UNIVERSITY OF KWAZULU-NATAL

SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL

ACTIVITY OF SUBSTITUTED 2-QUINOLINE

THIOSEMICARBAZONES

2017

SIBONISO SHEZI

SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF SUBSTITUTED 2-QUINOLINE THIOSEMICARBAZONES

SIBONISO SHEZI

2017

A thesis submitted to the School of Chemistry and Physics in the College of Agriculture, Engineering and Science for the fulfilment of the degree of Master of Science.

Preface

I hereby declare that the thesis entitled "**Synthesis, characterisation and antibacterial activity of substituted 2-quinoline thiosemicarbazones**" submitted to the University of KwaZulu-Natal for the award of the degree of Master of Science in Chemistry under the supervision of Professor Neil A. Koorbanally represents original work by the author and has not been submitted in full or part for any degree or diploma at this or any other University.

Where use was made of the work of others it has been duly acknowledged in the text. This work was carried out in the School of Chemistry and Physics, University of KwaZulu-Natal, Westville campus, Durban, South Africa.

Signed: _____   Date: _____

Siboniso Shezi, BSc(HONS)

As the candidate's supervisor, I have approved this dissertation for submission

Signed: _____   Date: _____

Professor Neil A. Koorbanally, PhD (Natal)

DECLARATIONS

DECLARATION 1 – PLAGIARISM

I, **Siboniso Shezi** declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a. Their words have been re-written but the general information attributed to them have been referenced.

    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed ………………………………………………………

ACKNOWLEDGEMENTS

List of Abbreviations

bd	broad doublet
bs	broad singlet
°C	degrees Celsius
^{1}H NMR	proton nuclear magnetic resonance spectroscopy
^{13}C NMR	carbon-13 nuclear magnetic resonance spectroscopy
COSY	correlation spectroscopy
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
FTIR	Fourier transform infrared
HMBC	heteronuclear multiple bond correlation
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
Hz	hertz
IR	infrared
LC-MS	liquid chromatography–mass spectrometry
MBC	miniumum bactericidal concentration
mp	melting point
MHA	Mueller-Hinton agar
MHz	megahertz
NHP	*N*-hydroxyphthalimide
NOESY	nuclear Overhauser effect spectroscopy
PIDA	phenyliodine(III) diacetate
rt	room temperature
s	singlet
t	triplet
TFA	trifluoroacetic acid
TLC	thin layer chromatography
TMS	tetramethylsilane
UV	ultraviolet

ABSTRACT

Fifteen quinoline-2-thiosemicarbazone hybrid derivatives were synthesised in a three step reaction involving formation of the quinoline, oxidation of the 2-methyl group and condensation to the thiosemicarbazones. The phenylhydrazines were prepared in an additional step prior to the condensation. Twelve of the fifteen hybrid molecules were novel. A full structural elucidation of all synthesised compounds were carried out using amongst others 1D and 2D NMR spectroscopy and mass spectrometry. The data presented here will provide a basis for the identification of further molecules of this type. The hybrid molecules were then subjected to antibacterial bioassays against Gram -ve and Gram +ve bacterial strains. Unfortunately, only one compound, the 6-bromo-4'-fluoro derivative on the (E)-2-quinoline-2-yl)methylene-N-phenylhydrazinecarbothiamide framework showed any antibacterial activity. They were active against both *Staphylococus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) at 387 μM.

Keywords: Quinoline-2-thiosemicarbazone, antibacterial, Doebner-von Miller, structural elucidation

STRUCTURES OF THE SYNTHESISED COMPOUNDS REPORTED IN THIS

THESIS

6a-o

	R_1	R_2		R_1	R_2		R_1	R_2
6a	F	H	**6f**	Cl	H	**6k**	Br	H
6b	F	F	**6g**	Cl	F	**6l**	Br	F
6c	F	Cl	**6h**	Cl	Cl	**6m**	Br	Cl
6d	F	NO_2	**6i**	Cl	NO_2	**6n**	Br	NO_2
6e	F	CH_3	**6j**	Cl	CH_3	**6o**	Br	CH_3

Table of Contents

List of Schemes

List of Figures

List of Tables

Chapter 1.   Introduction

Since the discovery of penicillin in 1928 by Sir Alexander Fleming, antibiotics have been used to treat bacterial infections, preventing secondary infections and even death. Even prior to this, there was evidence of antibiotics being used, with traces of tetracycline being found in human skeletal remains of Egypt and Sudanese Nubia populations dating back to 350-550 CE (Aminov, 2010). These antibiotics have also played an important role in medicine and surgery preventing bacterial infections from setting in after surgery (Ventola, 2015). However, over the years, overuse, inappropriate and incorrect prescribing, self-medicating and extensive agricultural use of antibiotics has led to antibiotic resistance (Ventola, 2015; Rather et al., 2017).

Antibiotic resistance occurs when bacteria modifies its structure and form in some way that reduces or eliminates antibiotic binding reducing or eliminating their effectiveness (Bisht et al., 2009). As a result, resistant bacteria are able to survive and mutate, leading to bacteria that are not susceptible to known antibiotics. The consequences are prolonged illnesses, greater risk of death, longer periods of hospitalization and an increase in infections. A huge percentage of bacteria (at least 70%) has become resistant to at least one of the most commonly used antibiotics used for treatment (Bisht et al., 2009).

To combat this problem, scientists and pharmaceutical companies have developed and introduced many new antibiotics. This was rampant from 1940-1980, but significantly declined thereafter, largely contributing to the antibiotic resistance crisis we find ourselves in today (Martens and Demain, 2017). This has been attributed to the merging of pharmaceutical companies, leading to less productive companies, a decline in Natural Products research, which inspired or contributed to the development of antibiotics and the cost involved in producing

these drugs (12-15 years and costing in the region of 1.2 billion US dollars) (Martens and Demain, 2017).

However, antibiotic resistance has now become a crisis, since the rate at which these antibiotics are being rendered inactive due to antibiotic resistance is alarming with more and more bacteria becoming resistant to commonly used drugs. For Gram positive bacteria, this has been described as a crisis that is under control and for Gram negative bacteria, a crisis going out of control (Rossolini et al., 2014). The inactivity of current antibiotics through antibiotic resistance will lead to loss of human lives if alternative antibiotics are not discovered soon. There is thus an urgent need for the development of new antimicrobials to treat bacteria that have become resistant to first line antibacterial agents used in treatment.

The current project aims to address this by synthesising new molecules, which have a good chance of having antibacterial activity and hence could be developed into alternative antibiotics to currently marketed drugs. We aim to use an approach of creating hybrid molecules.

Hybrid molecules are those incorporating the frameworks of two molecular scaffolds, each with bioactivity of their own. These compounds contain the basic framework of each of the individual structures. The desired effect of the hybrid molecule is to have a synergistic effect between the different pharmacophores, increasing the activity of either of them alone. The ultimate prize is one drug which can treat more than one disease or medical condition or enhance the therapeutic potential of an existing drug. These molecules are usually formed by an organic reaction with two functional groups, one on each molecular framework. Quinoline hybrids have produced compounds with increased bioactivity (Vandekerckhove and D'Hooghe, 2015), for example anti-malarial (Burgess et al., 2006), antitubercular (Jain et al., 2016),

anticancer (Solomon et al., 2010; Alegaon et al., 2017), antileishmanial (Coa et al., 2017) and antimicrobial activities (Desai et al., 2013) amongst others. Likewise, thiosemicarbazide hybrids have also shown good bioactivity such as antidiabetic (Taha et al., 2016), antibacterial, antitubercular (Rane et al., 2014) and anticancer agents (Rane et al., 2015).

When a quinoline with an aldehyde or ketone substituent reacts with a thiosemicarbazide moiety, the hybrid molecule that forms is termed a quinoline-thiosemicarbazone. These quinoline-thiosemicarbazone hybrids have shown anticancer (Huang et al., 2010), antimicrobial (Abdel-Moty et al., 2005; Kulkarni et al., 2010), anti-oxidant (Ramachandran et al., 2012), anti-HIV, antitubercular (Banerjee et al., 2010), anticonvulsant and analgesic activities (Aly et al., 2010).

1.1   Quinolines

Quinoline is an aromatic heterocyclic compound consisting of two fused rings, a benzene ring fused with a pyridine ring (**Figure 1.1**), and has the synonyms benzopyridine, benzo[b]pyridine, and benzazine. The quinoline nucleus occurs in several naturally occurring compounds (O'Donnell et al., 2006; Michael, 2008; Zhang et al., 2012) and pharmacologically active substances displaying a broad range of biological activity (Gopaul et al., 2015). These include antimalarial (Vandekerckhove and D'Hooghe, 2015), anti-cancer (Afzal et al., 2015; Gopaul et al., 2015), antioxidant (Maddela et al., 2015), anti-HIV (Luo et al., 2009), antimicrobial (Musiol et al., 2011), antileishmanial (Gopinath et al., 2013), antimycobacterial (Eswaran et al., 2010), and anticonvulsant activities (Siddiqui, 2010).
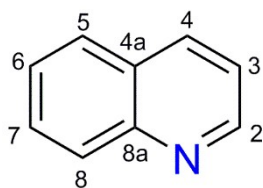
Figure 1.1 The general structure of the quinoline nucleus

Several natural occurring compounds that contain the quinoline moiety are either pharmaceuticals or employed as lead compounds for the development of new and more potent drugs (Kumar et al., 2009). One such example is quinine (**Figure 1.2**) isolated from the bark of the cinchona tree, used in the treatment of malaria (Kumar et al., 2009). This initial discovery was followed by the development of more potent drugs such as chloroquine, primaquine and mefloquine (**Figure 1.2**) (Kumar et al., 2009).

Quinine   Chloroquine   Primaquine   Mefloquine
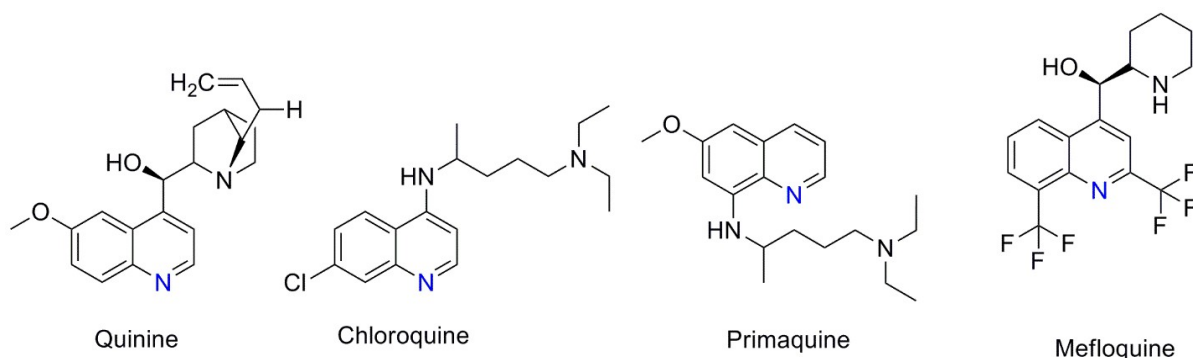
Figure 1.2 Biologically active compounds containing a quinoline nucleus

Synthesis

Many methods are used to synthesise quinolines (Yamashkin and Oreshkina, 2006; Madapa et al., 2008). Most start with simple anilines and make use of electrophiles, which form a heterocyclic ring from their reaction. Reactions used to synthesise quinolines include the Skraup, Conrad-Limpach, Combes, Pavarov, Doebner and Doebner-von Miller reaction (Li and Corey, 2004; Ramann and Cowen, 2016).

The Skraup synthesis involves anilines, such as *m*-toluidine reacting with glycerol in the presence of a sulphuric acid catalyst and nitrobenzene (an oxidising agent in the reaction) forming a mixture of 7-methylquinoline and 5-methylquinoline (**Scheme 1-1**) (Zibaseresht et al., 2013).
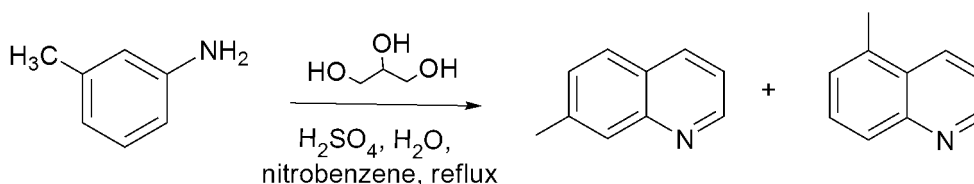
Scheme 1-1 Skraup quinoline synthesis

In the Combes synthesis, condensation of anline with *β*-diketones take place to form 2,4-substituted quinolines in the presence of an acid catalyst (**Scheme 1-2**) (Aribi et al., 2016).

Scheme 1-2 Combes quinoline syntheses

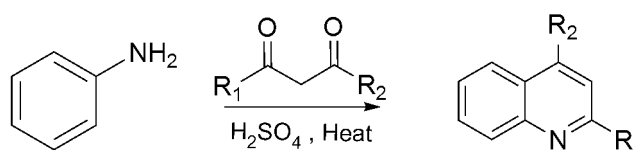The Conrad-Limpach synthesis conditions are like those of the Combes synthesis but uses *β*-ketoesters, which results in quinolones being formed (**Scheme 1-3**). A modification of this synthesis makes use of a vinyl ether instead of ethyl acetoacetate. With a deactivating *p*-nitro substituent on the aniline, the 4-hydroxyquinoline predominates (**Scheme 1-4**) (Brouet et al., 2009).
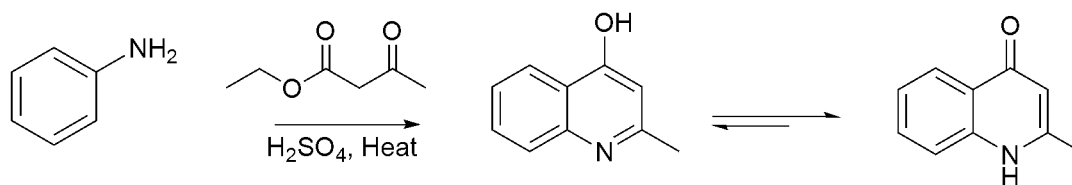
Scheme 1-3 Conrad-Limpach quinoline syntheses
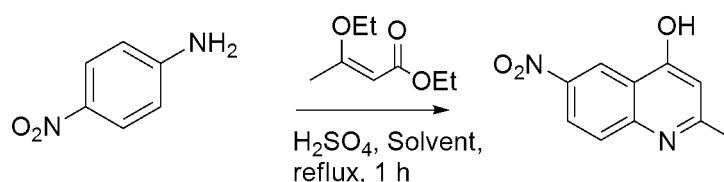
Scheme 1-4 Conrad-Limpach quinoline syntheses making use of a vinyl ether ester

Anilines reacted with substituted aldehydes and pyruvic acid forming quinoline-4-carboxylic acids in a reaction called the Doebner reaction (**Scheme 1-5**) (Wang et al., 2016). The resultant quinoline products have a carboxyl group at C-4 and substituent at C-2 depending on the aldehyde used in the synthesis.
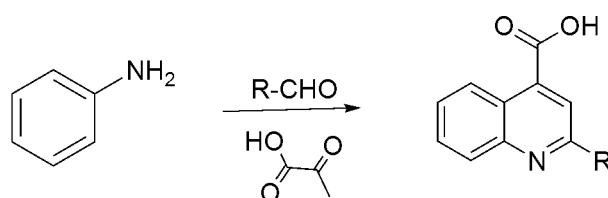
Scheme 1-5 Quinoline synthesis via the Doebner reaction

The Doebner-von Miller synthesis makes use of α,β-unsaturated ketones and aldehydes in the presence of an acid catalyst (**Scheme 1-6**) (Gopaul and Koorbanally, 2016). When aldehydes are used, 2-substituted quinolines are formed and when ketones are used, 2,4-disubstituted quinolines result (Chaskar et al., 2010).
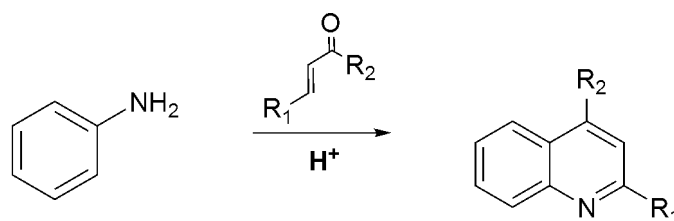
Scheme 1-6 Doebner-von Miller quinoline synthesis

In the Pavarov synthesis, condensation between an aniline and aromatic aldehyde first takes place forming an imine, which then undergoes a cycloaddition reaction with an alkene having an electron donating substituent such as ethoxyethene to form 2-phenylquinolines. This reaction uses boron trifluoride as a Lewis acid catalyst (**Scheme 1-7**) (Kouznetsov, 2009).
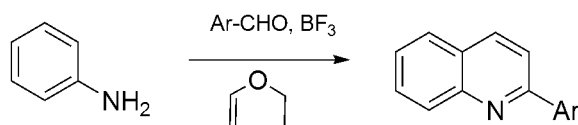
Scheme 1-7 Pavarov quinoline syntheses

This reaction proceeds by imine formation from a condensation reaction between aniline and benzaldehyde followed by a 2+2 cycloaddition reaction between the imine and ethoxyethene forming an unstable four membered ring intermediate. Ring opening and cyclisation to a six-membered intermediate then occurs, after which aromaticity is restored forming the product (**Scheme 1-8**).
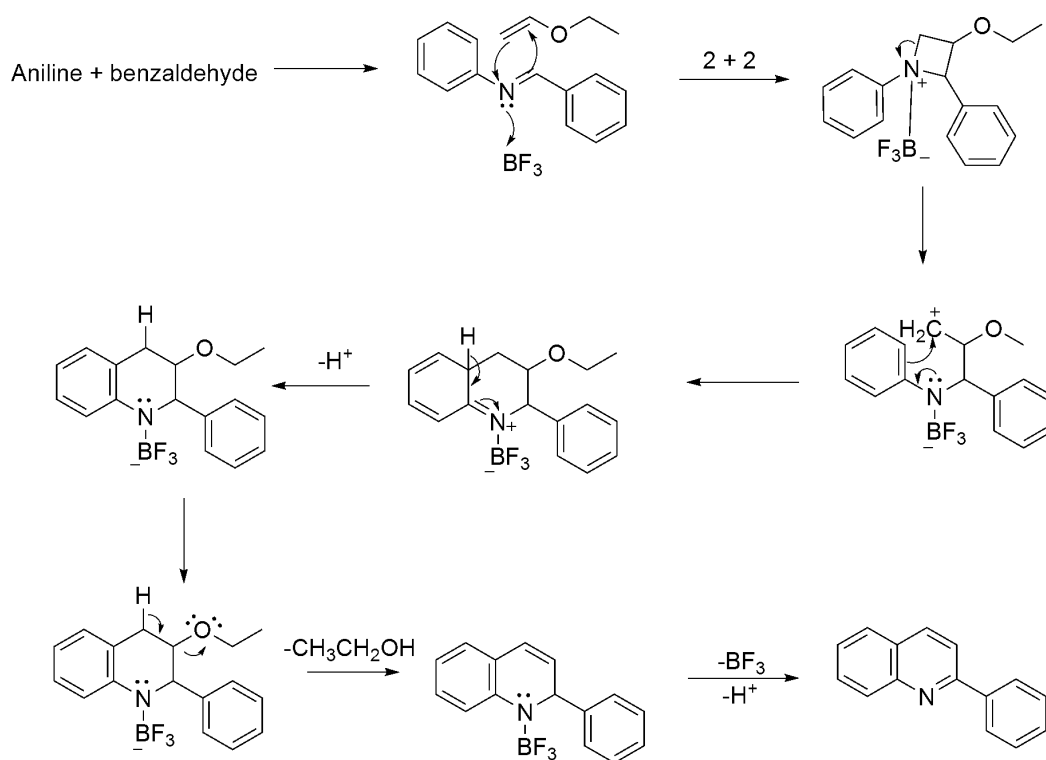
Scheme 1-8 Proposed reaction mechanism for the Pavarov synthesis

In the Friedlander synthesis, *o*-aminobenzaldehyde reacts with ketones, aldehydes or other carbonyl compounds containing reactive *α*-methylene groups to form 2,3-disubstituted quinoline derivatives (**Scheme 1-9**) (Shiri et al., 2011).
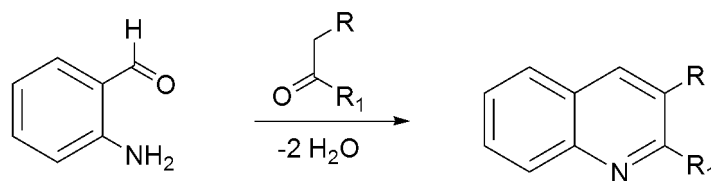
Scheme 1-9 Friedlander quinoline syntheses

The Niementowski quinoline synthesis is similar to the Friedlander synthesis, however instead of the aldehyde, a carboxylic acid group is present in the aniline derivative. When this reagent is reacted with a *β*-diketo ester, a pseudo coumarin skeleton occurs in an angular fashion on the quinoline framework (**Scheme 1-10**) (Poronik et al., 2017).
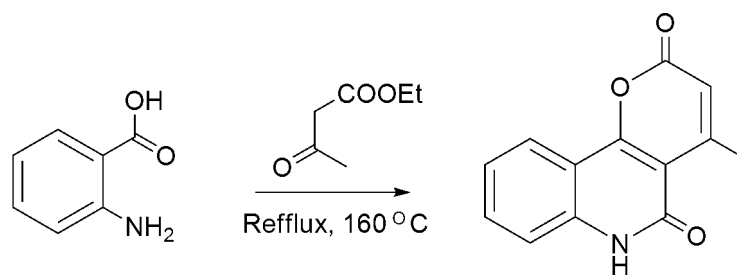
Scheme 1-10 Niementowski quinoline syntheses

The Pfitzinger quinoline synthesis uses isatin instead of aniline derivatives, which reacts with carbonyl compounds in the presence of strong bases such as potassium hydroxide. This reaction produces quinolines with a carboxyl group at C-4 (**Scheme 1-11**) (Sangshetti et al., 2014).

Scheme 1-11 Pfitzinger quinoline synthesis

In the present work, the Doebner-Miller reaction was used to synthesise quinolines since the design of the target molecules used crotonaldehyde as a reagent along with aniline to form 2-methylquinolines. This was an essential intermediate for subsequent oxidation to quinoline-2-carbaldehyde from which thiosemicarbazones were synthesised.

More about the Doebner-von Miller reaction
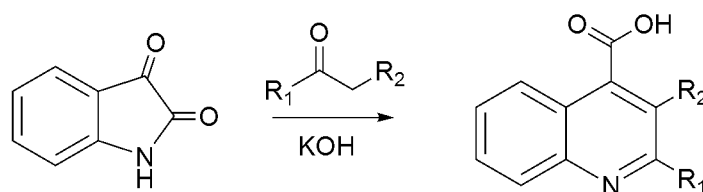
This reaction is normally conducted in a biphasic solvent system consisting of toluene and aqueous hydrochloric acid, in order to keep nucleophiles away from the aldehyde moiety, preventing polymerisation of the aldehyde. The biphasic system also helps in the isolation of the quinoline derivative in that the quinoline remains in the organic phase and other polar

compounds in the aqueous phase (Matsugi et al., 2000). This reaction has some limitations, such as being limited to sterically accessible aldehydes such as crotonaldehyde. Sterically bulky aldehydes such as cinnamaldehyde does not produce corresponding phenylquinolines under similar reaction conditions (Reynolds et al., 2010).

The Doebner-von Miller reaction involves cyclization of the amino group from aromatic amines with α,β unsaturated compounds (Reynolds et al., 2010). The four carbon crotonaldehyde (an α,β-unsaturated compound) can be formed by the reaction of two molecules of acetaldehyde (Snell et al., 2010). Aniline then condenses with crotonaldehyde forming an enol. Subsequent departure of the hydroxy group after protonation results in a conjugated imine. A 2+2 cycloaddition reaction of two molecules of the imine then forms a four-membered intermediate, which opens and cyclises to a six-membered intermediate. After aromatisation is restored, one of the nitrogen atoms (that which is not adjacent to the aromatic ring) is then protonated, catalysing the opening of the six-membered heterocyclic ring. Cyclisation then occurs with the adjacent alkene moiety and the aromatic amine is then released. The final step involves a dehydrogenation step to yield the quinoline (**Scheme 1-12**) (Eisch and Dluzniewski, 1989).

This reaction takes place at temperatures between 80 – 111 °C (Reynolds et al., 2010; Ramann and Cowen, 2015) and is usually catalysed by Brønsted acids such as hydrochloric acid (Gopaul and Koorbanally, 2016), sulfuric acid (Yadav et al., 2016) and heteropoly acids such as phosphomolybdic acid (Chaskar et al., 2010) and phosphotungstic acid (Sivaprasad et al., 2006). Ranu et al. (2003) also demonstrated that quinolines and dihydroquinolines could be synthesised using solvent free microwave reactions on the surface of silica gel impregnated with In (III).

Oxidation of 2-methylquinoline to quinoline-2-carboxaldehyde

The oxidation of 2-methylquinoline (quinaldine) to either quinoline-2-carbaldehyde or quinoline-2-carboxylic acid (quinaldinic acid) creates functionality at C-2 on the quinoline nucleus, which can be used for further nucleophilic and coupling reactions. Selenium dioxide (SeO_2) is a commonly used oxidant for the oxidation of quinaldine to quinoline-2-carboxaldehyde (**Scheme 1-13**) (Achremowicz, 1996; Pérez-Melero et al., 2004; Gopinath et al., 2013; Wang et al., 2015; Gopaul and Koorbanally, 2016).
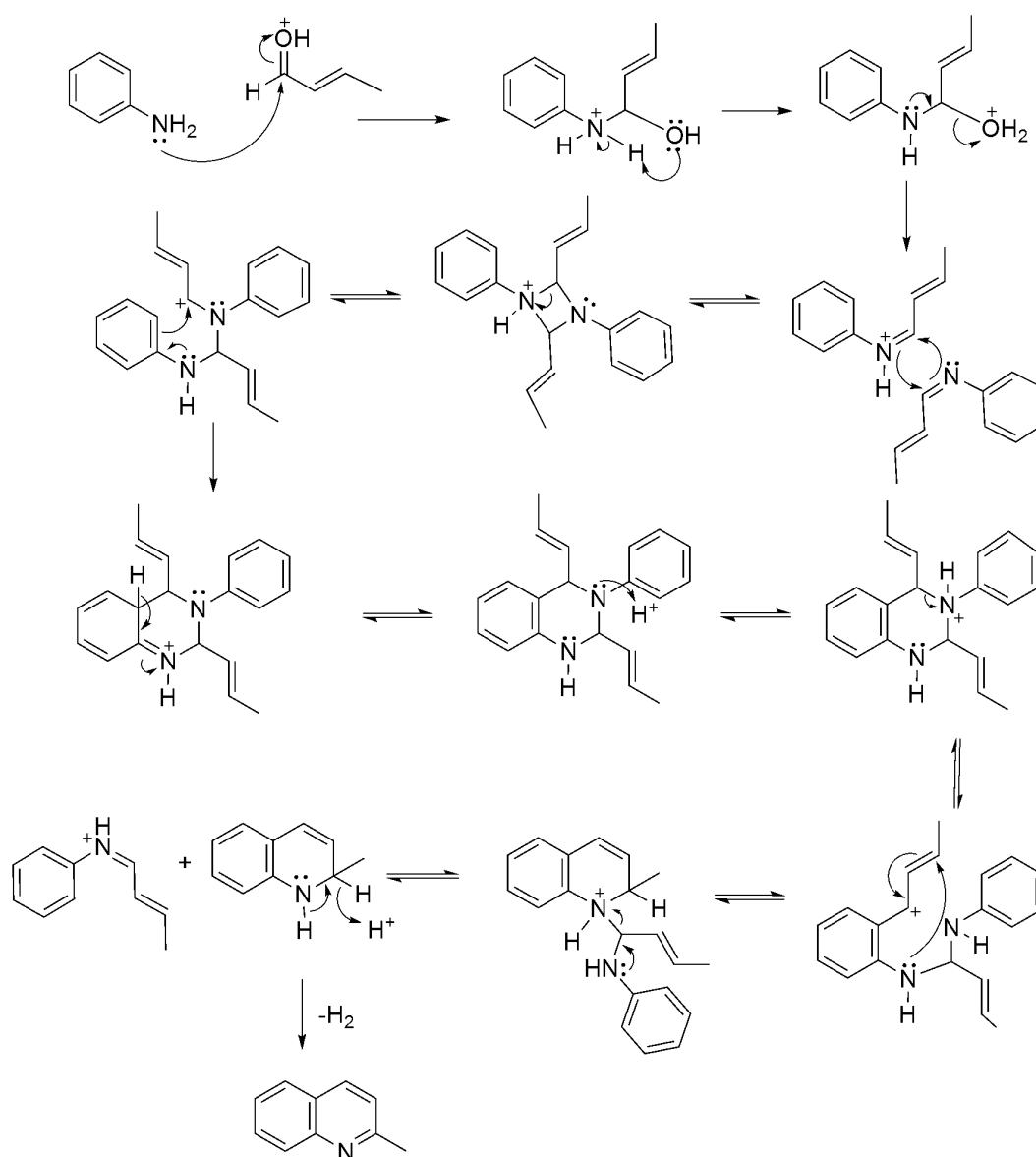
Scheme 1-12 Proposed mechanistic pathway for the Doebner-von Miller synthesis (Eisch and Dluzniewski, 1989)

Precautions are to be taken when working with SeO_2 as it is known to be toxic and to form selenous acid, a severe skin irritant when in contact with the body fluids such as perspiration and tears (Mlochowski and Wojtowicz-Mlochowska, 2015). The oxidation was carried out in boiling aprotic solvents such as dioxane with yields as high as 90% (Wang et al., 2015) or xylene (Pérez-Melero et al., 2004) with yields of between 50 – 80%. Using SeO_2 in the absence of reflux conditions produces yields of 70%, but only after two weeks (Achremowicz, 1996).
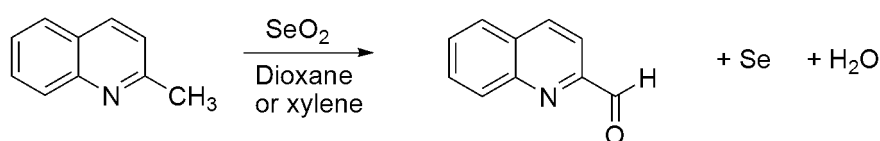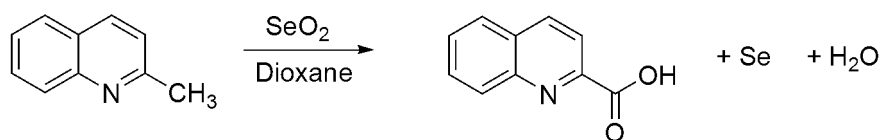

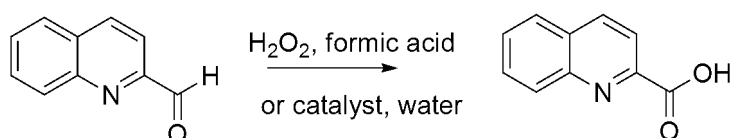**Scheme 1-13** Oxidation of 2-methylquinoline to quinoline-2-carbaldehyde

A much greener photo-oxidation of 2-methylquinoline (quinaldine) to quinoline-2-carboxaldehyde in good yield (65%) was reported where the methyl group was oxidised with TFA and I_2 by irradiation under an oxygen atmosphere for 20 hours (Nagasawa et al., 2016). Aerobic oxidation of 2-methylquinolines using copper catalysts in DMF at 130 °C in 12 hours was also carried out (Zheng et al., 2016). Microwave assisted oxidation of methylquinolines to quinoline aldehydes was demonstrated using phenyliodine(III) diacetate (PIDA) as a catalyst in DMSO in 18 to 42 minutes (Jiang et al., 2016).

Quinaldine (2-methylquinoline) can also be oxidized to quinoline-2-carboxylic acid (quinaldinic acid) when using selenium dioxide in dioxane refluxing for 2-8 hours (Musiol et al., 2009; Chandrasekhar et al., 2011) (**Scheme 1-14**). An aerobic oxidation of quinaldine to quinaldinic acid was also carried out using *N*-hydroxyphthalimide (NHP)/Co(OAc)$_2$/Mn(OAc)$_2$ catalysts with a NO_2 initiator (Sakaguchi et al., 2002).

Scheme 1-14 Oxidation of 2-methylquinoline to quinoline-2-carboxylic acid

Quinoline-2-carbaldehyde was easily oxidized to quinaldinic acid using hydrogen peroxide in formic acid (Dodd and Le Hyaric, 1993) or hydrogen peroxide in water catalysed by a sulfoxyalkyl-substituted *N*-heterocyclic carbene catalyst (**Scheme 1-15**) (Yoshida et al., 2009).


Scheme 1-15 Oxidation of quinoline-2-carbaldehyde to quinaldinic acid

Mechanism of oxidation

In the proposed mechanism by Trump and Zhou (1993) (**Scheme 1-16**), nucleophilic attack by methylquinoline to selenium dioxide followed by proton transfer, leads to an unstable four membered cyclic intermediate, resulting in the oxygen adding to the 2-methyl group to open up the four-membered ring by breaking the C-Se bond. In the final step, the oxygen bound to Se abstracts the proton from the 2-methylene group, forming quinoline-2-carbaldehyde and releasing selenium and water.

In SeO$_2$ oxidation of methylquinoline, Se^{4+} is reduced to Se, which is subsequently removed from the reaction mixture by filtration. The aldehyde formed is then separated by biphasic extraction and purified by column chromatography.
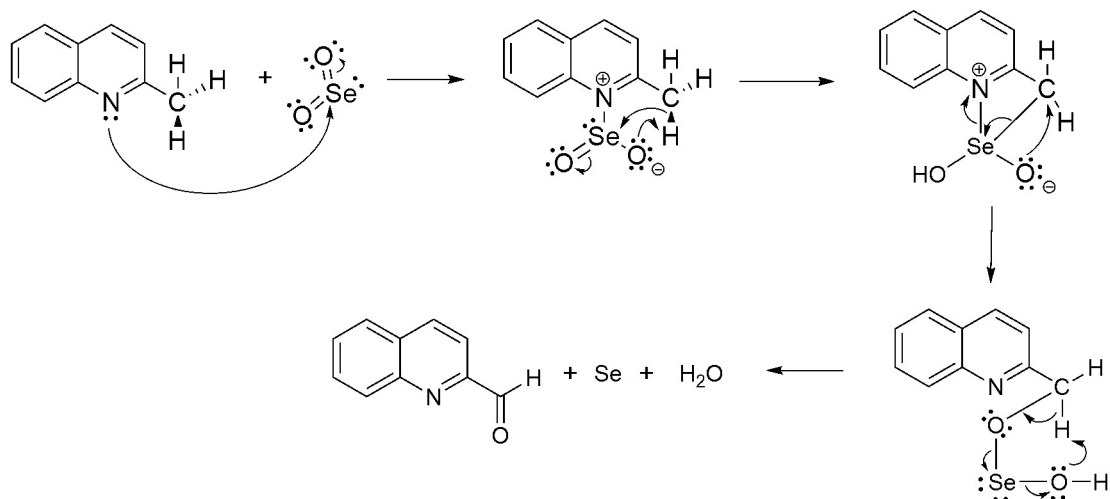
Scheme 1-16 Proposed mechanism for the oxidation of 2-methylquinoline by selenium dioxide (Trump and Zhou, 1993)

1.2   Thiosemicarbazides and thiosemicarbazones

Thiosemicarbazides are an important class of compounds, which together with their derivatives have good biological activity (Singhlal et al., 2013), including anticancer (Arora et al., 2014), antitubercular (Patel et al., 2014), antibacterial (Nevagi and Dhake, 2013), antiproliferative (Pitucha et al., 2016), and antifungal (Er et al., 2017) activity. When thiosemicarbazides form imines with aldehydes or ketones in an additional step, the resultant molecules are termed thiosemicarbazones (**Figure 1.3**) (Singh et al., 1978). Thus, thiosemicarbazones have an unsaturated *N*-1 whereas thiosemicarbazides have a fully saturated *N*-1. Thiosemicarbazones have shown antibacterial (Souza et al., 2013), anticancer (Thanigaimalai et al., 2012), antiparasitic (Glinma et al., 2014) and antioxidant activities (Nguyen et al., 2013).
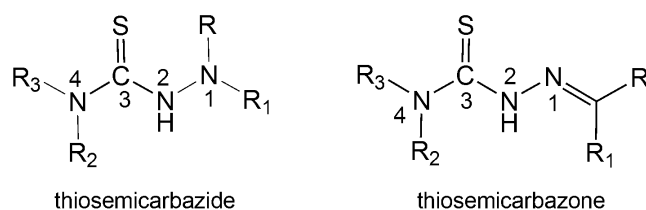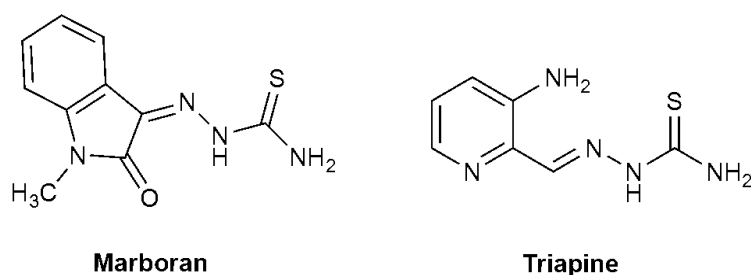

**Figure 1.3** The structures of thiosemicarbazides and thiosemicarbazones

Thiosemicarbazones have also demonstrated their ability to complex with metal ions, such as Fe(II), Cu(II), Pd (II) and Zn(II) resulting in pharmaceutically active compounds (Zeglis et al., 2011; Yu et al., 2009; Hernandez et al., 2013). The metallation has been shown to play a role in bioactivity such as topoiosomerase IIα inhibition and antiproliferative activity (Zeglis et al., 2011). Examples of commercially available thiosemicarbazone based drugs are Marboran® (methisazone), an antiviral used to treat smallpox, and Triapine® (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) which is an anti-cancer drug (**Figure 1.4**).
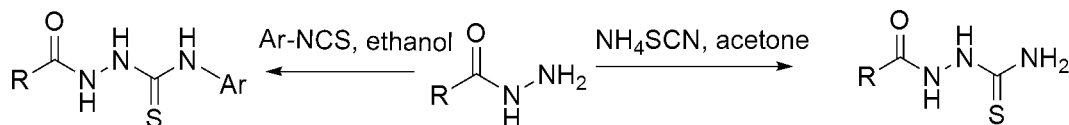
Synthesis

Thiosemicarbazide synthesis involves the preparation of isothiocyanates *in situ* by nucleophilic addition of amines to carbon disulfide, resulting in potassium arylcarbamodithioates. These intermediates then react with methyl iodide, which replaces the potassium to afford *N*-aryl methyldithiocarbamates, which undergo hydrazinolysis to give 4-arylthiosemicarbazides (**Scheme 1-17**). They can also be formed by starting directly from isothiocyanates or ammonium thiocyanates which undergo a condensation reaction with carbohydrazines (**Scheme 1-18**) (Metwally et al., 2011).

Figure 1.4 Examples of thiosemicarbazone based drugs available on the market
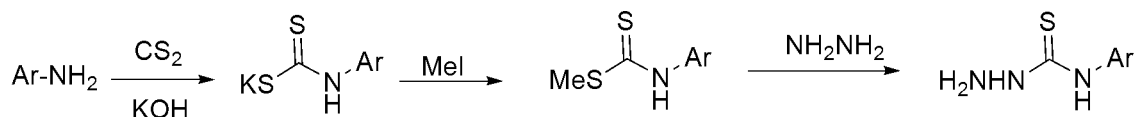
Scheme 1-17 Synthesis of 4-arylthiosemicarbazides

Scheme 1-18 Synthesis of thiosemicarbazide derivatives from carbohydrazines

Substituted phenylthiosemicarbazides were synthesiszed by nucleophilic addition between aromatic isothiocyanates and hydrazine (Bečka et al., 2017). A proposed mechanism for the reaction involves nucleophilic attack by hydrazine on the carbon of the isothiocyanate followed by proton transfer, resulting in the formation of phenylthiosemicarbazides (**Scheme 1-19**).
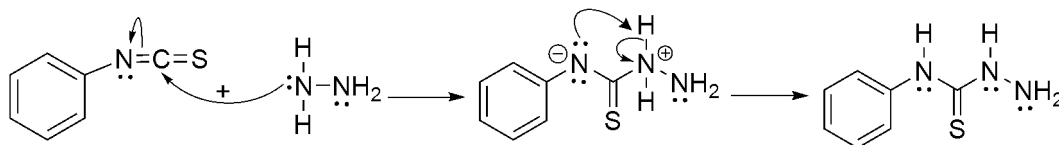
**Scheme 1-19** Postulated mechanism for thiosemicarbazide formation

This reaction was carried out in protic solvents such as ethanol under both under reflux (for 7 hours) (Foroughifar et al., 2014), and at room temperature (for 30 mins) (Benmohammed et al., 2014). Water can also be used with hydrochloric acid as a catalyst for selected thiosemicarbazides such as 1-(2-hydroxybenzoyl) thiosemicarbazide at 95 °C for 4 hours (Nurkenov et al., 2016).

This moiety also reacts with compounds containing C=O and C=N groups to form a variety of five or six membered heterocyclic molecules, including thiazoles, pyrazoles, thiadiazoles,

triazoles, oxadiazoles and thiazines, which have shown potential biological activities (Metwally et al., 2011; Gazieva and Kravchenko, 2012; Singhal et al., 2013).

## 1.3   Quinoline thiosemicarbazone hybrids

Quinoline thiosemicarbazide hybrid molecules contain both quinoline and thiosemicarbazone moieties (**Figure 1.5**). The thiosemicarbazone moiety can be linked to the quinoline framework at a variety of positions. These molecules have shown anti-tubercular (Patel et al., 2014), anticancer (Huang et al., 2010), antimalarial (Pingaew et al., 2010), antifungal (Abdel-Moty et al., 2005; Degola et al., 2015) and anticonvulsant (Singh et al., 2011) activity. The Cu (II) and Ni (II) complexes of quinoline-2-carboxaldehyde thiosemicarbazone derivatives were also shown to have anticancer and antifungal activity (Kulkarni et al., 2010; Bisceglie et al., 2015). 5-Acetyl (or 5 benzoyl)-8-hydroxyquinoline-4-phenyl thiosemicarbazones and 2-hydroxyquinoline-3-thiosemicarbazones were tested for the antibacterial activity, but proved to be mildy active or inactive against the bacterial strains tested against (Abdel-Moty et al., 2005; Kulkarni et al., 2010). There have been no reports on the antibacterial activity of quinoline-2-thiosemicarbazones in the literature.
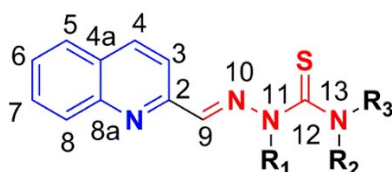
**Figure 1.5** The structure of a quinoline-thiosemicarbazone hybrid

These molecules have been prepared by a condensation reaction between quinoline-2-carboxaldehyde and thiosemicarbazides (**Scheme 1-20**) (Biscegli et al., 2015; Degola et al., 2015; Huang et al., 2010). The reaction is quick and simple and occurs by adding equimolar

quantities of the thiosemicarbazide and quinoline-2-carbaldehyde in methanol or ethanol at room temperature with a few drops of glacial acetic acid to catalyse the reaction and left to stir in an ice bath for 20 hours (Bisceglie et al., 2015; Degola et al., 2015). Variations of the synthesis involve changing the solvent to isopropanol and duration of the reaction (2-8 hours) (Huang et al., 2010).
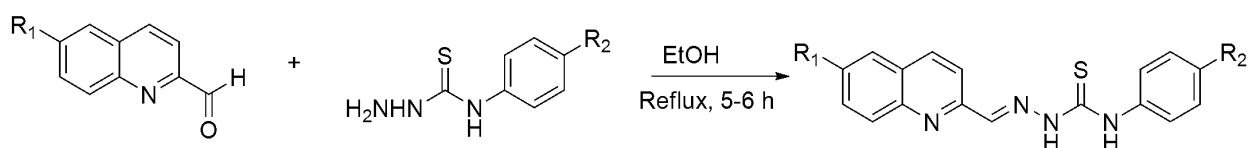
**Scheme 1-20** Condensation reaction of quinoline-2-carbaldehyde with thiosemicarbazides

A similar acid catalysed (HCl) condensation reaction between quinoline with ketone substituent at C-5 and thiosemicarbazide has also been reported (**Scheme 1-21**) (Abdel-Moty et al., 2005). In this reaction, alkanoyl chlorides were first reacted with 8-hydroxyquinoline to functionalise C-5 with a ketone from which quinoline-thiosemicarbazones were formed.
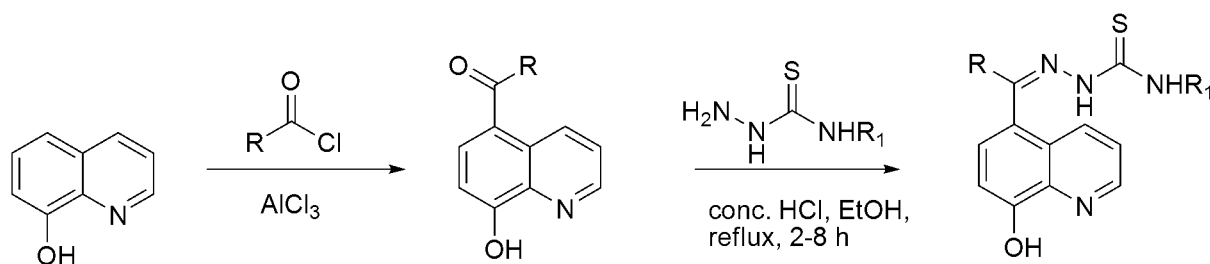
**Scheme 1-21** Synthesis of 8-hydroxyquinoline bearing a thiosemicarbazone moiety

Serda et al. (2010) used a microwave assisted reaction between quinolones and thiosemicarbazide in ethanol using polyphosphoric acid as a catalyst to synthesise quinoline-thiosemicarbazones where the thiosemicarbazone moiety was directly attached to the quinoline skeleton rather than a functional group on the ring. The quinoline substrate was first nitrated

18

in order to deactivate the aromatic quinoline system, making the amide carbonyl more reactive toward nucleophilic addition (**Scheme 1-22**).
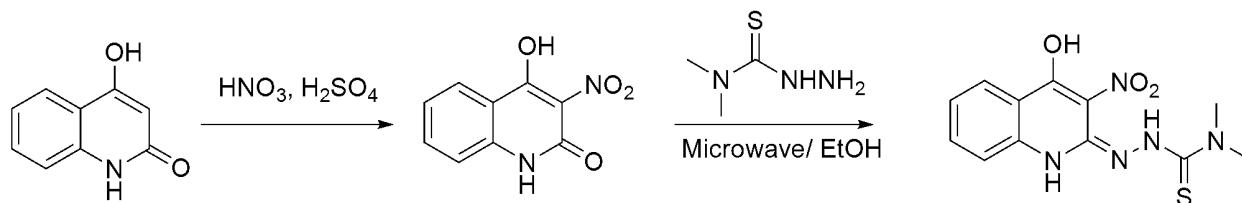
Scheme 1-22 Synthesis of quinoline-diylidene bearing a thiosemicarbazone moiety

The proposed mechanism of thiosemicarbazone formation proceeds by nucleophilic attack of the thiosemicarbazide on quinoline-2-carboxyaldehyde. This is followed by proton transfer and dehydration to form an imine (**Scheme 1-23**).
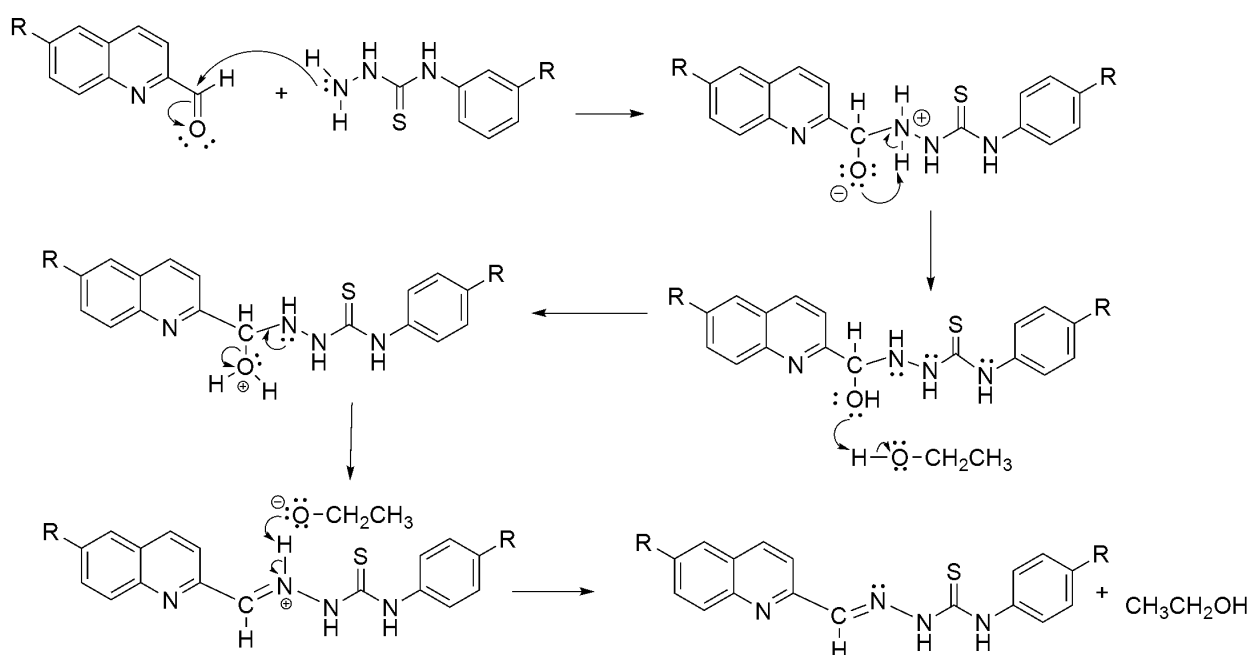
Scheme 1-23 Proposed mechanistic pathway for the synthesis of substituted quinoline-2-thiosemicarbazones

1.4   Hypothesis and aims

Hypothesis

Since both the quinoline and thiosemicarbazide moieties each have good biological activity on their own, combining these two structural motifs into one molecule may enhance the activity that each of them have on their own. Furthermore, synthesising novel molecules with each of these moieties may lead to a new drug with possible anticancer, anti-HIV, antimalarial, antitubercular and antibacterial activity.
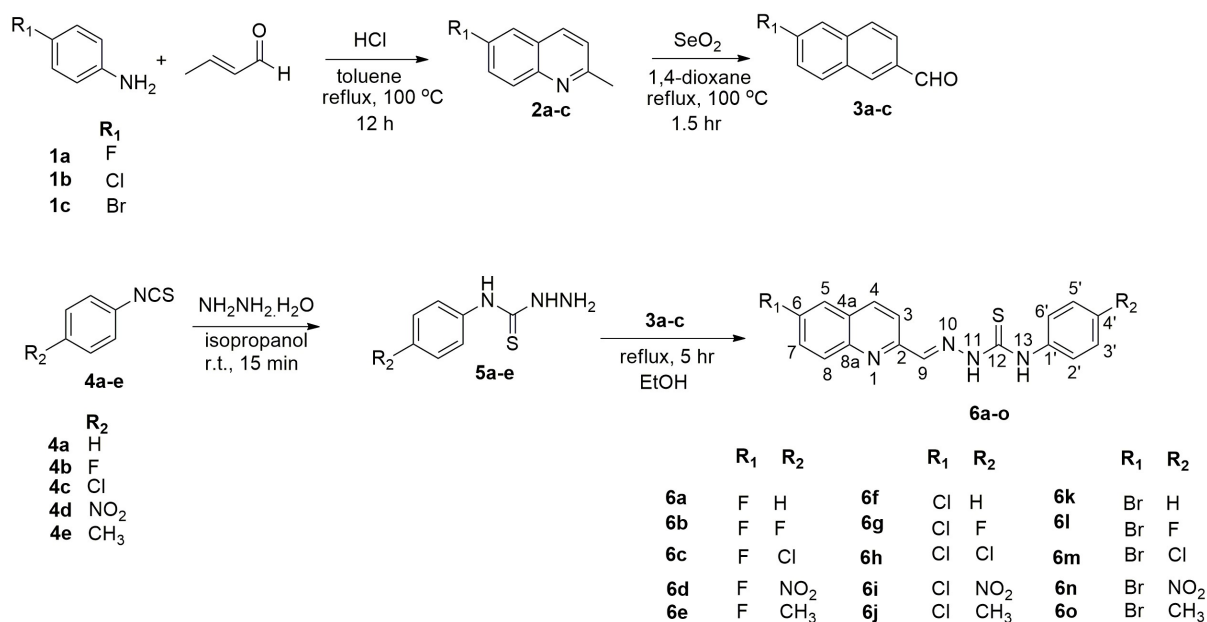
Aims

1.   To synthesise a small library of quinoline thiosemicarbazone derivatives, with varying functional groups on both the quinoline and thiosemicarbazide moieties.

2.   To characterise the synthesised compounds using NMR spectroscopy and mass spectrometry.

3.   To determine the antibacterial activity of the synthesised compounds.

4.   To conduct a structure activity relationship analysis of the synthesised compounds with regard to antibacterial activity.

Chapter 2.   Results and Discussion

2.1   Chemistry

Fifteen 2-quinoline thiosemicarbazones (**6a-o**), of which 12 were novel (**6b-e**, **g-j** and **l-o**) were synthesised in a four-step reaction (**Scheme 2-1**).   The 2-methylquinolines were first synthesised from fluoro, chloro and bromo substituted anilines (**1a-c**) and crotonaldehyde under acidic conditions using the Doebner-von Miller reaction.   The Doebner-von Miller reaction was used since this is the most popular method to synthesise 2-methylquinolines (Gopaul et al., 2015).   The resultant 2-methylquinolines were oxidised immediately without further purification to the quinoline-2-carbaldehydes (**3a-c**), which were then purified.   The thiosemicarbazide intermediates **5a-e** were formed by a nucleophilic addition reaction between hydrazine hydrate and *para* substituted isothiocynates and thereafter reacted with the carbaldehyde intermediates (**3a-c**) to form quinoline-2-thiosemicarbazone derivatives **6a-o** by a condensation reaction in yields of between 55 to 65%.


	R_1	R_2		R_1	R_2		R_1	R_2
6a	F	H	**6f**	Cl	H	**6k**	Br	H
6b	F	F	**6g**	Cl	F	**6l**	Br	F
6c	F	Cl	**6h**	Cl	Cl	**6m**	Br	Cl
6d	F	NO_2	**6i**	Cl	NO_2	**6n**	Br	NO_2
6e	F	CH_3	**6j**	Cl	CH_3	**6o**	Br	CH_3

Scheme 2-1 Reaction scheme for the synthesis of quinoline-2-carboxaldehyde
thiosemicarbazone derivatives **6a-o**

Characterisation of the 6-chloroquinoline-2-carbaldehyde intermediates (3a-c)

The intermediates were characterised by ^{1}H and ^{13}C NMR spectroscopy as well as 2D NMR spectroscopy. For example, the 6-chloroquinoline-2-carbaldehyde (**3b**) showed six proton resonances in the ^{1}H NMR spectrum, a sharp singlet at δ 10.17 assigned to the aldehyde group, and two olefinic doublets at δ 8.19 and δ 8.01 with J values of 8.5 Hz. The H-5, H-7 and H-8 resonances appeared at δ 7.86 (J = 2.2 Hz), 7.73 (J = 9.0, 2.2 Hz) and 8.16 (J = 9.0 Hz) as a doublet, double doublet and doublet respectively (**Figure 2.1**).
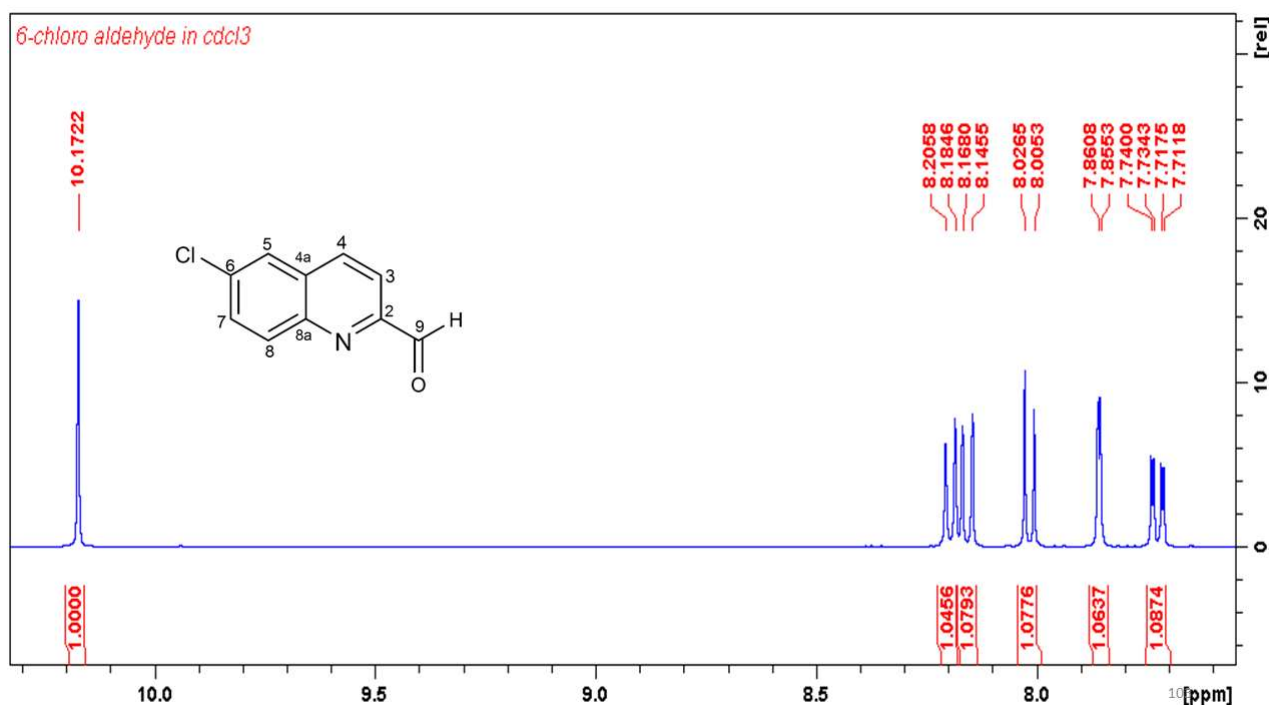
Figure 2.1 ^{1}H NMR spectrum for 6-chloroquinoline-2-carbaldehyde **3b**

The ^{13}C NMR spectrum showed the presence of ten carbon resonances, which includes the aldehyde carbon at δ 193.4 and five aromatic methine carbon resonances at δ 118.3 (C-3), 136.4 (C-4), 126.6 (C-5), 131.6 (C-7) and 132.0 (C-8). The remaining four carbon resonances were quaternary, the most deshielded being C-2 at δ 152.7, adjacent to the aldehyde and the

nitrogen atom, followed by C-8a at δ 146.3, adjacent to only the nitrogen and then followed by

C-4a and C-6 at δ 136.4 and δ 130.6 respectively (**Figure 2.2**).
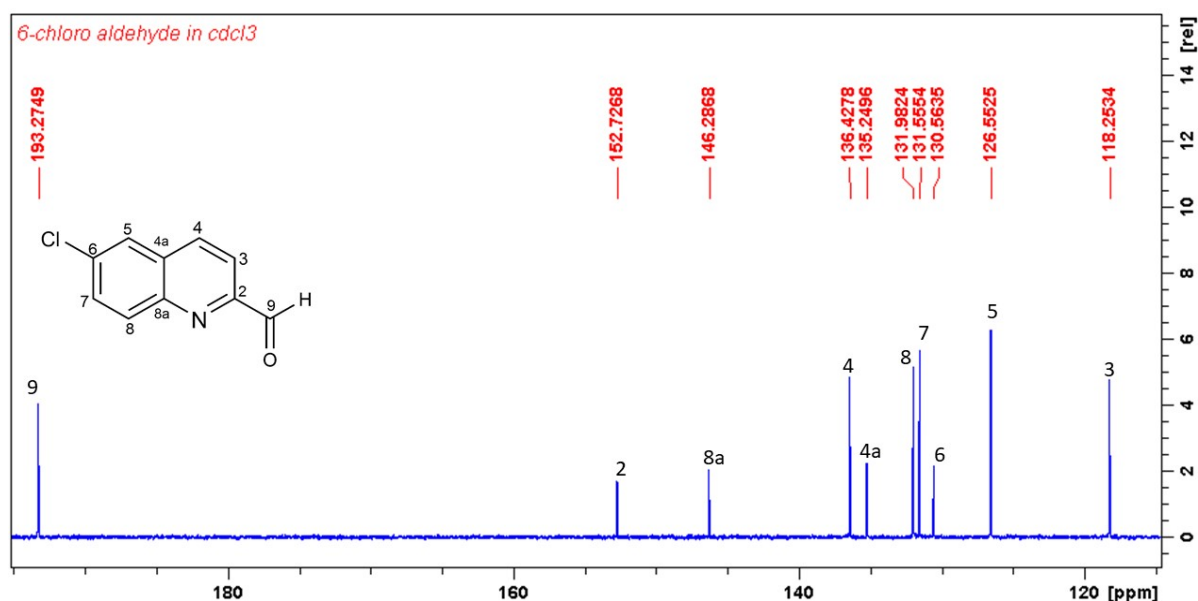
Figure 2.2 ^{13}C NMR spectrum of 6-chloroquinoline-2-carbaldehyde (**3b**)

H-5, H-7 and H-8 could be assigned by their splitting patterns and coupling constants. H-5 is

a doublet with a 2.2 Hz coupling to H-7, which appears as a double doublet with an additional

9.0 Hz coupling to H-8 appearing as a doublet. However, H-4 was assigned due to a HMBC

correlation to C-8a. Its coupled resonance was assigned to H-3. The quaternary C-4a was

assigned to δ 135.2 due to a HMBC with H-3 (**Figure 2.3**). The remaining quaternary

resonance at δ 130.6 was assigned to C-6. The other halogenated quinoline-2-carbaldehydes

were elucidated in a similar manner.
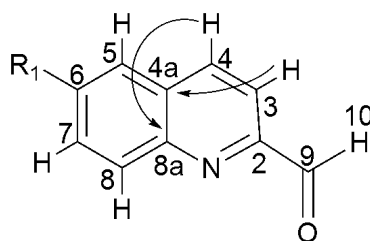
Figure 2.3 Selected HMBC correlations for **3b** used for the structural assignment

23

In **3a**, the splitting pattern of H-5, H-7 and H-8 becomes rather complex, due to the presence of the 6-fluoro group. H-5 is now a double doublet with $J_{H5,F}$ = 8.6 Hz and $J_{H5,H7}$ = 2.8 Hz, H-7 is a doublet of doublet of doublets (ddd) with $J_{H7,F}$ = 8.2 Hz, $J_{H7,H8}$ = 9.2 Hz and $J_{H5,H7}$ = 2.8 Hz and H-8 appeared as a double doublet ($J_{H7,H8}$ = 9.2 Hz and $J_{H8,F}$ = 5.0 Hz) (**Figure 2.4**). Changes in the ^{13}C NMR spectrum were also evident due to the presence of fluorine. The C-6 resonance was seen as a doublet with a large J value of 253.0 Hz at δ 162.0, the *ortho* carbon resonances were doublets with smaller coupling constants of 26.1 Hz (C-7 at δ 121.1) and 22.0 Hz (C-5 at δ 111.0) and the *meta* carbon resonances appeared at δ 133.2 and 131.0 as doublets with J values of 9.6 and 10.5 Hz respectively (**Figure 2.5**).
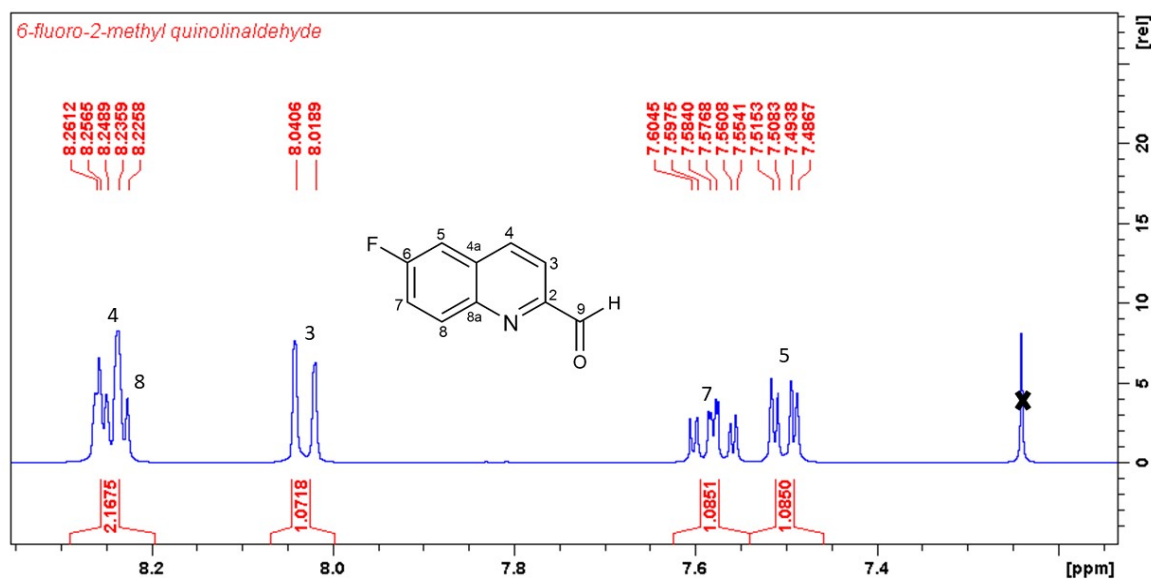
Figure 2.4 Aromatic region of the ^{1}H-NMR spectrum of compound **3a**
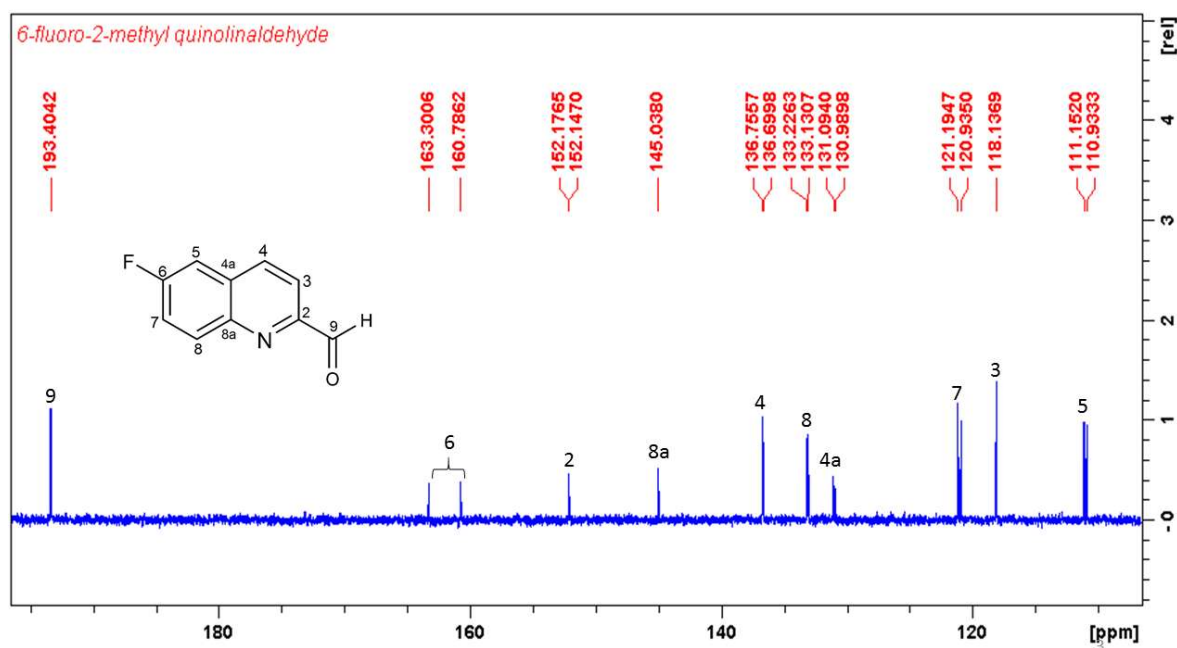
Figure 2.5 ^{13}C-NMR spectrum for compound **3a**

Since the quinolines were reaction intermediates, it was found that the yields could be maximised by using the methylquinoline reaction mixture directly (without isolating it) in the oxidation reaction with selenium dioxide as an oxidant to form the desired quinoline-2-carboxyaldehydes **3a-c**. The reaction was monitored to completion using thin layer chromatography. The yields of the substituted quinoline-2-carboxyaldehydes ranged from 55-65%. It was found that chloro and bromo substituents at C-6 on the quinoline moiety have higher oxidation yields (63-65%) than when a fluoro group is present at the same position (55%), probably due to the high electronegativity of fluorine, making the nitrogen on the quinoline ring less nucleophilic. This affects the nucleophilic addition to the selenium in the first instance. The mechanism for this reaction is shown in **Scheme 1-16** on page 12.

Synthesis and characterisation of the thiosemicarbazides 5a-e

The thiosemicarbazides **5a-e** were obtained in good yields (80 to 90%) by a straight forward nucleophilic addition reaction between hydrazine hydrate and various *para* substituted isothiocynates in isopropanol at ambient temperature. The mechanism for this reaction is shown in **Scheme 1-19** on page 14. These compounds crystallised out of solution upon completion of the reaction. Their ^{1}H NMR spectra were recorded and their structures confirmed by mass spectroscopy.

The NMR spectrum of a typical phenyl thiosemicarbazide, for example **5a** contained the aromatic resonances H-2/6 at δ 7.64 (d, *J* = 7.5 Hz), H-3/5 at δ 7.29 (t, *J* = 7.5 Hz) and H-4 at δ 7.09 (t, *J* = 7.5 Hz). The NH$_2$ proton resonance integrating to two protons appeared at δ 4.79 and H-1' and H-3', the two NH protons, appeared at δ 9.11 as a sharp singlet and 9.64 as a broad singlet. These two resonances are interchangeable. The HMBC spectrum showed no correlation between NH-1 and H-2/6, which would have allowed these two resonances to be distinguished (**Figure 2.6**).
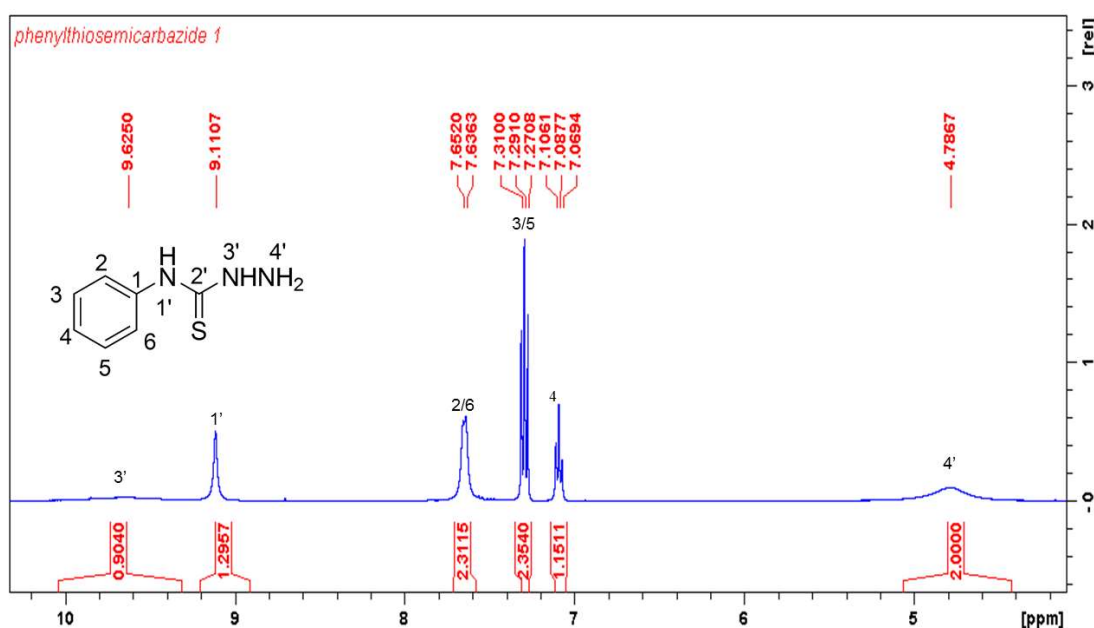
Figure 2.6 ^{1}H NMR spectrum for phenyl thiosemicarbazide **5a**

The mass spectra of all the compounds **5a-e** contained the molecular ion peak, which indicated that the hydrazide had formed. For example, in **5a**, a mass at *m/z* 168 was obtained.

The desired substituted quinoline-2-thiosemicarbazone derivatives **6a-o** were obtained via a simple condensation reaction between the quinoline aldehydes **3a-c** and the *para* substituted thiosemicarbazides **5a-e** (**Table 2-1**). The resulting quinoline-2-thiosemicarbazone derivatives were obtained in good yields (59 to 80%). Similar compounds were synthesised previously using the same condensation reaction (Biscegli et al., 2015; Huang et al., 2010), however the compounds in this work, **6a-o** differ from those synthesised previously in that they have substitution on the quinoline scaffold as well as *para* substituted phenylthiosemicarbazides used for the first time to form novel derivatives. The mechanism for this reaction is shown in **Scheme 1-23** on page 17.

Table 2-1 The yields of the synthesised quinoline-2-thiosemicarbazone derivatives

Compound 6	R_1	R_2	Melting point °C	Yield %
a	F	H	193-210	75
b	F	F	200-210	61
c	F	Cl	188-193	67
d	F	CH_3	185-195	82
e	F	NO_2	195-205	72
f	Cl	H	205-210	68
g	Cl	F	215-225	62
h	Cl	Cl	185-205	65
i	Cl	CH_3	198-205	80
j	Cl	NO_2	205-214	64
k	Br	H	190-205	70
l	Br	F	215-220	60
m	Br	Cl	205-220	66
n	Br	CH_3	212-220	76
o	Br	NO_2	208-219	59

In general, the yield for the thiosemicarbazides with electron withdrawing groups were lower than those with electron donating groups. The synthesised compounds were then characterised by NMR and mass spectroscopy.

The ^{1}H NMR spectrum of **6f** containing a chloro group at position 6, had resonances similar to both of the starting materials, **3b** and **5a**. Two of the resonances are however worth noting, H-9 and H-3. H-9 is the imine singlet proton and occurs as the result of the condensation between the aldehyde and thiosemicarbazone. This is an indication that the reaction was successful. H-3, which occurred at δ 8.01 in the carbaldehyde, now occurs more downfield at δ 8.66. This indicates that the thiosemicarbazide moiety has decreased the electron density at C-3, probably by induction after introducing all the electronegative groups present in the thiosemicarbazide moiety. The other resonance which had a slight shift was H-5, which shifted from δ 7.86 in **3b** to δ 8.12 in **6f** (**Figure 2.7**). The reason for this cannot be explained. Resonance and inductive effects were discussed, but a reasonable explanation could not be provided.

The ^{13}C NMR spectrum of **6f** contained similar resonances to that of its precursors, **3b** and **5a**. There were slight differences in the chemical shifts, however none were worth noting with the exception of the aldehyde carbon in **3b** at δ 193.3 now being absent and appearing as an imine carbon in **6f** at δ 142.6, also an indication that the reaction had occurred (**Figure 2.8**).
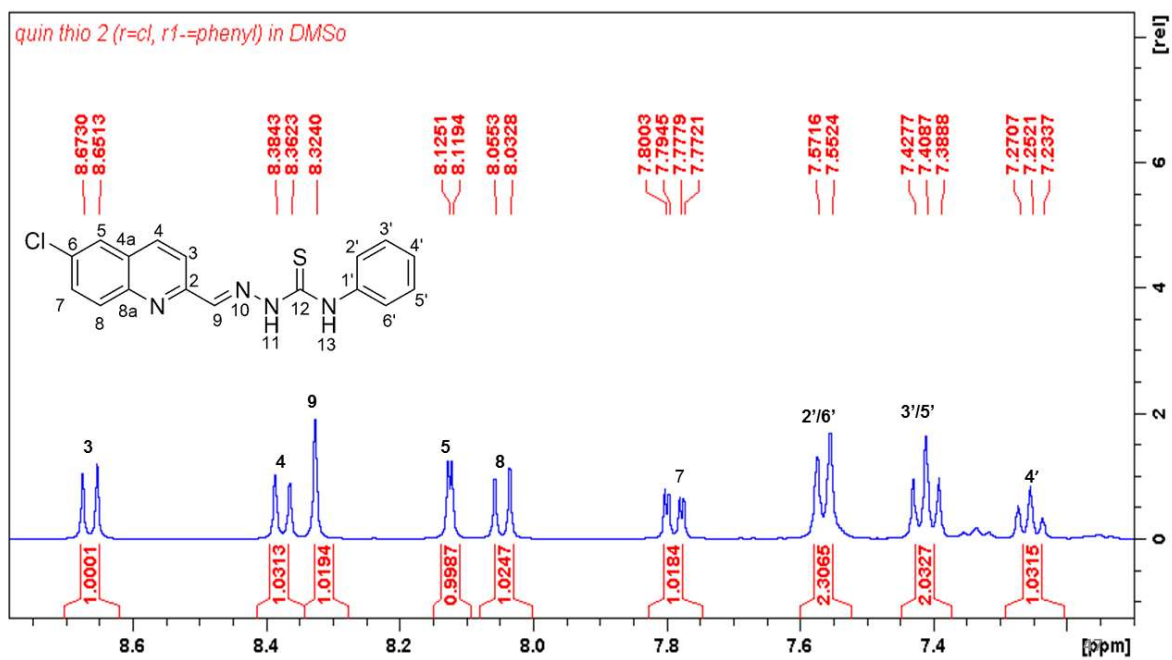
Figure 2.7 Aromatic region of the ^{1}H NMR spectrum for compound **6f**
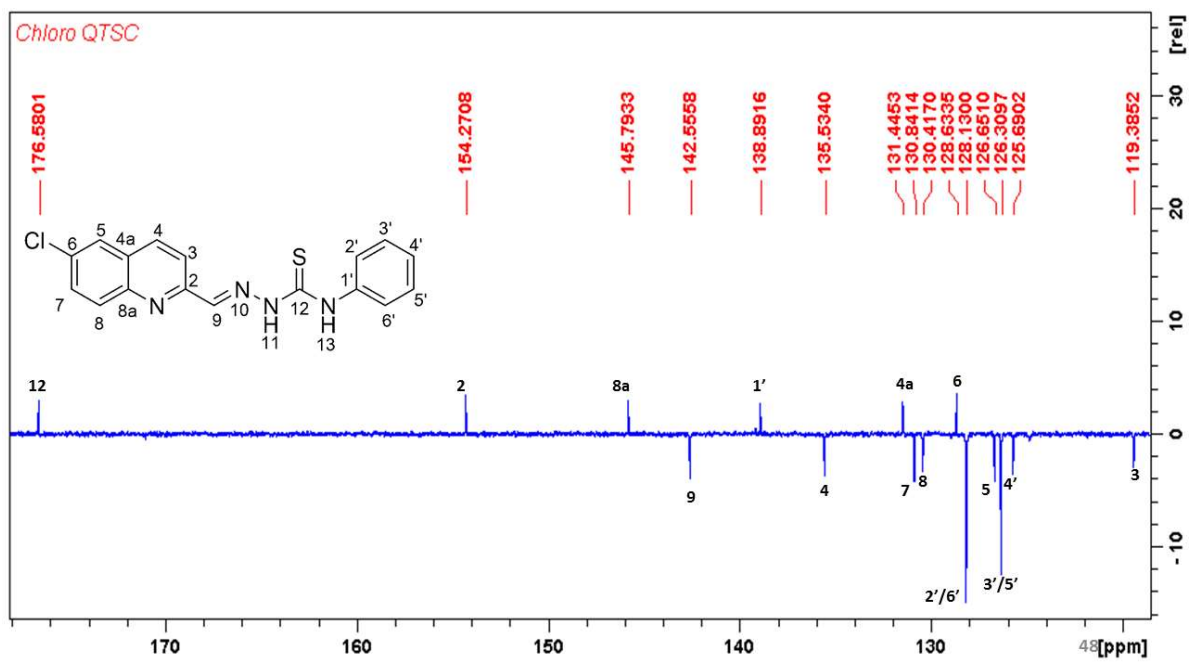
Figure 2.8 ^{13}C NMR spectrum for compound **6f**

2.2   Antibacterial activity of compounds 6a-o

The synthesised compounds were tested for their antibacterial activity against six bacterial strains, two Gram +ve strains, *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) and four Gram –ve strains, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Klebsiella pneumonia*.   These gram negative bacteria are on the WHOs priority 1 (critical) list and the *Staphylococcus* species on the priority 2 (high) list for bacterial strains where new drugs are needed (www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/). *Pseudomonas* is no. 2 on the critical list with the *Enterobacteriaceae,* such as *Escherichia*, *Salmonella* and *Klebsiella* being no. 3. *Staphylococcus aureus* and MRSA is no. 2 on the high priority list.

A disc diffusion assay was first used as a preliminary screening method to select compounds for further minimum bactericidal concentration (MBC) tests. Compounds **6c**, **6g**, **6h**, **6l** and **6o** showed activity against four or more strains (**Table 2-2**). This set contained all compounds with a *para* substituted fluoro group on the thiosemicarbazide portion of the molecule **6c**, **6g** and **6l**. These three compounds were also active against both *S. aureus* and MRSA.

Compound **6h** (with a 6-Cl, 4'-Cl substitution pattern) was active in all four of the Gram –ve bacterial strains. The activity was lost when a fluoro group replaced the chloro group at C-4' (**6g**), resulting in loss of activity with *E. coli*, however this resulted in added activity with MRSA. The compound with a bromo group at C-6 and methyl group at C-4' (**6o**) had the least activity among these five compounds, showing activity in four of the bacterial strains when the other four compounds showed activity in five of the bacterial strains.   Thus, the best lead for

antibacterial activity was fluoro substitution at the *para* position of the thiosemicarbazide moiety.

Table 2-2 Antibacterial activity of **6a-o** using the disc diffusion assay
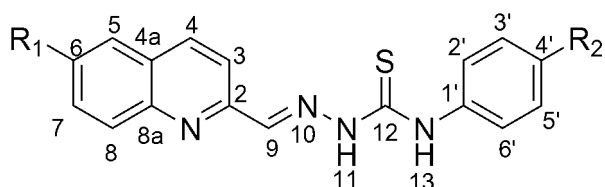
	Substituents		Gram-Positive		Gram-negative			
Compound	R$_1$	R$_2$	Sa	MRSA	Ec	Pa	St	Kp
6a	F	H	–	17	–	15	–	–
6b	F	F	–	15	–	14	–	–
6c	F	Cl	15	14	15	15	15	–
6d	F	NO$_2$	–	13	–	13	–	13
6e	F	CH$_3$	–	–	–	15	15	15
6f	Cl	H	–	15	–	15	–	–
6g	Cl	F	14	15	–	15	15	15
6h	Cl	Cl	14	–	14	16	15	15
6i	Cl	NO$_2$	–	–	–	–	–	–
6j	Cl	CH$_3$	14	15	–	–	–	15
6k	Br	H	–	15	–	–	15	–
6l	Br	F	15	15	–	13	16	14
6m	Br	Cl	–	–	–	–	14	14
6n	Br	NO$_2$	–	–	–	–	16	–
6o	Br	CH$_3$	15	–	–	16	14	15

Sa = *Staphyllococcus areus*; MRSA = Methicillin resistant *Staphyloccocus areus*; Ec = *Escherichia coli*; Ps = *Pseudomonas aeruginosa*; St = *Salmonella typhimurium*; Kp = *Klebsiella pneumonia*.

The five compounds (**6c**, **6l**, **6g**, **6h** and **6o**) showing activity in four or more bacterial strains were then diluted to different concentrations and spotted directly on agar plates containing the

different microorganisms to determine their MBC values. This method was used since the compounds were insoluble in aqueous media and precipitated out on trying the broth dilution method. Unfortunately, most of the compounds had MBC values of > 625 μM, with the exception of **6l**, containing a 6-Br substitutent and fluoro group at the *para* position of the thiosemicarbazide moiety (**Table 2-3**). Thus, the larger and more polarisable bromo group on the quinoline scaffold was essential for this antimicrobial activity as when the smaller chloro and fluoro groups occupy the same position, activity is decreased.

Table 2-3 Minimum bactericidal concentration (MBC in μM) of selected quinoline-2-thiosemicarbazone derivatives

			Gram +ve strain			Gram –ve strain		
Compound	R_1	R_2	Sa	MRSA	Ec	Pa	St	Kp
6c	F	Cl	> 625	> 625	> 625	> 625	> 625	> 625
6l	Br	F	**387**	**387**	> 625	> 625	> 625	> 625
6g	Cl	F	> 625	> 625	> 625	> 625	> 625	> 625
6h	Cl	Cl	> 625	> 625	> 625	> 625	> 625	> 625
6o	Br	CH₃	> 625	> 625	> 625	> 625	> 625	> 625
Ciprofloxacin			94.3	188.6	2.95	188.6	2.95	11.8
Levofloxacin			21.6	86.5	0.34	345	21.6	21.6

Sa = *Staphyllococcus areus*; MRSA = Methicillin resistant *Staphyloccocus areus*; Ec = *Escherichia coli*; Ps = *Pseudomonas aeruginosa*; St = *Salmonella typhimurium*; Kp = *Klebsiella pneumonia*.

It was already established from the disc diffusion assays that fluoro substitution on the thiosemicarbazide moiety resulted in antimicrobial activity. Thus, the 6-Br, 4'-F substitution pattern on these quinoline-2-thiosemicarbazides was the best substitution pattern on this particular quinoline-thiosemicarbazide scaffold for antibacterial activity against *Staphylococcus aureus* and MRSA to occur. However, the results were not comparable to the

standard antibiotics, ciprofloxacin and levofloxacin (**Table 2-3**), hence cell viability and cytotoxicity studies on **6l** were not carried out.

Our results corroborated findings by Kulkarni et al. (2010) and Abdel-Moty et al. (2005) who also found that 5-Acetyl (or 5 benzoyl)-8-hydroxyquinoline-4-phenyl thiosemicarbazones and 2-hydroxyquinoline-3-thiosemicarbazones showed weak to no activity in the bacterial strains tested against. However, 6l (6-Br, 4'-F derivative) did show interesting antibacterial activity and further research on this particular molecule, perhaps by adding other groups to the molecule using this as a basic scaffold can be determined in order to identify a compound that can be developed into a potential antibiotic.

Chapter 3.   Experimental

3.1   General Experimental Procedures

Chemicals and reagents used in this study were purchased from Sigma-Aldrich through Capital Laboratories, South Africa.   Organic solvents were redistilled according to standard procedures. TLC analysis was carried out on silica gel 60 F_{254} plates purchased from Merck South Africa. Purifications were carried out by column chromatography using silica gel (60-120 mesh) as the stationery phase and varying ratios of ethyl acetate and hexane as the mobile phase. IR spectra were recorded using a Perkin Elmer 100 FT-IR spectrometer. ^{1}H, ^{13}C and 2D NMR experiments were carried out in DMSO-d_6 using a Bruker Avance 400 MHz instrument. Tetramethylsilane (TMS) was used as the internal standard with chemical shifts reported in (ppm) and coupling constants (J) in Hz, referenced to the DMSO-d_6 solvent line (2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C). Spectra were analysed using Topspin 3.1 software (2013). High-Resolution mass spectral data (HRMS) were obtained at ambient temperature using a Bruker micro TOF-Q II ESI instrument. Melting points were carried out on a Stuart Scientific melting point apparatus SMP3 and UV data were obtained on a Shimadzu UV-3600 UV-Vis-IR spectrometer.

3.2   Synthesis

Synthesis of 6-substituted quinoline-2-aldehyde derivatives 3a-c

The respective *para* substituted anilines **1a-c** (40.0 mmol) were reacted with crotonaldehyde (60.0 mmol, 5.00 mL) in 40 mL of HCl:H_2O (1:1) under reflux at 100 °C for 12 h. The reaction was monitored by TLC. Upon completion, the mixture was basified with $NaHCO_3$ to pH 8 and NaCl added to the mixture, which was subsequently extracted with EtOAc (3 × 60 mL). The

mixture was then dried over anhydrous Na_2SO_4 and concentrated using a rotary evaporator to yield the crude 2-methylquinoline intermediates **2a-c** as brown gummy residues. These intermediates were not purified, but were oxidized directly using SeO_2 (5.00 g, 40.0 mmol) in 15 mL of 1,4-dioxane. Selenium dioxide was added gradually over a period of 5 min and the reaction left to stir under reflux at 110 °C for 1.5 h to produce the 6-substituted quinoline-2-aldehydes **3a-c** (**Scheme 3-1**). The reaction was monitored by TLC until completion. Once cooled, the reaction mixture was filtered through celite, diluted with water and extracted with ethyl acetate. The organic extract was dried over anhydrous $MgSO_4$, concentrated and subjected to column chromatography on silica gel using a mobile phase of hexane:ethyl acetate (95:5) in yields of between 55 to 65%.
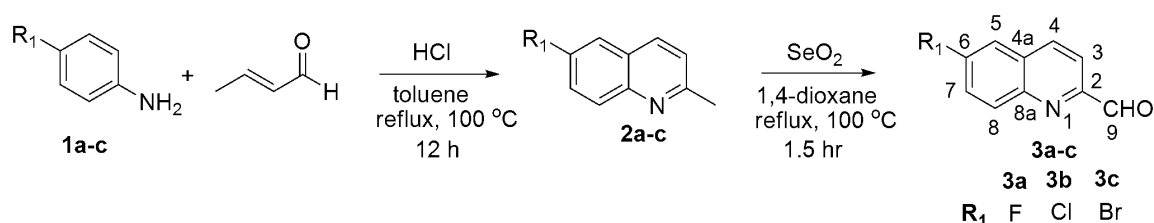
Scheme 3-1 Reactions for the synthesis of 6-substituted quinoline-2-aldehydes **3a-c**

6-fluoroquinoline-2-carbaldehyde (3a); Orange solid residue; 55% yield; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.18 (s, H-9), 8.25 (1H, d, J = 8.5 Hz, H-4), 8.24 (1H, dd, J = 9.2, 5.0 Hz, H-8), 8.03 (1H, d, J = 8.5 Hz, H-3), 7.58 (1H, ddd, J = 9.2, 8.2, 2.8 Hz, H-7), 7.50 (1H, dd, J = 8.6, 2.8 Hz, H-5); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 193.4 (C-9), 162.0 (d, J_{CF} = 253.0 Hz, C-6), 152.2 (d, J_{CF} = 2.9 Hz, C-2), 145.0 (C-8a), 136.7 (d, J_{CF} = 5.6 Hz, C-4), 133.2 (d, J_{CF} = 9.6 Hz, C-8), 131.0 (d, J_{CF} = 10.5 Hz, C-4a), 121.1 (d, J_{CF} = 26.1 Hz, C-7), 118.1 (C-3), 111.0 (d, J_{CF} = 22.0 Hz, C-5).
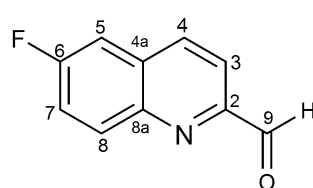
6-chloroquinoline-2-carbaldehyde (3b); Cream white; 65% yield; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.17 (s, H-9), 8.20 (1H, d, J = 8.5 Hz, H-4), 8.16 (1H, d, J = 9.0 Hz, H-8), 8.01 (1H, d, J = 8.5 Hz, H-3), 7.86 (1H, d, J = 2.2 Hz, H-5), 7.73 (1H, dd, J = 9.0, 2.2 Hz, H-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 193.3 (C-9), 152.7 (C-2), 146.3 (C-8a), 136.4 (C-4), 135.2 (C-4a), 132.0 (C-8), 131.6 (C-7), 130.6 (C-6), 126.6 (C-5), 118.3 (C-3).
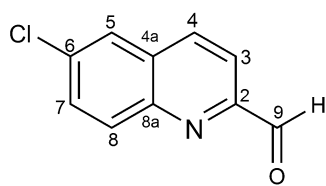
6-Bromoquinoline-2-carbaldehyde (3c); light brown solid residue; 63% yield; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.18 (s, H-9), 8.20 (1H, d, J = 8.5 Hz, H-4), 8.10 (1H, d, J = 9.0 Hz, H-8), 8.04 (1H, d, J = 2.1 Hz, H-5), 8.02 (1H, d, J = 8.5 Hz, H-3), 7.86 (1H, dd, J = 9.0, 2.3 Hz, H-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 193.3 (C-9), 152.8 (C-2), 146.5 (C-8a), 136.4 (C-4), 134.1 (C-8), 132.0 (C-7), 131.0 (C-4a), 130.0 (C-5), 123.6 (C-6), 118.3 (C-3).
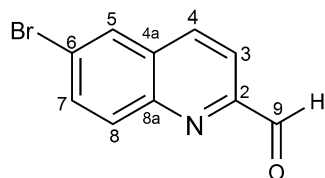
Synthesis of *para* substituted phenylisothiosemicarbazides 5a-e

Para substituted phenylisocynates **4a-e** (6.70 mmol) were dissolved in isopropanol (10 mL), to which hydrazine hydrate (0.66 mL, 13.40 mmol) was added and the mixture stirred for 15 min at room temperature and then placed in an ice-bath for 30 min. The crystals that formed were filtered and washed with isopropanol to yield the phenylisothiosemicarbazides **5a-e** in yields of between 82 and 90% (**Scheme 3-2**).
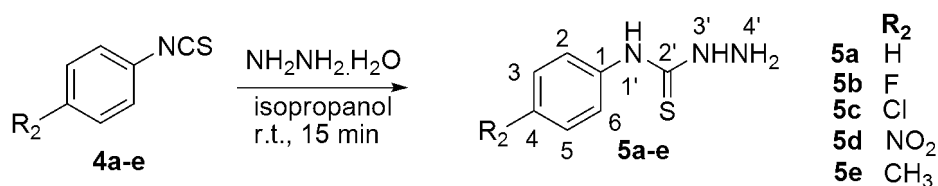
Scheme 3-2 The reaction of phenylisocyanates with hydrazine hydrate to produce 4-substituted thiosemicarbazides **5a-e**

N-phenylhydrazinecarbothioamide (5a); white solid residue: 90% yield: ^{1}H NMR (DMSO-*d6*,
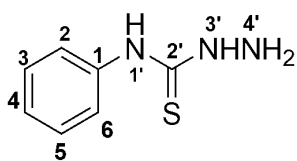
 400 MHz) δ 9.64 (s, 3'-NH), 9.11 (s, 1'-NH) 7.64 (2H, bd, *J* = 8.1 Hz, H-2/6), 7.29 (2H, dd, *J* = 8.1, 7.3 Hz, H-3/5), 7.09 (1H, t, *J* = 7.3 Hz, H-4), 4.79 (s, 4'-NH).

N-(4-fluorophenyl) hydrazinecarbothioamide (5b); white solid residue: 86% yield: ^{1}H NMR
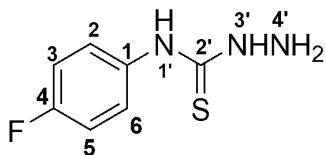
 (DMSO-*d6*, 400 MHz) δ 9.60 (s, 3'-NH), 9.11 (s, 1'-NH), 7.60 (bs, H-2/6), 7.12 (2H, t, *J* = 8.9 Hz, H-3/5), 4.76 (s, 4'-NH).

N-(4-chlorophenyl) hydrazinecarbothioamide (5c); white solid residue: 84% yield: ^{1}H NMR
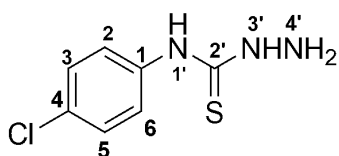
 (DMSO-*d6*, 400 MHz) δ 9.19 (s, 1'-NH), 7.68 (2H, bd, *J* = 8.7 Hz, H-2/6), 7.33 (2H, d, *J* = 8.7 Hz, H-3/5), 4.81 (s, 3'-NH).

N-(4-nitrophenyl) hydrazinecarbothioamide (5d); yellow solid residue: 80% yield: ^{1}H NMR
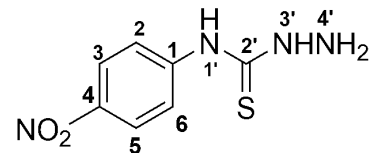
 (DMSO-*d6*, 400 MHz) δ 9.35 (s, 3'-NH), 8.13 (2H, d, *J* = 9.2 Hz, H-3/5), 7.80 (3H, bs, H-2/6, 1'-N-H), 4.45 (s, 4'-NH).

N-p-tolylhydrazinecarbothioamide (5e); yellow solid residue: 88% yield: ^{1}H NMR (DMSO-*d6*,
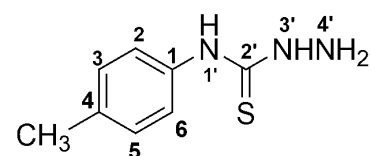
 400 MHz) δ 9.75 (s, 3'-NH), 7.41 (2H, d, *J* = 8.2 Hz, H-2/6), 7.10 (2H, d, *J* = 8.2 Hz, H-7/9), 2.26 (s, CH₃).

Synthesis of quinoline-2-carboxyaldehyde thiosemicarbazone derivatives 6a-o

Phenylthiosemicarbazides **5a-e** (0.482 mmol) were dissolved in ethanol by heating slightly while stirring in an oil bath. The substituted quinoline aldehydes **3a-c** (0.583 mmol) were then added separately to each of the dissolved phenylthiosemicarbazides (**5a-e**) and the resulting

mixture refluxed for 5 h at 110 °C. The reaction mixture was then placed in an ice bath for 30 min, where the product crystallised out and was filtered to yield the products **6a-o** in yields of between 59 and 80% (**Scheme 3-3**).
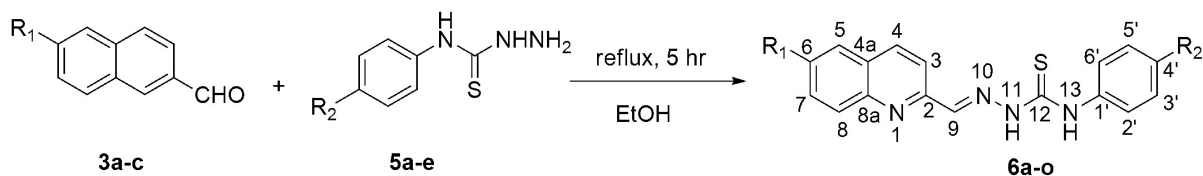
Scheme 3-3 The reaction of quinoline-2-carbaldehydes and thiosemicarbazides to form quinoline-2-carboxaldehyde thiosemicarbazone derivatives **6a-o**

(E)-2-((6-fluoroquinolin-2-yl)methylene)-N-phenylhydrazine carbothioamide (6a); light yellow solid: 75% yield: mp 193-210 °C: UV λ_{max} (EtOAc) nm (log ε) 250 (4.24), 287 (4.15), 326 (4.13), 341 (4.16), 355 (4.20); IR (KBr) υ_{max}: 3317 (N-H), 3105 (C-H), 2939 (C-H), 1542 (C=C), 1498 (C=C), 1181 (C-F) cm^{-1}; ^{1}H NMR (DMSO-$_{d6}$, 400 MHz) δ 12.20 (s, 11-NH), 10.38 (s, 13-NH), 8.65 (1H, d, J = 8.8 Hz, H-3), 8.38 (1H, d, J = 8.8 Hz, H-4), 8.33 (s, H-9), 8.10 (1H, dd, J = 9.2, 5.5 Hz, H-8), 7.80 (1H, dd, J = 9.3, 2.8 Hz, H-5), 7.70 (1H, ddd, J = 9.2, 8.9, 2.8 Hz, H-7), 7.56 (2H, d, J = 7.8 Hz, H-2'/6') , 7.41 (2H, t, J = 7.8 Hz, H-3'/5'), 7.25 (1H, t, J = 7.8 Hz, H-4'); ^{13}C NMR (DMSO-$_{d6}$, 100 MHz) δ 176.6 (C-12), 160.0 (d, J_{CF} = 245.2 Hz, C-6), 153.4 (d, J_{CF} = 2.5 Hz, C-2), 144.5 (C-8a), 142.7 (C-9), 138.9 (C-1'), 135.8 (d, J_{CF} = 5.0 Hz, C-4), 131.6 (d, J_{CF} = 9.4 Hz, C-8), 128.7 (d, J_{CF} = 10.3 Hz, C-4a), 128.1 (C-2'/6'), 126.3 (C-3'/5'), 125.7 (C-4'), 119.9 (d, J = 25.8 Hz, C-7), 119.2 (C-3), 111.2 (d, J = 21.9 Hz, C-5).

(E)-N-(4-fluorophenyl)-2-((6-fluoroquinolin-2-yl)methylene)hydrazine carbothioamide(6b);
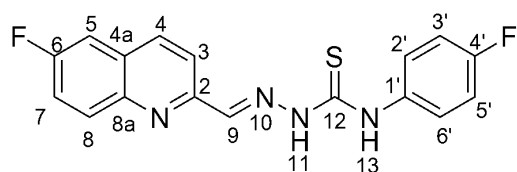
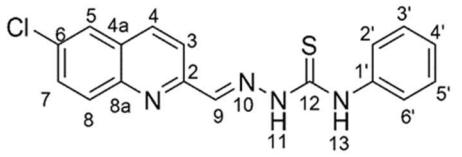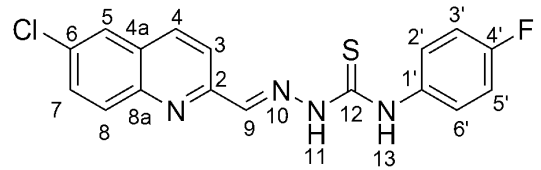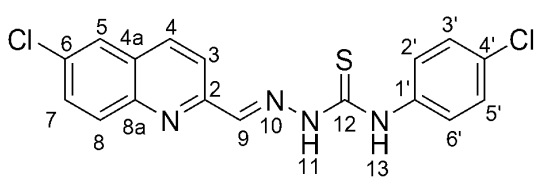yellow solid residue: 78% yield: mp 200-210 °C: UV λ_{max} (Ethylacetate)nm (log ε) 250 (4.24), 326 (4.31), 340 (4.35), 354 (4.37); IR (KBr) υ_{max}: 3302 (N-H), 3117 (C-H), 2977 (C-H), 1504 (C=C), 1176 (C-F) cm^{-1} ;^{1}H NMR (DMSO-$_{d6}$, 400 MHz) δ 12.22 (s, 11-NH), 10.37 (s, 13-NH), 8.64 (1H, d, J = 8.8 Hz, H-3), 8.39 (1H, d, J = 8.8 Hz, H-4), 8.32 (s, H-9), 8.10 (1H, dd, J = 8.8, 5.5, Hz, H-8), 7.80(1H, dd, J = 9.3, 2.8 Hz, H-5), 7.70 (1H, ddd, J = 8.8, 8.8, 2.9 Hz, H-7), 7.55 (2H, dd, J = 8.9 ,5.1 Hz, H-2'/6'), 7.24 (2H, dd, J = 8.8, 8.8 Hz, H-3'/5'); ^{13}C-NMR(DMSO-$_{d6}$, 100 MHz) δ 176.9 (C-12), 160.1 (d, J_{CF} = 245.1 Hz, C-6), 159.9 (d, J_{CF} = 241.3 Hz, C-4'), 153.4 (d, J_{CF} = 2.7 Hz, C-2), 144.5 (C-8a), 142.9 (C-9), 135.8 (d, J_{CF} = 5.1 Hz, C-4), 135.2 (C-1'), 131.6 (d, J_{CF} = 9.3 Hz, C-8), 128.7 (d, J_{CF} = 10.6 Hz, C-4a), 128.5 (d, J = 8.4 Hz, C-2'/6'), 119.9 (d, J_{CF} = 25.5 Hz, C-7), 119.1 (C-3), 114.8 (d, J = 22.3 Hz, C-3'/5'), 111.2 (d, J = 21.7 Hz, C-5); HRMS(pos) (*m/z*): calculated for $C_{17}H_{12}F_2N_4S$ (M + H)$^+$: 341.0672, found: 341.0680.

(E)-N-(4-chlorophenyl)-2-((6-fluoroquinolin-2-yl)methylene)hydrazine carbothioamide (6c);
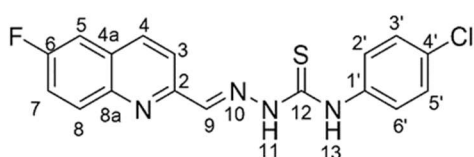
yellow solid residue: 67% yield: mp 188-193 °C: UV λ_{max} (Ethylacetate)nm (log ε) 249 (4.29), 326 (4.39), 340 (4.33), 354 (4.44); IR (KBr) υ_{max}: 3303 (N-H), 3118 (C-H), 2972 (C-H), 1503 (C=C), 1177 (C-F) cm^{-1}; ^{1}H NMR (DMSO-$_{d6}$, 400 MHz) δ 12.27 (s, 11-NH), 10.40 (s, 13-NH), 8.63 (1H, d, J = 8.8 Hz, H-3), 8.39 (1H, d, J = 8.8 Hz, H-4), 8.33 (s, H-9), 8.10 (1H, dd, J = 8.8, 5.5, Hz, H-8), 7.80 (1H, dd, J = 9.3, 2.8 Hz, H-5), 7.70 (1H, ddd, J = 8.8, 8.8, 2.9 Hz, H-7), 7.61 (1H, dd, J = 8.9 ,5.1 Hz, H-2'/6') , 7.46 (1H, dd, J = 8.8, 8.8 Hz, H-3'/5'); ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ 176.6 (C-12), 160.1 (d, J_{CF} = 245.1 Hz, C-6), 153.3 (C-2), 144.5 (C-8a), 143.1 (C-9), 137.9 (C-1'), 135.8 (d, J_{CF} = 4.9 Hz, C-4), 131.7 (d, J_{CF} = 9.3 Hz, C-8), 129.7 (C-4'), 128.7 (d, J_{CF} =

10.6 Hz, C-4a), 128.0 (C-3'/5'), 127.9 (C-2'/6'), 119.9 (d, J_{CF} = 25.6 Hz, C-7), 119.2 (C-3), 111.2 (d, J_{CF} = 22.0 Hz, C-5); HRMS(pos) (*m/z*): calculated for: $C_{17}H_{12}ClFN_4S$ (M-H)$^+$: 358.05, found: 357.081.

(E)-2-((6-fluoroquinolin-2-yl)methylene)-N-(4-nitrophenyl)hydrazine carbothioamide (6d); yellow solid residue: 82% yield: mp 200-210 °C:
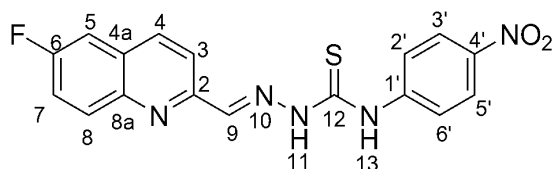
UV λ_{max} (Ethylacetate) nm (log ε) 249 (4.30), 330 (4.67), 344 (4.62); IR υ_{max} 3359 (N-H), 3083 (C-H), 2927 (C-H), 1702*, 1596 (C=C), 1529, 1499, 1485, 1322, 1299, 1222, 1153, 1106 (C-F) cm^{-1}; ^{1}H-NMR(DMSO-*d6*, 400 MHz) δ 11.49 (s, 11-NH), 9.71 (s, 13-NH), 8.56 (1H, d, *J* = 8.8 Hz, H-3), 8.42 (1H, d, *J* = 8.8 Hz, H-4), 8.24 (d, *J* = 9.3 Hz, H-3'/5'), 8.17 (s, H-9), 8.08 (1H, dd, *J* = 9.1, 5.4 Hz, H-8), 8.01 (d, *J* = 9.3, Hz, H-2'/6'), 7.82 (1H, dd, *J* = 9.4, 2.9 Hz, H-5) , 7.69 (1H, ddd, *J* = 9.1, 8.9, 2.9 Hz, H-7); ^{13}C-NMR (DMSO-*d6*, 100 MHz) δ 160.0 (d, J_{CF} = 244.7 Hz, C-6), 153.2 (d, J_{CF} = 2.8 Hz, C-2), 152.4 (C-12), 145.6 (C-4'), 144.5 (C-8a), 142.3 (C-9), 141.7 (C-1'), 135.8 (d, J_{CF} = 5.0 Hz, C-4), 131.6 (d, J_{CF} = 9.4 Hz, C-8), 128.6 (d, J_{CF} = 10.4 Hz, C-4a), 124.7 (C-2'/6'), 119.9 (d, J_{CF} = 25.6 Hz, C-7), 119.2 (C-3'/5'), 118.9 (C-3), 111.2 (d, J_{CF} = 22.0 Hz, C-5). *These absorption bands only appear for the nitro substituted compounds, however the frequency is higher than usual for a nitro stretch, typically occurring at 1535-1600 cm^{-1}.

(E)-2-((6-fluoroquinolin-2-yl)methylene)-N-p-tolylhydrazine carbothioamide(6e); yellow solid residue: 72% yield: mp 186-195 °C: UV λ_{max} (Ethylacetate)nm (log ε) 250 (4.27), 326 (4.38), 340 (4.42), 354 (4.44); IR υ_{max} 3300 (N-H), 3113 (C-H), 2962 (C-H), 1509 (C=C), 1178 (C-F) cm^{-1}; ^{1}H-NMR (DMSO-*d6*, 400 MHz) δ 12.14 (s, 11-NH), 10.31 (s, 13-NH), 8.65 (1H, d, *J* = 8.8 Hz, H-3), 8.38 (1H, d, *J* = 8.8 Hz, H-4), 8.31 (s, H-9), 8.10 (1H,dd, *J* = 9.1, 4.9, Hz,
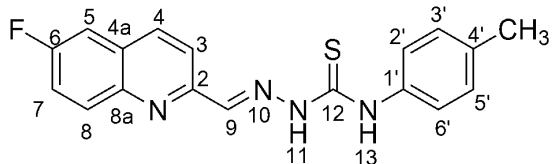
H-8), 7.80 (1H, dd, J = 9.3, 2.8 Hz, H-5), 7.70 (1H, ddd, J = 9.1, 8.9, 2.9 Hz, H-7), 7.41 (1H, d, J = 8.3, Hz, H-2'/6'), 7.20 (1H,d, J = 8.2 Hz, H-3'/5'); ^{13}C-NMR (DMSO-$d6$, 100 MHz) δ 176.6 (C-12), 160.0 (d, J_{CF} = 246.5 Hz, C-6), 153.4 (d, J_{CF} = 2.7 Hz, C-2), 144.5 (C-8a), 142.6 (C-9), 136.3 (C-4'), 135.8 (d, J_{CF} = 5.1 Hz, C-4), 134.8 (C-1'), 131.6 (d, J_{CF} = 9.3 Hz, C-8), 128.7 (C-4a), 128.6 (C-2'/6'), 126.2 (C-3'/5'), 119.9 (d, J_{CF} = 25.9 Hz, C-7), 119.2 (C-3), 111.2 (d, J = 21.7 Hz, C-5), 20.6 (C-7'); HRMS (m/z): calculated for: $C_{18}H_{15}FN_4S$ (M + Na)$^+$: 338.10, found: 361.0901.

(E)-2-((6-chloroquinolin-2-yl)methylene)-N-phenylhydrazine carbothioamide (**6f**); light

yellow solid residue: 62% yield: mp 200-210 °C: UV λ_{max} (Ethylacetate)nm (log ε) 250 (4.19), 270 (4.10), 330 (4.16), 344 (4.22), 359 (4.25); IR υ_{max} 3313 (N-H), 3110 (C-H), 2962 (C-H), 1532 (C=C), 1505, 1487, 1250, 1186 (C-F) cm^{-1};^{1}H-NMR (DMSO-$d6$, 400 MHz) δ 12.22 (s, 11-NH), 10.39 (s, 13-NH), 8.66 (1H, d, J = 8.8 Hz, H-3), 8.37 (1H, d, J = 8.8 Hz, H-4), 8.32 (s, H-9), 8.12 (1H,d, J = 2.3, H-5), 8.04 (1H, d, J = 9.0 Hz, H-8), 7.79 (1H, dd, J = 9.0, 2.3 Hz, H-7), 7.56 (2H, d, J = 7.7 Hz, H-2'/6'), 7.41 (2H, t, J = 7.8 Hz, H-3'/5'), 7.25 (1H, t, J = 7.4 Hz, H-4'); ^{13}C NMR (DMSO-$d6$, 100 MHz) δ 176.6 (C-12), 154.3 (C-2), 145.8 (C-8a), 142.6 (C-9), 138.9 (C-1'), 135.5 (C-4), 131.4 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.6 (C-6), 128.1 (C-2'/6'), 126.7 (C-5), 126.3 (C-3'/5'), 125.7 (C-4'), 119.4 (C-3).

(E)-2-((6-chloroquinolin-2-yl)methylene)-N-(4-fluorophenyl)hydrazine carbothioamide (**6g**);
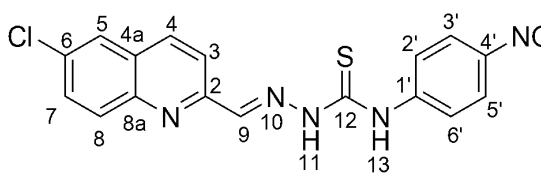
yellow solid residue: 65% yield: mp 215-225°C: UV λ_{max}(Ethylacetate) nm (log ε) 250 (4.42), 295 (4.33), 384 (4.31); IR υ_{max} 3301 (N-H), 3055 (C-H), 1511 (C=C), 1492, 1171 (C-F), 1067 cm^{-1};^{1}H NMR (DMSO-$d6$, 400 MHz) δ 12.24 (s, 11-NH), 10.38 (s, 13-NH), 8.65 (1H, d, J = 8.8

Hz, H-3), 8.38 (1H, d, *J* = 8.8 Hz, H-4), 8.32 (s, H-9), 8.12 (1H, d, *J* = 2.4 Hz, H-5), 8.04 (1H, d, *J* = 9.0 Hz, H-8), 7.79 (1H, dd, *J* = 9.0, 2.4 Hz, H-7), 7.55 (1H, dd, *J* = 8.9 ,5.1 Hz, H-2'/6') , 7.24 (1H, t, *J* = 8.8 Hz, H-3'/5'); ^{13}C NMR (DMSO-*d6*, 100 MHz) δ 176.9 (C-12), 159.9 (d, J_{CF} = 242.5 Hz, C-4'), 154.2 (C-2), 145.8 (C-8a), 142.7 (C-9), 135.5 (C-4), 135.2 (d, J_{CF} = 2.6 Hz, C-1'), 131.5 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.6 (C-6), 128.5 (d, J_{CF} = 8.3 Hz, C-2'/6'), 126.7 (C-5), 119.4 (C-3), 114.8 (d, J_{CF} = 22.4 Hz, C-3'/5'); HRMS (pos) (*m/z*): calculated for $C_{17}H_{12}ClFN_4S$ (M + H)$^+$: 358.05, found: 358.0416.

(E)-N-(4-chlorophenyl)-2-((6-chloroquinolin-2-yl)methylene)hydrazine carbothioamide (6h);

yellow solid residue: 80% yield: mp 185-205°C: UV λ$_{max}$ (Ethylacetate) nm (log ε) 250 (4.32), 330 (4.44), 343 (4.50), 359 (4.53); IR υ$_{max}$ 3316 (N-H), 3133 (C-H), 2989 (C-H), 1588 (C=C), 1536, 1509, 1488, 1190 (C-F), 1086 cm^{-1};^{1}H-NMR (DMSO-*d6*, 400 MHz) δ 12.29 (s, 11-NH), 10.41 (s, 13-NH), 8.64 (1H, d, *J* = 8.8 Hz, H-3), 8.38 (1H, d, *J* = 8.8 Hz, H-4), 8.32 (s, H-9), 8.12 (1H, d, *J* = 2.4 Hz, H-5), 8.04 (1H, d, *J* = 9.0 Hz, H-8), 7.79 (1H, dd, *J* = 8.5, 2.3 Hz, H-7), 7.60 (1H, d, *J* = 8.7 Hz, H-2'/6'), 7.46 (1H, d, *J* = 8.7 Hz, H-3'/5'); ^{13}C-NMR (DMSO-*d6*, 100 MHz) δ 176.6 (C-12), 154.2 (C-2), 145.8 (C-8a), 142.9 (C-9), 137.9 (C-1'), 135.6 (C-4), 131.5 (C-4a), 130.8 (C-7), 130.4 (C-8), 129.7 (C-4'), 128.6 (C-6), 128.0 (C-2'/6'), 127.9 (C-3'/5'), 126.7 (C-5), 119.4 (C-3); HRMS (pos) (*m/z*): calculated for :$C_{17}H_{12}Cl_2N_4S$ (M + H)$^+$: 374.02, found: 374.0122.

(E)-2-((6-chloroquinolin-2-yl)methylene)-N-(4-nitrophenyl)hydrazine carbothioamide (6i);
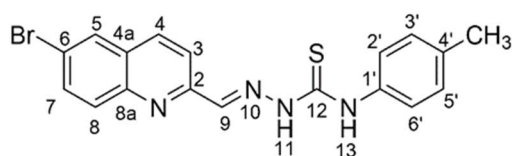

yellow solid residue: 64% yield: mp 198-215 °C: UV λ$_{max}$ (Ethylacetate) nm (log ε) 249 (4.47), 321 (4.58), 334 (4.61), 349 (4.55); IR υ$_{max}$ 3364 (N-H), 3072 (C-H), 2921 (C-H), 1711*,

1598 (C=C), 1539, 1504, 1491, 1332, 1302, 1223, 1144, 1110 (C-F) cm^{-1};^{1}H NMR (DMSO-$_{d6}$, 400 MHz) δ 11.52 (s, 11-NH), 9.71 (s, 13-NH), 8.57 (1H, d, J = 8.8 Hz, H-3), 8.42 (1H, d, J = 8.8 Hz, H-4), 8.24 (1H, d, J = 9.3 Hz, H-3'/5'), 8.17 (s, H-9), 8.14 (1H, d, J = 2.4 Hz, H-5), 8.03 (1H, d, J = 8.8 Hz, H-8), 8.02 (1H, d, J = 9.3 Hz, H-2'/6') , 7.79 (1H, dd, J = 9.0, 2.4 Hz, H-7); ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ 154.1 (C-12), 152.2 (C-4'), 145.7 (C-2), 145.5 (C-8a), 142.1 (C-9), 141.7 (C-1'), 135.6 (C-4), 131.4 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.5 (C-6), 126.7 (C-5), 124.7 (C-3'/5'), 119.2 (C-2'/6'), 119.1 (C-3). *These absorption bands only appear for the nitro substituted compounds, however the frequency is higher than usual for a nitro stretch, typically occurring at 1535-1600 cm^{-1}.

(E)-2-((6-chloroquinolin-2-yl)methylene)-N-p-tolylhydrazine carbothioamide (6j); yellow solid residue: 80% yield: mp 198-205 °C: UV
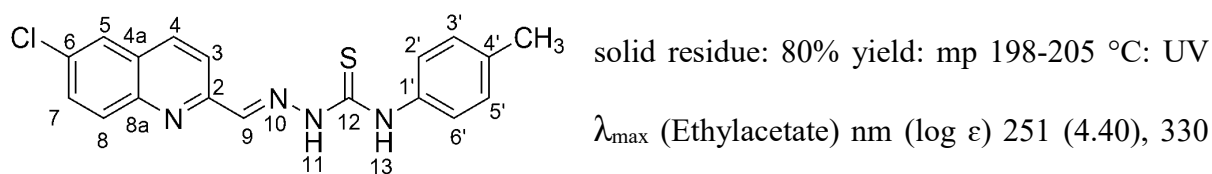
λ$_{max}$ (Ethylacetate) nm (log ε) 251 (4.40), 330 (4.26), 360 (4.37); IR υ$_{max}$ 3348 (N-H), 3308, 3114 (C-H), 2964 (C-H), 1513 (C=C), 1250, 1177 (C-F) cm^{-1} ;^{1}H NMR (DMSO-$_{d6}$, 400 MHz) δ 12.17 (s, 11-NH), 10.32 (s, 13-NH), 8.66 (1H, d, J = 8.8 Hz, H-3), 8.37 (1H, d, J = 8.8 Hz, H-4), 8.31 (s, H-9), 8.12 (1H, d, J = 2.4 Hz, H-5), 8.04 (1H, d, J = 9.0 Hz, H-8), 7.79 (1H, dd, J = 9.0, 2.4 Hz, H-7), 7.41 (1H, d, J = 8.3 Hz, H-2'/6'), 7.20 (1H, d, J = 8.3 Hz, H-3'/5'); ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ 176.6 (C-12), 154.3 (C-2), 145.8 (C-8a), 142.4 (C-9), 136.3 (C-1'), 135.5 (C-4), 134.9 (C-4'), 131.4 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.6 (C-6), 128.0 (C-3'/5'), 126.6 (C-5), 126.2 (C-2'/6'), 119.4 (C-3), 20.6 (C-7'); HRMS (pos) (*m/z*): calculated for C$_{17}$H$_{12}$F$_2$N$_4$S (M + Na)$^+$: **354.07,** found: 377.0609.

(E)-2-((6-bromoquinolin-2-yl)methylene)-N-phenylhydrazine carbothioamide(**6k**); light yellow solid residue: 70% yield: mp 190-205 °C: UV
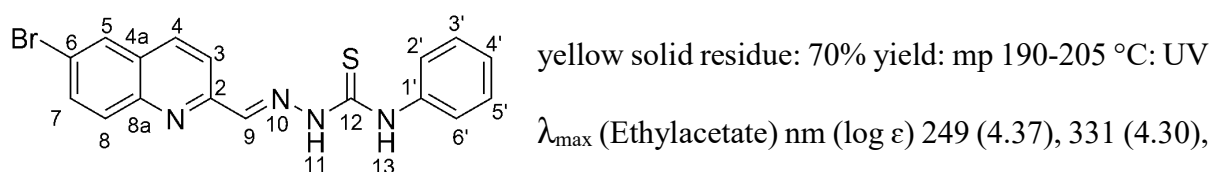
λ_{max} (Ethylacetate) nm (log ε) 249 (4.37), 331 (4.30), 347 (4.35), 360 (4.40); IR υ_{max} 3327 (N-H), 3139 (C-H), 2993 (C-H), 1533 (C=C), 1511, 1245, 1188 (C-F), cm^{-1} ;^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ 12.22 (s, 11-NH), 10.39 (s, 13-NH), 8.66 (1H, d, J = 8.8 Hz, H-3), 8.37 (1H, d, J = 8.8 Hz, H-4), 8.32 (s, H-9), 8.28 (1H, d, J = 2.1 Hz, H-5), 7.97 (1H, d, J = 9.0 Hz, H-8), 7.89 (1H, dd, J = 9.0, 2.3 Hz, H-7), 7.56 (2H, d, J = 7.6 Hz, H-2'/6'), 7.41 (2H, t, J = 7.8 Hz, H-3'/5'), 7.25 (1H, t, J = 7.4 Hz, H-4'); ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ 176.6 (C-12), 154.3 (C-2), 146.0 (C-8a), 142.6 (C-9),138.9 (C-1'), 135.4 (C-4), 133.0 (C-7), 130.9 (C-8), 129.9 (C-5), 129.1 (C-4a), 128.1 (C-2'/6'), 126.3 (C-3'/5'), 125.7 (C-4'), 120.1 (C-6), 119.4 (C-3).

*(E)-2-((6-bromoquinolin-2-yl)methylene)-N-(4-fluorophenyl)hydrazine carbothioamide (**6l**);*
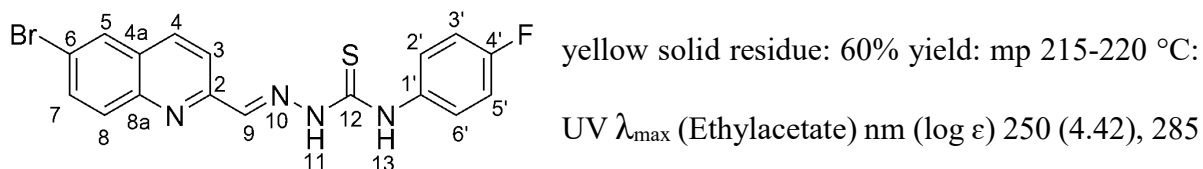
yellow solid residue: 60% yield: mp 215-220 °C: UV λ_{max} (Ethylacetate) nm (log ε) 250 (4.42), 285 (4.17), 310 (4.23), 361 (4.37); IR υ_{max} 3303 (N-H), 3107 (C-H), 2973 (C-H), 1505 (C=C), 1176 (C-F) cm^{-1} ;^{1}H NMR (DMSO-$_{d6}$, 400 MHz) δ 12.24 (s, 11-NH), 10.38 (s, 13-NH), 8.65 (1H, d, J = 8.8 Hz, H-3), 8.38 (1H, d, J = 8.8 Hz, H-4), 8.31 (s, H-9), 8.28 (1H, d, J = 2.1 Hz, H-5), 7.97 (1H, d, J = 9.0 Hz, H-8), 7.90 (1H, dd, J = 9.0, 2.1 Hz, H-7), 7.54 (1H, dd, J = 9.0, 5.1 Hz, H-2'/6'), 7.24 (1H, t, J = 8.7 Hz, H-3'/5'); ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ 176.9 (C-12), 159.9 (d, J_{CF} = 240.2 Hz, C-4'), 154.3 (C-2), 146.0 (C-8a), 142.7 (C-9), 135.4 (C-4), 134.2 (C-1'), 133.0 (C-7), 130.9 (C-8), 129.9 (C-5), 129.1 (C-4a), 128.5 (d, J_{CF} = 8.3 Hz, C-2'/6'), 120.1 (C-6), 119.3 (C-3), 114.8 (d, J_{CF} = 22.3 Hz,C-3'/5'); HRMS (pos) (*m/z*): calculated for $C_{17}H_{12}BrFN_4S$ (M + H)$^+$: 402.00, found: 402.9850.

(E)-2-((6-bromoquinolin-2-yl)methylene)-N-(4-chlorophenyl)hydrazine *carbothioamide*
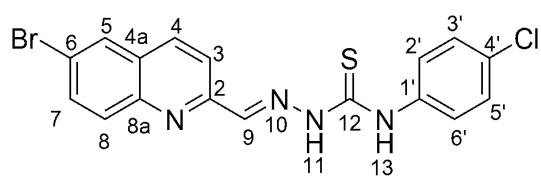
(6m); yellow solid residue: 66% yield: mp 205-220 °C: UV λ_{max} (Ethylacetate) nm (log ε) 228 (4.70), 275 (4.58), 333 (4.48); IR υ_{max} 3313 (N-H), 3284, 3129 (C-H), 2990 (C-H), 1510 (C=C), 1176 (C-F) cm^{-1}; ^{1}H-NMR (DMSO-$d6$, 400 MHz) δ 12.29 (s, 11-NH), 10.41 (s, 13-NH), 8.64 (1H, d, J = 8.8 Hz, H-3), 8.38 (1H, d, J = 8.8 Hz, H-4), 8.32 (s, H-9), 8.29 (1H, d, J = 2.1 Hz, H-5), 7.97 (1H, d, J = 9.0 Hz, H-8), 7.90 (1H, dd, J = 9.0, 2.3 Hz, H-7), 7.61 (1H, d, J = 9.0 Hz, H-2'/6'), 7.46 (1H, d, J = 8.7 Hz, H-3'/5'); ^{13}C NMR (DMSO-$d6$, 100 MHz) δ 176.6 (C-12), 154.2 (C-2), 146.0 (C-8a), 142.9 (C-9), 137.9 (C-1'), 135.5 (C-4), 133.0 (C-7), 130.9 (C-8), 129.9 (C-5), 129.7 (C-4'), 129.1 (C-4a), 128.0 (C-3'/5'), 127.9 (C2'/6'), 120.1 (C-6), 119.3 (C-3); HRMS (pos) (*m/z*): calculated for C$_{17}$H$_{12}$BrClN$_4$S (M + H)$^+$: 417.97, found: 418.9957.

(E)-2-((6-bromoquinolin-2-yl)methylene)-N-(4-nitrophenyl)hydrazine carbothioamide *(6n)*;
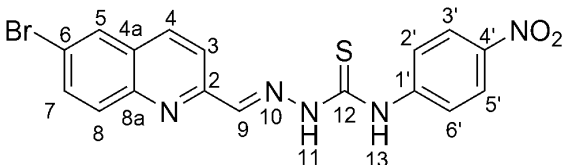
yellow solid residue: 76% yield: mp 195-205 °C: UV λ_{max} (Ethylacetate) nm (log ε) 249 (4.32), 323 (4.63), 334 (4.67), 349 (4.61); IR υ_{max} 3366 (N-H), 3071 (C-H), 2914 (C-H), 1721*, 1598 (C=C), 1539, 1504, 1333, 1145 (C-F) cm^{-1}; ^{1}H-NMR (DMSO-$d6$, 400 MHz) δ 11.52 (s, 11-NH), 9.71 (s, 13-NH), 8.57 (1H, d, J = 8.8 Hz, H-3), 8.41 (1H, d, J = 8.8 Hz, H-4), 8.30 (1H, d, J = 2.1 Hz, H-5), 8.24 (1H, d, J = 9.4 Hz, H-3'/5'); 8.16 (s, H-9), 8.01 (1H, d, J = 9.2 Hz, H-2'/6'), 7.96 (1H, d, J = 9.0 Hz, H-8), 7.89 (1H, dd, J = 9.0, 2.2 Hz, H-7), ^{13}C-NMR (DMSO-$d6$, 100 MHz) δ 154.1 (C-12), 152.4 (C-4'), 145.9 (C-2), 145.6 (C-8a), 142.1 (C-9), 141.7 (C-1'), 135.5 (C-4), 133.0 (C-7), 130.9 (C-8), 130.0 (C-5), 129.0 (C-4a), 124.7 (C-3'/5'), 120.0 (C-6), 119.2 (C-2'/6'), 119.1 (C-3). *These absorption bands only appear for the nitro substituted compounds, however the frequency is higher than usual for a nitro stretch, typically occurring at 1535-1600 cm^{-1}.

(E)-2-((6-bromoquinolin-2-yl)methylene)-N-p-tolylhydrazine carbothioamide (6o); yellow solid residue: 59% yield: mp 212-220 °C: UV λ_{max} (Ethylacetate) nm (log ε) 229 (4.71), 280 (4.64), 330 (4.57); IR υ_{max} 3309 (N-H), 3123 (C-H), 2963 (C-H), 1592 (C=C), 1537, 1514, 1485, 1249, 1177 (C-F) cm^{-1}; ^{1}H NMR (DMSO-$d6$, 400 MHz) δ 12.17 (s, 11-NH), 10.32 (s, 13-NH), 8.66 (1H, d, J = 8.8 Hz, H-3), 8.37 (1H, d, J = 8.8 Hz, H-4), 8.31 (s, H-9), 8.28 (1H, d, J = 2.2 Hz, H-5), 7.97 (1H, d, J = 9.0 Hz, H-8), 7.90 (1H, dd, J = 9.0, 2.3 Hz, H-7), 7.41 (1H, d, J = 8.3 Hz, H-2'/6'), 7.20 (1H, d, J = 8.2 Hz, H-3'/5'); ^{13}C-NMR (DMSO-$d6$, 100 MHz) δ 176.6 (C-12), 154.4 (C-2), 146.0 (C-8a), 142.4 (C-9), 136.3 (C-1'), 135.4 (C-4), 134.9 (C-4'), 133.0 (C-7), 130.9 (C-8), 129.9 (C-5), 129.1 (C-4a), 128.6 (C-3'/5'), 126.2 (C2'/6'), 120.1 (C-6), 119.4 (C-3), 20.6 (C-7'); HRMS (pos) (*m/z*): calculated for $C_{17}H_{12}F_2N_4S$ (M + Na)$^+$: **398.02**, found: 421.0108.

3.3 In-vitro antimicrobial studies

The antimicrobial activities of the synthesised compounds **6a-o** were tested against two Gram +ve strains (*Staphylococcus aureus* ATCC 25923 and Methicillin resistant *Staphylococcus aureus* ATCC BAA-1683) and four Gram –ve strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14026 and *Klebsiella pneumonia* ATCC 314588) using levofloxacin and ciprofloxacin as standards for comparison. The disc diffusion method was used for initial screening. Those compounds that showed a broad spectrum of activity across all the bacterial strains, showing activity in at least four of the six strains were chosen to determine their minimum bactericidal activity (MBC).

For the disc diffusion assay, the bacterial micro-organisms were grown overnight at 37 °C in nutrient broth (Biolab, South Africa) and adjusted to a 0.5 McFarland standard using distilled water. Mueller-Hinton agar (MHA) (Biolab, South Africa) plates were prepared by dissolving 38 g of agar in 1 L of water and pouring these into sterile petri dishes, which were then allowed to set and dry at room temperature. They were then inoculated with the respective strains of bacteria by streaking a swab (dipped into the micro-organism solution) evenly over the entire sterile agar surface. A volume of 5 μL of a 10 mg mL^{-1} solution of each test compound was then impregnated onto antibiotic test discs (12 mm) and placed onto the Mueller-Hinton plates. They were then left to incubate for 24 hours at 37 °C, and the zones of inhibition measured in millimeters.

For the determination of MBCs, a swab of the microbial cultures (adjusted to 0.5 McFarland) prepared as described previously were again evenly streaked over sterile agar plates. The test compounds were dissolved in DMSO and serially diluted in 1 mL Eppendorf tubes. A volume of 5 μL of concentrations of 19.5 to 625 μg mL^{-1} were directly spotted on MHA plates containing the respective bacterial strains and incubated at 37 °C for 20 h to determine the MBCs. The MBC was the lowest concentration showing a zone of inhibition around the spotted compound. DMSO was used as a control and showed no zones of inhibition to any of the bacterial strains tested against. Levofloxacin and ciprofloxacin served as the standard drugs for all antimicrobial studies. All experiments were conducted in triplicate.

Chapter 4.   Conclusion

Fifteen 6-substituted quinoline-2-thiosemicarbazones were synthesised in good yields from a Doebner-von Miller reaction between *para*-substituted anilines and crotonaldehyde to form quinoline intermediates. Oxidation with SeO_2 to form quinoline-2-carbaldehydes proved to be a useful method to functionalise the 2-position for further reactions and to hybridise the quinoline framework. Formation of thiosemicarbazones was an easy derivatisation and way in which the quinolines could form quinoline thiosemicarbazone hybrid molecules. Thus, the reaction sequence carried out was a good way of forming quinoline thiosemicarbazone hybrids which have the attributes of both quinolines and thiosemicarbazones.

A full structural elucidation of all the compounds synthesised was carried out and will provide a basis for the identification of similar molecules in future research. The different substitution patterns were found to have an effect on the antibacterial activity of the compounds with compound **6l**, containing a 6-Br substituent on the quinoline moiety and fluoro group at the *para* position of the thiosemicarbazide moiety showing the best antibacterial activity at 387 μM. Unfortunately, the antibacterial activity of the synthesised compounds were inactive as antibacterial agents even though the individual constituents, quinolines and thiosemicarbazones have previously demonstrated good antibacterial activity. This could partly be due to the insolubility of the compounds in aqueous media.

Future work will involve modifying the structure of **6l** (6-Br, 4'-F derivative), by adding other groups to the molecule using this as a basic scaffold. Compound **6l** was the only compound that showed good antibacterial activity. It is hoped that modifications of the structure will identify a compound that can be developed into a potential antibiotic. The modifications can include groups that will increase the solubility of the product.

Chapter 5.   References

Abdel-Moty, S., Abdel-Rahman, M., Elsherief, H., Kafafy, A. Synthesis of some quinoline thiosemicarbazone derivatives of potential antimicrobial activity. *Bull. Pharm. Sci.* **2005**, 28, 79-93.

Achremowicz, L. A new approach to the oxidation of methylquinolines with selenium dioxide. *Synth. Commun.* **1996**, 26 (9), 1681-1684.

Afzal, O., Kumar, S., Haider, M.R., Ali, M.R., Kumar, R., Jaggi M., Bawa, S. A review on anticancer potential of bioactive heterocycle quinoline. *Eur. J. Med. Chem.* **2015**, 97, 871-910.

Alegaon, S. G., Parchure, P., Araujo, L.D., Salve, P.S., Alagawadi, K.R., Jalalpure, S.S., Kumbar, V.M. Quinoline-azetidinone hybrids: Synthesis and *in vitro* antiproliferation activity against Hep G2 and Hep 3B human cell lines. *Bioorg. Med. Chem. Lett.* **2017**, 27 (7), 1566-1571.

Aly, M. M., Mohamed, Y.A., El-Bayouki, K. A. M., Basyouni, W. M., Abbas, S. Y. Synthesis of some new 4(3*H*)-quinazolinone-2-carboxaldehyde thiosemicarbazones and their metal complexes and a study on their anticonvulsant, analgesic, cytotoxic and antimicrobial activities - Part-1. *Eur. J. Med. Chem.*, **2010**, 45 (8), 3365-3373.

Aminov, R. I. A brief history of the antibiotic era: lessons learned and challenges for the future. *Front. Microbiol*., **2010**, Article 134, doi: 10.3389/fmicb.2010.00134, 7 pages.

Aribi, F., Schmitt, E., Panossian, A., Vors, J. P., Pazenok, S., Leroux, F. R. A new approach toward the synthesis of 2,4-bis(fluoroalkyl)-substituted quinoline derivatives using fluoroalkyl amino reagent chemistry. *Org. Chem. Front.* **2016**, 3 (11), 1392-1415.

Arora, S., Agarwal, S., Singhal, S. Anticancer activities of thiosemicarbazides/thiosemicarbazones: a review. *Int. J. Pharm. Pharm. Sci.*, **2014**, 6 (9), 34-41.

Banerjee, D., Yogeeswari, P., Bhat, P., Thomas, A., Srividya, M., Sriram, D. Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. *Eur. J. Med. Chem.* **2010**, 46 (1), 106-121.

Bečka, M., Vilková, M., Salem, O., Kašpárková, J., Brabec, V., Koţurková, M. 3-[(*E*)-(acridin-9'-ylmethylidene)amino]-1-substituted thioureas and their biological activity. *Spectrochim. Acta, Part A*., **2017**, 180, 234-241.

Benmohammed, A., Khoumeri, O., Djafri, A., Terme, T., Vanelle, P. Synthesis of novel highly functionalized 4-thiazolidinone derivatives from 4-phenyl-3-thiosemicarbazones. *Molecules* **2014**, 19 (3), 3068-3083.

Bisceglie, F., Musiari, A., Pinelli, S., Alinovi, R., Menozzi, I., Polverini, E., Tarasconi, P., Tavone, M., Pelosi, G. Quinoline-2-carboxaldehyde thiosemicarbazones and their Cu(II) and Ni(II) complexes as topoisomerase IIα inhibitors. *J. Inorg. Biochem*. **2015**, 152, 10-19.

Bisht, R., Katiyar, A., Singh, R., Mittal, P. Antibiotic resistance-A global issue of concern. *Asian J. Pharm. Clin. Res*. **2009**, 2 (2), 189.

Brouet, J. C., Gu, S., Peet, N. P., Williams, J. D. A survey of solvents for the Conrad-Limpach synthesis of 4-hydroxyquinolones. *Synth. Commun.* **2009**, 39 (9), 5193-5196.

Burgess, S. J., Selzer, A., Kelly, J. X., Smilkstein, M. J., Riscoe, M. K., Peyton, D. H. A chloroquine-like molecule designed to reverse resistance in *Plasmodium falciparum*. *J. Med. Chem.* **2006**, 49 (18), 5623.

Chandrasekhar, R., Gopalan, B., Nanjan, M. J. Synthesis and characterization of 3-hydroxy-4,5-dihydro[1,2,3]oxadiazolo[3,4-a]quinolin-10-ium and its fluoro derivative. *Int. J. ChemTech Res.* **2011**, 3 (3), 1125-1128.

Chaskar, A., Padalkar, V., Phatangare, K., Langi, B., Shah, C. Micellar-mediated phosphomolybdic acid: highly effective reusable catalyst for synthesis of quinoline and its derivatives. *Synth. Commun.* **2010**, 40 (15), 2336-2340.

Coa, J. C., Garcia, E., Carda, M., Agut, R., Velez, I. D., Munoz, J. A., Yepes, L. M., Robledo, S. M., Cardona, W. I. Synthesis, leishmanicidal, trypanocidal and cytotoxic activities of quinoline-chalcone and quinoline-chromone hybrids. *Med. Chem. Res.* **2017**, 1-10.

Degola, F., Morcia, C., Bisceglie, F., Mussi, F., Tumino, G., Ghizzoni, R., Pelosi, G., Terzi, V., Buschini, A., Restivo, F. M., Lodi, T. *In vitro* evaluation of the activity of thiosemicarbazone derivatives against mycotoxigenic fungi affecting cereals. *Int. J. Food Microbiol*. **2015**, 200, 104-111.

Desai, N. C., Rajpara, K. M., Joshi, V. V., Vaghani, H. V., Satodiya, H. M. Synthesis, characterization and antimicrobial screening of hybrid molecules containing quinoline, pyrimidine and morpholine analogs. *J. Chem. Sci.* **2013**, 125 (2), 321-333.

Dodd, R. H., Le Hyaric, M. The oxidation of aromatic aldehydes to carboxylic acids using hydrogen peroxide in formic acid. *Synthesis* **1993**, 3, 295-297.

Eisch, J. J, Dluzniewski, T. Mechanism of the Skraup and Doebner-von Miller quinoline syntheses. Cyclization of α,β-unsaturated *N*-aryliminium salts via 1,3-diazetidinium ion intermediates. *J. Org. Chem*. **1989**, 54 (6), 1269-1274.

Er, M., Erguven, B., Tahtaci, H., Onaran, A., Karakurt, T., Ece, A. Synthesis, characterization, preliminary SAR and molecular docking study of some novel substituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives as antifungal agents. *Med. Chem. Res.* **2017**, 1-16.

Eswaran, S., Adhikari, A. V., Chowdhury, I. H., Pal, N. K., Thomas, K. D. New quinoline derivatives: Synthesis and investigation of antibacterial and antituberculosis properties. *Eur. J. Med. Chem.* **2010**, 45 (8), 3374-3383.

Foroughifar, N., Mobinikhaledi, A., Rafiee, A. Efficient synthesis of some novel symmetrical and unsymmetrical pyridine *bis*-1,2,4-triazoles and *bis*-1,3,4-thiadiazoles. *J. Chem. Res.* **2014**, 38 (2), 111-114.

Gazieva, G. A., Kravchenko, A.N. Thiosemicarbazides in the synthesis of five- and six-membered heterocyclic compounds. *Russ. Chem. Rev.* **2012**, 81 (6), 494-523.

Glinma, B., Kpoviessi, S. D. S., Gbaguidi, F. A., Kapanda, C. N., Bero, J., Quetin-Leclercq, J., Moudachirou, M., Poupaert, J., Accrombessi, G. C., Gachomo, E. W., Baba-Moussa, L., Kotchoni, S. O. Trypanocidal and

cytotoxic evaluation of synthesized thiosemicarbazones as potential drug leads against sleeping sickness. *Mol. Biol. Rep.* **2014**, 41 (3), 1617-1622.

Gopaul, K. and Koorbanally, N. A. Synthesis and structure elucidation of a series of chloroquinoline-2-chalcones by the Doebner-Miller reaction. *Magn. Reson. Chem.*, **2016**, 54 (8), 677-683.

Gopaul, K., Shintre, S. A., Koorbanally, N. A. A Review on the synthesis and anti-cancer activity of 2-substituted quinolines. *Anti-Cancer Agents Med. Chem.* **2015**, 15 (5), 631-646.

Gopinath, V. S., Pinjari, J., Dere, R. T., Verma, A., Vishwakarma, P., Shivahare, R., Moger, M., Kumar Goud, P.S., Ramanathan, V., Bose, P., Rao, M. V. S., Gupta, S., Puri, S. K., Launay, D., Martin, D. Design, synthesis and biological evaluation of 2-substituted quinolines as potential antileishmanial agents. *Eur. J. Med. Chem.* **2013**, 69, 527-536.

Hernandez, W., Paz, J., Carrasco, F., Vaisberg, A., Spodine, E., Manzur, J., Hennig, L., Sieler, J., Blaurock, S., Beyer, L. Synthesis and characterization of new palladium(II) thiosemicarbazone complexes and their cytotoxic activity against various human tumor cell lines. *Bioinorg. Chem. Appl.* **2013**, Article ID 524701, 12 pages.

Huang, H., Chen, Q., Xin, K., Meng, L., Lin, L., Wang, X., Zhu, C., Wang, Y., Chen, Z., Li, M., Jiang, H., Chen, K., Ding, K., Liu, H. A series of α-heterocyclic carboxaldehyde thiosemicarbazones inhibit topoisomerase II*α* catalytic activity. *J. Med. Chem.* **2010**, 53 (8), 3048-3064.

Jain, P. P., Degani, M. S., Raju, A., Anantram, A., Seervi, M., Sathaye, S., Ray, M., Rajan, M. G. R. Identification of a novel class of quinoline-oxadiazole hybrids as anti-tuberculosis agents. *Bioorg. Med. Chem. Lett.* **2016**, 26 (2), 645-649.

Jiang, L., Huang, Y., Yan, Y., Xie, Y. Metal-free C(*sp3*)-H oxidation of 2-methylquinolines with PIDA under microwave irradiation. *Tetrahedron Lett.* **2016**, 57 (37), 4149-4151.

Kouznetsov, V. V. Recent synthetic developments in a powerful imino Diels–Alder reaction (Povarov reaction): application to the synthesis of *N*-polyheterocycles and related alkaloids. *Tetrahedron* **2009**, 65 (14), 2721-2750.

Kulkarni, N. V., Hegde, G. S., Kurdekar, G .S., Budagumpi, S., Sathisha, M. P., Revankar, V. K. Spectroscopy, electrochemistry, and structure of 3*d*-transition metal complexes of thiosemicarbazones with quinoline core: evaluation of antimicrobial property. *Spectrosc. Lett.* **2010**, 43 (3), 235-246.

Kumar, S., Bawa, S., Gupta, H. Biological activities of quinoline derivatives. *Mini-Rev. Med. Chem.* **2009**, 9 (14), 1648-1654.

Li, J. J., Corey, E. J. (Eds) Name reactions in heterocyclic chemistry. *John Wiley & Son*s, Hoboken, New Jersey, **2005**.

Luo, Z. G., Zeng, C. C., Wang, F., He, H. Q., Wang, C. X., Du, H. G., Hu, L. M. Synthesis and biological activities of quinoline derivatives as HIV-1 integrase inhibitors. *Chem. Res.* **2009**, 25 (6), 841-845.

Madapa, S., Tusi, Z., Batra, S. Advances in the syntheses of quinoline and quinoline-annulated ring systems. *Curr. Org. Chem.* **2008**, 12 (13), 1116-1183.

Maddela, S., Venugopal, M., Maddela, R., Ajitha, M. Design and synthesis of new *N'*-substituted-2-methylquinoline-3-carbohydrazides with antioxidant and antimicrobial activity. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2015**, 54 (7), 930-935.

Martens, E., Demain, A.L. The antibiotic resistance crisis, with a focus on the United States. *J. Antibiot.*, **2017**, 70 (5), 520-526.

Matsugi, M., Tabusa, F., Minamikawa, J. I. Doebner–Miller synthesis in a two-phase system: practical preparation of quinolines. *Tetrahedron Lett.*, **2000**, 41 (44), 8523-8525.

Metwally, M. A., Bondock, S., El-Azap, H., Kandeel, E. E. M. Thiosemicarbazides: Synthesis and reactions. *J. Sulfur Chem.* **2011**, 32 (5), 489-519.

Michael, J. P. Quinoline, quinazoline, and acridone alkaloids. *Nat. Prod. Rep.* **2008**, 25 (1), 166-187.

Mlochowski, J., Wojtowicz-Mlochowska, H. Developments in synthetic application of selenium(IV) oxide and organoselenium compounds as oxygen donors and oxygen-transfer agents. *Molecules* **2015**, 20 (6), 10205-10243.

Musiol, R., Jampilek, J., Podeszwa, B., Finster, J., Tabak, D., Dohnal, J., Polanski, J. RP-HPLC determination of lipophilicity in series of quinoline derivatives. *Cent. Eur. J. Chem.* **2009**, 7 (3), 586-597.

Musiol, R., Magdziarz, T., Kurczyk, A. Quinoline scaffold as a privileged substructure in antimicrobial drugs. *in* Science against microbial pathogens: communicating current research and technological advances. Mendez-Vilas, A. (Ed.), Formatex **2011**, 72-83.

Nagasawa, Y., Tachikawa, Y., Yamaguchi, E., Tada, N., Miura, T., Itoh, A. Catalytic aerobic photo-oxidation of a methyl group on a heterocycle to produce an aldehyde *via* homolytic C-I bond cleavage caused by irradiation with visible light. *Adv. Synth. Cat*. **2016**, 358 (2), 178-182.

Nevagi, R. J., Dhake, A. S. Antibacterial activity of thiosemicarbazide derivatives. *Pharm. Chem.* **2013**, 5 (2), 45-49.

Nguyen, D. T., Le, T. H., Bui, T. T. T. Antioxidant activities of thiosemicarbazones from substituted benzaldehydes and *N*-(tetra-*O*-acetyl-*β*-D-galactopyranosyl) thiosemicarbazide. *Eur. J. Med. Chem.* **2013**, 60, 199-207.

Nurkenov, O. A., Fazylov, S. D., Zhivotova, T. S., Satpayeva, Z. B., Akhmetova, S. B., Kurmanbayeva, Z. Z., Karipova, G. K., Isayeva, A. Z. Synthesis, chemical transformations and antimicrobial activity of some thiosemicarbazides of *o*- and *p*-hydroxybenzoic acid. *Izv. Nats. Akad. Nauk Resp. Kaz., Ser. Khim. Tekhnol.* **2015**, (1), 9-14.

O'Donnell, F., Ramachandran, V. N., Smyth, W. F., Hack, C. J., Patton, E. A study of the analytical behaviour of selected synthetic and naturally occurring quinolines using electrospray ionization ion trap mass spectrometry, liquid chromatography and gas chromatography and the construction of an appropriate database for quinoline characterization. *Anal. Chim. Acta*. **2006**, 572 (1), 63-76.

Patel, S. R., Gangwal, R., Sangamwar, A. T., Jain, R. Synthesis, biological evaluation and 3*D*-QSAR study of hydrazide, semicarbazide and thiosemicarbazide derivatives of 4-(adamantan-1-yl)quinoline as anti-tuberculosis agents. *Eur. J. Med. Chem.* **2014**, 85, 255-267.

Pérez-Melero, C., Maya, A. B. S., Del Rey, B., Peláez, R., Caballero, E., Medarde, M. A new family of quinoline and quinoxaline analogues of combretastatins. *Bioorg. Med. Chem. Lett.* **2004**, 14 (14), 3771-3774.

Pingaew, R., Prachayasittikul, S., Ruchirawat, S. Synthesis, cytotoxic and antimalarial activities of benzoyl thiosemicarbazone analogs of isoquinoline and related compounds. *Molecules* **2010**, 15, 988-996.

Pitucha, M., Wos, M., Miazga-Karska, M., Klimek, K., Miroslaw, B., Pachuta-Stec, A., Gladysz, A., Ginalska, G. Synthesis, antibacterial and antiproliferative potential of some new 1-pyridinecarbonyl-4-substituted thiosemicarbazide derivatives. *Med. Chem. Res.* **2016**, 25 (8), 1666-1677.

Poronik, Y. M., Klajn, J., Borzęcka, W., Gryko, G.T. The Niementowski reaction of anthranilic acid with ethyl acetoacetate revisited: a new access to pyrano [3,2-*c*] quinoline-2,5-dione. *Org. Chem.* **2017**, 7-11.

Ramachandran, E., Thomas, S. P., Poornima, P., Kalaivani, P., Prabhakaran, R., Padma, V. V., Natarajan, K. Evaluation of DNA binding, antioxidant and cytotoxic activity of mononuclear Co(III) complexes of 2-oxo-1,2-dihydrobenzo[*h*]quinoline-3-carbaldehyde thiosemicarbazones. *Eur. J. Med. Chem.* **2012**, 50, 405-415.

Ramann, G. A. Cowen, B. J. Quinoline synthesis by improved Skraup–Doebner–von Miller reactions utilizing acrolein diethyl acetal. *Tetrahedron Lett.* **2015**, 56 (46), 6436-6439.

Ramann, G. A., Cowen, B. J. Recent advances in metal-free quinoline synthesis. *Molecules* **2016**, 21 (8), 986.

Rane, R. A., Naphade, S. S., Bangalore, P. K., Palkar, M. B., Patel, H. M., Shaikh, M. S., Alwan, W. S., Karpoormath, R. Synthesis of novel hybrids inspired from bromopyrrole alkaloids inhibiting mmp-2 and -12 as antineoplastic agents. *Chem. Biol. Drug Des.* **2015**, 86 (2), 210-222.

Rane, R. A., Naphade, S. S., Bangalore, P. K., Palkar, M.B., Shaikh, M.S., Karpoormath, R. Synthesis of novel 4-nitropyrrole-based semicarbazide and thiosemicarbazide hybrids with antimicrobial and anti-tubercular activity. *Bioorg. Med. Chem. Lett.* **2014**, 24 (14), 3079-3083.

Ranu, B. C., Hajra, A., Dey, S. S., Jana, U. Efficient microwave-assisted synthesis of quinolines and dihydroquinolines under solvent-free conditions. *Tetrahedron* **2003**, 59 (6), 813-819.

Rather, I. A., Kim, B., Bajpai, V. K., Park, Y. Self-medication and antibiotic resistance: Crisis, current challenges, and prevention. *Saudi J. Biol. Sci*. **2017**, 24, 808-812.

Reynolds, K. A., Young, D. J., Loughlin, W.A. Limitations of the 'two-phase' Doebner-Miller reaction for the synthesis of quinolines. *Synthesis* **2010**, (21), 3645-3648.

Rossolini, G.M., Arena, F., Pecile, P., Pollini, S. Update on the antibiotic crisis. *Curr. Opin. Pharmacol*. **2014,** 18, 56-60.

Sakaguchi, S., Shibamoto, A., Ishii, Y. Remarkable effect of nitrogen dioxide for *N*-hydroxyphthalimide-catalyzed aerobic oxidation of methylquinolines. *Chem. Commun.* **2002**, (2), 180-181.

Sangshetti, J. N., Zambare, A. S., Gonjari, I., Shinde, D. B. Pfitzinger reaction in the synthesis of bioactive compounds - a review. *Mini-Rev. Org. Chem.* **2014**, 11 (2), 225-250.

Serda, M., Musiol, R., Polanski, J. New thiosemicarbazones based on quinoline scaffold as anticancer iron chelators. Proceedings of the 14th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-14). **2010**.

Shiri, M., Zolfigol, M. A., Kruger, H. G., Tanbakouchian, Z. Friedlander annulation in the synthesis of aza heterocyclic compounds. *Adv. Heterocycl. Chem.* **2011**, 102, 139-227.

Siddiqui, N. An updated review: emerging anticonvulsants. *Int. J. Pharm. Bio. Arch.* **2010**, 1 (5), 404-415.

Singh, P., Jain, J., Sinha, R., Samad, A., Kumar, R., Malhotra, M. Synthesis and screening of substituted thiosemicarbazone derivatives: an approach towards novel anticonvulsant search. *Cent. Nerv. Syst. Agents Med. Chem.* **2011**, 11 (1), 60-65.

Singh, R. B., Garg, B. S., Singh, R. P. Analytical applications of thiosemicarbazones and semicarbazones: a review. *Talanta* **1978**, 25 (11-12), 619-632.

Singhal, S., Arora, S., Agarwal, S., Sharma, R., Singhal, N. A review on potential biological activities of thiosemicarbazides. *World J. Pharm. Pharm. Sci.* **2013**, 2 (6), 4661-4681.

Sivaprasad, G., Rajesh, R., Perumal, P. T. Synthesis of quinaldines and lepidines by a Doebner–Miller reaction under thermal and microwave irradiation conditions using phosphotungstic acid. *Tetrahedron Lett.* **2006**, 47 (11), 1783-1785.

Snell, R. W., Combs, E., Shanks, B. H. Aldol condensations using bio-oil model compounds: the role of acid-base bi-functionality. *Top. Catal.* **2010**, 53, 1248-1253.

Solomon, V. R., Hu, C., Lee, H. Design and synthesis of anti-breast cancer agents from 4-piperazinylquinoline: A hybrid pharmacophore approach. *Bioorg. Med. Chem.* **2010**, 18 (4), 1563-1572.

Souza, M. A., Johann, S., dos Santos Lima, L. A. R., Campos, F. F., Mendes, I. C., Beraldo, H., de Souza-Fagundes, E. M., Cisalpino, P. S., Rosa, C. A., de Almeida Alves, T. M., de Sa, N. P., Zani, C. L. The antimicrobial activity of lapachol and its thiosemicarbazone and semicarbazone derivatives. *Mem. Inst. Oswaldo Cruz.* **2013**, 108 (3), 342-351.

Taha, M., Ismail, N. H., Imran, S., Wadood, A., Ali, M., Rahim, F., Khan, A. A., Riaz, M. Novel thiosemicarbazide-oxadiazole hybrids as unprecedented inhibitors of yeast α-glucosidase and in silico binding analysis. *RSC Adv.* **2016**, 6 (40), 33733-33742.

Thanigaimalai, P., Rao, E. V., Lee, K. C., Sharma, V. K., Roh, E., Kim, Y., Jung, S. H. Structure-activity relationship of naphthaldehydethiosemicarbazones in melanogenesis inhibition. *Bioorg. Med. Chem. Lett.* **2012**, 22 (2), 886-889.

Trump, E. L., Zhou, M. X. The use of selenium (IV) oxide to oxidize aromatic methyl groups. *Trans. Kansas Acad. Sci.* **1993**, 96 (3), 167-180.

Ventola, C. L. The antibiotic resistance crisis: part 1: Causes and threats. *Pharm. Therapeutics*, **2015**, 40 (4), 277-283.

Vandekerckhove, S., D'Hooghe, M. Quinoline-based antimalarial hybrid compounds. *Bioorg. Med. Chem.* **2015**, 23 (16), 5098-5119.

Wang, L., Hou, X., Fu, H., Pan, X., Xu, W., Tang, W., Fang, H. Design, synthesis and preliminary bioactivity evaluations of substituted quinoline hydroxamic acid derivatives as novel histone deacetylase (HDAC) inhibitors. *Bioorg. Med. Chem.* **2015**, 23 (15), 4364-4374.

Wang, X., Xie, X., Cai, Y., Yang, X., Li, J., Li, Y., Chen, W., He, M. Design, synthesis and antibacterial evaluation of some new 2-phenyl-quinoline-4-carboxylic acid derivatives. *Molecules* **2016**, 21 (3), 340.

Yadav, M. B., Kulkarni, S., Joshi, R. A., Kulkarni, A. A. Continuous flow Doebner-Miller reaction and isolation using continuous stirred tank reactors. *Org. Process Res. Dev.* **2016**, 20 (9), 1621-1625.

Yamashkin, S. A., Oreshkina, E. A. Traditional and modern approaches to the synthesis of quinoline systems by the Skraup and Doebner-Miller methods. *Chem. Heterocycl. Compd.* **2006**, 42 (6), 701-718.

Yoshida, M., Katagiri, Y., Zhu, W.B., Shishido, K. Oxidative carboxylation of arylaldehydes with water by a sulfoxylalkyl-substituted *N*-heterocyclic carbene catalyst. *Org. Biomol. Chem.* **2009**, 7 (19), 4062-4066.

Yu, Y., Kalinowski, D. S., Kovacevic, Z., Siafakas, A. R., Jansson, P. J., Stefani, C., Lovejoy, D. B., Sharpe, P. C., Bernhardt, P. V., Richardson, D. R. Thiosemicarbazones from the old to new: iron chelators that are more than just ribonucleotide reductase inhibitors. *J. Med. Chem.* **2009**, 52 (17), 5271-5294.

Zeglis, B. M., Divilov, V., Lewis, J. S. Role of metalation in the topoisomerase IIα inhibition and antiproliferation activity of a series of α-heterocyclic-*N*4-substituted thiosemicarbazones and their Cu(II) complexes. *J. Med. Chem.* **2011**, 54 (7), 2391-2398.

Zhang, Y., Han, T., Ming, Q., Wu, L., Rahman, K., Qin, L. Alkaloids produced by endophytic fungi: a review. *Nat. Prod. Commun.* **2012**, 7 (7), 963-968.

Zheng, G., Liu, H., Wang, M. Copper-catalyzed aerobic oxidation of azinylmethanes for access to trifluoromethylazinylols. *Chin. J. Chem.* **2016**, 34 (5), 519-523.

Zibaseresht, R., Karimi, P., Mohit-Azar, S., Amirloo, M. R., Azimi, M. An improved synthesis of 2-(pyrid-2'-yl)-1,10-phenanthroline tridentate terpyridyl ligand. *Int. J. Chem. Res.* **2013**, 5 (1), 153-158.

Supporting Information

SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF SUBSTITUTED 2-QUINOLINE THIOSEMICARBAZONES

Siboniso Shezi

Supervisor: Prof. N. Koobarnally

¹H-NMR spectrum of compound 3a

6-fluoro-2-methyl quinolinaldehyde
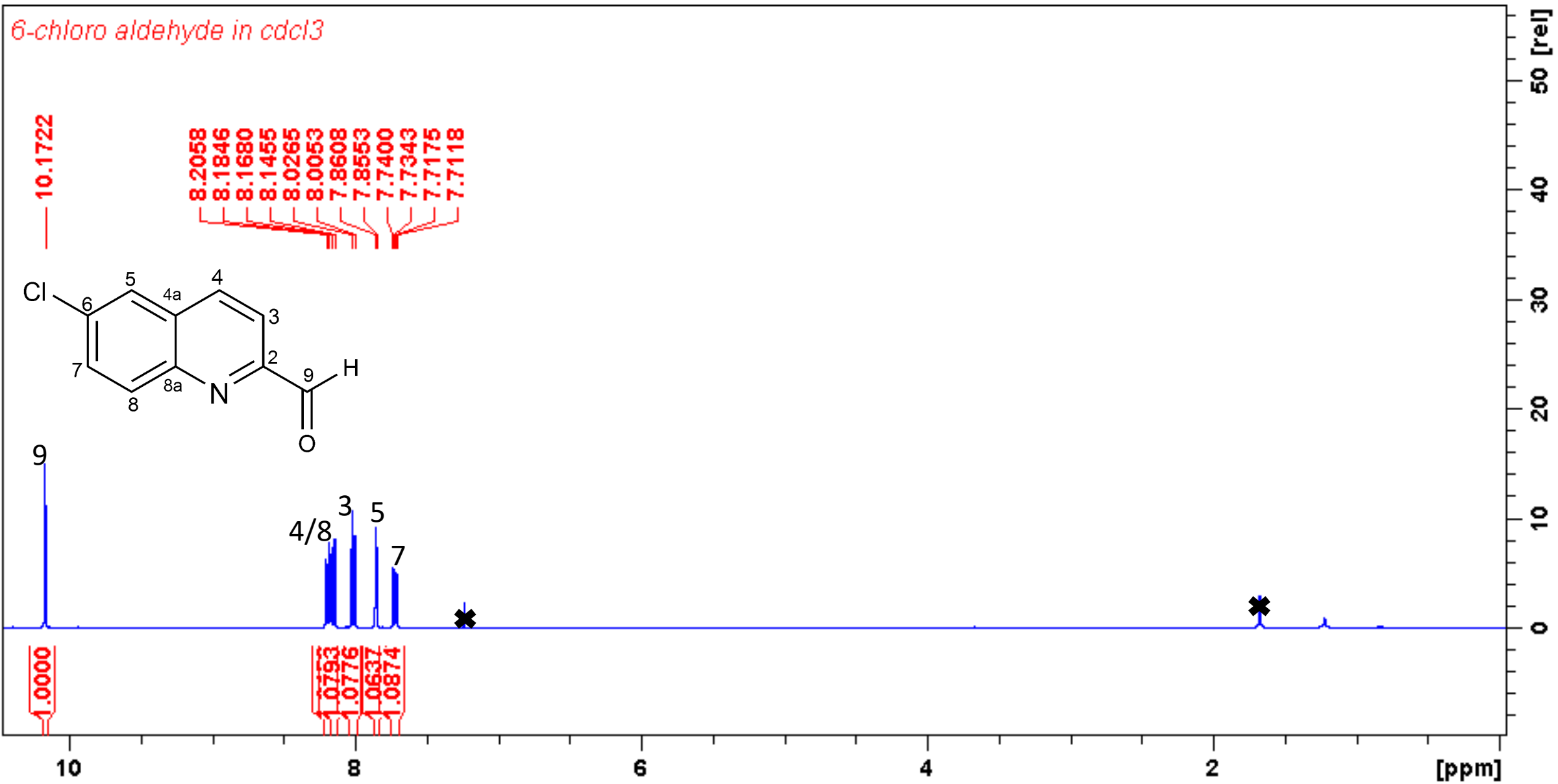
Peak labels: 8.2612, 8.2565, 8.2489, 8.2359, 8.2258; 8.0406, 8.0189; 7.6045, 7.5975, 7.5840, 7.5768, 7.5608, 7.5541, 7.5153, 7.5083, 7.4938, 7.4867

Integrations: 2.1675, 1.0718, 1.0851, 1.0850

Aromatic region ^{1}H-NMR spectrum of compound 3a

2

¹³C-NMR spectrum of compound 3a

¹H-NMR spectrum of compound 3b
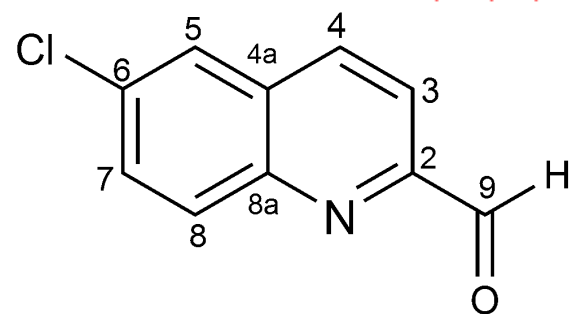
4

Aromatic region ¹H-NMR spectrum of compound 3b

5

^{13}C-NMR spectrum of compound 3b

6-chloro aldehyde in cdcl3

193.2749
152.7268
146.2868
136.4278
135.2496
131.9824
131.5554
130.5635
126.5525
118.2534
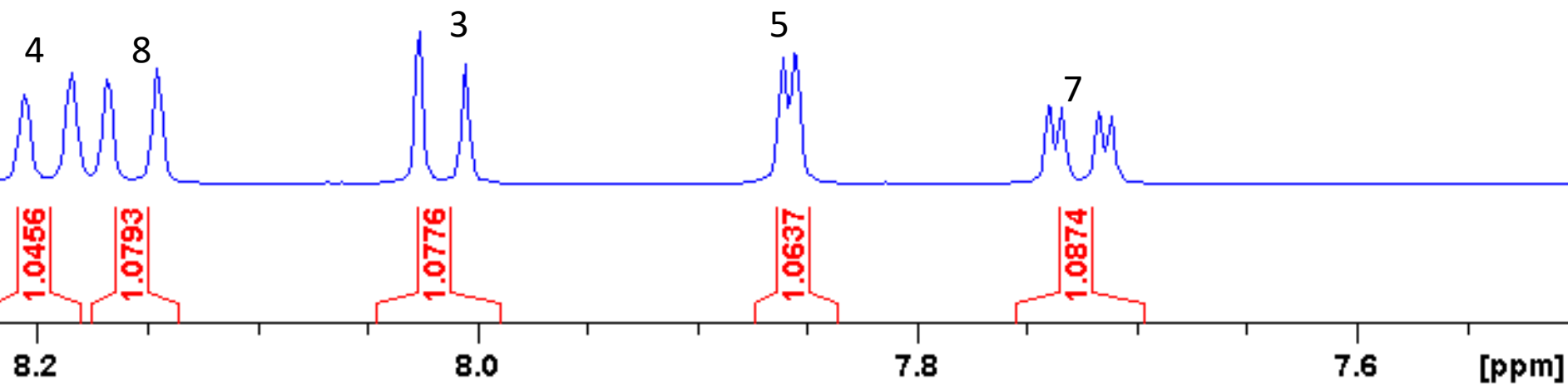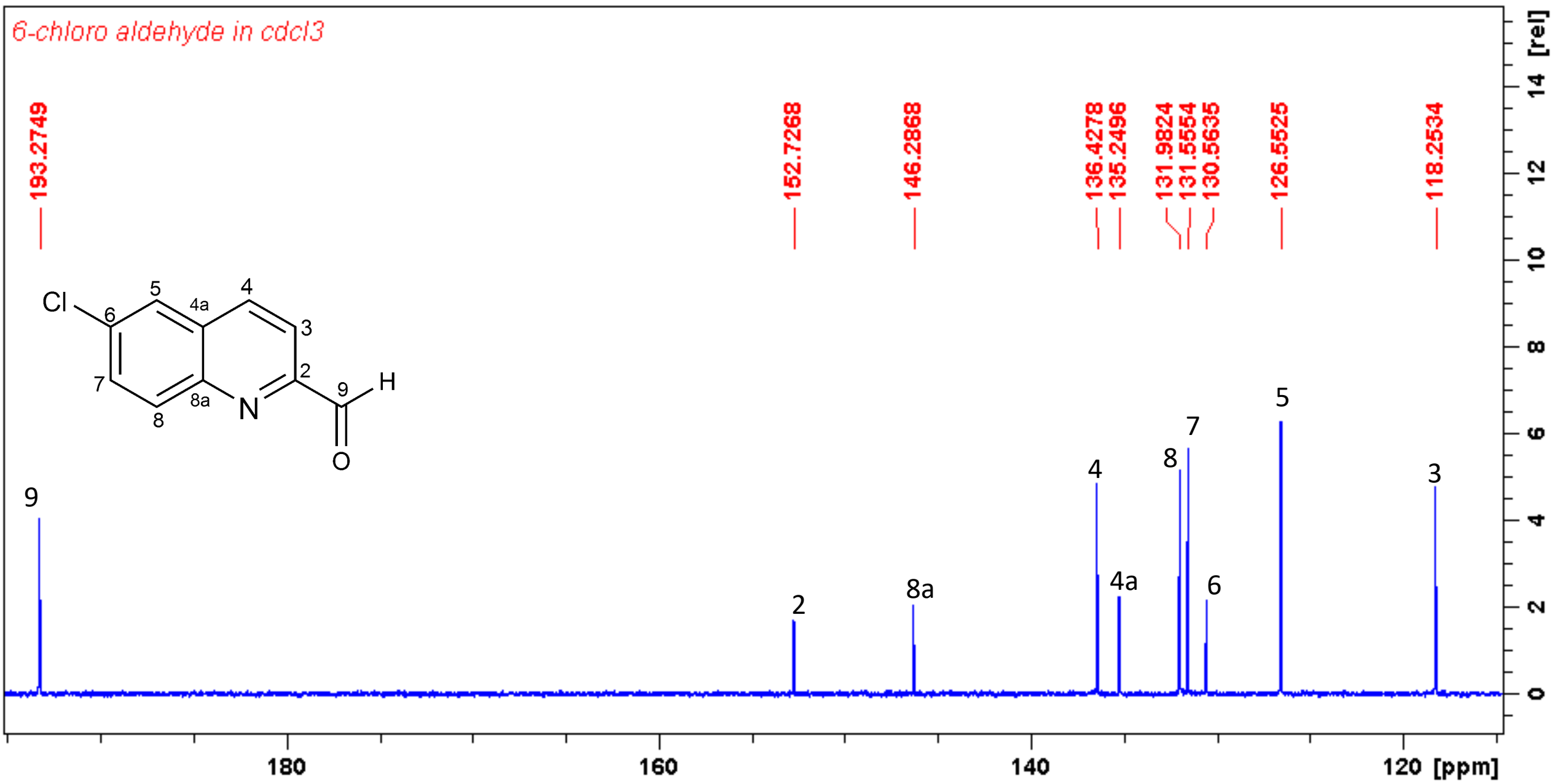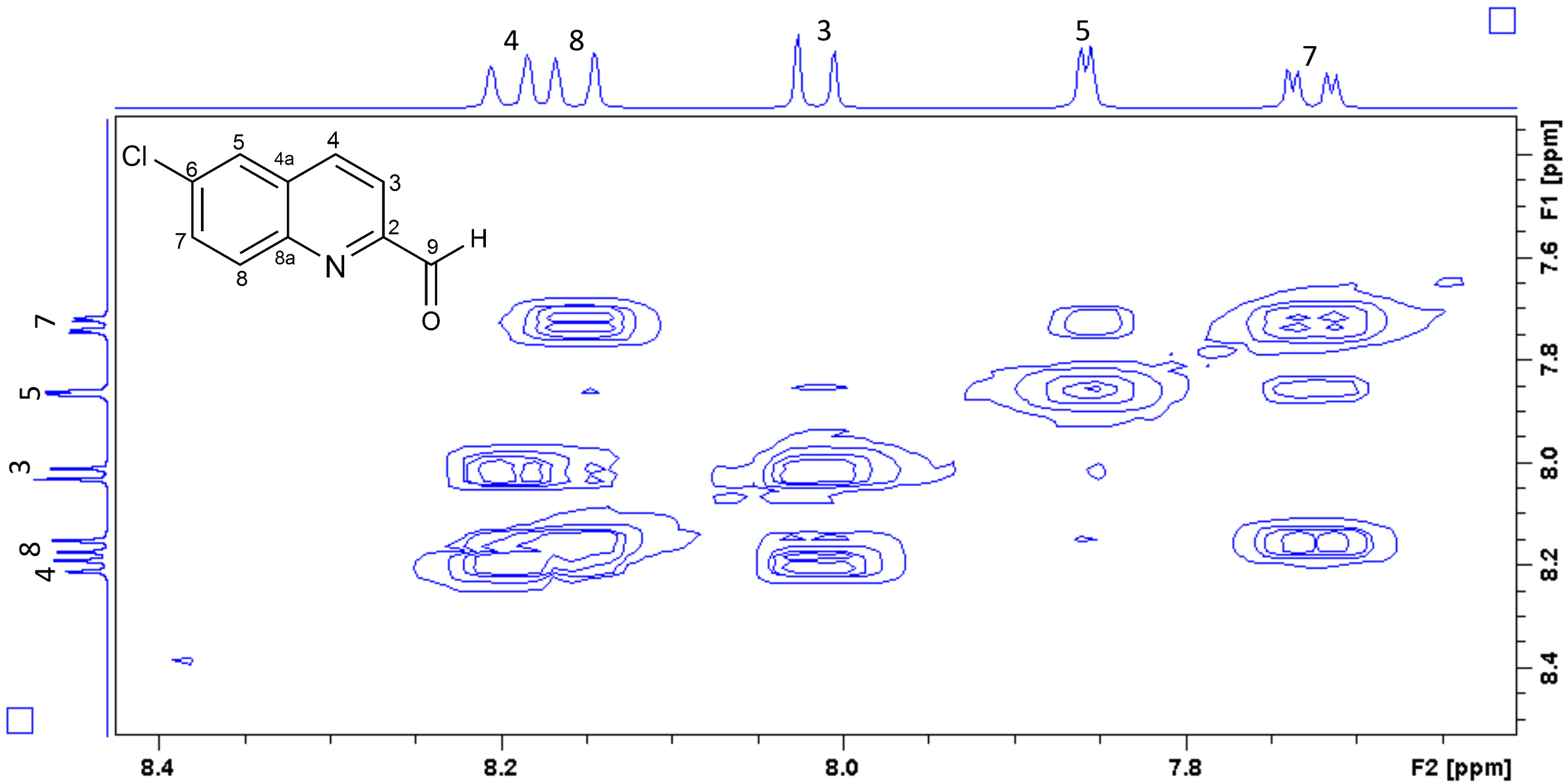
COSY spectrum of compound 3b

HSQC spectrum of compound 3b
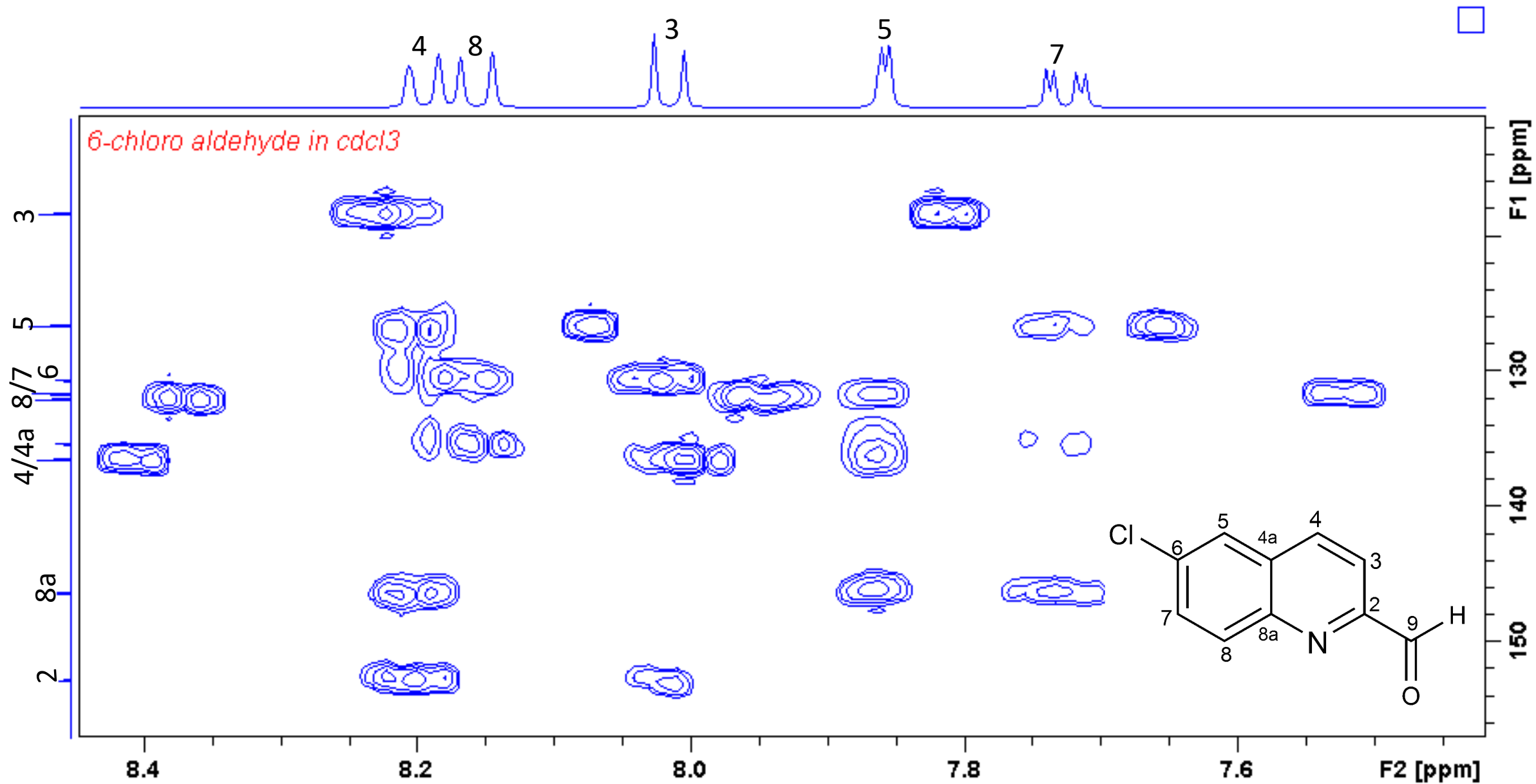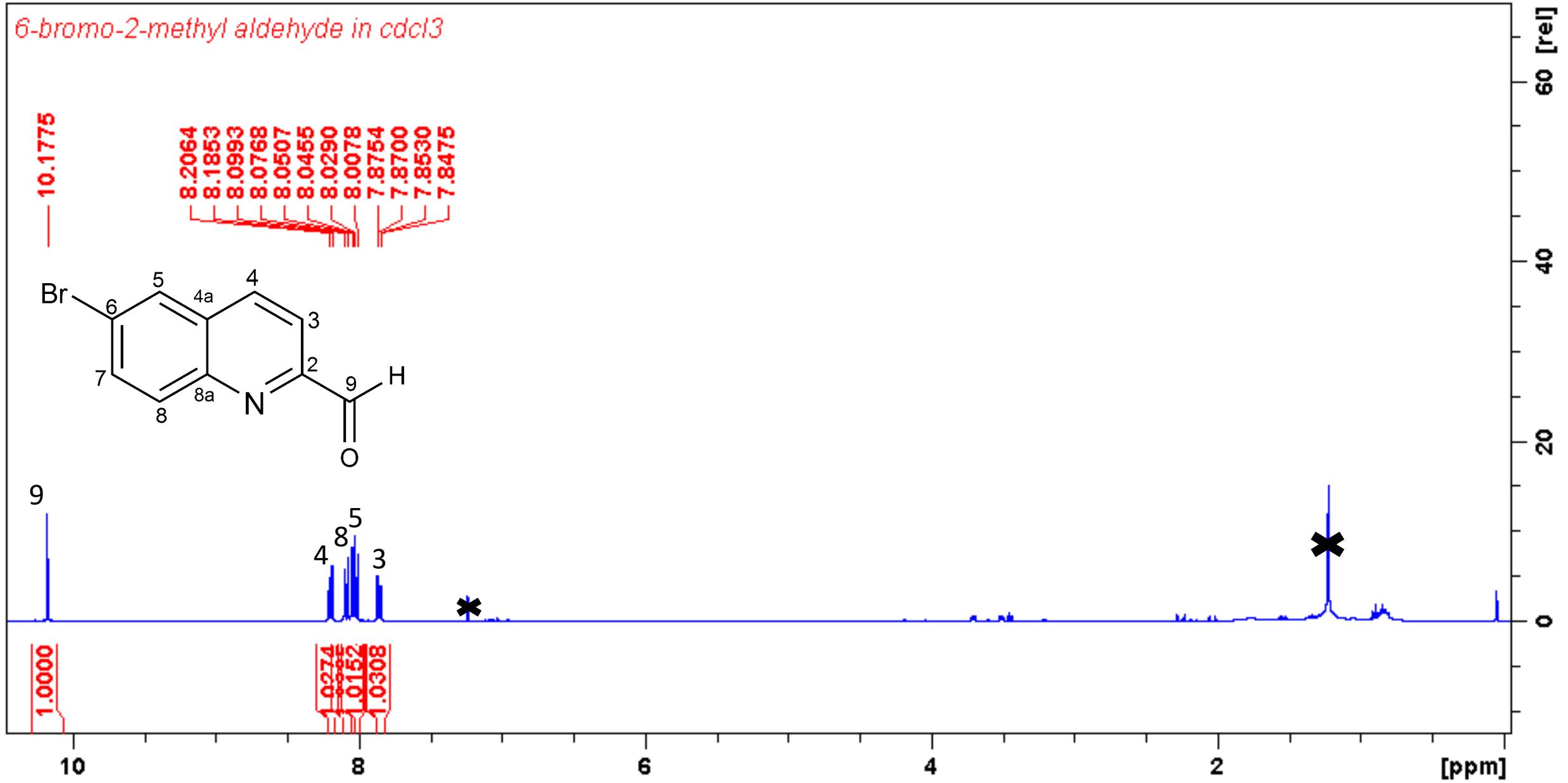
6-chloro aldehyde in cdcl3

HMBC spectrum of compound 3b

9

1H-NMR spectrum of compound 3c

10

6-bromo-2-methyl aldehyde in cdcl3

8.2064
8.1853

8.0993
8.0768
8.0507
8.0455
8.0290
8.0078

7.8754
7.8700
7.8530
7.8475

4

8

5

3

7

1.0274

1.0365

1.0328

1.0152

1.0308

^{1}H-NMR spectrum of compound 3c

11

6-bromo-2-methyl aldehyde in cdcl3

193.2952
152.7799
146.4749
136.3675
134.1277
132.0056
130.9780
129.9645
123.6241
118.2550

¹³C-NMR spectrum of compound 3c

12
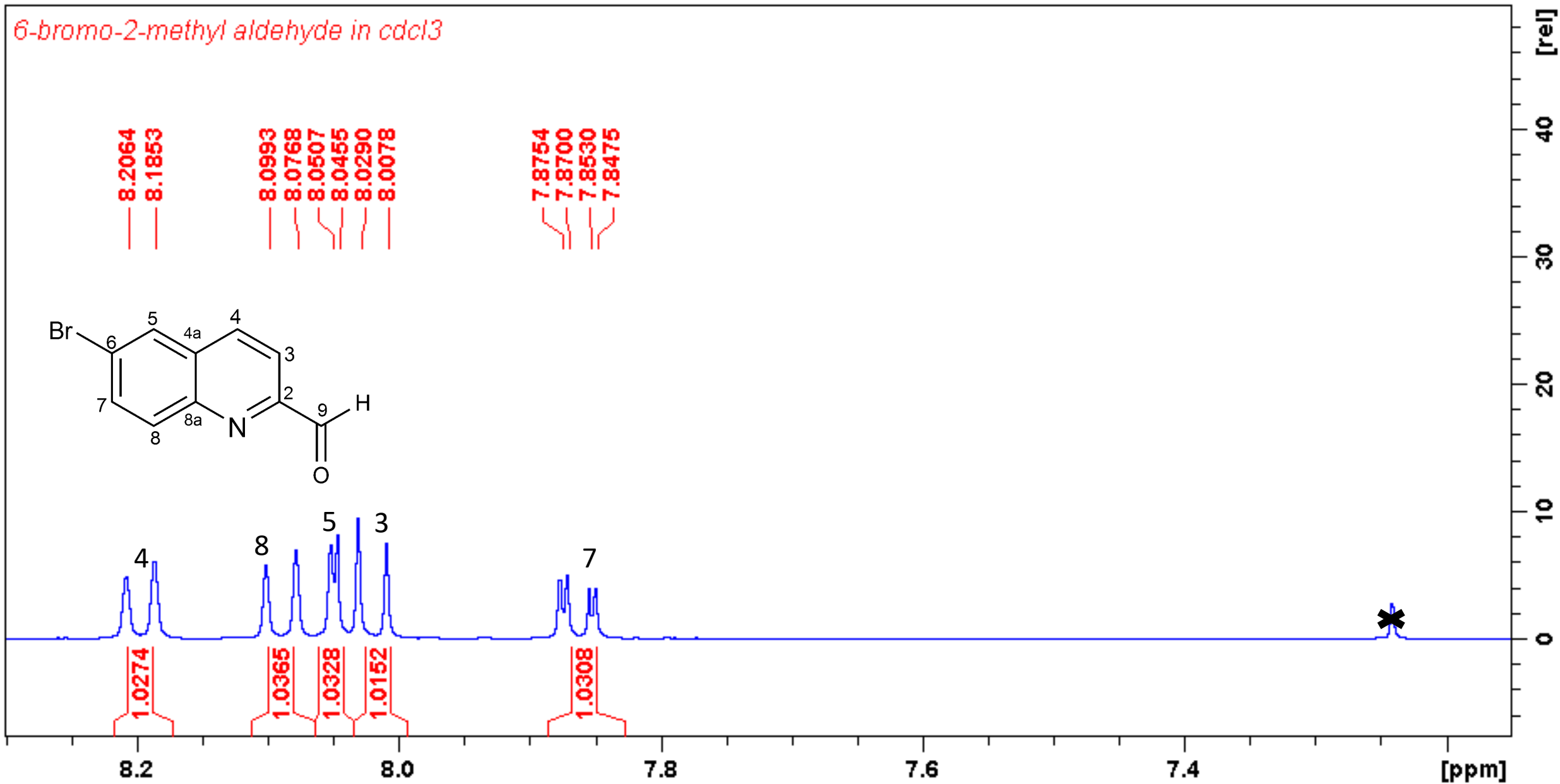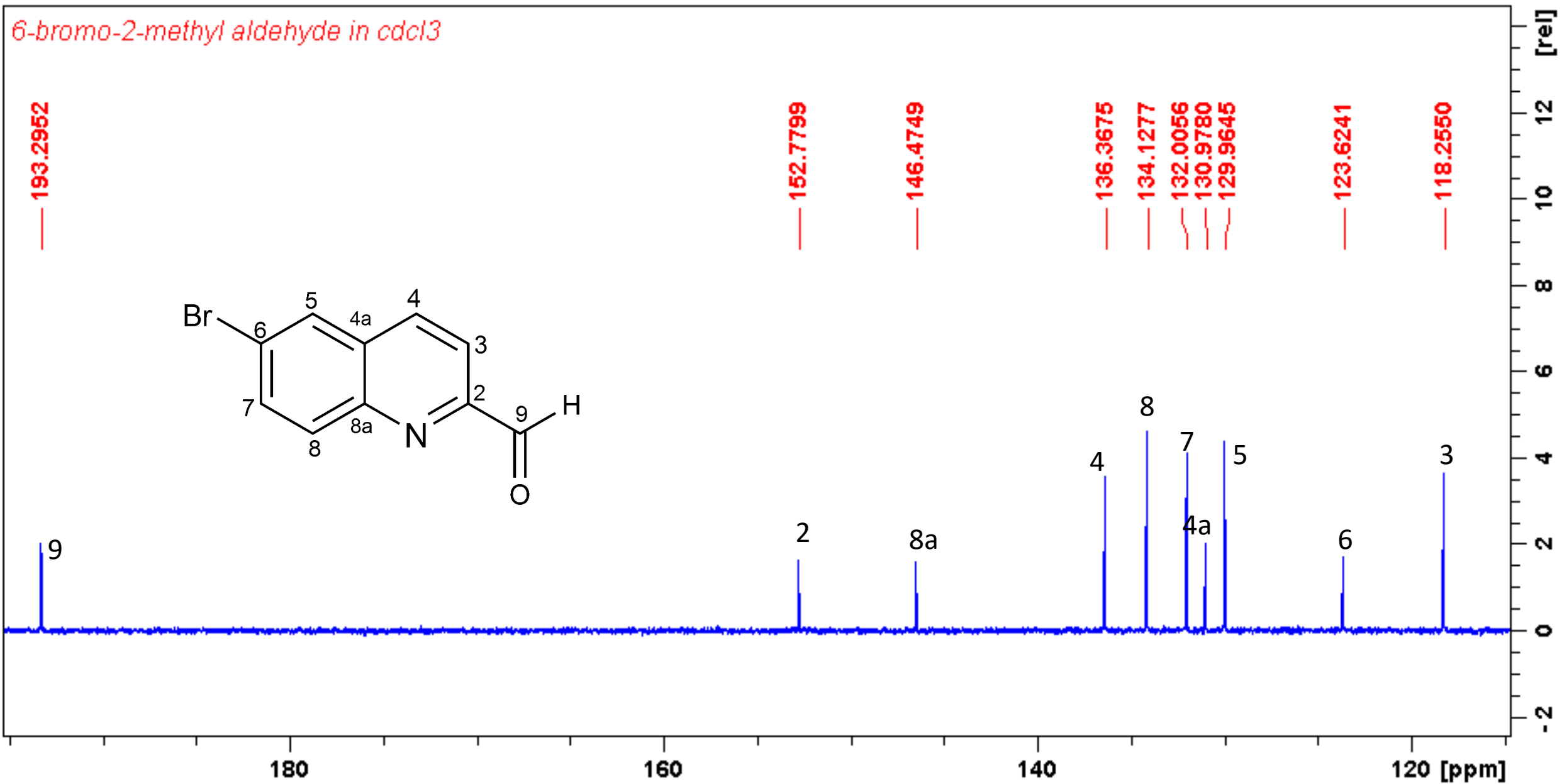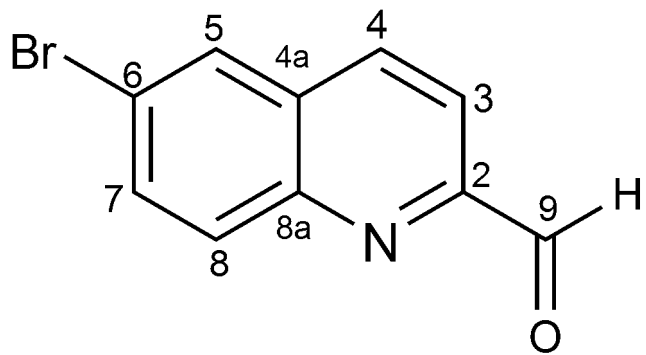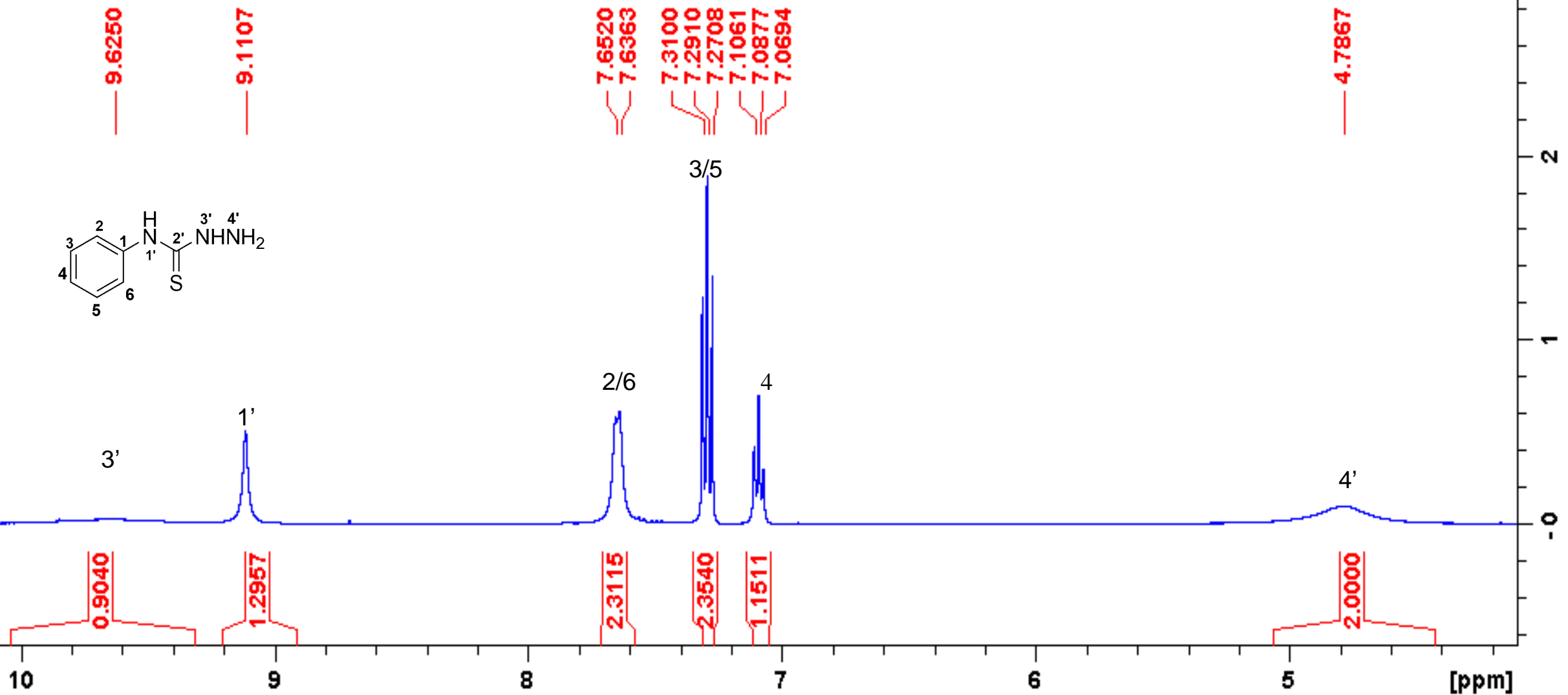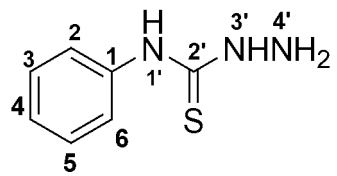
¹H-NMR spectrum of compound 5a

^{1}H-NMR spectrum of compound 5b
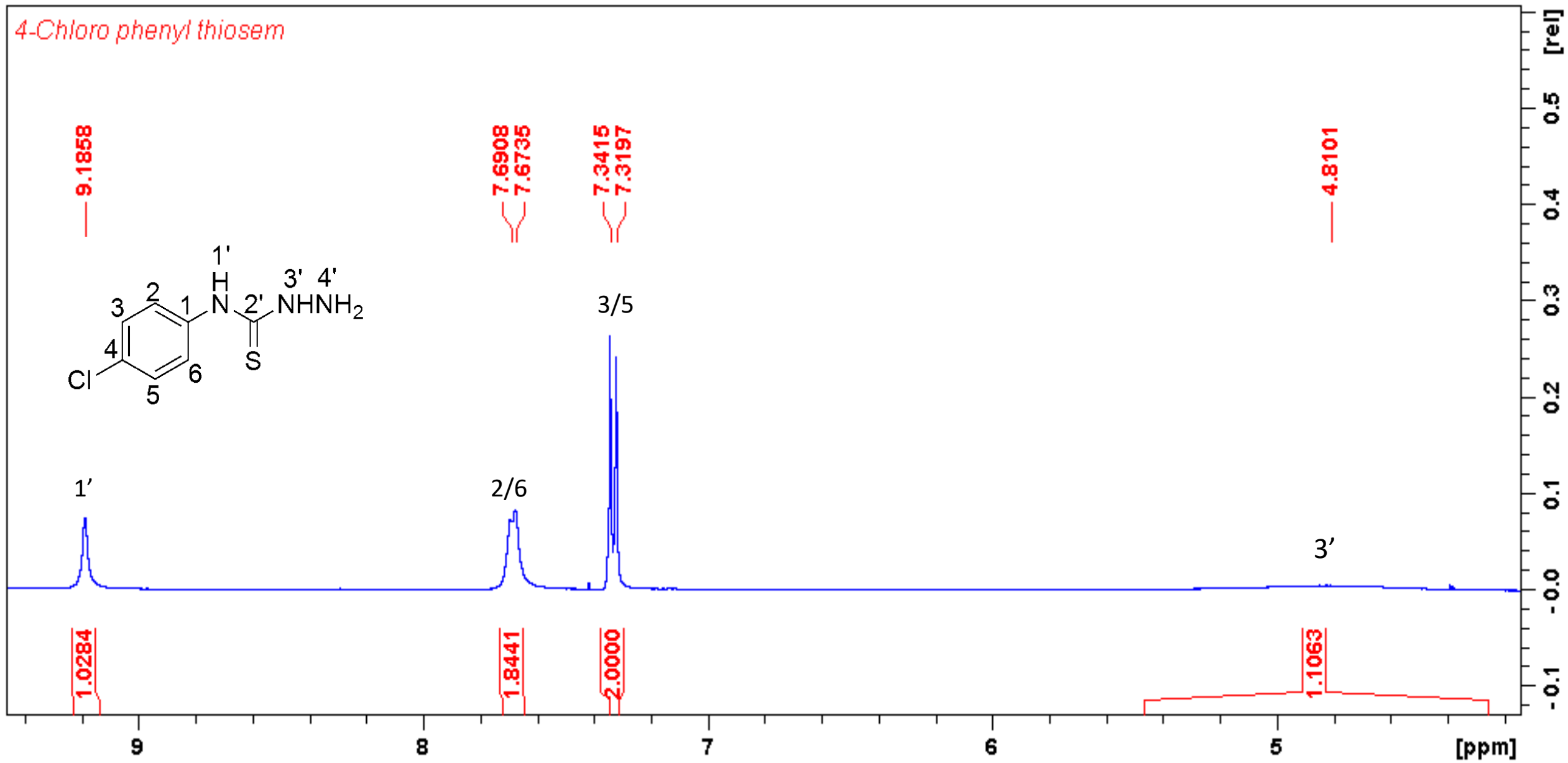
15

¹H-NMR spectrum of compound 5c
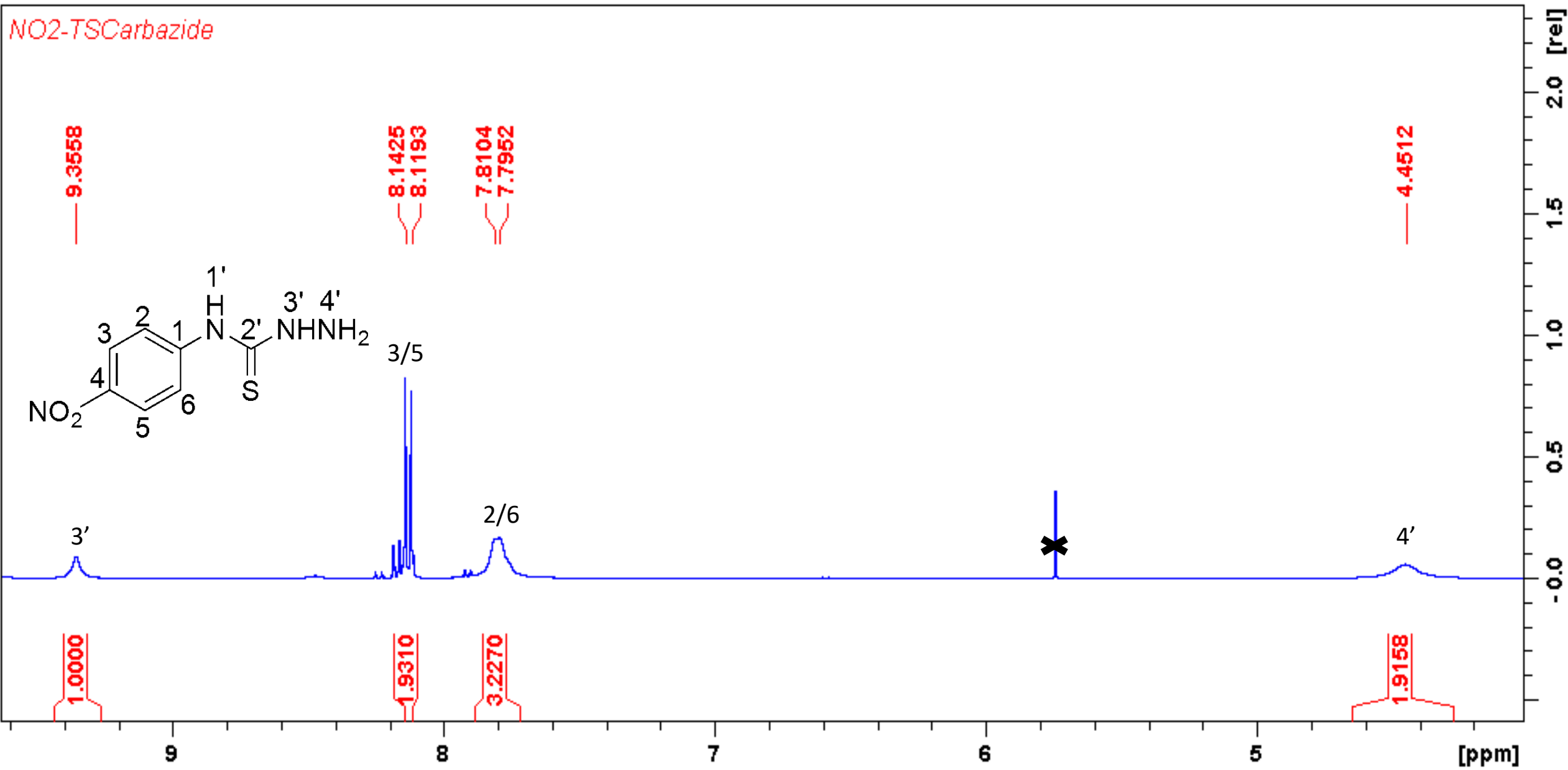
¹H-NMR spectrum of compound 5d

17
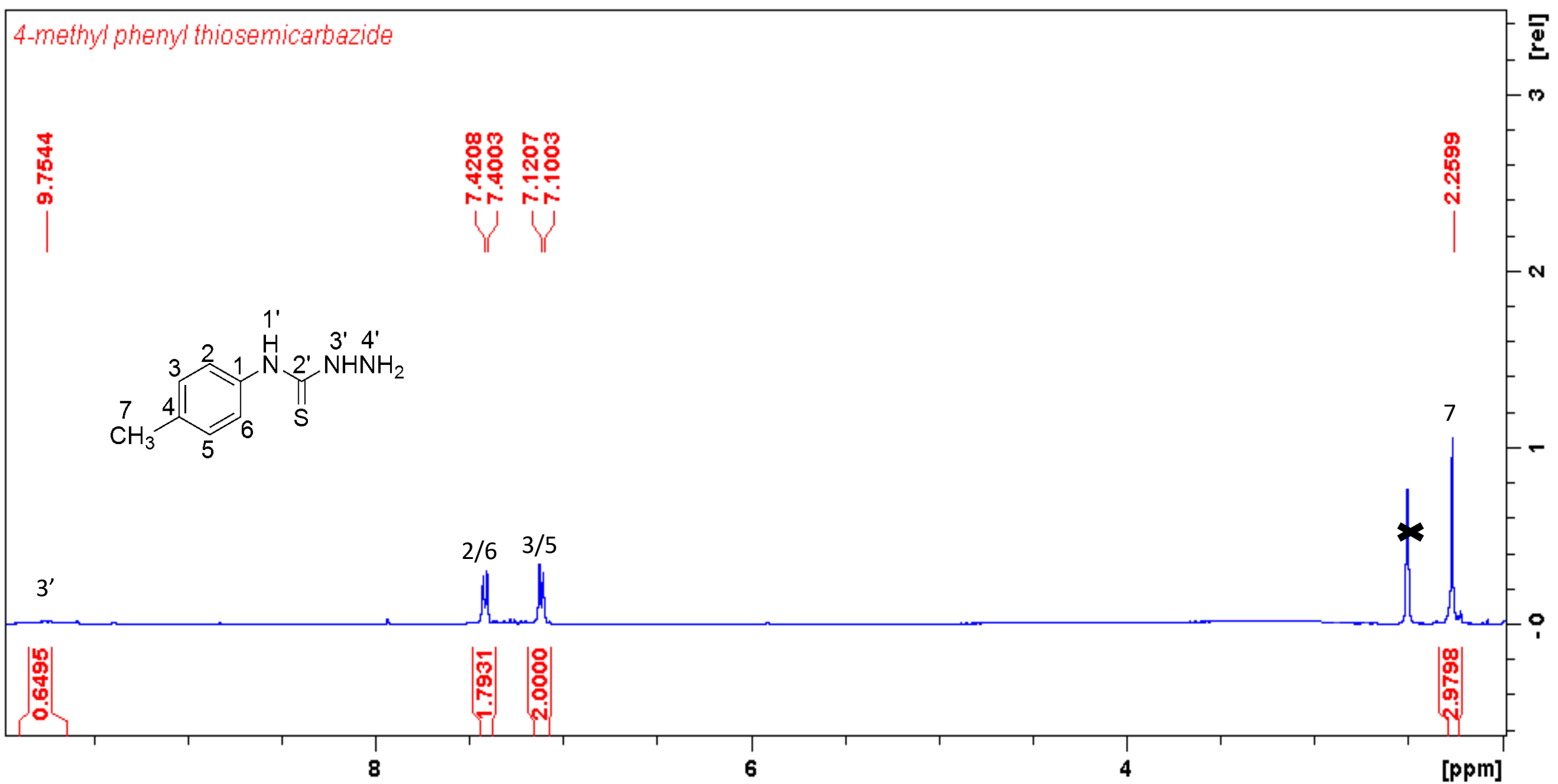
¹H-NMR spectrum of compound 5e

^{1}H-NMR spectrum of compound 6a

13

Aromatic region ¹H-NMR spectrum of compound 6a

14
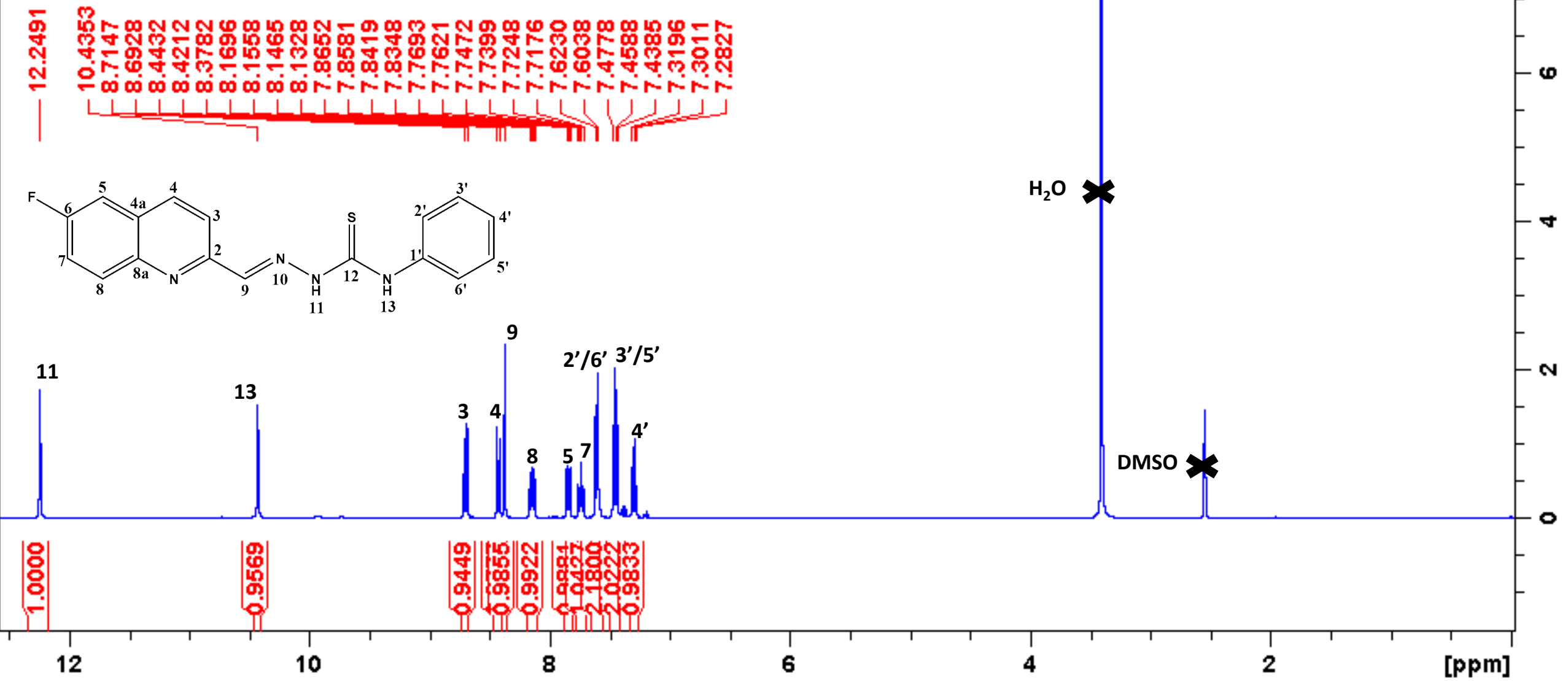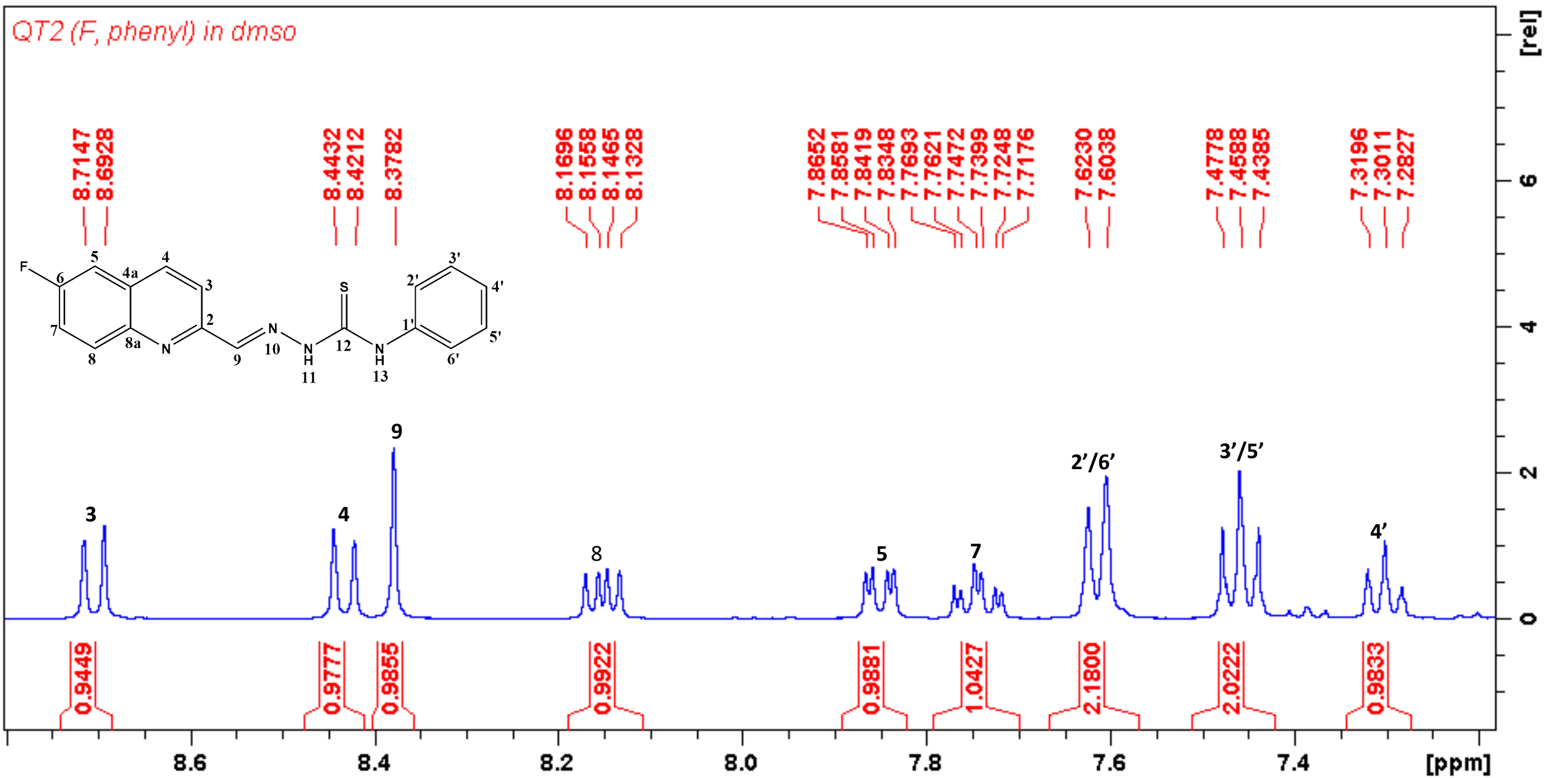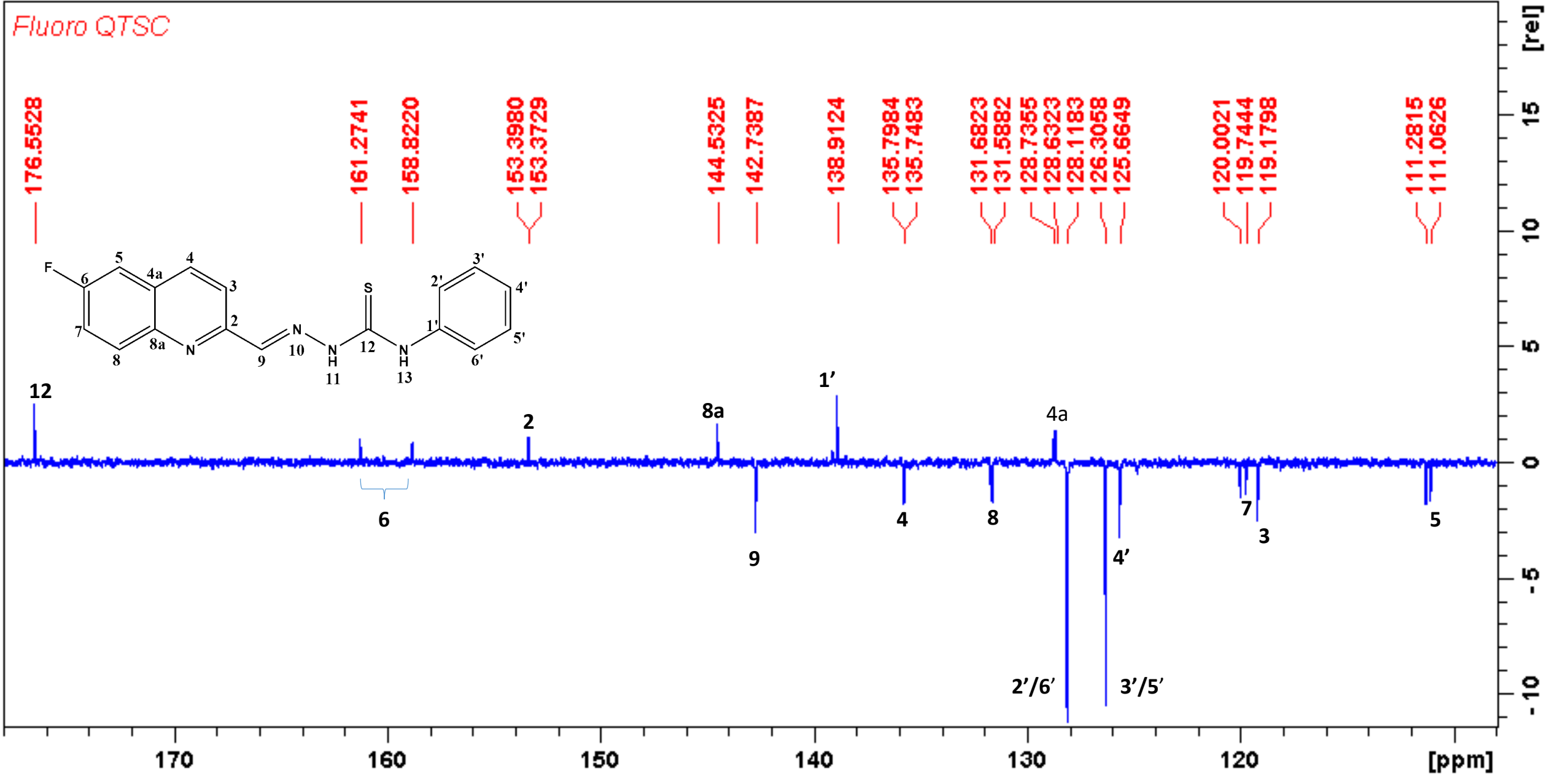
^{13}C-NMR spectrum of compound 6a
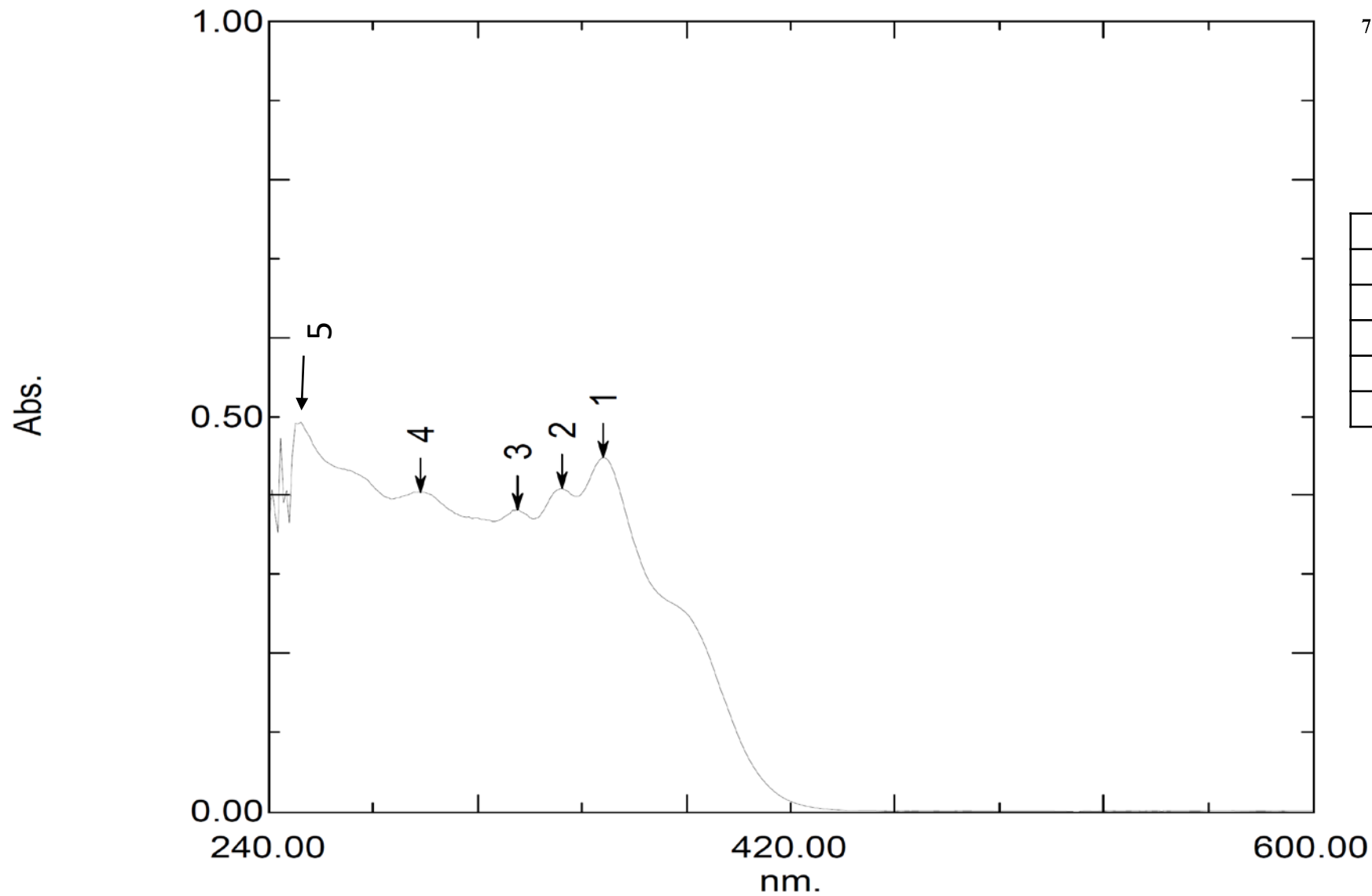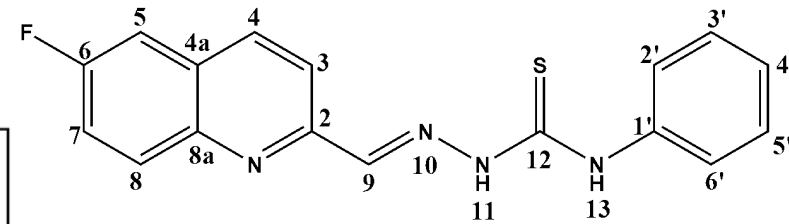
λmax	Absobance	Log ε
250	0.49	4.24
287	0.40	4.15
326	0.38	4.13
341	0.41	4.16
355	0.45	4.20

UV spectrum of compound 6a

16

IR spectrum of compound 6a

17

^{1}H-NMR spectrum of compound 6b

18
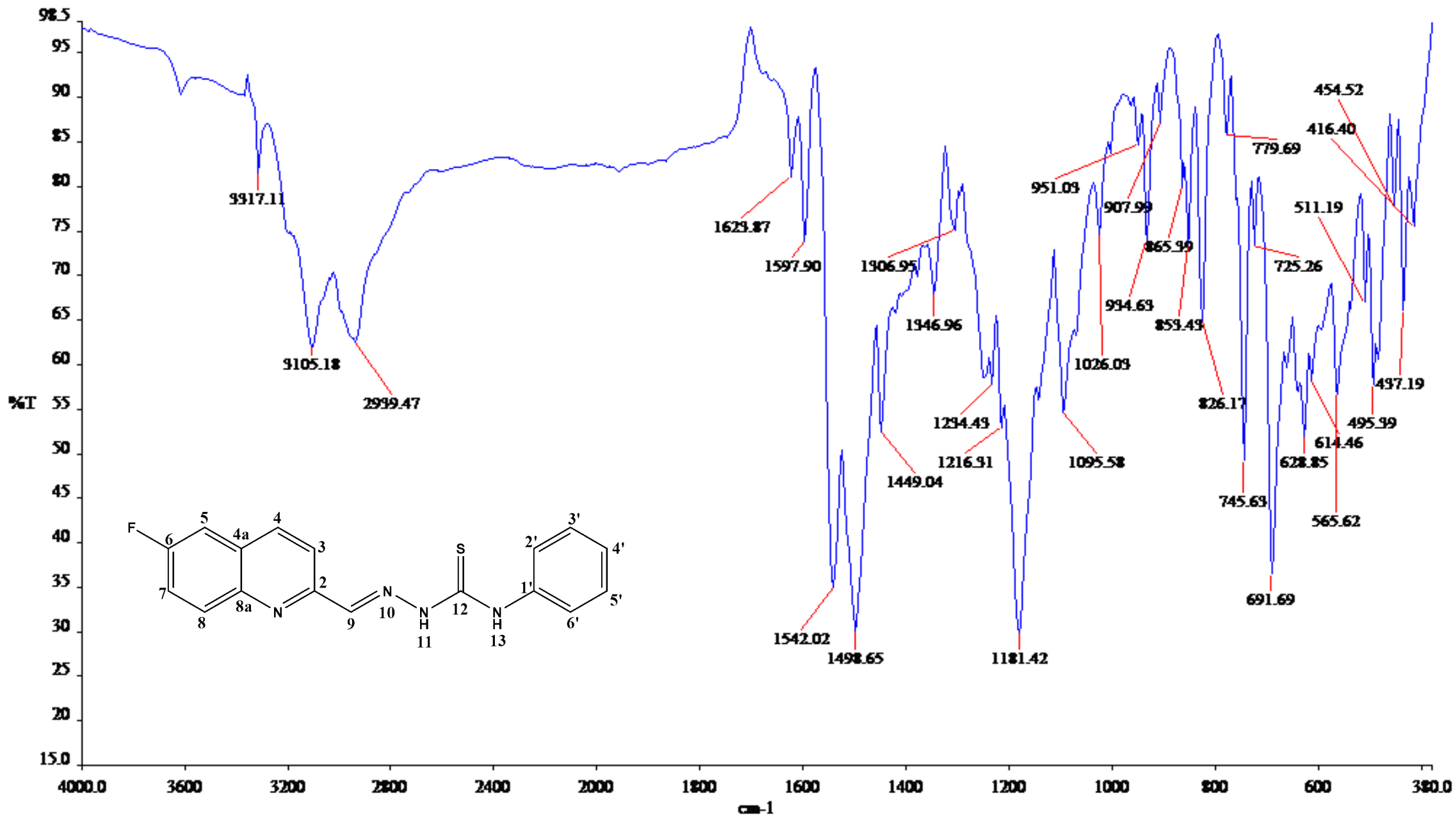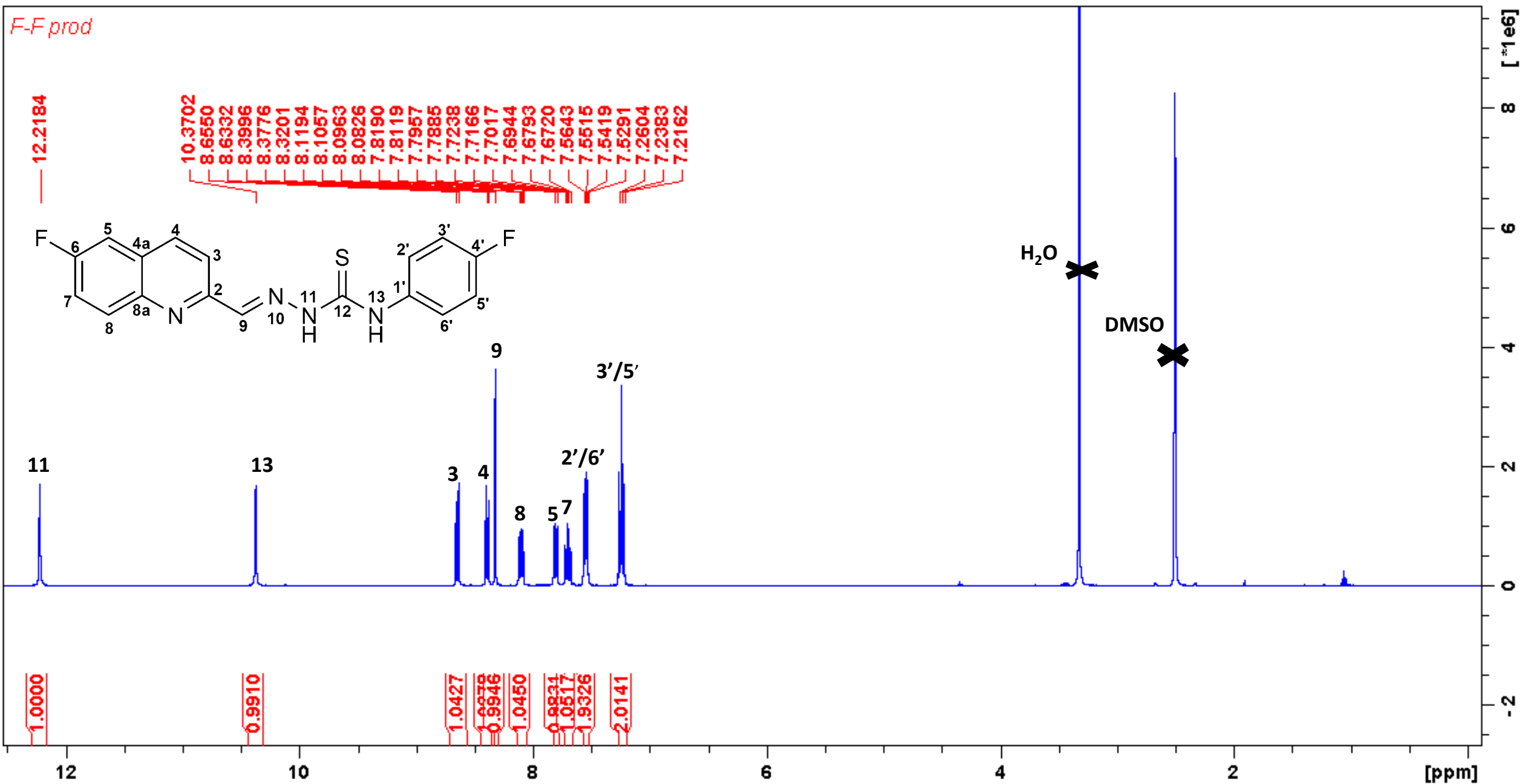
Aromatic region ^{1}H-NMR spectrum of compound 6b
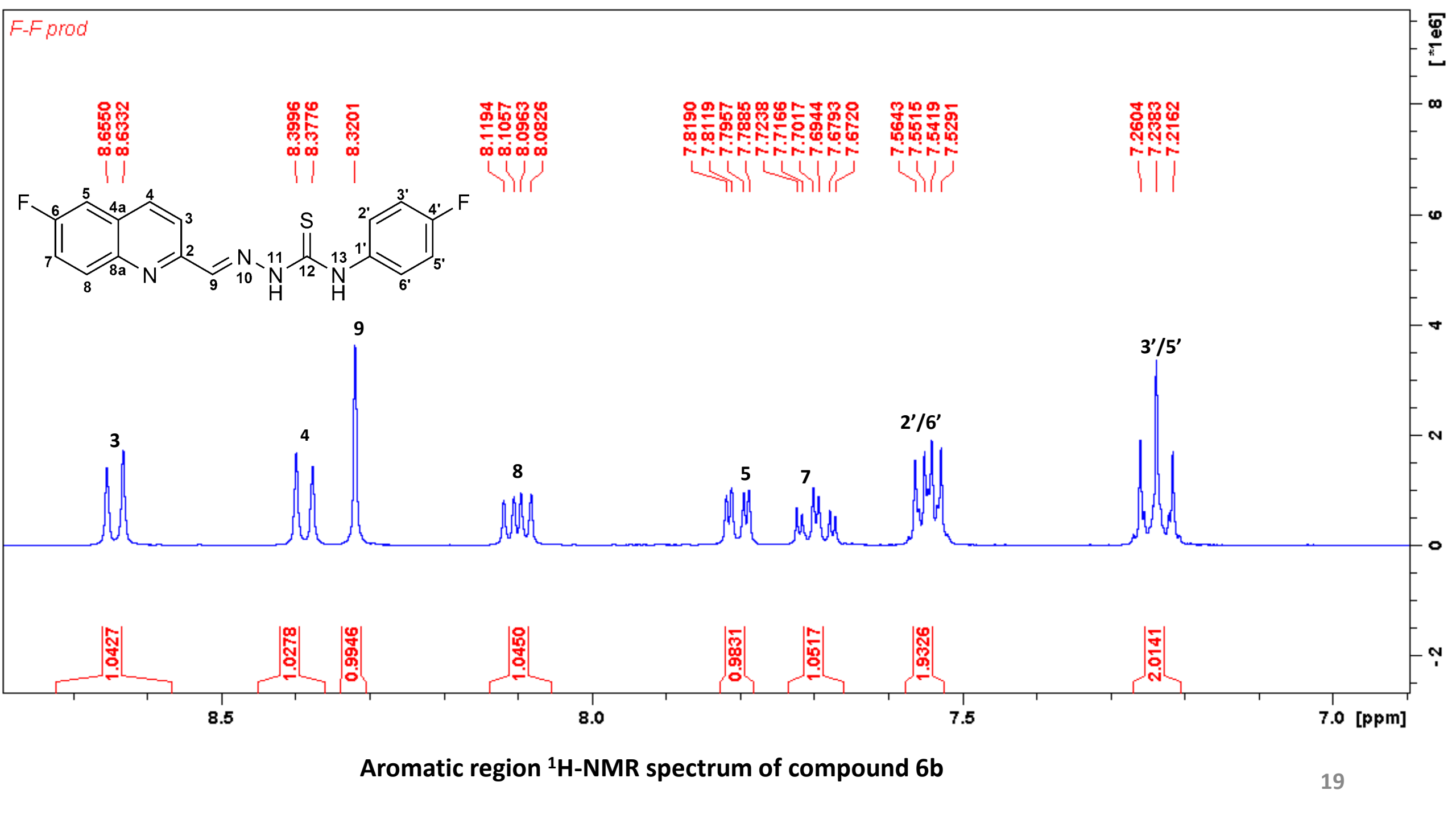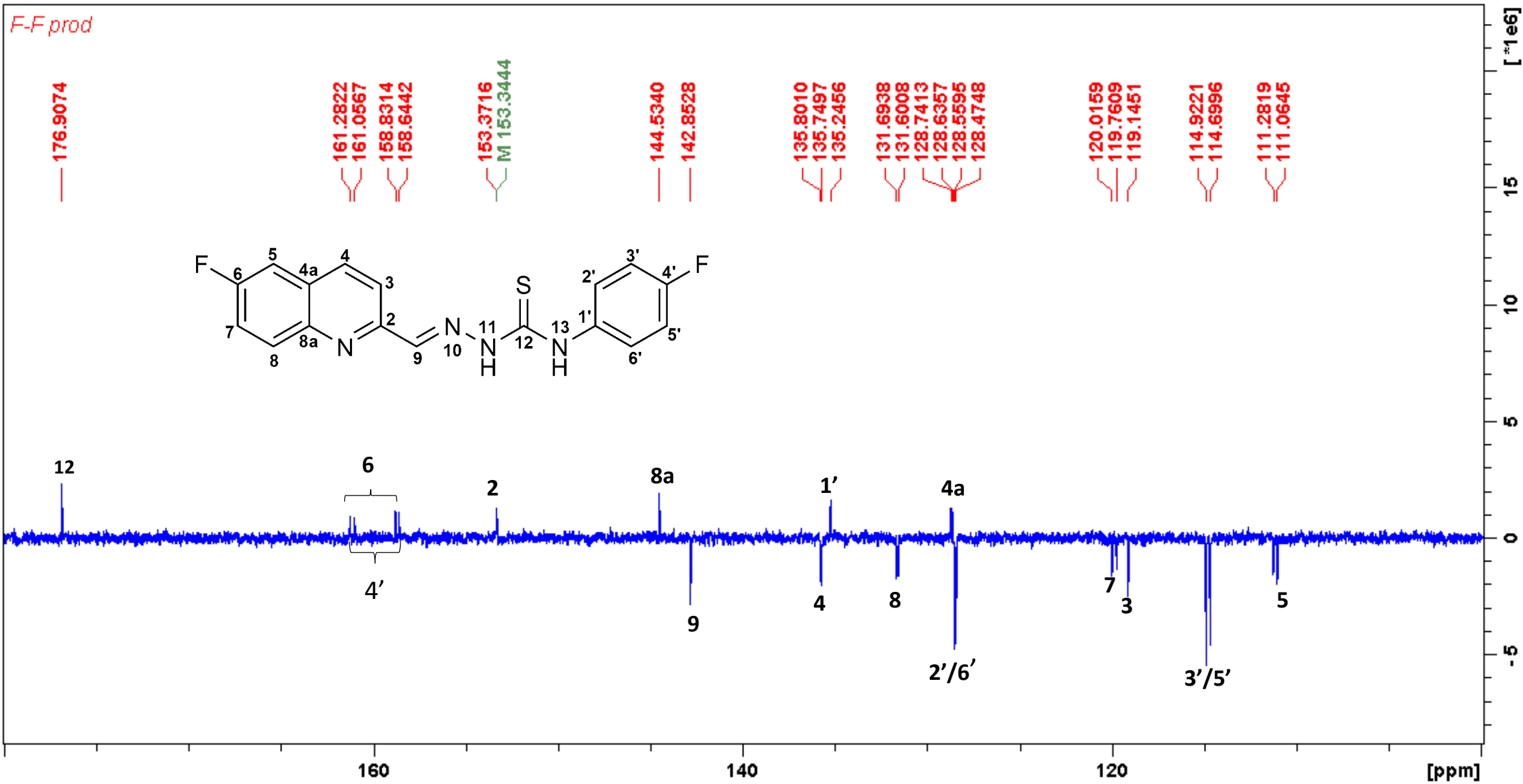
^{13}C-NMR spectrum of compound 6b

COSY spectrum of compound 6b

21

Aromatic region COSY spectrum of compound 6b

HSQC spectrum of compound 6b

23
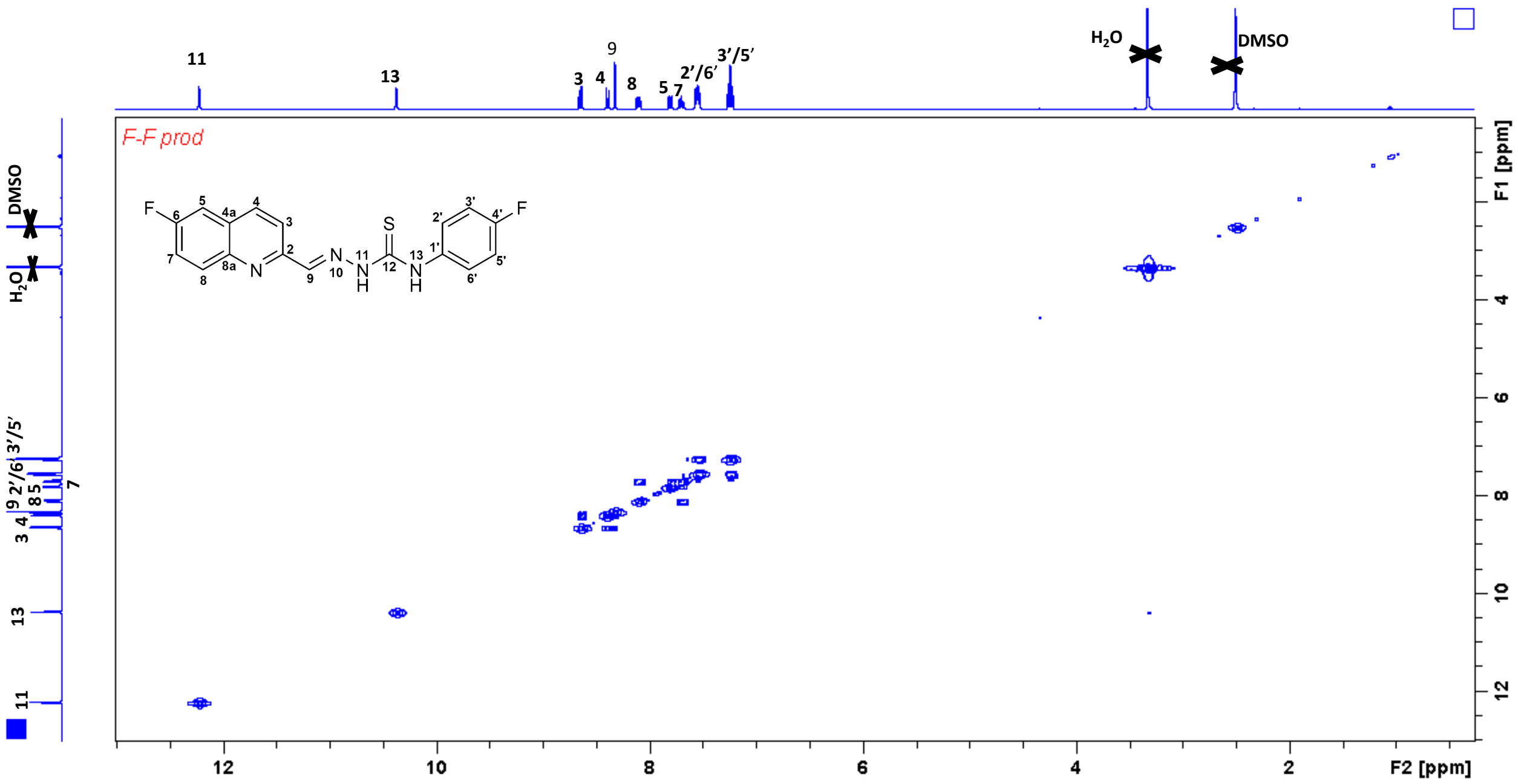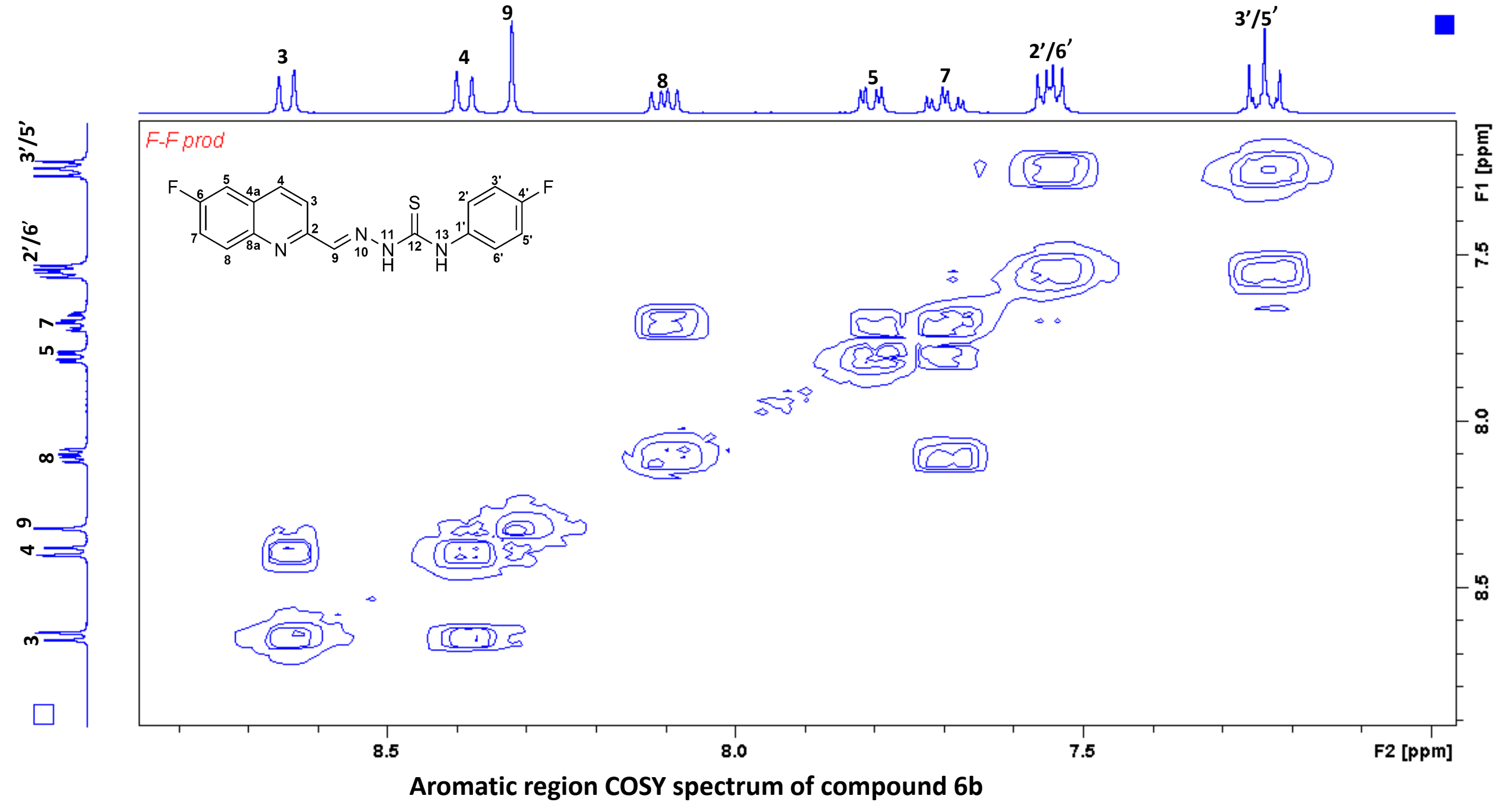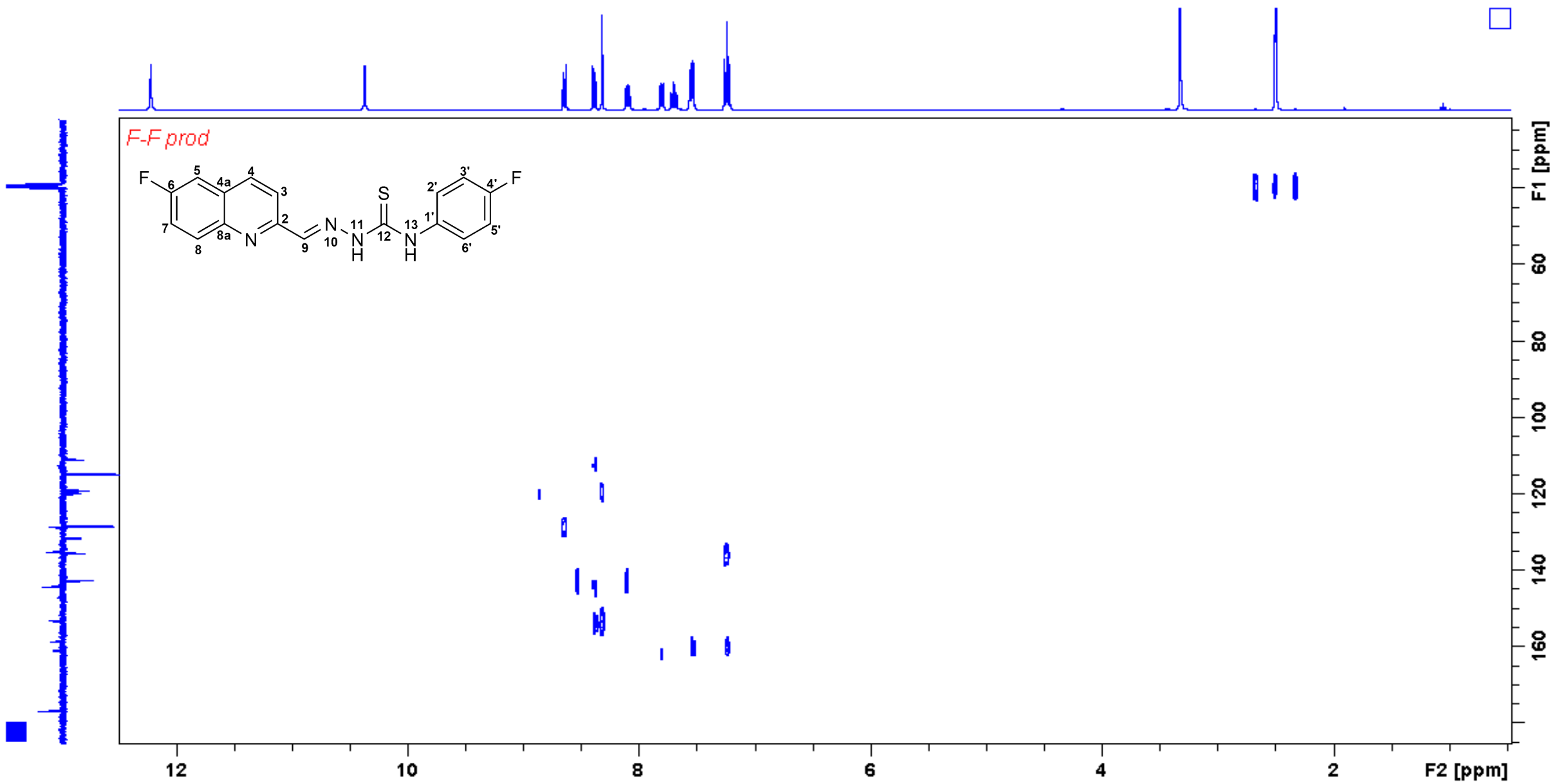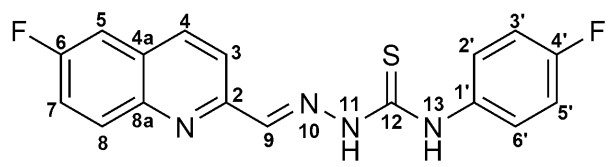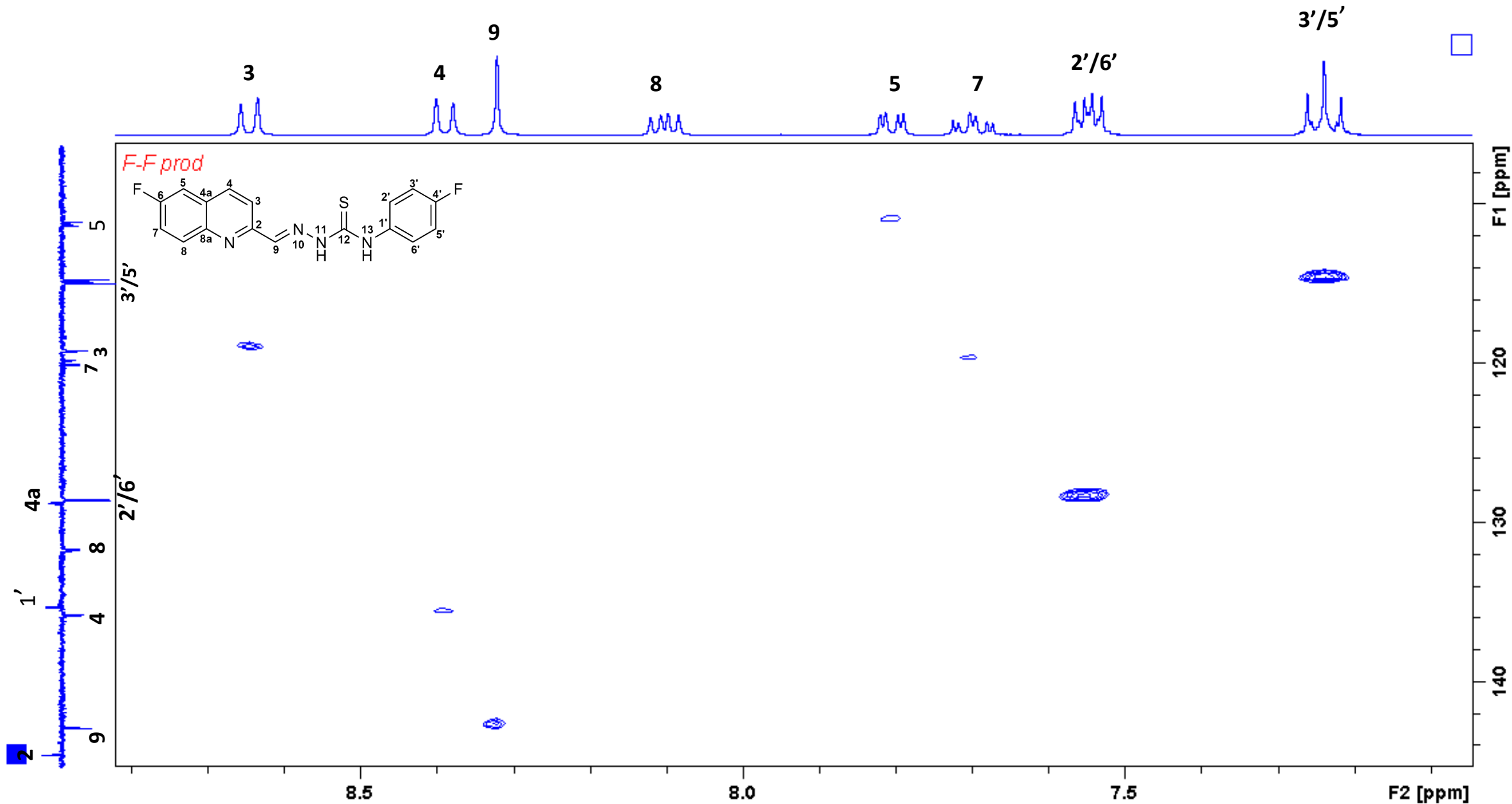
Aromatic region HSQC spectrum of compound 6b
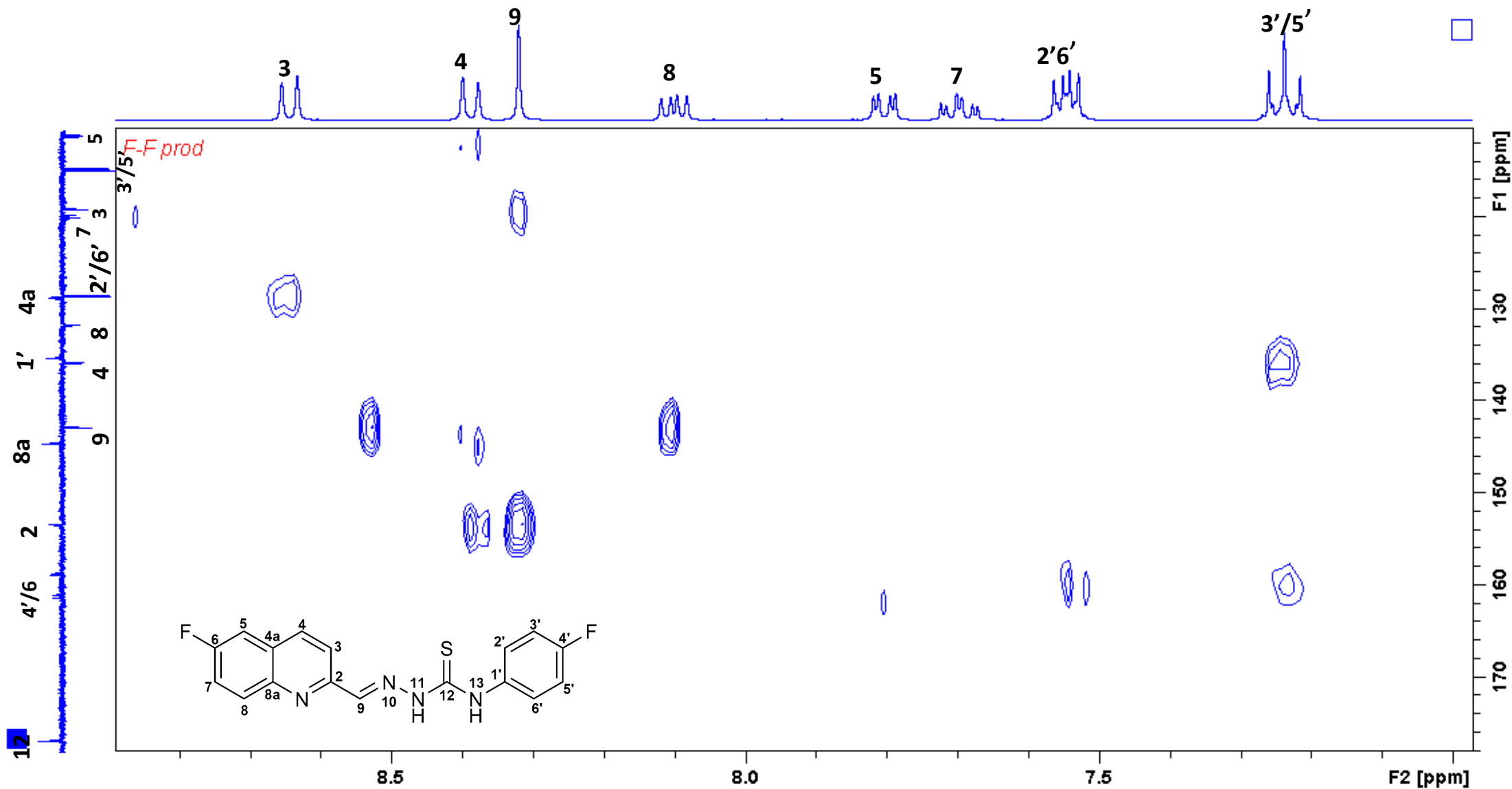
**Aromatic region HMBC spectrum of compound 6b**

25

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
14 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20   H: 10-15   N: 0-5   F: 0-2   S: 0-1
QTSC 2a 54 (1.788) Cm (1:61)
TOF MS ES-

2.86e+005
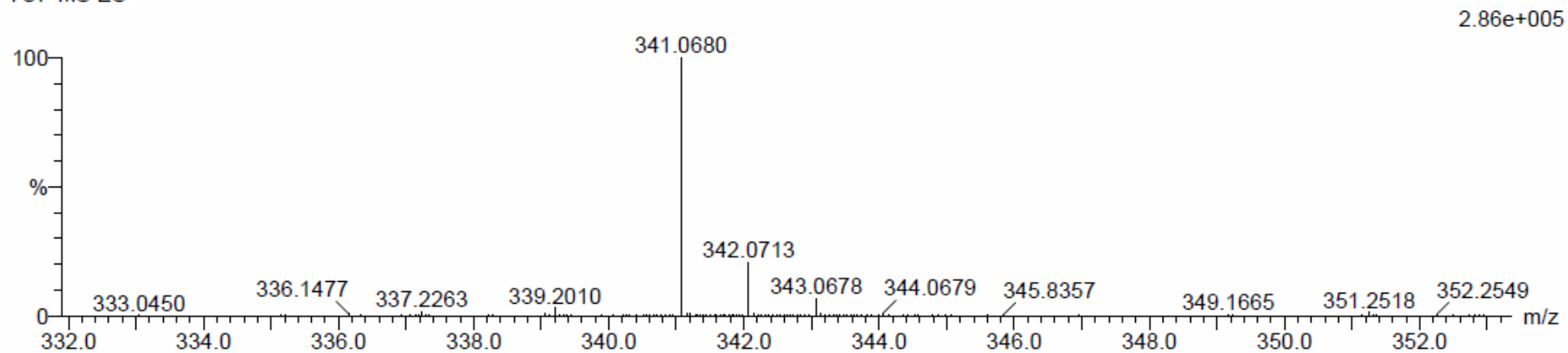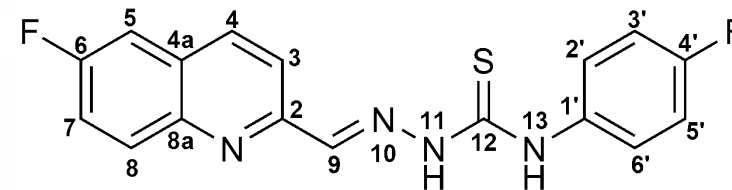
Minimum:			-1.5			
Maximum:	5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
341.0680	341.0672	0.8	2.3	13.5	661.1	0.0	C17 H11 N4 F2 S

HRMS of compound 6b

26
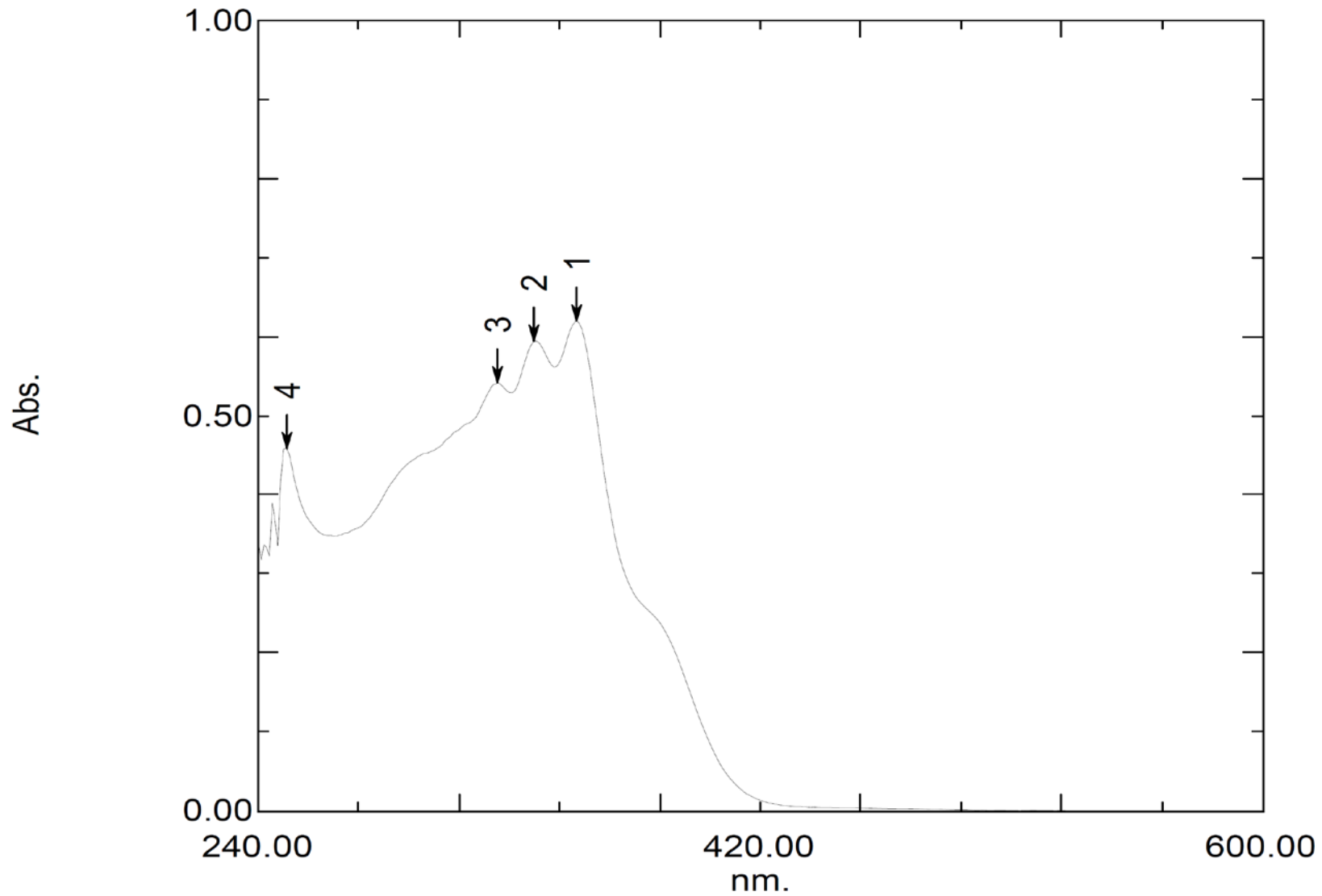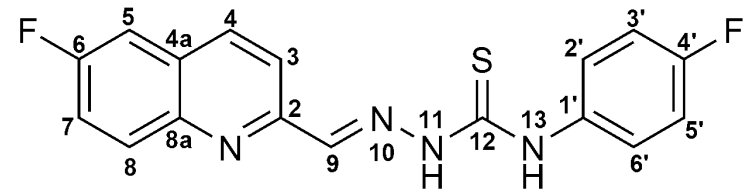
λmax	Absobance	Log ε
250	0.46	4.24
326	0.54	4.31
340	0.59	4.35
354	0.62	4.37

UV spectrum of compound 6b

27
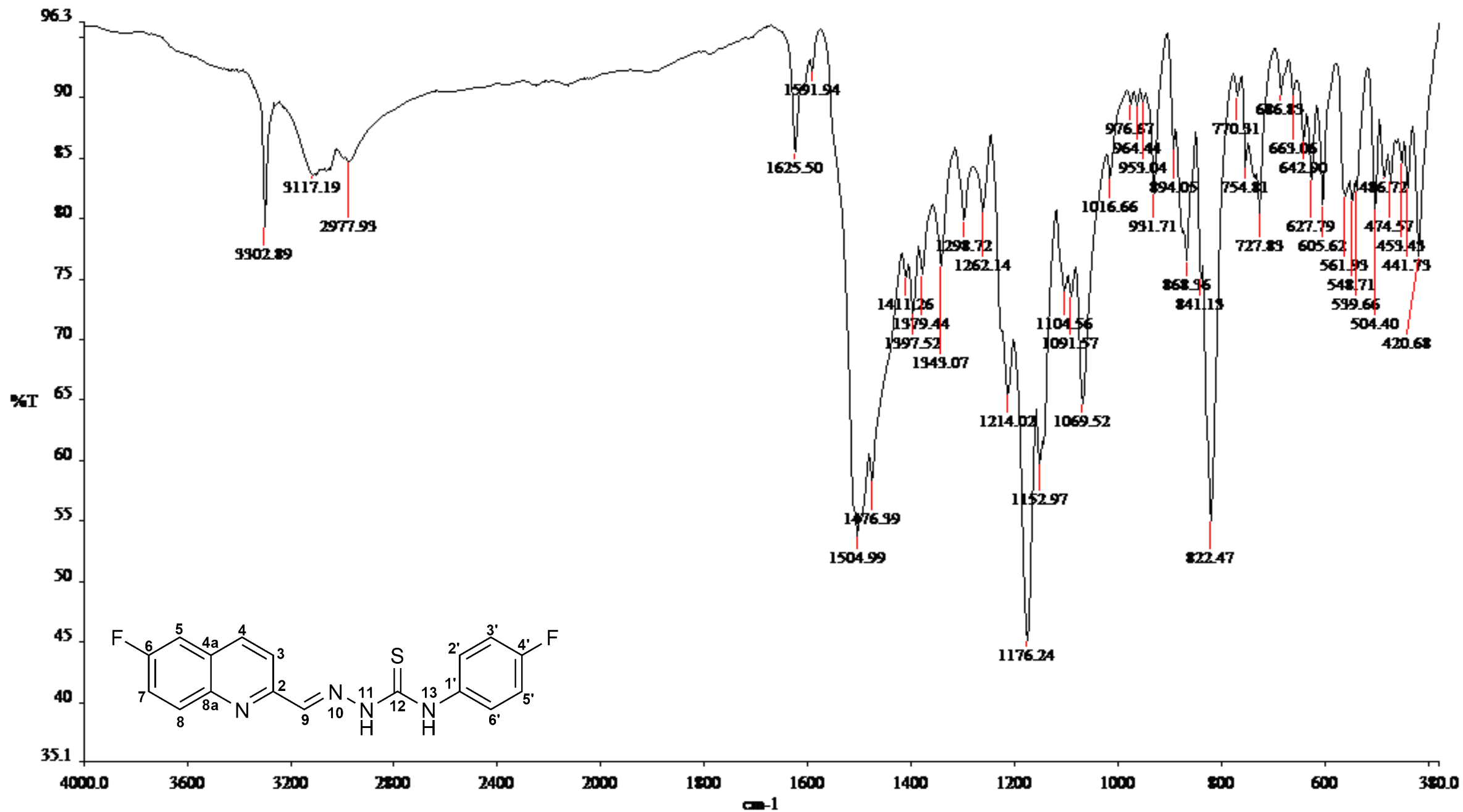
IR spectrum of compound 6b
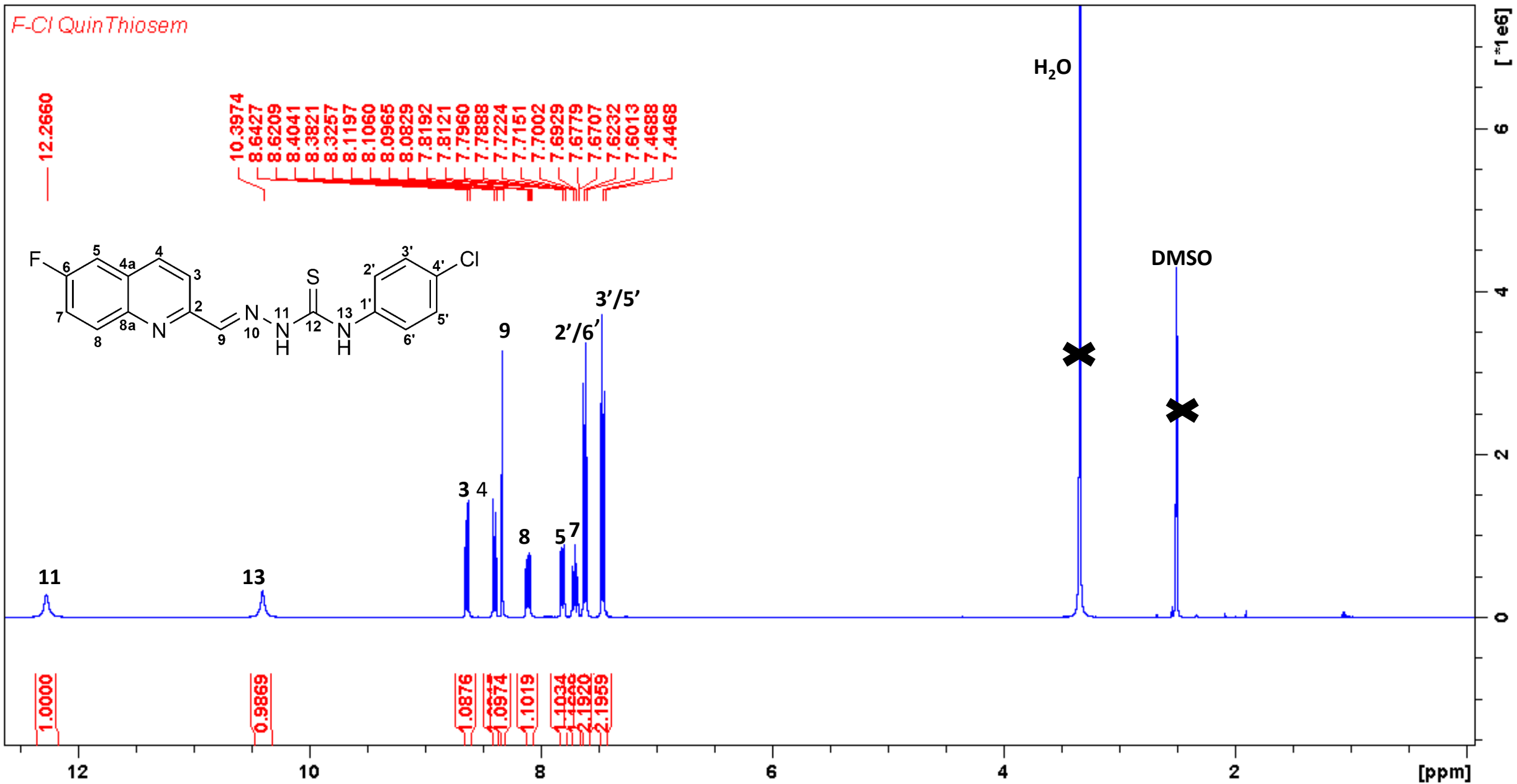
^{1}H-NMR spectrum of compound 6c
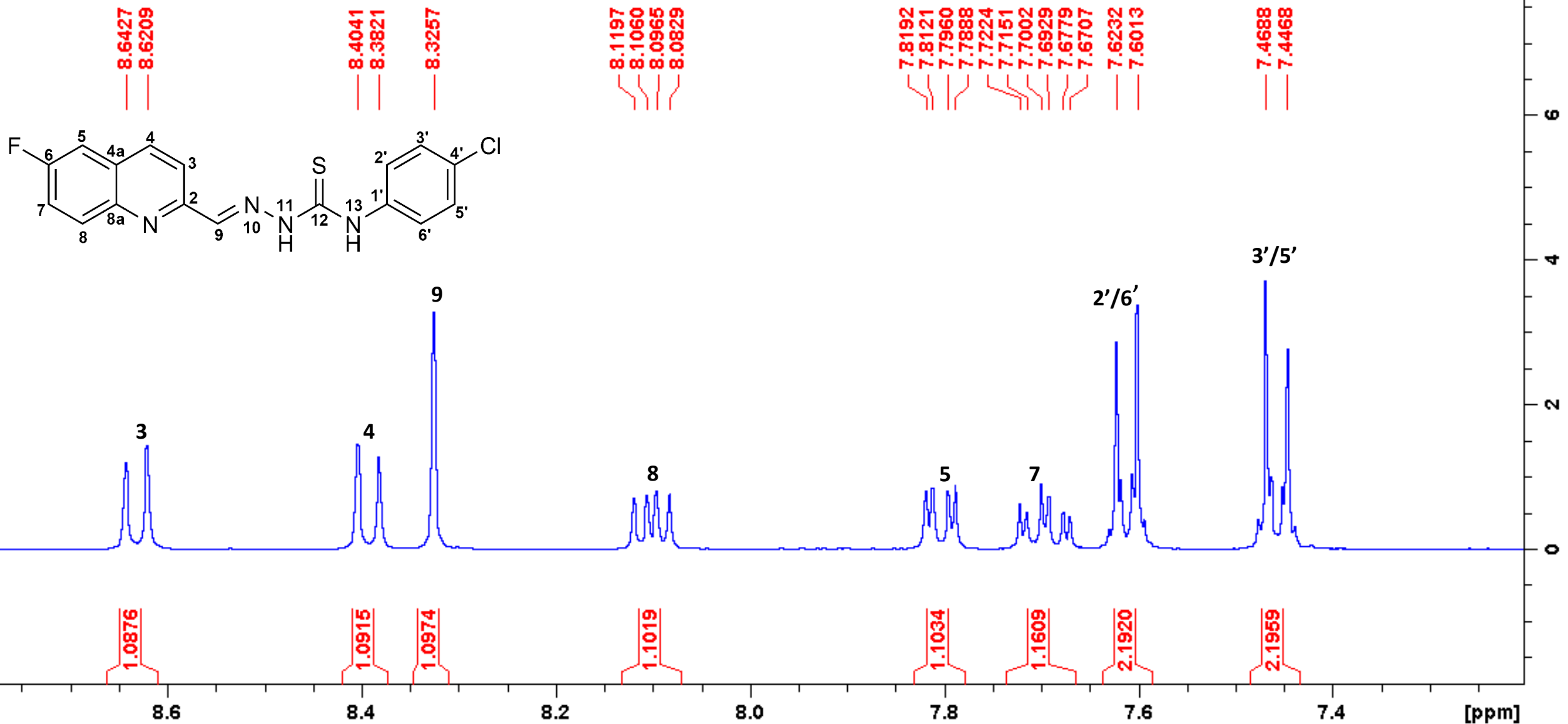
Aromatic region ^{1}H-NMR spectrum of compound 6c
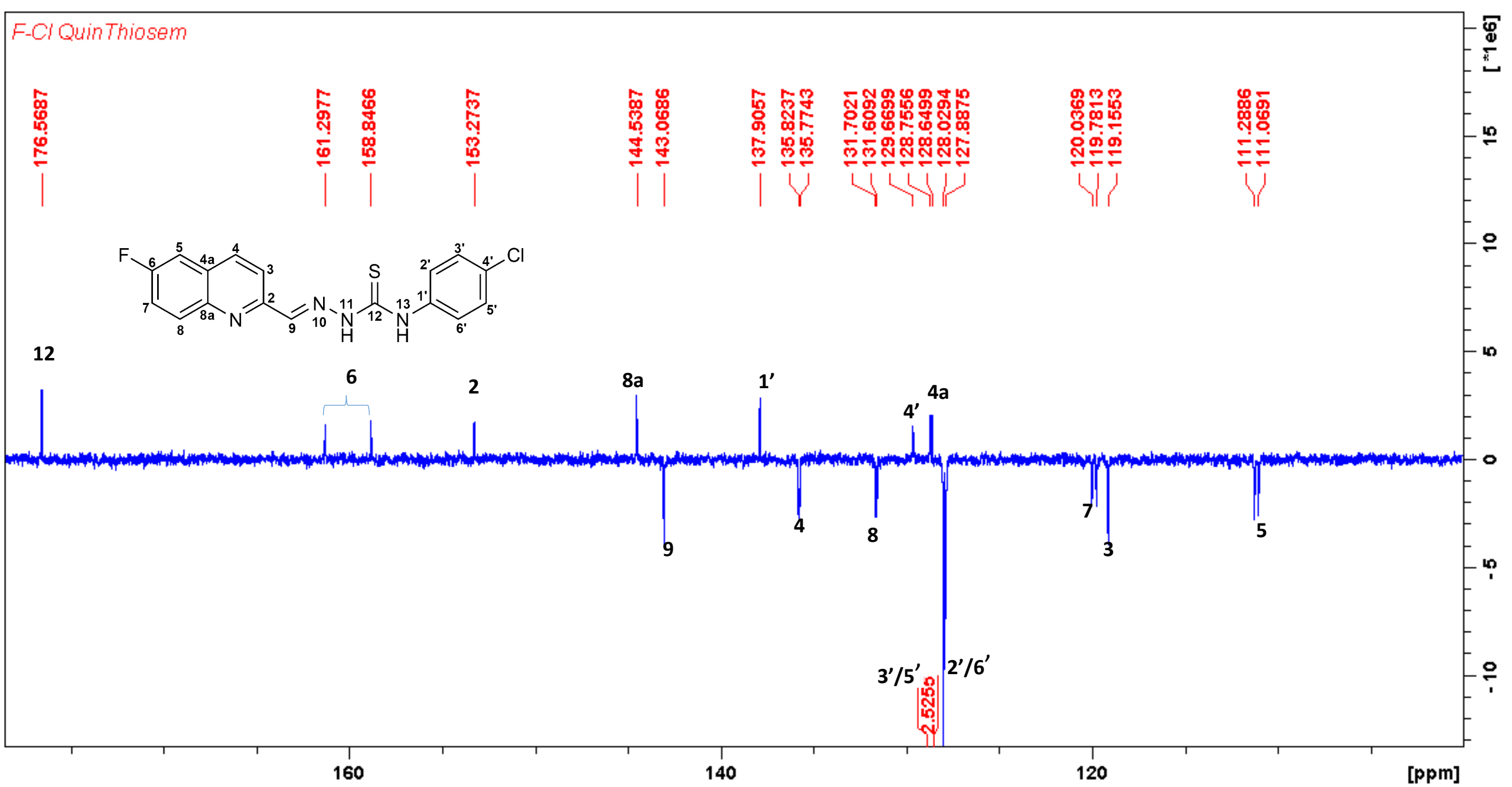
^{13}C-NMR spectrum of compound 6c
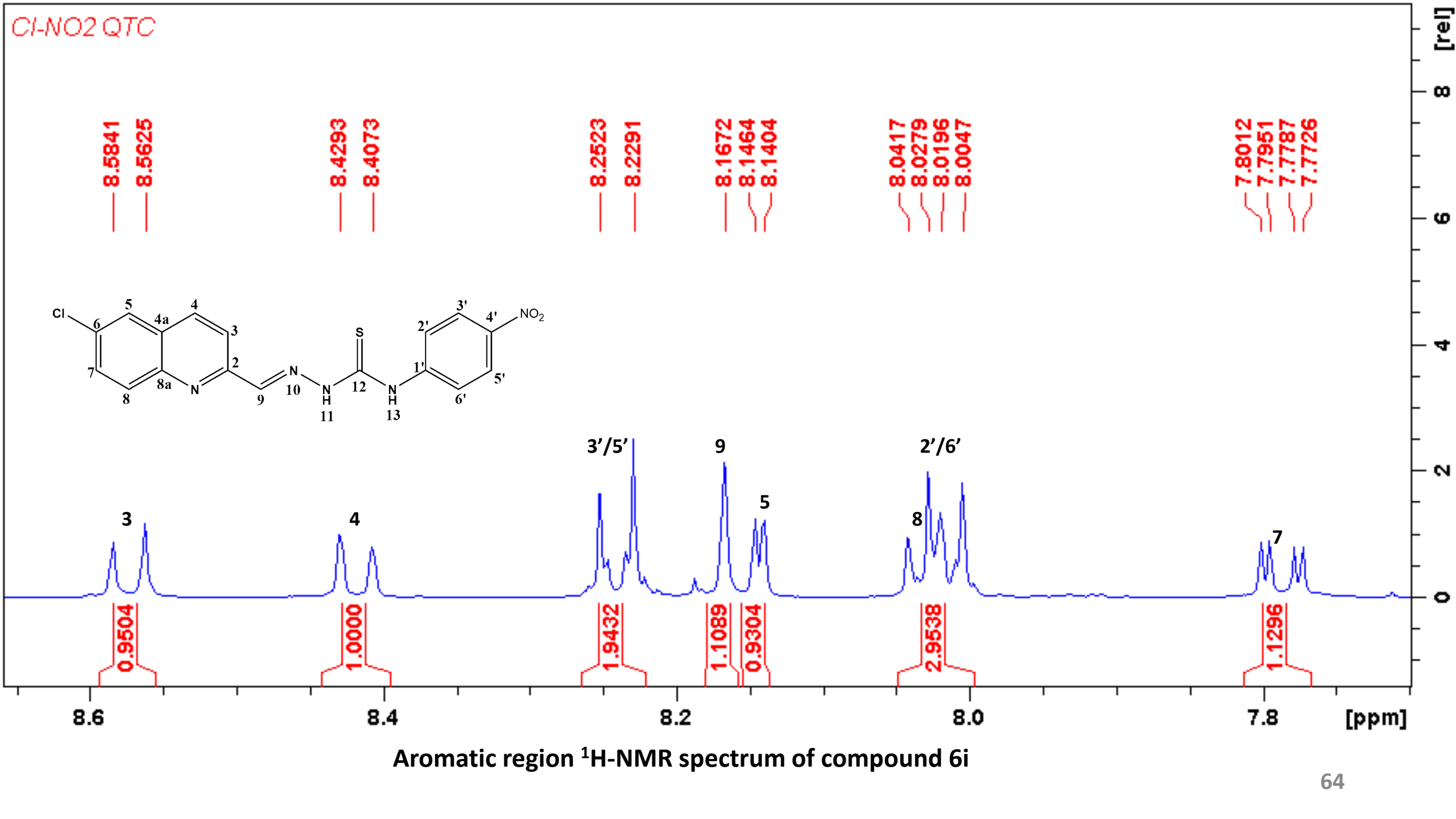
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
45 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20   H: 10-15   N: 0-5   F: 0-2   S: 0-1   Cl: 0-2

QTSC 2b 2 (0.068) Cm (1:60)
TOF MS ES-
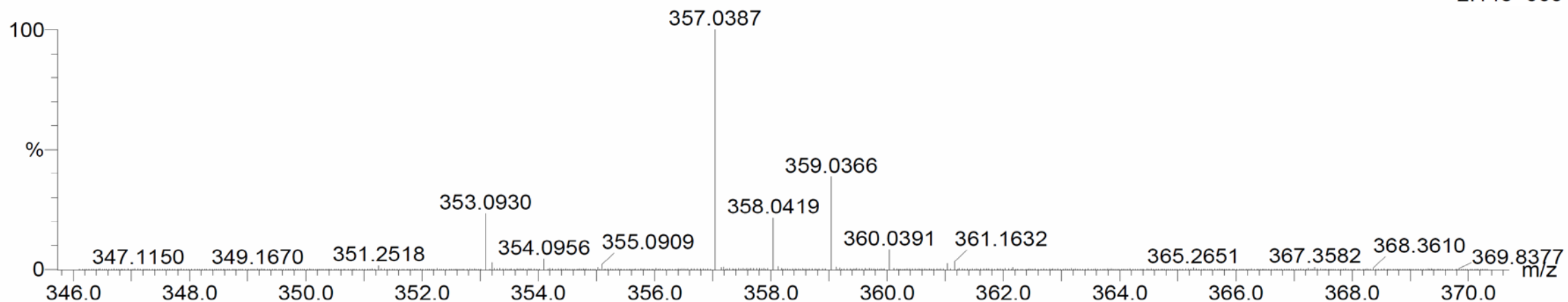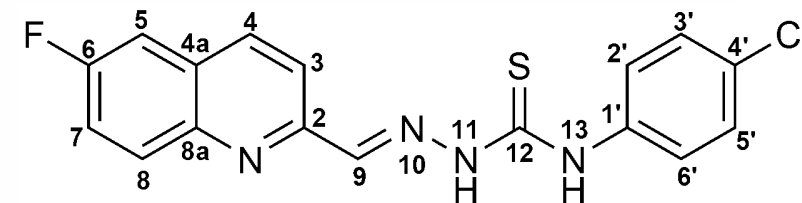
2.14e+005

| Minimum: | | | | -1.5 | | | |
| Maximum: | | 5.0 | 5.0 | 100.0 | | | |

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
357.0387	357.0377	1.0	2.8	13.5	631.8	0.0	C17 H11 N4 F S Cl

HRMS of compound 6c

32
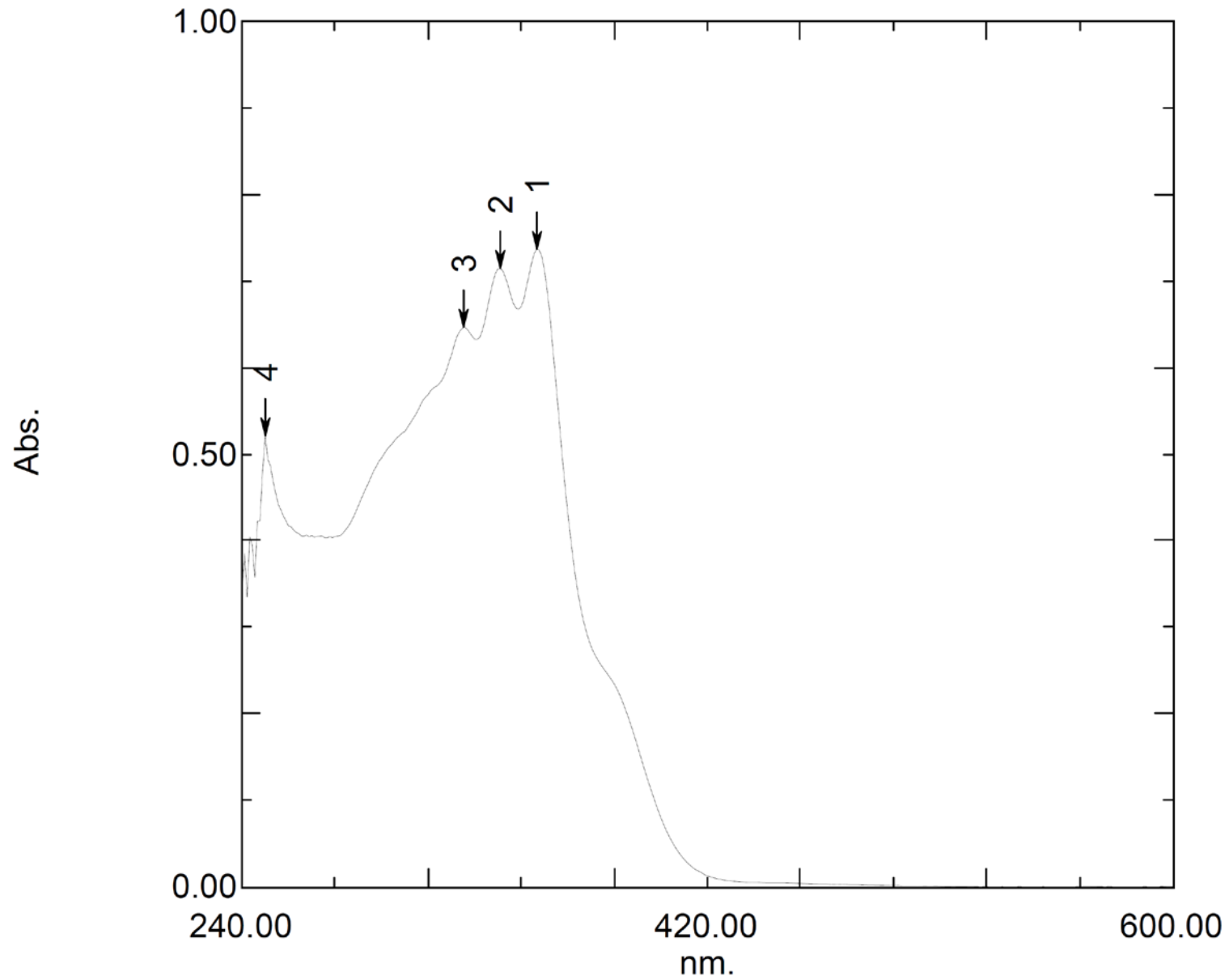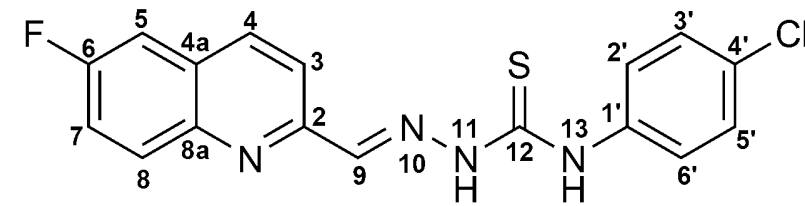
λmax	Absobance	Log ε
250	0.64	4.42
285	0.36	4.17
310	0.41	4.23
361	0.57	4.37

UV spectrum of compound 6c
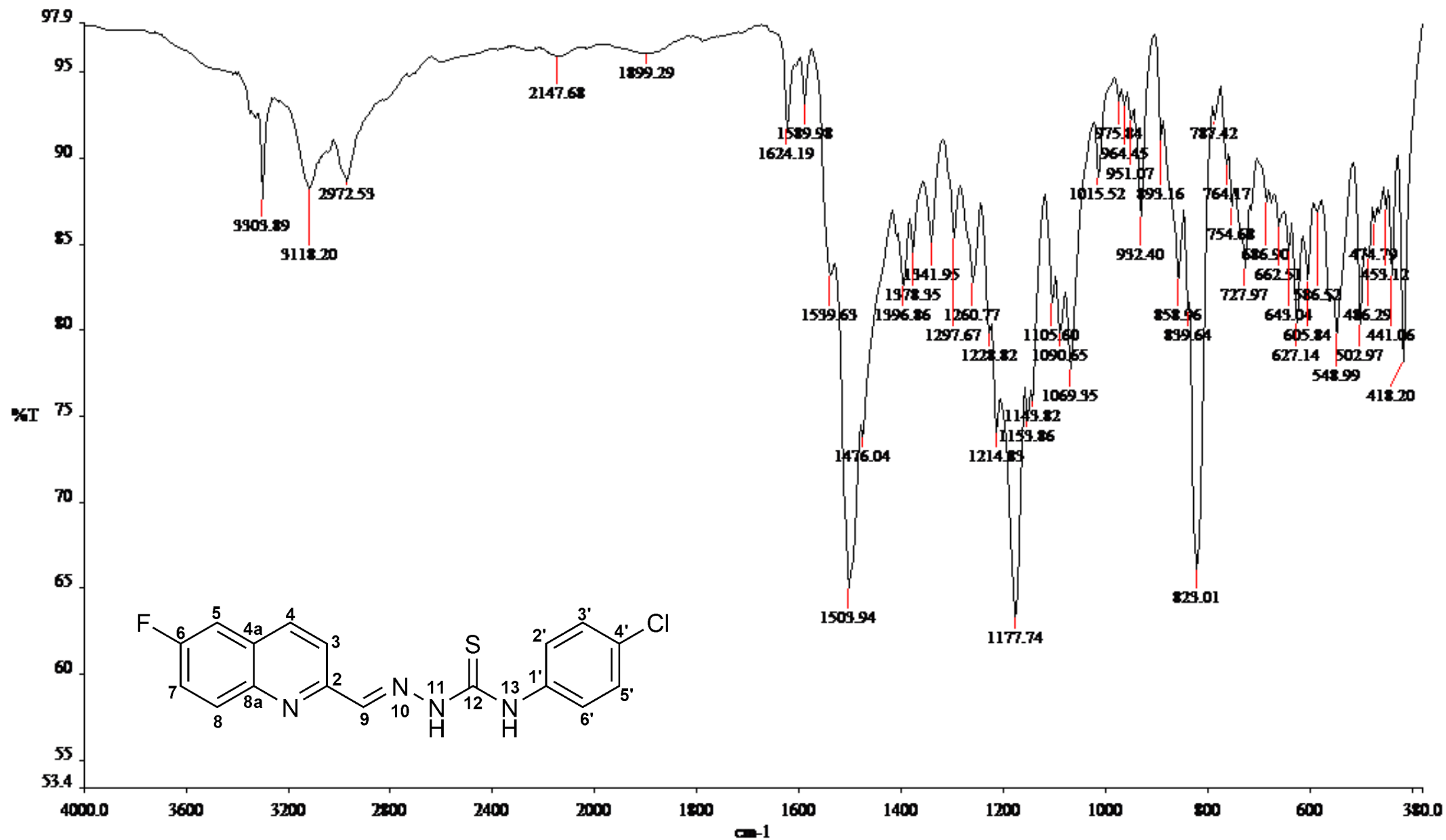
IR spectrum of compound 6c

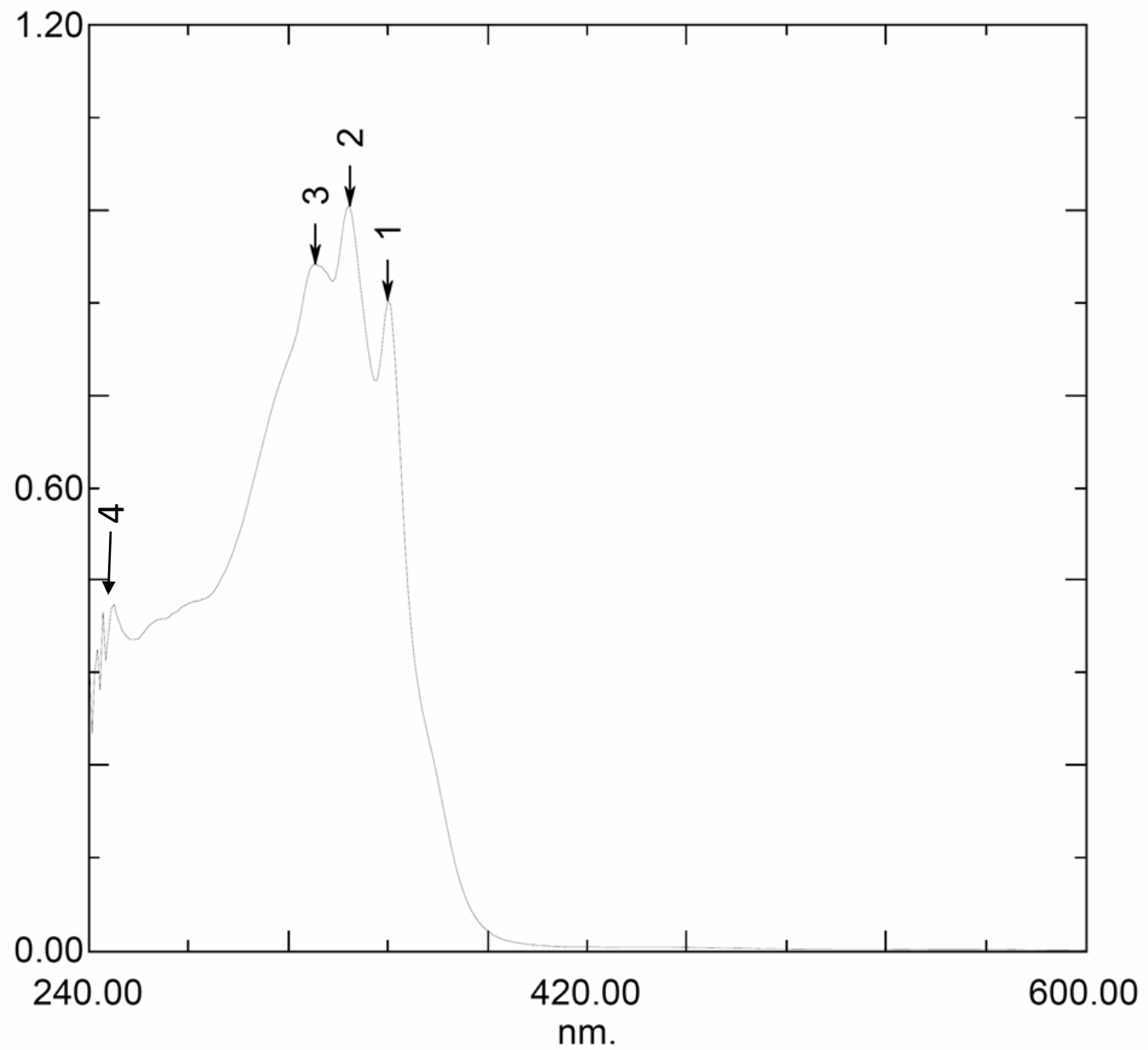^{1}H-NMR spectrum of compound 6d

35
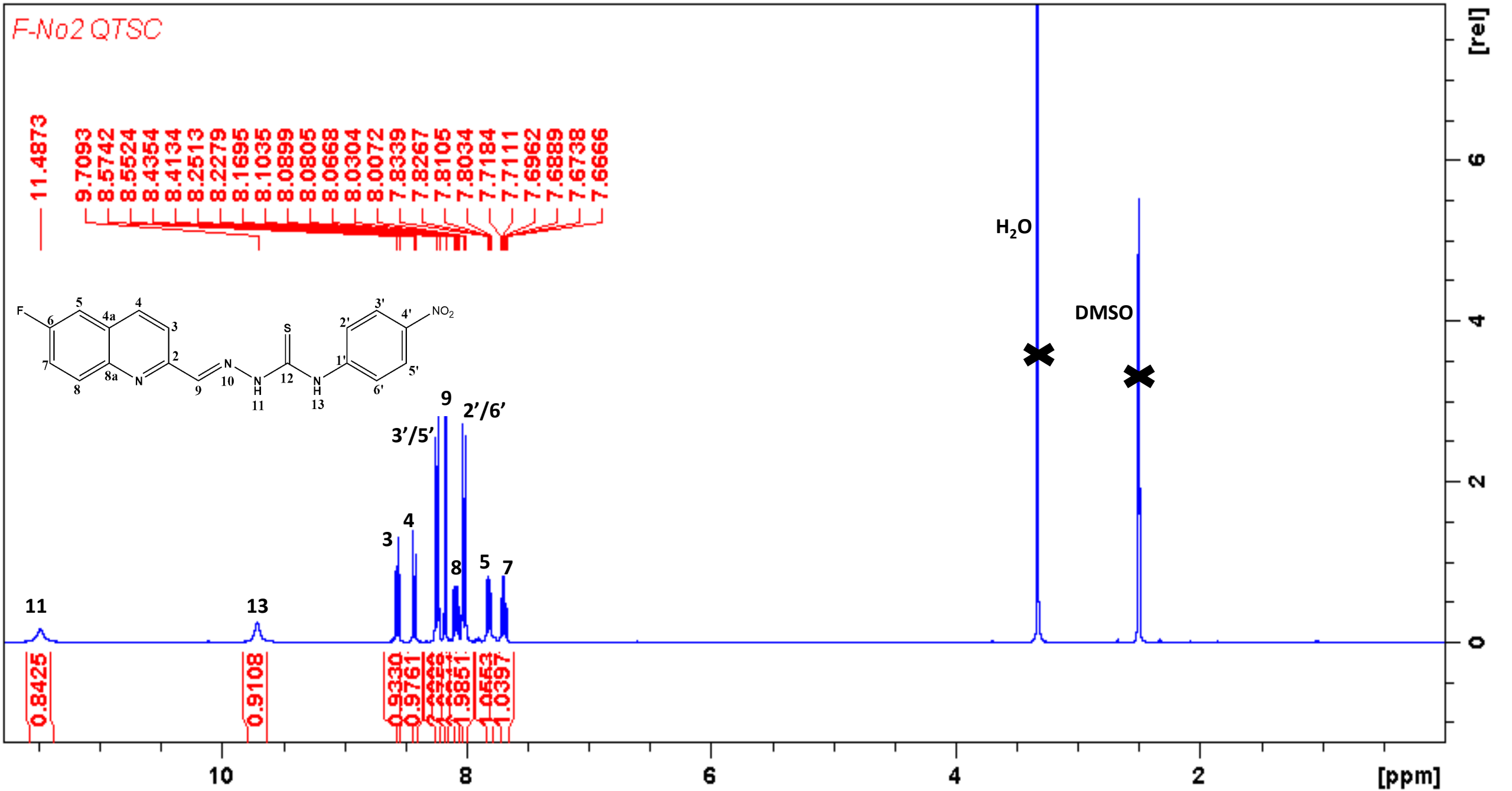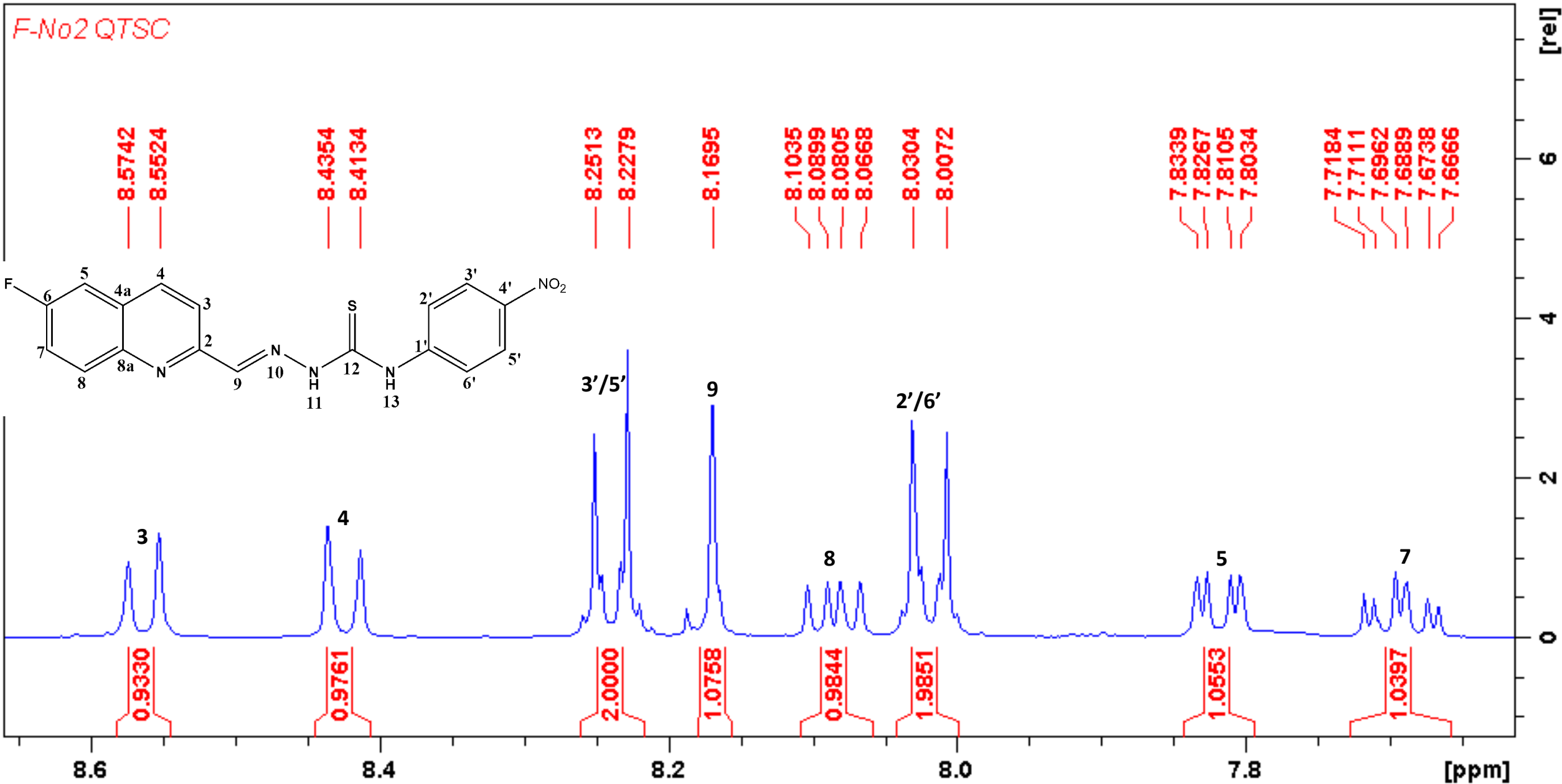
Aromatic region ¹H-NMR spectrum of compound 6d
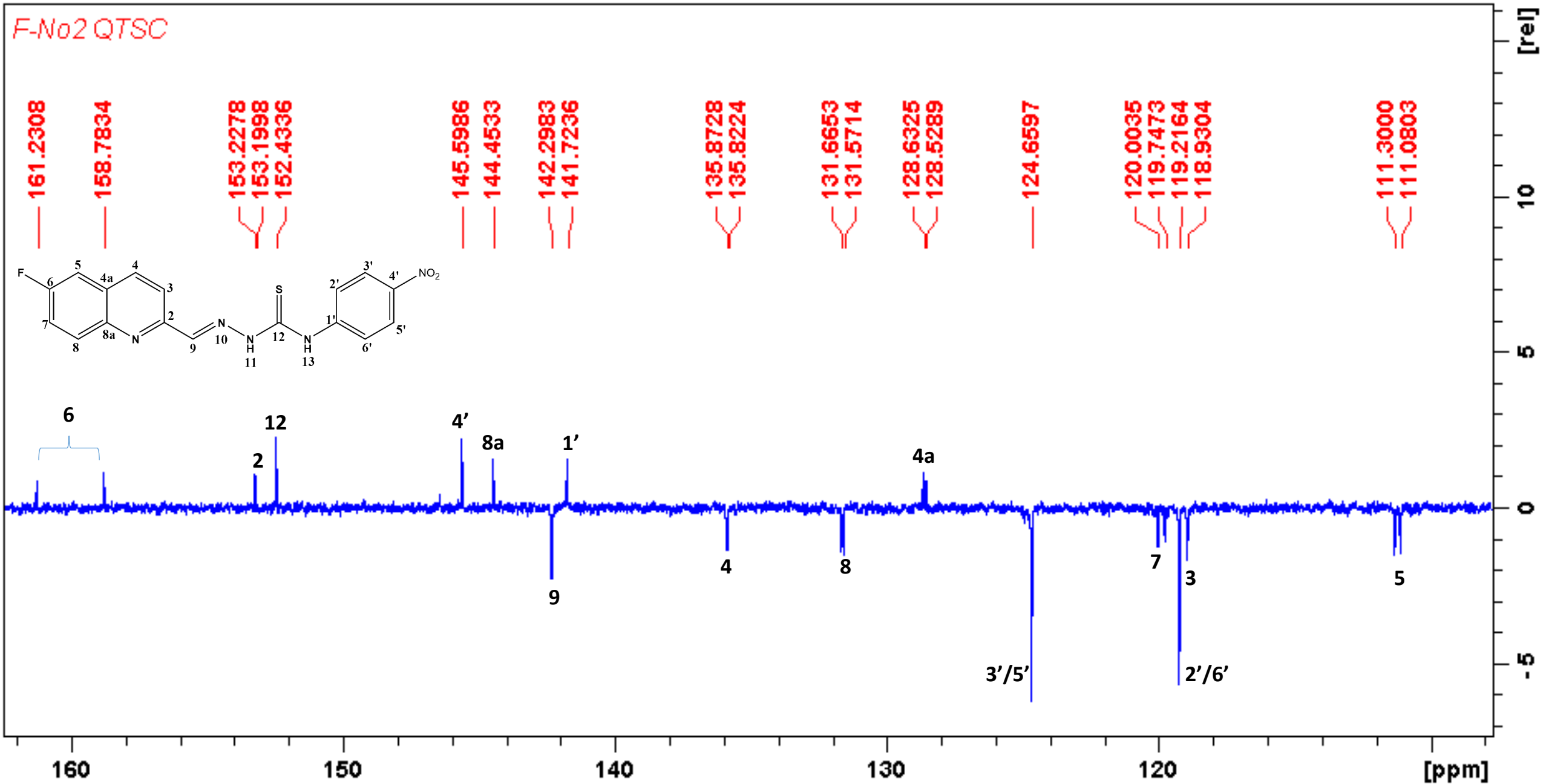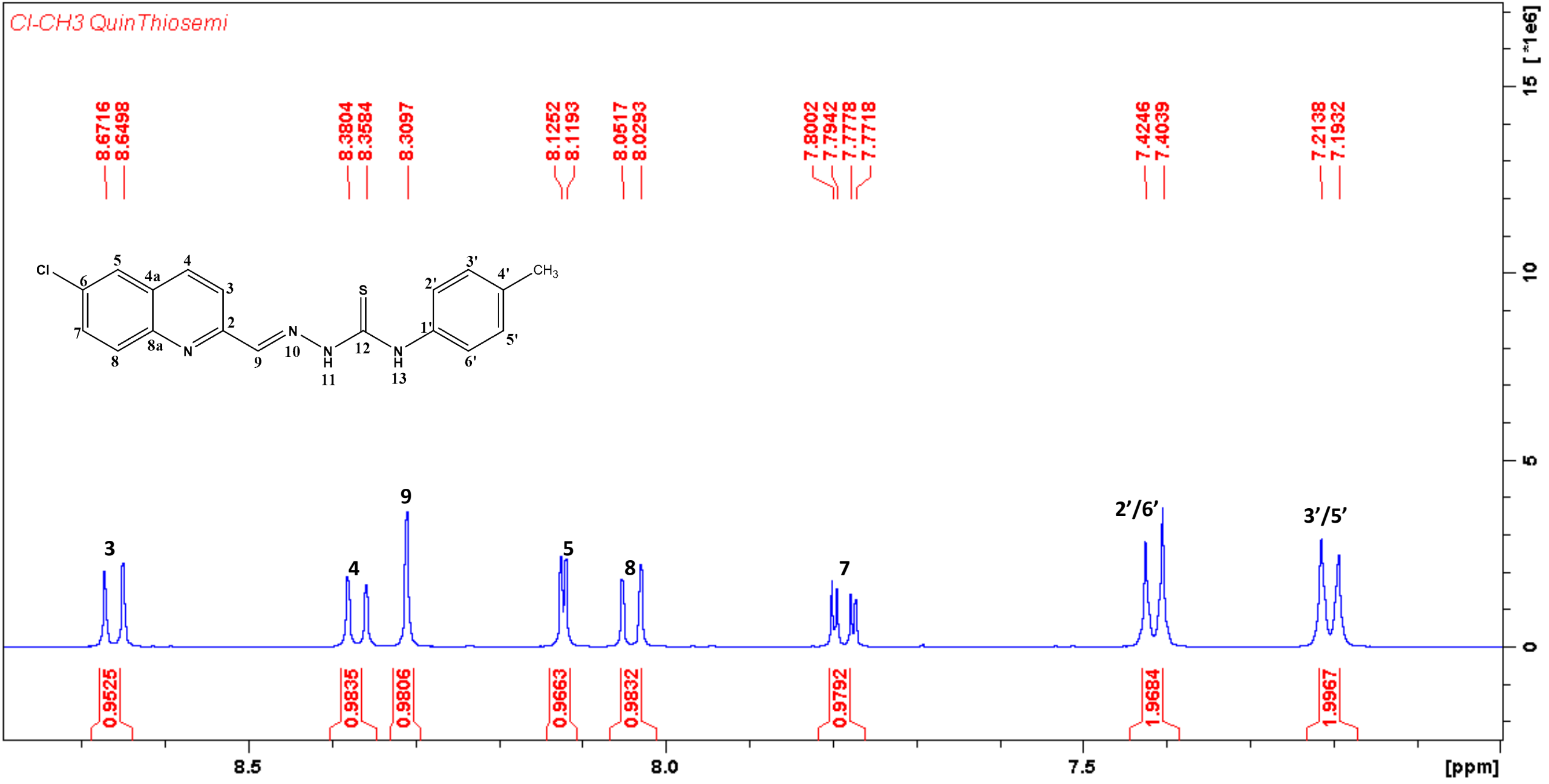
¹³C-NMR spectrum of compound 6d
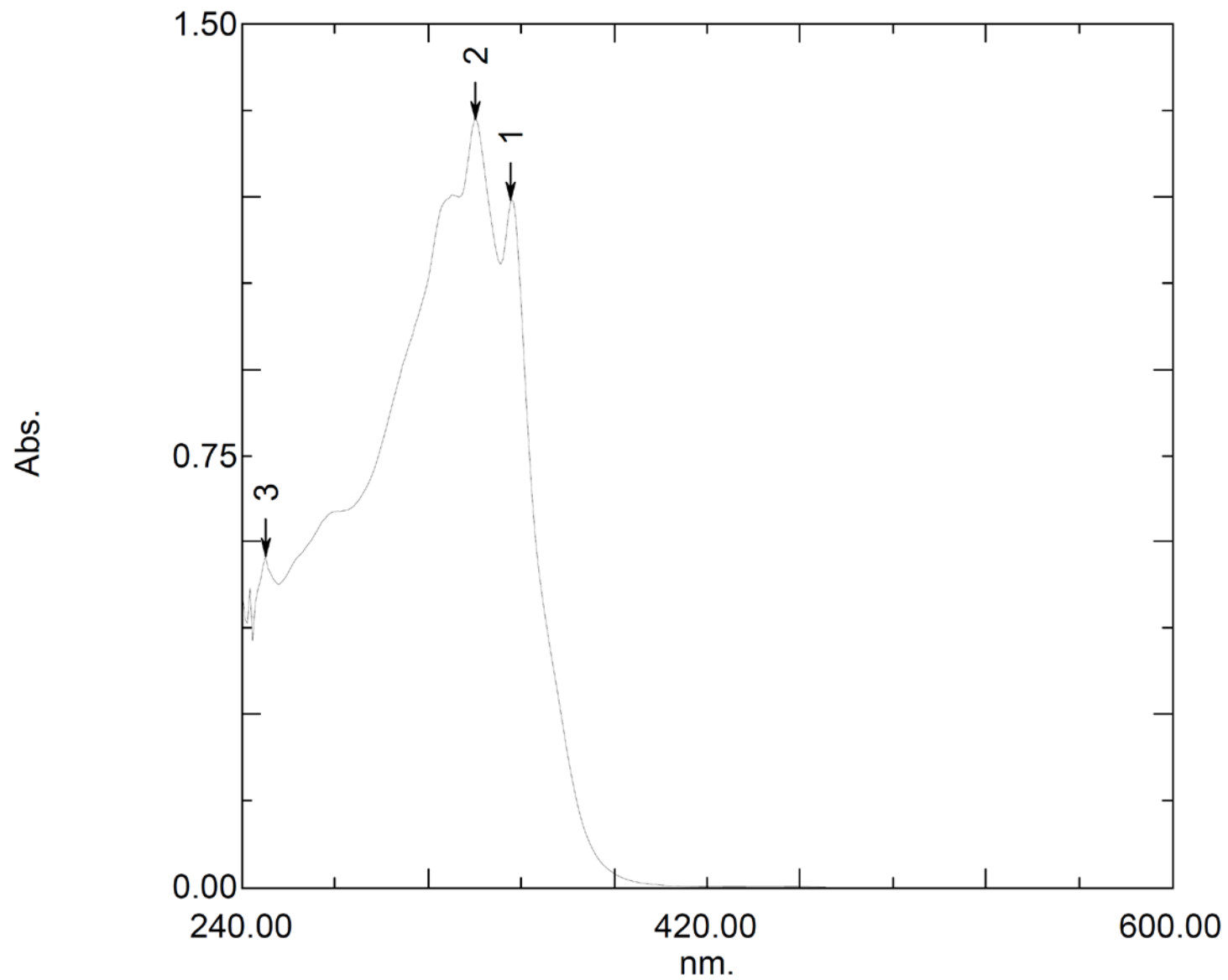
UV spectrum of compound 6d
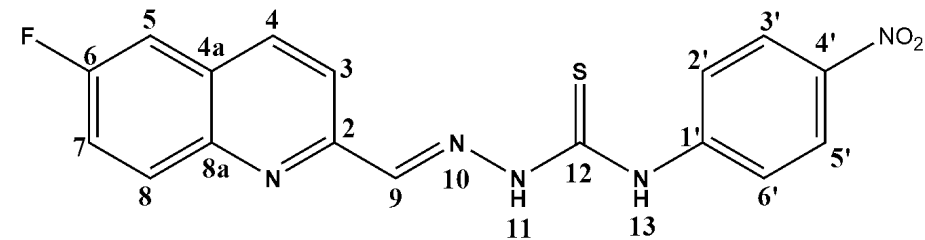
λmax	Absorbance	Log ε
249	0.57	4.30
330	1.33	4.67
344	1.19	4.62
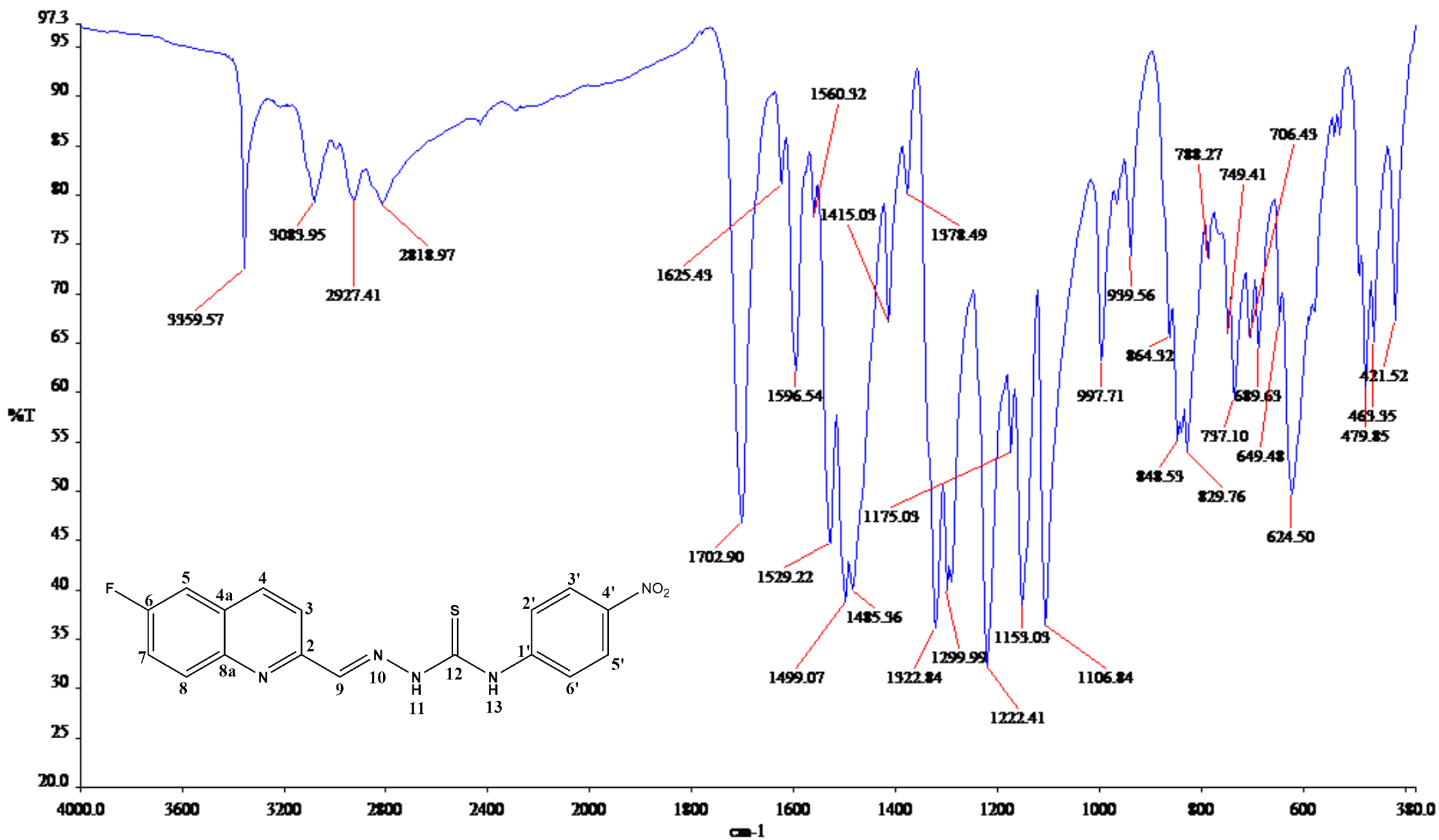
IR spectrum of compound 6d
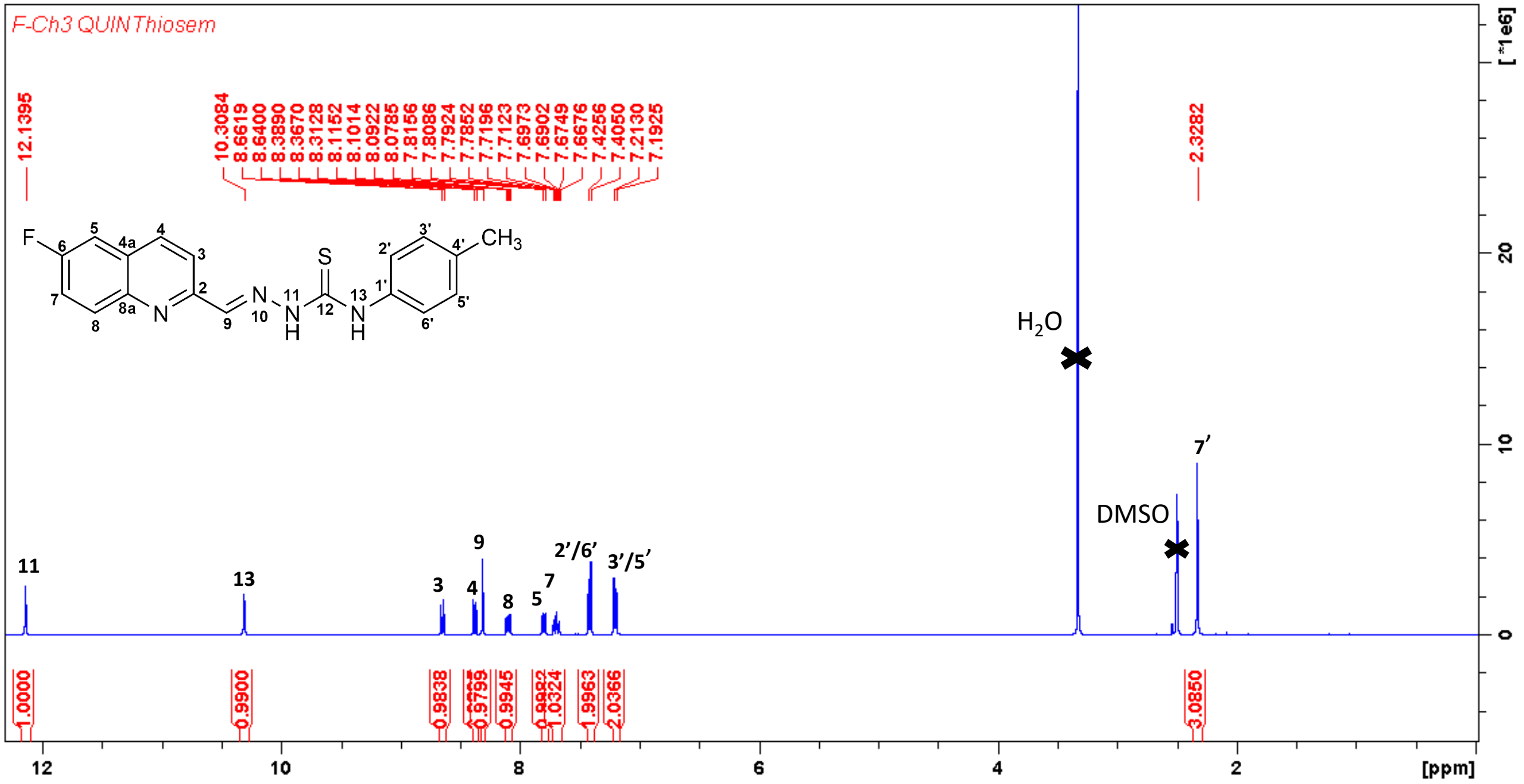
^{1}H-NMR spectrum of compound 6e
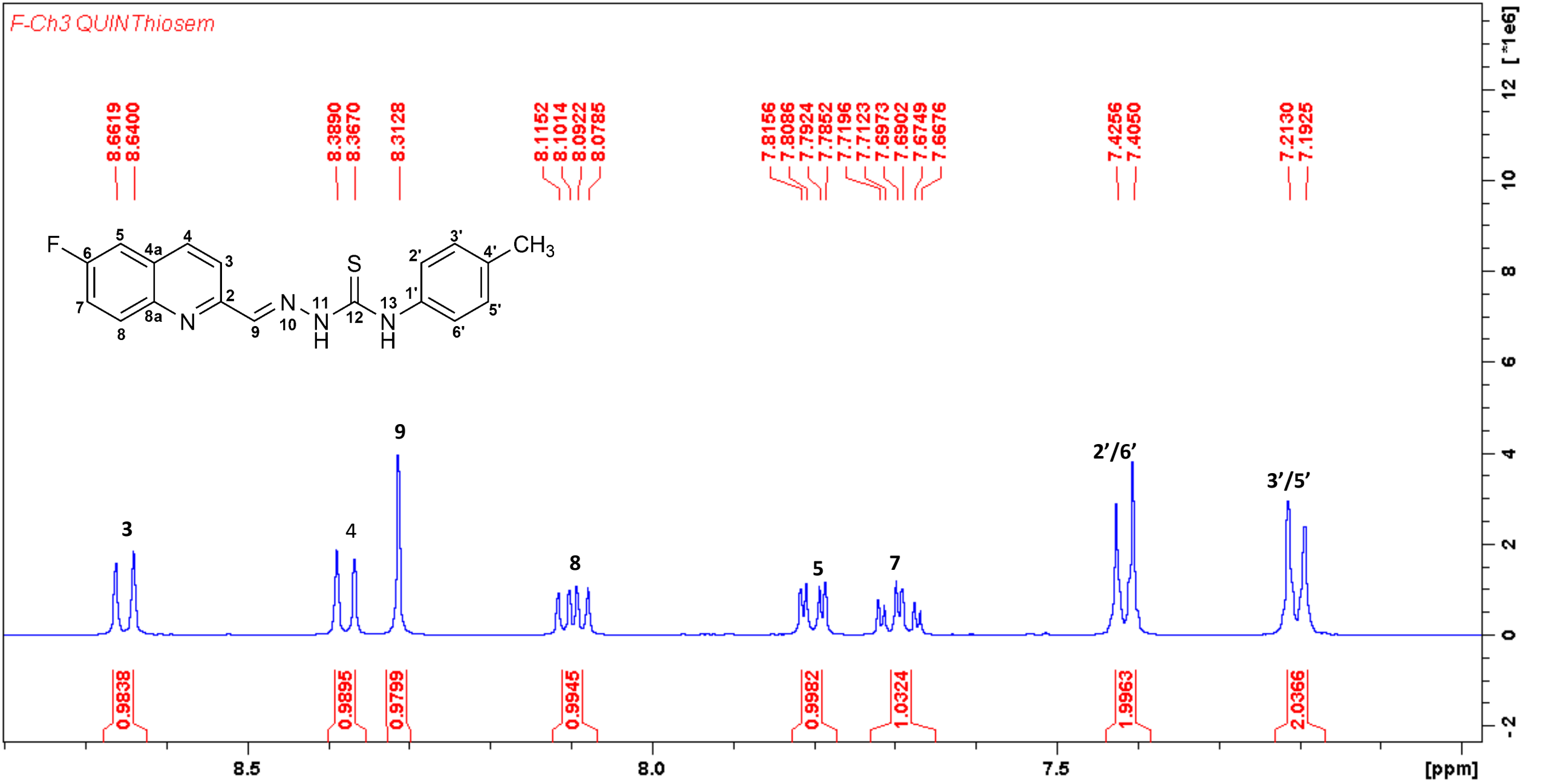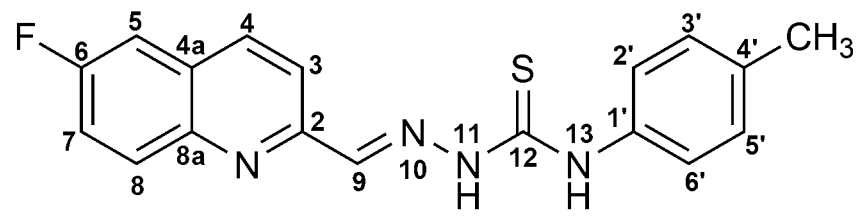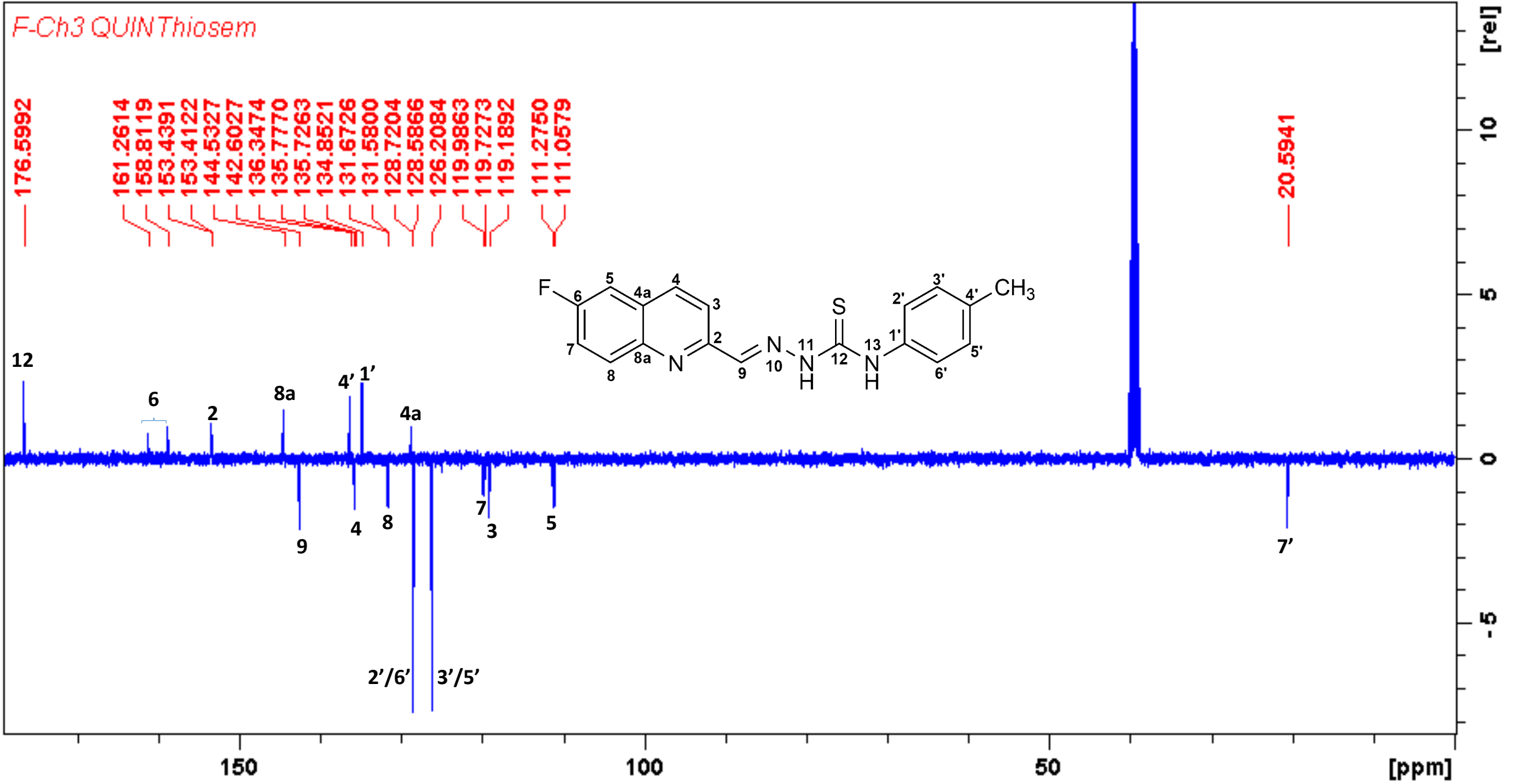
40

Aromatic region ^{1}H-NMR spectrum of compound 6e

41

^{13}C-NMR spectrum of compound 6e

42

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20   H: 15-20   N: 0-5   F: 0-1   Na: 0-1   S: 0-1
QTSC 4a 61 (2.024) Cm (1:61)
TOF MS ES+
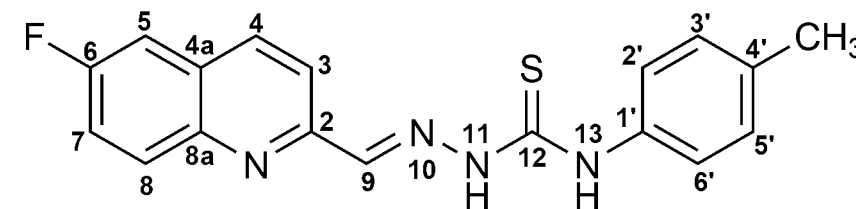
9.71e+004
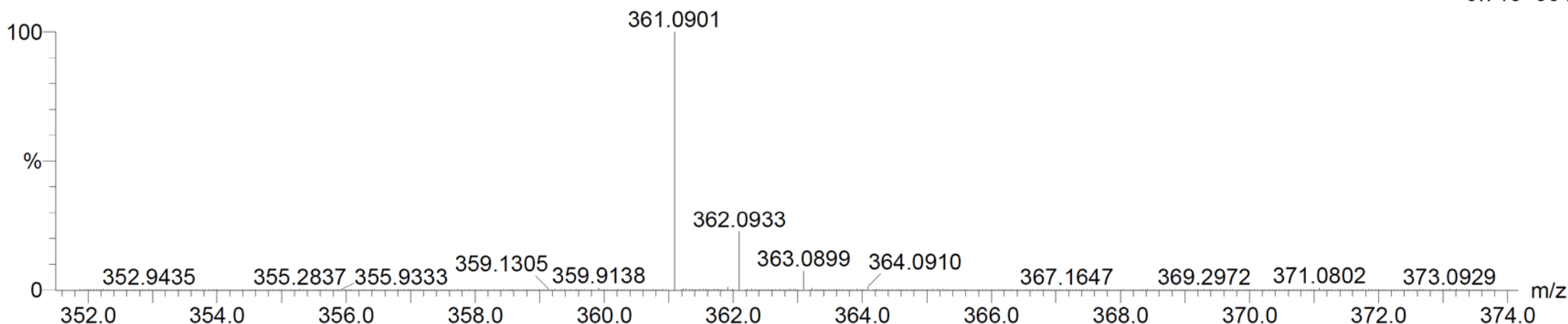
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
361.0901	361.0899	0.2	0.6	12.5	517.5	0.0	C18 H15 N4 F Na S

HRMS of compound 6e
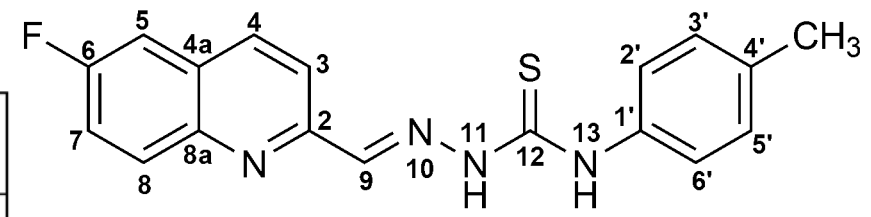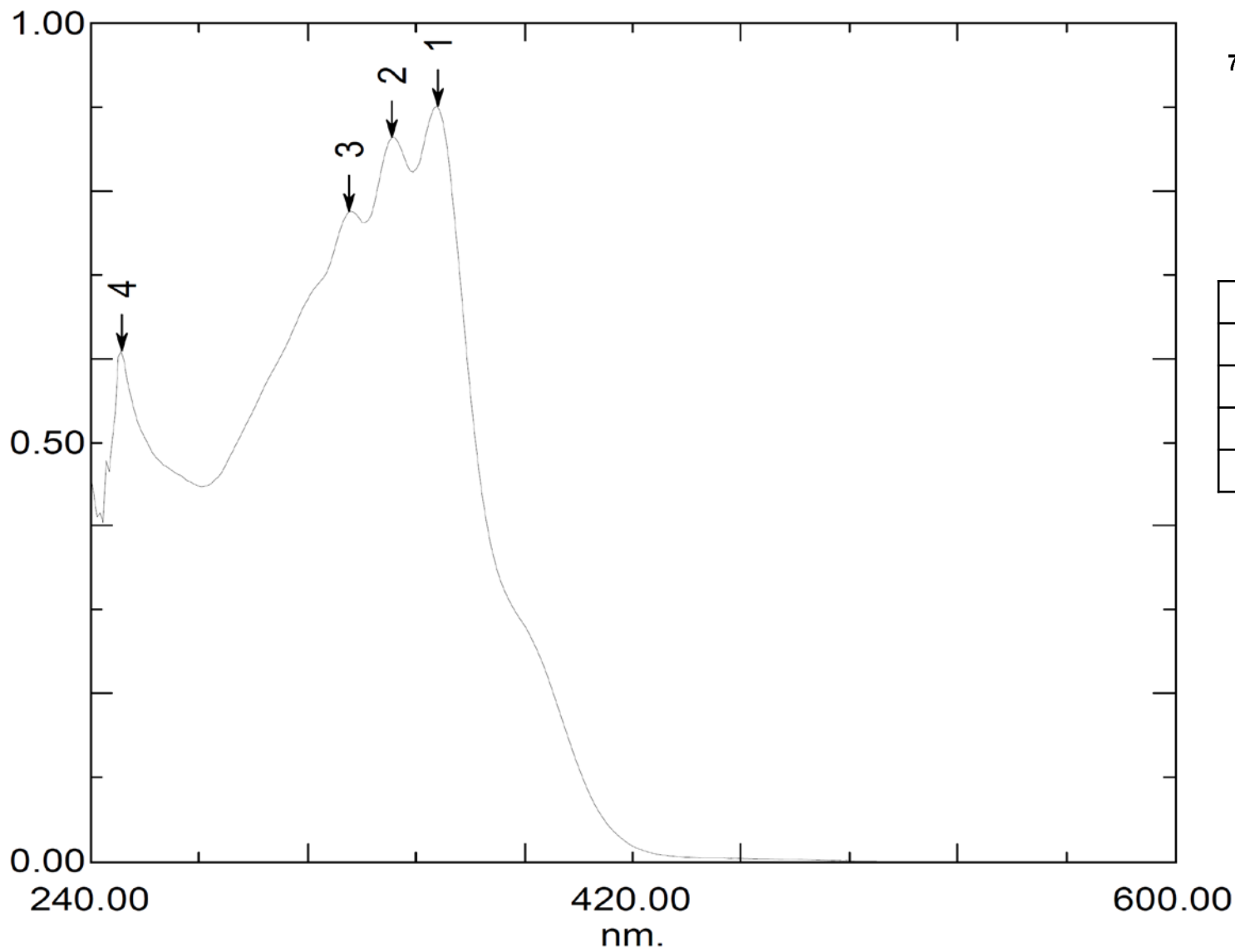
λmax	Absobance	Log ε
250	0.61	4.27
326	0.78	4.38
340	0.86	4.42
354	0.90	4.44

UV spectrum of compound 6e
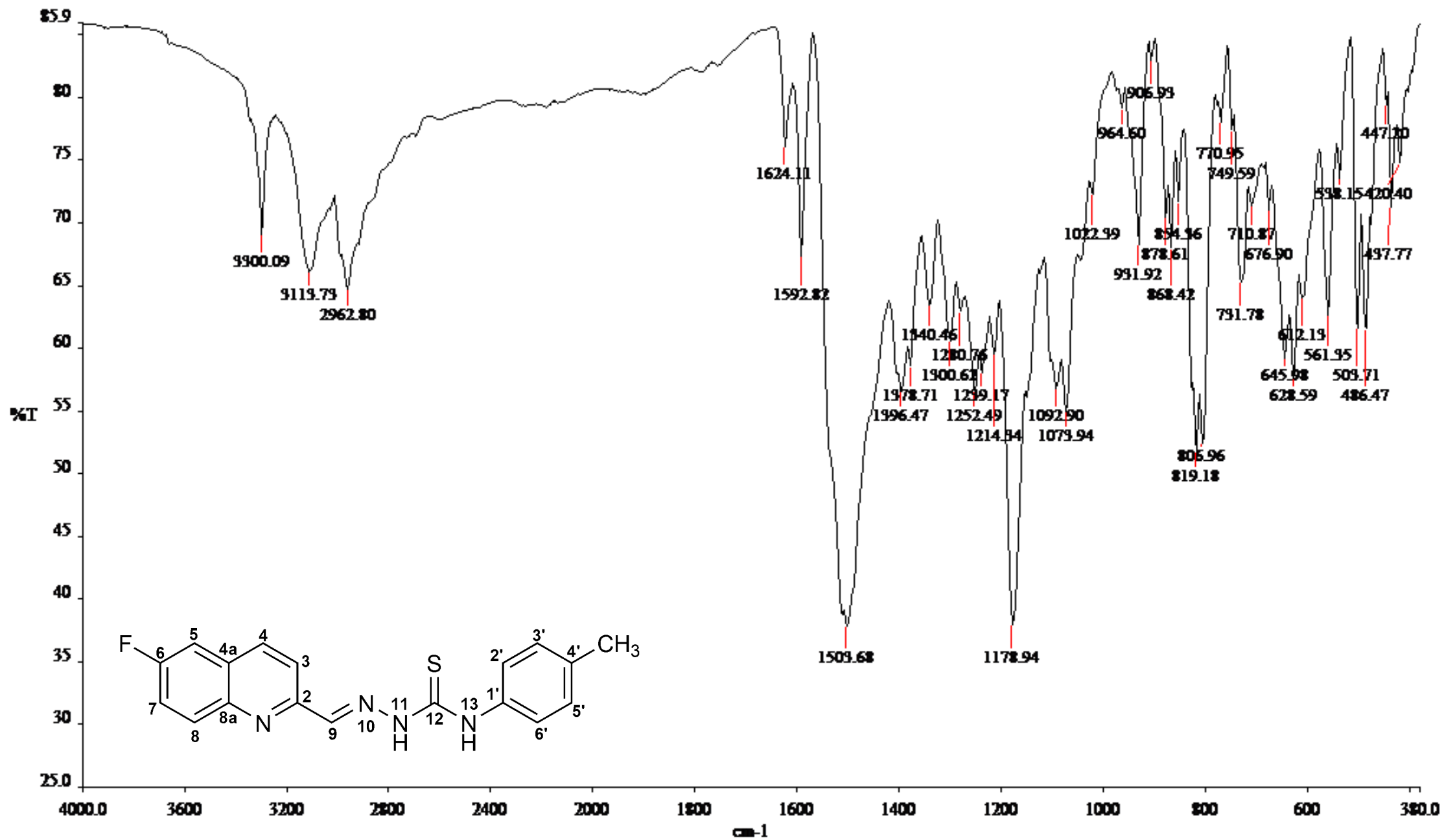
IR spectrum of compound 6e
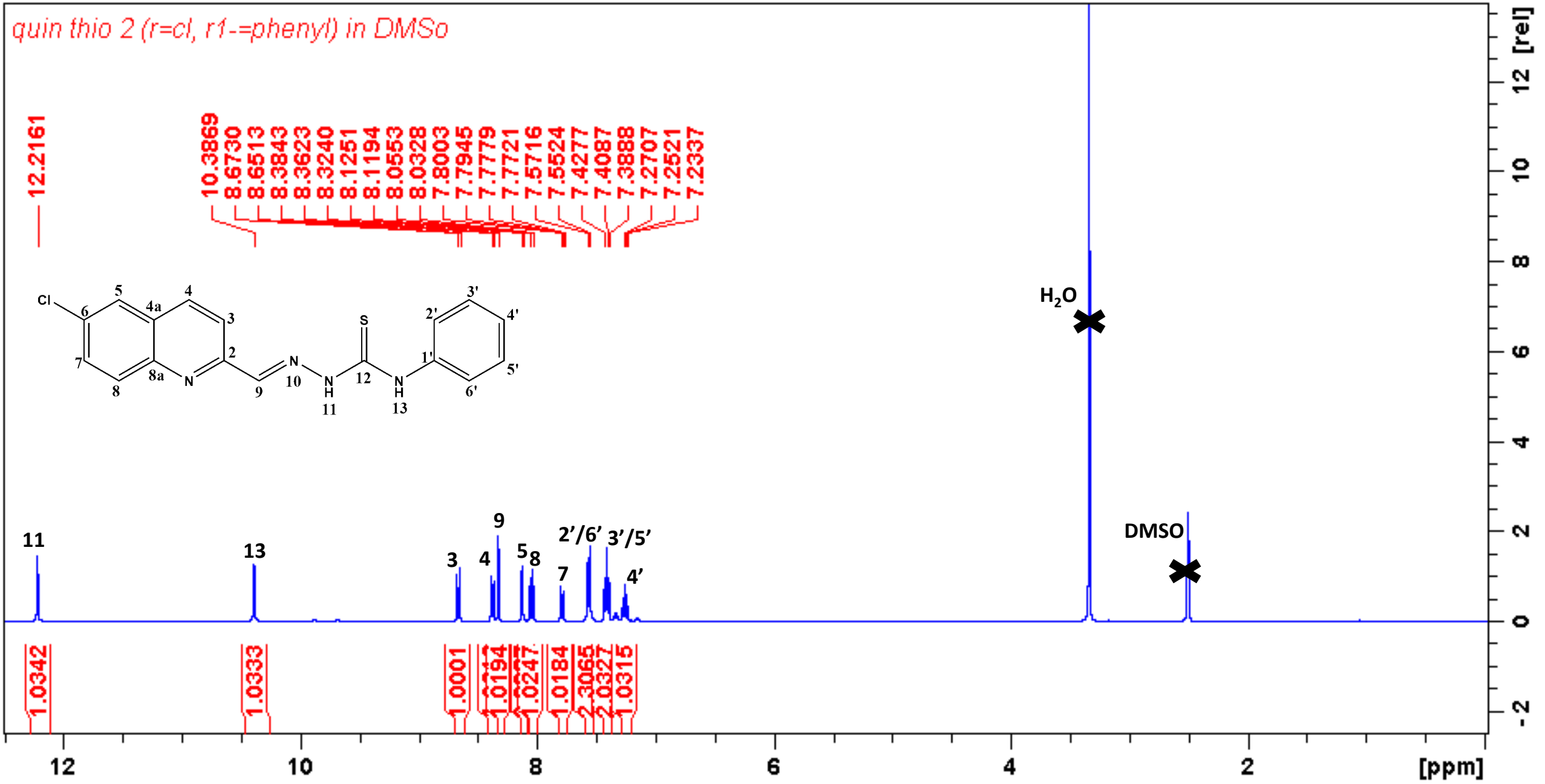
¹H-NMR spectrum of compound 6f
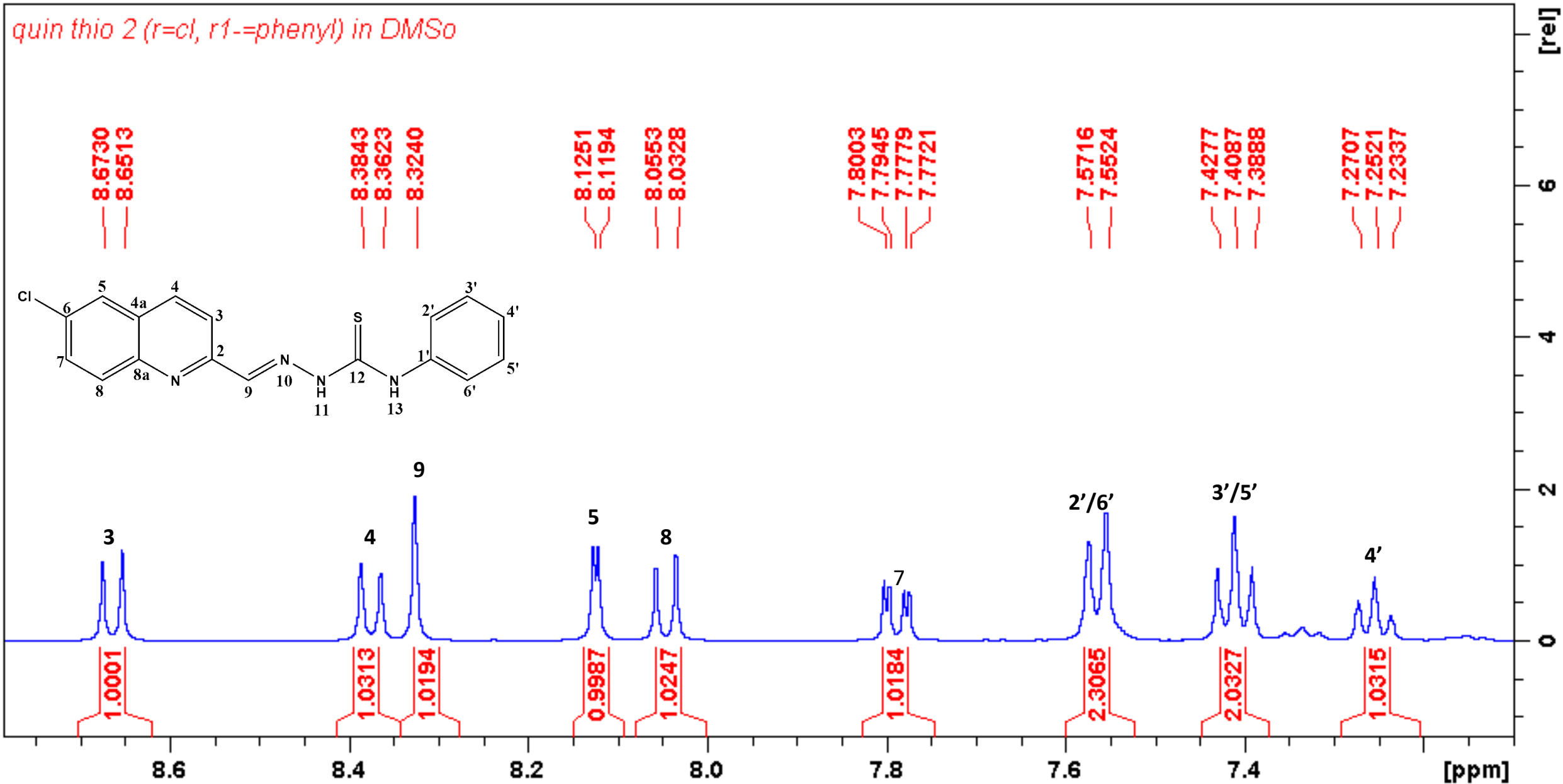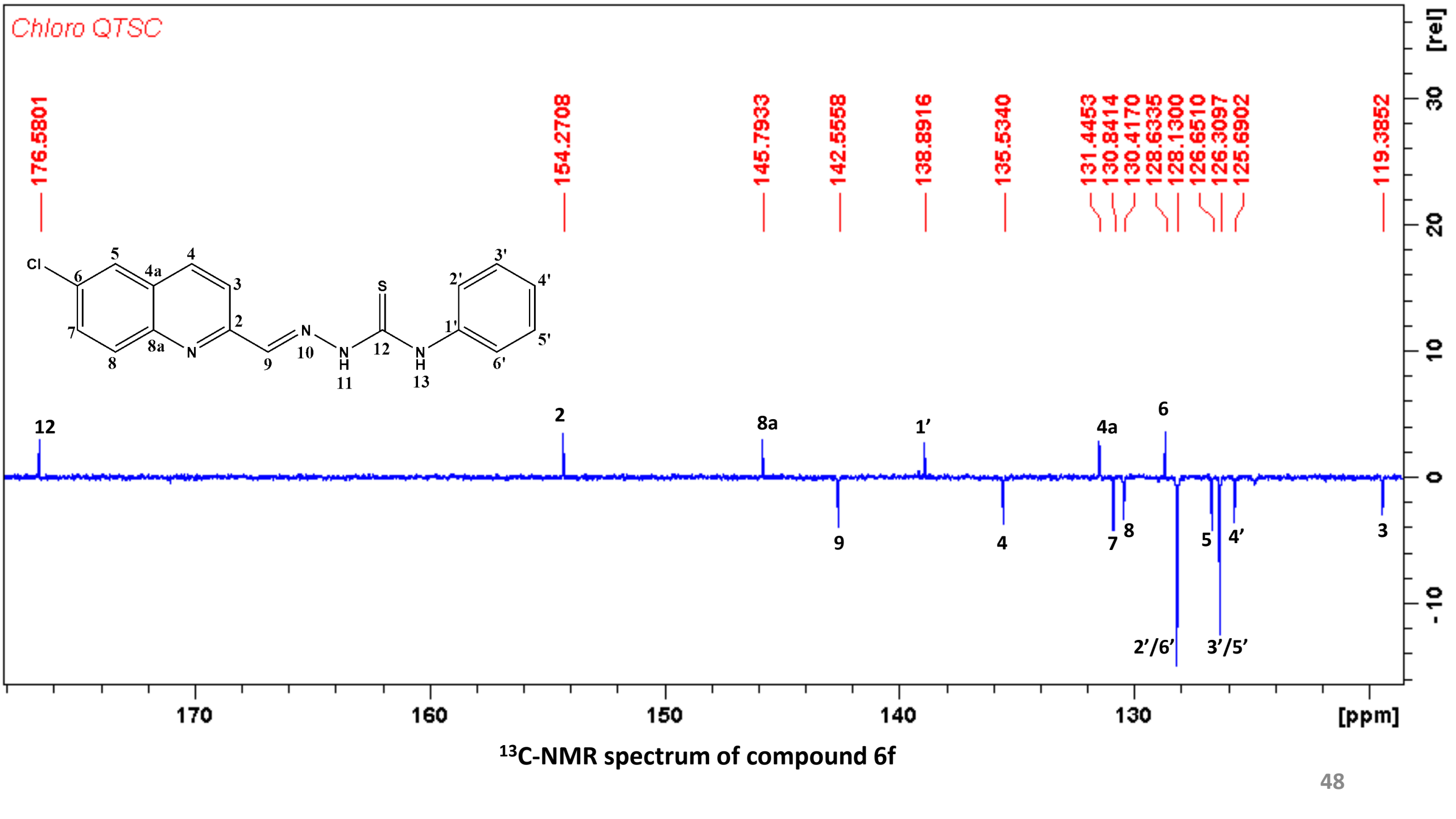
46

Aromatic region ¹H-NMR spectrum of compound 6f

47
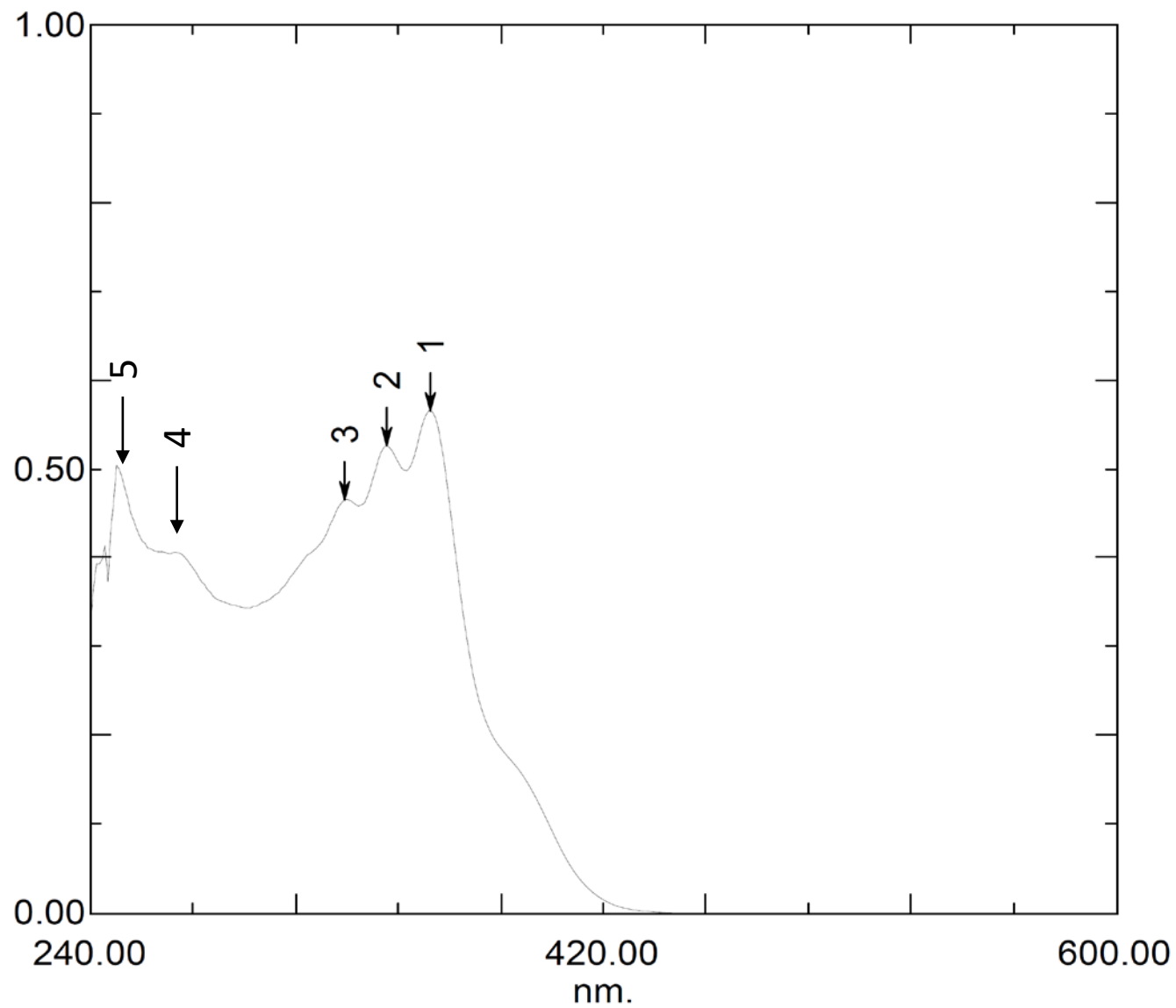
13C-NMR spectrum of compound 6f
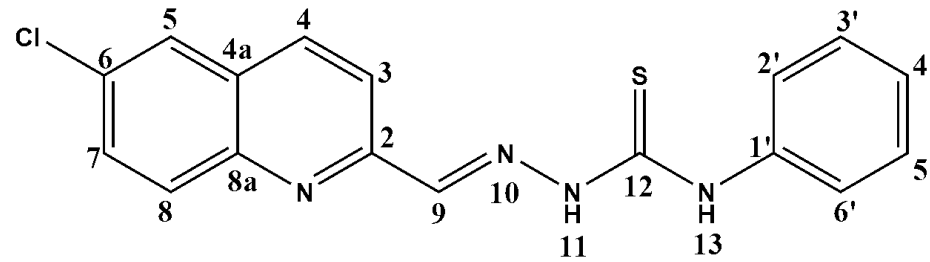
λmax	Absorbance	Log ε
250	0.50	4.19
270	0.41	4.10
330	0.47	4.16
344	0.53	4.22
359	0.57	4.25
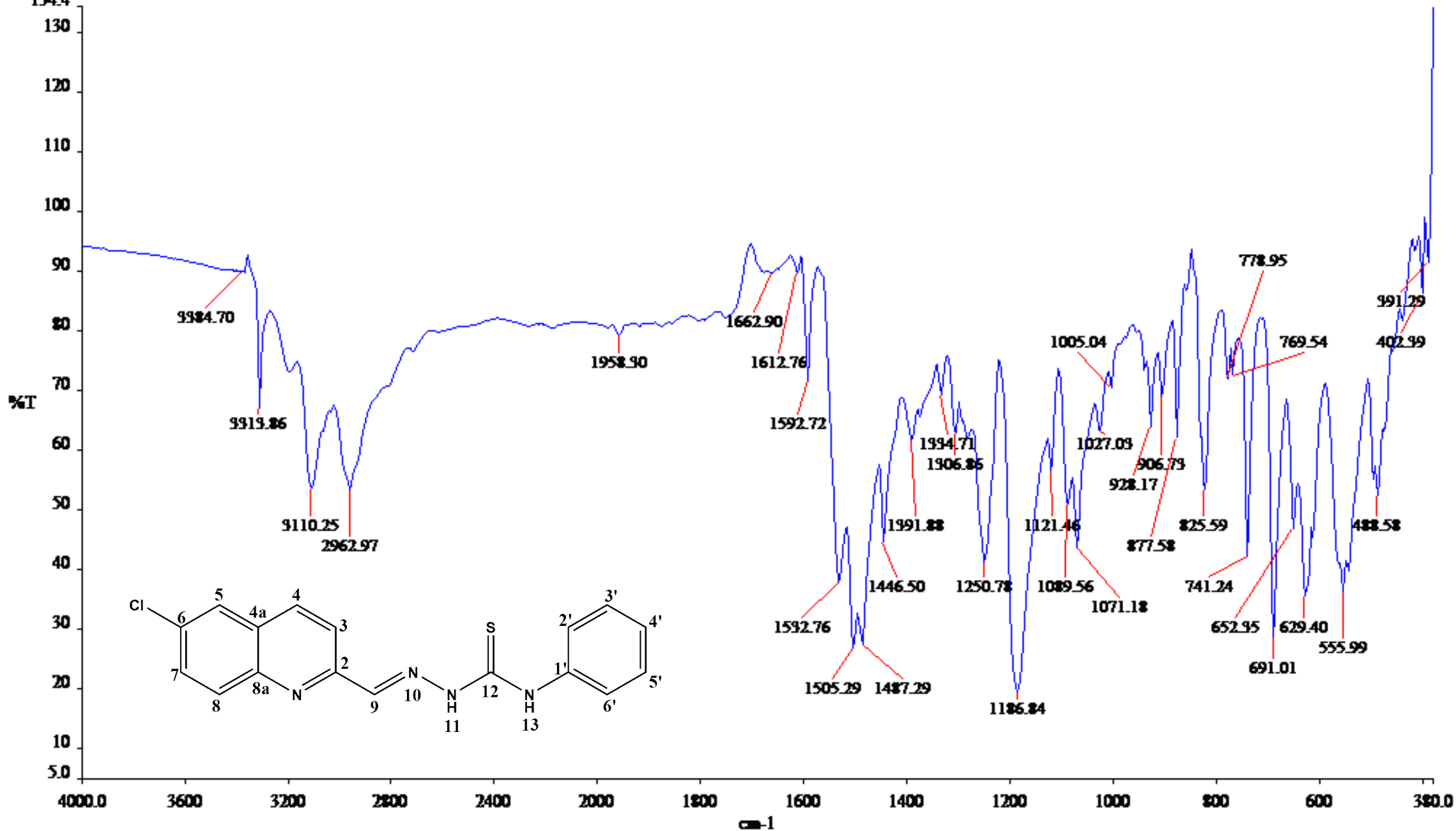
UV spectrum of compound 6f
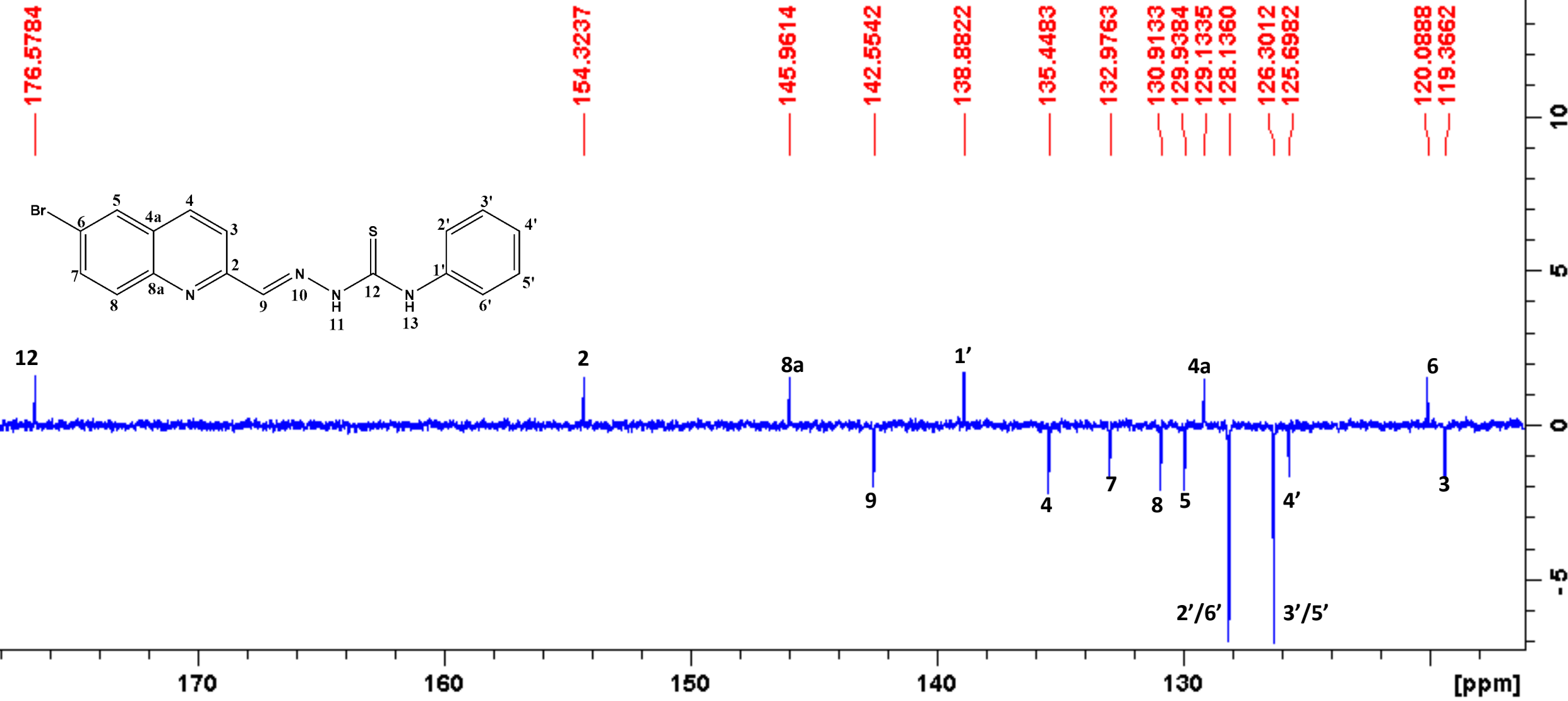
IR spectrum of compound 6f
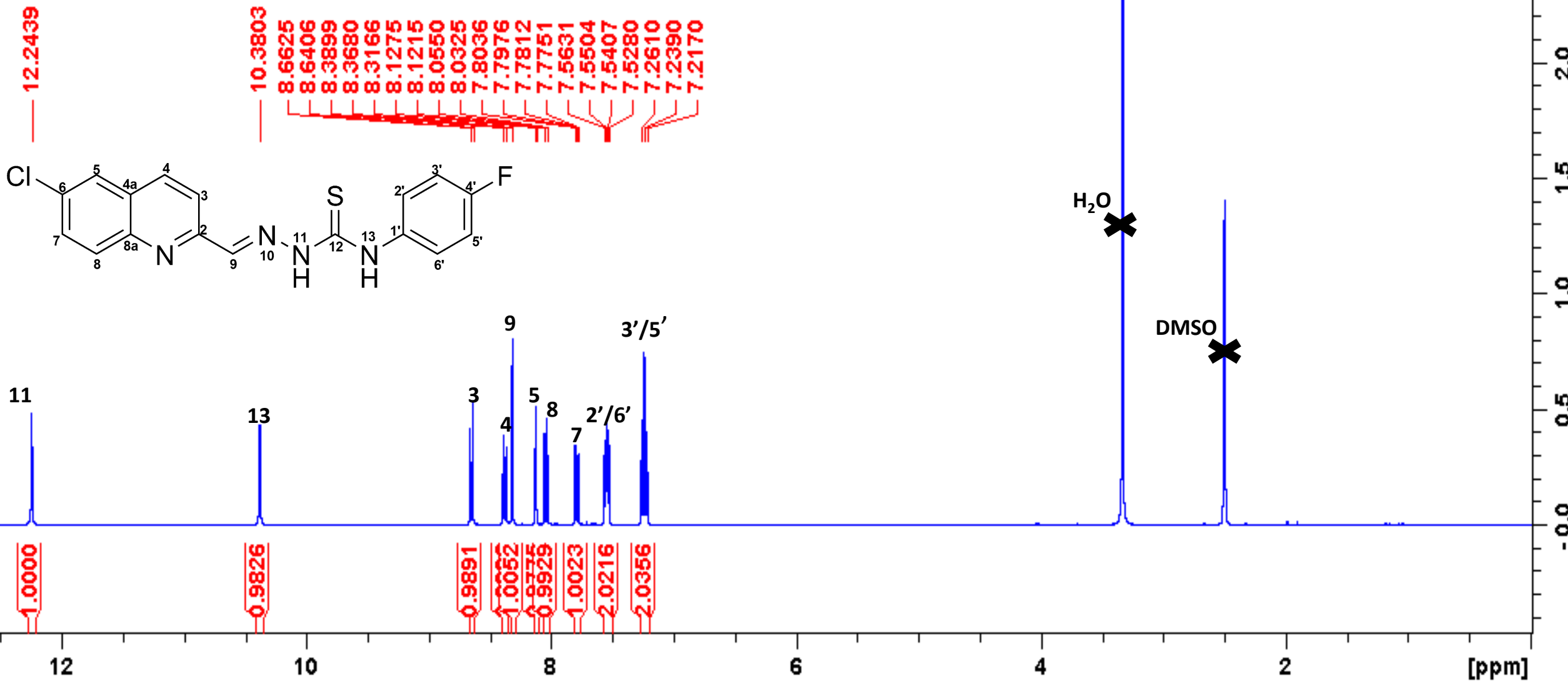
^{1}H-NMR spectrum of compound 6g
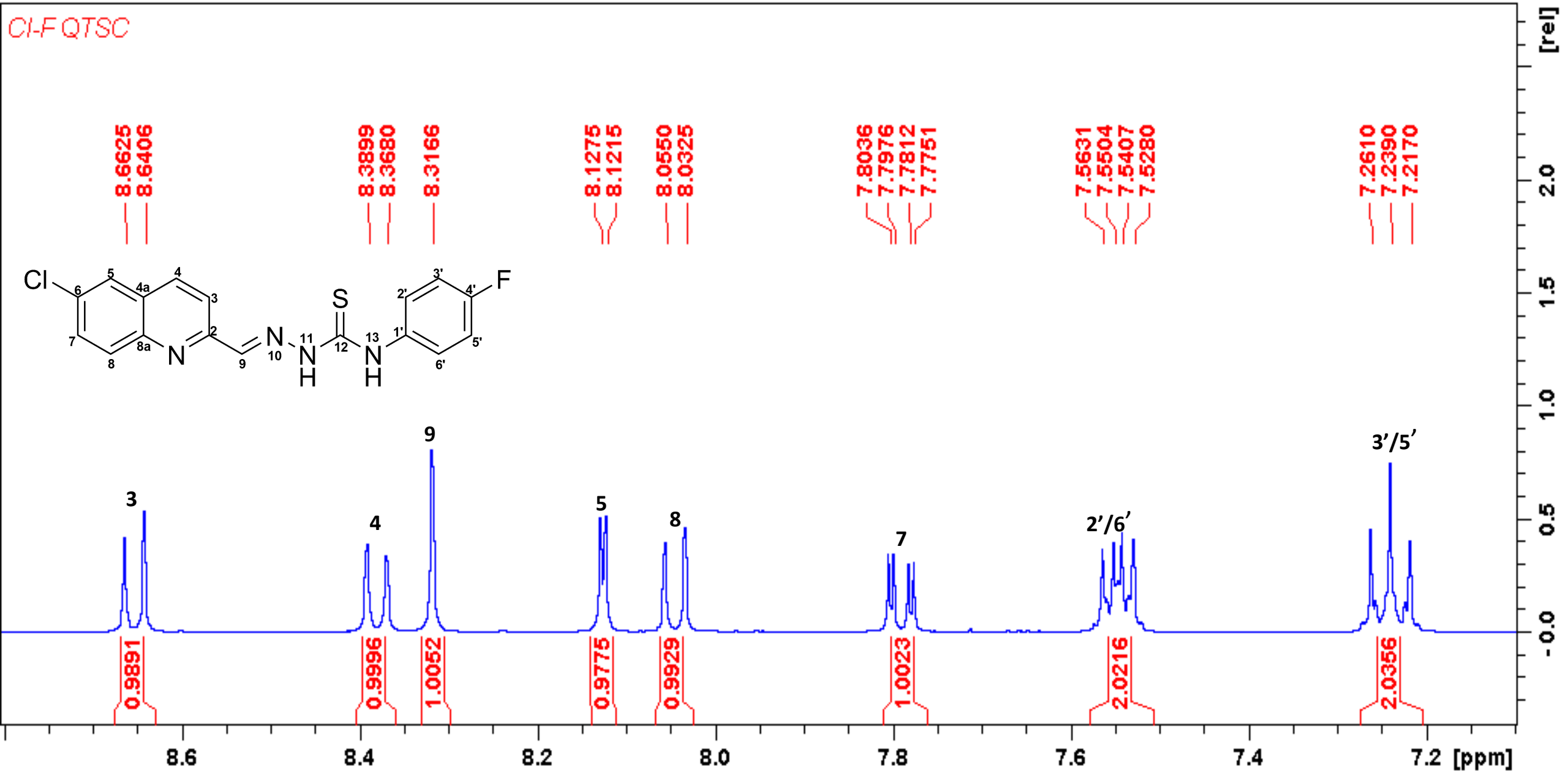
Aromatic region ^{1}H-NMR spectrum of compound 6g
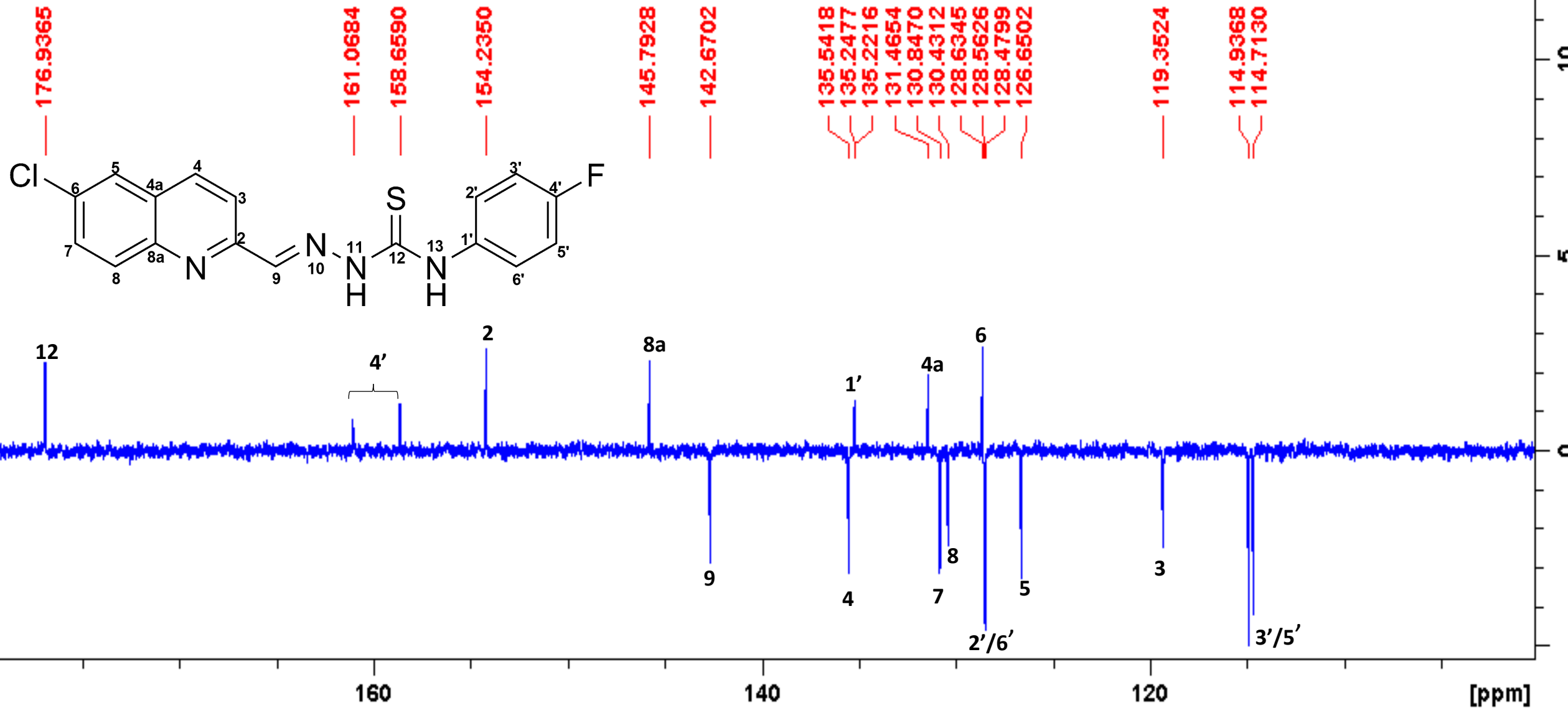
^{13}C-NMR spectrum of compound 6g

Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
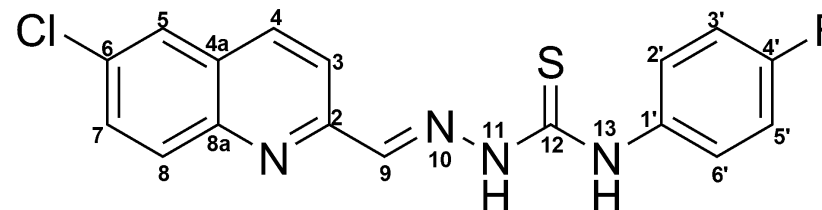
Monoisotopic Mass, Even Electron Ions
45 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20   H: 10-15   N: 0-5   F: 0-2   S: 0-1   Cl: 0-2
QTSC 1b 61 (2.025) Cm (1:61)
TOF MS ES-

2.50e+005
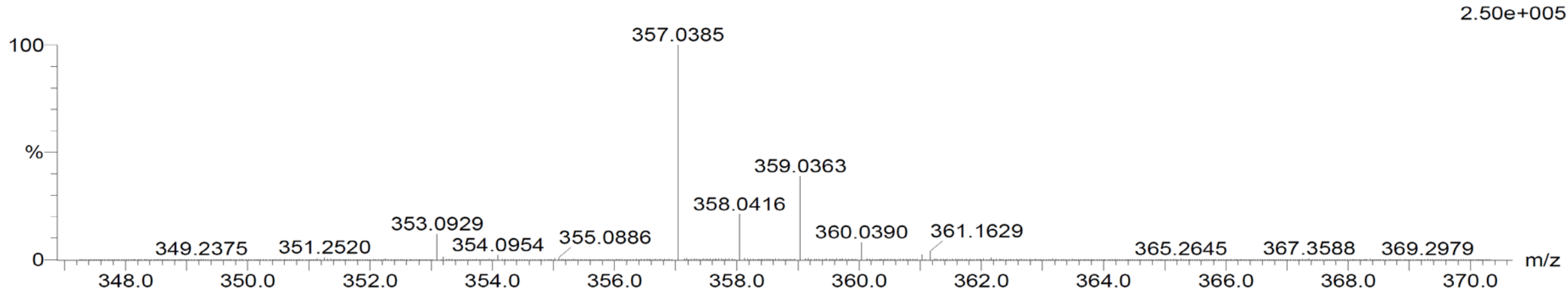
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
357.0385	357.0377	0.8	2.2	13.5	659.4	0.0	C17 H11 N4 F S Cl
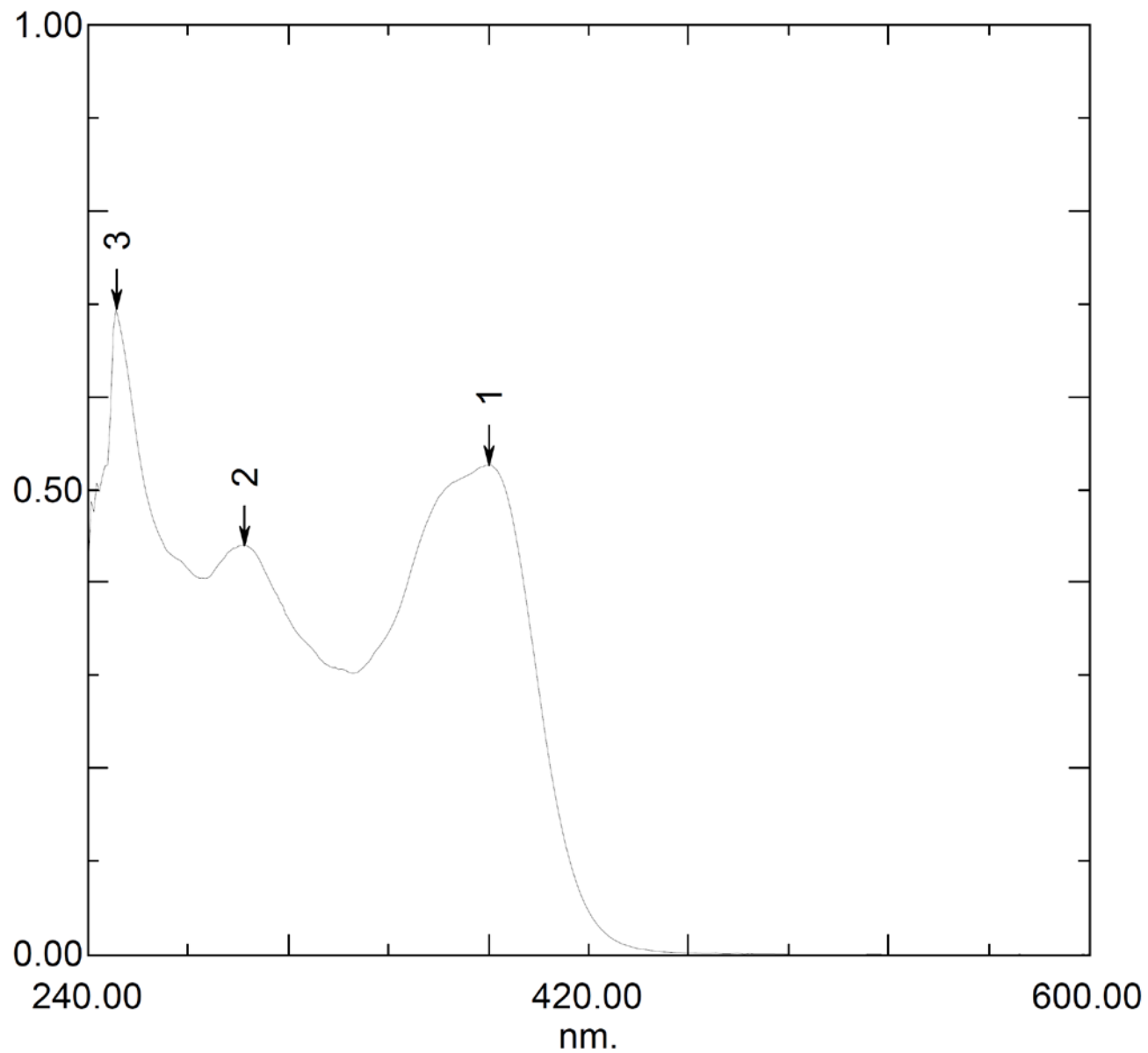
HRMS of compound 6g
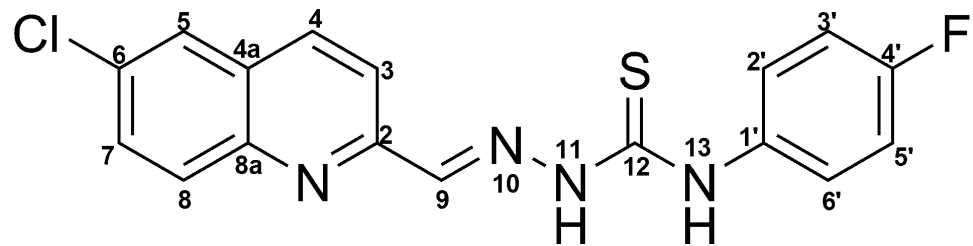
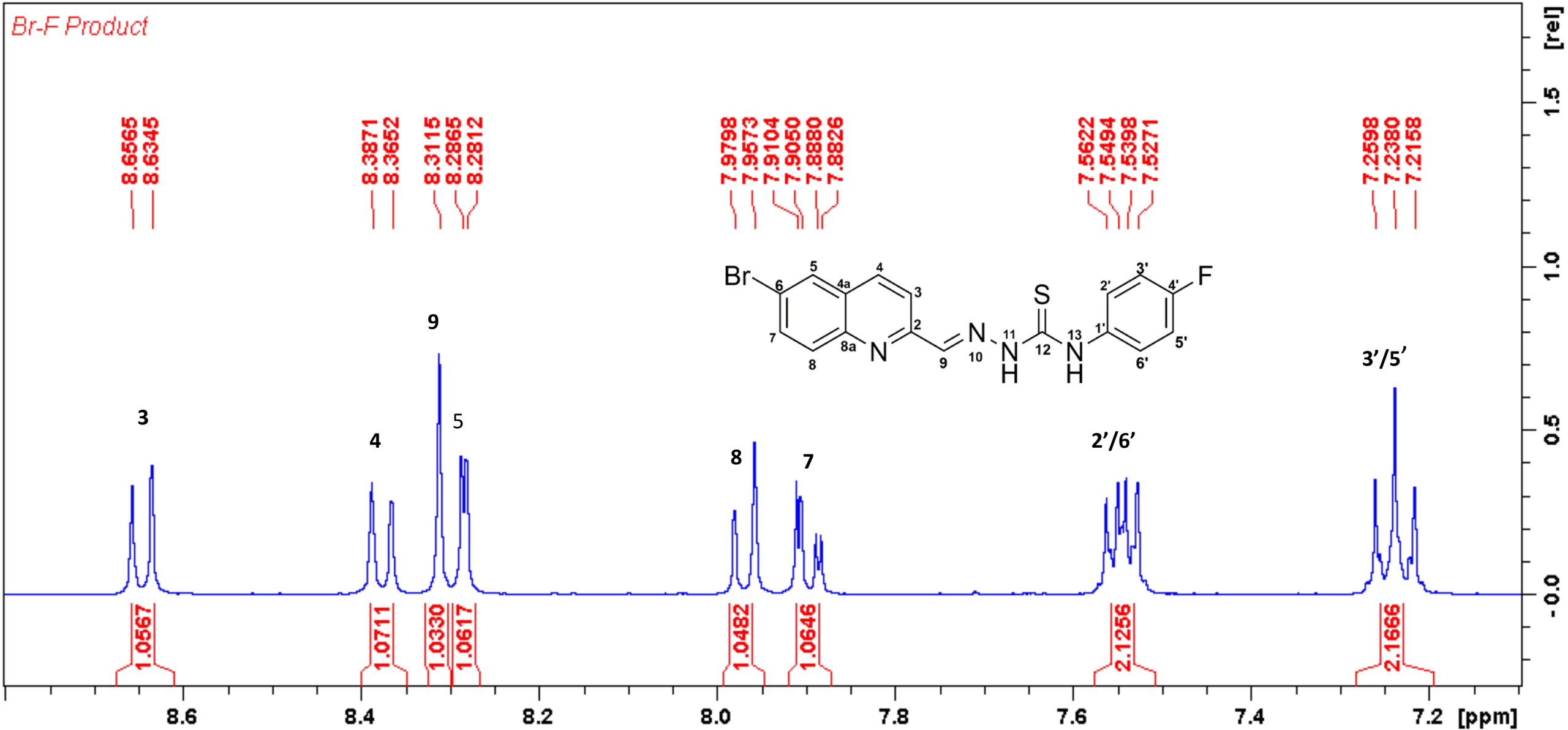| λmax	Absorbance	Log ε
250	0.69	4.42
295	0.44	4.33
384	0.53	4.31

UV spectrum of compound 6g
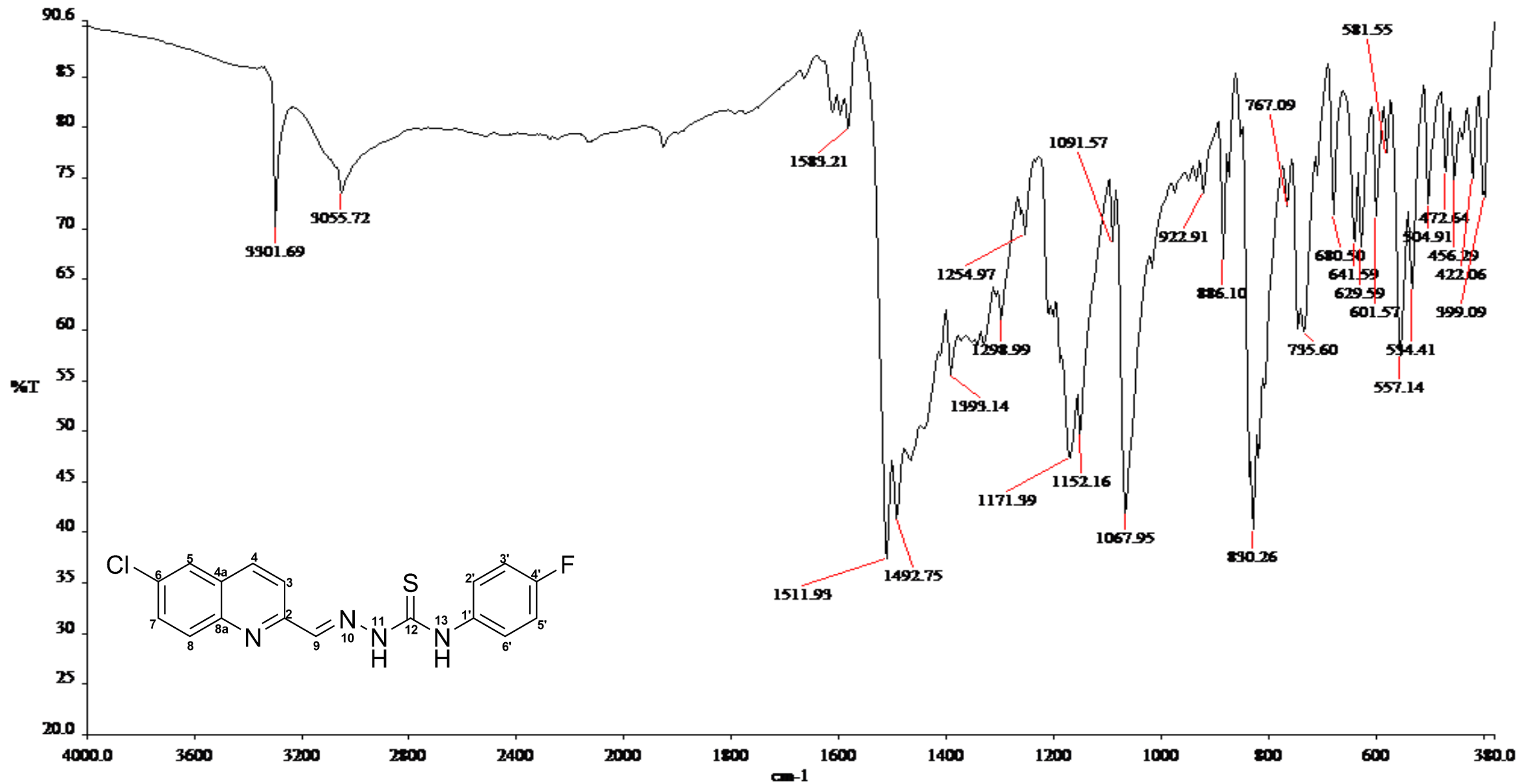
IR spectrum of compound 6g

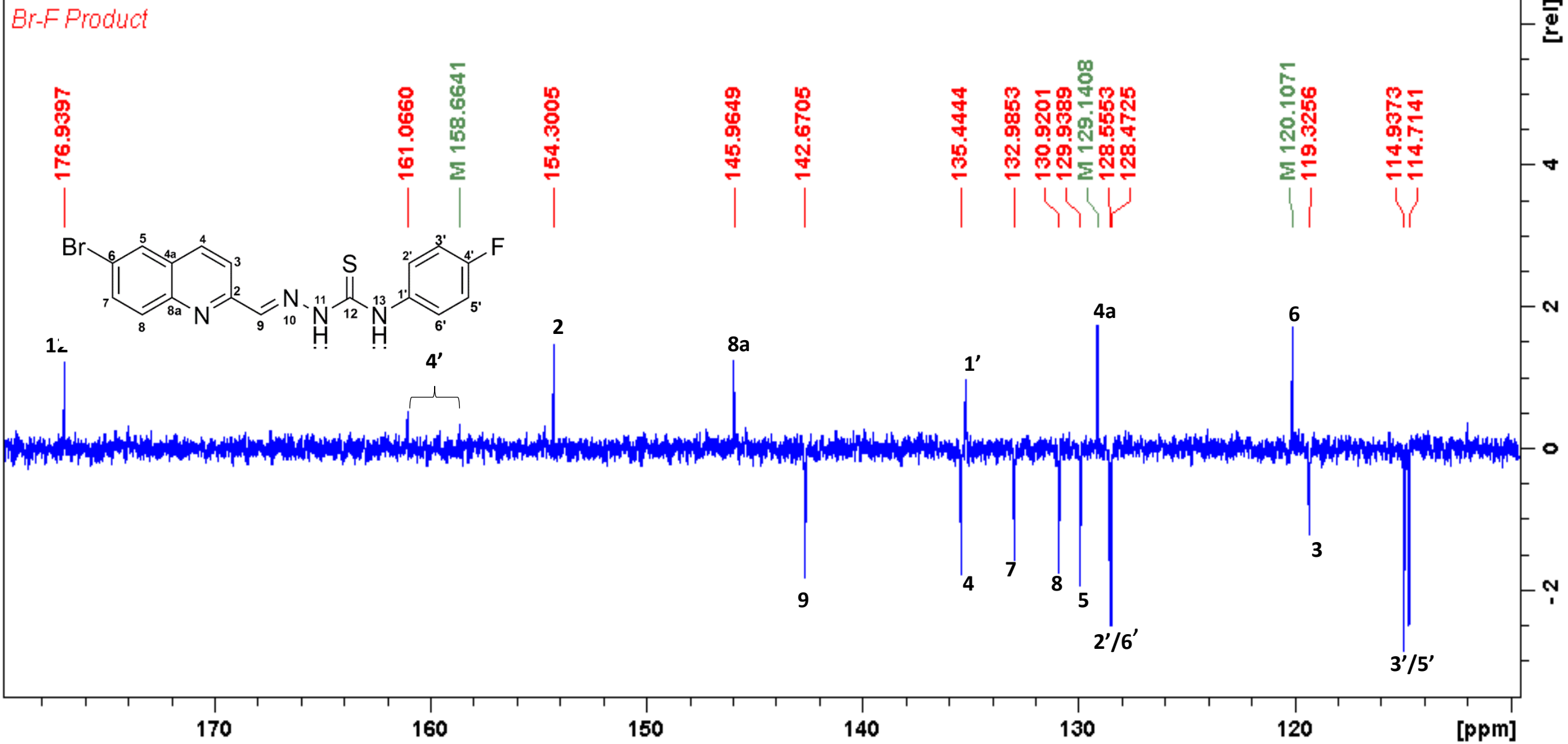**^{1}H-NMR spectrum of compound 6h**
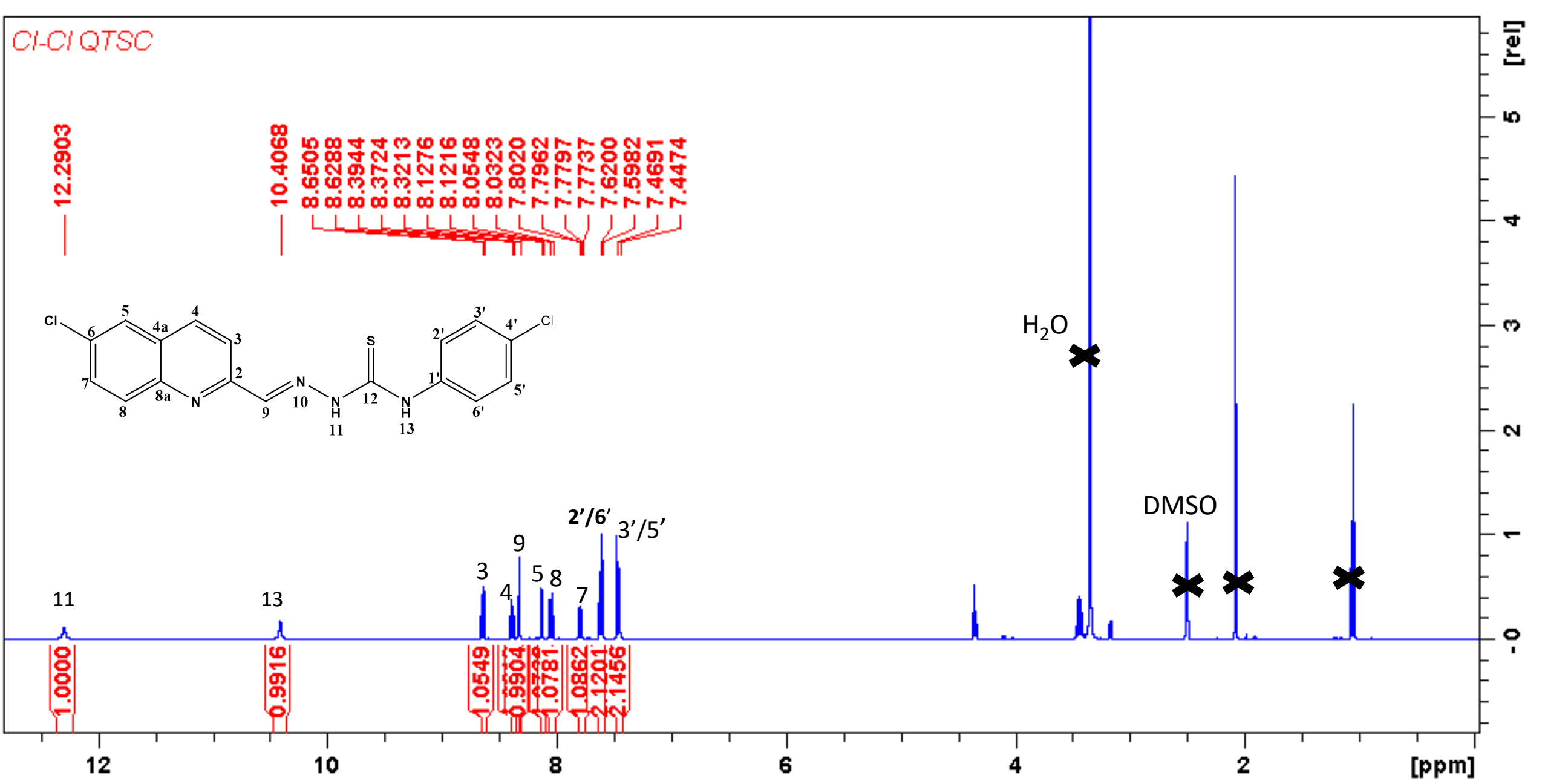
Aromatic region ¹H-NMR spectrum of compound 6h
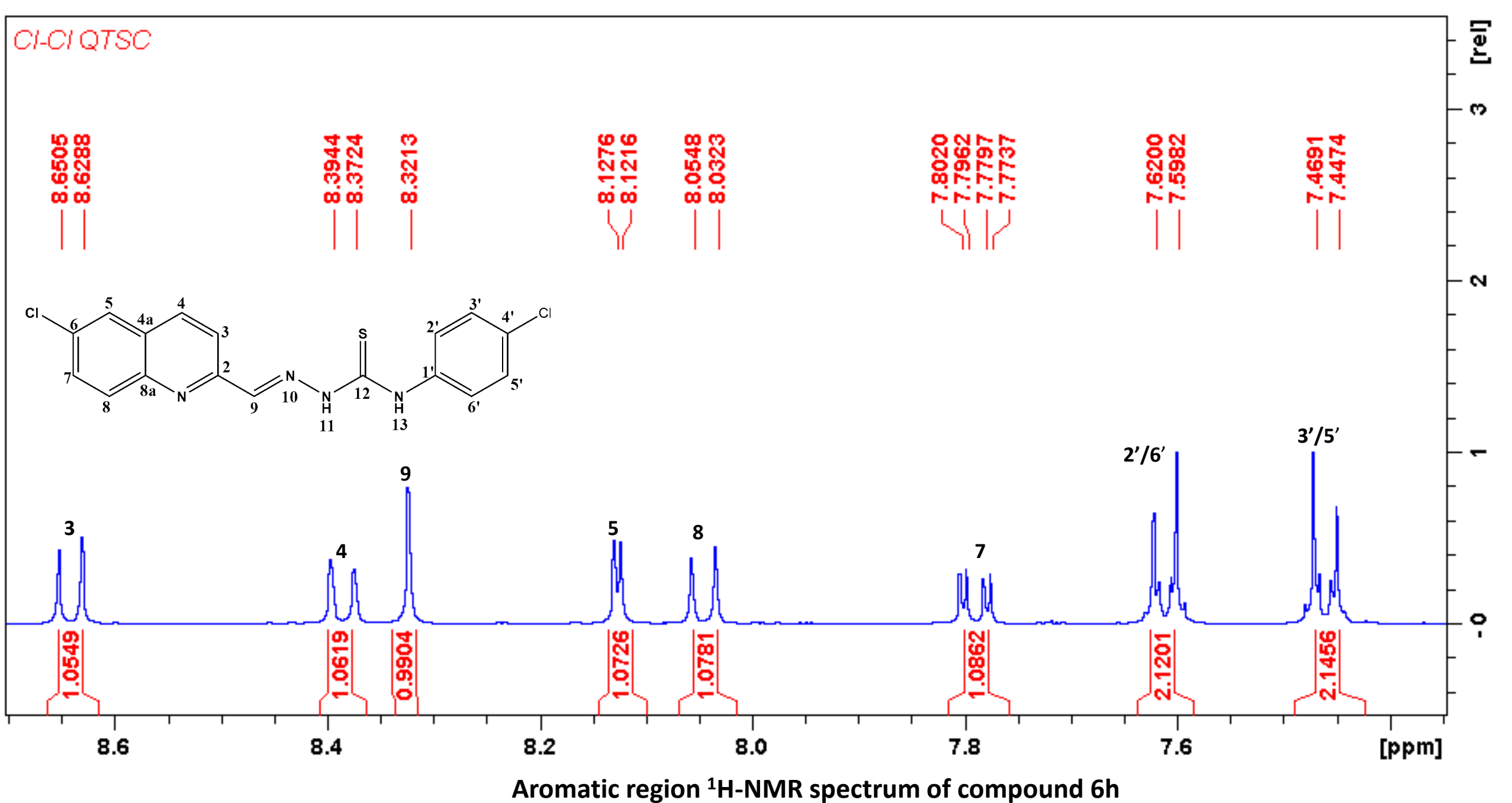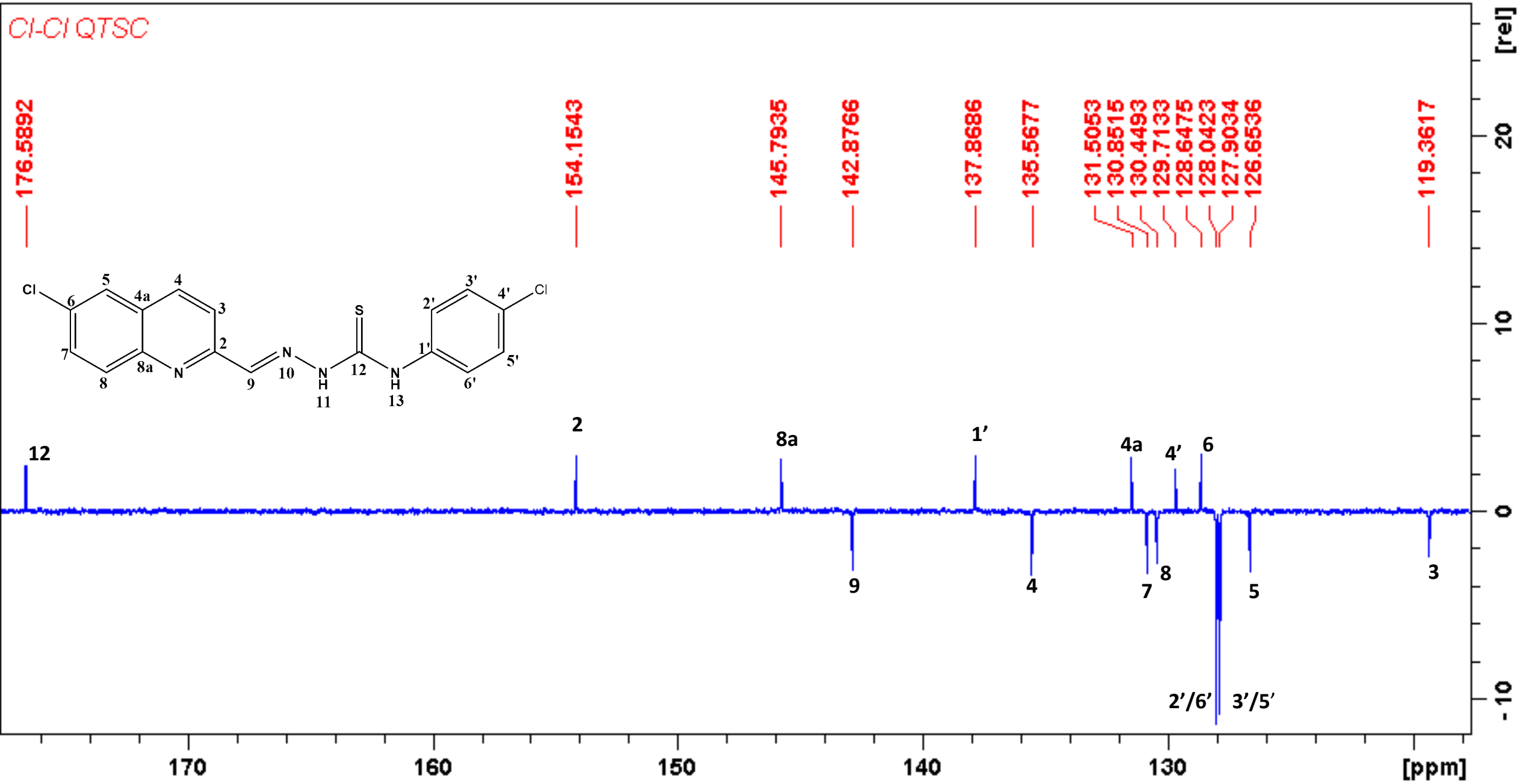
^{13}C-NMR spectrum of compound 6h

Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM   /   DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
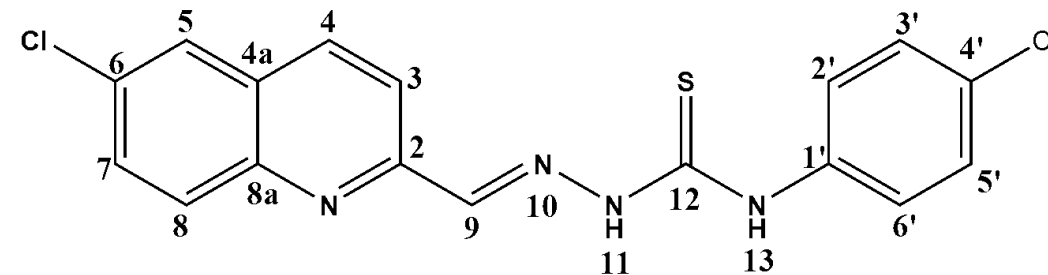
Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20   H: 10-15   N: 0-5   S: 0-1   Cl: 0-2
QTSC 1a 2 (0.034) Cm (1:61)
TOF MS ES-

2.15e+00!
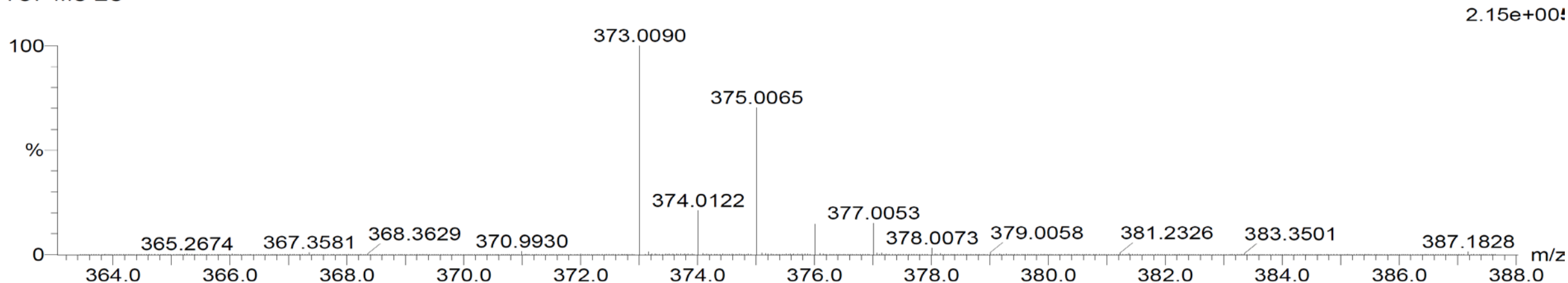
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
373.0090	373.0081	0.9	2.4	13.5	645.4	0.0	C17 H11 N4 S Cl2	

HRMS of compound 6h

60
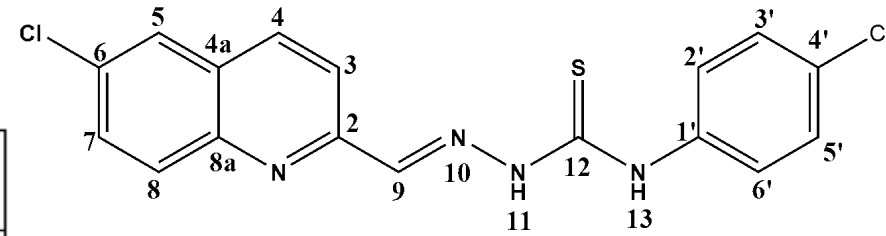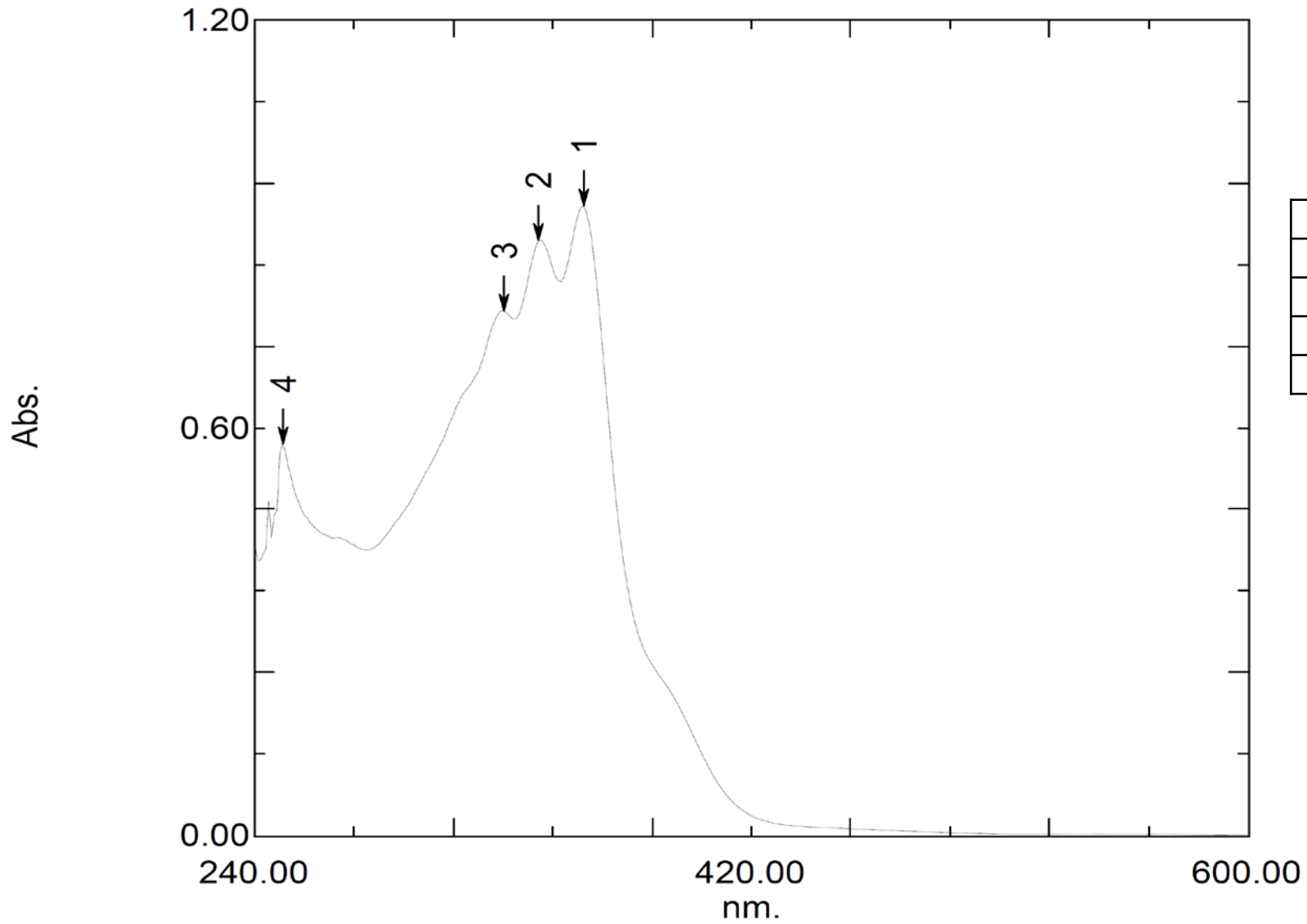
λmax	Absorbance	Log ε
250	0.58	4.32
330	0.77	4.44
343	0.88	4.50
359	0.93	4.53

UV spectrum of compound 6h
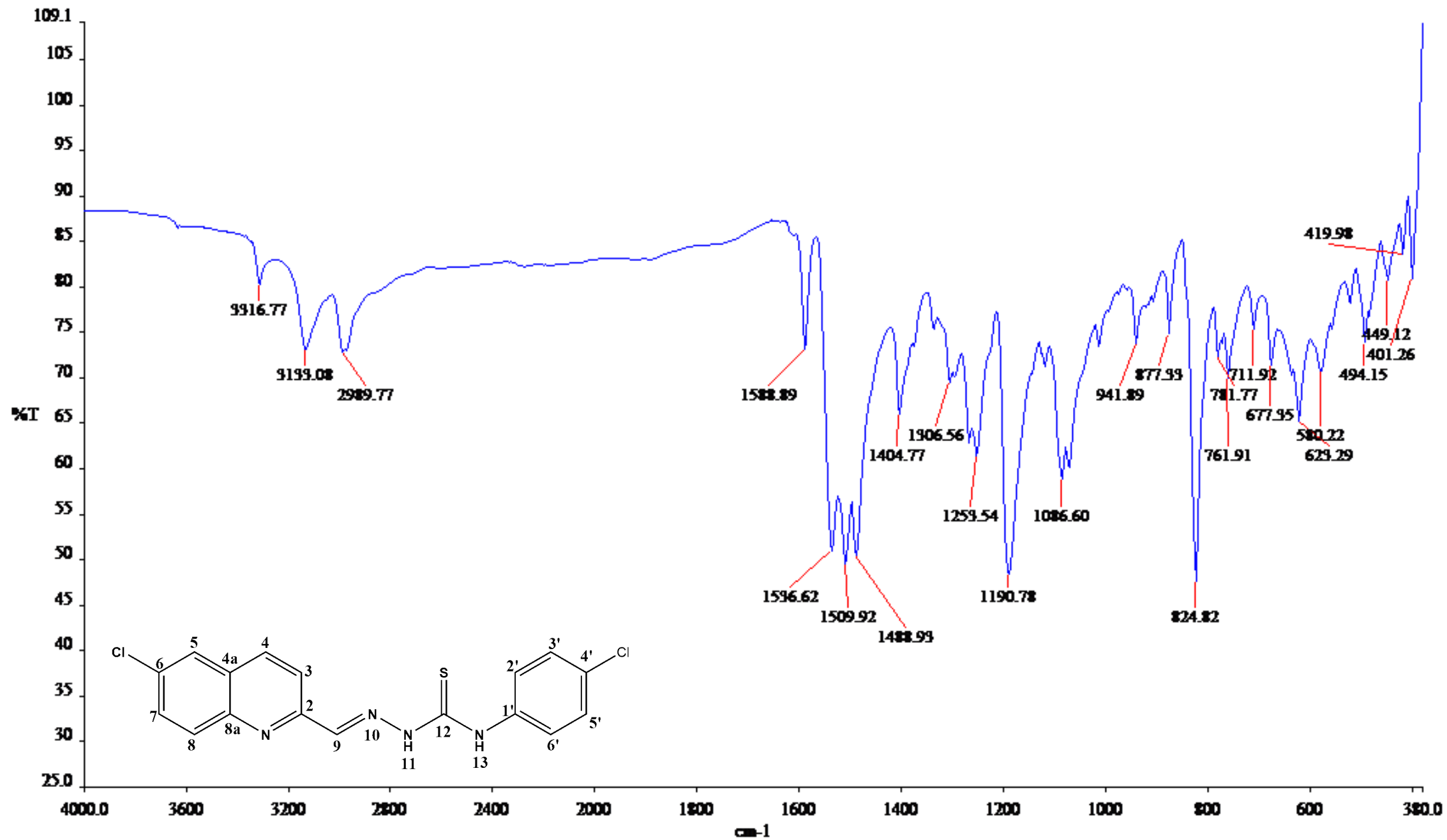
IR spectrum of compound 6h
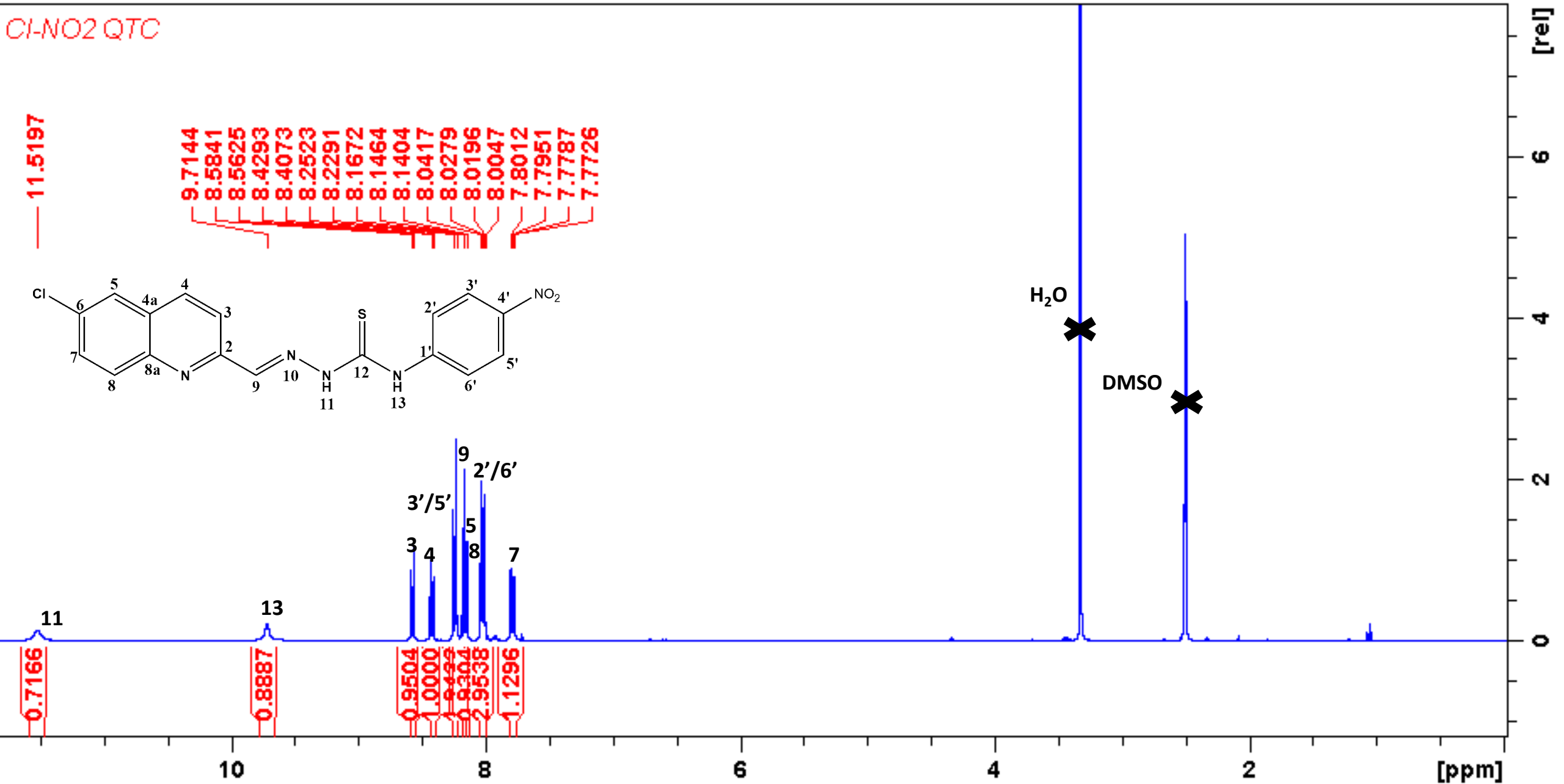
^{1}H-NMR spectrum of compound 6i

Aromatic region ¹H-NMR spectrum of compound 6i
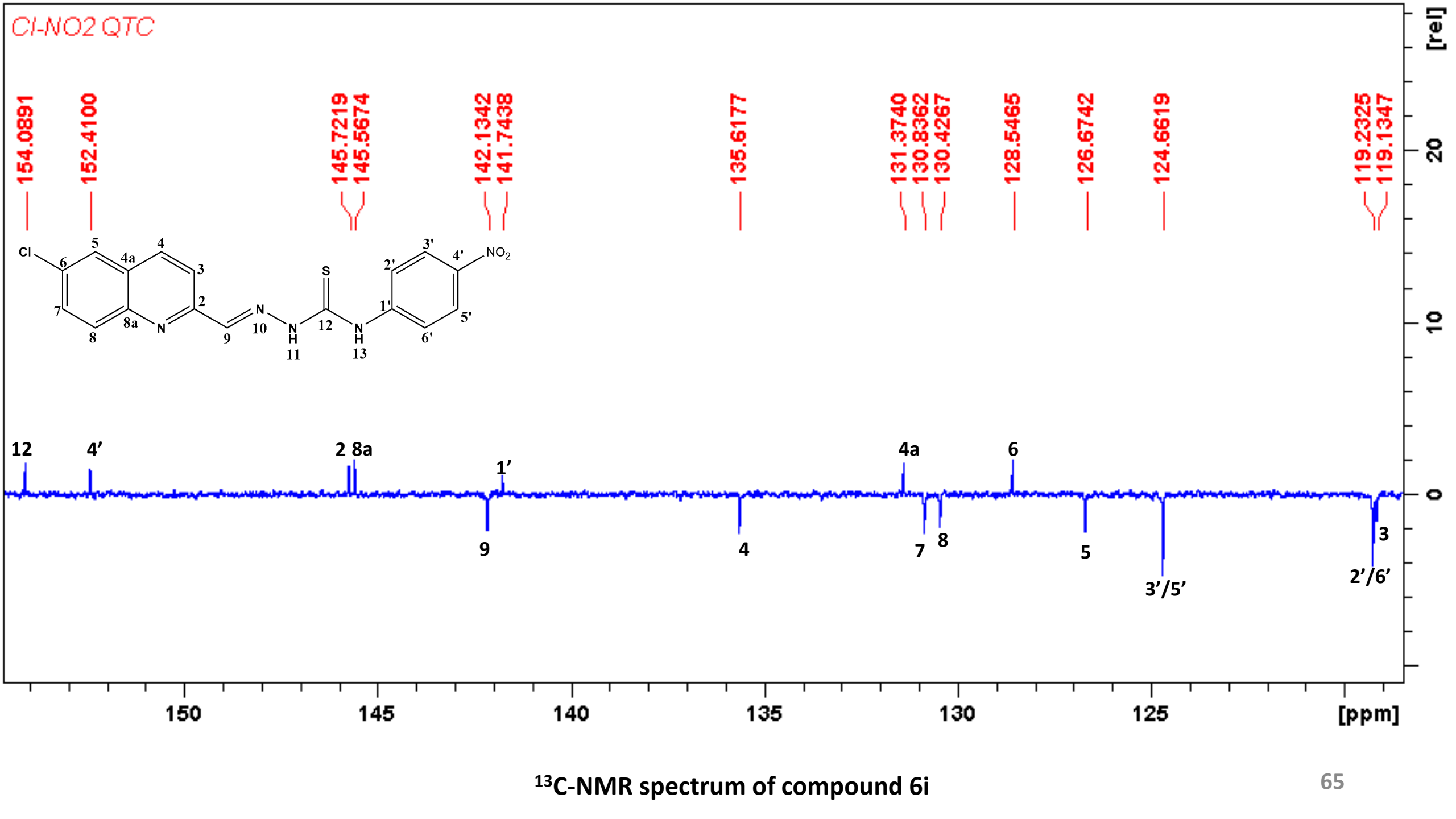
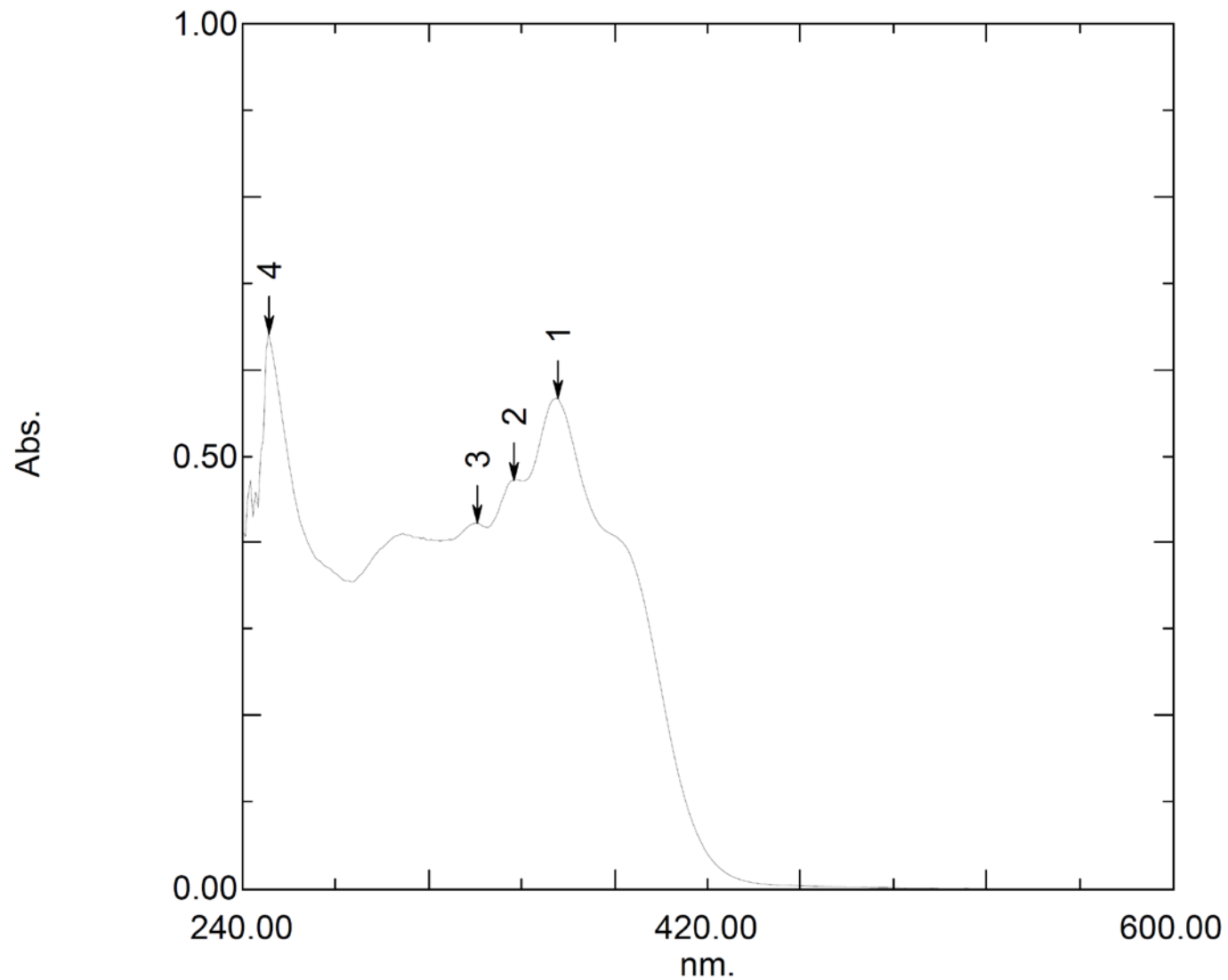**¹³C-NMR spectrum of compound 6i**
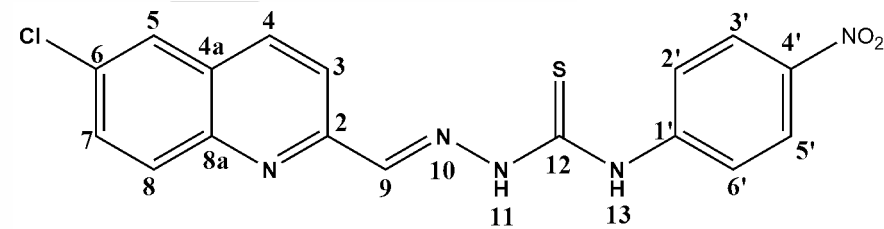
λmax	Absorbance	Log ε
249	0.69	4.47
321	0.89	4.58
334	0.97	4.61
349	0.84	4.55

UV spectrum of compound 6i
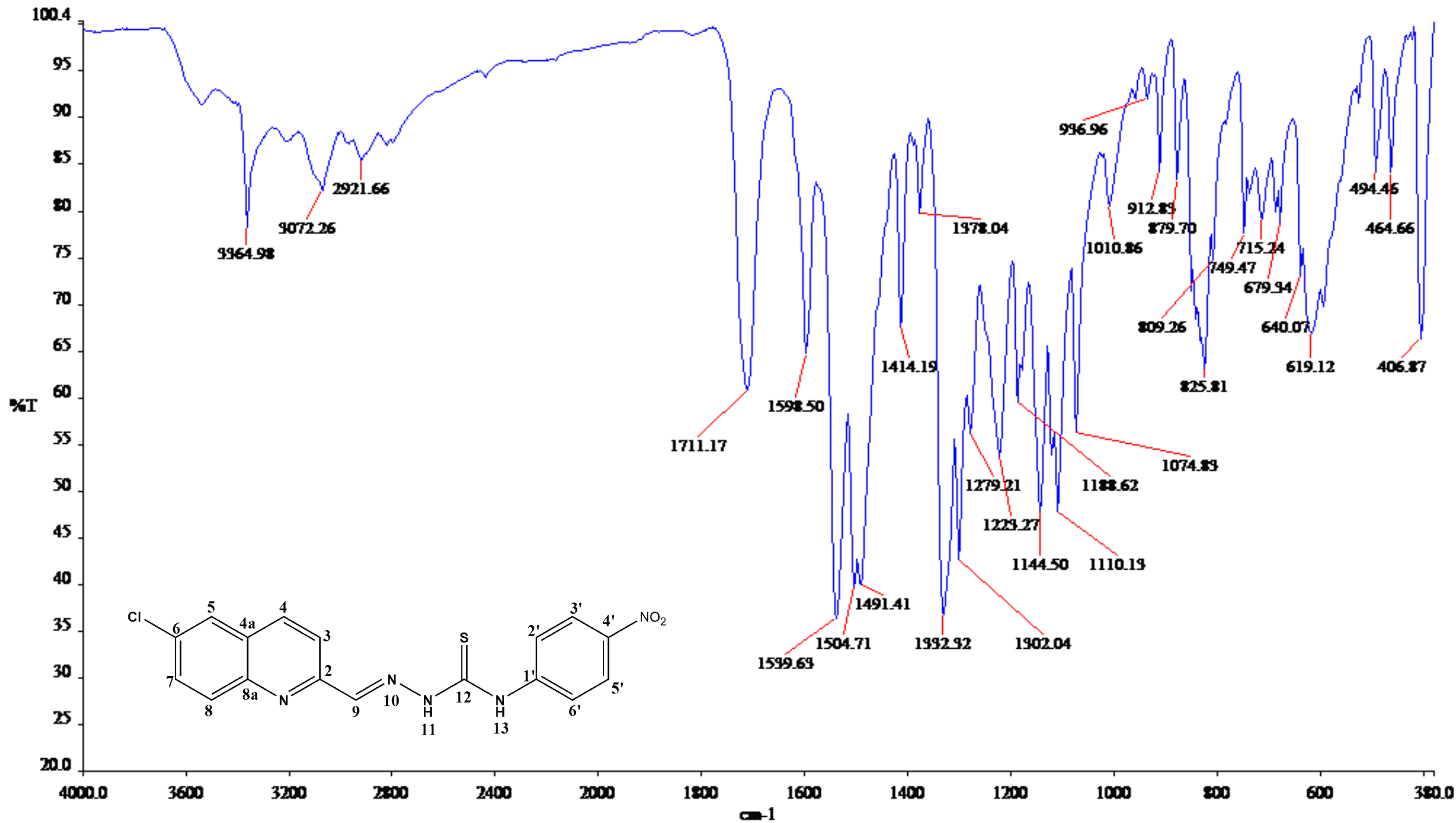
IR spectrum of compound 6i
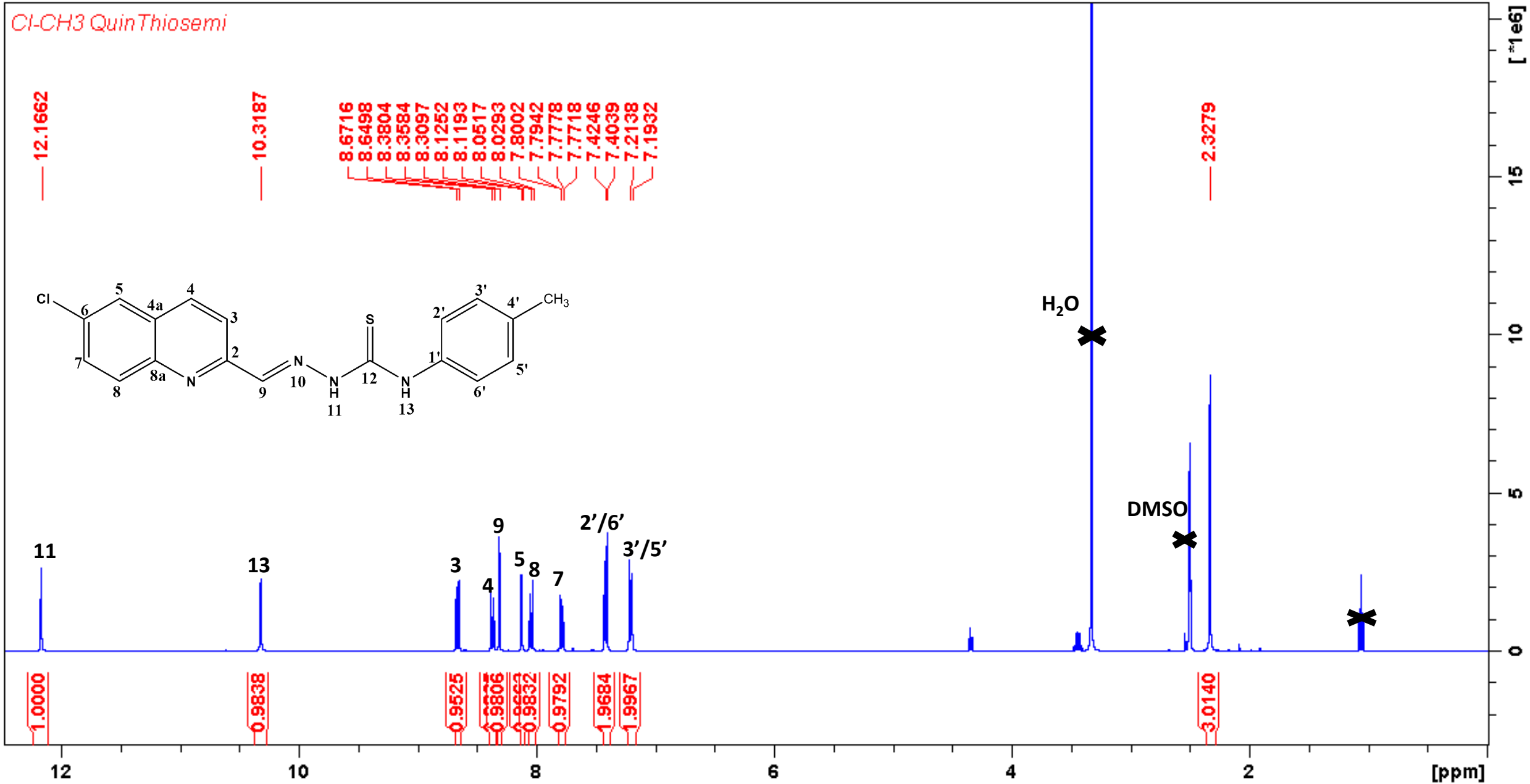
^{1}H-NMR spectrum of compound 6j

Aromatic region ¹H-NMR spectrum of compound 6j
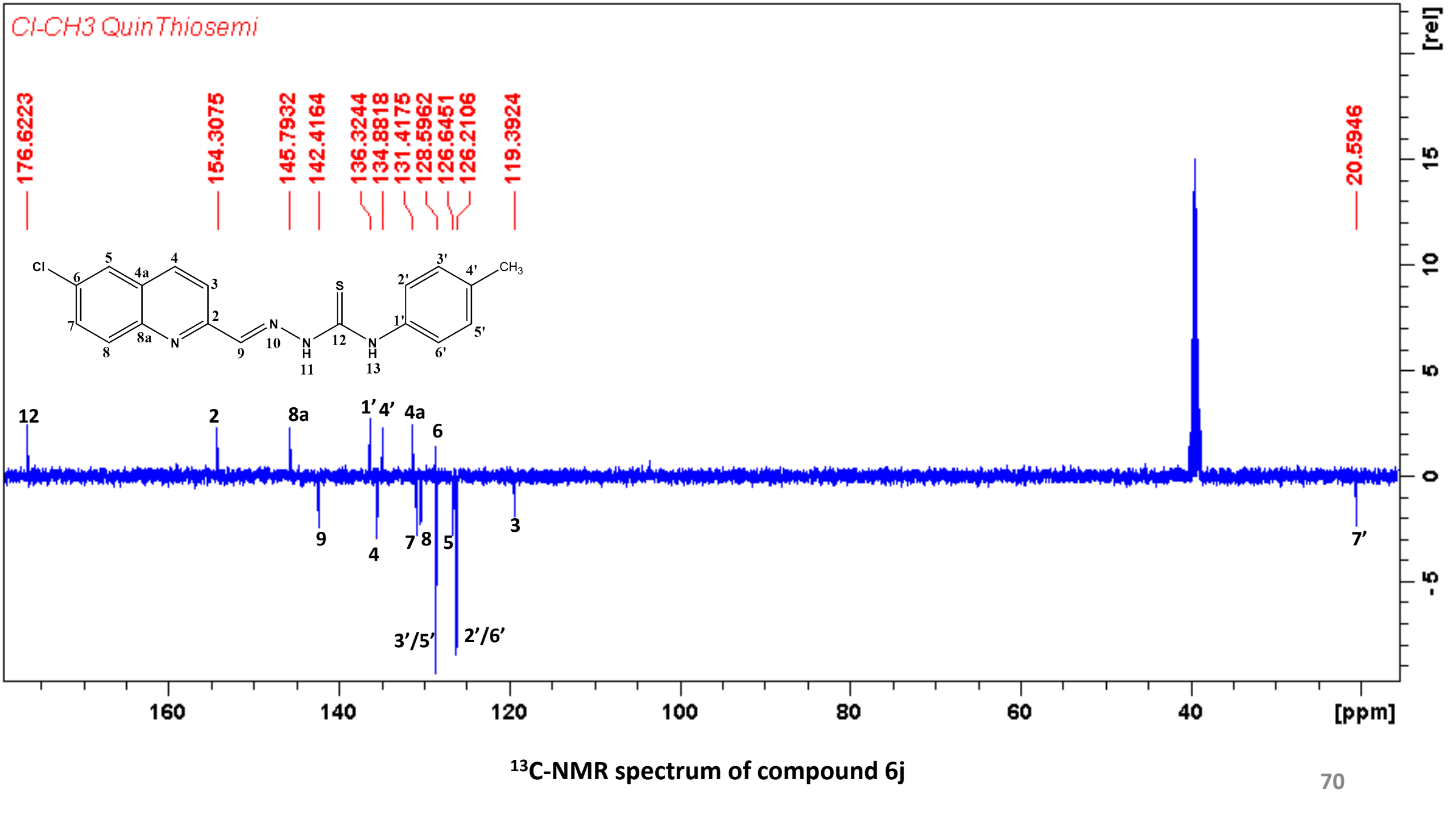
^{13}C-NMR spectrum of compound 6j

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM   /   DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
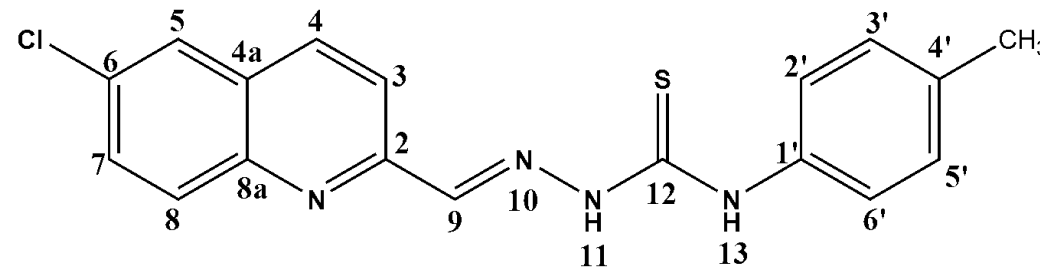
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20   H: 15-20   N: 0-5   Na: 0-1   S: 0-1   Cl: 0-1
QTSC 4b 21 (0.675) Cm (1:61)
TOF MS ES+

1.30e+005
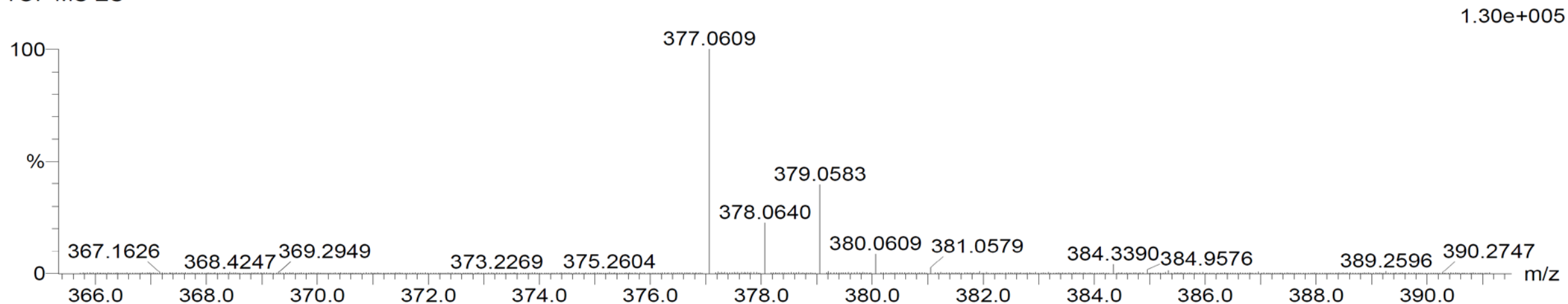
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
377.0609	377.0604	0.5	1.3	12.5	538.4	0.0	C18 H15 N4 Na S Cl

HRMS of compound 6j

71
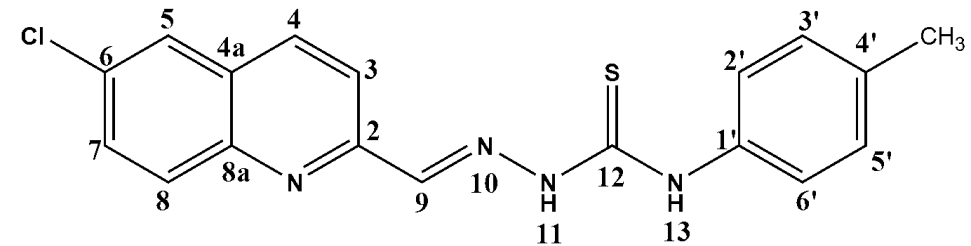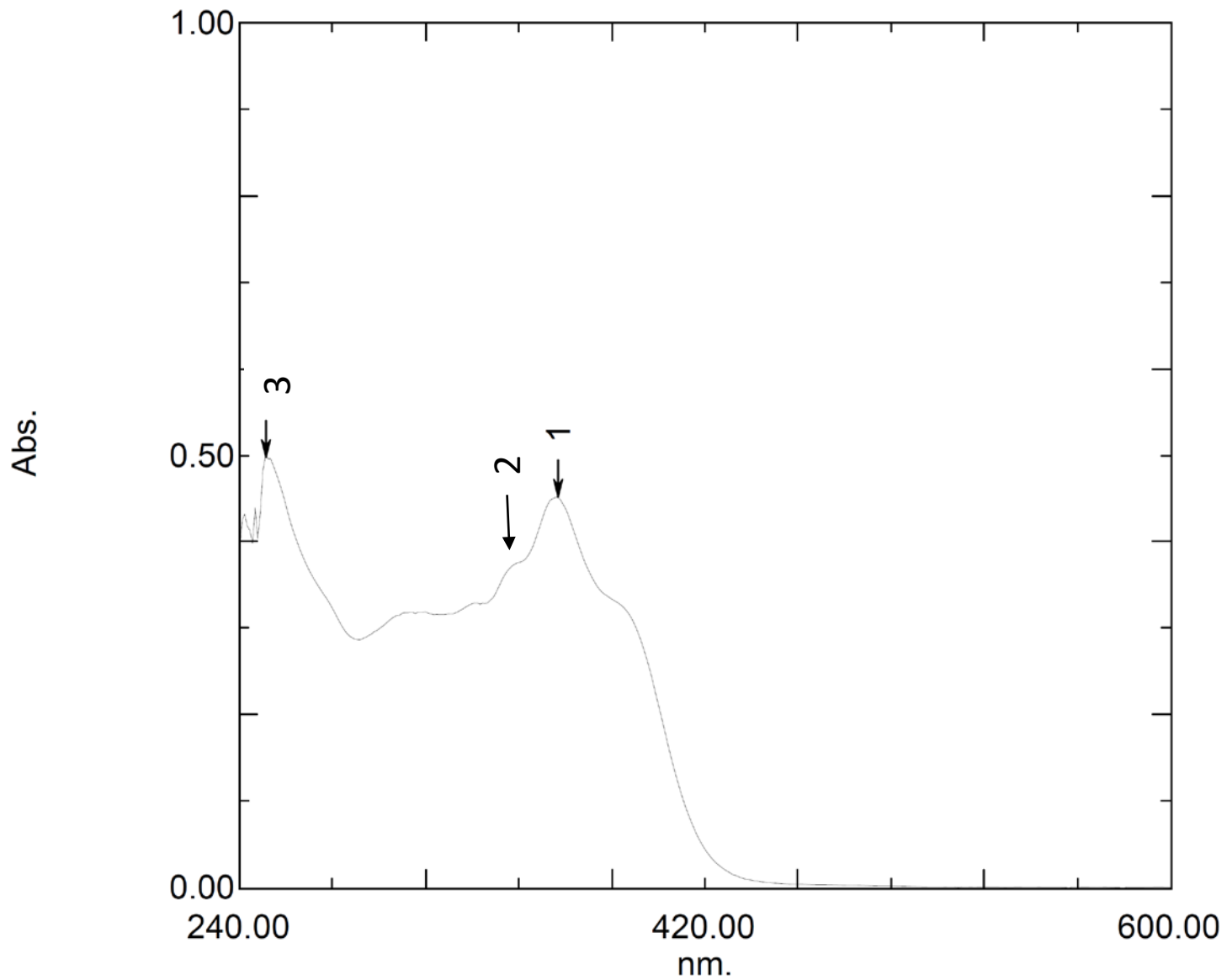
λmax	Absorbance	Log ε
251	0.70	4.40
330	0.51	4.26
360	0.66	4.37

UV spectrum of compound 6j
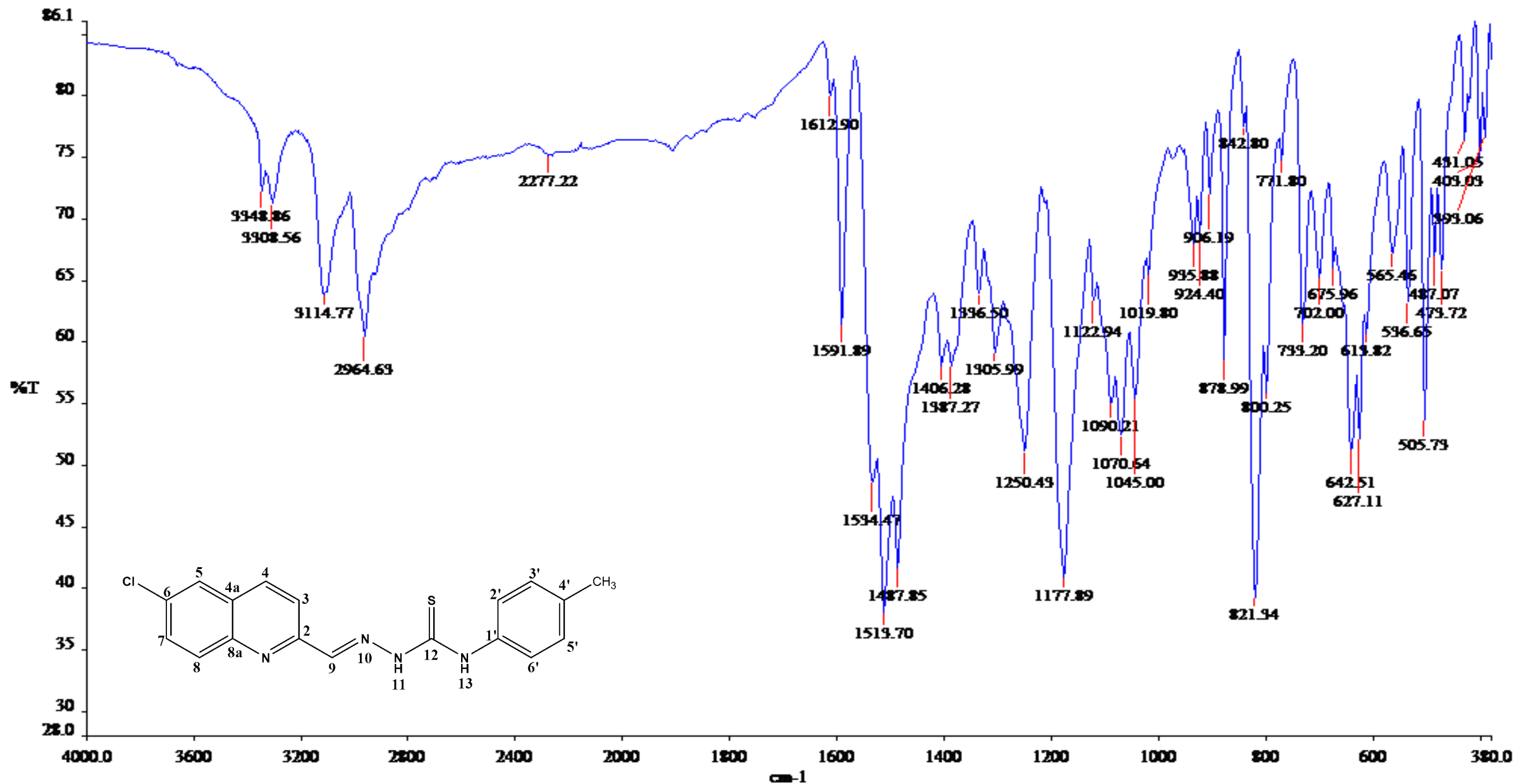
IR spectrum of compound 6j
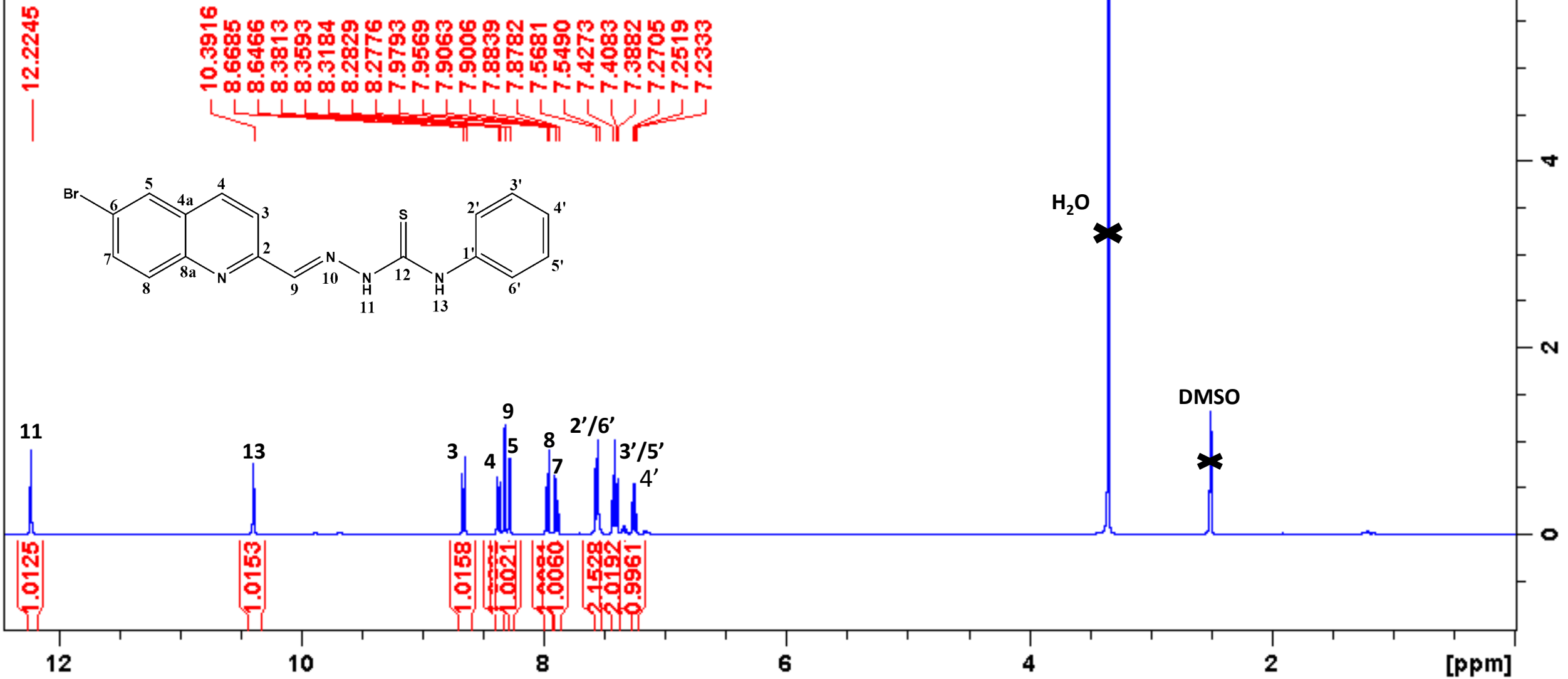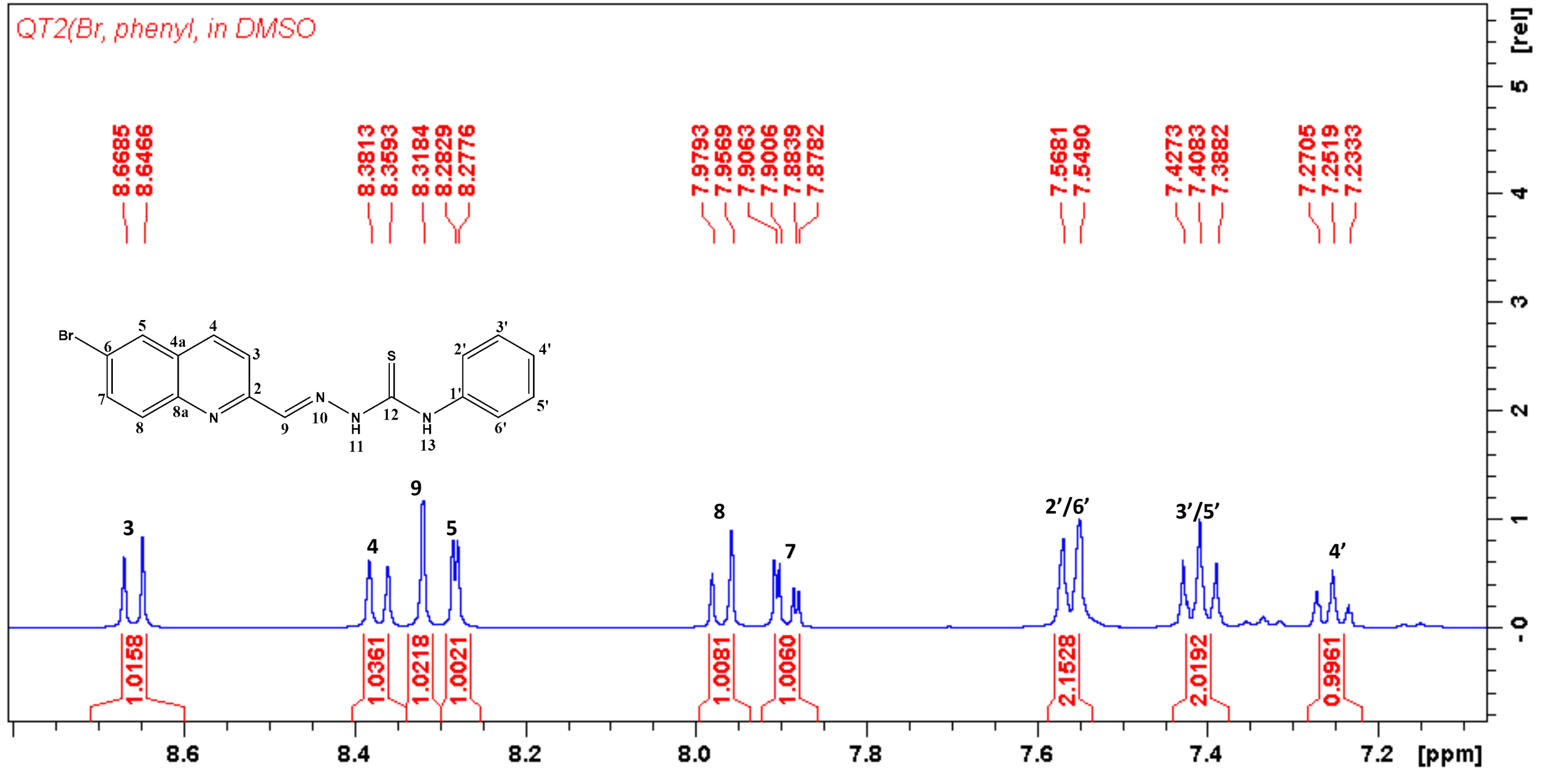
¹H-NMR spectrum of compound 6k

Aromatic region ¹H-NMR spectrum of compound 6k

^{13}C-NMR spectrum of compound 6k

76
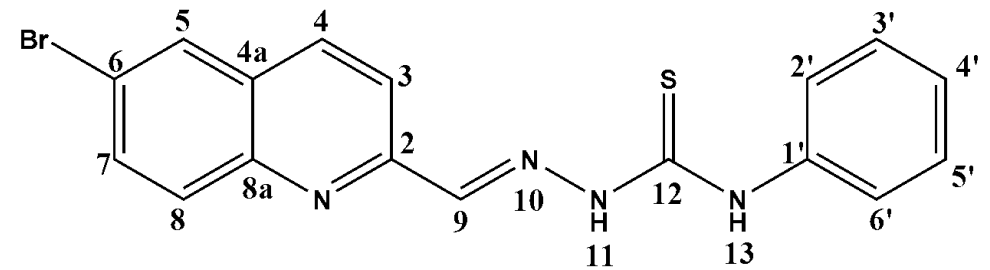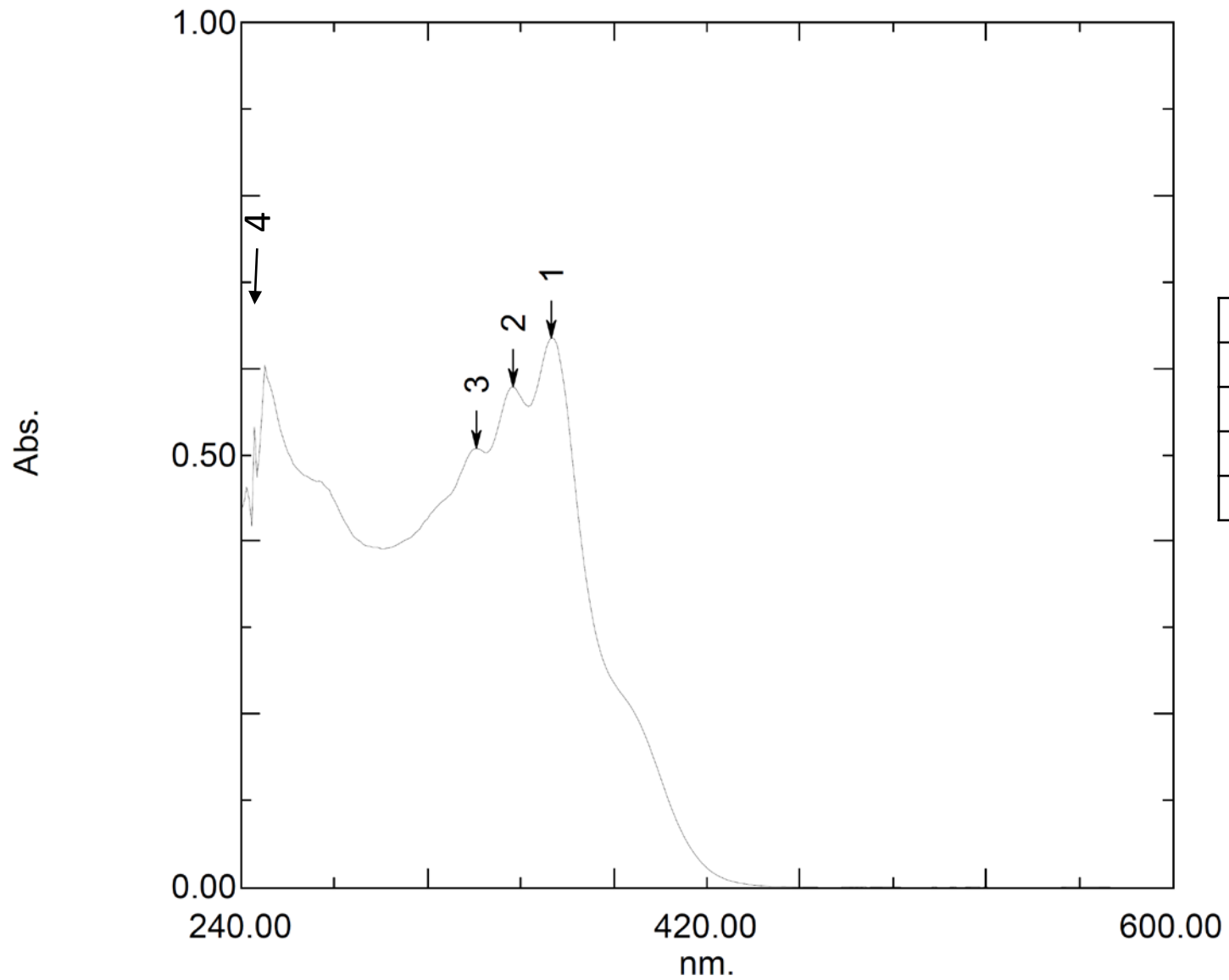
λmax	Absorbance	Log ε
249	0.60	4.37
331	0.51	4.30
347	0.57	4.35
360	0.64	4.40

UV spectrum of compound 6k

IR spectrum of compound 6k

78

^{1}H-NMR spectrum of compound 6l

79
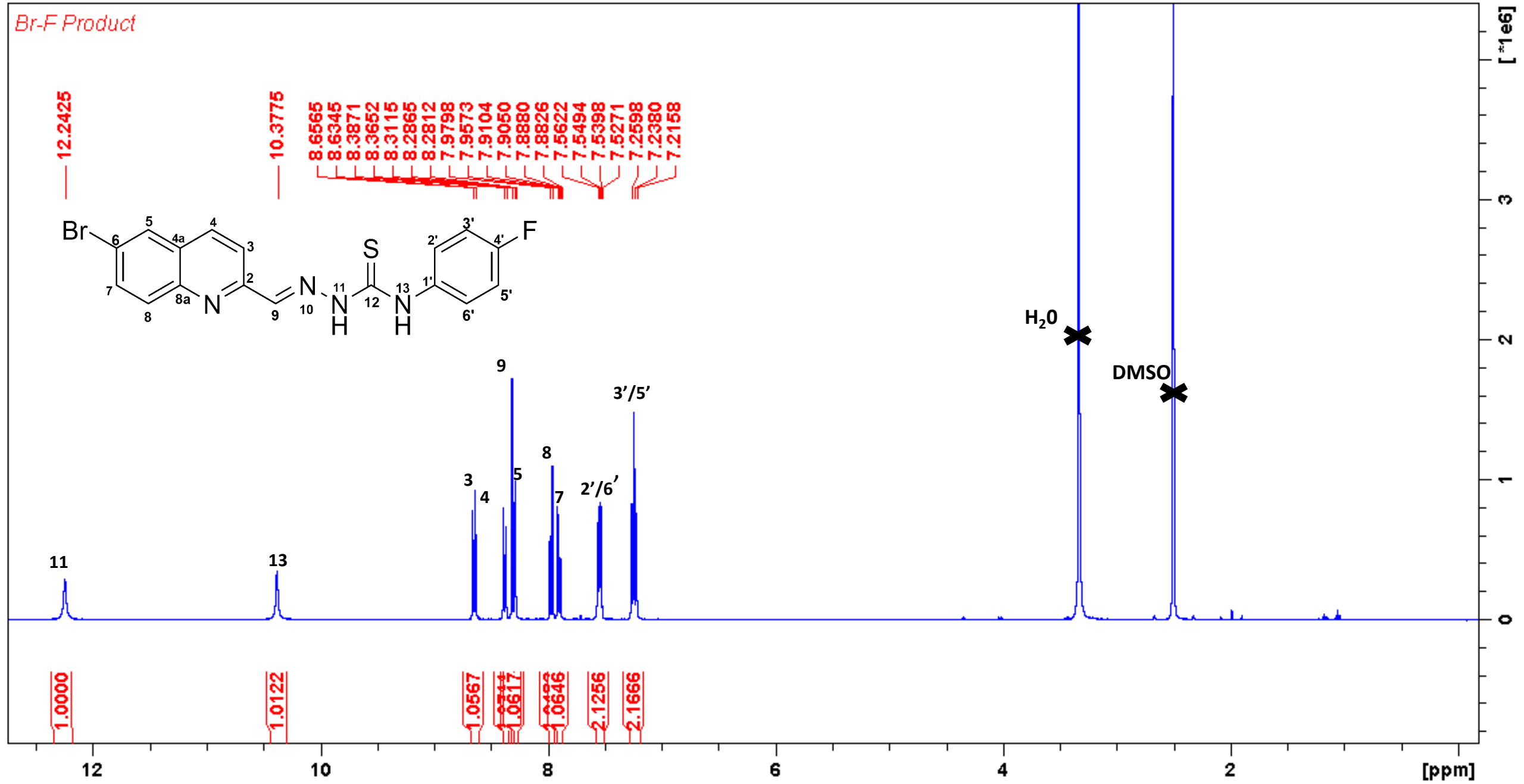
Aromatic region ¹H-NMR spectrum of compound 6l

¹³C-NMR spectrum of compound 6l
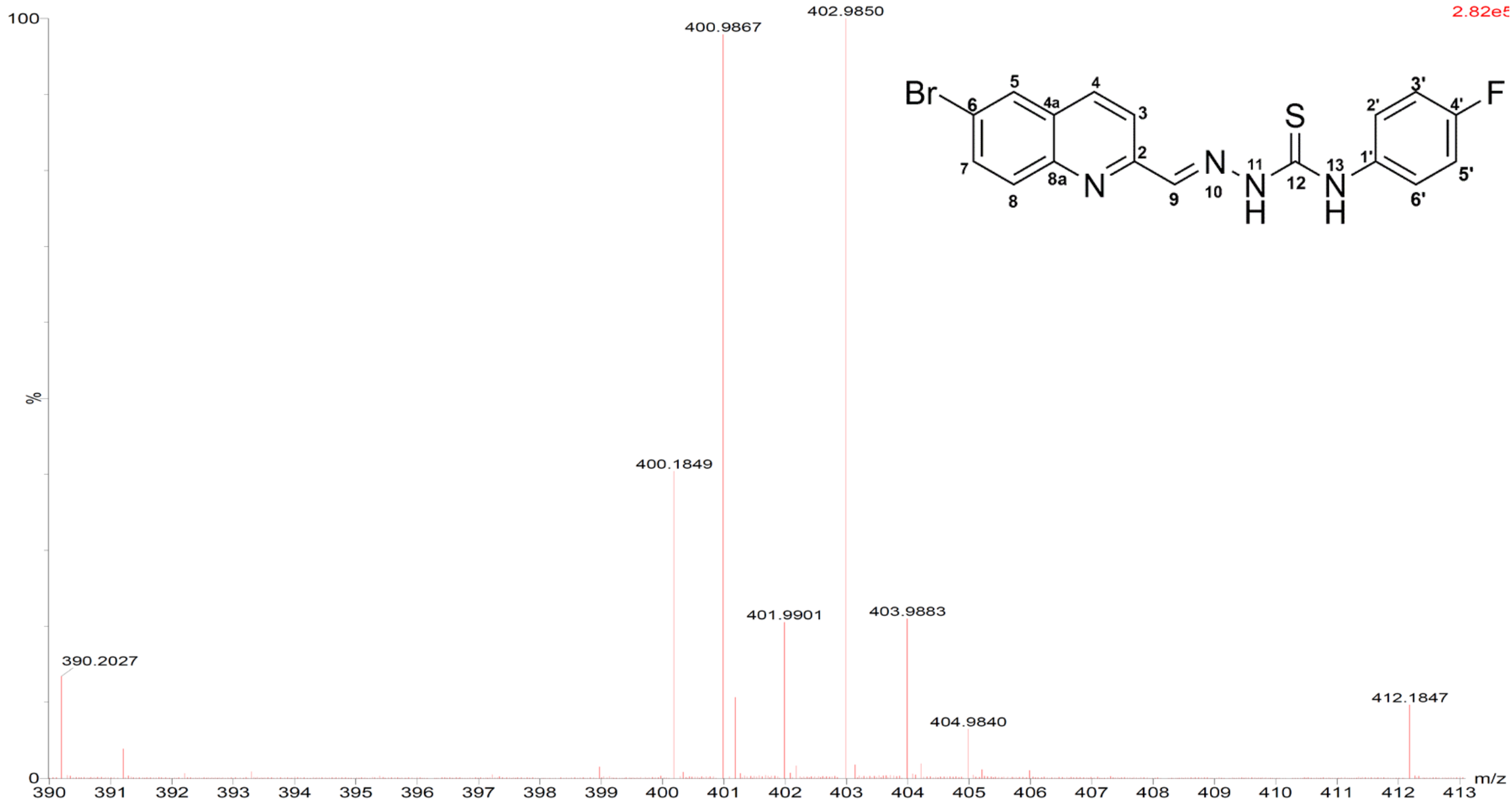
HRMS of compound 6l
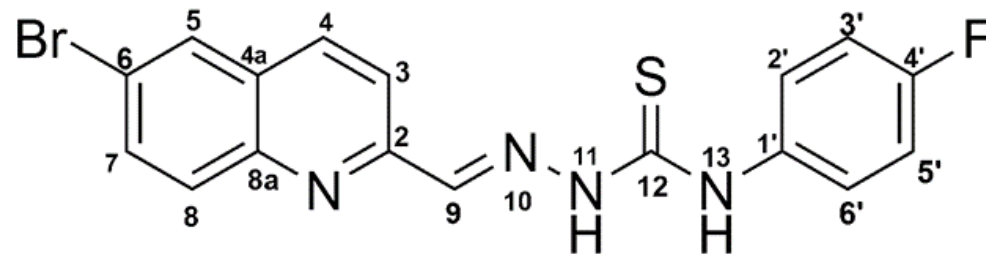
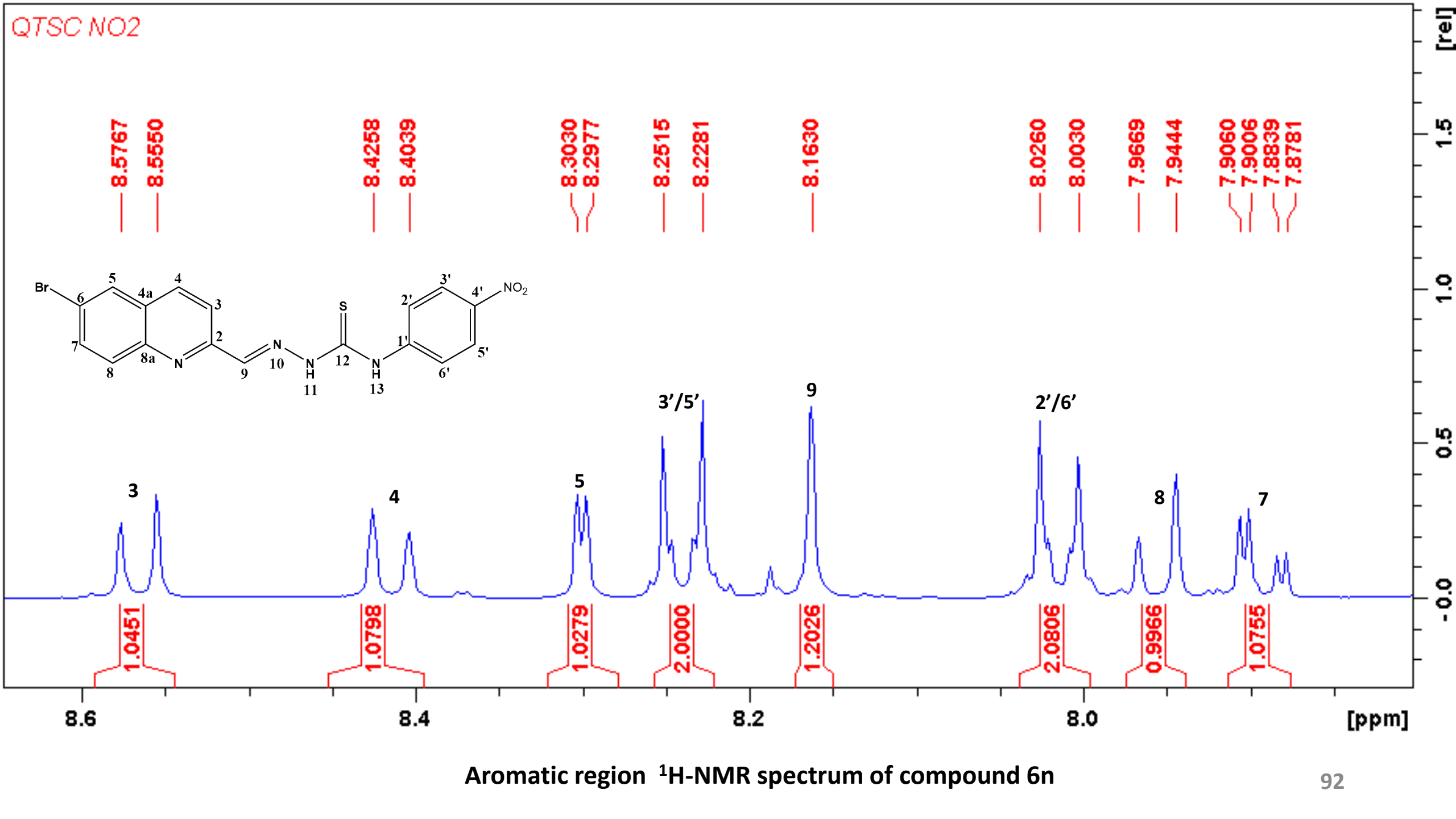| λmax	Absorbance	Log ε
249	0.52	4.29
326	0.65	4.39
340	0.71	4.43
354	0.74	4.44

UV spectrum of compound 6l

IR spectrum for compound 6l

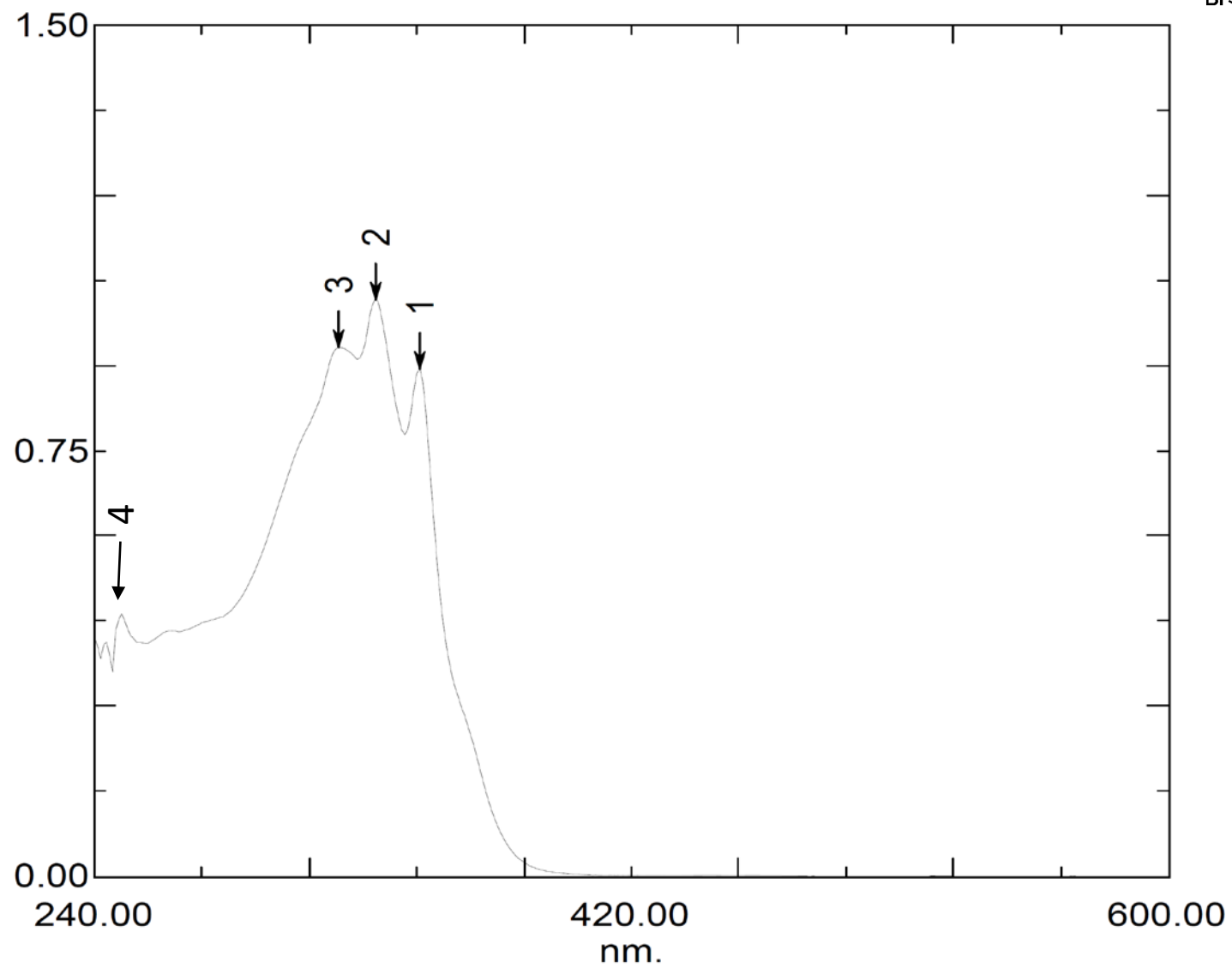^{1}H-NMR spectrum of compound 6m

Aromatic region ^{1}H-NMR spectrum of compound 6m
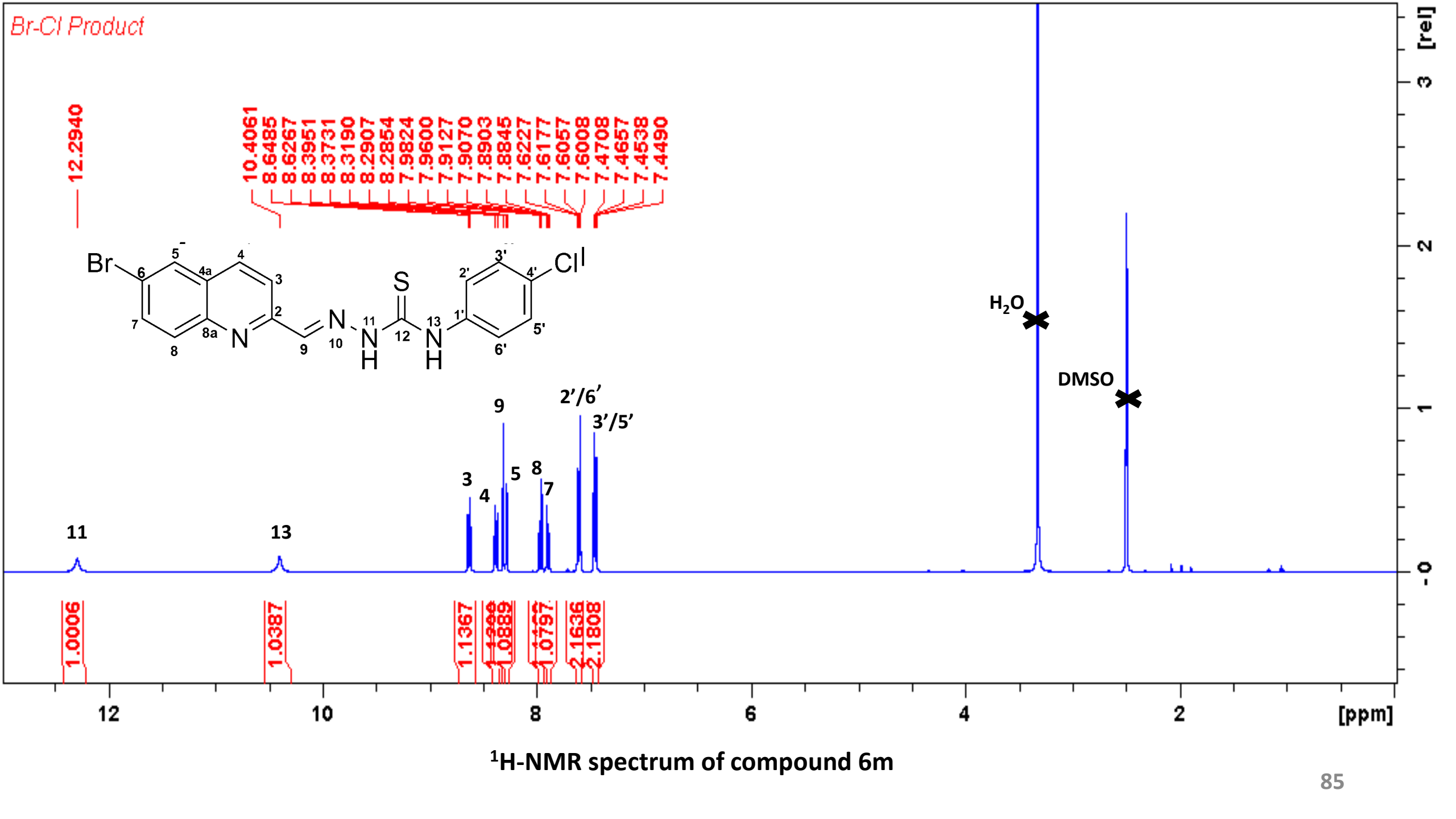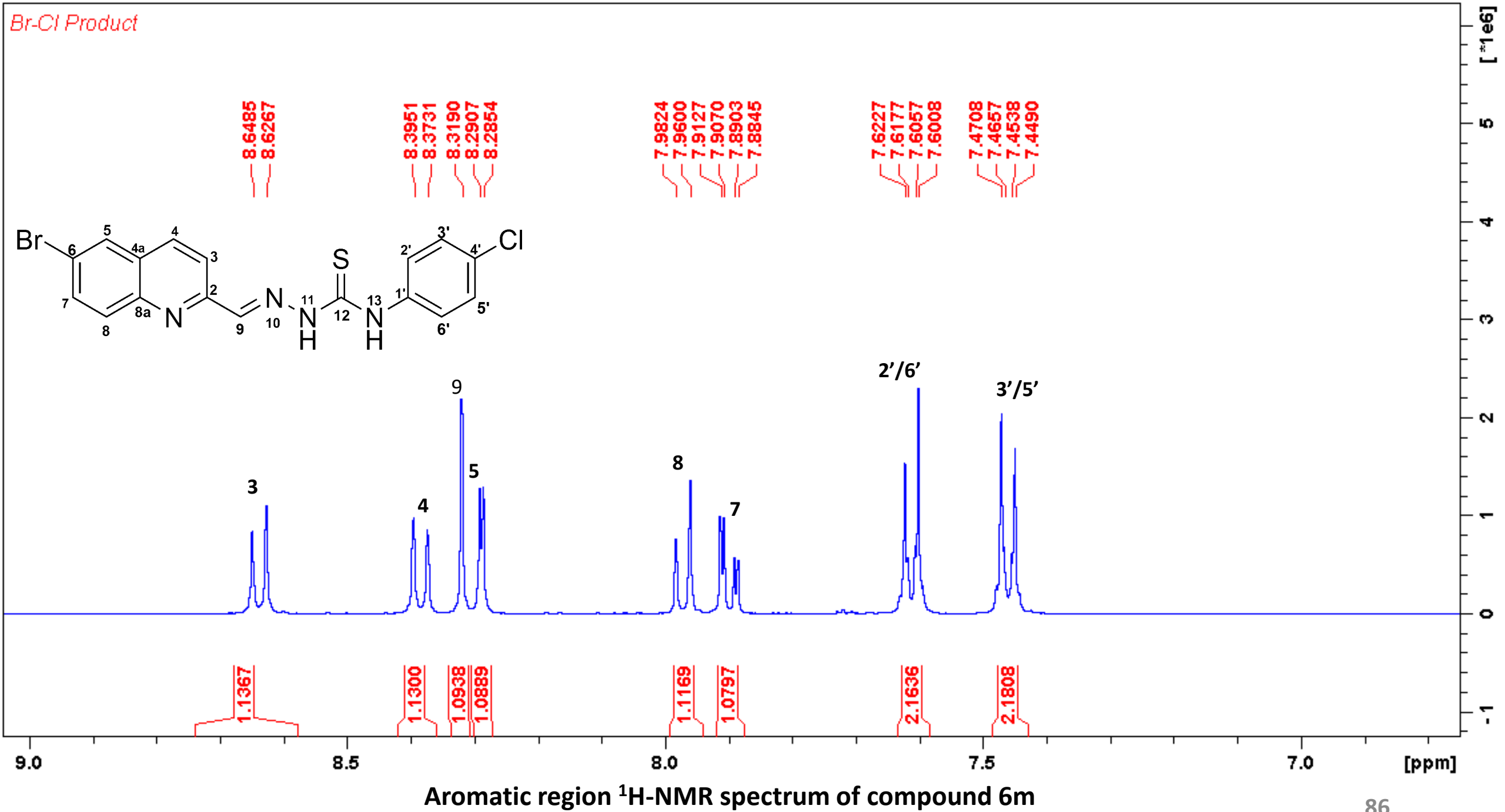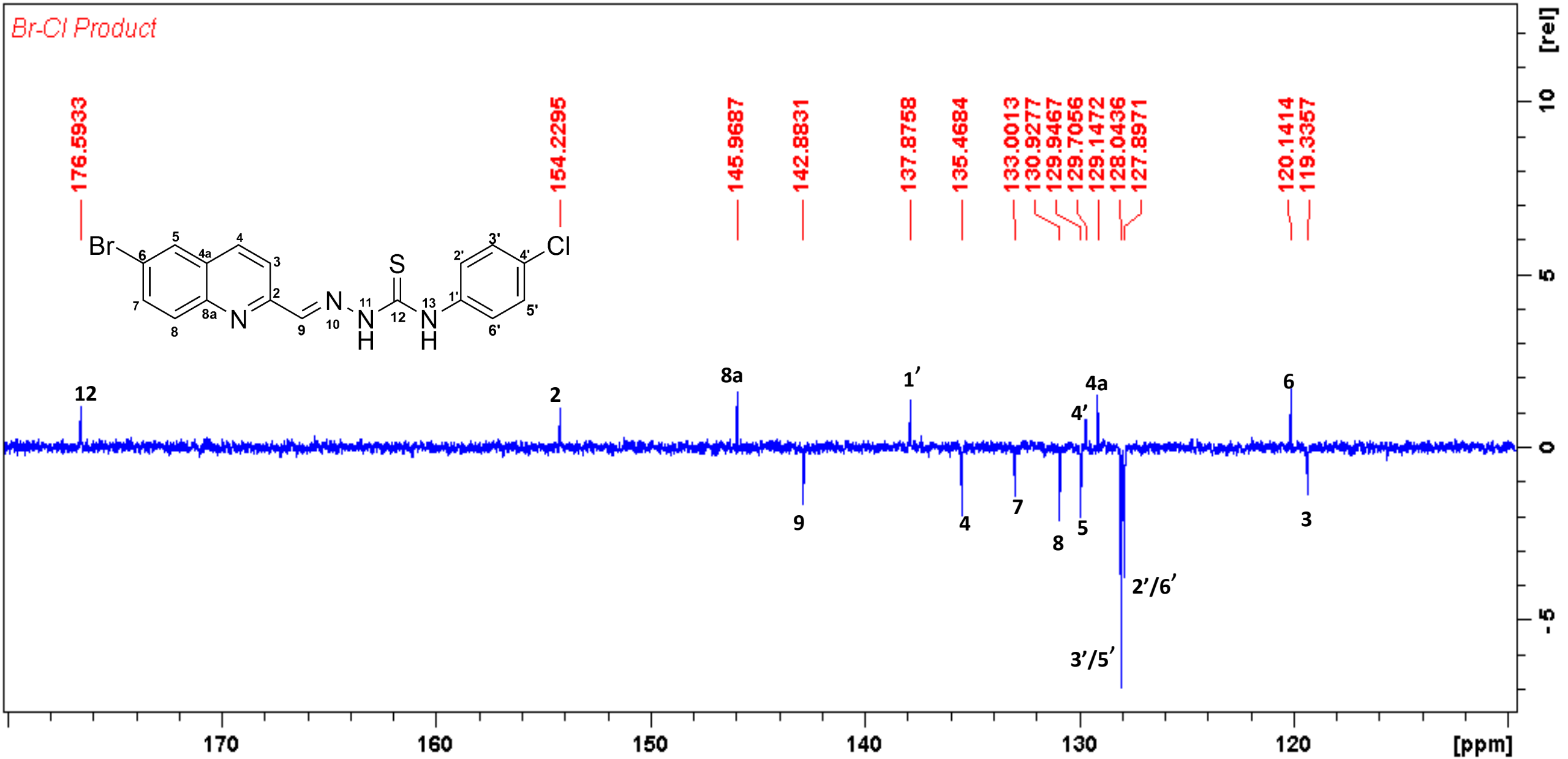
^{13}C-NMR spectrum of compound 6m

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM   /   DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
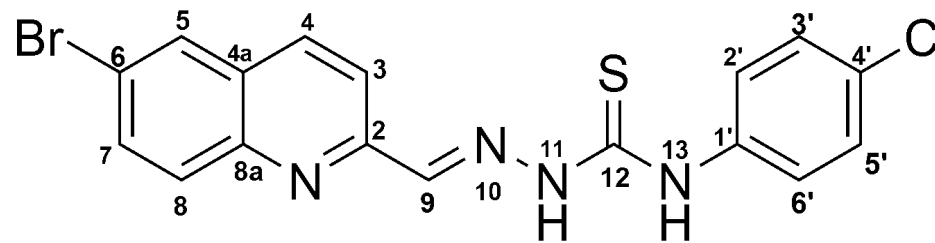
Monoisotopic Mass, Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20   H: 10-15   N: 0-5   S: 0-1   Cl: 0-1   Br: 0-1

QTSC 3a 37 (1.214) Cm (1:61)
TOF MS ES-
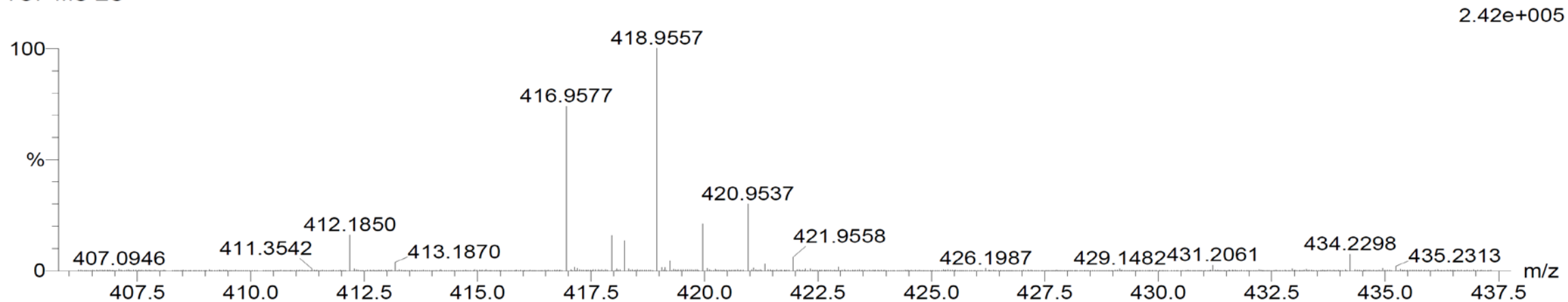
2.42e+005

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:	5.0	5.0	100.0				
416.9577	416.9576	0.1	0.2	13.5	604.6	0.0	C17 H11 N4 S Cl Br
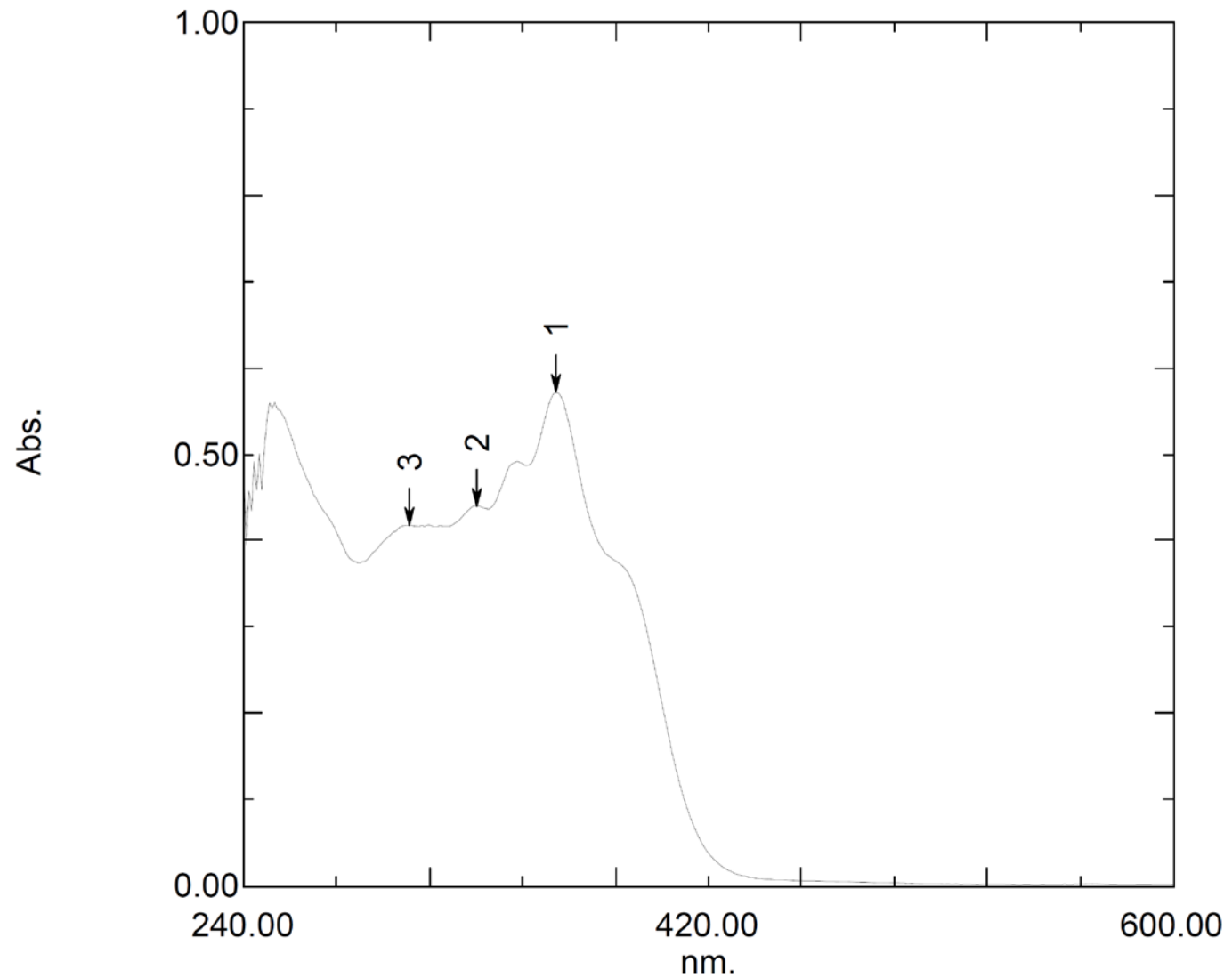
HRMS of compound 6m
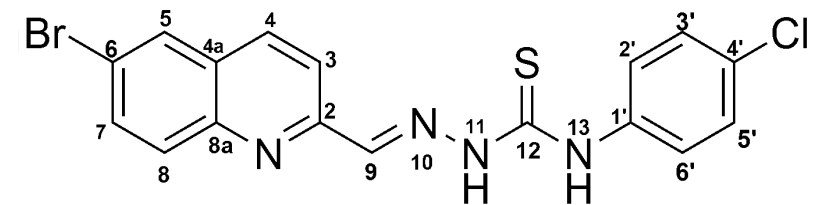
| λmax	Absorbance	Log ε
228	1.16	4.70
275	0.87	4.58
333	0.70	4.48
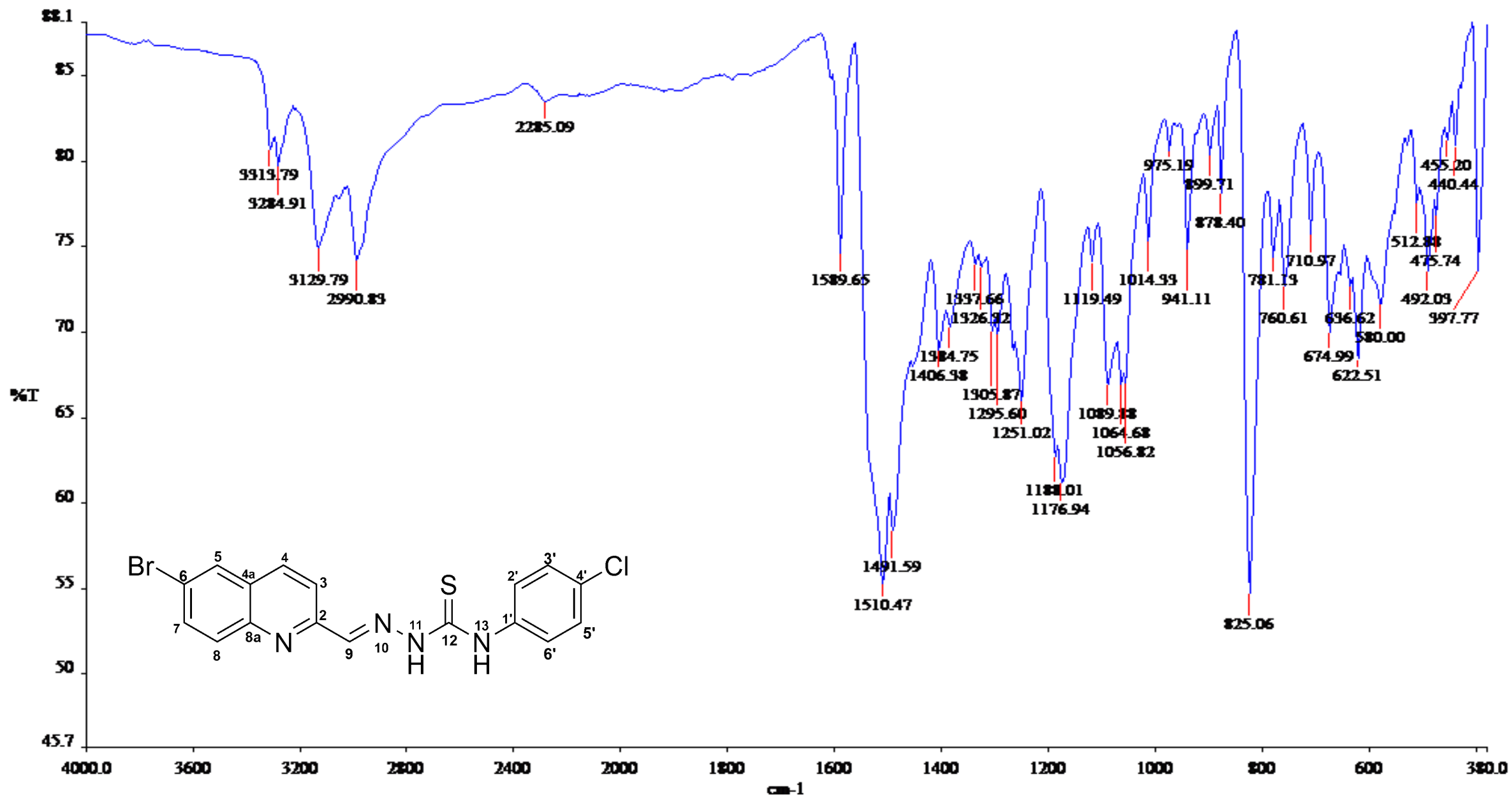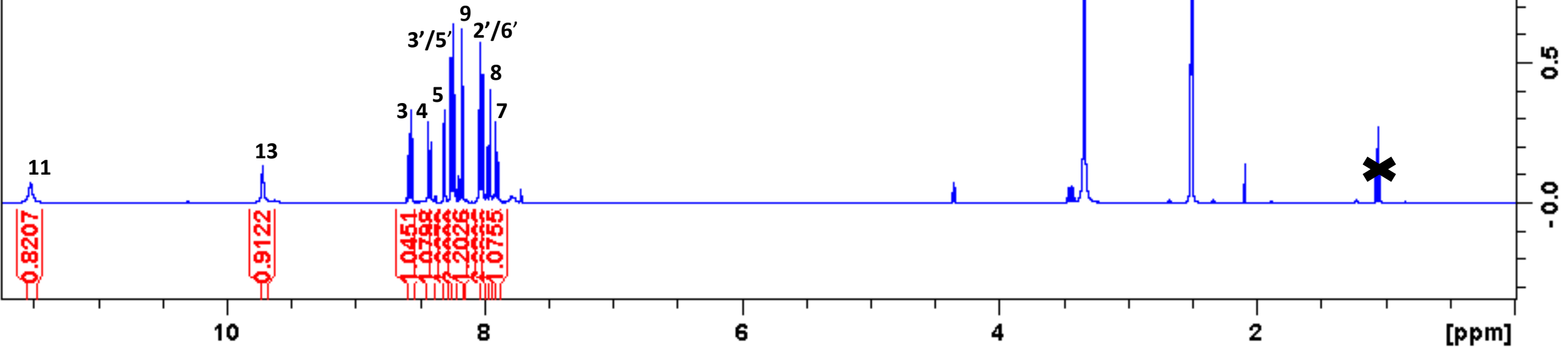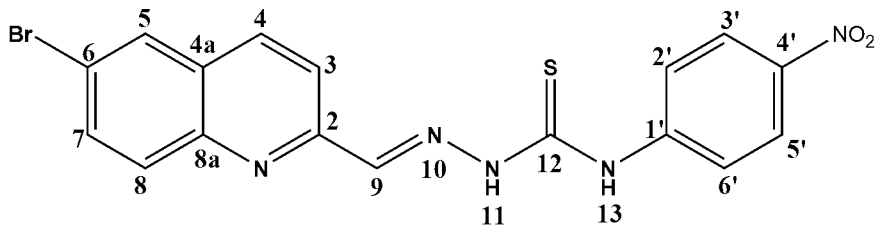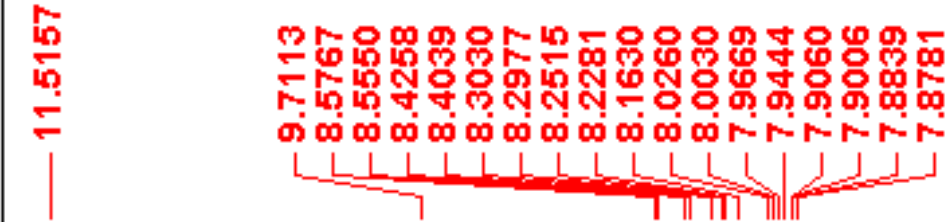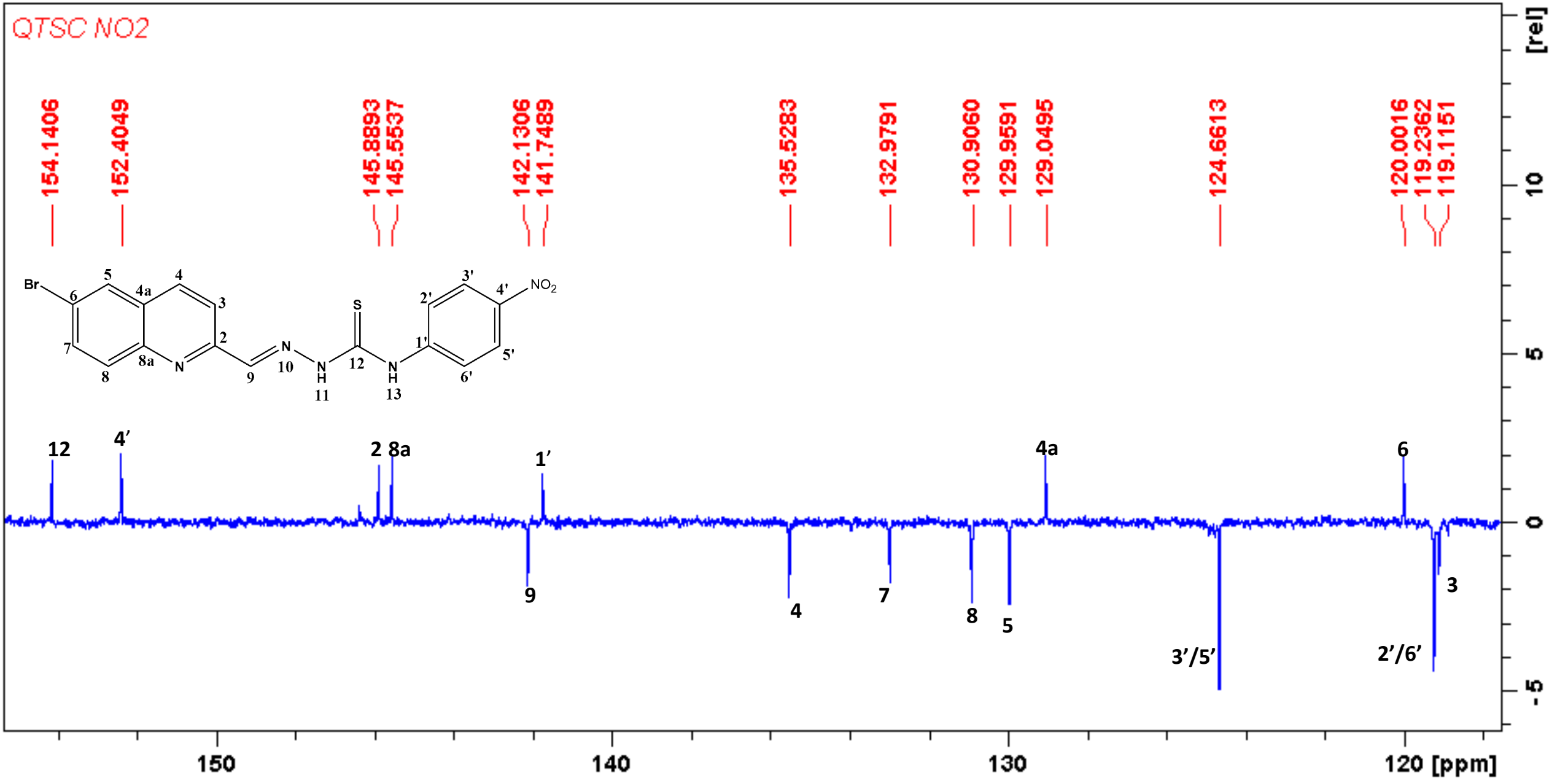
UV spectrum of compound 6m

IR spectrum of compound 6m

¹H-NMR spectrum of compound 6n

91

Aromatic region ¹H-NMR spectrum of compound 6n

92

^{13}C-NMR spectrum of compound 6n
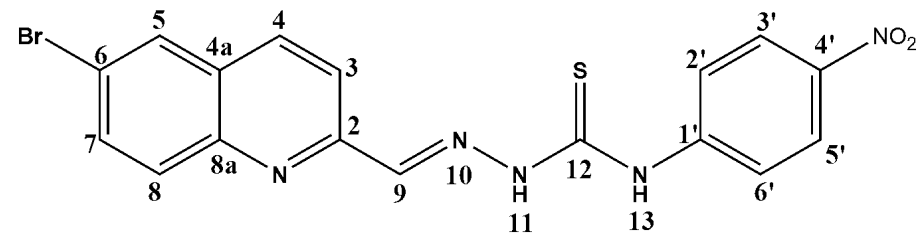
λmax	Absorbance	Log ε
249	0.46	4.32
323	0.93	4.63
334	1.02	4.67
349	0.90	4.61
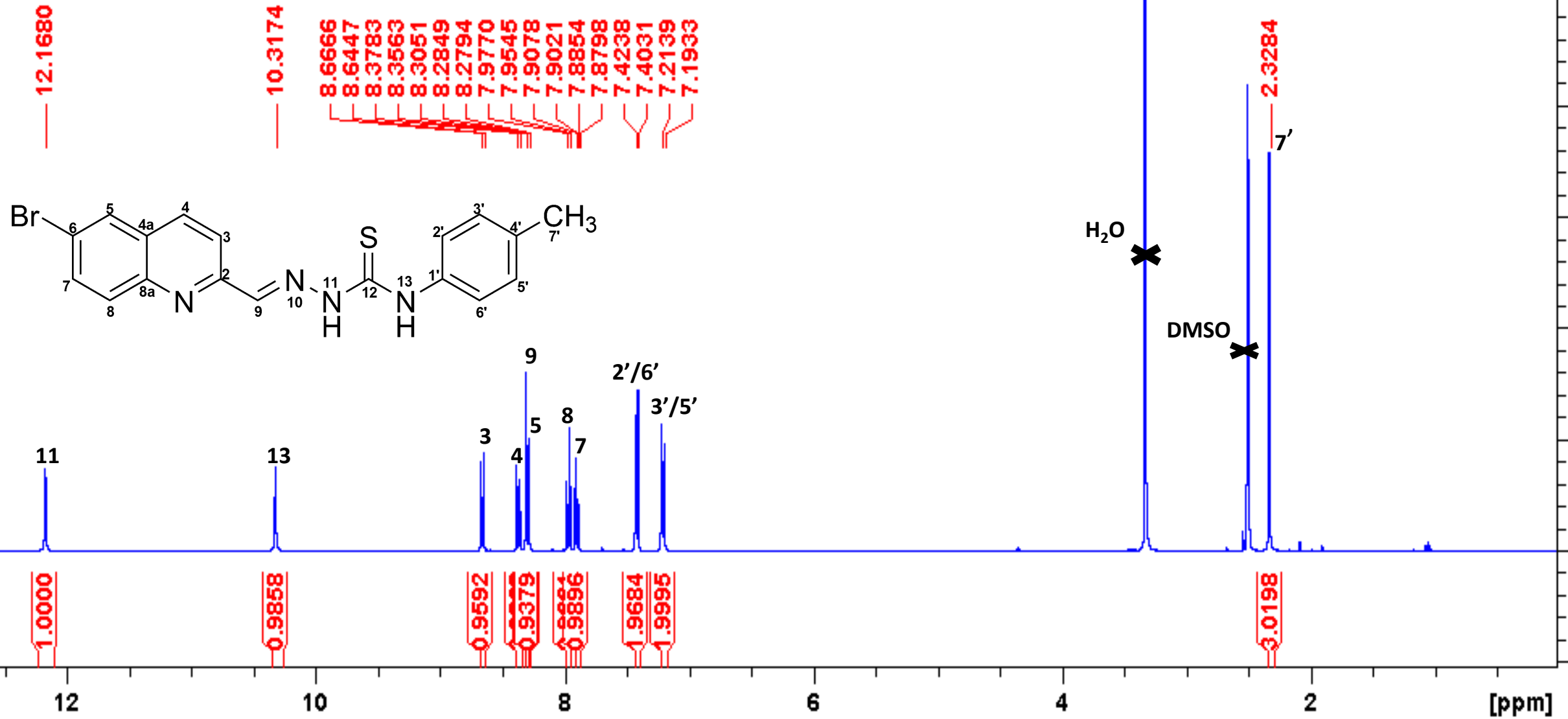
UV spectrum of compound 6n

IR spectrum of compound 6n

^{1}H-NMR spectrum of compound 6o
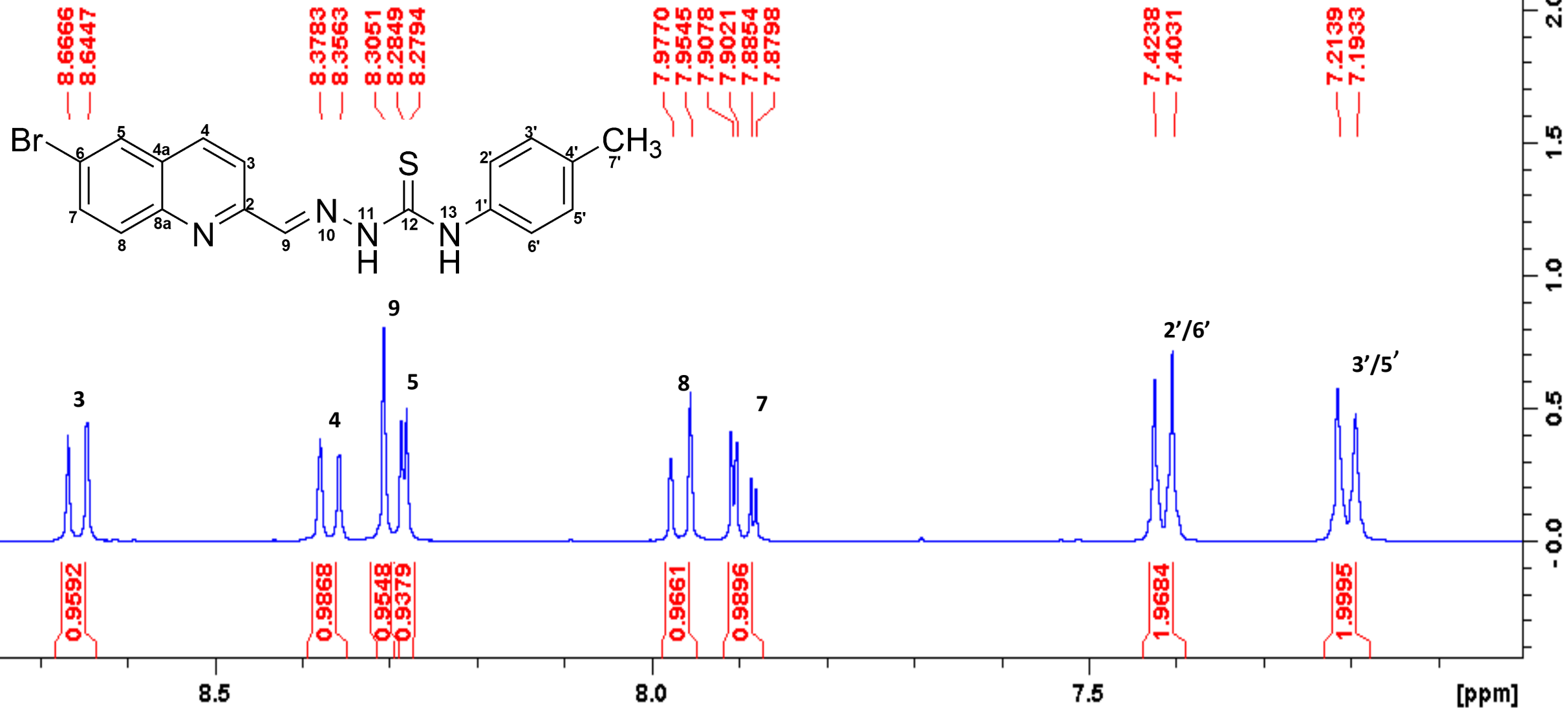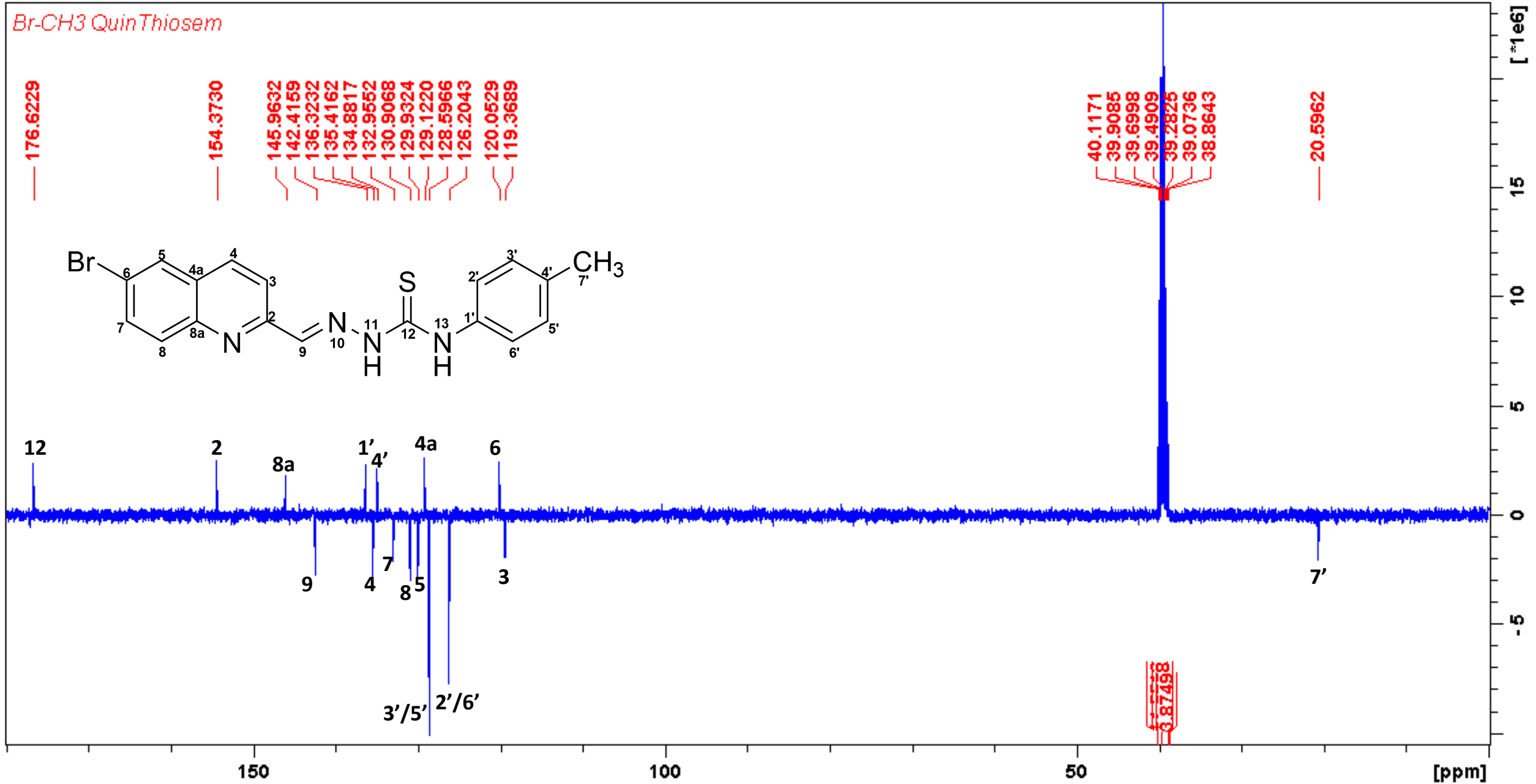
96

Br-CH3 QuinThiosem

Aromatic region^{1}H-NMR spectrum of compound 6o

¹³C-NMR spectrum of compound 6o

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM   /   DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
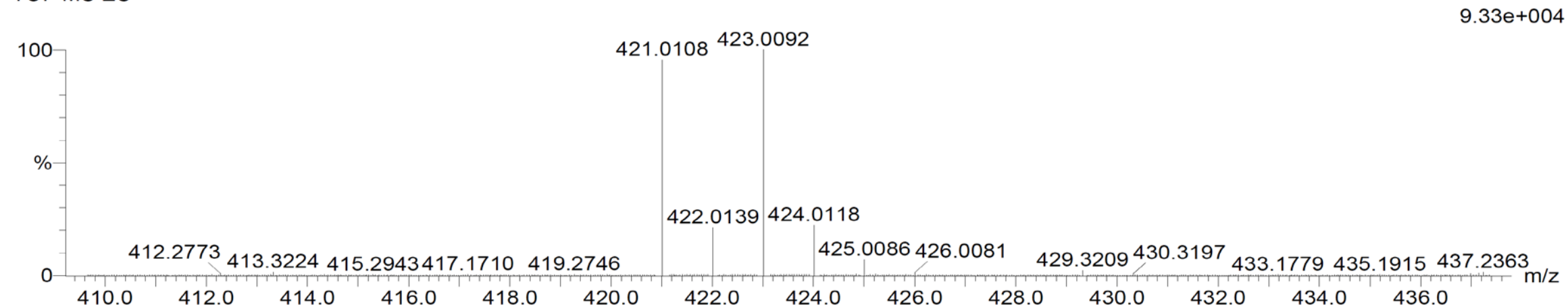
Monoisotopic Mass, Even Electron Ions
32 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20   H: 15-20   N: 0-5   Na: 0-1   S: 0-1   Br: 0-1
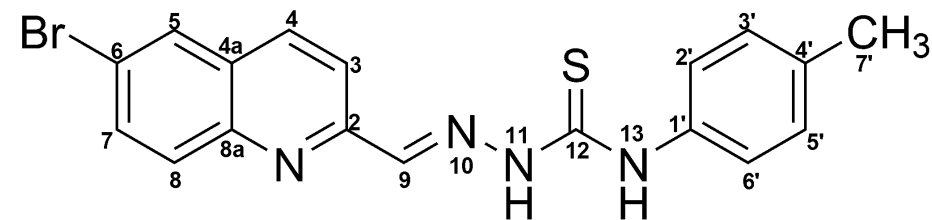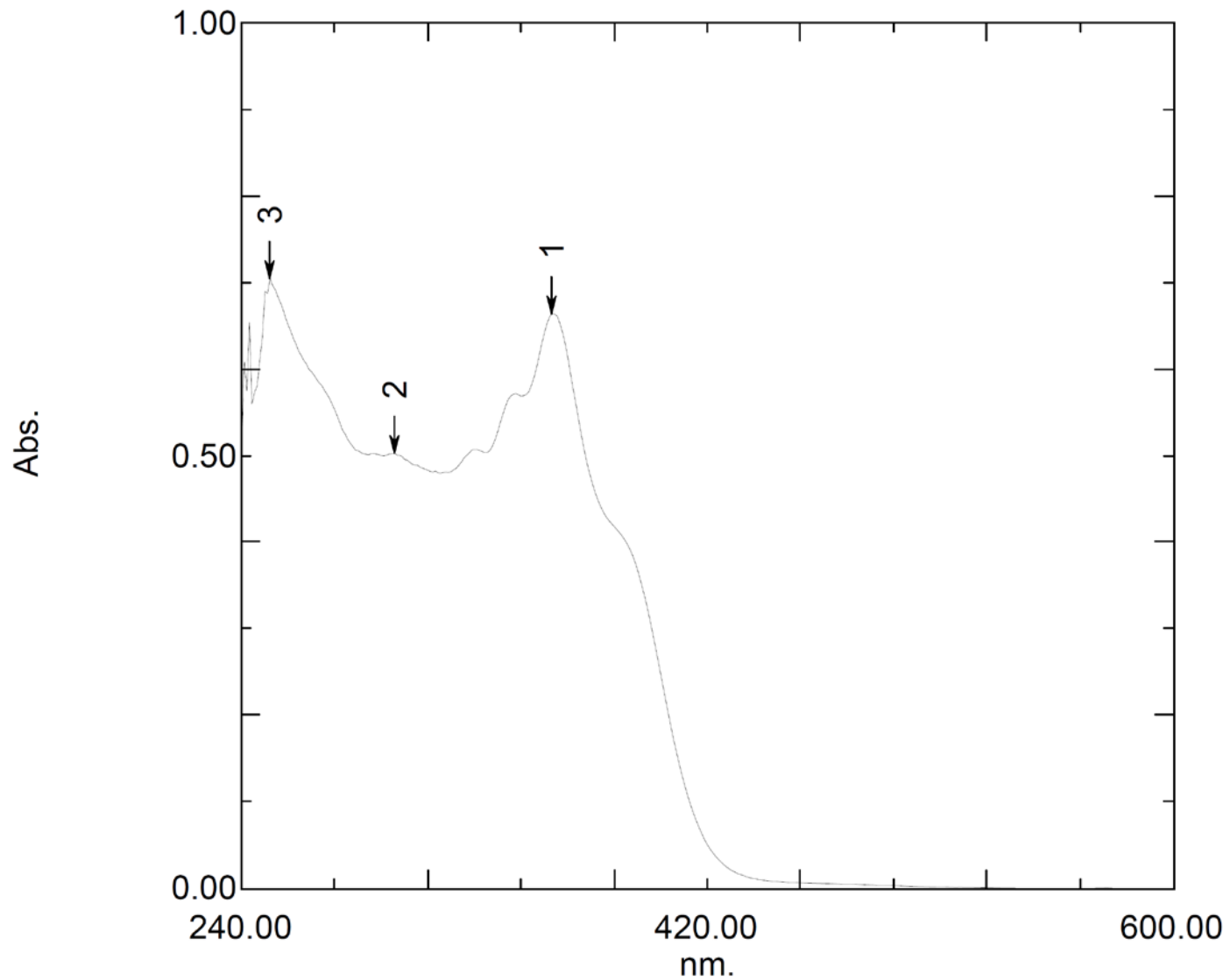
QTSC 4c 31 (1.013) Cm (1:61)
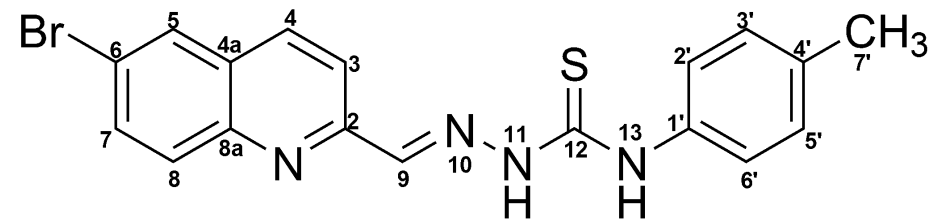TOF MS ES+
9.33e+004

Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
421.0108	421.0098	1.0	2.4	12.5	492.1	0.0	C18 H15 N4 Na S Br

HRMS of compound 6o

λmax	Absorbance	Log ε
229	0.73	4.71
280	0.62	4.64
330	0.52	4.57

UV spectrum of compound 6o

IR spectrum of compound 6o