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Evaluation of Novel 4-Aminoquinolines for Treatment of Drug-Resistant Malaria

by

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DISSERTATION

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Acknowledgement

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Abstract

Evaluation of Novel 4-Aminoquinolines for Treatment of Drug-Resistant Malaria

Peter B. Madrid

Malaria is a devastating disease that kills over a million children every year. Resistance to many of the most potent and affordable drugs has led to a renewed need for new antimalarials therapeutics. The tools of combinatorial synthesis were used to create several libraries of quinoline compounds with diversity at various positions around the 4-aminoquinoline scaffold. The structure-activity relationships for both the quinoline ring and basic side chains were defined based on *in vitro* activity against multiple strains of *Plasmodium falciparum*. Substitutions around the quinoline ring did not lead to substantial gains in activity against the drug-resistant W2 parasite strain. Conversely, the addition of substituents to the terminal nitrogen in the basic side chain led to several compounds with potent activities against drug-resistant parasite strains. In particular, the addition of an aromatic group with a hydrogen-bond accepting functional group led to several compounds with IC_{50} values less than 10 nM against the drug-resistant W2 strain of *P. falciparum*. This series of potent compounds was found to have poor metabolic stability, but conversion of the tertiary amine side chain into an analogous secondary amine, led to a set of potent and metabolically stable lead compounds.

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Chapter 1

Introduction

History of Antimalarial Chemotherapy

Malaria is one of the most devastating diseases to ever affect mankind. The role of malaria in effecting important historical events cannot be understated. Many conquests and wars have been decided based on the effect of the microscopic protazoal parasite that causes malaria. It is impossible to retrospectively diagnose malaria with any certainty, but the unique symptoms of the cyclic malarial fever lead historians to believe that many of the illnesses described in historical texts were most likely malaria.[1, 2] The characteristic recurring fever that comes and goes on a three or four day cycle has been described in writings for centuries is now almost certainly believed to be malaria. In fact, malarial DNA has even been found in an unearthed body discovered in a fifth-century villa near Rome.[3] Evidence suggests that several historical figures of great consequences such as Alexander the Great and several Popes died prematurely due to malaria.[2, 3]

The devastating consequences of malaria led to a historically desperate search for a cure. Ancient Rome, center of the Catholic Church and one of the most malarious cities on the earth, was one early center of the search for malaria cures. Treatments based on religious and superstitious medicine including bloodlettings and bizarre rituals involving fruits and chanting were common.[4] Finally, after the deaths of several important church figures, Pope UrbanVIII helped support the development of a modern apothecary in Rome known as the Santo Spirito.[5] The director of the apothecary was Jesuit university professor, Juan de Lugo, who was the first to treat malaria patients the dried bark from the South American Cinchona tree. (Fig 1.1)

Figure 1.1 Package of cinchona bark.

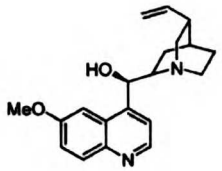
The Incas in Peru treated different ailments with preparations made from the local plants. Many of the colonial Jesuit priests became fascinated with the Inca medical treatments, especially Brother Augustino Salumbrino, who set up a major pharmacy in Lima in 1604.[4] Salumbrino learned from the Incas to prepare the bark from the Cinchona trees that was used to treat chills and shivering. The fevers in Rome were widely known at that time so Brother Salumbrino sent samples of the bark preparation back to Rome.[6] Indeed, the Cinchona bark was effective and this serendipitous discovery revolutionized malaria chemotherapy.

Early Use of Quinine

The use of the powdered Peruvian bark became the standard treatment for fevers in Rome by the end of the seventeenth century, but it took many more years before its use became more widely accepted. The use of a "Jesuit powder" was resisted in protestant England until the self-proclaimed fever specialist James Talbor marketed a proprietary concoction with extracts from cinchona bark and was credited for saving the life of King Charles II with his cure.[1, 7]

The isolation of the active component of the Cinchona bark challenged chemists for many years to come. Without realizing the alkaline nature of quinine, several scientists were able to isolate mixtures of components from doing extractions, but were unable to obtain the pure active ingredient.[8] The German chemist Friedrich Wilhelm

A



1.1
Quinine

B

Figure 1.2 A) Structure of quinine. B) Bottle of quinine sulphate pills with 5 grams of the pure alkaloid in each pill.

Sertürner then developed a process for isolating the alkaloid morphine by making salts with acid leading to the isolation of quinine.[9] The French chemists Pelletier and Caventou were the first to report the isolation of the pure active ingredient of the Peruvian bark, quinine (**1.1**). (Fig 1.2) Their procedure involved extracting into alcohol, diluting with water, then basifying to form a gummy precipitate of quinine. This precipitate could then be recrystallized in the presence of sulphuric acid to afford the pure quinine sulfate salt. Soon quinine was being isolated on an industrial scale by extracting pulverized bark with toluene in the presence of base, then extracting into an aqueous acid solution that was then slowly basified to form the quinine crystals.

In the New World, Rosengarten & Sons in Philadelphia began isolating Quinine on a commercial scale.[10] They sold the quinine to the Missouri doctor John Sappington who marketed "Dr. Sappington's Fever Pills."[11] These pills consisted of:

Sulphate of quinine 2 lb
Pulv Extract of Liquerice 1 ½ lb
Pulv Gum myrrh ½ lb
Oil of Sassafras
Acqua Pura
Make 240 boxes
24 pills to a box

Dr. Sappington had put together a formulation of quinine including liquorice to mask the bitter taste, oil for moistening and gum of myrrh to bind the ingredients together.[12, 13] This recipe for production is not very far off from modern day pill formulations.

From politics to synthesis

The colonialism of the late 19th century coupled with medical experiments demonstrating the efficacy of quinine against malarial fever quickly drove up the demand for quinine. The natural forests in South America could no longer adequately supply an abundant supply of the Cinchona bark. The quinine supply problem led to two important solutions: 1) the establishment of new Dutch plantations of cinchona in Java and 2) the use of the emerging science of organic chemistry to produce synthetic quinine.

The establishment of the Dutch cinchona plantations used a species of the tree called *Cinchona calisaya* that produced bark with up to 14% quinine content.[14] The seeds for this ideal species were smuggled illegally out of Peru by the Englishman Charles Ledger and his Bolivian servant Manuel Incra Mamani.[15, 16] The Java plantations produced 102,000 kg of quinine annually, but with the onset of World War I, the demands of the Allied and German armies could not be met. In World War II, the situation became much worse when the Japanese captured Java and completely cut off the Allies supply of quinine. The huge demand caused by both wars led both the Allies and German scientists towards massive synthetic chemistry efforts.

The synthetic attempts by chemists to make quinine in the last half of the 19th century were futile, but serendipitously led to major advances in the art of synthetic organic chemistry. With a limited understanding of the atomic structure of matter, early

synthetic chemists experimented with reactions to construct quinine by combining smaller organic structures together. Most notably, the products of some of this early experimentation by August Wilhelm von Hofmann and William H. Perkin produced the first synthetic dyes.[17, 18] The synthetic dye industry marked the beginning of industrial organic synthesis and many of the largest modern pharmaceutical companies such as BASF, Hoechst (Aventis), Ciba-Geigy (Novartis), and ICI (Astra-Zeneca and Syngenta) all began as dye companies.

Long after the development of superior antimalarial compounds, the quest to complete a total synthesis of quinine continued. Much of pioneering work was carried out by Paul Rabe in 1918, however he lacked knowledge of the stereochemistry of the structure and only could produce a mixture of epimers starting from quinotoxine.[19] With many advances in the science of organic synthesis over the next few decades, R. B. Woodward and Doering completed the final total synthesis of quinine in 1945.[20] The cumbersome synthesis procedure is of no practical utility but remains a classic in the art of organic synthesis.[21]

Synthetic Antimalarial Drugs

The early synthetic work by Hofmann, Perkin and others gave birth to the dye industry and oddly enough the dye industry is what produced the first synthetic antimalarial drug. Guttman and Ehrlich reported the clinical antimalarial efficacy of the dye methylene blue (**1.2**) in 1891 making it the first synthetic drug.(Fig 1.3)[22-24] This phenothiazine dye had very low activity, but acted as a lead compound in the design of more potent analogs. (Figure 2) [25] It was noted that addition of a basic side chain to the

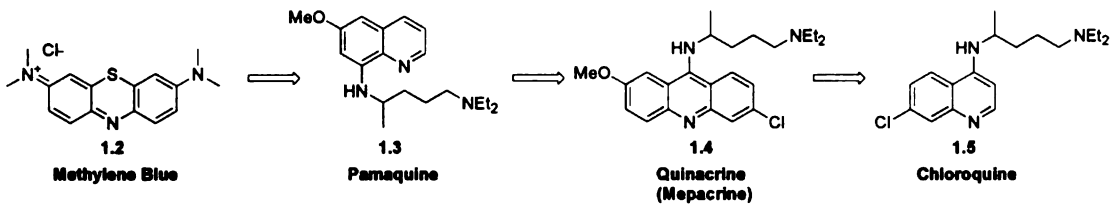


Figure 1.3 Progression of synthetic antimalarial drugs from methylene blue to chloroquine.

heterocyclic scaffold of methylene blue improved the antimalarial activity. This observation led to the development of the 8-aminoquinoline drug pamaquine (**1.3**) in the Bayer labs of the company IG Farbenindustrie.[26]

The drug pamaquine was found to have toxicity problems, so chemists in the Bayer labs attached the basic side chain of pamaquine to several other heterocyles. This pursuit led to the development of the drug quinacrine (**1.4**) (also known as atabrine or mepacrine).(Fig 1.3)[27] Quinacrine has an acridine heterocyclic scaffold and is also a dye with a bright yellow color. At the beginning of World War II, British and American scientists obtained the German patents on quinacrine and began industrial scale synthesis of the drug. The bright yellow drug became the primary antimalarial drug used by the US during the World War II period, particularly in the Pacific. Quinacrine was not popular amongst soldiers due to the fact that it turned one's skin yellow and caused impotency.[28] The drug was quite successful though and due to its long half-life it could be used for prophylaxis as well as treatment.[4]

The 4-Aminoquinolines

Prior to WWII, the Winthrop Company was the US corporate partner of IG Farbenindustrie in Germany. A scientist in the Bayer labs at IG Farben had given the Winthrop Company several potential antimalarial compounds including two 4-

aminoquinolines called Sontochin and Resochin.[29] The compounds were both patented in the US by the Winthrop Company (US Patent 2,233,970), but were not heavily pursued due to a report of a German psychiatrist that they were more toxic than quinacrine.[27] However, when the Allied forces in WWII captured Tunisia, they discovered a German clinical trial program for a new antimalarial drug called Sontochin.[27] The German doctor, J. Schneider, surrendered his supply of Sontochin to the US Army along with all of data from the outcome of their clinical trials. The promising clinical trial data caused the US Army to urge the Winthrop company to re-evaluate Sontochin and similar compounds. They then discovered that Sontochin and another compound Resochin, were several times more potent than quinine and significantly less toxic that quinacrine. This led to the US survey of antimalarial drugs from 1941 – 1946.[30] The drug Resochin was then taken into US clinical trials in 1944 and approved with the new name Chloroquine (CQ) (**1.5**).(Fig 1.3)[29]

In many ways, CQ was the ideal antimalarial drug leading to its heavy usage for the next fifty years. First of all, CQ had fewer side effects than any of the previous antimalarial drugs and its high potency allowed low doses to be given. The drug has a relatively long half-life such that one dose weekly produced complete suppression of suppression. This made it useful for both prophylaxis and treatment. Another important feature of CQ is that its production cost was low, making the drug very affordable. This would later be important for the drug to be used in the developing world, where malaria is most prevalent. CQ seemed like the "magic bullet" in man's incessant battle with malaria.

Emergence of Chloroquine Resistance

Interest in malaria and antimalarial drug research waned in the 1950s with the belief that global malaria eradication was imminent. That was until the first reports of CQ drug-resistance in South America came around 1960.[31] The high efficacy and low toxicity of CQ lead to huge quantities being used throughout South America, Africa and Asia. As with drugs for all infectious diseases, the presence of sub-lethal concentrations of drug creates a strong selection process for strains that are drug-resistant. Given the heavy use of CQ, it is not surprising that drug-resistance emerged. Since CQ was frequently used irregularly as a prophylactic, many people had sub-cidal concentrations of drug in their blood while infected. There were even worldwide "salt malaria control projects" in which chloroquine (and other drugs) were put in the table salt.[32] Such salt projects were carried out on each of the three major continents with endemic malaria. The negative impact of these poorly designed projects was increased by the fact that CQ is extremely bitter and can only be put in salt at low concentrations without noticeably effecting the taste. The table salt experiments as well as other poorly planned malaria eradication plans eventually would lead to worldwide CQ drug-resistance. The irresponsible use of CQ arguably lead to the unnecessary suffering from malaria for many years to come.

Second Generation Antimalarial Drugs

Renewed interest in antimalarial drugs began as the US army became involved in a conflict in Southeast Asia. It was eventually noted that CP tablets, containing a combination of chloroquine and primaquine, were no longer effective at preventing

malaria in soldiers.[33, 34] The Walter Reed Army Institute for Research (WRAIR) (Washington, DC) reinstated a large scale screening effort to find new potential antimalarial drugs.[35] The screening was done using multiple avian models of malaria, which are quite different from human malaria, but were useful for discovering lead compounds. WRAIR screened 25,000 compounds a year into the early 1980s.[36] Out of this effort, two classes of compounds emerged as the most promising candidates for development, both of which were initially identified in the 1940's US Army screening effort.

The first class of compounds are referred to as quinolinemethanols and are structural homologs of quinine (Fig 1.4). Several of these compounds had potent activities such as compounds **1.6 – 1.8**, but most of the members of this class exhibited photosensitizing side effects.[37-39] Out of this class, medicinal chemistry efforts eventually lead to the synthesis of Mefloquine (**1.9**).[40, 41] Mefloquine (trade name Larium) has proven to be an effective antimalarial drug and was very potent against CQ-resistant infections.[42] The negative features of mefloquine are that there are neurological side-effects and the drug is expensive to produce.[43] The cost of the drug has made it essentially unavailable to those in the developing world.

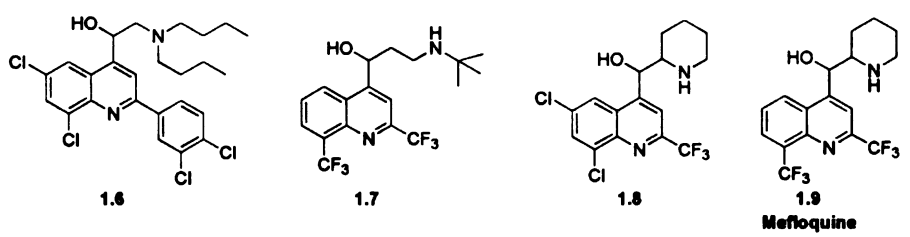


Figure 1.4 Early quinolinemethanols eventually leading to the development of the drug mefloquine.

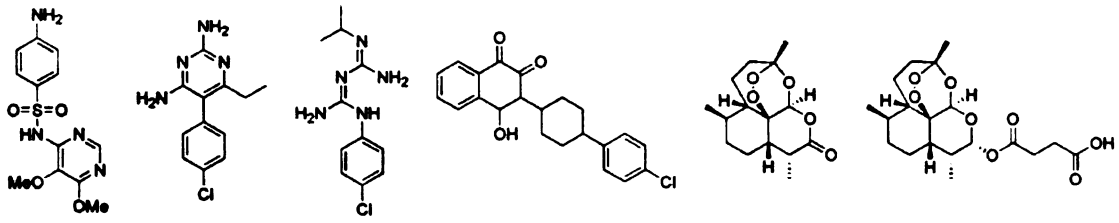


Figure 1.6 Representative members of important drug classes used for malaria chemotherapy.

The second class of compounds that came out of the WRAIR screen are called aryl(amino)carbinols. These compounds are similar to the quinolinemethanols in having a hydroxyl group α to the aromatic ring but contain a non-quinoline aromatic ring.(Fig 1.5) Out of this class the compounds emerged the drug Halofantrine (**1.10**).[44] This compound was eventually reported to have cardiotoxicity problems, but was modified into the drug Lumefantrine (**1.11**) that remains a heavily used antimalarial.[45]

Mechanistically Distinct Antimalarial Drugs

The quinoline antimalarials have been the most important antimalarial compounds to date, but several other mechanistically distinct drugs have also been developed. Each of these classes of drugs has advantages and disadvantages that will be discussed briefly. (Fig 1.6)

Folate Biosynthesis Inhibitors

The folate biosynthesis inhibitors were the second class of antimalarial drugs to be developed.[46] These enzyme inhibitors are some of the oldest synthetic drugs, developed first as antibiotics. The compounds most widely used as antimalarials are sulfadoxine (**1.12**) and pyrimethamine (**1.13**), which are usually co-administered as a

single pill formulation called Fansidar®. Sulfadoxine is a sulfonamide antibiotic that targets the protein dihydropteroate synthase (DHPS) involved in folate biosynthesis. Pyrimethamine targets the enzyme dihydrofolate reductase (DHFR), an essential enzyme in the same biosynthetic pathway. The targeting of two enzymes in the same biosynthetic pathway gives efficacy synergism and slows down the development of resistance. Each of these inhibitors is almost always used in some form of combination therapy since it was observed early on that resistance to each individual agent develops alarmingly fast.[47, 48] There is now reported resistance to Fansidar in most parts of the world, especially Africa and Southeast Asia.

Respiration Inhibitors

Another distinct class of antimalarial compounds are respiration inhibitors, such as the compound Atovaquone (**1.15**).(Fig 1.6)[49] This compound targets the mitochondrial electron transport process and is one of the most potent known antimalarials. Not long after use in the field, resistance to Atovaquone was reported and is believed to derive from a mutation in the target enzyme of the drug, cytochrome bc_1.[50] Currently, Atovaquone is used primarily in combination with another compound proguanil (**1.14**) under the trade name Malarone®. Proguanil is a prodrug DHFR inhibitor and shows synergy when used with Atovaquone through an unclear mechanism.[51] Malarone remains as one of the most effective antimalarial drugs, but is expensive and therefore unavailable to a large percentage of patients. There are reports of resistance to Malarone, which can be developed by two single-point mutations.[52, 53]

Artemisinins

The newest addition to the antimalarial drug arsenal are the artemisinins. This class of drug derives from a natural product found in sweet wormwood (*Artemisia annua*) called artemisinin (**1.16**). (Fig 1.6) Crude extracts of the natural source have been used in traditional Chinese medicine for more than 1500 years. Chinese researchers isolated the isolation active component in 1971 and synthetic efforts have produced many derivatives with improved solubility and bioavailability.[54] The mode of action of the artemisinins is still not completely understood, but it is notably distinct from that of CQ. While the pharmacological properties of most of the artemisinins are less than ideal, their strong potency and fast onset of action make them useful compounds, especially in combination therapies. The water-soluble derivative, artesunate (**1.17**), is now recommended for use by the World Health Organization (WHO) and is used extensively. The cost of the artemisinin compounds is prohibitive for much of the world's population and there are reports of severe neurological side effects.[55, 56]

Current Malaria Epidemiology

Malaria remains one of the most devastating infectious diseases in the world despite over a hundred years of drug development in this area.[57] In 2000, it was estimated that 803,000 children under the age of five died from malaria in Africa, while the worldwide mortality lies between 1.1 – 1.3 million people. The total number of reported case is between 350 – 500 million annually, with a large number of cases presumably going unreported. Despite all of our technological advances in the field, there is no

evidence that the overall burden in terms of morbidity and mortality has changed significantly between 2000 and 2004.

The reason for this continued suffering is primarily due to the high cost of the effective antimalarial drugs and alternative preventative measures. The economic state of most of the malarious countries makes concerted efforts to reduce the transmission of malaria difficult. Vaccines are often more cost-effective, but current the prospects for a malaria vaccine are unpromising.[58] Financial investment in the developing world is absolutely necessary to combat malaria, but the creation of new inexpensive antimalarial drugs, like chloroquine once was, would help alleviate much of the suffering in Africa and Southeast Asia, where the worst cases of malaria are found.

Modes of Action for Quinoline Antimalarial Drugs

Malaria is a disease caused by a protazoal organism of the genus *Plasmodium* and is transmitted by the bite of the Anopheles mosquito. There have been over 150 species of *Plasmodia* identified so far but only four of these species infect humans and these are *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium falciparum.* The most deadly of these species is *Plasmodium falciparum*, which is responsible for nearly all of the malaria deaths.

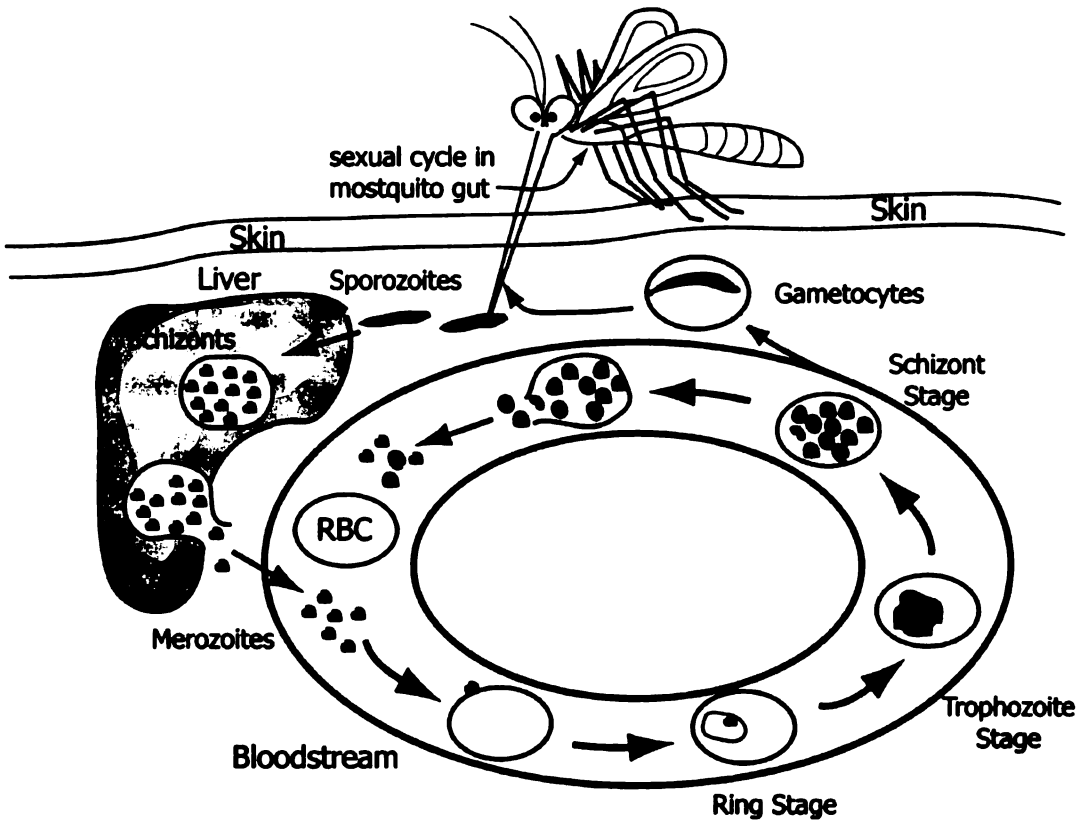


Figure 1.7 Lifecycle of the malaria parasite within both its arthropod and mammalian hosts.

The lifecycle of *P. falciparum* is complex, involving several distinct stages within both its mosquito and human hosts. A simplified diagram showing the various stages of the parasite lifecycle is show in Figure 1.7. When an infected mosquito bites a human, prior to sucking blood, it first injects a small volume of saliva and the sporozoite stage of the parasite into the human's bloodstream. The sporozoites make their way into the human liver where they multiply within hepatic cells. The parasites are eventually released back into the blood stream as merozoites and begin invading the host's erythrocytes (red blood cells (RBCs)).

The cyclic intra-erythrocytic stage of the parasitic infection begins with the merozoites invading an uninfected RBC. The parasite is endocytosed into the

parasitophorus vacuole (PV) where it begins to mature into a ring stage parasite. The rings establish themselves inside the RBC and begin to express many proteins required for growth. As the late rings mature into trophozoites, the parasite begins to consume hemoglobin as a source of energy and biosynthetic materials. This unique process occurs in the acidic food vacuole (FV) that is the target of the quinoline antimalarial drugs. The mature trophozoites will then begin cell replication until they become schizonts, a cluster of progeny parasites. The schizont will eventually rupture the membrane of the RBC and release 8-32 merozoites into the bloodstream. The free merozoites will them attempt to invade new non-infected RBCs, beginning the process over again.

A small percentage of the merozoites that invade RBCs will take a different path and develop into diploid gametocytes. Gametocytes can be either male or female and are the stage of the parasite that is taken up into the gut of the mosquito, while a mosquito is ingesting a meal of blood. The gametocytes can then undergo a sexual reproduction cycle within the gut of the female anopheles mosquito. Finally, new sporozoites are formed in the salivary glands of the mosquito and are ready to infect the next human host.

P. falciparum is particularly aggressive in the intra-erythrocytic stage of the infection, which is also the stage responsible for the majority of malaria symptoms. This will be the stage of the life cycle that the remainder of the discussion will be focused on since it is also the stage at which most of the quinoline antimalarial drugs work (except the 8-aminoquinolines which work primarily on the liver stage).

Site of Action of the 4-Aminoquinolines

Despite decades of research on the subject, CQ's mode of action still remains a mystery. Many important details have been uncovered, but the exact mechanism and targets of CQ are still not completely resolved. What has been made clear is that CQ only works on the intra-erythrocytic stage of the parasite and more specifically during the stages that are actively involved in hemoglobin degradation.(Fig 1.8)[59] CQ has been described as targeting an organelle or process, rather than a specific enzyme.[60] The morphological changes seen after chloroquine treatment were the first clue to suggest that the drugs were targeting the FV.[61, 62] Eventually it was realized that CQ interferes with the unique process occurring in the food vacuole where free heme, or ferriprotoporphyrin IX (FPIX), is polymerized into the insoluble polymer hemazoin.[63, 64]

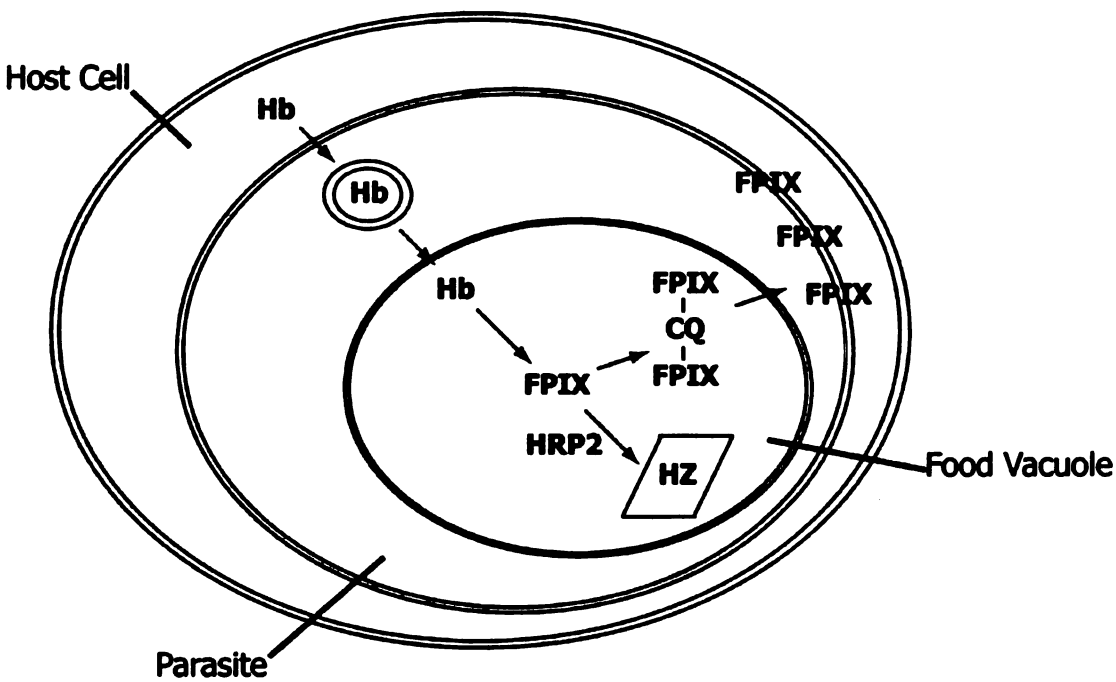


Figure 1.8 Inhibition of the formation of hemazoin (HZ) by CQ. Free heme (FPIX) is released into the food vacuole following hemoglobin (Hb) degradation. This is facilitated by histidine-rich protein 2 (HRP2). CQ can bind to the free FPIX, preventing the formation of HZ and allowing the toxic FPIX to accumulate in the membranes of the parasite, leading to death.

One of the first hypotheses was that CQ must inhibit an enzyme that is responsible for polymerizing the free heme. This putative "heme polymerase" enzyme was believed to exist because the rate of *in vitro* heme polymerization was shown to increase upon addition of trophozoite cellular extract.[65, 66] The "heme polymerase" hypothesis was later called into question when it was noted that purified hemazoin could also seed the formation of more hemazoin, similar to that of a crystal formation process or the formation of amyloid plaques in prion disease. Sullivan et al. then used immunological selection to identify a protein called Histidine-rich protein-2 (PfHRP2) that was shown to catalyze the formation of hemazoin and CQ was shown to inhibit this process.[67, 68] More detailed biochemical experiments on PfHRP2 showed that the protein had a rather weak affinity for FPIX (K_d = 1μM) but could bind up to 18 equivalents.[69, 70] Data from binding experiments suggests that CQ binds directly to the FPIX and not to PfHRP2. These combined data support a model in which PfHRP2 works to sequester heme and "seed" the hemazoin crystal formation process. CQ appears to inhibit this process by binding to FPIX units and driving the equilibrium away from hemazoin formation. The binding mode of CQ to FPIX has been shown to be through π-π stacking of the quinoline ring with the porphyrin ring of FPIX.[63, 71]

The ability of CQ to interfere with the heme polymerization process explains the biophysical mechanism of drug action, but this is only a partial picture of the CQ function. While CQ has been shown to accumulate 3-fold in uninfected RBCs, it is known to be concentrated several thousand-fold inside the Plasmodia, specifically within the acidic food vacuole.[72, 73] The primary mechanism of accumulation is due to the properties of CQ being a diprotic weak base (pKa_1 = 8.1, pKa_2 = 10.2). At intracellular

pH (7.4), a significant percentage of the drug will be monoprotonated and can more easily pass through membranes into the FV. The acidic FV environment (pH = 5.2) will keep CQ almost entirely in its diprotonated state, rendering it membrane impermeable, and concentrating the drug within the FV. This "pH trapping" process can be described by the Henderson-Hasselbach equation and is consistent with the observation that removal of the basic center of the drug gives a total abolishment of activity. The binding of CQ to the heme within the FV will also further drive the equilibrium in favor of more CQ in the FV. Other experiments have shown that CQ uptake is an energy dependent process, however this energy may be used for maintaining the pH gradient and not for the direct transport of CQ.[73-75] It is clear though that the total activity of CQ is due to a combination of two essential properties: 1) the ability to inhibit FPIX polymerization and 2) the ability to accumulate within the FV.[76, 77]

Additional proposed modes of action

The enigma surrounding the mode of action of CQ is partially due to the wide range of other reported activities for CQ. One of the earliest explainations of the mechanism of CQ was that it killed parasites through DNA intercalation.[78, 79] While it is true that CQ can intercalate into DNA, it only occurs at high concentrations (> 1 mM) and there is no basis for selective toxicity against the parasite without also harming the host.[80, 81]

Another possible mode of action for CQ is through the alkalinization of the acidic food vacuole.[61, 82] If high concentrations of basic CQ diffuse into the food vacuole, then the pH would be expected to rise within the FV. The pH could then be raised above the

optimal pH for the FV proteases digesting hemoglobin and thereby interfering with this process. The problem with this explanation is that *P. falciparum* has been shown to survive non-specific alkalinization agents and several of the proteases were shown to have broad pH optima.[83, 84]

It has also been demonstrated that CQ can inhibit the glutathione reduction of FPIX. Despite the ability of Plasmodia to sequester FPIX into hemazoin, greater than 60% of the heme released is not incorporated into hemazoin and is released into the cell.[85] The majority of this free FPIX is reduced by glutathione (GSH), but PfHRP2 has also been shown to further modulate the redox activity of FPIX.[86, 87] It has been suggested that the modulation of the reductive state of FPIX is equally important as the formation of hemazoin.[64] Either way, CQ also has shown to inhibit the GSH reduction of FPIX and is believed to do so similarly by binding to FPIX, thereby preventing its reduction.[85, 88]

Given the complexity of the mode(s) of action of CQ, it is very difficult to employ target-based drug design strategies. Whereas most drugs depend on binding to a single macromolecular receptor for activity, CQ activity requires a combination of binding affinities for multiple receptors and physiochemical properties that allow it to accumulate in its target organelle. The prospects of designing new agents with a similar mechanism to CQ is even further complicated after consideration of what is necessary to evade the drug-resistance mechanism.

Resistance to Quinoline Antimalarial Drugs

While antimalarial drugs have never been effective in every patient, it became apparent that true drug-resistance was becoming a major clinical problem around 1961 when there were reports of drugs no longer working from both South America and Southeast Asia.[31, 33, 34] Perhaps related to our incomplete understanding of the CQ mode of action, we also have a somewhat fragmented understanding of the mechanism of CQ drug-resistance. In contrast to the anti-folate drugs, where single point-mutations in the macromolecular targets confer resistance, CQ resistance appears to be a multi-genic phenomenon.[59, 60, 89] The fact that CQ resistance did not appear for many years after its widespread use, further argues that the resistance mechanism has a multi-genic basis brought about by extensive and prolonged drug use.[90, 91]

The first major reports on the mechanism of resistance to CQ established that CQ-resistant *P. falciparum* accumulate significantly less drug than susceptible parasites.[74, 91, 92] It was also reported that verapamil and other calcium channel blockers were able to reverse the drug-resistance phenotype.[91, 93] This result suggests similarities to multi-drug resistance (MDR) in tumor cells, which was already known to be caused by over-expression of an ATP-dependent transporter known as P-glycoprotein.[94] Verapamil had already been established as a drug-resistance reversal agent and was shown to bind directly to the mammalian MDR transporter protein suggesting that a similar mechanism might exist for drug-resistance in *P. falciparum*.[95]

Two homologs to the mammalian MDR transporters were found in *P. falciparum* and increased expression levels and copy number of one of the genes, pfmdr1, were found in drug-resistant parasite strains.[96, 97] The gene product of *pfmdr1* was the *P.*

falciparum P-glycoprotein homolog (Pgh1) and seemed like the likely causes of drug resistance. The transporter protein is also localized to the membrane of the digestive food vacuole, the site of action for CQ.[98] Ultimately, a genetic cross experiment showed that the increase CQ efflux phenotype was independent of either of the mdr-like genes.[99] Despite this genetic evidence that Pgh1 can not solely explain CQ drug-resistance, their is still valid evidence that the Pgh1 does play some role in modulating sensitivity to several of the drugs functioning at the food vacuole.[100-104]

Genetic mapping techniques mapped the CQ-resistance locus to a 400-kilobase region on chromosome 7.[105] In this region two candidate genes, *cg1* and *cg2*, were identified that showed mutations having high correlation with the CQ drug-resistance phenotype.[106] After advancements in genetic Transfection techniques it was shown that allelic modification of the *cg1* and *cg2* genes had no effect on the chloroquine response of *P. falciparum*.[107] This puzzling result was demystified when a highly interrupted and previously undetected gene, *pfcrt*, was identified in the CQ-resistance locus.[108] Transfection with the *pfcrt* gene confers the CQ-resistance phenotype and sequencing of over 40 strains of *P. falciparum* from around the world showed the importance of a few key mutations, particularly the K76T mutation, in developing the CQ-resistance phenotype. Currently, the K76T is the primary genetic marker for CQ resistance. The protein product of this gene, *P. falciparum* chloroquine-resistance transporter (PfCRT), is thought to be a membrane-bound transporter protein and was shown to localize to the food vacuole membrane.[108] PfCRT shows sequence homology to a known drug/metabolite transporter superfamily, but little is known about its native function.[109-111] Sequence analysis of PfCRT predicts that it has 10 transmembrane-spanning regions

and the key lysine residue at position 76 is positioned within one of the membrane spanning regions.[108] There is no ATP-binding domain in PfCRT so the driving force of this transporter is still unknown.

While there is now much known about the mechanism of resistance to CQ, there is still much unknown. Several factors seem to play a role in CQ-resistance. (Fig 1.9) It has also been proposed that changes to the digestive vacuole pH are important for drug-resistance.[112] The evidence that changes in pH are responsible for drug-resistance is weak, but this may play some additional minor role in determining sensitivity to CQ. Along this same line, it has been reported that a Na^+/H^+ exchanger may be responsible for importing CQ.[113] This theory is also based on inconclusive evidence, but it is expected that the accumulation would be indirectly related to the action of ion transporters. If the

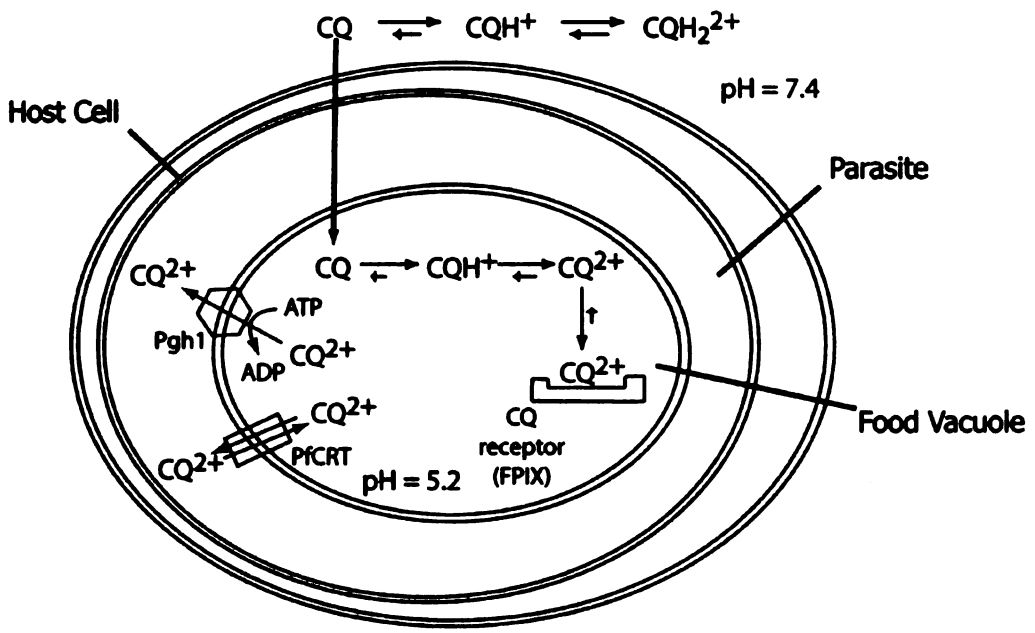


Figure 1.9 Mechanisms effecting CQ drug resistance. Unprotonated CQ can enter the food vacuole through either a transport mechanism or passive diffusion. Then it becomes predominantly doubly protonated and trapped within the FV. Factors effecting the concentration of CQ are the pH in the FV, the activity of the P-glycoprotein homolog-1 (Pgh-1), and the activity of the chloroquine resistance transporter (PfCRT).

cause of drug-resistance is due to a protein transporter, than drug-resistance can be overcome by creating new agents with weakened affinity for the efflux transporter while retaining binding to the targets in the FV.

Potential for New 4-Aminoquinoline Antimalarials

The emergence of drug-resistant *P. falciparum* has once again renewed research efforts to find new effective antimalarial drugs. Improvements in genetics and molecular biology techniques for *P. falciparum* have increased our understanding of the fundamental biology in recent years, but despite these advances few new validated drug targets have been elucidated. Also, history has taught us that drug-resistance can develop extremely quickly for agents that target a single protein, such as the anti-folate drugs. Most likely, any new molecular therapies will have to be administered in combinations with other drugs, leading to much higher costs. Despite this pessimistic view, it is also possible that another promising "magic bullet" new drugs could be just around the corner.

The rational for continuing research on 4-aminoquinolines is based on a history of tremendous success from within the drug class. The following are important points to consider:

1. 4-aminoquinolines have historically been the most effective antimalarial drugs for both the treatment and prophylaxis of malaria.
2. Drug-resistance to CQ was slow to develop, despite irresponsible massive worldwide use.
3. The 4-aminoquinolines have a time-tested acceptable toxicity profile.
4. 4-Aminoquinolines are generally inexpensive to produce, thereby they could practically be used on a wide-scale in developing countries.
5. New CQ-analogs have proven to be effective in treating CQ-resistant strains of *P. falciparum*.

Each of these important considerations underlies the motivation to continue research in the potential of this important class of antimalarial drugs. In order to develop the next-generation of 4-aminoquinoline antimalarials, it is important to first thoroughly examine the first hundred years of work in the field.

Antimalarial test systems

The long history of work on the development of quinoline antimalarials gives the medicinal chemist a large amount of *a priori* knowledge about the drug class, but the wealth of this knowledge is somewhat diminished by the major differences in screening systems. Prior to the landmark 1978 paper by Trager and Jensen describing the *in vitro* culturing of *Plasmodium*, all antimalarial screening was done *in vivo*.[114] The activity data published was therefore from a combination of the intrinsic activity of the compound along with its pharmacological properties. More importantly, screening had to be done against different species of *Plasmodia* since the human species would not infect the RBCs of another species of mammalian host. This makes direct comparisons of activities difficult. Most of the early antimalarial work was all done using an avian model of malaria.[115] These models involved usually involved the species *Plasmodium lophurae*, *Plasmodium cathemerium,* and *Plasmodium gallinaceum* infecting either a duck or chicken, respectively. Eventually, there was a shift toward using a rodent model for antimalarial testing using the species *Plasmodium yoelii*, *Plasmodium berghei* and *Plasmodium chabaudi chabaudi*. Since these *in vivo* models involve a completely different species of *Plasmodia* along with a very different host species, it is difficult to know how well the activity data will correlate with activity against *P. falciparum*. There

are clearly some reports of very poor correlation between the systems.[116] Given this disclaimer, the activities of the compounds tested only in the avian or rodent models should be interpreted cautiously.

Ring Substitutions on 4-Aminoquinolines

Modifications to the 7-chloroquinoline ring found in CQ can have quite drastic effects of the activity of a compound. The quinoline ring is believed to be the primary determinant of binding to free heme (ferriprotoporphyrin IX). This interaction involves π-π aromatic ring stacking and is very sensitive to the ring electronics and protonation state of the ring nitrogen. The original 7-chloroquinoline ring was reasoned to be a logical simplification of the tricyclic acridine scaffold of quinacrine.[27] Following the procurement of chloroquine in 1943, the synthetic literature is full of synthetic schemes for substituted quinoline rings for testing as antimalarial drugs.[117-130] Of the over 60 compounds made in this series, the only ones showing potency better than or equal to quinine were those containing a halogen at position 7. The activity pattern for this series is shown in Figure 1.10. Other active compounds contained a combination of a halogen at position 7 with other substitutions, but none of these were superior to the simple 7-chloroquinoline ring.[116]

More recent work on modifications to the quinoline ring also has focused on

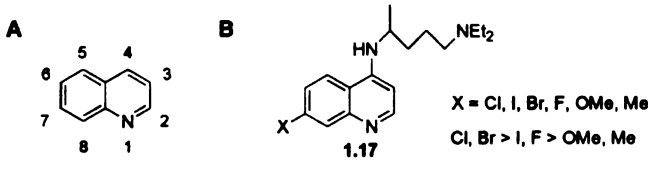


Figure 1.10 A) Numbering of positions around the quinoline ring. B) SAR of CQ-analogs with variations at the 7-position of the ring.

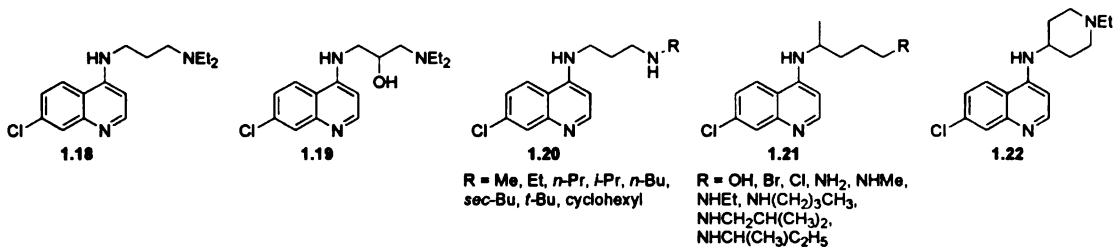


Figure 1.11 Early examples of side-chain modifications made during WWII antimalarial effort.

changes to the 7-position of the ring. De et al. reported a direct comparison of CQ analogs with a series of ring substitutions at the 7-position and showed an *in vitro* activity pattern against CQ-sensitive *P. falciparum* of I, Br > Cl > F > CF_3 > OMe.[131] This work also showed that I and CF_3 substitutions at the 7-position gave a 2-fold increase in activity against a CQ-resistant parasite strain. In a similar experiment, Kashula et al. made a similar series with a shortened basic side chain and showed a similar activity pattern where I > Cl, Br, NO_2 > CF_3 > F >> OCH_3, OH, H > NH_2.[132] This result is consistent with the notion that electron withdrawing groups are superior to electron donating groups. This group also showed in a Hammett plot how the electronic character of the substituent on the quinoline ring directly effects the pKa of the ring nitrogen. Given the strong evidence that pH trapping is necessary for activity, this pKa value is expected to be of considerable importance. These two studies demonstrate that there is potential to increase potency through modifications to the quinoline ring, though relatively little work has focused on developing the structure-activity relationship of this region of the molecule.

Basic Side Chain Modifications

The basic side-chain of CQ has been the primary focus of medicinal chemistry efforts since it is essential for activity and is the primary site of metabolism. While this

region of the molecule has shown a lot of tolerance for modification it is clear that the presence of a basic nitrogen is absolutely necessary for activity. Since all of the early testing was done using *in vivo* models, a lot of the activity may soley reflect on changes to the bioavailability of the molecules.

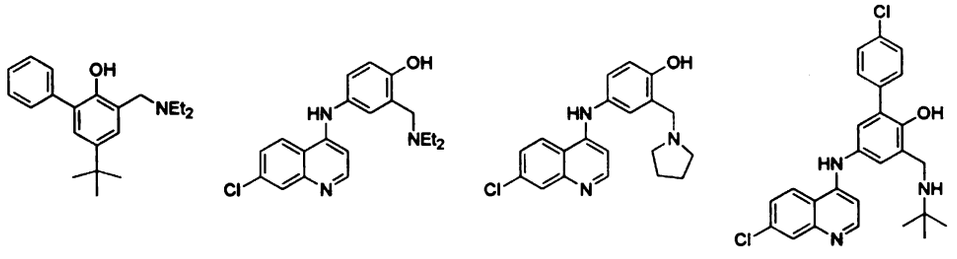
During the final developmental stages of CQ in the US, the final decision of which drug to move forward was between CQ and oxychloroquine (**1.19**).[27](Fig 1.11) CQ showed slightly higher toxicity (still though with a very high therapeutic index) while oxychloroquine had a much shorter half life. Based on this data, CQ was determined to be superior since its longer half-life makes it better suited for prophylaxis use. The 1940s antimalarial drug literature contains several other examples of side-chain modifications, including examples of shortening the length of the alkyl group in the side-chain, modifying the alkyl substitutions on the terminal nitrogen and incorporating cyclic groups into the basic side-chain.[121, 124, 133] (Fig 1.11)

In 1996, two groups independently reported that CQ analogs with a shortened alkyl side-chain have potent activity against CQ-resistant *P. falciparum*.[134, 135] De et al. synthesized CQ-analogs with alkyl sidechains ranging from two to twelve methylene units long. They showed that compounds with either shorter (< 4 methylenes) or longer (> 9 methylenes) side-chains had good potency (< 20 nM IC_{50}) against a drug-resistant parasite strain. Other groups have reported similar results as well.[136] Amazingly, one of these simple derivatives called AQ-13 has been taken all the way into phase II clinical trials.[137]

Amodiaquine-Related Compounds

In 1943, Burckhalter reported the discover of a new pharmacophore a having antimalarial activity that consisted of a α-diethylamino-*o*-cresol group.[138](Fig 1.12) Looking to improve the potency of his lead compounds he attached the 7-chloroquinoline ring found in chloroquine to his pharmacophore and created the potent compound amodiaquine (**1.24**). Though the potentcy and low toxicity of amodiaquine were noted, the success of CQ drew away most of the attention from amodiaquine. Once the development of CQ-resistance was noted in the late 1960's, there became a renewed interest in amodiaquine. It was realized that amodiaquine remained effective against *Plasmodium* strains that were resistant to CQ.[139]

In vitro testing of amodiaquine demonstrated its activity against several CQ-resistant parasite strains, but *in vivo* the drug was quickly metabolized to its desethyl derivative, which is significantly less potent against drug-resistant parasite strains.[140, 141] To circumvent this problem, many analogs of amodiaquine were synthesized and tested for activity against CQ-resistant strains of *P. falciparum* and the compound amopyroquine (**1.25**), which contains a pyrrolidine ring on the terminal nitrogen, showed the greatest potency.[142] A further increase in potency was accomplished by Werbel et al.



1.23 | **1.24** Amodiaquine | **1.25** Amopyroquine | **1.26** Tebuquine

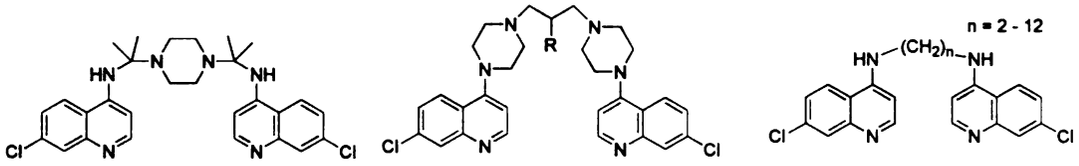
Figure 1.12 The development of the α-aminocresol motif into the drugs amodiaquine, amopyroquine and tebuquine.

when they synthesized a set of structures that were hybrids between amodiaquine and some of the earlier α-diethylamino-*o*-cresols that had been reported.[143] These hybrid structures resulted in the creation of Tebuquine (**1.26**), which was 25-times more potent than amopyroquine. Tebuquine remains one the most potent 4-aminoquinolines reported to date, but preclinical toxicology studies revealed lymphocyte toxicity problems. Many derivatives of tebuquine were subsequently made, but all had decreased activity and most still had the reported toxicity problem.[76, 144]

Of all the amodiaquine-like compounds produced, the parent compound itself has seen the most clinical use and is reported to have high efficacy in the field against CQ-resistant strains of *P. falciparum*.[145] Unfortunately, its use is limited to treatment of acute infections of drug-resistant *P. falciparum* due to rare cases of agranulocytosis and hepatotoxicity.[145, 146] While this class of drugs has only seen limited clinical use, it remains as a promising possible line of defense against drug-resistant *P. falciparum*.

Bisquinoline series

Evidence suggesting the mechanism of CQ-resistant is due to less accumulation within the FV lead to the proposition that compounds with a bisquinoline structure would be extruded less efficiently. Several compounds within this series have been prepared, many of which indeed retain activity against CQ-resistant *P. falciparum* infections.[147-150] (Fig 1.13) The compound hydroxypiperaquine (**1.28**, R = OH) has been shown to be effective in clinical trials against CQ-resistant strains, but the further development of this class of drugs has waned due to toxicity of an unknown molecular basis.[149]



1.27
Dichlorquinazine X

1.28
R = H Piperaquine
R = OH Hydroxypiperaquine

1.29

Figure 1.13 Structures of bis-quinoline antimalarials.

Quinlinemethanols

The quinolinemethanols gained renewed interest when many of the compounds found in the US Army 1940s screening program proved to be still active against CQ-resistant parasite strains. Unfortunately,animal studies showed that many compounds within this class produced severe photsensitivity.[38, 39] Medicinal chemistry efforts eventually led to a set of highly potent compounds without the photosensitivity side-effects.[37, 38] (Fig 1.4) Of this class of compounds, mefloquine was found to be the most effective against CQ-resistant strains of *P. falciparum* and its extremely long half-life made it an ideal candidate for prophylaxis as well as treatment.

Mefloquine does appear to function similarly to CQ by inhibiting the formation of hemazoin, but there are striking differences between the two classes of compounds, suggesting slightly different mechanisms of action.[80] There are also key differences in the structure-activity relationship (SAR) between the 4-aminoquinolines and the quinolinemethanols to further suggest important differences. The SAR for the ring substituents is quite different for the quinolinemethanols, probably due in part to the lack of the nitrogen in the 4-position that is conjugated to the aromatic ring system. This nitrogen significantly raises the pKa of the quinoline nitrogen and changes the ring electronics through resonance. Therefore the most potent of the quinolinemethanols all

contain multiple ring substitutions of strong electron withdrawing character. (Fig 1.4) The side-chains of the two classes of drugs seem to follow similar SARs. Both the 4-aminoquinolines and the quinolinemethanols have been shown to be cytotoxic and irreversible.[151]

Despite some reports of resistance to mefloquine, it remains a valuable drug for prophylaxis or treatment of CQ-resistant *P. falciparum*. The two major problems with mefloquine have been the expensive manufacturing costs and the possible CNS toxicity. The cost of mefloquine has made the drug virtually unobtainable by those in the developing world. The CNS toxicity of the drug is somewhat rare, but has gained attention recently. The drug is still not recommended for individuals with a history of epilepsy or psychiatric disease.[43]

Guidelines for Design

With an understanding of the rich history of antimalarial drugs, we can apply the important lessons learned to the design of new quinoline antimalarial compounds with activity against drug-resistant strains of *P. falciparum*. To accomplish this, we need to consider all that is now known about possible mechanisms of action and possible mechanisms of drug-resistance. The following design principles will need to be considered:

1) Retain aromatic ring capable of binding hemazoin and inhibiting hemazoin formation.
2) Retain basic functionalities to allow accumulation within the food vacuole.
3) Perturb structure enough to disrupt any acquired efflux mechanism in the food vacuole of drug-resistant strains.
4) Retain necessary bioavailability properties.

The remainder of this work will focus on the design and testing of such compounds. This work will contribute significantly to the greater understanding of the molecular properties that are important for developing a safe quinoline antimalarial active against drug-resistant strains of *P. falciparum*.

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Chapter 2

Quinoline Ring Substitution Structure-Activity Relationships

Overview

The synthesis of quinoline rings dates back to the early work published by Skraup in 1880.[1] There now exist several new methods for quinoline synthesis, but they all have the commonality of beginning with an aniline starting material and then going through a high-temperature cyclization. This chapter will discuss the development of a robust new set of conditions for the formation of quinoline rings with substitutions around the quinoline B-ring.(Fig 2.1) This method is used to create a screening library of chloroquine-analogs to further develop the structure-activity relationship for compounds with variations on the quinoline ring. The activities of these compounds and a discussion of the potential for such compounds to treat drug-resistant *P. falciparum* will be discussed.

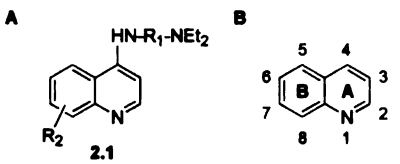


Figure 2.1 A) Generalized structure of compound library 2.1. B) Lettering of rings and numbering of positions around the quinoline ring.

Background

The search for improved synthetic alternatives to quinine led to the synthesis of many quinoline compounds. Synthetic modifications to the substitutions around the aromatic ring have lead to several important antimalarial drugs. (Fig 2.2) The emergence of worldwide CQ-resistance renewed much of the synthetic effort towards new quinoline antimalarials.[2-5] Most of these contain the 7-chloroquinoline nucleus of chloroquine, and vary in the length and nature of their basic amine side chain. Currently, compounds such as amodiaquine and AQ-13 are promising leads for the development of new drugs.[6]

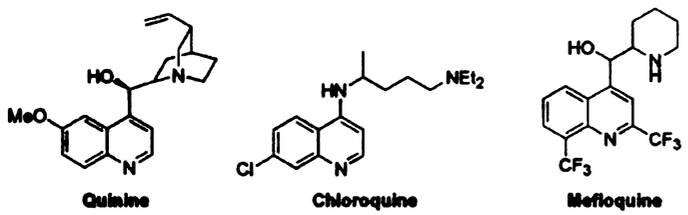


Figure 2.2 Quinoline antimalarial drugs with diverse substitutions around the quinoline ring.

While it is known that modification of the basic amine side chain can produce compounds active against drug-resistant *P. falciparum* strains, it has generally been assumed that changes to the quinoline nucleus itself will not. Changes to the ring system affect the pKa's of both the quinoline ring nitrogen and the side-chain nitrogen as well as other physical parameters such as lipophilicity, sterics, and electronegativity but have not significantly correlated with activity in drug resistant strains.[7] Recent work on ring substitutions has focused almost exclusively on the 7-position of the ring, so conclusions drawn from these data are limited. In this study, we explore the effects of B ring substitutions (Fig 2.1) on antimalarial activity against drug-resistant parasite strains.

The search for new antimalarial drugs also instigated the search for better synthetic methods to prepare quinolines. The first synthesis of quinoline by Skraup was done by reacting aniline with glycerol at very high temperatures with acid catalysis.[1] (Fig 2.3) Subsequent synthetic methods toward the synthesis of functionalized quinolines

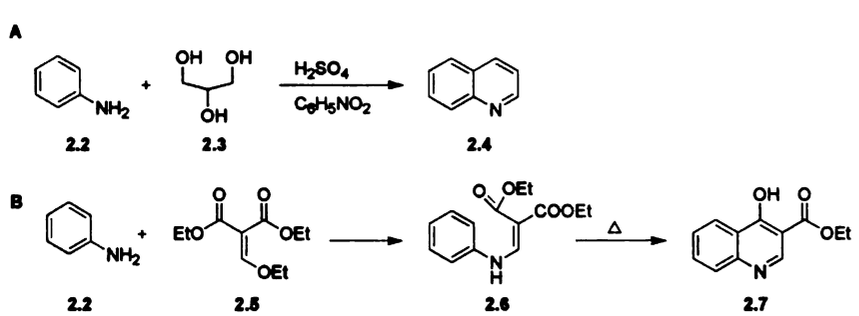
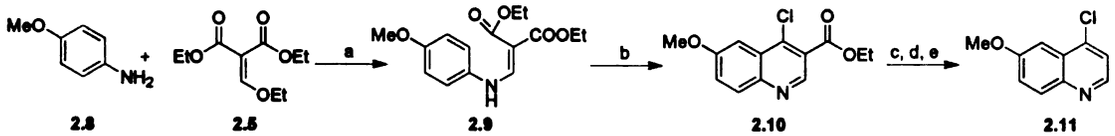


Figure 2.3 Classical quinoline syntheses. a) The original Skraup method of quinoline synthesis. b) The Gould and Jacobs method of quinoline synthesis that is used to make most of the 4-aminoquinolines.

include the Doebner method, the Beyer method and the Gould-Jacobs method.[8] Each of these methods involves starting from an aniline and forming an intermediate that undergoes cyclization by a Friedel-crafts acylation reaction. The Gould-Jacobs method is the most useful for synthesis of 4-substituted quinolines, since it leaves a hydroxyl group at the 4-position of the quinoline ring that can be selectively reacted. (Fig 2.3) Each of these methods involves a very high-temperature (> 250 °C) cyclization step that is catalyzed by an acid.

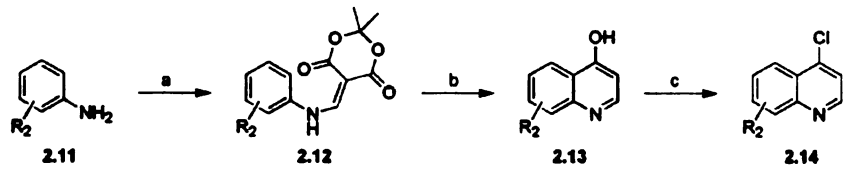
Synthesis of Substituted Quinolines

Originally, we set out to make substituted quinoline rings using the method of Price and Roberts, where we could functionalize the quinoline B-ring was functionalized by using appropriately substituted anilines as starting materials.[9] *P*-anisidine was chosen as a model aniline and reacted with diethyl ethoxymethylenemalonate to quantitatively form the ene-amine intermediate. (Scheme 2.1) The thermal cyclization step was attempted by refluxing in three different solvents; phenyl ether (B.P. 259 °C), *o*-dichlorobenzene (B.P. 180 °C), and toluene (B.P. 110 °C). Reactions were monitored by TLC and the cyclized product is easily visualized as a bright blue spot under short-wave UV light (254 nM). Only the reaction in phenyl ether yielded the cyclized product in a yield of 25%. The crude product of the high temperature reaction was a black tar and



2.8 2.5 2.9 2.10 2.11

Scheme 2.1 Synthesis of 4-chloro-6-methoxyquinoline using the Price and Roberts method. a. toluene, reflux, 5 min., (100%); b. $POCl_3$ (5 eq.), toluene, reflux, 30 min., (40%); c. 2N NaOH, reflux, 1h.; d. 1N HCl; e. Ph_2O, 250 °C, 30 min. (25% overall, 3 steps)



2.11 2.12 2.13 2.14

Scheme 2.2 Synthesis of substituted 4-chloroquinolines. a. Meldrum's acid, $CH(OCH_3)_3$, reflux, 1 h, then 2.11, DMF, reflux 2 h; b. diphenyl ether, 300 °C, 300 W, 5 min; c. $POCl_3$ (neat), reflux, 3 h.

purification of this resulted in low yields. In order to improve the yield and simplify the purification, the cyclization reaction was repeated in toluene using phosphorous oxychloride ($POCl_3$) as a Lewis acid catalyst. This raised the yield to 40% and gave a much cleaner product. Mass spectrometry analysis of the product confirmed that the $POCl_3$ also converted the 4-hydroxyquinoline directly to the 4-chloroquinoline product, eliminating the need for an additional reaction to convert the hydroxyl to a halide. Removal of the ethyl ester at the 3-position of the quinoline ring was accomplished by hydrolyzing the ester, converting it back to the free acid and then doing a thermal decarboxylation. The overall yield for these three steps was 25%. The high temperature decarboxylation step also resulted in a black tar that had to be column purified and led to significant loss of product. This entire sequence gave an overall yield of 10% over 5 steps, proving to be non-ideal for production of a library of compounds.

In order to produce a library of compounds with quinoline ring substitutions, modifications to the above procedure were made to try to raise the overall yield and use fewer steps. The first step in the sequence is the condensation of an aniline with Meldrum's acid and trimethylorthoformate (Scheme 2.2). The Meldrum's acid is refluxed in trimethylorthoformate to form methoxymethylene Meldrum's acid *in situ*. The aniline **2.11** is then added to this solution where it enters into an addition-elimination reaction with the methoxymethylene moiety to afford an ene-amine intermediate. Strongly electron deficient anilines, such as nitro anilines, did not quantitatively form the intermediate under the standard conditions. Adding an equal volume of DMF to the reaction allowed for sufficient increase in the reaction temperature to overcome the

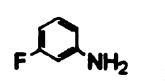
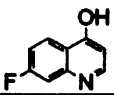
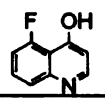
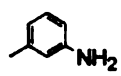
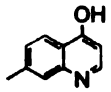
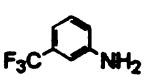
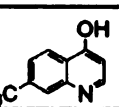
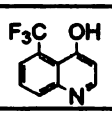
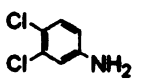
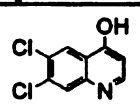
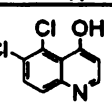
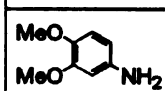
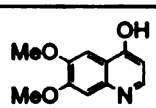
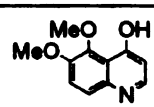
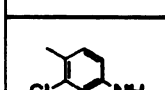
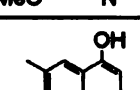
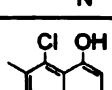
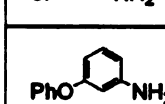
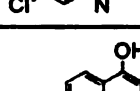
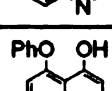
Starting Material Aniline	Quinoline Isomer 1	Isomer 1 Relative Ratio	Quinoline Isomer 2	Isomer 2 Relative Ratio
		1		1
		1		1.9
		1		2.4
		1		2.7
		1		2.8
		1		3.4
		1		6.3

Table 2.1 Ratio of regioisomers formed after microwave cyclization step.

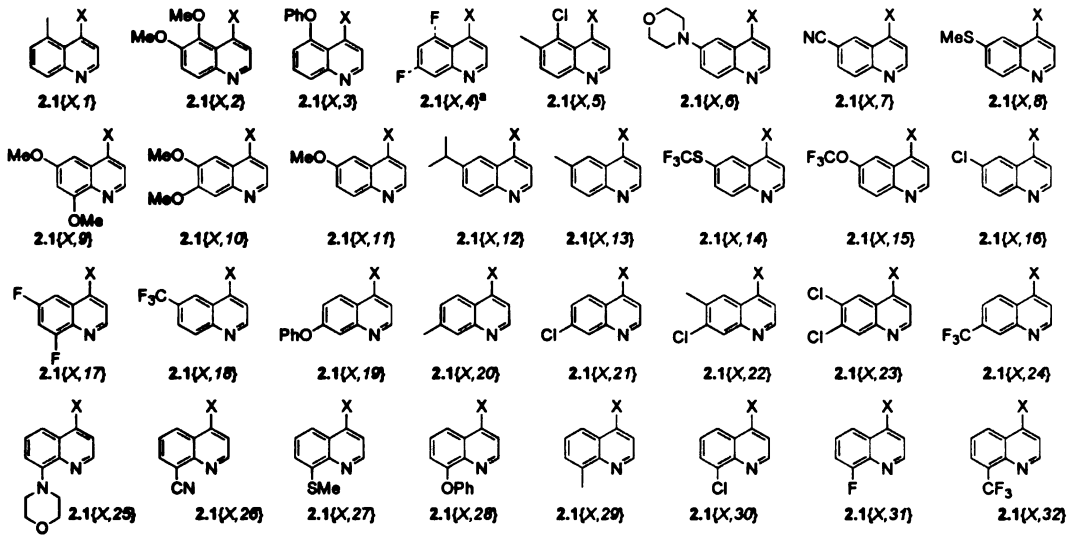
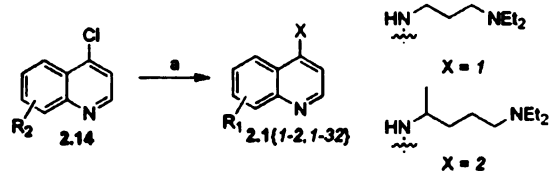


Figure 2.4 Structures of quinoline rings synthesized for this study and chemset nomenclature for the resulting library. [[a]Regioisomers were inseparable by reversed-phase HPLC. The 5-fluoro and 7-fluoro isomers were present in a ratio of 1:1.]

sluggish reactivity of the electron deficient anilines. The ene-amine intermediates were then sealed into a glass reaction tube with a small volume of phenyl ether as solvent and subjected to microwave irradiation for 5 minutes at 300 °C.

The crude reaction mixtures were then directly purified by silica chromatography to afford the desired pure 4-hydroxyquinolines in 20 – 70% overall yield. In certain cases a mixture of isomers were formed upon cyclization. All but one pair of the isomers could be separated by HPLC and the ratios of isomers formed are indicated in Table 2.1. (The isomers formed from the reaction of *m*-fluoroaniline were inseparable) The 4-hydroxyquinolines (**2.13**) were converted to 4-chloroquinolines (**2.14**) using $POCl_3$.



Scheme 2.3 Synthesis of library 2.1. a) X = 1: 3-diethylaminopropylamine (neat), 135–155 °C, 2 h. or X = 2: 2-amino-5-diethylaminopentane, 135–155 °C, 2 h.

In order to better understand the structure–activity relationship (SAR) for relative effects of side chain structure and substitutions on the quinoline ring, two different amine side chains were attached to the four position of the ring.(Scheme 2.3) One of the side chains was (N,N-diethyl)-1,4- diaminopentane (X = 2) the side chain found in chloroquine, a series where there is substantial drug-resistance among clinical isolates. The other side chain was (N,Ndiethyl)- 1,3-diaminopropane (X = 1), a shortened analog that is known to restore activity against drug resistant strains for the 7-chloroquinoline series. Both of these side chains were attached to the 4-chloroquinoline rings by a simple nucleophilic substitution. The final products were obtained cleanly in 70–90% yields after workup. Compounds with purity below 80% were purified by reversed-phase preparative HPLC to give the final library **2.1**{1–2,1–32} with an average purity of 95%. Purity and identity was verified for all samples by LC/MS (Fig 2.5); additionally, ^{1}H NMR were obtained for 10% of the library. Many of the reactions utilizing side chain X = 2 failed due to substantial impurities found in the commercially available amine starting material.

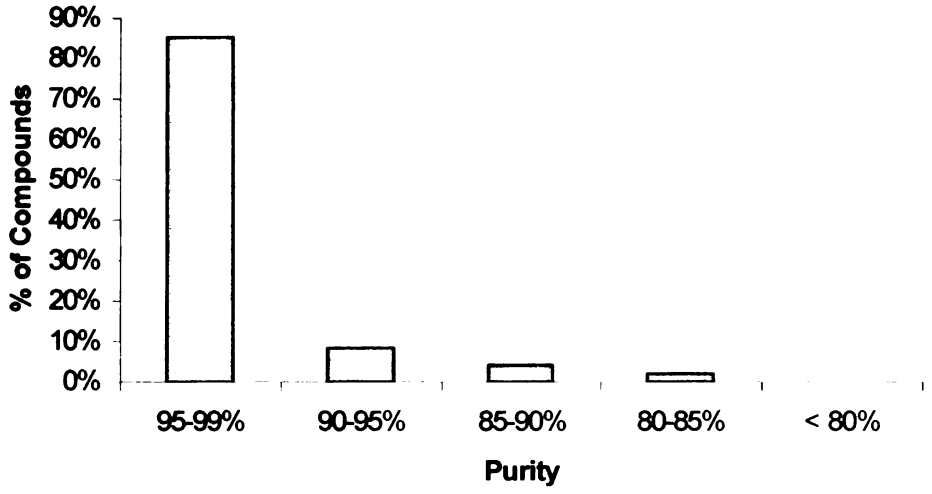


Figure 2.5 Histogram of purities of product library 2.1. Purity was assessed by analytical HPLC analysis monitored at 220 nm on a reversed-phase C18 column (Xterra C18, 3.5 μM).

Biological Activities

Compound library **1.2**{1–2,1–32} (Fig 2.5) was screened for growth inhibition activity against P. falciparum using a fluorescent-active cell sorting (FACS) assay (Fig 2.6). We chose to screen at two concentrations (30 nM and 200 nM) against both a drug-sensitive strain, 3D7, and a highly drug-resistant strain, W2. As expected, all compounds were more active against 3D7 than the drug-resistnat W2 strain and compounds with the

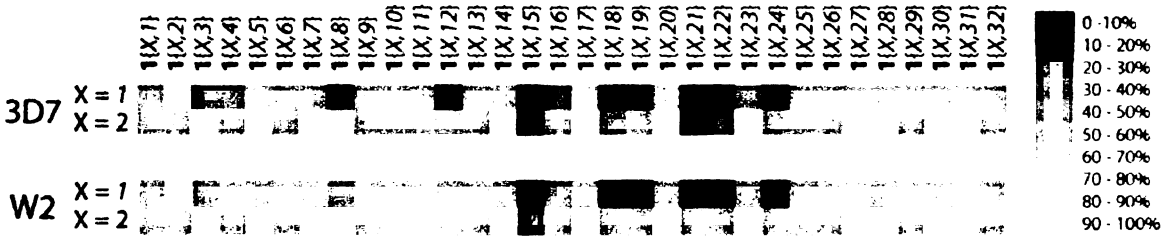
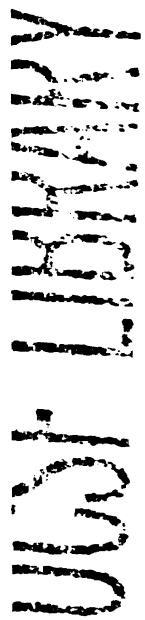


Figure 2.6 Colormetric representationof P. falciparum growth inhibition results for quinoline ring substitution library at 30 nM concentration. Colorimetric scale represents the percentage of growth of parasite relative to an untreated control population. White squares indicate a library member that could not be made or purified. Data represents the average of three separate measurements with standard deviation <10% of value.

shorter side chain were consistently more potent, especially against the drug-resistant W2 strain. It should also be noted that the most active compounds had substituents located at either the 6- or 7-position on the quinoline ring. In general, active substitutions tended to be small electron withdrawing groups with the notable exception of the 7-OPh compound **2.1{1,19}**[10], which proved to be one of the most active compounds. After screening the activities of these compounds, the six most active compounds were chosen for full dose–response analysis. Along with this set, we also included the 6-chloro-2-methoxyacridine ring system found in quinacrine to compare a non-quinoline ring against the set of quinolines. (Table 2.2) The shorter side chain variants consistently give higher potency than the chloroquine side chain variants against both 3D7 andW2. Interestingly, the pattern in EC50s for the propyl side chain compounds on W2 mirrors that for 3D7 with the EC50s being elevated by 1–2-fold, except in the case of the compounds containing a 7-chloroquinoline substructure. The 7-chloroquinoline and the 7-chloro-6-methylquinoline rings had EC_{50}s elevated by 11.5-foldand 5.5-fold, respectively.



When comparing the activities of the compounds with the chloroquine side chain against the two strains, it can be seen that the EC_{50}s increase, consistent with the notion that drug resistance is primarily modulated through the side chain identity. A feature worth noting is that the only compound that is notably less potent against W2 than the others is chloroquine (**2.1{2,21}**), which contains the 7-chloroquinoline ring. This indicates that perhaps the evolved resistance mechanism does have some specificity for the 7-chloroquinoline ring and therefore changes to the ring could be useful activity modulators against drug-resistant parasite strains. Another interesting feature of these data is that quinacrine retains its strong potency against the W2 strain despite the fact that

it contains the side chain of chloroquine. The 7-OPh quinoline **2.1**{1,19} also contains an additional third ring, but perhaps because of its flexible nature as opposed to the rigid three ring system of the acridine, the drug-resistant parasite is able to survive in the presence of this compound.

Table 2.2 Growth inhibitory activity, EC50 (nM)[a], of selected compounds against P. falciparum strains.

Chemset Number, substitution	3D7		W2	
	1{*1,X*}	**1{*2,X*}**	**1{*1,X*}**	**1{*2,X*}**
***21*, 7-Cl (CQ)**	2	17	23	382
***24*, 7-CF_3**	33	62	62	309
***19*, 7-OPh**	34	115	71	267
***22*, 7-Cl, 6-Me**	9	32	50	270
***18*, 6-CF_3**	90	51	125	290
***15*, 6-OCF_3**	82	62	102	287
quinacrine[b]	5	8	8	32

[a]Values are means of three experiments.
[b]acridine

Conclusions

In conclusion, we have used a simple two-step method to synthesize quinoline rings with diverse substitutions at the C-5, C-6, C-7, and C-8 positions. Modifications to the substituents around the ring led to several new active antimalarials, but their activity against the drugresistantW2 strain of P. falciparum was weak when they contained the chloroquine side chain. This further supports the belief that the length and nature of the basic side chain is the primary modulator of activity against drug-resistant parasite strains. Despite this fact, modifications to the quinoline ring do somewhat improve the activity of this series of compounds against the W2 parasite strain. Also, exchanging the

quinoline ring system with an acridine ring system has a profound affect onthe activity of these compounds against drug-resistant strains. Further studies elucidating these trends are underway.

Experimental

General Procedure A: Trimethyl orthoformate (10mL, 1 mol) and isopropylidine malonate (Meldrum's acid, 11 mmol, 1.1 eq.) were refluxed for 1 hour then cooled slightly. An appropriate aniline (10 mmol) was then added to the mixture along with 10 mL of DMF and reheated to reflux for 2 hours. The mixture was cooled to room temperature, then added into 150 mL of cold water and a crystalline solid was filtered and allowed to dry in the open air. Without any purification, a portion of the solid material (200 mg) was sealed in a glass tube with 2.0 mL of phenyl ether and a small magnetic stir bar. The sealed tube was heated to 300 °C for 5 minutes with 300 Watts of power, then cooled down to room temperature. Methylene chloride (4 mL) was then added to the glass tube and the tube was briefly sonicated to break up some of the chunky particles formed during the cylcization. This dark colored suspension was then loaded directly onto a silica column and purified with an isocratic mobile phase of 95% DCM, 4% MeOH and 1% TEA to yield pure quinoline **2.13**. All rings were characterized by ^{1}H-NMR and HPLC.

General Procedure B: In a 48-position Bohdan Miniblock with borosilicate glass reactions vessels, we added each 4-hydroxy quinoline **2.13**, $POCl_3$ (25μL, 0.27 mmol, ~3eq.) and 1 mL of NMP as solvent. The block was heated 3 hours at 110 °C then cooled to 0 °C. To each well in the cold block, 2.5 mL of 10% NaOH was slowly added and kept

at 0 °C for 1 hour to precipitate the solid 4-chloroquinoline **2.14**. The supernatant was drained and 2 mL of the appropriate side chain was added to redissolve the solid material and heated to 140 °C for 4 hours. The final compounds were then collected into test tubes and dried in a Genevac HT4 speedvac to remove most of the excess amine solvent. The samples were then dissolved in DMSO and injected onto a preparative HPLC. All compounds were then run on a Xterra C-18 column (Waters) running a 10 – 100% gradient of 0.5%TEA/Methanol with a 25 mL/min flowrate on a Parallex Flex HPLC system.

Representative Quality Control Data for Library 2.1.

^{1}H-NMR data and LC/MS data for 10 members of library **2.1**. All ^{1}H-NMR data is for HCl salts of the compounds in dimethyl sulfoxide-D_6 and is recorded on a 400 MHz Varian NMR spectrometer. Chemical shifts are measured in parts per million (δ) relative to tetramethylsilane (TMS) as the internal standard. LC/MS data is recorded on a Waters Alliance HPLC and Waters ZQ4000 mass spectrometer with an electrospray probe and a single quadrapole detector operating in positive ion mode.

N,N-Diethyl-N'-(5-methyl-quinolin-4-yl)-propane-1,3-diamine (2.1{1,1}) LCMS (ESI) *m/z* Calcd for $C_{17}H_{25}N_3$ $[M + H]^+$ 272.20. Found: 272.64. ^{1}H NMR ($CDCl_3$): δ 8.44 (d, J = 5.5, 1H), δ 7.85 (d, J = 7.0, 1H), δ 7.44 (dd, J = 7.3, 7.0, 1H), δ 7.12 (d, J = 7.0, 1H), δ 6.37 (d, J = 5.5, 1H), δ 3.32 (m, 2H), δ 2.94 (s, 3H), δ 2.62 (t, J = 6.6, 2H), δ 2.58 (q, J = 6.9, 4H), δ 1.60 (H_2O), δ 1.91 (t, J = 6.2, 2H), δ 1.25 (solvent), δ 1.01 (t, J = 7.3, 6H).

N'-(5-Chloro-6-methyl-quinolin-4-yl)-N,N-diethyl-propane-1,3-diamine (2.1{1,5}) LCMS (ESI) *m/z* Calcd for $C_{17}H_{24}ClN_3$ $[M + H]^+$ 306.17. Found: 306.57. ^{1}H NMR ($CDCl_3$): δ 8.49 (d, J = 8.7, 1H), 8.41 (d, J = 5.9, 1H), 7.78 (d, J = 8.4, 1H), 7.61 (m, 1H),

7.43 (d, J = 8.4, 1H), 6.39 (d, J = 5.9, 1H), 3.31 (dt, J = 5.9, 0.7, 2H), 2.63 (t, J = 6.9, 2H), 2.58 (q, J = 6.9, 4H), 2.49 (s, 3H),), 1.70 (H_20), 1.92 (t, J = 6.9, 2H), 1.25 (solvent), 1.04 (t, J = 7.3, 6H).

N,N-Diethyl-N'-(6-trifluoromethylsulfanyl-quinolin-4-yl)-propane-1,3-diamine (2.1{1,14}) LCMS (ESI) *m/z* Calcd for $C_{17}H_{22}F_3N_3S$ $[M + H]^+$ 358.15. Found: 358.57. ^1H NMR (d_6-DMSO): δ 10.44 (br-s, 1H), 9.82 (d, *J* = 5.6, 1H), 9.14 (d, J = 1.9, 1H), 8.66 (d, J = 7.2, 1H), 8.20 (dd, J = 8.8, 2.0, 1H), 8.10 (d, J = 8.8, 1H), 7.05 (d, J = 7.2, 1H), 3.67 (m, 2H), 3.14 (m, 6H), 2.11 (m, 2H), 1.23 (t, J = 7.3, 6H).

N,N-Diethyl-N'-(6-trifluoromethoxy-quinolin-4-yl)-propane-1,3-diamine (2.1{1,15}) LCMS (ESI) *m/z* Calcd for $C_{17}H_{22}F_3N_3O$ $[M + H]^+$ 342.17. Found: 342.60. ^1H NMR (d_6-DMSO): δ 10.51 (br-s, 1H), 9.75 (d, *J* = 5.6, 1H), 8.81 (d, J = 1.8, 1H), δ 8.64 (d, J = 7.2, 1H), δ 8.17 (d, J = 9.2, 1H), δ 7.99 (dd, J = 9.2, 1.8, 1H), δ 7.00 (d, J = 7.2, 1H), δ 3.67 (m, 2H), δ 3.12 (m, 6H), δ 2.12 (m, 2H), δ 1.22 (t, J = 7.3, 6H).

N,N-Diethyl-N'-(7-trifluoromethyl-quinolin-4-yl)-propane-1,3-diamine (2.1{1,18}) LCMS (ESI) *m/z* Calcd for $C_{17}H_{22}F_3N_3$ $[M + H]^+$ 326.18. Found: 326.18. ^1H NMR (d_6-DMSO): δ 10.55 (br-s, 1H), 9.83 (d, *J* = 5.6, 1H), 9.16 (d, J = 1.8, 1H), 8.66 (d, J = 7.2, 1H), 8.21 (m, 2H), 7.04 (d, J = 7.2, 1H), 3.68 (m, 2H), 3.17 (m, 2H), 3.10 (q, *J* = 7.3, 4H), 2.11 (m, 2H), 1.22 (t, J = 7.3, 6H).

N,N-Diethyl-N'-(7-phenoxy-quinolin-4-yl)-propane-1,3-diamine (2.1{1,19}) LCMS (ESI) *m/z* Calcd for $C_{22}H_{27}N_3O$ $[M + H]^+$ 350.22. Found: 350.63. ^1H NMR (d_6-DMSO): δ 10.62 (br-s, 1H), 9.61 (s, 1H), 8.75 (d, *J* = 9.2, 1H), 8.47 (d, J = 6.8, 1H), 7.55 (d, *J* = 8.0, 1H), 7.44 (m, 2H), 7.34 (m, 3H), 6.88 (d, J = 6.8, 1H), 3.64 (m, 2H), 3.16 (m, 2H), 3.09 (q, *J* = 7.3, 4H), 2.11 (m, 2H), 1.22 (t, J = 7.3, 6H).

N'-(7-Chloro-6-methyl-quinolin-4-yl)-N,N-diethyl-propane-1,3-diamine (2.1{1,22}) LCMS (ESI) *m/z* Calcd for $C_{17}H_{27}ClN_3$ $[M + H]^+$ 306.17. Found: 306.59. ^1H NMR ($CDCl_3$): 8.45 (d, J = 5.3, 1H), 8.12 (m, 1H), 7.94 (s, 1H), 7.62 (s, 1H), 6.26 (d, J = 5.3, 1H), 3.38 (m, 2H), 2.70 (t, J = 5.1, 2H), 2.67 (q, J = 7.3, 4H), 2.51 (s, 3H), 1.68 (H_20), 1.93 (t, J = 5.8, 2H), 1.25 (solvent), 1.13 (t, J = 7.3, 6H).

N'-(8-Chloro-quinolin-4-yl)-N,N-diethyl-propane-1,3-diamine (2.1{1,30}) LCMS (ESI) *m/z* Calcd for $C_{16}H_{22}ClN_3$ $[M + H]^+$ 292.15. Found: 292.56. ^1H NMR (d_6-DMSO): δ 10.55 (br-s, 1H), 10.00 (m, 1H), 8.78 (d, J = 8.4, 1H), 8.46 (d, J = 6.8, 1H), 8.17 (d, J = 7.6, 1H), 7.71 (t, J = 7.6, 1H), 7.04 (d, J = 7.2, 1H), 3.68 (m, 2H), 3.17 (m, 2H), 3.09 (q, J = 6.8, 4H), 2.12 (m, 2H), 1.22 (t, J = 6.8, 6H).

N'-(7-trifluoromethylquinolin-4-yl)-N,N-diethyl-pentane-1,4-diamine (2.1{2,24}) LCMS (ESI) *m/z* Calcd for $C_{19}H_{26}F_3N_3$ $[M + H]^+$ 354.21. Found: 354.63. ^1H NMR (d_6-DMSO): δ 10.17 (br-s, 1H), 9.29 (m, 1H), 9.05 (d, J = 8.8, 1H), 8.67 (d, J = 6.8, 1H), 6.38 (d, J = 2.0, 1H), 8.04 (dd, *J* = 8.8, 2.0, 1H), 7.05 (d, *J* = 6.8, 1H), 4.15 (m, 2H), 3.20 - 3.50 (solvent overlap), 3.05 (m, 6H), 1.90 (m, 2H), 1.72 (m, 4H), 1.34 (d, *J* = 6.4, 3H), 1.18 (t, J = 7.2, 6H).

4-(4-Diethylamino-1-methyl-butylamino)-quinoline-8-carbonitrile (2.1{2,26}) LCMS (ESI) *m/z* Calcd for $C_{19}H_{26}N_4$ $[M + H]^+$ 311.22. Found: 311.60. ^1H NMR (d_6-DMSO): δ 10.31 (br-s, 1H), 9.50 (m, 1H), 9.25 (d, J = 8.4, 1H), 8.52 (d, J = 6.8, 1H), 6.42 (d, J = 7.2, 1H), 7.80 (t, *J* = 7.6, 1H), 7.05 (d, *J* = 7.2, 1H), 4.15 (m, 2H), 3.20 - 3.50 (solvent overlap), 3.05 (m, 6H), 1.93 (m, 2H), 1.73 (m, 4H), 1.34 (d, *J* = 6.4, 3H), 1.19 (t, J = 7.2, 6H).

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Chapter 3

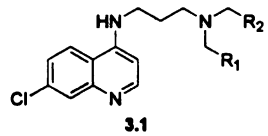
Basic Side Chain Structure-Activity Relationships

Overview

Due to growing problems with drug resistance, there is an outstanding need for new, cost-effective drugs for the treatment of malaria. We report herein the development of an efficient method for producing libraries of 4-aminoquinolines variant in the side chain portion of the molecule. The effects of these substitutions were evaluated by screening this library for activity against *P. falciparum* revealing four potent compounds active in drug resistant strains.

Background

Quinoline containing drugs, particularly 4-aminoquinolines, have a long and successful history as antimalarials[1, 2]. One of these, chloroquine, has been in worldwide use since the Second World War. However, resistance to chloroquine has become clinically significant in several areas of the world.[3, 4] Prior work has shown that either shortening or lengthening the linker of the alkyl amine side chain in chloroquine leads to compounds that remain effective against drug-resistant strains of *P. falciparum*.[5-7] Unfortunately many of these modifications have led to disadvantageous changes in the metabolism of these compounds, especially increased dealkylation of the distal amine. Dealkylation causes reduced lipid solubilities of the drugs and increased cross-resistance with chloroquine.[8] Contrawise, modifications of the substitutions on the distal amine side chain to bulkier groups have been shown to increase *in vivo* efficacy and decrease cross-resistance with chloroquine, presumably by circumventing metabolic dealkylation.[9] Bis-quinoline derivatives have also shown activity against chloroquine resistant strains of *P. falciparum*, but these compounds are known DNA intercalators and often cytotoxic.[10] In



3.1

Figure 3.1. Structure of 7-chloro-4-aminoquinoline library 3.1.

consideration of these issues, we wished to explore the tolerance for various bulky substitutions on the distal amine group of shortened side-chain chloroquine analogs.

Our current aim is to synthesize a new series of chloroquine analogs with a shortened side chain and diverse functionalities on the alkyl amine side chain to develop a structure-activity relationship (SAR) for these modifications (Fig 3.1). Synthetic methods that allow access to substituted amine groups are of high interest for medicinal chemistry since alkyl amine side chains are found in many drugs. We have developed a robust synthetic method that allows the introduction of two independent points of diversity to the amine of the side chain by sequential indirect reductive aminations.

Chemistry

Previous studies have shown that replacing the side chain of chloroquine with shorter analogs affords derivatives that are active against chloroquine resistant strains of *P. falciparum*.[5, 6, 9] Therefore, we fixed both the 7-chloroquinoline nucleus found in chloroquine and the shorter propyl amine side chain, while substituting the distal basic group to give a virtual library **3.1** (Fig 3.1). Retrosynthetically, we envisaged this library as arising from the double reductive amination of a pendant amine. A set of potential substitutions of the terminal amine group were chosen based on the commercial availability of the aldehyde reagents and used to populate a virtual library of all the theoretically possible products giving roughly 300,000 potential compounds. This virtual library was filtered to remove any poor screening candidates with non-organic atom

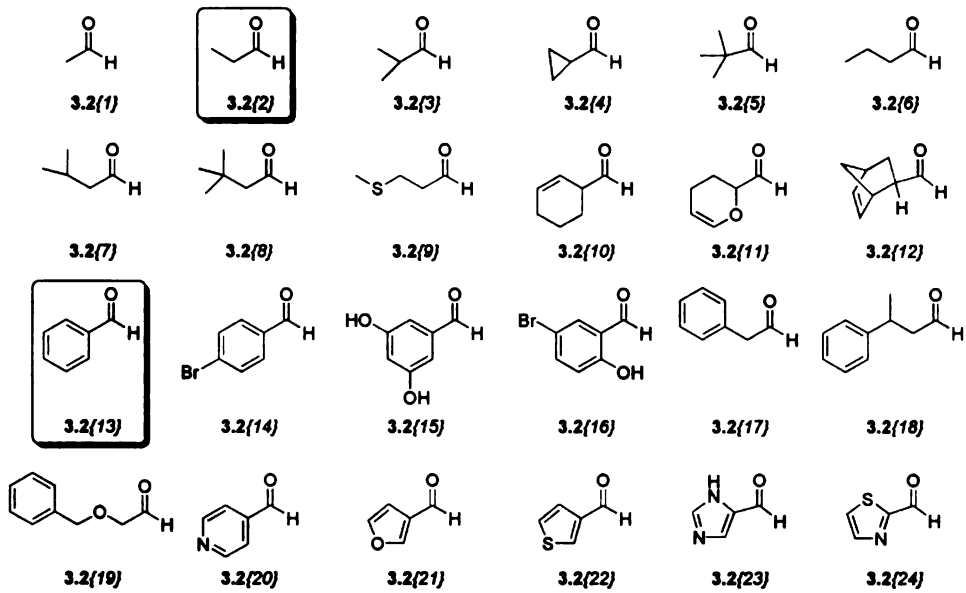
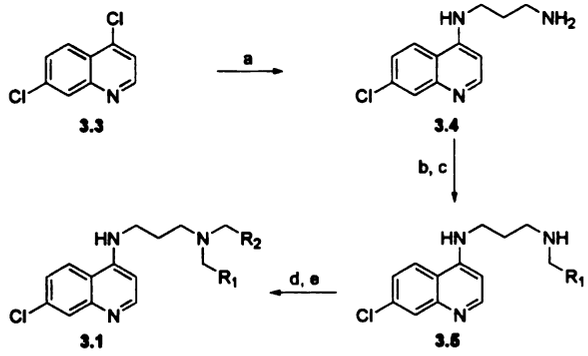


Figure 3.2 Structures of aldehyde diversity element 3.2*{1-24}*. Boxed elements represent the base structure used for R_2. All elements were used for R_1.

types; reactive substructures; or compounds not obeying Lipinski's "Rule of Five". Many of the compounds were relatively large by drug-likeness standards, therefore they were ranked by molecular weight. This procedure reduced the targeted library to roughly 850 members which were deconvoluted down to 97 aldehydes. When executed using protocols built in the Pipeline Pilot operating system, this filtering procedurerequired <10 min of computational time. Out of the top 50 aldehydes that passed through the screening filter as originators of this virtual library, 24 aldehydes were chosen by hand based upon chemistry compatibility, structural diversity and inclusion of drug-like substructures. (Fig 3.2) These 24 aldehydes were picked to sample the tolerance for diversity at this location and were combined with a second diversity element that represented either a small alkyl chain (propyl) or a bulky aromatic chain (benzyl). We report herein the synthesis and initial testing of the proofing library for this study.

Our synthesis strategy was developed to quickly generate a series of high-purity compounds for biological screening. This first proofing library was made both to test the scope of the chemistry and to optimize handling procedures larger libraries. We chose to employ parallel chemistry techniques so that we could increase our library size using the same handling procedures as in our proofing library. Since different reactions in the library give mixed purities, we elected to develop a general purification strategy so that we can purify any compounds of less than satisfactory purity.

The proofing library for this approach was constructed as outlined in Scheme 3.1. The bulk common intermediate (**3.4**) was prepared using a modification of De's method in which 4,7-dichloroquinoline was allowed to react with neat diaminopropane to afford **3.4.**[11] In initial studies we consistently achieved lower yields then reported due to the formation of a coarse particulate precipitate during the wash step of the work-up. Analysis of this precipitate revealed it was made up predominately of the desired product, co-precipitated with a tarry byproduct. The precipitate was recovered by filtration and purified by continuous solid-liquid extraction in a Soxhlet extractor to leach the desired product from the side products. When both crops of the product were recombined, the



Scheme 3.1. Synthesis of 4-amino-7-chloroquinoline library 3.1*{1-24;2,13}*. a) 1,3-diaminopropane (neat), reflux, 2 h, 83% yield; b) R_1CHO (3.2*{1-24}*, 4 eq.), MeOH, rt, 24 h; c) $NaBH_4$ (5 eq.), rt., 1 h; d) R_2CHO (3.2*{2,13}*, 4 eq.), MeOH, rt, 24 h; e) $NaBH_4$ (5 eq.), rt, 1 h.

procedure proceeded in an overall yield of 83%.

The primary amine intermediate (**3.4**) was subjected to two sequential reductive aminations to introduce two points of chemical diversity. Reductive aminations have been commonly used in combinatorial chemistry due to the reliability of the reaction, commercial availability of aldehyde building blocks, and process simplicity.[12] Although reductive aminations are a commonly employed reaction in combinatorial chemistry, we are not aware of any examples of doing two successive reductive aminations to make dissymmetrically substituted tertiary amines. The challenge of doing two sequential reductive aminations lies in the fact that reductive aminations of primary amines usually afford a tertiary amine side product from double addition of the aldehyde under the reducing conditions due to the increased nucleophilicity of the secondary amine product. While this side product is minor and usually can be separated, our final products are also all tertiary amines and were difficult to separate from the side products using the common conditions imposed logistically by the library purification process.

In order to avoid the need to purify our secondary amine intermediate, we explored numerous reductive amination conditions to eliminate the formation of the tertiary amine side product. A survey of reducing conditions, in the presence or absence of various dehydrating reagents, revealed no conditions that produced only the monosubstituted product. Previous reports in the literature have circumvented this problem by carrying out a stepwise reductive amination procedure with a "preformed imine" and no excess aldehyde.[12] This procedure did eliminate tertiary amine formation, but in many cases resulted in incomplete reaction progress leaving a mixture of the secondary amine and the primary amine starting material. The conditions that provided

the best combination of yield of desired product and suppression of the undesired symmetrically substituted amine were: 1) formation of the imine with excess aldehyde and no dehydrating reagent in anhydrous methanol, followed by 2) concomitant reduction of the imine and the excess aldehyde using sodium borohydride. This procedure produced a primary alcohol side product, arising from reduction of the excess aldehyde, in the crude reaction mixture. However, this side product did not interfere with the subsequent reductive amination in our reaction sequence and was easily separated from our desired tertiary amine products by our final purification procedure. We have also explored the use of solid-phase extraction (SPE) methods using a strong-cation exchange (SCX) resin to remove the alcoholic byproducts. This optional prepurification method was generally successful in partially purifying our desired product, but we achieved comparable purities without this intermediate purification step (data not shown). Depending on purity standards for screening libraries, such a SPE technique could potentially eliminate the need for preparative HPLC purification. The use of excess aldehyde in the reductive amination also made the reaction setup more convenient since maintaining an exact stoichiometric ratio between the amine and aldehyde was no longer necessary. Also, methanol was found to be the optimal solvent choice since all of our reagents were sufficiently soluble in it and it has been shown to facilitate the most rapid formation of the imine.[12] Thus in library production, intermediate **3.4** was split into 48 portions and treated in parallel with aldehyde diversity reagents **3.2{1-24}** then the reducing reagent, to give the crude chloroquine derivatives **3.5{1-24,H}.** The imine formation step was allowed to go for 24 hours since literature measurements of the kinetics of this reaction show reaction times varying from minutes to several hours, especially for sterically

crowded aldehydes, imine formation can be quite slow.[12] Finally, each of these intermediates was treated in parallel with aldehyde diversity reagents **3.2{2,13}** to give the crude chloroquine derivatives **3.1{1-24;2,13}.**

After the sequential amination procedure, the crude reaction mixtures were filtered of any remaining inorganic salts and the solutions were dried by evaporation *in vacuuo*. The crude mixtures were then directly purified by automated reversed-phase HPLC.[13] The crude materials were dissolved into a loading buffer containing a small amount of the aqueous mobile phase in order to equilibrate the products to a constant pH. We explored several different loading conditions, including non-aqueous acid-workups, salt formation, and loading solvents. To optimize the chromatographic separation quality, we found that it was beneficial to pre-equilibrate the compounds to the high pH used for the separations. Other changes in loading conditions showed little or no effect on the purity and yields post-chromatography. Fraction collection was triggered by a threshold UV absorption at 254 nm. The resulting fractions were analyzed by flow injection electrospray mass spectrometry and fractions with the desired products were pooled together and dried by evaporation *in vacuuo*. Each purified product was then re-dissolved in a saturated methanolic solution of HCl to afford the HCl salts. Finally, solvent was removed to give the purified water-soluble forms of each compound. The final yield of each product was determined at this point by weighing.

All compounds were analyzed to determine purity and yields prior to biological testing. The overall yields were moderate with a 29% average yield, with losses primarily attributed to purification and handling processes. The overall yields could be improved by more careful and time-consuming handling and purification procedures, but our

ultimate goal was to quickly and efficiently produce compounds of high purity in sufficient quantity for biological testing. Purity was assessed by analytical HPLC (C18 column) and monitored by a photodiode array (PDA) detector from 210 nm – 490 nm (**Fig 3.3**) . The average purity for the compound library was 91% with only 4 of the 48 compounds failing to meet our purity standard of 80%. Of these, one **5.1{2,3}** was determined to be of insufficient purity for assay.

The total amount of time spent creating this 48-member library, including purification and analysis, was about five days. Two days were spent carrying out the synthesis, four hours were spent doing the purification, and two days were spent doing fraction identification and sample drying. The actual number of man-hours spent working on the compounds is minimal since most of the steps are easily automated by commonly used robotics.

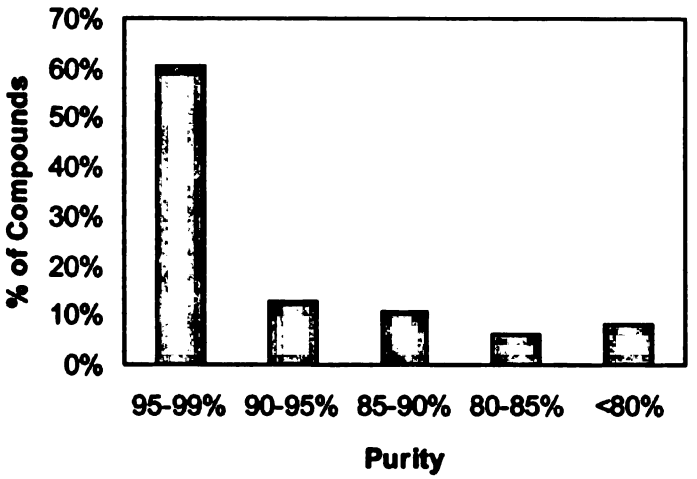


Figure 3.3. Histogram of purities of product library 5.1{2,13;1–24} after purification by reversed-phase HPLC. Purity was assessed by analytical HPLC analysis monitored at 220 nm on a reversed-phase C18 column (Column Engineering, ReliaSil C18 BDX, 5 μM).

Activities

All compounds were screened for antimalarial activity using *in vitro* cultures of *Plasmodium falciparum* strain 3D7 at two doses: 30 nM and 200 nM.[14, 15] One compound **3.1{2,2}** was lost during the screening process to a liquid handling error, therefore data are reported for 46 of the targeted 48 members of chemset **3.1**. Chloroquine, which has a reported EC_{50} of about 20 nM,[16] was used as a positive control. 4-amino-7-chloroquinoline, which is a structurally related inactive compound,[17] was used as a negative control. The data is summarized in **Figure 3.4**. The members of the library exhibited a wide range of antimalarial activity, completely spanning that defined by the controls. When one alkyl group of the side chain was held constant as a propyl, a number of derivatives, including several with bulky aliphatic or aromatic rings, were at least as active as chloroquine. In contrast, when one alkyl group was held constant as the more

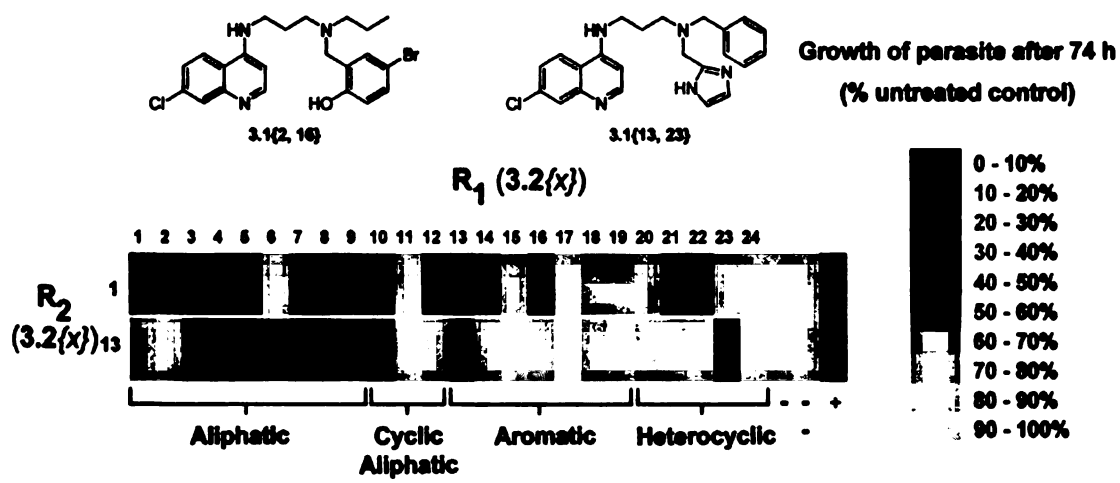


Figure 3.4. Relative activity of library 3.1{2,13;1–24} against Plasmodium falciparum 3D7 in cultured human erythrocytes. Substituents for R_1 are arrayed on the X-axis, broken into groups representing the general structural class of substituents. Substituents for R_2 are shown on the Y-axis. Grayscale shade represents the percent of parasite growth at 30 nm drug concentration relative to untreated cultures. "-" refers to cultures treated with no drug. "- -" refers to cultures treated with 30 nM negative control compound 4-amino-7-chloroquinoline. "+" refers to cultures treated with 30 nM chloroquine. The structures of two representative active library members are shown above the graph with their activities indicated by the filled triangles.

sterically demanding benzyl, almost no derivatives had significant activity. Strikingly one compound, **3.1**{*13,23*} exhibited activity with two bulky benzylic groups attached to the amine. The inactivity of the corresponding propyl derivative points to an unexpected synergy between the benzyl and imidazolylmethyl substituents and highlights the potential for cooperativity. A number of the compounds were active to within 2-fold of the potency of chloroquine.

	Strains		
Compound	3D7	W2	Dd2
CQ	25	250	250
3.1{16,2}	45	45	45
3.1{21,2}	50	70	45
3.1{22,2}	50	50	45
3.1{23,13}	50	130	150

Table 3.1. IC_{50} values of the four most active compounds and CQ against the 3D7, W2 and Dd2 *P. falciparum* strains.

The four most active compounds from our initial screen were assayed against a panel of three *P. falciparum* strains with varying degrees of drug resistance. The drug-resistant strains we used were W2 and Dd2, which are both originally from Indochina. Both of these strains are resistant to all known quinoline drugs. The IC50's clearly show the superior activity of the new compounds, as compared to chloroquine, against both of the drug-resistant strains. Interestingly, the compound **3.1**{*23,13*} containing two bulky aromatic groups had substantially inferior activity in W2 and Dd2 when compared with the other three compounds containing a small propyl group, despite having comparable

activity against the 3D7 strain. Given the rather small number of compounds included in this study and the fact that they were designed to enforce maximal diversity rather than to probe congeneric series carefully to reveal detailed structure activity relationships, it is premature to comment generally upon the function of the alkyl groups decorating the amine. Two unexpected features do deserve comment. First, the only compound with two bulky groups that had strong activity included a third basic site on one of the alkyl groups. This leads one to believe that two bulky groups tends to suppress activity but that this trend can be overcome by biasing the equilibrium partition of the compound into the acidic food vacuole where the drugs ct. Perhaps the most interesting feature is that all of the most active compounds against drug-resistant strains contain a hydrogen bond acceptor on the alkyl substituent attached to the distal basic group. The basic group will be protonated at physiological pH, allowing it to form an intramolecular hydrogen bond with the groups substituted on the amine. Both of these features will be included as design elements in subsequent studies.

Conclusions

The goal of this study was to develop a robust parallel synthesis / purification procedure for the production of moderate sized libraries (100's) of quinoline derivatives that will ultimately be coupled to similar approaches that allow variation of substituents on the A and B rings of the quinoline nucleus to afford large diverse screening libraries. The procedure reported herein is efficient, giving access to greater than 90% of the targeted compounds in greater than 80% purity using generalized procedures that have been adapted to the parallel format and automation. This should be sufficiently robust to

allow production of the desired screening libraries. Biological testing of the compounds produced in this proofing study revealed that a number of previously unaddressed substitutions at the distal base of chloroquine afford active antimalarials, even against multiple drug resistant strains. Additionally, this study revealed a previously unrecognized potential for synergistic advantage to the addition of two benzylic groups to the distal amine that is worthy of further study. The production of larger libraries of quinolines is underway and their synthesis and activity shall be reported in due course.

Experimental

All reagents and starting materials were purchased from commercial sources and used without further purification; solvents were anhydrous HPLC grade. All parallel synthesis steps were carried out in polypropylene fritted FlexChem© 48-well reaction blocks. ^{1}H-NMR spectra were recorded using a Varian 400 MHz spectrometer. Chemical shifts were measured in parts per million (δ) relative to tetramethylsilane (TMS) as the internal standard. Coupling constants (*J* values) were measured in hertz (Hz).

N1-(7-Chloro-quinolin-4-yl)-propane-1,3-diamine (3.4). A solution of 4,7-dichloroquinoline (25 g, 0.126 mol) in 1,3-diaminopropane (47 mL, 0.568 mol) was heated to reflux for 4 hours with stirring, and then allowed to cool to room temperature. The solution was diluted with methylene chloride (400 mL) and the resulting mixture was washed with sodium hydroxide (1 *N*, 400mL) and brine (400 mL), to give an aqueous layer, an organic layer, and a white course particulate precipitate (9.5 g). The organic layer was dried *in vacuuo* to give the product **3.4** as on off-white solid. The precipitate

from the wash was filtered and extracted in a Soxhlet extractor with methylene chloride for 3 days. The methylene chloride was then removed *in vacuuo* to yield a second crop of **3.4** as an off-white solid (8.6 g). ^{1}H-NMR analysis indicated the two solids were identical and pure, so they were combined and used without further purification (24.6 g, 0.104 mol, 83% yield); ^{1}H NMR (CD_3OD): δ 8.33 (d, J = 5.9 Hz, 1H), δ 8.05 (d, J = 8.8 Hz, 1H), δ 7.76 (d, J = 2.0 Hz, 1H), δ 7.36 (dd, J = 8.8, 2.0 Hz, 1H), δ 6.50 (d, J = 5.9, 1H), δ 4.90 (s, 2H), δ 3.40 (t, J = 6.8, 2H), δ 2.81 (t, J = 6.8, 2H), δ 1.90 (dt, J = 5.9, 5.9, 2H). ^{13}C NMR (CD_3OD): δ 152.9, 152.6, 149.9, 136.5, 127.8, 126.1, 124.5, 119.0, 99.9, 42.0, 40.5, 32.2. MS: ESI+ 236.3 [M + 1] Calc. Mass: 235.1.

General Procedure for Preparation of Chemset 3.1. N1-(7-Chloro-quinolin-4-yl)-propane-1,3-diamine (**3.4**) (1.44 g, 6.11 mmol) was dissolved in dry methanol (120 mL) to make a 50.9 mM stock solution. The stock solution was aliquoted to the wells of the 48-well reaction block (2.5 mL/well, 0.13 mmol/well). To each well was then added aldehydes **3.2** (4 equiv., 0.52 mmol). After clamping the block shut and rotating the block for 24 hours at room temperature, solid sodium borohydride (5 equiv.) was added to each well resulting in copious evolution of gas. CAUTION: Explosion hazard. The block was left open for 30 minutes to allow complete evolution of gas, then clamped shut and rotated for 2 hours to yield the secondary amine intermediate (**3.5**). The procedure was then repeated with a second aldehyde to yield the final the tertiary amine **3.1**. The crude reaction mixtures were then collected into a 48-position deep well plate and solvent was removed on a GeneVac HT-4 (50 mbar, 3.5 hours, 35 °C).

General Procedure for Purification of Chemset 3.1. Crude library members were dissolved in 1.8 mL of a loading buffer (90% methanol, 10% ammonium acetate buffer,

20 mM, pH 6.8) then purified with a preparative YMC ODS-AQ reversed-phase column (20 mm x 50 mm, particle size S-5) running a 10–100% gradient of ammonium acetate buffer (20 mM, pH 6.8)/ Methanol with a 25 mL/min flow rate on a Parallex Flex™ HPLC System. Chromatographs were monitored with a dual wavelength UV detector at 220 nm and 254 nm. Fraction collection was automatically triggered by UV absorption above 0.20 AU at 254 nm. Sample and fraction data was then transferred to the Waters OpenLynx operating software, which coordinated the injection, mass spectrometric analysis, and data processing for each fraction. A Gilson 215 liquid handler and a Gilson 208 injection module were used to inject samples into a Waters ZQ 4000 mass spectrometer rigged for flow injection with an electrospray probe and single quadrapole detector operating in positive ion mode. All fraction plates were then dried down using GeneVac Mega 980 solvent evaporator. Fractions that exhibited a peak in the mass spectra of the correct molecular weight were then dissolved in 1mL of freshly prepared saturated HCl in methanol and like fractions were combined. Combined fractions were then transferred to pre-weighed vials, solvent was removed on the GeneVac HT-4, and each vial was weighed to calculate final yields of the HCl salts of each compound.

N'-(7-Chloro-quinolin-4-yl)-N-ethyl-N-propyl-propane-1,3-diamine (3.1{1,2}) LCMS (ESI) *m/z* Calcd for $C_{17}H_{24}ClN_3$ $[M + H]^+$ 306.2. Found: 306.0.^{1}H NMR (CD_3OD): δ 8.49 (d, J = 8.7, 1H), 8.46 (d, J = 6.8, 1H), 7.90 (d, J = 1.9, 1H), 7.72 (dd, J = 8.7, J = 1.9, 1H), δ 6.98 (d, J = 6.8, 1H), 3.74 (t, J = 6.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.14 (m, 2H), 2.22 (m, 2H), 1.77 (m, 2H), 1.35 (t, J = 6.8, 3H), 1.03 (t, J = 6.8, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-isobutyl-N-propyl-propane-1,3-diamine (3.1{3,2}) LCMS (ESI) *m/z* Calcd for $C_{19}H_{28}ClN_3$ $[M + H]^+$ 334.2. Found: 333.9. ^{1}H NMR

(CD_3OD): δ 8.57 (d, J = 8.8, 1H), 8.47 (d, J = 6.8, 1H), 7.91 (d, J = 1.9, 1H), 7.72 (dd, J = 8.8, J = 1.9, 1H), 7.02 (d, J = 6.8, 1H), 3.75 (m, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.16 (m, 2H), 3.04 (d, J = 5.8, 2H), 2.28 (m, 2H), 2.15 (m, 1H), 1.75 (m, 2H), 1.06 (d, J = 5.8, 6H), 1.00 (t, J = 6.8, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-cyclopropylmethyl-N-propyl-propane-1,3-diamine (3.1{4,2}) LCMS (ESI) *m/z* Calcd for $C_{19}H_{26}ClN_3$ $[M + H]^+$ 332.2. Found: 332.0. ^1H NMR (CD_3OD): δ 8.52 (d, J = 8.8 Hz, 1H), 8.46 (d, J = 5.9 Hz, 1H), 7.90 (d, J = 1.9, 1H), 7.00 (dd, J = 5.9, J = 1.9, 1H), 3.75 (t, J = 4.88, 2H), 3.42 (m, 2H), 3.42 (m, 2H), 3.20 – 3.35 (m, overlap w/solvent), 3.13 (d, J = 6.8, 2H), 2.26 (m, 2H), 1.80 (m, 2H), 1.16 (m, 1H), 1.03 (t, J = 6.8, 3H), 0.75 (m, 2H), 0.46 (m, 2H).

N'-(7-Chloro-quinolin-4-yl)-N-(3-methyl-butyl)-N-propyl-propane-1,3-diamine (3.1{7,2}) LCMS (ESI) *m/z* Calcd for $C_{20}H_{30}ClN_3$ $[M + H]^+$ 348.2. Found: 348.1. ^1H NMR (CD_3OD): δ 8.52 (d, J = 9.7, 1H), 8.46 (d, J = 6.8, 1H), 7.90 (d, J = 1.9, 1H), 7.72 (dd, J = 8.8, J = 1.9, 1H), 7.00 (d, J = 6.8, 1H), 3.74 (t, J = 6.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.18 (m, 4H), 2.25 (m, 2H), 1.77 (m, 2H), 1.64 (m, 3H), 1.02 (t, J = 6.8, 3H), 0.96 (d, J = 6.8, 6H).

N'-(7-Chloro-quinolin-4-yl)-N-(3,3-dimethyl-butyl)-N-propyl-propane-1,3-diamine (3.1{8,2}) LCMS (ESI) *m/z* Calcd for $C_{21}H_{32}ClN_3$ $[M + H]^+$ 362.2. Found: 361.9. ^1H NMR (CD_3OD): δ 8.57 (d, J = 9.7, 1H), 8.47 (d, J = 5.8, 1H), 7.91 (d, J = 1.9, 1H), 7.72 (dd, J = 8.8, J = 1.9, 1H), 7.02 (d, J = 6.8, 1H), 3.76 (m, 2H), 3.25 – 3.40 (m, overlap w/solvent), 3.21 (m, 4H), 2.26 (m, 2H), 1.79 (m, 2H), 1.65 (m, 2H), 0.93 – 1.04 (m, 12H).

N-(4-Bromo-benzyl)-N'-(7-chloro-quinolin-4-yl)-N-propyl-propane-1,3-diamine (3.1{14,2}) LCMS (ESI) *m/z* Calcd for $C_{22}H_{25}BrClN_3$ $[M + H]^+$ 446.1. Found: 446.0. ^1H NMR (CD_3OD): δ 8.45 (d, J = 6.8, 1H), 8.40 (d, J = 8.8, 1H), 7.92 (d, J = 1.9, 1H), 7.74 (dd, J = 8.8, J = 1.9, 1H), 7.45 (m, 4H), 6.91 (d, J = 6.8, 1H), 4.43 (d, J = 12.6, 1H), 4.32 (d, J = 12.6, 1H), 3.69 (t, J = 4.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.16 (t, J = 8.8, 2H), 2.27 (m, 1H), 2.20 (m, 1H), 1.85 (m, 2H), 1.01 (t, J = 7.8, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-propyl-N-thiophen-3-ylmethyl-propane-1,3-diamine (3.1{22,2}) LCMS (ESI) *m/z* Calcd for $C_{20}H_{24}ClN_3S$ $[M + H]^+$ 374.1. Found: 373.8. ^1H NMR (CD_3OD): δ 8.47 (d, J = 8.8, 1H), 8.45 (d, J = 6.8, 1H), 7.91 (d, J = 1.9, 1H), 7.72 (m, 2H), 7.48 (m, 1H), 7.27 (d, J = 4.8, 1H), 6.95 (d, J = 6.8, 1H), 4.44 (s, 2H), 3.71 (t, J = 5.7, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.11 (m, 2H), 2.27 (m, 2H), 1.83 (m, 2H), 0.99 (t, J = 6.8, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-(3H-imidazol-4-ylmethyl)-N-propyl-propane-1,3-diamine (3.1{23,2}) LCMS (ESI) *m/z* Calcd for $C_{19}H_{24}ClN_5$ $[M + H]^+$ 358.2. Found: 358.0. ^1H NMR (CD_3OD): δ 9.07 (s, 1H), 8.58 (d, J = 8.8, 1H), 8.46 (m, 1H), 8.01 (m, 1H), 7.91 (d, J = 1.9, 1H), 7.70 (dd, J = 8.8, J = 1.9, 1H), 7.04 (d, J = 8.8, 1H), 4.67 (s, 2H), 3.78 (m, 2H), 3.44 (m, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.21 (m, 2H), 2.38 (m, 2H), 1.89 (m, 2H), 1.03 (t, J = 5.8, 3H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-isobutyl-propane-1,3-diamine (3.1{3,13}) LCMS (ESI) *m/z* Calcd for $C_{23}H_{28}ClN_3$ $[M + H]^+$ 382.2. Found: 381.9. ^1H NMR (CD_3OD): δ 8.45 (m, 2H), 7.91 (d, J = 1.9, 1H), 7.73 (dd, J = 8.8, J = 1.9, 1H), 7.53 (m, 2H),7.40 (m, 3H), 6.96 (d, J = 6.8, 1H), 4.43 (sb, 2H), 3.72 (t, J = 6.8, 2H), 3.25 – 3.25

(m, overlap w/solvent), 2.33 (m, 2H), 2.10 (m, 1H), 1.04 (d, J = 6.8, 3H), 0.96 (d, J = 6.8, 3H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-cyclopropylmethyl-propane-1,3-diamine (3.1{4,13}) LCMS (ESI) *m/z* Calcd for $C_{23}H_{26}ClN_3$ $[M + H]^+$ 380.2. Found: 380.0. ^1H NMR (CD_3OD): δ 8.44 (m, 2H), 7.91 (d, J = 1.95, 1H), 7.71 (dd, J = 8.8, J = 1.9, 1H), 7.54 (m, 2H), 7.35 (m, 3H), 6.94 (d, J = 6.8, 1H), 4.54 (d, J = 12.7, 1H), 4.40 (d, J = 12.7, 1H), 3.71 (t, J = 6.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.13 (m, 2H), 2.28 (m, 2H), 1.23 (m, 1H), 0.76 (d, J = 7.8, 2H), 0.43 (m, 2H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-(2,2-dimethyl-propyl)-propane-1,3-diamine (3.1{5,13}) LCMS (ESI) *m/z* Calcd for $C_{24}H_{30}ClN_3$ $[M + H]^+$ 396.2. Found: 396.0. ^1H NMR (CD_3OD): δ 8.48 (d, J = 8.8, 1H), 8.47 (d, J = 6.8, 1H), 7.91 (d, J = 1.9, 1H), 7.74 (dd, J = 8.8, J = 1.9, 1H), 7.58 (m, 2H), 7.44 (m, 3H), 6.99 (d, J = 6.8, 1H), 4.50 (d, J = 12.6, 1H), 4.39 (d, J = 12.6, 1H), 3.74 (t, J = 6.8, 2H), 3.43 (t, J = 7.8, 2H), 3.15 – 3.30 (m, overlap w/solvent), 2.40 (m, 2H), 0.98 (s, 9H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-(3-methyl-butyl)-propane-1,3-diamine (3.1{7,13}) LCMS (ESI) *m/z* Calcd for $C_{24}H_{30}ClN_3$ $[M + H]^+$ 396.2. Found: 396.1. ^1H NMR (CD_3OD): δ 8.44 (d, J = 6.8, 1H), 8.43 (d, J = 8.8, 1H), 7.91 (d, J = 1.9, 1H), 7.73 (dd, J = 8.8, J = 1.9, 1H), 7.53 (m, 2H), 7.35 (m, 3H), 6.93 (d, J = 6.8, 1H), 4.41 (d, J = 3.9, 2H), 3.70 (t, J = 6.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.19 (m, 2H), 2.27 (m, 2H), 1.67 (m, 3H), 0.92 (d, J = 4.8, 6H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-(3,3-dimethyl-butyl)-propane-1,3-diamine (3.1{8,13}) LCMS (ESI) *m/z* Calcd for $C_{25}H_{32}ClN_3$ $[M + H]^+$ 410.2. Found: 409.9. ^1H NMR (CD_3OD): δ 8.45 (m, 2H), 7.91 (d, J = 1.9, 1H), 7.73 (dd, J = 8.8, J = 1.9, 1H), 7.54

(m, 2H), 7.37 (m, 3H), 6.94 (d, J = 6.8, 2H), 4.41 (sb, 2H), 3.71 (t, J = 6.8, 2H), 3.25 – 2.35 (m, overlap w/solvent), 3.21 (m, 2H), 2.26 (m, 2H), 1.70 (m, 2H), 0.92 (s, 9H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-(3-methylsulfanyl-propyl)-propane-1,3-diamine (3.1{9,13}) LCMS (ESI) *m/z* Calcd for $C_{23}H_{28}ClN_3S$ $[M + H]^+$ 414.2. Found: 413.7. ^1H NMR (CD_3OD): δ 8.45 (d, J = 6.8, 1H), 8.41 (d, J = 8.8, 1H), 7.91 (d, J = 1.9, 1H), 7.73 (dd, J = 8.8, J = 1.9, 1H), 7.53 (m, 2H), 7.37 (m, 3H), 6.94 (d, J = 6.8, 1H), 4.42 (d, J = 3.9, 2H), 3.71 (t, J = 5.8, 2H), 3.20 – 3.35 (m, overlap w/solvent), 2.56 (t, J = 6.8, 2H), 2.26 (m, 4H), 2.12 (m, 2H), 2.07 (s, 3H).

N,N-Dibenzyl-N'-(7-chloro-quinolin-4-yl)-propane-1,3-diamine (3.1{13,13}) LCMS (ESI) *m/z* Calcd for $C_{26}H_{26}ClN_3$ $[M + H]^+$ 416.2. Found: 415.6. ^1H NMR (CD_3OD): δ 8.43 (d, J = 6.8, 1H), 8.35 (d, J = 8.8, 1H), 7.91 (d, J = 1.9, 1H), 7.71 (dd, J = 8.8, J = 1.9, 1H), 7.52 (m, 4H), 7.37 (m, 6H), 6.87 (d, J = 6.8, 1H), 4.42 (sb, 4H), 3.64 (t, J = 6.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.26 (m, 2H), 2.27 (m, 2H).

N-Benzyl-N-(4-bromo-benzyl)-N'-(7-chloro-quinolin-4-yl)-propane-1,3-diamine (3.1{14,13}) LCMS (ESI) *m/z* Calcd for $C_{26}H_{25}BrClN_3$ $[M + H]^+$ 494.1. Found: 494.0. ^1H NMR (CD_3OD): δ 8.45 (d, J = 6.8, 1H), 8.30 (d, J = 9.7, 1H), 7.91 (d, J = 1.9, 1H), 7.74 (dd, J = 8.8, J = 1.9, 1H), 7.53 (m, 2H), 7.48 (d, J = 8.8, 2H), 7.41 (d, J = 8.8, 2H), 7.39 (m, 3H), 6.86 (d, J = 6.8, 1H), 4.44 (sb, 2H), 4.30 (d, J = 16.6, 2H), 3.64 (t, J = 6.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.21 (m, 2H), 2.26 (m, 2H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-(3-phenyl-butyl)-propane-1,3-diamine (3.1{18,13}) LCMS (ESI) *m/z* Calcd for $C_{29}H_{32}ClN_3$ $[M + H]^+$ 458.2. Found: 457.5. ^1H NMR (CD_3OD): δ 8.44 (d, J = 6.8, 1H), 8.40 (d, J = 8.8, 1H), 7.91 (d, J = 1.9, 1H), 7.77 (dd, J = 8.8, J = 1.9, 1H), 7.17 – 7.40 (m, 10H), 6.90 (d, J = 6.8, 1H), 4.40 (d, J = 4.8,

2H), 3.66 (t, J = 6.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 3.15 (m, 1H), 2.84 (m, 1H), 2.73 (m, 1H), 2.10 (m, 3H), 1.28 (d, J = 6.8, 3H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-pyridin-4-ylmethyl-propane-1,3-diamine (3.1{20,13}) LCMS (ESI) *m/z* Calcd for $C_{25}H_{25}ClN_4$ $[M + H]^+$ 417.2. Found: 417.0. ^1H NMR (CD_3OD): δ 8.87 (d, J = 4.8, 2H), 8.44 (dd, J = 8.8, J = 6.8, 2H), 8.38 (d, J = 4.8, 2H), 7.91 (d, J = 1.9, 1H), 7.72 (dd, J = 8.8, J = 1.9, 1H), 7.55 (m, 2H), 7.29 (m, 3H), 6.93 (d, J = 6.8, 1H), 4.45 (m, 4H), 3.69 (t, J = 6.8, 2H), 3.25 – 3.35 (m, overlap w/solvent), 2.40 (m, 2H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-(3H-imidazol-4-ylmethyl)-propane-1,3-diamine (3.1{23,13}) LCMS (ESI) *m/z* Calcd for $C_{23}H_{24}ClN_5$ $[M + H]^+$ 406.2. Found: 405.6. ^1H NMR (CD_3OD): δ 9.036 (s, 1H), 8.46 (d, J = 8.8, 1H), 8.43 (d, J = 6.8, 1H), 7.97 (s, 1H), 7.92 (d, J = 1.9), 7.70 (dd, J = 8.8, J = 1.9, 1H), 7.60 (m, 2H), 7.30 (m, 3H), 6.92 (d, J = 6.8, 1H), 4.69 (s, 2H), 4.49 (s, 2H), 3.69 (m, 2H), 3.34 (m, 2H), 2.34 (m, 2H).

N-Benzyl-N'-(7-chloro-quinolin-4-yl)-N-thiazol-2-ylmethyl-propane-1,3-diamine (3.1{24,13}) LCMS (ESI) *m/z* Calcd for $C_{23}H_{23}ClN_4S$ $[M + H]^+$ 423.1. Found: 422.5. ^1H NMR (CD_3OD): δ 8.45 (m, 2H), 7.93 (d, J = 1.9, 1H), 7.86 (d, J = 2.9, 1H), 7.72 (m, 2H), 7.59 (m, 2H), 7.39 (m, 3H), 6.94 (d, J = 6.8, 1H), 4.58 (m, 2H), 3.71 (t, J = 6.8, 2H), 3.44 (t, J = 6.8, 2H), 3.25 – 2.35 (m, overlap w/solvent), 2.38 (m, 2H).

Measurement of in Vitro Antimalarial Activity. The effects of compounds in library **3.1** on the growth of *Plasmodium falciparum* cultures *in vitro* were measured using flow cytometry.[18] Synchronous cultures of ring stage parasites (500 μL, 0.8% parasitemia, 2% hematocrit) were grown in 24-well or 96-well tissue culture plates (Falcon) with 30 nm and 200 nm concentrations of experimental compounds. Cultures were grown in

atmospherically regulated (6 % CO2, 5 %O2) incubators (Sanyo) at 37 °C for a total of 4 days. Each day additional media and drug were added to the cultures (300 μL on day 2, 700 μL on day 3, and 500 μL on day 4) to maintain nutrient-rich conditions necessary for rapid parasite growth. Aliquots of 100 μL were removed from each well at 74 hours post drug treatment and resuspended in 900 μL of 1 % paraformaldehyde, 1 nM YOYO-1 (Molecular Probes) in PBS. Each sample was then incubated for 16 h in the dark at 4 °C before being analyzed on a Becton-Dickenson LSR2 flow cytometer to measure the percent of parasitized red blood cells (RBCs). Percent of parasitized RBCs was directly read and subsequently growth inhibition values were calculated as the fraction of parasitized RBCs relative to cultures without drug. All screening was done in triplicate with two negative controls 1) no drug addition and 2) addition of non-active 4-amino-7-chloroquinoline compound) and a positive control, chloroquine. Growth inhibition was then measured using the same conditions as in the screen for the five most active compounds and chloroquine at 1, 5, 10, 15, 20, 30, 50, and 200 nm drug concentrations. Fifty percent inhibitory concentrations (IC_{50}s) were estimated by analyzing the resulting log dose response curves via visual extrapolation.

Virtual Library Filtering. All computations were carried out using the commercially available software Pipeline Pilot (SciTegic™, San Diego, CA) and were done in 6 minutes on a 1.2 GHz Pentium processor with 384 MB of RAM. The Lipinski filter was set to allow compounds with the following properties: Sum of N and O atoms ≤ 10, Molecular Weight ≤ 550, H-bond donors ≤ 5, and ALOGP ≤ 5. ALOGP was calculated using the Ghose/Crippen group-contribution estimate method.

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Chapter 4

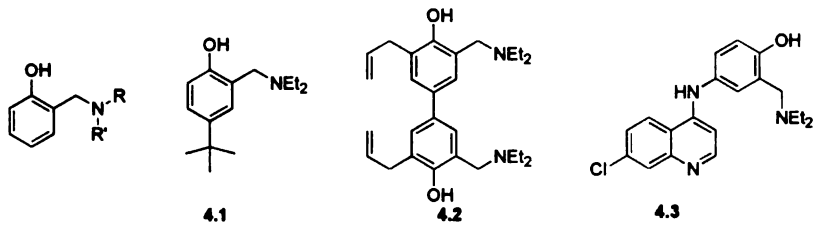
Evaluation of Side Chain Intramolecular Hydrogen-Bonding Motif

Overview

The previous finding that several chloroquine analogs containing an intramolecular hydrogen bonding motif on the side chain moiety were potent against multidrug-resistant *P. falciparum,* led to the exploration of the general importance of this motif. A series of 116 compounds were synthesized containing four different alkyl linkers and various aromatic substitutions with hydrogen bond accepting capability. The series showed broad potency against the drug-resistant W2 strain of *P. falciparum.* A novel subseries containing variations of the α-aminocresol motif gave 8 compounds with EC50's more potent 5 nM against the multidrug-resistant W2 strain. Such simple modifications significantly altering the pKa and sterics of the basic side chain in chloroquine analogs may prove to be part of a strategy for overcoming the problem of worldwide resistance to affordable antimalarial drugs.

Background

Prior work in our laboratory[1, 2] and others'[3, 4] systematically looked at modifications to both the quinoline ring and to the basic side chain of 4-aminoquinolines. While the structure-activity relationships showed reasonable tolerance for both types of modifications, the basic side chain modifications produced compounds with the greatest increase in potency towards drug-resistant parasite strains. Our studies revealed that a previously unappreciated structural motif -- the presence of a single aromatic ring containing a hydrogen-bond acceptor attached to the basic nitrogen --gave the most potent compounds. Interestingly, we noted a similar motif in the class of antimalarial compounds called α-aminocresols, first reported in the mid 1940's.[5, 6] (Fig 4.1)



α-aminocresol motif | 4.1 SN 7744 | 4.2 SN 6771 | 4.3 Amodiaquine

Figure 4.1. Intramolecular hydrogen bonds found in quinoline antimalarial compounds active against CQ-resistant *P. falciparum*.

Burckhalter et al. discovered that 4-*tert*-butyl-2-dimethylaminomethyl-phenol (4.1) was effective against trophozoite-induced avian malaria and made a library of over 100 analogs using the Mannich reaction.[5, 7] Since only *in vivo* screening was available at the time, it is difficult to compare the reported activities with those of modern *in vitro* screening methods. While bioavailability and metabolic stability strongly bias measurements of activity in any *in vivo* assay, qualitative comparisons amongst similar compounds are reasonable for interpreting a structure-activity relationship (SAR). The early work on α-aminocresols showed the importance of having the hydroxy group ortho to the α-amino group (*para*- α-aminocresols showed no activity) and the benefit of an additional hydrophobic substituent on the ring. Optimization of these compounds led to the more potent bis-α-aminocresols such as 4.2.[5] (Fig 4.1) Burckhalter later went on to attach his pharmacophore motif to the 7-chloroquinoline ring found in chloroquine to create the drug amodiaquine (4.3) which was far more potent than any of the original α-aminocresols.[7] Since the development of amodiaquine, little or no further work has been done on the α-aminocresols apart from modification to amodiaquine.

The success of chloroquine diverted attention away from Amodiaquine until the emergence of drug-resistance was reported by Moore and Lanier in 1961.[8-10] The US Army then renewed its antimalarial screening program and re-discovered amodiaquine

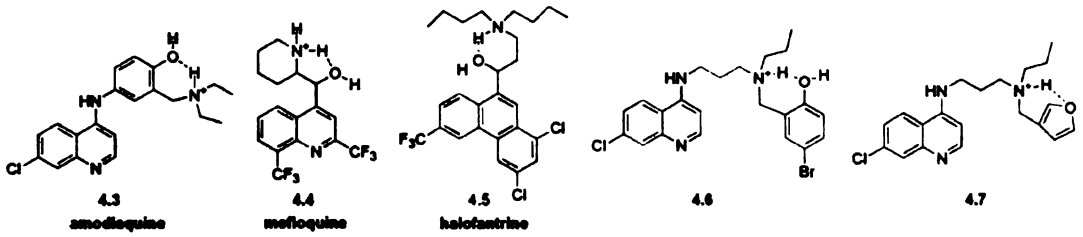


Figure 4.2. Intramolecular hydrogen-bonding of antimalarials active against drug-resistant *P. falciparum*.

along with the quinolinemethanol class of drugs. The quinolinemethanols are derived from quinine and were optimized into the drug mefloquine (4.4).[11] The common structural feature shared by both these compounds is a basic nitrogen within hydrogen-bonding proximity to a hydroxyl group. Since at physiological pH the basic nitrogen will be protonated, it is possible that the intramolecular hydrogen bonding between the protonated amine (H-bond donor) and the hydroxyl (H-bond acceptor) may be an important feature for activity against chloroquine-resistant *P. falciparum*.

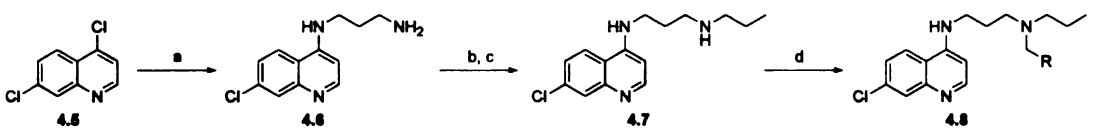
We hypothesized that this motif might provide a general approach to the discovery of novel and potent compounds and therefore synthesized a larger set of compounds containing an intramolecular hydrogen-bonding motif in the basic side-chain of the 4-aminoquinoline nucleus in order to test the hypothesis. These compounds were synthesized as a parallel library of purified discrete compounds which were then subjected to screening, followed by more detailed studies for active compounds.

Chemistry

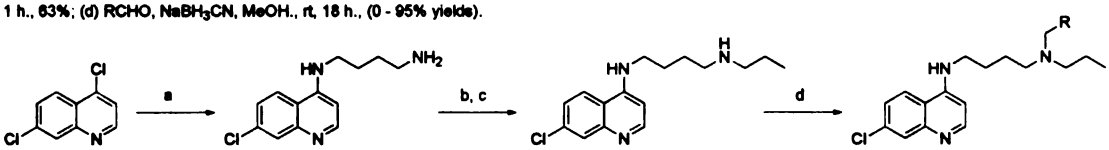
We elected to fix the scaffold as a 7-chloroquinoline ring since it remains the most generally potent ring system choice and other modifications do little to increase potency against the drug-resistant strains. We designed our compounds to have four

different alkyl groups linking together the basic nitrogen center to the quinoline ring. It has been shown that the length of this linker region is important for potency against CQ-resistant *P. falciparum*, with side-chains less than four methylene units or greater than six methylene units having the greatest potency.[4, 12] Out of the four alkyl linkers, we chose two that are linear chains of three and four methylene groups and the other two are cyclic chains containing either a pyrrolidinyl- or piperidinyl- group. For each of these four linkers, a key secondary amine intermediate was synthesized in bulk and then the nitrogen was reductively alkylated by a set of aldehydes. (Scheme 4.1)

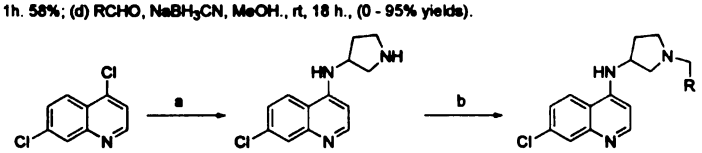
The synthesis of the secondary amine intermediates for the compounds with the propyl and butyl linkers were done similarly. First the 4,7-dichloroquinoline ring was



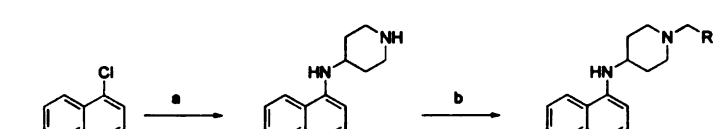
Reagents and conditions: (a) 1,3-diaminopropane, reflux, 1h, 84%; (b) propionic anhydride, $CHCl_3$, pyridine, 0°C, 20 min., 90%; (c) BH_3·DMS, THF, reflux, 1 h., 63%; (d) RCHO, $NaBH_3CN$, MeOH., rt, 18 h., (0 - 95% yields).



Reagents and conditions: (a) 1,4-diaminobutane, reflux, 1h, 78%; (b) propionic anhydride, $CHCl_3$, pyridine, 0 °C, 20 min., 92%; (c) BH_3·DMS, THF, reflux, 1h. 58%; (d) RCHO, $NaBH_3CN$, MeOH., rt, 18 h., (0 - 95% yields).



Reagents and conditions: (a) 3-aminopyrrolidine dihydrochloride (2 eq.), iPr_2Et, reflux, 4h. 70%; (b) RCHO, $NaBH_3CN$, MeOH, rt, 18 h., (0 - 95% yields).



Reagents and conditions: (a) 4-aminopiperidine (8 eq.), iPr_2Et, 100 °C, 20 h., 65%; (b) RCHO, $NaBH_3CN$, MeOH, rt, 18 h., (0 - 95% yields).

Scheme 4.1 Synthesis of side-chain modified 4-aminoquinolines.

reacted with either 1,3-diaminopropane or 1,4-diaminobutane by refluxing in the neat amine. The doubly arylated diamine side-products were easily removed by an acidic wash step. The primary amine products were then reacted with propionic anhydride to rapidly form the amide, which conveniently precipitated out of solution. The amide was reduced using borane-dimethylsulfide in THF to give the key secondary amine intermediate. The two intermediates with the cyclic side chains were synthesized in a single step through direct coupling of 4,7-dichloroquinoline with the necessary diamine. Both of these cyclic diamines have two reactive amine groups, but in each case, the main product in the direct reaction of the diamine with the 4,7-dichloroquinoline was the desired product and could be purified from the side product by column chromatography.

The diversity-enhancing step of our library synthesis was the reaction of each of the four secondary amine intermediates with a set of aldehydes containing an aromatic ring with a hydrogen bond accepting functional group (Fig 4.3). The reductive amination

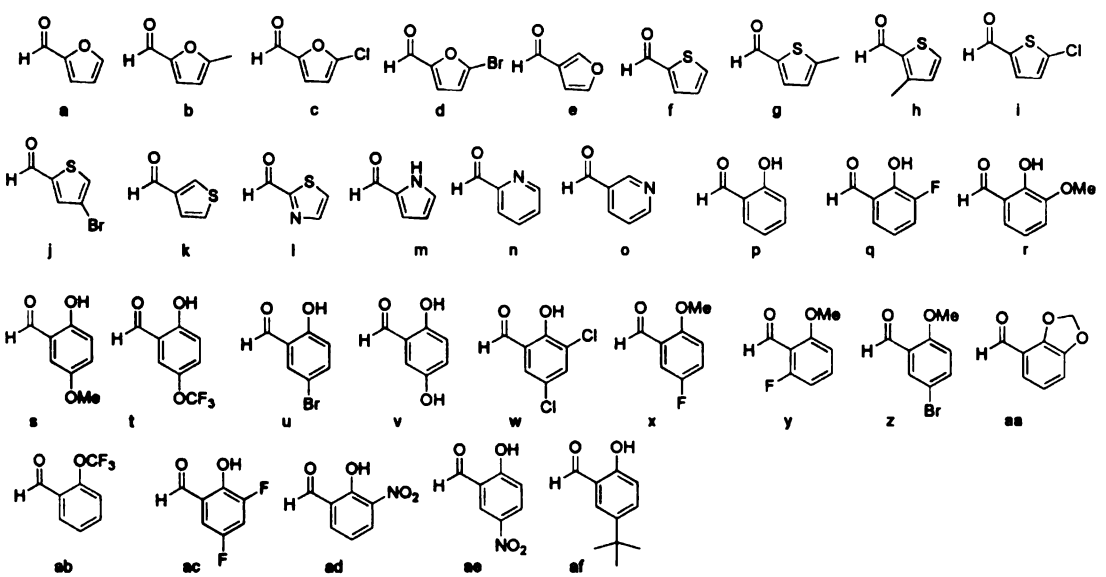


Figure 4.3 Aldehyde diversity elements used for side chain nitrogen substitutions.

was done *in situ* using sodium cyanoborohydride and excess aldehyde to drive the reaction to completion and reduce the amount of unreacted secondary amine byproduct. Each of the final products was then easily worked up using a two-step scavenge and solid-phase extraction (SPE) procedure. First, an equivalent of resin-bound thionyl chloride was added to each well to scavenge unreacted amine starting material. This step was important because separation of the desired products and amine starting material proved to be difficult. Each compound was then purified using a capture and release SPE strategy using a strong-cation exchange (SCX) resin. The crude reaction mixtures were first acidified with HCl in methanol to completely protonate the amine products and break up any boron complexes formed during the reduction step. This mixture was then added to a short column of SCX-SPE resin and washed with methanol to remove any non-protonated materials. Elution of the final products was done using a solution of 5% triethylamine in methanol. In greater than 90% of the reactions, this procedure alone led to products with > 80% purity. The products with less than 80% purity were individually purified using preparative HPLC. The reactions where the aldehyde starting materials contained a basic functional group could not be purified by the SCX-SPE method and so these were all purified by preparative HPLC.

Of the 124 compounds targeted in our library synthesis, we successfully synthesized 116 compounds (93%). The compounds were on average 89% pure following our SCX-SPE procedure. All compounds with purity less than 80% were purified by preparative HPLC, raising the average purity to 93% for the final set of compounds screened. (Fig 4.4) The primary impurity found in the compounds was triethylamine hydrochloride resulting from the incomplete evaporation of the triethylamine used in the

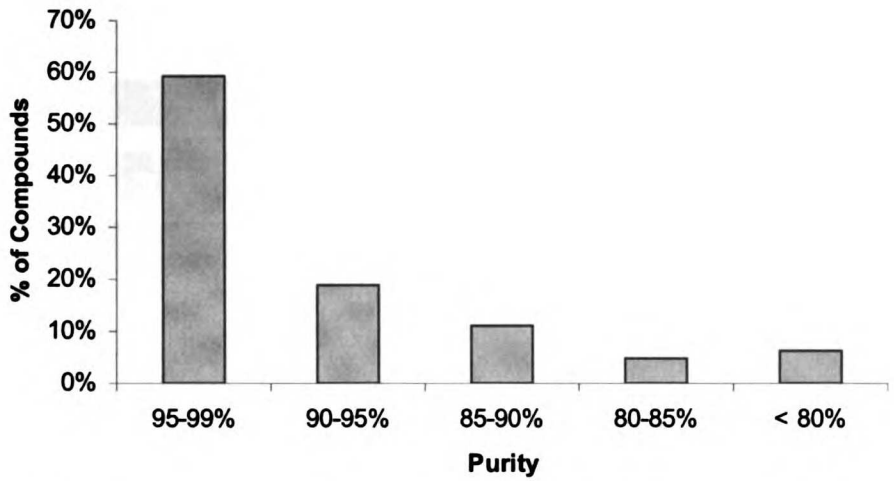


Figure 4.4 Histogram of purities of product libraries 4.8, 4.11, 4.13 and 4.15. Purity was assessed by analytical HPLC analysis monitored at 220 nm on a reversed-phase C18 column (Xterra C18, 3.5 μM).

purification processes. Each compound was initially synthesized on a 0.2 mmol scale and for compounds not requiring HPLC purification, the average yield for the final reaction and SPE purification procedure was 78%. The products requiring purification by prep-HPLC had much lower overall yields bringing the average yield for the final 112 compounds down to 55%. Since our initial screening library was synthesized on a relatively small scale, each compound was accurately quantitated using a chemiluminescent nitrogen detector (CLND). Equimolar stock solutions of each compound in DMSO were prepared using the data from the CLND analysis, ensuring accurate measurement of our final compound concentrations tested.

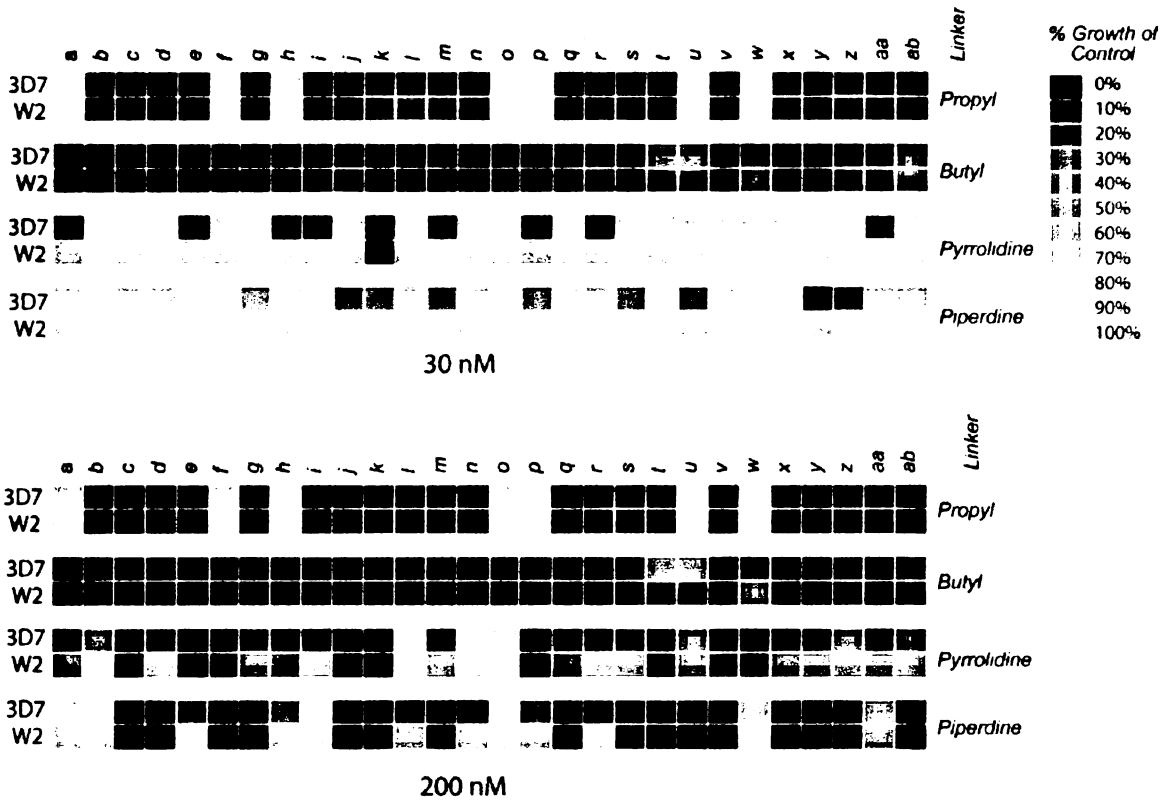


Figure 4.5 Activity screening data for all compounds against the 3D7 (CQ-sensitive) and W2 (CQ-resistant) parasite strains.

Activities

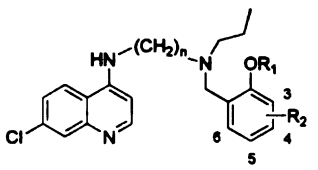
All of the compounds were initially assayed for growth inhibition in a cell-based assay against two strains of *P. falciparum* at two fixed concentrations (30 nM and 200 nM) to give a survey of all the activities (Fig 4.5). The 3D7 strain is a representative CQ-sensitive strain while the W2 strain is a multi-drug resistant parasite strain. Immediately, it can be seen that the majority of compounds within this class are active at 30 nM. This indicates that the aminocresol motif, when attached to the distal basic center of quinolines, does lead to potent compounds against both the 3D7 and W2 strains. A comparison of the different linkers between the distal basic center and the aromatic nucleus reveals that the compounds with the propyl and butyl alkyl linkers are more generally potent than those with either of the two cyclic linkers. It was hoped that rigidity

from the cyclic groups linking the basic center to the heterocyclic nucleus would introduce a conformational constraint leading to a more potent and/or selective compound. Conformational restraints are known to influence the thermodynamics of ligand/receptor interactions and have been statistically shown to have improved bioavailability over more flexible compounds.[13] It is a possibility that the compounds with the conformationally restricted linkers are no longer able to access the optimal conformation for binding to a particular receptor. Such was the case in the development of the drug cromakalim. The class of β-blocking agents it was derived from were known to have antihypertensive activity side effects, but the conformationally contrained analog cromakalim possessed only the antihypertensive activity but was devoid of any β-blocking activity.[14, 15] A broader evaluation of conformationally restricted linker regions is necessary to further understand the uniform loss of potency in the series of compounds with cyclic linkers between the basic center and aromatic core. Interestingly, only one compound with a structurally rigid linker (**4.13k**, Fig 4.3) showed growth inhibition against the drug-resistant W2 strain at the 30 nM dose. When the compounds were screened at 200 nM, several of the compounds with cyclic linkers began to show activity. Earlier work on modifications to the side chain of CQ-analogs had shown that a compound with the propyl diaminoalkane side chain was 10-times more potent than that with the butyl diaminoalkane side chain against a CQ-resistant parasite strain.[12] Surprisingly, in our study more compounds with the butyl linker were active at 30 nM than those with the propyl linker. The butyl linker has four methylene groups separating the two side chain nitrogens, similar to CQ, so we expected this set of compounds with

greater structural similarity to CQ to be less active against the drug-resistant *P. falciparum* strain.

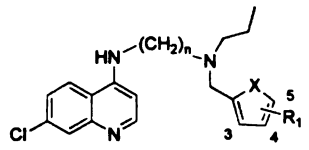
Full dose-response curves were done for compounds showing the greatest potency against the multi-drug resistant W2 strain. The data for compounds containing the aminocresol motif are shown in Table 4.1. Compounds with both the propyl and butyl linkers produced several compounds with low nanomolar IC50s against both parasite strains. A few of the compounds such as entries 6 and 18 had significantly weaker activities against the W2 strain, but all of the other compounds showed activity against W2 within 4-fold of that for 3D7. Interestingly, it appears that both electron-donating and the electron-withdrawing groups on the ring increase give similar activities, but both compounds with nitro substitutions (**4.8ad** & **4.8ac**) and the difluorosubstituted compound (**4.8ac**) had IC_{50}s at least an order of magnitude higher. This indicates that stronger electron withdrawing groups on the ring begin to decrease activity. Several of the compounds with heterocycle aromatic substitutions at the distal basic center, analogous in H-bonding capacity to the aminocresol motif, were selected and their IC_{50} values are shown in Table 2. The inhibitory activities of these compounds were slightly weaker than the benzyl substituted compounds, but still in the potent < 50 nM range that we were hoping to achieve. The methyl and chloro groups on the 5-position of the furan ring had little effect on the activity of the compounds. Within this series, the compounds with a 4 carbon diaminoalkyl side chains actually showed slightly higher potency than the 3 carbon linker equivalents.

Table 4.1. Inhibitory activities for 3D7 and W2 of benzyl substituted compounds.



					3D7 IC_{50} (nM)		W2 IC_{50} (nM)	
entry	no.	n	R_1	R_2	average	SD	average	SD
CQ					17	0.48	394	67
1	**4.8p**	3	H	H	0.6	0.02	1.8	0.05
2	**4.8q**	3	H	3-F	0.9	0.03	2.6	0.82
3	**4.8r**	3	H	3-OMe	3.0	0.59	4.5	0.42
4	**4.8s**	3	H	5-OMe	1.0	0.02	2.0	0.03
5	**4.8t**	3	H	5-OCF_3	2.5	2.0	8.0	0.04
6	**4.8v**	3	H	5-OH	2.9	0.54	22.5	3.7
7	**4.8x**	3	Me	5-F	5.2	1.6	13.3	0.77
8	**4.8y**	3	Me	6-F	8.3	0.64	16	1.8
9	**4.8aa**	3	-OCH_2O-		1.9	0.02	3.3	0.61
10	**4.11p**	4	H	H	7.0	0.57	20	0.86
11	**4.11q**	4	H	3-F	1.4	0.39	4.6	0.19
12	**4.11r**	4	H	3-OMe	1.0	0.80	4.3	0.27
13	**4.11s**	4	H	5-OMe	6.8	0.44	27	1.7
14	**4.11t**	4	H	5-OCF_3	2.4	0.04	5.9	1.7
15	**4.11v**	4	H	5-OH	2.2	1.0	8.9	0.27
16	**4.11x**	4	Me	5-F	1.9	0.21	4.9	0.15
17	**4.11y**	4	Me	6-F	10	0.53	11	1.3
18	**4.11aa**	4	-OCH_2O-		4.2	0.16	23	5.3
19	**4.8ac**	3	H	3-F, 5-F	21	9.8	34	4.0
20	**4.8ad**	3	H	3-NO_2	320	29	427	45
21	**4.8ae**	3	H	5-NO_2	38	3.4	125	0.41
22	**4.8af**	3	H	5-tBu	41	5.0	64	0.76

Table 4.2. Inhibitory activities for 3D7 and W2 of heterocycle substituted compounds.



					3D7 IC_{50} (nM)		W2 IC_{50} (nM)	
entry	no.	n	X	R_2	average	SD	average	SD
1	**4.8a**	3	O	H	12	2.1	33	3.7
2	**4.8b**	3	O	5-Me	14	0.03	39	4.0
3	**4.8c**	3	O	5-Cl	7.4	0.29	10.8	1.0
4	**4.8f**	3	S	H	32	1.7	136	5.9
6	**4.11a**	4	O	H	3.1	0.33	3.8	0.13
7	**4.11b**	4	O	5-Me	6.8	0.26	30	0.05
8	**4.11c**	4	O	5-Cl	4.3	1.3	6.4	0.52
9	**4.11f**	4	S	H	7.2	0.51	22	5.4

The parent drug compound CQ is thought to work by interfering with the parasites ability to polymerize toxic free heme into a non-toxic heme polymer called hemozoin.[16-18] The mechanism of resistance to CQ is now known to be primarily due to the PfCRT protein which alter the accumulation of drug within the parasite food vacuole, where heme polymerization takes place.[19, 20] The PfCRT protein shows homology to known drug/metabolite transporters and can confer CQ-resistance with a K76T mutation.[21, 22] It has been hypothesized the wild-type K76 residue in PfCRT is positively charged at physiological pH and the electrostatic repulsion with CQ may disfavor the transport of CQ out of the food vacuole, causing the toxic effects that lead to parasite death.[23, 24] The K76T mutation eliminates this positive charge and CQ is then transported out of the food vacuole, where it can no longer exert its activity. This mechanistic understanding further demonstrates the importance of the positively charged basic side chain of CQ-analogs in

determining the level of activity against CQ-resistant parasite strains. *In vitro* selection of CQ-resistant *P. falciparum* mutants resistant against halofantrine 4.5, which contains an intramolecular hydrogen bond to its basic center, selects a PfCRT mutant S163R that has renewed sensitivity to CQ despite having the K76T mutation.[23, 24] The S163R mutation reinstates a positive charge into the PfCRT protein providing further evidence that an electrostatic binding interaction is a key determinant in the level of drug efflux. This new insight into the mechanism of resistance to CQ and other heme-binding antimalarials, first of all show that cross-resistance among the current battery of heme-binding antimalarials is complex and resistance to one drug can renew sensitivity to another. Also, there must be a structure-activity relationship for binding to PfCRT that is dependent on steric and electrostatic properties of the basic side chains of the heme-binding antimalarials. The addition of substitutions to the basic nitrogen could renew activity against the drug-resistant strains by altering the binding affinity to the PfCRT binding site, causing renewed activity against CQ-resistant *P. falciparum*. The addition of an aromatic group on the basic nitrogen introduces substantial steric bulk to the basic side chain, significantly changing the surface area of the ligand. The addition of a hydrogen-bond acceptor in proximity to the protonated basic nitrogen center raises the pKa of the nitrogen, significantly changing the electrostatic character of the basic side chain at physiological pH. This hypothesis is consistent with our data showing that electron-donating substitutions on the ring tend to increase activity, since electron donating groups should further increase the pKa of the basic center. This would also be consistent with the sharp decrease in activity for the compounds with the strong electron withdrawing substitutions. Given that our primary screen is a cell-based assay, it is difficult to

correlate small shifts in pKa with observed activity, but the general trends fit the hypothesis that changing the pKa of the basic center is important for activity.

Conclusions

It remains unknown what the possible mode of action was for the original α-aminocresols since more attention was focused on the quinoline hybrids such as amodiaquine. It is possible that the α-aminocresols have a different mode of action from chloroquine and hybrid 4-aminoquinoline-α-aminocresol hybrid compounds such as amodiaquine exert their activity through interaction with multiple targets. This possibility would partially explain their continued activity against strains of *P. falciparum* that have grown resistant to chloroquine. It is also possible that the steric and electrostatic changes to the side chain of the CQ-analogs simply reduce binding to the PfCRT efflux protein that infers CQ-resistance. Further experiments to address this hypothesis are warranted by the strong activity of this class of quinolines

Experimental

All reagents and starting materials were purchased from commercial sources and used without further purification. Dichloromethane, tetrahydrofuran, and methanol were dried using the solvent purification system manufactured by Glass Contour Inc. (Laguna Beach, CA). ^{1}H and ^{13}C NMR were recorded on the Varian Utility 400 MHz spectrometer, in $CDCl_3$, CD_3OD, or *d*-DMSO solvent. Chemical shifts were reported as parts per million (ppm) downfield from an internal tetramethylsilane (TMS) standard (δ = 0.0 for ^{1}H NMR) or from solvent reference. Coupling constants (*J* values) were measured

in hertz (Hz). Electrospray mass spectra (ES-MS) were collected on a Waters ZQ 4000 mass spectrometer. Crude products were purified by either flash chromatography using 230-400 mesh silica gel (SiliCycle). All parallel synthesis steps were carried out in polypropylene fritted Bohdan 48-well MiniBlocks™. HPLC analysis was done using two orthogonal HPLC methods. HPLC method A: using an Xterra reversed phase C18 column (4.6 x 20 mm, 3 μm) running a binary gradient with water (w/ 0.05% TFA) and acetonitrile (w/ 0.05% TFA). Purity was measured at 220 nM on a 10 minute gradient was used running from 0 - 100% acetonitrile/TFA at 1 mL/min on a Waters Alliance HPLC. HPLC method B: using an Xterra reversed phase phenyl column (4.6 x 20 mm, 3 μm) running a binary gradient with water (w/ 0.05% TFA) and acetonitrile (w/ 0.05% TFA). Purity was measured at 220 nM on a 10 minute gradient was used running from 0 - 100% acetonitrile/TFA at 0.5 mL/min on a Waters Alliance HPLC.

N-(7-Chloro-quinolin-4-yl)-N'-propyl-propane-1,3-diamine (4.8). To a stirring solution of **4.6** (prepared as described by De et al.[9]) (10 g, 42 mmol) in chloroform (200 mL) and pyridine (3.83 mL, 47 mmol), propionic anhydride (6.0 mL, 47 mmol) was added dropwise by a syringe at 0 °C under an inert atomosphere. The reaction was allowed to warm until room temperature and then stirring was continued for 1 hour. Excess anhydride was then quenched by addition of several drops of water. The crude reaction mixture was then dried down under vacuum, redissolved in ethyl acetate (300 mL) and washed with brine 5-times (200 mL). Solvent was again removed under vacuum and the placed on a high-vaccum for 18 hours to remove the trace amounts of pyridine remaining. The remaining 11.14 g (90%) of fluffy white solid amide was then immediately dissolved in dry THF for the reduction. The flask was cooled to 0 °C and

Borane-methyl sulfide (15.4 mL, 4 eq.) was added slowly while stirring under an inert atomosphere. The reaction was then heated to reflux for 1 hour, before cooling to room temperature and careful quenching with water. After the addition of water no longer evolved bubbles, 25 mL of 37% HCl was carefully added to the reaction re-heated to reflux for 1 hour to break up any boron complexes formed with the product. After cooling this mixture, the reaction was basified with solid K_3PO_4 (pH > 10), and extracted into chloroform (200 mL). The organic solvent was removed under vacuum and the crude product was purified using silica gel with CH_2Cl_2/MeOH/Et_3N (9 : 1 : 0.1) as an eluent to give 7.5 g (63%) of **4.8** as a pure white solid, 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{15}H_{20}ClN_3$ $[M + H]^+$ 278.1. Found: 278.4. ^1H NMR (400 MHz, $CDCl_3$): δ 8.48 (d, *J* = 5.6, 1H), 7.91 (d, *J* = 2.0, 1H), 7.88 (br-s, 1H), 7.71 (d, *J* = 8.9, 1H), 7.30 (dd, *J* = 8.9, 2.0, 1H), 6.29 (d, *J* = 5.6, 1H), 3.48 (s, 1H), 3.78 (q, *J* = 5.6, 2H), 2.91 (t, *J* = 5.2, 2H), 2.64 (t, *J* = 6.8, 2H), 1.90 (m, 2H), 1.60 (m, 2H), 0.98 (t, *J* = 7.2, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): 151.9, 150.5, 148.8, 134.5, 128.2, 124.8, 122.3, 117.5, 98.2, 51.9, 49.3, 43.8, 27.1, 23.2, 11.8.

N-(7-Chloro-quinolin-4-yl)-N'-propyl-butane-1,4-diamine (4.10). To a stirring solution of **4.9** (prepared as described by De et al.[9]) (10 g, 40 mmol) in chloroform (200 mL) and pyridine (3.83 mL, 47 mmol), propionic anhydride (6.0 mL, 47 mmol) was added dropwise by a syringe at 0 °C under an inert atomosphere. The reaction was allowed to warm until room temperature and then stirring was continued for 1 hour. Excess anhydride was then quenched by addition of several drops of water. The crude reaction mixture was then dried down under vacuum, redissolved in ethyl acetate (300 mL) and washed with brine 5-times (200 mL). Solvent was again removed under vacuum

and the placed on a high-vaccum for 18 hours to remove the trace amounts of pyridine remaining. 11.26 g (92%) of the amide was then immediately dissolved in dry THF for the reduction. The flask was cooled to 0 °C and Borane-methyl sulfide (15.4 mL, 4 eq.) was added slowly while stirring under an inert atomosphere. The reaction was then heated to reflux for 1 hour, before cooling to room temperature and careful quenching with water. After the addition of water no longer evolved bubbles, 25 mL of 37% HCl was carefully added to the reaction re-heated to reflux for 1 hour to break up any boron complexes formed with the product. After cooling this mixture, the reaction was basified with solid K_3PO_4 (pH > 10), and extracted into chloroform (200 mL). The organic solvent was removed under vacuum and the crude product was purified using silica gel with CH_2Cl_2/MeOH/Et_3N (9 : 1 : 0.1) as an eluent to give 76.2 g (58 %) of **4.10** as an off-white solid, 100% pure by HPLC Method A; 93% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{16}H_{22}ClN_3$ $[M + H]^+$ 292.2. Found: 292.4. ^1H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, J = 5.2, 1H), 7.94 (d, J = 2.0, 1H), 7.71 (d, J = 8.9, 1H), 7.32 (dd, J = 8.9, 2.0, 1H), 6.36 (d, J = 5.2, 1H), 6.10 (s, 1H), 3.48 (s, 1H), 3.29 (q, J = 4.4, 2H), 2.71 (t, J = 6.4, 2H), 2.60 (t, J = 7.2, 2H), 1.86 (quin, J = 6.4, 2H), 1.69 (m, 2H), 1.55 (m, 4H), 0.94 (t, J = 7.2, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 152.0, 150.3, 149.2, 134.8, 128.5, 125.0, 121.9, 117.5, 98.9, 52.0, 49.2, 43.3, 27.9, 26.4, 23.2, 11.9.

(7-Chloro-quinolin-4-yl)-pyrrolidin-3-yl-amine (4.12). A solution of 4,7-dichloroquinoline (5 g, 25 mmol) and 3-aminopyrrolidine dihydrochloride (7.95 g, 50 mmol) in diisopropylethylamine (100 mL) was heated at reflux for 4 h. After concentration under vacuum, the crude reaction mixture was purified on silica gel with CH_2Cl_2/MeOH/Et_3N (9 : 1 : 0.1) as an eluent to give 4.3 g (70%) of **4.12**. 100% pure by

HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{13}H_{14}ClN_3$ $[M + H]^+$ 248.1. Found: 248.3. ^1H NMR (400 MHz, $CDCl_3$): δ 8.69 (d, *J* = 4.8, 1H), 8.02 (d, *J* = 2.0, 1H), 7.90 (d, *J* = 8.8, 1H), 7.42 (dd, *J* = 8.8, 2.0; 1H), 6.82 (d, *J* = 4.8, 1H), , 2H), 2.60 (t, *J* = 7.2, 2H), 1.86 (quin, *J* = 6.4, 2H), 1.69 (m, 2H), 1.55 (m, 4H), 0.94 (t, *J* = 7.2, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 152.2, 150.4, 149.7, 134.2, 128.0, 126.3, 123.7, 119.1, 102.8, 60.3, 51.1, 50.2, 34.5.

(7-Chloro-quinolin-4-yl)-piperidin-4-yl-amine (4.14). A solution of 4,7-dichloroquinoline (10 g, 50 mmol) and 4-aminopiperidine (8.4 mL, 250 mmol) in diisopropylethylamine (200 mL) was heated at 100 °C for 20 hours. After partial concentration under vacuum, the crude reaction mixture was purified on silica gel with CH_2Cl_2/MeOH/Et_3N (9 : 1 : 0.1) as an eluent to give 8.6 g (65%) of **4.12**. 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{14}H_{16}ClN_3$ $[M + H]^+$ 262.1. Found: 262.3. ^1H NMR (400 MHz, $CDCl_3$): δ 8.62 (d, *J* = 5.2, 1H), 7.96 (d, *J* = 2.0, 1H), 7.85 (d, *J* = 8.9, 1H), 7.35 (dd, *J* = 8.9, 2.0, 1H), 6.75 (d, *J* = 5.2, 1H), 5.241 (s, 1H), 3.48 (d, *J* = 12.0, 2H), 2.89 (m, 1H), 2.82 (t, *J* = 11.6, 2H), 1.97 (d, *J* = 12.0, 2H), 1.61 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 157.2, 151.6, 150.0, 134.5, 128.4, 125.6, 125.3, 121.9, 108.8, 51.1, 48.4, 35.5.

General Procedure for Reductive Alkylation Diversity Step. A stock solution (36 mM) of the appropriate secondary amine intermediate (**4.8**, **4.11**, **4.13** or **4.15**) was made in dry MeOH and 2 mL (0.07 mmol) was aliquoted to the wells of a 48-well reaction block (2 mL/well; 0.07 mmol/well). To each well was then added an aldehyde (Fig 3) (0.22 mmol, 4 eq.) with care taken to reduce exposure to moisture in the air. The reaction block was sealed with a rubber gasket and 220 μL (0.22 mmol) of a dry solution of

$NaBH_3CN$ in THF (1 M) was added by syringe to each well through the gasket and the block was shaken at room temperature for 18 hours. Each reaction was then quenched with 0.4 mL of a saturated solution of HCl in MeOH. CAUTTION: explosion hazard. After bubbling had ceased, 100 mg of polymer-bound sulfonyl chloride (Aldrich, 100-200 mesh, 1.5 mmol/g) was added to each well and shaken for 4 hours. The solution remaining in each well was transferred into another 48-position reaction block containing cartridges loaded with SCX-SPE medium. Each well was washed with 1% TFA in MeOH (1 mL, 2X), MeOH (2 mL, 2X) and eluted with 5% TEA in MeOH (2 mL, 2X). The eluted products were collected into glass test tubes and solvent was removed on a GeneVac HT-4 (10 mbar, 4.0 h, 35 °C). The partially purified products were then dissolved in saturated HCl/MeOH (1 mL) and dried again to form the HCl salts of each compound. The dry HCl salts were then each dissolved in 1mL d_6-DMSO and subjected to LC/MS/CLND for analysis of purity, identity and yield. A 10 mM stock solutions of each compound was made in d_6-DMSO based on the CLND quantitation data and selected compounds were analyzed by ^{1}H-NMR.

General Procedure for purification of library members. Compounds with purity < 80% by analytical HPLC were dissolved in a total of 1 mL DMSO and purified with a semi-preparative reversed-phase Xterra column (19 x 50 mm, particle size 5 μm) running a 10 - 100% gradient of 0.5%TEA-H_2O/methanol with a 20 mL/min flow rate on a Parallex Flex HPLC system. Fraction collection was automatically triggered by UV absorbance above 0.1 AU at 254nm. Sample and fraction data were then transferred to the Waters OpenLynx operating software, which coordinated the injection, mass spectrometric analysis, and data processing for each fraction. A Gilson 215 liquid handler

and a Gilson 208 injection module were used to inject samples into a Waters ZQ 4000 mass spectrometer rigged for flow injection with an electrospray probe and single quadrapole detector operating in positive ion mode. All fraction plates were then dried down using a GeneVac Mega 980 solvent evaporator. Fractions that exhibited a peak in the mass spectra of the correct molecular weight were then dissolved in 1 mL of freshly prepared saturated HCl in methanol, and like fractions were combined. Combined fractions were then dissolved in 0.5 mL of d_6-DMSO and were analyzed by LC/MS/CLND for purity, identity and yield. 10 mM stock solutions in d_6-DMSO were prepared based on the CLND quantitation data.

N'-(7-Chloro-quinolin-4-yl)-N-furan-2-ylmethyl-N-propyl-propane-1,3-diamine (4.8a) 89% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{20}H_{24}ClN_3O$ $[M + H]^+$ 358.2. Found: 358.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.93 (br, 0.5 H), 9.75 (t, J = 5.2, 1H), 8.70 (d, J = 9.2, 1H), 8.56 (d, J = 7.2, 1H), 8.05 (d, J = 1.6, 1H), 7.74 (dd, J = 9.2, 1.6, 1H), 7.70 (d, J = 3.2, 1H), 6.88 (d, J = 7.2, 1H), 6.72 (d, J = 3.2, 1H), 6.44 (d, J = 3.2, 1H), 5.27 (d, J = 2.2, 1H), 4.36 (br-s, 2H), 3.59 (m, 2H), 3.32 (H_20 peak), 3.04 (m, 2H), 2.87 (m, 2H), 2.13 (dd, J = 6.4, 6.4, 2H), 1.70 (m, 2H), 0.83 (t, J = 7.6, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 164.9, 154.5, 152.5, 148.0, 147.5, 136.4, 135.5, 128.6, 125.1, 120.7, 108.2, 63.3, 61.5, 59.2, 56.7, 31.7, 26.3, 20.4, 16.7.

N'-(7-Chloro-quinolin-4-yl)-N-(5-methyl-furan-2-ylmethyl)-N-propyl-propane-1,3-diamine (4.8b) 59% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{21}H_{26}ClN_3O$ $[M + H]^+$ 372.2. Found: 372.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 9.96 (br, 1H), 9.88 (t, J = 5.2, 1H), 8.83 (d, J = 9.2, 1H),

8.60 (d, J = 7.2, 1H), 8.12 (d, J = 1.6, 1H), 7.78 (dd, J = 9.2, 1.6, 1H), 6.93 (m, 1H), 6.61 (d, J = 3.2, 1H), 6.08 (d, J = 3.2, 1.2, 1H), 5.32 (d, J = 1.6, 1H), 4.43 (d, J = 4.8, 2H), 3.61 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.74 (m, 2H), 2.09 (s, 3H), 1.65 (m, 4H), 0.87 (t, J = 7.2, 3H).

N-(5-Chloro-furan-2-ylmethyl)-N'-(7-chloro-quinolin-4-yl)-N-propyl-propane-1,3-diamine (4.8c) 76% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{20}H_{23}Cl_2N_3O$ 391.1218. Found: 391.1214. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 9.96 (br, 1H), 9.88 (t, J = 5.2, 1H), 8.83 (d, J = 9.2, 1H), 8.60 (d, J = 7.2, 1H), 8.12 (d, J = 1.6, 1H), 7.78 (dd, J = 9.2, 1.6, 1H), 6.93 (m, 1H), 6.61 (d, J = 3.2, 1H), 6.08 (d, J = 3.2, 1.2, 1H), 5.32 (d, J = 1.6, 1H), 4.43 (d, J = 4.8, 2H), 3.61 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.74 (m, 2H), 2.09 (s, 3H), 1.65 (m, 4H), 0.87 (t, J = 7.2, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 157.1, 144.5, 143.8, 140.6, 139.4, 128.4, 126.5, 119.9, 119.0, 116.6, 109.3,109.2, 100.0, 56.0, 51.8, 49.2, 41.7, 24.0, 18.5, 11.3.

N'-(7-Chloro-quinolin-4-yl)-N-propyl-N-thiophen-2-ylmethyl-propane-1,3-diamine (4.8f) 90% yield; 82% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{20}H_{24}ClN_3S$ $[M + H]^+$ 374.1. Found: 374.4. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 11.16 (br, 0.5 H), 9.83 (t, J = 5.2, 1H), 8.75 (d, J = 9.2, 1H), 8.59 (d, J = 7.2, 1H), 8.13 (d, J = 1.6, 1H), 7.78 (dd, J = 9.2, 1.6, 1H), 7.61 (d, J = 5.2, 1H), 7.41 (d, J = 3.2, 1H), 7.03 (dd, J = 3.2, 3.2; 1H), 6.93 (d, J = 7.2, 1H), 4.56 (d, J = 2.2, 1H), 3.63 (m, 2H), 3.32 (H_20 peak), 3.14 (m, 2H), 2.93 (m, 2H), 2.18 (m, 2H), 1.76 (m, 2H), 0.87 (t, J = 7.6, 3H).

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-phenol (4.8p) 78% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS

(ESI) *m/z* Calcd for $C_{22}H_{26}ClN_3O$ $[M + H]^+$ 384.2. Found: 384.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.34 (br, 0.5 H), 10.06 (br, 1H), 9.77 (t, *J* = 5.2, 1H), 8.73 (d, *J* = 9.2, 1H), 8.59 (d, *J* = 7.2, 1H), 8.11 (d, *J* = 1.6, 1H), 7.77 (dd, *J* = 9.2, 1.6, 1H), 7.46 (d, *J* = 7.2, 1H), 7.17 (d, *J* = 7.2, 1H), 6.91 (m, 2H), 6.75 (d, *J* = 7.2, 1H), 5.32 (d, *J* = 2.4, 1H), 4.23 (br-s, 2H), 3.61 (m, 2H), 3.33 (H_20 peak), 3.15 (m, 2H), 2.96 (m, 2H), 2.18 (m, 2H), 1.77 (m, 2H), 0.86 (t, *J* = 7.6, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 165.8, 164.7, 152.3, 147.8, 147.2, 145.5, 142.2, 140.2, 136.2, 135.3, 128.4, 125.4, 124.9, 124.8, 108.0, 62.9, 61.3, 58.7, 54.7, 31.2, 25.8, 20.2, 17.7, 16.5.

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-6-fluoro-phenol (4.8q) 30% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{22}H_{25}ClFN_3O$ $[M + H]^+$ 402.2. Found: 402.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.38 (br-s, 1H), 9.75 (t, *J* = 5.2, 1H), 8.70 (d, *J* = 9.2, 1H), 8.58 (d, *J* = 7.2, 1H), 8.10 (d, *J* = 1.6, 1H), 7.78 (dd, *J* = 9.2, 1.6, 1H), 7.37 (d, *J* = 7.6, 1H), 7.17 (t, *J* = 9.2, 1H), 6.91 (d, *J* = 7.2, 1H), 6.87 (d, *J* = 7.2, 1H), 6.80 (m, 1H), 4.29 (s, 2H), 3.61 (m, 2H), 3.35 (H_20 peak), 3.15 (m, 2H), 2.97 (m, 2H), 2.16 (m, 2H), 1.76 (m, 2H), 0.87 (t, *J* = 7.2, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 153.7, 153.5, 152.4, 148.0, 147.5, 137.8, 136.4, 135.5, 129.4, 129.0, 128.5, 126.7, 126.5, 125.0, 108.1, 63.2, 61.5, 59.5, 58.9, 31.4, 26.0, 20.4, 16.7.

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-6-methoxy-phenol (4.8r) 77% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{23}H_{28}ClN_3O_2$ 413.1870. Found: 413.1871. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.13 (br, 1H), 9.75 (t, *J* = 5.2, 1H), 9.39 (br, 1H), 8.70 (d, *J* = 9.2, 1H), 8.57 (d, *J* = 7.2, 1H), 8.11 (d, *J* = 1.6, 1H), 7.78 (dd, *J* = 9.2, 1.6, 1H), 7.06 (d, *J* =

8.4, 1H), 6.95 (d, J = 8.4, 1H), 6.90 (d, J = 7.2, 1H), 6.75 (t, J = 8.4, 1H), 4.24 (br-s, 2H), 3.61 (m, 2H), 3.33 (H_20 peak), 3.14 (m, 2H), 2.95 (m, 2H), 2.17 (m, 2H), 1.76 (m, 2H), 0.86 (t, J = 7.6, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 157.3, 149.0, 147.1, 143.8, 140.8, 139.7, 128.5, 126.5, 124.7, 120.8, 120.1, 117.0, 116.8, 113.9, 100.0, 56.6, 53.1, 51.7, 41.7, 23.7, 18.4, 11.3.

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-4-methoxy-phenol (4.8s) 67% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{23}H_{28}ClN_3O_2$ 413.1870. Found: 413.1882. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 10.20 (br-s, 1H), 9.76 (br-s, 1H), 9.68 (t, J = 5.2, 1H), 8.69 (d, J = 9.2, 1H), 8.59 (d, J = 6.8, 1H), 8.08 (d, J = 1.6, 1H), 7.78 (dd, J = 9.2, 1.6, 1H), 7.15 (d, J = 2.9, 1H), 6.90 (d, J = 7.2, 1H), 6.75 (m, 2H), 4.20 (br-s, 2H), 3.65 (s, 3H), 3.60 (m, 2H), 3.33 (H_20 peak), 3.15 (m, 2H), 2.97 (m, 2H), 2.16 (m, 2H), 1.77 (m, 2H), 0.87 (t, J = 7.6, 3H).

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-4-trifluoromethoxy-phenol (4.8t) 70% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{23}H_{25}ClF_3N_3O_2$ 467.1587. Found: 467.1599. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 10.77 (br-s, 1H), 10.03 (br-s, 1H), 9.57 (t, J = 5.2, 1H), 8.61 (d, J = 9.2, 1H), 8.03 (d, J = 1.6, 1H), 7.80 (dd, J = 9.2, 1.6, 1H), 7.55 (d, J = 2.9, 1H), 7.20 (m, 1H), 6.97 (d, J = 8.9, 1H), 6.92 (d, J = 7.2, 1H), 4.26 (br-s, 1H), 3.62 (m, 2H), 3.33 (H_20 peak), 3.17 (m, 2H), 2.99 (m, 2H), 2.15 (m, 2H), 1.75 (m, 2H), 0.87 (t, J = 7.6, 3H).). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 157.3, 156.8, 143.9, 142.6, 140.8, 139.7, 128.6, 126.6, 125.6, 123.0, 120.1, 118.7, 117.5, 116.7, 100.0, 56.4, 53.0, 52.1, 41.8, 23.8, 18.2, 11.2.

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-benzene-1,4-diol (4.8v) 84% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{22}H_{26}ClN_3O_2$ $[M + H]^+$ 400.2. Found: 400.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.12 (br-s, 1H), 9.95 (br-s, 1H), 9.79 (t, *J* = 5.2, 1H), 9.56 (br-s, 1H), 8.98 (m, 1H), 8.74 (d, *J* = 9.2, 1H), 8.59 (d, *J* = 7.2, 1H), 8.11 (d, *J* = 1.6, 1H), 7.78 (dd, *J* = 9.2, 1.6, 1H), 6.91 (d, *J* = 7.2, 1H), 6.86 (d, *J* = 2.9, 1H), 6.71 (d, *J* = 8.6, 1H), 6.64 (dd, *J* = 8.6, 2.9; 1H), 4.15 (br-s, 2H), 3.60 (m, 2H), 3.33 (H_20 peak), 3.15 (m, 2H), 2.97 (m, 2H), 2.16 (m, 2H), 1.74 (m, 2H), 0.86 (t, *J* = 7.6, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-(5-fluoro-2-methoxy-benzyl)-N-propyl-propane-1,3-diamine (4.8x) 94% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{23}H_{27}ClFN_3O$ 415.1826. Found: 415.1839. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.40 (br-s, 1H), 9.81 (t, *J* = 5.2, 1H), 8.76 (d, *J* = 9.2, 1H), 8.59 (d, *J* = 6.8, 1H), 8.11 (d, *J* = 1.6, 1H), 7.79 (dd, *J* = 9.2, 1.6, 1H), 7.56 (dd, *J* = 9.1, 3.1; 1H), 7.21 (dt, *J* = 3.1, 8.2, 1H), 7.05 (m; 1H), 6.95 (d, *J* = 7.2, 1H), 4.26 (br-s, 2H), 3.79 (s, 2H), 3.61 (m, 2H), 3.33 (H_20 peak), 3.17 (m, 2H), 2.95 (m, 2H), 2.17 (m, 2H), 1.77 (m, 2H), 0.87 (t, *J* = 7.6, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 158.8, 157.2, 156.4, 155.8, 143.8, 140.7, 139.6, 128.5, 126.5, 120.1, 119.0, 116.7, 113.5, 112.0, 100.0, 59.6, 56.9, 53.0, 52.1, 41.7, 23.7, 18.2, 11.2.

N'-(7-Chloro-quinolin-4-yl)-N-(2-fluoro-6-methoxy-benzyl)-N-propyl-propane-1,3-diamine (4.8y) 32% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{23}H_{27}ClFN_3O$ 415.1826. Found: 415.1829. ^1H NMR (d_6-DMSO, 400 MHz): δ 9.89 (m, 2H), 8.79 (d, *J* = 9.2, 1H), 8.60 (d, *J* = 7.2, 1H), 8.12 (d, *J* = 1.6, 1H), 7.78 (dd, *J* = 9.2, 1.6, 1H), 7.47 (dd, *J* = 7.5, 8.2; 1H), 6.95 (m,

2H), 6.84 (m, 1H), 4.26 (br-s, 2H), 3.86 (s, 3H), 3.65 (m, 2H), 3.33 (H_20 peak), 3.20 (m, 2H), 2.99 (m, 2H), 2.20 (m, 2H), 1.79 (m, 2H), 0.89 (t, J = 7.6, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 160.7, 157.3, 143.8, 140.7, 139.6, 134.1, 131.6, 128.5, 126.6, 120.1, 116.7, 108.9, 108.4, 106.8, 100.1, 62.7, 57.2, 52.4, 46.2, 41.8, 23.7, 18.0, 11.3.

N-Benzo[1,3]dioxol-4-ylmethyl-N'-(7-chloro-quinolin-4-yl)-N-propyl-propane-1,3-diamine (4.8aa) 31% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{23}H_{26}ClN_3O_2$ 411.1713. Found: 411.1718. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 10.95 (br-s, 1H), 9.80 (t, J = 5.2, 1H), 8.73 (d, J = 9.2, 1H), 8.59 (d, J = 6.8, 1H), 8.10 (d, J = 1.6, 1H), 7.78 (dd, J = 9.2, 1.6, 1H), 7.13 (d, J = 7.6; 1H), 6.91 (m, 2H), 6.83 (t, J = 7.6, 1H), 6.05 (s, 2H), 4.23 (br-s, 2H), 3.62 (m, 2H), 3.33 (H_20 peak), 3.15 (m, 2H), 2.97 (m, 2H), 2.18 (m, 2H), 1.78 (m, 2H), 0.88 (t, J = 7.6, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 157.3, 149.1, 148.8, 143.8, 140.8, 139.7, 128.6, 126.5, 125.4, 123.5, 120.1, 116.7, 111.5, 108.5, 103.0, 100.0, 59.2, 56.0, 51.7, 41.7, 23.9, 18.3, 11.3.

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-4,6-difluoro-phenol (4.8ac) 65% yield; 92% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{22}H_{24}ClF_2N_3O$ 419.1564. Found: 419.1562. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 8.38 (d, J = 5.3, 1H), 8.21 (d, J = 8.9, 1H), 7.78 (d, J = 1.6, 1H), 7.42 (dd, J = 8.9, 1.6, 1H), 7.28 (m, 1H), 7.01 (m, 1H), 6.83 (m, 1H), 6.44 (d, J = 5.3, 1H), 3.77 (m, 2H), 3.35 (m, 2H), 3.15 (m, 2H), 2.60 (q, J = 6.0, 2H), 1.47 (m, 2H), 0.82 (t, J = 7.6, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 157.2, 143.8, 140.7, 139.6, 128.5, 126.5, 120.8, 120.1, 116.7, 115.2, 115.0, 107.1, 106.9, 106.6, 100.0, 56.4, 52.3, 51.8, 41.7, 23.7, 18.3, 11.3.

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-6-nitro-phenol (4.8ad) 57% yield; 90% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{22}H_{25}ClN_4O_3$ $[M + H]^+$ 429.2. Found: 429.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 8.39 (d, J = 5.3, 1H), 8.21 (d, J = 8.9, 1H), 7.78 (d, J = 1.6, 1H), 7.70 (d, J = 8.4, 1H), 7.44 (dd, J = 8.9, 1.6, 1H), 7.33 (t, J = 5.3, 1H), 7.29 (d, J = 7.2, 1H), 6.61 (m, 1H), 6.48 (d, J = 5.3, 1H), 4.01 (s, 2H), 3.29 (m, 2H), 2.79 (m, 2H), 2.63 (m, 2H), 1.96 (m, 2H), 1.55 (m, 2H), 0.84 (t, J = 7.2, 3H).

2-({[3-(7-Chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-4-nitro-phenol (4.8ae) 54% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{22}H_{25}ClN_4O_3$ $[M + H]^+$ 429.2. Found: 429.5. ^1H NMR (d_6-DMSO, 400 MHz):: δ 8.37 (d, J = 5.2, 1H), 8.19 (d, J = 9.2, 1H), 8.09 (d, J = 1.6, 1H), 7.95 (dd, J = 9.2, 1.6, 1H), 7.77 (d, J = 1.5, 1H), 7.41 (dd, J = 8.9, 1.5; 1H), 7.31 (m, 1H), 6.74 (t, J = 8.9, 1H), 6.45 (d, J = 5.2, 1H), 3.85 (s, 2H), 3.37 (m, 2H), 3.17 (m, 2H), 2.70 (t, J = 5.8; 2H), 1.91 (dd, J = 5.8, 5.8; 2H), 1.51 (dd, J = 7.2, 7.2; 2H), 0.84 (t, J = 7.2, 3H).

4-tert-Butyl-2-({[3-(7-chloro-quinolin-4-ylamino)-propyl]-propyl-amino}-methyl)-phenol (4.8af) 74% yield; 85% pure by HPLC Method A; 84% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{26}H_{34}ClN_3O$ $[M + H]^+$ 440.2. Found: 440.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 8.37 (d, J = 5.2, 1H), 8.19 (d, J = 9.2, 1H), 7.77 (d, J = 1.6, 1H), 7.41 (dd, J = 9.2, 1.6; 1H), 7.31 (m, 1H), 7.09 (d, J = 1.6, 1H), 7.05 (dd, J = 8.9, 1.6; 1H), 6.60 (d, J = 8.9, 1H), 6.43 (d, J = 5.2, 1H), 3.70 (s, 2H), 3.26 (m, w/H_20 overlap), 2.60 (t, J = 5.8; 2H), 1.88 (m; 2H), 1.49 (dd, J = 7.2, 7.2; 2H), 1.19 (s, 9H), 0.81 (t, J = 7.2, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-furan-2-ylmethyl-N-propyl-butane-1,4-diamine (4.11a) 64% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{21}H_{26}ClN_3O$ $[M + H]^+$ 372.2. Found: 372.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.54 (br, 0.5 H), 9.72 (t, J = 5.2, 1H), 8.78 (d, J = 9.2, 1H), 8.55 (m, 1H), 8.08 (d, J = 1.6, 1H), 7.76 (dd, J = 9.2, 1.6, 1H), 6.89 (d, J = 3.2, 1H), 6.88 (d, J = 7.2, 1H), 6.63 (d, J = 3.2, 1H), 6.10 (d, J = 3.2, 1H), 4.31 (d, J = 4.8, 2H), 3.56 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.90(m, 2H), 1.86 (m, 2H), 1.73 (m, 4H), 0.87 (t, J = 7.2, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-(5-methyl-furan-2-ylmethyl)-N-propyl-butane-1,4-diamine (4.11b) 59% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{22}H_{28}ClN_3O$ $[M + H]^+$ 386.2. Found: 386.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.54 (br, 1H), 9.74 (t, J = 5.2, 1H), 8.79 (d, J = 9.2, 1H), 8.54 (m, 1H), 8.08 (d, J = 1.6, 1H), 7.77 (dd, J = 9.2, 1.6, 1H), 6.88 (d, J = 3.4, 1H), 6.64 (d, J = 3.2, 1H), 6.13 (d, J = 3.2, 1.2, 1H), 4.32 (d, J = 4.8, 2H), 3.57 (m, 2H), 3.16 (solvent), 3.04 (m, 2H), 2.90 (m, 2H), 2.24 (s, 3H), 1.86 (dt, J = 7.5, 7.5, 2H), 1.72 (m, 4H), 0.87 (t, J = 7.2, 3H).

N-(5-Chloro-furan-2-ylmethyl)-N'-(7-chloro-quinolin-4-yl)-N-propyl-butane-1,4-diamine (4.11c) 13% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{21}H_{25}Cl_2N_3O$ $[M + H]^+$ 406.1. Found: 406.4. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.97 (br, 0.5 H), 9.83 (t, J = 5.2, 1H), 8.85 (d, J = 9.2, 1H), 8.53 (m, 1H), 8.12 (d, J = 1.6, 1H), 7.74 (dd, J = 9.2, 1.6, 1H), 6.89 (d, J = 3.4, 1H), 6.87 (d, J = 7.2, 1H), 6.56 (d, J = 3.2, 1H), 4.38 (d, J = 4.8, 2H), 3.58 (m, 2H), 3.16

(solvent), 3.04 (m, 2H), 2.92 (m, 2H), 1.86 (dt, J = 7.5, 7.5, 2H), 1.73 (m, 4H), 0.87 (t, J = 7.2, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-propyl-N-thiophen-2-ylmethyl-butane-1,4-diamine (4.11f) 44% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{21}H_{26}ClN_3S$ $[M + H]^+$ 388.2. Found: 388.4. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.79 (br-s, 0.5 H), 9.74 (t, J = 5.2, 1H), 8.78 (d, J = 9.2, 1H), 8.54 (m, 1H), 8.08 (d, J = 1.6, 1H), 7.76 (dd, J = 9.2, 1.6, 1H), 7.68 (d, J = 5.2, 1H), 7.42 (d, J = 3.2, 1H), 7.10 (dd, J = 3.2, 3.2; 1H), 6.89 (d, J = 7.2, 1H), 4.54 (d, J = 4.8, 2H), 3.54 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.90(m, 2H), 1.85 (m, 2H), 1.71 (m, 4H), 0.86 (t, J = 7.2, 3H).

2-({[4-(7-Chloro-quinolin-4-ylamino)-butyl]-propyl-amino}-methyl)-phenol (4.11p) 83% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{23}H_{28}ClN_3O$ $[M + H]^+$ 398.2. Found: 398.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 9.72 (t, J = 5.2, 1H), 8.77 (d, J = 9.2, 1H), 8.55 (d, J = 6.8, 1H), 8.07 (d, J = 1.6, 1H), 7.76 (dd, J = 9.2, 1.6, 1H), 7.46 (d, J = 7.6, 1H), 7.24 (t, J = 7.6, 1H), 6.97 (d, J = 7.6, 1H), 6.88 (d, J = 7.2, 1H), 6.82 (t, J = 7.6, 1H), 4.22 (d, J = 4.8, 2H), 3.54 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.90(m, 2H), 1.85 (m, 2H), 1.71 (m, 4H), 0.86 (t, J = 7.2, 3H).

2-({[4-(7-Chloro-quinolin-4-ylamino)-butyl]-propyl-amino}-methyl)-6-fluoro-phenol (4.11q) 39% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{23}H_{27}ClFN_3O$ $[M + H]^+$ 416.2. Found: 416.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 9.72 (t, J = 5.2, 1H), 8.77 (d, J = 9.2, 1H), 8.55 (d, J = 7.2, 1H), 8.07 (d, J = 1.6, 1H), 7.76 (dd, J = 9.2, 1.6, 1H), 7.46 (d, J = 7.6, 1H), 7.24 (t, J = 7.6,

1H), 6.96 (d, J = 7.6, 1H), 6.87 (d, J = 7.2, 1H), 6.82 (t, J = 7.6, 1H), 4.23 (d, J = 4.8, 2H), 3.56 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.94(m, 2H), 1.86 (dt, J = 6.4, 6.4, 2H), 1.70 (m, 4H), 0.86 (t, J = 7.2, 3H).

2-({[4-(7-Chloro-quinolin-4-ylamino)-butyl]-propyl-amino}-methyl)-6-methoxy-phenol (4.11r) 50% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{24}H_{30}ClN_3O_2$ 427.2026. Found: 427.2036. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 9.92 (br-s, 1H), 9.76 (t, J = 5.2, 1H), 8.80 (d, J = 9.2, 1H), 8.54 (m, 1H), 8.10 (d, J = 1.6, 1H), 7.75 (dd, J = 9.2, 1.6, 1H), 7.09 (dd, J = 7.8, 1.2, 1H), 7.02 (dd, J = 7.8, 1.2, 1H), 6.87 (d, J = 7.2, 1H), 6.81 (dd, J = 7.8, 7.8; 1H) 4.24 (d, J = 4.8, 2H), 3.81 (s, 3H), 3.55 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.93 (m, 2H), 1.85 (dt, J = 7.6, 7.6, 2H), 1.72 (m, 4H), 0.85 (t, J = 7.2, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 157.4, 149.1, 147.2, 143.7, 140.8, 139.8, 128.5, 126.5, 124.8, 120.9, 120.1, 117.3, 116.8, 114.1, 99.9, 56.6, 56.1, 54.1, 53.3, 44.1, 26.2, 22.4, 18.3, 11.2.

2-({[4-(7-Chloro-quinolin-4-ylamino)-butyl]-propyl-amino}-methyl)-4-methoxy-phenol (4.11s) 55% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{24}H_{30}ClN_3O_2$ $[M + H]^+$ 428.2. Found: 428.5. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 10.01(br-s, 1H), 9.72 (t, J = 5.2, 1H), 8.78 (d, J = 9.2, 1H), 8.5 (m, 1H), 8.08 (d, J = 1.6, 1H), 7.76 (dd, J = 9.2, 1.6, 1H), 7.16 (d, J = 2.9, 1H), 6.89 (d, J = 4.8, 1H), 6.87 (d, J = 2.9, 1H), 6.81 (m, 1H) 4.21 (d, J = 4.8, 2H), 3.67 (s, 3H), 3.55 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.95 (m, 2H), 1.86 (dt, J = 7.6, 7.6, 2H), 1.72 (m, 4H), 0.85 (t, J = 7.2, 3H).

2-({[4-(7-Chloro-quinolin-4-ylamino)-butyl]-propyl-amino}-methyl)-4-trifluoromethoxy-phenol (4.11t) 33% yield; 100% pure by HPLC Method A; 100%

pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{24}H_{27}ClF_3N_3O_2$ $[M + H]^+$ 482.2. Found: 482.4. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.07(br-s, 1H), 9.73 (t, *J* = 5.2, 1H), 8.78 (d, *J* = 9.2, 1H), 8.5 (m, 1H), 8.08 (d, *J* = 1.6, 1H), 7.77 (dd, *J* = 9.2, 1.6, 1H), 7.61 (d, *J* = 2.9, 1H), 7.26 (dd, *J* = 8.9, 2.9, 1H), 6.08 (d, *J* = 8.9, 1H), 6.87 (d, *J* = 7.2, 1H), 4.26 (d, *J* = 4.8, 2H), 3.55 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.95 (m, 2H), 1.85 (dt, *J* = 7.6, 7.6, 2H), 1.72 (m, 4H), 0.85 (t, *J* = 7.2, 3H).

2-({[4-(7-Chloro-quinolin-4-ylamino)-butyl]-propyl-amino}-methyl)-benzene-1,4-diol (4.11v) 52% yield; 87% pure by HPLC Method A; 83% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{23}H_{28}ClN_3O_2$ $[M + H]^+$ 414.2. Found: 414.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 9.76 (t, *J* = 5.2, 1H), 9.67 (br-s, 1H), 8.79 (d, *J* = 9.2, 1H), 8.54 (m, 1H), 8.09 (d, *J* = 1.6, 1H), 7.76 (dd, *J* = 9.2, 1.6, 1H), 6.88 (d, *J* = 7.2, 1H), 6.86 (d, *J* = 2.9, 1H), 6.78 (d, *J* = 8.6, 1H), 6.69 (dd, *J* = 8.6, 2.9; 1H), 4.15 (d, *J* = 4.8, 2H), 3.535 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.95 (m, 2H), 1.85 (dt, *J* = 7.5, 7.5, 2H), 1.72 (m, 4H), 0.86 (t, *J* = 7.2, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-(5-fluoro-2-methoxy-benzyl)-N-propyl-butane-1,4-diamine (4.11x) 39% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; LCMS (ESI) *m/z* Calcd for $C_{24}H_{29}ClFN_3O$ $[M + H]^+$ 430.2. Found: 430.5. ^1H NMR (d_6-DMSO, 400 MHz): δ 10.13 (br-s, 1H), 9.80 (t, *J* = 5.2, 1H), 8.82 (d, *J* = 9.2, 1H), 8.54 (m, 1H), 8.09 (d, *J* = 1.6, 1H), 7.76 (dd, *J* = 9.2, 1.6, 1H), 7.56 (dd, *J* = 9.1, 3.1; 1H), 7.26 (dt, *J* = 3.1, 8.2, 1H), 7.10 (dd, *J* = 4.4, 9.1; 1H), 6.88 (d, *J* = 7.2, 1H), 4.25 (d, *J* = 4.8, 2H), 3.81 (s, 3H), 3.55 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.95 (m, 2H), 1.85 (dt, *J* = 7.6, 7.6, 2H), 1.71 (m, 4H), 0.86 (t, *J* = 7.2, 3H).

N'-(7-Chloro-quinolin-4-yl)-N-(2-fluoro-6-methoxy-benzyl)-N-propyl-butane-1,4-diamine (4.11y) 22% yield; 100% pure by HPLC Method A; 87% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{24}H_{29}ClFN_3O$ 429.1983. Found: 429.1972. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 9.90 (t, *J* = 5.2, 1H), 9.75 (br-s, 1H), 8.85 (d, *J* = 9.2, 1H), 8.54 (m, 1H), 8.12 (d, *J* = 1.6, 1H), 7.75 (dd, *J* = 9.2, 1.6, 1H), 7.49 (dd, *J* = 7.5, 8.2; 1H), 6.97 (d, *J* = 8.6, 1H), 6.89 (m, 2H), 4.26 (d, *J* = 4.8, 2H), 3.88 (s, 3H), 3.55 (m, 2H), 3.16 (solvent), 3.05 (m, 2H), 2.95 (m, 2H), 1.88 (m, 2H), 1.74 (m, 4H), 0.86 (t, *J* = 7.2, 3H). ^{13}C NMR (d_4-MeOD, 400 MHz): δ 159.6, 156.2, 142.5, 140.7, 139.6, 138.5, 133.0, 127.3, 125.4, 118.9, 115.6, 107.9, 107.6, 107.3, 106.0, 56.0, 55.2, 53.3, 44.9, 42.9, 25.0, 21.1, 16.8, 10.1.

N-Benzo[1,3]dioxol-4-ylmethyl-N'-(7-chloro-quinolin-4-yl)-N-propyl-butane-1,4-diamine (4.11aa) 47% yield; 100% pure by HPLC Method A; 100% pure by HPLC Method B; HRMS (EI) *m/z* Calcd for $C_{24}H_{28}ClN_3O_2$ 425.1870. Found: 425.1857. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 10.68 (br-s, 1H), 9.79 (t, *J* = 5.2, 1H), 8.80 (d, *J* = 9.2, 1H), 8.54 (m, 1H), 8.10 (d, *J* = 1.6, 1H), 7.75 (dd, *J* = 9.2, 1.6, 1H), 7.12 (d, *J* = 7.6; 1H), 6.97 (d, *J* = 7.6, 1H), 6.88 (m, 2H), 6.06 (s, 2H), 4.24 (d, *J* = 4.8, 2H), 3.53 (m, 2H), 3.16 (solvent), 3.07 (m, 2H), 2.95 (m, 2H), 1.90 (dt, *J* = 7.5, 7.5, 2H), 1.74 (m, 4H), 0.86 (t, *J* = 7.2, 3H).

Measurement of *in vitro* antimalarial activity. The effects of experimental compounds on the growth of *Plasmodium falciparum* cultures *in vitro* were measured using flow cytometry.[25] Synchronous cultures of ring stage parasites (500 μL, 0.8% parasitemia, 2% hematocrit) were grown in 24-well tissue culture plates (Falcon) with 30 nm and 200 nm concentrations of experimental compounds. Cultures were grown in atmospherically

regulated (6 % CO2, 5 %O2) incubators (Sanyo) at 37 °C for a total of 4 days. Aliquots of 100 μL were removed from each well at 74 hours post drug treatment and resuspended in 900 μL of 1 % paraformaldehyde, 1 nM YOYO-1 (Molecular Probes) in PBS. Each sample was then incubated for 16 h in the dark at 4 °C before being analyzed on a Becton-Dickenson LSR2 flow cytometer to measure the percent of parasitized red blood cells (RBCs). Percent of parasitized RBCs was directly read and subsequently growth inhibition values were calculated as the fraction of parasitized RBCs relative to cultures without drug. All screening was done in triplicate with a no drug negative control and a positive control, chloroquine. Dose response studies followed the same general methodology with the exception that each drug was used at doses ranging from 0.1 to 200 nM in triplicate. Fifty percent inhibitory concentrations (IC_{50}'s) were calculated by fitting the data to a variable slope sigmoidal dose response curve using SigmaPlot graphing software.

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Chapter 5

Optimization of Pharmacokinetic Properties

Overview

Attaining an appropriate pharmacological profile for a compound is essential in the development of any drug. The discovery of potent compounds active against drug-resistant *P. falciparum* strains is an important first step, but it remains equally important to attain a compound that is has good Adsorption, Distribution, Metabolism, Excretion and Toxicology (ADMETox) properties. We focused our synthesis around the general scaffold of chloroquine, which has a very good and well-understood pharmacological profile. Starting from this "priveledged scaffold" gives us a distinct advantage in the development of compounds with good ADMETox properties, but small structural changes could lead to drastic changes in ADMETox properties. Any new chemical entitity (NCE) must be evaluated for its pharmacological properties and optimized in combination with optimization of biological activity. This chapter will discuss the evaluation of several ADMETox properties of some of our most potent compounds and efforts to improve their pharmacological profile.

Background

In order to evaluate the pharmacological properties of the compounds discussed in the preceeding chapters, we set up a collaboration with SRI International (Menlo Park, CA). All of the data presented regarding mutagenicity, cytotoxicity, metabolic stability, RBC partitioning, adsorption and bioavailability was measured in the lab of Dr. Jon Mirsalis and Dr. Carol Green at SRI International. Initially, we chose to focus on evaluating the pharmacological properties that we expected would cause the greatest number of compounds to fail, which were mutagenicity and metabolic stability. CQ was

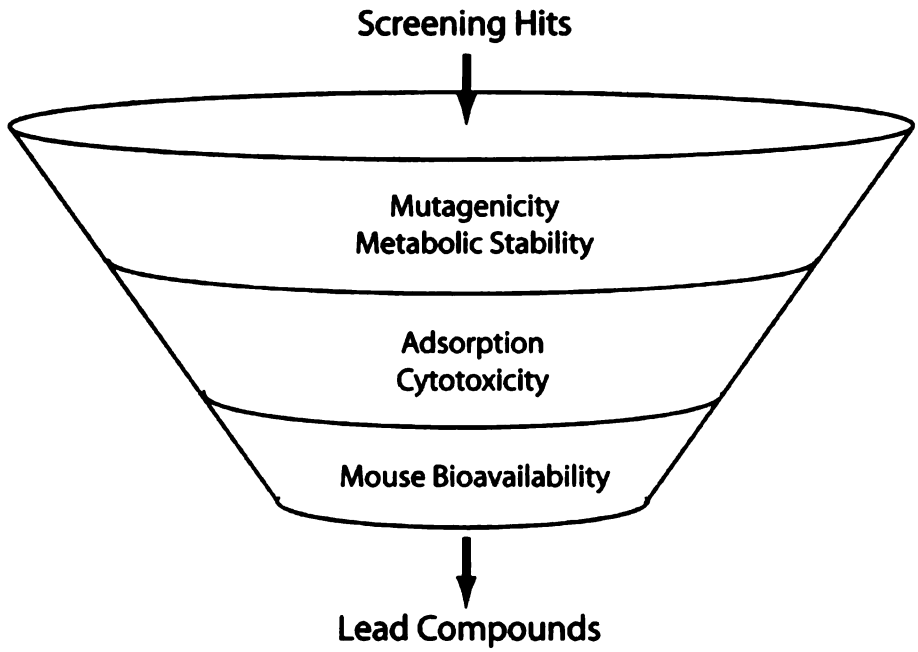


Figure 5.1 Development funnel for selecting lead compounds from our active hits.

already known to bind to DNA and be a weak mutagen so we expected that some of our compounds would share this property. It was also known that CQ was primarily metabolized on its side chains by N-dealkylations and many of our modification were to this region of the molecule, so we expected significant changes in the metabolism of our compounds. After measuring these properties, we would then go ahead and measure other pharmacokinetic (PK) properties to aid in the selection of lead candidates.(Fig 5.1)

Mutagenicity

CQ is known to intercalate with DNA at high concentrations and be a weak mutagen.[1, 2] The Ames II assay was used to assess the mutagenicity of the screening hits. This assay measures the ability of a compound to cause a revertant mutation in the bacterial strain *Salmonella typhimurium*.[3, 4] The Ames assay is useful for giving a guideline as to what might be mutagenic, but in no way guarantees that a compound is

not mutagenic. Eighteen compounds, containing both ring substitutions and side-chain substitutions, were tested using the Ames II assay. All of the compounds showed no mutagenic activity in the assay against either the TA98 (frame shift mutation) or TA100 (base substitution) strains. This data indicates that the class of compounds we have worked on do not have notably high mutagenicity potential.

Metabolic Stability

Assessment of the metabolic stability of compounds was assessed using a human microsomal degradation assay. In this assay, pooled human liver microsomes were incubated with each test compound in the presence of NADPH and the disappearence of parent compound was monitored by LC/MS/MS.[5, 6] Time points were taken at 0, 15, 30 and 60 minutes and the amount of parent compound relative to time 0 was plotted to estimate the half-life. This *in vitro* assay has shown to give excellent predictive value for real metabolic clearence in humans.[6, 7] Data for eighteen representative compounds (Fig

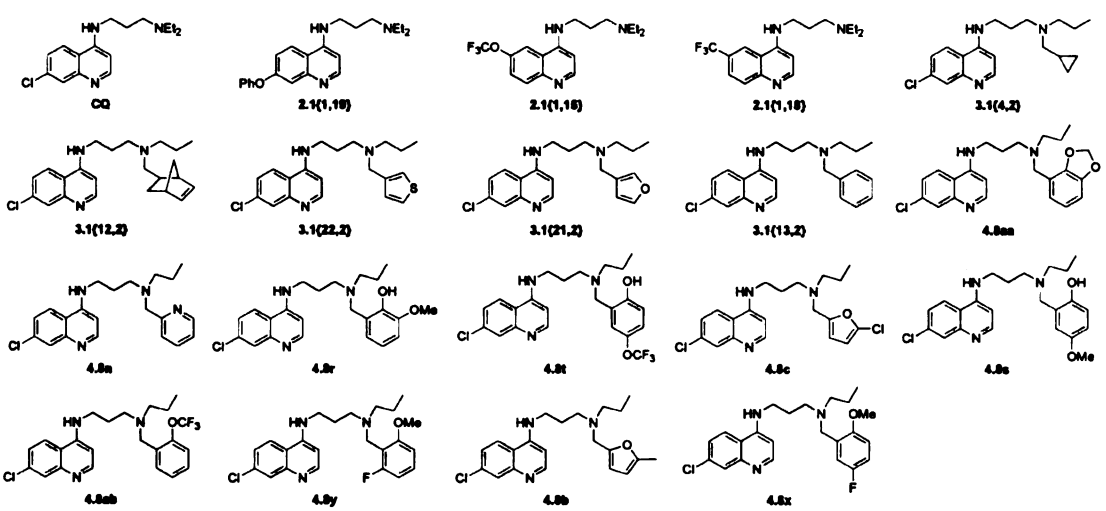


Figure 5.2 Stuctures of CQ and 18 synthetic compounds used for modeling PK properties. Compounds selected were the most potent members from each library.

5.2) is shown in Table 4.1.

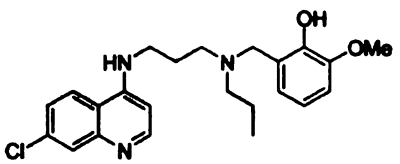
Compound	Region of Modification	$t_{½}$, 1 μM	$t_{½}$, 10 μM
CQ		749.0	NC
2.1{1,19}	Ring	374.7	255.6
2.1{1,15}	Ring	116.2	NC
2.1{1,18}	Ring	116.3	NC
3.1{4,2}	Side chain	48.2	108.9
3.1{12,2}	Side chain	13.8	13.0
3.1{22,2}	Side chain	12.0	17.7
3.1{21,2}	Side chain	11.8	29.3
3.1{13,2}	Side chain	10.3	6.7
4.8aa	Side chain	8.5	29.2
4.8n	Side chain	7.7	14.1
4.8r	Side chain	7.5	37.1
4.8t	Side chain	6.9	8.7
4.8c	Side chain	6.9	8.2
4.8s	Side chain	6.7	8.2
4.8ab	Side chain	6.4	29.5
4.8y	Side chain	6.3	8.8
4.8b	Side chain	5.6	38.4
4.8x	Side chain	5.5	8.6

Table 5.1 Half lives of representative compounds in human microsomal stability assay at two concentrations 1 μM and 10 μM. NC = Not calculated due to insufficient decrease in parent compound.

None of the compounds synthesized had the long half-life of chloroquine. The ring analogs had significantly longer half-lives than all the side-chain analogs tested. This fits our expectation since chloroquine is known to be primarily metabolized at the side-chain.[8] The modifications to the basic side chain did indeed have a very significant affect on the metabolism of the compounds. More importantly, all of the compounds with IC_{50} values less than 10 nM againstW2 (**4.8c, 4.8r, 4.8s, 4.8t, 4.8aa**) had half-lives at 1 μM

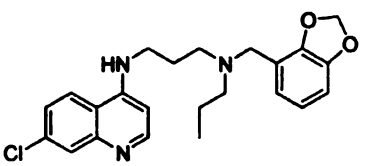
less than 10 minutes. The structures in Figure 5.2 are listed in order of decreasing half-lives and clearly show that all the structures containing an aromatic group in the side chain have much shorter half lives. The shortened half lives of the compounds with benzylic substituted nitrogens was somewhat expected since the protons at the benzylic position are significantly more acidic than those of an alkyl substitution on the nitrogen. The rate limiting step of most N-dealkylation reactions is the abstraction of the proton α to the nitrogen, so it was expected that increasing the acidity of this proton would increase the rate of metabolism. The measurements were done at two concentrations, 1 μM and 10 μM, to see if saturation of the metabolizing enzymes would be achieved over this concentration range. The data does show longer half lives at 10 μM for many compounds, indicating that metabolizing enzyme is close to saturation at this concentration range. The data does not allow us to extrapolate the measurement to an approximate half-life in humans, but clearly the metabolic stability of this set of compounds was less than desirable. Ideally, we would like to produce compounds with a half-life of at least 60 minutes in this assay.

In order to obtain more information about the metabolism of many of our most potent compounds, LC/MS was used to identify what metabolites were being formed in the incubation with human microsomes. (Fig 5.3) As expected, the primary metabolites formed were due to N-dealkylation reactions, of both the propyl group (N-des-Prop) and the aromatic groups (N-des-R). It was expected that the N-dealkylation of the aromatic groups would be much faster due to the increased acidity of the proton at the benzylic position as mentioned earlier. In each case where the metabolites were analyzed, the



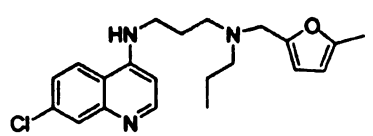
4.8r

Product	% metabolite
parent	63.4
N-des-R	11.2
N-des-Prop	12.1
O-desmethyl	9.4
"+1 oxygen"	2.5
"+1 oxygen"	1.4



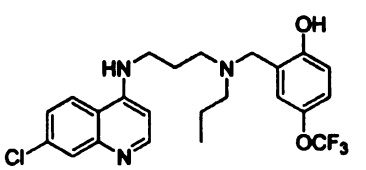
4.8aa

Product	% metabolite
parent	49.4
"- methylene"	23.9
N-des-Prop	19.4
N-des-R	7.3



4.8b

Product	% metabolite
N-des-Prop+O	27.8
N-des-Prop	26.8
N-des-R	18.5
parent	11.0
N-des-Prop+O	6.0
4-amino-7-chloroquin	6.0
unknown (305)	3.0
N-des-Prop+O	0.9



4.8t

Product	% metabolite
parent	46.2
N-des-Prop	21.0
N-des-linker	11.8
"+1 oxygen"	8.3
O-des-CF3	2.9
"+1 oxygen"	2.9
N-dealkyloxide	2.4
N-des-R	2.3
4-amino-7-chloroquine	1.4
N-des-Prop+O	0.7

Figure 5.3 Metabolites identified by LC/MS after incubation with pooled human microsomes.

products resulting from the N-dealkylation of the propyl group was just as abundant or more abundant that the product resulting from removal of the benzyl group (N-des-R). This data was encouraging since it is known that the alkyl substitution on the nitrogen is more tolerant to modifications than the aromatic group, so perhaps the metabolic stability could be increased by changes to the propyl group. Also, the methoxy group in **4.8r** and the methylene group in **4.8aa** proved to be metabolically labile. The metabolism at the aromatic groups was the most worrisome since this feature of the structure affords the greatest activity against the drug-resistant *P. falciparum* strains. This leaves us the challenge of modifying the compounds in such a way as to slow down the metabolism while retaining the activity.

RBC Partitioning

The stage of the parasite targeted by 4-aminoquinolines is inside human red blood cells (RBCs) so we decided to measure the partitioning between human plasma and RBCs. In this experiment, test compound is incubated with 40% RBCs in human serum for 30 minutes at 37 °C. The RBCs are then pelleted and the concentration of the compound in the serum is quantified by LC/MS/MS. This measurement allows measurement of the RBC to plasma partition ratio (B/P ratio), which was measured at two initial compound concentrations (1 μM and 10 μM). The results of these measurements are shown in Table 5.2.

Compound	Region of Modification	B/P, 1 μM	B/P, 10 μM
CQ		3.76	4.08
2.1{1,19}	Ring	4.79	21.31
2.1{1,15}	Ring	6.12	6.78

2.1{1,18}	Ring	4.94	5.45
3.1{4,2}	Side chain	2.58	3.67
3.1{12,2}	Side chain	3.28	5.16
3.1{22,2}	Side chain	3.20	3.67
3.1{21,2}	Side chain	5.69	5.36
3.1{13,2}	Side chain	IC	IC
4.8aa	Side chain	1.55	2.58
4.8n	Side chain	2.44	1.59
4.8r	Side chain	2.85	2.52
4.8t	Side chain	3.01	1.92
4.8c	Side chain	2.00	1.89
4.8s	Side chain	3.13	2.48
4.8ab	Side chain	3.20	3.36
4.8y	Side chain	2.44	2.36
4.8b	Side chain	2.74	2.23
4.8x	Side chain	2.34	2.13

Table 5.2 Ratios of partitioning between RBCs and plasma (B/P) at 1 μM and 10 μM.

These data show that all the compounds partition into RBCs over plasma with ratios ranging from 1.55 to 6.12 at 1 μM. This data partially reflects that all the compounds tested showed *in vitro* activity and a high B/P ratio should lead to higher activity. Although, our *in vitro* testing is done at 2% RBCs while the partitioning experiment is done at 40% RBCs, which more accurately simulates the concentration found in an average human blood stream. This data is interesting in that it adds to our knowledge of why this class of compounds shows broad potency, but the ability to accumulate in RBCs is indirectly captured in our *in vitro* activity assay.

Absorption

The 4-aminoquinolines we have synthesized all have a basic center and are relatively lipophilic. Given these properties, we wanted to analyze the permeability at

various pH values to get an idea of whether the compounds would have oral absorption in the intestinal tract. An *in vitro* permeability modeling system called Parallel Artificial Membrane Permeability Assay (PAMPA) was used to measure absorption at three pH values. This assay was performed by *p*ION Inc.©. The assay works by measuring the transfer of compound between a donor compartment, through a lipid bilayer membrane, into an acceptor compartment. These values were measured for 5 compounds and CQ at three pH values and the results are shown in Table 4.3.

Compound	pH (5.0 / 7.4) P_e (x 10^{-6}cm/s)	pH (6.2 / 7.4) P_e (x 10^{-6}cm/s)	pH (7.4 / 7.4) P_e (x 10^{-6}cm/s)	R% pH 5.0	R% pH 6.2	R% pH 7.4
CQ	< 0.1	3.3	99	18	23	31
2.1{1,19}	0.6	44	1078	22	35	84
2.1{1,15}	2.1	59	1202	21	37	62
4.8aa	4.1	393	1040	32	50	60
4.8b	3.3	340	1030	15	47	66
4.8s	7.8	111	688	25	40	61

Table 5.3 PAMPA permeability measurements (in x10^{-6} cm/s) and percent of compound retained within the membrane (%R) at three pH values.

It is generally accepted that any compound with greater that 1 x 10^{-5} cm/s permeability is considered highly permeable. All of the compounds tested show moderate permeability at pH 5.0, but high permeability at pH 6.2 and 7.4. This type of profile predicts absorption primarily in the lower gastrointestinal tract (jejunum and ileum) where the pH range is from 6.0 -7.0.[9] This is a logical pattern given the basic nature of all the compounds in this series. It should also be noted that all of the compounds tested showed better permeability than the CQ control. The percent of compound retained within the lipid bilayer membrane is also calculated and shows that all of the compounds partition heavily into the membrane significantly. At pH 7.4, all of the compounds tested are > 50% partitioned

into the membrane, except for chloroquine. Overall, this data indicates that our 4-aminoquinoline series have an acceptable permeability profile in this *in vitro* model.

Cytochrome P450 Inhibition

Inhibition of the cytochrome P450 enzymes can cause important drug-drug interactions and toxicity implications.[10] We measured the ability of a representative set of our compounds (Fig 5.2) to inhibit a panel of six common P450 isoforms. In this assay, crude P450 preparations were incubated simultaneously with specific substrates for each compound and a test compound. Primary metabolites for each of the P450 isoforms was detected by LC/MS/MS and used to quantitate the percentage of inhibition due to the test compound. The results of this experiment can be seen in Table 5.4. Most of the compounds showed very little P450 inhibition, but many of the compounds with side chain modifications did show inhibition of CYP2D6. The CYP2D6 enzyme is responsible for metabolizing about 25% of all known drugs, especially many alkaloid drugs.[11] Some of the known inhibitors of CYP2D6 include bufuralol, quinidine and thioridazine, which arguably have some structural similarity to some of the compounds in our libraries. All of the compounds containing either an oxygen or sulfur atom showed greater than 20% inhibition of CYP2D6, while those lacking either of these atoms all showed less than 20% inhibition. This could possible present a challenge since all of our most potent compounds contain additional hydrogen-bond accepting functional groups involving oxygen or sulfur. The data does show a broad range of inhibition though with many potent compounds showing only around 30% inhibition at 1 μM. The only compounds showing greater than 50% inhibition of CYP2D6 are **4.8aa** (77%) and **4.8t** (62%), which

unfortunately are also some of the most potent compounds. Interestingly, the compounds **4.8aa** and **4.8r** differ only by a single bond but have almost a 2-fold difference in inhibition of CYP2D6. Overall, this data indicates that there is some potential for drug-drug interactions with drugs metabolized by the CYP2D6 enzyme. Many of the same compounds that show inhibition of CYP2D6 also have shown poor metabolic stability, suggesting that possibly many of these compounds are metabolized by CYP2D6. Further investigations will be needed to verify this and determine what an acceptable level of CYP2D6 inhibition is.

Compound	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP3A4[a]	CYP3A4[b]
CQ	0	0	0	1	0	0
2.1{1,19}	0	0	0	0	0	0
2.1{1,15}	1	0	0	19	0	0
2.1{1,18}	3	0	7	6	6	4
3.1{4,2}	5	0	0	17	0	0
3.1{12,2}	0	0	0	39	11	24
3.1{22,2}	2	2	0	45	0	0
3.1{21,2}	0	0	3	40	0	1
3.1{13,2}	0	0	0	17	0	0
4.8aa	0	0	0	77	0	0
4.8n	0	0	0	15	0	0
4.8r	4	3	0	44	0	6
4.8t	2	4	0	62	3	20
4.8c	4	2	10	39	10	13
4.8s	6	15	0	47	3	11
4.8ab	2	7	0	36	0	8
4.8y	0	0	0	27	0	1
4.8b	0	0	0	6	0	0
4.8x	1	0	6	21	11	16

Table 5.4 Inhibition of five cytochrome P450 isoforms at 1μM. Data expressed as % inhibition of metabolism of known substrates. Known substrates are the following for each isoform: CYP2B6 (Bupropion), CYP2C9 (Diclofenac), CYP2C19 (Mephenytoin), CYP2D6 (Bufuralol), CYP3A4[a] (Midazolam), CYP3A4[b] (Testosterone).

Mouse PK Parameters

Six compounds were orally administered to male mice to measure the pharmacokinetic (PK) parameters of this compound series. A set was chosen containing structures with both ring substitutions and side chain substitutions to examine the PK parameters of both of these series of compounds. The data is presented below in Table 5.5.

		20 μmol/kg			100 μmol/kg	
Compound	$t_{1/2}$ (min.)	C_{max} (pm/mL)	AUC	$t_{1/2}$ (min.)	C_{max} (pm/mL)	AUC
CQ	2.6	81.4	425.1	5.5	444.3	2898.3
2.1{1,15}	3.4	249.6	1842.9	4.4	1353.7	9334.6
2.1{1,19}	4.9	55.0	327.6	3.1	261.4	1091.2
3.1{4,2}	8.8	126.1	1982.3	15.9	436.6	7029.2
4.8s	3.2	737.1	3147.8	3.4	3165.5	10652.8
4.8aa[a]	-	-	-	-	-	-
4.8b[a]	-	-	-	-	-	-

Table 5.5 PK parameters in male mice after oral administration at two doses, 20 μmol/kg and 100 μmol/kg. [a] none of the samples contained detectable drug.

The two compounds with ring substitutions (**2.1{1,15}** and **2.1{1,19}**) showed similar half lives ($t_{1/2}$) but very different peak plasma concentration (C_{max}) values. Compound **2.1{1,15}** actually had a longer half life and much greater C_{max} and AUC values than CQ. Both of these two compounds showed significantly shorter half-lives than CQ in the human microsomal degradation assay, but have slightly longer half-lives in the mouse. This data clearly shows that these compounds are indeed making it to the blood stream effectively after oral administration. Interestingly, the mouse PK data for the compounds with side chain variations show two compounds (**4.8aa** and **4.8b**) having undetectable amounts of compound and two others (**3.1{4,2}** and **4.8s**) with half-lives similar to or greater than CQ. The human metabolic stability data showed all of these four compounds

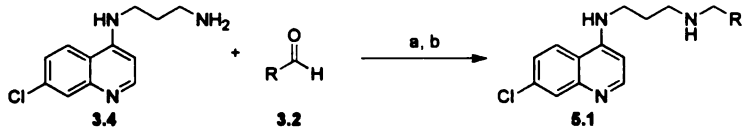
having short half-lives, yet in the mouse two of the four appear to be relatively metabolically stable. The most interesting compounds is **4.8s** which had a half live in human microsomes of 6.7 minutes, but had the longest half life of all the compounds tested by oral administration to a mouse. This compound also showed the greatest peak plasma concentration and AUC value. This discrepancy between metabolic stability in the human microsomes compared to in the mouse highlight the major differences in metabolism between mammalian species and the importance of using the human microsomal degradation assay.

Improvement of PK properties

Preliminary measurements of PK properties showed a good profile for our class of compounds with the exception of the metabolic stability and possibly the inhibition of CYP2D6. The short half-lives reported in the human microsomal degradation assay indicated that compound modifications are needed to increase the metabolic stability. The remainder of this chapter involves the synthesis and testing of new compounds based on the activity data reported in the previous chapters, trying to increase the half-life in the human microsomal degradation assay.

Chemistry

The analysis of metabolites in the human microsome assay showed that *N*-dealkylation was the primary route of metabolism for our compounds. The detection of the secondary amine products of this metabolism indicated that these metabolites possessed some metabolic stability themselves. It had previously been shown that the



Scheme 5.1 Synthesis of side chain substituted secondary amines. a) RCHO (3.2, 4 eq.), MeOH, rt, 24 h; b) $NaBH_4$ (5 eq.), rt., 1 h.

addition of an aromatic group with a hydrogen-bonding acceptor benefited the *in vitro* activity against drug-resistant *P. falciparum*, so we needed to preserve this moiety in our compounds. To this end, we elected to make the simple modification of making the analogous secondary amines to many of our most potent compounds by simply removing the propyl group from the terminal nitrogen group. Using a previously described intermediate, a set of compounds was synthesized by doing a simple one-step reductive amination. (Scheme 5.1) In order to prevent the formation of a tertiary amine double adduct, the imine intermediate was preformed before the reduction by sodium borohydride. The reactions yielded the final products shown in Figure 5.4. In addition, a secondary amine product was made using propionaldehyde to form the mono-propyl product that was also a major metabolite in each of the four compounds for which

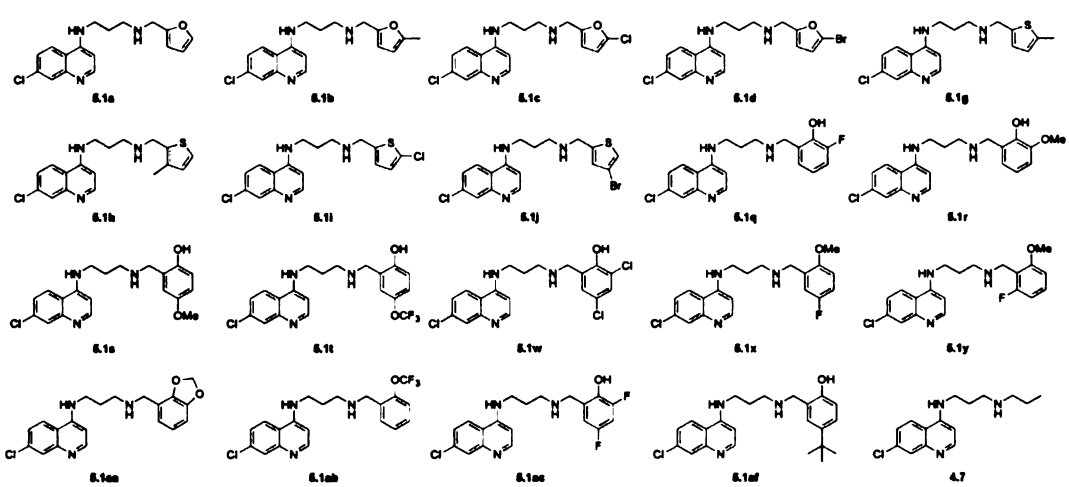


Figure 5.4 Structures of secondary amine products from reductive amination.

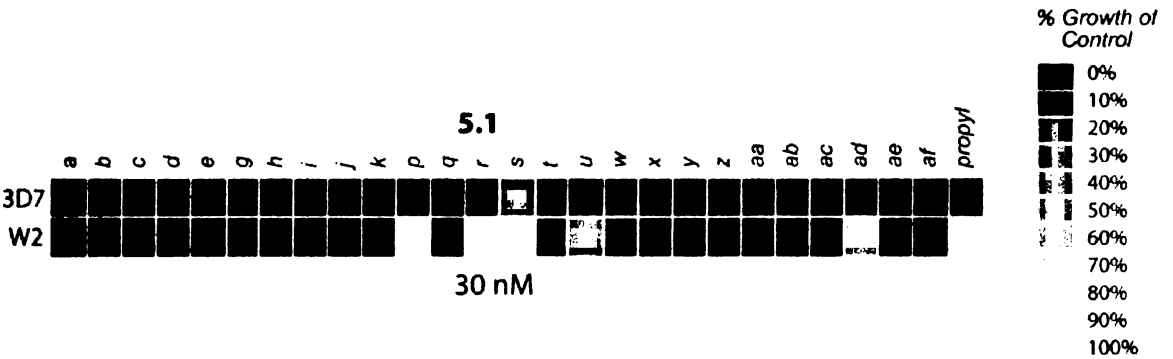


Figure 5.5 Colormetric representation of activity data for secondary amine compounds screened at 30 nM against the 3D7 (drug-sensitive) and W2 (drug-resistant) strains of *P. falciparum*.

metabolites were identified.(Fig 5.2) Each of these reactions were purified by a solid-phase extraction procedure similar to that described in the previous chapter, but without the scavenging step since our desired products contain a nucleophilic secondary amine center. Of the 33 targeted compounds, 27 were successfully synthesized (82%) in this library. The simplicity of this one step reaction allowed for each of the synthesized compounds to be made with greater than 95% purity by HPLC and an average yield of 70% using no chromatography purification.

Activities

Each of the secondary products synthesized were initially screened against the drug-sensitive 3D7 strain and the drug-resistant W2 strain of *P. falciparum*. The compounds were screened at two concentrations, 30 nM and 200 nM, and the majority of the compounds showed activity at the lowest concentration screened. (Fig 5.4) Some of the compounds did show less activity against the drug-resistant W2 strain, but generally the secondary amine compounds were nearly as potent as the tertiary amine compounds.

Dose response curves were made for several of the compounds and IC50 values were measured.(Table 5.6)

Compound	3D7 IC50 (nM)	W2 IC50 (nM)
5.1a	1.8	7.3
5.1b	4.2	11.3
5.1c	5.0	10.0
5.1d	6.0	9.4
5.1g	3.7	7.9
5.1h	6.6	16.1
5.1i	13.6	16.1
5.1j	10.4	10.8
5.1q	2.0	29.6
5.1r	4.7	45.3
5.1s	13	183.0
5.1t	1.9	25.3
5.1w	7.8	32.4
5.1x	3.5	4.3
5.1y	2.9	8.5
5.1aa	2.5	5.7
5.1ab	6.5	7.9
5.1ac	4.0	16.3
5.1af	7.2	23.0
mono-propyl (4.7)	1.0	100.0

Table 5.6 IC_{50} values of secondary amine compounds against 3D7 and W2 strains of *P. falciparum*.

The IC50 values show that most of the secondary amine compounds remain highly active against both strains whereas a few compounds, such as **5.1q, 5.1r, 5.1s, 5.1t,** and the mono-propyl compound show substantially less activity against the W2 strain than the 3D7 strain. Interestingly, when an additional propyl group on the amine converts these compounds into a tertiary amine, they become highly potent against the W2 strain. It is not clear why this small structural change results in such a substantial change in *in vitro* potency.

Given that many of the secondary amines still have significant activity against the drug-resistant parasite strains, we then proceeded to test their metabolic stability in the

human microsomal stability assay. The compounds **5.1s**, **5.1r** and **5.1aa** were initially chosen for testing and their half-lives and RBC partitioning are reported in Table 5.7.

Compound	$t_{1/2}$, 1 μM	$t_{1/2}$, 10 μM	B/P, 1 μM	B/P, 10 μM
5.1r	147	160	5.73	8.77
5.1s	409	ND[a]	2.66	0.91
5.1aa	94	276	5.15	3.33

Table 5.7 Half-lives of secondary amine compounds in the human microsomal stability assay and blood-plasma partitioning ratios at 1 μM and 10 μM compound concentrations. [a]Insufficient depletion of parent compound after 60 minutes to calculate half-life.

These data show that each of the three secondary amine compounds tested had metabolic stability half lives at least 10-times as long as for the tertiary amine compounds.

This data is analogous to the metabolic stability of the drug amodiaquine. This compound is actually a prodrug and is rapidly metabolized to des-ethylamodiaquine (DEAQ), the secondary amine metabolite resulting from the N-dealkylation reaction. The metabolism of amodiaquine to DEAQ is known to be mediated specifically by CYP2C8, which was not used for our set of P450 isoforms.[12] Interestingly, the compounds **5.1r**, **5.1s** and **5.1aa** were all tested for P450 inhibition against the same 6 isoforms shown in Table 5.4 and showed no inhibition of any of the isoforms. The analogous tertiary amines, **4.8r**, **4.8s**, and **4.8aa**, inhibited the CYP2D6 enzyme at 1 μM by 44%, 47% and 77% respectively. This dramatic change in metabolism mirrors the trend seen for the same three compounds in the human microsomal stability assay. This small data set is too small to draw any general conclusions, but it makes it appear that secondary amines tend to be more metabolically stable and this may be resulting from reduced metabolism by CYP2D6. Further studies on a larger set of compounds are now underway.

Conclusions

Only three compounds have currently been tested for metabolic stability, but each of these compounds showed significantly improved stability over their tertiary amine counterpart. Although the secondary amine compounds show slightly weaker potencies compared to the tertiary amines, the increase in metabolic stability more than offsets the slight loss in potency. Several of these compounds will be further evaluated in *in vivo* models to better assess the potential of these new lead compounds.

Experimental

General Procedure for Reductive Alkylation Diversity Step. A stock solution of N1-(7-Chloro-quinolin-4-yl)-propane-1,3-diamine (**3.4**) was made in dry MeOH and 1.5 mL (0.04 mmol) was aliquoted to the wells of a 48-well reaction block. To each well was then added an aldehyde (Fig 4.3) (0.17 mmol, 4 eq.) with care taken to reduce exposure to moisture in the air. The reaction block was sealed with a rubber gasket and shaken for 16 hours at 300 rpm. Then sodium borohydride (6.4 mg, 0.17 mmol) was added to each well. CAUTTION: explosion hazard. After bubbling had ceased, the solution remaining in each well was transferred into another 48-position reaction block containing cartridges loaded with SCX-SPE medium. Each well was washed with 1% TFA in MeOH (1 mL, 2X), MeOH (2 mL, 2X) and eluted with 5% TEA in MeOH (2 mL, 2X). The eluted products were collected into glass test tubes and solvent was removed on a GeneVac HT-4 (10 mbar, 4.0 h, 35 °C). The partially purified products were then dissolved in saturated HCl/MeOH (1 mL) and dried again to form the HCl salts of each compound. The dry HCl salts were then each dissolved in 1mL d_6-DMSO and subjected to LC/MS/CLND for

analysis of purity, identity and yield. A 10 mM stock solution of each compound was made in d_6-DMSO based on the CLND quantitation data and selected compounds were analyzed by ^{1}H-NMR.

N-(7-Chloro-quinolin-4-yl)-N'-furan-2-ylmethyl-propane-1,3-diamine (5.1a) 16% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{17}H_{18}ClN_3O$ $[M + H]^+$ 316.11. Found: 316.47. ^{1}H NMR (400 MHz, d_4-MeOD): δ 8.53 (d, J = 9.2, 1H), 8.44 (d, J = 7.2, 1H), 7.89 (d, J = 2.0, 1H), 7.64 (dd, J = 9.2, 2.0, 1H), 7.61 (d, J = 1.2, 1H), 6.98 (d, J = 7.2, 1H), 6.74 (d, J = 3.6, 1H), 6.51 (dd, J = 1.6, 3.2, 1H), 4.36 (s, 2H), 3.77 (t, J = 6.8, 2H), 3.25 (t, J = 7.6, 2H), 2.25 (m, 2H). ^{13}C NMR (400 MHz, d_4-MeOD): δ 157.1, 145.9, 145.4, 143.6, 140.5, 139.4, 128.3, 126.1, 119.8, 116.5, 113.5, 111.7, 99.6, 45.3, 43.9, 41.3, 25.4.

N-(7-Chloro-quinolin-4-yl)-N'-(5-methyl-furan-2-ylmethyl)-propane-1,3-diamine (5.1b) 41% yield;100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{18}H_{20}ClN_3O$ $[M + H]^+$ 330.13. Found: 330.46. ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.56 (d, J = 8.4, 1H), 8.54 (d, J = 6.4, 1H), 7.96 (d, J = 2.0, 1H), 7.70 (dd, J = 8.4, 2.0, 1H), 6.81 (d, J = 6.4, 1H), 6.48 (d, J = 2.8, 1H), 6.10 (d, J = 2.8, 1H), 4.15 (s, 2H), 3.56 (t, J = 6.4, 2H), 3.30 (H_2O), 3.01 (t, J = 7.2, 2H), 2.53 (DMSO), 2.25 (s, 3H), 2.08 (acetone).

N-(5-Chloro-furan-2-ylmethyl)-N'-(7-chloro-quinolin-4-yl)-propane-1,3-diamine (5.1c) 34% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{17}H_{17}Cl_2N_3O$ $[M + H]^+$ 350.07. Found: 350.41. ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.68 (d, J = 9.2, 1H), 8.58 (d, J = 6.4, 1H), 8.05 (d, J = 2.0, 1H), 7.75 (dd, J = 9.2, 2.0, 1H), 6.91 (d, J = 6.4, 1H), 6.73 (d, J = 3.2, 1H), 6.53 (d, J = 3.2, 1H), 4.21 (s, 2H), 3.63 (t, J = 6.4, 2H), 3.32 (H_2O), 3.03 (t, J = 7.2, 2H), 2.53 (DMSO), 2.25 (s, 3H), 2.08 (acetone), 1.28 (m, 2H).

N-(5-Bromo-furan-2-ylmethyl)-N'-(7-chloro-quinolin-4-yl)-propane-1,3-diamine (5.1d) 40% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{17}H_{18}ClN_3O$ [M + H]$^+$ 394.02. Found: 394.39. ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.61 (d, J = 9.2, 1H), 8.56 (d, J = 6.4, 1H), 8.00 (d, J = 2.0, 1H), 7.72 (dd, J = 9.2, 2.0, 1H), 6.86 (d, J = 6.4, 1H), 6.68 (d, J = 3.2, 1H), 6.62 (d, J = 3.2, 1H), 4.22 (s, 2H), 3.60 (t, J = 6.4, 2H), 3.32 (H_2O), 3.03 (t, J = 7.2, 2H), 2.53 (DMSO), 2.25 (s, 3H), 2.08 (acetone), 1.27 (m, 2H).

N-(7-Chloro-quinolin-4-yl)-N'-(5-methyl-thiophen-2-ylmethyl)-propane-1,3-diamine (5.1g) 37% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{18}H_{20}ClN_3S$ [M + H]$^+$ 346.11. Found: 346.45. ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.57 (m, 2H), 7.98 (d, J = 2.0, 1H), 7.74 (dd, J = 9.2, 2.0, 1H), 7.07 (d, J = 3.2, 1H), 6.85 (d, J = 6.8, 1H), 6.74 (d, J = 3.2, 1H), 4.28 (s, 2H), 3.60 (t, J = 6.4, 2H), 3.30 (H_2O), 3.16 (s, 3H), 3.03 (t, J = 7.2, 2H), 2.53 (DMSO), 2.25 (s, 3H), 2.08 (acetone).

N-(7-Chloro-quinolin-4-yl)-N'-(5-chloro-thiophen-2-ylmethyl)-propane-1,3-diamine (5.1i) 48% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{17}H_{17}Cl_2N_3S$ [M + H]$^+$ 366.05. Found: 366.41. ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.52 (m, 2H), 7.93 (d, J = 2.0, 1H), 7.67 (dd, J = 9.2, 2.0, 1H), 7.15 (d, J = 3.6, 1H), 7.07 (d, J = 3.6, 1H), 6.78 (d, J = 6.8, 1H), 4.27 (s, 2H), 3.55 (t, J = 6.4, 2H), 3.31 (H_2O), 2.98 (t, J = 7.2, 2H), 2.53 (DMSO), 2.08 (acetone).

N-(4-Bromo-thiophen-2-ylmethyl)-N'-(7-chloro-quinolin-4-yl)-propane-1,3-diamine (5.1j) 39% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for C17H17BrClN$_3$S [M + H]$^+$ 410.00. Found: 410.33. ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.52 (m, 2H), 7.93 (d, J = 2.0, 1H), 7.71 (d, J = 1.2, 1H), 7.66 (dd, J = 9.2, 2.0, 1H), 7.31 (d, J = 1.2, 1H), 6.76 (d,

J = 6.8, 1H), 4.31 (s, 2H), 3.55 (t, J = 6.4, 2H), 3.31 (H_2O), 2.99 (t, J = 7.2, 2H), 2.53 (DMSO), 2.08 (acetone).

5-{[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-methyl}-2-fluoro-phenol (5.1q) 32% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{19}H_{19}ClFN_3O$ $[M + H]^+$ 360.12. Found: 360.49. ^1H NMR (400 MHz, d_6-DMSO): δ 8.66 (d, J = 8.8, 1H), 8.58 (d, J = 6.8, 1H), 8.04 (d, J = 2.0, 1H), 7.78 (dd, J = 8.8, 2.0, 1H), 7.25 (d, J = 8.0, 1H), 7.21 (t, J = 8.0, 1H), 6.91 (d, J = 6.8, 1H), 6.85 (m, 1H), 4.14 (s, 2H), 3.64 (t, J = 6.4, 2H), 3.31 (H_2O), 3.06 (t, J = 7.2, 2H), 2.53 (DMSO), 2.08 (acetone) 1.26 (m, 2H).

2-{[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-methyl}-6-methoxy-phenol (5.1r) 68% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{20}H_{22}ClN_3O_2$ $[M + H]^+$ 372.14. Found: 372.49. ^1H NMR (400 MHz, d_4-MeOD): δ 8.38, (d, J = 6.4, 1H), 8.17 (d, J = 9.2, 1H), 7.81 (d, J =2.0, 1H), 7.50 (dd, J = 9.2, 2.0, 1H), 6.96 (dd, J = 7.6, 1.2, 1H), 6.86 (dd, J = 7.6, 1.2, 1H), 6.79 (t, J = 7.6, 1H), 6.67 (d, J = 6.4, 1H), 4.19 (s, 2H), 3.83 (s, 3H), 3.56 (t, J = 6.4, 2H), 3.14 (t, J = 7.2, 2H), 2.13 (m, 2H).

2-{[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-methyl}-4-methoxy-phenol (5.1s) 46% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{20}H_{22}ClN_3O_2$ $[M + H]^+$ 372.14. Found: 372.48. ^1H NMR (400 MHz, d_4-MeOD): δ 8.43, (m, 2H), 7.91 (d, J =2.0, 1H), 7.71 (dd, J = 9.2, 2.0, 1H), 6.92 (m, 2H), 6.81 (m, 2H), 4.20 (s, 2H), 3.71 (s, 3H), 2.21 (m, 2H).

2,4-Dichloro-6-{[3-(7-chloro-quinolin-4-ylamino)-propylamino]-methyl}-phenol (5.1w) 53% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{19}H_{18}Cl_3N_3O$ $[M + H]^+$ 410.05. Found: 410.39. ^1H NMR (400 MHz, d_6-DMSO): δ 8.51, (m, 2H), 7.93 (d, J =2.0, 1H), 7.65 (dd, J = 9.2, 2.0, 1H), 7.50 (d, J = 3.2, 1H), 7.42 (d, J = 3.2, 1H), 6.75 (d,

J = 6.8, 1H), 4.10 (s, 2H), 3.53 (t, J = 6.4, 2H), 2.98 (t, J = 7.2, 2H), 2.53 (DMSO), 2.08 (acetone), 2.00 (m, 2H).

N-(7-Chloro-quinolin-4-yl)-N'-(5-fluoro-2-methoxy-benzyl)-propane-1,3-diamine (5.1x) 31% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{20}H_{21}ClFN_3O$ [M + H]$^+$ 374.14. Found: 374.48. ^{1}H NMR (400 MHz, d_4-MeOD): δ 8.55 (d, J = 9.2, 1H), 8.44 (d, J = 7.2, 1H), 7.91 (d, J = 2.0, 1H), 7.67 (dd, J = 9.2, 2.0, 1H), 7.27 (dd, J = 2.4, 8.0, 1H), 7.07 (m, 2H), 6.98 (d, J = 7.2, 1H), 4.26 (s, 2H), 3.90 (s, 3H), 3.77 (t, J = 6.8, 2H), 3.25 (t, J = 7.6, 2H), 2.28 (m, 2H). ^{13}C NMR (400 MHz, d_4-MeOD): δ 156.3, 154.4, 142.7, 139.7, 138.7, 127.5, 125.2, 120.4, 119.0, 118.3, 117.3, 115.7, 112.1, 98.8, 56.8, 47.9, 47.3, 41.7, 25.7.

N-(7-Chloro-quinolin-4-yl)-N'-(2-fluoro-6-methoxy-benzyl)-propane-1,3-diamine (5.1y) 38% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{20}H_{21}ClFN_3O$ [M + H]$^+$ 374.14. Found: 374.48. ^{1}H NMR (400 MHz, d_4-MeOD): δ 8.37 (d, J = 6.4, 1H), 8.28 (d, J = 9.2, 1H), 7.79 (d, J = 2.0, 1H), 7.49 (dd, J = 9.2, 2.0, 1H), 7.41 (m, 1H), 6.90 (d, J = 8.4, 1H), 6.76 (m, 2H), 4.25 (s, 2H), 3.91 (s, 3H), 3.62 (t, J = 6.8, 2H), 3.24 (m, 2H), 2.23 (m, 2H). ^{13}C NMR (400 MHz, d_4-MeOD): δ 160.6, 155.0, 148.1, 144.6, 138.6, 133.2, 133.1, 127.4, 125.5, 123.8, 117.8, 109.0, 108.7, 108.1, 99.8, 55.9, 47.9, 41.5, 40.5.

N-Benzo[1,3]dioxol-4-ylmethyl-N'-(7-chloro-quinolin-4-yl)-propane-1,3-diamine (5.1aa) 82% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{20}H_{20}ClN_3O_2$ [M + H]$^+$ 370.12. Found: 370.47. ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.31, (d, J = 6.4, 1H), 7.97 (d, J = 9.2, 1H), 7.74 (d, J =2.0, 1H), 7.32 (dd, J = 9.2, 2.0, 1H), 6.72-6.82 (m, 3H), 6.50 (d, J = 6.4, 1H), 5.86 (s, 2H), 3.83 (s, 2H), 3.42 (t, J = 6.4, 2H), 3.28 (t, J = 7.2, 2H), 2.84 (t, J = 6.8, 2H), 1.97 (m, 2H). ^{13}C NMR (400 MHz, d_4-MeOD): δ.

N-(7-Chloro-quinolin-4-yl)-N'-(2-trifluoromethyl-benzyl)-propane-1,3-diamine (5.1ab) 39% yield; 100% pure by HPLC. LCMS (ESI) *m/z* Calcd for $C_{20}H_{19}ClF_3N_3$ [M + H]$^+$ 410.12. Found: 410.47. ^{1}H NMR (400 MHz, d_4-MeOD): δ 8.56 (d, J = 8.8, 1H), 8.46 (d, J = 6.8, 1H), 7.91 (d, J = 2.0, 1H), 7.83 (dd, J = 7.6, 0.8, 1H), 7.67 (dd, J = 8.8, 2.0, 1H), 7.58 (t, J = 7.6, 1H), 7.46 (m, 2H), 7.00 (d, J = 7.2, 1H), 4.39 (s, 2H), 3.78 (t, J = 6.8, 2H), 3.33 (m, 2H), 2.30 (m, 2H). ^{13}C NMR (400 MHz, d_4-MeOD): δ 162.3, 157.6, 144.1, 141.0, 139.9, 136.1, 133.3, 132.8, 128.8, 128.7, 126.5, 124.9, 121.6, 120.2, 116.9, 100.0, 46.6, 46.3, 41.7, 25.8.

2-{[3-(7-Chloro-quinolin-4-ylamino)-propylamino]-methyl}-4,6-difluoro-phenol (5.1ac) 22% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{19}H_{18}ClF_2N_3O$ [M + H]$^+$ 378.11. Found: 378.47. ^{1}H NMR (400 MHz, d_6-DMSO): δ

4-tert-Butyl-2-{[3-(7-chloro-quinolin-4-ylamino)-propylamino]-methyl}-phenol (5.1af) 23% yield; 100% pure by HPLC; LCMS (ESI) *m/z* Calcd for $C_{23}H_{28}ClN_3O$ [M + H]$^+$ 398.19. Found: 398.53. ^{1}H NMR (400 MHz, d_6-DMSO): δ

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Chapter 6

Conclusions

The work presented here outlines an exploration of 4-aminoquinolines as agents for the treatment of drug-resistant *P. falciparum*. The structure-activity relationships for both the quinoline ring and basic side chains were defined based on *in vitro* activity against drug-sensitive and drug-resistant parasite strains. Furthermore, both of these classes of compounds were evaluated for pharmacokinetic and early toxicity properties.

All of the compounds synthesized with diversity on the quinoline B-ring (**2.1**) showed diminished activity against the drug-sensitive *P. falciparum* strain 3D7 relative to CQ. (Fig 6.1) The 7-chloroquinoline ring found in chloroquine continues to give compounds with the greatest potency. When tested against the drug-resistant W2 strain of *P. falciparum*, the most potent of the ring-modified compounds were all in the 50 - 100 nM IC_{50} range. The potency of this series is only moderate, but all of the compounds submitted for preclinical testing showed good metabolic stability and no P450 inhibition. Both **2.1{1,15}** and **2.1{1,19}** showed acceptable mouse PK parameters ($t_{½}$, C_{max}, and AUC) similar to chloroquine. Currently, the potential of this series is limited only by the potency and possibly complimentary substitutions on the quinoline A-ring could be an area worth exploring.

Modification to the basic side chain of chloroquine proved to be more fruitful in generating potent compounds (**3.1**) against drug-resistant *P. falciparum*. It was known that shortening or lengthening the alkyl side chain of CQ-analogs gave improved potency against drug-resistant parasite strains, but now it has also been shown that substitutions to the

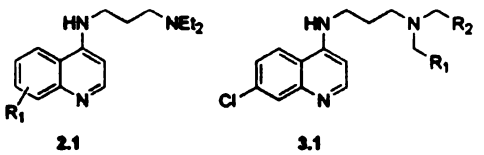
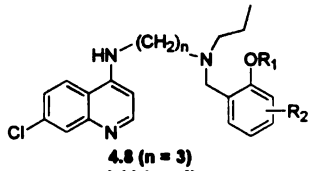


Figure 6.1 Initial series of compounds for expanding the SAR for ring substations (2.1) and basic side chain substitutions (3.1).

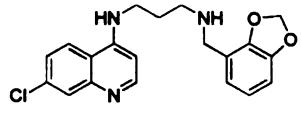


4.8 (n = 3)
4.11 (n = 4)

Figure 6.2 Generalized structure of the most potent compounds.

terminal nitrogen group on the basic side-chain can achieve similar increases in potencies. The most potent compounds discovered were those containing a previously unappreciated intramolecular hydrogen bonding motif in the basic side chain. (Fig 6.2) The presence of a benzylic substitution on the basic nitrogen along with a hydrogen bond accepting functional group generated 12 compounds with IC_{50} values less than 10 nM against the multi-drug resistant W2 strain of *P. falciparum*. Preclinical testing of several of the compounds with side chain modifications showed poor metabolic stability and some inhibition of the P450 isoform, CYP2D6. In the human microsomal stability assay many of the most potent compounds within this series were subjected to rapid N-dealkylation and had half lives of less than 10 minutes.

The elucidation of the metabolism products led to the synthesis of secondary amine analogs of several of our most potent compounds. Several of these compounds have similar potencies and greatly improved metabolic stabilities. The secondary amine analogs also no longer showed inhibition of CYP2D6 or any of the other major P450 isoforms. Currently, our top lead compound, **5.1aa** (Fig 6.3), has a combination of good potency and PK parameters. Several other compounds from library **5.1** that have similar potencies are now undergoing further evaluation for metabolic stability and mouse pharmacokinetics.



5.1aa

W2 IC_{50} = 5.7 nM
3D7 IC_{50} = 2.5 nM
$t_{1/2}$ (1 μM) = 94 min.
no P450 inhibition

Figure 6.3 Structure, activity and PK properties of lead candidate 5.1aa.

The next step towards evaluating our lead compound series will involve *in vivo* efficacy

testing against animal models of malaria. Through collaboration, we now plan to test our compounds against a mouse model of malaria. The mouse model involves a different species of *Plasmodia*, but will nevertheless verify that oral administration of the drug will allow it to reach the blood stream and the parasite. Further development will be based on progress in this area.

Appendix

Yields, Purity and Spectral Data

Table A.1 Screening data for all compounds against two strains of *P. falciparum*, 3D7 (drug-sensitive) and W2 (drug-resistant), at two concentrations 30 nM and 200 nM.

Compound	30 nM 3D7	200 nM W2	30 nM W2	200 nM W2
2.1{1,1}	1.00	0.28	1.00	0.99
2.1{1,3}	0.58	0.54	0.86	0.83
2.1{1,4}	0.51	0.23	1.00	0.94
2.1{1,5}	0.97	0.90	0.92	0.87
2.1{1,6}	0.94	0.36	1.00	0.88
2.1{1,7}	1.00	0.65	1.00	0.83
2.1{1,8}	0.18	0.15	0.73	0.30
2.1{1,9}	1.00	0.95	1.00	1.00
2.1{1,10}	1.00	0.99	1.00	1.00
2.1{1,11}	1.00	0.99	1.00	1.00
2.1{1,12}	0.21	0.37	0.87	0.95
2.1{1,12}	1.00	0.17	1.00	0.12
2.1{1,13}	1.00	0.95	1.00	1.00
2.1{1,14}	1.00	0.16	0.98	0.10
2.1{1,15}	0.20	0.14	0.13	0.09
2.1{1,16}	1.00	0.15	1.00	0.11
2.1{1,17}	1.00	0.91	1.00	1.00
2.1{1,18}	0.20	0.53	0.22	0.69
2.1{1,19}	0.17	0.14	0.08	0.09
2.1{1,20}	1.00	0.14	1.00	1.00
2.1{1,20}	0.20	0.15	0.08	0.07
2.1{1,23}	0.73	0.14	1.00	0.09
2.1{1,24}	0.17	0.14	0.08	0.10
2.1{1,25}	1.00	0.98	0.99	0.93
2.1{1,26}	1.00	1.00	1.00	1.00
2.1{1,27}	1.00	0.94	1.00	0.98
2.1{1,28}	1.00	0.93	0.91	0.93
2.1{1,29}	1.00	1.00	1.00	1.00
2.1{1,30}	1.00	0.96	1.00	0.88
2.1{1,31}	1.00	1.00	1.00	1.00
2.1{1,32}	1.00	0.99	0.96	0.98
2.1{2,2}	1.00	1.00	1.00	1.00
2.1{2,4}	1.00	1.00	1.00	1.00
2.1{2,6}	1.00	0.95	1.00	0.98
2.1{2,9}	1.00	1.00	1.00	1.00
2.1{2,10}	1.00	1.00	1.00	1.00
2.1{2,11}	1.00	0.52	1.00	1.00
2.1{2,12}	0.96	0.14	1.00	1.00
2.1{2,13}	1.00	0.21	1.00	1.00
2.1{2,15}	0.18	0.70	0.35	0.98
2.1{2,16}	0.99	0.15	1.00	0.97
2.1{2,18}	0.77	0.14	0.97	0.09
2.1{2,19}	0.96	1.00	1.00	1.00
2.1{2,22}	0.39	0.12	0.95	0.09
2.1{2,24}	0.90	0.16	1.00	0.11
2.1{2,25}	1.00	0.95	1.00	1.00
2.1{2,26}	0.99	0.95	1.00	1.00
2.1{2,29}	0.96	1.00	1.00	1.00
2.1{2,32}	1.00	1.00	0.96	1.00
3.1{1,2}	0.63	0.05		
3.1{3,2}	0.16	0.09		

3.1{4,2}	0.90	0.05		
3.1{5,2}	0.08	0.06		
3.1{6,2}	0.93	0.05		
3.1{7,2}	0.70	0.19		
3.1{8,2}	0.82	0.32		
3.1{9,2}	0.75	0.19		
3.1{10,2}	0.46	0.06		
3.1{11,2}	0.91	0.24		
3.1{12,2}	0.95	0.34		
3.1{13,2}	0.43	0.04		
3.1{14,2}	0.54	0.05		
3.1{15,2}	0.69	0.27		
3.1{16,2}	0.53	0.12		
3.1{17,2}	0.87	0.27		
3.1{18,2}	0.68	0.02		
3.1{19,2}	0.88	0.29		
3.1{20,2}	0.93	0.08		
3.1{21,2}	0.05	0.05		
3.1{22,2}	0.77	0.05		
3.1{23,2}	0.64	0.27		
3.1{24,2}	0.10	0.07		
3.1{1,13}	0.95	0.52		
3.1{2,13}	0.16	0.06		
3.1{3,13}	0.79	0.07		
3.1{4,13}	0.60	0.24		
3.1{5,13}	0.35	0.06		
3.1{6,13}	0.87	0.06		
3.1{7,13}	0.06	0.05		
3.1{8,13}	0.69	0.26		
3.1{9,13}	0.63	0.05		
3.1{10,13}	0.20	0.06		
3.1{11,13}	0.68	0.17		
3.1{12,13}	0.82	0.24		
3.1{13,13}	0.77	0.05		
3.1{14,13}	0.94	0.06		
3.1{15,13}	1.00	0.16		
3.1{16,13}	0.81	0.23		
3.1{17,13}	1.00	0.94		
3.1{18,13}	0.59	0.05		
3.1{19,13}	0.93	0.42		
3.1{20,13}	0.98	0.86		
3.1{21,13}	0.93	0.20		
3.1{22,13}	0.40	0.04		
3.1{23,13}	0.74	0.04		
3.1{24,13}	0.99	0.29		
4.8a	1.00	0.96	0.99	1.00
4.8b	0.08	0.06	0.10	0.09
4.8c	0.08	0.04	0.07	0.08
4.8d	0.09	0.05	0.07	0.08
4.8e	0.14	0.15	0.14	0.10
4.8f	1.00	0.93	1.00	1.00
4.8g	0.06	0.05	0.09	0.06
4.8h	0.97	1.00	0.86	0.90
4.8i	0.05	0.05	0.09	0.08
4.8j	0.06	0.07	0.12	0.07
4.8k	1.00	0.14	0.20	0.15

4.8l	0.15	0.12	0.25	0.10
4.8m	0.12	0.16	0.20	0.15
4.8n	0.15	0.15	0.16	0.08
4.8o	1.00	0.95	1.00	1.00
4.8p	0.97	0.97	1.00	0.99
4.8q	0.05	0.05	0.09	0.10
4.8r	0.07	0.06	0.14	0.11
4.8s	0.07	0.06	0.11	0.11
4.8t	0.05	0.08	0.12	0.09
4.8u	1.00	0.96	0.99	1.00
4.8v	0.08	0.07	0.11	0.08
4.8w	1.00	0.99	0.98	0.95
4.8x	0.16	0.17	0.13	0.10
4.8y	0.06	0.06	0.08	0.07
4.8z	0.18	0.10	0.10	0.08
4.8aa	0.12	0.13	0.14	0.09
4.8ab	0.09	0.05	0.12	0.06
4.8ac	0.08	0.07	0.10	0.09
4.8ad	1.00	0.28	0.89	0.95
4.8ae	0.10	0.08	0.90	0.20
4.8af	0.06	0.08	0.13	0.13
4.11a	0.12	0.10	0.07	0.05
4.11b	0.09	0.10	0.05	0.05
4.11c	0.10	0.12	0.06	0.05
4.11d	0.12	0.10	0.07	0.07
4.11e	0.10	0.11	0.07	0.05
4.11f	0.09	0.10	0.02	0.03
4.11g	0.09	0.09	0.07	0.06
4.11h	0.17	0.70	0.15	0.95
4.11i	0.09	0.10	0.09	0.07
4.11j	0.12	0.10	0.16	0.11
4.11k	0.09	0.10	0.06	0.05
4.11l	0.10	0.10	0.06	0.05
4.11m	0.10	0.09	0.06	0.79
4.11n	0.10	0.14	0.06	0.10
4.11o	0.09	0.10	0.06	0.05
4.11p	0.13	0.14	0.07	0.08
4.11q	0.15	0.14	0.07	0.08
4.11r	0.18	0.14	0.15	0.07
4.11s	0.15	0.15	0.07	0.08
4.11t	0.29	0.46	0.16	0.15
4.11u	0.29	0.50	0.22	0.18
4.11v	0.12	0.10	0.09	0.06
4.11w	0.18	0.20	0.32	0.32
4.11x	0.10	0.11	0.09	0.05
4.11y	0.10	0.11	0.05	0.04
4.11z	0.14	0.17	0.20	0.15
4.11aa	0.20	0.14	0.16	0.06
4.11ab	0.22	0.21	0.26	0.16
4.13a	0.15	0.13	0.79	0.41
4.13b	1.00	0.46	1.00	1.00
4.13c	0.97	0.17	0.89	0.22
4.13d	0.92	0.24	0.97	0.73
4.13e	0.07	0.07	0.88	0.17
4.13f	0.93	0.11	0.90	0.10
4.13g	0.93	0.25	1.00	0.35

4.13i	0.15	0.09	0.94	0.68
4.13j	0.89	0.12	0.92	0.11
4.13k	0.05	0.06	0.15	0.13
4.13l	1.00	1.00	0.93	1.00
4.13m	0.15	0.13	0.94	0.62
4.13n	1.00	0.92	0.90	0.90
4.13o	0.96	0.78	0.92	0.90
4.13p	0.10	0.11	0.07	0.08
4.13q	0.92	0.12	0.93	0.25
4.13r	0.12	0.13	0.86	0.59
4.13s	0.88	0.19	0.91	0.52
4.13t	0.85	0.11	0.85	0.10
4.13u	0.88	0.29	0.84	0.32
4.13v	0.96	0.09	0.86	0.20
4.13w	0.83	0.11	0.93	0.15
4.13x	0.87	0.12	0.96	0.30
4.13y	0.95	0.12	0.91	0.35
4.13z	0.85	0.36	0.90	0.47
4.13aa	0.25	0.09	0.97	0.42
4.13ab	0.96	0.28	0.99	0.58
4.15a	0.90	1.00	0.87	0.88
4.15b	1.00	1.00	0.89	0.94
4.15c	0.79	0.12	0.99	0.11
4.15d	0.77	0.11	1.00	0.12
4.15e	1.00	0.32	0.99	1.00
4.15f	1.00	0.11	1.00	0.12
4.15g	0.61	0.11	1.00	0.09
4.15h	1.00	0.42	0.90	0.92
4.15i	1.00	0.91	0.83	0.93
4.15j	0.40	0.13	0.97	0.10
4.15k	0.44	0.11	0.89	0.17
4.15l	0.86	0.15	0.88	0.68
4.15m	0.41	0.13	0.91	0.10
4.15n	0.86	0.16	0.88	0.78
4.15o	0.96	0.99	0.92	0.94
4.15p	0.48	0.32	0.97	0.78
4.15q	0.91	0.09	1.00	0.10
4.15r	0.75	0.16	0.93	0.88
4.15s	0.44	0.12	1.00	0.17
4.15t	1.00	0.15	1.00	0.09
4.15u	0.33	0.12	0.83	0.09
4.15v	0.85	0.13	1.00	0.08
4.15w	1.00	0.67	0.98	0.98
4.15x	0.95	0.15	1.00	0.07
4.15y	0.11	0.11	0.88	0.10
4.15z	0.23	0.12	1.00	0.09
4.15aa	0.81	0.60	0.94	0.63
4.15ab	0.75	0.11	1.00	0.10
5.1a	0.05	0.05	0.03	0.04
5.1b	0.03	0.05	0.02	0.03
5.1c	0.02	0.02	0.03	0.01
5.1e	0.04	0.05	0.03	0.02
5.1g	0.06	0.07	0.06	0.06
5.1h	0.02	0.03	0.02	0.02
5.1i	0.10	0.07	0.04	0.03
5.1j	0.05	0.06	0.03	0.03

5.1k	0.04	0.04	0.03	0.03
5.1p	0.03	0.04	0.95	0.08
5.1q	0.07	0.05	0.07	0.04
5.1r	0.07	0.14	0.96	0.08
5.1s	0.31	0.12	0.91	0.66
5.1t	0.07	0.08	0.17	0.10
5.1u	0.05	0.04	0.73	0.04
5.1x	0.08	0.10	0.05	0.03
5.1y	0.04	0.04	0.02	0.01
5.1z	0.06	0.05	0.06	0.07
5.1aa	0.06	0.15	0.04	0.04
5.1ab	0.14	0.10	0.05	0.03
5.1ac	0.03	0.02	0.03	0.04
5.1ad	0.12	0.11	0.58	0.08
5.1ae	0.08	0.10	0.10	0.14
5.1af	0.08	0.11	0.09	0.08

Table A.2 Yields and purity for all compounds.

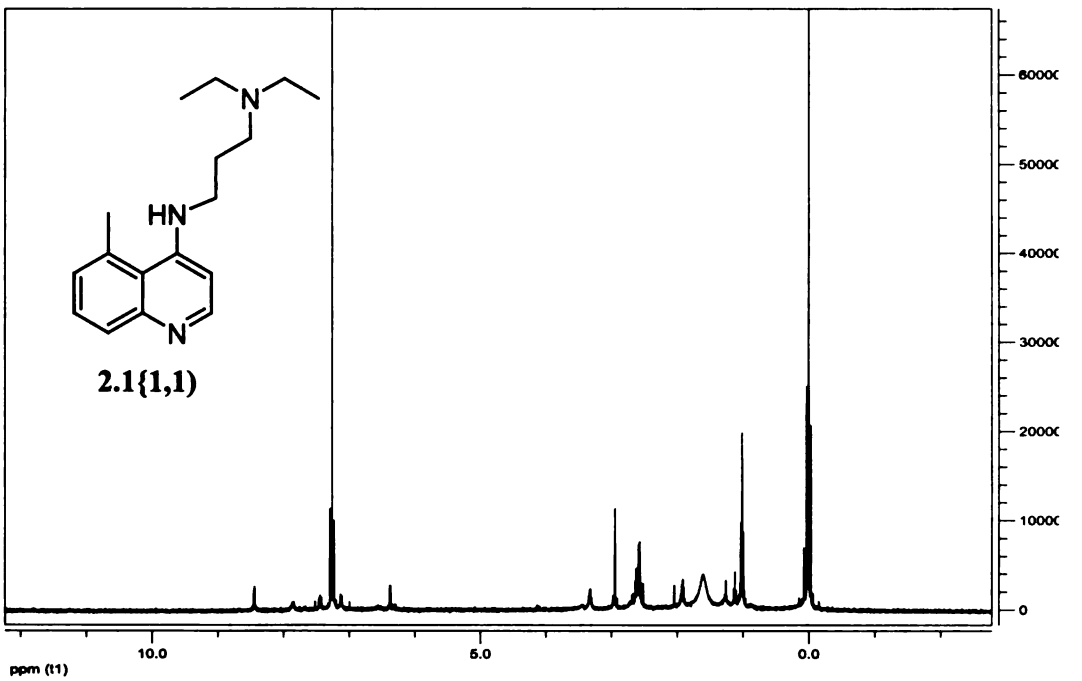
Compound	Yield	Purity
2.1{1,1}	83%	83%
2.1{1,3}	12%	95%
2.1{1,4}	21%	95%
2.1{1,5}	29%	95%
2.1{1,6}	19%	95%
2.1{1,7}	43%	95%
2.1{1,8}	35%	95%
2.1{1,9}	91%	87%
2.1{1,10}	27%	95%
2.1{1,11}	94%	95%
2.1{1,12}	61%	94%
2.1{1,13}	41%	95%
2.1{1,14}	39%	95%
2.1{1,15}	90%	95%
2.1{1,16}	42%	95%
2.1{1,17}	12%	95%
2.1{1,18}	73%	95%
2.1{1,19}	44%	88%
2.1{1,20}	83%	94%
2.1{1,22}	90%	95%
2.1{1,23}	35%	95%
2.1{1,24}	40%	95%
2.1{1,25}	47%	95%
2.1{1,26}	90%	95%
2.1{1,27}	9%	95%
2.1{1,28}	48%	95%
2.1{1,29}	95%	95%
2.1{1,30}	63%	95%
2.1{1,31}	31%	95%
2.1{1,32}	76%	95%
2.1{2,1}	11%	79%
2.1{2,2}	8%	95%
2.1{2,4}	14%	95%
2.1{2,6}	38%	95%
2.1{2,9}	10%	95%
2.1{2,10}	8%	95%
2.1{2,11}	12%	95%
2.1{2,12}	12%	95%
2.1{2,13}	3%	95%
2.1{2,15}	6%	95%
2.1{2,16}	13%	92%
2.1{2,18}	17%	95%
2.1{2,19}	29%	73%
2.1{2,22}	69%	95%
2.1{2,24}	19%	95%
2.1{2,25}	37%	93%
2.1{2,26}	78%	95%
2.1{2,29}	6%	95%
2.1{2,32}	12%	95%
3.1{1,2}	10%	100%
3.1{2,2}	24%	31%
3.1{3,2}	30%	95%

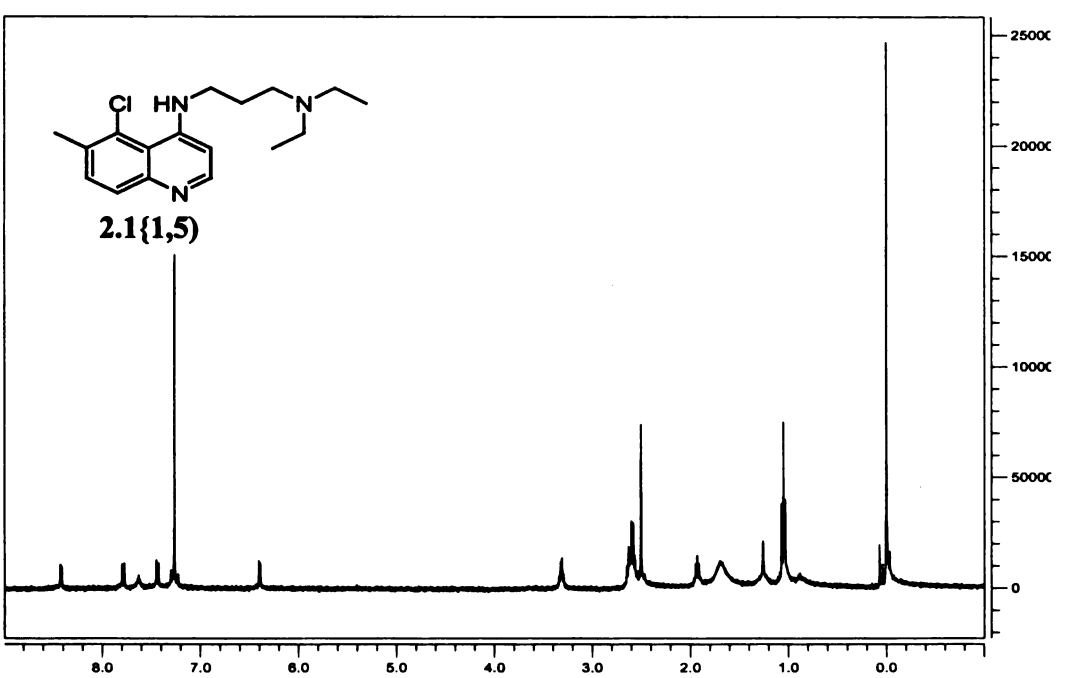
3.1{4,2}	25%	100%
3.1{5,2}	20%	88%
3.1{6,2}	5%	80%
3.1{7,2}	22%	100%
3.1{8,2}	41%	100%
3.1{9,2}	12%	81%
3.1{10,2}	27%	95%
3.1{11,2}	15%	88%
3.1{12,2}	26%	86%
3.1{13,2}	56%	90%
3.1{14,2}	24%	98%
3.1{15,2}	79%	100%
3.1{16,2}	53%	100%
3.1{17,2}	11%	84%
3.1{18,2}	12%	97%
3.1{19,2}	3%	72%
3.1{20,2}	67%	91%
3.1{21,2}	40%	99%
3.1{22,2}	29%	99%
3.1{23,2}	48%	100%
3.1{24,2}	83%	90%
3.1{1,13}	25%	88%
3.1{2,13}	6%	71%
3.1{3,13}	13%	100%
3.1{4,13}	34%	100%
3.1{5,13}	12%	100%
3.1{6,13}	8%	77%
3.1{7,13}	24%	98%
3.1{8,13}	32%	99%
3.1{9,13}	9%	95%
3.1{10,13}	21%	100%
3.1{11,13}	10%	87%
3.1{12,13}	19%	90%
3.1{13,13}	44%	100%
3.1{14,13}	15%	100%
3.1{15,13}	75%	88%
3.1{16,13}	39%	98%
3.1{17,13}	5%	85%
3.1{18,13}	13%	97%
3.1{19,13}	8%	86%
3.1{20,13}	16%	100%
3.1{21,13}	52%	100%
3.1{22,13}	40%	100%
3.1{23,13}	74%	84%
3.1{24,13}	40%	100%
4.8a	89%	100%
4.8b	59%	100%
4.8c	76%	100%
4.8f	90%	82%
4.8p	78%	100%
4.8q	30%	100%
4.8r	77%	100%
4.8s	67%	100%
4.8t	70%	100%
4.8v	84%	100%
4.8x	94%	100%

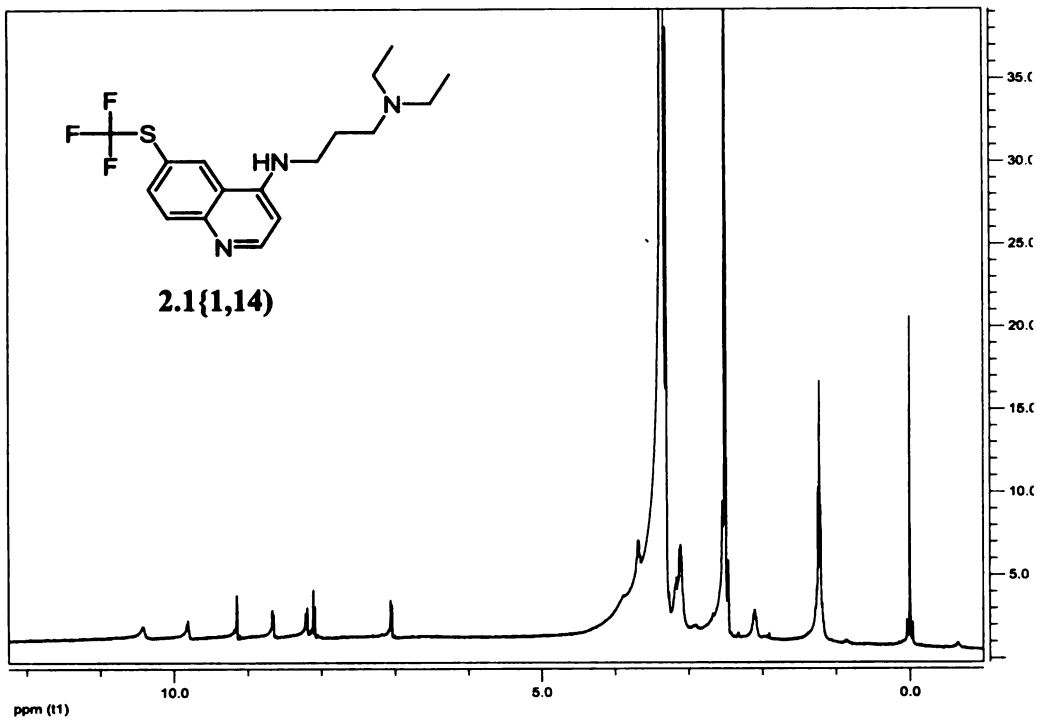
4.8y	32%	100%
4.8aa	31%	100%
4.8ac	65%	92%
4.8ad	57%	90%
4.8ae	54%	100%
4.8af	74%	85%
4.11a	64%	100%
4.11b	59%	100%
4.11c	13%	100%
4.11f	44%	100%
4.11p	83%	100%
4.11q	39%	100%
4.11r	50%	100%
4.11s	55%	100%
4.11t	33%	100%
4.11v	52%	87%
4.11x	39%	100%
4.11y	22%	100%
4.11aa	47%	100%
5.1a	16%	100%
5.1b	41%	100%
5.1c	34%	100%
5.1d	40%	100%
5.1g	37%	100%
5.1i	48%	100%
5.1j	39%	100%
5.1q	32%	100%
5.1r	68%	100%
5.1s	46%	100%
5.1w	53%	100%
5.1x	31%	100%
5.1y	38%	100%
5.1aa	82%	100%
5.1ab	39%	100%
5.1ac	22%	100%
5.1af	23%	100%

Table A.3 High-resolution mass spectrometry (EI) data for selected compounds.

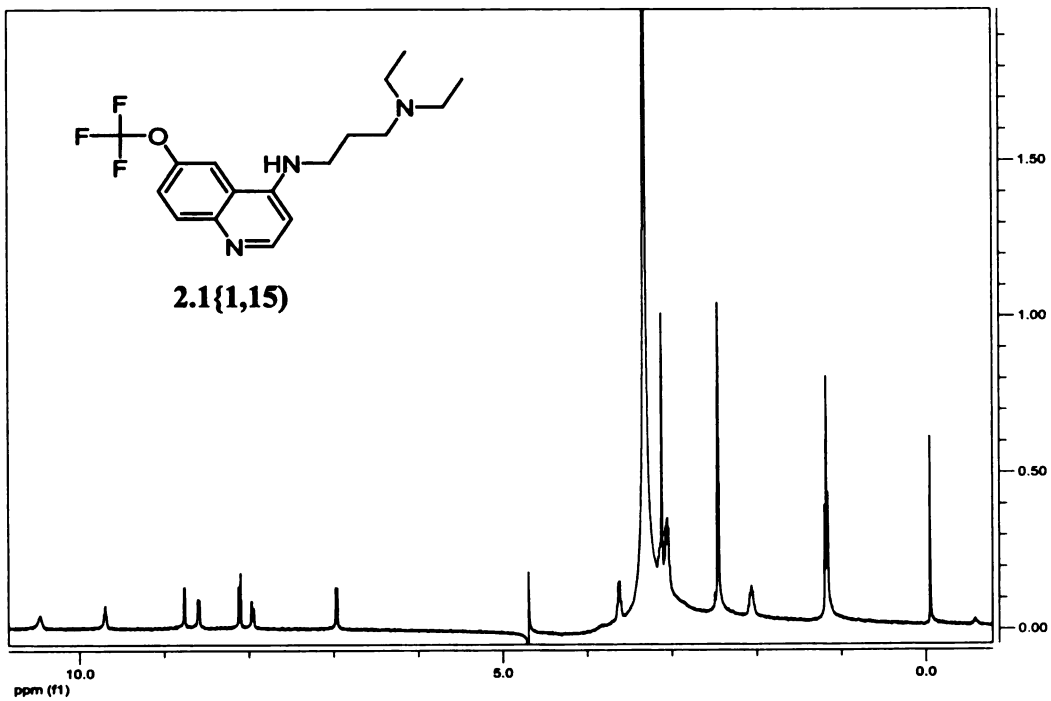
Compound	Formula	Calc. Mass	Mass
4.8c	$C_{20}H_{23}Cl_2N_3O$	391.1218	391.1214
4.8t	$C_{23}H_{25}ClF_3N_3O_2$	467.1587	467.1599
4.8x	$C_{23}H_{27}ClFN_3O$	415.1826	415.1839
4.8aa	$C_{23}H_{26}ClN_3O_2$	411.1713	411.1718
4.8s	$C_{23}H_{28}ClN_3O_2$	413.1870	413.1882
4.8r	$C_{23}H_{28}ClN_3O_2$	413.1870	413.1871
4.8y	$C_{23}H_{27}ClFN_3O$	415.1826	415.1829
4.8ac	$C_{22}H_{24}ClF_2N_3O$	419.1564	419.1562
4.11y	$C_{24}H_{29}ClFN_3O$	429.1983	429.1972
4.11aa	$C_{24}H_{28}ClN_3O_2$	425.1870	425.1857
4.11r	$C_{24}H_{30}ClN_3O_2$	427.2026	427.2036
5.1y	$C_{20}H_{21}ClFN_3O$	373.1357	373.1357
5.1x	$C_{20}H_{21}ClFN_3O$	373.1357	373.1359
5.1a	$C_{17}H_{18}ClN_3O$	315.1138	315.1138
5.1aa	$C_{20}H_{20}ClN_3O_2$	369.1244	369.1247
5.1ab	$C_{20}H_{19}ClF_3N_3O$	409.1168	409.1176


N
HN
N
2.1{1,1)
60000
50000
40000
30000
20000
10000
0
10.0
5.0
0.0
ppm (t1)

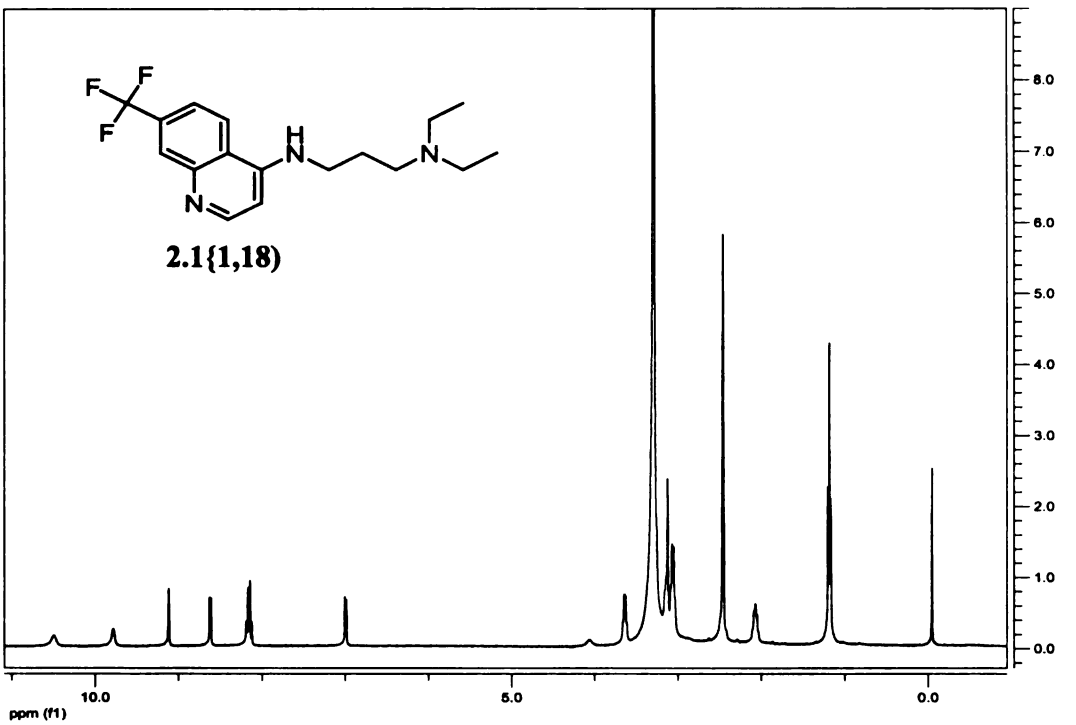

Cl
HN
N
N
2.1{1,5)
25000
20000
15000
10000
5000
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


F
F
S
F
HN
N
N
35.0
30.0
25.0
20.0
15.0
10.0
5.0
10.0
5.0
0.0
ppm (t1)

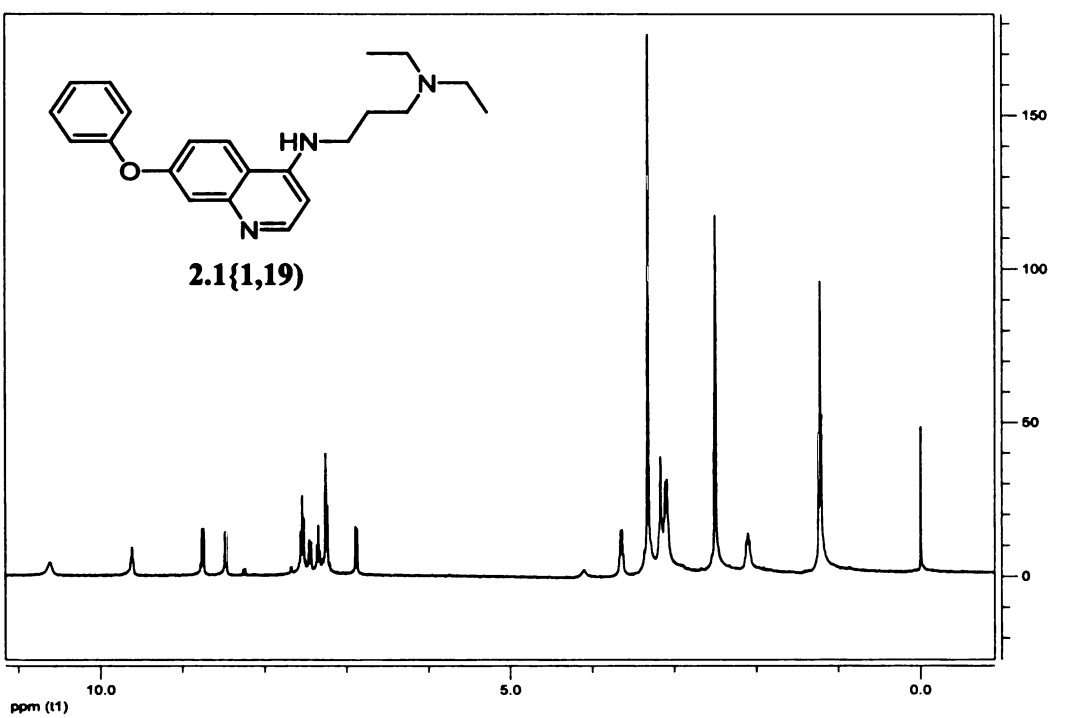
2.1{1,14)


F
F
O
F
HN
N
N
1.50
1.00
0.50
0.00
10.0
5.0
0.0
ppm (f1)

2.1{1,15)


F
F
F
H
N
N
N
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
10.0
5.0
0.0
ppm (f1)

2.1{1,18)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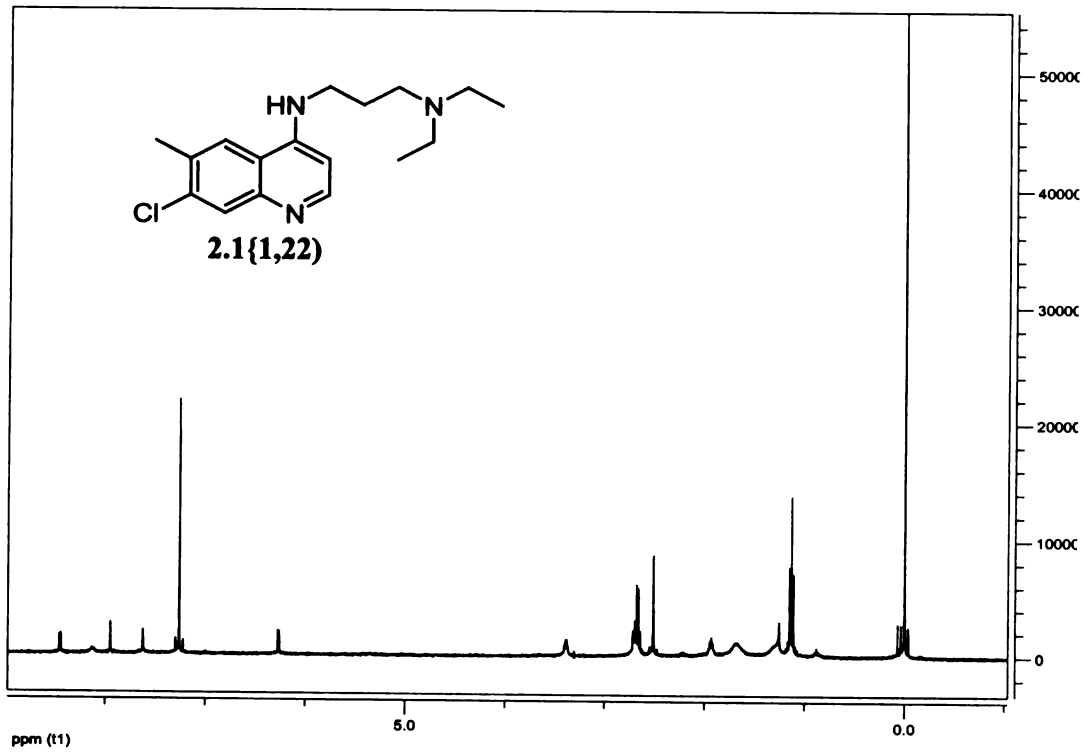
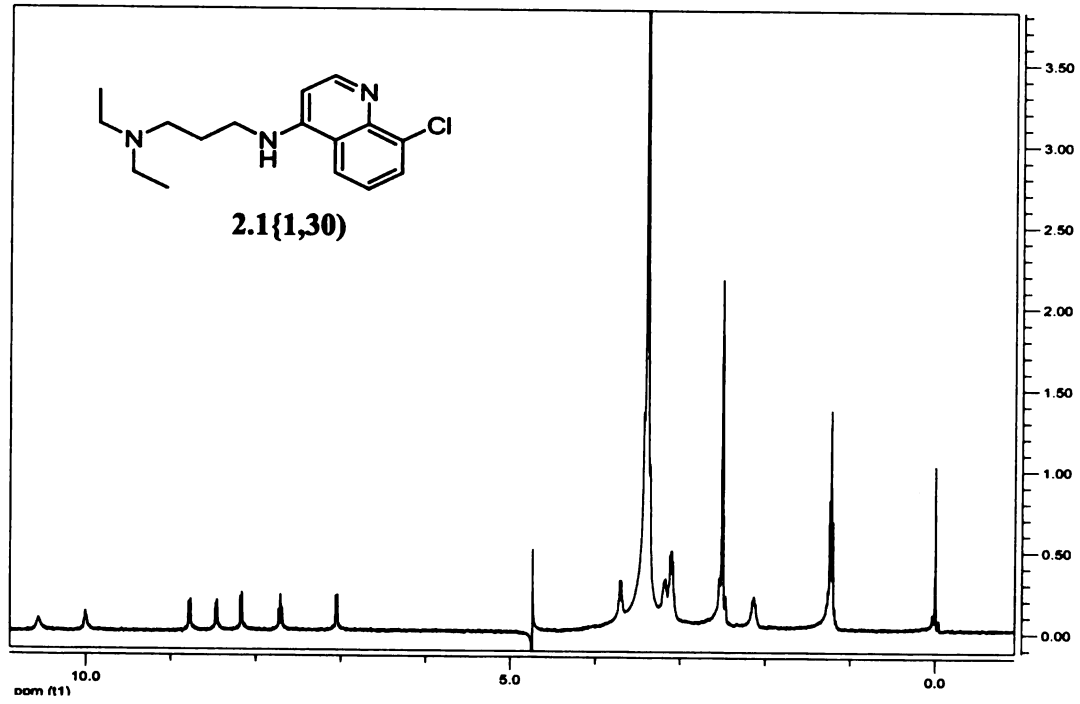
HN
O
N
150
100
50
0
10.0
5.0
0.0
ppm (t1)

2.1{1,19)


HN
N
Cl
N
5000
4000
3000
2000
1000
0
5.0
0.0
ppm (t1)

2.1{1,22)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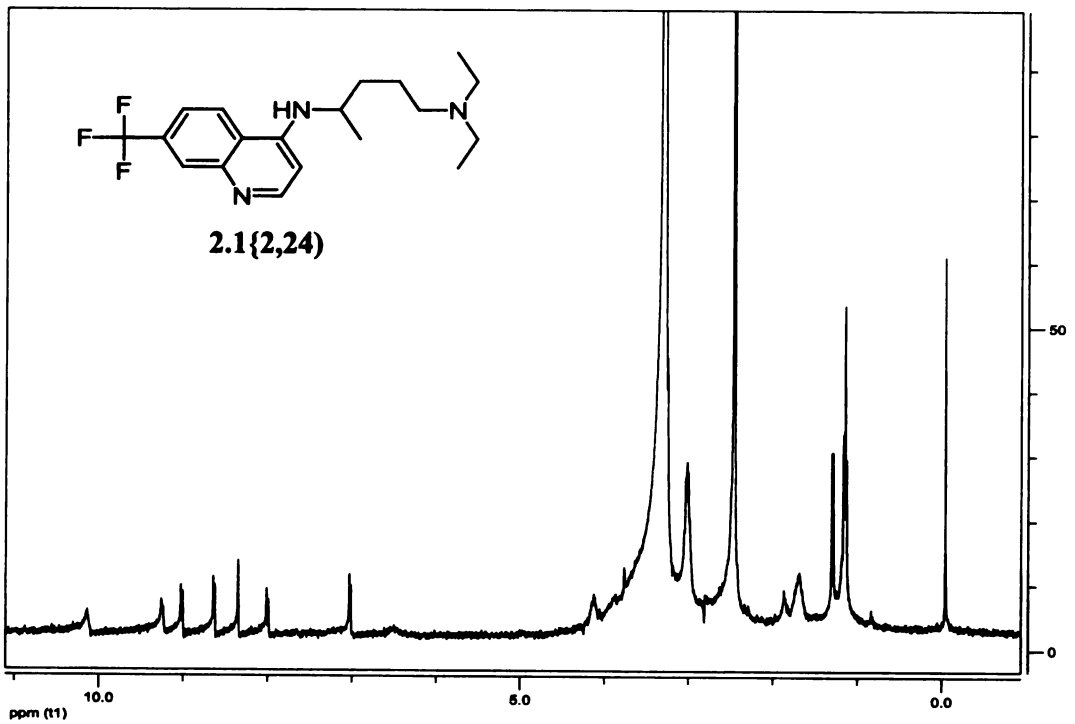
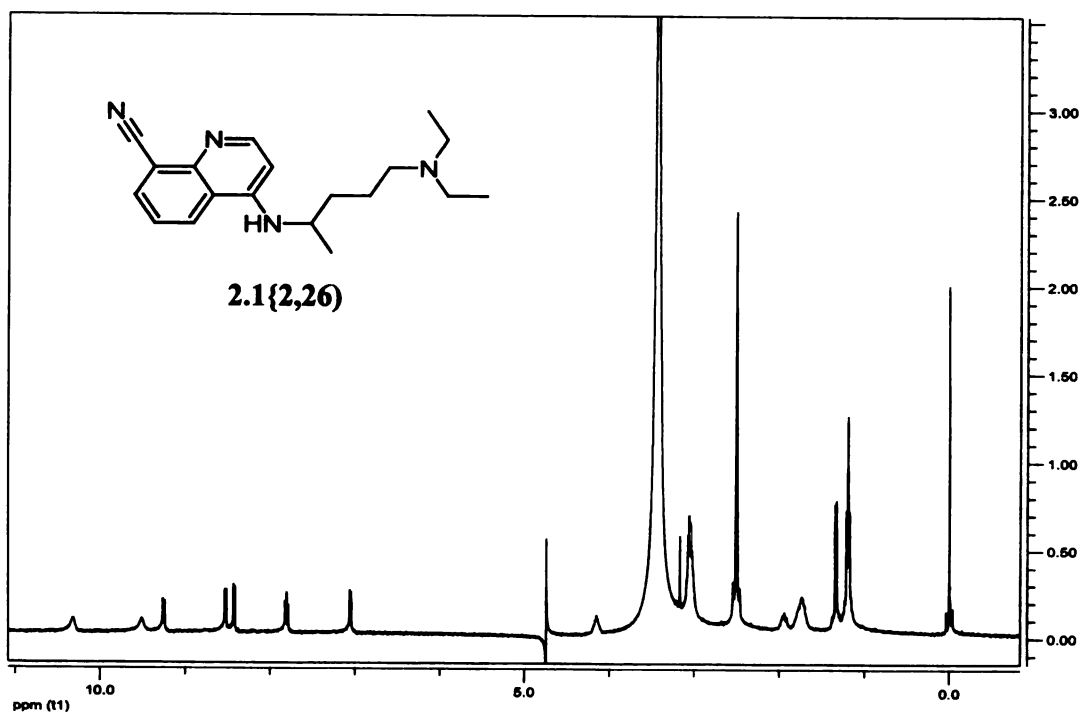
N
H
Cl
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
10.0
5.0
0.0
ppm (t1)

2.1{1,30)


F
F
F
HN
N
N
50
0
10.0
5.0
0.0
ppm (t1)

2.1{2,24)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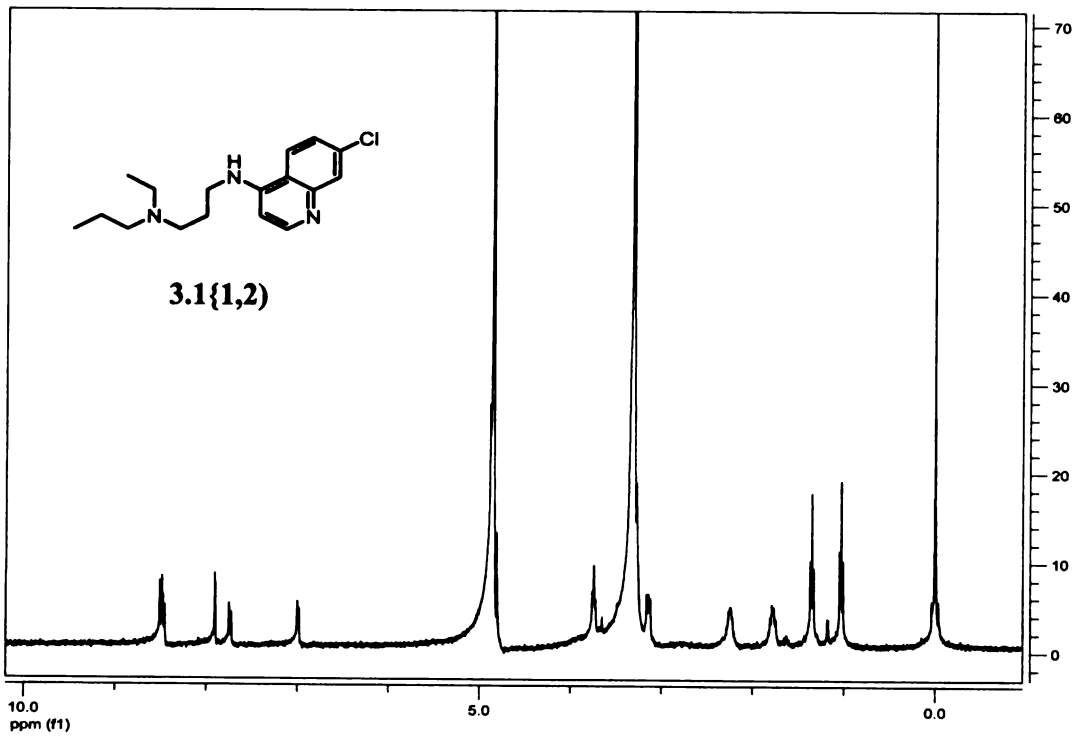
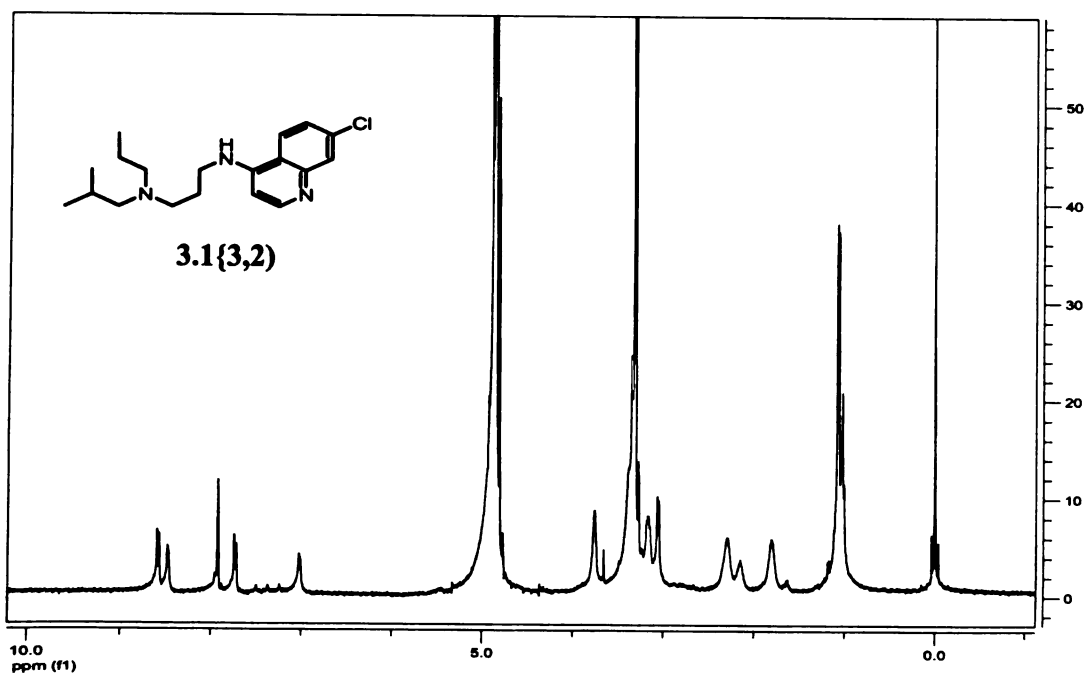
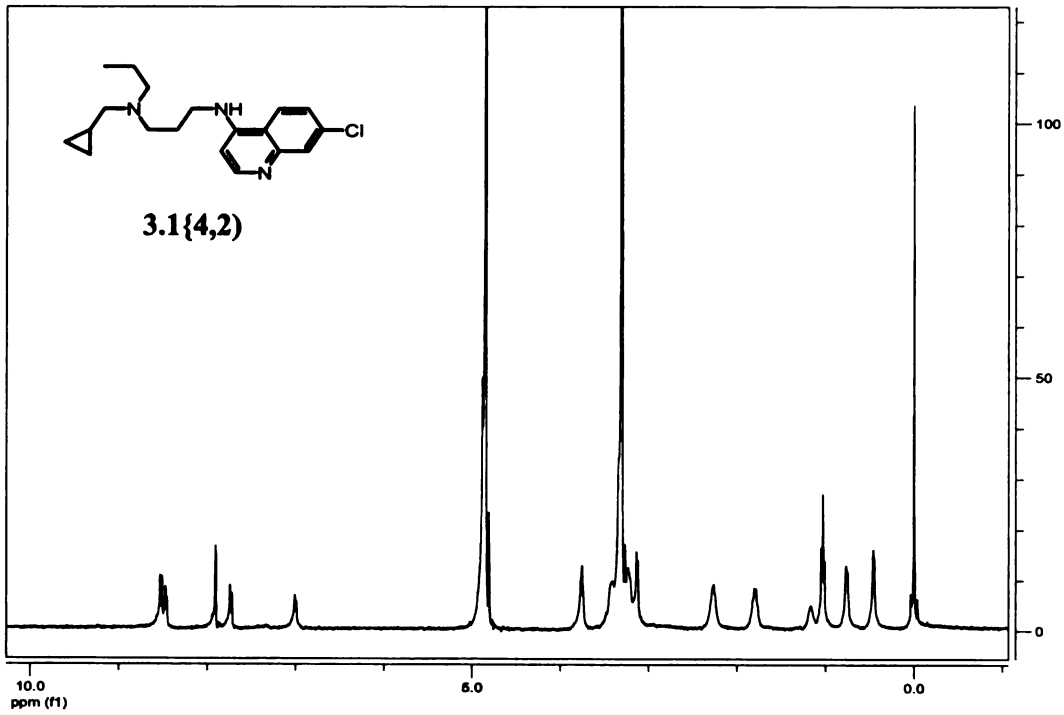
N
HN
N
3.00
2.50
2.00
1.50
1.00
0.50
0.00
10.0
5.0
0.0
ppm (t1)

2.1{2,26)


Cl
H
N
N
N
3.1{1,2)
70
60
50
40
30
20
10
0
10.0
ppm (f1)
5.0
0.0


Cl
H
N
N
N
3.1{3,2)
50
40
30
20
10
0
10.0
ppm (f1)
5.0
0.0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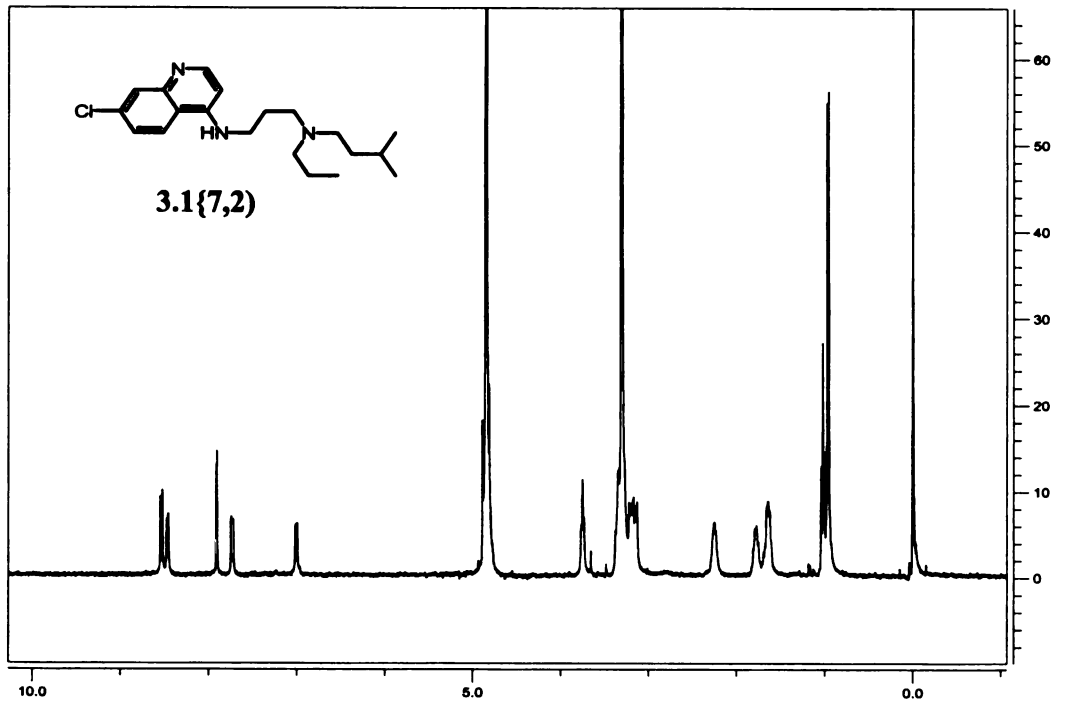
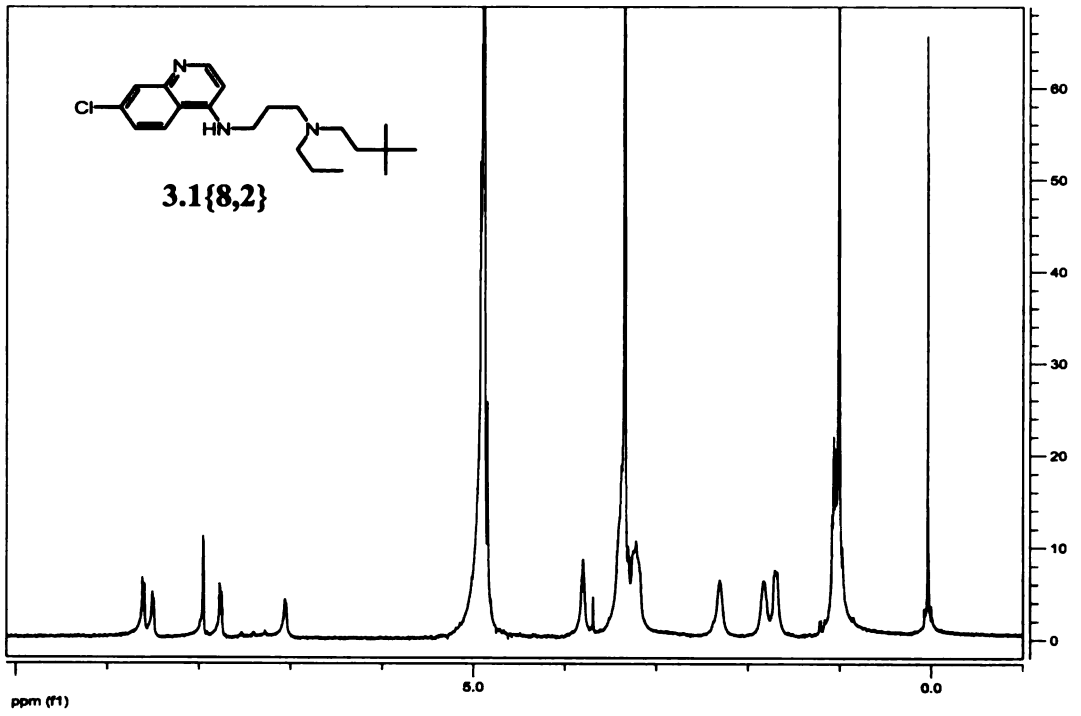
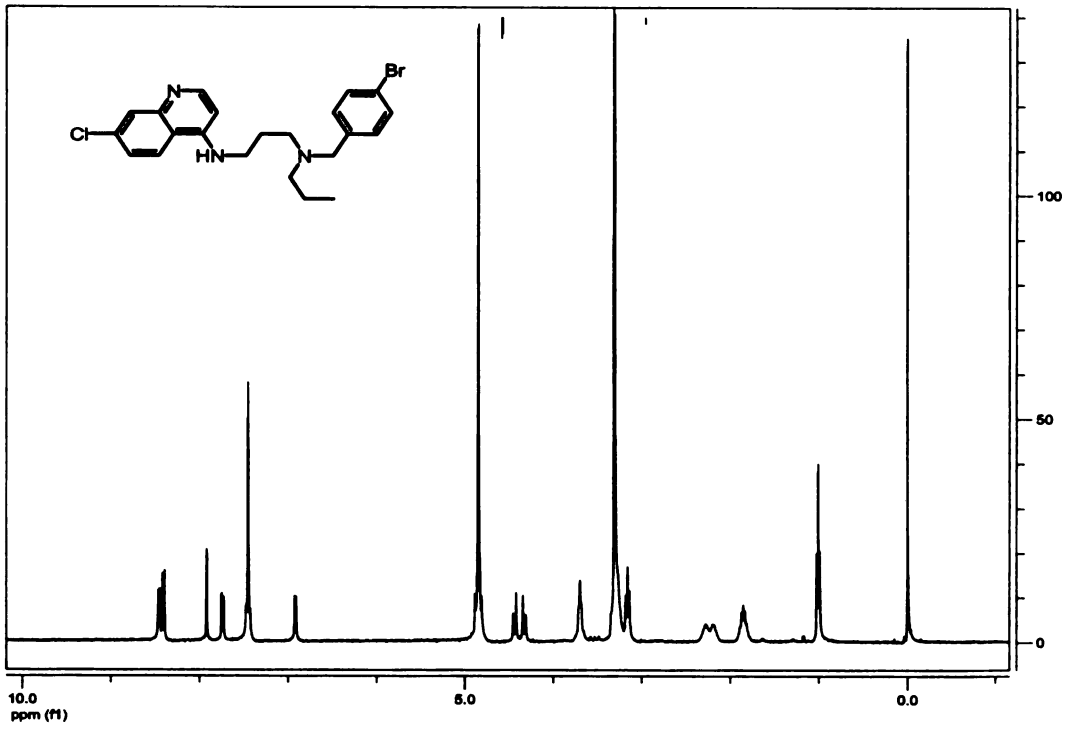
NH
Cl
N
100
50
0
10.0
ppm (f1)
5.0
0.0

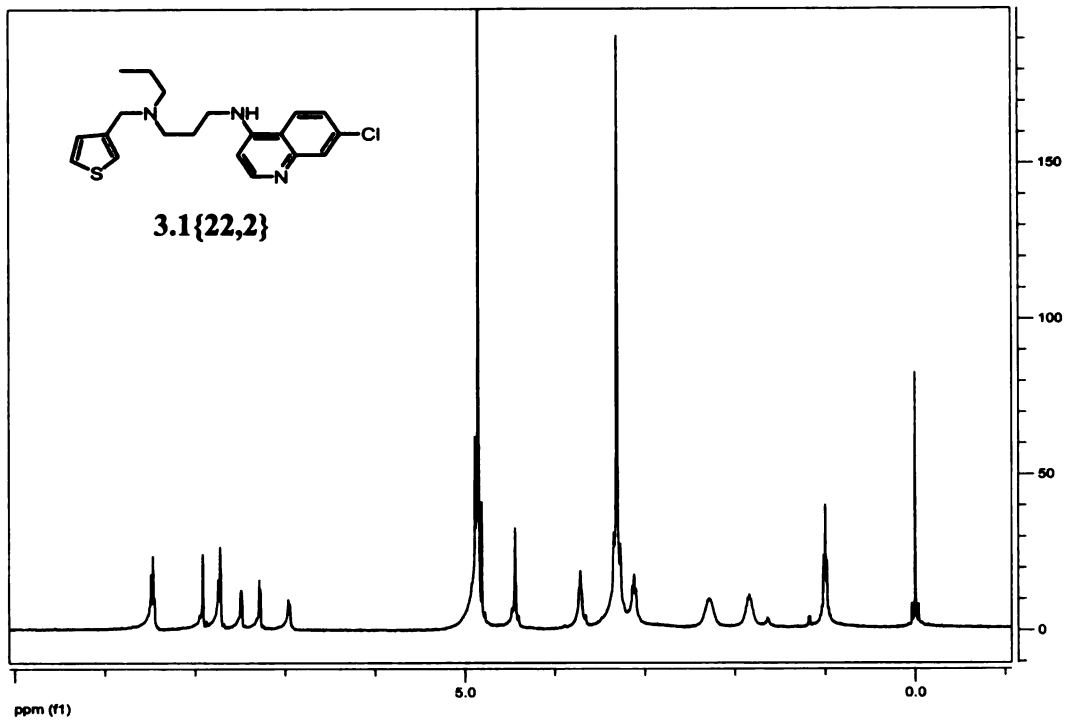
3.1{4,2)


N
Cl
HN
N
60
50
40
30
20
10
0
10.0
5.0
0.0

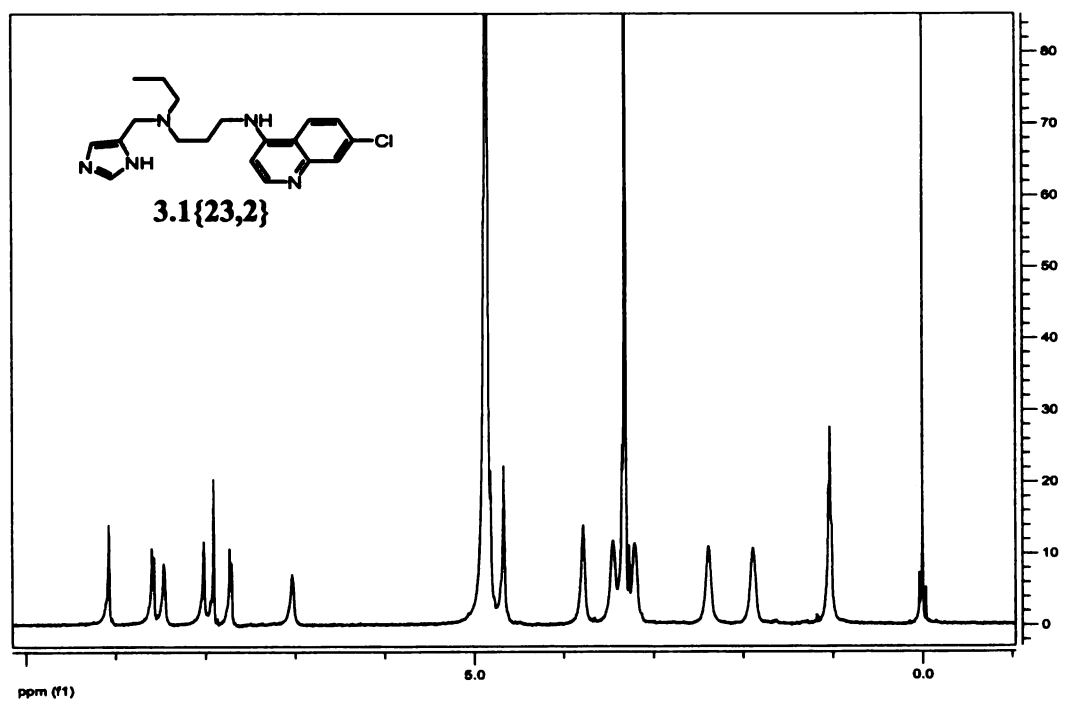
3.1{7,2)


Cl
N
HN
N
3.1{8,2}
60
50
40
30
20
10
0
5.0
0.0
ppm (f1)


Cl
N
HN
N
Br
100
50
0
10.0
5.0
0.0
ppm (f1)


NH
N
Cl
S
N
150
100
50
0
5.0
0.0
ppm (f1)

3.1{22,2}


N
NH
NH
N
Cl
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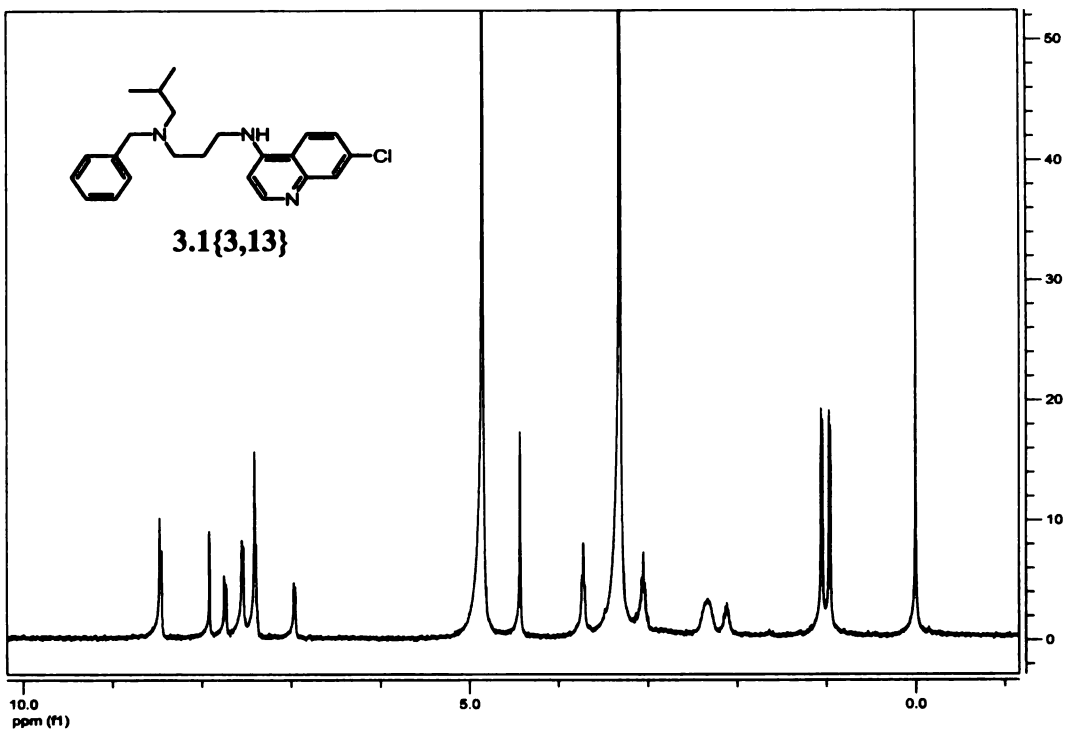
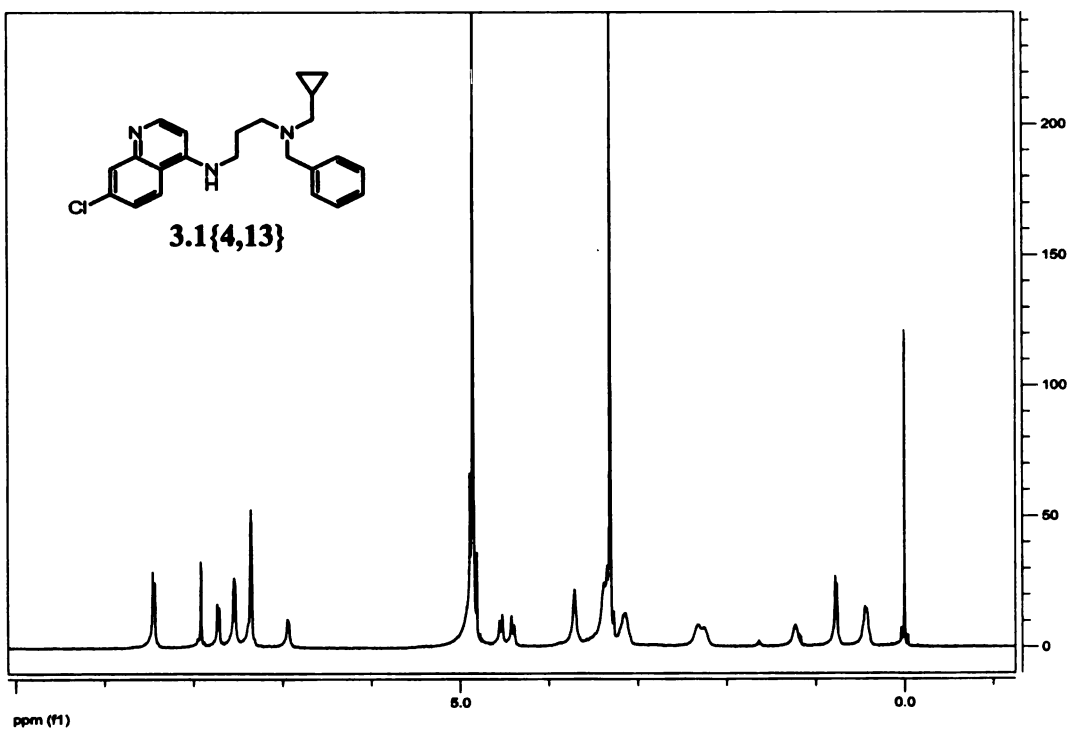
70
60
50
40
30
20
10
0
5.0
0.0
ppm (f1)

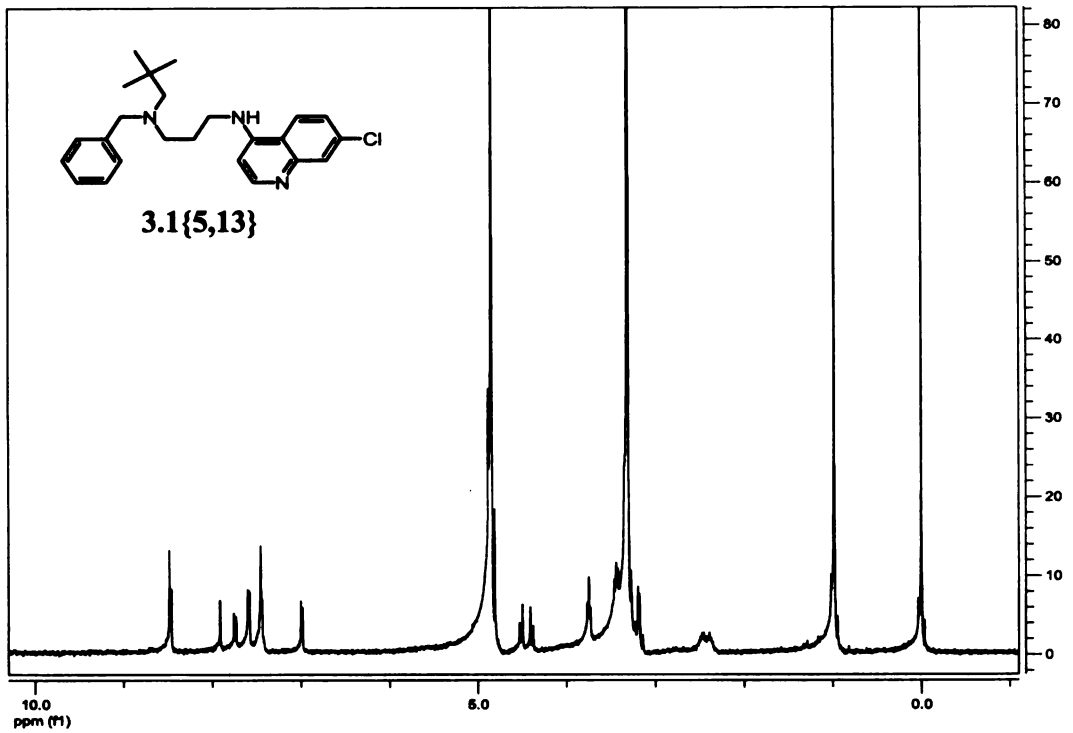
3.1{23,2}


NH
Cl
N
50
40
30
20
10
0
10.0
ppm (f1)
5.0
0.0

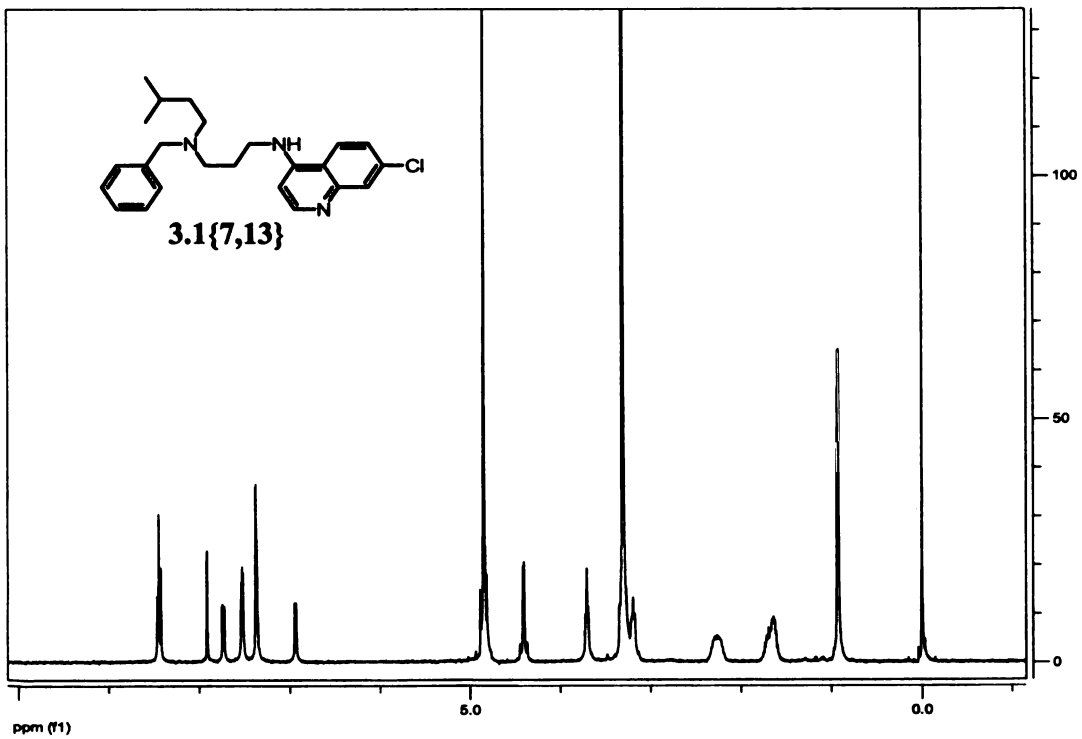
3.1{3,13}


N
H
N
Cl
200
150
100
50
0
5.0
0.0
ppm (f1)

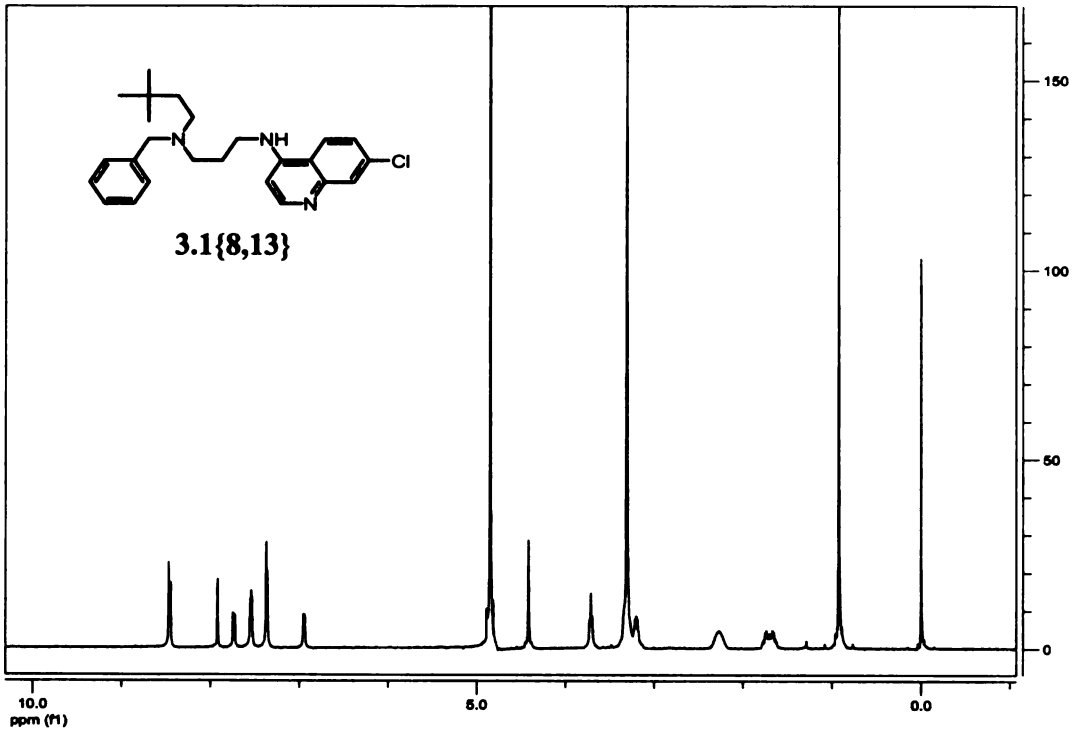
3.1{4,13}


NH
Cl
N
80
70
60
50
40
30
20
10
0
10.0
ppm (f1)
5.0
0.0

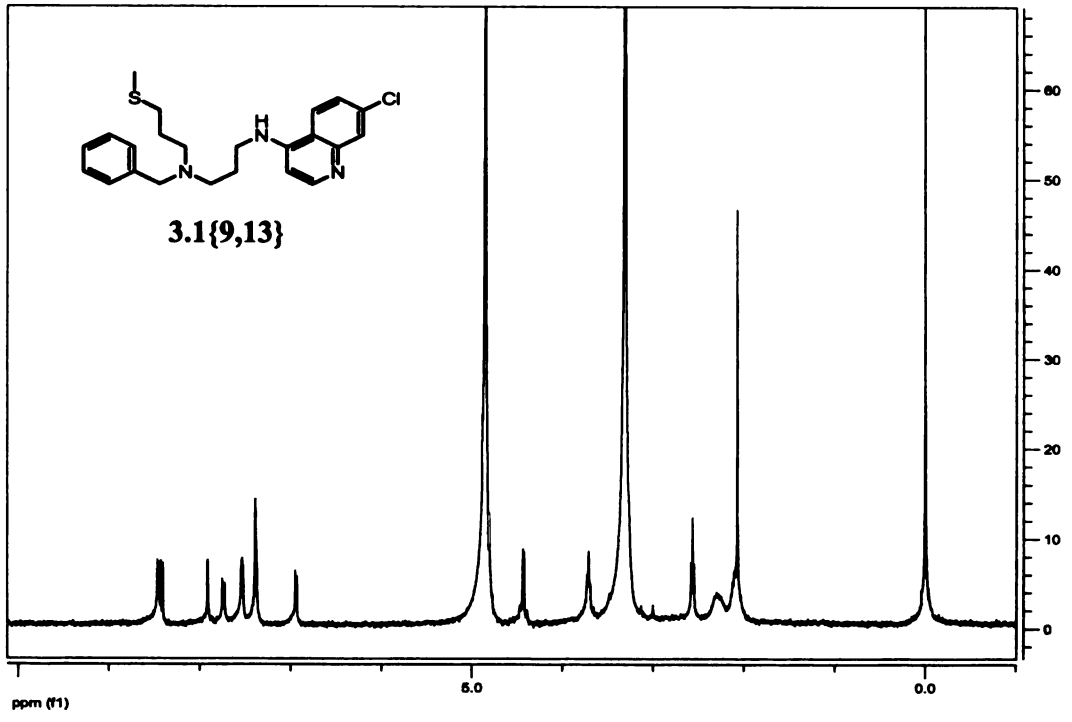
3.1{5,13}


NH
Cl
N
100
50
0
5.0
0.0
ppm (f1)

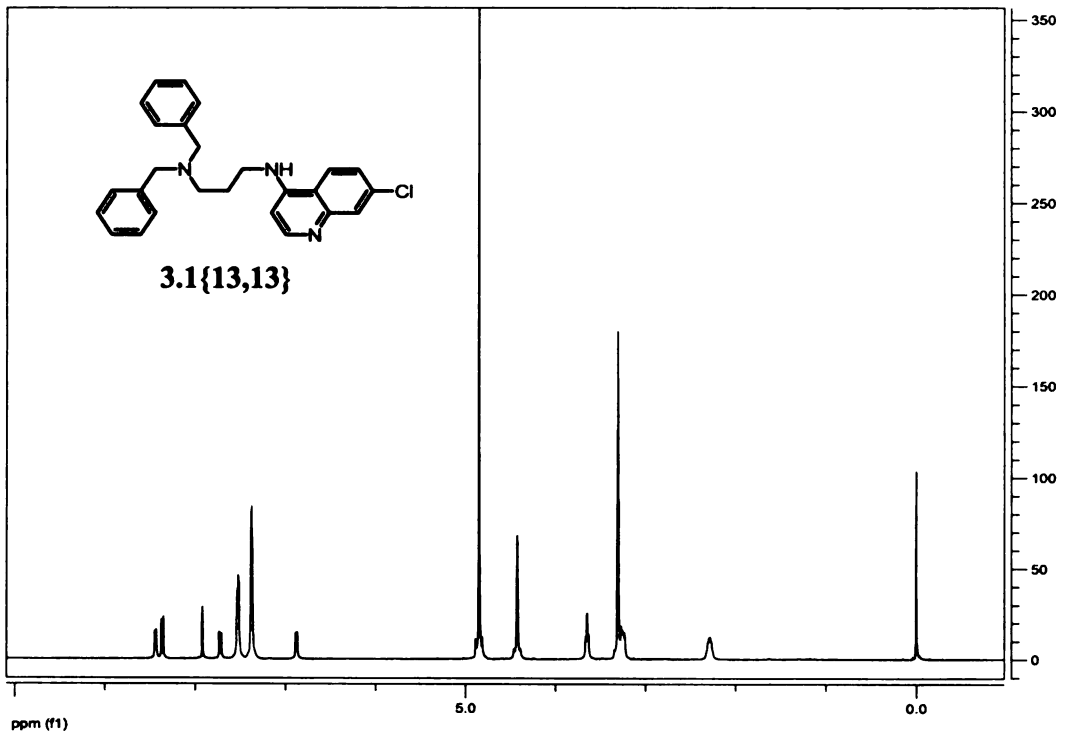
3.1{7,13}


NH
Cl
N
150
100
50
0
10.0
ppm (f1)
5.0
0.0

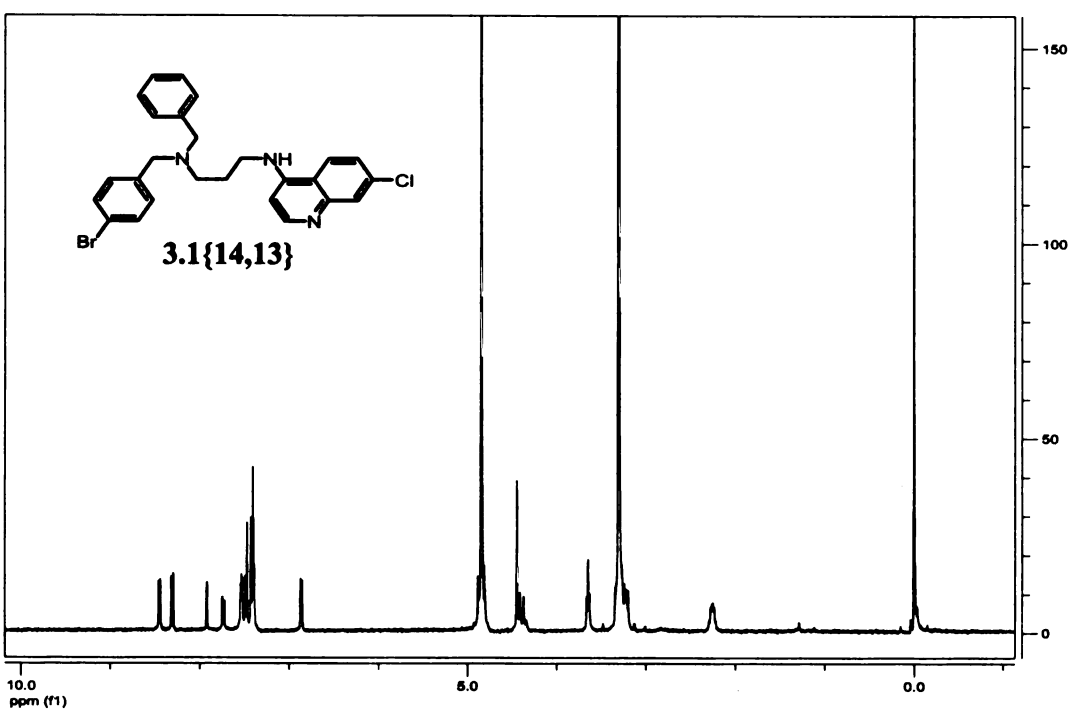
3.1{8,13}


S
N
H
N
Cl
60
50
40
30
20
10
0
5.0
0.0
ppm (f1)

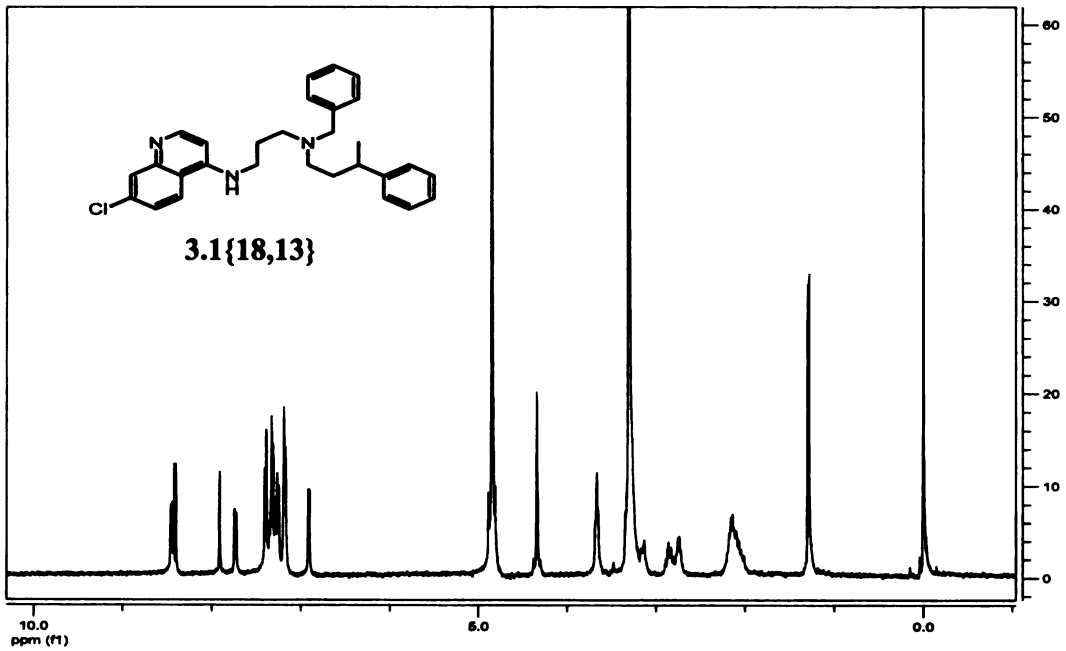
3.1{9,13}



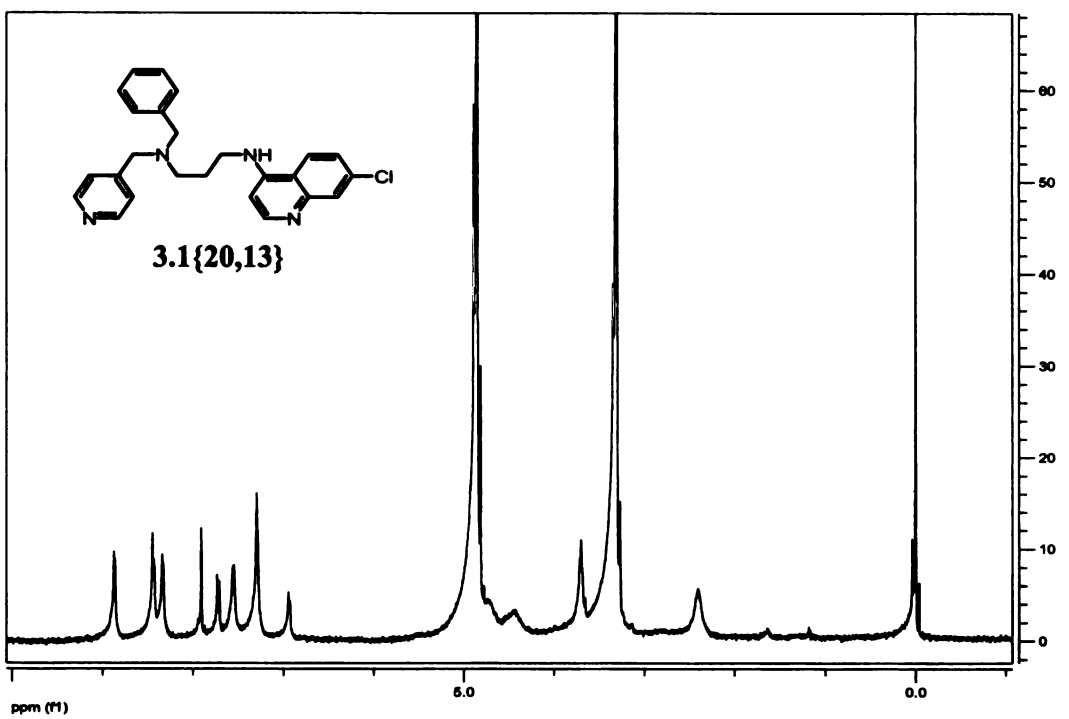
3.1{13,13}



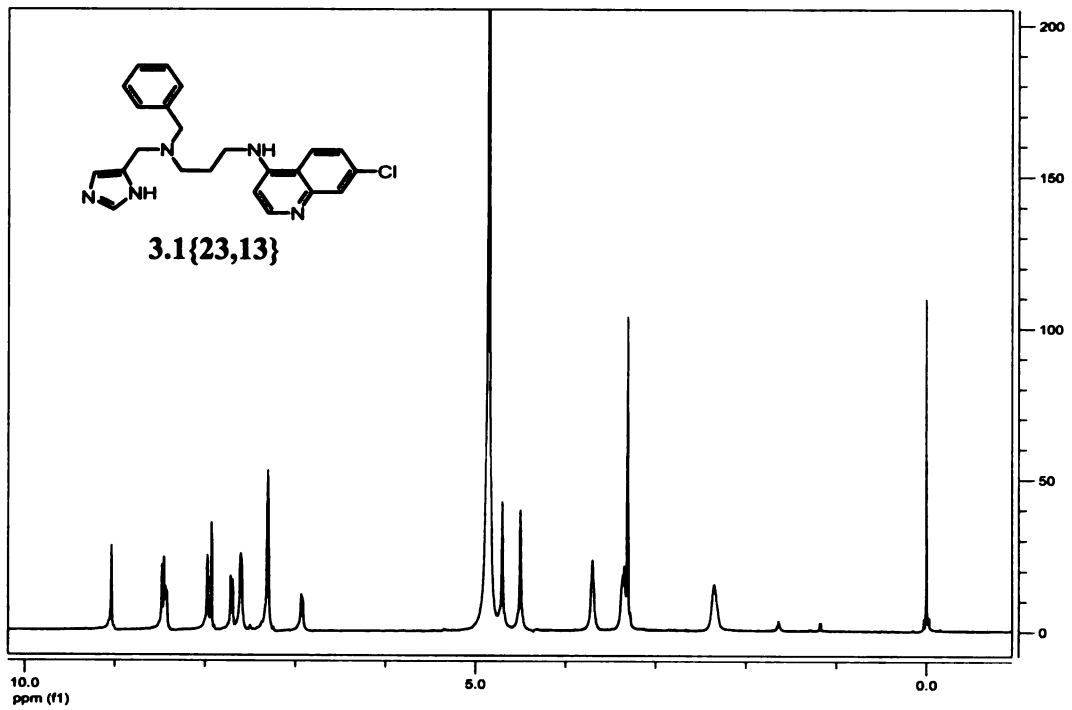
3.1{14,13}



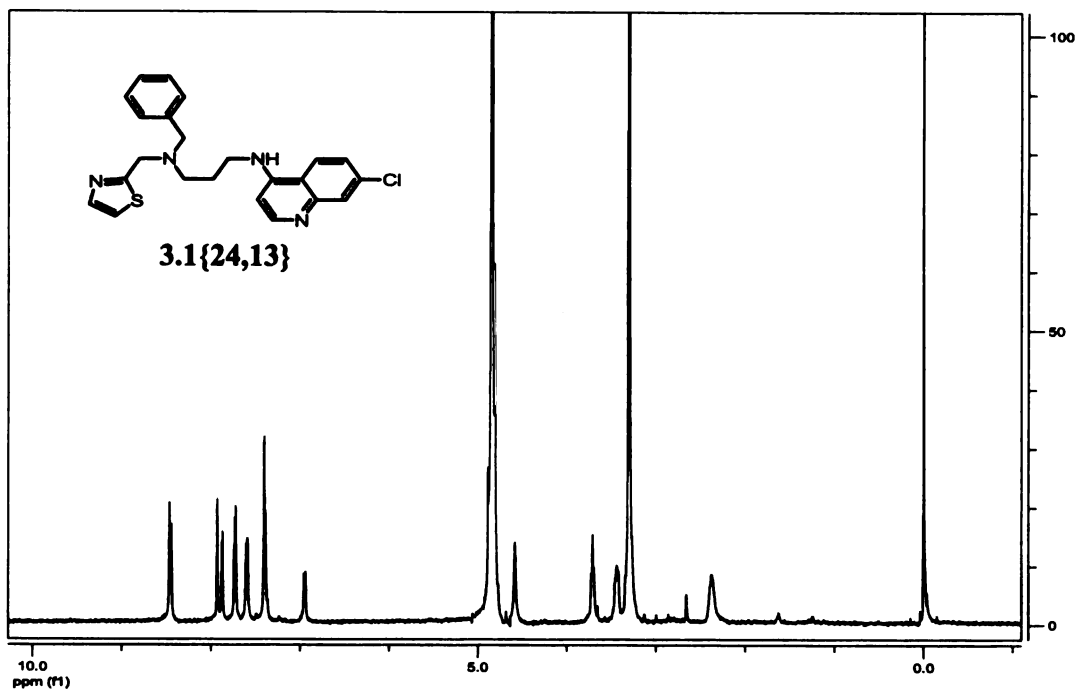
3.1{18,13}



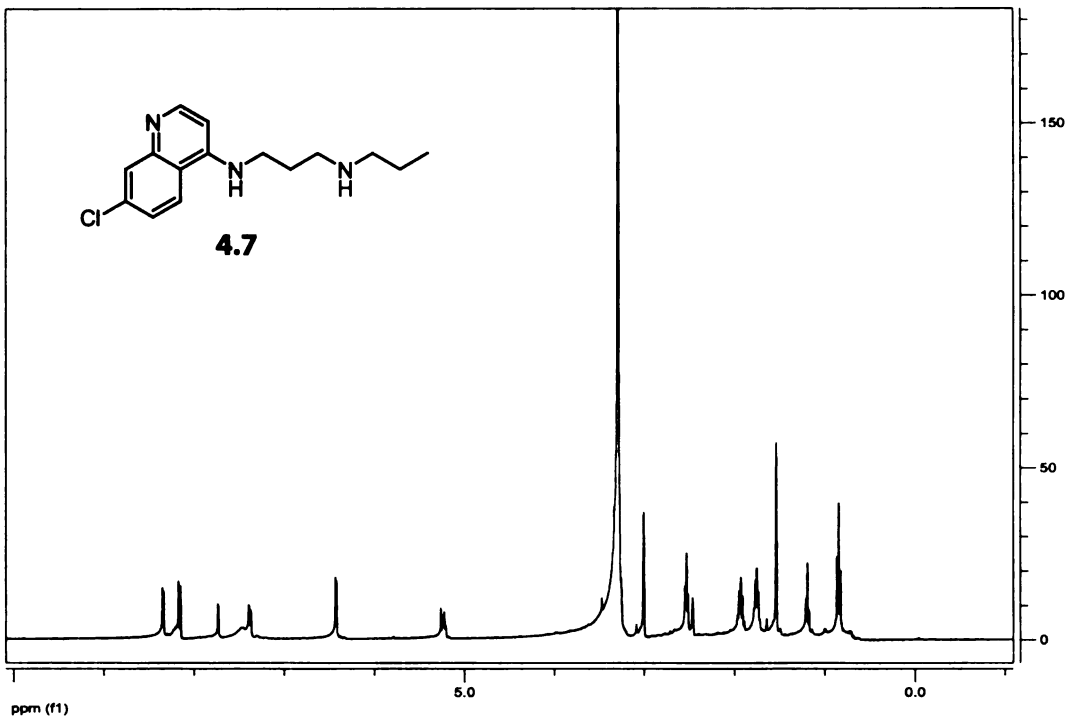
3.1{20,13}

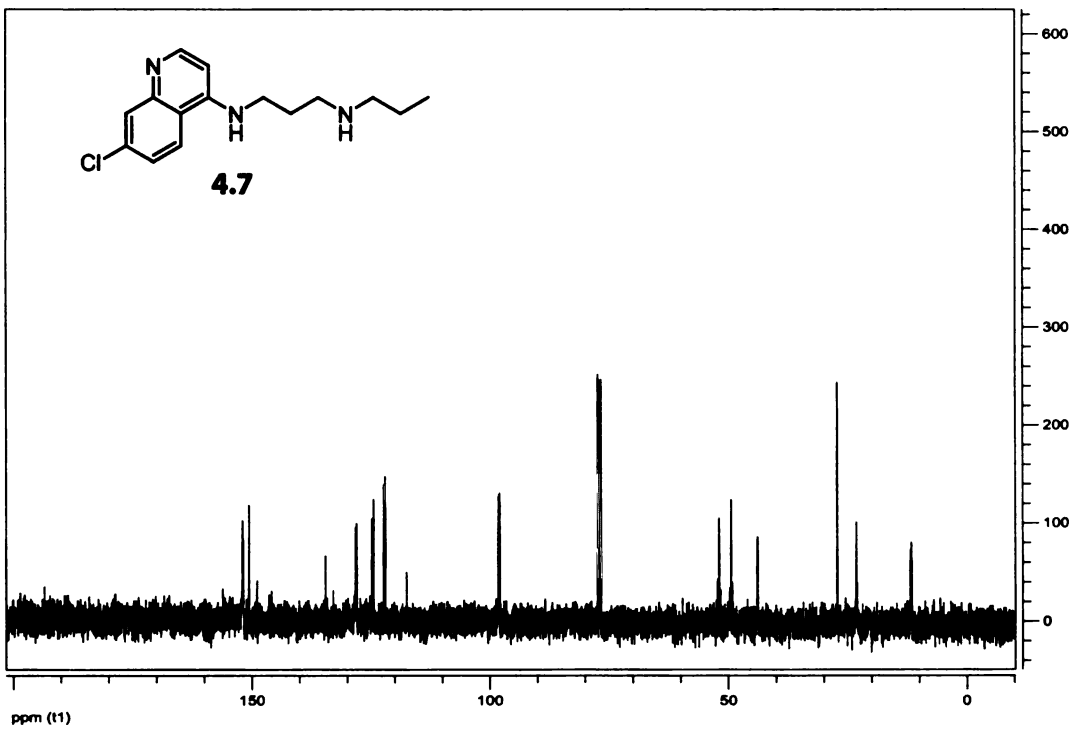


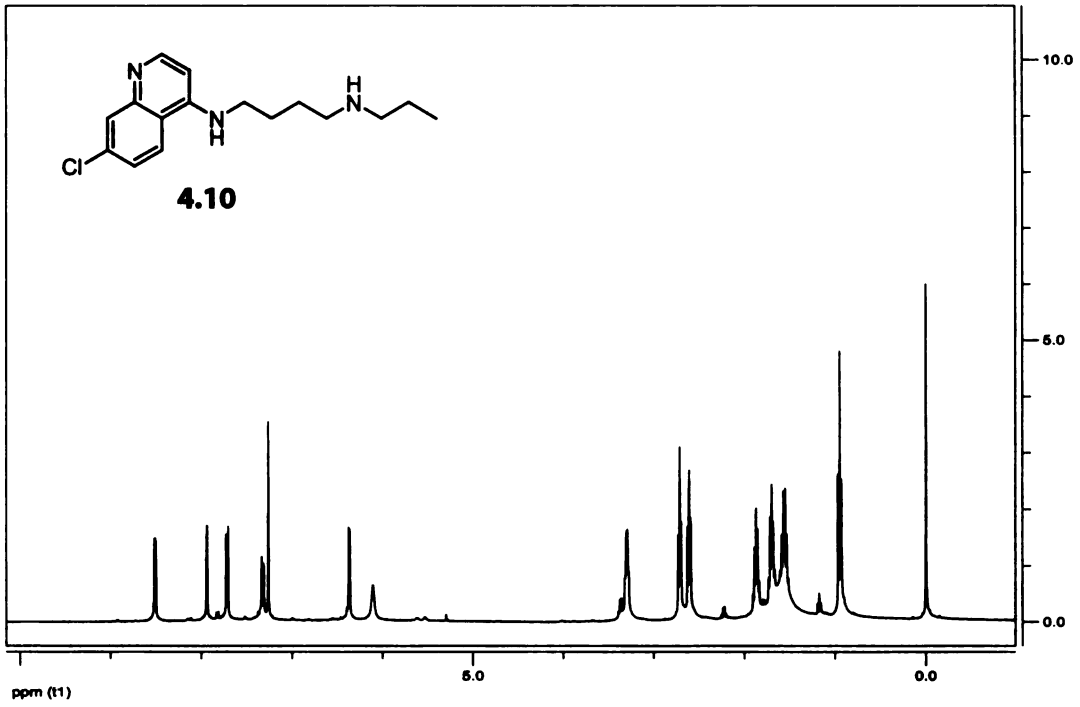
3.1{23,13}

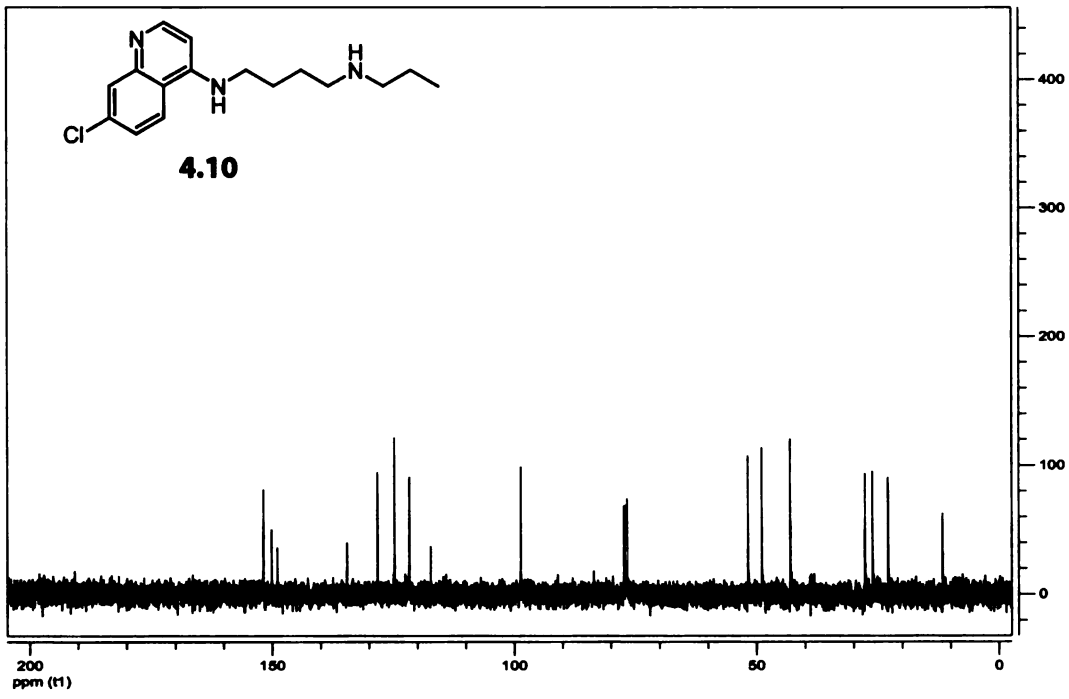


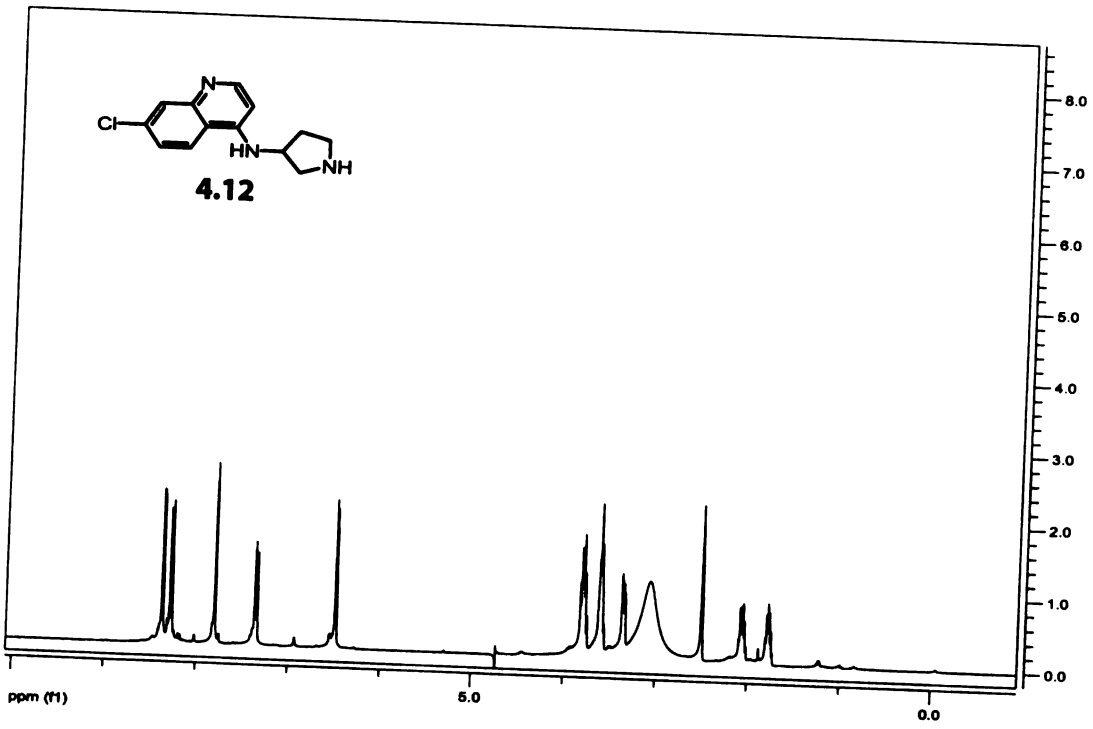
3.1{24,13}

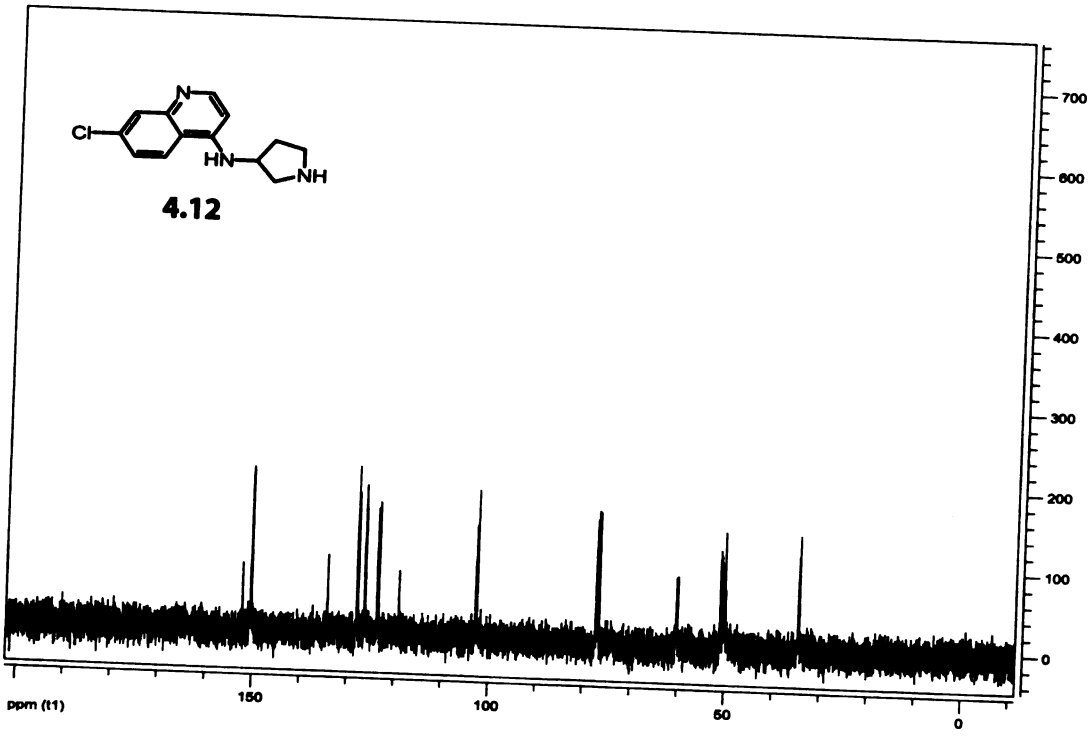

4.7
150
100
50
0
5.0
0.0
ppm (f1)

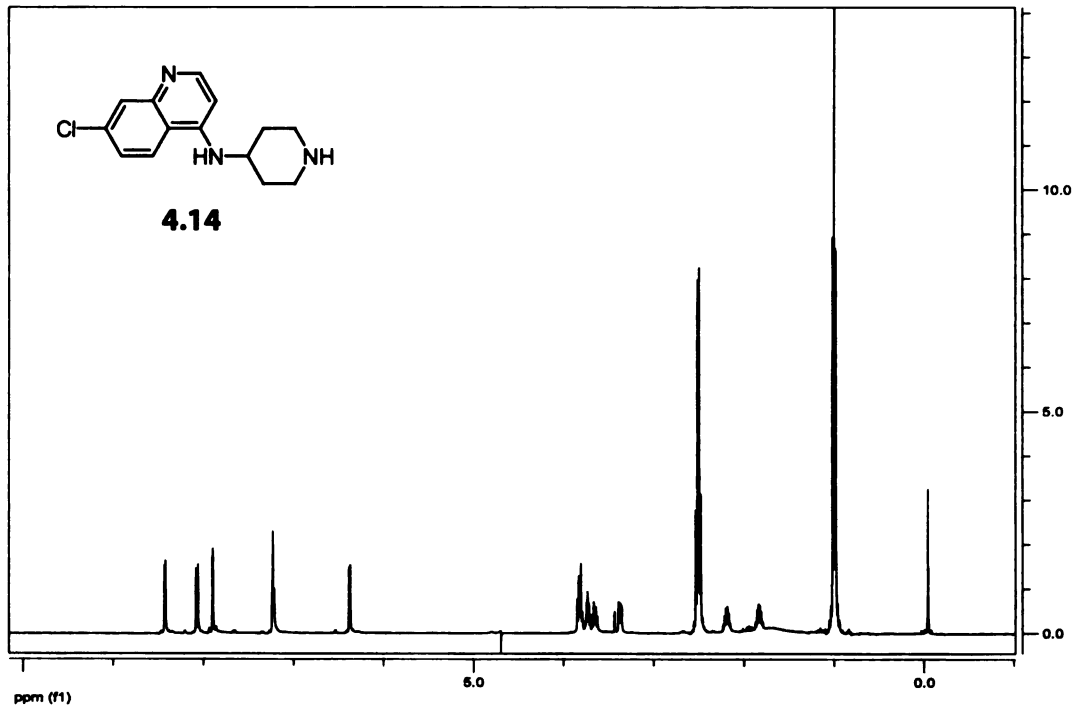

4.7
600
500
400
300
200
100
0
150
100
50
0
ppm (t1)


4.10
10.0
5.0
0.0
5.0
0.0
ppm (t1)

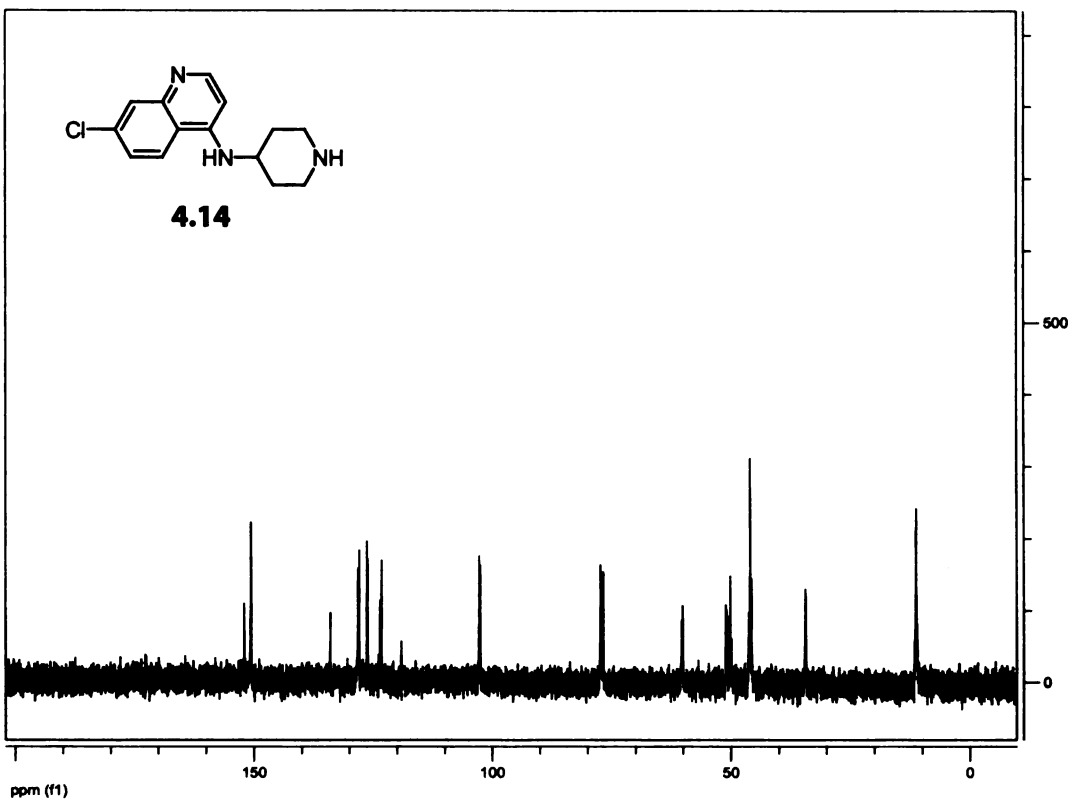

4.10
400
300
200
100
0
200
150
100
50
0
ppm (t1)


Cl
N
HN
NH
4.12
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)
5.0
0.0

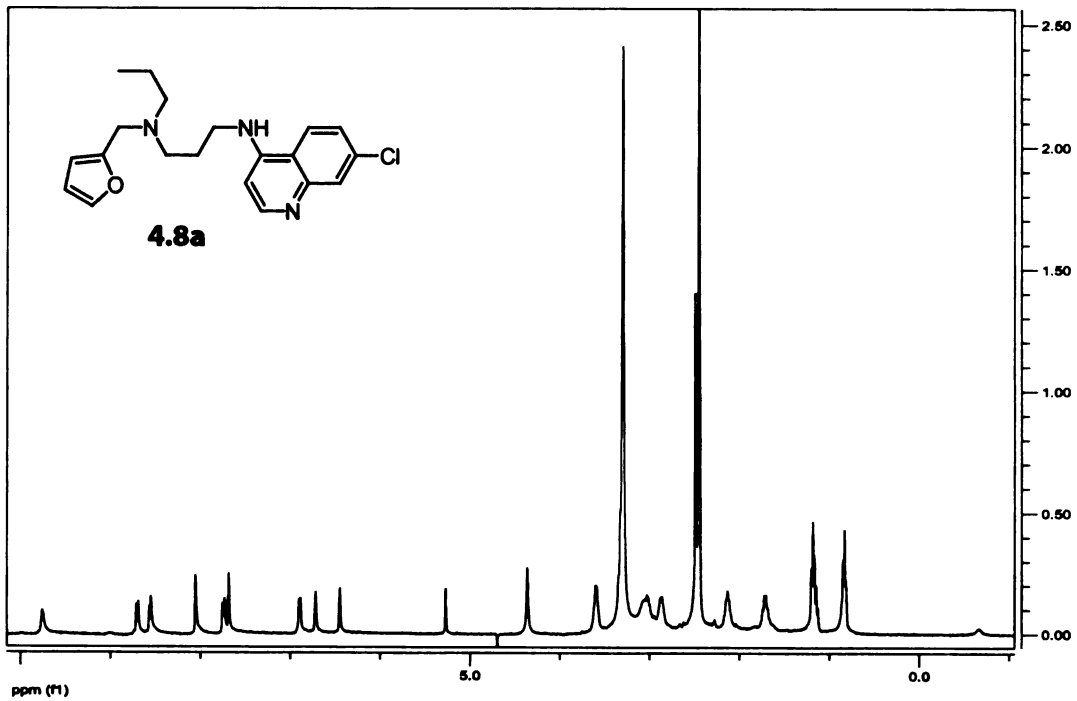

Cl
N
HN
NH
4.12
700
600
500
400
300
200
100
0
ppm (t1)
150
100
50
0

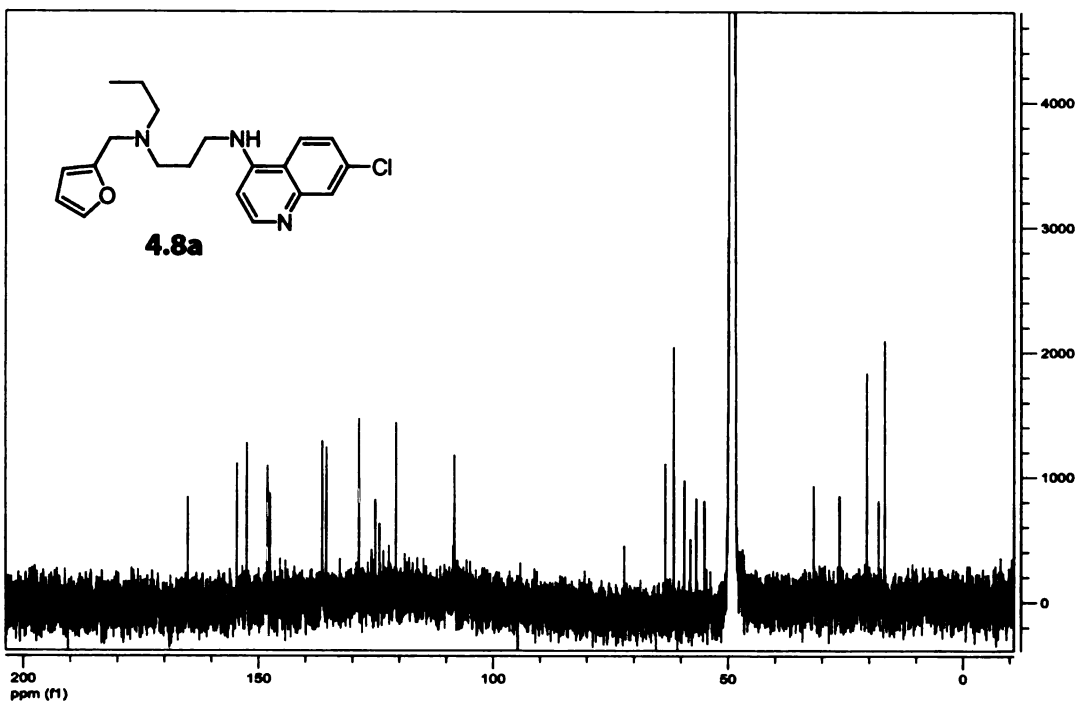


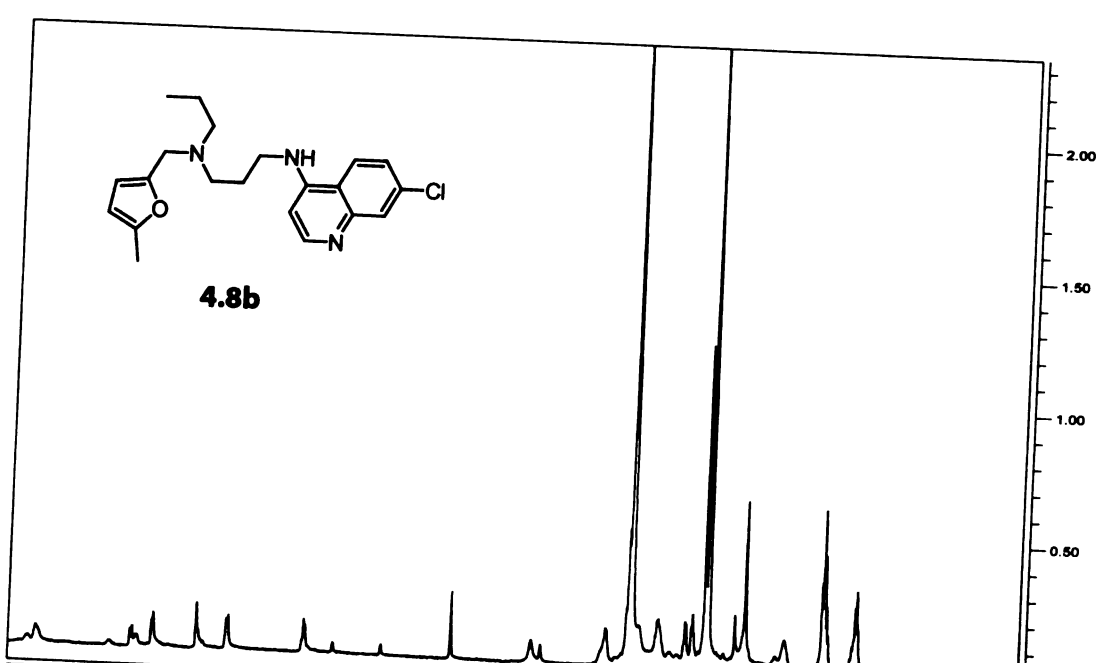
4.14



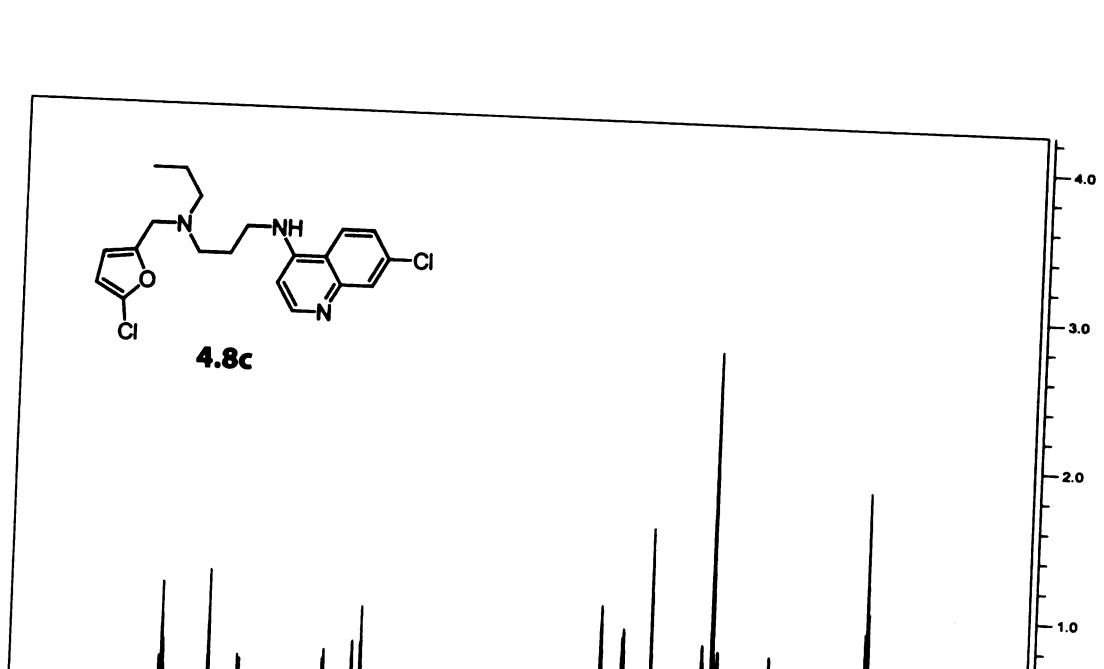
4.14


N
NH
Cl
O
N
4.8a
2.50
2.00
1.50
1.00
0.50
0.00
5.0
0.0
ppm (f1)

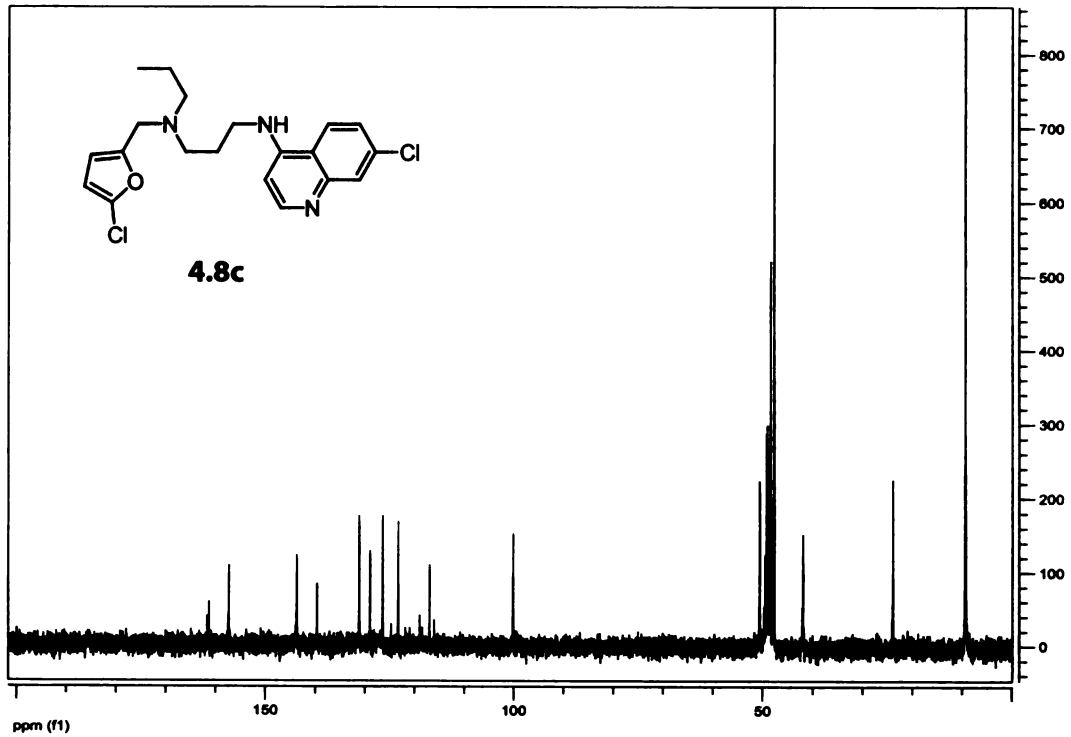

N
NH
Cl
O
N
4.8a
4000
3000
2000
1000
0
200
150
100
50
0
ppm (f1)


N
NH
Cl
O
N
2.00
1.50
1.00
0.50
0.00
ppm (f1)
5.0
0.0

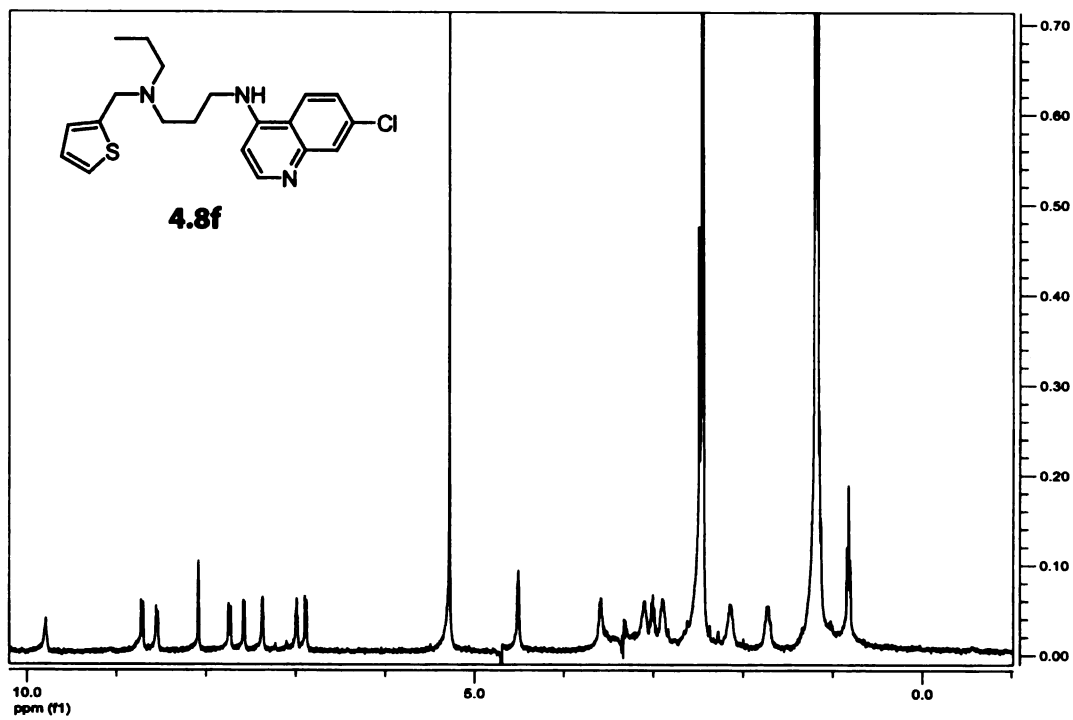
4.8b


N
NH
Cl
O
Cl
N
4.0
3.0
2.0
1.0
0.0
ppm (f1)
5.0
0.0

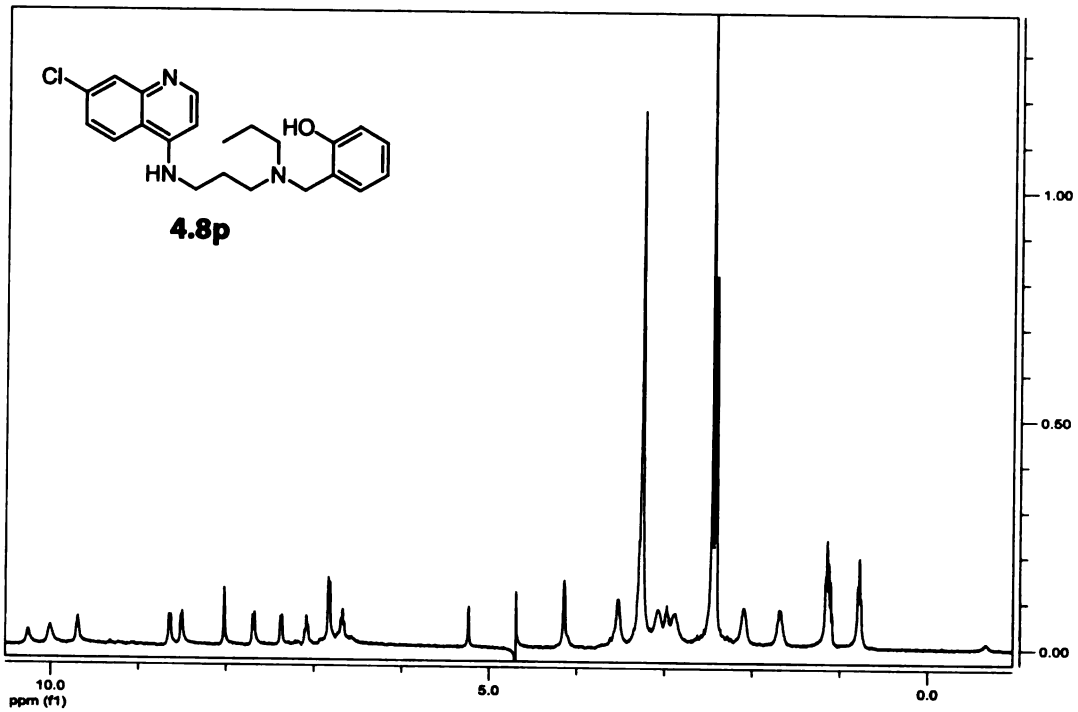
4.8c

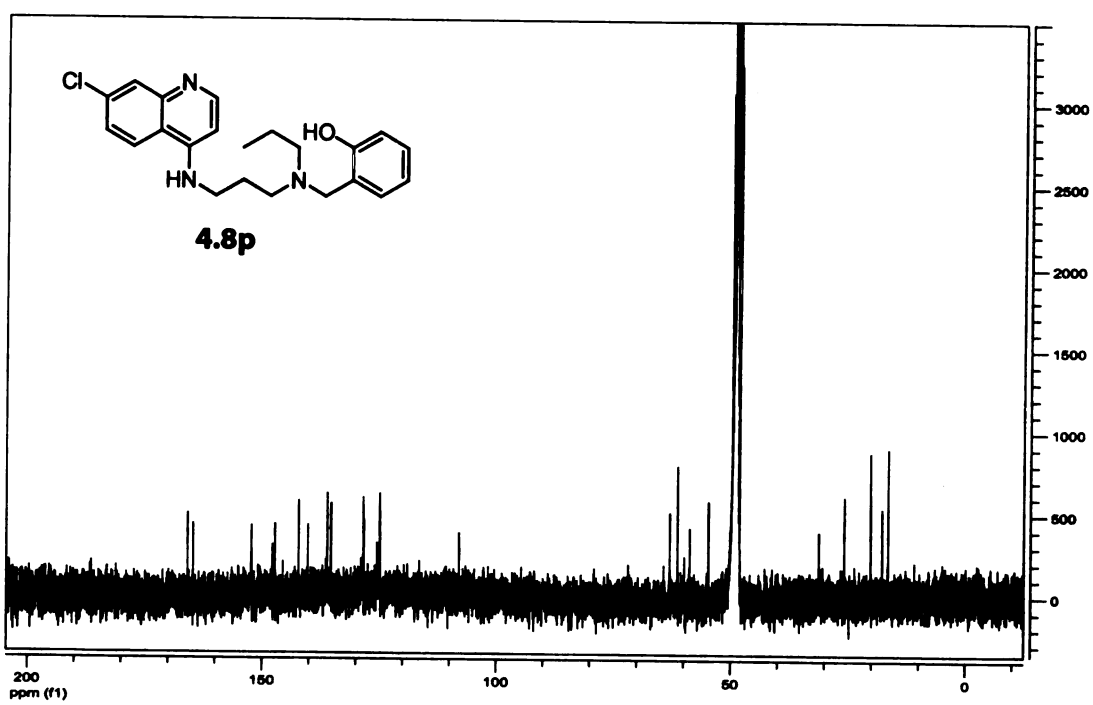

N
NH
Cl
O
Cl
N
800
700
600
500
400
300
200
100
0
150
100
50
ppm (f1)

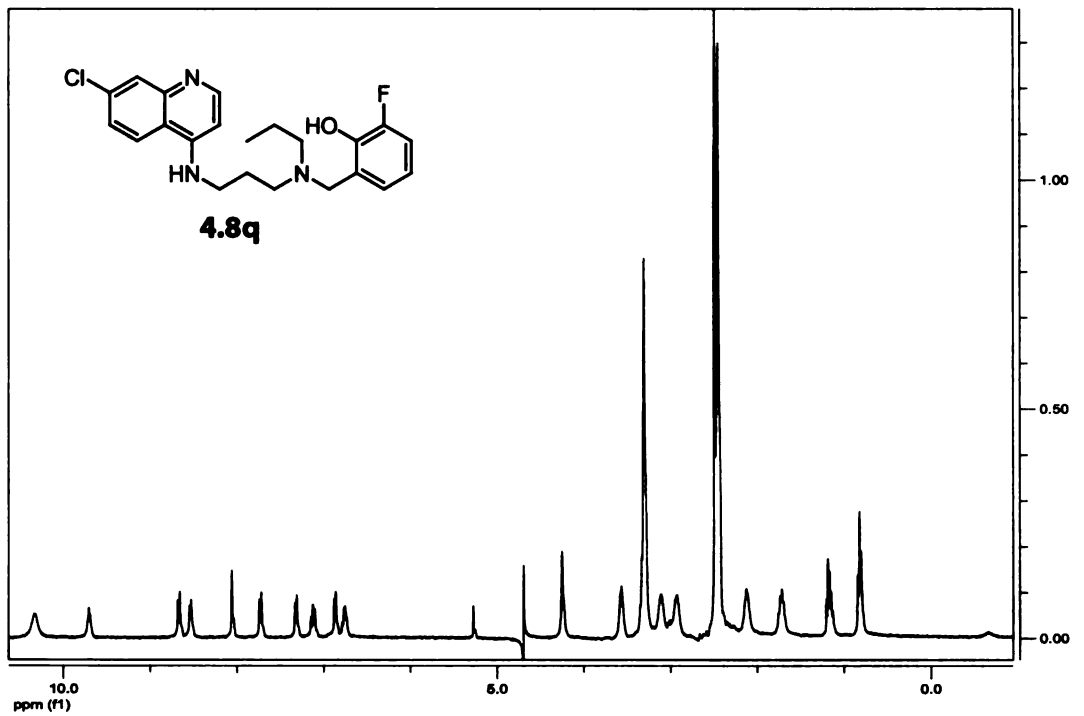
4.8c

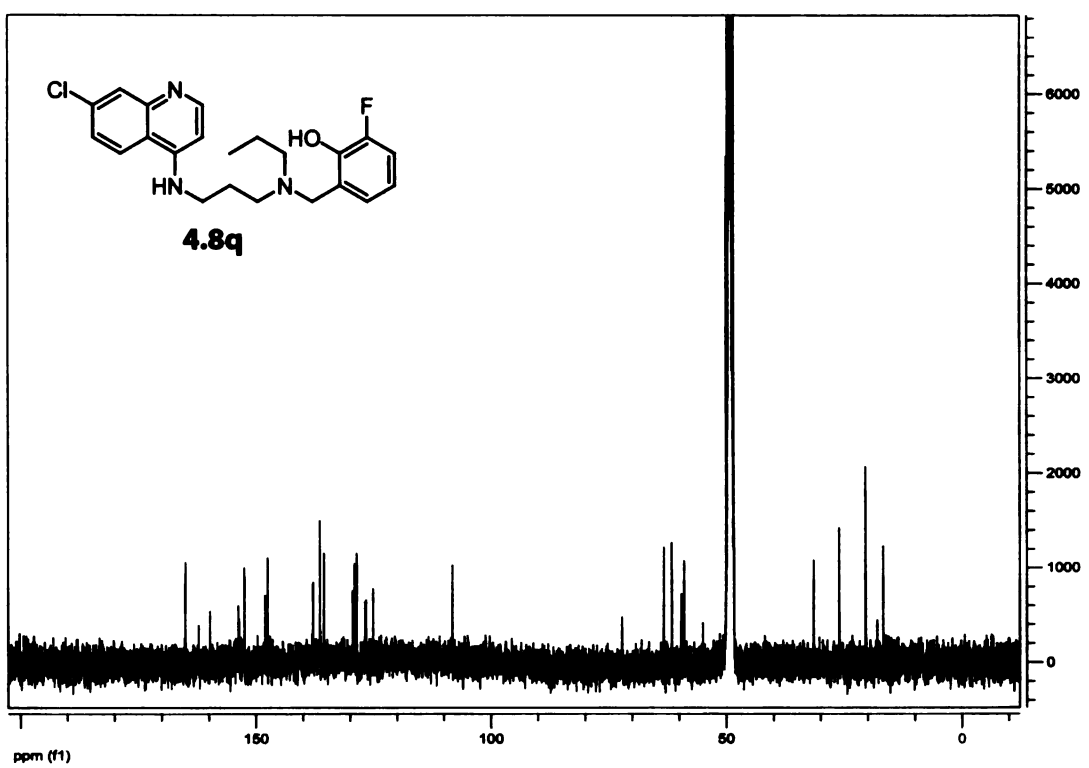

N
NH
Cl
S
N
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
10.0
5.0
0.0
ppm (f1)

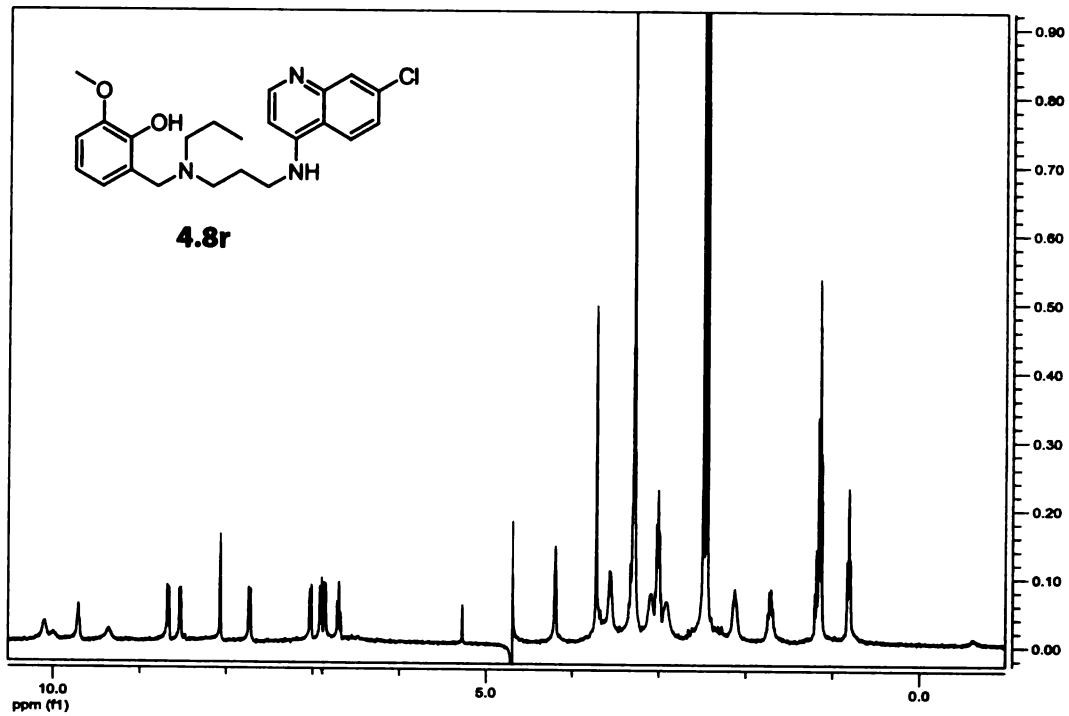
4.8f

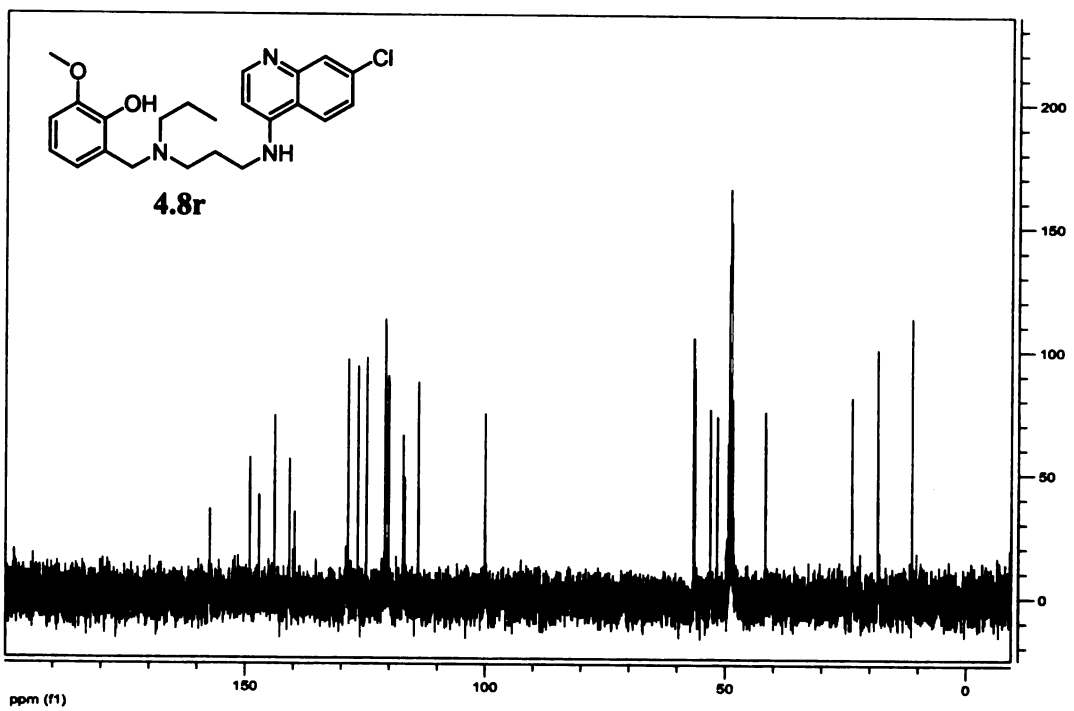

Cl
N
HO
HN
N
4.8p
1.00
0.50
0.00
10.0
ppm (f1)
5.0
0.0

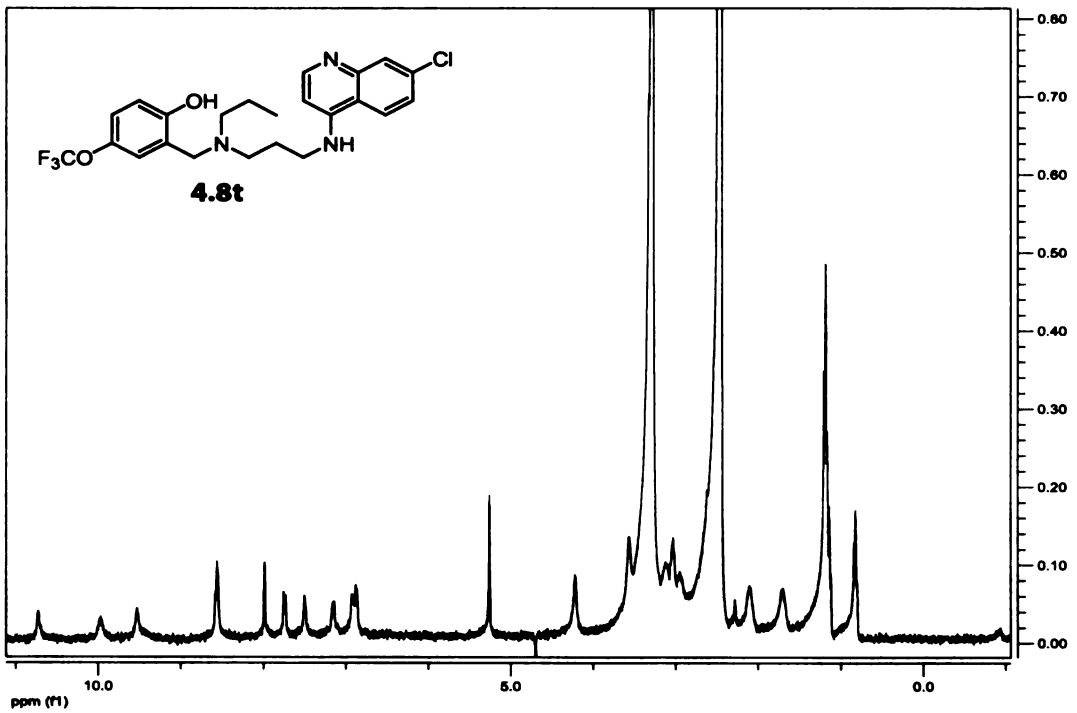

Cl
N
HO
HN
N
4.8p
3000
2500
2000
1500
1000
500
0
200
ppm (f1)
150
100
50
0

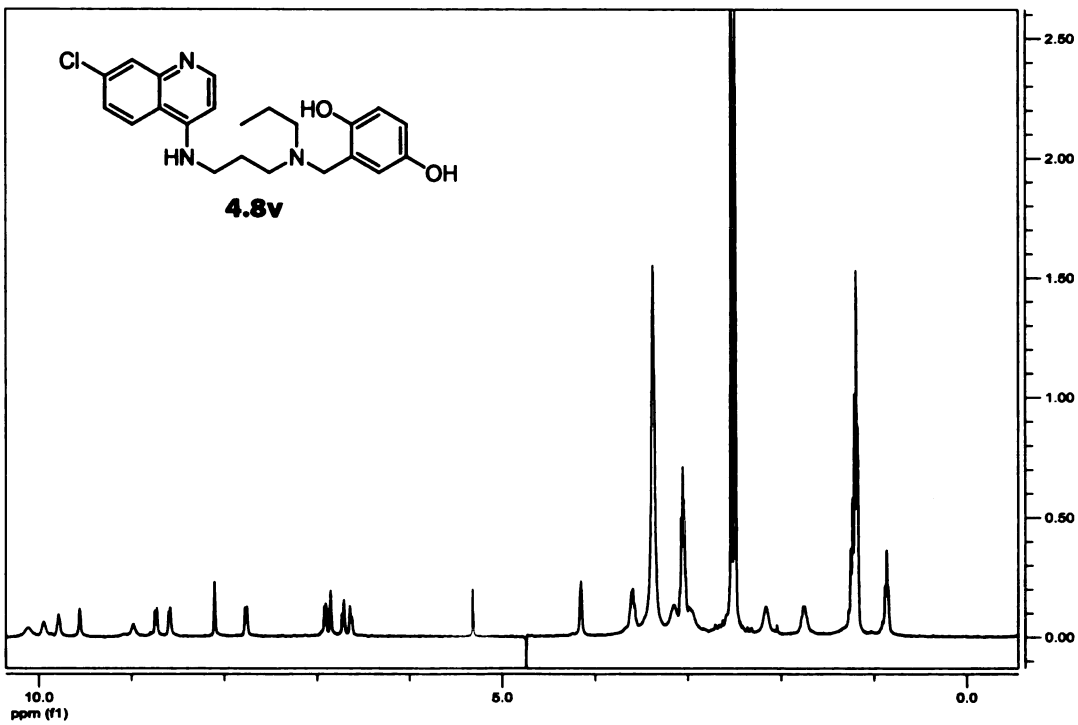

Cl
N
F
HO
HN
N
4.8q
1.00
0.50
0.00
10.0
5.0
0.0
ppm (f1)

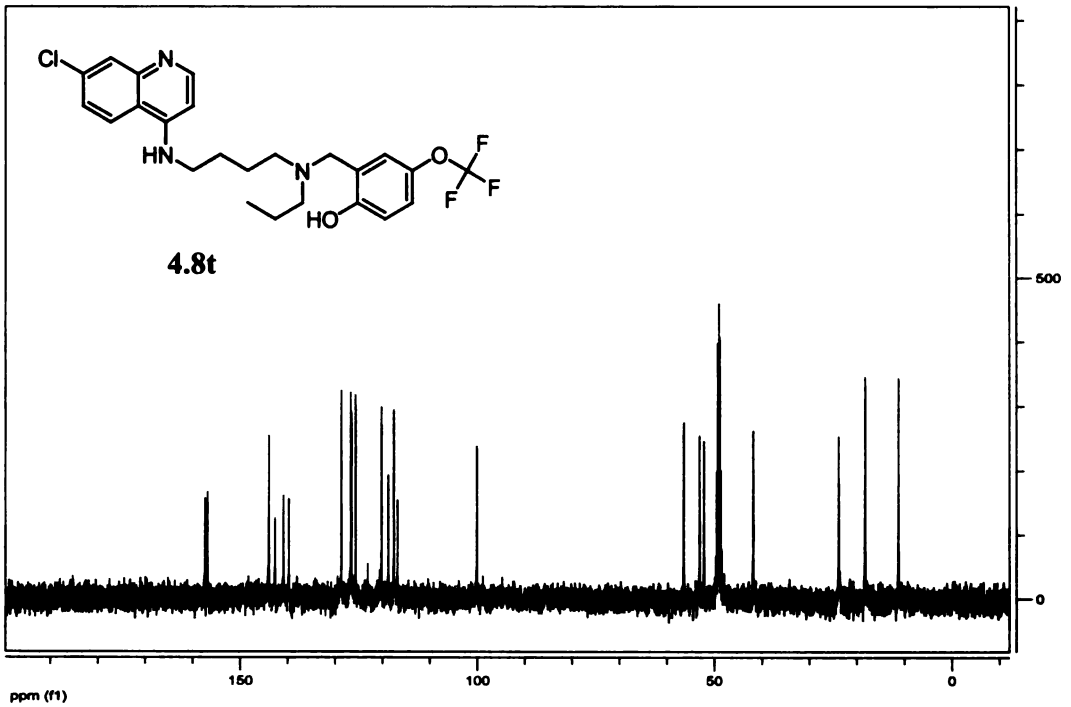

Cl
N
F
HO
HN
N
4.8q
6000
5000
4000
3000
2000
1000
0
150
100
50
0
ppm (f1)

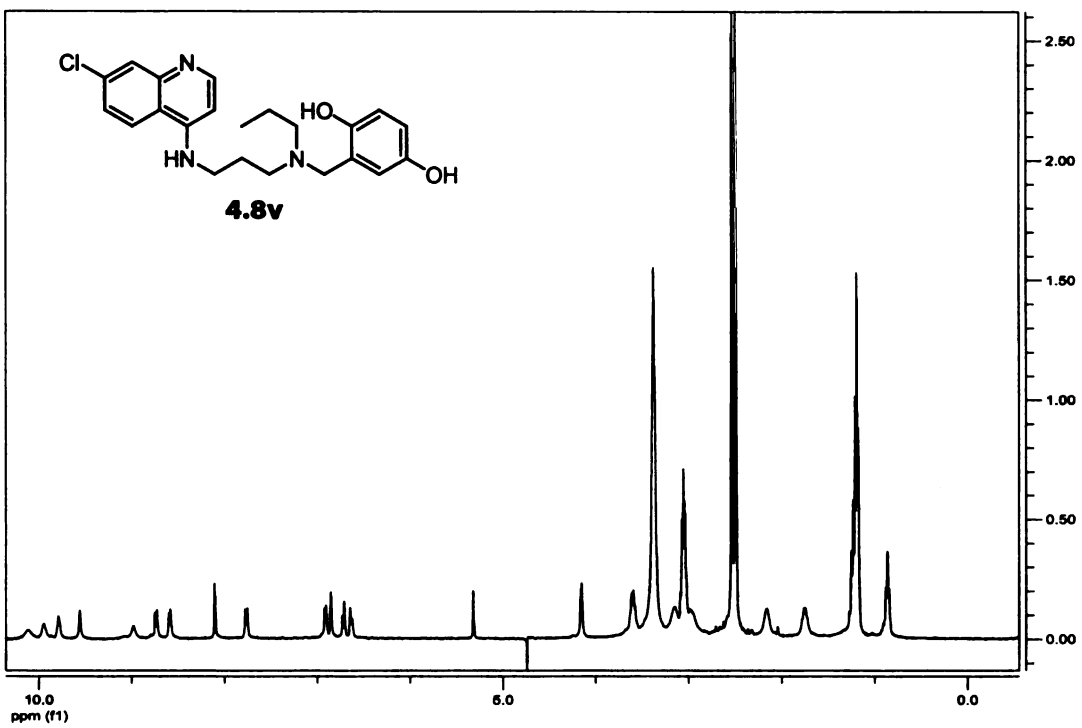

O
OH
N
Cl
N
NH
4.8r
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
10.0
ppm (f1)
5.0
0.0

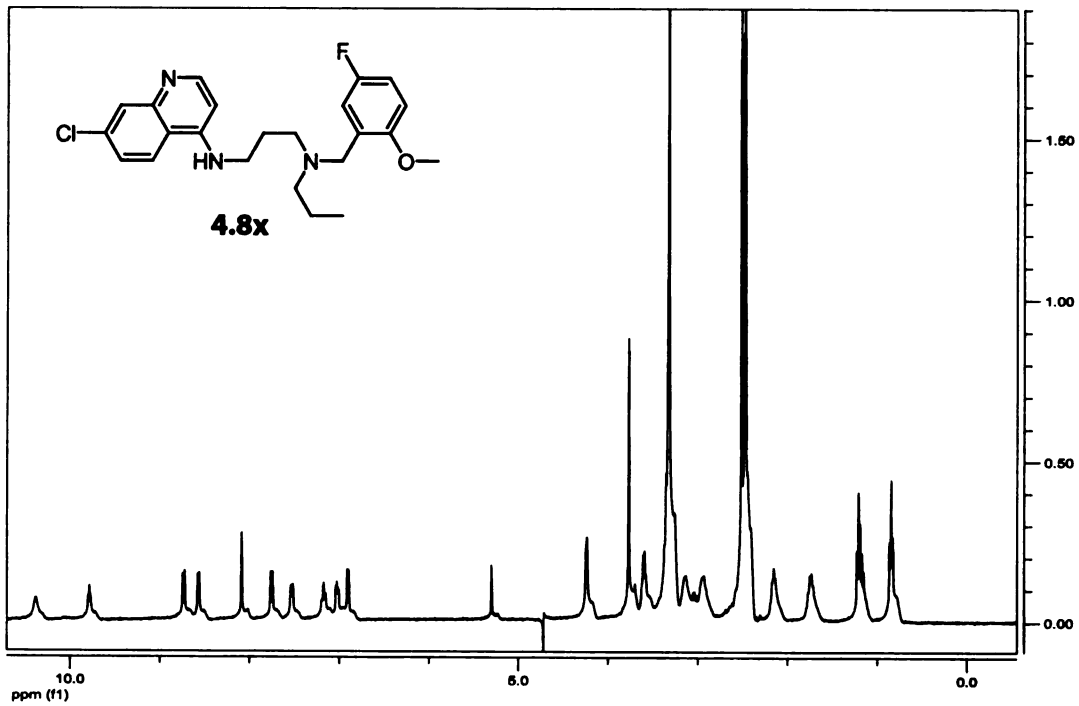

O
OH
N
Cl
N
NH
4.8r
200
150
100
50
0
ppm (f1)
150
100
50
0

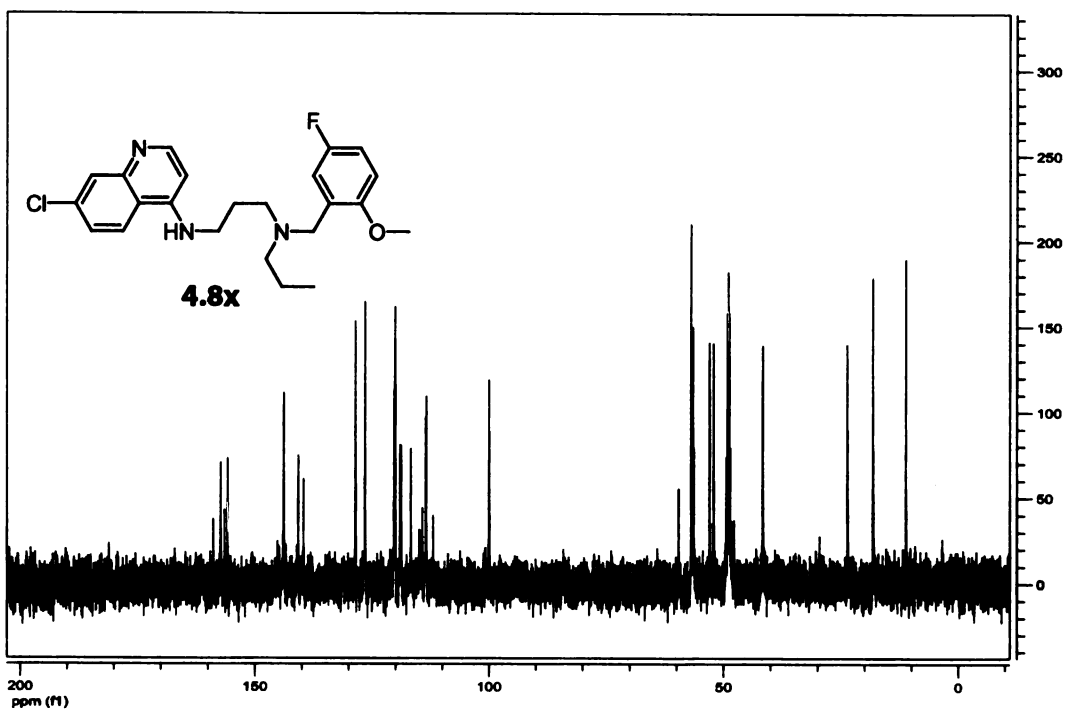

OH
N
Cl
F3CO
NH
4.8t
0.60
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
10.0
5.0
0.0
ppm (f1)

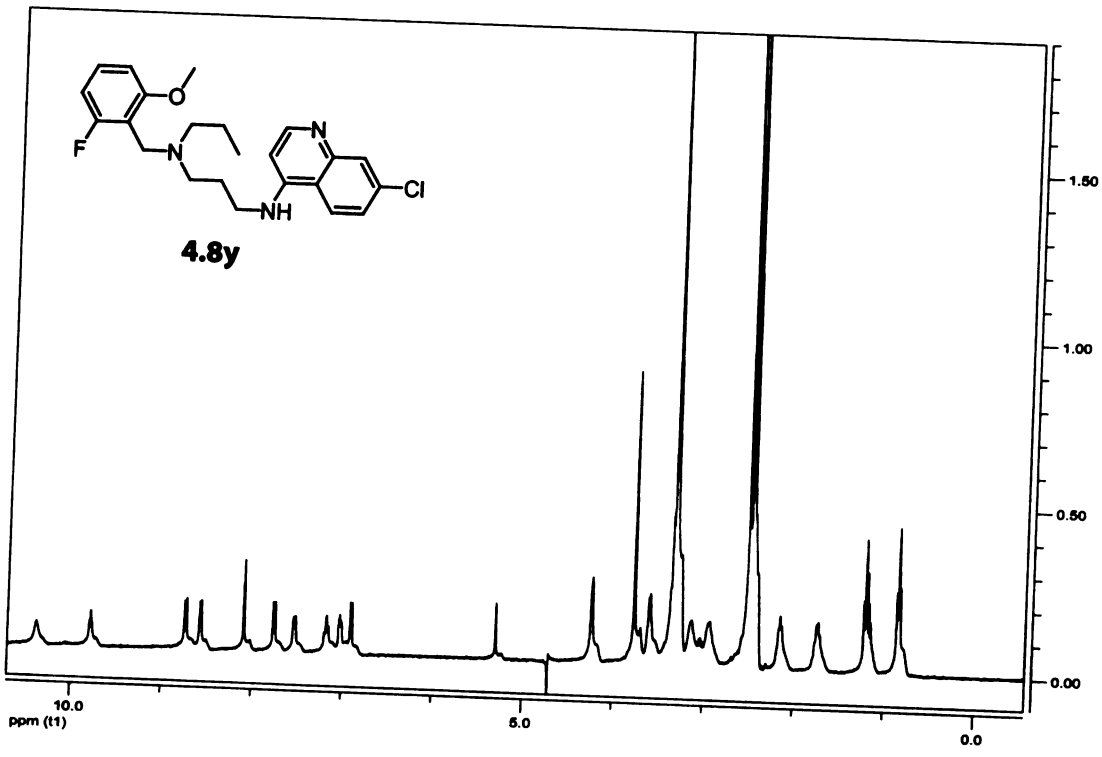

Cl
N
HO
HN
N
OH
4.8v
2.50
2.00
1.50
1.00
0.50
0.00
10.0
5.0
0.0
ppm (f1)

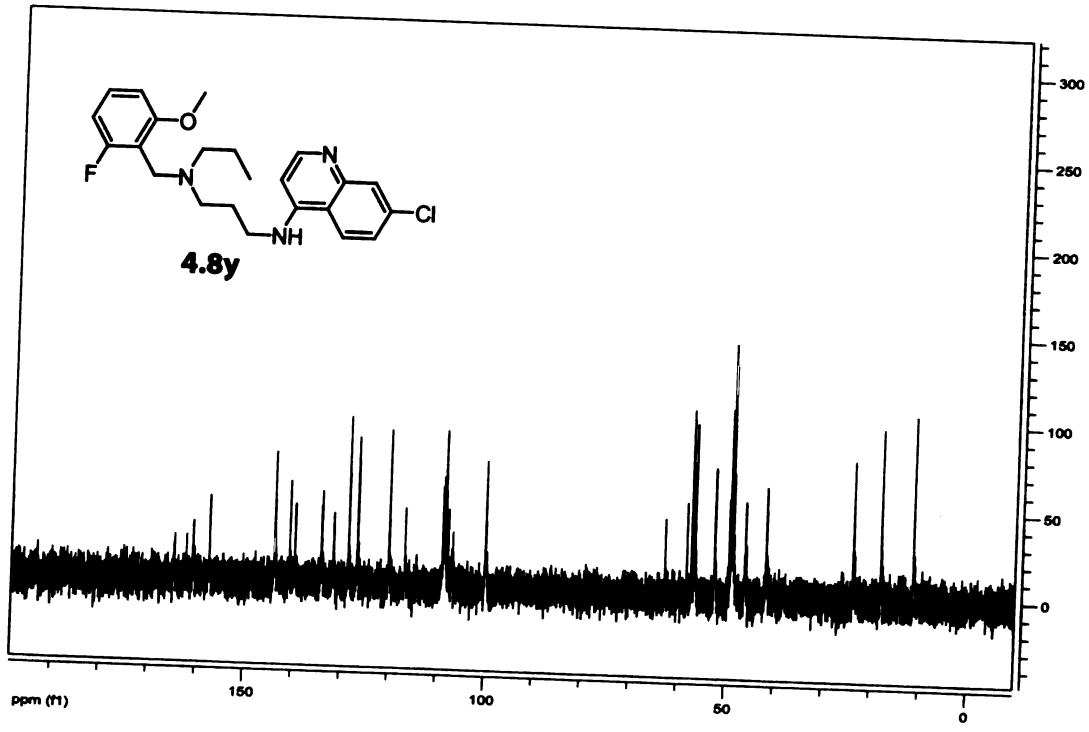

Cl
N
HN
N
O
F
F
F
HO
4.8t
500
0
150
100
50
0
ppm (f1)

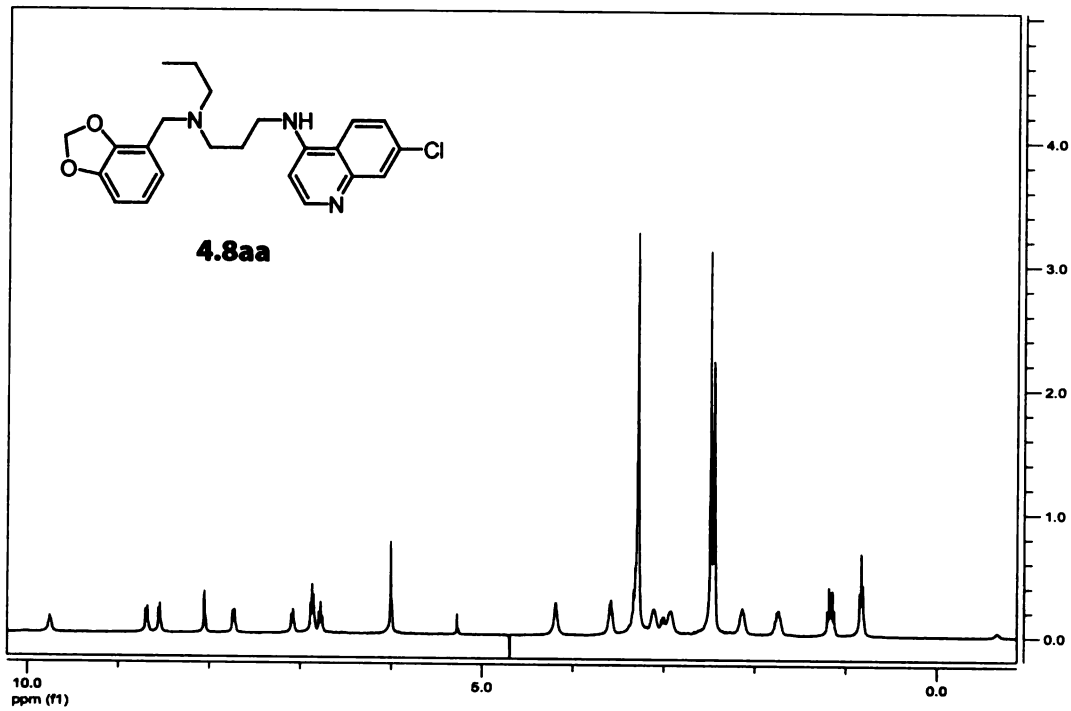

Cl
N
HO
HN
N
OH
4.8v
2.50
2.00
1.50
1.00
0.50
0.00
10.0
5.0
0.0
ppm (f1)

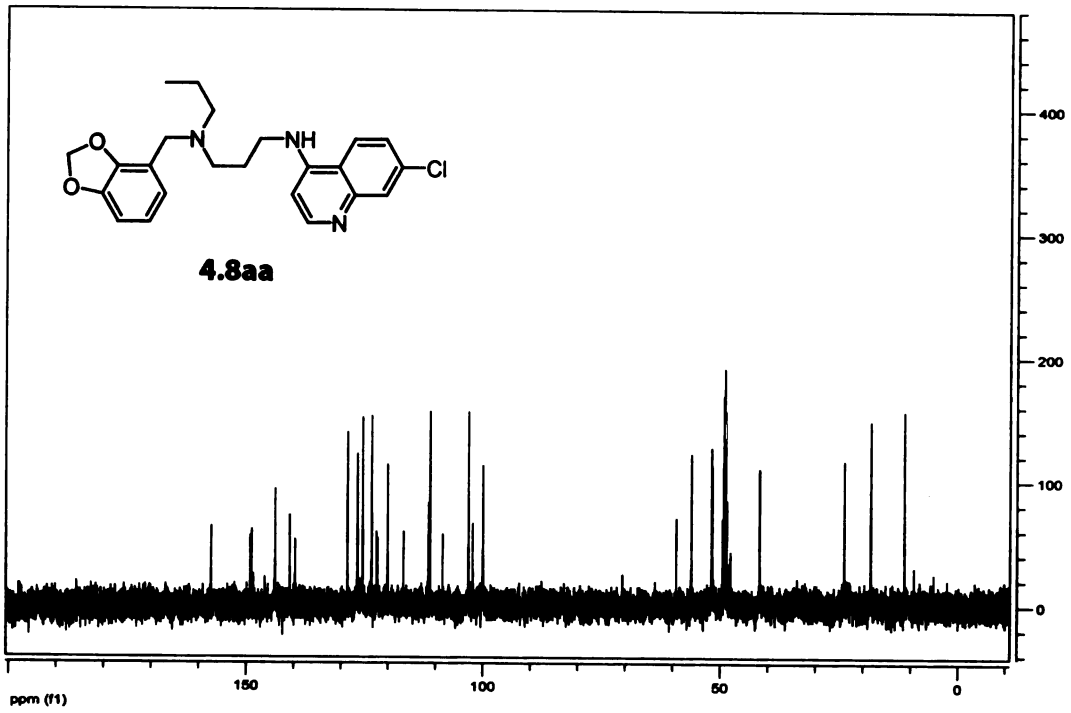

F
N
Cl
HN
N
O
4.8x
1.50
1.00
0.50
0.00
10.0
ppm (f1)
5.0
0.0

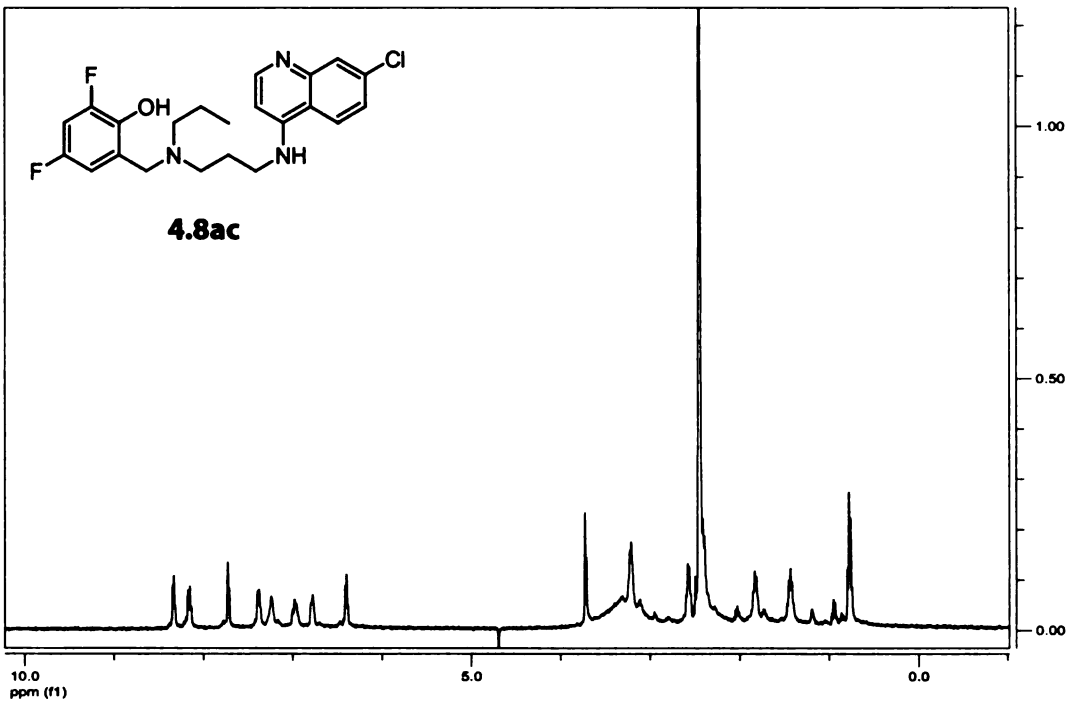

F
N
Cl
HN
N
O
4.8x
300
250
200
150
100
50
0
200
ppm (f1)
150
100
50
0


F
O
N
N
NH
Cl
4.8y
1.50
1.00
0.50
0.00
10.0
ppm (t1)
5.0
0.0

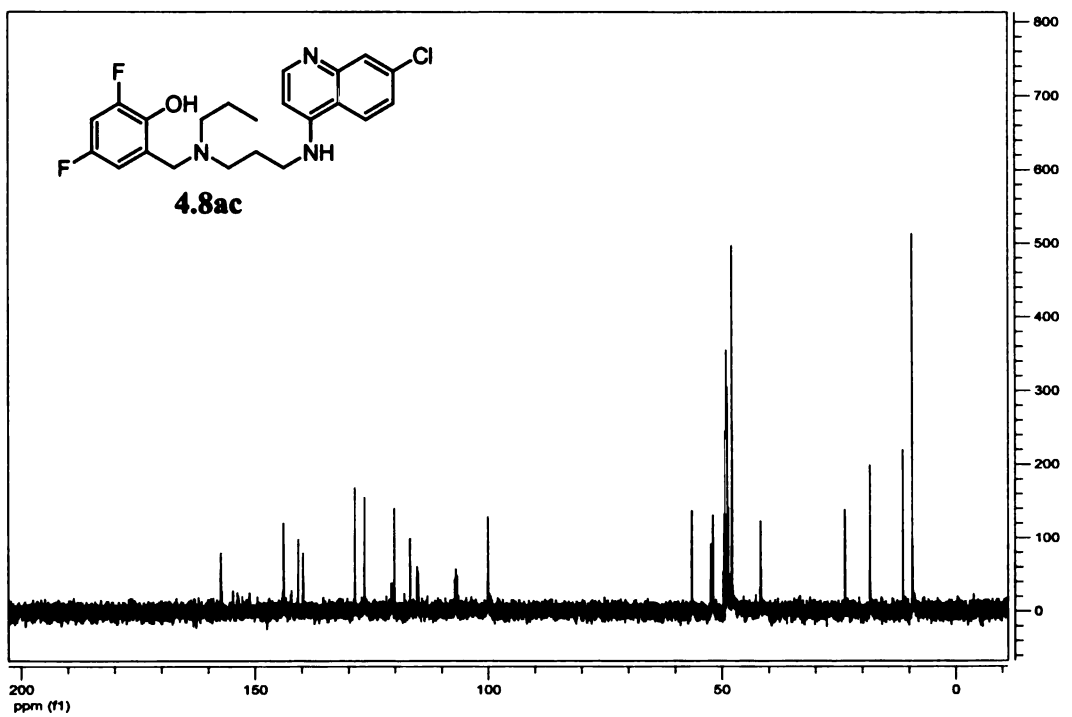

F
O
N
N
NH
Cl
4.8y
300
250
200
150
100
50
0
ppm (t1)
150
100
50
0


O
O
N
NH
Cl
N
4.8aa
4.0
3.0
2.0
1.0
0.0
10.0
ppm (f1)
5.0
0.0

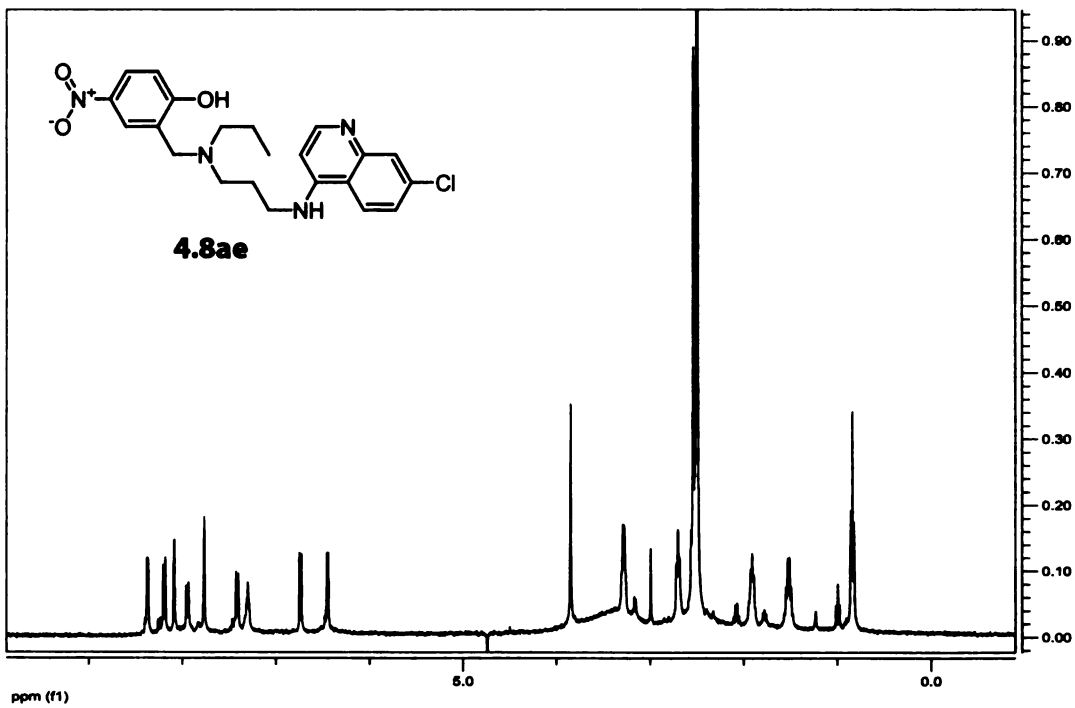

O
O
N
NH
Cl
N
4.8aa
400
300
200
100
0
ppm (f1)
150
100
50
0

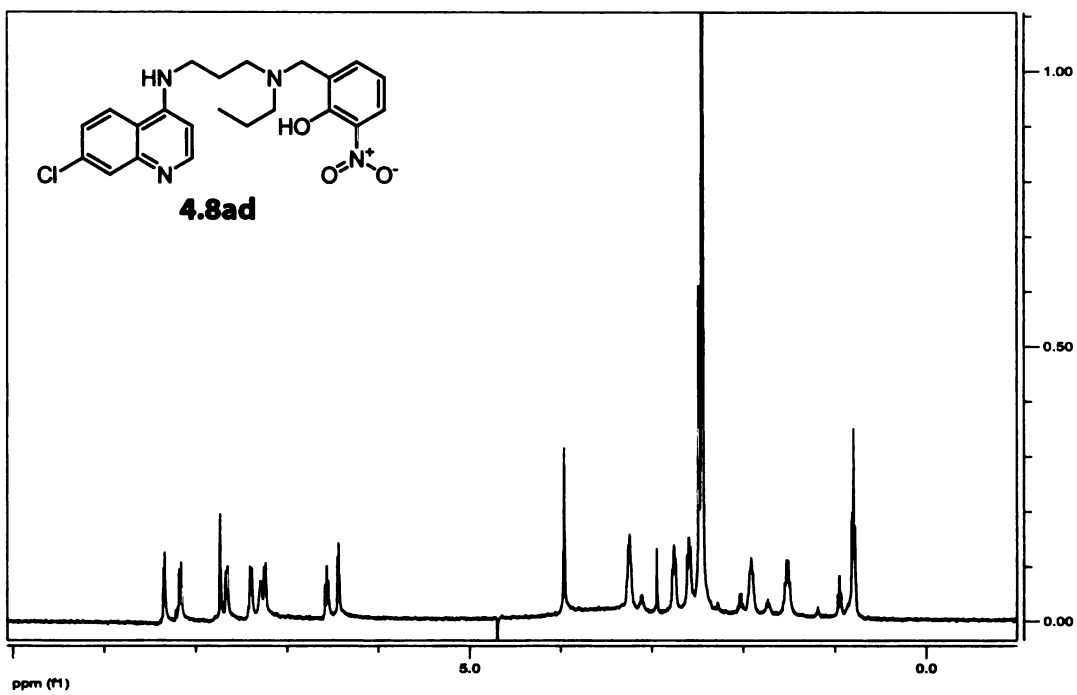

F
OH
N
Cl
F
N
NH
1.00
0.50
0.00
10.0
ppm (f1)
5.0
0.0

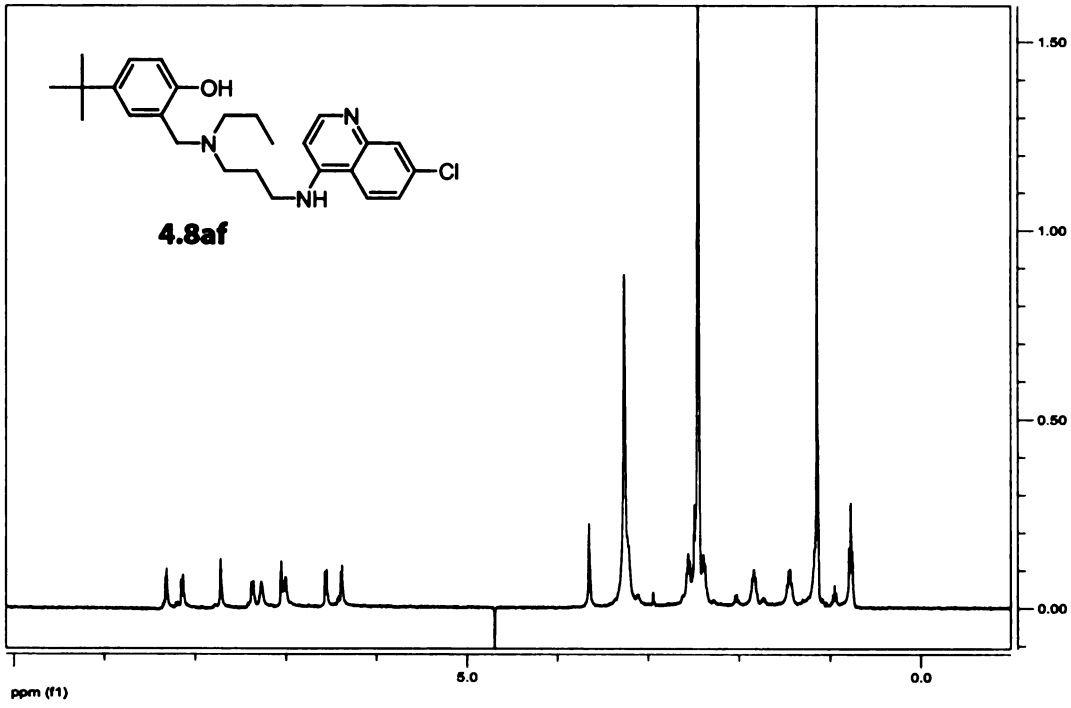
4.8ac

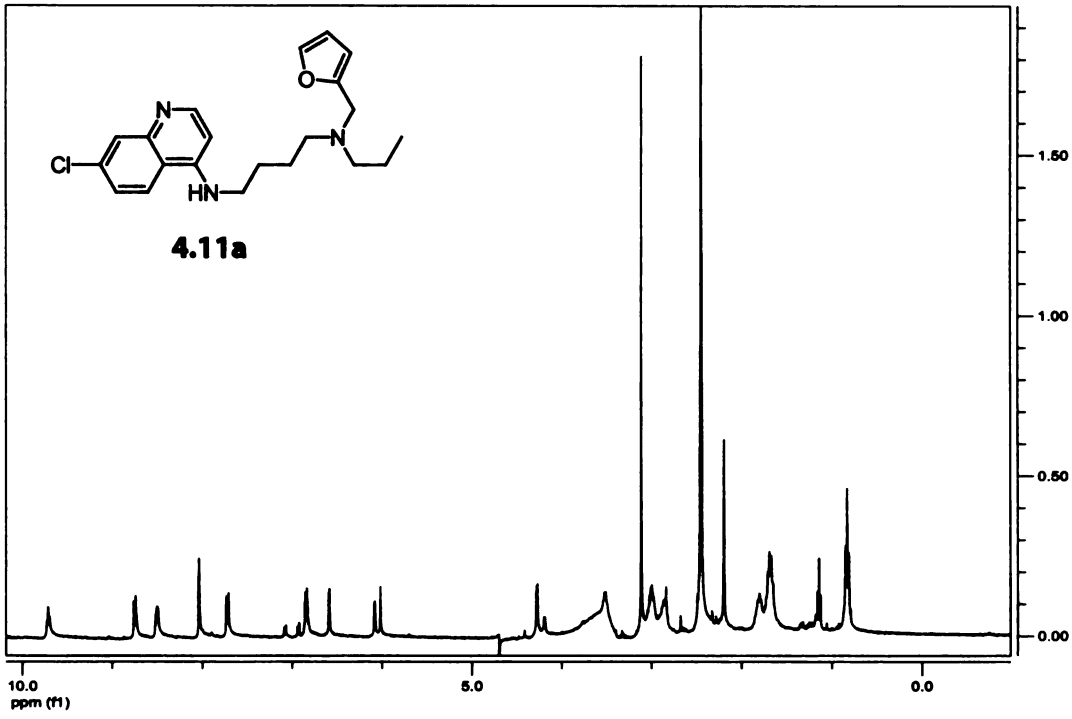

F
OH
N
Cl
F
N
NH
800
700
600
500
400
300
200
100
0
200
ppm (f1)
150
100
50
0

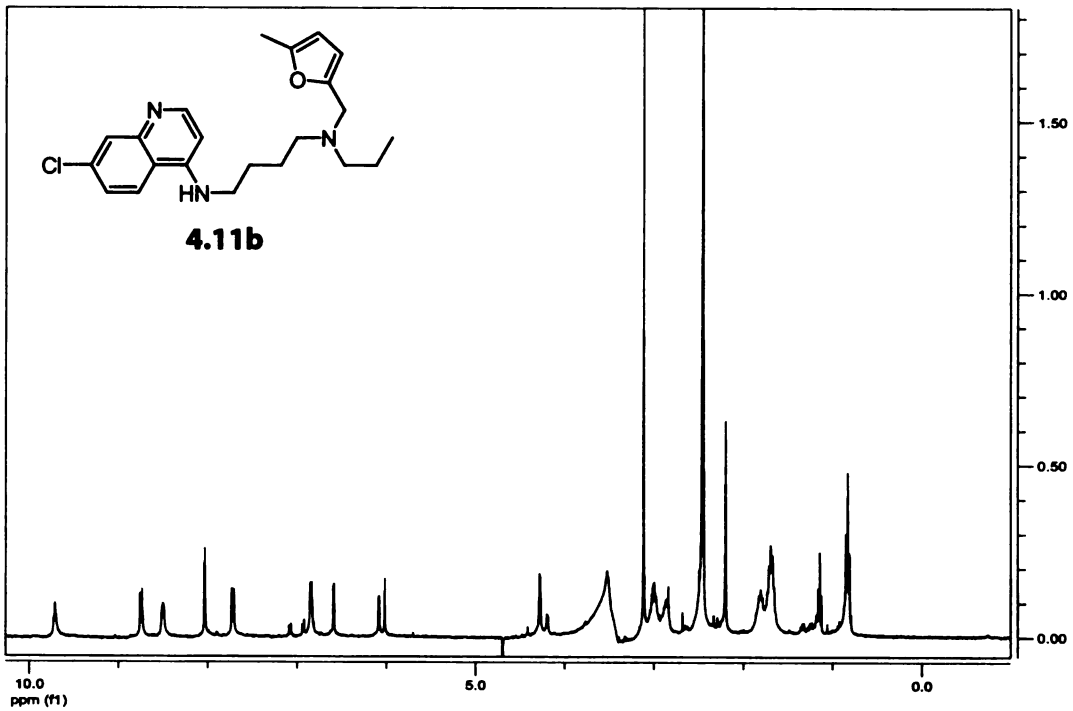
4.8ac

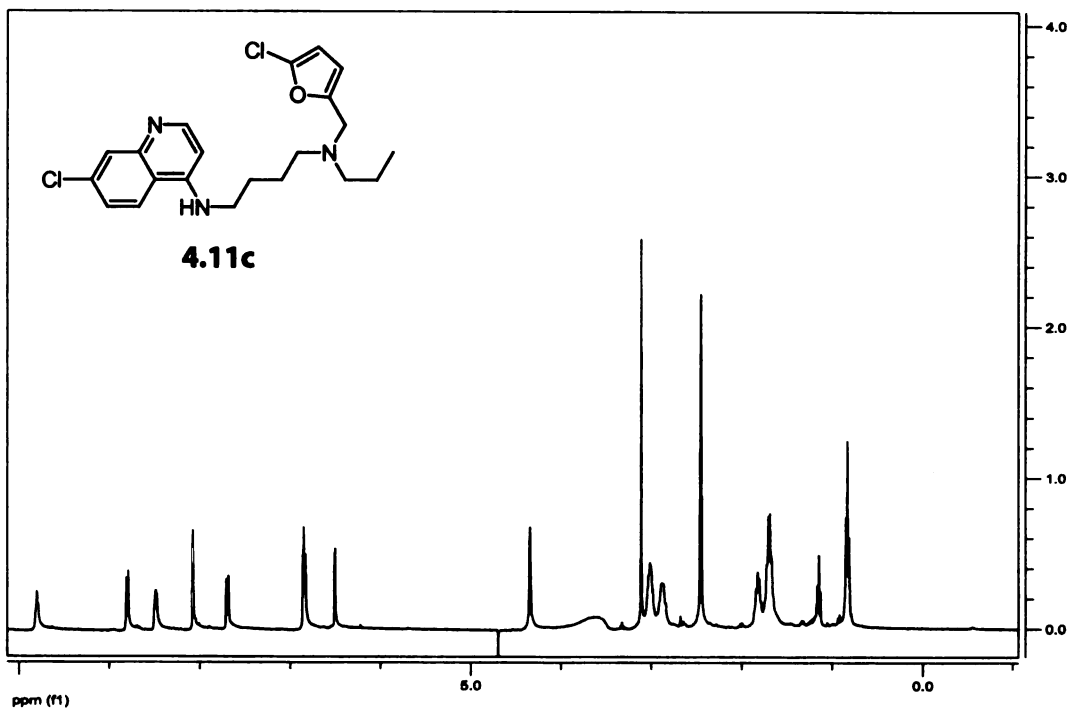

OH
N
Cl
NH
4.8ae
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
5.0
0.0
ppm (f1)

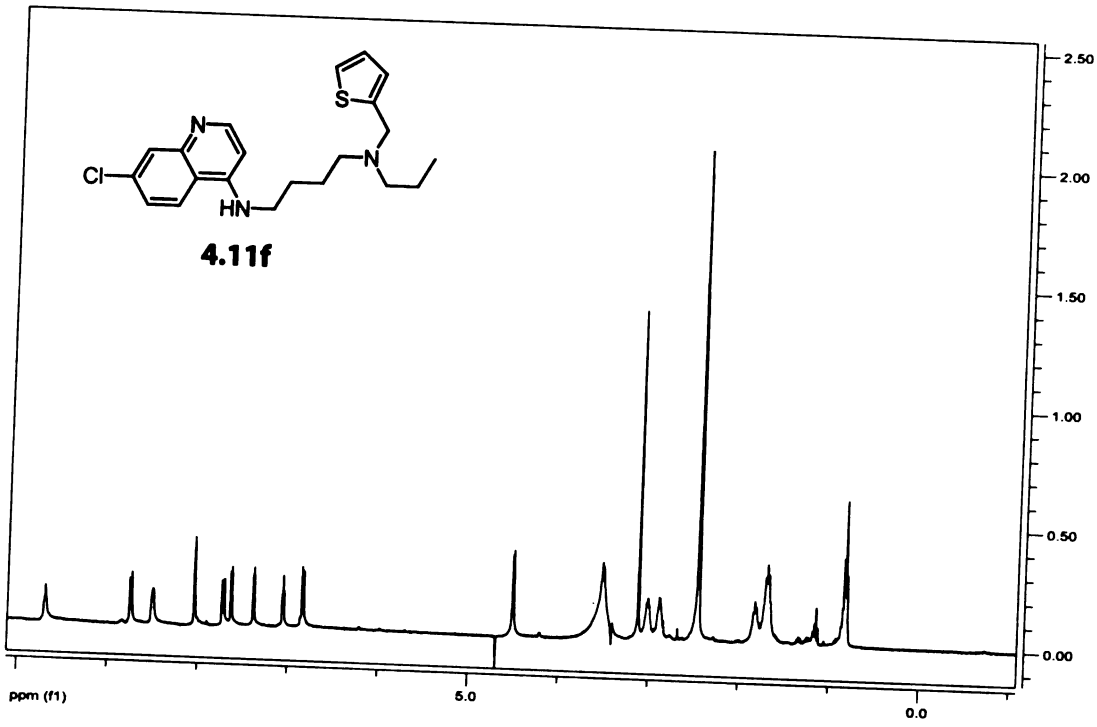

HN
N
HO
Cl
N
4.8ad
1.00
0.50
0.00
5.0
0.0
ppm (f1)

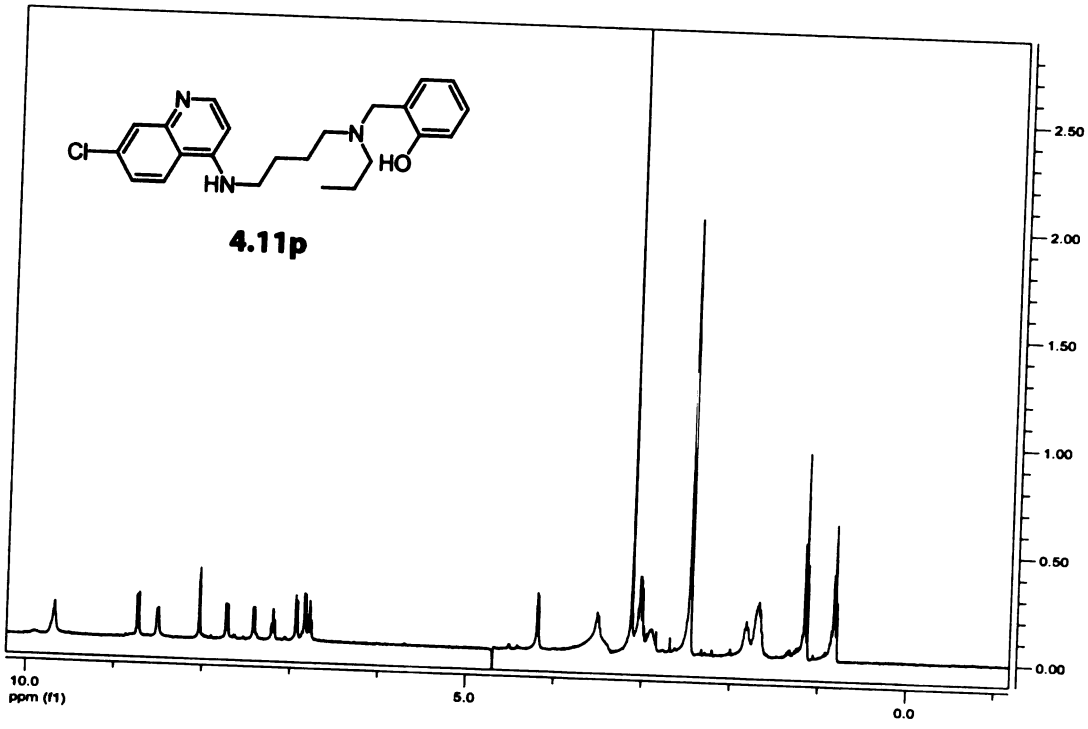

OH
N
N
NH
Cl
4.8af
1.50
1.00
0.50
0.00
5.0
0.0
ppm (f1)

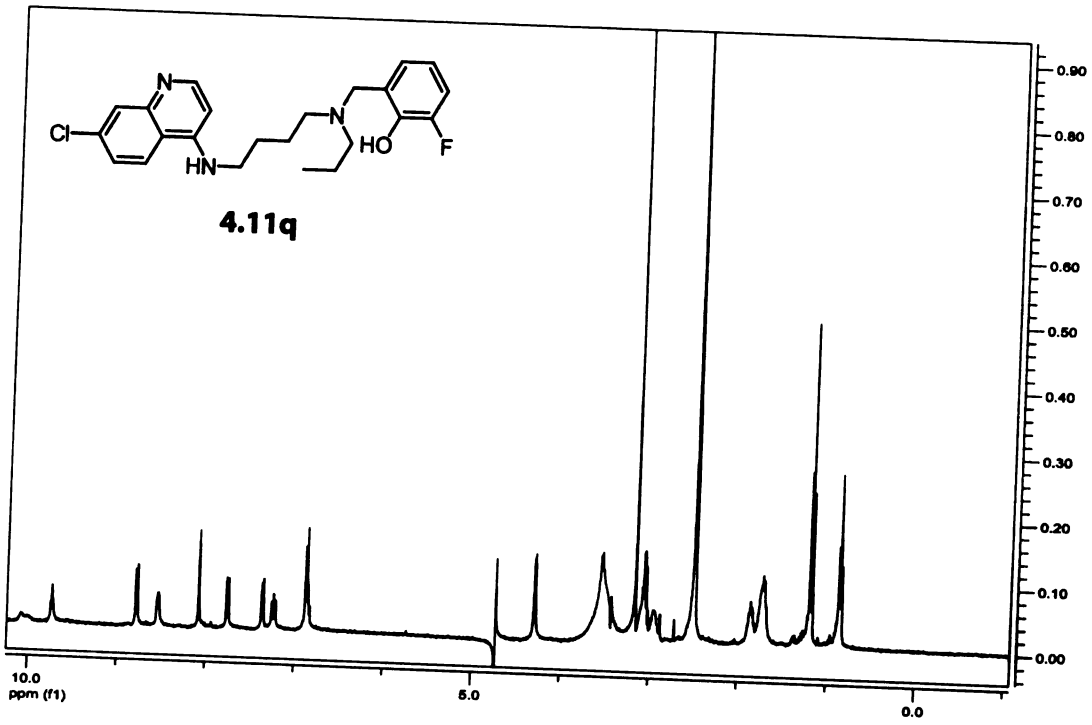

O
N
N
Cl
HN
4.11a
1.50
1.00
0.50
0.00
10.0
5.0
0.0
ppm (f1)


Cl
N
HN
O
N
4.11b
1.50
1.00
0.50
0.00
10.0
ppm (f1)
5.0
0.0

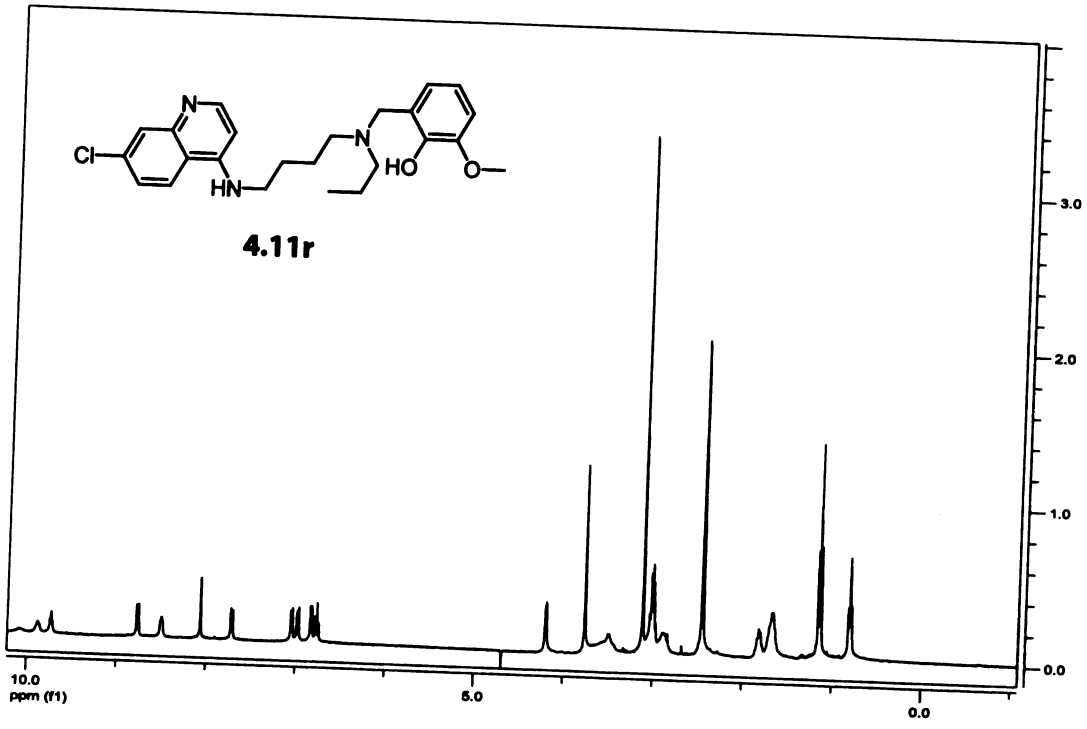

Cl
Cl
N
HN
O
N
4.11c
4.0
3.0
2.0
1.0
0.0
ppm (f1)
5.0
0.0


Cl
N
HN
S
N
4.11f
2.50
2.00
1.50
1.00
0.50
0.00
ppm (f1)
5.0
0.0

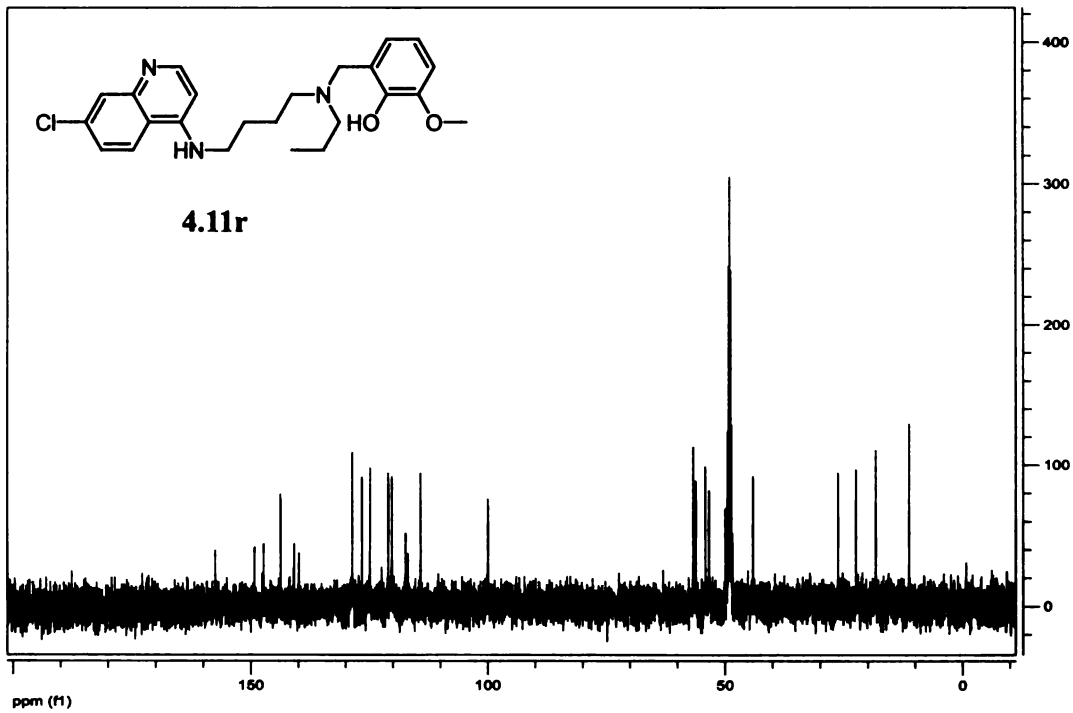

Cl
N
HN
N
HO
4.11p
2.50
2.00
1.50
1.00
0.50
0.00
10.0
ppm (f1)
5.0
0.0


Cl
N
HN
N
HO
F
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
10.0
ppm (f1)
5.0
0.0

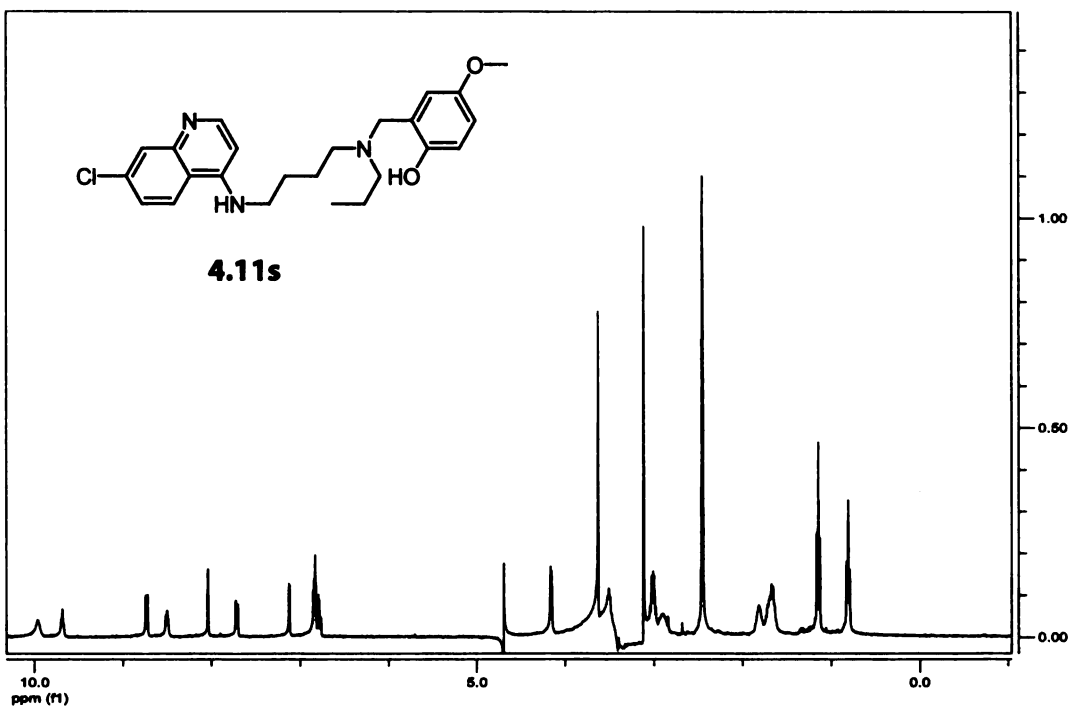
4.11q


Cl
N
HN
N
HO
O
3.0
2.0
1.0
0.0
10.0
ppm (f1)
5.0
0.0

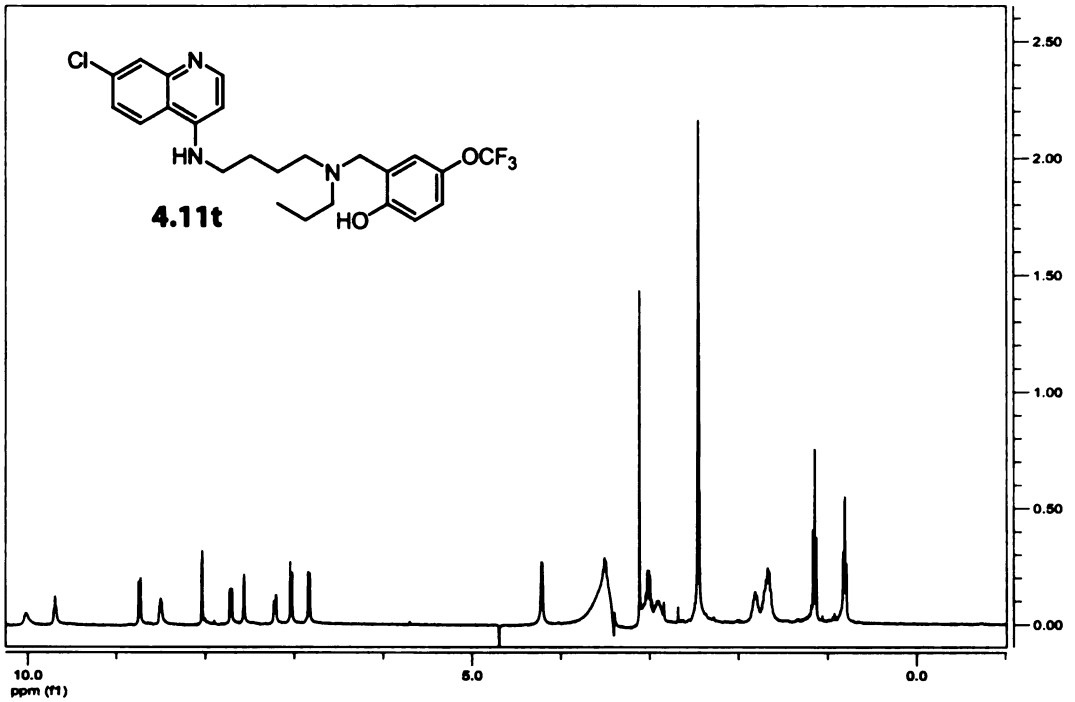
4.11r

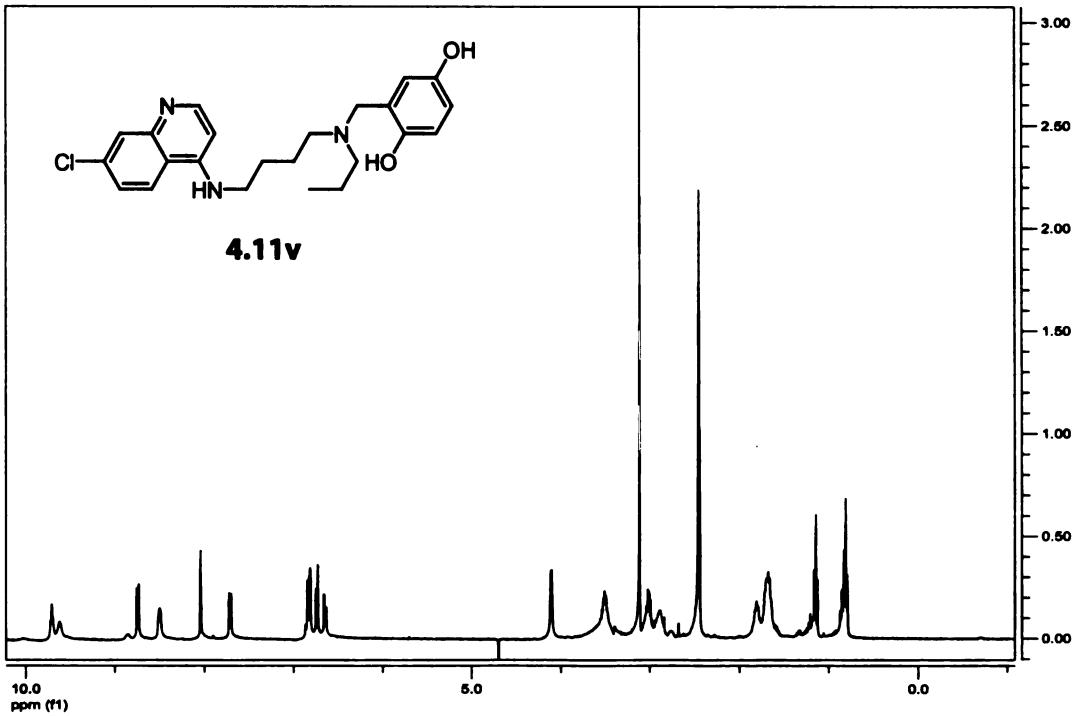

Cl
N
HN
N
HO
O
400
300
200
100
0
150
100
50
0
ppm (f1)

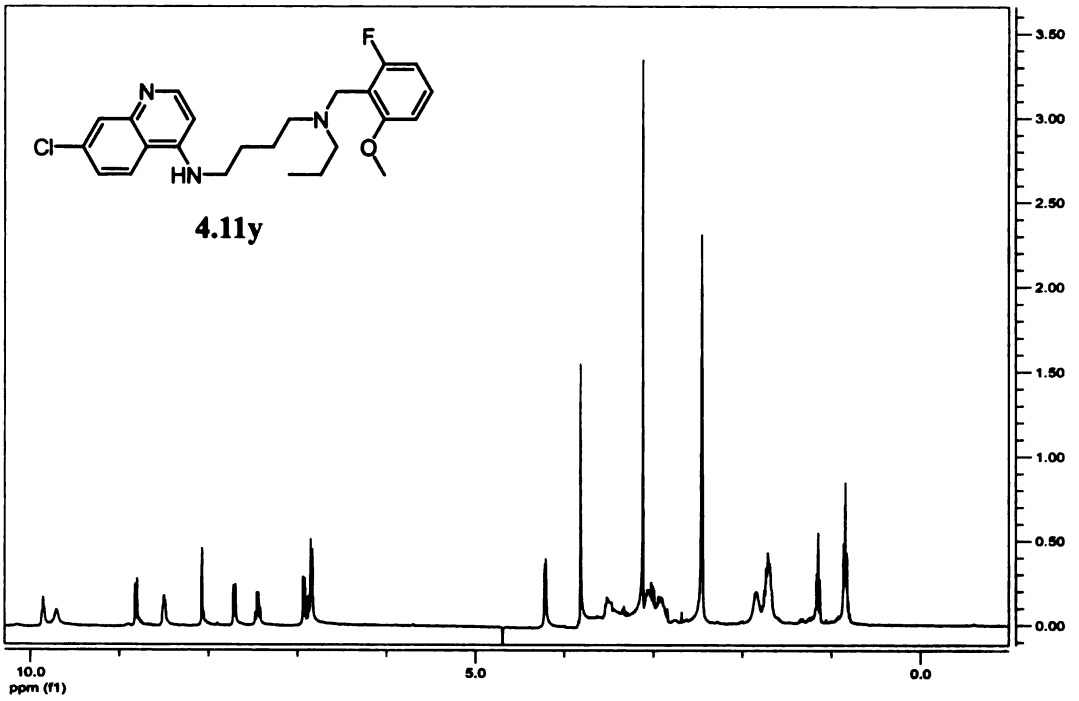
4.11r


Cl
N
HN
N
HO
O
1.00
0.50
0.00
10.0
5.0
0.0
ppm (f1)

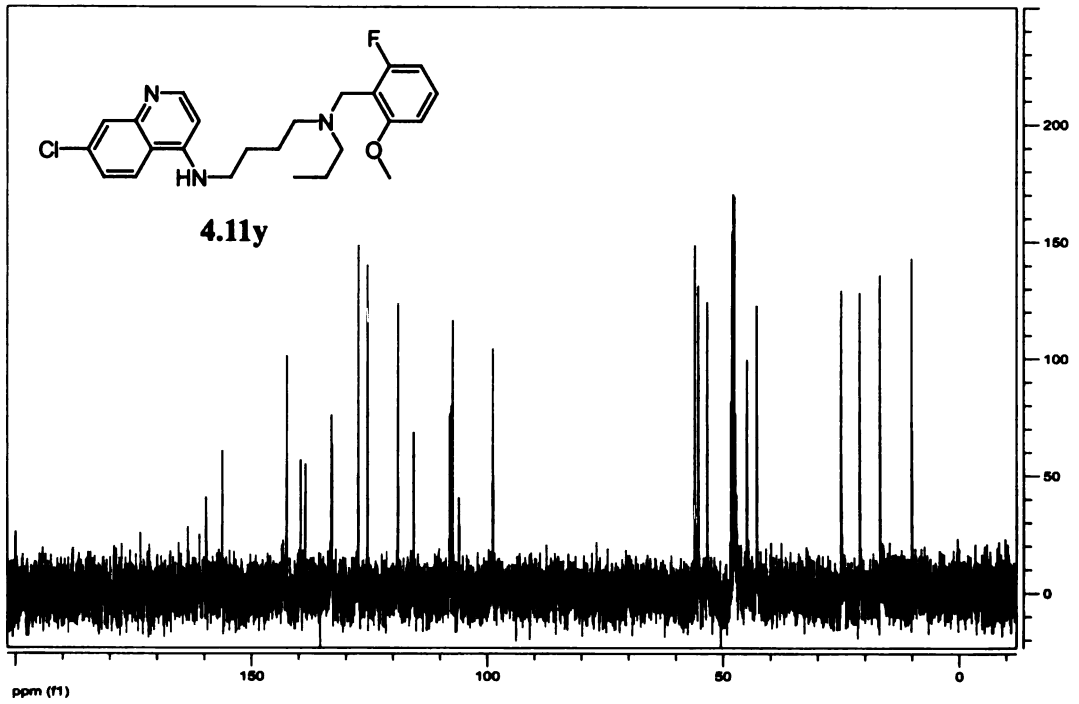
4.11s


Cl
N
HN
N
OCF3
HO
4.11t
2.50
2.00
1.50
1.00
0.50
0.00
10.0
ppm (f1)
5.0
0.0

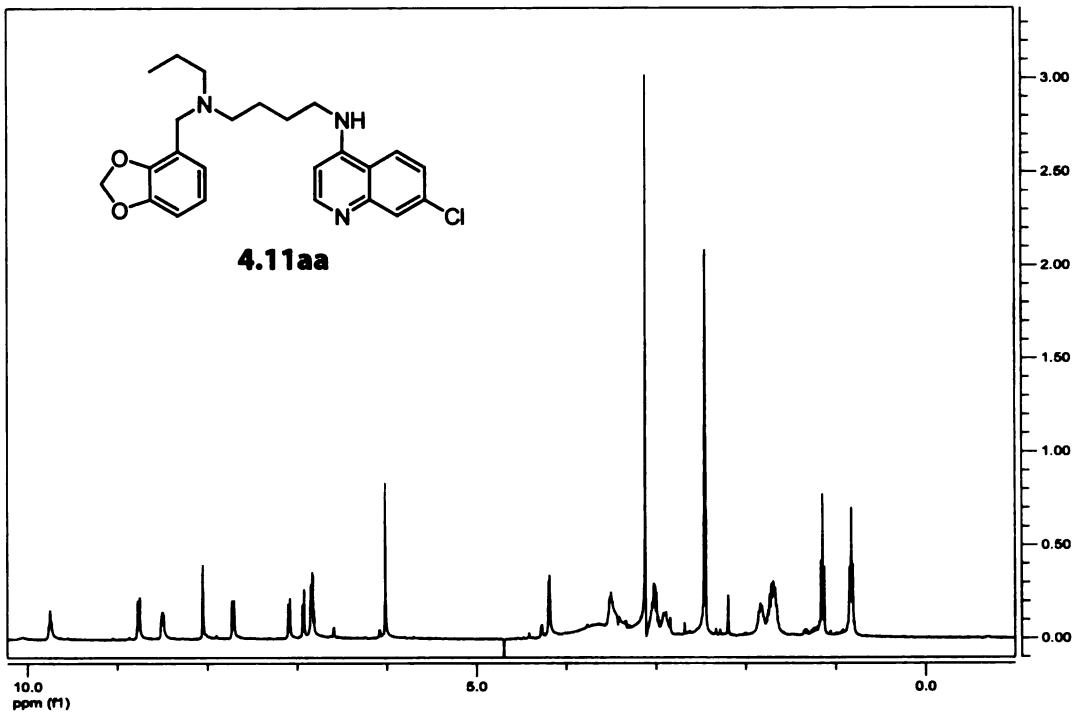

OH
N
Cl
HN
N
HO
4.11v
3.00
2.50
2.00
1.50
1.00
0.50
0.00
10.0
ppm (f1)
5.0
0.0

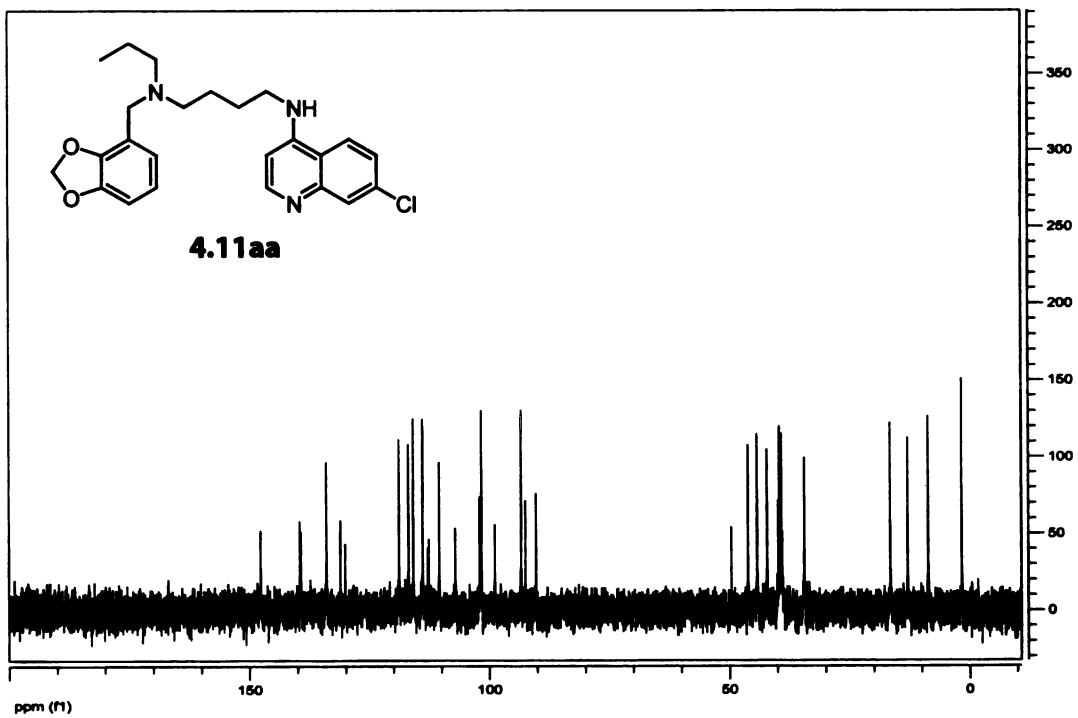

F
N
Cl
HN
O
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
10.0
ppm (f1)
5.0
0.0

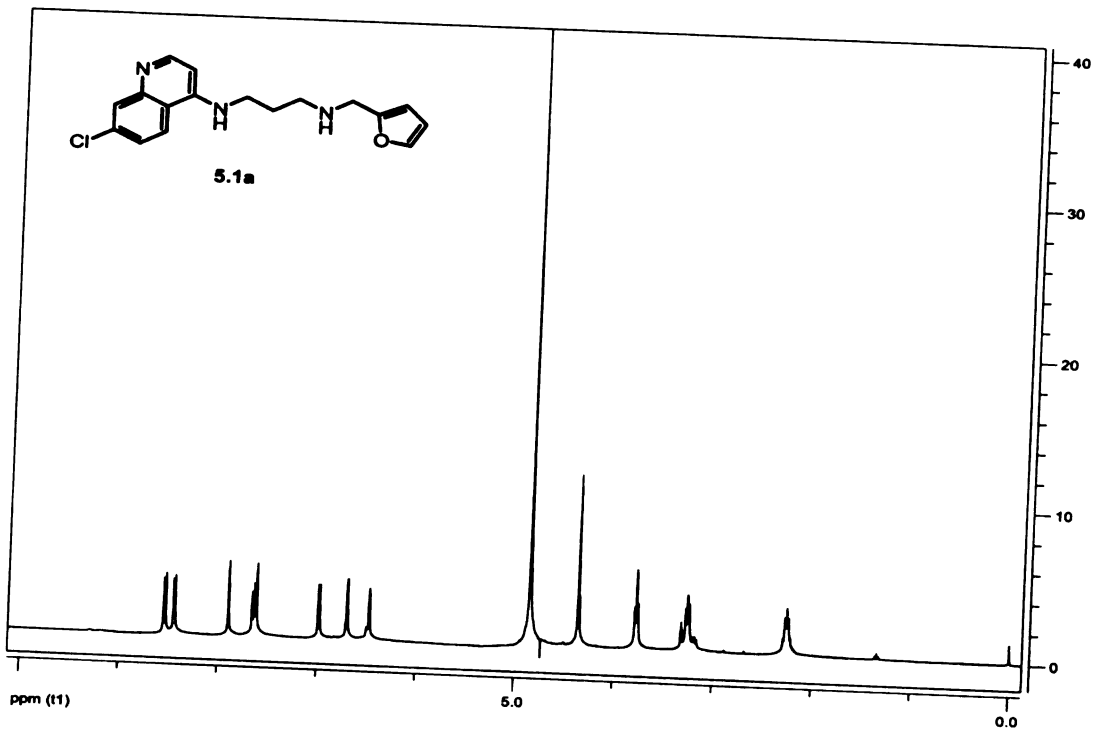
4.11y

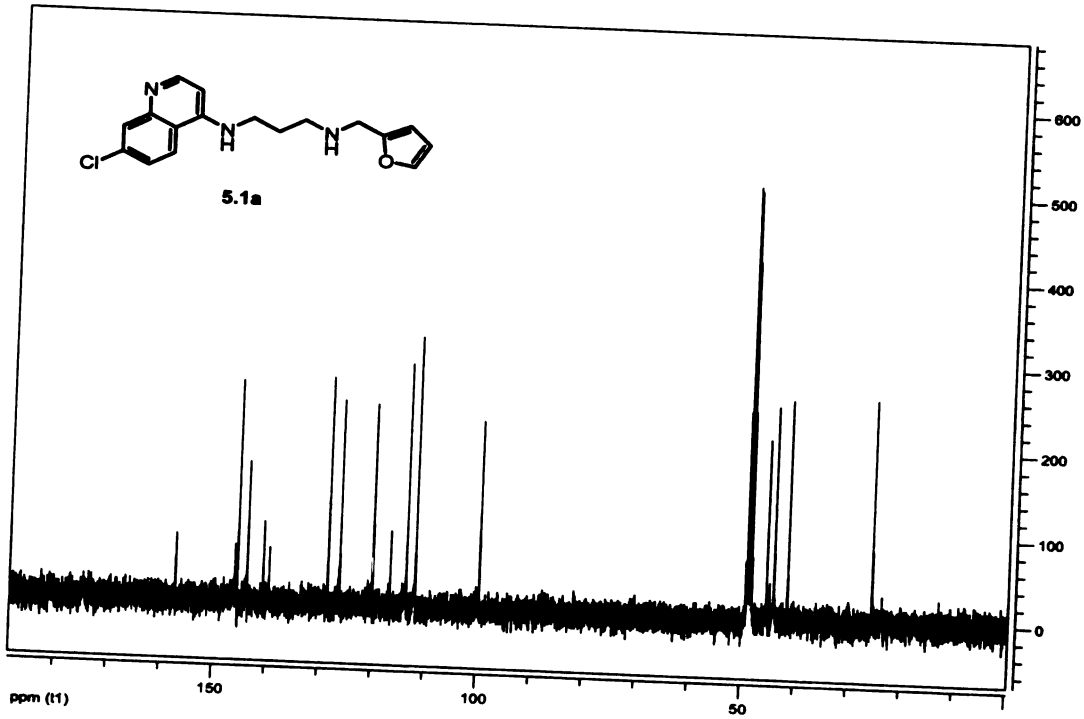

F
N
Cl
HN
O
200
150
100
50
0
150
100
50
0
ppm (f1)

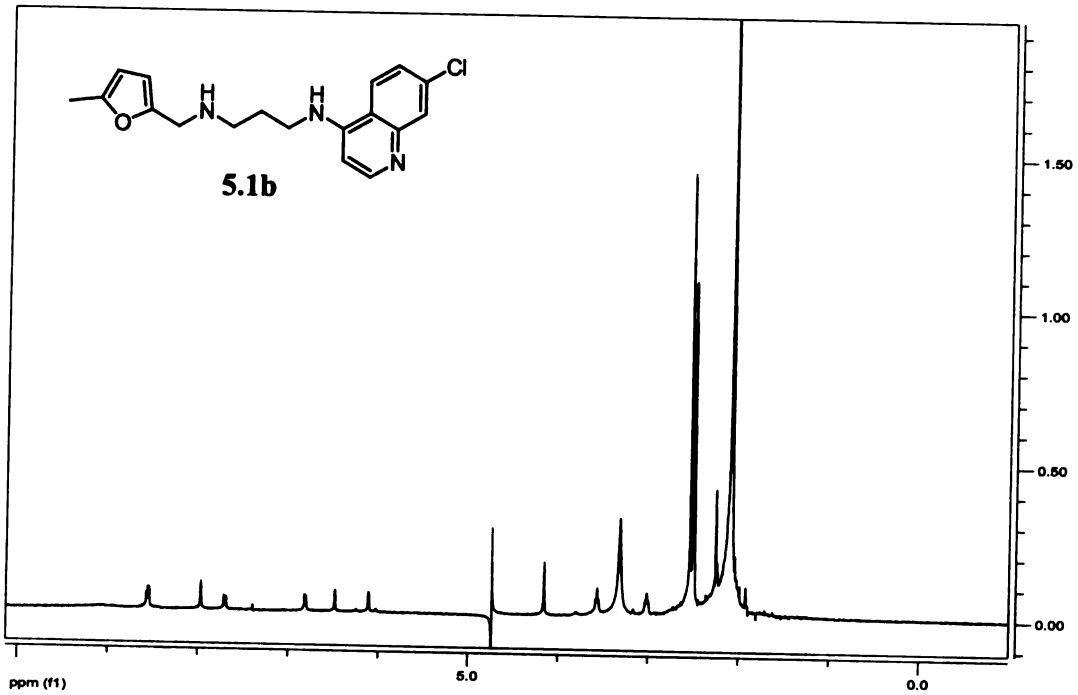
4.11y

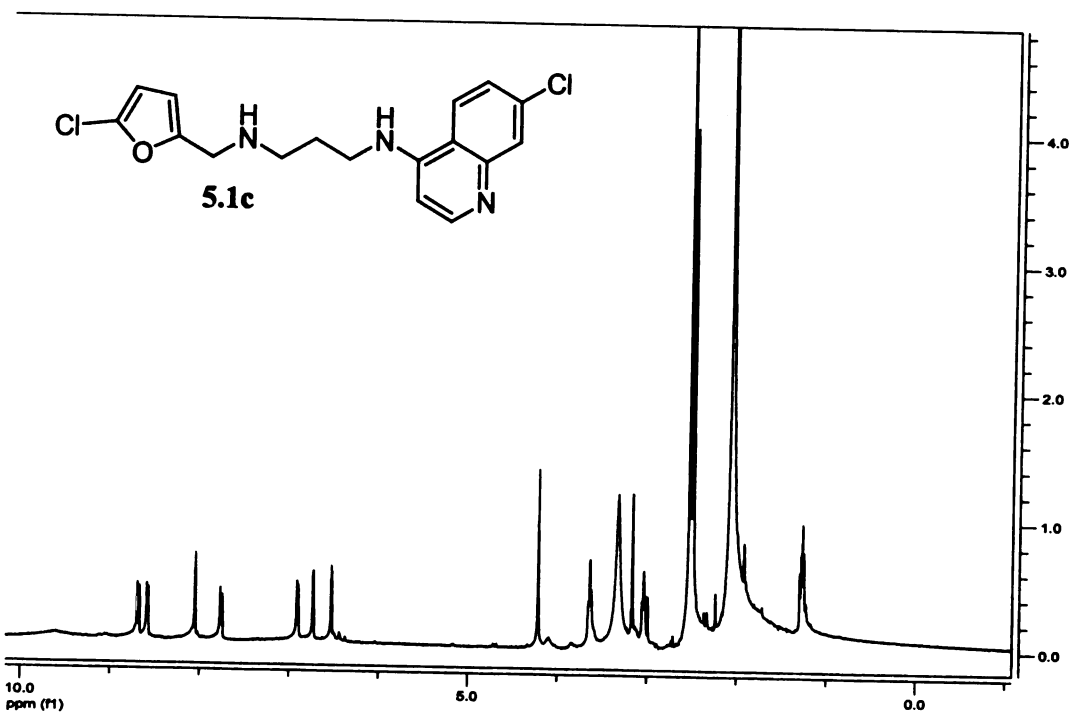

N
NH
O
O
N
Cl
4.11aa
3.00
2.50
2.00
1.50
1.00
0.50
0.00
10.0
5.0
0.0
ppm (f1)

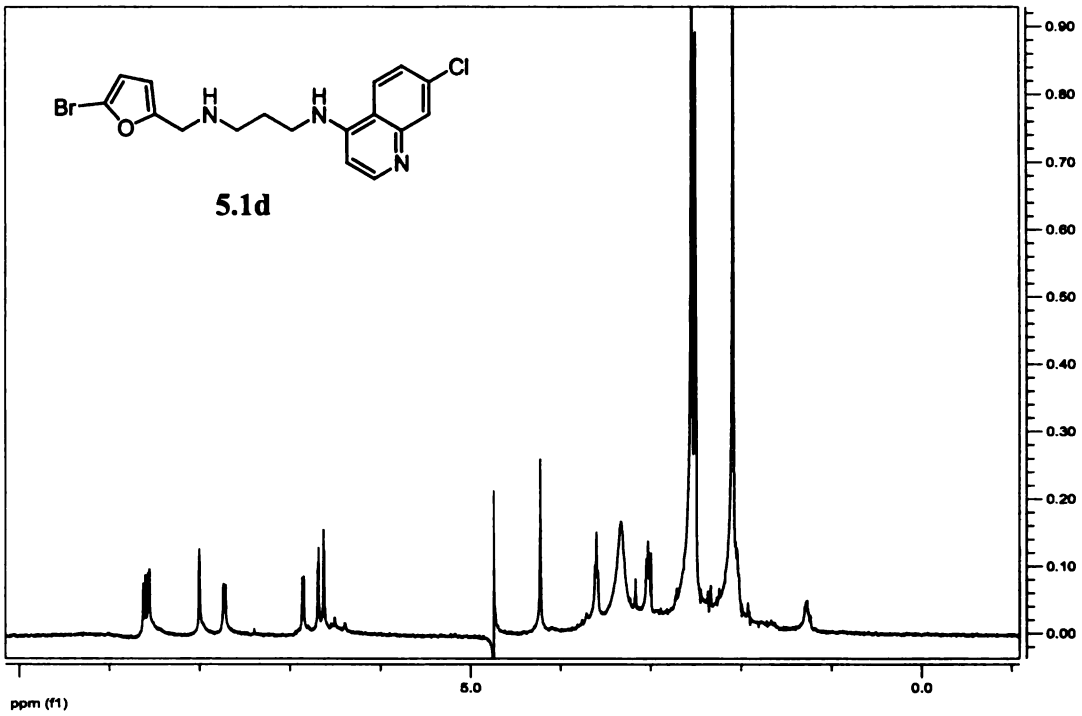

N
NH
O
O
N
Cl
4.11aa
350
300
250
200
150
100
50
0
150
100
50
0
ppm (f1)

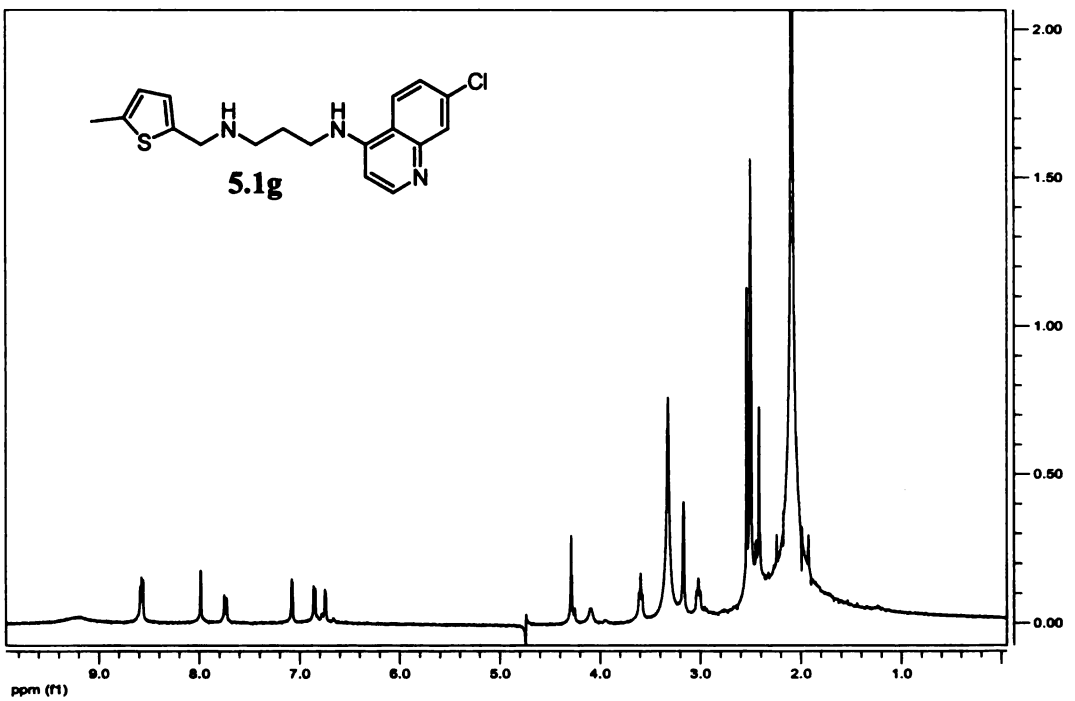

Cl
N
H
N
H
O
5.1a
40
30
20
10
0
ppm (t1)
5.0
0.0

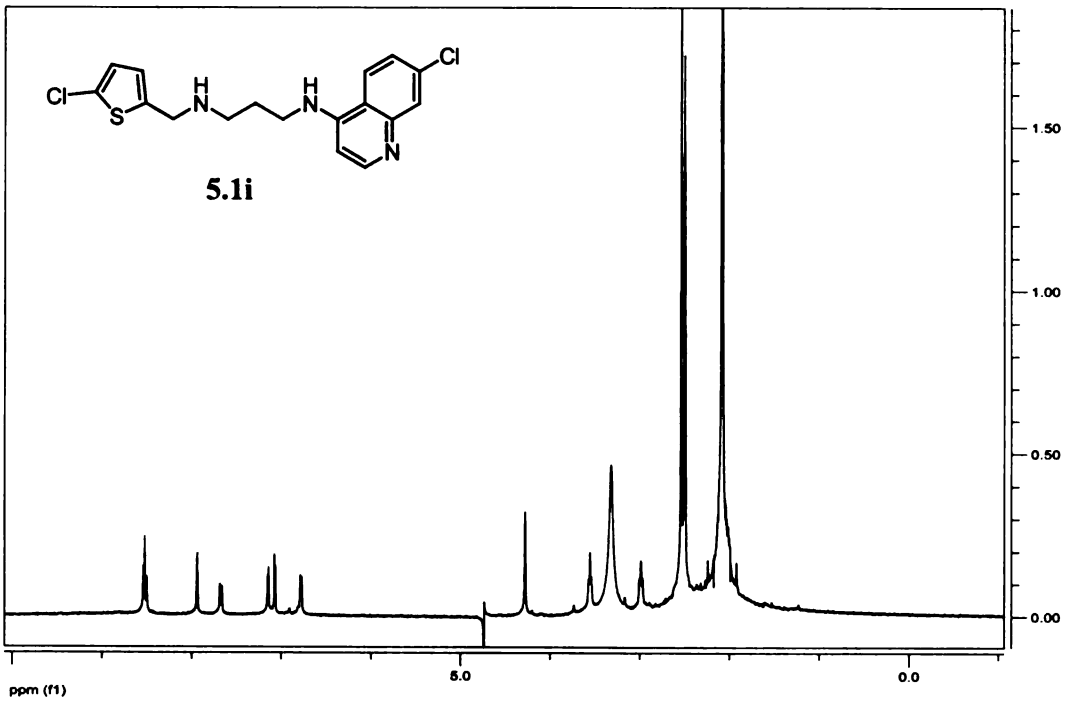

Cl
N
H
N
H
O
5.1a
600
500
400
300
200
100
0
ppm (t1)
150
100
50


Cl
H
N
H
N
O
N
5.1b
1.50
1.00
0.50
0.00
ppm (f1)
5.0
0.0

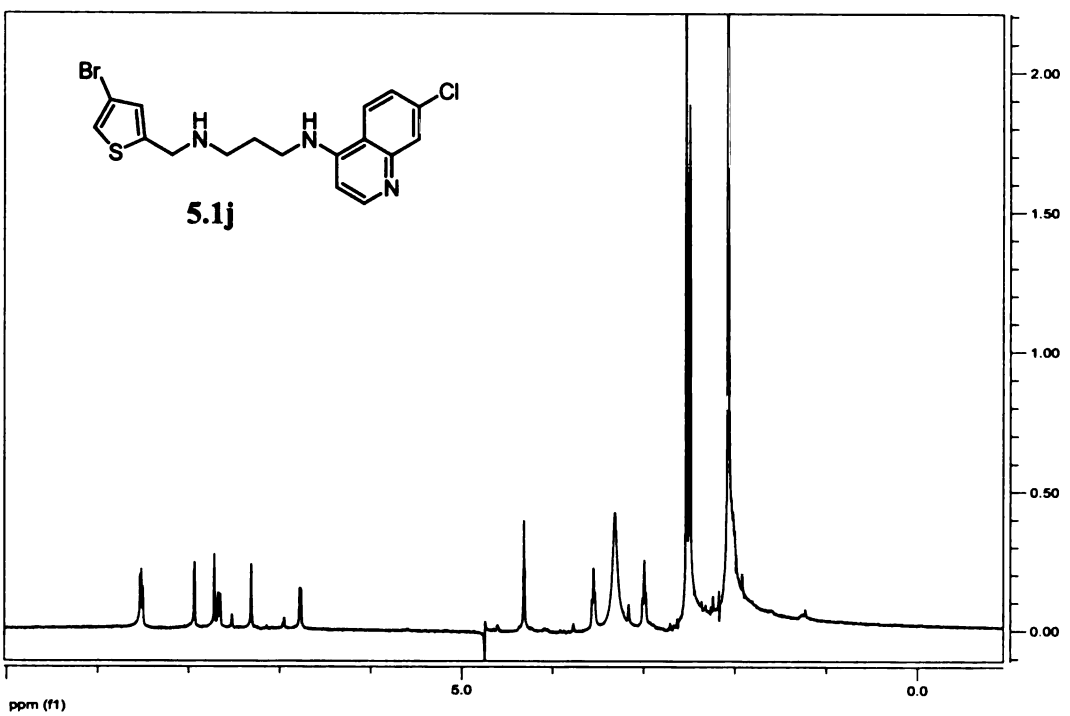

Cl
Cl
H
N
H
N
O
N
5.1c
4.0
3.0
2.0
1.0
0.0
10.0
ppm (f1)
5.0
0.0


Br
O
H
N
H
N
Cl
N
5.1d
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
5.0
0.0
ppm (f1)

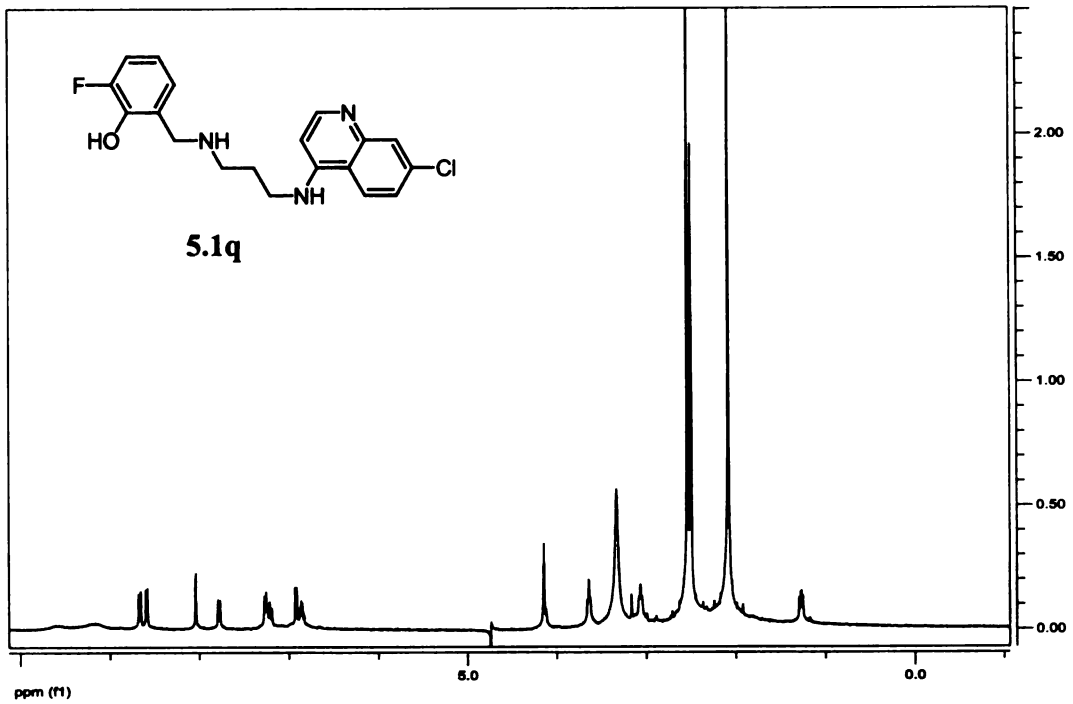

S
H
N
H
N
Cl
N
5.1g
2.00
1.50
1.00
0.50
0.00
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

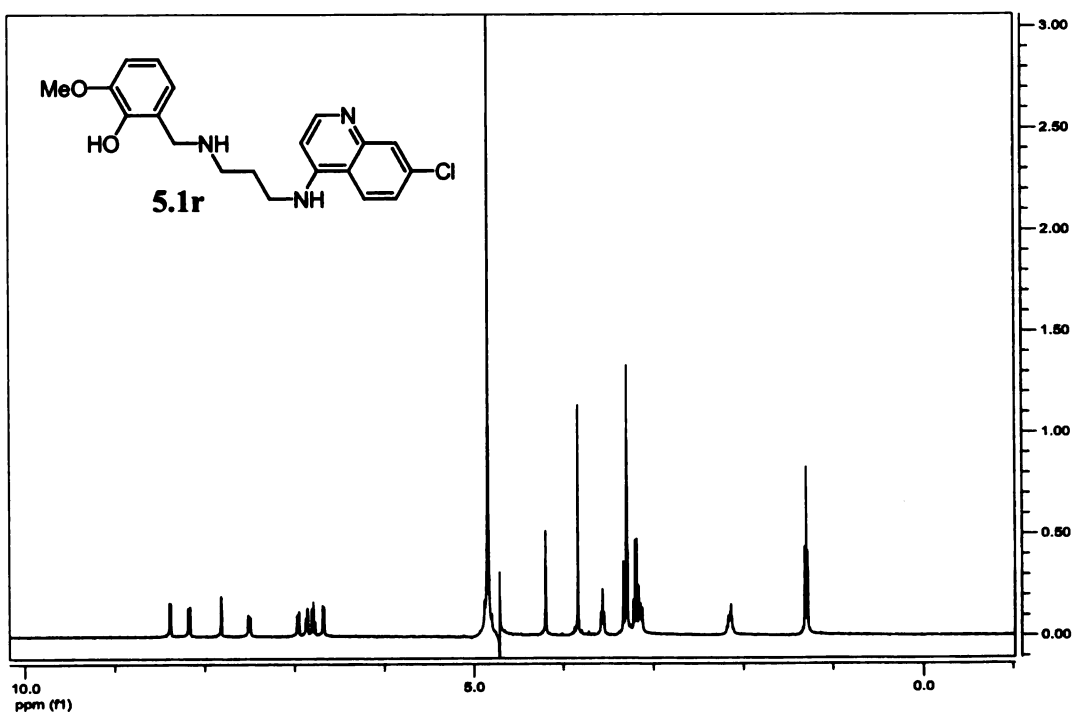

Cl
S
H
N
H
N
Cl
N
1.50
1.00
0.50
0.00
5.0
0.0
ppm (f1)

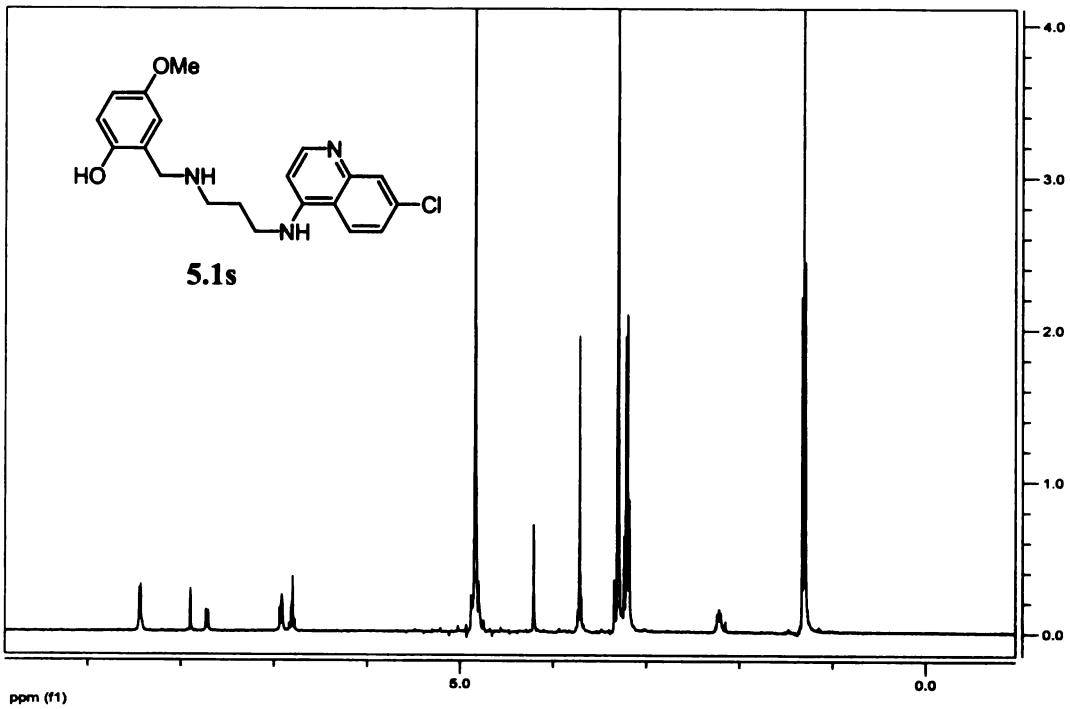
5.1i

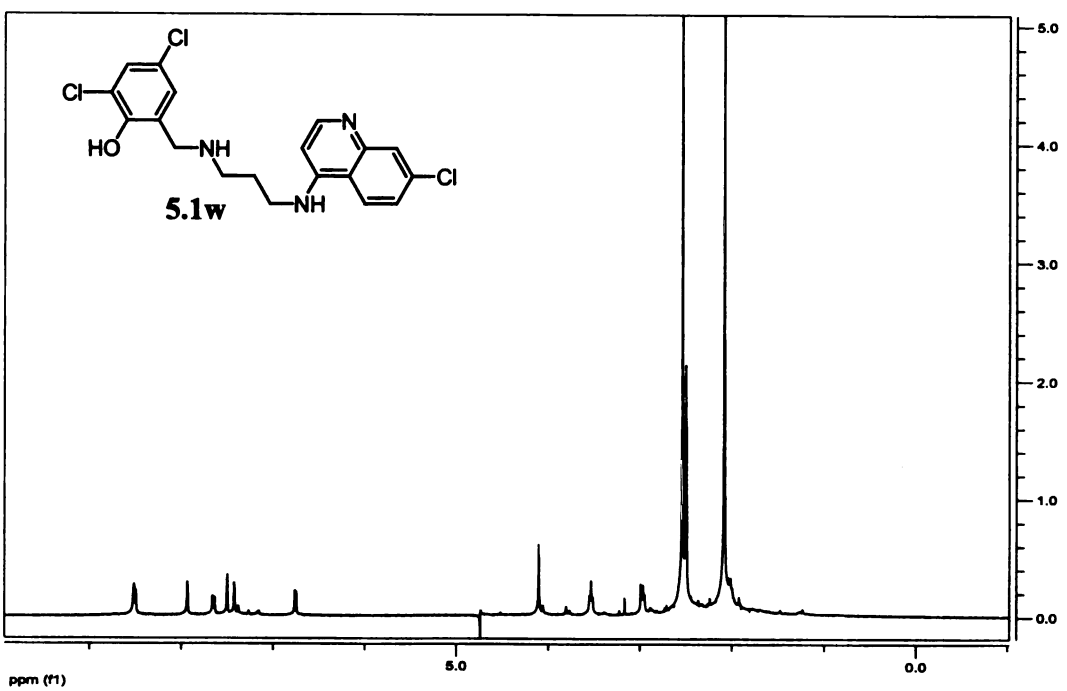

Br
S
H
N
H
N
Cl
N
2.00
1.50
1.00
0.50
0.00
5.0
0.0
ppm (f1)

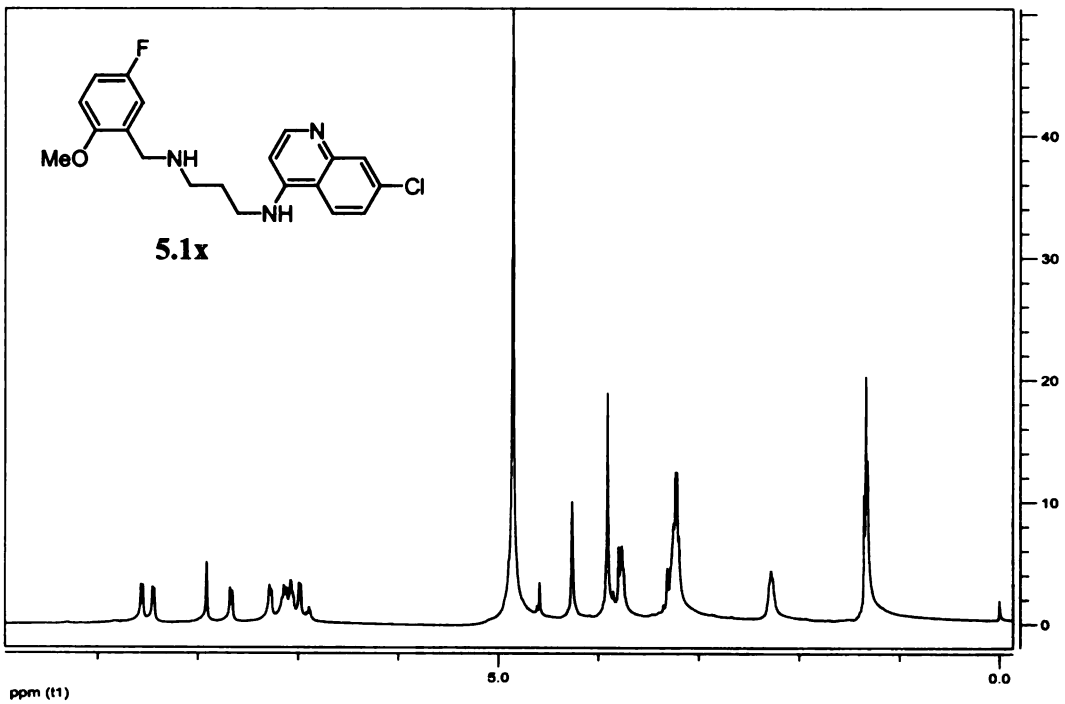
5.1j

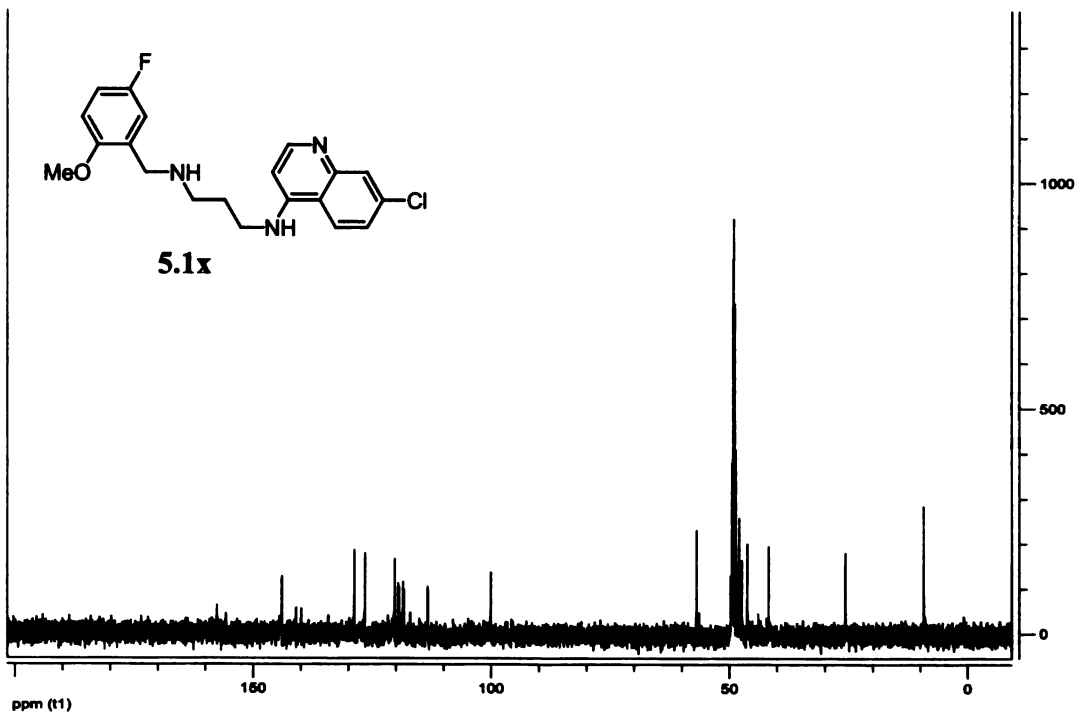

F
HO
NH
N
NH
Cl
5.1q
2.00
1.50
1.00
0.50
0.00
5.0
0.0
ppm (f1)

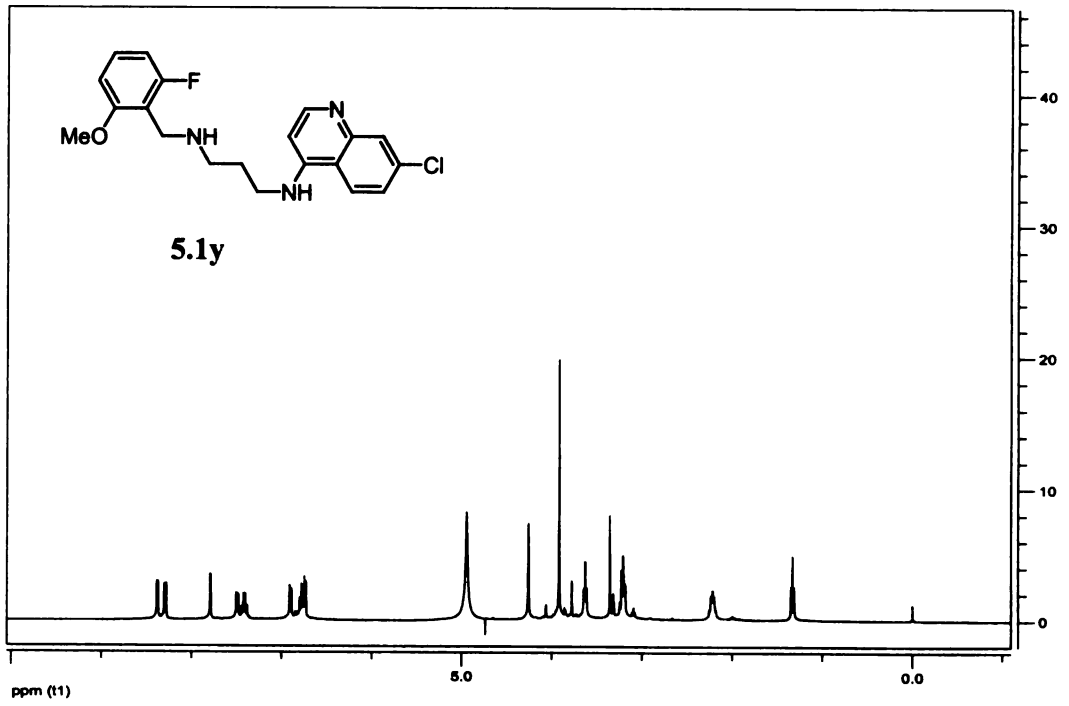

MeO
HO
NH
N
NH
Cl
5.1r
3.00
2.50
2.00
1.50
1.00
0.50
0.00
10.0
5.0
0.0
ppm (f1)

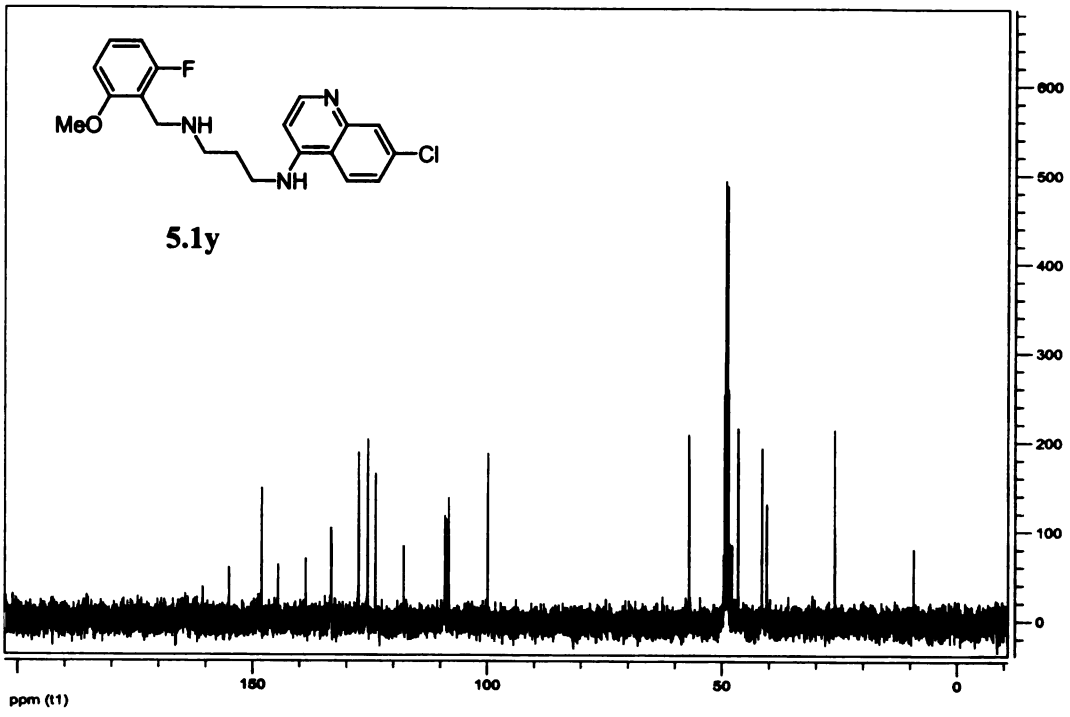

OMe
HO
NH
N
NH
Cl
5.1s
4.0
3.0
2.0
1.0
0.0
5.0
0.0
ppm (f1)

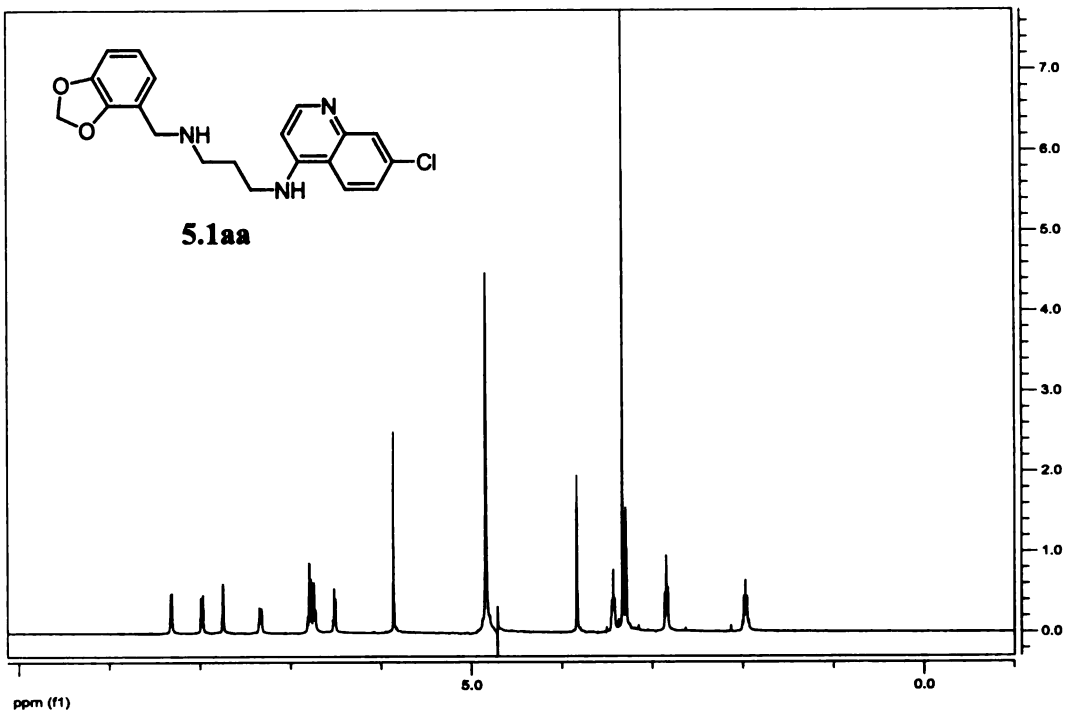

Cl
Cl
HO
NH
N
NH
Cl
5.1w
5.0
4.0
3.0
2.0
1.0
0.0
5.0
0.0
ppm (f1)


F
MeO
NH
N
Cl
NH
5.1x
40
30
20
10
0
5.0
0.0
ppm (t1)

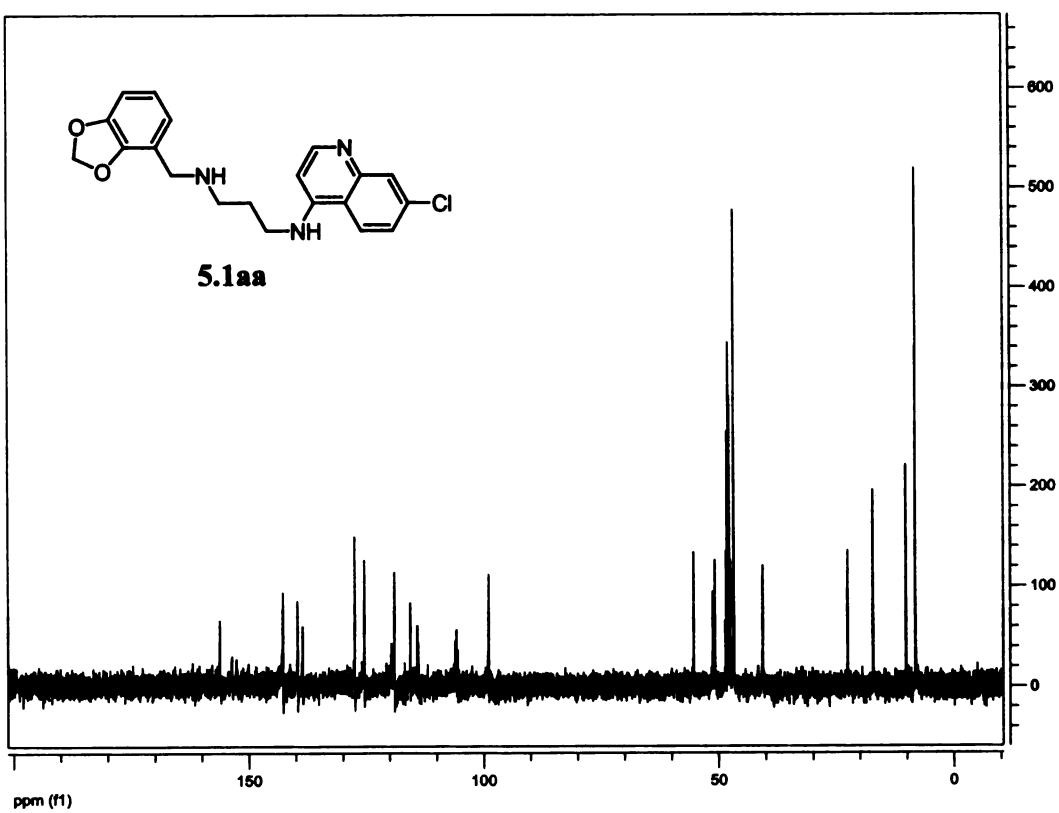

F
MeO
NH
N
Cl
NH
5.1x
1000
500
0
150
100
50
0
ppm (t1)


F
MeO
NH
N
Cl
NH
5.1y
40
30
20
10
0
5.0
0.0
ppm (t1)

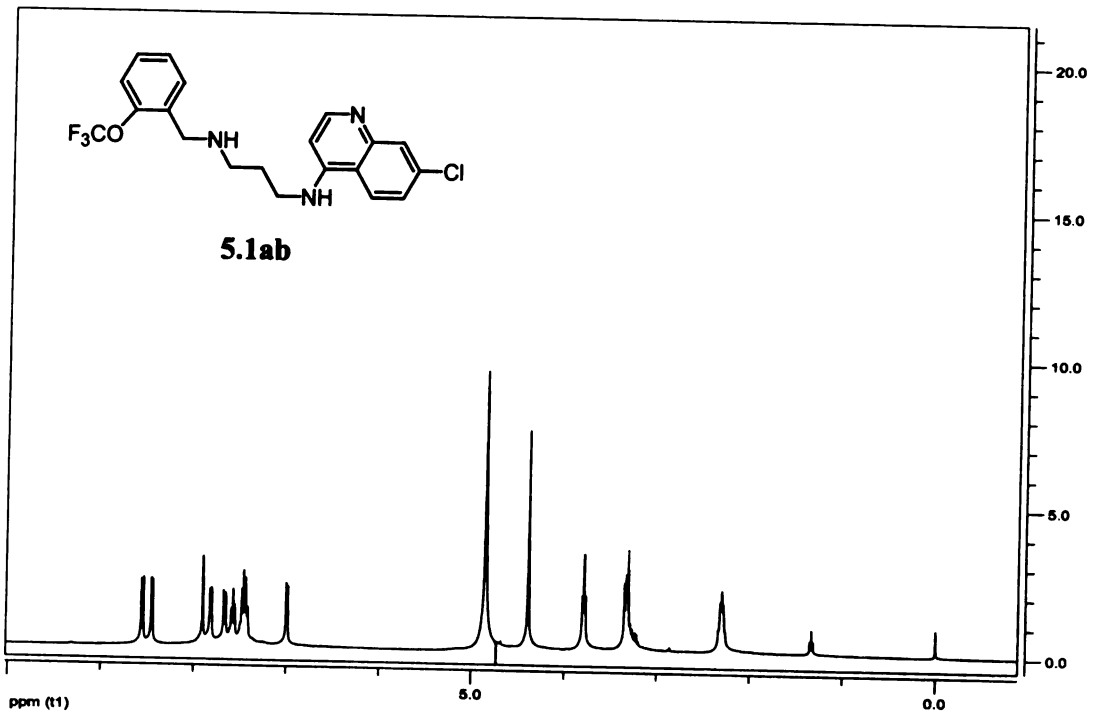

F
MeO
NH
N
Cl
NH
5.1y
600
500
400
300
200
100
0
150
100
50
0
ppm (t1)

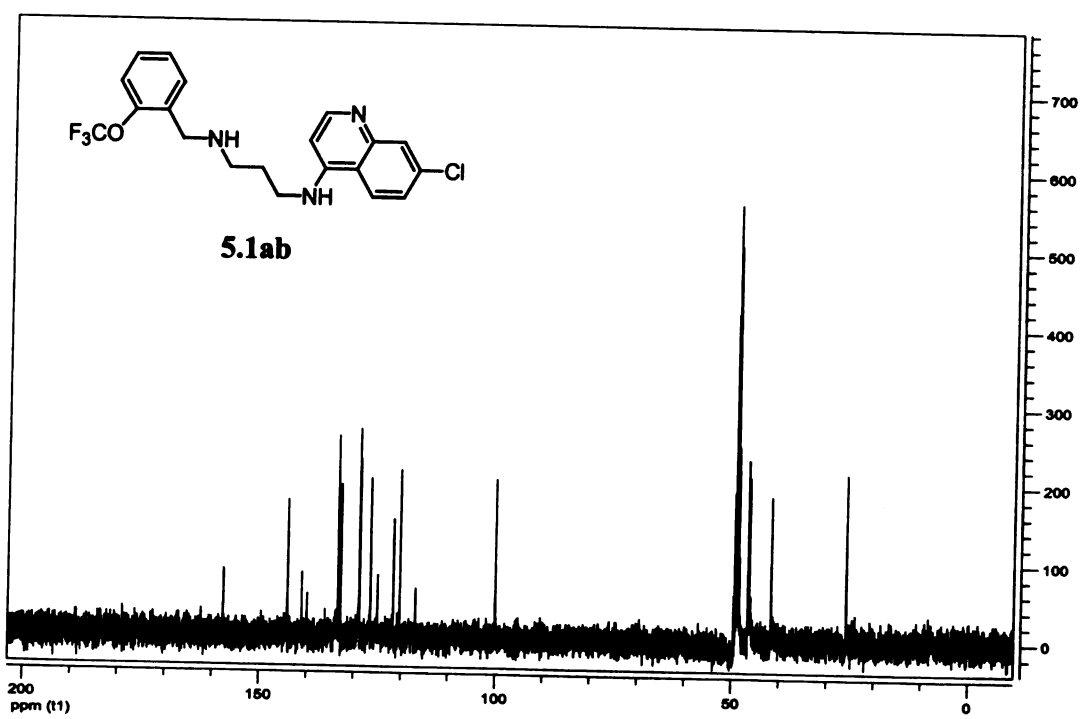

O
O
NH
N
NH
Cl
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
5.0
0.0
ppm (f1)

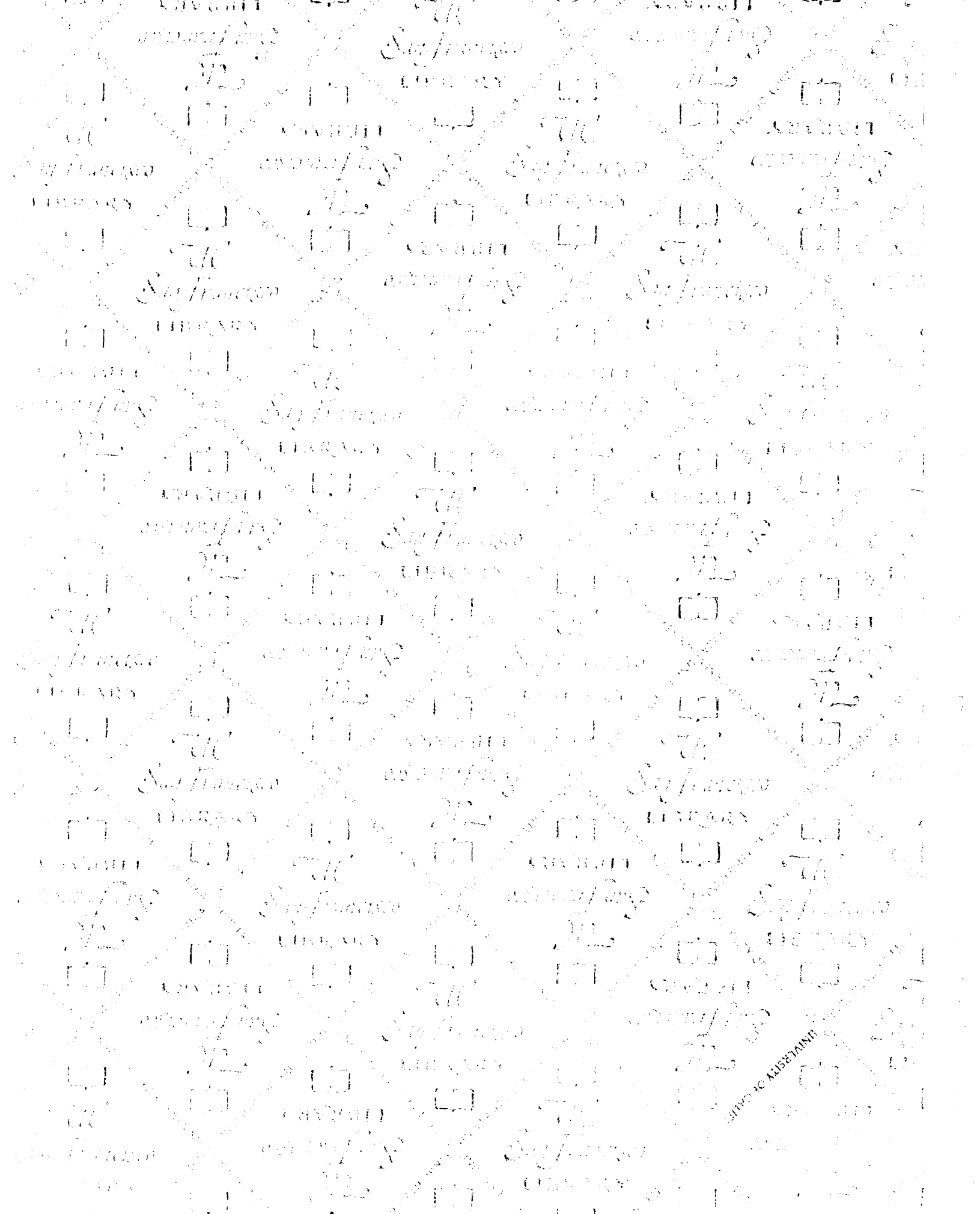
5.1aa


O
O
NH
N
NH
Cl
600
500
400
300
200
100
0
150
100
50
0
ppm (f1)

5.1aa


F3CO
NH
N
NH
Cl
5.1ab
20.0
15.0
10.0
5.0
0.0
ppm (t1)
5.0
0.0


F3CO
NH
N
NH
Cl
5.1ab
700
600
500
400
300
200
100
0
200
ppm (t1)
150
100
50
0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