Design and Synthesis of Trifluoromethyltriazoline-4-aminoquinoline hybrids as Antimalarial Agents

A Project Report submitted for the Partial fulfillment of the requirement for the degree of

Master of Science

in

CHEMISTRY (ORGANIC CHEMISTRY)

by

HISANA NASREEN K

School of Chemical Sciences

Mahatma Gandhi University

Kottayam - 686560, Kerala, India

Under the guidance of

Dr. KISHOR MOHANAN

Principal Scientist
Medicinal & Process Chemistry division

CSIR-CENTRAL DRUG RESEARCH INSTITUTE

LUCKNOW – 226031, INDIA

JUNE 2020

SCHOOL OF CHEMICAL SCIENCES

Mahatma Gandhi University
Priyadarsini Hills P. O., Kottayam-686 560
Kerala, India

Certificate

This is to certify that the work incorporated in this project entitled **"Design and Synthesis of Trifluoromethyltriazoline-4-aminoquinoline hybrids as Antimalarial Agents"**, submitted by **Ms. Hisana Nasreen K** for the partial fulfillment of the requirements for the degree of Master of Science in Chemistry (Organic Chemistry), to the Director, School of Chemical Sciences, Mahatma Gandhi University, Kottayam, was carried out under the supervision of **Dr. Kishor Mohanan,** Principal Scientist, Medicinal & Process Chemistry Division, at Central Drug Research Institute, Lucknow, in the year 2020.

Devlys

Dr. K. S. Devaky

Director
School of Chemical Sciences,
MG University, Kottayam

Place: Priyadarsini Hills
Dated: 22.7.2020

सी.एस.आई.आर.-केन्द्रीय औषधि अनुसंधान संस्थान, लखनऊ
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद्)
सेक्टर 10, जानकीपुरम विस्तार, सीतापुर रोड, लखनऊ – 226 031 (भारत)
CSIR - Central Drug Research Institute
(Council of Scientific & Industrial Research)
Sector 10, Janakipuram Extension, Sitapur Road, Lucknow - 226 031 (India)

Certificate

This is to certify that work embodied for project report "**Design and Synthesis of Trifluoromethyltriazoline-4-aminoquinoline hybrids as Antimalarial Agents**" submitted by Ms. Hisana Nasreen K towards the fulfillment of the course requirement of IV Semester of M.Sc. (Organic Chemistry) has been carried out under my supervision. She has put in one semester as prescribed in the ordinance, governing the award of the degree.

Dr. Kishor Mohanan

Principal Scientist, MPC division
CSIR-CDRI, Lucknow

Place: Lucknow

Dated: 13.07.2020

Declaration

I **Hisana Nasreen K**, hereby declare that the work presented in the report entitled **"Design and Synthesis of Trifluoromethyltriazoline-4-aminoquinoline hybrids as Antimalarial Agents"**submitted to School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala in partial fulfillment of the requirements for the degree of Master of Science in Chemistry (Organic Chemistry) is carried out by me under the supervision of Dr. Kishor Mohanan, Principal Scientist, Medicinal & Process Chemistry, CSIR-Central Drug Research Institute, Lucknow. In keeping with the general practice of reporting scientific observations, due acknowledgement has been made whenever work described is based on the findings of other investigators. Any omission which might have been occurred by oversight or error of misjudgment is regretted.

Hisana Nasreen K

Date: 13/07/2020

Acknowledgement

To commence with, I pay my obeisance to God, the almighty, to have bestowed upon me good health, courage, zeal and light.

I take this opportunity to express my deep sense of gratitude and respectful regards to my supervisor, ***Dr. Kishor Mohanan****, Principal Scientist, Central Drug Research Institute, Lucknow, for giving me the opportunity to carry out my M.Sc. project under his guidance. I really admire his continuous support, ceaseless motivation and the patience he had shown throughout my period of work. His excellent knowledge and valuable comments really helped me to improve my work as well as thesis.*

I express my sincere gratitude to ***Prof. Tapas Kumar Kundu****, Hon. Director, CSIR-CDRI, for granting me the permission to conduct my project work at this prestigious institution.*

I extend my sincere gratitude to Head of the MPC division, ***Dr. Atul Kumar****, for allowing me to proceed smoothly and institutionalizing my work so as to complete it within the stipulated time period.*

I owe my special thanks to ***Prof. K. S. Devaki****, Director, School of Chemical Sciences, Mahatma Gandhi*

University, Kottayam, all the faculties and staffs of my department. I would also like to thank ***Prof. G.Anil Kumar*** *for giving me all sorts of courage and support to carry out this project.*

I take this opportunity to thank all the members of Sophisticated Analytical Instrumentation Facility (SAIF), CDRI, Lucknow, for providing me the spectroscopic and analytical data of the compounds. I am also thankful to all the staffs of Glass Bowling section, stores and Library for their help and cooperation during my project period. I express my sincere to gratitude to ***Dr. Renu Tripathi*** *for doing the biological screening of my compounds. I am obliged to* ***Dr. Anil Kumar K S****, for his assistance in qualitative analysis, storage and distribution of our compounds to biologists through CBRS and for his reliable source of practical scientific knowledge.*

I am thankful to ***Mr. Raju Arora*** *for his technical assistance throughout my project.*

I am deeply indebted to ***Mr. Anuj Kumar****, without whom, my project would not have been a success. His expertise, invaluable guidance, constant encouragement, affectionate attitude, understanding, patience and healthy criticism added considerably to my experience. His professional and personal attitude has made my research period, a very rewarding and memorable experience. I would be ungrateful if I do not thank* ***Mr. Narendra Kumar Vaishanv*** *and* ***Mr. Khalid Zaheer*** *for their constant love, affection and relentless help they gave me since the day I have been here. I am deeply obliged to* ***Mr. Muhammad Fahad Jamali****,* ***Mr. Sanoop P.C*** *and* ***Mr. Sandeep Kumar Chaurasiya*** *for their timely motivation, healthy criticisms and*

for sharing their valuable knowledge throughout my work period. I am indeed grateful to ***Ms. Anamika Dhami****, whose constant support, patience, care, love and guidance helped me withstand bad times during my period of work. She was is like a sister for me who helped me since the day I came to CDRI in all aspects.*

I owe my deepest sense of gratitude to ***Ms. Anjalin Joy****,* ***Ms. Mary Merlin*** *and* ***Mr.Sameer Misra*** *whose fantastic friendship, love and positive attitude was reflected throughout my work period. I would also like to thank all my dear friends in CDRI for their valuable friendship and love.*

I am grateful to ***Mr. Mohammed Rafi K.P*** *and family who gave me all courage and motivation which remained the driving force during the entire period of my journey to complete this project successfully.*

No words can suffice my feelings of gratitude towards my father ***Mr. Abdu Rahiman K*** *and my mother* ***Mrs. Sameera V*** *for their unconditional love and support. I am indebted to their unwavering moral, emotional and financial support, without whom it was near impossible to fulfill my objective. Besides this, I am thankful from the core of my heart to my loving sister* ***Febin Rehman K*** *and brother* ***Aadil Swalah*** *for the care and affection.*

I also recall the cheerful companionship of all my friends and all my classmates at MG University.

Finally, I would like to thank everybody who was important to the successful realization of this thesis, as well as express my apology that I could not mention personally one by one.

Thank you all…

Hisana Nasreen K

List of abbrevations

%	:	Percentage
δ	:	Delta, chemical shift
s	:	Singlet
d	:	Doublet
dd	:	Double doublet
t	:	Triplet
°C	:	Degree Celsius
^{1}H	:	Proton
^{13}C	:	Carbon -13
^{19}F	:	Fluorine -19
$CDCl_3$	:	Deuterated chloroform
Conc.	:	Concentration
NaOH	:	Sodium hydroxide
DMF	:	Dimethylformamide
DCM	:	Dichloromethane
DMSO-d_6	:	Deuterated dimethyl sulfoxide
Et_3N	:	Triethylamine
EtOH	:	Ethanol
MeOH	:	Methanol
EtOAc	:	Ethyl acetate
Ag_2CO_3	:	Silver carbonate
Pf AQP	:	*Plasmodium falciparum* aquaporins
CQ	:	Chloroquine
Equiv	:	Equivalent
ESMS	:	Electron spray mass spectroscopy
g	:	Gram
h	:	Hour
Hz	:	Hertz
J	:	Coupling constant
m	:	Multiplet
mg	:	Milligram

mL	:	Mililitre
mmol	:	Millimole
Mp	:	Melting point
MW	:	Molecular weight
m/z	:	Mass-to-charge ratio
NMR	:	Nuclear magnetic resonance
ppm	:	Parts per million
R_f	:	Retention factor
TLC	:	Thin layer chromatography
UV	:	Ultra violet
IC_{50}	:	Inhibitory concentration
CC_{50}	:	Cytotoxic concentration
MHz	:	Mega hertz
µg	:	Microgram

Table of contents

1. Introduction

Malaria is a potential life threatening parasitic disease spread in tropical and subtropical regions which include Sub-Saharan Africa, Asia, and Latin America. The term malaria originates from the Italian word: *mala aria*- bad air.[1] It is caused by protozoan parasites known as *Plasmodium vivax (P. vivax), Plasmodium falciparum (P. falciparum), Plasmodium malariae (P. malariae), Plasmodium ovale (P. ovale)* and *Plasmodium knowlesi (P. knowlesi).* It is transmitted by the infective bite of female *Anopheles* mosquito. Human malaria caused by parasites *P. falciparum* and *P. vivax* are reported from India. Infection with *P. falciparum* is most deadly for humans.[2] According to the latest World malaria report, released in December 2019, there were 228 million cases of malaria in 2018 compared to 231 million cases in 2017.[3] A French army doctor Charles Louis Alphonse Laveran discovered parasites in the blood of a malaria infected person for the first time, for which he was awarded the Nobel Prize in 1907.[4]

1.1 Life cycle of parasite

The human malaria parasite has a complex life cycle as shown in Figure 1. The life cycle of malaria parasite involves two hosts viz. human and mosquito.

1. The malaria infection in human begins when the mosquito bites the skin and the motile infectious form, Plasmodium sporozoite, is passed to a blood vessel from which it feed. When sporozoites invade blood vessels, half of them remain in the skin for up to seven hours and, surprisingly, that between 15% and 20% of the sporozoites enter the lymphatic system, causing enlargement of the draining lymph node.
2. Within 30–60 min of inoculation, the thread-like sporozoites which entered the blood stream are carried to the liver hepatocytes through Kupffer cells. Kupffer cell is the liver's main line of defense against foreign bodies, it is exceedingly surprising that this resident macrophage is unable to eliminate the sporozoite.
3. Over a period of 7–12 days, the sporozoites grow into schizonts and can develop up to 30,000 merozoites, which rupture the hepatocytes and release into blood stream. Up to this stage is called **pre-erythrocytic stage**. On the other hand, some *vivax* and *ovale* sporozoites turn into hypnozoites, a form that can remain latent in the liver for months or years and cause relapses in infected people as a dormant stage.[1]

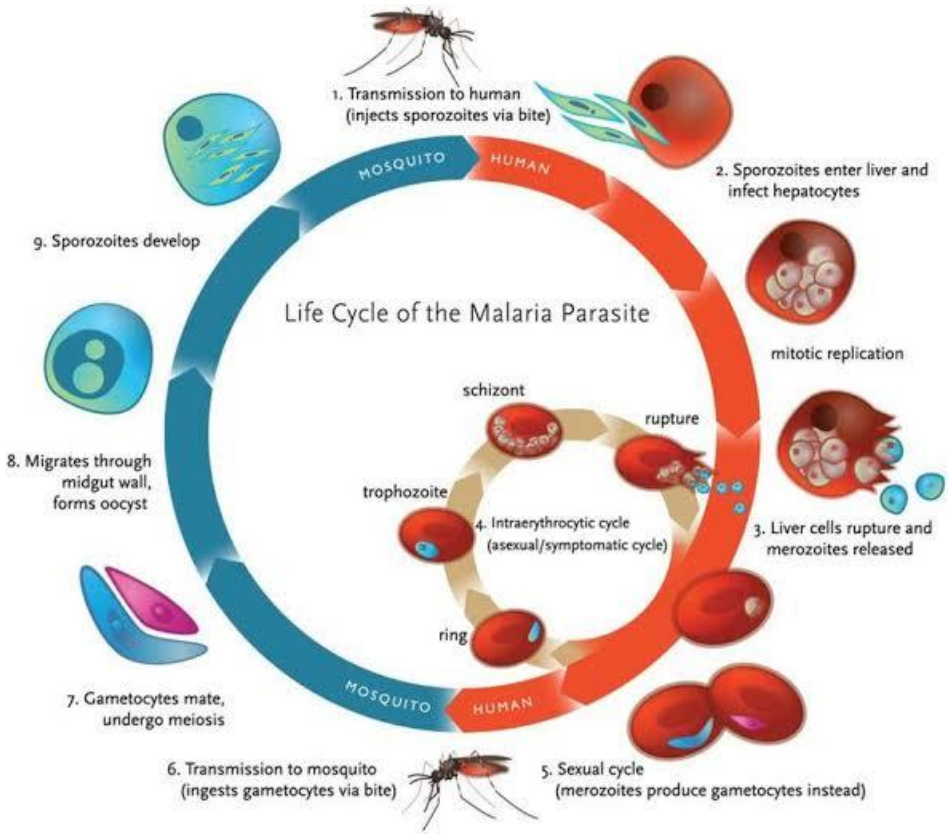


Figure 1: Life cycle of malaria parasite.

4. Then, the asexual cycle begins with the merozoites invading RBC to grow by consuming hemoglobin. Within the host RBC, the parasite undergoes development from the early ring stage to late trophozoite and then after mitotic divisions to the schizont stage, which contains 6 to 32 merozoites, depending on the parasite species. When the erythrocytic schizont ruptures, the released merozoites continue the life cycle by invading other RBCs. This stage called asexual **erythrocytic stage** and parasite at this stage shows clinical symptoms leading to illness and complications of malaria.
5. During this repeated cycle, some merozoites differentiate into male and female sexual forms known as erythrocytic gametocytes with one nucleus and then awaiting the arrival of a blood-seeking female Anopheles mosquito.
6. Then intake of gametocytes by the mosquito occurs through a blood meal.
7. Next is gametogenesis, the flagellated forms of microgametes, formed by exflagellation, penetrate or fertilize the macrogametes generate zygotes.

8. The zygotes change into ookinetes and then become a round oocyst. Inside the oocyst, the nucleus divides repeatedly, with the formation of a large number of sporozoites and enlargement of the oocyst.
9. When the sporozoites are fully formed, the oocyst bursts, releasing the sporozoites into the haemocoel (the mosquito's body cavity). The sporozoites migrate to the salivary glands, thus completing the life cycle.

Entrance of the sporozoites from the mosquito's salivary glands into a new human host perpetuates the malaria life cycle.

1.2 Clinical symptoms

The first symptoms of malaria are nonspecific and similar to those of a minor systemic viral illness. They comprise headache, lassitude, fatigue, abdominal discomfort and muscle and joint aches, usually followed by fever, chills, perspiration, anorexia, vomiting and worsening malaise. In young children, malaria may also present with lethargy, poor feeding and cough.[5]

At this early stage of disease progression, with no evidence of vital organ dysfunction, a rapid, full recovery is expected, provided prompt, effective antimalarial treatment is given. If ineffective or poor-quality medicines are given or if treatment is delayed, particularly in *P. falciparum* malaria, the parasite burden often continues to increase and the patient may develop potentially lethal severe malaria. Disease progression to severe malaria may take days but can occur within a few hours.

Malaria infection during pregnancy caused severe maternal complications like abortion (9.7%), premature labour (59.6%), and still-births (5.7%), which were higher in P. falciparum infection. Microcytic anaemia combined with dimorphic anaemia was predominant in the infected group (89.5%).[6]

Severe malaria usually manifests with one or more of the following: coma (cerebral malaria), metabolic acidosis, severe anaemia, hypoglycaemia, acute renal failure or acute pulmonary oedema. If left untreated, severe malaria is fatal in the majority of cases.

1.3 Diagnosis of malaria

Prompt diagnosis and accurate treatment is critical to the effective management of malaria. Delays in diagnosis and treatment are leading causes of death in many countries. Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood.

The diagnostic efficacy is subject to many factors. The different forms of the 5 malaria species; the different stages of erythrocytic schizogony, the endemicity of different species, the interrelation between levels of transmission, population movement, immunity, and signs and symptoms; drug resistance, the problems of recurrent malaria, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues, and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis, can all influence the identification and interpretation of malaria parasitemia in a diagnostic test. Diagnosis of malaria can be classified broadly into **clinical and parasitological diagnosis.**[7]

- Clinical diagnosis is based on the patient's symptoms and on signs at physical examination. There is no combination of signs or symptoms that reliably distinguishes malaria from other causes of fever; diagnosis based only on clinical features has very low specificity and results in overtreatment.
- Parasitological diagnosis methods are;
    1) Light microscopy
    2) Rapid Diagnostic Tests (RDTs)
    3) Polymerase Chain Reaction techniques(PCRs)
    4) Serology test

Detection of the parasites on giemsa-stained peripheral blood smears by light microscopy is used as the gold standard for diagnosis of malaria. A*s knowlesi* and *malariae* have almost similar morphology, microscopy alone is insufficient to diagnose *knowlesi*. In case of *vivax*, *ovale*, and *malariae*, all development stages subsequent to the liver cycle can be seen in the peripheral blood. However, in *falciparum*, only ring forms and banana-like gametocytes are usually present in the peripheral blood since mature parasites become sequestered.

In areas where microscopy is not readily available, RDTs can be used and are based on the detection of antigens or enzymatic activities associated with the parasites. The most common antigens for RDTs are *P. falciparum* histidine-rich protein-2(PfHRP2), specific for *falciparum* malaria, and two enzymes of the parasite glycolytic pathways, namely plasmodial lactate dehydrogenase (pLDH) and aldolase. RDTs can also measure parasite antigens when mature parasites are sequestered.

PCR-based methods are the most sensitive test able to identify low levels of parasitemia, parasite species, or mixed infections, but not a suitable method for routine use. A species-specific loop mediated isothermal amplification (LAMP) method has become widely accepted for identifying *knowlesi* infections. Besides, PCR is helpful as are search tool in epidemiological studies, clinical trials, and for detection of molecular markers of drug resistance to antimalarial agents.

Serology test is based on detection of antibodies against malarial parasites, using either indirect immune fluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology does not detect current infection but rather measures past exposure.[8]

1.4 Preventive and control measures

- ✓ Vector control: use of insecticide-treated bed nets (ITNs) and indoor spraying of residual insecticides (IRSs) in which pyrethroids, organochlorines (e.g., DDT), organophosphates, and carbamates are used as insecticide. Attractive toxic sugar bait (ATSB) methods.
- ✓ Use of chemicals for individual bite protection.
- ✓ Vaccines
- ✓ Environmental control- reduction of mosquito breeding sites.

1.4.1 Vaccines

The emergence and spread of drug and insecticide resistance has been limiting the current malaria control measures. People living in endemic areas develop clinical protective immunity despite the morphological changes and antigenic variations during the parasite life cycle allows them to escape the protective immune responses of the host. Thus safe and effective vaccine is required to achieve the world malaria eradication.

So far, three types of vaccine candidates have been intensively investigated:

1. **Pre-erythrocytic vaccines to prevent blood-stage infection**: Some vaccines of pre-erythrocytic group are RTS, S/AS01 in phase IV clinical trial, *falciparum* sporozoite vaccine(PfSPZ) in phase II trials, vivax malaria protein 1 (VMP001/AS01B) cell-traversal protein for ookinetes and sporozoites (CelTOS) [FMP012/ GLA-SE or AS01] under phase I/II a clinical trial, genetically attenuated parasite (GAP) vaccines and chemoprophylaxis vaccination (CVac) in Phase I clinical trial. Using in vitro studies, *falciparum* liver-stage

antigens (PfLSA-1, 2 & 3) and *vivax* liver-stage antigens (PvLSAs) are recognized as a novel candidate vaccine targeting infected hepatocytes.

2. **Blood-stage vaccines to clear parasitaemia and prevent clinical disease:** Candidates for erythrocyte-stage vaccine are AMA1, erythrocyte binding antigen (EBA-175), MSP-1, MSP-119, MSP-2, MSP-3, and serine repeat antigen 5 (SERA5).
3. **Transmission-blocking vaccines to prevent infection of mosquitoes and interrupt malaria transmission in populations:** Transmission-blocking vaccines (TBVs) target surface proteins expressed on gametocytes, zygotes, and ookinetes to prevent parasite development in the mosquito mid gut. This group include the gametocyte antigens (Pfs48/45andPfs230), *falciparum* ookinete surface antigens (Pfs25 and Pfs28), and their *vivax* homologues (Pvs25 and Pvs28).[1]

1.5 Treatment of malaria

Malaria can lead to fatal outcomes within few days, thus treatment should be started as soon as possible.

1.5.1 Traditional medicine

More than 1,200 plants that possess antimalarial activities are reported worldwide. A fifth of patients use traditional herbal remedies in endemic countries. Quinine, extracted from the bark of cinchona tree *(Cinchona officinalis)* was most effective.[9] *Ampelozyziphus amazonicus, Strychnopsis thouarsii, Phytolacca dodecandra, Justicia, Vernonia amygdalina, Buddleja Polystachya, Strychnos mitis, Aloe trichosantha, Cadaba rotundifolia, Adhatoda schimperiana, Piper capense* and *Gardenia ternifolia* were commonly used in endemic areas.

1.5.2 Conventional medicine

The main targets of current antimalarial drugs are asexual blood stages of the parasite, responsible for the malarial symptoms. Nowadays, the available antimalarials can be grouped into five classes according to their chemical structure and biological activity.

1.5.3 Classification of antimalarial drugs

Antimalarials are classified on the basis of their molecular structure and biological activity and are discussed as under:

1.5.3.1 Based on chemical structure:

- ✓ Quinoline based antimalarials: 4-aminoquinolines (chloroquine, amodiaquine, and piperaquine) and 8-aminoquinolines (primaquine and tafenoquine).
- ✓ Aryl amino alcohols—quinine, mefloquine, halofantrine, and lumefantrine.
- ✓ Antifolate compounds (pyrimethamine, proguanil, dapsone, and sulfadoxine).
- ✓ Artemisinin and its derivatives: first generation (dihdyroartemisinin, artesunate, arteether, and artemether) and second generation (artemisone).
- ✓ Hydroxynapthoquinone-atovaquone.[10]

1.5.3.2 Based on biological activity:

- ✓ Tissue schizonticidal agent: Primaquine is effective against the hypnozoites of vivax and ovale malaria. Proguanil is a biguanide compound that is active against all stages of *Plasmodium*.
- ✓ Blood schizonticidal agent: Chloroquine is a blood schizonticidal agent and the drug of choice for all malarial parasites except for chloroquine resistant Plasmodium strains. Primaquine can kill gametocytes and consequently block the malaria transmission. Quinine kills large ring and tropozoite asexual parasites and is gametocidal against *vivax, ovale* and *malariae* but not *falciparum* malaria. Mefloquine is also a blood schizonticide, active against the erythrocytic stages of all malaria parasites. Artemisinin (endoperoxide sesquiterpene lactone) is a potent and fast acting blood schizonticidal killing all parasite stages.

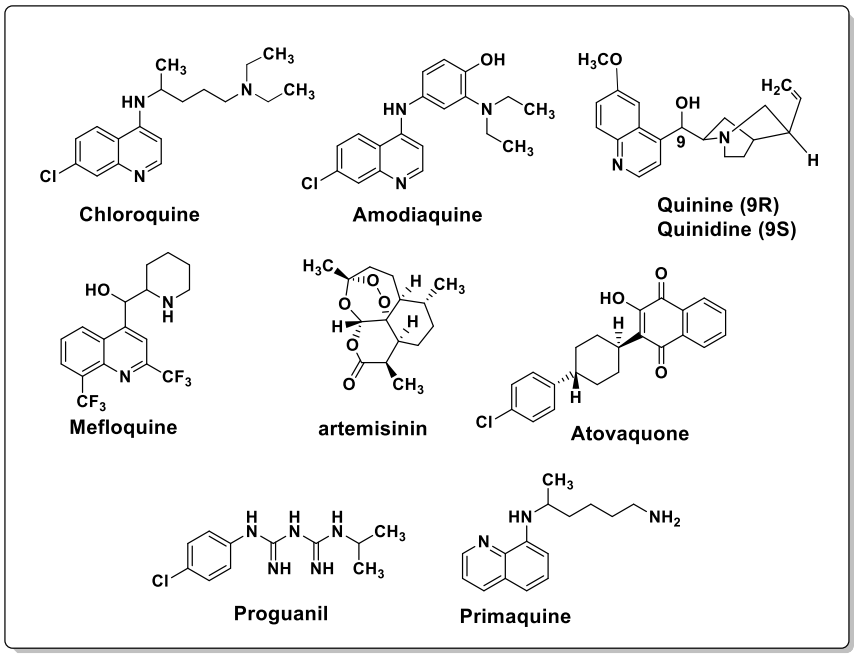


Figure 2: Synthetic and natural antimalarial drugs used as models.

1.6 Antimalarial drug resistance

According to WHO Antimalarial drug resistance has been defined as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject". This definition was later modified to specify that the drug in question must "gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action".

Genetic, molecular and pharmacological approaches have shown that different targets of older drugs are resistant due to spontaneous mutations or gene duplication on their key enzymes or transporters. For example, chloroquine resistance is caused by mutation of Pfmdr1 and Pfcrt. Atovaquone resistance is caused by mutation of cytochrome b gene. Point mutation of dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) are responsible for resistance to antifolates.[11] The most potent and safe anti-malarial drug, artemisinin and their derivatives are also non-effective to *P. falciparum* due to point mutation in *P. falciparum* kelch-like protein which is a primary marker those drugs.[12]

Many antimalarial drugs in current usage are chemically close related and development of resistance to one can facilitate development of resistance to others. Chloroquine and amodiaquine are both 4-aminoquinolines and they got cross-resistance. Development of resistance to mefloquine also lead to resistance to halofantrine and quinine. Resistance to antifolate combination drugs like sulfadoxine/ pyrimethamine may lead to increased parasitological resistance to other antifolate combination drugs. Development of high levels of SP resistance through continued accumulation of DHFR mutations may cause resistance against newer antifolate combination drugs such as chlorproguanil/dapsone (LapDap) even before they are brought into use. The incomplete cure due to non-adherence to antimalarial drugs by the patients also resulted in appearance of drug-resistant mutant.

1.6.1 Prevention of drug resistance

Although effective antimalarials are currently available the potential emergence of resistance to those drugs is a great problem in malaria treatment and eradication. Resistance is developed even against artemisinin and its derivatives, which are the most effective drugs available today. Artemisinins comprise the only known drug class that works effectively against multidrug resistant

parasites. Therefore, a strategy of combination of antimalarial drugs called "**Combination Therapies"** is established for delaying the emergence of multidrug-resistant *Plasmodium falciparum*. Use of combination therapy has been linked to slowing of the development of mefloquine resistance and reductions in overall malaria transmission rates in some parts of Thailand and has been recommended for widespread use in sub Saharan Africa.[13] Combination therapy include non-artemisinin combinations and artemisinin-based combinations:

1.6.1.1 Non-artemisinin-based combinations

- ✓ Chloroquine-Sulfadoxine -Pyrimethamine(CQ+SP)
- ✓ Amodiaquine-Sulfadoxine -Pyrimethamine (AQ+SP)
- ✓ Chloroproguanil-Dapsone (LAPDAP)
- ✓ Atovaquone-Proguanil (Malarone) is for treatment of uncomplicated malaria in travelers outside malaria-endemic areas.
- ✓ Quinine-clindamycin is for uncomplicated malaria treatment in the first trimester of pregnancy.[5]
- ✓ Mefloquine-Sulfadoxine/pyrimethamine (Fansimef)

1.6.1.2 Artemisinin-based combination therapy(ACTs)

The treatment of uncomplicated malaria caused by *falciparum* parasite or by chloroquine-resistant *vivax, ovale, malariae*, and *knowlesi* using a combination of fast acting artemisinin-based compounds with a drug from a different class are called ACTs. Artemisinin drugs are highly efficacious, rapidly active, and have action against a broader range of parasite developmental stages. Artemisinin is commercially produced by extract from sweet wormwood *(Artemisia annua).*[14] This action apparently yields two notable results. First, artemisinin compounds, used in combination with a longer acting antimalarial, can rapidly reduce parasite densities to very low levels at a time when drug levels of the longer acting antimalarial drug are still maximal. Second, the use of artemisinins has been shown to reduce gametocytogenesis by 8- to 18-fold. This reduces the likelihood that gametocytes carrying resistance genes are passed onwards and potentially may reduce malaria transmission rates. ACT combinations are:

- ✓ artemether-lumefantrine (AM+LM) (Coartem) is suggested as the first-line drug for uncomplicated falciparum malaria.[15]
- ✓ artesunate-amodiaquine (AS-AQ)

- ✓ artesunate-sulphadoxine-pyrimethamine (AS-SP)
- ✓ artesunate-mefloquine (AS-MQ)
- ✓ dihydroartemisinin-piperaquine (DH-PP), (Eurartesim)
- ✓ artesunate-chlorproguanil-dapsone (AS-CD)
- ✓ artesunate-pyronaridine
- ✓ artesunate-atovaquone-proguanil.

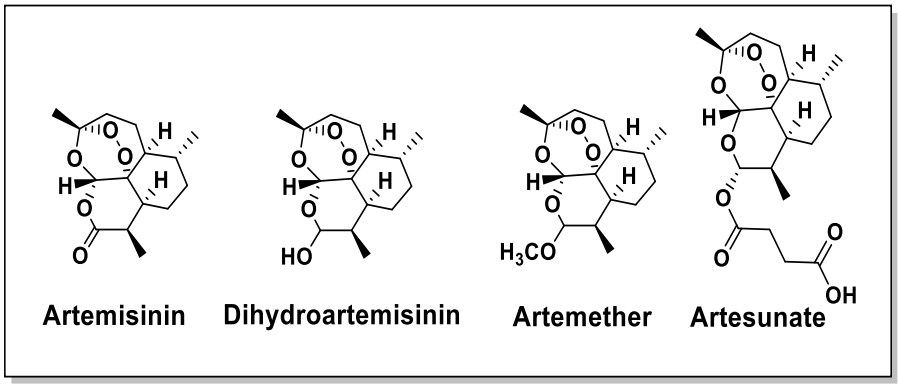


Figure 3: Artemisinin derivatives.

Any benefits of combination therapy in preventing development or intensification of resistance may be lost due to unofficial and incorrect use of the component drugs outside of official health services.[16] In the future, antimalarial therapy may be expanded by combining chemotherapy with vaccines (or other drugs) specifically designed to inhibit transmission of malaria.

1.7 New targets in malaria parasite chemotherapy

Identification of novel drug targets in the parasite and design of new chemical compounds acting on new targets is nowadays widely used approach all over the world to combat issue raised by emergence of resistance to existing drugs. New antimalarial targets are the prime need for the discovery of potent drug candidates. In order to fulfill this objective, antimalarial drug researches are focusing on promising targets in order to develop new drug candidates.

Basic metabolism and biochemical process in the malaria parasite, i.e. *Plasmodium falciparum* can play an indispensable role in the identification of these targets. An overview of the different drug targets for malaria is shown in Figure 4.

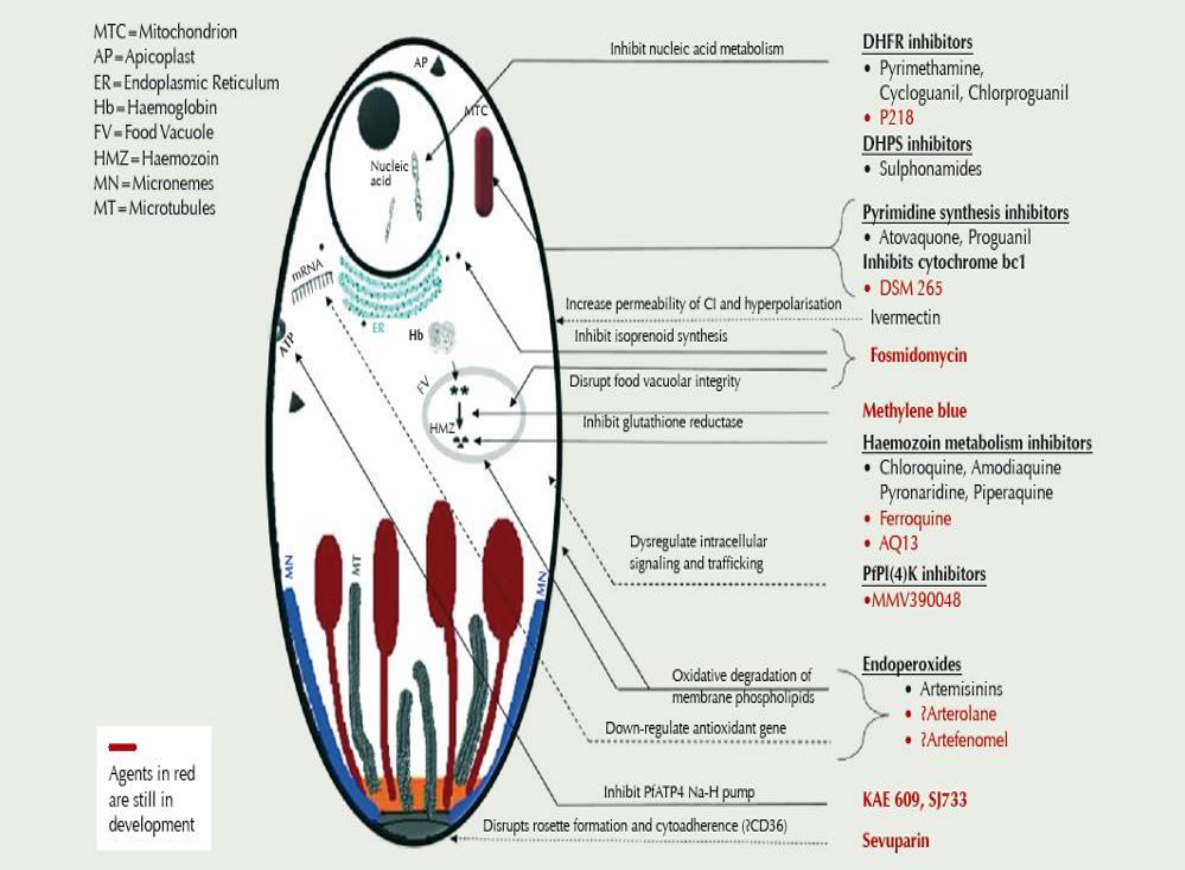


Figure 4: Antimalarial targets in plasmodium parasite and candidate drugs.

1.7.1 Food vacuole of malaria parasite as drug target

Food vacuole of malaria parasite is responsible for the degradation of 60–80% of the host red cell hemoglobin, providing a pool of amino acids required for its growth and development. This pathway is initiated by a series of proteases which digest hemoglobin into small peptides. So, inhibiting these proteases will stop this peptide synthesis through which parasite development can be stopped, thus, protease enzymes are a good target (Figure 5).

It has also been studied that digestion of hemoglobin release peptides which are transported to cytoplasm, where further proteolytic cleavage leads to the production of free amino acids. Therefore, a transporter that exports the peptides for terminal degradation to amino acids in the cytoplasm must exist. Inhibition of this transporter system could be a valid target to develop antimalarial agents.[17] Food vacuole nutrition of the malaria parasite can be checked by the following two ways:

a) Targeting protease enzymes

Hemoglobin hydrolysis in the Plasmodium digestive vacuole is thought to be a semi-ordered process mediated by the action of a series of proteases like plasmepsins (aspartic proteases) and falcipains (cysteine proteases). Cysteine protease inhibitors such as E64, leupeptin, chymostatin, fluoromethyl ketones, vinyl sulfones, and chalcones which have potency to protect plasmodium-infected mice against lethal malaria appear to be a valuable template for the development of new inhibitors specific to individual plasmodial proteases.[18]

The major food vacuole-resident hemoglobin degrading proteases are papain-like cysteine proteases falcipains, aspartic proteases plasmepsins, the metalloprotease falcilysin, dipeptidyl aminopeptidase I, and a M1-family alanyl aminopeptidase. PfSUB1 is a serine protease involved in both schizont rupture and erythrocyte reinvasion in the *P. falciparum* life cycle and its human enzyme homolog is available. Maslinic acid (MA), a low toxic natural pentacyclic triterpene has an ability to hinder the maturation from ring to schizont stage which terminate the release of merozoites and its subsequent invasion.[19]

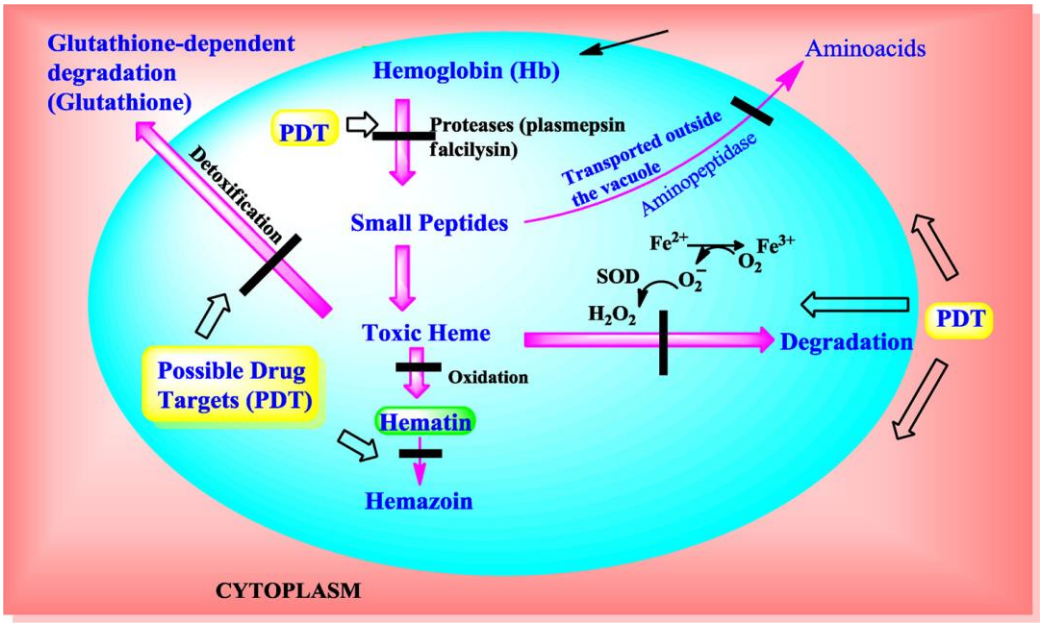


Figure 5: Food vacuole of intra-erythrocytic malarial parasite as drug target.

b) Targeting hemozoin formation

Formation of hemozoin pigment is the most vital way of detoxification of heme group in *P. falciparum* which involves oxidation of heme (Fe(II)PPIX) to hematin (Fe(III)PPIX) and then

conversion of hematin to hemozoin by Histidine-rich protein. Hematin is known to be the target of chloroquine and other blood schizonticidal like chloroquine, halofantrine, mefloquine, quinidine, and 8-aminoquiniolines analogues like tafequine as well as bisquinoline analogues, and previous studies suggest that these drugs act by preventing the detoxification of hematin, by converting them to a very insoluble microcrystalline dimer of Fe(III)PPIX called hemozoin. Some study reported that artemisinin binds to heme for antimalarial activity.[12]

1.7.2 Targeting apicoplast

The apicoplast is a non-photosynthetic plastid which is vital for the malarial parasite which covers a large number of important metabolic biochemical pathways for the *P. falciparum* like biosynthesis of fatty acid, isoprenoid precursors and heme synthesis.[20] However, these pathways are absent in the human host but present in various types of bacteria, plants, and apicomplexan parasites. Hence, these parasite-specific metabolic pathways can be considered as an ideal drug targets.

a) Targeting fatty acid pathway

Fatty acid biosynthesis pathways of the parasite (FAS II) and the human host (FAS I), have some inherent difference thus making them a promising target for the development of antimalarials. Various enzymes, which are involved in the synthesis of fatty acids in FASII pathway as a potential drug target includes acetyl-CoA carboxylase, β-ketoacyl-ACP reductase, malonyl transacetylase, β-hydroxyacyl-ACP dehydrase, β-hydroxyacyl-ACP dehydrase -ketoacyl-acyl carrier protein synthase and enoyl-ACP reductase.

Thiolactomycin (a natural antibiotic inhibits fatty acid and mycolic acid synthesis) has exhibited inhibition of β-ketoacyl-ACP synthases of FAS II (FabH, FabB/F) in the culture of *P. falciparum*. The antimicrobial Triclosan, a specific inhibitor of the enoyl-ACP-reductases (PfENR or FabI) of FAS II has exhibited inhibition of P. falciparum in in-vitro tests as well as plasmodium infected mice model.

b) Targeting isoprenoid pathway

Isoprenoid is one of the important requisite for parasite multiplication in human erythrocytes mainly to *P. falciparum*. Isoprenoid metabolism is critical to *P. falciparum*. Plasmodium synthesizes isoprenoids using 1-deoxy-Dxylulose-5phosphate (DOXP) as precursor which is not present in humans. This pathway is a non-mevalonate pathway or methyl erythritol

phosphate biosynthetic pathway which is also found in eubacteria and plants but not in humans (mavelonate pathway). So it is expressed a good target for antimalarial drugs. Genes encoding two enzymes in this pathway are DOXP reductoisomerase and DOXP synthase by P. falciparum. The antibiotic fosmidomycin and its derivative FR900098 inhibited the activity of recombinant DOXP reductoisomerase which resulted in the inhibition of the growth of cultured *P. falciparum* parasites and cured murine malaria.

c) Targeting heme biosynthesis

Malarial parasite synthesizes haem de novo for metabolic use. For that δ-aminolevulinic acid (ALA) is a main precursor which is synthesized from glycine and succinyl-CoA in animals using ALA dehydratase enzymes. The *P. falciparum* imports host ALA dehydratase and other subsequent enzymes from the host red cell and inhibition of this import process is another valid target which could lead to the inhibition of heme synthesis and death of the parasite. Chloroquine, artemisinin and other schizonticidal drugs act by interfering heme metabolism of the parasite.

1.7.3 Targeting mitochondria

The mitochondrial electron transport chain (ETC) is critical for parasite survival which regenerate mitochondrial coenzyme Q. It is an important drug target for blocking parasite transmission and prophylaxis outside the host erythrocytes. Compounds such as, 4(1H)-pyridones, acridones, acridinediones, myxothiazol, antimycin, and cyanide and 4(1H)-quinolones are reported drugs targeting ETC.[21] Decoquinate was found to be potent, selective, and specific inhibitor of *P. falciparum* mitochondrial bc1 complex.

1.7.4 Targeting plasmodium sugar transporters

Intra-erythrocytic stages of the parasite are entirely dependent on host glucose for energy. The main source of ATP production in asexual blood stages is glycolysis, followed by anaerobic fermentation of pyruvate to lactate, which provides fast ATP production, and is required for the rapid multiplication of intraerythrocytic parasite. Glucose is delivered to the intera-erythrocytic parasite by both host and plasmodium sugar transporters. Glucose is first transported to the host erythrocytes by the glucose transporter GLUT1 facilitatively, which is highly abundant in the erythrocyte plasma membrane. There is another *P. falciparium* Hexose transporter (PFHT), is a

sodium-independent, saturable, facilitative hexose transporter which shares some of the typical sugar transporter features with GLUT1. The diference between the above two is; GLUT1 is selective for D-glucose whereas PFHT can transport both D-glucose and D-fructose. Hence, selective inhibition of PFHT may be possible because mechanistic differences were observed between GLUT1 and *P. Falciparium* Hexose transporter (PFHT) in terms of their interaction with substrates.[22]

1.7.5 Targeting the Sarcoplasmic/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA)

P-type ATPase also known as E1-E2 ATPases, form a superfamily of cation transporters that catalyze the selective transport of various ions like H^+, Na^+, K^+, Ca^{2+} Zn^{2+} and Cu^{2+} across diverse biological membrane systems and hence maintain steep electrochemical gradients and cell homeostasis.[23] Prominent examples of P-type ATPases are the sodium-potassium pump (Na^+, K^+-ATPase), the plasma membrane proton pump (H^+-ATPase), the proton-potassium pump (H^+, K^+-ATPase), and the calcium pump (Ca^{2+}-ATPase). The SERCA belongs to the family of calcium pump P-type ATPases. Thapsigargin is a sesquiterpene lactone and the most widely used SERCA inhibitor that inhibits SERCA in the nanomolar concentration range. It is highly selective because it does not affect other Ca^{2+}-ATPase. Another compound extracted from traditional Chinese medicinal herb is Alisol B bind best to the trans-membrane domain at the same site occupied by thapsigargin.[24]

1.7.6 Targeting the lipid metabolic pathways

Infection by plasmodium causes a marked increase in the phospholipid content and a significant change in the lipid composition of the infected erythrocyte. So, phospholipids (PL) metabolism is an attractive target for new malaria chemotherapy due to its vital importance to the parasite.[25] Phospholipids metabolism is absent in normal mature human erythrocytes but the erythrocyte phospholipids content increases by as much as 500% after infection, specifically due to the metabolic machinery of the parasite. Malaria parasites need large amounts of phospholipids. Hexadecyl-trimethyl-ammonium-bromide was identified as an inhibitor of *P. falciparum* Choline kinase PFCK. Bis-quaternary ammonium salts, structurally analogue to the phospholipids precursor choline, have been shown to be the target of *P. falciparum* membrane biogenesis by blocking the biosynthesis of Choline kinase (PC).

1.7.7 Targeting eukaryotic protein kinases and proteasome

Eukaryotic Protein Kinases and proteasome of malaria parasite are also targeted. Compounds such as Flavopiridol and olomoucine have exhibited inhibition to protein kinases.[26] Proteasome is a multi-stage target in malaria therapy. Compounds such as lactacystin, epoxomicin, fellutamide B and designed compounds (MG-132, bortezomib) inhibits proteasome metabolic pathway of malaria parasite.[27]

1.7.8 Targeting aquaporins

Aquaporins (AQPs) are a class of membrane water channels whose primary function is to facilitate the passive transport of water across the plasma membrane of the cell in response to osmotic gradients that are created by the active transport of solutes. Since *Plasmodium falciparum* aquaglyceroporin (PfAQP) is able to transport other small solutes the parasites are sensitive to other compounds which are harmless to the human host. The human malarial parasite that resides within erythrocytes relies on AQP3 and AQP9 in the erythrocyte plasma membrane to obtain glycerol. Glycerol is important during malarial infection. The malarial parasites incorporate host plasma glycerol into lipid and membrane during the asexual intraerythrocytic stages of infection. A single aquaglyceroporin identified in plasmodium has been shown to be the major pathway for glycerol uptake from the erythrocyte cytoplasm into the parasite. In parasite lacking the aquaglyceroporin, PfAQP, grows more slowly and is less virulent, indicating that glycerol may be important for the proliferation of the parasite. Therefore, the inhibition of PfAQP may severely affect parasite proliferation.

Four classes of AQP-targeted small molecules have been described: cysteine-reactive heavy metal-based inhibitors (eg. Pcmbs, Au(phen), Silver sulphadiazine); small-molecule scaffolds that are reported to inhibit water conductance (eg; TEA^+, NCS168597, Acetazolamide); small molecules that target the interaction between AQP4 and the NMO autoantibody (eg; Berbamine, Arbidol); and agents that act as chemical chaperones (eg; Glycerol, Geldanamycin) to facilitate the cellular processing of NDI-causing AQP2 mutants.[28] Development of resistance against a potential AQP blocker is very limited, this increases the scope.

Due to the resistance of antimalarial drugs worldwide, finding incipient cellular targets and developing new agents targeting old targets is both imperative aspects in fighting drug-resistant malaria. In 2009, the Medicines for Malaria Venture (MMV) was established as a not-for-profit

organization to develop novel approaches to combat malaria by collaborating with industry and academic. The Malaria Genome Project has provided new scope to the identification and optimization of new targets.

1.8 Chloroquinoline derivatives as an antimalarial agent

Quinoline and its related derivatives comprise a class of heterocycles, which has been exploited immensely than any other nucleus for the development of potent antimalarial agents. Among these those derivatives with amino alkyl substitution at 4th position and chlorine substitution at 7th positions are of importance. From various structure–activity relationship (SAR) studies, the following can be concluded:

a. The 4-amino quinoline nucleus is essential for complexation with hematin,
b. The amino alkyl side chain helps in the accumulation of the drug inside the food vacuole and assists in the complexation of the quinoline nucleus with the porphyrin system and
c. The presence of chlorine at the 7-position is essential for the inhibition of hemozoin formation.[29]

Some of antimalarial drugs with chloroquinoline moiety are showcased in Figure 6.

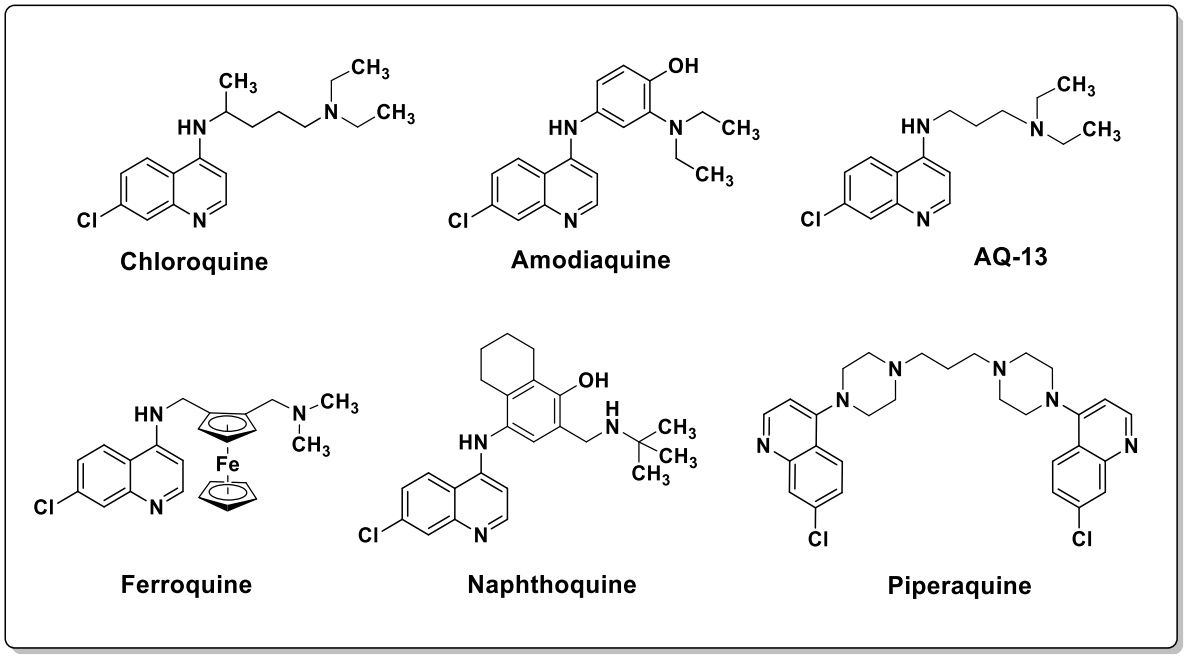


Figure 6: Antimalarial drugs with chloroquinoline moiety.

1.9 Antimalarial drugs in the pipeline

The portfolio of the antimalarial drugs that are in pipeline arises from the collaboration between Medicines for Malaria Venture with its partners in both academia and the pharmaceutical industry, with support from donors mainly include government agencies and philanthropic foundations (Figure 7,8).

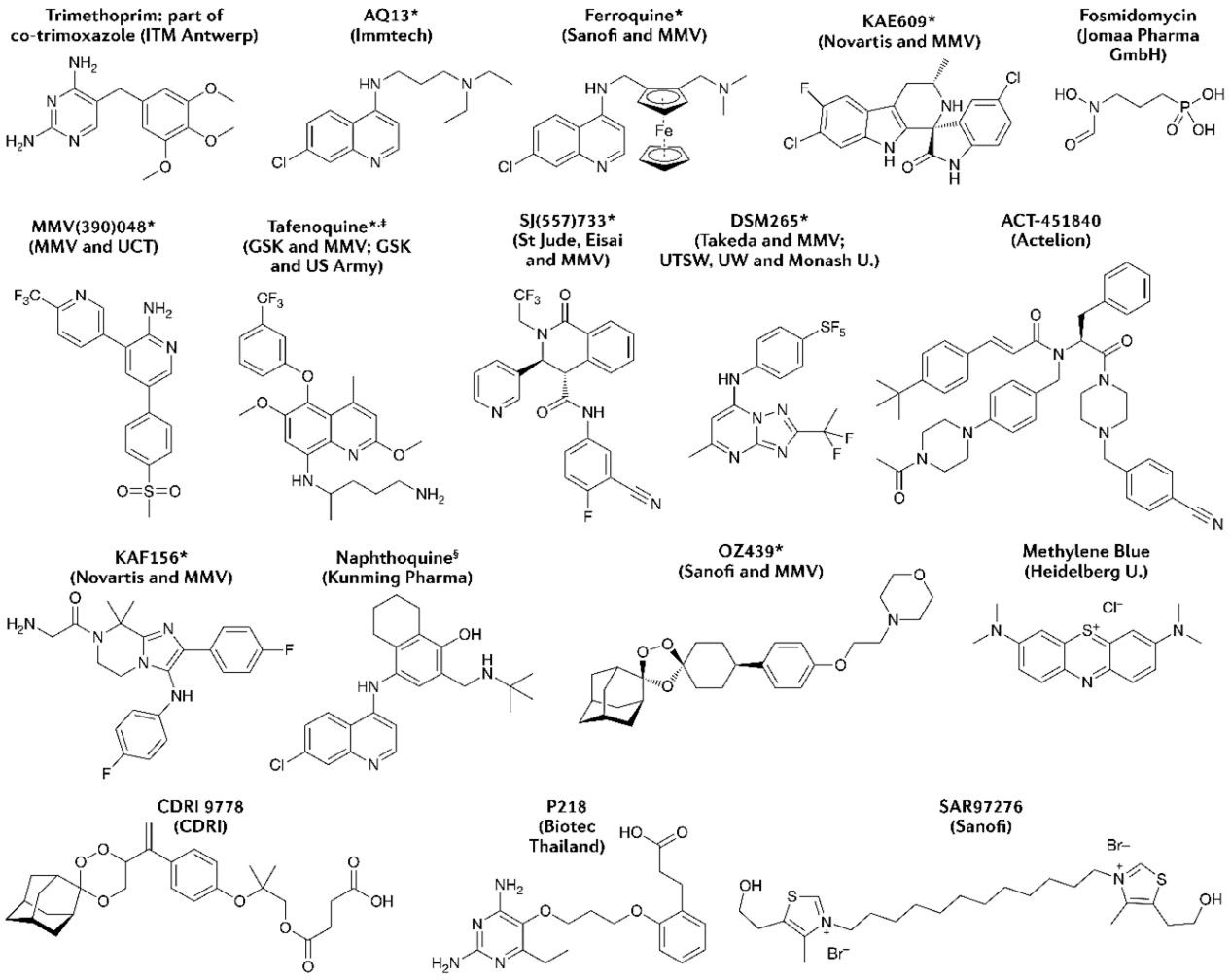


Figure 7: Antimalarial drugs in pipeline.

Sl.No	Drug Name	Manufacturer	Chemical Class	Target	Mechanism Of Action	Clinical Trial
1.	Ferroquine	Sanofi and MMV	4-aminoquinoline	Haematin	Hemozoin formation	Phase IIa
2.	OZ277 (aretrolane)	Ranbaxy and MMV	1,2,4-trioxolane	Asexual blood stage of *P.falciparum*	Haemoglobin digestion	Phase III
3.	OZ439 (artefenomel)	Sanofi and MMV	1,2,4-trioxolane	Asexual blood stage of *P.falciparum*	Haemoglobin digestion	Phase IIa
4.	KAF156	Novartis and MMV	Imidazolopiperazine	PfCarl	Unknown	Phase II
5.	KAE608 (cipargamin)	Novartis	Spiroindolone	PfATP4	PfATP4 inhibition	Phase IIa
6.	DSM265	University of Texas	Triazolopyrimidine	DHOD	Inhibition of DHOD	Phase I
7.	CDRI-97/78	CDRI	Trioxane	-	-	Phase II
8.	MMV(390)048	MMV	2-aminopyridine	PfPI(4)K	PfPI(4)K inhibition	Phase I
9.	SJ(557)733	MMV	Dihydroisoquinoline	PfATP4	PfATP4 inhibitor	Phase I
10.	P218	MMV	Pyrimidine side chain and carboxylate group	DHFR	Dihydrofolate reductase inhibitor	Phase I
11.	PA92	MMV	Aminopyrazole	PfATP4	PfATP4 inhibitor	Preclinical
12.	GSK030	MMV	Thiotriazole	PfATP4	PfATP4 inhibitor	Preclinical
13.	Tafenoquine	GlaxoSmithKline	8-aminoquinoline	unknown	unknown	Phase III
14.	M5717	MMV	-	PfEF2	PfEF2 inhibitor	Preclinical
15.	MMV253	MMV	triaminopyrimidine	V-ATP synthase subunit D	Unknown	Preclinical
16.	AN13762	MMV	Oxaborole	Unknown	Unknown	Preclinical
17.	JPC3210	Jacobus Pharmaceuticals	Aminocresols	Unknown	Unknown	Preclinical

Figure 8: Antimalarial drugs in pipeline.

2. Basis of work

2.1 Statement of problem

The widespread emergence and dissemination of *Plasmodium falciparum* (Pf) strains resistant to conventional antimalarial drugs has intensified the efforts to discover and develop novel, structurally diverse drugs against multidrug resistant Plasmodium strains. These drugs should be efficacious against liver-stage and blood-stage infections and active against resistant strains. There is also a need for next-generation drugs that kill gametocytes as well as the vector stages and thus can be used to prevent disease transmission. These desirable features would need to be incorporated into new molecules with longer half-lives for chemoprophylaxis and to provide long-term protection against reinfection. New, innovative drugs should also be fast acting, be safe for children and pregnant women, and ideally be amenable to a single-dose administration.

Food vacuole of malaria parasite is an important target for antimalarial drugs. Inhibition of protease enzymes and conversion of heme to hemozoin are the two ways for interrupting the parasite life cycle. Hematin or heme is known to be the target for chloroquine and other blood schizonticidal drugs like halofantrine, mefloquine, quinidine, and 8-aminoquiniolines analogues like tafequine as well as bisquinoline analogues. These drugs act by preventing the detoxification of hematin, by converting them to a very insoluble microcrystalline dimer of Fe(III)PPIX called hemozoin and thus stopping survival of the parasite.

Chloroquine is a typical fast acting schizonticidal drug. It has all the structural requirements as an antimalarial agent targeting food vacuole of parasite. Its size, presence of 4-aminoquinoline nucleus, amino alkyl sidechain and chlorine substitution are the key features for its activity. A schematic diagram of mechanism of action of chloroquinoline derivatives is discussed in Figure 9. In the context of our interest in developing novel antimalarials agents, we envisioned to develop a new class of 4-aminoquinoline hybrids which might exhibit potent antimalarial activity addressing the same target as that of chloroquine. Several classes of triazole tethered 4-aminoquinoline derivatives have been shown to possess excellent antiplasmodial activities. However, there have been no report on the utility of triazoline derivatives as antimalarial agents, presumably due to the difficulty in accessing them. Keeping this in mind, we thought to explore the possibility of combining triazoline with a known pharmacophore such as 4-aminoquinoline to develop new antimalarial agents. Our efforts in this direction culminated in the synthesis of a

unique class of trifluoromethyltriazoline-4-aminoquinoline hybrids, and this work forms the subject matter of this thesis.

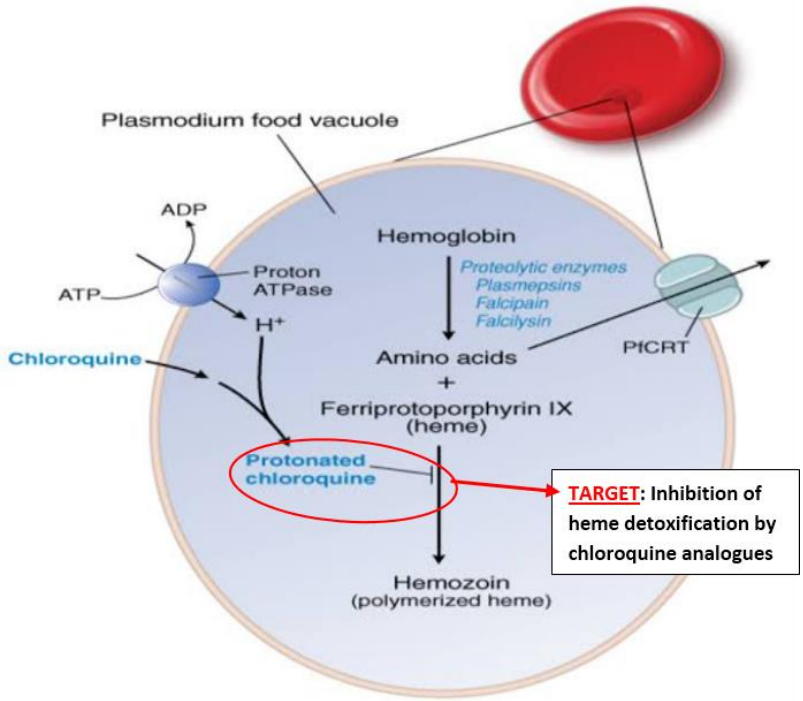


Figure 9: Mechanism of action of chloroquinoline analogues.

2.2 Retrosynthetic approach towards trifluoromethyltriazoline-4-aminoquinoline hybrids

The retrosynthetic pathway for the synthesis of trifluoromethyltriazoline-4-aminoquinoline derivatives is outlined in Figure 10. The target triazoline moiety can be obtained by [3+2] cycloaddition reaction of *in situ* generated imine with trifluorodiazoethane. The required imine is formed by condensation reaction of an aldehyde with *N*1-(7-chloroquinolin-4-yl) diamine. The retrosynthetic pathway for the synthesis of *N*1-(7-chloroquinolin-4-yl) diamine follows the nucleophilic aromatic substitution of 4,7-dichloroquinoline by a diaminoalkane.

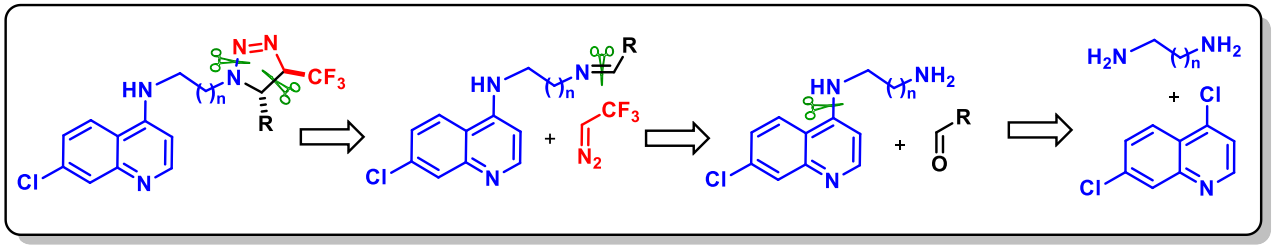


Figure 10: Retrosynthetic approach towards trifluoromethyltriazoline-4-aminoquinoline hybrids.

The results of our efforts in this direction are presented in the following pages.

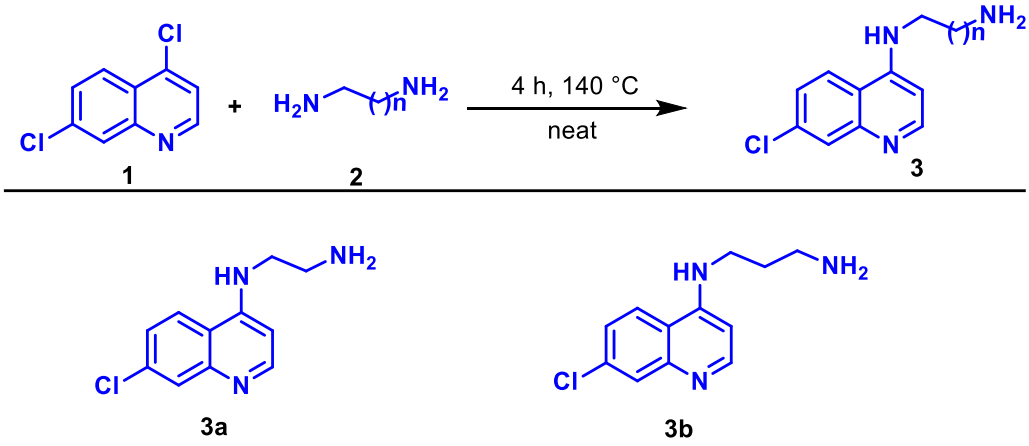
3. Results and discussion

The strategy adopted for the trifluoromethyl triazoline derivative of chloroquinoline synthesis includes two steps:

1. Synthesis of *N*1-(7-chloroquinolin-4-yl) diamine.
2. Formation of trifluoromethyl triazoline derivative of chloroquinoline by a domino-Schiff base formation/[3+2] cycloaddition reaction sequence.

3.1 Synthesis of *N*1-(7-chloroquinolin-4-yl) diamines

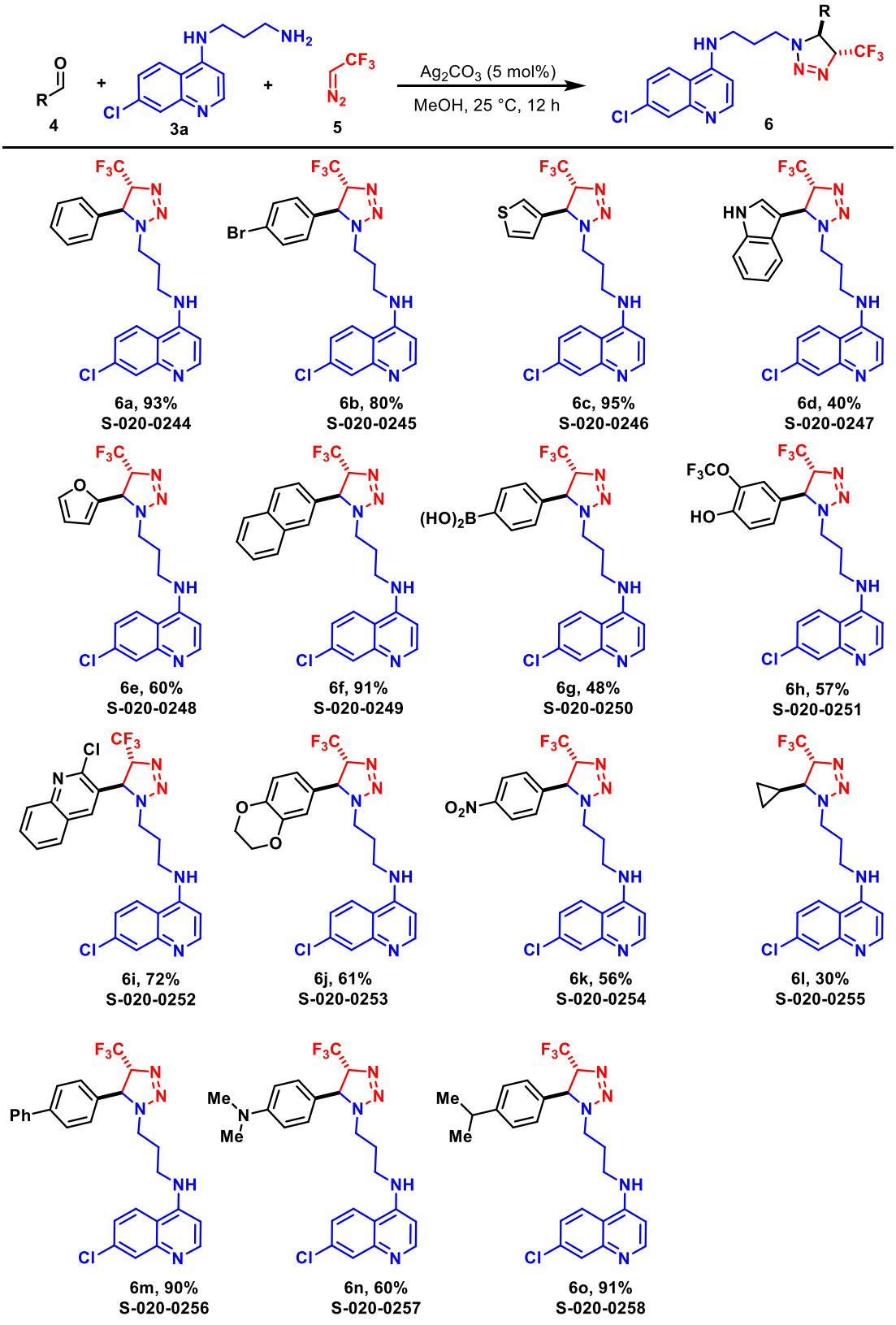
The synthesis of *N*1-(7-chloroquinolin-4-yl) diamine is shown in Scheme 1. Commercially available 4,7-dichloro quinoline **1** and terminal diaminoalkanes **2** were treated under neat conditions to furnish *N*1-(7-chloroquinolin-4-yl) diamine derivatives **3**. The strategy was applied to terminal diaminoalkanes containing two and three carbon chains as shown below.



Scheme 1: Synthesis of *N*1-(7-chloroquinolin-4-yl) diamines.

3.2 Synthesis of trifluoromethyl triazoline derivatives of chloroquinoline

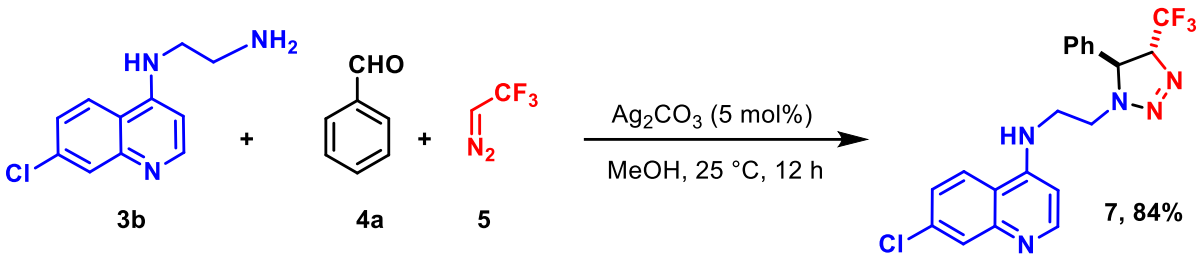
The synthesis of trifluoromethyl triazoline derivatives of chloroquinoline is shown in Scheme 2a and Scheme 2b. The protocol involves two steps; initially the N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3** and aldehyde **4** undergo condensation to form imine intermediate. The *in situ* formed imine subsequently undergoes a silver catalyzed [3+2] cycloaddition reaction with trifluorodiazoethane **5** to afford trifluoromethyltriazoline derivatives of chloroquinoline. The strategy was applied to a series of aldehydes (Scheme 2a). Pleasingly, aldehydes bearing electronically varied substituents underwent the reaction efficiently to afford the corresponding



Scheme 2a: Synthesis of trifluoromethyl triazoline derivatives of N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine.

hybrid derivatives in excellent yields. In addition to that, the reaction was compatible for medicinally relevant heteroaryl aldehydes such as thiophene, indole, furan, and quinoline. Notably, aldehydes bearing synthetic handles such as boronic acid and hydroxyl groups underwent the reaction, offering a suitable platform for further derivatization.

Besides this, a triazoline derivative using N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine **3b** and benzaldehyde **4a** was also synthesized employing the similar reaction conditions (Scheme 2b).



Scheme 2b: Synthesis of trifluoromethyl triazoline derivatives of N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine.

3.3 Biological screening

The synthesized compounds have been submitted to CBRS, CDRI and the biological evaluation for antimalarial activity is currently underway.

4. Experimental data

4.1 General information

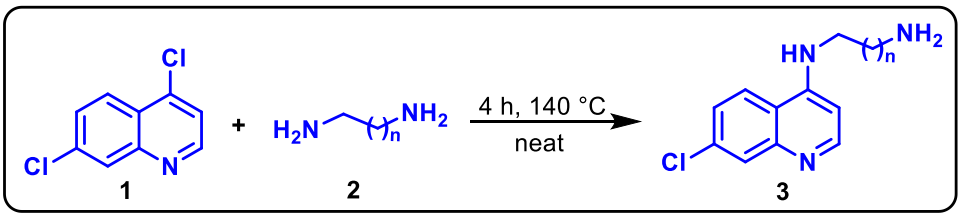
Unless otherwise specified, all reactions were carried out under air atmosphere in oven-dried round bottom flasks. The reactions were monitored by TLC visualized by UV (254 nm and 365 nm) and/or with iodine. Column chromatography was performed on 100-200 mesh silica gels using the gradient system ethyl acetate/hexane and methanol/dichloromethane. NMR data were recorded at Bruker AV 400 MHz in $CDCl_3$ and DMSO-d_6 using as internal standards. The residual $CDCl_3$ signal for ^1H NMR (δ = 7.26 ppm) and for ^{13}C (δ = 77.16 ppm). The signal of DMSO-d_6 for ^1H NMR (δ = 2.50 ppm) and for ^{13}C (δ = 39.50 ppm). Coupling constants are given in hertz (Hz) and the classical abbreviations are used to describe the signal multiplicities. Melting points were measured with a Büchi B-540 and are uncorrected. Mass spectra were obtained using Q-TOF mass spectrometer. All commercially available reagents were used as received. Trifluorodiazoethane were prepared by following a literature procedure [34].

Note: CF_3CHN_2 must be handled with care, and the diazo solutions must be stored at temperatures < 0 °C.

4.2 General reaction procedures

4.2.1 General procedure for the synthesis of *N*1-(7-chloroquinolin-4-yl) diamines

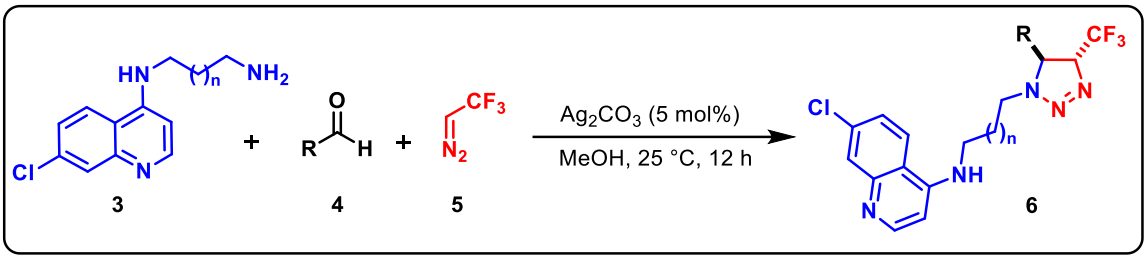
To an oven-dried round bottom flask, diaminoalkane **2** (4.5 equiv) and 4,7-dichloroquinoline **1** (1 equiv) were added and the resulting reaction mixture was allowed to stir at reflux for 4 h. After the completion of the reaction, as indicated by the TLC, the reaction mixture was cooled down to room temperature. The reaction mixture was extracted using DCM and 1N NaOH, and the organic layer.was washed with saturated brine. Finally, the organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give the product **3**.



Scheme 1: Synthesis of *N*1-(7-chloroquinolin-4-yl) diamines.

4.2.2 General procedure for the synthesis of trifluoromethyltriazoline-4-aminoquinoline hybrids

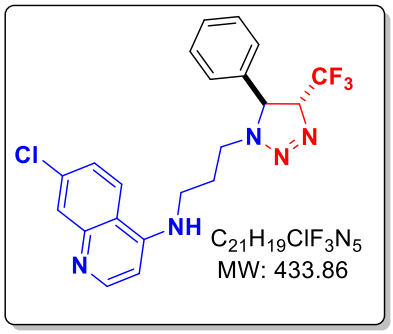
To an oven-dried 25 mL round bottom flask was added aldehyde **4** (0.5 mmol, 1.0 equiv), amine **3** (0.6 mmol, 1.2 equiv) and methanol (2.0 mL), and was allowed to stir for 10 minutes. The reaction mixture was subsequently charged with trifluorodiazoethane **5** stock solution in toluene (1.5 mmol, 3.0 equiv) and Ag_2CO_3 (5 mol%). Then, the resulting reaction mixture was stirred for 12 h at 25 °C. After the completion of reaction, as indicated by TLC, the solvent was evaporated under reduced pressure. The residue was purified using column chromatography (100-200 mesh silica gel) using acetone/dichloromethane as the eluent to afford the desired product **6**.



Scheme 1: Synthesis of trifluoromethyltriazoline-4-aminoquinoline hybrids.

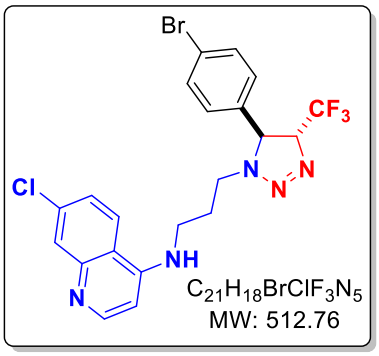
5. Characterization data of compounds 6a-6o and 7

Compound 6a: 7-chloro-*N*-(3-(5-phenyl-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine



Following the general procedure, treatment of benzaldehyde **4a** (53.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **5** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6a** (202.0 mg, 93%). **Major isomer:** brownish viscous compound, $\mathbf{R_f}$ (acetone/dichloromethane: 10/90) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 152.0 (CH), 149.5 (C), 149.2 (C), 137.1 (C), 135.1 (C), 129.7 (CH), 129.7 (CH), 129.4 (CH), 128.8 (CH), 127.0 (CH), 127.0 (CH), 125.5 (CH), 123.6 (q, $J_{C\text{-}F}$ = 276.9 Hz, C), 121.2 (CH), 117.3 (C), 99.1 (CH), 85.3 (q, $J_{C\text{-}F}$ = 28.4 Hz, CH), 62.5 (d, J = 2.2 Hz, CH), 45.7 (CH_2), 40.3 (CH_2), 26.6 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.46 (t, J = 5.2 Hz, 1H), 7.93 (t, J = 2.6 Hz, 1H), 7.60-7.56 (m, 1H), 7.37-7.35 (m, 1H), 7.32 (dd, J = 8.8, 2.8 Hz, 1H), 7.19-7.16 (m, 1H), 6.32-6.30 (m, 1H), 5.51 (s, 1H), 4.83-4.74 (m, 1H), 4.47 (d, J = 10.4 Hz, 1H), 3.73-3.66 (m, 1H), 3.55-3.34 (m, 3H), 2.14-2.01 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.1 (s). **ESMS** for $C_{21}H_{20}ClF_3N_5^+$: calcd. $[M+H]^+$: 434.86, found: 434.30.

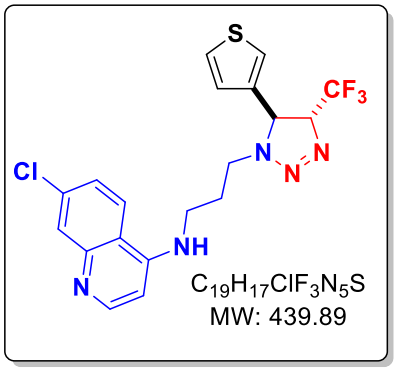
Compound 6b: N-(3-(5-(4-bromophenyl)-4-(trifluoromethyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)propyl)-7-chloroquinolin-4-amine



Following the general procedure, treatment of 4-bromobenzaldehyde **4b** (93.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **5** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6b** (205.0 mg, 80%). **Major isomer:** pale yellow solid compound, **Mp** 118 °C. $\mathbf{R_f}$ (Acetone/dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.6 (CH), 149.8 (C), 148.8 (C), 136.1 (C), 135.3 (C), 132.9 (CH), 132.9 (CH), 128.7 (CH), 128.7 (CH), 128.4 (CH), 125.7 (CH), 123.5 (C), 123.5 (q, $J_{C\text{-}F}$ = 276.7 Hz, C), 121.4 (CH), 117.2 (C), 99.1 (CH), 85.3 (q, $J_{C\text{-}F}$ = 28.4

Hz, CH), 62.0 (CH), 45.8 (CH_2), 40.3 (CH_2), 26.6 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.48 (d, *J* = 5.6 Hz, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.62 (d, *J* = 9.2 Hz, 1H), 7.49-7.46 (m, 2H), 7.32 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.06-7.03 (m, 2H), 6.34 (d, *J* = 5.6 Hz, 1H), 5.45 (s, 1H), 4.74-4.70 (m, 1H), 4.43 (d, *J* = 10.8 Hz, 1H), 3.71-3.64 (m, 1H), 3.52-3.35 (m, 3H), 2.13-2.05 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.0 (s). **ESMS** for $C_{21}H_{19}BrClF_3N_5^+$: calcd. $[M+H]^+$: 513.76, found: 514.20.

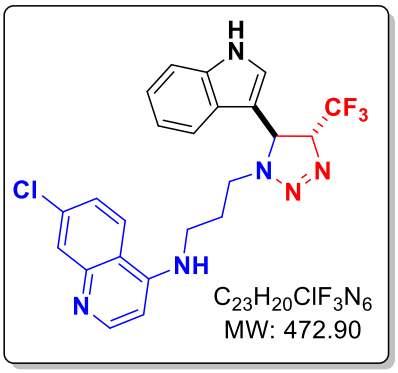
Compound 6c: 7-chloro-*N*-(3-(5-(thiophen-3-yl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine



Following the general procedure, treatment of thiophene-3-carboxaldehyde **4c** (56.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6c** (209.0 mg, 95%). **Major isomer:** brownish viscous compound. **R_f** (Acetone/dichloromethane: 30/70) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.5 (CH), 150.0 (C), 148.7 (C), 137.5 (C), 135.1 (C), 128.5 (CH), 128.1 (CH), 125.4 (CH), 125.0 (CH), 124.1 (CH), 123.6 (q, J_{C-F} = 277.1 Hz, C), 121.7(CH), 117.3 (C), 99.0 (CH), 83.9 (q, J_{C-F} = 28.4 Hz, CH), 57.9 (CH), 45.6 (CH_2), 40.3 (CH_2), 26.7 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.43 (d, *J* = 5.2 Hz, 1H), 7.88 (d, *J* = 2.0 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.34 (dd, *J* = 5.2, 3.2 Hz, 1H), 7.26 (dd, *J* = 8.8, 2,0 Hz, 1H), 7.20 (dd, *J* = 3.2, 1.2 Hz, 1H), 6.86 (dd, *J* = 5.2, 1.6 Hz, 1H), 6.31 (d, *J* = 5.6 Hz, 1H), 5.69 (t, *J* = 5.6 Hz, 1H), 4.79-4.71 (m, 1H), 4.62 (d, *J* = 10.4 Hz, 1H), 3.70-3.63 (m, 1H), 3.52-3.46 (m, 1H), 3.42-3.30 (m, 2H), 2.10-2.00 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.1 (s). **ESMS** for $C_{19}H_{18}ClF_3N_5S^+$: calcd. $[M+H]^+$: 440.89, found: 440.20.

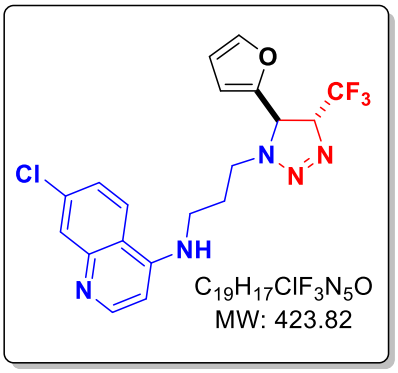
Compound 6d: *N*-(3-((4*R*,5*S*)-5-(1*H*-indol-3-yl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)-7-chloroquinolin-4-amine

Following the general procedure, treatment of indole-3-carboxaldehyde **4d** (73.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6d** (95.0 mg, 40%). **Major isomer:** brownish viscous compound. **R_f**



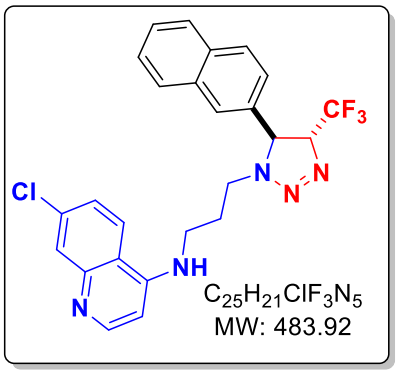
(Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/DMSO-d_6): 151.9 (CH), 150.7 (C), 148.9 (C), 137.3 (C), 134.1 (C), 127.5 (CH), 126.1 (CH), 124.9 (q, $J_{C\text{-}F}$ = 277.9 Hz, C), 124.8 (C), 124.6 (d, J = 8.1 Hz, CH), 124.6 (d, J = 8.1 Hz, CH), 122.2(CH), 119.9 (CH), 118.4 (CH), 117.8 (C), 112.7 (CH), 110.3 (C), 99.0 (CH), 80.8 (q, $J_{C\text{-}F}$ = 27.2 Hz, CH), 56.3 (CH), 45.1 (CH_2), 30.1 (CH_2), 26.9 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/DMSO-d_6): 11.29 (s, 1H), 8.35 (d, J = 4.8 Hz, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.78 (s, 1H),7.56 (s, 1H), 7.43 (t, J = 7.8 Hz, 1H), 7.30 (s, 1H), 7.20-7.11 (m, 2H), 6.99 (t, J = 7.2 Hz, 1H), 6.26 (d, J = 5.2 Hz, 1H), 5.25 (t, J = 8.6 Hz, 1H), 5.13 (d, J = 9.6 Hz, 1H), 3.67-3.35 (m, 4H), 1.97-1.93 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -67.7 (s). **ESMS** for $C_{23}H_{21}ClF_3N_6^+$: calcd. $[M+H]^+$: 473.90, found: 473.30.

Compound 6e: 7-chloro-*N*-(3-((4*R*,5*R*)-5-(furan-2-yl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine



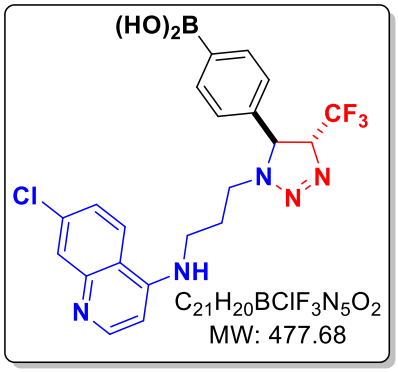
Following the general procedure, treatment of furfural **4e** (48.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6e** (127.0 mg, 60%). **Major isomer:** white solid compound, **Mp** 100 °C. **R$_f$** (Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.7 (CH), 149.7 (d, J = 3.3 Hz, C), 148.9 (C), 147.9 (C), 144.0 (CH), 135.0 (C), 128.4 (d, J = 3.4 Hz, CH), 125.4 (CH), 123.4 (q, $J_{C\text{-}F}$ = 276.9 Hz, C), 121.3(CH), 117.2 (C), 111.0 (CH), 110.3(CH), 99.0 (CH), 81.1 (q, $J_{C\text{-}F}$ = 28.8 Hz, CH), 55.8 (d, J = 2.4 Hz, CH), 45.6 (CH_2), 40.2 (CH_2), 26.7 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.47 (d, J = 5.2 Hz, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.66 (d, J = 8.8 Hz, 1H), 7.35 (dd, J = 2.0, 0.8 Hz, 1H), 7.29 (dd, J = 9.2, 2.4 Hz, 1H), 6.36 (dd, J = 3.6, 0.8 Hz, 1H), 6.32 (dd, J = 3.6, 2.0 Hz, 2H), 5.47 (t, J = 5.6 Hz, 1H), 5.06-4.99 (m, 1H), 4.57 (d, J = 10.0 Hz, 1H), 3.64-3.58 (m, 2H), 3.41-3.34 (m, 2H), 2.07-1.95 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.0 (s). **ESMS** for $C_{19}H_{18}ClF_3N_5O^+$: calcd. $[M+H]^+$: 424.82, found: 424.30.

Compound 6f: 7-chloro-*N*-(3-((4*R*,5*S*)-5-(naphthalen-2-yl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine



Following the general procedure, treatment of 2-naphthaldehyde **4f** (78.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6f** (220.0 mg, 91%). **Major isomer:** pale yellow solid compound, **Mp** 120 °C. $\mathbf{R_f}$ (Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.5 (CH), 149.9 (C), 148.7 (C), 135.0 (C), 134.0 (C), 133.4 (C), 133.1 (C), 129.9 (CH), 127.9-127.8 (m, CH), 127.9-127.8 (m, CH), 127.9-127.8 (m, CH), 127.1 (d, J = 5.7 Hz, CH), 125.2 (CH), 125.2 (CH), 125.2 (CH), 123.7 (q, $J_{C\text{-}F}$ = 277.7 Hz, C), 123.2 (CH), 121.7 (CH), 117.3 (C), 98.9 (CH), 84.8 (q, $J_{C\text{-}F}$ = 28.2 Hz, CH), 62.8 (CH), 45.7 (CH_2), 40.1 (CH_2), 26.6 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.35 (d, J = 5.2 Hz, 1H), 7.85 (d, J = 2.4 Hz, 1H), 7.80-7.77 (m, 2H), 7.74-7.71 (m, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.51-7.48 (m, 2H), 7.44 (d, J = 9.2 Hz, 1H), 7.18 (dd, J = 8.4, 1.6 Hz, 1H), 7.06 (dd, J = 8.8, 2.0 Hz, 1H), 6.22 (d, J = 5.6 Hz, 1H), 5.67 (t, J = 5.6 Hz, 1H), 4.91-4.83 (m, 1H), 4.63 (d, J = 10.4 Hz, 1H), 3.70-3.63 (m, 1H), 3.56-3.50 (m, 1H), 3.41-3.29 (m, 2H), 2.10-1.98 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -72.9 (s). **ESMS** for $C_{25}H_{22}ClF_3N_5^+$: calcd. $[M+H]^+$: 484.92, found: 484.30.

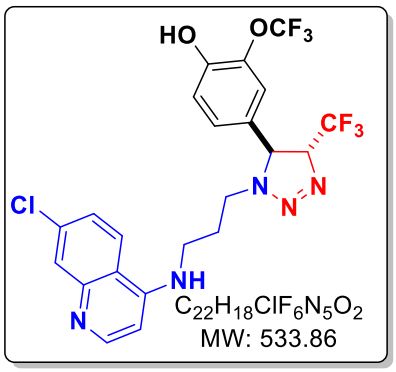
Compound 6g: (4-((4*R*,5*S*)-1-(3-((7-chloroquinolin-4-yl)amino)propyl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-5-yl)phenyl)boronic acid



Following the general procedure, treatment of 4-carboxyphenylboronic acid **4g** (75.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6g** (115.0 mg, 48%). **Major isomer:** yellow solid compound, **Mp** 144 °C. $\mathbf{R_f}$ (Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.1 (d, J = 8.4 Hz, C), 149.7 (d, J = 7.7 Hz, CH), 146.6 (d, J = 7.9 Hz, C), 137.1 (C), 136.2 (d, J = 2.8 Hz, C), 129.7 (CH), 129.7 (CH),

129.4 (CH), 127.0 (CH), 127.0 (CH), 126.2 (d, J = 11.6 Hz, CH), 126.0 (CH), 123.7 (q, $J_{C\text{-}F}$ = 277.0 Hz, C), 122.2 (C), 116.9 (C), 98.8 (CH), 85.1 (q, $J_{C\text{-}F}$ = 28.4 Hz, CH), 62.4 (CH), 45.7 (CH_2), 40.5 (CH_2), 26.5 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.40 (d, J = 5.6 Hz, 1H), 7.85 (s, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.40-7.35 (m, 3H), 7.31 (dd, J = 9.2, 2.0 Hz, 1H), 7.21-7.16 (m, 2H), 6.36 (d, J = 6.0 Hz, 1H), 5.83 (s, 1H), 4.83-4.74 (m, 1H), 4.51 (d, J = 10.4 Hz, 1H), 3.75-3.68 (m, 1H), 3.56-3.36 (m, 4H), 2.18-2.05 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.1 (s). **ESMS** for $C_{21}H_{21}BClF_3N_5O_2^+$: calcd. $[M+H\text{-}B(OH)_2]^+$: 434.86, found: 434.30.

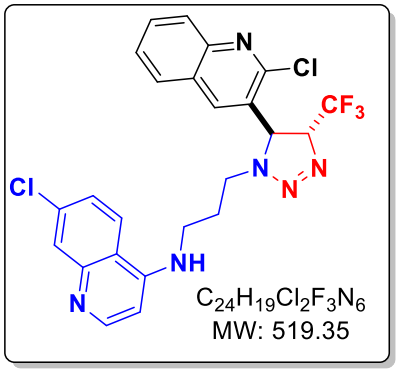
Compound 6h: 4-((4*R*,5*S*)-1-(3-((7-chloroquinolin-4-yl)amino)propyl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-5-yl)-2-(trifluoromethoxy)phenol



Following the general procedure, treatment of 4-hydroxy-3-(trifluoromethoxy)benzaldehyde **4h** (103.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6h** (152.0 mg, 57%). **Major isomer:** yellow solid compound, **Mp** 135 °C. **R_f** (Acetone/ dichloromethane: 30/70) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.2 (C), 150.6 (C), 150.5 (CH), 147.5 (C), 137.5 (C), 135.8 (C), 127.7 (CH), 126.9 (CH), 126.5 (CH),125.8 (CH), 123.8 (q, $J_{C\text{-}F}$ = 271.7 Hz, C), 121.7(CH), 121.3 (CH),119.1 (CH), 117.0 (C), 98.7 (CH), 84.8 (q, $J_{C\text{-}F}$ = 22.4 Hz, CH), 62.0 (CH), 45.9 (CH_2), 40.3 (CH_2), 26.6 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.31 (d, J = 4.4 Hz, 1H), 7.81 (s, 1H), 7.67 (d, J = 7.2 Hz, 1H), 7.29-7.26 (m, 1H), 6.98 (s, 1H), 6.91 (d, J = 6.8 Hz, 1H), 6.87-6.85 (m, 1H), 6.26 (d, J = 4.8 Hz, 1H), 4.72-4.67 (m, 1H), 4.38 (d, J = 8.4 Hz, 1H), 3.63-3.58 (m, 1H), 3.41-3.30 (m, 5H), 2.08-2.00 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -58.0 (s), -73.0 (s). **ESMS** for $C_{22}H_{19}ClF_6N_5O_2^+$: calcd. $[M+H]^+$: 534.86, found: 534.30.

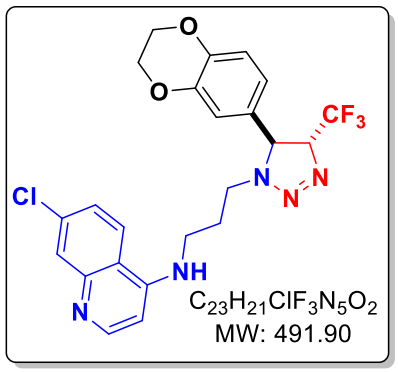
Compound 6i: 7-chloro-*N*-(3-((4*R*,5*S*)-5-(2-chloroquinolin-3-yl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine

Following the general procedure, treatment of 2-chloro-3-quinolinecarboxaldehyde **4i** (96.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87



mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6i** (187.0 mg, 72%). **Major isomer:** white solid compound, **Mp** 160 °C. **R_f** (Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.5 (CH), 149.8 (C), 148.6 (C), 148.3 (C), 147.8 (C), 135.4 (C), 132.0 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 127.6 (CH), 127.0 (CH), 125.7 (CH), 123.4 (q, $J_{C\text{-}F}$ = 277.8 Hz, C), 121.2(CH), 117.1 (C), 99.1 (CH), 84.4 (q, $J_{C\text{-}F}$ = 29.5 Hz, CH), 59.7 (CH), 45.8 (CH_2), 40.3 (CH_2), 26.8 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.43 (d, *J* = 5.6 Hz, 1H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.93 (s, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.77 (t, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.58-7.48 (m, 2H), 7.23 (dd, *J* = 9.2, 2.4 Hz, 1H), 6.32 (d, *J* = 5.6 Hz, 1H), 5.42 (s, 1H), 5.13-5.06 (m, 2H), 3.86-3.79 (m, 1H), 3.59-3.53 (m, 1H), 3.48-3.38 (m, 2H), 2.20-2.09 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.0 (s). **ESMS** for $C_{24}H_{20}Cl_2F_3N_6^+$: calcd. $[M+H]^+$: 520.35, found: 519.30.

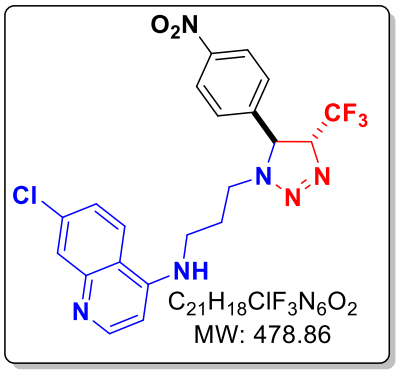
Compound 6j: 7-chloro-N-(3-((4R,5S)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-(trifluoromethyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)propyl)quinolin-4-amine



Following the general procedure, treatment of 1,4-benzodioxan-6-carboxaldehyde **4j** (82.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6j** (150.0 mg, 95%). **Major isomer:** pale yellow solid compound, **Mp** 136 °C. **R_f** (Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 152.0 (CH), 149.5 (C), 149.1 (C), 144.5 (C), 144.4 (C), 135.2 (C), 130.0 (C), 128.8 (CH), 125.6 (CH), 125.1 (CH), 123.7 (q, $J_{C\text{-}F}$ = 276.5 Hz, C), 121.1(CH), 120.0 (CH), 118.5 (CH), 117.3 (C), 115.9 (CH), 99.2 (CH), 85.1 (q, $J_{C\text{-}F}$ = 27.9 Hz, CH), 64.4 (CH_2), 64.4 (CH_2), 62.1 (CH), 45.5 (CH_2), 40.3 (CH_2), 26.7 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.52 (d, *J* = 5.6 Hz, 1H), 7.95 (d, *J* = 2.0 Hz, 1H), 7.55 (d, *J* = 9.2 Hz, 1H), 7.35 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.69 (d, *J* = 2.0 Hz, 1H), 6.65 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.36 (d, *J* = 5.6 Hz, 1H), 5.13 (t, *J* = 5.6 Hz, 1H), 4.79-4.70 (m, 1H), 4.36 (d, *J* = 10.4 Hz, 1H), 4.25-4.18 (m, 4H), 3.71-3.64 (m, 1H), 3.54-

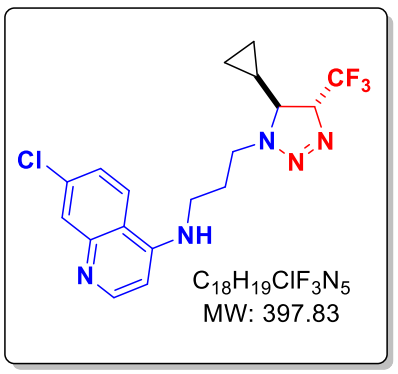
3.35 (m, 3H), 2.11-2.04 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.1 (s). **ESMS** for $C_{23}H_{22}ClF_3N_5O_2^+$: calcd. $[M+H]^+$: 492.90, found: 492.30.

Compound 6k: 7-chloro-*N*-(3-((4*R*,5*S*)-5-(4-nitrophenyl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine



Following the general procedure, treatment of 4-nitrobenzaldehyde **4k** (76.0 mg, 0.50 mmol), *N*^{1}-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6k** (134.0 mg, 56%). **Major isomer:** orange solid compound, **Mp** 140 °C. **R_f** (Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.9 (CH), 149.6 (C), 149.0 (C), 148.5 (C), 144.1 (C), 135.3 (C), 128.7 (CH), 128.0 (CH), 128.0 (CH), 125.7 (CH), 124.9 (CH), 124.9 (CH), 123.3 (q, J_{C-F} = 277.0 Hz, C), 121.1(CH), 117.3 (C), 99.1 (CH), 85.7 (q, J_{C-F} = 28.7 Hz, CH), 61.8 (CH), 46.3 (CH_2), 40.4 (CH_2), 26.7 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.48 (d, *J* = 5.2 Hz, 1H), 8.19-8.16 (m, 2H), 7.91 (d, *J* = 2.0 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.36-7.34 (m, 2H), 7.30 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.35 (d, *J* = 5.2 Hz, 1H), 5.36 (t, *J* = 5.6 Hz, 1H), 4.80-4.72 (m, 1H), 4.57 (d, *J* = 10.8 Hz, 1H), 3.79-3.72 (m, 1H), 3.55-3.36 (m, 3H), 2.14-2.09 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -72.8 (s). **ESMS** for $C_{21}H_{19}ClF_3N_6O_2^+$: calcd. $[M+H]^+$: 479.86, found: 479.30.

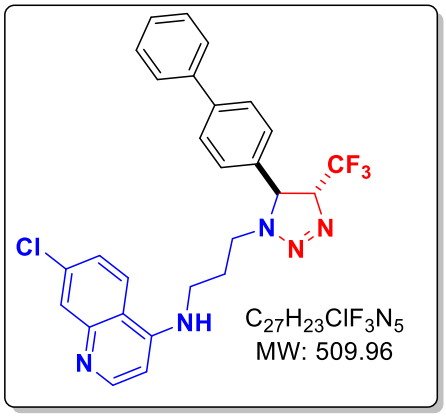
Compound 6l: 7-chloro-*N*-(3-((4*R*,5*S*)-5-cyclopropyl-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine



Following the general procedure, treatment of cyclopropanecarboxaldehyde **4l** (35.0 mg, 0.50 mmol), *N*^{1}-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6l** (60.0 mg, 30%). **Major isomer:** white solid compound, **Mp** 108 °C. **R_f** (Acetone/dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 150.7 (CH), 148.9 (C), 147.8 (C), 134.3 (C), 127.5 (CH), 124.7 (CH), 122.6 (q, J_{C-F} = 276.8 Hz, C), 120.3(CH), 116.3 (C), 98.1 (CH),

80.9 (q, J_{C-F} = 28.5 Hz, CH), 62.1 (CH), 44.6 (CH_2), 39.5 (CH_2), 25.9 (CH_2), 12.9 (CH_2), 4.3 (CH_2), 0.01 (CH). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.50 (d, *J* = 5.6 Hz, 1H), 7.93 (d, *J* = 2.4 Hz, 1H), 7.70 (d, *J* = 9.2 Hz, 1H), 7.35 (dd, *J* = 9.2, 2.4 Hz, 1H), 6.40 (d, *J* = 5.2 Hz, 1H), 5.46 (s, 1H), 4.72-4.64 (m, 1H), 3.93-3.88 (m, 1H), 3.80-3.73 (m, 1H), 3.52-3.40 (m, 2H), 2.80 (t, *J* = 9.2 Hz, 1H), 2.20-2.17 (m, 2H), 0.76-0.71 (m, 1H), 0.64-0.57 (m, 1H), 0.44-0.38 (m, 1H), 0.30-0.24 (m, 1H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.6 (s). **ESMS** for $C_{18}H_{20}ClF_3N_5^+$: calcd. $[M+H]^+$: 398.83, found: 398.30.

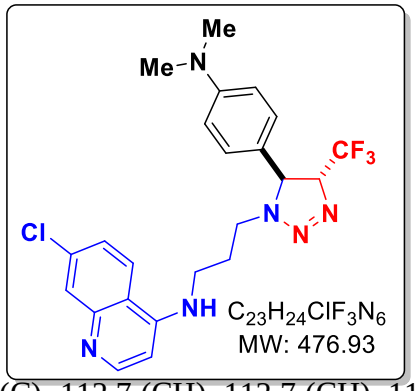
Compound 6m: *N*-(3-((4*R*,5*S*)-5-([1,1'-biphenyl]-4-yl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)-7-chloroquinolin-4-amine



Following the general procedure, treatment of biphenyl-4-carboxaldehyde **4m** (91.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6m** (230.0 mg, 90%). **Major isomer:** pale yellow solid compound, **Mp** 128 °C. **R_f** (Acetone/ dichloromethane: 10/90) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.7 (CH), 149.7 (C), 148.9 (C), 142.5 (C), 139.8 (C), 135.9 (C), 135.2 (C), 129.1 (CH), 129.1 (CH), 128.5 (CH), 128.3 (CH), 128.3 (CH), 128.1 (CH), 127.1 (CH), 127.1 (CH), 125.6 (CH), 124.0 (q, J_{C-F} = 278.3 Hz, C), 121.2(CH), 117.2 (C), 99.1 (CH), 85.3 (q, J_{C-F} = 22.8 Hz, CH), 62.3 (CH), 45.7 (CH_2), 40.3 (CH_2), 26.7 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.48 (d, *J* = 5.2 Hz, 1H), 7.92 (d, *J* = 2.4 Hz, 1H), 7.59-7.51 (m, 5H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.24-7.23 (m, 1H), 6.35 (d, *J* = 5.2 Hz, 1H), 5.30 (t, *J* = 5.6 Hz, 1H), 4.87-4.79 (m, 1H), 4.53 (d, *J* = 10.4 Hz, 1H), 3.77-3.70 (m, 1H), 3.60-3.36 (m, 3H), 2.25-2.20 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.0 (s). **ESMS** for $C_{27}H_{24}ClF_3N_5^+$: calcd. $[M+H]^+$: 510.96, found: 510.30.

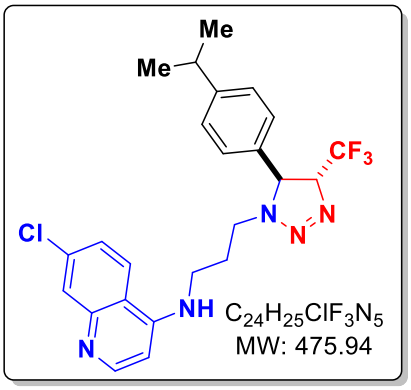
Compound 6n: 7-chloro-*N*-(3-((4*R*,5*S*)-5-(4-(dimethylamino)phenyl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine

Following the general procedure, treatment of 4-(dimethylamino)benzaldehyde **4n** (75.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL,



1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6n** (143.0 mg, 60%). **Major isomer:** brownish viscous compound. $\mathbf{R_f}$ (Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.0 (CH), 150.8 (C), 150.3 (C), 148.0 (C), 135.4 (C), 128.0 (CH), 128.0 (CH), 125.5 (CH), 123.8 (q, $J_{C\text{-}F}$ = 276.9 Hz, C), 123.5 (CH), 122.0 (CH), 117.1 (C), 112.7 (CH), 112.7 (CH), 111.9 (C), 98.9 (CH), 84.5 (q, $J_{C\text{-}F}$ = 28.1 Hz, CH), 62.3 (CH), 45.2 (CH_2), 40.3 (CH_3), 40.3 (CH_3), 40.2 (CH_2), 26.6 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.44 (d, *J* = 3.6 Hz, 1H), 7.92 (d, *J* = 2.4 Hz, 1H), 7.63 (d, *J* = 12.8 Hz, 1H), 7.30-7.27 (m, 1H), 7.01 (dd, *J* = 8.4, 1.6 Hz, 2H), 6.62 (dd, *J* = 8.4, 1.6 Hz, 2H), 6.32 (d, *J* = 2.8 Hz, 1H), 5.58 (s, 1H), 4.78-4.72 (m, 1H), 4.39 (d, *J* = 10.4 Hz, 1H), 3.54-3.41 (m, 4H), 2.93-2.92 (m, 6H), 2.14-2.02 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.2 (s). **ESMS** for $C_{23}H_{25}ClF_3N_6^+$: calcd. $[M+H]^+$: 477.93, found: 477.30.

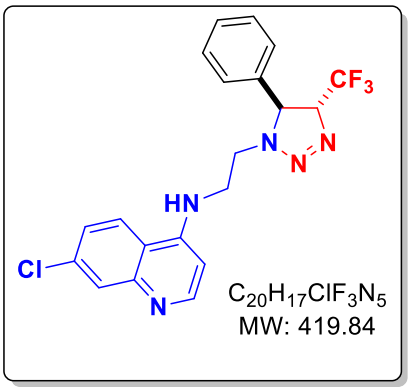
Compound 6o: 7-chloro-*N*-(3-((4*R*,5*S*)-5-(4-isopropylphenyl)-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)propyl)quinolin-4-amine



Following the general procedure, treatment of 4-isopropylbenzaldehyde **4o** (74.0 mg, 0.50 mmol), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine **3a** (141.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **6o** (217.0 mg, 91%). **Major isomer:** pale yellow solid compound, **Mp** 113 °C. $\mathbf{R_f}$ (Acetone/ dichloromethane: 10/90) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.9 (CH), 150.3 (C), 149.7 (C), 149.1 (C), 135.0 (C), 134.2 (C), 128.6 (CH), 127.7 (CH), 127.7 (CH), 126.9 (CH), 126.9 (CH), 125.4 (CH), 123.7 (q, $J_{C\text{-}F}$ = 276.9 Hz, C), 121.4 (CH), 117.3 (C), 99.0 (CH), 85.0 (q, $J_{C\text{-}F}$ = 28.1 Hz, CH), 62.1 (CH), 45.5 (CH_2), 40.2 (CH_2), 33.8 (CH), 26.6 (CH_2), 23.8 (d, *J* = 5.3 Hz, CH_3), 23.8 (d, *J* = 5.3 Hz, CH_3). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.48 (dd, *J* = 3.6, 2.0 Hz, 1H), 7.92 (d, *J* = 2.4 Hz, 1H), 7.61-7.57 (m, 1H), 7.32-7.27 (m, 1H), 7.16 (d, *J* = 7.6 Hz, 2H), 7.07 (dd, *J* = 8.4, 2.0 Hz, 2H), 6.34 (d, *J* = 4.8 Hz, 1H), 5.34 (s, 1H), 4.80-4.72 (m, 1H), 4.46 (d, *J* = 10.4 Hz, 1H), 3.73-3.66 (m, 1H), 3.50-3.34 (m, 3H), 2.91-2.84 (m, 1H)

2.12-2.02 (m, 2H), 1.21 (d, *J* = 6.8 Hz, 6H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.1 (s). **ESMS** for $C_{24}H_{26}ClF_3N_5^+$: calcd. $[M+H]^+$: 476.94, found: 477.30.

Compound 7: 7-chloro-*N*-(2-((4*R*,5*S*)-5-phenyl-4-(trifluoromethyl)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl)ethyl)quinolin-4-amine



Following the general procedure, treatment of benzaldehyde **4a** (53.0 mg, 0.50 mmol), *N*^{1}- (7-chloroquinolin-4-yl)ethane-1,2-diamine **3b** (133.0 mg, 0.60 mmol) and Ag_2CO_3 (7.0 mg, 0.025 mmol) with trifluorodiazoethane stock solution **3** in toluene (1.87 mL, 1.50 mmol) in methanol (2 mL) at 25 °C for 12 h followed by column chromatography afforded the product **7** (176.0 mg, 84%). **Major isomer:** pale yellow solid compound, **Mp** 125 °C. **R_f** (Acetone/ dichloromethane: 20/80) = 0.20. **^{13}C NMR** (100 MHz, δ ppm/$CDCl_3$): 151.8 (CH), 149.1 (C), 149.0 (C), 136.6 (C), 135.1 (C), 129.6 (CH), 129.6 (CH), 129.4 (CH), 128.7 (CH), 126.9 (CH), 126.9 (CH), 125.7 (CH), 123.4 (q, J_{C-F} = 276.8 Hz, C), 121.1 (CH), 117.2 (C), 99.0 (CH), 85.3 (q, J_{C-F} = 28.4 Hz, CH), 62.7 (CH), 46.5 (CH_2), 41.3 (CH_2). **^{1}H NMR** (400 MHz, δ ppm/$CDCl_3$): 8.36 (d, *J* = 5.2 Hz, 1H), 7.85 (s, 1H), 7.51 (d, *J* = 9.2 Hz, 1H), 7.26-7.18 (m, 4H), 7.06 (d, *J* = 7.2 Hz, 2H), 6.16 (d, *J* = 5.2 Hz, 1H), 5.45 (s, 1H), 4.75-4.66 (m, 1H), 4.41 (d, *J* = 10.8 Hz, 1H), 3.78-3.69 (m, 2H), 3.59-3.42 (m, 2H). **^{19}F NMR** (376 MHz δ ppm/$CDCl_3$): -73.0 (s). **ESMS** for $C_{20}H_{18}ClF_3N_6^+$: calcd. $[M+H]^+$: 420.84, found: 420.30.

6. Spectral data for compounds 6a-6o and 7

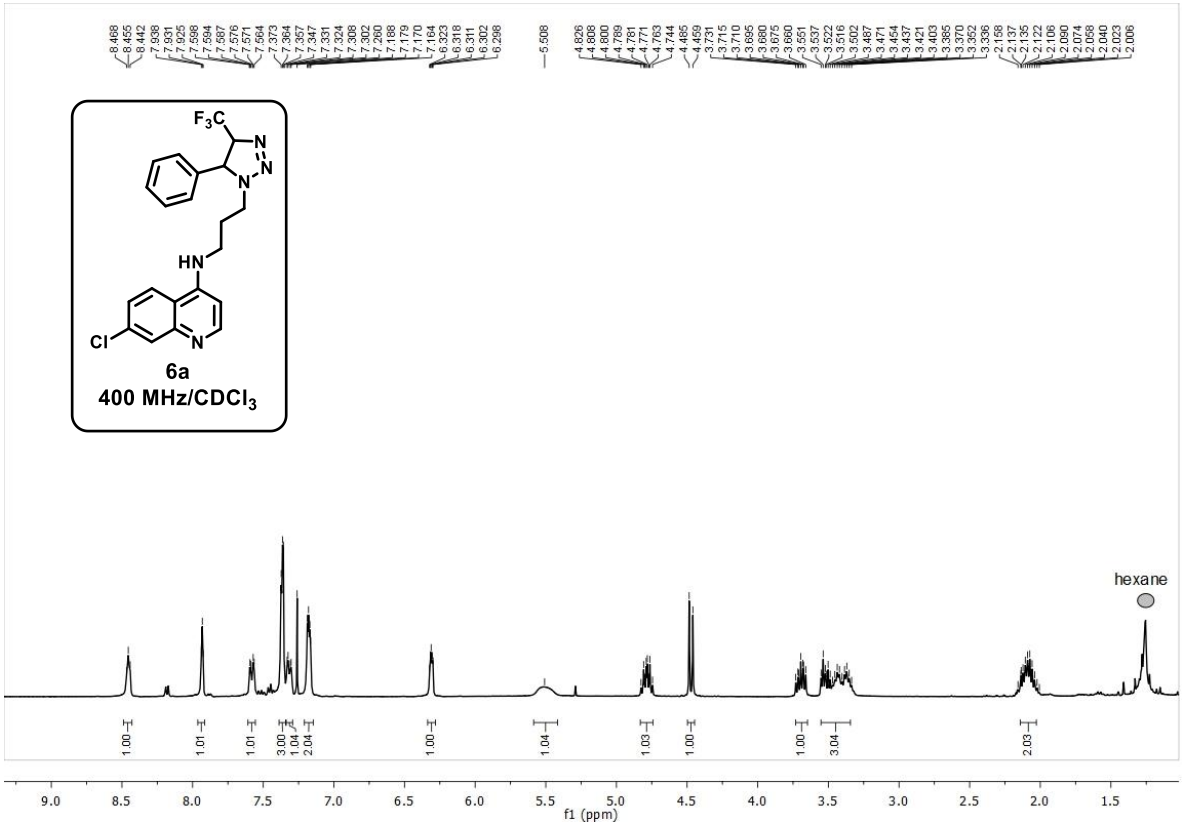


Figure 10: 1H NMR spectrum of 6a

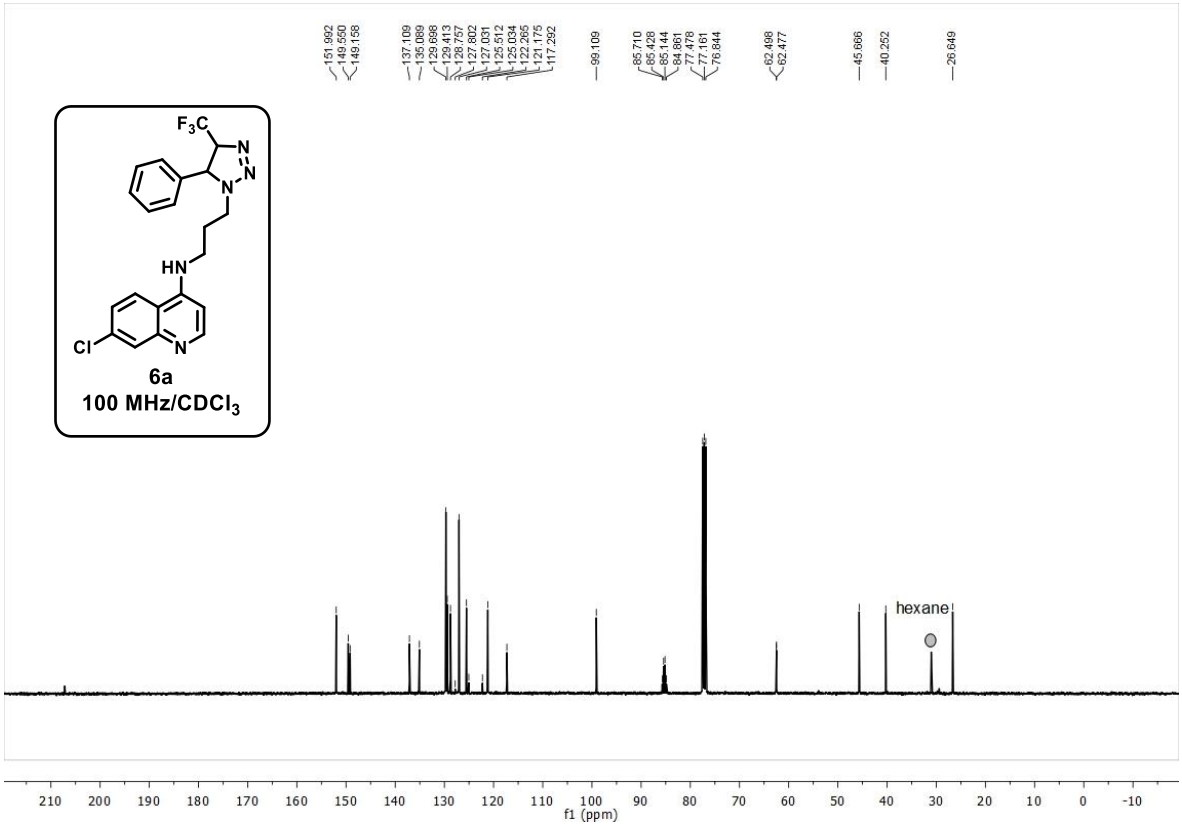


Figure 11: 13C NMR spectrum of 6a

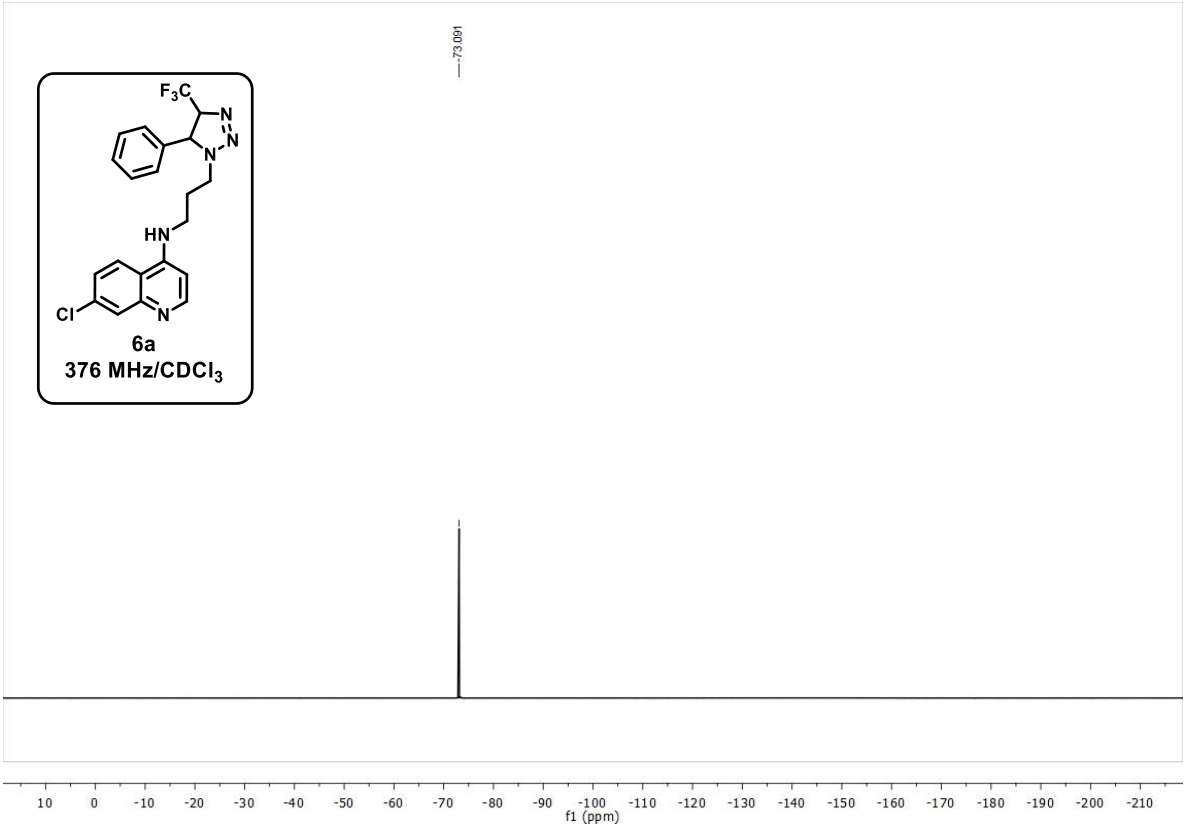


Figure 12: 19F NMR spectrum of 6a

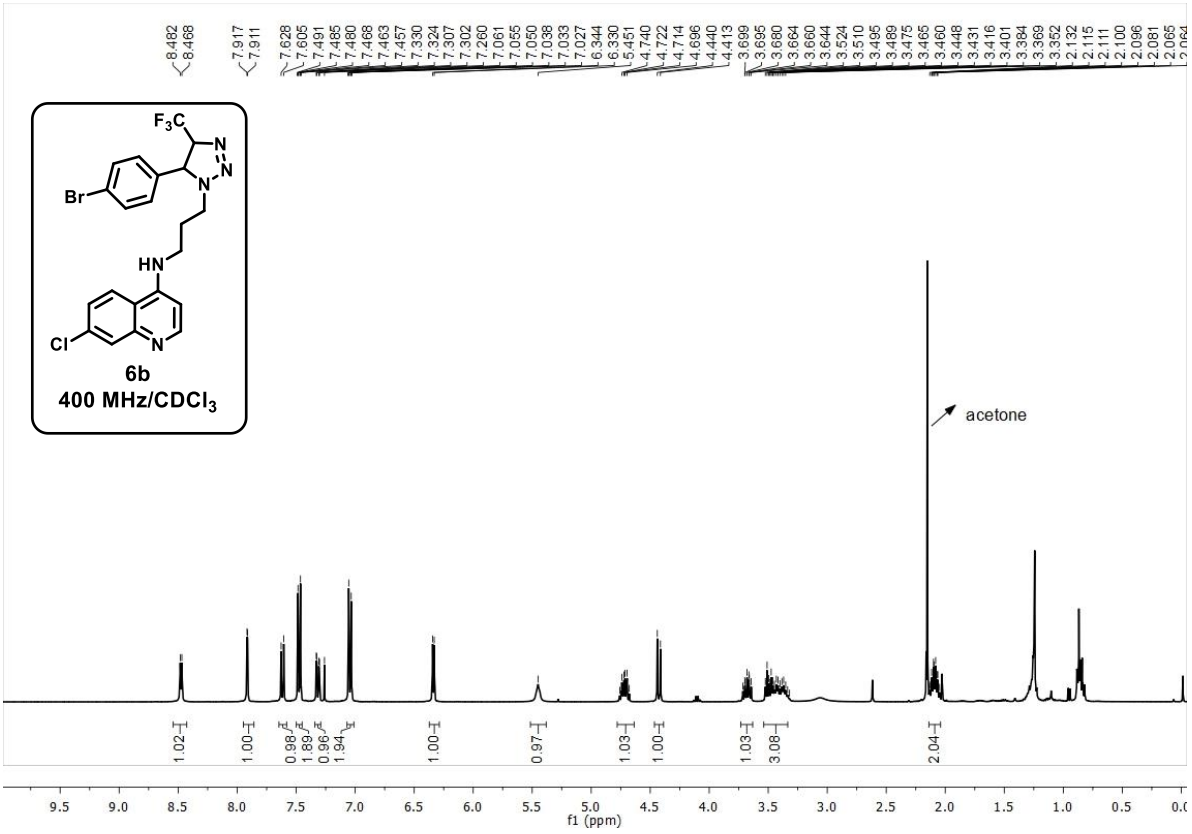


Figure 13: 1H NMR spectrum of 6b

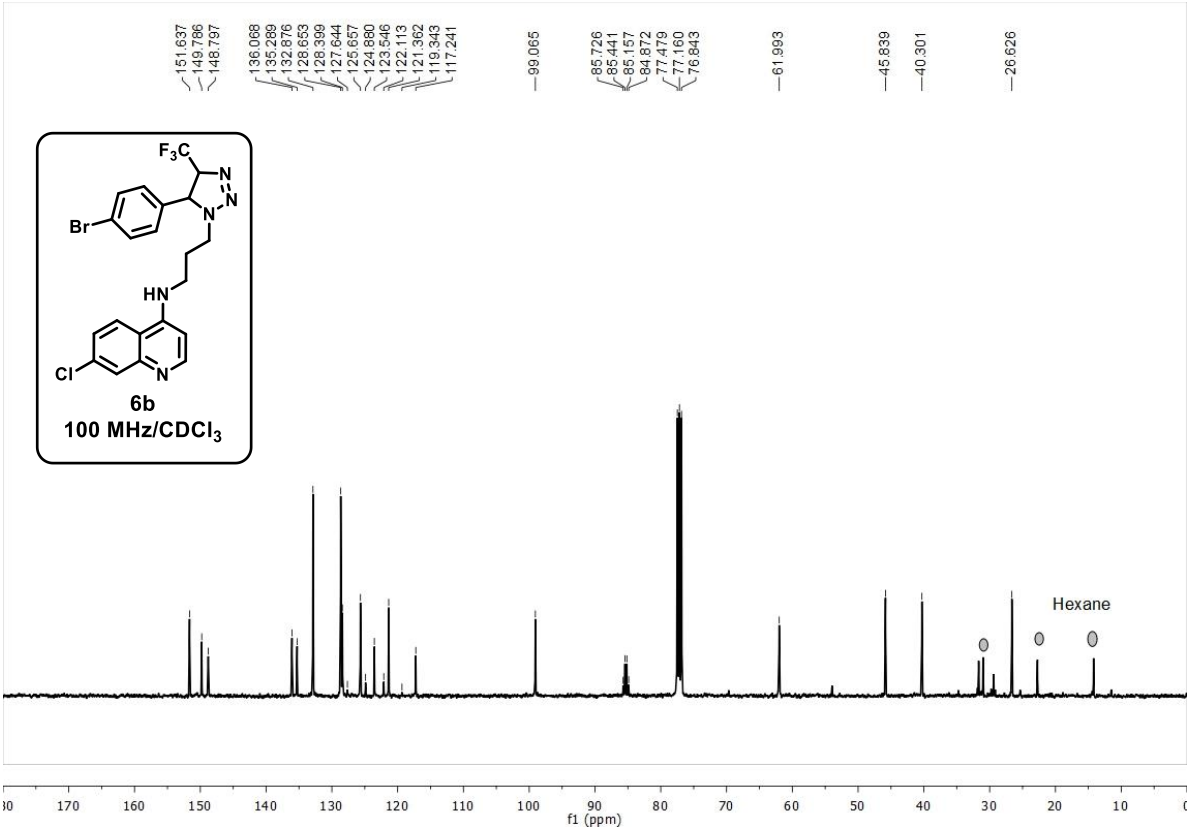


Figure 14: 13C NMR spectrum of 6b

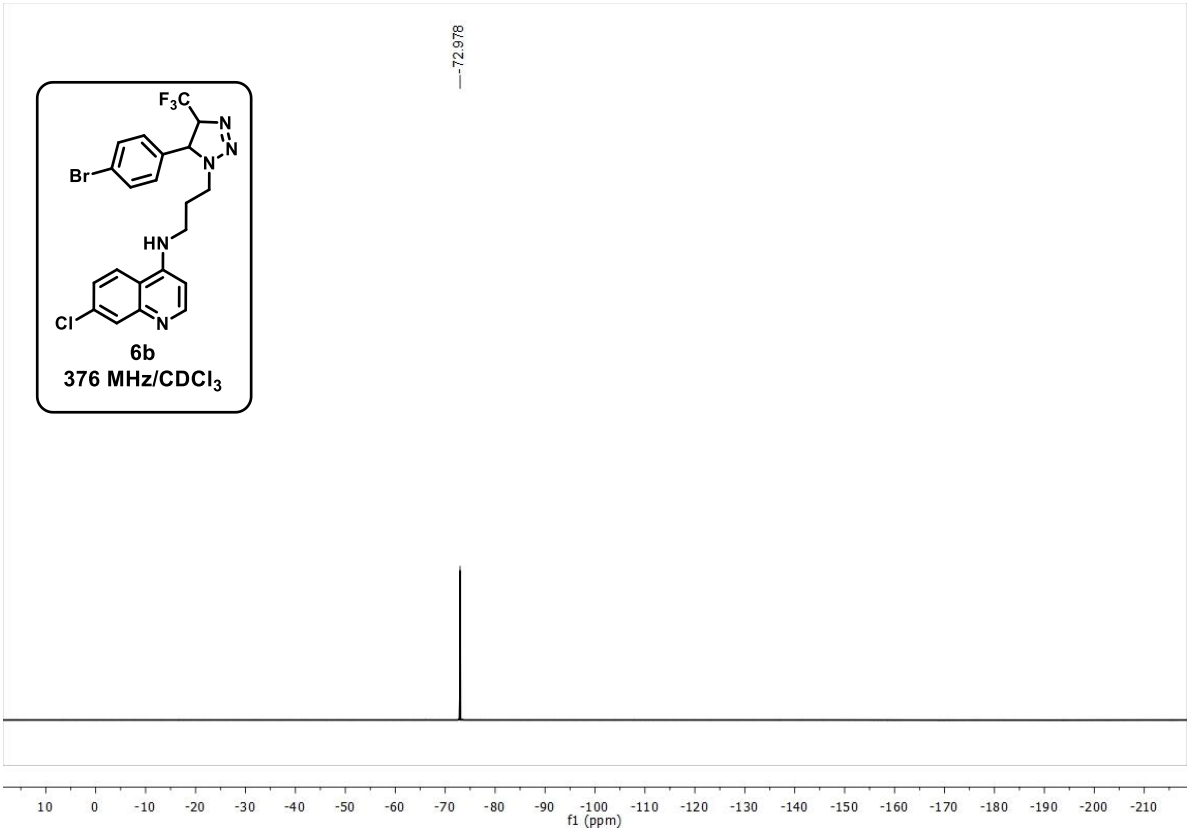


Figure 15: 19F NMR spectrum of 6b

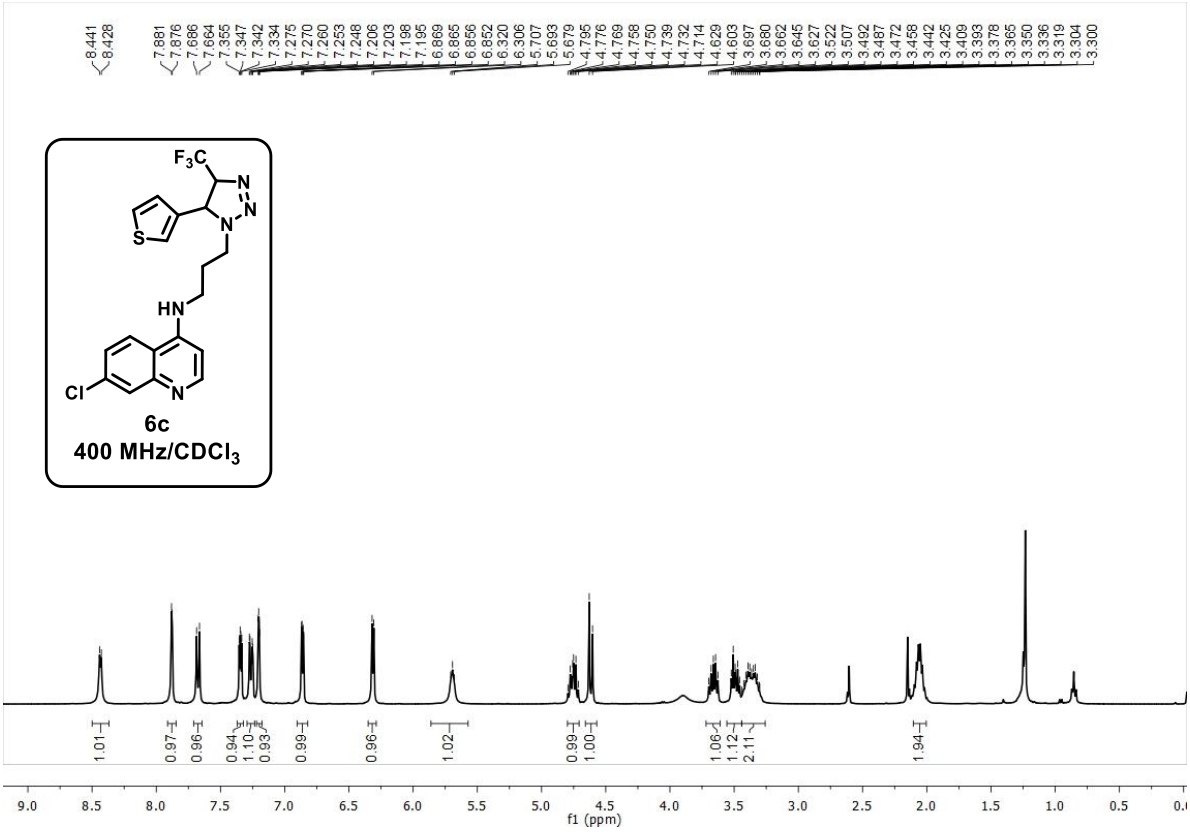


Figure 16: 1H NMR spectrum of 6c

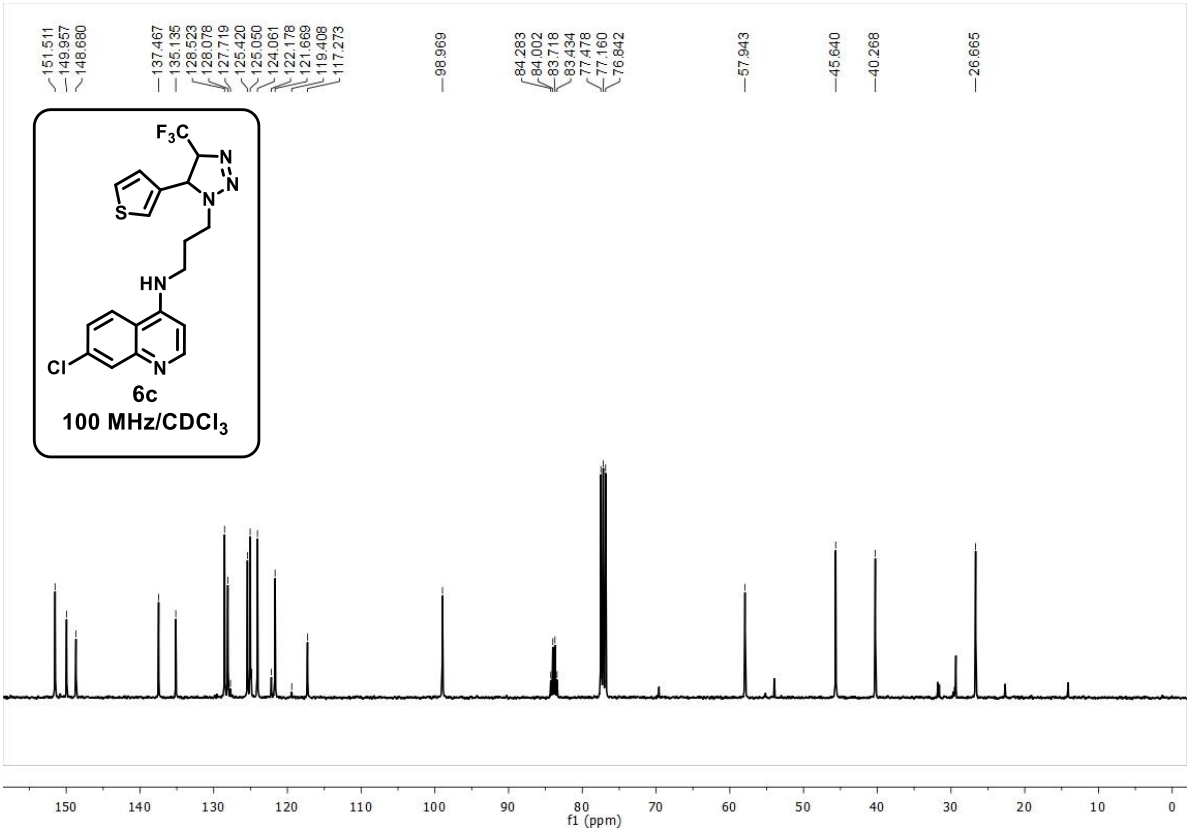


Figure 17: 13C NMR spectrum of 6c

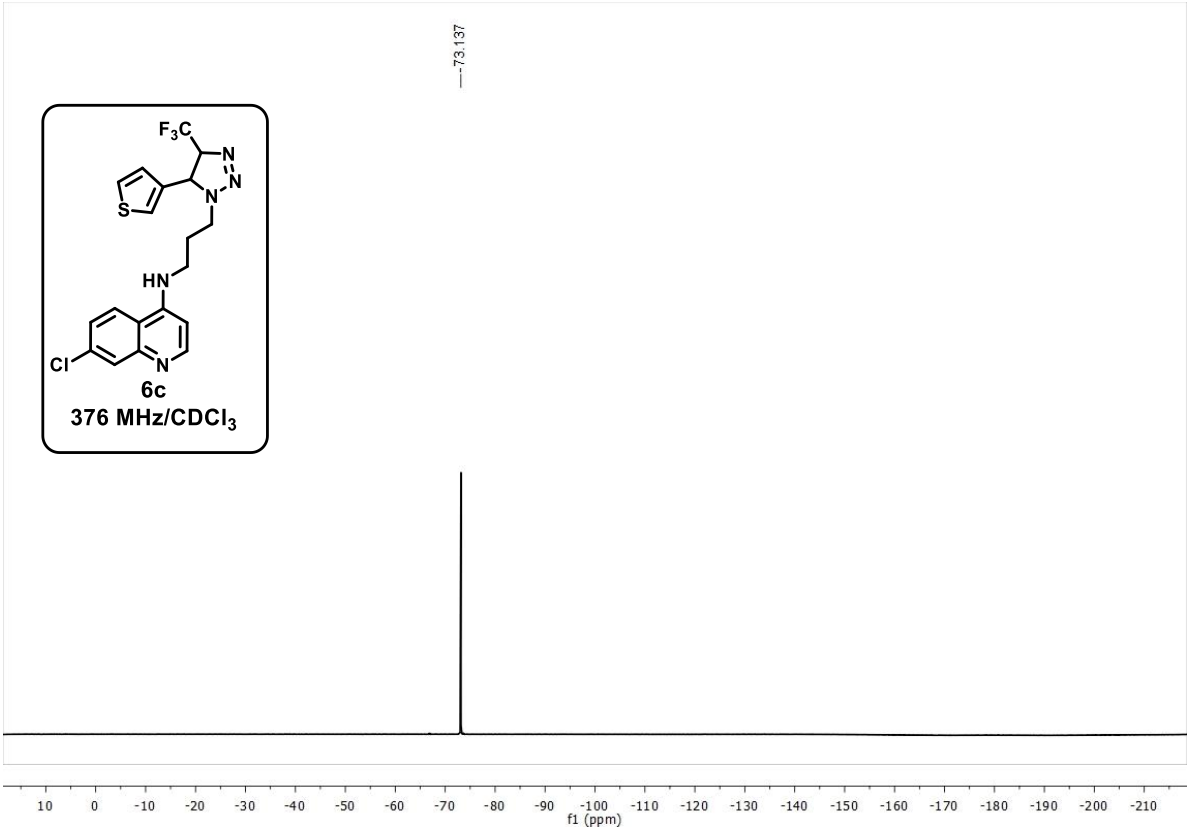


Figure 18: 19F NMR spectrum of 6c

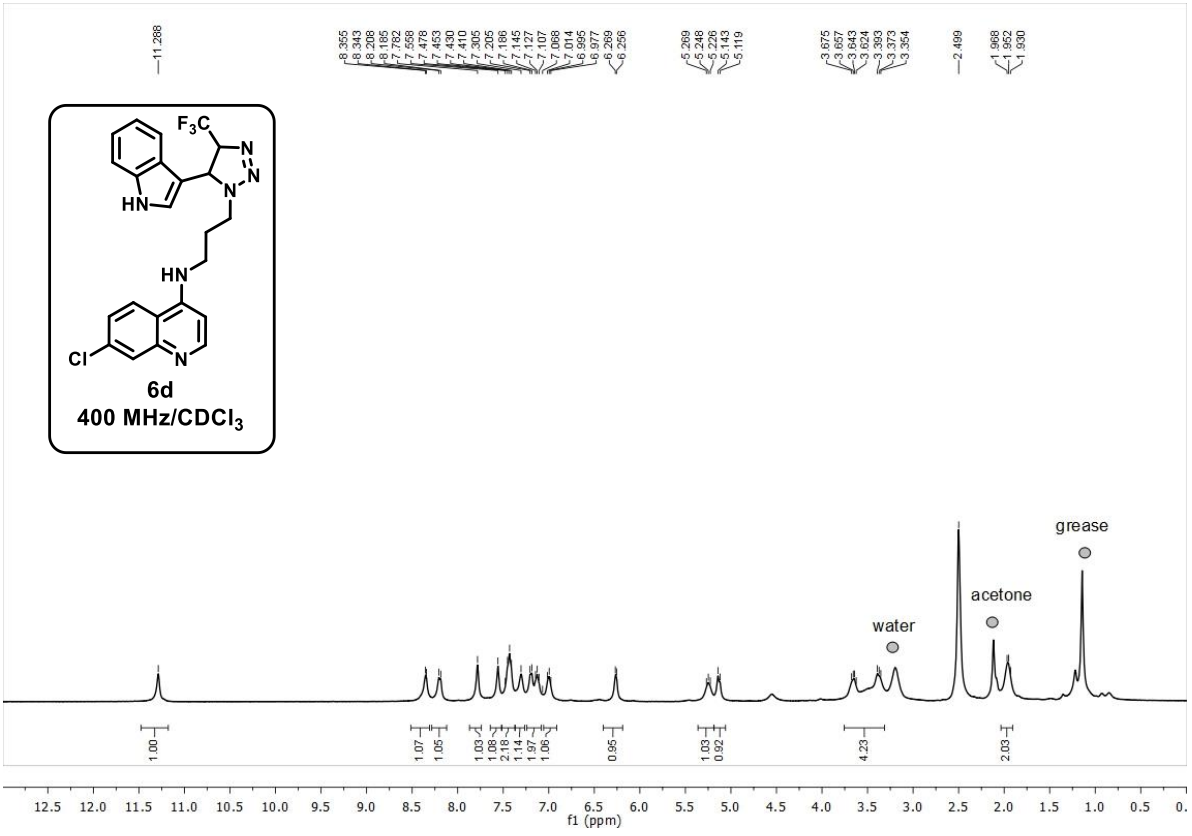


Figure 19: 1H NMR spectrum of 6d

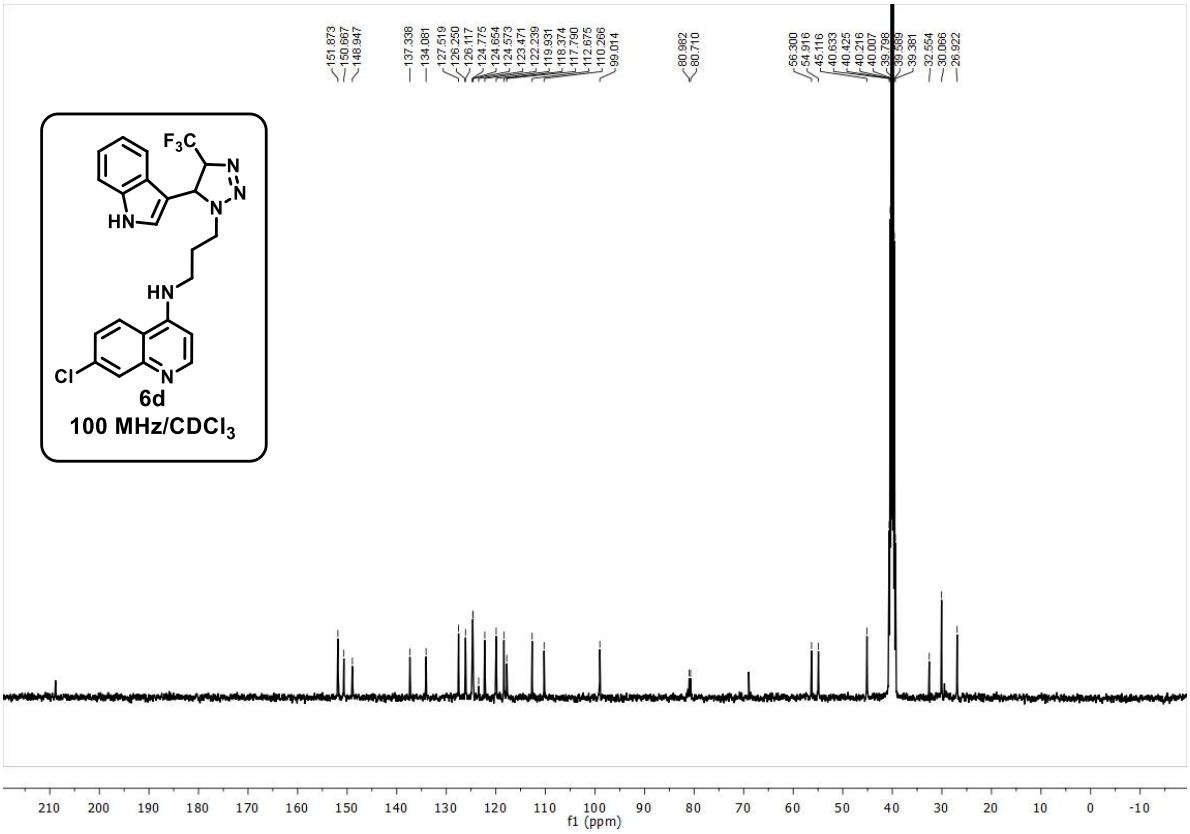


Figure 20: 13C NMR spectrum of 6d

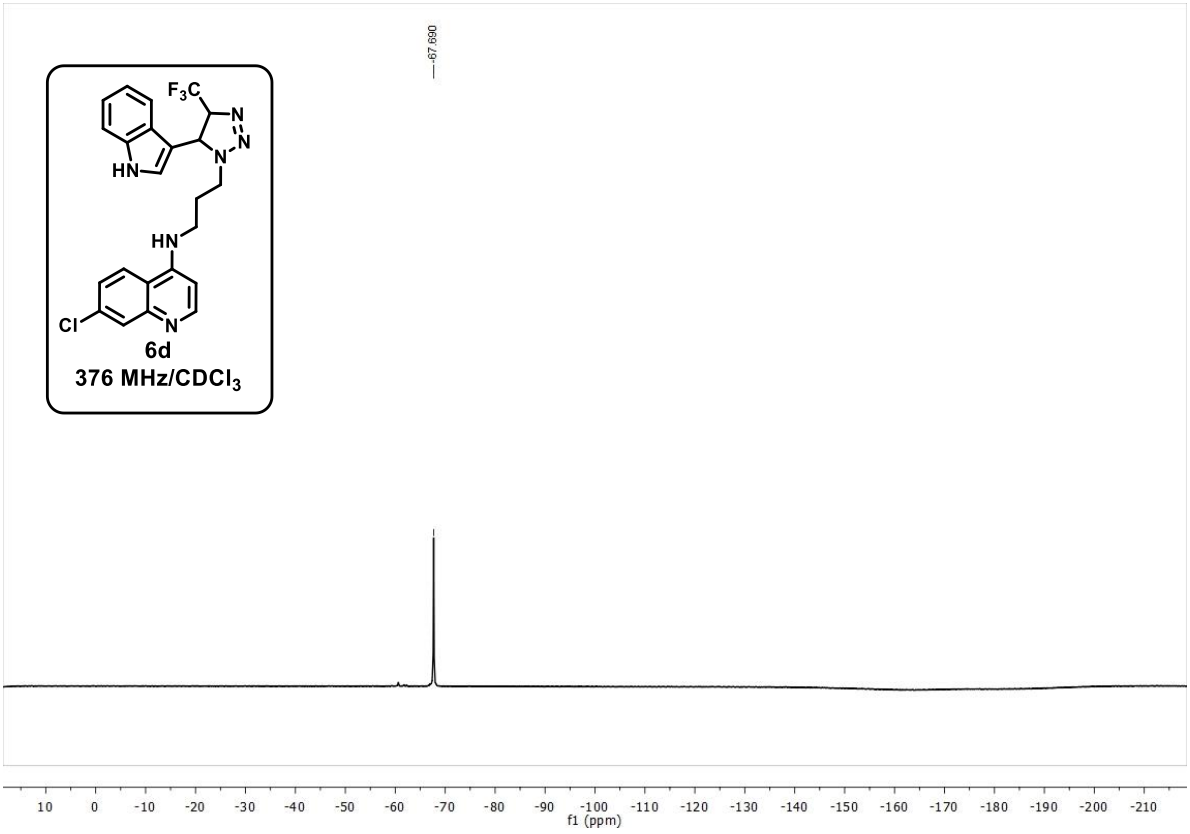


Figure 21: 19F NMR spectrum of 6d

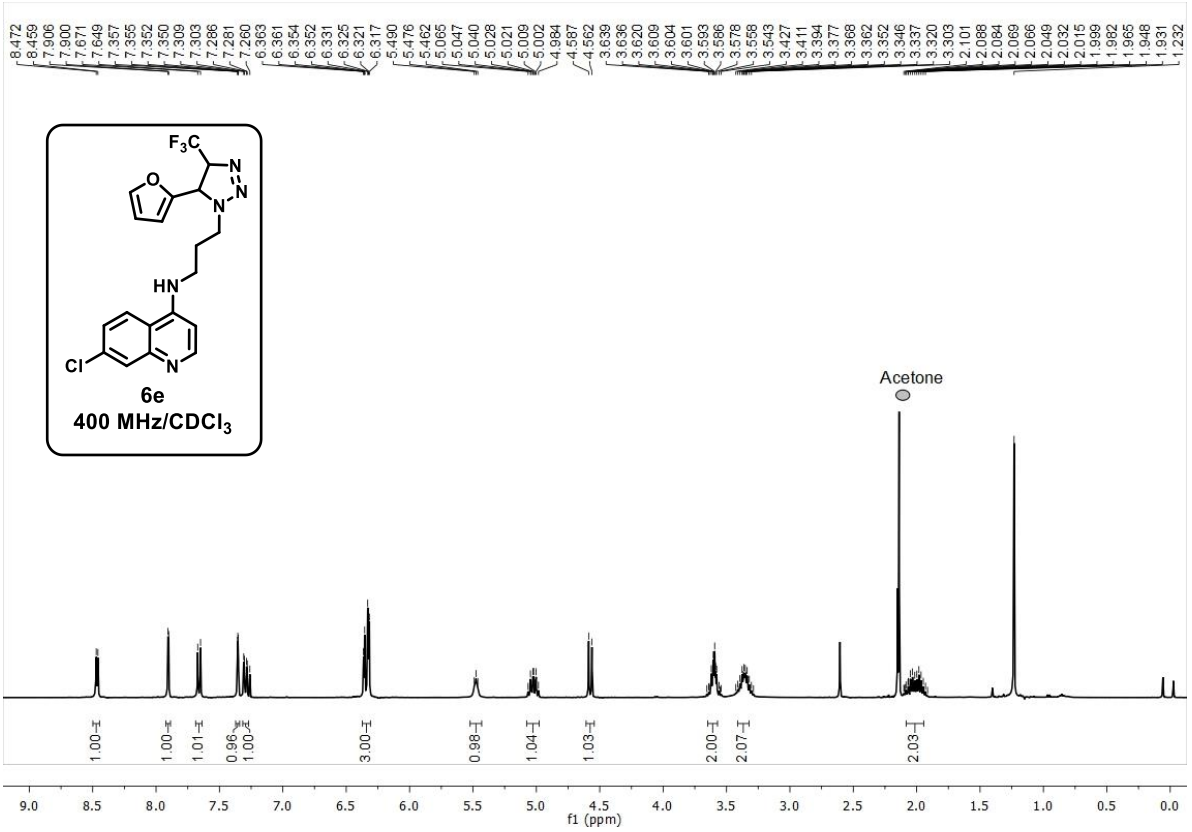


Figure 22: 1H NMR spectrum of 6e

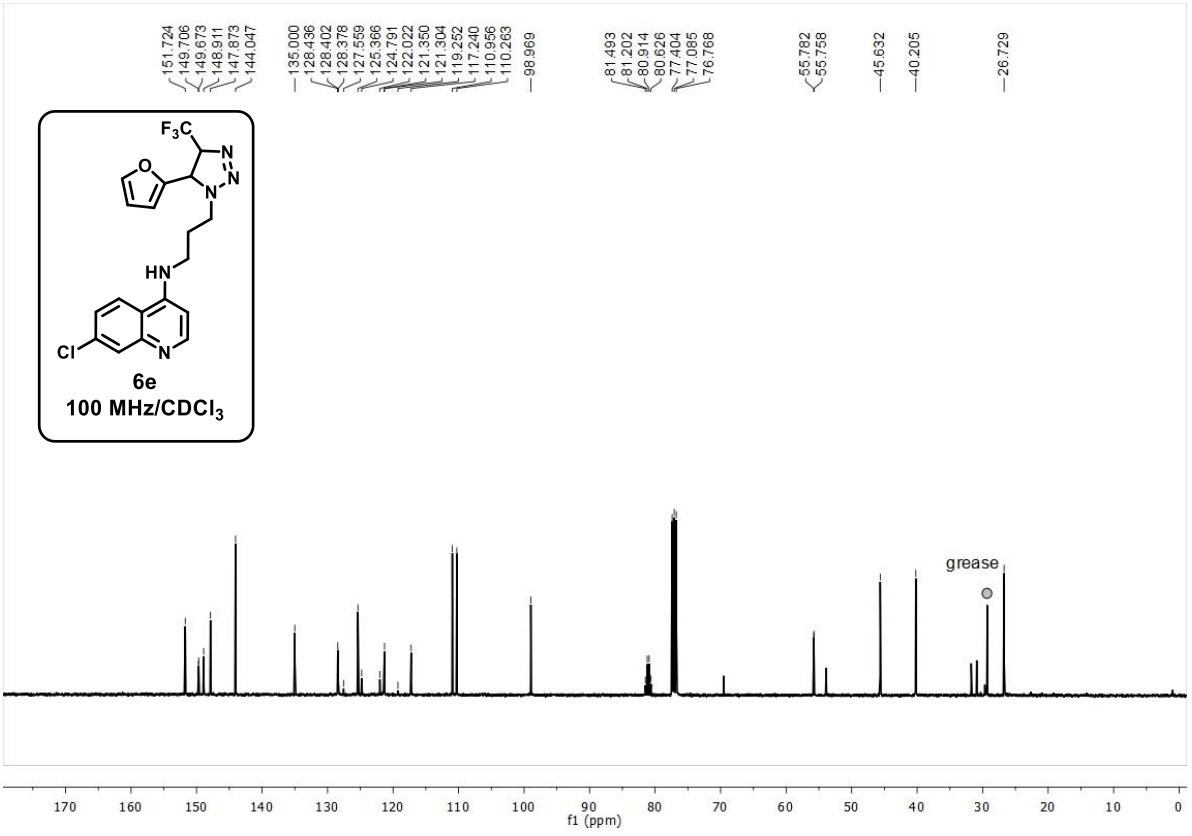


Figure 23: 13C NMR spectrum of 6e

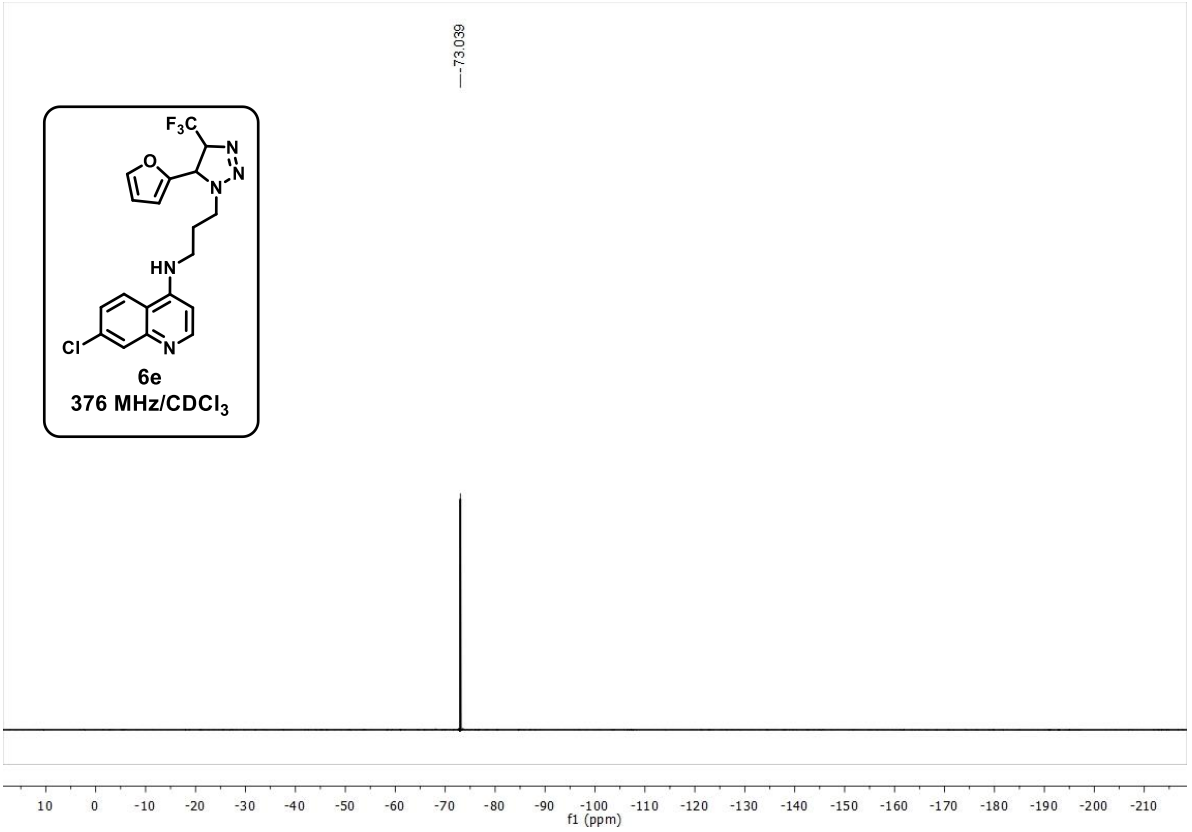


Figure 24: 19F NMR spectrum of 6e

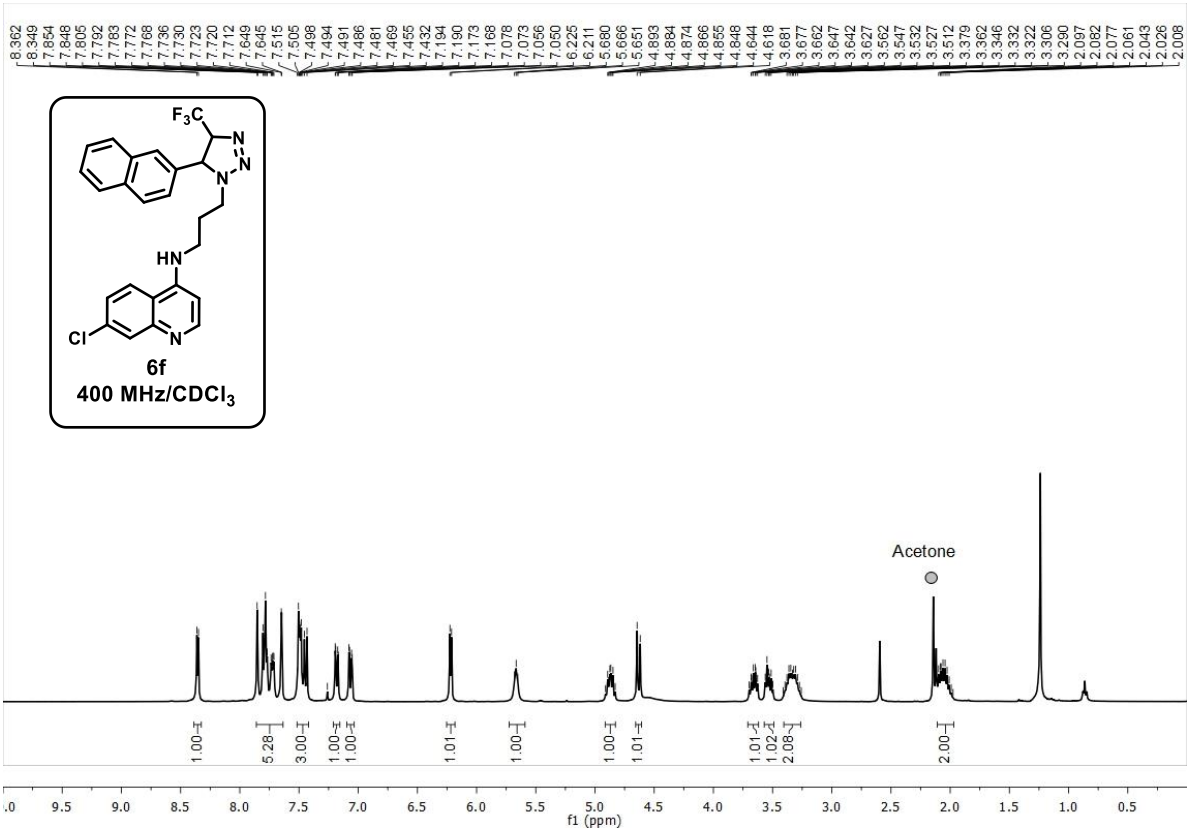


Figure 25: 1H NMR spectrum of 6f

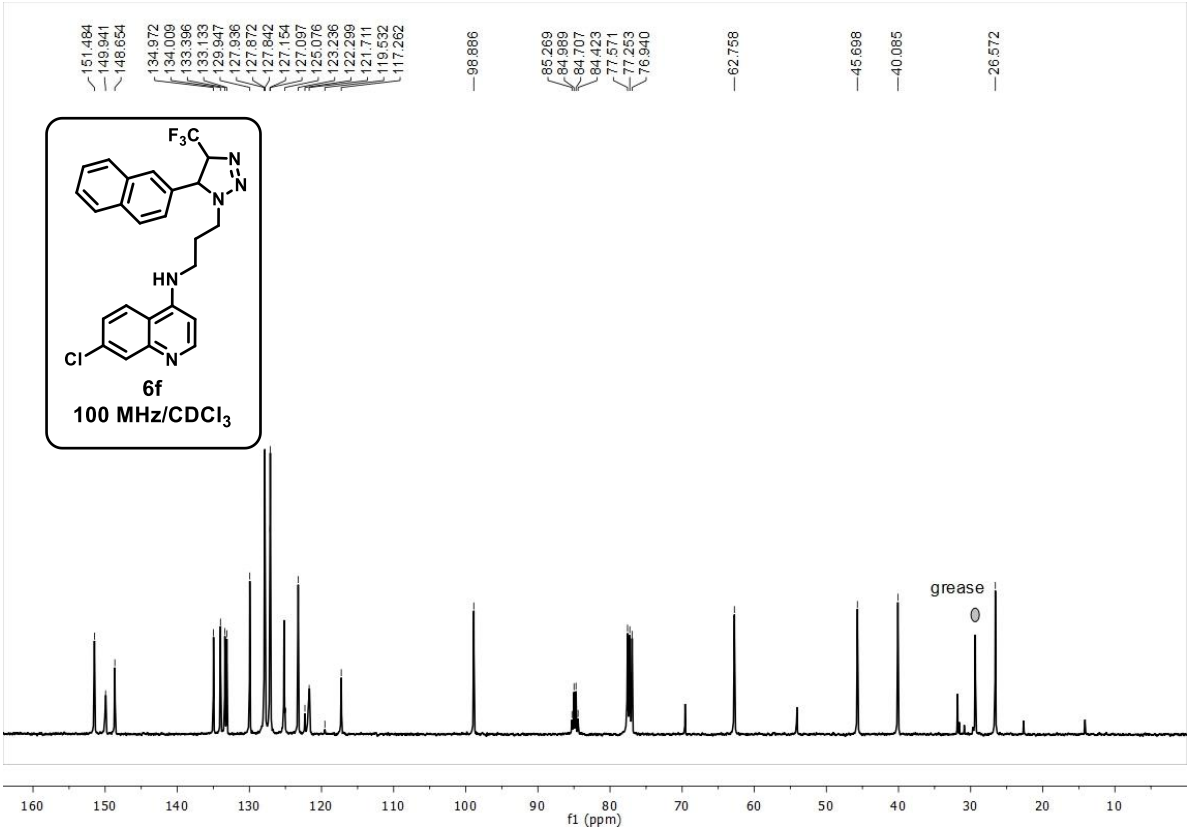


Figure 26: 13C NMR spectrum of 6f

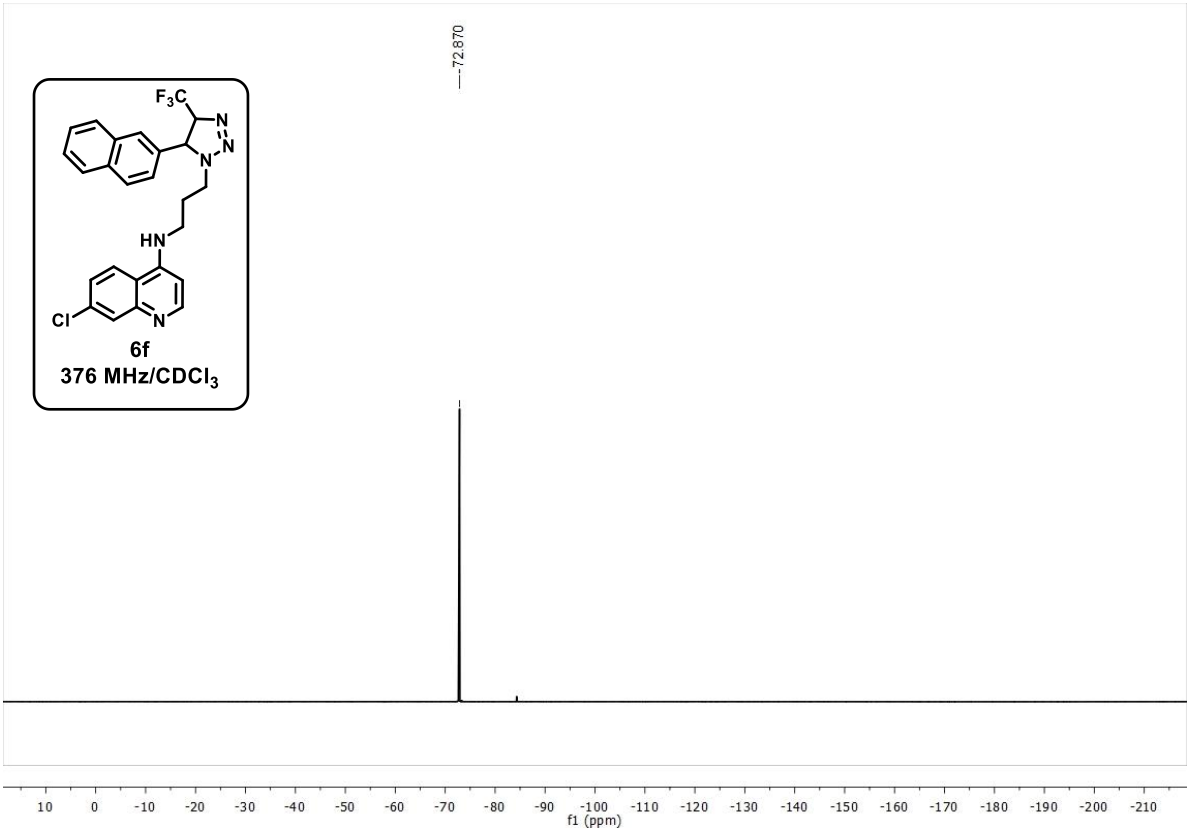


Figure 27: 19F NMR spectrum of 6f

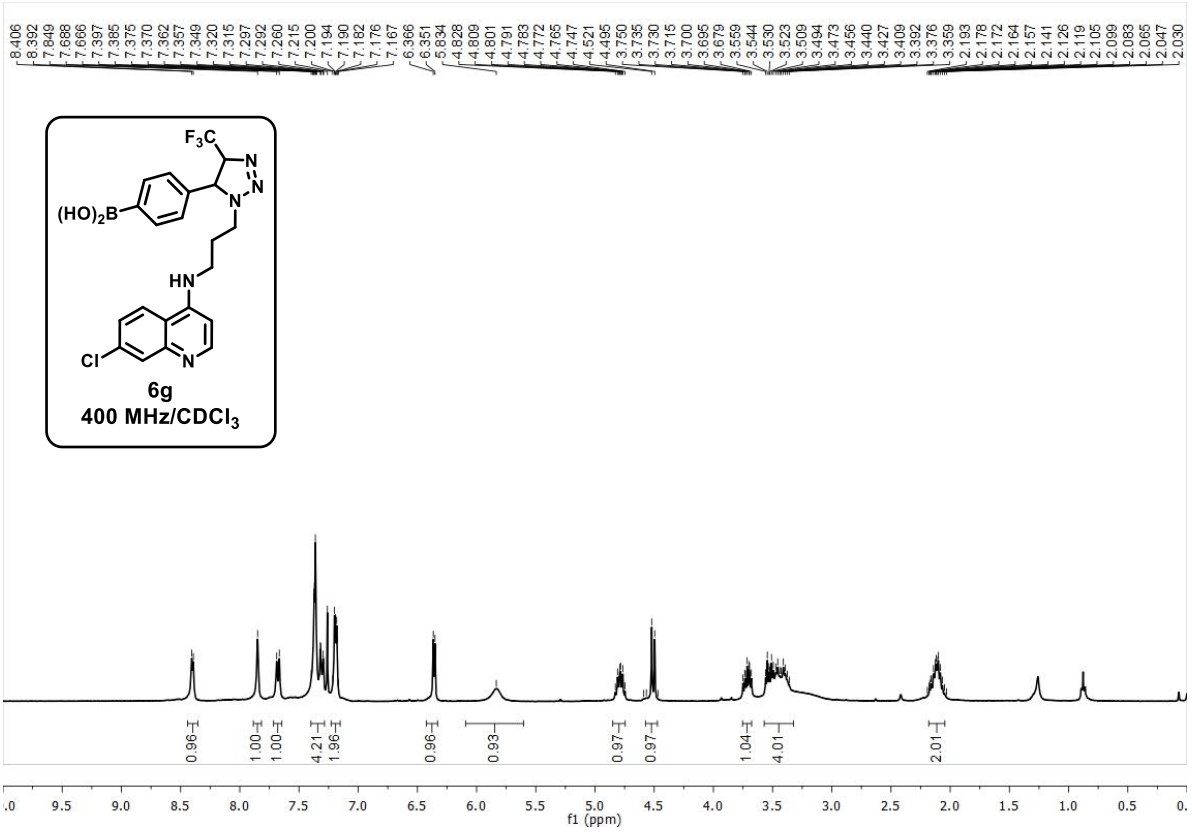


Figure 28: 1H NMR spectrum of 6g

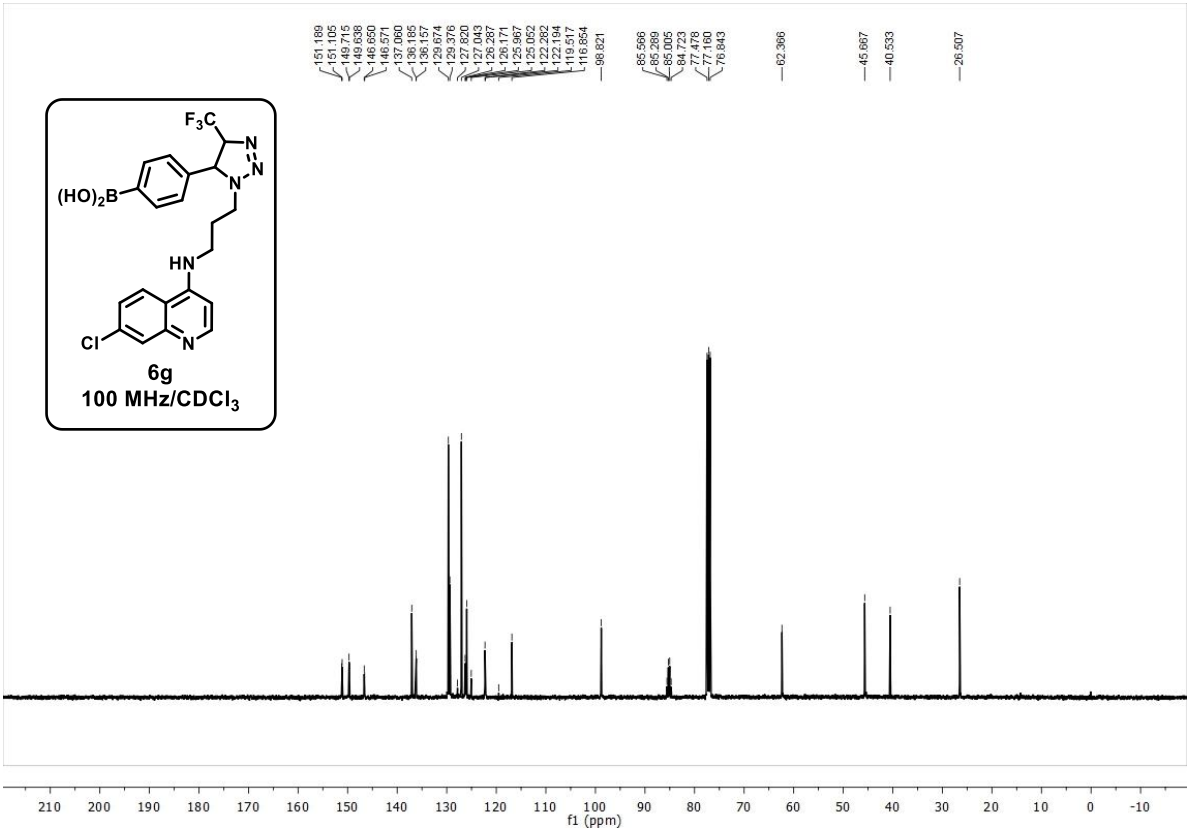


Figure 29: 13C NMR spectrum of 6g

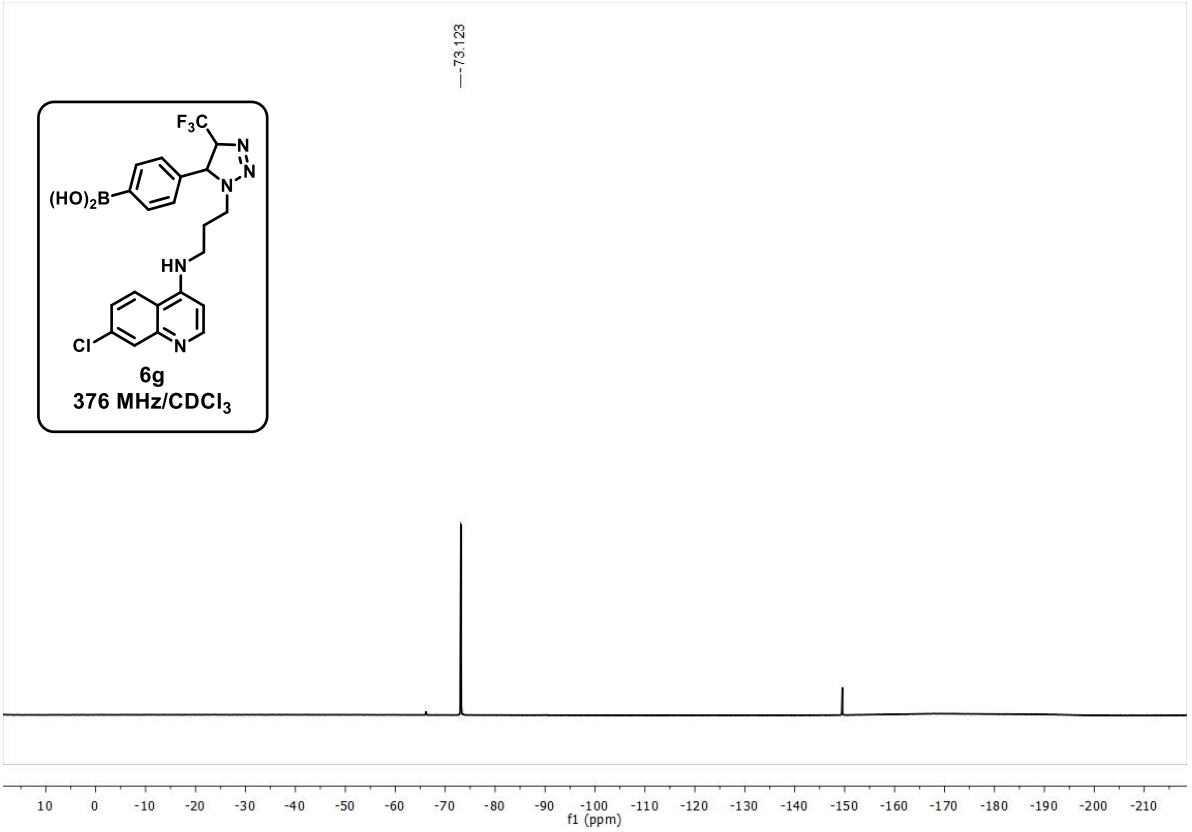


Figure 30: 19F NMR spectrum of 6g

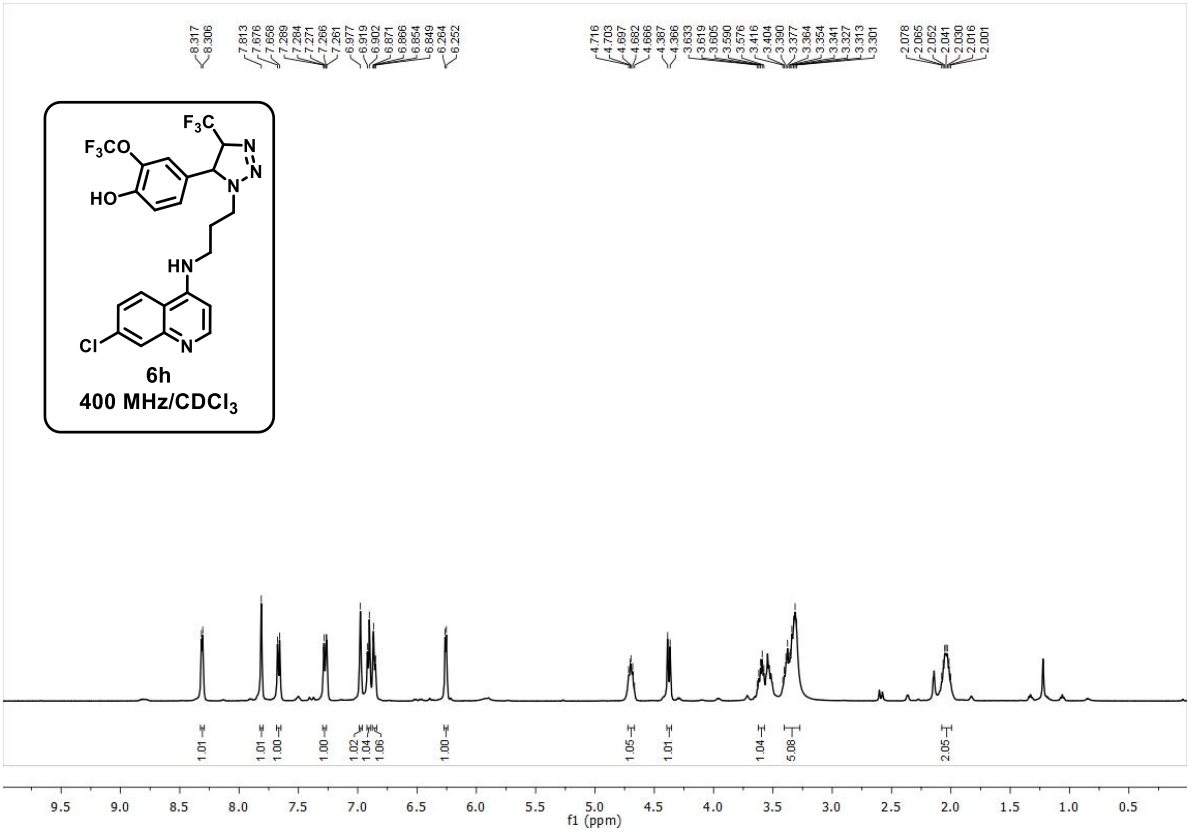


Figure 31: 1H NMR spectrum of 6h

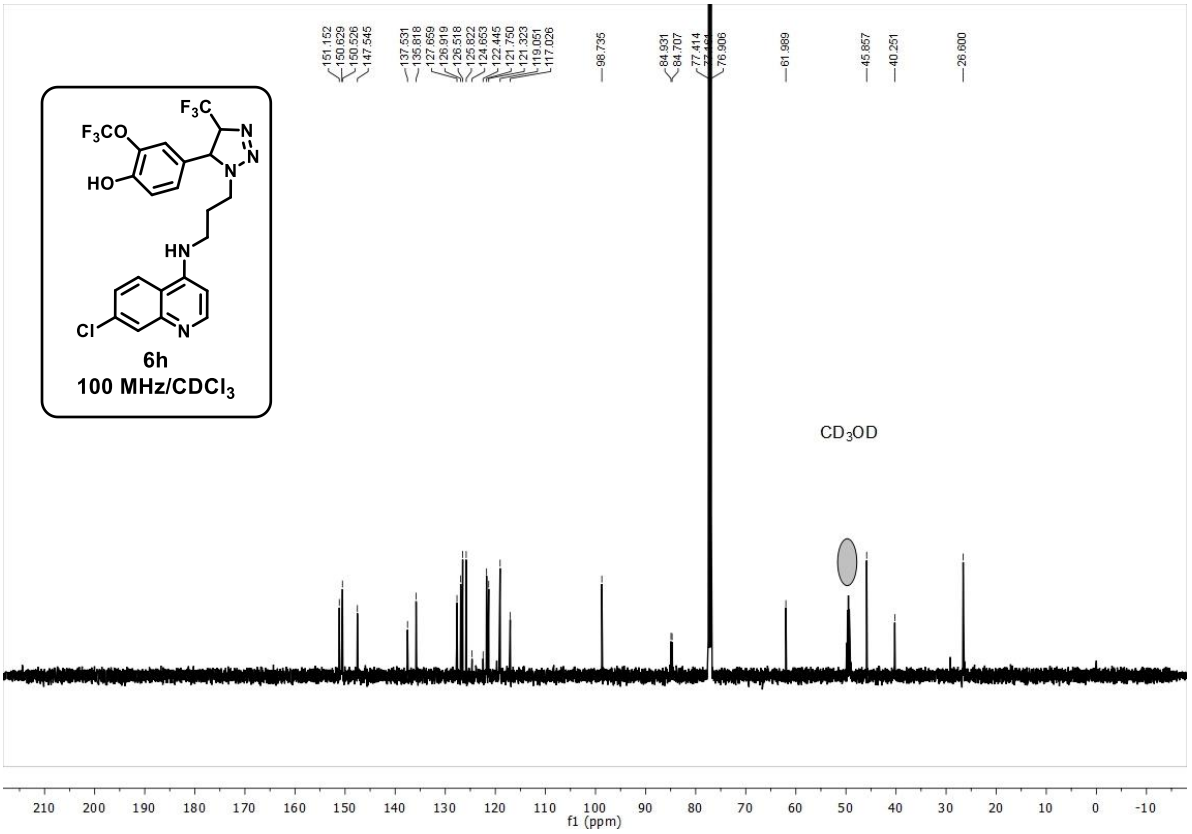


Figure 32: 13C NMR spectrum of 6h

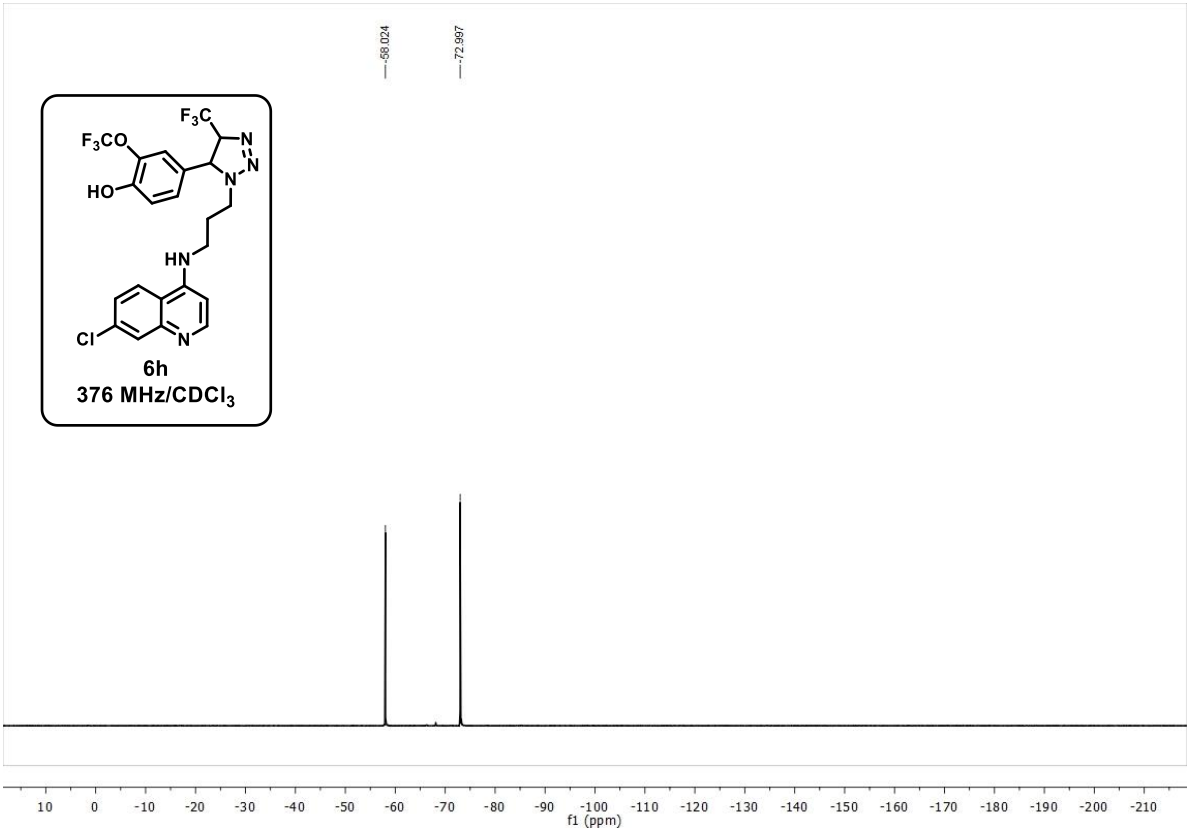


Figure 33: 19F NMR spectrum of 6h

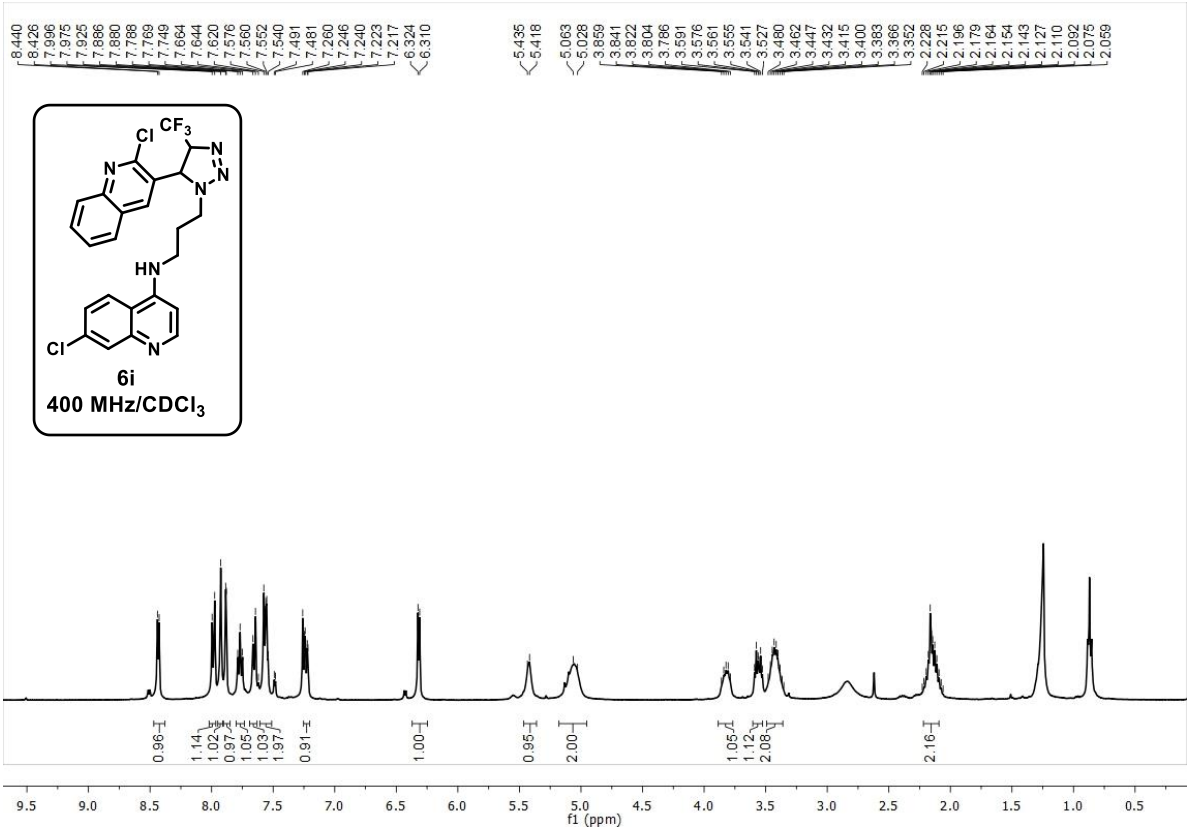


Figure 34: 1H NMR spectrum of 6i

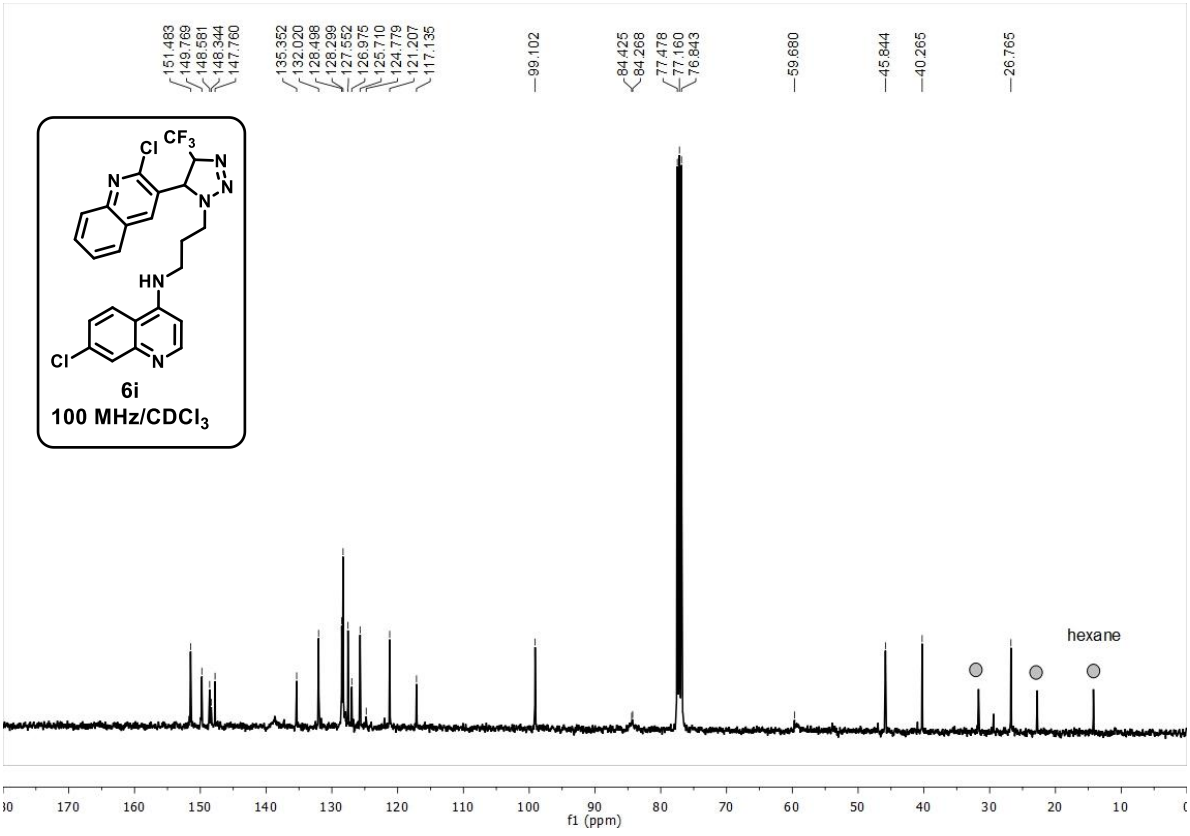


Figure 35: 13C NMR spectrum of 6i

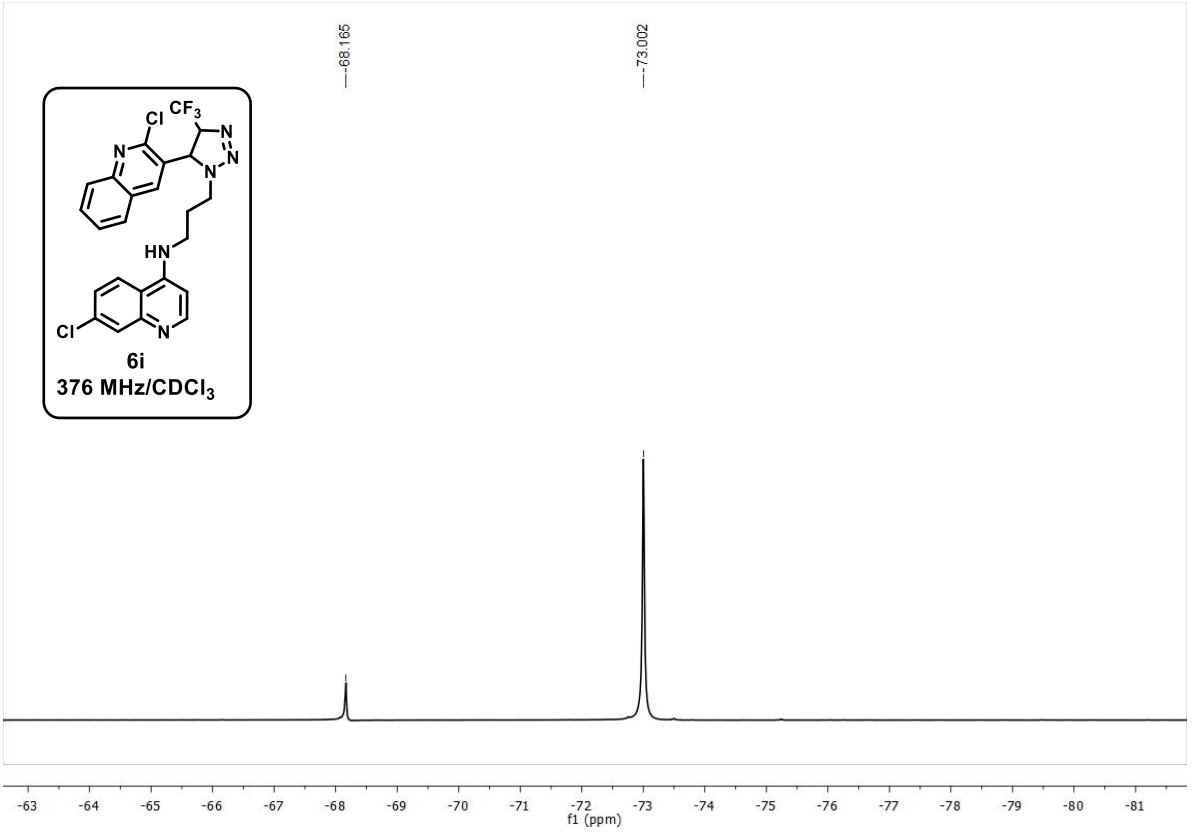


Figure 36: 19F NMR spectrum og 6i

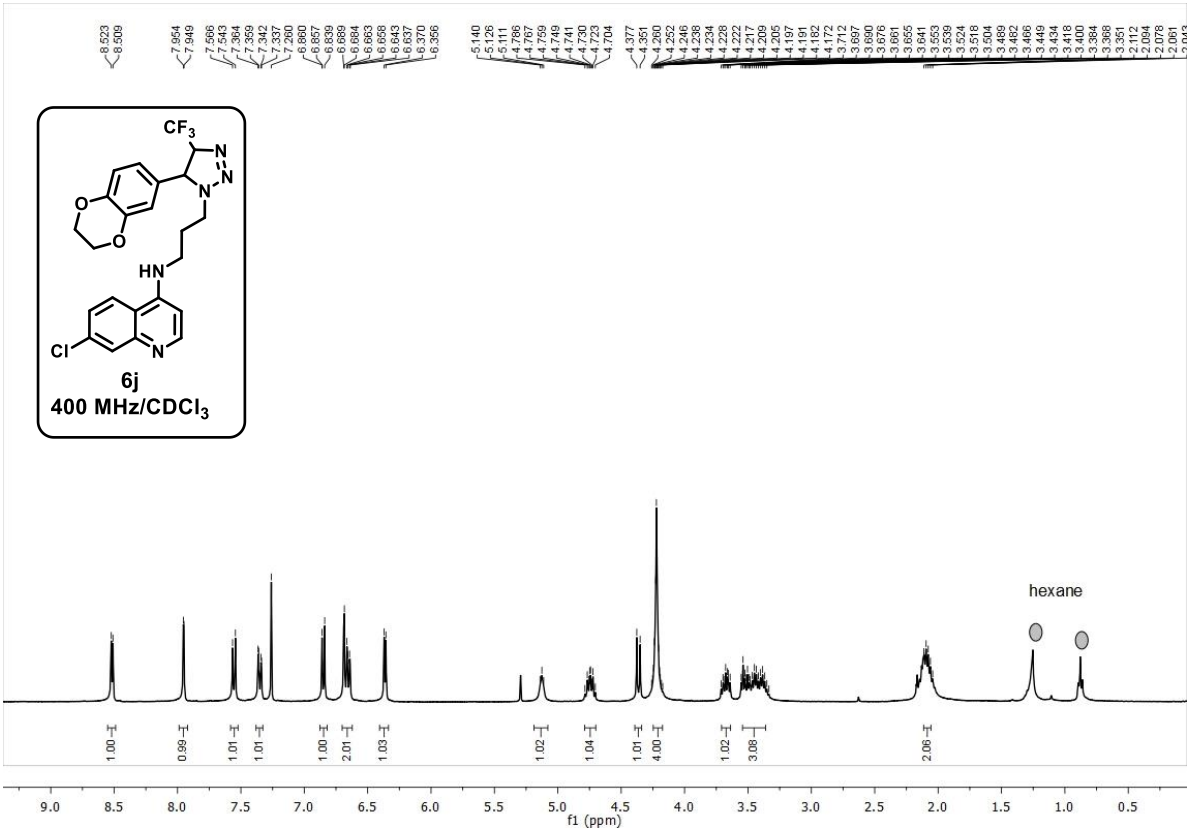


Figure 37: 1H NMR spectrum of 6j

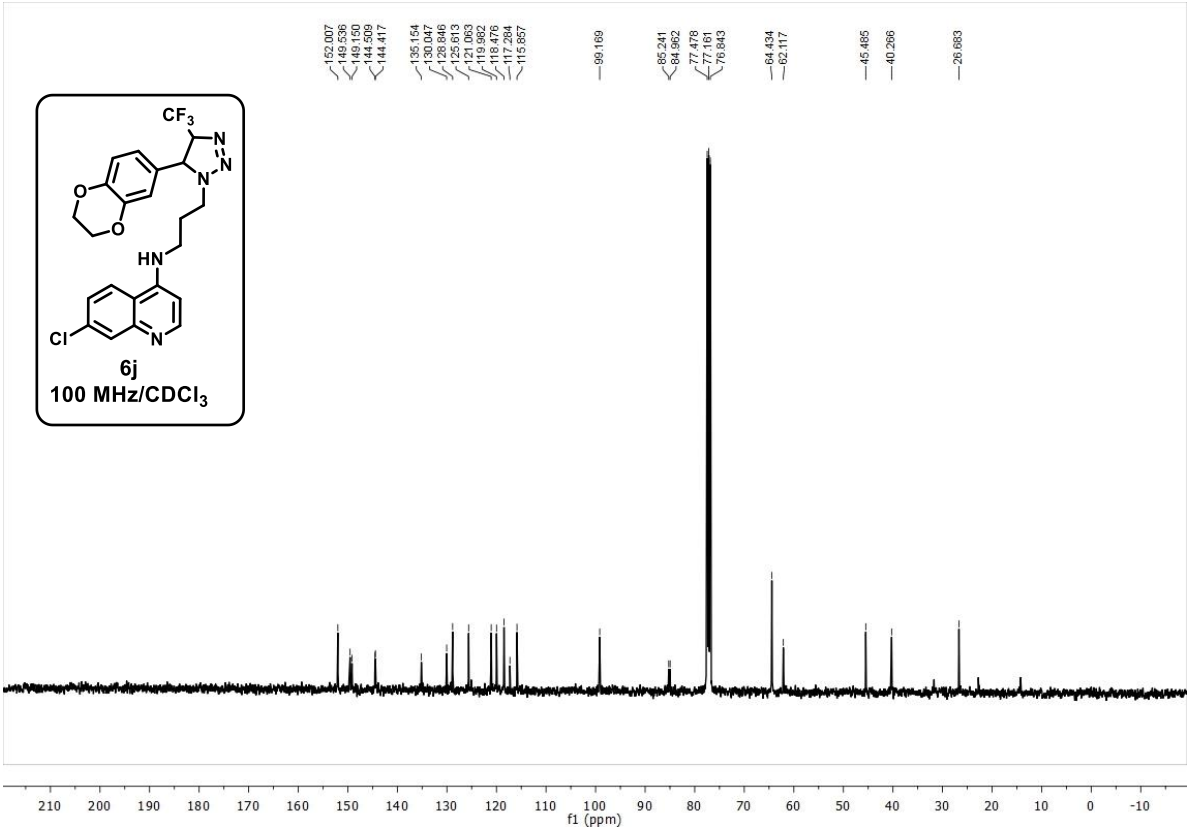


Figure 38: 13C NMR spectrum og 6j

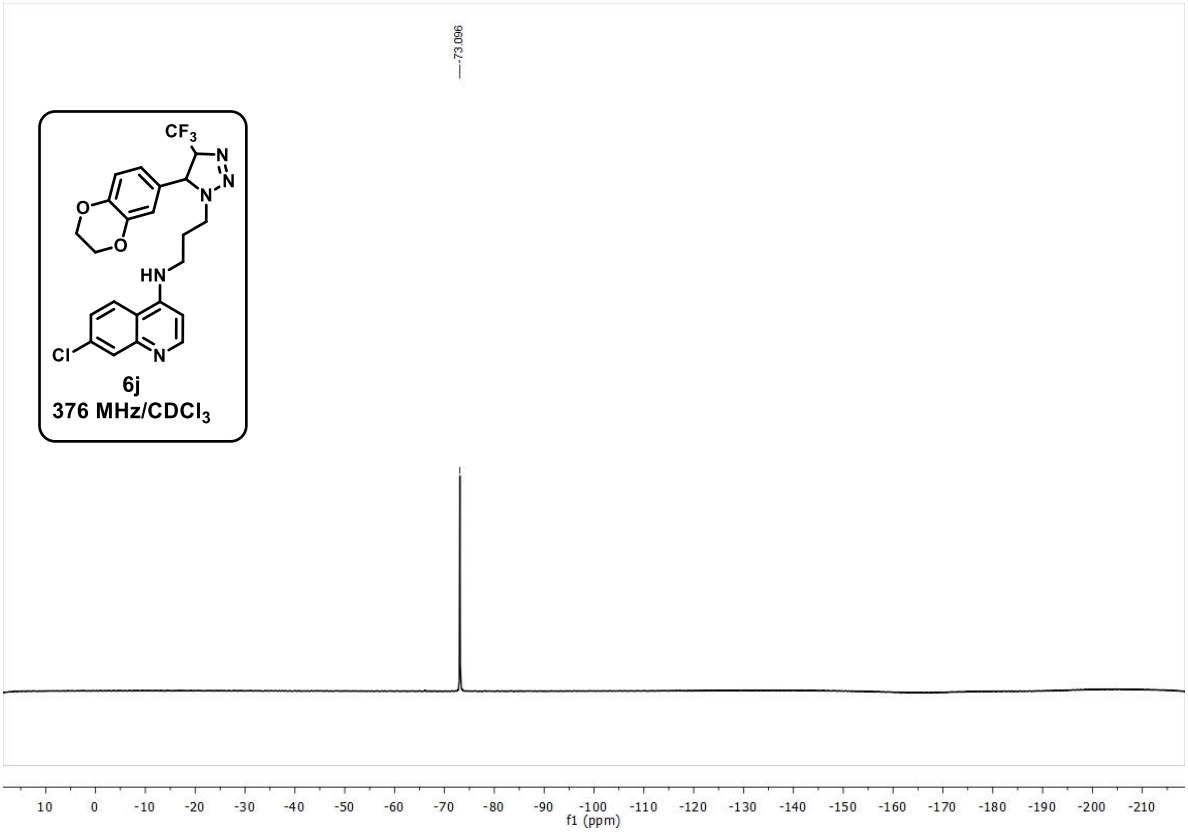


Figure 39: 19F NMR spectrum of 6j

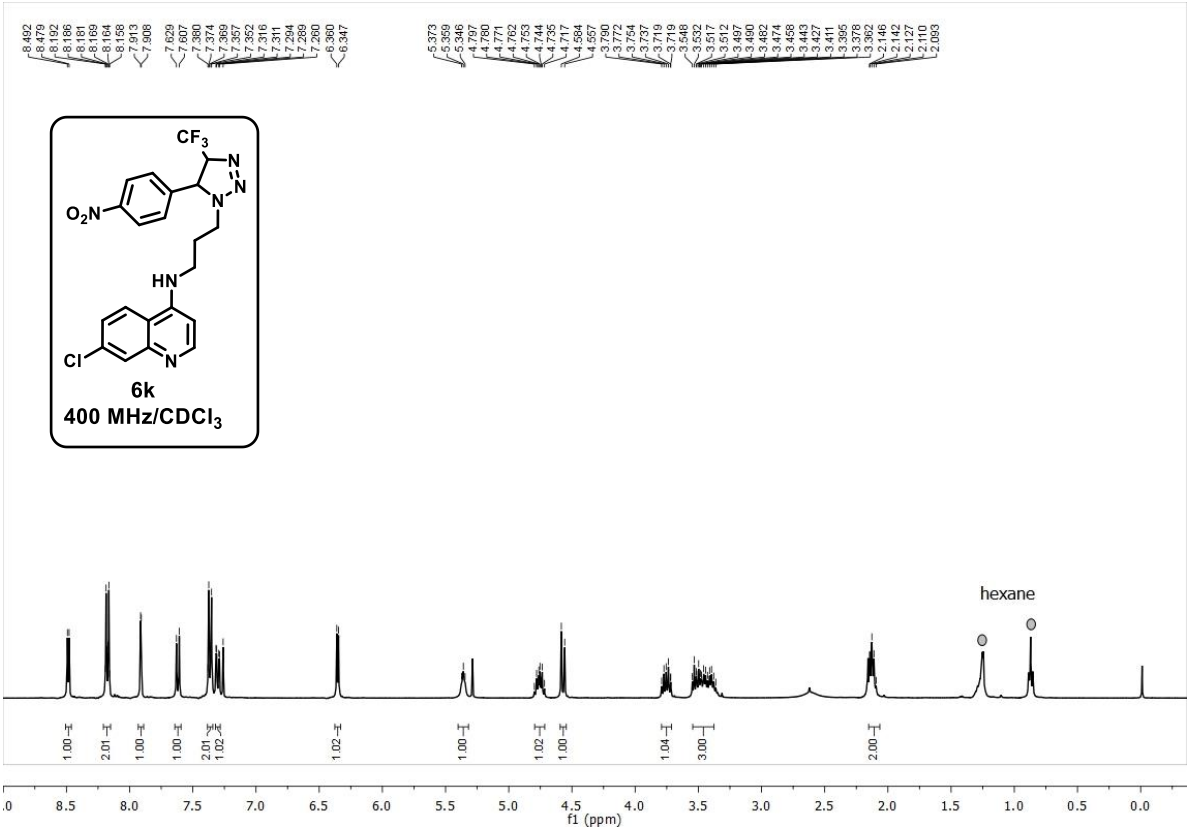


Figure 40: 1H NMR spectrum of 6k

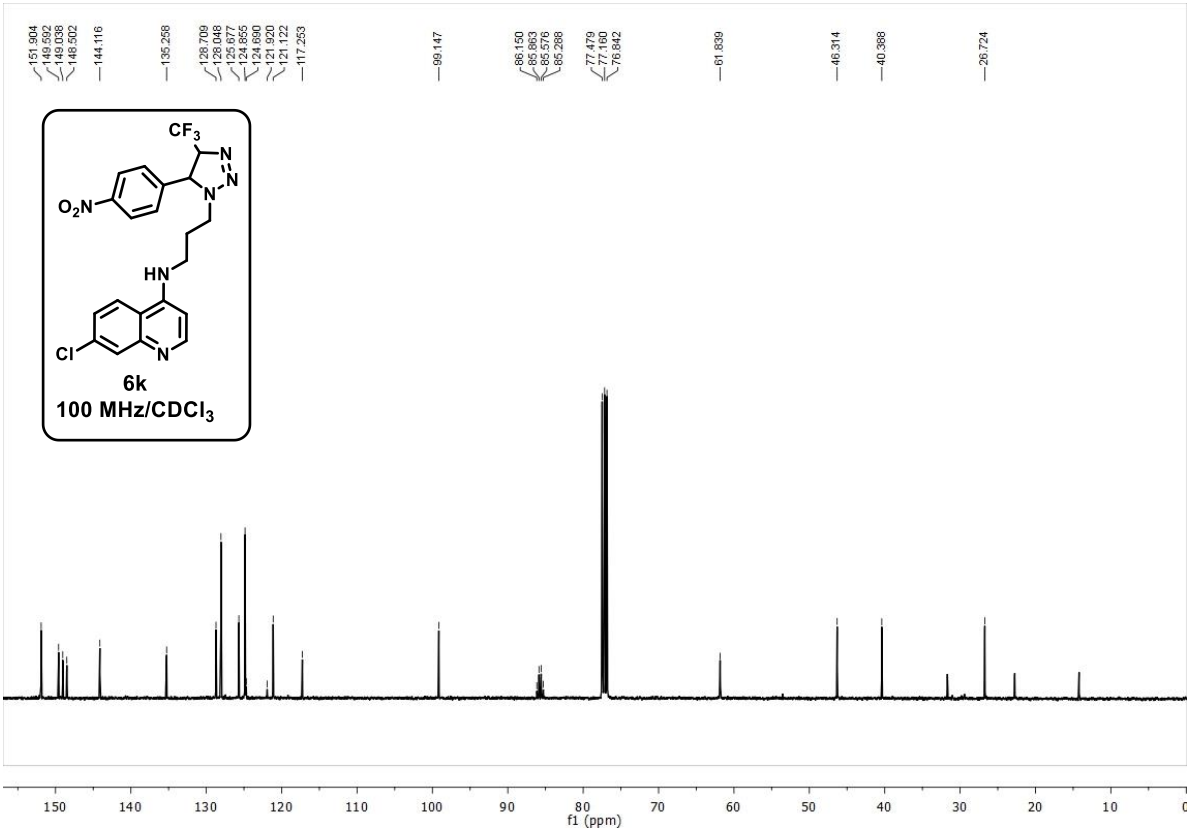


Figure 41: 13C NMR spectrum of 6k

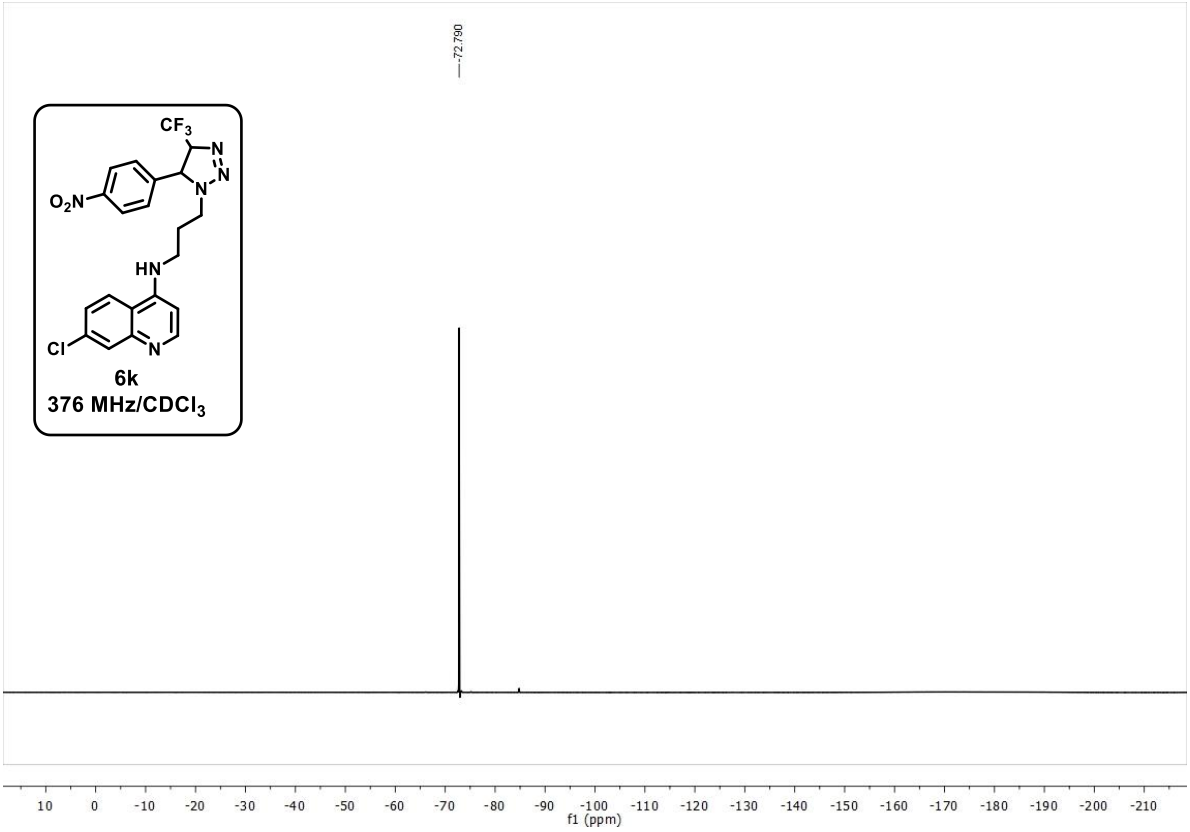


Figure 42: 19F NMR spectrum of 6k

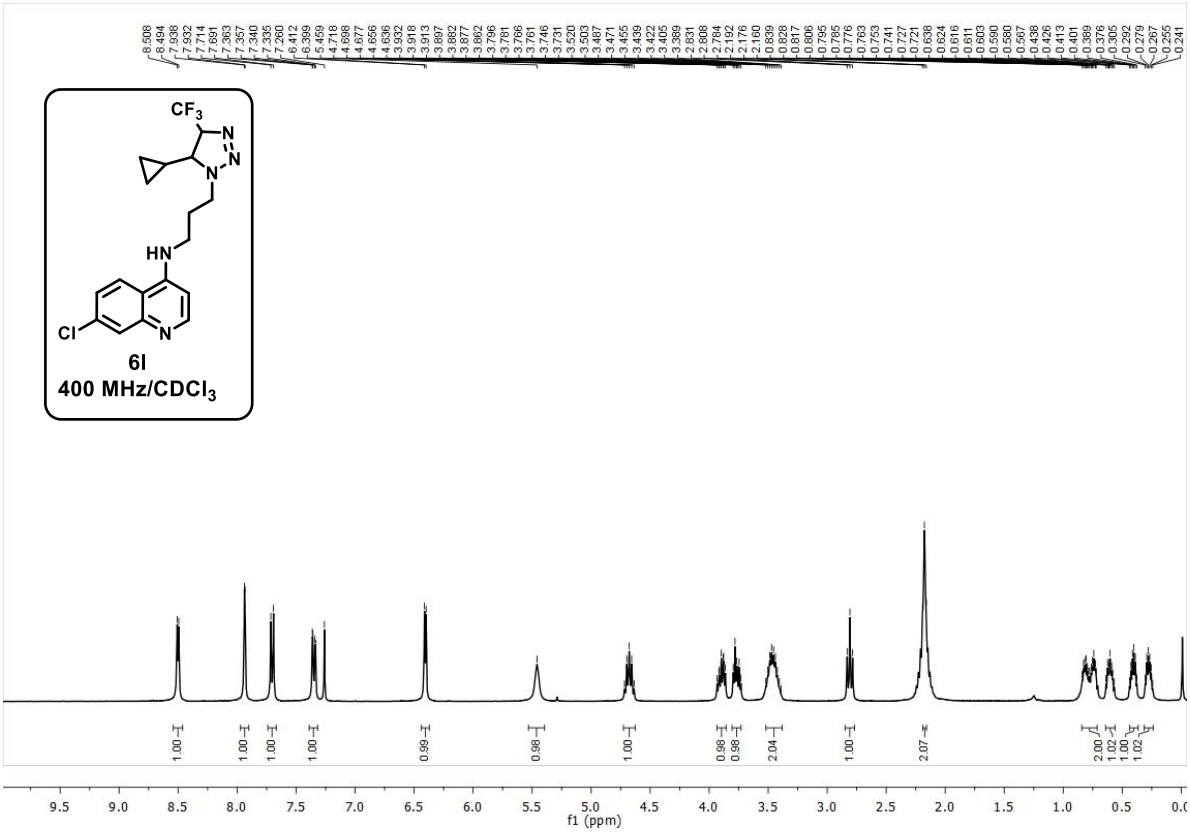


Figure 43: 1H NMR spectrum of 6l

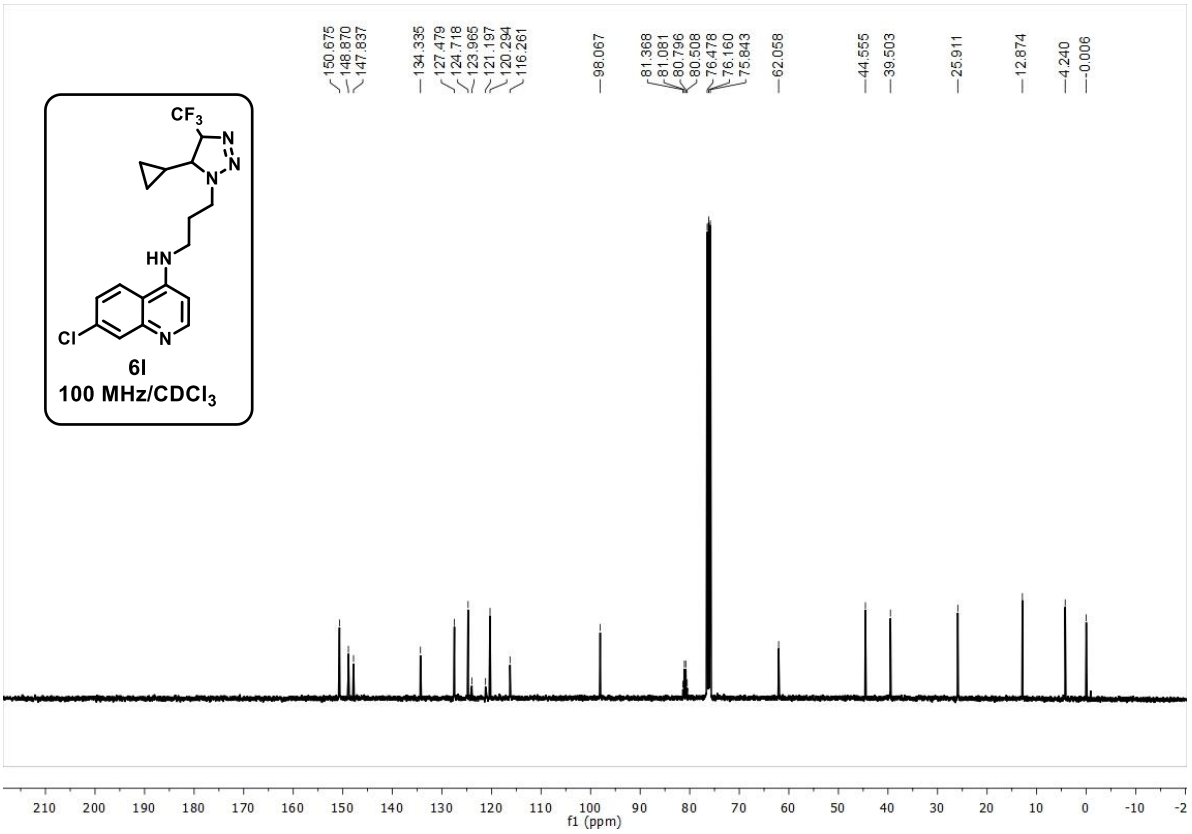


Figure 44: 13C NMR spectrum of 6l

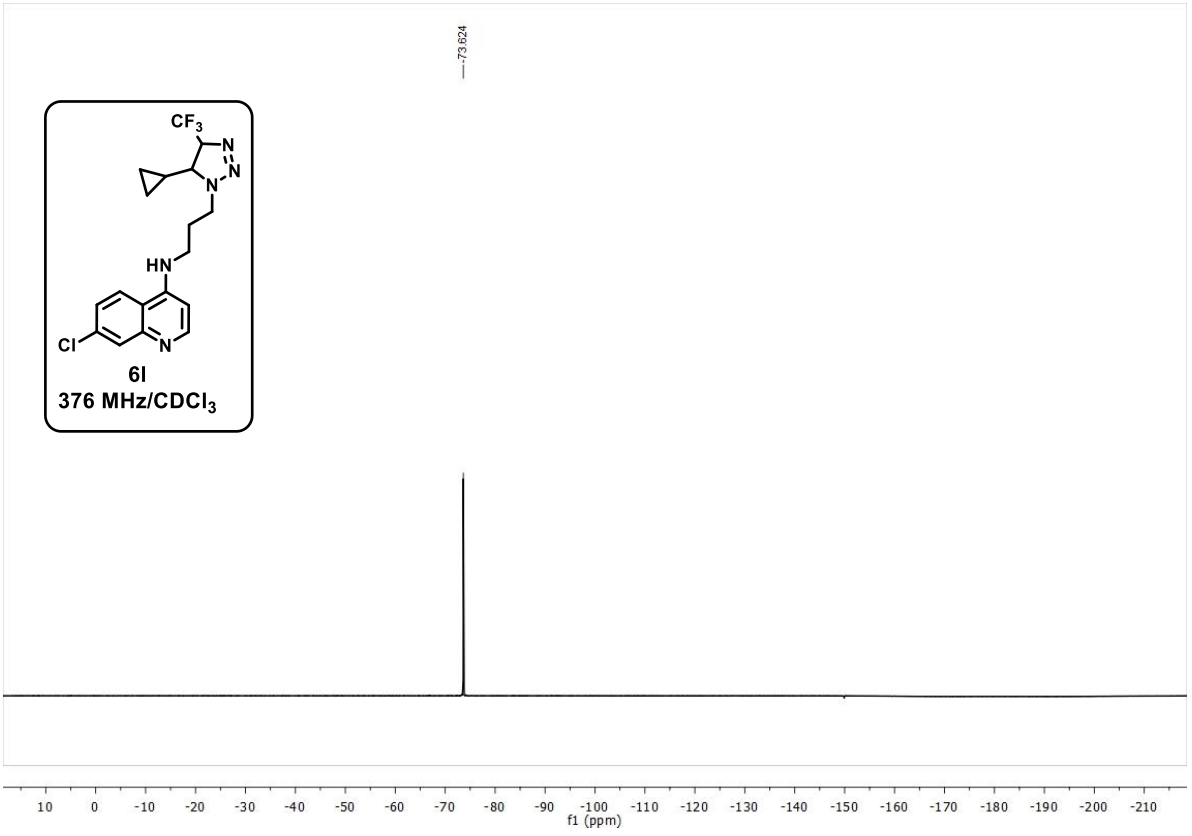


Figure 45: 19F NMR spectrum of 6l

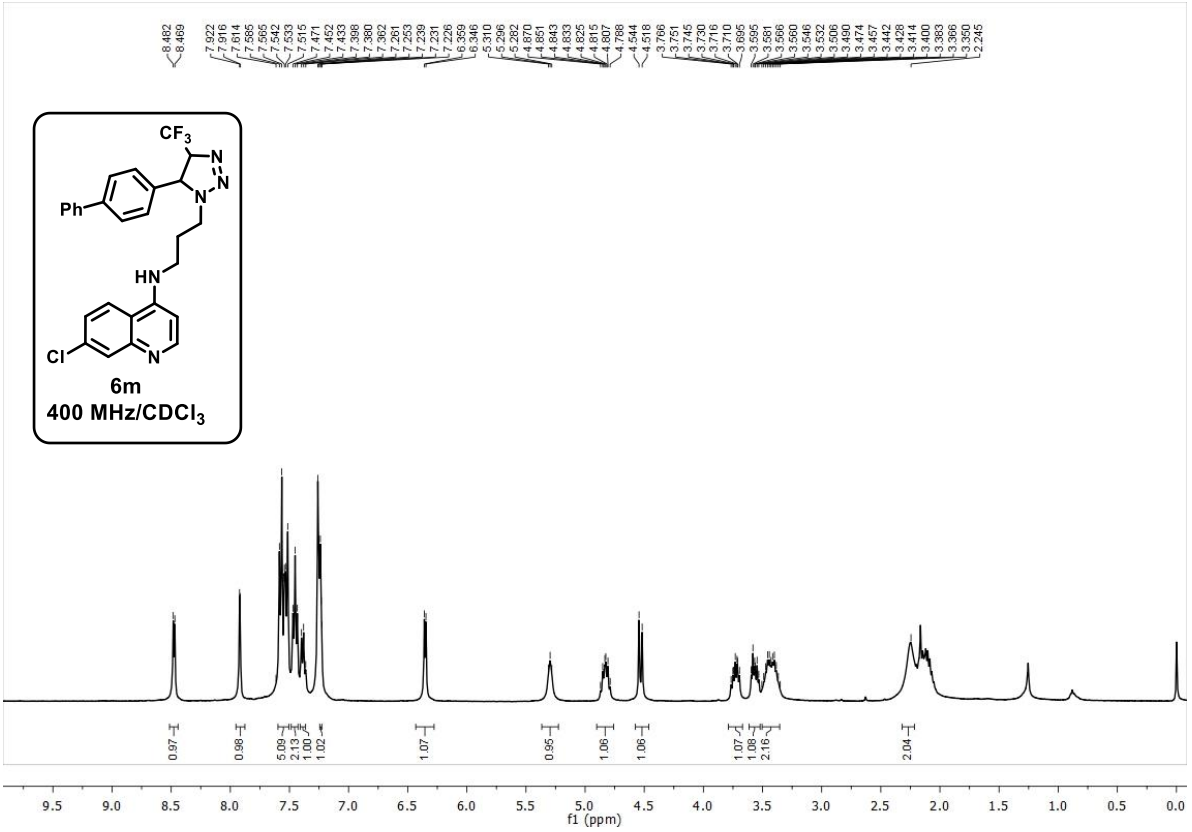


Figure 46: 1H NMR spectrum of 6m

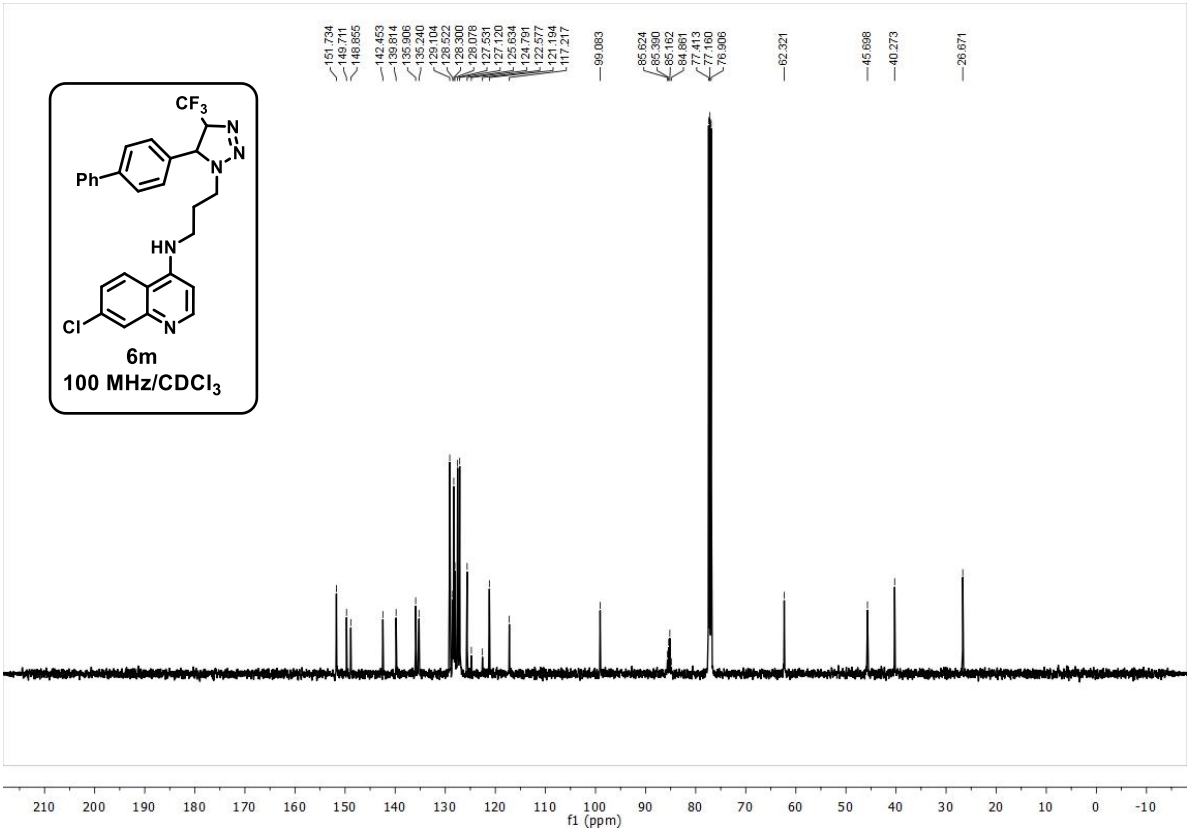


Figure 47: 13C NMR spectrum of 6m

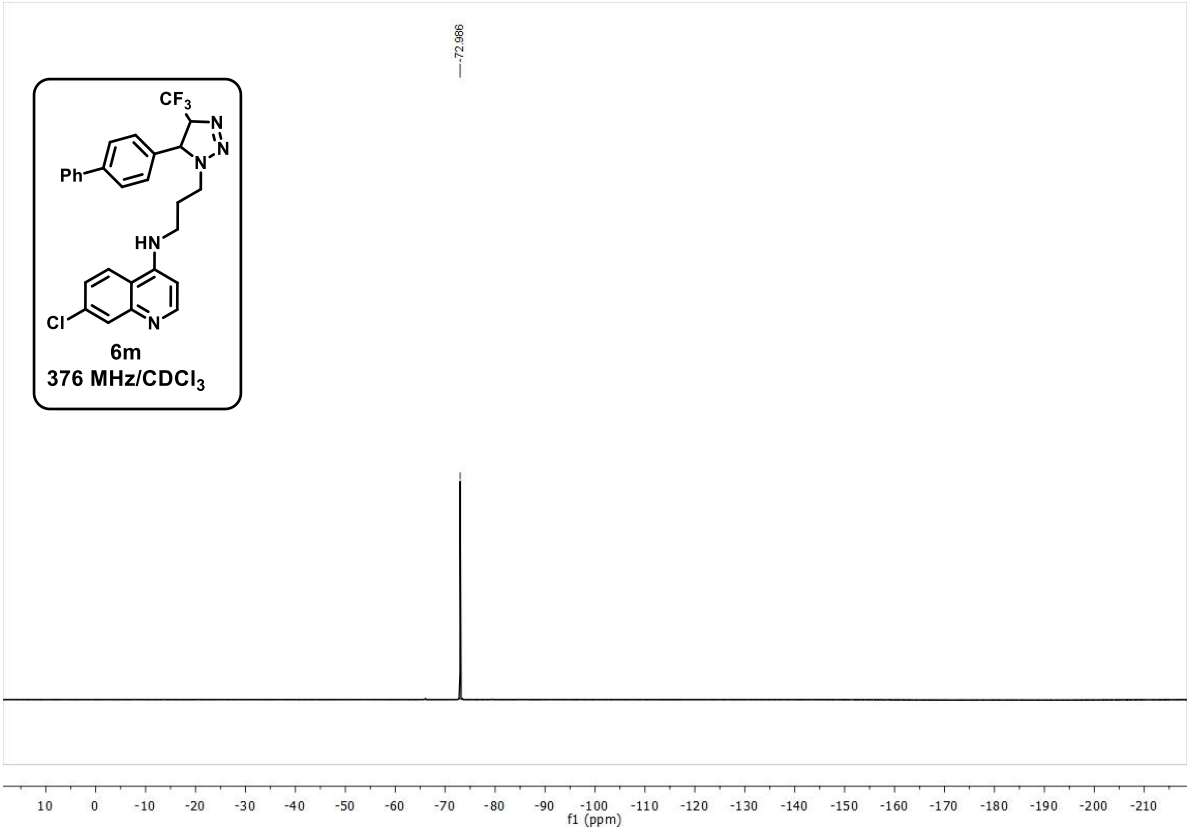


Figure 48: 19F NMR spectrum of 6m

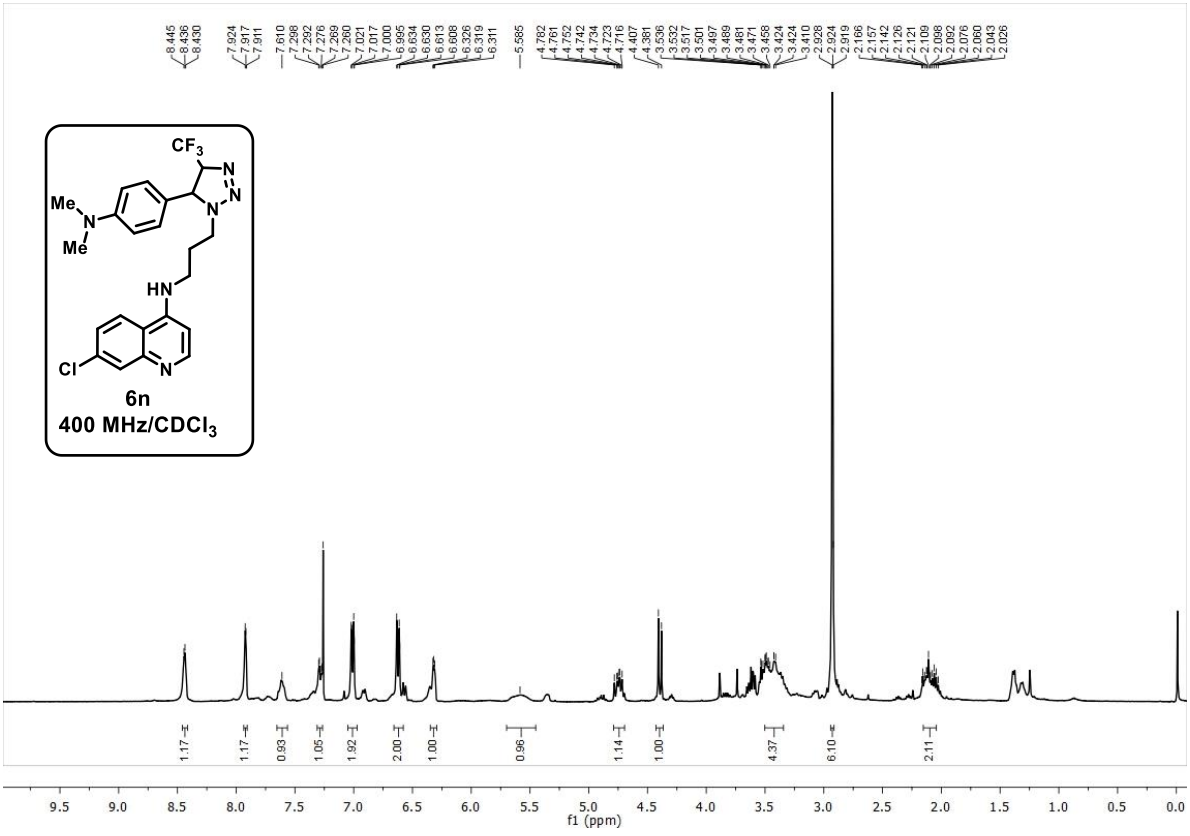


Figure 49: 1H NMR spectrum of 6n

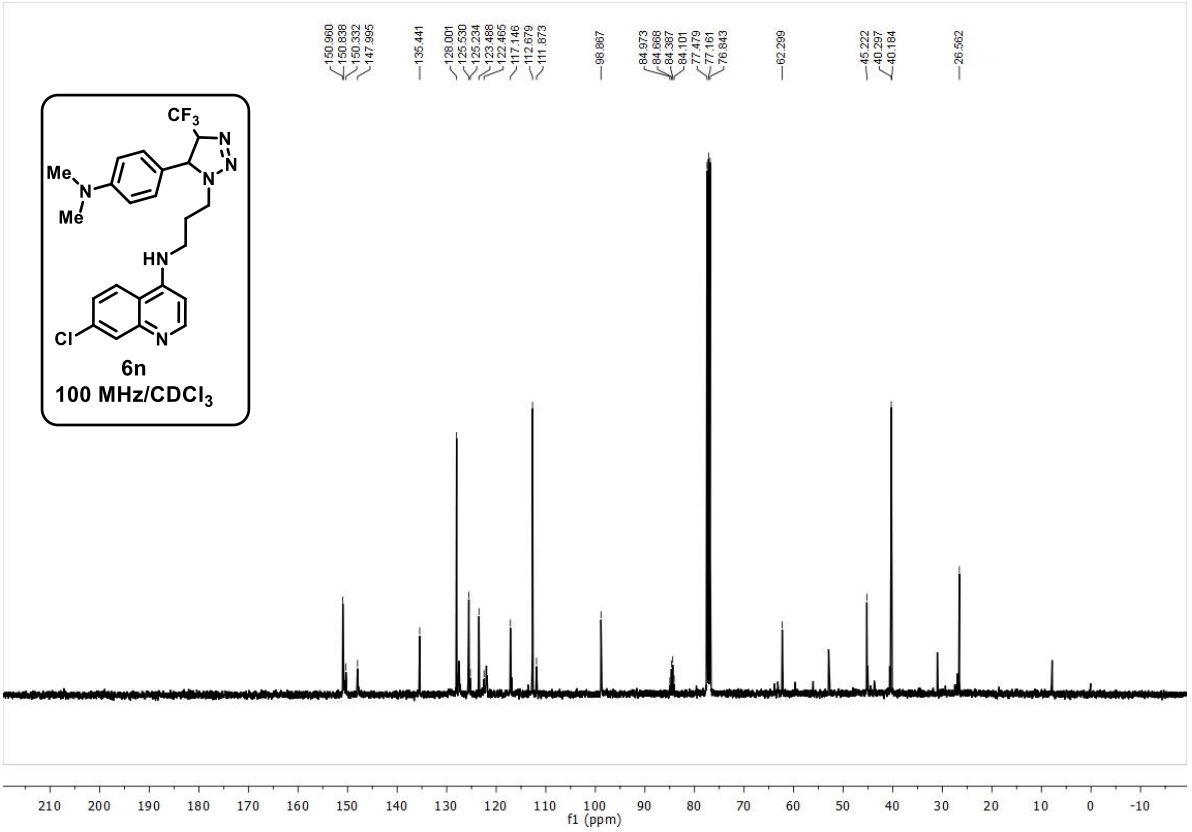


Figure 50: 13C NMR spectrum of 6n

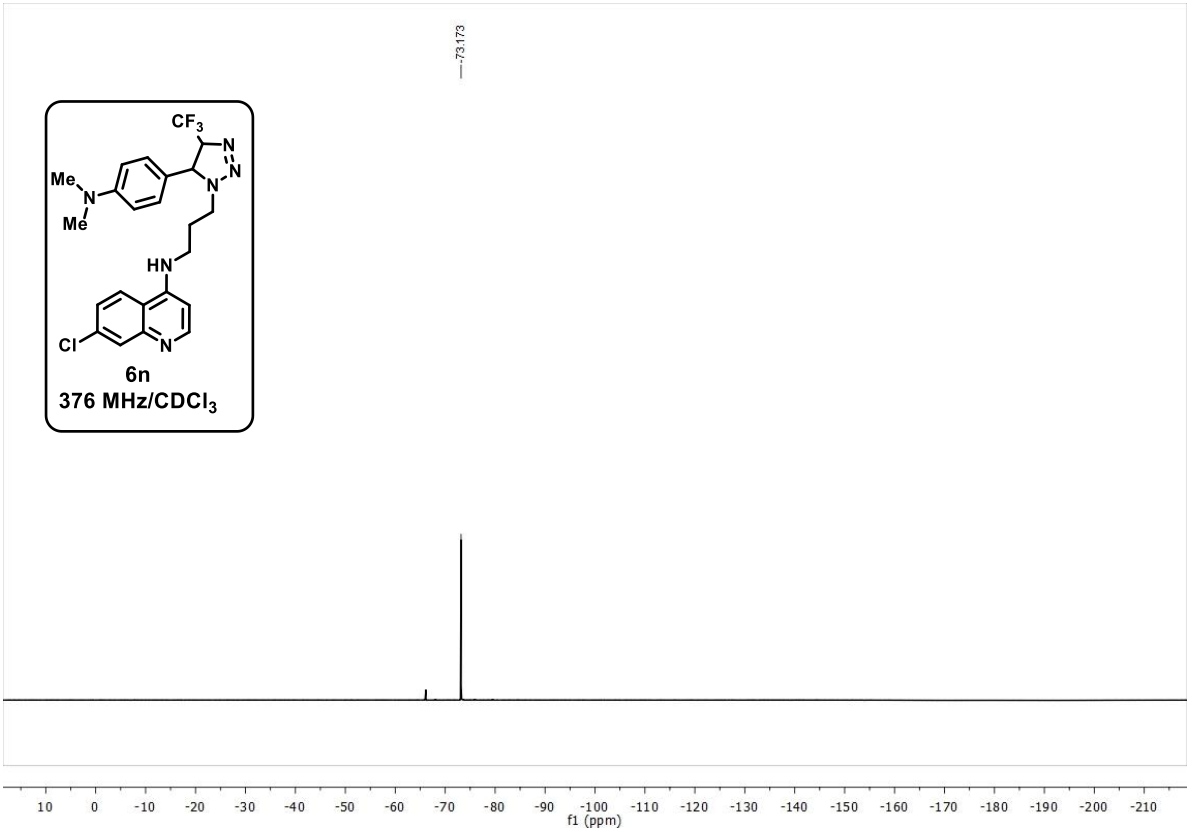


Figure 51: 19F NMR spectrum of 6n

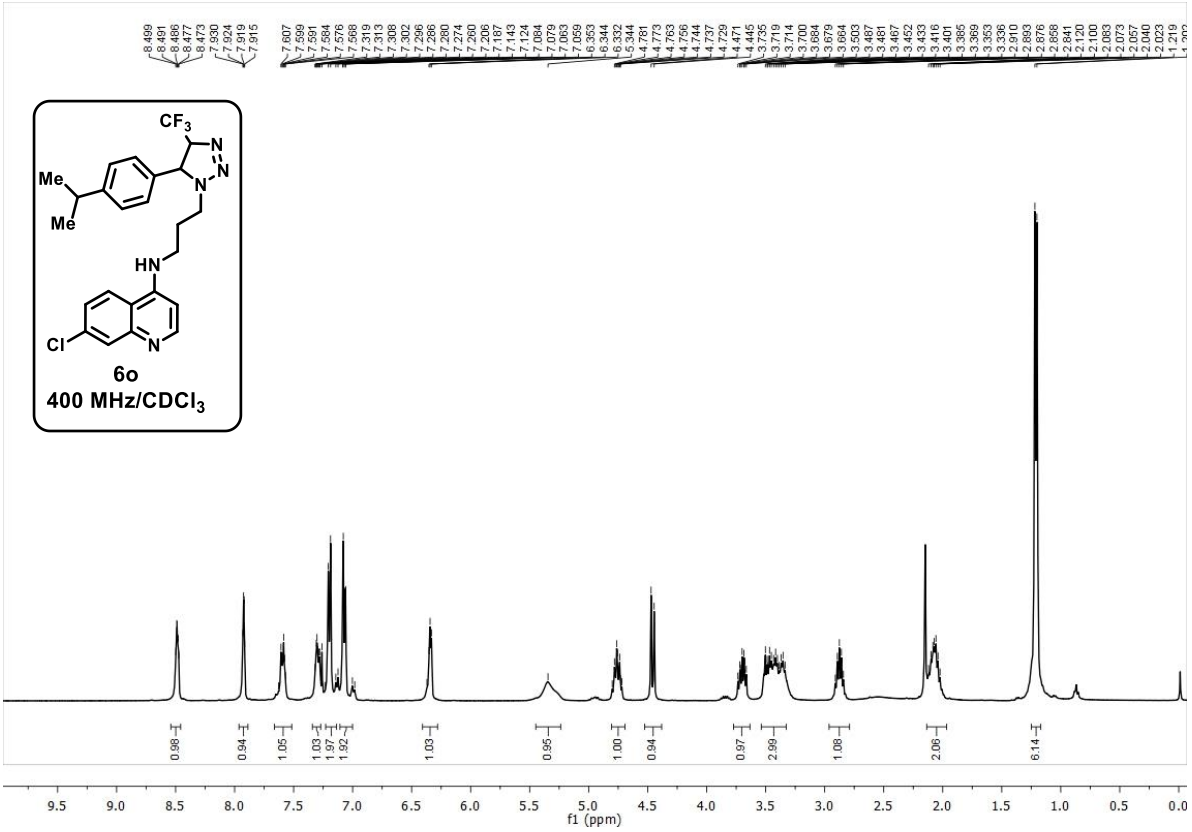


Figure 52: 1H NMR spectrum of 6o

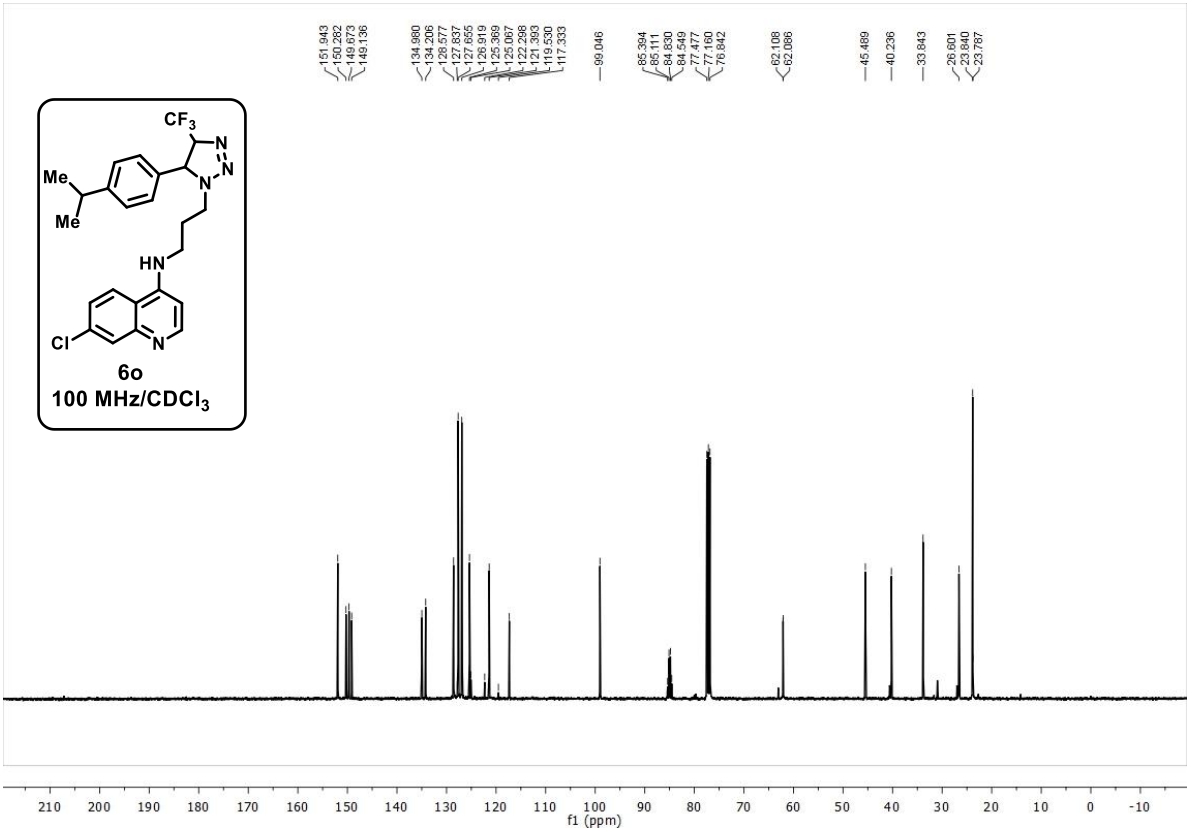


Figure 53: 13C NMR spectrum of 6o

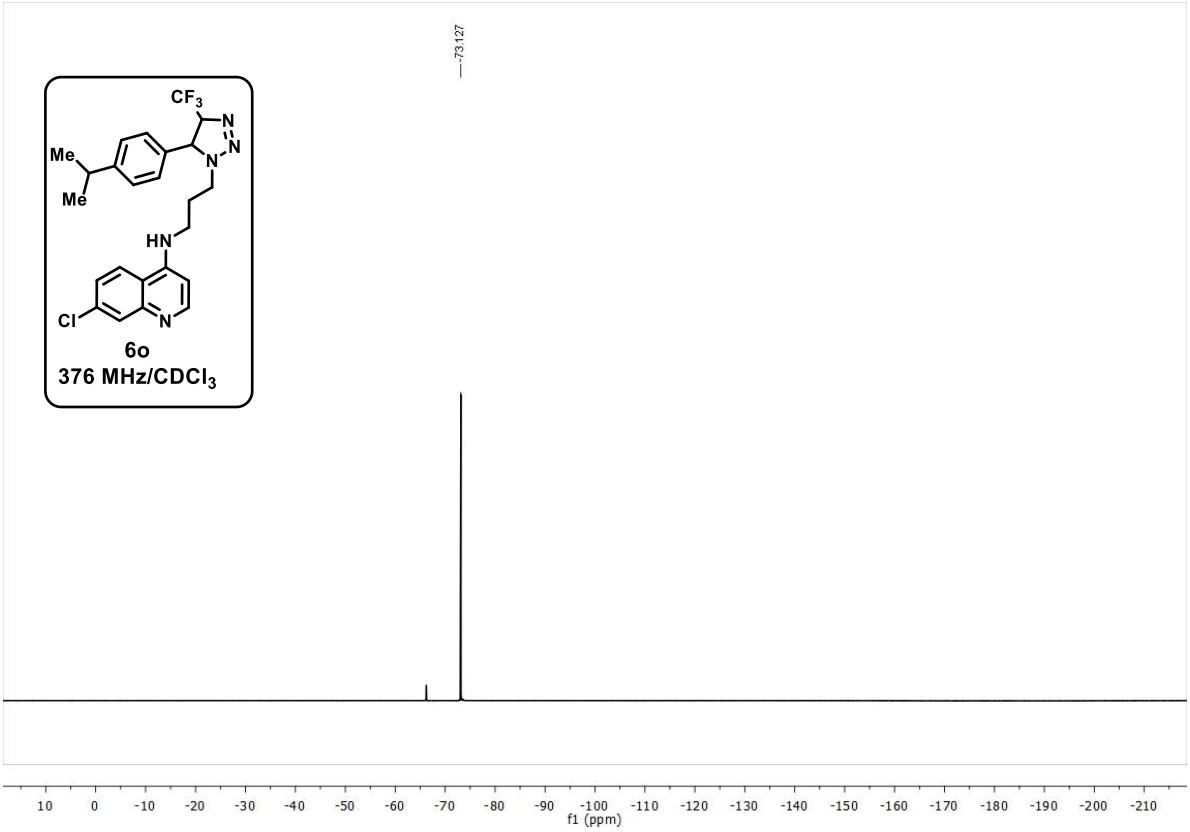


Figure 54: 19F NMR spectrum of 6o

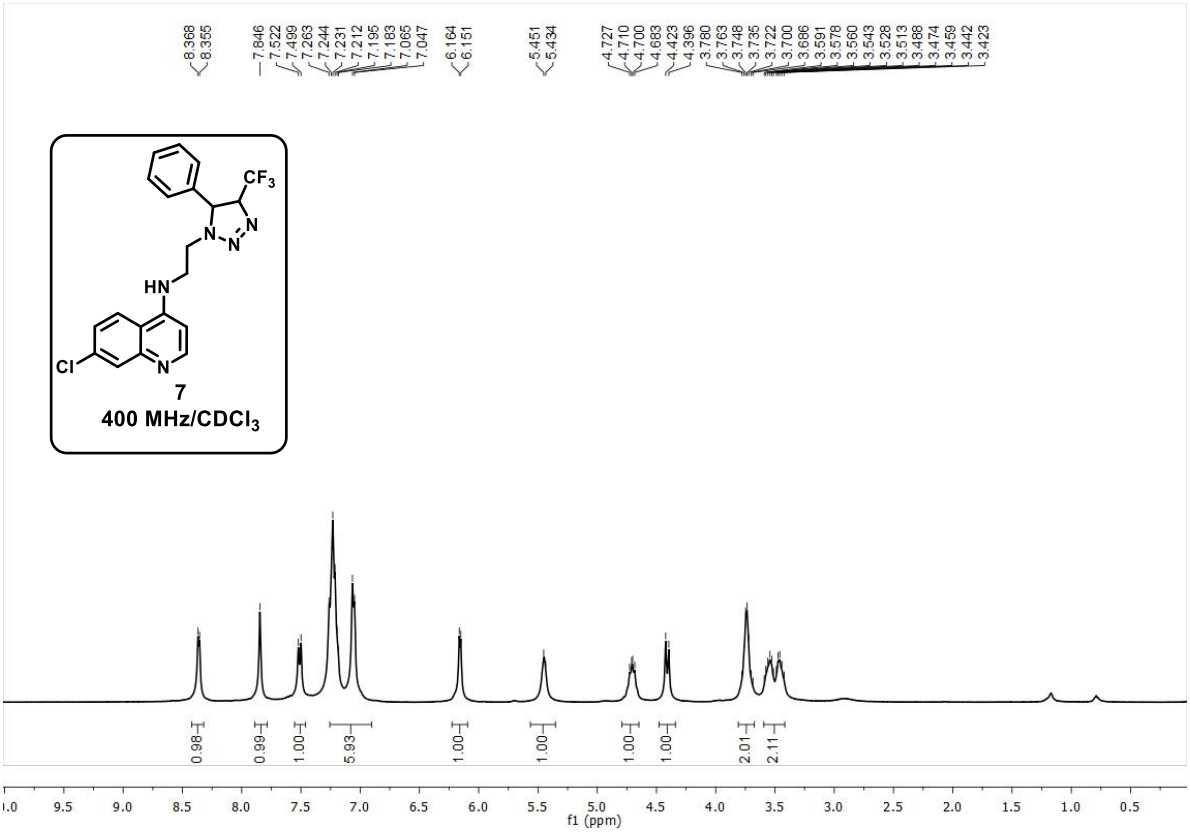


Figure 55 : 1H NMR spectrum of 7

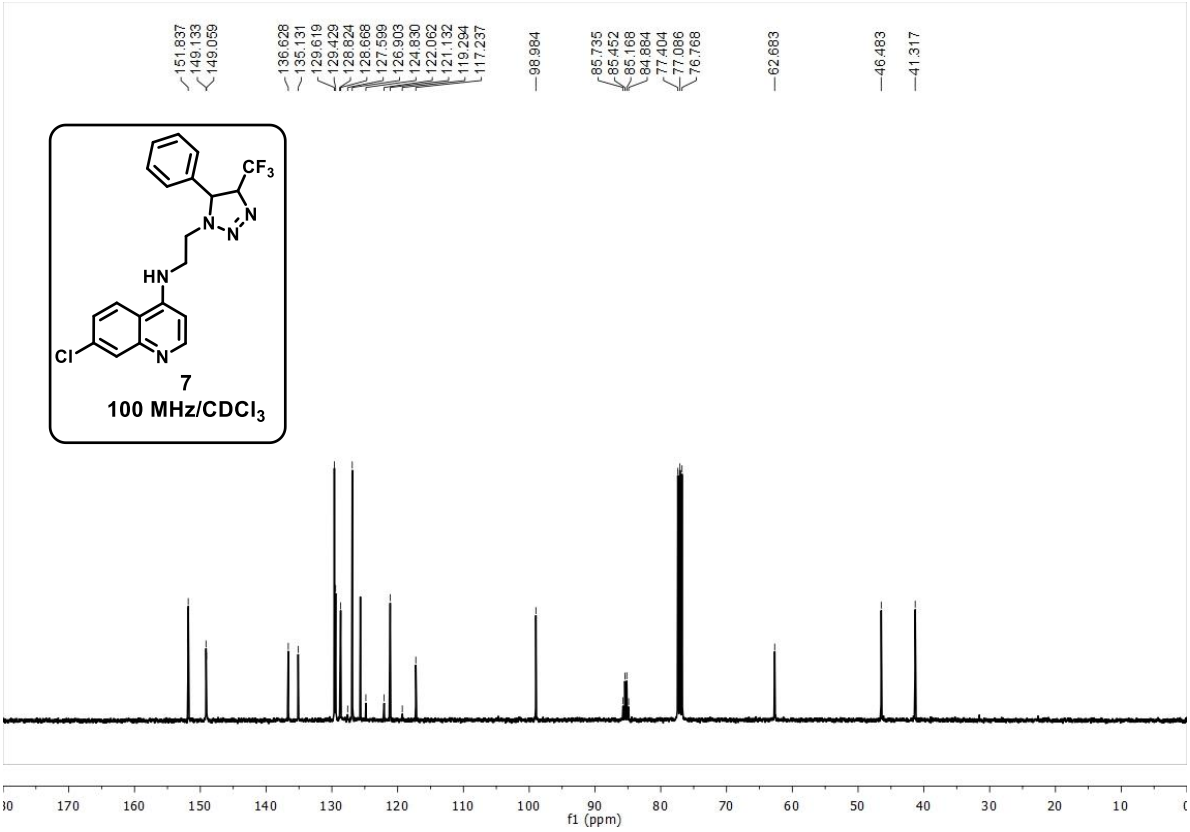


Figure 56: 13C NMR spectrum of 7

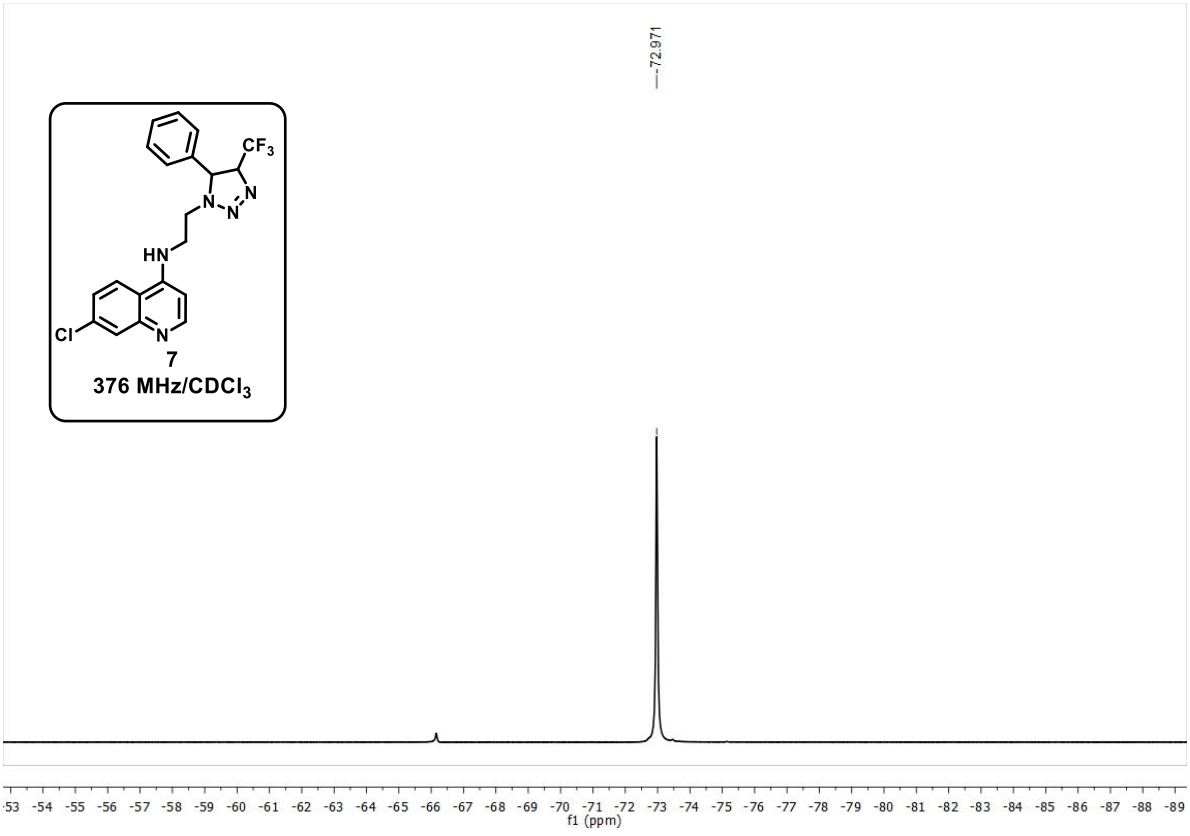


Figure 57: 19F NMR spectrum of 7

7. Conclusion

Malaria remains as the deadliest infectious disease across Africa, Asia and America so far. Antimalarial drug discovery is of paramount importance as part of eradication of this parasitic disease. It has been proved that food vacuole of *P. falciparum* consists of its major metabolic pathways, digestion of hemoglobin that provide pool of amino acid for parasite growth and detoxification of heme by various methods in different sites of the target. All these routes are promising targets for parasitic inhibition. By considering this fact, a novel class of antimalarials compounds, trifluoromethyltriazoline-4-aminoquinoline hybrids, have been synthesized which might target food vacuole of malaria parasite. The synthesis of the requisite compounds was carried by a silver catalyzed [3+2] cycloaddition reaction of trifluorodiazoethane with imines formed after condensation of N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine or N^1- (7-chloroquinolin-4-yl)ethane-1,2-diamine with aldehydes. The biological screening of these compounds as antimalarial agent is currently ongoing.

8. References

1. Nureye, D.; Assefa, S. Old and Recent Advances in Life Cycle, Pathogenesis, Diagnosis, Prevention, and Treatment of Malaria Including Perspectives in Ethiopia. *Sci. World J.* **2020**, *2020*, 1–17.
2. Alam, A. Serine Proteases of Malaria Parasite Plasmodium Falciparum : Potential as Antimalarial Drug Targets. *Interdiscip. Perspect. Infect. Dis.* **2014**, *2014*, 1–7.
3. World Malaria Report, WHO, Geneva, **2019**, **ISBN:** 978-92-4-156572-1.
4. Aly, A. S. I.; Vaughan, A. M.; Kappe, S. H. I. Malaria Parasite Development in the Mosquito and Infection of the Mammalian Host. *Annu. Rev. Microbiol.* **2009**, *63*, 195–221.
5. Guidelines for the Treatment of Malaria, 3rd edition. Global Malaria Programme, WHO, Geneva, **2015**, 24.
6. Nair, L. S.; Nair, A. S. Effects of Malaria Infection on Pregnancy. *Indian J Malariol*, **1993**, *30*, 207-214.
7. World Malaria Report, WHO, Geneva, **2015**, **ISBN:** 978-92-4-156515-8.
8. CDC, *8e History of Malaria, an Ancient Disease*. Division of Parasitic Diseases and Malaria, USA, 2016.
9. Wernsdorfer, W. H. The Development and Spread of Drug-Resistant Malaria. *Parasitol. Today* **1991**, *7*, 297–303.
10. Na-Bangchang, K.; Karbwang, J. Current Status of Malaria Chemotherapy and the Role of Pharmacology in Antimalarial Drug Research and Development. *Fundam. Clin. Pharmacol.* **2009**, *23*, 387–409.
11. Plowe, C. V.; Kublin, J. G.; Doumbo, O. K. P. Falciparum Dihydrofolate Reductase and Dihydropteroate Synthase Mutations: Epidemiology and Role in Clinical Resistance to Antifolates. *Drug Resist. Updat.* **1998**, *1*, 389–396.
12. Kumar, S.; Bhardwaj, T. R.; Prasad, D. N.; Singh, R. K. Drug Targets for Resistant Malaria: Historic to Future Perspectives. *Biomed. Pharmacother.* **2018**, *104*, 8–27.
13. Avery, M. A.; Mehrotra, S.; Johnson, T. L.; Bonk, J. D.; Vroman, J. A.; Miller, R. Structure-Activity Relationships of the Antimalarial Agent Artemisinin. 5. Analogs of 10-Deoxoartemisinin Substituted at C-3 and C-9. *J. Med. Chem.* **1996**, *39*, 4149–4155.
14. Biamonte, M. A.; Wanner, J.; Le Roch, K. G. Recent Advances in Malaria Drug Discovery. *Bioorganic Med. Chem. Lett.* **2013**, *23*, 2829–2843.
15. Nosten, F.; White, N. J. Artemisinin-Based Combination Treatment of Falciparum Malaria. *Am. J. Trop. Med. Hyg.* **2007**, *77*, 181–192.
16. Bloland, P. B. Drug resistance in malaria. WHO, Geneva, **2001**, 22.
17. Kolakovich, K. A.; Gluzman, I. Y.; Duffin, K. L.; Goldberg, D. E. Generation of Hemoglobin Peptides in the Acidic Digestive Vacuole of Plasmodium Falciparum Implicates Peptide Transport

in Amino Acid Production. *Mol. Biochem. Parasitol.* **1997**, *87*, 123–135.

18. Rosenthal, P. J. Antimalarial Drug Discovery: Old and New Approaches. *J. Exp. Biol.* **2003**, *206*, 3735–3744.
19. Philippe, G.; Christiane, D.; Isabelle, F. Advances in Antimalarial Drug Evaluation and New Targets for Antimalarials. In *Malaria Parasites*; InTech, 2012.
20. Lim, L.; McFadden, G. I. The Evolution, Metabolism and Functions of the Apicoplast. *Philos. Trans. R. Soc. B Biol. Sci.* **2010**, *365*, 749–763.
21. Medicus-Bringa, M. Book Review: Adverse Drug Reactions. *Ann. Pharmacother.* **2001**, *35*, 1499–1499.
22. Law, B. Y. K.; Wang, M.; Ma, D.-L.; Al-Mousa, F.; Michelangeli, F.; Cheng, S.-H.; Ng, M. H. L.; To, K.-F.; Mok, A. Y. F.; Ko, R. Y. Y.; Lam, S. K.; Chen, F.; Che, C.-M.; Chiu, P.; Ko, B. C. B. Alisol B, a Novel Inhibitor of the Sarcoplasmic/Endoplasmic Reticulum Ca2+ ATPase Pump, Induces Autophagy, Endoplasmic Reticulum Stress, and Apoptosis. *Mol. Cancer Ther.* **2010**, *9*, 718–730.
23. Kühlbrandt, W. Biology, Structure and Mechanism of P-Type ATPases. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 282–295.
24. Nigussie, D.; Beyene, T. New Targets in Malaria Parasite Chemotherapy: A Review. *Malar. Control Elimin.* **2015**.
25. Sonda, S.; Hehl, A. B. Lipid Biology of Apicomplexa: Perspectives for New Drug Targets, Particularly for Toxoplasma Gondii. *Trends Parasitol.* **2006**, *22*, 41–47.
26. M. Zhang, V.; Chavchich, M.; C. Waters, N. Targeting Protein Kinases in the Malaria Parasite: Update of an Antimalarial Drug Target. *Curr. Top. Med. Chem.* **2012**, *12*, 456–472.
27. Wells, T. N.; van Huijsduijnen, R. H. Ferroquine: Welcome to the next Generation of Antimalarials. *Lancet Infect. Dis.* **2015**, *15*, 1365–1366.
28. Verkman, A. S.; Anderson, M. O.; Papadopoulos, M. C. Aquaporins: Important but Elusive Drug Targets. *Nat. Rev. Drug Discov.* **2014**, *13*, 259–277.
29. Muraleedharan, K. M.; Avery, M. A. Advances in the Discovery of New Antimalarials. In *Comprehensive Medicinal Chemistry II*; Elsevier, 2007; Vol. 7, pp 765–814.