Triazole-linked 1,4-naphthoquinone derivatives: Synthesis and antiplasmodial activity

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PREFACE

This thesis is submitted in an article format in accordance with the General Academic Rules (A.13.7.3) of the North-West University. An article in the form of a manuscript is included in this dissertation:

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ABSTRACT

Malaria is a protozoan disease transmitted to humans through female *Anopheles* mosquitoes. The malaria parasite thrives in tropical areas, thus people there are at higher risk, especially in third-world countries like Africa and Asia. Even with preventative measures, like insecticide-treated nets (ITNs) and indoor residual spraying (IRS), taken to help control transmission, malaria still ravages through countries, killing adults and children alike.

World Health Organisation recommends artemisinin-based combination therapies (ACTs) as first-line treatment against uncomplicated malaria. However, recent statistics show an increase in resistance towards artemisinins that warrants the search for more efficient, safe and cheaper drug classes.

Molecular hybridisation has recently been in the lime light for medicinal chemists. In this study the hybridisation of two pharmacological active chemical moieties, namely 1,4-napthoquinone and 1,2,3-triazole, were utilised to develop a series of novel compounds. This series was further divided into two sub-series based on the major structural difference that is the linker between the pharmacophores. This structural difference was necessary to gauge the impact that the tether might have on the biological activity of these hybrids.

Sub-series 1 hybrids are structurally rigid as a result of direct linkage of the pharmacophores. The synthesis of these hybrids followed a two-step synthetic route involving firstly, an aromatic nucleophilic substitution resulting in an azide intermediate, and secondly, Huisgen copper alkyne-azide cycloaddition of the intermediate with various alkynes.

Sub-series 2 hybrids are flexible owing to an oxymethylene tether between the naphthoquinone and the triazole moieties. A three-step process was used to synthesise these hybrids. (1) a napthoquinolylalkyne intermediate was synthesised using a slightly modified version of Mitsunobu reaction for nucleophilic substitution SN2; (2) benzylazides were obtained in another nucleophilic substitution SN2 involving sodium azide and commercial benzyl bromides; (3) Huisgen copper alkyne-azide cycloaddition ‘‘click chemistry’’ of the napthoquinolylalkyne intermediate with the benzylazide afforded the target hybrids.

The CLog*P* values of the synthesised compounds were estimated to be in the two - five range, suggesting that the hybrids were drug-likeable, thus were expected to be endowed with enhanced biological activities.

The cytotoxicity of the compounds was evaluated using normal human embryonic kidney cells (HEK-293) and were found to be generally non-toxic.

The antimalarial activity of the hybrids was evaluated *in vitro* by determining the percentage growth inhibition of asexual stage *P. falciparum* NF54 strain parasites, at 5 µM and 1 µM concentrations, using SYBR Green I based assays. Sub-series 1 was completely inactive. Sub-series 2 on the other hand, was found to be very active with the percentage growth inhibition of hybrids ranging from 70 - 90 % regardless of the concentration, validating this sub-series for further investigation. Of particular interest is hybrid **22,** bearing *tert-butyl* substituent that showed 90 % parasite growth inhibition at 1 µM and moderate cytotoxicity with an IC_{50}-value of 36 µM. This hybrid compared well with atovaquone that had 96% parasite growth inhibition at 1 µM, and an IC_{50}-value of 56 µM. Compound **22** stands as a good candidate for further evaluation.

Keywords: *Plasmodium*, malaria, 1,4-naphthoquinone, 1,2,3-triazole, click-chemistry, hybrids

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ABBREVIATIONS

µm - Micromolar
ACT – Artemisinin-Based Combination Therapy
BBB - Blood Brain Barrier
BCS - Blantyre Coma Score
BM - Bone Marrow
CDC - Centers For Disease Control
$CDCl_3$ - Chloroform-*D*
CHO - Chinese Hamster Ovary Cell Line
CM - Cerebral Malaria
DBP - Duffy Binding Protein
DHF - Dihydrofolate
DHFR - Dihydrofolate Reductase
DHPS - Dihydropteroate Synthase
DMF - Dimethylformamide
DMSO - Dimethyl Sulfoxide
DV – Digestive Vacuole
EAD – Early After Depolarisation
E-B - *Epstein-Barr*
EI – Erythroblastic Island
FPPIX - Ferriprotoporphyrine IX
FST - Fluorescent Spot Test
G6PD - Glucose-6-Phosphate Dehydrogenase
Hb - Haemoglobin
Hbs – Sickle Haemoglobin
HCM - Human Cerebral Malaria
HEK-293 - Human Embryonic Kidney Cells
HPLC - High-Performance Liquid Chromatography
HRMS - High Resolution Mass Spectrometry
HZ - Hemozoin
IC_{50} - 50% Inhibitory Concentration
IPT - Intermittent Preventive Treatment
IR - Infrared

IRS - Indoor Residual Spraying

ITN - Insecticide Treated Nets

KINET- Kilombero Valley Insecticide-Treated Net

LAMP - Loop-Mediated Isothermal Amplification

MeOH - Methanol

$MgSO_4$ - Magnesium Sulphate

MIC - Minimum Inhibitory Concentration

M.P. - Melting Point

MS - Mass Spectrometry

Na-Asc - Sodium Ascorbate

NADPH - Nicotinamide Adenine Dinucleotide Phosphate

NAI - Naturally Acquired Immunity

NaN_3 - Sodium Azide

NCEs - New Chemical Entities

NH_4Cl - Ammonium Chloride

NMR - Nuclear Magnetic Resonance

NRF - South African National Research Foundation

PCR - Polymerase Chain Reaction

*Pf*CRT - *Plasmodium falciparum* Chloroquine Resistance Transporter

Pfemp1 - *Plasmodium falciparum* Erythrocyte Membrane Protein 1

PK - Pyruvate Kinase

PPPK – Pyrophosphokinase

Q_o - Quinol Oxidation

QT-Interval Start Of The Q-Wave And The End Of The T-Wave In The Hearts Electrical Cycle

RACD - Reactive Case Detection

RDT - Rapid Diagnostic Test

SAR - Structure-Activity Relationship

THF - Tetrahydrofolate

TLC - Thin Layer Chromatography

TS - Thymidylate Synthase

WHO- World Health Organisation

CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

1.1 Background

Malaria, a mosquito-borne disease caused by parasites of the genus *Plasmodium*, is transferred from infected female *Anopheles* mosquitoes to humans during blood meals. In Italian the term '*mala aria*' means 'bad air', this was used to describe the symptoms and associated circumstances in the 1700's (Uddin, 2017).

There are currently five known species of malaria that can infect humans *viz. P. falciparum*, *P. vivax*, *P. malariae, P. ovale*, and *P. knowlesi.* Long-tailed and pig-tailed macaques are the hosts for the fifth species*, P. knowlesi* that causes zoonotic malaria infection in humans (Singh & Daneshvar, 2013). Among the five pathogenic species, *P. falciparum* and *P. vivax* are responsible for more than 95 % of malaria cases in the world (Jourdan *et al.*, 2018b). New and improved treatment paradigms could be provided by learning more about the mechanisms and life cycle of malaria (Gilson *et al.*, 2018a).

In 2016 a total of 216 million cases of malaria were reported in 91 countries, 5 million cases higher than the previous year, and malaria deaths reached 445 000 (WHO, 2017b). Young children are particularly affected with cases reported in sub-Saharan Africa (90%), Southeast Asia (7%) and the Eastern Mediterranean Region (2%) (Krungkrai & Krungkrai, 2016). Endemic malaria regions show signs of seasonality, e.g. in tropical Africa where rainfall patterns affect transmission statistics (Greenwood *et al.*, 2017). Non-endemic or ectopic cases are also prevalent, with France and the UK receiving the highest number of cases averaging at more than 4 000 reported cases per year (Tatem *et al.*, 2017). The West African region accounts for 56 % of reported cases of ectopic infections.

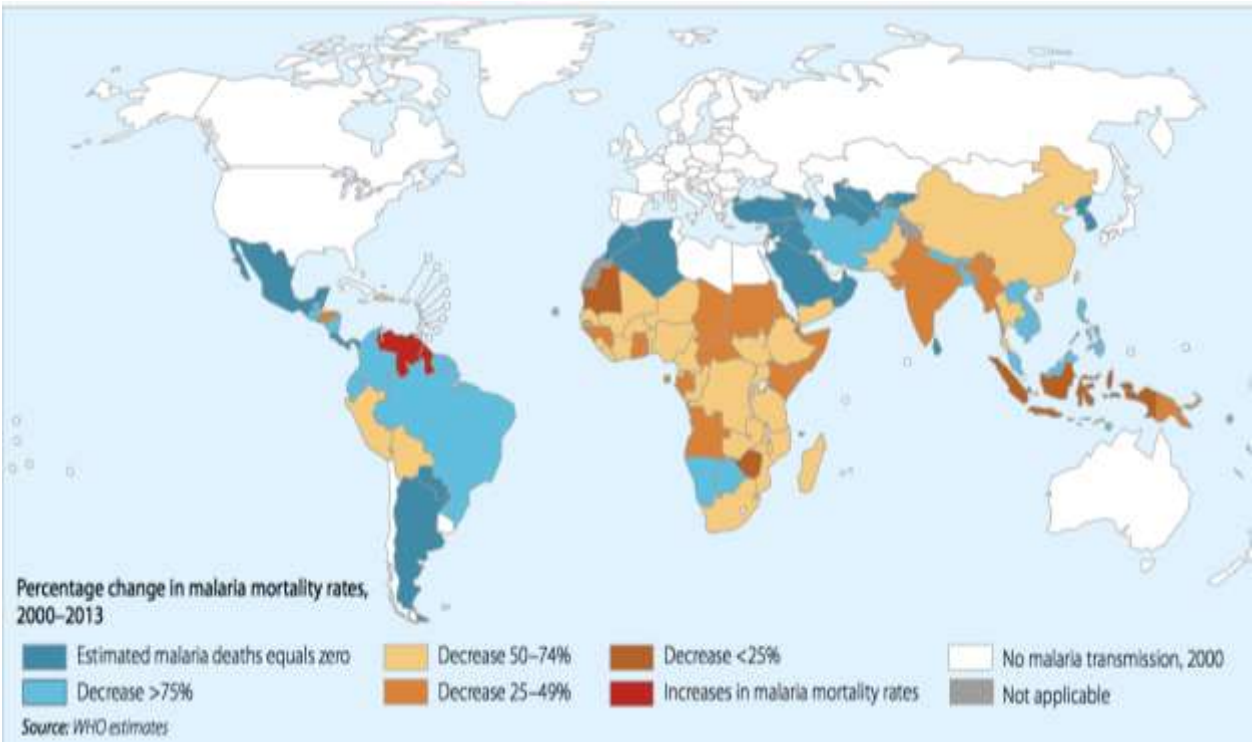


Figure 1-1: The UN Dispatch map of percentage change in malaria mortality rate between 2000-2013 (Dispatch, 2014)

Currently there are a few vaccines undergoing trials, but none have officially been allocated for antimalarial use (Ouattara *et al.*, 2015). A new generation of vaccines, based on recombinant antigens, can cover many of the necessary characteristics for a malaria vaccine, e.g. overcoming the poor immunogenicity of *Plasmodium* recombinant antigens. Researchers are currently exploring the application of numerous strategies with different antigens to achieve an effective vaccine, including the addition of adjuvants which have been used during the development of vaccine RTS,S/AS01 (Mehrizi *et al.*, 2018).

RTS,S/AS01 completed Phase III clinical testing in 2014. Notable progress is being made in the characterisation of possible regulatory pathways to fast-track timelines, including for vaccines designed to interrupt transmission of parasites from humans to mosquitoes. However, with the absence of financial support, investment in malaria vaccine development implies continued heavy reliance on public and philanthropic funding (Birkett, 2016a).

The World Health Organisation's (WHO*)* recommended first-line treatment regimens against uncomplicated malaria is artemisinin-based combination therapies (ACTs) (WHO, 2016c) . The success of malaria prevention, control, cure and elimination is, therefore, currently highly dependent on the sustained clinical efficacy of first-line ACTs, for which the emergence and spread of drug resistance in *P. falciparum* poses a constant threat (Zhang, 2016). The widespread

ACT resistance in malaria-endemic countries has been predicted to have an impact of >100,000 additional deaths per year (Blasco *et al.*, 2017). Antimalarial drug resistance in *P. falciparum* tends to emerge in low transmission settings, particularly in Southeast Asia or South America, before expanding to high-transmission settings in sub-Saharan Africa. Resistance to chloroquine and later to sulfadoxine–pyrimethamine have followed this route and have contributed to millions of malaria-attributable mortalities in African children. This emphasizes the urgent need for a new generation of either prophylactic and/or curative antimalarial drugs.

The emergence and geographic spread of artemisinin resistant *P. falciparum* in the Greater Mekong sub-region (GMS) signifies a severe threat to global malaria control and goals to eliminate the disease. Artemisinin resistance results in reduced ring stage exposure and manifests early as slow parasite clearance which has been attributed to both components of the ACT regimen, and ultimately leads to substantial reductions in cure rates. ACTs containing mefloquine and piperaquine are now failing across increasing areas of the GMS (Imwong *et al.*, 2017). Artemisinin resistance has also been linked with resistance to ACT partner drugs, resulting in high late treatment failure rates with dihydroartemisinin–piperaquine in Cambodia (Menard & Dondorp, 2017).

The artemisinins form one of seven main classes of antimalarial drugs and most of them have shown decline in therapeutic effects in drug-resistant parasites. Thus, new antimalarial drugs are urgently needed. An established strategy in the discovery of new therapeutic agents is molecular hybridisation. This entails the chemical-binding of two different biologically active moieties (pharmacophores) into a single new molecular entity which possesses a dual mode of action (Guantai *et al.*, 2010). With the two components working synergistically, this strategy may possibly result in a new treatment for malaria. Cost-effectiveness and decreased probability of drug-drug interactions are only a few of the important advantages of a hybrid drug over a multicomponent combination drug (Muregi & Ishih, 2010a). The down-side of a hybrid molecule is the possibility of transferring negative traits of one or both of the components into the target drug (Guantai *et al.*, 2010).

Naphthoquinones have broad-spectrum antiprotozoal activities by generating reactive oxygen species (ROS) that lead to oxidative stress and subseq.uently to parasite death (Guimarães *et al.*, 2013). Hydroxy-naphthoquinone is a structural analogue of protozoan ubiquinone, a protein found in the mitochondria and plays a role in electron transport (Baggish & Hill, 2002b). Atovaquone (Figure 1-2) is a well-known naphthoquinone derivative and is currently used in combination with proguanil for malaria prophylaxis (Dinter *et al.*, 2011). Atovaquone is very lipophilic (log*P*: 5.07) and poorly water soluble (0.43 mg/ml). Despite being an acid, a high pK_a causes it to essentially be neutral at normal physiological pH values. However, the drug shows

an increase as much as double the bioavailability when taken with fatty food (Baggish & Hill, 2002b) The absolute bioavailability of atovaquone tablets has been determined to be roughly 10% in the fasted state and 30% in the fed state (Dressman & Reppas, 2000).

Improving the solubility of poly-substituted 1,4-naphthoquinone derivatives can be achieved by introducing nitrogen in two different positions of the naphthoquinone pharmacophore (Lanfranchi *et al.*, 2012). In addition, complexation with cyclodextrin, a highly amphiphilic molecule, is also an effective method to enhance solubility (Shin *et al.*, 2012a). Atovaquone shows extremely high levels of plasma protein binding (approximately 99.5 %) but does not show displacement of other protein bound drugs (Baggish & Hill, 2002b; Nixon *et al.*, 2013).

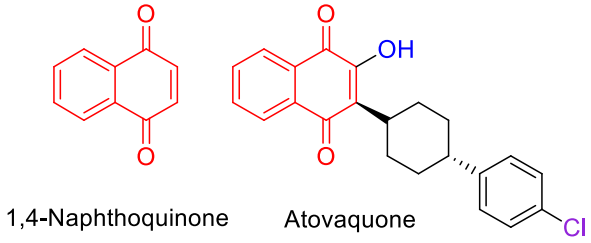


Figure 1-2: Structures of 1,4-naphthoquinone and atovaquone

Triazoles are another class of compounds that exhibit a variety of uses (Manohar *et al.*, 2011), including pharmaceutical agents, agrochemicals, industrial applications such as dyes, corrosion inhibition, photo stabilisers, and photographic materials (Sharghi *et al.*, 2009). A 1,2,3-Triazole (Figure 1-3) core is stable against acidic and basic hydrolysis as well as against oxidative and reductive conditions. It also has excellent water solubility (Log P: -0.27) (Lauria *et al.*, 2014b) and could possibly improve the solubility of naphthoquinones like atovaquone when both pharmacophores are chemically coupled.

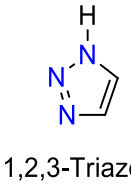


Figure 1-3: Structure of 1,2,3-triazole

In this study, **the** 1,4-naphtoquinone scaffold will be linked to the 1,2,3-triazole moiety to produce hybrid molecules. Naphthoquinone-triazole hybrids containing selenium have been synthesised following click methodology and were evaluated against six types of cancer cell lines. The naphthoquinone-triazole hybrids (Figure 1-4) were considered moderately anticancer active (da Cruz *et al.*, 2016) but were not tested against *Plasmodia*.

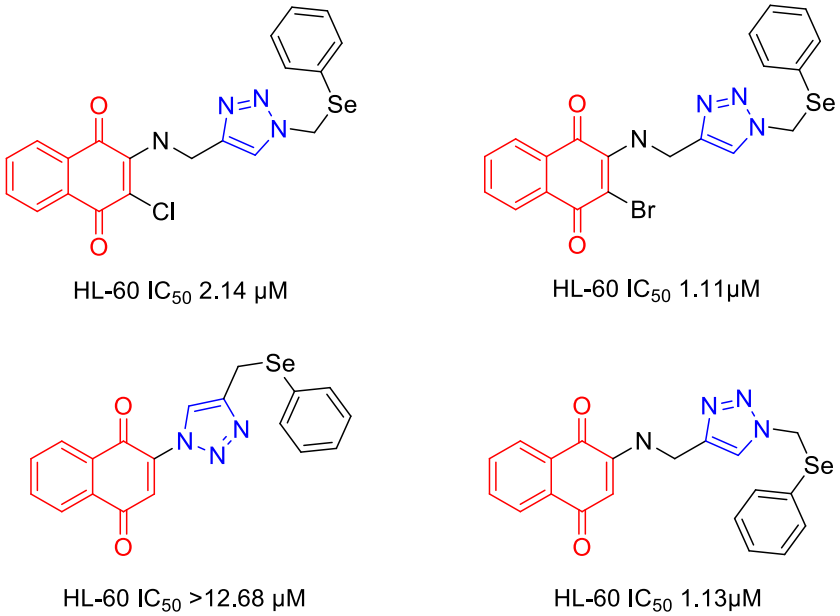


Figure 1-4: Selenium-containing naphthoquinone-based 1, 2, 3-triazoles tested against human promyelocytic leukemia (HL-60) cancer cells

In another study, a series of 2-bromo-1,4-naphthoquinone and 1,2,3-triazole hybrid compounds (Figure 1-5) have been synthesised and evaluated against the infective form of *Trypanosoma cruzi*, the etiological agent of Chagas disease. Two of these compounds showed a selectivity index worthy of further studies (da Silva *et al.*, 2012). Again, none of these compounds were screened for antimalarial activity. These potential anticancer and antiprotozoal properties, therefore, indicate that the molecular hybridisation of 1,4-naphthoquinones and 1,2,3-triazoles may produce significant antimalarial compounds.

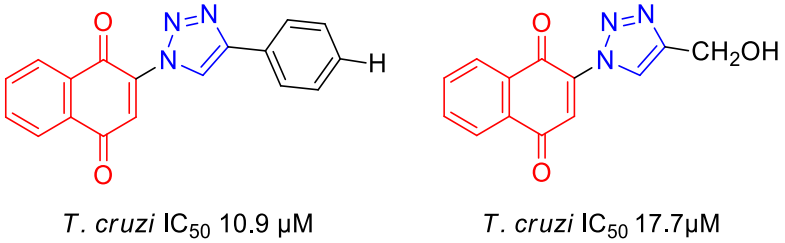


Figure 1-5: 1,4-Naphthoquinone-1,2,3-triazole hybrid compounds tested against infective form of Trypanosoma cruzi

1.2 Aim

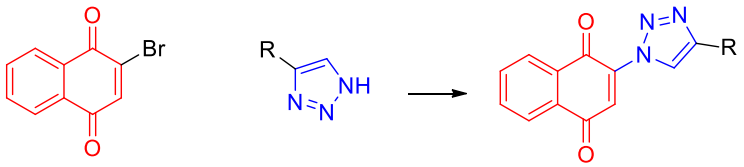
The aim of this study is to investigate naphthoquinone-1,2,3-triazole derivatives obtained through hybridisation of both pharmacophores in employing click chemistry, with the ultimate goal to produce a new antimalarial drug with improved efficacy and less cytotoxicity.

1.3 Objectives

The objectives of this study are:

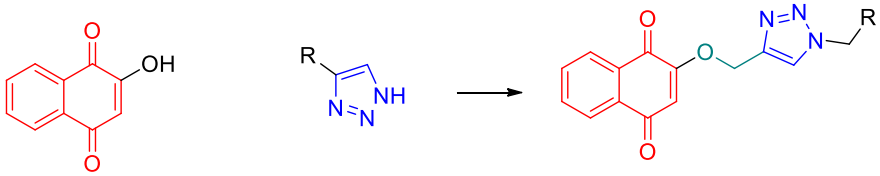
- To synthesise and characterise two series of novel naphthoquinone-triazole hybrids.

Series 1. Naphthoquinone directly linked triazole hybrids



2-Bromo-1,4-naphthoquinone 1,2,3-Triazole Hybrids

Series 2: Naphthoquinone oxymethylene chain linked triazole hybrids



2-Hydroxy-1,4-naphthoquinone 1,2,3-Triazole Hybrids

Where R is aryl

Figure 1-6: Schematic representation of the synthetic routes for the target naphthoquinone-triazole hybrids

- To assess *in vitro* antimalarial activity of the synthesised compounds against chloroquine-resistant (CQR) and chloroquine-sensitive (CQS) strains of *P. falciparum*.
- To assess the *in vitro* cytotoxicity of compounds using mammalian cell lines.

BIBLIOGRAPHY

Allison, A.C. 2009. Genetic control of resistance to human malaria. *Current Opinion in Immunology*, 21:499-505.

Baggish, A.L. & Hill, D.R. 2002. Antiparasitic agent atovaquone. *Antimicrobial Agents and Chemotherapy*, 46:1163-1173.

Birkett, A.J. 2016. Status of vaccine research and development of vaccines for malaria. *Vaccine*, 34:2915-2920.

Blasco, B., Leroy, D. & Fidock, D.A. 2017. Antimalarial drug resistance: linking Plasmodium falciparum parasite biology to the clinic. *Nature Medicine*, 23:917.

da Cruz, E.H.G., Silvers, M.A., Jardim, G.A.M., Resende, J.M., Cavalcanti, B.C., Bomfim, I.S.*, et al.* 2016. Synthesis and antitumor activity of selenium-containing quinone-based triazoles possessing two redox centres, and their mechanistic insights. *European Journal of Medicinal Chemistry*, 122:1-16.

da Silva, E.N., de Melo, I.M.M., Diogo, E.B.T., Costa, V.A., de Souza Filho, J.D., Valenca, W.O.*, et al.* 2012. On the search for potential anti-Trypanosoma cruzi drugs: Synthesis and biological evaluation of 2-hydroxy-3-methylamino and 1, 2, 3-triazolic naphthoquinoidal compounds obtained by click chemistry reactions. *European Journal of Medicinal Chemistry*, 52:304-312.

Dinter, D., and, G.G. & Garaj-Vrhovacb, V. 2011. An alkaline comet assay study on the antimalarial drug atovaquone in human peripheral blood lymphocytes: a study based on clinically relevant concentrations. *Journal of Applied Toxicology*, 33:56-62.

Dispatch, U. 2014. World Malaria Map. *https://www.undispatch.com/map-day-good-news-malaria/*, Date of access: 15 June 2018.

Dressman, J.B. & Reppas, C. 2000. In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs. *European Journal of Pharmaceutical Sciences*, 11:S73-S80.

Gilson, R.C., Deissler, R.J., Bihary, R.F., Condit, W.C., Thompson, M.E., Blankenship, D.A.*, et al.* 2018. Growth of Plasmodium falciparum in response to a rotating magnetic field. *Malaria Journal*, 17:190.

Greenwood, B., Dicko, A., Sagara, I., Zongo, I., Tinto, H., Cairns, M.*, et al.* 2017. Seasonal vaccination against malaria: a potential use for an imperfect malaria vaccine. *Malaria Journal*, 16:182.

Guantai, E.M., Ncokazi, K., Egan, T.J., Gut, J., Rosenthal, P.J., Smith, P.J.*, et al.* 2010. Design, synthesis and in vitro antimalarial evaluation of triazole-linked chalcone and dienone hybrid compounds. *Bioorganic & Medicinal Chemistry*, 18:8243-8256.

Guimarães, T.T., Pinto, M.d.C.F.R., Lanza, J.S., Melo, M.N., do Monte-Neto, R.L., de Melo, I.M.M.*, et al.* 2013. Potent naphthoquinones against antimony-sensitive and -resistant Leishmania parasites: Synthesis of novel a- and nor-a-lapachonebased 1,2,3-triazoles by copper-catalyzed azide-alkyne cycloaddition. *European Journal of Medicinal Chemistry*, 63:523-530.

Imwong, M., Suwannasin, K., Kunasol, C., Sutawong, K., Mayxay, M., Rekol, H.*, et al.* 2017. The spread of artemisinin-resistant Plasmodium falciparum in the Greater Mekong subregion: a molecular epidemiology observational study. *The Lancet Infectious Diseases*, 17:491-497.

Jourdan, J., Schneider, J., Dassonville-Klimpt, A. & Sonnet, P. 2018. Recent advances in antimalarial drugs: structures, mechanisms of action and clinical trials. *(In* Méndez-Vilas, A., *ed.* Microbiology Book Series Formatex Research Center. p. 599-609.

Krungkrai, J. & Krungkrai, S.R. 2016. Antimalarial qinghaosu/artemisinin: The therapy worthy of a Nobel Prize. *Asian Pacific Journal of Tropical Biomedicine*, 6:371-375.

Lanfranchi, D.A., Cesar-Rodo, E., Bertrand, B., Huang, H., Day, L., Johann, L.*, et al.* 2012. Synthesis and biological evaluation of 1,4-naphthoquinones and quinoline-5,8-diones as antimalarial and schistosomicidal agents. *Organic & Biomolecular Chemistry*, 10:6375-6387.

Lauria, A., Delisi, R., Mingoia, F., Terenzi, A., Martorana, A., Barone, G.*, et al.* 2014. 1,2,3-Triazole in Heterocyclic Compounds, Endowed with Biological Activity, through 1,3-Dipolar Cycloadditions. *European Journal of Organic Chemistry*, 2014:3289-3306.

Manohar, S., Khan, S.I. & Rawat, D.S. 2011. Synthesis of 4-aminoquinoline-1,2,3-triazole and 4-aminoquinoline-1,2,3-triazole-1,3,5-triazin, Hybrids as Potential Antimalarial Agents. *Chemical Biology & Drug Design*, 78:124-136.

Mehrizi, A.A., Rezvani, N., Zakeri, S., Gholami, A. & Babaeekhou, L. 2018. Poly (I: C) adjuvant strongly enhances parasite-inhibitory antibodies and Th1 response against Plasmodium falciparum merozoite surface protein-1 (42-kDa fragment) in BALB/c mice. *Medical Microbiology and Immunology*, 207:151-166.

Menard, D. & Dondorp, A. 2017a. Antimalarial Drug Resistance: A Threat to Malaria Elimination. *Cold Spring Harbor Perspectives in Medicine*, 7:p.a.025619.

Menard, D. & Dondorp, A. 2017b. Antimalarial drug resistance: a threat to malaria elimination. *Cold Spring Harbor Perspectives in Medicine*, 7:a025619.

Muregi, F.W. & Ishih, A. 2010. Next-Generation Antimalarial Drugs: Hybrid Molecules as a New Strategy in Drug Design. *Drug development research*, 71:20-32.

Nixon, G.L., Moss, D.M., Shone, A.E., Lalloo, D.G., Fisher, N., O'Neill, P.M.*, et al.* 2013. Antimalarial pharmacology and therapeutics of atovaquone. *Journal of Antimicrobial Chemotherapy*, 68:977-985.

Ouattara, A., Barry, A.E., Dutta, S., Remarque, E.J., Beeson, J.G. & Plowe, C.V. 2015. Designing malaria vaccines to circumvent antigen variability. *Vaccine*, 33:7506-7512.

Sharghi, H., Khalifeh, R. & Doroodmand, M.M. 2009. Copper Nanoparticles on Charcoal for Multicomponent Catalytic Synthesis of 1,2,3-Triazole Derivatives from Benzyl Halides or Alkyl Halides, Terminal Alkynes and Sodium Azide in Water as a “Green” Solvent. *Advanced Synthesis & Catalysis*, 351:207-218.

Shin, J.-A., Lim, Y.-G. & Lee, K.-H. 2012. Copper-Catalyzed Azide−Alkyne Cycloaddition Reaction in Water Using Cyclodextrin as a Phase Transfer Catalyst. *The Journal of Organic Chemistry*, 77:4117-4122.

Singh, B. & Daneshvar, C. 2013. Human infections and detection of Plasmodium knowlesi. *Clinical Microbiology Reviews*, 26:165-184.

Tatem, A.J., Jia, P., Ordanovich, D., Falkner, M., Huang, Z., Howes, R.*, et al.* 2017. The geography of imported malaria to non-endemic countries: a meta-analysis of nationally reported statistics. *The Lancet Infectious Diseases*, 17:98-107.

Uddin, T. 2017. Drug targets in the apicoplast of malaria parasites*, Doctoral Dissertation.*

WHO (World Health Orginization). 2016. World Malaria Report 2015. *http://www.who.int/malaria/publications/world_malaria_report/en/*, Date of access: 13 February 2017.

WHO. 2017. World Malaria Report 2016. *http://www.who.int/malaria/publications/world-malaria-report-2017/en/*, Date of access: 25 January 2018.

Zhang, R.-W. 2016. Artemisinin (Qinghaosu), Nobel Prize, anti-malaria, and beyond. *Chinese Journal of Natural Medicines*, 14:1.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Despite ongoing efforts to advance existing control strategies and the development of vaccines, malaria continues to be a heavy burden of illness worldwide (Thakur *et al.*, 2018b). Malaria is a protozoan disease that introduces *Plasmodium* sporozoites into the mammalian host through the bite of an infected female *Anopheles* mosquito (Ocaña-Morgner *et al.*, 2003). The WHO identified malaria as the second leading cause of death in the tropical and subtropical regions of the world in 2016, with HIV/AIDS being the biggest killing infectious disease and TB being third (Gilson *et al.*, 2018a; WHO, 2017a). Africa carries 90 % of the annual malaria victims, 74 % of which are children under five years of age (Dieye *et al.*, 2016).

The significant expansion in the reach of human travel across the world, specifically air travel in the past few decades, has a severe effect on the epidemiology of malaria. The total funding for malaria control and elimination reached an estimated US$ 2.7 billion in 2016. Donations from governments of endemic countries amounted to $ 800 million, representing 31 % of funding (WHO, 2016a). Malaria numbers are declining and this is expected to continue with the help of the WHO Global Technical Strategy for malaria 2016-2030 that targets to decrease its global incidence and mortality by at least 90 % by 2030 (Dieye *et al.*, 2016). Key interventions recommended by the WHO for the control of malaria are the use of insecticide treated nets (ITNs) and/or indoor residual spraying (IRS) for vector control, early access to diagnostic testing of suspected malaria and the treatment of confirmed cases (WHO, 2012).

In this chapter, an overview is given on the different *Plasmodium* species and the life-cycle of malaria. The symptoms, accompanying complications and the different methods of diagnosis will be discussed. Control, preventative methods and current treatment options will also be discussed in detail. Natural immunity and the effect of biochemical defects on malaria protection will also be briefly summarised. Molecular hybridisation will be discussed, with particular focus on naphthoquinone and triazole hybrids, to set the tone as a possible solution to the dire need for safe and effective malaria treatment without the burden of resistance.

2.2 Plasmodium Species

The genus *Plasmodium* is comprised of more than a 170 different species that infect mammals, reptiles, birds, and amphibians. Five of these species, *P. falciparum*, *P. vivax*, *P. malariae, P. ovale*, and *P. knowlesi,* can infect humans (Singh & Daneshvar, 2013). *P. falciparum* infection is predominant in sub-Saharan Africa, Southeast Asia and some parts of the Caribbean, especially Haiti and the Dominican Republic. *P. falciparum* and *P. knowlesi* infections have also been

reported in Borneo and Southeast Asia (Cannella & Archibald, 2017). *P. knowlesi* can be found throughout Southeast Asia as a natural pathogen of long-tailed and pig-tailed macaques, but has recently been shown to be a noteworthy cause of zoonotic malaria in that region, mostly in Malaysia. *P. knowlesi* has a 24 hour replication cycle and can quickly progress from an uncomplicated to a severe infection with reported lethal cases (CDC, 2018). *P. vivax* is found commonly in Asia, Latin America, and in some parts of Africa, because of the high population densities in these countries, particularly in Asia.

P. vivax req.uires binding to the Duffy glycoprotein in order to enter erythrocytes and individuals that do not express the Duffy protein are immune to *P. vivax* infection (Dean, 2005). The prevalence and effects of Duffy blood groups are discussed in paragraph 2.7. *P. ovale* is typically found in Africa, predominantly West Africa, and the islands of the western Pacific. It is biologically and morphologically very similar to *P. vivax*, with the exception that it can infect individuals who are negative for the Duffy blood group, specifically in sub-Saharan Africa where for many residents, the Duffy blood group is a common occurrence (CDC, 2018).

P. malariae, found globally, is the only human malaria parasite species that has a quartan cycle (three-day life cycle). If untreated, *P. malariae* causes a chronic infection that in some cases can lead to severe complications such as nephrotic syndrome (CDC, 2018).

2.3 Life cycle

The incubation period for malaria is usually between 7 - 30 days depending on the specific *Plasmodium* species involved. Shorter periods are most freq.uently observed with *P. falciparum* with an average of 12 days and the longer periods with *P. vivax* stretching between 13 - 17 days (CDC, 2016). Outbreaks differ between the species with 24 hours for *P. knowlesi,* 48 hours for *P. falciparum*, *P. vivax*, and *P. ovale,* and 72 hours for *P. malariae* (Cannella & Archibald, 2017). The malaria life cycle (Figure 2-1) involves two hosts, humans and female *Anopheles* mosquitoes. At the time of a blood meal an infected mosquito injects *Plasmodia* sporozoites into the dermis of the human's skin. The sporozoites then enter the bloodstream and reach the liver cells within minutes, where they multiply and get released as merozoites after one to two weeks to infect erythrocytes. In humans, a malaria infection can be established by as few as ten sporozoites (Ocaña-Morgner *et al.*, 2003).

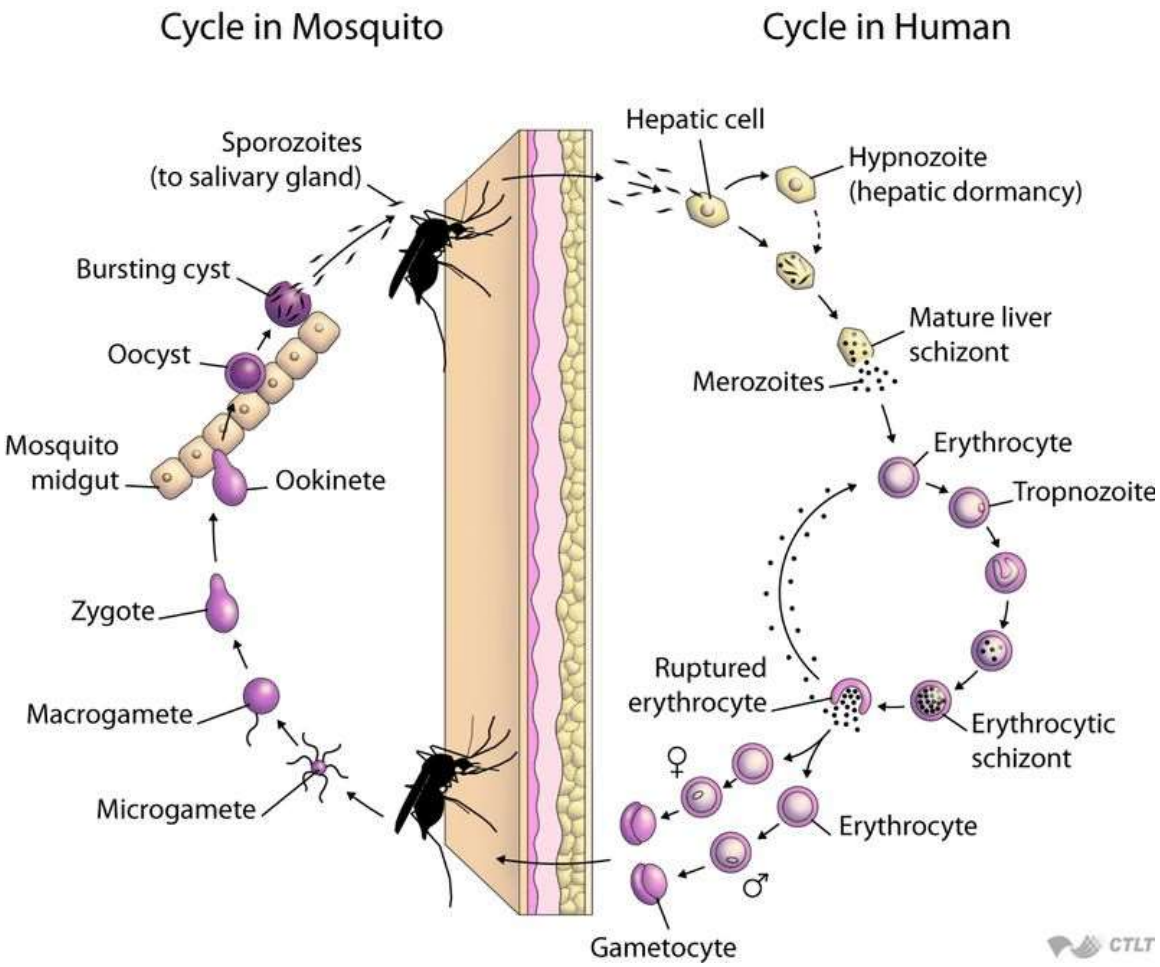


Figure 2-1: The malaria life cycle (Malwest, 2016).

During the liver-stage of malaria, also known as the asymptomatic period, or exo-erythrocytic stage of infection (Akhtar *et al.*, 2016), sporozoites infect liver cells (hepatocytes) and mature into schizonts (the multi-nucleate stage of the cell during asexual reproduction), each containing 10 000 - 30 000 merozoites (SchliTZer, 2008b). Upon maturity, these schizonts cause the infected hepatocyte to rupture, releasing the merozoites into the bloodstream that, in turn, infect erythrocytes (Cannella & Archibald, 2017). This marks the beginning of the asexual blood-stage of the disease with merozoites maturing in the erythrocytes (erythrocytic schizogony), translating into the symptomatic manifestation of malaria. After the initial exo-erythrocytic stage, *P. falciparum* and *P. malariae* are no longer seen in the liver. On the other hand, with *P. vivax* and *P. ovale* strains, some sporozoites turn into hypnozoites. These latent forms remain in the liver and are responsible for relapses months or even years after the primary infection (Jourdan *et al.*, 2018c).

During the blood-stage, two possible developmental routes exist for merozoites. Firstly, the ring stage trophozoites mature into schizonts that rupture, releasing new merozoites that can continue multiplying asexually (CDC, 2016; MMV, 2017). Secondly, merozoites can differentiate into

gametocytes, i.e., male (microgametocytes) and female (macrogametocytes). The reason for this differentiation is unknown, but these gametocytes are the link that causes the cycle of transmission to continue back to the mosquito. Male and female gametocytes fuse within the mosquito, forming diploid zygotes which in turn, become ookinetes that invade the midgut wall of the mosquito where they develop into oocysts. Meiotic division of the oocysts occur until maximum capacity then burst to release sporozoites that move to the mosquito's salivary glands from where the malaria transmission cycle can continue (CDC, 2016; MMV, 2017).

2.4 Symptoms and Complications

The primary symptoms of malaria (Figure 2-2) are flu-like and include high, spiking fevers (with or without periodicity), chills, headaches, myalgia, malaise, and gastro-intestinal symptoms. Severe headaches are a distinctive early symptom of malaria caused by all *Plasmodium* spp., usually preceding the fever and chills (VineTZ *et al.*, 2011). Acute symptoms due to *P. vivax* infection may appear severe due to high fever and prostration. Undeniably, the pyrogenic threshold of this parasite is lower than that of *P. falciparum*. Most severe forms and deaths from malaria are caused by *P. falciparum*, whereas *P. vivax* and *P. ovale* rarely produce serious complications, debilitating relapses, or even death.

Figure 2-2: Symptoms of malaria

The most common complications of severe malaria include cerebral malaria, pulmonary oedema, acute renal failure, severe anaemia, and/or bleeding. Acidosis and hypoglycaemia are the most common metabolic complications (Trampuz *et al.*, 2003).

Human cerebral malaria (HCM) is a severe form of malaria characterised by seq.uestration of infected erythrocytes in brain micro-vessels, increased levels of circulating free haem, pro-inflammatory cytokines and chemokines, brain swelling, vascular dysfunction and coma. HCM has a mortality rate of 15 – 20 % with treatment, it can rise above 30 % with multiple vital organ failure if untreated (Dondorp *et al.*, 2005).

Diminished cerebral-vascular integrity caused by leaks in the blood brain barrier (BBB) allows increased seeping of toxins into the brain parenchyma leading to the exacerbation of neurological function loss (Liu *et al.*, 2018). Cerebral malaria causes a fast developing coma and remains a foremost contributor to the morbidity and mortality rate of 15 – 20 % (this is defined as a Blantyre Coma Score (BCS) of two or less) (Thakur *et al.*, 2018b). Retinal vessel deviations and whitening (Figure 2-3) are common signs of malarial retinopathy which can be directly observed during routine eye examination in children with *P. falciparum* cerebral malaria (Barrera *et al.*, 2018). These signs have been found to be over 95 % sensitive and specific for pre-morbid identification (Thakur *et al.*, 2018b).

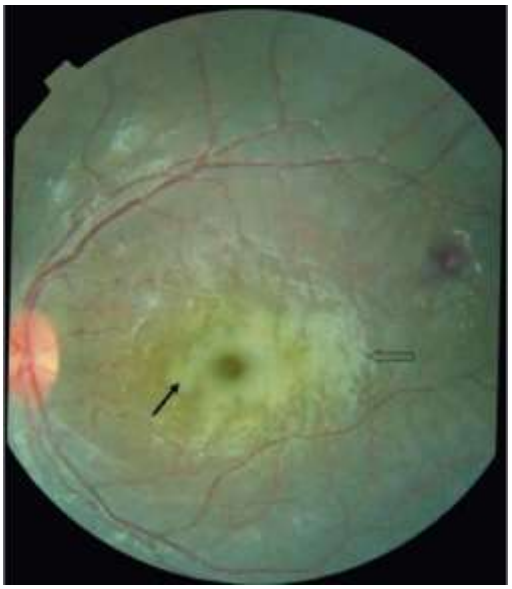


Figure 2-3: Severe macular whitening (solid arrow) surrounding the foveola of a Malawian child with cerebral malaria. The open arrow indicates glare (Beare *et al.*, 2006).

Individuals living in regions of high and stable transmission gradually acquire immunity after experiencing and surviving numerous infections (Dieye *et al.*, 2016). A long-term and usually unquantified consequence of malaria infection is the effect on cognitive function, schooling and social capital development (Chen *et al.*, 2016). Immune responses to *P. falciparum* infection are facilitated by the production of pro-inflammatory cytokines, chemokines and growth factors whose actions are critical for the control of the parasites. Following this response, the induction of anti-inflammatory immune mediators down-regulates the inflammation, thus preventing its adverse

effects such as damage to several organs and death (Dieye *et al.*, 2016). Disruptions in this balance of pro- and anti-inflammatory activities can be detrimental, as malaria can cause kidney or liver failure and spleen rupture. Unfortunately, such disruption may arise from the WHO's strategy of decreasing malaria prevalence in endemic areas. This will reduce the sustained transmission of malaria in the population, resulting in a decrease in immunity and an increase in individuals susceptible to severe cases. Aberrant immune response to repeated or chronic *P. falciparum* malarial infection may also serve as a disruption, resulting in tropical splenomegaly syndrome, a proportion of which show clonal proliferation of B lymphocytes (Ghosh & Ghosh, 2007).

Anaemia is also a common complication in malarial infections and a leading cause of death in children and pregnant women, the consequences being more pronounced with *P. falciparum* malaria (Ghosh & Ghosh, 2007; Skorokhod *et al.*, 2010). In severe malaria, anaemia is characterised by changed erythropoiesis and the presence of hemozoin-(HZ)-laden bone-marrow macrophages.

Important considerations for the selection of treatment for each individual include: (i) co-morbidities with other parasitic infestations; (ii) iron, folate, Vitamin B12, and other nutrient deficiencies; (iii) anaemia, which is aggravated by antimalarial drugs both through immune and non-immune mechanisms. In different endemic areas factors such as β-thalassemia, α-thalassemia, Haemoglobin (Hb) S, Hb E, Glucose-6-phosphate dehydrogenase deficiency (G6PD), or ovalocytosis can interact with malaria infection. This adds to special considerations when treating patients with malaria in areas where these conditions occurs (Ghosh & Ghosh, 2007).

2.5 Diagnosis

According to the WHO a patient must comply with at least one of the following criteria to be diagnosed with a *P. falciparum* infection (WHO, 2015):

- Plasma base excess less than –3.3 mmol/L
- Glasgow coma scale less than 11 of 15, or Blantyre coma scale less than 3 of 5 in preverbal children
- Haemoglobin less than 50 g/L and parasitaemia greater than 100 000 parasites per µL
- Blood urea greater than 10 mmol/L
- Compensated shock (capillary refill ≥3 s or temperature gradient on legs, but no hypotension)
- Decompensated shock; systolic blood pressure less than 70 mm Hg and cool peripheries

- Asexual parasitaemia more than 10%
- Visible jaundice and more than 100 000 parasites per µL
- Plasma glucose less than 3 mmol/L
- Respiratory distress, defined as costal in drawing, use of accessory muscles, nasal alar flaring, deep breathing, or severe tachypnoea

The identification of blood parasites in blood samples is the most definite means of diagnosis. However, the traditional visual identification, based on the examination of Giemsa-stained thick and thin blood smears under a microscope, has been regarded as an unsuccessful method of diagnosis since the 1990s due to insufficient microscopes and/or trained personnel to read and interpret the slides (Makler *et al.*, 1998). Even though several new diagnostic tests have been developed in an attempt to improve the diagnosis of malaria, microscopy has remained the gold standard against all other tests used.

Other tests include quantitative buffy coat, rapid diagnostic test, genetic probes and Polymerase Chain Reaction (PCR) (Vishruti Gandhi *et al.*, 2017). Patients who are suspected of having malaria should have a combination of parasitological tests done, e.g. PCR or a Rapid Diagnostic Test (RDT) plus a microscopic smear test to confirm diagnosis.

PCR is a pleasant addition to microscopy for validated identification of *Plasmodium* spp. in clinical specimens. Many PCR assays have been developed for the laboratory diagnosis of malaria, including conventional and real-time PCR techniques, that allow for the identification of all four species of *Plasmodium* infecting humans (Johnston *et al.*, 2006). RDT is a visual, rapid immunoassay for the qualitative differential detection of *P*. *falciparum* and *P*. *vivax* malarial antigens of human blood, based on sandwich immunoassay principle (WHO, 2015). Sensitivity and specificity of RDT for *P. falciparum* is 96.2 % and 90 %, respectively (Vishruti Gandhi *et al.*, 2017).

Loop-mediated isothermal amplification-(LAMP)-based methods have recently been introduced as an alternative procedure for the sensitive detection of malaria parasites in human blood samples (Frickmann *et al.*, 2018). LAMP has been regarded as an innovative gene amplification technology and emerged as an alternative to PCR-based methodologies in both clinical laboratory and food safety testing. The high sensitivity of LAMP allows detection of the pathogens in sample materials even without time consuming sample preparation (Li *et al.*, 2017).

2.6 Control and prevention

The Global Technical Strategy for Malaria sets ambitious but attainable goals for 2030, with milestones along the way to track progress. The milestones for 2020 include:

- Reducing malaria case incidence by at least 40 %;
- Reducing malaria mortality rates by at least 40 %;
- Eliminating malaria in at least ten countries;
- Preventing a resurgence of malaria in all countries that are malaria-free (WHO, 2016a).

Figure 2-4: Prevention of malaria (Primus, 2016)

Four randomised trials in Africa, targeting children under five years of age and introducing the use of insecticide treated nets (ITNs) or insecticide-treated curtains (Figure 2-4) found a decline in deaths among the children. However, most of the observers argue that neither a government nor an individual can be expected to foot the entire bill for an ITN programme, which include the buying of nets, distribution, education, and regular re-impregnation. At present ITN's and spraying cannot be exclusively relied upon for protection due to a rise in resistance against insecticides (Jourdan *et al.*, 2018c). With parasite resistance growing rapidly, there is a crucial need for new antimalarial drugs with chemo-types that are safe and effective against multiple stages of highly resistant parasites (Neelarapu *et al.*, 2018).

The Kilombero Valley Insecticide-Treated Net (KINET) project in the Ifakara district of Tanzania endorses the use of ITNs through social marketing, a partnership between the public sector and the private sector in a rural population of nearly half a million people. The project has already shown a 60% decline in the frequency of infection and of anaemia in children under two years of age with a six-fold increase in ownership of ITNs (Mathanga & Molyneux, 2016).

The development of a malaria vaccine that can prevent infection will diminish malaria morbidity and mortality and accelerate malaria eradication efforts. However, parasite genetic diversity poses a major difficulty to malaria vaccine efficacy. In recent pre-clinical and field trials, vaccines based on polymorphic *P. falciparum* antigens have shown efficacy only against homologous strains, raising the spectre of allele-specific immunity such as plaguing vaccines against influenza

and HIV. The most advanced malaria vaccine, RTS,S/AS01, targets *P*. *falciparum* circum-sporozoite protein and moderately reduces the risk of clinical malaria (efficacy 40 – 70 % in different populations) but did not completely prevent infection in field trials (Ouattara *et al.*, 2015).

Substantial progress has been made in the development of malaria vaccines during the past decade and RTS,S/AS01, has received a positive endorsement from the European Medicines Authority and will soon be deployed in large-scale, pilot implementation projects in sub-Saharan Africa. However, it only provides a fairly short period of high-level protection and flawed immunological memory. A probable reason for the latter is linked to the difficulty to develop natural effective immunological memory to malaria antigens in subjects exposed previously to the infection (Greenwood *et al.*, 2017).

A second vaccine, the irradiated sporozoites vaccine (*Pf*SPZ), is near to critical phase 3 trials. Various other pre-erythrocytic and blood stage vaccines have shown efficacy in trial experiments in volunteers and in endemic populations, but general efficacy remains limited (Greenwood *et al.*, 2017).

2.7 Biochemical defects and malaria protection

The Duffy glycoprotein is a receptor for substances that are released by erythrocytes during inflammation. It was named after a patient with haemophilia who had received several blood transfusions and was the first known producer of anti-Fya. The Duffy glycoprotein is a transmembrane protein that spans the erythrocyte membrane seven times and has an extracellular N-terminal field and a cytoplasmic C-terminal field. The frequency of the Duffy phenotypes varies in different populations, occurring in over two-thirds of the Black population (Dean, 2005).

Duffy antigens also happen to be a receptor for *P*. *vivax* and to cause disease *P. vivax* must first enter human erythrocytes by binding to the N-terminal extracellular domain of the Duffy glycoprotein through the cysteine-rich region of the Duffy binding protein (DBP). Individuals with the Duffy null phenotype do not express the Duffy protein on their erythrocytes and consequently are immune to *P. vivax* infection (Dean, 2005).

The parasite binds on the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) to erythrocytes surfaces through the common Duffy binding-like area. Binding to the erythrocytes of non-O blood groups is statistically significantly amplified when compared with those of group O(H). This binding even compares with variants of ABO glycosyl-transferase genes and that the soluble form of these enzymes themselves are engaged in the binding process (Arend, 2018). This defends against severe, life threatening cases of malaria but does not confer a sterile protection (Dieye *et al.*,

2016). Individuals with blood group O(H) in contrast to blood group A individuals not only have a considerably higher risk of developing certain types of cancer but also tend to show high predisposition to malaria *tropica* or infection by *P*. *falciparum.* (Arend, 2018).

Typically, erythrocytes are flexible and round, moving easily through the blood vessels. In sickle cell anaemia, the erythrocytes become rigid and adhesive (Figure 2-5). They are shaped like sickles or crescent moons, these abnormal shaped cells accumulate in the small blood vessels and thus cause constricted blood flow and oxygen to the body (Mayo, 2018). The high frequency of the sickle-cell haemoglobin (HbS) gene in malaria endemic regions is believed to be due to a heterozygote (HbAS) benefit against fatal malaria (Aidoo *et al.*, 2002).

Sickle-cell disease is one of the most common severe monogenic disorders in the world. Haemoglobin polymerisation, leading to erythrocyte rigidity and vaso-occlusion, is central to the pathophysiology of this disease, although the importance of chronic anaemia, haemolysis, and vasculopathy has been established. HbS is caused by a mutation in the β-globin gene in which the 17th nucleotide is changed from thymine to adenine and the sixth amino acid in the β-globin chain becomes valine instead of glutamic acid. This mutation produces a hydrophobic motif in the deoxygenated HbS tetramer that results in binding between β1 and β2 chains of two haemoglobin molecules. The mechanism of sickle cells promoting protection against malaria is yet to be fully understood (Rees *et al.*, 2010).

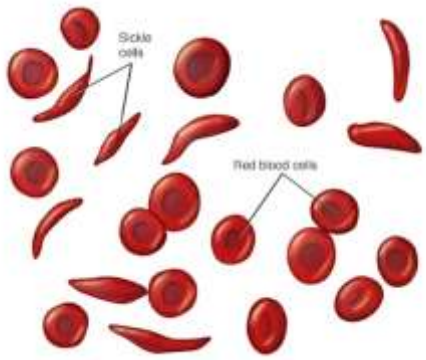


Figure 2-5: Normal erythrocytes and sickle cell erythrocytes (Mayo, 2018)

The human Pyruvate kinase (PK) gene PK-LR encodes for erythrocyte PK, which catalyses the conversion of phosphor-enolate to pyruvate. This is an important step in glycolysis and the manufacturing of ATP in red cells. Studies in mice show that mice lacking in PK are protected against malaria and in *P. falciparum* cultures of human erythrocytes deficient in PK growth is delayed (Allison, 2009).

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked, hereditary genetic defect caused by mutations in the *G6PD* gene, resulting in protein substitutes with different levels of enzyme activity. The remarkable resemblance between the areas where G6PD deficiency is common and *P. falciparum* malaria is endemic provides contingent evidence that G6PD deficiency confers resistance against malaria. Further discussed in paragraph 2.9.3 is the development of haemolytic anaemia, where individuals presented with very low levels of G6PD activity in their erythrocytes, caused by the antimalarial drug primaquine (Cappellini & Fiorelli, 2008).

2.8 Natural Immunity

Naturally acquired immunity (NAI) to *P. falciparum* protects millions of people regularly exposed to infection from severe disease and death. NAI should be respected as being virtually 100% effective against severe disease and death among exposed adults (Doolan *et al.*, 2009)

Asymptomatic malaria is often characterised by the sub-microscopic presence of parasites in the blood of patients that present with insignificant or no symptoms, due to partial immunity. The disease is, therefore, believed to be under control, but with the survival of enduring parasites. An asymptomatic state like this has been regarded as a constructive type of infection by reducing the risk of severe disease (Chen *et al.*, 2016). As mentioned in paragraph 2.4, a balance of pro- and anti-inflammatory activities prevents the development of severe complications in those partially immune to the disease. However, current studies show the contrary. Low-level “asymptomatic” malaria can result in chronic, low-grade haemolysis as well as recurrent, higher density symptomatic relapses. Each repetitive episode of symptomatic malaria causes a further bout of haemolysis, with 8 % – 14 % loss of erythrocyte mass that may, therefore, lead to anaemia (Chen *et al.*, 2016).

2.9 Chemotherapy – current treatment options

There are seven main classes of antimalarial drugs: i) aryl-aminoalcohols, ii) 4-aminoquinolines, iii) 8-aminoquinolines, iv) artemisinins, v) antifolates, vi) antimicrobial and vii) inhibitors of the respiratory chain (Jourdan *et al.*, 2018c). Antimalarials are devided into classes according to their chemical structure and mode of action and the target within the human host (Arrow *et al.*, 2004).

2.9.1 Aryl-amino alcohols

Aryl-amino-alcohol derivatives (Figure 2-6) such as quinine, quinidine, mefloquine, halofantrine, lumefantrine, are some of the oldest compounds used against malaria. These drugs target the *Plasmodium* erythrocytic stage although their mechanisms of action are not identical. Aryl-amino-alcohols have been shown to interfere with haem digestion (SchliTZer, 2008b)

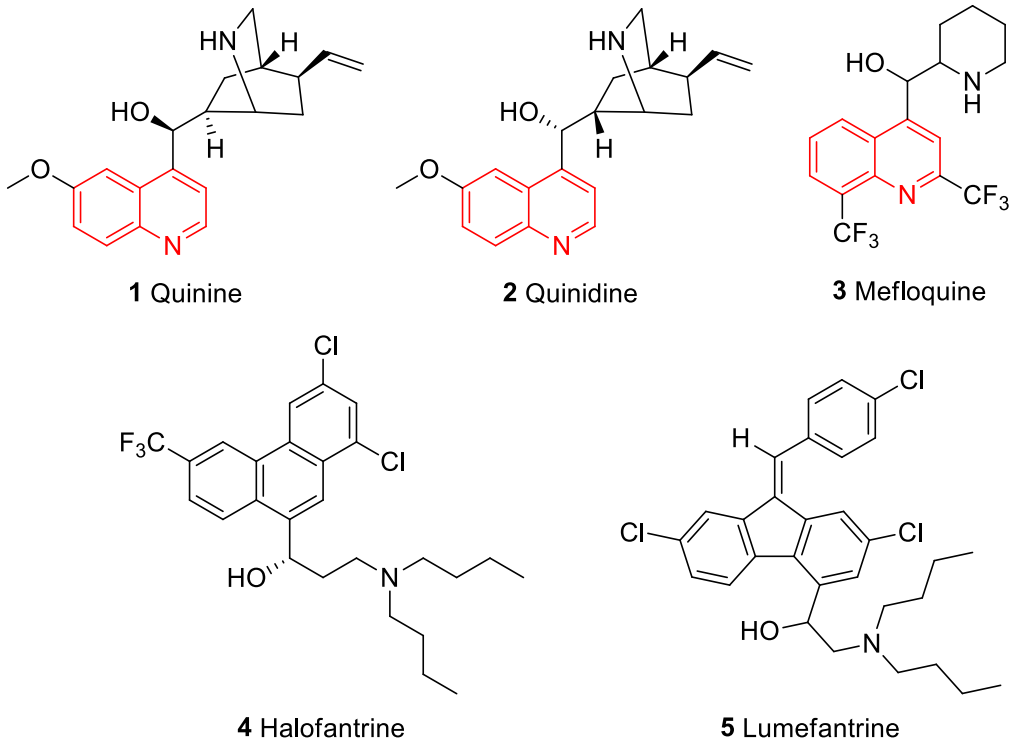


Figure 2-6: Arylaminoalcohol antimalarials

Quinine (**1**) has been the cornerstone for the treatment of severe malaria since the introduction of Cinchona Bark to European medicine in the 1630s (Dondorp *et al.*, 2005). Quinine, as an element of the bark of the cinchona tree, was used to treat malaria from as early as the 1600s, when it was referred to as the "Jesuits' bark," "cardinal's bark," or "sacred bark" (Achan *et al.*, 2011). Quinine (human $t_{1/2}$ = 10-12 hours) inhibits the formation of hemozoin in the parasite's digestive vacuole (DV). Many cases of quinine resistance have been reported due principally to mutations of genes encoding for transporter proteins such as *P. falciparum* chloroquine resistance transporter (*Pf*CRT) (Jourdan *et al.*, 2018c). It also has analgesic, but not antipyretic properties.

In the treatment of severe malaria, parenteral quinine is given either by intramuscular injection or as slow rate-controlled intravenous infusions with a dose every eight hours. Intramuscular administration is painful, and can cause sterile abscesses, sciatic nerve damage, and predispose the patient to lethal tetanus. Blindness and deafness can result after self-poisoning, but these adverse effects are rare in severe malaria, whereas quinine induced hyper-insulinemic hypoglycaemia is a common and serious complication (Dondorp *et al.*, 2005; Dondorp *et al.*, 2010). The side-effects commonly seen at therapeutic concentrations are referred to as cinchonism, with mild forms including tinnitus, slight impairment of hearing, headache and nausea. More severe manifestations include vertigo, vomiting, abdominal pain, diarrhoea, marked auditory loss, and visual symptoms, including loss of vision. Quick administration can cause

hypotension, and venous thrombosis may occur following intravenous injections (Achan *et al.*, 2011).

A variety of quinine preparations are available, including the hydrochloride, dihydrochloride, sulphate, bisulphate, and gluconate salts. Of these, the dihydrochloride form is the most regularly used. Quinine has rapid schizonticidal action against intra-erythrocytic malaria parasites. It is also gametocytocidal for *P*. *vivax* and *P*. *malariae*, but not for *P*. *falciparum*.

Cinchona alkaloids quinidine (**2**) and quinine are commonly used as antimalarials and able to suppress ionic conductance through K^+, Na^+ and Ca^{2+} channels in the membranes of a range of different cells. Blocking of the outward membrane repolarising K+ current by quinidine can produce early after depolarisations (EADs) and trigger rhythm in presence of low extracellular potassium thus causing cardiac complications and QT prolongation (Sharma *et al.*). Quinidine is the diastereomer of quinine and shows different antimalarial activity (Frosch *et al.*, 2007). The CDC recommends a loading dose of quinidine gluconate 10 mg/kg over one – two hours followed by a continuous infusion of 0.02 mg/kg/min (Wroblewski *et al.*, 2012). Intravenous quinidine doses for resistant malaria are two – three times higher than those used for arrhythmias (Wroblewski *et al.*, 2012).

Mefloquine (**3**) was developed in the 1970s to counter quinine and chloroquine resistance. Mefloquine is a blood schizonticide, killing the parasitic schizont during the blood stage, and is structurally related to quinine. It also impedes haem detoxification in the parasite. However, treatment failures started to occur within only six years of use in Cambodia and Thailand (Price *et al.*, 2004). It is absorbed with a half-life of one to four hours and has a peak-time concentration of 7 - 24 hours usually 16.7 hours (Karbwang & White, 1990).

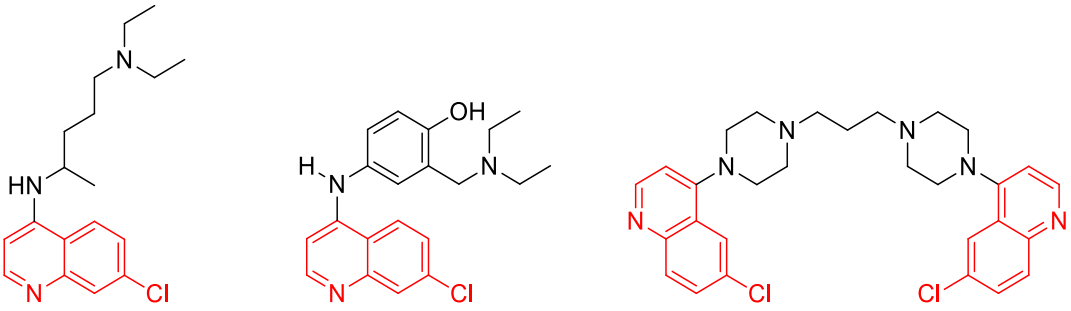
Halofantrine (**4**), a phenanthrene methanol derivative of amino-alcohol, was first marketed in 1988. Halofantrine has activity against the blood stages of the malaria parasite (Arrow *et al.*, 2004). It was considered effective and safe for treating malaria, including multidrug resistant *P falciparum* strains until 1993, when reports of severe and sometimes fatal cardiotoxicity associated with the use of halofantrine led the WHO to limit its use. Since 2002 around 20 reports of fatal cardiac complications relating to use of the drug was reported (Kinoshita *et al.*, 2010). Later, multiple studies have confirmed cardiac toxicity in both adults and children, and numerous cases of death (Bouchaud *et al.*, 2009).

Lumefantrine (**5**) (also known as benflumetol) show less antimalarial activity than halofantrine. This is a highly lipophilic drug and taking it with fatty meals increases the oral absorption by 16-fold (SchliTZer, 2008b). In contrast to halofantrine, lumefantrine is not associated with dangerous

cardiac side effects. Lumefantrine displays *in vitro* synergism with artemether and is currently used in combination under the brand name *Riamet* (SchliTZer, 2008b).

2.9.2 4-Aminoquinolines

Quinolines have been the basis of antimalarial chemotherapy starting with quinine nearly 400 years ago. 4-Aminoquinolines (Figure 2-7) form complexes with ferriprotoporphyrin IX (FPPIX), thereby inhibiting its polymerisation into non-toxic hemozoin. One of the major challenges in advancing these quinolines into antimalarial drugs is the poor aqueous solubility of these scaffolds, which limits the oral bioavailability.



6 Chloroquine **7** Amodiaquine **8** Piperaquine

Figure 2-7: 4-Aminoquinolines

Resistance against 4-aminoquinolines results from a mutation K76T in the gene of a transport protein positioned in the membrane of the DV that facilitates the removal of 4-aminoquinolines from the DV (SchliTZer, 2008b). Cross resistance with atovaquone (discussed in paragraph 2.9.7) is also a concern for any new antimalarial chemotherapy because cytochrome bc1 is known to be the biological target of antimalarial 4-aminoquinolines (Neelarapu *et al.*, 2018).

In the 20th century major impacts on global public health was made with the development of Chloroquine (**6**) as an antimalarial drug and the following evolution of drug-resistant *Plasmodium* strains (Wellems & Plowe, 2001). Chloroquine has been the most successful single drug for the treatment and prophylaxis of malaria as a safe and affordable drug. However, resistant strains to chloroquine began to appear in the 1960s (SchliTZer, 2008b). Chloroquine resistance is linked to multiple mutations in *P. falciparum* chloroquine resistance transporter (PfCRT), a protein that possibly functions as a transporter in the parasite's DV membrane.

Chloroquine's efficacy is hypothesised to rely on its capability to interrupt haematin detoxification in malaria parasites as they grow within human erythrocytes. This drug binds with haematin in its mu-oxodimer form and also adsorbs to the growing faces of the hemozoin crystals, disrupting detoxification thus poisoning the parasite (Wellems & Plowe, 2001). Studies show that more than

80 % of field isolates are resistant to chloroquine, in several regions this number can reach 100 %. In contrast, most strains of *P. vivax, P. ovale,* and *P. malariae* are still sensitive to chloroquine (SchliTZer, 2008b).

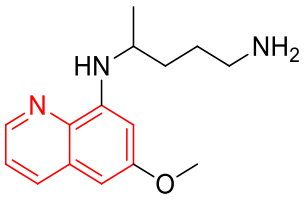
Amodiaquine (**7**) was developed by integrating an aromatic structure into chloroquine's side chain. This drug is generally effective against chloroquine-resistant *P*. *falciparum* infections with variable efficacy. Amodiaquine-induced toxic effects *viz.* idiosyncratic hepatotoxicity and agranulocytosis, have been reported with use as prophylaxis, but shows good tolerance when used as treatment (Adjuik *et al.*, 2002; Zhang *et al.*, 2018). The therapeutic value of amodiaquine is considerably reduced by the biotransformation of its *p*-aminophenol moiety into a quinoneimine, which is responsible for severe hepatotoxicity and life-threatening agranulocytosis. Amodiaquine is no longer sold in western countries (SchliTZer, 2008b).

Sulfadoxine-pyrimethamine (also known as Fansidar, discussed in paragraph 2.9.4) and amodiaquine is the standard drug combination for seasonal malaria chemoprevention and has been highly effective in reducing malaria morbidity and mortality in the Sahel and sub-Sahel regions of Africa, where seasonal malaria chemoprevention is commonly applied (Dicko *et al.*, 2018).

Piperaquine (**8**) is a bisquinoline antimalarial drug that was first synthesized in the 1950s.This is a highly lipid-soluble compound and it is proposed that its absorption can be increased by a high-fat meal (Sim *et al.*, 2005). In China and Indochina, it was mostly used as prophylaxis and treatment, but its use declined during the 1980s. However, during the following decade, piperaquine was identified by Chinese scientists as a suitable drug for combination with an artemisinin derivative. The rationale for the ACTs was to provide an inexpensive, short-course treatment regimen with a high cure rate and good tolerability that would reduce transmission and protect against the development of parasite resistance (Davis *et al.*, 2005). Due to the long chemo prophylactic period offered by piperaquine and the activity against juvenile *P falciparum* gametocytes, dihydroartemisinin-piperaquine combination therapy is advantageous in mass treatment settings, resulting in a decline of human to mosquito transmission (Dicko *et al.*, 2018).

2.9.3 8-Aminoquinoline

Primaquine (**9,** Figure 2-8) is the only widely available drug that is effective against *P*. *vivax* hypnozoites, the latent forms that emerge from the liver to produce relapses of *P*. *vivax* malaria (Chu *et al.*, 2017). The method of action suggests that it interferes with the mitochondrial function of *Plasmodium.* Because of its short half-life of four hours, the drug needs to be administered more often, even though it has good oral absorption (Robert *et al.*, 2001).



9 Primaquine

Figure 2-8: 8-Aminoquinoline

Primaquine has potent activity against mature *P. falciparum* gametocytes, but can induce haemolysis in individuals with G6PD deficiency. While G6PD deficiency is very common in malaria-endemic areas, G6PD testing is commonly unavailable because the standard point-of-care test requires suitable reagents, electricity, trained staff, and quality controls. The point-of-care diagnosis of G6PD deficiency is usually made by a phenotypic screening test in which the G6PD-mediated reduction of NADP+ to nicotinamide adenine dinucleotide phosphate (NADPH) is measured semi-quantitatively. The average test is the fluorescent spot test (FST), which assesses the fluorescence of NADPH in a blood spot under ultraviolet light. This recognises blood samples with ≳30%± 40% activity as abnormal (Chu *et al.*, 2017). A single dose of 0.25 mg/kg is safe and well tolerated, even in individuals with a G6PD-deficiency, and still highly effective in reducing transmissibility (Price & White, 2018).

2.9.4 Artimisinins

The rediscovery of qinghaosu in China in 1972 and the subsequent synthesis of artemether and artesunate have provided highly effective alternatives to quinine. Compared to all the antimalarial drugs artemisinin derivatives (Figure 2-9) are the most rapidly acting and potent (Dondorp *et al.*, 2005). Only three - four cycles (six – eight days) of treatment are required to have total removal of parasites from the blood.

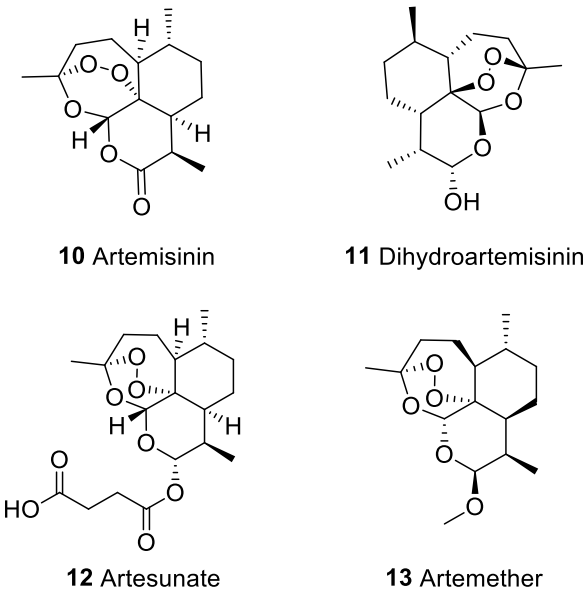


Figure 2-9: Artemisinin, dihydroartemisinin, artesunate and artemether

Artimisinins (**10**) both kill parasites and inhibit their major metabolic processes, such as glycolysis and nucleic acid and protein synthesis (Brunton, 2014; Haynes & Krishna, 2004).

For decades after the discovery of artemisinins, the proposed mechanism of action was founded on their unusual core chemical structure including a peroxide bridge, and the absolute requirement of the peroxide for antimalarial activity. Artemisinins that lack a peroxidic oxygen atom, such as desoxo compounds in which one of the peroxide oxygen atoms is substituted by carbon, show no activity. There is a possible link between the assumed mechanism of action of artemisinins and the generation of reactive oxygen species (ROS) *viz.* hydroxyl, alkoxyl, superoxide or peroxyl radicals within the parasitised erythrocyte. (Haynes & Krishna, 2004). Reductive scission of the peroxide bridge by reduced haem-iron, produced inside the highly acidic DV of the parasite as it digests haemoglobin, can result in the generation of free radicals that alkylate and oxidize proteins and possibly lipids in infected erythrocytes (Brunton, 2014).

It is thought that Fe^{2+}- catalysed reductive cleavage of the peroxide might give rise to O-centred, radicals and then C-centred radicals and neutral products. These reactive intermediates have been recommended to be the actual killing agents in reacting with ‘sensitive’ or ‘vital’ biomolecules in the parasite (Haynes & Krishna, 2004).

A third hypothesis for the anti-parasitic properties of artemisinins include parasite haem, electron transport chain in mitochondria or other proteins as targets, but these cannot coherently accommodate other observations. These include ring-stage death of parasites, killing of non-haem generating parasites, perturbations in calcium metabolism that are seen with artemisinins and genetic association studies between polymorphisms in PfATP6 and artemisinin susceptibilities (Krishna *et al.*, 2010).

Oral preparations of artemisinin and its derivatives are absorbed rapidly but incompletely with inter-individual factors playing a substantial role. Peak plasma concentrations are reached in one – two hours and most of these compounds have a short elimination half-life of one – three hours following oral consumption. Artesunate (**12**) acts like a pro-drug with fast conversion into dihydroartemisinin (**11**) and has an elimination half-life of less than 30 minutes (van Agtmael *et al.*, 1999).

A group of semisynthetic artemisinin derivatives have been formulated for oral (dihydroartemisinin artesunate, and artemether (**13**)), intramuscular (artesunate and artemether), intravenous (artesunate), and rectal (artesunate) routes, where the bioavailability after oral dosing characteristically is ≤ 30% (Brunton, 2014). Artemisinin itself is available in a few countries in Asia. It is five – ten times less active than the derivatives, and it is not metabolized to dihydroartemisinin (Arrow *et al.*, 2004).

Artesunate and artemether both have limited levels of plasma protein binding that range from 43 % to 82 %. Artemether is more lipophilic compared to artemisinin and is thus better absorbed from the gastrointestinal (GI) tract, allowing for an oral administration (SchliTZer, 2008b).

Repeated dosing cause artemisinin and artesunate to induce their own CYP-mediated metabolism, primarily *via* CYPs 2B6 and 3A4 enzymes, this could enhance clearance by up to five-fold. When treating severe malaria, intravenous artesunate is more rapidly acting than intravenous quinine in terms of parasite clearance, is safer, and is simpler to administer (Brunton, 2014).

Artemether is an oil-based formulation that releases the drug slowly and unpredictably from the injection site, compared to artesunate, which can be given intravenously and is absorbed reliably and rapidly after intra-muscular injection with peak concentrations arising within one hour. (Dondorp *et al.*, 2005).

The hypothesis that artemisinins might act as antimalarial agents by inhibiting a single target, the *Plasmodium falciparum* sarcoplasmic, endoplasmic, calcium ATPase PfATP6, led to the forecast that amino acid polymorphisms in this sequence might be associated with resistance to these drugs. These predictions were based on biochemical assays after heterologous expression and molecular modelling techniques. The PfATP6/SERCA hypothesis has created numerous testable outcomes and continues to be useful in design of experiments in the field of epidemiology, biology and drug development as well as areas such as oncology (Krishna *et al.*, 2010). Artemisinin-resistant *P. falciparum* malaria has emerged in western Cambodia. Resistance starts with prolonged *in vivo* parasite clearance times after artesunate treatment. The hypothesis that

delayed parasite clearance results from a stage-specific reduction in artemisinin sensitivity of the circulating young asexual parasite ring stages was examined (Saralamba *et al.*, 2011).

One of the limitations of artemisinin is that in order to produce more effective semi-synthetic derivatives, it has to be chemically altered for better pharmacological properties and this escalates the cost of ACTs (Eastman & Fidock, 2009). ACTs include artemether-lumefantrine, artesunate-mefloquine, artesunate-amodiaquine, artesunate-sulfoxadine-pyrimethamine, and dihydroartemisinin-piperaquine. Artemether-lumefantrine and artesunate-amodiaquine are the ACTs most used in Africa. For intermittent preventive treatment (IPT) of pregnant women the antifolate sulfadoxine-pyrimethamine is the main present option (Dahlström *et al.*, 2014b).

Studies in pregnant rats and rabbits show that artemisinins can cause increased embryo death or malformations early after conception. Preclinical toxicity studies have identified the brain including the brainstem, liver, bone marrow as the primary target organs. In patients, the numerous neurological changes that accompany severe malaria confound the evaluation of drug neurotoxicity. However, no systematic neurological changes were attributable to treatment in patients > five years of age (Brunton, 2014).

These drugs are selectively toxic in a dose-dependent and time-dependent manner to original foetal erythroblasts. Pregnant women are at higher risk of severe malaria than non-pregnant women of the same age. Reduced birth-weight is caused by both *P. falciparum* and *P. vivax* malaria in this setting. No reliable measures for the prevention of malaria exist for pregnant women and their treatment options are limited by drug resistance (McGready *et al.*, 2012b). A chemoprophylaxis trial in children younger than five years old also demonstrated a reduction in all-cause morbidity, including lower rates of gastrointestinal and respiratory illness, although the effect was only apparent after three to four years of chemo-protection and was less than the change in malaria-specific morbidity (Chen *et al.*, 2016).

2.9.5 Antifolates

Antifolates target two enzymes of tetrahydrofolate bio-synthesis , dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) (SchliTZer, 2008b). Tetrahydrofolates are crucial cofactors for DNA synthesis and methionine metabolism. Malaria parasites are capable of both synthesizing both tetra-hydrofolates and precursors *de novo* and of salvaging them from the environment (Salcedo-Sora *et al.*, 2011). Resistance to DHFR and DHPS inhibitors is conferred by single mutations of the gene encoding for the respective enzyme, resulting in substitutions in the amino acid chain. There are areas of the DHFR and DHPS genes with identified mutations that are found in isolates that fail to respond to pyrimethamine/sulfadoxine treatment (Olliaro, 2001).

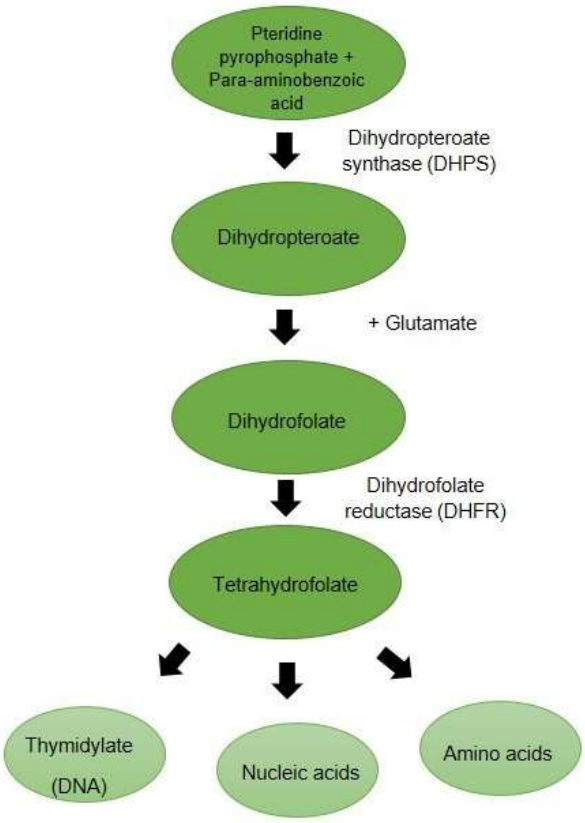
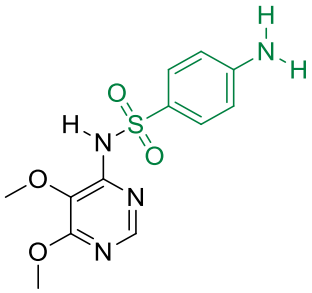


Figure 2-10: Biosynthetic pathway of tetrahydrofolate

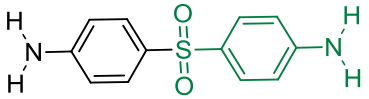
The antifolate antimalarials are divided into two classes, I and II, according to the enzymes they inhibit.

Class I

Class I antifolates include sulfonamides and sulfones (Figure 2-11), e.g. sulphadoxine (**13**) and dapsone (**14**) (Triglia *et al.*, 1997). They imitate p-aminobenzoic acid (PABA) activity by competing for the active site of dihydropteroate synthase. These compounds prevent the formation of dihydropteroate from hydroxylmethyl-dihydropterin, catalysed by dihydropteroate synthase. This is a bifunctional enzyme in *Plasmodia* which is coupled with 2-amino-4-hydroxy-6-hydroxymethyl-dihydropteridine pyrophosphokinase (PPPK) (Olliaro, 2001). DHPS inhibitors have feeble antimalarial activity but are synergistic with dihydrofolate reductase inhibitors. Stepwise accumulation of mutations has led to significant resistance against DHPS inhibitors (SchliTZer, 2008b).



13 Sulphadoxine



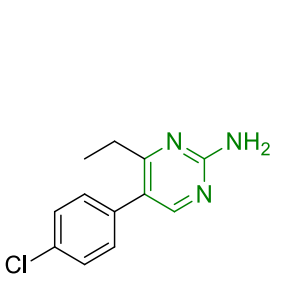
14 Dapsone

Figure 2-11: Class I antifolates

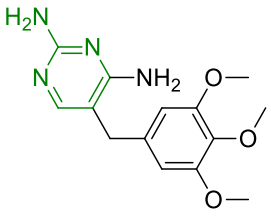
Class II

Class II antifolates (Figure 2-12) include pyrimethamine (**15**), biguanides and triazine metabolites *viz.* proguanil (**17**), chlorproguanil (**18**), pyrimethamine, and trimethoprim (**16**). Quinazolines (**15** and **16)** inhibit DHFR, a bifunctional enzyme in *Plasmodia* coupled with thymidylate synthase (TS). This causes inhibition of the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) by DHFR. THF is a necessary cofactor for the biosynthesis of thymidylate, purine nucleotides, and certain amino acids. DHFR inhibitors mimic the pteridine ring of the natural substrate DHF and compete with it for the active site of the enzyme. (Olliaro, 2001).

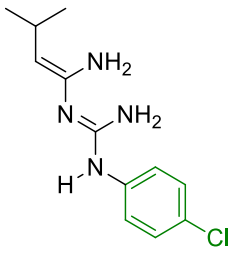
The combination of the sulfonamide sulfadoxine with the DHFR inhibitor pyrimethamine, is known under its brand name Fansidar. This compound replaced chloroquine as first-line antimalarial therapy (SchliTZer, 2008b). Proguanil-mediated enhancement is particular to atovaquone (compound discussed in paragraph 2.9.7) since the effects of other mitochondrial electron transport inhibitors, such as myxothiazole and antimycin, were not altered by the inclusion of proguanil (Srivastava & Vaidya, 1999).



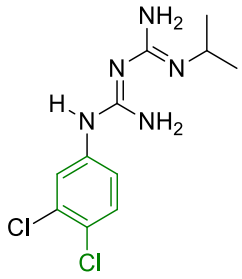
15 Pyrimethamine



16 Trimethoprim



17 Proguanil

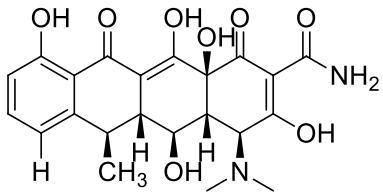


18 Chlorproguanil

Figure 2-12: Class II antifolates

2.9.6 Antimicrobial

Of the tetracycline class doxycycline (**19,** Figure 2-13) is the most widely used antibiotic against malaria. In therapy, it is combined with quinine or artesunate for the treatment of uncomplicated and severe malaria (SchliTZer, 2008b). Doxycycline come in multiple dosage forms and strengths with 100 mg tablets or capsules being most often used for malaria prophylaxis or treatment. The protective efficacy of doxycycline has been shown in three randomized placebo-controlled trials to be between 92 % and 96 % for *P. falciparum* and 98 % for primary *P. vivax* infection. When used in combination with a fast acting schizontocide, treatment efficacy of doxycycline has been shown to be 96 – 100 % in three open-label trials. Doxycycline's restricted activity in the liver stages of *Plasmodium* also means that it does not kill *P. vivax* hypnozoites. It is, therefore, recommended that doxycycline prophylaxis be continued for four weeks post exposure (Tan *et al.*, 2011).

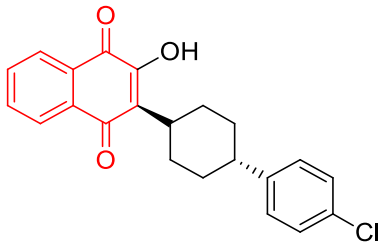


19 Doxycycline

Figure 2-13: Doxycycline

2.9.7 Inhibitors of the respiratory chain

Atovaquone (**20,** Figure 2-14) is a highly lipophilic analogue of ubiquinone and was developed as a promising synthetic hydroxynaphthoquinone derivative with potent activity against *Plasmodium* species and the opportunistic pathogens *Pneumocystis jiroveci, Pneumocystis carinii* and *Toxoplasma gondii* (Brunton, 2014).



20 Atovaquone

Figure 2-14: Atovaquone

Atovaquone stay unchanged by the first pass effect of the human liver microsomes (Nixon *et al.*, 2013). Because of this molecule's high lipophilic nature it is almost water insoluble but shows an increase as much as double the bioavailability when taken with fatty foods (Baggish & Hill, 2002b). Atovaquone has a good safety profile, and was shown to be non-toxic from cyto- and genotoxicity aspects (Brunton, 2014). In contrast with artemisinin, atovaquone has a much longer half-life of 50 – 84 hours (Nixon *et al.*, 2013).

This antimalarial drug kills both the blood and liver stages of malaria (Goodman *et al.*, 2016). Atovaquone is highly active against *P. falciparum* asexual blood stage parasites *in vitro* with nano-molar activity and *in vivo* in primates. Clinical studies in patients with uncomplicated *P. falciparum* malaria revealed that atovaquone produced good preliminary responses. A combination of atovaquone and proguanil produced high cure rates with minimal toxicity (Brunton, 2014).

This compound is a competitive inhibitor of the quinol oxidation (Q_o) site of the mitochondrial cytochrome bc_1 complex. Its action results in the failure of the mitochondrial respiratory chain of malaria parasites, specifically at the cytochrome c reductase complex, which leads to a collapse of the mitochondrial membrane potential and the inhibition of electron transport (Dinter *et al.*, 2011; Fisher *et al.*, 2012).The major function of mitochondrial electron transport in *P. falciparum* is to regenerate ubiquinone, which is the electron acceptor for parasite dihydroorotate dehydrogenase, an enzyme vital for pyrimidine biosynthesis.

Cytochrome *b* mutations lead to atovaquone resistance, resulting in antimalarial drug failures. Resistance to atovaquone in the field is associated with point mutations in the Q_o pocket of cytochrome *b*, especially near the conserved Pro^{260}-Glu^{261}-Trp^{262}-Tyr^{263} (PEWY) region**.** Atovaquone has also recently been shown to affect the conversion of fumarate to aspartate, further connecting mitochondrial function with pyrimidine biosynthesis and also possibly purine metabolism (Fisher *et al.*, 2012). Human *P. falciparum* malaria parasites carrying atovaquone resistant mutations in cytochrome b are unable to successfully transmit resistance to other mammals (Goodman *et al.*, 2016).

Atovaquone and the non-metabolized biguanides, proguanil and chlorproguanil show a strong synergism (SchliTZer, 2008b). Reports of atovaquone-proguanil treatment failures are rare. A tablet containing a fixed dose of 250 mg atovaquone and 100 mg proguanil hydrochloride, taken orally, is highly effective and safe in a three-day regimen for treating mild-to-moderate attacks of chloroquine- or sulfadoxine-pyrimethamine-resistant malaria (Brunton, 2014).

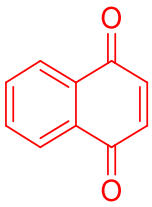
The enhanced solubility of poly-substituted 1,4-naphthoquinone derivatives was attained by adding nitrogen in two different positions of the naphthoquinone pharmacophore (Lanfranchi *et al.*, 2012).

2.10 Molecular hybridisation

Molecular hybridization, based on the combination of chemical moieties has emerged as an important strategy for the development of new drugs that are able to act as multi-target ligands (Bahia *et al.*, 2016a). Hybrid molecules offer various advantages including dosage compliance, minimized toxicity, ability to design better drug combinations, and cheaper preclinical evaluation while achieving the ultimate objective of delaying or circumventing the development of resistance (Muregi & Ishih, 2010a).

2.10.1 1,4-Naphthoquinones

Derivatives of 1,4- and 1,2-naphthoquinones are expansively distributed in nature, and many plants containing these substances have been used in folk medicine for the treatment of various ailments (Munday *et al.*, 2007a). Naphthoquinones (**21)** are known for their ability to transform DNA and thus belong to an essential class of natural products with wide-ranging biological applications (Bala *et al.*, 2014b). The favourable cytotoxic, antibacterial, antifungal, antiprotozoal, insecticidal and anti-inflammatory effects have attracted great interest in the poly-substituted 1,4-naphthoquinones in recent years as final compounds or synthetic intermediates.



21 1,4-Naphthoquinone

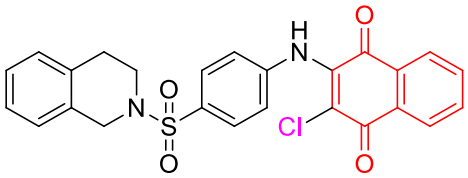
Figure 2-15: Structure of 1,4-Naphthoquinone

1,4-Naphthoquinones (Figure 2-15) are principally effective at inducing apoptosis and as such provide a rich source of unique cytotoxic reagents that can be used. They are often involved in bio-reductive activation and induce generic oxidative stress in both cancer cell lines and healthy cells (Bao *et al.*, 2018).

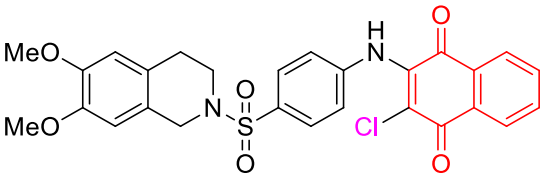
Previously studied 1,4-naphthoquinone derivatives have been suggested to display antimalarial activity by involving a cascade of redox reactions in the parasite. The extended studies to structural variations of the naphthoquinones revealed that the presence of amino- or chloro-substituents on the 1,4-naphthoquinone contribute to their redox potentials (Pingaew et al., 2015).A range of 1,4-naphthoquinone derivatives were synthesized and evaluated for their in vitro anti-proliferative activity against four cancer cells and a normal cell line as well as antimalarial activity toward P. falciparum. The synthesized quinones (Figure 2-16) with closed chain

sulfonamides showed significant antimalarial activity against K1 strain of P. falciparum with IC50 values of 14.74 and 2.80 µM for the para-compounds 22 and 23, respectively, and 7.27 and 8.79 µM for the meta-compounds 24 and 25, respectively (Pingaew et al., 2015).

PARA- CLOSED CHAIN

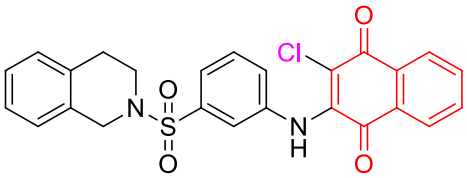


22 *Pf* K1 IC_{50} 14.74 µM

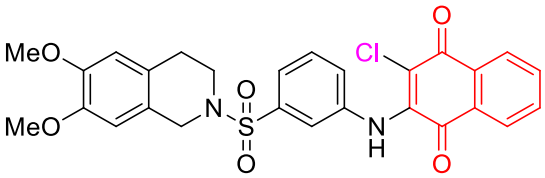


23 *Pf* K1 IC_{50} 2.80 µM

META-, CLOSED CHAIN



24 *Pf* K1 IC_{50} 7.27 µM

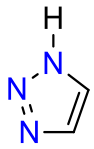


25 *Pf* K1 IC_{50} 8.79 µM

Figure 2-16: Structures of synthesized quinones with closed chain sulphonamides tested against P. falciparum (K1, multidrug resistant strain) for antimalarial activity.

2.10.2 Triazoles

Huisgen was the first to do an in-depth study of the synthesis of 1,2,3-triazoles (**26,** Figure 2-17) in the 1960s giving rise to the Huisgen 1,3-dipolar cycloaddition which involves the reaction of an alkyne and an azide to give 1,4- and 1,5-disubstituted-triazole regioisomers (Totobenazara & Burke, 2015).



26 1,2,3-Triazole

Figure 2-17: Structure of 1,2,3-Triazole

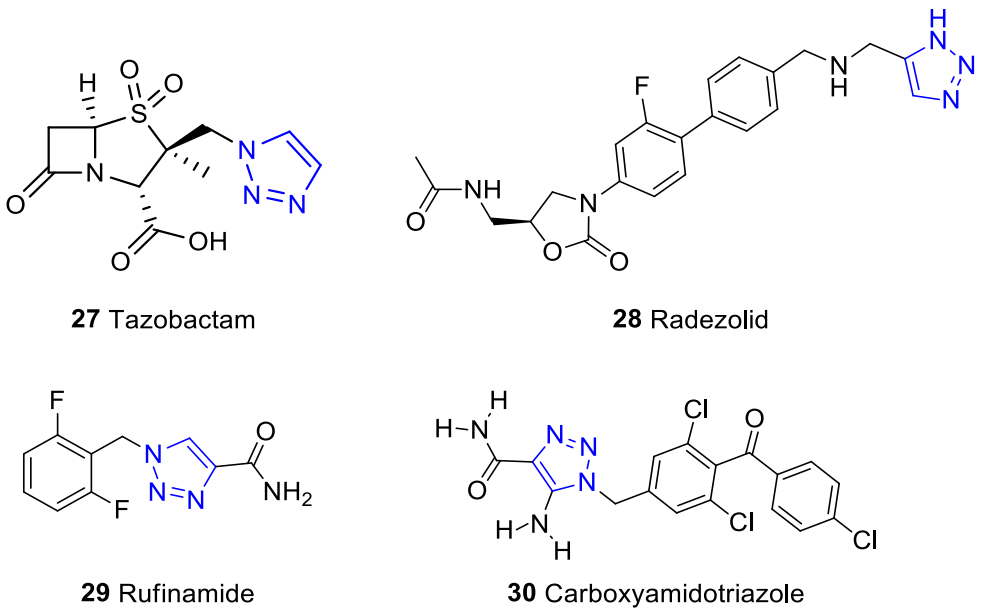
Triazoles are a class of compounds that also display an assortment of biological activities such as antimicrobial, analgesic, anti-inflammatory, local anaesthetic, anticonvulsant, antineoplastic, antimalarial, antiviral agents, anticancer activity and is used as agrochemicals, industrial applications such as dyes, corrosion inhibition, photo stabilisers, and photographic materials (Holla *et al.*, 2005; Sharghi *et al.*, 2009). Their metal chelating properties were also recently

studied. They behave as stabilising ligands for a range of metals, including Palladium, Copper, Zinc, in catalysis, as well as application as radiopharmaceuticals (Totobenazara & Burke, 2015).

Structural features of 1,2,3-triazoles, such as polarity, rigidity, and ability to act as both hydrogen bond donors (HBD) and acceptors (HBA), enable them to mimic the features of different functional groups. These compounds also show notable stability under hydrolytic, oxidative, and reductive conditions (Bonandi *et al.*, 2017).

Some 1,2,3-triazoles are used as DNA cleaving agents and potassium channel activators. Introduction of CF_3 group in the moiety vastly increases the pharmacological activity as well as the liphophilicity (Holla *et al.*, 2005).

The triazole products are more than just passive linkers, they readily associate with biological targets through hydrogen-bonding and dipole interactions (Agalave *et al.*, 2011). Some of the important 1,2,3-triazole-based therapeutic agents **27**-**30** (Figure 2-18) are tazobactam (antibiotic to stop bacterial infections), radezolid (antibiotic for the treatment of abscess, bacterial skin diseases, *Streptococcal* infections, infectious skin diseases, and *Staphylococcal* skin infections), rufinamide (anticonvulsant), carboxyamidotriazole orotate (known as CTO, anticancer drug), and tert-butyldimethylsilyls piroaminooxathioledioxide (known as TSAO, HIV reverse transcriptase inhibitor) (Prasad *et al.*, 2018). The synthesis of triazole-conjugated compounds accessed through click chemistry reactions continue to attract the attention of chemists (Bala *et al.*, 2014b).

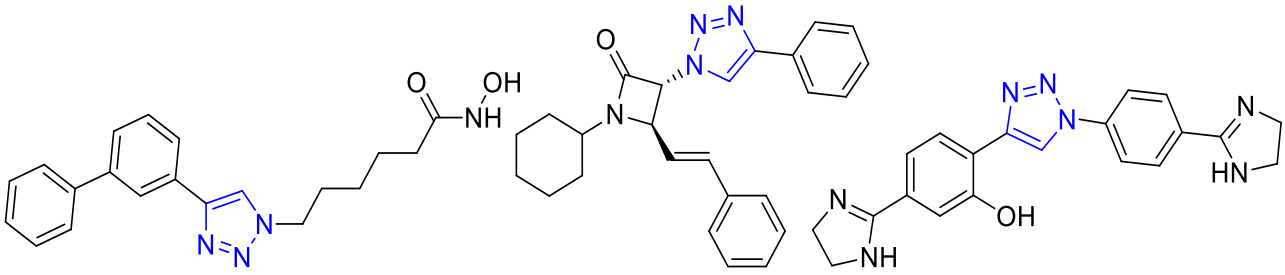


27 Tazobactam **28** Radezolid

29 Rufinamide **30** Carboxyamidotriazole

Figure 2-18: Structures of 1,2,3-Triazole based therapeutic agents, tazobactam, radezolid, rufinamide and carboxyamidotriazole orotate.

There are numerous reports on the antimalarial activity of compounds containing the 1,2,3-triazole motif. The activity of three 1,2,3-triazole derivatives **31**-**33** (Figure 2-19) tested against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* were reported with IC_{50} values ranging from 1.4 to 46 µM (Boechat *et al.*, 2014).



31 *Pf* W2 IC_{50} 29 µM **32** *Pf* W2 IC_{50} 18 µM **33** *Pf* W2 IC_{50} 26 µM

Figure 2-19: Structures of 1,2,3-triazole derivatives tested against P. falciparum chloroquine resistant W2 strain.

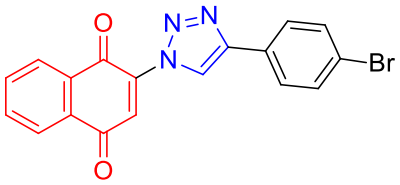
2.10.3. Naphthoquinone-Triazole hybrids

The development of new methodologies for the efficient synthesis of heterocycles with favourable medicinal properties are of great importance. 1,2,3-Triazoles derived from Huisgen's 1,3-dipolar cycloaddition of azides and alkynes is one of the most fundamental click reactions (Bala *et al.*, 2014b). Hybridisation is a logical alternative for preparing small molecules that are able to interfere with the vital targets on parasites metabolism and, by modulating multiple protein targets simultaneously, it can be beneficial for treating complex diseases (Bahia *et al.*, 2016a).

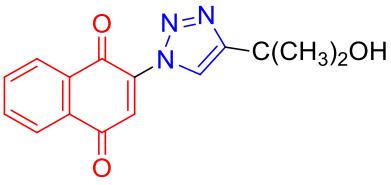
Click chemistry reactions possess exclusive characteristics which include, a rapid process in mild reaction conditions, high yields, better tolerance, producing stable products, simple reactions and impervious to humidity and oxygen (Zou *et al.*, 2018).

Copper catalysts exhibit high catalytic activity and 1,4-regioselectivity for the [3+ 2] Huisgen cycloaddition (Sharghi *et al.*, 2009). Thus, obtaining a 1,4-napthoquinone-1,2,3-triazole hybrid and not a 1,4-napthoquinone-1,2,4-triazole hybrid is because of regioselectivity.

In a 2012 study a series of 2-bromo-1, 4-naphthoquinone and 1, 2, 3-triazole hybrid compounds (Figure 2-20) were synthesised through copper-catalysed click chemistry and evaluated against the infective form of *Trypanosoma cruzi*, the etiological agent of Chagas disease. These compounds were, however, not screened for antimalarial activity (da Silva *et al.*, 2012). Triazole-naphthoquinone hybrids have also recently stood out in research on medicinal chemistry because of their structural diversity and biological properties (Oliveira *et al.*, 2017a).



T. cruzi IC_{50} *492.2* µM



T. cruzi IC_{50} 80.2 µM

Figure 2-20: Structures of 2-bromo-1, 4-naphthoquinone and 1, 2, 3-triazole hybrid compounds

The following chapter (Chapter 3) will include a manuscript which reports details of the synthesis of 1,4-naphthoquinone-1,2,3-triazole hybrids. The analytical and biological work will also be discussed as well as the results, analysis and conclusions of the project.

BIBLIOGRAPHY

Achan, J., Talisuna, A.O., Erhart, A., Yeka, A., Tibenderana, J.K., Baliraine, F.N., *et al.* 2011. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malaria Journal*, 10:144.

Adjuik, M., Agnamey, P., Babiker, A., Borrmann, S., Brasseur, P., Cisse, M., *et al.* 2002. Amodiaquine-artesunate versus amodiaquine for uncomplicated Plasmodium falciparum malaria in African children: a randomised, multicentre trial. *The Lancet*, 359:1365-1372.

Agalave, S.G., Maujan, S.R. & Pore, V.S. 2011. Click Chemistry: 1,2,3-Triazoles as Pharmacophores. *Chemistry – An Asian Journal*, 6:2696-2718.

Aidoo, M., Terlouw, D.J., Kolczak, M.S., McElroy, P.D., ter Kuile, F.O., Kariuki, S., *et al.* 2002. Protective effects of the sickle cell gene against malaria morbidity and mortality. *The Lancet*, 359:1311-1312.

Akhtar, M.N., Jamil, S., Amjad, S.I., Butt, A.R. & Farooq, M. 2016. Association of malaria with thrombocytopenia. *Annals of King Edward Medical University*, 11(4).

Allison, A.C. 2009. Genetic control of resistance to human malaria. *Current Opinion in Immunology*, 21:499-505.

Arend, P. 2018. Position of human blood group O (H) and phenotype-determining enzymes in growth and infectious disease. *Annals of the New York Academy of Sciences*, 1425:5-18.

Arrow, K.J., Panosian, C.B. & Gelband, H. 2004. Antimalarial drug classes. Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance. Washington, DC: The National Academies Press. pp. 252-299.

Baggish, A.L. & Hill, D.R. 2002. Antiparasitic agent atovaquone. *Antimicrobial Agents and Chemotherapy*, 46:1163-1173.

Bahia, S.B.B., Reis, W.J., Jardim, G.A., Souto, F.T., de Simone, C.A., Gatto, C.C., *et al.* 2016. Molecular hybridization as a powerful tool towards multitarget quinoidal systems: synthesis, trypanocidal and antitumor activities of naphthoquinone-based 5-iodo-1, 4-disubstituted-, 1, 4-

and 1, 5-disubstituted-1, 2, 3-triazoles. *The European Federation for Medicinal Chemistry*, 7:1555-1563.

Bala, B.D., Muthusaravanan, S., Choon, T.S., Ashraf Ali, M. & Perumal, S. 2014. Seq.uential synthesis of amino-1,4-naphthoquinone-appended triazoles and triazole-chromene hybrids and their antimycobacterial evaluation. *European Journal of Medicinal Chemistry*, 85:737-746.

Bao, N., Ou, J., Shi, W., Li, N., Chen, L. & Sun, J. 2018. Highly Efficient Synthesis and Structure-Activity Relationships of a Small library of Substituted 1, 4-Naphthoquinones. *European Journal of Organic Chemistry*, 19:2254-2258.

Barrera, V., MacCormick, I.J.C., Czanner, G., Hiscott, P.S., White, V.A., Craig, A.G.*, et al.* 2018. Neurovascular seq.uestration in paediatric P. falciparum malaria is visible clinically in the retina. *eLife*, 7:e32208.

Beare, N.A., Taylor, T.E., Harding, S.P., Lewallen, S. & Molyneux, M.E. 2006. Malarial retinopathy: a newly established diagnostic sign in severe malaria. *The American Journal of tropical Medicine and Hygiene*, 75:790-797.

Boechat, N., Ferreira, M.d.L.G., Pinheiro, L., Jesus, A.M.L., Leite, M.M.M., Junior, C.C.S.*, et al.* 2014. New Compounds Hybrids 1H-1,2,3-Triazole-Quinoline Against Plasmodium falciparum. *Chemical Biology & Drug Design*, 84:325-332.

Bonandi, E., Christodoulou, M.S., Fumagalli, G., Perdicchia, D., Rastelli, G. & Passarella, D. 2017. The 1,2,3-triazole ring as a bioisostere in medicinal chemistry. *Drug Discovery Today*, 22:1572-1581.

Bouchaud, O., Imbert, P., Touze, J.E., Dodoo, A.N., Danis, M. & Legros, F. 2009. Fatal cardiotoxicity related to halofantrine: a review based on a worldwide safety data base. *Malaria Journal*, 8(1):289-307.

Brunton, L.L. 2014. Antimalarial Drug Therapy. Goodman and Gilman's manual of pharmacology and therapeutics. Mcgraw-hill New York. p. 1383-1418.

Cannella, A.P. & Archibald, L. 2017. Plasmodium species - Infectious Diseases. *https://www.infectiousdiseaseadvisor.com/infectious-diseases/plasmodium-species-malaria/article/609978/*, Date of Access: 20 September 2018.

Cappellini, M.D. & Fiorelli, G. 2008. Glucose-6-phosphate dehydrogenase deficiency. *The Lancet*, 371:64-74.

CDC, (Centers For Disease Control and Prevention). 2016. Malaria Life Cycle. *https://www.cdc.gov/malaria/about/index.html*, Date of Access: 25 August 2017.

CDC. 2018. Malaria Parasites. *https://www.cdc.gov/malaria/about/biology/parasites.html*, Date of Access: 25 Spetember 2018.

Chen, I., Clarke, S.E., Gosling, R., Hamainza, B., Killeen, G., Magill, A.*, et al.* 2016. "Asymptomatic" malaria: a chronic and debilitating infection that should be treated. *PLoS Medicine*, 13:e1001942.

Chu, C., Bancone, G., Moore, K., Win, H., Thitipanawan, N., Po, C.*, et al.* 2017. Primaquine induced haemolysis in G6PD heterozygous females treated for the radical cure of Plasmodium vivax malaria: a nested cohort study. *PLos Medicine*, 14:1-19.

da Silva, E.N., de Melo, I.M.M., Diogo, E.B.T., Costa, V.A., de Souza Filho, J.D., Valenca, W.O.*, et al.* 2012. On the search for potential anti-Trypanosoma cruzi drugs: Synthesis and biological evaluation of 2-hydroxy-3-methylamino and 1, 2, 3-triazolic naphthoquinoidal compounds obtained by click chemistry reactions. *European Journal of Medicinal Chemistry*, 52:304-312.

Dahlström, S., Aubouy, A., Maïga-Ascofaré, O., Faucher, J.-F., Wakpo, A., Ezinmègnon, S.*, et al.* 2014. Plasmodium falciparum polymorphisms associated with ex vivo drug susceptibility and clinical effectiveness of artemisinin-based combination therapies in Benin. *Antimicrobial Agents and Chemotherapy*, 58:1-10.

Davis, T.M.E., Hung, T.-Y., Sim, I.-K., Karunajeewa, H.A. & Ilett, K.F. 2005. Piperaquine. *Drugs*, 65:75-87.

Dean, L. 2005. The Duffy Blood Group. Blood groups and red cell antigens. National Center for Biotechnology Information. p. 1-6.

Dicko, A., Roh, M.E., Diawara, H., Mahamar, A., Soumare, H.M., Lanke, K.*, et al.* 2018. Efficacy and safety of primaquine and methylene blue for prevention of Plasmodium falciparum transmission in Mali: a phase 2, single-blind, randomised controlled trial. *The Lancet Infectious Diseases*, 18:627-639.

Dieye, Y., Mbengue, B., Dagamajalu, S., Fall, M.M., Loke, M.F., Nguer, C.M., *et al.* 2016. Cytokine response during non-cerebral and cerebral malaria: evidence of a failure to control inflammation as a cause of death in African adults. *PeerJ*, 4:e1965.

Dinter, D., and, G.G. & Garaj-Vrhovacb, V. 2011. An alkaline comet assay study on the antimalarial drug atovaquone in human peripheral blood lymphocytes: a study based on clinically relevant concentrations. *Journal of Applied Toxicology*, 33:56-62.

Dondorp, A., Nosten, F., Stepniewska, K., Day, N. & White, N. 2005. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet*, 366:717-725.

Dondorp, A.M., Fanello, C.I., Hendriksen, I.C., Gomes, E., Seni, A. & Chhaganlal, K.D. 2010. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet*, 376:1647-1657.

Doolan, D.L., DobaÃ±o, C. & Baird, J.K. 2009. Acquired immunity to malaria. *Clinical microbiology Reviews*, 22:13-36.

Eastman, R.T. & Fidock, D.A. 2009. Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. *Nature Reviews Microbiology*, 7(12):864-874.

Fisher, N., Majid, R.A., Antoine, T., Al-Helal, M., Warman, A.J., Johnson, D.J., *et al.* 2012. Cytochrome b mutation Y268S conferring atovaquone resistance phenotype in malaria parasite results in reduced parasite bc1 catalytic turnover and protein expression. *Journal of Biological Chemistry*, 287:9731-9741.

Frickmann, H., Hinz, R., Rojak, S., Bonow, I., Ruben, S., Wegner, C., *et al.* 2018. Evaluation of automated loop-mediated amplification (LAMP) for routine malaria detection in blood samples of German travelers – A cross-sectional study. *Travel Medicine and Infectious Disease*, 24:25-30.

Frosch, T., Schmitt, M. & Popp, J. 2007. In situ UV resonance Raman micro-spectroscopic localization of the antimalarial quinine in cinchona bark. *The Journal of Physical Chemistry B*, 111:4171-4177.

Ghosh, K. & Ghosh, K. 2007. Pathogenesis of anemia in malaria: a concise review. *Parasitology Research*, 101:1463-1469.

Gilson, R.C., Deissler, R.J., Bihary, R.F., Condit, W.C., Thompson, M.E., Blankenship, D.A.*, et al.* 2018. Growth of Plasmodium falciparum in response to a rotating magnetic field. *Malaria Journal*, 17:190-223.

Goodman, C.D., Siregar, J.E., Mollard, V., Vega-Rodríguez, J., Syafruddin, D., Matsuoka, H.*, et al.* 2016. Parasites resistant to the antimalarial atovaquone fail to transmit by mosquitoes. *Science*, 352:349-353.

Greenwood, B., Dicko, A., Sagara, I., Zongo, I., Tinto, H., Cairns, M.*, et al.* 2017. Seasonal vaccination against malaria: a potential use for an imperfect malaria vaccine. *Malaria Journal*, 16:182-192.

Haynes, R.K. & Krishna, S. 2004. Artemisinins: activities and actions. *Microbes and Infection*, 6:1339-1346.

Holla, B.S., Mahalinga, M., Karthikeyan, M.S., Poojary, B., Akberali, P.M. & Kumari, N.S. 2005. Synthesis, characterization and antimicrobial activity of some substituted 1,2,3-triazoles. *European Journal of Medicinal Chemistry*, 40:1173-1178.

Johnston, S.P., Pieniazek, N.J., Xayavong, M.V., Slemenda, S.B., Wilkins, P.P. & da Silva, A.J. 2006. PCR as a confirmatory technique for laboratory diagnosis of malaria. *Journal of Clinical Microbiology*, 44:1087-1089.

Jourdan, J., Schneider, J., Dassonville-Klimpt, A. & Sonnet, P. 2018. Recent advances in antimalarial drugs: structures, mechanisms of action and clinical trials. *(In* Méndez-Vilas, A., *ed.* Microbiology Book Series Formatex Research Center. p. 599-609.

Karbwang, J. & White, N.J. 1990. Clinical pharmacokinetics of mefloquine. Clinical pharmacokinetics. pp. 264-279.

Kinoshita, A., Yamada, H., Kotaki, H. & Kimura, M. 2010. Effects of anti-malarial drugs on the electrocardiographic QT interval modelled in the isolated perfused guinea pig heart system. *Malaria Journal*, 9:318-348.

Krishna, S., Pulcini, S., Fatih, F. & Staines, H. 2010. Artemisinins and the biological basis for the PfATP6/SERCA hypothesis. *Trends in Parasitology*, 26:517-523.

Lanfranchi, D.A., Cesar-Rodo, E., Bertrand, B., Huang, H., Day, L., Johann, L.*, et al.* 2012. Synthesis and biological evaluation of 1,4-naphthoquinones and quinoline-5,8-diones as antimalarial and schistosomicidal agents. *Organic & Biomolecular Chemistry*, 10:6375-6387.

Li, Y., Fan, P., Zhou, S. & Zhang, L. 2017. Loop-mediated isothermal amplification (LAMP): a novel rapid detection platform for pathogens. *Microbial Pathogenesis*, 107:54-61.

Liu, M., Solomon, W., Cespedes, J.C., Wilson, N.O., Ford, B. & Stiles, J.K. 2018. Neuregulin-1 attenuates experimental cerebral malaria (ECM) pathogenesis by regulating ErbB4/AKT/STAT3 signaling. *Journal of Neuroinflammation*, 15:104-170.

Makler, M.T., Palmer, C.J. & Ager, A.L. 1998. A review of practical techniques for the diagnosis of malaria. *Annals of tropical medicine and parasitology*, 92:419-434.

Malwest. 2016. Plasmodium Life cycle. *http://www.malwest.gr/en-us/malaria/informationforhealthcareprofessionals/plasmodiumlifecycle.aspx.*, Date of Access : 31 August 2018l

Mathanga, D. & Molyneux, M.E. 2016. Bednets and malaria in Africa. *Malawi Medical Journal*, 28:92-93.

Mayo, C, (Mayo Clinic). 2018. Sickle Cell Anemia. *https://www.mayoclinic.org/diseases-conditions/sickle-cell-anemia/symptoms-causes/syc-20355876/,* Date of Access: 25 June 2018.

McGready, R., Lee, S.J., Wiladphaingern, J., Ashley, E.A., Rijken, M.J., Boel, M.*, et al.* 2012. Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. *The Lancet Infectious Diseases*, 12:388-396.

MMV, (Medicines for Malaria Venture). 2017. Parasite Lifecycle. *https://www.mmv.org/malaria-medicines/parasite-lifecycle/,* Date Of Access: 11 August 2017.

Munday, R., Smith, B.L. & Munday, C.M. 2007. Structure-activity relationships in the haemolytic activity and nephrotoxicity of derivatives of 1, 2-and 1, 4-naphthoquinone. *Journal of Applied Toxicology*, 27:262-269.

Muregi, F.W. & Ishih, A. 2010. Next-Generation Antimalarial Drugs: Hybrid Molecules as a New Strategy in Drug Design. *Drug Development Research*, 71:20-32.

Neelarapu, R., Maignan, J.R., Lichorowic, C.L., Monastyrskyi, A., Mutka, T.S., LaCrue, A.N.*, et al.* 2018. Design and Synthesis of Orally Bioavailable Piperazine Substituted 4 (1 H)-Quinolones with Potent Antimalarial Activity: Structure–Activity and Structure–Property Relationship Studies. *Journal of Medicinal Chemistry*, 61:1450-1473.

Nixon, G.L., Moss, D.M., Shone, A.E., Lalloo, D.G., Fisher, N., O'Neill, P.M.*, et al.* 2013. Antimalarial pharmacology and therapeutics of atovaquone. *Journal of Antimicrobial Chemotherapy*, 68:977-985.

Ocaña-Morgner, C., Mota, M.M. & Rodriguez, A. 2003. Malaria Blood Stage Suppression of Liver Stage Immunity by Dendritic Cells. *The Journal of Experimental Medicine*, 197:143-151.

Oliveira, R.N.d., Silva, M.G.d., Silva, M.T.d., Melo, V.N., Valença, W.O., Paz, J.A.d.*, et al.* 2017. Strategies for Molecular Diversification of 2-[Aminoalkyl-(1H-1, 2, 3-triazol-1-yl)]-1, 4-naphthoquinones Using Click Chemistry. *Journal of the Brazilian Chemical Society*, 28:681-688.

Olliaro, P. 2001. Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacology & Therapeutics*, 89:207-219.

Ouattara, A., Barry, A.E., Dutta, S., Remarque, E.J., Beeson, J.G. & Plowe, C.V. 2015. Designing malaria vaccines to circumvent antigen variability. *Vaccine*, 33:7506-7512.

Pingaew, R., Prachayasittikul, V., Worachartcheewan, A., Nantasenamat, C., Prachayasittikul, S., Ruchirawat, S.*, et al.* 2015. Novel 1,4-naphthoquinone-based sulfonamides: Synthesis, QSAR, anticancer and antimalarial studies. *European Journal of Medicinal Chemistry*, 103:446-459.

Prasad, C.V., Nayak, V.L., Ramakrishna, S. & Mallavadhani, U.V. 2018. Novel menadione hybrids: Synthesis, anticancer activity, and cell-based studies. *Chemical biology & drug design*, 91:220-233.

Price, R.N., Uhlemann, A.-C., Brockman, A., McGready, R., Ashley, E., Phaipun, L.*, et al.* 2004. Mefloquine resistance in Plasmodium falciparum and increased pfmdr1 gene copy number. *The Lancet*, 364:438-447.

Price, R.N. & White, N.J. 2018. Drugs that reduce transmission of falciparum malaria. *The Lancet Infectious Diseases*, 18:585-586.

Primus, H. 2016. Prevention of Malaria. *https://plus.google.com/+PrimusHospitalprimushospital/posts/RosNYbN3d69/*, Date of Access: 3 July 2018.

Rees, D.C., Williams, T.N. & Gladwin, M.T. 2010. Sickle-cell disease. *The Lancet*, 376:2018-2031.

Robert, A., Benoit-Vical, F., Dechy-Cabaret, O. & Meunier, B. 2001. From classical antimalarial drugs to new compounds based on the mechanism of action of artemisinin. *Pure and Applied Chemistry*, 73:1173-1188.

Salcedo-Sora, J.E., Ochong, E., Beveridge, S., Johnson, D., Nzila, A., Biagini, G.A*., et al.* 2011. The molecular basis of folate salvage in Plasmodium falciparum characterization of two folate transporters. *Journal of Biological Chemistry*, 286:44659-44668.

Saralamba, S., Pan-Ngum, W., Maude, R.J., Lee, S.J., Tarning, J., Lindegårdh, N*., et al.* 2011. Intrahost modeling of artemisinin resistance in Plasmodium falciparum. *Proceedings of the National Academy of Sciences*, 108:397-402.

SchliTZer, M. 2008. Antimalarial drugs–what is in use and what is in the pipeline. *Archiv der Pharmazie*, 341:149-163.

Sharghi, H., Khalifeh, R. & Doroodmand, M.M. 2009. Copper Nanoparticles on Charcoal for Multicomponent Catalytic Synthesis of 1,2,3-Triazole Derivatives from Benzyl Halides or Alkyl Halides, Terminal Alkynes and Sodium Azide in Water as a “Green” Solvent. *Advanced Synthesis & Catalysis*, 351:207-218.

Sharma, S.K., Mohanty, P.K., Rath, B. & Sahoo, S.C. 2018. Ventricular tachyarrhythmia in falciparum malaria. *Journal of Evidence Based Medicine and Healthcare*, 5:1571-1576.

Sim, K., Davis, T.M. & Ilett, K.F. 2005. Effects of a high-fat meal on the relative oral bioavailability of piperaquine. *Antimicrobial Agents and Chemotherapy*, 49:2407-2411.

Singh, B. & Daneshvar, C. 2013. Human infections and detection of Plasmodium knowlesi. *Clinical Microbiology Reviews*, 26:165-184.

Skorokhod, O.A., Caione, L., Marrocco, T., Migliardi, G., Barrera, V., Arese, P.*, et al.* 2010. Inhibition of erythropoiesis in malaria anemia: role of hemozoin and hemozoin-generated 4-hydroxynonenal. *Blood*, 27:4328-4332.

Srivastava, I.K. & Vaidya, A.B. 1999. A mechanism for the synergistic antimalarial action of atovaquone and proguanil. *Antimicrobial Agents and Chemotherapy*, 43:1334-1339.

Tan, K.R., Magill, A.J., Parise, M.E. & Arguin, P.M. 2011. Doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. *The American Journal of Tropical Medicine and Hygiene*, 84:517-531.

Thakur, K.T., Vareta, J., Carson, K.A., Kampondeni, S., Potchen, M.J., Birbeck, G.L.*, et al.* 2018. Cerebrospinal fluid Plasmodium falciparum histidine-rich protein-2 in pediatric cerebral malaria. *Malaria Journal*, 17:125-141.

Totobenazara, J. & Burke, A.J. 2015. New click-chemistry methods for 1,2,3-triazoles synthesis: recent advances and applications. *Tetrahedron Letters*, 56:2853-2859.

Trampuz, A., Jereb, M., Muzlovic, I. & Prabhu, R.M. 2003. Clinical review: Severe malaria. *Critical Care*, 7:315-323.

Triglia, T., Menting, J.G., Wilson, C. & Cowman, A.F. 1997. Mutations in dihydropteroate synthase are responsible for sulfone and sulfonamide resistance in Plasmodium falciparum. *Proceedings of the National Academy of Sciences*, 94:13944-13949.

van Agtmael, M.A., Eggelte, T.A. & van Boxtel, C.J. 1999. Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. *Trends in Pharmacological Sciences*, 20:199-205.

VineTZ, J.M., Clain, J., Bounkeua, V., Eastman, R.T.a. & Fidock, D. 2011. Goodman & Gilman's the pharmacological basis of therapeutics. *Chemotherapy of Malaria*, 12:1383-1418.

Vishruti Gandhi, V.G., Muley, P., Parikh, N., Gandhi, H. & Mehta, A. 2017. Is rapid diagnostic test (malaria Pv/Pf Ag card test) reliable in diagnosing malaria. *International Journal Of Contemporary Pediatrics*, 5:92-96.

Wellems, T.E. & Plowe, C.V. 2001. Chloroquine-resistant malaria. *The Journal of Infectious Diseases*, 184:770-776.

WHO. 2012. WHO Policy Recommendation: Seasonal Malaria Chemoprevention (SMC) for Plasmodium falciparum malaria control in highly seasonal transmission areas of the Sahel sub-region in Africa. *http://www.who.int/malaria/publications/world_malaria_report/en/*, Date of Access: 11 August 2017.

WHO. 2015. WHO Guidelines for the treatment of malaria. *https://books.google.co.za/books?hl=en&lr=&id=IVo0DgAAQBAJ&oi=fnd&pg=PP1&dq=Malaria+current+chemotherapy+drug+regimens&ots=9Tie5nL2iO&sig=BeETIjvua7Opa_AG6bill6vvkGU#v=onepage&q&f=false/*, Date Of Access: 15 March 2018.

WHO. 2016. Fact Sheet: World Malaria Report 2016. *http://www.who.int/malaria/media/world-malaria-report-2016/en/*, Date of Access: 12 March 2018.

WHO. 2017. The top 10 causes of death. *http://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death/*, Date of Access: 20 September 2018.

Wroblewski, H.A., Kovacs, R.J., Kingery, J.R., Overholser, B.R. & Tisdale, J.E. 2012. High Risk of QT Interval Prolongation and Torsades de Pointes Associated with Intravenous Quinidine Used for the Treatment of Resistant Malaria or Babesiosis. *Antimicrobial Agents and Chemotherapy*:AAC. 06396-06311.

Zhang, R.-W. 2016. Artemisinin (Qinghaosu), Nobel Prize, anti-malaria, and beyond. *Chinese Journal of Natural Medicines*, 14:1.

Zhang, Y., den Braver-Sewradj, S.P., den Braver, M.W., Hiemstra, S., Vermeulen, N.P.E., van de Water, B.*, et al.* 2018. Glutathione S-transferase P1 protects against amodiaquine quinoneimines-induced cytotoxicity but does not prevent activation of endoplasmic reticulum stress in HepG2 cells. *Frontiers in Pharmacology*, 9:388-411.

Zou, Y., Zhang, L., Yang, L., Zhu, F., Ding, M., Lin, F., Wang, Z. & Li, Y. 2018. Click chemistry in polymeric scaffolds: Bioactive materials for tissue engineering. *Journal of Controlled Release*, 273:160-179.

CHAPTER 3: ARTICLE FOR SUBMISSION

Chapter 3 consists of an article for submission to the European Journal of Medicinal Chemistry. This article comprises of a graphical abstract, an abstract, an introduction, results and discussion, *in vitro* biological evaluations, conclusion, materials and methods and synthesis of devised compounds. This article is prepared according to the author's guidelines, available in the Author Information pack on the journal's homepage (Annexure B): https://www.elsevier.com/journals/european-journal-of-medicinal-chemistry/0223-5234/guide-for-authors.

Triazole-linked 1,4-naphthoquinone derivatives: synthesis and antiplasmodial activity

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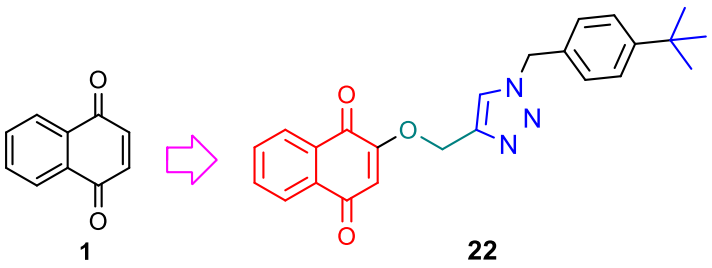
The synthesis was conducted by C Erasmus under the guidance of Dr FJ Smit and Prof DD N'Da. The cytotoxicity was assessed by Dr J Aucamp while the antiplasmodial activity determination was conducted by Dr D CoerTZen and Prof L-M BirkholTZ.

GRAPHICAL ABSTRACT



ClogP 4.2
P. falciparum
asexual 90 % inhib. at 1 µM:
NF54 90
Cytotoxicity: IC_{50} 36.42 µM
HEK-293 36

HIGHLIGHTS

- Synthesis of a series of naphthoquinone-triazole hybrids.
- Evaluation of cytotoxicity and antimalarial activity.
- Hybrids with oxymethylene linker were only active ones.
- Hybrid **22** with *tert*-butyl chain was most active with 90 % parasite growth inhibition at 1 µM.

Abstract

Malaria is a protozoan disease ravaging the world, having killed 445 000 people in 2016 and currently affecting 216 million people annually. The increasing incidence of multi-drug resistant strains of the pathogen, *Plasmodium* sp. stresses the need for the development of new antimalarial drugs. While resistance has risen autonomously in *P. falciparum* around the world, chloroquine resistance in *P. vivax* does not involve *P. vivax* chloroquine-resistance transporter and its source remains unknown. Recently hybridisation of different moieties has been in the spotlight because of their numerous advantages, including compliance, minimized toxicity, ability to design better drug combinations, and more cost-effective preclinical assessment. We synthesised a series of hybrid compounds containing 1,4-naphthoquinone and 1,2,3-triazole pharmacophores, using click-chemistry, and determined their ability to inhibit chloroquine-sensitive NF54 strain *Plasmodia*, as well as their cytotoxicity using human embryonic kidney cell line (HEK-293) alongside atovaquone as reference drug. The hybrid **22** featuring 4-(*tert*-butyl) benzyl moiety was the most active with 90 % *plasmodia* inhibition at 1 μM, comparable to the reference atovaquone (96 %). It was also found to have moderate cytotoxicity against chloroquine-sensitive NF54 *P. falciparum* parasites.

Keywords: *Plasmodium*, malaria, 1,4-naphthoquinone, 1,2,3-triazole, click-chemistry, hybrids

3.1 Introduction

Malaria is a protozoan disease that continues to affect millions of lives around the world every year. WHO statistics of 2016 identified HIV/AIDS as the number one killing infectious disease, with malaria the second leading cause of death in the tropical and subtropical regions of the world and TB being third [1, 2]. There are five species of the genus *Plasmodium* that can cause malaria in humans, namely *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and, in parts of Southeast Asia, *P. knowlesi* [3]. In 2016, a total of 216 million cases of malaria were reported in 91 countries. This is an increase of five million cases over the previous year and malaria deaths reached 445 000 [2] in 2016. The manifestation of parasite resistance is the primary reason why malaria remains a major worldwide public health problem and requires new innovative solutions [4].

The parasitic cycle is divided in two stages: i) a sexual stage in the female Anopheles mosquito, ii) an asexual stage in the human host. The sexual stage activates in the mosquito, where gametes fuse to form the human infesting form, liver-stage sporozoites [4]. The asexual or blood-stage begins when the parasite exits the liver cells and begin infecting erythrocytes. A good approach for new malaria drugs would be to target the liver-stage of the disease, thereby eliminating transmission and preventing the disease to progress to the blood-stage.

Artemisinin combination therapies (ACTs) are recommended by WHO for the treatment of all *P. falciparum* malaria, but not in the first trimester of pregnancy because results from animal studies suggest artemisinins to be embryotoxic [7]. ACTs currently used (Figure 3-1) include artemether-lumefantrine, artesunate-mefloquine, artesunate-amodiaquine, artesunate-sulfoxadine-pyrimethamine, and dihydroartemisinin-piperaquine [8]. Even though ACTs are recommended by WHO, it is a reality that artemisinin resistance is growing. Resistance to partner drugs is causing high treatment failure of ACTs, e.g. in the case of resistance to sulphadoxine-pyrimethamine in some African countries, ACT failure rates are high even in the absence of artemisinin resistance [9].

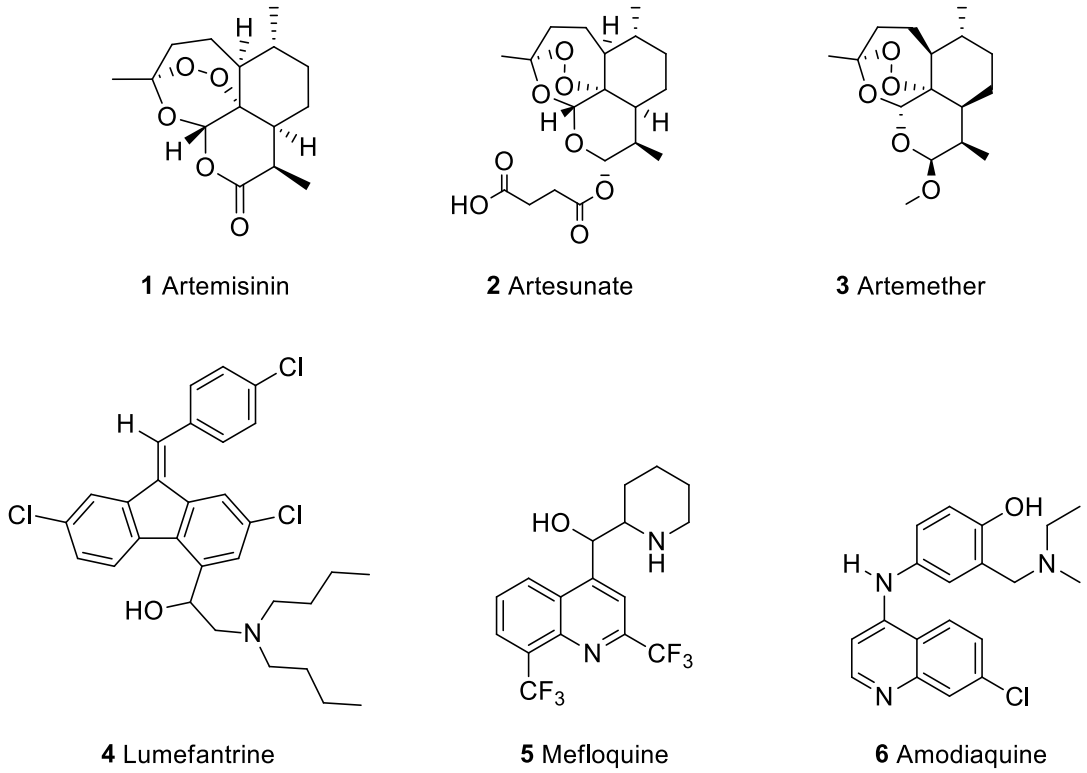
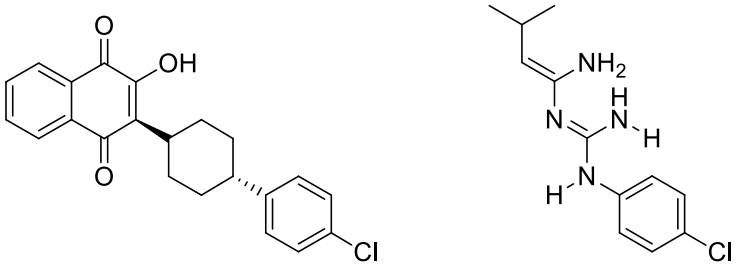


Figure 3-1: Chemical structures of artemisinin, artesunate, artemether, Arylaminoalcohols (Lumefantrine and Mefloquine) and 4-Aminoquinolines (Amodiaquine) used in ACT therapy.

Naphthoquinones are molecules widely distributed in nature that possess a broad spectrum of biological activities, such as anti-bacterial, anti-fungal, anti-parasitic, anti-viral, anti-inflammatory, and cytotoxic effects [10]. This has encouraged their study and recognition as privileged structures in medicinal chemistry. The cytotoxic activity exhibited by these quinone derivatives is facilitated by several mechanisms, including the inhibition of DNA topoisomerase-II (an enzyme vital in the replication of DNA and the condensation and segregation of chromosomes) and through the formation of semi-quinones and superoxide radicals that contribute to the production of hydroxyl radical (the main source of oxidative generated damage to cellular DNA) [11].

Atovaquone is a hydroxy-1,4-naphthoquinone, as well as a structural analogue of protozoan ubiquinone and a co-enzyme Q analogue. Atovaquone has been shown to specifically bind to the ubiquinone binding site of the cytochrome bc1 complex of the mitochondrial respiratory chain in the malarial parasite and other protozoa [6, 12, 13]. It is a key drug used in a fixed-dose combination with proguanil for the treatment of uncomplicated malaria in adults and children, for malaria chemoprophylaxis, and as alternative therapy in case of resistances against chloroquine or artemisinin-based therapies [14]. Atovaquone inhibits the mitochondrial electron transport chain, specifically targeting the cytochrome bc1 complex in the *Plasmodium* respirational system, and interferes in many processes that are important for its survival, including protein synthesis

and haem biogenesis. New studies suggest a “cross-relation” between the respiratory chain and pyrimidine biosynthesis [15].



7 Atovaquone **8** Proguanil

Figure 3-2: Chemical structures of Atovaquone and Proguanil

Proguanil by itself has weak anti-*Plasmodium* activity, but it is converted to the active metabolite cycloguanil, an effective dihydrofolate reductase inhibitor, following absorption and hepatic breakdown by cytochrome P450 3A and 2C subfamilies.

A possible theory regarding the mechanism of the synergism between atovaquone and proguanil suggests that when electron transport, which is central to establishing the mitochondrial membrane potential, is inhibited by atovaquone, it triggers an alternative pathway involving ATP hydrolysis and exchange of generated ADP3- against ATP4- by the ATP/ADP transporter. The new pathway is then inhibited by proguanil, resulting in a quick breakdown of membrane potential. Through this synergism the selection of resistant strains during therapy is reduced [6].

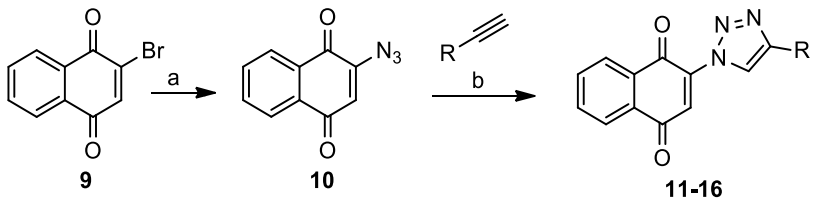
1,2,3-Triazole is a versatile moiety found in a large variety of bioactive molecules, *viz.* tazobactam (α-lactamase inhibitor), cefatriazine (orally active semisynthetic cephalosporin antibiotic) and rufinamide (anticonvulsant). This moiety is also found in antitubercular, antibacterial, antifungal, anticancer, antioxidant, antimalarial, antidiabetic medication [16]. The 1,2,3-triazole core is stable against acidic and basic hydrolysis, as well as against oxidative and reductive conditions, reflecting a high aromatic stabilisation and relative resistance to metabolic degradation [17].

The development of new antimalarials with novel mechanisms of action, i.e. active against new targets, are needed to fight this battle [18]. Molecular hybridisation involves linking two chemical moieties with separate intrinsic activity into a single agent, thus packaging dual activity into a single hybrid molecule. This is a key strategy for the development of new drugs that are able to act as multi-target ligands [19, 20]. Triazole-naphthoquinone hybrids have recently been a popular topic in research on medicinal chemistry because of their structural diversity and biological properties [21]. Reported herein are the synthesis, antimycobacterial activity and cytotoxicity of a series of on 1,4-naphthoquinone and 1,2,3-triazole hybrids.

3.2 Results and discussion

3.2.1 Chemistry

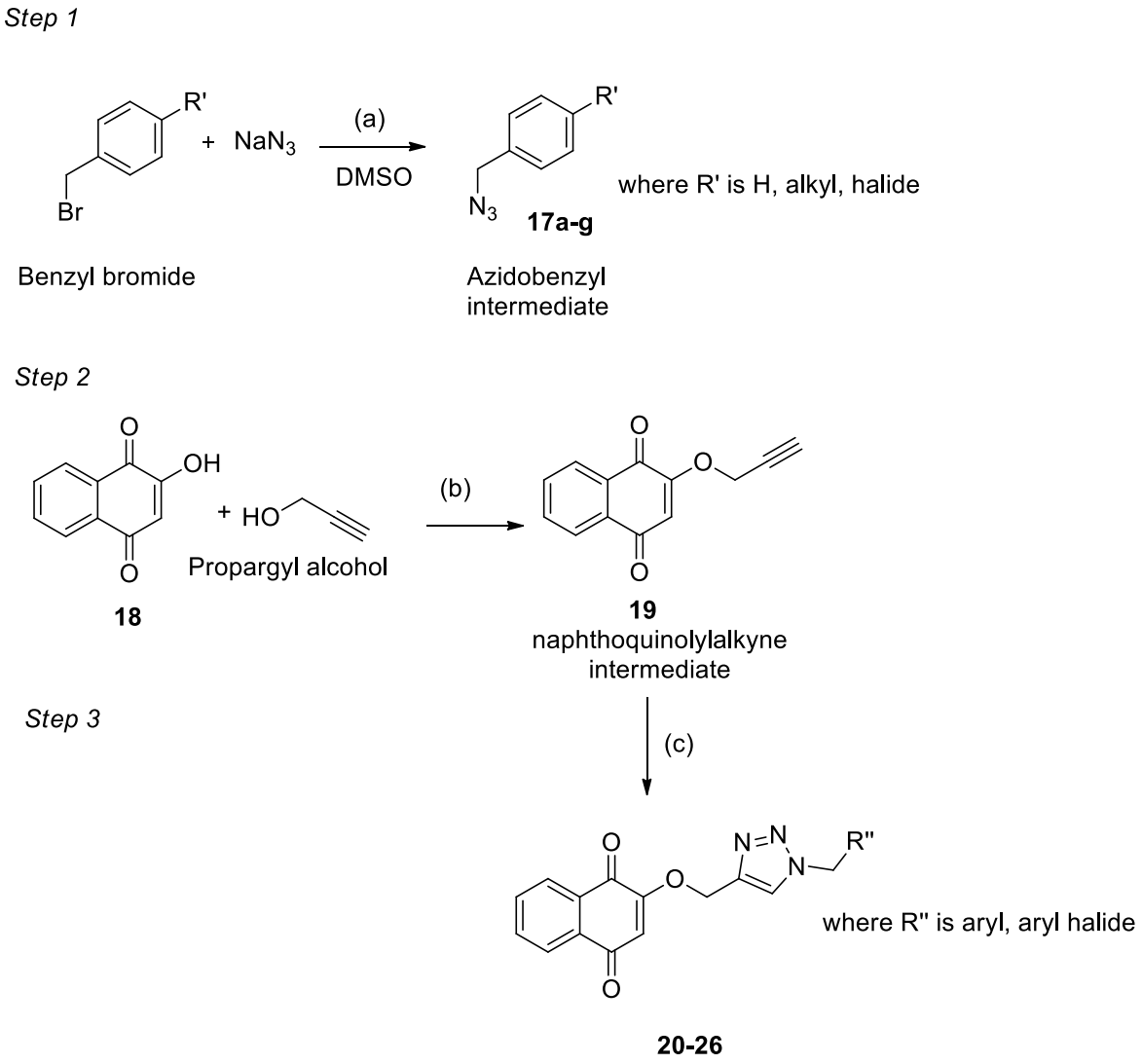
A series of 13 hybrids was synthesised by covalently linking 1,4-naphthoquinone and 1,2,3-triazole antimalarial pharmacophores. It comprises two Sub-series. In Sub-series 1 (six compounds) the two moieties were directly linked, while in sub-series 2 (seven compounds) they were separated by an oxymethylene tether. Sub-series 1 hybrids were synthesised in a two-step process, starting with the formation of azido intermediate **10** from commercially available 2-bromo-1,4-naphthoquinone *via* nucleophilic aromatic substitution with sodium azide [22] Subsequent treatment of this intermediate with various alkynes, followed by Huisgen copper alkyne-azide cycloaddition in a regioselective manner [23], afforded Sub-series 1 hybrids **11**-**16** (Scheme 3-1) after purification by column chromatography on silica gel with poor to low yields (2 – 22 %). Purification of these compounds by column chromatography caused break-down leading to the poor yields. Recrystallisation was tried as an alternative purification method to no avail.



Scheme 3-1: Synthesis of Sub-series 1 hybrids

Reagents and conditions: (a) **10**: **9** (1 eq.), sodium azide (5 eq.), DMF, 60°C, 1.5 hr; (b) **11-16**: alkyne (1.2 eq.), β-cyclodextrin (0.02 eq.), sodium ascorbate (0.2 eq.), **10** (1 eq.), $CuSO_4$ (0.1 eq.), DMF, 45°C, overnight.

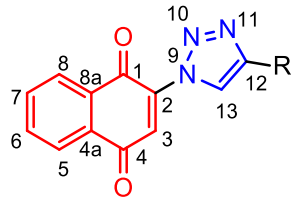
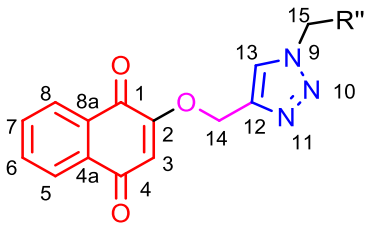
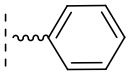
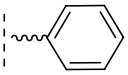
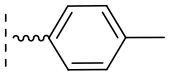
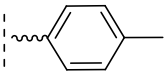
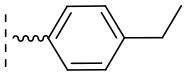
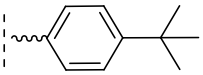
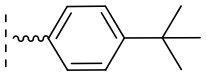
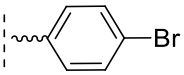
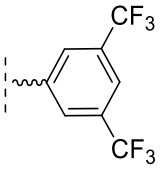
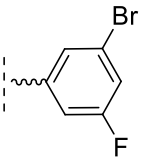
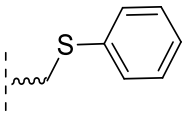
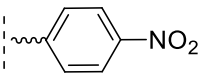
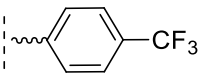
The synthesis of Sub-series 2 hybrids, on the other hand, followed a three-step process, involving two nucleophilic reactions and a cycloaddition. First, commercial benzyl bromides were azido functionalised using sodium azide in SN2 reaction to afford intermediates **17a-g**. In parallel, commercial 2-hydroxy-1,4-naphthoquinone underwent SN2 Mitsunobu reaction with readily available propargyl alcohol to give naphthoquinoly lalkyne intermediate **19** [24]. Click chemistry of intermediates **17** and **19** resulted in the target hybrids (Scheme 3-2) in poor to high yields (13 – 96%) after purification. The major variation in yield could be attributed to the instability of certain compounds especially during the purification process. Indeed, it was observed that compound **24**, obtained in the lowest yield (13%) was di-halide aryl substituted with 2 EWG (Br and F). These electron-withdrawing groups rendered the compound less stable to withstand the flush chromatography purification process, which ultimately resulted in the poor yield.



Scheme 3-2: Sub-series 2 synthesis route of the azidobenzyl intermediates and the target hybrids

Reagents and conditions: (a) **17a-g**: benzyl bromide or/and *para*-substituted (1.0 eq.), sodium azide (1.5 eq.), DMSO, rt, overnight. (b) **19**: 2-hydroxy-naphthoquinone (1 eq.), propargyl alcohol (2 eq.), PPh_3 (2 eq.), DIAD (2 eq.), ice-bath, DCM, 2 hr, rt then 40 °C, overnight. (c) **20 - 26**: alkyne (1 eq.), β-cyclodextrin (0.02 eq.), sodium ascorbate (0.2 eq.), 17 (1.2 eq., $CuSO_4$ (0.2 eq.), DMF, 45 ºC, 25 hr.

Table 3-1: Synthesised hybrids.

 **Sub-series 1**	 **Sub-series 2**
Compound R	**Compound R"**
11 	**20** 
12 	**21** 
13 	**22** 
14 	**23** 
15 	**24** 
16 	**25** 
	26 

The formation of the target compounds was confirmed by routine chemical structure characterisation techniques, IR, NMR, and HRMS. The ^{1}H spectra of all title compounds were thoroughly examined for characteristic peaks, evidence of the presence of the naphthoquinone and triazole scaffolds, while the IR-spectra were also inspected for the presence of characteristic absorptions, allowing for the identification of functional groups.

Thus, the ^{1}H NMR spectra of all Sub-series 1 hybrids commonly showed a singlet in the 8.08-9.08 ppm range that is attributed to the triazolyl proton resonance (H-13). The naphthoquinolyl protons, H-5 and H-8, were represented by two doublets at 8.0 ppm and 8.10 ppm, respectively.

In ^{13}C spectra, there were two pronounced signals representing the resonance of naphthoquinone carbonyl C-4 and C-1 at 181.17 and 176.31 ppm, respectively.

For Sub-series 2 compounds, the singlet at 5.60 ppm was assigned to the resonance of H-14 protons of methylene CH_2 in the linker. In correlation to the first Sub-series, the resonance of the triazolyl H-13 proton presented a singlet at 8.37 ppm. The ^{13}C spectra showed distinctive carbonyl carbon C-4 and C-1 peaks at 185.00 and 179.94 ppm, respectively. The resonance of the methylene carbon C-14 was observed at 62.82 ppm, and the singlet at 53.4 ppm was characteristic of carbon benzyl methylene C-15.

The compounds underwent HRMS with the APCI source and were confirmed to be present. In Sub-series 1, there was M+NH fragment for the six compounds. This is suspected to be an extra NH from ammonia breakdown from this process. Sub-series 2 were more stable and were confirmed with a normal fragment of M+1.

In the IR-spectra of all compounds the presence of the double carbonyl C=O was confirmed with strong peaks, *ca*. 1620 – 1700 cm^{-1}, the =C-H stretches of the triazole showed *ca*. 3500-3000 cm^{-1} and 3500 cm^{-1} and C-H stretches of the aromatic rings are between 1600-1450 cm^{-1}.

3.2.2 Physiochemical properties

The *n*-octanol/water partition factor (logP) is an important indicator used for the measurement of the balance between hydrophilicity and lipophilicity. This gives a good prediction of the transport characteristics of a substance across bio-membranes through passive diffusion [25] . LogP values between 1 and 5 are usually targeted, while values between 1 and 3 are ideal [26]. In Sub-series 1, compounds **11 - 16** exhibited ClogP values between 3 - 5, which were slightly higher than those of sub-series 2, **20** – **26,** in the 2- 4 range. This implies that hybrids in Sub-series 2 have more drug-like partition coefficients and are thus expected to possess good antimalarial activity as a result of efficient bio-membrane permeation.

3.2.3 Biological activity

Dual-point screening experiments at 5 µM and 1 µM concentrations were performed to assess the parasite inhibition potential of each compound against the chloroquine-sensitive NF54 *P. flaciparum* parasites. Classification of the results in table 3-2 below, relate that a compound with more than 50 % inhibition at 1 µM concentration has good activity and has a predicted IC_{50}-value under 1 µM and, therefore, warrants further investigation.

The results in sub-series 1 showed one compound, **11**, with a moderate activity of 55% growth inhibition at 5 µM. In contrast, sub-series 2 uncovered five compounds, **20**, **21**, **22**, **23** and **26**, with significant activity of 69 – 90% growth inhibition, with compound **22** showing the best activity. Permeability through a bio-membrane requires a multifunctional interaction of factors such as molecular size, lipophilicity, polar van der Walls surface, and the molecular flexibility [27] . Thus, it could be suggested that the flexible linker endowed sub-series 2 hybrids with the observed activity.

Comparing the structure activity relationship (SAR) of sub-series 1 and 2 suggests that the more rigid structure of sub-series 1 lowers activity and the more flexible structure of sub-series 2 improves activity. Comparison of the activities of hybrids **11, 12** and **14** in Sub-series 1 with those of their counterparts, **20**, **21** and **22** in Sub-series 2 reveals the latter has high inhibition of parasite, while the former is devoid of this ability. This is attributed to the oxymethylene tether between the naphthoquinone and triazole scaffolds resulting in more flexible structures in sub-series 2. This further supports the importance of flexibility as a crucial and influential parameter of the activity of these compounds.

Furthermore, Sub-series 1 compounds were 99% pure but showed no antiplasmodial activity. In contrast Sub-series 2 compounds had purity in the 88 - 95% range. Yet, compound **20** (90% purity) showed 80% parasite inhibition, **22** (93% purity) displayed 90% parasite inhibition and **26** (94 % purity) possessed 82% parasite inhibition. This may suggest that the limited impurity in the later Sub-series could not reasonably instil the observed biological activity which infers that Sub-series 2 compounds was endowed with intrinsic activity.

Table 3-2: *In vitro* antimalarial activities of hybrid compounds against asexual stage *P. falciparum* NF54 strain using SYBR Green I based assay.

Compd	Clog*P*[a]	NF54 % growth inhibition ± SD[b]		Cytotoxicity IC_{50} (µM)[c]
		5 µM	1 Mm	HEK-293
AV **7**	6.4	97.89 ± 0.65	96.05 ± 0.95	55.85 ± 0.05
11	3.3	55.27 ± 5.46	1.019±1.313	> 100
12	3.8	8.12 ±1.4	0.10 ± 0.17	> 100
13	4.3	8.18 ± 5.15	0	> 100
14	5.2	4.48 ± 6.23	0	> 100
15	5.1	5.94 ± 1.25	1.42 ± 2.46	> 100
16	3.3	15.91 ± 6.31	2.84 ±2.54	> 100
20	2.4	71.67 ± 2.09	80.36 ± 2.02	32.43 ± 5.52
21	2.9	78.79 ± 1.4	68.95 ± 1.22	> 100
22	4.2	80.45 ± 0.78	89.93 ± 1.03	36.42 ± 2.32
23	3.3	82.10 ± 1.19	72.581±3.484	> 100
24	3.4	89.32 ± 0.45	29.42 ± 4.80	> 100
25	2.4	82.98 ± 1.69	17.11 ± 7.55	> 100
26	3.3	78.08 ± 1.24	82.18 ± 1.37	> 100
EM				0.02 ± 0.002

[a] cLogP values calculated using ChemDraw Ultra Version 12; [b]compounds screened, *P*fNF54; [c] HEK-293 cell line of normal human embryonic kidney cells; AV: atovaquone

The data allows the analysis of the potential impact the electronic effect of the substituent on the aryl ring could have on the activity in Sub-series 2, considering the inhibitory percentages at 5 µM concentration. In Sub-series 2, hybrids **20**, **21** and **22** are of increasing electron donating (ED) effect of substituent, the electron donating groups (EDGs) being in the order H, CH_3 and 3 (CH_3) or hydrogen, methyl and tert-butyl. The electron withdrawing groups (EWGs) are F, Br, NO_2, CF_3 with the increasing electronic effect order F<Br<NO_2<CF_3. Compounds **20** - **22** are more ED and show good activity, **23** show good antimalarial activity as well, but has a relatively neutral electronegativity. Compound **26** has an EWG but shows good activity, thus electronegativity doesn't seem to play an important role in the activity of the compounds.

The cytotoxicity of the 13 compounds were tested in human embryonic kidney (HEK-293) cells. Only two compounds were found to be moderately cytotoxic in contrast to the other non-cytotoxic compounds. The first sub-series showed no cytotoxicity (IC_{50} > 100 µM) against HEK-293 cells. The second sub-series presented with two compounds, 20 and 22, with mild to moderate cytotoxicity, IC_{50}: 32 µM and 36 µM, respectively. This is comparable to the cytotoxicity of atovaquone, IC_{50}: 56 µM.

3.3 Conclusion

Two sub-series of 1,4-naphthoquinone-triazole compounds were synthesised using a slightly modified version of Huisgen's "click-chemistry" method, *viz.* sub-series 1 (six hybrids directly linked between the naphthoquinone and the triazole) and sub-series 2 (an oxymethylene linker was introduced between the naphthoquinone and the triazole of seven hybrids).

These compounds were tested for antimalarial activity against the asexual stage of the chloroquine sensitive, *P. falciparum* NF54 strain using SYBR Green I based assay. Only one compound in sub-series 1 showed moderate activity with 55 % growth inhibition at 5 µM, compared to sub-series 2 where all the compounds had moderate to good activity at 5 µM with the percentage growth inhibition between 71 – 82 %. The percentage growth inhibition at a concentration of 1 µM of this sub-series showed good activities of 68 – 89 % inhibition. The second sub-series will undergo further analysis and the IC_{50} values will be determined, it is predicted to be in the nanomolar range.

Electronegativity does not play a significant role in the antimalarial activity. This is confirmed in the second sub-series where compounds bearing either EDGs or EWG were active. On the other hand, comparing the flexibility of the two sub-series indicated that flexibility is an important parameter governing the activity of the compounds. Cytotoxicity data showed most hybrids to be non-toxic to kidney HEK-293 cells.

Hybrid **22,** which features the *tert*-butyl moiety and showed 90 % parasite growth inhibition at 1 µM concentration, was as active as the standard drug, atovaquone. It stands as a good candidate for further investigation in the search for novel effective antimalarials.

3.4 Materials and Methods

3.4.1 Materials

2-bromo-1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, sodium azide, dimethylformamide (DMF), β-cyclodextrin, sodium ascorbate, copper sulphate, 2-hydroxy-1,4-naphthoquinone, triphenylphosphine (PPh_3), propargyl alcohol, diisopropyl azodicarboxylate (DIAD),

phenylacetylene, 4-ethynyltoluene, phenyl propyl sulphate, 1-ethynyl-3,5-bis(trifluoromethyl)benzene, 1-ethynyl-4-ethylbenzene, 4-tert-butylphenylacetylene, 4-methylbenzyl bromide, 4-bromobenzyl bromide, 4-benzyl bromide, 4-tert-butyl benzyl bromide, 4-trifluoro benzyl bromide, 4-nitrobenzyl bromide, 4-bromo-2-fluorobenzyl bromide and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (South-Africa). All solvents used were purchased from Associated Chemical Enterprises (ACE, South Africa). All chemicals and reagents were of analytical grade and were used without further purification. Dichloromethane (DCM), ethyl acetate, petroleum ether was distilled over calcium hydride and kept on molecular sieves prior to use in reactions.

3.4.2 General procedures

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance™ III 600 spectrometer at a freq.uency of 600 MHz and 150.913 MHz, respectively, in DMSO-d_6 or $CDCl_3$. Chemical shifts δ are reported in parts per million (ppm), with the residual protons of the solvent as reference. The splitting pattern abbreviations are as follows: singlet (s), doublet (d), doublet of doublet (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), doublet of quartets (dq), triplet (t), triplet of doublets (td), triplet of triplets (tt), quartet of doublets (qd) and multiplet (m).

HRMS was recorded on a Bruker MicroTOF Q II mass spectrometer, eq.uipped with an APCI or an ESI source, set at 200°C or 180°C, respectively, using Bruker Compass DataAnalysis 4.0 software. A full scan from 50 - 1500 m/z was performed at a capillary voltage of 4500 V, an end plate offset voltage of -500 V, with the nebulizer set at 1.6 Bar and 0.4 Bar, respectively, and a collision cell RF voltage of 100 Vpp.

IR spectra were recorded on a Bruker Alpha-P FTIR instrument. Thin layer chromatography (TLC) was performed, using silica gel plates ($60F_{254}$), obtained from Merck (Johannesburg, South Africa). Column chromatography was performed, using MN silica gel 60, 70 - 230 mesh ASTM, supplied by Macherey-Nagel (Germany).

High performance liquid chromatography (HPLC) analysis of the final compounds were performed to determine purity. An Agilent 1100 HPLC system eq.uipped with a quaternary pump and an Agilent 1100 series diode array detector was utilized. HPLC grade acetonitrile (Merck) and Milli-Q water (Millipore) were used for chromatography. A Venusil XBP C18 column (4.60 x 150 mm, 5 µm) with an initial mobile phase (70 % MilliQ water: 30 % acetonitrile) was employed at a flow rate of 1ml/min. The concentration of acetonitrile in the mobile phase was linearly increased over a period of five minutes to a final concentration of 85 %. The time allowed for equilibration between runs was five minutes and the duration of each HPLC run was 15 minutes. The concentration of

the test compounds injected varied (20 µl of 1 mM to 20 µl of 0.25 mM). The eluent was monitored at wavelengths of 210, 254, and 300 nm.

Melting points (m.p.) were determined with a BÜCHI melting point B-545 instrument and were uncorrected.

3.4.3 General procedure for the synthesis of azidonaphthalene-1,4-dione, 10

10: 2-bromo-1,4-naphthoquinone (2.1 mmol, 0.5 g, 1eq.) and sodium azide (10.6 mmol, 0.7 g, 5 eq.) was added to DMF (8 ml) at 60 °C and was left to stir for 90 min to give a dark red liquid. The solvent was evaporated, the residue dissolved in DCM (150 ml) and washed three times with NH_4Cl (50 ml). The resulting organic layer was then dried over magnesium sulphate ($MgSO_4$). This mixture was purified by column chromatography on silica gel eluting with Pet.Ether/EtOAc (7:3, v/v).

Red crystals. Yield: 292 mg (40 %). IR v_{max}: 2922 (N-N, azide), 1682 (C=O, 1), 1658 (C=O, 4). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.06 (d, 2H, H-5, H-8), 7.73 – 7.71 (m, 2H, H-6, H-7), 7.19 (s, 1H, H-3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.27 (C-4), 176.06 (C-1), 134.48 - 127.45 (Ar), 126.76 (C-3)

3.4.4 Synthesis of Sub-series 1 hybrids, 11 - 16

The compounds were prepared in accordance with the general procedure depicted in Scheme 3-1 and is described as follows:

11 - 16: DMF (8 ml) in a ball flask was heated in a sand bath to 45 °C. The alkyne (1 eq.) was added, followed by β-cyclodextrin (0.3 mmol, 39 mg, 0.02 eq.), sodium ascorbate (0.25 mmol, 61 mg, 0.2 eq.), azido-intermediate **10** (1.25 mmol, 300 mg, 1.2 eq.) and $CuSO_4$ (0.16 mmol, 42 mg, 0.1 eq.), forming a brown mixture. The reaction was left to stir for 24 hr. The completed reaction was confirmed with TLC. The solvent was evaporated and the residue suspended in DCM (150 ml) and washed with H_2O (3 x 50 ml). The resulting organic layer was then dried over magnesium sulphate ($MgSO_4$). The mixture was purified by column chromatography on silica gel eluting with Pet.Ether /EtOAc (7:3 v/v).

3.4.4.1 2-(4-phenyl-1H-1,2,3-triazol-1-yl) naphthalene-1,4-dione, 11

The reaction with phenylacetylene afford hybrid **11** as yellow-orange fine crystals. Yield**:** 108 mg (3%), Purity (HPLC): 99%. m.p. 76.1-77.1 °C; IR v_{max}: 3302 (=C-H, TZ), 1608 (C=O, 1), 1564 (C=O, 4) cm^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.68 (s,1H, H-13), 8.08 (dd, *J* = 22.4, 7.7 Hz, 2H, H-5, H-8), 7.97 – 7.87 (m, 3H, H-3, H-6, H-7), 7.86 – 7.76 (m, 2H, H-15, H-19), 7.50 (dd, *J* = 10.7,

4.8 Hz, 2H, H-16, H-18), 7.39 – 7.33 (m, 1H, H-17). ^{13}C NMR (151 MHz, DMSO) δ 181.05 (C-4), 176.32 (C-1), 46.36 (C-12), 135.95 - 126.4 (Ar), 111.97 (C-3), 40.48 (C-18). HRMS (APCI) *m/z*: $[M+NH]^+$ 317.0930 (calcd for $C_{18}H_{11}N_3O_2$: 317.1044)

3.4.4.2 2-(4-(*p*-tolyl)-1H-1,2,3-triazol-1-yl) naphthalene-1,4-dione, 12

The reaction with 4-ethynyltoluene produced hybrid **12** as bright orange paper-like crystals; Yield: 85 mg (4%). Purity (HPLC): 99%. m.p. 76.3-76.9 ℃; IR v_{max}: 3311 (=C-H, TZ), 1694 (C=O, 1), 1681 (C=O, 4) cm^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.61 (s, 1H, H-13), 8.07 (dd, *J* = 23.7, 7.4 Hz, 2H, H-5, H-8), 7.92 (t, *J* = 7.1 Hz, 1H, H-3), 7.86 – 7.82 (m, 2H, H-6, H-7), 7.81 (d, *J* = 10.2 Hz, 2H, H-15, H-19), 7.30 (d, *J* = 7.9 Hz, 2H, H-16, H-18), 2.36 (s, 1H, H-20). ^{13}C NMR (151 MHz, DMSO) δ 181.19 (C-4), 176.26 (C-1), 146.46 (C-12), 137.59 - 124.70 (Ar), 112.03 (C-3), 21.37(C-20). HRMS (APCI) *m/z*: $[M+NH]^+$ 331.1224 (calcd for $C_{19}H_{13}N_3O_2$: 331.1086)

3.4.4.3 2-(4-(4-ethylphenyl)-1H-1,2,3-triazol-1-yl)naphthalene-1,4-dione, 13

The reaction with 1-ethynyl-4-ethynylbenzene formed hybrid 13 as bright orange paper-like crystals. Yield: 55 mg (22%), Purity (HPLC): 99%. m.p. 76.1-76.8 ℃; IR v_{max}: 3338 (=C-H, TZ), 1679 (C=O, 1), 1625 (C=O, 4) cm^{-1}^{1}H NMR (600 MHz, DMSO) δ 8.61 (s, 1H, H-13), 8.07 (dd, *J* = 21.9, 7.6, 2H, H-5, H-8), 7.91 (t, *J* = 13.1, 6.5 Hz, 2H, H-6, H-7), 7.88 (d, 2H, H-15, H-21), 7.32 (d, *J* = 8.2 Hz, 2H, H-16, H-20), 2.66 (q, *J* = 7.6 Hz, 2H, H-18), 1.23 (t, *J* = 7.6 Hz, 3H, H-19). ^{13}C NMR (151 MHz, DMSO) δ 181.16 (C-4), 176.42 (C-1), 146.42 (C-12), 143.84 (C-17), 135.94 - 124.72 (Ar), 112.00 (C-3), 28.45 (C-14), 15.82 (C-19). HRMS (APCI) *m/z*: $[M+NH]^+$ 345.1347 (calcd for $C_{20}H_{15}N_3O_2$: 345.1243)

3.4.4.4 2-(4-(4-(tert-butyl) phenyl)-1H-1,2,3-triazol-1-yl)naphthalene-1,4-dione, 14

The reaction with 4-tert-butylphenylacetylene afforded hybrid **14** as bright yellow-orange paper-like crystals. Yield: 51 mg (20 %), Purity (HPLC): 99%. m.p. 85.9-86.3 ℃; IR v_{max}: 3322 (=C-H, TZ), 1686 (C=O, 1), 1630 (C=O, 4) cm^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.62 (s, 1H, H-13), 8.09 (dd, 2H, H-5, H-8), 7.92 (d, *J* = 7.6, 1.1 Hz, 1H, H-3), 7.87 (d, *J* = 8.4 Hz, 2H, H-6, H-7), 7.82 (d, 2H, H-15, H-21), 7.50 (d, 2H, H-16, H-20), 1.33 (s, 9H, H-19). ^{13}C NMR (151 MHz, DMSO) δ 181.17 (C-4), 176.31 (C-1), 150.75 (C-12), 146.46 (C-17), 135.95 (C-8a), 133.44 - 125.46 (Ar), 112.00 (C-3), (C-18), 31.60 (C-19). HRMS (APCI) *m/z*: $[M+NH]^+$ 373.1664 (calcd for $C_{22}H_{19}N_3O_2$: 373.1556)

3.4.4.5 2-(4-(3,5-bis(trifluoromethyl)phenyl)-1H-1,2,3-triazol-1-yl)naphthalene-1,4-dione, 15

The reaction with 1-ethynyl-3,5-bis(trifluoromethyl)benzene produced hybrid **15** as bright orange paper-like crystals. Yield: 134 mg (9%), Purity (HPLC): 98%. m.p. 74.1-75.0 °C; IR ν_{max}: 3305 (=C-H, TZ), 1682 (C=O, 1), 1620 (C=O, 4) cm^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 9.08 (s, 1H, H-13), 8.62 (s, 2H, H-5, H-8), 8.08 (dd, *J* = 25.7, 7.3 Hz, 4H, H-15, H-18, H-3, H-21), 7.95 – 7.78 (m, *J* = 13.1, 6.5 Hz, 2H, H-6, H-7). ^{13}C NMR (151 MHz, DMSO) δ 180.97 (C-4), 176.21 (C-1), 146.83 (C-12), 143.83 (C-8), 135.95 – 121.60 (Ar), 111.54 (C-3). HRMS (APCI) *m/z*: $[M+NH]^+$ 453.0803 (calcd for $C_{20}H_9F_6N_3O_2$: 453.0677)

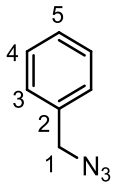
3.4.4.6 2-(4-((phenylthio)methyl)-1H-1,2,3-triazol-1-yl)naphthalene-1,4-dione, 16

The reaction with phenyl-propyl-sulphate resulted hybrid **16** as orange wet-looking crystals. Yield: 131 mg (14%), Purity (HPLC): 91%. m.p. 110.1-112.3 °C; IR ν_{max}: 3307 (=C-H, TZ) 1684 (C=O, 1), 1618 (C=O, 4) cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 (s, 1H, H-13), 7.66 (dd, *J* = 45.8, 7.5 Hz, 3H, H-5, H-8, H-3), 7.26 (dt, *J* = 61.9, 7.5 Hz, 2H, H-6, H-7), 7.07 – 6.87 (m, 2H, H-17, H-18), 6.88 – 6.65 (m,2H, H-16, H-20), 3.85 (s, 2H, H-14). ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.58 (C-4), 175.71 (C-1), 141.10 (C-12), 135.51-125.18 (Ar), 112.65 (C-3), 29.00 (C-14). HRMS (APCI) *m/z*: $[M+NH]^+$ 363.0943 (calcd for $C_{19}H_{13}N_3O_2S$: 363.0807)

3.4.5 Synthesis of benzylazide intermediates, 17a-g

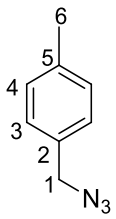
Benzyl bromide or benzyl bromide *para*-substituted **17** (1.0 eq.) was dissolved in DMSO (15 ml) together with sodium azide (NaN_3, 1.5 eq.). The reaction mixture was stirred at room temperature overnight. The resulting reaction mixture was diluted with water (30 ml) and the aqueous phase extracted with diethyl ether (3 x 40 ml). The combined organic layers were washed with brine (3 x 50 ml) and dried over $MgSO_4$, leaving a clear oil.

3.4.5.1 (azidomethyl) benzene, 17a



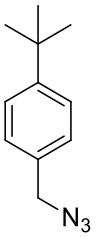
Light yellow oil. Yield: 1.43 g (70%); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.42 – 7.32 (m, 5H, Bz), 4.35 (s, 2H, H-1); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 135.49 (C-4), 128.96 (C-3), 128.43 (C-5), 128.34, 54.93 (C-1).

3.4.5.2 1-(azidomethyl)-4-methylbenzene, 17b



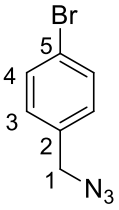
Light yellow oil, Yield: 2.15 g (95%); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.23 – 7.18 (m, 4H, Bz), 4.30 (s, *J* = 8.1 Hz, 2H, H-1), 2.37 (s, 3H, H-6); ^{13}C NMR (151 MHz, CDCl3) δ (ppm): 138.28 (C-3), 132.41 (C-4), 128.40 (C-5), 54.75 (C-1), 21.31 (C-6).

3.4.5.3 1-(azidomethyl)-4-(*tert*-butyl) benzene, 17c



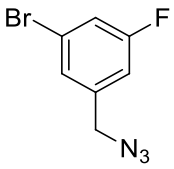
Light yellow liquid, Yield: 2.62 g (90%). This compound was synthesised using the method described above and was used directly in subsequent reaction without purification.

3.4.5.4 1-(azidomethyl)-4-bromobenzene, 17d



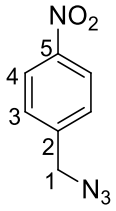
Light yellow liquid, Yield: 3.23 g (99%); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.54 (m, 2H, H-4), 7.22 (d, *J* = 8.2 Hz, 2H, H-3), 4.33 (d, *J* = 8.4 Hz, 2H, H-1); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 134.52 (C-2), 132.13 (C-4), 129.94 (C-3), 54.23 (C-1).

3.4.5.5 1-(azidomethyl)-3-bromo-5-fluorobenzene, 17e



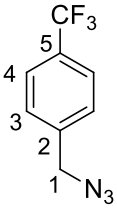
Light yellow liquid, Yield: 3.56 g (99%). This compound was synthesised using the method described above and was used directly in subsequent reaction without purification.

3.4.5.6 1-(azidomethyl)-4-nitrobenzene, 17f



Yellow oil; Yield: 2.19 g (80%); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.24 (d, *J* = 7.8 Hz, 2H, H-3), 7.50 (d, *J* = 8.5 Hz, 2H, H-4), 4.50 (s, 2H, H-1); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 147.88 (C-5), 142.83 (C-2), 128.72 (C-3), 124.18 (C-4), 53.86 (C-1).

3.4.5.7 1-(azidomethyl)-4-(trifluoromethyl) benzene, 17g



Light yellow oil, Yield: 2.32 g (75%), ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.15 (d, *J* = 7.7 Hz, 2H, H-3), 6.95 (d, *J* = 7.8 Hz, 2H, H-4); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 125.92 (C-4), 54.20 (C-1).

3.4.6 Synthesis of naphthoquinolylalkyne intermediate, 19

A slightly modified version of Mitsunobu reaction was used to synthesise the naphthoquinone-alkyne intermediate **19**. 2-hydroxy-1,4-naphthoquinone (11.5 mmol, 2 g, 1 eq.) together with propargyl alcohol (23 mmol, 1.4 ml, 2 eq.), PPh_3 (23 mmol, 6.020 g, 2 eq.) and DIAD (drop-wise) (23 mmol, 4.5 ml, 2 eq.) was added to dried DCM (200 ml) in an ice bath and was left to stir for two hrs with an Argon balloon. The mixture was removed and stirred until it reached RT. The reaction was then heated in a sand bath at 40 °C for two hours after which it was left to stir at RT overnight. The mixture was washed three times with H_2O (50 ml). The resulting organic layer was then dried over magnesium sulphate ($MgSO_4$). The mixture was purified by recrystallisation in EtOAc (50 ml) and Hexane (120 ml).

Light beige crystals. Yield: 2.49 g (80%). ^{1}H NMR (600 MHz, DMSO) δ 8.06 – 7.95 (m, 2H, H-5, H-8), 7.95 – 7.75 (m, 2H, H-6, H-7), 6.42 (s, 1H, H-3), 4.95 (d, *J* = 2.4 Hz, 1H, H-14), 2.58 – 2.42 (s, 1H, H-12). ^{13}C NMR (151 MHz, $CDCl_3$) δ 184.73 (C-4), 179.85 (C-1), 158.05 (C-2), 134.42 - 126.25 (Ar), 111.68 (C-3), 77.36 (C-12), 56.77 (C-14).

3.4.7 Synthesis of Sub-series 2 hybrids, 20 - 26

20 – 26: DMF (10 ml) was heated in a sand bath to 45 °C. **19** (400 mg, 1 eq.) was added, followed by β-cyclodextrin (0.03 mmol, 42 mg, 0.02 eq.), sodium ascorbate (0.1 mmol, 75 mg, 0.2 eq.), azido-intermediate **17** (1.2 eq.) and $CuSO_4$ (0.34 mmol, 92 mg, 0.2 eq.) forming a brown mixture. The reaction was left to stir for 24 hours. The completed reaction was confirmed with thin layer chromatography (TLC). The solvent was evaporated, the residue suspended in DCM (150 ml) and washed three times with NH_4Cl (50 ml). The resulting organic layer was then dried over magnesium sulphate ($MgSO_4$). The mixture was purified by recrystallisation in EtOAc (30 ml) and Hexane (80 ml), giving a range of light yellow to orange crystals.

3.4.7.1 2-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)naphthalene-1,4-dione, 20

The reaction of **19** with **17a** resulted in hybrid **20**, light yellow-orange crystalline solid. Yield: 386 mg (59%). Purity (HPLC): 73%. m.p. 179.1-179.4 °C; IR v_{max}: 3146 (=C-H, TZ), 3061 (C-H, Bn), 1679 (C=O, 1), 1647 (C=O, 4) cm^{-1}. ^1H NMR (600 MHz, DMSO) δ 8.39 (s, 1H, H-14), 7.98 (t, *J* = 8.2 Hz, 2H, H-5, H-8), 7.84 (d, *J* = 23.9 Hz, 2H, H-6, H-7), 7.59 (d, *J* = 7.7 Hz, 2H, H-17, H-21), 7.30 (d, *J* = 7.8 Hz, 2H, H-8, H-20), 6.61 (s, 1H, H-3), 5.64 (s, 1H, H-9), 5.25 (s, 1H, H-15). ^{13}C NMR (151 MHz, DMSO) δ 185.00 (C-4), 179.93 (C-1), 159.40 (C-2), 141.63 (C-10), 135.77 - 126.07 (Ar), 122.00 (C-19), 111.38 (C-3), 62.81 (C-9), 52.65 (C-15). HRMS (APCI) *m/z*: $[M+H]^+$ 346.1207 (calcd for $C_{20}H_{15}N_3O_3$: 346.1192)

3.4.7.2 2-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)naphthalene-1,4-dione, 21

The reaction of **19** with **17b** afforded hybrid **21**, light orange powder. Yield: 443 mg (96%). Purity (HPLC): 90%. m.p.173.6-174.5 °C; IR v_{max}: 3051 (=C-H, TZ), 1683 (C=O, 1), 1652 (C=O, 4) cm^{-1}. ^1H NMR (600 MHz, DMSO) δ 8.34 (s, 1H, H-14), 7.98 (t, *J* = 7.4 Hz, 2H, H-5, H-8), 7.84 (dt, *J* = 24.2, 7.3 Hz, 2H, H-6, H-7), 7.24 (d, *J* = 7.9 Hz, 2H, H-18, H-21), 7.18 (d, *J* = 7.9 Hz, 2H, H-17, H-22), 6.60 (s, 1H, H-3), 5.58 (s, 2H, H-9), 5.22 (s, 2H, H-15), 2.28 (s, 3H, H-20). ^{13}C NMR (151 MHz, DMSO) δ 185.02 (C-4), 179.94 (C-1), 159.39 (C-2), 141.54 (C-10), 138.10 (C-19), 135.01 – 126.58 (Ar), 111.33 (C-3), 62.80 (C-9), 53.22 (C-15), 21.18 (C-20). HRMS (APCI) *m/z*: $[M+H]^+$ 360.1323 (calcd for $C_{21}H_{17}N_3O_3$: 360.1348)

3.4.7.3 2-((1-(3-(tert-butyl) benzyl)-1H-1,2,3-triazol-4-yl)methoxy)naphthalene-1,4-dione, 22

The reaction of **19** with **17c** produced hybrid **22**, light yellow paper-like crystals. Yield: 324 mg, (43%). Purity (HPLC): 92%. m.p. 178.4-180.3 °C; IR v_{max}: 2960 (=C-H, TZ), 1684 (C=O, 1), 1649 (C=O, 4) cm^{-1} ^1H NMR (600 MHz, DMSO) δ 8.37 (s, 1H, H-14), 7.98 (t, *J* = 7.4 Hz, 2H, H-5, H-8),

7.89 – 7.78 (d, 2H, H-6, H-7), 7.40 (d, *J* = 8.3 Hz, 2H, H-17, H-22), 7.28 (d, *J* = 8.2 Hz, 2H, H-18, H-23), 6.62 (s, 1H, H-3), 5.60 (s, 1H, H-13), 5.23 (s, 1H, H-15), 1.25 (s, 3H, H-21). ^{13}C NMR (151 MHz, DMSO) δ 185.00 (C-4), 179.94 (C-1), 159.40 (C-2), 151.20 (C-18), 141.56 (C-10), 135.01 – 125.85 (Ar), 111.36 (C-3), 62.82 (C-9), 53.12 (C-15), 34.78 (C-19), 31.52 (C-21). HRMS (APCI) *m/z*: $[M+H]^+$ 402.1848 (calcd for $C_{24}H_{23}N_3O_3$: 402.1818)

3.4.7.4 2-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) naphthalene-1,4-dione, 23

The reaction of **19** with **17d** afforded hybrid **23**, beige crystals. Yield: 100 mg (58%). Purity (HPLC): 94%. m.p.180.7 - 181.5 °C; IR v_{max}: 3122 (=C-H, TZ), 1684 (C=O, 1), 1648 (C=O, 4) cm^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.45 (s, 1H, H-14), 7.98 (t, *J* = 8.1 Hz, 2H, H-5, H-8), 7.84 (d, *J* = 7.4 Hz, 2H, H-6, H-7), 7.76 (d, *J* = 8.1 Hz, 2H, H-17, H-22), 7.54 (d, *J* = 8.1 Hz, 2H, H-18, H-21), 6.62 (s, 1H, H-3), 5.79 (s, 1H, H-9), 5.26 (s, 1H, H-15). ^{13}C NMR (151 MHz, DMSO) δ 185.01 (C-4), 179.94 (C-1), 159.39 (C-2), 141.71 (C-10), 141.01 (C-16), 135.01 - 126.21 (Ar), 111.38 (C-3), 62.78 (C-9), 52.73 (C-15). HRMS (APCI) *m/z*: $[M+H]^+$ 424.0289 (calcd for $C_{20}H_{14}BrN_3O_3$: 424.0297)

3.4.7.5 2-((1-(3-bromo-5-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)naphthalene-1,4-dione, 24

The reaction of **19** with **17e** afford hybrid **24**, light brown powder. Yield: 179 mg (13%). Purity (HPLC): 94%. m.p.179.4 - 181.1 °C; IR v_{max}: 3122 (=C-H, TZ), 1682 (C=O, 1), 1644 (C=O, 4) cm^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.47 (s, 1H, H-14), 7.97 (q, *J* = 8.4 Hz, 2H, H-5, H-8), 7.84 (d, *J* = 7.2 Hz, 2H, H-6, H-7), 7.57 (d, *J* = 8.6 Hz, 2H, H-18, H-19), 6.62 (s, 1H, H-3), 5.84 (s, 1H, H-9), 5.27 (s, 1H, H-15). ^{13}C NMR (151 MHz, DMSO) δ 185.01 (C-4), 179.94 (C-1), 159.38 (C-2), 147.75 (C-19), 143.74 (C-10), 141.75 (C-23), 135.01 - 124.44 (Ar), 111.39 (C-3), 62.77 (C-9), 52.49 (C-15). HRMS (APCI) *m/z*: $[M+H]^+$ 442.0223 (calcd for $C_{20}H_{13}BrFN_3O_3$: 442.0203)

3.4.7.6 2-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)naphthalene-1,4-dione, 25

The reaction of **19** with **17f** afford hybrid **25**, light orange powder. Yield: 500 mg (22%). Purity (HPLC): 95%. m.p. 172.6 - 175.2 °C; IR v_{max}: 3121.87 (=C-H, TZ), 1675 (C=O, 1), 1650 (C=O, 4), 1579 (NO_2) cm^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.38 (s, 1H, H-14), 7.98 (t, *J* = 8.2 Hz, 2H, H-5, H-8), 7.84 (d, *J* = 7.3 Hz, 2H, H-6, H-7), 7.62 (d, *J* = 9.5 Hz, 1H, H-19), 7.47 (d, *J* = 8.2 Hz, 1H, H-21), 7.36 (t, *J* = 8.1 Hz, 1H, H-17), 6.61 (s, 1H, H-3), 5.69 (s, 1H, H-9), 5.24 (s, 1H, H-15). ^{13}C NMR (151 MHz, DMSO) δ 185.00 (C-4), 179.93 (C-1), 161.37 (C-2), 159.54 (C-10), 141.54 (C-23), 135.01 - 119.62 (Ar), 111.36 (C-3), 62.72 (C-9), 47.09 (C-15). HRMS (APCI) *m/z*: $[M+H]^+$ 391.1073 (calcd for $C_{20}H_{14}N_4O_5$: 391.1042)

3.4.7.7 2-((1-(4-(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)methoxy)naphthalene-1,4-dione, 26

The reaction of 19 with 17g afford hybrid **26**, light yellow powder. Yield: 450 mg (68%). Purity (HPLC): 88%. m.p. 175.6 - 178.0 ℃; IR v_{max}: 3061 (N-H, TZ), 1685 (C=O, 1), 1649 (C=O, 4), cm^{-1}. ^{1}H NMR (600 MHz, DMSO) δ 8.34 (s, 1H, H-14), 7.98 (t, *J* = 7.4 Hz, 2H, H-5, H-8), 7.84 (dt, *J* = 24.2, 7.3 Hz, 2H, H-6, H-7), 7.24 (d, *J* = 7.9 Hz, 2H, H-18, H-21), 7.18 (d, *J* = 7.9 Hz, 2H, H-17, H-22), 6.60 (s, 1H, H-3), 5.58 (s, 1H, H-9), 5.22 (s, 1H, H-15), 2.28 (s, 1H, H-20). ^{13}C NMR (151 MHz, DMSO) δ 185.02 (C-4), 179.94 (C-1), 159.39 (C-2), 141.54 (C-10), 138.10 (C-19), 135.01 - 125.95 (Ar).111.33 (C-3), 62.80 (C-9), 53.22 (C-15), 21.18 (C-20). HRMS (APCI) *m/z*: $[M+H]^+$ 414.1101 (calcd for $C_{21}H_{14}F_3N_3O_3$: 414.0166)

3.4.8 *In-vitro* biological evaluation

3.4.8.1 Antimalarial activity assessment

P. falciparum drug-sensitive NF54 strain were maintained at 37 °C in human erythrocytes suspended in complete medium, consisting of 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 (Sigma Aldrich), 24 µg/mL gentamycin (Sigma Aldrich) and 0.5 % Albumax II (Thermofisher Scientific) in a gaseous environment of 90% N_2, 5 %O_2 and 5 % CO_2 [28].

Prior to assay setup, the parasites were synchronised using 5 % D-sorbitol to provide a parasite population of at least 95 % rings. The parasites were then seeded on 96 well pates (100 µL per well at 1 % haematocrit and 1 % parasitaemia) with 100 µL of compound dissolved in complete medium. For the dual point screen 1 µM and 5 µM concentrations were tested. Controls for the assay included 1 µM chloroquine disulphate as positive control and complete medium as negative control. The treated parasites were incubated for 96 hours at 37 °C under 90% N_2, 5% O_2 and 5 % CO_2. After incubation, the plates were frozen at -20 °C for 24 – 48 hours in order to promote erythrocyte lysis upon thawing.

For the assay, 100 µL of SYBR Green I lysis buffer (0.2 µL/mL 10 000x SYBR Green I [Invitrogen], 20 mM Tris, pH 7.5 [Sigma Aldrich], 5 mM EDTA [Sigma Aldrich], 0.008 % saponin [Sigma Aldrich] and 0.08 % Triton X-100 [Sigma Aldrich]) was added to new 96 well plates. An eq.ual volume of thawed parasite samples (100 µL) was then added to the SYBR Green I lysis buffer. The fluorescence was measured using the GloMax®-Multi+ Detection System (excitation 490 nm, emission 520 nm). Results were analysed using Microsoft Excel and GraphPad software and expressed as percentage growth inhibition ± SD.

Percentage growth inhibition was calculated as follows:

Growth inhibition % = (1 – [Δ Abs sample – Δ Abs positive control] / [Δ Abs neg control – Δ Abs positive control]) X 100.

3.4.8.2 Cytotoxicity assay

HEK-293 cells were cultured in Hyclone Dulbecco's modified Eagle's medium with high glucose, supplemented with 10 % fetal bovine serum [Thermofisher Scientific] and 1 % L-glutamine [Lonza], penicillin-streptomycin [Lonza], amphotericin B [Lonza] and non-essential amino acids [Lonza]. The cells are maintained in a humidified atmosphere at 37 °C and 5 % CO_2. For the MTT assay, 96 well plates were prepared with 200 µL of cell suspension (25 000 cells/mL) and incubated for 24 hours. The cells were then treated with: (1) 100 µL of emetine dihydrochloride

solution diluted with growth medium to the necessary concentrations (positive control); (2) 80 µL of growth medium and 20 µL of solvent (negative control to compensate for possible solvent effects); (3) 80 µL of growth medium and 20 µL of experimental compound solutions. Blanks contained growth medium without cells. The treated plates were incubated for 48 hours.

To initiate the MTT assay, 20 µL of sterile-filtered MTT solution (1 mg/mL in PBS) was added and the plates incubated for four hours. The growth medium-MTT mixture was then aspirated and 100 µL of 2-propanol added to dissolve purple formazan crystals. Absorbance was measured at 560 and 650 nm using the Thermofisher Scientific GO Multiscan plate reader. Due to light sensitivity of MTT reagent, the assay was performed in the dark. Thus, the plates were covered with aluminium foil and the contents gently mixed for five minutes at room temperature. Data analysis was performed for each biological replicate using SkanIt 4.0 Research Edition software. Background absorbance (650 nm) was subtracted from absorbance values (560 nm), the mean absorbance calculated and the percentage cell viability was determined by the following equation:

Cell viability % = (Δ Abs sample – Δ Abs blank) / (Δ Abs neg control – Δ Abs blank) X 100

The IC_{50} was determined for each compound's biological replicate using GraphPad Prism 5. For the final IC_{50} of each compound, the mean IC_{50} of the biological replicates were calculated.

Disclaimer

Any opinions, findings and conclusions, or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regard thereto.

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BIBLIOGRAPHY

[1] R.C. Gilson, R.J. Deissler, R.F. Bihary, W.C. Condit, M.E. Thompson, D.A. Blankenship, K.O. Grimberg, R.W. Brown, B.T. Grimberg, Growth of plasmodium falciparum in response to a rotating magnetic field, Malar J, 17 (2018) 190.

[2] WHO, World Malaria Report 2016, http://www.who.int/malaria/publications/world-malaria-report-2017/en/, [Date of access: January 2018]. (2017).

[3] M. Samprathi, S. Angurana, Managing malaria in the pediatric intensive care unit, Br J Anaesth, 4 (2017) 60-71.

[4] J. Jourdan, J. Schneider, A. Dassonville-Klimpt, P. Sonnet, Recent advances in antimalarial drugs: structures, mechanisms of action and clinical trials, in: A. Méndez-Vilas (Ed.) Microbiology Book Series Formatex Research Center, 2018, pp. 599-609.

[5] M. SchliTZer, Antimalarial drugs–what is in use and what is in the pipeline, Arch Pharm, 341 (2008) 149-163.

[6] Institute of Medicine Committee on the Economics of Antimalarial Drugs, in: K.J. Arrow, C. Panosian, H. Gelband (Eds.) Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance, National Academies Press (US), 2004.

[7] S. Satish, J. Puttur, E. Coutinho, P. Ambre, S.R. Nandan, Recent advances in the treatment of malaria, Biomed Res Int, 3 (2016) 216-228.

[8] R. McGready, S.J. Lee, J. Wiladphaingern, E.A. Ashley, M.J. Rijken, M. Boel, Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study, Lancet Infect Dis, 12 (2012) 388-396.

[9] S. Dahlström, A. Aubouy, O. Maïga-Ascofaré, J.-F. Faucher, A. Wakpo, S. Ezinmègnon, A. Massougbodji, P. Houzé, E. Kendjo, P. Deloron, J.L. Bras, S. Houzéb, Plasmodium falciparum polymorphisms associated with ex vivo drug susceptibility and clinical effectiveness of artemisinin-based combination therapies in Benin, Antimicrob Agents Chemother, 58 (2014) 1-10.

[10] A.M. Dondorp, F.M. Smithuis, C. Woodrow, L. von Seidlein, How to contain artemisinin-and multidrug-resistant falciparum malaria, Trends Parasitol, 33 (2017) 353-363.

[11] A.H. Uc-Cachón, R. Borges-Argáez, S. Said-Fernández, J. Vargas-Villarreal, F. González-Salazar, M. Méndez-González, M. Cáceres-Farfán, G.M. Molina-Salinas, Naphthoquinones

isolated from diospyros anisandra exhibit potent activity against pan-resistant first-line drugs mycobacterium tuberculosis strains, Pulm Pharmacol Ther, 27 (2014) 114-120.

[12] J. Acuña, J. Piermattey, D. Caro, S. BannwiTZ, L. Barrios, J. López, Y. Ocampo, R. Vivas-Reyes, F. Aristizábal, R. Gaitán, Synthesis, anti-proliferative activity evaluation and 3d-qsar study of naphthoquinone derivatives as potential anti-colorectal cancer agents, Molecules, 23 (2018) 186.

[13] A.L. Baggish, D.R. Hill, Antiparasitic agent atovaquone, Antimicrob Agents Chemother, 46 (2002) 1163-1173.

[14] J.E. Siregar, G. Kurisu, T. Kobayashi, M. Matsuzaki, K. Sakamoto, F. Mi-ichi, Y.-i. Watanabe, M. Hirai, H. Matsuoka, D. Syafruddin, S. Marzuki, K. Kita, Direct evidence for the atovaquone action on the Plasmodium cytochrome bc1 complex, Parasitol Int, 64 (2015) 295-300.

[15] D. Birth, W.-C. Kao, C. Hunte, Structural analysis of atovaquone-inhibited cytochrome bc 1 complex reveals the molecular basis of antimalarial drug action, Nat Commun, 5 (2014) 4029.

[16] G.C. Brandao, F.C.R. Missias, L.M. Arantes, L.F. Soares, K.K. Roy, R.J. Doerksen, A.B. de Oliveira, G.R. Pereira, Antimalarial naphthoquinones. Synthesis via click chemistry, in vitro activity, docking to PfDHODH and SAR of lapachol-based compounds, Eur J Med Chem, 145 (2018) 191-205.

[17] K.M. Faraz, V. Garima, A. Wasim, M. Akranth, A.M. Mumtaz, A. Mymoona, H. Asif, H.S. Misbahul, S. Mohammad, H.S. Rashiduddin, Synthetic trends followed for the development of 1, 2, 3-triazole derivatives, Int J Drug Dev Res, 9 (2017) 22-25.

[18] A. Lauria, R. Delisi, F. Mingoia, A. Terenzi, A. Martorana, G. Barone, A.M. Almerico, 1,2,3-Triazole in heterocyclic compounds, endowed with biological activity, through 1,3-dipolar cycloadditions, Eur J Org Chem, 2014 (2014) 3289-3306.

[19] S.B.B. Bahia, W.J. Reis, G.A. Jardim, F.T. Souto, C.A. de Simone, C.C. Gatto, R.F. Menna-Barreto, S.L. de Castro, B.C. Cavalcanti, C. Pessoa, Molecular hybridization as a powerful tool towards multitarget quinoidal systems: synthesis, trypanocidal and antitumor activities of naphthoquinone-based 5-iodo-1, 4-disubstituted-, 1, 4-and 1, 5-disubstituted-1, 2, 3-triazoles, MedChemComm, 7 (2016) 1555-1563.

[20] F.W. Muregi, A. Ishih, Next-generation antimalarial drugs: Hybrid molecules as a new strategy in drug design, Drug Develop Res, 71 (2010) 20-32.

[21] R.N.d. Oliveira, M.G.d. Silva, M.T.d. Silva, V.N. Melo, W.O. Valença, J.A.d. Paz, C.A. Camara, Strategies for molecular diversification of 2-[aminoalkyl-(1h-1, 2, 3-triazol-1-yl)]-1, 4-naphthoquinones using click chemistry, J Brazil Chem Soc, 28 (2017) 681-688.

[22] W.S. do Nascimento, C.A. Camara, R.N. de Oliveira, Synthesis of 2-(1H-1, 2, 3-Triazol-1-yl)-1, 4-naphthoquinones from 2-Azido-1, 4-naphthoquinone and Terminal Alkynes, Synthesis, 2011 (2011) 3220-3224.

[23] J.-A. Shin, Y.-G. Lim, K.-H. Lee, Copper-catalyzed azide–alkyne cycloaddition reaction in water using cyclodextrin as a phase transfer catalyst, J Org Chem, 77 (2012) 4117-4122.

[24] G.S. Prakash, S. Chacko, S. Alconcel, T. Stewart, T. Mathew, G.A. Olah, Stereoselective monofluoromethylation of primary and secondary alcohols by using a fluorocarbon nucleophile in a Mitsunobu reaction, Angew Chem, 119 (2007) 5021-5024.

[25] V.K. Gombar, K. Enslein, Assessment of n-octanol/water partition coefficient: when is the assessment reliable?, J Chem Inf Comput Sci, 36 (1996) 1127-1134.

[26] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv Drug Deliv Rev, 23 (1997) 3-25.

[27] M.N. Martinez, G.L. Amidon, A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals, J Clin Pharmacol, 42 (2002) 620-643.

[28] B.K. Verlinden, J. Niemand, J. Snyman, S.K. Sharma, R.J. Beattie, P.M. Woster, L.-M. BirkholTZ, Discovery of novel alkylated (bis) urea and (bis) thiourea polyamine analogues with potent antimalarial activities, J Med Chem, 54 (2011) 6624-6633.

CHAPTER 4: SUMMARY AND CONCLUSION

Malaria is a protozoan disease that continues to affect millions of lives around the world every year, causing substantial morbidity and mortality (Thakur *et al.*, 2018a). Five species of *Plasmodium* genus are responsible of human malaria. These include *viz. P. vivax*, *P. ovale*, *P. malariae*, *P. falciparum* and *P. knowlesi* (Singh & Daneshvar, 2013). Severe malaria was once believed to be mainly caused by *P. falciparum* and this has been increasingly contradicted by the growth in *P. vivax* disease incidence over the past few decades (Samprathi & Angurana, 2017a). *P. falciparum* still reigns as the most lethal form of malaria in humans and it is described as a public health disaster, causing increased morbidity and mortality (Chandralekha *et al.*, 2018).

The latest WHO statistics revealed mortality rates of 445 000 deaths and 91 countries reported a total of 216 million cases of malaria (WHO, 2016b) in 2016. More than 60 % of deaths occurred in children under the age of five years in high transmission areas. Malaria is a significant killer in this age group, claiming one child every two minutes (Samprathi & Angurana, 2017a).

ACTs currently remain the first-line drug of choice for uncomplicated *P. falciparum* malaria (Zhang, 2016). However, growing resistance in *P. falciparum* and *P. vivax* parasites to artemisinins suggests that the current antimalarial drug regimens may not remain active for long (Satish *et al.*, 2016a). The continuous changing of malarial parasites (and thus their ability to develop resistance against current drugs) and the unavailability of malaria vaccines emphasises the development of new and more effective drugs (Birkett, 2016b; Satish *et al.*, 2016b)

Derivatives of 1,4- and 1,2-naphthoquinone are widely spread in nature and many plants containing these substances have been used in folk medicine for treatment of a number of diseases (Munday *et al.*, 2007b). Great interest has been shown in the poly-substituted 1,4-naphthoquinones in recent years as final compounds or synthetic intermediates because of their encouraging cytotoxic, antibacterial, antifungal, antiprotozoal, insecticidal and anti-inflammatory properties. Pharmacological derivatives like *β*-lapachone, an *ortho*-quinone lapachol isomer is currently in clinical phase II studies in the United States for the treatment of progressive solid tumors (Bala *et al.*, 2014a; Suárez-Rozas *et al.*, 2018). Atovaquone is a hydroxy-1,4-naphthoquinone derivative and is a structural equal of protozoan ubiquinone (Baggish & Hill, 2002b).

Atovaquone is a competitive inhibitor of ubiquinol, inhibiting the mitochondrial electron transport chain at the bc1 complex. Inhibition of bc1 activity results in a loss of mitochondrial function. For the duration of the intra-erythrocytic stage of infection, a crucial role of the parasite mitochondrion is to provide orotate for pyrimidine biosynthesis through the activity of dihydroorotate dehydrogenase (DHODH). Consistent with this, inhibition of the bc1 complex by atovaquone

affects the concentrations of metabolites in the pyrimidine biosynthetic pathway (Nixon *et al.*, 2013). Currently, atovaquone is used as a fixed-dose mixture with proguanil (Malarone) for the treatment of uncomplicated malaria or as a chemoprophylactic agent for malaria in travellers.

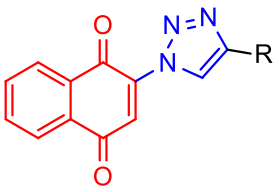
Triazoles are five-membered heterocyclic molecules, bearing three nitrogen atoms in the ring. Triazole scaffold has the molecular formula $C_2H_3N_3$ and exists in two isomeric forms namely 1,2,3-triazoles and 1,2,4-triazoles. Due to high aromatic stabilization of 1,2,3- ring, triazoles are impervious to oxidation, reduction and hydrolysis in both acidic and basic conditions (Faraz *et al.*, 2017b).

Molecular hybridization is based on the combination of two active chemical moieties to produce one chemical entity, and has emerged as an important strategy for the development of new drugs that are able to act as multi-target ligands (Bahia *et al.*, 2016a). Hybrids have a lower risk of drug–drug adverse interactions compared to multicomponent drugs (Muregi & Ishih, 2010a).

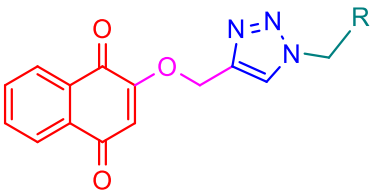
The aim of this study was to synthesise 1,4-naphthoquinone-1,2,3-triazole hybrids, linking the pharmacophores of 1,4-naphthoquinone and 1,2,3-triazole through click-chemistry, with the ultimate goal to produce a new antimalarial drug endowed with better efficacy and less toxic effects.

To achieve this aim, the following objectives were set:

- Synthesis of novel analogues of 1,4-naphthoquinone and 1,2,3-triazole hybrids, as shown below, through click-chemistry and confirmation of structures using IR, NMR, MS and melting point analysis.



Sub-series **1** hybrids



Sub-series **2** hybrids

- Cytotoxicity assessment of the synthesised compounds using mammalian cell lines
- *In vitro* evaluation of antimalarial activity of the hybrids against chloroquine - sensitive (CQS) and -resistant (CQR) strains of *P. falciparum*.

Synthesis of the two sub-series of 1,4-naphthoquinone-1,2,3-triazole hybrids was achieved using similar methods. The first sub-series include compounds **11** - **16**. The synthesis took place in two steps. The azido intermediate **10** was first synthesised using a method described by do Nascimento *et al.* (2011). Secondly, the hybrids were synthesised using the copper catalysed Huisgen alkyne-azide cycloaddition reaction with an alkyne (Shin *et al.*, 2012b). Sub-series 1

hybrids were isolated in poor yields (2-22 %) after purification by column chromatography on silica gel and recrystallization from hexane.

Three steps were followed to synthesise sub-series 2 hybrids. Firstly, the benzyl-azide derivatives **17a-g** were synthesised through nucleophilic substitution involving sodium azide and commercial benzyl bromides. In the second step naphthoquinolylalkyne **19** was synthesised with a slightly modified version of the Mitsunobu reaction (Prakash *et al.*, 2007a). Hybrids **20** - **26** in sub-series 2 were obtained following "click chemistry" between intermediates **17** and **19** (do Nascimento *et al.*, 2011a; Shin *et al.*, 2012b).Yields in this sub-series were low to good (12 – 96 %) after purification by recrystallisation in hexane.

Sub-series 1 and sub-series 2 are structurally different in that sub-series 2 hybrids are flexible while sub-series 1 compounds are rigid, the former having an oxymethylene linker between the naphthoquinone and the triazole scaffolds. The successful synthesis of the target compounds was confirmed by routine chemical structure characterisation techniques such as IR, NMR and HRMS.

The hydrophilicity and lipophilicity of compounds were determined by calculating the estimated cLogP values of the compounds. Ideal cLogP values are aimed to be between one and three (Lipinski *et al.*, 1997). The estimated cLogP values of the synthesised compounds ranged between two and five that showed encouraging drug-likeness.

The cytotoxicity of the hybrids was assessed using the human embryonic kidney (HEK-293) cell line, alongside emetine as control and atovaquone as reference. The hybrids were found to be generally non-toxic. However, hybrids **20** and **22** in sub-series 2, featuring benzyl and *p*-tert-butylbenzyl substituents, respectively, displayed moderate toxicity to the kidney cells.

Dual point (5 µM and 1µM) SYBR Green I based assays were performed to evaluate the *in vitro* antimalarial activity of the hybrids through determination of parasite growth inhibition percentage against asexual stage chloroquine sensitive *P. falciparum* NF54 strain. In the first sub-series compound **11** showed moderate activity (55 %) at the 5 µM concentration. None of the other compounds in sub-series 1 had any activity, this is believed to be a result of the rigid structure of these compounds. The second sub-series showed good activity (69 – 90 %) for five compounds (**20**, **21**, **22**, **23**, and **26**) at 1 µM concentration. This result suggests that the more flexible structure of sub-series 2 will have more activity and gives justification for further study. Thus, the oxymethylene tether had an impact on the biological activity of these hybrids.

Compound **22** was the most active having the highest percentage growth inhibition at 1 µM (90 %) and a moderate cytotoxicity (IC_{50} 36 µM). Comparatively, the reference atovaquone had 96 %

parasite growth inhibition at 1 µM and IC_{50} 56 µM cytotoxicity. Subsequently, hybrid **22** stands as a good candidate for further investigation against chloroquine resistant parasites. Due to time limitations, the cellular potency of the compounds was not assessed against drug-sensitive and drug-resistant strains of *P. falciparum* as initially envisaged". Thus, assessment of the cellular antimalarial activity of sub-series hybrids against these strains through IC_{50} determination will be future endeavours.

BIBLIOGRAPHY

Achan, J., Talisuna, A.O., Erhart, A., Yeka, A., Tibenderana, J.K., Baliraine, F.N.*, et al.* 2011. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malaria Journal*, 10:144.

Adjuik, M., Agnamey, P., Babiker, A., Borrmann, S., Brasseur, P., Cisse, M.*, et al.* 2002. Amodiaquine-artesunate versus amodiaquine for uncomplicated Plasmodium falciparum malaria in African children: a randomised, multicentre trial. *The Lancet*, 359:1365-1372.

Agalave, S.G., Maujan, S.R. & Pore, V.S. 2011. Click Chemistry: 1,2,3-Triazoles as Pharmacophores. *Chemistry – An Asian Journal*, 6:2696-2718.

Aidoo, M., Terlouw, D.J., Kolczak, M.S., McElroy, P.D., ter Kuile, F.O., Kariuki, S.*, et al.* 2002. Protective effects of the sickle cell gene against malaria morbidity and mortality. *The Lancet*, 359:1311-1312.

Akhtar, M.N., Jamil, S., Amjad, S.I., Butt, A.R. & Farooq, M. 2016. Association of malaria with thrombocytopenia. *Annals of King Edward Medical University*, 11(4).

Allison, A.C. 2009. Genetic control of resistance to human malaria. *Current Opinion in Immunology*, 21:499-505.

Arend, P. 2018. Position of human blood group O (H) and phenotype-determining enzymes in growth and infectious disease. *Annals of the New York Academy of Sciences*, 1425:5-18.

Arrow, K.J., Panosian, C.B. & Gelband, H. 2004. Antimalarial drug classes. Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance. Washington, DC: The National Academies Press. pp. 252-299.

Baggish, A.L. & Hill, D.R. 2002. Antiparasitic agent atovaquone. *Antimicrobial Agents and Chemotherapy*, 46:1163-1173.

Bahia, S.B.B., Reis, W.J., Jardim, G.A., Souto, F.T., de Simone, C.A., Gatto, C.C.*, et al.* 2016. Molecular hybridization as a powerful tool towards multitarget quinoidal systems: synthesis, trypanocidal and antitumor activities of naphthoquinone-based 5-iodo-1, 4-disubstituted-, 1, 4- and 1, 5-disubstituted-1, 2, 3-triazoles. *The European Federation for Medicinal Chemistry*, 7:1555-1563.

Bala, B.D., Muthusaravanan, S., Choon, T.S., Ashraf Ali, M. & Perumal, S. 2014. Seq.uential synthesis of amino-1,4-naphthoquinone-appended triazoles and triazole-chromene hybrids and their antimycobacterial evaluation. *European Journal of Medicinal Chemistry*, 85:737-746.

Bao, N., Ou, J., Shi, W., Li, N., Chen, L. & Sun, J. 2018. Highly Efficient Synthesis and Structure-Activity Relationships of a Small library of Substituted 1, 4-Naphthoquinones. *European Journal of Organic Chemistry*, 19:2254-2258.

Barrera, V., MacCormick, I.J.C., Czanner, G., Hiscott, P.S., White, V.A., Craig, A.G.*, et al.* 2018. Neurovascular seq.uestration in paediatric P. falciparum malaria is visible clinically in the retina. *eLife*, 7:e32208.

Beare, N.A., Taylor, T.E., Harding, S.P., Lewallen, S. & Molyneux, M.E. 2006. Malarial retinopathy: a newly established diagnostic sign in severe malaria. *The American Journal of tropical Medicine and Hygiene*, 75:790-797.

Boechat, N., Ferreira, M.d.L.G., Pinheiro, L., Jesus, A.M.L., Leite, M.M.M., Junior, C.C.S.*, et al.* 2014. New Compounds Hybrids 1H-1,2,3-Triazole-Quinoline Against Plasmodium falciparum. *Chemical Biology & Drug Design*, 84:325-332.

Bonandi, E., Christodoulou, M.S., Fumagalli, G., Perdicchia, D., Rastelli, G. & Passarella, D. 2017. The 1,2,3-triazole ring as a bioisostere in medicinal chemistry. *Drug Discovery Today*, 22:1572-1581.

Bouchaud, O., Imbert, P., Touze, J.E., Dodoo, A.N., Danis, M. & Legros, F. 2009. Fatal cardiotoxicity related to halofantrine: a review based on a worldwide safety data base. *Malaria Journal*, 8(1):289-307.

Brunton, L.L. 2014. Antimalarial Drug Therapy. Goodman and Gilman's manual of pharmacology and therapeutics. Mcgraw-hill New York. p. 1383-1418.

Cannella, A.P. & Archibald, L. 2017. Plasmodium species - Infectious Diseases. *https://www.infectiousdiseaseadvisor.com/infectious-diseases/plasmodium-species-malaria/article/609978/*, Date of Access: 20 September 2018.

Cappellini, M.D. & Fiorelli, G. 2008. Glucose-6-phosphate dehydrogenase deficiency. *The Lancet*, 371:64-74.

CDC, (Centers For Disease Control and Prevention). 2016. Malaria Life Cycle. *https://www.cdc.gov/malaria/about/index.html*, Date of Access: 25 August 2017.

CDC. 2018. Malaria Parasites. *https://www.cdc.gov/malaria/about/biology/parasites.html*, Date of Access: 25 Spetember 2018.

Chen, I., Clarke, S.E., Gosling, R., Hamainza, B., Killeen, G., Magill, A.*, et al.* 2016. "Asymptomatic" malaria: a chronic and debilitating infection that should be treated. *PLoS Medicine*, 13:e1001942.

Chu, C., Bancone, G., Moore, K., Win, H., Thitipanawan, N., Po, C.*, et al.* 2017. Primaquine induced haemolysis in G6PD heterozygous females treated for the radical cure of Plasmodium vivax malaria: a nested cohort study. *PLos Medicine*, 14:1-19.

da Silva, E.N., de Melo, I.M.M., Diogo, E.B.T., Costa, V.A., de Souza Filho, J.D., Valenca, W.O.*, et al.* 2012. On the search for potential anti-Trypanosoma cruzi drugs: Synthesis and biological evaluation of 2-hydroxy-3-methylamino and 1, 2, 3-triazolic naphthoquinoidal compounds obtained by click chemistry reactions. *European Journal of Medicinal Chemistry*, 52:304-312.

Dahlström, S., Aubouy, A., Maïga-Ascofaré, O., Faucher, J.-F., Wakpo, A., Ezinmègnon, S.*, et al.* 2014. Plasmodium falciparum polymorphisms associated with ex vivo drug susceptibility and clinical effectiveness of artemisinin-based combination therapies in Benin. *Antimicrobial Agents and Chemotherapy*, 58:1-10.

Davis, T.M.E., Hung, T.-Y., Sim, I.-K., Karunajeewa, H.A. & Ilett, K.F. 2005. Piperaquine. *Drugs*, 65:75-87.

Dean, L. 2005. The Duffy Blood Group. Blood groups and red cell antigens. National Center for Biotechnology Information. p. 1-6.

Dicko, A., Roh, M.E., Diawara, H., Mahamar, A., Soumare, H.M., Lanke, K.*, et al.* 2018. Efficacy and safety of primaquine and methylene blue for prevention of Plasmodium falciparum transmission in Mali: a phase 2, single-blind, randomised controlled trial. *The Lancet Infectious Diseases*, 18:627-639.

Dieye, Y., Mbengue, B., Dagamajalu, S., Fall, M.M., Loke, M.F., Nguer, C.M., *et al.* 2016. Cytokine response during non-cerebral and cerebral malaria: evidence of a failure to control inflammation as a cause of death in African adults. *PeerJ*, 4:e1965.

Dinter, D., and, G.G. & Garaj-Vrhovacb, V. 2011. An alkaline comet assay study on the antimalarial drug atovaquone in human peripheral blood lymphocytes: a study based on clinically relevant concentrations. *Journal of Applied Toxicology*, 33:56-62.

Dondorp, A., Nosten, F., Stepniewska, K., Day, N. & White, N. 2005. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet*, 366:717-725.

Dondorp, A.M., Fanello, C.I., Hendriksen, I.C., Gomes, E., Seni, A. & Chhaganlal, K.D. 2010. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet*, 376:1647-1657.

Doolan, D.L., DobaÃ±o, C. & Baird, J.K. 2009. Acquired immunity to malaria. *Clinical microbiology Reviews*, 22:13-36.

Eastman, R.T. & Fidock, D.A. 2009. Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. *Nature Reviews Microbiology*, 7(12):864-874.

Fisher, N., Majid, R.A., Antoine, T., Al-Helal, M., Warman, A.J., Johnson, D.J., *et al.* 2012. Cytochrome b mutation Y268S conferring atovaquone resistance phenotype in malaria parasite results in reduced parasite bc1 catalytic turnover and protein expression. *Journal of Biological Chemistry*, 287:9731-9741.

Frickmann, H., Hinz, R., Rojak, S., Bonow, I., Ruben, S., Wegner, C., *et al.* 2018. Evaluation of automated loop-mediated amplification (LAMP) for routine malaria detection in blood samples of German travelers – A cross-sectional study. *Travel Medicine and Infectious Disease*, 24:25-30.

Frosch, T., Schmitt, M. & Popp, J. 2007. In situ UV resonance Raman micro-spectroscopic localization of the antimalarial quinine in cinchona bark. *The Journal of Physical Chemistry B*, 111:4171-4177.

Ghosh, K. & Ghosh, K. 2007. Pathogenesis of anemia in malaria: a concise review. *Parasitology Research*, 101:1463-1469.

Gilson, R.C., Deissler, R.J., Bihary, R.F., Condit, W.C., Thompson, M.E., Blankenship, D.A.*, et al.* 2018. Growth of Plasmodium falciparum in response to a rotating magnetic field. *Malaria Journal*, 17:190-223.

Goodman, C.D., Siregar, J.E., Mollard, V., Vega-Rodríguez, J., Syafruddin, D., Matsuoka, H.*, et al.* 2016. Parasites resistant to the antimalarial atovaquone fail to transmit by mosquitoes. *Science*, 352:349-353.

Greenwood, B., Dicko, A., Sagara, I., Zongo, I., Tinto, H., Cairns, M.*, et al.* 2017. Seasonal vaccination against malaria: a potential use for an imperfect malaria vaccine. *Malaria Journal*, 16:182-192.

Haynes, R.K. & Krishna, S. 2004. Artemisinins: activities and actions. *Microbes and Infection*, 6:1339-1346.

Holla, B.S., Mahalinga, M., Karthikeyan, M.S., Poojary, B., Akberali, P.M. & Kumari, N.S. 2005. Synthesis, characterization and antimicrobial activity of some substituted 1,2,3-triazoles. *European Journal of Medicinal Chemistry*, 40:1173-1178.

Johnston, S.P., Pieniazek, N.J., Xayavong, M.V., Slemenda, S.B., Wilkins, P.P. & da Silva, A.J. 2006. PCR as a confirmatory technique for laboratory diagnosis of malaria. *Journal of Clinical Microbiology*, 44:1087-1089.

Jourdan, J., Schneider, J., Dassonville-Klimpt, A. & Sonnet, P. 2018. Recent advances in antimalarial drugs: structures, mechanisms of action and clinical trials. *(In* Méndez-Vilas, A., *ed.* Microbiology Book Series Formatex Research Center. p. 599-609.

Karbwang, J. & White, N.J. 1990. Clinical pharmacokinetics of mefloquine. Clinical pharmacokinetics. pp. 264-279.

Kinoshita, A., Yamada, H., Kotaki, H. & Kimura, M. 2010. Effects of anti-malarial drugs on the electrocardiographic QT interval modelled in the isolated perfused guinea pig heart system. *Malaria Journal*, 9:318-348.

Krishna, S., Pulcini, S., Fatih, F. & Staines, H. 2010. Artemisinins and the biological basis for the PfATP6/SERCA hypothesis. *Trends in Parasitology*, 26:517-523.

Lanfranchi, D.A., Cesar-Rodo, E., Bertrand, B., Huang, H., Day, L., Johann, L.*, et al.* 2012. Synthesis and biological evaluation of 1,4-naphthoquinones and quinoline-5,8-diones as antimalarial and schistosomicidal agents. *Organic & Biomolecular Chemistry*, 10:6375-6387.

Li, Y., Fan, P., Zhou, S. & Zhang, L. 2017. Loop-mediated isothermal amplification (LAMP): a novel rapid detection platform for pathogens. *Microbial Pathogenesis*, 107:54-61.

Liu, M., Solomon, W., Cespedes, J.C., Wilson, N.O., Ford, B. & Stiles, J.K. 2018. Neuregulin-1 attenuates experimental cerebral malaria (ECM) pathogenesis by regulating ErbB4/AKT/STAT3 signaling. *Journal of Neuroinflammation*, 15:104-170.

Makler, M.T., Palmer, C.J. & Ager, A.L. 1998. A review of practical techniques for the diagnosis of malaria. *Annals of tropical medicine and parasitology*, 92:419-434.

Malwest. 2016. Plasmodium Life cycle. *http://www.malwest.gr/en-us/malaria/informationforhealthcareprofessionals/plasmodiumlifecycle.aspx.*, Date of Access : 31 August 2018l

Mathanga, D. & Molyneux, M.E. 2016. Bednets and malaria in Africa. *Malawi Medical Journal*, 28:92-93.

Mayo, C, (Mayo Clinic). 2018. Sickle Cell Anemia. *https://www.mayoclinic.org/diseases-conditions/sickle-cell-anemia/symptoms-causes/syc-20355876/,* Date of Access: 25 June 2018.

McGready, R., Lee, S.J., Wiladphaingern, J., Ashley, E.A., Rijken, M.J., Boel, M.*, et al.* 2012. Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. *The Lancet Infectious Diseases*, 12:388-396.

MMV, (Medicines for Malaria Venture). 2017. Parasite Lifecycle. *https://www.mmv.org/malaria-medicines/parasite-lifecycle/,* Date Of Access: 11 August 2017.

Munday, R., Smith, B.L. & Munday, C.M. 2007. Structure-activity relationships in the haemolytic activity and nephrotoxicity of derivatives of 1, 2-and 1, 4-naphthoquinone. *Journal of Applied Toxicology*, 27:262-269.

Muregi, F.W. & Ishih, A. 2010. Next-Generation Antimalarial Drugs: Hybrid Molecules as a New Strategy in Drug Design. *Drug Development Research*, 71:20-32.

Neelarapu, R., Maignan, J.R., Lichorowic, C.L., Monastyrskyi, A., Mutka, T.S., LaCrue, A.N., *et al.* 2018. Design and Synthesis of Orally Bioavailable Piperazine Substituted 4 (1 H)-Quinolones with Potent Antimalarial Activity: Structure–Activity and Structure–Property Relationship Studies. *Journal of Medicinal Chemistry*, 61:1450-1473.

Nixon, G.L., Moss, D.M., Shone, A.E., Lalloo, D.G., Fisher, N., O'Neill, P.M., *et al.* 2013. Antimalarial pharmacology and therapeutics of atovaquone. *Journal of Antimicrobial Chemotherapy*, 68:977-985.

Ocaña-Morgner, C., Mota, M.M. & Rodriguez, A. 2003. Malaria Blood Stage Suppression of Liver Stage Immunity by Dendritic Cells. *The Journal of Experimental Medicine*, 197:143-151.

Oliveira, R.N.d., Silva, M.G.d., Silva, M.T.d., Melo, V.N., Valença, W.O., Paz, J.A.d., *et al.* 2017. Strategies for Molecular Diversification of 2-[Aminoalkyl-(1H-1, 2, 3-triazol-1-yl)]-1, 4-naphthoquinones Using Click Chemistry. *Journal of the Brazilian Chemical Society*, 28:681-688.

Olliaro, P. 2001. Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacology & Therapeutics*, 89:207-219.

Ouattara, A., Barry, A.E., Dutta, S., Remarque, E.J., Beeson, J.G. & Plowe, C.V. 2015. Designing malaria vaccines to circumvent antigen variability. *Vaccine*, 33:7506-7512.

Pingaew, R., Prachayasittikul, V., Worachartcheewan, A., Nantasenamat, C., Prachayasittikul, S., Ruchirawat, S., *et al.* 2015. Novel 1,4-naphthoquinone-based sulfonamides: Synthesis, QSAR, anticancer and antimalarial studies. *European Journal of Medicinal Chemistry*, 103:446-459.

Prasad, C.V., Nayak, V.L., Ramakrishna, S. & Mallavadhani, U.V. 2018. Novel menadione hybrids: Synthesis, anticancer activity, and cell-based studies. *Chemical biology & drug design*, 91:220-233.

Price, R.N., Uhlemann, A.-C., Brockman, A., McGready, R., Ashley, E., Phaipun, L., *et al.* 2004. Mefloquine resistance in Plasmodium falciparum and increased pfmdr1 gene copy number. *The Lancet*, 364:438-447.

Price, R.N. & White, N.J. 2018. Drugs that reduce transmission of falciparum malaria. *The Lancet Infectious Diseases*, 18:585-586.

Primus, H. 2016. Prevention of Malaria. *https://plus.google.com/+PrimusHospitalprimushospital/posts/RosNYbN3d69/*, Date of Access: 3 July 2018.

Rees, D.C., Williams, T.N. & Gladwin, M.T. 2010. Sickle-cell disease. *The Lancet*, 376:2018-2031.

Robert, A., Benoit-Vical, F., Dechy-Cabaret, O. & Meunier, B. 2001. From classical antimalarial drugs to new compounds based on the mechanism of action of artemisinin. *Pure and Applied Chemistry*, 73:1173-1188.

Salcedo-Sora, J.E., Ochong, E., Beveridge, S., Johnson, D., Nzila, A., Biagini, G.A*., et al.* 2011. The molecular basis of folate salvage in Plasmodium falciparum characterization of two folate transporters. *Journal of Biological Chemistry*, 286:44659-44668.

Saralamba, S., Pan-Ngum, W., Maude, R.J., Lee, S.J., Tarning, J., Lindegårdh, N*., et al.* 2011. Intrahost modeling of artemisinin resistance in Plasmodium falciparum. *Proceedings of the National Academy of Sciences*, 108:397-402.

SchliTZer, M. 2008. Antimalarial drugs–what is in use and what is in the pipeline. *Archiv der Pharmazie*, 341:149-163.

Sharghi, H., Khalifeh, R. & Doroodmand, M.M. 2009. Copper Nanoparticles on Charcoal for Multicomponent Catalytic Synthesis of 1,2,3-Triazole Derivatives from Benzyl Halides or Alkyl Halides, Terminal Alkynes and Sodium Azide in Water as a “Green” Solvent. *Advanced Synthesis & Catalysis*, 351:207-218.

Sharma, S.K., Mohanty, P.K., Rath, B. & Sahoo, S.C. 2018. Ventricular tachyarrhythmia in falciparum malaria. *Journal of Evidence Based Medicine and Healthcare*, 5:1571-1576.

Sim, K., Davis, T.M. & Ilett, K.F. 2005. Effects of a high-fat meal on the relative oral bioavailability of piperaquine. *Antimicrobial Agents and Chemotherapy*, 49:2407-2411.

Singh, B. & Daneshvar, C. 2013. Human infections and detection of Plasmodium knowlesi. *Clinical Microbiology Reviews*, 26:165-184.

Skorokhod, O.A., Caione, L., Marrocco, T., Migliardi, G., Barrera, V., Arese, P.*, et al.* 2010. Inhibition of erythropoiesis in malaria anemia: role of hemozoin and hemozoin-generated 4-hydroxynonenal. *Blood*, 27:4328-4332.

Srivastava, I.K. & Vaidya, A.B. 1999. A mechanism for the synergistic antimalarial action of atovaquone and proguanil. *Antimicrobial Agents and Chemotherapy*, 43:1334-1339.

Tan, K.R., Magill, A.J., Parise, M.E. & Arguin, P.M. 2011. Doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. *The American Journal of Tropical Medicine and Hygiene*, 84:517-531.

Thakur, K.T., Vareta, J., Carson, K.A., Kampondeni, S., Potchen, M.J., Birbeck, G.L.*, et al.* 2018. Cerebrospinal fluid Plasmodium falciparum histidine-rich protein-2 in pediatric cerebral malaria. *Malaria Journal*, 17:125-141.

Totobenazara, J. & Burke, A.J. 2015. New click-chemistry methods for 1,2,3-triazoles synthesis: recent advances and applications. *Tetrahedron Letters*, 56:2853-2859.

Trampuz, A., Jereb, M., Muzlovic, I. & Prabhu, R.M. 2003. Clinical review: Severe malaria. *Critical Care*, 7:315-323.

Triglia, T., Menting, J.G., Wilson, C. & Cowman, A.F. 1997. Mutations in dihydropteroate synthase are responsible for sulfone and sulfonamide resistance in Plasmodium falciparum. *Proceedings of the National Academy of Sciences*, 94:13944-13949.

van Agtmael, M.A., Eggelte, T.A. & van Boxtel, C.J. 1999. Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. *Trends in Pharmacological Sciences*, 20:199-205.

VineTZ, J.M., Clain, J., Bounkeua, V., Eastman, R.T.a. & Fidock, D. 2011. Goodman & Gilman's the pharmacological basis of therapeutics. *Chemotherapy of Malaria*, 12:1383-1418.

Vishruti Gandhi, V.G., Muley, P., Parikh, N., Gandhi, H. & Mehta, A. 2017. Is rapid diagnostic test (malaria Pv/Pf Ag card test) reliable in diagnosing malaria. *International Journal Of Contemporary Pediatrics*, 5:92-96.

Wellems, T.E. & Plowe, C.V. 2001. Chloroquine-resistant malaria. *The Journal of Infectious Diseases*, 184:770-776.

WHO. 2012. WHO Policy Recommendation: Seasonal Malaria Chemoprevention (SMC) for Plasmodium falciparum malaria control in highly seasonal transmission areas of the Sahel sub-region in Africa. *http://www.who.int/malaria/publications/world_malaria_report/en/*, Date of Access: 11 August 2017.

WHO. 2015. WHO Guidelines for the treatment of malaria. *https://books.google.co.za/books?hl=en&lr=&id=IVo0DgAAQBAJ&oi=fnd&pg=PP1&dq=Malaria+current+chemotherapy+drug+regimens&ots=9Tie5nL2iO&sig=BeETIjvua7Opa_AG6bill6vvkGU#v=onepage&q&f=false/*, Date Of Access: 15 March 2018.

WHO. 2016. Fact Sheet: World Malaria Report 2016. *http://www.who.int/malaria/media/world-malaria-report-2016/en/*, Date of Access: 12 March 2018.

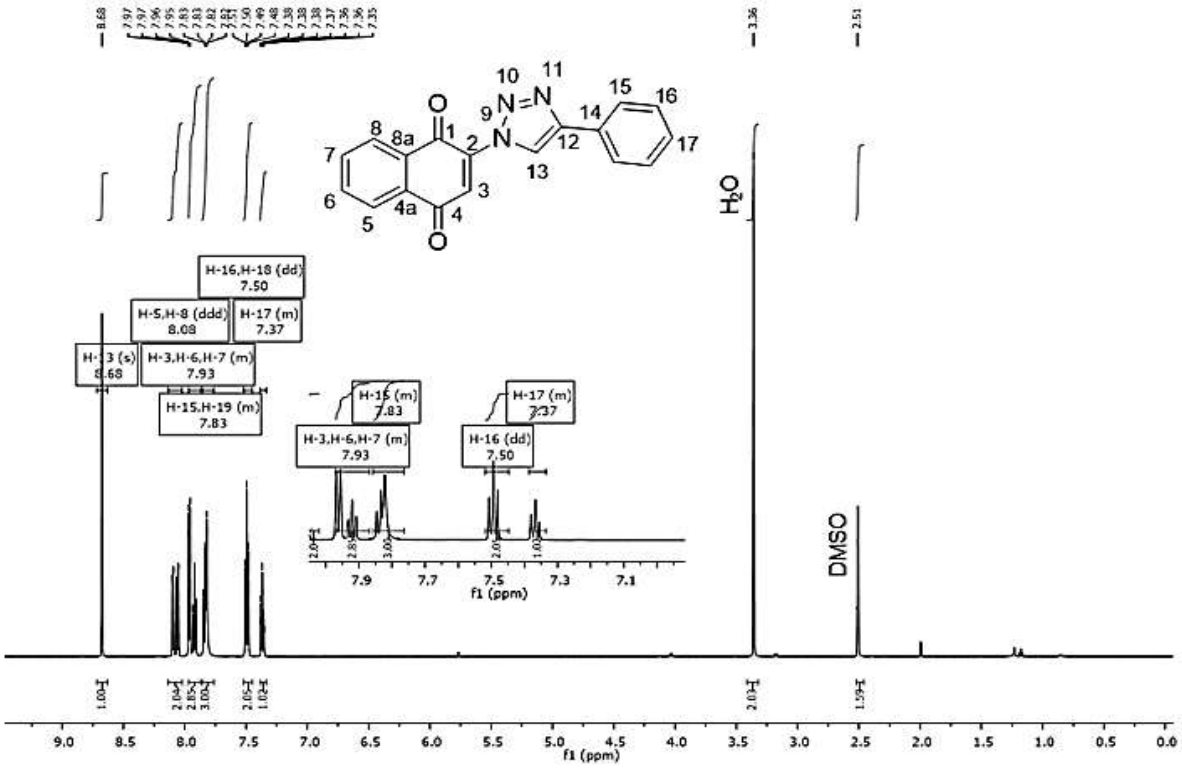
WHO. 2017. The top 10 causes of death. *http://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death/*, Date of Access: 20 September 2018.

Wroblewski, H.A., Kovacs, R.J., Kingery, J.R., Overholser, B.R. & Tisdale, J.E. 2012. High Risk of QT Interval Prolongation and Torsades de Pointes Associated with Intravenous Quinidine Used for the Treatment of Resistant Malaria or Babesiosis. *Antimicrobial Agents and Chemotherapy*:AAC. 06396-06311.

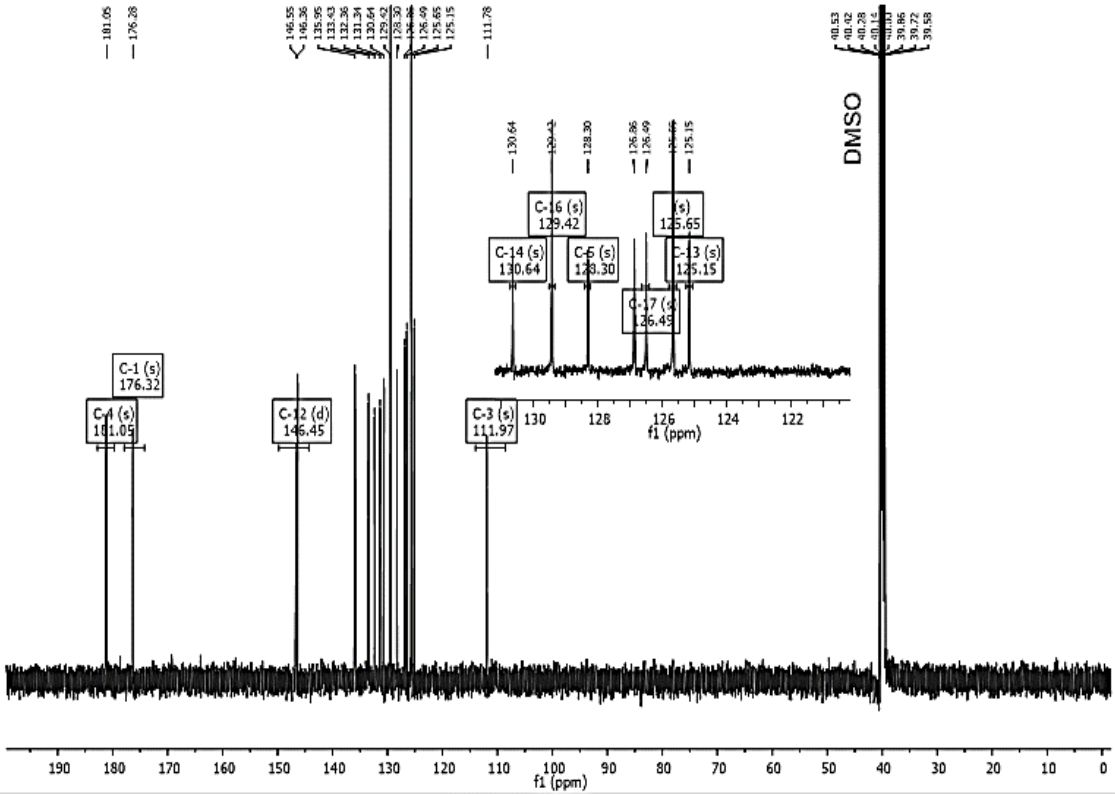
Zhang, Y., den Braver-Sewradj, S.P., den Braver, M.W., Hiemstra, S., Vermeulen, N.P.E., van de Water, B.*, et al.* 2018. Glutathione S-transferase P1 protects against amodiaquine quinoneimines-induced cytotoxicity but does not prevent activation of endoplasmic reticulum stress in HepG2 cells. *Frontiers in Pharmacology*, 9:388-411.

ANNEXURE A

Compound 11
NMR ^{1}H DMSO



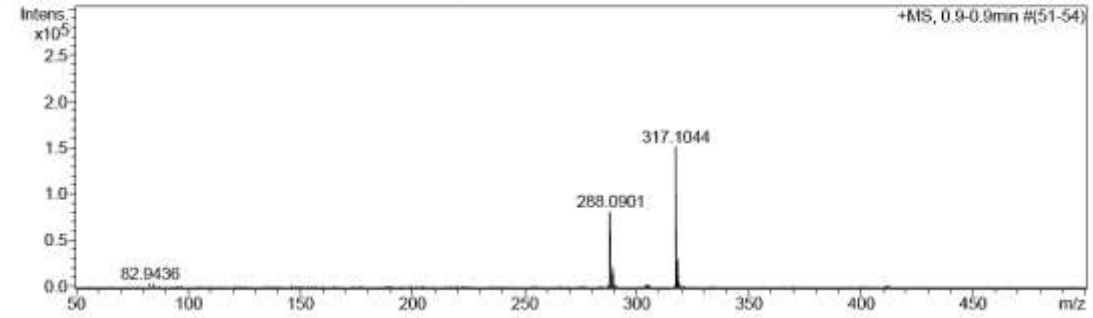
NMR ^{13}C in DMSO



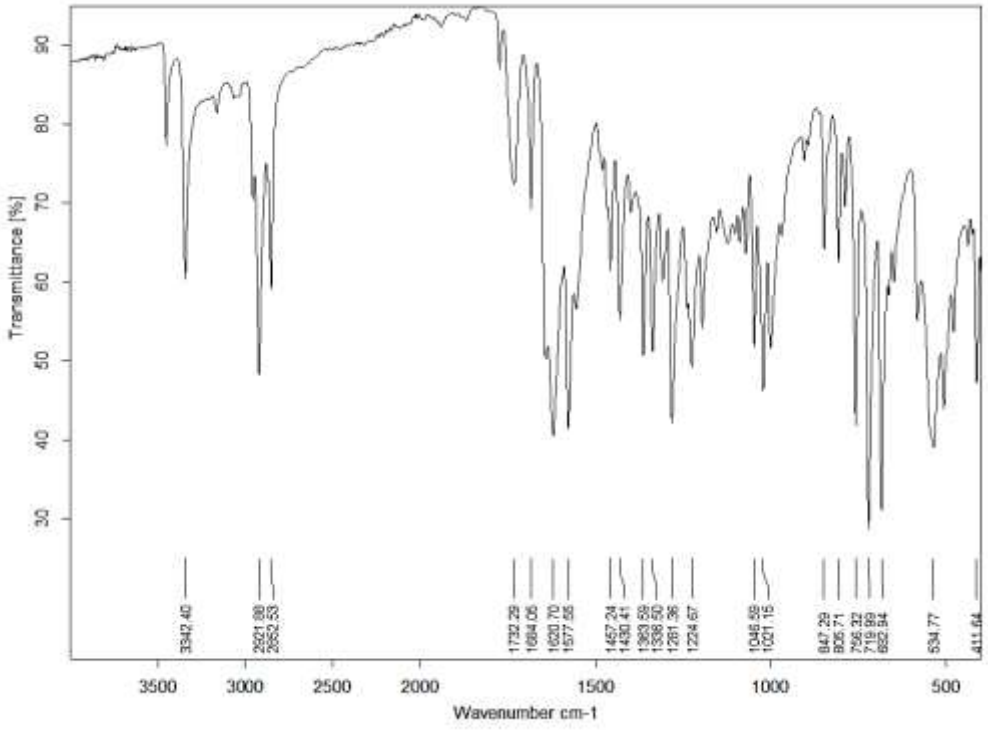
HRMS

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	150.0 Vpp	Set Divert Valve	Waste

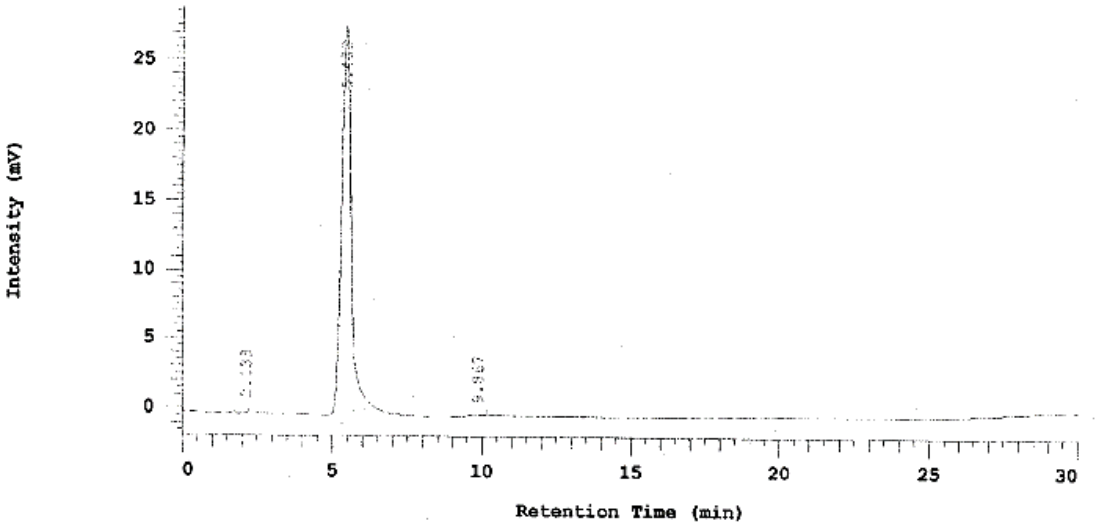


IR



HPLC

Chrom Type: Chromaster Channel : 1

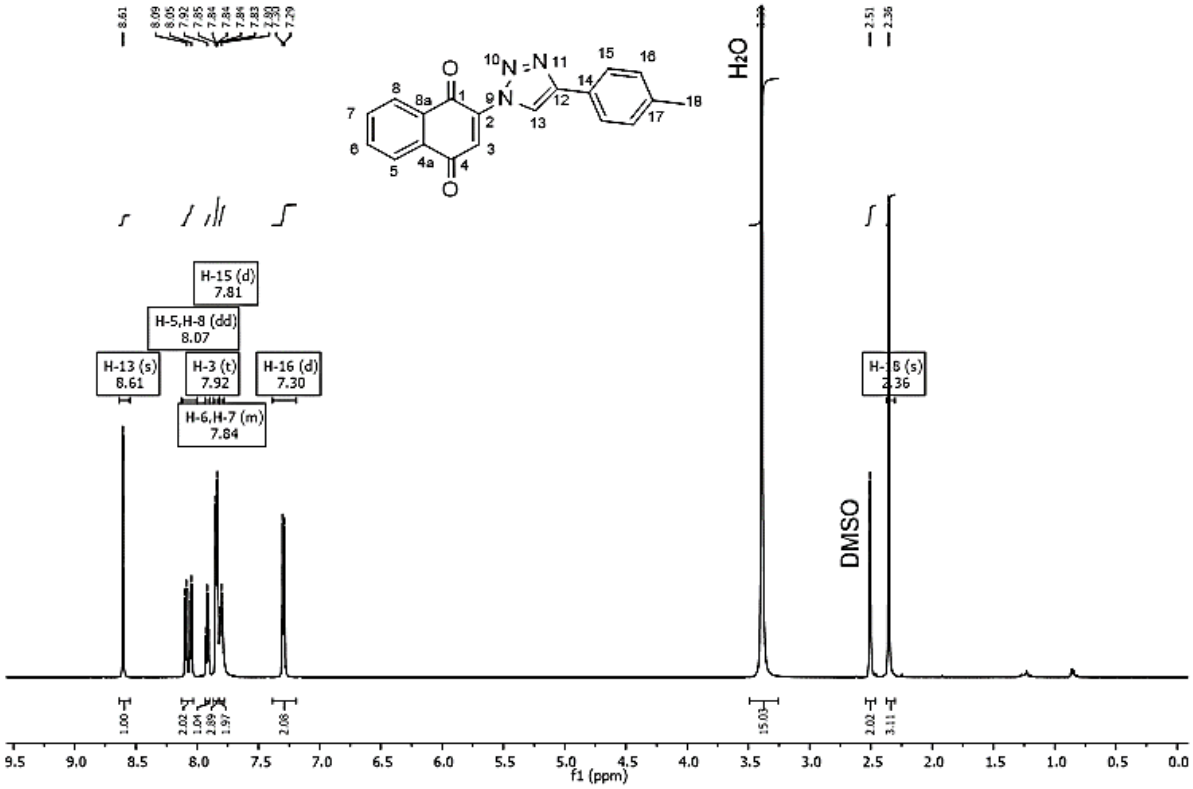


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A:
Pump 1 Solvent B:
Pump 1 Solvent C:
Pump 1 Solvent D:
Method Description: Malaria

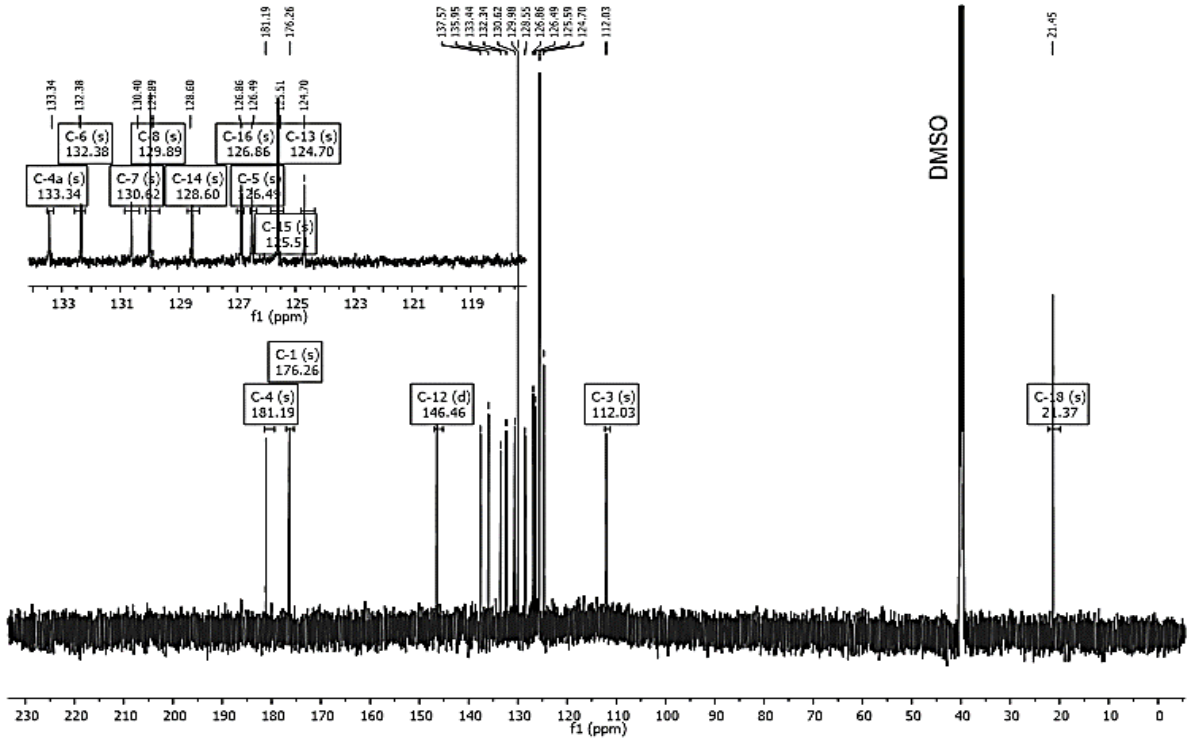
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	2.133	2705	0.445	BB
2	5.433	604070	99.324	BB
3	9.867	1404	0.231	BB
		608179	100.000	

Compound 12
NMR ^{1}H in DMSO



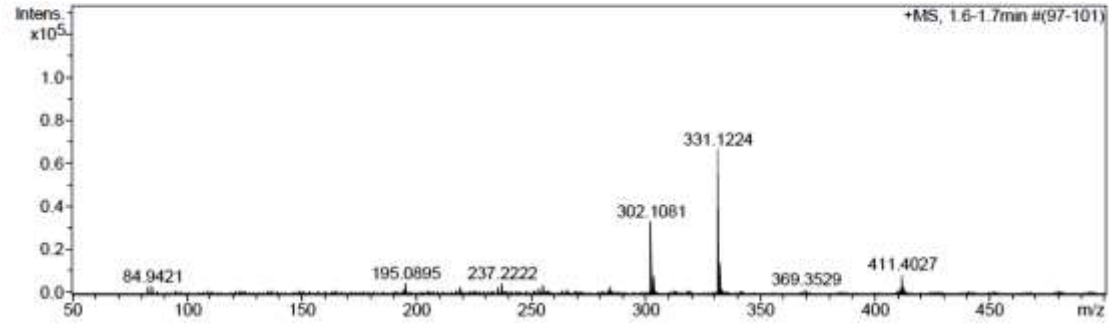
NMR ^{13}C in DMSO



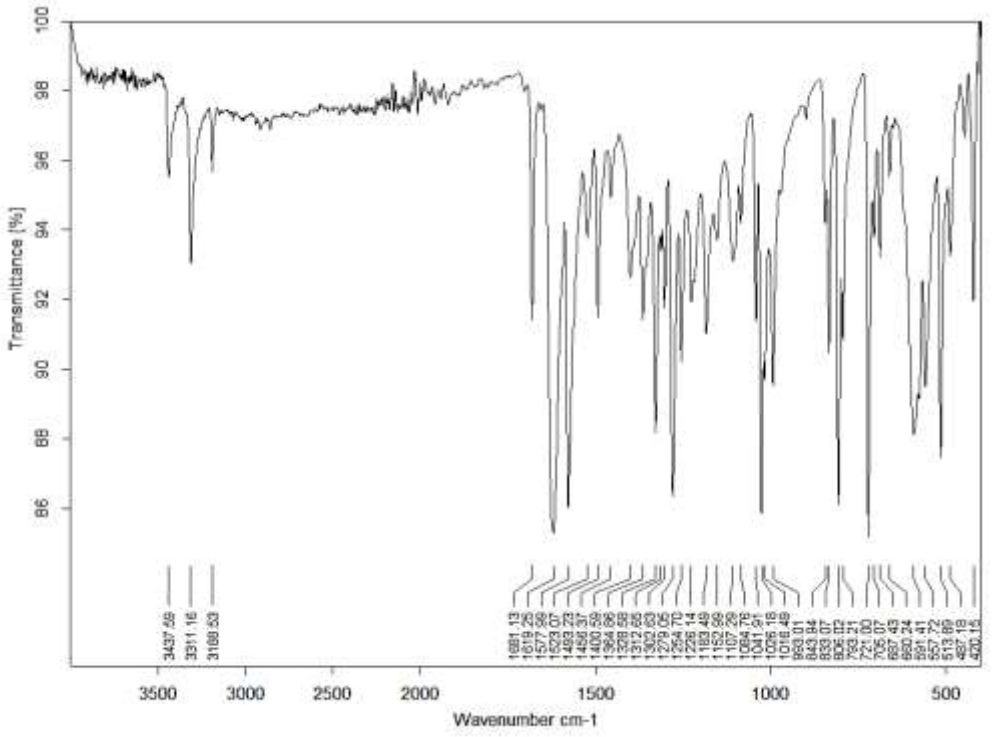
HRMS

Acquisition Parameter

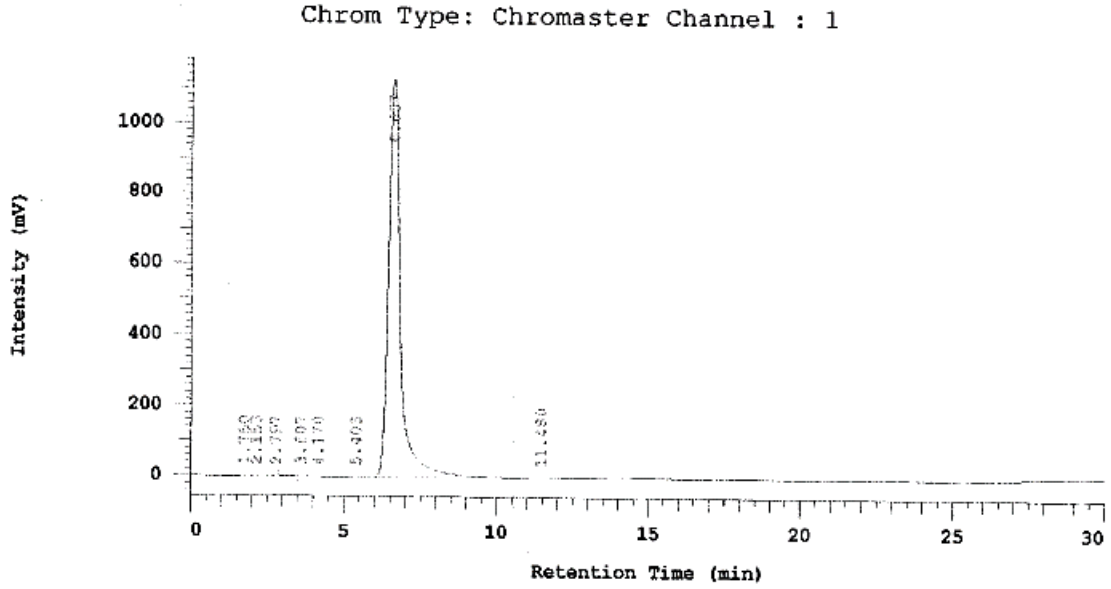
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



IR



HPLC

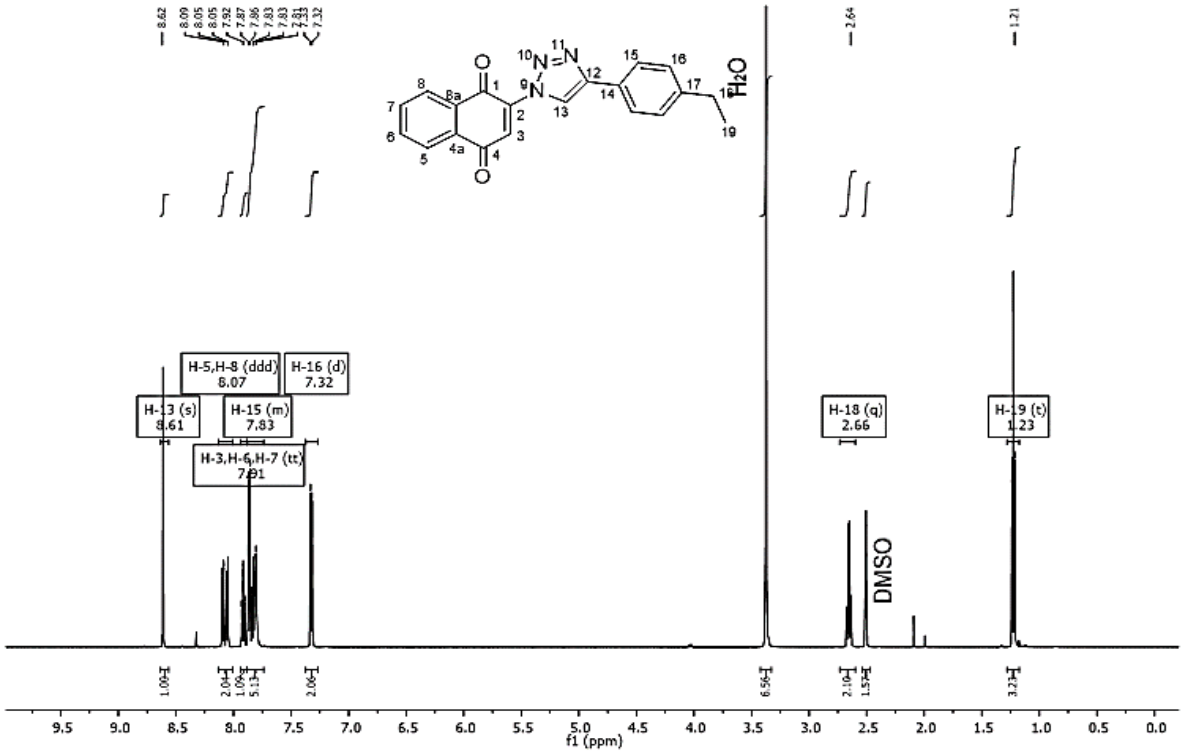


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A:
Pump 1 Solvent B:
Pump 1 Solvent C:
Pump 1 Solvent D:
Method Description: Malaria

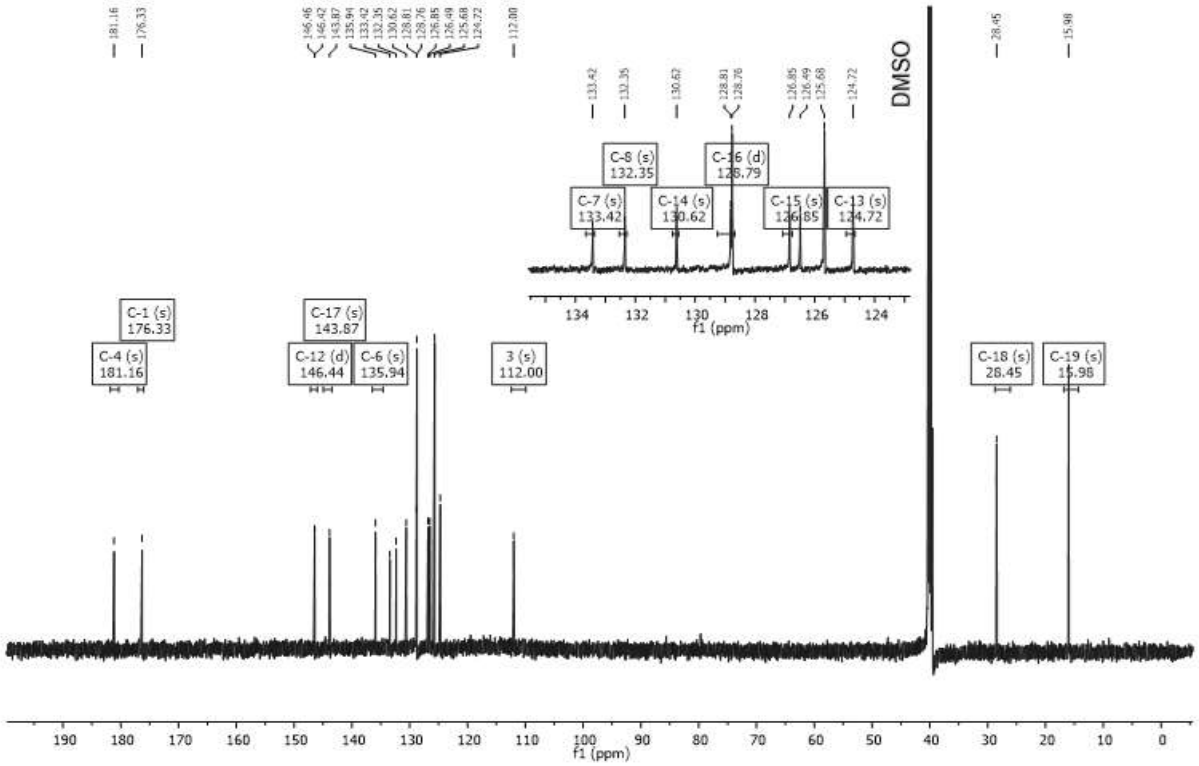
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.750	2450	0.008	BB
2	2.153	3428	0.011	BB
3	2.797	468	0.002	BB
4	3.607	857	0.003	BB
5	4.170	312	0.001	BB
6	5.403	745	0.002	BB
7	6.607	30596341	99.967	BB
8	11.480	1753	0.006	BB
		30606354	100.000	

Compound 13
NMR ^{1}H in DMSO

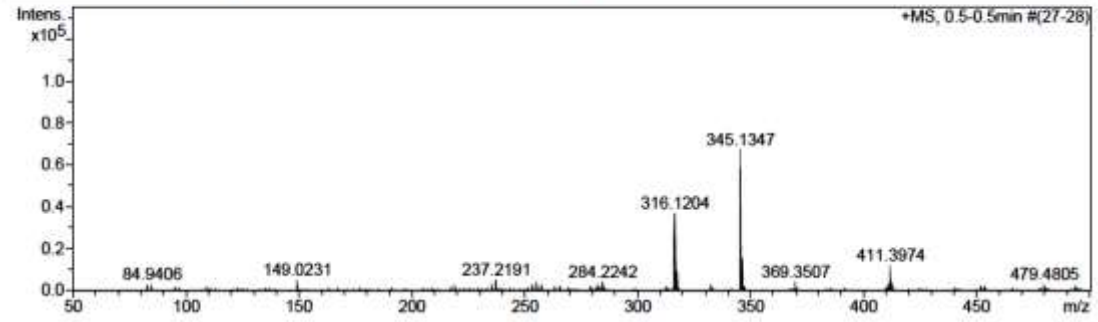


NMR ^{13}C in DMSO

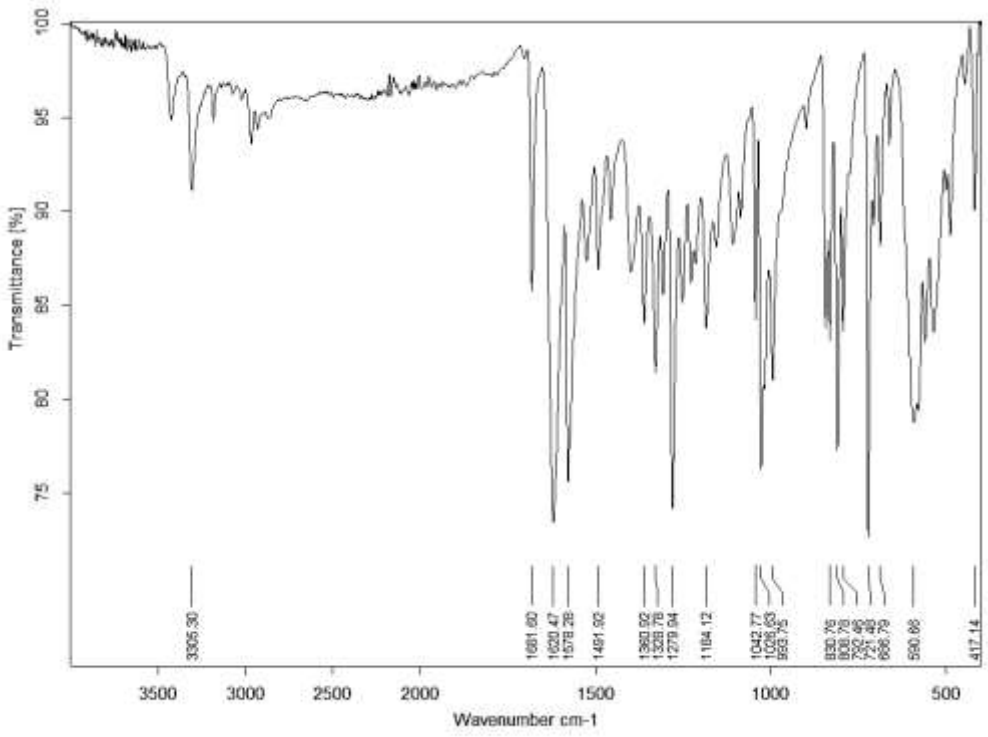


HRMS

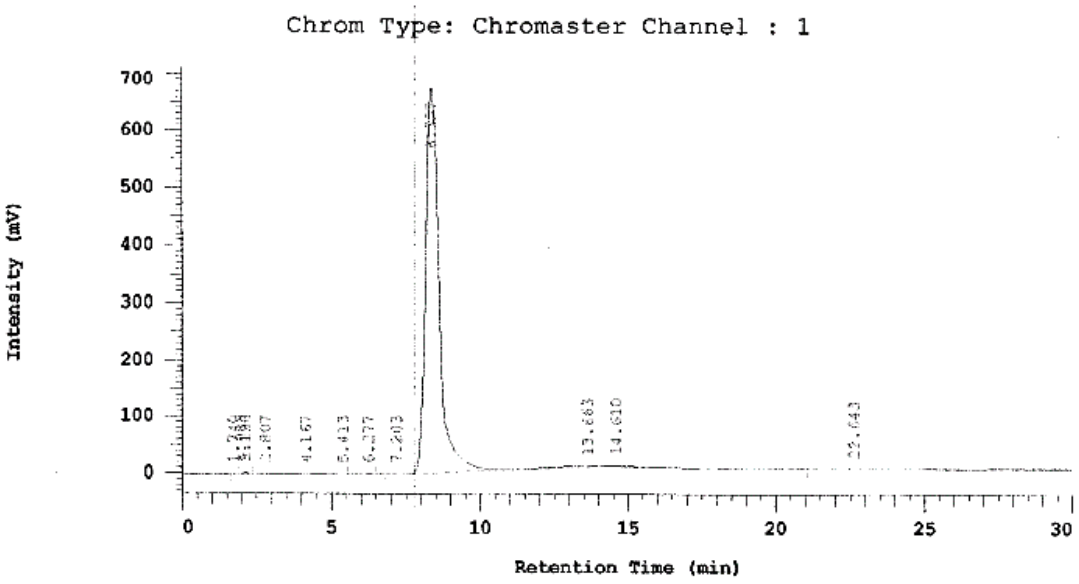
Acquisition Parameter					
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



IR



HPLC

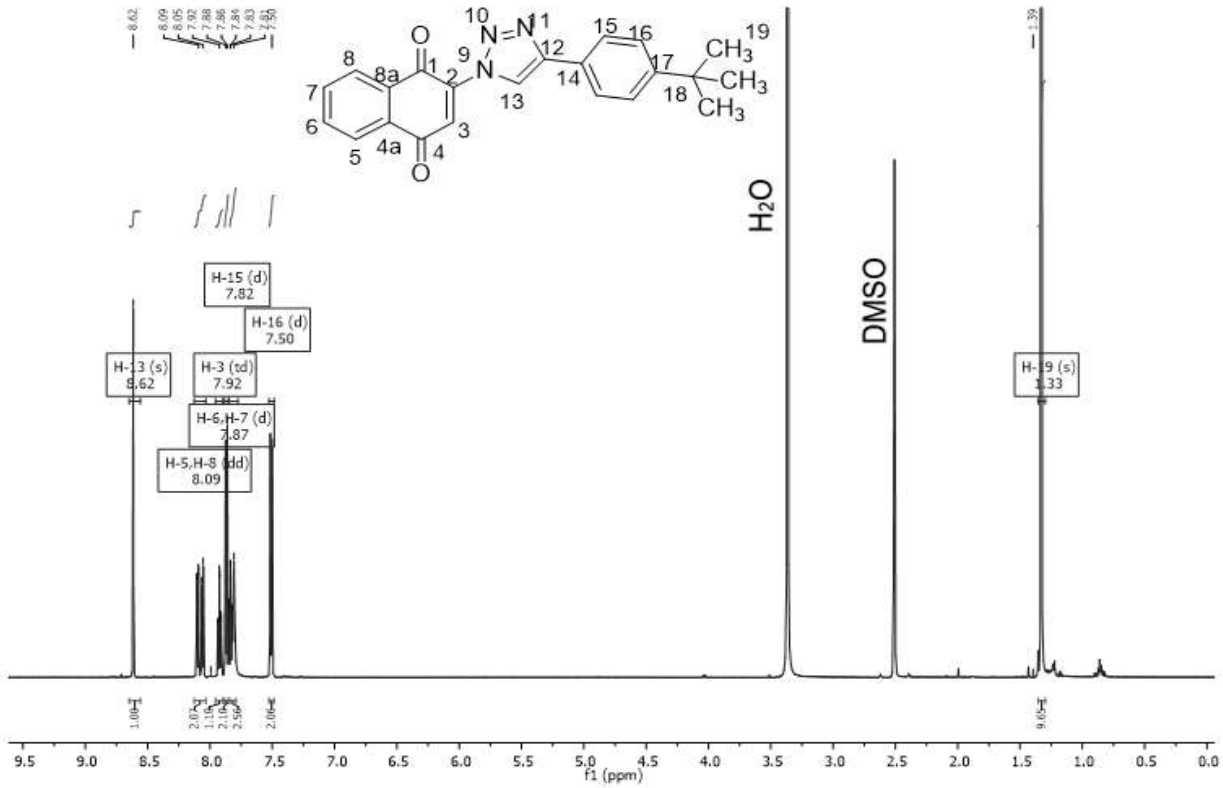


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A:
Pump 1 Solvent B:
Pump 1 Solvent C:
Pump 1 Solvent D:
Method Description: Malaria

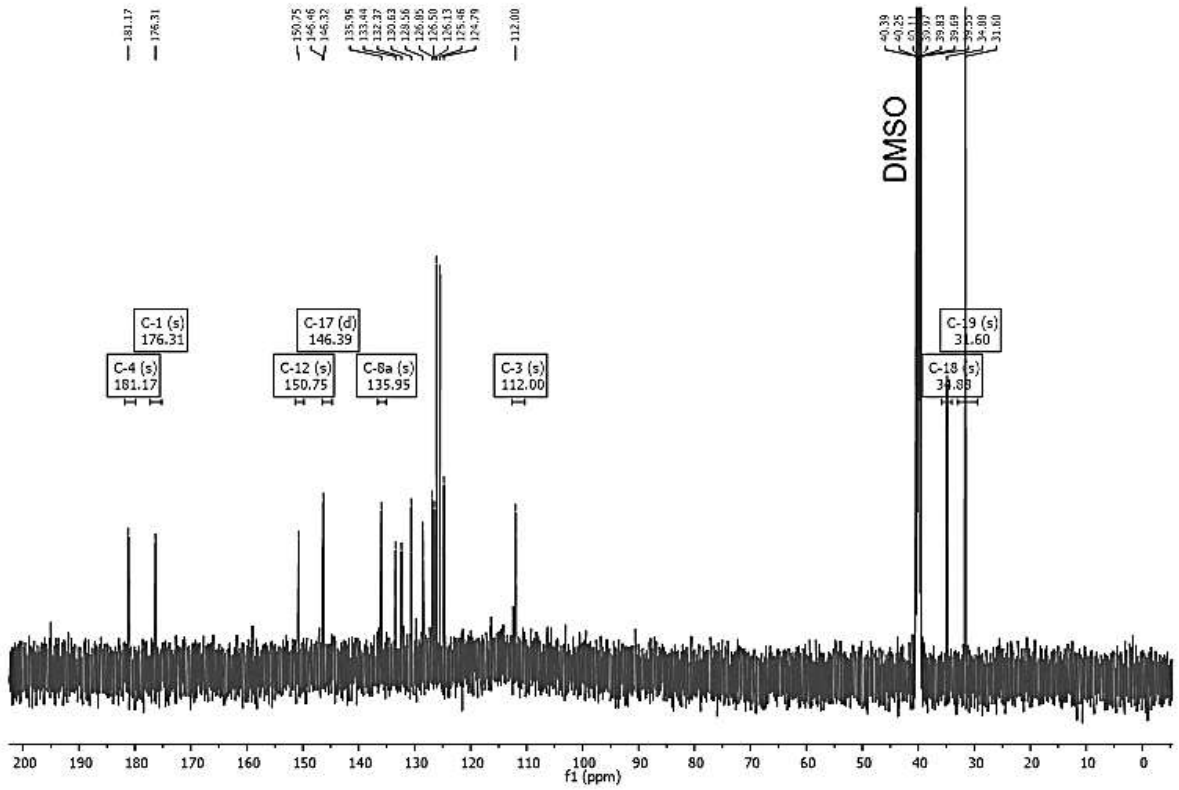
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.740	1226	0.006	BB
2	1.983	3607	0.017	BV
3	2.130	13011	0.060	VB
4	2.807	521	0.002	BB
5	4.167	421	0.002	BB
6	5.413	637	0.003	BB
7	6.277	4710	0.022	BB
8	7.203	4853	0.022	BB
9	8.357	21273190	98.413	BB
10	13.663	215800	0.998	BB
11	14.610	68736	0.318	BB
12	22.643	29616	0.137	BB
		21616328	100.000	

Compound 14
NMR ^{1}H in DMSO



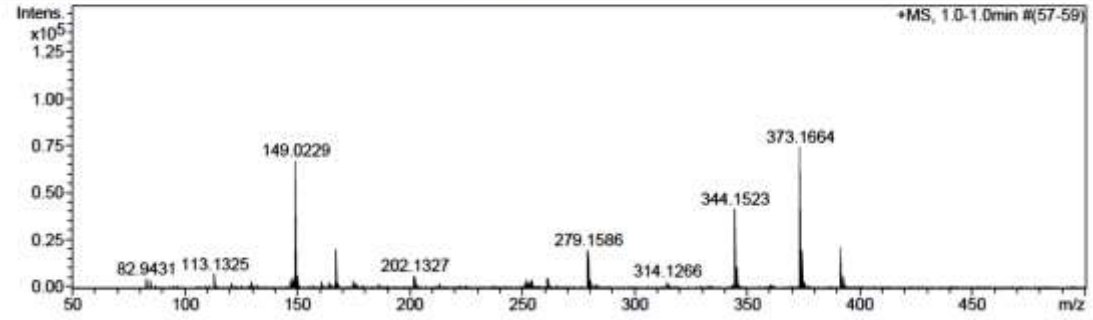
NMR ^{13}C in DMSO



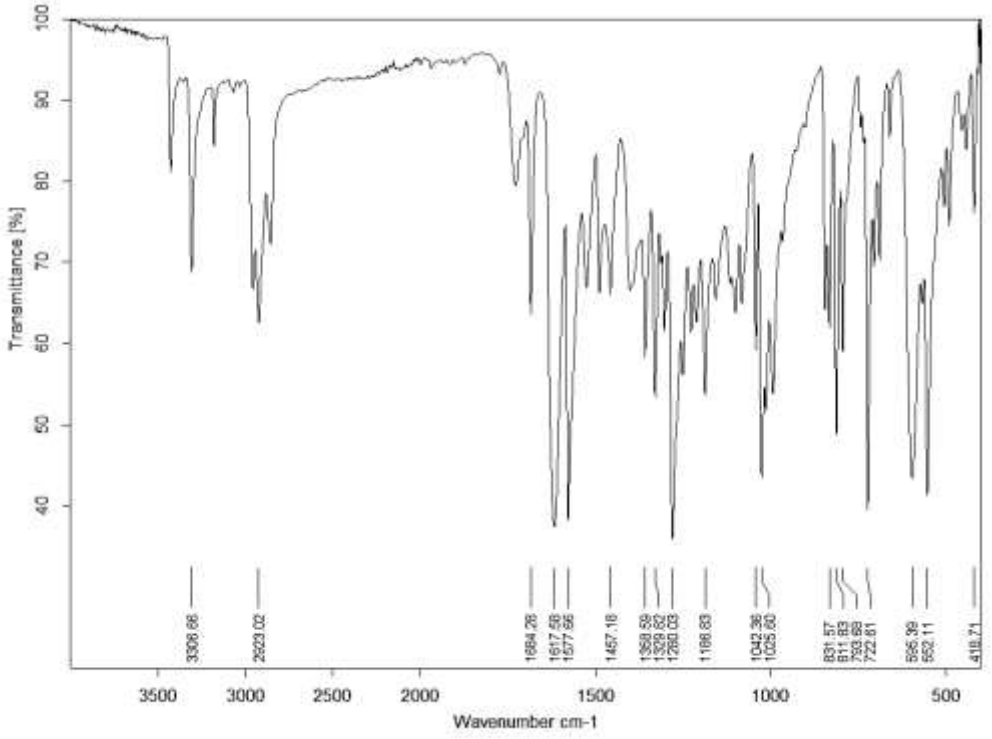
HRMS

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	150.0 Vpp	Set Divert Valve	Waste

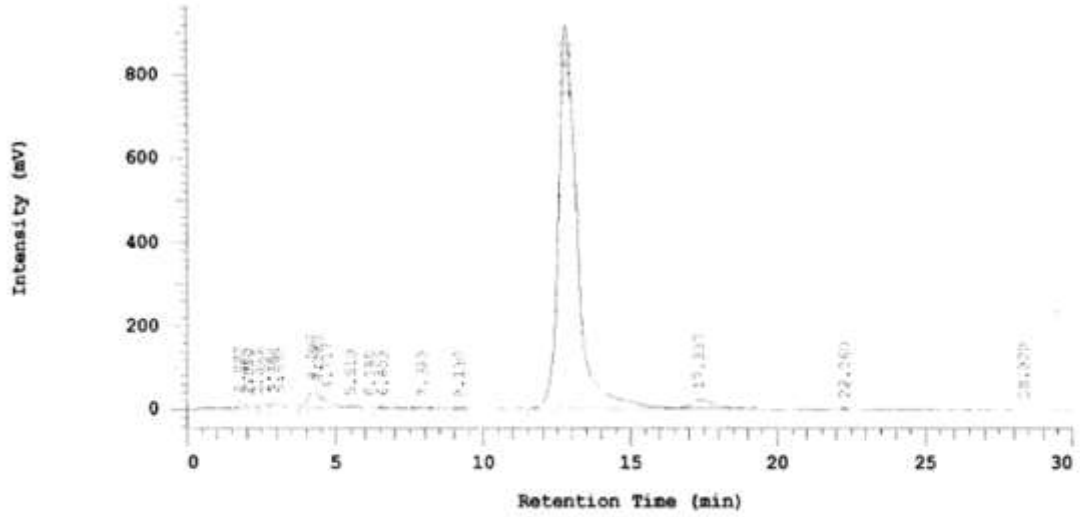


IR



HPLC

Chrom Type: Chromaster Channel : 1

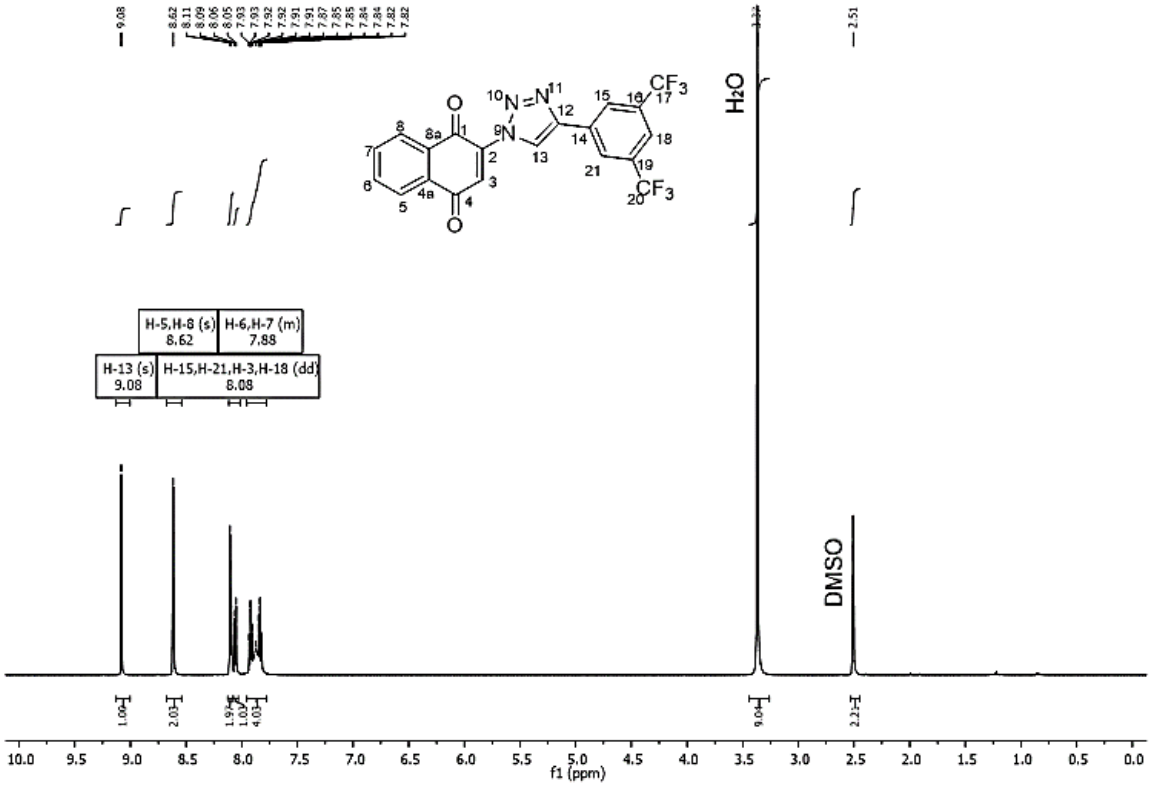


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A: Pump 1 Solvent B:
Pump 1 Solvent C: Pump 1 Solvent D:
Method Description: Malaria

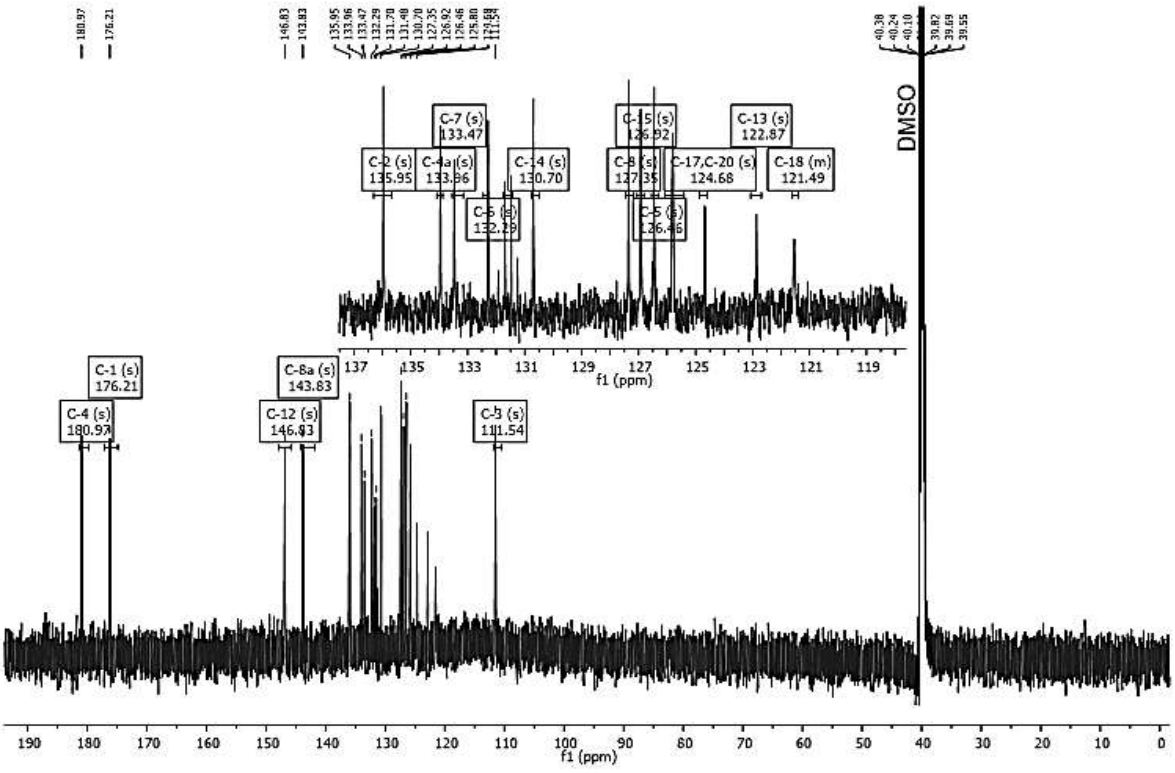
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.777	154447	0.351	BV
2	1.963	45977	0.104	VV
3	2.070	66290	0.150	VV
4	2.373	35054	0.080	VV
5	2.627	170791	0.388	VV
6	2.950	112410	0.255	VV
7	3.093	238734	0.542	VV
8	4.207	642226	1.458	VV
9	4.380	511640	1.161	VV
10	4.717	524623	1.191	VV
11	5.510	25421	0.058	TBB
12	6.180	1850	0.004	BV
13	6.603	27033	0.061	VB
14	7.923	62914	0.143	BB
15	9.180	3366	0.008	BB
16	12.813	40379230	91.640	BV
17	17.337	1040543	2.361	TBB
18	22.260	15418	0.035	BB
19	28.320	4984	0.011	BB
		44062951	100.000	

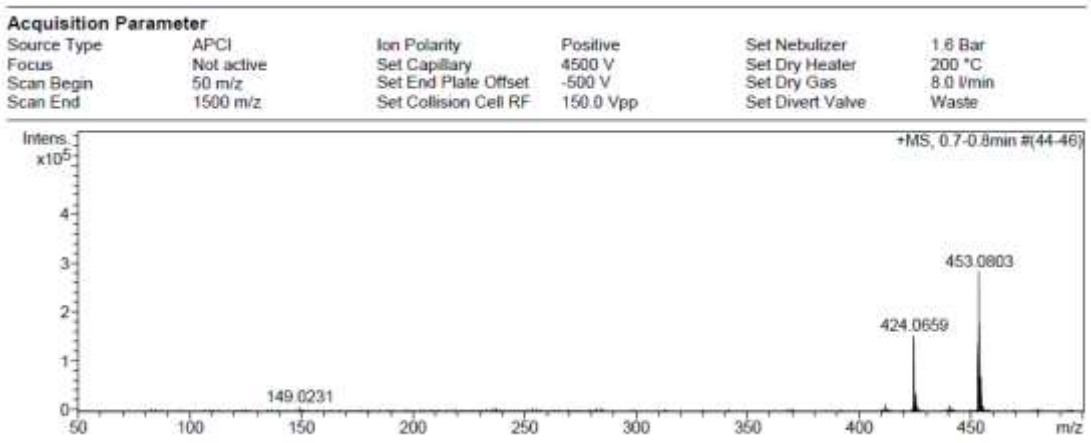
Compound 15
NMR ^{1}H in DMSO



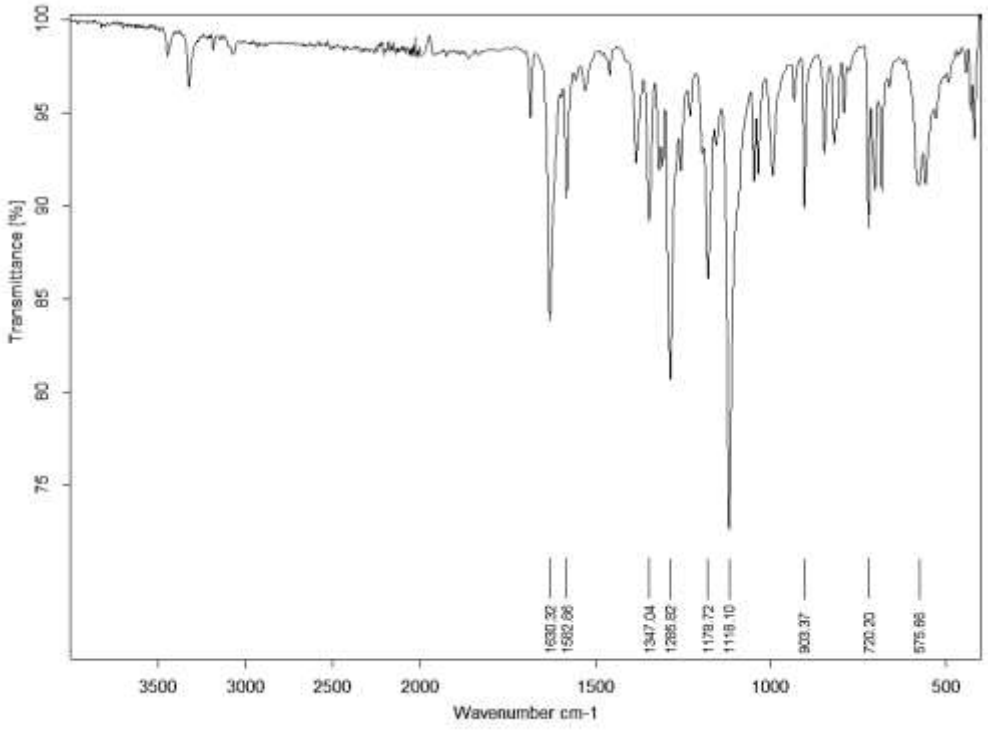
NMR ^{13}C in DMSO



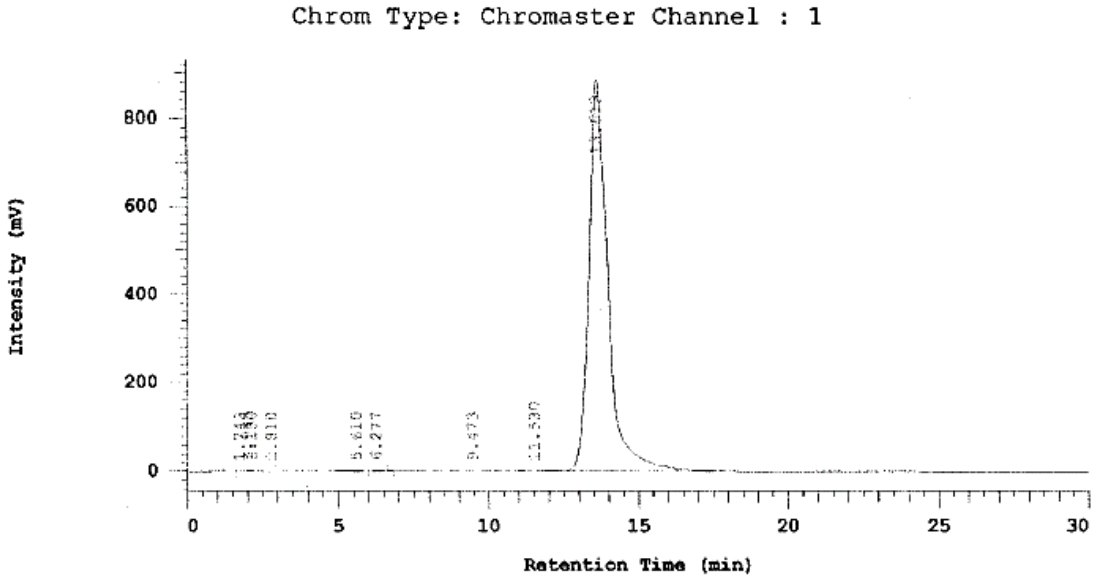
HRMS


Acquisition Parameter
Source Type APCI
Focus Not active
Scan Begin 50 m/z
Scan End 1500 m/z
Ion Polarity Positive
Set Capillary 4500 V
Set End Plate Offset -500 V
Set Collision Cell RF 150.0 Vpp
Set Nebulizer 1.6 Bar
Set Dry Heater 200 °C
Set Dry Gas 8.0 l/min
Set Divert Valve Waste
Intens.
x10^5
+MS, 0.7-0.8min #(44-46)
453.0803
424.0659
149.0231
m/z

IR


Transmittance [%]
Wavenumber cm-1
1630.32
1582.88
1347.04
1285.82
1178.72
1118.10
903.37
720.20
576.66

HPLC

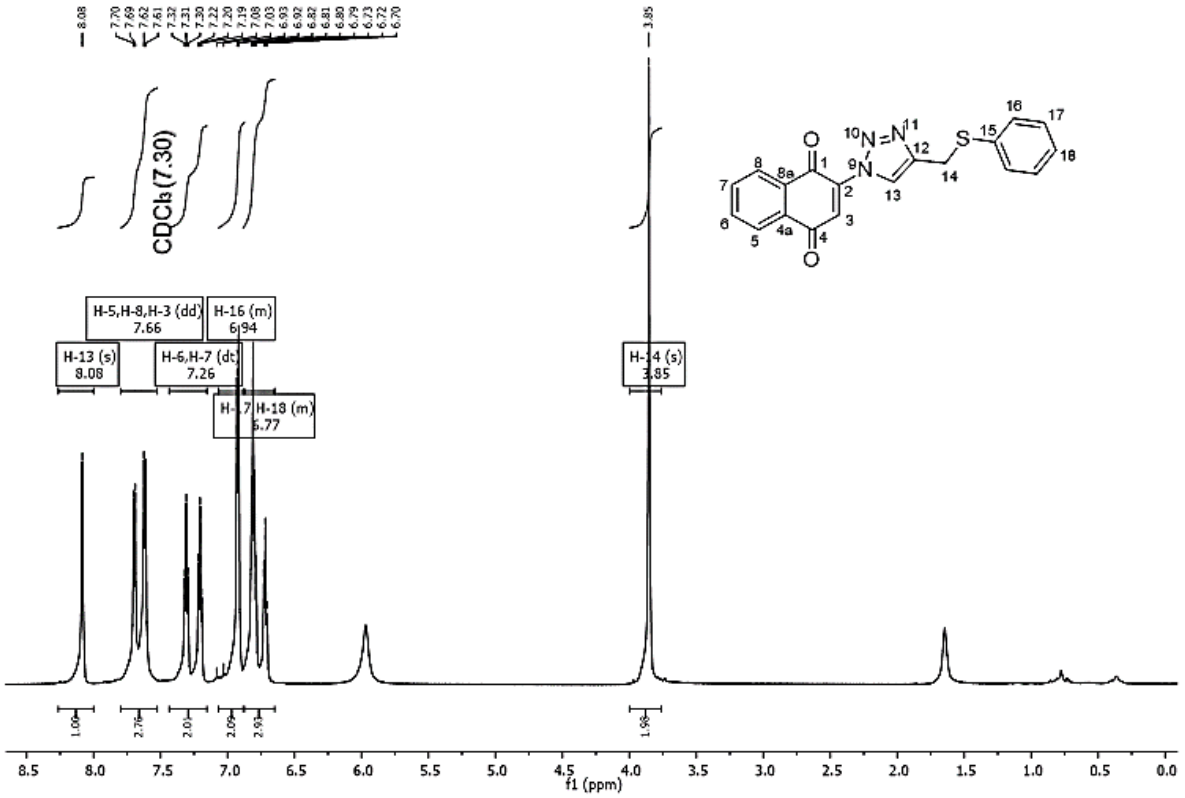


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A: Pump 1 Solvent B:
Pump 1 Solvent C: Pump 1 Solvent D:
Method Description: Malaria

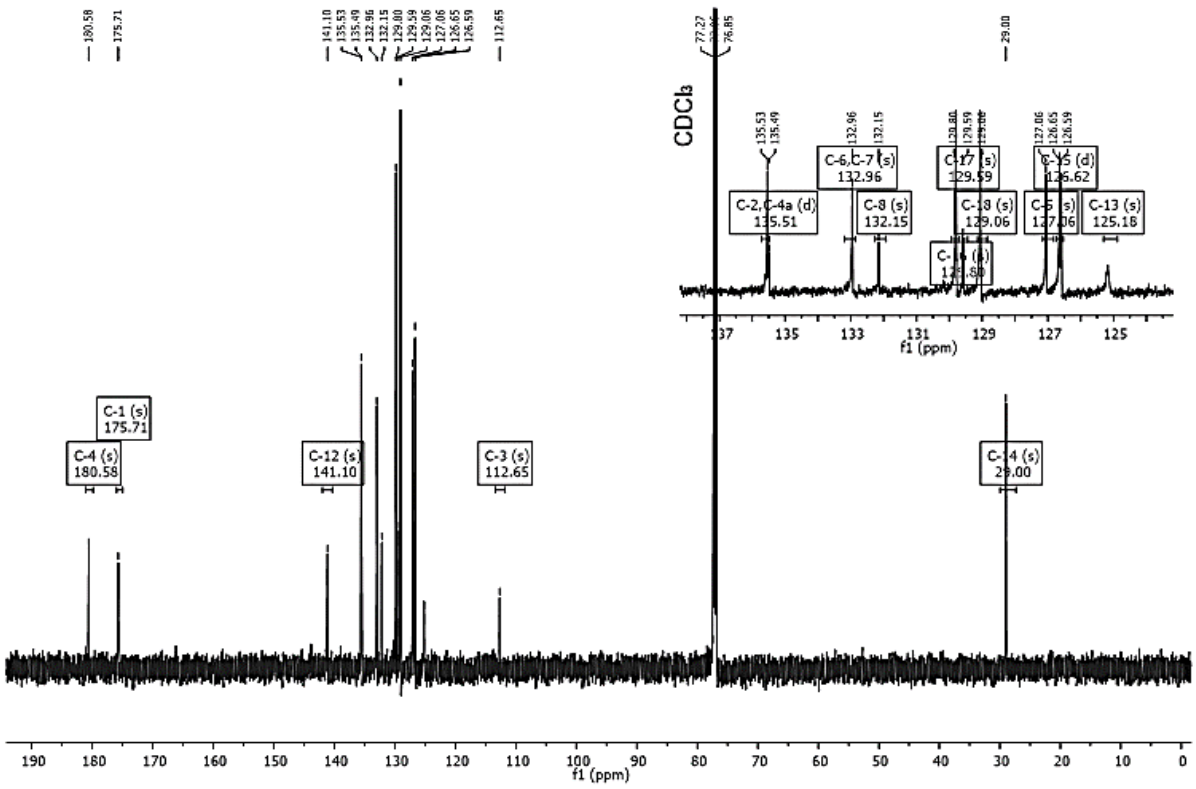
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.743	1359	0.004	BB
2	1.983	3759	0.010	BV
3	2.130	13777	0.036	VB
4	2.810	473	0.001	BB
5	5.610	12328	0.032	BB
6	6.277	5571	0.014	BB
7	9.473	22125	0.057	BB
8	11.530	1665	0.004	BB
9	13.613	38744414	99.843	BB
		38805471	100.000	

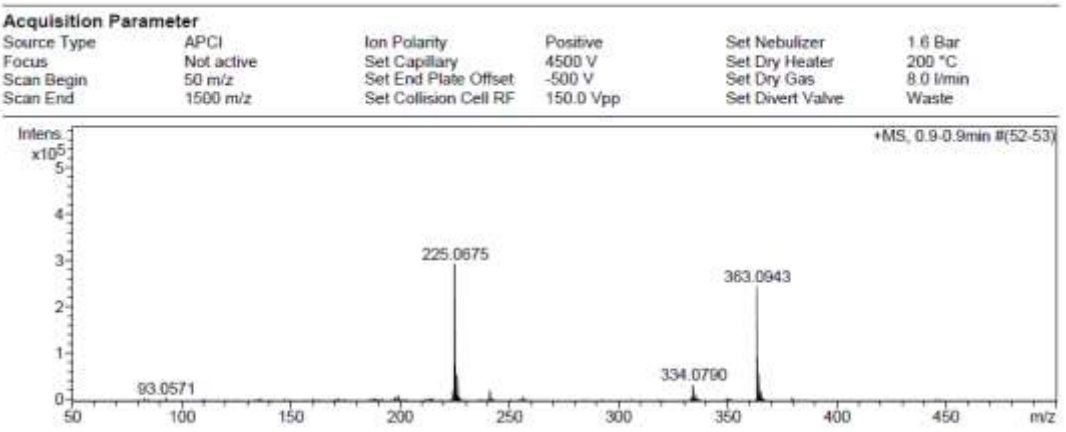
Compound 16
NMR ^{1}H in $CDCl_3$



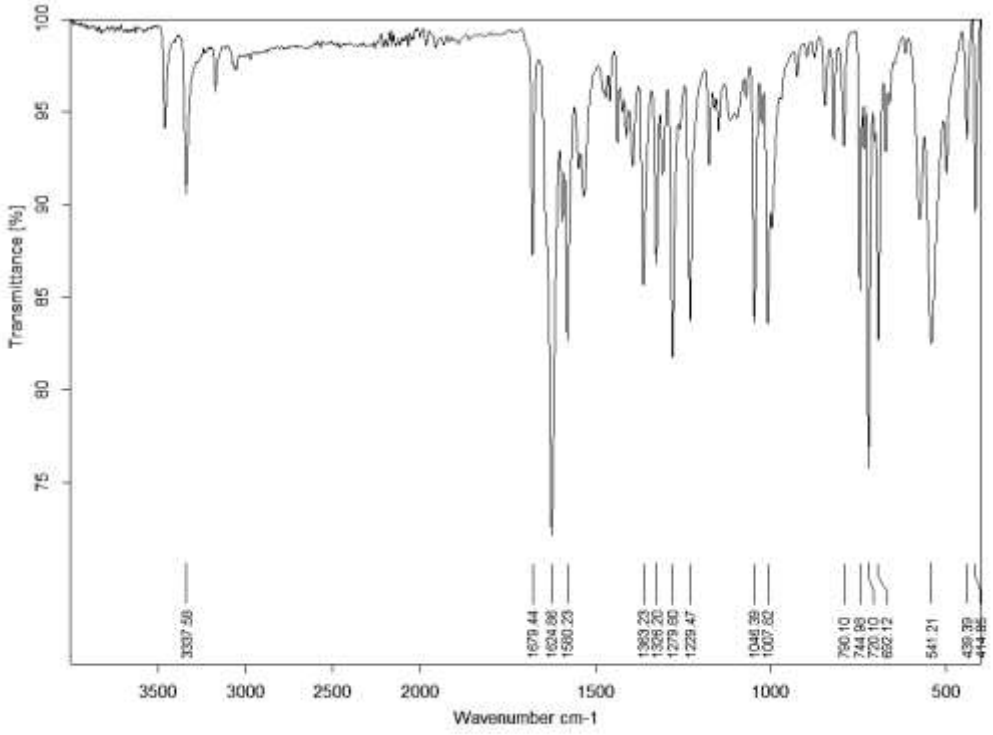
NMR ^{13}C in $CDCl_3$



HRMS

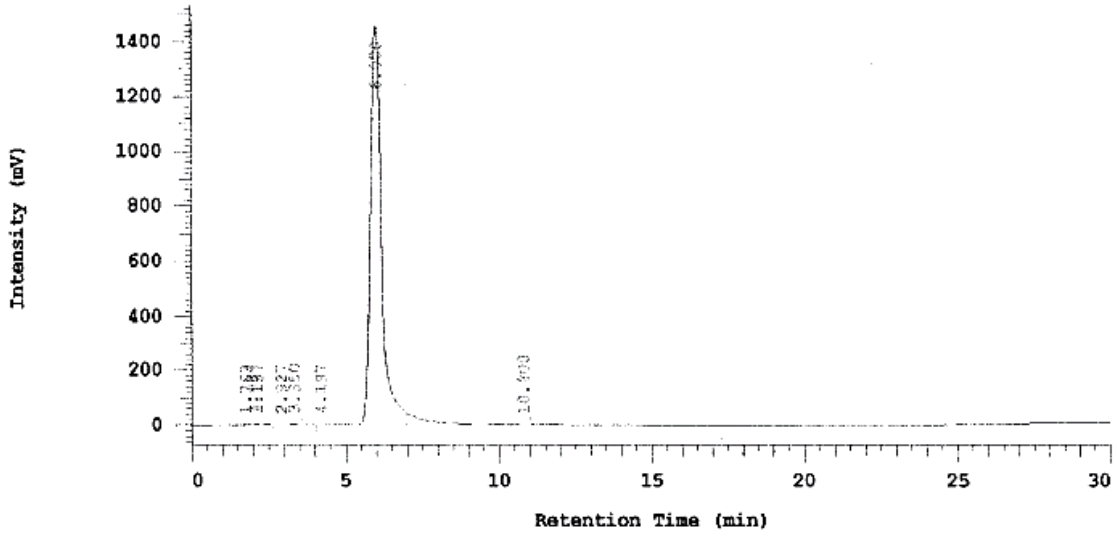

Acquisition Parameter
Source Type APCI
Focus Not active
Scan Begin 50 m/z
Scan End 1500 m/z
Ion Polarity Positive
Set Capillary 4500 V
Set End Plate Offset -500 V
Set Collision Cell RF 150.0 Vpp
Set Nebulizer 1.6 Bar
Set Dry Heater 200 °C
Set Dry Gas 8.0 l/min
Set Divert Valve Waste
Intens.
x10^5
+MS, 0.9-0.9min #(52-53)
225.0675
363.0943
334.0790
93.0571
m/z

IR


Transmittance [%]
Wavenumber cm-1
3337.58
1679.44
1624.86
1580.23
1363.23
1329.20
1279.92
1229.47
1046.39
1007.82
790.10
744.88
720.10
682.12
541.21
439.39
414.85

HPLC

Chrom Type: Chromaster Channel : 1

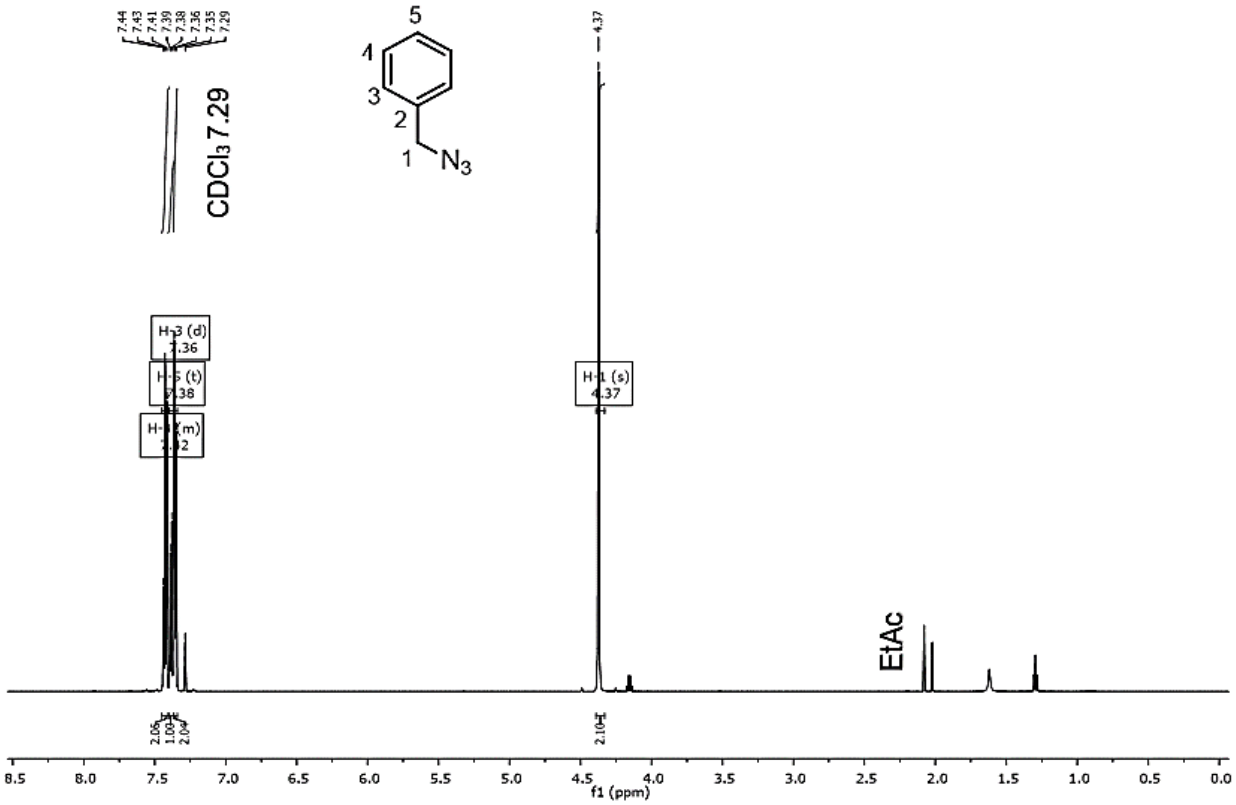


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A:
Pump 1 Solvent B:
Pump 1 Solvent C:
Pump 1 Solvent D:
Method Description: Malaria

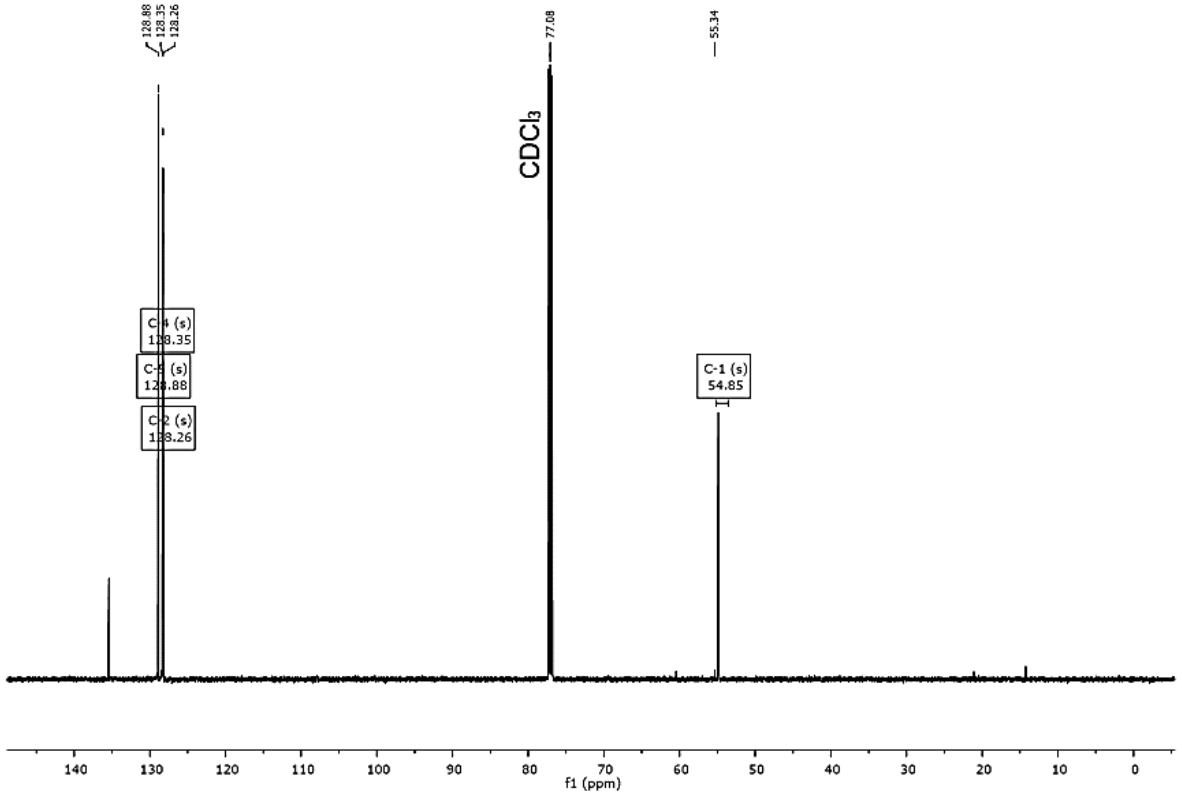
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.763	1274	0.003	BB
2	1.987	2615	0.007	BV
3	2.137	8944	0.024	VB
4	2.927	5786	0.015	BV
5	3.350	16127	0.043	VB
6	4.197	284	0.001	BB
7	6.000	37436771	99.905	BB
8	10.800	748	0.002	BB
		37472549	100.000	

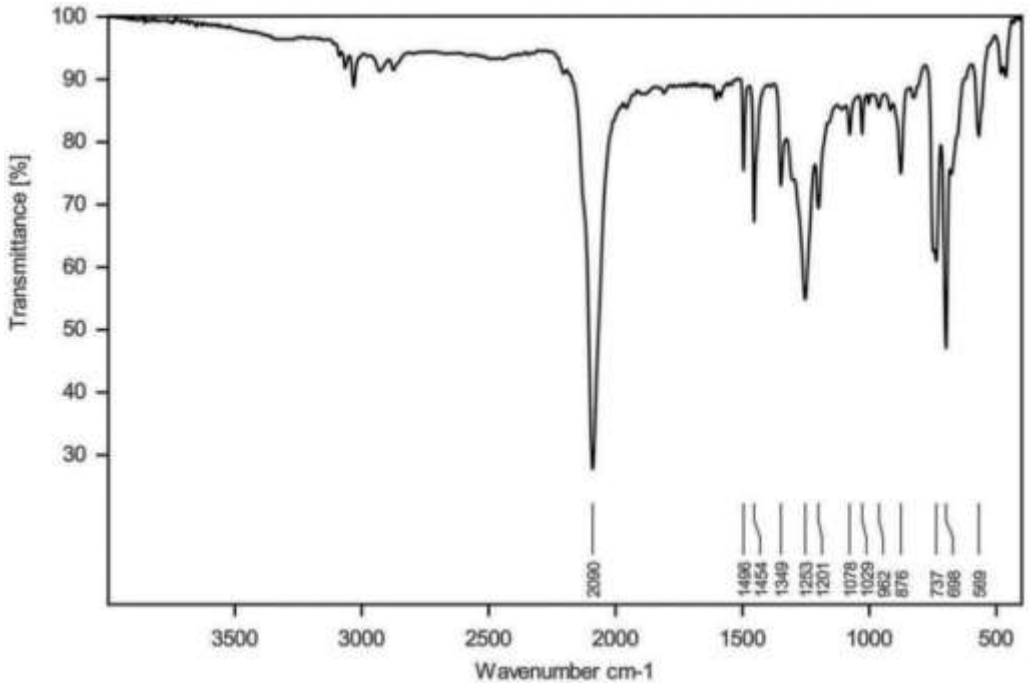
Intermediate 17a
NMR ^{1}H in $CDCl_3$



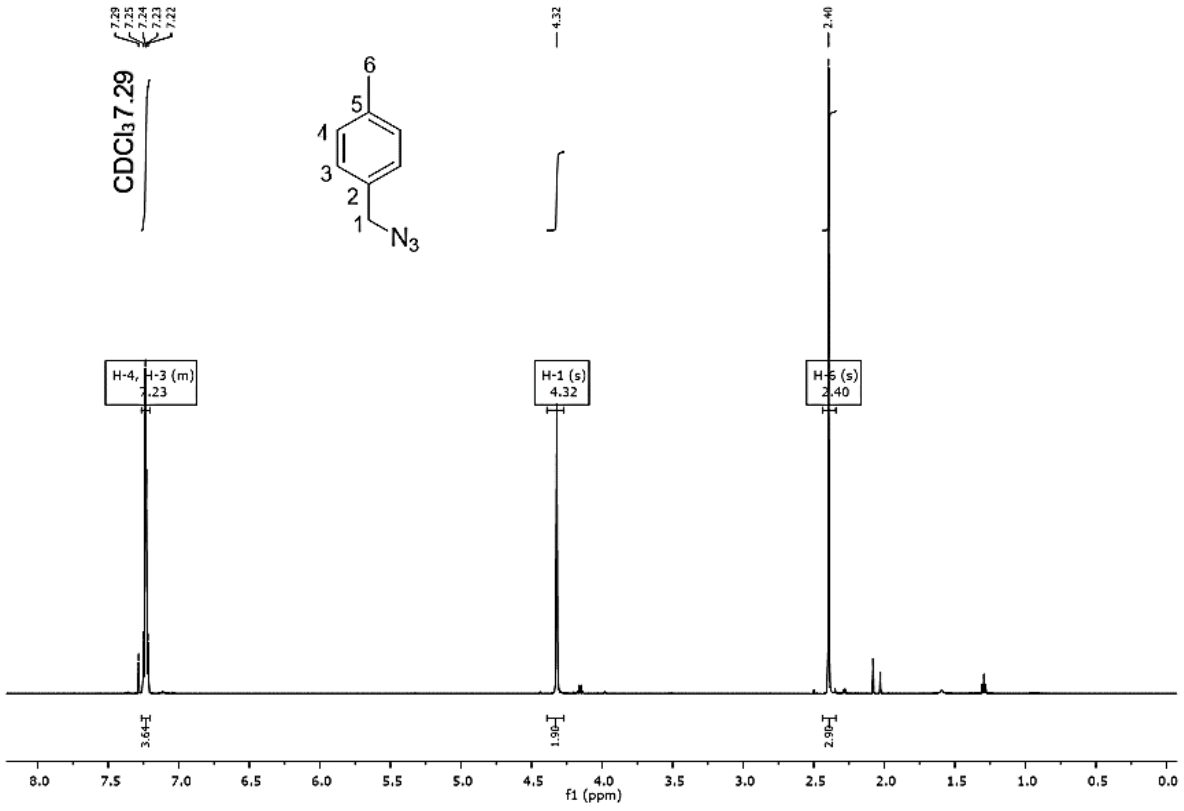
NMR ^{13}C in $CDCl_3$



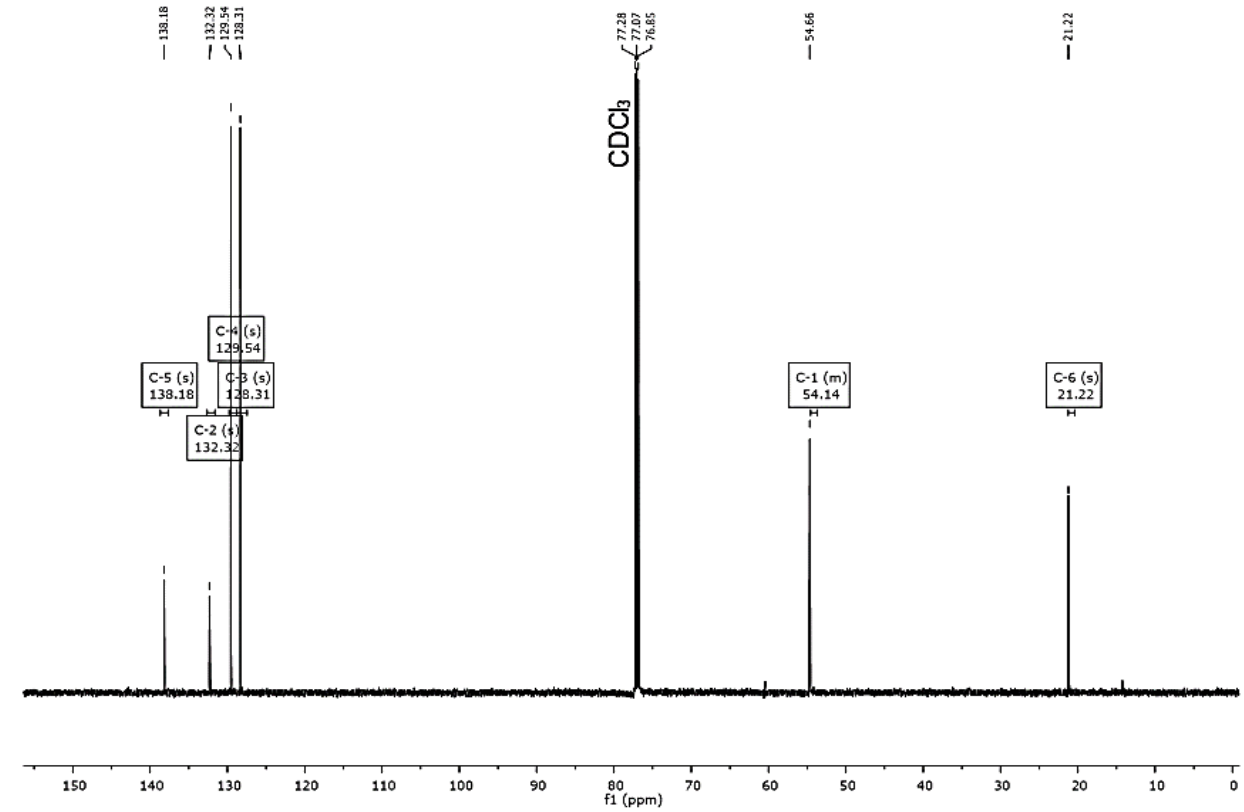
IR



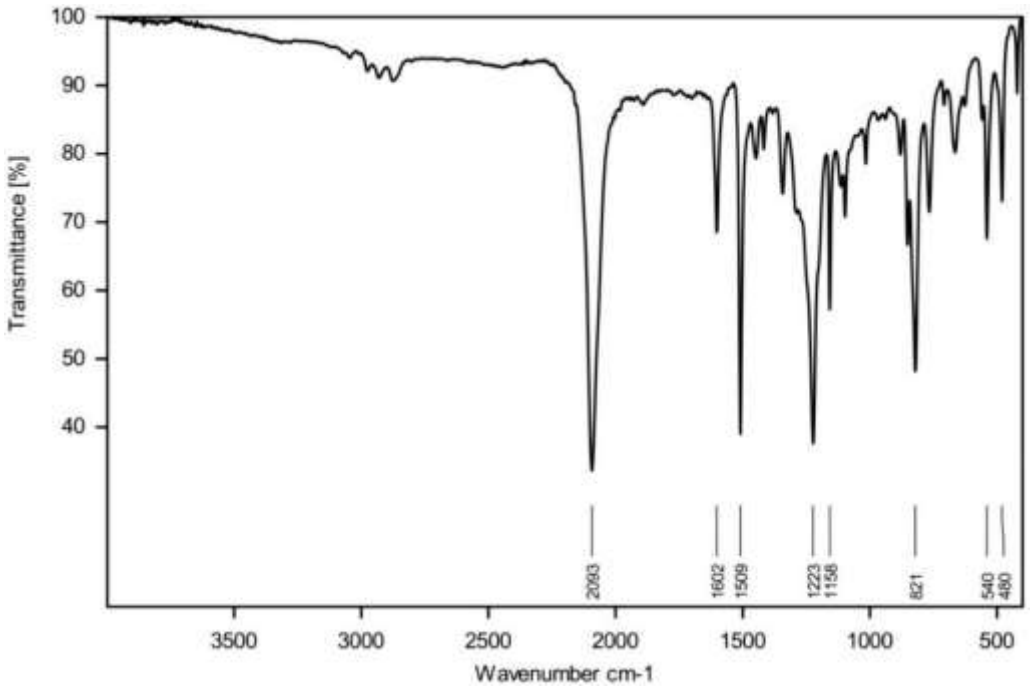
Intermediate 17b
NMR ^{1}H in $CDCl_3$



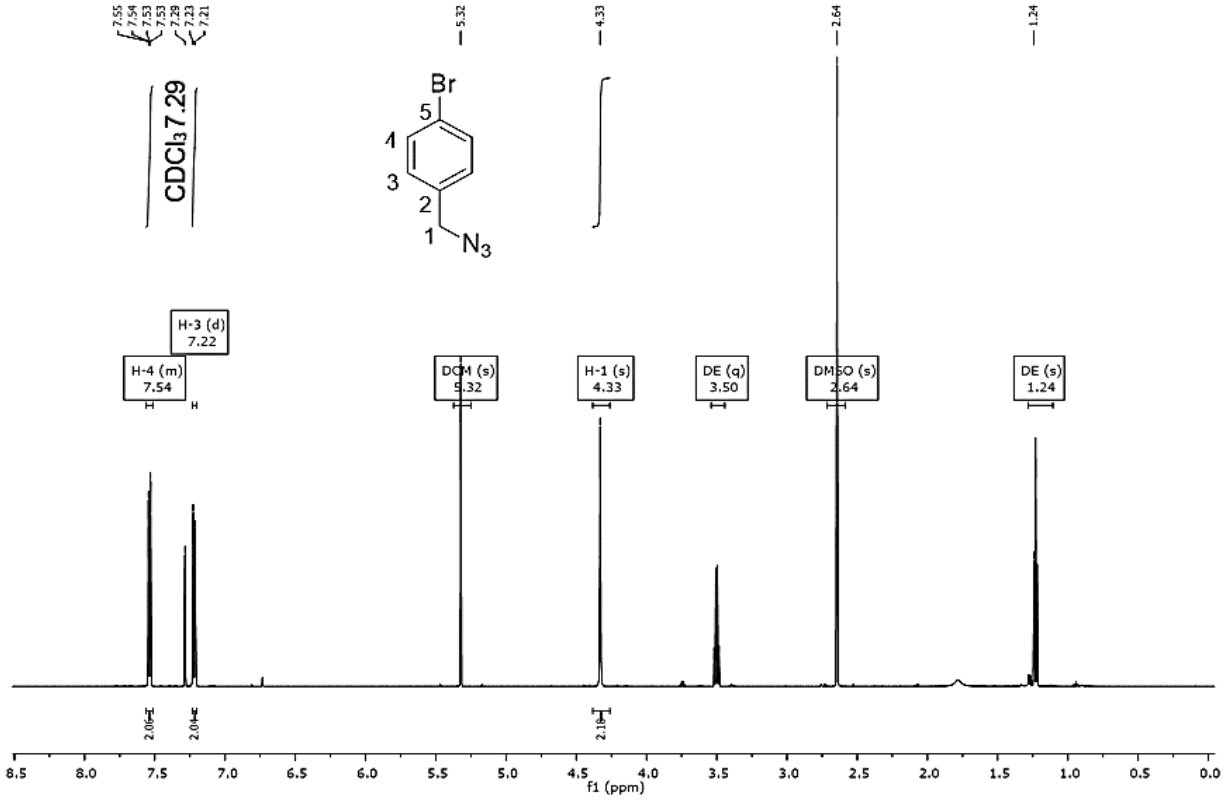
NMR ^{13}C in $CDCl_3$



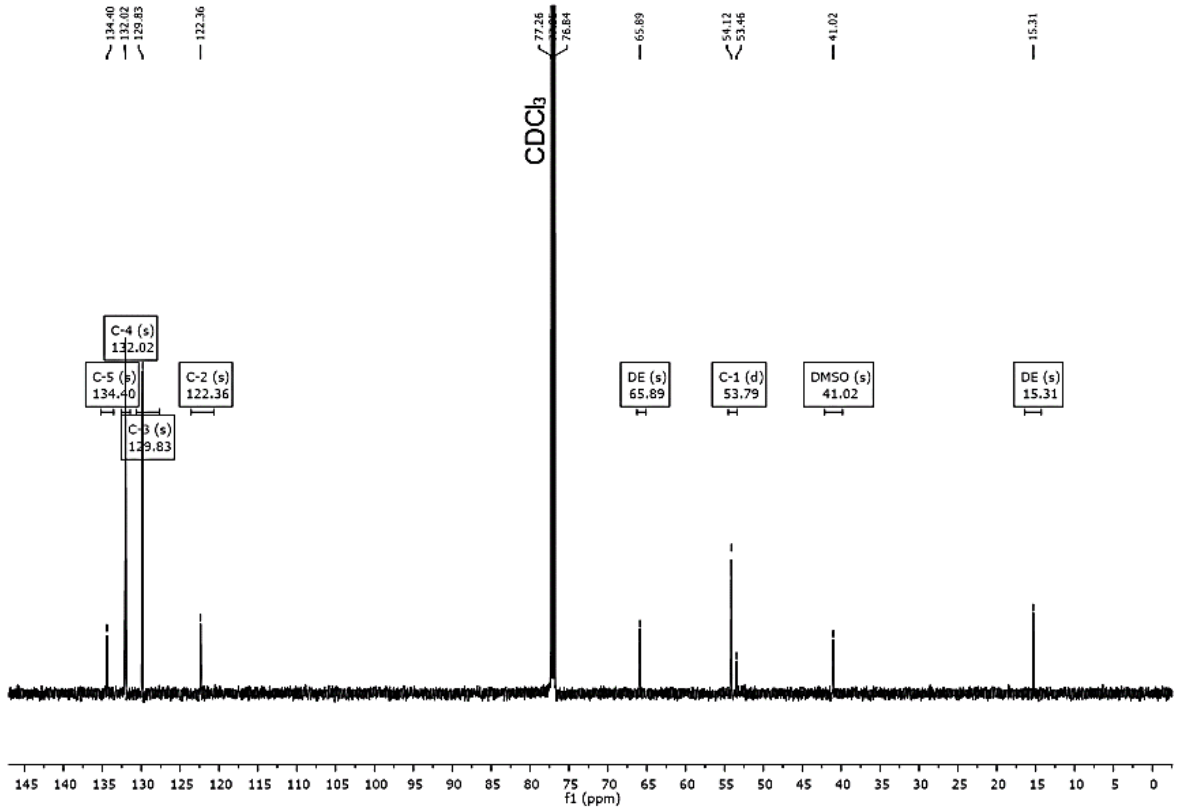
IR



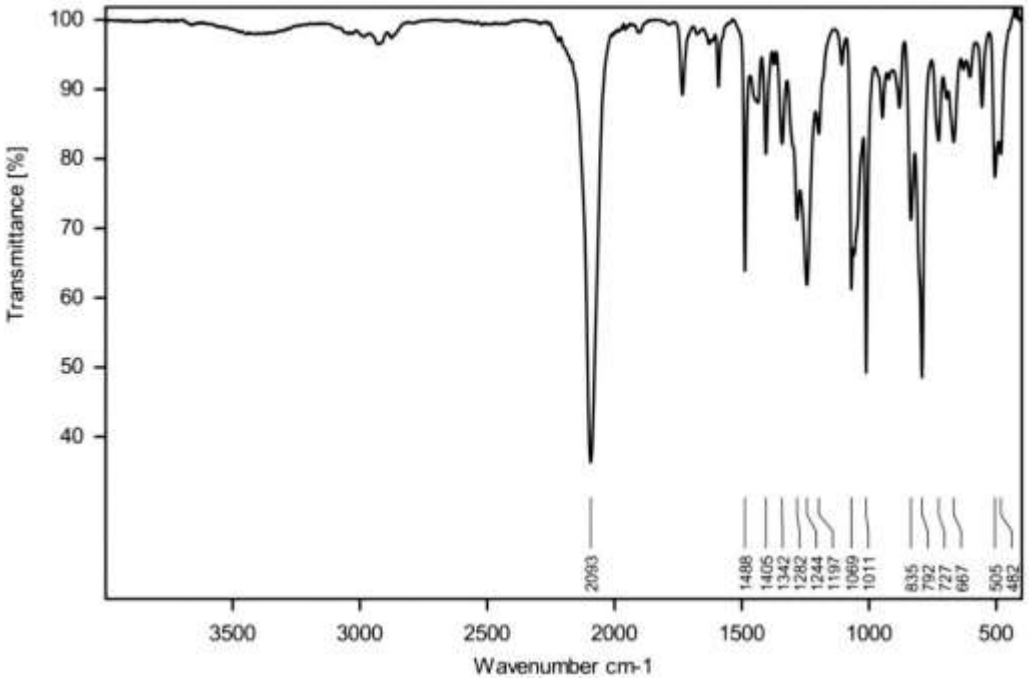
Intermediate 17d
NMR ^{1}H in $CDCl_3$



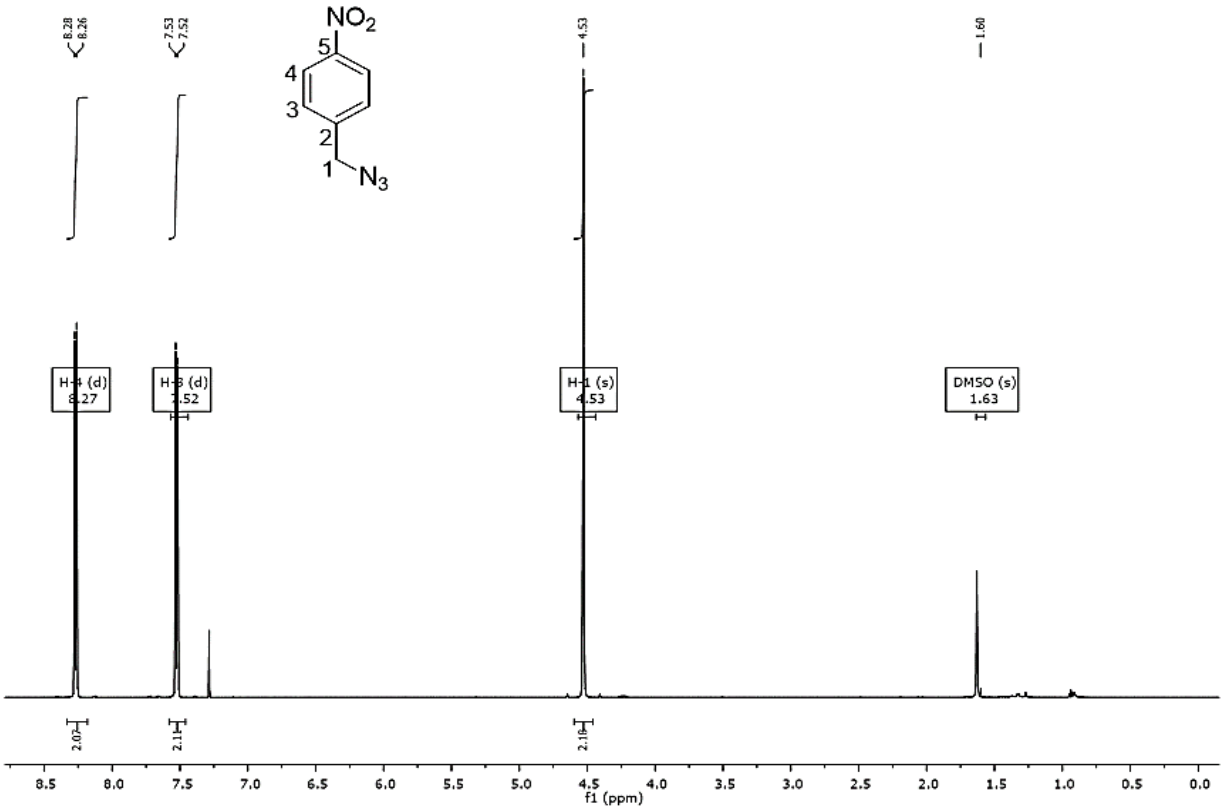
NMR ^{13}C in $CDCl_3$



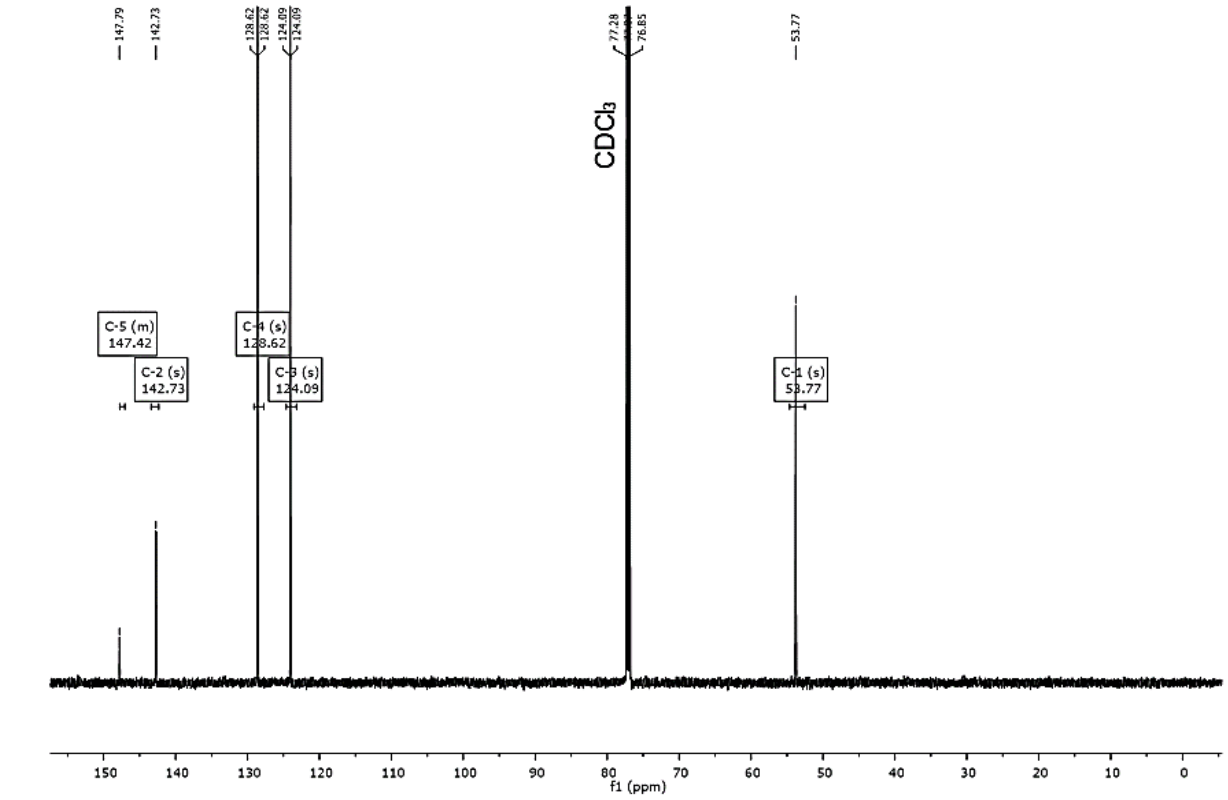
IR



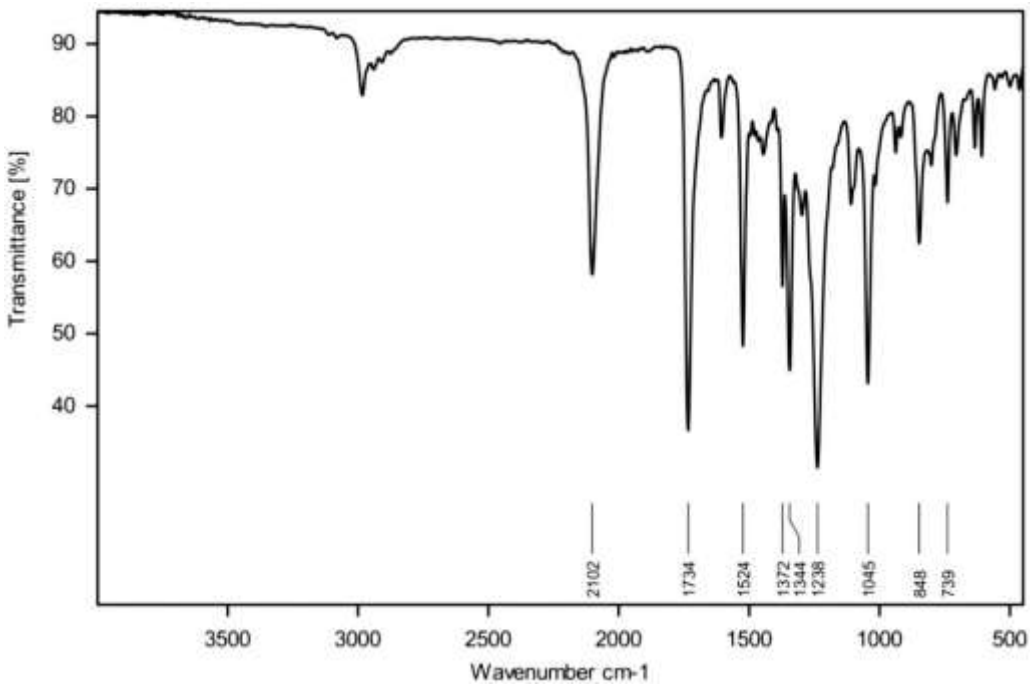
Intermediate 17f
NMR ^{1}H in $CDCl_3$



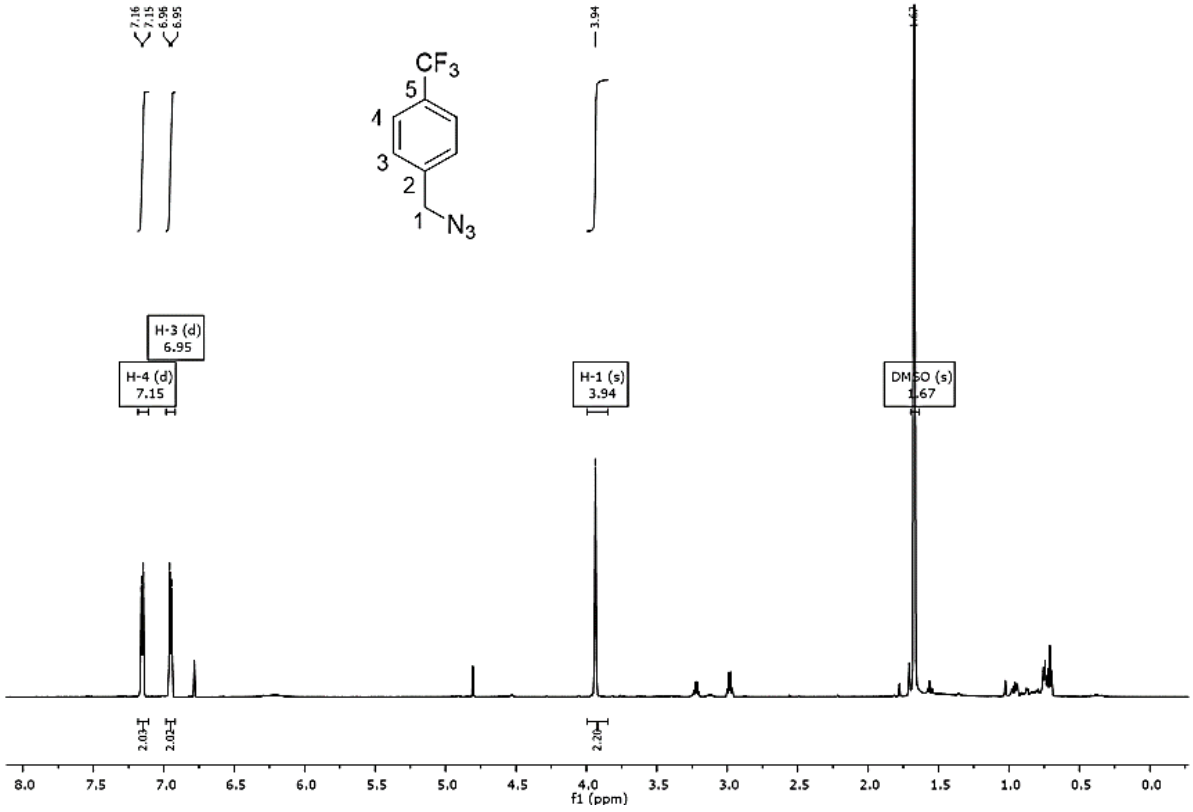
NMR ^{13}C in $CDCl_3$



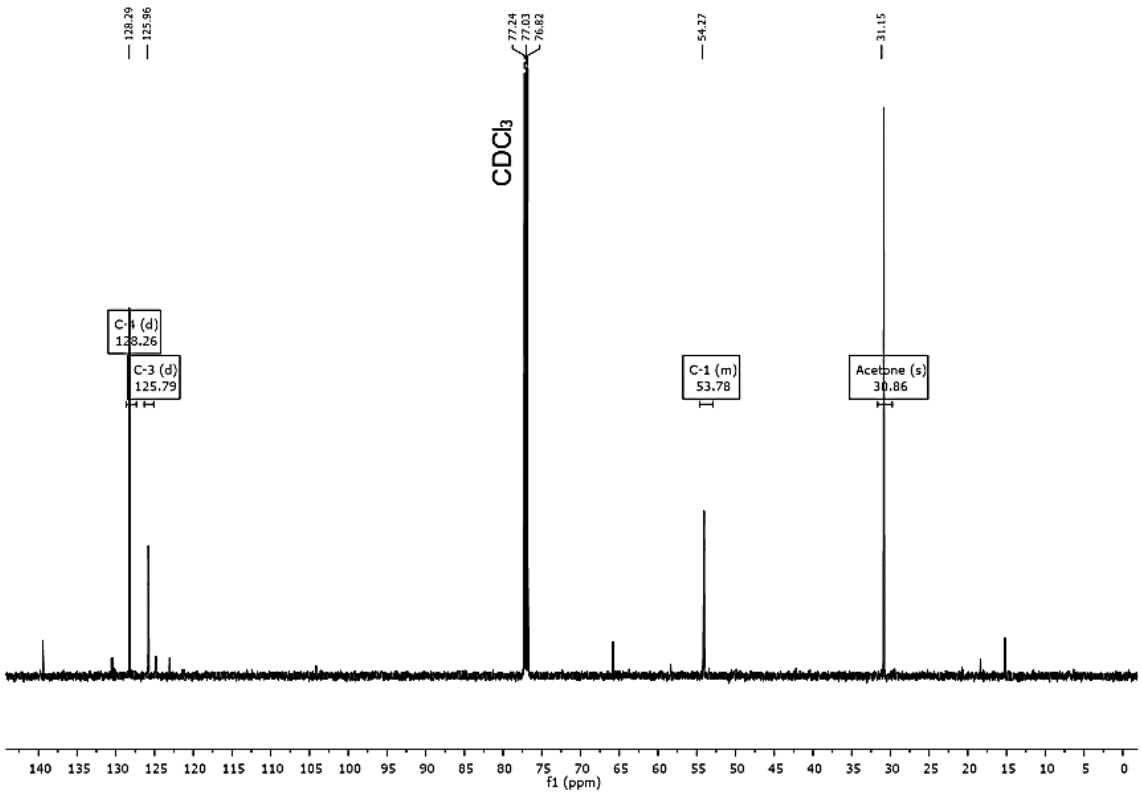
IR



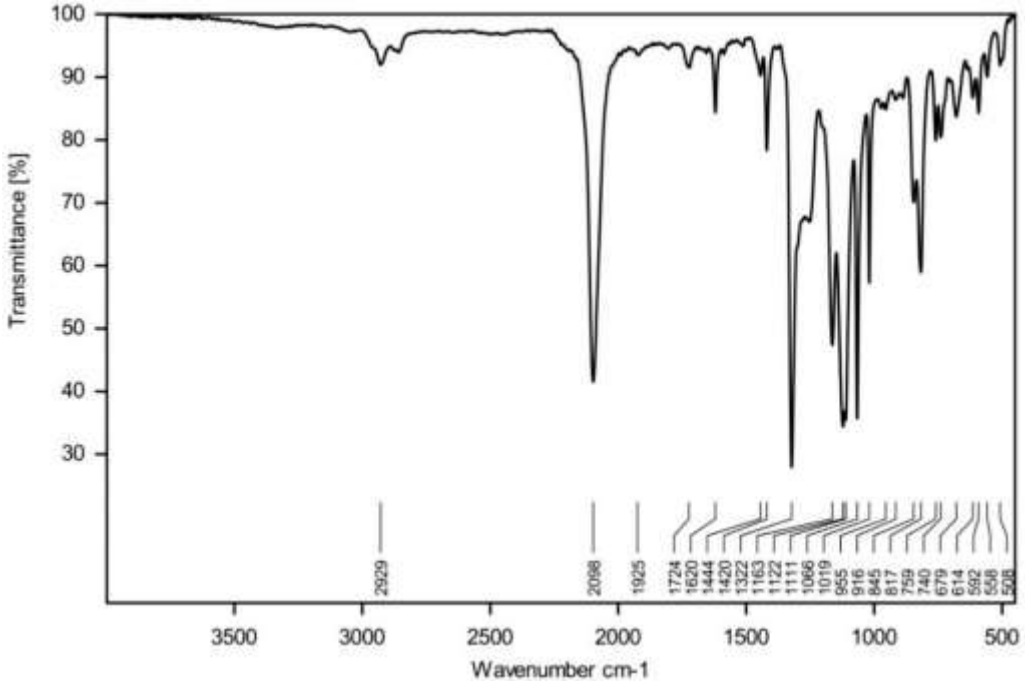
Intermediate 17.g
NMR ^{1}H in $CDCl_3$



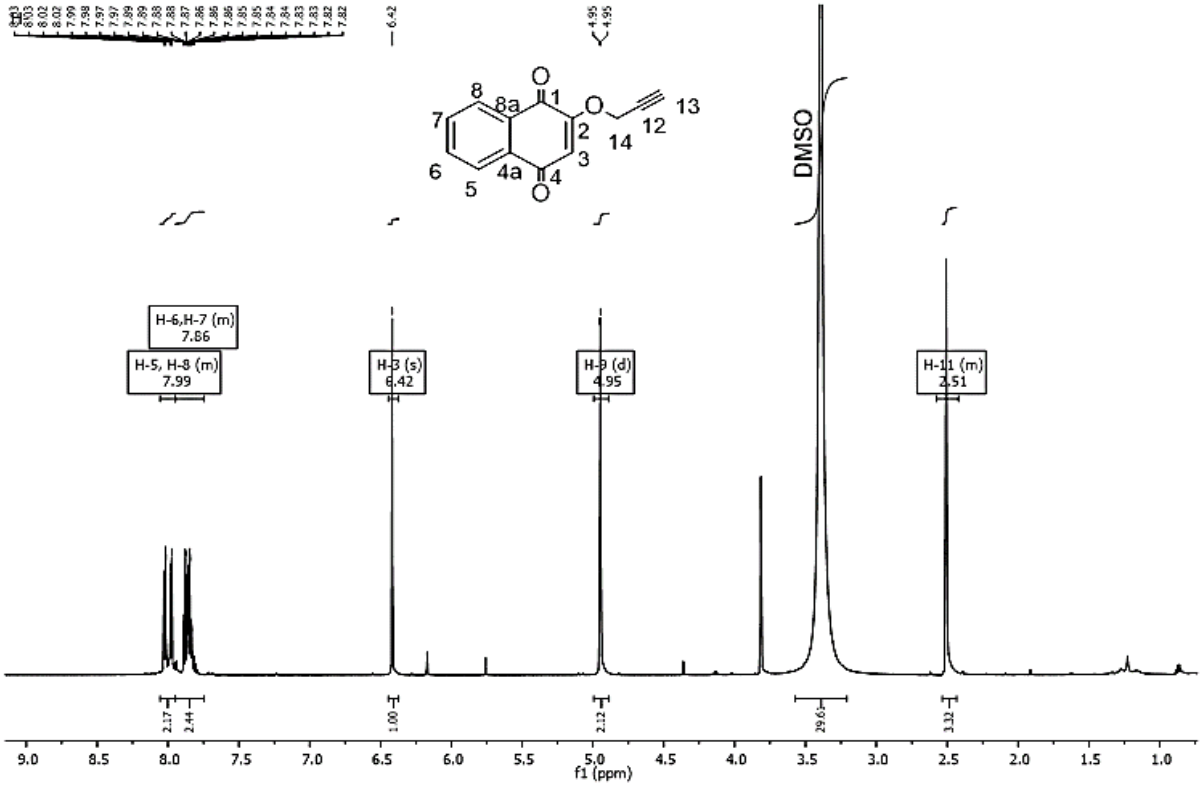
NMR ^{13}C in $CDCl_3$



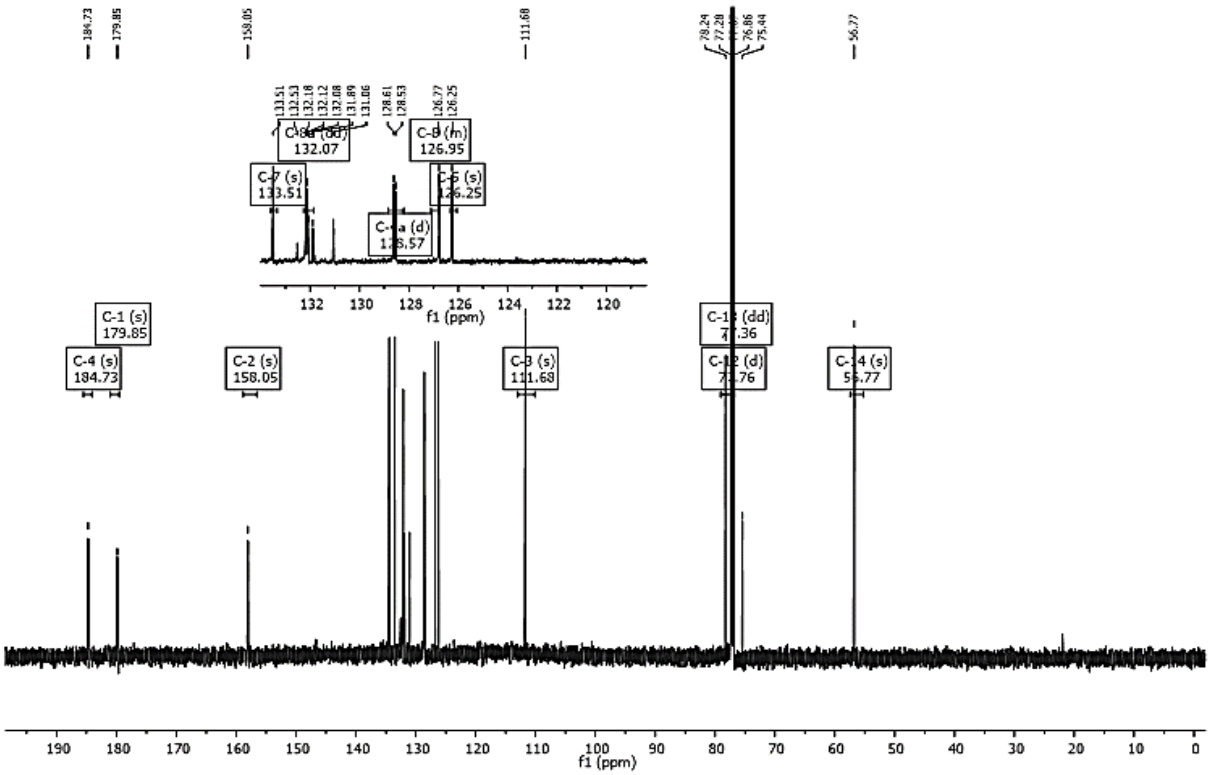
IR



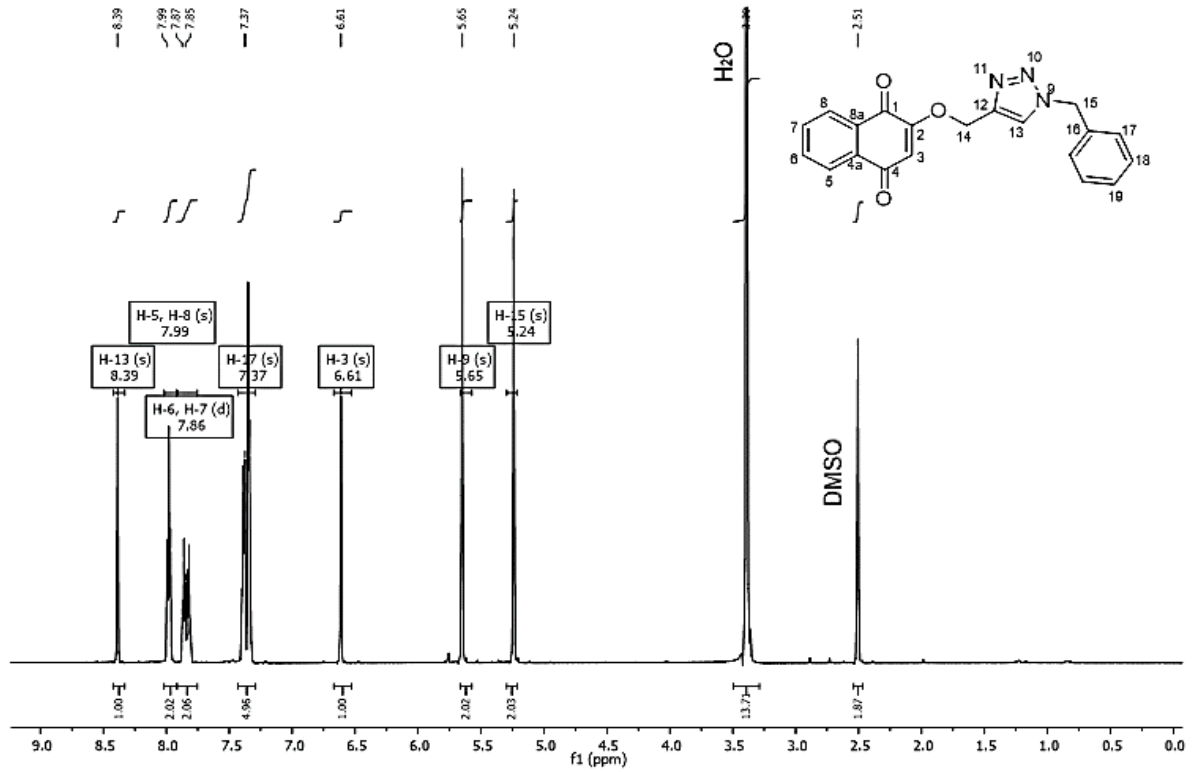
Intermediate 19
NMR ^{1}H in DMSO



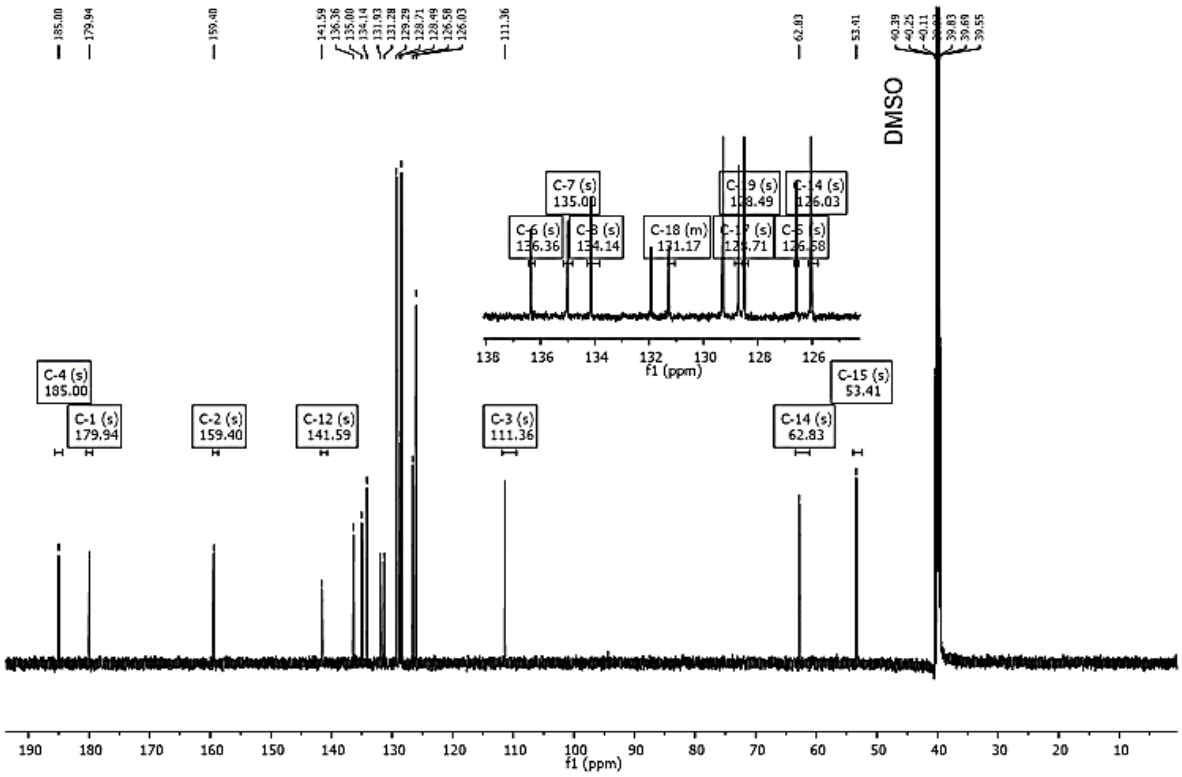
NMR ^{13}C in DMSO



Compound 20
NMR ^{1}H in DMSO

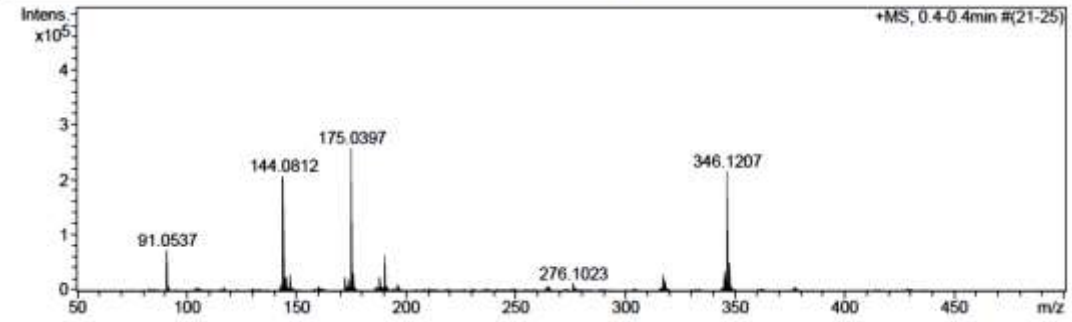


NMR ^{13}C in DMSO

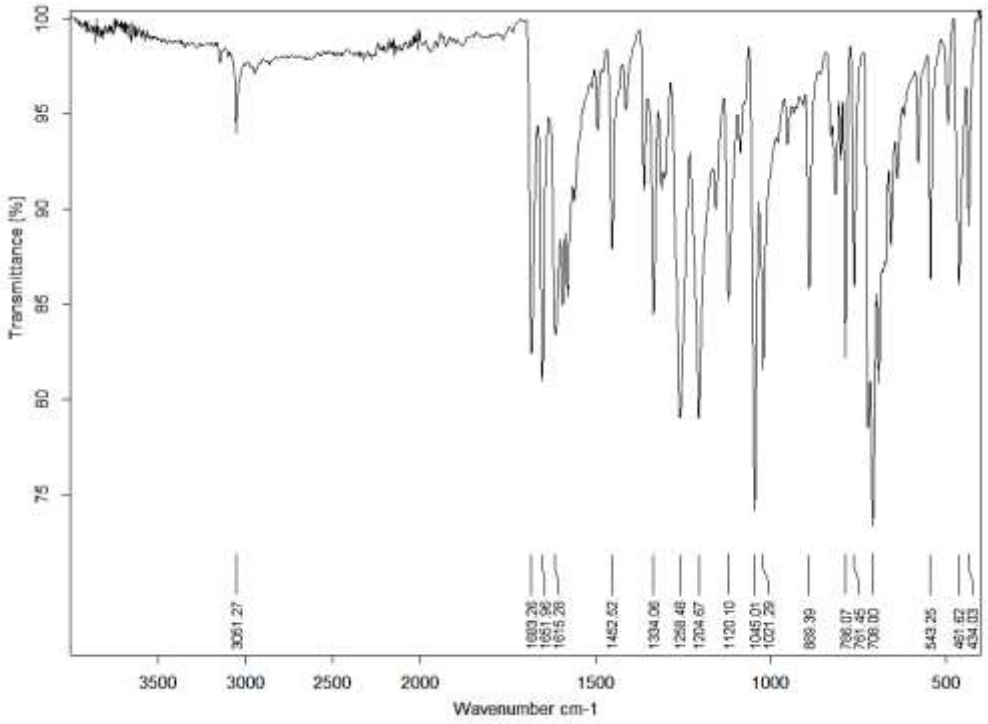


HRMS

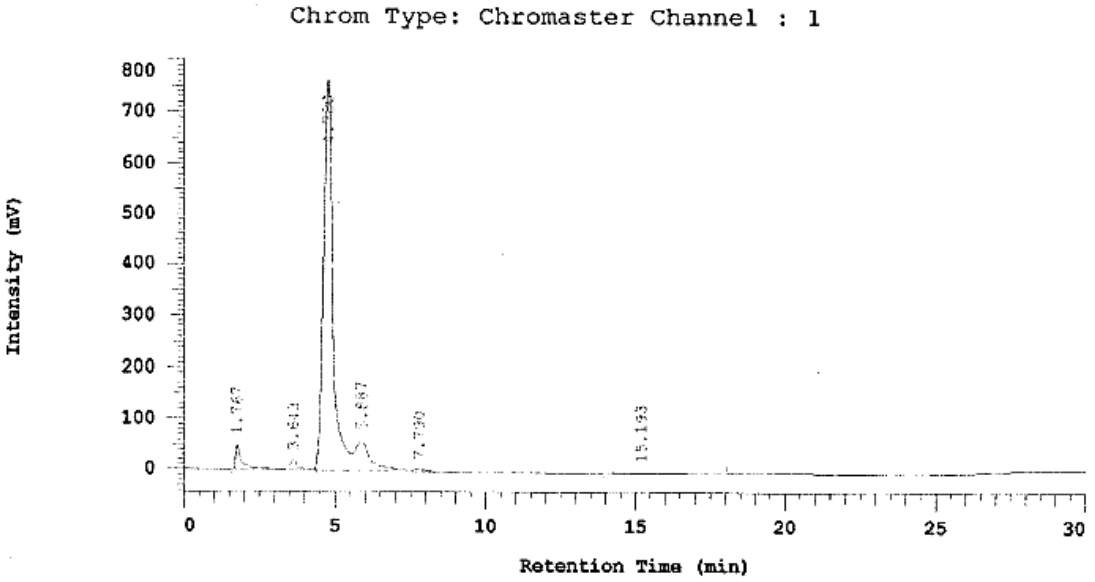
Acquisition Parameter					
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



IR


Transmittance [%]
100
95
90
85
80
75
3061.27
1683.26
1651.98
1615.26
1452.52
1334.06
1288.48
1204.67
1120.10
1045.01
1021.29
898.39
766.07
751.45
708.00
543.25
461.62
434.03
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

HPLC

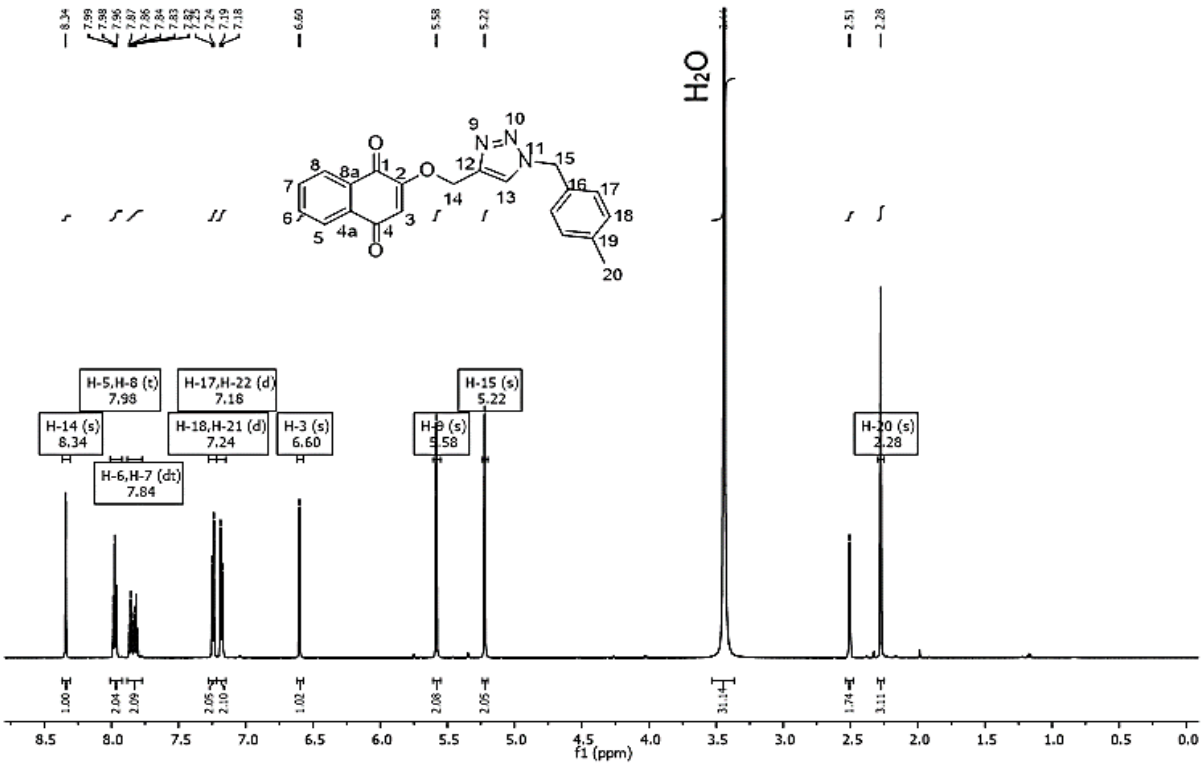


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A:
Pump 1 Solvent B:
Pump 1 Solvent C:
Pump 1 Solvent D:
Method Description: Malaria

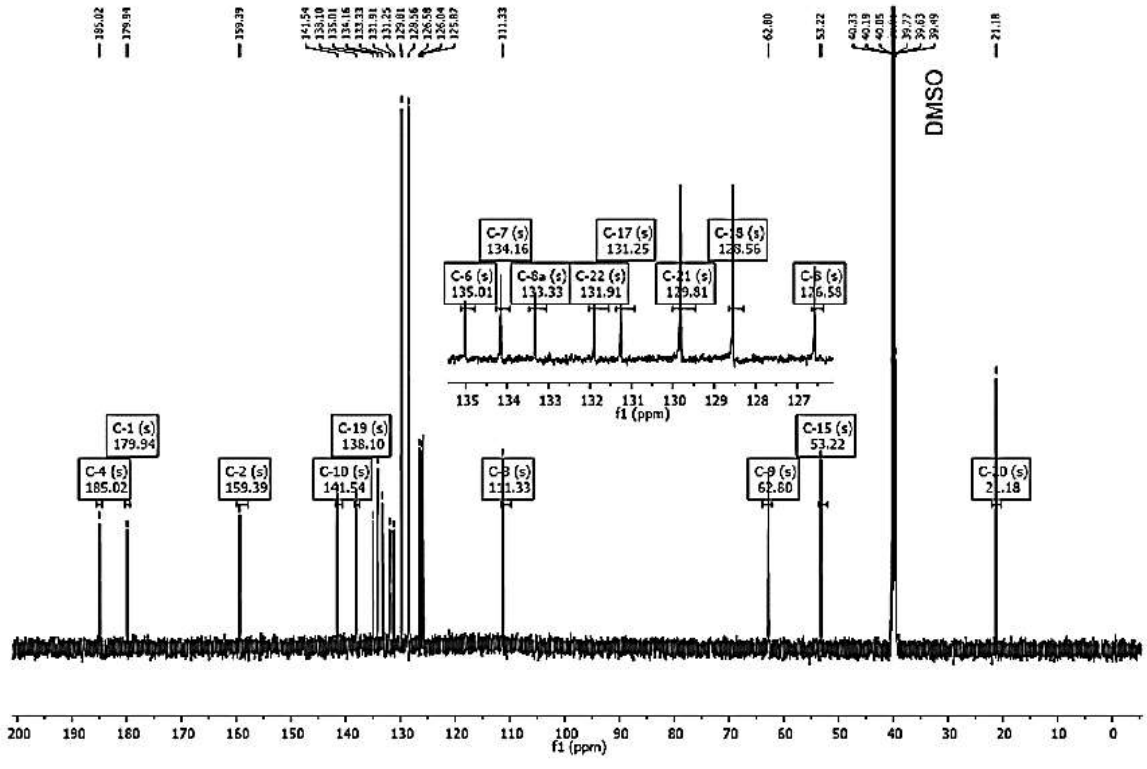
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.767	771647	3.848	BV
2	3.643	361501	1.803	VV
3	4.780	18088414	90.209	VV
4	5.887	724455	3.613	TBB
5	7.790	65825	0.328	TBB
6	15.193	39876	0.199	BB
		20051718	100.000	

Compound 21
NMR ^{1}H in DMSO



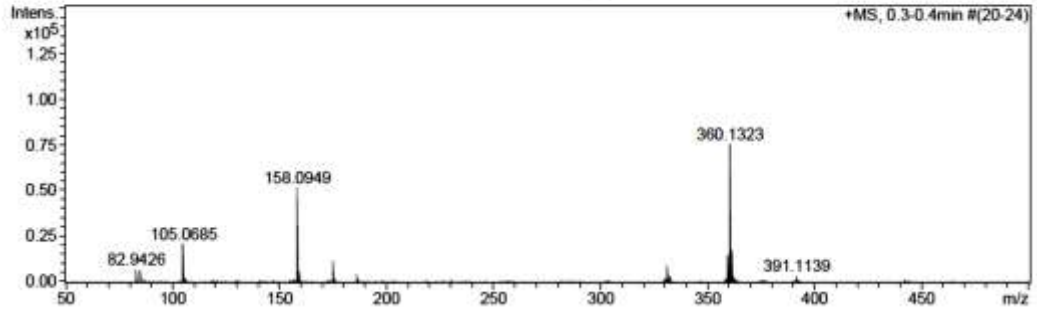
NMR ^{13}C in DMSO



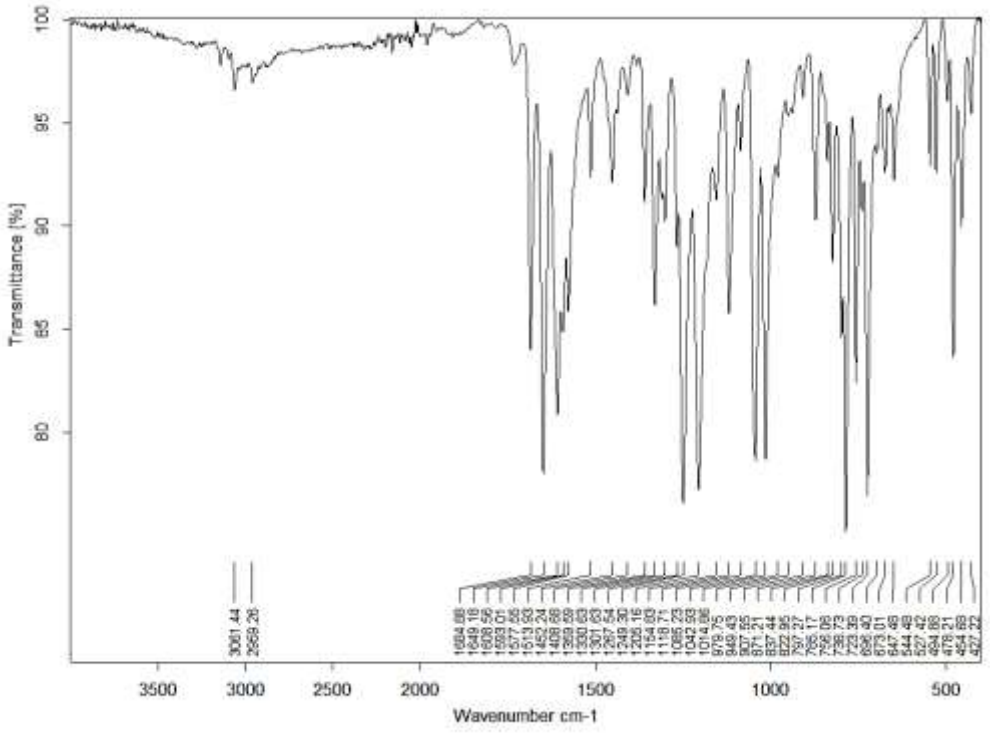
HRMS

Acquisition Parameter

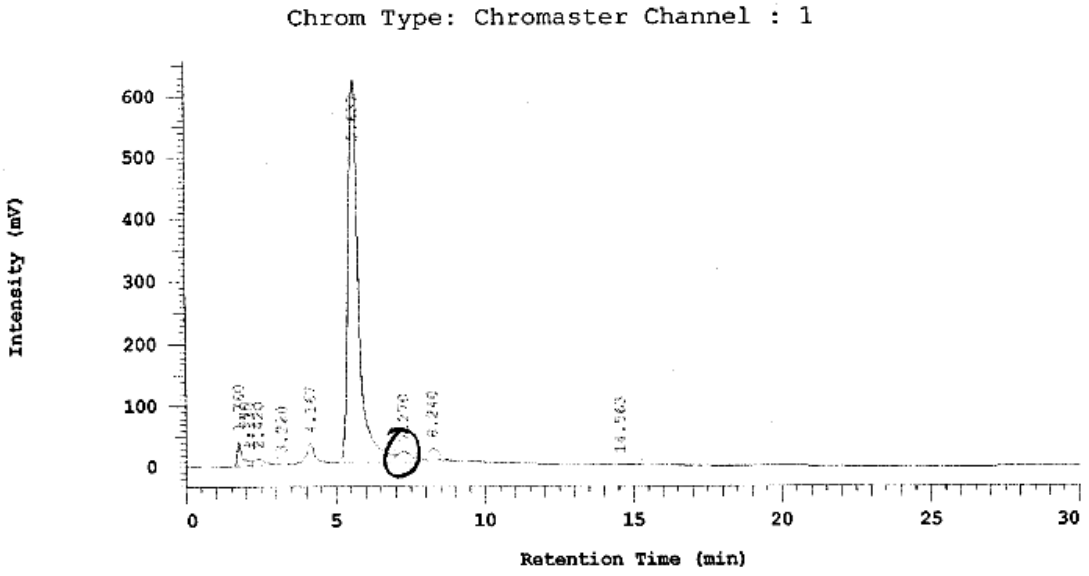
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



IR



HPLC

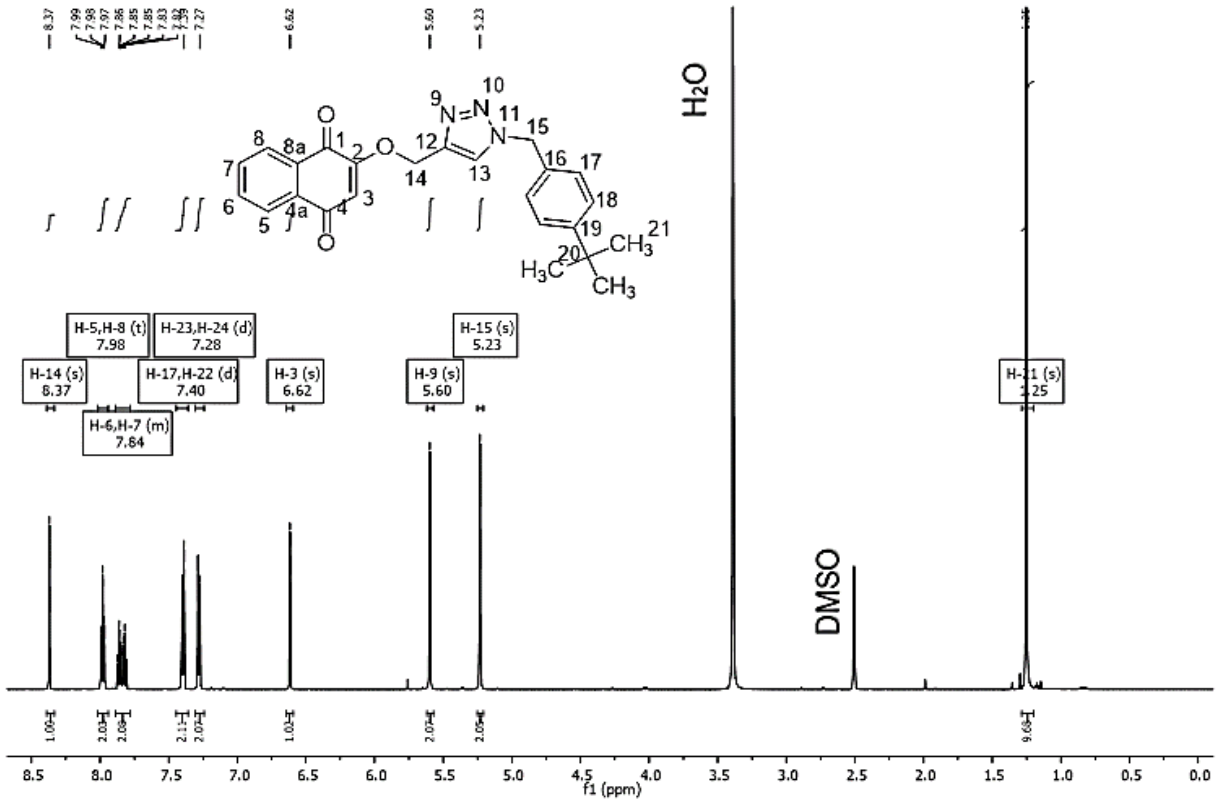


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A:
Pump 1 Solvent B:
Pump 1 Solvent C:
Pump 1 Solvent D:
Method Description: Malaria

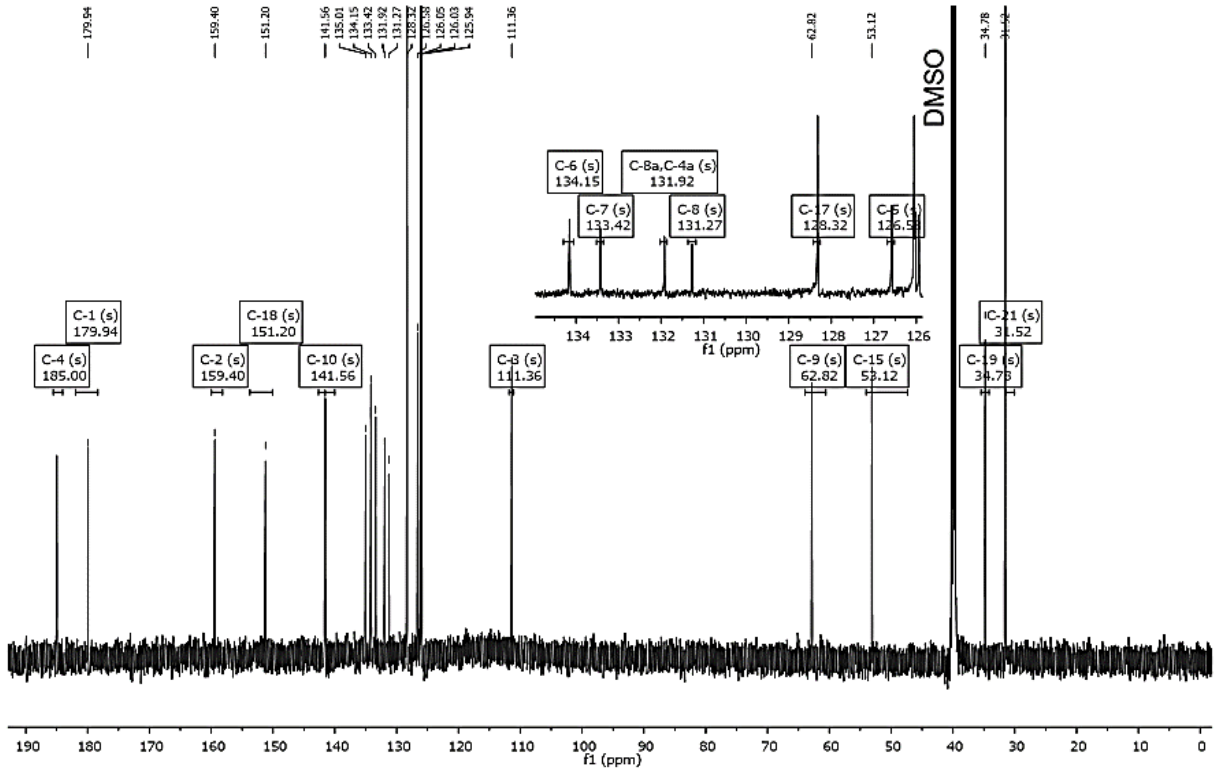
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.760	490517	2.725	BV
2	1.970	4040	0.022	TBV
3	2.130	10818	0.060	TVB
4	2.420	206330	1.146	VB
5	3.220	730	0.004	BB
6	4.167	643015	3.576	BV
7	5.637	15919942	88.425	VV
8	7.270	185996	1.033	TBB
9	8.240	517767	2.876	TBB
10	14.563	23916	0.133	BB
		18003871	100.000	

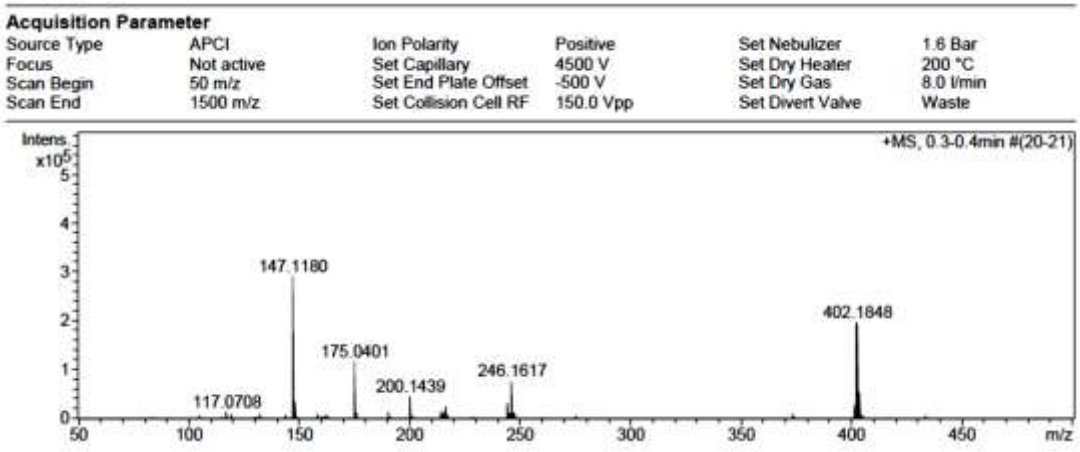
Compound 22
NMR ^{1}H in DMSO



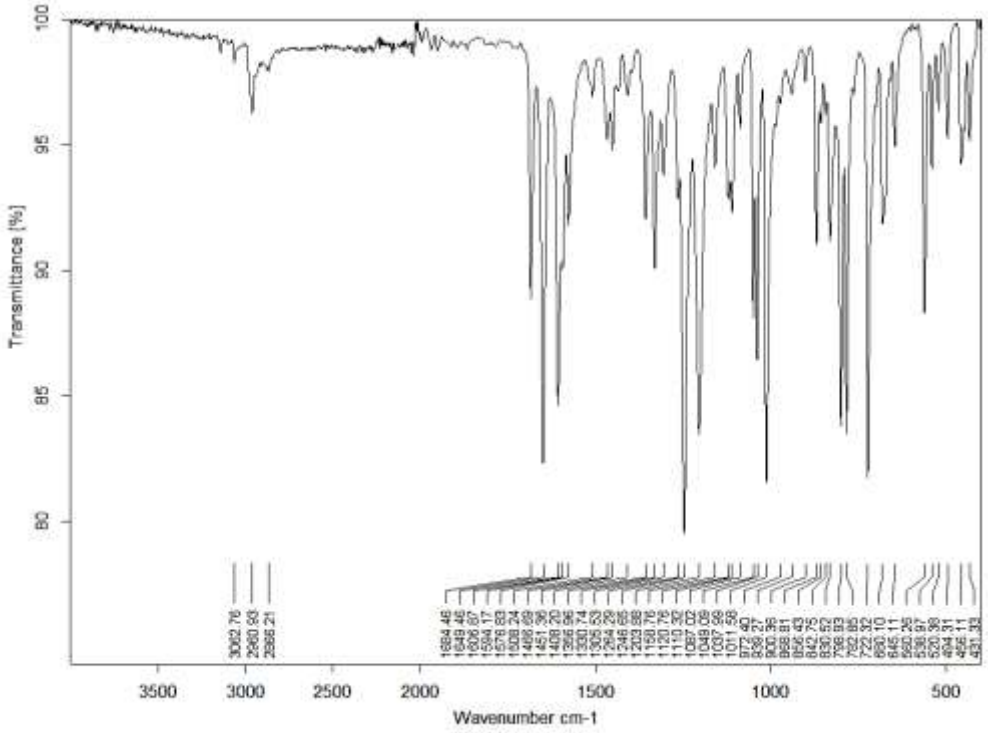
NMR ^{13}C in DMSO



HRMS

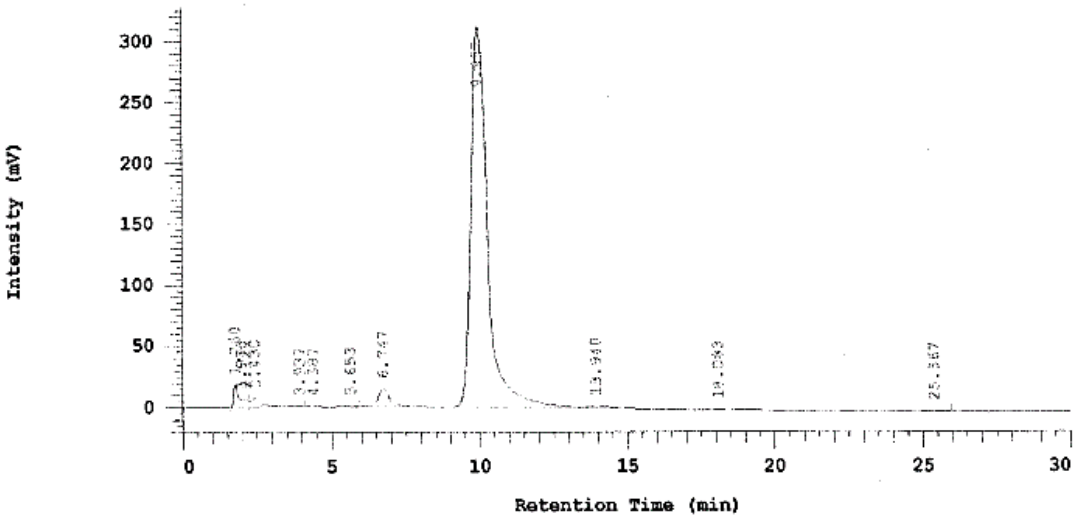

Acquisition Parameter
Source Type APCI
Focus Not active
Scan Begin 50 m/z
Scan End 1500 m/z
Ion Polarity Positive
Set Capillary 4500 V
Set End Plate Offset -500 V
Set Collision Cell RF 150.0 Vpp
Set Nebulizer 1.6 Bar
Set Dry Heater 200 °C
Set Dry Gas 8.0 l/min
Set Divert Valve Waste
Intens.
x10^5
+MS, 0.3-0.4min #(20-21)
147.1180
402.1848
175.0401
246.1617
200.1439
117.0708
m/z

IR


Transmittance [%]
Wavenumber cm-1

HPLC

Chrom Type: Chromaster Channel : 1

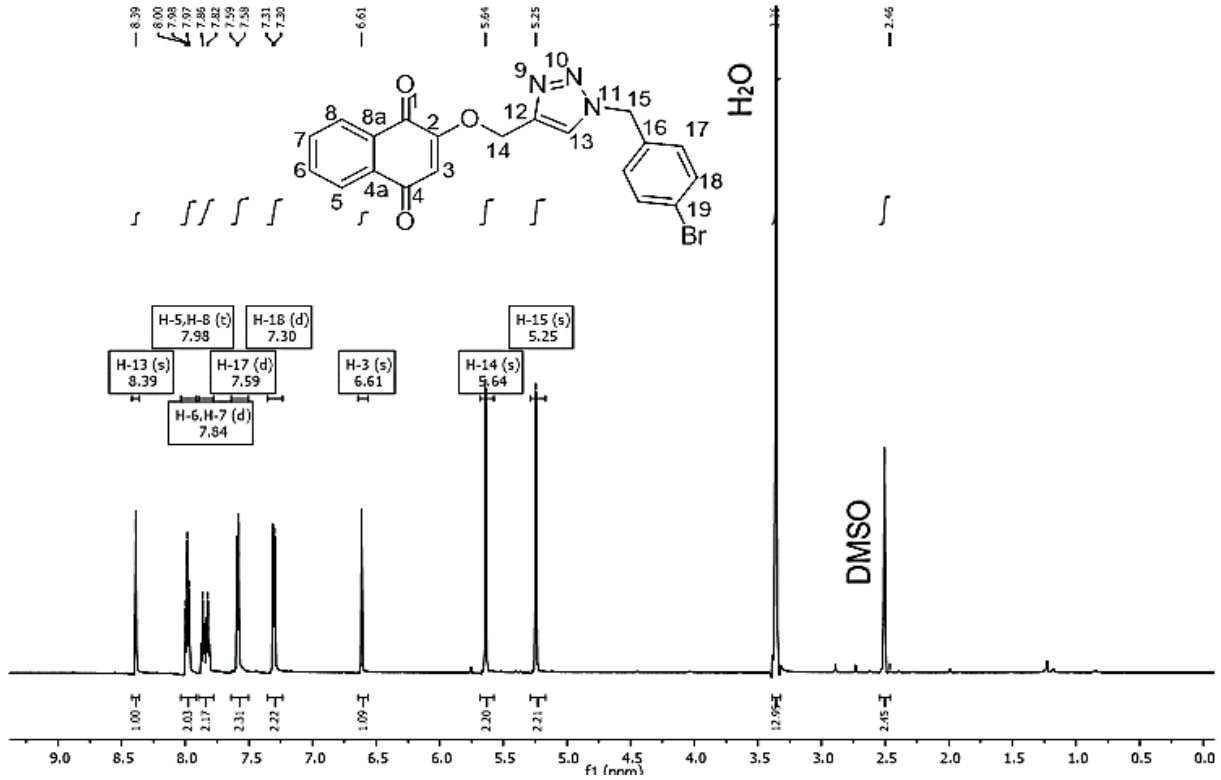


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A: Pump 1 Solvent B:
Pump 1 Solvent C: Pump 1 Solvent D:
Method Description: Malaria

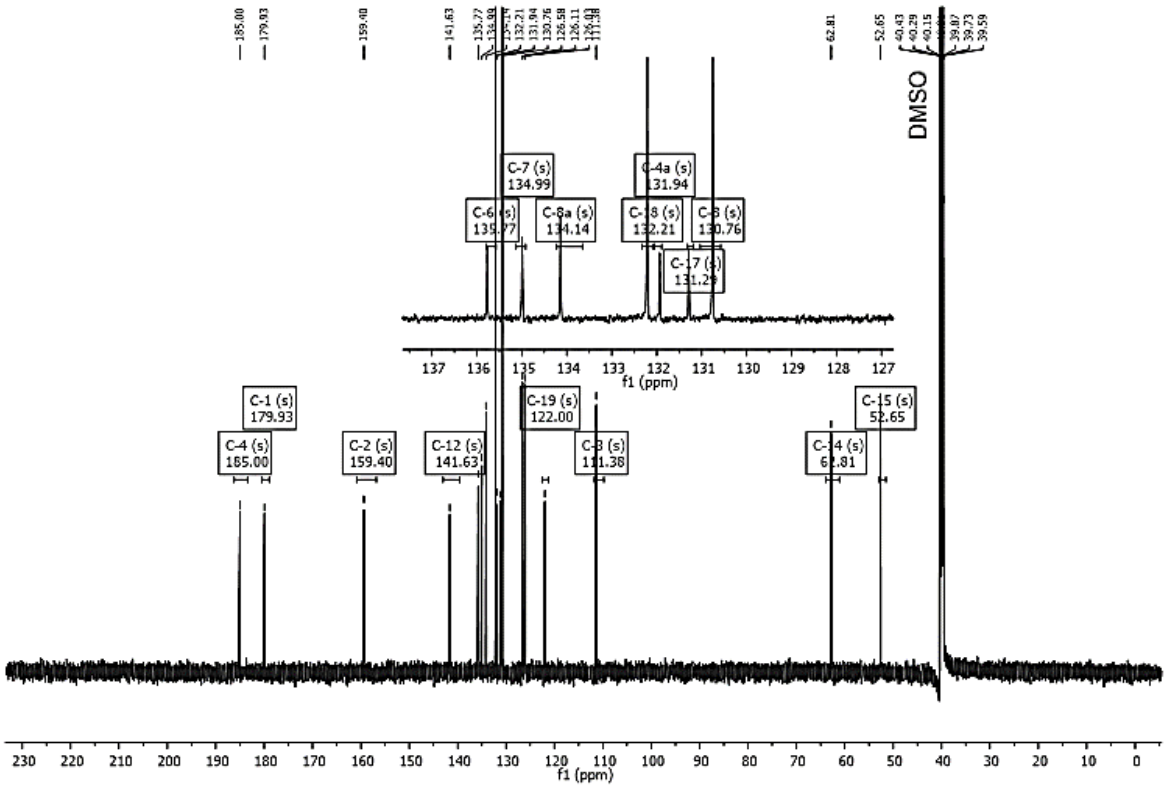
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.760	173403	1.334	BV
2	1.973	38807	0.298	VV
3	2.127	79910	0.615	VV
4	2.430	170750	1.313	VB
5	3.937	1686	0.013	BB
6	4.387	1648	0.013	BB
7	5.653	17175	0.132	BB
8	6.747	379522	2.919	BB
9	9.957	12062812	92.780	BV
10	13.940	60938	0.469	TBB
11	18.083	5334	0.041	BB
12	25.367	9554	0.073	BB
		13001539	100.000	

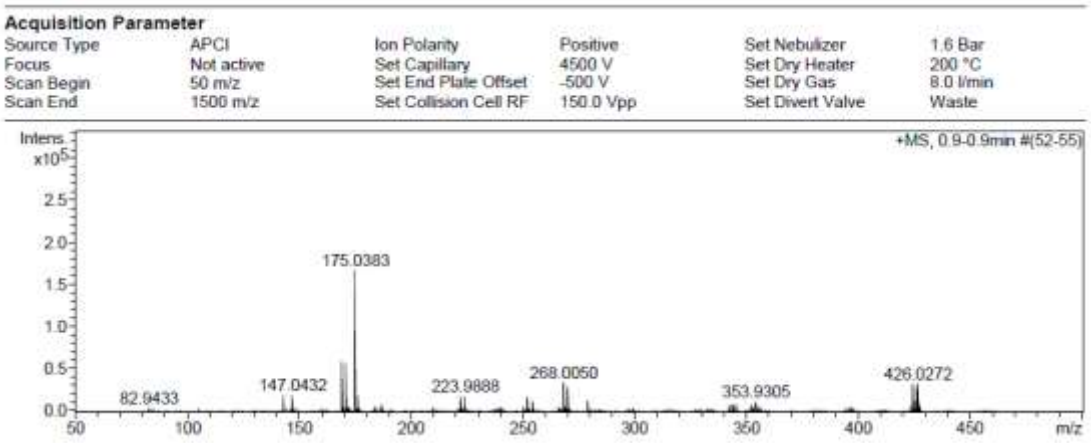
Compound 23
NMR ^{1}H in DMSO



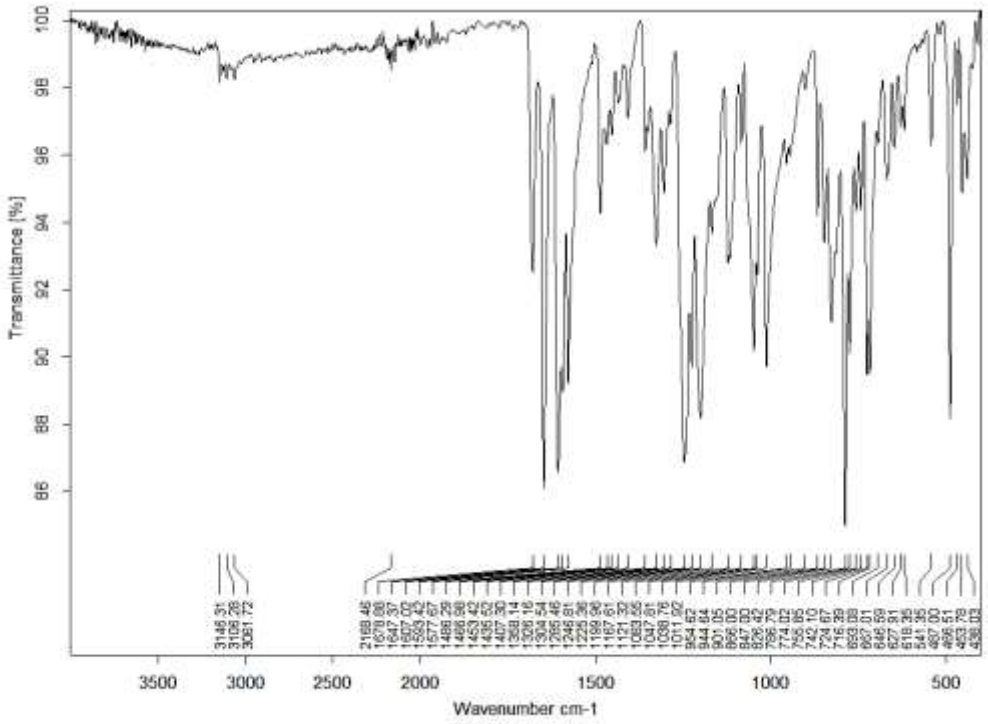
NMR ^{13}C in DMSO



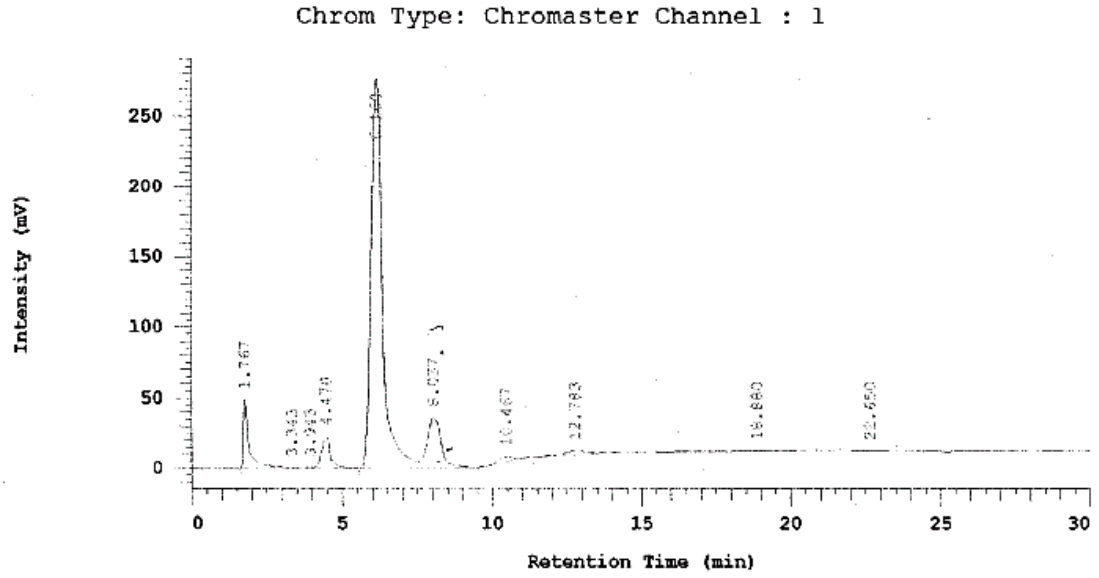
HRMS


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 150.0 Vpp
Set Divert Valve Waste
Intens.
x10^5
+MS, 0.9-0.9min #(52-55)
175.0383
82.9433
147.0432
223.9888
268.0050
353.9305
426.0272
m/z

IR


Transmittance [%]
Wavenumber cm-1

HPLC

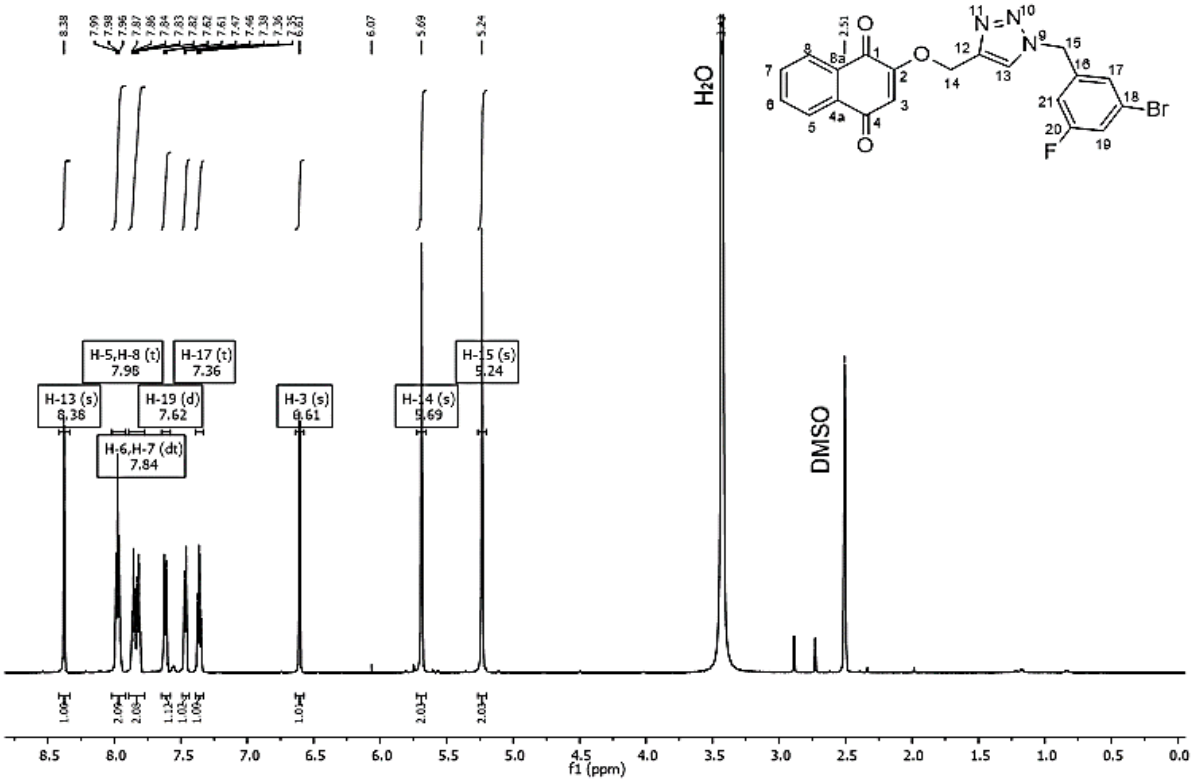


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A:
Pump 1 Solvent B:
Pump 1 Solvent C:
Pump 1 Solvent D:
Method Description: Malaria

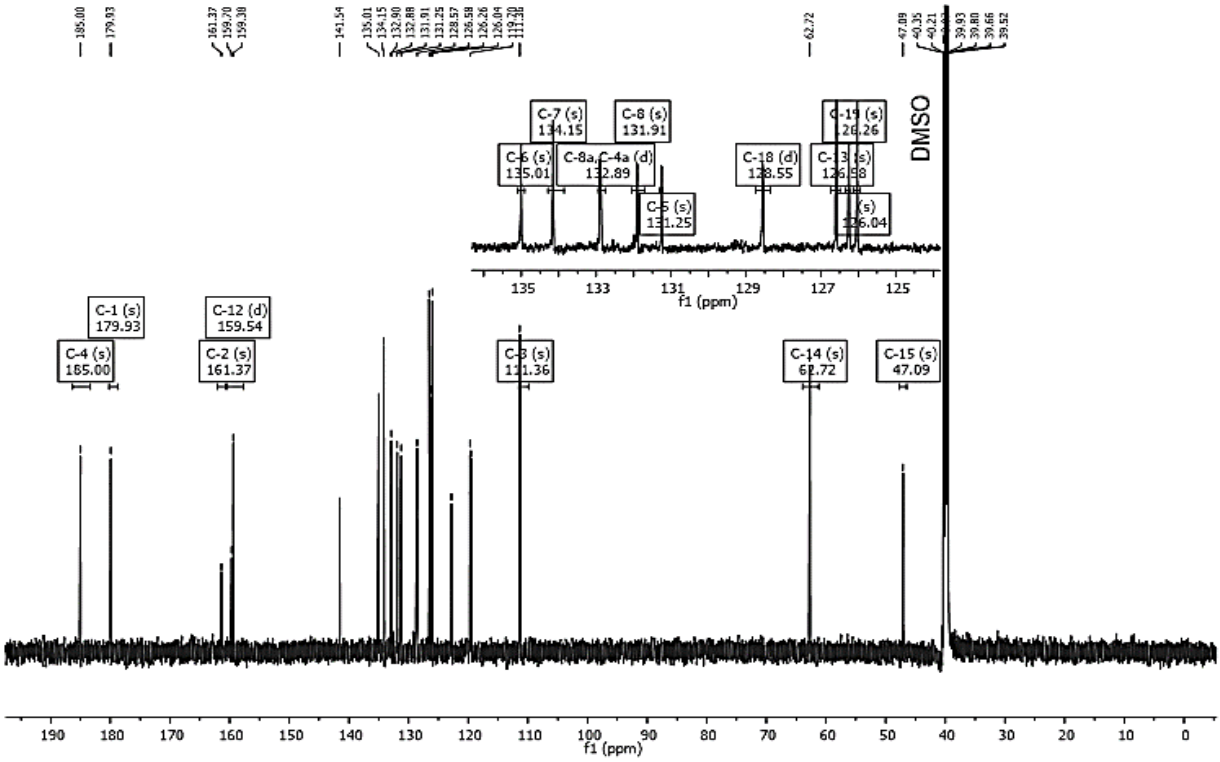
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.767	655826	6.648	BV
2	3.343	1601	0.016	TBB
3	3.943	7693	0.078	BV
4	4.470	433267	4.392	VB
5	6.160	7234954	73.336	BV
6	8.037	1147974	11.636	VB
7	10.467	115829	1.174	BB
8	12.783	150325	1.524	BB
9	18.880	106754	1.082	BB
10	22.650	11269	0.114	BB
		9865492	100.000	

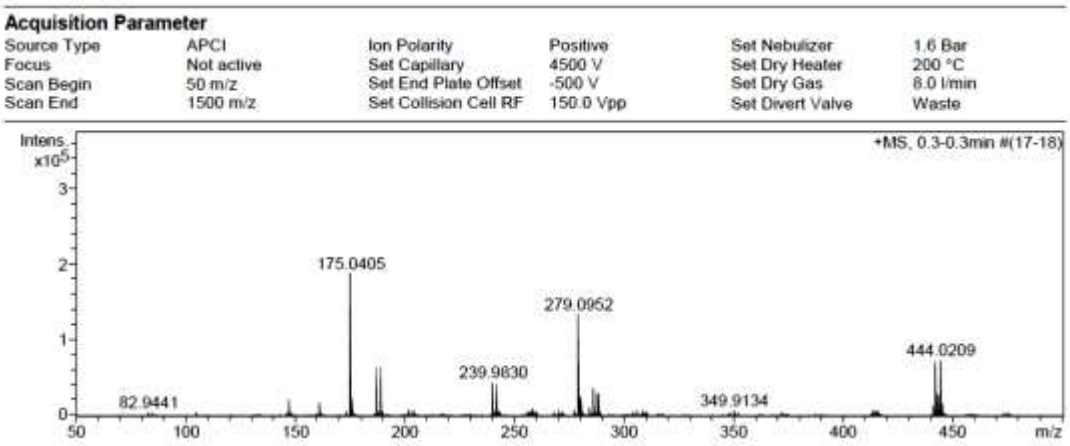
Compound 24
NMR ^{1}H in DMSO



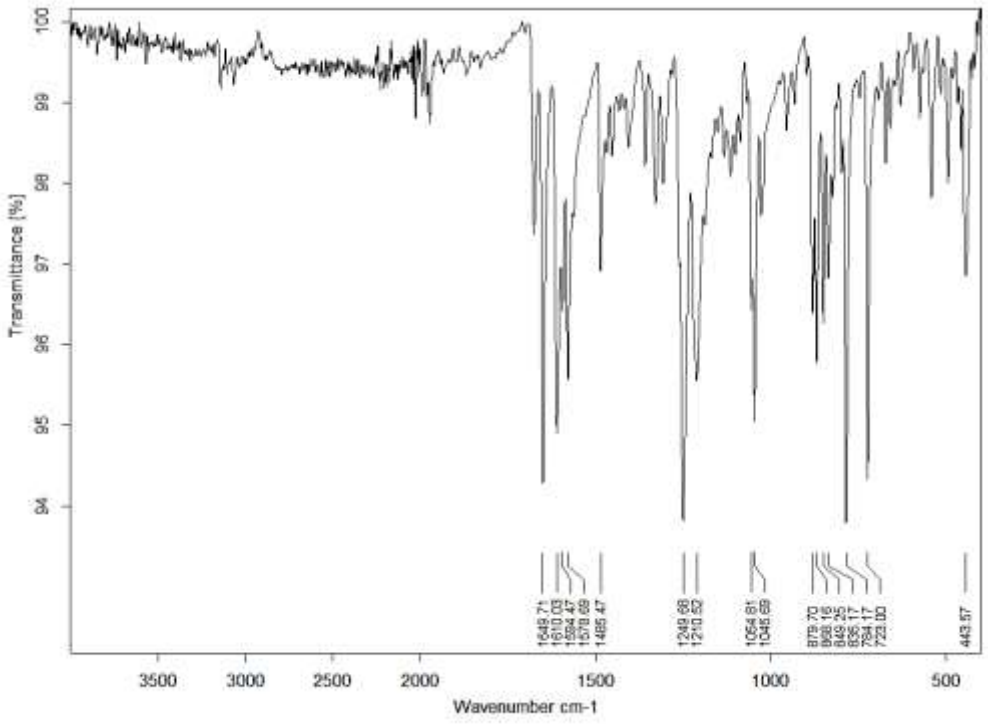
NMR ^{13}C in DMSO



HRMS

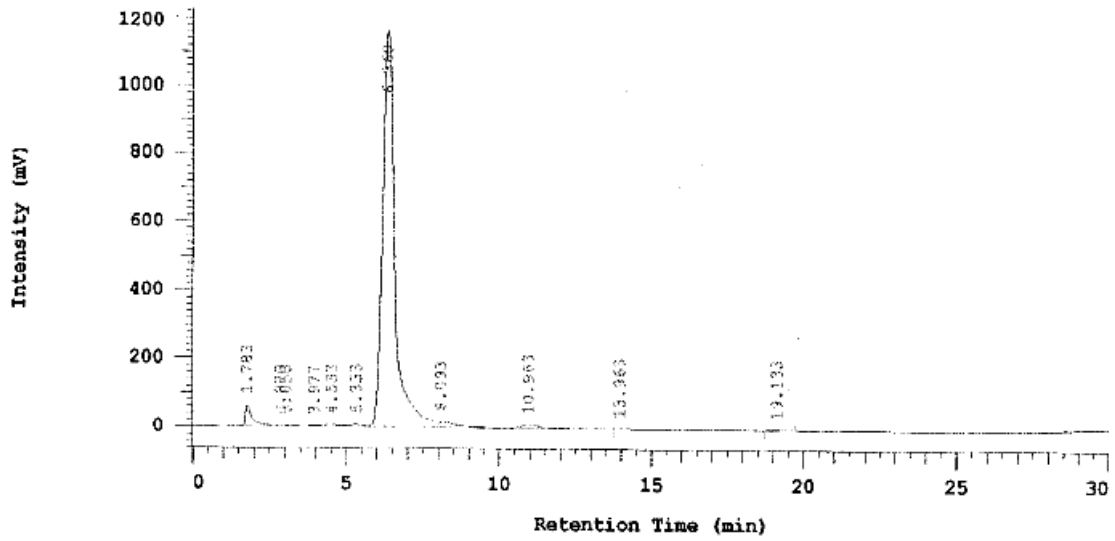

Acquisition Parameter
Source Type APCI
Focus Not active
Scan Begin 50 m/z
Scan End 1500 m/z
Ion Polarity Positive
Set Capillary 4500 V
Set End Plate Offset -500 V
Set Collision Cell RF 150.0 Vpp
Set Nebulizer 1.6 Bar
Set Dry Heater 200 °C
Set Dry Gas 8.0 l/min
Set Divert Valve Waste
Intens.
x10^5
+MS, 0.3-0.3min #(17-18)
175.0405
279.0952
444.0209
239.9830
82.9441
349.9134
m/z

IR


Transmittance [%]
Wavenumber cm-1
1649.71
1610.03
1594.47
1578.69
1485.47
1249.66
1210.52
1064.81
1045.69
879.70
846.15
836.17
784.17
723.00
443.57

HPLC

Chrom Type: Chromaster Channel : 1

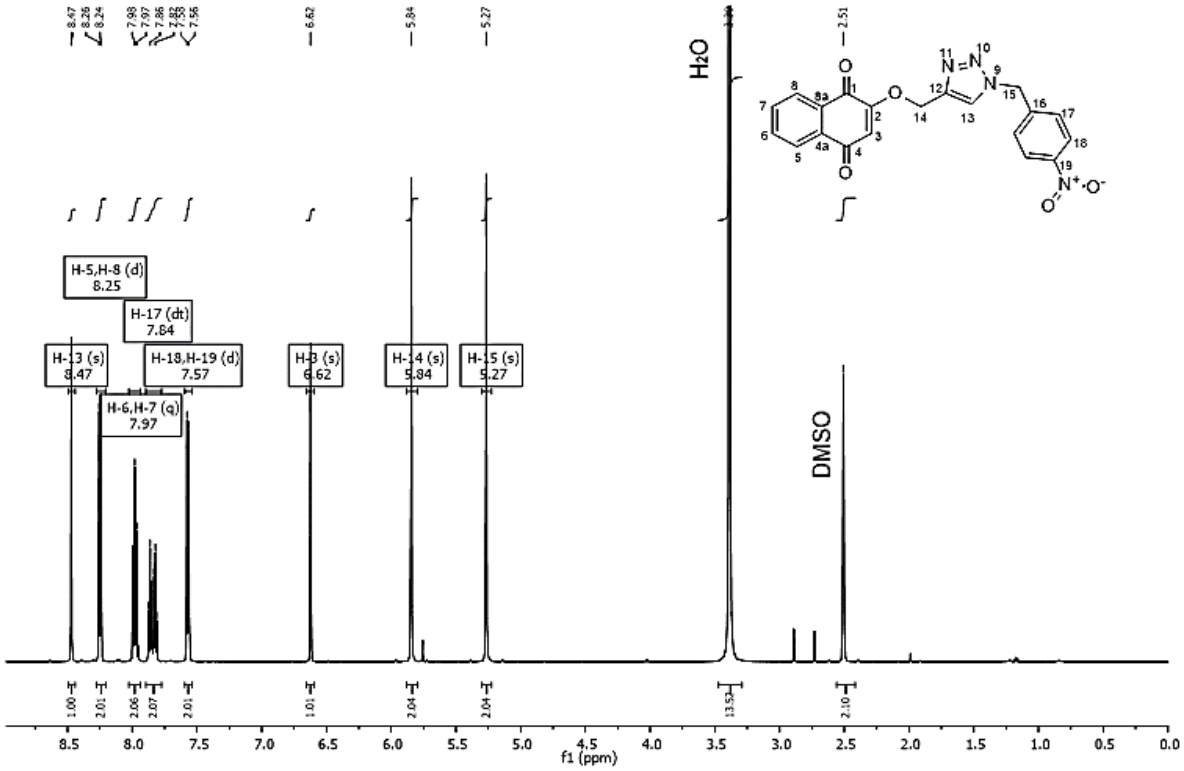


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A:
Pump 1 Solvent B:
Pump 1 Solvent C:
Pump 1 Solvent D:
Method Description: Malaria

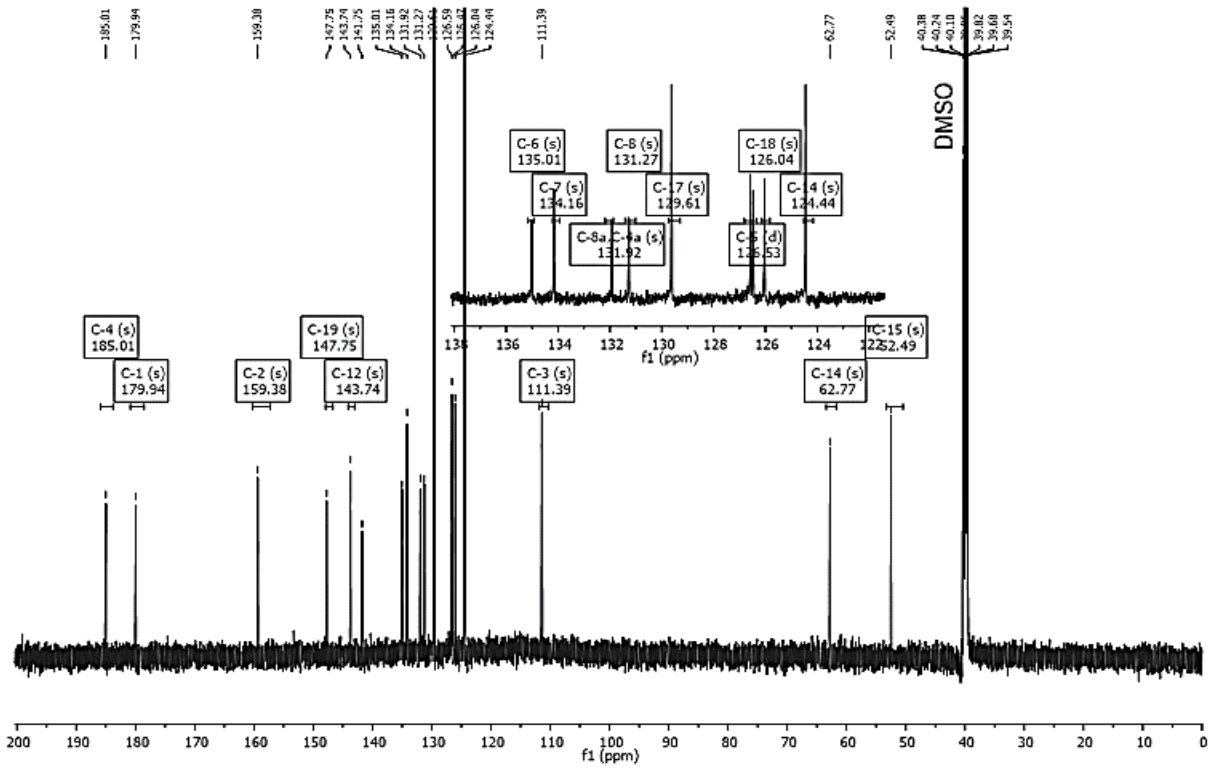
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.783	949377	2.748	BB
2	2.870	2690	0.008	BV
3	3.050	7605	0.022	VB
4	3.977	15206	0.044	BV
5	4.533	146116	0.423	VV
6	5.333	136866	0.396	VV
7	6.360	32799431	94.930	VV
8	8.093	63412	0.184	TBB
9	10.963	409066	1.184	BB
10	13.963	988	0.003	BB
11	19.133	20301	0.059	BB
		34551058	100.000	

Compound 25
NMR ^{1}H in DMSO



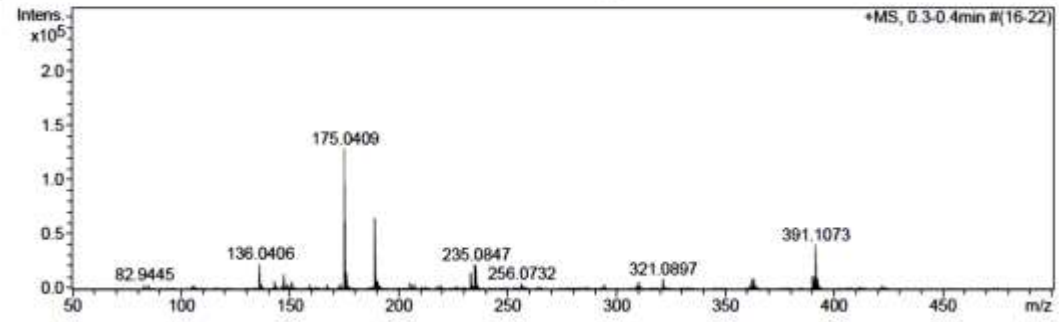
NMR ^{13}C in DMSO



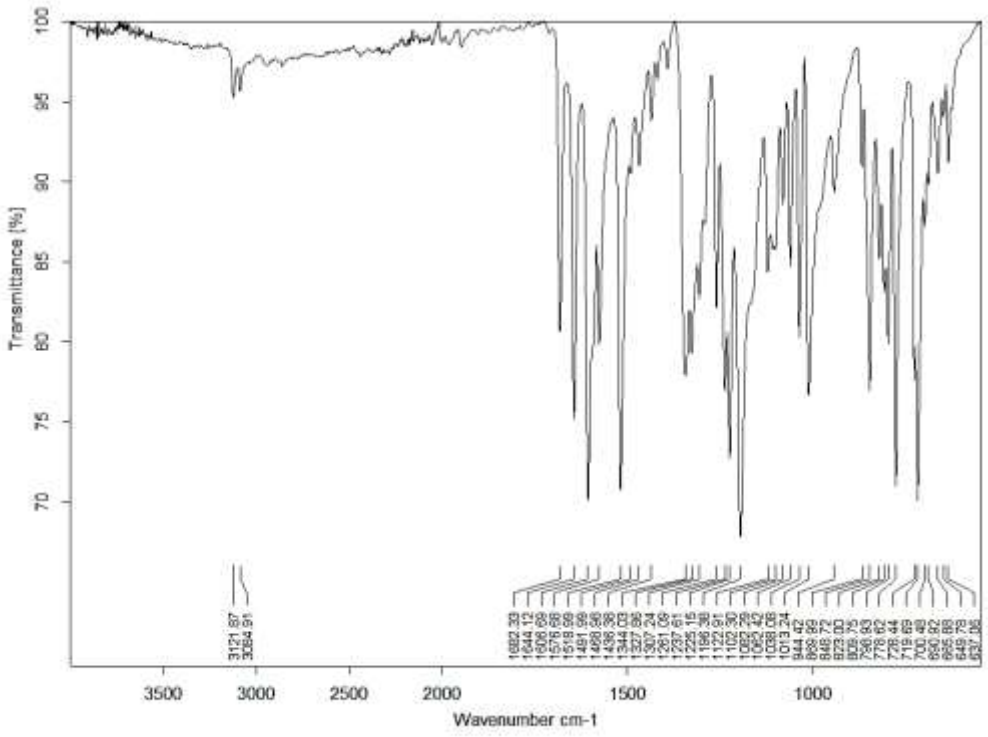
HRMS

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	150.0 Vpp	Set Divert Valve	Waste

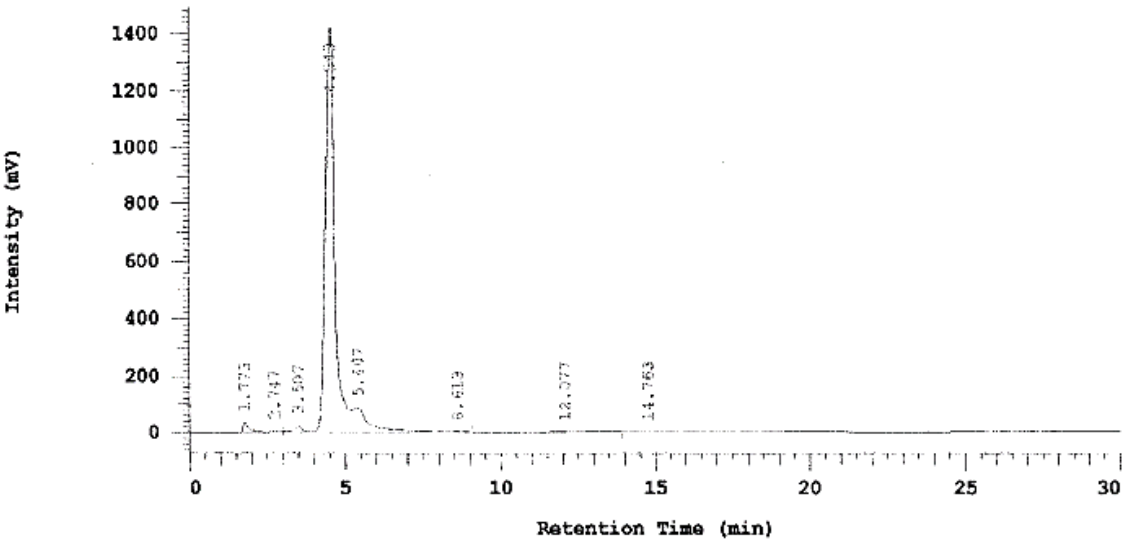


IR



HPLC

Chrom Type: Chromaster Channel : 1

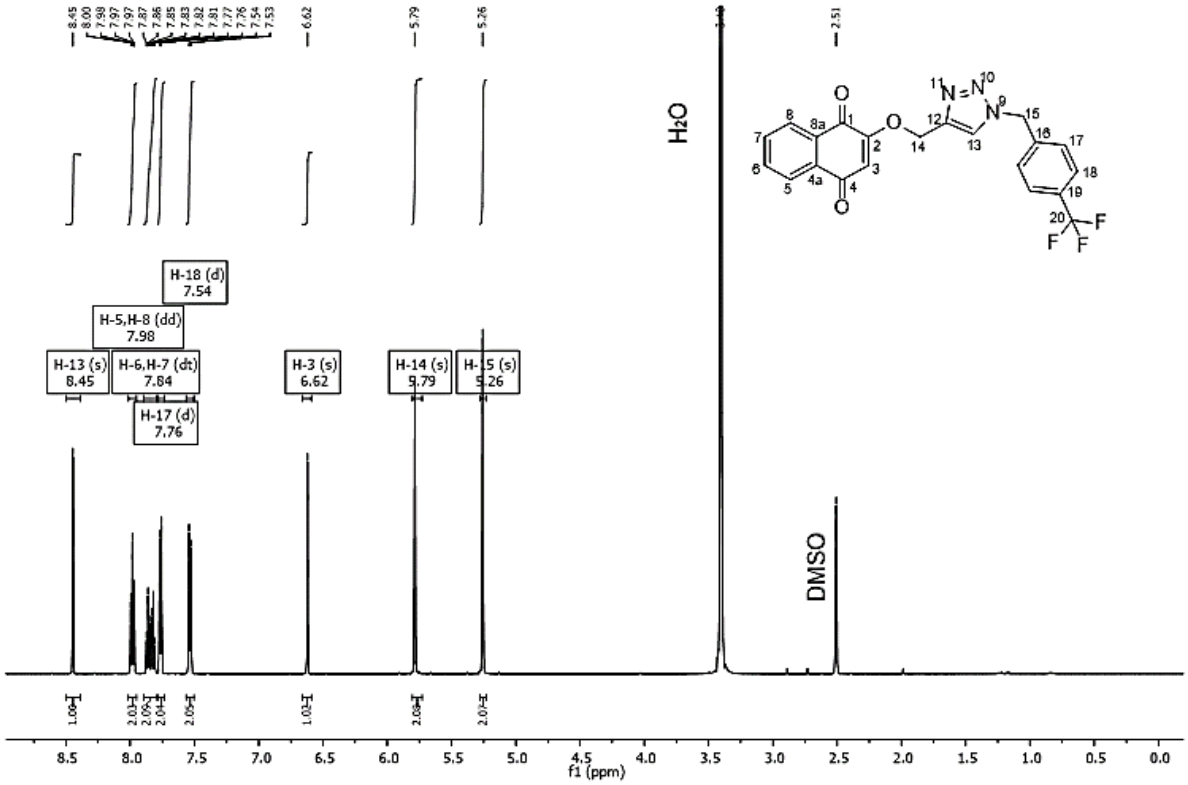


Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A: Pump 1 Solvent B:
Pump 1 Solvent C: Pump 1 Solvent D:
Method Description: Malaria

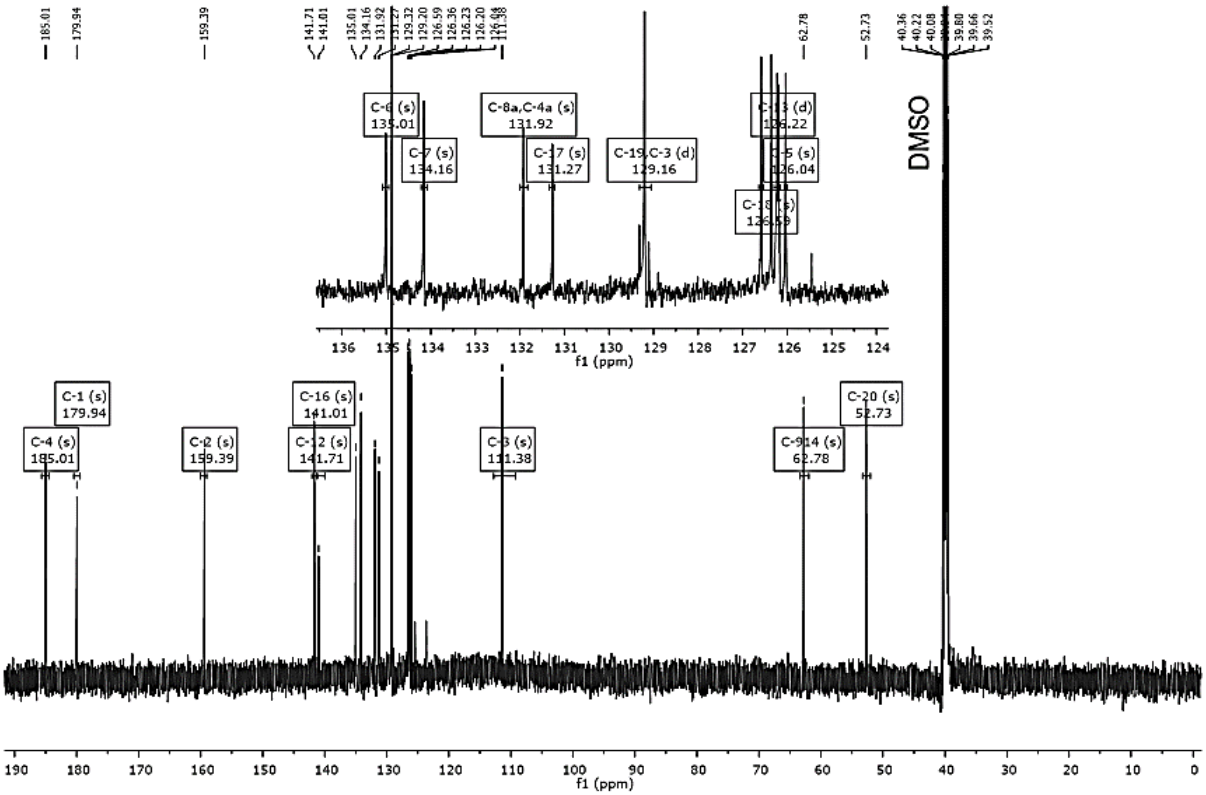
Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.773	546123	1.650	BV
2	2.747	133064	0.402	VV
3	3.507	514027	1.553	VV
4	4.543	31258252	94.434	VV
5	5.407	492109	1.487	TBB
6	8.613	36015	0.109	BB
7	12.077	19007	0.057	BB
8	14.763	102177	0.309	BB
		33100774	100.000	

Compound 26
NMR ^{1}H in DMSO



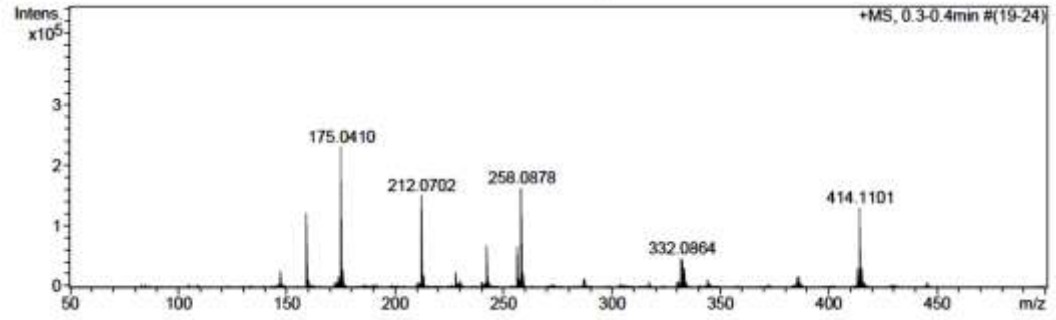
NMR ^{13}C in DMSO



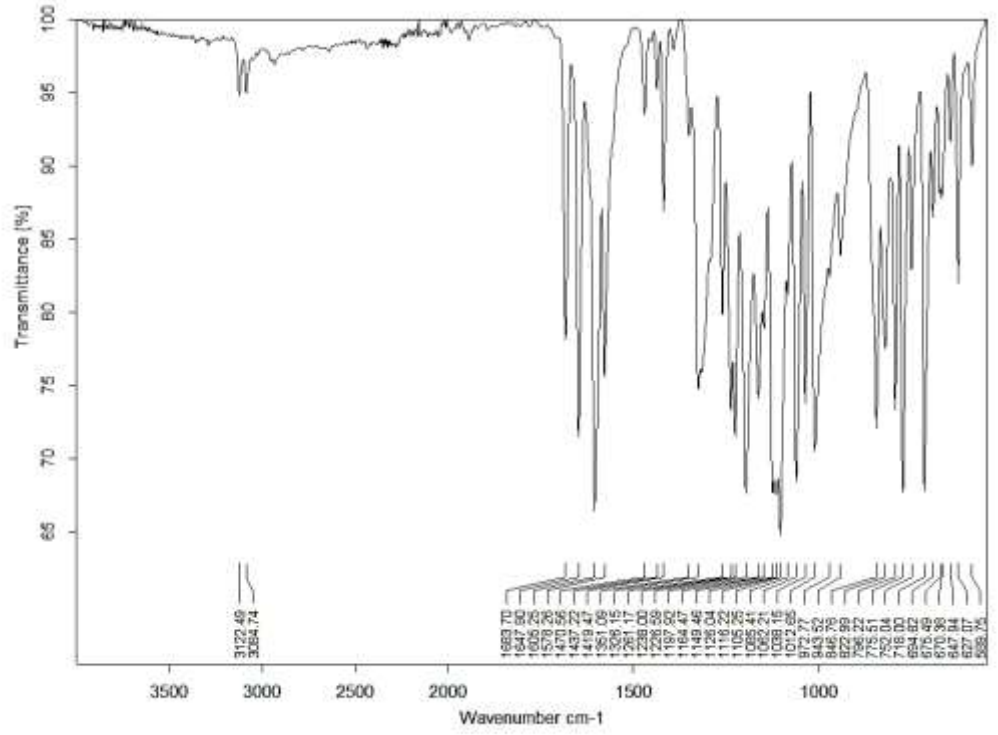
HRMS

Acquisition Parameter

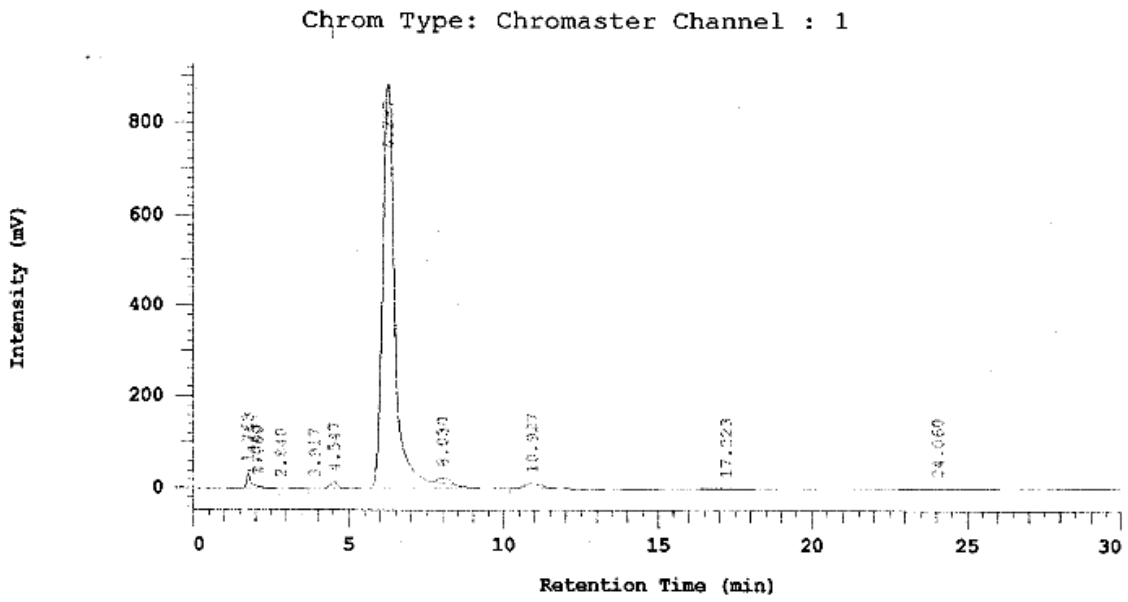
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



IR



HPLC



Processing Method: Malaria
Method Developer:
Pump 1: 5110
Pump 1 Solvent A: Pump 1 Solvent B:
Pump 1 Solvent C: Pump 1 Solvent D:
Method Description: Malaria

Chrom Type: Chromaster Channel : 1
Peak Quantitation: AREA
Calculation Method: AREA%

No.	RT	Area	Conc 1	BC
1	1.763	435715	1.644	BV
2	1.963	2281	0.009	TBB
3	2.080	8555	0.032	TBB
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9	10.927	428339	1.616	BB
10	17.223	89295	0.337	BB
11	24.060	36955	0.139	BB
		26507665	100.000	

ANNEXURE B

GUIDE FOR AUTHORS

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

Published under the auspices of the French Société de Chimie Thérapeutique (SCT)

AUTHOR INFORMATION PACK

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ISSN: 0223-5234

DESCRIPTION

The *European Journal of Medicinal Chemistry* is a global journal that publishes studies on all aspects of medicinal chemistry. It provides a medium for publication of original papers and also welcomes critical review papers.

A typical paper would report on the organic synthesis, characterization and pharmacological evaluation of compounds. Other topics of interest are drug design, QSAR, molecular modeling, drug-receptor interactions, molecular aspects of drug metabolism, prodrug synthesis and drug targeting.

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[dataset] [5] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/xwj98nb39r.1.

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