SYNTHESIS AND EXPLORATION OF QUINOLINO-BENZOXABOROLES AS POTENTIAL THERAPEUTIC AGENTS

A THESIS

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Greeshma P. Kumpati

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Advisor: Joseph L. Johnson

Co-Advisor: Venkatram R. Mereddy

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ABSTRACT

Benzoxaborole structure contains a phenyl ring fused with a heterocyclic oxaborole ring moiety. Benzoxaboroles are considerably more stable and exhibit high hydrolytic resistance compared with corresponding phenylboronic acids. The enhanced acidity of benzoxaboroles allows them to be predominantly in anionic forms in aqueous solution at physiological pH, which causes them to exhibit higher water solubility and better pharmacokinetic properties than phenylboronic acids. Increasing interest in benzoxaborole compounds is mainly due to their broad-spectrum biological activity including antimicrobial, anti-inflammatory and other medicinal properties. Quinoline is a highly privileged nitrogen containing a bicyclic ring system where a benzene ring is fused to a pyridine ring. The quinoline moiety is found in many natural products and has been traditionally used as a medicine for treating a wide variety of diseases. Quinoline-based molecules have been found to exhibit a diverse range of pharmacological properties with uses as antimalarial, antibacterial, anticonvulsant, cardiotonic, anticancer, anthelmintic, antifungal, anti-inflammatory and analgesic agents.

In this regard, we envisioned that introduction of aminobenzoxaborole unit on quinolines would result in novel molecular entities with favorable pharmacological and pharmaceutical properties for developing therapeutic agents for a wide variety of diseases. The aims of the current work include: 1) Develop a new synthetic methodology for the rapid creation of aminobenzoxaborole containing quinolines; and 2) Explore the efficacy of synthesized candidate compounds as antibacterial, antifungal, antiviral, anti-inflammatory, and antimalarial agents.

As a part of this thesis, we developed a novel synthetic methodology for preparing quinolino aminobenzoxaboroles. The synthesized compounds were initially evaluated for

their cytotoxic properties against various human and murine proliferating cancer cells. All the compounds were found to be well tolerated did not display toxicity even at high concentrations. Encouraged by their lack of toxicity, the test compounds were evaluated for their antibacterial activity against *E. coli, B. subtilis, and M. smegmatis* and for their antifungal activity against *C. neoformans* and *C. albicans.* Some of the synthesized derivatives exhibited good and selective activity against *M. smegmatis.* Future studies will involve evaluation of synthesized candidate compounds as antitubercular, antiviral, antimalarial, and anti-inflammatory agents.

Table of Contents

List of schemes

List of Figures

List of Abbreviations

IC_{50}	inhibitory concentration where 50% of biological response is inhibited
MeOH	Methanol
EtOH	Ethanol
µM	Micromolar
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
K_2CO_3	Potassium carbonate
$NaBH_4$	Sodium borohydride
THF	Tetrahydrofuran
NaH	Sodium hydride
$B(iPrO)_3$	Triisopropyl borate
Et3N or TEA	Triethyl amine
PCC	Pyridinium chlorochromate
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
$Pd(dppf)Cl_2$	(1,1'-Bis(diphenylphosphino)ferrocene)palladium(II) dichloride
$Pd(PPh3)_4$	tetrakis(triphenylphosphine)palladium(0)
EtOAc	Ethyl acetate
TLC	Thin layer chromatography
HCl	Hydrochloric acid
HNO_3	Nitric acid
Br_2	Bromine
Pd-C	Palladium Carbon
NBS	N-bromo succinimide
DHP	Dihydropyran
BuLi	Butyl lithium
$POBr_3$	Phosphoryl bromide

Chapter 1

Introduction

Boron is used in many day-to-day applications, from cleaning materials such as borates and perborates to being utilized in glass manufacturing like boric acid.[1] Additionally, boron continues to play an important role in chemical synthesis and has medicinal uses as anticancer agents,[2] antibacterial agents,[3] etc. For example, boron containing reagents, sodium borohydride ($NaBH_4$) and borane (BH_3) are extensively used as important reducing agents in chemical synthesis. Bortezomib is a boron containing anti-cancer medication used for the treatment of multiple myeloma.[4] Many organic and inorganic boron compounds have been utilized in boron neutron capture therapy to treat cancers.[5]

Boron is a group 13 metalloid element. The chemical reactivity of boron reflects its position in the periodic table. It has $1s^2 2s^2 2p^1$ as a ground state electronic configuration with three valence electrons and thus can form trivalent compounds such as boron trifluorides (BF_3). Boron typically undergoes sp^2-hybridization and has one vacant p orbital adopting trigonal planar geometry. The empty p orbitals on boron make trivalent boron compounds electron deficient and thus act as Lewis acids. The electrophilic trivalent boron reacts with incoming nucleophiles and can form tetravalent compounds adopting tetrahedral geometry (Figure 1.1).

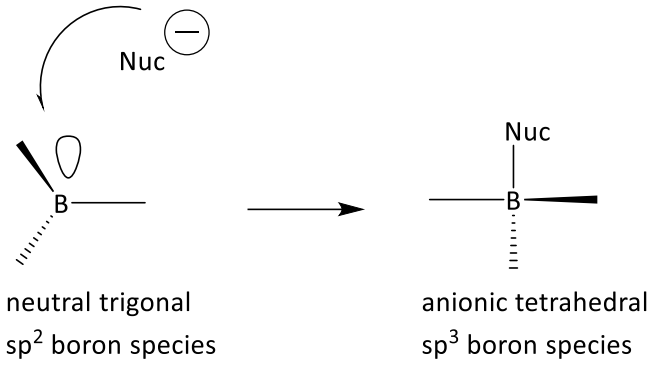


Figure 1.1 Conversion of a sp^2 trigonal boron species to an anionic sp^3 tetrahedral boron species upon reaction with a nucleophile.

The ability of boron to form trigonal and tetrahedral species is consistent in reaction mechanisms where boron is involved. The boron trigonal species can be regarded as neutral counterparts of carbocation and tetrahedral boron species as equivalents of sp^3 hybridized carbon compounds.[6] This similarity prompted researchers to focus on synthesis and diverse applications of organoboron compounds.

1.1 Boronic acids

Boronic acids are important class of organoboron compounds. These are trivalent compounds that contain one alkyl/aryl substituent and two hydroxyl groups on the boron atom.[7] The six outermost electrons and the resulting two electron deficiency helps sp^2 boron to maintain/retain an empty p orbital. This vacant orbital is orthogonal to the other three substituents that are aligned in trigonal planar geometry.[6,7] Boronic acid containing compounds have many applications including their use in Suzuki-Miyaura coupling, in optical and electrochemical sensors for a broad range of biologically relevant materials, and therapeutic uses for the treatment and prevention of several diseases (Figure1.2).[5-7]

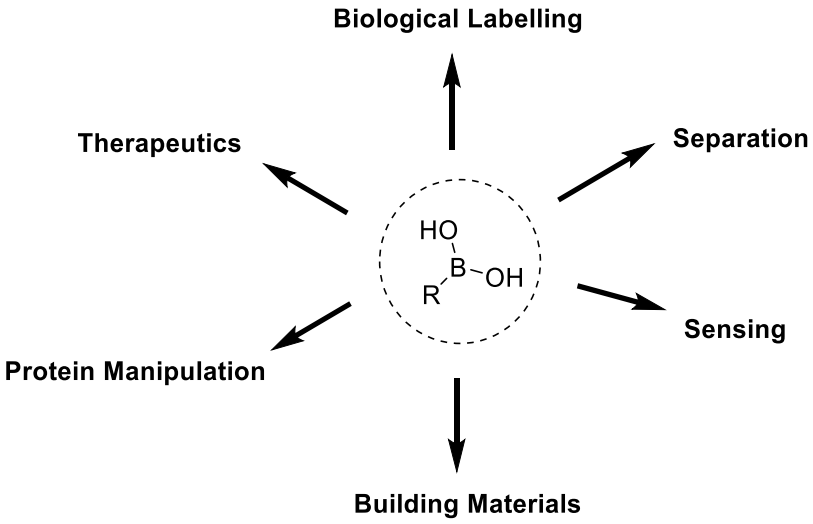
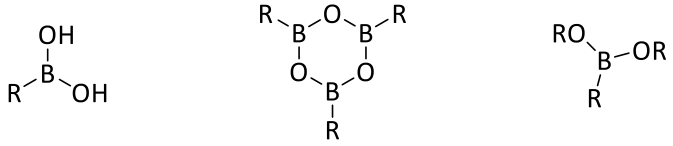


Figure 1.2 Broad-spectrum applications of boronic acids.

The distinct properties of boronic acids **1** are due to the presence of two free hydroxyl groups. For example, boronic acids readily form cyclic boroxines[8] **2** undergoing dehydration. This process is reversible; hence, boroxines and boronic acids are often utilized interchangeably. Correspondingly, the reversible interaction of the boronic acids with other hydroxyl groups result in the formation of boronic esters. In general, boronic esters **3** are more widely used than the corresponding boronic acids **1** because of their increased stability and solubility.[8,9]



1 **2** **3**

Figure 1.3 Structures of generalized organoboron compounds.

1.2 Benzoxaboroles

The internal hemiesters of 2-(hydroxymethyl)phenylboronic acids are called benzoxaboroles **4**, a unique class of boronic compounds known for their significant applications in chemical synthesis, materials, and drug development.[10] These compounds are considerably more stable and exhibit high hydrolytic resistance compared with the corresponding boronic acids. Benzoxaborole **4** contains a benzene ring fused with stable heterocyclic oxaborole ring moiety. Benzoxaboroles have a lower pK_a compared to the corresponding phenyl boronic acids **5**.[9-11] This difference is due to the ring strain that is introduced by the five-membered oxaborole ring. The ring strain is released upon addition of water leading to the pK_a depression (Figure 1.4).[9-11]

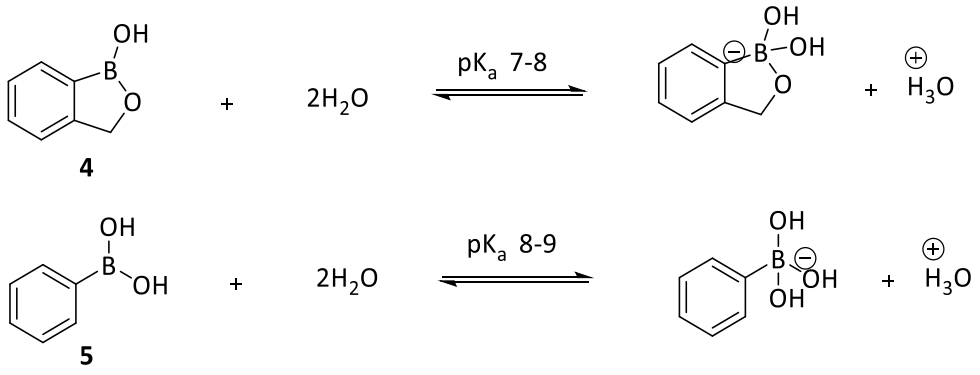
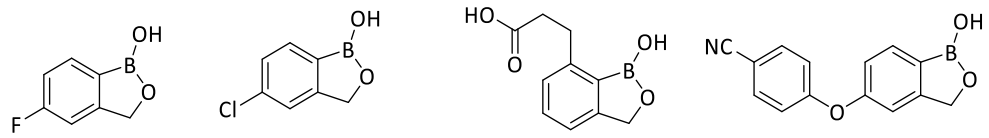


Figure 1.4 The pK_a values of benzoxaborole and phenylboronic acid.

Benzoxaboroles are more acidic in nature compared to the corresponding phenylboronic acids. This enhanced acidity of benzoxaboroles allows them to be predominantly in anionic forms in aqueous solution at physiological pH, which causes benzoxaboroles to exhibit higher water solubility and better pharmacokinetic properties than phenylboronic acids.[9-11]

Increasing interest in benzoxaborole compounds is mainly due their broad-spectrum biological activity, with 5-fluorobenzoxaborole **6** (tavaborole) identified and approved by the FDA as an anti-fungal medication for the treatment of onychomycosis (toe and fingernail infection). Likewise, there are many benzoxaborole derivatives with anti-bacterial, anti-fungal, and anti-inflammatory properties (Figure 1.5).[9-11]



Tavaborole
Activity against onychomycosis

6

AN2718
Activity against Tenia Pedis

7

AN3661
Activity against Plasmodium falciparum

8

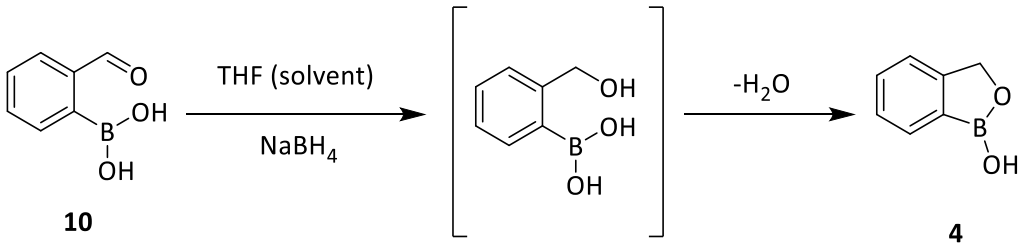
Crisaborole
anti-inflammatory activity against Psoriasis

9

Figure 1.5 Functionalized benzoxaboroles with medicinal uses.

1.3 General synthetic methodologies to prepare functionalized benzoxaboroles:

Mereddy et al. found that benzoxaboroles could be synthesized by reacting 2-formylphenylboronic acid **10** with sodium borohydride ($NaBH_4$) in THF to give unsubstituted stable benzoxaborole **4** in 90% yield (Scheme 1.1).[12]

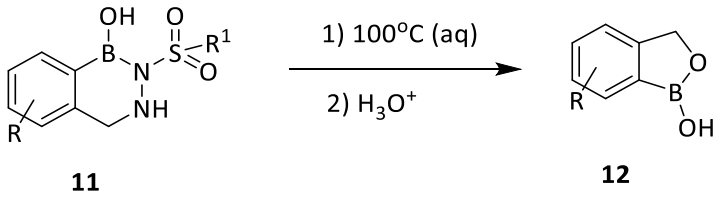


10

4

Scheme 1.1 Synthesis of Benzoxaborole.

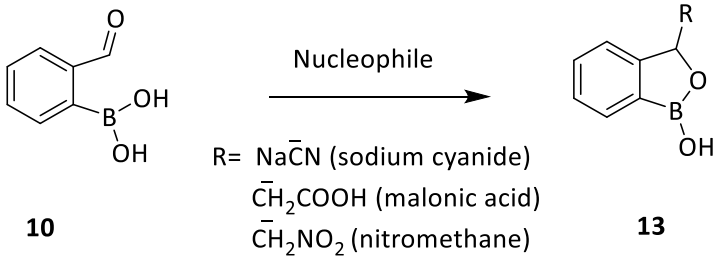
An alternative approach to synthesize monosubstituted benzoxaborole **12** via hydrolysis of 1,2-dihydro-1-hydroxy-2,3,1-benzodiazaborines **11** in the presence of aqueous sodium hydroxide was reported by Grassberger et al. (Scheme 1.2).[13]



11 **12**

Scheme 1.2 Synthesis of aryl-substituted benzoxaboroles.

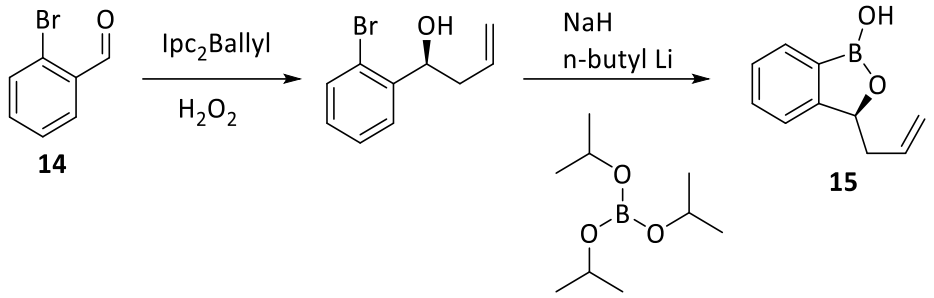
Synder and coworkers synthesized 3-substituted benzoxaboroles **13** upon reacting ortho-formylphenylboronic acid **10** with different nucleophilic substrates such as nitromethane, malonic acid, and sodium cyanide (Scheme 1.3).[14]



10 **13**

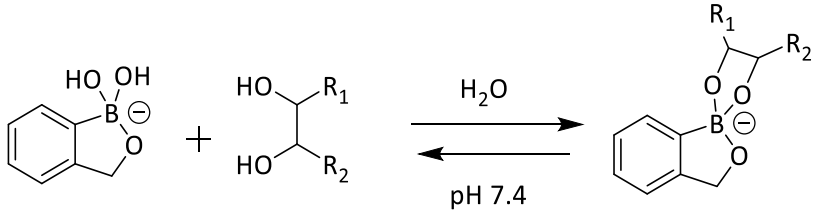
Scheme 1.3 Synthesis of benzylic carbon substituted benzoxaboroles.

Molecular chirality plays a significant role in chemistry, biology, and medicine. It is important to control the chirality of a drug candidate to achieve proper interaction with chiral biomolecules. Chiral benzoxaborole **15** was synthesized in two step reaction protocol by Mereddy et al. Firstly, bromobenzaldehyde **14** was reacted with the asymmetric allylating agent $Ipc_2Ballyl$ to form chiral homoallylic alcohol, which then underwent cyclization upon debromination, borylation, and hydrolysis (Scheme 1.4).[15]



Scheme 1.4 Stereoselective synthesis of benzoxaborole via asymmetric allylboration.

Boronic acids form cyclic esters upon reaction with diols. This occurs when there is a second hydroxy group present on the anionic form of benzoxaborole (Scheme 1.5).[16]

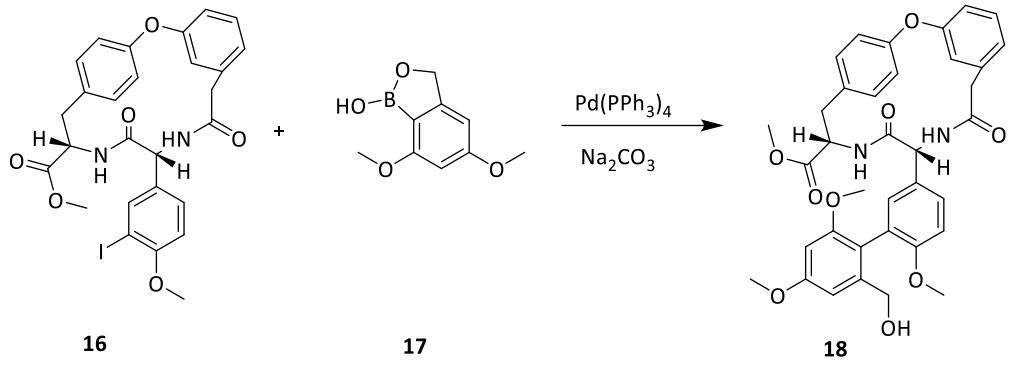


Scheme 1.5 Formation of cyclic borate esters with benzoxaboroles.

1.4 Applications of Benzoxaborole Derivatives in Organic and Medicinal Chemistry:

1.4.1 Applications in Organic synthesis:

Benzoxaborole played an important role in the total synthesis of vancomycin via Suzuki-Miyaura cross coupling reported by Nicolaou et al. The methoxy substituted benzoxaborole **17** was cross coupled with an aryl iodide **16** in the presence of the $Pd(PPh_3)_4$ catalyst and Na_2CO_3 to obtain the benzyl alcohol intermediate in the synthesis of the key vancomycin synthon **18** (Scheme 1.6).[17]



16 17 18

Scheme 1.6 Suzuki cross-coupling reaction approach to bicyclic system of vancomycin with dimethoxybenzoxaborole.

1.4.2 Application as Antifungal agents

The benzoxaborole derivative 5-fluorobenzoxaborole (tavaborole) **6** targets and blocks fungal leucyl -tRNA synthetase. The boron atom in benzoxaborole irreversibly binds to the 2′and 3′ hydroxy groups of the t-RNA's 3'-terminal adenosine and thus trap the t-RNA (Figure 1.6).[18] This affects the charging of the t-RNA to its corresponding amino acid and the transportation of resultant aminoacyl t-RNA to ribosomes for protein synthesis.[19]

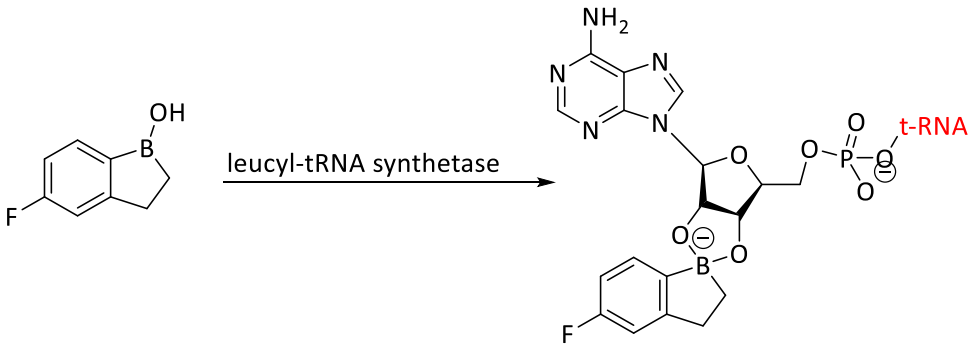
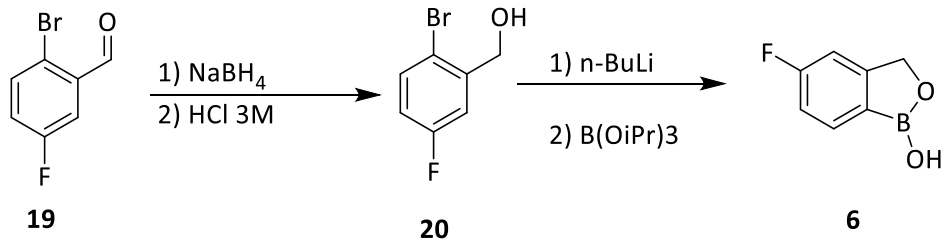


Figure 1.6 The stable "ate" complex of tRNA and tavaborole.

Tavaborole was approved by the FDA as an anti-fungal agent for the topical treatment of onychomycosis. Tavaborole **6** was obtained synthetically via a twostep

protocol. The first step was $NaBH_4$ reduction of 2-bromo-5-fluorobenzaldehyde **19** to obtain 2-bromo-5-fluorobenzyl alcohol **20**, followed by reaction of the obtained product **20** with *n*-BuLi and $B(O^iPr)_3$ (Scheme 1.7).[18,19]

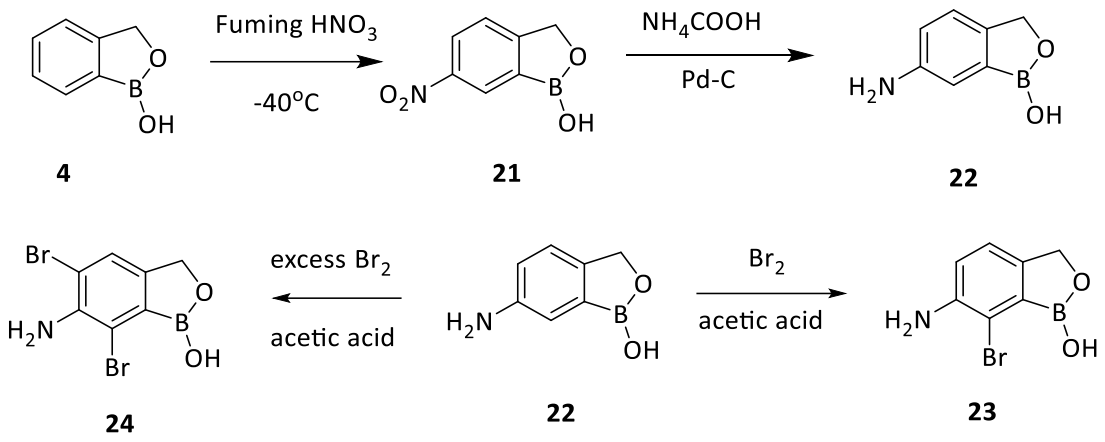


19 **20** **6**

Scheme 1.7 Synthesis of 5-fluorobenzoxabole tavaborole.

1.4.3 Application as Antibacterial agents

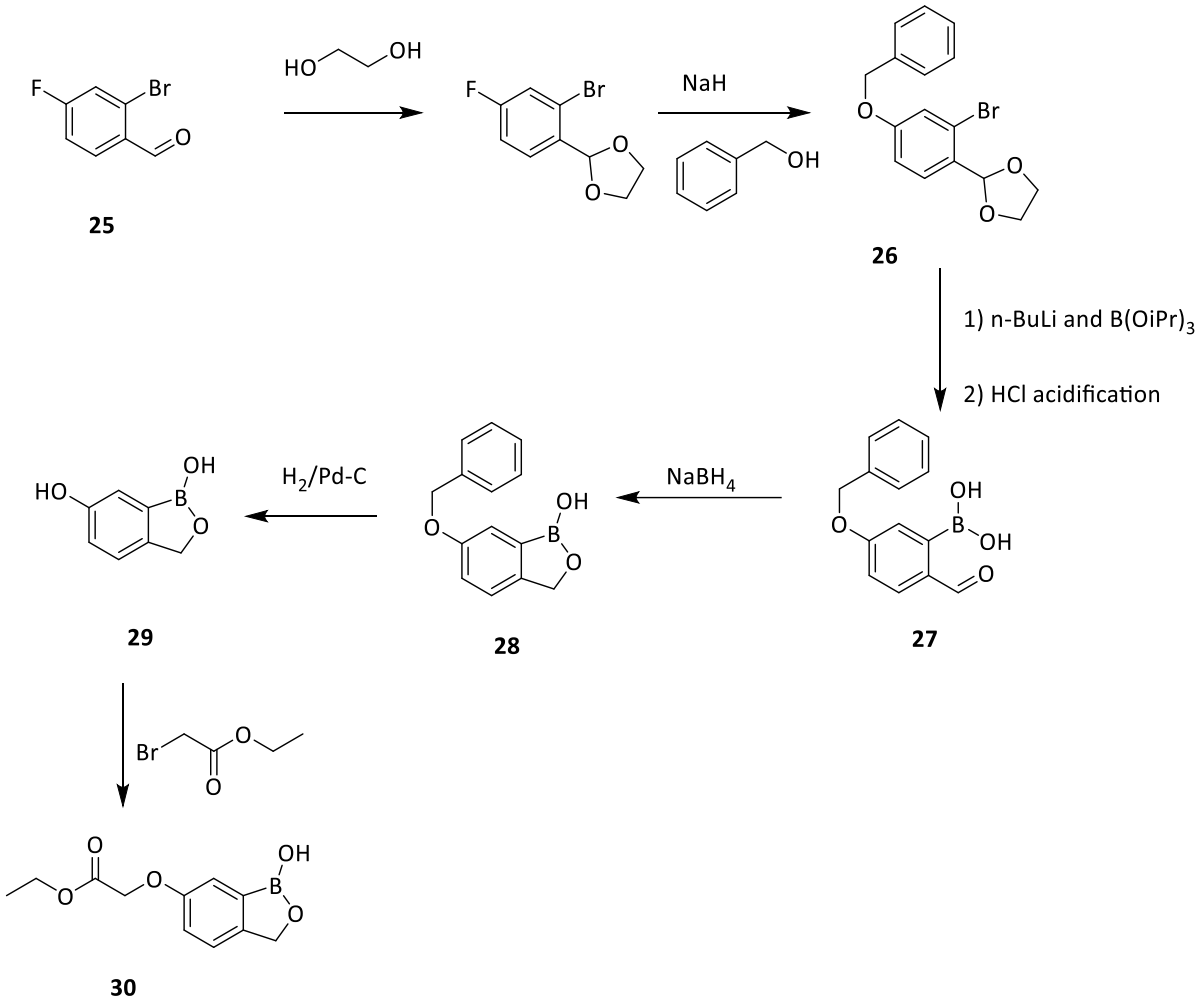
Benzoxaboroles were shown to inhibit bacterial aminoacyl tRNA synthetase by an oxaborole tRNA trapping mechanism.[18] Similar to tavaborole, brominated aminobenzoxaborole **22** has been evaluated by Mereddy et al. as a lead derivative for its anti-mycobacterial activity against *Mycobacterium tuberculosis*.[12] The chemical synthesis of brominated aminobenzoxaborole **22** was achieved via nitration of benzoxaborole using fuming nitric acid to form 6-nitrobenzoxaborole **21** followed by reduction of the nitro group with ammonium formate in the presence of palladium-carbon resulting in the formation of the aminobenzoxaborole **22** in 90% yield. This amine was further brominated using bromine in acetic acid to obtain mono and dibrominated products **23** & **24**, respectively (Scheme 1.8).[12]



Scheme 1.8 Synthesis of brominated aminobenzoxaboroles.

1.4.4 Application as antiparasitic agents

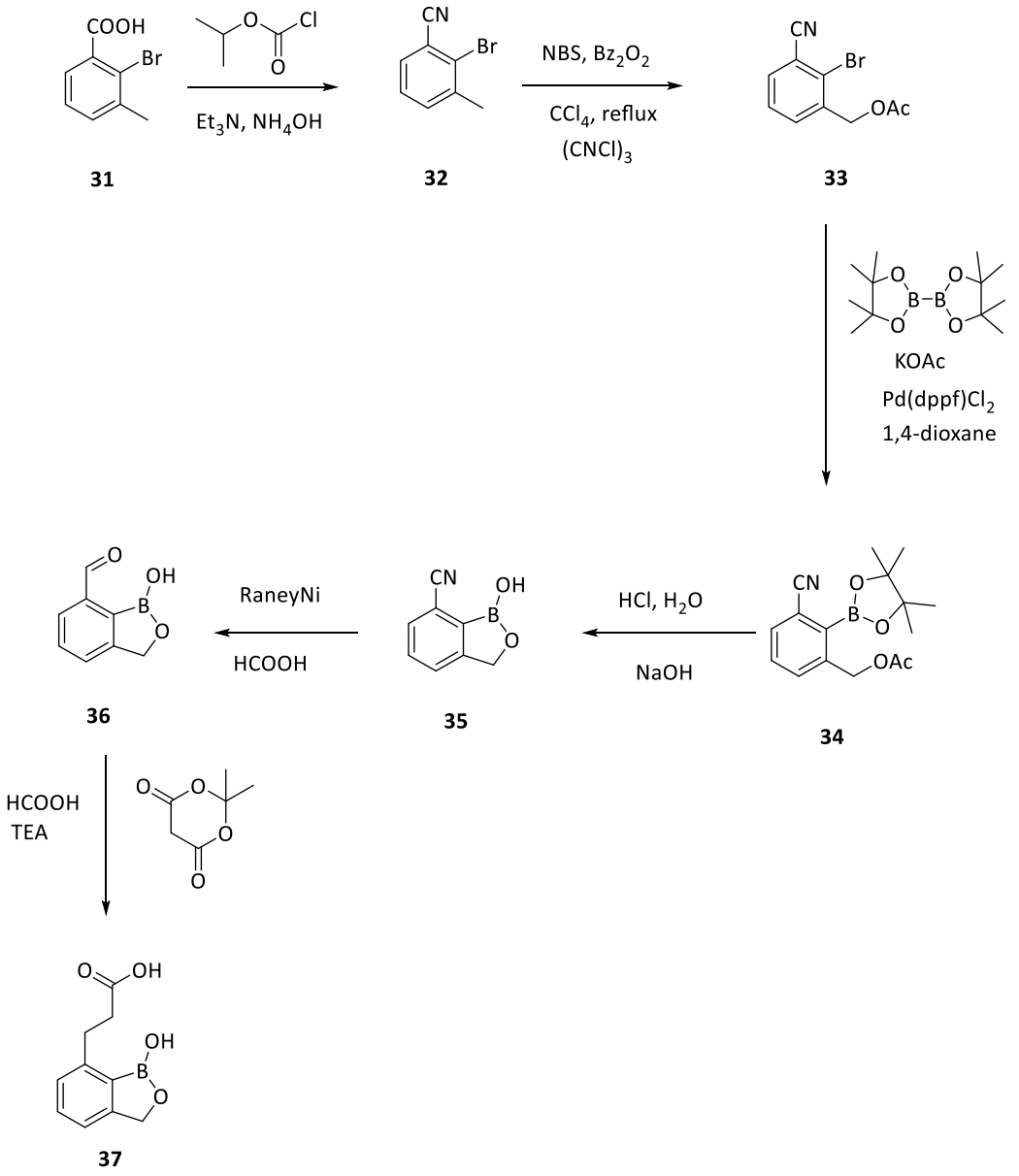
Benzoxaboroles represent a new class of anti-parasitic agents. Aryl ether, synthesized from 6-hydroxybenzoxaborole **29**, has been evaluated as anti-trypanosomal agent.[20] The reaction proceeded through protection of 2-bromo-4-fluorobenzaldehyde **25** with ethylene glycol followed by nucleophilic displacement of fluoride with benzyl alcohol to obtain 2-(4(benzyloxy)-2-bromophenyl)-1,3-dioxolane **26.** The product **26** was later reacted with *n*-butyl lithium and triisopropylborate to form boronic acid which, upon acidification, retained the aldehyde **27**. Reduction of 6-(O-benzyl)benzoxaborole using $NaBH_4$, followed by Pd-C reduction gave 6-hydroxybenzoxaborole **29**. The product **29** was coupled to ethyl-2-bromoacetate to form alkoxyacetate **30** (Scheme 1.9).[20]



Scheme 1.9 Synthesis of benzoxaborole alkoxyacetate derivative with anti-trypanosomal activity.

Boron containing small molecules (oxaboroles) were found to have potent antimalarial activity against *Plasmodium falciparum* with the lead derivative **37** exhibiting an IC_{50} of 0.026μM.[21] This was obtained from 2-bromo-1,3-dimethyl benzene **31** which was converted to the cyano compound **32** followed by dehydration. Later the compound was subjected to bromination followed by acetate formation **34** upon reaction with potassium acetate. This product underwent borylation to yield arylboronate. Hydrolysis of acetate and acidification of the resultant product generated cyanobenzoxabole, which upon treatment with Raney nickel gave the aldehyde **36**. This aldehyde was subsequently

reacted with 2,2 dimethyl-1,3 dioxane-4,6-dione to obtain the target compound **37** (Scheme 1.10).[21]

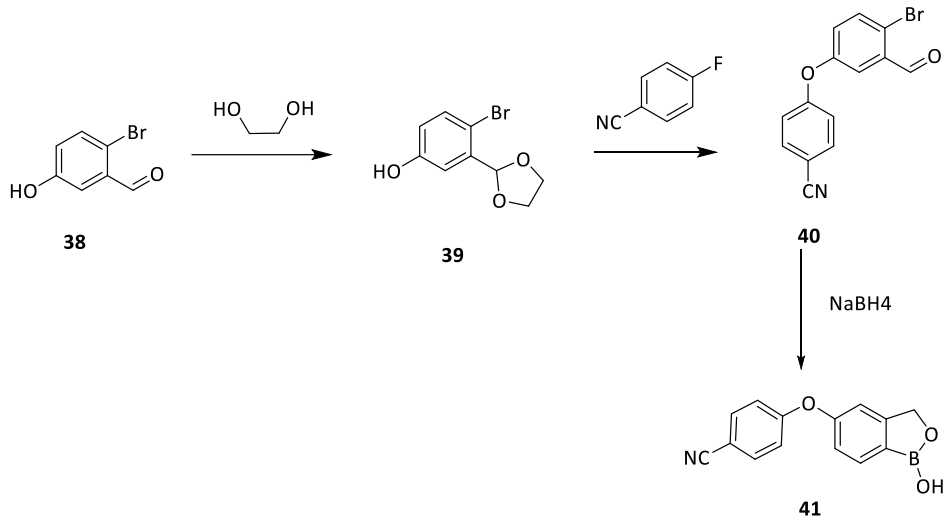


31 **32** **33** **34** **35** **36** **37**

Scheme 1.10 Synthesis of novel benzoxaborole as antimalarial agents.

1.4.5 Application as anti-inflammatory agents

Cyanophenoxy benzoxaborole derivative (Crisaborole) **41** was found to exhibit inhibitory activity against phosphodiesterase 4 (PDE_4), which is an anti-inflammatory target. It also blocks the release of cytokines such as tumor necrosis factor- α (TNF-α) and interferon-gamma (IFN-γ).[22] The cyanophenoxy benzoxaborole derivative was obtained by reacting **39** with 4-fluorobenzonitrile to form 4-(4-bromo-3-3-formylphenoxy) benzonitrile **40**. The resulting aldehyde was then reduced and protected as the tetrahydropyranyl (THP) ether. The boron atom was then introduced by metal-halogen exchange reaction using *n*-butyl lithium and triisopropylborate followed by deprotection of THP acetal to obtain alcohol which underwent cyclization to form 4-((1-hydroxy-1,3-dihydrobenzo [c][1,2]oxaborol-5-yl)oxy)benzonitrile **41** (Scheme 1.11).[22]

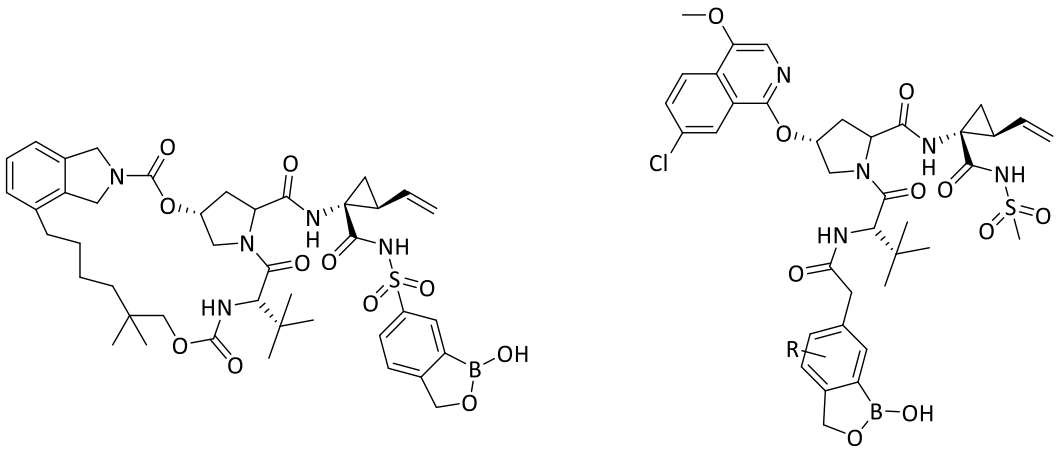


Scheme 1.11 Synthesis of anti-inflammatory agent for skin inflammatory disease.

1.4.6 Application as antiviral agents

Hepatitis C virus (HCV) is one of the main causes of liver cirrhosis. It was reported that the HCV NS3 serine protease plays an important role in viral replication.[23-25]

Benzoxaborole containing macrocyclic **42** and acyclic **43** compounds were found to have excellent inhibitory activity against the HCV NS3 serine protease (Figure 1.7).[23-25]

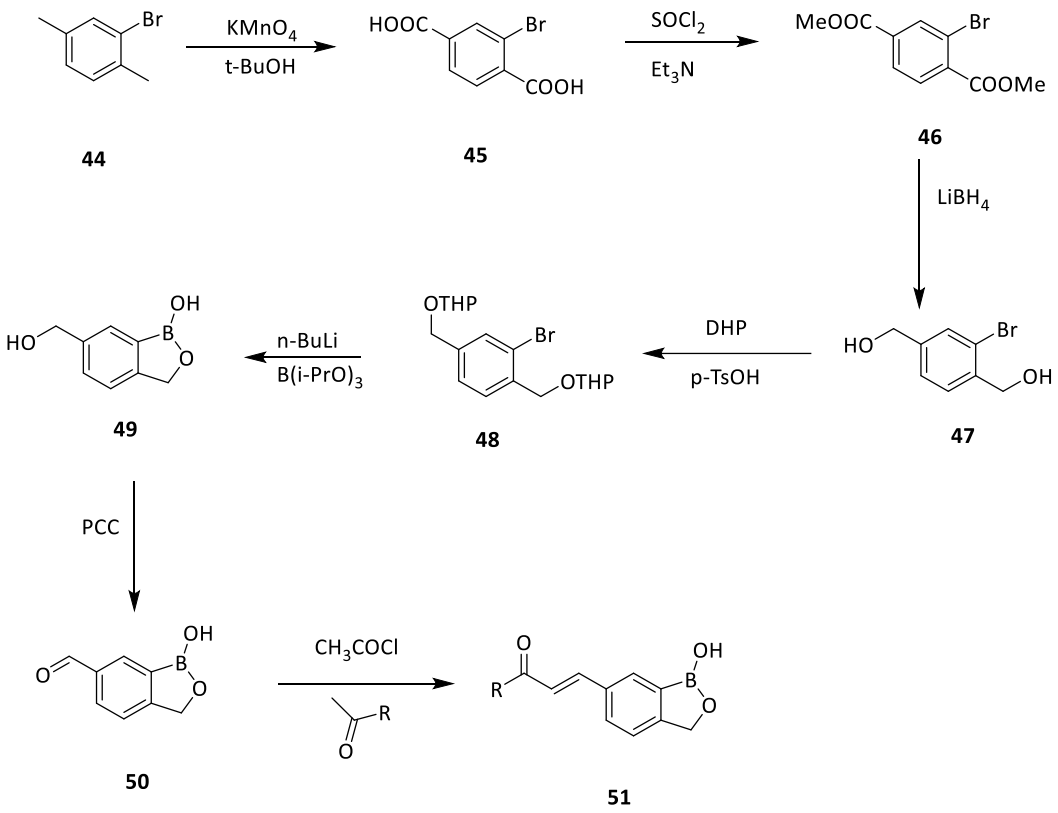


42 **43**

Figure 1.7 Benzoxaborole containing HCV NS3 protease inhibitors (**42 & 43**).

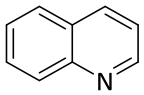
1.4.7 Application as anticancer agents:

Chalcone-benzoxaborole compounds were found to exhibit anticancer potency and toxicity against the human ovarian cancer cell line SKOV3 with an IC_{50} value of 1.4μM.[26] Compound **51** was obtained by oxidizing 2,6-dimethylbromobenzene **44** to 2-bromoterepthalic acid **45** which was eventually converted to methyl ester **46** followed by reduction of the ester group to give benzyl alcohol **47**.[26] The benzyl alcohol was then protected with di-tetrahydropyranyl ether **48** followed by deprotection and cyclization to give benzoxaborole **49**. Pyridinium chlorochromate (PCC) oxidation of alcohol gave the aldehyde **50** which underwent aldol condensation to yield the product **51** (Scheme 1.12).[26]



Scheme 1.12 Synthesis of chalcone-benzoxaborole hybrid molecules.

1.5 Quinolines as pharmacologically privileged structural units



Quinoline

Quinoline is a highly privileged nitrogen containing a bicyclic ring system where a benzene ring is fused to a pyridine ring. Other names for quinoline include bezo[b]pyridine, benzazine, 1-benzazine, and bezopyridine.[27] The quinoline moiety is found in many natural products and has been traditionally used as a medicine for treating a wide variety of diseases.[27] Some of the quinoline-based natural products include quinine, quinidine, cinchonidine, and cinchonine that have been used as traditional

antimalarial agents (Figure1.8).[27] Quinoline-based molecules have been found to exhibit a diverse range of pharmacological properties with uses as antimalarial, antibacterial, anticonvulsant, cardiotonic, anticancer, anthelmintic, antifungal, anti-inflammatory, and analgesic agents.

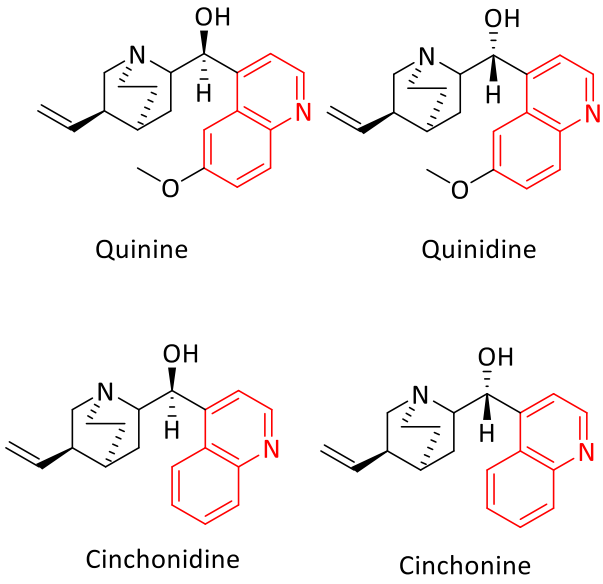


Figure 1.8 Chemical structures of quinoline based natural products.

1.5.1 Application of quinoline containing compounds as antimalarial agents

Traditionally, the quinoline containing natural product quinine (Figure1.8) has been used as an antimalarial agent. It was isolated from cinchona tree bark in early 1600's.[24] Later, researchers focused on developing synthetic antimalarial drugs centered on modification of quinine structure, which resulted in development of 4-aminoquinoline derivatives as anti-malarial drugs.[28] Examples of aminoquinoline antimalarial drugs include chloroquine, piperaquine and quinacrine (Figure 1.9).[29]

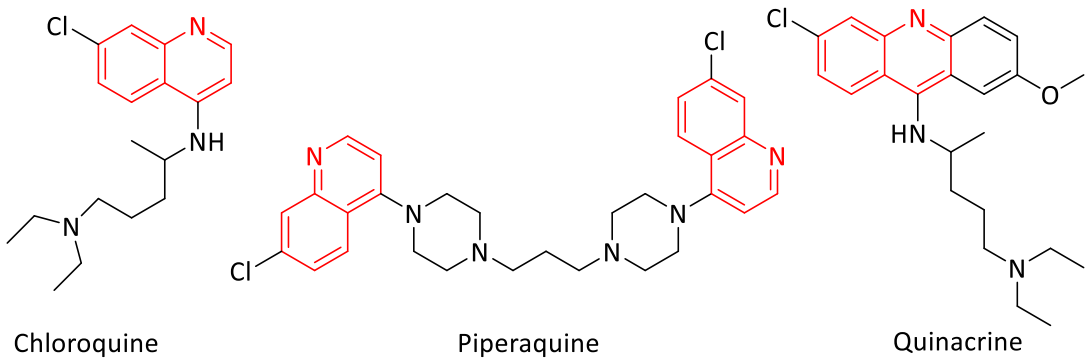


Figure 1.9 Antimalarial drugs with aminoquinoline moiety.

1.5.2 Application of quinoline containing compounds as anticancer agents

Quinoline derivatives have been found to exhibit cytotoxic properties as DNA interacting agents. Some of the quinoline containing anticancer agents are berberine, campotothecin, chelidonine, chelerythrine, dictamine, lophocereine, and nitidine. (Figure 1.10).[30]

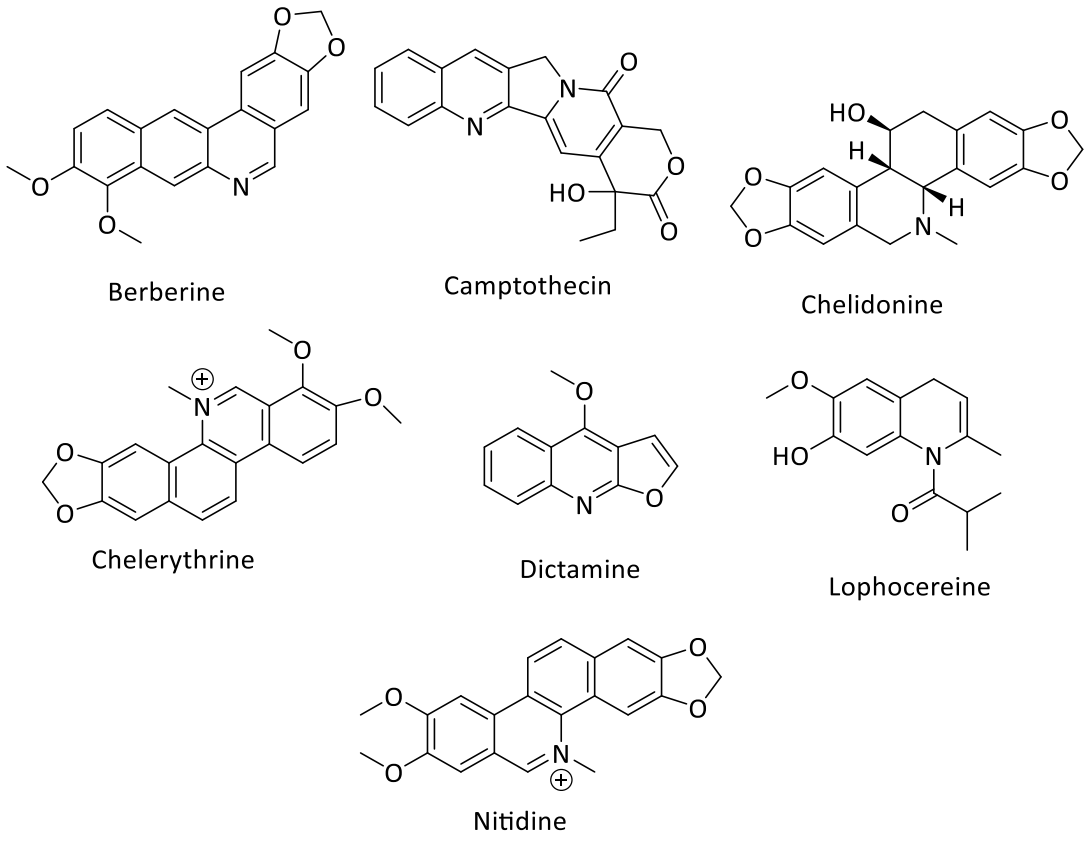


Figure 1.10 Quinoline containing compounds with anticancer activity.

1.5.3 Application of quinolones as antimicrobial agents

Modified quinolines have found important applications as antimicrobial agents. Several fluorinated quinolones are clinically used as important antibacterial agents for the treatment of a wide variety of bacterial infections.[27,31] Some of the examples in this category include ciprofloxacin, ofloxacin, gatifloxacin, and moxifloxacin. (Figure 1.11).[27,31]

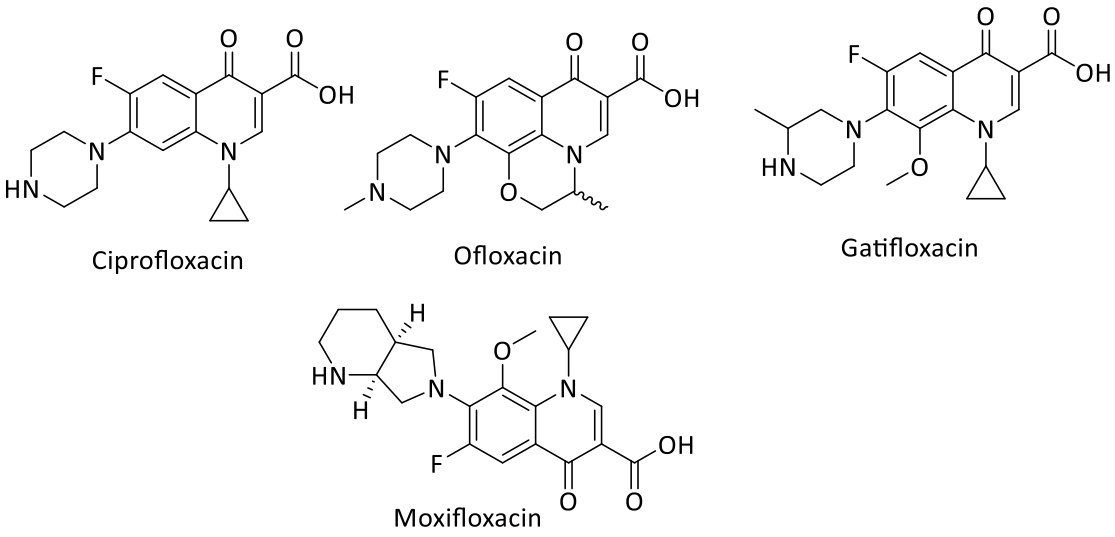


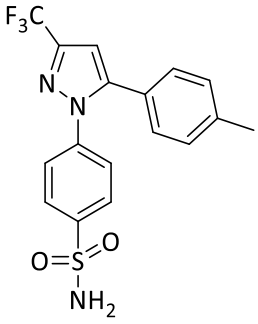
Figure 1.11 Quinoline containing antibacterial agents.

1.5.4 Application of quinoline containing compounds as anti-inflammatory agents

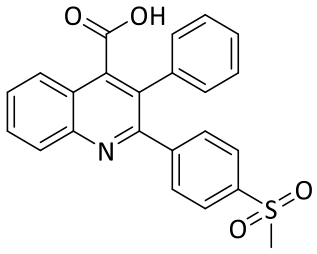
Some of the prominent inflammatory diseases are rheumatoid arthritis, inflammatory bowel disease, asthma, cardiovascular disease, diabetes, chronic obstructive pulmonary disease (COPD), Alzheimer's disease, and age-related macular degeneration (AMD). Quinolines have been used as anti-inflammatory agents targeting phosphodiesterase 4 (PDE4), transient potential vanilloid receptor (TRPV1), TNF-alpha converting enzyme (TACE), and cyclooxygenase (COX).[32]

Non-steroidal anti-inflammatory drugs (NSAIDS) are known competitive inhibitors of cyclooxygenase (COX). COX-1 is involved in the supply of necessary arachidonic metabolites and COX-2 is responsible for inducing inflammatory responses.[27] The therapeutic anti-inflammatory action of NSAIDS also leads to undesired side effects due to the non-selective nature of NSAIDS towards COX-2.[32] Hence, researchers focused on developing various drug derivatives that selectively inhibit COX-2. Godsi et al. synthesized many quinoline derivatives and 2-(4-(methylsulfonyl) phenyl) quinoline 4-

carboxylic acid having lipophilic substituents at carbon-7 and carbon-8 positions. The lead derivative **52** exhibited higher selectivity for COX-2 compared to the reference drug celecoxib (Figure 1.12).[32]



Celecoxib



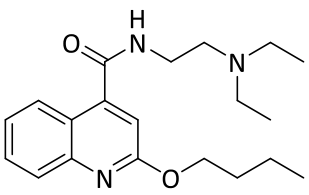
52

Compound	COX-1 IC_{50}(μM)	COX-2 IC_{50}(μM)	Selectivity index (SI) (COX1 IC_{50})/(COX2 IC_{50})
52	48.1	0.07	687.1
celecoxib	24.3	0.06	405

Figure 1.12 Quinoline based compound with COX-2 selectivity.

1.5.5 Application of quinoline containing compounds as antiviral agents

Quinoline derivatives **53** & **54** (Figure 1.13) were found to exhibit antiviral activity against viral strains such as enterovirus (EV-D68) and coxsackievirus (CVB3) by targeting viral 2C protein and inhibiting viral replication.[33] On the other hand, compounds **55** & **56** (figure 1.13) were found to be active against HIV-infection.[33]



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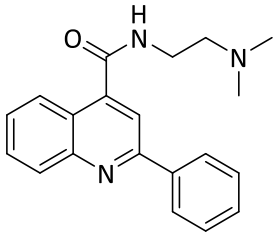

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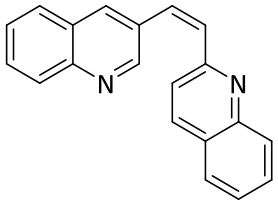

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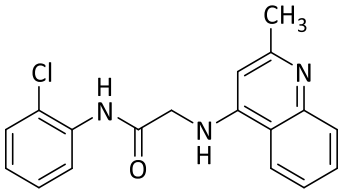


56

Figure 1.13 Quinoline containing antiviral agents.

Summary

Boron-containing stable compounds benzoxaboroles have garnered a lot of attention in recent years because of their diverse applications in chemistry, materials science, and medicine. Benzoxaborole-based compounds have been found to exhibit a wide variety of therapeutic applications including antimicrobial, antiparasitic, and anti-inflammatory agents. The unique structural characteristics of benzoxaboroles coupled with their potential as broad-spectrum therapeutics inspired us to develop novel methodologies for the synthesis of functionalized benzoxaboroles. As discussed above, quinoline-containing compounds have a wide variety of therapeutic applications and quinoline has been recognized as a pharmacologically privileged structural template in medicinal chemistry. Thus, we sought to synthesize and evaluate novel quinolino benzoxaboroles with broad-spectrum therapeutic applications.

Chapter 2

Results and Discussion

2.1 Hypothesis and Objectives

As discussed in the Introduction, benzoxaboroles and quinolines have been found to be highly useful structural units with broad-spectrum applications as therapeutic agents (Figure 2.1). Quinoline-based clinically used drugs also exhibit favorable pharmaceutical properties such as metabolic stability, longer biological half-life, and oral bioavailability. For example, quinoline based antimalarial drugs chloroquine and mefloquine have a $t_{1/2}$ of approximately month and 70 to 100% oral bioavailability.

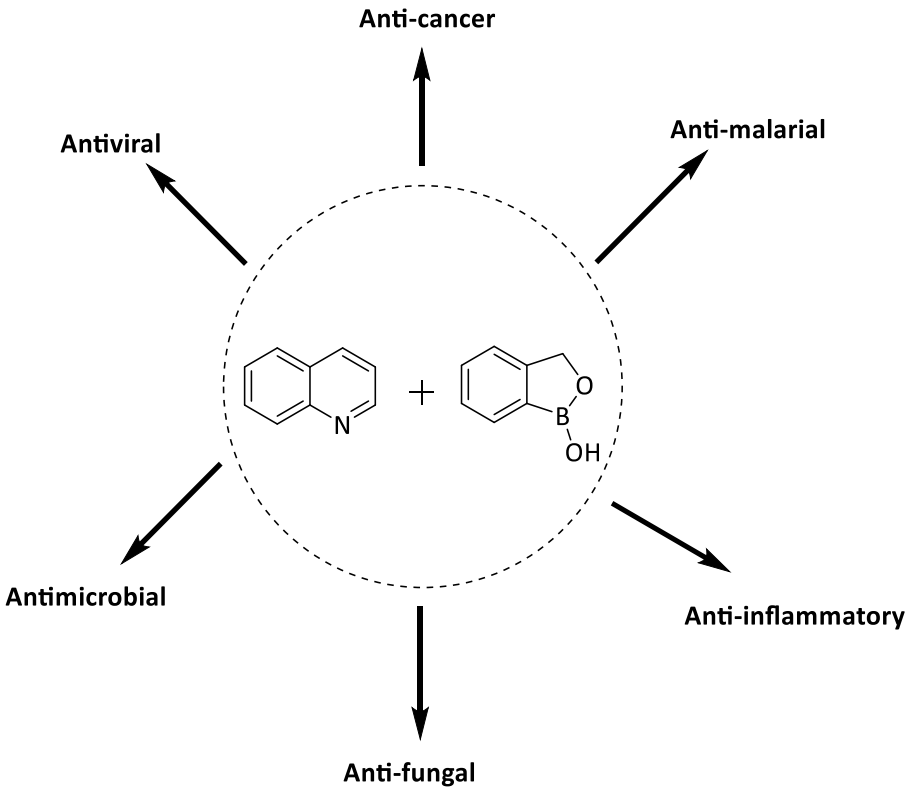


Figure 2.1 Benzoxaborole and quinoline conjugates for exploring a broad range of therapeutic applications.

We hypothesized that the introduction of aminobenzoxaborole unit on quinolines would result in novel molecular entities with favorable pharmacological and pharmaceutical properties for developing therapeutic agents targeting a wide variety of diseases. In this regard, the objectives of the current work include:

1. Develop a new synthetic methodology for the rapid creation of aminobenzoxaborole containing quinolines.

2. Explore the efficacy of synthesized candidate compounds as antibacterial, antiviral, antifungal, anti-inflammatory, and antimalarial agents.

2.2 Development of a novel protocol for the synthesis of quinolino-aminobenzoxaboroles

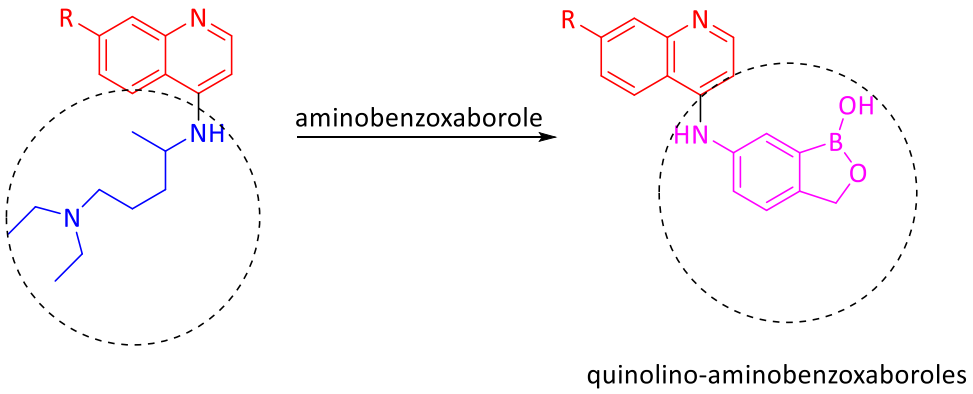
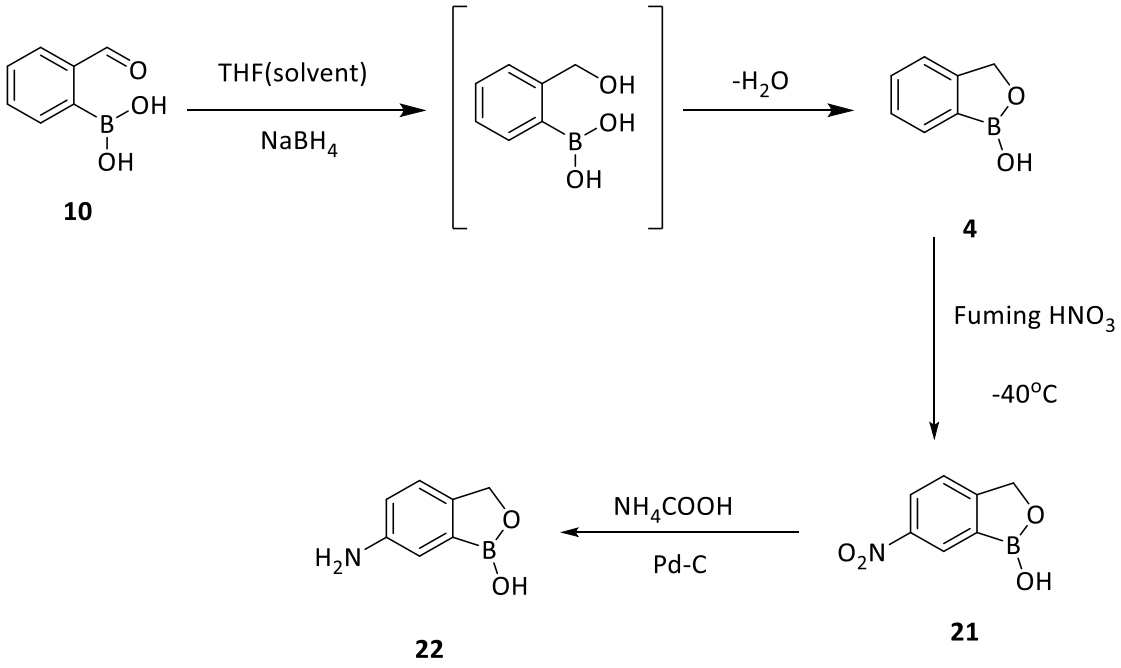


Figure 2.2 Schematic representation of quinolino-aminobenzoxaboroles

Aryl amination is one of the important reactions in introducing C-N bond between aryl halides and amines in one step. 7-halo substituted quinolines are known for an efficient nucleophilic displacement with alkyl and aryl amines. This reaction usually occurs under mild reaction conditions without the need for organometallic catalyst or strong bases. We envisioned 7-halo substituted quinolines as excellent starting materials for

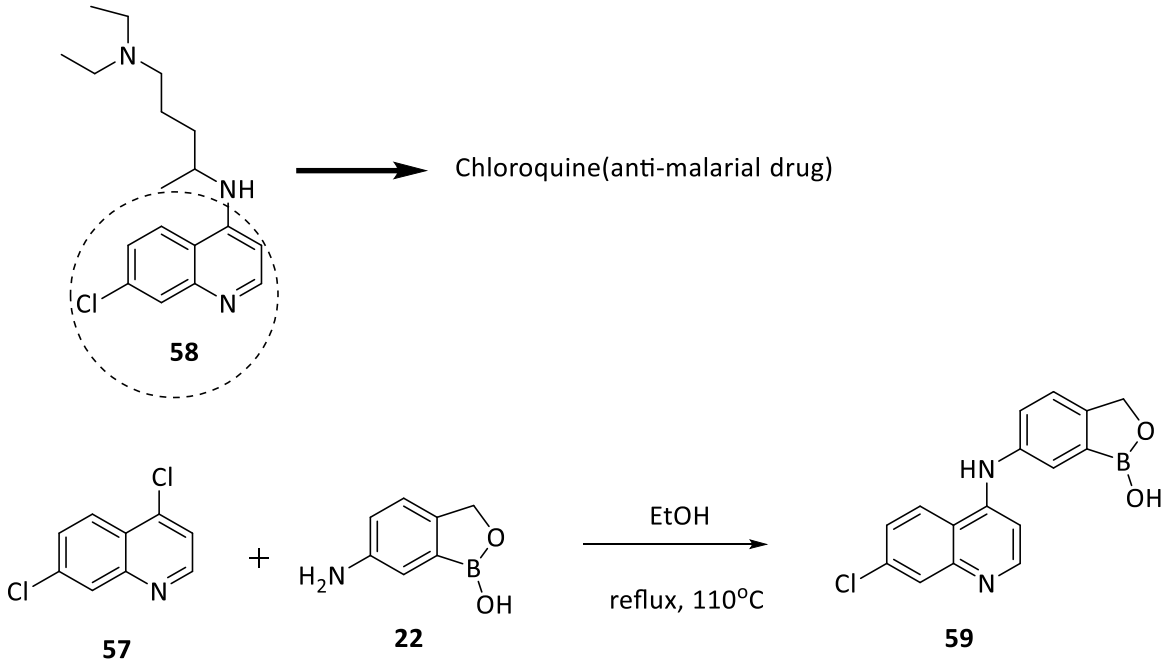
nucleophilic aromatic substitution with aminobenzoxaborole **22**. If the nucleophilic displacement with **22** occurred, as is the case with normal aromatic amines, then it would lead to an efficient synthetic protocol for creating novel quinoline containing aminobenzoxaboroles.

Initially, we explored the reaction of commercially available 4,7-dichloroquinoline **57** with aminobenzoxaborole **22**. The required **22** was synthesized in three steps starting with 2-formylphenylboronic acid **10**. Sodium borohydride reduction of **10** resulted in benzoxaborole **22**, which was nitrated using fuming nitric acid at -40^{o}C. No solvent was used in this reaction; nitric acid served as the nitrating agent as well as the solvent. The reaction mixture was poured into a water/ice mixture, the resulting yellow precipitate was filtered using a Buchner funnel and washed several times with water to remove trace nitric acid. The solid was further washed with hexanes, which resulted in 6-nitrobenzoxaborole **21** in 90% yield (Scheme 2.1). The nitro group in **21** was reduced with ammonium formate using Pd/C (5 mol %) as the catalyst. The reduction took place smoothly and the 6-aminobenzoxaborole **22** was obtained in 93% yield after filtration over silica gel pad and washing with THF (Scheme 2.1).[16] Nitrobenzoxaborole **21** and aminobenzoxaborole **22** were thoroughly characterized using ^{1}H NMR, ^{13}C NMR, and mass spectral analysis.



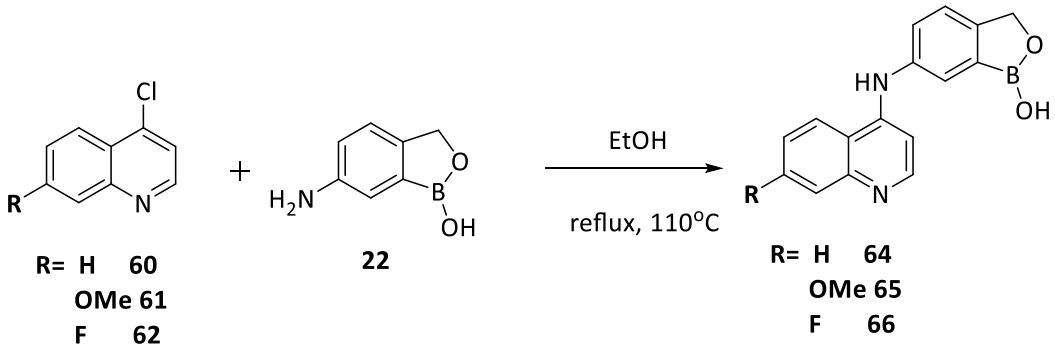
Scheme 2.1 Synthesis of 6-aminobenzoxaborole **22** from formylphenylboronic acid **10** in three steps reaction protocol

With 6-aminobenoxaborole **22** in hand, we then explored the nucleophilic displacement reaction of 7-haloquinolines. Initially, we utilized 4,7-dichloroquinoline **57**, which is found in antimalarial drug chloroquine **58**, as a representative example. The reaction of 6-aminobenzoxaborole **22** with **57** was carried out at room temperature in EtOH. No reaction occurred so it was then carried out under refluxing conditions at 100°C for 10 hrs. Gratifyingly, the reaction took place smoothly as observed by TLC analysis of a more polar spot than 4,7-dichloroquinoline **57** and disappearance of both reactants. The reaction was processed by removing EtOH under reduced pressure using a rotary evaporator. The crude solid product was washed with cold acetone to obtain the 7-chloro-d-quinolino-4-aminobenzoxaborole conjugate **59** (Scheme 2.2). This compound was thoroughly characterized using ^{1}H NMR, ^{13}C NMR, and mass spectral analysis.



Scheme 2.2 Synthesis of 7-chloro substituted quinoline-aminobenzoxaborole conjugate via aryl amination

Encouraged by the successful nucleophilic displacement reaction, we also carried out the reactions with another 7-halo substituted quinolines. Examples include 4-chloroquinoline **60**, 4-chloro-7-methoxyquinoline **61**, 4-chloro-7-fluoroquinoline **62**, and 4-chloro-7-(trifluoromethyl) quinoline **63** (scheme 2.3). For these cases, refluxing EtOH conditions was sufficient to obtain 4-aminobenzoxaborole substituted quinolines **64**, **65**, and **66,** (Scheme 2.3). For all these synthesized compounds, usage of base or transition metal catalyst was not required. The synthesized products were thoroughly characterized using ^{1}H NMR, ^{13}C NMR, and mass spectral analysis.



Scheme 2.3 Synthesis of 7-halosubstituted quinoline-aminobenzoxaborole conjugates

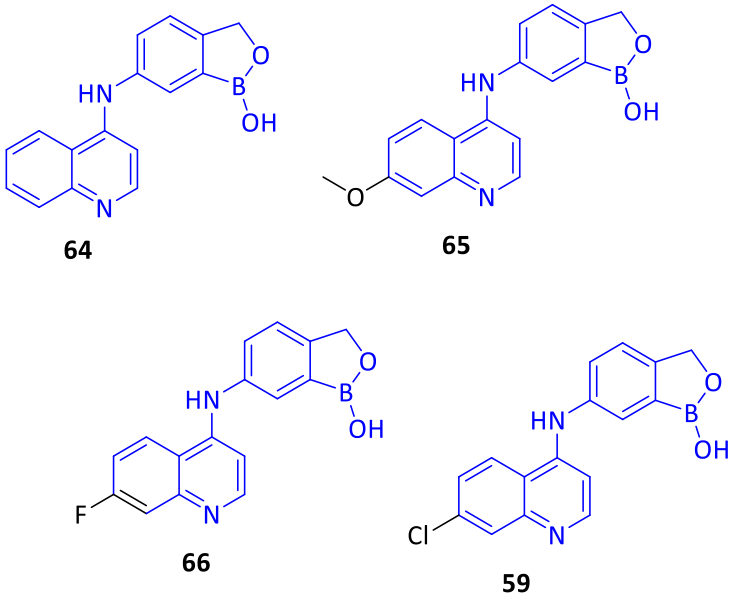
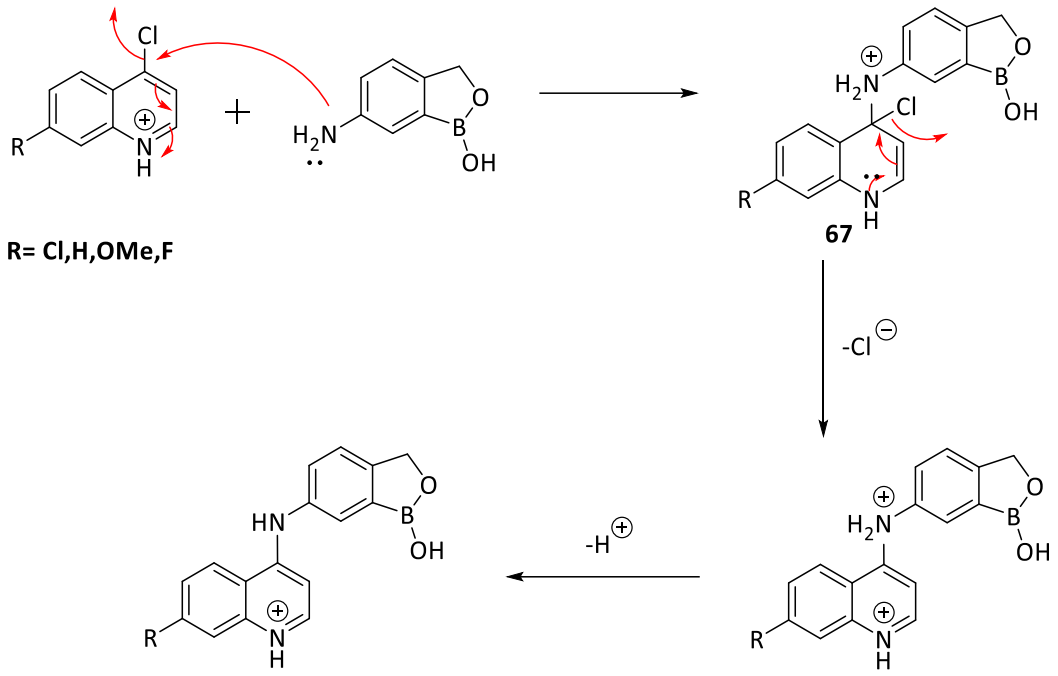


Figure 2.3 synthesis of novel quinolino aminobenzoxaboroles

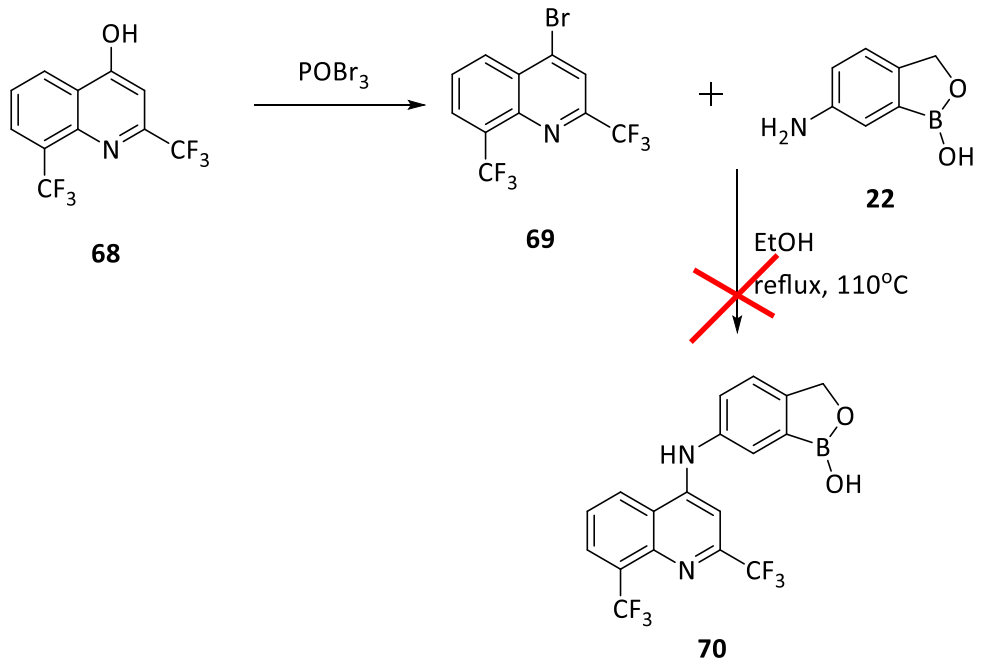
The probable reaction mechanism to synthesize 7-halo substituted quinoline-aminobenzoxaboroles is depicted below (Scheme 2.4).



Scheme 2.4 Probably mechanism of nucleophilic displacement reaction of 4-chloroquinolines with aminobenzoxaborole

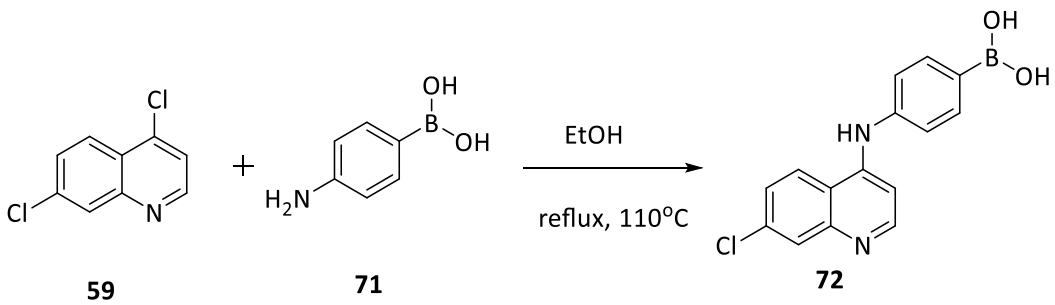
We expect that the pyridine nitrogen is protonated under the reaction conditions and that subsequent electron delocalization creates a positive environment at the 4-carbon which allows nucleophilic attack by the amine to yield the intermediate **67** (Scheme 2.4). The displacement of the leaving group chlorine gives the aminated quinoline products (Scheme 2.4). This nucleophilic aromatic substitution mechanism can only occur in electron-deficient aromatic compounds such as pyridines or pyrimidines with attached electron withdrawing and potential leaving groups. The proposed mechanism (Scheme 2.4) is in part supported by the lack of product formation using 4-bromo-2,8-(trifluoromethyl) quinoline **69** and **22**. In this case, 4-bromo-2,8-(trifluoromethyl) quinolines **69** did not undergo nucleophilic amine substitution with aminobenzoxaborole **22** nor with simple fluoro and chloro anilines. This could be due to the highly electron withdrawing nature of CF3 groups deactivating the pyridyl nitrogen

for initial protonation necessary for facilitating nucleophilic displacement reaction. Prolonged heating for three days, in the presence of acid or base also did not result in the product formation (Scheme 2.5).



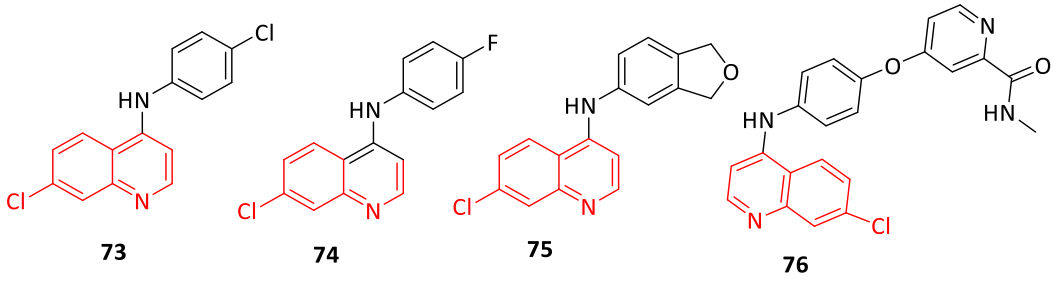
Scheme 2.5 Synthesis of 2,8 -(trifluoromethyl)quinolines-aminobenzoxaborole

To further understand the structure activity relationship (SAR) studies and evaluate the role of the benzoxaborole unit, we have synthesized quinolino-aminophenylboronic acid derivative **72**. Reaction of 4,7-dichloroquinoline **59** with 4-aminophenylboronic acid **71** resulted in **72** in 90% yield (Scheme 2.6).



Scheme 2.6 Synthesis of chloroquinoline-aminophenylboronic acid

To explore the role of the boronated unit itself, non-boronyl derivatives **73** and **74** were synthesized by reaction of 4,7-dichloroquinoline **59** with 4-chloro and 4-fluoro anilines. The benzofuran containing aniline was used as a surrogate for aminobenzoxaborole, and the product quinolino-benzofuran **75** was synthesized to understand the role of benzoxaborole unit. Similarly, a pharmacologically privileged non-boron containing pyridinyloxy aniline was utilized to synthesize pyridinyl quinoline **76**.



73 **74** **75** **76**

Figure 2.9 Synthesis of SAR of novel chloroquine derivatives

2.3 *In vitro* biological evaluation as potential antimicrobial agents

2.3.1 Cell proliferation inhibition studies on cancer cell lines.

All the synthesized compounds were tested for their *in vitro* cell proliferation inhibition properties using the 3-(4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. For this, we utilized human triple negative breast cancer (MDA-MBA-231), human pancreatic cancer cell line (MIAPaCa-2), human colorectal adenocarcinoma (WiDr), human breast cancer (MCF-7), murine breast cancer (67NR), and murine metastatic breast cancer cell line (4T1) cell lines. Cells were cultured in 96-well plates and were incubated with the test compound for 78 hours, at which time

MTT was added. MTT was reduced to MTT formazan by mitochondrial succinate dehydrogenase with an absorbance at 570 nm. The half maximal inhibitory concentration (IC_{50}) was calculated for each compound using GraphPad Prism 6 software.

2.3.2 *In vitro* evaluation of synthesized compounds on bacterial strains.

From the cell proliferation inhibition assay carried out on the compounds, none of our compounds showed general cytotoxicity at 100 µM. Encouraged by their lack of cytotoxicity in proliferating cells, we screened all the compounds for their biological activity against bacterial strains such as *Escherichia coli(E. coli), Bacillus subtilis (B. subtilis)* , and *Mycobacterium smegmatis (M. smegmatis).* We employed Kirby-Bauer disk method for preliminary investigation of biological activity of test compounds. All of the synthesized derivatives were tested at 100 µM and were compared against 4 controls such as 6-aminobenzoxaborole **22**, streptomycin, ampicillin, and DMSO solvent (Figure 2.10).

For gram-negative *E. coli*, having a neutral or electron donating substituent on the quinolino-aminobenzoxaborole template such as hydrogen in **64** or methoxy in **65** gave similar zone of inhibition (1.0 cm – 1.3 cm respectively) slightly above the DMSO controls (Figure 2.10). Adding an electron withdrawing substituent on the quinolino-benzoxaborole template such as **59**, **66** (Figure 2.10). Utilizing the Cl and F substituted aniline derivatives **73** and **74** in place of the benzoxaborole template resulted in abolishment of efficacy to the level of the DMSO control. Using phenylboronic acid in place of benzoxaborole moiety showed no increase in efficacy of the synthesized derivative **72** (figure 2.10). Benzofuran derivative **75** was screened as a surrogate to investigate the effect of boron in the 5-membered ring. The benzofuran derivative **75** also

displayed zone of inhibition identical to that of DMSO control (Figure 2.10). The pharmacological derivative containing the pyridinyloxy aniline **76** also showed no activity towards *E. coli* (Figure 2.10).

We then screened the test compounds against the gram-positive bacterial strain *B. subtilis* and found that overall inhibitory activity was equal to or slightly above DMSO control for all derivatives. For the benzoxaborole containing derivatives, having a neutral hydrogen substituent on the quinoline template such as in **64** gave a slightly larger zone of inhibition than that of DMSO control. Increased zone of inhibition values were observed for electron withdrawing substituents found in chlorine containing **59** (Figure 2.10). Interestingly, the electron withdrawing fluorine containing benzoxaborole derivative **66** had no observed zone of inhibition changes compared to DMSO control. No inhibitory activity was observed for the phenylboronic acid containing **72** relative to benzoxaborole. Like *E. coli*, these results indicate that benzoxabrole unit is required on the quinoline for the observed activity (Figure 2.10).

Additionally, we screened the test compounds on the acid-fast bacterial strain *M. smegmatis* and found an increase in efficacy for some derivatives compared to positive controls ampicillin and streptomycin (2.3 cm and 2.4 cm respectively) (Figure 2.10). Hydrogen containing quinoline-aminobenzoxaborole **64** and electron donating methoxy substituted **65** had increased zones of inhibition of 3.2 cm and 2.2 cm respectively (Figure 2.10). The electron withdrawing substituted and aniline-based derivatives had a loss of efficacy and were equal to the DMSO control. Future synthesized derivatives will explore if further functionalization utilizing an unsubstituted quinoline template or additional electron donating substituents will increase efficacy against *M. smegmatis*.

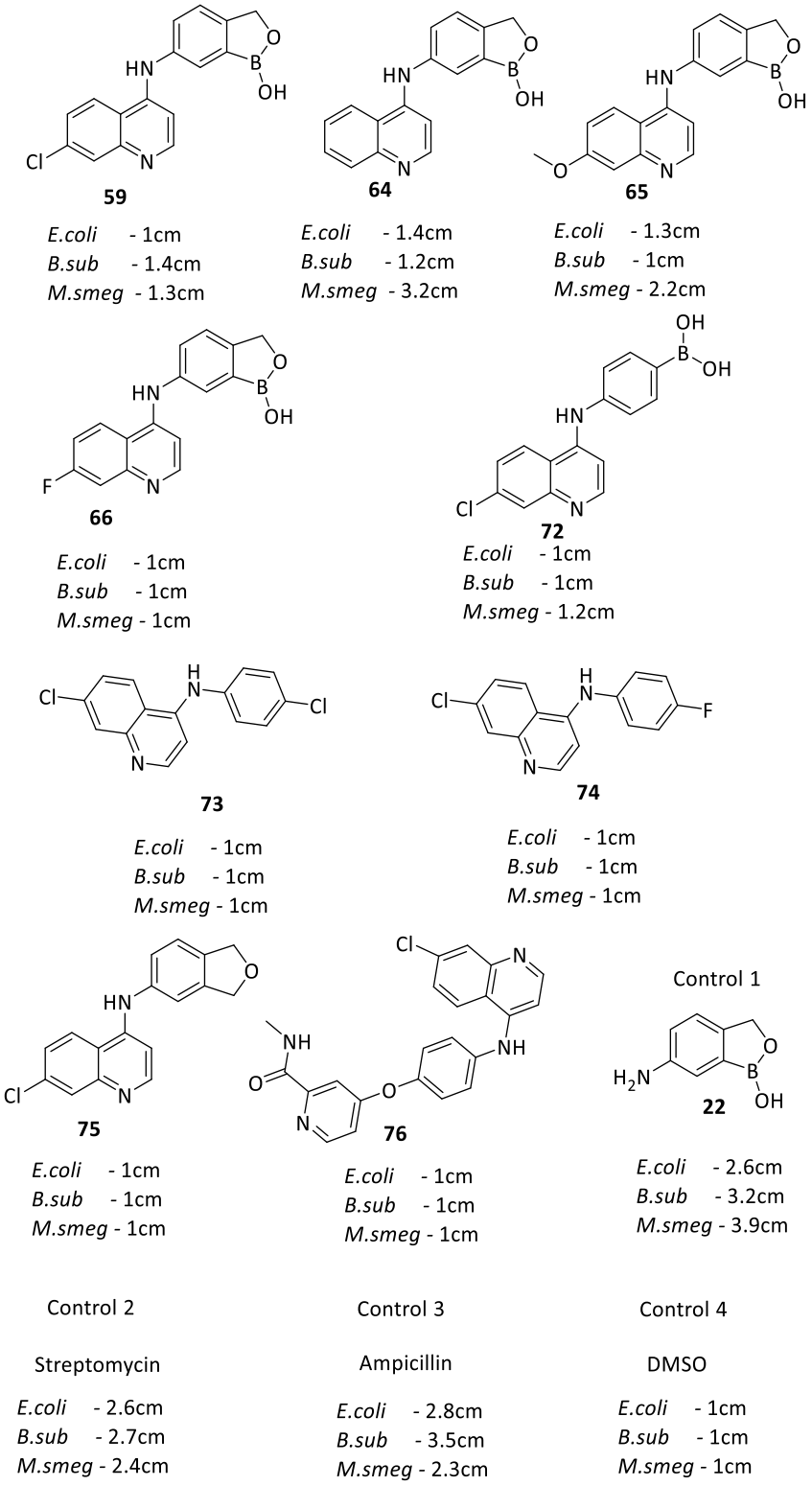


Figure 2.10 Zone of inhibition values (cm) of the novel synthesized compounds against bacterial strains.

The synthesized quinolino benzoxaborolederivatives were also tested against two fungal strains *Cryptococus neoformans* and *Candida albicans* to explore their antifungal activity. The activity of the synthesized derivates were compared against four controls for their efficacy. The controls used for this study were 6-aminobenzoxaborole **22**, fluconazole (antifungal drug), itraconazole (antifungal drug), and DMSO solvent. All the synthesized derivatives were tested at 100 μM in DMSO and were compared against controls for their antifungal efficacy.

Unfortunately, none of the compounds including the controls 6-aminobenzoxaborole **22** and DMSO exhibited antifungal activity against *C. neoformans* and *C. albicans* whereas, one of the controls fluconazole exhibited antifungal activity against *C. neoformans* and *C. albicans* with zone of inhibition values 5.5 cm and 5.2 cm respectively (Figure 2.11). Correspondingly, the other control antifungal drug itraconazole exhibited antifungal activity against *C. neoformans* and *C. albicans* with zone of inhibition values 5.5 cm and 3 cm, respectively (Figure 2.11). The absence of activity of the 6-aminobenzoxaborole **22** control suggests that the aminobenzoxaborole moiety itself does not have any intrinsic antifungal activity (Figure 2.11). Further functionalization of the benzoxaborole moiety with other structural units such as triazole and imidazole will be carried out to investigate if these substitutions provide antifungal activity.

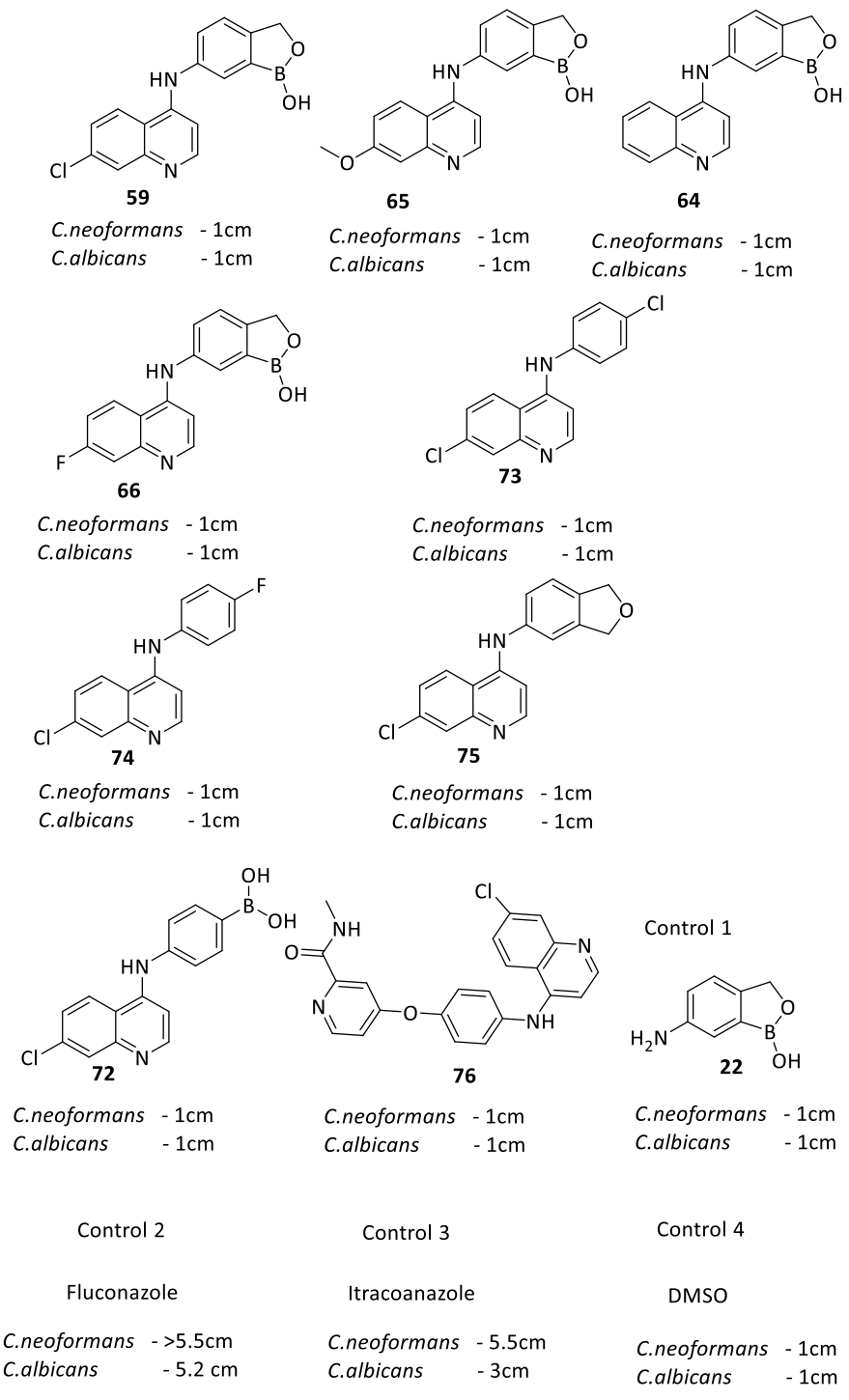


Figure 2.11 Zone of inhibition values (cm) of novel quinoline-amine based derivatives against fungal strains.

Conclusions and Future Directions:

In conclusion, we developed a novel synthetic methodology for preparing quinolino aminobenzoxaboroles. The synthesized compounds have been thoroughly characterized by various spectral means. Initially, these compounds were evaluated for their cytotoxic properties against various human and murine proliferating cancer cells. All the compounds were found be well tolerated as evidenced by their nontoxic nature even at 100 micromolar concentrations. Encouraged by the nontoxic nature, the test compounds were evaluated for their antibacterial activity against *E. coli, B. subtilis, and M. smegmatis.* Some of the synthesized derivatives exhibited modest to good activity against *M. smegmatis.* Further SAR and biological studies are required to identify the lead compound with high potency and favorable pharmacological properties. The test compounds were also evaluated against *two* fungal species, *C. neoformans* and *C. albicans,* but none of the compounds exhibited any activity.

Our future studies include:

1) Evaluate the efficacy of synthesized candidate compounds as antiviral agents including SARS-Cov-2.

Chloroquine and hydroxychloroquine exhibit high potency against a wide variety of viruses including SARS-Cov-2. However, due to their cytotoxic nature (IC_{50} against proliferating cancer cells ~ 10 micromolar), they failed in clinical trials to be repurposed as antiviral agents. Benzoxaboroles have been reported to exhibit good activity against a wide range of viruses. These boron-containing heterocycles also exhibit excellent safety profiles, along with high metabolic stability. Our quinolino aminobenzoxaboroles are not cytotoxic even at 100 micromolar concentration in six cancer cell lines that we tested. If

these compounds exhibit encouraging activity, they can be further optimized to be developed as antiviral agents. In collaboration with University of Tennessee Health Sciences Center, we are planning to evaluate if our candidate compounds exhibit activity against SARS-Cov-2.

2) Evaluate the efficacy of synthesized candidate compounds as antimalarial agents.

Quinoline based clinical drugs such as chloroquine, hydroxychloroquine, mefloquine are highly used antimalarial agents. However, clinical resistance and toxicity are some of the problems associated with these drugs. Recently benzoxaboroles have been reported to exhibit potent antimalarial properties. In this regard, we envision that quinoline containing aminobenzoxaboroles would have promise as antimalarial agents. In collaboration with Walter Reed Army Institute of Research, we are planning to evaluate the efficacy of the candidate compounds as antimalarial agents.

3) Evaluate the efficacy of synthesized candidate compounds as anti-inflammatory agents.

Functionalized benzaoxaboroles (e.g., crisaborole) exhibit good anti-inflammatory properties. If quinolino benzoxaboroles exhibit good anti-inflammatory properties along with favorable pharmaceutical properties, they can be further studied for development as systemic agents for a wide variety of inflammatory diseases. We are planning to carry out these studies inhouse at UMD.

Chapter 3

Experimental Section

3.1 Materials and Methods:

4,7-dichloroquinoline, 4-chloroquinoline, 4-chloro-7-methoxyquinoline, 4-chloro-7-fluoroquinoline,1,3-dihydroisobenzofuran-5-amine, 4-(4-aminophenoxy)-N-methylpicolinamide and (4-aminophenyl)boronic acid were purchased from Ambeed chemicals. 4-chloroaniline (Sigma-Aldrich), 4-fluoroaniline (Sigma-Aldrich), (2-formylphenyl)boronic acid (oakwood chemicals) were purchased from respective vendors. All other chemical reagents utilized for chemical synthesis were high grade quality and were purchased from Sigma-Aldrich.

The ^{1}H NMR and ^{13}C NMR spectral analysis was carried out using Bruker Ascend TM 400 MHz spectrometer. High resolution Mass spectrometry (HRMS) were recorded using Bruker micrOTOP-Q III ESI mass spectrometer.

3.2 General procedure for synthesis of 7-Substituted quinoline -4-amines:

To the solution of 7-substituted 4-chloroquinolines (1.2 eq, 4 mmol) in absolute ethanol (7 ml/mmol), amine (1 eq, 3 mmol) was added and the mixture was refluxed (24 hours). Reaction progress was monitored via thin layer chromatography (TLC) (30% of EtOAc & hexane). Upon completion of the reaction the reaction mixture was brought to room temperature. The formed precipitate was filtered using Buchkner funnel and rinsed with acetone to remove impurity traces. The precipitate was dried under vacuum to yield the corresponding 7-substituted quinoline-4-amines in 90% yield.

3.3 Cell culture and Cytotoxicity Assay:

MDA-MB-231 cells (ATCC) were grown in DMEM media supplemented with 10% FBS (Fetal Bovine Serum) and 1% penicillin-streptomycin. MIAPaCa-2 cells (ATCC) were grown in DMEM media supplemented with 10% FBS, 2.5% Horse serum and 1% penicillin-streptomycin. WiDr cells (ATCC) were grown in MEM media supplemented with 10% FBS (Fetal Bovine Serum) and 1% penicillin-streptomycin. MCF-7 cells (ATCC) were grown in α-MEM supplemented with FBS(5%), insulin(10 µg/ml), non-essential amino acids (0.1 mM), sodium pyruvate (1 mM), EGF (100 µg/mL), hydrocortisone (10 µg/ml), HEPES (10 mM), and penicillin-streptomycin (1%). 4T1 cells (ATCC) and 67NR cells (ATCC) were cultured in RPMI-1640 supplemented with 10% FBS and penicillin-streptomycin (50 U/ml; 50 µg/ml).

Cells were seeded ($5x10^3$cells/well) in 96 well plates and incubated overnight (37^oC, 5% CO_2). Test compounds were then added to 96-well plates in duplicate followed by serial dilution and were incubated (37^oC, 5% CO_2). After incubation for 72hrs, 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/ml) was added and the plates were incubated for four hours. The formazan precipitate was then solubilized using sodium lauryl sulfate (SDS 10% w/v, 0.01 N HCl) and incubated for four hours. Later absorbance readings were recorded at 570 nm for each well and concentrations at which 50% of cell growth was inhibited (IC_{50}) were tabulated, using control wells as 100% cell survival.

3.4 Cell culture and Antimicrobial Assay:

The compounds were screened against Escherichia *coli* (gram-), *Bacillus subtilis* (gram+), and *Mycobacterium smegmatis* (acid-fast). Although *E. coli* and *B. subtilis* are not highly pathogenic, initial screening of test compounds against these microbes aimed to verify potential activity towards highly pathogenic strains. Similarly, *M. smegmatis* is non-pathogenic in nature but shares a 90% genetic similarity to tuberculosis which is a severe disease. We also screened the synthesized aminobenzoxaborole quinoline conjugates against two fungal species *Candida albicans* and Cryptococcus *neoformans* to evaluate the broad-spectrum applications of these compounds. These two fungal species, C. *albicans* and *C. neoformans,* can be pathogenic for immunocompromised patients.

3.4.1 Zone of inhibition protocol

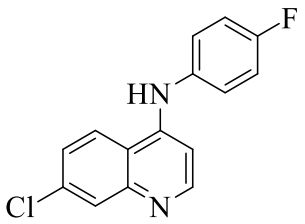
Zones of inhibition values were determined for the two fungal stains (*Candida albicans* and Cryptococcus *neoformans)* and three bacterial strains against *Escherichia coli*, *Bacillus subtilis*, and *Mycobacterium smegmatis*. Difco Luria-Bertani (LB) broth and Difco Sabouraud Dextrose Broth were made following widely reported procedures. Agar plates were poured using the respective broth containing 5% agar (25 mL per culture dish). All media and their components were sterilized via autoclave at 250^{o}C for 15 minutes. Experiments were carried out using aseptic technique in a biohazard level 2 fume hood.

M. smegmatis cells were grown in 7H9 with Tween 80 (0.05% w/v) media maintained at pH 7, *E.coli* and *B. subtilis* cells were grown in LB broth with tryptone, yeast extract and NaCl maintained at pH 7.9, *C. Neoformans* cultures were grown in SB growth

media maintained at pH 6.9 and *C. Albicans* were grown in LB broth containing tryptone, yeast extract and NaCl maintained at pH 7.

Bacterial and fungal strains of interest were cultured in their respective broths. Inoculated broth was spread evenly over the face of sterile agar plate. The white discs were placed on the sterile surface and 5 µl of the test compound was absorbed into disks. The disk was placed in specific coordinate on the petri dish using sterile tweezers. These tests were incubated and allowed to grow for a couple of days until culture is visible. The zone of inhibition values of the test compounds were recorded and compared with controls.

3.5 Spectral Characterization

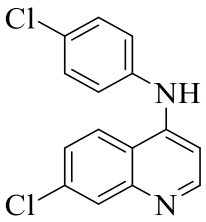


7-chloro-N-(4-fluorophenyl)quinoline-4-amine

^{1}H NMR (400MHz, DMSO-d_6): δ 11.19 (s,1H), 8.87 (d, J=9Hz,1H), 8.53 (d, J=7Hz, 1H), 8.18 (d, J=1.6Hz, 1H), 7.88 (m, 1H), 7.55 (m, 2H), 7.43 (m, 2H), 6.73 (d, J=7Hz, 1H)

^{13}C NMR (100 MHz, DMSO-d_6): 161.36 (d, J=243Hz), 155.65, 143.78, 139.49,138.85, 133.75 (d, J=2Hz), 128.41(d, J=8Hz), 127.8, 126.76, 119.62, 117.3 (d, J=22Hz), 116.34, 100.66

HRMS (ESI) m/z: calc'd for C15H10ClFN2 [M]+:273.0589, found at 273.0598

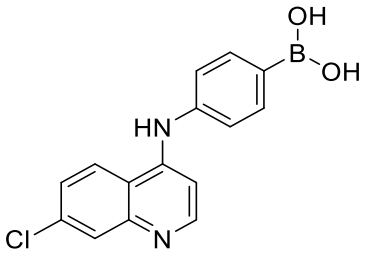


7-chloro-N-(4-chlorophenyl)quinoline-4-amine

^{1}H NMR (400MHz, DMSO-d_6): δ 11.12 (s, 1H), 8.85 (d, J= 9.2Hz, 1H), 8.54 (d, J=2Hz, 1H), 8.17 (s, 1H), 7.87 (d, J=9.2Hz, 1H), 7.64 (s, 1H), 7.62 (d, J=6.8Hz, 1H), 7.53 (m, 2H), 6.84 (d, J=6.8Hz, 1H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 155.22, 144.16, 139.65, 138.91, 136.53, 132.12, 130.43, 127.94, 127.68, 126.60, 119.86, 116.55, 101.00

HRMS (ESI) m/z: calc'd for C15H10Cl2N2 [M]+:289.0294, found at 289.0298

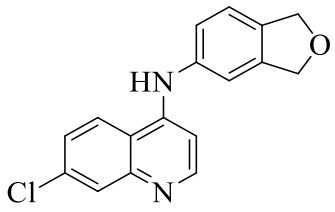


((7-chloroquinolin-4-yl)amino)phenyl)boronic acid

^{1}H NMR (400MHz, DMSO-d_6): δ 11.18 (s, 1H), 8.87 (d, J = 5.2Hz, 1H), 8.54 (d, J = 11.2Hz, 1H), 8.19 (m, 2H), 7.99 (d, J=2Hz, 2H), 7.89 (dd, J=2.4 Hz, 5.2Hz, 1H), 7.47 (d, J=2Hz, 2H), 6.88 (d, s, 1H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 155.10, 143.80, 139.55, 139.04, 138.85, 136.15, 133.91, 127.81, 126.73, 124.47, 119.64, 116.52, 100.93

HRMS (ESI) m/z: calc'd for C15H12BClN2O2 [M]+:299.0753, found at 299.0752

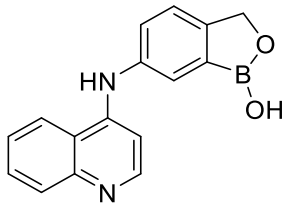


7-chloro-N-(1,3-dihydroisobenzofuran-5-yl)quinoline-4-amine

^{1}H NMR (400MHz, DMSO-d_6): δ 11.26 (s, 1H), 8.91 (d, J=9.2Hz, 1H), 8.51(d, J=6.8Hz, 1H), 8.19 (d, J=2Hz, 1H), 7.86 (dd, J=2Hz, 9.2 Hz, 1H), 7.50 (d, J=8Hz, 1H), 7.44-7.37 (m, 2H), 6.77 (d, J=6.8Hz, 1H), 5.07 (s, 4H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 155.57, 143.78, 141.59, 139.54, 138.99, 138.84, 136.61, 127.80, 126.67, 125.17, 123.07, 119.68, 119.00, 116.36, 100.78, 72.95, 72.90

HRMS (ESI) m/z: calc'd for C17H13ClN2O [M]+:297.0789, found at 297.0802

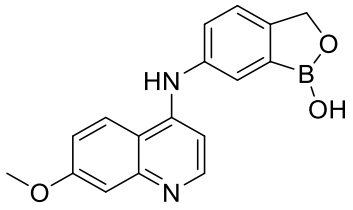


6-(quinoline-4-ylamino)benzo[c][1,2]oxaborol-1(3H)-ol

^{1}H NMR (400MHz, DMSO-d_6): δ 11.16 (s, 1H), 9.47 (s, 1H), 8.88 (d, J=7.6Hz, 1H), 8.51 (s, 1H), 8.13-8.04 (m, 2H), 7.86-7.83 (m, 2H), 7.64-7.60 (m, 2H), 6.74 (s, 1H), 5.09 (s, 2H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 155.77, 153.50, 143.00, 138.67, 136.46, 134.31, 132.90, 128.82, 128.11, 127.45, 124.30, 123.53, 120.63, 117.52, 100.03, 70.39

HRMS (ESI) m/z: calc'd for C16H13BN2O2 [M]+:277.114, found at 277.116

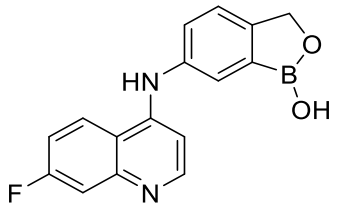


6-(7-methoxyquinoline-4-ylamino)benzo[c][1,2]oxaborol-1(3H)-ol

^{1}H NMR (400MHz, DMSO-d_6): δ 10.95 (s, 1H), 9.42 (s, 1H), 8.75 (d, J=8.8Hz, 1H), 8.39 (d, J=6.8Hz, 1H), 7.81 (s, 1H), 7.62-7.55 (m, 2H), 7.45-7.43 (m, 2H), 6.61 (d, J= 7.2 Hz, 1H), 5.07 (s, 2H), 3.97 (s, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 163.47, 155.29, 153.33, 142.55, 141.05, 136.53, 132.81, 128.80, 128.04, 126.14, 123.46, 118.59, 111.82, 100.43, 99.39, 70.38, 56.50

HRMS (ESI) m/z: calc'd for C17H15BN2O3 [M]+:307.125, found at 307.126

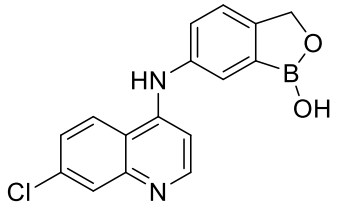


6-((7-fluroquinoline-4-yl)amino)benzo[c][1,2]oxaborol-1(3H)-ol

^{1}H NMR (400MHz, DMSO-d_6): δ 9.24 (s, 1H), 9.14 (s, 1H), 8.52-8.43 (m, 2H), 7.72 (s, 1H), 7.57 (dd, J=2.4Hz, 10.4 Hz 1H), 7.50-7.44 (m, 3H) 6.79 (d, J=5.2Hz, 1H), 5.02 (s, 2H)

^{13}C NMR (100 MHz, DMSO-d_6): 162.89 (d, J=245 Hz), 152.30 (d, J=14Hz), 150.57, 150.43 (d, J=4Hz), 149.20, 139.40, 132.22, 126.85, 125.57 (d, J=10Hz), 125.27, 122.96, 117.09, 114.76 (d, J=24Hz), 112.67 (d, J=20Hz), 110.21, 70.32

HRMS (ESI) m/z: calc'd for C16H12BFN2O2 [M]+:295.105, found at 295.106

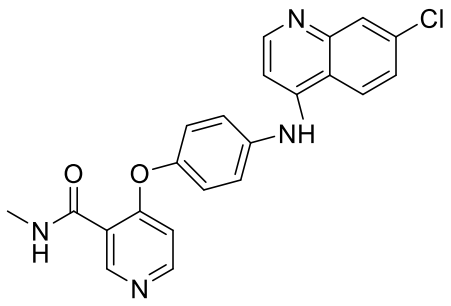


6-((7-chloroquinoline-4-yl)amino)benzo[c][1,2]oxaborol-1(3H)-ol

^{1}H NMR (400MHz, DMSO-d_6): δ 11.25 (s, 1H), 9.43 (s, 1H), 8.89 (d, J=9.2Hz, 1H), 8.51 (d, J=7.2Hz, 1H), 8.17(d, J=2.16 Hz, 1H), 7.9 (m, 1H), 7.82 (s, 1H), 7.64-7.57 (m, 2H), 6.73(d, J=7.2Hz, 1H), 5.08 (s, 2H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 155.69, 153.65, 143.73, 139.49, 138.89, 136.23, 132.96, 128.68, 127.94, 127.82, 126.67, 123.59, 119.64, 116.35, 100.57, 70.40

HRMS (ESI) m/z: calc'd for C16H12BClN2O2 [M]+:311.075, found at 311.076



4-(4-((7-chloroquinolin-4-yl)amino)phenoxy)-N-methylnicotinamide

^{1}H NMR (400MHz, DMSO-d_6): δ 11.29 (s, 1H), 8.92 (d, J=9.2Hz, 1H), 8.81 (d, J=5.2Hz, 1H), 8.57(d, J=6Hz, 2H), 8.22 (d, J=2.4Hz, 1H), 7.89 (dd, J=2Hz, 9.2 Hz, 1H), 7.65(d, J=8.8Hz, 2H), 7.46-7.44 (m, 3H), 7.26 (dd, 2.8 Hz, 5.6Hz, 1H), 6.89 (d, J=6.8, 1H), 2.81 (s, 3H)

^{13}C NMR (100 MHz, DMSO-d_6): δ 165.74, 164.18, 155.38, 153.07, 152.69, 151.07, 143.93, 139.57, 138.89, 134.95, 128.17, 127.88, 126.68, 122.82, 119.72, 116.47, 114.99, 109.69, 100.88, 26.51

HRMS (ESI) m/z: calc'd for C22H17ClN4O2 [M]+:405.1113, found at 405.1097

CHAPTER 4: SUPPORTING INFORMATION

4.1 References

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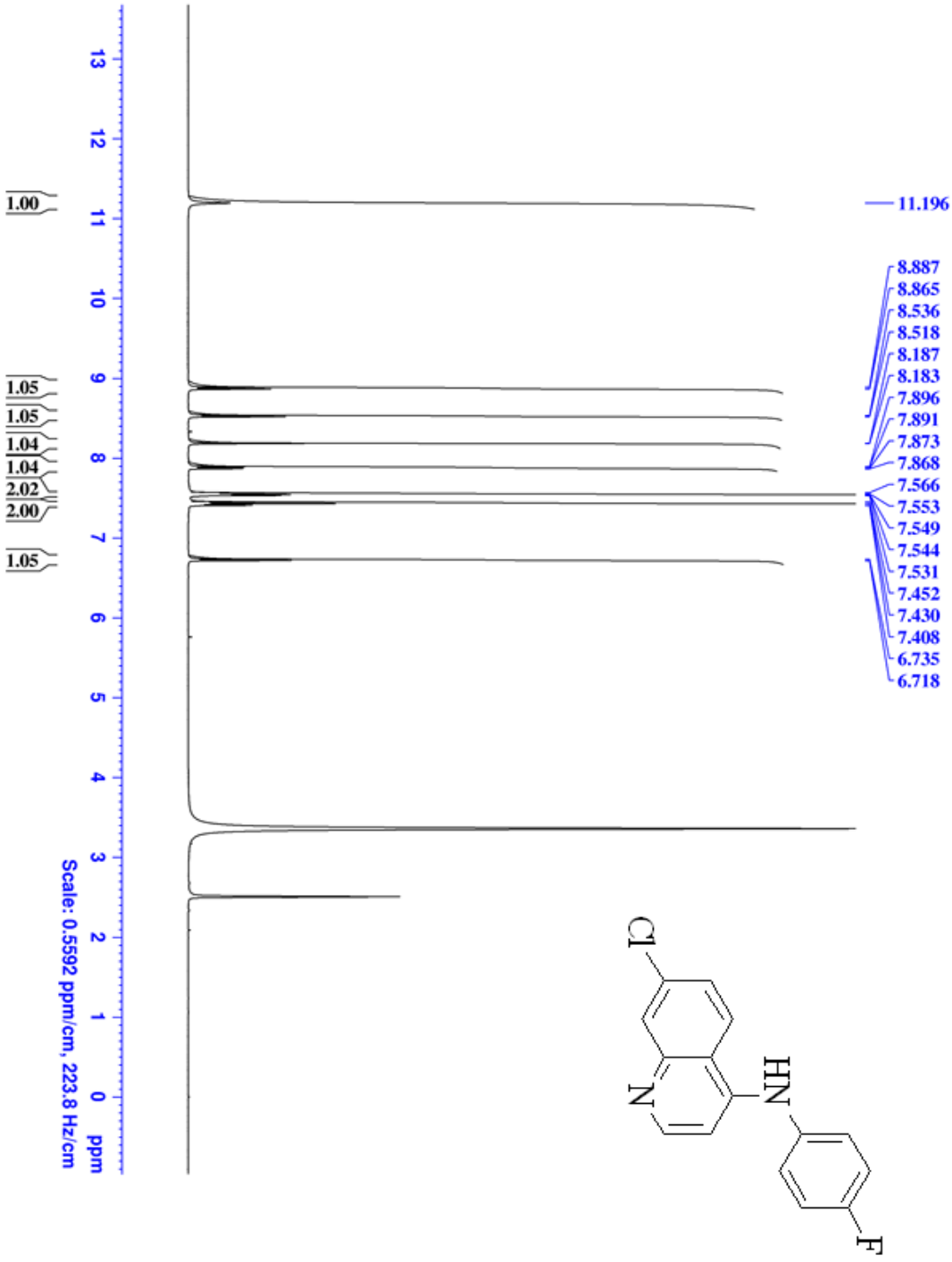
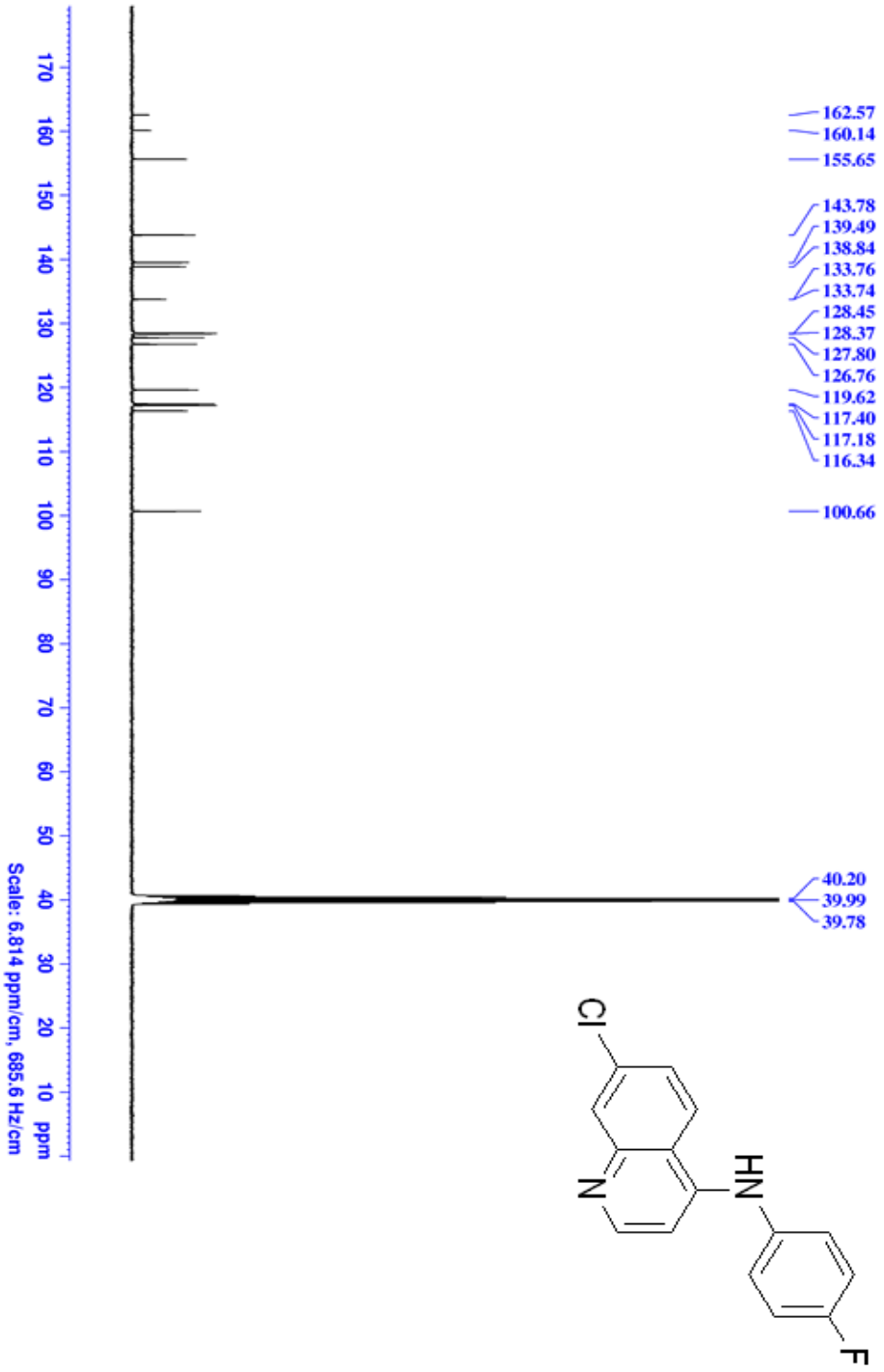
4.2 Appendix – NMR Spectra

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116.34
100.66
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Cl
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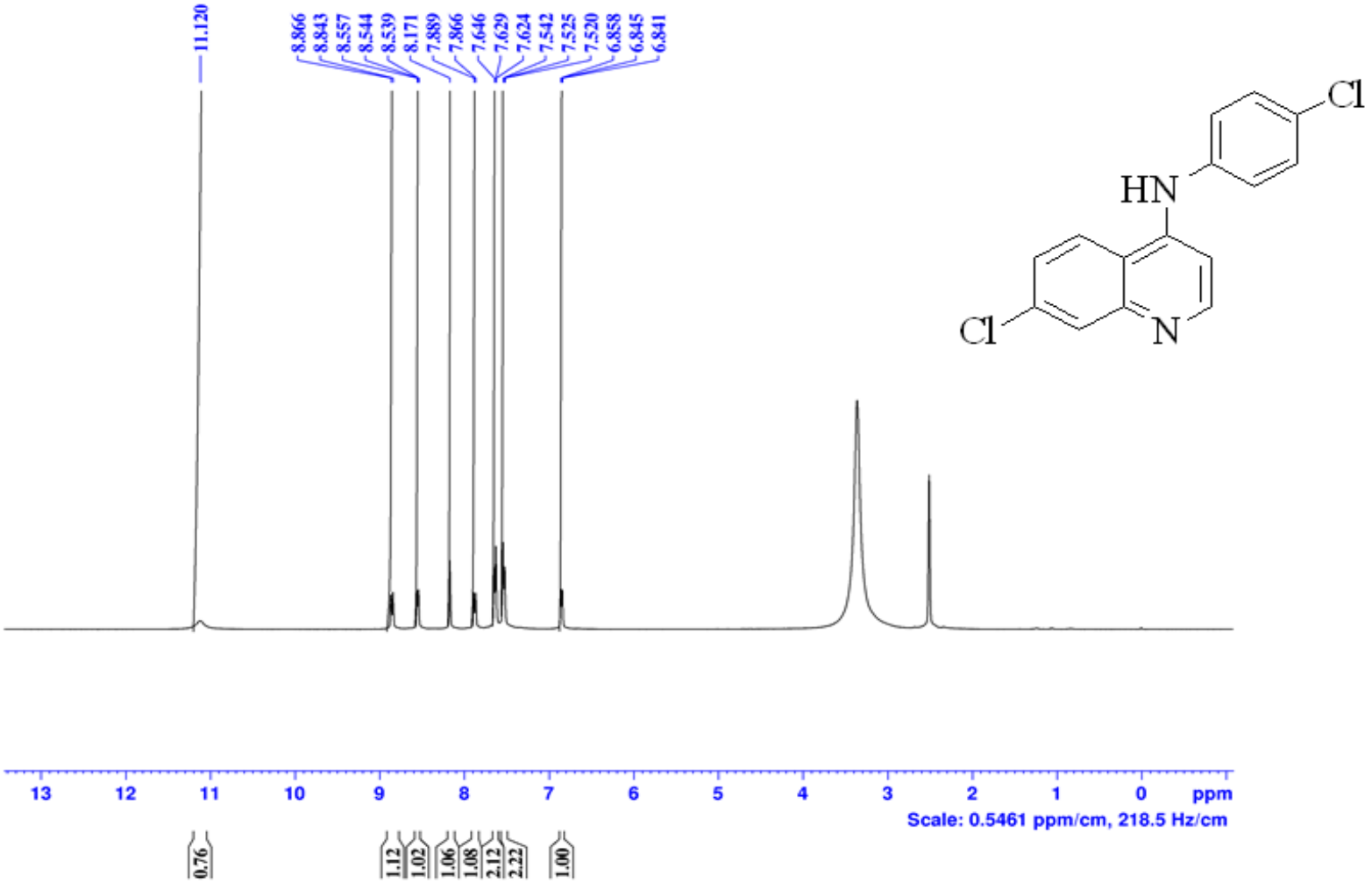
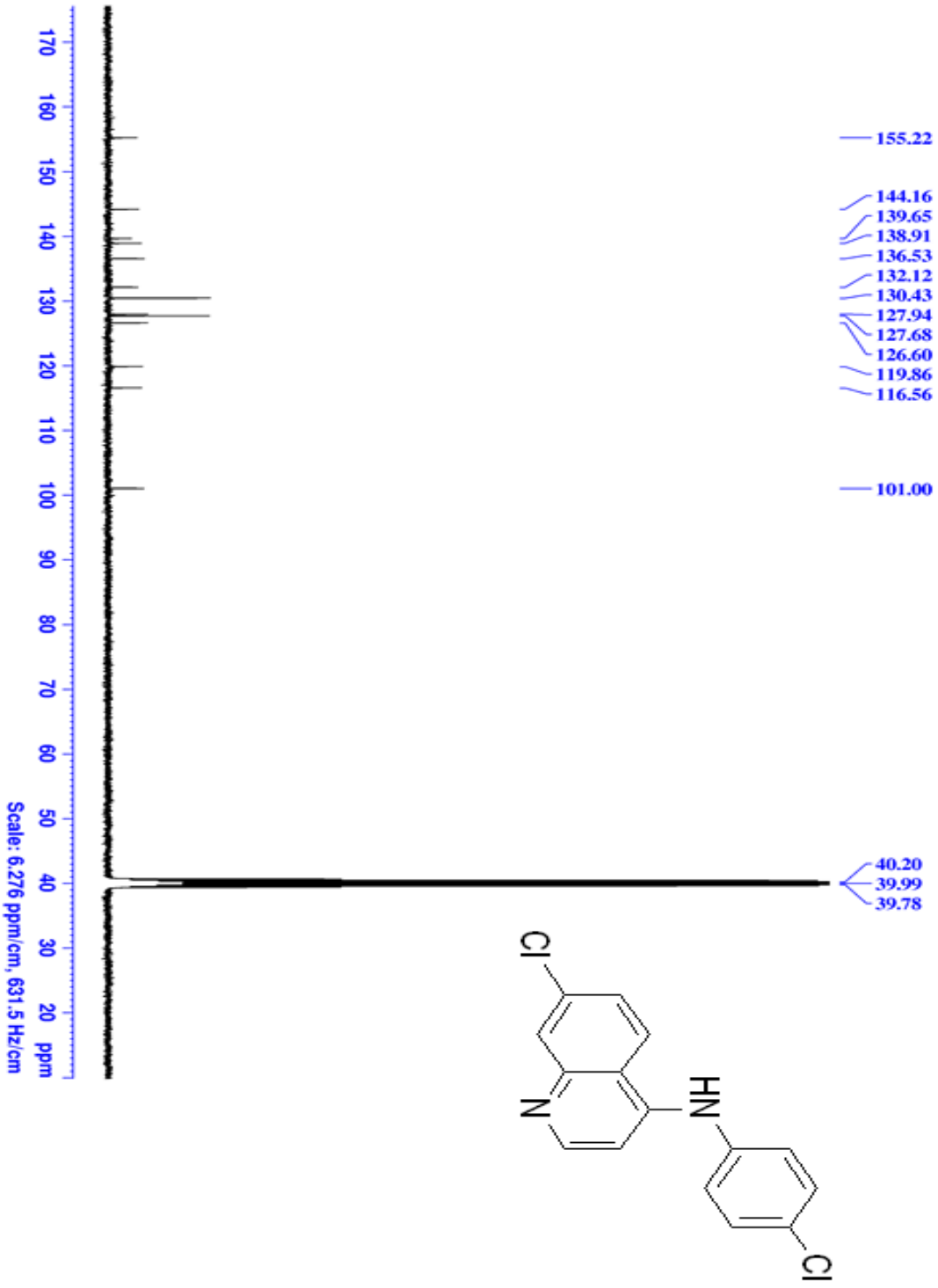
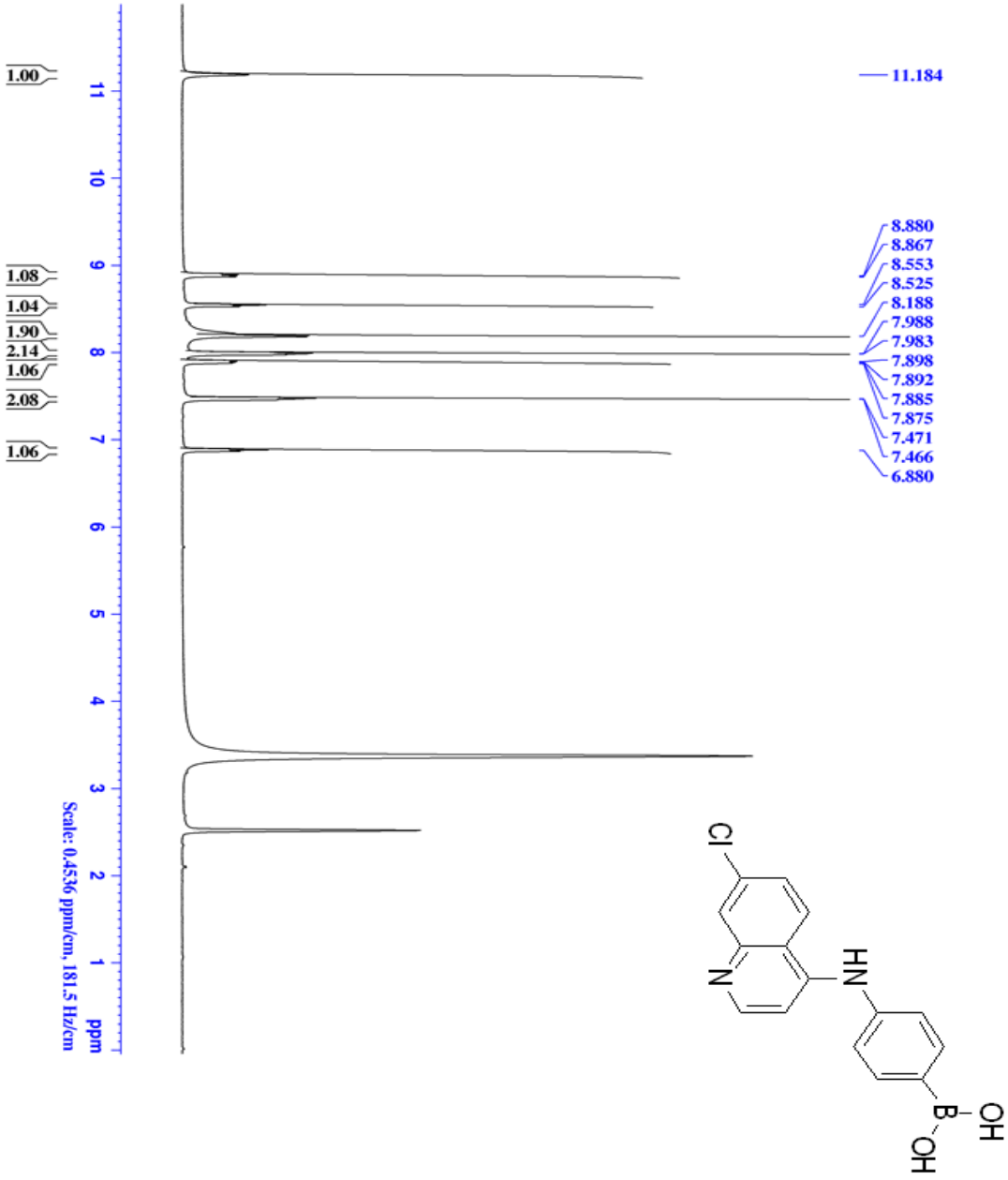
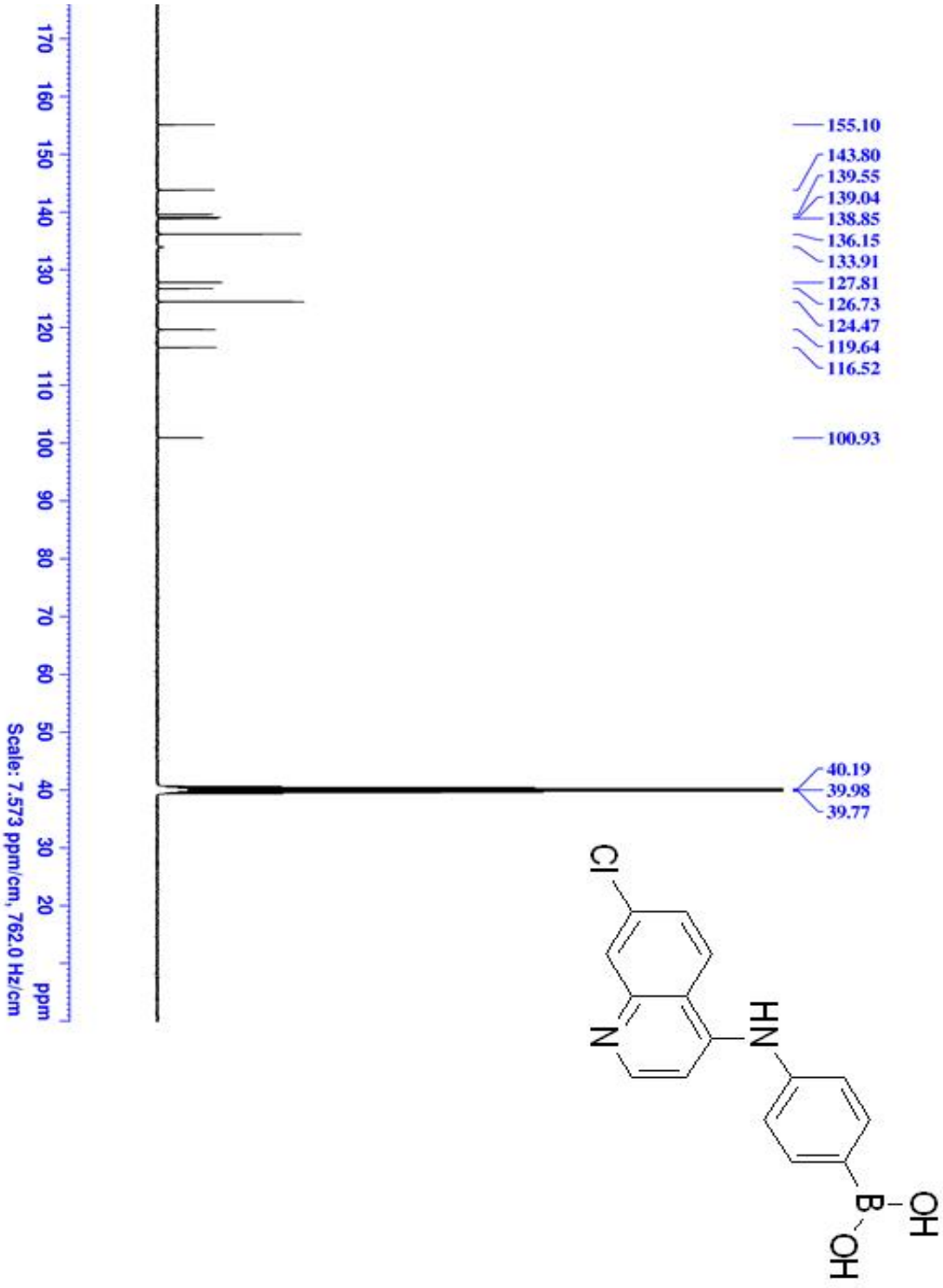
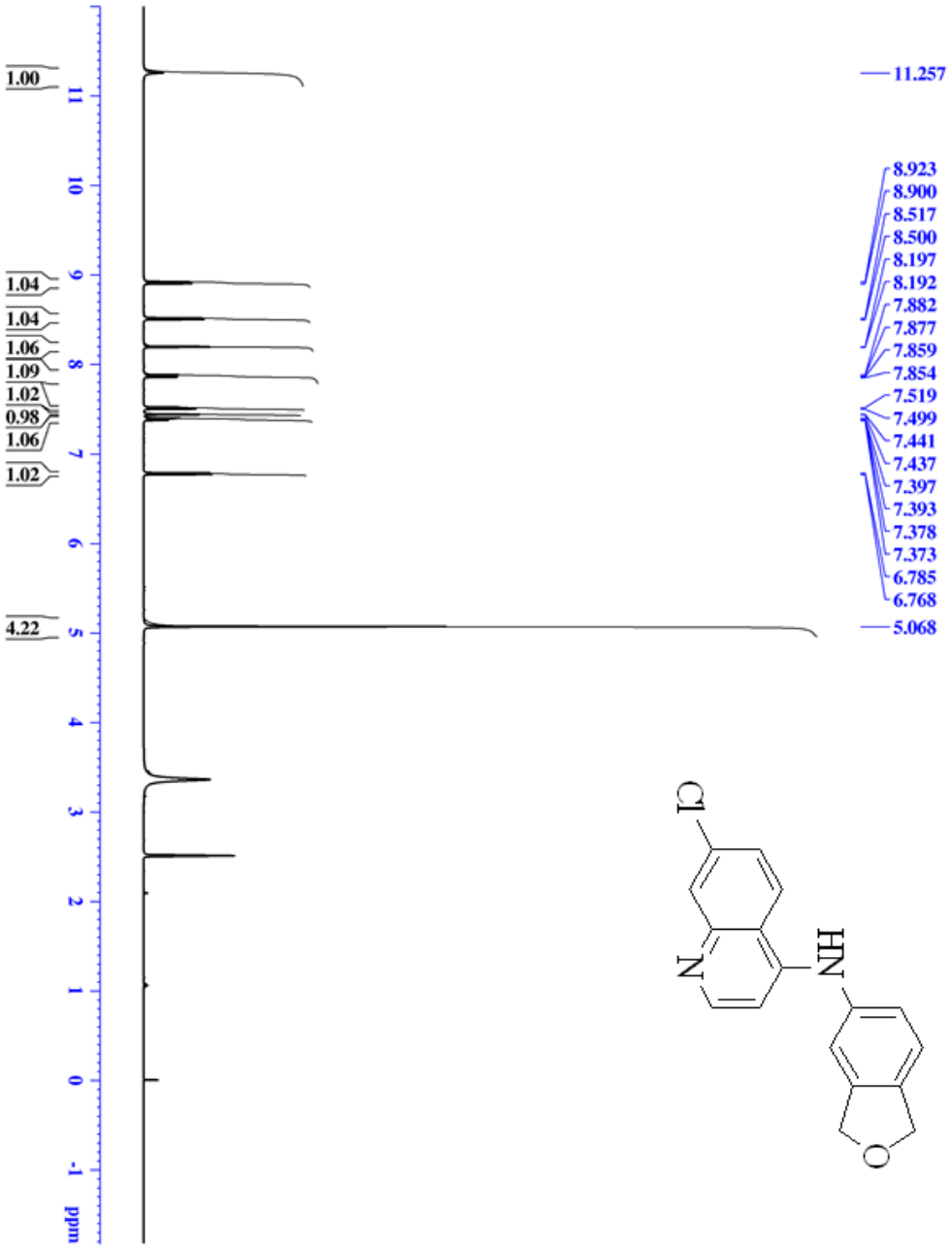
HN
F
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
Scale: 6.814 ppm/cm, 685.6 Hz/cm

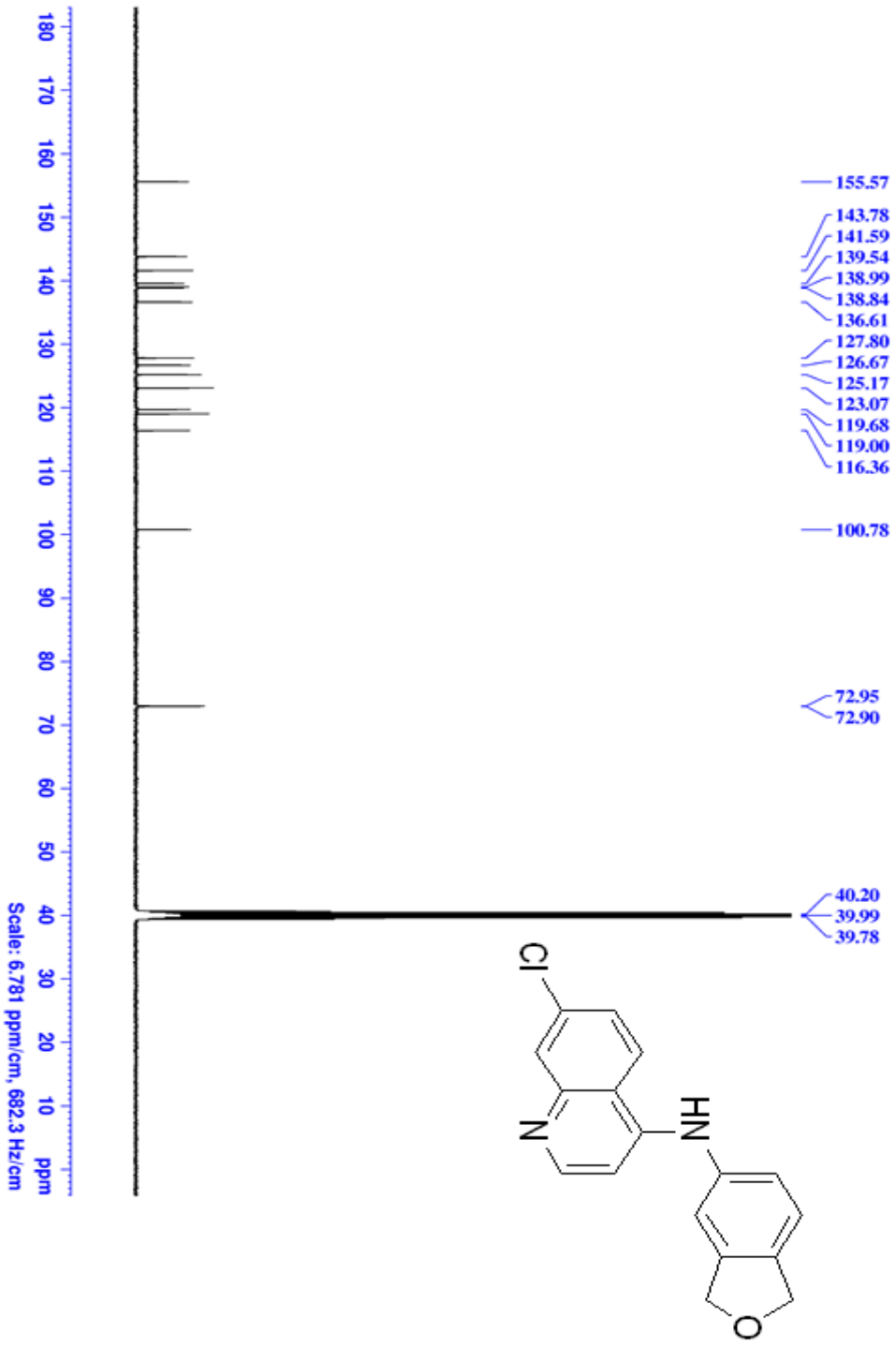

11.120
8.866
8.843
8.557
8.544
8.539
8.171
7.889
7.866
7.646
7.629
7.624
7.542
7.525
7.520
6.858
6.845
6.841
Cl
HN
Cl
N
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
Scale: 0.5461 ppm/cm, 218.5 Hz/cm
0.76
1.12
1.02
1.06
1.08
2.12
2.22
1.00

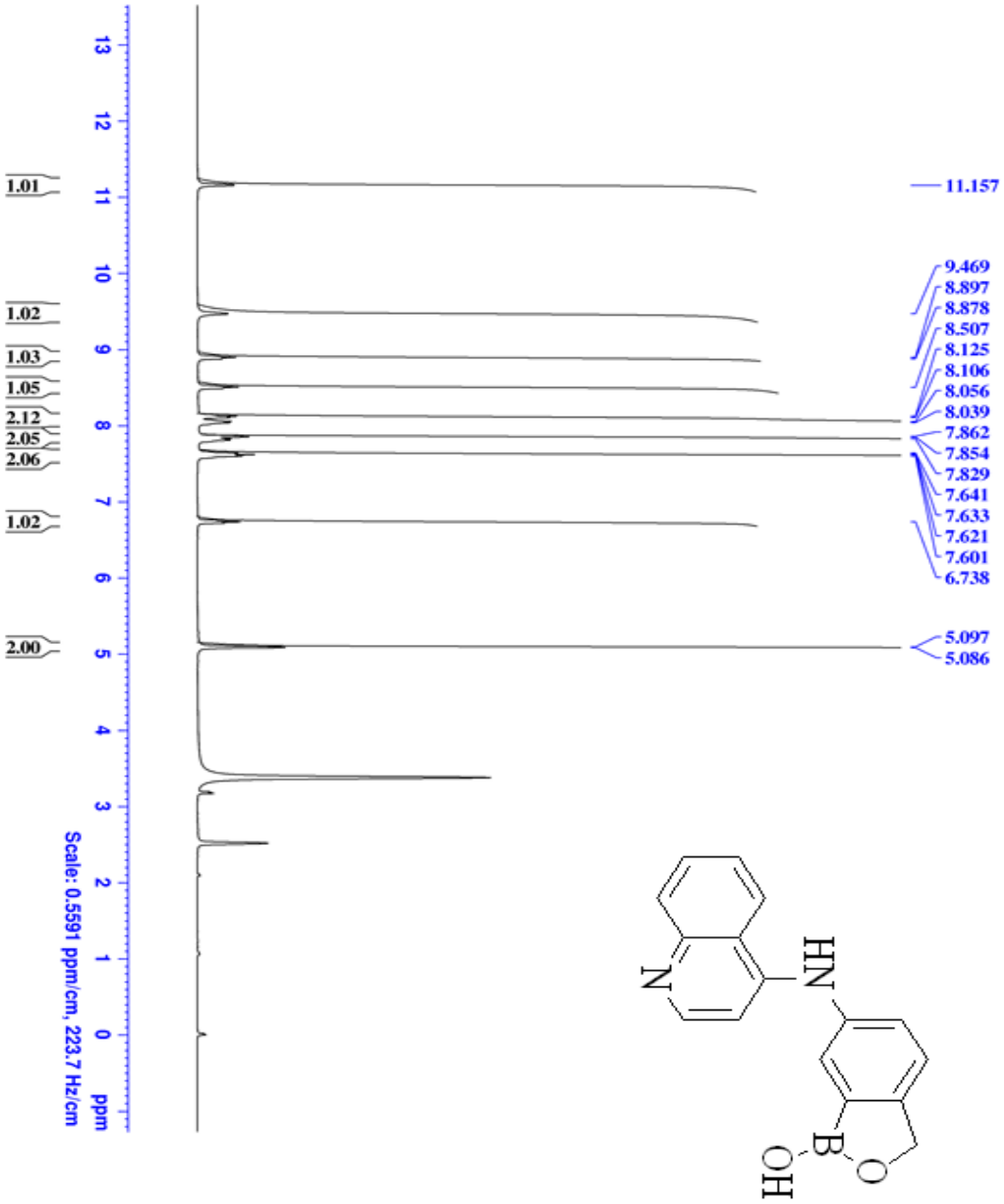

155.22
144.16
139.65
138.91
136.53
132.12
130.43
127.94
127.68
126.60
119.86
116.56
101.00
40.20
39.99
39.78
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
Scale: 6.276 ppm/cm, 631.5 Hz/cm
Cl
N
HN
Cl

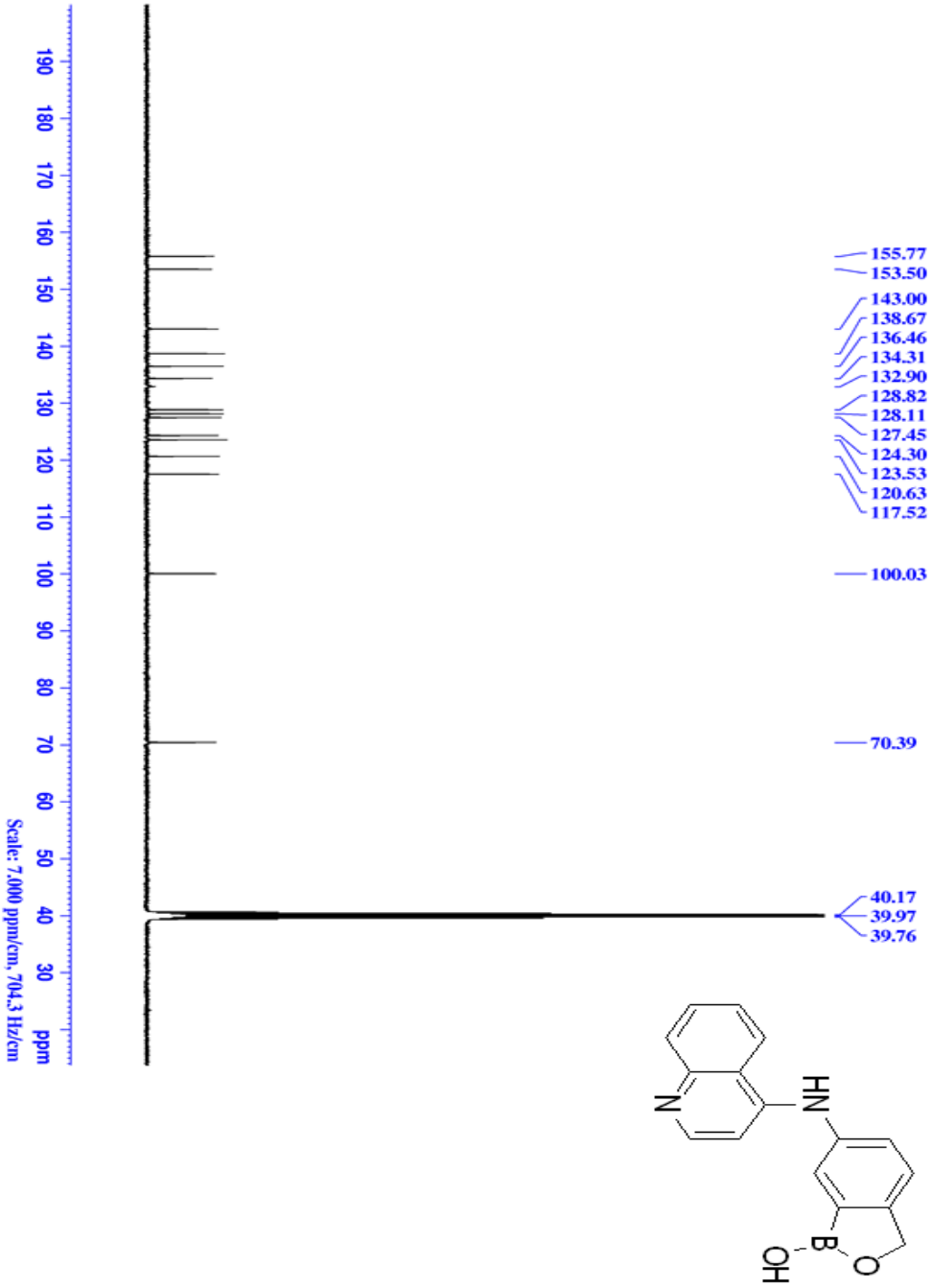

1.00
1.08
1.04
1.90
2.14
1.06
2.08
1.06
11.184
8.880
8.867
8.553
8.525
8.188
7.988
7.983
7.898
7.892
7.885
7.875
7.471
7.466
6.880
11
10
9
8
7
6
5
4
3
2
1
ppm
Scale: 0.4536 ppm/cm, 181.5 Hz/cm
Cl
N
HN
B
OH
OH

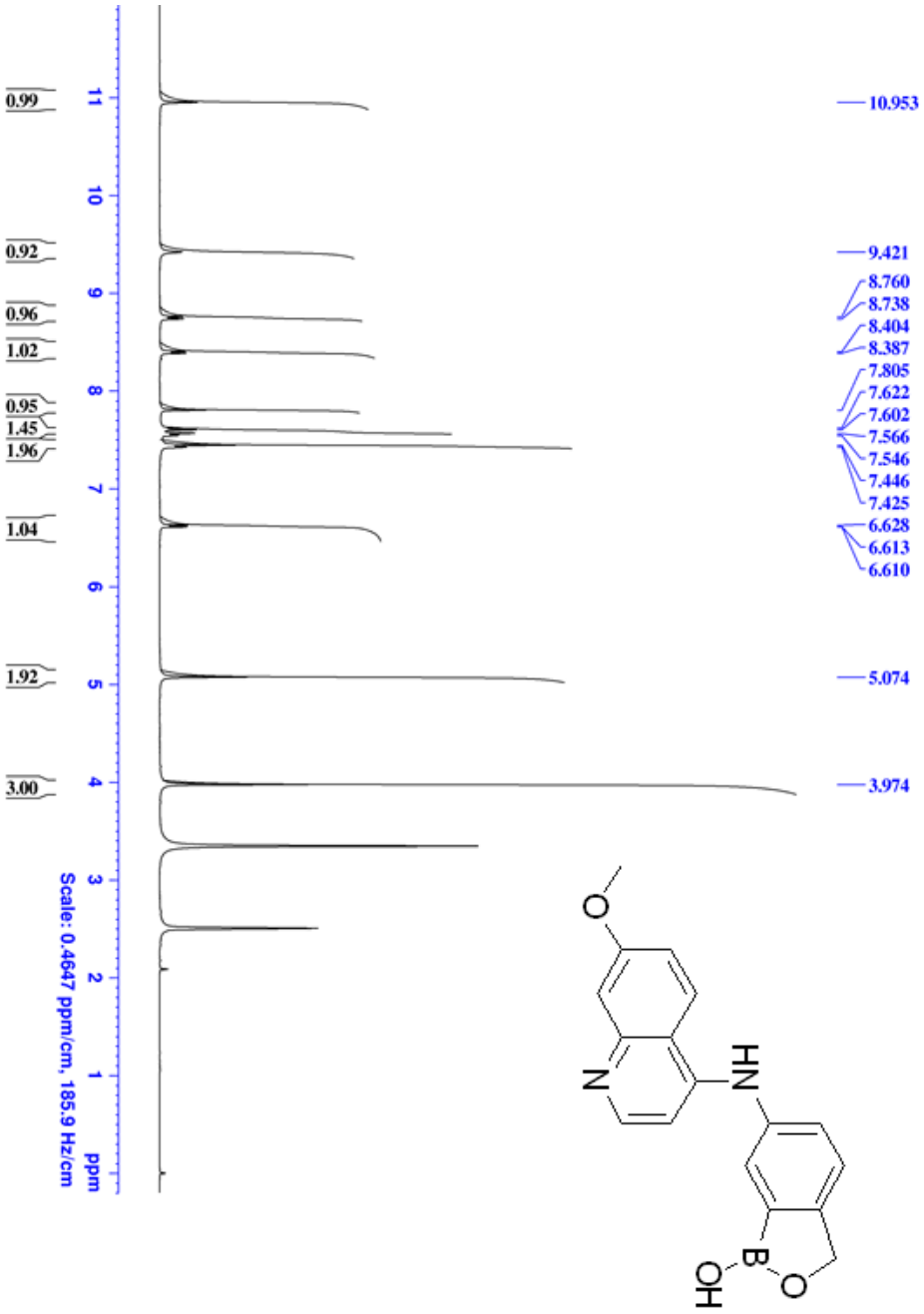

155.10
143.80
139.55
139.04
138.85
136.15
133.91
127.81
126.73
124.47
119.64
116.52
100.93
40.19
39.98
39.77
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
Scale: 7.573 ppm/cm, 762.0 Hz/cm
Cl
N
HN
B
OH
OH

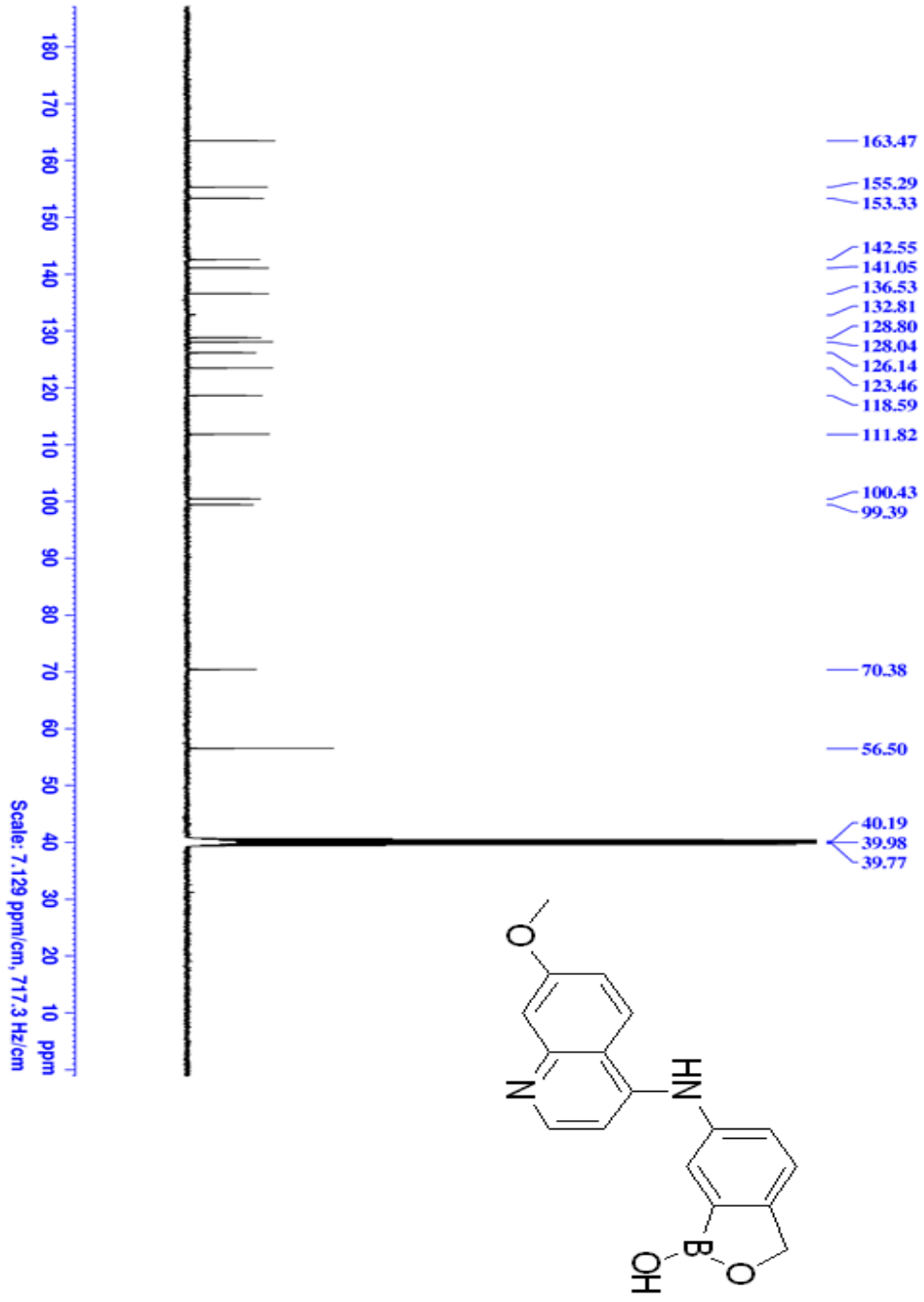

1.00
1.04
1.04
1.06
1.09
1.02
0.98
1.06
1.02
4.22
11.257
8.923
8.900
8.517
8.500
8.197
8.192
7.882
7.877
7.859
7.854
7.519
7.499
7.441
7.437
7.397
7.393
7.378
7.373
6.785
6.768
5.068
ppm
Cl
N
HN
O

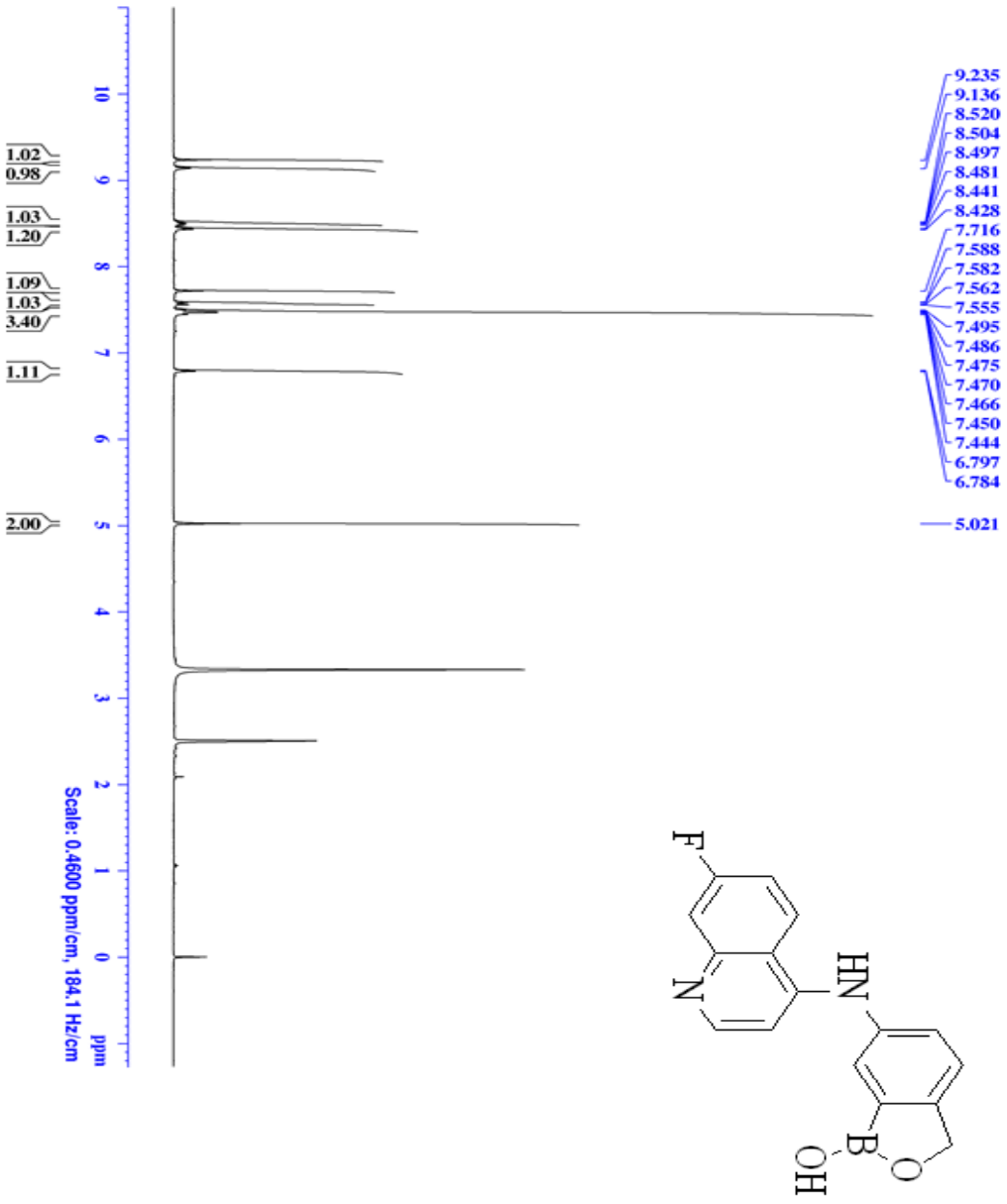

155.57
143.78
141.59
139.54
138.99
138.84
136.61
127.80
126.67
125.17
123.07
119.68
119.00
116.36
100.78
72.95
72.90
40.20
39.99
39.78
Cl
N
HN
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
Scale: 6.781 ppm/cm, 682.3 Hz/cm

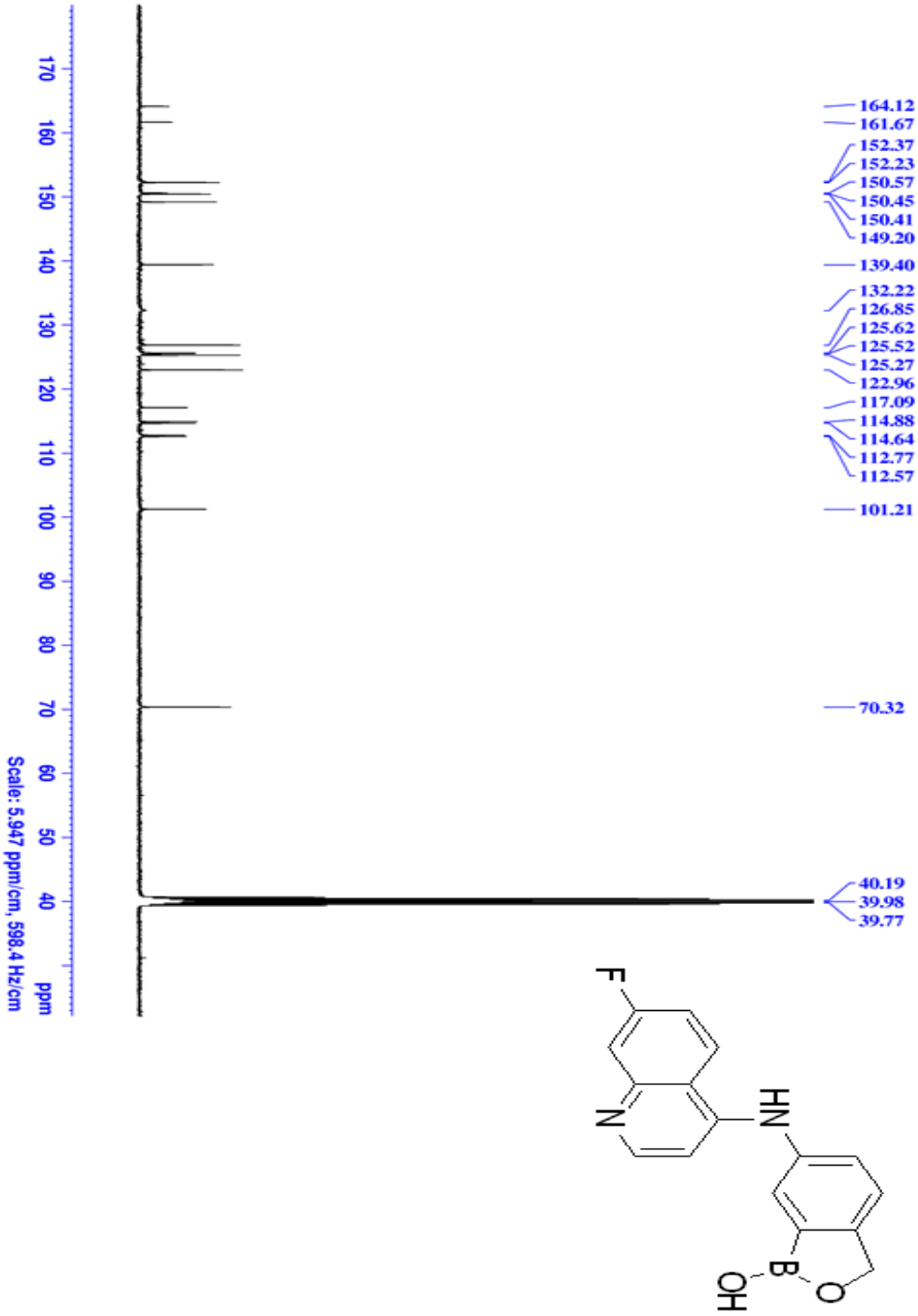

11.157
9.469
8.897
8.878
8.507
8.125
8.106
8.056
8.039
7.862
7.854
7.829
7.641
7.633
7.621
7.601
6.738
5.097
5.086
1.01
1.02
1.03
1.05
2.12
2.05
2.06
1.02
2.00
HN
N
B
O
OH
Scale: 0.5591 ppm/cm, 223.7 Hz/cm
ppm

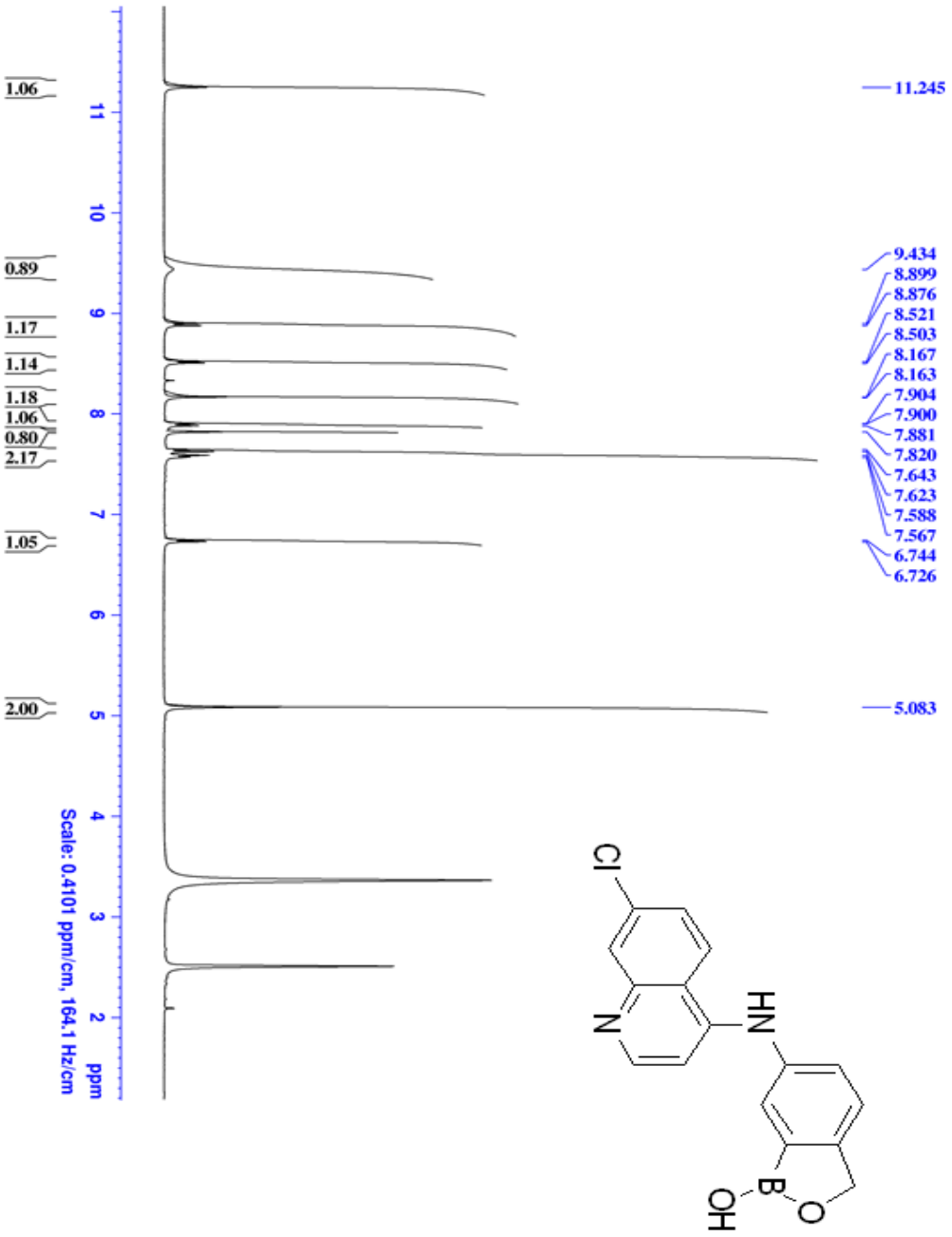

155.77
153.50
143.00
138.67
136.46
134.31
132.90
128.82
128.11
127.45
124.30
123.53
120.63
117.52
100.03
70.39
40.17
39.97
39.76
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm
Scale: 7.000 ppm/cm, 704.3 Hz/cm
N
HN
B
OH
O

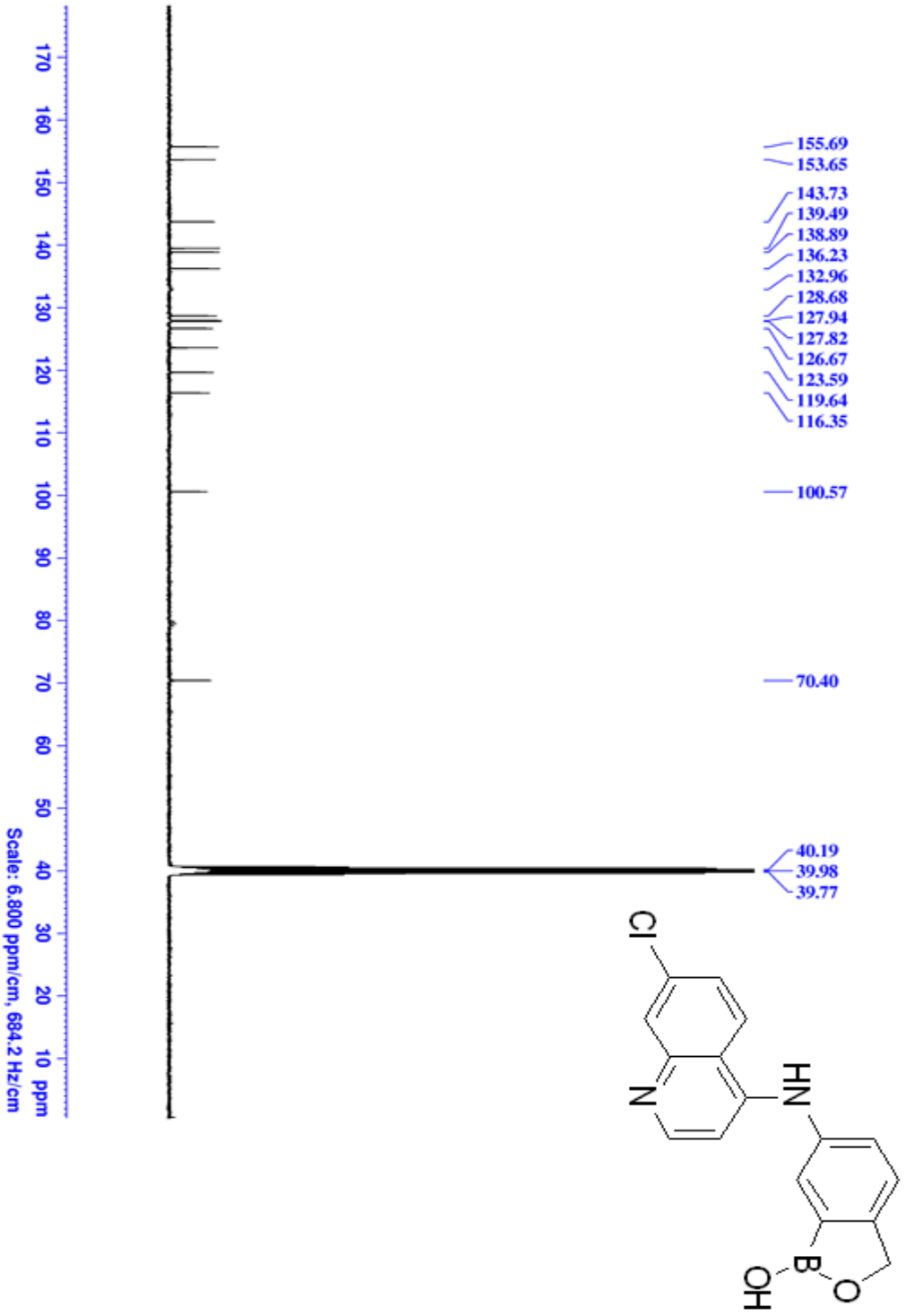

0.99
0.92
0.96
1.02
0.95
1.45
1.96
1.04
1.92
3.00
10.953
9.421
8.760
8.738
8.404
8.387
7.805
7.622
7.602
7.566
7.546
7.446
7.425
6.628
6.613
6.610
5.074
3.974
Scale: 0.4647 ppm/cm, 185.9 Hz/cm
ppm
O
N
HN
B
HO
O

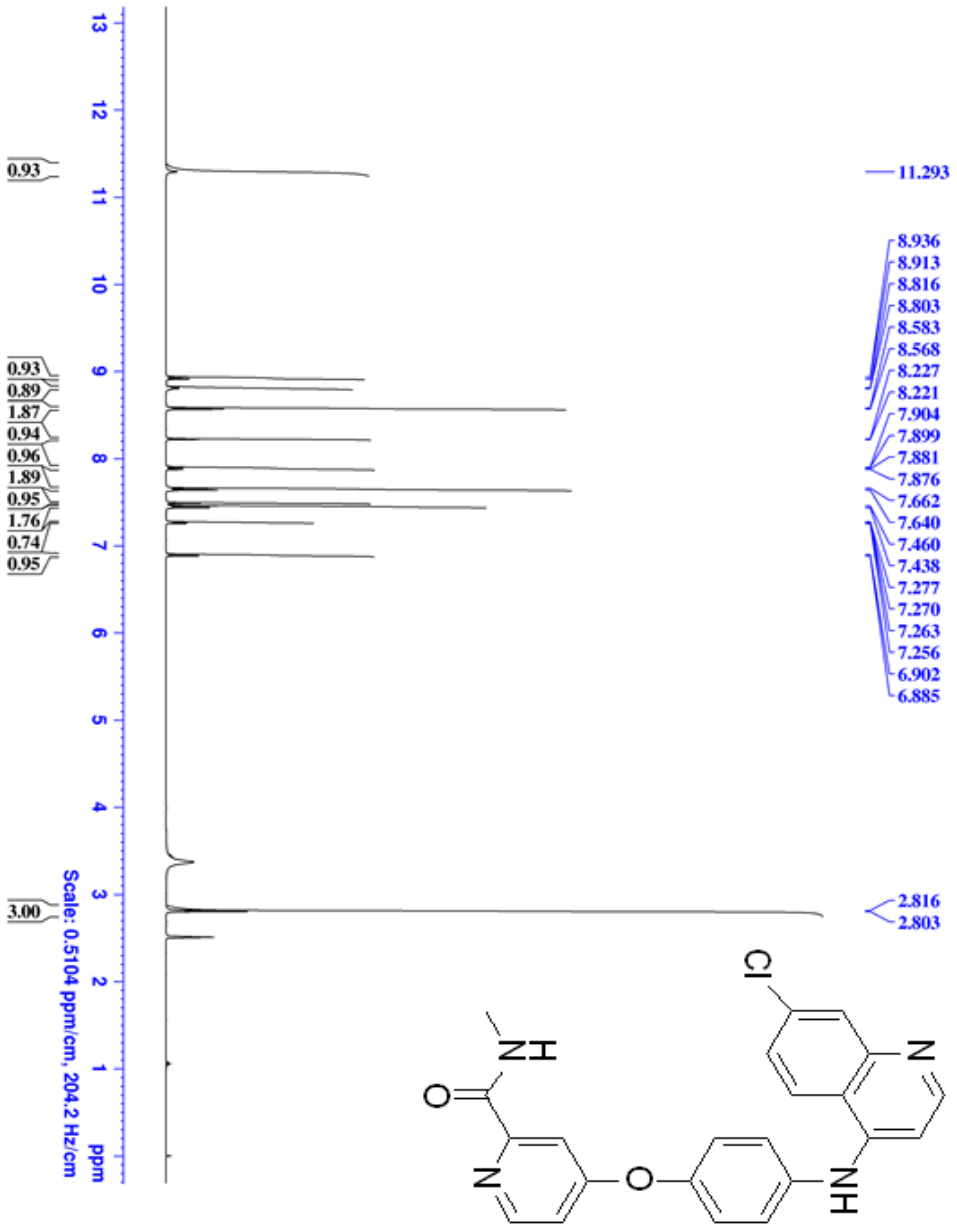

163.47
155.29
153.33
142.55
141.05
136.53
132.81
128.80
128.04
126.14
123.46
118.59
111.82
100.43
99.39
70.38
56.50
40.19
39.98
39.77
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
Scale: 7.129 ppm/cm, 717.3 Hz/cm
O
N
HN
B
OH
O

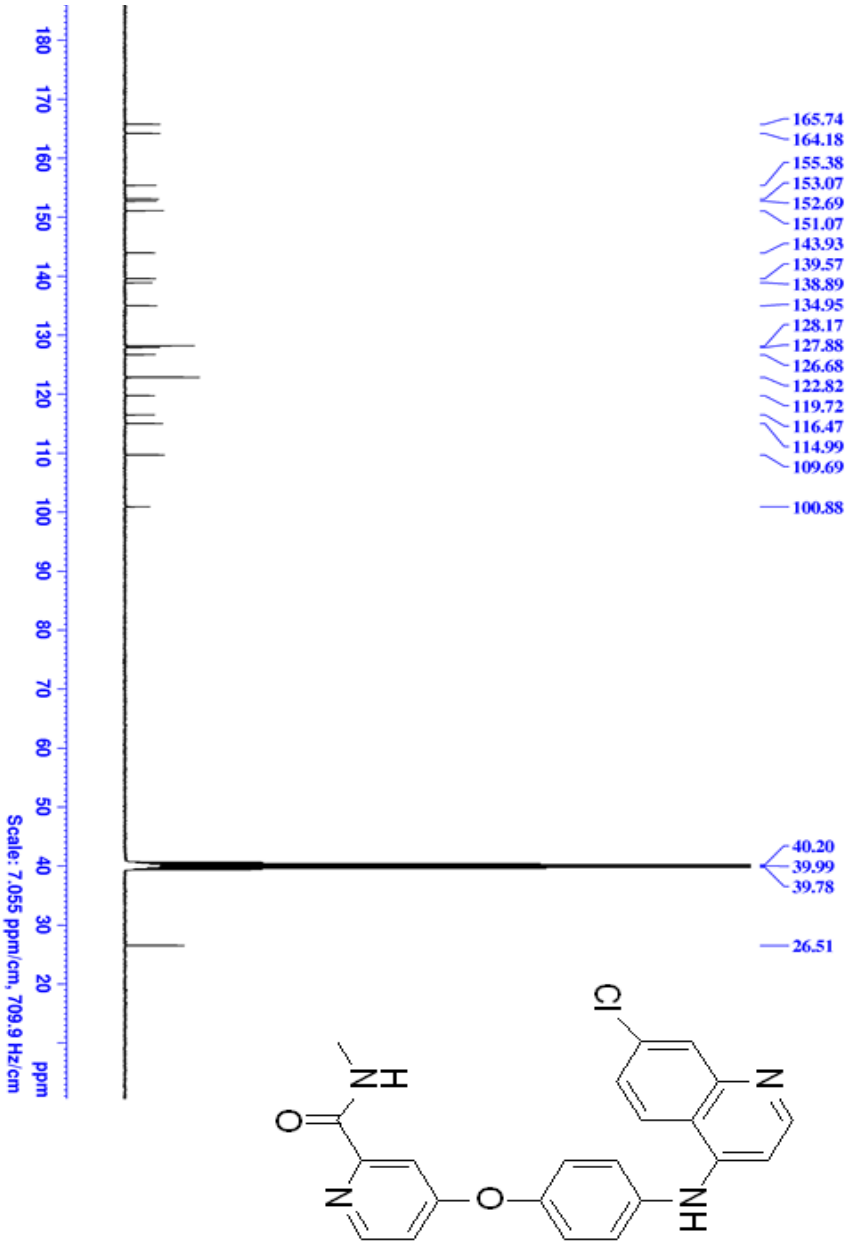

9.235
9.136
8.520
8.504
8.497
8.481
8.441
8.428
7.716
7.588
7.582
7.562
7.555
7.495
7.486
7.475
7.470
7.466
7.450
7.444
6.797
6.784
5.021
1.02
0.98
1.03
1.20
1.09
1.03
3.40
1.11
2.00
Scale: 0.4600 ppm/cm, 184.1 Hz/cm
ppm
F
N
HN
B
O
OH


164.12
161.67
152.37
152.23
150.57
150.45
150.41
149.20
139.40
132.22
126.85
125.62
125.52
125.27
122.96
117.09
114.88
114.64
112.77
112.57
101.21
70.32
40.19
39.98
39.77
170
160
150
140
130
120
110
100
90
80
70
60
50
40
ppm
Scale: 5.947 ppm/cm, 598.4 Hz/cm
F
N
HN
B
OH
O


1.06
0.89
1.17
1.14
1.18
1.06
0.80
2.17
1.05
2.00
11.245
9.434
8.899
8.876
8.521
8.503
8.167
8.163
7.904
7.900
7.881
7.820
7.643
7.623
7.588
7.567
6.744
6.726
5.083
Cl
N
HN
B
OH
O
11
10
9
8
7
6
5
4
3
2
ppm
Scale: 0.4101 ppm/cm, 164.1 Hz/cm


155.69
153.65
143.73
139.49
138.89
136.23
132.96
128.68
127.94
127.82
126.67
123.59
119.64
116.35
100.57
70.40
40.19
39.98
39.77
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
Scale: 6.800 ppm/cm, 684.2 Hz/cm
Cl
N
HN
B
OH
O


11.293
8.936
8.913
8.816
8.803
8.583
8.568
8.227
8.221
7.904
7.899
7.881
7.876
7.662
7.640
7.460
7.438
7.277
7.270
7.263
7.256
6.902
6.885
2.816
2.803
0.93
0.93
0.89
1.87
0.94
0.96
1.89
0.95
1.76
0.74
0.95
3.00
Scale: 0.5104 ppm/cm, 204.2 Hz/cm
ppm
Cl
N
NH
O
N
O
NH


165.74
164.18
155.38
153.07
152.69
151.07
143.93
139.57
138.89
134.95
128.17
127.88
126.68
122.82
119.72
116.47
114.99
109.69
100.88
40.20
39.99
39.78
26.51
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
Scale: 7.055 ppm/cm, 709.9 Hz/cm
Cl
N
NH
O
N
O
NH