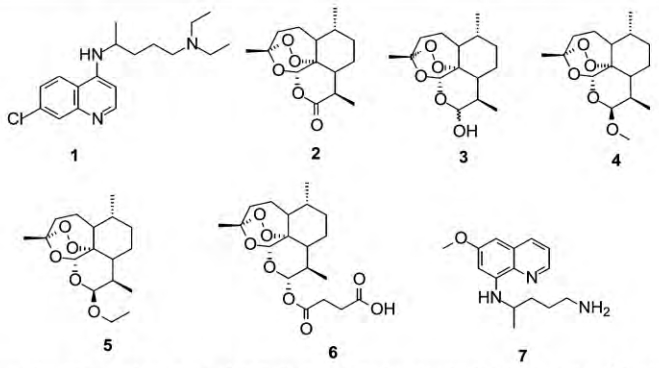


Figure 2 Structures of chloroquine (1), artemisinin (2) and its derivatives: dihydroartemisinin (3), artemether (4), arteether (5), artesunate (6), and primaquine (7).

pinpointing of the rapid spread of this phenotype, the search for new anti-malarial drugs becomes of utmost importance [13].

The anti-malarial drugs discussed above are more effective against the blood stage than any other stage of the malarial parasite. Primaquine is the only clinically proven drug that effectively kills hypnozoites (2) (the liver stage of the *P. vivax* parasite), and is also active against gametocytes (8) (the transmission stage of the parasite). Primaquine, however, causes fatal haemolysis in patients with glucose-6-phosphate dehydrogenase deficiency, an adverse side effect that has significantly limited its use [14].

Overall, resistance and tolerance associated with currently available anti-malarial drugs have created a driving force to the search for new chemical entities having novel modes of action, being readily available and meeting the Medicines for Malaria Venture (MMV) requirements for the next generation drugs needed to eradicate malaria. According to MMV, a suitable drug candidate for malaria eradication should be able to kill gametocytes, hypnozoites and other liver stages, thereby inhibiting transmission, relapse, as well as providing prophylaxis against the disease. Ideally, such a potential candidate should also have a minimum half-life of three days [15], although in practice such a property may be difficult to achieve.

Review

One way of uncovering new compounds involves evaluating highly efficacious compounds in other therapeutic fields. It is apparent that some of these compounds contain the quinolone scaffold and are active against the malaria parasite.

The quinolone scaffold

Quinolones are synthetic compounds containing the 4-oxo-1,4-dihydroquinoline skeleton that may be written as the tautomeric 4-hydroxyquinoline (Figure 3). They are mostly used as antibiotics [16]. The first anti-bacterial quinolone, nalidixic acid, was discovered as a by-product during the synthesis of CQ [17]. Since the discovery of nalidixic acid and its anti-bacterial properties against gram negative bacteria, several structural modifications have been made to widen its spectrum of activity to also include gram positive bacteria [18].

Besides possessing bactericidal properties, the quinolone scaffold is present in the structures of certain

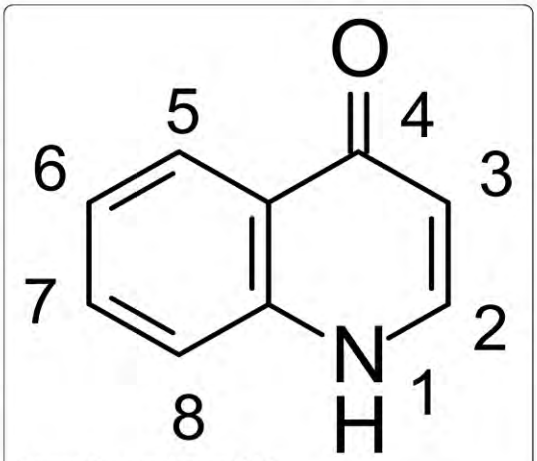


Figure 3 The quinolone scaffold.

anti-cancer [19] and anti-viral drugs [20], and also in anti-oxidants [21]. The scaffold is also incorporated into compounds that display anti-malarial activity [22].

Reports on the anti-malarial properties of quinolones, compared to their anti-bacterial properties, are relatively limited. Recent research involving the evaluation of anti-malarial properties of quinolones, however, indicates that these compounds demonstrate promising potential. They show very good efficacies and target more than one stage of the malaria parasite life cycle, including the blood, liver and gametocyte stages. They also seem to have novel modes of action, different from those of most of the current, clinically used drugs. Thus, in this review are presented details of anti-malarial quinolones documented within the past six years.

Quinolones

Endochin and its analogues

The anti-malarial properties of endochin (**8**, Figure 4) have been known since 1948, when its activity against avian malaria was demonstrated [23]. Further research on this molecule has established that it is active against both the liver (phase A, Figure 1) and blood (phase B, Figure 1) stages of the parasites. It targets the cytochrome bc_1 complex of the parasite [22]. However, endochin has proven to be ineffective *in vivo* against human malaria [24]. Its failure in humans has been ascribed to the fact that endochin is easily metabolized to inactive metabolites in the presence of cytochrome P450 (CYP450) enzymes [25].

Recent advances in understanding of both the drug mechanism of action and the manner in which resistance may arise suggest that previously abandoned lead molecules may in fact be viable anti-malarial drug candidates. This awareness has led to both a re-examination of many abandoned molecules, including quinolone endochin, and the activation of synthetic campaigns, aimed at generating more robust and potent analogues. These activities are exemplified by the development of the following new endochin analogues that have improved therapeutic properties, compared to the parent endochin.

2-Methyl-3-(n-heptyl)-5,7-difluoroquinolone (ELQ-121)

Replacement of the methoxyl group at C-7 by fluorine and the insertion of fluorine at C-5 result in the compound, ELQ-121 (**9**, Figure 4). It has a half-maximal inhibitory concentration (IC_{50}) value of 0.1 nM against the CQ sensitive (D6) and CQ resistant (Dd2) strains of *P. falciparum*. It is stable in the presence of microsomal elements (CYP450). The parent endochin has an IC_{50} value of 4 nM against the D6 and Dd2 strains and is unstable in the presence of CYP450 enzymes [25]. Thus, relative to endochin, the derivative ELQ-121 shows an approximate forty-fold improvement in IC_{50} for the inhibition of *P. falciparum in vitro* and exhibits an enhanced metabolic stability.

2-Methyl-3-(n-heptyl)-6-cloroquinolone (ELQ-130)

Insertion of chlorine at C-6 results in the compound, ELQ-130 (**10**, Figure 4). It has an IC_{50} value of 16 nM against the TM90-C2B strain of *P. falciparum*. Although

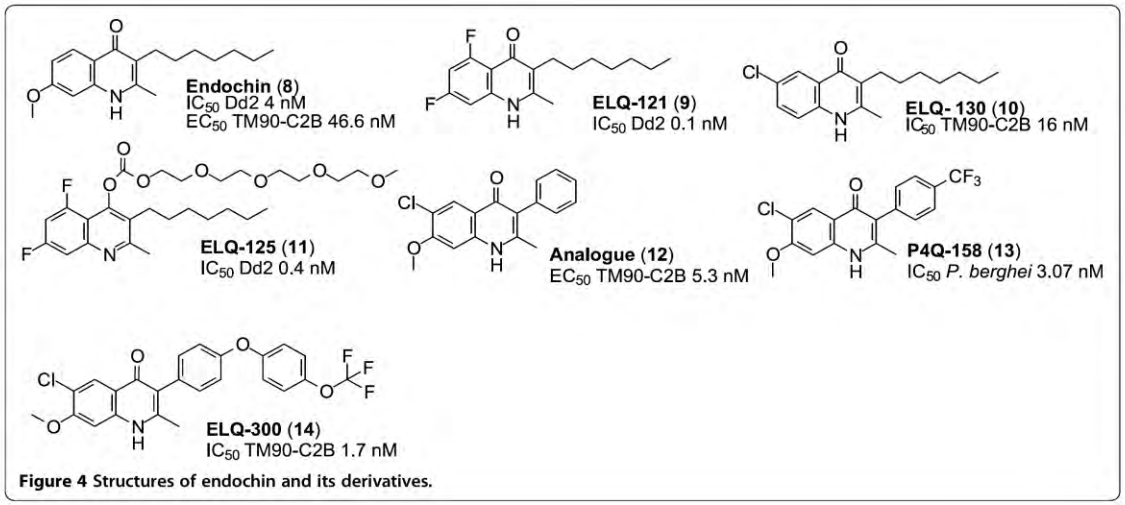


Figure 4 Structures of endochin and its derivatives.

this is a less potent IC_{50} value compared to endochin (IC_{50} value of 11 nM against the same strain), ELQ-130 has enhanced metabolic stability than endochin and is devoid of cross-resistance with atovaquone [25].

4-(2,5,8,11-Tetraoxatridecan-13-yl-carbonate)-2-methyl-3-(n-heptyl)-5,7-difluorquinolone (ELQ-125)

ELQ-125 (**11**, Figure 4) is a polyethylene glycol (PEG) conjugate of ELQ-121. It has, in addition to the two fluorine atoms at positions 5 and 7, a PEG moiety linked to the oxygen of the quinolone nucleus. It has an IC_{50} value of 0.4 nM against the D6 and Dd2 strains and is hence ten-fold (0.4 nM *vs* 4 nM) more active than the parent endochin. ELQ-125 displays higher oral bioavailability than both ELQ-121 and endochin. This property is attributed to the presence of the PEG moiety, which also enhances the aqueous solubility of the compound. As such, ELQ-125 does not have the solubility problem of endochin and its other analogues. ELQ-125 completely removed parasites from the blood stream of mice infected with *Plasmodium yoelii* on the third day of treatment, at a dose of 50 mg/kg/day, whereas endochin showed no activity in infected mice [25].

3-Phenyl-4(1H)-quinolone

This analogue (**12**, Figure 4) has the *n*-heptyl chain at C-3 of endochin replaced by a phenyl ring, whilst a chlorine atom replaces the H atom at C-6. These modifications confer better aqueous solubility and microsomal stability relative to the parent endochin. This compound has an EC_{50} value of 15.3 nM against the TM90-C2B strain of *P. falciparum in vitro*. Compound **12** is therefore more potent than endochin, with an EC_{50} value of 46.6 nM against the same strain. It is important to note that this analogue shows no cross-resistance with atovaquone, contrary to endochin [26]. More importantly, results from *in vivo* studies indicated that the compound completely prevented exflagellation of microgametocytes at a dose of 10 μM, administered for fourteen days post infection. As such, this analogue has the same transmission blocking potential as primaquine [27].

6-Chloro-7-methoxy-2-methyl-3-[4-(trifluoromethyl)phenyl] quinolin-4(1H)-one (P4Q-158)

In P4Q-158 (**13**, Figure 4), the *n*-heptyl chain of endochin is replaced with a trifluorotoluene moiety at C-3, whereas a chlorine atom replaces the H atom at C-6. P4Q-158 has potent anti-malarial activity *in vitro*, with an IC_{50} value of 3.07 nM against the liver stage of the *Plasmodium berghei* parasite. However, its potency is slightly less than that of atovaquone that has an IC_{50} value of 1.42 nM against the liver stage of *P. berghei*. P4Q-158 also demonstrates strong activity against the liver stage of *P. berghei in vivo*, as mice treated with this compound at a dose of 10 mg/kg displayed a more than 60% survival rate, compared to untreated mice [28].

6-Chloro-7-methoxy-2-methyl-3-{4-[4-(trifluoromethyl) phenoxy]phenyl}quinolin-4(1H)-one (ELQ-300)

ELQ-300 (**14**, Figure 4) has a diaryl ether moiety replacing the C-3 *n*-heptyl substituent of endochin. Besides being metabolically more stable than endochin, ELQ-300 exhibits potent *ex vivo* anti-malarial activity against MDR *P. falciparum*, having IC_{50} values of 1.8 nM and 1.7 nM against the W2 and TM90-C2B strains, respectively. The drug is thus substantially more potent than CQ that has IC_{50} values of 126 nM and 96.2 nM against the W2 and TM90-C2B strains respectively.

Furthermore, ELQ-300 shows no cross-resistance with atovaquone, displays a high selectivity for the parasite respiratory bc_1 complex (selectivity index ≥ 20,000), and is very potent against the early and late stages of *P. falciparum* gametocytes. At a concentration of 0.1 μM, ELQ-300 completely stopped further development of stage I and II gametocytes, and it is active against stage IV gametocytes, with an IC_{50} value of 79.1 nM. It also has an ED_{50} value of 0.02 mg/kg/day against murine *P. yoelii*. At a dose of 0.3 mg/kg/day, administered for 30 days post infection, ELQ-300 completely cleared parasites in infected murine models [29]. Presently, ELQ-300 is undergoing formulation studies in preparation for clinical studies [30].

Hydroxy-2-dodecyl-4-(1H)-quinolone (HDQ) and its analogues

HDQ (**15**, Figure 5) is the only compound known to inhibit both the NADH:ubiquinone oxidoreductase enzyme (*Pf*NHD-2) and the bc_1 complex in the respiratory chain of *P. falciparum*. This multi-target inhibition confers a benefit over the single target inhibition in that the former delays the onset of drug resistance [31]. Because of this advantage, HDQ had been used as a starting point for a drug discovery project that has led to several new quinolone analogues with promising anti-malarial activities. Examples of the most effective of these analogues are discussed next.

7-Chloro-3-methyl-2-{4-[4-(trifluoromethoxy)benzyl]phenyl} quinolin-4(1H)-one (CK-2-68)

This HDQ derivative (**16**, Figure 5) bears a [4-(4-trifluoromethoxy)benzyl]phenyl group at C-2 of the quinolone nucleus instead of the metabolically vulnerable *n*-dodecyl unit present in HDQ. This 2-bisaryl quinolone has an IC_{50} of 31 nM against the 3D7 strain of *P. falciparum in vitro*. When given orally at a concentration of 20 mg/kg, CK-2-68 completely cleared *P. berghei* parasites in mice. It is stable in the presence of human

microsomal elements. It inhibits NADH:ubiquinone oxidoreductase of *P. falciparum* with an IC_{50} value of 16 nM. This compares to a value higher than 1,000 nM for atovaquone. CK-2-68 and its phosphate pro-drug (**17**, Figure 5) have activities against *P. falciparum* microgametocytes similar to that of atovaquone (IC_{50} ~ 10 μM) [31-33].

7-Fluoro-3-methyl-2-{6-[4-(trifluoromethoxy)phenyl]pyridin-3-yl}quinolin-4(1H)-one (SL-2-64)

In SL-2-64 (**18**, Figure 5), a 2-[(4-trifluoromethoxy)benzyl)]pyridine moiety replaces the *n*-dodecyl unit at C-2 in HDQ. The pyridine unit in this molecule lowers lipophilicity and increases aqueous solubility relative to CK-2-68. SL-2-64 has activity comparable to that of some clinically used anti-malarial drugs against the 3D7 strain of *P. falciparum*. *In vivo*, this derivative has an ED_{50} value of 3.3 mg/kg against the 3D7 strain in murine models. This activity is similar to that of artemether, which has an ED_{50} value of 3.1 mg/kg against the same strain. This HDQ analogue is listed in the MMV discovery pipeline [31].

Acridinones

Acridinones are tricyclic compounds incorporating the 4-oxo-1,4-dihydroquinolone skeleton, and are thus structurally closely related to quinolones. The anti-malarial activity of a compound containing the acridinone scaffold (**19**, Figure 6) was reported in 1947 [34]. This compound garnered little interest until 1970 when the anti-malarial prophylactic property of floxacrine came (an analogue of acridinone) was discovered [35]. Shortly after floxacrine came, WR 243251 (**20**, Figure 6) with improved therapeutic properties. However, these compounds were not further evaluated, due to their poor aqueous solubility and metabolic instability [36]. Recent studies aimed at re-evaluating the acridinone scaffold through structure–property relationships (SPR) and optimization by using structure-activity relationships (SAR) have led to the discovery of promising anti-malarial analogues.

6-{4-[4-(Trifluoromethyl)phenoxy]phenyl}-1,3,4,4a,9a,10-hexahydroacridin-9(2H)-one (THA 118)

THA 118 (**21**, Figure 6) has a diphenyl ether moiety replacing the phenyl moiety at C-6 in WR 243251. THA 118 possesses more potent anti-malarial activity and has improved metabolic stability and solubility relative to WR 243251. Indeed, THA 118 displays anti-malarial activity *in vitro* with EC_{50} values of 12.2 nM and 9.1 nM against the W2 and TM90-C2B strains of *P. falciparum* respectively, compared to WR 243251, which has EC_{50} values of 25.0 nM and 300.0 nM against W2 and TM90-C2B strains [37].

7-Chloro-3-(2,4-dichlorophenyl)-3,4-dihydroacridine-1,9(2H,10H)-di-one (WR 249685)

This analogue (**22**, Figure 6) possesses potent *in vitro* anti-malarial activity (IC_{50} 15 nM) comparable to that of CQ (IC_{50} 7.4 nM) against the D6 strain of *P. falciparum* [38]. WR 249685 has a higher selectivity index for the parasite bc_1 complex than does atovaquone. This was established by comparing their *in vitro* therapeutic indices (IVTI) for the human bc_1 complex, i.e. IVTI was 4,600 for WR 249685 and 24 for atovaquone. In addition to targeting the parasite bc_1 complex, WR 249685 also binds to haem, although it has a low affinity for haem compared to CQ [39]. Thus, it is unlikely to exert an anti-malarial effect in this manner relative to CQ.

Haloalkoxyacridinones

This is a relatively new class of acridinone [38]. The most active analogues in this class have at C-3 of the acridone ring an alkoxy moiety terminating in one or more trifluoromethyl units. The acridinone (**23**, Figure 6) is an example of such an analogue. It exhibits extraordinarily strong anti-malarial activity *in vitro*, with IC_{50} values of 1 pM against both the D6 and Dd2 strains of *P. falciparum*. With such favourable IC_{50} values, this compound appears to be the most effective anti-malarial ever synthesized and tested in a laboratory. Although its mode of action is still unknown, it is assumed that it

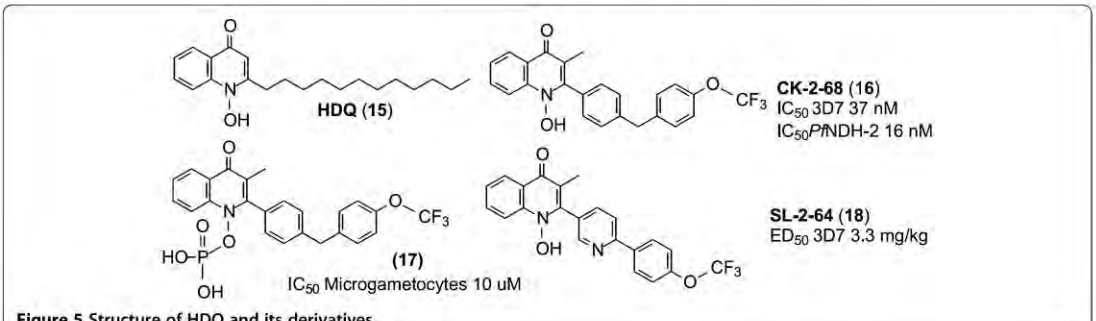


Figure 5 Structure of HDQ and its derivatives.

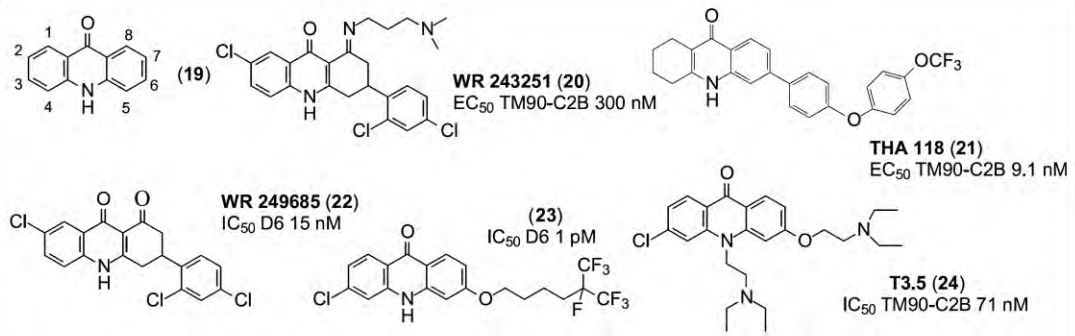


Figure 6 Structure of acridinone and analogues.

binds to the bc_1 complex due to its structural similarity with known anti-malarial drugs targeting that complex. This acridinone analogue has an *in vitro* therapeutic index higher than 100,000 [39] and has been patented in the US as an anti-malarial drug [40].

3-Chloro-6-(2-diethylamino-ethoxy)-10-(2-diethylamino-ethyl)-acridinone (T3.5)

This 10-*N* substituted acridinone analogue (**24**, Figure 6) incorporates two potential anti-malarial features, namely, a haem-targeting unit through the tricyclic acridone core, and a quinoline resistance-reversal unit, due to the *N*-(2-diethylamino-ethyl) unit. T3.5 has *in vitro* IC_{50} values of 77.3 nM and 71.3 nM against the Dd2 and TM90-C2B strains of *P. falciparum,* respectively. In comparison, CQ has IC_{50} values of 124.7 nM and 122.7 nM against the same strains respectively. This acridinone analogue is non-toxic to mammalian cell lines both *in vitro* and *in vivo* and exhibits synergism with each of piperaquine, CQ and amodiaquine against the Dd2 strain [41].

Carboxyquinolones

Since the discovery of the anti-malarial activity of ICI56-780 (**25**, Figure 7) [42], recent research on quinolone anti-malarial drugs has focused on carboxyl derivatives of quinolones. Due to the need for cheap anti-malarial drugs that have novel modes of action, commercially available compounds with antiparasitic activity had been screened for anti-malarial activity. This has led to the discovery of various 3-carboxylquinolones as potential anti-malarial drugs.

Ethyl 2-(1,3-benzodioxol-5-yl)-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (TDR 42098)

This compound demonstrates improved potency against the blood stages of the CQ-R K1 and CQ-S NF54 strains of *P. falciparum*, and has better physicochemical properties, than endochin analogues, bearing a lipophilic side chain [43]. Derivatization of TDR 42098 (**26**, Figure 6) produced the analogue (**27**, Figure 7) bearing a meta-substituted aromatic ring at C-2. This derivative has mid-range EC_{50} values of 0.13 μM and 0.10 μM against the K1 and 3D7 strains of *P. falciparum*, respectively [44].

6-Deoxy-7-ethoxy-4-oxo-1H-quinoline-3-carboxylic acid ethyl ester (decoquinate (DQ))

The anti-malarial activities of this long used anti-coccidial drug were recently discovered during screening conducted against the liver stages of *P. falciparum. In vitro*, decoquinate (DQ) (**28**, Figure 7) is potent against the liver (IC_{50} 2.6 nM), blood (IC_{50} 10 nM), and sexual (IC_{50} 36 nM) stages of the malaria parasite. It reportedly

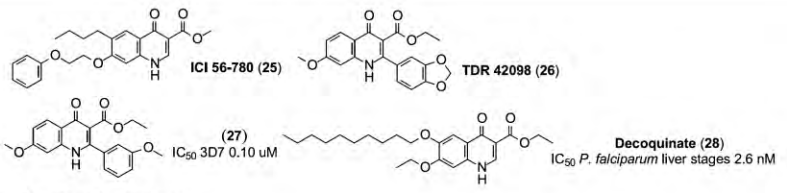


Figure 7 Structures of carboxylquinolones.

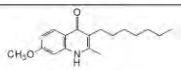
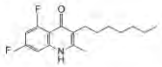
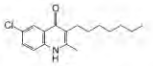
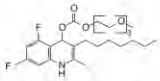
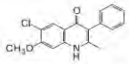
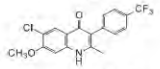
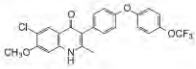
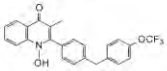
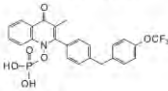
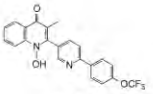
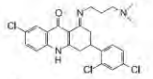
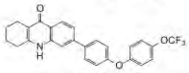
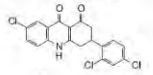
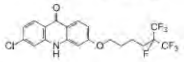
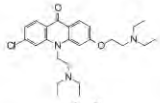
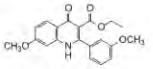
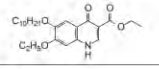
Structure		IC_{50} (nM)	EC_{50} (nM)	ED_{50} (mg/kg)	Target	Ref.
	**8**	4	-	-	Dd2	[25]
	**9**	0.1	-	-	Dd2	[25]
	**10**	16	-	-	TM90-C2B	[25,29b]
	**11**	0.4	-	-	Dd2	[25]
	**12**	-	5.3	-	TM90-C2B	[26,27]
	**13**	3.07	-	-	*P. berghei*	[28]
	**14**	1.7	-	-	TM90-C2B	[29]
	**16**	16 37	-	-	*Pf*NDH-2 3D7	[31-33]
	**17**	10,000	-	-	Microgametocytes of *P. falciparum*	[31-33]
	**18**	-	-	3.3	3D7	[31]
	**20**	-	300	-	TM90-C2B	[36]
	**21**	-	9.1	-	TM90-C2B	[37]
	**22**	15	-	-	D6	[38,39]
	**23**	0.001	-	-	D6	[38-40]
	**24**	71	-	-	TM90-C2B	[41]
	**27**	10,000	-	-	3D7	[44]
	**28**	2.6	-	-	*P. falciparum* liver stages	[45,46]

Figure 8 All structures, and their potencies.

targets the bc_1 complex of *P. falciparum* and shows no cross-resistance with atovaquone [45,46].

According to the results of the above studies, it is apparent that DQ meets the MMV requirements for the next generation drugs needed for malaria eradication [15]. Despite demonstrating good anti-malarial activity, DQ has the major liability of poor aqueous solubility. It also undergoes facile metabolism to the free carboxylic acid. However, DQ is a very cheap compound and is readily available. Thus, it is apparent that new derivatives of DQ can be prepared through reducing the lipophilicity, enhancing both the solubility and metabolic stability by replacing the ester group by appropriate amide groups.

The important features of each compound are summarized in Figure 8.

Conclusion

The quinolone nucleus is a chemotype common to classes of chemotherapeutic agents including antibiotic, anti-viral and anti-cancer drugs. More recent research on the anti-malarial activities of quinolones indicates that such compounds are relatively potent against the blood, liver and transmission stages of the malarial parasite and act on one or more targets of the parasite. These findings overall indicate the importance of the quinolone nucleus in the development of drugs aimed at eradicating malaria. Nevertheless, more research is required in order to address the specific difficulties associated with quinolone lead compounds, including those of relatively poor aqueous solubility and metabolic instability.

Abbreviations

WHO: World Health Organization; MMV: Medicines for Malaria Venture; RBC: Red blood cell; CQ: Chloroquine; ACT: Artemisinin combination therapy; IC_{50}: Half maximal inhibitory concentration; ED_{50}: Half maximal effective dose; EC_{50}: Half maximal effective concentration; HDQ: Hydroxy-2-dodecyl-4-(1H)-quinolone; DQ: Decoquinate; SPR: Structure property relationship; SAR: Structure activity relationship; IVTI: *In vitro* therapeutic index; nM: Nano-molar; μM: Micro-molar; USPTO: United States Patent and Trademark Office.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RMB drafted this manuscript, and FJS, DDN and RKH critically revised it. All authors read and approved the final manuscript.

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Chapter 4

Straightforward conversion of decoquinate into inexpensive tractable new derivatives with significant antimalarial activities.

This chapter contains a research article published in Bioorganic and Medicinal Chemistry Letters. It presents syntheses and anti-malarial activities of a series of decoquinate derivatives.

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Straightforward conversion of decoquinate into inexpensive tractable new derivatives with significant antimalarial activities

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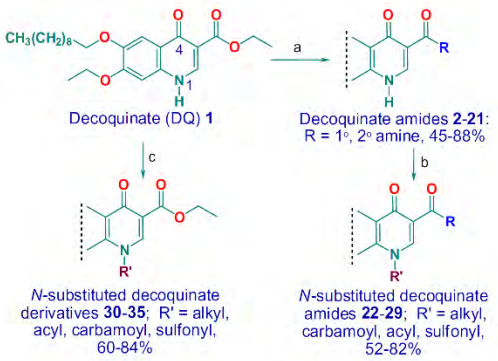
ABSTRACT

As part of a programme aimed at identifying rational new triple drug combinations for treatment of malaria, tuberculosis and toxoplasmosis, we have selected quinolones as one component, given that selected examples exhibit exceptionally good activities against the causative pathogens of the foregoing diseases. The quinolone decoquinate (DQ), an old and inexpensive coccidiostat, displays anti-malarial activity in vitro against *Plasmodium falciparum* (*Pf*). However, because of its exceedingly poor solubility in water or organic solvents, development of DQ as a drug is problematical. We have therefore converted DQ in straightforward fashion into tractable new derivatives that display good activities in vitro against chloroquine-sensitive NF54 and multidrug-resistant K1 and W2 *Pf*, and relatively low toxicities against human fibroblast cells. The most active compound, the *N*-acetyl derivative **30**, is 5-fold more active than DQ against NF54 and K1 and equipotent with DQ against W2. It possesses an activity profile against all strains comparable with that of the artemisinin derivative artesunate. Overall, this compound and the other accessible and active derivatives serve as an attractive template for development of new and economic lead quinolones.

Under a programme designed to develop new triple drug combinations for the treatment of malaria, tuberculosis, and toxoplasmosis,[1] we are preparing and evaluating efficacies of compound sets based on combinations of oxidant and redox drugs[2] coupled with a third partner with a different mode of action. In the case of malaria, the need to develop new drug combinations is particularly pressing.[3] Chemotherapy coupled with vector control and inculcation of public awareness has reduced mortality due to malaria by over 66% since 2000.[4] However, the emergence of malaria parasites resistant to the current clinically-used artemisinin derivatives[5] mandates the urgent development of newer artemisinin derivatives. Such derivatives must be incapable of providing the active metabolite dihydroartemisinin (DHA) common to the current clinical artemisinins, and should be rationally combined with the redox drug and a third combination partner to counter resistance and inhibit spread of resistant phenotypes by blocking transmission.[6] The third partner is logically



Scheme 1. Conversion of decoquinate (DQ) into DQ amide derivatives. Reagents and conditions: (a) 1° or 2° amine (5 equiv), DBU (0.9 equiv), $CHCl_3$, reflux 24–72 h; (b) DQ amide, alkyl, acyl or sulfonyl halide (5 equiv), DBU (0.6 equiv), $CHCl_3$, reflux, 15 h; (c) DQ, as for (b).

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constructed about the 4(1*H*)-quinolone scaffold. In addition to being used clinically against a variety of infectious diseases including tuberculosis,[7] certain quinolones have acquired lead status for development of drugs for treatment of toxoplasmosis[8,9] and malaria[10–12] respectively.

Our attention is drawn to decoquinate (DQ, **1**) that has been used for many years in veterinary medicine largely co-administered with poultry feed for treatment of coccidiosis wherein it displays negligible toxicity.[13,14] It is also used against other apicomplexan infections in animals[15] and is highly active in a murine model against *Toxoplasma gondii*.[16] Activity of DQ against malaria including *Plasmodium berghei*[17] in mice and *Plasmodium cynomolgi*[18] in monkeys has been known for some time. DQ is active in vitro against the liver, blood and gametocyte parasite stages of *Plasmodium falciparum* (*Pf*).[19,20] Although such multistage activity matches the profile required for blocking transmission of the malaria parasite, 6 DQ like other 4(1*H*)-quinolones including the advanced lead compound ELQ 300,[10,12] is highly lipophilic with a Log *P* of 8.19,[21] has exceedingly low solubility in water (0.06 mg L^{-1})[14] and other solvents,[12] and has low bioavailability.[19,22] The decisive benefit of DQ is its low cost, ⩽$10 per kg,[23] which renders it substantially cheaper than the other 4(1*H*)-quinolones proposed for further development as antimalarial drugs. However, the poor solubility markedly complicates screening both in vitro and in vivo and hampers evaluation of pharmacokinetic parameters, thereby restricting its further development as a drug. Because of this, resort has been made to preparation of nanoparticle formulations that appear to enhance efficacy against malaria in an animal model.[22] Our approach consistent with the overall aims of our work is to convert DQ into accessible and more tractable derivatives that in the first instance are amenable to screening in vitro. We report here the preparation and preliminary in vitro antimalarial activity and cytotoxicity of such derivatives.

Chemistry. The proposed steps involve conversion of the ethyl ester into the less readily metabolized amide[24] and replacement of H-1 largely by acyl, carbamoyl or sulfonyl groups, operations that should improve physicochemical properties associated with enhanced polarity (Scheme 1). *N*-Alkyl derivatives are also prepared based on structure–activity considerations vis-à-vis the *N*-acyl and *N*-sulfonyl derivatives.

For the ester aminolysis (step a, Scheme 1), DQ is acidic with a pK_a of 9.81,[21] and thus a basic amine nucleophile under standard aminolysis conditions[25] is likely to undergo competitive equilibrating proton transfer with H-1 of the 4(1*H*)-quinolone (cf. Fig. 2 below). Whilst this problem may be countered by the use of Lewis acidic reagents,[26] base-catalyzed aminolysis with an amine and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[27] attracts because of its economy. Although the reactions may be conducted with the neat reactants and reagents, the most expeditious involved the heating of DQ and excess of primary or secondary amine with DBU in a solvent. We use chloroform here because of lack of solubility of DQ in other solvents, although it is emphasized that our initial concern is to obtain the new derivatives in the first place and then to optimize preparation for hit compounds using industrially acceptable solvents. Amides **2**–**21** (Fig. 1) were obtained in

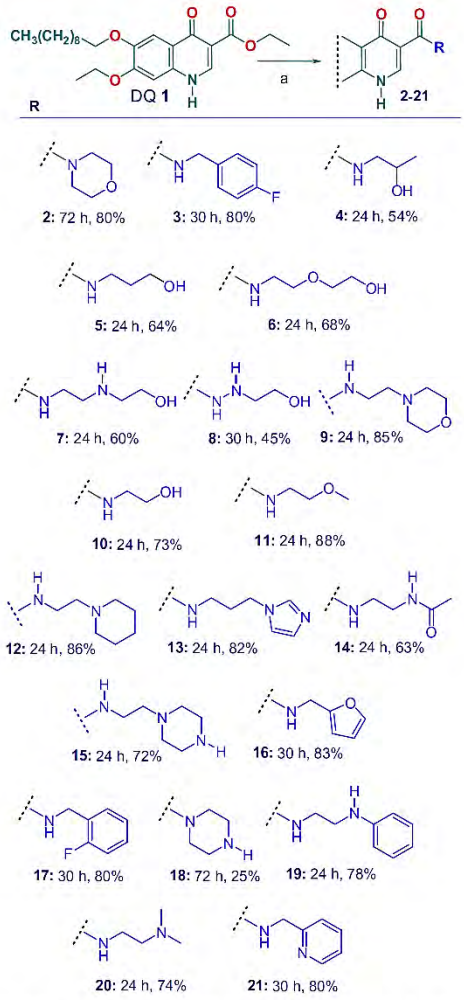

Figure 1. Amides obtained from direct aminolysis of decoquinate **1** with primary and secondary amines in chloroform according to method a, Scheme 1; reaction time (h), overall yields given.

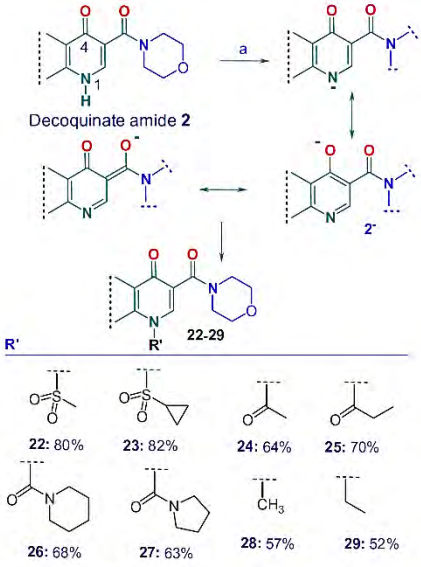

Figure 2. *N*-Substituted amide derivatives obtained from decoquinate amide **2** by treatment with DBU in the presence of sulfonyl, acyl and alkyl halides in chloroform with a reaction time of 15 h according to method b, Scheme 1; overall yields given.

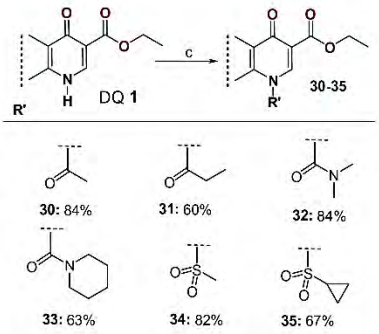

Figure 3. *N*-Substituted decoquinate derivatives obtained from decoquinate **1** and DBU in the presence of acyl, carbamoyl, and sulfonyl chlorides in chloroform, with a reaction time of 15 h according to method c, Scheme 1; overall yields given.

good yields. Characterization data for the compounds are given in the Supplementary material.

For functionalization at N-1 according to route b, Scheme 1, the decoquinate morpholino amide **2** was selected. Although the pK_a of amide **2** with respect to deprotonation at N-1 is unknown, it is likely this will be efficiently deprotonated by DBU in aprotic solvents to give the conjugate base **2**$^-$ that as an ambident anion may react through N-1 or O-4. In the event, the reactions with various acyl and sulfonyl chlorides, and alkyl halide proceeded smoothly through N-1 to give the products **22–29** in good yields (Fig. 2). Whereas the IR spectrum of compound **2** shows an absorption at 3162 cm^{-1} due to the N–**H** stretching vibration in the quinolone nucleus, the peak is absent in derivatives **22–29**, and a signal due to C-4 bearing an oxo group is evident at ca. 170 ppm in the ^{13}C NMR spectra.

Decoquinate **1** was also functionalized at N-1 according to Scheme 1 route c to give the *N*-substituted decoquinate derivatives **30–35** in good yields (Fig. 3). Data for derivatives **30, 31** and **33** appear in the Supplementary Material. As above, reaction through N-1 occurs in all cases. This is evident from the ^{13}C NMR spectra, which contain signals due to C-4 bearing an oxo group at ca. 170 ppm and the ester carbonyl at 160–165 ppm.

In summary, decoquinate **1** is converted readily via aminolysis of the ethyl ester with primary and secondary amines in the presence of DBU in chloroform under reflux into the corresponding secondary and tertiary amides. Decoquinate **1** and the morpholino amide **2** under the foregoing conditions react with acyl and sulfonyl halides and alkyl halides through N-1 to provide new *N*-substituted derivatives in good yields.

Antimalarial activities: Decoquinate and the new derivatives were screened in vitro for antimalarial activity with the SYBR Green I-based fluorescence assay[28] against *Pf* NF54 asexual blood stage parasites to determine the % inhibition of proliferation at initial compound concentrations of 1 and 5 μM (Fig. S1, Supplementary material). Compounds showing the highest activity (>50% inhibition at 5 μM and >70% inhibition at 1 μM) were submitted to a second dual point assay at 100 nM and 500 nM (Fig. S2, Supplementary material). Compounds showing near complete inhibition of parasite proliferation were carried forward for IC_{50} determination against chloroquine (CQ)-sensitive *Pf* NF54 and multidrug resistant K1 and W2. CQ, the artemisinins artemether and artesunate, and decoquinate **1** were used as controls. Cytotoxicity of the compounds was also evaluated with the sulforhodamine B (SRB) assay on the WI-38 cell line[29] using emetine as reference standard. IC_{50} values are summarized in Table 1. Of all compounds, only five, namely the decoquinate amides **3** and **6** (Fig. 1) and the N-substituted decoquinate derivatives **30**, **31** and **33** (Fig. 3) showed near complete inhibition at 1 and 5 μM regardless of the parasite strain (Fig. S2, Supplementary material). Decoquinate **1** and the derivatives showed no appreciable toxicity to mammalian WI-38 cells; their IC_{50} values are all substantially greater than the IC_{50} of 0.05 μM of emetine. The derivatives are thus selective in their antimalarial action as evidenced by the SI values $\geqslant$400 (Table 1). The amides **3** and **6** (Fig. 1) are as active as DQ, but less active than both artemether and artesunate against all three parasite strains. The *N*-acyl derivative **30** (Fig. 3) on the other hand is equipotent with the two clinically used artemisinins against all strains, and is 5-fold more potent than DQ against *Pf* NF54 and K1, whilst being equipotent with DQ against W2. In general according to the resistance indices (Table 1), the other compounds are less active against K1 with respect to drug sensitive NF54, and essentially equipotent with DQ against W2. Although derivative **30** shows slight differences in activities between the three parasite strains, the selectivity indices indicate it is more selective than DQ in its antimalarial activity. Thus the balance between strain susceptibility, efficacy and safety favors derivative **30** over the parent decoquinate. It is to be noted that with the exception of compound **3**, *c* Log *P* values indicate that all compounds are less lipophilic than decoquinate, and were able to be screened by preparing solutions in DMSO.

Table 1
Calculated Log *P* and in vitro biological data of selected derivatives

Compound	*c* Log *P*[a]	Antimalarial activity IC_{50} ± SEM, nM[b]			Resistance index		Cytotoxicity IC_{50}, μM	Selectivity index
		NF54	K1	W2	RI[c]	RI[d]	WI-38[e]	SI[f]
CQ	nd	10 ± 3	154.73 ± 14.96	233.18 ± 49.49	15.4	23.3	nd	nd
Artemether	nd	5.77 ± 1.33	7.26 ± 2.34	4.5 ± 0.78	1.3	0.8	nd	nd
Artesunate	nd	7.88 ± 1.35	8.97 ± 2.26	6.77 ± 1.13	1.4	0.8	>100	>12690
DQ **1**	8.2	26.6 ± 1.4	64.9 ± 8.8	15.4 ± 3.4	2.4	0.6	>100	>3759
3	8.0	33.1 ± 2.5	63.1 ± 9.8	8.6 ± 4.3	1.9	0.2	>100	>3021
6	6.0	40.4 ± 1.3	64.8 ± 7.2	18.5 ± 4.5	1.6	0.5	43.9	1087
30	5.9	4.9 ± 0.9	12.0 ± 3.5	8.5 ± 6.3	2.5	1.7	>100	>20408
31	6.5	15.4 ± 0.5	48.9 ± 9.9	11.5 ± 1.2	3.2	0.8	>100	>6494
33	6.5	18.3 ± 4.8	89.1 ± 6.0	28.2 ± 11.7	4.9	1.5	8.5	465
Emetine	nd	nd	nd	nd	nd	nd	0.05	nd

[a] *c* Log *P* values calculated with ACD/ChemSketch Version 14.02.
[b] Data from at least three independent biological repeats, each performed in triplicate; CQ = chloroquine; nd = not determined.
[c] Resistance index (RI) = IC_{50} K1/IC_{50} NF54.
[d] Resistance index (RI) = IC_{50} W2/IC_{50} NF54.
[e] WI-38 cell line of normal human fetal lung fibroblast (HFLF).
[f] Selectivity index (SI) = IC_{50} WI-38-HFLF/IC_{50} NF54.

To conclude, the derivative **30** that has an in vitro activity profile comparable to that of artesunate coupled with a favorable incipient toxicity profile, and others of Table 1 represent easily accessible and economic hit compounds to guide the preparation of new and potent derivatives. As foreshadowed in the introduction, activities of these and related compounds against *Mycobacterium tuberculosis* and *T. gondii* have also been recorded, and will be reported elsewhere.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.024.

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Chapter 5

New decoquinate derivatives with potent anti-mycobacterial activities

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Chapter 5 is a manuscript to be submitted to European journal of medicinal chemistry. The manuscript contains the background, aim, syntheses, in vitro anti-tubercular and cytotoxicity evaluation of all compounds synthesised. This manuscript is prepared according to author's guidelines from the journal homepage at https://www.elsevier.com/journals/european-journal-of-medicinal-chemistry/0223-5234/guide-for-authors. Supporting data for chapter 5 are found in addendum B.

New decoquinate derivatives with potent anti-mycobacterial activities

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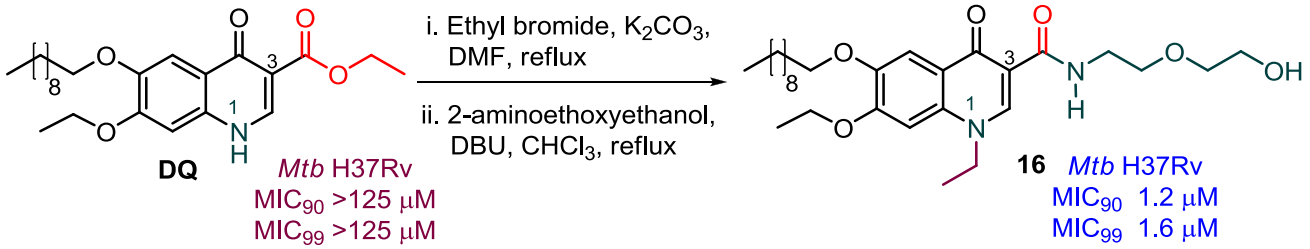
Abstract

Tuberculosis (TB) presents an enormous worldwide disease burden that is becoming even greater given that current TB drugs are fast losing their efficacy in the face of emergent multidrug resistant *Mycobacterium tuberculosis* (*Mtb*), the principal pathogen of TB. Newer drugs are therefore urgently needed, especially those that in some way may obviate the difficult and protracted treatment regimens required of the current drugs. That is, the new drugs should be economic to prepare and use, and above all be relatively non-toxic. Decoquinate (DQ), a coccidiostat that has long been used to treat coccidiosis and other important veterinary infections, is a cheap, non-toxic and lipophilic quinolone. However, DQ is insoluble in water and other solvents, and is inactive against *Mtb*. One strategy to render DQ active is to attach polar groups to generate derivatives that are less lipophilic and more soluble than DQ such that these may emulate certain physicochemical properties of the fluoroquinolone ciprofloxacin, a second-line TB drug. Thus, twenty five new DQ derivatives were prepared and evaluated *in vitro* for their anti-mycobacterial activity and cytotoxicity. Twenty-one active hits emerged from this series with the most active derivative **16** featuring an ethyl group attached to N-1 of the quinolone nucleus, and a (2-hydroxyethoxy)ethylamino group replacing the ethoxy of the ester at C-3 of DQ. This derivative has an MIC_{99} of 1 μM against rifampicin sensitive H37Rv *Mtb* and a selectivity index of 45 with respect to normal human fetal lung fibroblast cell lines. Thus, compound **16** represents a convenient starting point for preparation of economic new quinolones that have the potential to serve as safe and highly potent TB drugs.

Key words: Tuberculosis, TB drugs, fluoroquinolones, decoquinate, derivatives

Graphical abstract



Introduction

Tuberculosis (TB), caused principally by the bacterium *Mycobacterium tuberculosis* (*Mtb*),[1] is one of the oldest known diseases. [2] The number of deaths due to TB now outweighs that due to any other infectious disease including AIDS and malaria.[3] In 2014, 9.6 million people were infected with TB of which 1.4 million succumbed to the disease.[3] Unlike malaria, morbidity and mortality due to TB are not limited to tropical zones,[4] but are even higher in temperate zones wherein Russia and Eastern Europe account for 58% of the total global TB burden.[3, 5] In essence, TB is endemic to both temperate and tropical zones, with Russia, India, China, and South Africa being the hot spots for the disease.[6, 7]

The first line treatment regimen for TB consists of a cocktail of four drugs, namely isoniazid, ethambutol, pyrazinamide, and rifampicin, which have to be administered daily at a total dose of 5 grams for two months followed by isoniazid and rifampicin daily for another four months.[5, 8] The quantity and duration of administration of the drugs may lead to severe side effects.[9] Isoniazid and rifampicin cause hepatotoxicity, renal failure, metabolic acidosis and coma.[10] Not surprisingly, there has been poor patient compliance [11, 12] that in turn has led to the emergence of resistant strains of *Mtb*.[13] This has aggravated the control and management of TB.[14] Three resistance types of *Mtb* based on drug susceptibility have been documented, namely multi-drug resistant (MDR),[15] extensively drug resistant (XDR),[16] and totally drug resistant (XXDR) TB.[17] Overall in 2014 for MDR TB there were 480,000 cases and 190,000 mortalities, and for XDR TB, this was reported in 105 countries and comprised 9.7% of MDR TB infections; there were some 600 000 XDR TB cases in 2014.[11] MDR strains are not susceptible to isoniazid and rifampicin, but are susceptible to fluoroquinolones used as second line anti-TB drugs.[18, 19] XDR strains are resistant to isoniazid and rifampicin, fluoroquinolone and at least one of the three second line injectable TB drugs amikacin, kanamycin and capreomycin,[20] while XXDR strains are resistant to all first and second line TB drugs.[21] Overall, the intensive and cumbersome nature of first line treatment and the growing resistance to current TB drugs underscore the need for new drugs coupled with improved regimens for treatment of TB and especially to limit the spread of drug resistant TB.

A number of efficacious anti-bacterial agents have been constructed about the 4-(1*H*)-quinolone scaffold (Fig. 1) .[22, 23] The introduction of a fluorine atom at C-6 of the quinolone creates a unique subclass of quinolones called fluoroquinolones (FQs) exemplified by

ciprofloxacin (Fig. 1). The fluorine atom greatly enhances potency, as well as broadening the activity spectrum of FQs.[24] FQs have proven to be effective in treating TB cases where the first line treatment regimen have failed.[25] On-going studies suggest that using FQs in first line treatment against TB reduces the number of drugs and duration required to achieved an effective cure.[25-27]

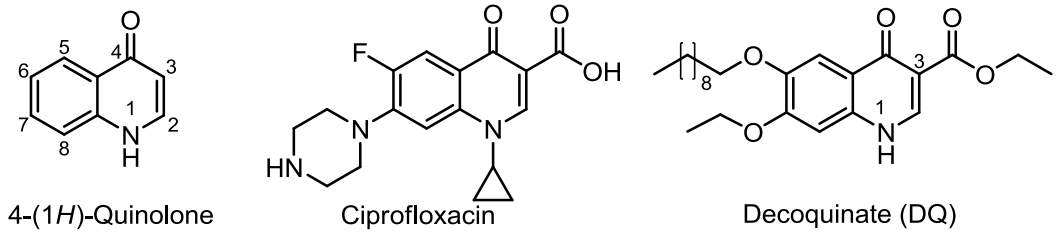


Figure 1: Structures of 4-(1*H*)- quinolone, ciprofloxacin and decoquinate

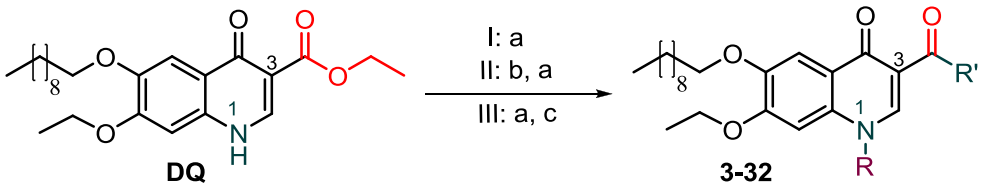
Despite the merits associated with the use of FQs to fight TB, these drugs suffer from two major setbacks. Firstly, there is reduced susceptibility of MDR *Mtb* strains to FQs. This is evident through the increasing prevalence of XDR strains emerging to FQs.[28, 29] Secondly, FQs cause rapid onset of neuropathy, hepatotoxicity and other side effects which may last for years or become permanent.[30-33] Both the merits and drawbacks of FQs suggest that an alternative drug based on the quinolone scaffold is urgently needed to treat both FQ-sensitive and resistant strains of *Mtb*. In the search for such a drug, we have considered using decoquinate (DQ) (Fig. 1) as an incipient template. DQis an FDA-approved quinolone that has been used in animal farming to treat coccidiosis for many years without any noticeable side effects.[34] DQ has a good safety profile in livestock even at repeated dosing or at dose levels some five times higher than the effective curative dose. Thus, possibly, a DQ-based TB drug may be better tolerated during prolonged administration to humans.[35, 36] Although it appears to have a good safety profile, DQ is very lipophilic.[37] Owing to this high lipophilicity, DQ should easily penetrate the outer lipophilic mycolic acid wall of *Mtb*. However, DQ itself is inactive against *Mtb*. Further, from a drug development perspective, it has the disadvantage of being almost insoluble in water (0.6 µg/L) and in other solvents. It is difficult to use this compound in screening, for example against the malaria parasite wherein resort has to be made to formulation to enhance efficacy.[38] However, it should be possible to activate DQ by attaching polar groups that should enhance

aqueous solubility and antimycobacterial activity. We now report on the preparation of twenty five decoquinate derivatives and the results of the evaluation of their antimycobacterial activity against *Mtb* and cytotoxicity against the normal human fetal lung fibroblast WI-38 cell line (HFLF WI-38) *in vitro*.

Results

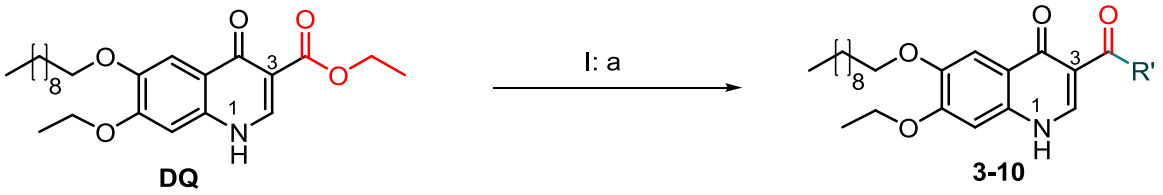
According to the methods outlined in Scheme 1, DQ was modified either at the C-3 carbethoxy group, or at both the C-3 carbethoxy group and N-1 in the quinolone nucleus to generate three series of compound sets I, II and III whose structures are depicted in Figures 2-4.

Scheme 1. Three synthetic routes (I, II, III) used for preparation of the derivatives.



Reagents and conditions: a. amine, DBU (0.9 equiv), $CHCl_3$, reflux, 24-72 h; b. alkyl halide, K_2CO_3 (5 equiv.), DMF, reflux, 10 h; c. carbamoyl or acyl chloride, DBU (0.7 mmol), $CHCl_3$, reflux, 15 h.

Aminolysis of the ester wherein DQ was treated with the appropriate amine and DBU as a base (route I, Scheme 1) gave amide derivatives **3-10** in good yields (Fig. 2). The secondary amides **3-9** contain a triplet (J = 5.3 Hz) at *ca* δ 11.0 ppm in their ^{1}HNMR spectra, which is due to H-1' in the amide chain (Fig. 2). The tertiary amide **10** shows a nine-proton multiplet between 3.76 – 3.68 ppm due to the presence of the piperazinyl unit. Compounds **3-10** all show a singlet at *ca* 12.0 ppm in their ^{1}H NMR spectra due to H-1 attached to N-1 in the quinolone nucleus.



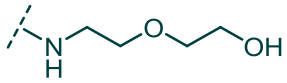
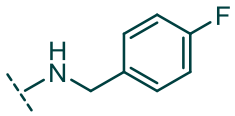
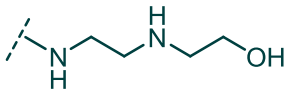
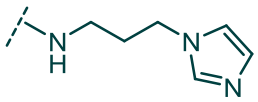
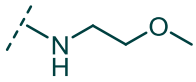
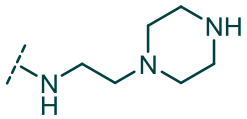
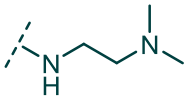
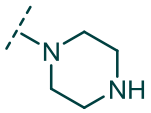
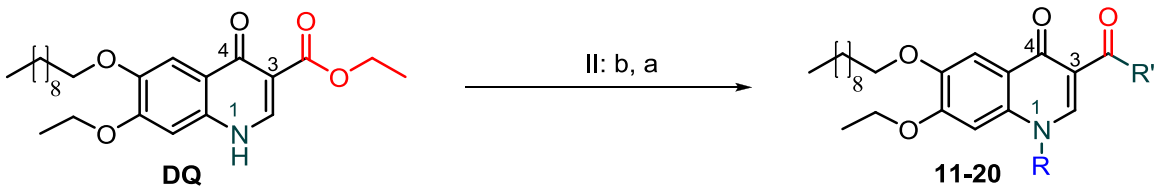
Cpd	R'	Cpd	R'
3		**7**	
4		**8**	
5		**9**	
6		**10**	

Figure 2: Amides obtained from direct aminolysis of decoquinate (DQ**)** with primary and secondary amines in chloroform according to method a, Scheme 1.

DQ was treated with each of ethyl and propyl bromide in the presence of K_2CO_3 (route II, Scheme 1) to give the *N*-alkylated intermediates, which without further purification were submitted to aminolysis in the presence of the primary amine and DBU as a base to generate compound **11-20** (Fig. 3**)**. The absence of a singlet at *ca* δ 12.0 ppm in the ^{1}H NMR spectra (due to H-1 that appears in the spectra of **3-10**) of compound **11-20** confirms N-1 was successfully alkylated. Moreover, the triplet (J = 5.3 Hz) at *ca* 11.0 ppm indicating the presence of H-1' confirms that the ester was successfully transformed to a secondary amide.



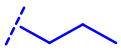
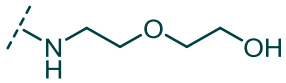
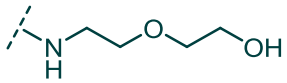
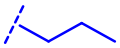
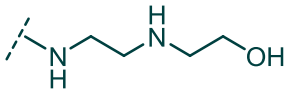
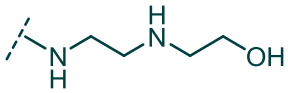
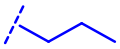
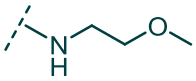
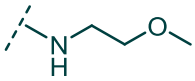
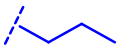
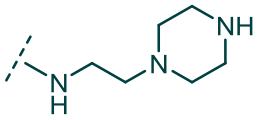
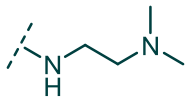
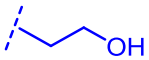
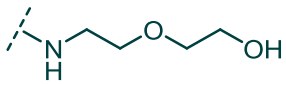
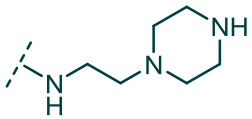
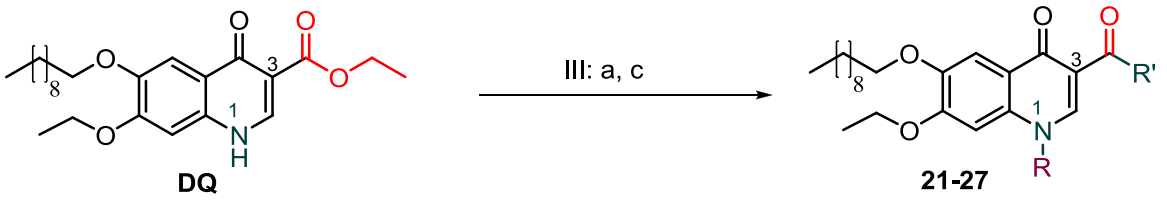
Cpd	R	R'	Cpd	R	R'
11			**16**		
12			**17**		
13			**18**		
14			**19**		
15			**20**		

Figure 3: *N*-alkylated amide derivatives obtained from decoquinate (DQ) by treatment with K_2CO_3 and alkyl halides, followed by treatment with a primary amine and DBU in chloroform according to route II, Scheme 1.

DQ was treated with a carbamoyl chloride in the presence of DBU to generate the *N*-carbamoyl intermediate which was submitted to aminolysis with a primary amine **(**route III Scheme 1) to give derivatives **21-23**, (Fig. 4**)**. Besides the absence of a singlet at *ca* δ 12.0 ppm in the ^{1}H NMR spectrum of compound **21- 23**, the ^{13}C NMR spectra of these compounds show a signal at *ca.* 153 ppm, which is assigned to the carbonyl carbon (C-a) of the urea. This data indicates that N-carbamoylation, rather that formation of a carbamate via reaction through O-4 took place. DQ was treated with carbamoyl or acyl chlorides in the presence of DBU to generate the N-carbamoylated or *N*-acylated intermediates which were then treated with a cyclic secondary amine in the presence of DBU (route III Scheme 1) to give the tertiary amides **24-27**

(Fig. 4). That carbamoylation or acylation had taken place at N-1 in the quinolone nucleus was again indicated by the absence of the singlet due to H-1 *ca* 12.0 ppm (*cf.* compounds 3-10) in their ^{1}H spectra.



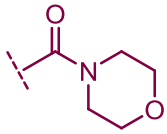
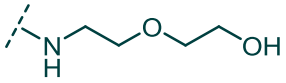
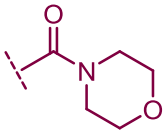
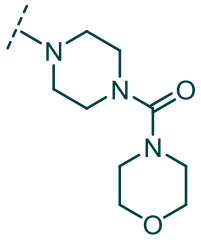
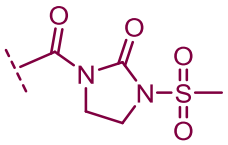
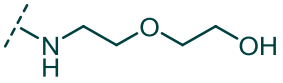
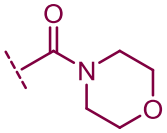
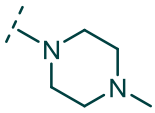
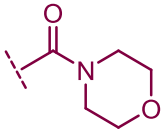
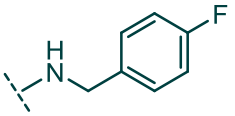
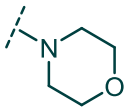
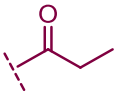
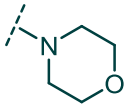
Cpd	R	R'	Cpd	R	R'
21			**24**		
22			**25**		
23			**26**		
			27		

Figure 4: *N*-carbamoylated and *N*-acyl amide derivatives obtained from decoquinate (DQ) by treatment with DBU and carbamoyl or acyl chloride, followed by treatment with amine and DBU in chloroform according to route III, Scheme 1.

The ^{1}H spectra of the products show a singlet at *ca.* δ 8.2 ppm assigned to H-2, two singlets in the range δ 6.8 – 7.5 ppm assigned to H-5 and H-8 of the of the quinolone nucleus, two triplets (J = 7.0, 6.9 Hz) in the range 4.0 – 4.4 ppm ascribed to H-13 and H-12 in the two alkoxy chains attached to C-6 and C-7. The ^{13}C NMR spectra of all compounds contain a signal

at ~175 ppm assigned to the ketone carbonyl at C-4 and a signal at ~165 ppm assigned to the carbonyl carbon atom C-23. The signals at ~ 69 and 65 ppm are due to C-13 and C-12 in the alkoxy chains attached to the quinolone nucleus. The identity of all compounds in this study was also confirmed by high resolution mass spectrometry (HRMS). All compounds were established to have greater than 95% purity using analytical HPLC methods. These were more soluble in common organic solvents such as ethanol, acetone or dimethyl sulfoxide and less lipophilic (cLogP < 8) than DQ.

All compounds were evaluated *in vitro* for antimycobacterial activity and cytotoxicity. They were screened in a broth microdilution assay against the rifampicin susceptible strain H37Rv of *Mtb* using rifampicin as the standard drug alongside the parent DQ. The sulforhodamine B (SRB) assay was used to assess the cytotoxicity against the normal human fetal lung fibroblast WI-38 cell line (HFLF WI-38) with emetine as standard. The antimycobacterial activities are reported as minimum inhibitory concentration (MIC) required to inhibit 90%, and 99% of the parasites while their cytotoxic activities are reported as IC_{50} values - the concentration required to reduce the viability of the cell line by 50%. The selectivity index (SI) is obtained by dividing the IC_{50} value by the MIC_{90} value. The biological activities of all compounds are presented in Table 1 below.

Discussion

Whilst DQ showed no antimycobacterial activity (MIC_{90}>125 μM) and no cytotoxicity, compounds **3-10** (Fig. 2) showed antimycobacterial activity (MIC_{90}) in the range of 10-40 μM. A comparison within this series suggests that a flexible unit attached to the C-3 side chain promotes activity (*e.g.* **3**, MIC_{90} 17 μM) whereas a rigid unit does not (*e.g.* **7**, MIC_{90} >125 μM). The compounds **11-20** (Fig. 3) were active with MIC_{90} values ranging between 1.2 – 72.3 μM. Structure-activity comparison within this series reveals that compounds with an ethyl group at *N*-1 (*e.g.* **17**, MIC_{90} 3 μM) are generally more active against *Mtb* H37Rv than those with a propyl group at *N*-1 (*e.g.* **12**, MIC_{90} 6 μM). The most active compounds of all are **15** (MIC_{90} 1.44, MIC_{99} 1.88 μM) and **16** (MIC_{90} 1.25, MIC_{99} 1.61 μM). Importantly, compounds **15** and **16** exhibited low cytotoxicities against the WI-38 cell line, with selectivity indices of 67 and 45 respectively. The compounds **22-27** (Fig. 4) are generally inactive against *Mtb* H37Rv, with compound **22** (MIC_{90} 6.09 μM; MIC_{99} 7.97 μM) and **25** (MIC_{90} 3.32 μM; MIC_{99} 4.89 μM) being

the only active compound in this sub-series. With respect to cytotoxicity against the normal human fetal lung fibroblast WI-38 cell line, emetine has an IC_{50} value of 0.050 μM, whereas most decoquinate derivatives low to moderate cytotoxicity, having IC_{50} value greater than 30 μM.

Table 1. *In vitro* antimycobacterial, cytotoxicity values and selectivity indices of DQ derivatives.

Compound[a]	cLogP[b]	Activity *Mtb* H37Rv		Cytotoxicity	Selectivity
		MIC_{90}, μM	MIC_{99}, μM	WI-38, IC_{50} μM	Index SI[d]
RIF	nd[c]	0.00315	0.00386	nd	nd
DQ	8.0	>125	> 125	> 125	nd
3	6.0	17.5	21	43.9	2.5
4	5.9	18	34.4	> 100	> 5.5
5	6.8	33.9	64.9	48.7	1.4
6	7.2	19	22.1	13.3	0.7
7	8.0	>125	> 125	nd	nd
8	6.8	38.3	49	3.08	0.08
9	6.7	10.5	14.5	> 100	> 9.5
10	6.5	22.9	36.9	5.88	0.3
11	5.5	72.3	81.3	54.9	0.7
12	5.3	6.49	8.67	41.3	6.4
13	6.2	6.07	7.92	32.4	5.4
14	6.1	12.40	17.90	70.6	5.7
15	3.8	1.44	1.88	> 100	> 69.4
16	4.9	1.25	1.61	56.2	45.0
17	4.7	3.64	4.16	40.1	11.0
18	5.6	8.00	18.90	83.4	10.4
19	6.0	12.10	16.5	49.1	4.1
20	5.6	8.50	10.80	37.8	4.4
21	3.1	>125	> 125	> 100	nd
22	1.8	6.1	7.97	64.2	10.5

Compound[a]	cLogP[b]	Activity *Mtb* H37Rv MIC_{90}, µM	MIC_{99}, µM	Cytotoxicity WI-38, IC_{50} µM	Selectivity Index SI[d]
23	5.7	>125	> 125	> 100	nd
24	3.2	15.20	20.30	40.1	2.6
25	3.9	3.32	4.89	36.2	10.9
26	4.3	> 125	> 125	nd	nd
27	5.0	7.63	8.51	> 100	> 13
EM	nd	nd	nd	0.05	nd

[a]RIF Rifampicin, DQ decoquinate, EM emetine; [b]cLogP values calculated with ACD/ChemSketch Version 14.02; [c]nd not determined, [d]SI Selectivity index = IC_{50}/MIC_{90}.

Conclusion

Twenty five new DQ derivatives bearing amido groups at C-3 and alkyl and acyl groups attached to N-1 were prepared using straightforward synthetic transformations. In contrast to DQ, all derivatives in this study were either soluble in chloroform, dimethyl sulfoxide, or ethanol. DQ has no antimycobacterial activity, whilst most of the derivatives showed antimycobacterial activity against H37Rv *Mtb*. A preliminary structure activity consideration suggests that the conversion of the carbethoxy group of DQ into an amide bearing a flexible chain, and/or an alkyl group at N-1 position are the most effective modifications for imprinting antimycobacterial activity on to DQ. On the other hand, conversion of the C-3 carbethoxy group into cyclic amides or introduction of a sulfonyl group at N-1 failed to impart appreciable antimycobacterial activity. Overall, derivatives **15**, **16** and **17** are the most active compounds, of which compound **16** featuring an ethyl group at N-1 and an *N*-[2-(2-hydroxyethoxy)ethyl]acetamido group at C-3 is the most active. These compounds will be taken forward for more advanced screening *in vitro*, for example against MDR and XDR *Mtb*, and will be used as a template upon which to base the preparation of newer DQ derivatives in the search for new TB drugs.

Experimental section

General procedures

Amines, acyl/carbamoyl/alkyl halides in this study were bought from either Sigma Aldrich or AKSci and were used as supplied, decoquinate was bought from Hohance chemical. All NMR spectra of synthesized compounds were obtained on a 600 MHz Bruker Avance™ III spectrometers as solutions in deuterated solvents ($CDCl_3$, or DMSO-d_6). All chemical shifts (δ) are reported in parts per million (ppm) values. ^1H chemical shifts are reported downfield of tetramethylsilane (TMS), and were internally referenced to the residual proton in $CDCl_3$ (7.26 ppm), or DMSO-d_6 (2.50 ppm). ^{13}C chemical shifts were internally referenced to resonances in $CDCl_3$ (δ 77.16 ppm), or DMSO-d_6 (δ 39.51 ppm). Peak multiplicities are abbreviated as follows: s (singlet), d (doublet), dd(doublet of doublet), t (triplet), q (quartet), and m (multiplet). Coupling constant (*J*) are reported in Hz. NMR data were analyzed using MestReNova Software, version 5.3.2-4936.

Exact mass measurements were recorded on Bruker MicroTOF Q II mass spectrometer that has an APCI/ESI source set at 300^oC, using Bruker Compass Data analysis 4.0 software. A full scan, ranging between 50–1500 m/z, was generated at a capillary voltage of 4500 V, end plate offset voltage of -500 V, and a collision cell RF voltage of 100 V.

Melting points (mp) were determined with a BÜCHI melting point B-545 instrument and were uncorrected. Thin layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$), acquired from Merck, and components were visualized by ultraviolet light (254 nm). Silica gel 230-400 (particle size 40-63μm) mesh was used for all flash column chromatography. Purity was determined by HPLC using a solvent gradient of 30% ACN/ 70% 0.1% H_3PO_4 and a Venusil XBP C18 column of size 150 × 4.6 mm.

***In vitro* antimycobacterial assay**

Determination of minimum inhibitory concentration (MIC_{90})

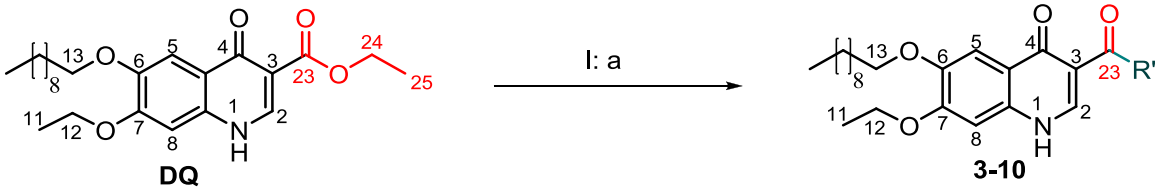
The broth microdilution method was used to assess the MIC_{90} - the minimum concentration of the compound that is required to inhibit 90% of the bacterial population growth. This procedure involves using a range of concentrations of the test compound in one 96-well microtitre plate. Here a culture of mutant Mtb (H37RvMA::*gfp*) strain expressing recombinant green fluorescent protein (GFP) off a plasmid integrated at the *attB* locus is grown to OD_{600}

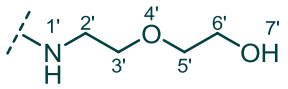
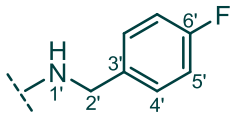
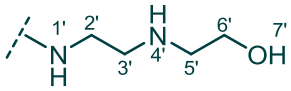
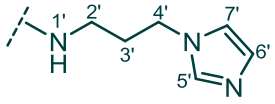
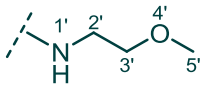
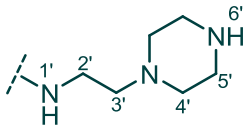
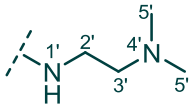
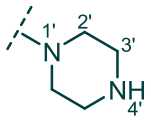
between 0.6 and 0.7. This culture is diluted 1:100 in GAST/Fe medium. A total volume of 50 µL of the GAST/Fe medium is added to rows 2-12 of the plate. To row 1, 640 µM of the compound is added in duplicate. In addition to the test compound in row 1, other wells contain GAST/Fe medium, 5% DMSO, rifampicin and kanamycin as controls. From there two-fold serial dilution will be performed by transferring 50 µL solution from each preceding row to the next ensuring that all the wells contain 50 µL (the dilution is exclusively for compound containing wells from row 1 and not controls). Then this plate is incubated at 37 °C (humid conditions) for 14 days. MIC_{90} values are scored by quantitative fluorescence using a Fluostar Optima microplate reader (BMG Labtech) at 7-days and 14-days post inoculation. Digital images are captured and stored. Furthermore, the efficacy of the compounds can be determined as required during growth in different carbon sources.

***In vitro* cytotoxicity**

The WI-38 cell line (normal human fetal lung fibroblast) from ECACC was routinely maintained as a monolayer cell culture at 37 °C, 5% CO_2, 95% air and 100% relative humidity in EMEM containing 10% fetal bovine serum, 2 mM L-glutamine and 50 µg/mL gentamicin. For this screening experiment, the cells (21 - 50 passages) were inoculated in 96-well microtiter plates at plating densities of 10 000 cells/well and incubated for 24 hours. After 24 hours, the cells were treated with the experimental drugs; which had previously been dissolved in DMSO and diluted in medium to produce five concentrations. Neat cells served as the control. The blank contained complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 hours after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein bound dye was extracted with 10 mM Tris base for optical density determination at a wavelength of 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression.

Table 2. General procedure for synthesis of C-3 amide derivatives 3-10 of DQ



Cpd	R'	Cpd	R'
3		**7**	
4		**8**	
5		**9**	
6		**10**	

To a 100 mL two neck flask containing decoquinate **1** (1 g, 2.34 mmol) and DBU (320 µL, 0.33 g, 2.1 mmol, 0.9 eq.) in chloroform (25 mL) was added the primary or secondary amine (5 eq.). The resulting mixture was stirred under reflux for 24-72 h. Progress of the reactions was monitored by TLC. Upon completion, the reaction mixture was concentrated *in vacuo*. The concentrated residue was dissolved in chloroform (20 mL) and washed with distilled water (4 x 20 mL). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product. This was submitted to chromatography on silica gel. Elution with dichloromethane/methanol (10:1, v/v) provided the fraction containing the product that was isolated by evaporation of solvent. The product was recrystallized from ethyl acetate and air dried.

6-Decoxy-7-ethoxy-N-[2-(2-hydroxyethoxy)ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxamide ***(3)***

White powder, 0.680 g (68%), m.p. 133-136 °C; HPLC 30% ACN 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 8.79 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 11.99 (s, 1H, H-1), 10.73 (t, *J* = 5.3 Hz, 1H, H-1'), 8.64 (s, 1H, H-2), 7.39 (s, 1H, H-5), 6.80 (s, 1H, H-8), 4.43 (s, 1H, H-7'), 4.01 (q, *J* = 7.0 Hz, 2H, H-13), 3.86 (t, *J* = 6.8 Hz, 2H, H-12), 3.83 – 3.78 (t, *J* = 5.3 Hz, 2H, H-2'), 3.71 – 3.60 (m, 6H, H-3', H-5', H-6'), 1.76 (p, *J* = 7.0 Hz, 2H, H-14), 1.46 – 1.19 (m, 17H, H-11, H-15,-H-21), 0.87 (dt, *J* = 21.8, 7.3 Hz, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.76 (C-4), 166.62 (C-23), 153.66 C-6), 147.86 (C-7), 141.49 (C-2), 134.73 (C-9), 120.40 (C-10), 110.13 (C-3), 105.39 (C-5), 99.88 (C-8), 72.38 (C-6'), 69.80 (C-5'), 68.54 (C-5'), 64.63 (C-13), 61.73 (C-12), 31.96 (C-2'), 31.90 (C-14), 29.65 (C-15, C-16) , 29.27 (C-17, C-18), 28.92 (C-19, C-20), 22.67 (C-21), 14.44 (C-11), 14.10 (C-22). APCI-HRMS *m/z* calcd for $C_{26}H_{41}N_20_6$ 477.2956, found 477.2911 $[M+H]^+$.

6-Decoxy-7-ethoxy-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-1,4-dihydroquinoline-3-carbox-amide ***(4)***

White powder, 0.506 g (50%), m.p. 122.4-125.1 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 95% pure, RT 2 min; ^{1}H NMR (600 MHz, DMSO-*d6*) δ (ppm) : 10.27 (t, *J* = 5.6 Hz, 1H, H-1'), 8.58 (s, 1H, H-2), 7.55 (s, 1H, H-5), 7.08 (s, 1H, H-8), 4.62 (s, 1H, H-7'), 4.12 (q, *J* = 6.9 Hz, 2H, H-13), 4.03 (t, *J* = 6.6 Hz, 2H, H-12), 3.48 (dt, *J* = 5.6, 6.2 Hz, 2H, H-2'), 3.41 (q, *J* = 6.2 Hz, 3H, H-6' overlap with HDO peak), 2.75 (t, *J* = 6.2 Hz, 2H, H-3'), 2.67 (t, *J* = 6.2 Hz, 2H, H-5'), 1.74 (p, *J* = 6.8 Hz, 2H, H-14), 1.41 (dt, *J* = 17.4, 6.8 Hz, 5H, H-11, H-15), 1.36 – 1.17 (m, 15H, H-16,-H-22). ^{13}C NMR (151 MHz, DMSO-d6) δ (ppm): 174.74 (C-4), 165.05 (C-23), 152.91 (C-6), 146.99 (C-7), 141.98 (C-2), 137.50 (C-9), 134.34 (C-10), 109.99 (C-3) , 107.61 (C-5) , 105.61 (C-8) 68.39 (C-13), 64.20 (C-12), 59.72 (C-6'), 48.54 (C-3'), 40.04 (C-5‘), 31.96 (C-2'), 31.34 (C-14), 29.03 (C-15) 28.99 (C-16), 28.74(C-17), 28.46 (C-18), 25.49 (C-19), 22.14 (C-20), 22.14 (C-21), 14.41 (C-11), 14.00 (C-22) . APCI-HRMS *m/z* calcd for $C_{26}H_{42}N_30_5$ 476.3119, found 476.3048 $[M+H]^+$.

*6-Decoxy-7-ethoxy-N-(2-methoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**5**)*

Light yellow powder, 0.620 g (62%), m.p. 167-170 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 97% pure, RT 11.4 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 12.01 (s, 1H, H-1), 10.83 (t, *J* = 5.6 Hz, 1H, H-1'), 8.67 (s, 1H, H-2), 7.68 (s, 1H, H-5), 6.89 (s, 1H, H-8), 4.11 – 4.01 (m, 4H, H-13, H-12), 3.70 (dd, *J* = 5.6, 5.6 Hz, 2H, H-2'), 3.56 (t, *J* = 5.6 Hz, 2H, H-3'), 3.33 (s, 3H, H-5'), 1.83 (p, *J* = 7.1 Hz, 2H, H-14), 1.44 (dt, *J* = 24.6, 7.1 Hz, 4H, H-15, H-16), 1.36 – 1.19 (m, 13H, H-11, H-17,-H-21), 0.85 (t, *J* = 6.9 Hz, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.06 (C-4), 166.97 (C-23), 153.84 (C-6), 148.04 (C-7), 141.18 (C-2), 134.87 (C-9), 120.73 (C-10), 110.16 (C-3), 105.93 (C-5), 99.98 (C-8), 71.18 (C-3'), 69.22 (C-13), 64.76 (C-12), 58.91 (C-5'), 39.30 (C-2'), 31.88 (C-14), 29.54 (C-15), 29.53 (C-16), 29.35 (C-17), 29.31 (C-18), 28.88 (C-19), 25.91 (C-20), 22.66 (C-21), 14.44 (C-11), 14.10 (C-22). APCI-HRMS *m/z* calcd for $C_{25}H_{39}N_20_5$ 447.2853, found 447.2814$[M+H]^+$.

*6-Decoxy-N-[2-(dimethylamino)ethyl]-7-ethoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (**6**)*

White powder, 0.928 g (92.8%), m.p. 151.-155 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 95% pure, RT 4.9 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 12.01 (s, 1H, H-1), 10.64 (t, *J* = 5.6 Hz, 1H, H-1'), 8.47 (s, 1H, H-2), 7.36 (s, 1H, H-5), 6.73 (s, 1H, H-8), 4.15 – 4.06 (m, 2H, H-13), 3.90 (q, *J* = 7.2 Hz, 2H, H-12), 3.65 (dt, *J* = 5.6, 6.0 Hz, 2H, H-2'), 2.60 (t, *J* = 6.0 Hz, 2H, H-3'), 2.32 (s, 6H, H-5'), 1.90 – 1.76 (m, 2H, H-14), 1.53 – 1.42 (m, 2H, H-15), 1.30 – 1.19 (m, 15H, H-11, H-16,-H-21), 0.87 (dt, *J* = 14.0, 7.3 Hz, 3H, H-22). ^{13}C NMR (151 MHz, CDCl3) δ (ppm): 175.63 (C-4), 166.52 (C-23), 153.42 (C-6), 147.66 (C-7), 141.41 (C-2), 134.63 (C-9), 120.29 (C-10), 110.01 (C-3), 105.26 (C-5), 99.93 (C-8), 68.92 (C-13), 64.60 (C-12), 58.99 (C-3'), 45.28 (C-5', C-6'), 36.64 (C-2'), 31.88 (C-14), 29.58 (C-15), 29.55 (C-16), 29.32 (C-17), 28.92 (C-18), 28.92 (C-19), 25.91 (C-20), 22.65 (C-21), 14.50 (C-11), 14.09 (C-22). APCI-HRMS *m/z* calcd for $C_{26}H_{42}N_30_4$ 460.3170, found 460.3102 $[M+H]^+$.

*6-Decoxy-7-ethoxy-N-(4-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**7**)*

White powder, 0.82 g (80%), m.p. 202-203 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution >96% pure, RT 16.5 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 11.85 (s, 1H, H-1), 11.11 (t, *J* = 5.8 Hz, 1H, H-1'), 8.67 (s, 1H, H-2), 7.67 (s, 1H, H-5), 7.28 (dd, *J* = 8.4, 5.3 Hz, 2H, H-5',), 6.94 (t, *J* = 8.5 Hz, 2H, H-4',), 6.81 (s, 1H, H-8), 4.66 (d, *J* = 5.8 Hz, 2H, H-2'),

4.05 (t, *J* = 6.9 Hz, 2H, H-13), 3.92 (t, *J* = 6.9 Hz, 2H, H-12), 1.84 (dd, *J* = 7.4, 6.9 Hz, 2H, H-14,), 1.47 – 1.18 (m, 17H, H-11, H-15,-H-21,), 0.92 – 0.82 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.11 (C-4), 166.87 (C-23), 162.83 (C-6'), 153.82 (C-6,) , 148.18 (C-7), 141.18 (C-2), 134.77 (C-9), 133.94 (C-3'), 128.93 (C-4'), 120.70 (C-10), 115.51 (C-5'), 110.05 (C-3), 105.92 (C-5), 99.85 (C-8), 69.26 (C-13), 64.70 (C-12) , 42.72 (C-2'), 31.87 (C-14), 29.53 (C-15, C-16), 29.31 (C-17, C-18), 28.85 (C-19), 25.90 (C-20) , 22.66 (C-21), 14.38 (C-11) , 14.10 (C-22). APCI-HRMS *m/z* calcd for $C_{29}H_{38}FN_20_4$ 497.2810, found 497.2734 $[M+H]^+$.

6-Decoxy-7-ethoxy-N-[3-(1H-imidazol-1-yl)propyl]-4-oxo-1,4-dihydroquinoline-3-carboxamide ***(8)***

White powder, 0.956 g (95.6%), m.p. 124.-127 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 5.0 min; ^{1}H NMR (600 MHz, DMSO-*d6*) δ (ppm): 12.01 (s, 1H, H-1), 10.29 (t, *J* = 5.8 Hz, 1H, H-1'), 8.60 (s, 1H, H-2), 7.65 (s, 1H, H-6'), 7.56 (s, 1H, H-5), 7.21 (s, 1H, H-9'), 7.08 (s, 1H, H-8), 6.89 (s, 1H, H-8'), 4.12 (q, *J* = 7.0 Hz, 2H, H-4'), 4.02 (dt, *J* = 13.7, 6.7 Hz, 4H, H-13, H-12), 3.27 (dt, *J* = 5.8, 6.9 Hz, 2H, H-2'), 1.95 (dt, *J* = 6.9, 7.0 Hz, 2H, H-3'), 1.74 (p, *J* = 13.7 Hz, 2H, H-14), 1.46 – 1.17 (m, 17H, H-11, H-15,-H-21), 0.87 – 0.81 (m, 3H, H-22). ^{13}C NMR (151 MHz, DMSO-*d6*) δ (ppm): 174.84 (C-4), 165.04 (C-23), 152.94 (C-6), 147.03 (C-7), 141.75 (C-2), 137.30 (C-6'), 134.45 (C-9), 128.43 (C-9'), 120.84 (C-10), 119.35 (C-8'), 109.89 (C-3), 105.57 (C-5), 100.53 (C-8), 68.38 (C-13), 64.19 (C-12), 43.76 (C-4'), 39.08 (C-2'), 35.42 (C-3'), 31.32 (C-14), 31.03 (C-15), 29.02 (C-16), 28.98 (C-17), 28.73 (C-18), 28.44 (C-19), 25.48 (C-10), 22.12 (C-21), 14.39 (C-11), 13.98 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{41}N_40_4$ 497.3170, found 497.3081$[M+H]^+$.

*6-Decoxy-7-ethoxy-4-oxo-N-[2-(piperazin-1-yl)ethyl]-1,4-dihydroquinoline-3-carboxamide (**9**)*

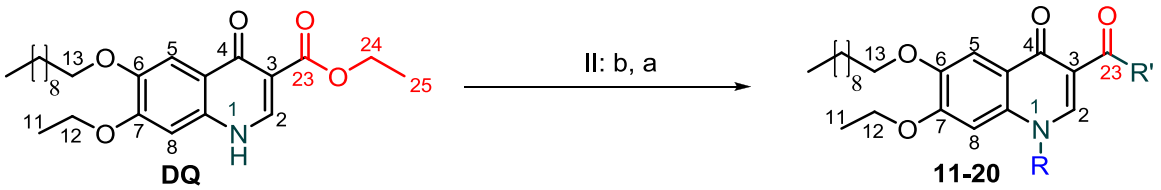
Off white powder, 0.750 g (75%), m.p. 168.-169 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 4.8 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 12.01 (s, 1H, H-1), 10.62 (t, *J* = 5.6 Hz, 1H, H-1'), 8.60 (s, 1H, H-2), 7.58 (s, 1H, H-5), 6.83 (s, 1H, H-8), 4.12 – 3.97 (m, 4H, H-13, H-12), 3.62 (dt, *J* = 5.6, 6.4 Hz, 2H, H-2'), 2.89 – 2.77 (m, 9H, H-4'- H-6'), 2.62 (t, *J* = 6.4 Hz, 2H, H-3'), 1.83 (p, *J* = 7.0 Hz, 2H, H-14), 1.49 – 1.18 (m, 17H, H-11, H-15,-H-21), 0.92 – 0.83 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.94 (C-4), 166.60 (C-23), 153.72 (C-6), 147.95 (C-7), 141.15 (C-2), 134.71 (C-9), 120.65 (C-10), 110.26 (C-3), 105.84 (C-5), 99.91 (C-8), 69.16 (C-13), 64.74 (C-12), 58.03 (C-3'), 54.15 (C-4'), 45.76 (C-5'),

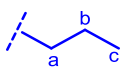
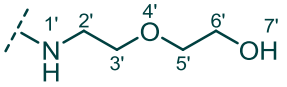
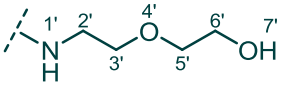
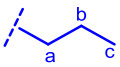
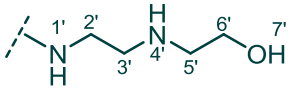
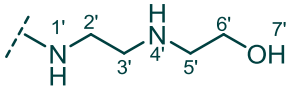
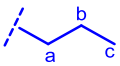
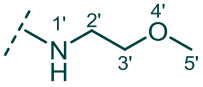
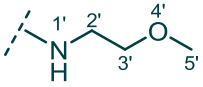
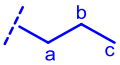
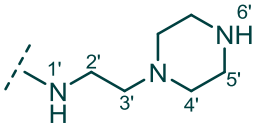
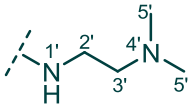
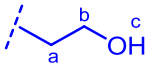
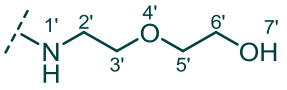
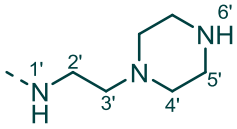
36.34 (C-2'), 31.88 (C-14), 29.56 (C-15), 29.54 (C-16), 29.38 (C-17), 29.32 (C-18), 28.91 (C-19), 25.93 (C-20), 22.66 (C-21), 14.50 (C-11), 14.11 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{45}N_40_4$ 501.3435, found 501.3360$[M+H]^+$.

*6- Decoxy -7-ethoxy-3-(piperazin-1-ylcarbonyl)quinolin-4(1H)-one (**10**)*

Yellow powder, 0.28 g (28%), m.p. 182-184 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 98% pure, RT 4.38 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) : 12.00 (m, 1H, H-1), 7.67 (s, 1H, H-2), 7.52 (s, 1H, H-5), 6.70 (s, 1H, H-8), 4.01 – 3.92 (m, 4H, H-13, H-12), 3.76 – 3.68 (m, 9H, H-2',-H-4'), 1.81 (m, 2H, H-14), 1.45 – 1.35 (m, 4H, H-15, H-16), 1.37 – 1.18 (m, 13H, H-11, H-17,-H-21), 0.85 – 0.68 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm) : 172.75 (C-4), 167.79 (C-23), 153.61 (C-6), 147.80 (C-7), 138.63 (C-2), 135.00 (C-9), 120.22 (C-10), 115.47 (C-3), 105.57 (C-5), 99.84 (C-8), 69.17 (C-13), 67.23 (C-2'), 64.61 (C-12), 48.08 (C-3'), 31.88 (C-14), 29.54 (C-15, C-16), 29.35 (C-17, C-18), 28.97 (C-19), 25.93 (C-20), 22.66 (C-21), 14.45 (C-11), 14.10 (C-22). APCI-HRMS *m/z* calcd for $C_{26}H_{40}N_30_4$ 458.3013, found 458.2942 $[M+H]^+$.

Table 3. General procedure for synthesis of *N*-alkylated amide derivatives 11-20 of DQ



Cpd	R	R'	Cpd	R	R'
11			**16**		
12			**17**		
13			**18**		
14			**19**		
15			**20**		

N-alkylation: A mixture of DQ (1.19 mmol, 0.5 g, 1 eq), K_2CO_3 (5.0 mmol, 0.69 g), alkyl halide (5 eq.) in DMF (10 mL) was refluxed for 15 h, after which the mixture was evaporated to dryness, dissolved in chloroform and washed with distilled water (20 mL × 4). The organic phase was evaporated to dryness to obtain the crude N-alkylated product which was used in the ensuing step without purification.

Carboxyamidation: A mixture of the crude N-alkylated product (0.5 g, 1 eq.), DBU (320 µL, 0.33 g, 2.1 mmol), the appropriate amine (5 eq.), and chloroform (15 mL) in a 100 mL round bottom flask was stirred under reflux for 24-72 h. Progress of the reaction was monitored by

TLC. Upon completion, the reaction mixture was treated with additional chloroform (20 mL) and washed with distilled water (20 mL × 4). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product. This was submitted to chromatography on silica gel. Elution with $CH2Cl_2$/methanol (10:1, v/v) provided the fraction containing the product that was isolated by evaporation of solvent. The product was recrystallized from ethyl acetate and air dried.

*6-Decoxy-7-ethoxy-N-[2-(2-hydroxyethoxy)ethyl]-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxamide (**11**)*

White powder, 0.38 g (76%), m.p. 162-164 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 98% pure, RT 4.30 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.45 (t, *J* = 5.4 Hz, 1H, H-1'), 8.61 (s, 1H, H-2), 7.80 (s, 1H, H-5), 6.76 (s, 1H, H-8), 4.33 – 3.96 (m, 6H, H-12, H-13, H-a), 3.74 – 3.48 (m, 4H, H-6', H-2'), 2.95 – 2.75 (m, 4H, H-5', H-3'), 2.37 (s, 1H, H-7'), 2.00 – 1.76 (m, 4H, H-b, H-14), 1.59 – 1.37 (m, 4H, H-15, H-16), 1.35 – 1.17 (m, 13H, H-c, H-17 - H-21), 0.98 (t, *J* = 7.4 Hz, 3H, H-11), 0.91 – 0.67 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.35 (C-4), 165.77 (C-23), 153.54 (C-6), 147.78 (C-7), 145.88 (C-2), 134.32 (C-9), 122.22 (C-10), 110.87 (C-3), 107.40 (C-5), 98.59 (C-8), 69.60 (C-13), 64.98 (C-12), 60.94 (C-6'), 55.79 (C-a), 50.66 (C-5'), 48.49 (C-3'), 38.89 (C-2'), 31.86 (C-b), 29.52 (C-14), 29.49 (C-15), 29.30 (C-16), 29.28 (C-17), 28.87 (C-18), 25.92 (C-19), 22.64 (C-20), 22.09 (C-21), 14.51 (C-11), 14.08 (C-c), 11.08 (C-22). APCI-HRMS *m/z* calcd for $C_{29}H_{47}N_2O_6$ 519.3429, found 519.3431 $[M+H]^+$.

*6-Decoxy-7-ethoxy-N-[2-(2-hydroxyethoxy)ethyl]-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**12**)*

White powder, 0.4 g (80%), m.p. 160-162 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 98% pure, RT 4.00 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.46 (t, *J* = 5.4 Hz, 1H, H-1'), 8.62 (s, 1H, H-2), 7.64 (s, 1H, H-5), 6.83 (s, 1H, H-8), 4.35 (s, 2H, H-c, H-7'), 4.22 – 4.09 (m, 2H, H-13), 4.06 – 3.97 (m, *J* = 6.9 Hz, 4H, H-12, H-a), 3.73 (d, *J* = 6.5, 2H, H-6'), 3.63 – 3.57 (m, *J* = 6.5, 6H, H-3', H-5', H-b), 3.44 (dd, *J* = 5.4, 6.5 Hz, 2H, H-2'), 1.88 – 1.78 (m, 2H, H-14), 1.56 – 1.42 (m, 6H, H-15 - H-17), 1.32 – 1.20 (m, 11H, H-18 - H-22), 0.85 (t, *J* = 6.9 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.12 (C-4), 166.27 (C-23), 153.58 (C-6),

147.69 (C-7), 146.69 (C-2), 134.75 (C-9), 121.79 (C-10), 110.34 (C-3), 107.12 (C-5), 98.56 (C-8), 72.62 (C-6'), 69.66 (C-b), 69.16 (C-13), 65.02 (C-12), 61.67 (C-5'), 59.78 (C-3'), 54.42 (C-a), 38.87 (C-2'), 31.86 (C-14), 29.49 (C-15), 29.30 (C-16), 29.28 (C-17), 28.87 (C-18), 25.92 (C-19), 22.64 (C-20), 22.09 (C-21), 14.50 (C-11), 14.09 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{45}N_2O_7$ 521.3221, found 521.3168 $[M+H]^+$.

6-Decoxy-7-ethoxy-N-(2-methoxyethyl)-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxamide ***(13)***

White powder, 0.45 g (90%), m.p. 152-153 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 97% pure, RT 6.70 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.35 (t, *J* = 5.3 Hz, 1H, H-1'), 8.62 (s, 1H, H-2), 7.84 (s, 1H, H-5), 6.77 (s, 1H, H-8), 4.21 – 4.00 (m, 6H, H-13, H-12, H-a), 3.66 (dt, *J* = 5.3, 5.5 Hz, 2H, H-2'), 3.56 (t, *J* = 5.5 Hz, 2H, H-3'), 3.38 (s, 3H, H-4'), 2.00 – 1.86 (m, 2H, H-b), 1.86 – 1.82 (m, 2H, H-14), 1.49 (dt, *J* = 16.2, 5.1 Hz, 4H, H-15, H-16), 1.48 – 1.37 (m, 2H, H-17), 1.30 – 1.17 (m, 11H, H-c, H-18 - H-21), 0.98 (t, *J* = 7.4 Hz, 3H, H-11), 0.91 – 0.71 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.35 (C-4), 165.53 (C-23), 153.54 (C-6), 147.71 (C-7), 145.92 (C-2), 134.31 (C-9), 122.31 (C-10), 110.84 (C-3), 107.52 (C-5), 98.55 (C-8), 71.35 (C-3') 69.19 (C-13), 64.99 (C-12), 58.82 (C-4'), 55.74 (C-a), 38.91 (C-2'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-b), 25.92 (C-19), 22.64 (C-20), 22.09 (C-21), 14.51 (C-11), 14.08 (C-c), 11.08 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{45}N_2O_5$ 489.3323, found 489.3302 $[M+H]^+$.

6-Decoxy-7-ethoxy-4-oxo-N-[2-(piperazin-1-yl)ethyl]-1-propyl-1,4-dihydroquinoline-3-carboxamide ***(14)***

White powder, 0.47 g (94%), m.p. 168-169 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 5.18 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.32 (t, *J* = 5.8 Hz, 1H, H-1'), 8.61 (s, 1H, H-2), 7.82 (s, 1H, H-5), 6.77 (s, 1H, H-8), 4.13 (ddd, *J* = 21.8, 13.9, 6.9 Hz, 6H, H-13, H-12, H-a), 3.58 (dd, *J* = 5.8, 4.8 Hz, 2H, H-2'), 2.90 (t, *J* = 4.8 Hz, 2H, H-3'), 2.71 – 2.36 (m, 9H, H-4', H-5', H-6'), 1.94 – 1.72 (m, 4H, H-b, H-14), 1.48 (dt, *J* = 33.4, 7.3 Hz, 4H, H-15, H-16), 1.50 – 1.37 (m, 2H, H-17), 1.27 – 1.21 (m, 11H, H-c, H-18 - H-21), 0.97 (t, *J* = 6.9 Hz, 3H, H-11), 0.84 (t, *J* = 7.0 Hz, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.33 (C-4), 165.40 (C-23), 153.45 (C-6), 147.70 (C-7), 145.88 (C-2), 134.31 (C-9),

122.27 (C-10), 110.96 (C-3), 107.49 (C-5), 98.52 (C-8), 69.17 (C-13), 64.97 (C-12), 58.01 (C-4'), 55.73 (C-a), 54.37 (C-5'), 45.95 (C-3'), 36.34 (C-2'), 31.83 (C-b), 29.50 (C-14), 29.47 (C-15), 29.27 (C-16), 29.26 (C-17), 28.84 (C-18), 25.89 (C-19), 22.61 (C-20), 22.19 (C-21), 14.50 (C-11), 14.06 (C-c), 11.06 (C-22). APCI-HRMS *m/z* calcd for $C_{31}H_{51}N_4O_4$ 543.3905, found 543.3872 $[M+H]^+$.

*6-Decoxy-7-ethoxy-1-(2-hydroxyethyl)-N-[2-(2-hydroxyethoxy)ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxamide (**15**)*

White powder, 0.47 g (94%), m.p. 155-157 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 4.02 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.46 (t, 1H, H-1'), 8.62 (s, 1H, H-2), 7.64 (s, 1H, H-5), 6.83 (s, 1H, H-8), 4.35 (s, 2H, H-c, H-7'), 4.22 – 4.09 (m, 2H, H-13), 4.06 – 3.97 (m, *J* = 6.9 Hz, 4H, H-12, H-a), 3.73 (d, *J* = 16.5, 2H, H-6'), 3.63 – 3.57 (m, *J* = 16.5, 6H, H-3', H-5', H-b), 3.44 (dd, 2H, H-2'), 1.88 – 1.78 (m, 2H, H-14), 1.56 – 1.42 (m, 6H, H-15 - H-17), 1.32 – 1.20 (m, 11H, H-18 - H-22), 0.85 (t, *J* = 6.9 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.12 (C-4), 166.27 (C-23), 153.58 (C-6), 147.69 (C-7), 146.69 (C-2), 134.75 (C-9), 121.79 (C-10), 110.34 (C-3), 107.12 (C-5), 98.56 (C-8), 72.62 (C-6'), 69.66 (C-b), 69.16 (C-13), 65.02 (C-12), 61.67 (C-5'), 59.78 (C-3'), 54.42 (C-a), 38.87 (C-2'), 31.86 (C-14), 29.49 (C-15), 29.30 (C-16), 29.28 (C-17), 28.87 (C-18), 25.92 (C-19), 22.64 (C-20), 22.09 (C-21), 14.50 (C-11), 14.09 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{45}N_2O_7$ 521.3221, found 521.3168 $[M+H]^+$.

*6-Decoxy-7-ethoxy-1-ethyl-N-[2-(2-hydroxyethoxy)ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxamide (**16**)*

White powder, 0.42 g (84%), m.p. 158-160 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 4.18 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.50 (t, *J* = 5.8 Hz, 1H, H-1'), 8.65 (s, 1H, H-2), 7.81 (s, 1H, H-5), 6.78 (s, 1H, H-8), 4.18- 4.00 (ddd, *J* = 21.9, 13.0, 6.9 Hz, 6H, H-a, H-13, H-12), 3.86 – 3.71 (m, 2H, H-6'), 3.69 – 3.53 (m, 6H, H-2', H-3', H-5'), 3.30 (s, 1H, H-7'), 1.92 – 1.72 (m, 2H, H-14), 1.56 – 1.38 (m, 8H, H-11, H-22, H-15), 1.36 – 1.16 (m, 12H, H-16 - H-21), 0.84 (t, *J* = 6.9 Hz, 3H, H-b). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.33 (C-4), 165.50 (C-23), 153.64 (C-6), 147.79 (C-7), 145.26 (C-2), 134.15 (C-9), 122.28 (C-10), 111.31 (C-3), 107.56 (C-5), 98.27 (C-8), 72.51 (C-5'), 69.85 (C-3'), 69.18 (C-13), 65.01 (C-12), 61.79 (C-6'), 49.11 (C-a), 38.81 (C-2'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-19), 25.92 (C-20), 22.63 (C-21), 14.54 (C-b), 14.50 (C-11), 14.07 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{45}N_2O_6$ 505.3272, found 505.3254 $[M+H]^+$.

*6-Decoxy-7-ethoxy-1-ethyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-1,4- dihydroquinoline-3-carboxamide (**17**)*

White powder, 0.38 g (76%), m.p. 154-156 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 4.09 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.50 (t, *J* = 5.8 Hz, 1H, H-1'), 8.65 (s, 1H, H-2), 7.81 (s, 1H, H-5), 6.78 (s, 1H, H-8), 4.18- 4.00 (ddd, *J* = 28.9, 13.0, 6.9 Hz, 6H, H-a, H-13, H-12), 3.86 – 3.71 (m, 2H, H-6'), 3.69 – 3.53 (m, 6H, H-2', H-3', H-5'), 3.30 (s, 1H, H-7'), 1.92 – 1.72 (m, 2H, H-14), 1.56 – 1.38 (m, 8H, H-11, H-22, H-15), 1.36 – 1.16 (m, 12H, H-16 - H-21), 0.84 (t, *J* = 6.9 Hz, 3H, H-b). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.33 (C-4), 165.50 (C-23), 153.64 (C-6), 147.79 (C-7), 145.26 (C-2), 134.15 (C-9), 122.28 (C-10), 111.31 (C-3), 107.56 (C-5), 98.27 (C-8), 72.51 (C-5'), 69.85 (C-3'), 69.18 (C-13), 65.01 (C-12), 61.79 (C-6'), 49.11 (C-a), 38.81 (C-2'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-19), 25.92 (C-20), 22.63 (C-21), 14.54 (C-b), 14.50 (C-11), 14.07 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{46}N_3O_5$ 504.3432, found 504.3426 $[M+H]^+$.

*6-Decoxy-7-ethoxy-1-ethyl-N-(2-methoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**18**)*

White powder, 0.4 g (80%), m.p. 157-159 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 4.33 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.35 (t, *J* = 5.5 Hz, 1H, H-1'), 8.66 (s, 1H, H-2), 7.84 (s, 1H, H-5), 6.79 (s, 1H, H-8), 4.32 – 4.20 (m, 2H, H-a), 4.17 (q, *J* = 6.9 Hz, 2H, H-12), 4.10 (t, *J* = 6.8 Hz, 2H, H-13), 3.64 (t, *J* = 5.5 Hz, 2H, H-3'), 3.56 (dt, *J* = 5.5, 5.5 Hz, 2H, H-2'), 3.38 (s, 3H, H-5'), 1.90 – 1.80 (m, 2H, H-14), 1.60 – 1.40 (m, 10H, H-15- H-19), 1.30 – 1.18 (m, 10H, H-a, H-20 - H-22), 0.85 (t, *J* = 6.9 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.34 (C-4), 165.55 (C-23), 153.54 (C-6), 147.70 (C-7), 145.30 (C-2), 134.13 (C-9), 122.30 (C-10), 111.29 (C-3), 107.57 (C-5), 98.30 (C-8), 71.34 (C-4'), 69.19 (C-13), 65.05 (C-12), 58.82 (C-3'), 49.06 (C-a), 38.93 (C-2'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-19), 25.92 (C-20), 22.63 (C-21), 14.54 (C-b), 14.51 (C-11), 14.08 (C-22). APCI-HRMS *m/z* calcd for $C_{27}H_{43}N_2O_5$ 475.3166, found 475.3169 $[M+H]^+$.

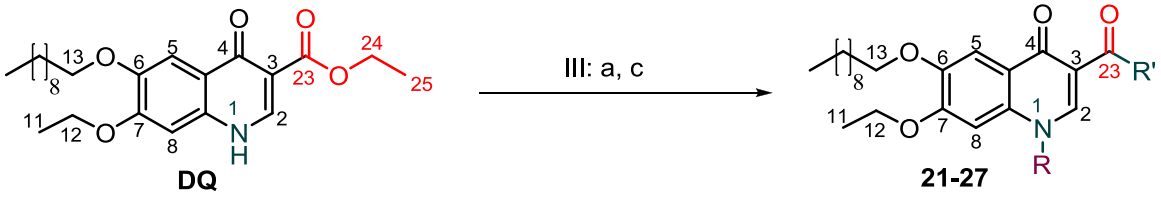
*6-Decoxy-N-[2-(dimethylamino)ethyl]-7-ethoxy-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (**19**)*

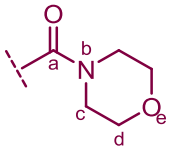
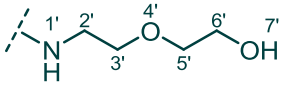
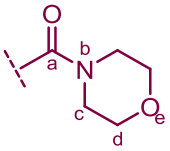
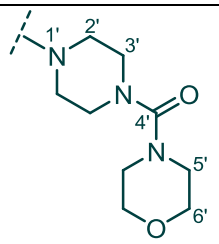
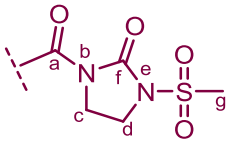
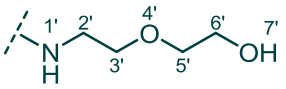
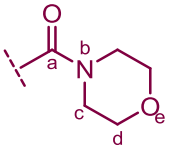
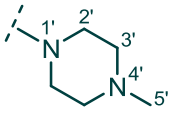
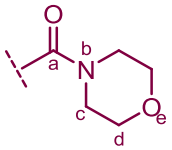
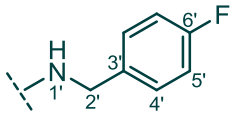
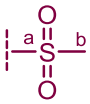
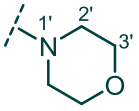
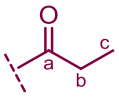
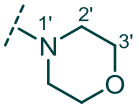
White powder, 0.25 g (51%), m.p. 155-156 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 4.11 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.35 (t, *J* = 5.8 Hz, 1H, H-1'), 8.65 (s, 1H, H-2), 7.83 (s, 1H, H-5), 6.80 (s, 1H, H-8), 4.25 (q, *J* = 7.2 Hz, 2H, H-a), 4.18 (q, *J* = 7.0 Hz, 2H, H-13), 4.11 (t, *J* = 6.8 Hz, 2H, H-12), 3.70 (dt, *J* = 5.8, 6.1 Hz, 2H, H-2'), 2.73 (t, *J* = 6.1 Hz, 2H, H-3'), 2.44 (s, 6H, H-5'), 1.90 – 1.82 (m, 2H, H-14), 1.60 – 1.42 (m, 8H, H-15 - H-18), 1.34 – 1.16 (m, 12H, H-b, H-19 - H-22), 0.93 – 0.75 (m, *J* = 6.8 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.34 (C-4), 165.55 (C-23), 153.54 (C-6), 147.70 (C-7), 145.30 (C-2), 134.13 (C-9), 122.30 (C-10), 111.29 (C-3), 107.57 (C-5), 98.33 (C-8), 69.23 (C-13), 65.04 (C-12), 58.13 (C-3'), 49.13 (C-a), 44.93 (C-5', C-6'), 36.44 (C-2'), 31.87 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-19), 25.92 (C-20), 22.63 (C-21), 14.54 (C-b), 14.51 (C-11), 14.08 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{46}N_3O_4$ 488.3483, found 488.3490 $[M+H]^+$.

*6-Decoxy-7-ethoxy-1-ethyl-4-oxo-N-[2-(piperazin-1-yl)ethyl]-1,4-dihydroquinoline-3-carboxamide (**20**)*

Yellow pellet, 0.3 g (60%), m.p. 145-147 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 97% pure, RT 3.96 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.35 (t, *J* = 5.8 Hz, 1H, H-1'), 8.64 (s, 1H, H-2), 7.83 (s, 1H, H-5), 6.79 (s, 1H, H-8), 4.32 – 4.20 (m, 2H, H-a), 4.17 (q, *J* = 6.9 Hz, 2H, H-12), 4.10 (t, *J* = 6.8 Hz, 2H, H-13), 3.64 (t, *J* = 5.5 Hz, 2H, H-3'), 3.56 (dt, *J* = 5.5, 5.8 Hz, 2H, H-2'), 3.38 – 2.86 (m, 9H, H-4'- H-6'), 1.90 – 1.80 (m, 2H, H-14), 1.60 – 1.40 (m, 10H, H-15- H-19), 1.30 – 1.18 (m, 10H, H-a, H-20 - H-22), 0.85 (t, *J* = 6.9 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.34 (C-4), 165.55 (C-23), 153.54 (C-6), 147.70 (C-7), 145.30 (C-2), 134.13 (C-9), 122.30 (C-10), 111.29 (C-3), 107.57 (C-5), 98.30 (C-8), 69.19 (C-13), 65.05 (C-12), 58.82 (C-4'), 55.73 (C-a), 54.37 (C-5',), 38.93 (C-3'), 36.43 (C-2'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-19), 25.92 (C-20), 22.63 (C-21), 14.54 (C-b), 14.51 (C-11), 14.08 (C-22). APCI-HRMS *m/z* calcd for $C_{30}H_{49}N_4O_4$ 529.3748, found 529.3760 $[M+H]^+$.

Table 4: General procedure for synthesis of *N*-acylated amide derivatives 21-27 of DQ



Cpd	R	R'	Cpd	R	R'
21			**24**		
22			**25**		
23			**26**		
			27		

Aminolysis: To a 100 mL two neck flask containing decoquinate (0.5 g, 1.19 mmol) and DBU (320 µL, 0.33 g, 2.1 mmol, 0.9 eq.) in chloroform (25 mL) was added the amine (5 eq.). The resulting mixture was stirred under reflux for 72 h. Progress of the reactions was monitored by TLC. Upon completion, the reaction mixture was treated with additional chloroform (20 mL) and washed with distilled water (20 mL × 4). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product, which was used in the next step without further purification.

N-acylation: To a 100 mL two neck flask was added 0.5 g of the above crude product, chloroform (15 mL) , DBU (0.7 mmol, 100 µL), and the acyl or carbamoyl chloride (5 eq.). The mixture was stirred under reflux for 15 h, and then washed with aqueous saturated NH_4Cl

solution (20 mL × 3). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to obtain the crude product which was purified by silica gel column chromatography. Elution with CH_2Cl_2/methanol (20:1, v/v) provided the fraction containing the product that was isolated by evaporation of solvent. Derivatives **9-12** were thereby obtained in 50-80% yield.

*6-Decoxy-7-ethoxy-N-[2-(2-hydroxyethoxy)ethyl]-1-(morpholin-4-ylcarbonyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**21**)*

White powder, 0.25 g (48%), m.p. 143-146 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 6.97 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.73 (t, *J* = 5.3 Hz, 1H, H-1'), 8.64 (s, 1H, H-2), 7.39 (s, 1H, H-5), 6.80 (s, 1H, H-8), 4.43 (s, 1H, H-7'), 4.22– 4.17 (m, 8H, H-c, H-d), 4.01 (q, *J* = 7.0 Hz, 2H, H-13), 3.86 (t, *J* = 6.8 Hz, 2H, H-12), 3.83 (t, *J* = 5.3 Hz, 2H, H-2'), 3.71 – 3.60 (m, 6H, H-3', H-5', H-6'), 1.76 (p, *J* = 7.0 Hz, 2H, H-14), 1.46 – 1.19 (m, 17H, H-22, H-15,-H-21), 0.87 (t, *J* = 6.8, Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.76 (C-4), 166.62 (C-23), 153.59 (C-a) 153.46 (C-6), 147.86 (C-7), 141.49 (C-2), 134.73 (C-9), 120.40 (C-10), 110.13 (C-3), 105.39 (C-5), 99.88 (C-8), 72.38 (C-6'), 69.80 (C-d), 68.54 (C-3', C-5'), 66.46 (C-c), 64.63 (C-13), 61.73 (C-12), 31.96 (C-2'), 31.90 (C-14), 29.65 (C-15, C-16) , 29.27 (C-17, C-18), 28.92 (C-19, C-20), 22.67 (C-21), 14.44 (C-11), 14.10 (C-22). ESI-HRMS *m/z* calcd for $C_{31}H_{48}N_30_8$ 590.3436, 590.3380 $[M+H]^+$.

*6-Decoxy-7-ethoxy-N-[2-(2-hydroxyethoxy)ethyl]-1-{[3-(methylsulfonyl)-2-oxoimidazolidin-1-yl]carbonyl}-4-oxo-1,4-dihydroquinoline-3-carboxamide (**22**)*

Yellow powder, 0.29 g (58%), m.p. 143-146 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 96% pure, RT 6.42 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.73 (t, *J* = 5.3 Hz, 1H, H-1'), 8.64 (s, 1H, H-2), 7.39 (s, 1H, H-5), 6.80 (s, 1H, H-8), 4.43 (s, 1H, H-7'), 4.12 (d, *J* = 6.6 Hz, 2H, H-c), 4.08 (d, *J* = 6.6 Hz, 2H, H-d), 4.01 (q, *J* = 7.0 Hz, 2H, H-13), 3.86 (t, *J* = 6.8 Hz, 2H, H-12), 3.83 (t, *J* = 5.3 Hz, 2H, H-2'), 3.71 – 3.60 (m, 6H, H-3', H-5', H-6'), 3.39 (s, 3H, H-g), 1.76 (p, *J* = 6.9 Hz, 2H, H-14), 1.46 – 1.19 (m, 17H, H-22, H-15,-H-21), 0.87 (t, *J* = 6.8, Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.76 (C-4), 166.62 (C-23), 153.59 (C-a) 153.46 C-6), 150.92 (C-f), 147.86 (C-7), 141.49 (C-2), 134.73 (C-9), 120.40 (C-10), 110.13 (C-3), 105.39 (C-5), 99.88 (C-8), 72.38 (C-6'), 68.54 (C-3', C-5'), 64.63 (C-13), 61.73 (C-

12), 41.25 (C-c), 40.69 (C-d), 39.93 (C-g), 31.96 (C-2'), 31.90 (C-14), 29.65 (C-15, C-16), 29.27 (C-17, C-18), 28.92 (C-19, C-20), 22.67 (C-21), 14.44 (C-11), 14.10 (C-22). ESI-HRMS *m/z* calcd for $C_{31}H_{47}N_4O_{10}S$ 667.3007, found 667.2959 $[M+H]^+$.

*6-Decoxy-7-ethoxy-N-(4-fluorobenzyl)-1-(morpholin-4-ylcarbonyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**23**)*

Yellow powder, 0.25 g (50%), m.p. 187-200 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution >95% pure, RT 13.15 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 11.11 (t, *J* = 5.8 Hz, 1H, H-1'), 8.67 (s, 1H, H-2), 7.67 (s, 1H, H-5), 7.28 (dd, *J* = 8.4, 5.3 Hz, 2H, H-5'), 6.94 (t, *J* = 8.5 Hz, 2H, H-4'), 6.81 (s, 1H, H-8), 4.66 (d, *J* = 5.8 Hz, 2H, H-2'), 4.55 (t, *J* = 6.9 Hz, 2H, H-13), 3.40 (t, *J* = 6.9 Hz, 2H, H-12), 4.12– 4.01 (m, 8H, H-c, H-d), 1.84 (q, *J* = 7.4, 6.7 Hz, 4H, H-14,-15), 1.47 – 1.18 (m, 15H, H-11, H-16,-H-21,), 0.92 – 0.82 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.11 (C-4), 166.87 (C-23), 162.83 (C-6'), 153.82 (C-6), 153.59 (C-a), 148.18 (C-7), 141.18 (C-2), 134.77 (C-9), 133.94 (C-3'), 128.93 (C-4'), 120.70 (C-10), 115.51 (C-5'), 110.05 (C-3), 105.92 (C-5), 99.85 (C-8), 69.26 (C-13), 69.19 (C-d), 66.46 (C-c), 64.70 (C-12), 42.72 (C-2'), 31.87 (C-14), 29.53 (C-15, C-16), 29.31 (C-17, C-18), 28.85 (C-19), 25.90 (C-20), 22.66 (C-21), 14.38 (C-11) , 14.10 (C-22). ESI-HRMS *m/z* calcd for $C_{34}H_{45}FN_3O_6$ 610.3287, found 610.3235 $[M+H]^+$.

*6-Decoxy-7-ethoxy-3-{[4-(morpholin-4-ylcarbonyl)piperazin-1-yl]carbonyl}-1-(morpholin-4-yl-carbonyl)quinolin-4(1H)-one (**24**)*

Yellow pellet, 0.37 g (74%), m.p. 167-169 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution >95% pure, RT 10.02 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.99 (s, 1H, H-2), 7.24 (s, 1H, H-5), 6.74 (s, 1H, H-8), 4.26 – 4.20 (m, 2H, H-13), 4.09 (t, *J* = 6.8, 2H, H-12), 3.85 – 3.20 (m, 24H, H-2', H-3', H-5', H-6', H-c, H-d), 1.88 – 1.80 (m, 2H, H-14), 1.54 – 1.42 (m, 6H, H-15 - H-17), 1.30 – 1.16 (m, 11H, H-18 - H-21), 0.91 – 0.79 (m, *J* = 6.8, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.63 (C-4), 165.28 (C-23), 163.53 (C-a), 155.86 (C-4'), 153.82 (C-6), 152.14 (C-7), 138.86 (C-2), 132.52 (C-9), 120.42 (C-10), 118.38 (C-3), 107.22 (C-5), 100.05 (C-8), 69.59 (C-13), 69.25 (C-12), 66.57 (C-c), 66.46 (C-d), 65.94 (C-6'), 49.42 (C-2'), 46.45 (C-3'), 44.65 (C-5'), 31.85 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-

18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 14.49 (C-22), 14.09 (C-11). ESI-HRMS *m/z* calcd for $C_{36}H_{54}N_5O_8$ 684.3967, found 684.3926 $[M+H]^+$.

*6-Decoxy-7-ethoxy-3-[(4-methylpiperazin-1-yl)carbonyl]-1-(morpholin-4-ylcarbonyl)quinolin-4(1H)-one (**25**)*

Brown powder, 0.3 g (60%), m.p. 172-174 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution >95% pure, RT 7.24 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.48 (s, 1H, H-2), 7.24 (s, 1H, H-5), 6.74 (s, 1H, H-8), 4.26 – 4.20 (m, 2H, H-13), 4.09 (t, *J* = 6.8, 2H, H-12), 3.65 – 3.20 (m, 8H, H-2', H-3'), 2.59 – 2.31 (m, 8H, H-c, H-d), 2.10 (s, 3H, H-5') 1.88 – 1.80 (m, 2H, H-14), 1.54 – 1.42 (m, 6H, H-15 - H-17), 1.30 – 1.16 (m, 11H, H-18 - H-21), 0.91 – 0.79 (m, *J* = 6.8, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.68 (C-4), 165.57 (C-23), 153.59 (C-a), 152.54 (C-6), 151.91 (C-7), 145.82 (C-2), 138.72 (C-9), 120.49 (C-10), 117.94 (C-3), 108.59 (C-5), 100.25 (C-8), 69.30 (C-13), 69.19 (C-d), 66.46 (C-c), 64.51 (C-12), 54.49 (C-2'), 46.98 (C-3'), 41.90 (C-5'), 31.85 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 14.49 (C-22), 14.09 (C-11). ESI-HRMS *m/z* calcd for $C_{32}H_{49}N_4O_6$ 585.3647, 585.3604 $[M+H]^+$.

*6-Decoxy-7-ethoxy-3-(morpholin-4-ylcarbonyl)-1-methylsulfonyl-4(1H)-one (**26**)*

Yellow powder, 0.2 g (50%), m.p. 142-144 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 97% pure, RT 7.2 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.00 (s, 1H, H-2), 7.71 (s, 1H, H-5), 6.92 (s, 1H, H-8), 4.18 – 4.11 (m, 4H, H-13, H-12), 4.03 (s, 3H, H-b), 3.74 – 3.03 (m, 8H, H-2', H-3'), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 11H, H-11, H-18,-H-21), 0.87 (m, 3H, H-22). ^{13}C NMR (151 MHz, CDCl3) δ (ppm) :172.83 (C-4), 165.61 (C-23), 153.25 (C-6), 147.71 (C-7), 144.17 (C-2), 140.83 (C-9), 134.49 (C-10), 120.37 (C-3), 107.01 (C-5), 99.63 (C-8), 69.24 (C-13), 67.32 (C-12), 66.79 (C-3'), 47.94 (C-2'), 38.67 (C-b), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 25.89 (C-20), 22.64 (C-21), 14.41 (C-11), 14.08 (C-22). APCI-HRMS *m/z* calcd for $C_{27}H_{41}N_20_7S$ 537.2629, found 537.2549 $[M+H]^+$.

*6-Decoxy-7-ethoxy-3-(morpholin-4-ylcarbonyl)-1-propanoylquinolin-4(1H)-one (**27**)*

Yellow powder, 0.2 g (50%), m.p. 144-146 °C; HPLC 30% ACN/ 70% 0.1% H_3PO_4 gradient elution > 97% pure, RT 8.2 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.00 (s, 1H, H-2), 7.71 (s, 1H, H-5), 6.92 (s, 1H, H-8), 5.75 (p, *J* = 6.9 Hz, 2H, H-b), 4.18 – 4.11 (m, 2H, H-13), 4.13 – 4.03 (m, 2H, H-12), 3.74 – 3.03 (m, 8H, H-2', H-3'), 2.47 – 2.38 (m, 3H, H-c), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 11H, H-11, H-18,-H-21), 0.87 (m, 3H, H-22). ^{13}C NMR (151 MHz, CDCl3) δ (ppm) :172.83 (C-4), 171.24 (C-a), 165.61 (C-23), 153.25 (C-6), 147.71 (C-7), 144.17 (C-2), 140.83 (C-9), 134.49 (C-10), 120.37 (C-3), 107.01 (C-5), 99.63 (C-8), 69.24 (C-13), 67.32 (C-12), 66.79 (C-3'), 47.94 (C-2'), 38.67 (C-b), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 27.03 (C-c), 25.89 (C-20), 22.64 (C-21), 14.41 (C-11), 14.08 (C-22). APCI-HRMS *m/z* calcd for $C_{29}H_{43}N_20_6$ 515.3024, found 515.3116 $[M+H]^+$.

Abbreviations

FDA Food and Drug Administration; DBU 1,8-diazabicycloundec-7-ene; DCM dichloromethane; TLC thin layer chromatography; MS mass spectrometry; NMR nuclear magnetic resonance; eq. equivalents; HRMS high resolution mass spectrometry; APCI atomic pressure chemical ionization; ESI electrospray ionization.

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Chapter 6

New decoquinate derivatives with improved solubilities and in vitro antimalarial activities

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Chapter 6 is a manuscript to be submitted to European journal of medicinal chemistry. The manuscript contains the background, aim, syntheses, in vitro anti-malarial and cytotoxicity evaluation of all compounds synthesised. This manuscript is prepared according to author's guidelines from the journal homepage at https://www.elsevier.com/journals/european-journal-of-medicinal-chemistry/0223-5234/guide-for-authors. Supporting data for chapter 6 are found in addendum C.

New decoquinate derivatives with improved solubilities and *in vitro* antimalarial activities

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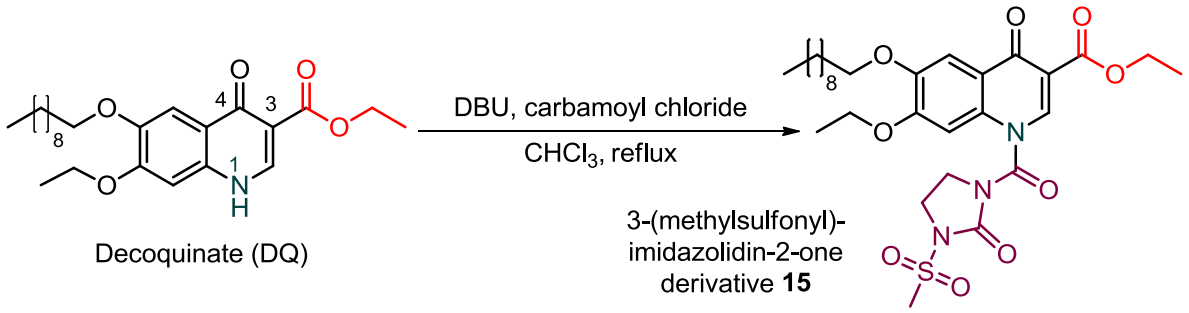
Abstract

Although the old coccidiostat decoquinate (DQ) possesses good anti-malarial activity both *in vitro* and *in vivo*, it suffers from very low solubility, a property that militates against development as an anti-malarial drug. We herewith report on the synthesis of new decoquinate derivatives that have improved solubilities and anti-malarial activities. Polar functional groups were incorporated into DQ in straightforward fashion to generate derivatives that are less lipophilic than DQ, and soluble in ethanol. These derivatives were screened *in vitro* against chloroquine-sensitive NF54 and multidrug-resistant K1 and W2 *Plasmodium falciparum*. Cytotoxicity was assessed by using the human fibroblast cell line (WI-38). The new DQ derivatives had *in vitro* anti-malarial activities superior to those of DQ, chloroquine, artemether, and artesunate. Compound **15**, featuring a 3-(methylsulfonyl)imidazolidin-2-one attached to N-1, is the most active with IC_{50} values of 1-1.4 nM against all three *P. falciparum* strains, a resistance index of less than 1 between all strains and a selectivity index greater than 7×10^5. This compound therefore will likely show no cross resistance with chloroquine and mefloquine.

Key word: malaria, anti-malarial drugs, quinolones, decoquinate, derivatives,

Graphical abstract



IC_{50} nM: *Pf* NF54 26.6; K1 64.9; W2 15.4
IC_{50} μM: WI-38 >100; SI >3759

IC_{50} nM: *Pf* NF54 1.4; K1 1.3; W2 1.1
IC_{50} μM WI-38 >100; SI >7 x 10^5

Introduction

There were 214 million malarial cases, and 438,000 reported deaths attributed to malaria in 2015. 88% of these cases and 90% of the deaths occurred in Sub-Sahara Africa, where a child under the age of five is lost to malaria every two minutes.[1] Although the number of people dying from malaria has reduced over the past fifteen years,[1-3] this disease still presents a great global health burden that has been aggravated with the emergence and spread of parasites resistant to all current treatment options, including artemisinins.[4-7]

The global malaria eradication program launched by WHO in 1955 and based essentially on the use of chloroquine (CQ) [8], resulted in substantial reduction of incidence of malaria. However, the emergence and spread of CQ resistant malaria parasites by 1970s greatly jeopardised the efficacy of this drug, leading to a resurgence of mortality and morbidity.[9-11] In a bid to overcome CQ resistance, piperaquine, an analogue of CQ, was later developed,[12] but resistance has also emerged against this analogue, [13] Newer drugs including mefloquine, pyronaridine, atovaquone, pyrimethamine/sulfadoxine were introduced, but here also, resistance has developed.[14-16]

Artemisinin and its derivatives, referred to as artemisinins, are highly active anti-malarial agents that are fast acting, also against parasites resistant to other drugs.[17] However, artemisinins have half-lives of less than three hours; this results in recrudescence of the disease if treatment regimens are too short to remove residual parasites.[18] To overcome this problem, and also to avoid the development of malaria parasites resistant to artemisinins, the WHO adopted and introduced artemisinins combined with partner drugs that have longer half-lives, so-called artemisinin combination therapies (ACTs) as the standard for malaria treatment.[19, 20, 21] There is now well documented resistance for most partner drugs in ACTs, most of which act on the asexual blood stages of the malaria parasite, which are prone to mutations.[22] Artemisinin derivatives on the other hand act by generating reactive oxygen species (ROS).[23] It is important to note that ROS causes dormancy of ring stage malaria parasites, which has also been linked to the development of malaria parasites resistant to ACTs.[24] Therefore, the deep-seated resistance against established drugs like chloroquine and fansidar and the reduced susceptibility of the parasite to ACTs place urgent demand upon the development of new drugs to control the disease.

It is important to adopt measures that assist in the elimination and eradication of malaria. Chemotherapeutic agents with activity against the liver and gametocyte stages have been

highlighted as tools necessary for malaria elimination and eradication. The malaria eradication agenda put forth in 2007 by the Bill and Melinda Gates Foundation highlighted the liver and gametocyte stages as important targets which need to be considered in malarial drug discovery projects aimed at complete eradication of the disease.[25] As hepatic stage parasites are less numerous and less rapidly replicating than the blood phase, it is assumed a drug that kills the liver stages parasites will prevent development of the ensuing blood stage parasites including gametocytes. This approach might prevent development of the disease and its transmission. The profile of such a drug is termed target candidate profile (TCP) 4 or 3a by the Medicines for Malaria Venture (MMV), Geneva.[26] As gametocytes are stages transmitted from infected humans to mosquitos, drugs that kill gametocytes will prevent transmission of the disease. The profile of such a candidate drug is termed target candidate profile 3b.[26] It is also been recommended that any new candidate drug be cheap (less than $5 per dose), safe, and have a novel structure and mode of action that differ from other anti-malarial drugs currently used in the clinic.[27, 28]

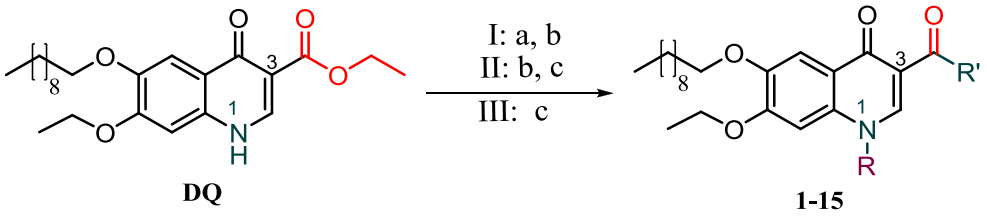
Decoquinate (DQ) is a 4(1*H*)-quinolone that has been used for many years in veterinary medicine to treat coccidiosis.[29] DQ is relatively non-toxic, is cheap, ≤$10 per kg,[30] and should be more affordable than other quinolones proposed for further development as anti-malarial drugs. It has potent activity *in vitro* against the liver, blood, and gametocytes stages of the malaria parasite.[31, 32] With this activity profile, DQ essentially meets the criteria for a drug candidate required for malaria eradication. However, DQ like other anti-malarial quinolone hit and lead compounds including ELQ 300,[33] suffers from exceedingly poor aqueous solubility (0.06 μg L^{-1}).[34] We therefore have embarked on an exploratory programme evaluating the effects of acylation of N-1 through attachment of polar groups, and through modification of the ester group in the C-3 carbethoxy group of DQ on the solubility and anti-malarial activity of DQ. We herein report the preparation, *in vitro* anti-malarial activities against the blood stages of the malaria parasite, and cytotoxicity of the new DQ derivatives.

Results

Chemistry

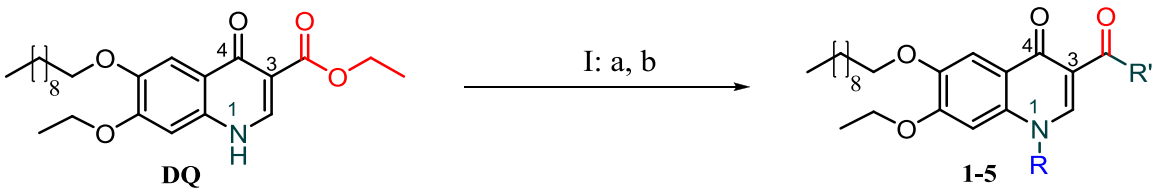
The three routes used for conversion of DQ into the new derivatives **1-15** are summarized in Scheme 1.

Scheme 1. Three synthetic routes (I, II, III) followed for preparation of the derivatives.



Reagents and conditions: a. alkyl halide, K_2CO_3, $CHCl_3$/THF (3:1 v/v), reflux, 10 h; b: amine, DBU, $CHCl_3$, reflux, 24 h; c: acyl chloride, DBU, $CHCl_3$, reflux, 24h.

DQ was treated with each of ethyl and propyl bromides in the presence of potassium carbonate (route I, Scheme 1) to give the *N*-alkylated intermediates,[35] which without purification were submitted to ester aminolysis by the primary amine in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [36] to provide the derivatives **1-5** (Fig. 1**)** in yields greater than 80%. The presence of the *N*-alkyl group and the secondary amide were readily recognized from spectroscopic data; in particular the presence of the secondary amide was confirmed by the presence of the triplet (J = 5.3 Hz) at δ *ca* 11.0 ppm in their ^{1}H NMR spectra.

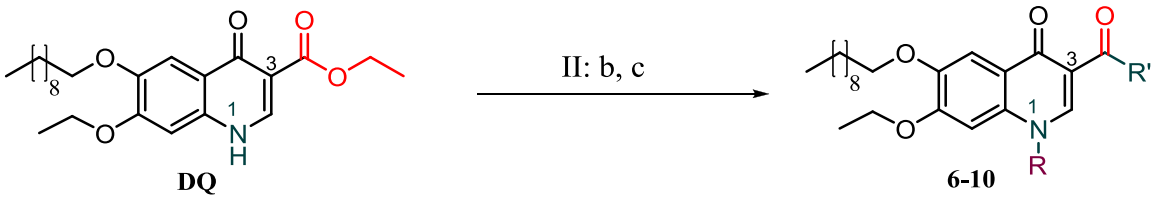


Cpd	R	R'
1		
2		
3		
4		
5		

Figure 1: *N*-alkylated amide derivatives obtained from decoquinate (**DQ**) by treatment with K_2CO_3 and alkyl halides, followed by treatment with a primary amine and DBU in chloroform according to route I, Scheme 1.

Next, compounds **6-10** were prepared via route II (Scheme 1). Ester aminolysis of DQ with primary or secondary amines in the presence of DBU [36] generated the secondary or tertiary amide intermediates that upon treatment with an acyl or carbamoyl chloride in the presence of DBU were converted into the products **6-10** in moderate overall yields (to 60%) (Fig. 2**)**. Whilst compounds were readily characterized by spectroscopic data, it is noted that the ^{13}C NMR spectra of these compounds show a signal at *ca.* δ 153 ppm. This is assigned to the carbonyl carbon (C-a) of the acyl or carbamoyl derivative; this indicates that *N*-acylation or carbamoylation, rather that formation of an ester or carbamate via reaction through O-4 took

place. Unlike the secondary amides **1-7**, the tertiary amides **8-10** lack the signal at 11.3 ppm in their ^{1}H NMR spectra.



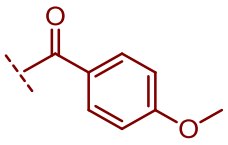
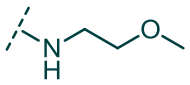
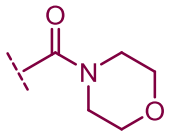
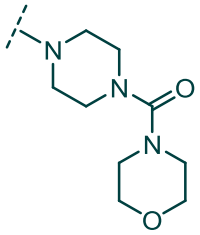
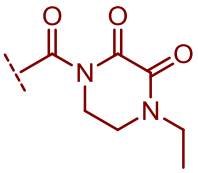
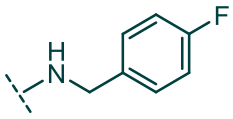
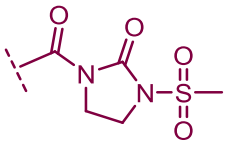
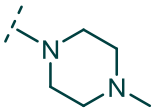
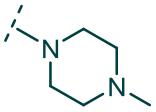
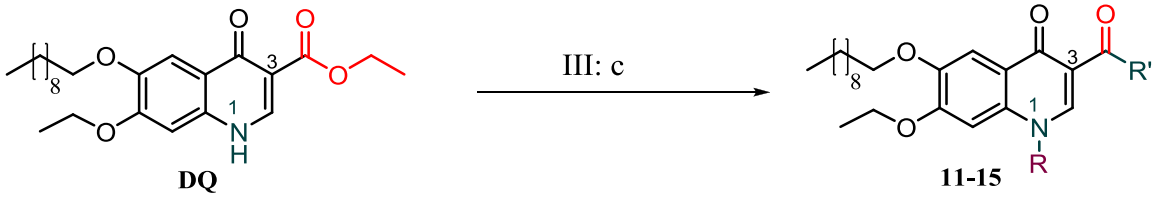
Cpd	R	R'	Cpd	R	R'
6			**8**		
7			**9**		
			10		

Figure 2: *N*-Acylated and *N*-carbamoylated amide derivatives obtained from decoquinate (DQ) by treatment with DBU and the acyl or carbamoyl chloride, followed by treatment with amine and DBU in chloroform according to route II, Scheme 1.

DQ was treated with 2- methoxyethanol in the presence of sulfuric acid to generate the transesterified intermediates. Without purification, these were treated with a carbamoyl chloride in the presence of DBU according to route III **(**Scheme 1) to give derivatives **11-13** (Fig. 3) in overall yields of approximately 50%. Alternatively, DQ itself was submitted to carbamoylation under the foregoing condition to generate derivatives **14** and **15** in yields greater than 75%. Once

again, the derivatives were readily characterized by spectroscopic data. Compounds **11-13** contain a singlet at approximately 3.4 ppm in their ^{1}H NMR spectra due to the methoxyl group at C-5' in the ester side chain. The ^{13}C NMR spectra of all compounds show a signal at δ *ca.* 153 ppm assigned to the carbonyl carbon (C-a) of the *N*-acyl or -carbamoyl derivative. As above, *N*-acylation or carbamoylation, rather that formation of an ester or carbamate via reaction through O-4 took place.



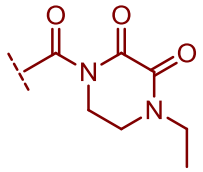
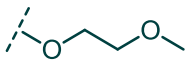
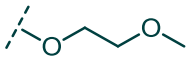
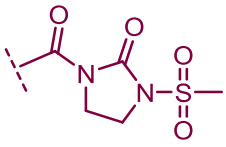
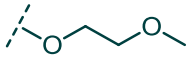
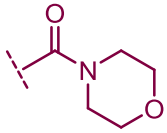
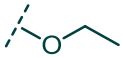
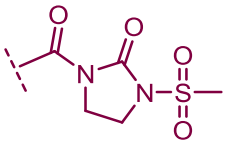
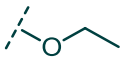
Cpd	R	R'	Cpd	R	R'
11			**13**		
12			**14**		
			15		

Figure 3: *N*-carbamoylated and acylated derivatives of decoquinate obtained from decoquinate (DQ) by treatment with DBU and acyl or carbamoyl chloride according to route III, Scheme 1 and transesterification (compounds **11-13**).

For all derivatives, the ^{1}H spectra display a singlet at *ca.* δ 8.2 ppm assigned to H-2, two singlets at δ 6.8-7.5 ppm assigned to H-5 and H-8 of the of the quinolone nucleus, two triplets (*J*

= 7.0, 6.9 Hz) at 4.0-4.4 ppm ascribed to H-13 and H-12 in the alkoxy chains attached to C-6 and C-7 respectively. The ^{13}C NMR spectra contain a signal at ~175 ppm assigned to the ketone carbonyl at C-4 and a signal at ~165 ppm assigned to the carbonyl carbon atom C-23. The signals at ~ 65 and 69 ppm are due to C-12 and C-13 in the alkoxy chains attached to the quinolone nucleus. All compounds were established to have greater than 95% purity using analytical HPLC methods.

In notably straightforward fashion, we have transformed DQ into new derivatives by aminolysis or ester exchange of the C-3 carbethoxy group and attachment of substituents to N-1. All derivatives, as adjudged by their ability to dissolve in common solvents like dichloromethane, ethanol and dimethyl sulfoxide are more tractable than DQ.

***In vitro* anti-malarial activity and cytotoxicity**

All compounds were screened *in vitro* against the intraerythrocytic stages of CQ sensitive NF54, CQ and antifolate resistant K1, and CQ and mefloquine resistant W2 *P. falciparum* (*Pf*). The IC_{50} values (concentrations resulting in 50% inhibition of parasite proliferation over 96 h) are presented in Table 1. Alkylated amide derivatives **1-7** of DQ were generally less active than DQ, while acylated tertiary amide derivatives **8-10** have activities comparable to that of DQ but were less active than artemether and artesunate. The *N*-acylated derivatives **11-15** retaining the carbethoxy group of DQ show anti-malarial activity superior to DQ, artemether, artesunate against sensitive and multi-drug resistant *Pf*. Cytotoxicity of the compounds was evaluated using a sulforhodamine B (SRB) assay on the WI-38 cell line with emetine as control. Biological data are summarized in Table 1. Also included in Table 1 are calculated Log P data for DQ and the new derivatives. It can be seen that all derivatives are appreciably less lipophilic than DQ.

Table 1. In vitro biological data of comparator drugs, DQ and the new derivatives and calculated LogP data. Data are from at least three independent biological experiments each performed in triplicate ±S.E**.**

Compd	ClogP	Antimalarial activity IC_{50}±SEM (nM)			Resistance Index		Cytotoxicity, IC_{50} (μM)	Selectivity Index
		NF54	K1	W2	RI[a]	RI[b]	WI-38	SI[d]
ARM	nd	5.77±1.33	7.26±2.34	4.5±0.78	1.3	0.8	nd	nd
ARS	nd	7.88±1.35	8.97±2.26	6.77±1.13	1.4	0.8	>100	>12690
CQ	nd	139±13.5	154.7±14.9	233.18±49	1.1	1.7	nd	nd
DQ	8.19	26.6±1.4	64.9±8.8	15.4±3.4	2.4	0.6	>100	>3759
1	5.5	>200	>200	>200	nd	nd	54.9	nd
2	5.3	156.9±10.6	197.7±41.4	194.5±48.3	1.3	1.2	41.3	263
3	6.3	159.6±20.3	>200	189.9±51.6	1.9	1.2	37.6	235
4	4.7	>200	>200	>200	nd	nd	40.1	nd
5	5.6	>200	>200	>200	nd	nd	83.4	nd
6	6.0	>200	>200	>200	nd	nd	64	nd
7	5.0	>200	>200	>200	nd	nd	>100	nd
8	3.2	92.1±49.3	65.35±22.3	19.8±1.2	1.2	0.2	40.1	435
9	2.7	21.9±11.7	16.8±5.9	9.1±1.1	0.7	0.4	56.5	2579
10	4.9	90.8±31.3	128.1±48.5	67.7±25.8	1.4	0.7	>100	>1111

Compd	ClogP	Antimalarial activity IC_{50}±SEM (nM)			Resistance Index		Cytotoxicity, IC_{50} (μM)	Selectivity Index
		NF54	K1	W2	RI[a]	RI[b]	WI-38	SI[d]
11	3.5	93.1±35.4	80.5±34.1	47.1±2.9	0.8	0.5	>100	>1074
12	2.9	9.6±2.9	15.0±5.6	7.4±1.9	1.5	0.7	>100	>10416
13	5.2	136.0±41.9	>200	84.4±28.1	2.0	0.6	>100	>735
14	5.0	6.8±3.9	14.4±6.3	5.7±3.5	2.0	0.8	>100	>14705
15	3.8	1.4±0.5	1.3±0.6	1.1±0.1	0.9	0.7	>100	>71428
Emetine	nd	nd	nd	nd	nd	nd	0.05	nd

ARM = artemether; ARS = artesunate; CQ= chloroquine; DQ = decoquinate;

[a] resistance index (RI) = IC_{50} K1/IC_{50} NF54;

[b] resistance index (RI) = IC_{50} W2/IC_{50} NF54;

[c] WI-38 cell line of normal human fetal lung fibroblast;

[d] Selectivity index (SI) = (IC_{50} WI-38-HFLF/IC_{50} NF54).

Discussion

In vitro anti-malarial activity and cytotoxicity

Initial screens were conducted against asexual NF54 *P. falciparum* parasites to determine their respective % inhibition at 5 μM and 1 μM. Compounds showing the highest % inhibition against parasite proliferation (>50% inhibition at 5 μM and >70% inhibition at 1 μM) were submitted for further screening with second dual point assay at 500 nM and 100 nM. At these concentrations, compounds showing near complete inhibition of parasite proliferation were selected for IC_{50} determination against asexual CQ-sensitive (NF54), and multidrug resistant–K1 (chloroquine, pyrimethamine, mefloquine, and cycloguanil) and W2 (chloroquine, quinine,

pyrimethamine, cycloguanil) stages of *P. falciparum*. Chloroquine, artemether, artesunate and decoquinate served as controls.

Despite the fact that *N*-alkylation coupled with conversion of DQ into an amide provides compounds having lower lipophicity (as expressed in cLogP values) than DQ (*e.g.* **2** and **4**, Table 1), these were generally less active than DQ against all of the *P. falciparum* strains. On the contrary, conversion of DQ into a tertiary amide and acylation at N-1 resulted in derivatives that are less lipophilic, and have varied anti-malarial activities in comparison with DQ. For example compound **8** was less active than DQ against NF54, but was equipotent with DQ against K1 and W2. Compound **9** was equipotent with DQ against NF54 but was superior against K1 and W2. *N*-Acylated derivatives retaining the C-3 carbethoxy group were generally more active than both DQ and the amides. Compound **15** (IC_{50} 1 nM) displayed superior activity against all strains, being 26-fold more active than DQ and five fold more potent than artesunate and artemether against NF54 (Table 1). Additionally, compounds with cLogP values <3.8 (Table 1) displayed overall the highest anti-malarial activity (**9, 12** and **15**).

Compounds **8**, **9** and **15** all have resistant indices below 1 indicating that these compounds display higher activity against multi-drug resistant *Pf* than CQ sensitive *Pf*. All active compounds (**2, 3, 8-15**) in this study have resistant indices below 2. This suggests that these compounds may have low propensity to develop cross resistance with chloroquine, mefloquine and the antifolates currently in clinical use.

Decoquinate and the active hits showed no appreciable toxicity to mammalian WI-38 cells. Their IC_{50} values are all substantially greater than the IC_{50} of 0.05 μM of emetine. The compounds are thus selective in their anti-malarial action as evidenced by the SI values ≥400 (Table 2). More importantly, the optimum compound **15** exhibits a selectivity index of $>7 \times 10^5$, a potentially important result in relation to future development of this compound as a potent and highly selective antimalarial drug.

Conclusion

Decoquinate shows potent activity against different stages of the malaria parasite. However, poor aqueous solubility of this compound prevents routine screening and evaluation of pharmacokinetic parameters. In an effort to circumvent the poor aqueous solubility and optimize anti-malarial activity, fifteen new decoquinate derivatives were prepared and screened *in vitro*

for anti-malarial activity and cytotoxicity. To summarize, all *N*-alkylated amide derivatives **1-5** are less active than decoquinate; the *N*-acylated amide derivatives **6-10** are generally more soluble in polar solvents and more active than decoquinate against all malaria parasite strains; the *N*-acylated esters **11-15** are soluble and generally the most active in this series. *N*-Carbamoylation with a sulfonyl containing moiety increases anti-malarial activities. Compounds **9, 12, 14**, and **15** will be evaluated for activity against liver and gametocytes stages of the malarial parasite and *in vivo* in rodent malarial models.

Experimental section

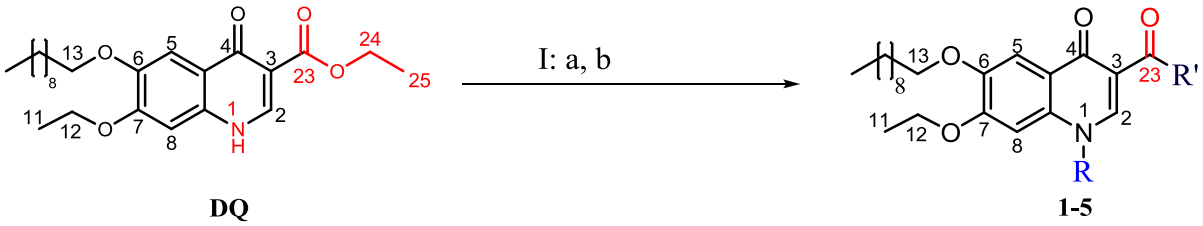
General procedures

Amines, 2-methoxyethanol, the acyl, carbamoyl and alkyl halides in this study were bought from either Sigma Aldrich or AKSci and were used as supplied, decoquinate was bought from Hohance chemical. NMR spectra were obtained on a 600 MHz Bruker Avance™ III spectrometer as solutions in deuterated solvents ($CDCl_3$, or DMSO-d_6). All chemical shifts (δ) are reported in parts per million (ppm) values. ^1H chemical shifts are reported downfield of tetramethylsilane (TMS), and were internally referenced to the residual proton in $CDCl_3$ (7.26 ppm), or DMSO-d_6 (2.50 ppm). ^{13}C chemical shifts were internally referenced to resonances in $CDCl_3$ (δ 77.16 ppm), or DMSO-d_6 (δ 39.51 ppm). Peak multiplicities are abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), and m (multiplet). Coupling constant (*J*) are reported in Hz. NMR data were analyzed using MestReNova Software, version 5.3.2-4936.

Exact mass measurements were recorded on Bruker MicroTOF Q II mass spectrometer with an APCI/ESI source set at 300 °C, using Bruker Compass Data analysis 4.0 software. A full scan, ranging between 50–1500 *m/z*, was generated at a capillary voltage of 4500 V, end plate offset voltage of -500 V, and a collision cell RF voltage of 100 V. Melting points (mp) were determined with a Büchi melting point B-545 instrument and are uncorrected.

Thin layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$), acquired from Merck, and components were visualized by ultraviolet light (254 nm). Silica gel 230-400 (particle size 40-63μm) mesh was used for all flash column chromatography. Calculated LogP (cLogP) values were generated using ACD/ChemSketch free ware version 14.02. Purity was determined by HPLC using a solvent gradient of 30% ACN/ 70% 0.1% H_3PO_4 and a Venusil XBP C18 column of size 150 × 4.6 mm.

Table 2. General procedure for synthesis of *N*-alkylated amide derivatives 1-5 of DQ



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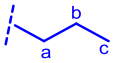
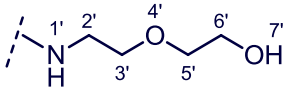
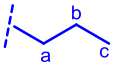
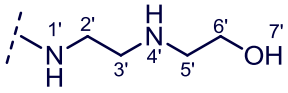
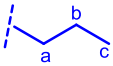
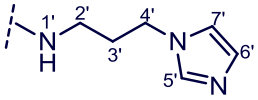
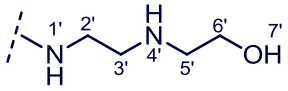
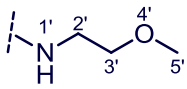
1		
2		
3		
4		
5		

N-alkylation: A mixture of DQ (1.19 mmol, 0.5 g, 1 eq), K_2CO_3 (5.0 mmol, 0.69 g), alkyl halide (5 eq.) in DMF (10 mL) was refluxed for 15 h, after which the mixture was evaporated to dryness, dissolved in chloroform and washed with distilled water (20 mL × 4). The organic phase was evaporated to dryness to obtain the crude *N*-alkylated product which was used in the ensuing step without purification.

Carboxyamidation: A mixture of the crude *N*-alkylated product (0.5 g, 1 eq.), DBU (320 µL, 0.33 g, 2.1 mmol), the appropriate amine (5 eq.), and chloroform (15 mL) in a 100 mL round bottom flask was stirred under reflux for 24-72 h. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was treated with additional chloroform (20 mL) and washed with distilled water (20 mL × 4). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product. This was submitted to

chromatography on silica gel. Elution with CH_2Cl_2/methanol (10:1, v/v) provided the fraction containing the product that was isolated by evaporation of solvent. The product was recrystallized from ethyl acetate and air dried.

*6-Decoxy-7-ethoxy-N-[2-(2-hydroxyethoxy)ethyl]-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxamide (**1**)*

White powder, 0.38 g (76%), m.p. 162-164 °C; Purity > 98%, RT 4.30 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.45 (t, *J* = 5.4 Hz, 1H, H-1'), 8.61 (s, 1H, H-2), 7.80 (s, 1H, H-5), 6.76 (s, 1H, H-8), 4.33 – 3.96 (m, 6H, H-12, H-13, H-a), 3.74 – 3.48 (m, 4H, H-6', H-2'), 2.95 – 2.75 (m, 4H, H-5', H-3'), 2.37 (s, 1H, H-7'), 2.00 – 1.76 (m, 4H, H-b, H-14), 1.59 – 1.37 (m, 4H, H-15, H-16), 1.35 – 1.17 (m, 13H, H-c, H-17 - H-21), 0.98 (t, *J* = 7.4 Hz, 3H, H-11), 0.91 – 0.67 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.35 (C-4), 165.77 (C-23), 153.54 (C-6), 147.78 (C-7), 145.88 (C-2), 134.32 (C-9), 122.22 (C-10), 110.87 (C-3), 107.40 (C-5), 98.59 (C-8), 69.60 (C-13), 64.98 (C-12), 60.94 (C-6'), 55.79 (C-a), 50.66 (C-5'), 48.49 (C-3'), 38.89 (C-2'), 31.86 (C-b), 29.52 (C-14), 29.49 (C-15), 29.30 (C-16), 29.28 (C-17), 28.87 (C-18), 25.92 (C-19), 22.64 (C-20), 22.09 (C-21), 14.51 (C-11), 14.08 (C-c), 11.08 (C-22). APCI-HRMS *m/z* calcd for $C_{29}H_{47}N_2O_6$ 519.3429, found 519.3431 $[M+H]^+$.

*6-Decoxy-7-ethoxy-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-1-propyl-1,4-dihydroquinoline-3-carboxamide (**2**)*

White powder, 0.4 g (80%), m.p. 152-153 °C; Purity > 98% , RT 4.70 min; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.21 (t, *J* = 5.5 Hz, 1H, H-1'), 8.68 (s, 1H, H-2), 7.66 (s, 1H, H-5), 7.13 (s, 1H, H-8), 4.47 (s, 1H, H-7'), 4.41 (t, *J* = 7.0 Hz, 2H, H-13), 4.23 (t, *J* = 6.9 Hz, 2H, H-12), 4.08 – 3.98 (m, 2H, H-a), 3.46 (dt, 2H, *J* = 5.5, 6.2 Hz, H-2'), 3.38 (t, *J* = 5.7 Hz, 2H, H-6'), 2.68 (t, *J* = 6.2 Hz, 2H, H-3'), 2.60 (t, *J* = 5.7 Hz, 2H, H-5'), 2.37 (s, 1H, H-4'), 1.83 – 1.71 (m, 4H, H-b, H-14), 1.49 – 1.33 (m, 4H, H-15, H-16), 1.30 – 1.18 (m, 13H, H-c, H-17 - H-21), 0.85 (dt, *J* = 13.8, 6.9 Hz, 6H, H-11, H-22). ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 175.35 (C-4), 165.77 (C-23), 153.54 (C-6), 147.78 (C-7), 145.88 (C-2), 134.32 (C-9), 122.22 (C-10), 110.87 (C-3), 107.40 (C-5), 98.59 (C-8), 69.60 (C-13), 64.98 (C-12), 60.94 (C-6'), 55.79 (C-a), 50.66 (C-5'), 48.49 (C-3'), 38.89 (C-2'), 31.86 (C-b), 29.52 (C-14), 29.49 (C-15), 29.30 (C-16),

29.28 (C-17), 28.87 (C-18), 25.92 (C-19), 22.64 (C-20), 22.09 (C-21), 14.51 (C-11), 14.08 (C-c), 11.08 (C-22). APCI-HRMS *m/z* calcd for $C_{29}H_{48}N_3O_5$ 518.3588, found 518.3573 $[M+H]^+$.

*6-Decoxy-7-ethoxy-N-[3-(1H-imidazol-1-yl)propyl]-4-oxo-1-propyl-1,4-ihydroquinoline-3-carboxamide (**3**)*

White powder, 0.25 g (50%), m.p. 168-169 °C; Purity > 96% , RT 7.10 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.39 (t, *J* = 5.8 Hz, 1H, H-1'), 8.72 (s, 1H, H-2), 7.82 (s, 1H, H-5'), 7.55 (s, 1H, H-7'), 7.24 (s, 1H, H-6'), 7.03 (s, 1H, H-5), 6.79 (s, 1H, H-8), 408 –4.30 (dt, *J* = 13.2, 7.4 Hz, 6H, H-13, H-12, H-a), 4.04 (t, *J* = 7.1 Hz, 2H, H-4'), 3.46 (dt, *J* = 5.8, 5.8 Hz, 2H, H-2'), 2.16 – 2.04 (dt, *J* = 7.1, 5.8 Hz, 2H, H-3'), 1.96 – 1.80 (m, 4H, H-b, H-14), 1.62 – 1.46 (m, 4H, H-15, H-16), 1.39 – 1.31 (m, 2H, H-17), 1.24–1.03 (m, 11H, H-c, H-18 - H-21), 0.99 (t, *J* = 7.4 Hz, 3H, H-11), 0.91 – 0.72 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.41 (C-4), 165.80 (C-23), 153.64 (C-6), 147.89 (C-7), 145.87 (C-2), 137.14 (C-5'), 134.38 (C-9), 129.37 (C-7'), 122.17 (C-10), 118.91 (C-6') 110.67 (C-3), 107.26 (C-5), 98.55 (C-8), 69.21 (C-13), 65.03 (C-12), 55.85 (C-a), 44.54 (C-4'), 35.87 (C-2'), 31.84 (C-3'), 31.44 (C-14), 29.51 (C-15), 29.49 (C-16), 29.28 (C-17), 29.27 (C-18), 28.83 (C-b), 25.90 (C-19), 22.63 (C-20), 22.24 (C-21), 14.50 (C-11), 14.07 (C-c), 11.08 (C-22). APCI-HRMS *m/z* calcd for $C_{31}H_{47}N_4O_4$ 539.3592, found 539.3551 $[M+H]^+$.

*6-Decoxy-7-ethoxy-1-ethyl-N-{2-[(2-hydroxyethyl)amino]ethyl}-4-oxo-1,4- dihydroquinoline-3-carboxamide (**4**)*

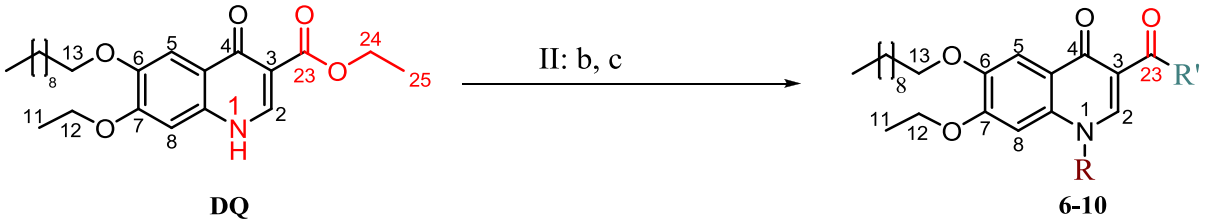
White powder, 0.38 g (76%), m.p. 154-156 °C; Purity > 96% , RT 4.09 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.50 (t, *J* = 5.8 Hz, 1H, H-1'), 8.65 (s, 1H, H-2), 7.81 (s, 1H, H-5), 6.78 (s, 1H, H-8), 4.18- 4.00 (ddd, *J* = 28.9, 13.0, 6.9 Hz, 6H, H-a, H-13, H-12), 3.86 – 3.71 (m, 2H, H-6'), 3.69 – 3.53 (m, 6H, H-2', H-3', H-5'), 3.30 (s, 1H, H-7'), 1.92 – 1.72 (m, 2H, H-14), 1.56 – 1.38 (m, 8H, H-11, H-22, H-15), 1.36 – 1.16 (m, 12H, H-16 - H-21), 0.84 (t, *J* = 6.9 Hz, 3H, H-b). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.33 (C-4), 165.50 (C-23), 153.64 (C-6), 147.79 (C-7), 145.26 (C-2), 134.15 (C-9), 122.28 (C-10), 111.31 (C-3), 107.56 (C-5), 98.27 (C-8), 72.51 (C-5'), 69.85 (C-3'), 69.18 (C-13), 65.01 (C-12), 61.79 (C-6'), 49.11 (C-a), 38.81 (C-2'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-19), 25.92 (C-

20), 22.63 (C-21), 14.54 (C-b), 14.50 (C-11), 14.07 (C-22). APCI-HRMS *m/z* calcd for $C_{28}H_{46}N_3O_5$ 504.3432, found 504.3426 $[M+H]^+$.

*6-Decoxy-7-ethoxy-1-ethyl-N-(2-methoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**5**)*

White powder, 0.4 g (80%), m.p. 157-159 °C; Purity > 96% , RT 4.33 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.35 (t, *J* = 5.5 Hz, 1H, H-1'), 8.66 (s, 1H, H-2), 7.84 (s, 1H, H-5), 6.79 (s, 1H, H-8), 4.32 – 4.20 (m, 2H, H-a), 4.17 (q, *J* = 6.9 Hz, 2H, H-12), 4.10 (t, *J* = 6.8 Hz, 2H, H-13), 3.64 (t, *J* = 5.5 Hz, 2H, H-3'), 3.56 (dt, *J* = 5.5, 5.5 Hz, 2H, H-2'), 3.38 (s, 3H, H-5'), 1.90 – 1.80 (m, 2H, H-14), 1.60 – 1.40 (m, 10H, H-15- H-19), 1.30 – 1.18 (m, 10H, H-a, H-20 - H-22), 0.85 (t, *J* = 6.9 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.34 (C-4), 165.55 (C-23), 153.54 (C-6), 147.70 (C-7), 145.30 (C-2), 134.13 (C-9), 122.30 (C-10), 111.29 (C-3), 107.57 (C-5), 98.30 (C-8), 71.34 (C-4'), 69.19 (C-13), 65.05 (C-12), 58.82 (C-3'), 49.06 (C-a), 38.93 (C-2'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-19), 25.92 (C-20), 22.63 (C-21), 14.54 (C-b), 14.51 (C-11), 14.08 (C-22). APCI-HRMS *m/z* calcd for $C_{27}H_{43}N_2O_5$ 475.3166, found 475.3169 $[M+H]^+$.

Table 3. General procedure for synthesis of *N*-acylated amide derivatives 6-10 of DQ



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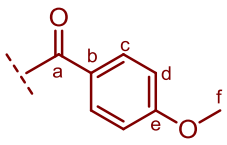
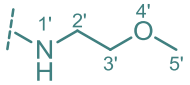
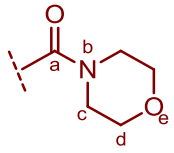
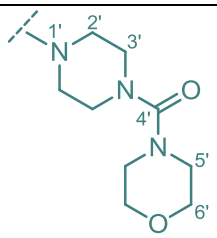
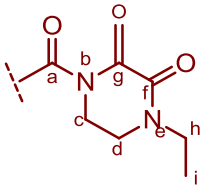
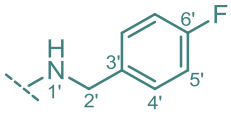
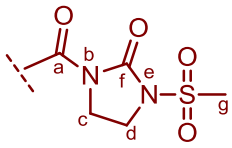
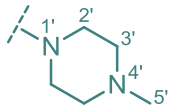
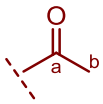
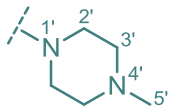
6			**8**		
7			**9**		
			10		

Carboxyamidation: To a 100 mL two neck flask containing decoquinate (0.5 g, 1.19 mmol) and DBU (320 µL, 0.33 g, 2.1 mmol, 0.9 eq.) in chloroform (25 mL) was added the amine (5 eq.). The resulting mixture was stirred under reflux for 72 h. Progress of the reactions was monitored by TLC. Upon completion, the reaction mixture was treated with additional chloroform (20 mL) and washed with distilled water (20 mL × 4). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product, which was used in the next step without further purification.

N- acylation: To a 100 mL two neck flask was added 0.5 g of the above crude product, chloroform (15 mL) , DBU (0.7 mmol, 100 µL), and the acyl or carbamoyl chloride (5 eq). The mixture was stirred under reflux for 15 h, and then washed with aqueous saturated NH_4Cl solution (20 mL × 3). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to obtain the crude product which was purified by silica gel column

chromatography. Elution with CH_2Cl_2/methanol (20:1, v/v) provided the fraction containing the product that was isolated by evaporation of solvent. Derivatives **6-10** were thereby obtained in 50-80% yield.

*6-Decoxy-7-ethoxy-1-(4-methoxybenzoyl)-N-(2-methoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**6**)*

White powder, 0.4 g (80%), m.p. 162-164 °C; Purity > 96% , RT 6.04 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 10.35 (t, *J* = 5.5 Hz, 1H, H-1'), 8.89 (s, 1H, H-2), 7.89 (dd, *J* = 8.4, 5.3 Hz, 4H, H-c, H-d), 7.28 (s, 1H, H-5), 6.79 (s, 1H, H-8), 4.17 (q, *J* = 6.9 Hz, 2H, H-12), 4.10 (t, *J* = 6.8 Hz, 2H, H-13), 3.90 (s, 3H, H-f), 3.64 (t, *J* = 5.5 Hz, 2H, H-3'), 3.56 (dt, *J* = 5.5, 5.5 Hz, 2H, H-2'), 3.40 (s, 3H, H-5'), 1.90 – 1.80 (m, 2H, H-14), 1.60 – 1.40 (m, 10H, H-15- H-19), 1.30 – 1.18 (m, 7H, H-20 - H-22), 0.85 (t, *J* = 6.9 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.38 (C-4), 169.87 (C-a), 165.30 (C-23), 164.42 (C-b), 153.54 (C-6), 148.70 (C-7), 143.30 (C-2), 138.06 (C-7), 133.72 (C-9), 133.49 (C-c), 132.11 (C-d), 122.30 (C-10), 111.29 (C-3), 107.57 (C-5), 98.30 (C-8), 71.34 (C-4'), 69.19 (C-13), 65.05 (C-12), 58.86 (C-f), 55.81 (C-3'), 38.93 (C-2'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.30 (C-17), 29.28 (C-18), 28.87 (C-19), 25.92 (C-20), 22.63 (C-21), 14.51 (C-11), 14.08 (C-22). ESI-HRMS *m/z* calcd for $C_{33}H_{45}N_2O_7$ 581.3221, found 581.3208 $[M+H]^+$.

*6-Decoxy-7-ethoxy-1-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]-N-(4-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**7**)*

Yellow powder, 0.25 g (50%), m.p. 182-184 °C; Purity > 95%, RT11.05 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 11.11 (t, *J* = 5.8 Hz, 1H, H-1'), 8.67 (s, 1H, H-2), 7.67 (s, 1H, H-5), 7.28 (dd, *J* = 8.4, 5.3 Hz, 2H, H-5'), 6.94 (t, *J* = 8.5 Hz, 2H, H-4'), 6.81 (s, 1H, H-8), 4.66 (d, *J* = 5.8 Hz, 2H, H-2'), 4.55 (t, *J* = 6.9 Hz, 2H, H-13), 3.40 (t, *J* = 6.9 Hz, 2H, H-12), 3.89– 3.61 (m, 4H, H-c, H-d), 3.51 (t, *J* = 6.0 Hz, 2H, H-h), 1.84 (q, *J* = 7.4, 6.7 Hz, 4H, H-14, H-15), 1.47 – 1.18 (m, 18H, H-11, H-i, H-16,-H-21), 0.92 – 0.82 (m, 3H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.11 (C-4), 166.87 (C-23), 162.83 (C-6'), 155.72 (C-a), 155.11 (C-g), 153.82 (C-f), 153.59 (C-6), 148.18 (C-7), 141.18 (C-2), 134.77 (C-9), 133.94 (C-3'), 128.93 (C-4'), 120.70 (C-10), 115.51 (C-5'), 110.05 (C-3), 105.92 (C-5), 99.85 (C-8), 69.26 (C-13), 64.70 (C-12), 48.79 (C-2'), 45.50 (C-d), 44.88 (C-c), 39.10 (C-h), 31.87 (C-14), 29.53 (C-15, C-16), 29.31 (C-17, C-

18), 28.85 (C-19), 25.90 (C-20), 22.66 (C-21), 14.32 (C-i), 14.38 (C-11), 14.10 (C-22). ESI-HRMS *m/z* calcd for $C_{36}H_{46}FN_40_7$ 665.3345, found 665.3282 $[M+H]^+$.

*6-Decoxy-7-ethoxy-3-{[4-(morpholin-4-ylcarbonyl)piperazin-1-yl]carbonyl}-1-(morpholin-4-ylcarbonyl)quinolin-4(1H)-one (**8**)*

Yellow viscous gum, 0.37 g (74%), m.p. 167-169 °C; Purity > 95% , RT 10.02 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.99 (s, 1H, H-2), 7.24 (s, 1H, H-5), 6.74 (s, 1H, H-8), 4.26 – 4.20 (m, 2H, H-13), 4.09 (t, *J* = 6.8, 2H, H-12), 3.85 – 3.20 (m, 24H, H-2', H-3', H-5', H-6', H-c, H-d), 1.88 – 1.80 (m, 2H, H-14), 1.54 – 1.42 (m, 6H, H-15 - H-17), 1.30 – 1.16 (m, 11H, H-18 - H-21), 0.91 – 0.79 (m, *J* = 6.8, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.63 (C-4), 165.28 (C-23), 163.53 (C-a), 155.86 (C-4'), 153.82 (C-6), 152.14 (C-7), 138.86 (C-2), 132.52 (C-9), 120.42 (C-10), 118.38 (C-3), 107.22 (C-5), 100.05 (C-8), 69.59 (C-13), 69.25 (C-12), 66.57 (C-d), 66.46 (C-6'), 65.94 (C-c), 49.42 (C-2'), 48.73 (C-5'), 46.45 (C-3'), 31.85 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 14.49 (C-22), 14.09 (C-11). ESI-HRMS *m/z* calcd for $C_{36}H_{54}N_5O_8$ 684.3967, found 684.3926 $[M+H]^+$.

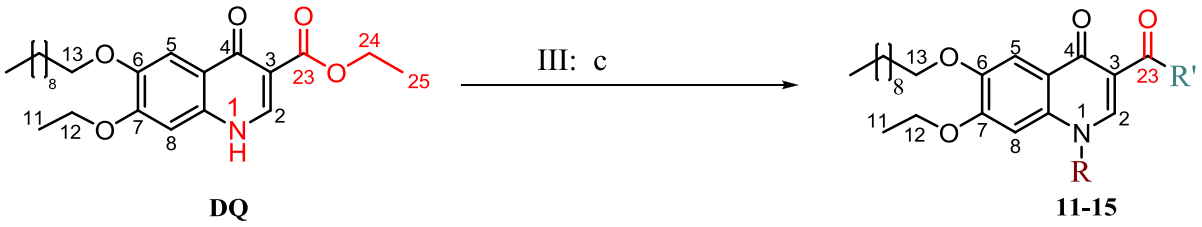
*6-Decoxy-7-ethoxy-3-[(4-methylpiperazin-1-yl)carbonyl-1-{[3-(methylsulfonyl)-2-oxoimidazolidin-1-yl]carbonyl}quinolin-4(1H)-one (**9**)*

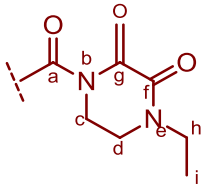
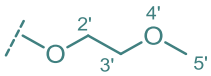
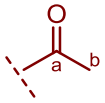
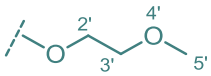
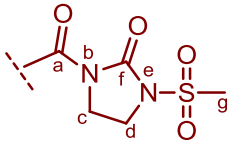
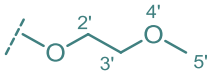
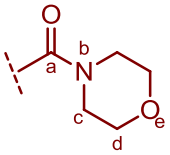
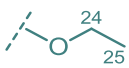
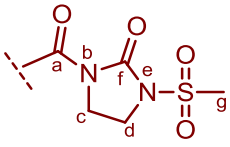
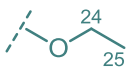
Brown powder, 0.28 g (56%), m.p. 170-173 °C; Purity > 96%, RT 6.94 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.48 (s, 1H, H-2), 7.24 (s, 1H, H-5), 6.74 (s, 1H, H-8), 4.26 – 4.20 (m, 2H, H-13), 4.09 (t, *J* = 6.8, 2H, H-12), 3.65 – 3.20 (m, 8H, H-2', H-3'), 3.09 (s, 3H, H-g), 2.59 – 2.31 (m, 4H, H-c, H-d,), 2.10 (s, 3H, H-5') 1.88 – 1.80 (m, 2H, H-14), 1.54 – 1.42 (m, 6H, H-15 - H-17), 1.30 – 1.16 (m, 11H, H-18 - H-21), 0.91 – 0.79 (m, *J* = 6.8, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.68 (C-4), 165.57 (C-23), 153.59 (C-a), 152.54 (C-f), 151.91 (C-6), 150.92 (C-7), 145.82 (C-2), 138.72 (C-9), 120.49 (C-10), 117.94 (C-3), 108.59 (C-5), 100.25 (C-8), 69.30 (C-13), 69.19 (C-g), 66.46 (C-c), 65.04 (C-d), 64.51 (C-12), 54.49 (C-2'), 46.98 (C-3'), 41.90 (C-5'), 31.85 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 14.49 (C-22), 14.09 (C-11). ESI-HRMS *m/z* calcd for $C_{32}H_{48}N_5O_8S$, 662.3218, found 662.3193 $[M+H]^+$.

1-Acetyl-6-Decoxy-7-ethoxy-3-[(4-methylpiperazin-1-yl)carbonyl]quinolin-4(1H)-one ***(10)***

Brown powder, 0.28 g (56%), m.p. 175-177 °C; Purity > 96% , RT 8.71 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.98 (s, 1H, H-2), 7.51 (s, 1H, H-5), 7.25 (s, 1H, H-8), 4.20 – 3.93 (m, 2H, H-13), 3.44 (t, *J* = 6.7 Hz, 2H, H-12), 3.39 – 3.26 (m, 8H, H-2', H-3', H-5', H-6'), 2.87 (s, 3H, H-7'), 2.10 (s, 3H, H-b), 2.02 – 1.90 (m, 2H, H-14), 1.63 – 1.46 (m, 6H, H-15 - H-17), 1.46 – 1.08 (m, 11H, H-18 - H-22), 0.82 – 0.77 (m, *J* = 6.7 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 169.47 (C-4), 164.88 (C-23), 155.13 (C-a), 154.24 (C-6), 151.43 (C-7), 149.14 (C-2), 147.74 (C-9), 116.85 (C-10), 112.19 (C-3), 107.61 (C-5), 100.62 (C-8), 69.09 (C-13), 64.79 (C-12), 54.42 (C-2'), 48.63 (C-6'), 37.87 (C-3'), 32.20 (C-5'), 30.84 (C-b), 30.20 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 19.39 (C-7'), 14.49 (C-22), 14.09 (C-11). ESI-HRMS *m/z* calcd for $C_{29}H_{44}N_3O_5$ 514.6692, found 514.6688 $[M+H]^+$.

Table 4. General procedure for synthesis of *N*-acylated DQ derivatives 11-15



Cpd	R	R'	Cpd	R	R'
11			**13**		
12			**14**		
			15		

A mixture of DQ (2.34 mmol, 1 g, 1 eq.) in chloroform (20 mL) containing conc. H_2SO_4 (320 µL, 0.33 g, 2.1 mmol, 0.9 eq) and 2-methoxyethanol (1 mL, 5 eq.) in a 100 mL round bottom flask was stirred under reflux for 72 h. The reaction mixture was washed with aqueous saturated $NaHCO_3$ solution (20 mL × 3). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product. This was submitted to chromatography on silica gel. Elution with CH_2Cl_2/methanol (20:1, v/v) provided the fraction containing the intermediate that was isolated by evaporation of solvent. This transesterified intermediate was submitted to *N*-acylation without further purification.

To a 100 mL two neck flask containing DQ (0.5 g, 1.19 mmol., 1 eq) or the transesterified intermediate as prepared above (0.54 g 1.19 mmol., 1 eq) in chloroform (15 mL) and DBU (0.7 mmol, 0.6 eq) was added the acyl chloride (5 eq). The mixture was stirred under reflux for 15 h, and then washed with aqueous saturated NH_4Cl solution (20 mL × 3). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product. This was submitted to chromatography on silica gel. Elution with CH_2Cl_2/methanol (20:1, v/v) provided the fraction containing the product that was isolated by evaporation of solvent. The product was recrystallized from acetone and air dried overnight. Derivatives **11-15** were thereby obtained in 50-80% yield.

*(2'-Methoxy)ethyl 6-decoxy-7-ethoxy-1-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]-4-oxo-1,4-dihydroquinoline-3-carboxylate (**11**)*

Yellow powder, 0.30 g (60%), m.p. 182-183 °C; Purity > 95%, RT 12.58 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.05 (s, 1H, H-2), 7.40 (s, 1H, H-5), 7.11 (s, 1H, H-8), 4.46 (d, *J* = 5.3 Hz, 2H, H-2'), 4.24 (q, *J* = 7.0 Hz, 2H, H-13), 4.10 (t, *J* = 6.7 Hz, 2H, H-12), 3.67 (d, *J* = 5.3, Hz, 2H, H-3'), 3.57 – 3.49 (m, 4H, H-c, H-d), 3.41 (s, 3H, H-5'), 1.92 – 1.82 (m, 2H, H-14), 1.58 – 1.46 (m, 6H, H-15 - H-17), 1.40 – 1.20 (m, 16H, H-h, H-i, H-18 - H-22), 0.93 (t, *J* = 6.7 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.47 (C-4), 163.99 (C-23), 158.85 (C-a), 157.07 (C-f, C-g), 155.07 (C-7), 151.50 (C-6), 146.38 (C-2), 128.74 (C-9), 122.21 (C-10), 120.29 (C-3), 106.97 (C-5), 103.58 (C-8), 70.17 (C-3'), 69.40 (C-13), 68.09 (C-12), 64.82 (C-2'), 59.05 (C-5'), 48.55 (C-c), 44.87 (C-d), 43.73 (C-h), 31.87 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 14.49 (C-22), 14.31 (C-i), 14.09 (C-11). HRMS-ESI *m/z* calcd for $C_{36}H_{54}N_5O_8$ 684.3967, found 684.3926 $[M+H]^+$.

*(2'-Methoxy)ethyl 6-decoxy-7-ethoxy-1-{[3-(methylsulfonyl)-2-oxoimidazolidin-1-yl]carbonyl}-4-oxo-1,4-dihydroquinoline-3-carboxylate (**12**)*

White powder, 0.20 g (40%), m.p. 170-173 °C; Purity > 96%, RT 10.13 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.03 (s, 1H, H-2), 7.50 (s, 1H, H-5), 7.23 (s, 1H, H-8), 4.58 – 4.50 (d, *J* = 5.3 Hz, 2H, H-2'), 4.24 (q, *J* = 7.0 Hz, 2H, H-13), 4.16 – 4.12 (t, *J* = 6.7 Hz, 2H, H-12), 3.95 – 3.90 (d, *J* = 5.3 Hz, 2H, H-3'), 3.79 – 3.71 (m, 4H, H-c, H-d), 3.42 (s, 3H, H-5'), 3.31 (s, 3H, H-g), 1.94 – 1.86 (m, 2H, H-14), 1.60 – 1.46 (m, 6H, H-15 - H-17), 1.38 – 1.23 (m, 11H, H-18 - H-22), 0.88 – 0.85 (t, *J* = 6.7 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.74 (C-4), 164.76 (C-23), 154.20 (C-7), 151.01 (C-6), 148.08 (C-2), 147.05 (C-a), 141.21 (C-f) 128.77 (C-9), 121.71 (C-10), 120.35 (C-3), 108.59 (C-5), 103.64 (C-8), 70.31 (C-3'), 69.28 (C-13), 68.12 (C-12), 64.62 (C-2'), 59.05 (C-5'), 40.80 (C-c), 40.52 (C-d), 39.92 (C-h), 31.87 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 14.49 (C-22), 14.09 (C-11). HRMS-ESI *m/z*) calcd for $C_{32}H_{48}N_5O_8S$ 662.3218, found 662.3193 $[M+H]^+$.

*(2'-Methoxy)ethyl 1-acetyl-6-Decoxy-7-ethoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (**13**)*

White powder, 0.26 g (52%), m.p. 175-178 °C; Purity > 96% , RT 12.8 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.21 (s, 1H, H-2), 7.40 (s, 1H, H-5), 7.11 (s, 1H, H-8), 4.46 (d, *J* = 5.3 Hz, 2H, H-2'), 4.24 (q, *J* = 7.0 Hz, 2H, H-13), 4.10 (t, *J* = 6.7 Hz, 2H, H-12), 3.77 – 3.67 (d, *J* = 5.3 Hz, 2H, H-3'), 3.41 (s, 3H, H-5'), 2.51 (s, 3H, H-b), 1.92 – 1.82 (m, 2H, H-14), 1.58 – 1.46 (m, 6H, H-15 - H-17), 1.40 – 1.20 (m, 11H, H-18 - H-22), 0.93 – 0.75 (m, *J* = 6.7 Hz, 3H, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.47 (C-4), 164.88 (C-23), 155.13 (C-a), 154.24 (C-6), 151.43 (C-7), 149.14 (C-2), 147.74 (C-9), 116.85 (C-10), 112.19 (C-3), 107.61 (C-5), 100.62 (C-8), 70.36 (C-3'), 69.21 (C-13), 64.72 (C-12), 64.28 (C-2'), 58.98 (C-5'), 31.87 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 21.01 (C-b), 14.49 (C-22), 14.09 (C-11). HRMS-ESI *m/z* calcd for $C_{27}H_{40}NO_7$ 490.2799, found 490.2818 $[M+H]^+$.

Ethyl 6-decoxy-7-ethoxy-1-(morpholin-4-ylcarbonyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate ***(14)***

White powder, 0.46 g (92%), m.p. 164-166 °C; Purity > 97%, RT 10.3 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 9.19 (s, 1H, H-2), 7.40 (s, 1H, H-5), 7.23 (s, 1H, H-8), 4.35 (d, *J* = 15.6 Hz, 2H, H-24), 4.25 – 4.20 (m, 2H, H-13), 4.20 – 4.16 (t, *J* = 6.6 Hz, 2H, H-12), 4.12– 4.01 (m, 8H, H-c, H-d,), 1.96 – 1.82 (m, 2H, H-14), 1.51 (t, *J* = 15.5 Hz, 3H, H-25), 1.42 – 1.36 (m, 6H, H-15 - H-17), 1.30 – 1.18 (m, 11H, H-18 - H-22), 0.91 – 0.79 (t, 3H, *J* = 6.6 Hz, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.68 (C-4), 165.57 (C-23), 153.59 (C-a), 152.54 (C-6), 151.91 (C-7), 145.82 (C-2), 138.72 (C-9), 120.49 (C-10), 117.94 (C-3), 108.59 (C-5), 100.25 (C-8), 69.30 (C-13), 69.19 (C-d), 66.46 (C-c), 64.51 (C-12), 61.40 (C-24), 31.85 (C-14), 29.67 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 14.37 (C-25), 14.49 (C-22), 14.09 (C-11). ESI-HRMS *m/z* calcd for $C_{29}H_{43}N_2O_7$ 531.3065, found 531.3035 $[M+H]^+$.

Ethyl 6-decoxy-7-ethoxy-1-{[3-(methylsulfonyl)-2-oxoimidazolidin-1-yl]carbonyl}-4-oxo-1,4-dihydroquinoline-3-carboxylate ***(15)***

White powder, 0.4 6 g (92%), m.p. 160-162 °C; Purity >97%, RT 9.8 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 9.19 (s, 1H, H-2), 7.40 (s, 1H, H-5), 7.23 (s, 1H, H-8), 4.35 (d, *J* = 15.6 Hz, 2H, H-24), 4.25 – 4.20 (m, 2H, H-13), 4.20 – 4.16 (t, *J* = 6.6 Hz, 2H, H-12), 4.12 (d, *J* = 6.6 Hz, 2H, H-c), 4.01 (d, *J* = 6.6 Hz, 2H, H-d), 3.39 (s, 3H, H-g), 1.96 – 1.82 (m, 2H, H-14), 1.51 (t, *J* = 15.5 Hz, 3H, H-25), 1.42 – 1.36 (m, 6H, H-15 - H-17), 1.30 – 1.18 (m, 11H, H-18 - H-22), 0.91 – 0.79 (m, 3H, *J* = 6.6 Hz, H-11). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm) 16.74 (C-4), 154.82 (C-23), 153.23 (C-a), 150.92 (C-f), 149.74 (C-6), 149.28 (C-7), 148.85 (C-2), 148.15 (C-9), 117.32 (C-10), 112.31 (C-3), 108.50 (C-5), 100.35 (C-8), 69.41 (C-13), 64.76 (C-12), 61.40 (C-24), 41.25 (C-c), 40.69 (C-d), 39.93 (C-g), 31.87 (C-14), 29.53 (C-15), 29.54 (C-16), 29.50 (C-17), 29.34 (C-18), 28.81 (C-19), 25.98 (C-20), 22.65 (C-21), 14.37 (C-25), 14.49 (C-22), 14.09 (C-11). ESI-HRMS *m/z* calcd for $C_{29}H_{42}N_3O_9S$ 608.2636, found 608.2609 $[M+H]^+$.

***In vitro* antimalarial assays**

P. falciparum parasites were maintained at 37 °C in human erythrocytes (O^+) suspended in complete culture medium [RPMI 1640 medium (Sigma-Aldrich) supplemented with 25 mM HEPES (Sigma-Aldrich), 20 mM D-glucose (Sigma-Aldrich), 200 μM hypoxanthine (Sigma-Aldrich), 0.2% sodium bicarbonate, 24 μg/mL Gentamycin (Sigma-Aldrich) and 0.5% AlbuMAX II] in a gaseous environment of 90% N_2, 5% O_2, and 5% CO_2.[1] *In vitro* ring-stage intra-erythrocytic *P. falciparum* parasite cultures (Genotyped drug sensitive strains; W2 (Chloroquine, quinine, pyrimethamine and cycloguanil resistant), K1 (chloroquine, pyrimethamine, mefloquine and cycloguanil resistant) and NF54 (drug sensitive) (200 μL at 1% haematocrit, 1% parasitaemia) were treated with the DQ derivatives series.

The controls for this assay included chloroquine disulphate (1 μM, as positive control) and complete RPMI media (as negative control) and grown for 96 h at 37 °C under the 90% N_2, 5% O_2, and 5% CO_2 gas mixture in 96-well plates.

At the end of the 96 h growth period, equal volumes (100 μL each) of the *P. falciparum* parasite cultures were combined with SYBR Green I lysis buffer (0.2 μL/mL 10 000 x SYBR Green I, Invitrogen; 20 mM Tris, pH 7.5; 5 mM EDTA; 0.008% (w/v) saponin; 0.08% (v/v) Triton X-100).

The samples were incubated at 37 °C for 1h after which the fluorescence was measured using a Fluoroskan Ascent FL microplate fluorometer (Thermo Scientific, excitation at 485 nm and emission at 538 nm). The ‗background' fluorescence (i.e. that measured in the samples derived from chloroquine-treated iRBC samples in which parasite proliferation was completely inhibited) was subtracted from the total fluorescence measured for each sample to provide a measure of parasite proliferation. Data obtained were analysed in Excel 2010 and sigmoidal dose-response curves were plotted using GraphPad 6.0.

Decoquinate **1** and the derivatives were screened at 5 μM and 1 μM against the NF54 strain of asexual *P. falciparum* parasites to determine their % inhibition. Compounds showing near complete inhibition at 5 μM and 1 μM (Figure S1), were further screened at 500 and 100 nM against NF54 parasites (Figure S2). Compounds were weighed (1 mg) and dissolved in 100% DMSO and made up to a 10 mM stock and stored at -20 °C.

Solutions of compounds were thawed at room temperature (~1 h) prior to an assay to prepare working solutions. The final starting concentrations (5, 1 μM and 500, 100 nM) were

prepared in complete RPMI-1640 media and aliquoted in triplicate into a 96-well plate. The %DMSO in the starting concentration was 0.05% for the 5 μM, 0.000125% for the 1 μM, 0.05% for the 500 nM and 0.00000125% for the 100 nM drug concentrations; these concentrations were below the parasite inhibition threshold as previously determined in our laboratory. Following dual point assays, compounds were subjected to *in vitro* IC_{50} determinations against asexual *P. falciparum* NF54, K1 and W2 parasites. The respective IC_{50} values for compounds in the series showing activity <200 nM were determined. The same methods and conditions were applied for dissolving and storing the compounds as described for the dual point assays. Fresh stock solutions of the compound used for the IC_{50} determinations were prepared prior to assays and stored at -20 °C. All assays were performed in triplicate for at least three independent biological repeats.

***In vitro* cytotoxicity**

The WI-38 cell line (normal human fetal lung fibroblast) from ECACC was maintained as a monolayer cell culture at 37 °C, 5% CO_2, 95% air and 100% relative humidity in EMEM containing 10% fetal bovine serum, 2 mM L-glutamine and 50 μg/mL gentamicin. For this screening experiment, the cells (21 - 50 passages) were inoculated in 96-well microtiter plates at plating densities of 10 000 cells/well and incubated for 24 hours. After 24 hours, the cells were treated with the experimental drugs; which had previously been dissolved in DMSO and diluted in medium to produce five concentrations. Neat cells served as the control. The blank contained complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 hours after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein bound dye was extracted with 10 mM Tris base for optical density determination at a wavelength of 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression.

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Chapter 7

Summary and conclusions

Malaria is the third leading cause of human deaths worldwide. Over 40% of the world's population currently inhabit endemic malaria areas in Africa, Asia, and in South and Central America. Sub-Saharan Africa alone makes up eighty per cent (80%) of this total endemic region (WHO, 2015). In 2015, 438,000 people died from the disease (WHO, 2015) . Pregnant women and children under the age of five are the most vulnerable (Shetty, 2012), as they either have reduced, or no acquired immunity against malaria (Roggelin & Cramer, 2014).

Malaria is caused by a protozoan, belonging to the genus, *Plasmodium*. Five species of *Plasmodium*, namely *P. falciparum, P. ovale, P. vivax, P. malariae* and *P. knowlesi* cause malaria in humans. Of these species, *P. falciparum* is responsible for the most severe form of the disease and its prevalence is highest in Sub-Saharan Africa than in any other region (Guerra *et. al*., 2010).

Chemotherapy currently is the sole option for treating malaria (Frevert, 2004). The challenging issue with treating malaria using chemotherapy is the widespread development of resistance towards existing anti-malarial drugs. This is evident from the worldwide spread of resistant *P. falciparum* strains towards prominent anti-malarial drugs, like chloroquine, Fansidar, mefloquine, amodiaquine and piperaquine (Ridley, 2002). Even more concerning is that the parasite has already developed resistance towards artemisinin combination therapies (ACTs) as well (Phyo *et. al*., 2012; Makam *et. al*., 2014). ACTs currently are the cornerstone of malaria treatments and form the last line of defence against the disease.

Tuberculosis (TB), a disease that is spread through the inhalation of droplets from an infected individual when he or she coughs, is caused by the bacterium, *Mycobacterium tuberculosis* (*Mtb*) (Rao *et. al*., 2013). Although TB is one of the oldest diseases known in human history (Smith, 2003), it is still the leading cause of human mortalities worldwide, as the number of deaths resulting from TB presently outweighs that being caused by any other disease, including AIDS and malaria (WHO, 2015). In 2014, 9.6 million people were infected with TB, of which 1.4 million had succumbed from it (WHO, 2015). Unlike malaria, the morbidity and

mortality numbers that result from TB are not limited to tropical zones (WHO, 2015), but are even higher in temperate zones, like Russia and the Eastern European region, which together account for 58% of the total TB burden (Wang *et. al*., 2015; WHO, 2015). In essence, TB is endemic to both temperate and tropical zones, with Russia, India, China, and South Africa being the hot spots of the disease (Churchyard *et. al*., 2014).

Chemotherapy is also the cornerstone for the control and treatment of TB. Besides the long treatment time of 2 to 6 months and the serious side effects (e.g. hepatotoxicity and neuropathy), associated with the use of current anti-tubercular drugs, *Mtb* strains resistant to current drugs like rifampicin, isoniazid, fluoroquinolones have emerged and spread globally (Yempalla *et. al*., 2015).

The growing resistance towards anti-malarial and anti-TB drugs emphasises the need to discover new chemotherapeutic agents for the treatment of these diseases. It is also imperative that these new therapies would be cost-effective, efficient and safe.

One of the fastest and cheapest approaches to discover new drugs is to start with a known therapeutic agent, identify its potentials and liabilities, then define strategies to address those liabilities to augment its therapeutic potential.

Decoquinate (DQ) is a known therapeutic agent in veterinary medicine, which was identified as a potent anti-malarial agent, having strong gametocytocidal and schizonticidal activities, with a unique mode of action that targets the cytochrome bc_1 complex (Da Cruz *et. al*., 2012). The outcomes of another study also confirmed that decoquinate had demonstrated strong activity against hypnozoites, both *in vivo* and *in vitro* (Bonamy *et. al*., 2011). From these studies, it has become obvious that decoquinate meets the Medicine for malarial ventures (MMV) requirements for possible qualification as a next generation drug, needed for the eradication of malaria (Burrows *et. al*., 2013). These requirements are that a drug candidate should be able to kill gametocytes, hypnozoites and other liver stages, thereby inhibiting transmission and relapse, as well as to provide prophylaxes for the disease. With respect to TB, DQ also has a flexible and lipophilic 10-carbon alkyl chain, and as such should easily penetrate macrophages and the lipophilic mycolic acid coating of the *Mtb* cells. However, the poor aqueous solubility of DQ represents the biggest disadvantage as a therapeutic agent for the treatment of systemic diseases, like malaria and tuberculosis. DQ also has a metabolically susceptible ester, which, if hydrolysed into carboxylic acid, may result in its low bio-availability *in vivo*.

This study was aimed at addressing the poor aqueous solubility of DQ, as well as replacing the metabolically susceptible ester group with amide groups that would be more resistant to hydrolysis. To achieve this, the secondary amine at the N-1 position and/or the ester at the C-3 position of DQ were transformed during syntheses. The secondary amine was either transformed by treating DQ with an alkyl bromide in the presence of potassium carbonate to obtain *N*-alkylated derivatives, or by treating DQ with an acyl chloride in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to generate N-acylated derivatives. The ester was transformed into amides by treating DQ with amines in the presence of DBU. Seventy-seven novel derivatives, with varied groups attached to either position 1 and/or 3 of DQ, were generated. These derivatives were screened *in vitro* against the H37Rv strain of *Mycobacterium tuberculosis* and against W1-cell lines for anti-TB activity and cytotoxicity, respectively, and also against NF54, K1, and W2 strains of *P. falciparum* for anti-malarial activity.

From the analysis of the biological data generated during this study, the following conclusions were made:

- The derivatisation of decoquinate at N-1 and the replacement of the ester had led to derivatives that were soluble in polar (ethanol, methanol, dimethylsulfoxide) and non-polar (dichloromethane, chloroform) solvents.
- The C-3 ethyl ester functional group of decoquinate was found important for anti-malarial activity, but not for anti-tubercular activity. While those derivatives wherein the ester had been transformed into an amide generally showed inferior, or no anti-malarial activities, compared to decoquinate, those same derivatives were generally more active than decoquinate against *Mtb*.
- Modifications at the N-1 position of the amide derivatives of decoquinate generally increased their anti-TB activities. The highest anti-TB activity was obtained from a tertiary amide, bearing an ethyl group attached to N-1
- Derivatisation at the N-1 position of the quinolone ring had variable impacts on anti-malarial activity. It was found that the attachment of an alkyl group to N-1 had led to derivatives with a loss in activity, while the conversion of the N-1 into an amide, or urea had resulted in increased activity, relative to decoquinate. The most outstanding anti-malarial activity was obtained from the conversion of the secondary amine into a urea, bearing a sulfonyl moiety.

In summary, this project has delivered a non-toxic hit compound with outstanding *in vitro* anti-malarial activities (IC_{50}: NF54 1 nM; K1 1 nM; W2 1 nM), as compared to DQ (IC_{50}: NF54 26 nM; K1 64 nM; W2:15 nM), chloroquine (IC_{50}: NF54 139 nM; K1 154 nM; W2 233 nM), artesunate (IC_{50}: NF54 7 nM; K1 8 nM; W2 6 nM) and artemether (IC_{50}: NF54 5 nM; K1 7 nM; W2 4 nM). The study has also led to the discovery of a safe and potent anti-tubercular hit (H37Rv: MIC_{90}: 1 μM; MIC_{99}: 1 μM). Both of these promising derivatives will undergo further evaluations in their respective medicinal areas.

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Addendum A: ^{1}H and ^{13}C NMR and mass spectra for chapter 4

Straightforward conversion of decoquinate into inexpensive tractable new quinolones with significant antimalarial activities.

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Supporting Information

Experimental Section

General procedures

The amines, acyl, sulfonyl, carbamoyl and alkyl halides in this study were purchased from either Sigma Aldrich or AKSci and were used as supplied. NMR spectra were obtained on a 600 MHz Bruker Avance™ III spectrometer as solutions in deuterated solvents ($CDCl_3$, or DMSO-d_6). All chemical shifts (δ) are reported in parts per million (ppm) values. ^{1}H chemical shifts are reported downfield of tetramethylsilane (TMS), and were internally referenced to the residual proton in $CDCl_3$ (7.26 ppm), or DMSO-d_6 (2.50 ppm). ^{13}C chemical shifts were internally referenced to resonances in $CDCl_3$ (δ 77.16 ppm), or DMSO-d6 (δ 39.51 ppm). Peak multiplicities are abbreviated as follows: s (singlet), d (doublet), dd(doublet of doublet), t (triplet), q (quartet), p (pentet), and m (multiplet). Coupling constant (*J*) are reported in Hz. NMR data were analyzed using MestReNova Software, version 5.3.2-4936. Exact mass measurements were recorded on Bruker MicroTOF Q II mass spectrometer with its APCI source set at 300 °C coupled chromatography. Calculated LogP (cLogP) values were generated using ACD/ChemSketch version 14.02.with Bruker Compass Data analysis 4.0 software. A full scan, ranging between 50–1500 m/z, was generated at a capillary voltage of 4500 V, end plate offset voltage of -500 V, and a collision cell RF voltage of 100 V. Infrared (IR) spectra were recorded on a Bruker Alpha-P FTIR instrument. Melting points (mp) were determined with a Büchi melting point B-545 instrument and were uncorrected. Thin layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$) (Merck) and components were visualized by ultraviolet light (254 nm). Silica gel 230-400 (particle size 40-63 μm) mesh was used for column

Syntheses

(a) To a 100 mL two neck flask containing decoquinate **1** (1 g, 2.34 mmol) and DBU (320 μL, 0.33 g, 2.1 mmol, 0.9 eq.) in chloroform (25 mL) was added the primary or secondary amine (5 eq.). The resulting mixture was stirred under reflux for 24-72 h. Progress of the reactions was monitored by tlc. Upon completion, the reaction mixture was concentrated *in vacuo*. The concentrated residue was taken into chloroform (20 mL) and washed with distilled water (4 x 20 mL). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product, which was subjected to flash column chromatography on

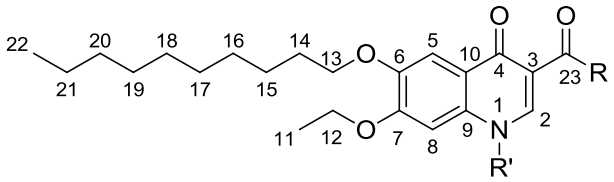
silica gel. The product was eluted with a mixture of dichloromethane/methanol (10:1, v/v). The product was recrystallized from ethyl acetate and air dried. The amide derivatives **2**-**21** were thereby obtained in good yields using this procedure. Data for derivatives **2**, **3** and **6** are given below.

(b) To a 100 mL two neck flask containing DQ **1** (0.5 g 1.22 mmol. 1 eq), DBU (100 µL 0.7 mmol, 0.6 eq.) in chloroform (15 mL) was added the appropriate acyl or sulfonyl halide, or alkyl halide (5 eq). The mixture was stirred under reflux for 15 h, and then washed with aqueous saturated NH_4Cl solution (3 x 20 mL). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product, which was submitted flash column chromatography on silica gel. Elution with dichloromethane/methanol (20:1, v/v) gave the product mixture, that was evaporated under reduced pressure to leave the crude product that was recrystallized from acetone and air dried overnight. The *N*-substituted derivatives **30**-**35** were obtained in 50-80% yields. Data for derivatives **30**, **31** and **33** are given below.

The foregoing procedure was applied to the morpholino amide **2** (0.35 g, 0.8 mmol, 1 eq.) to give the *N*-substituted derivatives **22**-**29**.

Supporting data for chapter 4

DQ and key derivatives in this study have the general structure below.



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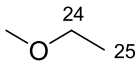
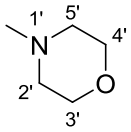
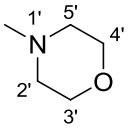
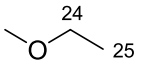
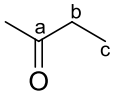
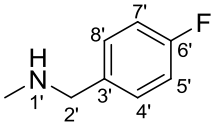
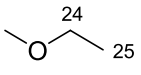
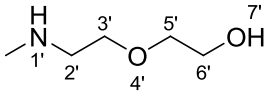
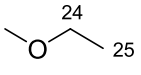
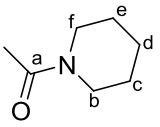
1		H	**24**		
2		H	**30**		
3		H	**31**		
6		H	**33**		

6-Decoxy-7-ethoxy-3-(morpholin-4-ylcarbonyl)quinolin-4(1H)-one (2)

White powder; yield: 80%, 0.87 g; m.p.: 182-184 °C; HPLC: 30% ACN/ 70% 0.1% H_3PO_4 gradient, >93% pure, rt = 5 min; APCI-HRMS (*m/z*): 459.2793 $[M+H]^+$ (calculated for $C_{26}H_{39}N_2O_5$: 459.2853); IR (ATR) $\upsilon_{max}cm^{-1}$: 3167, 3135, 2988, 2916, 1633, 1604, 1566, 1539, 1000-574; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 11.96 (s, 1H, H-1), 7.67 (s, 1H, H-2), 7.52 (s, 1H, H-5), 6.70 (s, 1H, H-8), 4.01 – 3.92 (m, 4H, H-12, H-13), 3.76 – 3.68 (m, 8H, H-2',-H-5'), 1.81 (m, *J* = 7.0 Hz, 2H, H-14), 1.45 – 1.35 (m, 4H, H-15, H-16), 1.37 – 1.18 (m, 13H, H-11, H-17,-H-21), 0.85 (t, *J* = 6.9 Hz, 3H, H-22); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.75 (C-4), 167.79 (C-23), 153.61 (C-6), 147.80 (C-7), 138.63 (C-2), 135.00 (C-9), 120.22 (C-10), 115.47 (C-3), 105.57 (C-5), 99.84 (C-8), 69.17 (C-13) , 67.23 (C-3', C-4') , 64.61 (C-12), 48.08 (C-2', C-5'), 31.88 (C-14), 29.54 (C-15, C-16), 29.35 (C-17, C-18), 28.97 (C-19), 25.93 (C-20), 22.66 (C-21), 14.45 (C-11), 14.10 (C-22).

Supporting data for chapter 4

6-Decoxy-7-ethoxy-N-(4-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (3)

White powder; yield: 80%, 0.82 g; m.p.: 202-203 °C; HPLC: 30% ACN/ 70% 0.1% H_3PO_4 gradient, >92% pure, rt = 16.5 min; APCI-HRMS (*m/z*): 497.2734 $[M+H]^+$ (calculated for $C_{29}H_{38}FN_20_4$: 497.2810); IR (ATR) υ_{max} cm^{-1}: 3167, 3135, 2988, 2916, 1648, 1627, 1614, 1580, 1531,1498; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 11.85 (s, 1H, H-1), 11.11 (t, *J* = 5.8 Hz, 1H, H-1'), 8.67 (s, 1H, H-2), 7.67 (s, 1H, H-5), 7.28 (dd, *J* = 8.4, 5.3 Hz, 2H, H-5', H-7'), 6.94 (t, *J* = 8.5 Hz, 2H, H-4', H-8'), 6.81 (s, 1H, H-8), 4.66 (d, *J* = 5.8 Hz, 2H, H-2'), 4.05 (t, *J* = 6.9 Hz, 2H, H-13), 3.92 (t, *J* = 6.9 Hz, 2H, H-12), 1.84 (q, *J* = 7.4, 6.7 Hz, 4H, H-14,-15), 1.47 – 1.18 (m, 15H, H-11, H-16,-H-21,), 0.92 – 0.82 (m, 3H, H-22); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.11 (C-4), 166.87 (C-23), 162.83 (C-6'), 153.82 (C-6,) , 148.18 (C-7), 141.18 (C-2), 134.77 (C-9), 133.94 (C-3'), 128.93 (C-4', C-8'), 120.70 (C-10), 115.51 (C-5', C-7'), 110.05 (C-3), 105.92 (C-5), 99.85 (C-8), 69.26 (C-13), 64.70 (C-12) , 42.72 (C-2'), 31.87 (C-14), 29.53 (C-15, C-16), 29.31 (C-17, C-18), 28.85 (C-19), 25.90 (C-20) , 22.66 (C-21), 14.38 (C-11) , 14.10 (C-22).

6-Decoxy-7-ethoxy-N-[2-(2-hydroxyethoxy)ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxamide (6)

White powder; yield: 68%, 0.68 g; m.p.: 133-136 °C; HPLC: 30% ACN/ 70% 0.1% H_3PO_4 gradient, > 96% pure, rt= 8.7 min; APCI-HRMS (*m/z*): 477.2911 $[M+H]^+$ (calculated for $C_{26}H_{41}N_20_6$: 477.2959); IR (ATR) υ_{max} cm^{-1}: 3336, 3178, 3135, 3085, 2922, 2849, 1639, 1581, 1561, 1523, 1499; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 11.99 (s, 1H, H-1), 10.73 (t, *J* = 5.3 Hz, 1H, H-1'), 8.64 (s, 1H, H-2), 7.39 (s, 1H, H-5), 6.80 (s, 1H, H-8), 4.43 (s, 1H, H-7'), 4.01 (t, *J* = 7.0 Hz, 2H, H-13), 3.86 (t, *J* = 6.8 Hz, 2H, H-12), 3.83 – 3.78 (m, 2H, H-2'), 3.71 – 3.60 (m, 6H, H-3', H-5', H-6'), 1.76 (t, *J* = 6.9 Hz, 2H, H-14), 1.46 – 1.19 (m, 17H, H-11, H-15,-H-21), 0.87 (dt, *J* = 21.8, 7.3 Hz, 3H, H-22); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 175.76 (C-4), 166.62 (C-23), 153.66 (C-6), 147.86 (C-7), 141.49 (C-2), 134.73 (C-9), 120.40 (C-10), 110.13 (C-3), 105.39 (C-5), 99.88 (C-8), 72.38 (C-2'), 69.80 (C-6'), 68.54 (C-3', C-5'), 64.63 (C-13), 61.73 (C-12), 31.90 (C-14), 29.65 (C-15, C-16), 29.27 (C-17, C-18), 28.92 (C-19, C-20), 22.67 (C-21), 14.44 (C-11), 14.10 (C-22).

Supporting data for chapter 4

1-Acetyl-6-decoxy-7-ethoxy-3-(morpholin-4-ylcarbonyl) quinolin-4(1H)-one (24)

White powder; yield: 64%, 0.24 g; m.p. 142-144°C; HPLC: 30% ACN/ 70% 0.1% H_3PO_4 gradient, > 83% pure, rt = 12.6 min; APCI-HRMS (*m/z*): 501.2895 $[M+H]^+$ (calculated for $C_{28}H_{41}N_20_6$: 501.2959); IR (ATR) υ_{max} cm^{-1}: 2988, 2916, 1633, 1604, 1566, 1539, 1000-574; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.57 (s, 1H, H-2), 7.3 (s, 1H, H-5), 6.92 (s, 1H, H-8), 4.18 – 4.11 (m, 2H, H-13), 4.09 – 4.03 (m, 2H, H-12), 3.74 – 3.30 (m, *J* = 16.1 Hz, 8H, H-2',-H-5'), 2.43 (s, 3H, H-b), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 11H, H-11, H-18,-H-21), 0.87 (dt, *J* = 14.0, 7.3 Hz, 3H, H-22); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm) :172.83 (C-4), 167.24 (C-a), 165.61 (C-23), 153.25 (C-6), 147.71 (C-7), 144.17 (C-2), 140.83 (C-9), 134.49 (C-10), 120.37 (C-3), 107.01 (C-5), 99.63 (C-8), 69.24 (C-13), 67.32 (C-12), 66.79 (C-3'), 64.83 (C-4'), 47.94 (C-2'), 42.85 (C-5'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 27.03 (C-b), 25.89 (C-20), 22.64 (C-21), 14.41 (C-11), 14.08 (C-22).

Ethyl-6-decoxy-7-ethoxy-4-oxo-1-propanoyl-1,4-dihydroquinoline-3-carboxylate, (30)

White powder; yield: 60%, 0.30 g; m.p.: 160-162°C; HPLC: 30% ACN, 70% 0.1% H_3PO_4 gradient, > 96% pure, rt= 9.3 min; APCI-HRMS (*m/z*): 474.2806 $[M+H]^+$ (calculated for $C_{27}H_{40}N0_6$: 474.2850); IR (ATR) υ_{max} cm^{-1}: 2988, 2916, 1741, 1604, 1566, 1539, 1000-574; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 9.15 (s, 1H, H-2), 7.41 (s, 1H, H-5), 7.12 (s, 1H, H-8), 4.47 – 4.30 (m, 2H, H-24), 4.31 – 4.21 (m, 2H, H-13), 4.13 – 4.03 (m, 2H, H-12), 2.91 – 2.81 (m, 2H, H-b), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 17H, H-11, H-18,-H-21, H-25, H-c), 0.87 (dt, *J* = 14.0, 7.3 Hz, 3H, H-22); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.83 (C-4), 165.61 (C-23), 154.40 (C-a), 154.06 (C-6), 150.44 (C-7), 149.37 (C-2), 130.86 (C-9), 117.55 (C-10), 113.16 (C-3), 108.72 (C-5), 100.60 (C-8), 69.15 (C-13), 68.11 (C-12), 64.19 (C-24), 38.68 (C-b), 31.87 (C-14), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 25.89 (C-20), 22.64 (C-21), 14.39 (C-c), 14.25 (C-25), 14.11 (C-11), 14.09 (C-22).

Supporting data for chapter 4

Ethyl-1-acetyl-6-decoxy-7-ethoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (31)

White powder; yield: 84%, 0.42 g; m.p.: 159-162 °C; HPLC: 30% ACN/ 70% 0.1% H_3PO_4 gradient, > 97% pure, rt= 8.2 min; APCI-HRMS (*m/z*): 460.2656 $[M+H]^+$ (calculated for $C_{26}H_{38}N0_6$: 460.2694); IR (ATR) υ_{max} cm^{-1}: 2988, 2916, 1743, 1604, 1566, 1539, 1000-574; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 9.15 (s, 1H, H-2), 7.41 (s, 1H, H-5), 7.12 (s, 1H, H-8), 4.47 – 4.30 (m, 2H, H-24), 4.31 – 4.21 (m, 2H, H-13), 4.13 – 4.03 (m, 2H, H-12), 2.51 (s, 3H, H-b), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 14H, H-11, H-18, -21, -25,), 0.87 (dt, *J* = 14.0, 7.3 Hz, 3H, H-22); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm):172.83 (C-4), 165.61 (C-23), 154.46 (C-a), 154.06 (C-6), 150.44 (C-7), 149.37 (C-2), 130.86 (C-9), 117.55 (C-10), 113.16 (C-3), 108.72 (C-5), 100.60 (C-8), 69.15 (C-13), 68.11 (C-12), 64.19 (C-24), 31.87 (C-14), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 25.89 (C-20), 22.64 (C-21), 21.13 (C-b), 14.39 (C-25), 14.27 (C-11), 14.09 (C-22).

Ethyl-6-decoxy-7-ethoxy-4-oxo-1-(piperidin-1-ylcarbonyl)-1,4-dihydroquinoline-3-carboxylate (33)

White powder; yield: 63.7%, 0.32 g; m.p.: 170-172° C; HPLC: 30% ACN/ 70% 0.1% H_3PO_4 gradient, > 96% pure, rt= 7.3 min; APCI-MS (*m/z*): 529.3236 $[M+H]^+$ (calculated for $C_{30}H_{45}N_20_6$: 529.3272); IR (ATR) υ_{max} cm^{-1}: 2988, 2916, 1733, 1604, 1566, 1539, 1000-574; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 9.15 (s, 1H, H-2), 7.42 (s, 1H, H-5), 7.12 (s, 1H, H-8), 4.47 – 4.30 (m, 2H, H-24), 4.31 – 4.21 (m, 2H, H-13), 4.13 – 4.03 (m, 2H, H-12), 2.71 – 2.01 (m, 10H, H-b, -H-f), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 14H, H-11, H-18,-H-21, H-25), 0.87 (dt, *J* = 14.0, 7.3 Hz, 3H, H-22).; ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 172.83 (C-4), 165.61 (C-23), 154.48 (C-a), 154.06 (C-6), 150.44 (C-7), 149.37 (C-2), 130.86 (C-9), 117.55 (C-10), 113.16 (C-3), 108.72 (C-5), 100.60 (C-8), 69.15 (C-13), 68.11 (C-12), 64.19 (C-24), 54.58 (C-b, C-f), 31.89 (C-c, C-e), 31.87 (C-14), 29.57 (C-d'), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 25.89 (C-20), 22.64 (C-21), 14.25 (C-25), 14.11 (C-11), 14.09 (C-22).

Supporting data for chapter 4

***In vitro* antimalarial assays**

P. falciparum parasites were maintained at 37 °C in human erythrocytes (O^+) suspended in complete culture medium [RPMI 1640 medium (Sigma-Aldrich) supplemented with 25 mM HEPES (Sigma-Aldrich), 20 mM D-glucose (Sigma-Aldrich), 200 μM hypoxanthine (Sigma-Aldrich), 0.2% sodium bicarbonate, 24 μg/mL Gentamycin (Sigma-Aldrich) and 0.5% AlbuMAX II] in a gaseous environment of 90% N_2, 5% O_2, and 5% CO_2.[1] *In vitro* ring-stage intra-erythrocytic *P. falciparum* parasite cultures (Genotyped drug sensitive strains; W2 (Chloroquine, quinine, pyrimethamine and cycloguanil resistant), K1 (chloroquine, pyrimethamine, mefloquine and cycloguanil resistant) and NF54 (drug sensitive) (200 μL at 1% haematocrit, 1% parasitaemia) were treated with the DQ derivatives series.

The controls for this assay included chloroquine disulphate (1 μM, as positive control) and complete RPMI media (as negative control) and grown for 96 h at 37 °C under the 90% N_2, 5% O_2, and 5% CO_2 gas mixture in 96-well plates.

At the end of the 96 h growth period, equal volumes (100 μL each) of the *P. falciparum* parasite cultures were combined with SYBR Green I lysis buffer (0.2 μL/mL 10 000 x SYBR Green I, Invitrogen; 20 mM Tris, pH 7.5; 5 mM EDTA; 0.008% (w/v) saponin; 0.08% (v/v) Triton X-100).

The samples were incubated at 37 °C for 1h after which the fluorescence was measured using a Fluoroskan Ascent FL microplate fluorometer (Thermo Scientific, excitation at 485 nm and emission at 538 nm). The ‗background' fluorescence (i.e. that measured in the samples derived from chloroquine-treated iRBC samples in which parasite proliferation was completely inhibited) was subtracted from the total fluorescence measured for each sample to provide a measure of parasite proliferation.

Data obtained were analysed in Excel 2010 and sigmoidal dose-response curves were plotted using GraphPad 6.0.

Decoquinate **1** and the derivatives were screened at 5 μM and 1 μM against the NF54 strain of asexual *P. falciparum* parasites to determine their % inhibition. Compounds showing near complete inhibition at 5 μM and 1 μM (**Figure S1**), were further screened at 500 and 100 nM against NF54 parasites (**Figure S2**). Compounds were weighed (1 mg) and dissolved in 100% DMSO and made up to a 10 mM stock and stored at -20°C. Some compounds, however, did not completely dissolve (**1**, **7**, **11**, **13**, **21**, **30**, **31**, **33**, **34** and **35**) despite being incubated at 37

°C for >1h. These were dissolved at lower stock concentrations (<5 mM) and treated with 1xPBS. Therefore, prior to assay these compounds were vortexed thoroughly and the suspensions were immediately used to make up the working solutions in complete RMPI-1640 media.

Solutions/suspensions of compounds were thawed at room temperature (~1 h) prior to an assay to prepare working solutions. The final starting concentrations (5, 1 μM and 500, 100 nM) were prepared in complete RPMI-1640 media and aliquoted in triplicate into a 96-well plate. The %DMSO in the starting concentration was 0.05% for the 5 μM, 0.000125% for the 1 μM, 0.05% for the 500 nM and 0.00000125% for the 100 nM drug concentrations; these concentrations were below the parasite inhibition threshold as previously determined in our laboratory. Following dual point assays, compounds were subjected to *in vitro* IC_{50} determinations against asexual *P. falciparum* NF54, K1 and W2 parasites. The respective IC_{50} values for compounds in the series showing activity <100 nM were determined (DQ **1** and compounds **2**, **5**, **30**, **31**, **33**). The same methods and conditions were applied for dissolving and storing the compounds as described for the dual point assays. Fresh stock solutions of the compound used for the IC_{50} determinations were prepared prior to assays and stored at -20 °C. All assays were performed in triplicate for at least three independent biological repeats.

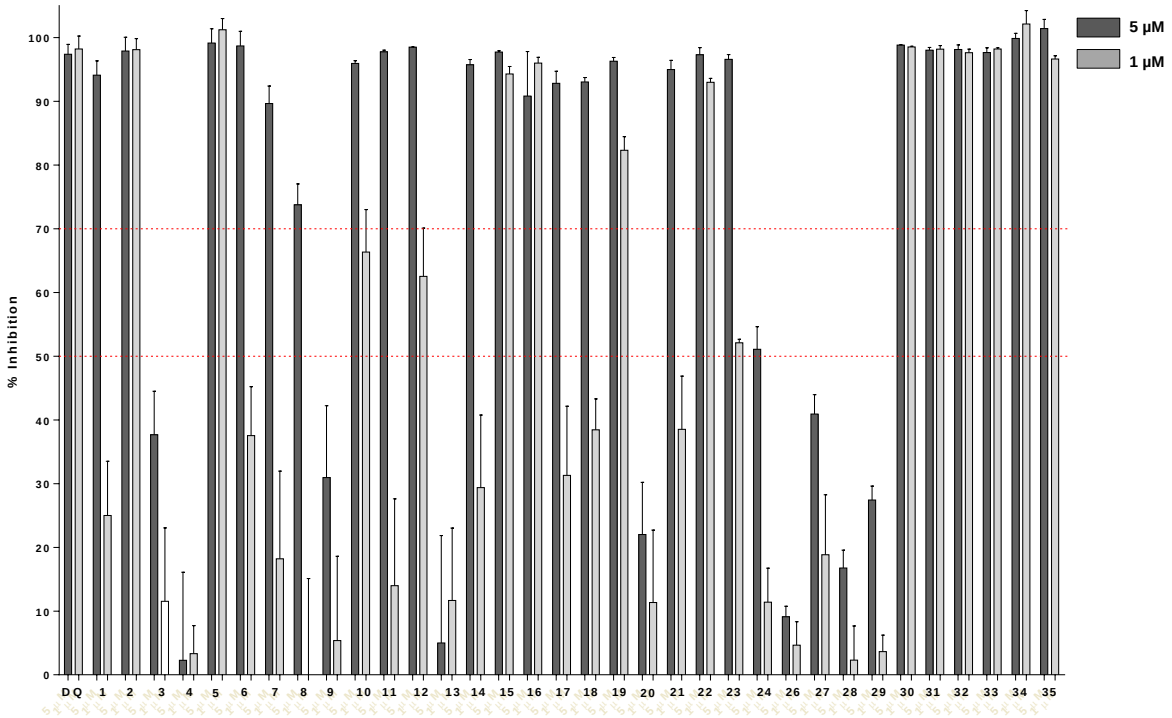


Figure S1: Inhibitory potential against asexual NF54 *P. falciparum* parasites.

Compounds were incubated at 5 µM and 1 µM against asexual NF54 *P. falciparum* parasites. Several compounds showed near complete inhibition of parasite proliferation as determined with the SYBR Green I fluorescence based assay following incubation at 37 ºC for 96 h with decoquinate **1** as control. Data analysis were performed in triplicate for n = 3 independent biological repeats with GraphPad 6.0; the average % inhibition is shown and error bars indicate SEM

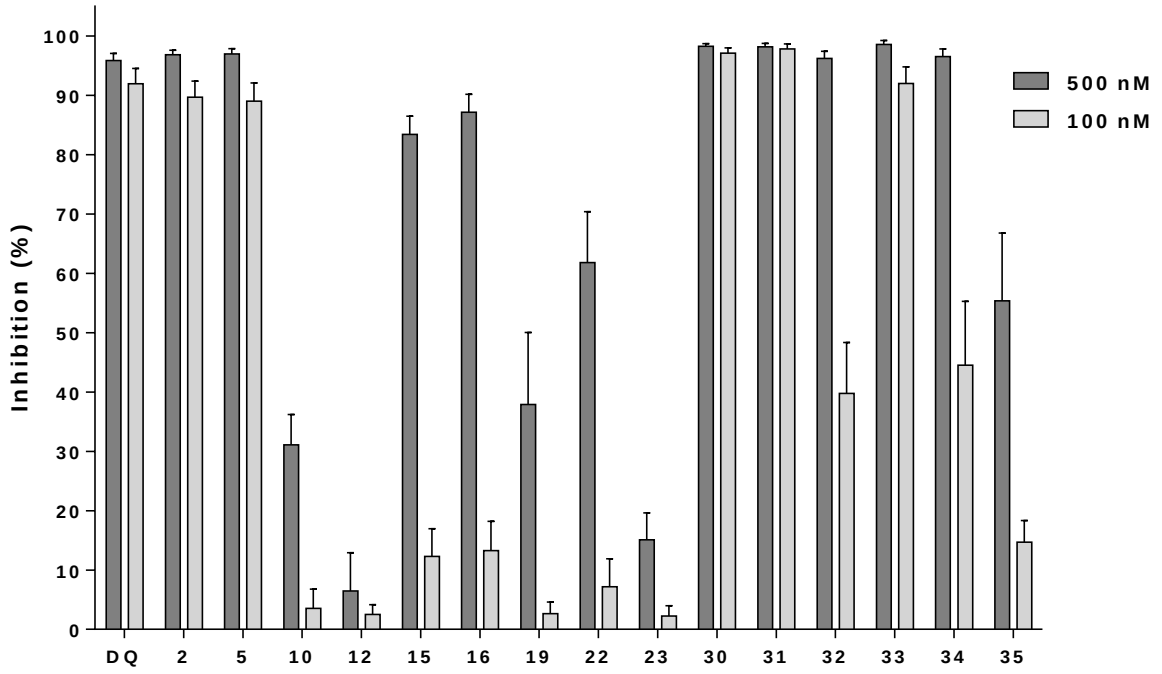


Figure S2: Inhibitory potential against asexual NF54 *P. falciparum* parasites**.**

The most active compounds at 5 μM and 1 μM were incubated at 500 nM and 100 nM against asexual NF54 *P. falciparum* parasites. Eight compounds showed near complete inhibition of parasite proliferation as determined with the SYBR Green I fluorescence based assay following incubation at 37 ºC for 96 h. Data analysis were performed in triplicate for n=3 independent biological repeats with GraphPad 6.0; the average % inhibition is shown and error bars indicate SEM.

***In vitro* cytotoxicity**

The WI-38 cell line (normal human fetal lung fibroblast) from ECACC was maintained as a monolayer cell culture at 37 °C, 5% CO_2, 95% air and 100% relative humidity in EMEM containing 10% fetal bovine serum, 2 mM L-glutamine and 50 μg/mL gentamicin. For this screening experiment, the cells (21 - 50 passages) were inoculated in 96-well microtiter plates at plating densities of 10 000 cells/well and incubated for 24 hours. After 24 hours, the cells were treated with the experimental drugs; which had previously been dissolved in DMSO and diluted in medium to produce five concentrations. Neat cells served as the control. The blank contained complete medium without cells. Parthenolide was used as a standard. The plates were incubated

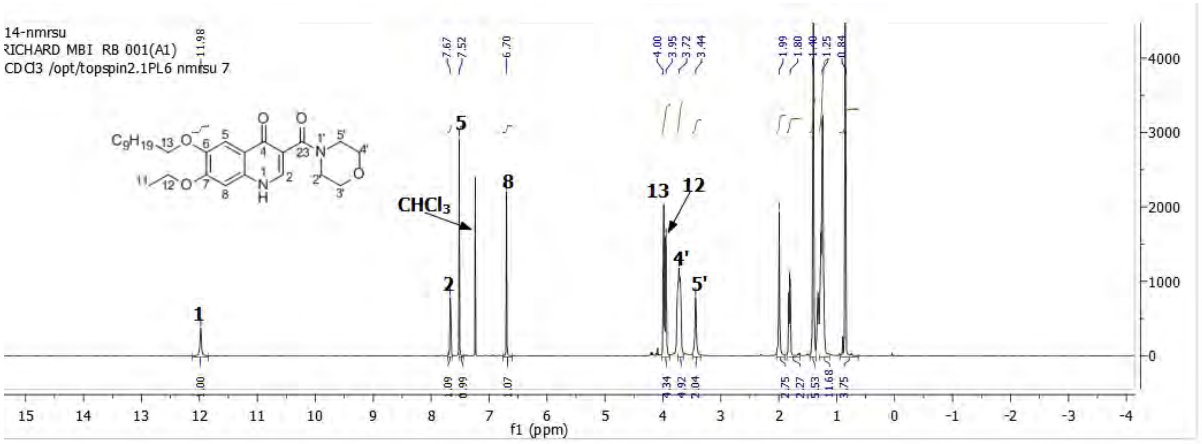
for 48 hours after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein bound dye was extracted with 10 mM Tris base for optical density determination at a wavelength of 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression.

Supporting data for chapter 4

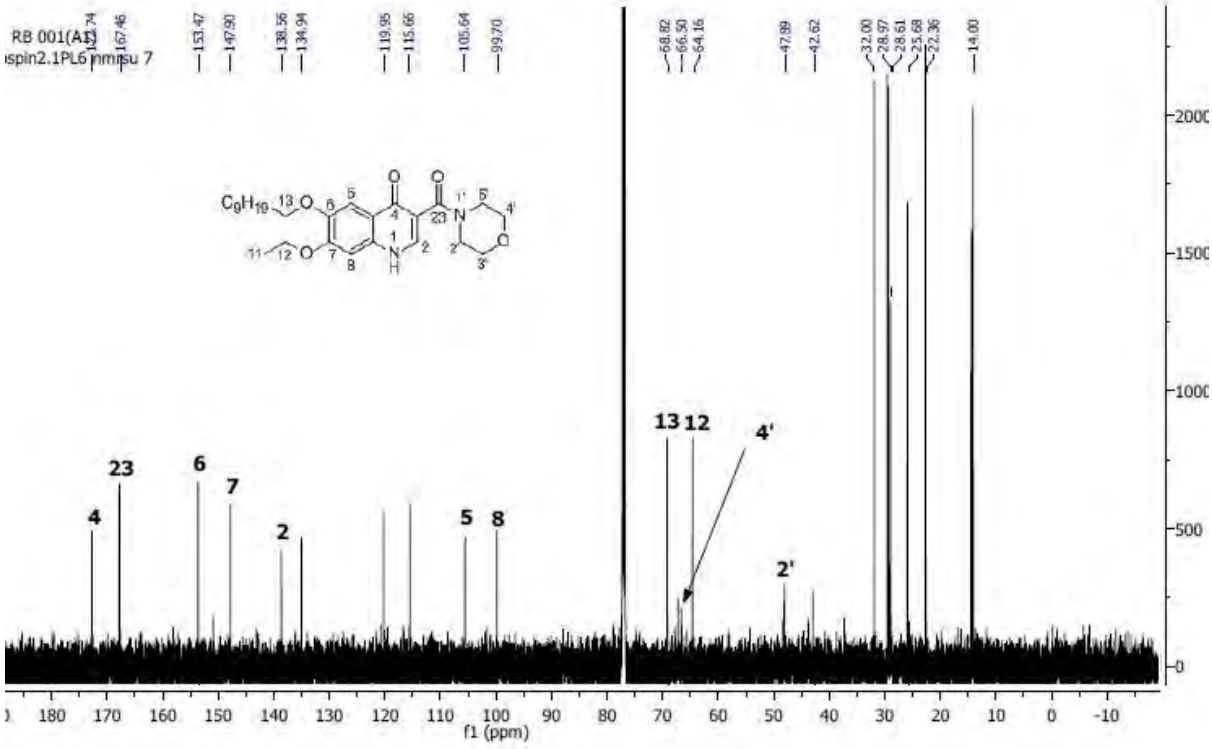
NMR and HRMS spectra of key derivatives

Compound 2

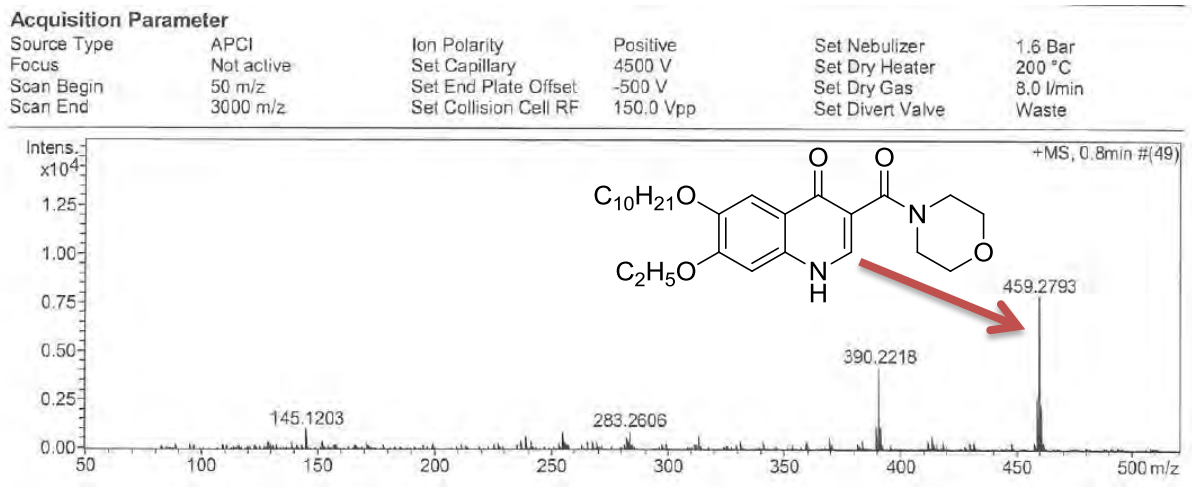
^{1}H NMR spectrum of Compound 2



^{13}C NMR spectrum of Compound 2



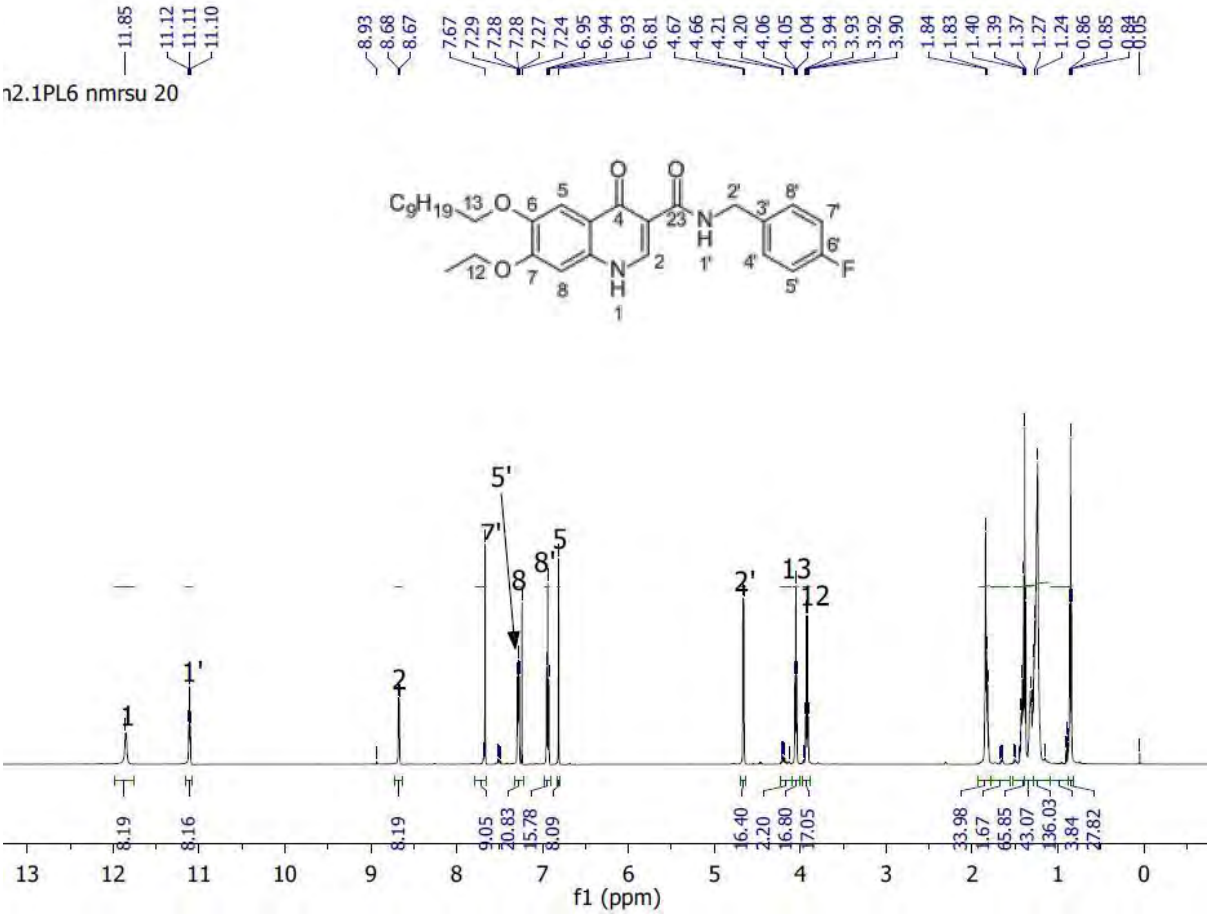
HRMS spectrum of Compound 2



Supporting data for chapter 4

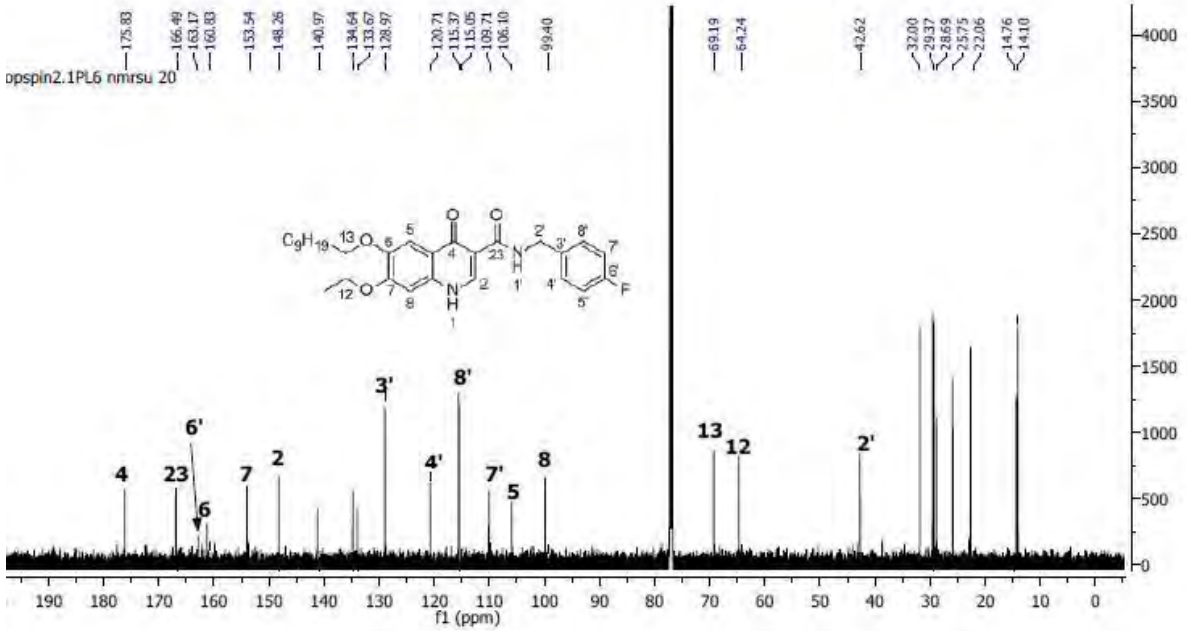
Compound 3

^{1}H NMR spectrum of Compound 3



Supporting data for chapter 4

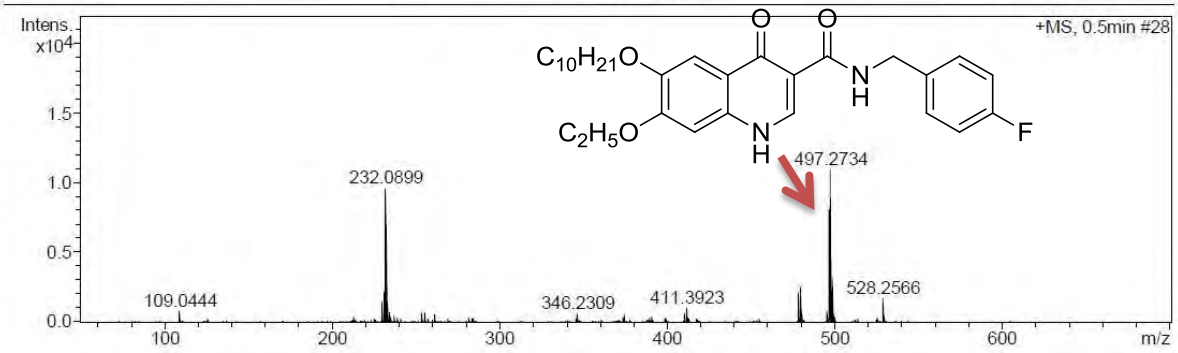
^{13}C NMR spectrum of Compound 3



HRMS Spectrum of Compound 3

Acquisition Parameter

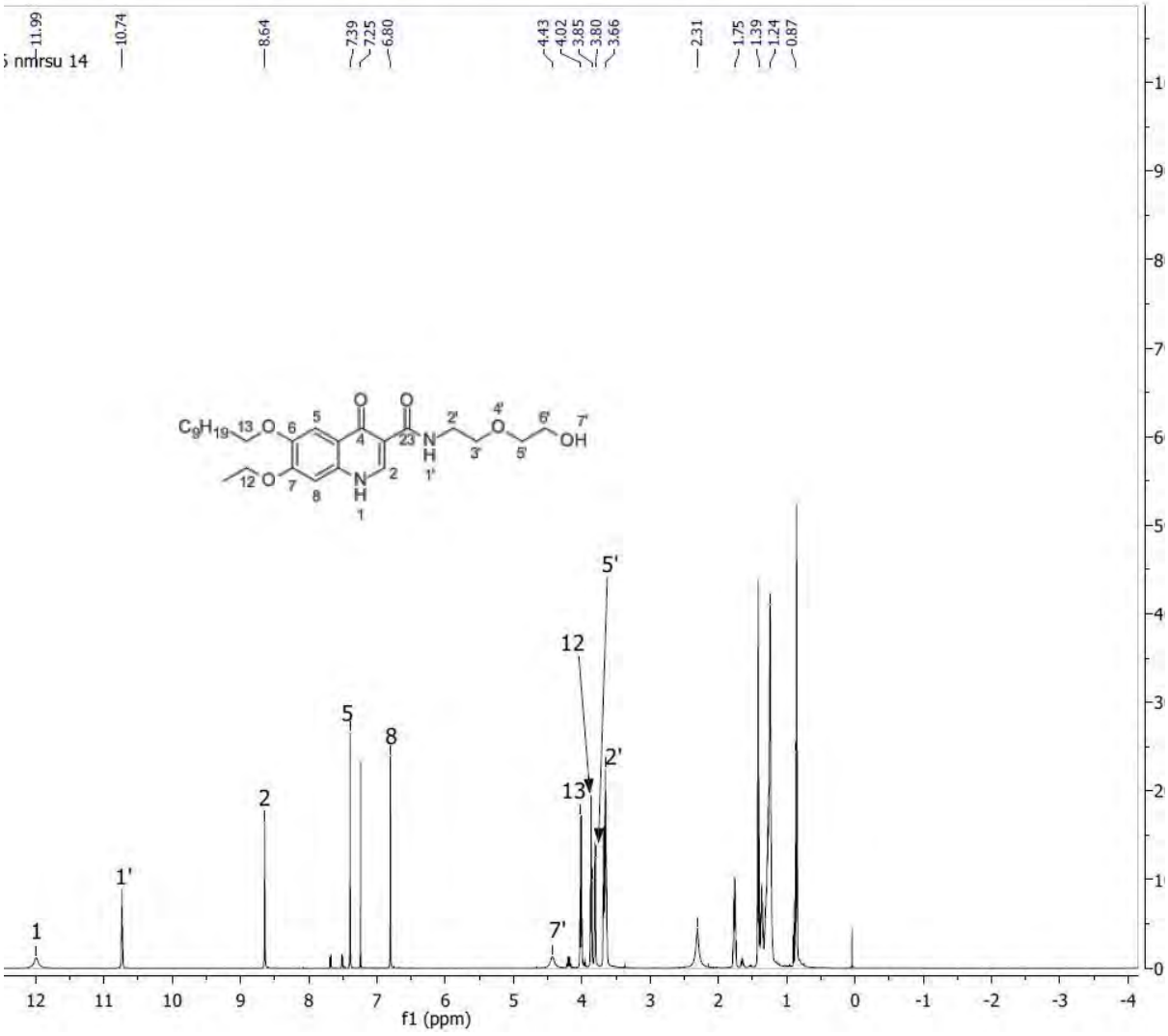
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	250 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Supporting data for chapter 4

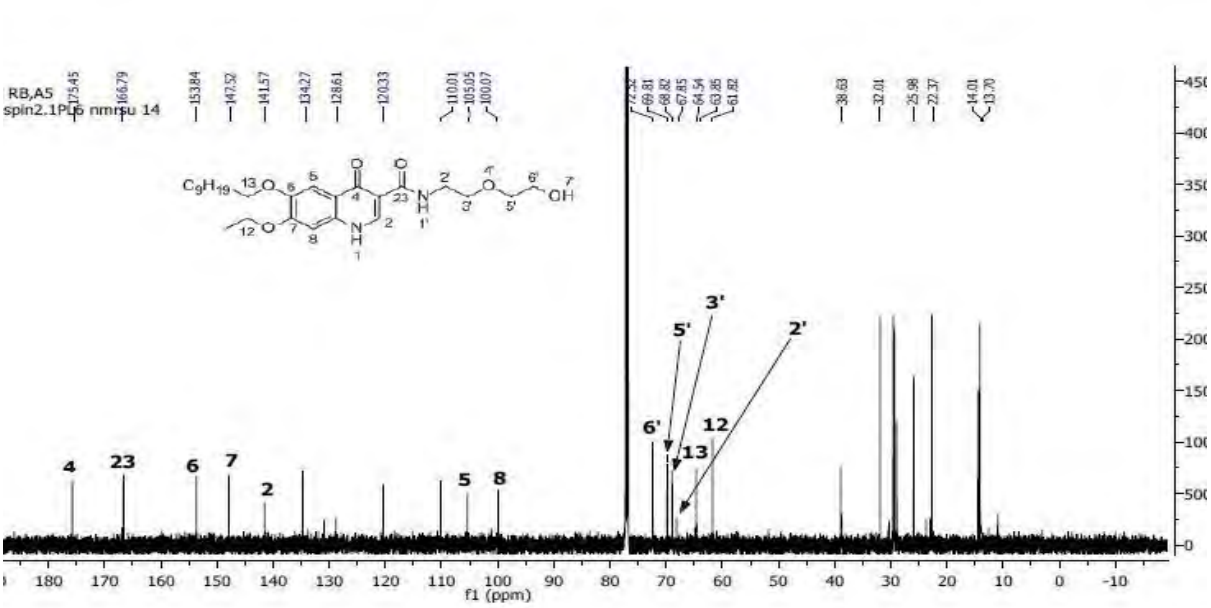
Compound 6

^{1}H NMR spectrum of Compound 6

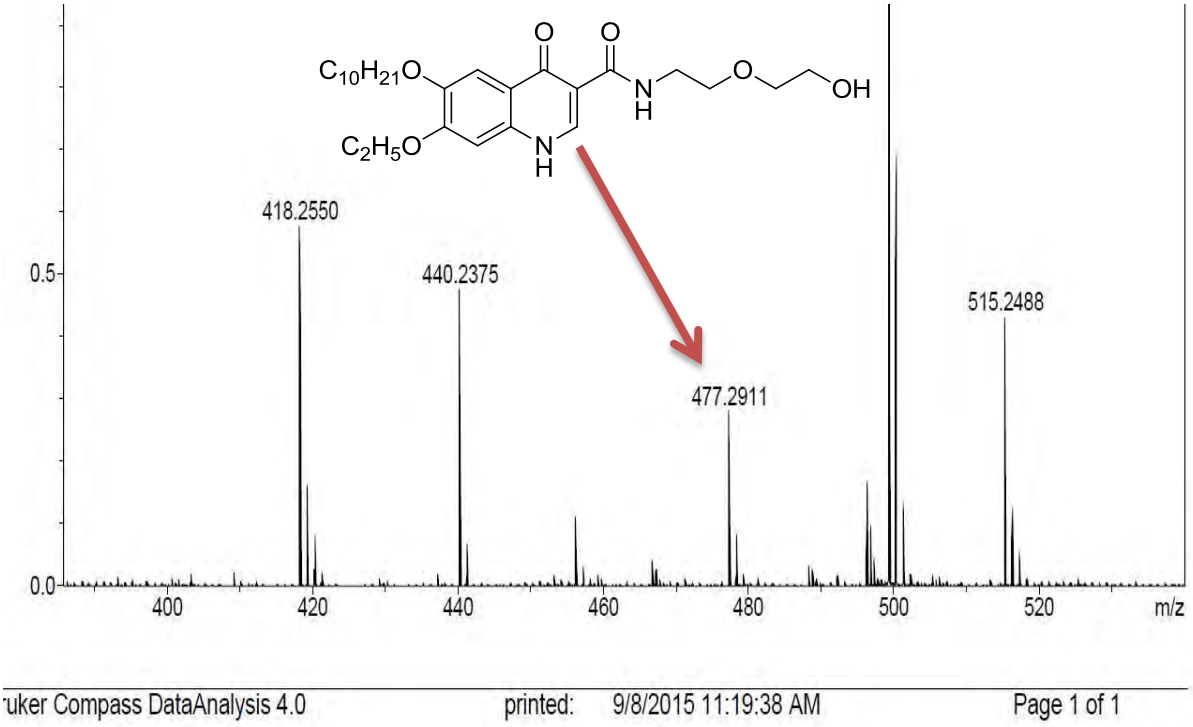


Supporting data for chapter 4

^{13}C NMR spectrum of Compound 6



HRMS spectrum of Compound 6



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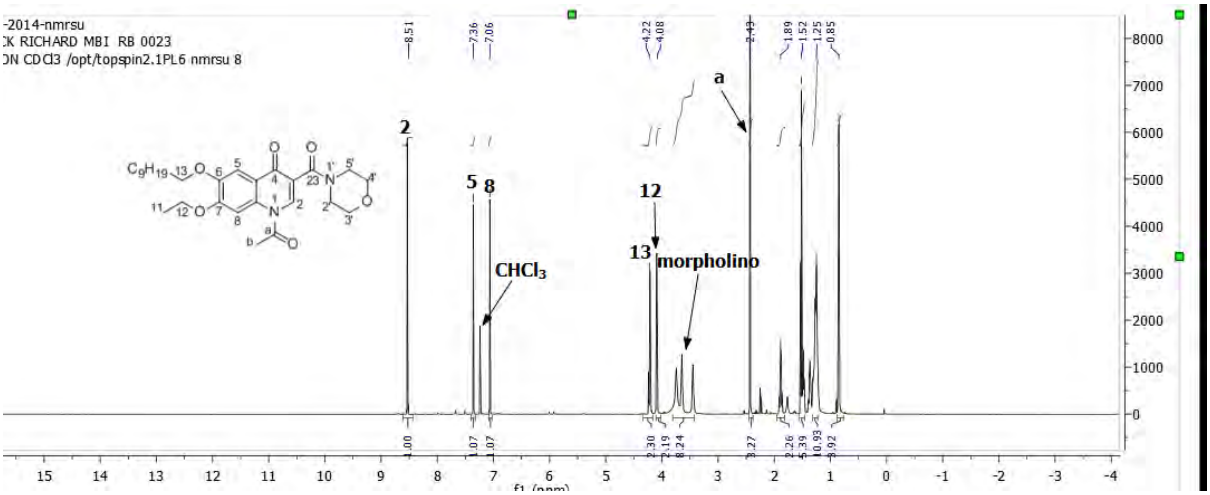
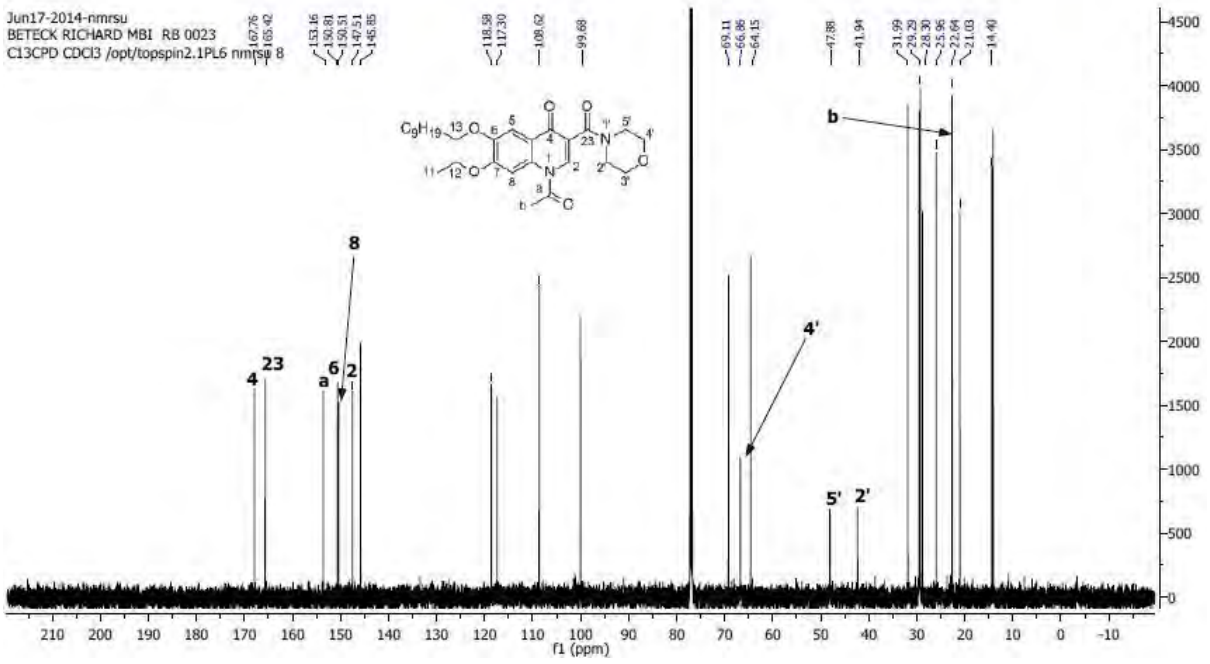
Supporting data for chapter 4

Compound 24

^{1}H NMR spectrum of Compound 24



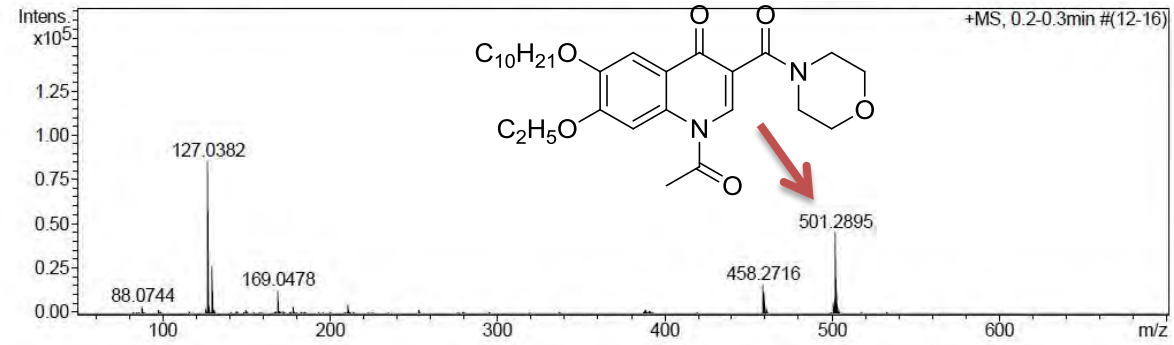
^{13}C NMR spectrum of Compound 24



HRMS spectrum of Compound 24

Acquisition Parameter

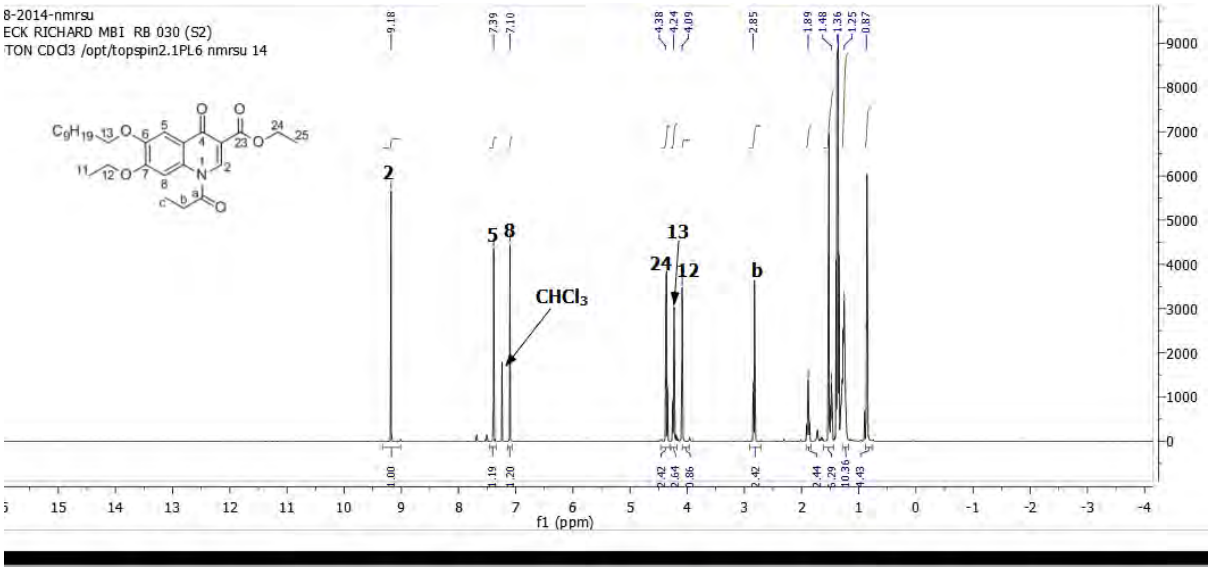
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



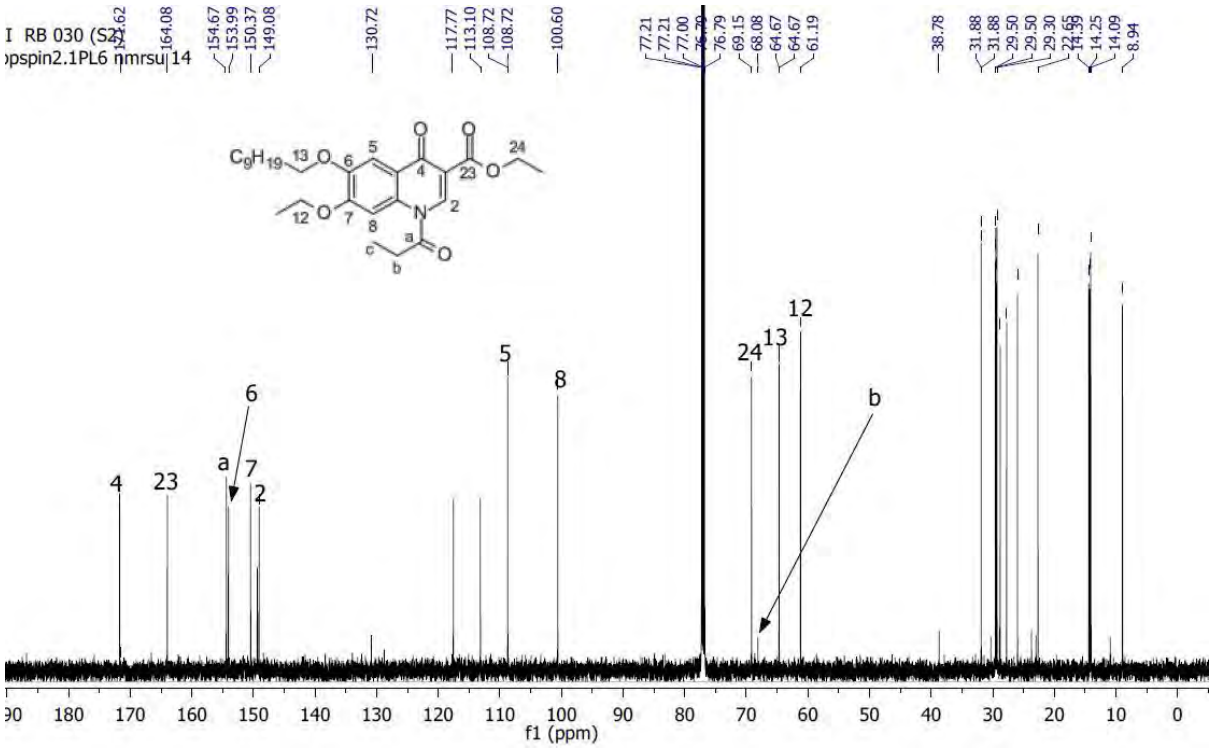
Supporting data for chapter 4

Compound 30

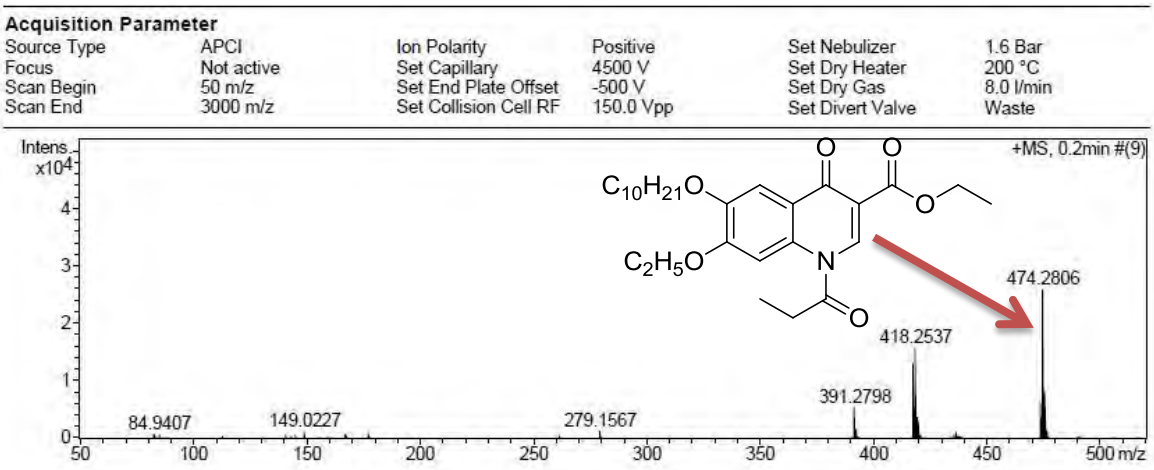
^{1}H NMR spectrum of Compound 30



^{13}C NMR spectrum of Compound 30



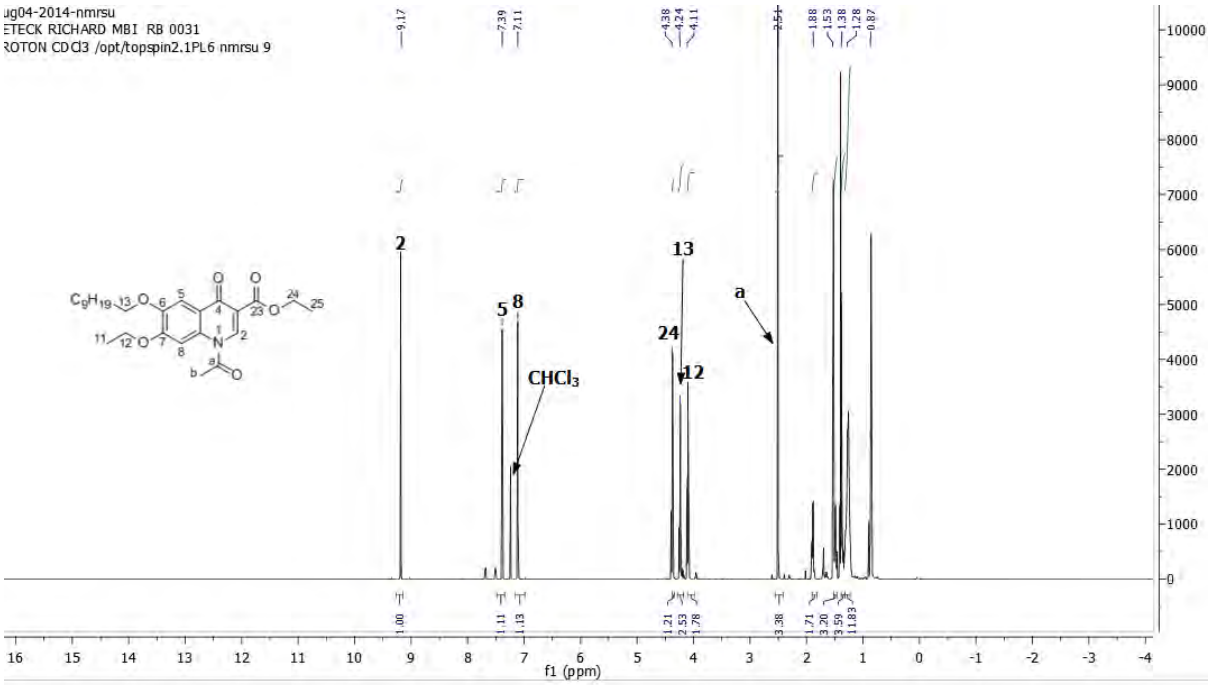
HRMS spectrum of Compound 30



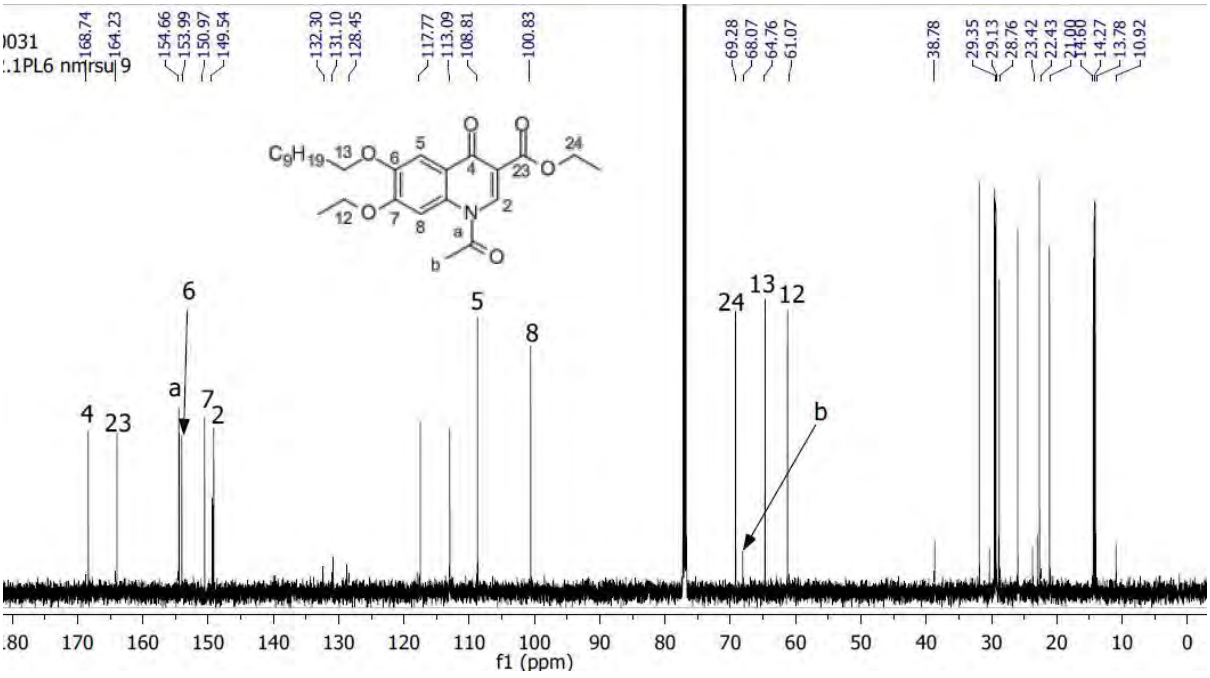
Supporting data for chapter 4

Compound 31

^{1}H NMR spectrum of Compound 31



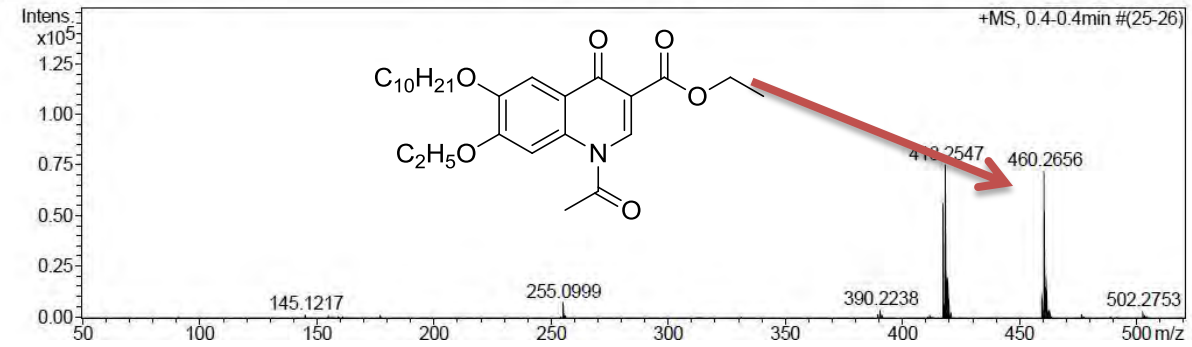
^{13}C NMR spectrum of Compound 31



HRMS spectrum of Compound 31

Acquisition Parameter

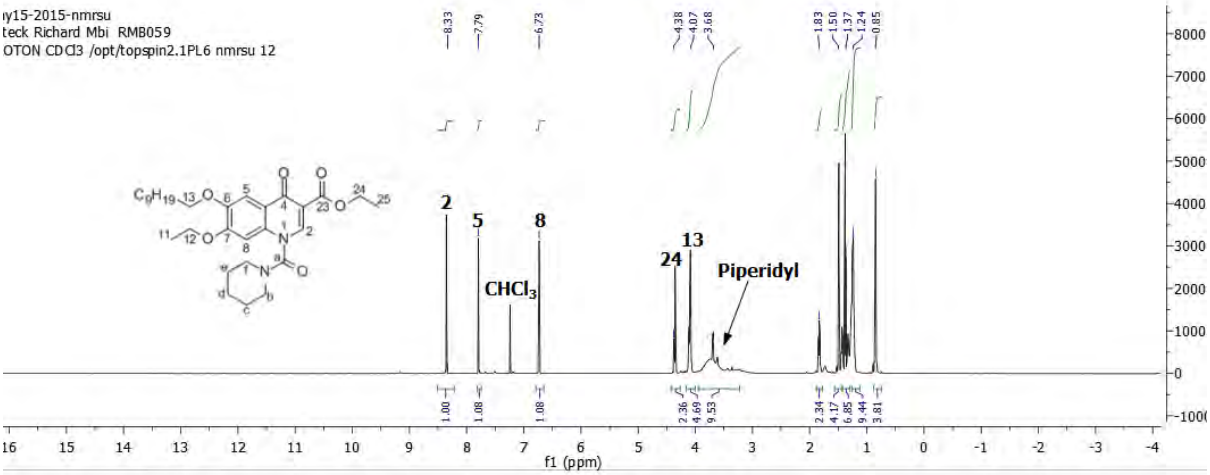
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



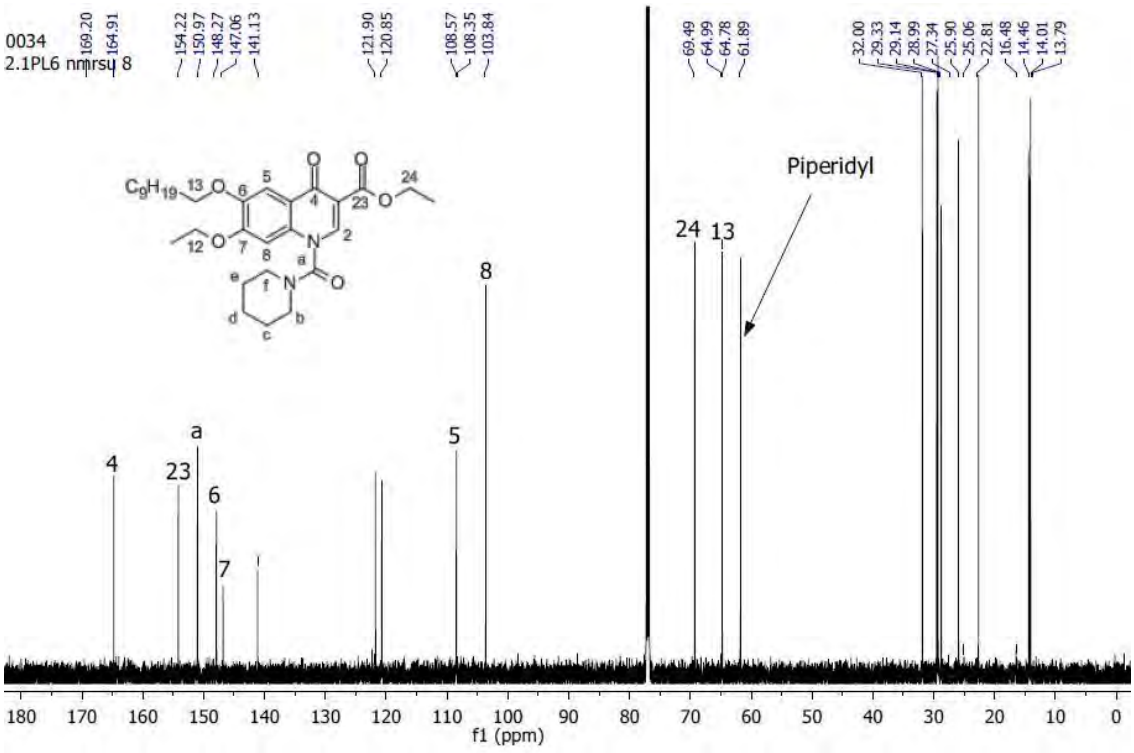
Supporting data for chapter 4

Compound 33

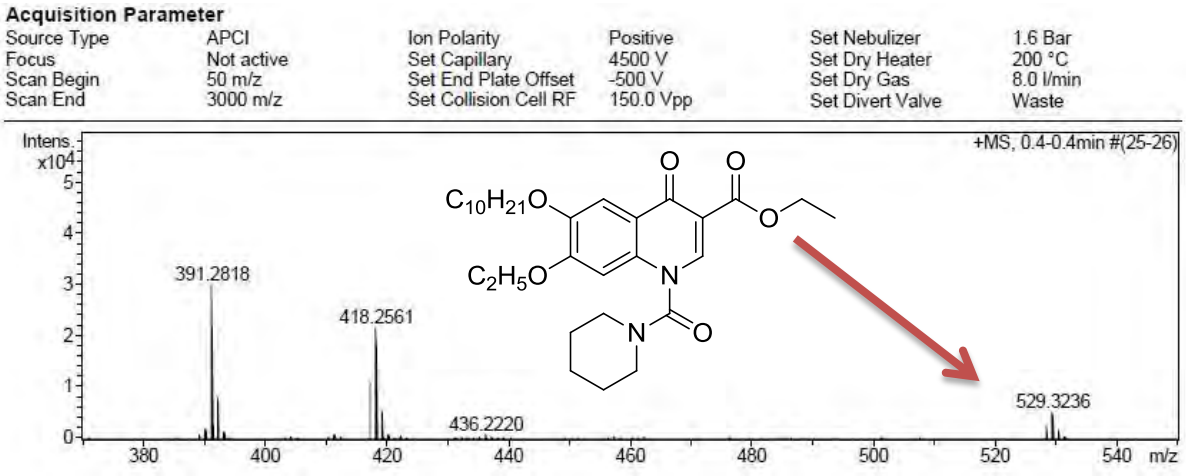
^{1}H NMR spectrum of Compound 33



^{13}C NMR spectrum of Compound 33



HRMS spectrum of Compound 33

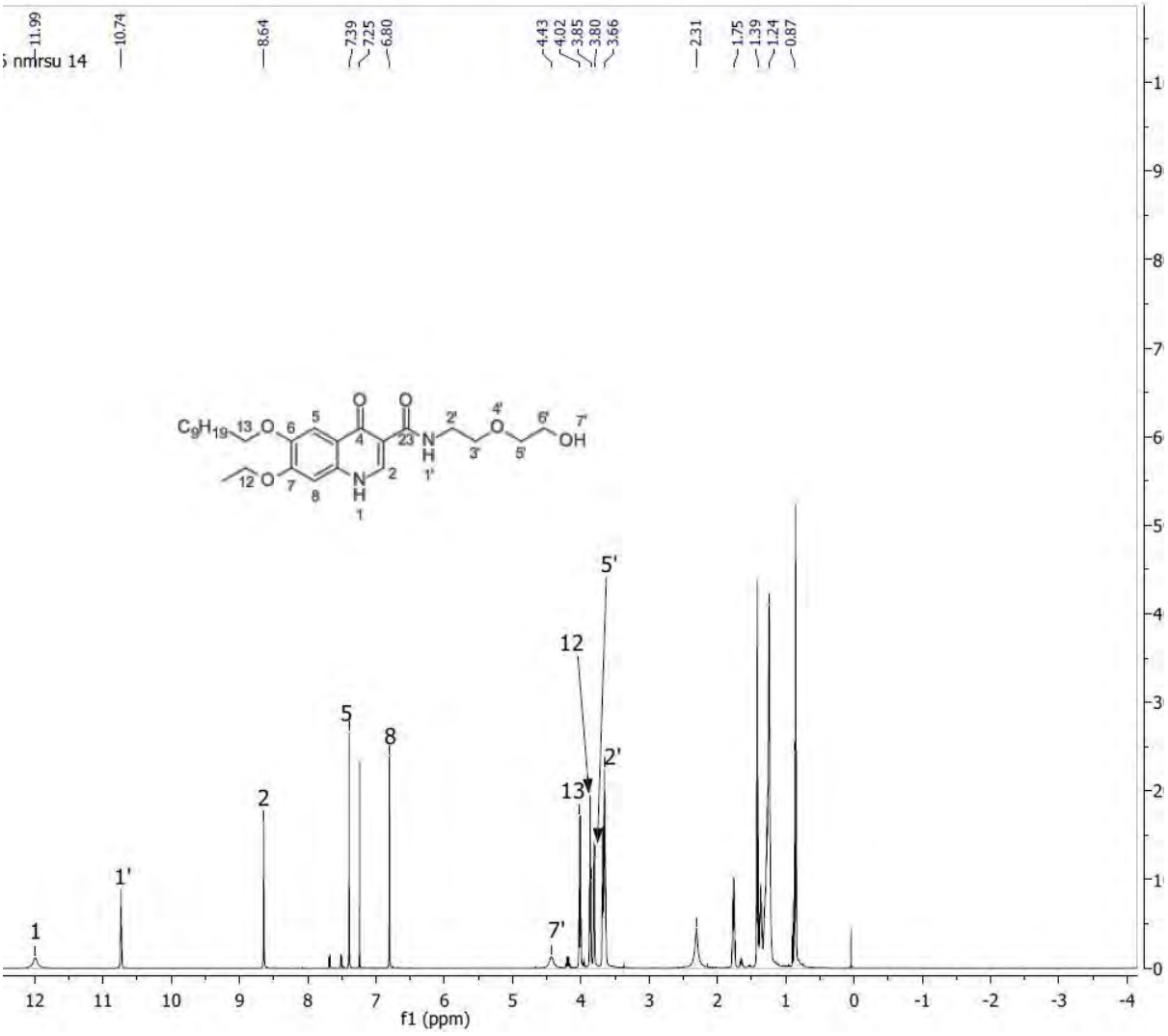


Addendum B: ^{1}H and ^{13}C NMR and mass spectra for chapter 5

SUPPORTING DATA FOR TB MANUSCRIPT

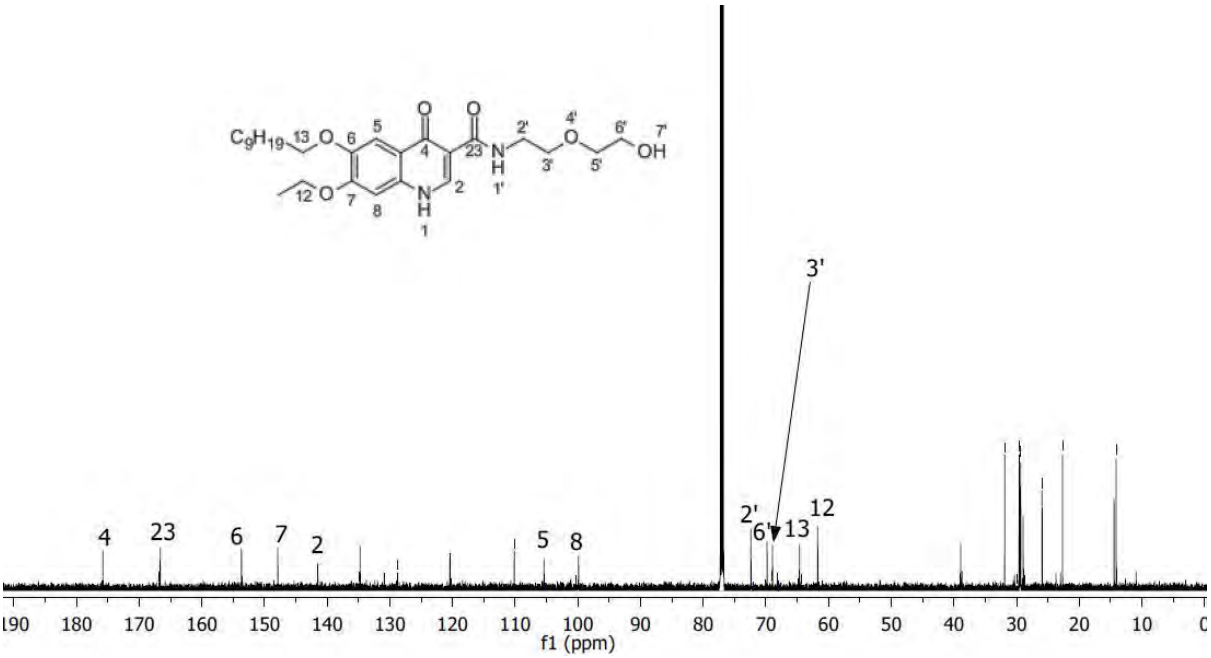
Compound 3

^{1}H NMR spectrum of compound 3

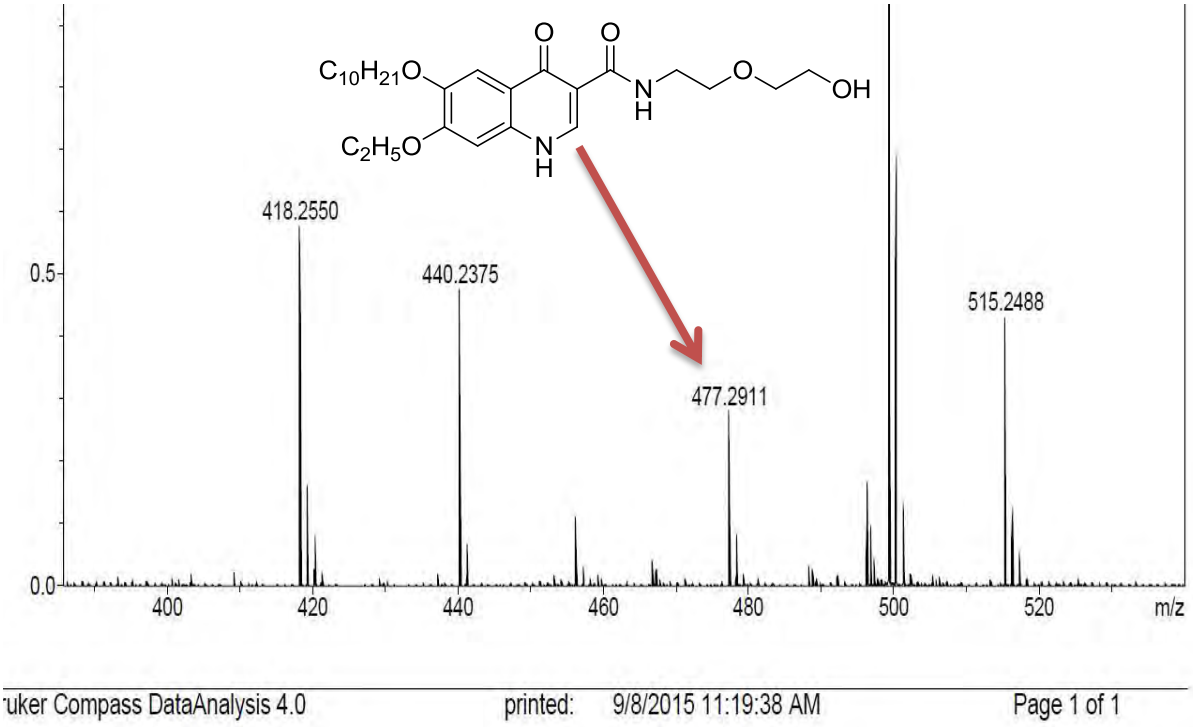


Supporting data for chapter 5

^{13}C NMR spectrum of compound 3



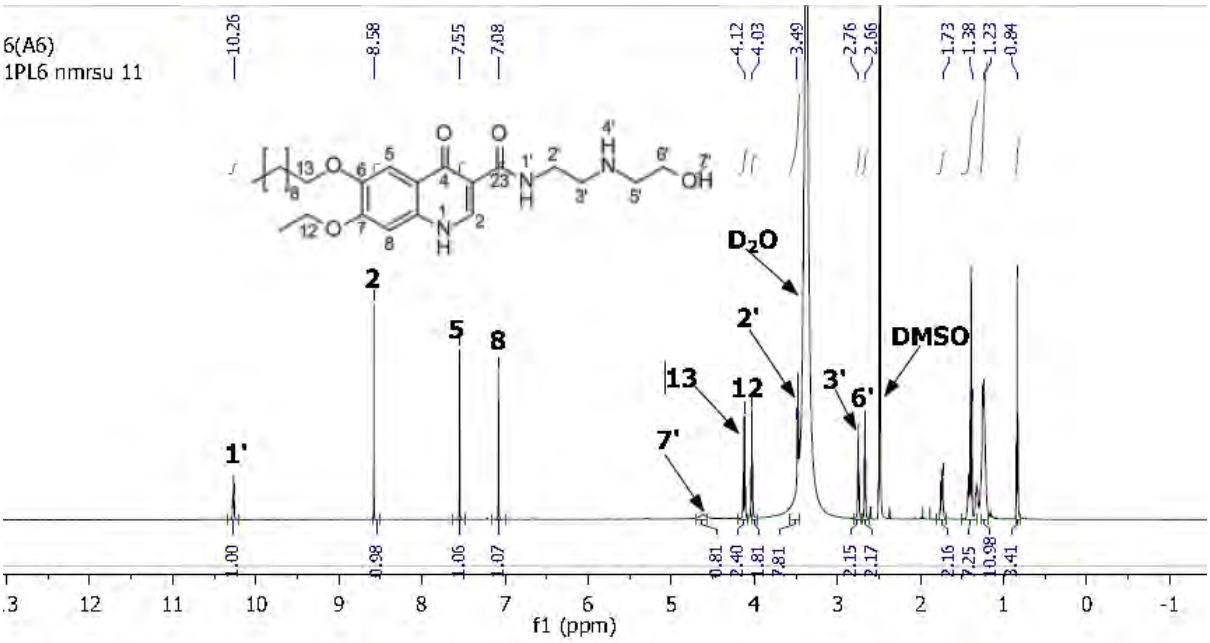
HRMS spectrum of compound 3



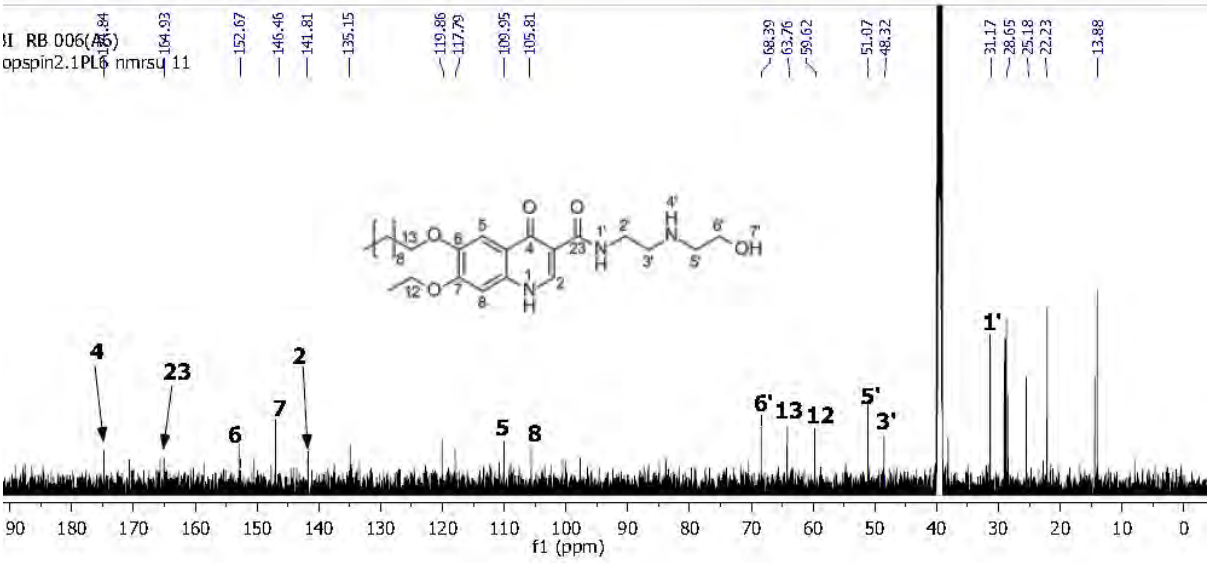
Supporting data for chapter 5

Compound 4

^{1}H NMR spectrum of compound 4



^{13}C NMR spectrum of compound 4



Supporting data for chapter 5

HRMS spectrum of compound 4

Acquisition Parameter

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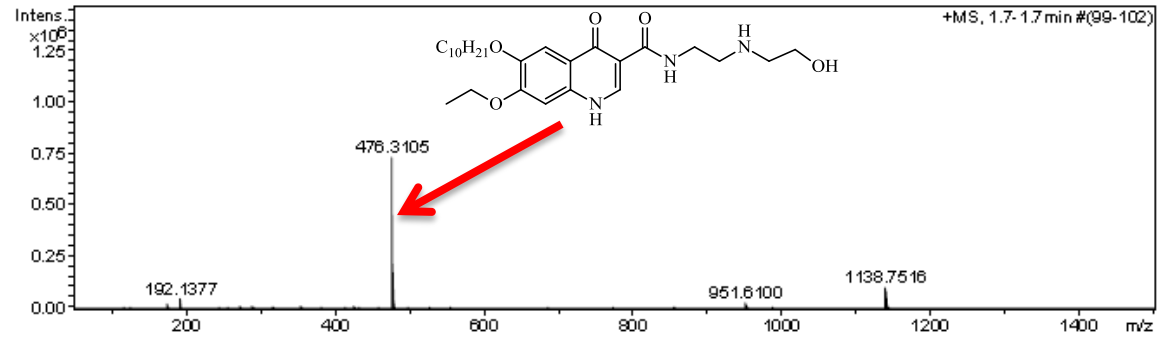
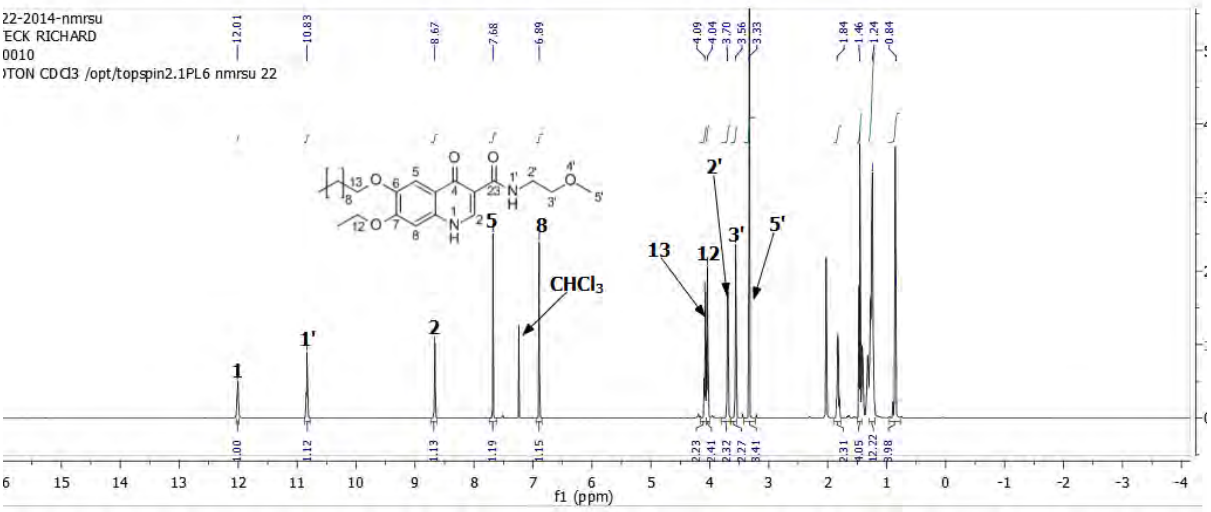


Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
476.3105	1	C 26 H 42 N 3 O 5	100.00	476.3119	1.4	2.9	0.9	7.5	ok	even

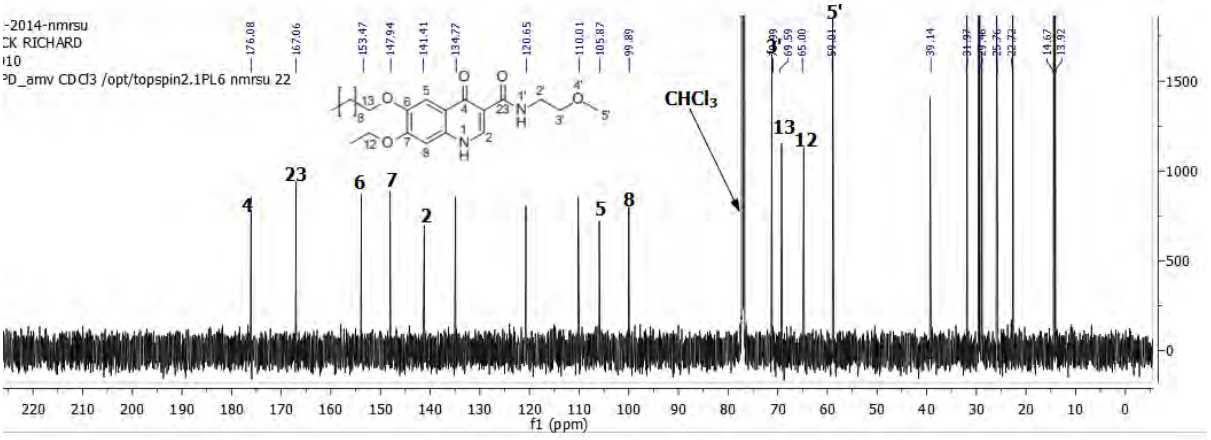
Supporting data for chapter 5

Compound 5

^{1}H NMR spectrum of compound 5

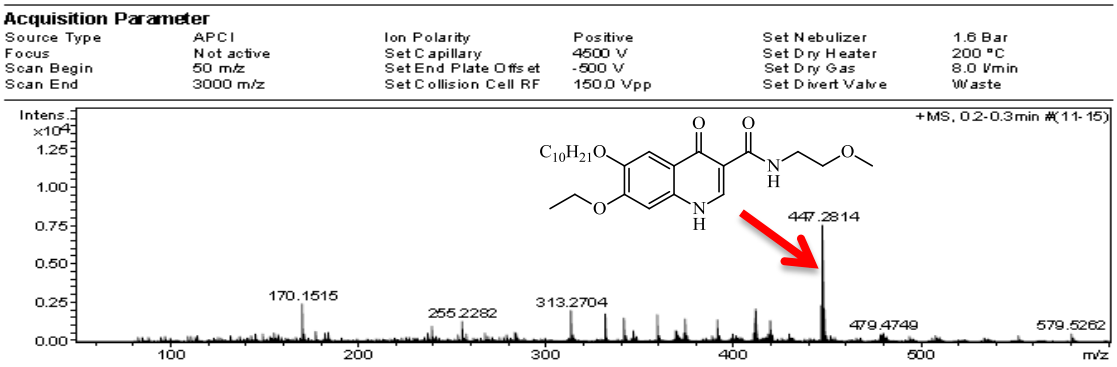


^{13}C NMR spectrum of compound 5



Supporting data for chapter 5

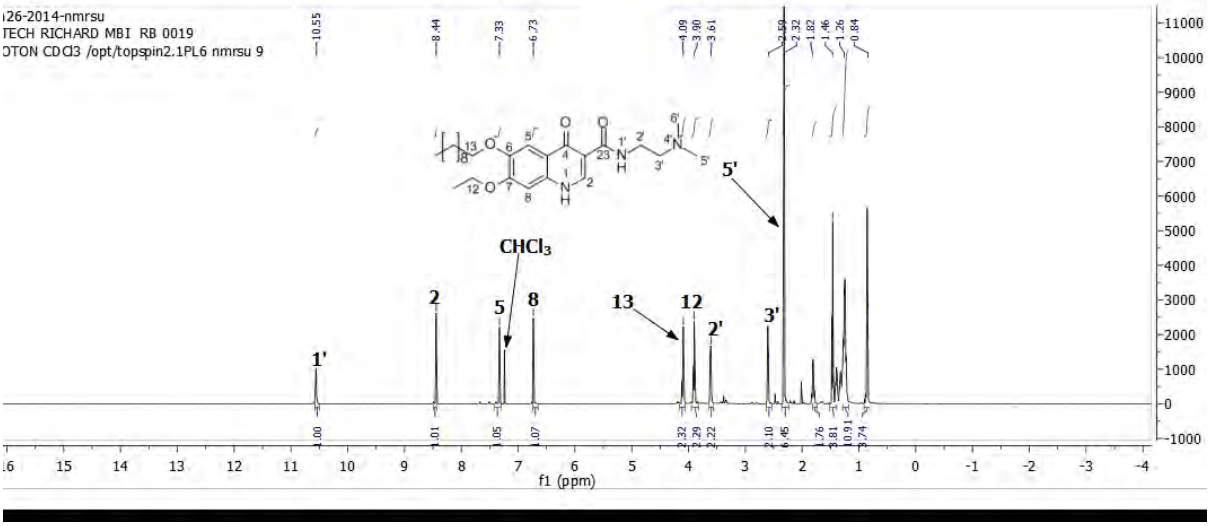
HRMS spectrum of compound 5



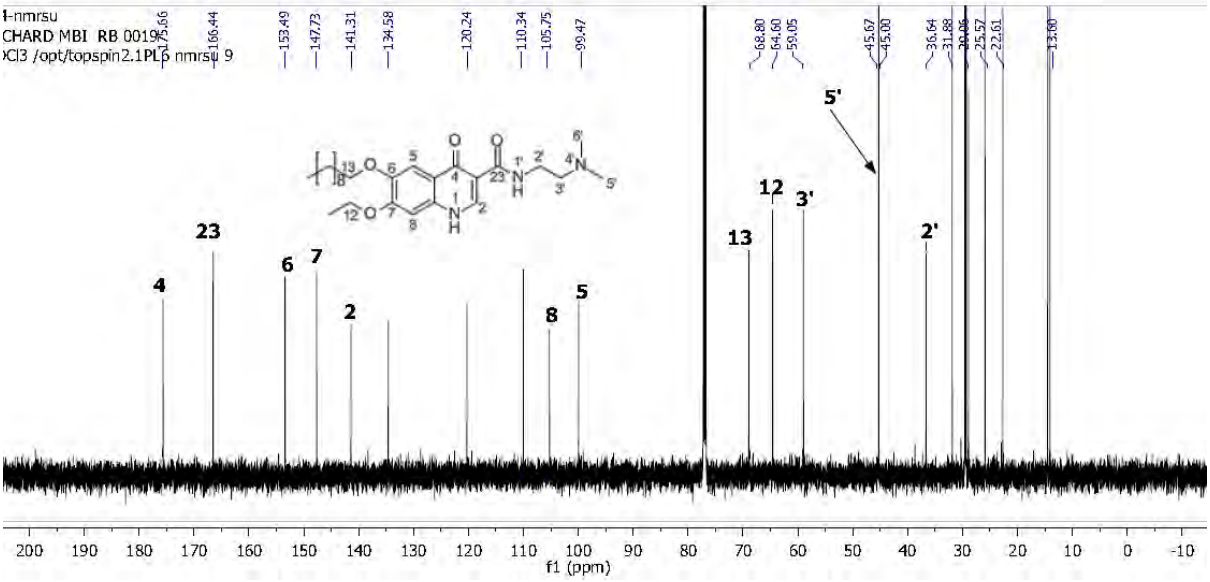
Supporting data for chapter 5

Compound 6

^{1}H NMR spectrum of compound 6



^{13}C NMR spectrum of compound 6

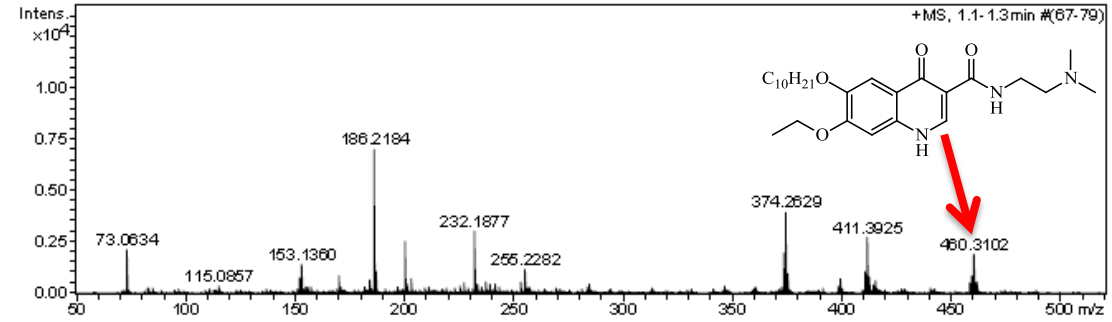


Supporting data for chapter 5

HRMS spectrum of compound 6

Acquisition Parameter

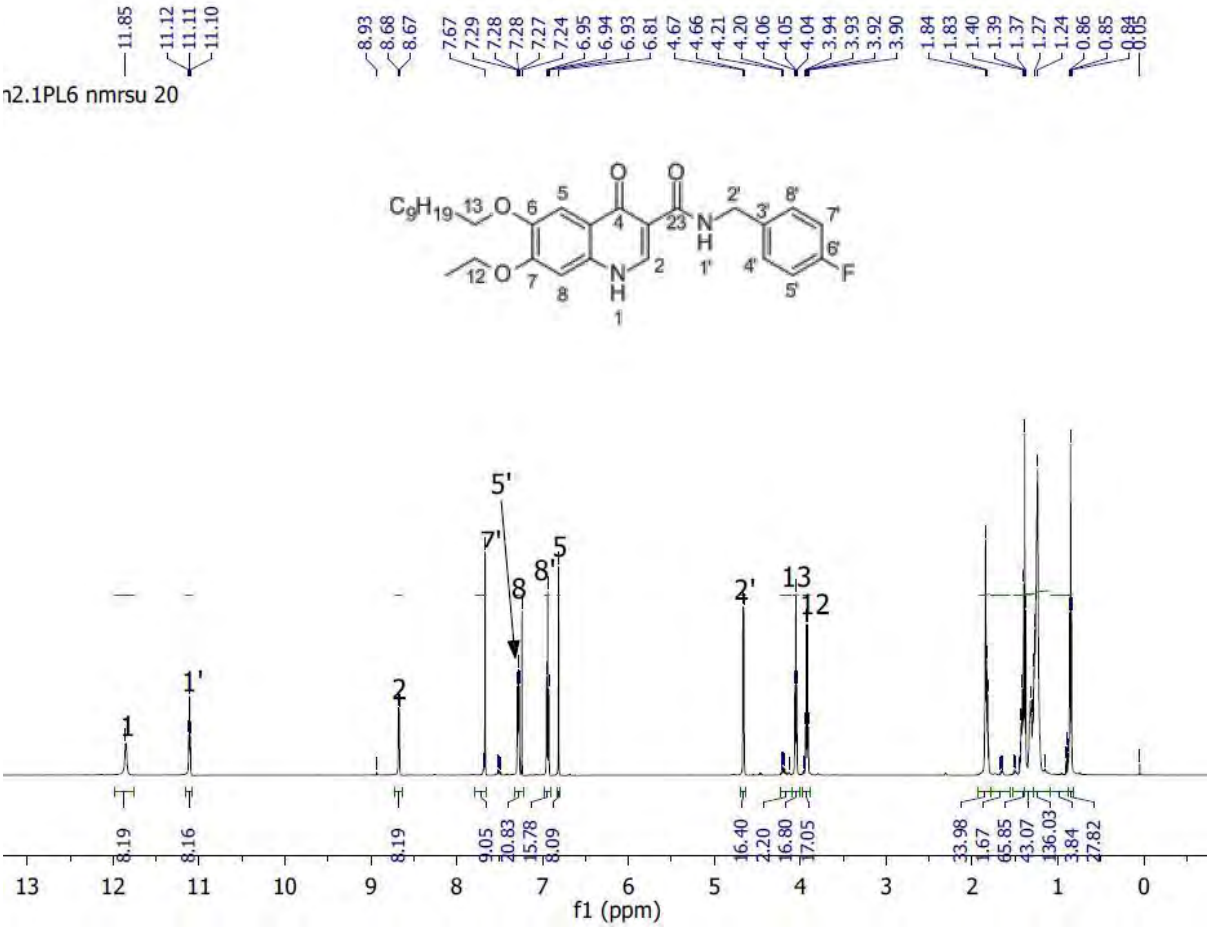
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Supporting data for chapter 5

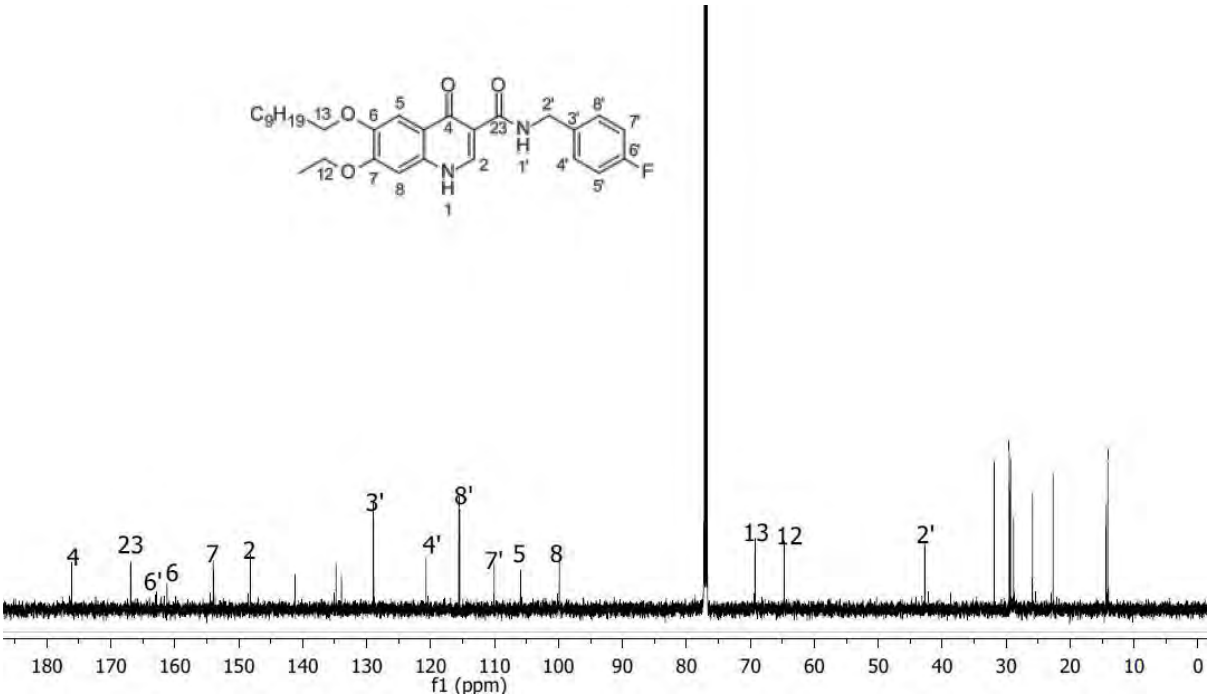
Compound 7

^{1}H NMR spectrum of compound 7



Supporting data for chapter 5

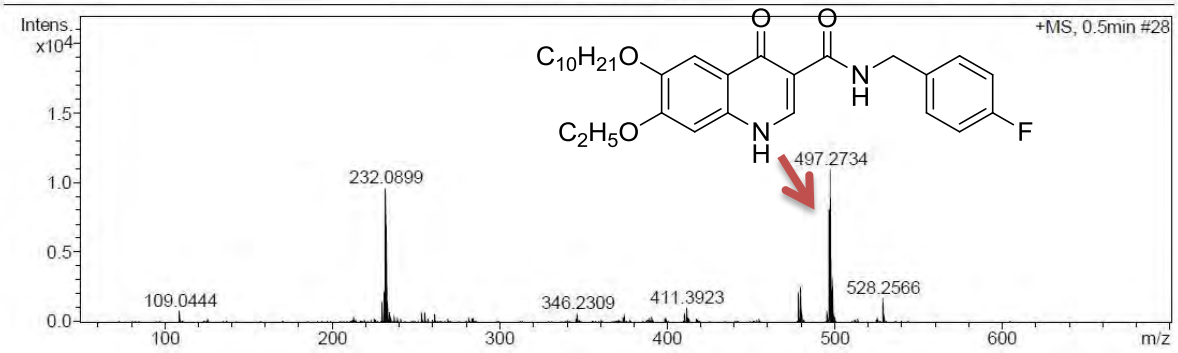
^{13}C NMR spectrum of compound 7



HRMS spectrum of compound 7

Acquisition Parameter

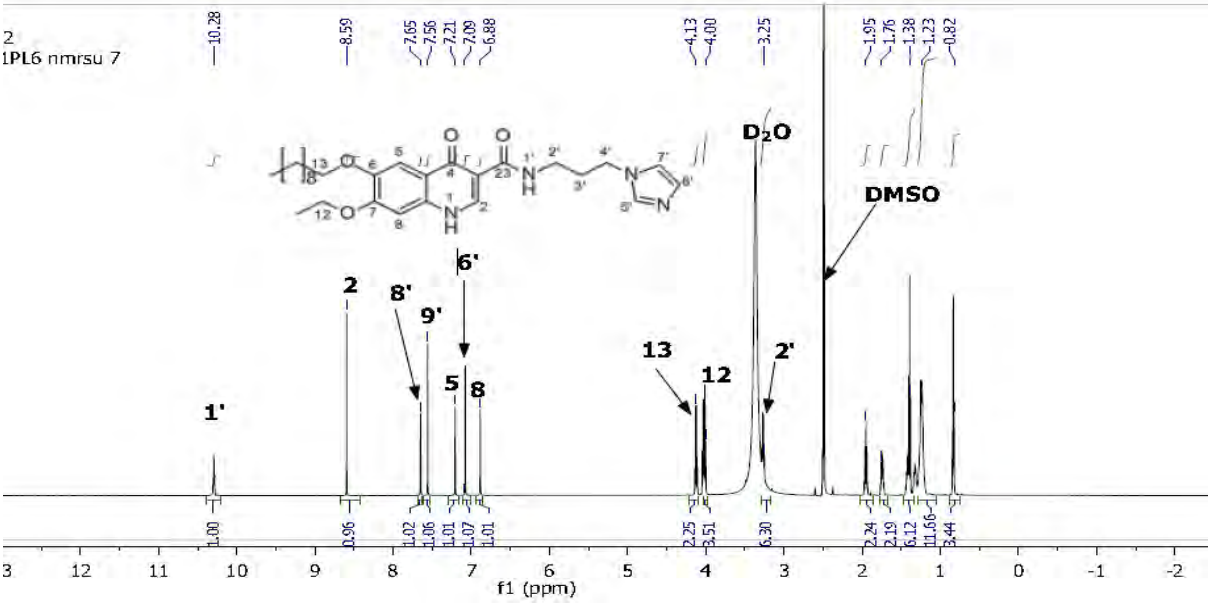
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	250 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



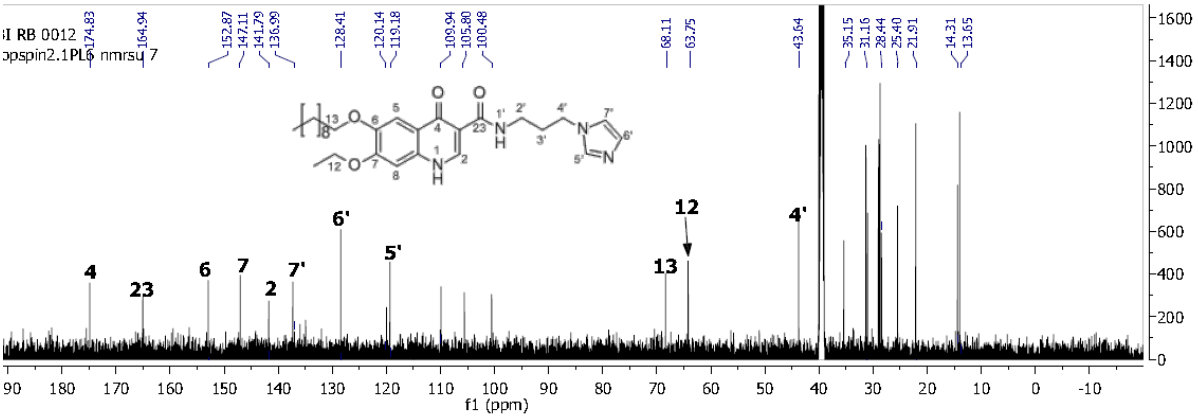
Supporting data for chapter 5

Compound 8

^{1}H NMR spectrum of compound 8



^{13}C NMR spectrum of compound 8

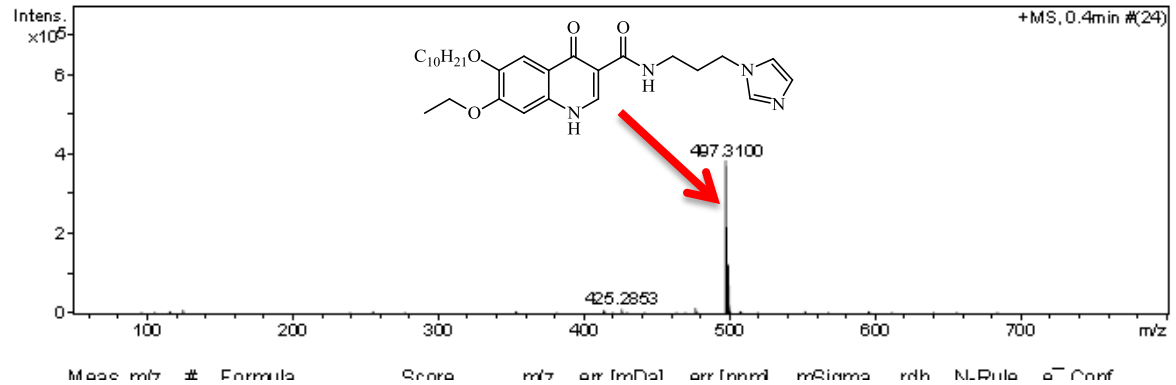


Supporting data for chapter 5

HRMS spectrum of compound 8

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste

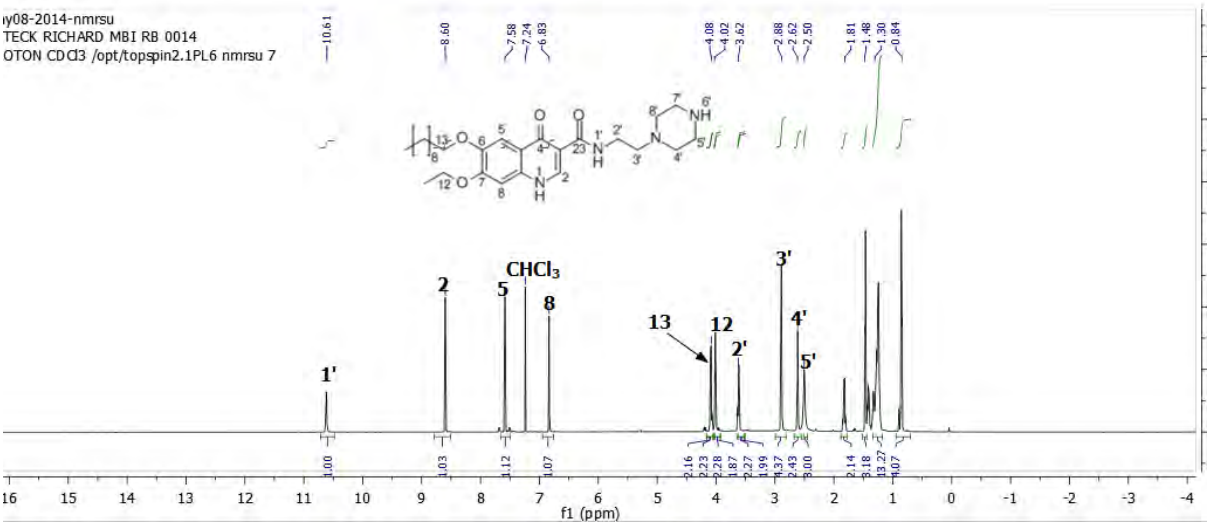


Meas. m/z # Formula Score m/z err [mDa] err [ppm] mSigma rdb N-Rule e⁻ Conf

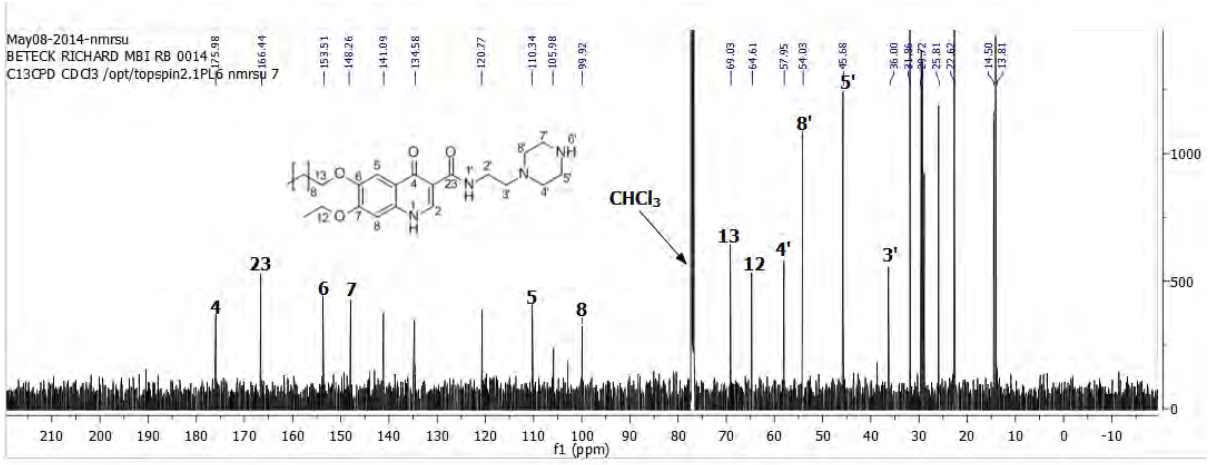
Supporting data for chapter 5

Compound 9

^{1}H NMR spectrum of compound 9

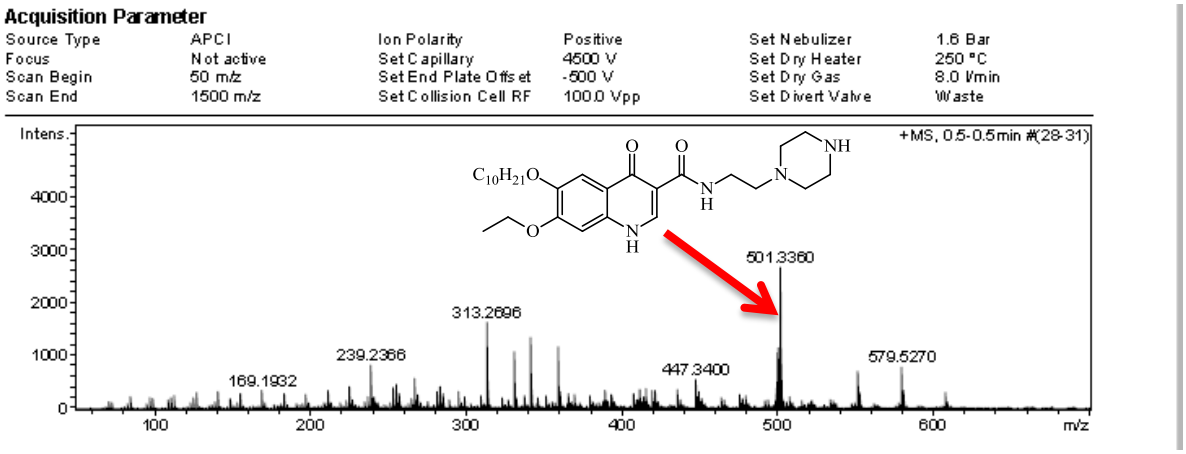


^{13}C NMR spectrum of compound 9



Supporting data for chapter 5

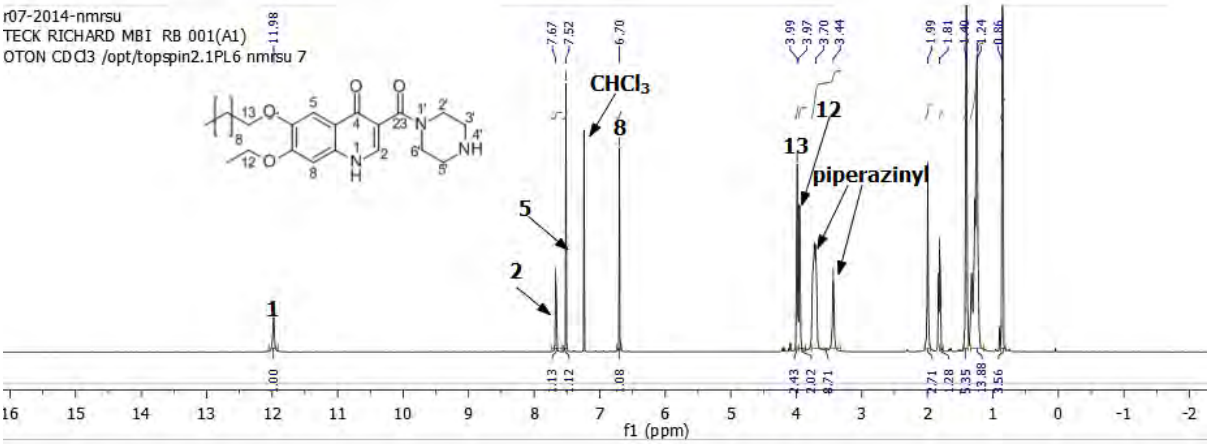
HRMS spectrum of compound 9



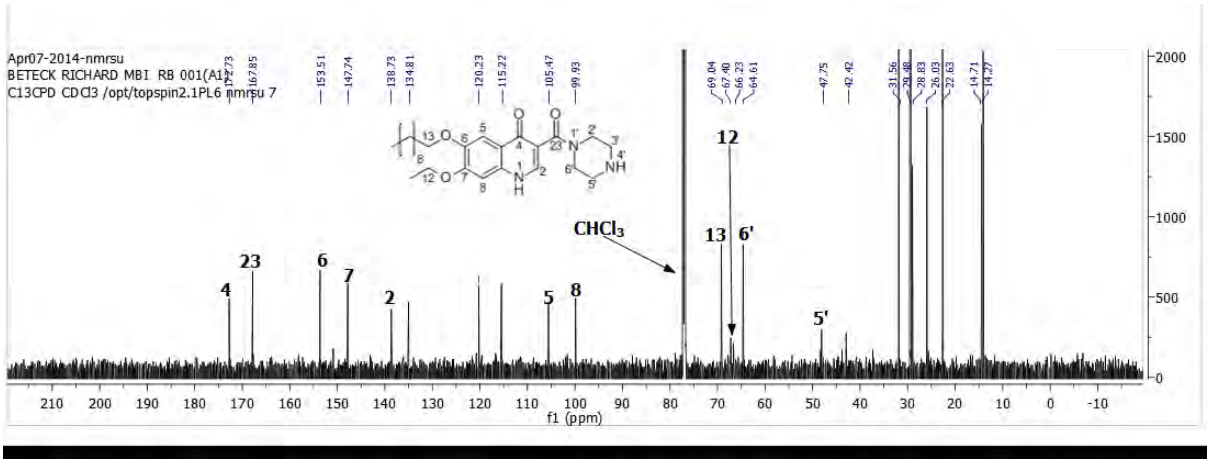
Supporting data for chapter 5

Compound 10

^{1}H NMR spectrum of compound 10



^{13}C NMR spectrum of compound 10

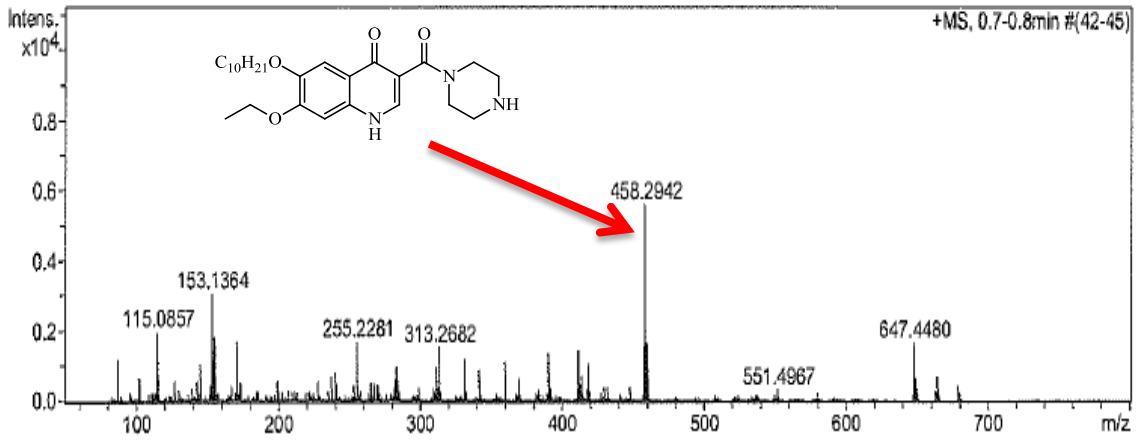


Supporting data for chapter 5

HRMS spectrum of compound 10

Acquisition Parameter

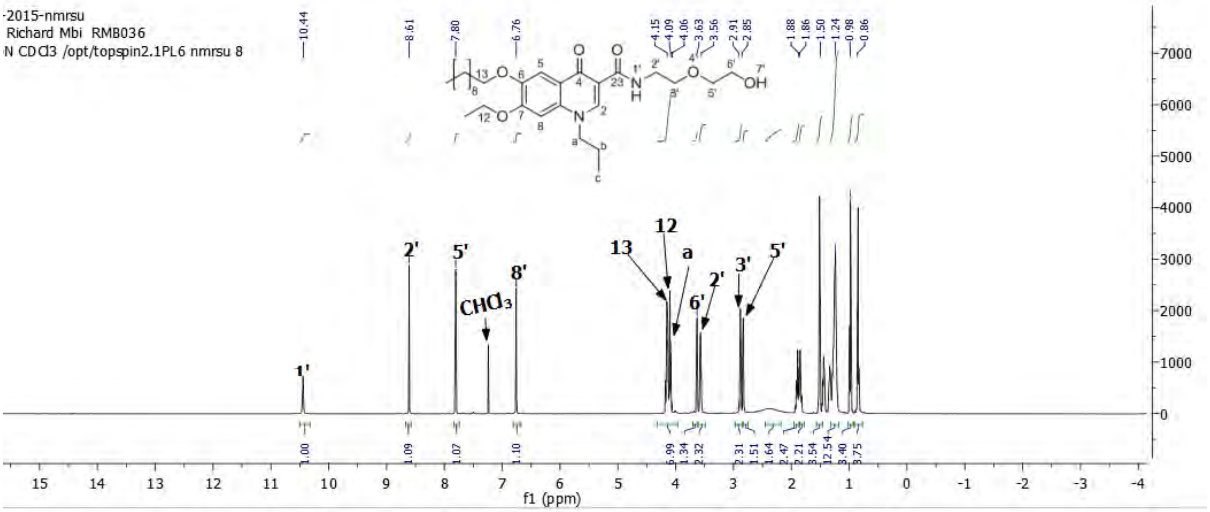
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



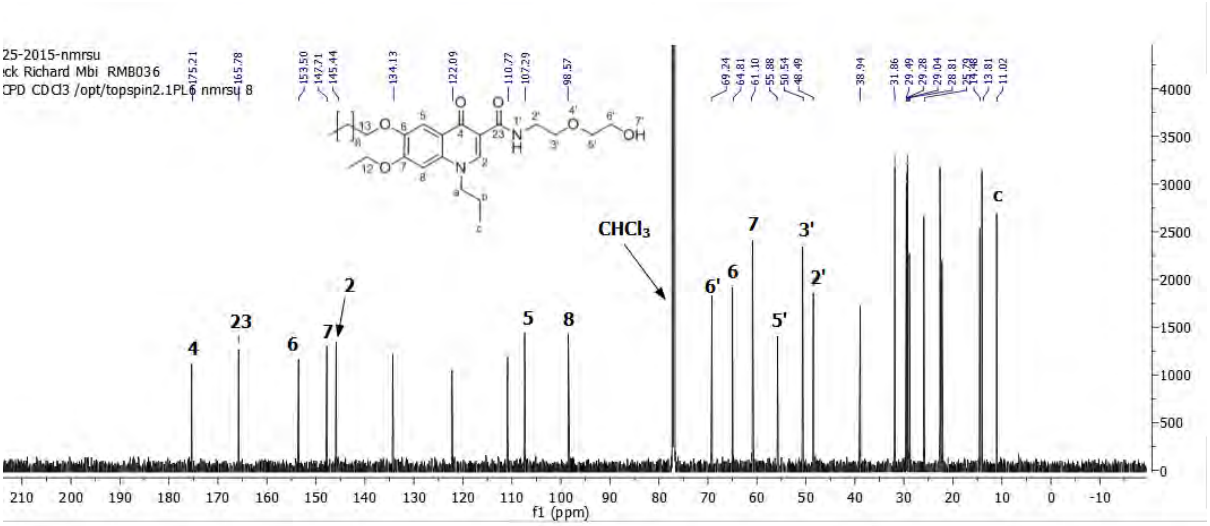
Supporting data for chapter 5

Compound 11

^{1}H NMR spectrum of compound 11

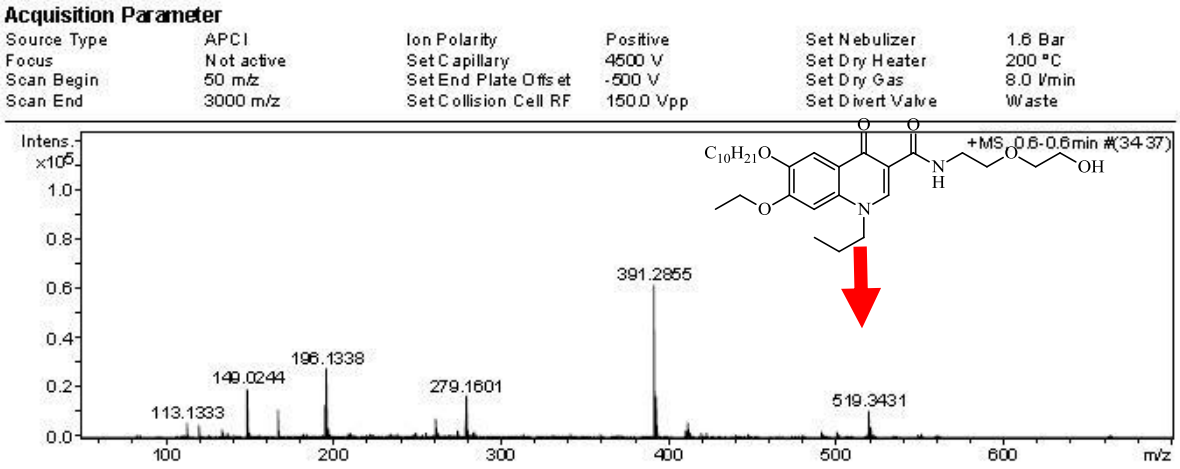


^{13}C NMR spectrum of compound 11



Supporting data for chapter 5

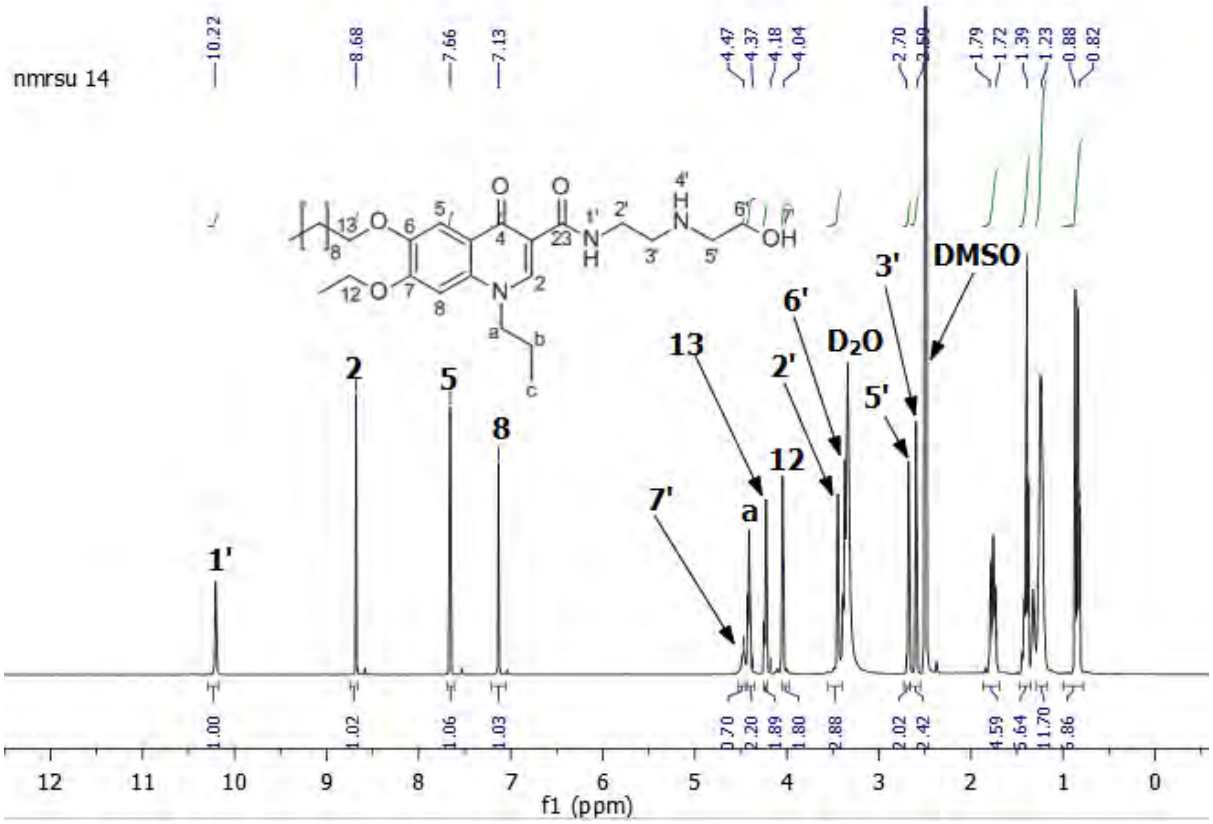
HRMS spectrum of compound 11



Supporting data for chapter 5

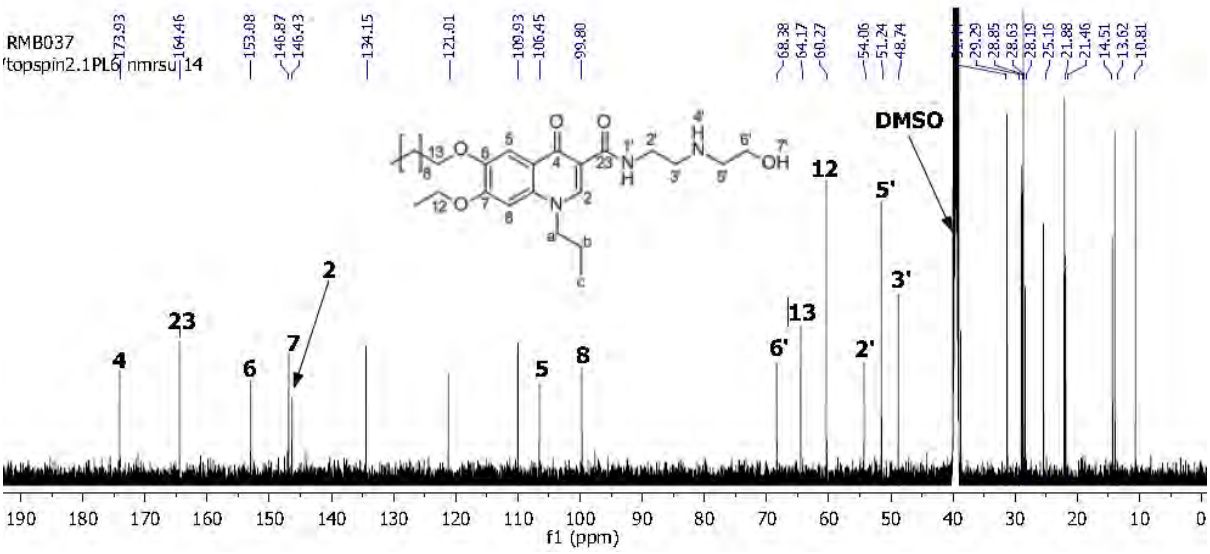
Compound 12

^{1}H NMR spectrum of compound 12

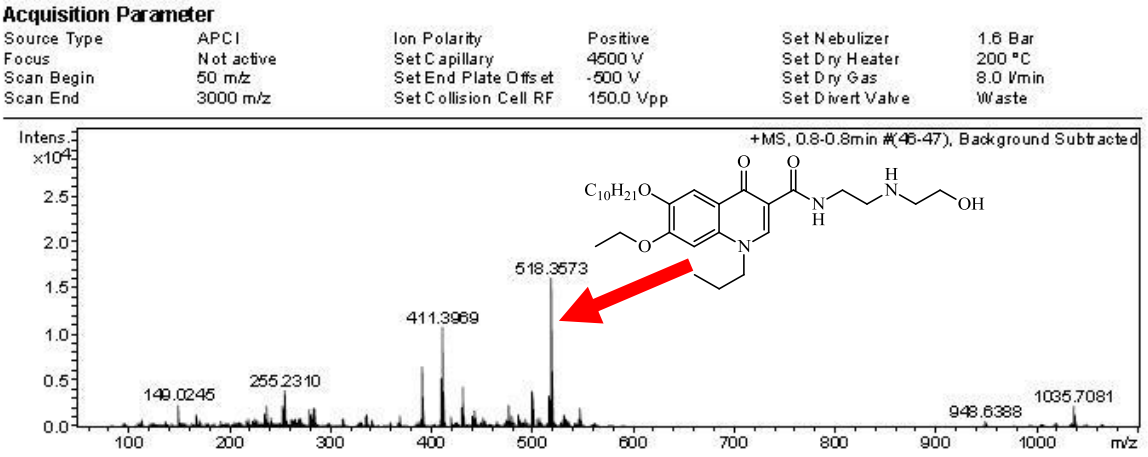


Supporting data for chapter 5

^{13}C NMR spectrum of compound 12



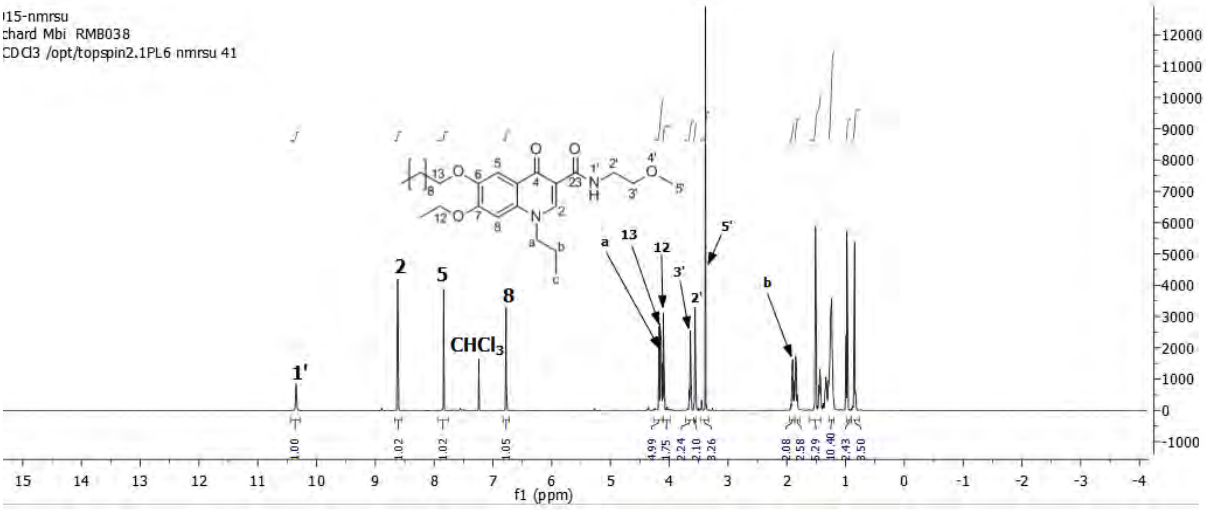
HRMS spectrum of compound 12



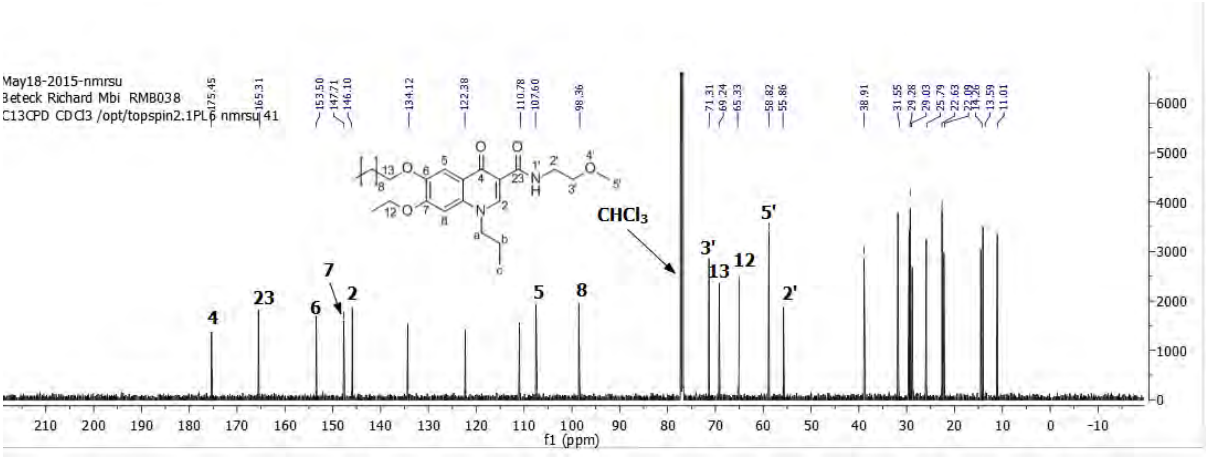
Supporting data for chapter 5

Compound 13

^{1}H NMR spectrum of compound 13

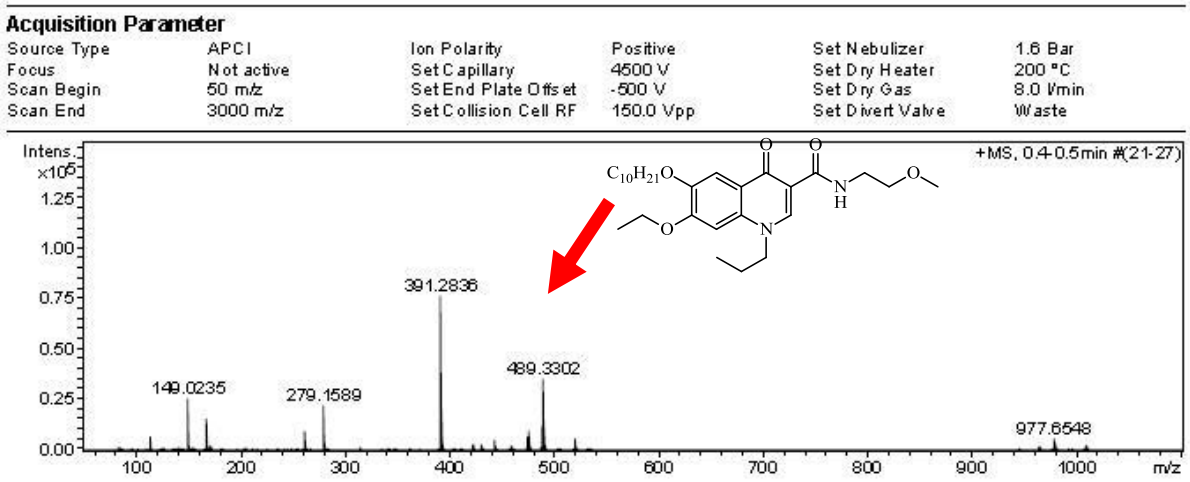


^{13}C NMR spectrum of compound 13



Supporting data for chapter 5

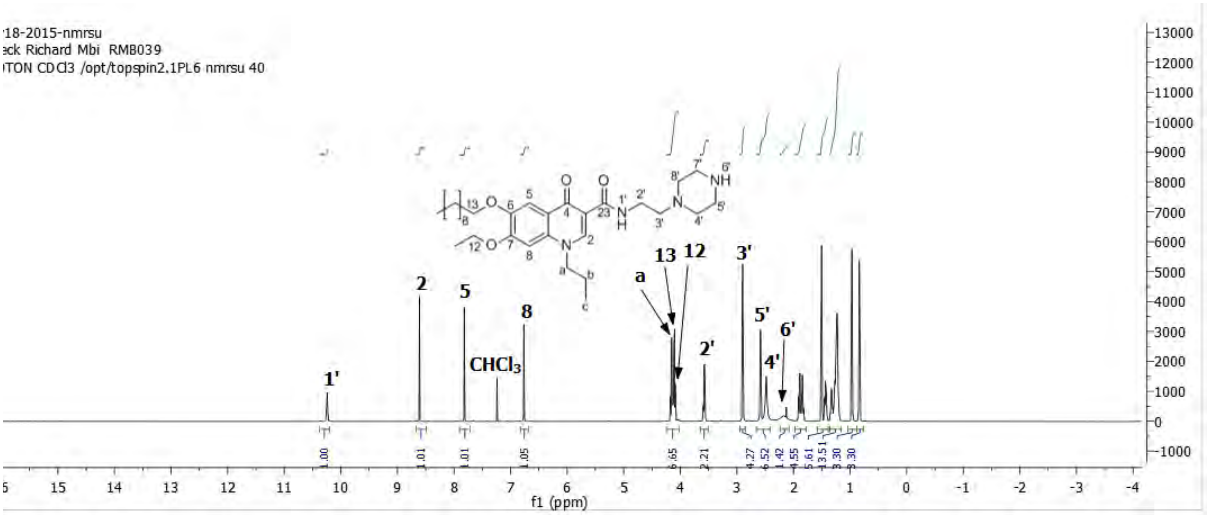
HRMS spectrum of compound 13



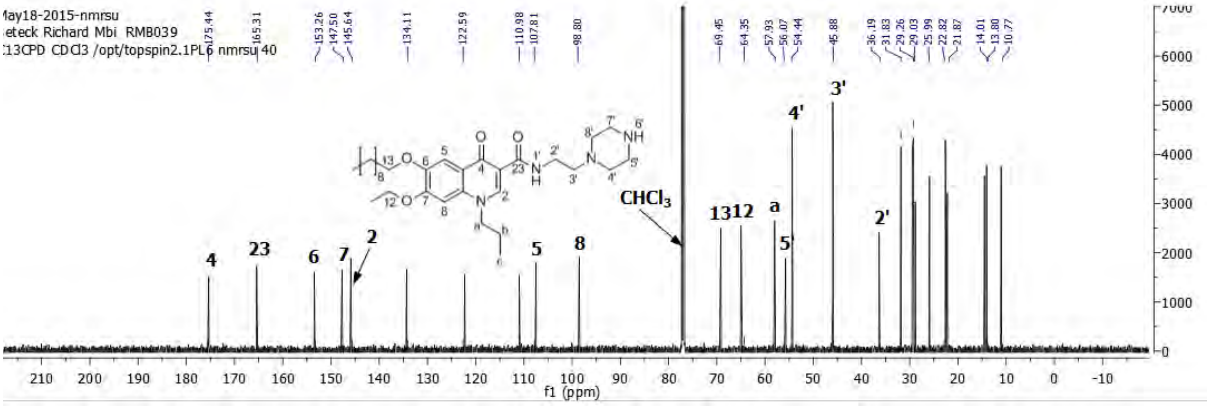
Supporting data for chapter 5

Compound 14

^{1}H NMR spectrum of compound 14



^{13}C NMR spectrum of compound 14

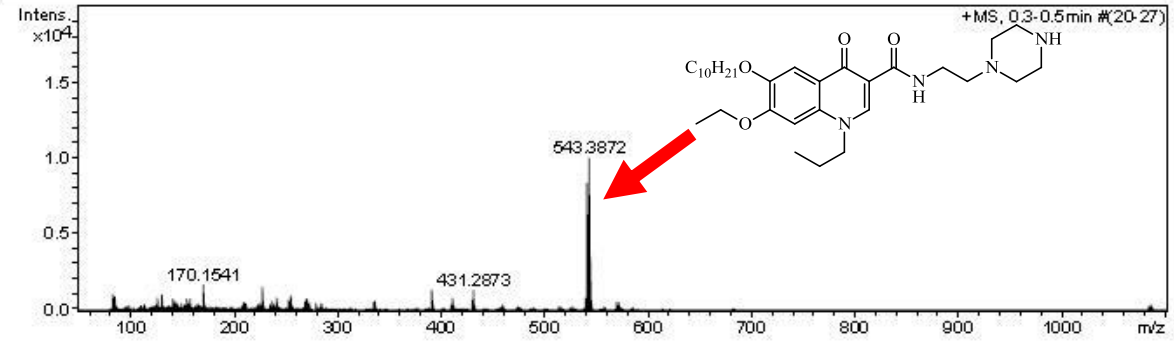


Supporting data for chapter 5

HRMS spectrum of compound 14

Acquisition Parameter

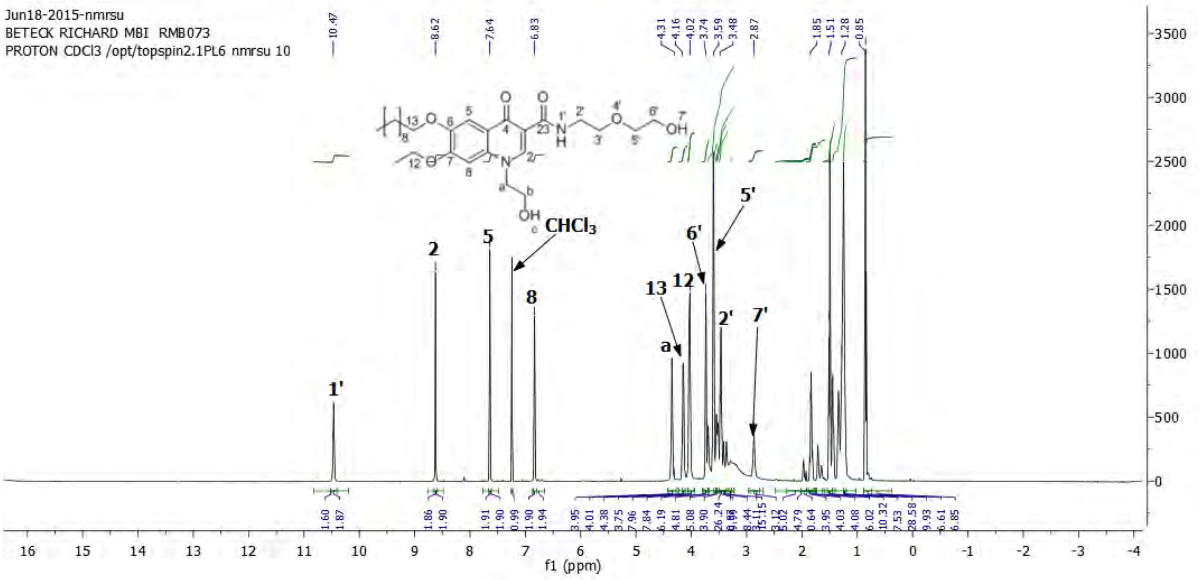
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



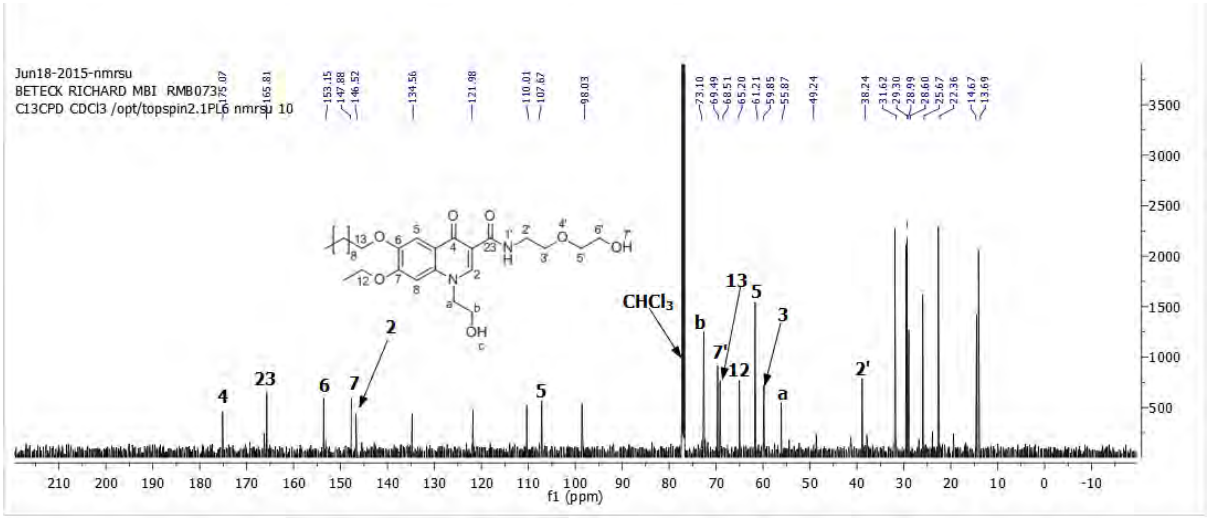
Supporting data for chapter 5

Compound 15

^{1}H NMR spectrum of compound 15

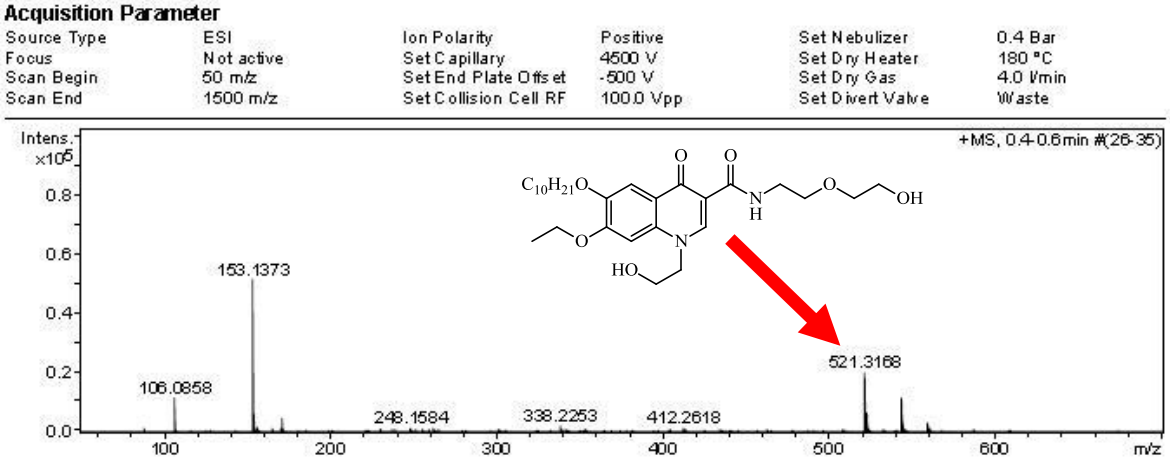


^{13}C NMR spectrum of compound 15



Supporting data for chapter 5

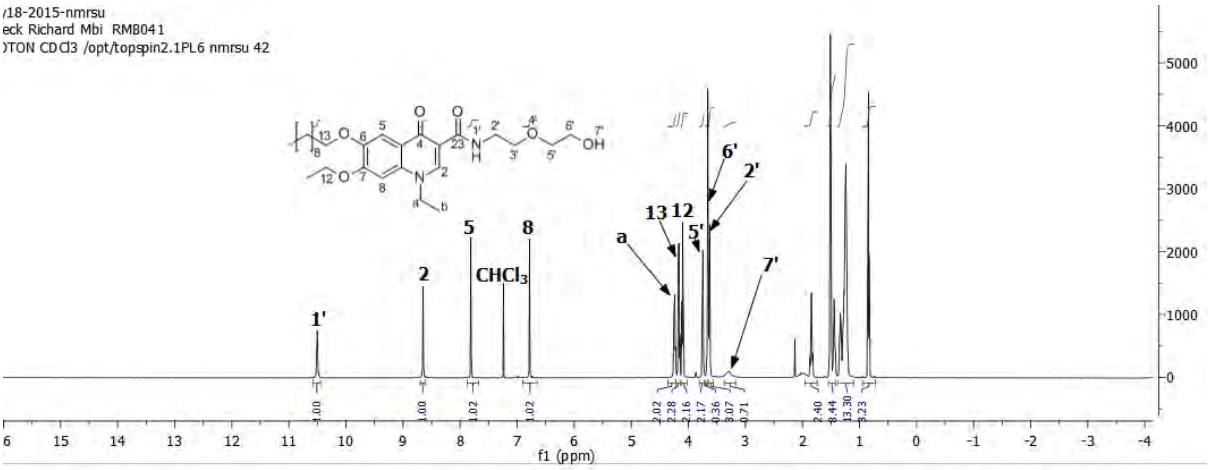
HRMS spectrum of compound 15



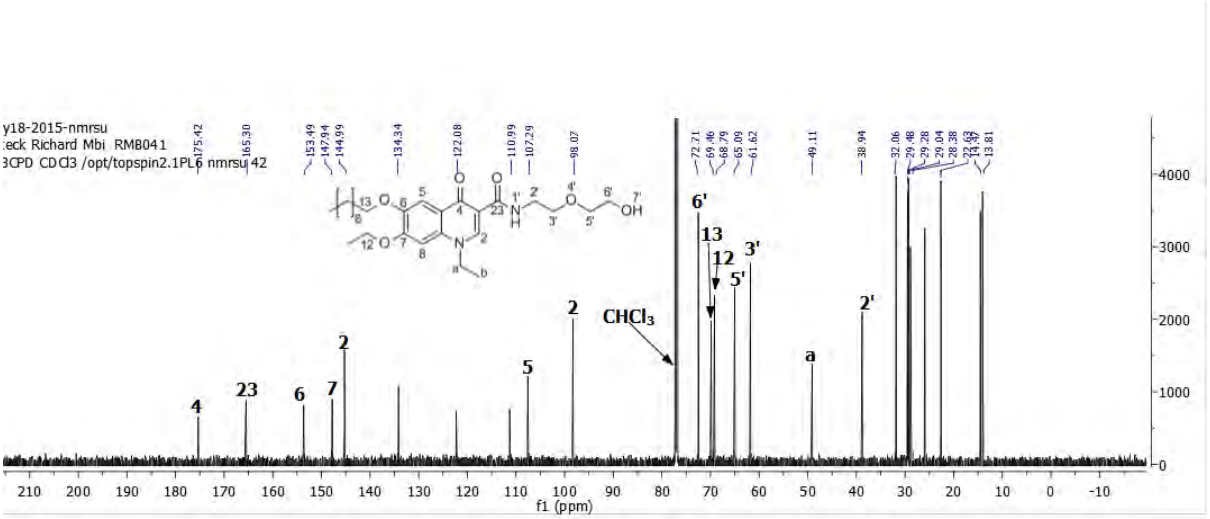
Supporting data for chapter 5

Compound 16

^{1}H NMR spectrum of compound 16



^{13}C NMR spectrum of compound 16

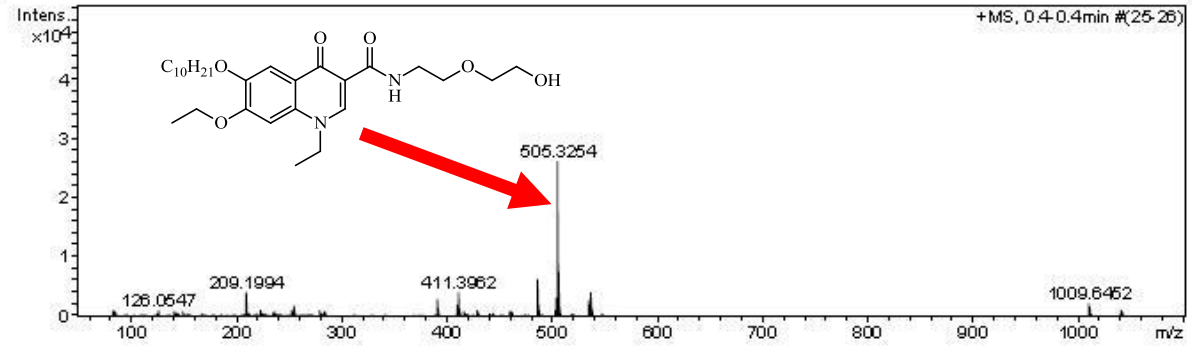


Supporting data for chapter 5

HRMS spectrum of compound 16

Acquisition Parameter

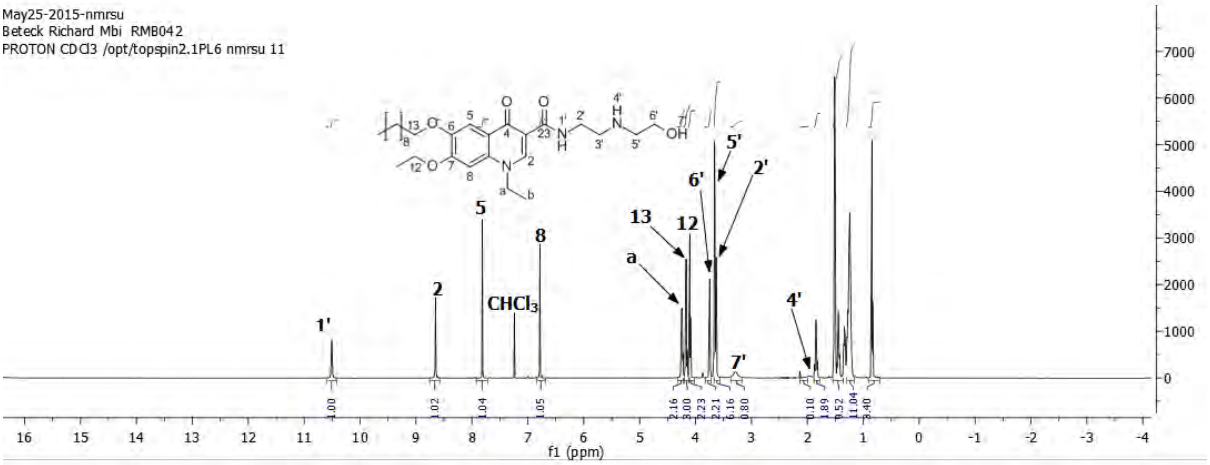
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



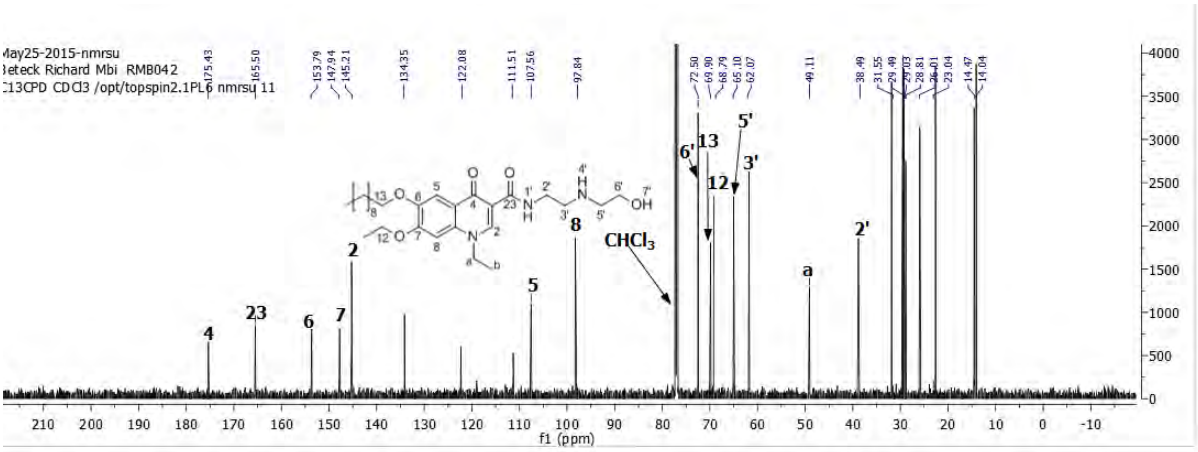
Supporting data for chapter 5

Compound 17

^{1}H NMR spectrum of compound 17

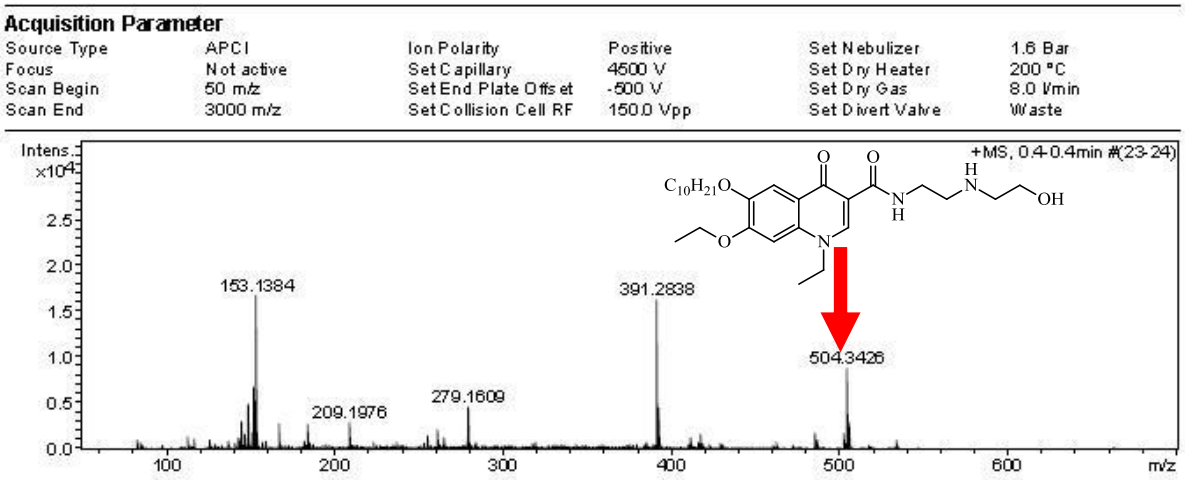


^{13}C NMR spectrum of compound 17



Supporting data for chapter 5

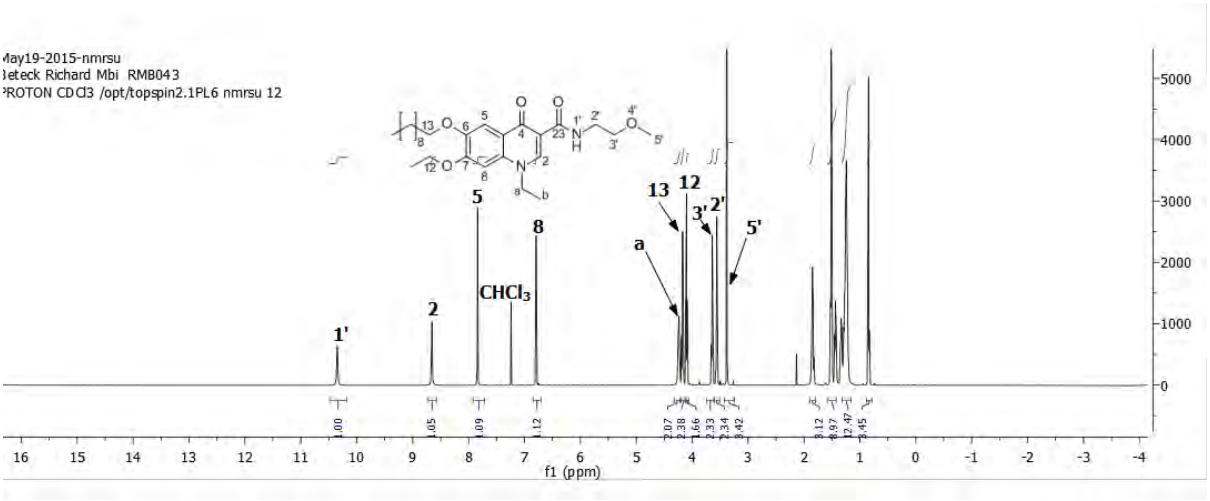
HRMS spectrum of compound 17



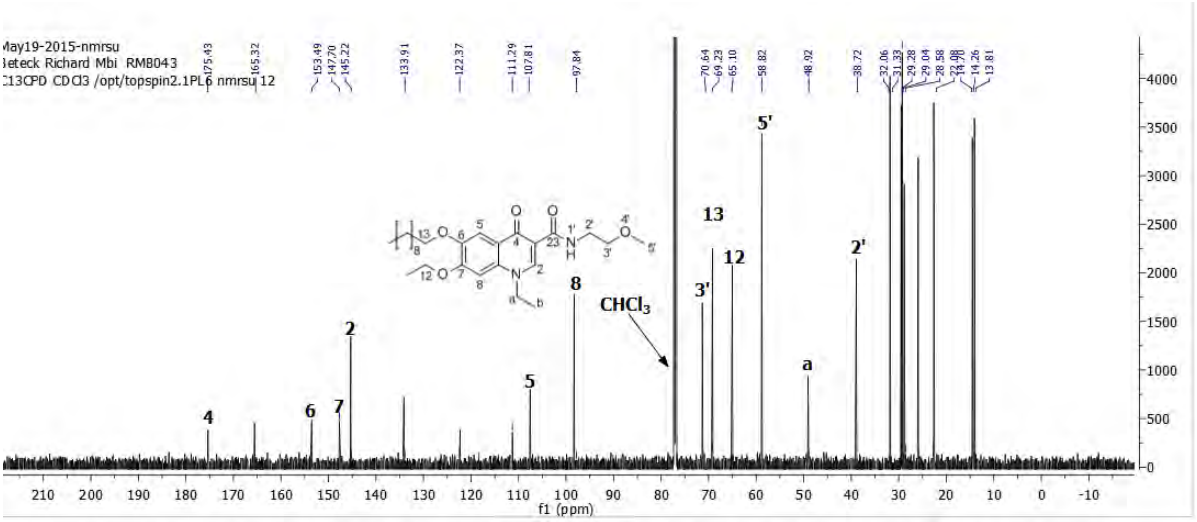
Supporting data for chapter 5

Compound 18

^{1}H NMR spectrum of compound 18

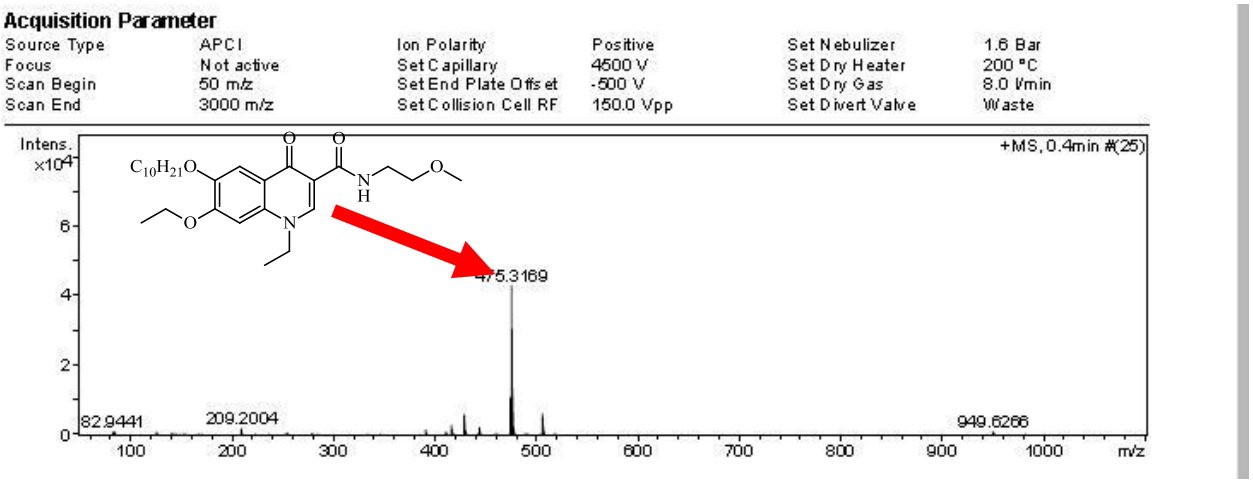


^{13}C NMR spectrum of compound 18



Supporting data for chapter 5

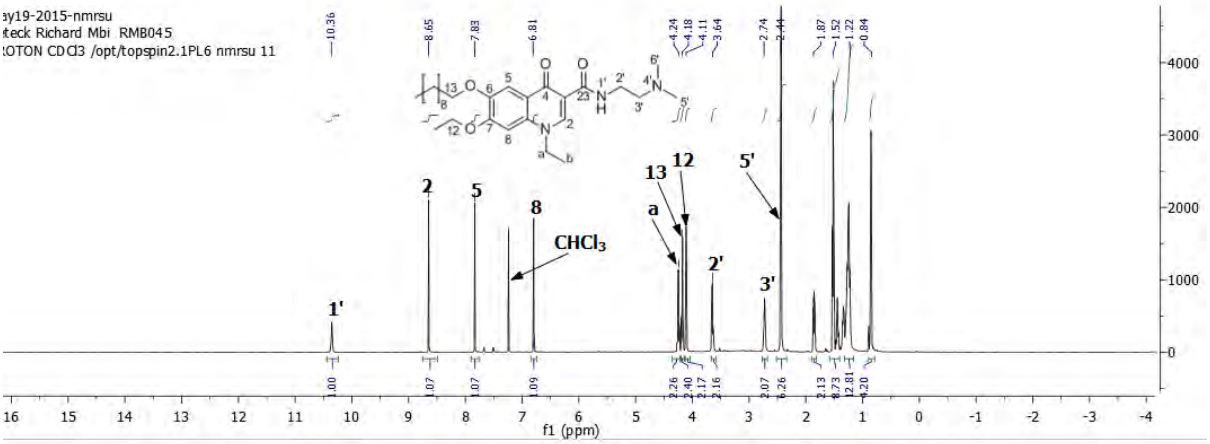
HRMS spectrum of compound 18



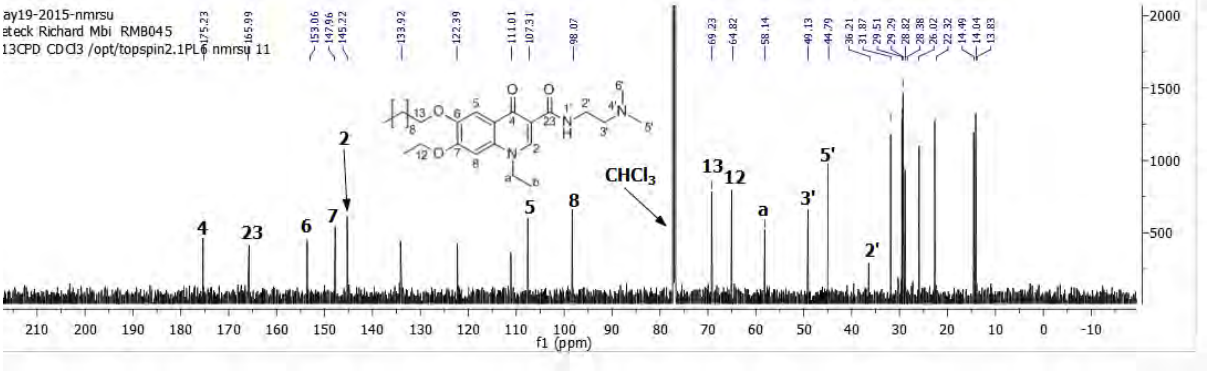
Supporting data for chapter 5

Compound 19

^{1}H NMR spectrum of compound 19



^{13}C NMR spectrum of compound 19

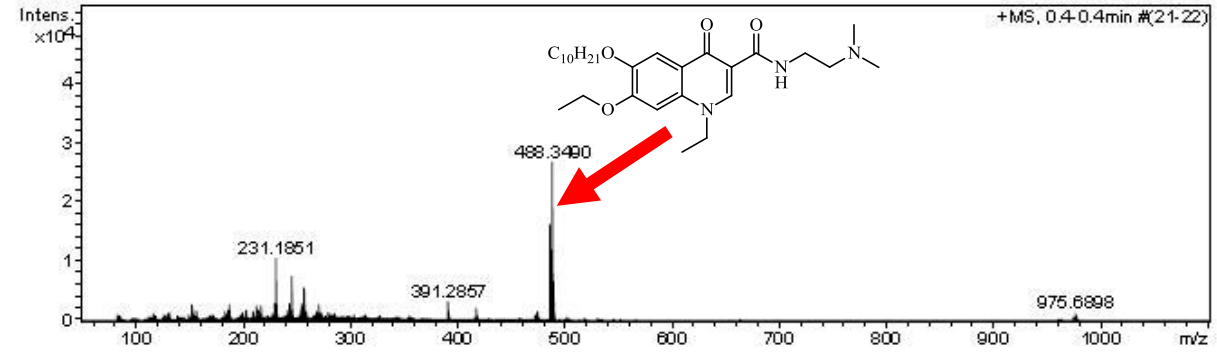


Supporting data for chapter 5

HRMS spectrum of compound 19

Acquisition Parameter

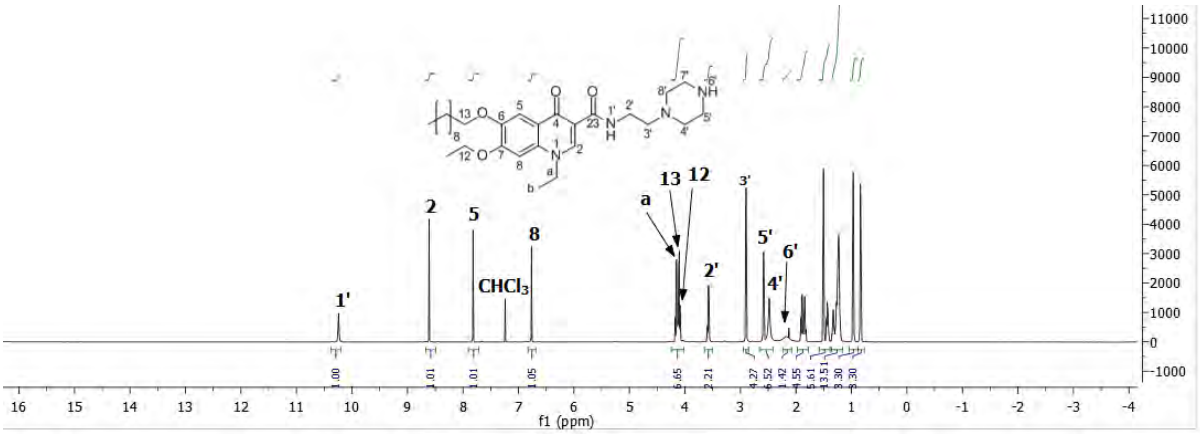
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



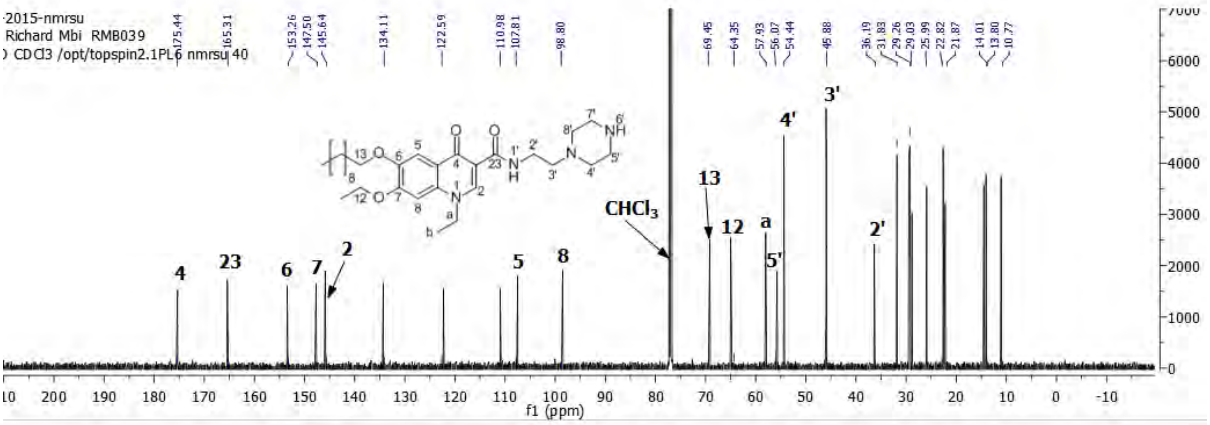
Supporting data for chapter 5

Compound 20

^{1}H NMR spectrum of compound 20

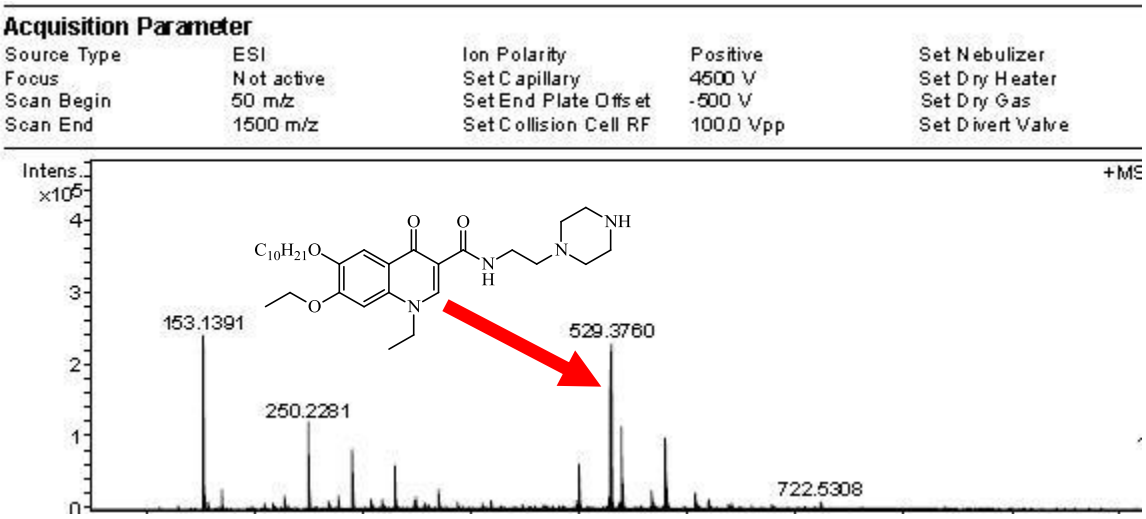


^{13}C NMR spectrum of compound 20



Supporting data for chapter 5

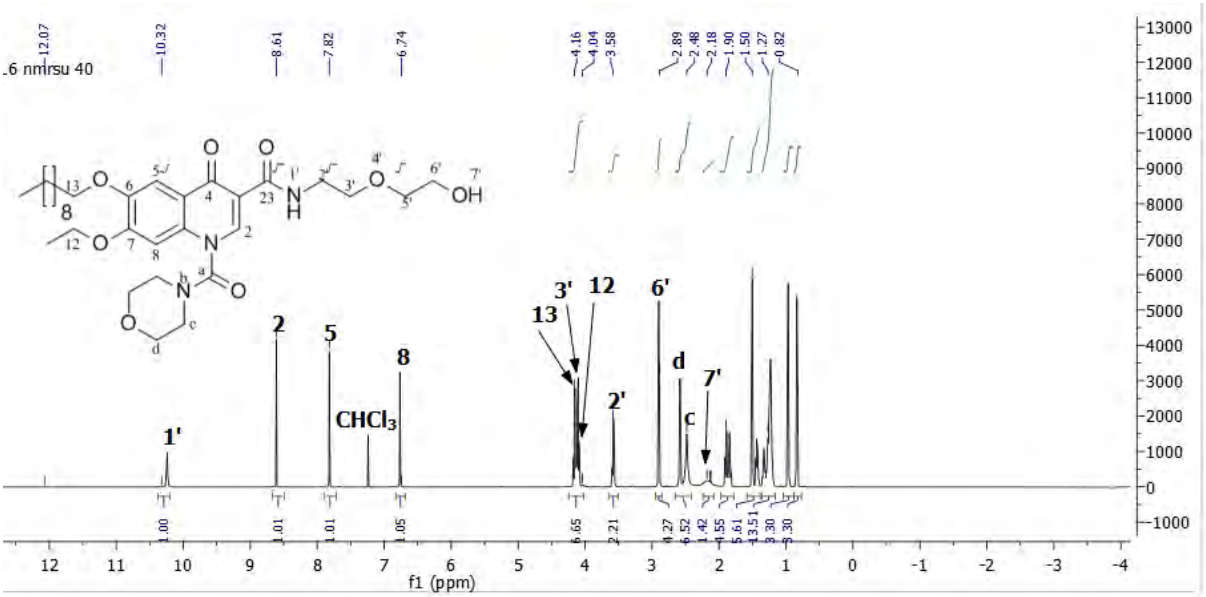
HRMS spectrum of compound 20



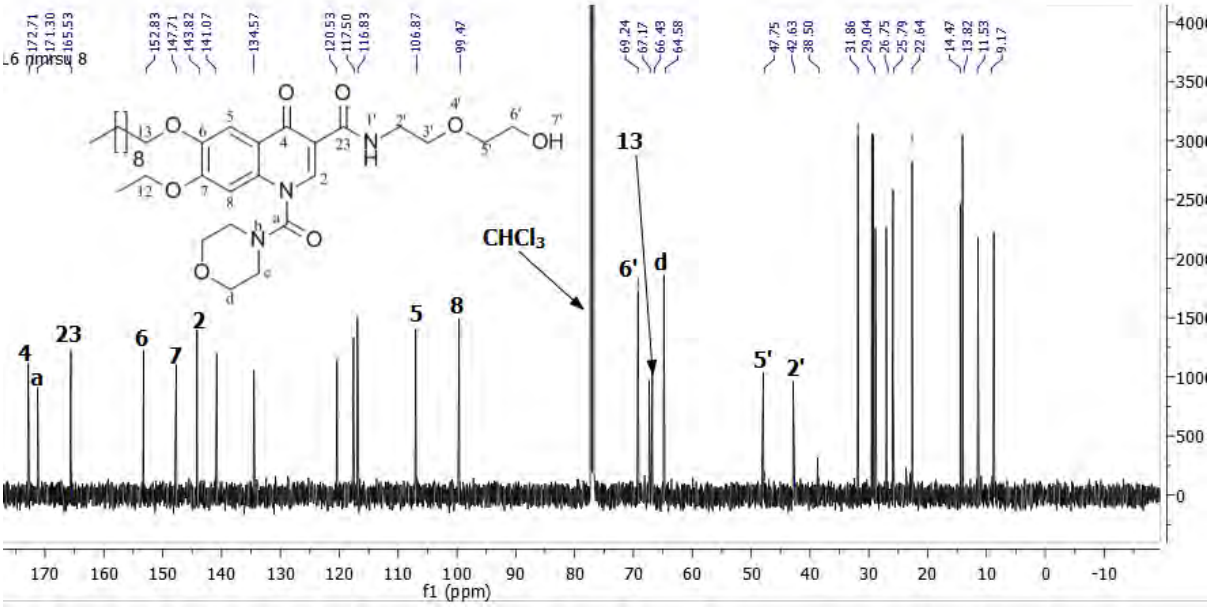
Supporting data for chapter 5

Compound 21

^{1}H NMR spectrum of compound 21

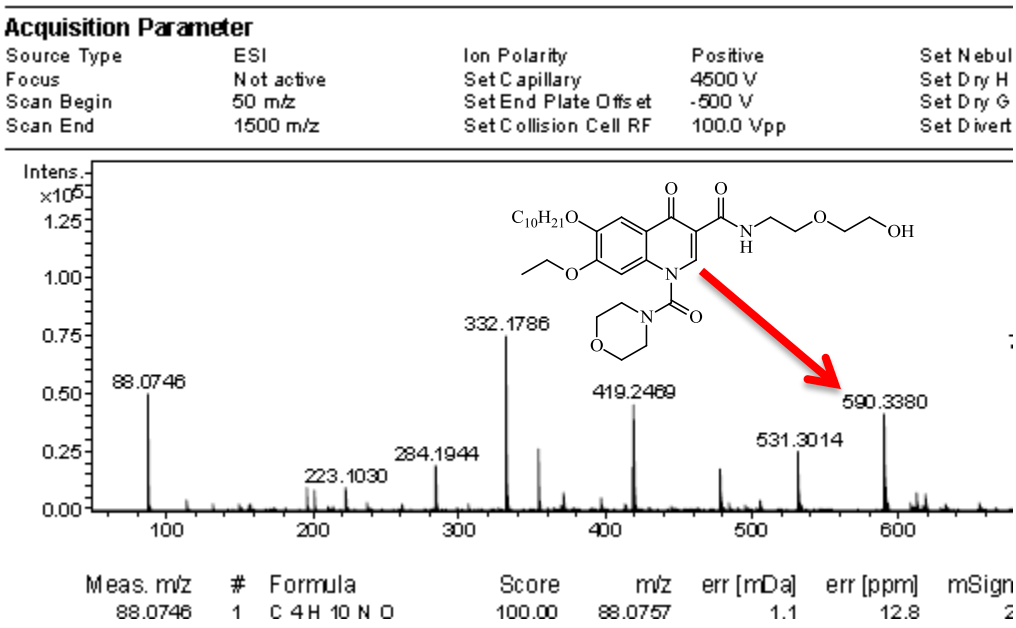


^{13}C NMR spectrum of compound 21



Supporting data for chapter 5

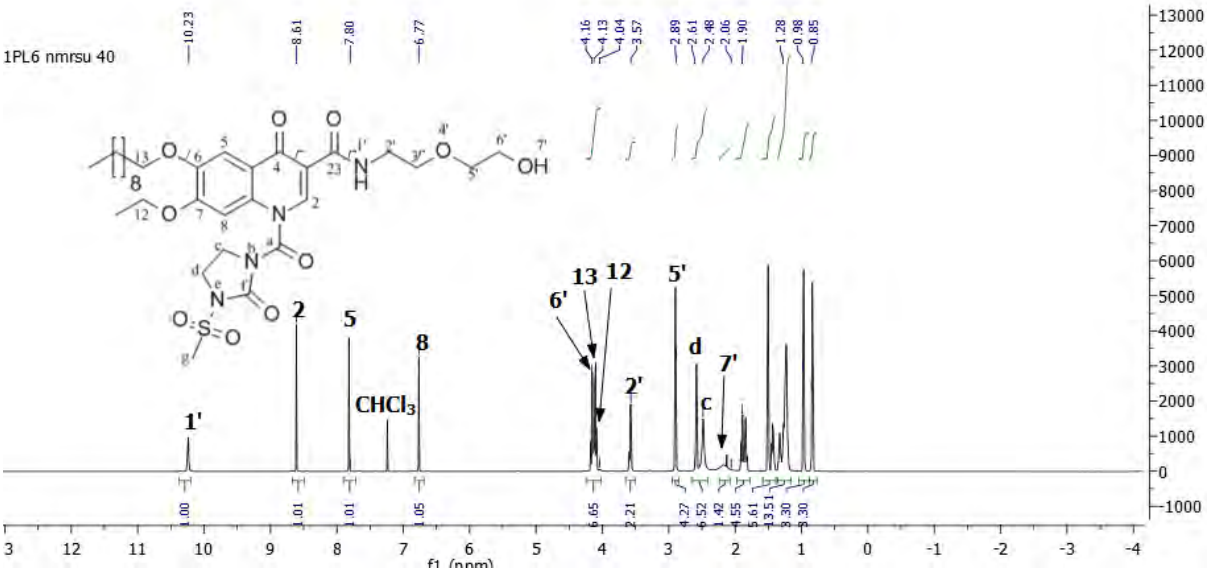
HRMS spectrum of compound 21



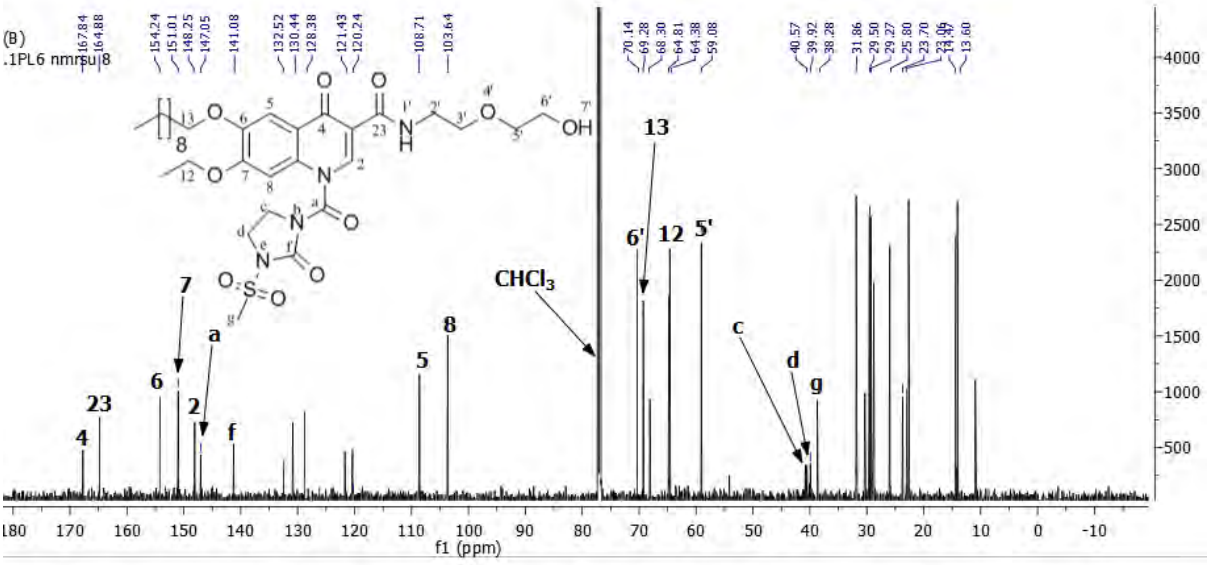
Supporting data for chapter 5

Compound 22

^{1}H NMR spectrum of compound 22

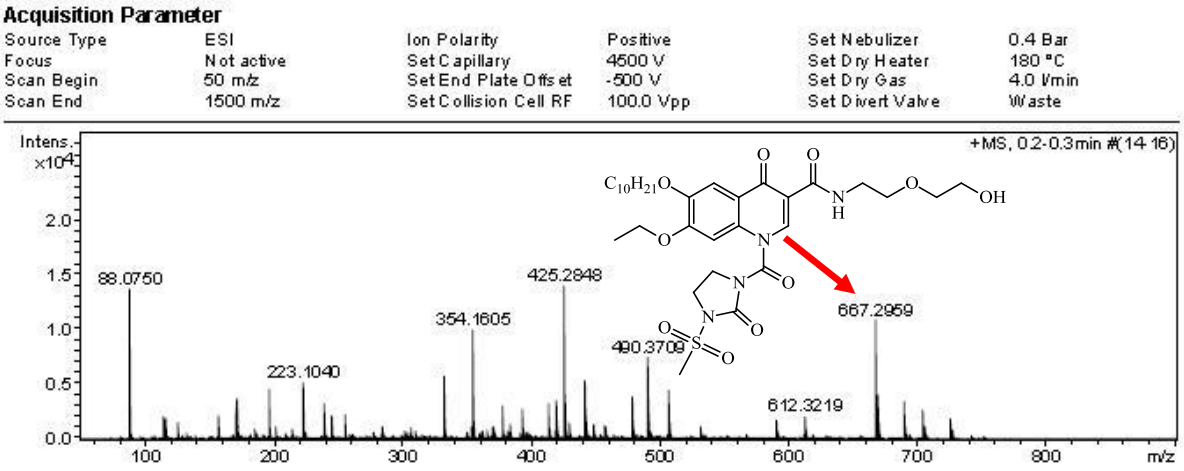


^{13}C NMR spectrum of compound 22



Supporting data for chapter 5

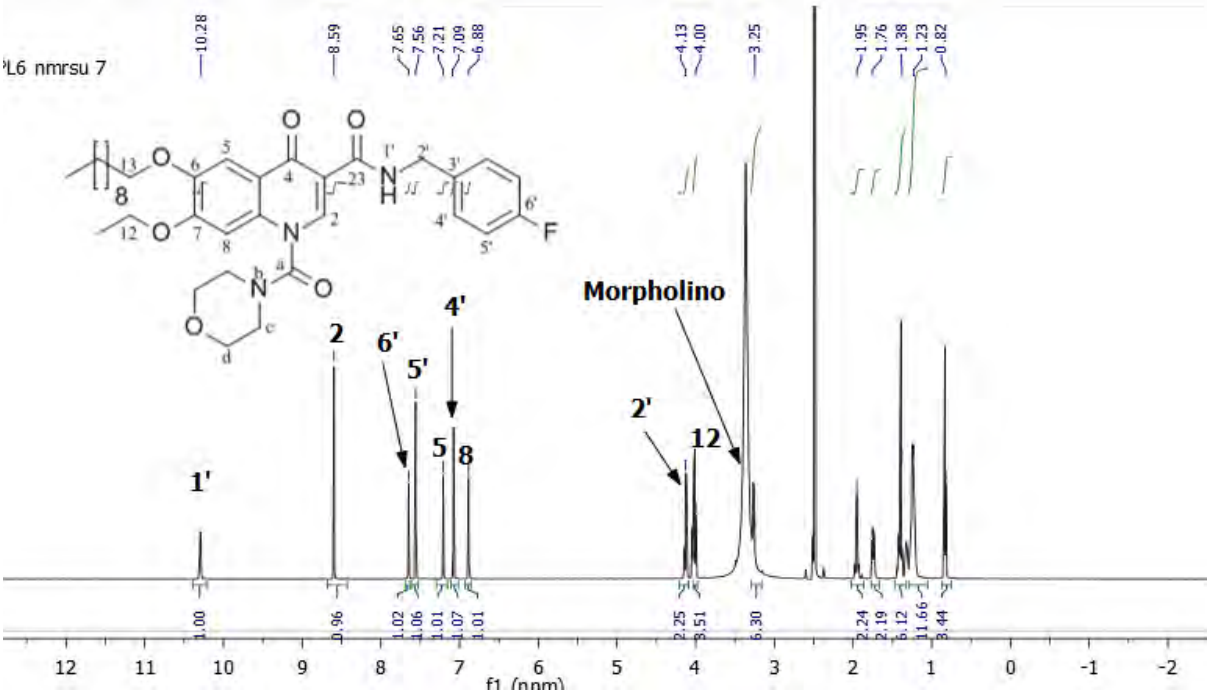
HRMS spectrum of compound 22



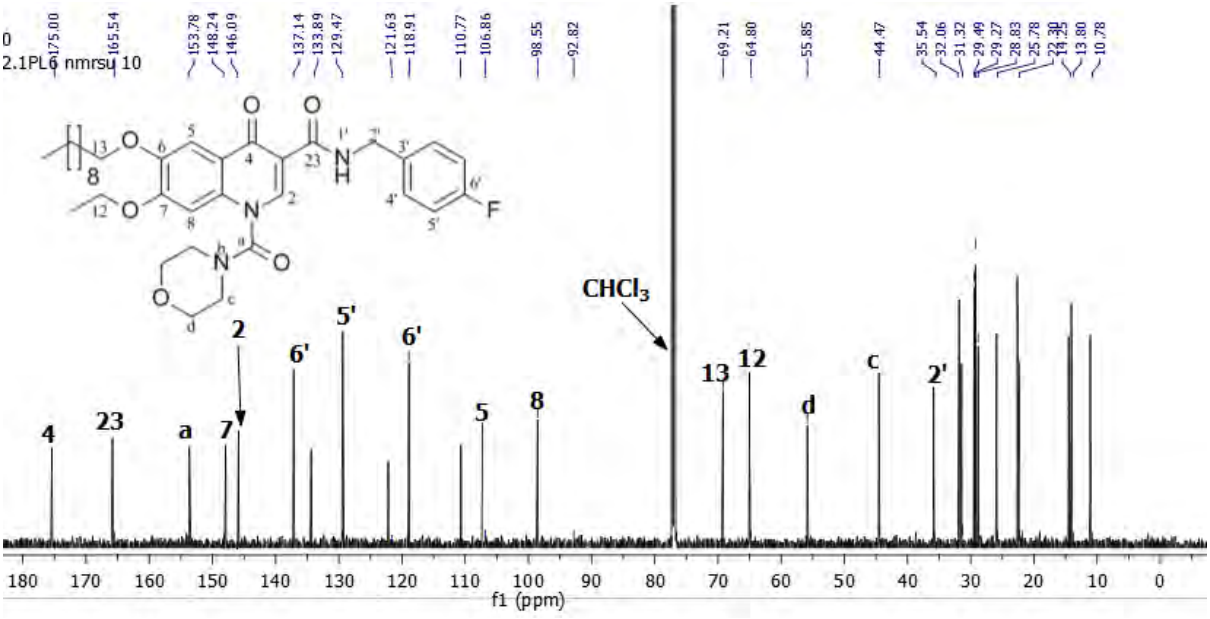
Supporting data for chapter 5

Compound 23

^{1}H NMR spectrum of compound 23

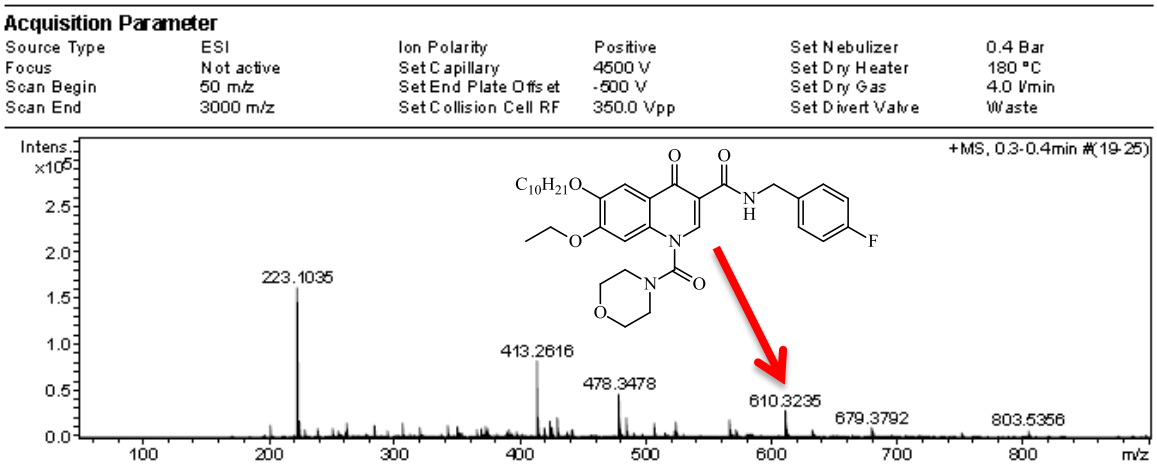


^{13}C NMR spectrum of compound 23



Supporting data for chapter 5

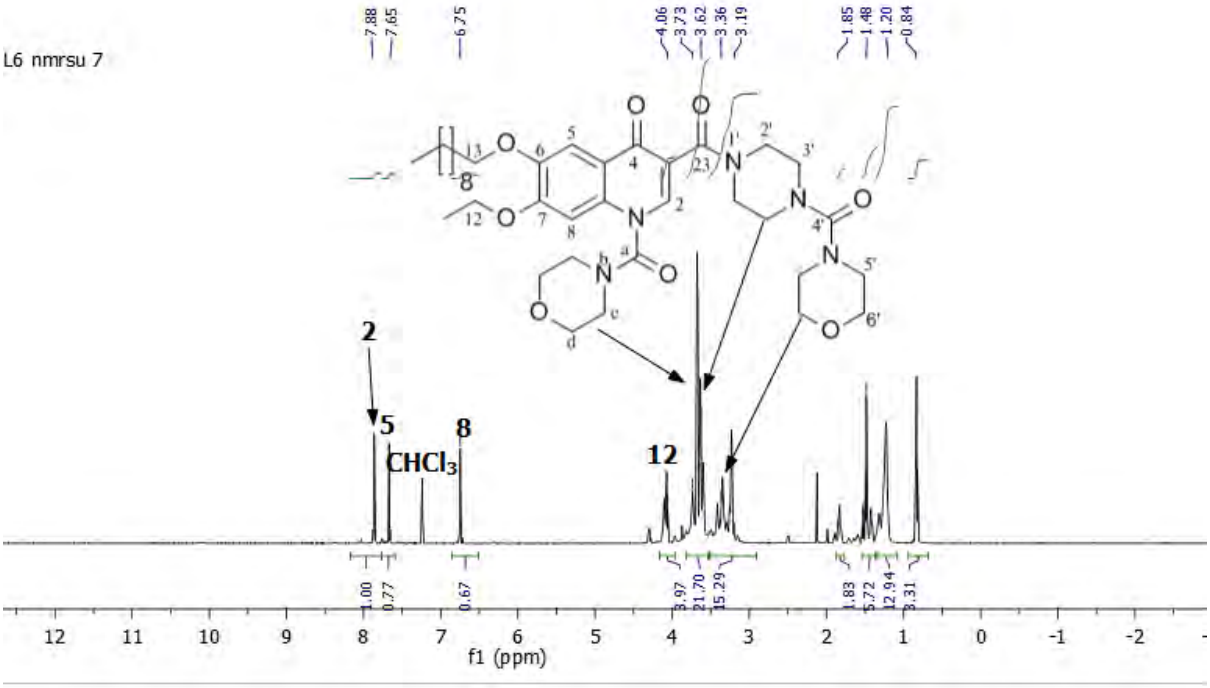
HRMS spectrum of compound 23



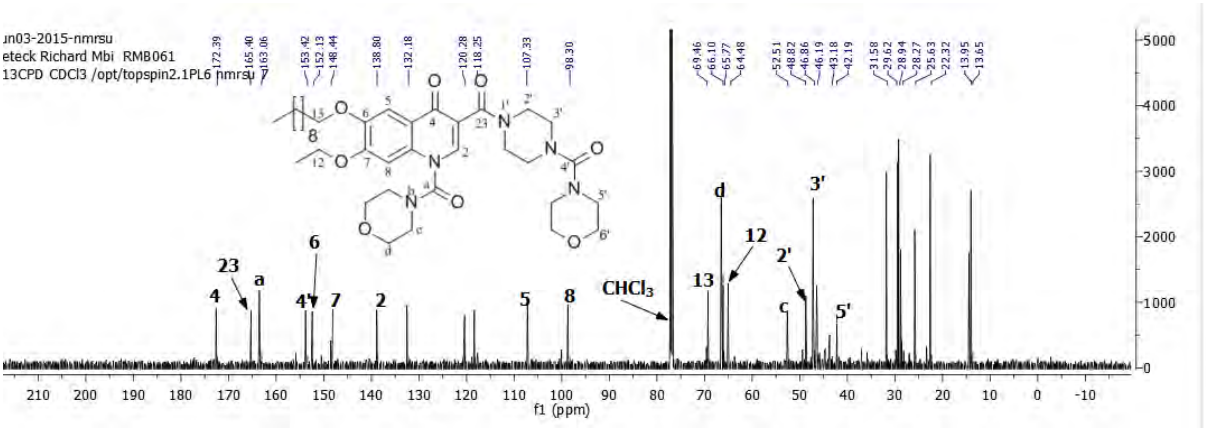
Supporting data for chapter 5

Compound 24

^{1}H NMR spectrum of compound 24

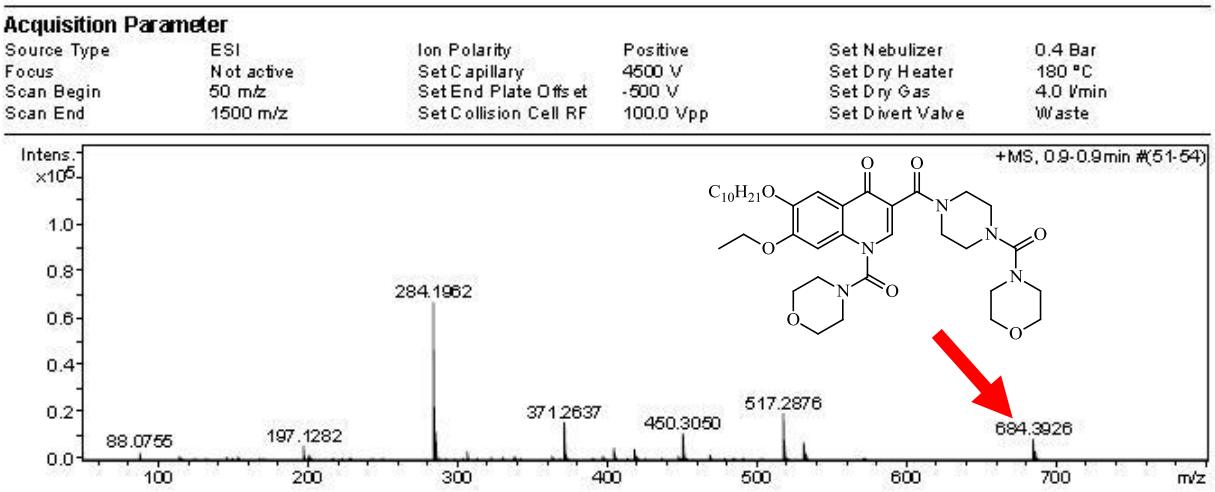


^{13}C NMR spectrum of compound 24



Supporting data for chapter 5

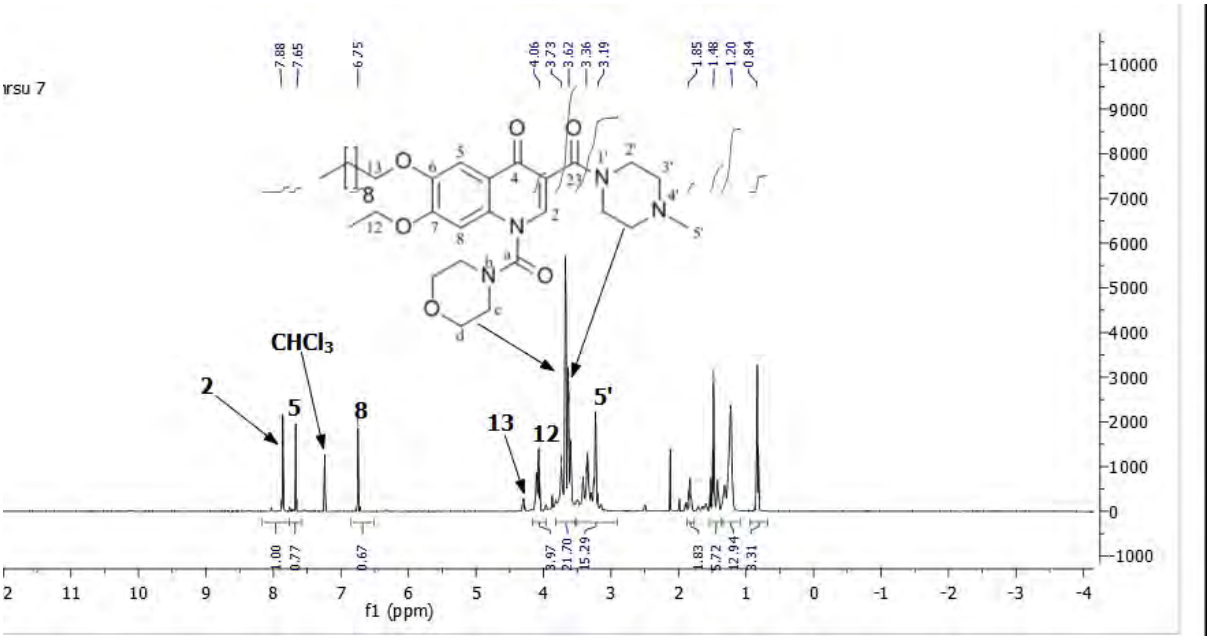
HRMS spectrum of compound 24



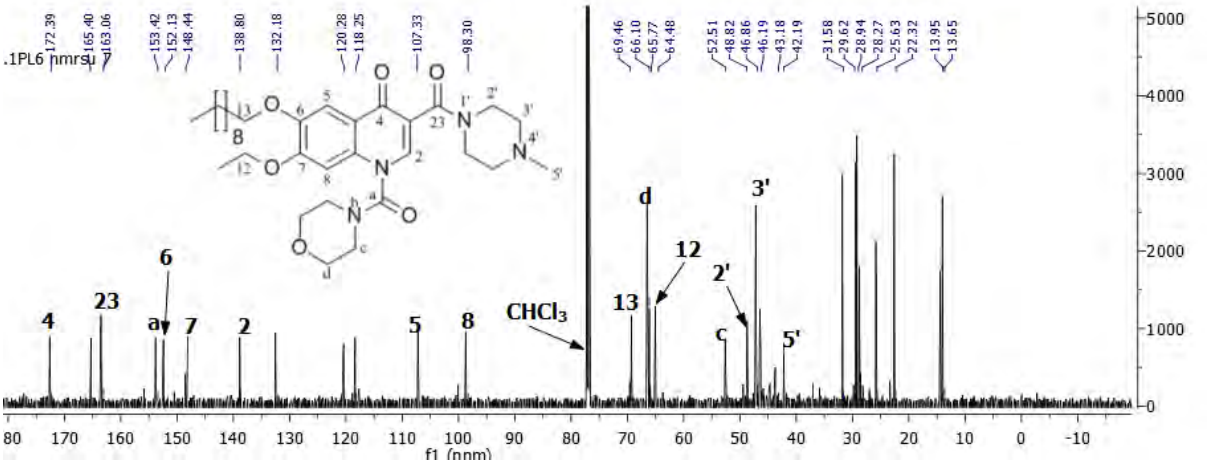
Supporting data for chapter 5

Compound 25

^{1}H NMR spectrum of compound 25

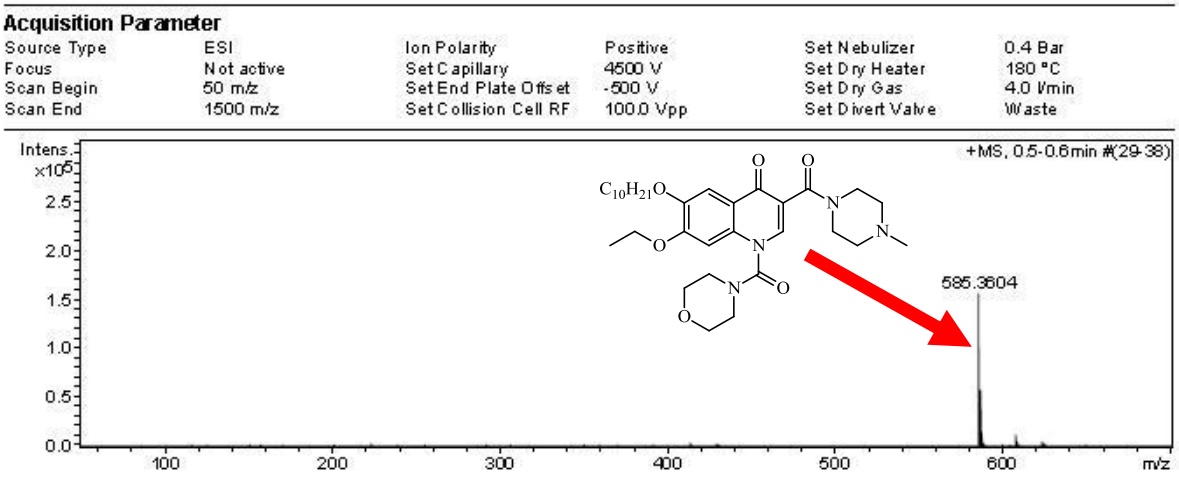


^{13}C NMR spectrum of compound 25



Supporting data for chapter 5

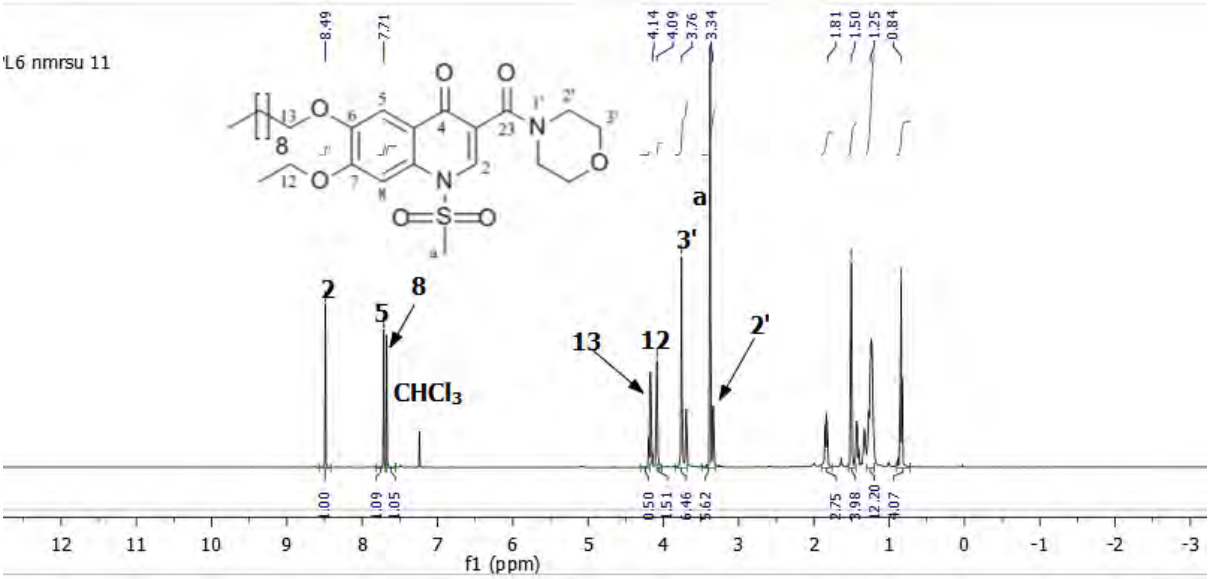
HRMS spectrum of compound 25



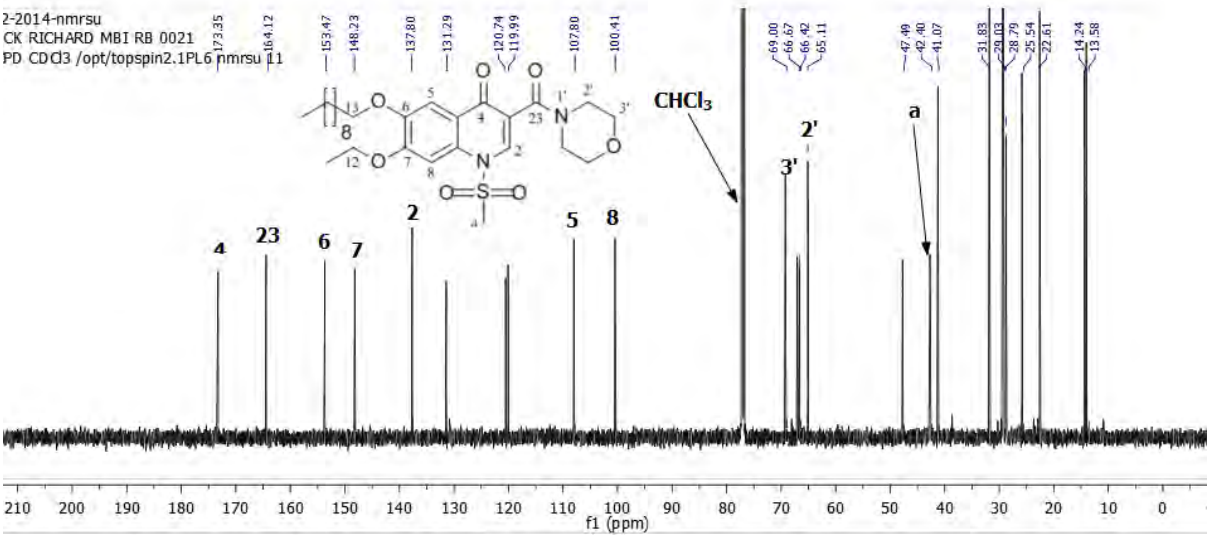
Supporting data for chapter 5

Compound 26

^{1}H NMR spectrum of compound 26



^{13}C NMR spectrum of compound 26

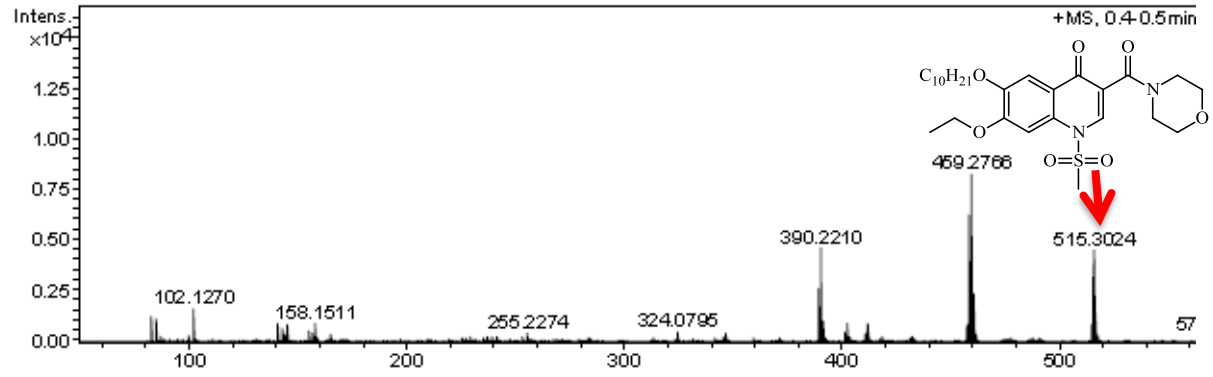


Supporting data for chapter 5

HRMS spectrum of compound 26

Acquisition Parameter

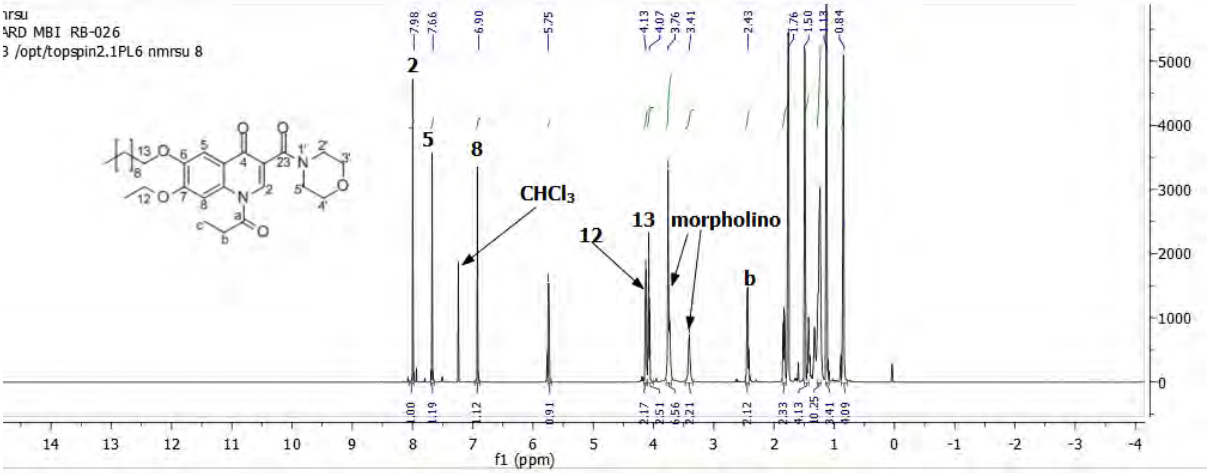
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



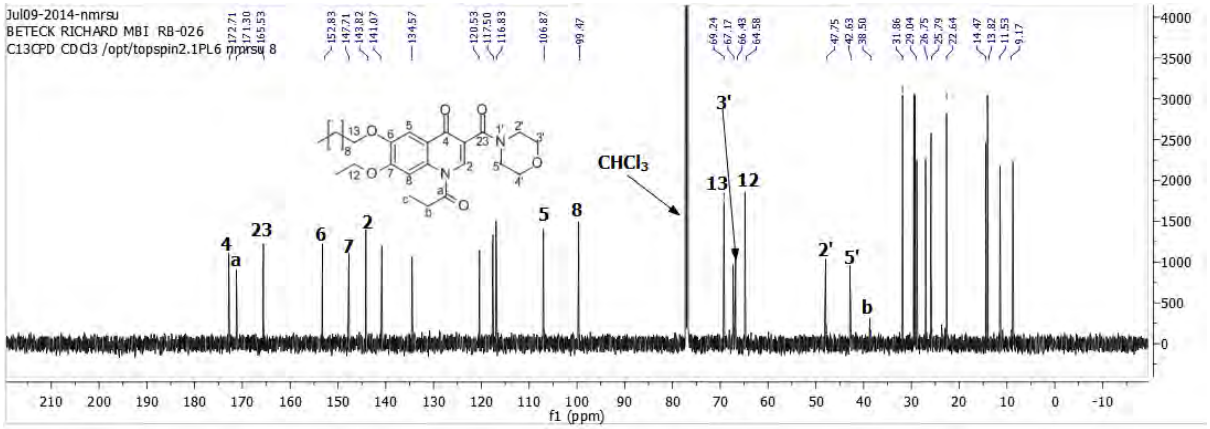
Supporting data for chapter 5

Compound 27

^{1}H NMR spectrum of compound 27

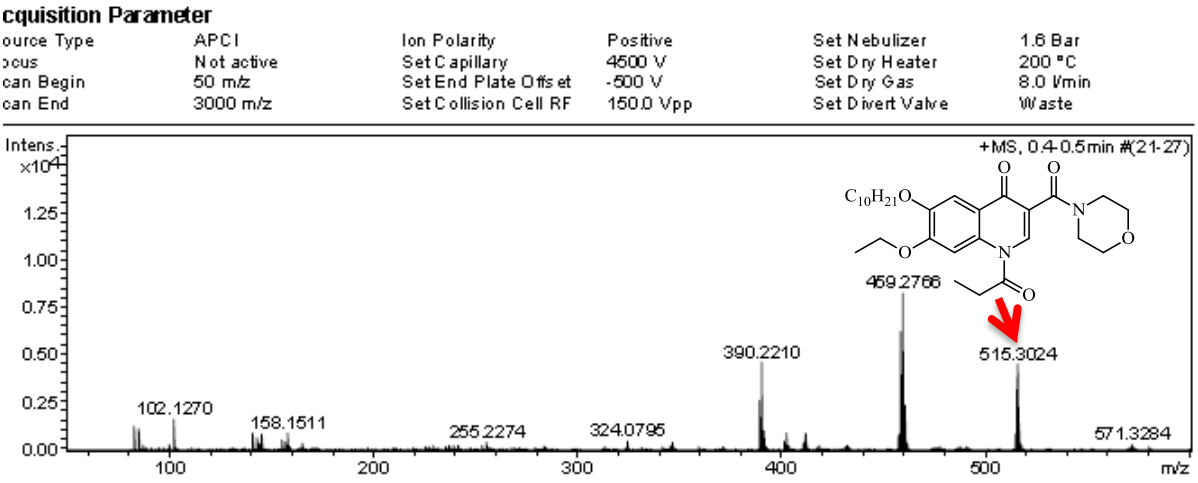


^{13}C NMR spectrum of compound 27



Supporting data for chapter 5

HRMS spectrum of compound 27



Addendum C: ^{1}H and ^{13}C NMR and mass spectra for Chapter 6

Substituted decoquinate derivatives with improved solubilities and *in vitro* antimalarial activities

Richard M. Beteck[1], Dina Coertzen[2], Frans J. Smit[3], Lyn-Marie Birkholtz[2], Richard K. Haynes[3], David D. N'Da[3]

[1] Pharmaceutical Chemistry, School of Pharmacy, North-West University, Potchefstroom 2520, South Africa.

[2] Department of Biochemistry, Centre for Sustainable Malaria Control, University of Pretoria, Pretoria 0002, South Africa

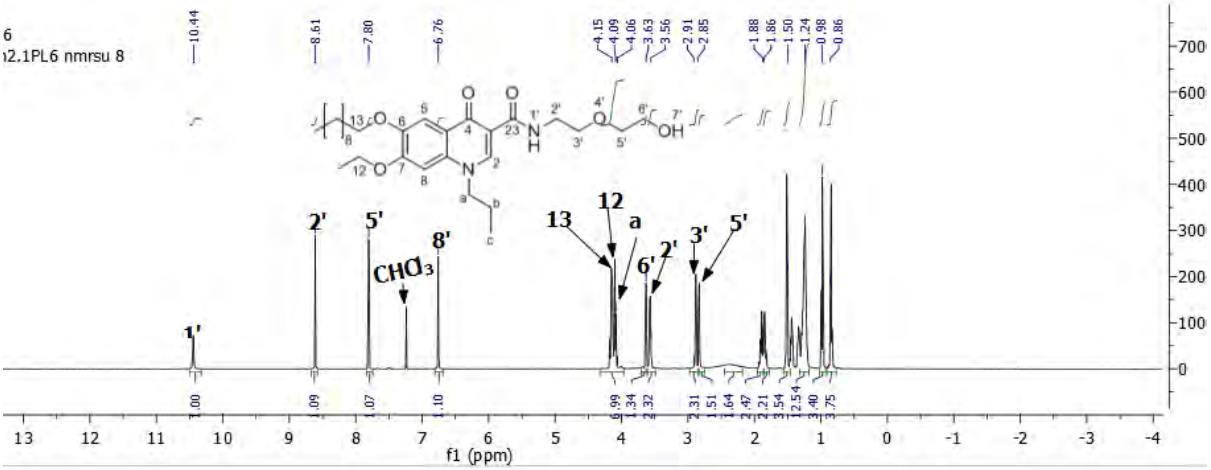
[3] Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom 2520, South Africa.

SUPPORTING DATA: ^{1}H AND ^{13}C NMR, and HRMS data for all compounds

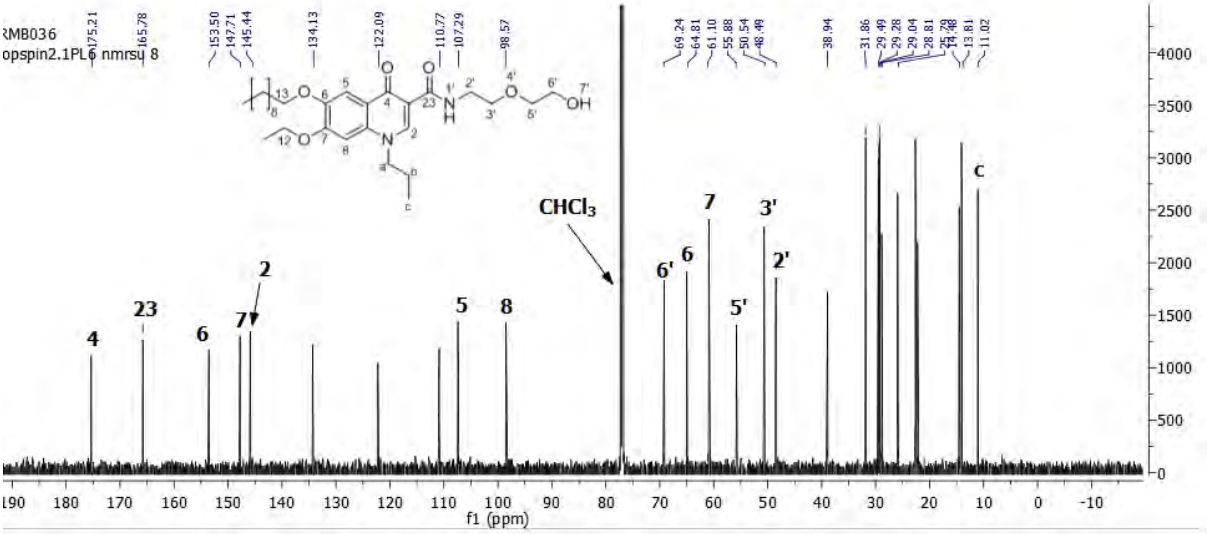
Supporting data for chapter 6

Compound 1

^{1}H NMR spectrum of compound 1

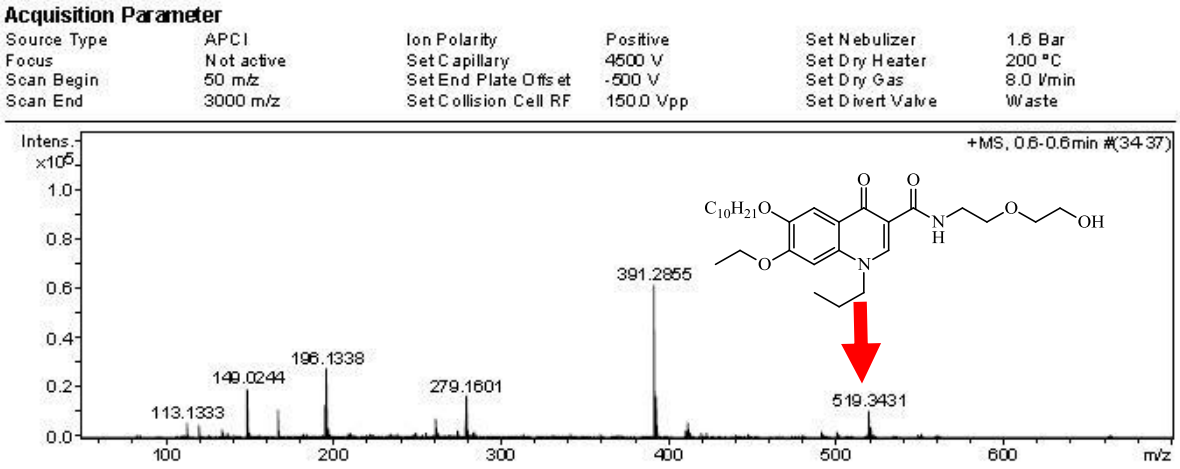


^{13}C NMR spectrum of compound 1



Supporting data for chapter 6

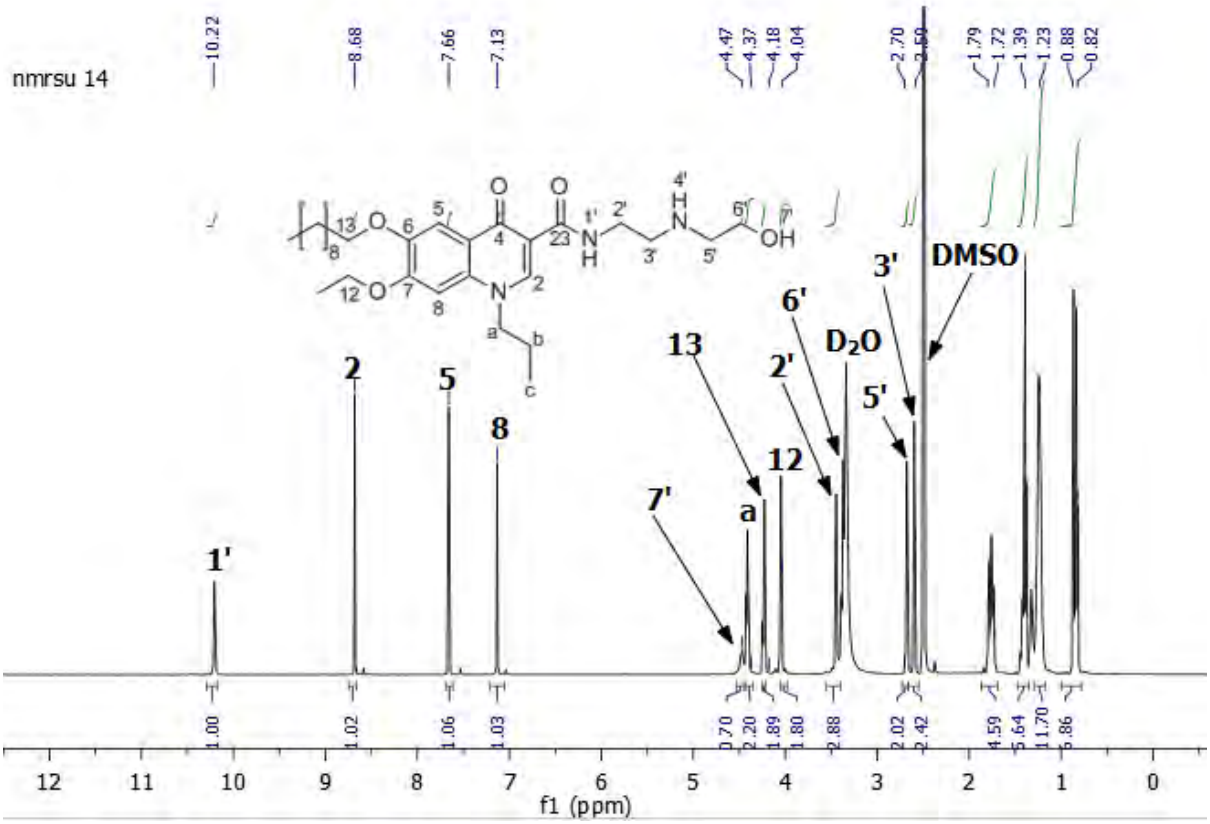
HRMS spectrum of compound 1



Supporting data for chapter 6

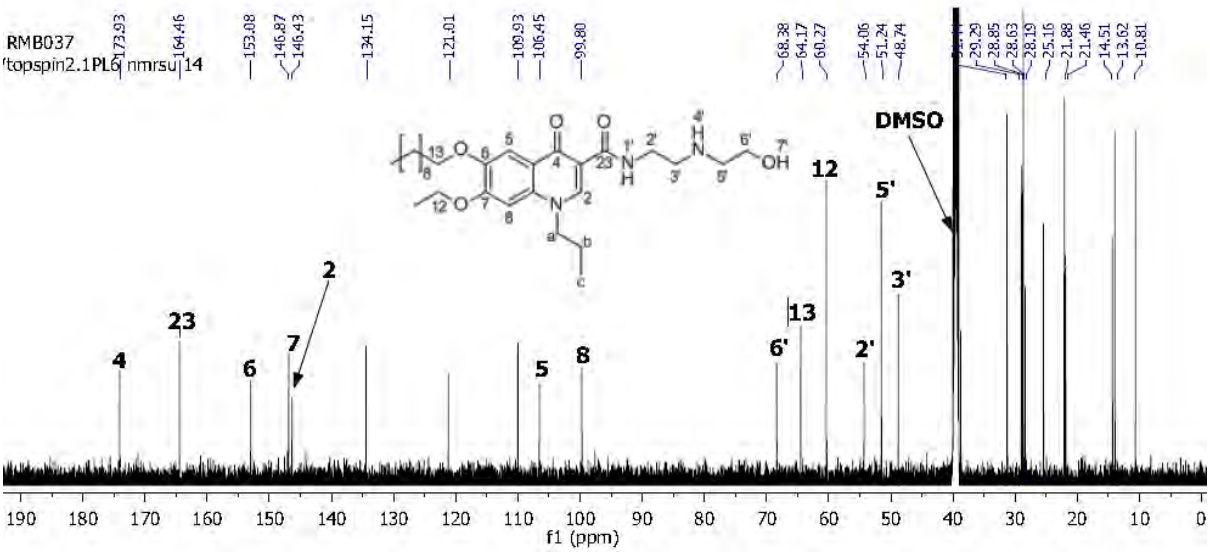
Compound 2

^{1}H NMR spectrum of compound 2

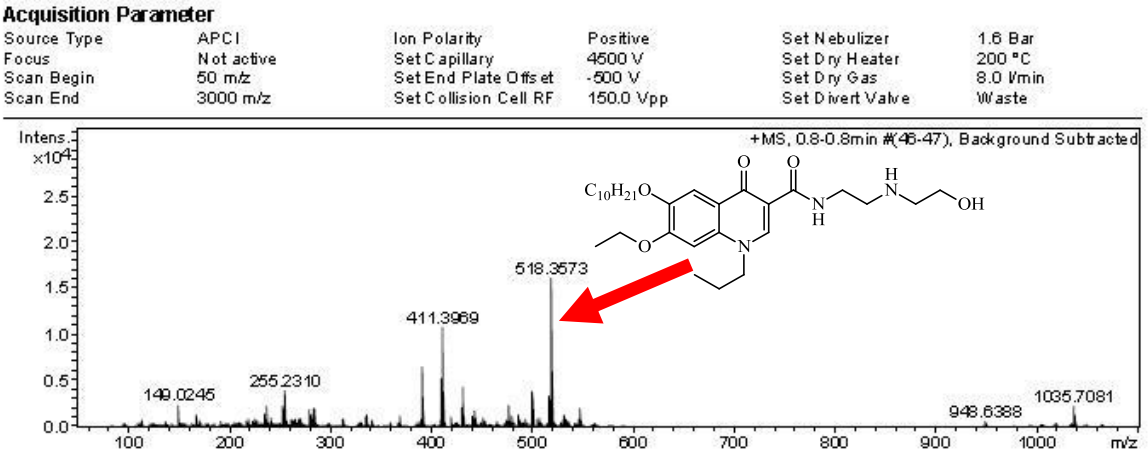


Supporting data for chapter 6

^{13}C NMR spectrum of compound 2



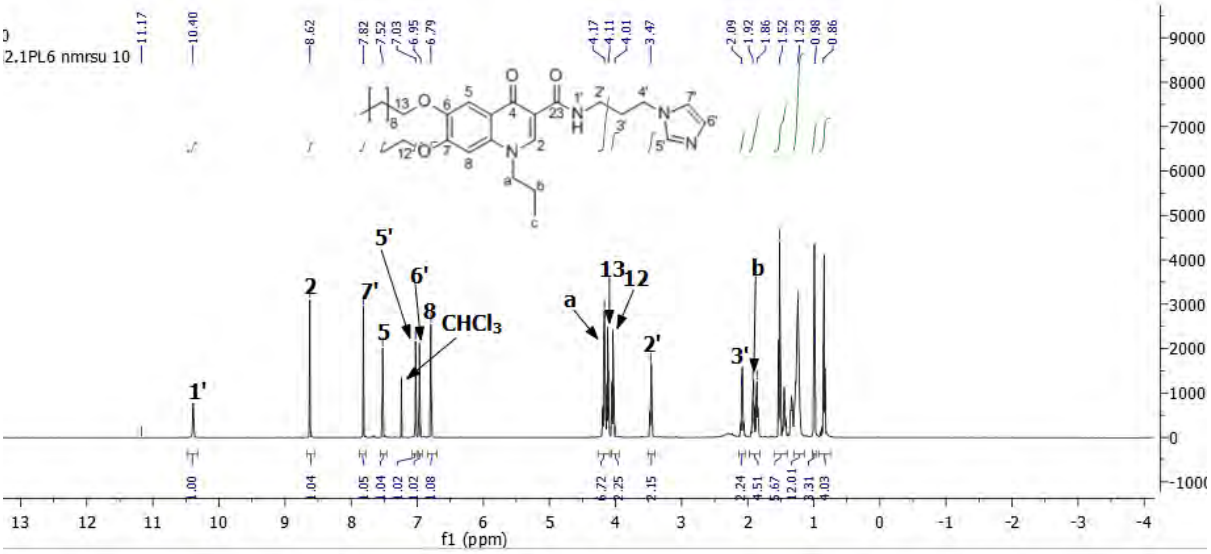
HRMS spectrum of compound 2



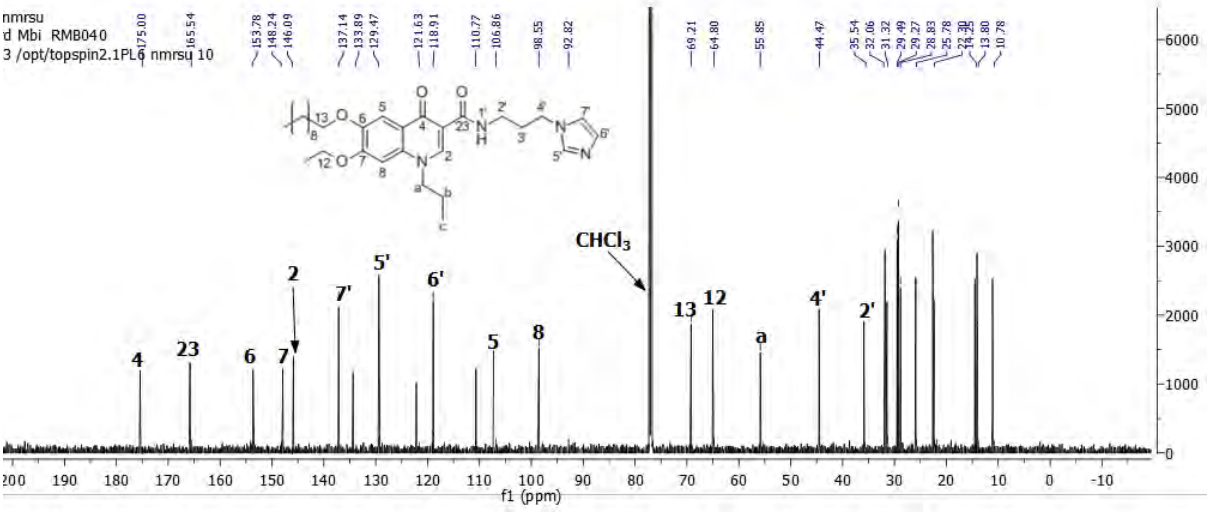
Supporting data for chapter 6

Compound 3

^{1}H NMR spectrum of compound 3

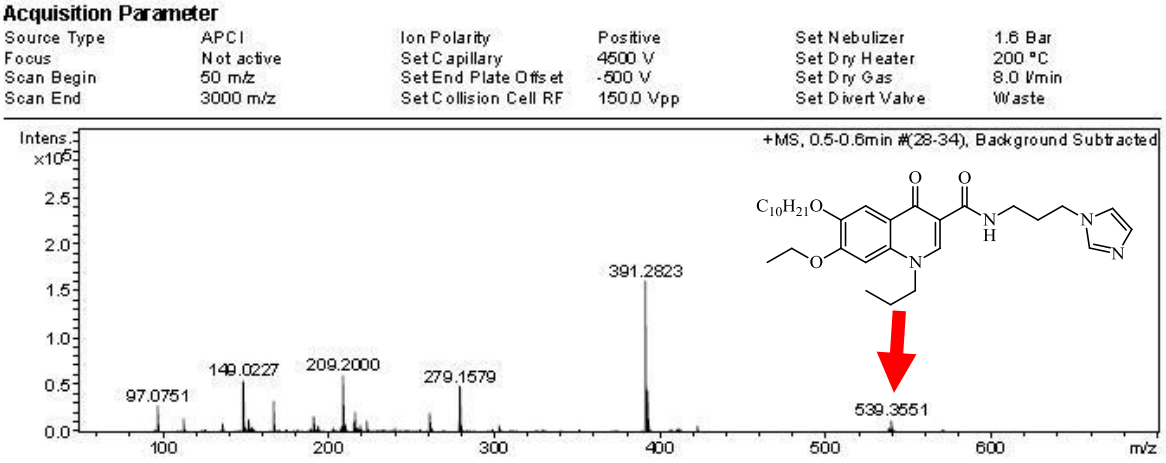


^{13}C NMR spectrum of compound 3



Supporting data for chapter 6

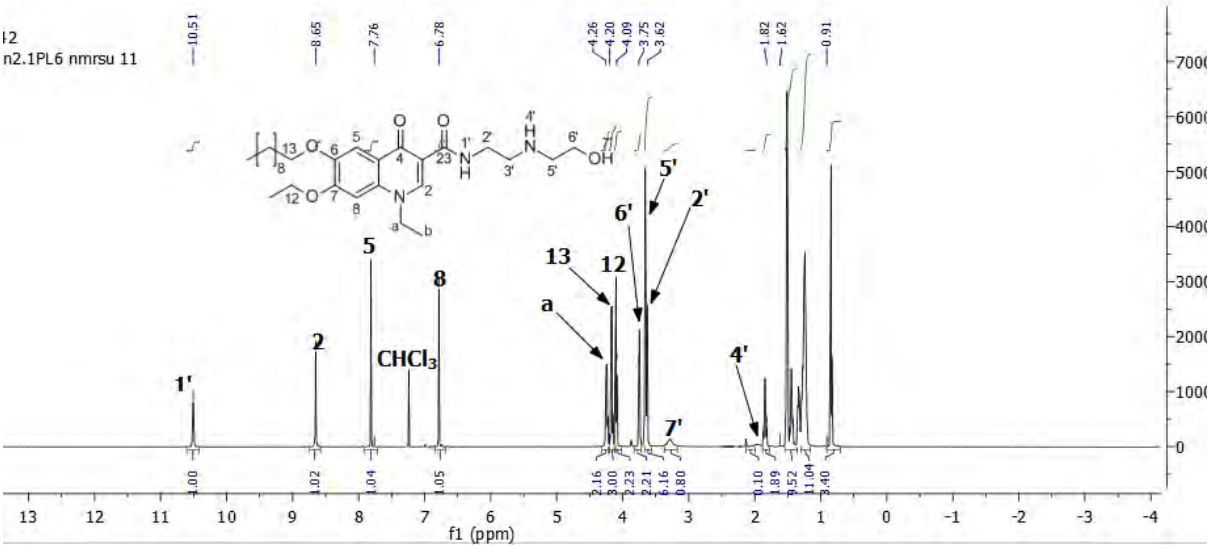
HRMS spectrum of compound 3



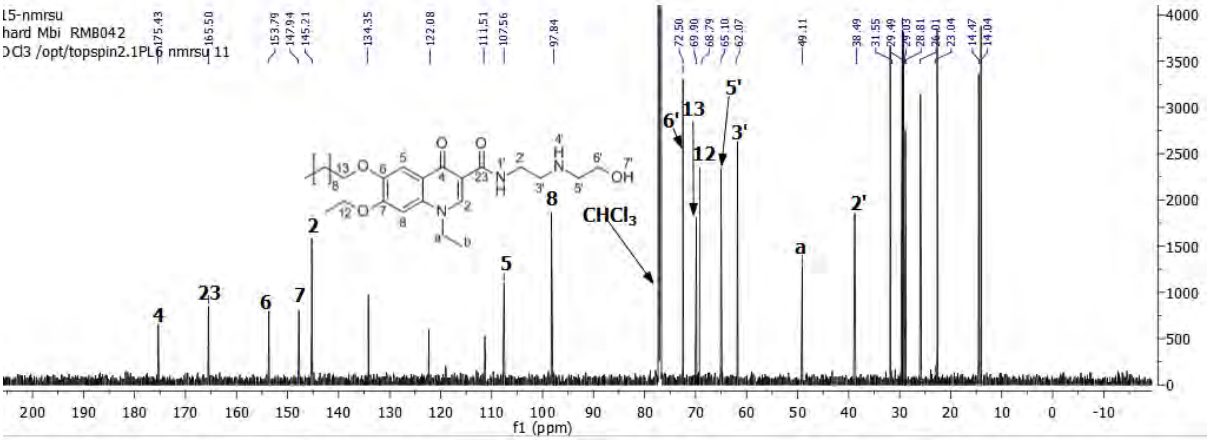
Supporting data for chapter 6

Compound 4

^{1}H NMR spectrum of compound 4

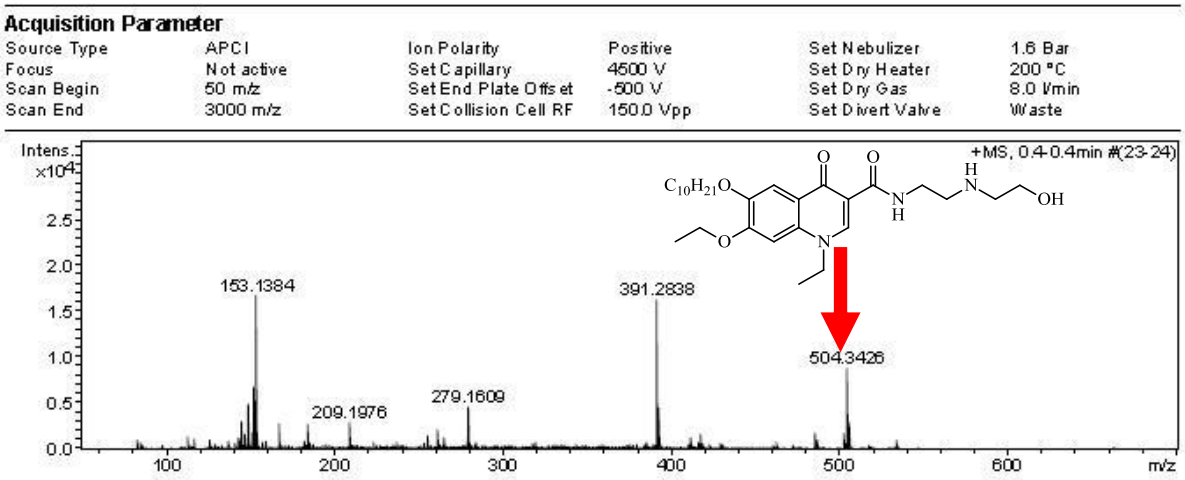


^{13}C NMR spectrum of compound 4



Supporting data for chapter 6

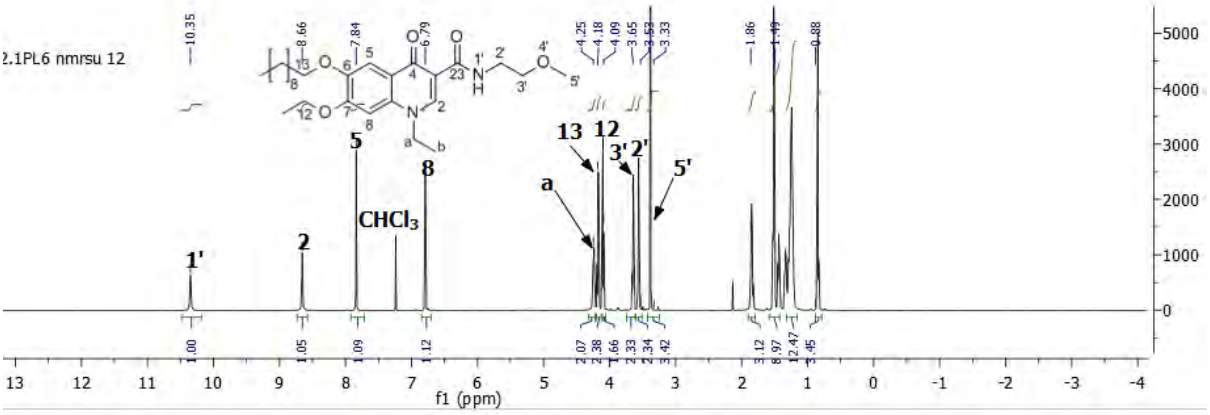
HRMS spectrum of compound 4



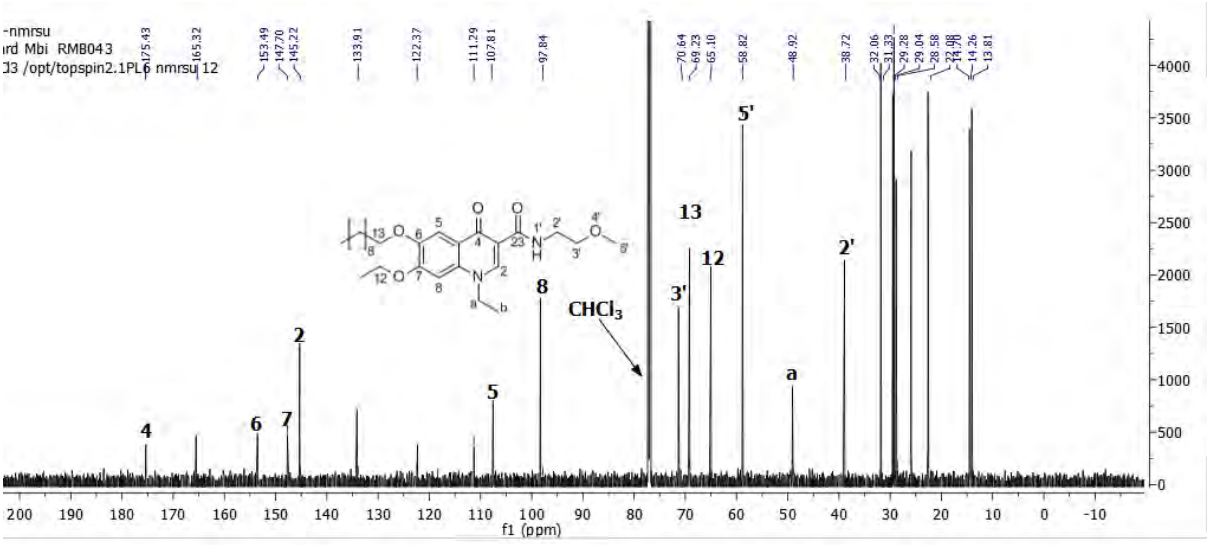
Supporting data for chapter 6

Compound 5

^{1}H NMR spectrum of compound 5

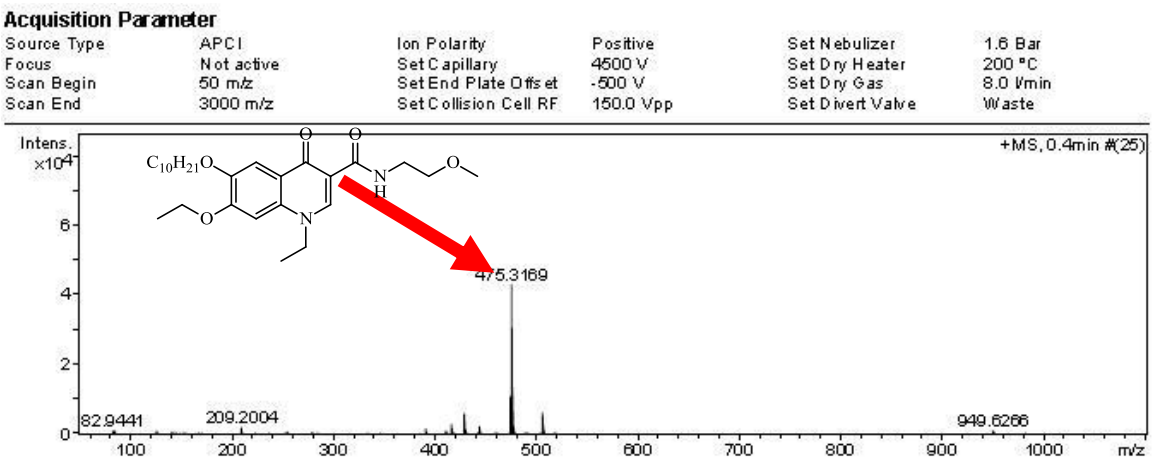


^{13}C NMR spectrum of compound 5



Supporting data for chapter 6

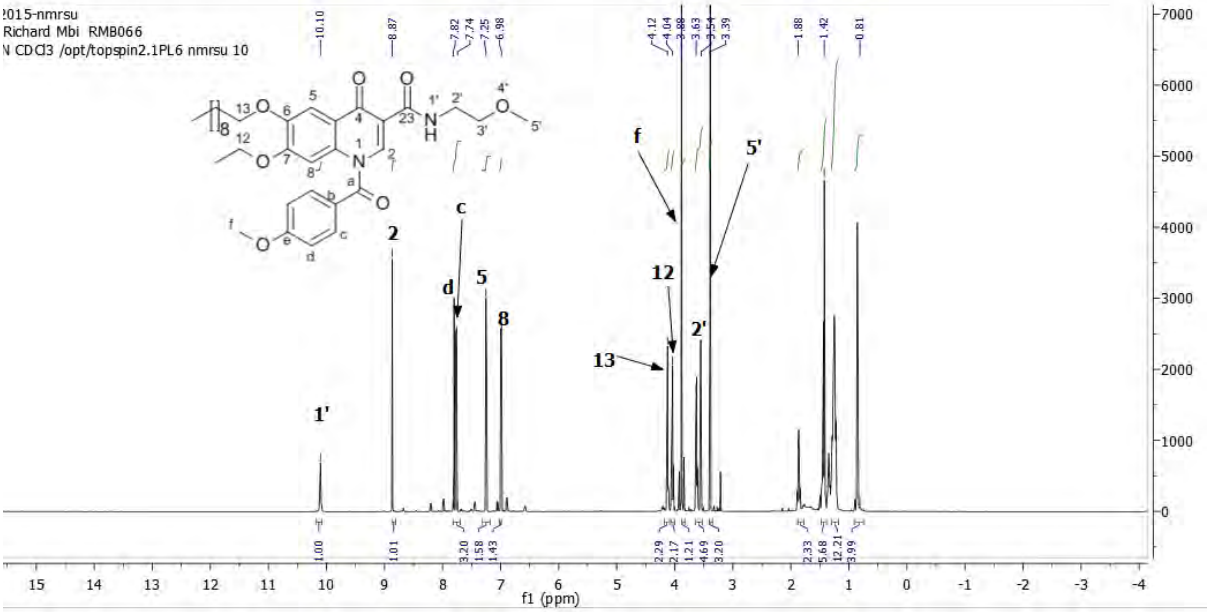
HRMS spectrum of compound 5



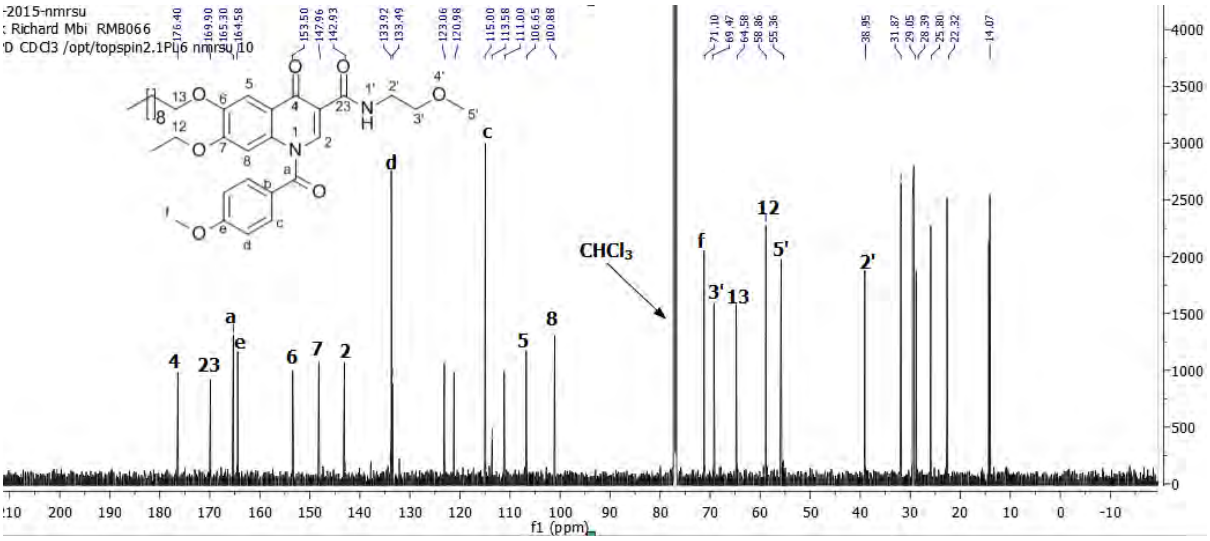
Supporting data for chapter 6

Compound 6

^{1}H NMR spectrum of compound 6



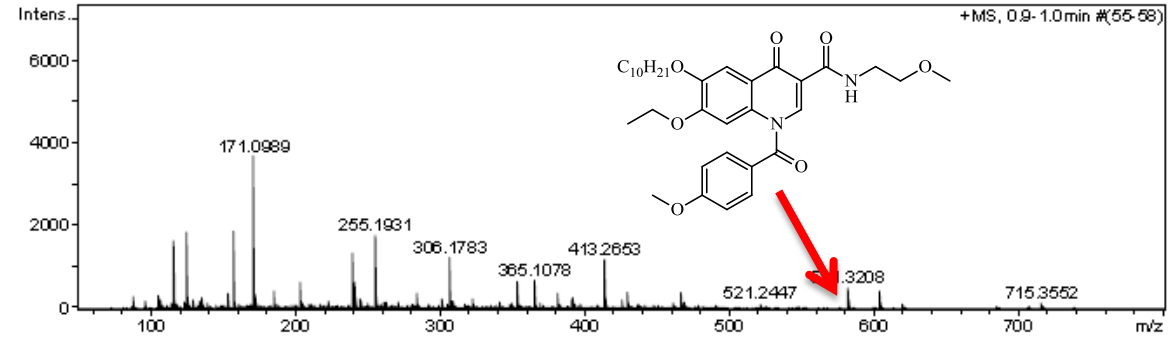
^{13}C NMR spectrum of compound 6



Supporting data for chapter 6

HRMS spectrum of compound 6

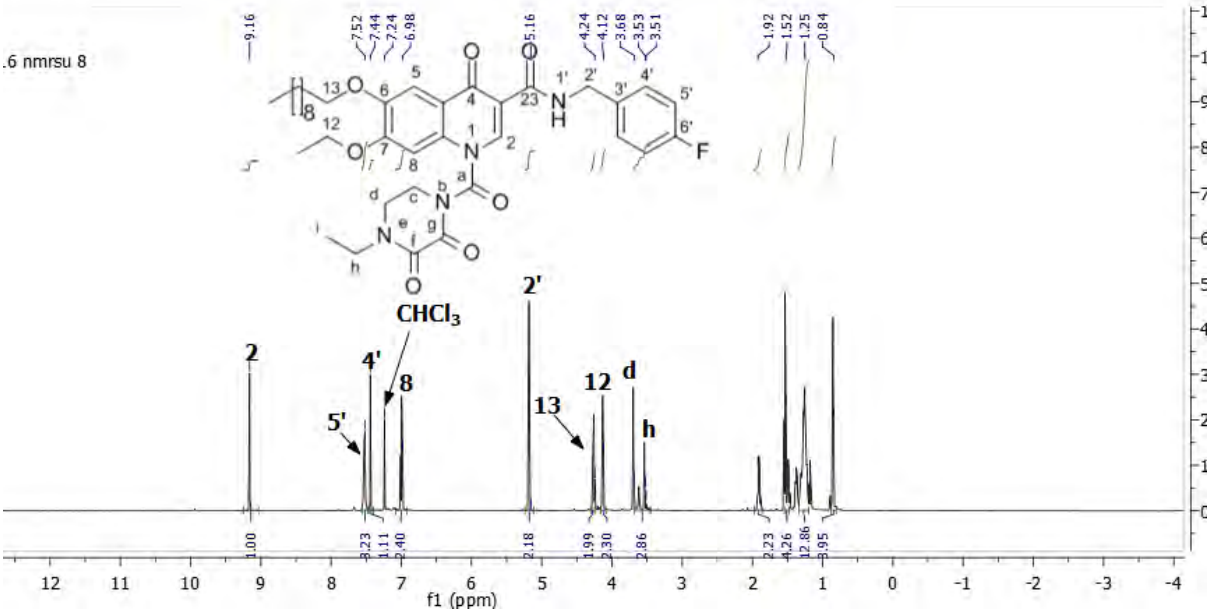
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



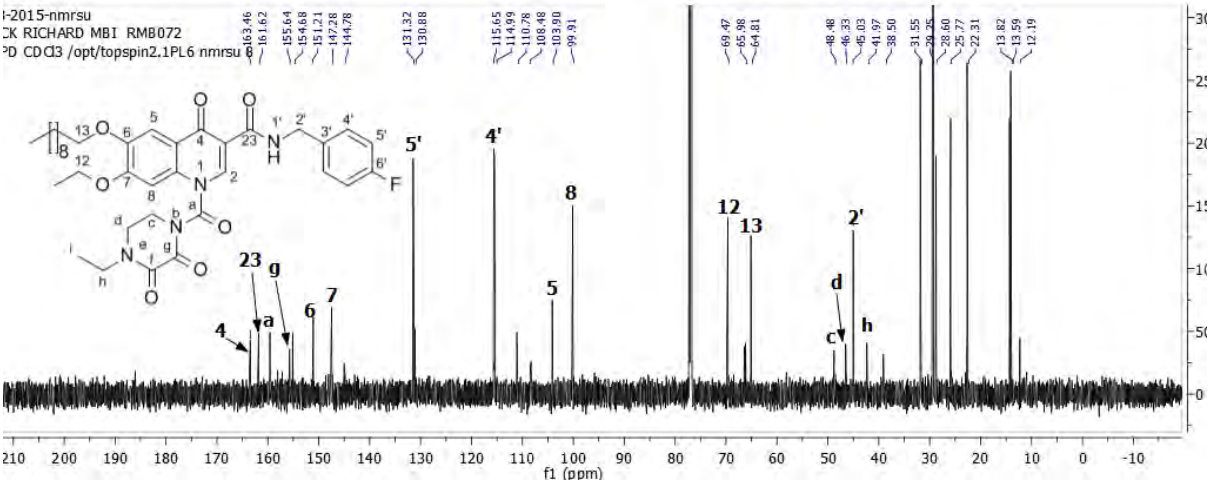
Supporting data for chapter 6

Compound 7

^{1}H NMR spectrum of compound 7

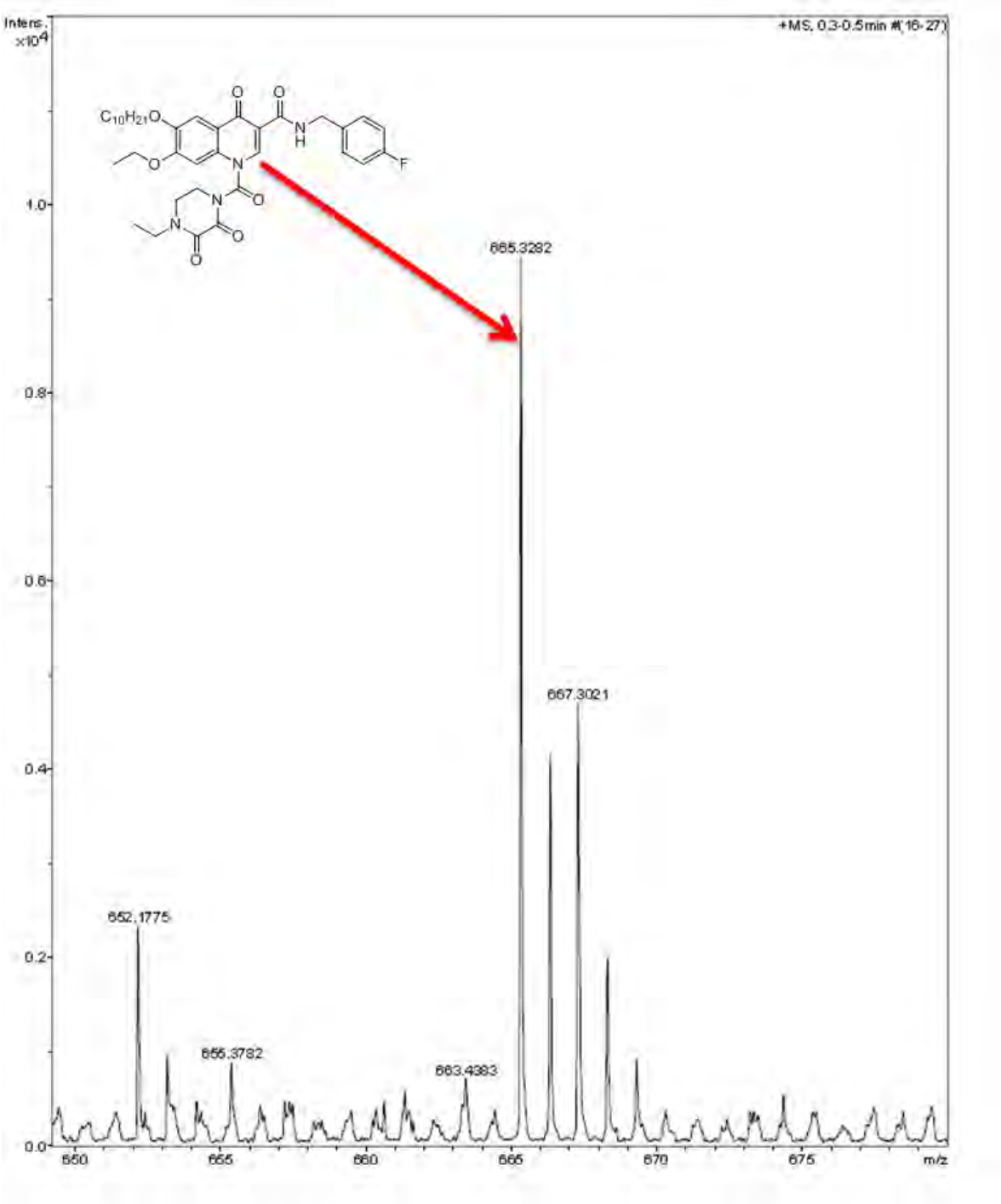


^{13}C NMR spectrum of compound 7



Supporting data for chapter 6

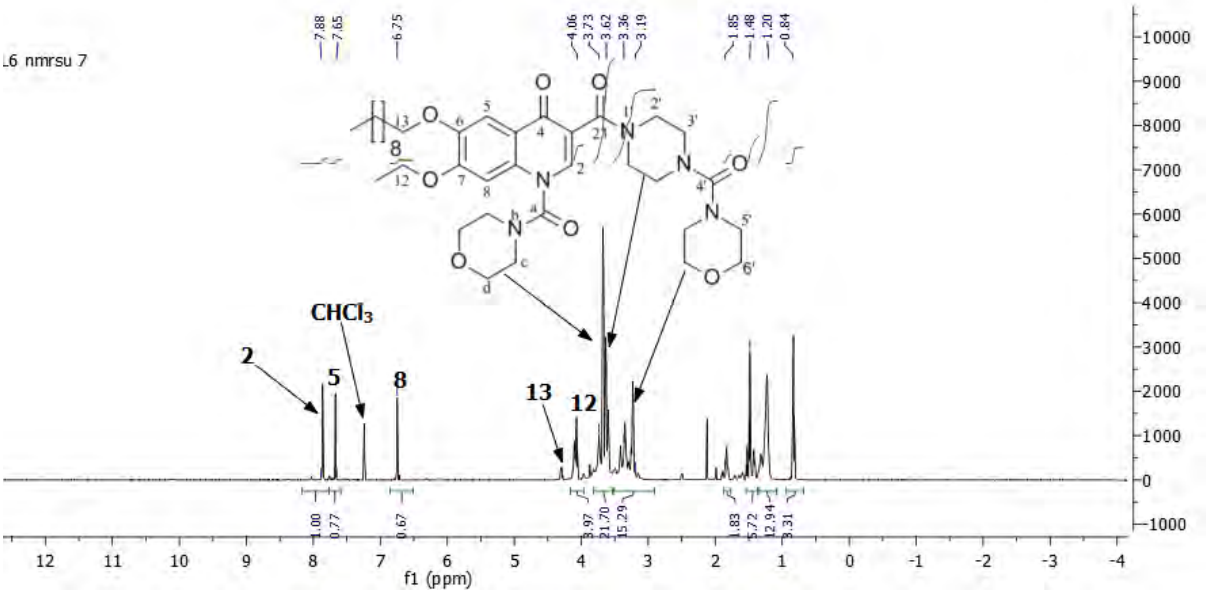
HRMS spectrum of compound 7



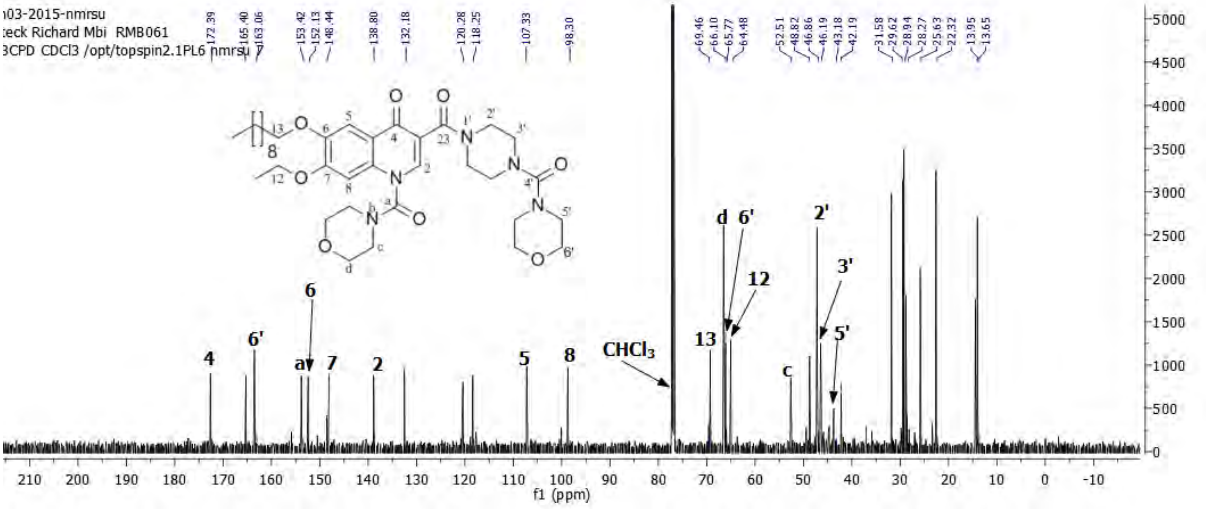
Supporting data for chapter 6

Compound 8

^{1}H NMR spectrum of compound 8

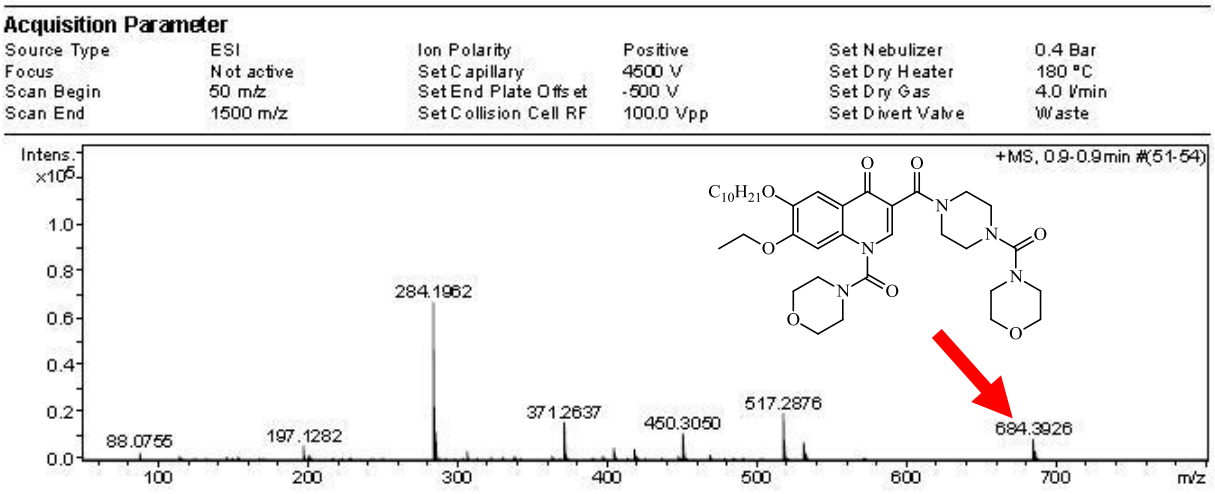


^{13}C NMR spectrum of compound 8



Supporting data for chapter 6

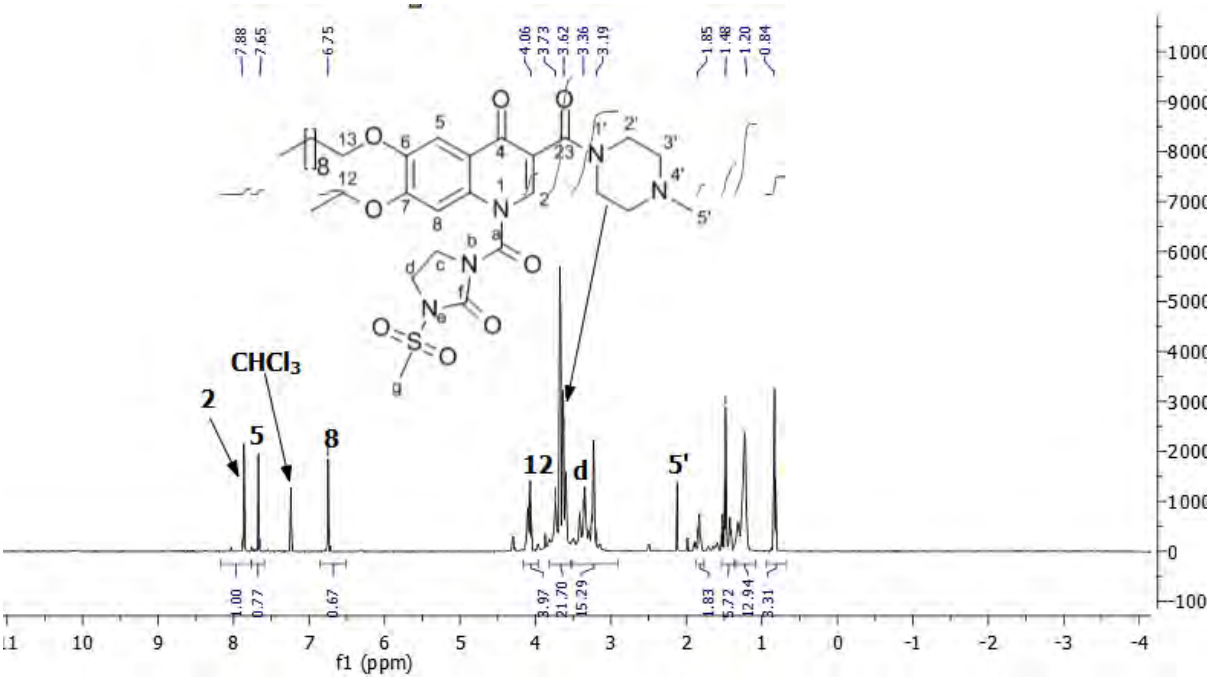
HRMS spectrum of compound 8



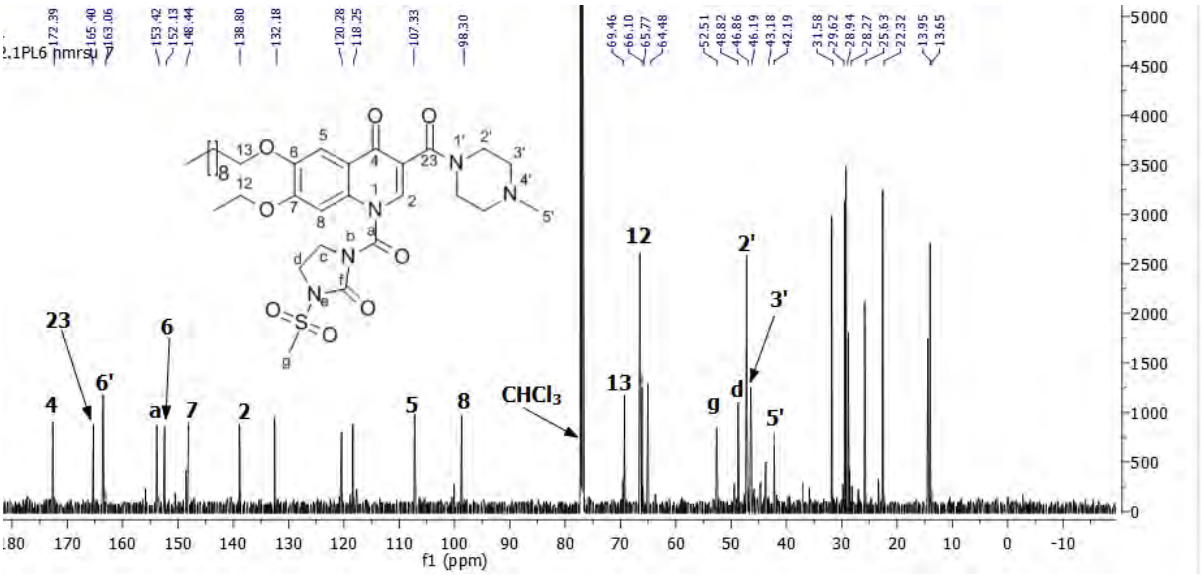
Supporting data for chapter 6

Compound 9

^{1}H NMR spectrum of compound 9

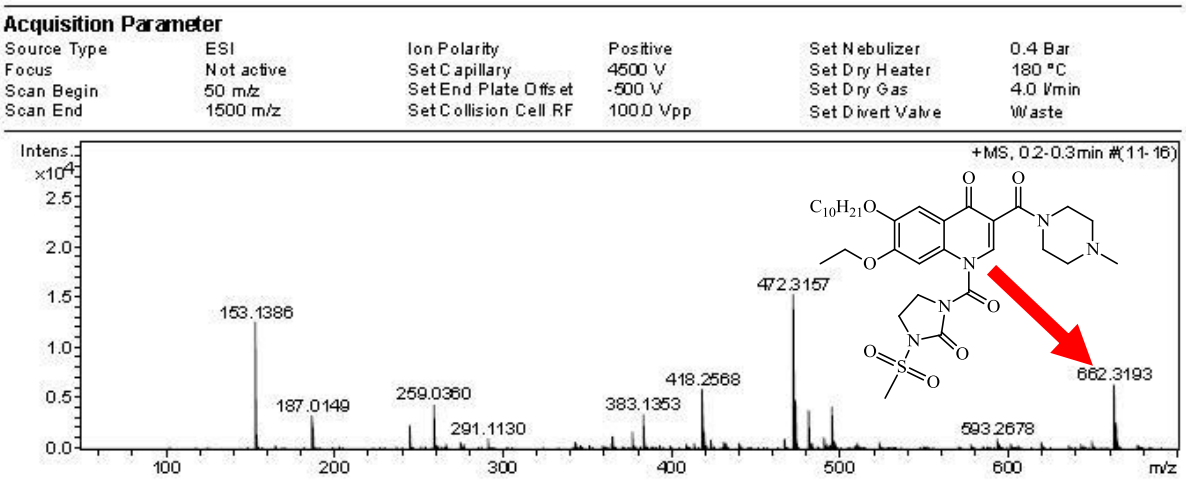


^{13}C NMR spectrum of compound 9



Supporting data for chapter 6

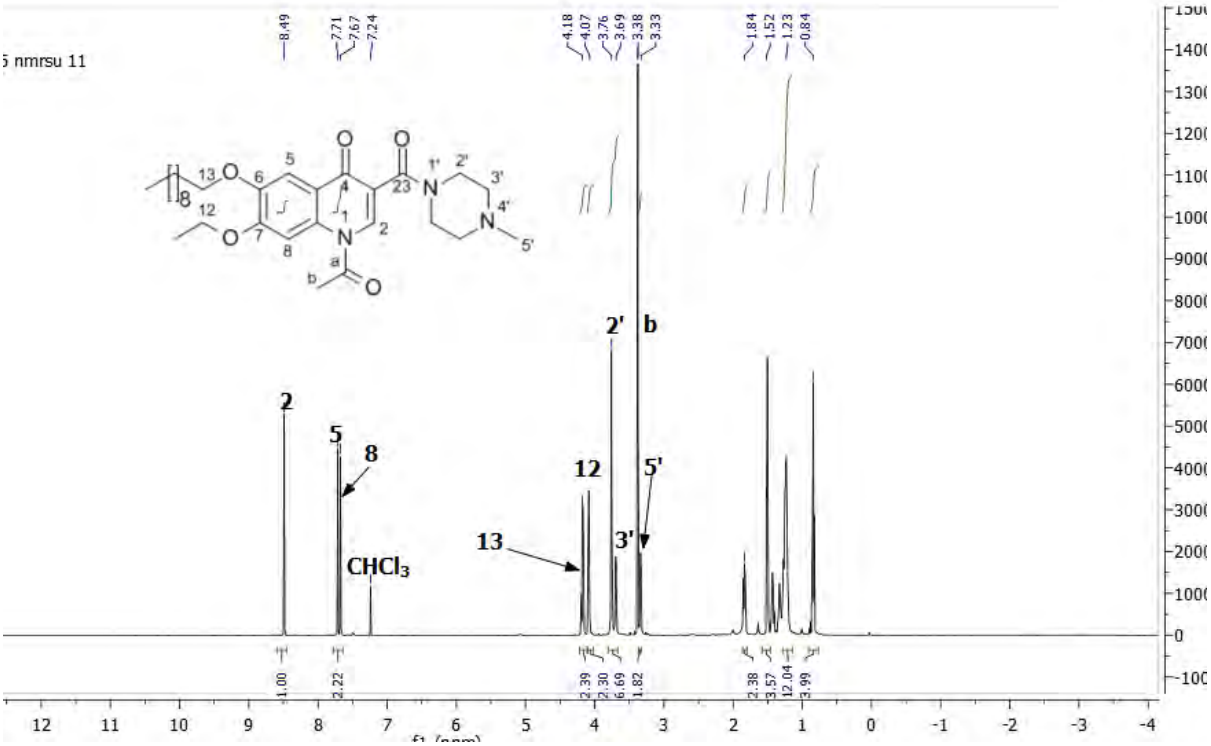
HRMS spectrum of compound 9



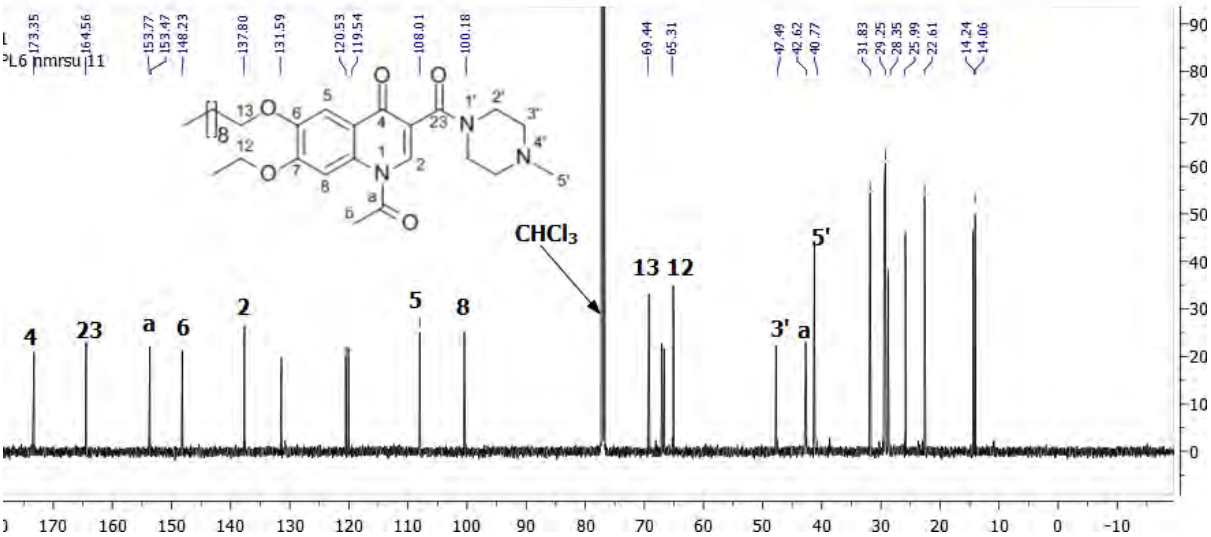
Supporting data for chapter 6

Compound 10

^{1}H NMR spectrum of compound 10

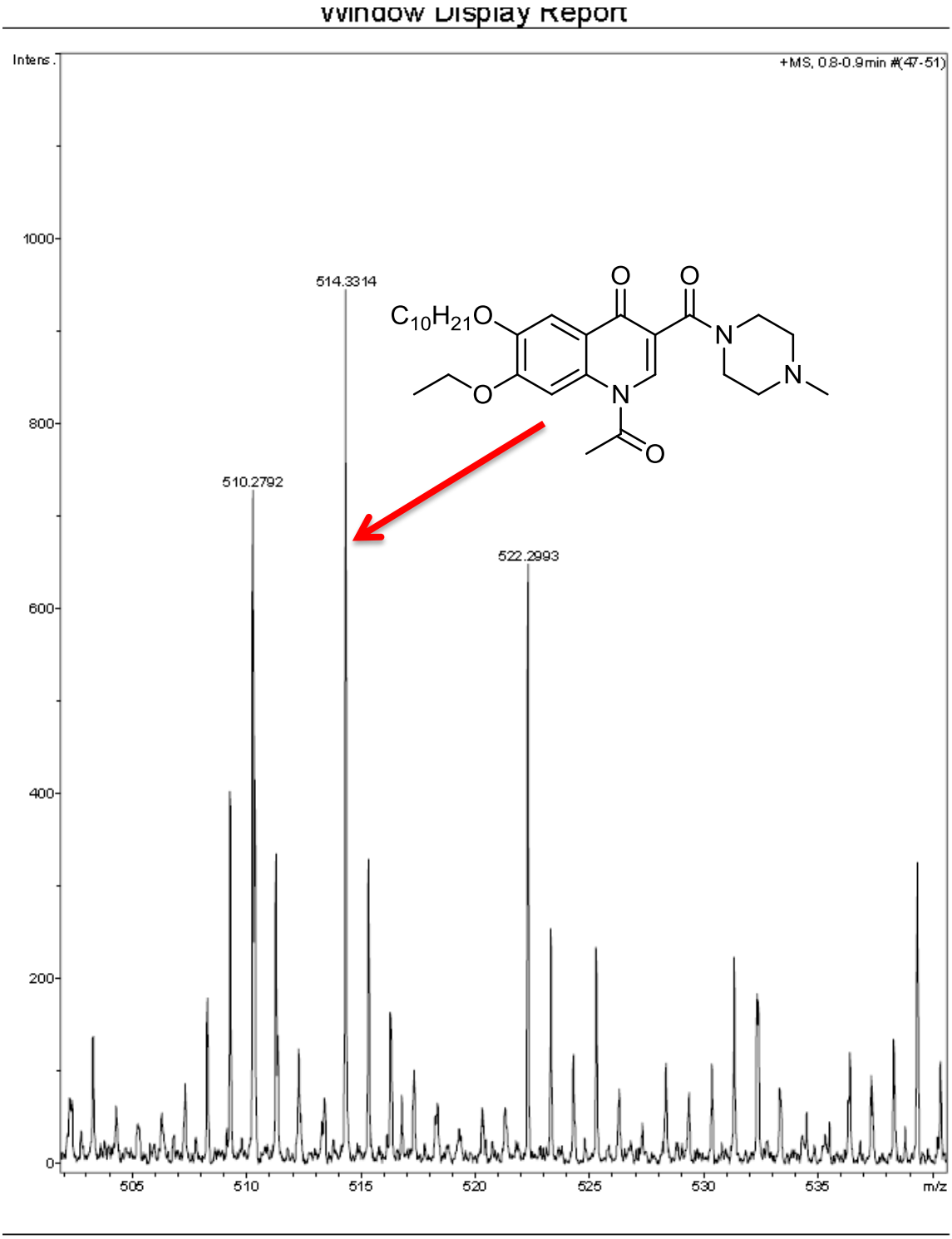


^{13}C NMR spectrum of compound 10



Supporting data for chapter 6

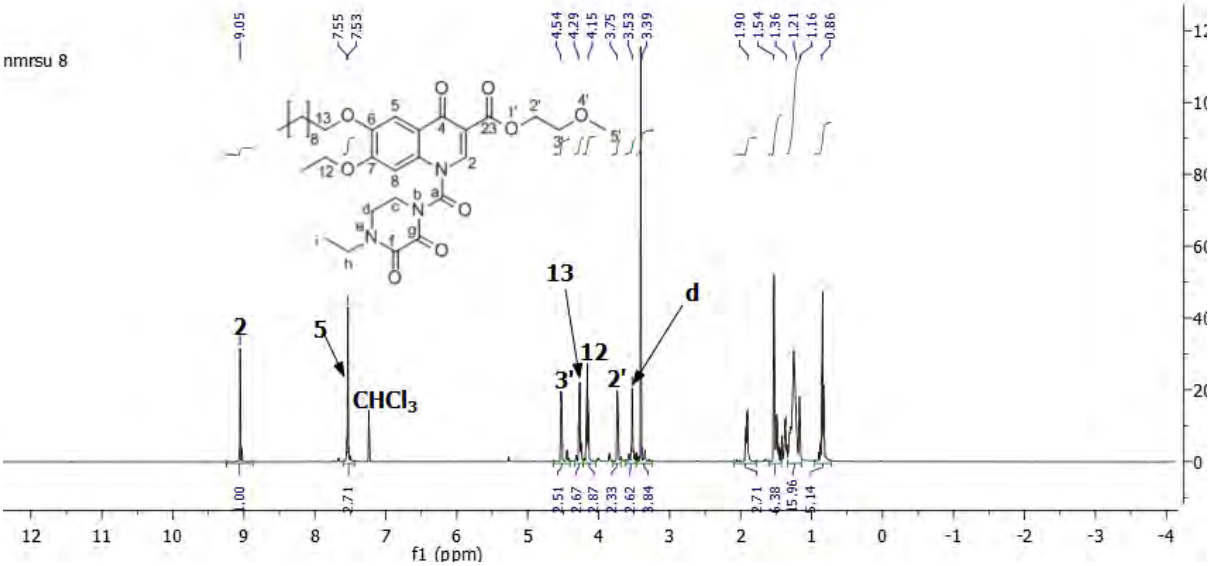
HRMS spectrum of compound 10



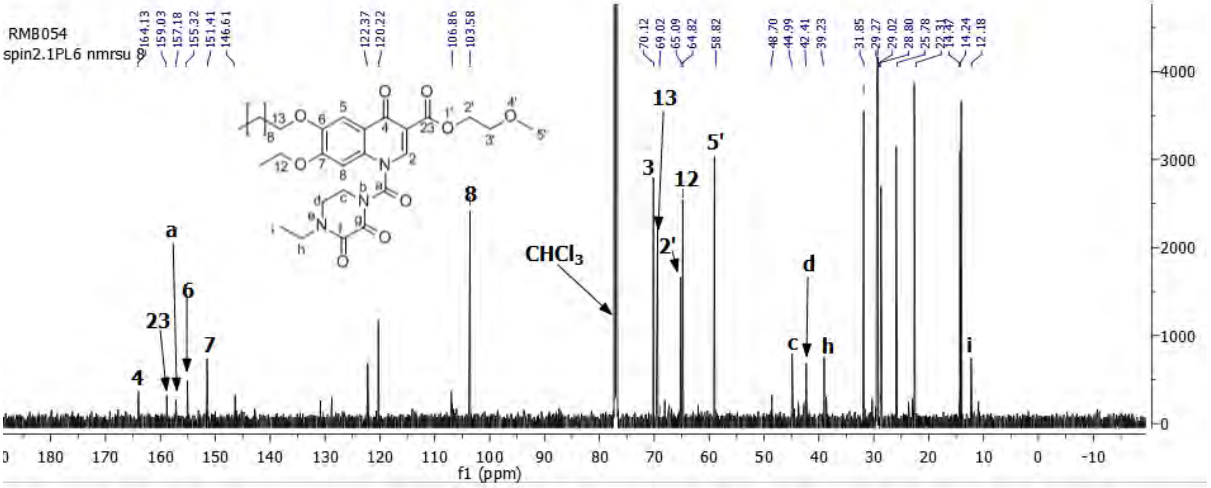
Supporting data for chapter 6

Compound 11

^{1}H NMR spectrum of compound 11

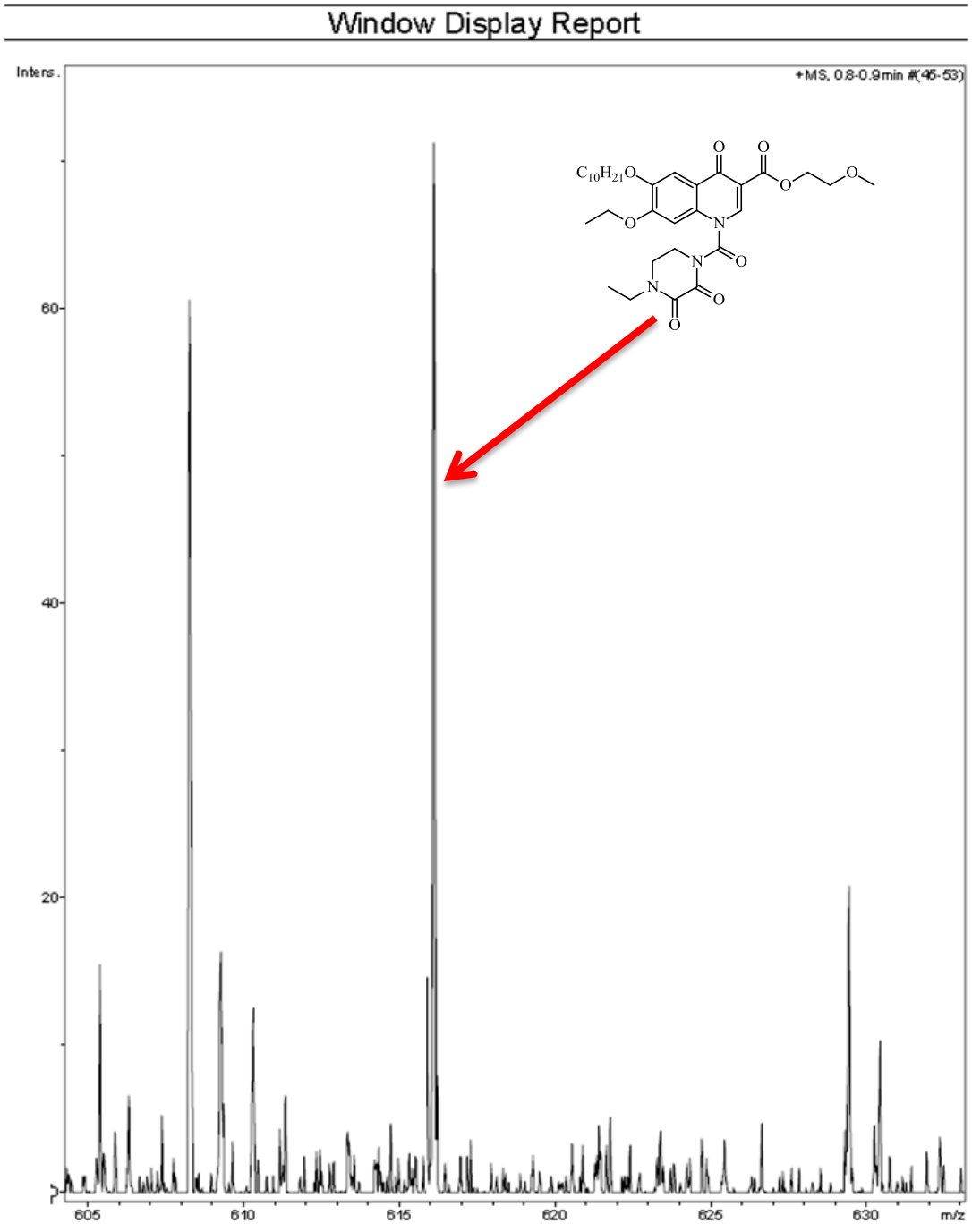


^{13}C NMR spectrum of compound 11



Supporting data for chapter 6

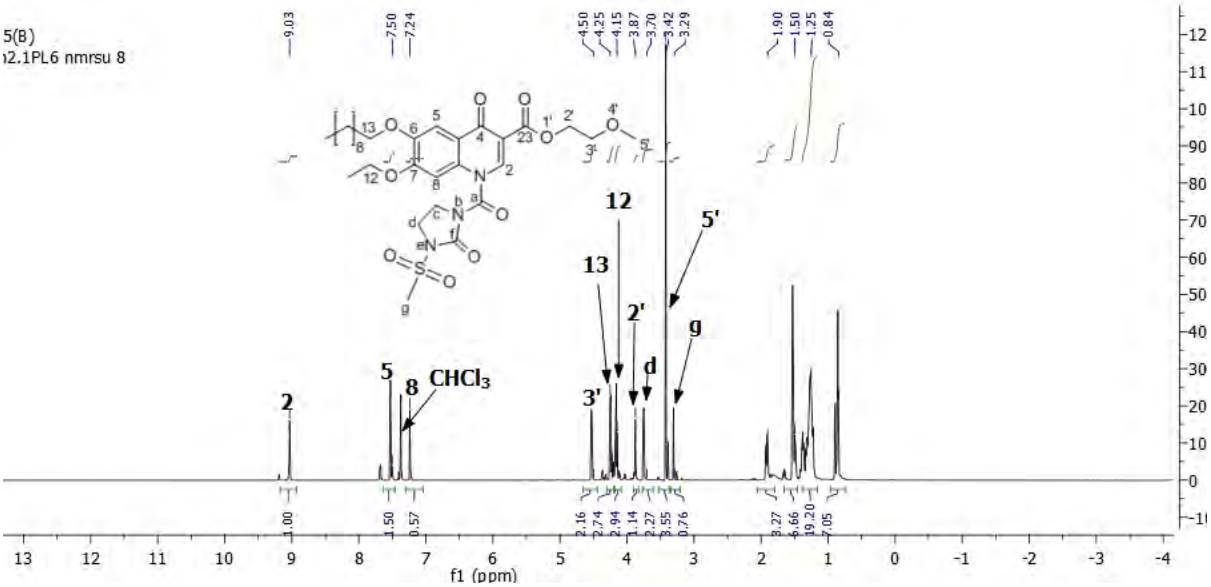
HRMS spectrum of compound 11



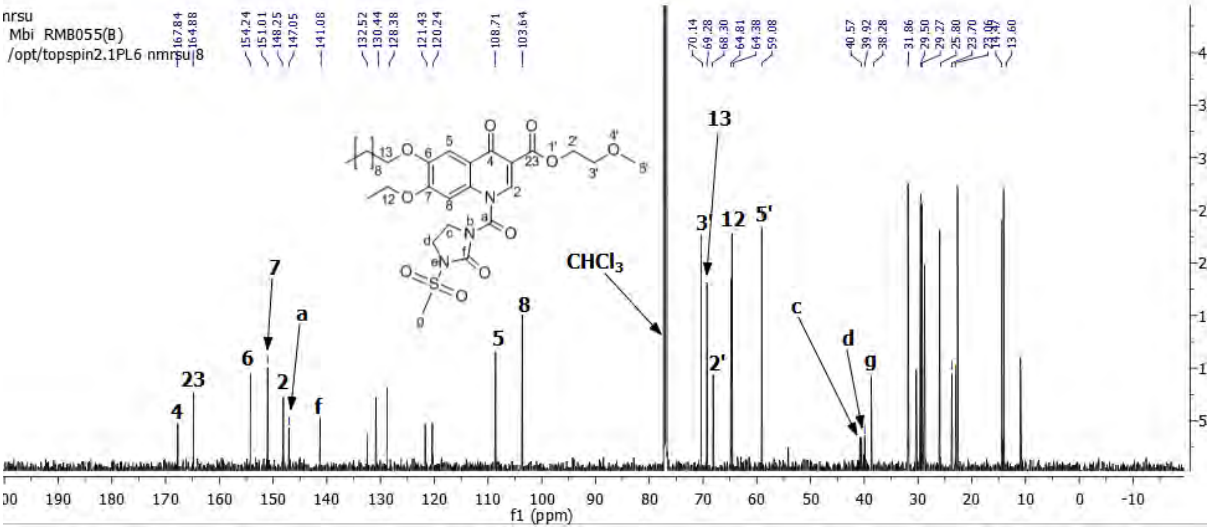
Supporting data for chapter 6

Compound 12

^{1}H NMR spectrum of compound 12

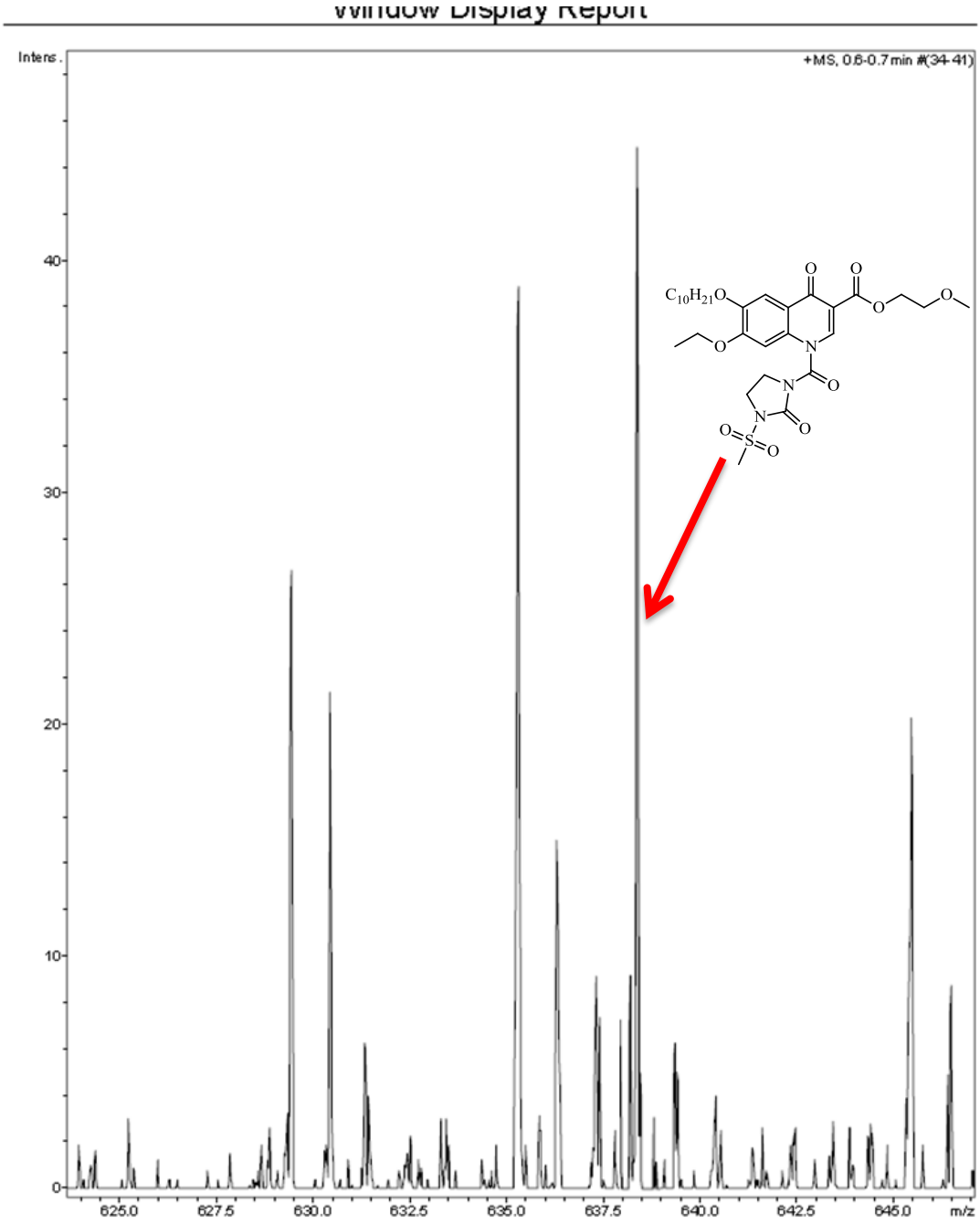


^{13}C NMR spectrum of compound 12



Supporting data for chapter 6

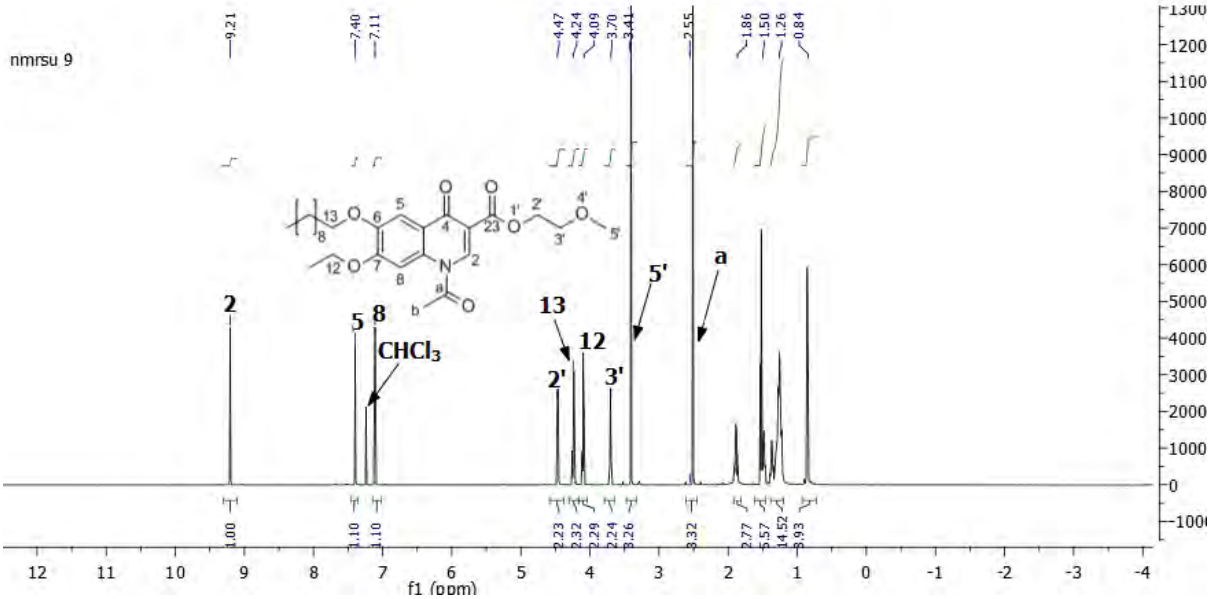
HRMS spectrum of compound 12



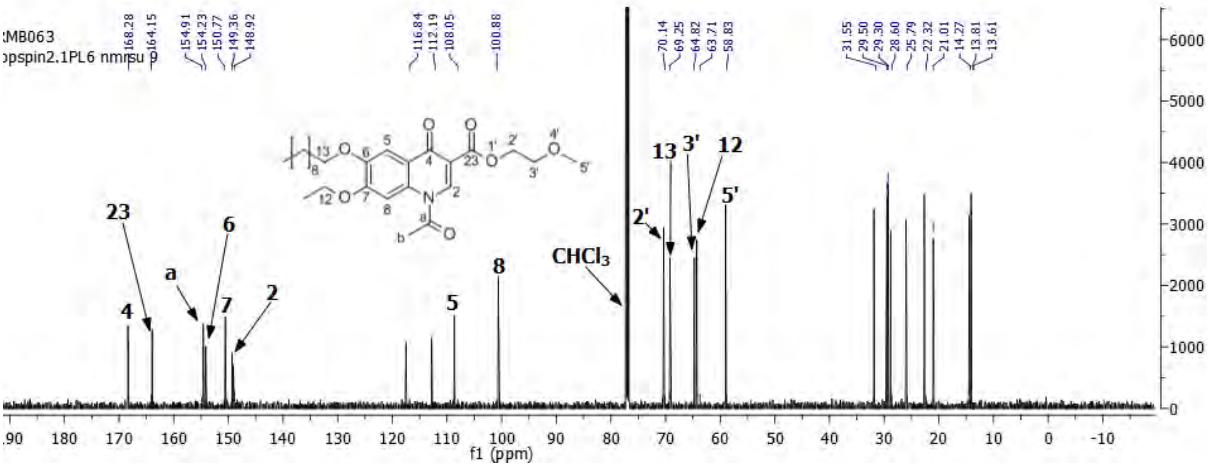
Supporting data for chapter 6

Compound 13

^{1}H NMR spectrum of compound 13

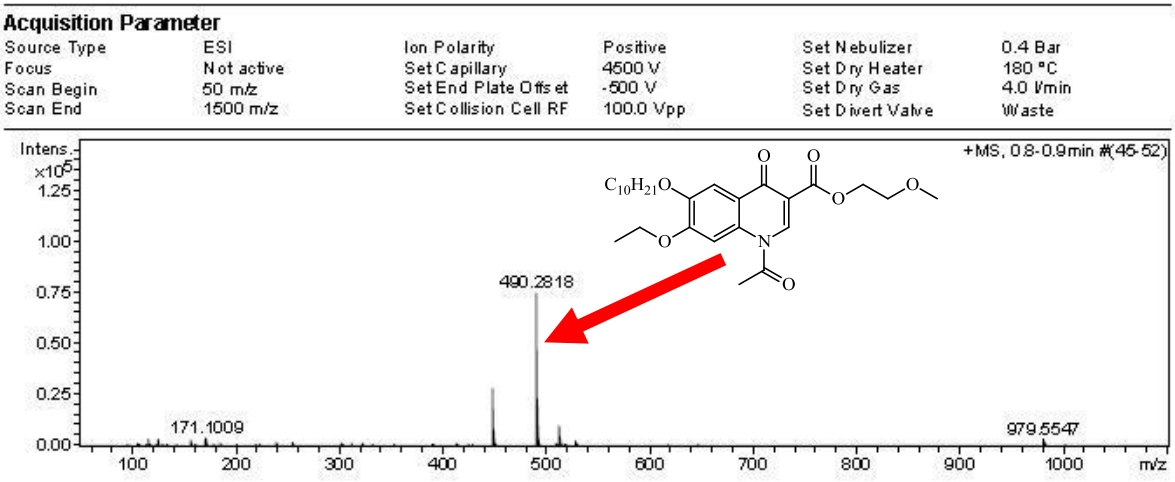


^{13}C NMR spectrum of compound 13



Supporting data for chapter 6

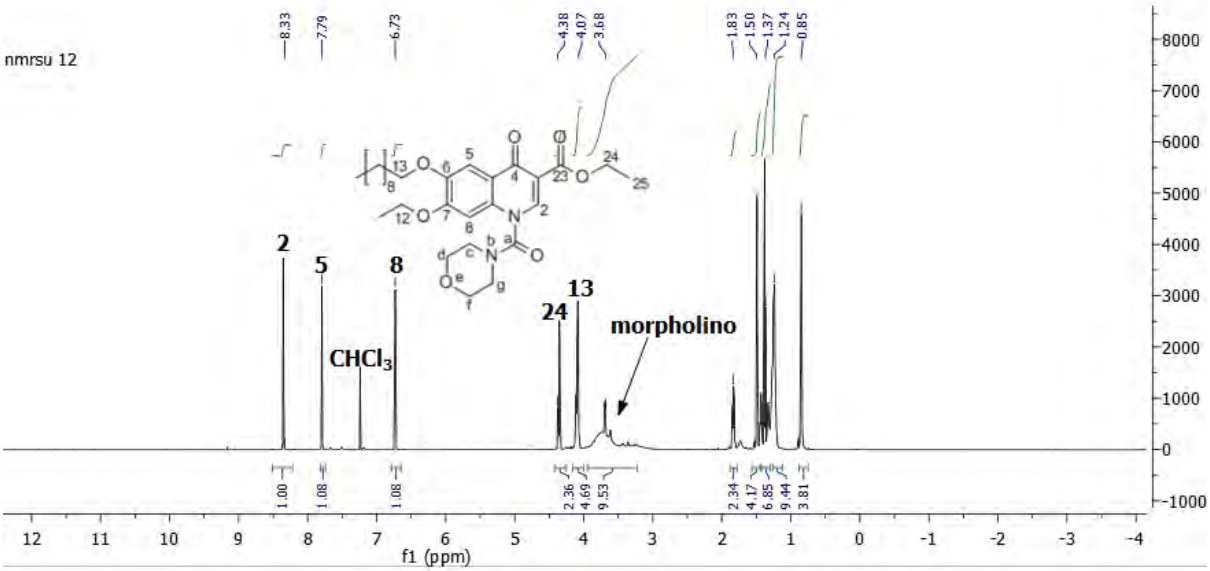
HRMS spectrum of compound 13



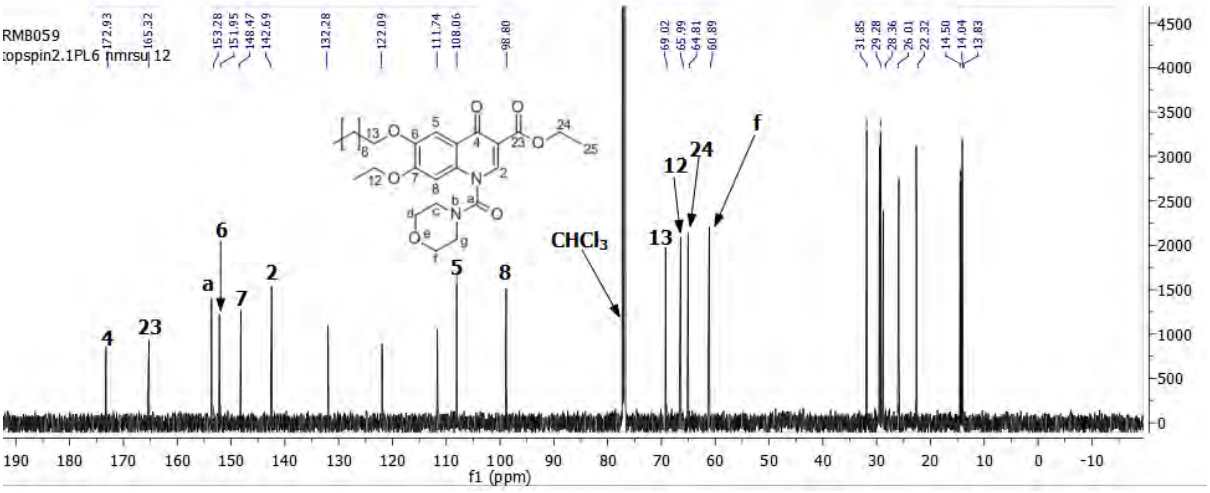
Supporting data for chapter 6

Compound 14

^{1}H NMR spectrum of compound 14

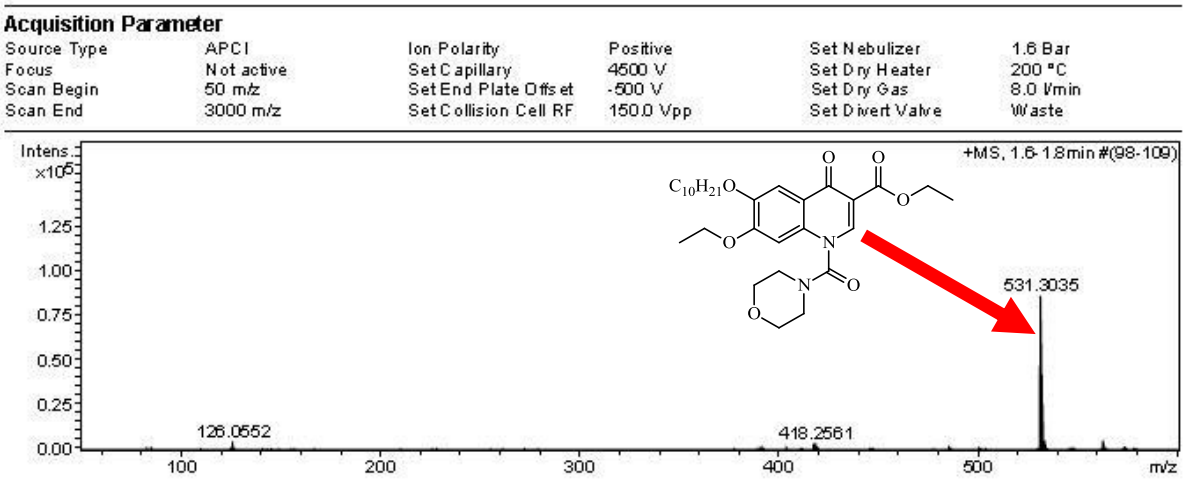


^{13}C NMR spectrum of compound 14



Supporting data for chapter 6

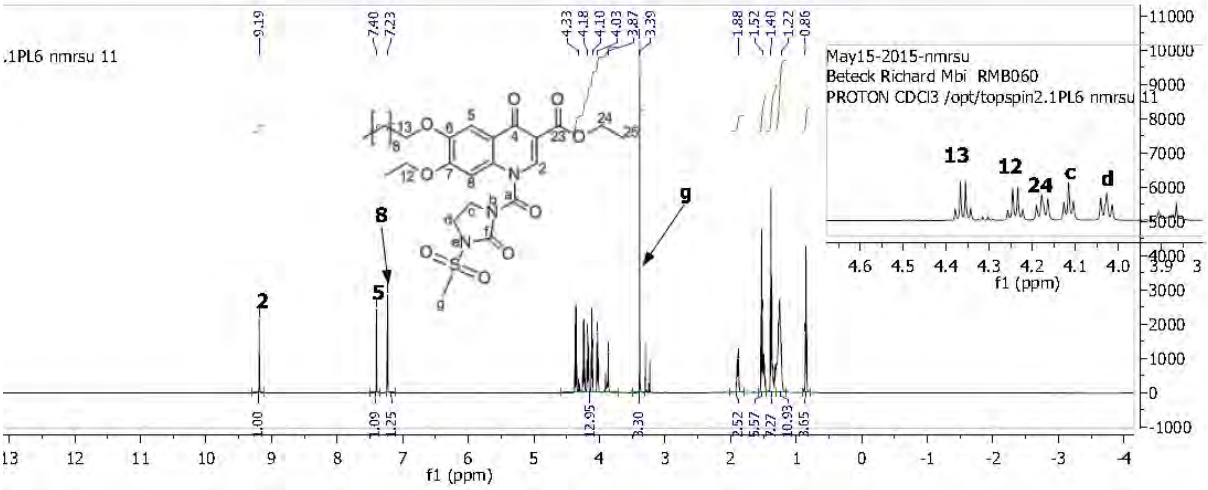
HRMS spectrum of compound 14



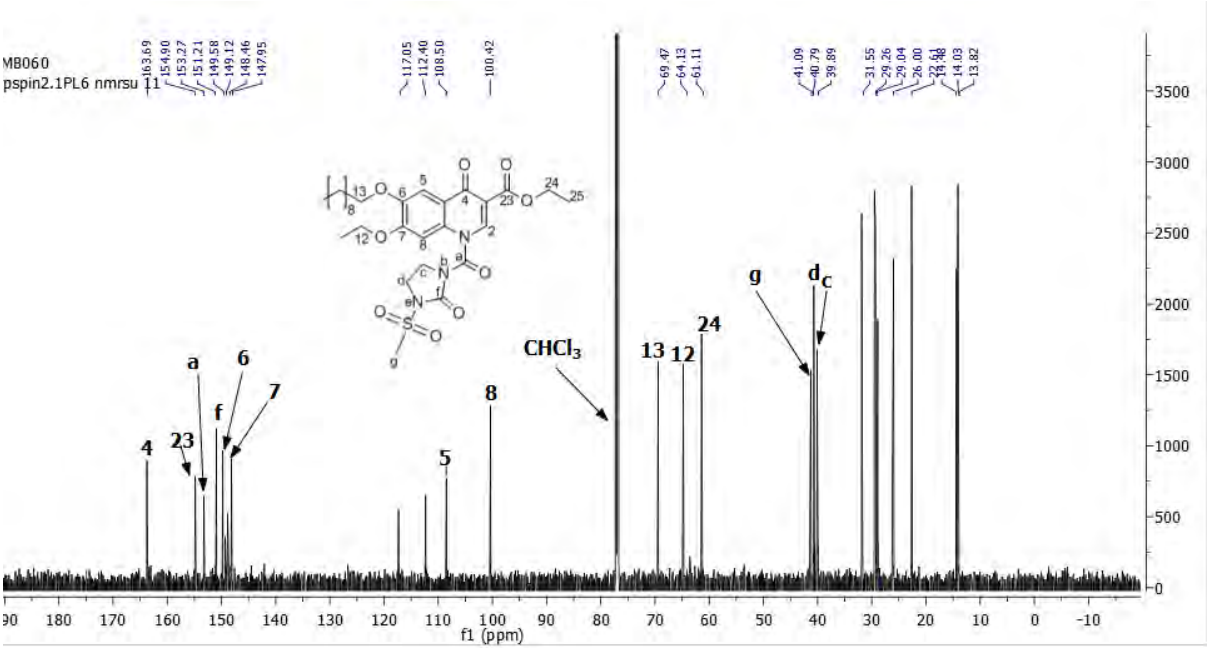
Supporting data for chapter 6

Compound 15

^{1}H NMR spectrum of compound 15



^{13}C NMR spectrum of compound 15



Supporting data for chapter 6

HRMS spectrum of compound 15