Synthesis of Triazole-Linked Chloroquinoline Derivatives as Novel Antimalarial Agents

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Declaration

I declare that the work presented in this thesis represent my own work that was carried out by myself under the supervision of Professor W.A.L van Otterlo. It is being submitted for the degree of Master of Chemistry in the University of Stellenbosch and the thesis has not been submitted previously for the academic examination towards any qualification. The thesis represents my own opinions and not necessarily those of the Stellenbosch University.

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Abstract

Aminoquinolines are important class of drugs that have been used for malaria chemotherapy for centuries. However, long-term exposure to these drugs leads to extensive spread of drug resistance. As such, modified chloroquinoline derivatives are being studied as alternative antimalarial agents with the possibility to overcome drug resistance associated with chloroquine analogues.

In this study, 15 aminoquinoline derivatives that are linked by a 1,4-disubstituted 1,2,3-triazole ring to an ethyl and propyl carbon spacer with a distal amine motif were designed and synthesized as novel antimalarial agents using the Cu(I)-promoted Huisgen reaction. The compounds have been synthesized from the 7-chloro-N-(prop-2-yn-1-yl)quinolin-4-amine alkyne precursor and the azides of ethyl and propyl amino moieties using a 1,3-dipolar cycloaddition-coupling in the presence of CuI catalyst to obtain moderate to good yields (53 – 85%). These compounds have been characterized by the combination of NMR, ESI+ HRMS and IR spectroscopic methods.

The antiplasmodial activity of the compounds was investigated \textit{in vitro} against \textit{P. falciparum} strain NF54 using chloroquine as a reference drug together with a standard antimalarial drug artemisin. Of the 15 novel chloroquinoline derivatives, 11 have demonstrated to possess promising potency by way of the inhibition concentrations less than 250 nM with the lowest being 28 nM. The observed activities have been ascribed to the overall modifications such as the introduction of a triazole linker and changing of carbon chain length as these were the variables. The compounds are accordingly under further biological investigations and only the chloroquine sensitive results are reported in this work.
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Preface

Thesis layout

This thesis is divided into five chapters. The first chapter consists of the background introduction to the impact of the disease malaria, then motivates and explains why the research question as to why synthesizing structurally novel aminoquinoline antimalarial drugs is important. The research objectives that this work is intended to achieve have been listed in this chapter.

The second chapter is a review of the previous research work in the subject research area related to antimalarial drugs and chemotherapy but leans towards the aminoquinoline drugs which are more significant in this study. The basic antimalarial biology of chloroquine analogues, an aminoquinoline derivative has been added to stress the importance of current understanding of drug resistance and developing the alternative antimalarial agents in an effort to overcome drug resistance.

The third chapter specifically discusses and analyses the successes and failures that have been encountered in the course of this work. It elaborates on the important experimental findings which which are in line with this work, mainly the chemistry of the synthetic work. The biological study performed in this work was intended to assess the potential of the synthesized compounds to inhibit the antimalarial activity and as such the discussion of antimalarial activity has been catered for in brief, and not to primarily formulate the biological reasons for the outcome.

The fourth chapter gives the summary and a conclusion of this study finding, the limitations of the methods employed, and suggests the implications based on the obtained results. Finally, the last chapter gives the detailed experimental methods as well as the spectrometric data collected during the analyses.

Numbering of captions and interpretations of spectroscopic data

Due to a variable number of figures per subtitle, the captions for figures and tables have the extended digit that displays the count of the figure or a table. The numbering of atoms in the
chemical structures is unconventional but the same numbering sequence has been maintained for the spectral references of the compounds.
Chapter 1

Brief background introduction and research objectives
1.1 Background introduction

The disease malaria is a haematozoan infection caused by sporozoites, transmitted by female *Anopheline* mosquitoes.\(^1\) It is a pernicious and most deadly parasitic infection whose impact ranks it the second cause of death in the African continent, after HIV/AIDS related infections. In addition, it is globally a leading cause of morbidity and mortality,\(^2\) mainly in the tropical and subtropical regions where this disease is endemic. It is now over a century since a Nobel laureate Robert Ross, in his pioneering work of malaria parasite transmission, considered malaria as a “million-murdering death”\(^3\) and today the disease continues to wreak havoc on millions annually. In 2010, the human burden caused by malaria approximated 216 million episodes in different territories around the world with most occurrences in sub-Saharan Africa affecting children without a protective immunity.\(^4\)

The nature of the burden caused by the disease malaria is a result of diversity in the parasite host species (*Plasmodium falciparum, P. vivax, P. malariae, and P. ovale* and *P. knowlesi*) that are responsible for the human malaria.\(^5\)\(^-\)\(^6\) Among these species, *P. falciparum* is responsible for the lethal human malaria; obstruction of the blood vessels of vital organs, premature delivery of infants, and the worst problem of developing resistive genetic mutations to antimalarial drugs.\(^7\)\(^-\)\(^8\) The emergence and spread of resistant parasite is a therapeutic challenge that has undermined generations of antimalarial drugs and the current grim death rate statistics could even rise higher should the resistance to the existing drugs develop further. The life-cycle of malaria causing parasites and their developmental stages are complex, each with a preferred cell-target in different hosts such as mosquitoes and humans or macaques, and thus creates a challenge in the management, control and elimination of the infection.\(^9\)

The reason for the continued survival of the *Plasmodium* parasites against antimalarial drugs lies in their genetic adaptability. The parasites have selective advantage mechanisms over drugs which are not fully understood, but certainly the parasite undergoes sexual recombination, a process of genetic cross-over that results in the offspring having different combinations of genes from those of their parents.\(^10\) This meiotic recombination process results in ‘genetic mutations’ that make parasites less sensitive to antimalarial drugs.\(^11\) Clinical experience and experimental evidence
indicates that drugs are generally effective when first introduced; however, their efficacies are curtailed by the emergence of resistant parasites. Coupled to the ever-present problem of controlling the disease vectors, is the lack of an effective vaccine. The problem has stimulated investigators to overturn the continuously evolving resistant parasites by developing analogues of once-effective and well-established antimalarial drugs, in addition to the identification of novel drug targets.

Despite the long-standing global efforts to eliminate malaria, no new classes of antimalarial drugs have been introduced into clinical practice since artemisinin combination therapy (ACT). Traditionally, antimalarial agents have been classified by the stages that are targeted by the drugs in the parasite’s life-cycle, but to date the chemotherapy arsenal represents three main structural categories, namely, quinolines, antifolate and artemisinin derivatives (figure 1.1.1). The primary discovery of antimalarial drugs began with the alkaloids comprised of a quinoline motif. Since then, quinoline derivatives such as the aminoquinolines, quinoline-methanols, acridines and others have evolved from these initial structures. The antifolate is a different class that encompasses mostly sulfur-containing and aminopyrimidine antimalarial drugs. Finally, the artemisinin derivatives are sesquiterpene lactone endoperoxides, and currently the World Health Organisation (WHO) “gold standard” drugs of malaria therapy.

The heterocyclic compounds with a quinoline motif are versatile, with a wide range of pharmacological activities and continue to be useful for antimalarial drug discovery. They have been a cornerstone for the synthesis of active antimalarial drugs for several decades and effective antimalarial drugs such as chloroquine (CQ), mefloquine (MQ), amodiaquine (AQ), pamaquine (PamQ) and others have been developed (refer to figure 1.1.1a). The essence of drug resistance has had a paradigm shifting effect in defining the molecular basis of antimalarial drug resistance and its relationship to therapeutic failure as guidance to design new effective antimalarial agents. The aminoquinoline moiety is the key pharmacophore on which the attachment of various ionizable amino side-chains have shown to enhance activity via increasing basicity, possible pH trapping, or as CQ resistance reversal motifs in CQ analogues.
a) Quinoline-based antimalarial agents

- Chloroquine (CQ)
- Pamaquine (PamQ)
- Mepacrine (MepQ)
- Anodinaquine (AQ)
- Quinine (QN)
- Mefloquine (MQ)
- Soutaquine (SQ)
- Tefenoquine (TQ)
- Pyronaridine (PQ)

b) Antifolate antimalarial agents

- Sulfadoxine
- Cycloguanil
- Pyrimethamine

c) Sesquiterpene lactones

- Artemisinin
- Artesunate
- Artemether

Figure 1.1.1 Three main categories of antimalarial drugs and examples thereof.
In a response to a burdensome challenge posed by malaria, the importance of generating new antimalarial drugs and maximizing therapy by improving the physicochemical properties of drugs cannot be overemphasized. A design based on the structure activity relationship (SAR) has reignited interest in discovering novel antimalarial agents by identifying discrete molecular components with recognition at a given target.\textsuperscript{13-14} In the process, the biological activity of potent antimalarial drugs is enhanced by the addition or repositioning of functionalities on a molecular fragment. The synthetic organic chemistry of antimalarial drugs was overturned in the late 19\textsuperscript{th} century after William Henry Perkin commenced to synthesise QN in 1856, but failed and the first total synthesis of quinine was accomplished almost a century later in 1944.\textsuperscript{15} The end synthetic chemistry of QN was found to be complex for commercial use and, even to this day, has not been accomplished on an economic scale.

The addition of an alkylamine side chain functionality to quinoline scaffolds in designing aminoquinoline antimalarial drugs was a result of Paul Ehrlich’s work in using haematological stains to diagnose blood diseases, including malaria itself.\textsuperscript{16} He observed that a synthetic dye, methylene blue (\textbf{figure 1.1.2}), was effective in staining and poisoning malaria parasites. Ehrlich’s intensions to enhance stain visibility led to the preparation of methylene blue analogues with alkylamine chains, in addition, the analogues were found to possess better antimalarial activity.\textsuperscript{16-17} On the basis of these observations, the alkylamine chain was viewed as beneficial for the antimalarial activity and thus the invention of potent antimalarial drugs were discovered through addition of alkylamine side chains on different quinoline scaffolds, most notably by the chemists at Bayer (this process is described schematically in \textbf{figure 1.1.2}).\textsuperscript{8,16,18} Nonetheless, it was only after the 2002 unveiling of the \textit{P. falciparum} genome in a joint global effort led by the Wellcome Trust Sanger Institute,\textsuperscript{19} that many intricate details about \textit{P. falciparum} drug resistance were obtained and significantly provided an opportunity to challenge therapeutic resistance with modified CQ analogues.
Figure 1.1.2 Historical perspective of aminoquinoline drugs discovery.
1.2 Research objectives

The structural function of quinoline antimalarial drugs remains definitively demonstrated. Recent short chain analogues of CQ have suggested chain variation as a recognition motif to overcome CQ resistance, explained in detail later in the thesis. The lateral amine functionality is another distinctive feature that has the ability to “reverse” CQ resistance. In general, modifications on the alkylamine side chain can be viewed as a feasible prospect to overcome the acquired parasite drug resistance to aminoquinoline agents. The application of click chemistry for the formation of a triazole-linked quinoline system, which possesses metabolic stability, to the lateral alkylamine chain is therefore justified. The perspectives for triazole-linked compounds stand on the threshold of the new era and the reported triazole pharmacological activity in various bioactive molecules is also remarkable.

Our particular study is intended at developing potent antimalarial agents through syntheses of new molecular entities that are structurally unrelated to CQ or AQ on the lateral side chain. Thus, we aimed at the creation of a small library of triazole-linked chloroquinoline derivatives having a short alkyl chain bearing amino functionalities and determination of the SAR for these modifications. The derivatives are characterized by an aminoquinoline ring that is linked by a 1,4-disubstituted 1,2,3-triazole to an ethyl and/or propyl spaced tertiary amino moiety (Figure 1.2.1). The work intends to use a Huisgen-Meldal-Sharpless reaction in creating a covalent triazole linkage of two molecular units by means of copper(I)-accelerated azide-alkyne 1,3-cycloaddition (CuAAC). This synthetic method tolerates various functionalized aminoalkyl azides to be integrated with the aminoquinoline parent compound and gives a broader structural horizon for exploring basic amines with the possibility of overcoming CQ resistance.
Figure 1.2.1 A representative structure for a library of triazole-linked CQ derivatives

The study explores the use of small amine functionalities such as diethylamine, metabolically stable cyclic amines (i.e. piperidinyl, pyrrolidinyl and morpholinyl)\textsuperscript{20} and some bulkier aromatic amines. Some of the latter analogues have been demonstrated to possess the ability to inhibit the efflux transport of drugs in multi-drug resistance diseases;\textsuperscript{21-22} as such their chemical structures are revealed in the next chapters to follow.

The following objectives for this study were therefore formulated:

- To synthesize the 7-chloro-N-(prop-2-yn-1-yl)quinolin-4-amine alkyne precursor, azides of ethyl amino and propyl amino moieties suitable for the triazole formation.
- To synthesize 1,4-disubstituted 1,2,3-triazole-linked chloroquinoline derivatives using the CuAAC reaction.
- To determine the antiplasmodial activity of the above synthesized compounds \textit{in vitro} against \textit{P. falciparum} strains.
- To compare the respective antimalarial activities to that of the conventional antimalarial drug CQ.

In the light of these objectives, the main theme of this work involves organic synthesis of the aminoquinoline alkyne, the aminoalkyl azides functionalized with a basic tertiary amine, and to
carry out the 1,3-cycloaddition of azides and alkyne promoted by CuI. The *in vitro* antimalarial activity investigations of the synthesized compounds should further assist in determining the SAR and the application of triazole moiety in the future development of antimalarial agents of chloroquinoline analogues. Should the introduction of a triazole linker be an important strategy in the modification of chloroquinoline analogues, it would follow that this moiety can critically influence pharmacologic bioavailability and potentially reverse CQ resistance. The synthesized compounds might have potent antimalarial activity, although it should not be ignored that the compounds can inhibit resistance mechanisms by some, as yet unidentified, protein interactions that are involved in the efflux of drugs by the malaria parasite.

After the short introduction provided in this section, the next chapter will give a more “in-depth” look of aminoquinoline derivatives and various strategies involved in their use as antimalarials.
Chapter 2

Literature review: Antimalarial activity of aminoquinoline derivatives, chemotherapeutic treatment of malaria and the synthesis of antimalarial agents.
2.1 Molecular mechanisms of action for CQ analogues and resistance

The exogenous supply of amino acids from haemoglobin degradation is absolutely essential for the parasite growth and survival. In the degradation process, toxic haematin moieties are generated as by-products and are removed by polymerization into an inert haemozoin.\textsuperscript{23-25} The haem accumulation is induced by the CQ analogues that bind to haematin moieties leading to inhibition of haemozoin formation.\textsuperscript{19} This inhibition of haematin polymerisation vitally sensitises the parasite and the antimalarial activity is subsequently manifested. Unlike other drug targets, haematin polymerisation process is non-enzymatic and the drug target remains vulnerable to drug’s bioavailability.\textsuperscript{26} The aminoquinoline target, haem-detoxification has been extensively studied, and continues to remain the most attractive area for developing 4-aminoquinoline antimalarial agents.\textsuperscript{25,27}

CQ accumulation in the parasite's digestive vacuole (DV) might occur via ion trapping, in other words, a passive diffusion of a basic CQ across the pH gradient into the acidic vacuole or binding to active transporters such as ATP or CQ receptors.\textsuperscript{28} In the DV, CQ becomes membrane impermeable after di-protonation (CQ\textsuperscript{2+}) and complexes with the intra-vacuolar free haematin or ferriprotoporphyrin IX (FP) receptor and thus inhibits the formation of crystalline haemozoin. The resistance of CQ is considered to be modulated by the protein membrane transporters such as P. falciparum CQ resistance transporters (PfCRT) or ATP-dependent P-glycoprotein homolog-I (Pgh-I) pumps which decrease drug concentration by way of drug efflux mechanisms.\textsuperscript{29-30} In addition, biochemical data in the literature indicates that resistance to CQ is a multi-genetic phenomenon and affects drug accumulation in the parasite’s food vacuole.\textsuperscript{17,31}

The molecular mechanisms of CQ resistance have remained enigmatic and theoretical models adopting reduced drug accumulation in the DV or carrier-mediated efflux have received much attention. The present evidence reveals that CQ resistance is primarily linked to an increased drug’s efflux as a result of mutations in the metabolite transporter of \emph{P. falciparum} (PfCRT). PfCRT is a membrane localized protein in the parasite’s digestive vacuole which is associated with the drug’s efflux from the site of action in the parasite’s DV.\textsuperscript{32-33} The key role of PfCRT in CQ resistance is beyond dispute\textsuperscript{34} and studies confirming the point mutations in this gene as
primary determinants of cross-resistance on various antimalarial drugs has been established.\textsuperscript{35} The molecular mechanism of CQ mode of action and resistance mechanism is summarized in \textbf{figure 2.1.1} adapted from the literature.\textsuperscript{28}

\textbf{Figure 2.1.1} Proposed molecular mechanisms for CQ mode of action and resistance.\textsuperscript{28}

\textbf{2.2 Structure function relationships in CQ derivatives}

The SAR of 4-aminoquinolines has been extensively elucidated in an attempt to restore antimalarial efficacy and overcome drug resistance. To this end, synthetic medicinal chemistry has developed a shortened-chain series of CQ analogues, organometallic antimalarial agents and also the hybrid antimalarial drugs, see \textbf{figure 2.2.1}. These classes of compounds have established the crucial role of the 7-chloro-4-aminoquinoline ring as well as the lateral side chain nitrogen for the antimalarial activity.\textsuperscript{24,36} The significance of alterations on the lipophilic carbon chain of CQ
analogues has also led to new analogues with improved efficacy and compounds which do not exhibit in vivo cross-resistance with CQ against P. falciparum.\textsuperscript{37-38}

\textbf{Figure 2.2.1} Shortened carbon chain series of CQ analogues that are active against CQ resistant \textit{P. falciparum} strains.

AQ-13 (refer to above figure 2.2.1) is an example of a short chain 4-aminoquinoline antimalarial drug that has undergone Phase I clinical trials and progressive dose-finding Phase II (efficacy) studies which are estimated to be complete by March 2013 in Mali.\textsuperscript{39}
Figure 2.2.2 Metabolic N-dealkylation of CQ antimalarial drug.

Of note is that CQ undergoes in vivo metabolic N-dealkylation and is transformed into a CQ metabolite (refer to figure 2.2.2) that has the same efficacy as CQ against sensitive *P. falciparum* strains except for the reduced activity in CQ resistant strains.\(^4\) AQ-13 has a similar metabolism to CQ but its modest activity against the MDR *P. falciparum* is believed to circumvent the parasite resistance mechanism as a result of reduced carbon chain length.\(^3\) The in vivo N-dealkylation and increased plasma clearance are key problems with this CQ analogue, but the replacements of the distal diethylamine with \(N\)-heterocyclic structures (i.e. piperidinyl, pyrrolidinyl, and morpholinyl groups) in AQ analogues is known to result in compounds that are metabolically stable to dealkylation and increased antimalarial activity (refer to figure 2.2.3).\(^2\) Of additional interest is that a large number of antimalarial drugs, for instance mefloquine and amopyroquine, having the \(N\)-heterocycle ring as part of their chemical structures, have potent antimalarial activity.
Another interesting rigid and more lipophilic CQ derivative is the antimalarial drug AQ with a 4-aminophenol linker joining chloroquinoline with an N-diethylamine short side-chain. This drug has been established as a superior alternative to CQ for the treatment of malaria infection in areas of high CQ resistance, see figure 2.2.4. AQ has a similar profile to CQ’s mechanism of action, but unfortunately, in vivo formation of toxic quinoneimine metabolites such as AQ metabolite 2, which is associated with liver toxicity and reduction of white blood cells, has severely restricted the clinical use of this promising candidate. Metabolic N-dealkylation of AQ further reduced the lipid solubility and increased cross-resistance with CQ.
From the chemistry point of view, the major limitation of the AQ drug’s potential is the inherent structural toxicity of the \( p \)-aminophenol linker that is subsequently transformed into a reactive quinoneimine (see AQ metabolite 2 on figure 2.2.4). The majority of the toxic effects are associated with the formation of covalent immunogenic aryl protein conjugates known to occur with interactions with the reactive quinoneimine metabolites.\(^{45}\) The isosteric replacement of the 4-aminophenol fragment with a piperazino or phenyl linker has been demonstrated to result in compounds with improved safety profile, observed during the formulation of hybrid molecules by Kumar and co-workers.\(^{46-47}\) A recent study has also demonstrated that benzyl-linked CQ derivatives of 4-aminquinoline utilised in a tandem with a reversal agent, result in the improved antimalarial activity against CQ resistant parasites and also the ability to inhibit the efflux action of PfCRT.\(^{48}\) Of importance is that these latter benzyl-linked CQ analogues have been alleged to be the first reversed-CQ antimalarial agents to inhibit haemoglobin formation and CQ resistance mechanisms \textit{in vivo} (refer to structures in figure 2.2.5).\(^{48-49}\)

\[ \text{Figure 2.2.5} \text{ Linker variants in reversed CQ resistance compounds.}^{46-48} \]
2.3 Reversed CQ resistance compounds

The multi drug resistance (MDR) in CQ analogues is associated with reduced drug uptake that is caused by enhanced drug efflux but this process can be mechanistically reversed by chemosensitizers.\textsuperscript{21} The chemosensitizers, also known as the reversal agents, have the capability of inhibiting certain lysosomal functions by disrupting accumulation-efflux mechanisms, and CQ resistance by PfCRT is thus reversible via these mechanisms.\textsuperscript{21,50} Imipramine and verapamil (figure 2.3.1) are amongst the better-studied PfCRT reversal agents, but have not been embraced for clinical use because their co-administration to potentiate efficacy require unacceptably high clinical doses.\textsuperscript{23,51} The clinical value of CQ sensitizers has also been impaired by their profound antipsychotic, antihistaminic, or cardiovascular effects.\textsuperscript{51}

![Chemical structures of imipramine, verapamil, and trifluoperazine]

**Figure 2.3.1** Some known chemosensitizers for CQ resistance reversal.

The CQ derivatives with bulkier and more lipophilic amino substitutions at the distal amino group have also been demonstrated to show remarkable efficacy against CQ resistant *P. falciparum* strains.\textsuperscript{22} Several structurally diverse motifs that are known to aid or partially inhibit the PfCRT efflux mechanism have been identified as important components of reversal agents (in fact, some are structural analogues of reversal agents) and these are shown in **figure 2.3.2**.\textsuperscript{52} Of interest is that these particular pharmacophores have demonstrated abilities to act as direct inhibitors of PfCRT when linked to a 7-chloroquinoline ring.\textsuperscript{49} The primary requirement of a reversal agent is to inhibit efflux and to enhance build-up of a CQ derivative in the parasite’s DV. The latter assignment to a reversal agent does not necessarily require a direct interaction with an efflux
effector, but the reversed-CQ molecule could have a lower binding to such transporters so that a reduced binding could lead to a higher drug accumulation.

Figure 2.3.2 Structural motifs recognised as CQ reversal agents.

2.4 Chemotherapy of malaria

Humanity has possessed the tools to tackle malaria for centuries, in fact even preceding the discovery of quinoline-containing pharmacophores in 1820s and before, the causative organisms of the disease were identified.\textsuperscript{53} Quinine (QN) and other \textit{cinchona} alkaloids were the first active ingredients to be used against human malaria\textsuperscript{54} which made malaria the first human disease to be controlled by a pure chemical,\textsuperscript{53} thus chemotherapy has been a reliable option for malaria control ever since. QN, a quinoline derivative, finds application in the intravenous treatment of uncomplicated malaria although it has been recently superceded by the drug artesunate.\textsuperscript{55-57} Due to clinical resistance, the monotherapeutic use of QN has been discontinued and a combination therapy with antibiotics such as tetracycline, doxycycline or clindamycin (see structures in figure 2.4.1), is now preferred for the treatment of uncomplicated malaria.\textsuperscript{73-74}
Antimalarial drugs

![Chemical structures of antimalarial drugs](image)

Antibiotics

![Chemical structures of antibiotics](image)

**Figure 2.4.1** Antimalarial drugs and a number of antibiotics used for antimalarial combination therapy.

The initial practice of applying clinical combinations of sulfadoxine, mefloquine and pyrimethamine (see chemical structures in **figure 2.4.2**) was developed in Thailand, with the specific intention to delay the onset of resistance to mefloquine; unfortunately these combinations failed to have the desired effect. The main reasons for the failure were the significant pharmacokinetic variations of individual drugs, such as absorption and distribution, metabolism, concentration achieved in the body, and subsequent elimination of the compounds. A pyrimethamine-sulfadoxine combination was, by then, coincidentally used in Africa (Malawi) to treat CQ resistant *P. falciparum* malaria. The rates of accelerated recoveries and reduced parasite transmission results were astonishing; however, a prolonged use this particular combination of compounds showed a decrease to 21% of the original efficacy and finally failed to prevent the emergence and spread of drug resistance.
Malaria therapy advocates a simultaneous use of different classes of antimalarial drugs with unrelated targets or mechanisms of action. This approach is geared towards delaying and preventing further emergence of drug resistance or even combating MDR P. falciparum malaria.\textsuperscript{62} The model underlying combination therapy is often designed to exploit synergy, shorten duration of treatment and increase patient compliance.\textsuperscript{60} A combination of drugs is therefore anticipated to effectively exploit multiple reductions in selection risks that arise from spontaneous genetic mutations of a parasite.\textsuperscript{60,63}

The idea of retarding resistance through combinations is a common solution to MDR and this concept was first pioneered in anti-tuberculosis therapy, later used on cancer chemotherapy, and more recently adapted to antiretroviral treatment.\textsuperscript{64} The notion has also been adopted as a standard care of P. falciparum malaria following extensive combination studies by Peters and his colleagues in the mouse model P. bergei.\textsuperscript{44,59} However, the current combinations lack synthetic simplicity which has prevented easy access in the Third World. In addition, their pharmacology has become a major economic disadvantage due to large and costly clinical profiling requirements (on average the current price of high quality artemisinin before combination is around US$ 450/kg).\textsuperscript{65}
The prophylactic use of CQ has been challenged by the widespread CQ resistant strains and has been replaced by artemisinin derivatives. However, the first signs of therapeutic resistance to artemisinin are subtle and have been very difficult to analyse.\textsuperscript{65-66} At the present moment, there is no convenient surveillance for detecting ACT resistance and the most reliable way is through rigorous clinical efficacy studies, which are very expensive and often lengthy.\textsuperscript{67} Clinical evidence gathered from Western Cambodia has recently warned that the emerging ACT resistance against the current first-line drugs would be catastrophic to initiatives aiming at global malaria control.\textsuperscript{66} The Southeast Asia region has, in the past, hatched ACT treatment-resistant malaria that has spread to the rest of tropical regions around the world.\textsuperscript{65} It is therefore imperative to prime the antimalarial pipeline with novel compounds that have been made by the application of facile, reliable and quick synthetic methods.

2.5 Rationale for selecting 1,2,3-triazoles as linkers

Apart from creating benzyl- or phenyl-linked chloroquinoline derivatives in creating “reversed” CQ compounds that might exhibit similar cross-resistance susceptibility patterns to AQ, it is envisaged that a triazole moiety can be used as a scaffold to broaden structural diversity. A 1,2,3-triazole is a five-membered heterocyclic aromatic system containing three consecutive nitrogen atoms connected to two carbon atoms in a ring, as illustrated in figure 2.5.1. Triazoles have been known for over a century\textsuperscript{68} and are stable to hydrolysis, as well as reductive or oxidative conditions, as a result of their aromatic stabilization. The triazole moiety is more than just a passive linker with \textit{in vivo} metabolic stability\textsuperscript{69} and rigidity, as it associates readily with biological targets through hydrogen bonding and dipole interactions.\textsuperscript{70} It is also an important structural feature that has inhibition activity against cytochrome p450 enzyme and/or peptide analogue in many biological compounds.\textsuperscript{71}
Figure 2.5.1 The 1,2,3-triazole structure and an example of a triazole-linked CQ-chalcone hybrid compound.\textsuperscript{72}

The use of the triazole functional group as a linker has been utilised in a wide variety of biological applications.\textsuperscript{73} A large volume of research has reviewed and disclosed the excellent biological activities such as the antibiotic,\textsuperscript{74} anticancer,\textsuperscript{75} antituberculosis,\textsuperscript{76} and antiviral properties\textsuperscript{77} associated with of the triazole-containing compounds. The pharmacological activities tailored by a triazole while acting as an effective linker of biomolecules is therefore difficult to ignore.\textsuperscript{70,78} At the moment, there are approximately 15 triazole-bearing compounds in the “Drug Bank” and the market, mostly with antifungal activities, and a further 24 are in different clinical trial stages.\textsuperscript{79-80} The significance of triazoles ability to effect the treatment of parasitic drug-resistant cutaneous leishmaniasis, Chaga’s diseases,\textsuperscript{81-82} and enhance the in vitro activity of protease inhibition against the strains of \textit{P. falciparum} has been documented.\textsuperscript{72,83}

The triazoles also successfully play pivotal roles as linkers and nonclassical bioisosteres of metabolically unstable groups to enhance pharmacokinetic properties of several drugs.\textsuperscript{67-68} This means that the triazole ring can replace many functional groups of structural diversity in potent compounds to produce viable clinical candidates. It is, therefore, a suitable bioisostere of many functional groups such as pyrazoles,\textsuperscript{84} aromatic rings,\textsuperscript{85} amides\textsuperscript{86} and others. The bioisosteric and linker functions offered by triazoles are more prominent in drug discovery. One of the challenges
of the malaria chemotherapy is to develop promising compounds that are innovative with chemical structures and mechanism of action.

2.6 New generation antimalarial agents

In the advent of the organic synthesis of new antimalarial drugs that are affordable, two or more ‘biologically privileged’ pharmacophores can be covalently joined into a single molecule by a linker.\(^{87-88}\) These derivatized compounds have the potential to offer an effective way of delivering a drug with favourable synergistic pharmacological activity.\(^{13-14}\) This approach can be regarded as supplementary to combinations, especially when combination has a marginal benefit that carries a significant toxicity. The formulation of “linked drugs” carries less risk of combination pharmacological interactions although inherent negative traits of individual pharmacophores is still possible.\(^{13}\) This is thus a cheaper development investment in drug discovery, especially where there are difficulties in sourcing natural metabolites or when the chemistry of synthesis is complex.

Another basis for hybridization is to exploit receptor binding capabilities by using linkers that can actively enhance active transport mechanisms and drug bioavailability. This is vital with respect to designing molecules which are intended to overcome cross-resistance under metabolic conditions. The 1,2,3-triazoles linkers are logical antiplasmodials as they are lipophilic nitrogen-containing heterocycles that tend to accumulate into the DV of a parasite.\(^{89}\) A critically important strategic element in generating such linkers is the reliability of the synthetic chemistry,\(^{88}\) which must proceed selectively to construct chemically stable bonds in a broad range of functional groups. For this reason, Kolb, Finn and Sharpless described the ideal class of reactions with organic transformations that satisfies this criteria, namely click chemistry reactions.\(^{90}\) The transformations performed with these reactions are versatile and have found applications in various fields of chemistry, materials, peptides, bioconjugation, radiolabeling of biomolecules for imaging, and importantly in the advancement of structure-based drug discovery.\(^{85,90-91}\)
2.7 Click chemistry

The energetically favoured click reactions generate stable molecules by irreversibly joining suitable molecular units or pharmacophores together, predominantly by way of heteroatomic linkages. Among the click reaction transformations, the 1,3-dipolar cycloadditions readily form five-membered rings at the aromatic oxidation level and this particular reaction has been shown to have had a significant impact in drug discovery. The 1,3-cycloaddition reaction that results in the formation of triazoles was discovered by Michael in 1893 and extensively studied by Dimroth in the early 1900s. The scope and diversity of the reaction was elucidated mechanistically in the 1960s by Huisgen. Azides and alkynes are energetically reactive species ($\Delta H^\circ = -45 \text{ to } -55 \text{ kcal/mol}$) that undergo a kinetically sluggish concerted cycloaddition reaction as a result of a high activation-energy barrier (ca 24 – 26 kcal/mol). The thermal promotion of the Huisgen reaction lacks regioselectivity and as a result generates a mixture of 1,4- and 1,5-disubstituted triazoles, as depicted in figure 2.7.1.

![Figure 2.7.1 Triazole formation via the Huisgen 1,3-dipolar cycloaddition reaction.](image)

The Huisgen $[3 + 2]$ cycloaddition reaction between a terminal alkyne and an azide that results in the generation of a disubstituted 1,2,3-triazole moieties is of popular interest and a key example in the click chemistry ‘area’. The central electron deficient nitrogen of an azide dipole influences a synchronous shift of electrons during the reaction with an alkyne, thereby generating the triazole heterocycles. The mesomerism of the 1,3-dipole, proposed by Pauling, results in a negative charge exchange between the two ends of a dipole which compellingly explains the reactivity and formation of two isomeric triazoles in the Huisgen reaction. Huisgen himself described these types of compounds as ambivalent since they displayed electrophilic and nucleophilic character in the positions 1 and 3, respectively, as shown in figure 2.7.2.
2.8 Copper(I)-catalysed alkyne-azide 1,3-cycloaddition reactions

The regioselective synthesis of the triazole ring following the Huisgen reaction remained a puzzle even after Kinugasa and Hashimoto had demonstrated the application of Cu(I)-acetylides and 1,3-dipolar nitrones in the enantioselective 1,3-dipolar cycloadditions reactions. The application of Cu(I)-acetylides with the 1,3-dipolar azides for the exclusive formation of 1,4-disubstituted triazoles in 1,3-dipolar cycloadditions was illustrated by independent reports of Meldal and Sharpless, see figure 2.8.1. A reaction resulting in the complementary 1,5-disubstituted isomer was later reported by Fokin and Sharpless, this time using ruthenium(II)-pentamethylcyclopentadienyl (Cp*) analogues, RuAAC. With the CuAAC reaction, the Cu(I) complex engages in the catalysis of terminal alkynes, while in the RuAAC reactions the ruthenium is also compatible with internal alkynes. Among the two transformations, the CuAAC reaction has been the more important reaction, and has been extensively utilised for the customisation and linking of the functional molecules.

Figure 2.8.1 Regioselective transformations of the Huisgen reaction under ruthenium and copper metal catalysis.
To date, for assembling disparate molecular fragments, the CuAAC reaction has demonstrated several advantages over the uncatalyzed Huisgen reaction.\textsuperscript{90,104} The application of copper catalyst significantly lowers the activation-energy barrier and thus accelerates the reaction rate ($10^7$ to $10^8$ faster) at room temperature. An important feature is that Cu(I)-accelerated reaction is not affected by the steric and stereoelectronic influences of the substrates utilised.\textsuperscript{102,105-106} A variety of experimental approaches have been effectively carried out with different Cu(I) sources, direct Cu(I) salts or the in situ reduction of Cu(II) salts, on a myriad substituted alkyne functional groups.\textsuperscript{102,106-107}

A nitrogenous base or copper ligands are sometimes added to the CuAAC reaction, with the most commonly used conditions being those reported by Sharpless group.\textsuperscript{106} Under those conditions, a catalytic Cu(II) salt and excess molar equivalents of a reducing ascorbate with the amine base additive are used to couple azides and alkynes with little or no co-solvent. The mechanism of the CuAAC reaction has been under several in-depth and ongoing investigations. According to kinetic studies and density functional theory calculations, the reaction is considered to be non-concerted in nature and proceeds in a stepwise fashion.\textsuperscript{107-108} The key steps in the reaction are highlighted in figure 2.8.2. The reaction proceeds by generating the copper(I)-acetylide, which occurs via alkyne $\pi$-coordination with a copper catalyst. The alkyne reactivity is thereby enhanced via a decrease in the electron density of the sp carbon atom and subsequently the pK$_a$ of the acetylene proton is lowered by about 10 log units.\textsuperscript{97,109-111} The reaction has been demonstrated to follow a second order rate law in copper when catalytic quantities are used.\textsuperscript{110} The intermediate Cu(I)-acetylide participates in a [3 + 2] dipolar cycloaddition with the azide. Due to a high potential energy barrier (ca 23.7 kcal/mol) of the 1,3-cycloaddition of azides to the copper(1)-acetylide, the azide is selectively activated by coordination to the acetylide-copper complex, thus forming a 6-membered metallocycle ring.\textsuperscript{109-111} A ring contraction then follows the thermo-neutral ligand exchange, after which a triazole ring is formed by protonation and release of the copper catalyst.
Figure 2.8.2 Stepwise CuAAC reaction between terminal azides and alkynes.\textsuperscript{97}

Given the modular synthesis of the above reaction, it was anticipated that its deployment in developing analogues of existing antimalarial drugs might create promising compounds with the novel chemical structures coupled with a different mechanism of action to the molecular target of the aminoquinolines.
Chapter 3

Results and discussion: Synthetic methods and *in vitro* antimalarial activity
3.1 Target compounds

The chemical structures shown below represent the final test compounds that were synthesized and evaluated for antiplasmodial activity. The first set of triazole linked chloroquinoline derivatives is the small aliphatic amines followed by the aryl amines.

![Chemical structures](image-url)

**Figure 3.1.1** Triazole-linked chloroquinoline test compounds.

A carbon spacer (n) corresponds to ethyl or propyl length in the respective order.
3.2 Synthesis of the amino chloroquinoline alkyne precursor

At the onset of our investigations, the synthesis of triazole-linked chloroquinoline derivatives by means of the Huisgen-Meldal-Sharpless reaction was the key chemical transformation because of its appealing regioselective formation of 1,4-disubstituted triazole products. More precisely, the Cu(I)-catalysed 1,3-cycloaddition reactions between the aminquinoline alkyne and azide building blocks were the central focus of synthesis that were then utilised to afford 1,4-disubstituted 1,2,3-triazole linked compounds with potential antimalarial activity.

The first step of the proposed strategy was to synthesise a terminal alkyne precursor derived from the well-known haematin-binding template, chloroquinoline. The synthesis of the key aminquinoline alkyne precursor 1 was carried out via a nucleophilic ipso substitution on the suitably substituted 4,7-dichloroquinoline scaffold. This was achieved by using propargyl amine as a nucleophile (refer to figure 3.2.1). It should be noted that the chloroquinoline ring can be considered as the prototypical electron poor aromatic system. The electronegative nitrogen substituted on the ring has its aromaticity disrupted through a strong permanent dipole by the inductive polarisation that causes fractional positive charges on the α- and γ-carbon atoms of the chloroquinoline ring. Therefore, the 4,7-dichloroquinoline ring can be pictured as experiencing mesomeric electron withdrawal by the nitrogen atom, and more importantly, experiences additional inductive withdrawal from the chlorine atom at the γ-position.\(^{112}\) This means that the chloroquinoline preferably undergoes nucleophilic aromatic substitution via an addition of a nucleophile at γ-carbon, followed by the elimination of chlorine atom (S\(_{\text{NAE}}\)).

![Figure 3.2.1 Synthesis of 7-chloro-N-(prop-2-yn-1-yl)quinolin-4-amine.](image)
The synthesis of \textit{I} has not been reported in the literature before, but the nucleophilic \textit{ipso} substitution reactions on 4,7-dichloroquinoline ring system using non-activated primary amines have been described in established literature methods.\textsuperscript{113-116} We therefore turned our attention to the development of this aminoquinoline alkyne precursor \textit{I}. A brief assessment of reagents used in these reactions show that amine nucleophiles play several additional roles, for instance, to basify and to solvate the reaction media. These methods thus predominantly make use of a large excess of amine nucleophile, up to 20 molar equivalents, to obtain moderate to good yields of the desired product (60\% \textendash 92\%). It should be considered that in our case, the high price of propargyl amine (5 mL > 1,300 ZAR Sigma Aldrich) would make this atom uneconomical approach extremely costly. The described literature conditions were however attempted with the initial 2 molar equivalents of propargyl amine,\textsuperscript{113} supplemented by another less nucleophilic base to decrease the amounts of propargyl amine required as described in the paragraphs that follow.

According to the cited experimental methods,\textsuperscript{113-115} and others reported elsewhere, a mixture of chloroquinoline and amine is usually homogenised at 80 °C, followed by a heating at reflux or at elevated temperature, i.e. 120 \textendash 140 °C. Similarly, in our hands, the experiments were stirred at 80 °C for 1 hour during which TLC monitoring displayed formation of the intended product. However, a follow-up reaction at 120 °C made it clear that at elevated temperature the product was undergoing decomposition into a brown solid of unknown composition (see \textbf{table 3.2.1}, entry 1). From this observation, it was concluded that higher temperature was detrimental to the reaction. Decomposition has been demonstrated as a major problem for similar reactions and milder reaction conditions have often been considered necessary for these reactions to be successful, i.e. reactions at 85 °C rather than high temperatures.\textsuperscript{24} In further modifications, the amounts of supplementary base was increased, but unfortunately the yields obtained were rather low (refer to \textbf{table 3.2.1}, entries 2 \textendash 6). In addition, it can be deduced from entry 3 from the same table that a proportional addition of excess propargyl amine, which was believed to perhaps escape from the reaction medium by vaporisation, did not have a significant impact on the reaction yield obtained and alternative conditions were thus investigated.
Table 3.2.1 The Attempted experimental conditions for N-alkylation of 4,7-dichloroquinoline

<table>
<thead>
<tr>
<th>Nucleophile equivalents</th>
<th>Base/Acid equivalents</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 2</td>
<td>Et₃N (0.5)</td>
<td>None</td>
<td>80 – 120</td>
<td>Decomposition</td>
</tr>
<tr>
<td>2) 2</td>
<td>Et₃N (0.5)</td>
<td>None</td>
<td>80 – 100</td>
<td>11</td>
</tr>
<tr>
<td>3) 2 + 2</td>
<td>Et₃N (2.0)</td>
<td>None</td>
<td>80 – 85</td>
<td>24</td>
</tr>
<tr>
<td>4) 2</td>
<td>Et₃N (4.0)</td>
<td>None</td>
<td>80 – 85</td>
<td>17</td>
</tr>
<tr>
<td>5) 2</td>
<td>K₂CO₃ (3.0)</td>
<td>DMSO</td>
<td>80 – 100</td>
<td>Insignificant product</td>
</tr>
<tr>
<td>6) 2</td>
<td>K₂CO₃ (3.0) + Et₃N (2.0)</td>
<td>DMSO</td>
<td>80 – 85</td>
<td>Insignificant product</td>
</tr>
<tr>
<td>7) 2</td>
<td>pTSA (0.1)</td>
<td>MeCN</td>
<td>80 – 85</td>
<td>37</td>
</tr>
<tr>
<td>8) 2</td>
<td>pTSA (0.1)</td>
<td>Dioxane</td>
<td>80 – 85</td>
<td>55 – 63</td>
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<tr>
<td>9) 2</td>
<td>None</td>
<td>Dioxane</td>
<td>80 – 85</td>
<td>6</td>
</tr>
</tbody>
</table>

Nucleophilic substitution reactions that proceed slowly at high temperature to give low yields might be found, in polar aprotic solvents like DMSO, to proceed rapidly and give better yields. Given the reputable advantages of DMSO in promoting such reactions together with a high boiling point advantage, it was expected that the yields of I would improve, unfortunately under these conditions, the reaction was found to have hardly progressed even after 24 hours, although decomposition was not observed (see table 3.2.1, entries 5 – 6).

In these type of reactions, it is known that the chlorine atom generally has poor leaving abilities when compared to other leaving groups due to strength of the C–Cl bond (D₀ ~ 79 kcal/mol). In order to carry out successful nucleophilic substitutions on the ring carrying this leaving group, the N-heterocycle’s propensity to undergo nucleophilic addition can be enhanced by inducing a formal positive charge on the ring nitrogen. Thus, N-oxides or N-quinolinium salts are established promoters of such reactions; nonetheless, N-oxides on quinoline can be viewed as disguised 1,3-
dipoles that will likely undergo Diels-Alder [3 + 2] cycloadditions with the alkyne of the propargyl amine, and this approach was not attempted.

A direct $N$-alkylation in the nucleophilic substitution of $N$-heterocyclic structures appears deceptively simple, but the synthetic value of this method is chiefly limited to employing a large excess of the alkylating amine. Chauhan’s group\textsuperscript{47} has demonstrated that the amination of 4,7-dichloroquinoline under $p$-toluenesulfonic acid ($p$TSA) catalysis using lower quantities of primary amine nucleophile, is viable, and such reactions have also been carried out on other substituted 4,7-dichloroquinolines using different catalytic acids such as HCl.\textsuperscript{117} Therefore, chloroquinoline was protonated with $p$TSA which supposedly forms a more polarised $N$-chloroquinolinium intermediate. The reaction was anticipated to undergo a known $S_{N}A_{E}$ process under the strong influence of a positive formal charge on the nitrogen as graphically depicted in Figure 3.2.2.

![Figure 3.2.2](image_url)

**Figure 3.2.2** $p$TSA catalysed $N$-alkylation of 4,7-dichloroquinoline.
Given the impact of the heterocyclic nitrogen atom in nucleophilic substitutions on the chloroquinoline ring, pTSA catalysis was therefore investigated in MeCN and dioxane solvents with the intention of promoting the substitution rates (see table 3.2.1, entries 7 – 10). Of significance was that a maximum yield of 63% was obtained when 1,4-dioxane was used as compared to 37% for MeCN as solvent. The importance of the pTSA catalysis was also investigated and a low yield of only 6% was obtained where this catalyst was omitted (refer to table 3.2.1, entry 9). Nonetheless, under acid catalysis, it appears that nucleophilic substitution on halogenated N-heterocycles remains the most feasible option, particularly when only utilising a single equivalent of a nucleophile, as the use of mild basic conditions with multiple equivalents of amine nucleophile were found not to be optimal.

In deuterated DMSO, the structural elucidation of compound 1 by $^1$H NMR spectroscopy confirmed that the signals representing the expected aromatic signals for the chloroquinoline ring in the region 6.6 – 8.5 ppm were present (refer to figure 3.2.3). The doublets at 8.5 ppm and 6.5 ppm with decreased coupling constant $J_{HH \text{ ortho}} = 5.7$ Hz coupling were assigned to the two protons H-2 and H-3 experiencing the mesomeric and inductive deshielding effect from the ring nitrogen, N-1 and the substituted N-4, respectively. A doublet at 8.2 ppm integrating for a single proton, was attributed to H-5 with $J_{HH \text{ ortho}} = 9.0$ Hz coupling to a doublet of doublets H-6, that was further coupled to a meta proton H-8 at 7.5 ppm with a $J_{HH \text{ meta}} = 2.2$ Hz. The aromatic coupling of protons ($J_{HH \text{ ortho}} = 5 – 9$ Hz between H-2 and H-3 as well as H-5 and H-6, $J_{HH \text{ meta}} = 2.2$ Hz between H-6 and H-8) and the splitting pattern of signals were definitive in deciding the assignments of the chloroquinoline protons.

![Figure 3.2.3](image)

**Figure 3.2.3** Structural elucidation of 7-chloro-N-(prop-2-yn-1-yl)quinolin-4-amine (1).
The most important signals were those of the newly installed propargylic amine group in addition to the expected aromatic signals for the chloroquine ring. Although amine protons are generally not considered suitable for diagnostic purposes due to lack of consistency in their shifts, a broad triplet at 7.8 ppm was assigned to the N–H proton at the γ-position with a $J = 5.7$ Hz corresponding to the allylic proton coupling. Moreover, a double of a doublet at 4.1 ppm was assigned to the allylic protons which were further coupled to the acetylenic proton triplet at 3.2 ppm with a $J = 2.4$ Hz. These signals were indicative of the successful substitution of the propargyl amine on the chloroquinoline ring system. $^{13}$C NMR spectroscopy was also used to substantiate the structural elucidation of $I$ and the three additional carbon signals were observed: namely, the high-field shifted carbon signals at 74 ppm and 81 ppm in comparison to the aromatic carbons (100 – 152 ppm) were characteristic of the sp hybridised acetylene carbons of a terminal alkyne and the additional carbon signal at 32 ppm provided the evidence of an aliphatic CH$_2$ carbon with an incremental downfield shift due to a proximal nitrogen atom.

In addition to NMR spectroscopy experiments, the positive electron spray ionisation mode in high resolution mass spectrometry (ESI$^+$ HRMS) was carried out to determine the isotopic mass and perhaps elemental composition of compound $I$. The protonated molecular ions ([M + H]$^+$; m/z) was the dominant species and the peak with the highest isotopic abundance was customarily assigned 100%. The determined experimental monoisotopic mass of $I$ [M + H]$^+$ = 217.0529 and was found to be in good agreement with the theoretical 217.0533 and within -1.84 ppm accuracy. In addition to the molecular ion peak, a peak at m/z 219.0503 with approximate abundance of 27% was considered to be due to the isotopic $^{37}$Cl which has about 24% relative abundance, thus confirming the atomic composition of $I$. Additional small clustered peaks around the base peak were ascribed to the compositional isotopic masses too.

The IR spectroscopic skeletal vibrations ($\nu$/cm$^{-1}$) were further used to confirm the important functional groups: 3391 (NH stretch) was assigned to the aliphatic secondary amine; 3205 (C≡CH) acetylenic proton stretch, 3094 (Ar CH) aromatic protons stretch in the ring, 2963 (CH$_2$) aliphatic methylene asymmetric stretch; 2019 (C≡C) was a very broad band of acetylene; and a very weak peak at 1567 frequency further confirmed the (C=C) aromaticity in reference to Ar CH aromatic protons observed at 3094.
3.3 Synthesis of lateral chain aliphatic azides

As aforementioned, the azide building blocks were the next requirements for the CuAAC reactions with compound 1. It was, therefore, necessary to synthesise aliphatic azide spacers which would later be functionalized with selected amines. This section describes the synthesis that was followed to obtain azides of ethyl and propyl linear chains. Several detailed models of the structure function relationships of CQ have established the importance of carbon chain length variation in restoring the antimalarial efficacy of 4-aminoquinolines. The azides of ethyl and propyl carbon chain length were therefore synthesized in two steps from ethane-1,2-diol and propane-1,3-diol starting materials, respectively, using a modified literature procedure. The diols were first converted into their corresponding bis-mesylates using mesyl chloride under basic conditions in THF as a solvent to afford compounds 2a and 2a′ in good yields, 94% and 99%, respectively.

The \(^1\)H NMR spectra for the compounds were in agreement with the data reported in the literature. The chemical structures of 2a – 2a′ were also supported by the IR spectrometry (\(\nu/cm^{-1}\)). The intensely sharp peaks at 1176 (S=O) and 1048 – 1045(O–S) distinctively confirmed the presence of the newly introduced, substituted sulfonate groups. The medium intensity peaks at 2963 – 2933 (CH\(_2\)) and 2884 – 2789 (CH\(_2\)) were assigned to the methylene asymmetric and symmetric stretches while the peaks at 1359 – 1352 (CH bend) were attributed to CH bend vibrations, respectively, confirming the presence of aliphatic carbon chain. In addition to the above mentioned peaks, a medium band at 748 (CH rocking), which can only appear in longer and linear aliphatic chains, was observed in the case of 2a′ to strengthen that 2a′ was of a longer propyl length (see figure 3.3.1).
These intermediates containing the mesylate leaving groups were then treated with sodium azide in acetonitrile solvent with the intention to displace one of the mesylate groups and therefore obtained compounds 3a and 3a'. The synthesis of the azidomesylates 3a and 3a' proceeded smoothly via a nucleophilic substitution (S_N2) in both steps, and moderate yields of 42 – 46% were obtained. It is obvious that the azide nucleophile has the potential to displace both mesylate groups in 2a and 2a' and bis-azidation was the cause of the overall decrease of yields. Azide disubstitution was confirmed on TLC by Staudinger reduction and ninhydrin staining that revealed formation of two major products in the reaction mixture.\textsuperscript{121} Again, the $^1$H NMR spectra of 3a and 3a' resembled the literature data.\textsuperscript{119,122} The IR spectra of the relevant compounds established the azide substitution with an intensely sharp peak at a frequency of 2093 cm$^{-1}$, which was otherwise not observed in the bis-mesylate intermediates. This was a conclusive confirmation of the presence of the azide functional group.

3.4 Functionalization of the aliphatic azides with amine groups

In continuation with the azides synthesis, the selected amine functional groups that are essential for optimising the biological activity were added to the aliphatic azide chains of 3a and 3a'. The aminoalkyl azides were thus prepared next via displacement of the unreacted mesylate group with a number of secondary amines. A series of aminoalkyl azides were therefore synthesized as illustrated in figure 3.4.1.
Figure 3.4.1 A general template for the synthesis of aminoalkyl azides.

The S_N2 N-alkylation reactions were performed under mildly basic conditions by heating starting material at reflux in MeCN for 16 hours to obtain compounds 4b, 4b' and 4d' – 4h' as the sole products in low to good yields of 34 – 73% (refer to table 3.4.1 for a summary of the secondary amine utilised and for all yields obtained).
<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Product</th>
<th>Yield (%)&lt;br&gt;n = 2</th>
<th>Yield (%)&lt;br&gt;n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4b and 4b'</td>
<td><img src="" alt="Cyclohexylamine" /></td>
<td><img src="" alt="Diazocyclohexylamine" /></td>
<td>72</td>
<td>70</td>
</tr>
<tr>
<td>4c and 4c'</td>
<td><img src="" alt="Cyclopentylamine" /></td>
<td><img src="" alt="Diazocyclopentylamine" /></td>
<td>64</td>
<td>73</td>
</tr>
<tr>
<td>4d and 4d'</td>
<td><img src="" alt="Cyclopropylamine" /></td>
<td><img src="" alt="Diazocyclopropylamine" /></td>
<td>48</td>
<td>36</td>
</tr>
<tr>
<td>4e and 4e'</td>
<td><img src="" alt="Benzylamine" /></td>
<td><img src="" alt="Diazobenzylamine" /></td>
<td>69</td>
<td>68</td>
</tr>
<tr>
<td>4f and 4f'</td>
<td><img src="" alt="Phenethylamine" /></td>
<td><img src="" alt="Diazophenethylamine" /></td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>4g and 4g'</td>
<td><img src="" alt="Diethylaminoethylamine" /></td>
<td><img src="" alt="Diazodiethylaminoethylamine" /></td>
<td>41</td>
<td>47</td>
</tr>
<tr>
<td>4h and 4h'</td>
<td><img src="" alt="Diphenylmethylamine" /></td>
<td><img src="" alt="Diazodiphenylmethylamine" /></td>
<td>61</td>
<td>56</td>
</tr>
<tr>
<td>4i'</td>
<td><img src="" alt="Hexylamine" /></td>
<td><img src="" alt="Diazohexylamine" /></td>
<td>—</td>
<td>61</td>
</tr>
</tbody>
</table>

'Synthesized using a different route illustrated later in figure 3.4.2'
In cases where lower yields were obtained, for instance with 4d and 4d', it was observed that the azides were semi-volatile and that most of the product was being lost \textit{in vacuo} during solvent removal. The rest of samples were therefore dried by passive evaporation in a vacuumed desiccator after short treatment with once off vacuum to remove most of the solvent entrapped in the sample. The majority of synthesized aminoalkyl azides, reported in \textbf{table 3.4.1}, are not reported in the literature and as such \textsuperscript{1}H NMR, \textsuperscript{13}C NMR, ESI\textsuperscript{+} HRMS and IR spectroscopic methods were used to confirm the structures of these compounds. Depending on whether the amine functional group was aliphatic or aromatic, the characteristic signals were verified by the appropriate spectroscopic methods; however, constantly common was the important azide peak at frequency 2093 – 2136 cm\textsuperscript{-1} in the IR spectra.

The \textsuperscript{1}H NMR spectra of compounds 4b – 4i varied from one compound to the other, but the aliphatic azide chain signals remained almost unchanged (see \textbf{figure 3.4.1}). The protons in the \(\alpha\)-position with respect to the azide at 3.3 – 3.8 ppm were equally split into a triplet signal by the neighbouring protons with a \(J\)-value of 6.3 – 7.9 Hz which denoted the free rotating aliphatic carbon chains. In the case of the propyl chain, an additional quintet at 1.7 – 1.8 ppm with a similar coupling constant, was observed, in the absence of other overlapping protons signals.

The efforts to achieve substitution with a pyrrolidine group (refer to \textbf{table 3.4.1}, entries 4c and 4c') using the described conditions (MeCN, K\textsubscript{2}CO\textsubscript{3},)\textsuperscript{119} proved to be futile presumably as a result of assumed decomposition. During the reaction progress, an instant change of colour to a brown solution was considered to indicate the unidentified side reactions, in this particular case. An alternative method was therefore employed\textsuperscript{123} and the reagents were thus homogenised at 0 °C first followed by overnight heating at reflux to afford compounds 4c and 4c' in good yields (64 – 73%). Furthermore, the \(N\)-alkylation of aromatic amines was promoted with potassium iodide (KI) in the light of their poor basicity, low nucleophilicity and problems associated with steric effects (see \textbf{table 3.4.1}, entries 4f – 4c'). Traditionally, KI is known to promote substitutions via the Finkelstein reaction\textsuperscript{124} in which a less labile halide or halide equivalent sulfonate is substituted by a better leaving iodine which is subsequently displaced by the amine.
The direct alkylation of the azidomesylates $3a$ and $3a'$ (see table 3.4.1, entry 4i) with a sterically hindered 10,11-dihydro-5H-dibenzo[b,f]azepine was also unsuccessful under the usual conditions described earlier. There are few similar reactions reported in the literature using the same nucleophilic amine and low yields are often obtained due to the poor basicity of this amine nucleophile.\(^{51}\) It should be noted that in the literature, similar reactions have been investigated by means of metal-mediated cross-coupling or palladium alkylation after which several steps, including hydroboration and the Appel reaction, were followed to produce several potential antimalarial agents with a linear chain (see figure 2.3.1 described earlier in the dissertation).\(^{125}\) In our work, we decided to investigate the two reaction routes shown in figure 3.4.2. The nucleophilic amine was thus deprotonated under strong basic conditions to carry out the substitution reaction with a suitable leaving group. It should also be noted that route B was utilised, after numerous failures when route A was pursued.

![Figure 3.4.2 Synthesis of 5-(3-azidopropyl)-10,11-dihydro-5H-dibenzo[b,f]azepine.](image)

Figure 3.4.2 Synthesis of 5-(3-azidopropyl)-10,11-dihydro-5H-dibenzo[b,f]azepine.

Of importance is that, strong bases are generally good nucleophiles and *vice versa*; thus β-elimination is also a common competing side-reaction under strongly basic conditions. Therefore, the N-alkylation of azepine in figure 3.4.2 was investigated on the azidomesylates ($3a$ and $3a'$), as well as 1-bromo-3-chloropropane under mild and strong basic conditions and the results are presented in table 3.4.2. Under mildly basic conditions, substitution reactions of nucleophilic
amines are known to be promoted by heating but such efforts were inefficient in our hands (refer to table 3.4.2, entries 1 – 3). Moreover, mesylate esters are good electrophiles for substitution reactions but can also undergo efficient elimination when reacted under strong base conditions. With this information at hand, it was realised that elimination reaction was the major side-reaction taking place (see table 3.4.2, entries 3 – 4) and that the sulfonate leaving group also favoured elimination over substitution under these conditions.

At this point, the sulfonate leaving group was swapped for a halide and 1-bromo-3-chloropropane was used as an alkylating agent. 10,11-Dihydro-5H-dibenzo[b,f]azepine was thus deprotonated using nBuLi in THF solvent (refer to figure 3.4.2); however, alkylations under these conditions gave the unwanted N-allyl product (refer to table 3.4.2, entry 5), obviously due to a subsequent E2 elimination. Reaction rate studies of similar reactions by Collum and co-workers described the formation of various diphenyl lithium aggregates to be the primary determinants of N-alkylation in the THF solvent. In neat THF solvent, the alkylation rates exhibited fractional dependence on the amide, first order on the alkyl halide and a seventh order dependence on THF. The alkylation success thus changes with the degree of amide solvation and aggregation states, and these factors thus influence the ratios of N-alkylation versus E2 elimination.

Table 3.4.2 N-alkylation of 10,11-dihydro-5H-dibenzo[b,f]azepine

<table>
<thead>
<tr>
<th>Leaving group</th>
<th>Solvents (Base)</th>
<th>Temp °C</th>
<th>Product (5a:5a')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. -OMs</td>
<td>MeCN (K₂CO₃)</td>
<td>80 – 110</td>
<td>Not observed</td>
</tr>
<tr>
<td>2. -OMs</td>
<td>Toluene (tBuOK)</td>
<td>80 – 140</td>
<td>Not observed</td>
</tr>
<tr>
<td>3. -OMs</td>
<td>DMF (NaH)</td>
<td>RT – 140</td>
<td>Not observed</td>
</tr>
<tr>
<td>4. -OMs</td>
<td>DMF (NaH, nBuLi)</td>
<td>0 – RT</td>
<td>Insignificant product</td>
</tr>
<tr>
<td>5. -X</td>
<td>THF (nBuLi)</td>
<td>0 – RT</td>
<td>43%, 5a</td>
</tr>
<tr>
<td>6. -X</td>
<td>THF: C₆H₆ (1:1; nBuLi)</td>
<td>RT – 50</td>
<td>Insignificant prod.(5a')</td>
</tr>
<tr>
<td>7. -X</td>
<td>THF: C₆H₆ (1:1; nBuLi)</td>
<td>0 – RT</td>
<td>44%, 5a/5a' ~(5:1)</td>
</tr>
<tr>
<td>8. -X</td>
<td>THF: C₆H₆ (1:4; nBuLi)</td>
<td>0 – RT</td>
<td>47%, 5a/5a' ~(1:1)</td>
</tr>
</tbody>
</table>
Under this paradigm, S_N2/E2 selectivity would then show propensity to favour S_N2 at low THF concentrations. However, in an attempt to perform the reaction using Collum’s conditions at 50°C, an insignificantly low amount of N-alkyl product was formed (see table 3.4.2, entry 6). The satisfactory S_N2 product 5a' was however isolated from an approximated 1:1 product ratio, when employing 20% THF in benzene solvent, and when the reaction was carried out at 0 °C – RT (refer to table 3.4.2, entry 7). From the chemistry point of view, the N-allyl product, 5a, could be converted into a desired terminally substituted azide via the known anti-Markovnikov hydroazidation in the presence of catecholborane,\textsuperscript{128} but in our case the direct halide substitution of 5a' with NaN\textsubscript{3} to produce 4i (61% yield) was followed. The structural analysis of 4i was carried out using the same spectroscopic methods described earlier in this section.

The identification of isolated compounds 5a and 5a' was established clearly by means of \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectroscopy. The structure of 5a was distinct since it had a unique ABX allylic splitting pattern at 5.1 – 5.8 ppm together with a diagnostic vinylic proton coupling system (\textsuperscript{2}J_{H,H} = 5.7 Hz, \textsuperscript{3}J_{H,H,cis} = 10.3 Hz, and \textsuperscript{3}J_{H,H,trans} = 17.3 Hz) to both cis and trans protons which could be differentiated from their J-values. In order to ascertain whether 5a' was bromo- or chloro-substituted, \textsuperscript{1}H NMR spectroscopy was again crucial in differentiating the two compounds using the protons chemical shifts. The protons at the \textalpha-position to Cl resonated at a much higher frequency of 4.4 ppm in 5a' compared to those of Br at 3.6 ppm. The ESI\textsuperscript{+} HRMS spectra data also supported the \textsuperscript{1}H NMR spectral evidence.
3.5 Synthesis of the 1,4-disubstituted 1,2,3-triazole-linked chloroquinoline derivatives

Having synthesized the azide building blocks and the aminoquinoline alkyne precursor \( I \), the final step in the synthesis was to perform a Cu(I)-promoted 1,3-cycloaddition reaction between these azides and the alkyne \( I \). There are many variations in the experimental approaches to the reaction as discussed under section 2.8 and many discrepancies in the literature centre on the presence or absence of a base ligand to have the resultant effect on the reactivity of Cu(I).\(^\text{109,129}\) The CuAAC reactions in this study were thus performed between \( I \) and \( 4b - 4i \) in THF solvent by means of a modified literature procedure as shown in figure 3.5.1.\(^\text{130}\) The reactions were performed under anhydrous nitrogen atmosphere for 30 – 90 minutes at room temperature using CuI, to afford the triazole-linked chloroquinoline derivatives in moderate to good yields (53 – 85\%) after purification by means of silica gel column chromatography.

![Figure 3.5.1 Synthesis of the triazole-linked chloroquinoline derivatives via CuAAC reaction.](image)

\[ \text{CuI (1 equiv.), THF} \]
\[ \text{RT, 0.5 - 1.5 hrs} \]
\[ (53 - 85\%) \]
### Table 3.5.1 The product yields of the respective triazole-linked chloroquinoline derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( n = 2 )</td>
<td>( n = 3 )</td>
</tr>
<tr>
<td>6b and 6b'</td>
<td><img src="image1.png" alt="Image" /></td>
<td>76</td>
<td>79</td>
</tr>
<tr>
<td>6c and 6c'</td>
<td><img src="image2.png" alt="Image" /></td>
<td>67</td>
<td>63</td>
</tr>
<tr>
<td>6d and 6d'</td>
<td><img src="image3.png" alt="Image" /></td>
<td>70</td>
<td>61</td>
</tr>
<tr>
<td>6e and 6e'</td>
<td><img src="image4.png" alt="Image" /></td>
<td>74</td>
<td>85</td>
</tr>
<tr>
<td>6f and 6f'</td>
<td><img src="image5.png" alt="Image" /></td>
<td>64</td>
<td>53</td>
</tr>
<tr>
<td>6g and 6g'</td>
<td>![Chemical Structure]</td>
<td>69</td>
<td>65</td>
</tr>
<tr>
<td>6h and 6h'</td>
<td>![Chemical Structure]</td>
<td>54</td>
<td>63</td>
</tr>
<tr>
<td>6i</td>
<td>![Chemical Structure]</td>
<td>—</td>
<td>64</td>
</tr>
</tbody>
</table>

Under the above CuAAC reaction conditions utilised (refer to figure 3.5.1), the reactions carried out for longer than 120 minutes became thick slurries, perhaps due to coordination of the THF, the substrates or perhaps the products to free CuI forming insoluble organometallic compounds. In some cases, the products yields were lowered by the same effect as the work up procedure became difficult to perform. In addition, the presence of significant amounts of a poorly soluble by-product which could not be readily identified by $^1$H NMR spectroscopy as a result of peak broadening in addition to poorly resolved $^{13}$C NMR spectra, also complicated the experimental procedures.

A competitive oxidative Glaser coupling reaction is known to occur during CuAAC reaction, especially when direct Cu(I) salts are employed, but in our case there was no positive identification of the bis-acetylates of the alkyne precursor and thus the Glaser reaction was ruled out. The by-product was only occurring in prolonged reaction conditions, hence a longer (4 hour)
reaction was carried out and it was found that the intended product deteriorated until no product spot could be identified on the TLC. The 1,4-disubstituted triazole-linked chloroquinoline products (see table 3.5.1) were very polar, but fortunately a solvent system consisting of 10% methanol in acetone successfully developed analytical TLC for the compounds, at least two TLC runs in some cases. However, the by-product did not move from the TLC baseline with this mobile phase and increasing mobile phase polarity often resulted in poorly resolved TLC due to trailing.

3.6 Structural elucidation of the final CuAAC compounds

The structures of all the compounds obtained, 6b – 6i, were established by $^1$H NMR, $^{13}$C NMR, ESI$^+$ HRMS and IR spectroscopic methods. A representative characterisation of compound 6f’ is described below. In a deuterated chloroform, a similar $^1$H NMR and $^{13}$C NMR spectra pattern of the chloroquinoline ring (described under section 3.2) was observed for all the compounds with the exception of signal migrations and swapping of the chemical environments between H-5 and H-8 (refer to figure 3.6.1). The order of chemical frequencies for H-5 and H-8 were swapped in the synthesized triazole derivatives as compared to the previously characterised aminoquinoline alkyne 1. The H-5 doublet had migrated to a lower frequency at 7.8 ppm in comparison to the initially observed 8.2 ppm in 1. In addition, the triazole proton H-5’ appeared as a singlet in all the cases although a weak spatial coupling to the neighbouring protons was observed, as discussed later.
Figure 3.6.1 A representative characterisation of 6f' by $^1$H NMR and $^{13}$C NMR spectroscopic methods.

With respect to compound 6f' in figure 3.6.1 above, the phenyl amine protons signals in the aromatic region were thus assigned as follows: 3 signals integrating in the ratios of 2:2:1 at 7.3 – 6.4 ppm were attributed to the aromatic protons H-2'' - H-6'' with $^3J_{HH \text{ ortho}} = 7.3 - 8.0$ Hz where the signals multiplicity could be clearly identified as a triplet or a doublet. The broadened singlets at 6.1 ppm and 4.6 ppm were attributed to the N–H and H-4'' protons, respectively. The triplets of the protons that appeared downfield were H-1'' at 4.4 ppm and H-2'' at 2.6 ppm compared to the chemical shifts of H-1'' and H-2'' (H-1'' at 3.4 ppm and H-2'' at 1.8 ppm) in the aliphatic azides chain, perhaps as a result of the triazole ring polarisation effects. The H-3'' and N-CH$_3$ protons appeared at chemical shifts at 3.4 ppm and 2.9 ppm, respectively.

The $^{13}$C NMR spectrum also supported the observations in the $^1$H NMR spectrum. The disappearance of the alkyne carbon signals in the starting material, at 81 ppm and 74 ppm, with the concomitant appearance of two additional carbon signals in the aromatic region at 136 ppm and 122 ppm of the product, were conclusive that an aromatic 1,4-disubstituted triazole ring had been synthesized.
A homonuclear correlations $^1\text{H}^1\text{H}$ COSY spectroscopy experiment was also performed on the related compound 6c (refer to figure 3.6.2) to confirm the observed interactions between the protons (coupling constants, $J$). As mentioned earlier that there were changes in the chemical environments upon installing the triazole ring, a proper differentiation of the protons through $^3J_{\text{H-H}}$ was made possible by the COSY experiment. The absolute interaction between the protons ($^3J_{\text{H-H}}$ ortho coupling of H-5 and H-6, $^3J_{\text{H-H}}$ meta coupling of H-6 and H-8 and a very weak $^3J_{\text{H-H}}$ para coupling of H-5 and H-8) in the chloroquinoline ring and the vicinal protons was confirmed. In addition to the chloroquine protons, H-3 and H-5, spatial coupling with the H-$4''$ protons was also observed. Of particular significance was that a weak cross-coupling between the triazole H-5' and both H-$4''$ and H-$1''$ protons hinted that a triazole ring was 1,4-regioisomer.

![Figure 3.6.2 $^1\text{H}^1\text{H}$ COSY correlations for compound 6c.](image)

As previously mentioned in section 2.7, the azides are ambivalent compounds and their reactivity is influenced by their mesomerism which can result in the formation of two possible product regioisomers. It was thus found necessary to confirm the synthesized compounds were indeed the
1,4-disubstituted triazole-linked chloroquinoline derivatives, and not the corresponding 1,5-derivatives.

To ascertain the 1,4-disubstitution and connectivity around the triazole ring, NMR spectroscopy experiments utilising gradient selected heteronuclear correlations over a single bond (gHSQCAD) and multiple bonds (gHMBC) were conducted for the compound 6b'. Having confirmed the proton correlations from the COSY NMR spectroscopy experiment, the gradient selected HSQCAD and HMBC spectroscopic experiments were used to trace the carbon skeleton of the identified triazole-linked chloroquinoline derivative. The HSQCAD spectra was used to selectively determine the single coherence between protons and carbons, $^1J_{C,H}$. From this experiment the carbon carrying the triazole proton (C-5') was clearly identified from the rest of the aromatic carbons, whose signals overlapped heavily (refer to figure 3.6). As it can be deduced from figure 3.6, it would have been very difficult to also distinguish C-5 at 121.3 ppm from the C-5' triazole carbon at 122.1 ppm. These two carbons could easily be misinterpreted when using normal APT and DEPT spectroscopic methods because of their equivalence on the $^{13}$C NMR spectra. Furthermore, the regioisomeric nature of the triazole isomer could already be partly deduced at this point when taking into consideration the chemical shifts of C-5' triazole carbon at 122 ppm versus C-2 at 152 ppm of the chloroquinoline. Because these chemical shifts must be controlled by the pyrrole or the pyrimidine character of the neighbouring nitrogen atoms, it was obvious that the triazole C-5 resembled the pyrrole character which reserved for the 1,4-regiosomers that are unsubstituted at this position.
To justify the connectivity and nature of the triazole isomer, additional heteronuclear correlations over multiple bonds could safely be performed ($^2J_{C,H}$ and $^3J_{C,H}$) and a gHMBC spectroscopy experiment was thus conducted. Using the data from the HSQC spectra, the $^2J_{C,H}$ correlations of CH$_2$-1" and the CH-5' triazole protons were then determined (see figure 3.6.4). The C-1" is connected directly to a nitrogen atom of the triazole and the aliphatic chain C-2" and as a result of such connectivity the $^2J_{C,H}$ for CH$_2$-1" only displayed the correlations with the C-2". On the other hand, the $^2J_{C,H}$ on the triazole H-5' displayed the cross-coupling to a C-4' at 144 ppm of the triazole. Therefore, further $^3J_{C,H}$ investigations on the CH$_2$-1" proton showed additional correlations between with an aliphatic chain C-3" and C-5' of the triazole ring which were definitive in concluding the absolute structure of the triazole-linked chloroquinoline compounds 6b – 6i as the 1,4 derivatives.
Figure 3.6.4 Multiple bond correlations (gHMBC) for $^1$H and $^{13}$C NMR spectra of compound 6b'.

As mentioned earlier, the ESI$^+$ HRMS spectra showed the protonated molecular ions ([M + H]$^+$; m/z) as dominant species. The base peak on the m/z was sometimes determined to be a fragment or an adduct depending on how heavily the compound was fragmented or made into an adduct during the ionisation process. The similar signal patterns of 1 Da and dominant 2 Da were usually observed as a result of isotopic abundances, but similar cluster patterns were also observed at lower m/z, mainly due to a fragmentation. For instance, the mass spectra of the 6g had 100% abundance molecular ion at m/z 407.1744, corresponding well to the theoretical m/z 407.1752. However, there was another m/z at 317.1293 with a similar cluster pattern as the molecular ion. The daughter peak was identified as a fragment resulting from secondary ionisation after molecular ion cleavage ion adjacent to the charged nitrogen atom as proposed in figure 3.6.5. These types of fragmentations, however, find a more important role when the elemental composition of compounds with unknown structure is of interest.
Finally, the use of IR spectroscopy was of limited use in the identification of the click products. For instance, in the IR spectra of the above compound, 6g, only a few dominant signals could be identified as peaks characteristic of functional groups found in the structure of this compound. These included the skeletal vibrations at frequencies (\textit{\text{\mu cm}^{-1}}) 3493 (N–H), 2970 (CH), 1740 (C=O), 1368 (C–N) which were the typical identification peaks that have already been discussed earlier.

With the 1,4-disubstituted chloroquinoline derivatives at hand, susceptibility of human malaria strain of \textit{P. falciparum} to these compounds was investigated \textit{in vitro}.

\textbf{3.7. Antimalarial activity}

Among the purposes of this study, it was necessary to assess the ability of the synthesized compounds’ antimalarial potency to inhibit \textit{P. falciparum} strains (chemical structures of the test compounds can be reviewed on the pages 29, 43 – 44). The antimalarial potency in this context was measured from the inhibition concentration of the test compounds that produced 50% reduction in maximum growth or survival of \textit{P. falciparum} cells in the culture media (IC\textsubscript{50}). Therefore, a comparison of the \textit{in vitro} activity of synthesized 6b – 6i with the reference drug CQ
and an artesunate standard was undertaken. The test compounds were evaluated on the asexual blood cycle stages, namely merozoites → trophozoites → schizonts that produce more merozoites of malaria parasitic infection. At this stage, the blood cells are invaded and the symptoms of malaria infection become evident in a person suffering from malaria infection as the cycle continues. These blood schizonts can be destroyed by drugs such as CQ to terminate a continuous infection that causes red blood cell invasion by the merozoites.

The human malaria parasites rely on the anaerobic carbohydrate metabolism, i.e. glycolysis, for the supply of glucose and the pLDH enzyme which is produced during the process has been used as an index of reduction in the parasite multiplication. The parasite lactate dehydrogenase (pLDH) levels have been used in this study to assess P. falciparum strain NF54 sensitivity as a marker of antimalarial activity.

The synthesized compounds exhibited notable antiplasmodial activity with few exceptions and the data for the test compounds are illustrated in figure 3.7.1. Among the 15 tested compounds, 11 exhibited potent antimalarial activity below the 250 nM concentration. The highest concentration beyond which the exposed parasites had no measurable inhibition in the blank was out of measurable range and it is not shown. In addition, the inhibition activity of compound 6h could not be measured under the concentration range investigated and a default IC₅₀ value was extrapolated from the highest test concentration. Compound 6e had a very low antiplasmodial activity but the complementary compounds 6e′ and 6h′ displayed good inhibition activities below 250 nM. The observation automatically made compounds 6e and 6h the qualified experimental outliers since the inhibition effect of the two compounds needed to produce 50% maximal inhibition effect was not certainly quantifiable under the concentration range investigated.
Figure 3.7.1 *In vitro* antiplasmodial activity of triazole-linked chloroquinoline derivatives 6b – 6i against NF54.

Compounds 6f and 6f′ are a related pair that had lower activity status with IC₅₀ values over 250 nM. These phenyl amine analogues, 6f and 6f′, with activity in the range 247 – 587 nM were clearly distinguished from the structurally related benzyl amines, 6g′ and 6g with activity range 32 – 163 nM. On some occasions the data for 6b – 6e, 6d′ and 6g′ compared closely to the standards; CQ, artesunate, and MMV 390048 (antimalarial drug in phase I clinical trials launched by the university of Cape Town), with the IC₅₀ values being below 100 nM. Apart from 6f and 6f′, the rest of aryl amine substituted compounds possessed inhibition activities below 250 nM while the aliphatic amines generally exhibited good inhibition activity below 100 nM, with the exception of the already mentioned outliers 6e and 6h. Compounds 6b and 6b′ were the aliphatic piperidinyl compounds with IC₅₀ values in the range of 27 – 42 nM. The activity unfortunately did not reveal conclusive information on the variation of carbon chain length. The influence of chain length on
the activity varied between the individual pairs, but on average the aliphatic amine derivatives bearing an ethyl spacer were more active (6b – 6d') than their counterparts with a propyl spacer. On the contrary, the activity order was reversed in the series of aryl amines, the compounds with a propyl spacer demonstrably being more active (6f – 6h').

Studies by Krogstad and co-workers, on the impact of the length of aliphatic chains in antimalarial activity have demonstrated that changes in the length have minimal impact on improving the activity in CQ sensitive strains of *P. falciparum*. These observations are, however, in agreement with our results although the impact cannot be directly linked to the change in chain length as the triazole ring is also an important variable concerning the observed activities of the compounds. However it is tempting to suggest that the modifications, either chain length or incorporation of a triazole ring, perhaps constitute a primary recognition motif for the undiminished antimalarial activity of the compounds. The findings are valuable because homologation in the side chain alone alters the lipophilic character and thus is expected to affect the spatial relationship of functional groups in the molecule, and thereby influencing activity.

Other studies, from the literature, on CQ resistance reversal activity have provided substantial insights into the function of the lateral chain amine. The essential features such as hydrogen bond acceptor capabilities, lipophilicity, and tertiary nitrogen atoms have been found to be optimal for activity in some CQ analogues. Therefore the distal amine functionalities have also been associated with CQ reversal activity and on such grounds the SAR profiles of the triazole-linked CQ derivatives will have to be substantiated with the CQ resistant strains assays which are currently in progress. As aforementioned, asexual recombination of malaria parasites plays a crucial role in the development of treatment-resistive *P. falciparum* malaria. Thus the current inhibition activity results serve as a valuable prognostic indicator of the application of compounds containing a triazole linker in the restoration of antimalarial efficacy of CQ analogues.
Chapter 4

Summary, conclusion and recommendations
4.1 Summary and conclusion

The primary objectives of this work were successfully achieved. Synthesis of the chloroquinoline amino alkyne precursor 1 was successfully accomplished in a moderate yield of 63%. The azide building blocks were then synthesized in various yields (34 – 73%) and were obtained using different reaction routes as described in the dissertation. The triazole-linked chloroquinoline derivatives were then synthesized from chloroquinoline amino alkyne 1 and various aminoalkyl azides building blocks using a modular CuAAC reaction to obtain the compounds 6b – 6i within 30 – 90 minutes at room temperature, in moderate to good yields (53 – 85%). The characterisation and structural elucidation of the novel compounds was carried out using inter alia $^1$H NMR, $^{13}$C NMR, and ESI$^+$ HRMS spectroscopic methods supported by the IR spectra. The antiplasmodial activity of the synthesized compounds was lastly investigated on the asexual blood stages of *P. falciparum* strain NF54. The *in vitro* biological investigations have showed the potential of a good antiplasmodial activity in some compounds which compared closely in activity to the standard antimalarial drugs.

In conclusion, a library of 15 4-aminoquinoline compounds, linked to a variety lipophilic aliphatic amino side chains by a 1,4-disubstituted 1,2,3-triazole has been created using the versatile CuAAC reaction. The compounds were synthesized in moderate to good yields and the *in vitro* antiplasmodial activity of the compounds is promising. The overall results have demonstrated that triazole-linked CQ analogues are potential inhibitors of *P. falciparum* with undiminished antimalarial activity. As there was more than one variable in the design of our compounds, including the introduction of a triazole linker as well as variation in chain lengths, the observed activity is attributable to the overall contribution of these modifications. Therefore, the results reported herein should be useful in guiding future efforts to discover CQ analogues with increased antimalarial activity. Consequently, the reported compounds are currently undergoing the *in vitro* antiplasmodial investigations.
4.2 Recommendations

Despite moderate to good synthetic results of the compounds, it has been observed that the use of excessive amounts of Cu(I) in the catalysis of amine bearing compounds can be disadvantageous even though it provides shorter reaction times and quick generation of compounds. Therefore, such syntheses should in future, be investigated using different Cu(I) sources at substoichiometric quantities. It has been shown that the triazole-linked chloroquinoline derivatives posses the antimalarial activity which justifies the compounds to be recognised as antimalarial agents.

In the recent literature, the investigations concentrating on the mode of action of CQ analogues and new targets for these compounds are evident. In terms of the latter research, these novel targets include proteases that are involved in haemoglobin degradation. \(^{33,135}\) These findings necessitate further investigations as to whether there is an additional role that the triazole ring can play to potentially affect the antimalarial activity when used as a linker to various haematin complexing scaffolds. The inhibition of haemozoin formation is likely the mechanism by which the synthesized compounds exerted their antimalarial activity. However, a β-haematin inhibition assay which must reveal correlations between parasite inhibition and haemozoin formation is necessary to determine the mode of the antimalarial activity. \(^{136}\) This should further serve in the SAR evaluation of the generated triazole-linked aminoquinoline derivatives in development on novel antimalarial compounds.
Chapter 5

Experimental data; chemical synthesis and *in vitro* biological investigations
5.1 General methods

Unless otherwise stated, all the reactions were carried out under inert nitrogen atmosphere in dried solvents using conventional distillation methods.

The $^1$H, $^{13}$C, COSY, gHMBC, and gHSQCAD NMR spectra were recorded on Varian Unity Inova spectrometers with $^1$H frequency of 400MHz or 300 MHz, and $^{13}$C frequency of 75 MHz or 150 MHz. All spectra were obtained on a 5mm dual broad band PFG probe with a probe temperature of 25 °C. The chemical shifts (δ) are reported in ppm relative to internal solvent peaks and the coupling constants (J) were measured in Hertz. The $^1$H signals splitting pattern abbreviation were as follows: singlet (s), (doublet), triplet (t), quartet (q), quintet (p) doublet of doublet (dd), triplet of a doublet (td),multiplet (m), and a broadened signal was prefixed (br).

The fingerprint identification of specific functional groups was achieved by assigning of group frequencies (cm$^{-1}$) of particular peaks within the IR spectra. The IR spectra were obtained from Nexus infra-red Pc spectrometer equipped with a DTGS KBr detector and the percentage (%) transmittance was recorded against the wave length (υ/cm$^{-1}$) for the reported signals. The high resolution mass spectra were recorded on electrospray ionisation on positive mode (ESI$^+$) using a quadrupole MALDI-TOF LC/MS instrument.

Preparative column chromatography was carried out on a silica gel 60; 70 – 230 mesh ASTM, Fluka. Thin layer chromatography (TLC) was performed using pre-coated aluminium silica gel plates (60F$_{254}$ Merck) and the developed TLCs for the compounds were viewed after staining or under UV (254nm) light. Aliphatic mesylate intermediates were detected by means of KMNO$_4$ stain and their corresponding azides were visualized after a contrast Ruhemann’s purple colour was developed with a ninhydrin stain following a TLC azide to amine reduction.$^{121}$

Finally, the melting points were determined on a variable heat Gallenkamp melting point apparatus (temperature range 20 – 350 °C) equipped with a laboratory thermometer, and are uncorrected.
5.2. Chemical syntheses

5.2.0.1 7-Chloro-N-(prop-2-yn-1-yl)quinolin-4-amine (I).

\[
\begin{align*}
\text{HN} & \quad \text{CH} \\
\text{Cl} & \quad \text{C} \\
\text{N} & \quad \text{C}
\end{align*}
\]

In a Schlenk reaction tube, a solution of 4,7-dichloroquinoline (500 mg, 2.52 mmol) and a catalytic amount of anhydrous pTSA (43 mg, 0.25 mmol, 10 mol %) in 1,4-dioxane (0.5 mL) was stirred at 50 °C for 30 minutes. To the solution, propargyl amine (0.35 mL, 5.1 mmol) was added and the Schlenk tube was sealed with a stopper; the reaction was then stirred for 24 hours at 85 °C. After cooling to a room temperature, 10% aqueous NaHCO₃ (20 mL) was added and the product was extracted into DCM (3 × 20 mL). The extract was dried with MgSO₄ and the organic solvent was evaporated under vacuum and the crude product was purified through a silica gel column (Hex/EtOAc; 6:4) to obtain compound I (344 mg, 63% yield) as an off-white to a pale yellow solid (mp: 186 – 190 °C).

IR \nu_{\text{max}} \ 3461 (\text{NH}), 3089 (\text{Ar CH}), 2947 & 2872 (\text{aliphatic CH}), 2161 (\text{C=O}), 1573 (\text{C=C}); \ ^1H NMR (300 MHz, DMSO) \delta 8.49 (d, J = 5.4 Hz, 1H), 8.19 (d, J = 9.0 Hz, 1H), 7.83 (d, J = 2.2 Hz, 1H), 7.76 (t, J = 5.7 Hz, 1H), 7.49 (dd, J = 9.0, 2.3 Hz, 1H), 6.59 (d, J = 5.4 Hz, 1H), 4.14 (dd, J = 5.7, 2.3 Hz, 2H), 3.19 (t, J = 2.3 Hz, 1H); \ ^13C NMR (75 MHz, DMSO) \delta 152.3, 149.7, 149.4, 134.0, 128.0, 125.0, 124.3, 118.0, 100.3, 80.9, 74.2, 32.0; MS (ESI⁺) 217.0533 calculated for C_{12}H_{9}ClN_{2} [M + H]^+, found 217.0529 (-1.84 ppm).

5.2.0.2 Ethane-1,2-diyl dimethanesulfonate (2a).

\[
\begin{align*}
\text{SO} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O}
\end{align*}
\]

Compounds 2a and 2a⁺ were prepared by the same method; a representative procedure for the synthesis 2a, carried out according a literature procedure,\textsuperscript{119} using THF as a solvent is described below. In the round-bottomed flask placed in water was added a THF solvent (200 mL), ethane-
1,2-diol (4.50 mL, 0.08 mol) and Et₂N (34.0 mL, 0.24 mmol). After stirring the solution for about 5 minutes, mesyl chloride (15.0 mL, 193.9 mmol) was added in a drop-wise manner via a syringe for about 10 minutes. The reaction was stirred for 1 h at 0 °C then warmed to room temperature under continuous stirring for another 3 h. The white precipitate removed by filtration and excess THF solvent was evaporated in vacuo to obtain a pale yellow oil. The oil was purified on a silica gel column using 20% ethyl acetate in hexane to afford 2a as colourless oil (16.5 g, 94% yield) that slowly solidified to an off-white solid after drying. The ¹H NMR and ¹³C NMR spectroscopic data for 2a was found to match cited literature values as shown below.¹¹⁹

IR \( \nu_{\text{max}} \) 2961 – 2789 (CH \text{ stretch}), 1352 (CH \text{ bend}), 1173 (S=O), 1045 (S–O); ¹H NMR (400 MHz, CDCl₃) \( \delta \) 4.47 (s, 4H), 3.08 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) \( \delta \) 66.6, 36.8.

5.2.0.3 Propane-1,3-diy dimethanesulfonate (2a').

![Propane-1,3-diy dimethanesulfonate (2a')]() Compound 2a' was obtained as a colourless oil (14.7 g, 99% yield) prepared from propane-1,3-diol and the spectroscopic data was found to be consistent with the literature.¹²⁰

IR \( \nu_{\text{max}} \) 3019 – 2944 (CH), 1359 (CH), 1176 (S–O), 1048 (S–O), 922, 743 (CH rocking); ¹H NMR (300 MHz, CDCl₃) \( \delta \) 4.33 (t, \( J = 7.2 \) Hz, 4H), 3.04 (s, 6H), 2.02 (p, \( J = 7.6, 4.5 \) Hz, 2H); ¹³C NMR (101 MHz, CDCl₃) \( \delta \) 66.8, 37.5, 28.9.

5.2.0.4 2-Azidoethyl methane sulfonate (3a).

![2-Azidoethyl methane sulfonate (3a)()]() As azidation reactions of both compounds 3a and 3a' were similar, a representative literature procedure is described here.¹¹⁹ Compound 2a (10.0 g, 0.05 mol) was dissolved in MeCN (200 mL), sodium azide (3.6 g, 0.06 mol) was added and the reaction mixture was heated at 80 °C for 12 h. The white precipitated was removed by filtration, excess solvent was evaporated in vacuo and the crude product was purified on a silica gel column using 30% ethyl acetate in hexane as
eluant to give 3a (3.18 g, 42% yield) as a colourless oil. The NMR spectroscopic data, referred to the cited literature.\textsuperscript{119}

IR $\nu_{\text{max}}$ 2963 – 2784 (CH), 2136 (N=\(N\)), 1347 (C–N), 1163 (S–O), 917 (S–O); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.28 (t, $J = 7.7$ Hz, 2H), 3.53 (t, $J = 6.5$ Hz, 2H), 3.02 (s, 1H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 66.4, 48.9, 36.8.

5.2.0.5 3-Azidopropyl methane sulfonate (3a').

\[
\begin{tabular}{c}
\text{O} \\
\text{N$_3$} \\
\end{tabular}
\]

A colourless oil of 3a' (5.00 g, 46% yield) was obtained from 2a' at 14 g scale. The $^1$H NMR spectroscopic data was analogous to the literature data.\textsuperscript{122}

IR $\nu_{\text{max}}$ 3029 – 2938 (CH), 2136 (N=\(N\)), 1347 (C–N), 1163 (S–O), 967 (S–O), 915, 780; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.34 (t, $J = 6.0$ Hz, 2H), 3.50 (t, $J = 6.5$ Hz, 2H), 3.05 (s, 3H), 2.02 (p, $J = 6.4$ Hz, 2H); $^{13}$C NMR (75MHz, CDCl$_3$) $\delta$ 66.5, 47.4, 37.4, 28.7.

5.2.0.6 1-(2-Azidoethyl)piperidine (4b).

\[
\begin{tabular}{c}
\text{N$_3$} \\
\text{N} \\
\end{tabular}
\]

Compounds 4b – 4e' were synthesized using a similar method, with the exception of 4c and 4c', obtained from literature\textsuperscript{119} and a representative procedure for compound 4b is described in detail below. The compounds were prepared from azido mesylates 3a and 3a' in excess amounts of 500 mg with minor modifications. To a stirred solution of 3a (500 mg, 3.03 mmol) and K$_2$CO$_3$ (1.25 mg, 9.09 mmol) in MeCN (15 mL) was added piperidine (0.25 mL, 2.55 mmol). The resulting mixture was heated at reflux overnight, the K$_2$CO$_3$ was removed by filtration, and excess solvent was removed by evaporating in vacuo to obtain a crude product. A colourless oil of 4b (282 mg, 72% yield) was obtained after purification by silica gel chromatography using 70% EtOAc/Hex as eluant. The $^1$H MNR data for 4b was found to be in good agreement with the data reported by Benalil et al.\textsuperscript{137}
IR $\nu_{\text{max}}$ 2943 – 2810 (CH), 2096 (N≡N), 1451, 1281 (C–N), 1115; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 3.34 (t, $J = 6.3$ Hz, 2H), 2.55 (t, $J = 6.3$ Hz, 2H), 2.43 (br t, $J = 6.2$ Hz, 4H), 1.57 (p, $J = 6.2$ Hz, 4H), 1.43 (ddd, $J = 9.0$, 6.2, 2.2 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 57.8, 54.6, 48.4, 25.7, 24.3.

5.2.0.7 1-(3-Azidopropyl)piperidine (4b').

![Image](image_url)

Compound 4b' was obtained as a pale yellow oil (273 mg, 70% yield) using the method already described for 4b. The NMR data was found to match the cited literature.

IR $\nu_{\text{max}}$ 2933 – 2799 (CH), 2093 (N≡N), 1445, 1278 (C–N), 1123; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 3.33 (t, $J = 6.8$ Hz, 2H), 2.37 (td, $J = 7.9$, 6.7 Hz, 6H), 1.79 (p, $J = 6.9$ Hz, 2H), 1.59 (td, $J = 10.8$, 5.6 Hz, 4H), 1.41 (dd, $J = 12.2$, 7.2 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 56.2, 54.6, 49.8, 26.4, 26.0, 24.4.

5.2.0.8 1-(2-Azidoethyl)pyrrolidine (4c).

![Image](image_url)

Compounds 4c and 4c' were synthesized by the same method the corresponding azido mesylates 3a and 4a' by adapting a literature method as follows: to a stirred solution of methane sulfonate 3a (500 mg, 3.03 mmol) in THF (15 mL) were added K$_2$CO$_3$ (1250 mg, 9.09 mmol) and pyrrolidine (0.21 mL, 2.53 mmol) at 0°C. The resulting mixture was allowed to warm to room temperature and then heated at reflux overnight. The reaction mixture was diluted with DCM (15 mL), the K$_2$CO$_3$ was removed by filtration and the excess organic solvent was evaporated to obtain a crude product that was purified by silica gel column using 50% EtOAc/Hex to afford 4c as a pale yellow oil (204 mg, 64%). The NMR data for 4c was consistent with the same compound reported in the literature.
IR \( \nu_{\text{max}} \) 2933 (CH), 2093 (N\equiv N), 1278 (C–N); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 3.38 (t, \( J = 6.4 \) Hz, 2H), 2.67 (t, \( J = 6.4 \) Hz, 2H), 2.56 – 2.52 (m, 4H), 1.79 (td, \( J = 6.4, 3.1 \), 4H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 54.9, 54.2, 50.2, 23.5.

5.2.0.9 \( \text{1-(3-Azidopropyl)pyrrolidine (4c’).} \)

\[
\text{N} \quad \text{N}_3
\]

Compound 4c’ was prepared using the experimental procedure already described above to afford a pale yellow oil (261 mg, 73\% yield).

Yield 73\%, IR \( \nu_{\text{max}} \) 2961 – 2789 (CH), 2093 (N\equiv N), 1456, 1276 (C–N), 1147; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 3.36 (t, \( J = 6.8 \) Hz, 2H), 2.57 – 2.45 (m, 6H), 1.87 – 1.72 (m, 6H); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \( \delta \) 54.2, 53.3, 49.7, 28.4, 23.4; MS (ESI\(^+\)) 155.1297 calculated for C\(_7\)H\(_{14}\)N\(_4\) [M + H]\(^+\), found 155.1297 (100\%, 0.00 ppm), 84.0182 (40\%).

5.2.1.0 \( \text{2-Azido-N,N-diethylenamine (4d).} \)

\[
\text{N} \quad \text{N}_3
\]

Compound 4d was synthesized from 3a using the same methods already described for 4b to obtain a colourless oil (189 mg, 48\%). The \(^1\)H NMR spectra was found to be similar with one reported in the literature.

IR \( \nu_{\text{max}} \) 2947 – 2791 (CH), 2092 (N\equiv N), 1453, 1253 (C–N); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 3.29 (t, \( J = 6.3 \) Hz, 2H), 2.66 (t, \( J = 7.4 \) Hz, 2H), 2.57 (q, \( J = 6.1 \) Hz, 4H), 1.04 (t, \( J = 7.1 \) Hz, 6H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 52.1, 49.5, 47.2, 11.8.
5.2.1.1 3-Azido-N,N-diethylpropan-1-amine (4d').

\[
\begin{array}{c}
\text{N} \\
\text{N}_3
\end{array}
\]

Compound 4d' was prepared as in 4d to afford a colourless oil (129 mg, 36% yield).

IR \(\nu_{max}\) 2942 (CH), 2097 (N\(\equiv\)N), 1343 (C–N), 1169; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 3.34 (t, \(J = 6.8\) Hz, 2H), 2.52 (ddd, \(J = 8.0, 7.3, 5.0\) Hz, 6H), 1.73 (p, \(J = 6.8\) Hz, 2H), 1.03 (t, \(J = 7.3\) Hz, 6H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 49.8, 46.8, 46.8, 31.8, 29.3, 11.5; MS (ESI\(^+\)) 157.1454 calculated for \(\text{C}_7\text{H}_{16}\text{N}_4\)[M + H]\(^+\), found 157.1446 (-5.09 ppm).

5.2.1.2 4-(2-Azidoethyl)morpholine (4e).

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{N}_3
\end{array}
\]

Compound 4e was also prepared similarly to 4d and the product was obtained as a colourless oil (271 mg, 69% yield).

IR \(\nu_{max}\) 2933 – 2789 (CH), 2093(N\(\equiv\)N), 1276 (C–N), 1147; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 3.35 (t, \(J = 4.8\) Hz, 4H), 3.35 (t, \(J = 6.4\) Hz, 2H), 2.60 (t, \(J = 6.0\) Hz, 2H), 2.51 (t, \(J = 4.8\) Hz, 2H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 66.9, 57.6, 53.6, 47.9.

5.2.1.3 4-(3-Azidopropyl)morpholine (4e').

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{N}_3
\end{array}
\]

Compound 4e' was prepared in a similar fashion to 4d to recover a product as a colourless oil (268 mg, 68% yield).\(^{141}\)

IR \(\nu_{max}\) 2947 – 2810 (CH), 2096 (N\(\equiv\)N), 1451, 1281 (C–N), 1115, 1069 (COC); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 3.71 (t, \(J = 4.5\) Hz, 4H), 3.35 (t, \(J = 6.7\) Hz, 2H), 2.50 – 2.32 (m, 6H), 1.77 (p, \(J = 6.0\) Hz, 2H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 66.9, 55.6, 53.6, 47.5, 25.9.
5.2.1.4 General procedure for alkylation of N-(benzyl or phenyl)amine (4f – 4h').

Compounds 4f – 4h' were prepared from 3a and 3a' by as described in the following procedure. To a solution of 3a (500 mg, 3.03 mmol) and K₂CO₃ (1250 mg, 9.09 mmol) in MeCN (15 mL) were added KI (38.2 mg, 0.20 mmol) and an appropriate aryl amine (2.02 mmol). The resulting mixture was continuously stirred at 90 °C until TLC displayed complete consumption of the starting material (28 h for benzylamines and 72 h for phenylamine). The K₂CO₃ was removed by filtration, after which the solvent was evaporated in vacuo, the crude was rinsed with 10% Na₂S₂O₄ (20 mL) and then extracted into DCM (3 × 20 mL). The extract was dried with MgSO₄ and the solvent was removed under vacuum to obtain a crude product that was purified on a silica gel column using hexane to afford the products described below.

5.2.1.5 N-(2-Azidoethyl)-N-methylaniline (4f).

A pale yellow oil (121 mg, 34% yield).

IR νmax 2947 – 2791 (aliphatic CH), 2092 (N=N), 1741 (C=C), 1453, 1253 (C–N), 1141;¹ H NMR (300 MHz, CDCl₃) δ 7.33 – 7.23 (m, 2H), 6.81 – 6.71 (m, 3H), 3.57 (t, J = 5.7 Hz, 2H), 3.47 (t, J = 5.7 Hz, 2H), 3.03 (s, 3H);¹³ C NMR (75 MHz, CDCl₃) δ 148.9, 129.3, 116.3, 111.9, 52.0, 49.8, 38.7, 29.7; MS (ESI⁺) 177.1142 calculated for C₉H₁₂N₄ [M + H]⁺, found 177.1142 (0.00 ppm).

5.2.1.6 N-(3-Azidopropyl)-N-methylaniline (4f').

A pale yellow oil (131 mg, 37% yield).
IR \( \nu_{\text{max}} \) 3140 – 3056 (Ar CH), 2945 – 2875 (aliphatic CH), 2092 (N=N), 1588 (C=C), 1492, 1243 (C–N); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.32 – 7.21 (m, 2H), 6.79 – 6.71 (m, 3H), 3.45 (t, \( J = 6.5 \) Hz, 2H), 3.40 (t, \( J = 6.5 \) Hz, 2H), 2.97 (s, 3H), 1.89 (p, \( J = 6.6 \) Hz, 2H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 149.3, 129.3, 116.5, 112.2, 49.7, 49.8, 49.0, 38.8, 26.3; MS (ESI\(^+\)) 191.1294 calculated for C\(_{10}\)H\(_{14}\)N\(_4\) [M + H]\(^+\), found 191.1294 (-1.57 ppm).

### 5.2.1.7 2-Azido-N-benzyl-N-methylethanamine (4g).

![Structure 4g](image1)

A colourless oil (157 mg, 41% yield).

IR \( \nu_{\text{max}} \) 2941 – 2791 (aliphatic CH), 2092 (N=N), 1588 (C=C), 1453, 1253 (C–N), 735; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.41 – 7.23 (m, 5H), 3.59 (s, 2H), 3.36 (t, \( J = 6.1 \) Hz, 2H), 2.67 (t, \( J = 6.1 \) Hz, 2H), 2.29 (s, 3H); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \( \delta \) 138.7, 128.8, 128.3, 127.1, 62.5, 56.2, 48.9, 42.2; MS (ESI\(^+\)) 191.1297 calculated for C\(_{10}\)H\(_{14}\)N\(_4\) [M + H]\(^+\), found 191.1300 (1.57 ppm).

### 5.2.1.8 3-Azido-N-benzyl-N-methylpropan-1-amine (4g').

![Structure 4g'](image2)

A colourless oil (178 mg, 47% yield).

IR \( \nu_{\text{max}} \) 3096 – 3059 (Ar CH), 2937 – 2873 (aliphatic CH), 2090 (N=N), 1598 and 1503 (C=C), 1366, 1258 (C–N); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 7.38 – 7.22 (m, 5H), 3.51 (s, 2H), 3.37 (t, \( J = 6.9 \) Hz, 2H), 2.48 (t, \( J = 6.9 \) Hz, 2H), 2.21 (s, 3H), 1.80 (p, \( J = 6.9 \) Hz, 2H); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \( \delta \) 139.0, 128.9, 128.2, 126.9, 62.4, 54.2, 49.5, 42.0, 25.5; MS (ESI\(^+\)) 205.1454 calculated for C\(_{11}\)H\(_{16}\)N\(_4\) [M + H]\(^+\), found 205.1457 (1.46 ppm).
5.2.1.9 2-Azido-N,N-dibenzylethamine (4h).

![Molecular structure of 2-Azido-N,N-dibenzylethamine (4h).]

A colourless oil (327 mg, 61% yield) that slowly solidified into an off-white solid at room temperature.

IR $\nu_{\text{max}}$ 3021 (Ar CH), 2947 – 2796 (aliphatic CH), 2089 (N=N), 1493 (C=C), 1449, 1294 (C–N); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.47 – 7.25 (m, 10H), 3.67 (s, 4H), 3.28 (t, $J$ = 6.1 Hz, 2H), 2.74 (t, $J$ = 6.1 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 139.02, 128.8, 128.4, 127.1, 58.9, 53.0, 49.3; MS (ESI$^+$) 267.1610 calculated for C$_{16}$H$_{18}$N$_4$ [M + H]$^+$, found 267.1610 (0.00 ppm).

5.2.2.0 3-Azido-N,N-dibenzylpropan-1-amine (4h').

![Molecular structure of 3-Azido-N,N-dibenzylpropan-1-amine (4h').]

A pale yellow oil (296 mg, 57% yield).

IR $\nu_{\text{max}}$ 3021 (Ar CH), 2947 – 2796 (aliphatic CH), 2093 (N=N), 1490 (C=C), 1449, 1294 (C–N); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.37 – 7.22 (m, 10H), 3.56 (s, 4H), 3.29 (t, $J$ = 7.0 Hz, 2H), 2.51 (t, $J$ = 6.8 Hz, 2H), 1.75 (p, $J$ = 6.9 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 138.6, 128.9, 128.3, 127.1, 62.6, 56.2, 48.9, 42.2; MS (ESI$^+$) 281.1767 calculated for C$_{17}$H$_{20}$N$_4$ [M + H]$^+$, found 281.1775 (2.85 ppm).
5.2.2.1 Synthesis of 5-(3-Chloropropyl)-10,11-dihydro-5H-dibenzo[b,f]azepine (5a').

Compound 5a' was synthesized by adapting a procedure from the literature methods as described.\textsuperscript{126-127} In a Schlenk reaction tube, 10,11-dihydro-5H-dibenzo[b,f]azepine (252 mg, 1.29 mmol) was dissolved in C\textsubscript{6}H\textsubscript{6}-THF (1:4, 10 mL) to which was added nBuLi (0.76 mL, 1.20 mmol) in a drop-wise fashion under a constant stirring for 30 minutes at 0 °C. The reaction was allowed to warm to room temperature after which 1-bromo-3-chloropropane (0.160 mL, 55 mmol) was added and stirring was continued for 4 hours. The resultant light brown solution was concentrated \textit{in vacuo} and the crude product was purified on a silica gel column using hexane eluant to isolate a mixture of 5a and 5a' with the amounts described below.

\textit{5-allyl-10,11-dihydro-5H-dibenzo[b,f]azepine (5a).}

\begin{center}
\includegraphics[width=0.2\textwidth]{fig}
\end{center}

A colourless oil (70 mg, 23 % yield).

IR \(\nu_{\text{max}}\) 3058 – 3022 (Ar CH), 2917 – 2842 (aliphatic CH), 1588 and 1485 (C=C), 1448, 1333 and 1227 (C–N), 1112, 742; \(^1\)H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 7.21 – 7.03 (m, 6H), 7.00 – 6.89 (m, 2H), 5.82 (ddt, \(J = 17.3, 10.3, 5.7\) Hz, 1H), 5.28 (dq, \(J = 17.3, 1.6\) Hz, 1H), 5.12 (dq, \(J = 10.3, 1.5\) Hz, 1H), 4.43 (dt, \(J = 5.7, 1.6\) Hz, 2H), 3.20 (s, 4H); \(^{13}\)C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta\) 148.0, 135.6, 134.1, 129.7, 126.2, 122.4, 120.4, 117.1, 54.4, 32.4.
5-(3-chloropropyl)-10,11-dihydro-5H-dibenzo[b,f]azepine (5a').

![Chemical structure of 5a']

A pale yellow solid (84 mg, 24 % yield).

Melting point: 43 – 45 °C; IR ν\textsubscript{max} 3056 (Ar CH), 2953 (aliphatic CH), 1587 (C=C), 1492, 1364, 1265 and 1244 (C–N), 747, 693 (C–Cl) \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.25 – 7.05 (m, 6H), 7.01 – 6.94 (m, 2H), 3.94 (t, J = 6.5 Hz, 2H), 3.60 (t, J = 6.5 Hz, 2H), 3.20 (s, 4H), 2.08 (p, J = 6.4 Hz, 2H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ 147.0, 133.3, 128.9, 125.4, 121.7, 118.8, 46.5, 41.9, 31.1, 29.6; MS (ESI\textsuperscript{+}) 272.1207 calculated for C\textsubscript{17}H\textsubscript{18}ClN [M + H]\textsuperscript{+}, found 272.1215 (-2.94 ppm).

5.2.2 5-(3-Azidopropyl)-10,11-dihydro-5H-dibenzo[b,f]azepine (4i).

![Chemical structure of 4i]

Compound 5a' (70 mg, 0.26 mmol) was dissolved in C\textsubscript{6}H\textsubscript{6} (2.5 mL) and to this was transferred NaN\textsubscript{3} (25 mg, 0.39 mmol) in DMSO (2.5 mL). The reaction was then stirred at 100 °C until TLC showed a complete consumption of the starting material (48 h). The reaction mixture was then diluted with distilled water (15 mL) and the product was extracted into DCM (3 × 15 mL). The extract was dried with MgSO\textsubscript{4} and the organic solvent was evaporated \textit{in vacuo} and 4i (61%, 44 mg) was obtained as a pale yellow oil after silica gel column purification using hexane as eluant.

IR ν\textsubscript{max} 3057 (Ar CH); 2921 – 2843 (aliphatic CH), 2091 (N=N), 1592 (C=C), 1485, 1229 (C–N), 1112; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.23 – 7.06 (m, 6H), 7.02 – 6.92 (m, 2H), 3.86 (t, J = 6.7 Hz, 2H), 3.36 (t, J = 6.7 Hz, 2H), 3.19 (s, 4H), 1.89 (p, J = 6.6 Hz, 2H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ
148.0, 134.3, 130.0, 126.5, 122.8, 119.8, 49.6, 47.5, 32.2, 27.2; MS (ESI⁺) 279.1610 calculated for C₁₇H₁₈N₄ [M + H]⁺, found 279.1619 (3.22 ppm).

5.2.2.3 General procedure for CuAAC reactions of chloroquine derivatives (6b – 6i).

Coupling reactions were carried out using a literature procedure with minor modifications. In a 2.5 mL round-bottomed flask, 1 (20 mg, 0.09 mmol) and CuI (17 mg, 0.09 mmol) were dissolved in anhydrous THF (0.5 mL). To this was added appropriate alkyl azide (0.08 mmol) and the reaction was then stirred for 30 – 90 minutes at room temperature, until the starting material was consumed. The reaction mixture was diluted with DCM (2 mL), the CuI was removed by filtration, and the crude product was purified on a silica gel column under gradient elution with 50% EtOAc:Hex to 10% MeOH:Me₂CO as eluant. The product quantities, as well as the nature and description that were obtained are given below.

5.2.2.4 7-Chloro-N-((1-(2-(piperidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6b).

A light yellow solid (22 mg, 76% yield, mp: 132 – 134 °C).

IR νmax 3336 (NH), 3145 (Ar CH), 2925 – 2854 (aliphatic CH), 1576 (C=C), 1451, 1370 (C–N);
¹H NMR (400 MHz, CDCl₃) δ 8.53 (d, J = 5.2 Hz, 1H), 7.96 (d, J = 2.1 Hz, 1H), 7.77 (s, 1H), 7.74 (d, J = 9.0 Hz, 1H), 7.38 (dd, J = 6.8, 2.0 Hz, 1H), 6.52 (d, J = 5.4 Hz, 1H), 5.95 (br s, 1H), 4.63 (d, J = 4.1 Hz, 2H), 4.45 (t, J = 6.2 Hz, 2H), 2.75 (t, J = 6.2 Hz, 2H), 2.48 – 2.36 (m, 4H), 1.51 (td, J = 9.7, 4.3 Hz, 4H), 1.46 – 1.38 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 150.3, 148.4, 147.7, 143.0, 134.2, 127.4, 124.6, 121.2, 120.4, 116.2, 98.4, 54.0, 53.3, 47.3, 38.0, 26.1, 24.6; MS (ESI⁺) 371.1752 calculated for C₁₉H₂₃ClN₆ [M + H]⁺, found 371.1751 (~0.27 ppm).
5.2.2.5 7-Chloro-N-((1-(3-(piperidin-1-yl)propyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6b').

A light yellow solid (23 mg, 79% yield, mp: 133 – 135).

IR $\nu_{\text{max}}$ 3489 – 3390 (NH), 3145 (Ar CH), 2922 – 2854 (aliphatic CH), 1461 (C=C), 1260 (C–N), 1022; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.53 (d, $J = 5.4$ Hz, 1H), 7.94 (d, $J = 2.1$ Hz, 1H), 7.78 (d, $J = 9.0$ Hz, 1H), 7.57 (s, 1H), 7.33 (dd, $J = 8.9$, 2.1 Hz, 1H), 6.48 (d, $J = 5.4$ Hz, 1H), 6.08 (br s, 1H), 4.64 (d, $J = 3.6$ Hz, 2H), 4.42 (t, $J = 6.9$ Hz, 2H), 2.35 – 2.14 (m, 6H), 2.06 (p, $J = 6.6$ Hz, 2H), 1.58 – 1.47 (m, 4H), 1.41 (br d, $J = 5.1$ Hz, 2H overlapping); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.1, 149.6, 149.1, 144.0, 135.3, 128.7, 125.7, 122.4, 121.6, 117.5, 99.6, 55.3, 54.6, 48.6, 39.1, 27.6, 26.2, 24.5; MS (ESI$^+$) 385.1876 calculated for C$_{20}$H$_{25}$ClN$_6$ [M + H]$^+$, found 385.1895 (4.9 ppm).

5.2.2.6 7-Chloro-N-((1-(2-(pyrrolidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6c).

A light yellow solid (18 mg, 67% yield, mp: 117 – 119 °C).

IR $\nu_{\text{max}}$ 3452 (NH), 2925 – 2854 (aliphatic CH), 1576 (C=C), 1461, 1370 (C–N); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.52 (d, $J = 5.4$ Hz, 1H), 7.95 (d, $J = 2.1$ Hz, 1H), 7.81 (d, $J = 9.0$ Hz, 1H), 7.75 (s, 1H), 7.35 (dd, $J = 8.9$, 2.1 Hz, 1H), 6.54 (d, $J = 5.4$ Hz, 1H), 6.17 (br s, 1H), 4.65 (s, 2H), 4.50
(t, J = 6.4 Hz, 2H), 2.98 (t, J = 6.4 Hz, 2H), 2.60 – 2.51 (m, 4H), 1.78 (td, J = 6.4, 3.1 Hz, 4H);
\(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 151.4, 149.3, 148.2, 143.5, 135.4, 128.0, 125.8, 122.5, 121.7, 117.1, 99.5, 55.7, 54.1, 49.7, 39.0, 23.6; MS (ESI\(^+\)) 357.1595 calculated for C\(_{18}\)H\(_{21}\)ClN\(_6\) [M + H]\(^+\), found 357.1581 (-1.40 ppm).

5.2.2.7 7-Chloro-N-((1-(3-(pyrrolidin-1-yl)propyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6c').

A light yellow solid (18 mg, 63% yield, mp: 115 – 117 °C).

IR \(\nu_{max}\) 3390 (NH), 2925 – 2854 (aliphatic CH), 1576 (C=C), 1451, 1370 (C–N); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.54 (s, 1H), 7.97 (s, 1H), 7.80 (d, J = 9.0 Hz, 1H), 7.60 (s, 1H), 7.37 (dd, J = 8.9, 1.6 Hz, 1H), 6.51 (d, J = 5.2 Hz, 1H), 6.02 (br s, 1H), 4.65 (br s, 2H), 4.44 (t, J = 6.4 Hz, 2H), 2.98 (t, J = 6.4 Hz, 2H), 2.62 – 2.53 (m, 4H), 1.83 – 1.76 (m, 4H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 150.4, 148.3, 147.9, 142.8, 134.1, 127.6, 124.6, 121.0, 120.3, 116.2, 98.5, 52.9, 51.4, 47.4, 38.0, 28.7, 22.4; MS (ESI\(^+\)) 371.1752 calculated for C\(_{19}\)H\(_{23}\)ClN\(_6\) [M + H]\(^+\), found 371.1751 (-0.27 ppm).
5.2.2.8 7-Chloro-N-((1-(diethylamino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6d).

A light yellow solid (19 mg, 70% yield, mp: 83 – 85 °C).

IR \( \nu_{max} \) 3493 (NH), 3054 (Ar CH), 2976 (aliphatic CH), 1581 (C=C bend), 1495, 1365, 1265 (C–N), 747; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.52 (br s, 1H), 7.94 (s, 1H), 7.75 (d, \( J = 8.9 \) Hz, 1H), 7.70 (s, 1H), 7.34 (d, \( J = 9.0 \) Hz, 1H), 6.50 (d, \( J = 5.2 \) Hz, 1H), 5.95 (br s, 1H), 4.63 (s, 2H), 4.39 (t, \( J = 6.2 \) Hz, 2H), 2.85 (t, \( J = 6.1 \) Hz, 2H), 2.52 (q, \( J = 6.9 \)Hz, 4H), 0.92 (t, \( J = 7.1 \) Hz, 6H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 151.7, 149.4, 148.8, 143.2, 135.4, 128.4, 125.7, 122.6, 121.3, 117.3, 99.5, 52.9, 49.2, 47.3, 39.0, 11.8; MS (ESI\(^+\) ) 359.1752 calculated for C\(_{18}\)H\(_{23}\)ClN\(_6\) [M + H]\(^+\), found 359.1758 (-1.67 ppm).

5.2.2.9 7-Chloro-N-((1-(diethylamino)propyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6d').

A light yellow solid (17 mg, 61% yield, mp: 82 – 84 °C).

IR \( \nu_{max} \) 3493 (NH), 3080 (Ar CH 2970), 2922 – 2852 (aliphatic CH), 1513 (C=C bend), 1336, 1216 (C–N), 856, 748; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.55 (d, \( J = 5.4 \) Hz, 1H), 7.98 (d, \( J = 2.1 \) Hz, 1H), 7.79 (d, \( J = 9.0 \) Hz, 1H), 7.61 (s, 1H), 7.39 (dd, \( J = 8.9, 2.2 \) Hz, 1H), 6.52 (d, \( J = 5.4 \) Hz,
1H), 5.92 (br s, 1H), 4.66 (br s, 2H), 4.45 (t, J = 6.9 Hz, 2H), 2.55 (q, J = 7.2 Hz, 4H), 2.47 (t, J = 6.9 Hz, 2H), 2.11 (p, J = 6.8 Hz, 2H), 1.00 (t, J = 7.2 Hz, 6H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 152.1, 149.7, 149.5, 144.2, 135.6, 129.1, 126.2, 122.6, 121.8, 117.8, 100.2, 50.2, 49.4, 47.7, 40.1, 28.9, 12.5. MS (ESI$^+$) 373.1908 calculated for C$_{13}$H$_{23}$ClN$_6$ [M + H]$^+$, found 373.1904 (1.07 ppm).

5.2.3.0 7-Chloro-N-((1-(2-morpholinoethyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6e).

A light yellow solid (21 mg, 74% yield, mp: 180 – 182 °C).

IR $\nu_{max}$ 3485 (NH), 2921 – 2849 (aliphatic CH), 1581 (C=C), 1460, 1374, 1260 (C–N), 795; $^1$H NMR (300 MHz, CDCl$_3$) δ 8.53 (s, 1H), 7.95 (d, J = 2.0 Hz, 1H), 7.78 (d, J = 9.0 Hz, 1H), 7.71 (s, 1H), 7.32 (dd, J = 8.9, 2.0 Hz, 1H), 6.51 (d, J = 5.3 Hz, 1H), 6.15 (br s, 1H), 4.63 (d, J = 3.6 Hz, 2H), 4.46 (t, J = 6.9 Hz, 2H), 3.66 (br t, J = 4.8 Hz, 4H), 2.81 (br t, J = 4.6 Hz, 4H), 2.47 (t, J = 6.8 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 150.4, 148.5, 147.8, 142.9, 134.2, 127.2, 124.6, 121.5, 120.5, 116.3, 98.8, 65.9, 53.8, 52.5, 47.2, 37.9; MS (ESI$^+$) 373.1544 calculated for C$_{18}$H$_{21}$ClN$_6$O [M + H]$^+$, found 373.1537 (~1.88 ppm).
5.2.3.1  7-Chloro-N-((1-(3-morpholinopropyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6e).

A light yellow solid (25 mg, 85% yield, mp: 153 – 155 °C).

IR $\nu_{max}$ 3452 – 3317 (NH), 3032 (Ar CH), 2932 – 2794 (aliphatic CH), 1576 (C=C), 1450, 1366; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.53 (s, 1H), 7.97 (d, $J = 2.0$ Hz, 1H), 7.77 (d, $J = 9.0$ Hz, 1H), 7.55 (s, 1H), 7.37 (dd, $J = 8.9$, 2.0 Hz, 1H), 6.51 (d, $J = 5.3$ Hz, 1H), 5.97 (br s, 1H), 4.66 (d, $J = 3.6$ Hz, 2H), 4.45 (t, $J = 6.9$ Hz, 2H), 3.66 (br t, $J = 4.8$ Hz, 4H), 2.37 (br t, $J = 4.6$ Hz, 4H), 2.32 (t, $J = 6.8$ Hz, 2H overlapping), 2.08 (p, $J = 6.9$ Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 150.4, 148.5, 147.8, 142.9, 134.2, 127.2, 124.6, 121.5, 120.5, 116.3, 98.8, 65.9, 53.8, 52.5, 47.2, 37.9, 25.9; MS (ESI$^+$) 387.1701 calculated for C$_{19}$H$_{23}$ClN$_6$O [M + H]$^+$, found 387.1707 (1.55 ppm).

5.2.3.2  7-Chloro-N-((1-(2-(methyl(phenyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6f).

An off-white solid (19 mg, 64% yield, mp: 167 – 169 °C).

IR $\nu_{max}$ 3493 (NH), 2970 – 2759 (aliphatic CH), 1740 (C=C), 1368 and 1217 (C–N); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.54 (d, $J = 5.3$ Hz, 1H), 7.97 (d, $J = 2.1$ Hz, 1H), 7.78 (d, $J = 8.9$ Hz, 1H), 7.62 (s, 1H), 7.36 (dd, $J = 8.7$, 2.2 Hz, 1H), 7.26 – 7.12 (m, 5H), 6.53 (d, $J = 5.3$ Hz, 1H), 6.01 (s,
1H), 4.65 (d, J = 1.6 Hz, 2H), 4.45 (t, J = 6.1 Hz, 2H), 3.54 (s, 2H), 2.78 (t, J = 6.1 Hz, 2H), 2.29 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 151.5, 149.5, 148.5, 144.1, 135.1, 129.4, 128.8, 125.6, 122.8, 121.1, 117.6, 112.3, 99.5, 53.1, 48.0, 38.9, 38.6; MS (ESI$^+$) 407.1752 calculated for C$_{21}$H$_{21}$ClN$_6$ [M + H]$^+$, found 407.1744 (-1.96 ppm).

5.2.3.3 7-Chloro-N-((1-(3-(methyl(phenyl)amino)propyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6f').

![Molecule structure]

An off-white solid (17 mg, 53% yield, mp: 163 – 165 °C).

IR $\nu_{\text{max}}$ 3452 – 3317 (NH), 2932 – 2847 (aliphatic CH), 1576 (C=C), 1450, 1366, 1323, 1220 (C–N), 1138, 738; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.50 (d, J = 5.4 Hz, 1H), 7.94 (d, J = 2.1 Hz, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.47 (s, 1H), 7.33 (dd, J = 9.0, 2.1 Hz 1H), 7.19 (td, J = 8.4, 1.2 Hz, 2H), 6.71 (t, J = 7.3 Hz, 1H), 6.65 (d, J = 8.0 Hz, 2H), 6.49 (d, J = 5.4 Hz, 1H), 6.09 (br s, 1H) 4.63 (s, 2H), 4.38 (t, J = 7.0 Hz, 2H), 3.37 (t, J = 6.9 Hz, 2H), 2.89 (s, 3H), 2.21 (p, J = 7.0 Hz, 2H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 152.1, 150.3, 149.8, 149.2, 144.7, 136.0, 130.0, 128.9, 126.4, 122.5, 122.2, 117.8, 113.4, 100.2, 50.2, 49.0, 39.7, 39.3, 30.4; MS (ESI$^+$) 407.1752 calculated for C$_{22}$H$_{23}$ClN$_6$ [M + H]$^+$, found 407.1743 (-2.21 ppm).
5.2.3.4 7-Chloro-N-((1-(2-(benzyl(methyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)methyl) quinolin-4-amine (6g).

A light yellow solid (22 mg, 69% yield, mp: 133 – 135 °C).

IR \( \nu_{\text{max}} \) 3444 – 3317 (NH), 3144 (Ar CH), 2932 – 2847 (aliphatic CH), 1579 (C=C), 1448, 1367, 1220 (C–N), 736; \(^1H\) NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.54 (s, 1H), 7.97 (s, 1H), 7.78 (d, \( J = 8.9 \) Hz, 1H), 7.62 (s, 1H), 7.36 (d, \( J = 7.6 \) Hz, 1H), 7.26 – 7.12 (m, 5H), 6.53 (d, \( J = 5.2 \) Hz, 1H), 6.01 (s, 1H), 4.65 (d, \( J = 2.8 \) Hz, 2H), 4.45 (t, \( J = 6.1 \) Hz, 2H), 3.54 (s, 2H), 2.87 (t, \( J = 6.1 \) Hz, 2H), 2.29 (s, 3H); \(^13C\) NMR (75 MHz, CDCl\(_3\)) \( \delta \) 151.5, 149.5, 148.6, 143.6, 138.2, 135.23, 129.0, 128.8, 128.3, 127.3, 125.9, 122.3, 121.4, 117.2, 99.4, 62.4, 56.4, 48.4, 42.23, 39.02; MS (ESI\(^+\)) 407.1752 calculated for C\(_{22}\)H\(_{23}\)ClN\(_6\) [M + H]\(^+\), found 407.1744 (100%, -1.96 ppm), 317.1293 (30%).

5.2.3.5 7-Chloro-N-((1-(3-(benzyl(methyl)amino)propyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6g').

An off-white solid (21 mg, 65% yield, mp: 131 – 133 °C).

IR \( \nu_{\text{max}} \) 3444 – 3317 (NH), 2932 (aliphatic CH), 1579 (C=C), 1448, 1367, 1220 (C–N), 1137, 736; \(^1H\) NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.56 (s, 1H), 7.98 (s, 1H), 7.77 (d, \( J = 7.7 \) Hz, 1H), 7.42 – 7.18 (m, 7H), 6.50 (s, 1H), 5.95 (s, 1H), 4.58 (s, 2H), 4.43 (t, \( J = 6.8 \) Hz, 2H), 3.46 (s, 2H), 2.35 (t, \( J = 6.4 \) Hz, 2H), 2.22 (s, 3H), 2.07 (p, \( J = 6.5 \) Hz, 2H); \(^13C\) NMR (75 MHz, CDCl\(_3\)) \( \delta \) 151.5, 149.5, 148.6,
143.6, 138.2, 135.2, 129.1, 128.8, 128.3, 127.3, 125.7, 122.3, 121.4, 117.2, 99.4, 62.4, 56.4, 48.4, 42.2, 39.0, 29.7; MS (ESI+) 421.1908 calculated for C_{23}H_{25}ClN_{6} [M + H]^+; found 421.1905 (-0.71 ppm).

5.2.3.6 7-Chloro-N-((1-(2-(dibenzylamino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6h).

\[
\text{[Diagram of the molecule here]}
\]

A yellow solid (20 mg, 54% yield, mp: 115 – 118 °C).

IR \nu_{max} \ 3493 (NH), 3004 (Ar CH), 2970 (aliphatic CH), 1740 (C=O), 1368, 1217 (C–N), 1054; \n^{1}H NMR (300 MHz, CDCl_{3}) \delta 8.43 (s, 1H), 7.94 (s, 1H), 7.88 (d, J = 9.0 Hz, 1H), 7.41 – 7.17 (m, 11H), 6.89 (s, 1H), 6.50 (br s, 1H), 4.54 (s, 2H), 4.34 (t, J = 6.1 Hz, 2H), 3.58 (s, 6H), 2.46 (t, J = 6.3 Hz, 2H); \n^{13}C NMR (75 MHz, CDCl_{3}) \delta 151.3, 150.1, 149.2, 143.8, 139.8, 136.1, 129.8, 129.1, 128.8, 127.8, 126.4, 122.8, 122.1, 117.8, 99.4, 59.5, 50.4, 48.7, 39.5; MS (ESI^+) 483.2065 calc for C_{28}H_{27}ClN_{6} [M + H]^+, found 483.2063 (-0.41 ppm)
5.2.3.7 7-Chloro-N-((1-(3-(dibenzylamino)propyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6h').

A yellow solid (24 mg, 64% yield, mp: 114 – 115 °C).

IR $\nu_{\text{max}}$ 3484 (NH), 3206 (Ar CH), 2994 – 2931 (aliphatic CH), 1580 and 1543 (C=C), 1431, 1365, 775; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.55 (d, $J$ = 4.8 Hz, 1H), 7.98 (d, $J$ = 1.8 Hz, 1H), 7.76 (d, $J$ = 9.0 Hz, 1H), 7.39 – 7.22 (m, 11H), 6.79 (s, 1H), 6.47 (d, $J$ = 5.4 Hz, 1H), 5.96 (br s, 1H), 4.49 (d, $J$ = 2.3 Hz, 2H), 4.35 (t, $J$ = 6.8 Hz, 2H), 3.56 (s, 4H), 2.46 (t, $J$ = 6.2 Hz, 2H), 2.05 (p, $J$ = 6.3 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 151.9, 150.3, 149.2, 143.9, 140.1, 136.1, 129.8, 129.1, 128.8, 127.9, 126.4, 122.8, 122.2, 117.9, 100.0, 59.5, 50.4, 48.7, 39.5, 28.8; MS (ESI$^+$) 497.2221 calculated for C$_{29}$H$_{29}$ClN$_6$ [M + H]$^+$, found 497.2214 (37%, -1.41 ppm), 407.1749 (40%), 249.1146 (100%).

5.2.3.8 7-Chloro-N-((1-(2-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)quinolin-4-amine (6i).

A light brown solid (24 mg, 64% yield, mp: 67 – 69 °C).

IR $\nu_{\text{max}}$ 3484 – 3371 (NH), 3147 (Ar CH), 2927 and 2851 (aliphatic CH), 1576 and 1544 (C=C), 1258 (C –N), 1058, 767; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.50 (d, $J$ = 5.4 Hz, 1H), 7.97 (d, $J$ = 2.1
Hz, 1H), 7.74 (d, J = 9.0 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H overlapping), 7.35 (d, J = 2.0 Hz, 1H overlapping), 7.16 – 6.90 (m, 8H), 6.44 (d, J = 5.5 Hz, 1H), 4.57 (d, J = 3.1 Hz, 2H), 4.41 (t, J = 6.9 Hz, 2H), 3.77 (t, J = 6.4 Hz, 2H), 3.18 (s, 4H), 2.20 (p, J = 5.7 Hz, 2H);\textsuperscript{13}C NMR (75 MHz, CDCl$_3$) $\delta$ 151.1, 150.6, 147.6, 143.8, 135.4, 134.1, 130.1, 129.5, 128.0, 126.6, 125.7, 123.1, 121.9, 121.4, 119.7, 117.0, 99.3, 48.1, 46.9, 38.9, 32.1, 28.3; MS (ESI$^+$) 495.2065 calculated for C$_{29}$H$_{27}$ClN$_6$ [M + H]$^+$, found 495.2067 (0.40 ppm).

5.3 \textit{In vitro} antimalarial activity investigations.

The biological analysis of the test compounds was performed by the Professor Peter Smith in Division of Pharmacology, University of Cape Town. Reported, herein, was the only received data by the time of writing a thesis and additional data is expected once the ongoing tests have been finalised.

5.3.1 \textit{Quantitative assessment of antiplasmodial activity}.

The antiplasmodial activity of 6b – 6l were carried out on asexual erythrocyte stages of a continuously maintained culture of NF54, a CQ-sensitive strain of \textit{P. falciparum}, by a modified literature method of parasite lactate dehydrogenase assay (pLDH).\textsuperscript{131,133} Briefly, the compounds were prepared to a 20 mg/mL stock solution or a suspension in DMSO and sonicated to enhance the solubility. Serial dilutions of stock solutions were carried out in triplicate in a 96-well plate using a multichannel pipette and the compounds were tested on a concentration range of 1000 $\mu$g/mL and 0.2 ng/mL. CQ was used as a reference compound while DMSO solvent was a blank. Additional positive controls such as artesunate standard antimalarial drug and MMV390048 currently in the clinical trials were included with triazole-linked chloroquinoline derivatives test compounds. The 50\% inhibition concentration value of parasite growth (IC$_{50}$) for each compound was deduced from a non-linear full-dose response curve performed by Graph Pad Prism v.4.0 software. Data were collected using a microplate reader and analyzed as described above The IC$_{50}$ activity values are shown in \textbf{table 5.3.0.1}. 83
Table 5.3.1 Antiplasmodial activity of triazole-linked chloroquinoline derivatives against CQ-sensitive NF 54

<table>
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<th>Compound</th>
<th>Chemical Structure</th>
<th>$IC_{50}$ (nM) NF54</th>
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<tr>
<td>artesunate</td>
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</table>
$MMV390048^*$

* Standard antimalarial drugs
References

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(9) Roepe, D. P. Biochem. 2011, 50, 163.


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(131) Trager, W.; Jensen, J. B. Science 1976, 193, 673.


Supporting information

$^1$H NMR spectra
$^{13}$C NMR spectra
$6f'$
$^1\text{H} - ^1\text{H}$ Homonuclear correlations
$^1\text{H} - ^{13}\text{C}$ Heteronuclear correlations
(ESI$^+$) HR MS spectra
C\textsubscript{7}H\textsubscript{14}N\textsubscript{4}  

[\text{M - I}]^{+} = 155.1297
$C_7H_{16}N_4$

$[M + H]^+ = 157.1454$
$4f$

$C_9H_{12}N_4$

$[M+H]^+ = 177.1142$
$4^f'\quad$  
$C_{10}H_{14}N_4$  
$[M + H]^+ = 191.1297$
N₃

4g
C₁₀H₁₄N₄

[M + H]⁻ = 191.1297
$4g'$

$C_{11}H_{16}N_4$

$[M + H]^+ = 205.1454$
$C_{16}H_{18}N_4$

$[M + H]^+ = 267.1610$
$4h'$

$\text{C}_{17}\text{H}_{20}\text{N}_4$

$[M+H]^+ = 281.1767$
4i

C_{17}H_{18}N_{4}

[M+H] = 279.1610
$5a'$

$C_{15}H_{18}N_4$

$[M + H]^+ = 272.1207$
$C_{20}H_{25}ClN_8$

$[M + H]^+ = 385.1876$
\[ \text{C}_{15}\text{H}_{21}\text{ClN}_6 \]

\[ [M + H]^+ = 357.1595 \]
$6c'$

$C_{19}H_{23}ClN_6$

$[M+H]^+ = 371.1752$
$6d'$

$C_{19}H_{25}ClN_6$

$[M + H] = 573.1908$
\[ C_{18}H_{21}ClN_6O \]

\[[M+1]^+ = 373.1544 \]
C_{13}H_{23}ClN_{6}O

[M + H]^+ = 387.1701
$6f$

$C_{21}H_{21}ClN_6$

$[M + H]^+ = 393.1595$
C_{22}H_{23}ClN_6
\[ [M \text{ II}]^+ = 407.1743 \]
C\textsubscript{22}H\textsubscript{23}ClN\textsubscript{6}

\[\text{[M + H]}^+ = 407.1743\]
$6^7$

$C_{23}H_{23}ClN_6$

$[M + 1]^+ = 424.1908$
6h

C_{21}H_{27}ClN_{6}

[M+1]^+ = 483.2065
$6h'$

$C_{29}H_{29}ClN_8$

$[M + H] = 497.2221$
$6i$

$\text{C}_{29}\text{H}_{29}\text{ClN}_{6}$

$[M + H]^+ = 495.2065$
IR Spectra
N,N-diethyl azide

$\text{N}_3\text{CH}_{\text{2}}\text{CH}_{\text{2}}\text{N}$

$4d$