UNIVERSITY OF KWAZULU-NATAL

SYNTHESIS AND BIOLOGICAL ACTIVITY OF QUINOLINE DERIVATIVES

2018

H GOVENDER

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF QUINOLINE DERIVATIVES

A thesis submitted in fulfillment for the requirements for the award of the degree of

Doctor of Philosophy

in the

School of Chemistry and Physics

College of Agriculture, Engineering & Science,

by

H GOVENDER

2018

Supervisor: Prof. N.A. Koorbanally

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

Supervisor:

Signed: ----------Name: ---------- Date: ----------

SYNTHESIS AND BIOLOGICAL ACTIVITY OF QUINOLINE DERIVATIVES

by

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2018

A thesis submitted to the School of Chemistry, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, for the degree of Doctor of Philosophy.

This thesis has been prepared according to **Format 4** as outlined in the guidelines from the College of Agriculture, Engineering and Science which states:

This is a thesis in which chapters are written as a set of discrete research papers, with an overall introduction and final discussion, where one (or all) of the chapters have either been submitted for publication or already been published. Typically, these chapters will have been published in internationally recognized, peer- reviewed journals.

DEDICATION

This study is dedicated to my Son

Teshan Govender

who passed away during my PhD study from a terminal illness,

Duchene Muscular Dystrophy


In Loving Memory
Teshan Govender
20 April 1993 - 13 May 2012
A Guiding Light

DECLARATIONS

DECLARATION 1-

PLAGIARISM

I, HOGANTHARANNI GOVENDER**,** declare that the experimental work described in this dissertation was carried out at the School of Chemistry and Physics, University of KwaZulu-Natal, Westville campus under the supervision of Prof. N. A. Koorbanally, and 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.
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Signed ……

DECLARATION 2- PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

Publication 1

Hogantharanni Govender, Chunderika Mocktar and Neil A. Koorbanally. Synthesis and bioactivity of quinoline-3-carboxamide derivatives. Journal of Heterocyclic Chemistry, 2018, 55, 1002-1009.

Publication 2

Hogantharanni Govender, Chunderika Mocktar and Neil A. Koorbanally. The design and biological activity of substituted thiosemicarbazones; prepared for publication.

Publication 3

Hogantharanni Govender, Chunderika Mocktar and Neil A. Koorbanally. Synthetic study and biological investigation of substituted phenylhydrazones; prepared for publication

For all the publications above, I conducted the experimental work and together with my supervisor contributed to the writing of the publications. The publications were edited by the co-authors for scientific content.

Signed: …….

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I would like to express my sincerest gratitude to my supervisor ***Prof. N. A. Koorbanally*** for his expert guidance, advice and support in this work. He was always available and willing to assist anytime.

I thank also Dr Chunderika Mocktar for her guidance and assistance in the antibacterial studies.

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To my family, my late father George, thank you for the values you instilled in me and for my becoming the person that I am. To my mother, Angel, I thank you for all the inspiration you have provided in my life. My brothers, Derek, Mark, Neville, Gavin, Alvin, Delon and sister,

Linda, I thank you for all the moral support you had given me during my loss of Teshan and the encouragement to continue with my studies.

Last, but not least, To My Husband Thagaraj and Son, Sherwyn, Chester, I thank you for your understanding, patience, tolerance and care you had given me during my difficult time after the loss of Teshan. Thanks for motivating me to pursue my PhD studies as a tribute to Teshan.

To my guiding light, my beloved son Teshan, I miss you……...

Preface

I hereby declare that the thesis entitled "Synthesis and biological activity of quinoline derivatives" submitted to the University of KwaZulu-Natal for the award of the degree of Doctor of Philosophy 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: _____
Hogantharanni Govender, MSc (UKZN)

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

Signed: _____
Prof. Neil A. Koorbanally, Ph. D (Natal)

LIST OF ABBREVIATIONS

^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
°C	Degrees Celsius
d	Doublet
dd	Double Doublet
DMF	Dimethylformamide
EDC	*N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide
HIV	Human Immuno-deficiency virus
HOBt	1-Hydroxybenzotrizole
HMBC	Heteronuclear Multiple Bond Coherence
h	Hours
HRESIMS	High Resolution Electrospray Ionisation Mass spectrometry
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
IR	Infrared Spectroscopy
m	Multiplet
MBC	Minimum Bactericidal Concentration
MeOH	Methanol
MH	Mueller-Hinton
MHA	Mueller-Hinton Agar
min	Minutes
mp	Melting Point
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
ORTEP	Oak Ridge Thermal Ellipsoid Plot
r.t	Room Temperature
s	Singlet
t	Triplet
td	Triplet of doublets
TLC	Thin Layer Chromatography
UV	Ultraviolet Spectroscopy

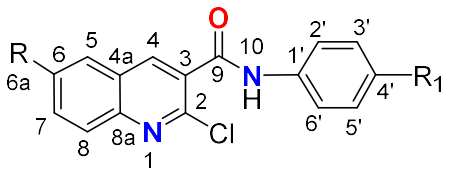
ABSTRACT

A total of 42 compounds, which include 12 quinoline-3-carboxamides (**A5a-l),** 15 quinolone thiosemicarbazones (**B5a-o**) and 15 tetrazoloquinoline phenylhydrazones (**C5a-o**) were successfully synthesized and characterised. Of these 42 compounds, 36 have been prepared for the first time in this work, with 4 thiosemicarbazones and 2 phenylhydrazones being previously reported.

The 2-chloroquinoline-3-carbaldehyde intermediate was the basic scaffold on which the three types of quinoline hybrids were based on. Various substituents were placed at C-6 on this substituent to create small libraries of compounds. This key intermediate was prepared using the Vilsmeier-Haack reaction, which resulted in a quinoline with a carbaldehyde at C-3. In the case of the quinoline-3-carboxamides, the aldehyde at position 3 was oxidized to the acid functionality via the Pinnick Oxidation. This was followed by the preparation of carboxamides using a coupling reaction with different substituted anilines and using the coupling reagents EDC·HCl and HOBt in the presence of triethylamine used as a base. The thiosemicarbazones were prepared by condensing the 2-quinolone carbaldehydes with thiosemicarbazides and the tetrazolophenylhydrazones prepared by first forming a tetrazolo ring with sodium azide on the quinoline and then forming phenylhydrazones from the carbaldehyde moiety and phenylhydrazines. The quinoline scaffold was varied at C-6 with Cl, F, Br and CH_3 groups and the various hybrids were varied again using different anilines, thiosemicarbazides and phenylhydrazines. The structures of the synthesised compounds were elucidated using 1D and 2D NMR spectroscopy.

The synthesised compounds were tested for their antibacterial activity against two Gram positive (*Staphylococcus aureus* and *S. aureus* Rosenbach (MRSA)) and four Gram negative species, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhimurium*. Although compounds in all three classes of hybrids showed antibacterial activity, these were not as good as current drugs being used as antibiotics. The synthesised compounds showed antibacterial activity in the range of 0.80 to 36.49 mM.

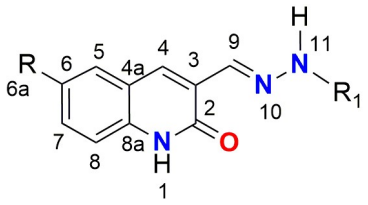
Structures of compounds reported in Chapter 2



2-chloro-*N*-phenylquinoline-3-carboxamides A5a-l

No.	R	R_1
A5a	H	H
A5b	H	CH_3
A5c	H	F
A5d	CH_3	H
A5e	CH_3	CH_3
A5f	CH_3	F
A5g	Cl	H
A5h	Cl	CH_3
A5i	Cl	F
A5j	F	H
A5k	F	CH_3
A5l	F	F

Structures of compounds reported in Chapter 3



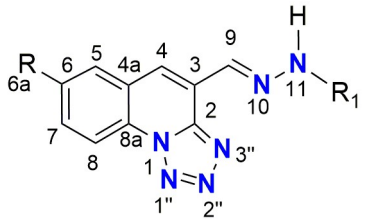
Substituted-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazine-1-carbothioamides

B5a-o

No.	R	R_1
B5a	H	TSC
B5b	CH_3	TSC
B5c	Br	TSC
B5d	Cl	TSC
B5e	F	TSC
B5f	H	*N*-MTSC
B5g	CH_3	*N*-MTSC
B5h	Br	*N*-MTSC
B5i	Cl	*N*-MTSC
B5j	F	*N*-MTSC
B5k	H	4-PHTSC
B5l	CH_3	4-PHTSC
B5m	Br	4-PHTSC
B5n	Cl	4-PHTSC
B5o	F	4-PHTSC

R_1 = TSC (thiosemicarbazide); *N*-MTSC (*N*-methylthiosemicarbazide); 4-PHTSC (4-phenylthiosemicarbazide)

Structures of compounds reported in Chapter 4



Substituted-(phenyl)hydrazono)methyl)-tetrazolo[1,5-a]quinolines

C5a-o

No.	R	R_1
C5a	H	PHYD
C5b	CH_3	PHYD
C5c	Br	PHYD
C5d	Cl	PHYD
C5e	F	PHYD
C5f	H	2F-PHYD
C5g	CH_3	2F-PHYD
C5h	Br	2F-PHYD
C5i	Cl	2F-PHYD
C5j	F	2F-PHYD
C5k	H	4F-PHYD
C5l	CH_3	4F-PHYD
C5m	Br	4F-PHYD
C5n	Cl	4F-PHYD
C5o	F	4F-PHYD

R_1 = PHYD (phenylhydrazone); 2F-PHYD (2-fluoro-phenylhydrazone; 4-F-PHYD (4-phenylhydrazone)

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Chapter 1. Introduction

The quality of human life has improved significantly since the introduction of antibiotics, used in the fight against bacterial infection (Badwaik et al., 2011). However, some bacteria have become drug resistant thereby reducing the effectiveness of the antibiotic (Badwaik et al., 2011). Therefore, a need arises to synthesise newer drugs to which these resistant pathogen are susceptible to (Badwaik et al., 2011). The two main contributors to the drug-resistance problem are drug-resistance genes (as well as their microbial hosts) and overuse of antibiotics (Levy, 1994). Drug resistance can be reduced by adjusting either one or both of these contributors (Levy, 1994). One approach would be to develop new antibiotics which the microorganisms would not have encountered before. A second approach would be to design drugs that block resistance mechanisms (Levy, 1994).

Presently, there is a growing increase in drug resistant bacteria. The large increase in bacterial resistance is responsible for community acquired infections, especially in *Staphylococci* and *Pneumococci* (Tran et al., 2012). This increase in community acquired infections and bacterial resistance has prompted the need to develop new drugs to combat the dramatic rise in bacterial infections. Therefore, this study is aimed at the synthesis of new hybrid compounds (molecules with two different pharmacophores) with antibacterial properties which could contribute to the development of new antibiotic drugs and hence attempt to address the problem of drug resistance antibiotics.

A hybrid molecule is the combination of different pharmacophores derived from biologically active compounds. It is hoped that the two entities produce a drug with higher bioactivity than the original parent drugs (Viegas-Junior et al., 2007). The hybridization strategy is considered to be a good approach to produce new drug-like molecules displaying bioactivity (Kouznetsov

et al., 2016). Hybrid molecules containing the quinoline core were found to display biological activity such as antimalarial (Vandekerckhove and D'Hooghe, 2015), anti-cancer (Kuo et al., 2016), anti-tubercular, antibacterial (Nayak et al., 2016) and antiviral activities (Held et al., 2017).

1.1 Quinolines

Quinoline is considered to be one of the most important heterocyclic aromatic compounds and has attracted interest in the medical field due to their vast range of bioactivities and widespread applications (Prajapati, 2014). Quinoline is a nitrogen containing aromatic compound possessing a benzene ring fused to pyridine at two adjacent carbon atoms in its structure (**Figure 1.1**). Quinoline is also known as 1-azanaphthalene and its core structure has been used in the synthesis of fungicides, biocides, alkaloids, dyes, rubber, chemicals, flavoring agents, antiseptics and antipyretics (Venkataraman et al., 2010).

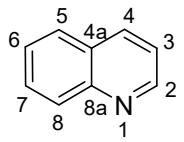


Figure 1.1 Chemical structure and numbering of quinoline

Interest in quinoline derivatives is due to their many pharmacological activities, such as antioxidant (Verbanac et al., 2016), anti-inflammatory, analgesic (Gupta and Mishra, 2016), antimalarial (Mahajan et al., 2007), antimicrobial (Mistry and Jauhari, 2010; Parab et al., 2011; Parab and Dixit, 2012), antifungal (Thumar and Patel, 2011), antimycobacterial (Upadhayaya et al., 2010), and anticancer activity (Solomon and Lee, 2011; Mahajan et. al., 2007).

Quinolines have also been employed extensively in the synthesis of drugs, especially antimalarial drugs such as quinine, chloroquine and mefloquine (**Figure 1.2**) (Thompson et al., 2007). Other quinoline containing drugs which are also available include the antiviral drug, saquinavir, (**Figure 1.3**) the antibacterial fluoroquinolones, ciprofloxacin, sparfloxacin and gatifloxacin, (**Figure 1.4**) and the anticancer drugs camptothecin and bosutinib (Afzal et al., 2015) (**Figure 1.5**). Other nitrogenous compounds related to quinoline, are also used as antihypertensive agents, for example prazosin and doxazosin (**Figure 1.6**), (Venkataraman et al., 2010).

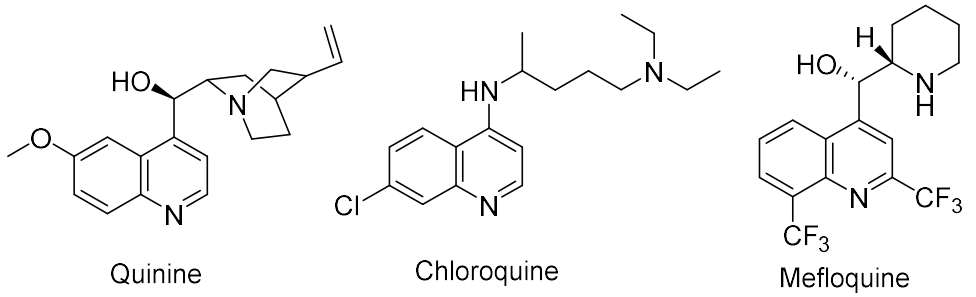


Figure 1.2 Structures of antimalarial drugs containing a quinoline scaffold

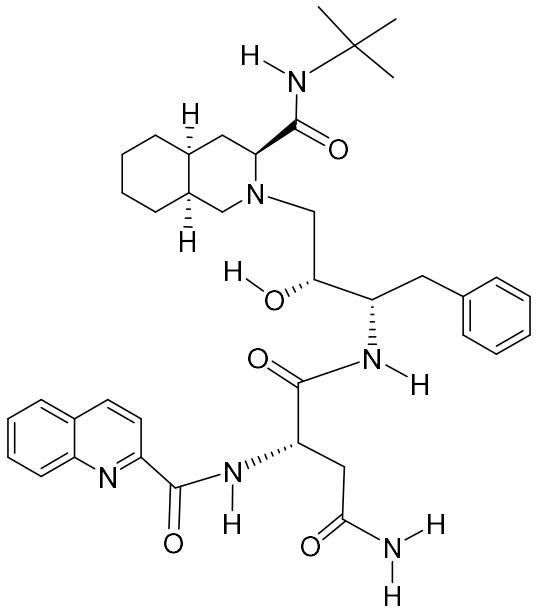


Figure 1.3 Structure of the antiviral drug, saquinavir

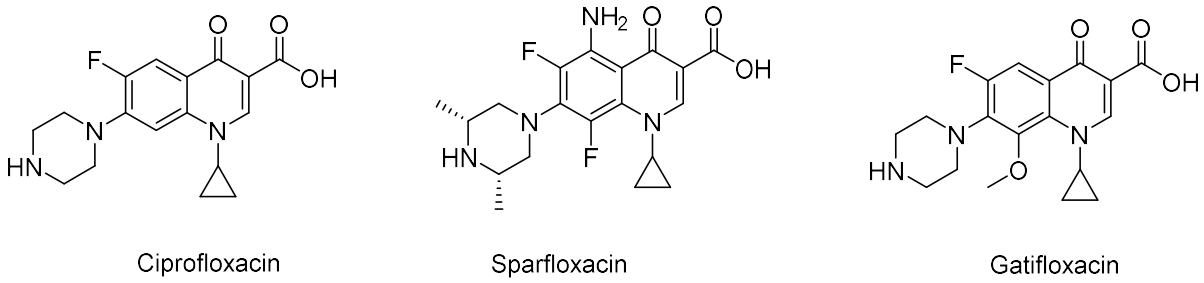


Figure 1.4 Structures of antibacterial fluoroquinolones

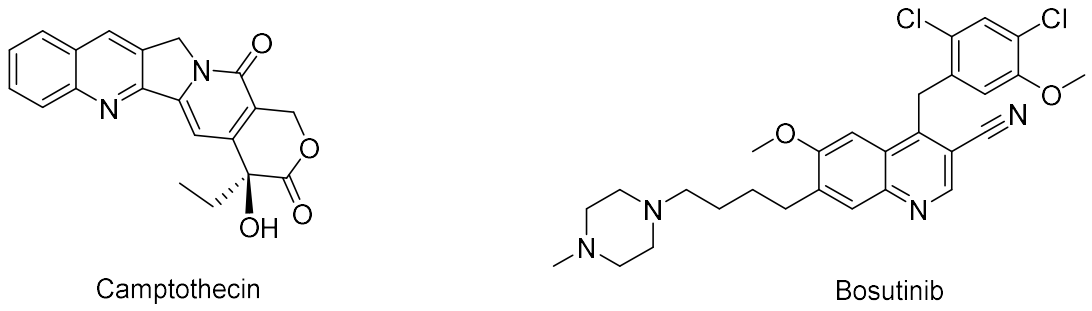


Figure 1.5 Structures of some anticancer drugs containing a quinoline scaffold

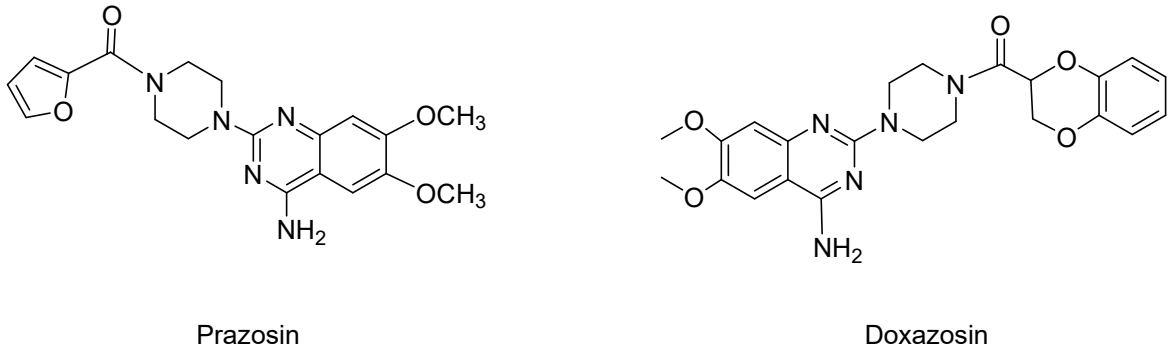


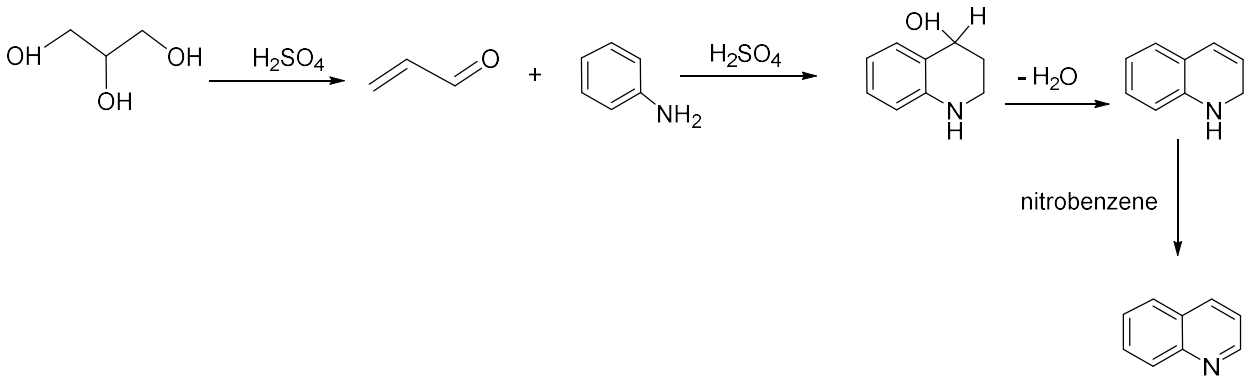
Figure 1.6 Structures of antihypertensive agents with structures related to quinoline

1.1.1 The synthesis of quinolines

Different methods such as the Doebner-von Miller, Skraup, Combes (Furniss et, al., 1989), Friedlander (Teimouri et al., 2016), Conrad-Limpach (Brouet et al., 2009), Knorr, Povarov,

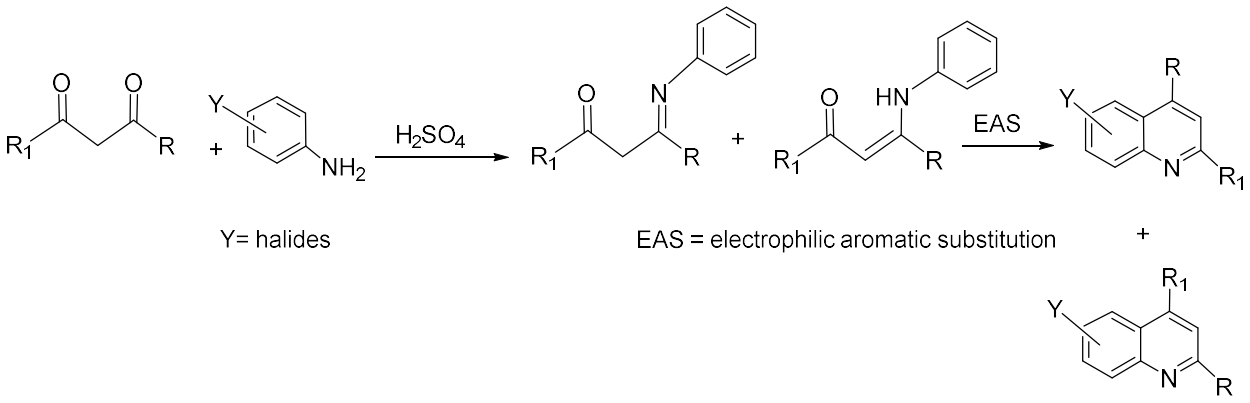
Camps, Gould-Jacobs, Niementowski and Pfitzinger have been used in the synthesis of quinolines and quinoline derivatives (Wang, et al., 2012).

The Skraup method involves the reaction of aniline and glycerol to form the quinoline (**Scheme 1.1**). Glycerol first undergoes a dehydration reaction using sulphuric acid as the catalyst to form acrolein, which then reacts with the aniline forming a hydroxy intermediate, which subsequently undergoes dehydration and oxidation to form the quinoline (Zibaseresht et al., 2013).



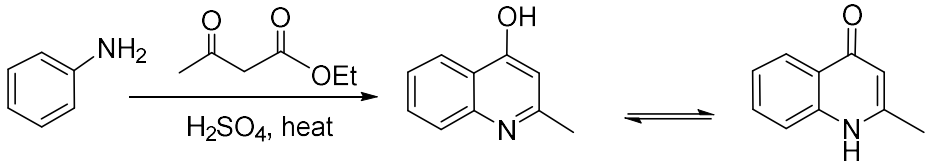
Scheme 1.1 Skraup synthesis of quinoline

The Combes synthesis makes use of a condensation reaction between substituted anilines and 1,3-diketones with an acid catalyst such as H_2SO_4 to form the quinolines (**Scheme 1.2**) (Sloop, 2009). The reaction proceeds via imine and enamine intermediates to form the desired products.



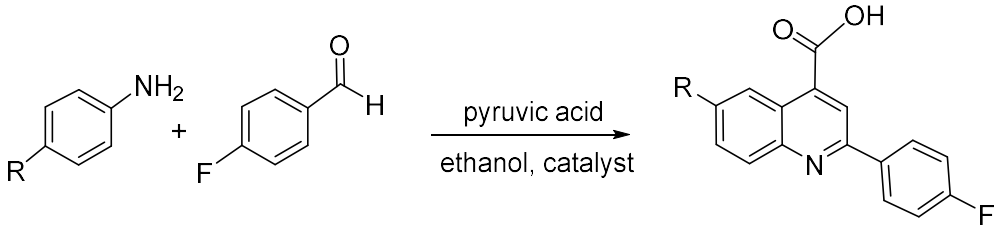
Scheme 1.2 Combes preparation of quinoline

The Conrad-Limpach synthesis is similar to the Combes synthesis except that *β*-ketoesters are used instead 1,3-diketones (**Scheme 1.3**). This resulted in the preparation of 4-quinolones.



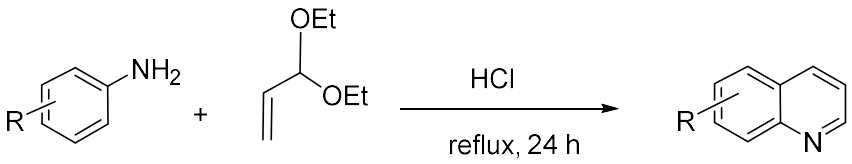
Scheme 1.3 Conrad-Limpach synthesis of quinolone

In the Doebner reaction, substituted anilines are reacted with substituted aldehydes and pyruvic acid, in the presence of an acid catalyst in ethanolic solution to form substituted-4-carboxyquinolines (**Scheme 1.4)** (Garudachari et al., 2012).



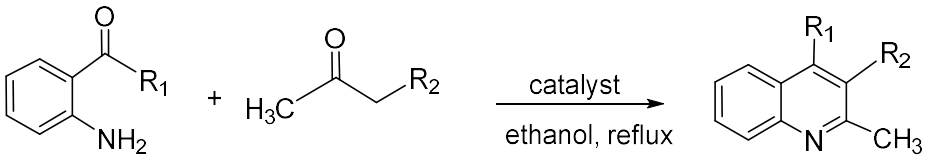
Scheme 1.4 Doebner reaction with substituted aldehydes

The Doebner-von Miller reaction is a modification of the Skraup reaction where an *α,β*-unsaturated ketone is used instead of glycerol (Matsugi et al., 2000). In the example below, substituted anilines are reacted with acrolein diethyl acetal to form substituted quinolines (**Scheme 1.5**) in what was called the Skraup–Doebner–von Miller reactions and was seen as an improvement on the Doebner-von Miller reactions (Ramann and Cowen, 2015).



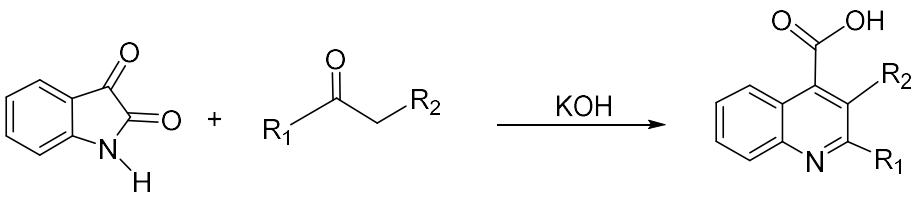
Scheme 1.5 Skraup–Doebner–von Miller reaction

The Friedlander condensation makes use of 2-aminoarylketones, which are reacted with *α*-methylene carbonyl compounds using heterogeneous solid acid catalysts including Montmorrilonite K-10, zeolite, and nano-crystalline sulfated zirconia (SZ) in ethanol medium (**Scheme 1.6**) (Teimouri et al., 2016). This results in quinolines that can be substituted at the 2, 3 and 4- positions on the quinoline ring.



Scheme 1.6 Friedlander condensation of 2-aminoarylketones and *α*-methylene carbonyl compounds

In the Pfitzinger reaction (**Scheme 1.7**), isatin is used instead of aniline and reacted with a methylketone under strong basic conditions to produce substituted quinoline-4-carboxylic acid derivatives (Sangshetti et al., 2014).



Scheme 1.7 Pfitzinger reaction using isatin and methylketones producing quinoline-4-carboxylic acid derivatives

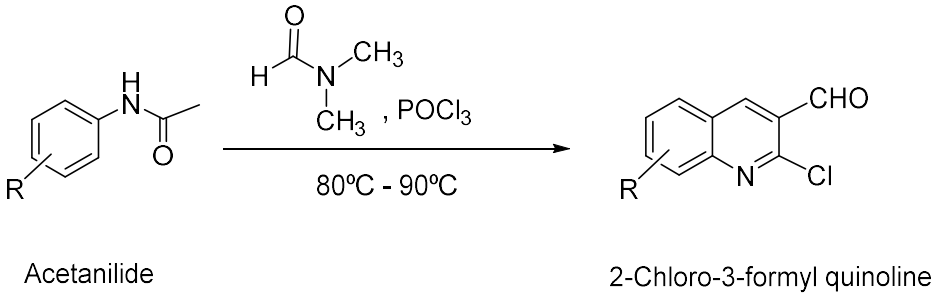
Other methods for the preparation of quinoline derivatives have also been used. These include making use of different metal catalysts such as Ni-containing compounds, NiF_2, $NiCl_2 \cdot 6H_2O$, $Ni(acac)_2$, $Ni(OAc)_2 \cdot 4H_2O$, and $Ni(NO_3)_2 \cdot 6H_2O$ (Khusnutdinov et al., 2012).

1.1.2 The synthesis of 2-chloroquinoline-3-carbaldehydes and their derivatives

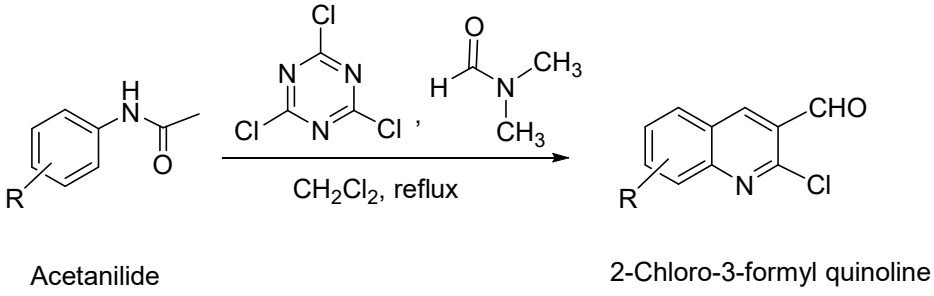
Quinoline compounds such as 2-chloroquinoline-3-carbaldehyde and their analogues are incorporated in many biologically active compounds having antibacterial, antifungal, antimalarial, anti-inflammatory, antiviral, analgesic and cardiovascular activity (Marella et al., 2013).

The Vilsmeier-Haack reagent has also been used in the preparation of a variety of synthetic compounds, including formylation (Roohi et al., 2013), cyclisation (Srivastava and Singh, 2005), cycloaddition and ring annulations (Beniwal and Jain, 2015). It has been used effectively in the preparation of 2-chloro-3-formyl quinolines (Abdel-Wahab et al., 2012; Abdel-Wahab and Khidre, 2013; Tekale et al., 2015; Venkanna et al., 2015). The procedure makes use of dimethyl formamide (DMF) added to phosphorus oxychloride ($POCl_3$) and then addition of acetanilide (**Scheme 1.8**). The resultant quinoline carbaldehdyes were formed after 6-8 hours of reflux (Abdel-Wahab and Khidre, 2013; Tekale et al., 2015). Venkanna et al.

(2015) synthesised the same molecules in a similar manner but used 2,4,6-trichloro-1,3,5-triazine in place of $POCl_3$ (**Scheme 1.9**).

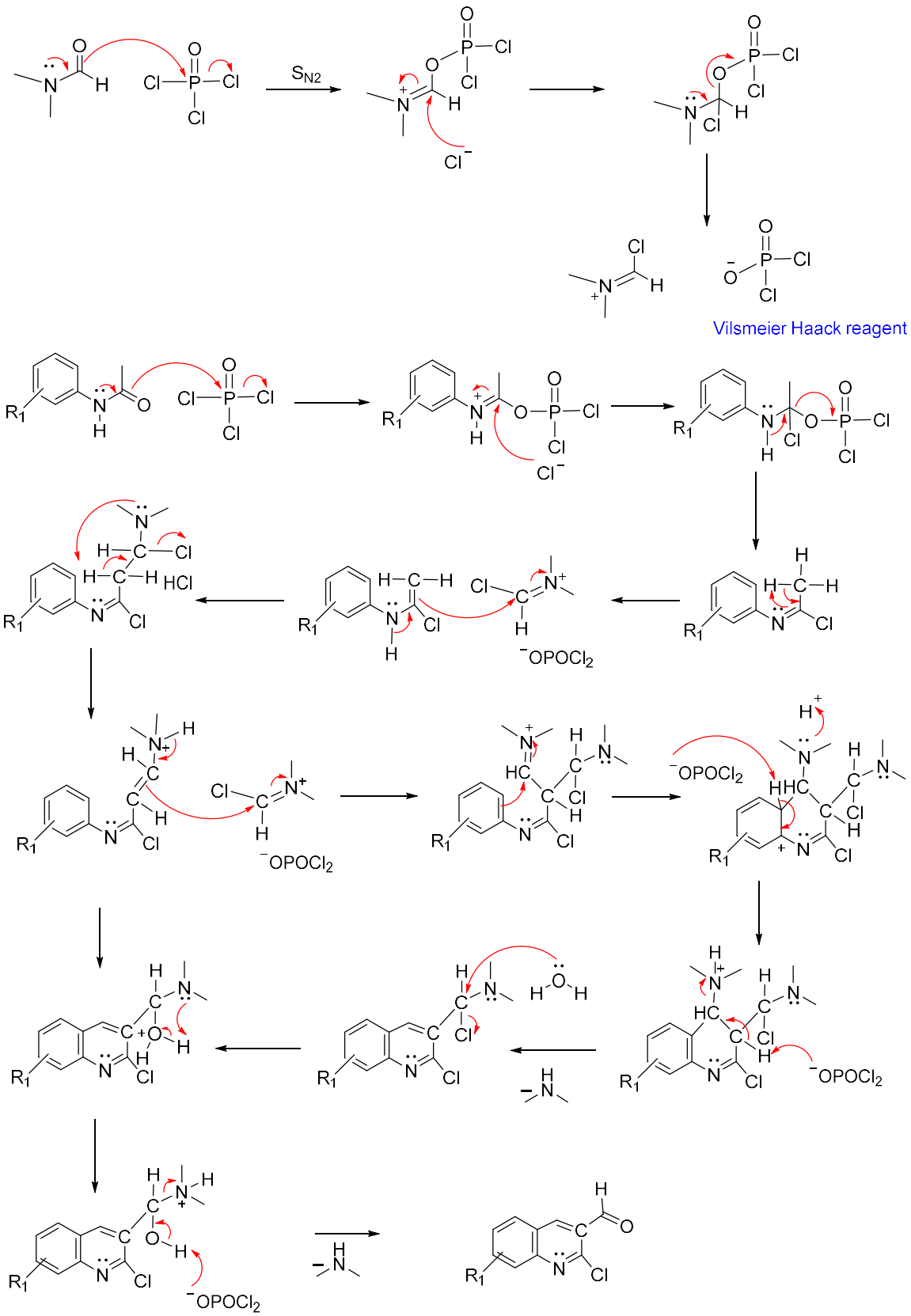


Scheme 1.8 Vilsmeier Haack reaction using DMF and $POCl_3$



Scheme 1.9 Preparation of 2-chloro-3-formyl quinoline with 2,4,6,-trichloro-1,3,5-triazine and DMF

The mechanism (Error! Reference source not found.) involves the reaction of dimethylformamide with phosphorus oxychloride producing a chloroiminium ion, referred to as the Vilsmeier reagent, used later in the mechanism. The substituted amides react with $POCl_3$, forming an activated imine, which rearranges to an enamine that reacts with two molecules of the Vilsmeier reagent mentioned earlier. Nucleophilic attack on the iminium carbon leads to the formation of the six-membered heterocyclic ring and expulsion of the dimethylamide anion, together with addition of water to the chlorinated carbon attached to the amine ultimately leads to formation of the 2-chloroquinoline-3-carbaldehyde (Li, 2006).

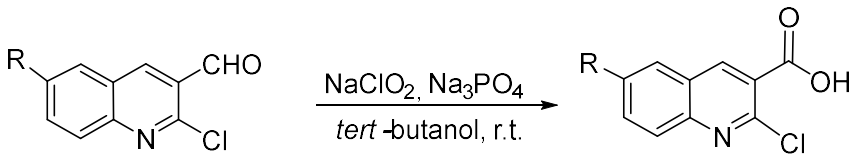


Scheme 1.10 Proposed mechanism of the Vilsmeier- Haack reaction to form aldehyde

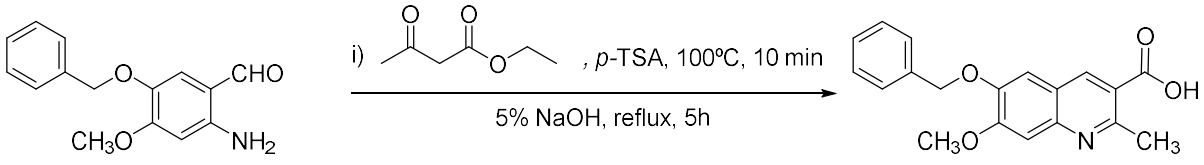
This reaction was used in this study to synthesise 2-chloroquinoline-3-carbaldehydes and their derivatives following the procedure by Toth et al. (2006). This intermediate was important for the formation of the final carboxamide, thiosemicarbazone and phenylhydrazone products.

1.2 Oxidation of 2-chloroquinoline-3-carbaldehydes to 2-chloroquinoline 3-carboxylic acids

In general, aromatic aldehydes can be oxidized to the carboxylic acid using reagents such as potassium permanganate, chromium (VI) oxide, pyridinium dichromate, pyridinium chlorochromate, silver nitrate in basic media, or hydrogen peroxide in formic acid (Dodd and Le Hyaric, 1993). George et al., (2013) used the Pinnick oxidation to oxidise 2-chloroquinoline-3-carbaldehdyes to carboxylic acids with a solution of sodium phosphate and sodium chlorite in *tert*-butanol (**Scheme 1.11**). Quinoline-3-carboxylic acids were synthesised directly from 3-formylanilines, ethyl acetoacetate and *p*-toluene sulfonic acid (*p*-TSA), followed by addition of sodium hydroxide (Li et al., 2012), (**Scheme 1.12**).



Scheme 1.11 Pinnick oxidation of 2-chloroquinoline-3-carbaldehyde



Scheme 1.12 Preparation of 6-benzyloxy-7-methoxyquinoline-3-carboxamide

The conversion to the acid provides a further functional group on the quinoline scaffold which can be used as a point of attachment for a second scaffold.

1.3 Synthesis of quinoline-3-carboxamide derivatives

Amide bond formation plays a key role in biological systems. It links amino acid building blocks together to form proteins. They are also present in commercially available drugs such as Atorvastatin, Lisinopril, Valsartan and Diltiazem (Valeur and Bradley, 2009). The quinoline-3-carboxamide, laquinimod is presently in clinical development for the treatment of multiple sclerosis (Haggiag et al., 2013), tasquinomod is used for prostate cancer (Osonto et al., 2013) and paquinimod for systemic lupus erythematosus (Bengtsson et al., 2012) (**Figure 1.7**).

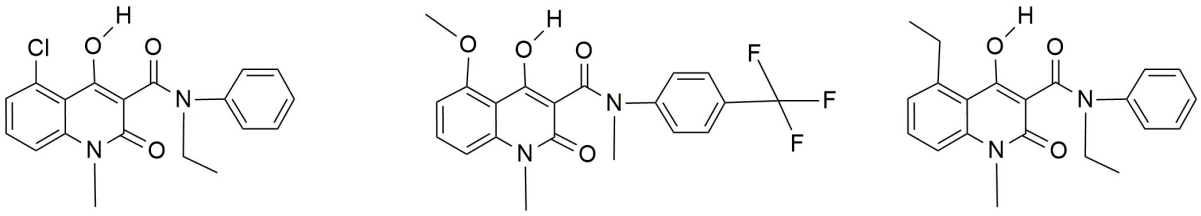
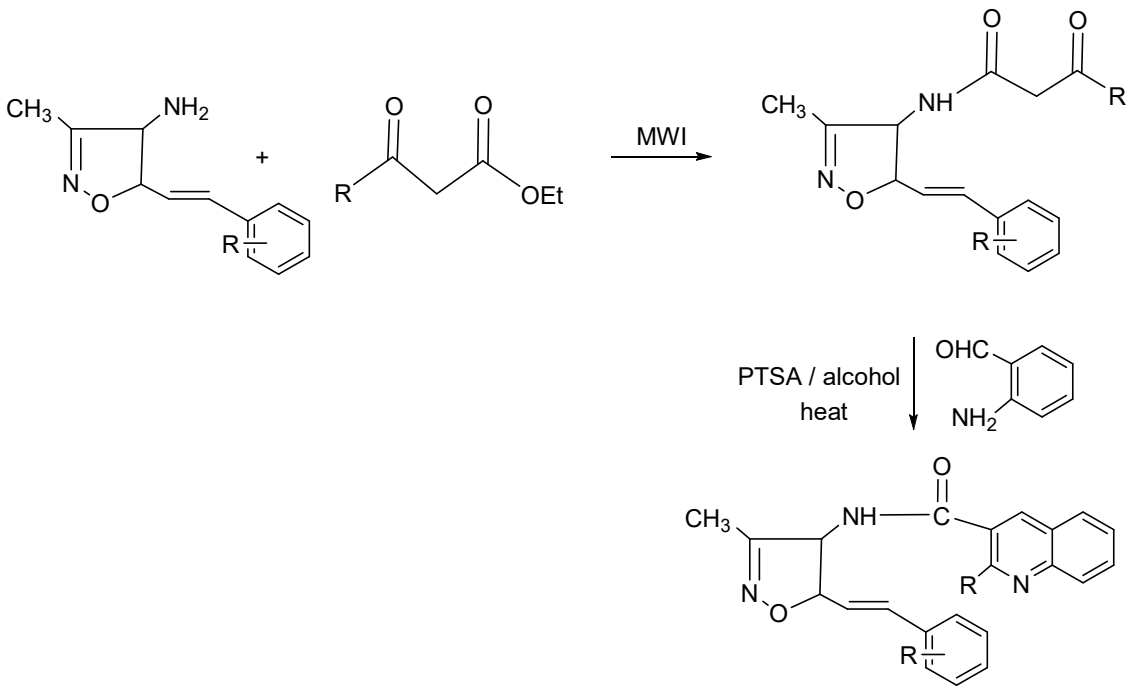


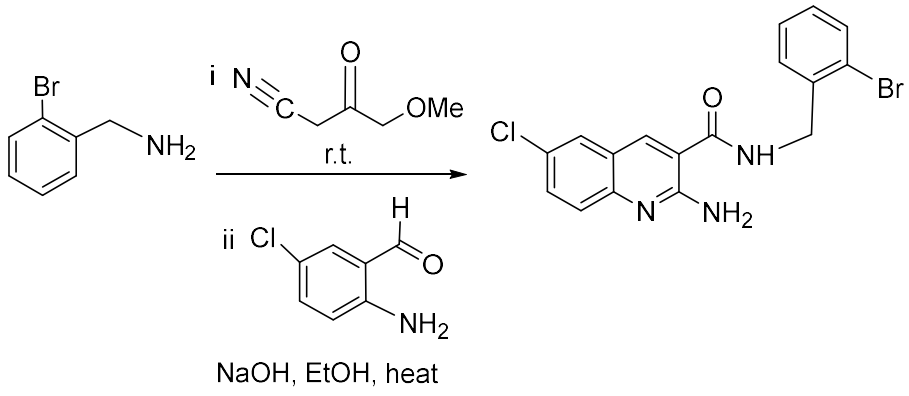
Figure 1.7 Examples of anti-cancer drugs containing the quinoline-3-carboxamide scaffold

The amide bond is often used as a linker between the quinoline moiety and another scaffold. For example, a number of isoxazolyl quinoline hybrids were formed by adding on a *β*-keto ester to isoxazole via the amino group using microwave reactions and then forming the quinoline with the keto moiety using *ortho*-aminobenzaldehyde (Rajanarendar et al., 2010) (**Scheme 1.13**).



Scheme 1.13 Synthesis of isoxazolyl-2-methyl-3-quinolinecarboxamide

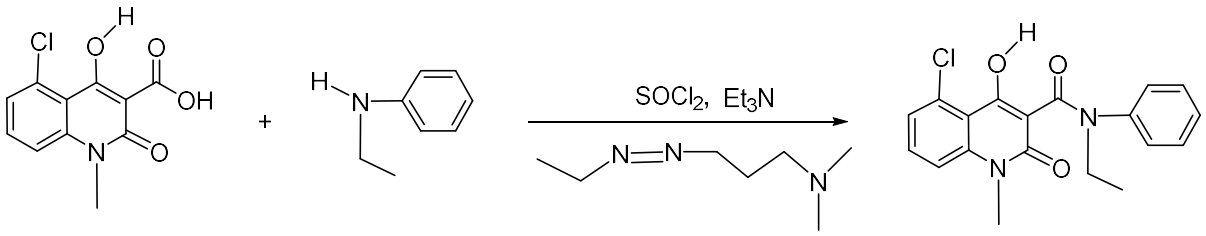
These molecules were also synthesised in a one-pot reaction using 2-amino-5-chlorobenzaldehyde, cyano acetic acid methyl ester and 2-bromobenzylamine in basic media (Wang et al., 2012) (**Scheme 1.14**).



Scheme 1.14 One-pot reaction of aminoquinoline-3-carboxamide

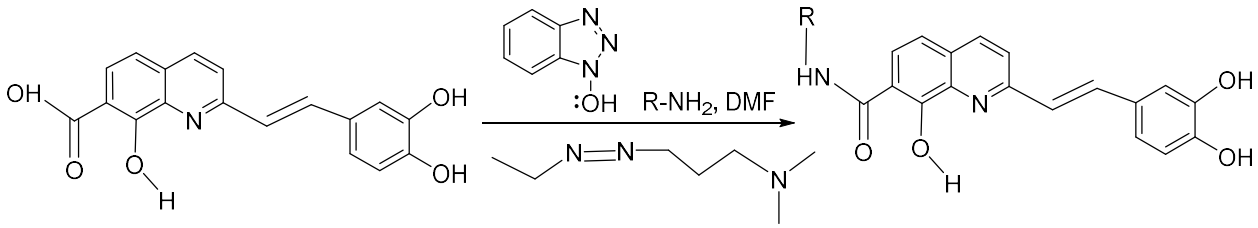
The quinoline-3-carboxamide drug, laquinimod was formed by amide coupling using EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), a coupling reagent with a quinolone 3-

carboxylic acid and a secondary aryl amine in the presence of thionyl chloride and triethylamine (Bjork et al., 2000) (**Scheme 1.15**).



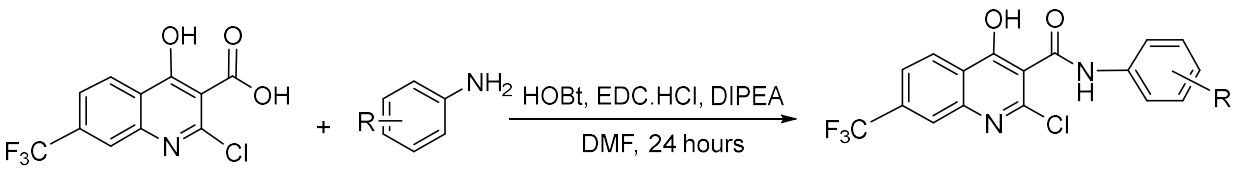
Scheme 1.15 Synthetic scheme to laquinimod from a quinolone 3-carboxylic acid

EDC and HOBt (hydroxybenzotriazole) were used as coupling reagents to couple styryl quinoline carboxylic acids and amines in a slow reaction taking 3 days to complete with low yields (10-37%) (Lee et al., 2006) (**Scheme 1.16**).



Scheme 1.16 Preparation of styrylquinoline carboxamides

The same reagents were used to couple 2-chloroquinoline-3-carboxylic acid derivatives with amines, which was much faster (24 h) and resulted in better yields (15-66%) (**Scheme 1.17**) (Narayan et al., 2016).

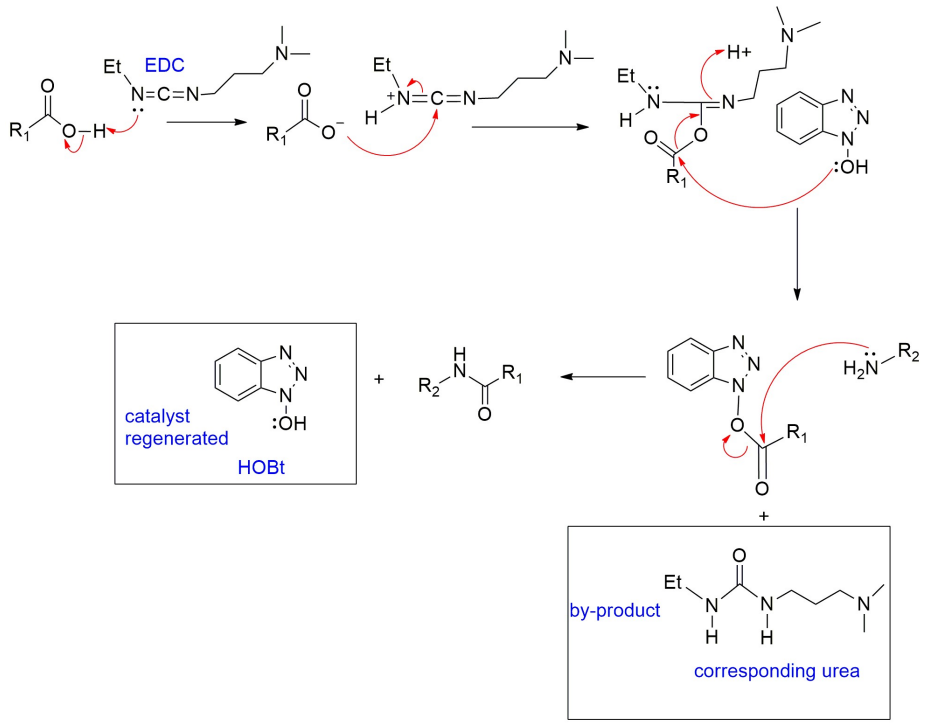


Scheme 1.17 Preparation of quinoline carboxamide derivatives using 2-chloroquinoline-3-carboxylic acid precursors

General mechanism for amide bond formation using EDC and HOBt

EDC and HOBt are two common coupling reagents used in amide bond formation. A general mechanism for the reaction is shown in

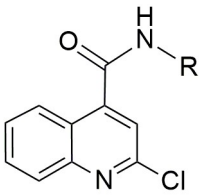
Scheme 1.18. In the first step of the mechanism, EDC abstracts a proton from the hydroxyl group of the carboxylic acid and the resultant carboxylate ion forms a bond with the carbon of the carbodiimide forming a quinoline urea ester. This activates the carbonyl carbon and enables HOBt to attack it via the lone pair on oxygen, which is subsequently attacked by the aniline forming the amide (Valeur and Bradley, 2009).



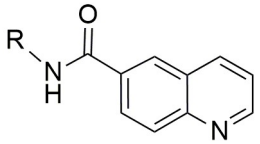
Scheme 1.18 General mechanism for amide bond formation with EDC and HOBt (Valeur and Bradley, 2009)

1.3.1 Antibacterial activity of quinoline-3-carboxamides

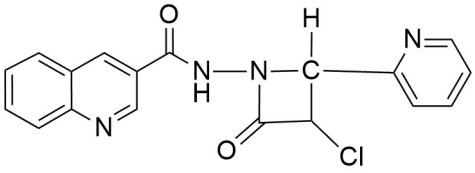
Quinoline carboxamide derivatives with alkyl groups showed good to moderate activity against several Gram +ve and Gram –ve strains of bacteria. It was shown that those compounds with longer alkyl groups attached to the quinoline carboxamide core showed better activity than when shorter chains were attached (Wang et al., 2016). Antibacterial activity was also shown by 2-chloro-quinoline-4-carboxamides and quinoline-6-carboxamide **(Figure 1.8)** against *Escherichia coli* and *Staphylococcus aureus* (Shivaraj et al., 2013) and a quinoline-3-carboxamide azetidine pyridine hybrid was shown to have better activity than streptomycin against *S. aureus* and *B. subtillis* (Singh and Kumar, 2016).



2-Chloroquinoline-4-carboxamide



Quinoline-6-carboxamide



Quinoline-3-carboxamide azetidine pyridine hybrid

Figure 1.8 Structures of quinoline carboxamides with antibacterial activity

1.4 Quinolone-thiosemicarbazone derivatives

Thiosemicarbazones (**Figure 1.9**) possess bioactivities such as antioxidant (Priyadarshini et al., 2016), antibacterial (Keshk et al., 2008; Ibrahim et al., 2011; Gupta and Gupta, 2015), antitumour (Zou et al., 2017), antitubercular (Asif, 2015), antiviral (Sebastian et al., 2008), antifungal (Sultana et al., 2010), anti-inflammatory (Hussein et al., 2009), anticonvulsant, (Aggarwal and Mishra, 2005), and anticancer (Khan et al., 2015). Thiacetazone and methisazone are two examples of drugs used on the market as antitubercular and antiviral agents respectively (**Figure 1.10**).

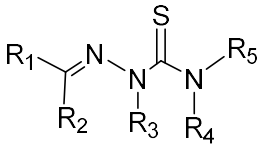
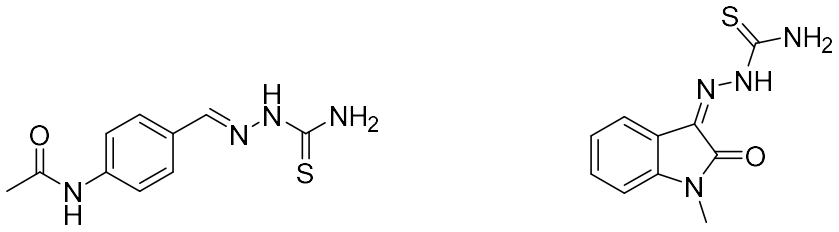


Figure 1.9 The basic structure of a thiosemicarbazone



Thiacetazone (Conteben) (antitubercular)

Methisazone (antiviral)

Figure 1.10 Thiosemicarbazone bioactive compounds

Pyridine and quinoline thiosemicarbazone derivatives depicted in **Figure 1.11** were shown to be anticancer iron chelators (Serda et al., 2010). Thiosemicarbazone metal complexes formed

from 6-methyl-2-oxo-quinoline-3-carbaldehyde (H-L) and Cu were shown to have good anticancer activity. In particular, a $[Cu(H\text{-}L)NO_3(O)]^+$ complex (**Figure 1.12**) was found to be more effective than cisplatin in *in vitro* SK-OV-3 and MGC80-3 tumour cell lines (Zou et al., 2017).

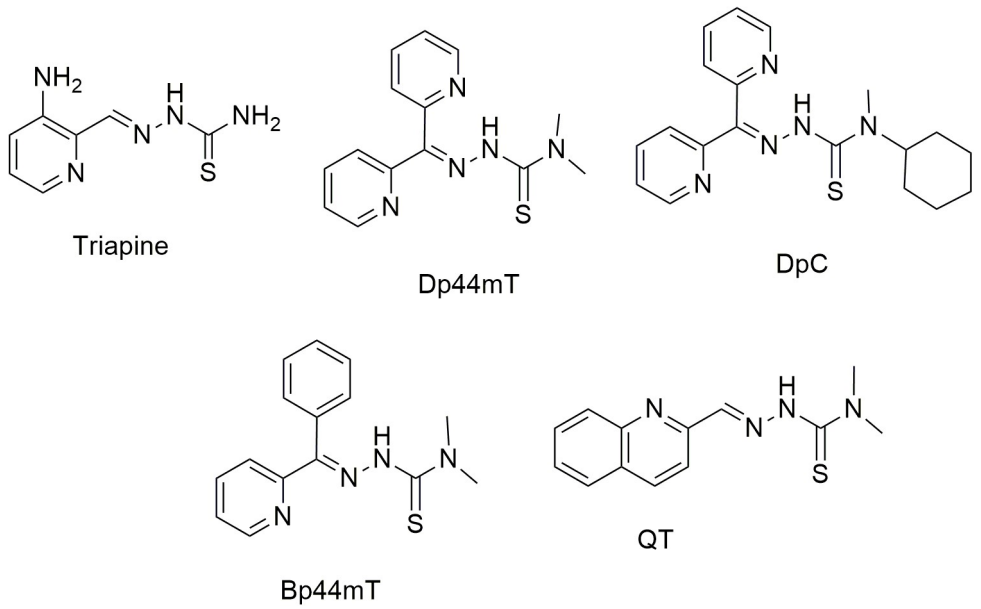


Figure 1.11 Anticancer chelating pyridine and quinoline thiosemicarbazones

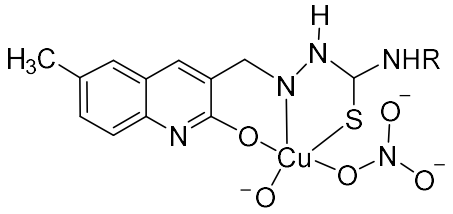
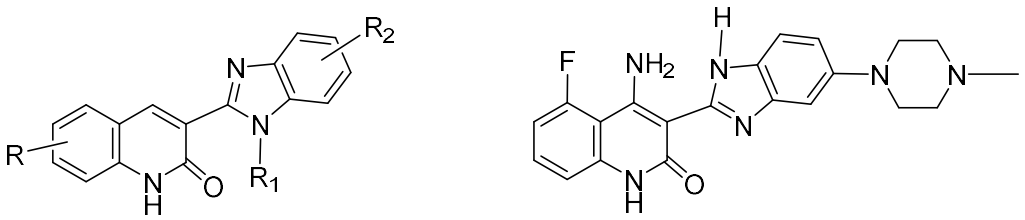


Figure 1.12 Anticancer quinoline $[Cu(H\text{-}L)NO_3(O)]^+$ complex

2-Quinolone derivatives were also found to possess various pharmacological activities such as antibacterial (Shoeb and Shaikh, 2016), anticancer (Hawtin et al., 2010), antiviral (Richter et al, 2005), anti-inflammatory (Abishek et al., 2014), antioxidant and antimicrobial activity (Al-Amiery et al., 2012). The 3-substituted quinolin-2-one is a particularly important moiety,

present in many compounds with anti-tumour activities such as quinoline-2-one imidazole hybrids (**Figure 1.13**) (Abonia et al., 2012).

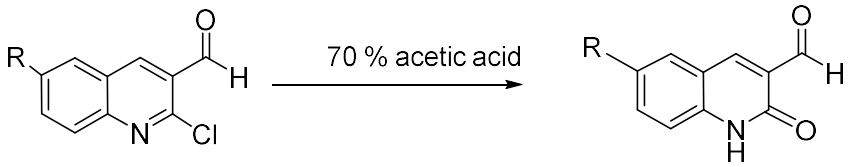


2-quinolone benzimidazole hybrids

Figure 1.13 2-Quinolone antitumour compounds

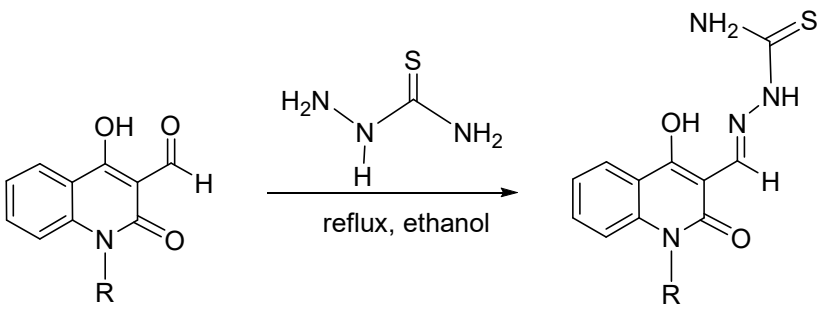
1.4.1 Synthesis of quinolone thiosemicarbazides

2-Chloroquinoline-3-carbaldehydes are easily converted to quinolone analogues using 70% acetic acid (**Scheme 1.19**) (Singh et al., 2007).



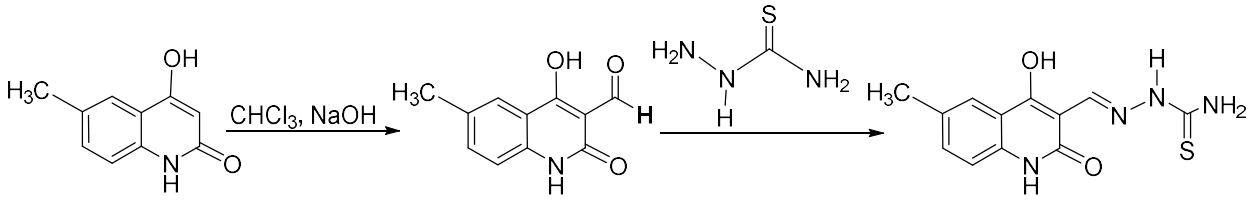
Scheme 1.19 Conversion of 2-chloro-3-formylquinolines to their quinolone analogues

Thiosemicarbazones can be prepared from these aldehydes by condensing them with thiosemicarbazides in the presence of a polar solvent such as 2-propanol, methanol or ethanol (**Scheme 1.20**) (Ukrainets et al., 2009; Huang et al., 2010; Bisceglie et al., 2015). The reaction is simple and occurs at room temperature with stirring.



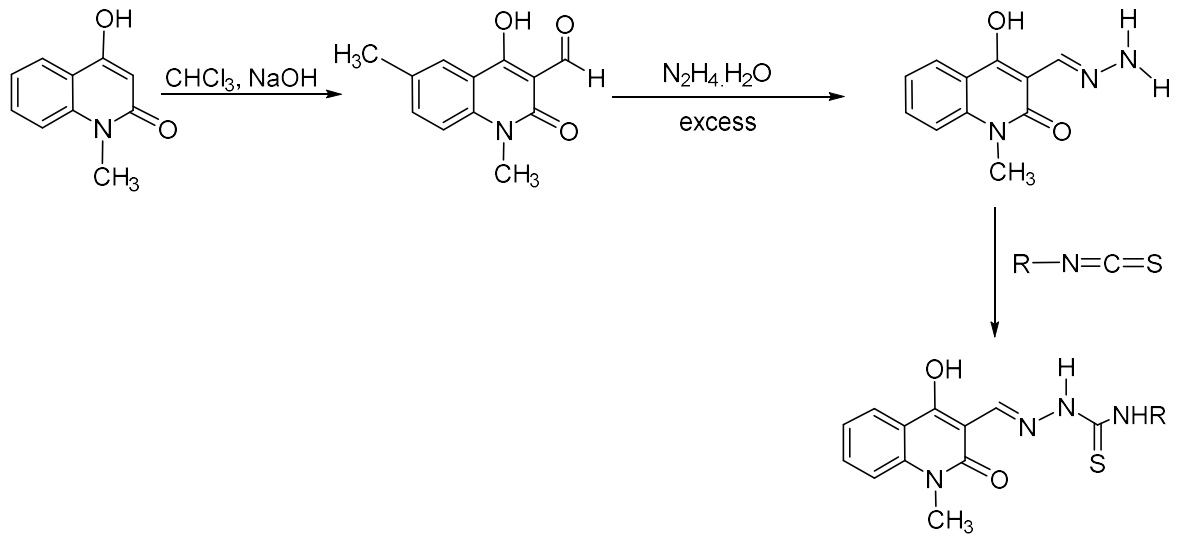
Scheme 1.20 Thiosemicarbazone condensation reaction done under reflux

The aldehyde group is added onto the quinolone core framework by a formylation reaction, and the formylated quinolone is subsequently reacted with thiosemicarbazides (**Scheme 1.21**) (Mohamed et al., 1994). The thiosemicarbazide reaction with quinoline aldehydes can also be carried out under microwave conditions (Serda et al., 2012).



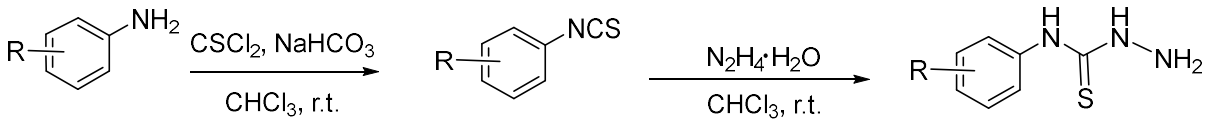
Scheme 1.21 Synthesis of quinolone thiosemicarbazone hybrids from quinolones via a formylation reaction

In another method, instead of using thiosemicarbazides to condense with the aldehyde, the quinolone-3-carbaldehyde was first reacted with hydrazine after the formylation reaction and then isothiocyanate to form the quinolone-thiosemicarbazone hybrids (**Scheme 1.22**) (Mohamed et al., 1994).



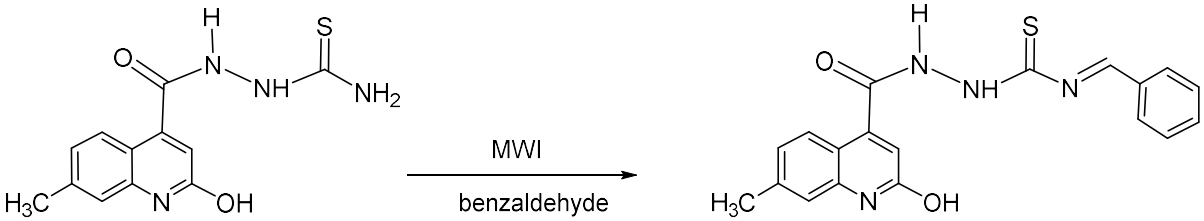
Scheme 1.22 Synthesis of quinolone-thiosemicarbazone hybrids using hydrazine and isothiocyanate

Anilines were also converted to isothiocyanates using thiophosgene under basic conditions and then reacted with hydrazine hydrate to form thiosemicarbazides (Qi et al., 2013). Formation of the isothiocyanate took 6 hours and the thiosemicarbazone formed within 3 hours (**Scheme 1.23**).



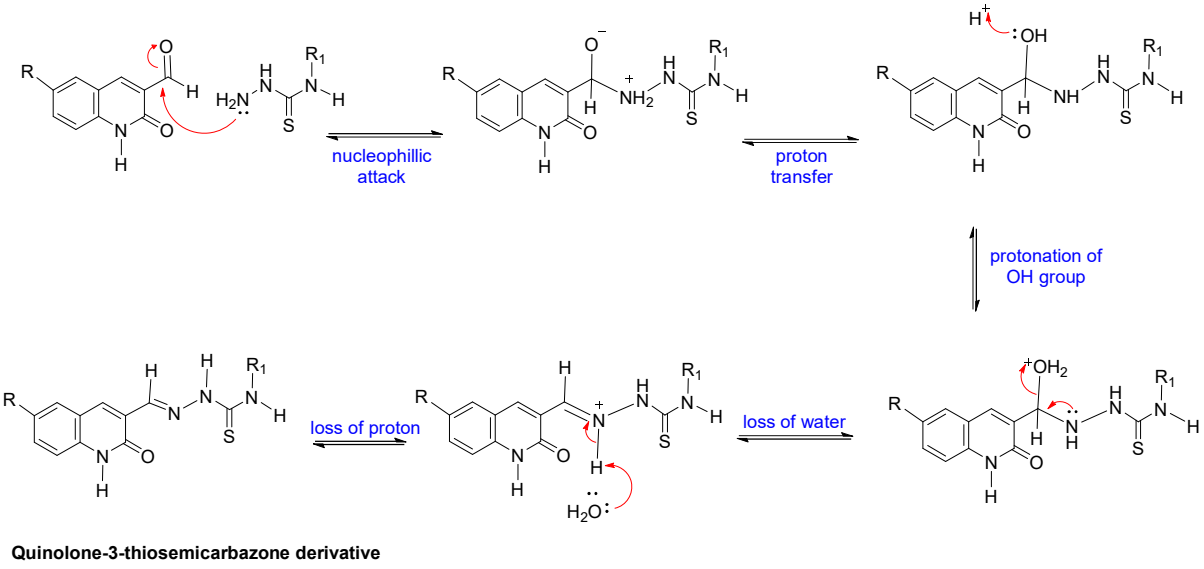
Scheme 1.23 Formation of thiosemicarbazones from anilines, thiophosgene and hydrazine hydrate.

The thiosemicarbazone moiety on the quinoline scaffold was also derivatised, forming imines with benzaldehyde under microwave conditions (**Scheme 1.24**) (Sudha and Selvi, 2015).



Scheme 1.24 Derivatisation of the thiosemicarbazone moiety under microwave conditions

The mechanism for quinolone thiosemicarbazone formation is the same as that for imine formation. The terminal amino group of the thiosemicarbazide attacks the aldehydic carbonyl group, forming an alcohol intermediate followed by elimination of water to produce the imine (**Scheme 1.25**).



Scheme 1.25 Proposed mechanism for thiosemicarbazone formation from aldehydes

1.4.2 Antibacterial activity of quinolone thiosemicarbazones

Some well known drugs containing the quinolone core include the fluoroquinolone antibiotics ciprofloxacin, norfloxacin, delafloxacin, sparfloxacin, temafloxacin, and difloxacin (**Figure 1.14**) (Kanani and Patel, 2014). The *N*-piperazinyl fluoroquinolone derivatives have been used

as antibacterial agents and for the treatment of *Mycobacterium tuberculosis* (Agrawal and Talele, 2013). In some cases, the *N*-piperazinyl fluoroquinolones showed better activity than the mother drugs to *E. coli* and *K. pneumonia* (Shamsa et al., 2011).

Thus far there have been few reports in the literature concerning quinolone thiosemicarbazides. Quinolone-3-thiosemicarbazone derivatives were synthesised by Radl et al. (1991) and did not show any significant antibacterial activity. Compounds with a similar backbone were also synthesised by Ukrainets et al. (2009) and tested against *M. tuberculosis* H37Rv, again showing no activity. These two cases are, however, not substantial enough to rule out quinoline thiosemicarbazides as antibacterial agents and more work needs to be carried out before this conclusion can be made.

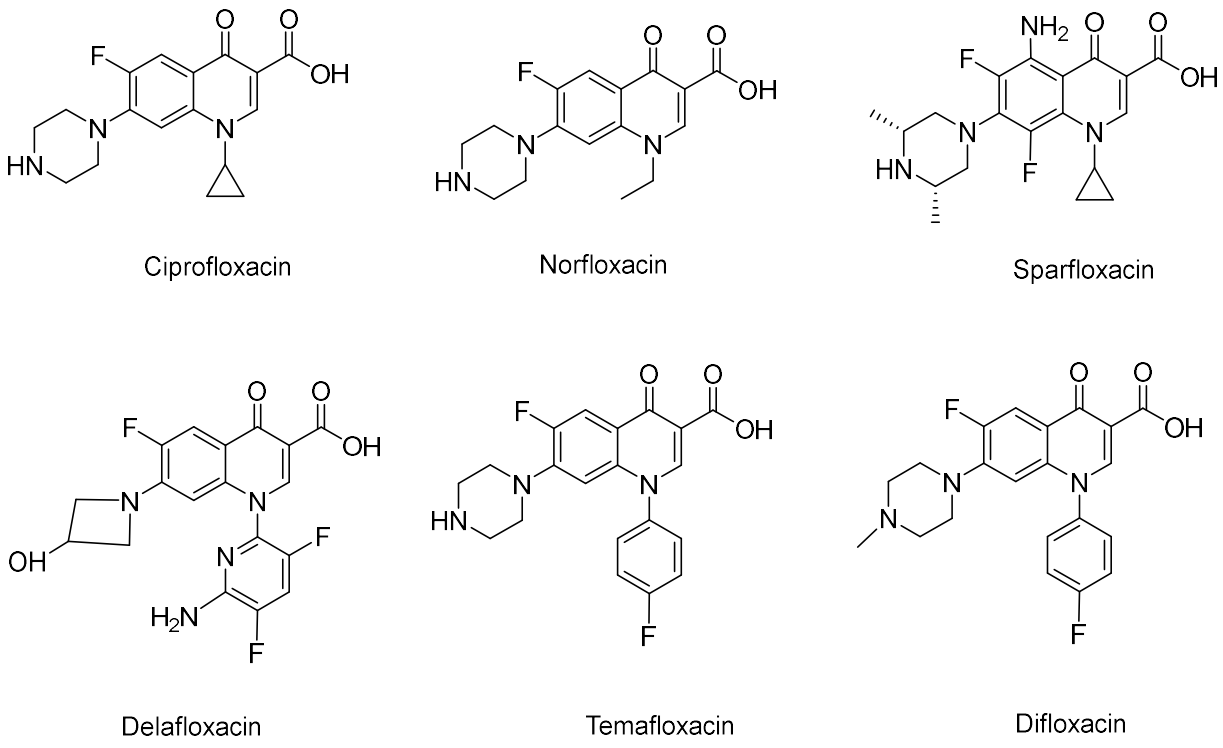


Figure 1.14 Fluoroquinolone antibiotics containing the quinolone core

1.5 Tetrazole Derivatives

The tetrazole moiety is a five-membered ring which has one carbon and four nitrogen atoms as shown in (**Figure 1.15**).

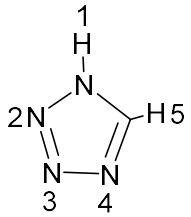
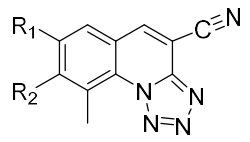


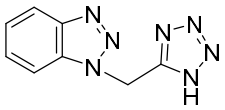
Figure 1.15 Structure of tetrazole

Tetrazoles play an important role in medicinal chemistry and is known to have antibacterial (Abdel-Rahman et al., 2013), antifungal (Dhayanithi et al., 2011), anticancer (Kumar et al., 2011), anti-inflammatory (Anu et al., 2013), anticonvulsant (Rostom et al., 2009) and antihypertensive activities (Wu et al., 2008). Tetrazoles have been added onto many organic scaffolds with an aim to increasing the bioactivity of these scaffolds (Mohamed, 2014; Madalageria et al., 2016, Joginder et al., 2017).

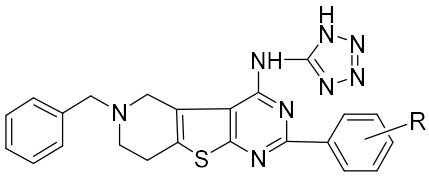
Tetrazolo quinoline derivatives showed antibacterial activity against *B. subtilis* and *E. coli* (Kategaonkar et al., 2010). Thieno pyrimidine derivatives having a tetrazole ring in its structure were also shown to have moderate antibacterial activity (Salahuddin et al., 2009) as did tetrazolo benzotriazole derivatives, which also shown good antifungal activity (Ali, 2012). These compounds are shown in **Figure 1.16**.



Tetrazolo quinolines



Tetrazolo benzotriazoles



Tetrazolo pyrido thieno pyrimidines

Figure 1.16 Tetrazolo derivatives on different scaffolds

1.5.1 Tetrazolo quinolines

Quinolines with a fused tetrazole moiety were found to have anti-inflammatory (Bawa and Kumar, 2009), antibacterial (Asif, 2014), antifungal (Ashok et al., 2016), cytotoxic (Bekhit et al., 2004a) and antitubercular activity (Bhuva and Patel, 2012). They also showed anticonvulsant activity and showed promise in treating depression (Deng et al., 2010). Some bioactive quinolines with fused tetrazole rings are shown in **Figure 1.17**, (Subhedar, et al., 2016).

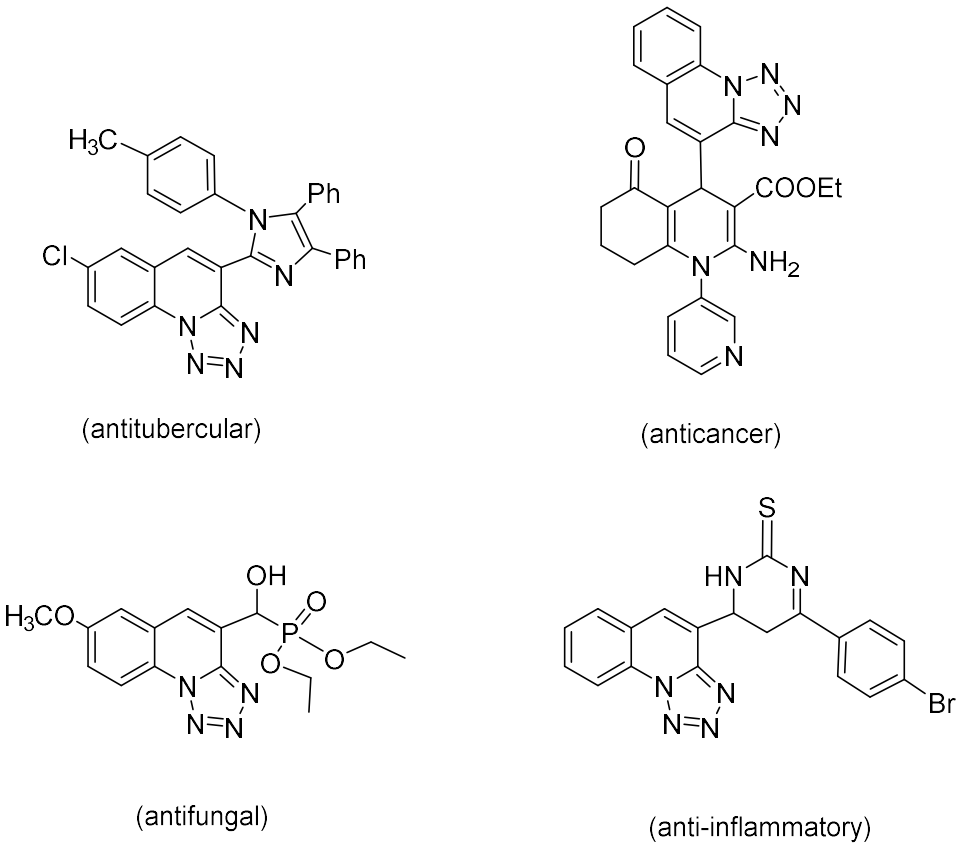
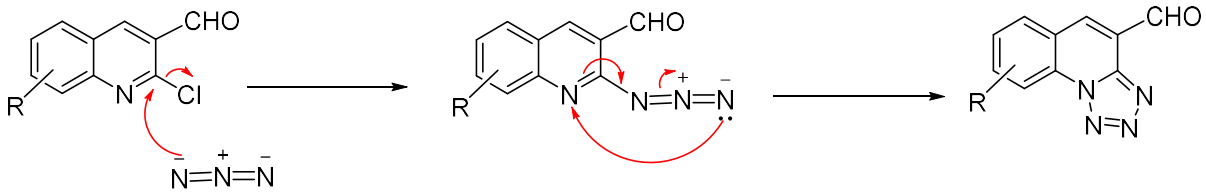


Figure 1.17 Bioactive compounds containing a fused tetrazoloquinoline scaffold

1.5.2 Synthesis of tetrazolo quinolines

Tetrazolo compounds are normally fused onto heterocyclic scaffolds, by reacting azides with 2-chloroquinoline (Bekhit et al., 2004b). The proposed mechanism for the fusion of tetrazoles onto the quinoline scaffold is shown in **Scheme 1.26** below (Marganakop et al., 2014).



Scheme 1.26 Proposed mechanism for the formation of the tetrazolo quinoline

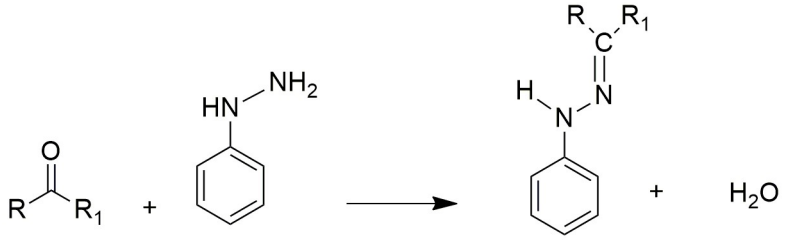
Azide substitution can occur at both C-2 and C-4 with the 4-chloro group being preferred over the 2-chloro (Ismail et al., 2000). The chloro group at C-4 is twice as reactive toward nucleophilic substitution compared to the chloro group at C-2. An acid catalyst changes this preference to the 2-chloro substituent forming a fused tetrazolo quinoline. Methane sulphonic acid, trichloroacetic acid or trifluoroacetic acid is used as the catalyst with either ethanol or dioxane as the solvent (Ismail et al., 2000). Acetic acid can also be used to catalyse the reaction (Ladani et al., 2009).

1.6 Phenylhydrazone derivatives

A Schiff base is a compound which has an imine or azomethine (–C=N–) functional group in its structure. They are formed by a condensation reaction between primary amines and carbonyl compounds and are found to be good intermediates in the design and synthesis of many bioactive hit compounds (Kajal et al., 2013). They have been known to have antimalarial (Rollas and Küçükgüzel, 2007), anticancer (Bingul et al., 2016), antibacterial and antifungal (Khan et al., 2014), antitubercular (Savini et al., 2002; Nayyer and Jain, 2008), and antimicrobial and anti-inflammatory activity (Abishek et al., 2014).

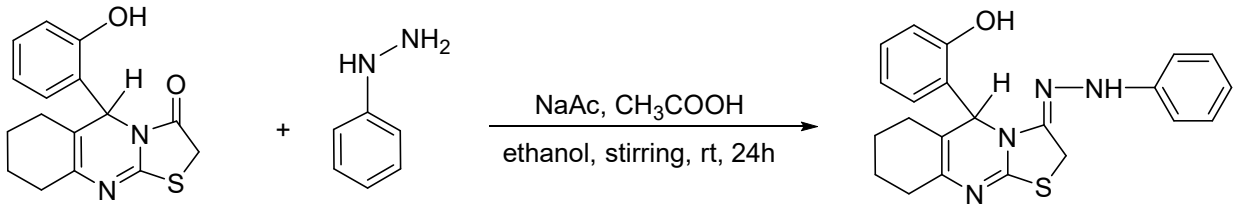
1.6.1 Preparation of phenylhydrazones

Phenylhydrazones are formed by the reaction of a (substituted) phenylhydrazine with a carbonyl compound (aldehyde or ketone). The general reaction is shown in **Scheme 1.27**.

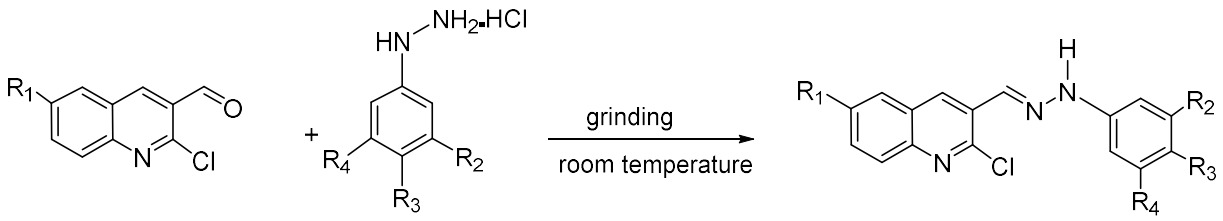


Scheme 1.27 General reaction for the preparation of phenylhydrazone

There are numerous examples in the literature where phenylhydrazone derivatives were synthesised from aldehyde or ketone groups, which formed part of organic frameworks. Examples of these are quinazoline thiazole phenylhydrazones (**Scheme 1.28**) (Selvam and Kumar, 2010) and 2-chloroquinoline-3-phenylhydrazones (**Scheme 1.29**) (Chandrika et al., 2013).

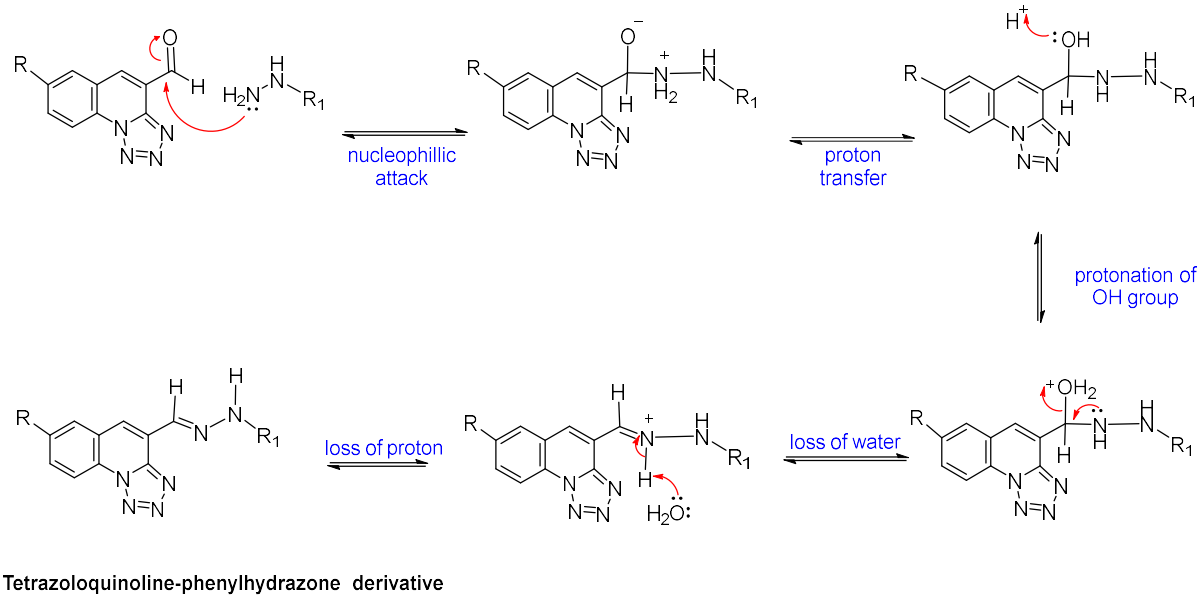


Scheme 1.28 Preparation of quinazoline thiazole phenylhydrazones



Scheme 1.29 Synthesis of 2-chloroquinoline-3-carbaldehyde phenylhydrazones

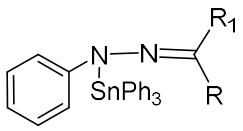
The mechanism for hydrazone formation is similar to the mechanism shown earlier for formation of quinolone-3-thiosemicarbazones and consists of a nucleophilic addition reaction of the hydrazine to the carbonyl carbon of an aldehyde or ketone, followed by loss of water to form the imine (**Scheme 1.30**).



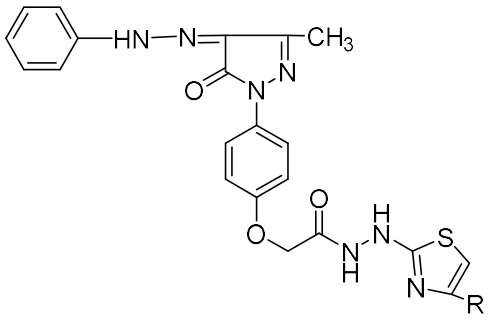
Scheme 1.30 Proposed mechanism for tetrazoloquinoline-phenylhydrazone formation

1.6.2 Antibacterial activity of phenylhydrazone derivatives

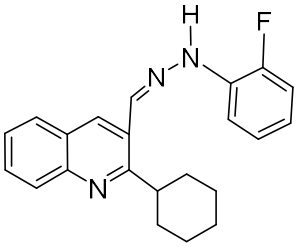
Metal complexes of phenylhydrazones (Khan et al., 2014) and pyrazoline-5-one-4-phenylhydrazone derivatives (**Figure 1.18**) (Krishna et al., 2013) showed good antibacterial activity (Hania et al., 2009). A 3-substituted quinoline fluorophenylhydrazone hybrid with a cyclohexyl moiety at C-2 was also shown to have good antitubercular activity (**Figure 1.18**) (Nayyar et al., 2007) and 2-chloroquinoline-3-carbaldehyde phenylhydrazones was found to have good chemical nuclease activity, being able to cleave DNA (Chandrika et al., 2013).



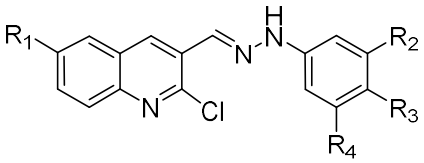
metal complex of phenylhydrazone



pyrazoline phenylhydrazone derivative



quinoline fluorophenylhydrazone hybrid



2-chloroquinoline-3-carbaldehyde phenylhydrazone derivatives

Figure 1.18 Structures of bioactive phenylhydrazones

1.7 Hypothesis, aims and objectives

Hypothesis

Compounds containing the quinoline scaffold have demonstrated bioactivity against a range of pathogens and have shown good activity in a number of biological assays. This scaffold is therefore an important framework on which hybrid molecules can be synthesised. It is hypothesized that joining other pharmacologically active scaffolds to this quinoline framework will result in bioactive molecules with enhanced activity compared to the quinolone core framework or molecules with fewer side effects than known drugs.

Aim

The aim of this project was to design and synthesise compounds with the quinoline framework as its core structure and to join other organic scaffolds to the quinoline nucleus in order to identify hit compounds that could be developed further into antibiotics.

Objectives

The objectives were to synthesize three quinoline based hybrid molecules: quinoline-3-carboxamides, quinolonethiosemicarbazones and tetrazoloquinoline- phenylhydrazones with different substituents at C-2 and C-6 on the quinoline ring and introduce other bioactive functionalities at C-3, and to evaluate these compounds for antibacterial activity.

Specific objectives

a) To synthesise small libraries of novel quinoline-3-carboxamides, quinolonethiosemicarbazones and tetrazoloquinolinephenylhydrazones and confirm their structures using NMR spectroscopy and Mass spectrometry.

b) To test the synthesised derivatives for bioactivity in available assays and determine whether these compounds could be hits for future generation antibiotics.

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Chapter 2. Synthesis and Bioactivity of Quinoline-3-carboxamide Derivatives

***** The compounds referred to in the chapter are referred to elsewhere in the thesis with **A** preceding the number of the compound. For example **5a** is referred to as **A-5a**.

Abstract

Twelve novel substituted 2-chloroquinoline-3-carboxamide derivatives were prepared from acetanilides using the Vilsmeier-Haack reaction, producing 2-chloro-3-carbaldehyde quinolines, followed by oxidation of the 3-carbaldehyde to the carboxylic acid and coupling this group with various anilines. The structures of the synthesized compounds were confirmed by NMR, mass spectrometry and single crystal XRD. The chemical shifts of H-5 and H-8 were influenced by the substituent at C-6. This substituent also affected the chemical shift of C-5, C-7 and C-8, with C-5 and C-7 being more shielded in **5j** (F substituted) in comparison to **5g** (Cl substituted) and **5d** (CH_3 substituted). The compounds showed weak activity in the mM range against Gram +ve and -ve bacteria of which **5b**, **5d** and **5f** showed the best activity with MBC values for **5b** being 3.79 mM against MRSA and **5d** and **5f** having MBC values of 3.77 and 1.79 mM against *S. aureus* ATCC 25923 respectively.

Keywords*:* quinoline carboxamides, Vilsmeier-Haack, antibacterial

2.1 Introduction

Quinoline derivatives have been known to have medicinal applications such as antibacterial (Kidwai et al. 2000; Shivaraj et al., 2013), antimalarial, anti-inflammatory (Marella, et al., 2013), antimicrobial (Desai et al., 2012; Gogoi et al., 2012), antifungal (Musiol, et al., 2006), antimycobacterial (Gonec et al., 2012; de Souza et al., 2009), antiviral (Ghosh et al., 2008), anticancer (Ilango et al., 2015), and cytotoxic activity (Murugesh et al., 2014). The antimalarial drugs, quinine, chloroquine and mefloquine are used for the effective treatment of malaria (WHO, 2015). Quinoline-3-carboxamides have been used in clinical trials for cancer treatment and autoimmune conditions (Deronic et al., 2014). The quinoline-3-carboxamide tasquinimod was found to be effective during the early stages of tumour development (Deronic et al., 2016) whilst paquinimod was used recently in clinical trials for the treatment of Systemic Sclerosis (Stenström et al., 2016).

Various methods such as the Doebner-von Miller, Skraup, Combes (Furniss, et al., 1989), Friedlander (Teimouri and Chermahini, 2016), Conrad-Limpach (Brouet et al., 2009) and Vilsmeier-Haack synthesis have been employed for the preparation of quinolines and their derivatives. Among these, the Vilsmeier-Haack reaction is popularly used for the synthesis of 2-chloro-3-formyl quinolines (Abdel-Wahab et al., 2012; Abdel-Wahab and Khidre, 2013; Tekale et al., 2015). Reagents used to oxidise the aldehyde further to carboxylic acids include potassium permanganate, chromium (VI) oxide, pyridinium dichromate, pyridinium chlorochromate, silver nitrate in basic media, via the nitrile with phosphoryl chloride and hydroxylamine and then conversion to the carboxylic acid with sodium hydroxide or hydrogen peroxide with sodium chlorite and formic acid (Dodd and Le Hyaric, 1993; Syniugin et al., 2016). This provides a further functional group on which to build on the quinoline scaffold.

Quinolines with a carboxamide group linked to an isoxazolyl group has been previously synthesised by first forming butanamides with 4-amino-5-styrylisoxazoles and *β*-keto esters under microwave conditions and then forming the quinoline portion with *o*-amino benzaldehydes (Rajanarendar et al., 2010). Quinoline carboxamides were also made in a one-pot reaction by reacting cyano acetic acid methyl ester, 2-amino benzaldehydes and 2-bromobenzylamines in good yields (Wang et al., 2012). Laquinimod, an experimental drug used for the treatment of multiple sclerosis was prepared from quinoline carboxylic acids, ethylamine, carbodiimides, thionyl chloride and triethylamine (Dixit et al., 2014). Carbodiimides such as EDC together with hydroxybenzotriazole (HOBt) were commonly used reagents to couple quinoline carboxylic acids with amines forming quinoline carboxamides (Lee et al. 2006; Narayan et al., 2016). Besides forming carboxamides, quinoline carboxylic acids were also used to synthesise benzodiazepin-12-ones and benzimidazoles with *o*-phenyldiamines (Abdel-Wahab and Khidre, 2013).

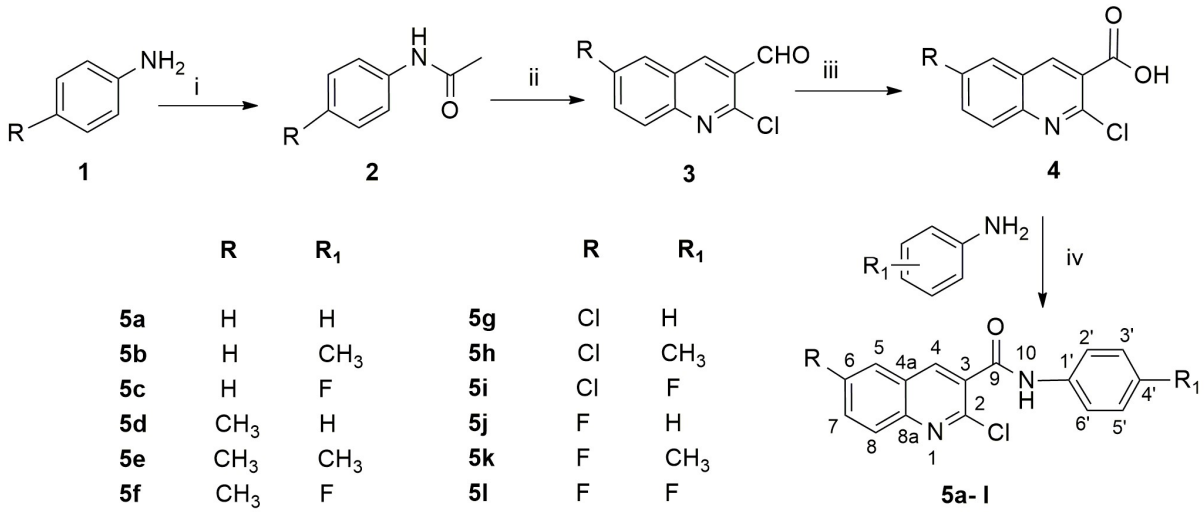
We herein report on the synthesis of twelve novel quinoline-3-carboxamide derivatives and their antibacterial activity.

2.2 Results and Discussion

Chemistry

Twelve novel 2-chloro-*N*-phenylquinoline-3-carboxamides were prepared, starting from anilines, forming acetanilides and using Vilsmeier reagents, DMF and phosphoryl chloride to produce the 2-chloroquinoline-3-carbaldehyde scaffold following the procedure in Toth et al. (2006). The Pinnick oxidation was then used to oxidise the 3-carbaldehydes to the carboxylic acids, which were then coupled to various anilines to produce 2-chloro-*N*-phenylquinoline-3-carboxamide derivatives in yields of 32-53%, following the procedures in George et al. (2013)

and Li et al. (2012) (**Scheme 2.1**). The structures of the synthesised compounds were elucidated by ^{1}H, ^{13}C and 2D NMR, mass spectrometry and single crystal X-Ray diffraction.



	R	R_1		R	R_1
5a	H	H	**5g**	Cl	H
5b	H	CH_3	**5h**	Cl	CH_3
5c	H	F	**5i**	Cl	F
5d	CH_3	H	**5j**	F	H
5e	CH_3	CH_3	**5k**	F	CH_3
5f	CH_3	F	**5l**	F	F

Scheme 2.1 Synthetic route to 2-chloroquinoline-3-carboxamides **5a-l**; i acetic anyhdride, acetic acid; 1 h; ii DMF, $POCl_3$, 82°C, reflux 24 h; iii $NaClO_2$, $NaH_2PO_4.2H_2O$, butan-1-ol, r.t., 2 h; iv EDC, Et_3N, HOBt, DMF, r.t., 24 h.

The ^{1}H NMR spectrum of **5a** showed two characteristic singlets at δ_H 10.73 and δ_H 8.74 attributed to the amide proton NH-10 and H-4 on the quinoline ring. H-5 and H-8 appeared as two doublets at δ_H 8.14 and 8.04 respectively with J = 8.4 Hz each and H-6 and H-7 were observed at δ_H 7.92 (H-7) and δ_H 7.73 (H-6) as triplets of doublets with J = 8.4 Hz for *ortho* coupling and 1.2 Hz for *meta* coupling. The aromatic protons on the *N*-phenyl ring were present as a doublet at δ_H 7.75 (H-2'/6') and two triplets at δ_H 7.39 (H-3'/5') and 7.15 (H-4') with *ortho* coupling of 7.6 Hz for each of the resonances. In the ^{13}C NMR spectrum, the amide carbonyl resonance could be seen at δ_C 163.5 and C-8a and C-2 adjacent to the nitrogen of the quinoline ring could be observed at δ_C 146.8 and δ_C 145.9. C-1' adjacent to the amide nitrogen appeared at δ_C 138.7 and C-4 at δ_C 138.2. The other aromatic carbon resonances were present

between δ_C 119.5 to 131.9 and assigned based on HSQC and HMBC correlations. For example C-3 was differentiated from C-4a due to HMBC correlations from C-4a to H-6 and H-8. C-8a with HMBC correlations to H-4, H-5 and H-7 allowed the distinction to be made between C-2 and C-8a. H-4 was also verified by a HMBC correlation to the carbonyl resonance at δ_C 163.5. The structures of the remaining derivatives were characterised similarly and apart from slight differences on the quinoline ring (when a substituent was present at C-6) and the *N*-phenyl ring (due to *para* substitution), most resonances were similar.

When methyl, chloro and fluoro groups were substituted at C-6, interesting observations were made in the ^{1}H NMR spectrum regarding the chemical shifts of H-5, H-7 and H-8. In **5d**, having a methyl group at C-6, H-8 appeared as a doublet at δ_H 7.94 (J = 8.6 Hz), H-5 slightly more shielded at δ_H 7.89 as a broad singlet and H-7 most shielded, overlapping with H-2'/6' as a double doublet at δ_H 7.75 (J = 8.8, 1.9 Hz). However, when the methyl group was replaced by a chloro group **5g**, H-5 was now the most deshielded resonance, a doublet at δ_H 8.28 followed by the more shielded H-8 doublet at δ_H 8.07 and H-7 still the most shielded appearing as a double doublet at δ_H 7.94 (J = 9.0, 2.4 Hz). With fluorine at this position (**5j**), the order of resonances was similar to **5d**. H-8 was the most deshielded and now a double doublet at δ_H 8.13 with J = 9.4 Hz (*ortho* coupling) and 5.2 Hz (coupling with F). This was followed by the more shielded H-5 at δ_H 7.96, now a double doublet due to coupling with F ($J_{H\text{-}5,F}$ = 9.0 Hz and $J_{H\text{-}5,H\text{-}7}$ = 3.0 Hz). The H-7 resonance was still the most shielded at δ_H 7.85 appearing as a triplet of doublets ($J_{H\text{-}7,H\text{-}8} = J_{H\text{-}7,F}$ = 8.9 Hz and $J_{H\text{-}7,H\text{-}5}$ = 3.0 Hz). Overlaid ^{1}H NMR spectra showing these resonances and their differences are presented in **Figure 2.1**.

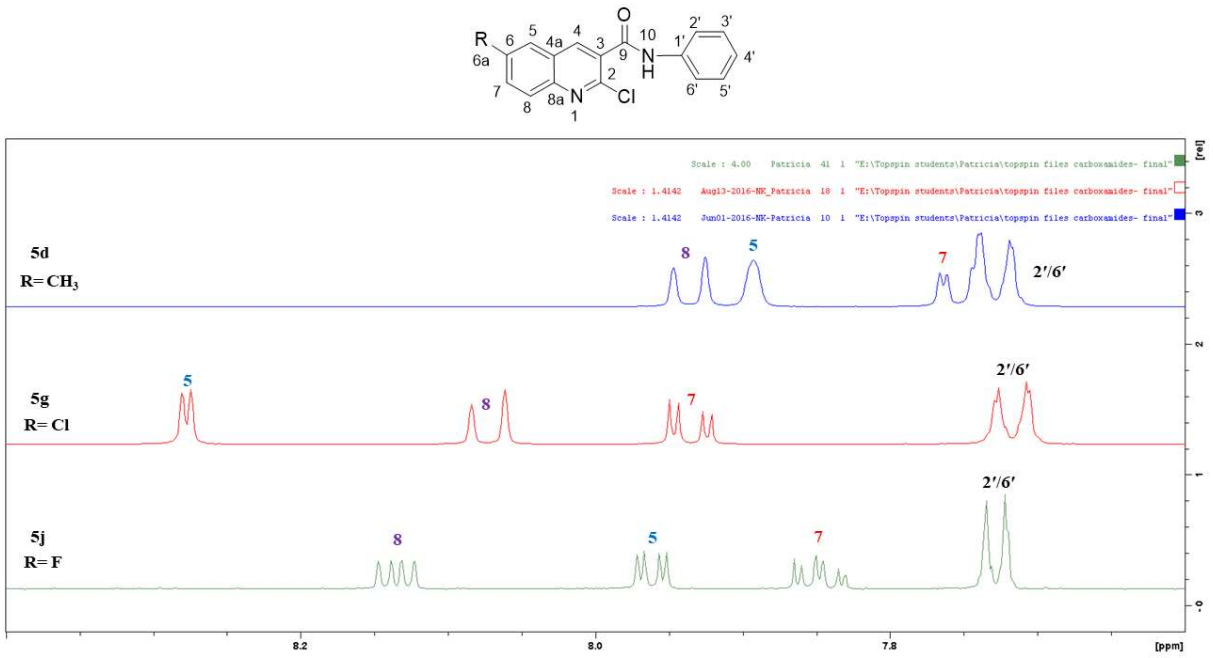


Figure 2.1 Overlay of ^{1}H NMR spectra showing the shifts in the H-5, H-7 and H-8 resonances as a result of substitution of H-6

The different substituents at C-6 also have a noticeable effect on the C-5, C-6, C-7 and C-8 resonances. Interestingly when the methyl group is present at C-6, it resonates at δ_C 137.7 and when the chloro group is substituted at C-6, this resonance is more shielded at δ_C 131.6, indicating that electron donation through resonance is much stronger than the inductive effect. However, when the highly electronegative fluoro group is placed at this position, the inductive effect is much stronger than electron donation by resonance, causing C-6 to appear at δ_C 160.8 as a doublet ($J_{C\text{-}F}$ = 246.2 Hz). The C-7, C-8 and C-5 resonances at δ_C 134.0, 127.4 and 127.0 respectively in **5d** (CH_3 at C-6) occur at similar chemical shifts in **5g** (Cl at C-6), however there is a distinct difference in the C-5 and C-7 resonances in **5j** (F at C-6), which appeared much more shielded at δ_C 112.2 and 122.4 respectively as doublets with $J_{C\text{-}F}$ of 22.3 and 25.7 Hz respectively. This indicates an increase in electron density at these particular carbon atoms, through electron donation by resonance from the fluorine. These results indicate that this

scaffold with fluorine at C-6 is ideal for nucleophilic substitution at C-6 and for electrophilic substitution at C-5 and C-7. These distinct differences are shown in the overlaid ^{13}C NMR spectra of **5d**, **5g** and **5j** (**Figure 2.2**).

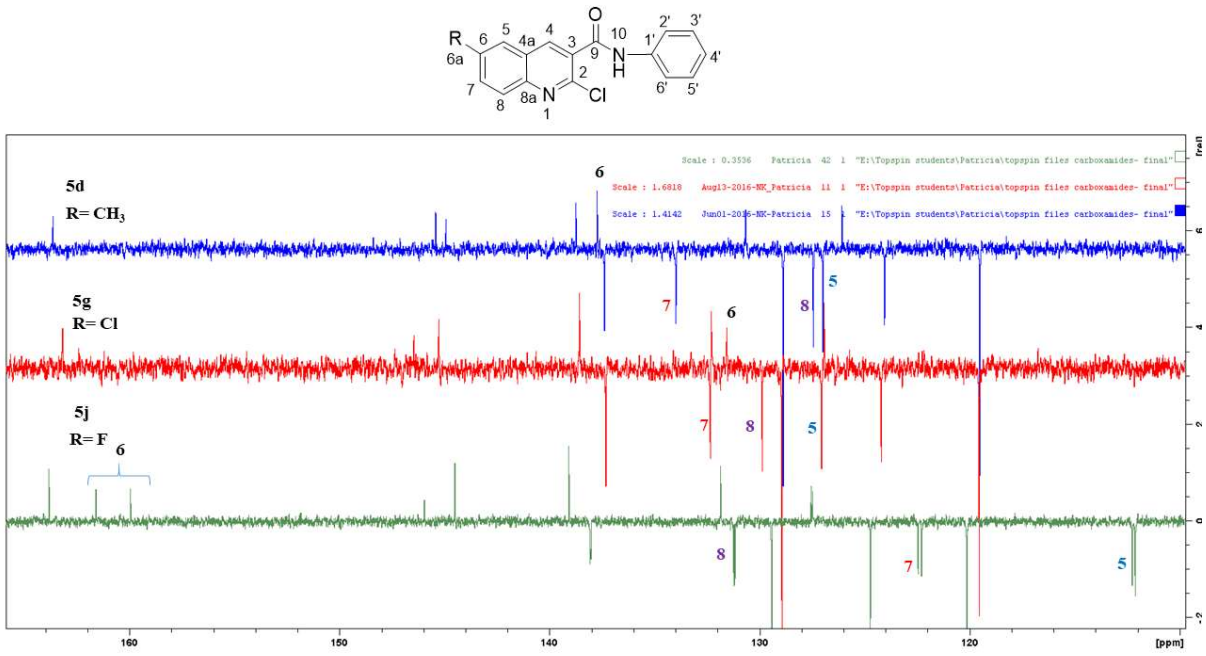


Figure 2.2 Overlay of ^{13}C NMR spectra for **5d**, **5g** and **5j** showing the shifts in resonances of C-5 to C-8 as a result of substitution at C-6

The crystal structure of the 4-fluorophenyl derivative **5f** was obtained to explore the three dimensional structure of the molecule. The molecule is essentially planar with all bond angles close to 120° and the *N*-phenyl group pointing away from the quinoline nucleus. The torsion angle for C8-C7-N1-C1 is 8.9(3) indicating that the *N*-phenyl group is slightly out of the plane of the quinoline nucleus. **Figure 2.3** shows an ORTEP diagram of **5f**. The refinement parameters are given in the supplementary material.

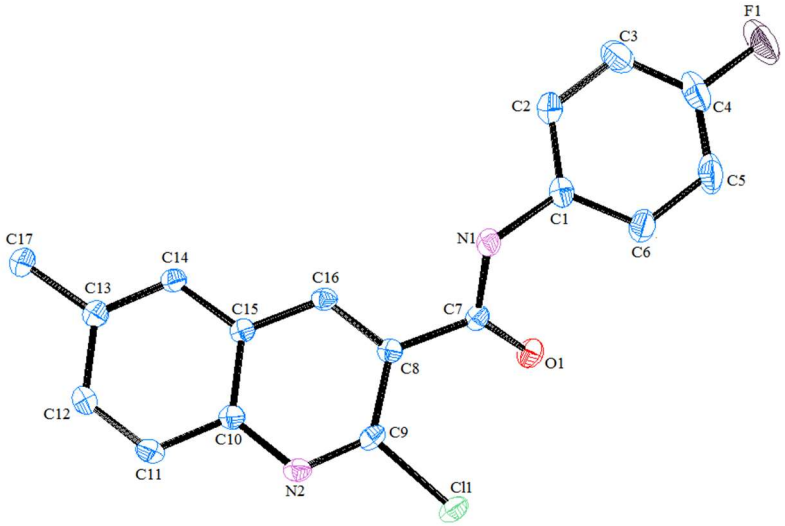


Figure 2.3 ORTEP diagram of **5f**

Antibacterial activity

The synthesised compounds **5a-5l** were screened against two Gram positive and four Gram negative strains (*Staphylococcus aureus*, MRSA, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhimurium*) using the disc diffusion assay. Compounds **5b-5f** showed broad spectrum activity in the disc diffusion assay having zones of inhibition greater than 7 mm in five or more of the bacterial strains and were subject to the broth microdilution method to determine their MBC values. However, none of the compounds showed significant antibacterial activity. The best activity was shown by **5b** (3.79 mM against MRSA), and **5d** and **5f** (3.77 and 1.79 mM against *S. aureus* ATCC 25923 respectively). The disc diffusion and MBC data for all compounds are given in the supplementary material.

2.3 Experimental

General

Chemical reagents and solvents were purchased from Sigma Aldrich, via Capital Laboratories, South Africa. Silica gel 60 (0.063-0.2 mm) was used for column chromatography. Alumina-

backed silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) was used for TLC and visualised under UV light (254 nm). Melting points were conducted on an Electrothermal IA 9100 Digital melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 100 instrument with Universal ATR sampling accessory. ^{1}H, ^{13}C and 2D NMR spectra were acquired on either Bruker Avance^{III} 400 or 600 MHz spectrometers (Bruker Co., Karlsruhe, Germany) at frequencies of 400.22 MHz for ^{1}H and 100.63 MHz for ^{13}C. Chemical shifts were reported as δ values (ppm) relative to an internal standard of tetramethylsilane (TMS) or to the solvent line of DMSO-d_6 (δ_H = 2.50, δ_C = 39.52). High-resolution mass data were obtained using a Waters Micromass LCT Premier TOF-MS instrument. Ultraviolet (UV) analyses were conducted with a UV-VIS-NIR Shimadzu series 3600 spectrophotometer using methanol.

Synthesis

*General procedure for synthesis of para substituted acetanilides **2***

Acetic anhydride (10 mL, 0.1 mol) and glacial acetic acid (10 mL, 0.175 mol) was added to substituted anilines (0.1 mole), heated gently under reflux for 1 h and poured into ice-water (200 mL), where crystals of the acetylated product was formed. The crystals were filtered and recrystallized with acetic acid:water (1:2), producing the acetanilides in yields of between 94-100%.

*General procedure for synthesis of 2-chloroquinoline-3-carbaldehydes **3***

Substituted 2-chloroquinoline-3-carbaldehydes (**3a-d**) were prepared according to the method in Toth et al. (2006). Briefly, dry *N*,*N*,-dimethylformamide (DMF) (9.6 mL; 0.125 mol) was transferred under inert conditions to a two-neck round bottom flask, placed in an ice-bath (0ºC)

to which phosphoryl chloride ($POCl_3$) (32.2 mL; 0.35 mol) was added dropwise with constant stirring. The appropriate acetanilide (0.05 mol) was then added and heated under reflux with a condenser fitted with a $CaCl_2$ drying tube at 82°C for 24 h. The reaction was monitored to completion using TLC. Upon completion, the mixture was cooled, poured into 300 mL ice water and stirred for 1 h at 0-10 °C. A pale yellow precipitate formed and was filtered off, washed with 100 mL water and dried producing 2-chloroquinoline-3-carbaldehydes in yields ranging from 43-74%.

General procedure for the oxidation of 2-chloroquinoline-3-carbaldehydes ***3*** to *2-chloroquinoline-3-carboxylic acids* ***4***

The procedure in George et al. (2013) was carried out with modifications. The substituted 2-chloroquinoline-3-carbaldehydes (4.68 mmol) was dissolved in *n*-butanol (89 mL) and a solution of sodium dihydrogen orthophosphate (5.56 g; 35.61 mmol) and sodium chlorite (4.29 g; 47.47 mmol) in water (39 mL) gradually added and stirred for 2 h at room temperature. The reaction was monitored to completion by TLC, concentrated in vacuo and diluted with water (68 mL) and ethyl acetate (50 mL). Sodium carbonate (5g) was added to the mixture to separate the two layers and the lower aqueous layer acidified with HCl to pH 4 where the 2-chloroquinoline-3-carboxylic acids **4** precipitated as cream coloured compounds with yields of between 54-72%.

*General procedure for synthesis of 2-chloro-N-phenylquinoline-3-carboxamides (****5a-l****)*

The procedure used by Li et al. (2012) for the synthesis of quinoline-3-carboxamides was followed with modifications. EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) (0.99 mmol) and 1-hydroxybenzotriazole (HOBt) (0.99 mmol) were added sequentially at room temperature to the substituted quinoline-3-carboxylic acids **4** (0.9 mmol)

dissolved in 10 mL DMF. The reaction mixture was stirred for 30 min before adding the appropriate substituted arlyamines (0.99 mmol) and triethylamine (1.895 mmol) to the mixture and stirring at room temperature for 24 h. Upon completion, the reaction mixture was poured into ice water, where it formed a cream coloured precipitate. This was washed with water, dried and purified using column chromatography on silica gel with hexane:ethyl acetate (9:1) producing 2-chloro-*N*-phenylquinoline-3-carboxamide derivatives **5a-l** in yields of between 32-53%.

*2-Chloro-N-phenylquinoline-3-carboxamide (**5a**)* cream-coloured solid (32% yield), mp 178-180 °C, IR υ_{max} (cm^{-1}): 3256 (N-H), 1660 (C=O); UV λ_{max} (MeOH) nm (log ε): 237 (3.95), 309 (3.14), 325 (2.93); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.73 (1H, s, H-10), 8.75 (1H, s, H-4), 8.14 (1H, d, *J* = 8.4 Hz, H-5), 8.04 (1H, d, *J* = 8.4 Hz, H-8), 7.92 (1H, td, *J* = 8.4, 1.2 Hz, H-7), 7.75 (2H, d, *J* = 7.6 Hz, H-2'/6'), 7.73 (1H, td, *J* = 8.4, 1.2, H-6), 7.39 (2H, t, *J* = 7.6 Hz, H-3'/5'), 7.15 (1H, t, *J* = 7.6 Hz, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.5 (C-9), 146.8 (C-8a), 145.9 (C-2), 138.7 (C-1'), 138.2 (C-4), 132.0 (C-7), 130.7 (C-3), 128.9 (C-3'/5'), 128.4 (C-5), 128.0 (C-6), 127.7 (C-8), 126.1 (C-4a), 124.1 (C-4′), 119.5 (C-2'/6'); HRESIMS (pos): (*m/z*) 304.0459 [M+Na] (calculated for $C_{16}H_{11}N_2OClNa$, 304.0458).

*2-Chloro-N-4-tolylquinoline-3-carboxamide (**5b**)* cream-coloured solid (44% yield), mp 138-140 °C, IR υ_{max} (cm^{-1}): 3238 (N-H), 1657 (C=O); UV λ_{max} (MeOH) nm (log ε): 237 (4.31), 310 (3.47), 325 (3.32); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.64 (1H, s, H-10), 8.73 (1H, s, H-4), 8.13 (1H, d, *J* = 8.3 Hz, H-5), 8.04 (1H, d, *J* = 8.3 Hz, H-8) 7.91 (1H, td, *J* = 8.3, 1.2 Hz, H-7), 7.74 (1H, td, *J* = 8.3, 1.2 Hz, H-6), 7.62 (2H, d, *J* = 8.3 Hz, H-2'/6'), 7.20 (2H, d, *J* = 8.3 Hz, H-3'/5'), 2.29 (3H, s, 4′-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.3 (C-9), 146.8 (C-

8a), 146.0 (C-2), 138.1 (C-4), 136.2 (C-1′), 133.1 (C-4′), 131.9 (C-7), 130.7 (C-3), 129.2 (C-3'/5'), 128.4 (C-5), 127.9 (C-6), 127.7 (C-8), 126.1 (C-4a), 119.5 (C-2'/6'), 20.5 (4′-CH_3); HRESIMS (pos): (*m/z*) 319.0617 [M+Na] (calculated for $C_{17}H_{13}N_2OClNa$, 319.0614).

*2-Chloro-N-(4-fluorophenyl)quinoline-3-carboxamide (**5c**)* cream-coloured solid (46% yield), mp 199-201 °C, IR υ_{max} (cm^{-1}): 3241 (N-H), 1669 (C=O); UV λ_{max} (MeOH) nm (log ε): 237 (3.98), 296 (3.48), 325 (3.18); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.82 (1H, s, H-10), 8.77 (1H, s, H-4), 8.17 (1H, d, *J* = 8.0 Hz, H-5), 8.07 (1H, d, *J* = 8.4 Hz, H-8), 7.95 (1H, td, *J* = 8.4, 1.3 Hz, H-7), 7.76-7.80 (3H, m, H-2'/6', H-6), 7.26 (2H, t, *J* = 8.8 Hz, H-3'/5'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.4 (C-9), 158.4 (d, *J* = 240.6 Hz , C-4′), 146.8 (C-8a), 145.9 (C-2), 138.2 (C-4), 135.1 (d, *J* = 2.3 Hz, C-1'), 132.0 (C-7), 130.5 (C-3), 128.4 (C-5), 128.0 (C-6), 127.7 (C-8), 126.0 (C-4a), 121.4 (d, *J* = 7.9 Hz, C-2'/6'), 116.0 (d, *J* = 22.1 Hz, C-3'/5'); HRESIMS (neg): (*m/z*) 299.0390 [M-H] (calculated for $C_{16}H_9N_2OFCl$, 299.0387).

*2-Chloro-6-methyl-N-phenylquinoline-3-carboxamide (**5d**)* cream-coloured solid (43% yield), mp 173-175 °C, IR υ_{max} (cm^{-1}): 3253 (N-H), 1660 (C=O); UV λ_{max} (MeOH) nm (log ε): 241 (4.13), 327 (3.17); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.71 (1H, s, H-10), 8.62 (1H, s, H-4), 7.94 (1H, d, *J* = 8.6 Hz, H-8), 7.89 (1H, br s, H-5), 7.75 (1H, dd, *J* = 8.8, 1.9 Hz, H-7), 7.72 (2H, d, *J* = 7.6 Hz, H-2'/6'), 7.39 (2H, t, *J* = 7.6 Hz, H-3'/5'), 7.15 (1H, t, *J* = 7.6 Hz, H-4'), 2.54 (3H, s, 6-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.6 (C-9), 145.4 (C-8a), 144.9 (C-2), 138.7 (C-1'), 137.7 (C-6), 137.4 (C-4), 134.0 (C-7), 130.7 (C-3), 128.9 (C-3'/5'), 127.4 (C-8), 127.0 (C-5), 126.1, (C-4a), 124.1 (C-4′), 119.5 (C-2'/6'), 21.1 (6-CH_3); HRESIMS (pos): (*m/z*) 319.0616 [M+Na] (calculated for $C_{17}H_{13}N_2OClNa$, 319.0614).

*2-Chloro-6-methyl-N-4-tolylquinoline-3-carboxamide (**5e**)* cream-coloured solid (38% yield), mp 224-226 °C, IR υ_{max} (cm^{-1}): 3244 (N-H), 1656 (C=O); UV λ_{max} (MeOH) nm (log ε): 240 (4.42), 311 (3.86), 328 (3.82); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.62 (1H, s, H-10), 8.60 (1H, s, H-4), 7.93 (1H, d, *J* = 8.6 Hz, H-8), 7.89 (1H, s, H-5), 7.75 (1H, dd, *J* = 8.6, 1.8 Hz, H-7), 7.61 (2H, d, *J* = 8.4 Hz, H-2′/6′), 7.19 (2H, d, *J* = 8.4 Hz, H-3′/5′), 2.53 (3H, s, 6-CH_3), 2.29 (3H, s, 4′-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.4 (C-9), 145.4 (C-8a), 145.0 (C-2), 137.7 (C-6), 137.3 (C-4), 136.3 (C-1′), 133.9 (C-7), 133.0 (C-4′), 130.8 (C-3), 129.2 (C-3′/5′), 127.4 (C-8), 127.0 (C-5), 126.1 (C-4a), 119.5 (C-2′/6′), 21.1 (6-CH_3), 20.5 (4′-CH_3); HRESIMS (pos): (*m/z*) 310.0770 [M+Na] (calculated for $C_{18}H_{15}N_2OClNa$, 310.0771).

*2-Chloro-N-(4-fluorophenyl)-6-methylquinoline-3-carboxamide (**5f**)* cream-coloured solid (53% yield), mp 196-198 °C, IR υ_{max} (cm^{-1}): 3244(N-H), 1658 (C=O); UV λ_{max} (MeOH) nm (log ε): 241 (4.44), 327 (3.40); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.78 (1H, s, H-10), 8.61 (1H, s, H-4), 7.93 (1H, d, *J* = 8.6 Hz, H-8), 7.88 (1H, br s, H-5), 7.74 (2H, d, *J* = 8.8 Hz, H-2′/6′), 7.72-7.74 (1H, m, H-7), 7.23 (2H, t, *J* = 8.8 Hz, H-3′/5′), 2.53 (3H, s, 6-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.6 (C-9), 158.4 (C-4′), 145.4 (C-8a), 144.9 (C-2), 137.8 (C-6), 137.4 (C-4), 135.1 (C-1′), 134.0 (C-7), 130.4 (C-3), 127.4 (C-8), 127.0 (C-5), 126.0 (C-4a), 121.4 (d, *J* = 7.9 Hz, C-2′/6′), 116.0 (d, *J* = 22.2 Hz, C-3′/5′), 21.1 (6-CH_3); HRESIMS (neg): (*m/z*) 279.0945 $[M-Cl]^+$; (calculated for $C_{17}H_{12}N_2OF$, 279.0934).

*2,6-Dichloro-N-phenylquinoline-3-carboxamide (**5g**)* cream-coloured solid (33% yield), mp 156-158 °C, IR υ_{max} (cm^{-1}): 3244 (N-H), 1660 (C=O); UV λ_{max} (MeOH) nm (log ε): 240 (4.41), 309 (3.28); 330 (3.19); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.77 (1H, s, H-10), 8.71 (1H, s, H-4), 8.28 (1H, d, *J* = 2.4 Hz, H-5), 8.07 (1H, d, *J* = 9.0 Hz, H-8), 7.94 (1H, dd, *J* = 9.0, 2.4 Hz,

H-7), 7.72 (2H, d, *J* = 7.5 Hz, H-2′/6′), 7.39 (2H, t, *J* = 7.5 Hz, H-3′/5′), 7.16 (1H, t, *J* = 7.5 Hz, H-4′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.2 (C-9), 146.5 (C-2), 145.3 (C-8a), 138.6 (C-1′), 137.3 (C-4), 132.4 (C-7), 132.3 (C-3), 131.6 (C-6), 129.9 (C-8), 128.9 (C-3′/5′), 127.0 (C-5), 126.9 (C-4a), 124.2 (C-4′), 119.5 (C-2′/6′); HRESIMS (neg): (*m/z*) 315.0098 [M-H] (calculated for $C_{16}H_9N_2OCl_2$, 315.0092).

*2,6-dichloro-N-4-tolylquinoline-3-carboxamide (**5h**)* cream-coloured solid (35% yield), mp 189-191 °C, IR υ_{max} (cm^{-1}): 3236 (N-H), 1656 (C=O); UV λ_{max} (MeOH) nm (log ε): 241 (4.31), 309 (3.22); 330 (3.05); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.66 (1H, s, H-10), 8.68 (1H, s, H-4), 8.26 (1H, d, *J* = 2.5 Hz, H-5), 8.06 (1H, d, *J* = 9.0 Hz, H-8), 7.92 (1H, dd, *J* = 9.0, 2.5 Hz, H-7), 7.59 (2H, d, *J* = 8.3 Hz, H-2′/6′), 7.19 (2H, d, *J* = 8.3 Hz, H-3′/5′), 2.29 (3H, s, 4′-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.5 (C-9), 147.0 (C-2), 145.8 (C-8a), 137.8 (C-4), 136.6 (C-1′), 133.8 (C-4′), 132.80 (C-7), 132.79 (C-3), 132.2 (C-4a), 130.4 (C-8), 129.8 (C-3′/5′), 127.55 (C-5), 127.46 (C-6), 120.1 (C-2′/6′); 21.0 (4′-CH_3); HRESIMS (neg): (*m/z*) 329.0247 [M-H] (calculated for $C_{17}H_{11}N_2OCl_2$, 329.0248).

*2,6-dichloro-N-(4-fluorophenyl)quinoline-3-carboxamide (**5i**)* cream-coloured solid (44% yield), mp 167-169 °C, IR υ_{max} (cm^{-1}): 3229 (N-H), 1656 (C=O); UV λ_{max} (MeOH) nm (log ε): 236 (4.18), 280 (3.64); 330 (3.22); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.84 (1H, s, H-10), 8.72 (1H, s, H-4), 8.27 (1H, d, *J* = 2.4 Hz, H-5), 8.07 (1H, d, *J* = 9.0 Hz, H-8), 7.93 (1H, dd, *J* = 9.0, 2.4 Hz, H-7), 7.74 (2H, dd, *J* = 8.9, 5.0 Hz, H-2′/6′), 7.24 (2H, t, *J* = 8.9 Hz, H-3′/5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.7 (C-9), 159.1 (d, *J* = 239.5 Hz , C-4′), 146.9 (C-2), 145.8 (C-8a), 137.9 (C-4), 135.4 (C-1′), 133.0 (C-7), 132.9 (C-4a), 131.9 (C-3), 130.4 (C-8), 127.6

(C-5), 127.4 (C-6), 122.0 (d, *J* = 7.8 Hz, C-2′/6′), 116.1 (d, *J* = 22.2 Hz, C-3′/5′); HRESIMS (neg): (*m/z*) 333.0006 [M-H] (calculated for $C_{16}H_9N_2OCl_2F$, 332.9998).

*2-Chloro-6-fluoro-N-phenylquinoline-3-carboxamide (**5j**)* cream-coloured solid (42% yield), mp 187-189 °C, IR υ_{max} (cm^{-1}): 3242 (N-H), 1655 (C=O); UV λ_{max} (MeOH) nm (log ε): 237 (4.20), 309 (3.32); 325 (3.32); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.76 (1H, s, H-10), 8.71 (1H, s, H-4), 8.13 (1H, dd, *J* = 9.4, 5.2 Hz, H-8), 7.96 (1H, dd, *J* = 9.0, 3.0 Hz, H-5), 7.85 (1H, td, *J* = 8.9, 3.0 Hz, H-7), 7.73 (2H, d, *J* = 7.6 Hz, H-2′/6′), 7.41 (2H, t, *J* = 7.6 Hz, H-3′/5′), 7.18 (1H, t, *J* = 7.6 Hz, H-4′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.8 (C-9), 160.8 (d, *J* = 246.2 Hz, C-6), 146.0 (d, *J* = 2.8 Hz, C-2), 144.5 (C-8a), 139.1 (C-1′), 138.1 (C-4), 131.9 (C-3), 131.2 (d, *J* = 9.1 Hz, C-8), 129.4 (C-3′/5′), 127.5 (d, *J* = 10.8 Hz, C-4a), 124.7 (C-4′), 122.4 (d, *J* = 25.7 Hz, C-7), 120.1 (C-2′/6′); 112.2 (d, *J* = 22.3 Hz, C-5); HRESIMS (neg): (*m/z*) 299.0392 [M-H] (calculated for $C_{16}H_9N_2OClF$, 299.0387).

*2-Chloro-6-fluoro-N-4-tolylquinoline-3-carboxamide (**5k**)* cream-coloured solid (51% yield), mp 195-197 °C, IR υ_{max} (cm^{-1}): 3233 (N-H), 1655 (C=O); UV λ_{max} (MeOH) nm (log ε): 237 (4.47), 311 (3.65), 325 (3.64); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.67 (1H, s, H-10), 8.69 (1H, s, H-4), 8.12 (1H, dd, *J* = 9.3, 5.3 Hz, H-8), 7.95 (1H, dd, *J* = 9.0, 2.9 Hz, H-5), 7.83 (1H, td, *J* = 9.0, 2.9 Hz, H-7), 7.60 (2H, d, *J* = 8.3 Hz, H-2′/6′), 7.19 (2H, d, *J* = 8.3 Hz, H-3′/5′), 2.29 (3H, s, 4′-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.1 (C-9), 160.2 (d, *J* = 245.8 Hz, C-6), 145.5 (d, *J* = 2.8 Hz , C-2), 144.0 (C-8a), 137.5 (d, *J* = 5.2 Hz, C-4), 136.1 (C-1′), 133.2 (C-4′), 131.4 (C-3), 130.7 (d, *J* = 9.5 Hz, C-8), 129.3 (C-3′/5′), 127.0 (d, *J* = 10.8 Hz, C-4a), 121.8 (d, *J* = 26.0 Hz, C-7), 119.6 (d, *J* = 14.1 Hz, C-2′/6′); 111.7 (d, *J* = 22.4 Hz, C-5), 20.5 (C-4′a); HRESIMS (neg): (*m/z*) 314.0544 [M-H] (calculated for $C_{17}H_{11}N_2OClF$, 314.0544).

*2-Chloro-6-fluoro-N-(4-fluorophenyl)quinoline-3-carboxamide (**5l**)* cream-coloured solid (35% yield), mp 183-185 °C, IR υ_{max} (cm^{-1}): 3236 (N-H), 1660 (C=O); UV λ_{max} (MeOH) nm (log ε): 236 (4.45), 310 (3.62), 330 (3.46); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.81 (1H, s, H-10), 8.71 (1H, s, H-4), 8.13 (1H, dd, *J* = 9.0, 5.2 Hz, H-8), 7.95 (1H, dd, *J* = 9.0, 2.8 Hz, H-5) 7.84 (1H, td, *J* = 9.0, 2.8 Hz, H-7), 7.74 (2H, dd, *J* = 9.0, 4.8 Hz, H-2'/6'), 7.24 (2H, t, *J* = 9.0 Hz, H-3'/5'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.7 (C-9), 162.5 (d, *J* = 237.2 Hz, C-4'), 160.8 (d, *J* = 274.0 Hz, C-6), 146.0 (C-2), 144.6 (C-8a), 138.2 (C-4), 135.5 (C-1′), 131.3 (C-8), 127.5 (d, *J* = 9.9 Hz, C-4a), 122.4 (d, *J* = 25.4 Hz, C-7) 122.0 (d, *J* = 7.7 Hz, C-2'/6'); 116.5 (d, *J* = 22.2 Hz, C-3'/5'), 112.2 (d, *J* = 22.8 Hz, C-5); HRESIMS (neg): (*m/z*) 317.0285 [M-H] (calculated for $C_{16}H_8N_2OClF_2$, 317.0293).

Antibacterial activity

The quinoline-3-carboxamides were screened for antibacterial activity against two Gram positive bacteria, *Staphylococcus aureus* ATCC 25923 and methicillin resistant *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (MRSA) and four Gram negative bacterial strains, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 31488*, Escherichia coli* ATCC 25922 and *Salmonella typhimurium ATCC 14026 (S.t).* The compounds were initially screened using the disc diffusion method. Briefly, bacterial strains were grown in nutrient broth (Biolab, South Africa) for 18 h at 37 ºC and diluted with sterile distilled water to achieve a final concentration equivalent to a 0.5 McFarland standard. Mueller-Hinton agar plates (Biolab, South Africa) were lawn inoculated with the different strains of bacteria. Discs were impregnated with 10 µL of each sample (10 mg mL^{-1} in 100% DMSO), placed onto Mueller-Hinton plates and incubated for 18 h at 37 °C. Compounds displaying antibacterial activity by showing a zone of inhibition were further subject to the broth microdilution method to determine their minimum bactericidal concentrations (MBC).

The selected compounds **5d-f** were active against MRSA and **5b-c** active against *Salmonella*. Experiments were done in triplicate and an average of the readings taken.

For the broth microdilution method, a 10 mg sample of the selected compounds were dissolved in 1 mL DMSO and serially diluted five times in Eppendorf tubes using Mueller Hinton broth (Johannesburg, South Africa) to achieve concentrations of between 312.5 µg mL^{-1} to 10 mg mL^{-1} such that each eppendorf tube contained 180 µL of the broth diluted compound. To each eppendorf, 20 µL of the bacterial strains (at a concentration equivalent to a 0.5 McFarland Standard) was then added and incubated for 24 h at 37 ºC. Thereafter, 10 µL of each concentration was spotted onto MHA plates and incubated at 37 ºC to determine the MBC. The MBC was determined as the lowest concentration of the compound that showed no bacterial growth after incubation. Levofloxacin was used as a standard antibiotic. Experiments were conducted in triplicate and an average of the readings taken.

X-Ray crystallography

Crystallographic data is included in the Supplementary material and has been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository number CCDC-1575908 (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).

2.4 Conclusions

A series of 2-chloroquinoline-3-carboxamides (**5a−l**) were successfully synthesized from substituted acetanilides using the Vilsmeier-Haack reaction, forming the quinoline 3-carbaldehydes, oxidation to the carboxylic acids and amide coupling with anilines using EDC

and HOBt. Substitution at C-6 resulted in either upfield or downfield shifts in the ^{1}H NMR spectra for H-5, H-7 and H-8 and in the ^{13}C NMR for C-5 to C-8. All synthesised compounds showed weak antibacterial activity with the best activity being seen by **5b** (6-H, 4'-CH_3), **5d** (6-CH_3, 4'-H) and **5f** (6-CH_3, 4'-F). Thus, the electron donating methyl groups either at C-6 or C-4' were shown to be responsible for the antibacterial activity of the quinoline-carboxamide scaffold. The DMSO was used as a blank standard and showed no activity against all the bacterial strains tested.

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Chapter 3. Synthesis, Antibacterial Activity and Docking Studies of Substituted Quinolone Thiosemicarbazones

***** The compounds referred to in the chapter are referred to elsewhere in the thesis with **B** preceding the number of the compound. For example **5a** is referred to as **B-5a**.

Abstract

Fifteen substituted 2-quinolone thiosemicarbazone hybrid derivatives of which eleven were new, were synthesised at room temperature by first synthesising 2-chloroquinoline-3-carbaldehyde derivatives, converting them to the quinolone and forming the thiosemicarbazone from the aldehyde. Electron withdrawing halogens and electron donating methyl groups were substituted at C-6 on the quinolone ring and the thiosemicarbazone moeity was substituted with methyl and phenyl groups to determine the best possible substitution pattern for antibacterial activity. The structures of the synthesised compounds were elucidated by 1D and 2D-NMR and mass spectrometry. The synthesised compounds showed antibacterial activity with MBCs in the range of 0.80 to 36.49 mM against *Staphylococcus aureus*, *S. aureus* Rosenbach (MRSA), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhimurium*.

Keywords*:* quinolone, thiosemicarbazone, *N*-methylthiosemicarbazones, phenylthiosemicarbazones, antibacterial activity.

3.1 Introduction

Quinolones are derived from hydroxyquinolines, heterocyclic molecules containing pyridine fused to a benzene ring. These quinolines are synthesised by a variety of methods including the Skraup, Combes, Doebner-von Miller, Knorr and Pfitzinger syntheses (Madapa et al., 2008). Quinolones are usually formed by the Conrad-Limpach synthesis (Brouet et al., 2009). The fluoroquinolones in particular are important antibiotics currently prescribed for bacterial infections (Redgrave, 2014). They are used in the treatment of respiratory, gastrointestinal, gynaecologic and skin infections, and pneumonia (Oliphant and Green, 2002; Grossman et al., 2014) and act by interfering with DNA replication (Aldred et al., 2014). Drugs such as ciprofloxacin and levofloxacin have broad spectrum activity and are drugs of choice against bacterial infections (Drago et al., 2001; Ren et al., 2017).

Thiosemicarbazones belong to a class of Schiff based ligands usually synthesised by condensing aldehydes or ketones with thiosemicarbazide (Hassan et al., 2012). They can also be prepared by first forming the imine between the aldehyde and hydrazine and then reacting this with isothiocyanate (Mohamed, 1994). Thiosemicarbazone derivatives have various pharmacological activities, including antibacterial (Siwek et al., 2011), antioxidant (Karaküçük-İyidoğan et al., 2014), antimalarial (Biot et al., 2007), antitumour (Hu et al., 2010); antiviral (Padmanabhan et al., 2017), anticancer (Hu et al., 2006; Hernández et al., 2013), anti-HIV and antitubercular activity (Banerjee et al., 2011). Thiosemicarbazones are also known to behave as chelating ligands by forming transitional metal complexes (Pelosi, 2010).

The combination of two structural scaffolds, each having pharmacological activity of their own could lead to molecules with enhanced therapeutic effects (Dave and Rahatgaonkar, 2016). These molecules are often referred to as "molecular hybrids" and extensive work has recently

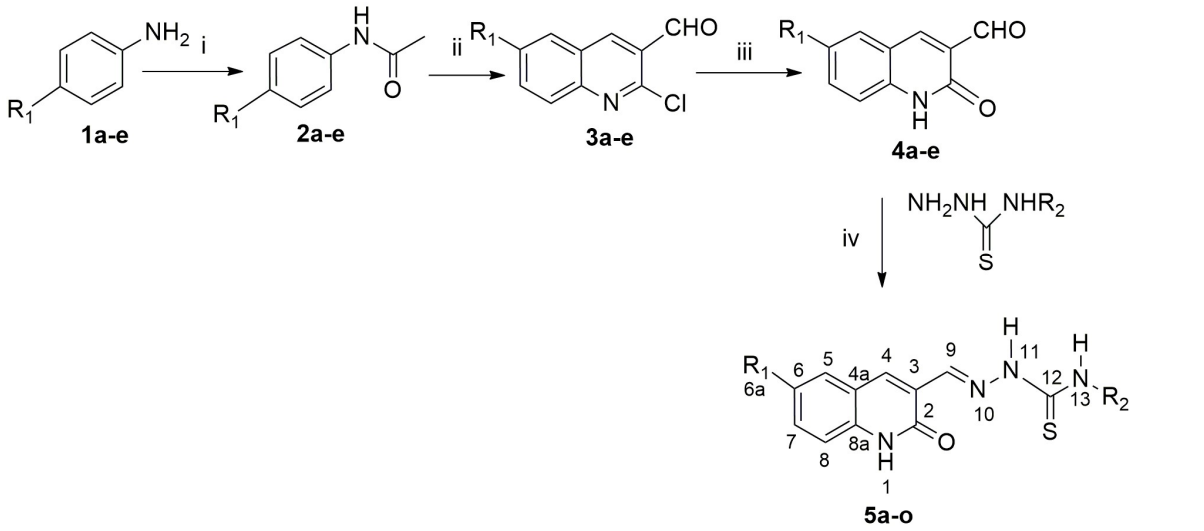
been carried out on quinoline hybrids (Vandekerckhove and D'hooghe, 2015). Quinoline-thiosemicarbazone hybrids have been prepared by reacting quinoline carbaldehydes with substituted thiosemicarbazides and have shown antibacterial, antifungal (Sudha and Selvi, 2015), antitubercular (Ukrainets et al., 2009), antioxidant (Liu et al., 2009), anti-inflammatory (Jayakumar Swamy et al., 2012) and anticancer activtiy (Serda et al., 2012; Bisceglie et al., 2015).

Due to the emergence of antibacterial resistance and the need for the development of new antibiotics against pathogens such as *Klebsiella* sp., *P. aeruginosa* and *E. coli* (Levy, 2002; Neu, 1992), a small library of 2-quinolone thiosemicarbazones were synthesised to be tested for their antibacterial activity

3.2 Results and Discussion

Chemistry

Fifteen derivatives of quinolone-thiosemicarbazone hybrids were prepared by first synthesising 3-formyl-2-quinolone and then condensing it with substituted hydrazines. The 2-quinolones were prepared by making substituted 2-chloroquinolines using the Vilsmeier-Haack reaction and converting this to the quinolone under acidic conditions (**Scheme 3.1**). The yields obtained for the final step of the synthesis of the compounds were between 69-96%. The synthesised compounds contained electron donating hydrogen and methyl groups and electron withdrawing halogens at C-6 on the quinolone scaffold and the thiosemicarbazone was varied with methyl and phenyl groups to determine the best possible functionalities for antibacterial activity. Of the 15 derivatives synthesised, only **5a**, **5b**, **5f** and **5k** were previously synthesised (Raja et al., 2011; Ye et al., 2013). The 6-halogenated quinolones and two of the 6-methylquinolones were new.



	R_1	R_2		R_1	R_2		R_1	R_2
5a	H	H	**5f**	H	CH_3	**5k**	H	Ph
5b	CH_3	H	**5g**	CH_3	CH_3	**5l**	CH_3	Ph
5c	Br	H	**5h**	Br	CH_3	**5m**	Br	Ph
5d	Cl	H	**5i**	Cl	CH_3	**5n**	Cl	Ph
5e	F	H	**5j**	F	CH_3	**5o**	F	Ph

Scheme 3.1 Synthetic route to the quinoline thiosemicarbazones **5a-o**; i. Acetic anyhdride, Acetic acid, reflux 1 h; ii. DMF, $POCl_3$, 82°C, reflux 24 h; iii. 70% acetic acid, reflux, 4-6 h; iv methanol, rt, 24 h

The synthesised compounds were characterised using ^1H, ^{13}C, 2D NMR spectroscopy and mass spectrometry. For example, the ^1H NMR spectrum of **5d**, the 6-chloro derivative, showed the presence of the NH-1 and NH-11 proton resonances at δ_H 12.12 and 11.69 respectively. Two broadened singlets attributed to NH-13a and NH-13b could be seen at δ_H 8.35 and 8.04. The H-4 and H-9 (imine proton) resonances were present as singlets at δ_H 8.70 and 8.25 respectively. The H-5, H-7 and H-8 proton resonances on the benzene portion of the quinoline ring showed a nice splitting pattern, consisting of two doublets at δ_H 7.67 (2.3 Hz, H-5) and δ_H 7.31 (8.8 Hz) and a double doublet at δ_H 7.53 (8.8, 2.3 Hz, H-7).

The ^{13}C NMR spectrum showed the presence of 11 carbon resonances consisting of 5 methine, 4 fully substituted aromatic carbons, a quinolone carbonyl resonance at δ_C 178.1 and a

thiosemicarbazone carbon at δ_C160.7. H-8 showed HMBC correlations to both C-6 and C-4a at δ_C 126.5 and 120.3 respectively, while H-9 showed an HMBC correlation to C-3 at δ_C 126.1. H-4, H-5 and H-7 all showed HMBC correlations to C-8a. C-2 at δ 160.7 was confirmed by HMBC correlations to H-4 and H-9.

Antibacterial study

The synthesised quinolone thiosemicarbazones were tested for their antibacterial activity against two Gram +ve (*S. aureus* and methicillin resistant *S. aureus* (MRSA)) and four Gram –ve strains (*P. aeruginosa*, *K. pneumoniae*, *E. coli* and *S. typhimurium*). In general, the *N*-phenyl thiosemicarbazones showed better activity than the *N*-methyl or unsubstituted thiosemicarbazone derivatives, with three of the compounds, **5l** (6-methyl), **5m** (6-bromo) and **5n** (6-chloro) showing activity of < 1 mM against MRSA, being just one order of magnitude less active than that of levofloxacin, a known antibacterial drug. However, all the other quinolones had MBC values with 2 orders of magnitude lower than levofloxacin against the strains tested with the exception of *E. coli* where the activity of the compounds were 4-5 orders of magnitude lower (**Table 3.1**). Comparing the five active compounds, **5l** and **5n** showed the broadest spectrum of activity, being active at < 10 mM against 5 of the 6 strains tested against. Both these compounds had a *N*-phenyl thiosemicarbazone group, with **5l** containing a methyl group at C-6, and **5n** a chloro group at the same position.

Our results were better than that reported in the literature for similar compounds. Most derivatives of 8-hydroxyquinoline thiosemicarbazones were inactive against *S. aureus*, *P. aeruginosa* and *Micrococcus luteus*, however all were active with small zones of inhibition in disc diffusion assays against *Serratia marcescens* and derivatisation of the thiosemicarbazone

moiety led to moderate activity against *Bacillus cereus*, *E. coli* and *S. marcescens* (Abdel-Moty et al., 2005; Hussein et al., 2009).

Table 3.1 Antibacterial activity of the quinolone-thiosemicarbazone hybrids (MBC in mM)

Compound			Gram +ve		Gram -ve			
No.	**R_1**	**R_2**	**MRSA**	***S.a***	***P.a***	***S.t***	***E.c***	***K.p***
5g	CH_3	NMTSC	18.24	9.12	9.12	9.12	4.561	36.49
5l	CH_3	PHYTSC	0.93	3.72	1.86	0.93	29.75	7.44
5m	Br	PHYTSC	0.80	3.13	25.00	6.25	6.25	25.00
5n	Cl	PHYTSC	0.88	1.76	7.02	7.02	7.02	28.08
5o	F	PHYTSC	14.70	1.84	29.40	14.70	14.70	29.41
Levofloxacin			0.0865	0.0216	0.345	0.0216	0.00034	0.0216

Molecular Docking

Quinolones such as ciprofloxacin bind to DNA gyrase (Jena et al., 2015), a type II topoisomerase enzyme, to inhibit negative supercoiling of DNA by preventing ligation of dsDNA breaks. The structure of the DNA gyrase complex was used as a starting point for modelling. The docking of substituted quinolone thiosemicarbazones was carried out using the potential binding sites of the receptor DNA gyrase. The main goal was to understand the ability of the synthesised molecules to interact with the target.

The docking study with the assistance of lig-plot (**Figure 3.1** to **Figure 3.3**) indicated that compound **5g** (**Figure 3.3**) showed a similar binding mode to Ciprofloxacin, a quinolone reported to inhibit negative supercoiling of DNA by preventing ligation of dsDNA breaks. The corresponding binding affinity for each antagonist is displayed in **Table 3.2**. The test

compounds show a high –ve binding affinity and hence suggest promising antimicrobial activity. The four compounds docked into the active site of DNA gyrase all showed a similar binding affinity and there were no notable differences between compounds with different substituents. However, improvements to these structures are still needed since none of the quinolone derivatives had more binding affinity than the reference ligand, levofloxacin. Of the five compounds, **5n** showed the highest binding affinity and this structure could be used as a starting point for modification. These results are encouraging due to a number of different hydrogen interactions observed from the different complexes. These interactions could provide a mechanism of action for the tested compounds and indicate the possibility of developing novel antibacterial agents targeting an early step in peptidoglycan biosynthesis.

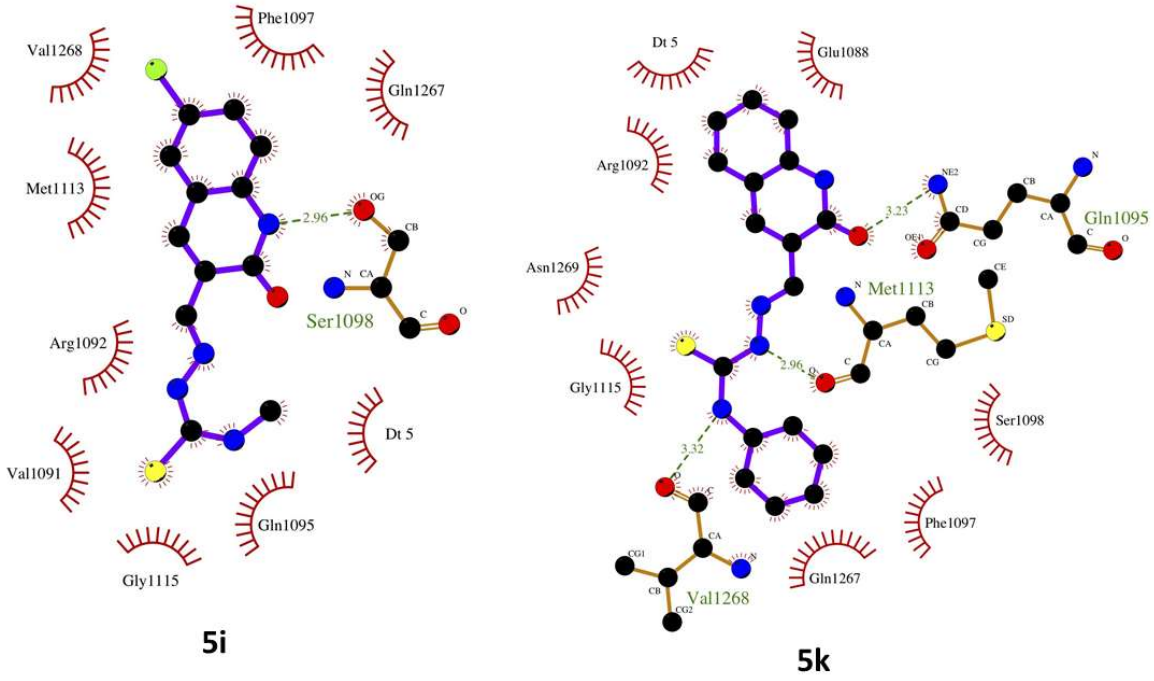


Figure 3.1 3D Ligand residue interactions for **5i** and **5k** against DNA Gyrase. Oxygen = red spheres, nitrogen = blue spheres, carbon = black spheres, sulfur = yellow spheres, chlorine = green sphere, red arcs = hydrophobic interactions and green dashed lines = hydrogen bonds.

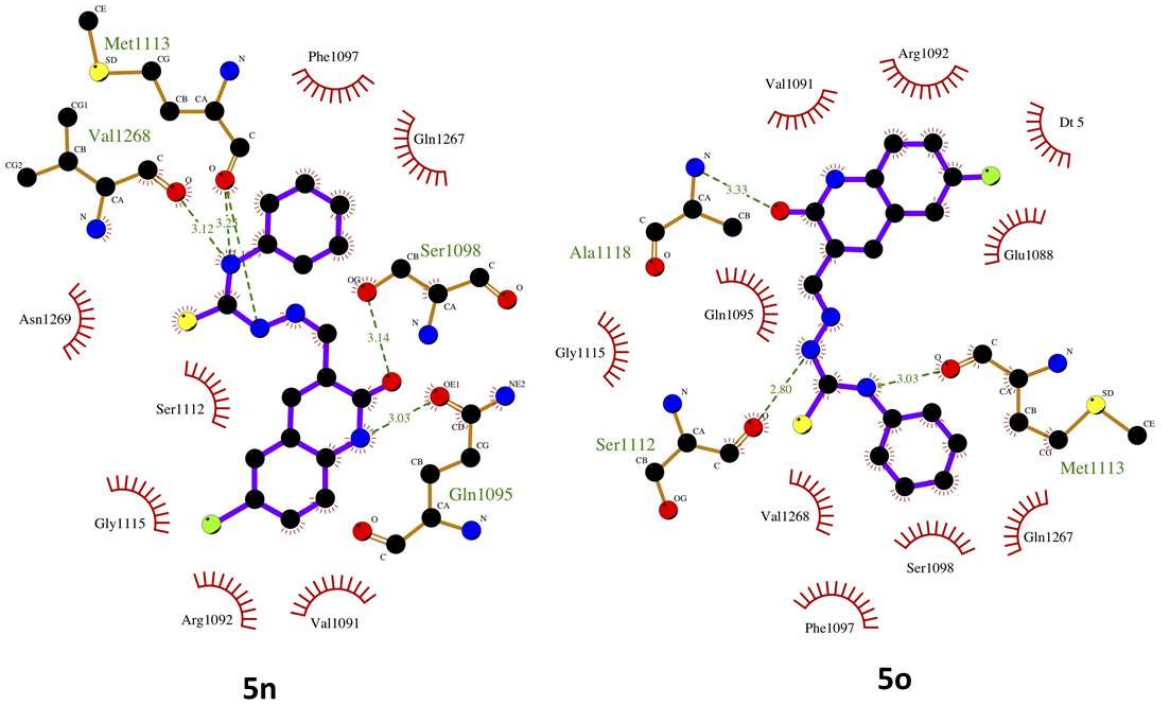


Figure 3.2 3D Ligand residue interactions for **5n** and **5o** against DNA Gyrase. Oxygen = red spheres, nitrogen = blue spheres, carbon = black spheres, sulfur = yellow spheres, chlorine (**5n**) and fluorine (**5o**) = green sphere, red arcs = hydrophobic interactions and green dashed lines = hydrogen bonds.

Table 3.2 Binding affinities for the quinolone-thiosemicarbazone hybrids against DNA Gyrase

Inhibitor	**Binding affinity (kcal/mole)**
5g	-9.6
5k	-11.1
5l	-10.9
5n	-11.3
5o	-9.1
Levofloxacin	-13.4

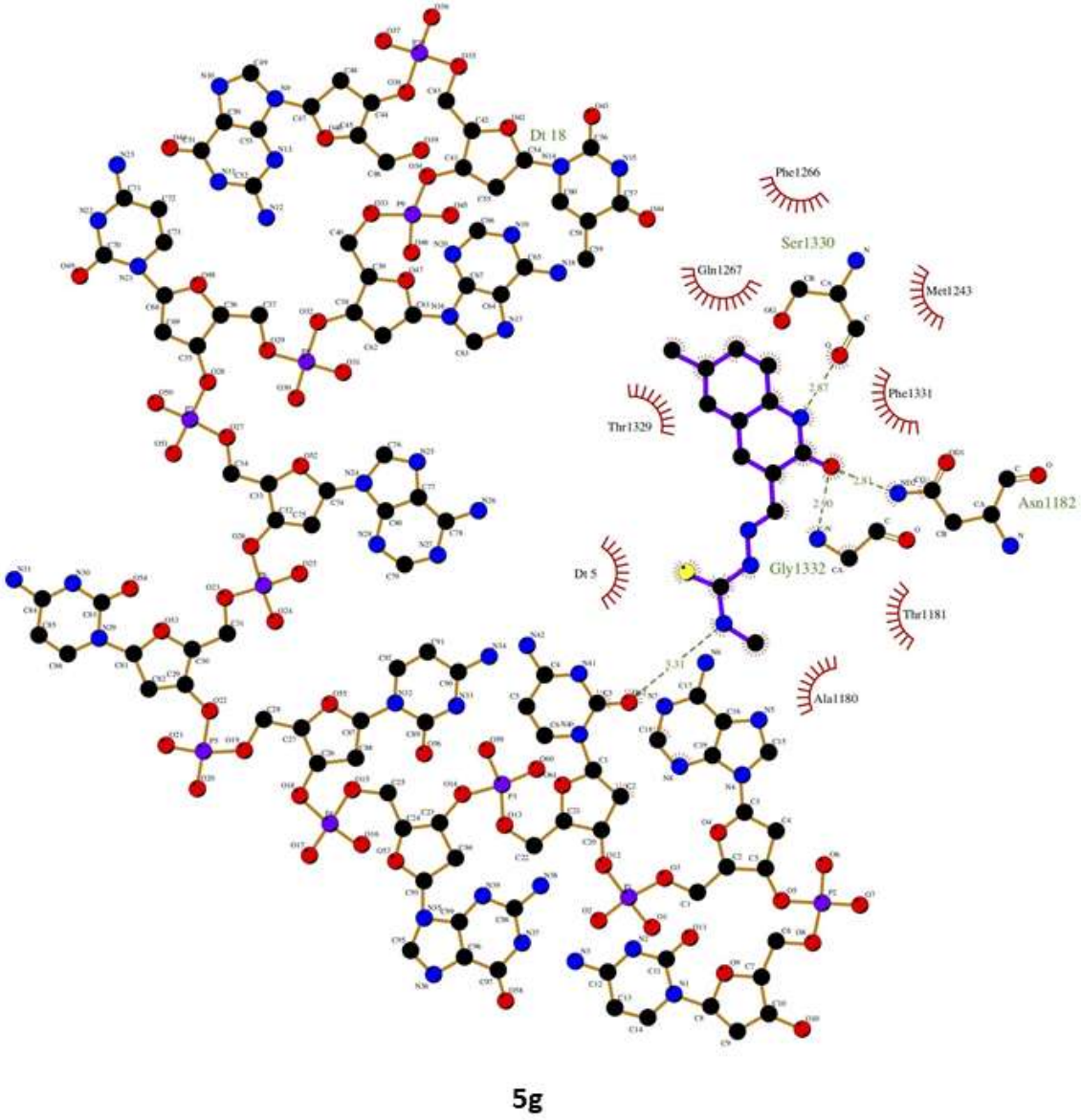


Figure 3.3 3D Ligand residue interactions for **5g** against DNA Gyrase. Oxygen = red spheres, nitrogen = blue spheres, carbon = black spheres, sulfur = yellow spheres, red arcs = hydrophobic interactions and green dashed lines = hydrogen bonds**.**

3.3 Experimental

General

Reagents and solvents were supplied by Sigma Aldrich, via Capital Laboratories, South Africa. Silica gel 60 (63-200 μm) was used for column chromatography. Alumina-backed silica gel

40 F_{254} plates (Merck) were used for TLC and visualised under a UV lamp at 254 nm. Melting points were determined on an Electrothermal IA 9100 Digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 instrument with Universal ATR sampling accessory. ^{1}H, ^{13}C and 2D NMR spectra were acquired on Bruker Avance^{III} 400 or 600 MHz spectrometers (Bruker Co., Karlsruhe, Germany) at frequencies of 400.22 MHz for ^{1}H and 100.63 MHz for ^{13}C and referenced to TMS. High-resolution mass spectral data was acquired on a Waters Micromass LCT Premier TOF-MS instrument. UV analysis was carried out on a UV-VIS-NIR Shimadzu series 3600 spectrophotometer using methanol as a solvent.

Acetylation of anilines

Substituted anilines (0.1 mol), acetic anhydride (10 mL, 0.1 mol) and glacial acetic acid (10 mL, 0.175 mol) were added and subject to gentle reflux for one hour. Upon completion, the mixture was poured into ice-water where the acetylated products (**2a-e**) precipitated out of solution in yields of between 94-100%. The acetylated compounds were recrystallised using acetic acid-water (1:2).

*General procedure for synthesis of 2-chloroquinoline-3-carbaldehydes (**3a-e**)*

The different derivatives were synthesised using the method in Toth et al. (2006). Phosphoryl chloride (32.2 mL; 0.35 mol) and the respective acetanilide (0.05 mol) was added slowly to dry DMF (9.6 mL; 0.125 mol) at 0 ºC under inert conditions in a two-necked round bottom flask fitted with a calcium chloride drying tube. The mixture was heated under reflux for 24 h at 82 °C and the reaction monitored by TLC. Upon completion, the mixture was cooled and added to 300 mL ice water, maintaining the temperature between 0-10 °C whilst stirring for 1 hour.

The pale yellow products were washed with 100 mL water and left to dry and in yields ranging from 43-74%.

*General procedure for the synthesis of 3-formyl-2-quinolone derivatives (**4a-e**)*

The substituted 2-chloroquinoline-3-carbaldehyde (1 mmol) was heated under reflux with 10 mL 70% acetic acid for 4-6 h and the reaction monitored by TLC. On completion, the reaction was cooled and a precipitate formed, which was filtered, washed with water and dried under vacuum to yield the 3-formyl-2-quinolone derivatives in yields of 68-94%.

*General procedure for the synthesis of N-methyl, N-phenyl and thiosemicarbazone derivatives (**5a-o**)*

The thiosemicarbazone derivatives were formed according to the method in Bisceglie et al. (2015). Substituted thiosemicarbazides, *N*-methyl thiosemicarbazides or *N*-phenylthiosemicarbazides (1.0 mmol) dissolved in methanol (80 mL) were added to an equivalent amount of quinolone-3-carboxyaldehyde (200 mg; 1.0 mmol) and the solution stirred at room temperature for 24 h. Upon completion, the flask was placed in an ice bath, where a solid formed, which was filtered, washed with ethanol and dried.

*(E)-2-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazine-1-carbothioamide (**5a**)* yellow solid (74% yield), mp 296-298 °C, IR υ_{max} (cm^{-1}): 3369 (Ar-N-H), 3267, 3148 (N-H), 1642 (C=O), 1519 (C=N), 824 (C=S); UV λ_{max} (MeOH) nm (log ε) 242 (4.11), 382 (4.15); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 11.99 (1H, s, H-1), 11.63 (1H, s, H-11), 8.74 (1H, s, H-4), 8.27 (2H, bs, H-9, H-13a), 8.09 (1H, bs, H-13b), 7.65 (1H, dd, *J* = 8.4, 1.0 Hz, H-5), 7.51 (1H, td, *J* = 8.4, 1.4 Hz, H-6), 7.31 (1H, d, *J* = 8.2 Hz, H-8), 7.31 (1H, td, *J* = 8.2, 1.0 Hz, H-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 178.0 (C-12), 160.9 (C-2), 138.8 (C-8a), 136.8 (C-9), 135.1 (C-4),

130.9 (C-6), 128.5 (C-5), 125.2 (C-3), 122.4 (C-7), 119.2 (C-4a), 115.1 (C-8), HRMS: (*m/z*) 269.0475 [M+Na] (calculated for $C_{11}H_{10}N_4ONaS$, 269.0473).

*(E)-2-((6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazine-1-carbothioamide (**5b**)* yellow solid (90% yield), mp 287-289 °C, IR υ_{max} (cm^{-1}): 3273 (Ar-N-H), 3155, 3015 (N-H), 1651 (C=O), 1531 (C=N), 810 (C=S); UV λ_{max} (MeOH) nm (log ε) 223 (3.90), 245 (3.82); 351 (4.10); 388 (4.22); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 11.92 (1H, s, H-1), 11.62 (1H, s, H-11), 8.69 (1H, s, H-4), 8.29 (1H, bs, H-13a), 8.26 (1H, s, H-9), 8.07 (1H, bs, H-13b), 7.42 (1H, bs, H-5), 7.35 (1H, dd, *J* = 8.4, 1.7 Hz, H-7), 7.22 (1H, d, *J* = 8.4 Hz, H-8), 2.34 (3H, s, H-6a); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 178.0 (C-12), 160.8 (C-2), 136.9 (C-8a), 136.8 (C-9), 134.8 (C-4), 132.3 (C-7), 131.3 (C-6), 128.8 (C-5), 125.2 (C-3), 119.1 (C-4a), 115.1 (C-8), 20.4 (C-6a).

*(E)-2-((6-bromo-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazine-1-carbothioamide (**5c**)* yellow solid (69% yield), mp 281-283 °C, IR υ_{max} (cm^{-1}): 3350 (Ar-N-H), 3214, 3146 (N-H), 1642 (C=O), 1514 (C=N), 821 (C=S); UV λ_{max} (MeOH) nm (log ε): 245 (4.37), 352 (4.05), 389 (4.17); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.13 (1H, s, H-1), 11.70 (1H, s, H-11), 8.72 (1H, s, H-4), 8.38 (1H, bs, H-13a), 8.24 (1H, s, H-9), 8.03 (1H, bs, H-13b), 7.80 (1H, d, *J* = 2.0 Hz, H-5), 7.66 (1H, dd, *J* = 8.8, 2.0 Hz, H-7), 7.26 (1H, d, *J* = 8.8 Hz, H-8); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 184.1 (C-12), 160.7 (C-2), 137.8 (C-8a), 136.1 (C-9), 133.7 (C-4), 133.3 (C-7), 130.1 (C-5), 126.5 (C-3), 120.9 (C-6), 117.3 (C-8), 113.8 (C-4a), HRMS: (*m/z*) 346.9578 [M+Na] (calculated for $C_{11}H_9N_4ONaSBr$, 346.9578).

(E)-2-((6-chloro-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazine-1-carbothioamide ***(5d)*** yellow solid (94% yield), mp 283-285 °C, IR υ_{max} (cm^{-1}): 3259 (Ar-N-H), 3192, 3151 (N-H), 1645 (C=O), 1519 (C=N), 824 (C=S); UV λ_{max} (MeOH) nm (log ε): 242 (4.43), 352 (4.08), 387 (4.20); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.12 (1H, s, H-1), 11.69 (1H, s, H-11), 8.70 (1H, s, H-4), 8.35 (1H, s, H-13a), 8.25 (1H, s, H-9), 8.04 (1H, s, H-13b), 7.67 (1H, d, *J* = 2.3 Hz, H-5), 7.54 (1H, dd, *J* = 8.8, 2.3 Hz, H-7), 7.32 (1H, d, *J* = 8.8 Hz, H-8); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 178.1 (C-12), 160.7 (C-2), 137.5 (C-8a), 136.2 (C-9), 133.8 (C-4), 130.7 (C-7), 127.1 (C-5), 126.5 (C-6), 126.1 (C-3), 120.3 (C-4a), 117.1 (C-8), HRMS: (*m/z*) 303.0086 [M+Na] (calculated for $C_{11}H_9N_4ONaSCl$, 303.0083).

(E)-2-((6-fluoro-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazine-1-carbothioamide ***(5e)*** yellow solid (94% yield), mp 284-286 °C, IR υ_{max} (cm^{-1}): 3273 (Ar-N-H), 3144, 3048 (N-H), 1642 (C=O), 1527 (C=N), 806 (C=S); UV λ_{max} (MeOH) nm (log ε): 223 (4.21), 351 (3.76), 387 (3.89); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.07 (1H, s, H-1), 11.69 (1H, s, H-11), 8.72 (1H, s, H-4), 8.35 (1H, bs, H-13a), 8.26 (1H, s, H-9), 8.05 (1H, bs, H-13b), 7.45-7.39 (2H, m, H-5, H-7), 7.34 (1H, dd, *J* = 8.6, 4.8 Hz, H-8);^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 178.0 (C-12), 160.5 (C-2), 157.0 (d, *J* = 237.3 Hz, C-6), 136.2 (C-9), 136.2 (C-8a), 133.9 (C-4), 126.4 (C-3), 119.7 (d, *J* = 8.9 Hz, C-4a), 118.9 (d, *J* = 24.5 Hz, C-7), 116.9 (d, *J* = 8.5 Hz, C-8), 112.6 (d, *J* = 22.4 Hz, C-5), HRMS: (*m/z*) 287.0380 [M+Na] (calculated for $C_{11}H_9N_4OFNaS$, 287.0379).

(E)-N-methyl-2-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazine-1-carbothioamide ***(5f)*** yellow solid (78% yield), mp 285-287 °C, IR υ_{max} (cm^{-1}) 3341 (Ar-N-H), 3156 (N-H), 1641 (C=O), 1526, (C=N), 809 (C=S), UV λ_{max} (MeOH) nm (log ε): 233 (4.30), 385 (4.06); ^{1}H NMR

(DMSO-d_6, 400 MHz) δ_H 12.02 (1H, s, H-1), 11.69 (1H, s, H-11), 8.67 (1H, s, H-4), 8.59 (1H, q, *J* = 4.5 Hz, H-13), 8.28 (1H, s, H-9), 7.66 (1H, d, *J* = 7.6 Hz, H-5), 7.52 (1H, td, *J* = 8.2, 0.9 Hz, H-7), 7.32 (1H, d, *J* = 8.2 Hz, H-8), 7.23 (1H, t, *J* = 7.6, Hz, H-6), 3.05 (3H, d, *J* = 4.5 Hz, H-14); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.7 (C-12), 160.9 (C-2), 138.8 (C-8a), 136.3 (C-9), 134.6 (C-4), 130.9 (C-6), 128.4 (C-7), 125.5 (C-3), 122.4 (C-5), 119.1 (C-4a), 115.2 (C-8), 30.8 (C-14); HRMS: (*m/z*) 283.0632 [M+Na]; (calculated for $C_{12}H_{12}N_4ONaS$, 283.0630).

*(E)-N-methyl-2-((6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazine-1-carbothioamide (**5g**)* yellow solid (88% yield), mp 340-342 °C, IR υ_{max} (cm^{-1}): 3342 (Ar-N-H), 3245, 3161 (N-H), 1648 (C=O), 1518, (C=N), 838 (C=S); UV λ_{max} (MeOH) nm (log ε): 245 (4.36), 390 (4.22); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 11.94 (1H, s, H-1), 11.68 (1H, s, H-11), 8.61 (1H, s, H-4), 8.57 (1H, q, *J* = 4.6 Hz, H-13), 8.27 (1H, s, H-9), 7.43 (1H, bs, H-5), 7.35 (1H, dd, *J* = 8.4, 1.7 Hz, H-7), 7.23 (1H, d, *J* = 8.4 Hz, H-8), 3.05 (3H, d, *J* = 4.6 Hz, H-14), 2.35 (3H, s, H-6a); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.7 (C-12), 160.8 (C-2), 136.9 (C-8a), 136.4 (C-9), 134.4 (C-4), 132.3 (C-7), 131.3 (C-6), 127.7 (C-5), 125.4 (C-3), 119.1 (C-4a), 115.1 (C-8), 30.8 (C-14), 20.4 (C-6a); HRMS: (*m/z*) 297.0785 [M+Na] (calculated for $C_{13}H_{14}N_4ONaS$, 297.0786).

*(E)-2-((6-bromo-2-oxo-1,2-dihydroquinolin-3-yl)methylene)-N-methylhydrazine-1-carbothioamide (**5h**)* yellow solid (72% yield), mp 332-334 °C, IR υ_{max} (cm^{-1}): 3379 (Ar-N-H), 3300, 3148 (N-H), 1652 (C=O), 1519, (C=N), 816 (C=S); UV λ_{max} (MeOH) nm (log ε): 247 (4.44), 392 (4.20); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.15 (1H, s, H-1), 11.76 (1H, s, H-11), 8.63 (1H, s, H-4), 8.55 (1H, q, *J* = 4.6 Hz, H-13), 8.25 (1H, s, H-9), 7.83 (1H, d, *J* = 2.2 Hz, H-5), 7.67 (1H, dd, *J* = 8.8, 2.2 Hz, H-7), 7.27 (1H, d, *J* = 8.8 Hz, H-8), 3.05 (3H, d, *J* =

4.6 Hz, H-14); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.8 (C-12), 160.7 (C-2), 137.8 (C-8a), 135.7 (C-9), 133.3 (C-7), 133.2 (C-4), 130.1 (C-5), 126.7 (C-3), 120.8 (C-6), 117.4 (C-8), 113.8 (C-4a), 30.8 (C-14); HRMS: (*m/z*) 336.9761 [M-H] (calculated for $C_{12}H_{10}N_4OSBr$, 336.9759).

*E)-2-((6-chloro-2-oxo-1,2-dihydroquinolin-3-yl)methylene)-N-methylhydrazine-1-carbothioamide (**5i**)* yellow solid (78% yield), mp 324-326 °C, IR υ_{max} (cm^{-1}): 3373 (Ar-N-H), 3165 (N-H), 1647 (C=O), 1520 (C=N), 818 (C=S); UV λ_{max} (MeOH) nm (log ε): 245 (4.41), 390 (4.29); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.15 (1H, s, H-1), 11.76 (1H, s, H-11), 8.63 (1H, s, H-4), 8.56 (1H, q, *J* = 4.6 Hz, H-13), 8.25 (1H, s, H-9), 7.69 (1H, d, *J* = 2.3 Hz, H-5), 7.56 (1H, dd, *J* = 8.8, 2.3 Hz, H-7), 7.33 (1H, d, *J* = 8.8 Hz, H-8), 3.05 (3H, d, *J* = 4.6 Hz, H-14); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.8 (C-12), 160.7 (C-2), 137.5 (C-8a), 135.8 (C-9), 133.4 (C-4), 130.6 (C-7), 127.0 (C-5), 126.8 (C-6), 126.1 (C-3), 120.3 (C-4a), 117.1 (C-8), 30.8 (C-14); HRMS: (*m/z*) 293.0273 [M-H] (calculated for $C_{12}H_{10}N_4OSCl$, 293.0264).

*E)-2-((6-fluoro-2-oxo-1,2-dihydroquinolin-3-yl)methylene)-N-methylhydrazine-1-carbothioamide (**5j**)* yellow solid (79% yield), mp 297-299 °C, IR υ_{max} (cm^{-1}): 3399 (Ar-N-H), 3172 (N-H), 1663 (C=O), 1501 (C=N), 825 (C=S); UV λ_{max} (MeOH) nm (log ε): 229 (4.31), 280 (4.37); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.09 (1H, s, H-1), 11.74 (1H, s, H-11), 8.62 (1H, s, H-4), 8.58 (1H, q, *J* = 4.5 Hz, H-13), 8.27 (1H, s, H-9), 7.44 (1H, d, *J* = 8.6 Hz, H-5), 7.43 (1H, td, *J* = 8.6, 2.8 Hz, H-7); 7.34 (1H, dd, *J* = 8.6, 4.8 Hz, H-8), 3.05 (3H, d, *J* = 4.5 Hz, H-14); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.8 (C-12), 160.6 (C-2), 157.1 (d, *J* = 237.2 Hz, C-6), 135.9 (C-9), 135.6 (C-8a), 133.7 (d, *J* = 3.8 Hz, C-4), 126.7 (C-3), 119.7 (d, *J* = 8.9 Hz, C-4a), 118.9 (d, *J* = 24.6 Hz, C-5), 117.1 (d, *J* = 8.5 Hz, C-8), 112.7 (d, *J* = 22.4 Hz, C-7), 30.8 (C-14); HRMS: (*m/z*) 301.0529 [M+Na] (calculated for $C_{12}H_{11}N_4OFNaS$, 301.0535).

(E)-2-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)-N-phenylhydrazine-1-carbothioamide ***(5k)*** yellow solid (94%), mp 331-333 °C, IR υ_{max} (cm^{-1}): 3306 (Ar-N-H), 3164 (N-H), 1650 (C=O), 1529 (C=N), 815 (C=S); UV λ_{max} (MeOH) nm (log ε): 238 (4.36), 388 (4.36); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.04 (2H, s, H-1/H-11), 10.16 (1H, s, H-13), 8.87 (1H, s, H-4), 8.40 (1H, s, H-9), 7.68 (1H, d, *J* = 7.7 Hz, H-8), 7.58 (2H, d, *J* = 7.6 Hz, H-2′/6′), 7.53 (1H, td, *J* = 7.7, 1.2 Hz, H-7), 7.39 (2H, t, *J* = 7.6 Hz, H-3′/5′), 7.33 (1H, d, *J* = 8.2 Hz, H-5), 7.20-7.25 (2H, m, H-4′, H-6); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 176.0 (C-12), 160.9 (C-2), 138.98 (C-8a), 138.96 (C-1′), 137.5 (C-9), 135.5 (C-4), 131.1 (C-7), 128.5 (C-5), 128.1 (C-3′/5′), 126.1 (C-2′/6′), 125.5 (C-4′), 125.1 (C-3), 122.4 (C-6), 119.1 (C-4a), 115.2 (C-8), HRMS: (*m/z*) 345.0777 [M+Na] (calculated for $C_{17}H_{14}N_4ONaS$, 345.0786).

(E)-2-((6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)methylene)-N-phenylhydrazine-1-carbothioamide ***(5l)*** yellow solid (96% yield), mp 344-346 °C; IR υ_{max} (cm^{-1}): 3312 (Ar-N-H), 3140 (N-H), 1651 (C=O), 1530 (C=N), 823 (C=S); UV λ_{max} (MeOH) nm (log ε): 246 (4.31), 357 (4.15), 393 (4.26); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.03 (1H, s, H-1), 11.97 (1H, s, H-11), 10.14 (1H, s, H-13), 8.80 (1H, s, H-4), 8.40 (1H, s, H-9), 7.58 (2H, d, *J* = 7.7 Hz, H-2′/6′), 7.46 (1H, bs, H-5), 7.35-7.41 (3H, m, H-3′/4′/5′); 7.21-7.25 (2H, m, H-7, H-8), 2.34 (3H, s, H-6a); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 176.0 (C-12), 160.9 (C-2), 138.9 (C-1′), 137.6 (C-9), 137.1 (C-8a), 135.3 (C-4), 132.4 (C-7), 131.3 (C-6), 128.1 (C-3′/5′), 127.9 (C-5), 125.9 (C-2′/6′), 125.5 (C-4′), 125.0 (C-3), 119.1 (C-4a), 115.1 (C-8), 20.4 (C-6a); HRMS: (*m/z*) 359.0943 [M+Na] (calculated for $C_{18}H_{16}N_4ONaS$, 359.0943).

*(E)-2-((6-bromo-2-oxo-1,2-dihydroquinolin-3-yl)methylene)-N-phenylhydrazine-1-carbothioamide (**5m**)* yellow solid (82% yield), mp 339-341 °C; IR υ_{max} (cm^{-1}): 3323 (Ar-N-H), 3141 (N-H), 1647 (C=O), 1526 (C=N), 815 (C=S); UV λ_{max} (MeOH) nm (log ε): 220 (4.37), 247 (4.36), 393 (4.21); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.17 (1H, s, H-1), 12.11 (1H, s, H-11), 10.10 (1H, s, H-13), 8.81 (1H, s, H-4), 8.37 (1H, s, H-9), 7.85 (1H, d, *J* = 2.2 Hz, H-5), 7.67 (1H, dd, *J* = 8.8, 2.2 Hz, H-7), 7.59 (2H, d, *J* = 7.6 Hz, H-2'/6'), 7.40 (2H, t, *J* = 7.6 Hz, H-3'/5'), 7.35 (1H, d, *J* = 8.8 Hz, H-8), 7.24 (1H, t, *J* = 7.6 Hz, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 176.1 (C-12), 160.7 (C-2), 138.8 (C-1'), 137.6 (C-8a), 136.8 (C-9), 134.1 (C-4), 133.4 (C-7), 130.2 (C-5), 128.2 (C-3'/5'), 126.3 (C-3), 125.9 (C-2'/6'), 125.5 (C-4'), 120.8 (C-6), 117.4 (C-8), 113.8 (C-4a); HRMS: (*m/z*) 422.9888 [M+Na] (calculated for $C_{17}H_{13}N_4ONaSBr$, 422.9891).

*(E)-2-((6-chloro-2-oxo-1,2-dihydroquinolin-3-yl)methylene)-N-phenylhydrazine-1-carbothioamide (**5n**)* yellow solid (86 % yield), mp 325-327 °C; IR υ_{max} (cm^{-1}): 3315 (Ar-N-H), 3146 (N-H), 1650 (C=O), 1527 (C=N), 819 (C=S); UV λ_{max} (MeOH) nm (log ε): 230 (4.17), 245 (4.25), 393 (4.12); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.17 (1H, s, H-1), 12.10 (1H, s, H-11), 10.11 (1H, s, H-13), 8.81 (1H, s, H-4), 8.37 (1H, s, H-9), 7.71 (1H, d, *J* = 2.4 Hz, H-5), 7.60 (2H, d, *J* = 7.6 Hz, H-2'/6'), 7.56 (1H, dd, *J* = 8.8, 2.4 Hz, H-7), 7.40 (2H, t, *J* = 7.6 Hz, H-3'/5'), 7.34 (1H, d, *J* = 8.8 Hz, H-8), 7.24 (1H, t, *J* = 7.6 Hz, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 176.1 (C-12), 160.7 (C-2), 138.9 (C-1'), 137.6 (C-8a), 136.9 (C-9), 134.2 (C-4), 130.8 (C-7), 128.2 (C-3'/5'), 127.2 (C-5), 126.4 (C-3), 126.1 (C-4a), 125.9 (C-2'/6'), 125.5 (C-4'), 120.3 (C-6); 117.1 (C-8), HRMS: (*m/z*) 379.0394 [M+Na] (calculated for $C_{17}H_{13}N_4ONaSCl$, 379.0396).

*(E)-2-((6-fluoro-2-oxo-1,2-dihydroquinolin-3-yl)methylene)-N-phenylhydrazine-1-carbothioamide (**5o**)* yellow solid (90% yield), mp 296-298 °C, IR υ_{max} (cm^{-1}): 3312 (Ar-N-H), 3156 (N-H), 1650 (C=O), 1531 (C=N), 826 (C=S); UV λ_{max} (MeOH) nm (log ε): 223 (4.42), 351 (4.45), 387 (4.46); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 12.12 (1H, s, H-1), 12.09 (1H, s, H-11), 10.14 (1H, s, H-13), 8.81 (1H, s, H-4), 8.39 (1H, s, H-9), 7.58 (2H, d, *J* = 7.6 Hz, H-2′/6′), 7.34-7.46 (5H, m, H-5, H-7, H-8, H-3′/5′), 7.23 (1H, t, *J* = 7.6, Hz, H-4′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 176.1 (C-12), 160.6 (C-2), 157.1 (d, *J* = 237.4 Hz, C-6), 138.9 (C-1′), 137.0 (C-9), 135.7 (C-8a), 134.5 (d, *J* = 3.4 Hz, C-4), 128.1 (C-3′/5′), 126.4 (C-3), 125.9 (C-2′/6′), 125.5 (C-4′), 119.7 (d, *J* = 8.9 Hz, C-4a), 119.2 (d, *J* = 24.7 Hz, C-7), 117.1 (d, *J* = 8.1 Hz, C-8), 112.8 (d, *J* = 22.4 Hz, C-5), HRMS: (*m/z*) 363.0686 [M+Na] (calculated for $C_{17}H_{13}N_4OFNaS$, 363.0692).

Antibacterial Activity

The thiosemicarbazones and its derivatives were tested for antibacterial activity against two Gram +ve strains, *S. aureus* ATCC 25923 *(S.a)*, *S. aureus* Rosenbach ATCC BAA-1683 (methicillin resistant *S. aureus,* MRSA*)* and four Gram -ve bacterial strains (*P. aeruginosa* ATCC 27853 *(P.a)*, *K. pneumoniae* ATCC 31488 *(K.p), E. coli* ATCC 25922 *(E.c)* and *S. typhimurium ATCC 14026 (S.t)).* The compounds were first subject to a preliminary screen using the disc diffusion assay (Andrews, 2001) and based on their activity, selected compounds were chosen to determine their minimum bactericidal concentration (MBC).

The different bacterial strains were grown in Nutrient Broth (Biolab, South Africa) at 37ºC in a shaking incubator at 115 rpm for 18 hours. The bacteria were diluted with sterile distilled water to achieve a final concentration equivalent to a 0.5 McFarland's standard using a McFarland's densitometer. Mueller-Hinton agar plates (Biolab, South Africa) were lawn

inoculated with the bacteria using a throat swab. A 5 µL sample of each compound (10 mg mL^{-1} in 100% DMSO) was placed onto the Mueller-Hinton plates in triplicate and incubated at 37 °C for 18 hours. After the incubation period, the plates were examined for activity, which was indicated by zones of inhibition around the area where the sample was placed. Those compounds that showed broad spectrum activity were selected for the MBC assay.

The MBCs were determined in triplicate using a modification of the Broth Dilution Method (Kalhapure et al., 2014). A 10 mg sample of the test compounds was dissolved in 1 mL DMSO and serially diluted in triplicate. Mueller-Hinton agar plates were lawn inoculated with the different bacterial strains which were prepared as described above. A 5 µL sample of each dilution of the selected compounds was spotted onto the Mueller-Hinton agar plates and incubated for 24 h at 37ºC. The experiment was conducted in triplicate and averaged. The MBC was the lowest concentration that showed a zone of inhibition around the area where the sample was placed.

Molecular Docking

The crystal structure (PDB ID: 2XCT) of the DNA gyrase complex was taken from the Protein from the RSCB Protein Data Bank (Zhou et al., 2009). The missing residues were added using a graphical user interface tool of molecular modeling, Chimera (Pettersen et al., 2004) and a ligand interaction map was generated using the web version of Pose View. The docking calculations were performed using Autodock Vina (Trott and Olsen, 2010). During docking, Geister partial chargers were assigned and the Autodock atom types were defined using the Autodock Graphical user interface supplied by MGL tools (Sanner, 1999). The docked conformations were generated using the Lamarckian Genetic Algorithm, which is considered to be one of the best docking methods available (Morris et al., 1998; Huey et al., 2007). The

files were converted into the pdbqt format required for docking using Raccoon software. The gridbox was defined using Autodock Vina with the grid parameters being X= 40, Y= 36 and Z= 24 for the dimensions and X= 54.750, Y= 39.250 and Z= -19.389 for the center grid box and the reports for each calculation were reported in (kcal mol^{-1}). This technique has been validated in previous studies (Kumalo and Soliman, 2016).

3.4 Conclusions

A series of 2-chloroquinolone-3-thiosemicarbazones (**5a-o**) were easily prepared from quinolone carbaldehyde precursors. Three compounds showed good activity against MRSA, only one order of magnitude lower than levofloxacin. Better activity was seen by those compounds with a *N*-phenyl group in the thiosemicarbazide moiety and with a halogen or methyl group at C-6 on the quinolone framework. Although the thiosemicarbazones had moderate activity, the results indicate that 6-methyl and 6-chloroquinolone *N*-phenyl thiosemicarbazone moieties could be a useful scaffold to synthesise antibacterial compounds. The DMSO was used as a blank standard and showed no activity against all the bacterial strains tested.

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Chapter 4. Synthetic Study and Biological Investigation of Quinoline Tetrazolo Phenylhydrazine Hybrids

***** The compounds referred to in the chapter are referred to elsewhere in the thesis with **C** preceding the number of the compound. For example **5a** is referred to as **C-5a**.

Abstract

A small library of fifteen quinoline tetrazolo phenylhydrazones, thirteen of which were new, were synthesised in a four-step reaction, which involved the formation of 2-chloroquinoline-3-carbaldehydes, adding a tetrazolo ring onto the quinoline and forming phenylhydrazones at the 3-carbaldehyde. Various electron donating and withdrawing groups were substituted at C-6 on the quinoline framework and the phenylhydrazone portion was also varied with 2- and 4-fluoro substituted phenyl groups in addition to the unsubstituted phenyl group. The yields for the final step in the synthesis ranged from 55 to 96%. The synthesised compounds were characterised by extensive 2D-NMR spectroscopy and confirmed by High resolution mass spectrometry. Compounds with a 2-phenylhydrazone moiety showed moderate activity against all bacterial strains tested against with MBC values in the range of 3.26-31.22 mM.

Keywords: quinoline, tetrazolo, Vilsmeier-Haack, phenylhydrazone, antibacterial.

4.1 Introduction

Recently, antibiotic resistance has become a global concern with multi resistant infections becoming difficult to treat (Levy, 2002). Bacteria causing respiratory infection, diarrhea and urinary tract infections have now become resistant to current antibiotics. Overuse of antibiotics and unnecessary prescribing of antibiotics has contributed to this crisis. There is thus a need for the development of new antibiotics to beat the "cat and mouse game" being played with these bacteria and prevent a medical crisis.

Heterocyclic compounds containing heteroatoms such as nitrogen, oxygen and sulphur are biologically active compounds (Al-Mulla, 2017). Amongst these, quinolines have been known to possess pharmacological activities such as anti-inflammatory (Wen et al., 2015), antimicrobial (Azad et al., 2007; Chikhalia et al., 2008; Jadhav et al., 2014), anticancer (Bingul et al., 2016), analgesic and anti-asthmatic (Chabukswar et al., 2016), antimalarial (Nqoro et al., 2017), and anti-Alzheimer's activity (Mantoani et al., 2016).

Many tetrazole compounds have also shown good biological activity and reported to have anti-inflammatory, anticancer, antifungal, antimicrobial, analgesic, antimycobacterial, antidiabetic, anticonvulsant, and antihypertensive activities (Mohite and Bhaskar, 2011; Asif, 2014). Quinoline tetrazolo compounds showed good antimicrobial (Mungra et al., 2009; Kategaonkar et al., 2010; Madalageri et al., 2016), antioxidant (Uttarwa et al., 2013; Nikam et al., 2015), anti-inflammatory (Bawa and Kumar, 2009) and anticancer activtiy (Marganakop et al., 2014). Hydrazones on the other hand contain an azomethine –NHN=CH group and have been documented to have analgesic, anti-inflammatory and antiplatelet aggregration activity (Asif and Husain, 2013), as well as antitumour, antibacterial, antiviral, antihypertensive, antipyretic,

antitubercular, anticonvulsant and antidepressant activity (Singh and Raghav, 2011; Kumar et al., 2017).

Quinolines are synthesised by a variety of methods, among which the Vilsmeier-Haack reaction resulting in 2-chloroquinoline-3-carbaldehdyes is quite popular (Srivastava and Singh 2005). These molecules can be used as intermediates to a variety of other organic molecules due to the reactive chloro and carbaldehyde moieties. Tetrazolo compounds are normally fused onto heterocyclic scaffolds, usually by reacting azides with 2-chloroquinoline (Bekhit et al., 2004), and hydrazones are formed by the reaction of hydrazine or hydrazide with aldehydes and ketones (Kumar et al., 2017). A combination of these reactions result in a hybrid molecule with all three functionalities and scaffolds in a single molecule, increasing its chances of becoming a hit compound for the pharmaceutical industry.

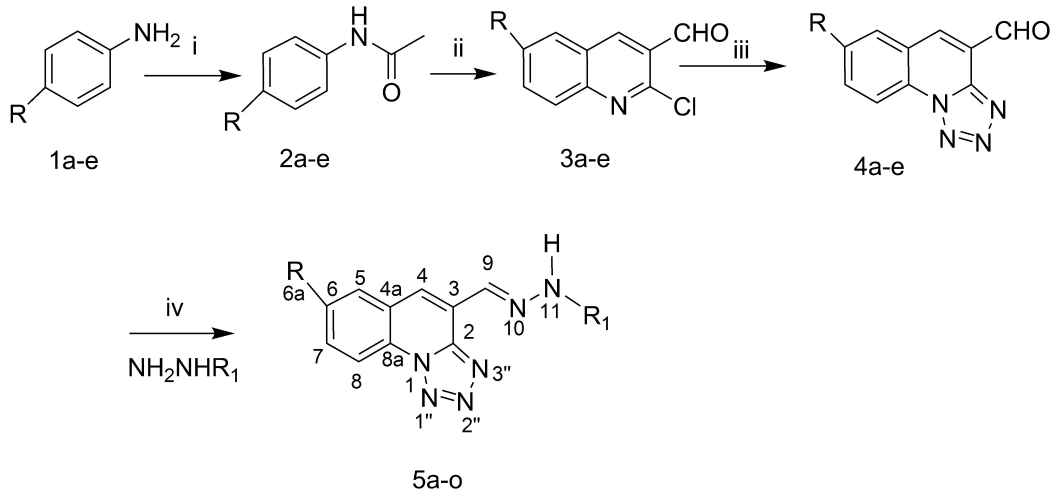
We herein report on the synthesis and antibacterial activity of a small library of quinoline tetrazolo phenylhydrazine hybrid molecules with variation on both the quinoline and hydrazone moieties.

4.2 Results and Discussion

Chemistry

A series of fifteen quinoline tetrazolo phenylhydrazones **5a-o** were synthesised in a four-step reaction, forming 2-chloroquinoline-3-carbaldehydes in the first two steps using the Vilsmeier-Haack reaction (Toth et al., 2006), then forming the tetrazolo ring onto the quinoline at the nitrogen (Ladani et al., 2009) and finally forming phenylhydrazones at the 3-carbaldehyde group (**Scheme 4.1**). The final compounds were prepared in yields ranging from 55 to 96%.

Of the fifteen compounds synthesised only **5a** and **5b** were previously synthesised. All other compounds synthesised were new.



R	R_1		R	R_1		R	R_1	
5a	H	PHYD	**5f**	H	2F-PHYD	**5k**	H	4F-PHYD
5b	Me	PHYD	**5g**	Me	2F-PHYD	**5l**	Me	4F-PHYD
5c	Br	PHYD	**5h**	Br	2F-PHYD	**5m**	Br	4F-PHYD
5d	Cl	PHYD	**5i**	Cl	2F-PHYD	**5n**	Cl	4F-PHYD
5e	F	PHYD	**5j**	F	2F-PHYD	**5o**	F	4F-PHYD

R_1 = PHYD (phenylhydrazone); 2F-PHYD (2-fluorophenylhydrazone; 4F-PHYD (4-fluorophenylhydrazone)

Scheme 4.1 Synthetic route to the phenylhydrazones **5a-o**; i. Acetic anyhdride, acetic acid; ii. DMF, $POCl_3$, 82°C; reflux 24 h; iii. NaN_3, acetic acid, ethanol; iv. Acetic acid, sodium acetate

The structures of the synthesised compounds were determined by ^1H, ^{13}C and 2D NMR as well as mass spectrometry. For example in **5a**, formation of the phenylhydrazone was indicated by the presence of the singlet H-9 resonance at δ 8.42. This H-9 resonance was differentiated from H-4 at δ8.57, since its corresponding carbon resonance, C-9 showed a HMBC correlation to the singlet resonance at δ11.08 attributed to NH-11, which also showed HMBC correlations to C-1' and C-2'/6'. Both H-4 and H-9 showed HMBC correlations to C-2 at δ 146.2. H-8 and H-5 on the quinoline ring each appeared as doublets at δ8.60 and 8.30 with J values of 7.9 Hz and H-7 and H-6 occurred as triplets at δ7.91 and 7.80 (J = 7.9 Hz). These two resonances were distinguished by an HMBC correlation from H-6 to C-4. Furthermore, H-5 and H-4 both

showed HMBC correlations to C-8a at δ 129.1 and H-9 showed an HMBC correlation to C-4a (**Figure 4.1**). The aromatic protons of the phenyl ring attached to N-11 appeared at δ 7.30 (t, H-3'/5'), 7.24 (d, H-2'/6') and 6.86 (t, H-4'). In the ^{13}C NMR spectrum it is worth noting that C-8 is more shielded at δ 116.1 than C-7, C-5 and C-6 at δ 130.5, 129.5 and 128.3 respectively.

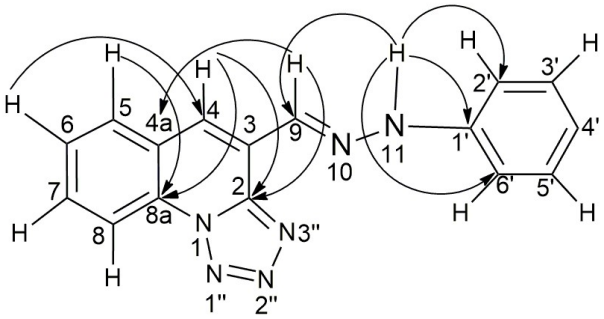


Figure 4.1 Key HMBC correlations used in the structural elucidation of **5a**

Antibacterial activity

Six bacterial strains, two Gram +ve, *Staphylococcus aureus* and *Staphylococcus aureus* Rosenbach (methicillin resistant *S. aureus* (MRSA)) and four Gram –ve strains, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhimurium* were tested against the novel derivatives synthesized to see whether the tetrazolo ring would enhance antibacterial activity. Our previous work where 2-quinolone thiosemicarbazides were synthesised showed weak activity in the mM range against the same set of bacteria. Also under investigation was the phenylhydrazone ring. Three subsets of compounds were synthesised with an unsubstituted phenylhydrazine ring (**5a-e**), 2-fluoro (**5f-j**) and 4-fluoro (**5k-o**) substitution. This was done to determine whether fluorination on the phenylhydrazone moiety would enhance antibacterial activity. Finally, the substituent at C-6 on the quinoline backbone

was varied with an electron donating methyl group and then three electron withdrawing halogens, bromine, chlorine and fluorine to compare with the unsubstituted molecule.

Preliminary disc diffusion assays were carried out to determine which compounds showed any activity against the strains used in the assays. Although the activity of some of the compounds were in the mM range, the results obtained were quite interesting. Neither the unsubstituted phenylhydrazine derivatives nor the 4-fluorophenylhydrazine derivatives showed any activity against the test strains. Only the 2-fluorophenylhydrazone tetrazolo quinolines were active in the mM range (**Table 4.1**). The exceptions were the 6-fluoroquinoline derivative, which was active at very high concentrations against all the bacterial strains and the 6-methyl derivative, following the same trend, but active against *S. aureus* at 3.90 mM. The unsubstituted, the 6-bromo and the 6-chloro derivatives all showed broad spectrum activity against the remaining five strains albeit activity in the mM range. The results indicated that a 2-fluoro substituent on the phenylhydrazone moiety was essential for antibacterial activity and that moving the fluorine to the 4-position or removing the fluorine substituent altogether led to loss of activity.

Table 4.1 Minimum Bactericidal Concentration (MBC in mM) of **5f-5j**

Compound			**Gram +ve**		**Gram -ve**			
No.	**R_1**	**R_2**	**MRSA**	***S. a.***	***P. a.***	***S. t.***	***E. c.***	***K. p.***
5f	H	2-F	16.34	4.08	8.17	4.08	16.34	32.67
5g	CH_3	2-F	31.22	3.90	31.22	31.22	31.22	31.22
5h	Br	2-F	3.26	6.52	6.53	26.11	13.05	26.11
5i	Cl	2-F	7.37	3.69	7.37	7.37	3.69	29.49
5j	F	2-F	30.95	30.95	30.95	30.95	30.95	30.95
Levoloxacin (standard)			0.0865	0.0216	0.345	0.0216	0.00034	0.0216

S. a. (*Staphylococcus aureus*); MRSA (methicillin resistant *Staphylococcus aureus*); *P. a*. (*Pseudomonas aeruginosa*); *S. t.* (*Salmonella typhimurium*); *E. c.* (*Escherichia coli*); *K. p*. (*Klebsiella pneumoniae*)

4.3 Experimental

General

Reagents were purchased from Sigma Aldrich and were reagent grade. Alumina-backed silica gel 40 F_{254} plates (Merck) were used for TLC and visualised under UV light (254 nm). Melting points were determined on Electrothermal IA 9100 Digital melting point apparatus and are uncorrected. Infrared (IR) spectra was obtained on a PerkinElmer Spectrum 100 with Universal ATR sampling accessory. ^{1}H, ^{13}C and 2D NMR spectra were acquired on a Bruker Avance^{III} 400 MHz spectrometer (Bruker Co., Karlsruhe, Germany) at frequencies of 400.22 MHz for ^{1}H and 100.63 MHz for ^{13}C and referenced to TMS. High-resolution mass data were obtained using a Waters Micromass LCT Premier TOF-MS instrument. Ultraviolet spectroscopy was performed using a UV-VIS-NIR Shimadzu series 3600 spectrophotometer using methanol as a solvent.

General procedure for acetylation of para substituted anilines

The para substituted acetanilides were acetylated according to the procedure in Mann and Saunders (1960). Briefly, 10 mL acetic anhydride (0.1 mole), 10 mL glacial acetic acid (0.175 mole) and aniline or substituted aniline (0.1 mole) was gently refluxed for 1 h. The contents were then added to 200 mL ice-water and stirred continuously. The precipitate that formed was filtered, washed with water and recrystallized with an acetic acid-water mixture (1:2). The yields of the acetylated product (**2a-e**) were in the range of 94-100%.

*General procedure for synthesis of 2-chloroquinoline-3-carbaldehydes (**3a-e**)*

The procedure published in Toth et al. (2006) was used with modifications. Phosphoryl chloride (32.2 mL; 0.35 mol) was added in a dropwise manner under inert conditions, whilst stirring to a solution of dry DMF (9.6 mL; 0.125 mol) contained in an ice-bath (0ºC). Various

acetanilides (0.05 mol) (each separate reactions) were then added with stirring. The reaction was then refluxed for 24 h at 82 °C using a condenser fitted with a calcium chloride drying tube. Upon completion, the mixture was cooled, poured into 300 mL ice water and stirred for 1 h at 0-10 °C. The products precipitated out as pale yellow powders and was filtered, washed with water and dried. The yields were recorded in the range of 43-74%.

*General procedure for synthesis of tetrazolo[1,5-a]quinoline-4-carbaldehydes (**4a-e**)*

The tetrazolo quinoline carbaldehydes were formed according to the procedure published in Ladani et al. (2009). Briefly, the 2-chloro-3-formyl quinolines (0.005 moles) were dissolved in 10 mL ethanol and 1.0 mL acetic acid and sodium azide (0.65 g; 0.01 moles) added to the mixture, which was then heated at 100 °C for 4 hr and monitored by TLC. The product precipitated out of solution upon being formed and was filtered, washed with ethanol and dried. Yields were recorded between 76-88%.

*General procedure for synthesis of phenylhydrazone derivatives (**5a-o**)*

Phenylhydrazone derivatives were prepared according to the procedure outlined in Mann and Saunders (1960). Phenylhydrazine hydrochloride (1.5 g) was dissolved in 6.0 mL water to which 1.5 g sodium acetate was already added. The solution was warmed gently and 0.44 g added to a solution of glacial acetic acid (0.3 mL in 2 mL water). The contents were mixed thoroughly and (0.001 moles) of the respective tetrazolo quinoline carbaldehydes added. The reaction was left to stir for 24 h after which the products precipitated out of solution. The mixture was filtered, washed with dilute acetic acid and water and dried. The yields of the final product were recorded in the range of 55 to 96%.

*(E)-4-((2-phenylhydrazono)methyl)tetrazolo[1,5-a]quinoline (**5a**)* yellow solid (96% yield), mp 257-259°C; IR υ_{max} (cm^{-1}): 3312 (N-H), 1658 (C=N); UV λ_{max} (MeOH) nm (log ε): 231 (4.40), 277 (4.12), 411 (4.25); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.08 (1H, s, H-11), 8.60 (1H, d, *J* = 7.9 Hz, H-8), 8.57 (1H, s, H-4), 8.42 (1H, s, H-9), 8.30 (1H, d, *J* = 7.9 Hz, H-5), 7.92 (1H, t, *J* = 7.9 Hz, H-7), 7.80 (1H, t, *J* = 7.9 Hz, H-6), 7.31 (2H, t, *J* = 7.7 Hz, H-3'/5'), 7.25 (2H, d, *J* = 7.7 Hz, H-2'/6'), 6.86 (1H, t, *J* = 7.7 Hz, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 146.2 (C-2), 144.4 (C-1'), 130.5 (C-7), 129.5 (C-5), 129.2 (C-3'/5'), 129.1 (C-8a), 128.6 (C-9), 128.3 (C-6), 125.3 (C-4), 124.4 (C-3), 121.1 (C-4a), 120.0 (C-4'), 116.1 (C-8), 112.6 (C-2'/6').

*(E)-7-methyl-4-((2-phenylhydrazono)methyl)tetrazolo[1,5-a]quinoline (**5b**)* yellow solid (96% yield), mp 210-212 °C, IR υ_{max} (cm^{-1}): 3254 (N-H), 1600 (C=N); UV λ_{max} (MeOH) nm (log ε): 242 (4.38), 277 (3.86), 308 (3.56); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.05 (1H, s, H-11), 8.48 (1H, d, *J* = 8.5 Hz, H-8), 8.47 (1H, s, H-4), 8.40 (1H, s, H-9), 8.07 (1H, s, H-5), 7.73 (1H, dd, *J* = 8.5, 1.2 Hz, H-7), 7.30 (2H, t, *J* = 7.6 Hz, H-3'/5'), 7.24 (2H, d, *J* = 7.6 Hz, H-2'/6'), 6.86 (1H, dd, *J* = 7.1, 7.1 Hz, H-4'), 2.54 (3H, s, H-6a); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 146.0 (C-2), 144.4 (C-1'), 137.9 (C-6), 131.8 (C-7), 129.2 (C-3'/5'), 128.8 (C-5), 128.6 (C-9), 127.2 (C-8a), 125.0 (C-4), 124.4 (C-3), 121.0 (C-4a), 119.9 (C-4'), 115.9 (C-8), 112.6 (C-2'/6'), 20.9 (C-6a); HRESIMS: (*m/z*) 325.1180 [M+Na] (calculated for $C_{17}H_{14}N_6Na$, 325.1178).

*(E)-7-bromo-4-((2-phenylhydrazono)methyl)tetrazolo[1,5-a]quinoline (**5c**)* yellow solid (58% yield), mp 263-265 °C, IR υ_{max} (cm^{-1}): 3258 (N-H), 1600 (C=N); UV λ_{max} (MeOH) nm (log ε): 244 (4.55), 325 (3.59); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.14 (1H, s, H-11), 8.56 (1H, d, *J* = 2.2 Hz, H-5), 8.50 (1H, s, H-4), 8.49 (1H, d, *J* = 8.7 Hz, H-8), 8.37 (1H, s, H-9), 8.00 (1H, dd, *J* = 8.7, 2.2 Hz, H-7), 7.30 (2H, t, *J* = 8.4 Hz, H-3'/5'), 7.23 (2H, d, *J* = 8.4 Hz, H-2'/6'), 6.87

(1H, t, *J* = 8.4 Hz, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 145.9 (C-2), 143.9 (C-1'), 132.5 (C-7), 131.0 (C-5), 128.9 (C-3'/5'), 127.70 (C-9), 127.69 (C-8a), 125.8 (C-6), 123.5 (C-4), 121.9 (C-3), 120.4 (C-4a), 119.8 (C-4'), 117.9 (C-8), 112.3 (C-2'/6'); HRESIMS: (*m/z*) 389.0129 [M+Na] (calculated for $C_{16}H_{11}N_6NaBr$, 389.0126).

*(E)-7-chloro-4-((2-phenylhydrazono)methyl)tetrazolo[1,5-a]quinoline (**5d**)* yellow solid (63% yield), mp 259-261 °C, IR υ_{max} (cm^{-1}): 3264 (N-H), 1600 (C=N); UV λ_{max} (MeOH) nm (log ε): 243 (4.49), 288 (4.07), 419 (4.22); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.15 (1H, s, H-11), 8.58 (1H, d, *J* = 8.9 Hz, H-8), 8.51 (1H, s, H-4), 8.42 (1H, d, *J* = 2.1 Hz, H-5), 8.38 (1H, s, H-9), 7.90 (1H, dd, *J* = 8.8, 2.2 Hz, H-7), 7.29 (2H, t, *J* = 8.3 Hz, H-3'/5'), 7.24 (2H, d, *J* = 8.3 Hz, H-2'/6'),), 6.87 (1H, t, *J* = 8.3 Hz, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 146.2 (C-2), 144.2 (C-1'), 132.4 (C-6), 130.2 (C-7), 129.2 (C-3'/5'), 128.3 (C-5), 128.1 (C-9), 127.7 (C-8a), 125.8 (C-3), 124.0 (C-4), 122.3 (C-4a), 120.2 (C-4'), 118.1 (C-8), 112.6 (C-2'/6'); HRESIMS: (*m/z*) 345.0628 [M+Na] (calculated for $C_{16}H_{11}N_6NaCl$, 345.0631).

*(E)-7-fluoro-4-((2-phenylhydrazono)methyl)tetrazolo[1,5-a]quinoline (**5e**)* yellow solid (73% yield), mp 262-264 °C, IR υ_{max} (cm^{-1}): 3260 (N-H), 1601 (C=N); UV λ_{max} (MeOH) nm (log ε): 238 (4.36), 286 (4.10), 399 (4.32);^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.14 (1H, s, H-11), 8.62 (1H, dd, *J* = 9.1, 4.8 Hz, H-8), 8.51 (1H, s, H-4), 8.38 (1H, s, H-9), 8.15 (1H, dd, *J* = 9.8, 2.3 Hz, H-5), 7.77 (1H, td, *J* = 9.1, 2.3 Hz, H-7), 7.30 (2H, t, *J* = 8.0 Hz, H-3'/5'), 7.23 (2H, d, *J* = 8.0 Hz, H-2'/6'), 6.87 (1H, t, *J* = 8.0 Hz, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.2 (d, *J* = 244.0 Hz, C-6), 145.6 (C-2), 143.8 (C-1'), 128.8 (C-3'/5'), 127.7 (C-9), 125.7 (C-8a), 125.5 (C-3), 123.7 (C-4), 121.8 (C-4a), 119.7 (C-4'), 118.3-118.1 (m, C-8, C-7), 113.7 (d, *J* = 23.6

Hz, C-5), 112.2 (C-2'/6'); HRESIMS: (*m/z*) 329.0928 [M+Na] (calculated for $C_{16}H_{11}N_6NaF$, 329.0927).

*(E)-4-((2-(2-fluorophenyl)hydrazono)methyl)tetrazolo[1,5-a]quinoline (**5f**)* yellow solid (71% yield), mp 236-238 °C, IR υ_{max} (cm^{-1}): 3235 (N-H), 1622 (C=N); UV λ_{max} (MeOH) nm (log ε): 238 (4.39), 291 (4.17), 414 (4.39); ^{1}H NMR (DMSO-d_6, 400 MHz) 10.99 (1H, s, H-11), 8.68 (1H, s, H-4), 8.60 (1H, s, H-9), 8.59 (1H, d, *J* = 7.8 Hz, H-8), 8.30 (1H, d, *J* = 7.3 Hz, H-5), 7.92 (1H, td, *J* = 7.3, 1.2 Hz, H-7), 7.80 (1H, dt, *J* = 7.8, 1.0 Hz, H-6), 7.77 (1H, dt, *J* = 8.5, 2.0 Hz, H5'), 7.22-7.17 (2H, m, H-3', H-6'), 6.89-6.84 (1H, m, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 149.3 (d, *J* = 238.5 Hz, C-2'), 146.2 (C-2), 132.6 (d, *J* = 9.5 Hz, C-1'), 131.6 (C-7), 130.8, (C-5), 129.6 (C-9), 129.3 (C-8a), 128.3 (C-6), 126.3 (C-4), 125.0 (d, *J* = 2.9 Hz, C-6'), 124.3 (C-3), 120.9 (C-4a), 119.9 (d, *J* = 6.8 Hz, C-4'), 116.1 (C-8), 115.2 (d, *J* = 17.4 Hz, C-3'), 114.5 (d, *J* = 2.7 Hz, C-5'); HRESIMS: (*m/z*) 329.0933 [M+Na]; (calculated for $C_{16}H_{11}N_6NaF$, 329.0927).

*(E)-4-((2-(2-fluorophenyl)hydrazono)methyl)-7-methyltetrazolo[1,5-a]quinoline (**5g**)* yellow solid (91% yield), mp 243-245 °C, IR υ_{max} (cm^{-1}): 3260 (N-H), 1619 (C=N); UV λ_{max} (MeOH) nm (log ε): 242 (4.36), 289 (4.02), 399 (4.25); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.96 (1H, s, H-11), 8.65 (1H, s, H-4), 8.48 (1H, s, H-9), 8.45 (1H, d, *J* = 8.6 Hz, H-8), 8.06 (1H, s, H-5), 7.75 (1H, dt, *J* = 7.9, 1.5 Hz, H-5'), 7.73 (1H, dd, *J* = 8.6, 1.7 Hz, H-7), 7.22-7.17 (2H, m, H-3', H-6'), 6.89-6.83 (1H, m, H-4'), 2.53 (3H, s, H-6a); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 149.3 (d, *J* = 238.3 Hz, C-2'), 145.9 (C-2), 138.0 (C-6), 132.7 (d, *J* = 9.6 Hz, C-1'), 132.1 (C-7), 131.6 (C-5), 128.9 (C-9), 127.4 (C-8a), 126.1 (C-4), 125.0 (d, *J* = 2.9 Hz, C-6'), 124.2 (C-3), 120.8 (C-4a), 119.8 (d, *J* = 6.7 Hz, C-4'), 115.9 (C-8), 115.2 (d, *J* = 17.4 Hz, H-3'), 114.5 (d, *J* = 2.6

Hz, C-5'), 20.9 (C-6a); HRESIMS: (*m/z*) 343.1088 [M+Na] (calculated for $C_{17}H_{13}N_6NaF$, 343.1083).

*(E)-7-bromo-4-((2-(2-fluorophenyl)hydrazono)methyl)tetrazolo[1,5-a]quinoline (**5h**)* yellow solid (71% yield), mp 261-263 °C, IR υ_{max} (cm^{-1}): 3253 (N-H), 1621 (C=N); UV λ_{max} (MeOH) nm (log ε): 245 (4.57), 291 (4.13), 409 (4.35); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.07 (1H, s, H-11), 8.67 (1H, s. H-4), 8.60 (1H, d, *J* = 2.1 Hz, H-5), 8.57 (1H, s, H-9), 8.53 (1H, d, *J* = 8.7 Hz, H-8), 8.05 (1H, dd, *J* = 8.7, 2.1 Hz, H-7), 7.76 (1H, td, *J* = 8.3, 1.4 Hz, H-5'), 7.24-7.18 (2H, m, H-3', H-6'), 6.91-6.86 (1H, m, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 149.3 (d, *J* = 238.8 Hz, C-2'), 146.1 (C-2), 133.1 (C-7), 132.5 (d, *J* = 9.6 Hz, C-1'), 131.5 (C-5), 131.0 (C-9), 128.2 (C-8a), 126.0 (C-6), 125.0 (d, *J* = 2.8 Hz, C-6'), 124.9 (C-4), 122.1 (C-4a), 120.7 (C-3), 120.0 (d, *J* = 6.6 Hz, C-4'), 118.2 (C-8), 115.3 (d, *J* = 17.5 Hz, C-3'), 114.5 (C-5'); HRESIMS: (*m/z*) 383.0067 [M-H] (calculated for $C_{16}H_9N_6FBr$, 383.0056).

*(E)-7-chloro-4-((2-(2-fluorophenyl)hydrazono)methyl)tetrazolo[1,5-a]quinoline (**5i**)* yellow solid (67% yield), mp 254-256 °C, IR υ_{max} (cm^{-1}): 3264 (N-H), 1600 (C=N); UV λ_{max} (MeOH) nm (log ε): 243 (4.48), 290 (4.08), 407 (4.29); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.05 (1H, s, H-11), 8.64 (1H, s, H-4), 8.57 (1H, d, *J* = 8.8 Hz, H-8), 8.53 (1H, s, H-9), 8.42 (1H, bs, H-5), 7.91 (1H, d, *J* = 8.8 Hz, H-7), 7.74 (1H, t, *J* = 8.1 Hz, H-5'), 7.19 (2H, m, H-3', H-6'), 6.90-6.85 (1H, m, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 149.3 (d, *J* = 238.7 Hz, C-2'), 146.2 (C-2), 132.5 (d, *J* = 9.6 Hz, C-1'), 132.4 (C-6), 131.1 (C-7), 130.4 (C-5), 128.4 (C-9), 127.9 (C-8a); 125.7 (C-3), 125.0 (2C, C-6', C-4), 122.2 (C-4a), 120.1 (d, *J* = 6.8 Hz, C-4'), 118.1 (C-8), 115.3 (d, *J* = 17.4 Hz, C-3'), 114.5 (d, *J* = 2.5 Hz, C-5'); HRESIMS: (*m/z*) 339.0560 [M-H] (calculated for $C_{16}H_9N_6FCl$, 339.0561).

(E)-7-fluoro-4-((2-(2-fluorophenyl)hydrazono)methyl)tetrazolo[1,5-a]quinoline (***5j***) yellow solid (63% yield), mp 256-258 °C, IR υ_{max} (cm^{-1}): 3236 (N-H), 1624 (C=N); UV λ_{max} (MeOH) nm (log ε): 238 (4.48), 285 (4.20), 405 (4.39); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.07 (1H, s, H-11), 8.68 (1H, s, H-4), 8.65 (1H, dd, *J* = 9.1, 4.8 Hz, H-8), 8.58 (1H, s, H-9), 8.18 (1H, dd, *J* = 8.4, 2.8 Hz, H-5), 7.80 (1H, dt, J = 8.7, 2.5 Hz, H-7), 7.76 (1H, dd, J = 8.2, 2.0 Hz, H-5'), 7.23-7.18 (2H, m, Hz, H-3', H-6'), 6.91-6.86 (1H, m, H-4'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.6 (d, *J* = 244.0 Hz, C-6), 149.3 (d, *J* = 238.7 Hz, C-2'), 145.9 (C-2), 132.5 (d, *J* = 2.5 Hz, C-1'), 131.1 (C-9), 126.1 (C-8a), 125.9 (C-3); 125.2 (d, *J* = 3.4 Hz, C-4), 125.0 (d, *J* = 2.9 Hz, C-6'), 122.0 (C-4a), 120.0 (d, *J* = 6.7 Hz C-4'), 118.9 (d, *J* = 25.4, C-7), 118.6 (d, *J* = 9.3 Hz, C-8), 115.3 (d, *J* = 17.5 Hz, C-3'), 114.5 (d, *J* = 2.7 Hz, C-5'), 114.2 (d, *J* = 23.8 Hz, C-5); HRESIMS: (*m/z*) 323.0861 [M-H] (calculated for $C_{16}H_9N_6F_2$, 323.0857).

(E)-4-((2-(4-fluorophenyl)hydrazono)methyl)tetrazolo[1,5-a]quinoline (***5k***) yellow solid (66% yield), mp 258-260 °C, IR υ_{max} (cm^{-1}): 3261 (N-H), 1605 (C=N); UV λ_{max} (MeOH) nm (log ε): 236 (4.37), 288 (4.14), 407 (4.31); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.07 (1H, s, H-11), 8.58 (1H, d, *J* = 7.8 Hz, H-8), 8.54 (1H, s, H-4), 8.38 (1H, s, H-9), 8.27 (1H, d, *J* = 7.8 Hz, H-5), 7.90 (1H, t, *J* = 7.8, Hz, H-7), 7.79 (1H, t, *J* = 7.8 Hz, H-6), 7.23 (2H, dd, *J* = 8.8, 4.8, Hz, H-2'/6'), 7.15 (2H, t, *J* = 8.8 Hz, H-3'/5'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 156.5 (d, *J* = 234.0 Hz, C-4'), 146.2 (C-2), 141.1 (d, *J* = 1.9 Hz, C-1'), 130.5 (C-7), 129.4 (C-5), 129.1 (C-8a), 128.6 (C-9), 128.3 (C-6), 125.4 (C-4), 124.3 (C-3), 121.0 (C-4a), 116.1 (C-8), 115.8 (d, *J* = 22.3 Hz, C-3'/5'), 113.6 (d, *J* = 7.7 Hz, C-2'/6'); HRESIMS: (*m/z*) 329.0932 [M+Na] (calculated for $C_{16}H_{11}N_6NaF$, 329.0927).

(E)-4-((2-(4-fluorophenyl)hydrazono)methyl)-7-methyltetrazolo[1,5-a]quinoline ***(5l)*** yellow solid (55% yield), mp 263-265 °C, IR υ_{max} (cm^{-1}): 3285 (N-H), 1605 (C=N); UV λ_{max} (MeOH) nm (log ε): 242 (4.46), 291 (4.15), 406 (4.30); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.03 (1H, s, H-11), 8.46 (1H, d, *J* = 8.6 Hz, H-8), 8.44 (1H, s, H-4), 8.36 (1H, s, H-9), 8.04 (1H, bs, H-5), 7.72 (1H, dd, *J* = 8.6, 1.4 Hz, H-7), 7.23 (2H, dd, *J* = 8.7, 4.8 Hz, H-2'/6'), 7.14 (2H, t, *J* = 8.7, H-3'/5'), 2.54 (3H, s, H-6a); ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* 156.5 (d, *J* = 234.0 Hz, C-4'), 145.9 (C-2), 141.1 (C-1'), 137.9 (C-6), 131.8 (C-7), 128.74 (C-5), 128.68 (C-9), 127.2 (C-8a), 125.2 (C-4), 124.3 (C-3), 120.9 (C-4a), 115.9 (d, *J* = 22.4 Hz, C-3'/5'), 115.8 (C-8), 113.6 (d, *J* = 7.5 Hz, C-2'/6'), 20.9 (C-6a); HRESIMS: (*m/z*) 319.1107 [M-H] (calculated for $C_{17}H_{12}N_6F$, 319.1107).

(E)-7-bromo-4-((2-(4-fluorophenyl)hydrazono)methyl)tetrazolo[1,5-a]quinoline ***(5m)*** yellow solid (67% yield), mp 264-266 °C, IR υ_{max} (cm^{-1}): 3273 (N-H), 1600 (C=N); UV λ_{max} (MeOH) nm (log ε): 245 (4.57), 294 (4.14), 421 (4.36); ^{1}H NMR (DMSO-d_6, 400 MHz) *δ* 11.14 (1H, s, H-11), 8.54 (1H, d, *J* = 2.0, H-5), 8.49 (1H, s, H-4), 8.48 (1H, d, *J* = 8.7 Hz, H8), 8.34 (1H, s, H-9), 8.00 (1H, dd, *J* = 8.9, 2.0 Hz, H-7), 7.22 (2H, dd, *J* = 8.8, 4.8 Hz, H-2'/6'), 7.15 (2H, t *J* = 8.8 Hz, H-3'/5'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 156.7 (d, *J* = 234.5 Hz, C-4'), 146.2 (C-2), 140.9 (C-1'), 132.8 (C-7), 131.3 (C-5), 128.1 (C-9), 128.0 (C-6), 126.1 (C-8a) 123.9 (C-4), 122.2 (C-3), 120.7 (C-4a), 118.2 (C-8), 115.8 (d, *J* = 22.5 Hz, C-3'/5'), 113.7 (d, *J* = 7.6 Hz, C-2'/6'); HRESIMS: (*m/z*) 383.0066 [M-H] (calculated for $C_{16}H_9N_6FBr$, 383.0056).

(E)-7-chloro-4-((2-(4-fluorophenyl)hydrazono)methyl)tetrazolo[1,5-a]quinoline ***(5n)*** yellow solid (57% yield), mp 255-257 °C, IR υ_{max} (cm^{-1}): 3259 (N-H), 1604 (C=N); UV λ_{max} (MeOH) nm (log ε): 243 (4.38), 294 (3.98), 418 (4.25); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.15 (1H, s,

H-11), 8.58 (1H, d, *J* = 8.8, H-8), 8.50 (1H, s, H-4), 8.40 (1H, d, *J* = 2.2, H-5), 8.35 (1H, s, H-9), 7.90 (1H, dd, *J* = 8.8, 2.2 Hz, H-7), 7.22 (2H, dd, *J* = 8.7, 4.8 Hz, H-2′/6′), 7.15 (2H, t, *J* = 8.7 Hz, H-3′/5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 156.7 (d, *J* = 234.3 Hz, C-4′), 146.2 (C-2), 140.9 (d, *J* = 1.9 Hz, C-1′), 132.4 (C-6), 130.2 (C-7), 128.3 (C-5), 128.1 (C-9), 127.7 (C-8a), 125.8 (C-3), 124.0 (C-4), 122.3 (C-4a), 118.1 (C-8), 115.8 (d, *J* = 22.3 Hz, C-3′/5′), 113.7 (d, *J* = 7.6 Hz, C-2′/6′); HRESIMS: (*m/z*) 339.0551 [M-H] (calculated for $C_{16}H_9N_6FCl$, 339.0561).

*(E)-7-fluoro-4-((2-(4-fluorophenyl)hydrazono)methyl)tetrazolo[1,5-a]quinoline (**5o**)* yellow solid (67% yield), mp 265-267 °C, IR υ_{max} (cm^{-1}): 3303 (N-H), 1626 (C=N); UV λ_{max} (MeOH) nm (log ε): 238 (4.39), 286 (4.12), 399 (4.35); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.12 (1H, s, H-11), 8.59 (1H, dd, *J* = 9.1, 4.8 Hz, H-8), 8.47 (1H, s, H-4), 8.33 (1H, s, H-9), 8.11 (1H, dd, *J* = 9.3, 2.7 Hz, H-5), 7.75 (1H, td, *J* = 9.1, 2.7 Hz, H-7), 7.20 (2H, dd, *J* = 8.8, 4.8 Hz, H-2′/6′), 7.14 (2H, t, *J* = 8.8 Hz, H-3′/5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.6 (d, *J* = 243.7 Hz, C-6), 156.6 (d, *J* = 234.2 Hz, C-4′), 145.9 (C-2), 140.9 (d, *J* = 1.91 Hz, C-1′), 128.1 (C-9), 126.1 (C-8a), 125.9 (C-3), 124.2 (d, *J* = 3.4 Hz, C-4), 122.1 (C-4a), 118.8-118.5 (2C, m, C-7, C-8), 115.8 (d, *J* = 22.5 Hz, C-3′/5′), 114.0 (d, *J* = 23.7 Hz, C-5), 113.6 (d, *J* = 7.7 Hz, C-2′/6′); HRESIMS: (*m/z*) 323.0860 [M-H] (calculated for $C_{16}H_9N_6F_2$, 323.0857).

Antibacterial Activity

The synthesied compounds were tested for their antibacterial activity against two Gram +ve bacteria (*Staphylococcus aureus* ATCC 25923 *(S.a)* and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (methicillin resistant *S. aureus*, MRSA)) and four Gram -ve bacteria (*Pseudomonas aeruginosa* ATCC 27853 *(P.a)*, *Klebsiella pneumoniae* ATCC 31488 *(K.p), Escherichia coli* ATCC 25922 *(E.c)* and *Salmonella typhimurium* ATCC *14026 (S.t))*.

A preliminary disc diffusion assay was carried out to determine which of the compounds were active against the test strains at a concentration of 10 mg mL^{-1}. The various bacterial strains were grown in nutrient broth (Biolab, South Africa) at 37 ºC for 18 hours in a shaking incubator at 115 rpm. Bacterial strains were diluted with sterile distilled water to obtain a concentration equivalent to 0.5 McFarland's standard. Mueller-Hinton agar plates (Biolab, South Africa) were lawn inoculated with the bacteria with the aid of a throat swab. A 5 µL sample of the test compounds (10 mg mL^{-1} in 100% DMSO) was spotted in triplicate onto the Mueller-Hinton plates and incubated at 37°C for 18 h. Antibacterial activity was indicated by zones of inhibition at the site of spotting.

Minimum Bactericidal Concentration Assay

A modification of the Broth Dilution Method (Kalhapure et al., 2014) was carried out in triplicate to determine the minimum bactericidal concentrations (MBCs) of the test samples. Modification was necessary as the diluted samples precipitated when added to Mueller-Hinton Broth as per the Broth Dilution Method. Test samples were prepared at a concentration of 10 mg mL^{-1} in DMSO and serially diluted with DMSO to obtain a concentration range from 0.3125-5.000 mg mL^{-1}. The Mueller-Hinton agar plates were lawn inoculated with the respective bacterial strains as described above. A 5 µL aliquot of each concentration of every sample was spotted onto the Mueller-Hinton agar plates in triplicate and the plates incubated at 37 ºC for 24 h. The lowest concentration that inhibited bacterial growth was determined as the minimum bactericidal concentration (MBC).

4.4 Conclusion

Thirteen novel 6-substituted tetrazolo-3-phenylhydrazone quinolines were synthesised in good yields from 55-96%. Only tetrazolo quinoline phenylhydrazones that had a 2-

fluorophenylhydrazone moiety was active against the bacterial strains tested. Activity was lost with a phenylhydrazone group and a 4-phenylhydrazone group. However, the activity shown by the 2-fluorophenylhydrazone derivatives were in the mM range and did not improve from our earlier reports where the quinoline thiosemicarbazone or the quinoline carboxamides also showed activity in the mM range. The best substituents at C-6 on the quinoline scaffold were H, Br and Cl. The DMSO was used as a blank standard and showed no activity against all the bacterial strains tested.

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Chapter 5. Conclusion

Three small libraries of hybrid molecules based on the quinoline scaffold with three different bioactive moieties, carboxamides, thiosemicarbazones and phenylhydrazones were successfully synthesised in good yields. The 3-carbaldehyde moiety proved to be a good functional group to build onto the quinoline scaffold, both to introduce variety into the scaffold and to join other bioactive moieties to a central scaffold, in this case quinoline. In this way, 36 new hybrid molecules based on the 2-chloroquinoline-3-carbaldehyde scaffold was synthesised.

The synthesis of substituted 2-chloroquinoline-3-carbaldehydes were successfully carried out using the Vilsmeier Haack reaction using various para-substituted anilines as well as the unsubstituted aniline. Variation at C-2 on the quinoline scaffold by converting the chloroquinoline to the quinolone and then by fusing a tetrazolo ring onto the quinoline between the nitrogen and C-2 was also carried out for the thiosemicarbazone and phenylhydrazone hybrids respectively.

For the synthesis of the carboxamides, the aldehyde group at position 3 on the quinoline ring was oxidised to the carboxylic acid functionality via the Pinnick oxidation reaction. For this reaction, a primary alcohol was used instead of the tertiary alcohol reported in literature and the results obtained were comparable. Thereafter, the acid was reacted via a coupling reaction with different *para* substituted anilines containing an activating group (methyl substituent), a deactivating group (fluoro substituent) as well as the unsubstituted aniline.

All twelve compounds showed weak antibacterial activity with the best activity displayed by compounds **5b, 5d** and **5f**. Therefore, it can be deduced that the electron donating methyl

groups either at position C-6 or C-4' most probably contributed to the antibacterial activity of the quinoline-carboxamide scaffold.

The synthesise of the fifteen quinolone thiosemicarbazones, eleven of which were new, was performed by first preparing 3-formyl-2-quinolones from the various 2-chloroquinoline-3-carbaldehydes under acidic conditions and this was then followed by a condensation reaction with substituted hydrazines to form the final products. The target compounds contained both electron donating hydrogen and methyl groups as well as electron withdrawing halogens at C-6 on the quinolone scaffold whilst the substituents on the thiosemicarbazone were varied in order to ascertain the best possible functionalities for antibacterial activity.

The antibacterial study showed that the *N*-phenyl thiosemicarbazones displayed better activity than the *N*-methyl or unsubstituted thiosemicarbazone derivatives. Furthermore, three of the compounds, **5l**, **5m** and **5n** showed activity against MRSA, being just one order of magnitude less active than that of levofloxacin.

The synthesis of the fifteen tetrazoloquinoline phenylhydrazones, thirteen of which were new was done successfully. After the formation of the various 2-chloroquinoline-3-carbaldehydes, the tetrazolo ring was formed onto the quinoline at the nitrogen and this was followed by the formation of the phenylhydrazones at C-3 on the quinolone ring.

The results from the antibacterial investigation indicated that only the tetrazolo quinoline phenylhydrazones with a 2-fluorophenylhydrazone moiety was found to be active against the bacterial strains tested.

The synthesised compounds could easily be verified by their ^{1}H and ^{13}C NMR spectrum as well as mass spectrometry along with a crystal structure for one of the compounds. It was shown for the carboxamides that the substituent at C-6 had a noticeable effect on the proton and carbon resonances at positions 5, 7 and 8 of the quinoline ring.

The quinoline carboxamides, thiosemicarbazones and phenylhydrazones all proved to be weak antibacterial agents with MBC values in the mM range. It is surprising that the fluoroquinolone thiosemicarbazones and the other fluorinated carboxamides and phenylhydrazones also showed this weak activity, since fluoroquinolones such as ciprofloxacin and levofloxacin are such potent antibacterial agents.

Future Work

From the present study, it is speculated that the insolubility of the compounds in water may have something to do with the inactivity of the compounds and therefore future work will concentrate on making derivatives which make the compounds soluble in aqueous media, such as sulphonamides.

Future work will also involve testing the three sets of quinoline hybrid molecules for other biological activity such as antioxidant, anti-diabetic and antitubercular activity. It is hoped that a good lead can be found in one of these assays that could provide some insight as to what types of bioactivity these particular scaffolds may be useful for. This will provide some guidance as to the types of bioassays one should carry out with related scaffolds of the quinoline type.

Other future work will involve conducting docking studies of the compounds in Chapters 2 and 4. This will allow one to make a more informed conclusion on the effect different types of scaffolds have in pockets within the enzyme.

In addition, cytoxicity studies will be undertaken for all the compounds to ascertain whether the limited antibacterial activity displayed by the compounds was due to their cytotoxicity.

Other subsituents such as bromo and methoxy will also be looked at in future work, to determine whether or not these substituents will have an effect on the antibacterial activity of the compounds.

Supplementary Information

Chapter 2 Synthesis and Bioactivity of Quinoline-3-carboxamide Derivatives

Contents

Table S1 Screening results of quinoline-3-Carboxamides (**5a-l**) using the disc diffusion assay (zones of inhibition in mm)

No.	Gram Positive Bacteria		Gram Negative Bacteria			
	MRSA*	*S. aureus*	*P. aeruginosa*	*S. typhimurium*	*E. coli*	*K. pneumoniae*
5a	-	-	7	7	7	9
5b	-	8	8	7	7	8
5c	-	9	7	7	7	8
5d	8	7	10	-	9	7
5e	7	10	7	-	7	8
5f	9	9	7	-	8	9
5g	-	7	7	-	-	7
5h	7	-	7	-	-	9
5i	7	7	8	-	7	8
5j	7	7	7	-	7	8
5k	7	-	7	7	-	7
5l	7	-	7	-	-	9

- denotes that no activity was observed by the test compounds

Table S2 Minimum Bactericidal Concentration (MBC in mM) of selected quinoline-3-carboxamides

Compound			Gram Positive Bacteria		Gram Negative Bacteria			
No.	R	R_1	MRSA*	*S. aureus*	*P. aeruginosa*	*S. typhimurium*	*E. coli*	*K. pneumoniae*
5b	H	CH_3	3.79	7.58	7.58	15.16	7.58	7.58
5c	H	F	7.48	7.48	7.48	14.96	7.48	11.22
5d	CH_3	H	7.53	3.77	7.53	15.06	15.06	7.53
5e	CH_3	CH_3	7.26	14.51	7.26	14.51	7.26	14.51
5f	CH_3	F	7.15	1.79	5.37	0.000	7.15	14.30
Levoloxacin			0.0865	0.0216	0.345	0.0216	0.00034	0.0216

Crystallographic data

A crystal of dimensions 0.25 x 0.28 x 0.48 mm^3, was selected and glued on to the tip of a glass fibre. The crystal evaluation and data collection were performed on a Bruker Smart APEXII diffractometer with Mo Kα radiation (λ = 0.71073 Å). The data collection method involved ω scans of width 0.5°. Data reduction was carried out using the program *SAINT+* and the structure solved by direct methods using SHELXS and refined. Non-H atoms were first refined isotropically and then anisotropically with full-matrix least-squares calculations based on F^2 using SHELXS. All H atoms were positioned geometrically and allowed to ride on their respective parent atoms. All H atoms were refined isotropically. Crystallographic data has been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository number CCDC-1575908 (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).

Table S3 Selected bond lengths/ **Å** and angles/**°**

	Bond lengths (Å)
N(1)-C(7)	1.353(2)
N(1)-C(1)	1.427(2)
N(1)-H(1)	0.8800
	Bond angles (°)
C(7)-N(1)-C(1)	123.97(16)
C(7)-N(1)-H(1)	118.0
C(1)-N(1)-H(1)	118.0
N(1)-C(7)-C(8)	113.82(16)
O(1)-C(7)-N(1)	123.89(17)
N(1)-C(7)-C(8)	113.82(16)
C(2)-C(1)-N(1)	117.79(18)
C(6)-C(1)-N(1)	121.88(19)
F(1)-C(4)-C(3)	118.0(2)
O(1)-C(7)-N(1)	123.89(17)

Table S4 Crystal data and structure refinement for 16zs_nk_gh_qcab_me_f_0m.

Identification code	shelx	
Empirical formula	C17 H12 Cl F N2 O	
Formula weight	314.74	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C c	
Unit cell dimensions	a = 25.6747(6) Å	α = 90°.
	b = 4.81190(10) Å	β = 94.7540(10)°.
	c = 11.6763(3) Å	γ = 90°.
Volume	1437.58(6) $Å^3$	
Z	4	
Density (calculated)	1.454 Mg/m^3	
Absorption coefficient	0.279 mm^{-1}	
F(000)	648	
Crystal size	0.220 x 0.140 x 0.120 mm^3	
Theta range for data collection	3.185 to 28.308°.	
Index ranges	-33<=h<=34, -6<=k<=6, -15<=l<=13	
Reflections collected	7137	
Independent reflections	2742 [R(int) = 0.0138]	
Completeness to theta = 25.242°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.976 and 0.935	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2742 / 2 / 201	
Goodness-of-fit on F^2	1.065	
Final R indices [I>2sigma(I)]	R1 = 0.0241, wR2 = 0.0655	
R indices (all data)	R1 = 0.0246, wR2 = 0.0660	
Absolute structure parameter	0.08(5)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.274 and -0.148 $e.Å^{-3}$	

Table S5 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 16zs_nk_gh_qcab_me_f_0m. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Cl(1)	73(1)	4607(1)	12339(1)	22(1)
F(1)	2327(1)	6793(4)	6882(2)	51(1)
N(1)	690(1)	8925(3)	9548(2)	18(1)
O(1)	512(1)	4487(3)	10051(2)	22(1)
N(2)	-711(1)	7899(3)	11930(2)	18(1)
C(17)	-2001(1)	15913(4)	9477(2)	22(1)
C(13)	-1646(1)	13816(4)	10103(2)	18(1)
C(14)	-1190(1)	12936(4)	9667(2)	17(1)
C(15)	-866(1)	10950(4)	10265(2)	15(1)
C(16)	-394(1)	9971(4)	9858(2)	16(1)
C(8)	-94(1)	8043(4)	10469(2)	16(1)
C(7)	400(1)	6949(4)	10020(2)	16(1)
C(1)	1123(1)	8341(4)	8894(2)	18(1)
C(6)	1496(1)	6370(5)	9241(2)	26(1)
C(5)	1905(1)	5856(5)	8552(3)	35(1)
C(4)	1929(1)	7326(5)	7560(2)	32(1)
C(12)	-1779(1)	12708(4)	11171(2)	22(1)
C(11)	-1474(1)	10782(4)	11762(2)	22(1)
C(10)	-1007(1)	9843(4)	11321(2)	17(1)
C(9)	-284(1)	7058(4)	11507(2)	16(1)
C(2)	1162(1)	9828(5)	7891(2)	26(1)
C(3)	1569(1)	9315(5)	7201(2)	32(1)

Table S6 Bond lengths [Å] and angles [°] for 16zs_nk_gh_qcab_me_f_0m.

Cl(1)-C(9)	1.741(2)
F(1)-C(4)	1.368(2)
N(1)-C(7)	1.353(2)
N(1)-C(1)	1.427(2)
N(1)-H(1)	0.8800
O(1)-C(7)	1.219(2)
N(2)-C(9)	1.303(2)
N(2)-C(10)	1.367(3)

C(17)-C(13)	1.508(3)
C(17)-H(17A)	0.9800
C(17)-H(17B)	0.9800
C(17)-H(17C)	0.9800
C(13)-C(14)	1.381(2)
C(13)-C(12)	1.423(3)
C(14)-C(15)	1.413(3)
C(14)-H(14)	0.9500
C(15)-C(10)	1.418(3)
C(15)-C(16)	1.418(2)
C(16)-C(8)	1.368(3)
C(16)-H(16)	0.9500
C(8)-C(9)	1.424(3)
C(8)-C(7)	1.507(2)
C(1)-C(2)	1.382(3)
C(1)-C(6)	1.385(3)
C(6)-C(5)	1.398(3)
C(6)-H(6)	0.9500
C(5)-C(4)	1.362(4)
C(5)-H(5)	0.9500
C(4)-C(3)	1.371(3)
C(12)-C(11)	1.364(3)
C(12)-H(12)	0.9500
C(11)-C(10)	1.417(3)
C(11)-H(11)	0.9500
C(2)-C(3)	1.394(3)
C(2)-H(2)	0.9500
C(3)-H(3)	0.9500
C(7)-N(1)-C(1)	123.97(16)
C(7)-N(1)-H(1)	118.0
C(1)-N(1)-H(1)	118.0
C(9)-N(2)-C(10)	117.68(16)

C(13)-C(17)-H(17A)	109.5
C(13)-C(17)-H(17B)	109.5
H(17A)-C(17)-H(17B)	109.5
C(13)-C(17)-H(17C)	109.5
H(17A)-C(17)-H(17C)	109.5
H(17B)-C(17)-H(17C)	109.5
C(14)-C(13)-C(12)	118.72(19)
C(14)-C(13)-C(17)	121.54(18)
C(12)-C(13)-C(17)	119.73(17)
C(13)-C(14)-C(15)	120.54(18)
C(13)-C(14)-H(14)	119.7
C(15)-C(14)-H(14)	119.7
C(14)-C(15)-C(10)	120.03(16)
C(14)-C(15)-C(16)	122.94(18)
C(10)-C(15)-C(16)	117.03(18)
C(8)-C(16)-C(15)	120.64(18)
C(8)-C(16)-H(16)	119.7
C(15)-C(16)-H(16)	119.7
C(16)-C(8)-C(9)	116.86(16)
C(16)-C(8)-C(7)	120.59(17)
C(9)-C(8)-C(7)	122.50(17)
O(1)-C(7)-N(1)	123.89(17)
O(1)-C(7)-C(8)	122.25(16)
N(1)-C(7)-C(8)	113.82(16)
C(2)-C(1)-C(6)	120.33(18)
C(2)-C(1)-N(1)	117.79(18)
C(6)-C(1)-N(1)	121.88(19)
C(1)-C(6)-C(5)	119.2(2)
C(1)-C(6)-H(6)	120.4
C(5)-C(6)-H(6)	120.4
C(4)-C(5)-C(6)	119.1(2)
C(4)-C(5)-H(5)	120.5

C(6)-C(5)-H(5)	120.5
C(5)-C(4)-F(1)	118.8(2)
C(5)-C(4)-C(3)	123.11(19)
F(1)-C(4)-C(3)	118.0(2)
C(11)-C(12)-C(13)	121.89(17)
C(11)-C(12)-H(12)	119.1
C(13)-C(12)-H(12)	119.1
C(12)-C(11)-C(10)	120.00(19)
C(12)-C(11)-H(11)	120.0
C(10)-C(11)-H(11)	120.0
N(2)-C(10)-C(11)	118.69(19)
N(2)-C(10)-C(15)	122.50(16)
C(11)-C(10)-C(15)	118.81(19)
N(2)-C(9)-C(8)	125.26(18)
N(2)-C(9)-Cl(1)	114.81(14)
C(8)-C(9)-Cl(1)	119.91(14)
C(1)-C(2)-C(3)	120.6(2)
C(1)-C(2)-H(2)	119.7
C(3)-C(2)-H(2)	119.7
C(4)-C(3)-C(2)	117.7(2)
C(4)-C(3)-H(3)	121.2
C(2)-C(3)-H(3)	121.2

Symmetry transformations used to generate equivalent atoms:

Table S7 Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 16zs_nk_gh_qcab_me_f_0m. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Cl(1)	26(1)	20(1)	18(1)	5(1)	-2(1)	3(1)
F(1)	36(1)	51(1)	69(1)	-8(1)	32(1)	6(1)
N(1)	19(1)	11(1)	24(1)	-2(1)	4(1)	1(1)
O(1)	28(1)	13(1)	27(1)	0(1)	4(1)	3(1)
N(2)	23(1)	18(1)	13(1)	1(1)	2(1)	-1(1)
C(17)	21(1)	22(1)	24(1)	2(1)	1(1)	4(1)
C(13)	19(1)	16(1)	18(1)	-2(1)	0(1)	-1(1)
C(14)	20(1)	15(1)	15(1)	0(1)	2(1)	-1(1)
C(15)	18(1)	14(1)	14(1)	-1(1)	0(1)	-1(1)
C(16)	20(1)	13(1)	14(1)	0(1)	3(1)	-2(1)
C(8)	19(1)	12(1)	16(1)	-2(1)	2(1)	-1(1)
C(7)	19(1)	14(1)	14(1)	-2(1)	0(1)	2(1)
C(1)	16(1)	15(1)	24(1)	-6(1)	1(1)	-1(1)
C(6)	22(1)	22(1)	35(1)	4(1)	2(1)	5(1)
C(5)	21(1)	27(1)	58(2)	-1(1)	9(1)	8(1)
C(4)	21(1)	30(1)	46(2)	-12(1)	12(1)	-2(1)
C(12)	21(1)	23(1)	22(1)	-1(1)	6(1)	3(1)
C(11)	25(1)	23(1)	17(1)	2(1)	7(1)	1(1)
C(10)	20(1)	15(1)	15(1)	-1(1)	2(1)	-1(1)
C(9)	20(1)	12(1)	16(1)	1(1)	-2(1)	0(1)
C(2)	19(1)	28(1)	32(2)	5(1)	4(1)	6(1)
C(3)	26(1)	42(1)	30(1)	3(1)	9(1)	-2(1)

Table S8 Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for 16zs_nk_gh_qcab_me_f_0m.

	x	y	z	U(eq)
H(1)	607	10677	9651	21
H(17A)	-1938	17753	9821	33
H(17B)	-2366	15380	9537	33
H(17C)	-1930	15969	8666	33
H(14)	-1094	13671	8960	20
H(16)	-285	10659	9153	19
H(6)	1474	5380	9940	32
H(5)	2163	4501	8772	42
H(12)	-2090	13328	11480	26
H(11)	-1574	10070	12470	26
H(2)	909	11211	7671	32
H(3)	1597	10309	6506	39

Table S9 Torsion angles [°] for 16zs_nk_gh_qcab_me_f_0m.

C(12)-C(13)-C(14)-C(15)	0.8(3)
C(17)-C(13)-C(14)-C(15)	-179.53(19)
C(13)-C(14)-C(15)-C(16)	179.9(2)
C(13)-C(14)-C(15)-C(10)	0.0(3)
C(14)-C(15)-C(16)-C(8)	-180.0(2)
C(10)-C(15)-C(16)-C(8)	0.0(3)
C(15)-C(16)-C(8)-C(9)	1.1(3)
C(15)-C(16)-C(8)-C(7)	178.39(18)
C(1)-N(1)-C(7)-O(1)	8.9(3)
C(1)-N(1)-C(7)-C(8)	-168.81(19)
C(16)-C(8)-C(7)-O(1)	-136.1(2)
C(9)-C(8)-C(7)-O(1)	40.9(3)
C(16)-C(8)-C(7)-N(1)	41.6(3)
C(9)-C(8)-C(7)-N(1)	-141.3(2)
C(7)-N(1)-C(1)-C(6)	-44.1(3)
C(7)-N(1)-C(1)-C(2)	136.1(2)
C(2)-C(1)-C(6)-C(5)	-1.5(3)
N(1)-C(1)-C(6)-C(5)	178.7(2)
C(1)-C(6)-C(5)-C(4)	0.6(4)
C(6)-C(5)-C(4)-F(1)	-179.3(2)

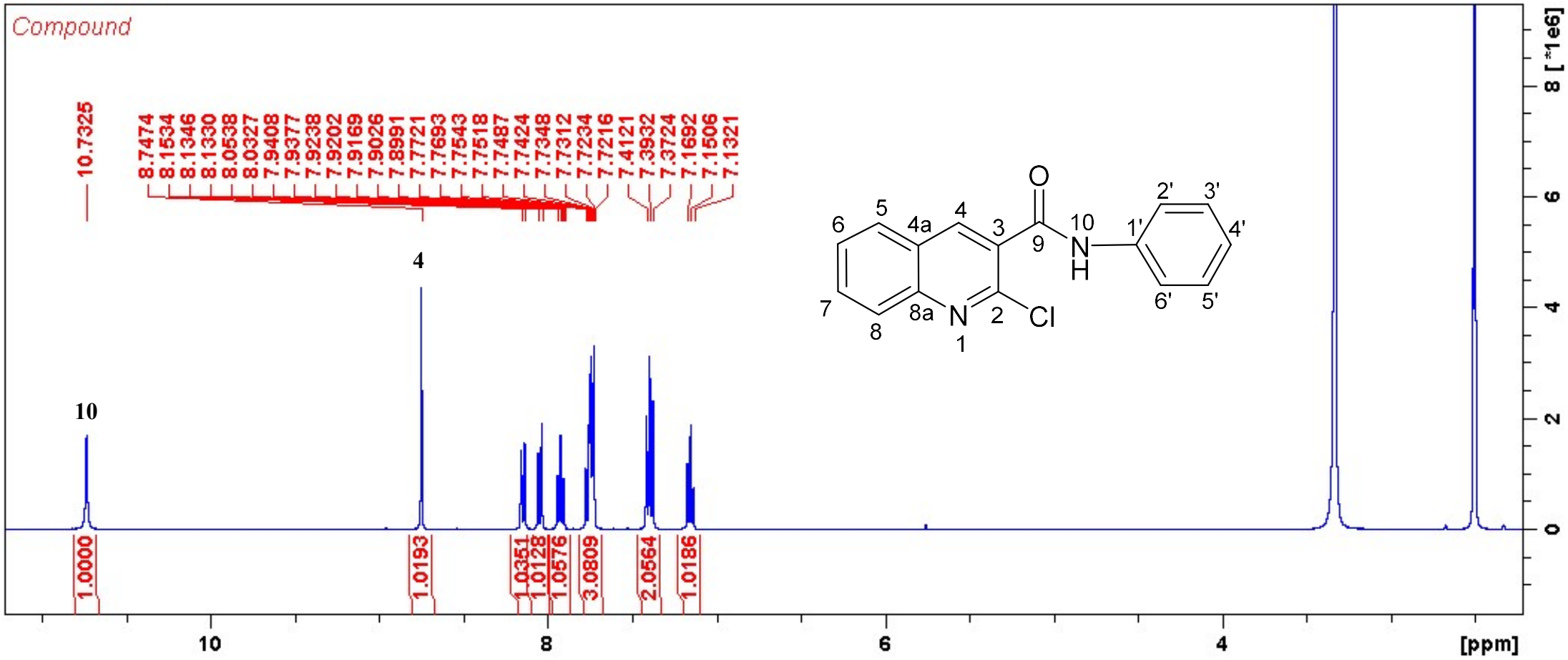
C(6)-C(5)-C(4)-C(3)	0.3(4)
C(14)-C(13)-C(12)-C(11)	-1.1(3)
C(17)-C(13)-C(12)-C(11)	179.2(2)
C(13)-C(12)-C(11)-C(10)	0.6(4)
C(9)-N(2)-C(10)-C(11)	179.7(2)
C(9)-N(2)-C(10)-C(15)	-0.1(3)
C(12)-C(11)-C(10)-N(2)	-179.6(2)
C(12)-C(11)-C(10)-C(15)	0.2(3)
C(14)-C(15)-C(10)-N(2)	179.36(19)
C(16)-C(15)-C(10)-N(2)	-0.6(3)
C(14)-C(15)-C(10)-C(11)	-0.5(3)
C(16)-C(15)-C(10)-C(11)	179.6(2)
C(10)-N(2)-C(9)-C(8)	1.4(3)
C(10)-N(2)-C(9)-Cl(1)	179.49(15)
C(16)-C(8)-C(9)-N(2)	-1.9(3)
C(7)-C(8)-C(9)-N(2)	-179.11(19)
C(16)-C(8)-C(9)-Cl(1)	-179.95(16)
C(7)-C(8)-C(9)-Cl(1)	2.9(3)
C(6)-C(1)-C(2)-C(3)	1.7(4)
N(1)-C(1)-C(2)-C(3)	-178.6(2)
C(5)-C(4)-C(3)-C(2)	-0.2(4)
F(1)-C(4)-C(3)-C(2)	179.4(2)
C(1)-C(2)-C(3)-C(4)	-0.8(4)

Symmetry transformations used to generate equivalent atoms:

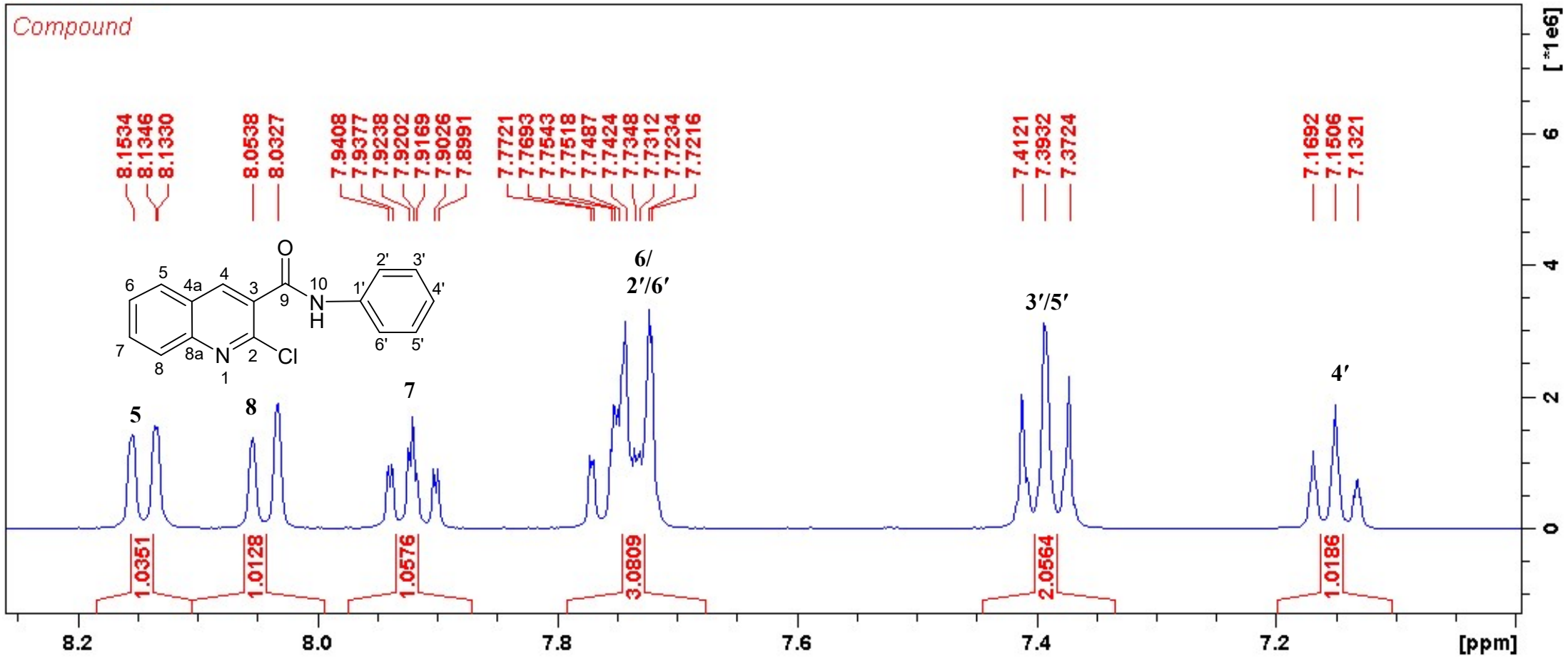
Table S10 Hydrogen bonds for gh [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1)...O(1)#1	0.88	1.92	2.788(2)	171

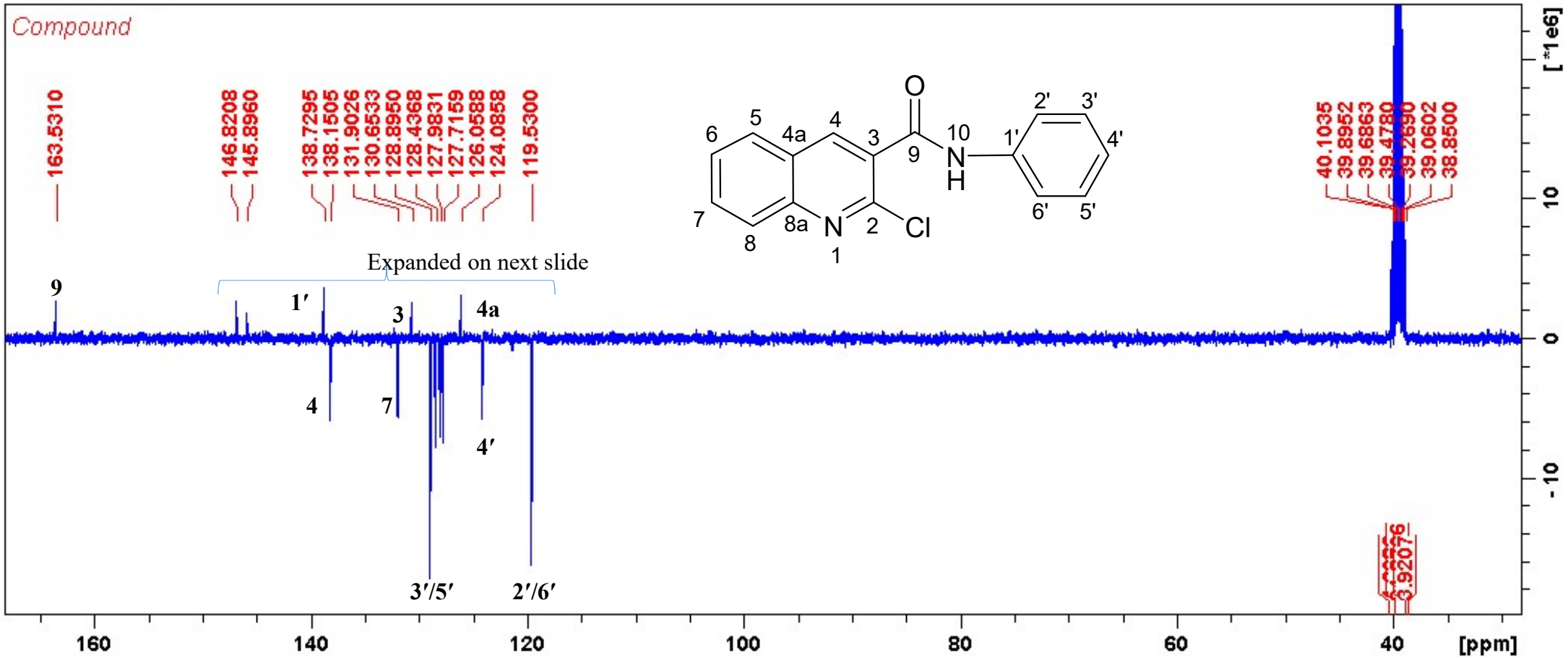
Symmetry transformations used to generate equivalent atoms:
#1 x,y+1,z



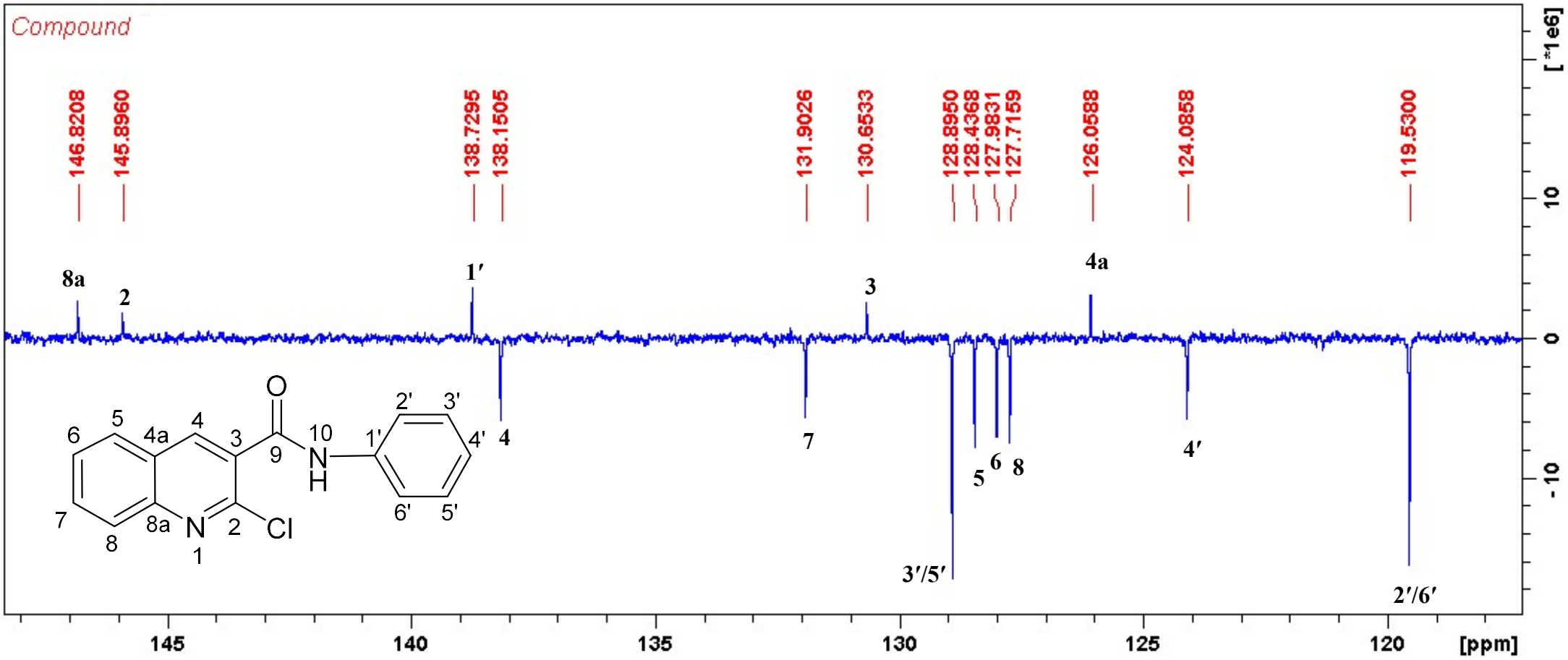
The ^{1}H NMR spectrum of compound 5a



The ^{1}H NMR spectrum of compound 5a (expanded)



The ^{13}C NMR spectrum of compound 5a

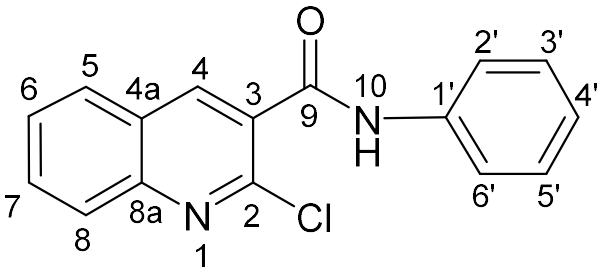


The ^{13}C NMR spectrum of compound 5a (expanded)

Elemental Composition Report

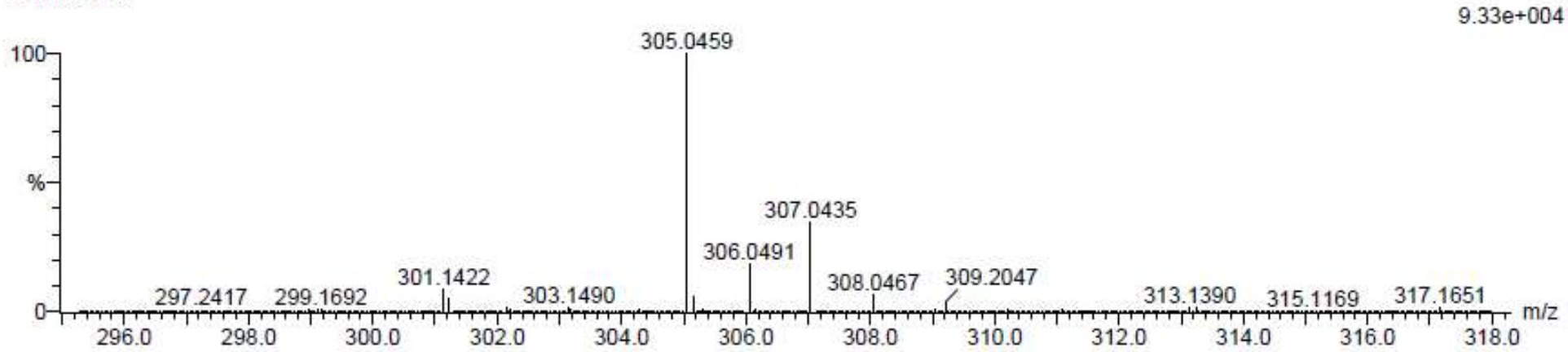
Single Mass Analysis

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



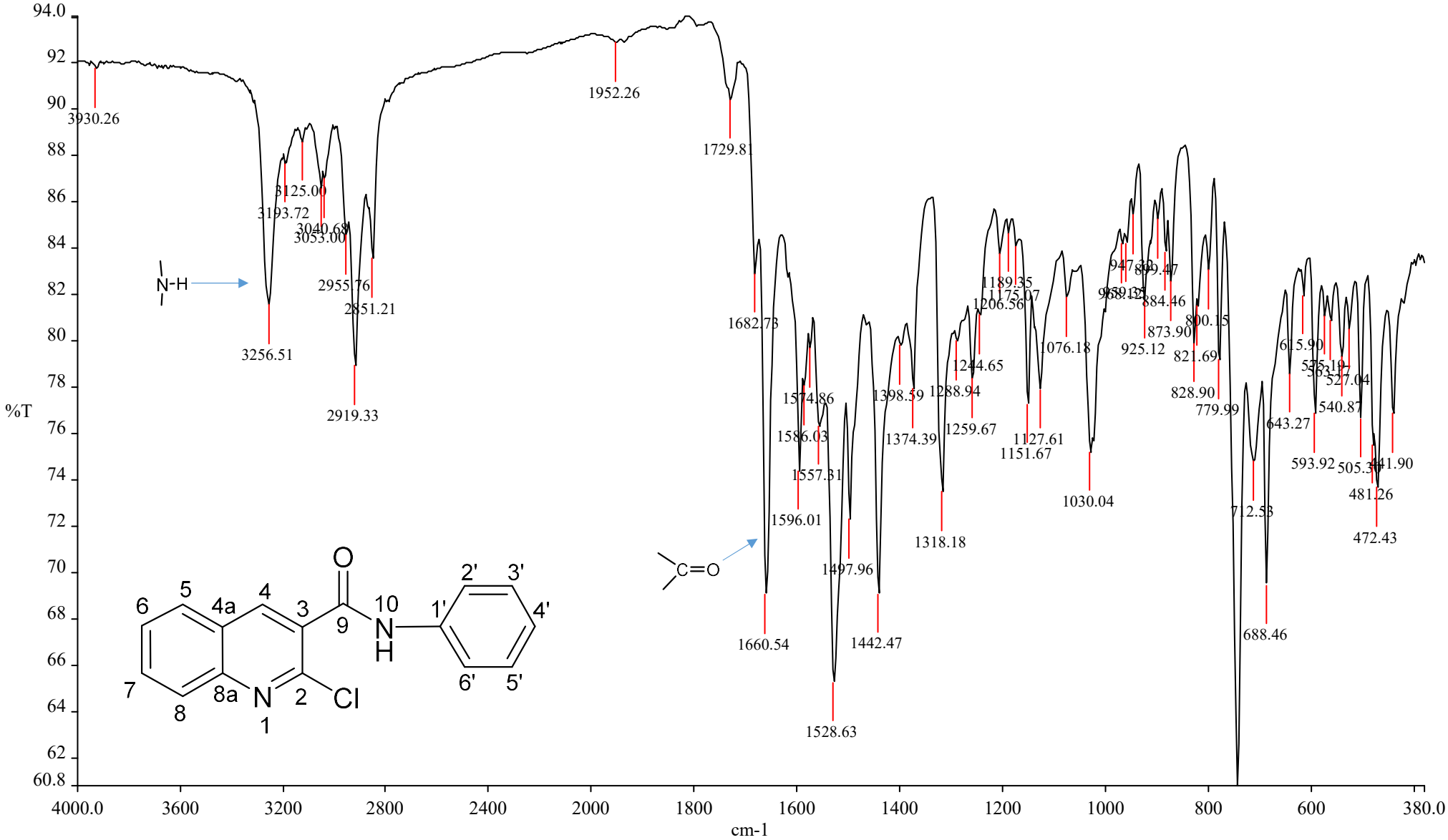
Monoisotopic Mass, Even Electron Ions
45 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-1

7a HG DUP 13 (0.405) Cm (1:61)
TOF MS ES+

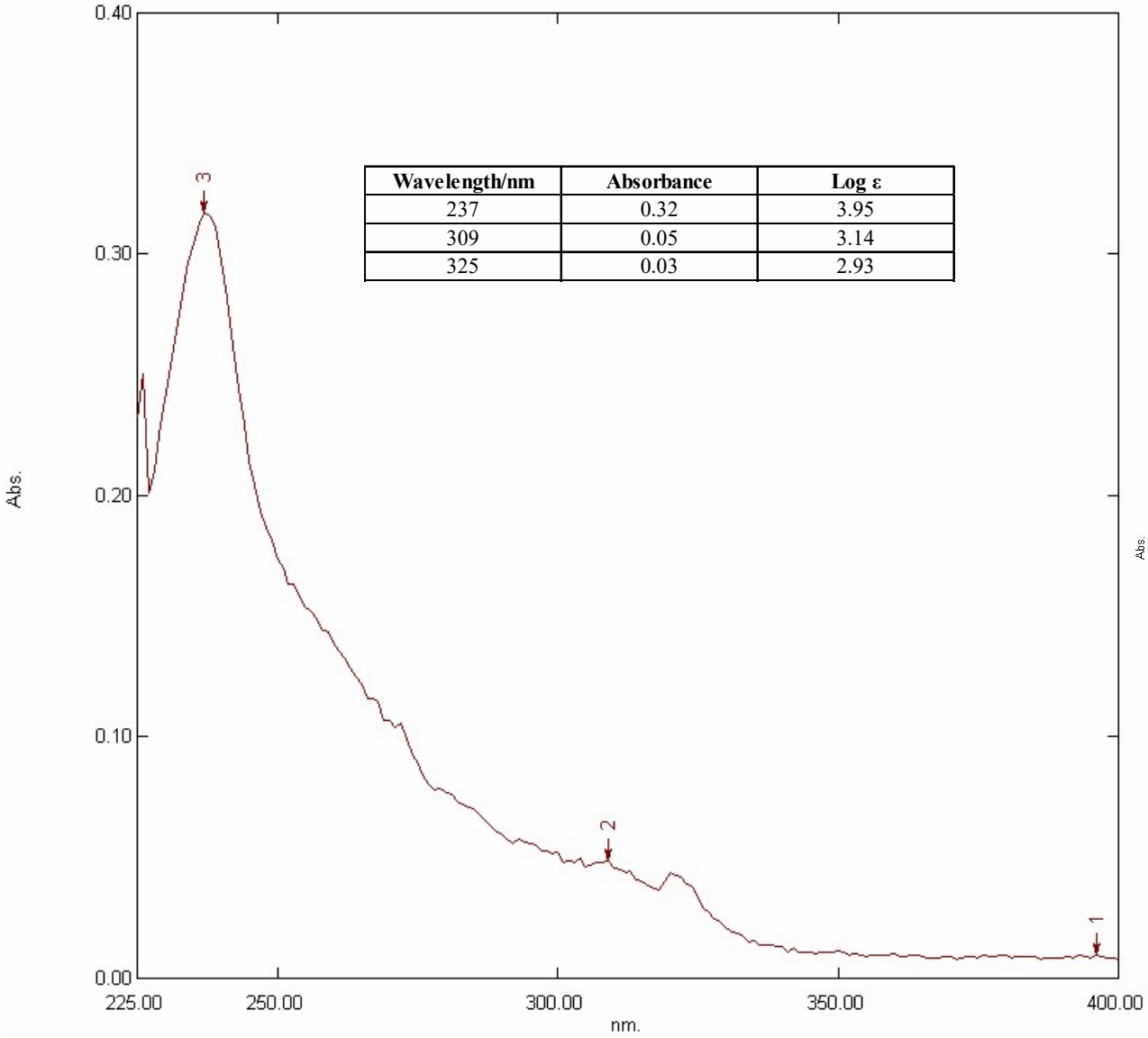


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
305.0459	305.0458	0.1	0.3	11.5	558.5	0.0	C16 H11 N2 O Na Cl

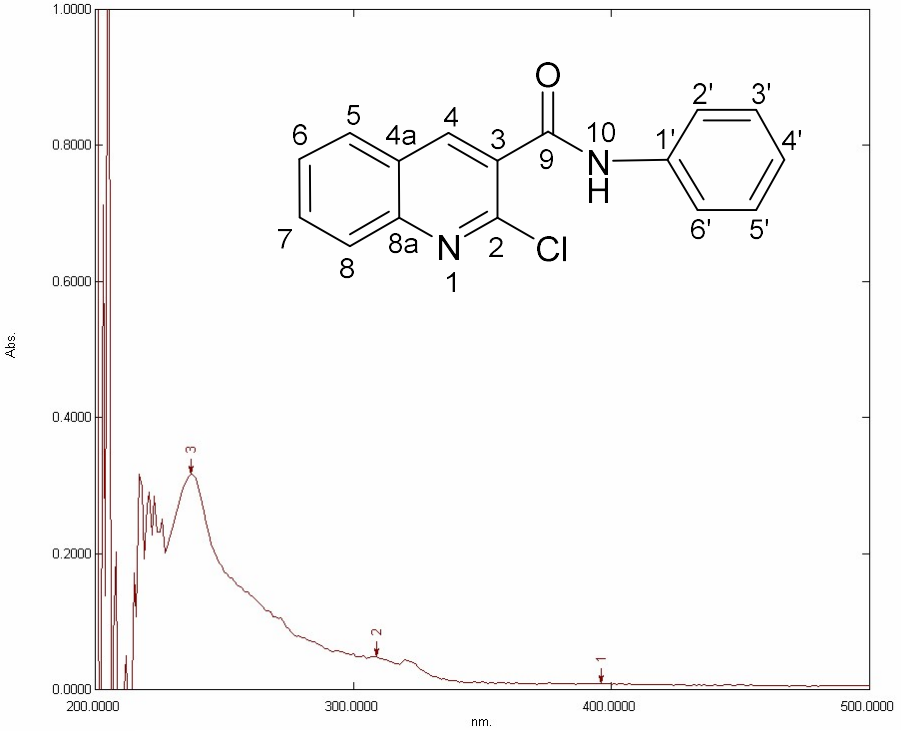
The HRMS spectrum of compound 5a



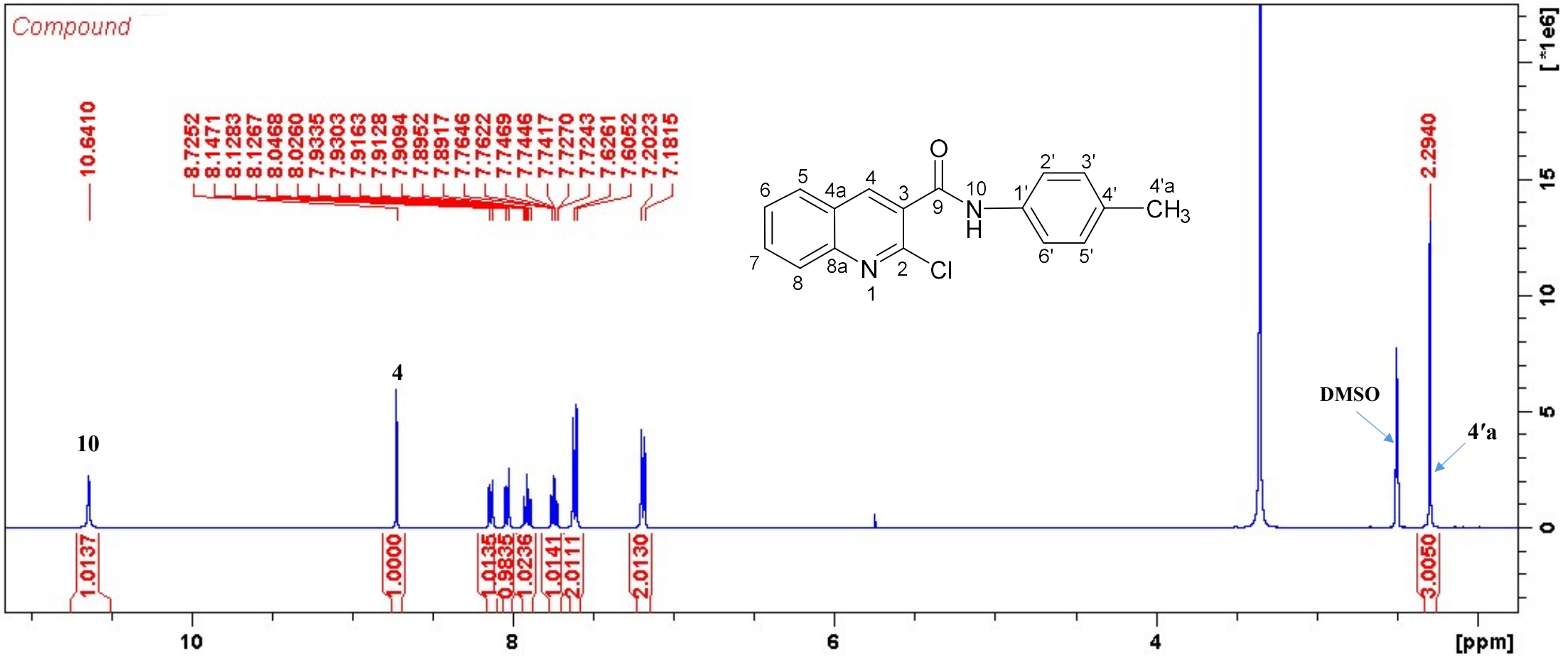
The IR spectrum of compound 5a



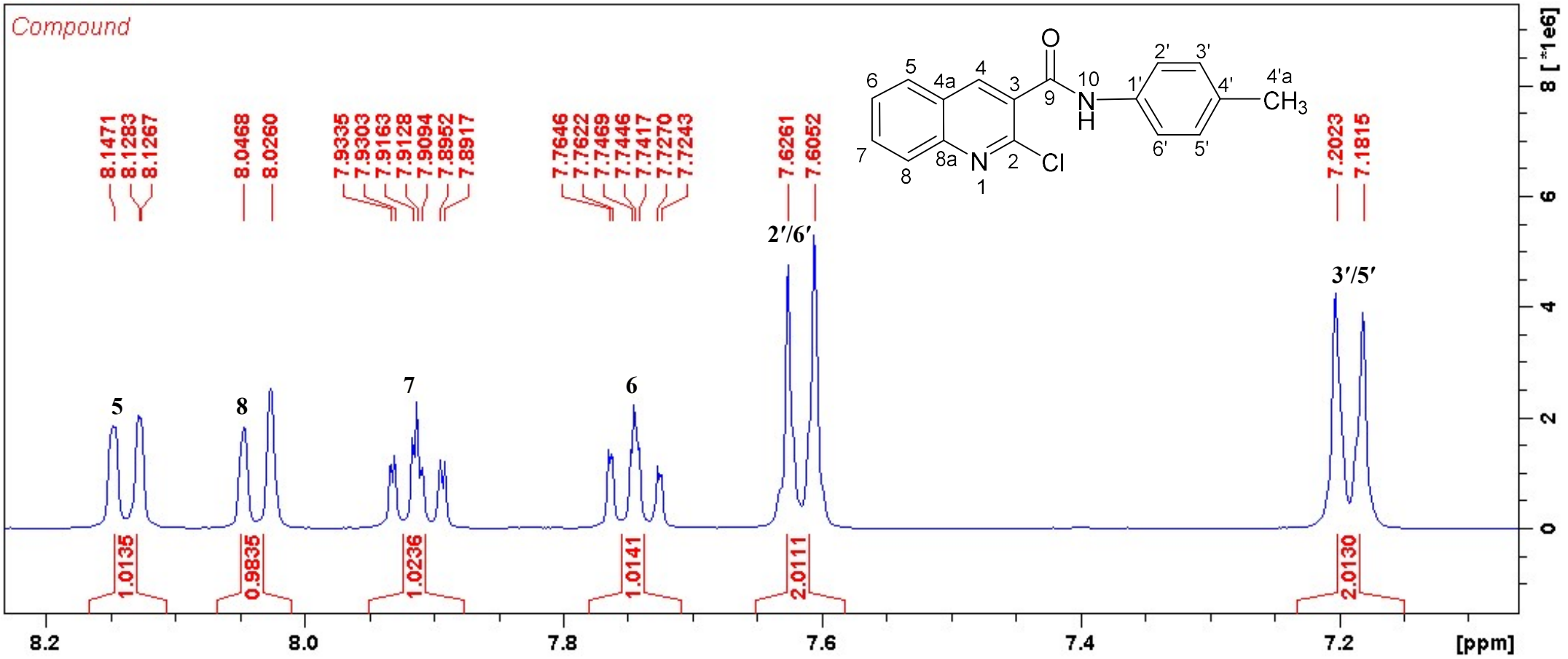
Wavelength/nm	Absorbance	Log ε
237	0.32	3.95
309	0.05	3.14
325	0.03	2.93



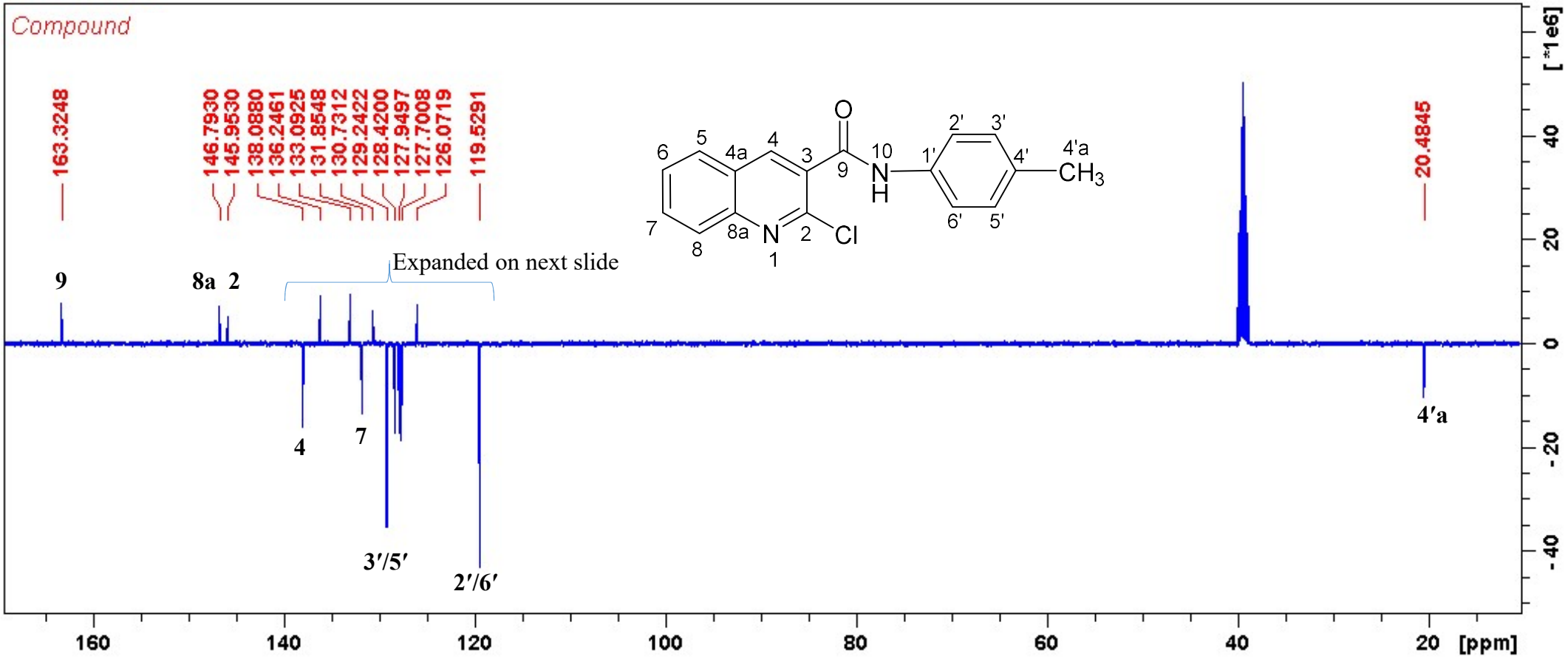
The UV-Vis spectrum of compound 5a



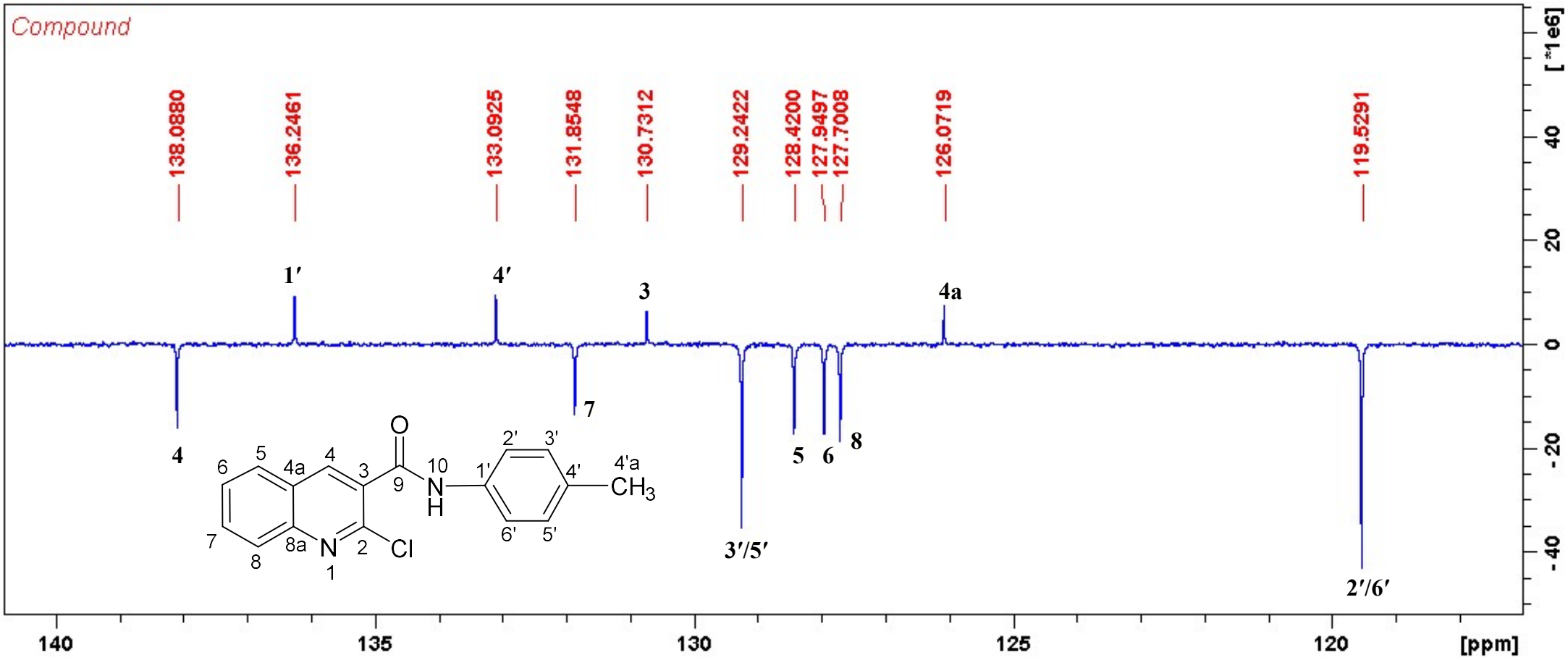
The ^{1}H NMR spectrum of compound 5b



The ^{1}H NMR spectrum of compound 5b (expanded)



The ^{13}C NMR spectrum of compound 5b

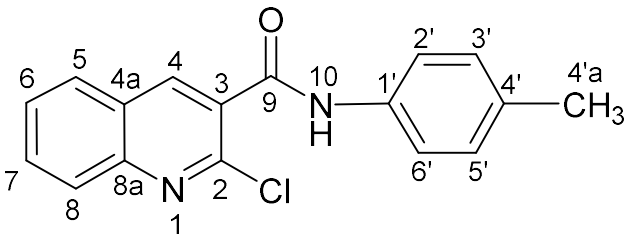


The ^{13}C NMR spectrum of compound 5b (expanded)

Elemental Composition Report

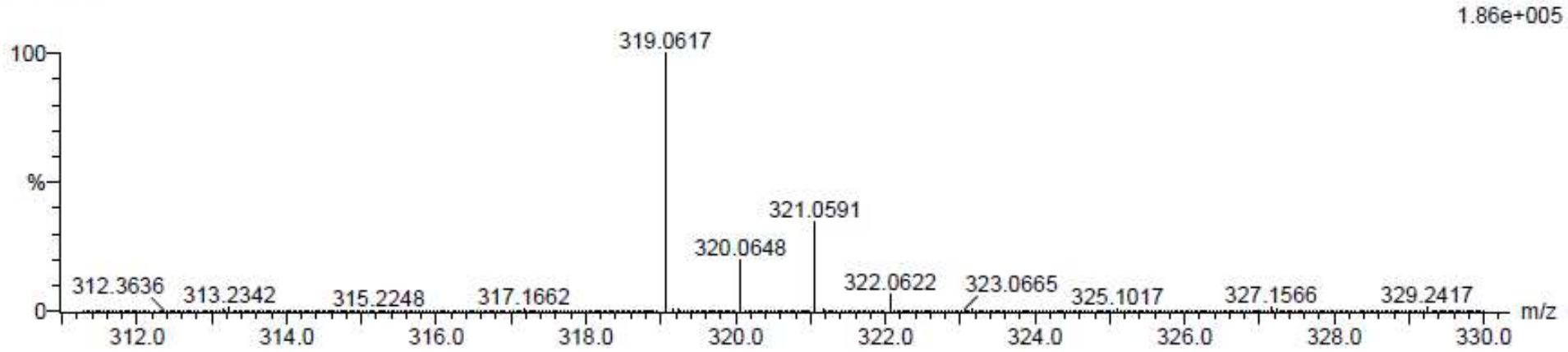
Single Mass Analysis

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



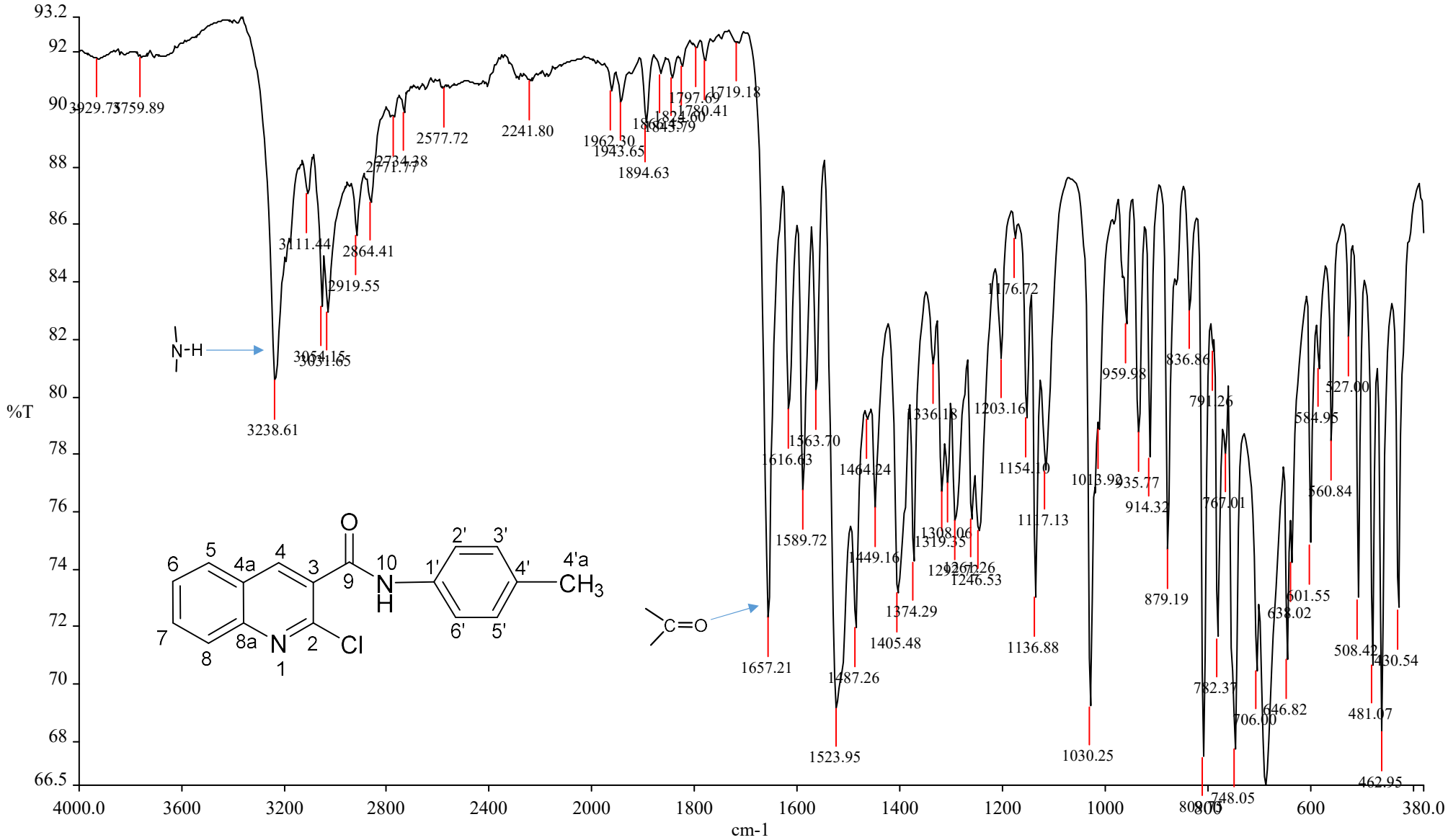
Monoisotopic Mass, Even Electron Ions
39 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-1

1b HG 60 (1.993) Cm (1:61)
TOF MS ES+

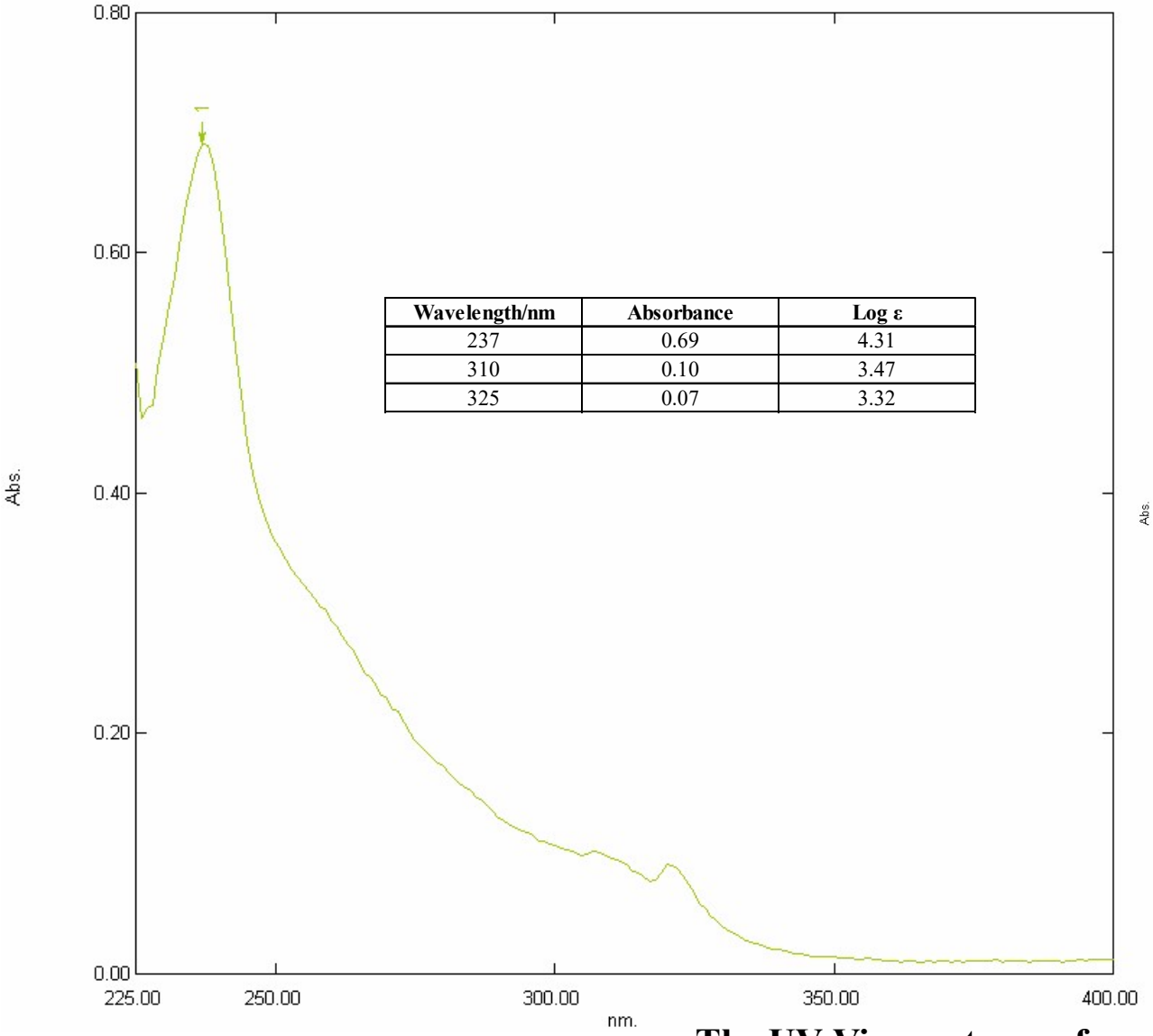


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
319.0617	319.0614	0.3	0.9	11.5	611.8	0.0	C17 H13 N2 O Na Cl

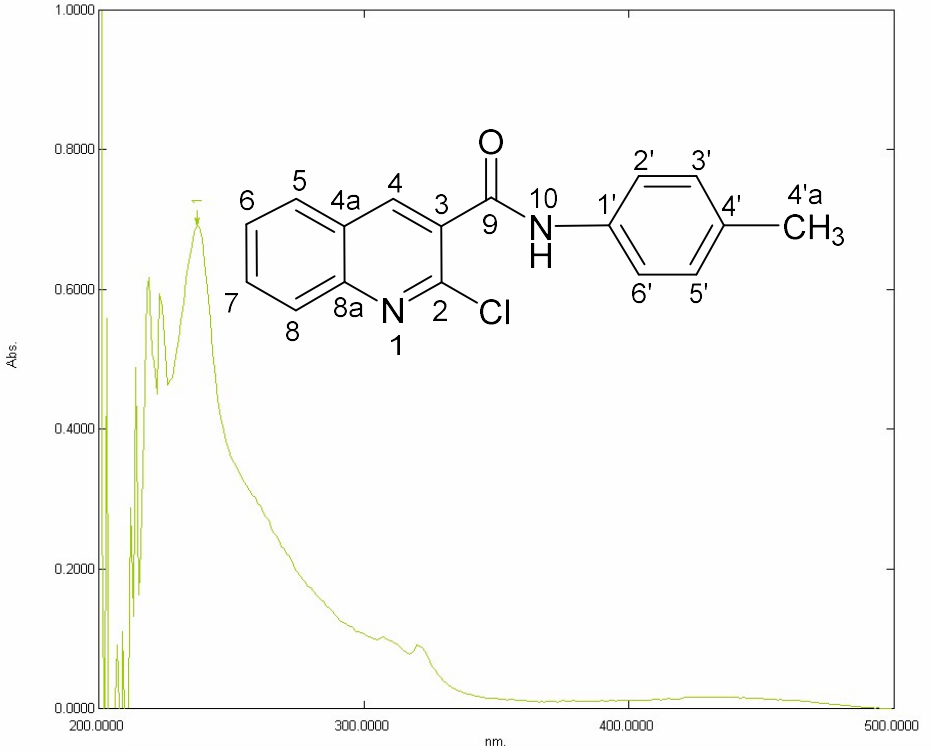
The HRMS spectrum of compound 5b



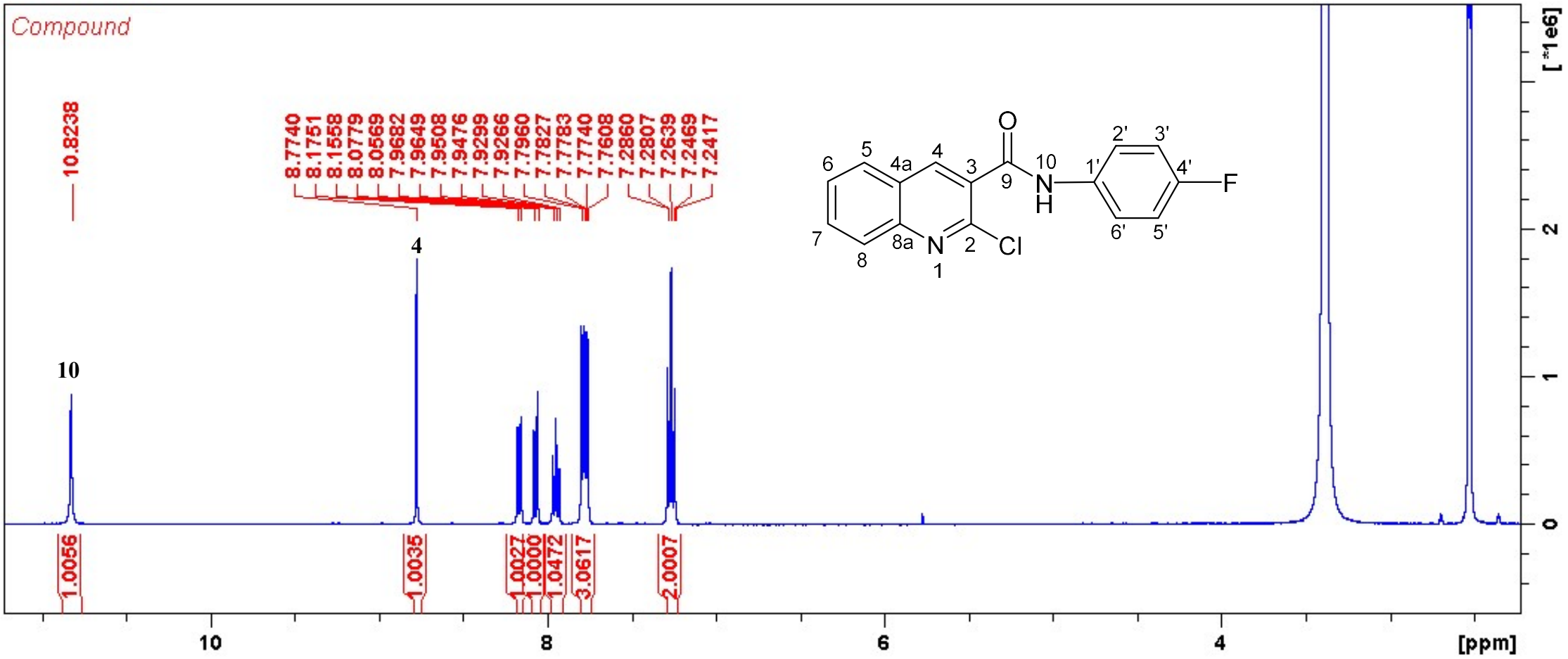
The IR spectrum of compound 5b



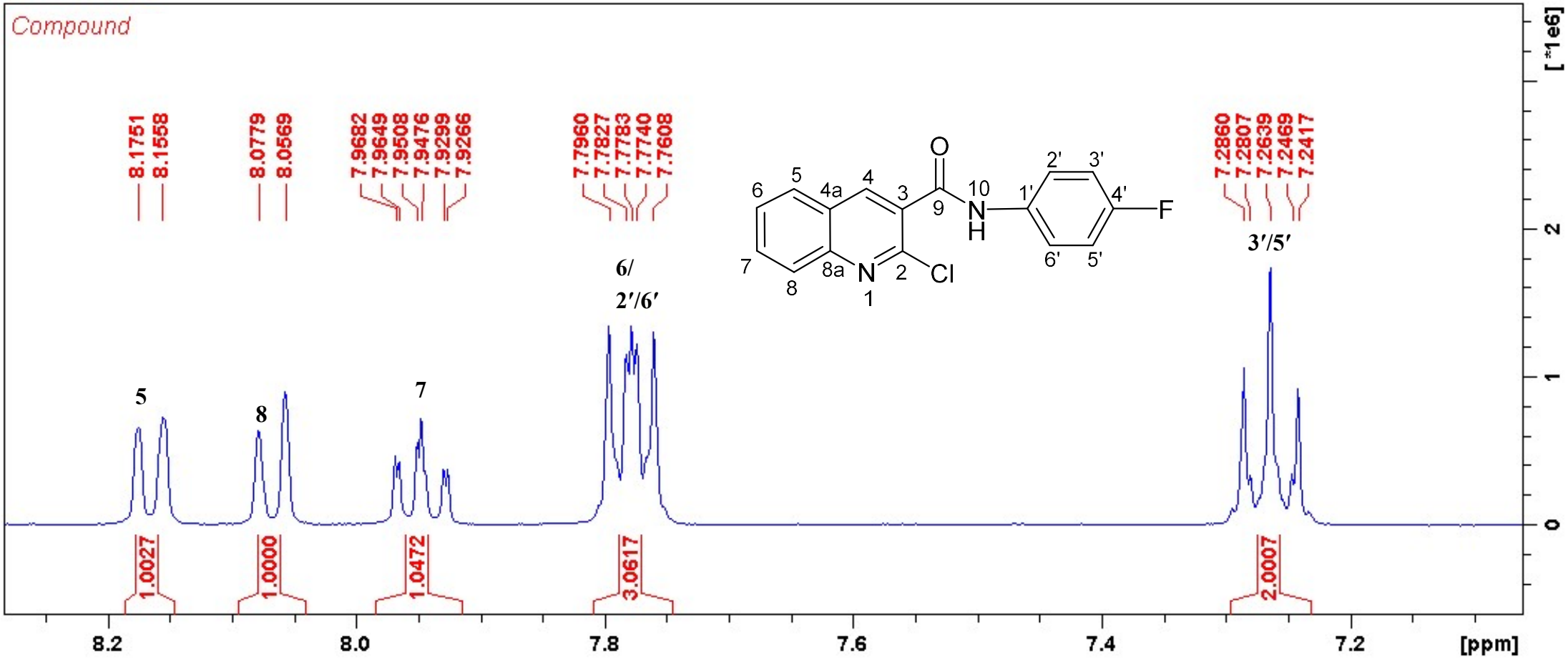
Wavelength/nm	Absorbance	Log ε
237	0.69	4.31
310	0.10	3.47
325	0.07	3.32



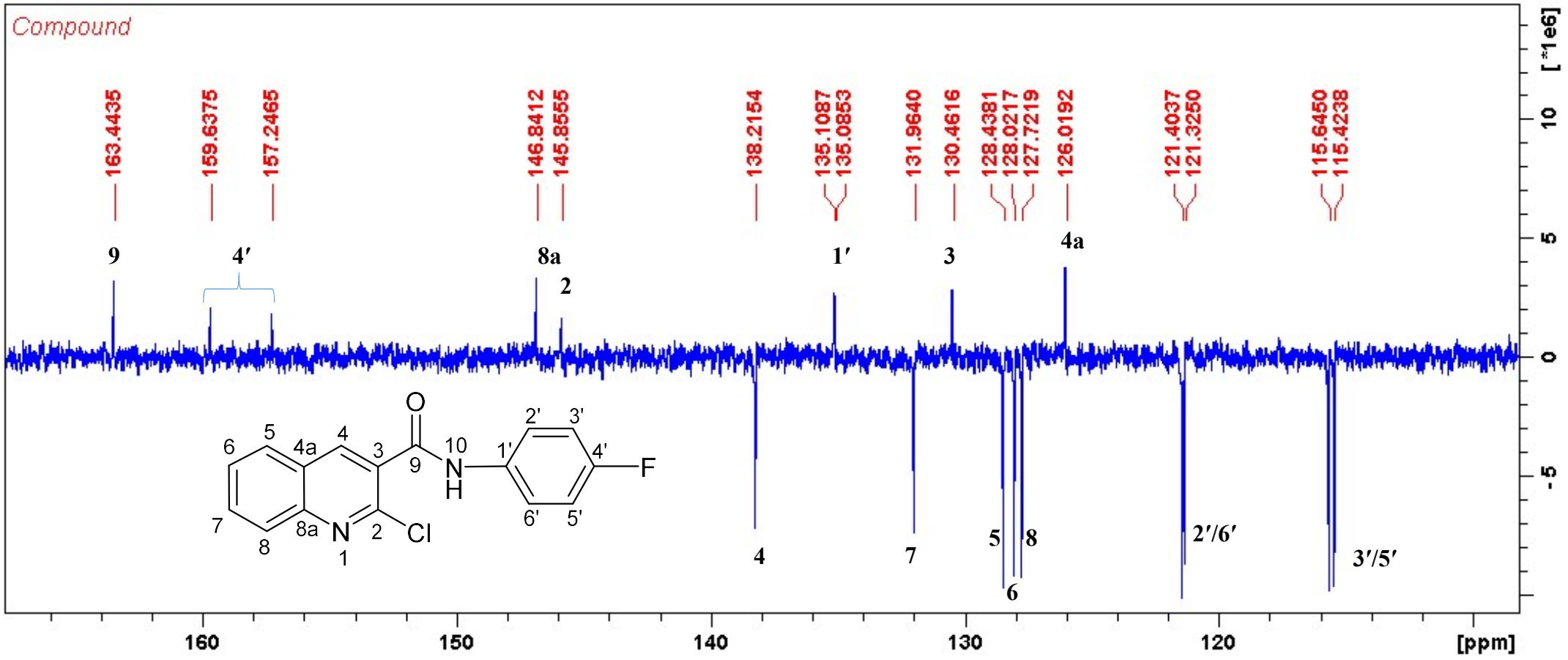
The UV-Vis spectrum of compound 5b



The ^{1}H NMR spectrum of compound 5c



The ^{1}H NMR spectrum of compound 5c (expanded)

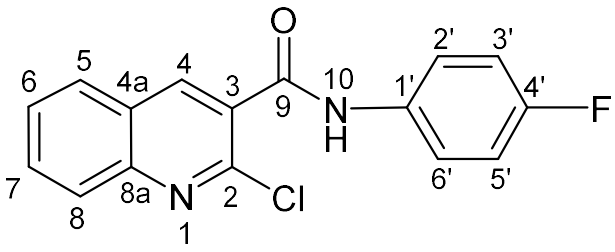


The ^{13}C NMR spectrum of compound 5c

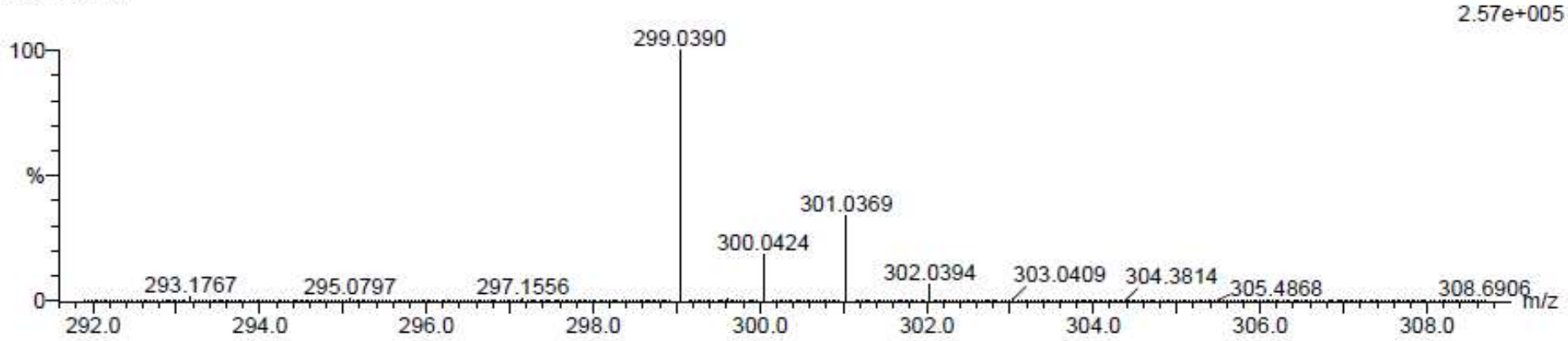
Elemental Composition Report **Page 1**

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

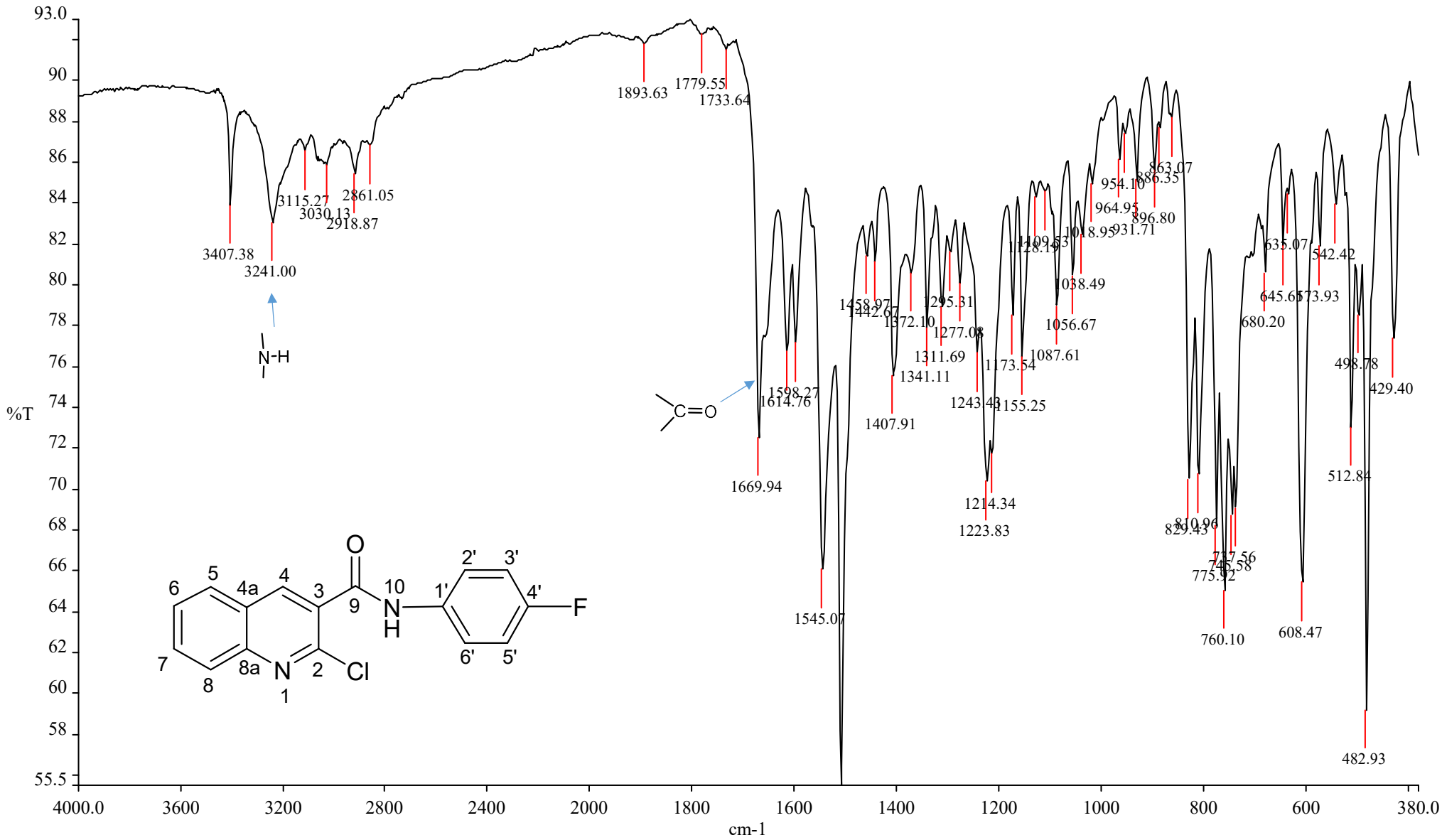


Monoisotopic Mass, Even Electron Ions
74 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 0-5 O: 1-5 F: 0-1 Cl: 0-1
Compound 4e-5c 25 (0.810) Cm (1:61)
TOF MS ES-

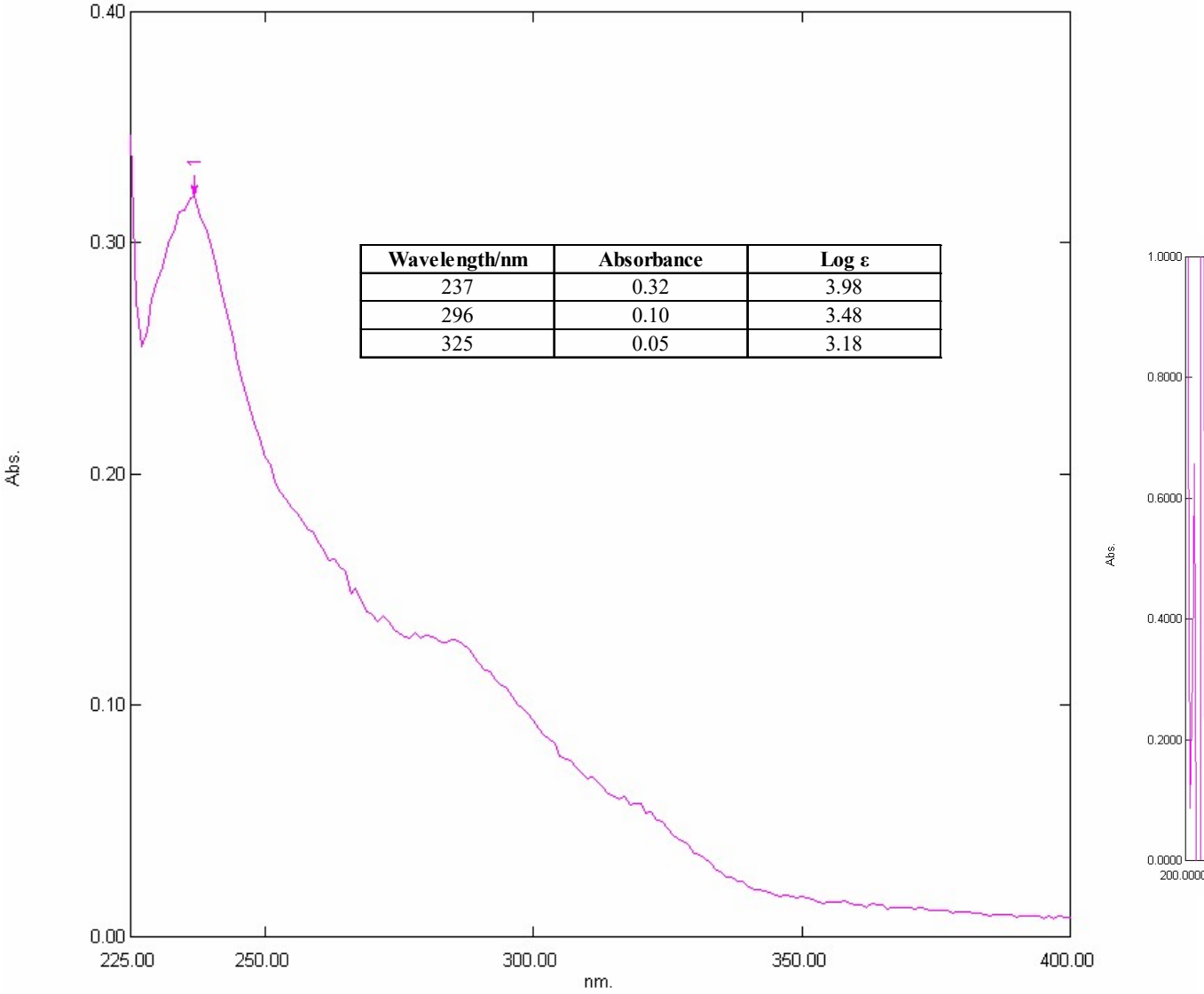


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
299.0390	299.0387	0.3	1.0	12.5	647.9	0.0	C16 H9 N2 O F Cl

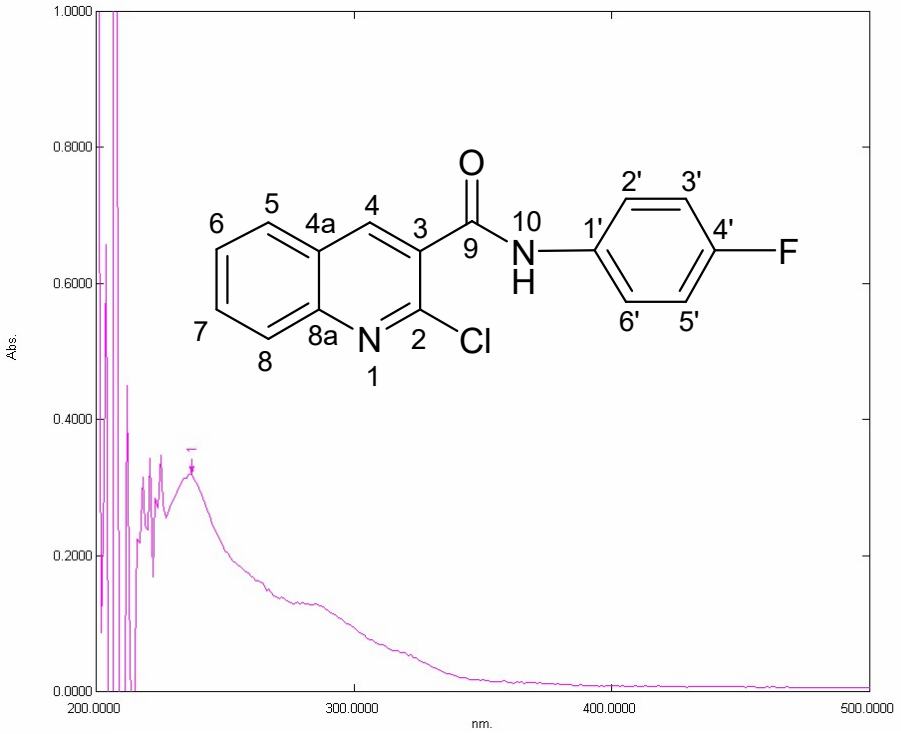
The HRMS spectrum of compound 5c



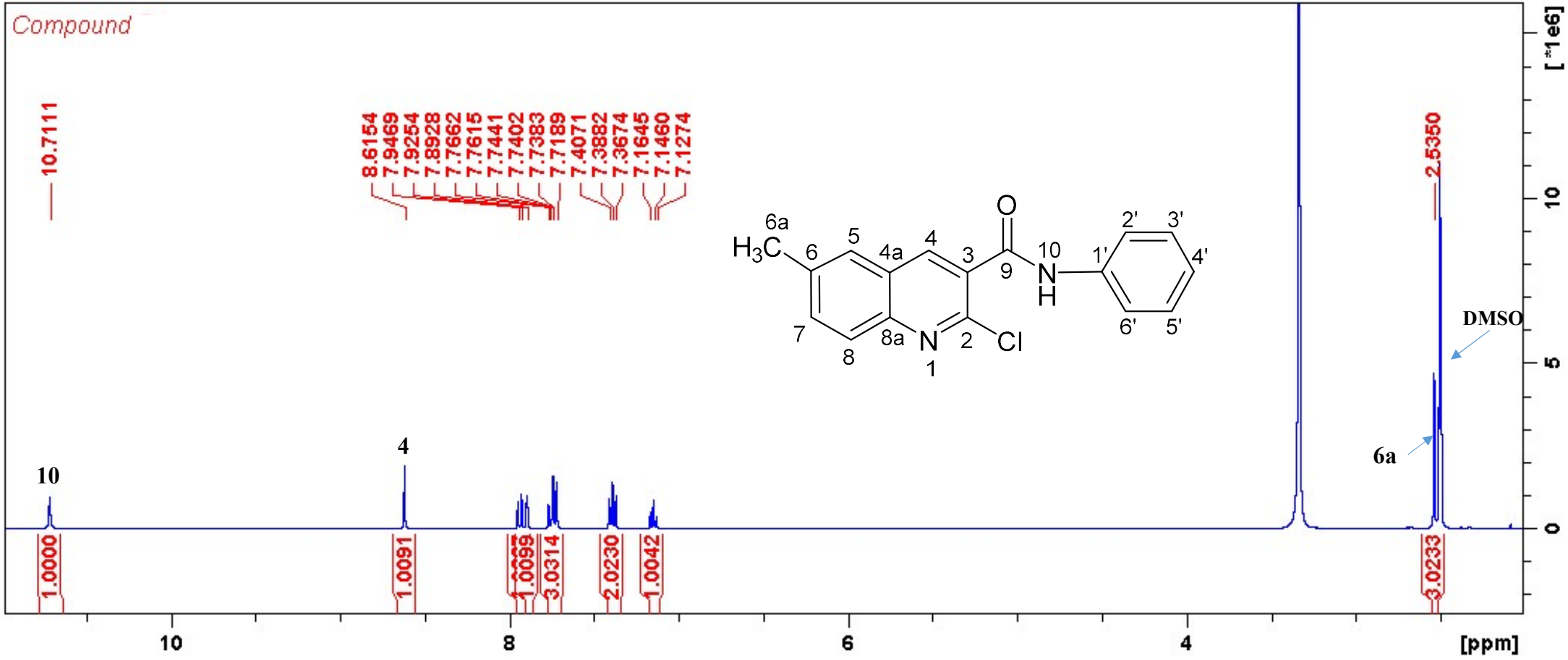
The IR spectrum of compound 5c



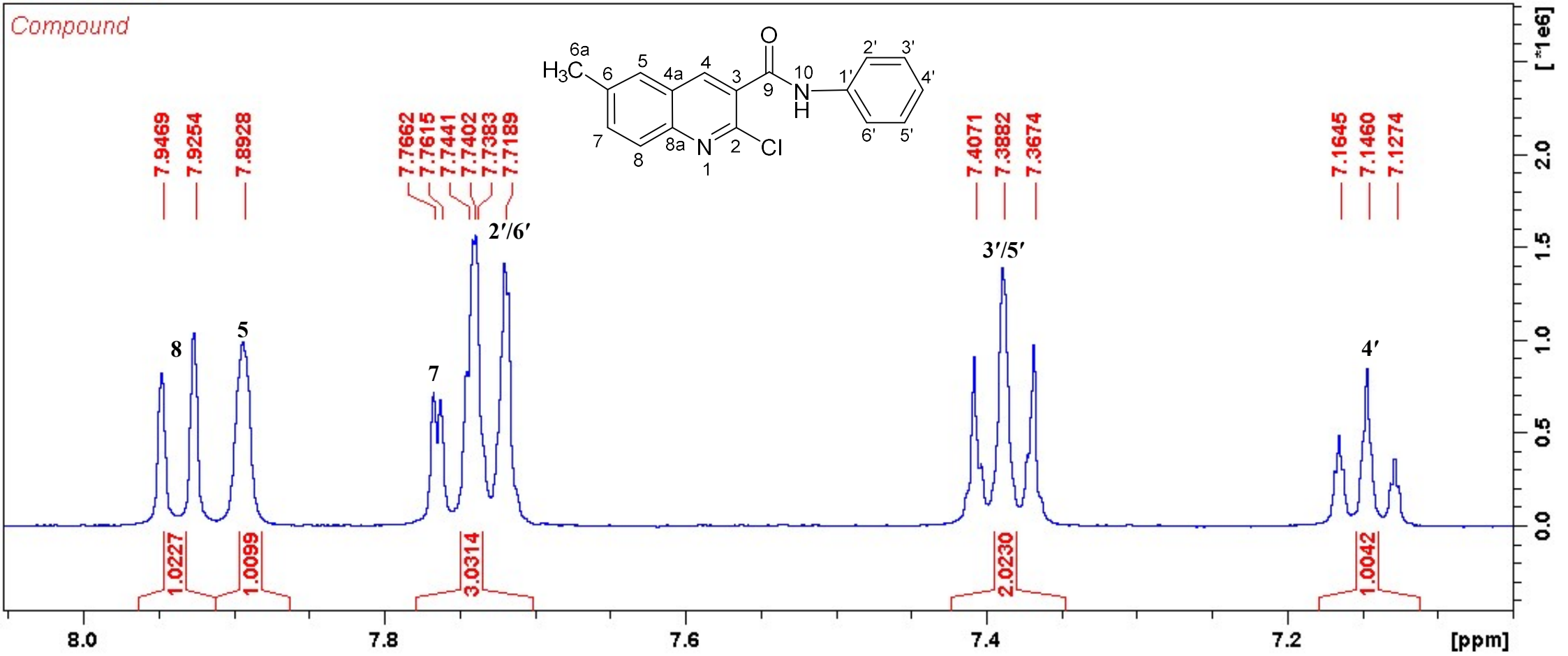
Wavelength/nm	Absorbance	Log ε
237	0.32	3.98
296	0.10	3.48
325	0.05	3.18



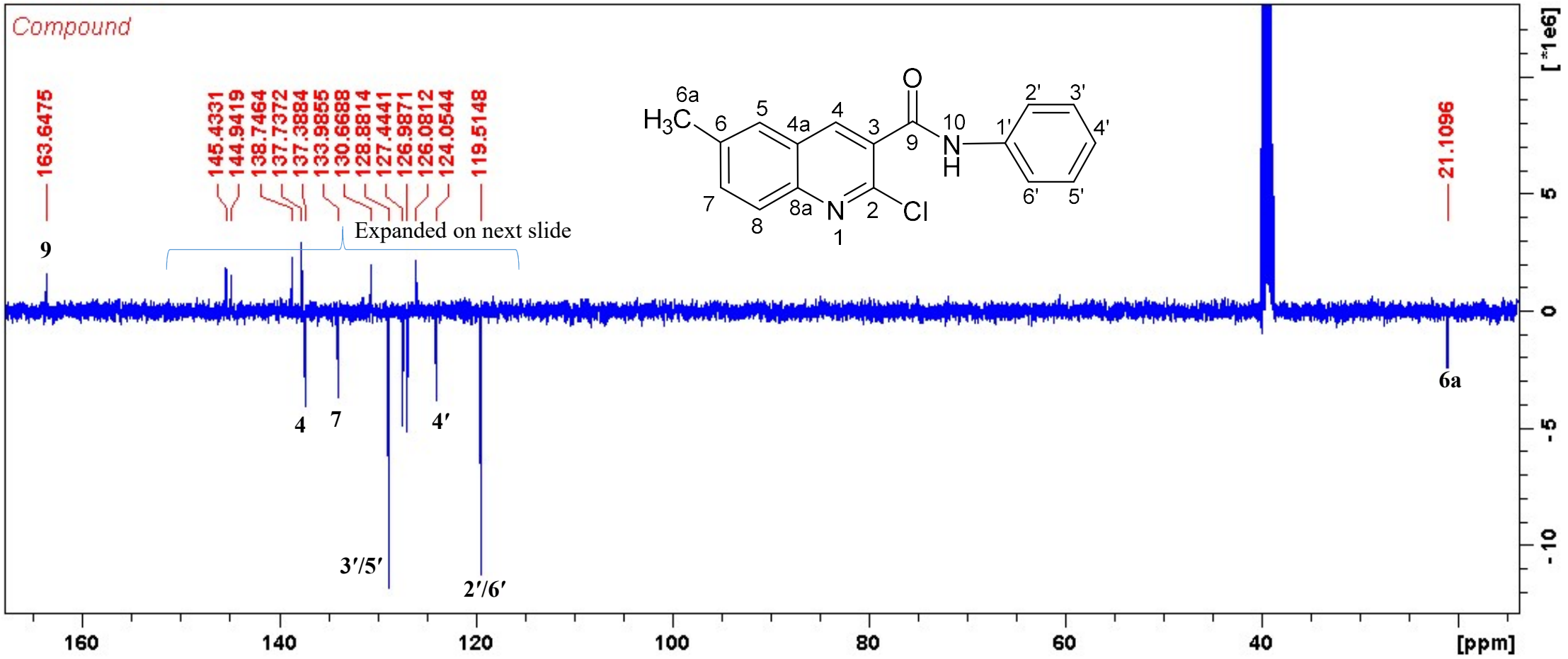
The UV-Vis spectrum of compound 5c



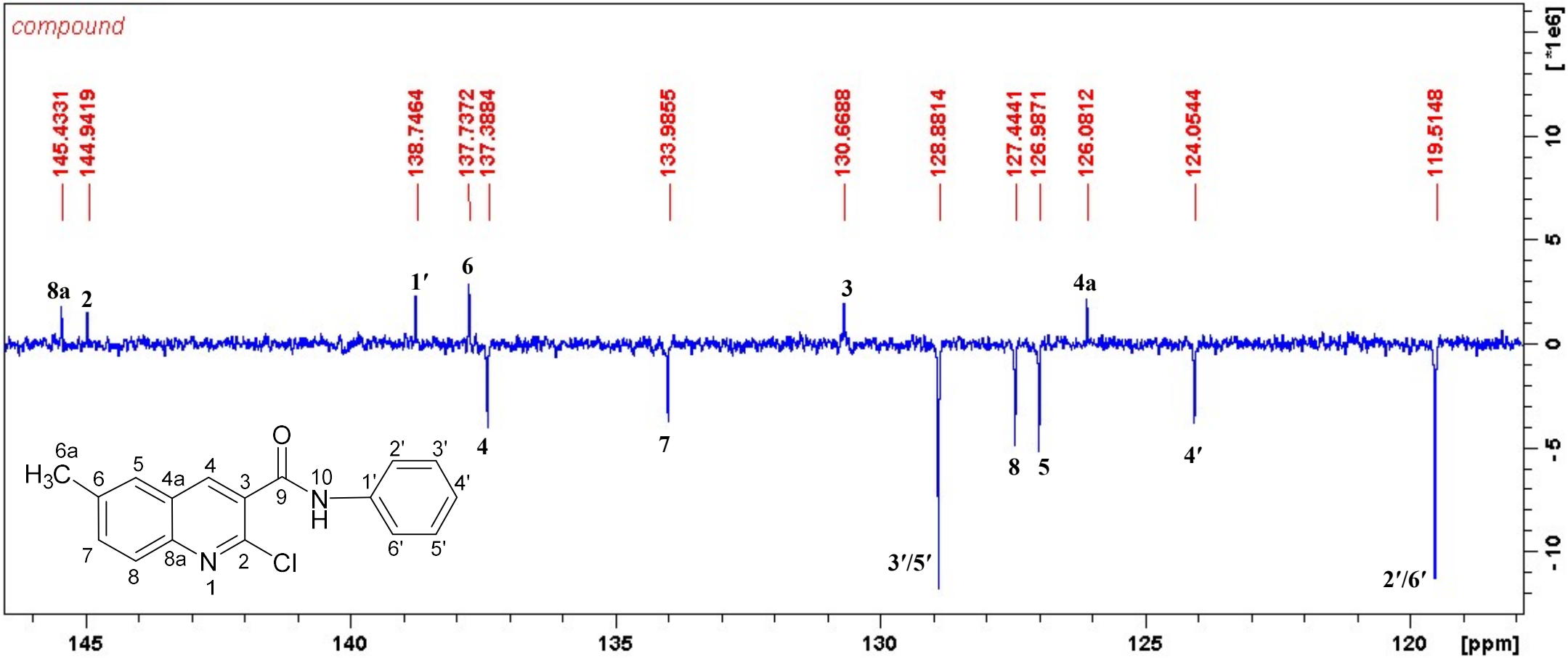
The ^{1}H NMR spectrum of compound 5d



The ^{1}H NMR spectrum of compound 5d (expanded)



The ^{13}C NMR spectrum of compound 5d

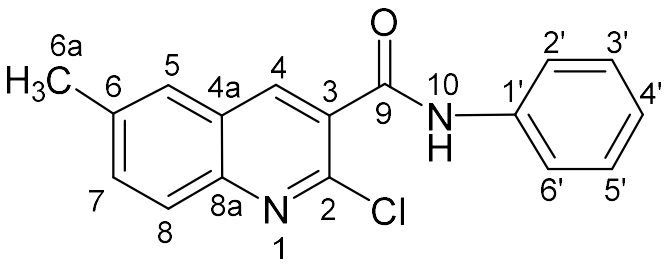


The ^{13}C NMR spectrum of compound 5d (expanded)

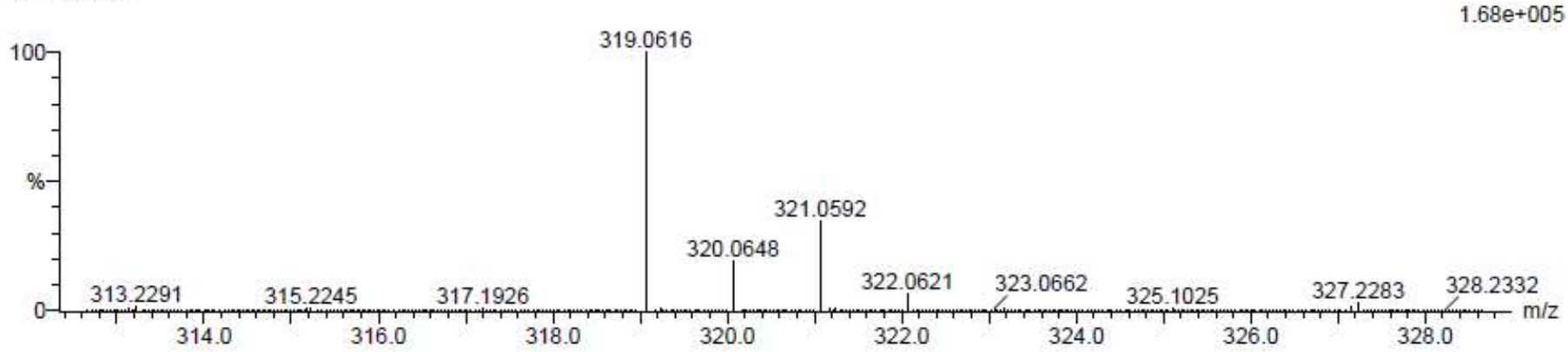
Elemental Composition Report **Page 1**

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

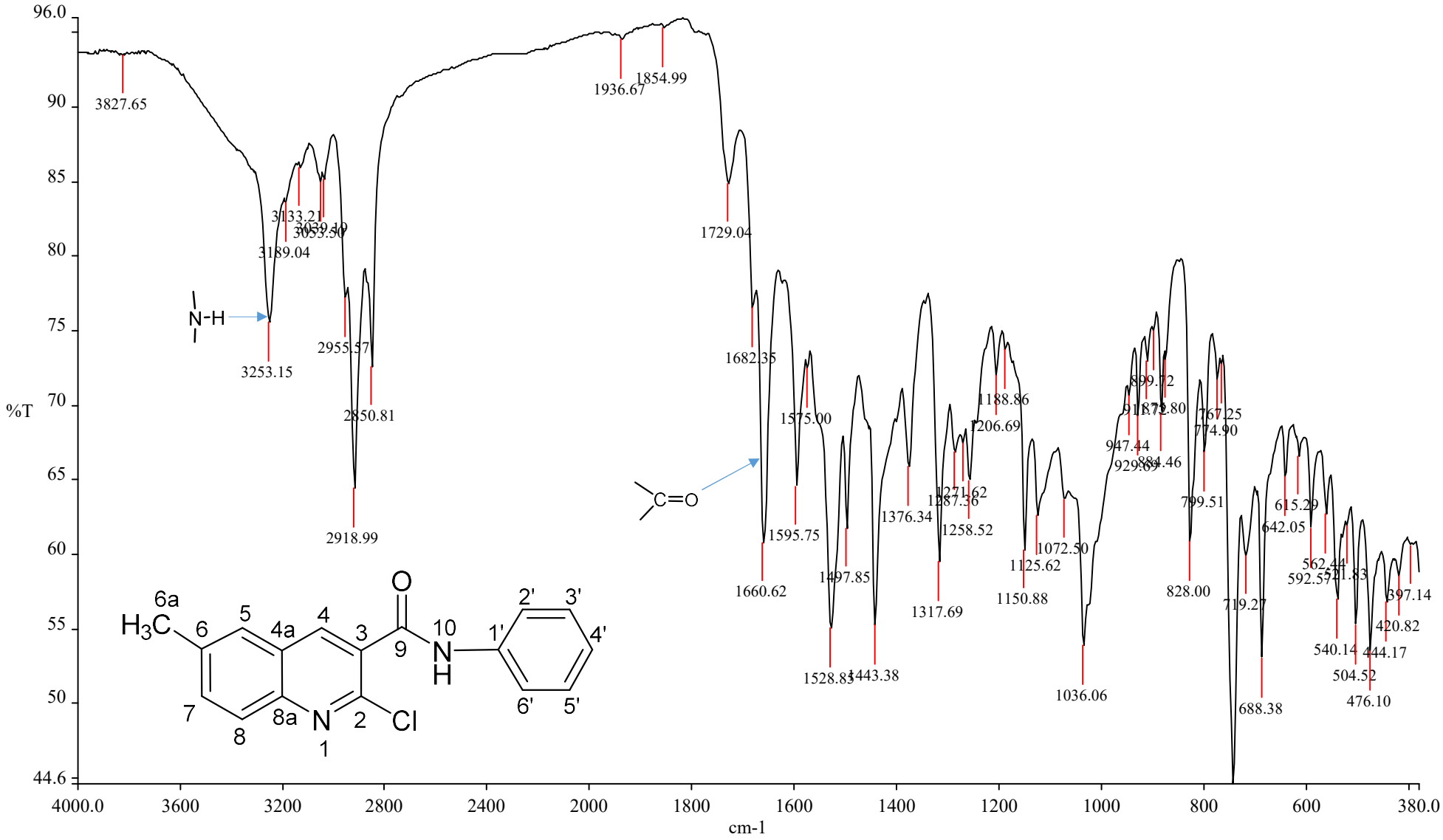


Monoisotopic Mass, Even Electron Ions
39 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-1
6a HG DUP 42 (1.384) Cm (1:61)
TOF MS ES+

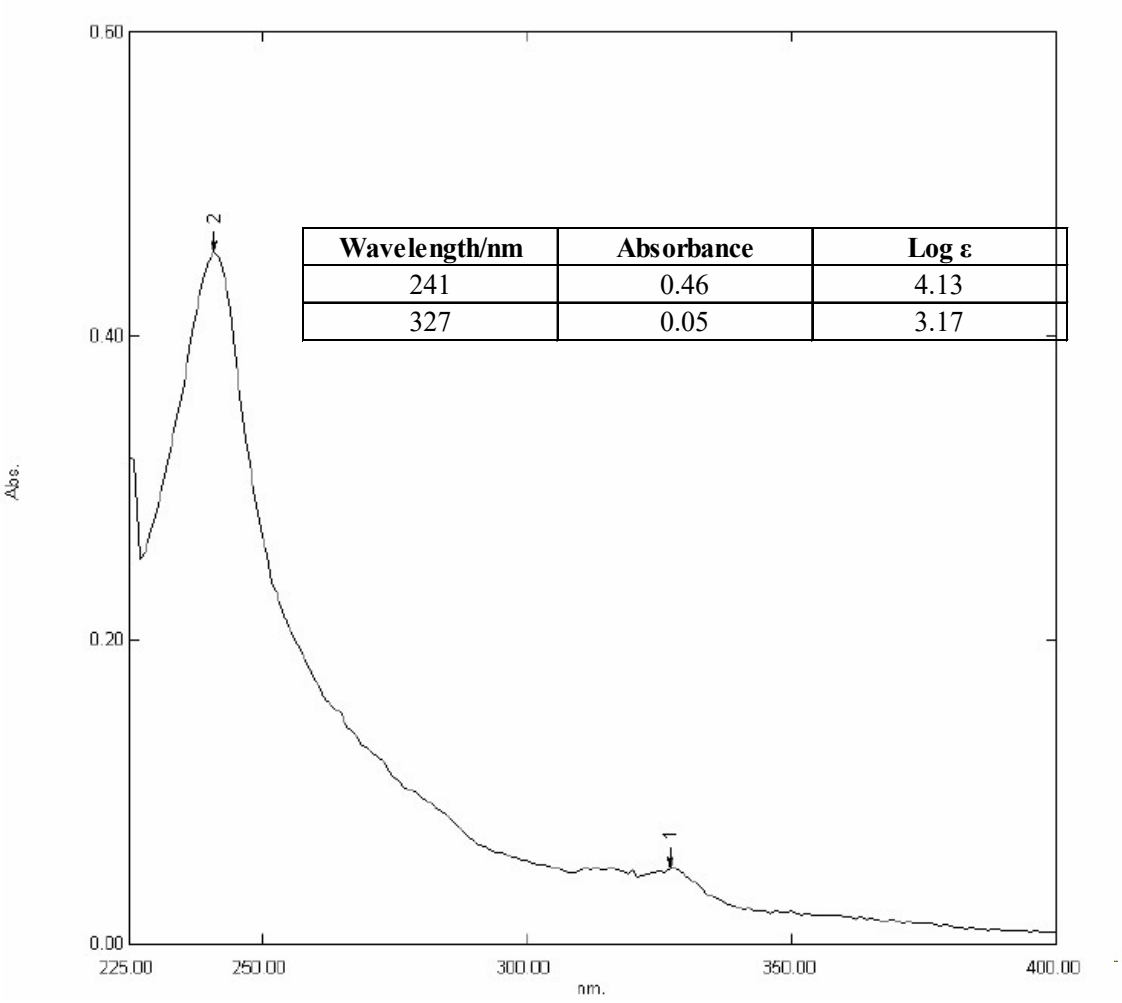


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
319.0616	319.0614	0.2	0.6	11.5	586.2	0.0	C17 H13 N2 O Na Cl

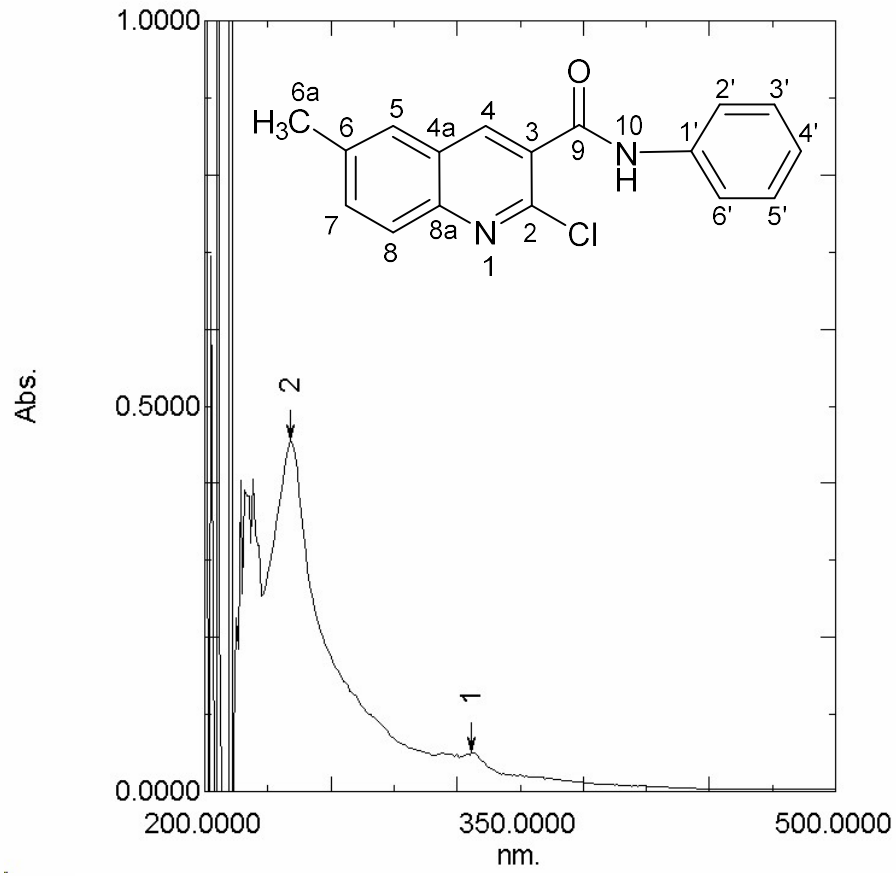
The HRMS spectrum of compound 5d



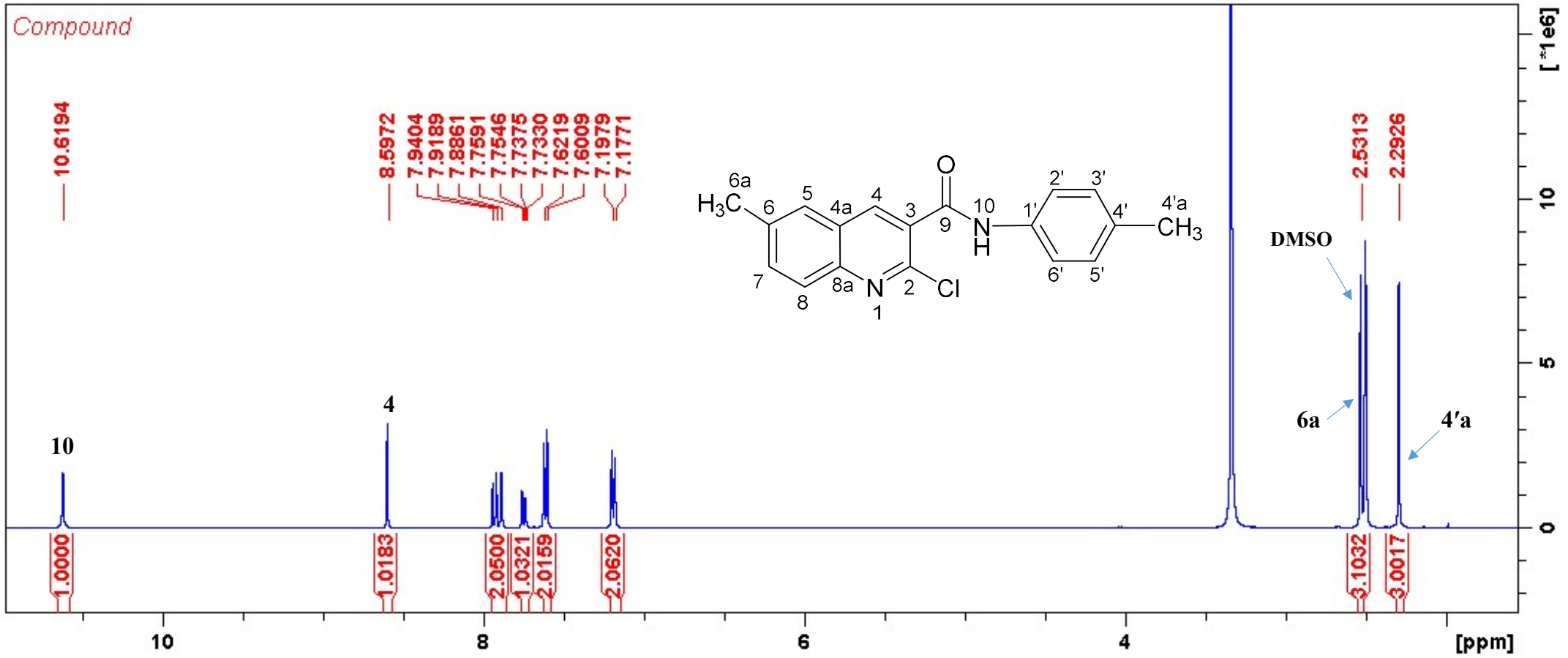
The IR spectrum of compound 5d



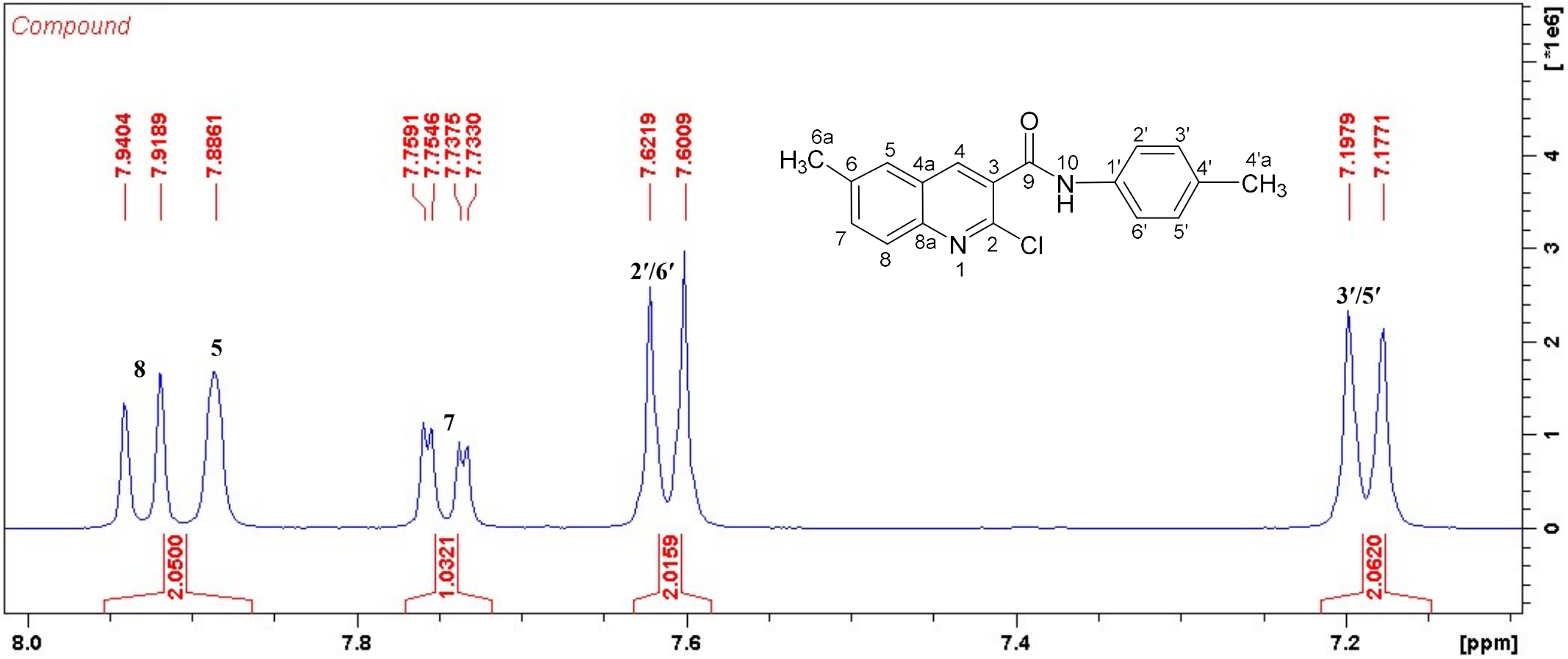
Wavelength/nm	Absorbance	Log ε
241	0.46	4.13
327	0.05	3.17



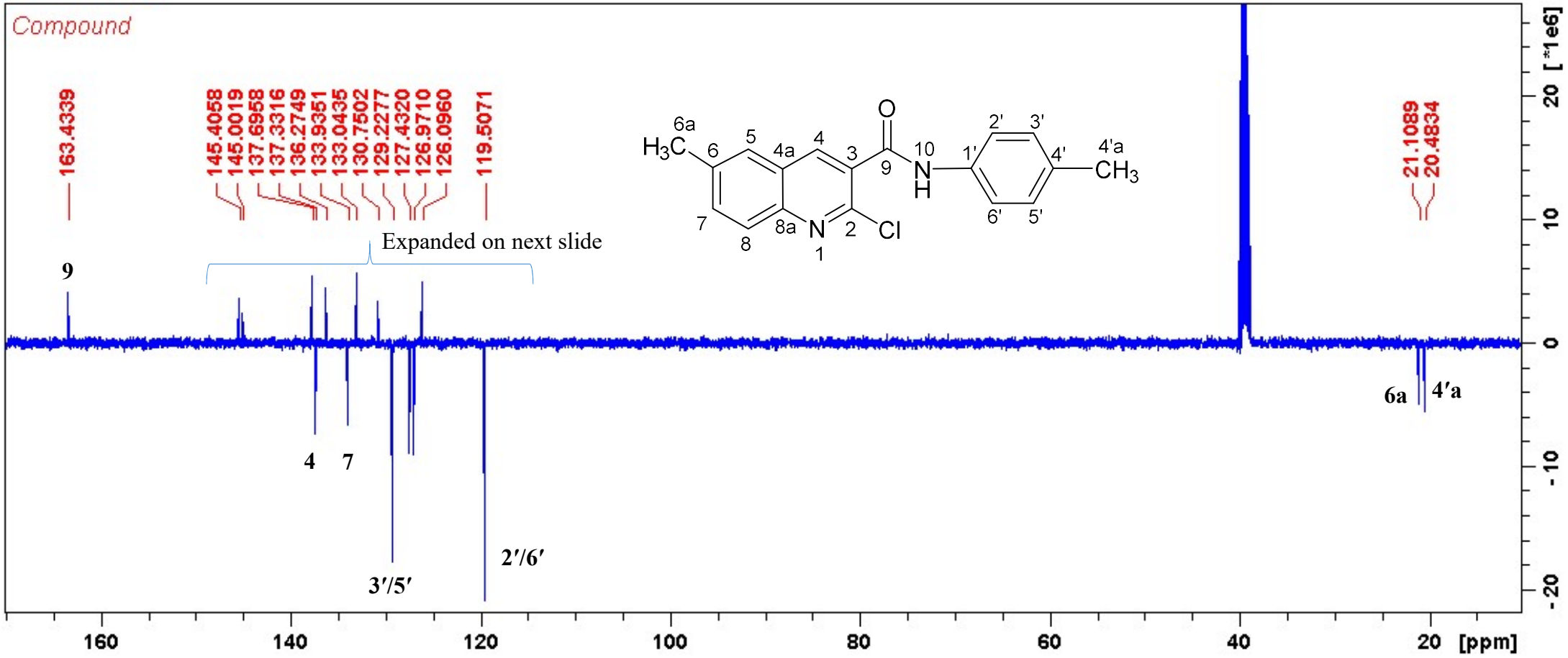
The UV-Vis spectrum of compound 5d



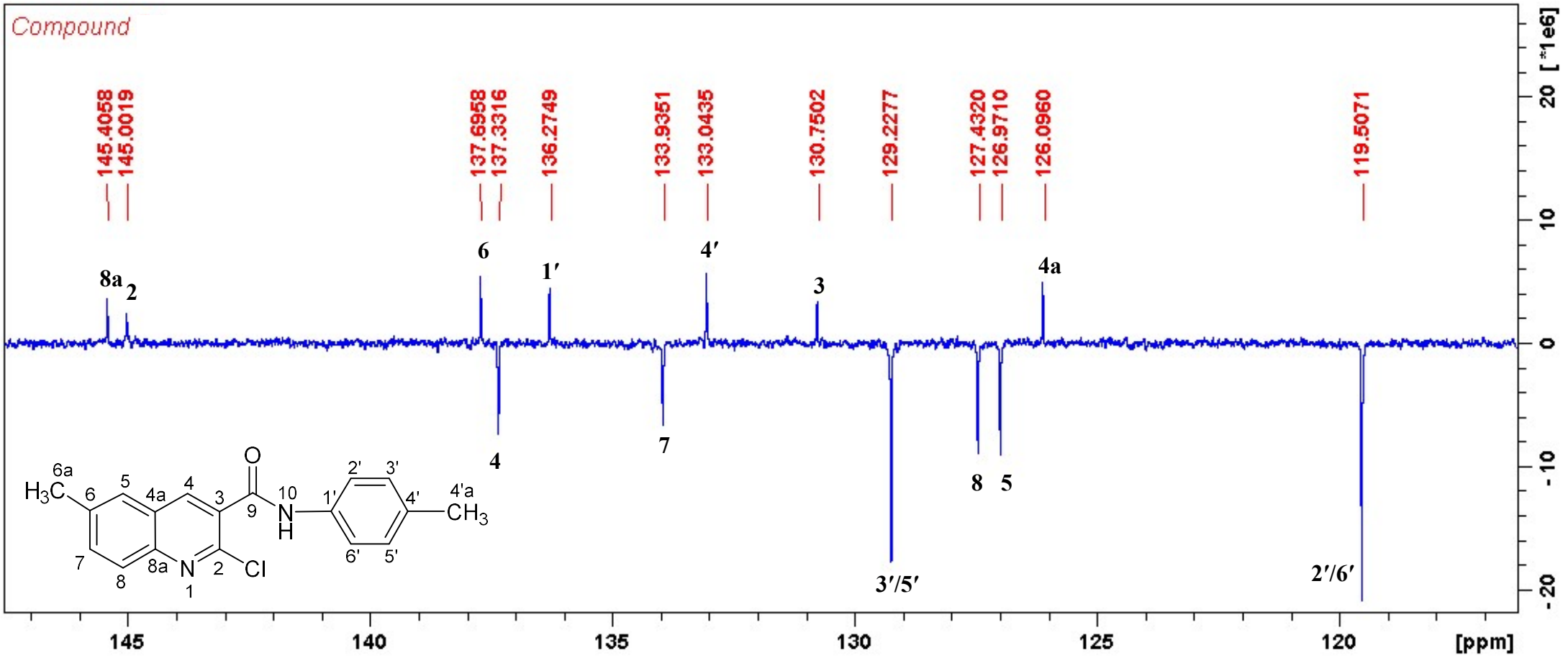
The ^{1}H NMR spectrum of compound 5e



The ^{1}H NMR spectrum of compound 5e (expanded)



The ^{13}C NMR spectrum of compound 5e

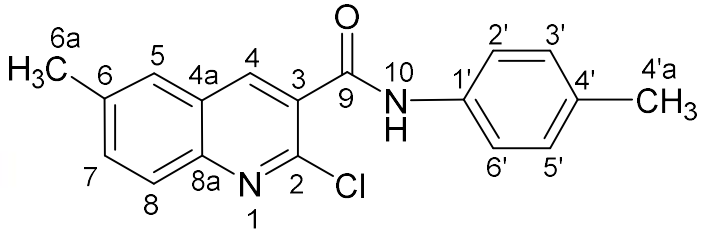


The ^{13}C NMR spectrum of compound 5e (expanded)

Elemental Composition Report

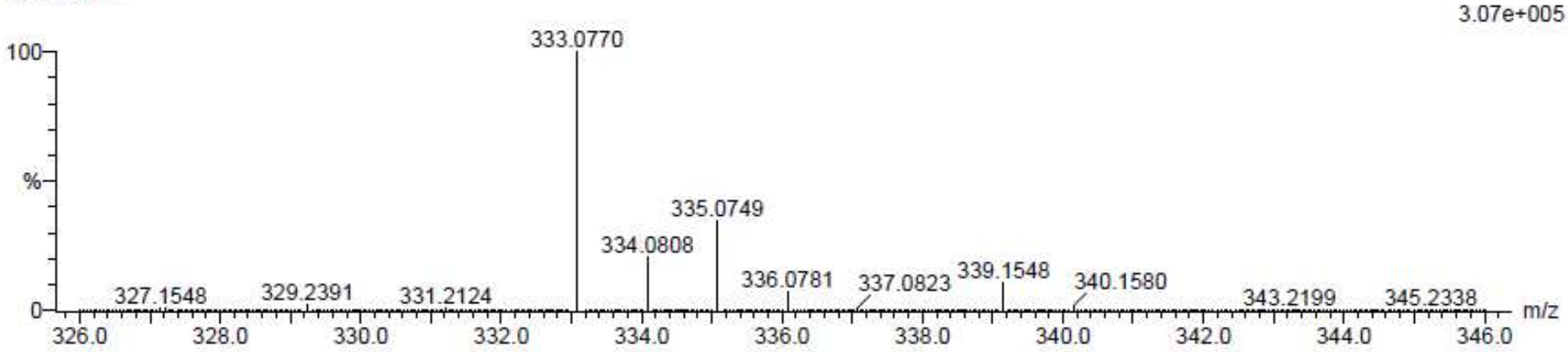
Single Mass Analysis

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



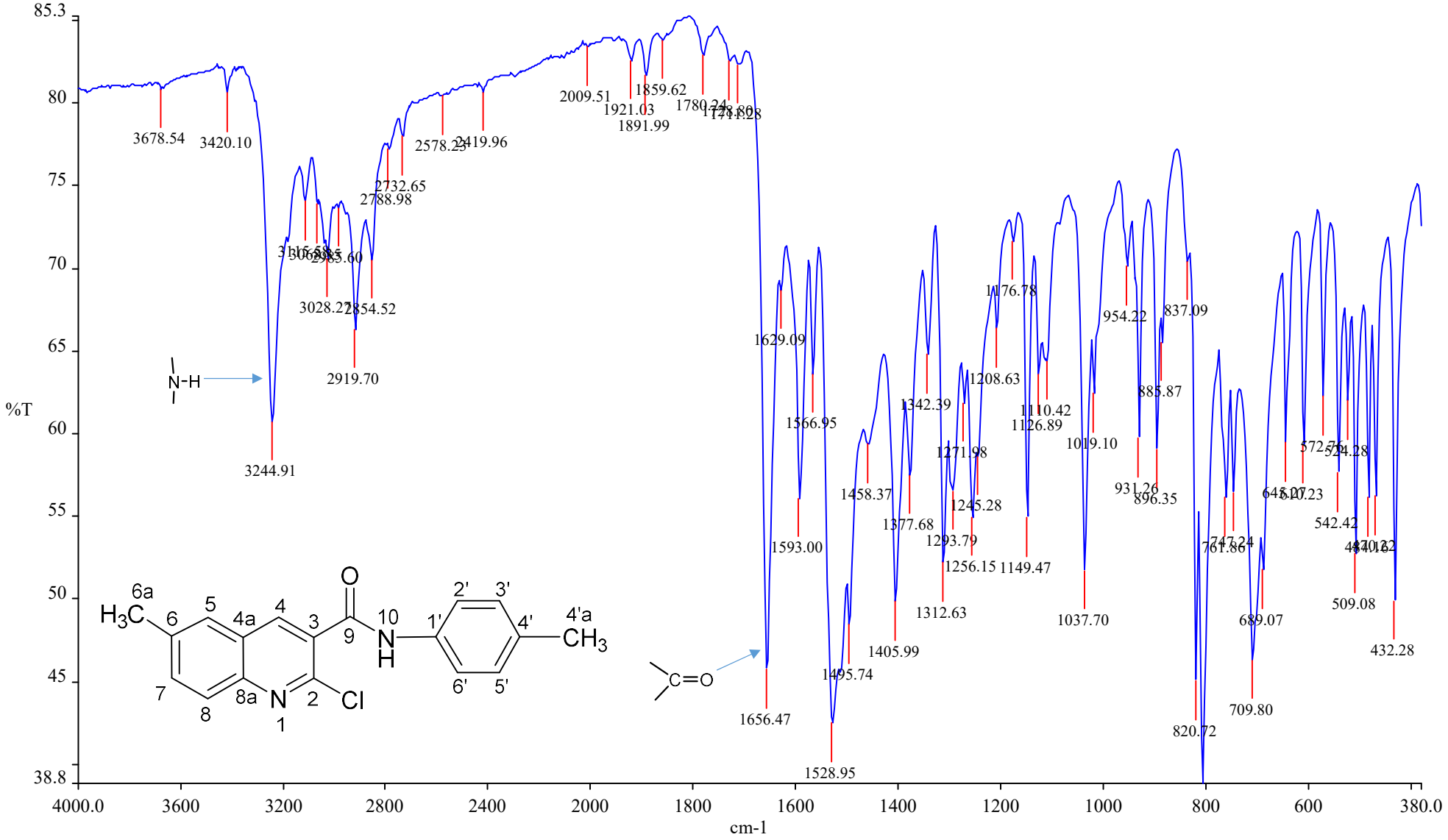
Monoisotopic Mass, Even Electron Ions
35 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-1

5a HG DUP 51 (1.688) Cm (1:61)
TOF MS ES+

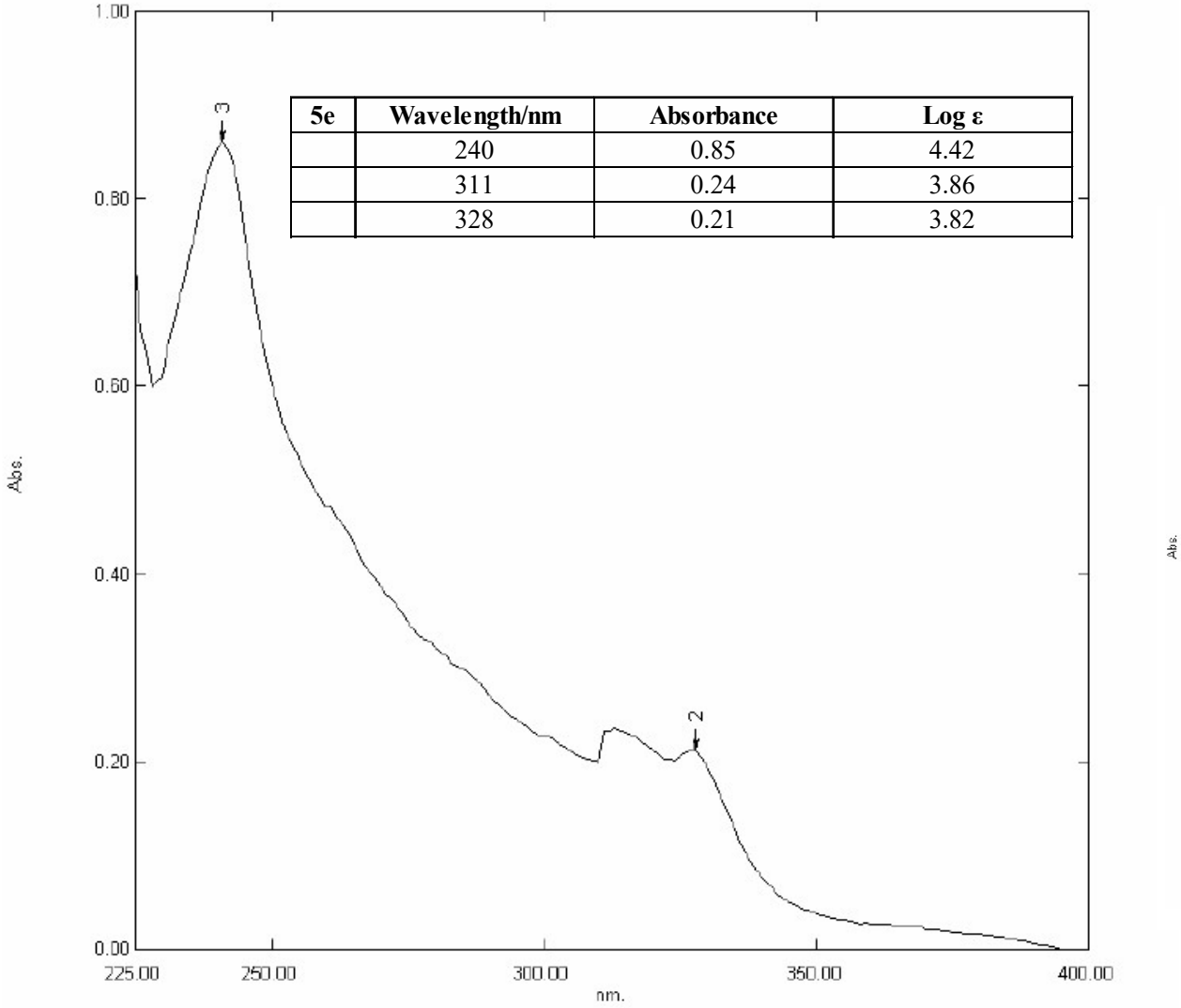


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
333.0770	333.0771	-0.1	-0.3	11.5	647.6	0.0	C18 H15 N2 O Na Cl

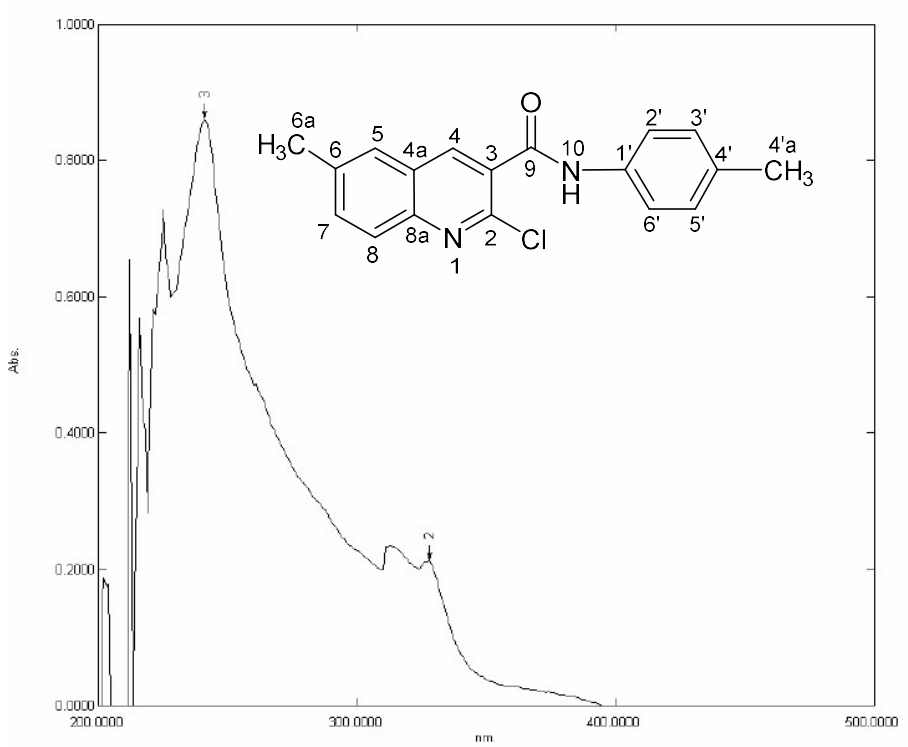
The HRMS spectrum of compound 5e



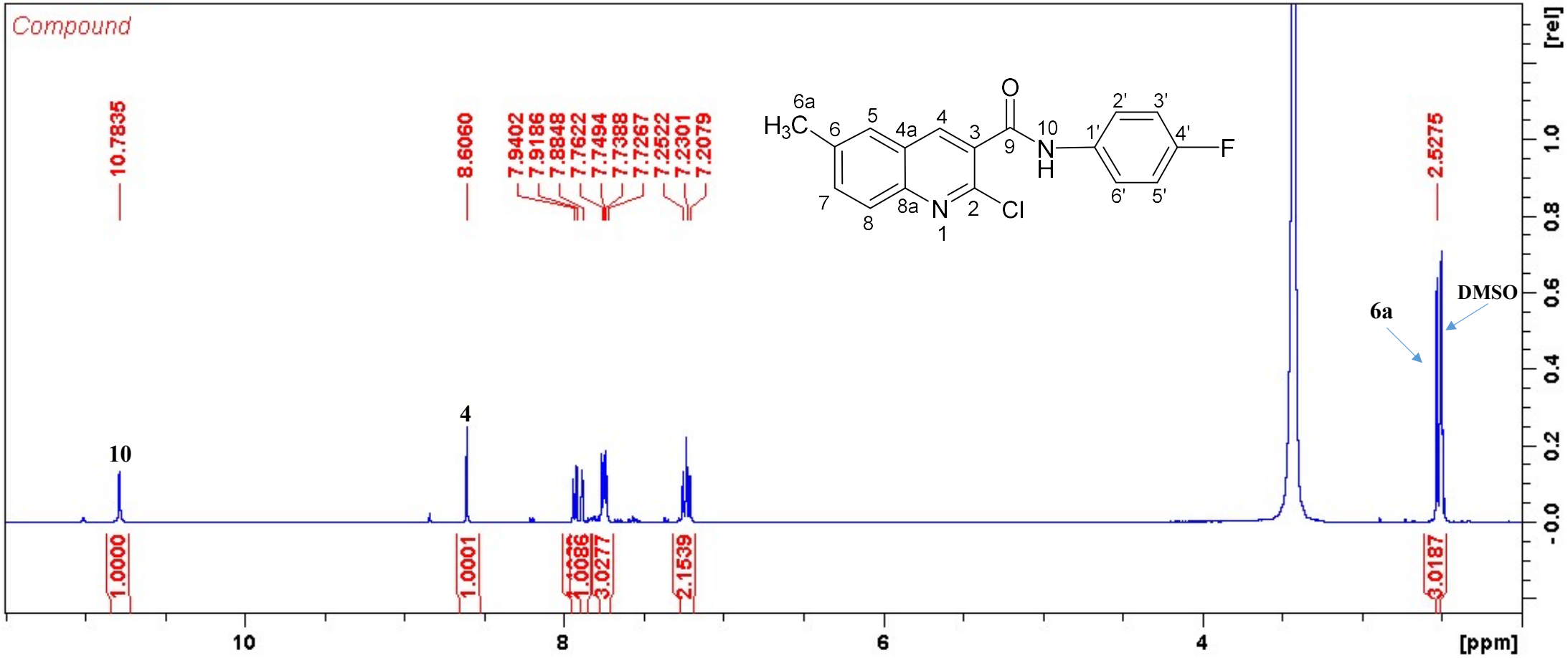
The IR spectrum of compound 5e



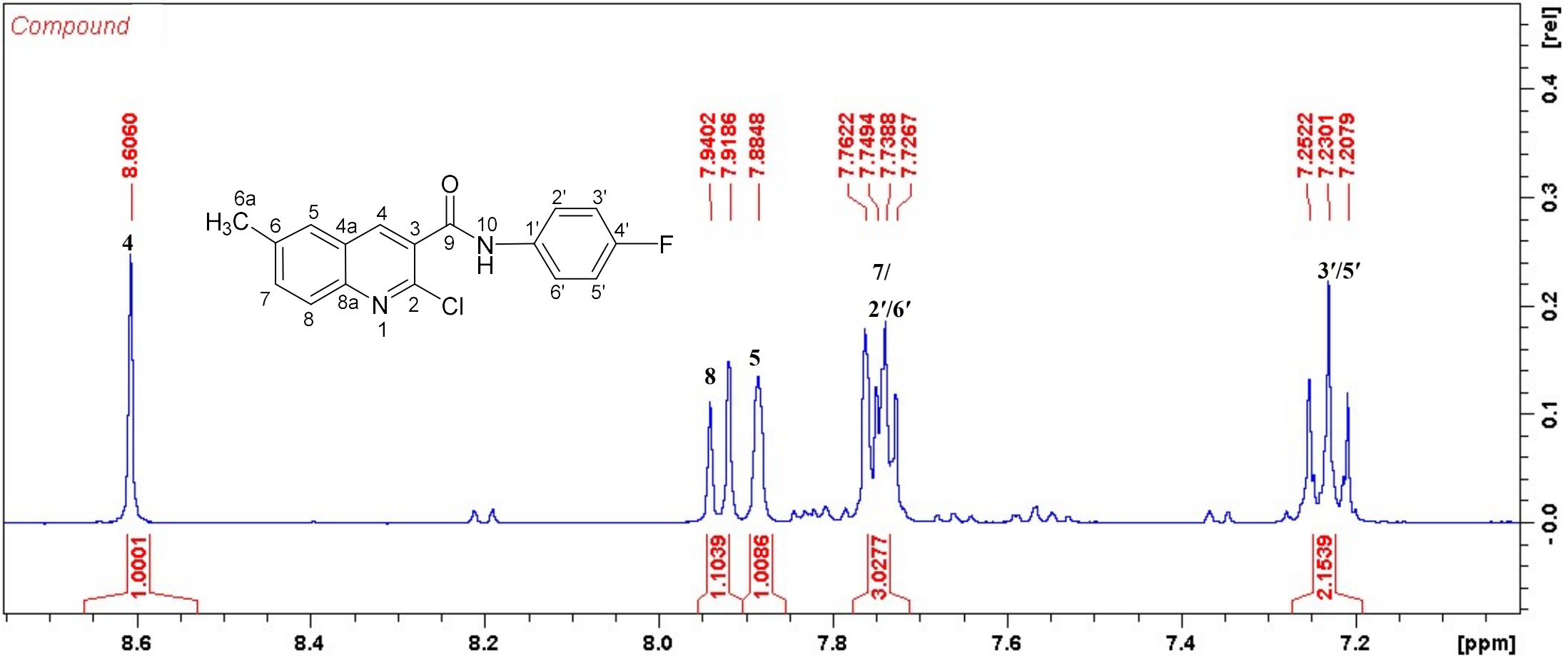
5e	Wavelength/nm	Absorbance	Log ε
	240	0.85	4.42
	311	0.24	3.86
	328	0.21	3.82



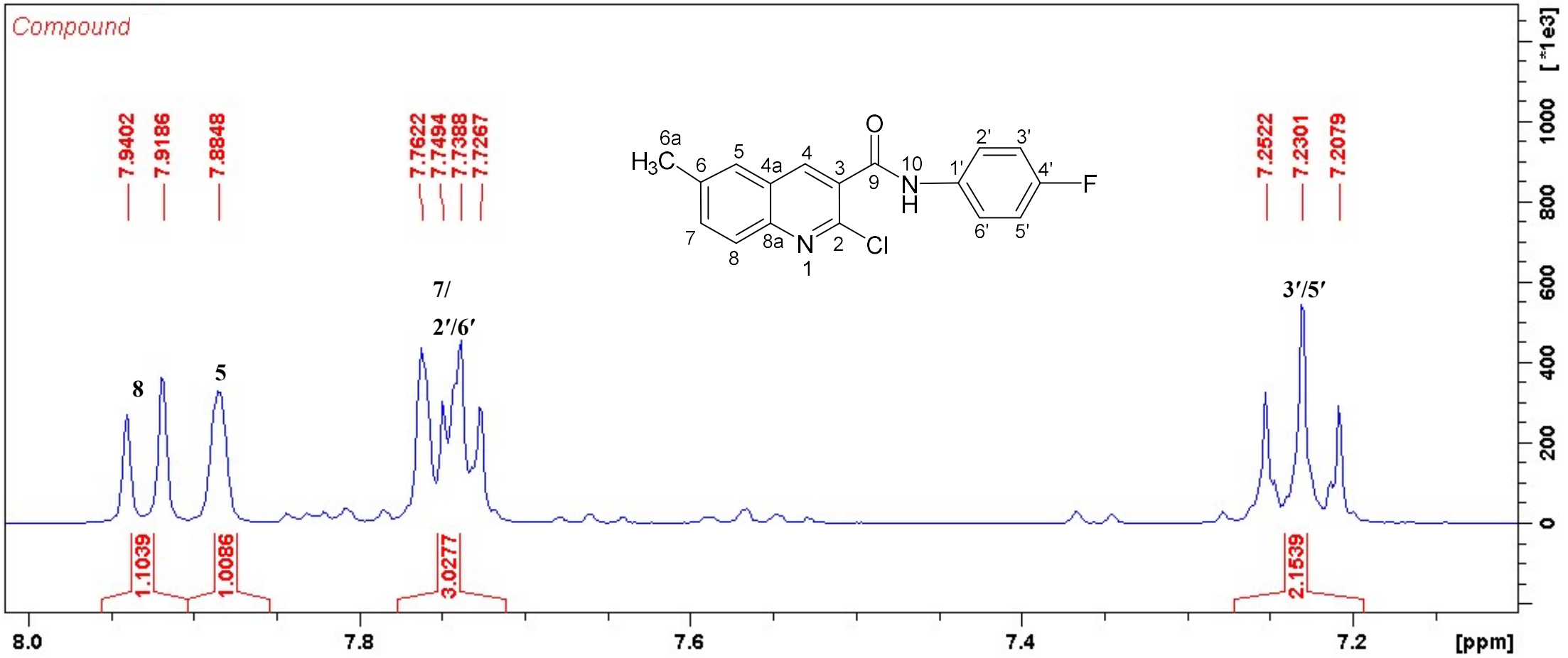
The UV-Vis spectrum of compound 5e



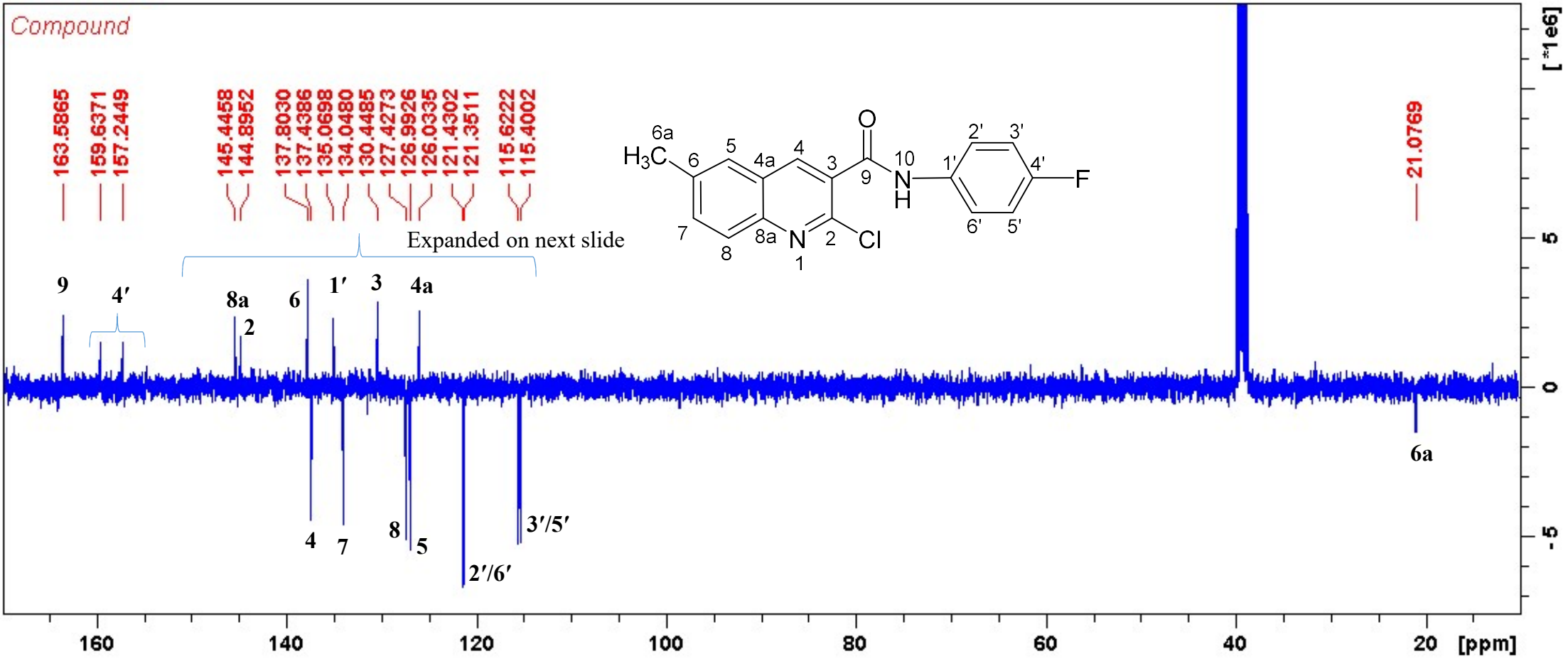
The ^{1}H NMR spectrum of compound 5f



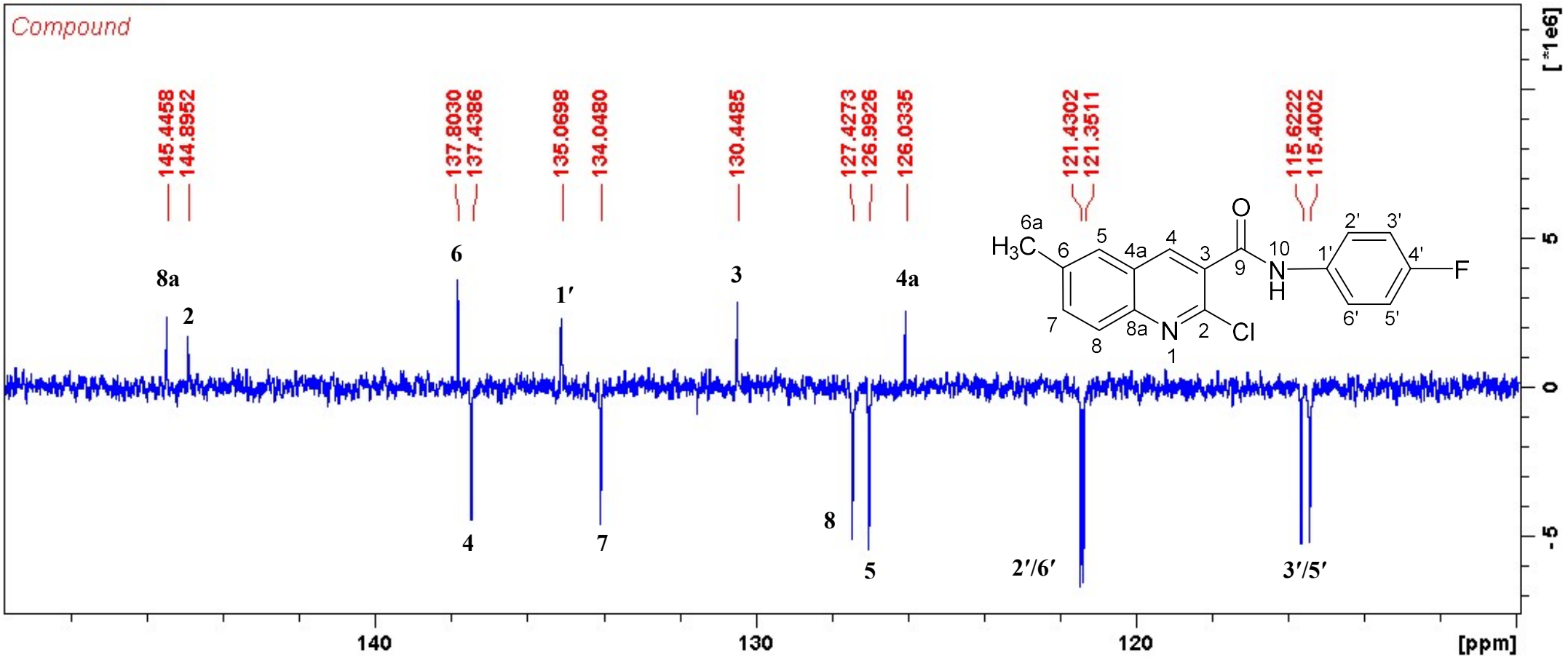
The ^{1}H NMR spectrum of compound 5f (expanded)



The ^{1}H NMR spectrum of compound 5f (expanded)



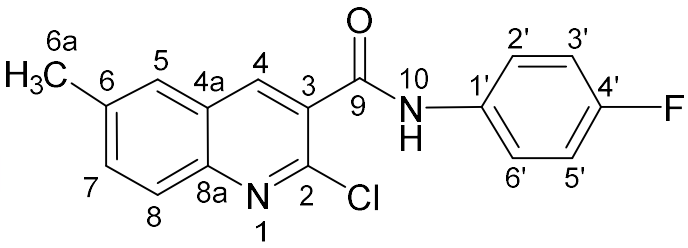
The ^{13}C NMR spectrum of compound 5f



The ^{13}C NMR spectrum of compound 5f (expanded)

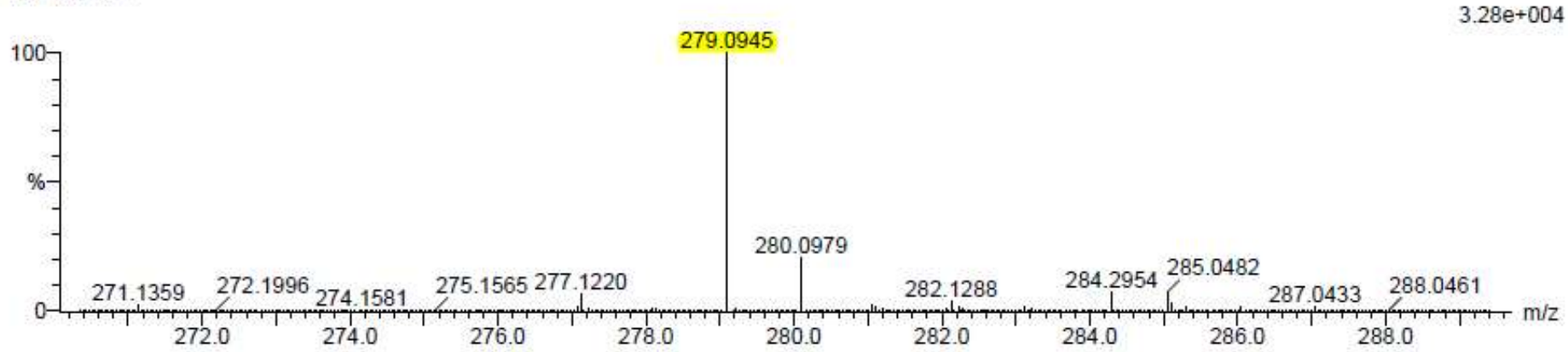
Elemental Composition Report **Page 1**

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



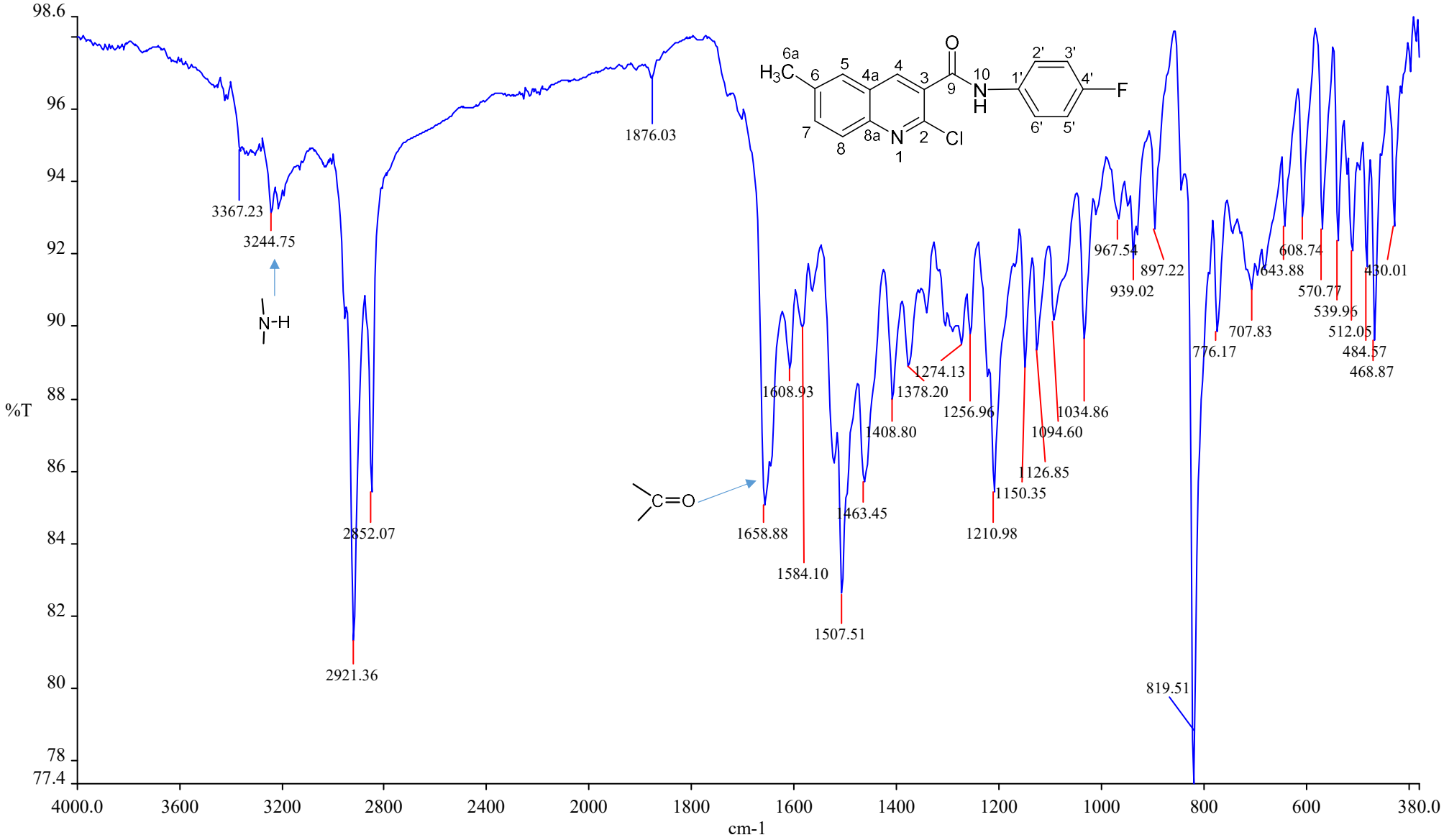
Monoisotopic Mass, Even Electron Ions
40 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 0-1

Compound 1 HG 31 (1.012) Cm (1:61)
TOF MS AP+



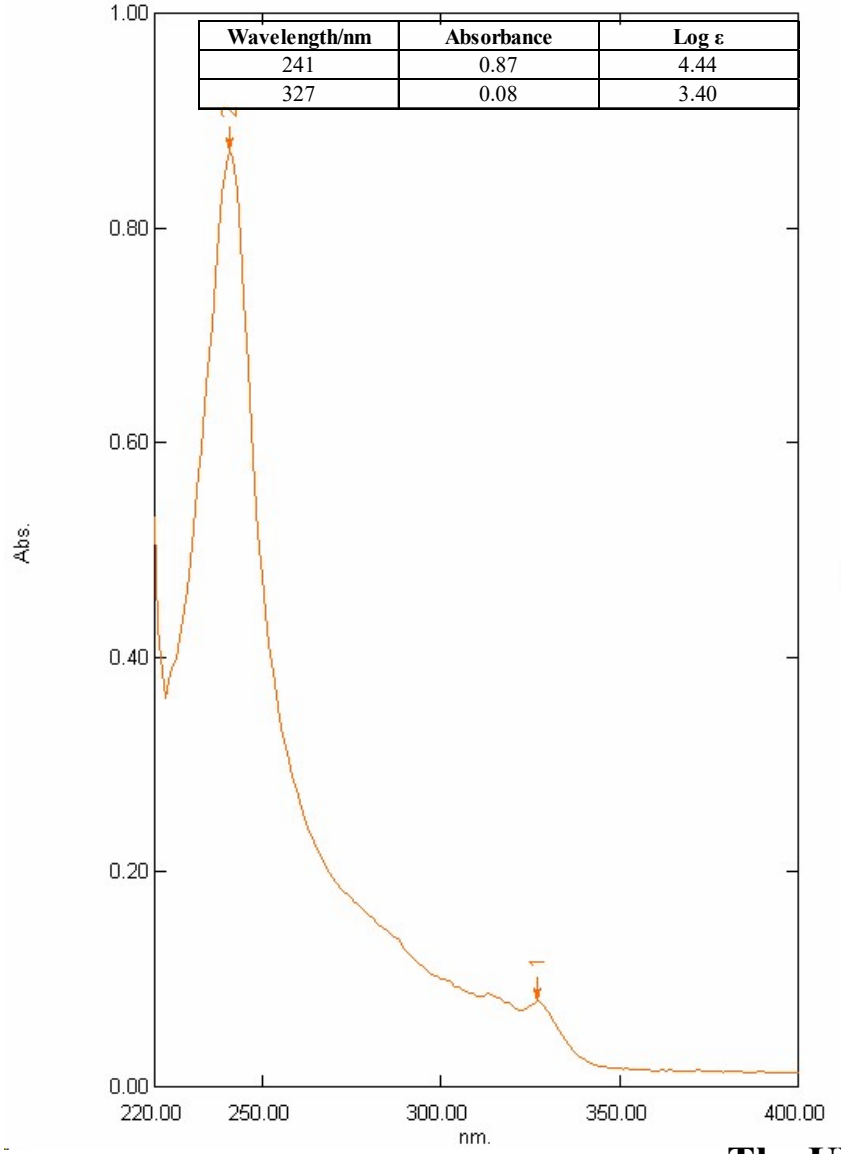
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
279.0945	279.0934	1.1	3.9	12.5	453.5	0.0	C17 H12 N2 O F

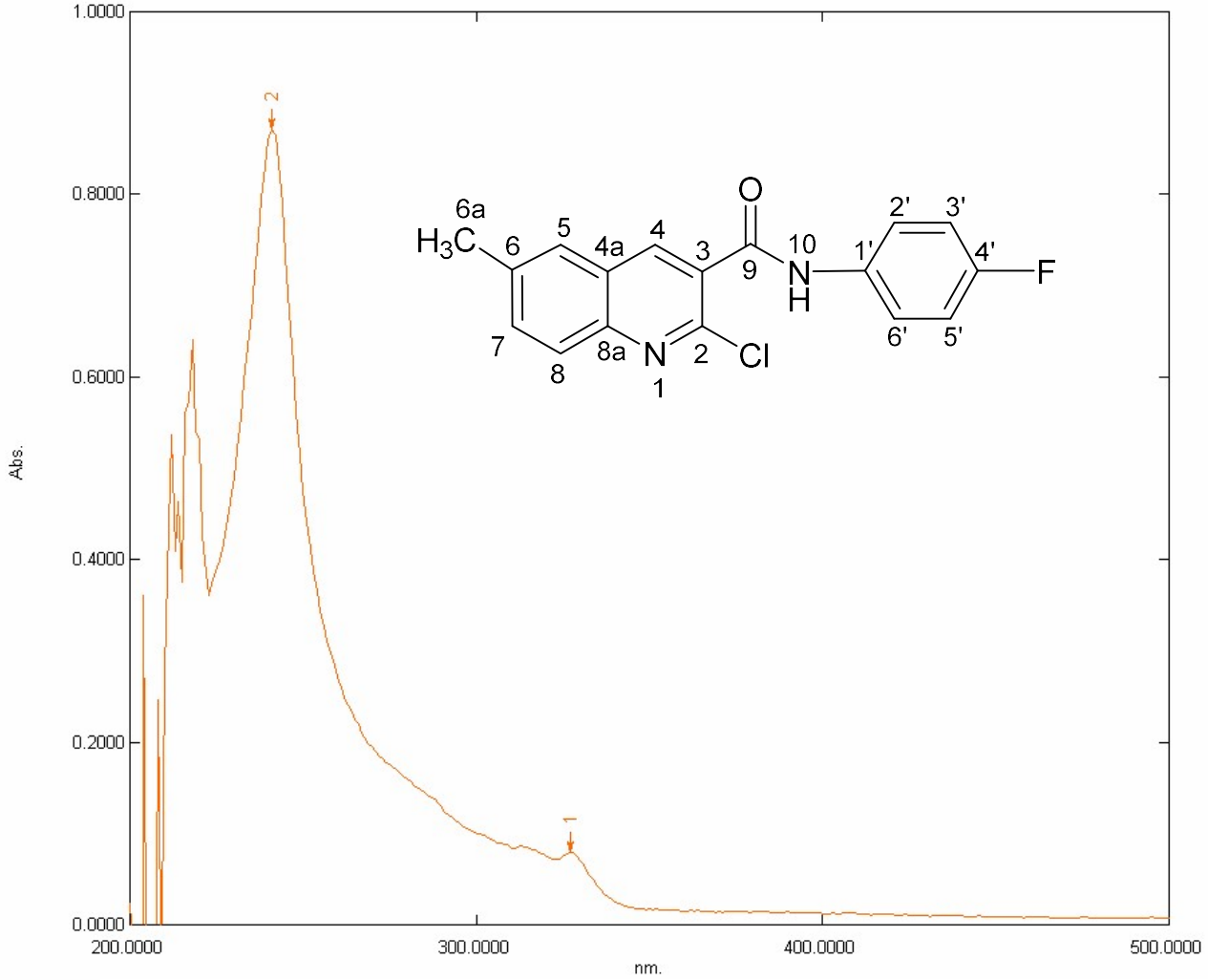
The HRMS spectrum of compound 5f



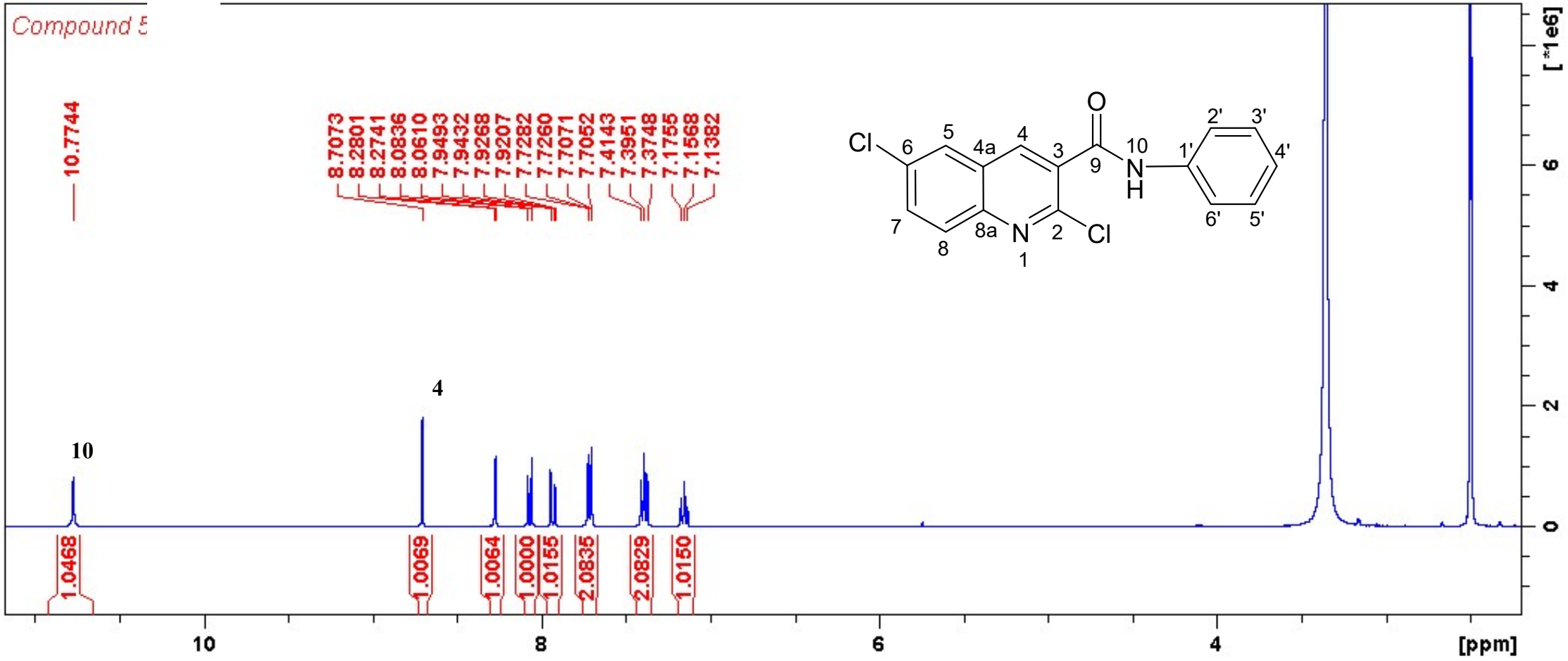
The IR spectrum of compound 5f

Wavelength/nm	Absorbance	Log ε
241	0.87	4.44
327	0.08	3.40

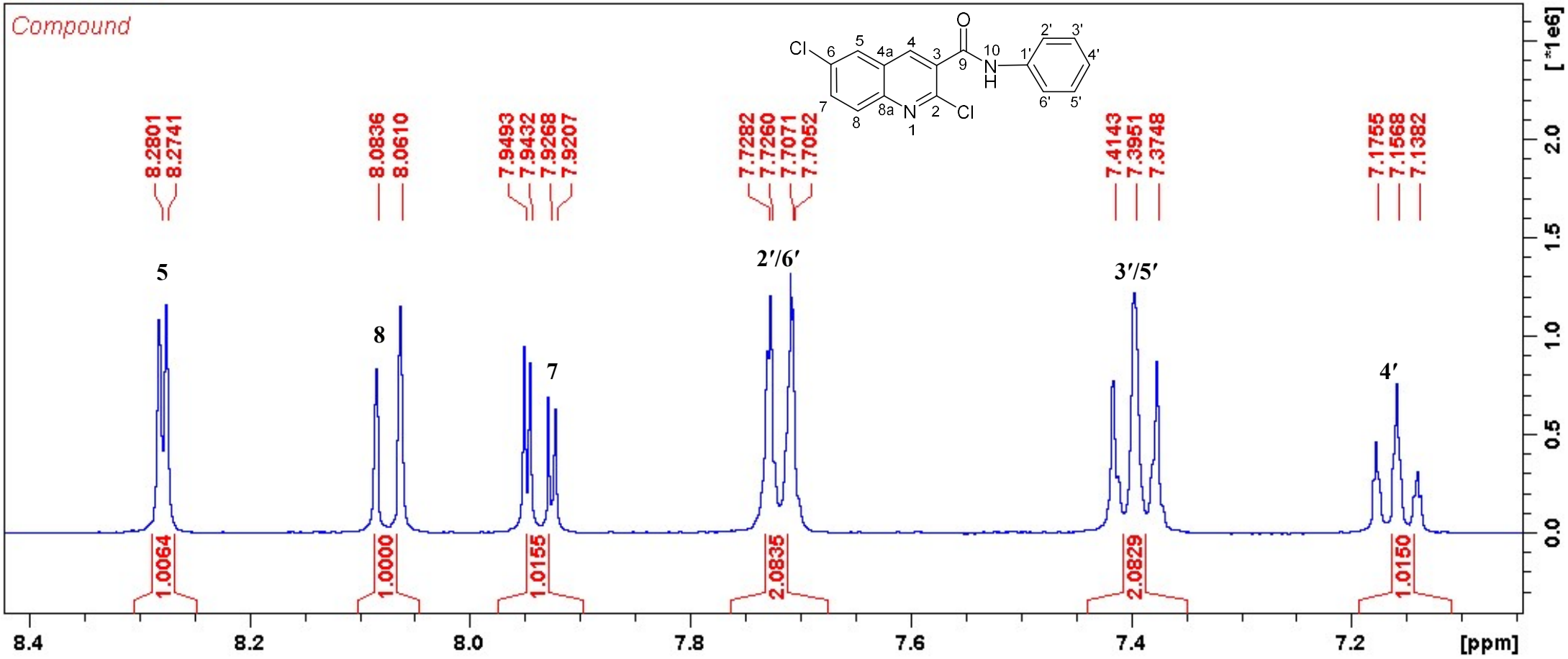




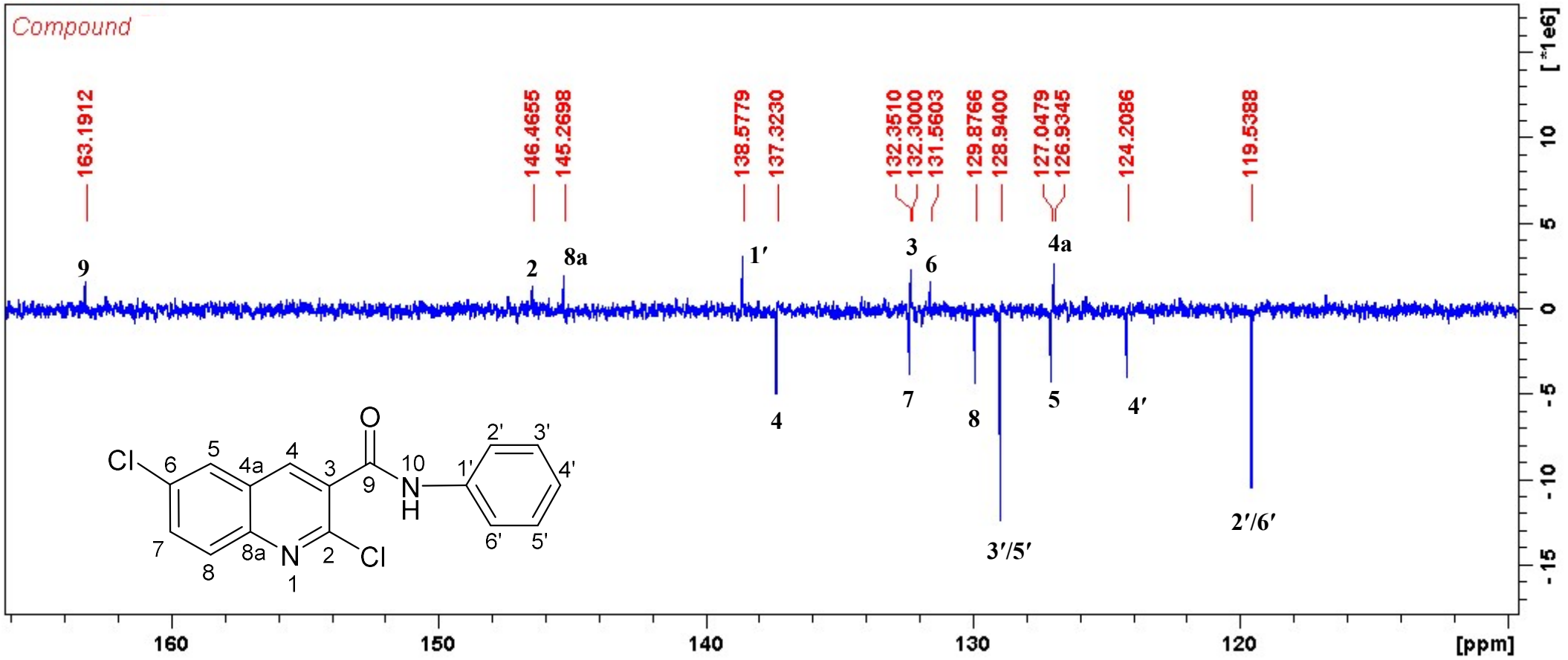
The UV-Vis spectrum of compound 5f



The ^{1}H NMR spectrum of compound 5g



The ^{1}H NMR spectrum of compound 5g (expanded)

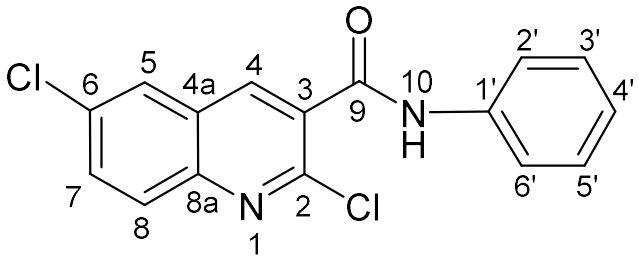


The ^{13}C NMR spectrum of compound 5g (expanded)

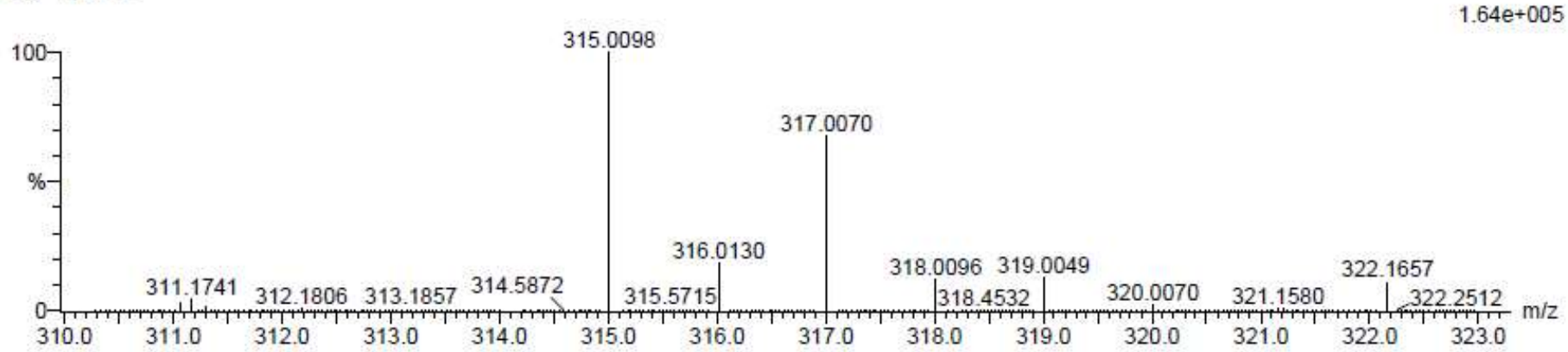
Elemental Composition Report **Page 1**

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

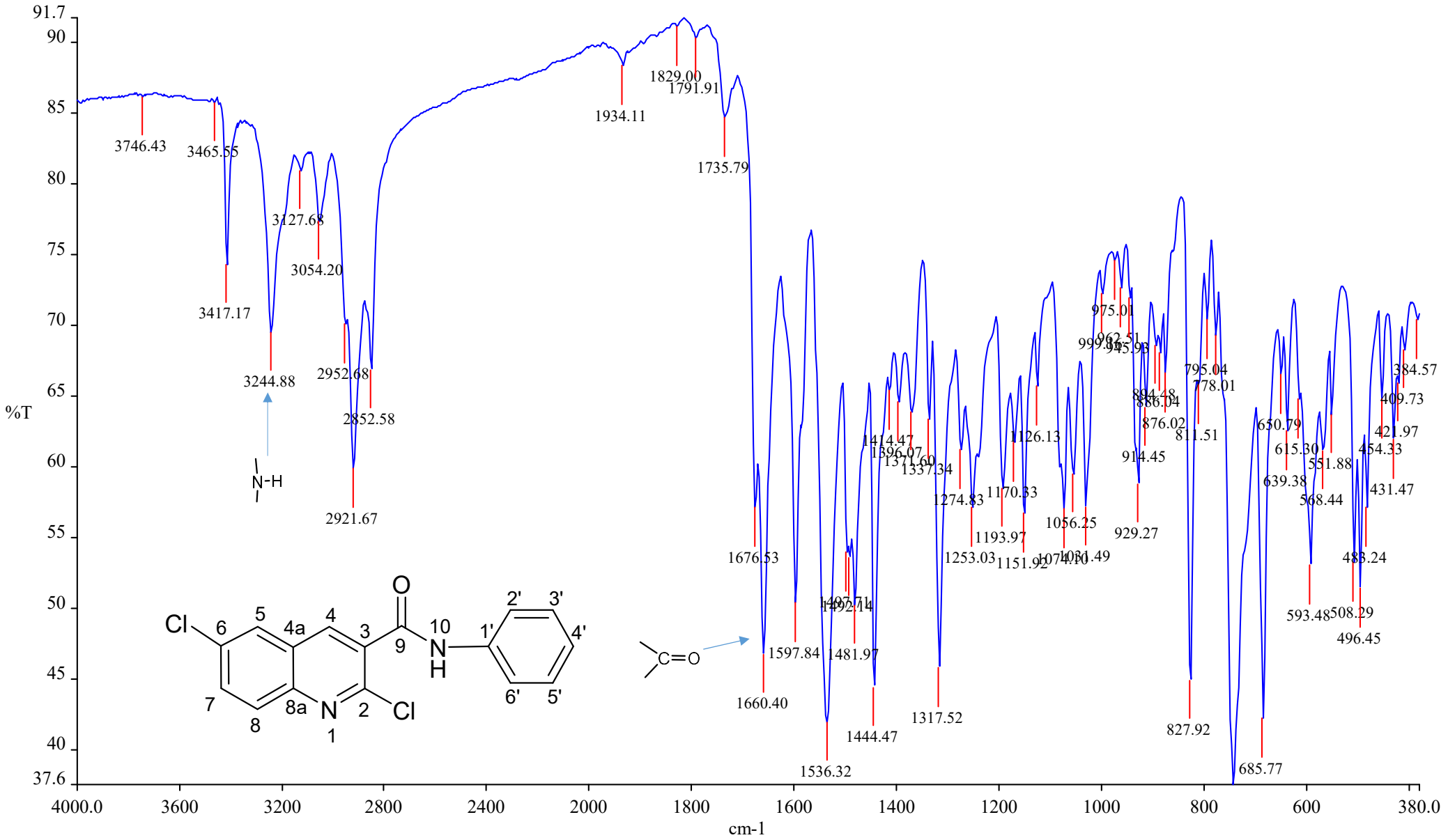


Monoisotopic Mass, Even Electron Ions
62 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 0-5 O: 0-5 Cl: 0-2
Compound 1d HG 13 (0.405) Cm (1:61)
TOF MS ES-

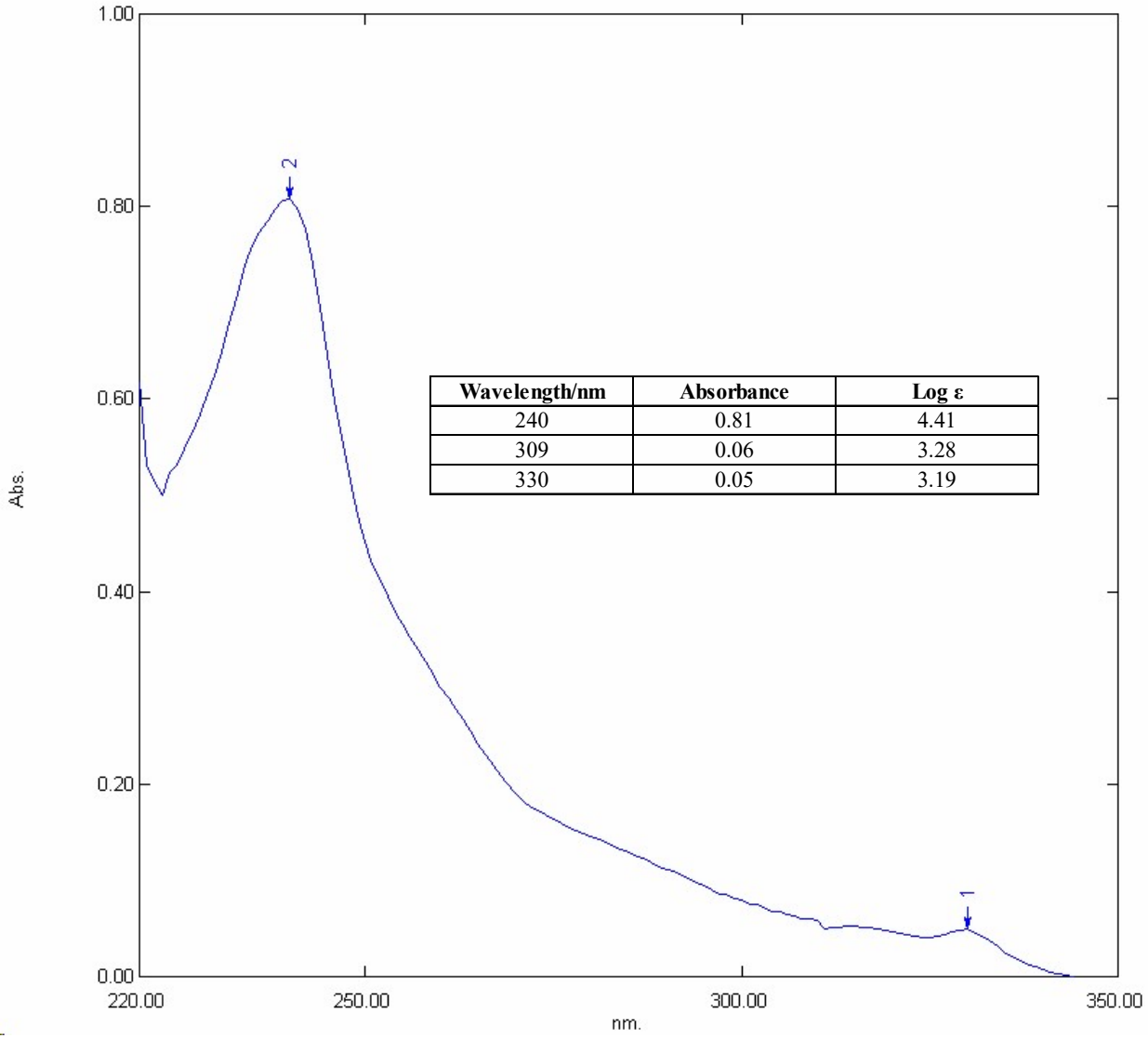


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
315.0098	315.0092	0.6	1.9	12.5	528.9	0.0	C16 H9 N2 O Cl2

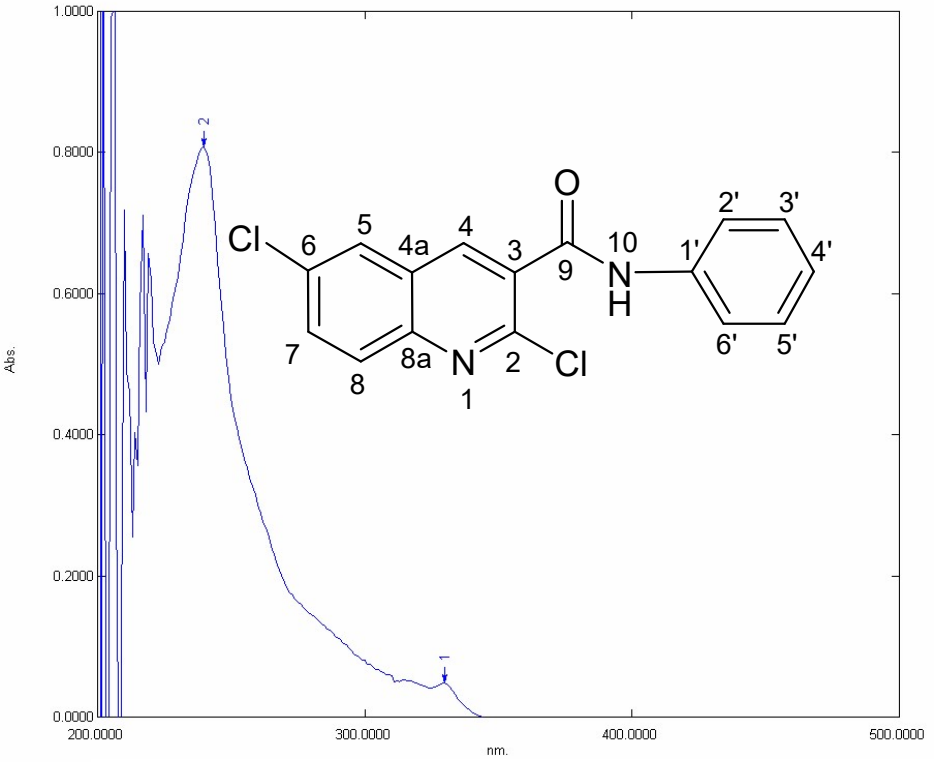
The HRMS spectrum of compound 5g



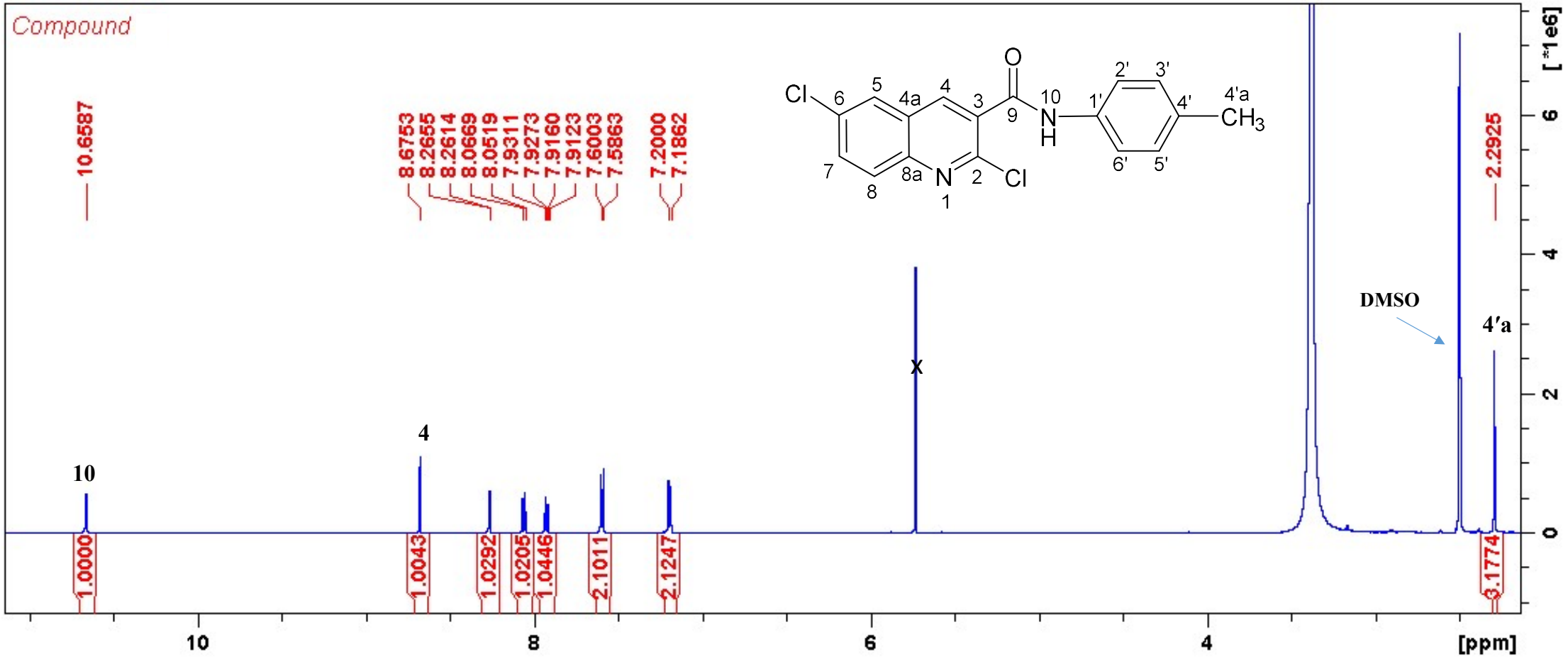
The IR spectrum of compound 5g



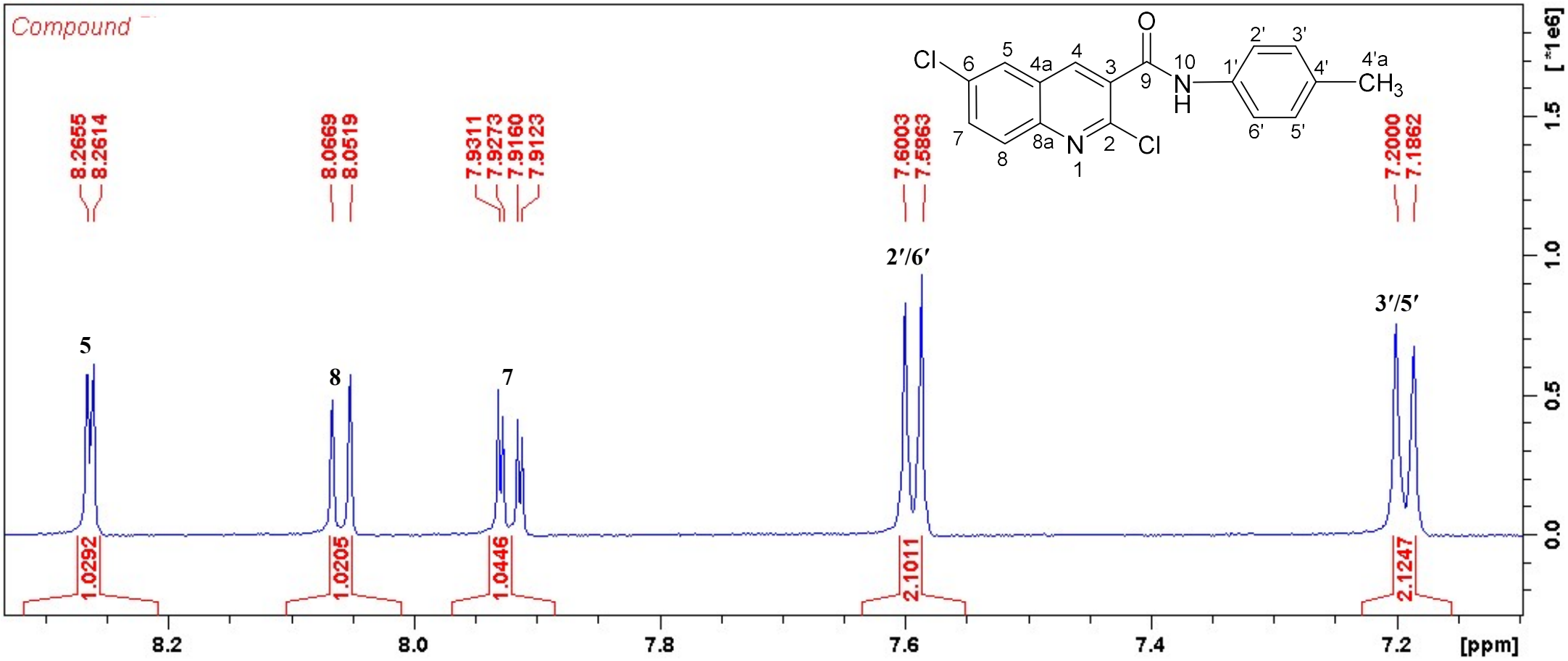
Wavelength/nm	Absorbance	Log ε
240	0.81	4.41
309	0.06	3.28
330	0.05	3.19



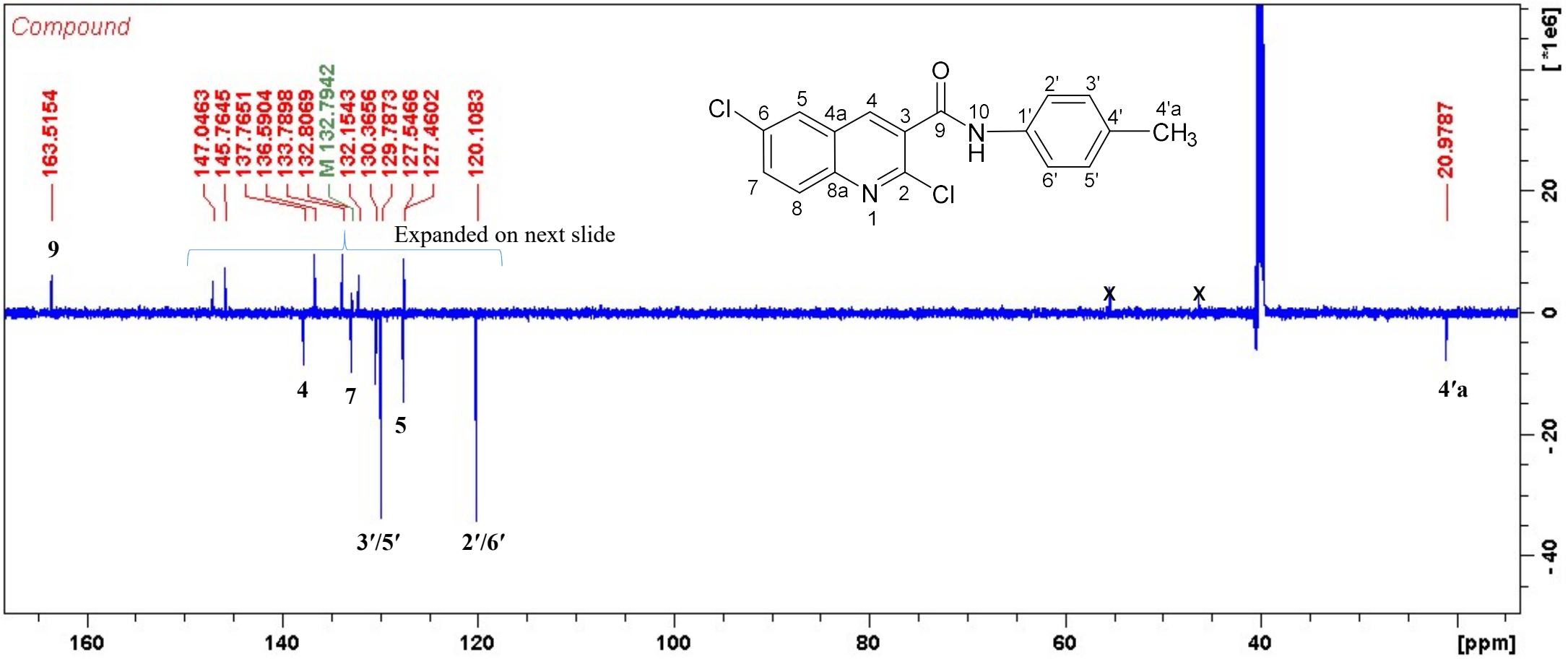
The UV-Vis spectrum of compound 5g



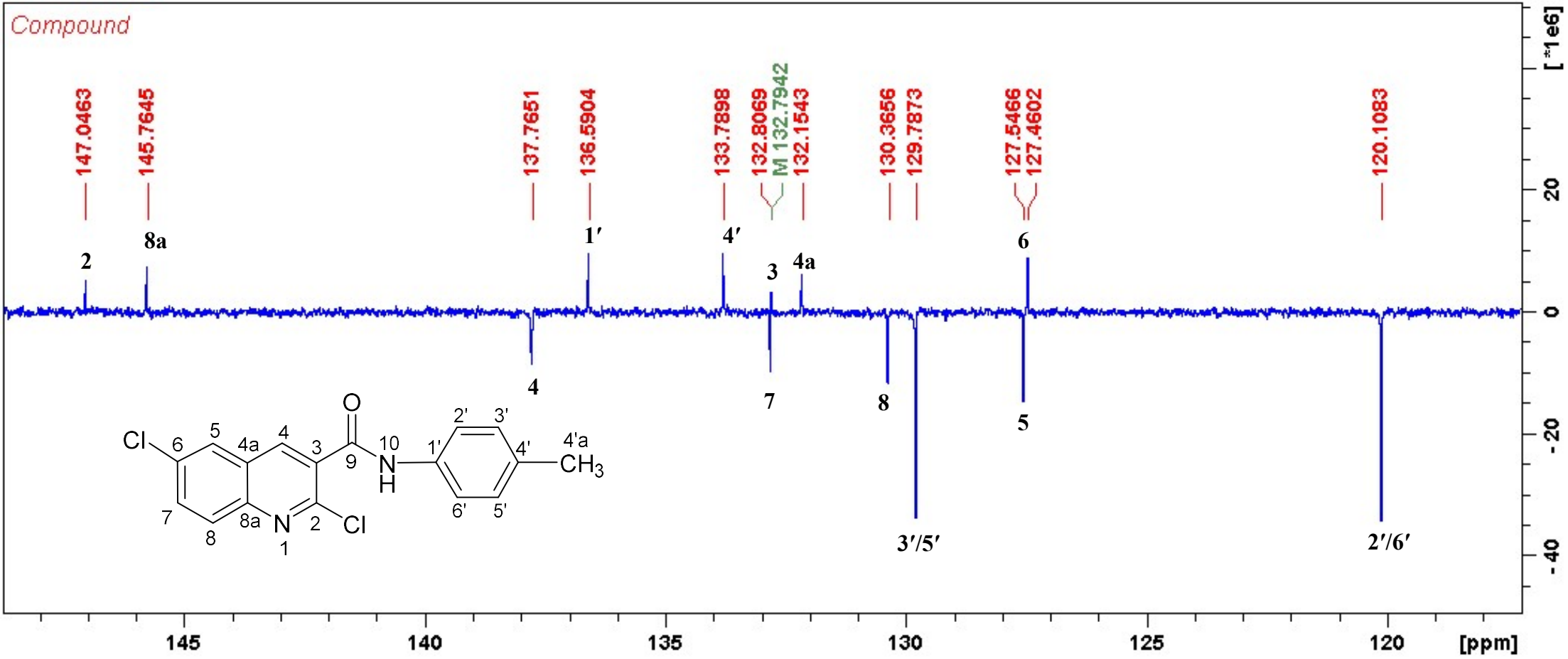
The ^{1}H NMR spectrum of compound 5h



The ^{1}H NMR spectrum of compound 5h (expanded)



The ^{13}C NMR spectrum of compound 5h

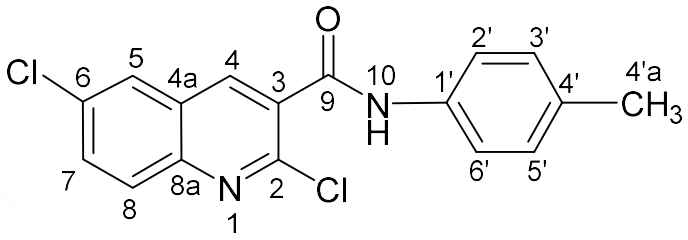


The ^{13}C NMR spectrum of compound 5h (expanded)

Elemental Composition Report

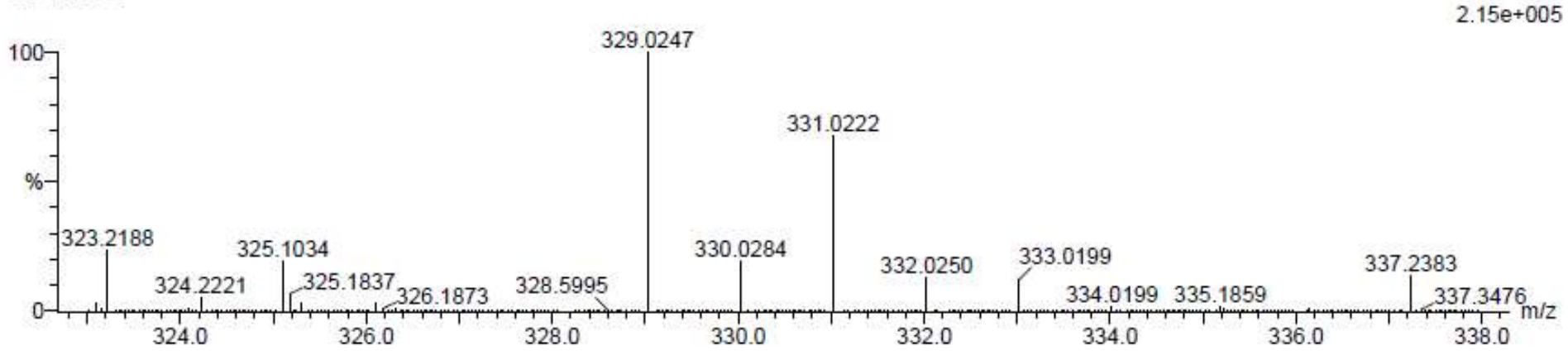
Single Mass Analysis

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



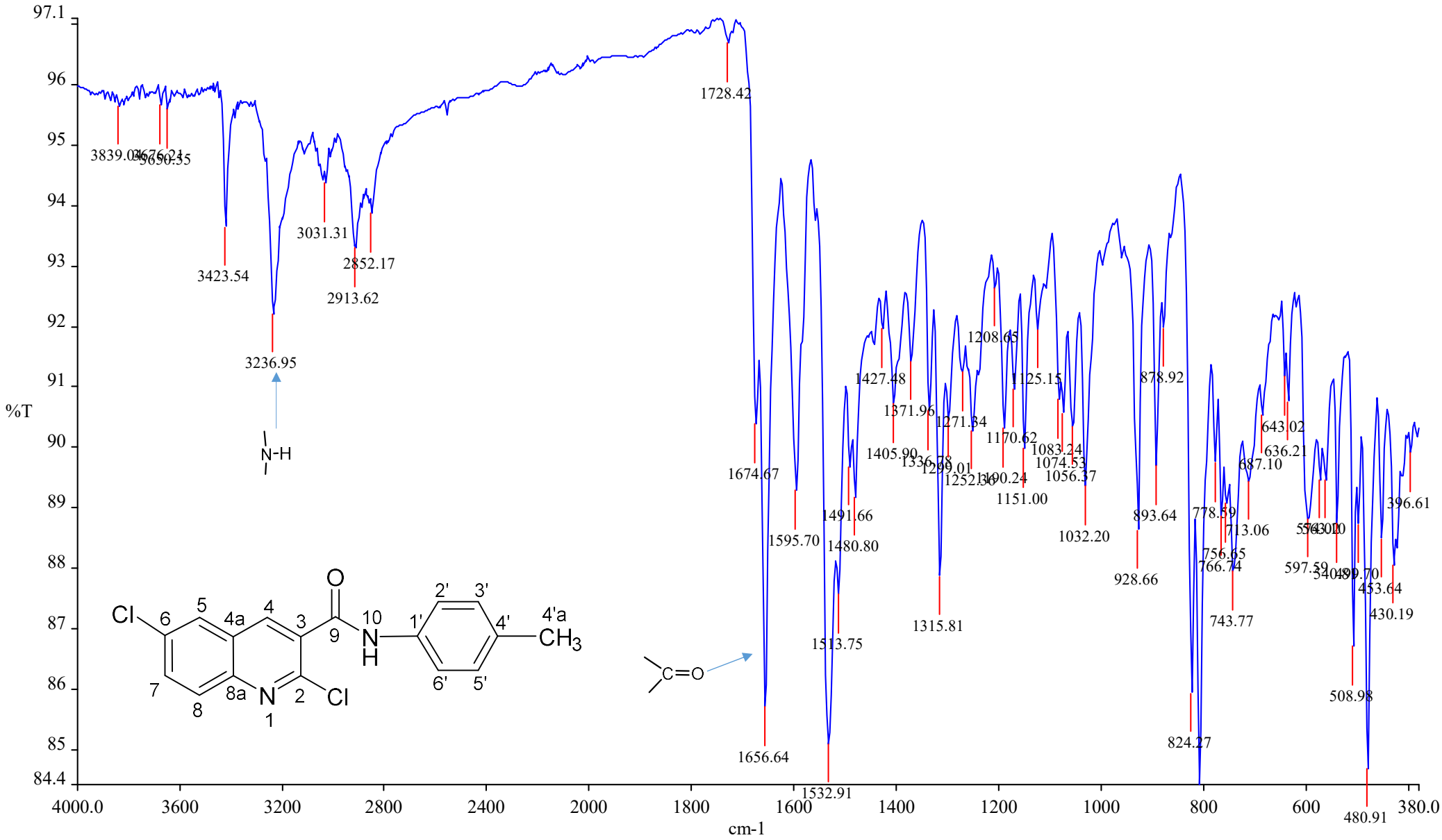
Monoisotopic Mass, Even Electron Ions
54 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 Cl: 0-2

Compound 2d HG 53 (1.753) Cm (1:61)
TOF MS ES-



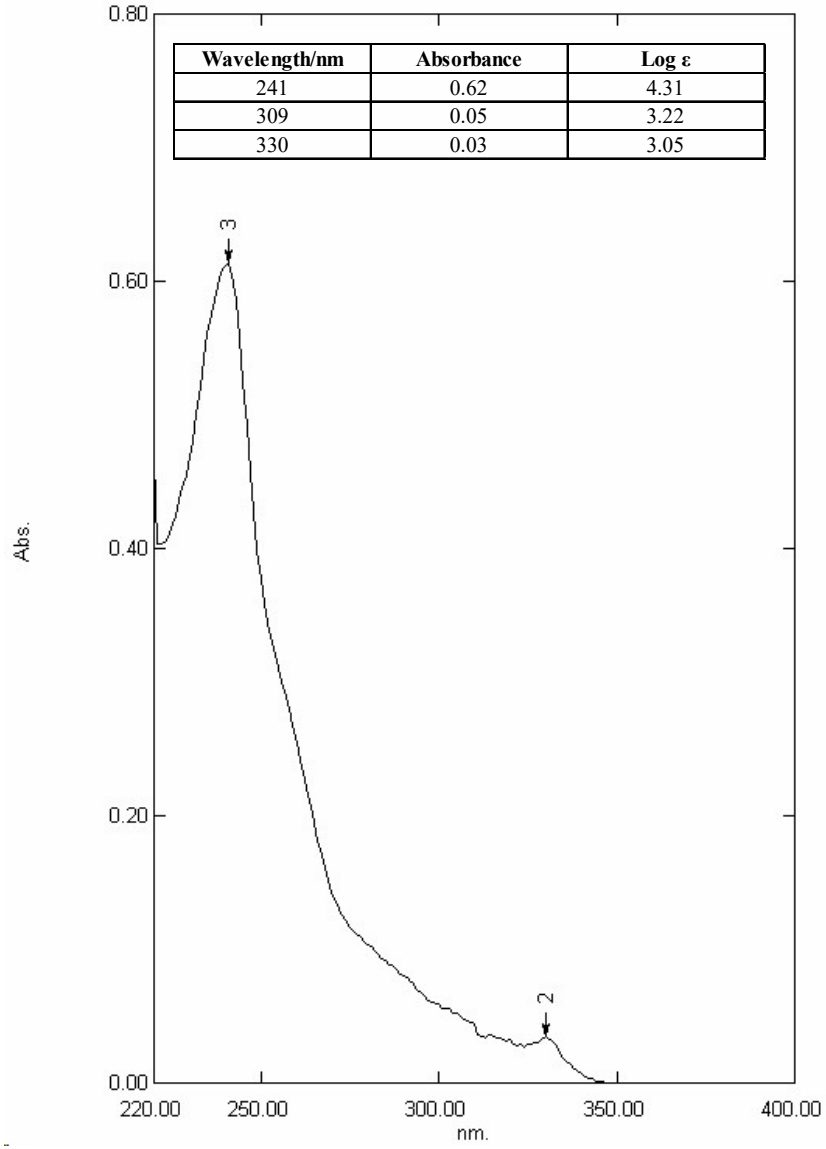
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
329.0247	329.0248	-0.1	-0.3	12.5	516.5	0.0	C17 H11 N2 O Cl2

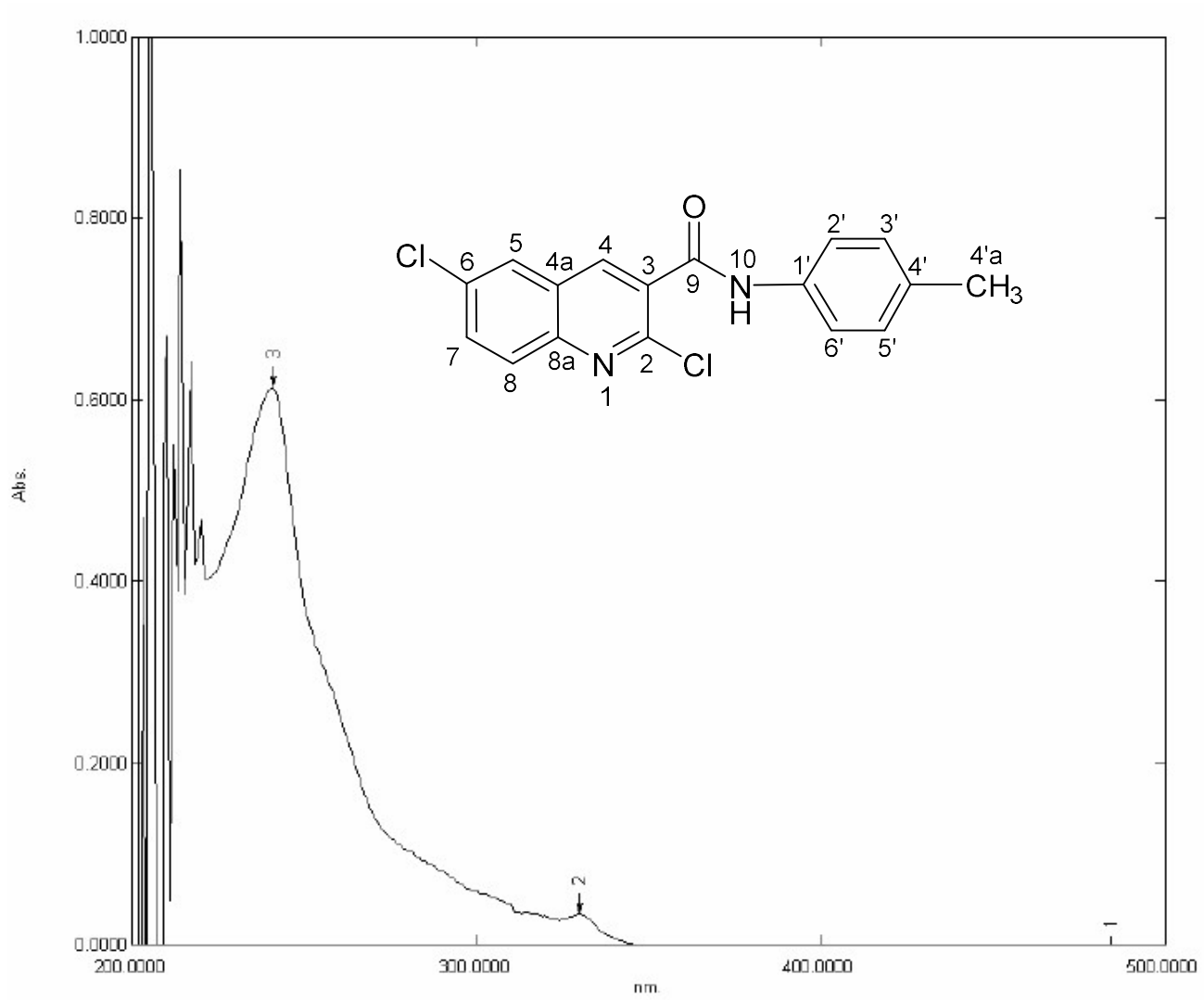
The HRMS spectrum of compound 5h



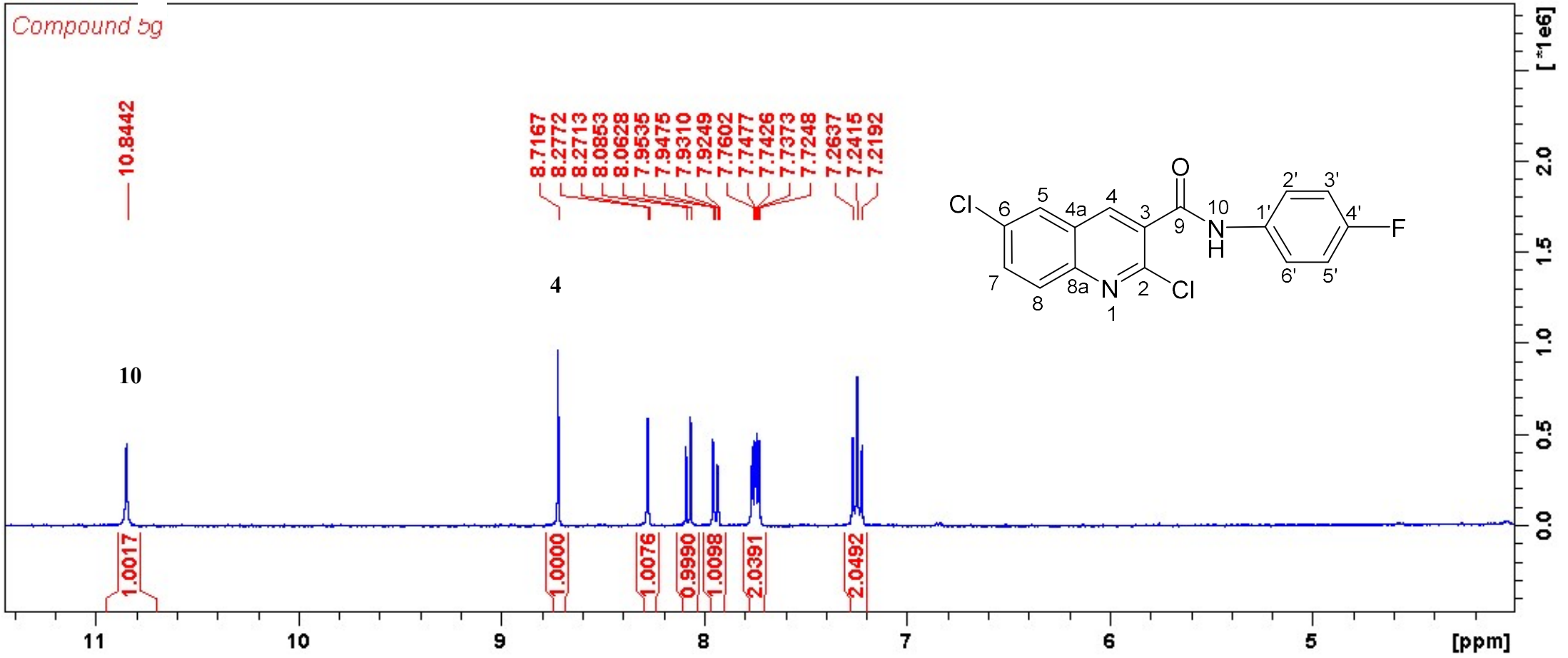
The IR spectrum of compound 5h

Wavelength/nm	Absorbance	Log ε
241	0.62	4.31
309	0.05	3.22
330	0.03	3.05

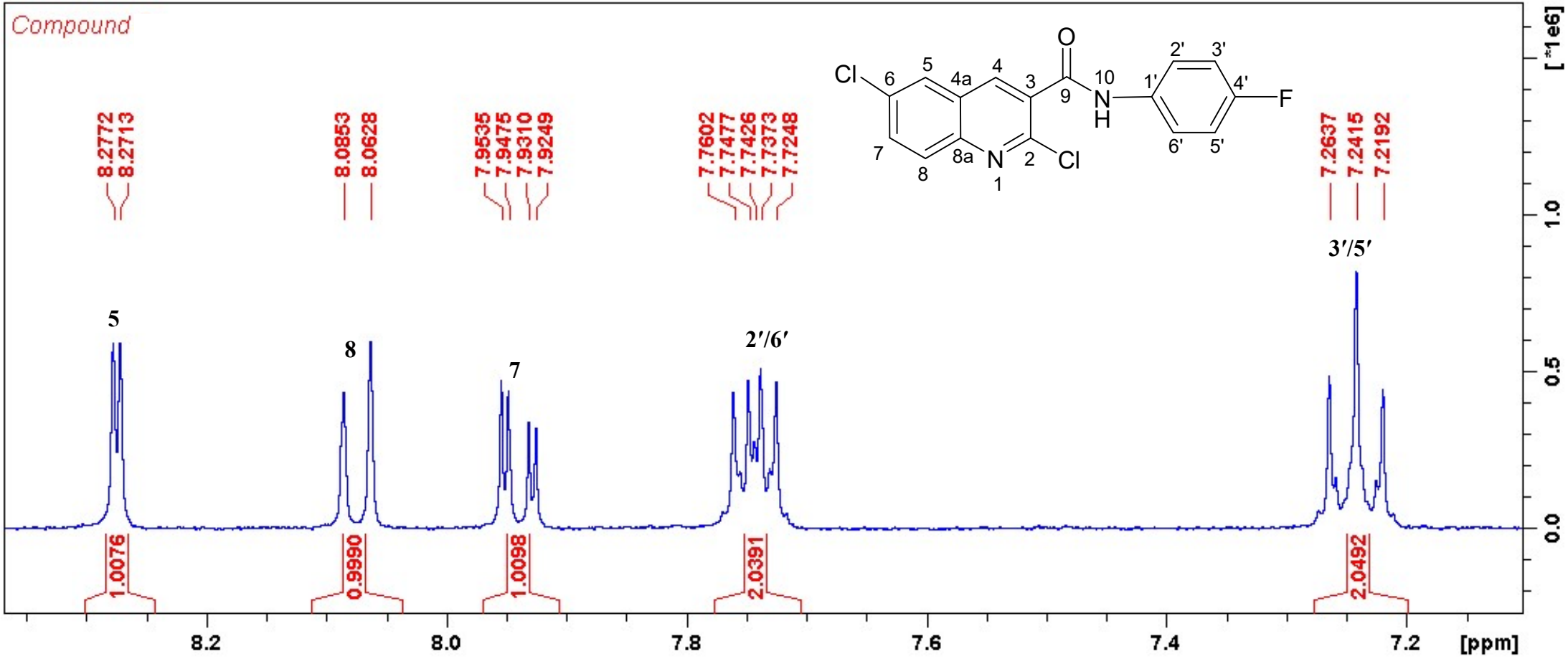




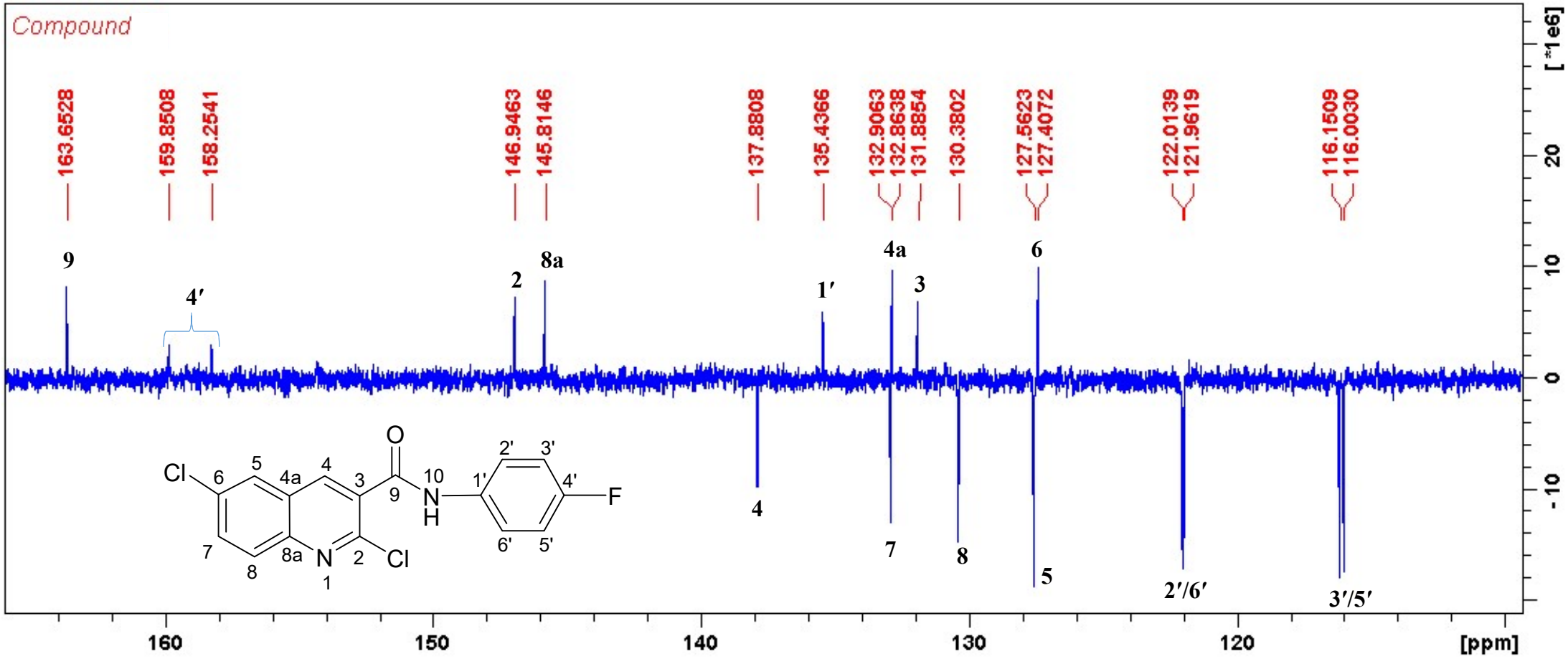
The UV-Vis spectrum of compound 5h



The ^{1}H NMR spectrum of compound 5i



The ^{1}H NMR spectrum of compound 5i (expanded)



The ^{13}C NMR spectrum of compound 5i (expanded)

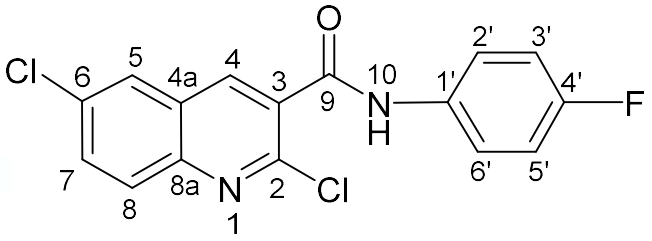
Elemental Composition Report **Page 1**

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

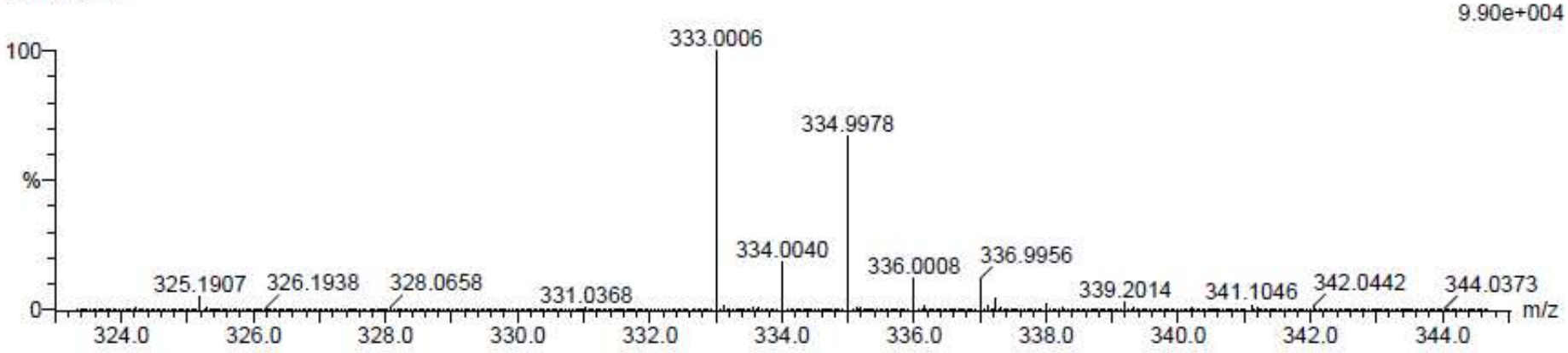
76 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)

Elements Used:

C: 15-20 H: 5-10 N: 0-5 O: 1-5 F: 0-1 Cl: 1-2

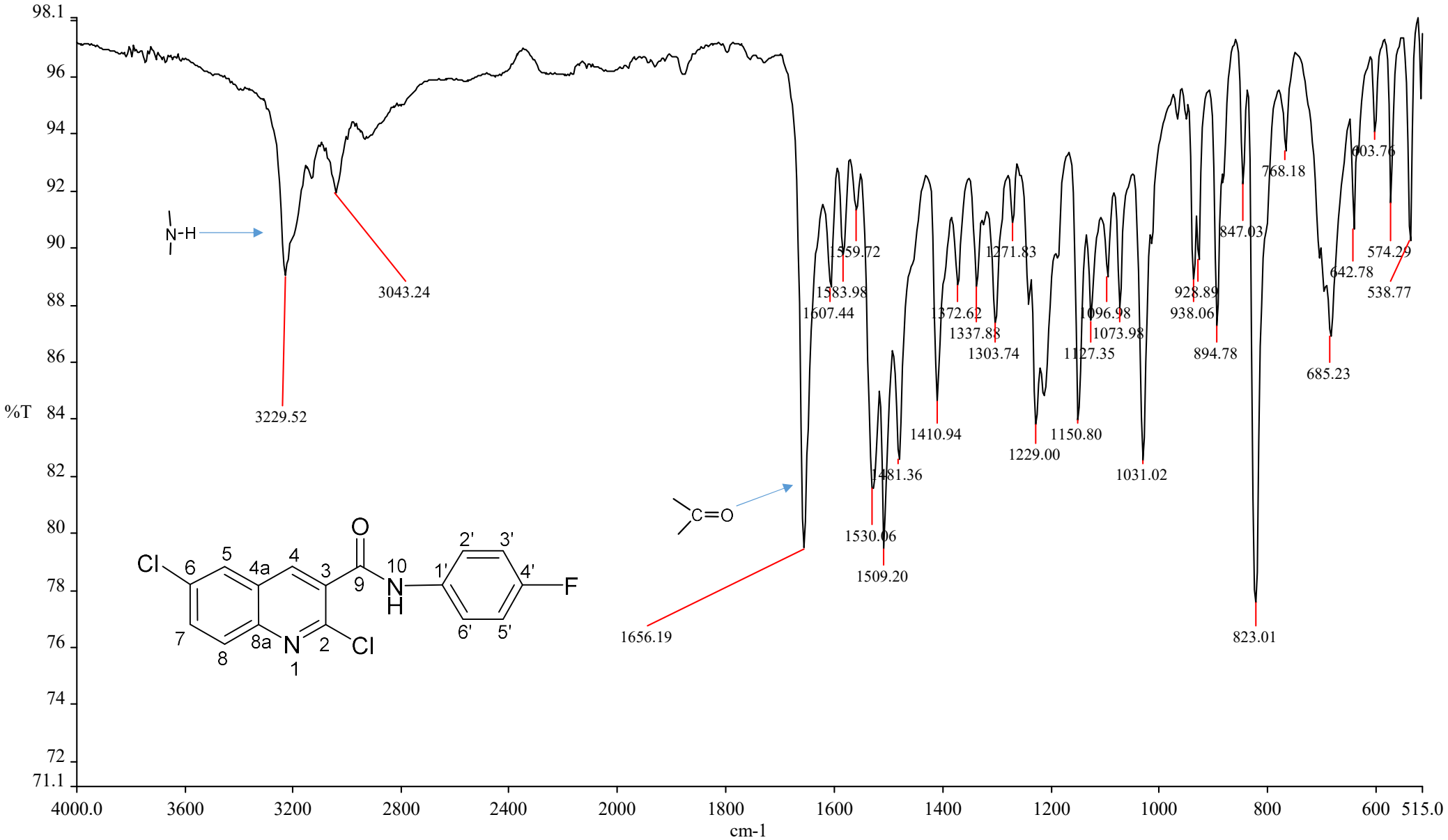
Compound 1c HG 61 (2.027) Cm (1:61)

TOF MS ES-

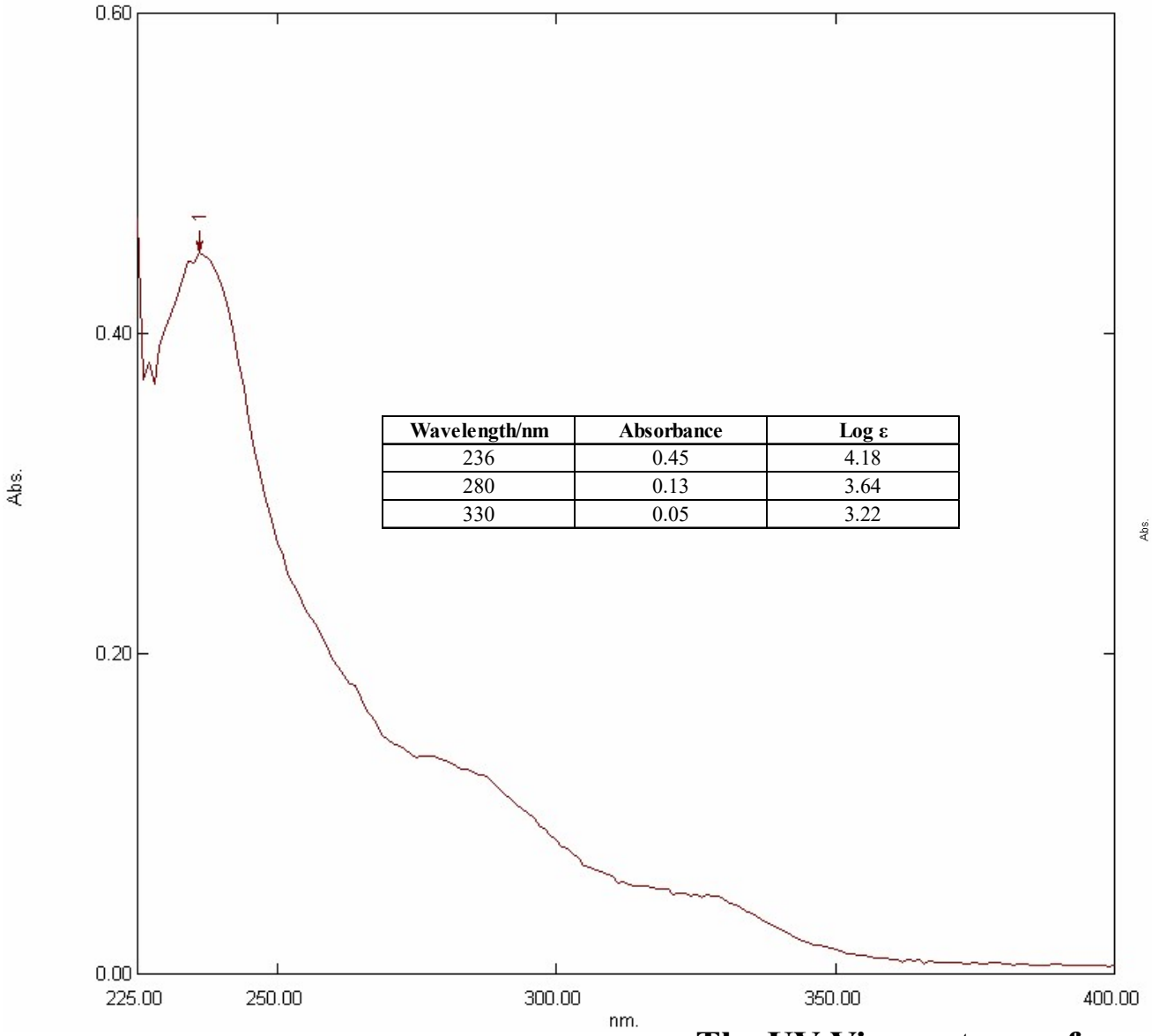


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
333.0006	332.9998	0.8	2.4	12.5	560.6	0.0	C16 H8 N2 O F Cl2

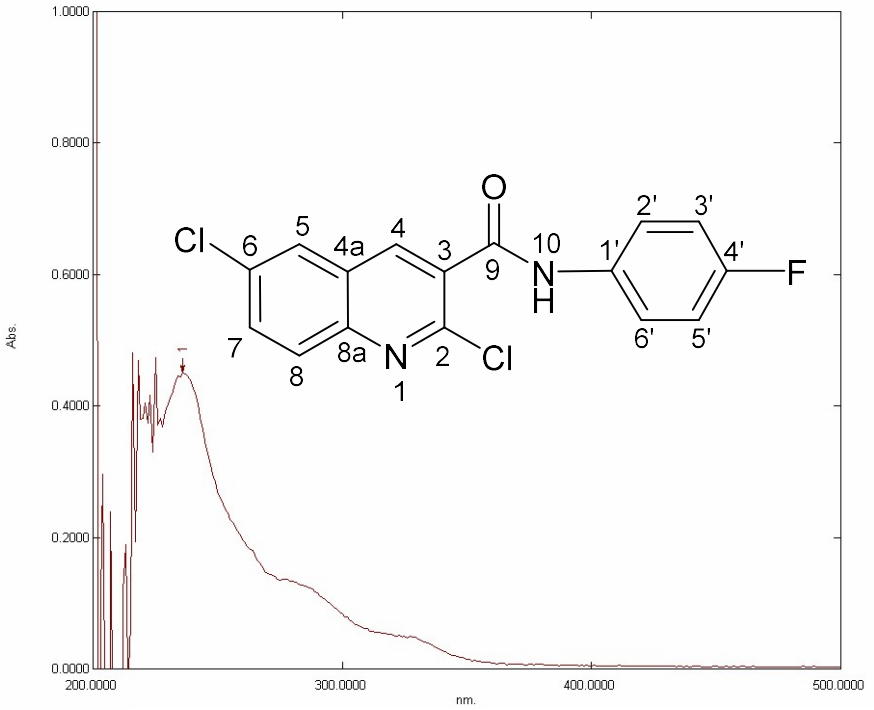
The HRMS spectrum of compound 5i



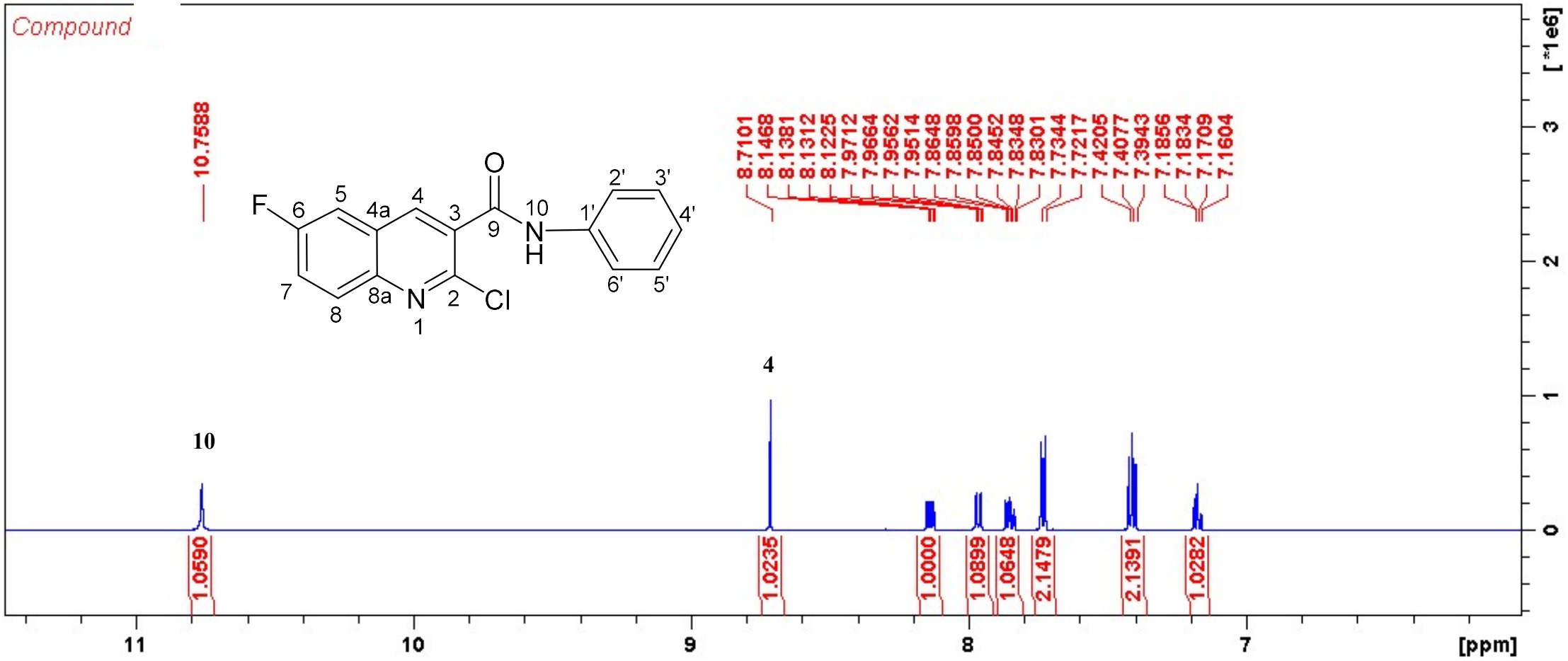
The IR spectrum of compound 5i



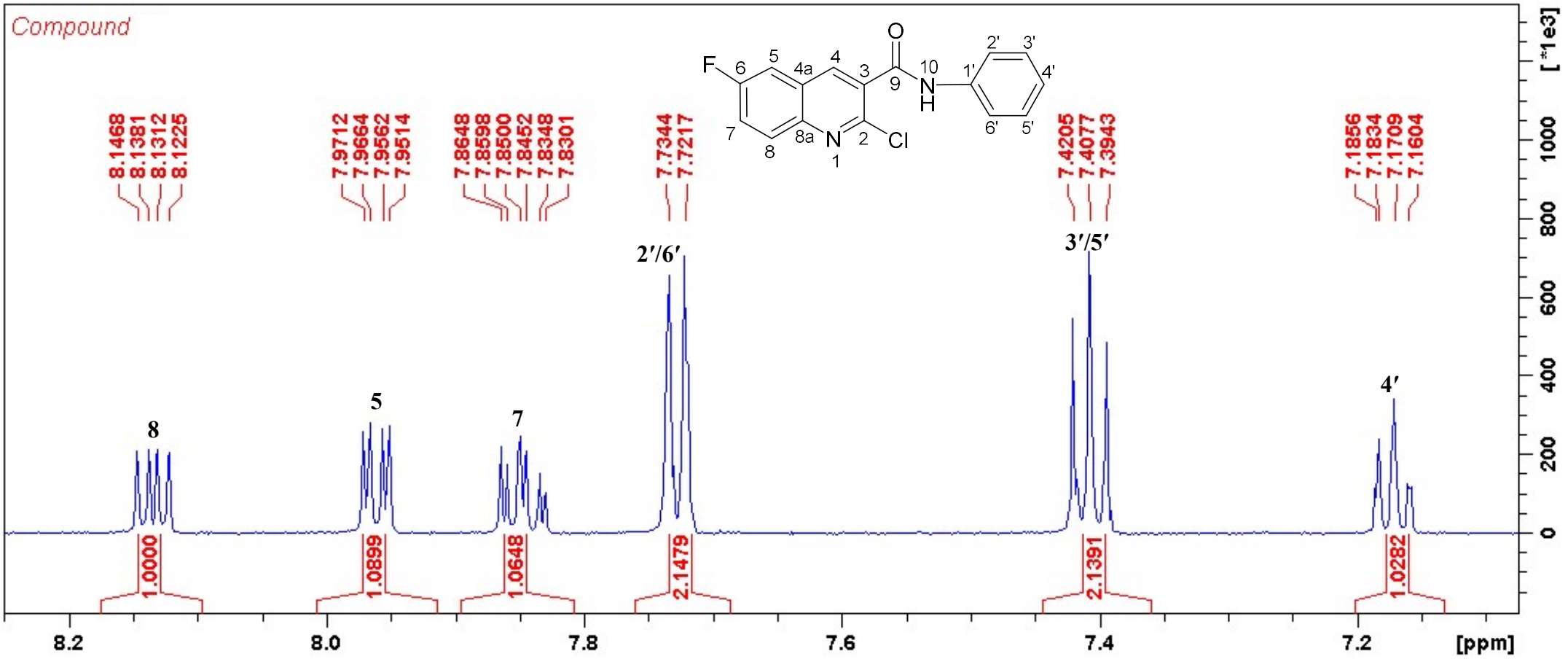
Wavelength/nm	Absorbance	Log ε
236	0.45	4.18
280	0.13	3.64
330	0.05	3.22



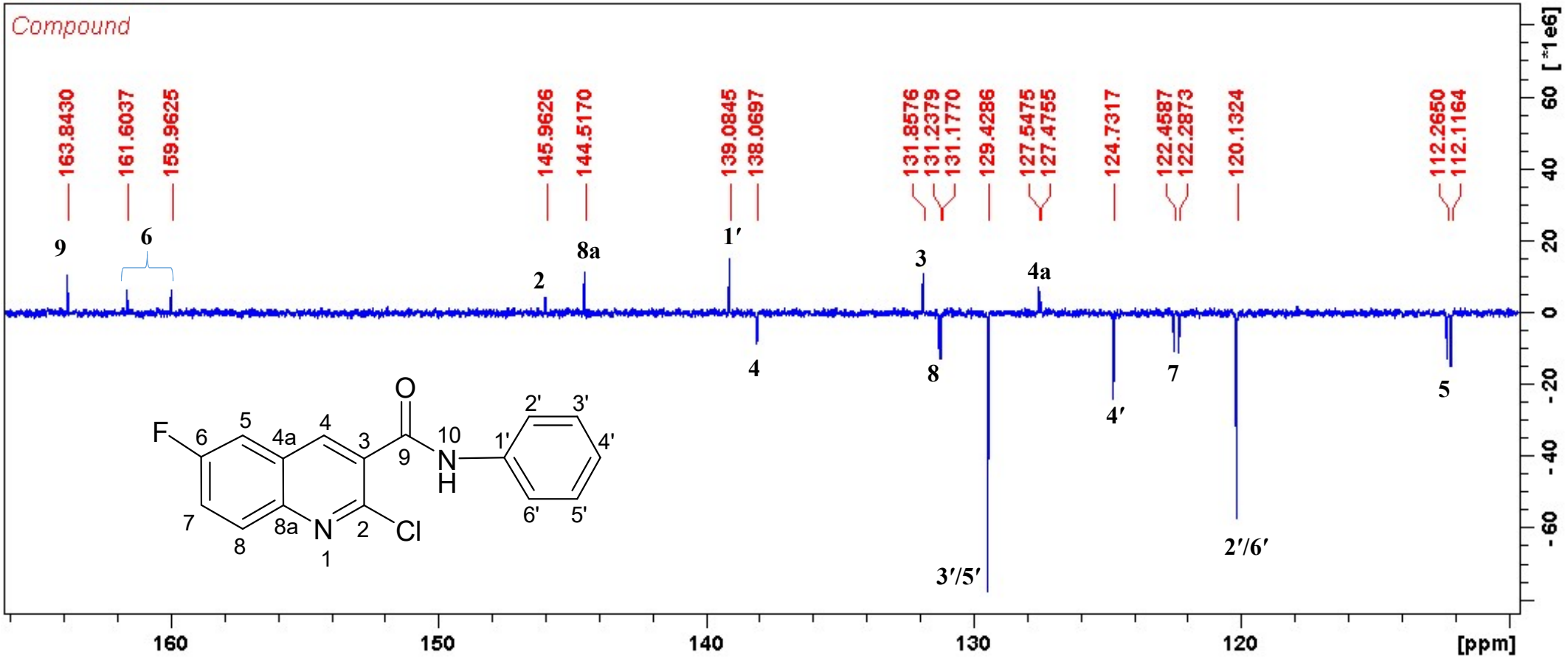
The UV-Vis spectrum of compound 5i



The ^{1}H NMR spectrum of compound 5j



The ^{1}H NMR spectrum of compound 5j (expanded)



The ^{13}C NMR spectrum of compound 5j

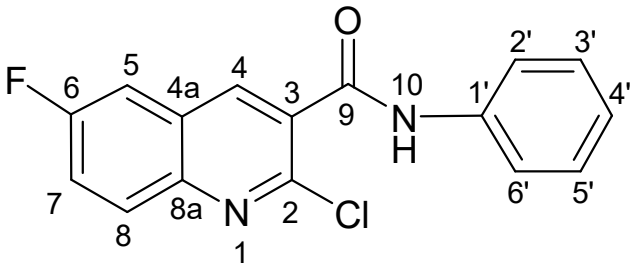
Elemental Composition Report **Page 1**

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

100 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)

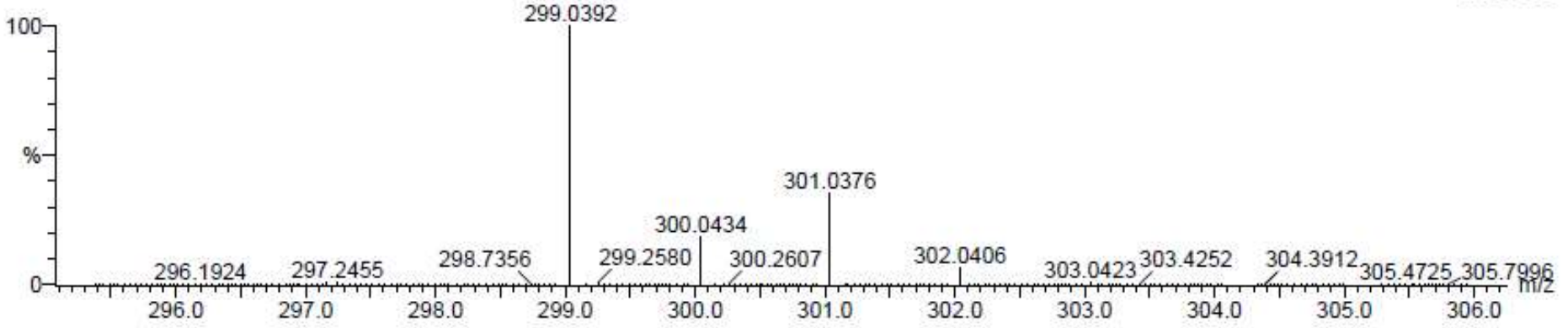
Elements Used:

C: 15-20 H: 5-10 N: 0-5 O: 1-5 F: 0-1 Cl: 1-2

Compound 3c HG 30 (0.979) Cm (1:61)

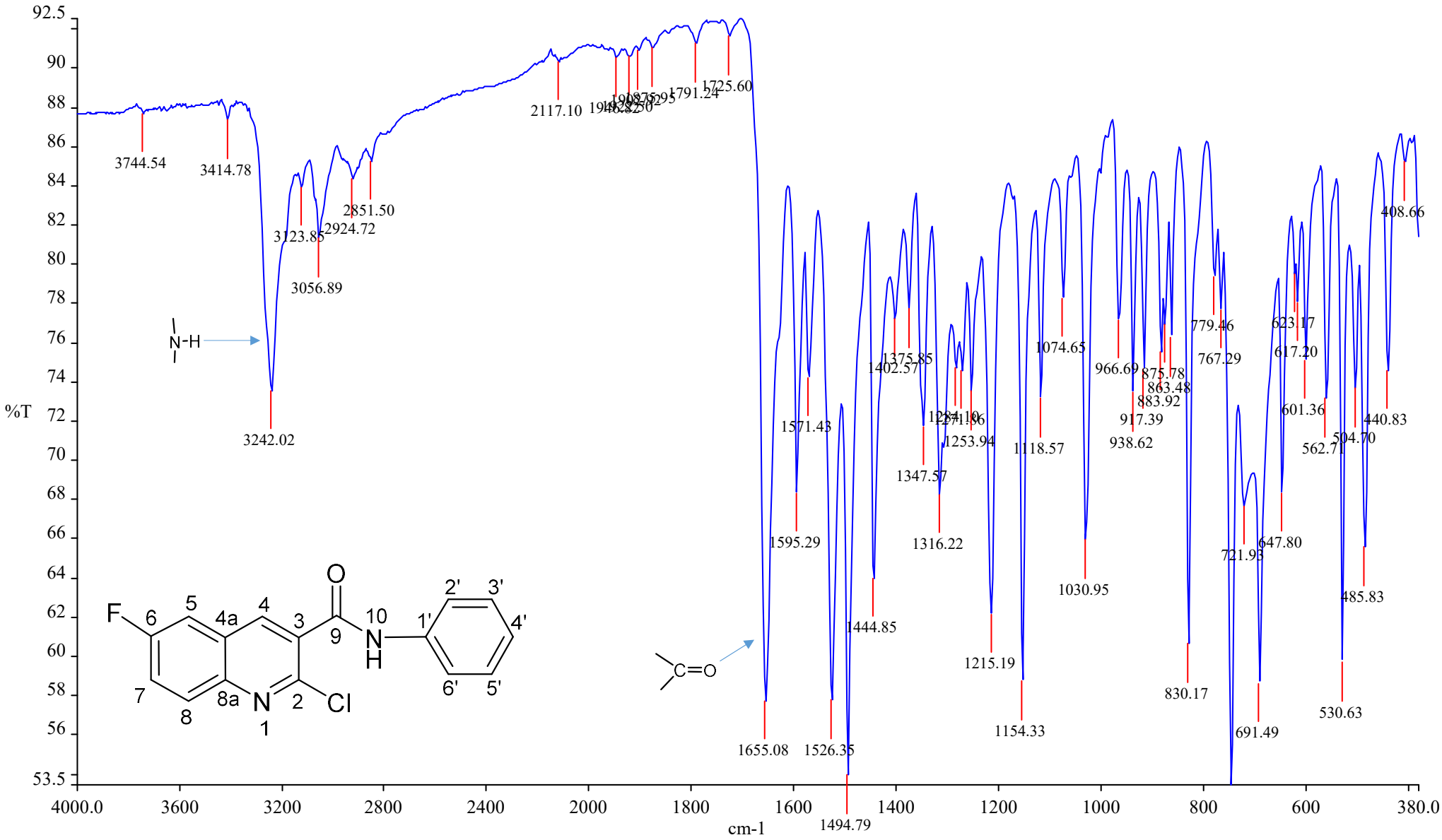
TOF MS ES-

8.52e+005

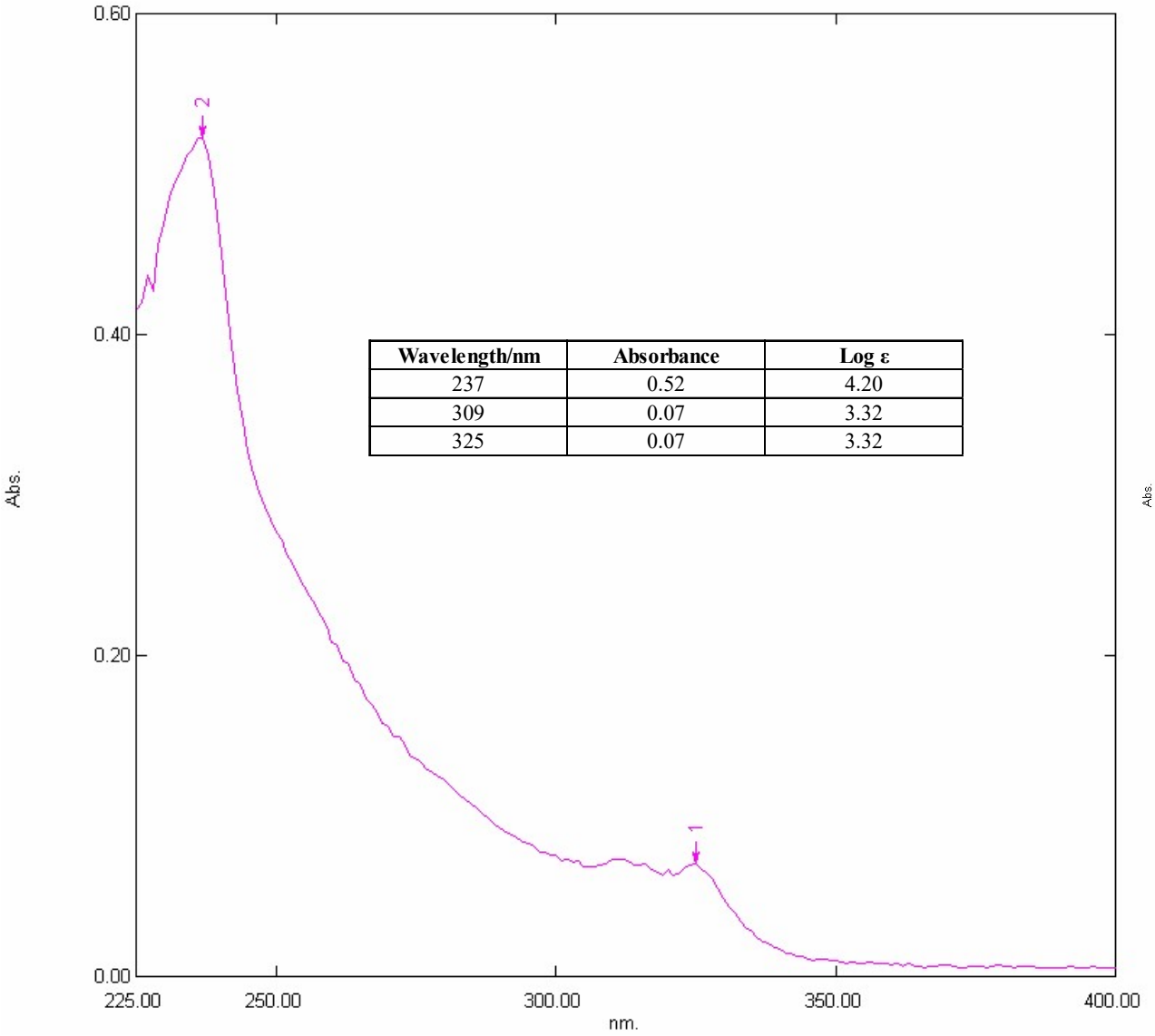


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
299.0392	299.0387	0.5	1.7	12.5	680.4	0.0	C16 H9 N2 O F Cl

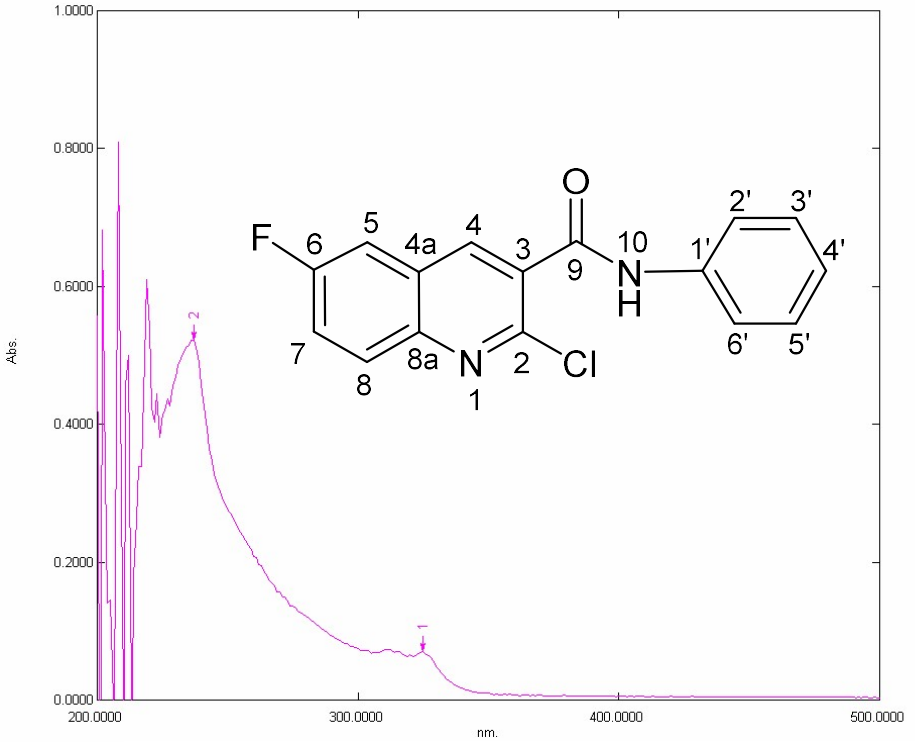
The HRMS spectrum of compound 5j



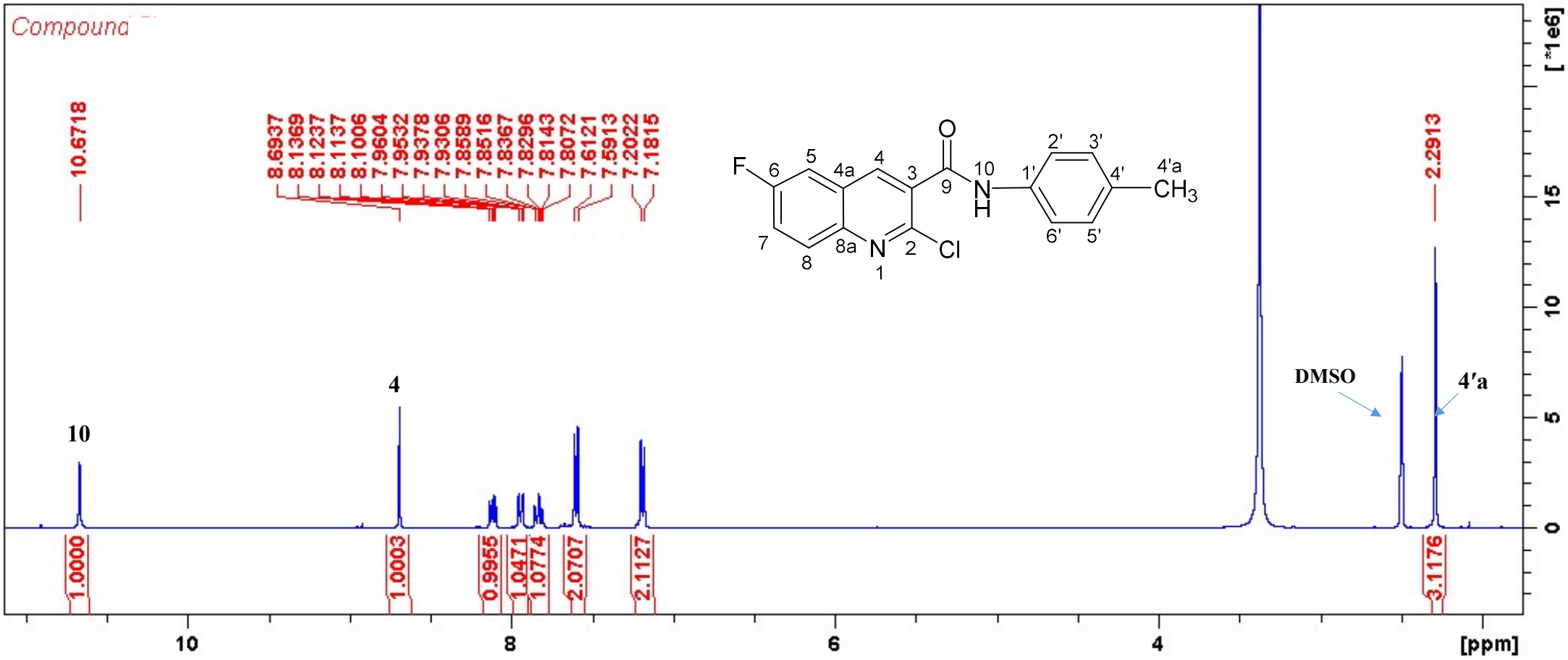
The IR spectrum of compound 5j



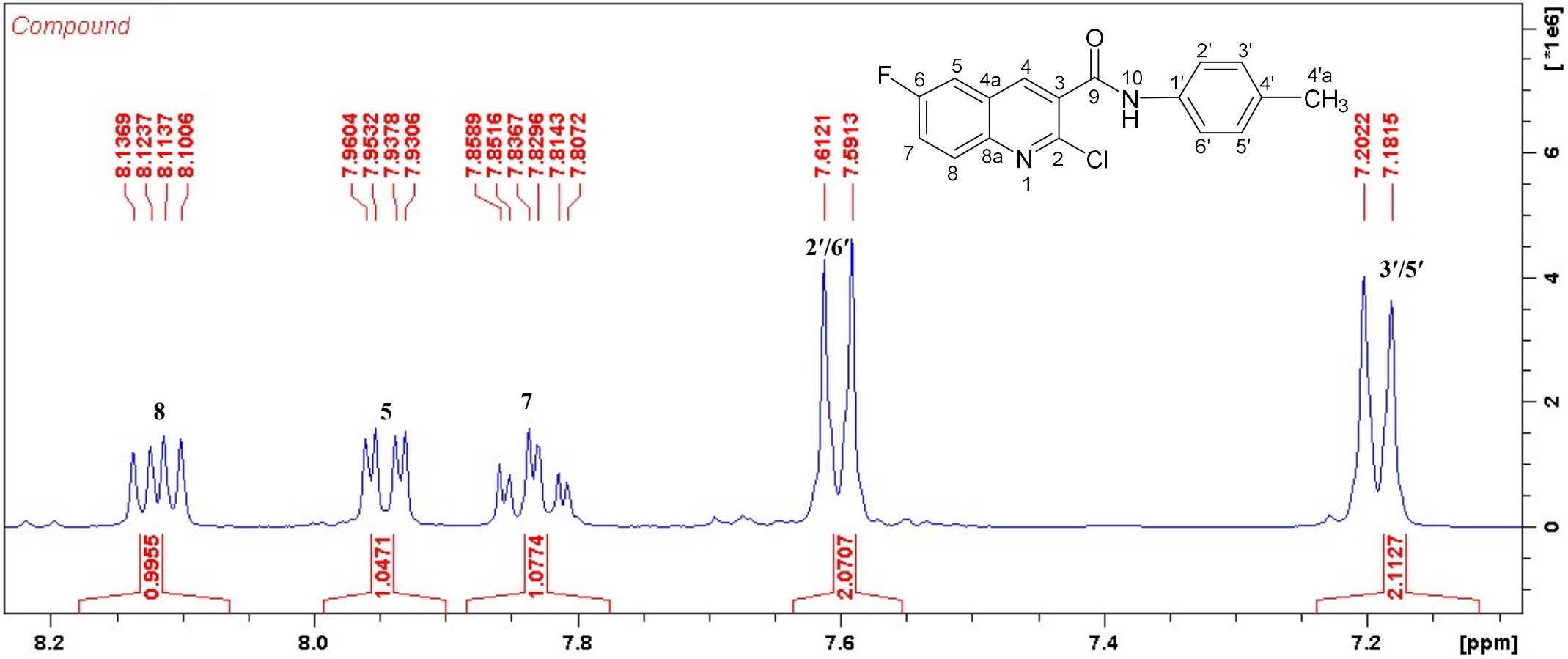
Wavelength/nm	Absorbance	Log ε
237	0.52	4.20
309	0.07	3.32
325	0.07	3.32



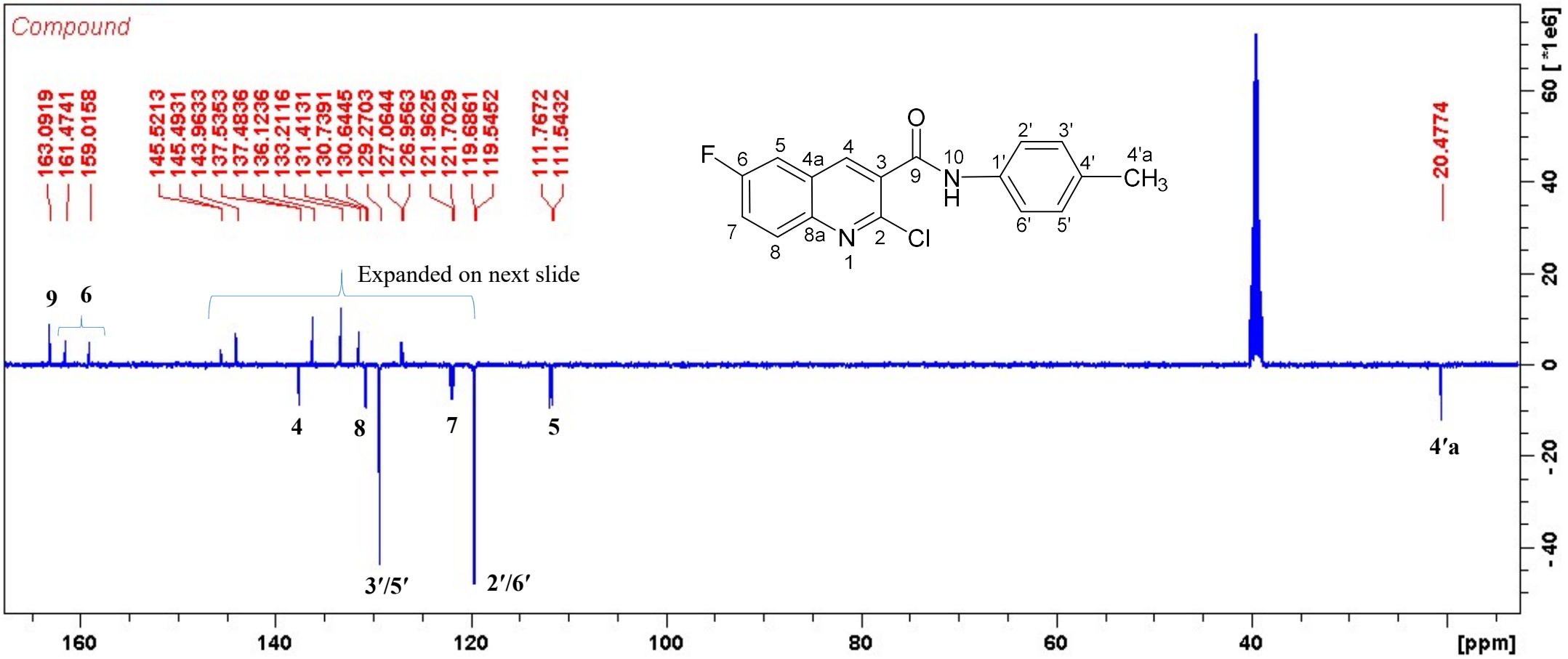
The UV-Vis spectrum of compound 5j



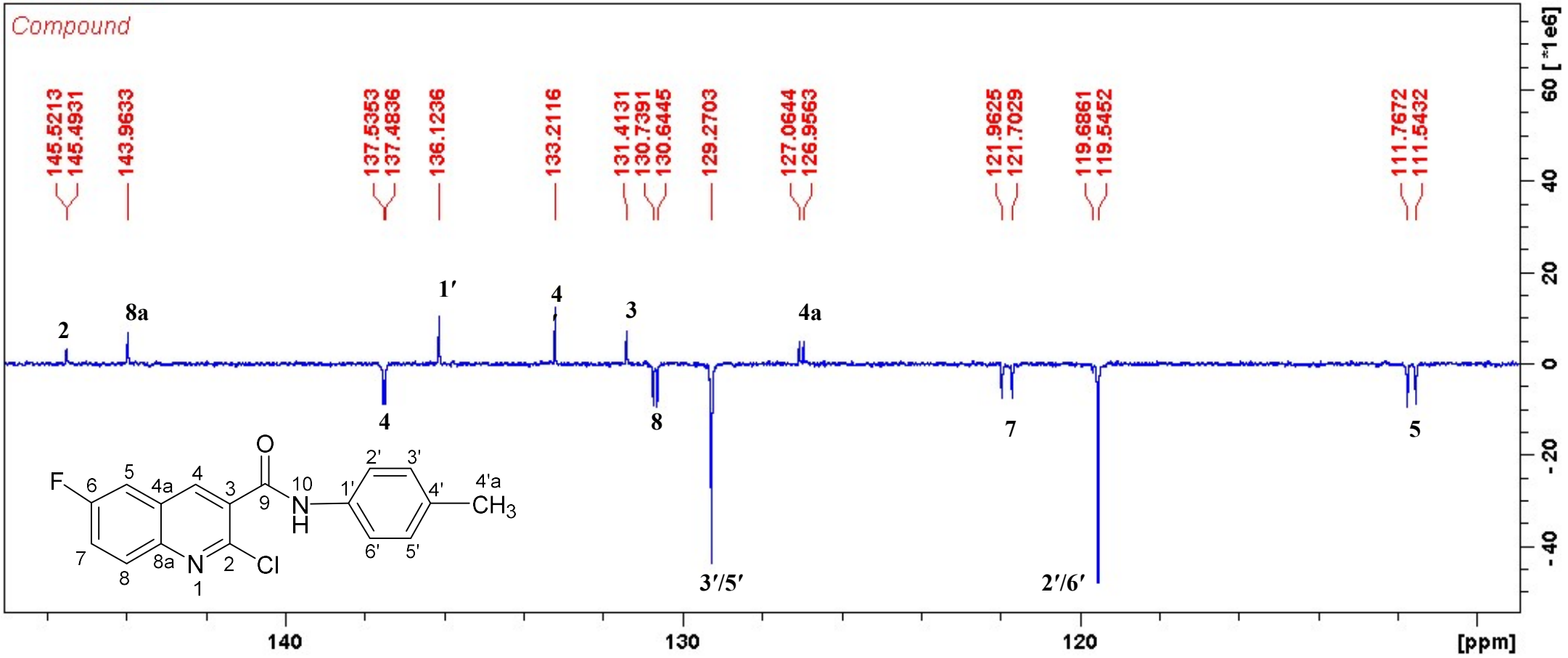
The ^{1}H NMR spectrum of compound 5k



The ^{1}H NMR spectrum of compound 5k (expanded)



The ^{13}C NMR spectrum of compound 5k

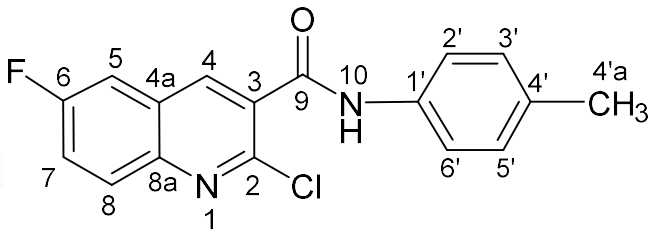


The ^{13}C NMR spectrum of compound 5k (expanded)

Elemental Composition Report

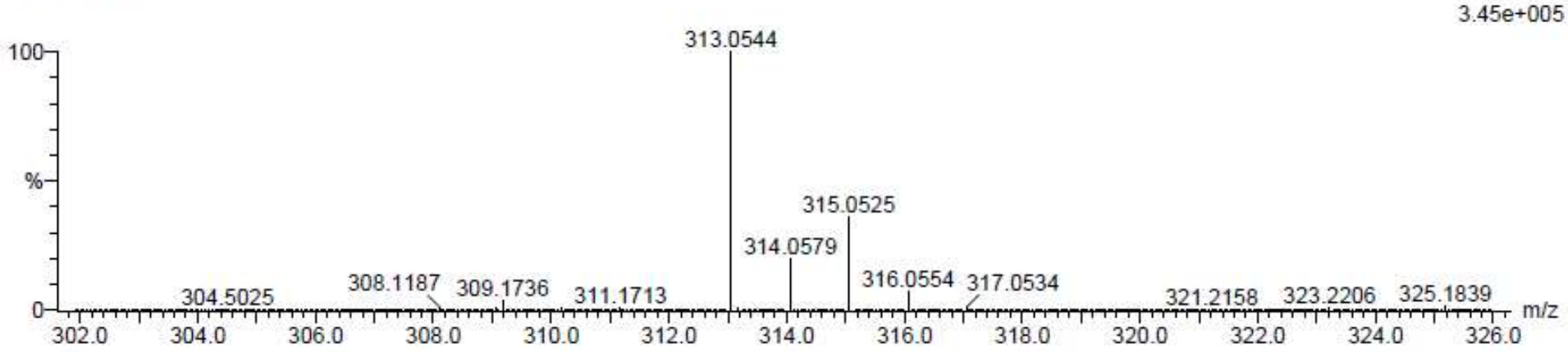
Single Mass Analysis

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



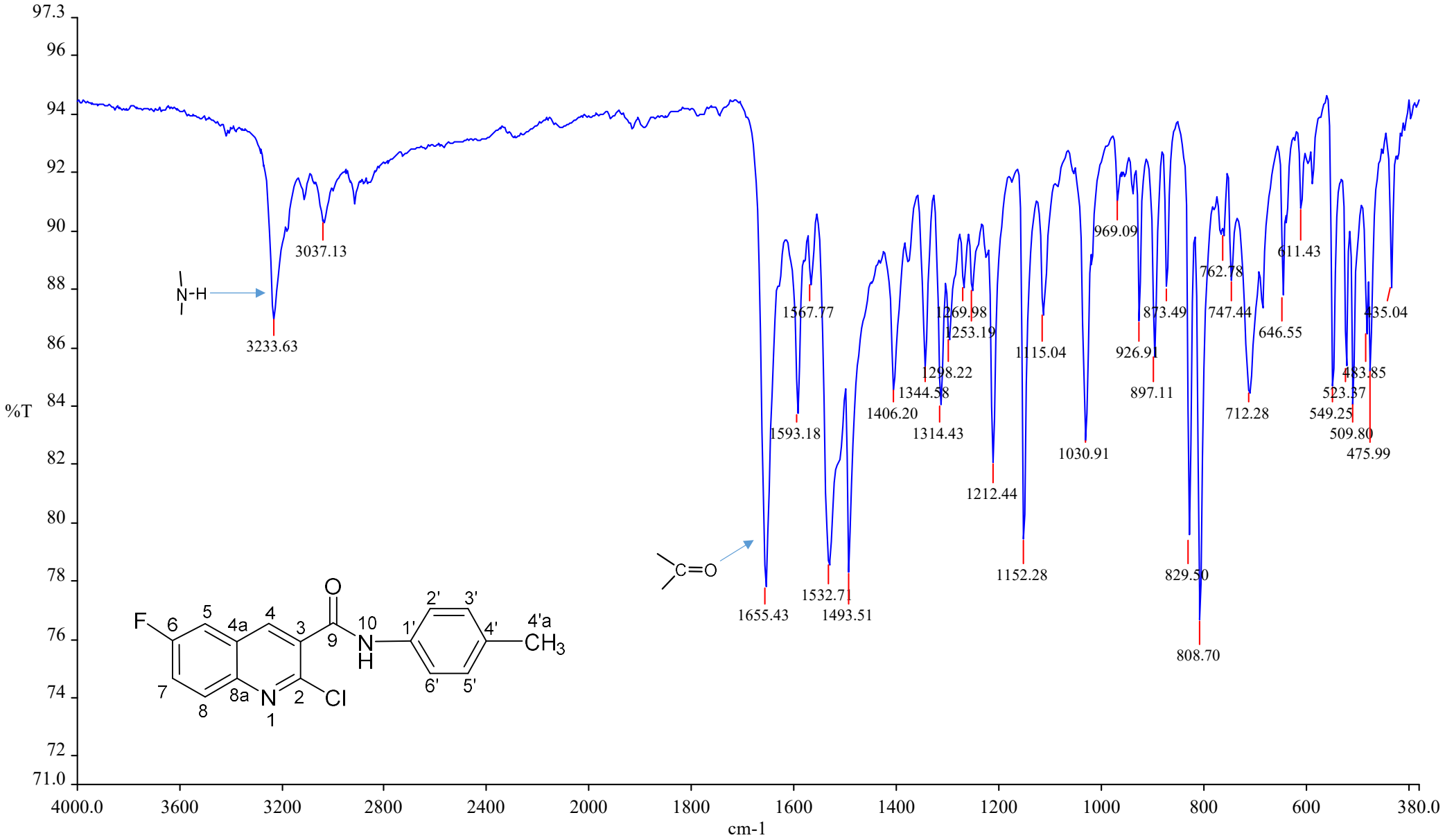
Monoisotopic Mass, Even Electron Ions
37 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 1-5 F: 0-1 Cl: 1-1

Compound 2c HG 41 (1.351) Cm (1:61)
TOF MS ES-

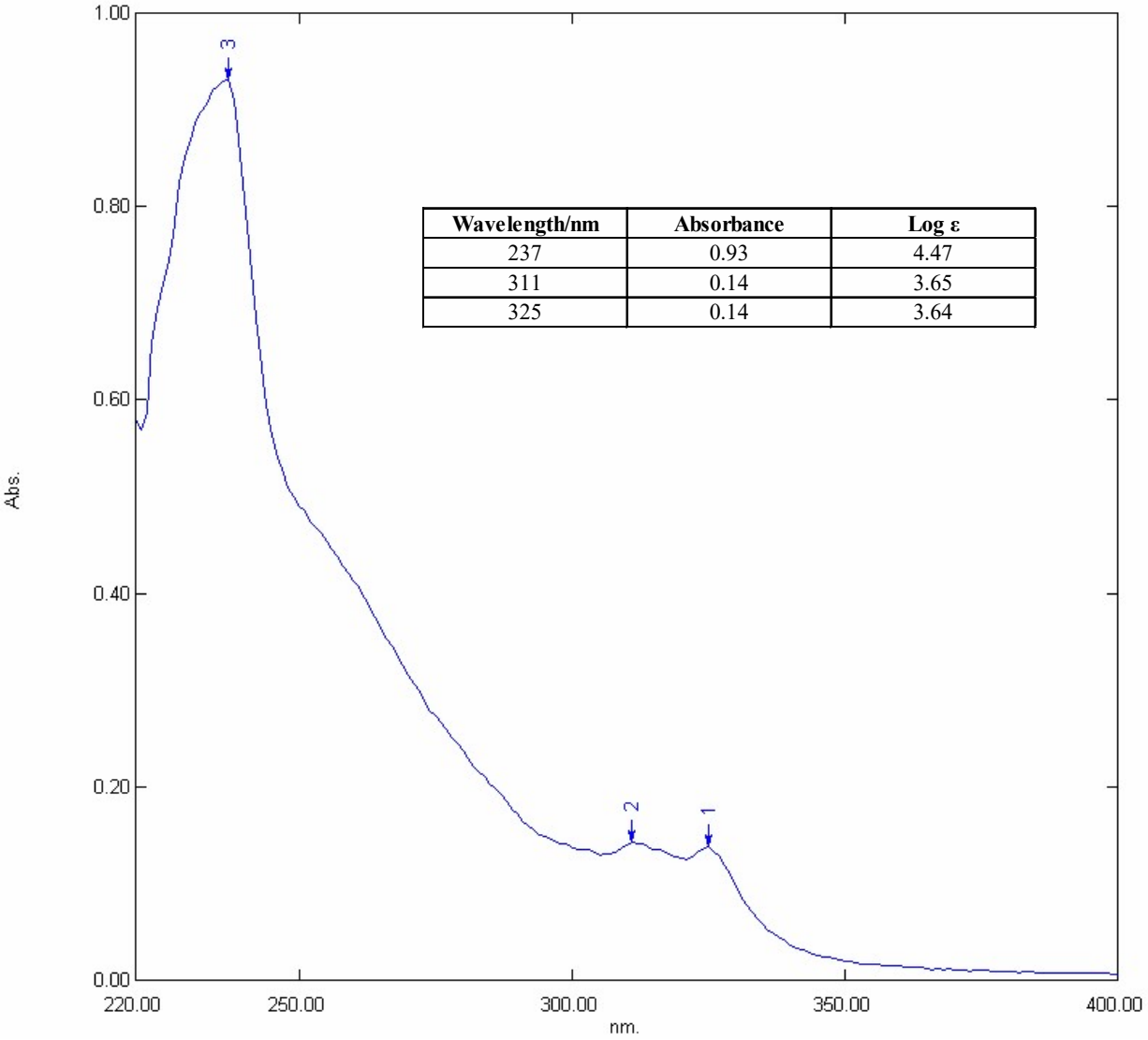


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
313.0544	313.0544	0.0	0.0	12.5	645.2	0.0	C17 H11 N2 O F Cl

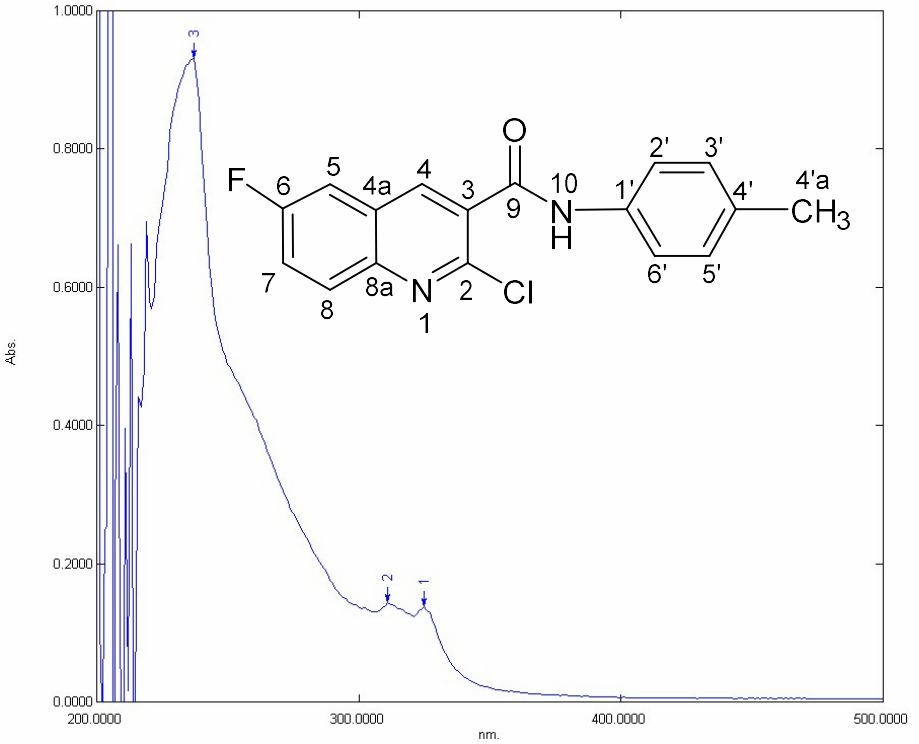
The HRMS spectrum of compound 5k



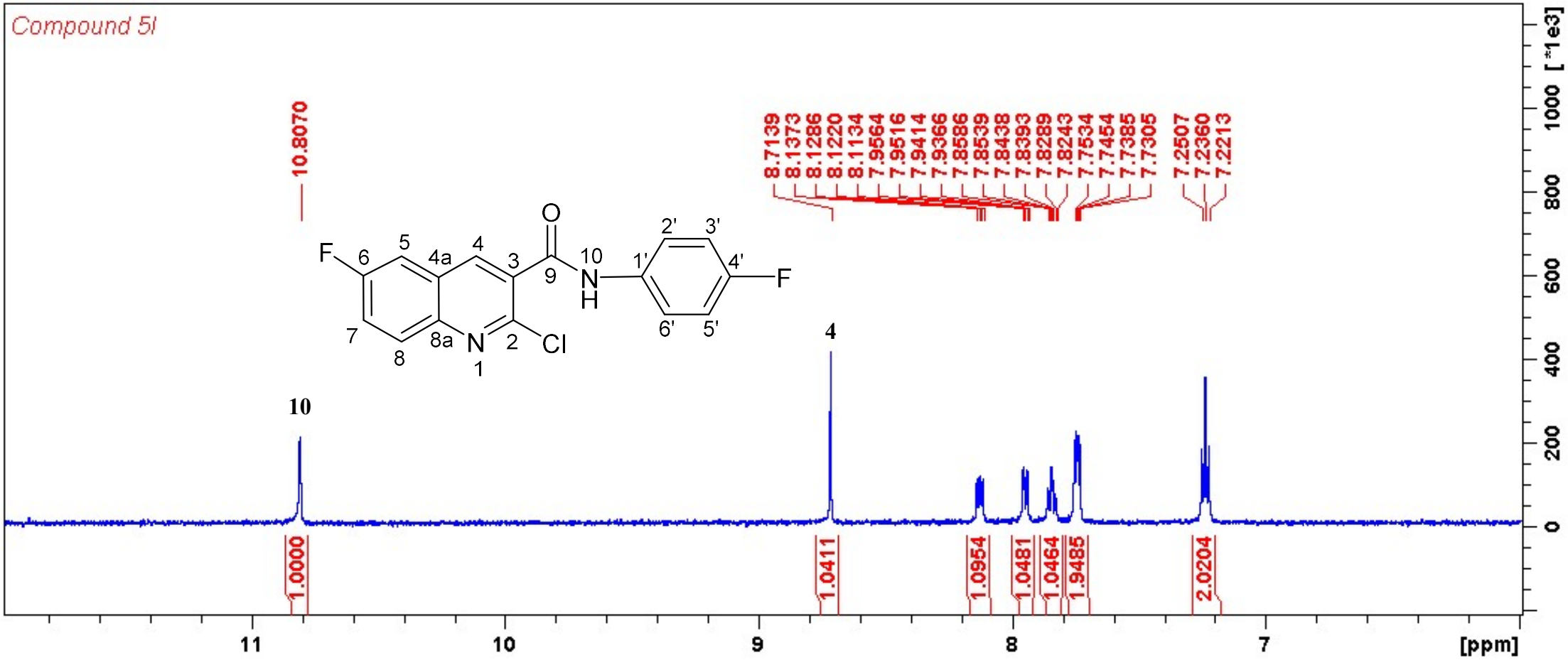
The IR spectrum of compound 5k



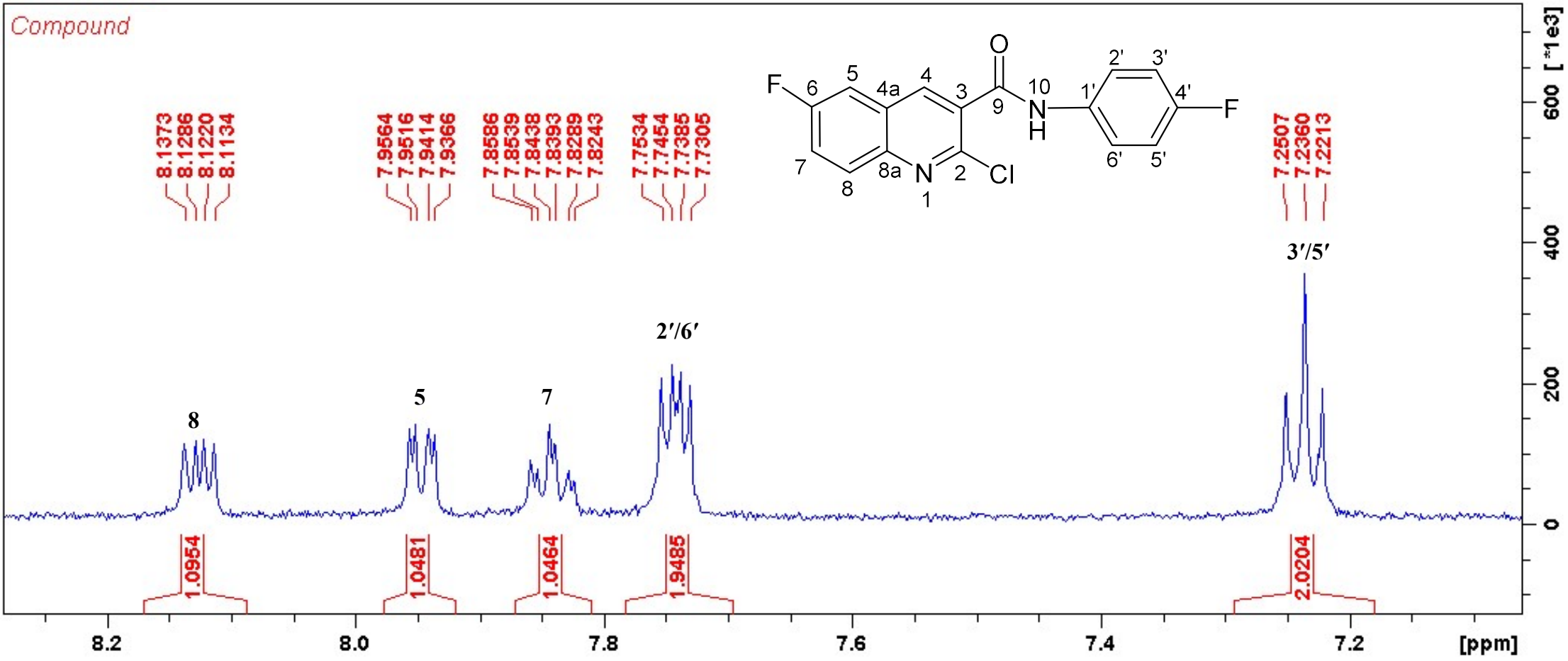
Wavelength/nm	Absorbance	Log ε
237	0.93	4.47
311	0.14	3.65
325	0.14	3.64



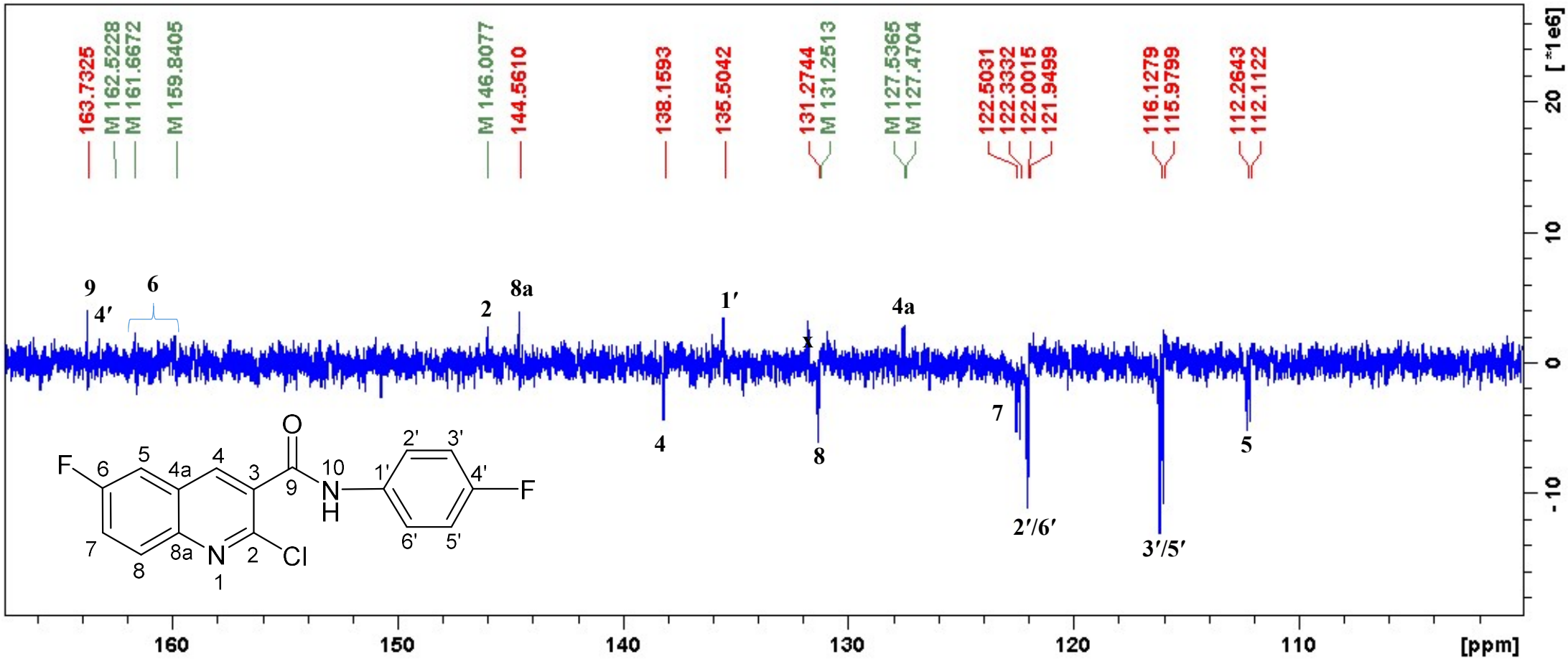
The UV-Vis spectrum of compound 5k



The ^{1}H NMR spectrum of compound 5l



The ^{1}H NMR spectrum of compound 5l (expanded)



The ^{13}C NMR spectrum of compound 5l

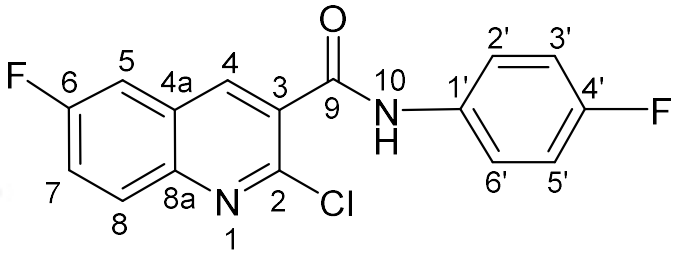
Elemental Composition Report **Page 1**

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

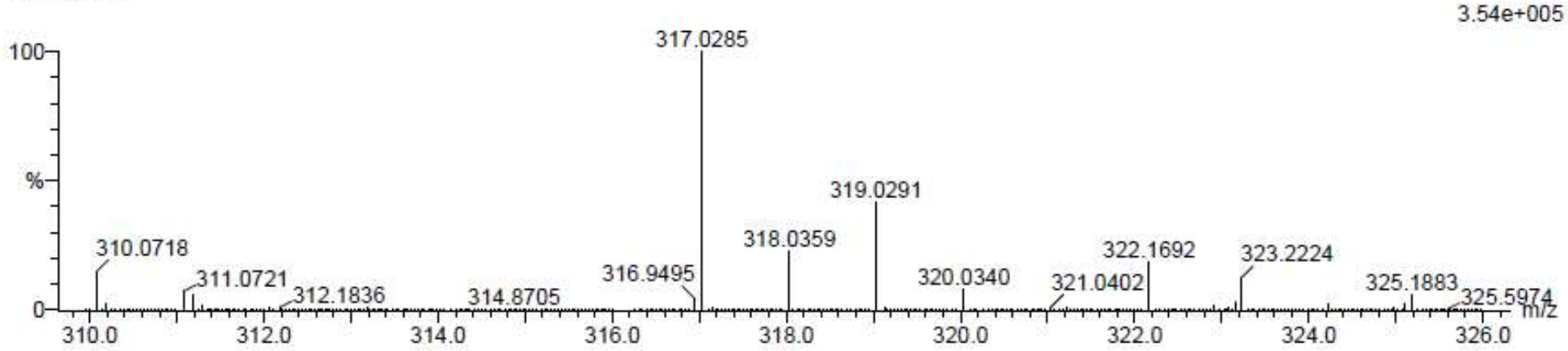
103 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)

Elements Used:

C: 15-20 H: 5-10 N: 0-5 O: 1-5 F: 0-2 Cl: 0-1

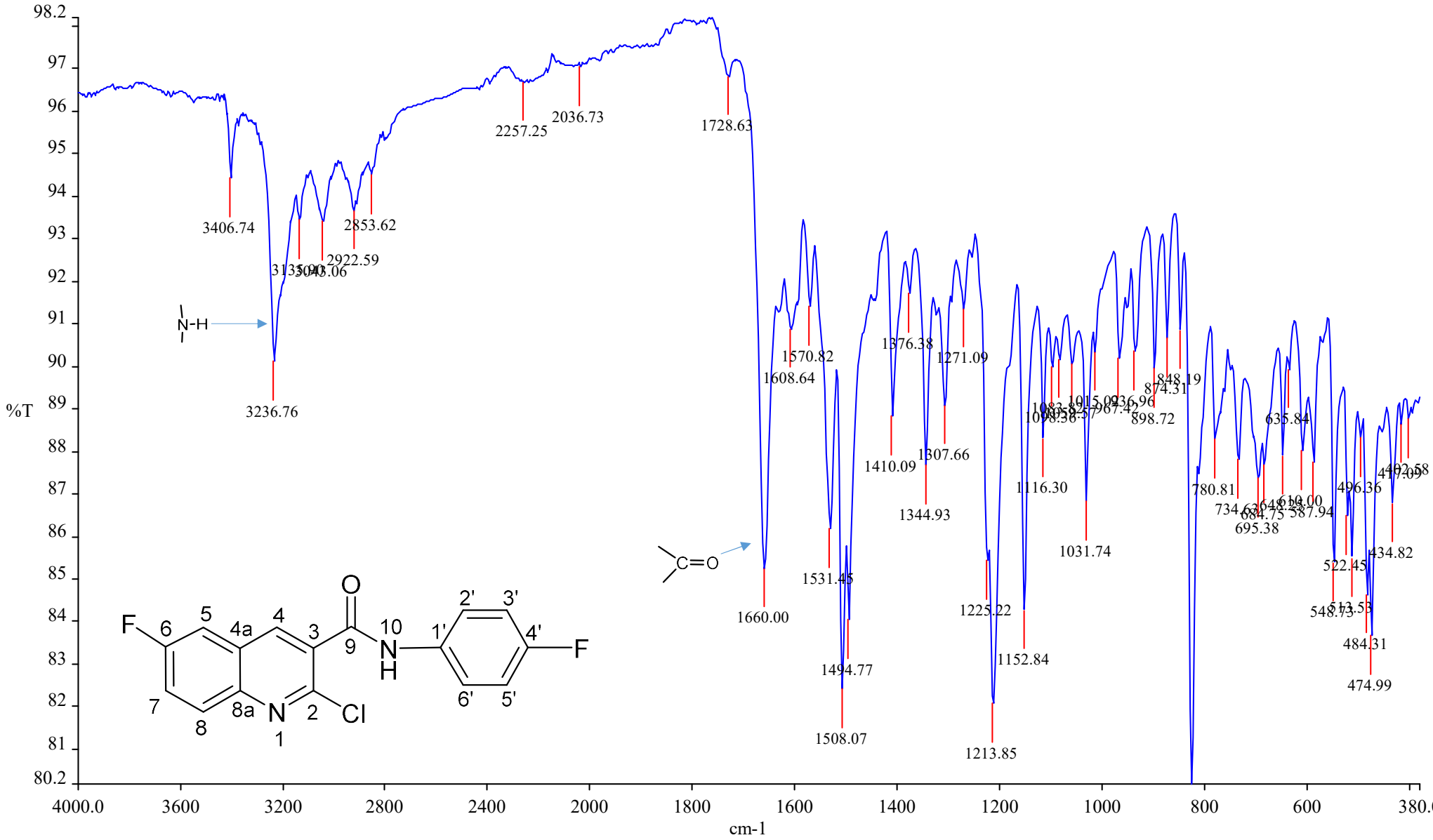
Compound 4c HG 10 (0.304) Cm (1:61)

TOF MS ES-

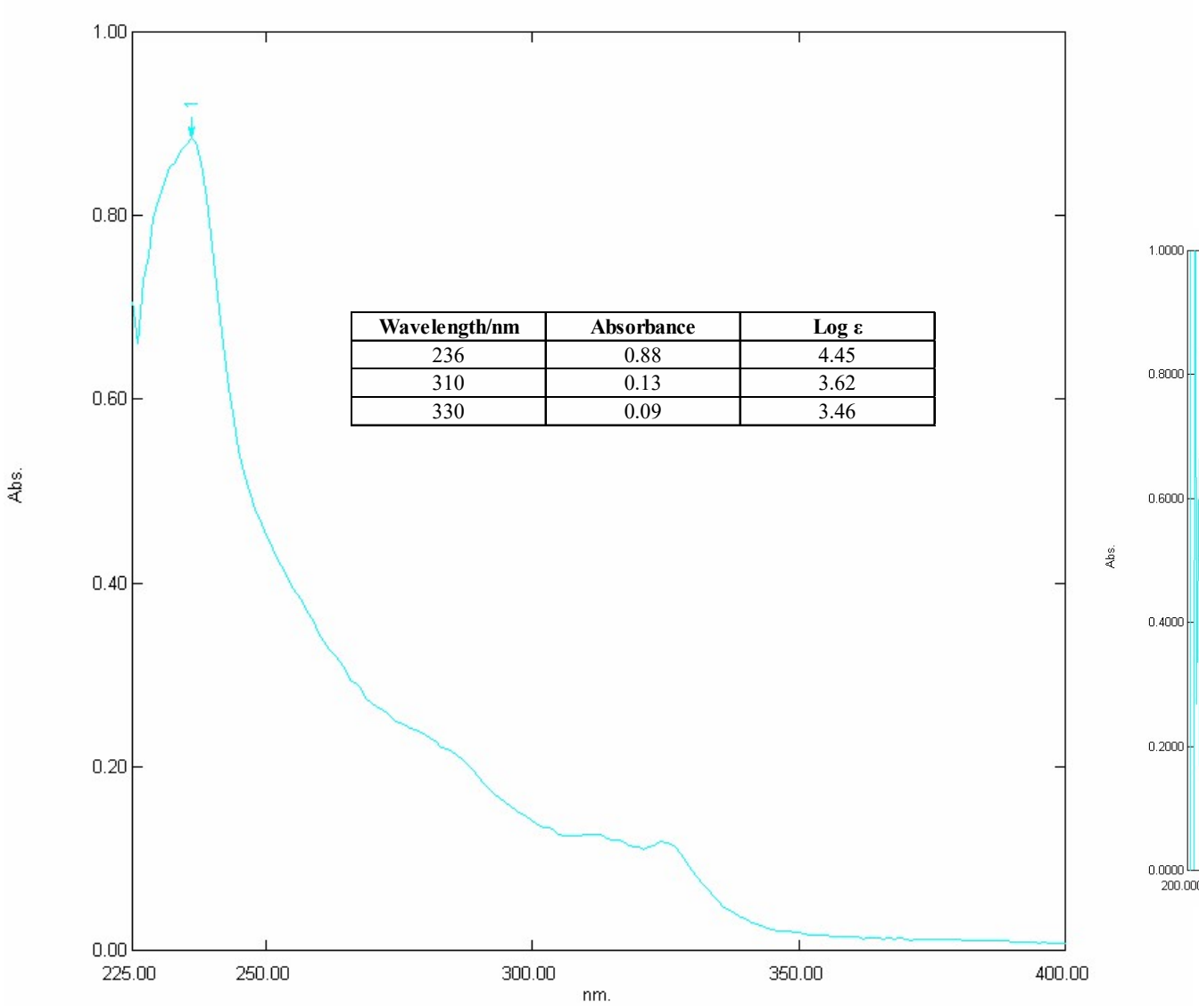


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
317.0285	317.0293	-0.8	-2.5	12.5	636.0	0.0	C16 H8 N2 O F2 Cl

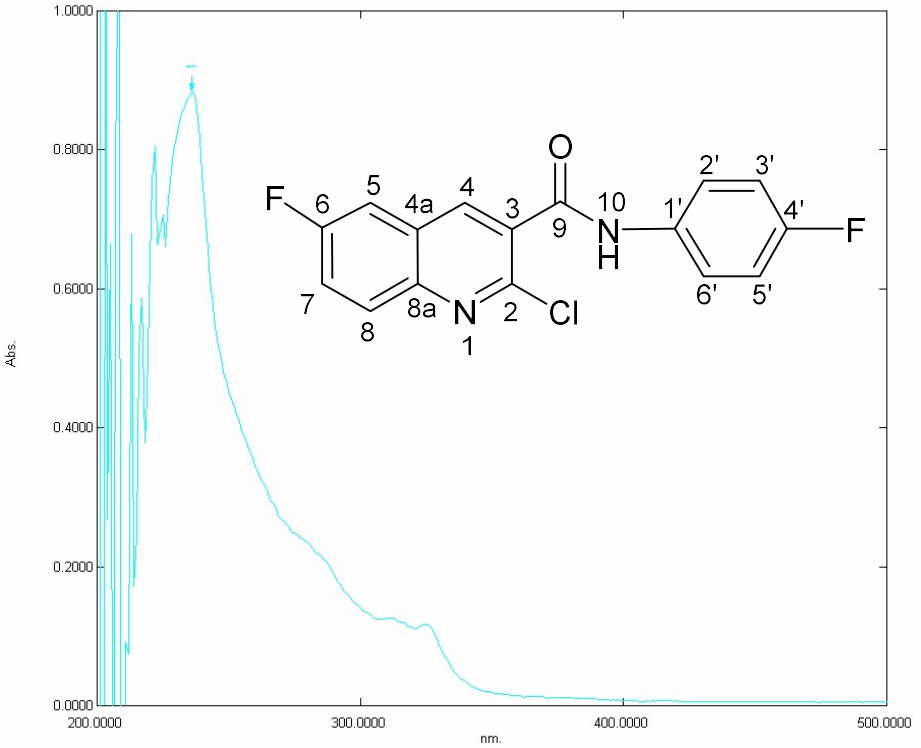
The HRMS spectrum of compound 5l



The IR spectrum of compound 5l



Wavelength/nm	Absorbance	Log ε
236	0.88	4.45
310	0.13	3.62
330	0.09	3.46

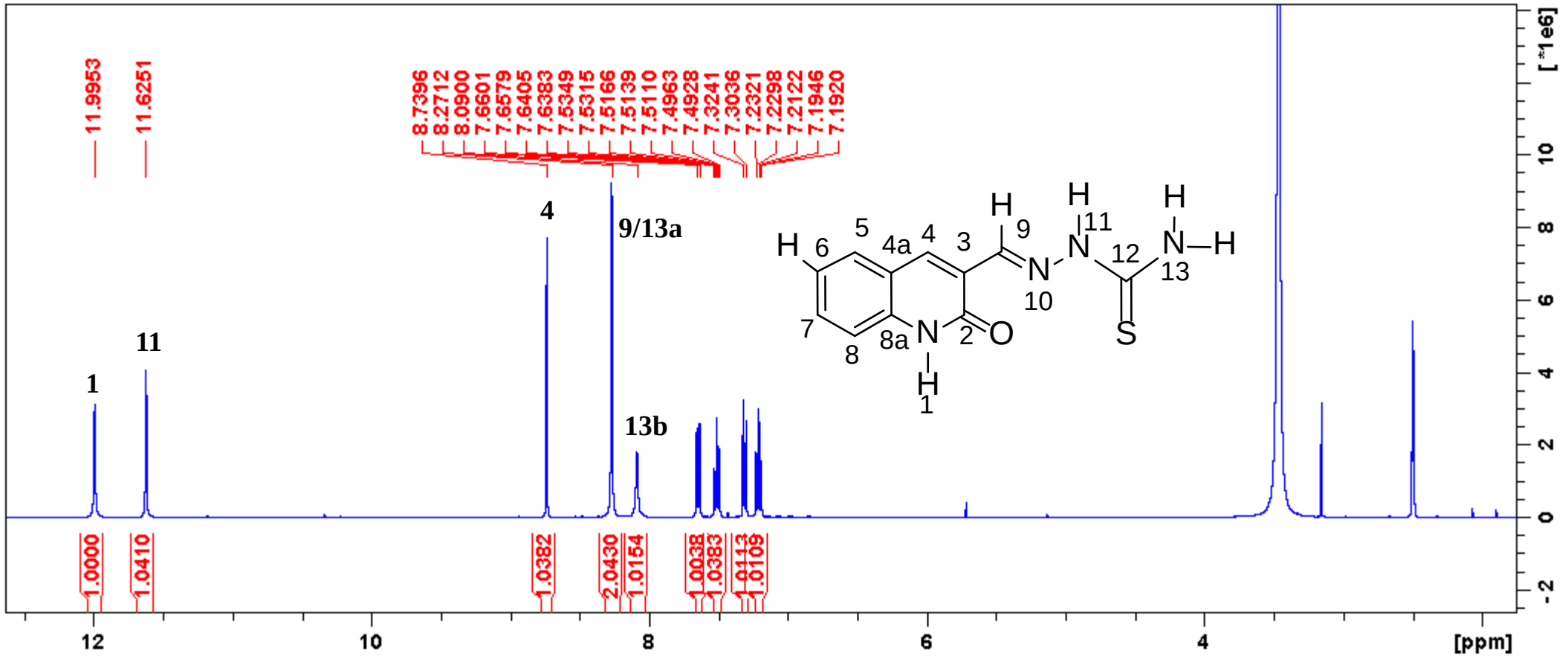


The UV-Vis spectrum of compound 5l

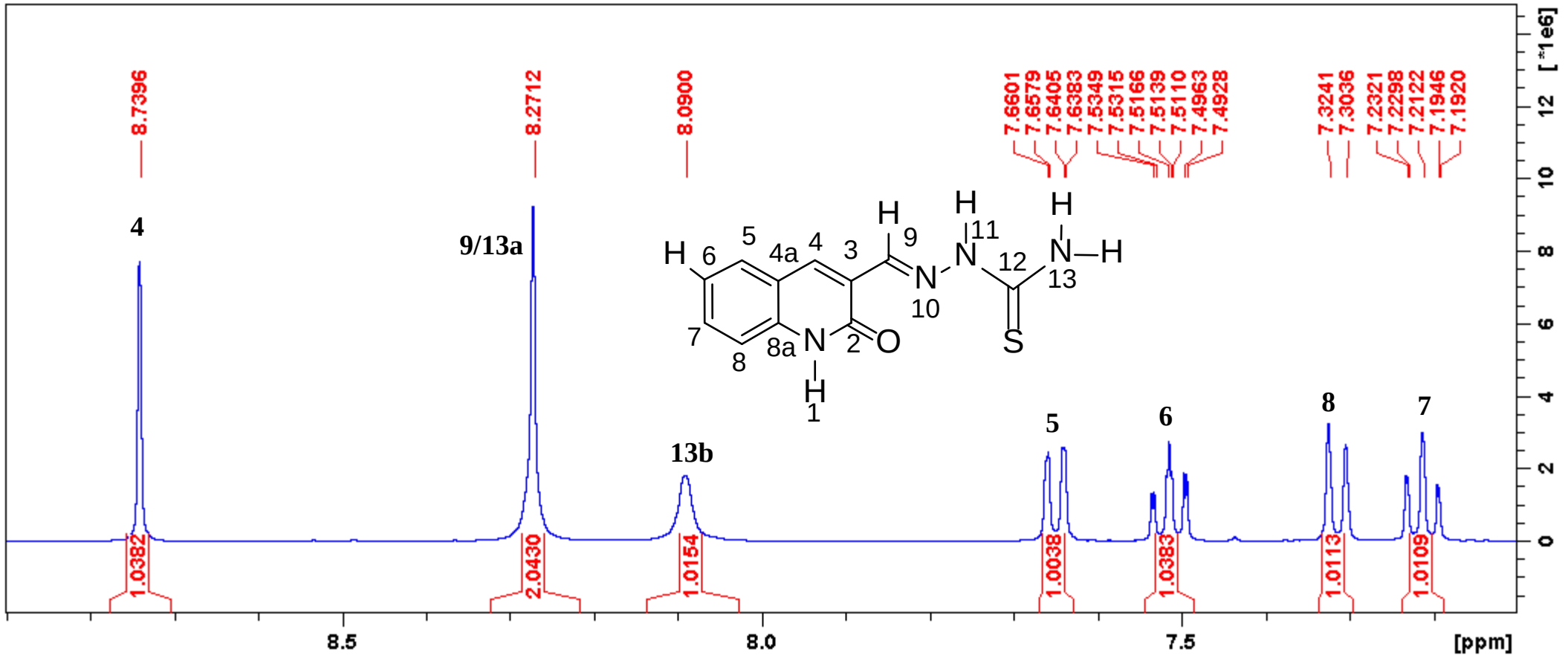
Supplementary Information

Chapter 3 Synthesis, Antibacterial Activity and Docking Studies of Substituted Quinolone Thiosemicarbazones

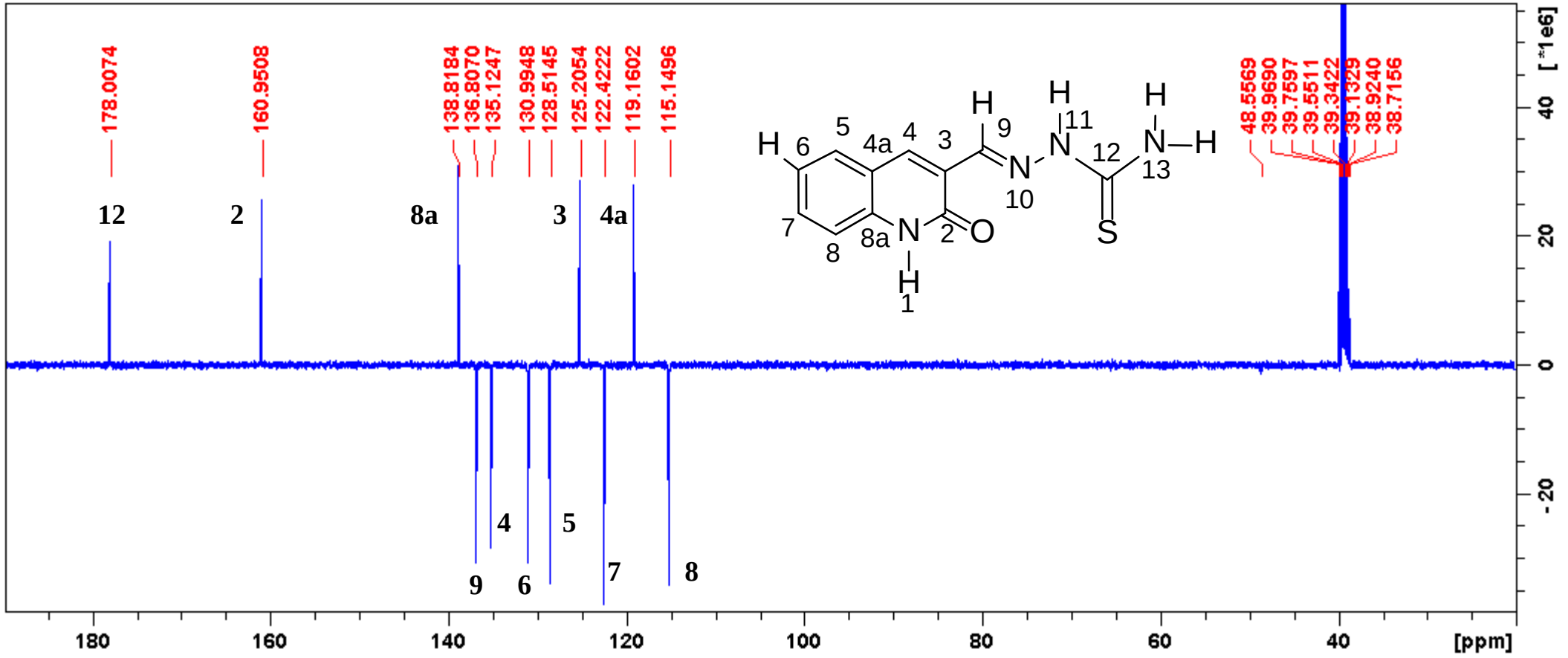
Contents



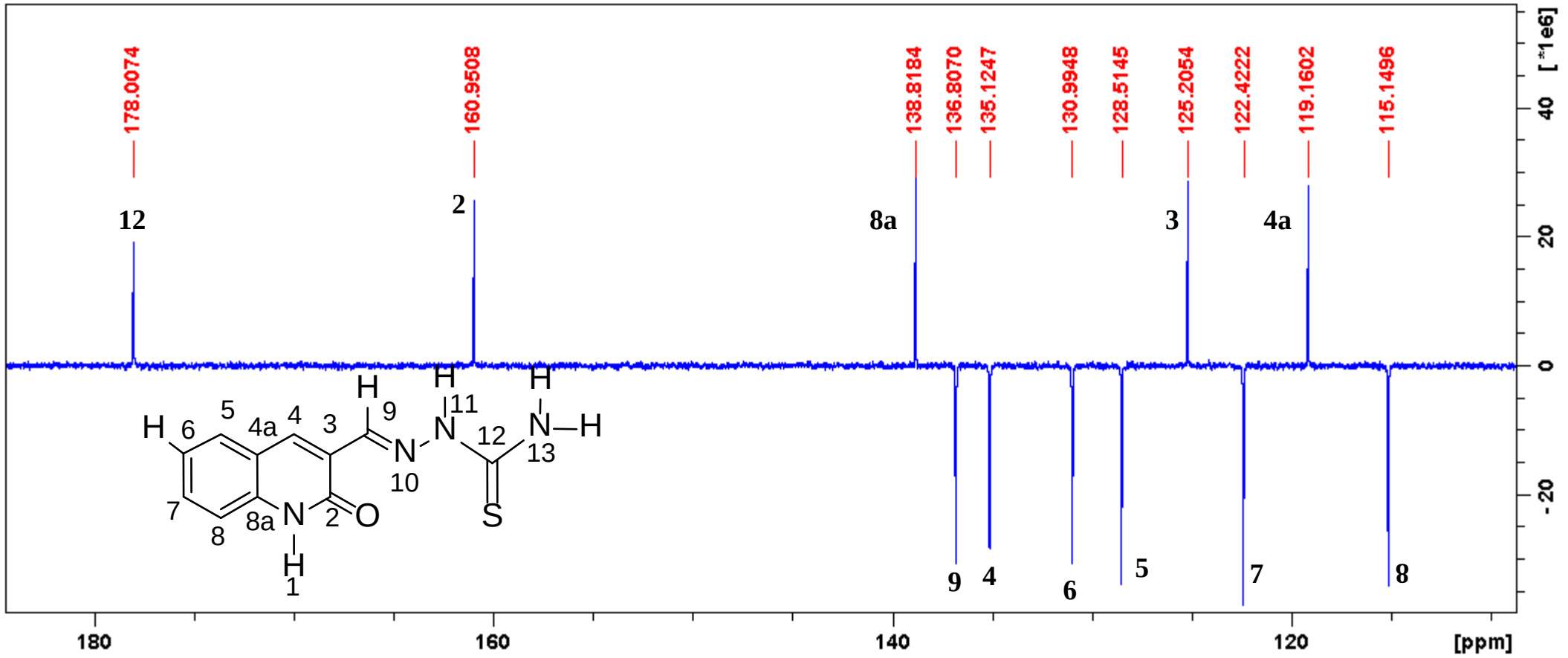
^{1}H NMR spectrum of compound 5a



^{1}H NMR spectrum of compound 5a (expanded)



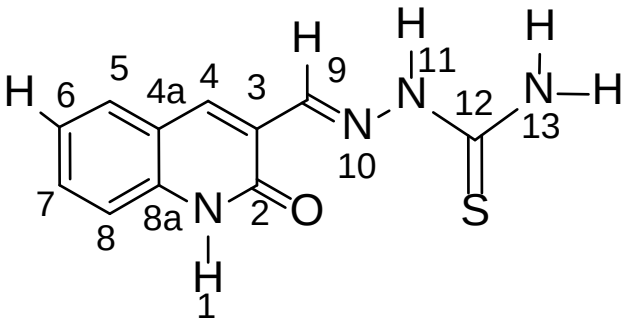
^{13}C NMR spectrum of compound 5a



^{13}C NMR spectrum of compound 5a (expanded)

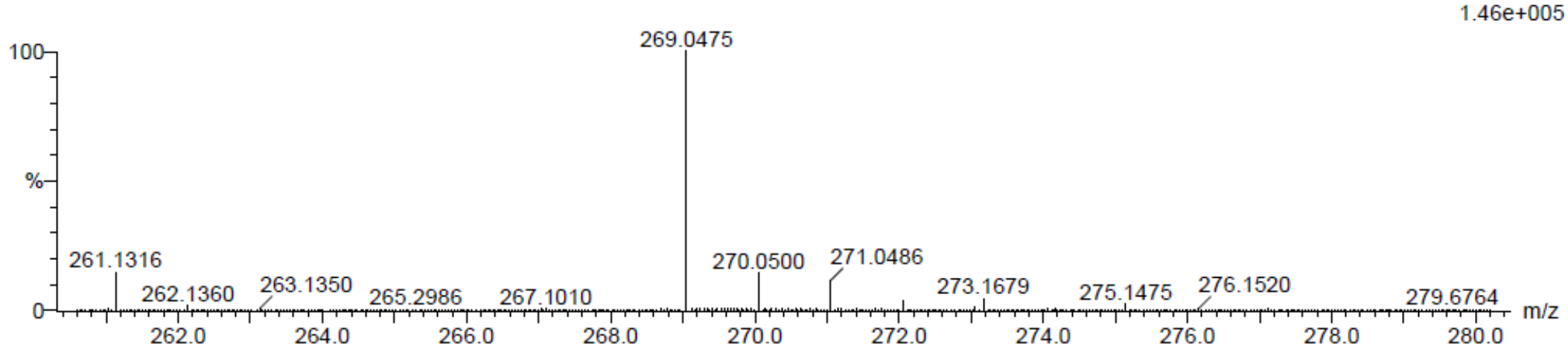
Single Mass Analysis

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



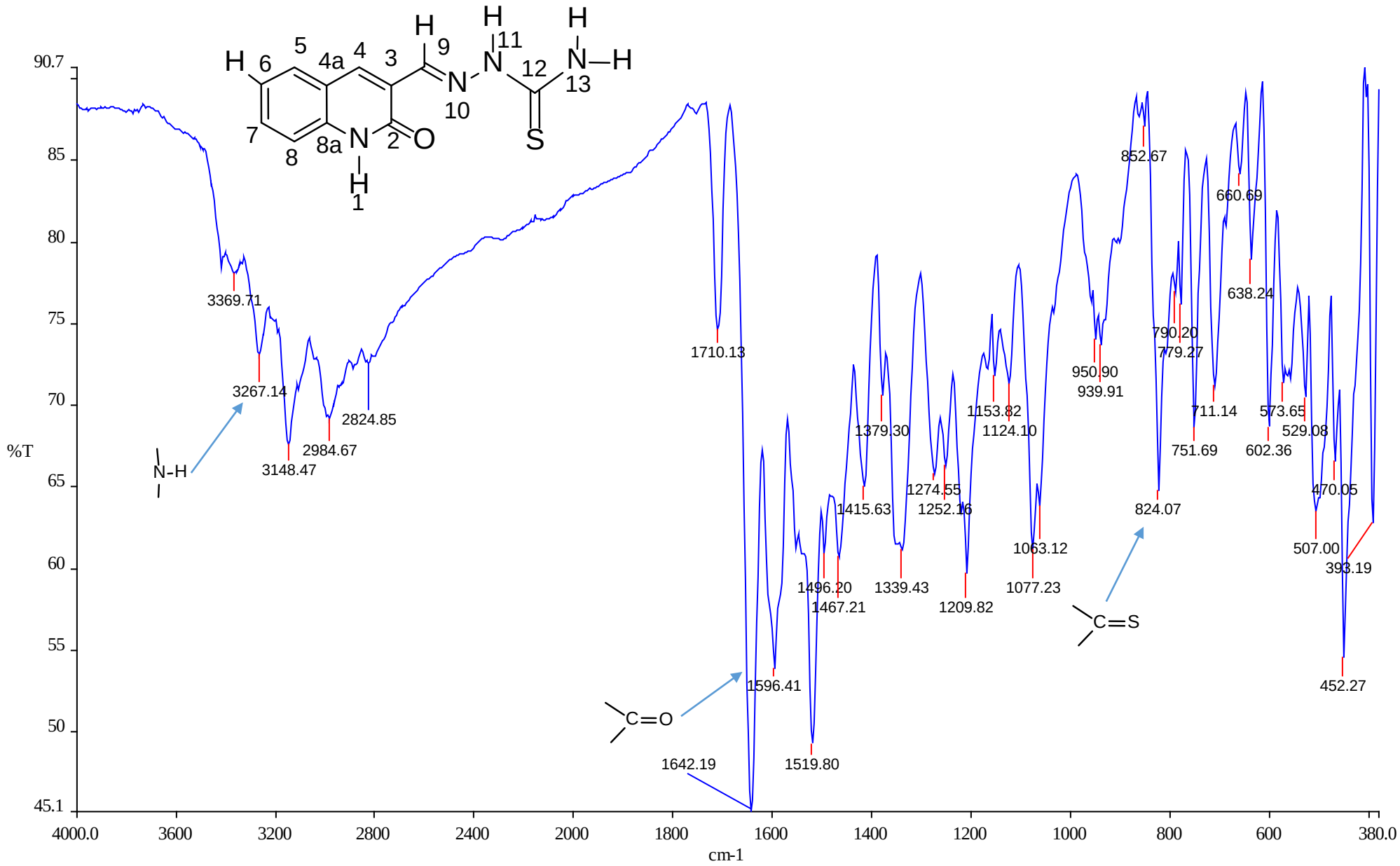
Monoisotopic Mass, Even Electron Ions
39 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 5-10 N: 0-5 O: 0-5 Na: 1-1 S: 0-1

Compound 5g HG 22 (0.709) Cm (1:61)
TOF MS ES+



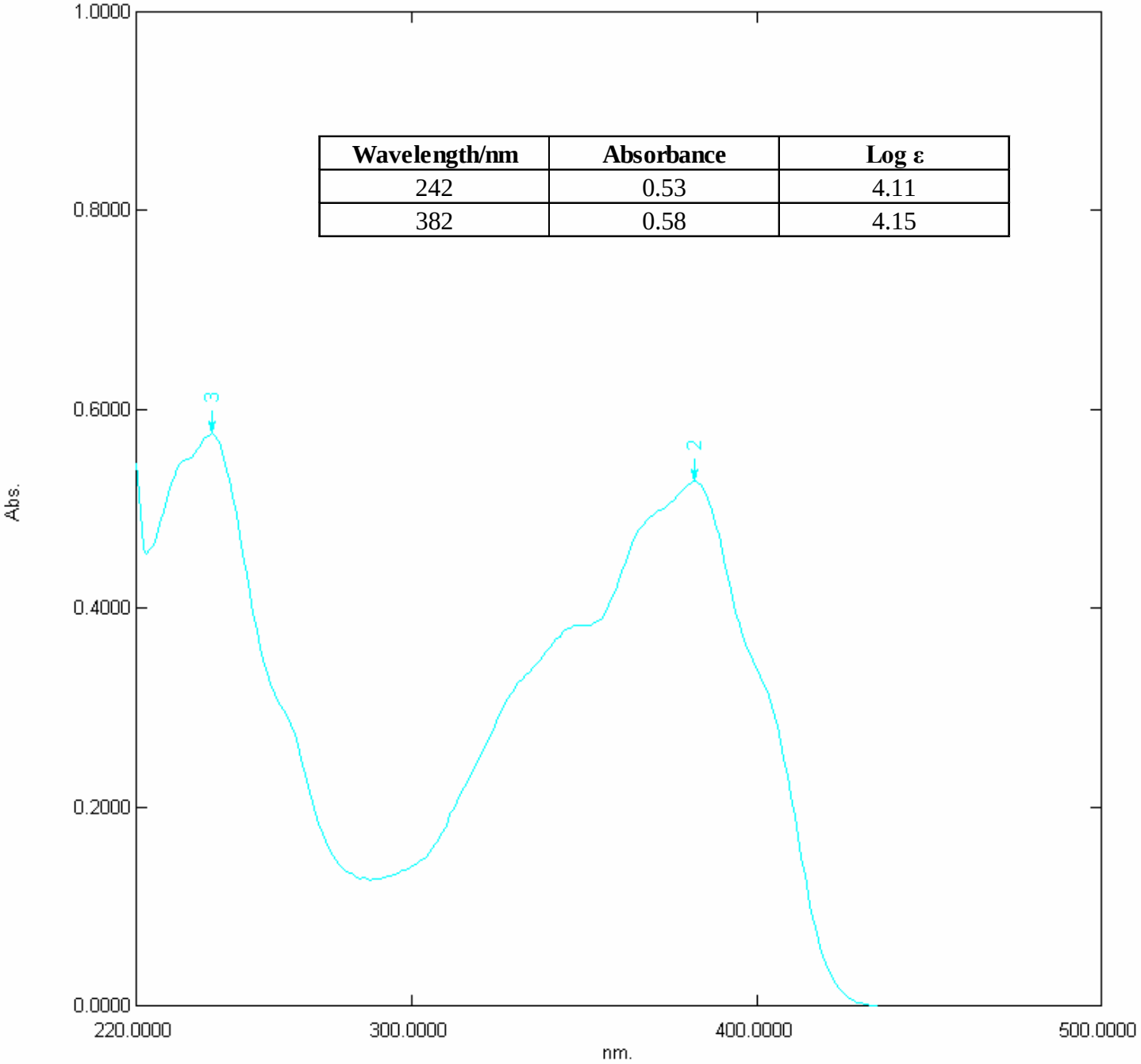
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
269.0475	269.0473	0.2	0.7	8.5	637.3	0.0	C11 H10 N4 O Na S

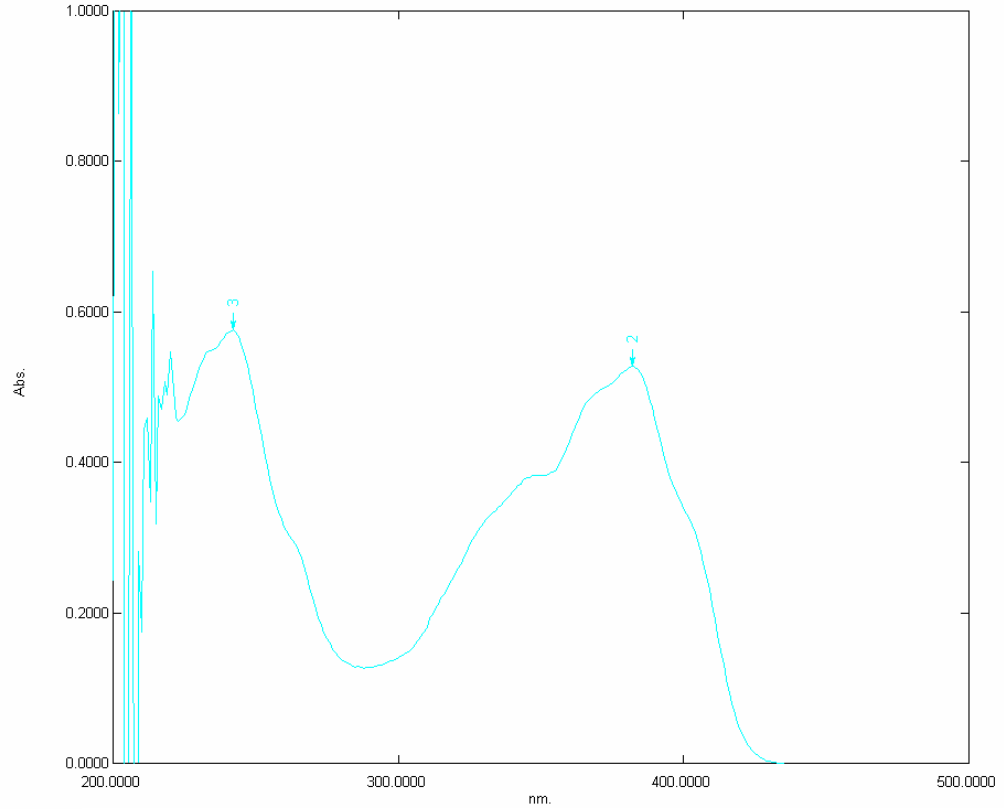
HRMS spectrum of compound 5a

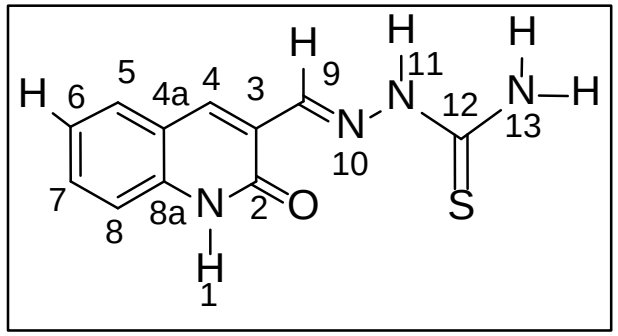


IR spectrum of compound 5a

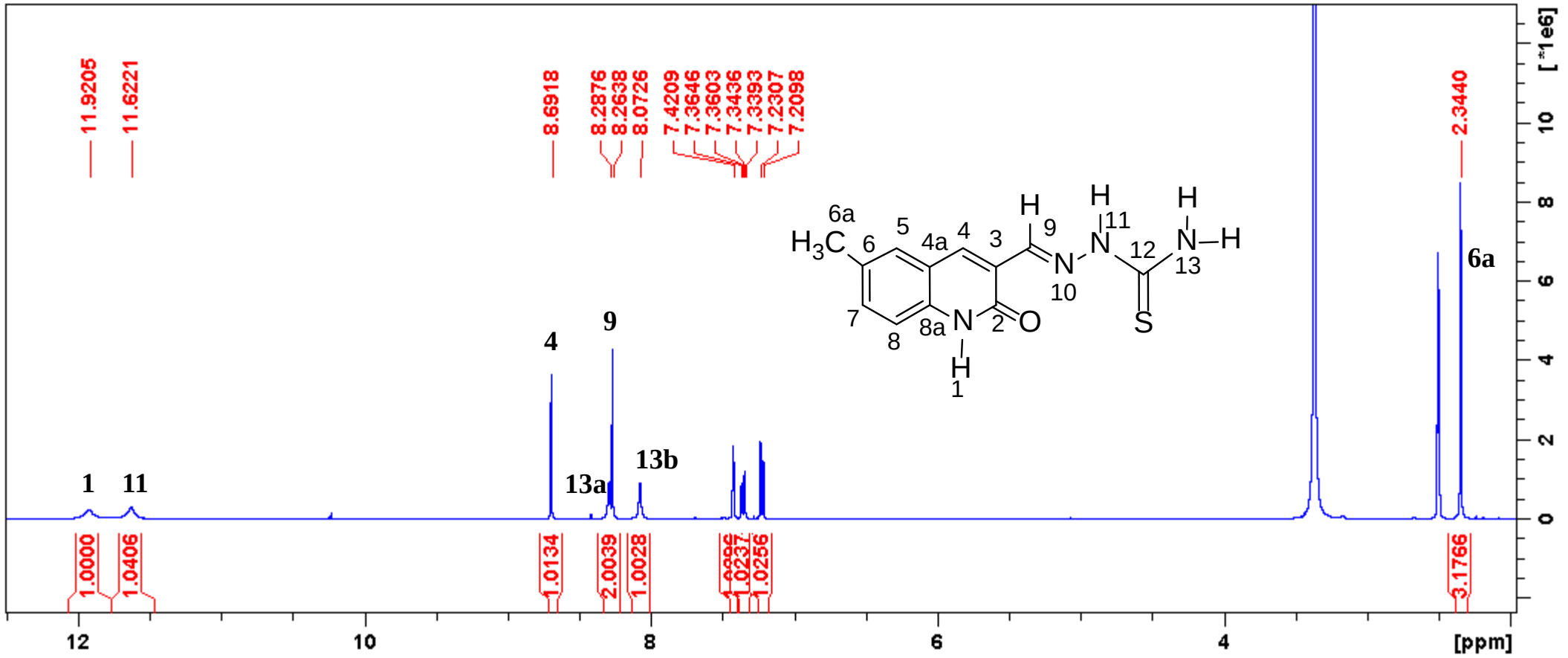
Wavelength/nm	Absorbance	Log ε
242	0.53	4.11
382	0.58	4.15



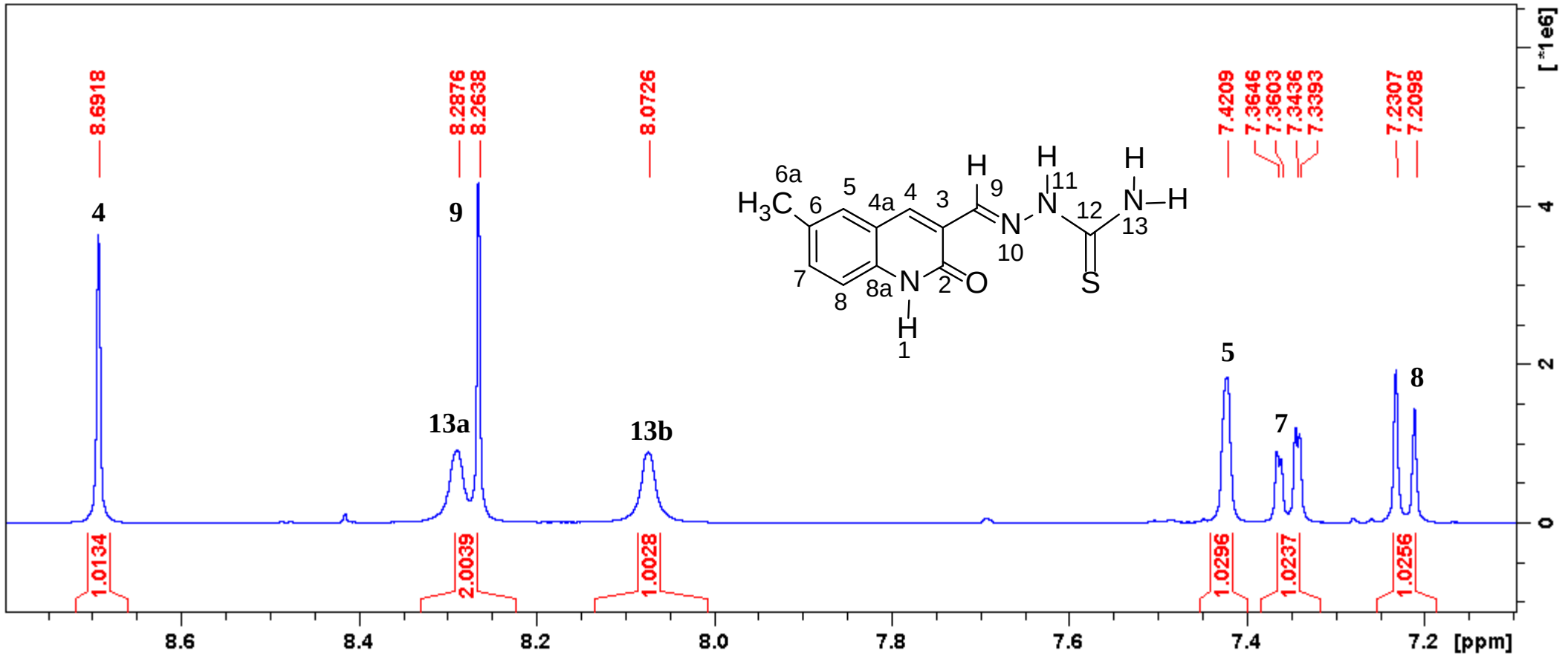




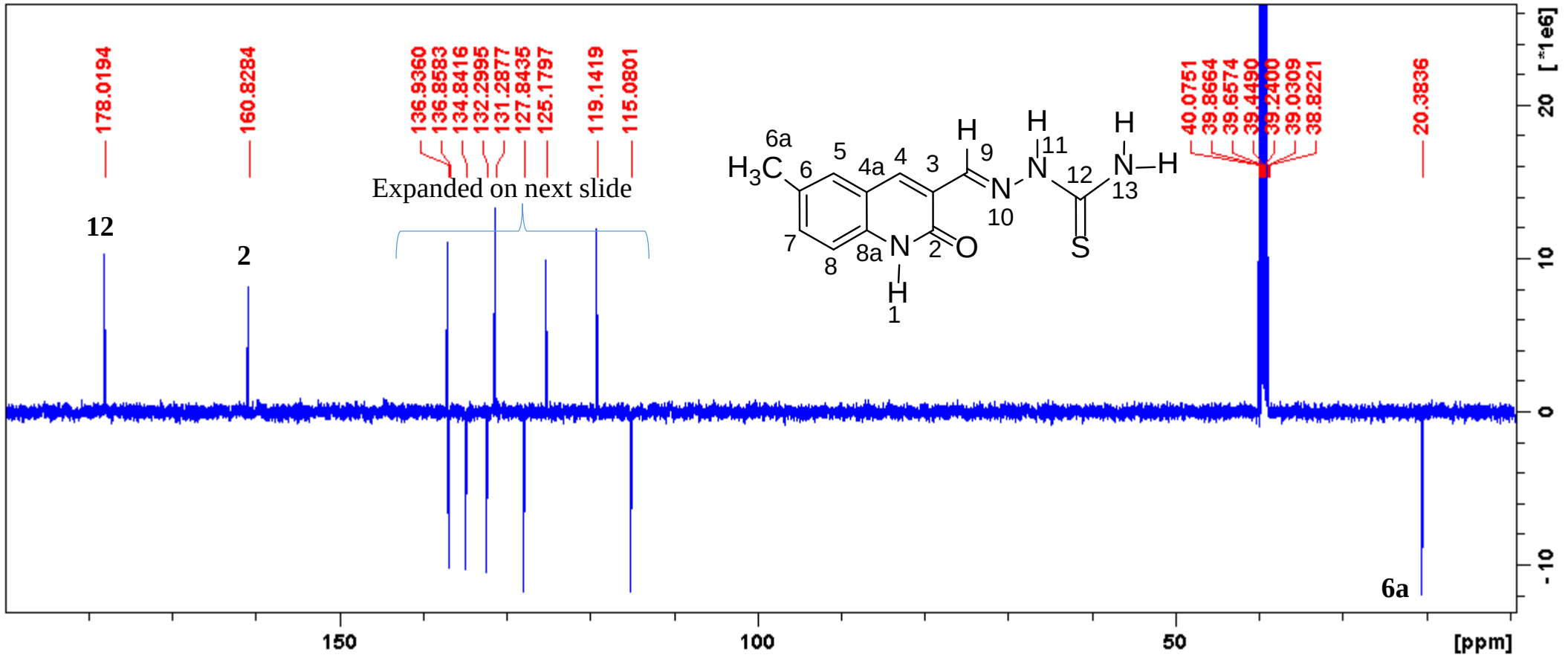
UV-Vis spectrum of compound 5a



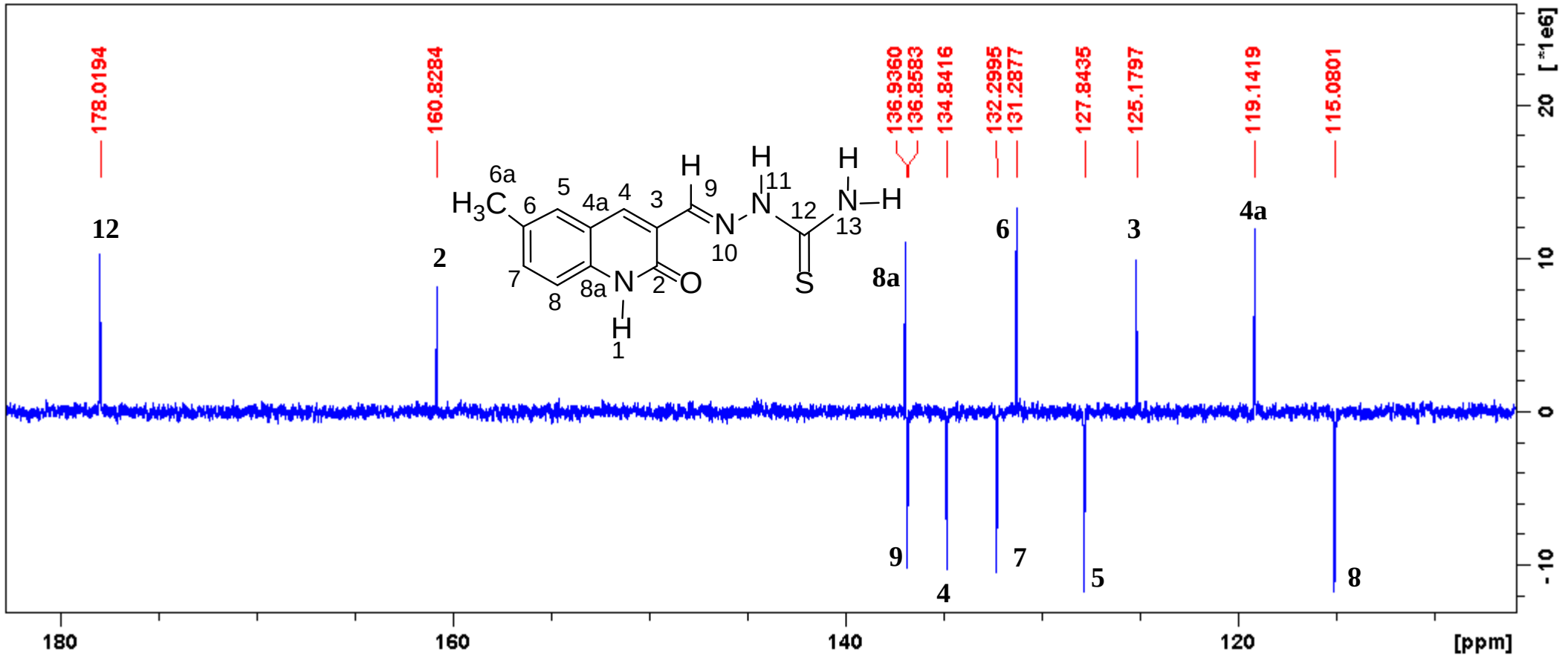
^{1}H NMR spectrum of compound 5b



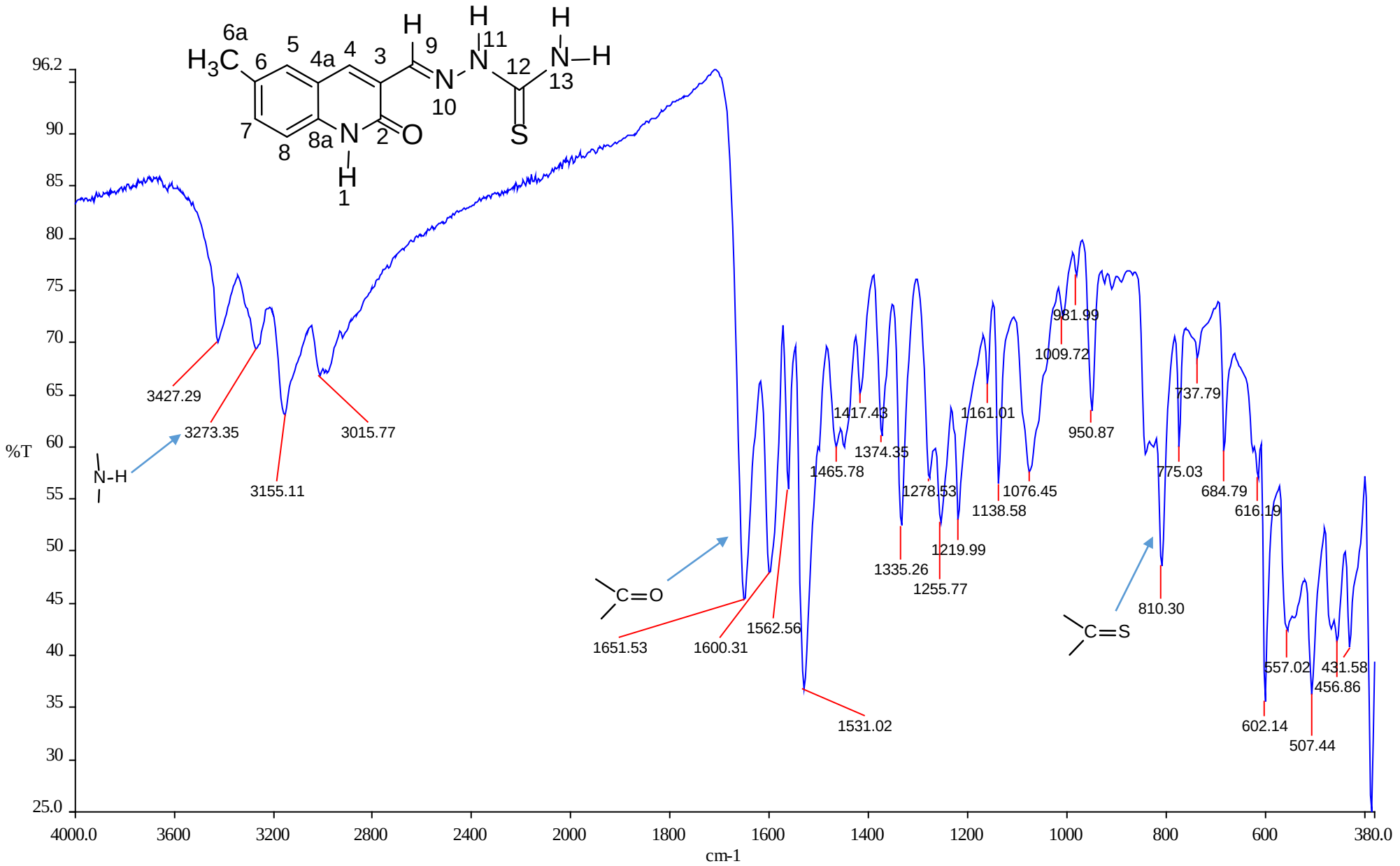
^{1}H NMR spectrum of compound 5b (expanded)



^{13}C NMR spectrum of compound 5b

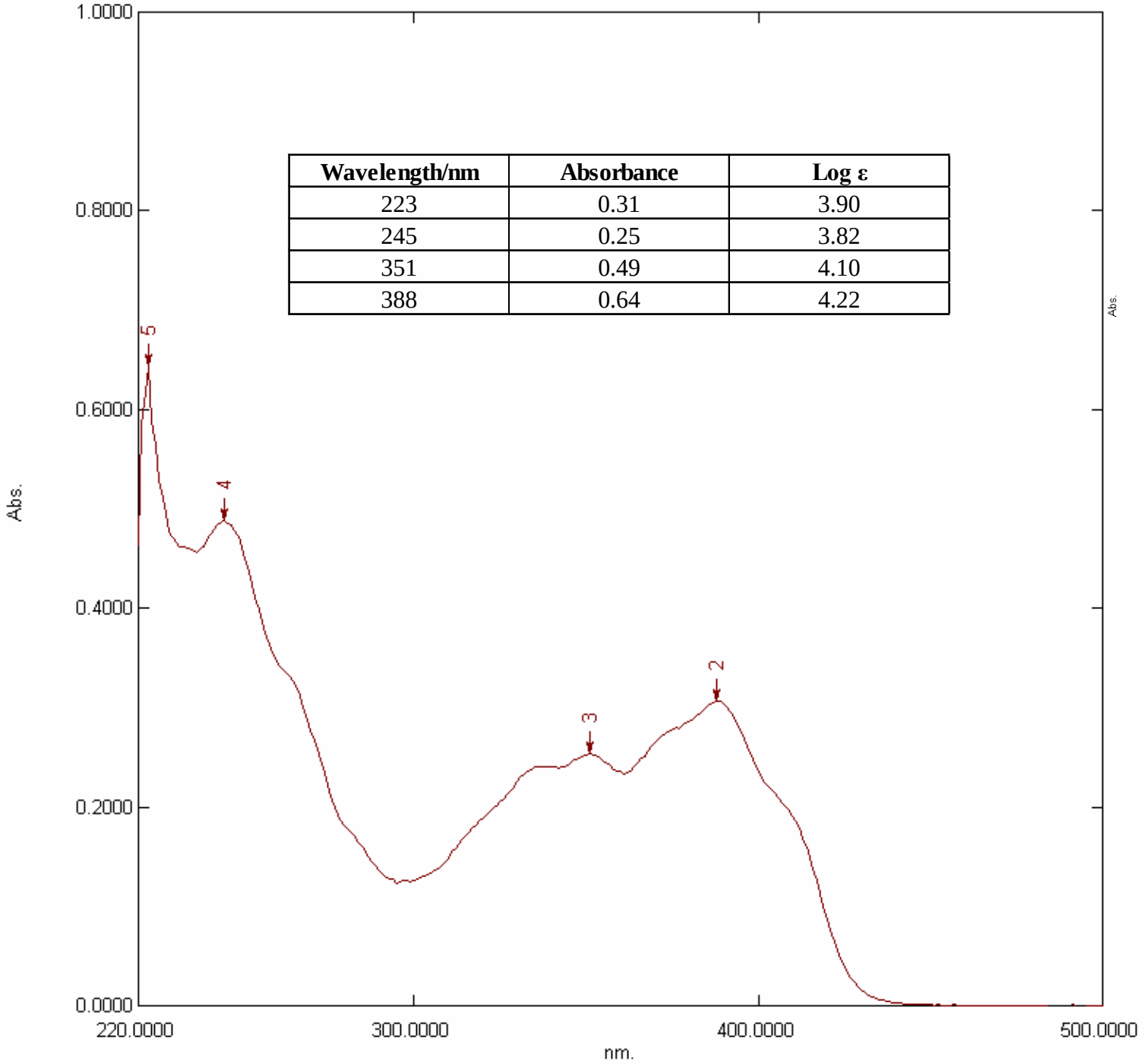


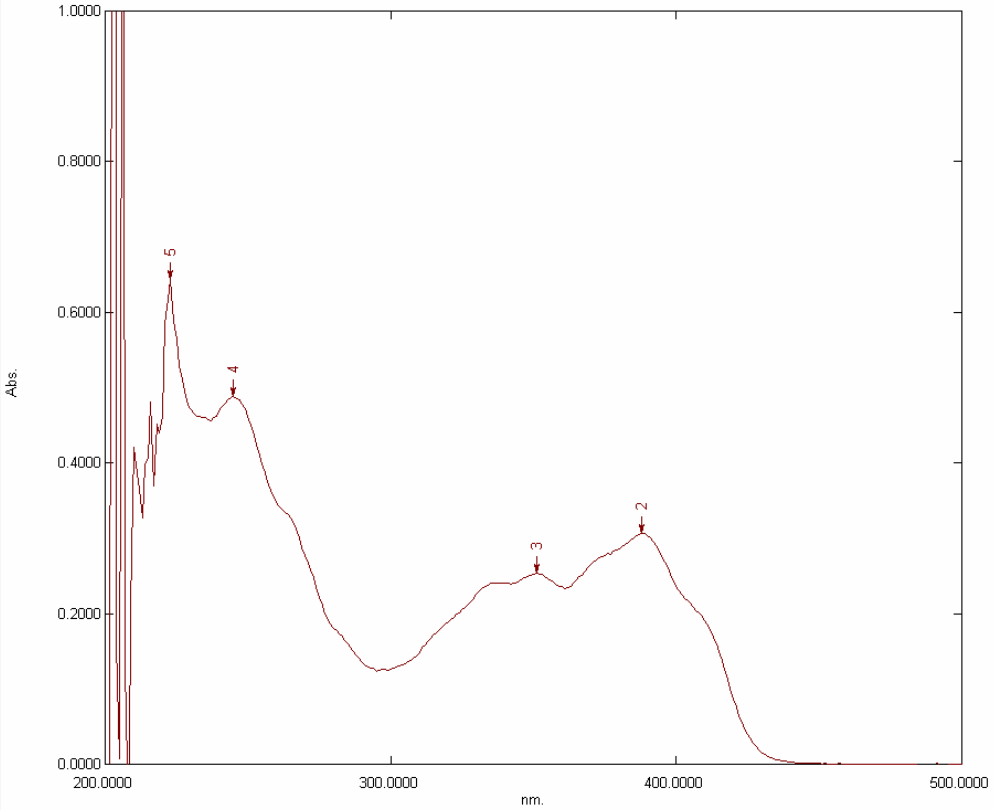
^{13}C NMR spectrum of compound 5b (expanded)

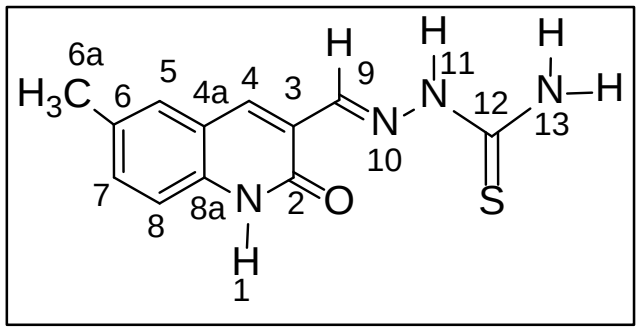


IR spectrum of compound 5b

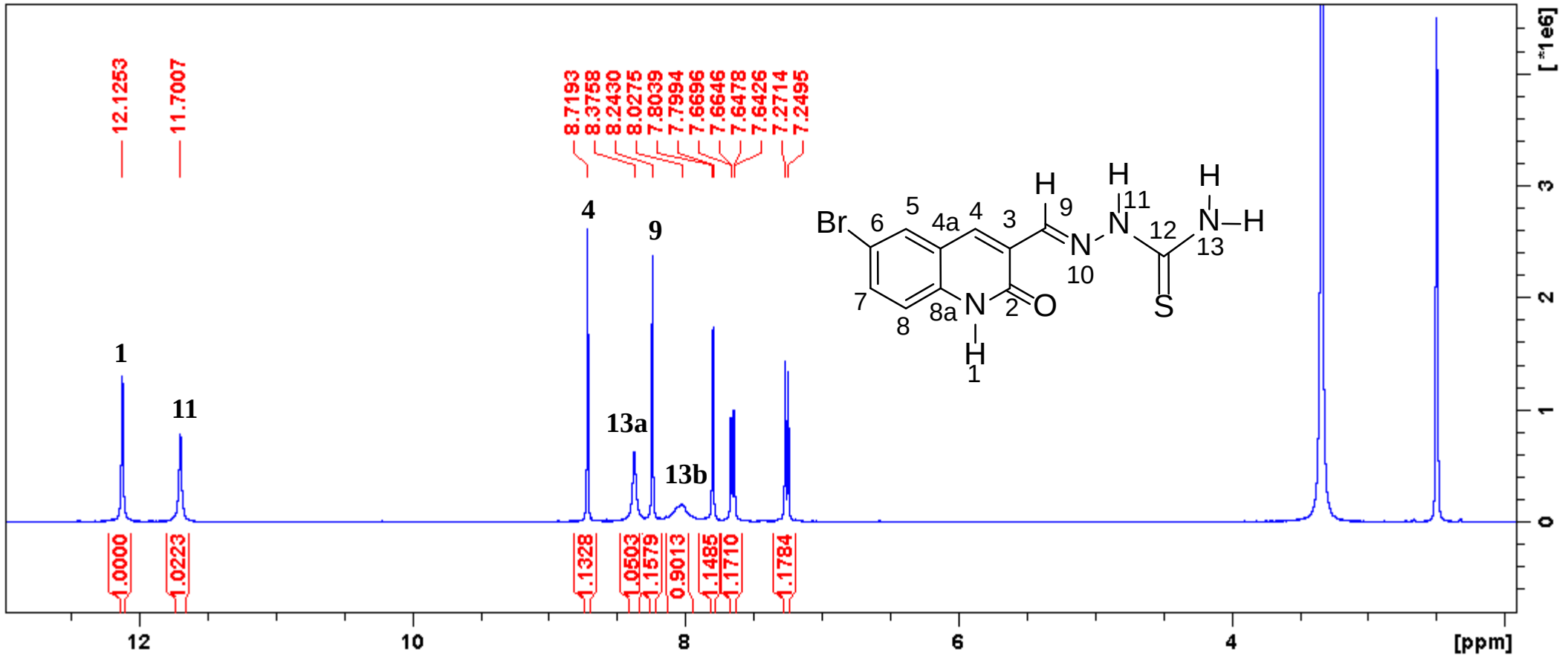
Wavelength/nm	Absorbance	Log ε
223	0.31	3.90
245	0.25	3.82
351	0.49	4.10
388	0.64	4.22



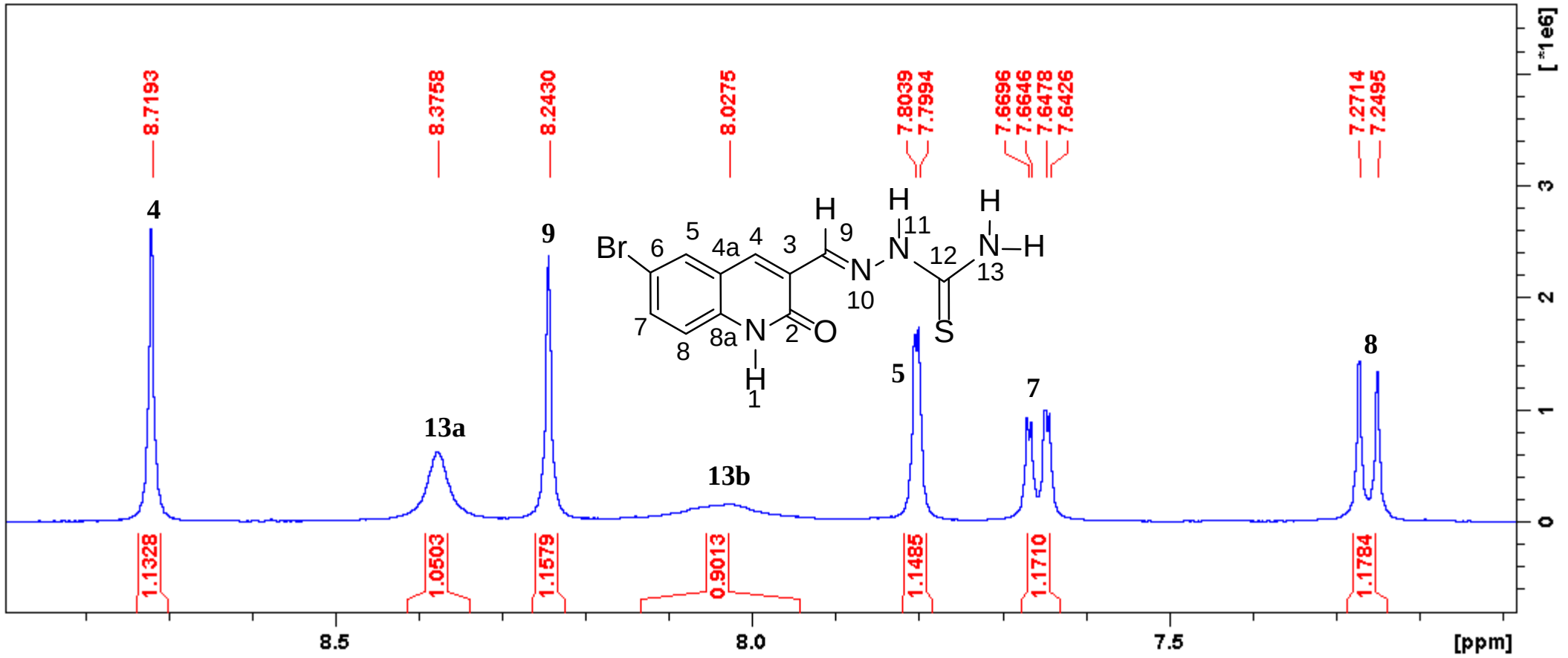




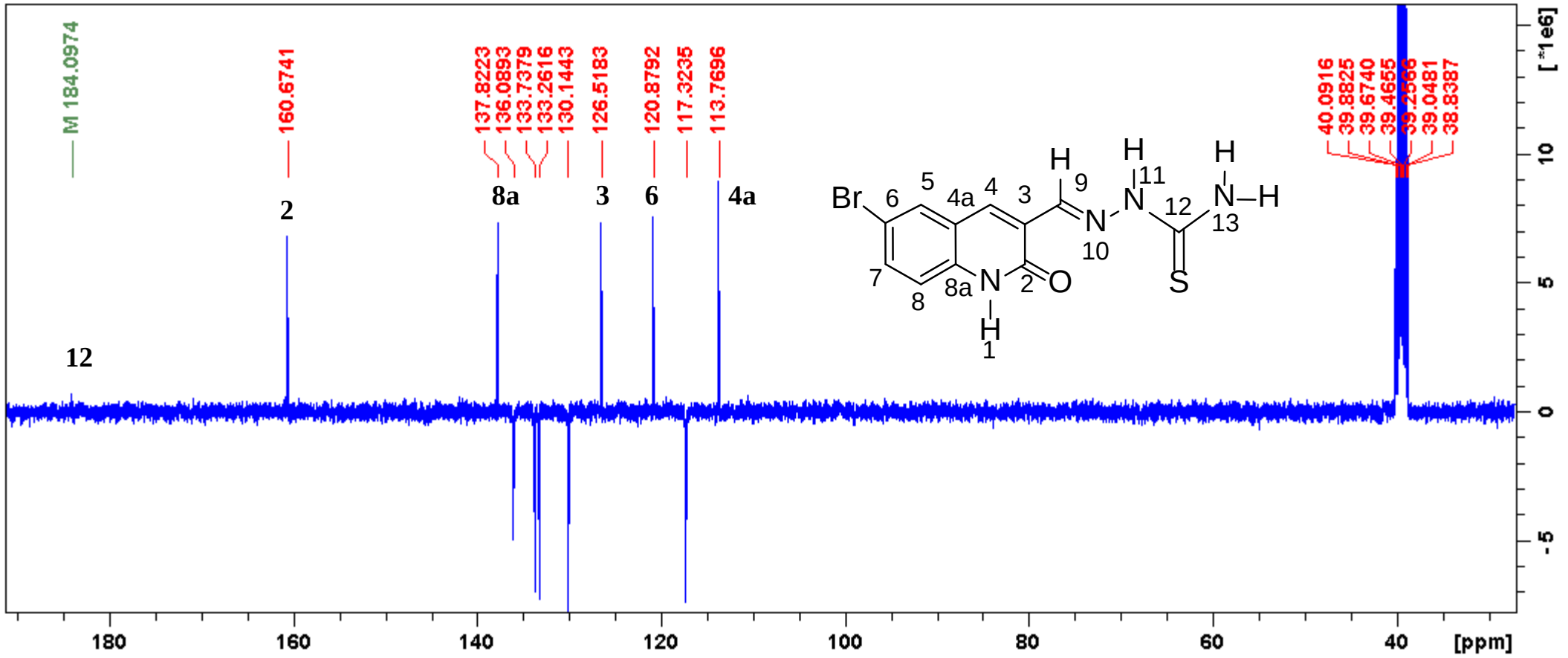
UV-Vis spectrum of compound 5b



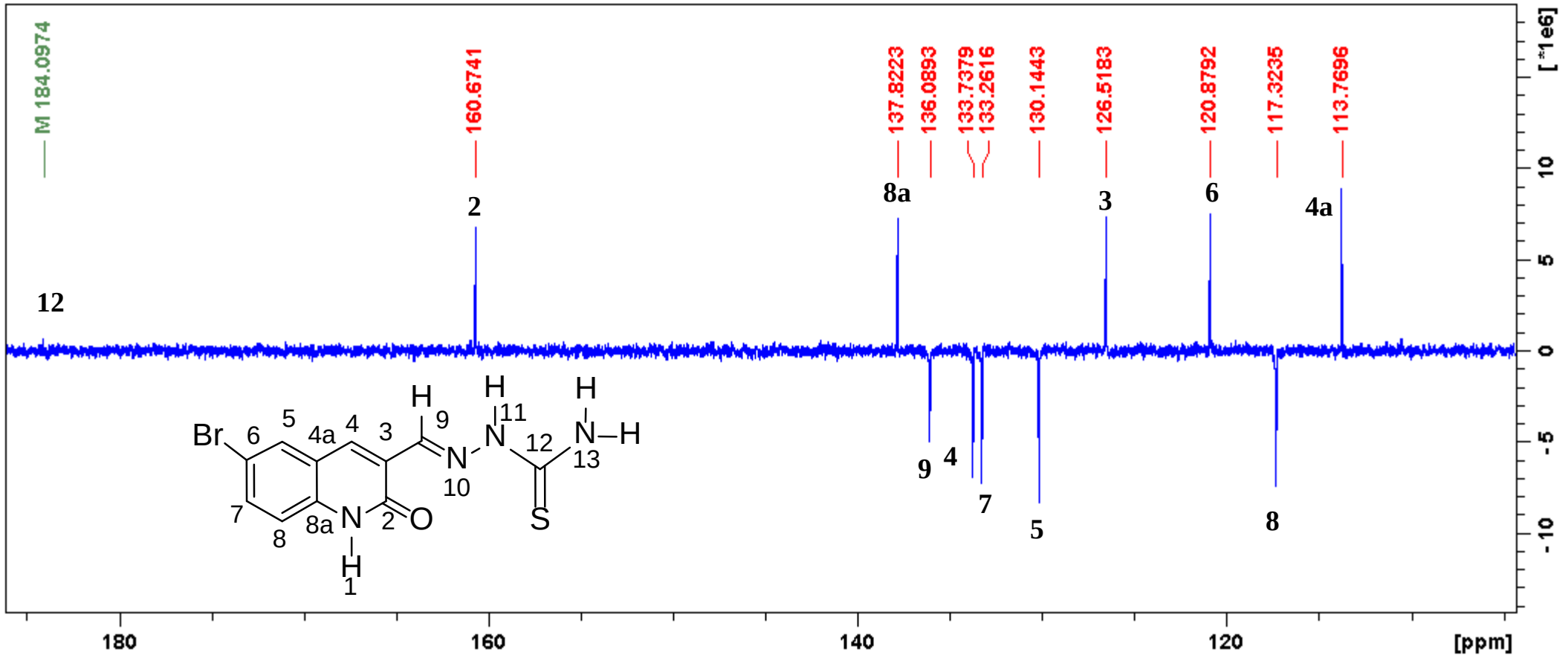
^{1}H NMR spectrum of compound 5c



^{1}H NMR spectrum of compound 5c (expanded)



^{13}C NMR spectrum of compound 5c



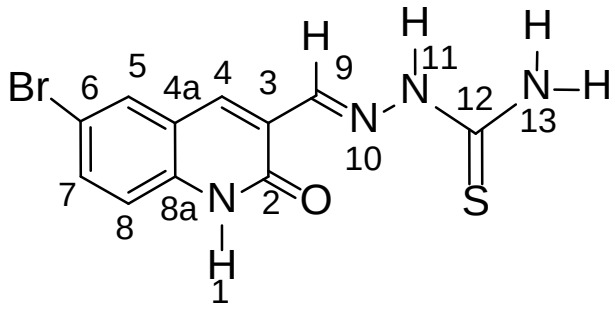
^{13}C NMR spectrum of compound 5c (expanded)

Elemental Composition Report

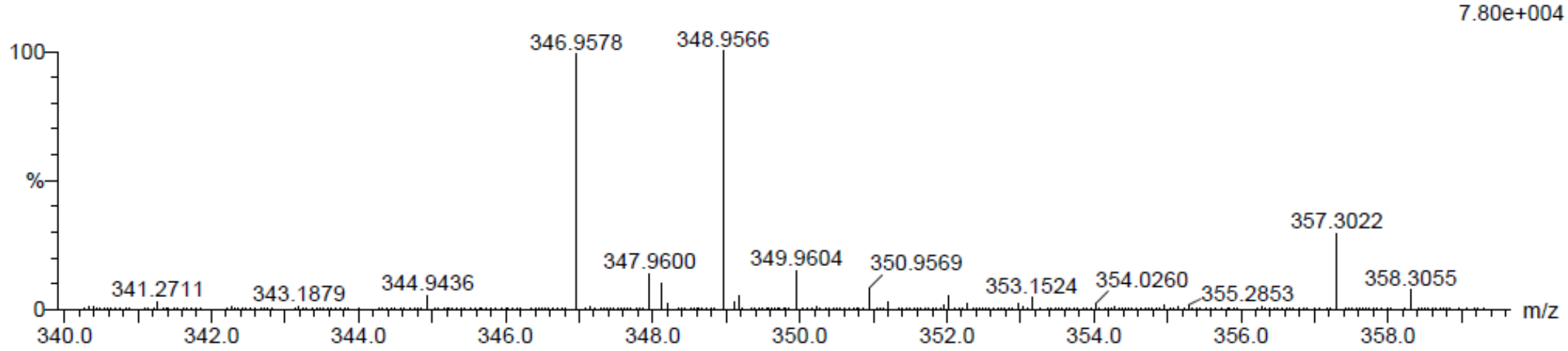
Single Mass Analysis

Tolerance = 4.0 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
177 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 5-10 N: 0-5 O: 0-5 Na: 0-1 S: 0-1 Br: 0-1

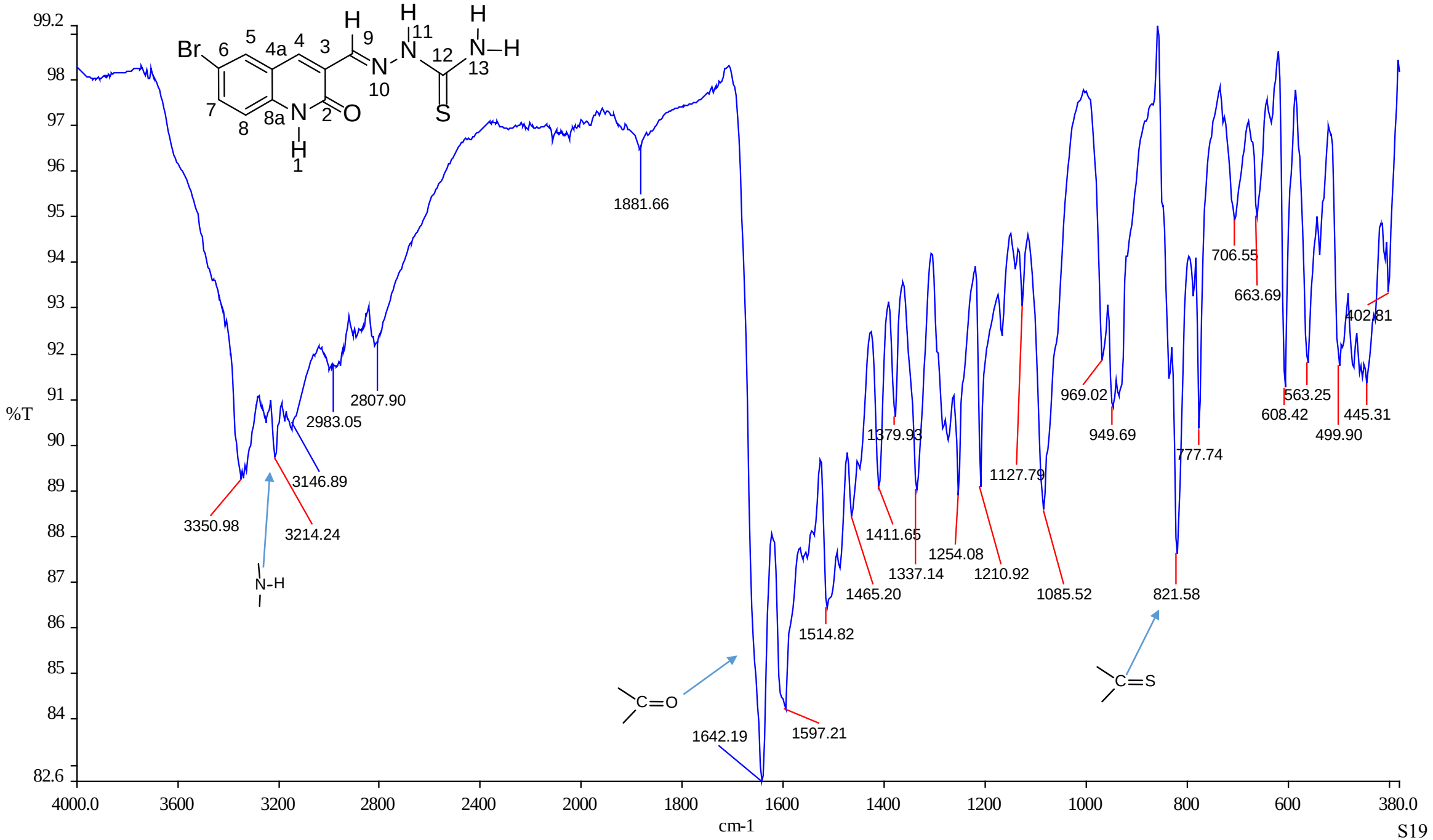


Compound 6 HG 18 (0.574) Cm (1:61)
TOF MS ES+



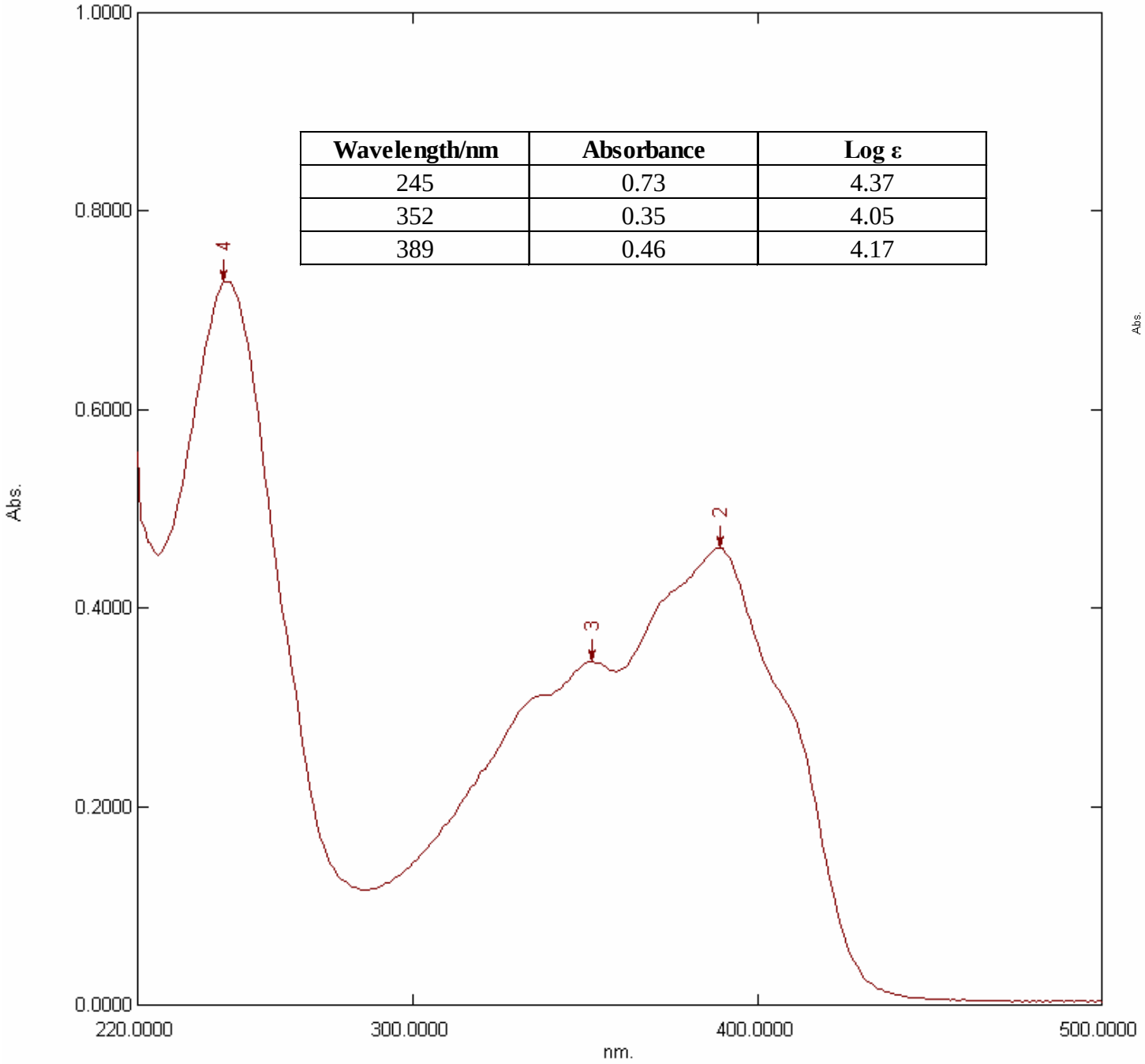
Minimum:				-1.5			
Maximum:		5.0	4.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
346.9578	346.9578	0.0	0.0	8.5	509.2	0.0	C11 H9 N4 O Na S Br

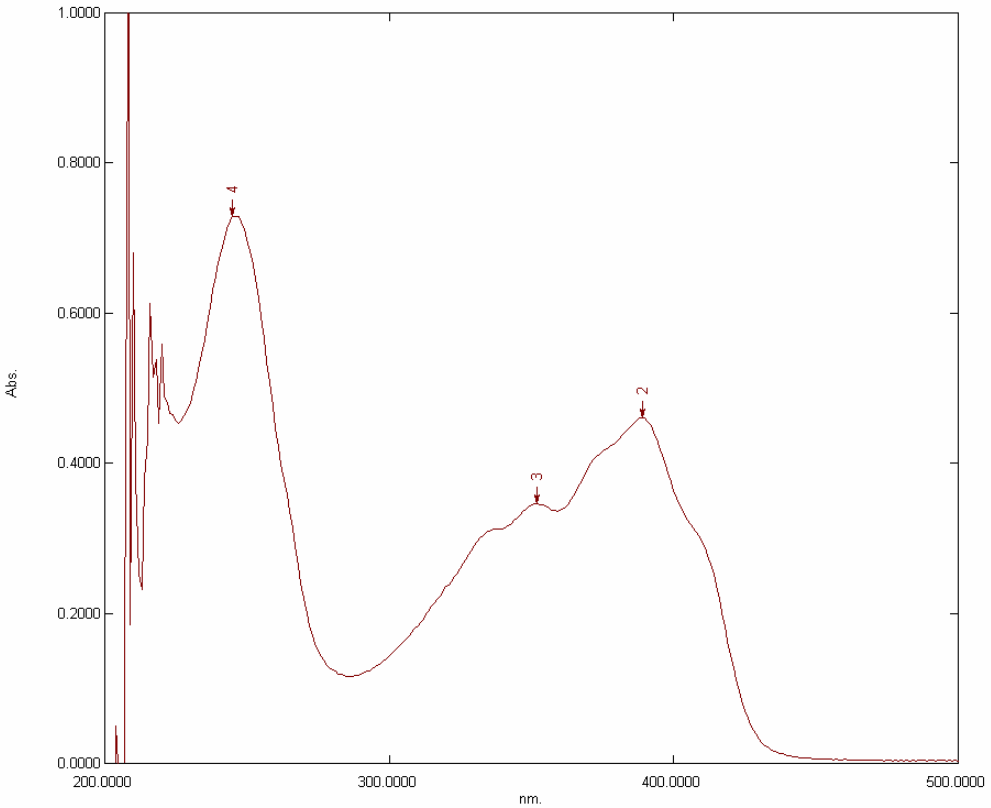
HRMS spectrum of compound 5c

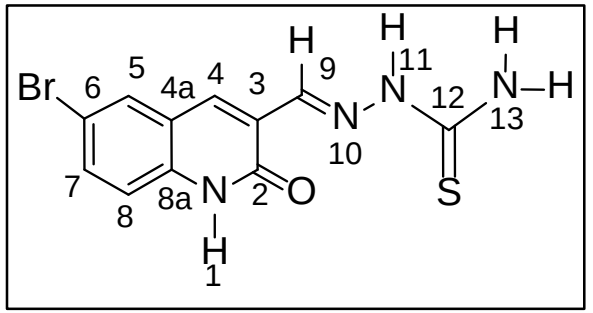


IR spectrum of compound 5c

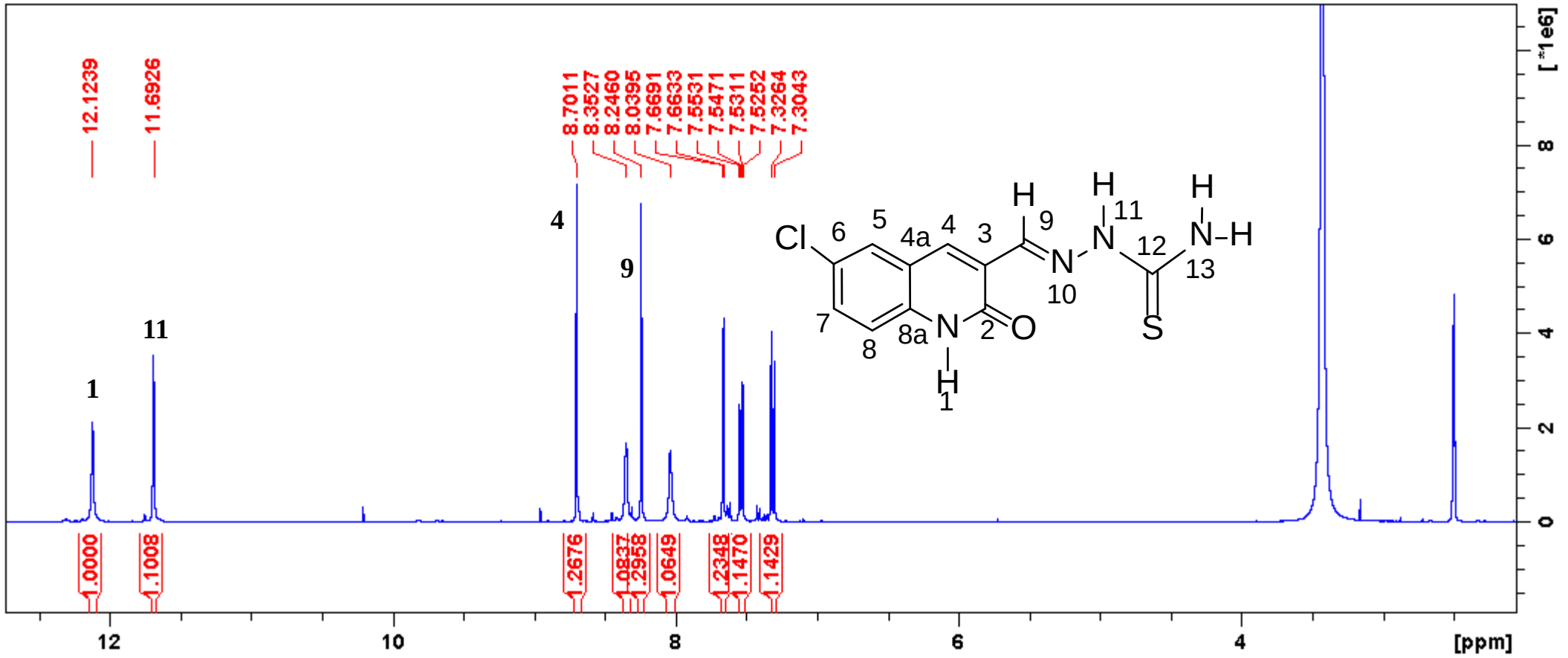
Wavelength/nm	Absorbance	Log ε
245	0.73	4.37
352	0.35	4.05
389	0.46	4.17



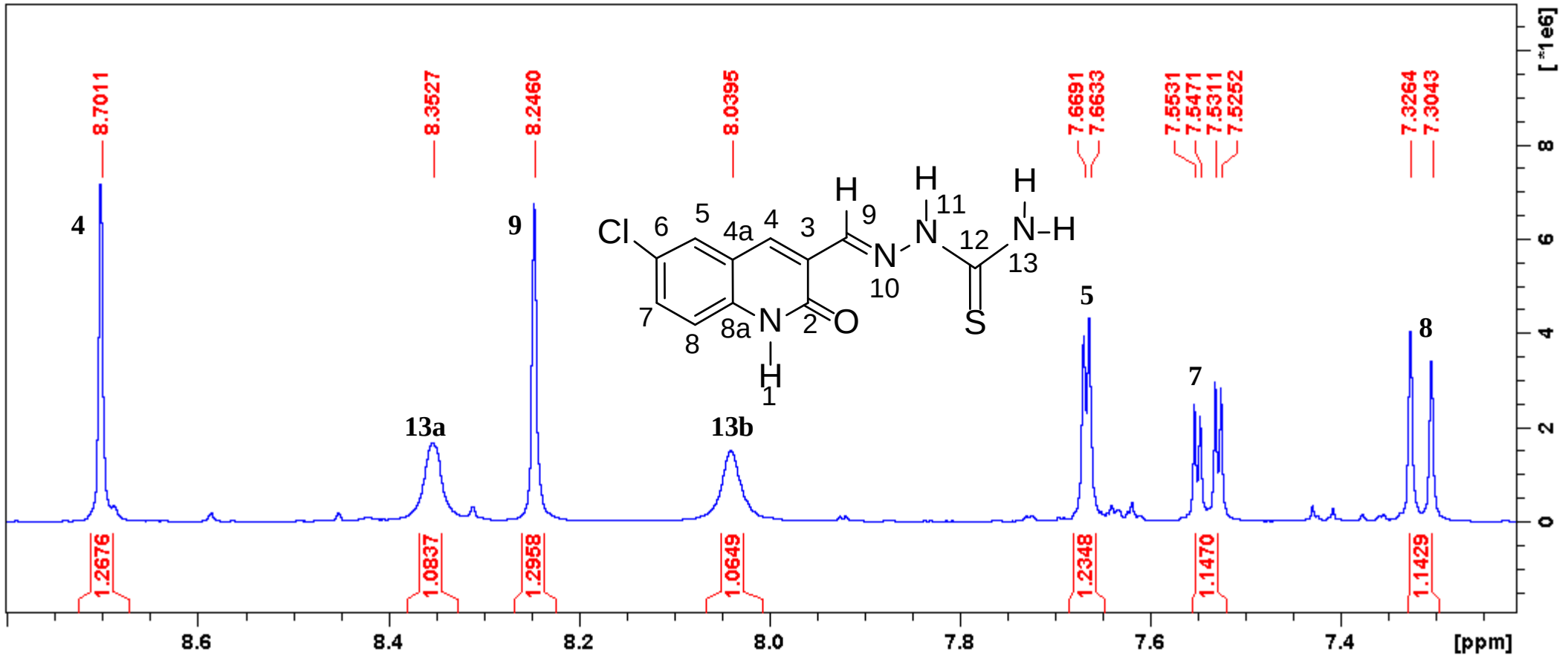




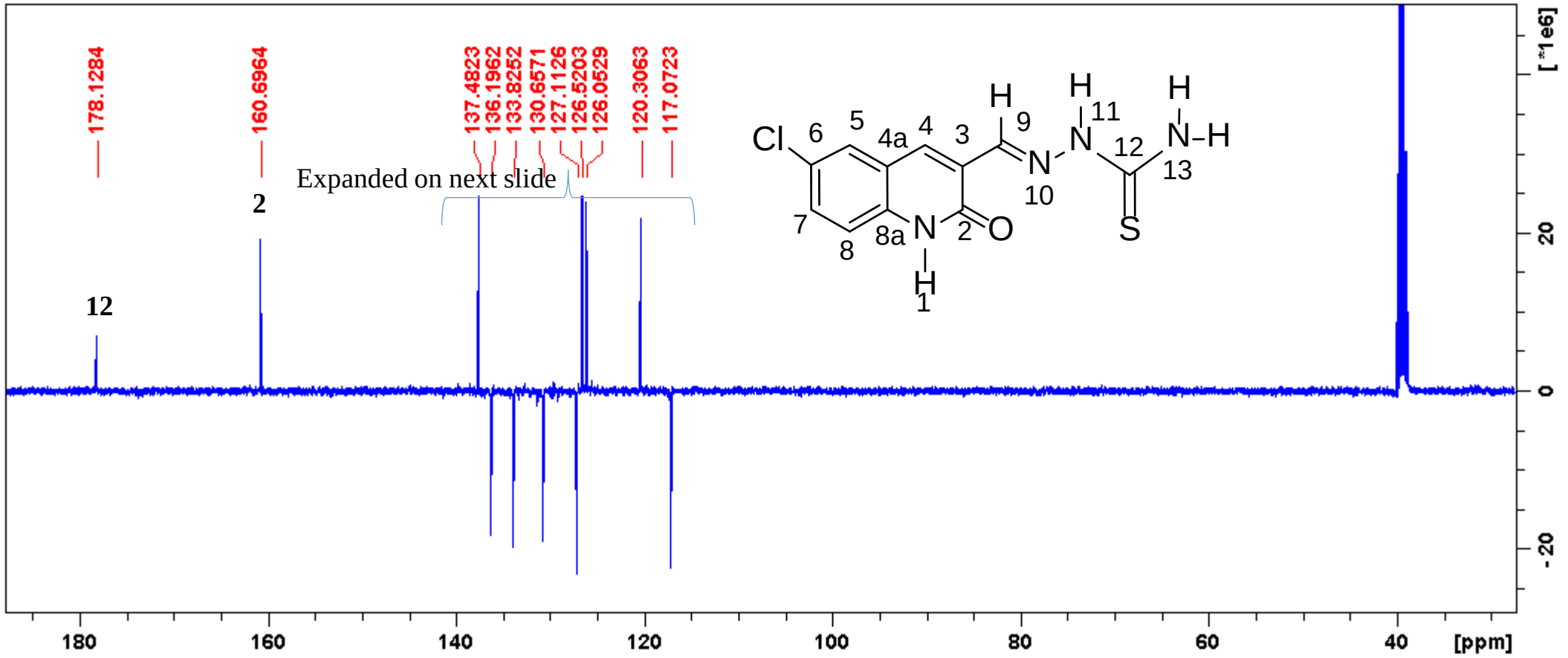
UV-Vis spectrum of compound 5c



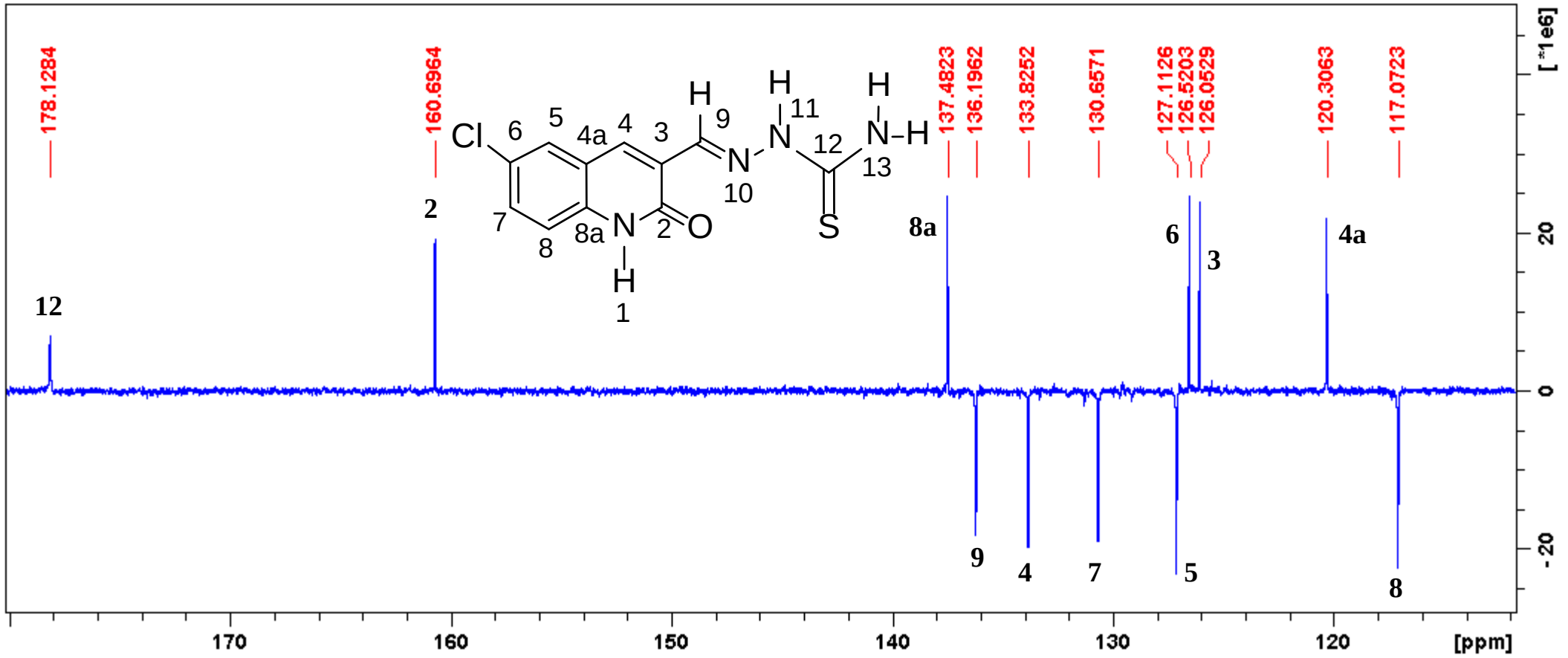
^{1}H NMR spectrum of compound 5d



^{1}H NMR spectrum of compound 5d (expanded)



^{13}C NMR spectrum of compound 5d

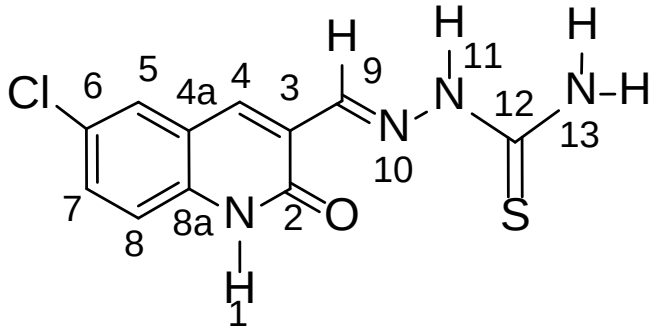


^{13}C NMR spectrum of compound 5d (expanded)

Elemental Composition Report

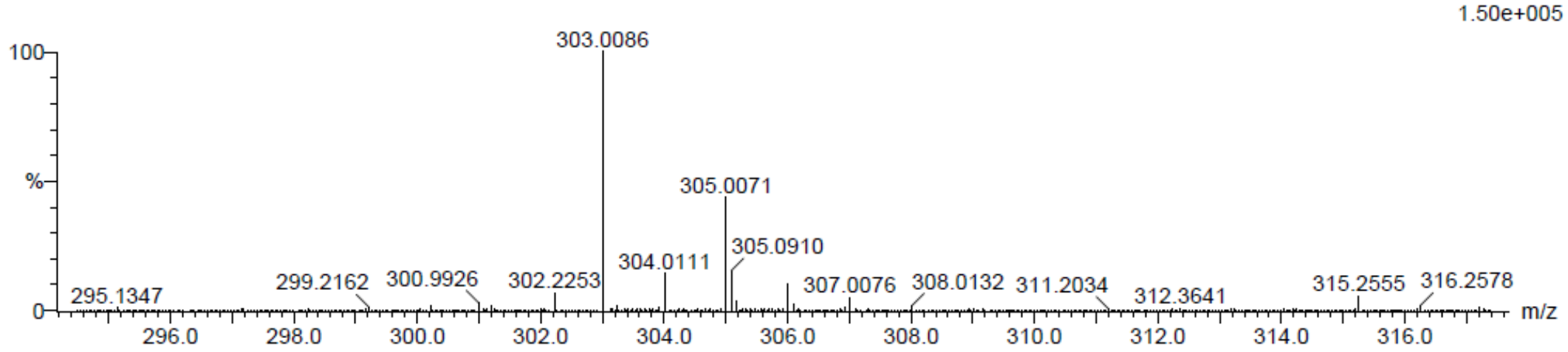
Single Mass Analysis

Tolerance = 4.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



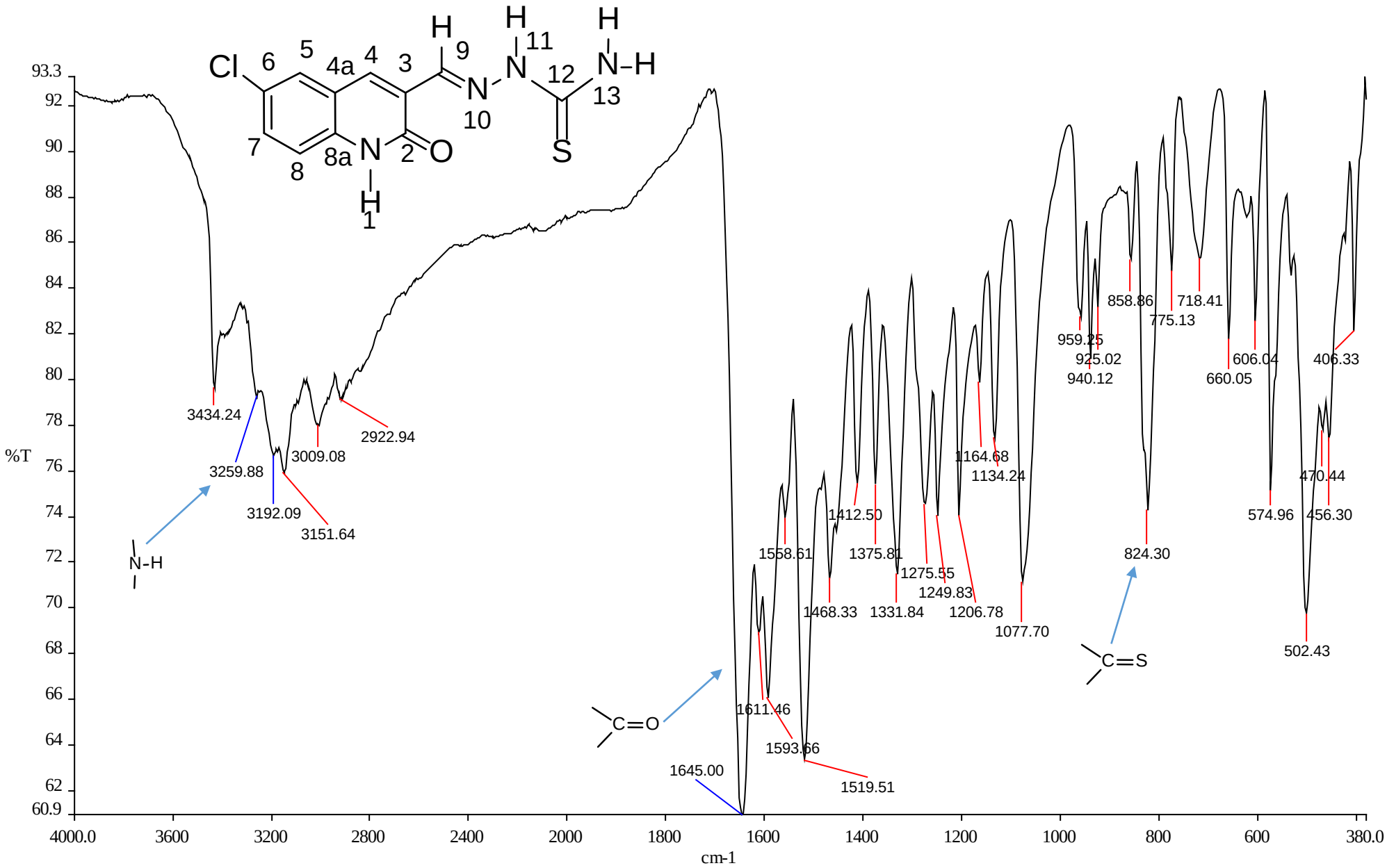
Monoisotopic Mass, Even Electron Ions
100 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 5-10 N: 0-5 O: 0-3 Na: 0-1 S: 0-1 Cl: 0-1

Compound 7 HG 49 (1.619) Cm (1:61)
TOF MS ES+



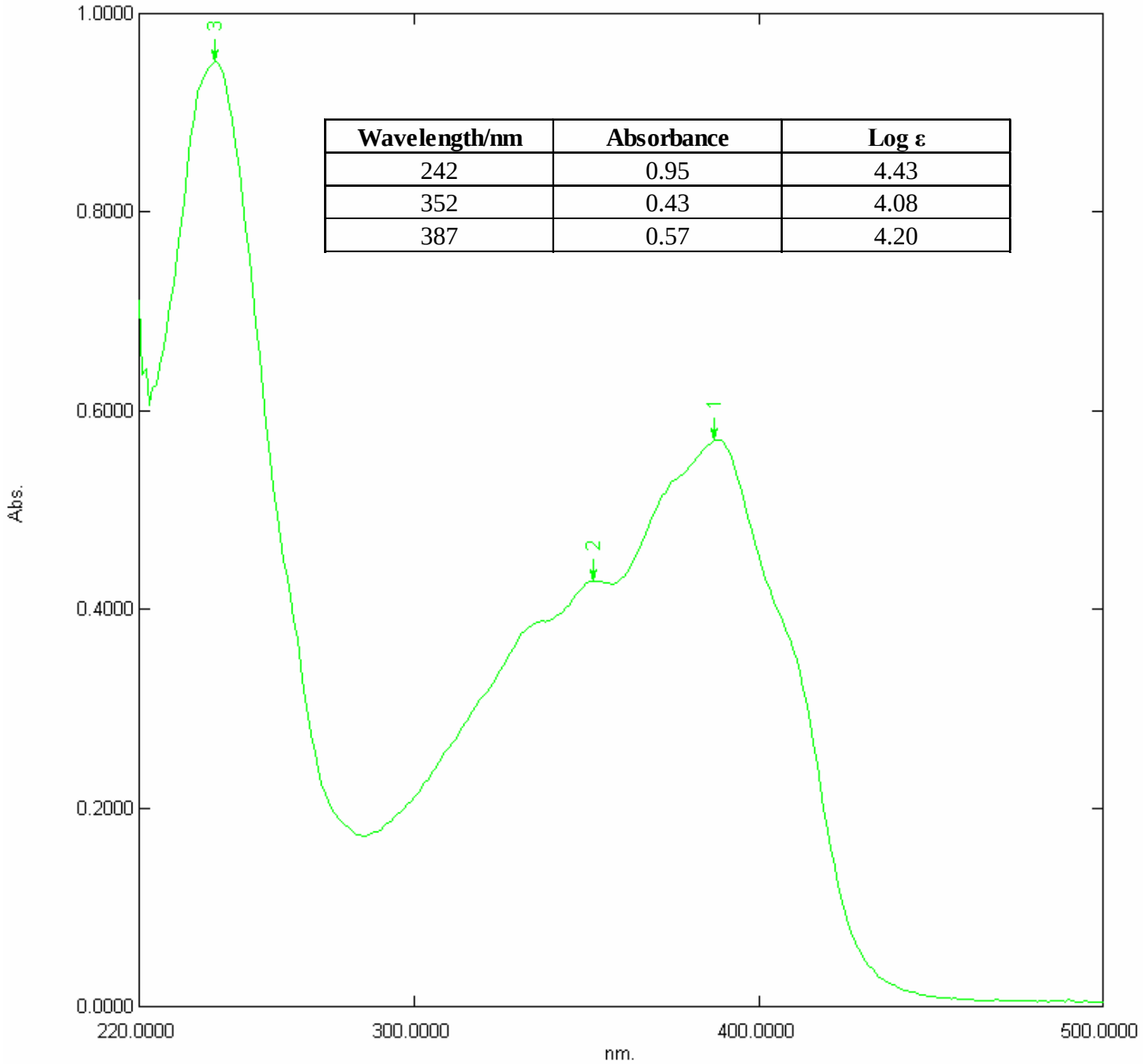
Minimum:				-1.5			
Maximum:		5.0	4.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
303.0086	303.0083	0.3	1.0	8.5	611.4	0.0	C11 H9 N4 O Na S Cl

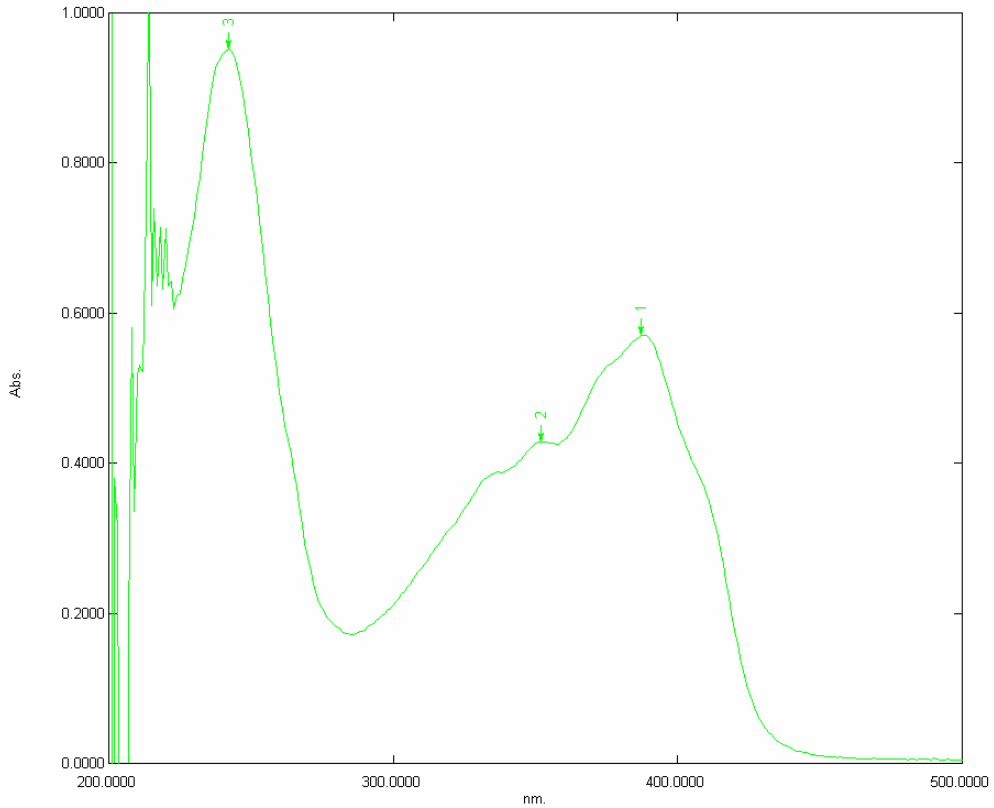
HRMS spectrum of compound 5d

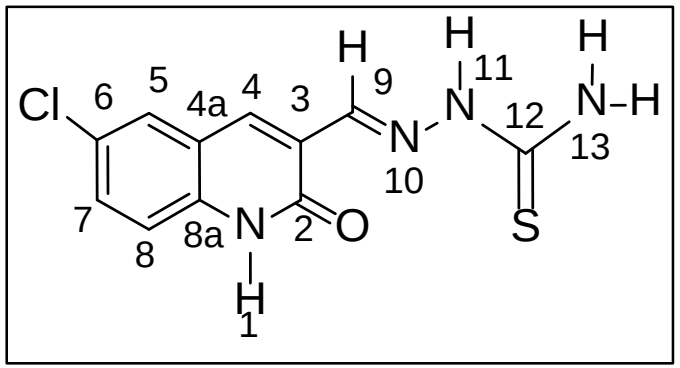


IR spectrum of compound 5d

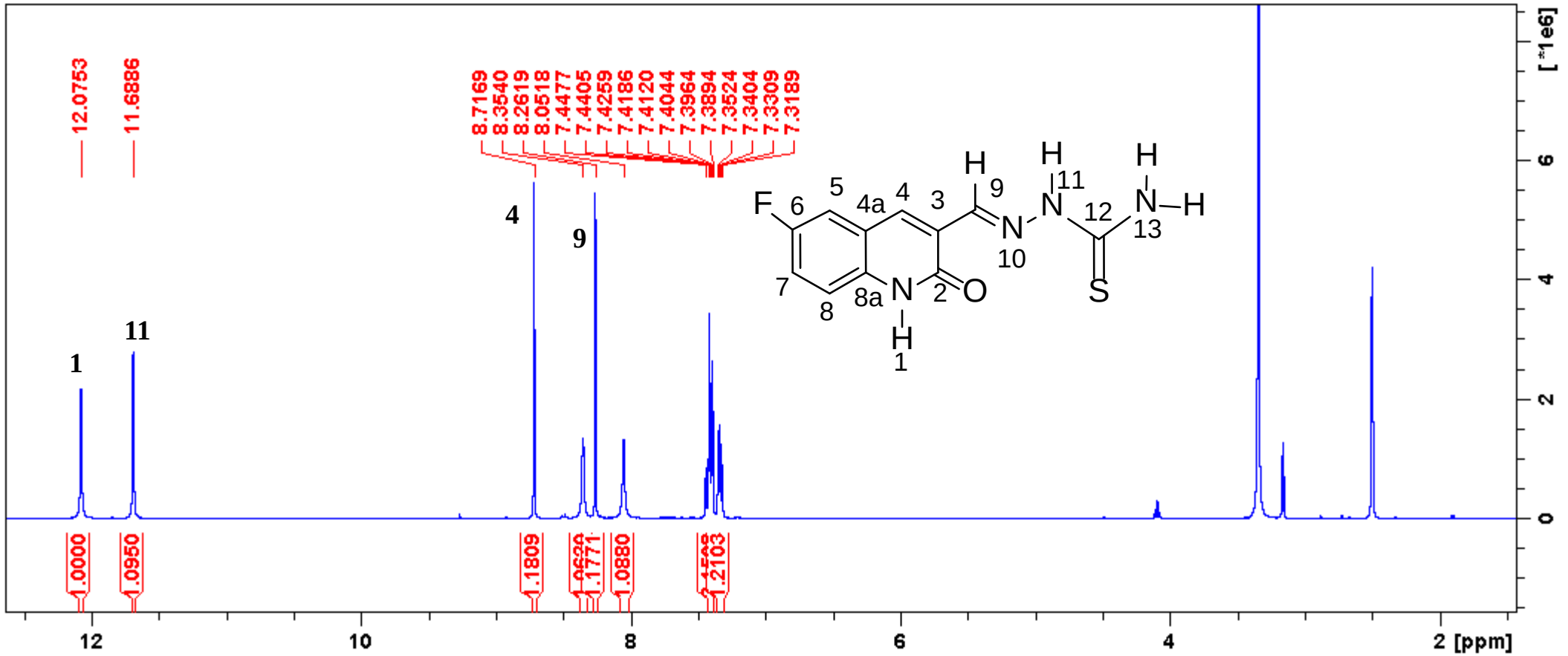
Wavelength/nm	Absorbance	Log ε
242	0.95	4.43
352	0.43	4.08
387	0.57	4.20



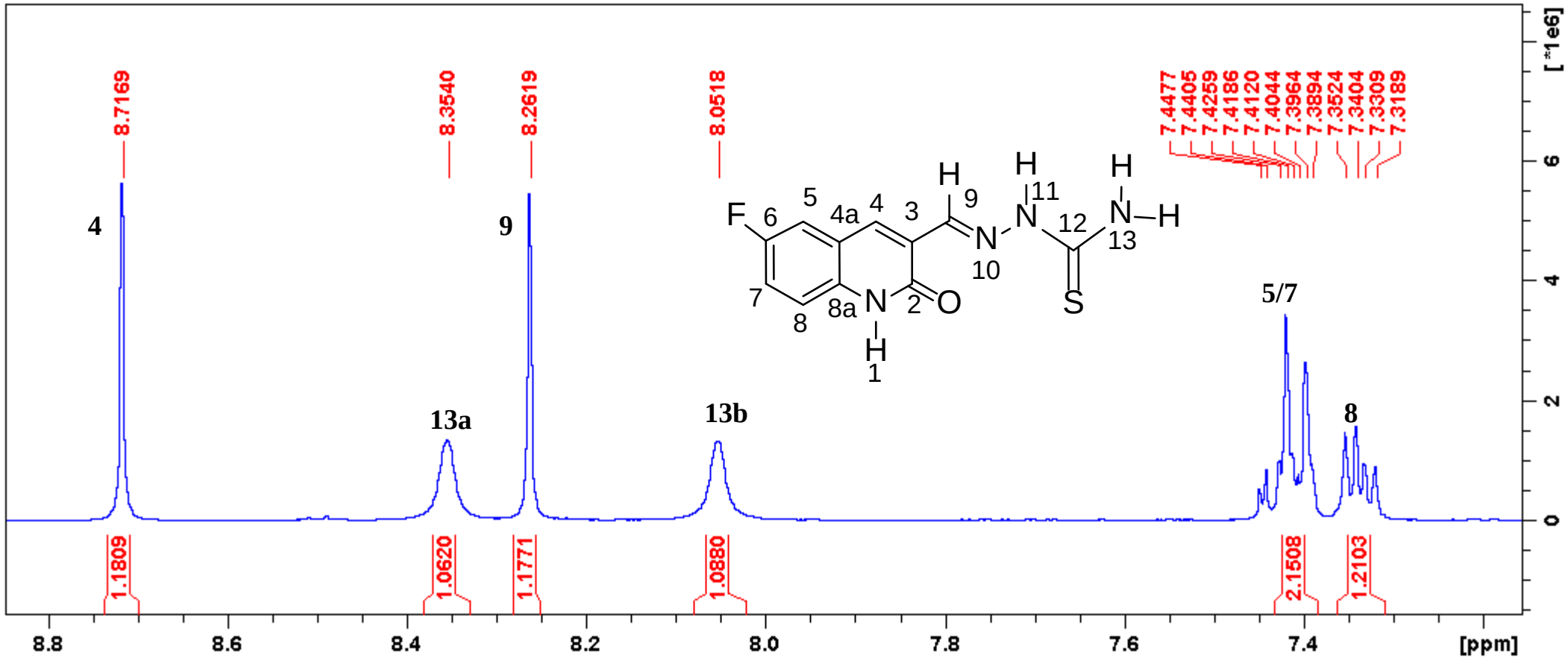




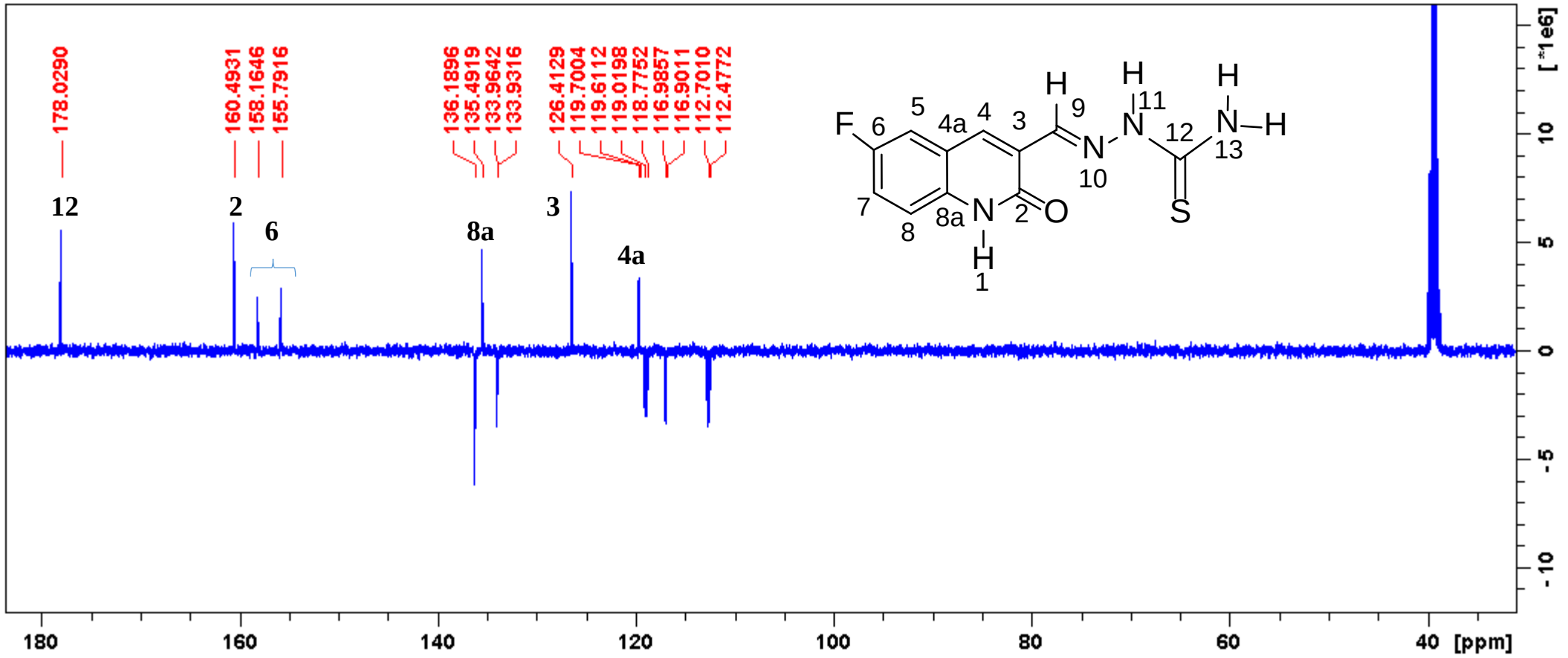
UV-Vis spectrum of compound 5d



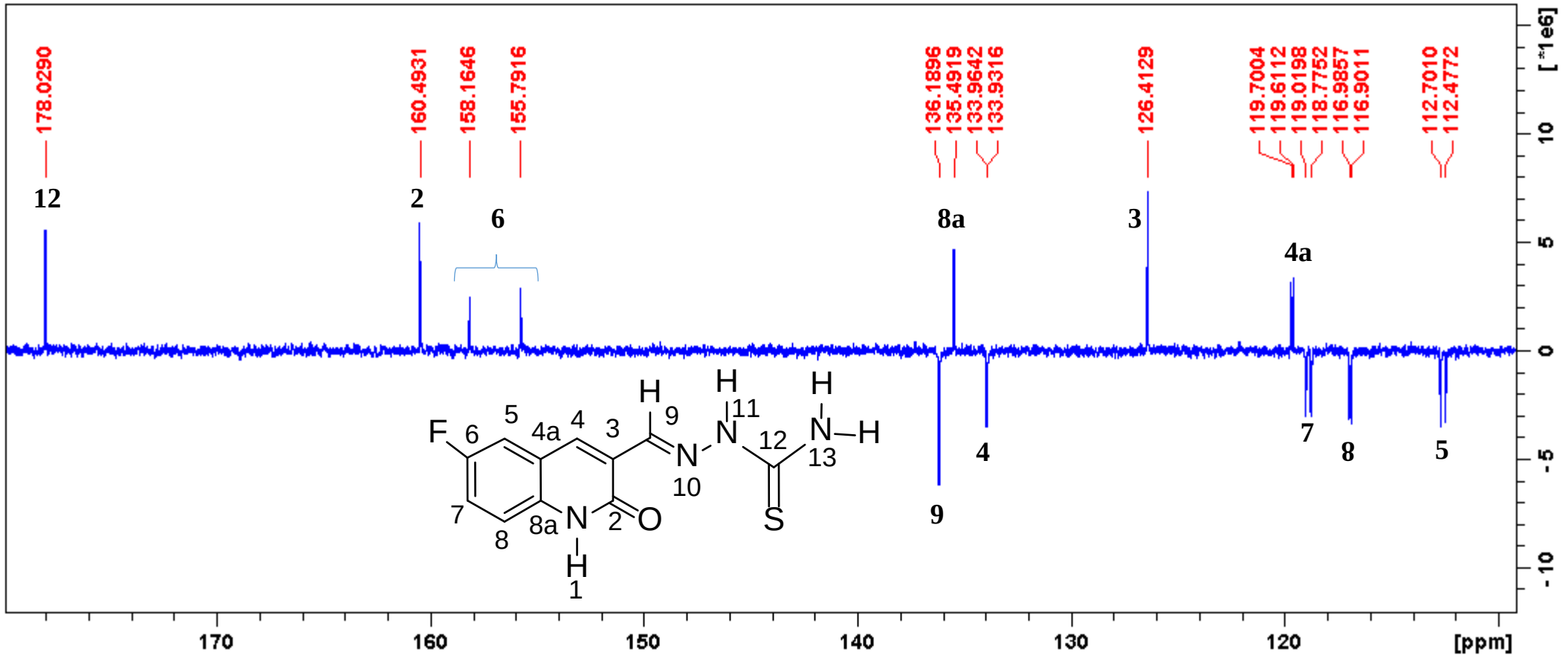
^{1}H NMR spectrum of compound 5e



^{1}H NMR spectrum of compound 5e (expanded)



^{13}C NMR spectrum of compound 5e



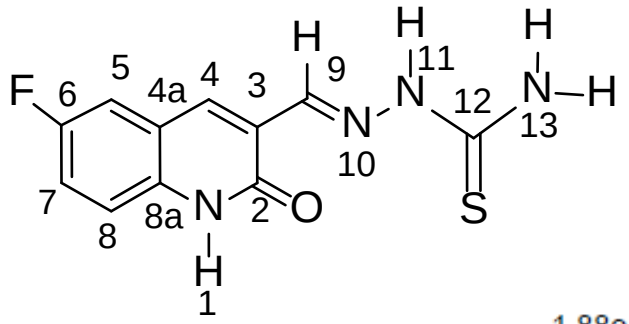
^{13}C NMR spectrum of compound 5e (expanded)

Elemental Composition Report

Single Mass Analysis

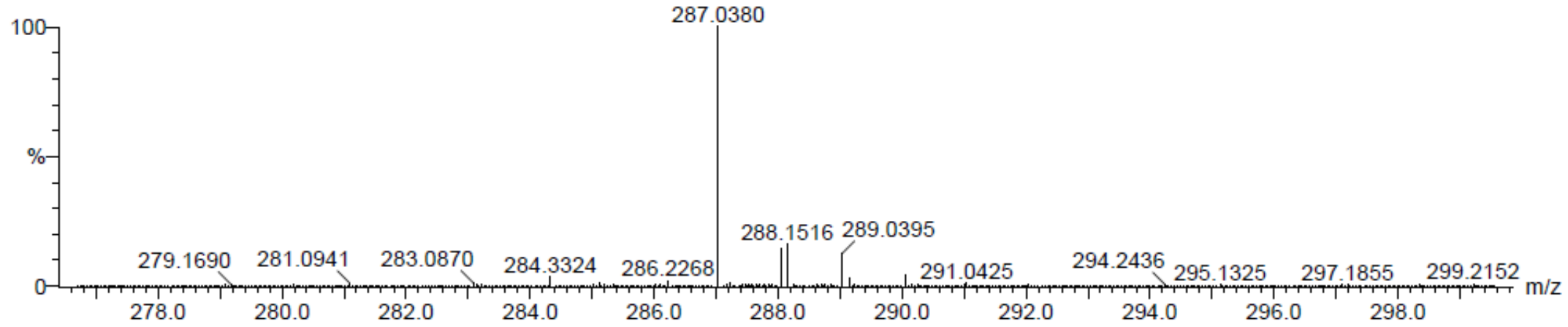
Tolerance = 4.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

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



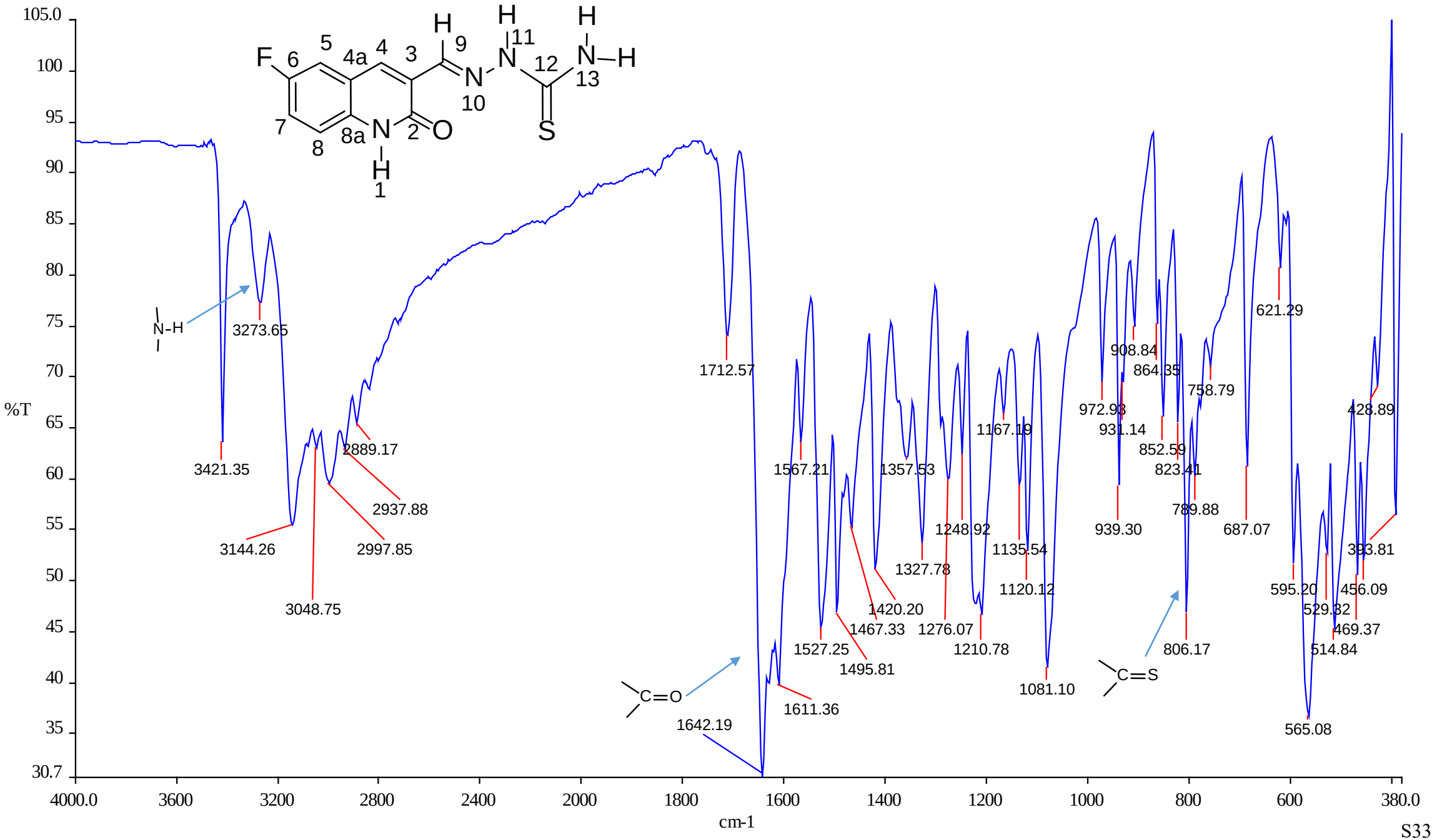
Compound 5 HG 21 (0.675) Cm (1:61)
TOF MS ES+

1.88e+005



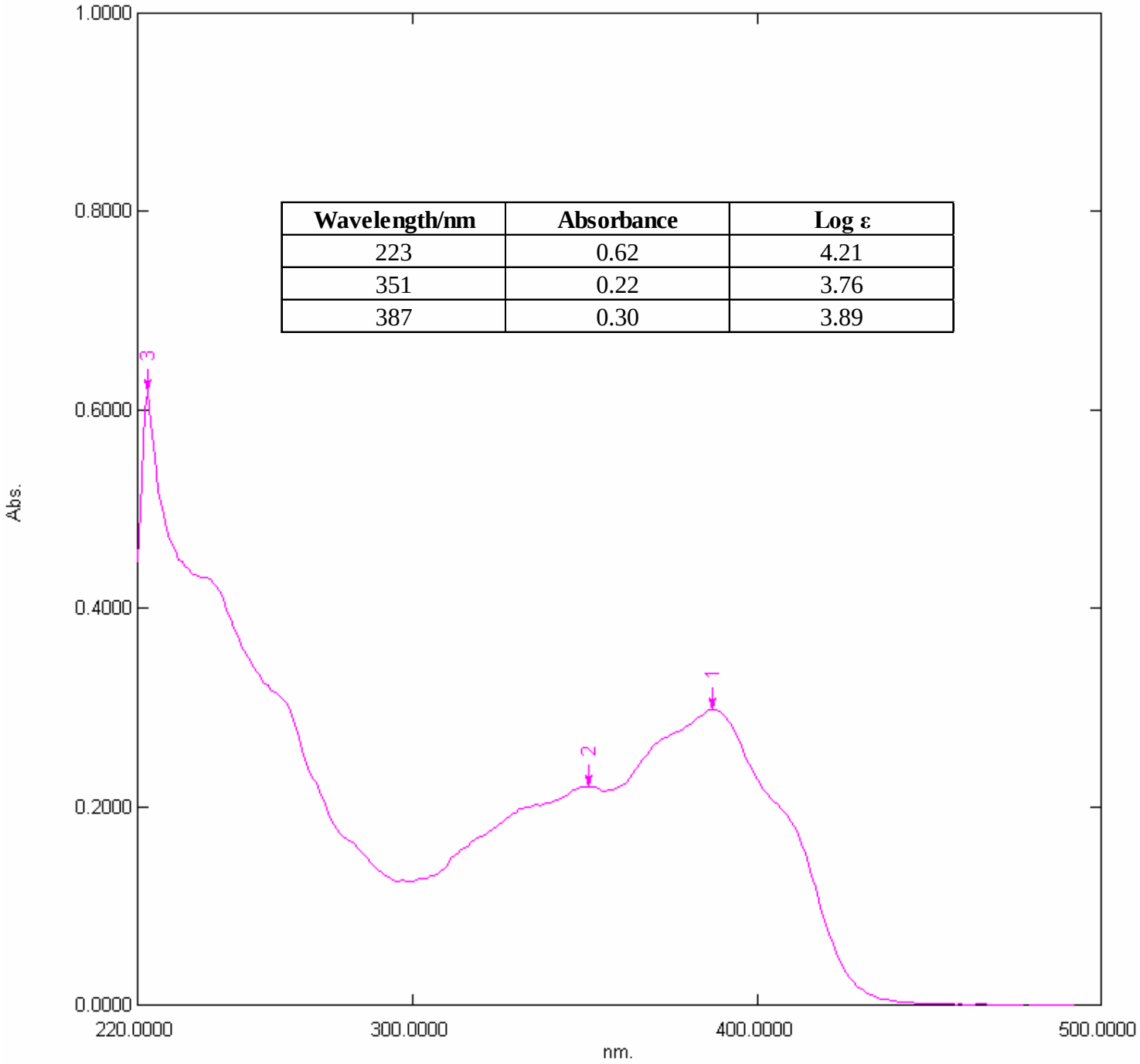
Minimum:				-1.5			
Maximum:		5.0	4.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
287.0380	287.0379	0.1	0.3	8.5	633.2	0.0	C11 H9 N4 O F Na S

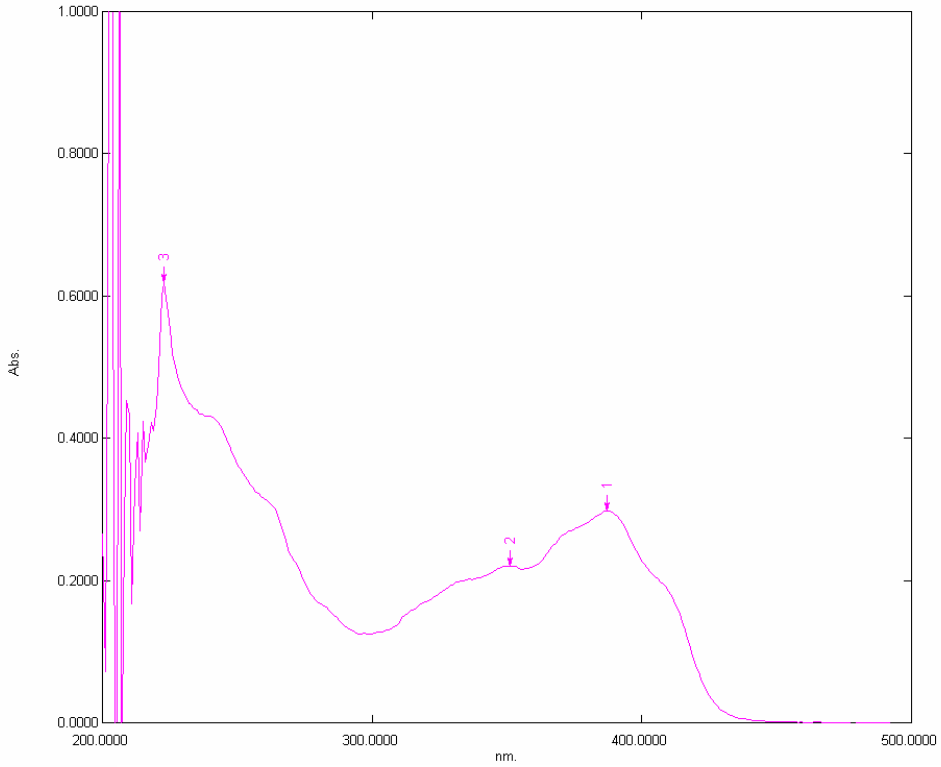
HRMS spectrum of compound 5e

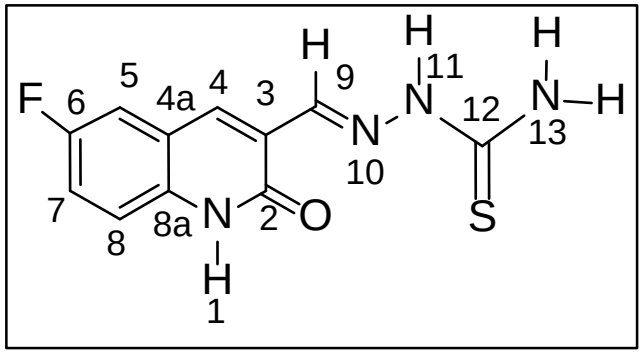


IR spectrum of compound 5e

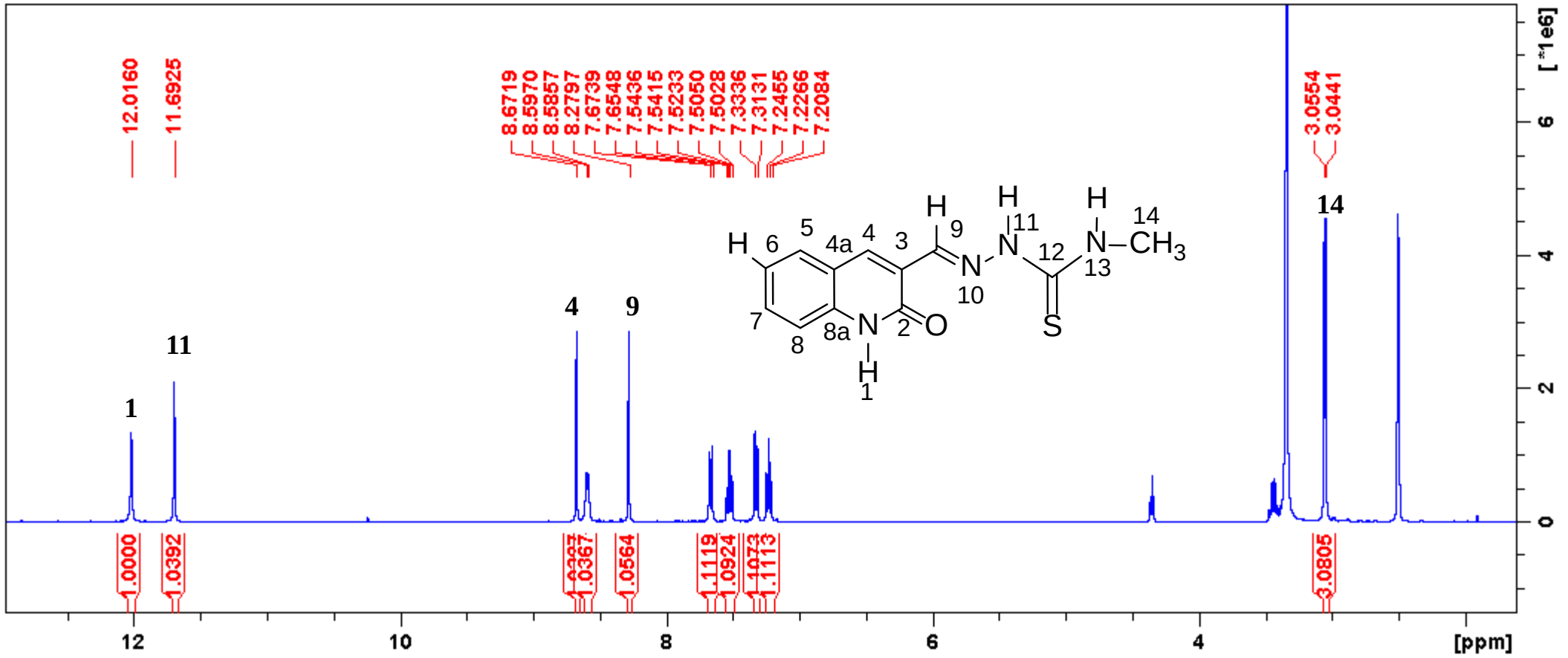
Wavelength/nm	Absorbance	Log ε
223	0.62	4.21
351	0.22	3.76
387	0.30	3.89



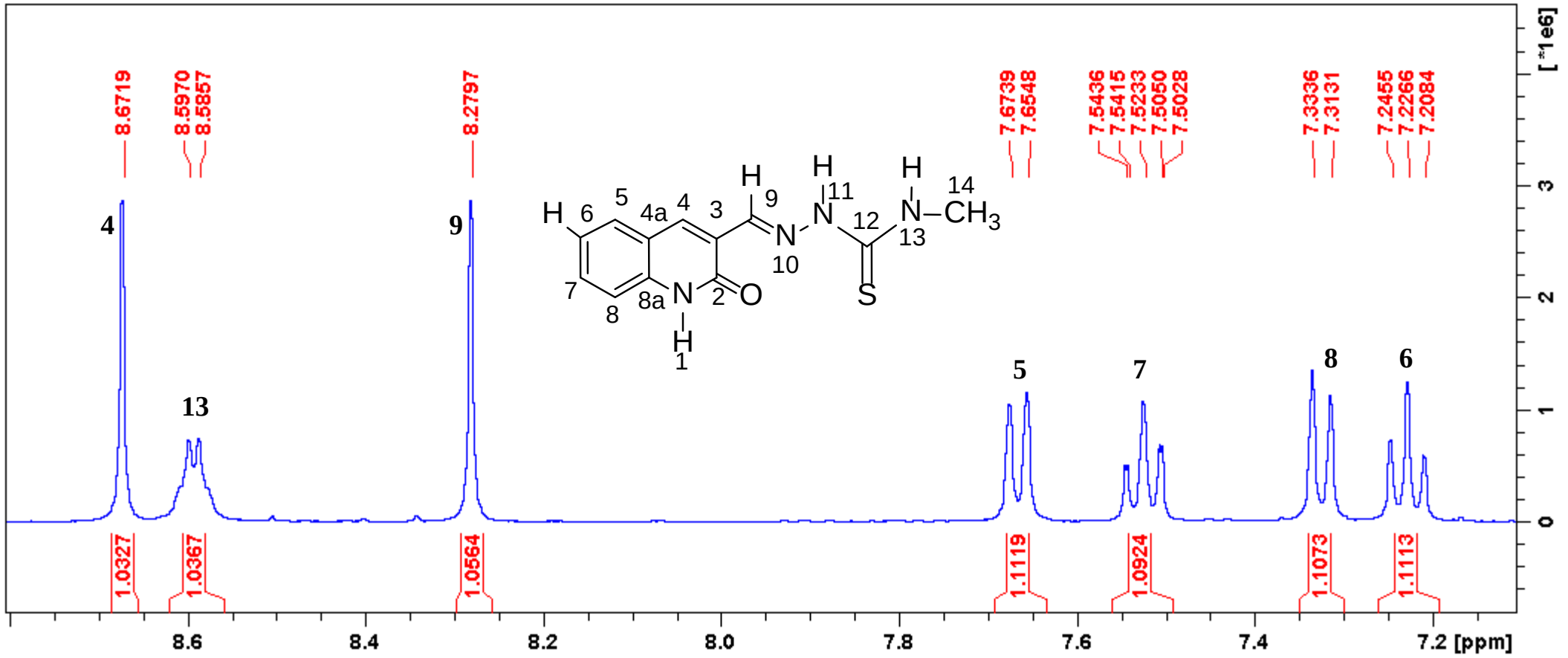




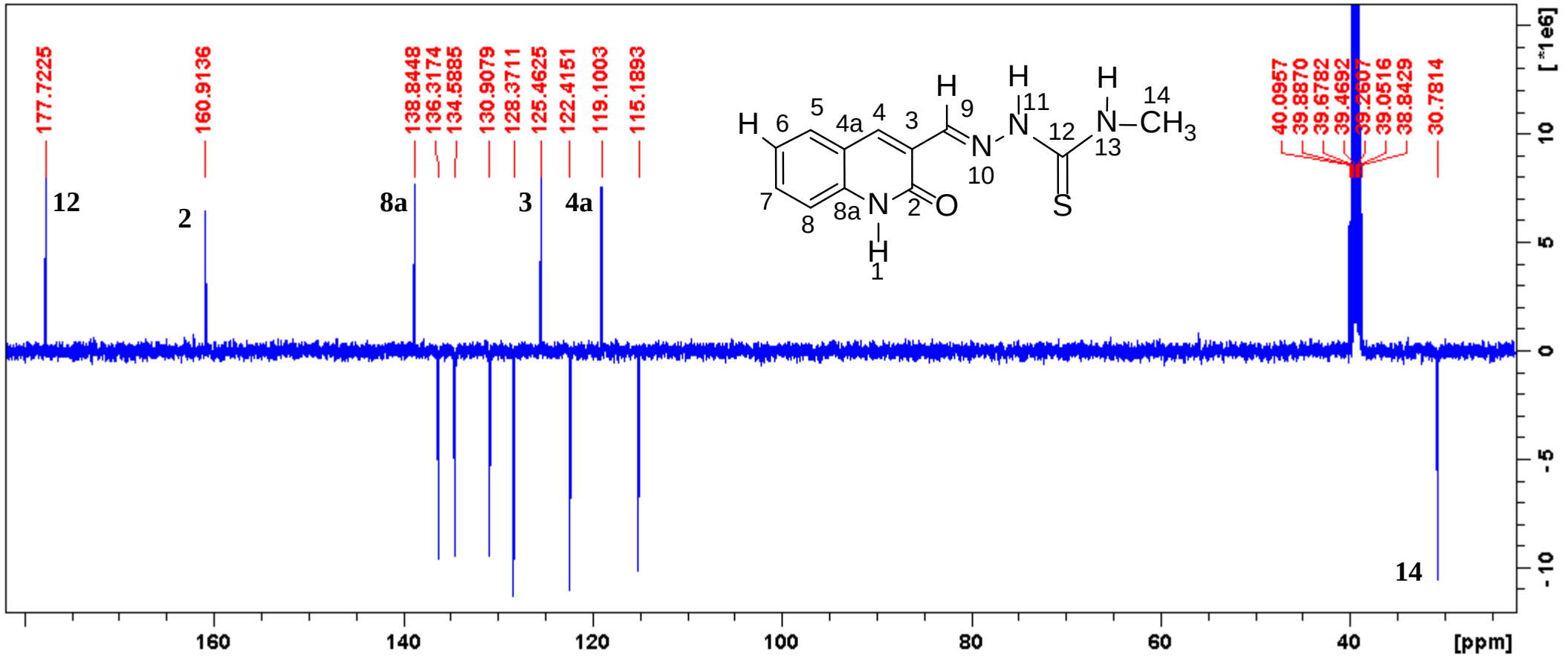
UV-Vis spectrum of compound 5e



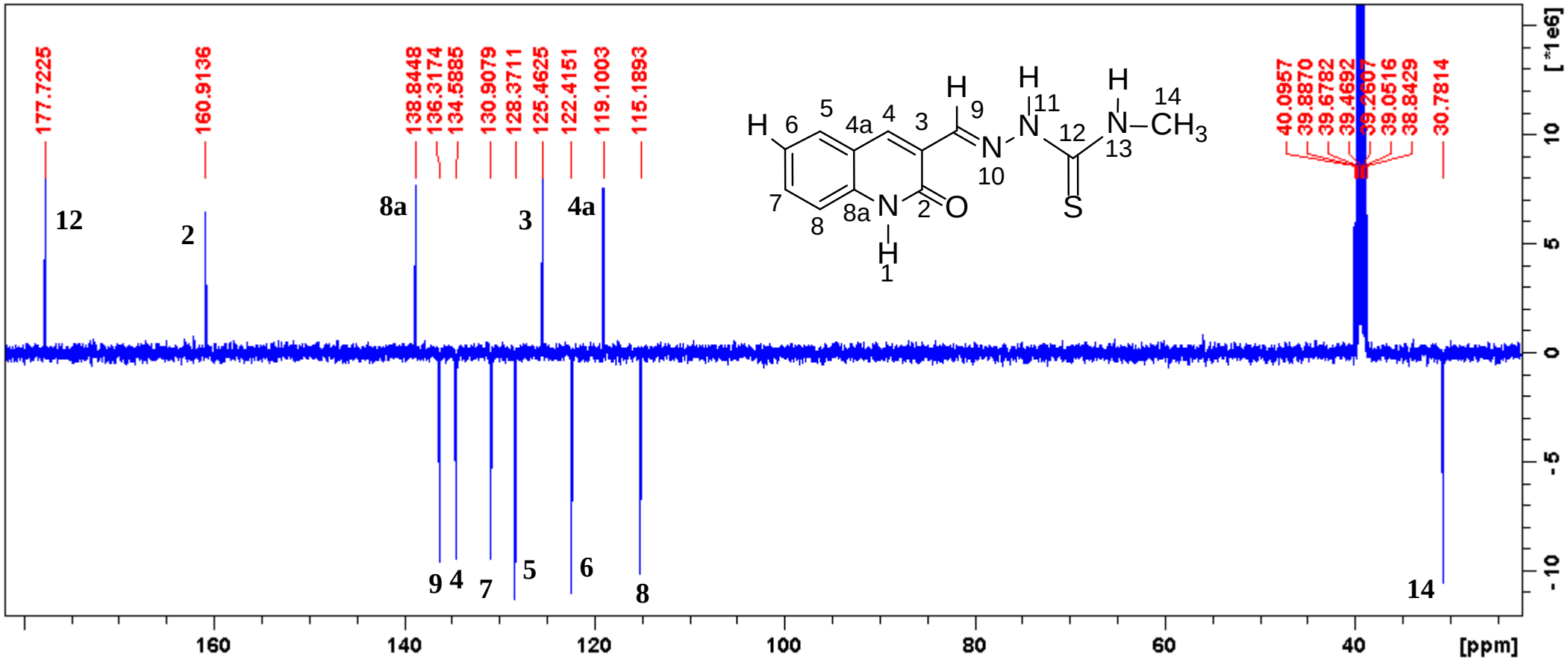
^{1}H NMR spectrum of compound 5f



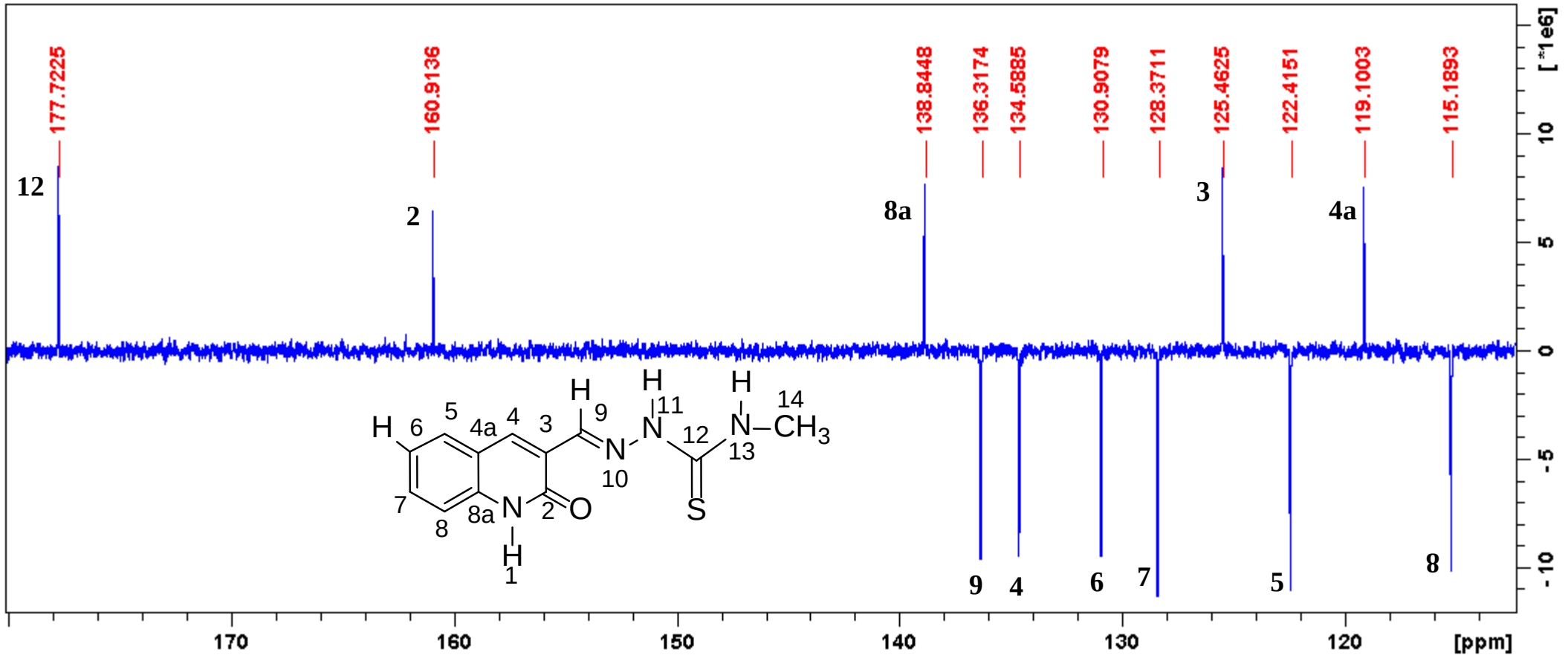
The ^{1}H NMR spectrum of compound 5f (expanded)



^{1}H NMR spectrum of compound 5f



^{13}C NMR spectrum of compound 5f

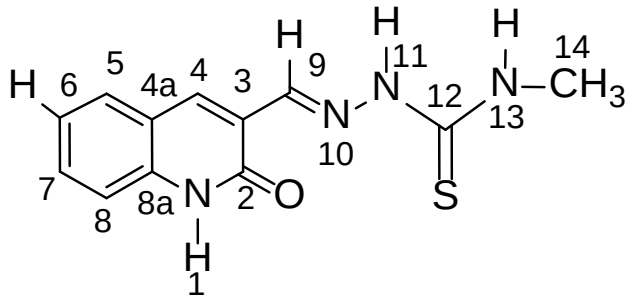


^{13}C NMR spectrum of compound 5f (expanded)

Elemental Composition Report

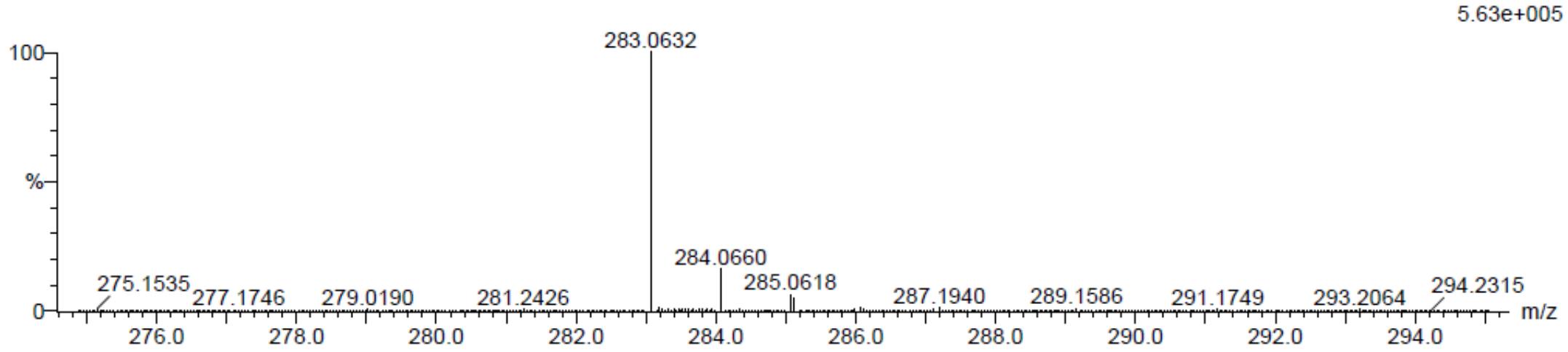
Single Mass Analysis

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



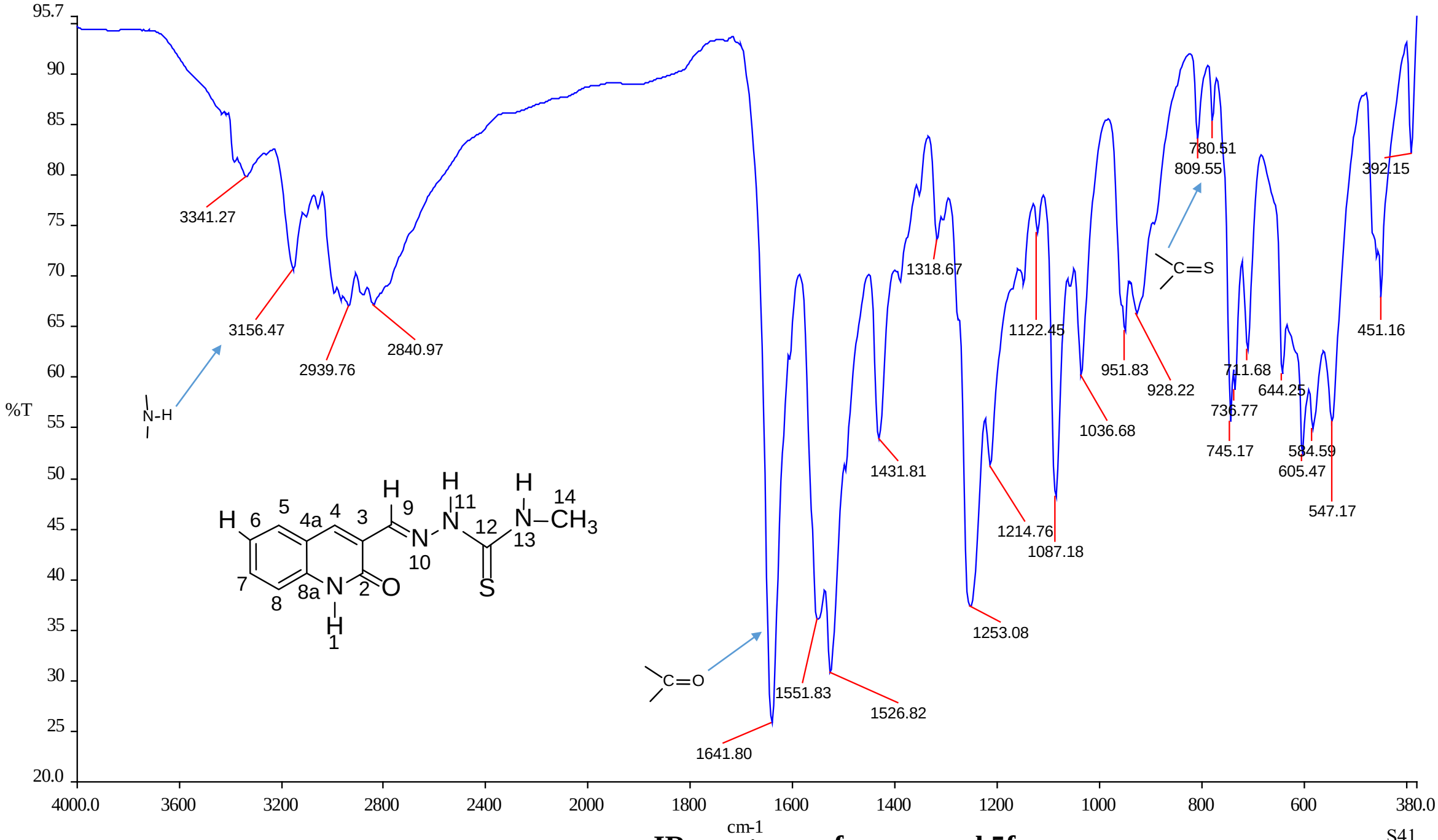
Monoisotopic Mass, Even Electron Ions
55 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 10-15 N: 1-5 O: 0-5 Na: 0-1 S: 0-1

Compound 5d HG 4 (0.101) Cm (1:61)
TOF MS ES+

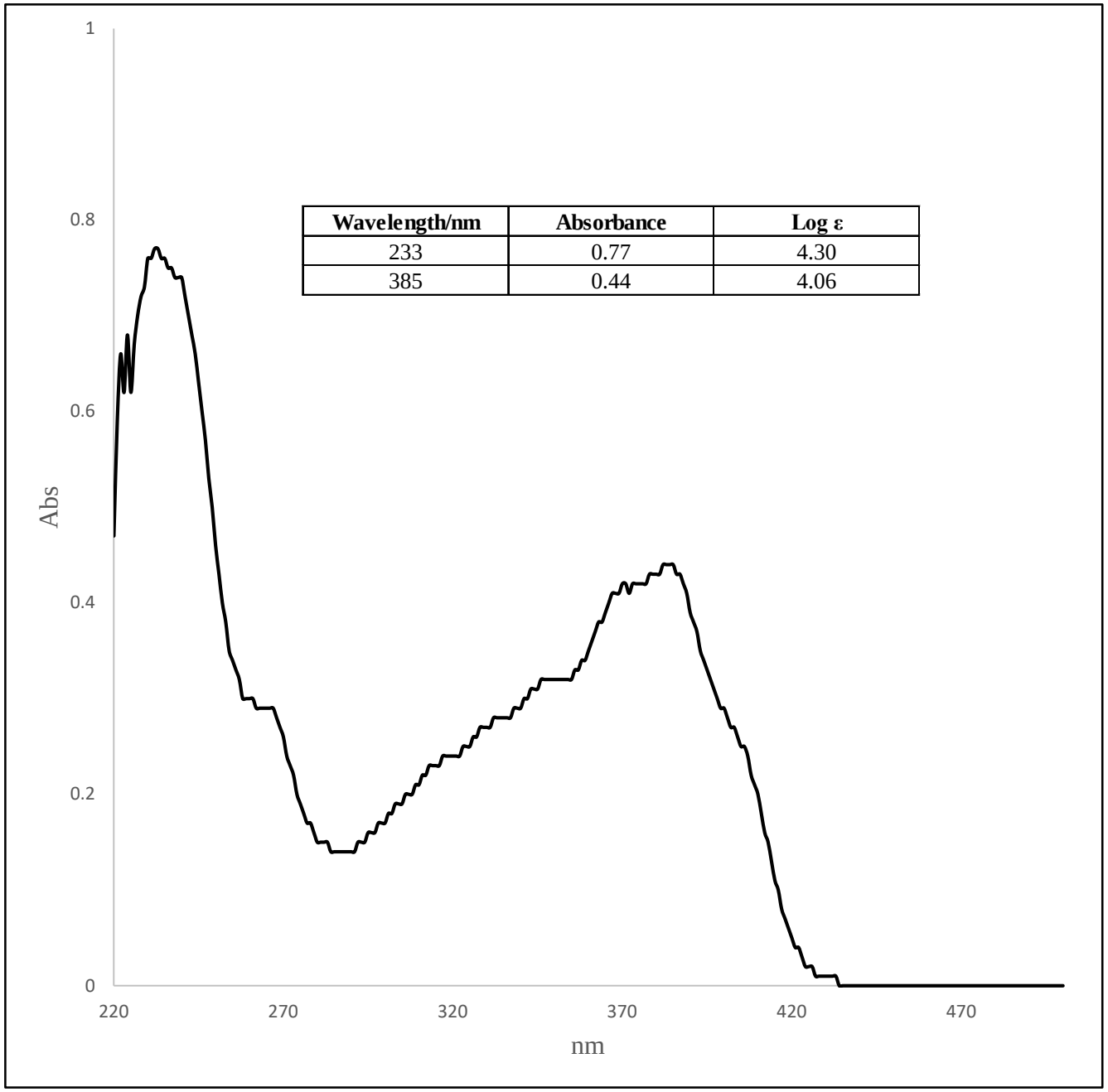


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
283.0632	283.0630	0.2	0.7	8.5	756.0	0.0	C12 H12 N4 O Na S

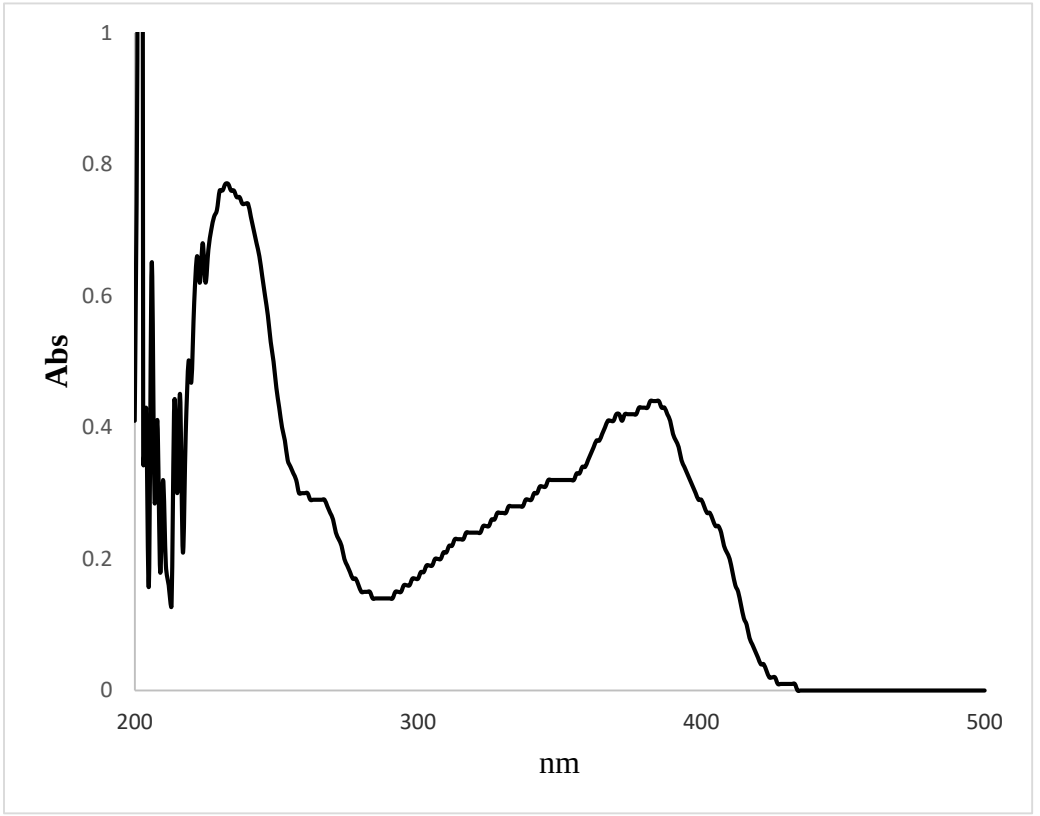
HRMS spectrum of compound 5f

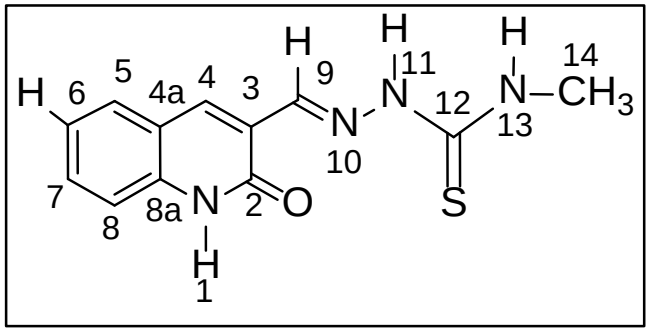


IR spectrum of compound 5f

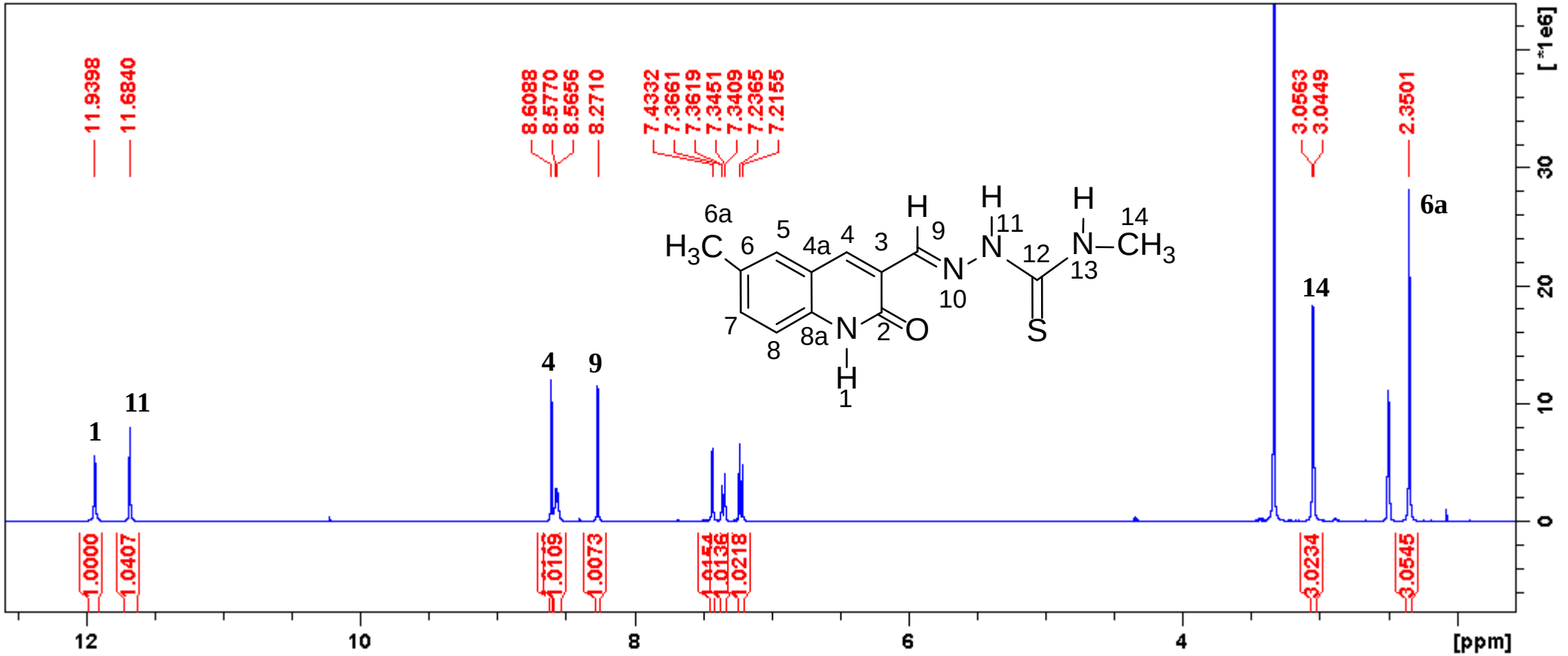


Wavelength/nm	Absorbance	Log ε
233	0.77	4.30
385	0.44	4.06

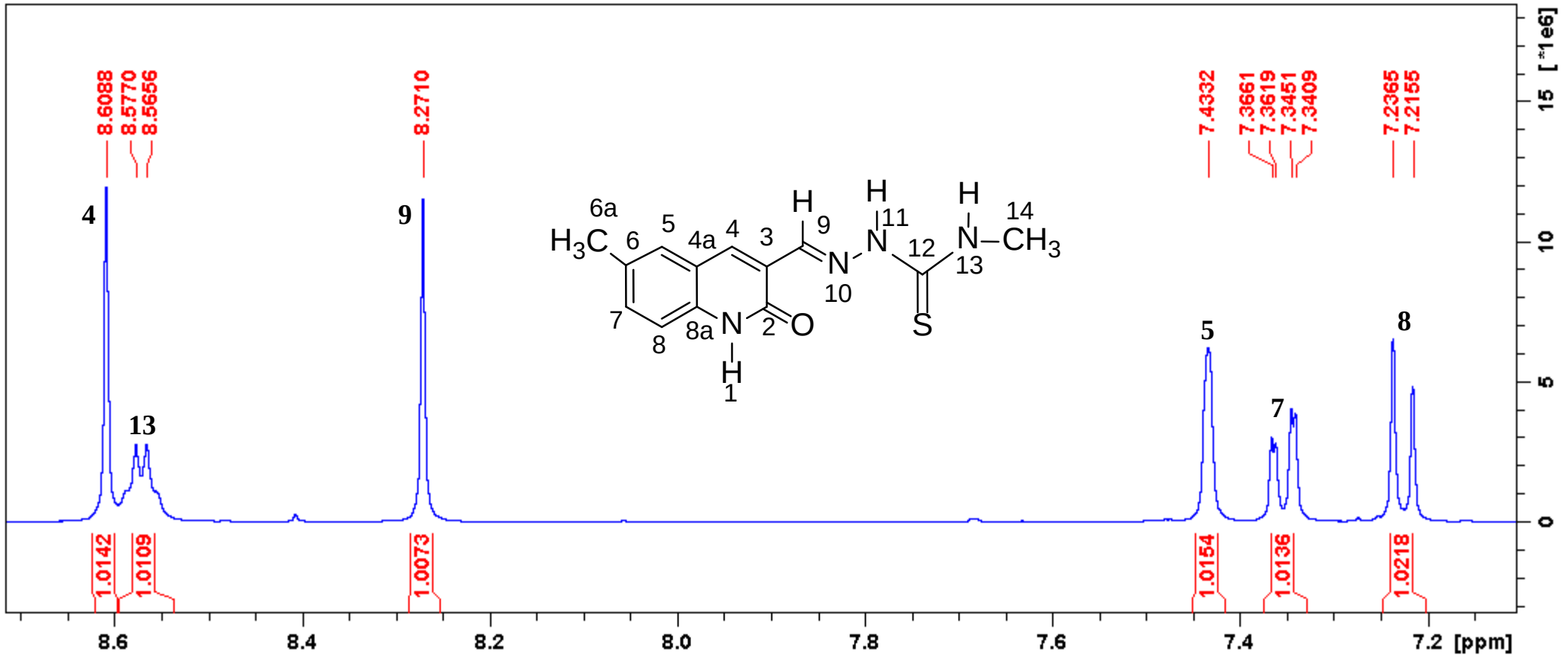




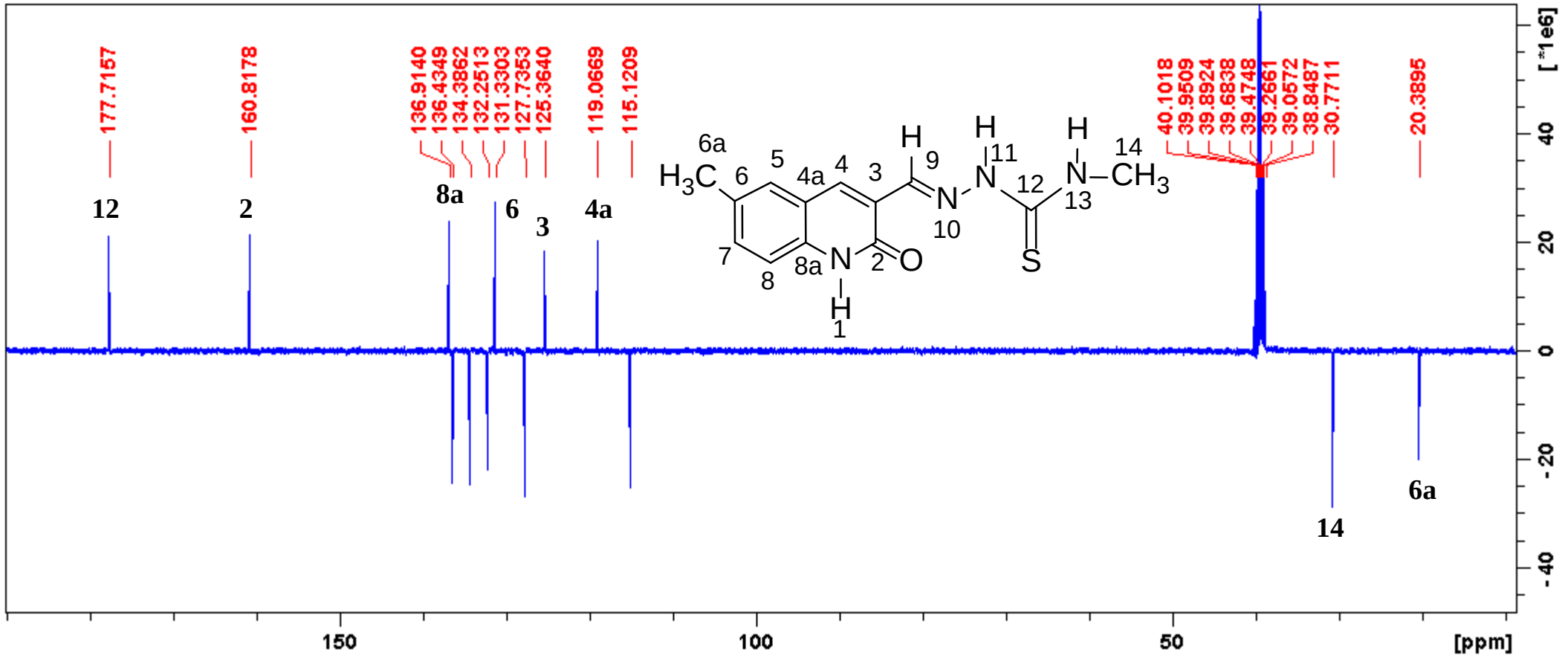
UV-Vis spectrum of compound 5f



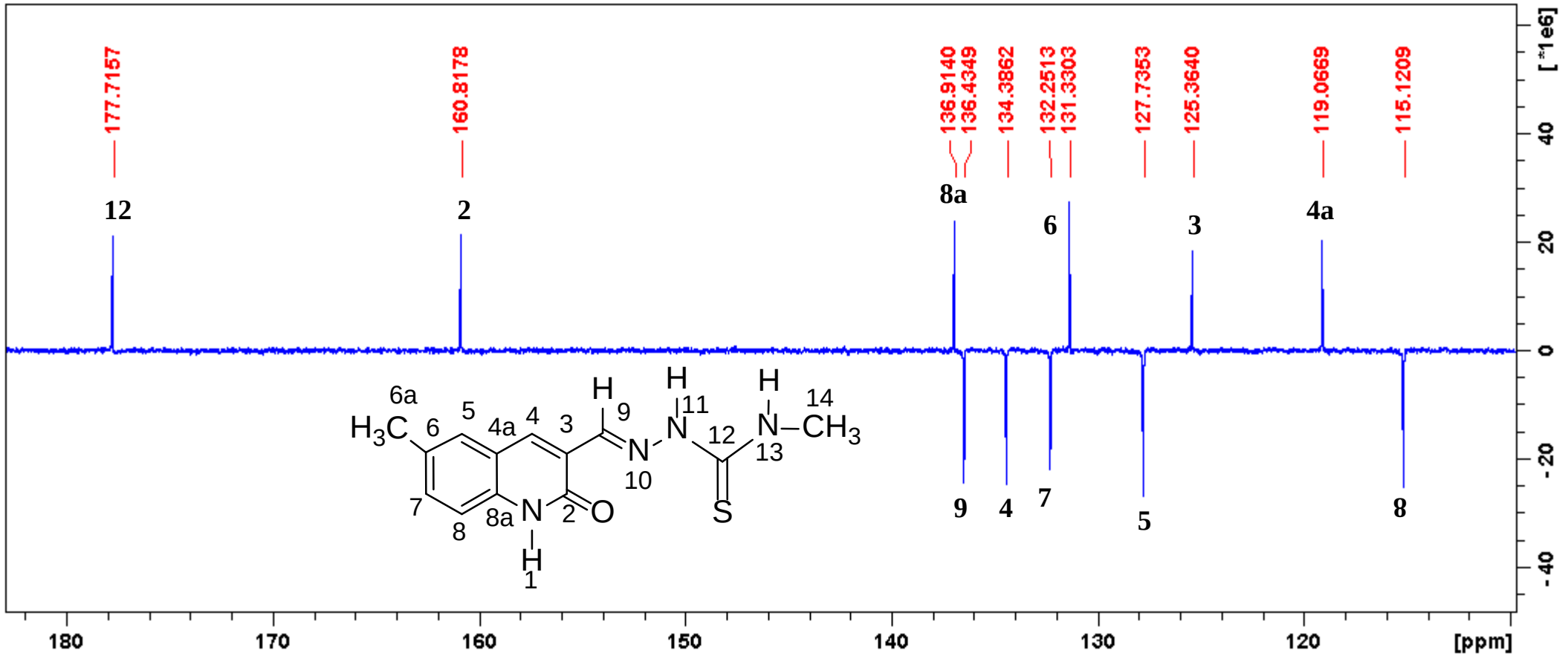
^{1}H NMR spectrum of compound 5g



^{1}H NMR spectrum of compound 5g (expanded)



^{13}C NMR spectrum of compound 5g

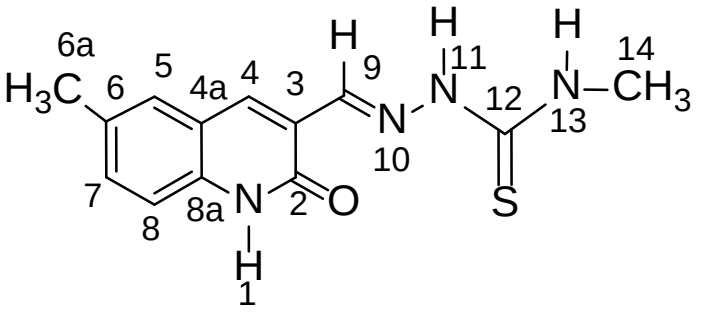


^{13}C NMR spectrum of compound 5g (expanded)

Elemental Composition Report

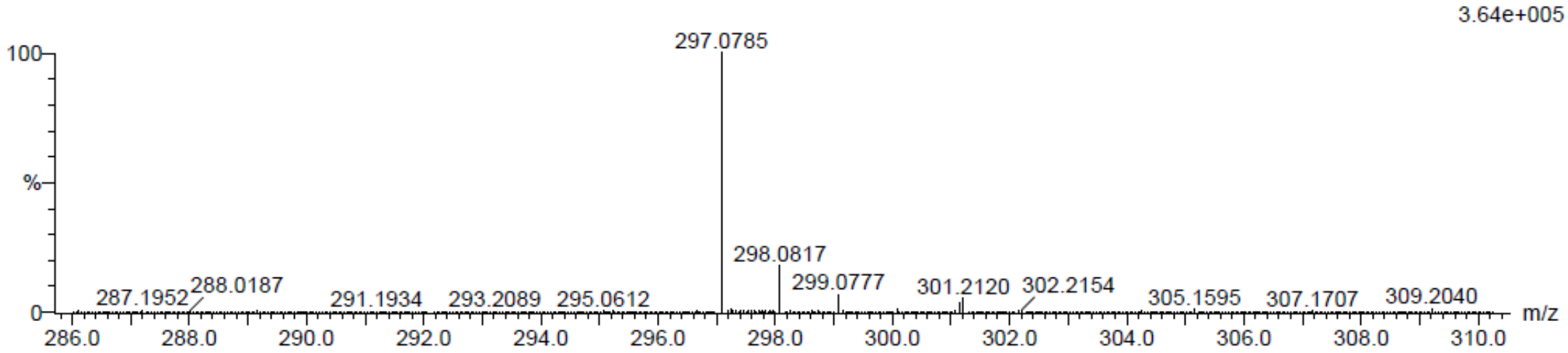
Single Mass Analysis

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



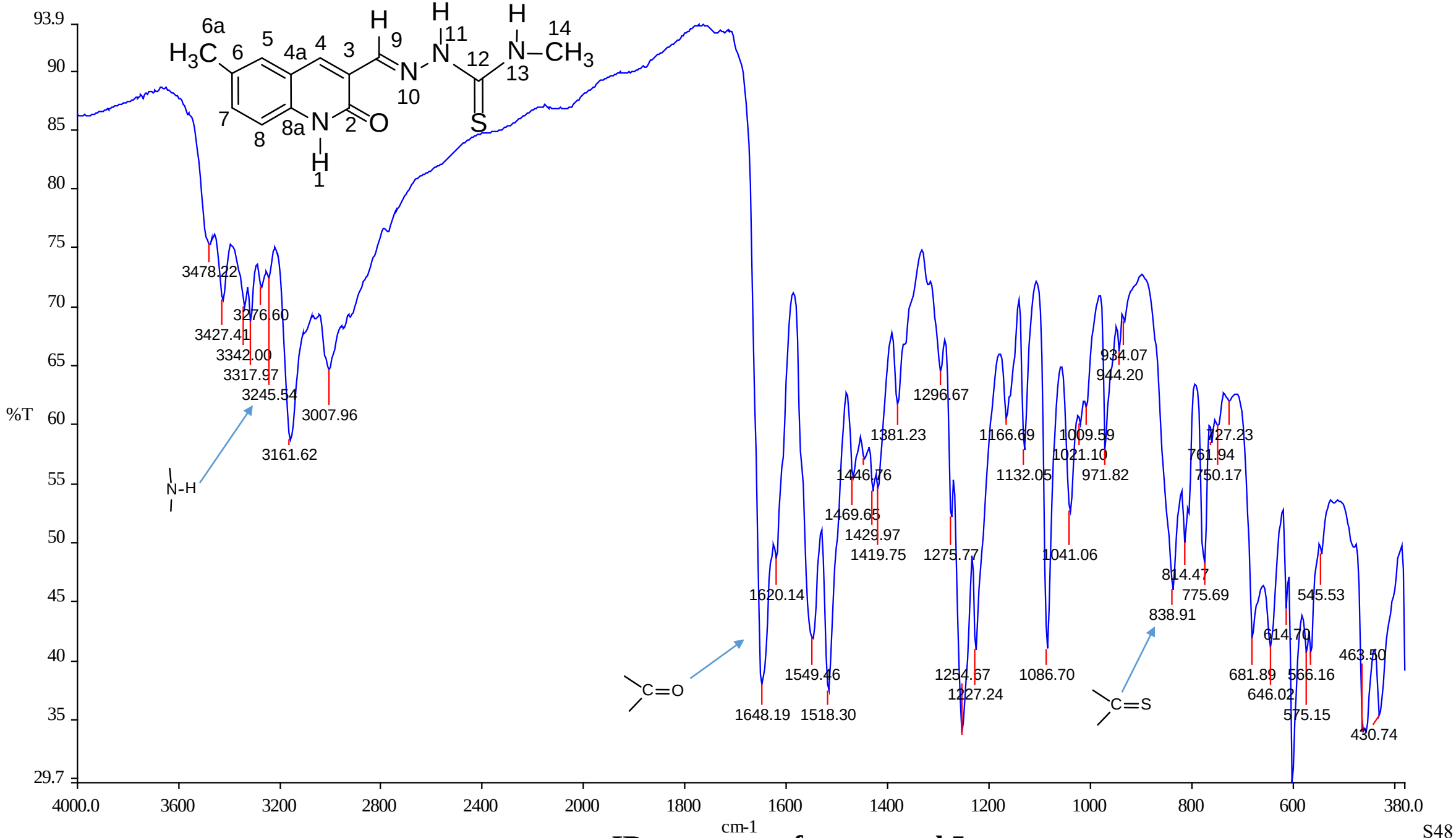
Monoisotopic Mass, Even Electron Ions
54 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 10-15 N: 1-5 O: 0-5 Na: 0-1 S: 0-1

Compound 6d HG 43 (1.417) Cm (1:61)
TOF MS ES+

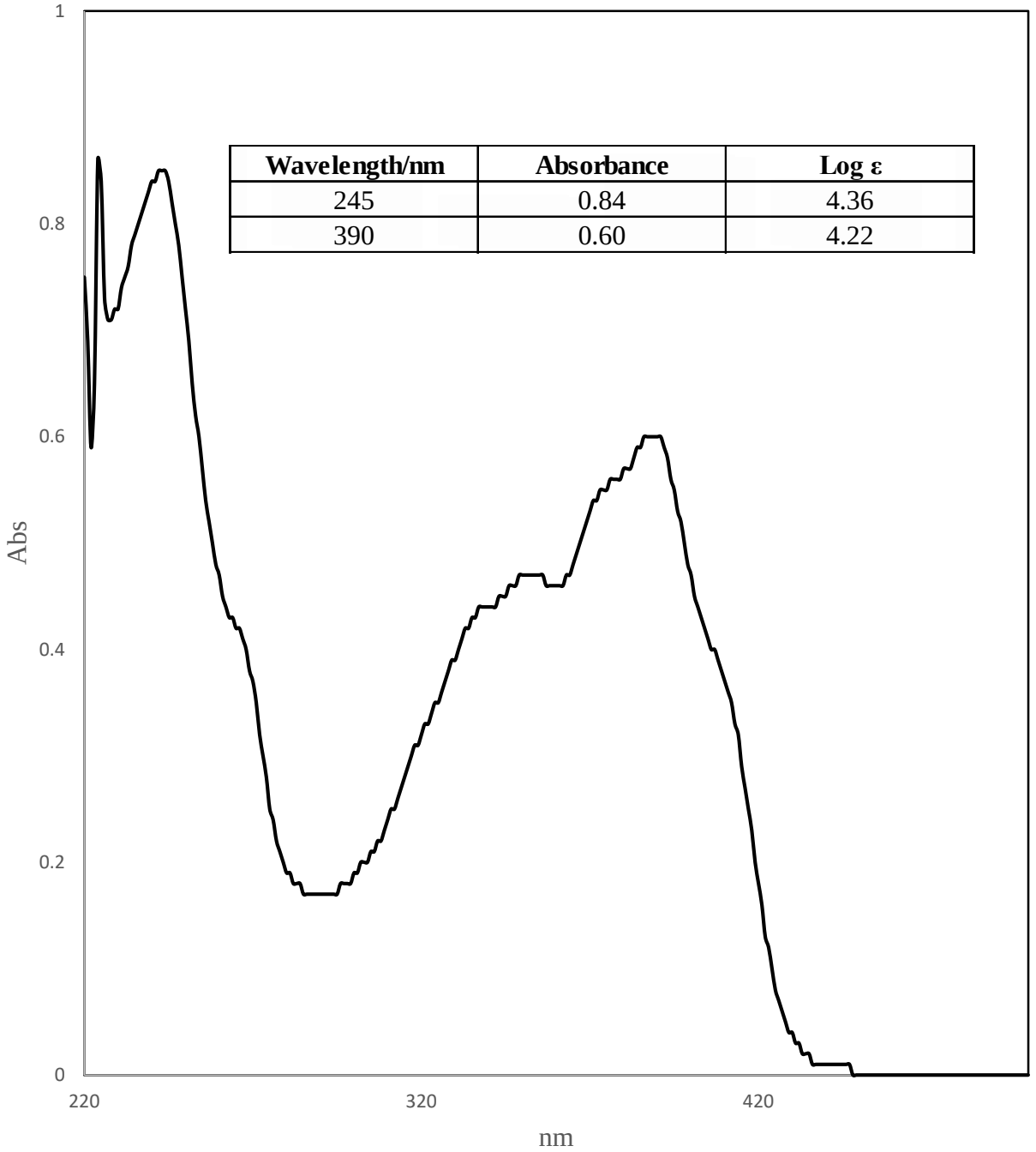


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
297.0785	297.0786	-0.1	-0.3	8.5	716.9	0.0	C13 H14 N4 O Na S

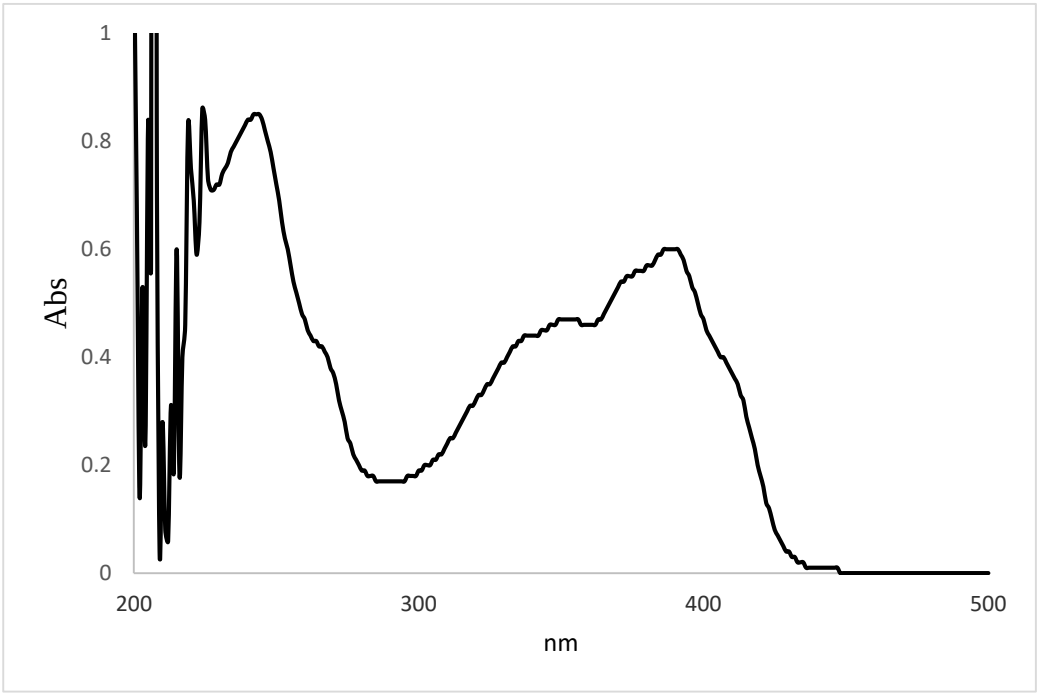
HRMS spectrum of compound 5g

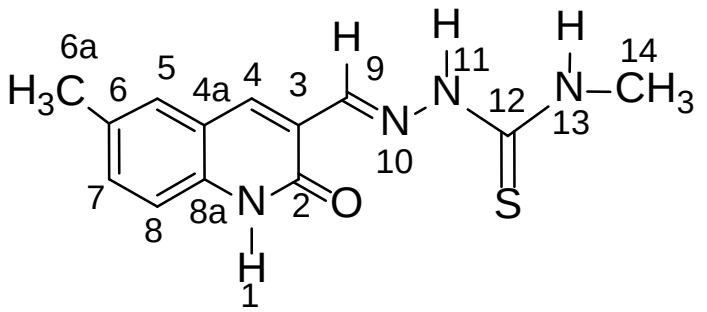


IR spectrum of compound 5g

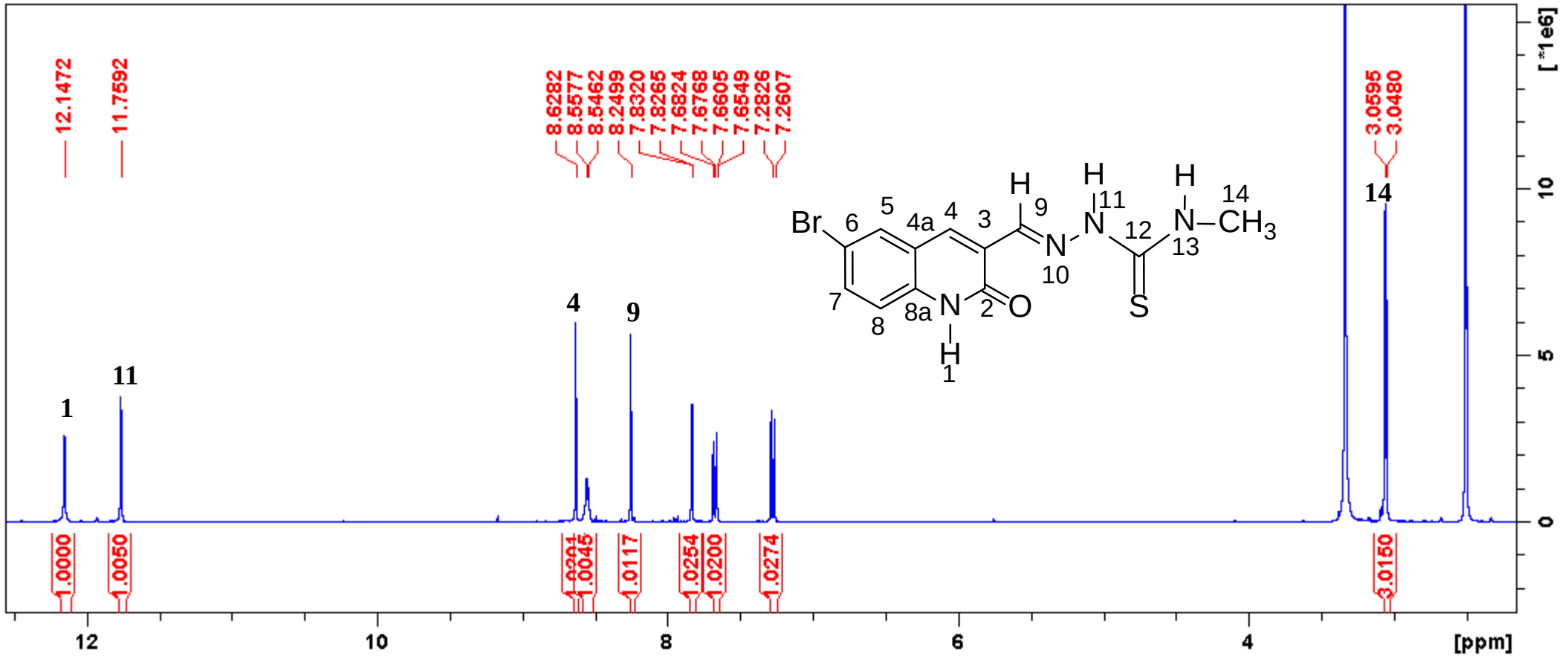


Wavelength/nm	Absorbance	Log ε
245	0.84	4.36
390	0.60	4.22

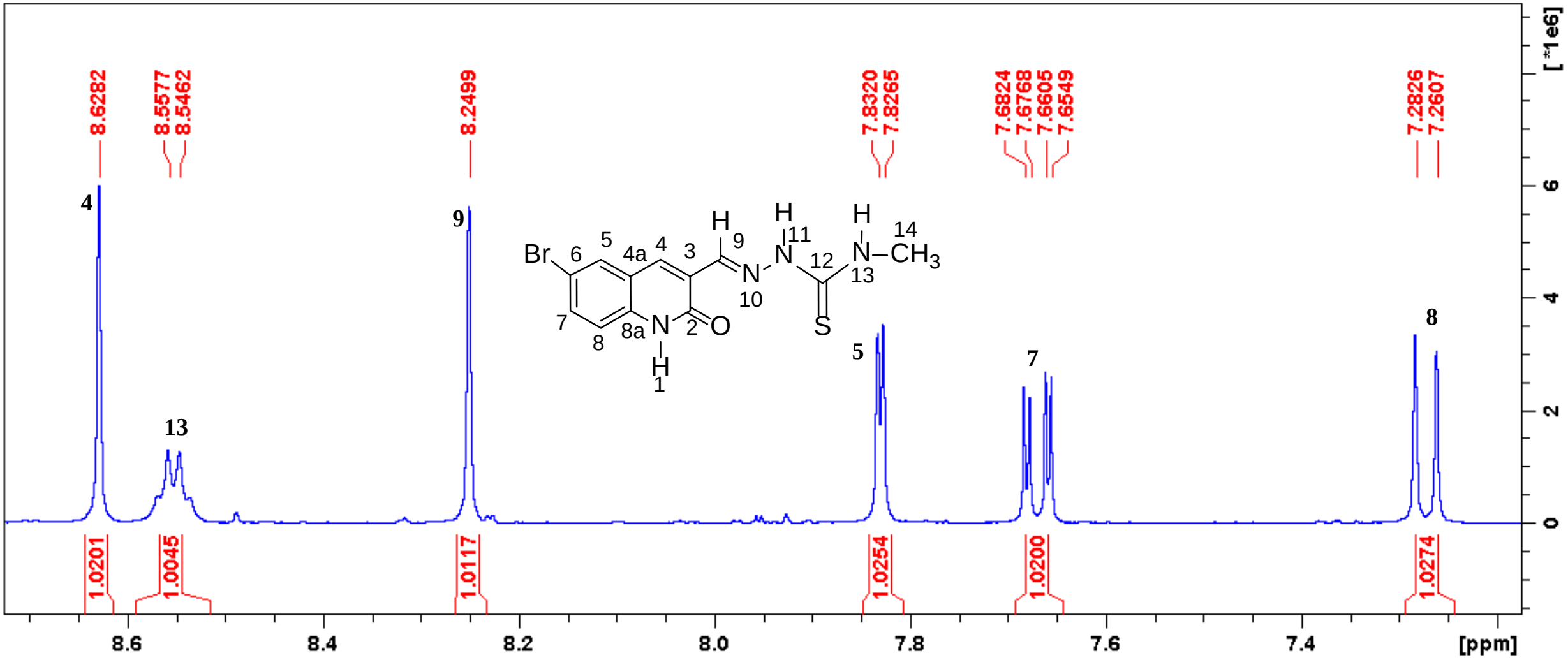




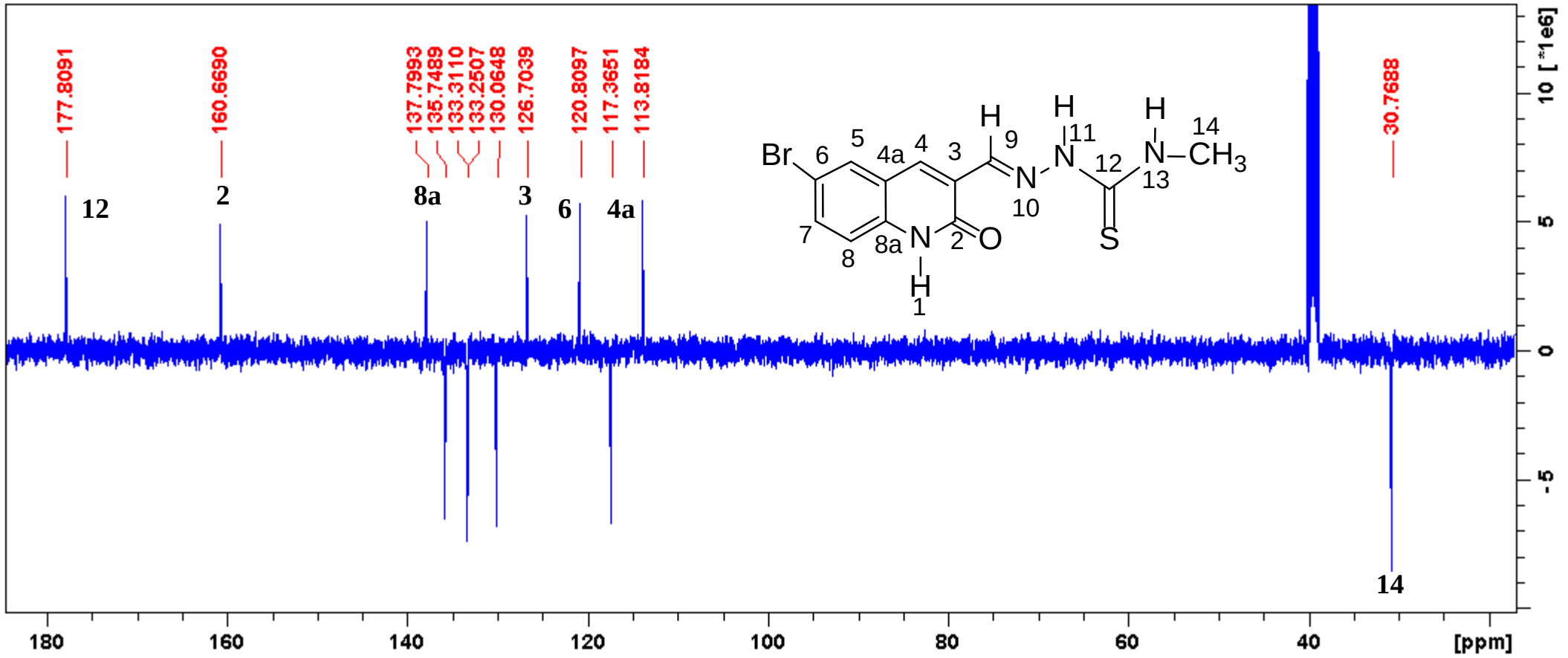
UV-Vis spectrum of compound 5g



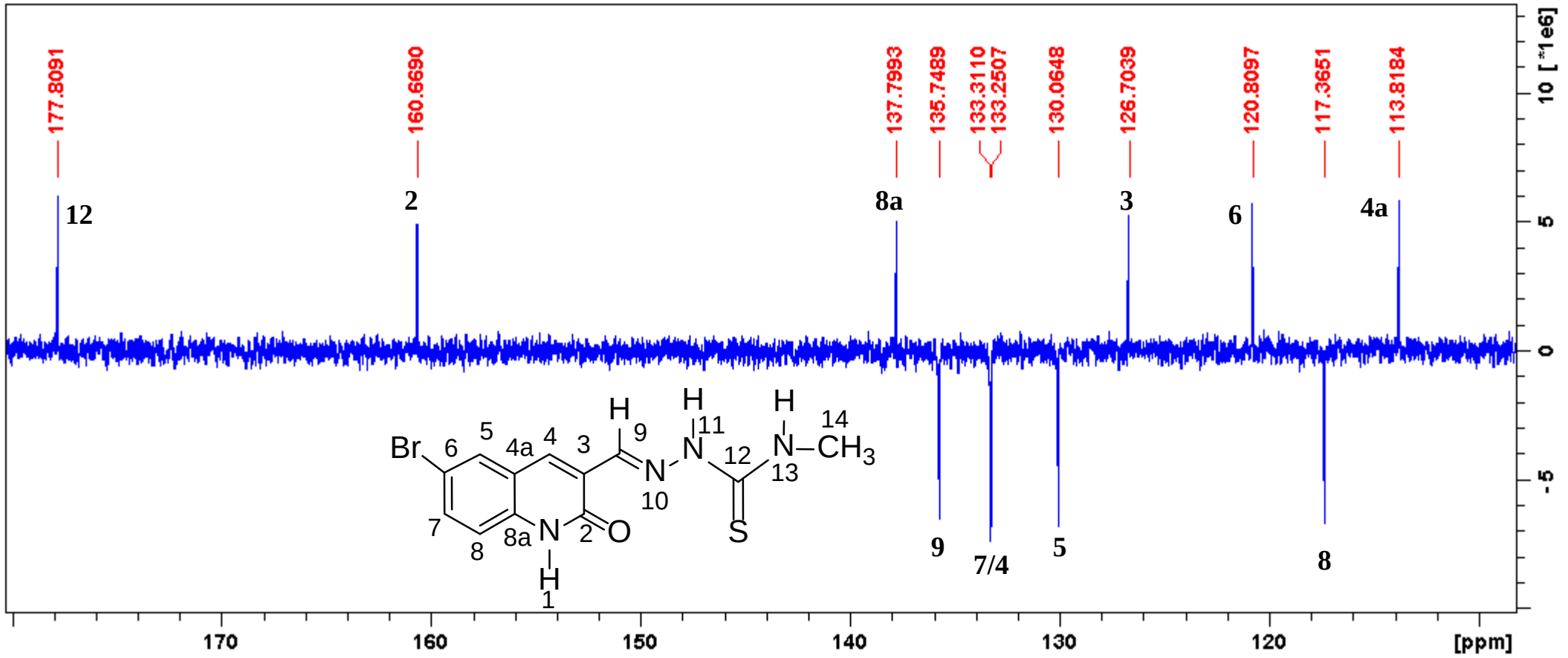
^{1}H NMR spectrum of compound 5h



^{1}H NMR spectrum of compound 5h (expanded)



^{13}C NMR spectrum of compound 5h

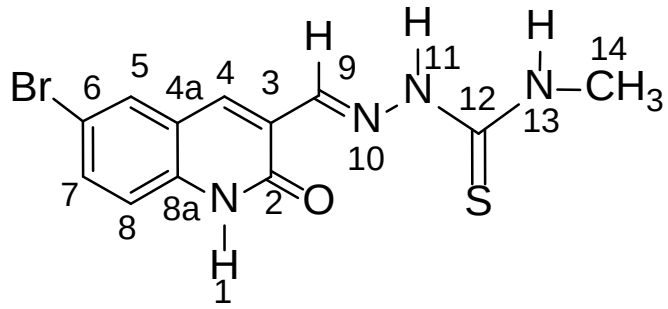


^{13}C NMR spectrum of compound 5h (expanded)

Elemental Composition Report

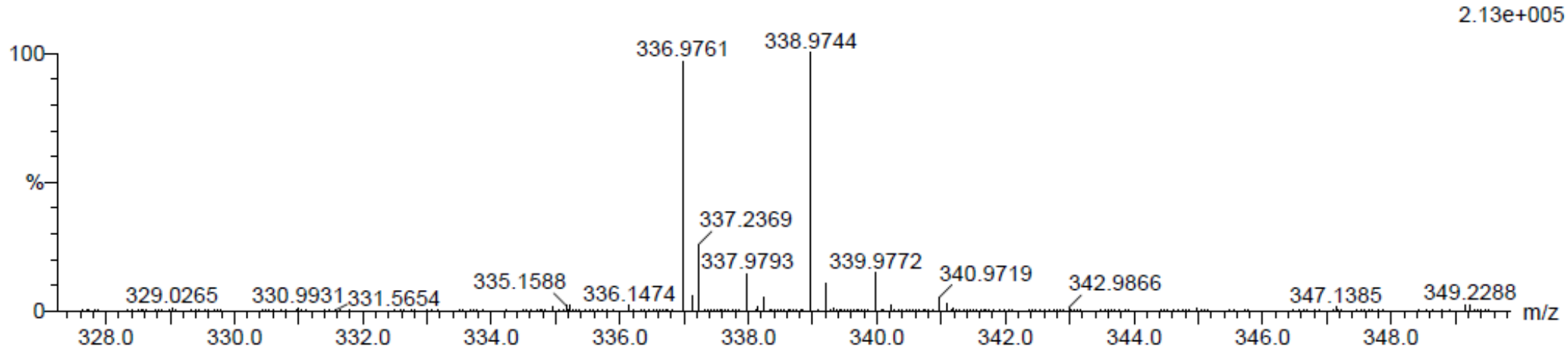
Single Mass Analysis

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



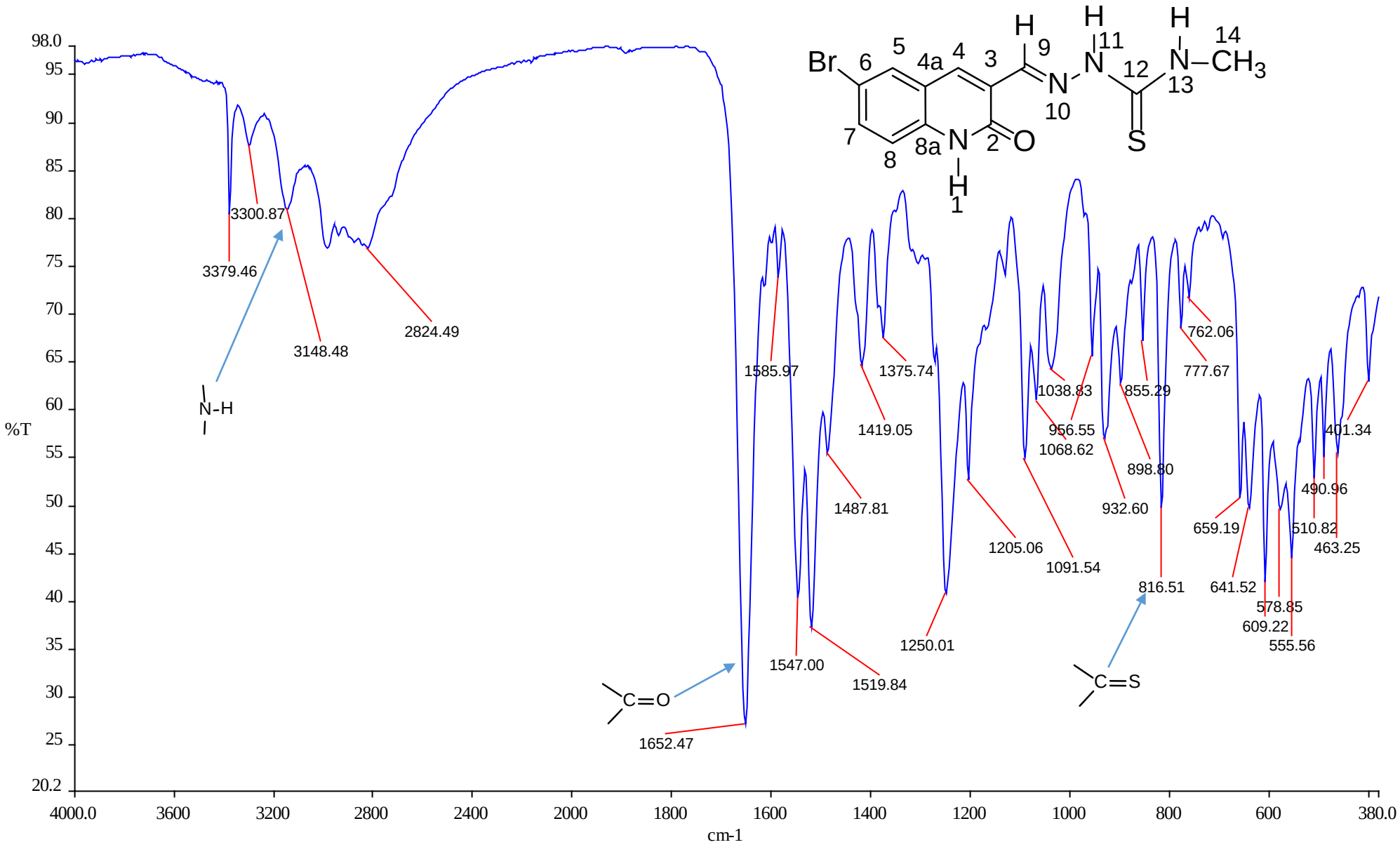
Monoisotopic Mass, Even Electron Ions
68 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 10-15 N: 1-5 O: 0-5 S: 0-1 Br: 0-1

Compound 3d HG 60 (1.989) Cm (1:61)
TOF MS ES-



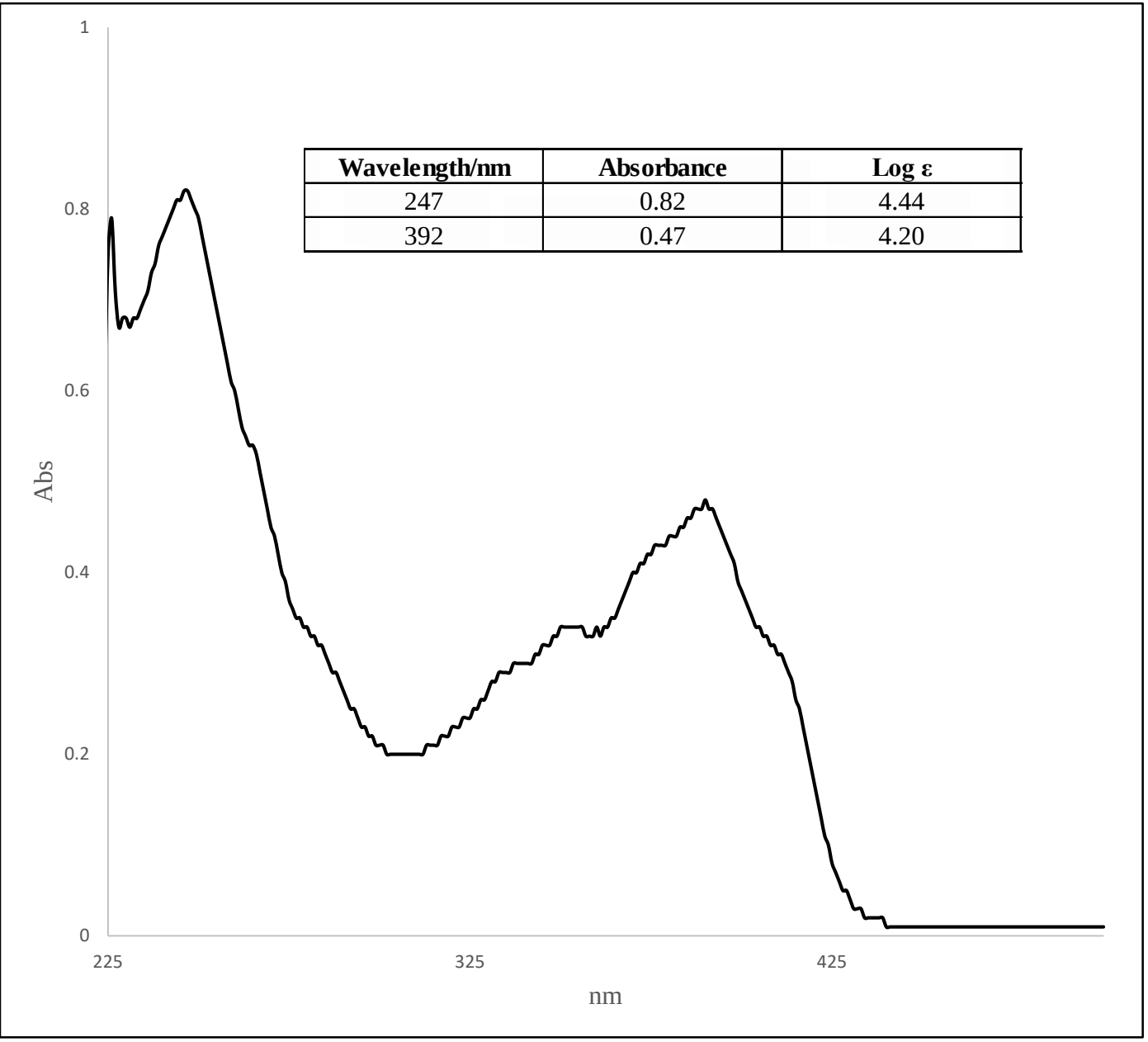
Minimum:				-1.5							
Maximum:		5.0	5.0	100.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula				
336.9761	336.9759	0.2	0.6	9.5	573.3	0.0	C12	H10	N4	O	S Br

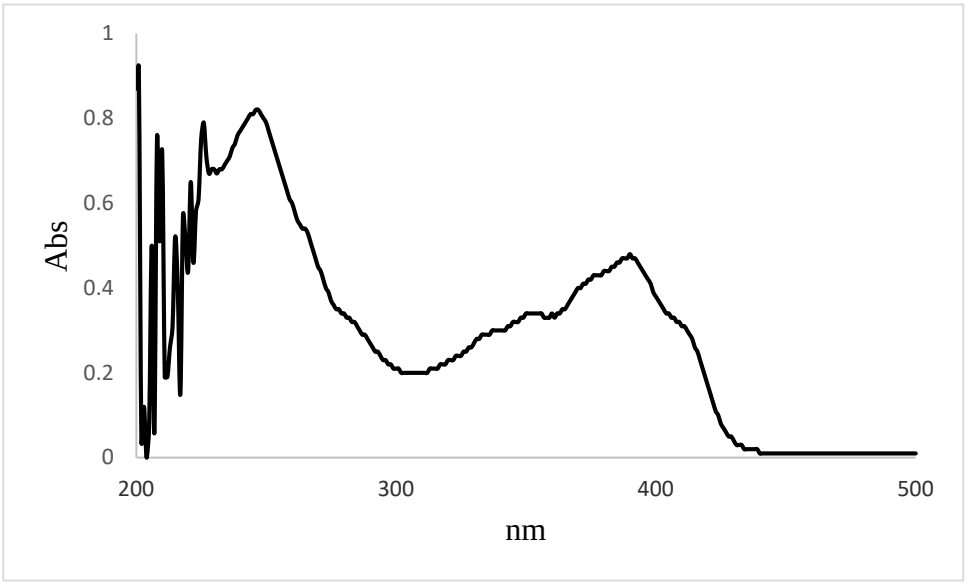
HRMS spectrum of compound 5h

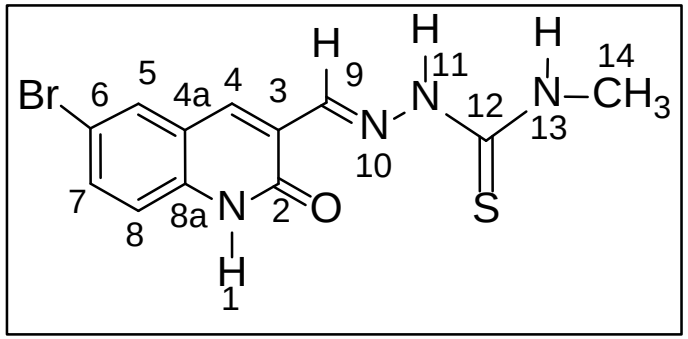


IR spectrum of compound 5h

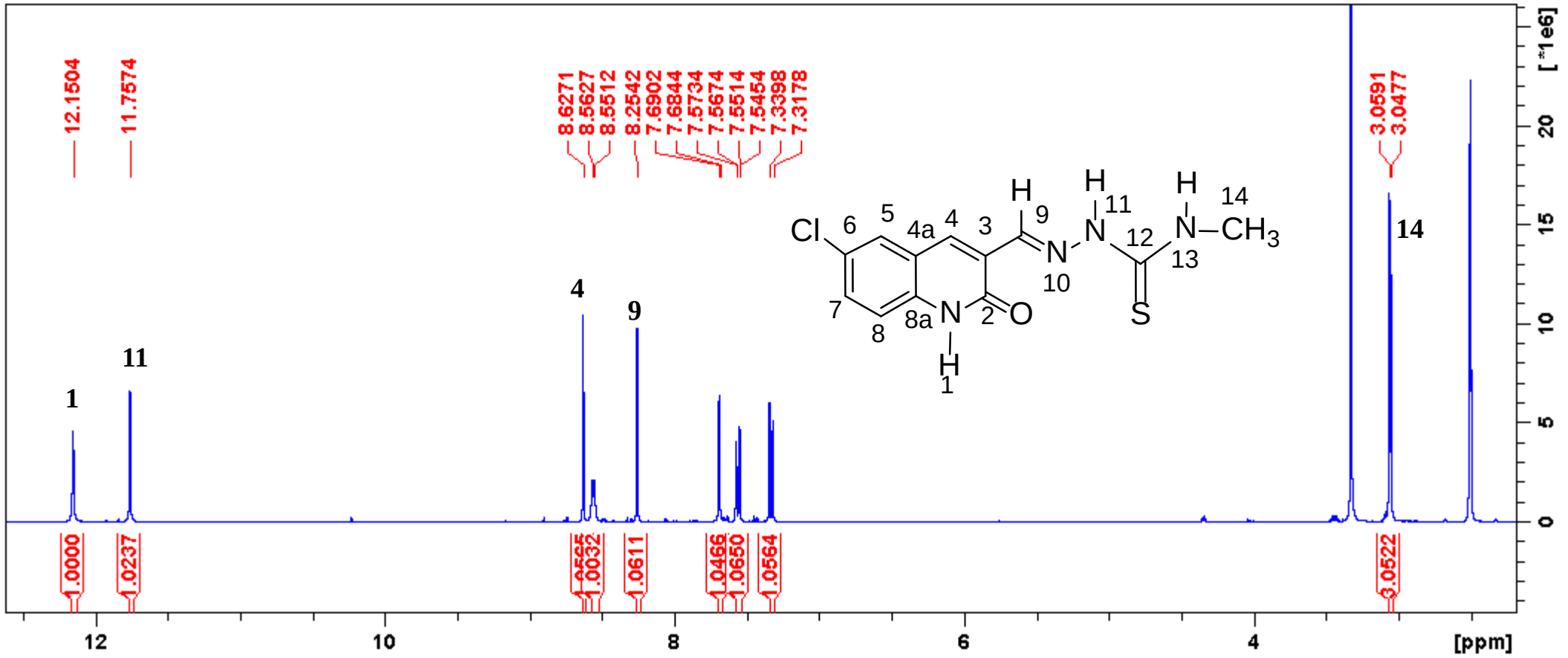
Wavelength/nm	Absorbance	Log ε
247	0.82	4.44
392	0.47	4.20



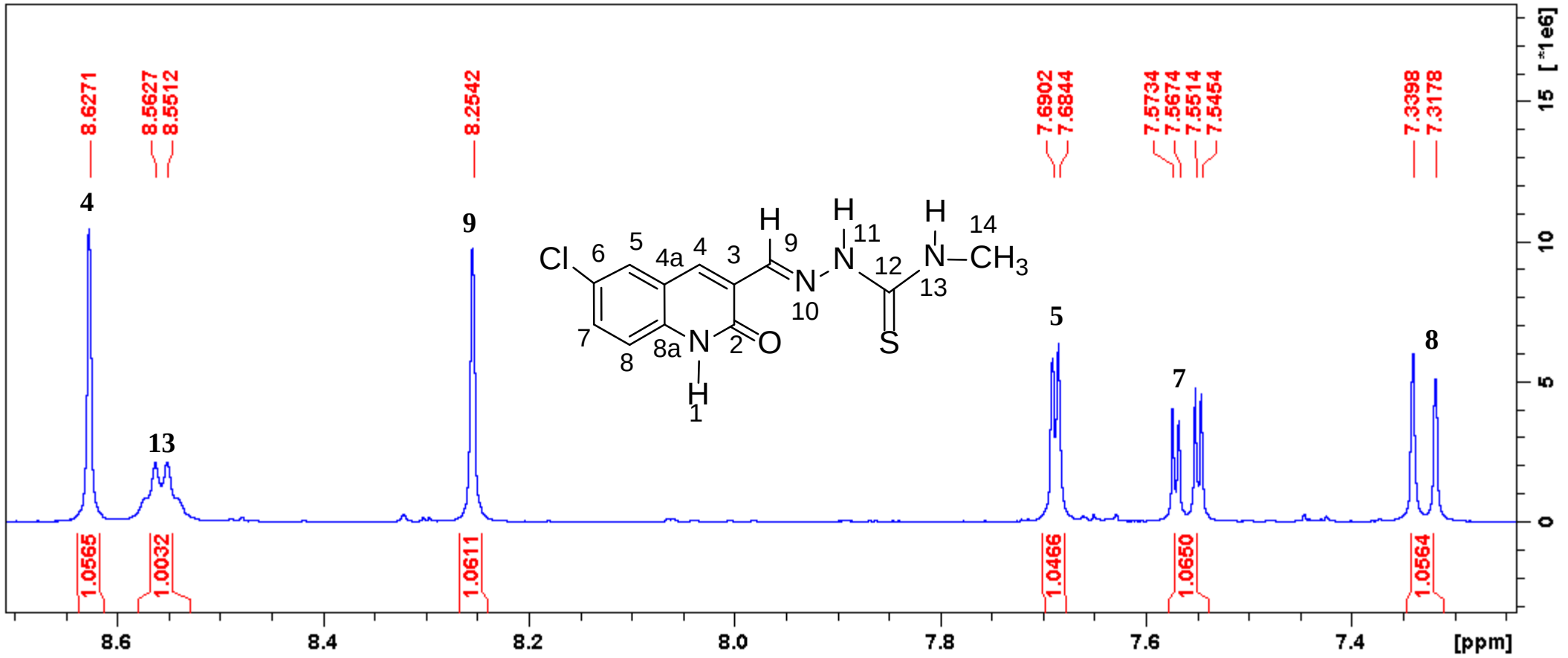




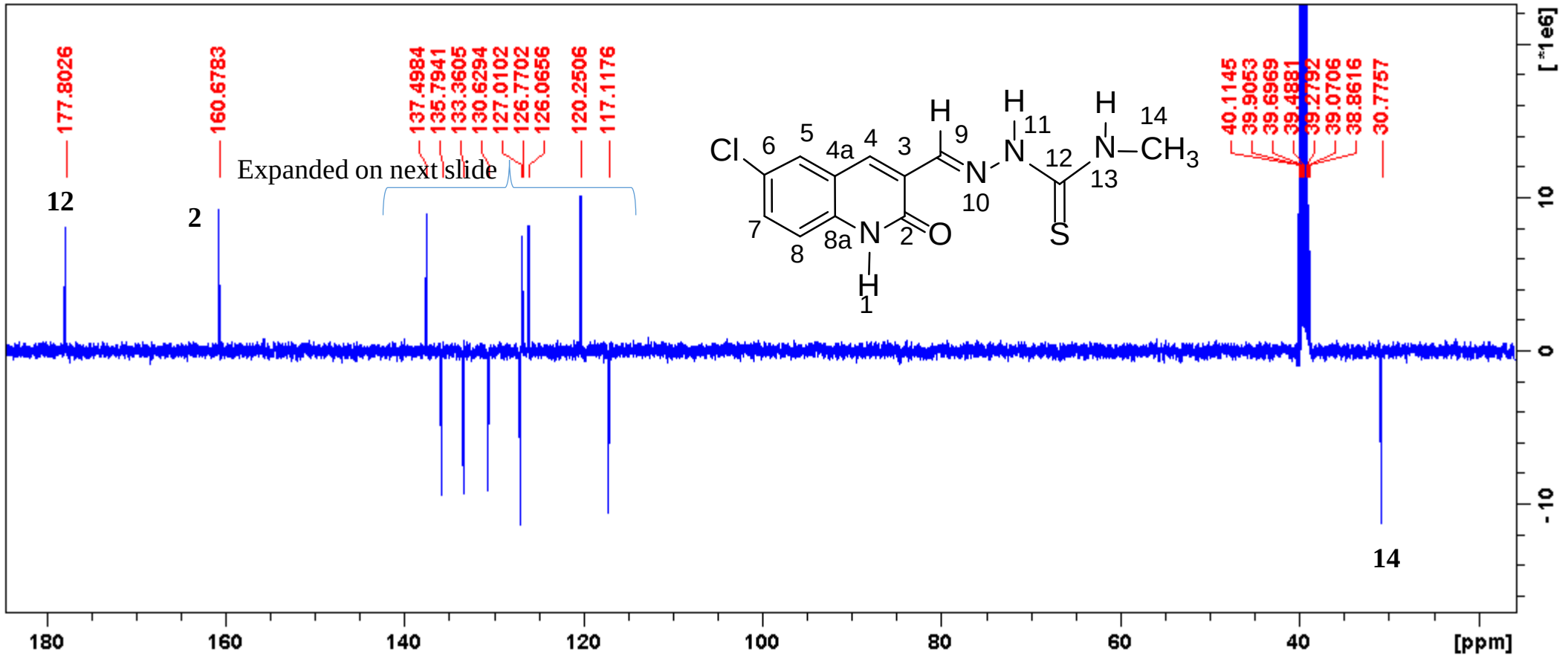
UV-Vis spectrum of compound 5h



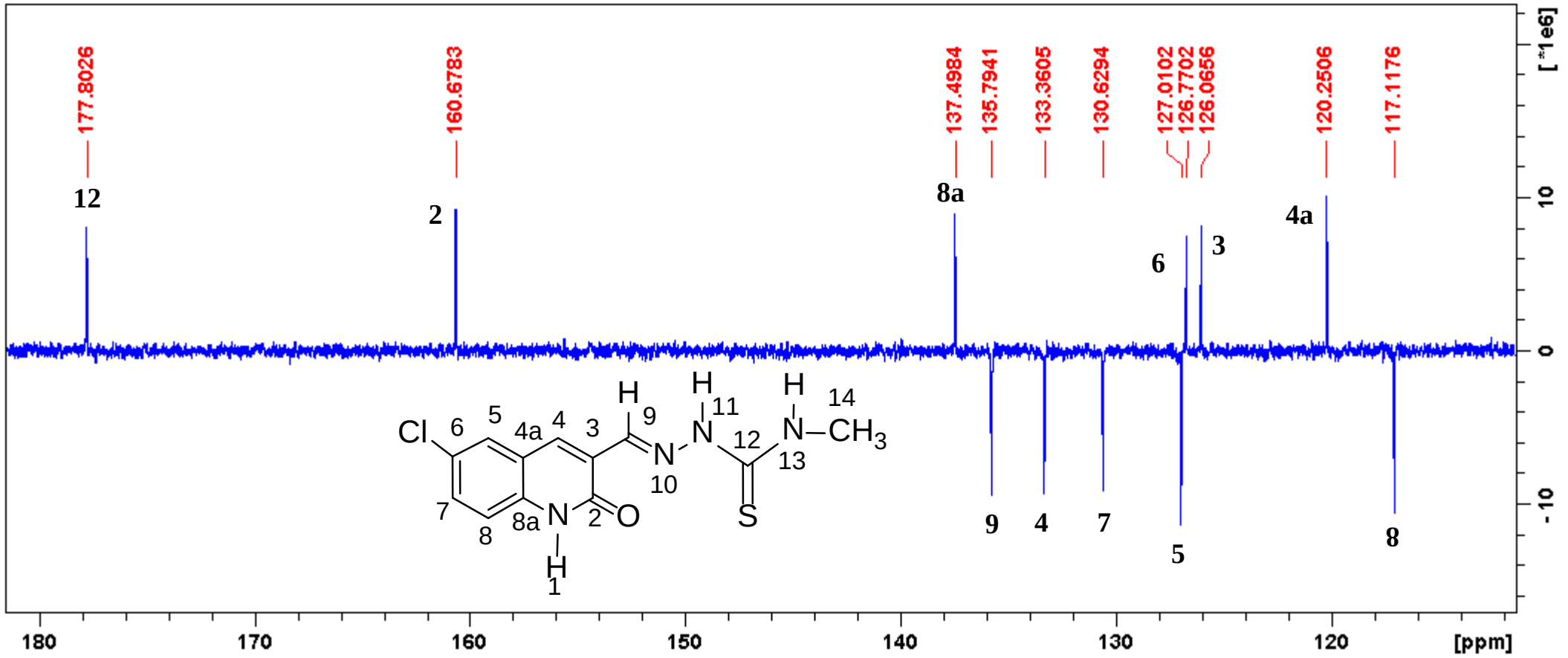
^{1}H NMR spectrum of compound 5i



^{1}H NMR spectrum of compound 5i (expanded)



^{13}C NMR spectrum of compound 5i



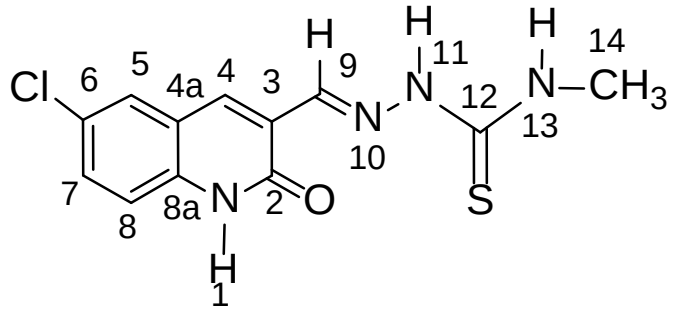
^{13}C NMR spectrum of compound 5i (expanded)

Elemental Composition Report

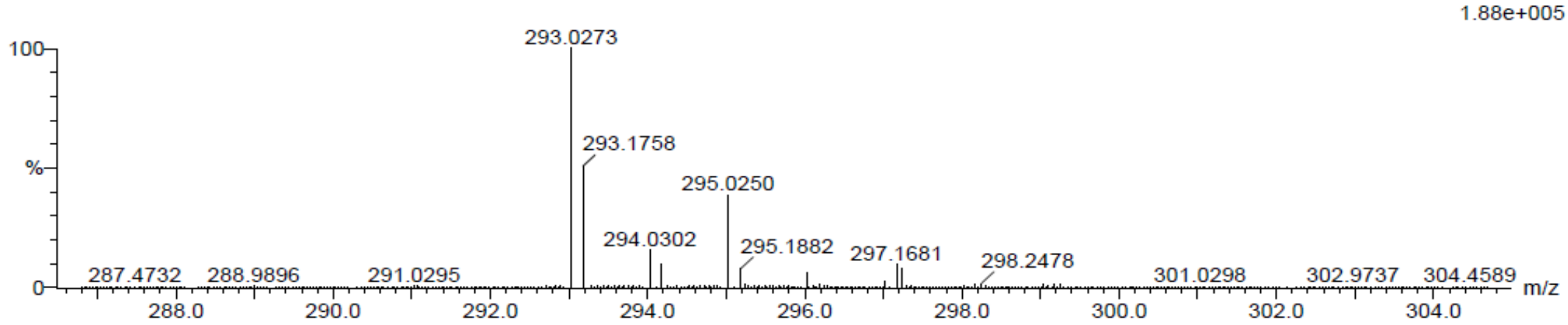
Single Mass Analysis

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
54 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 10-15 N: 1-5 O: 0-5 S: 0-1 Cl: 0-1

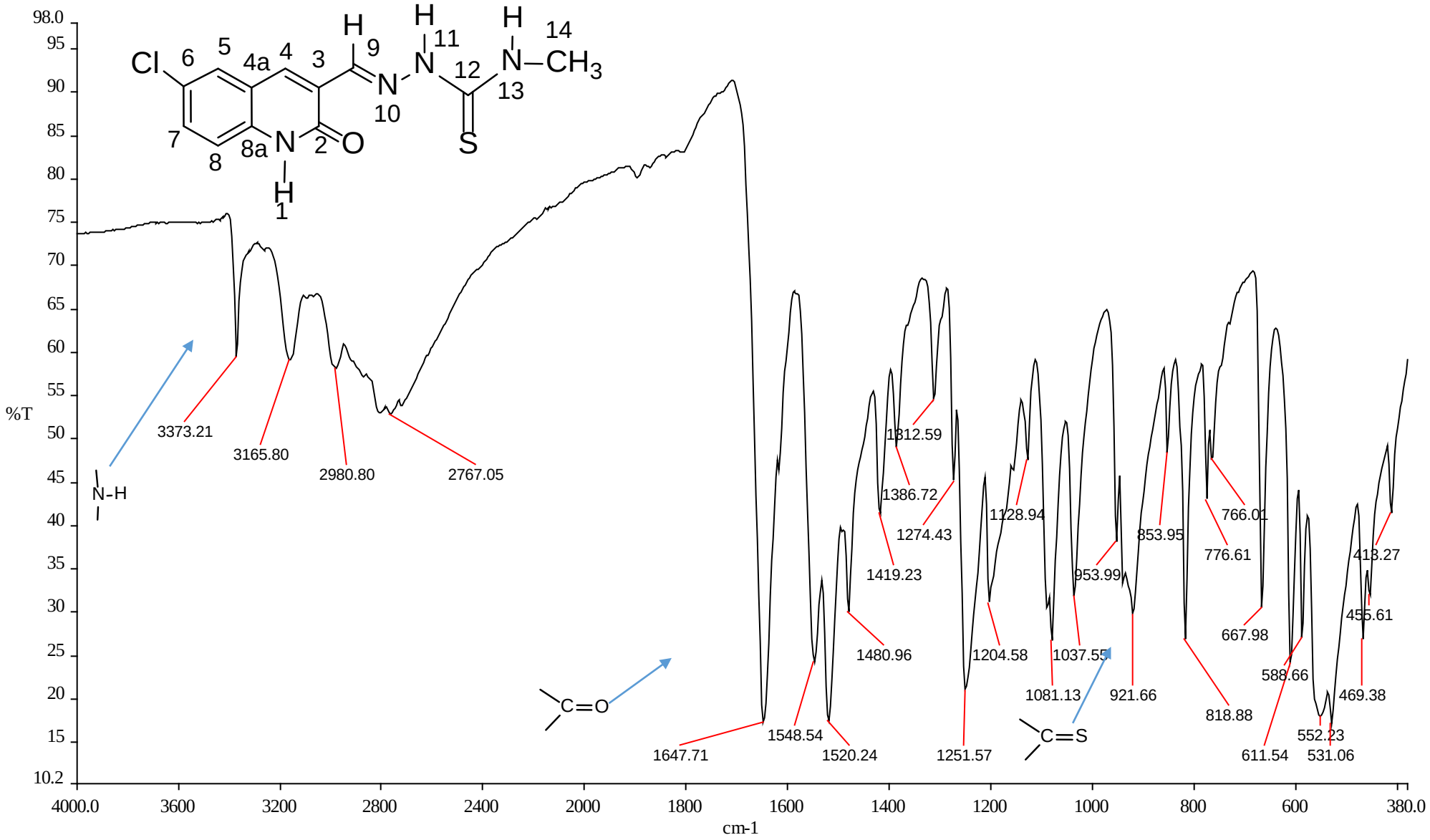


Compound 4d HG 34 (1.113) Cm (1:61)
TOF MS ES-



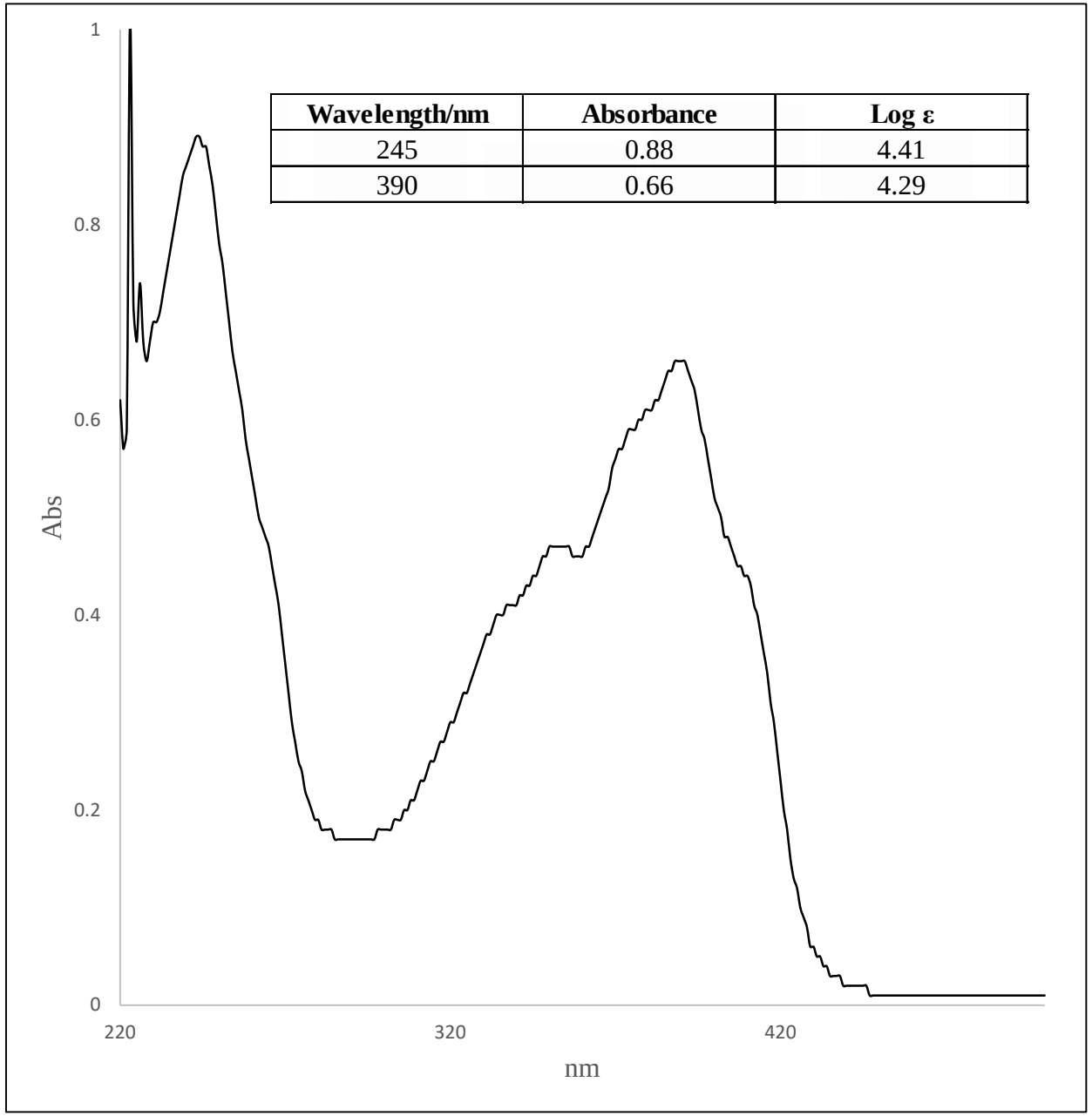
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
293.0273	293.0264	0.9	3.1	9.5	619.4	0.0	C12 H10 N4 O S Cl

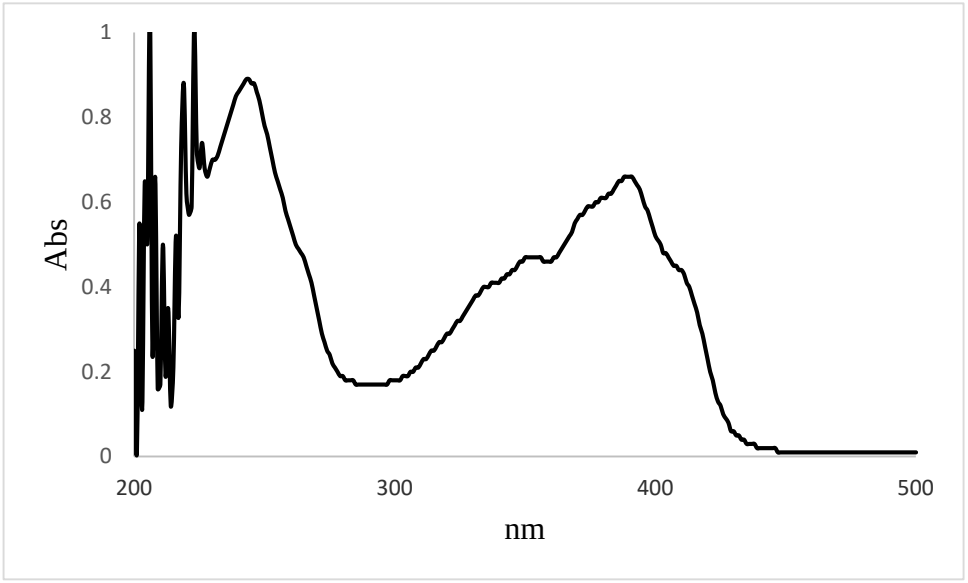
HRMS spectrum of compound 5i

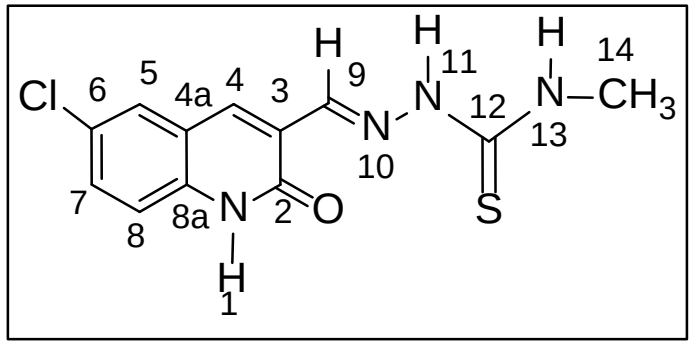


IR spectrum of compound 5i

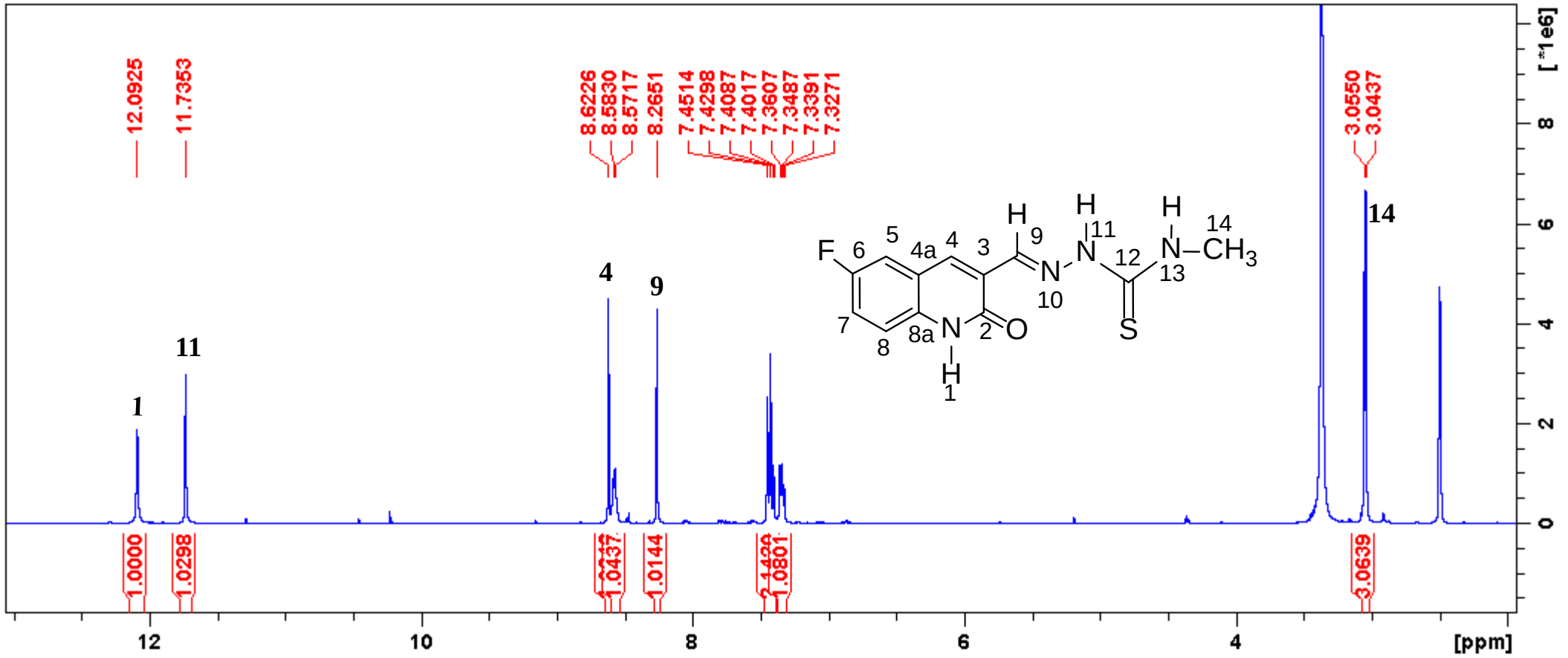
Wavelength/nm	Absorbance	Log ε
245	0.88	4.41
390	0.66	4.29



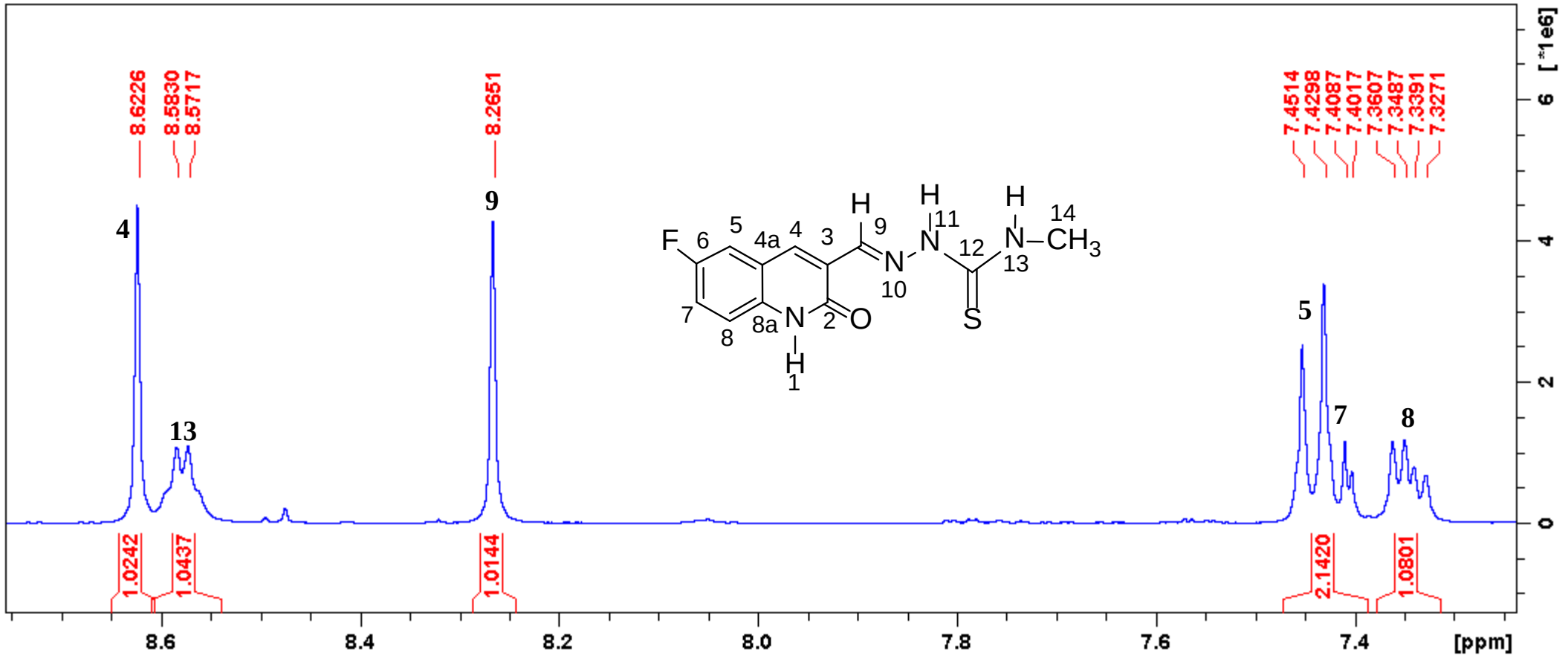




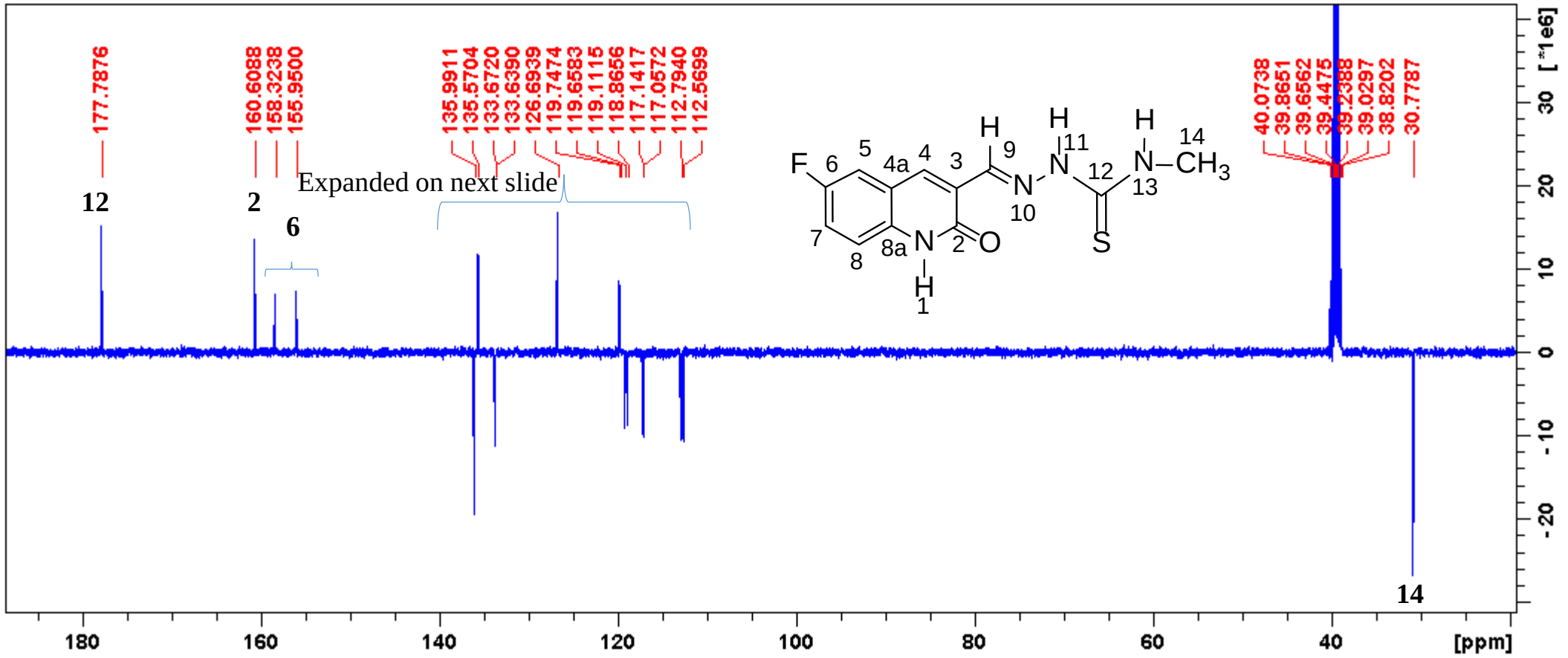
UV-Vis spectrum of compound 5i



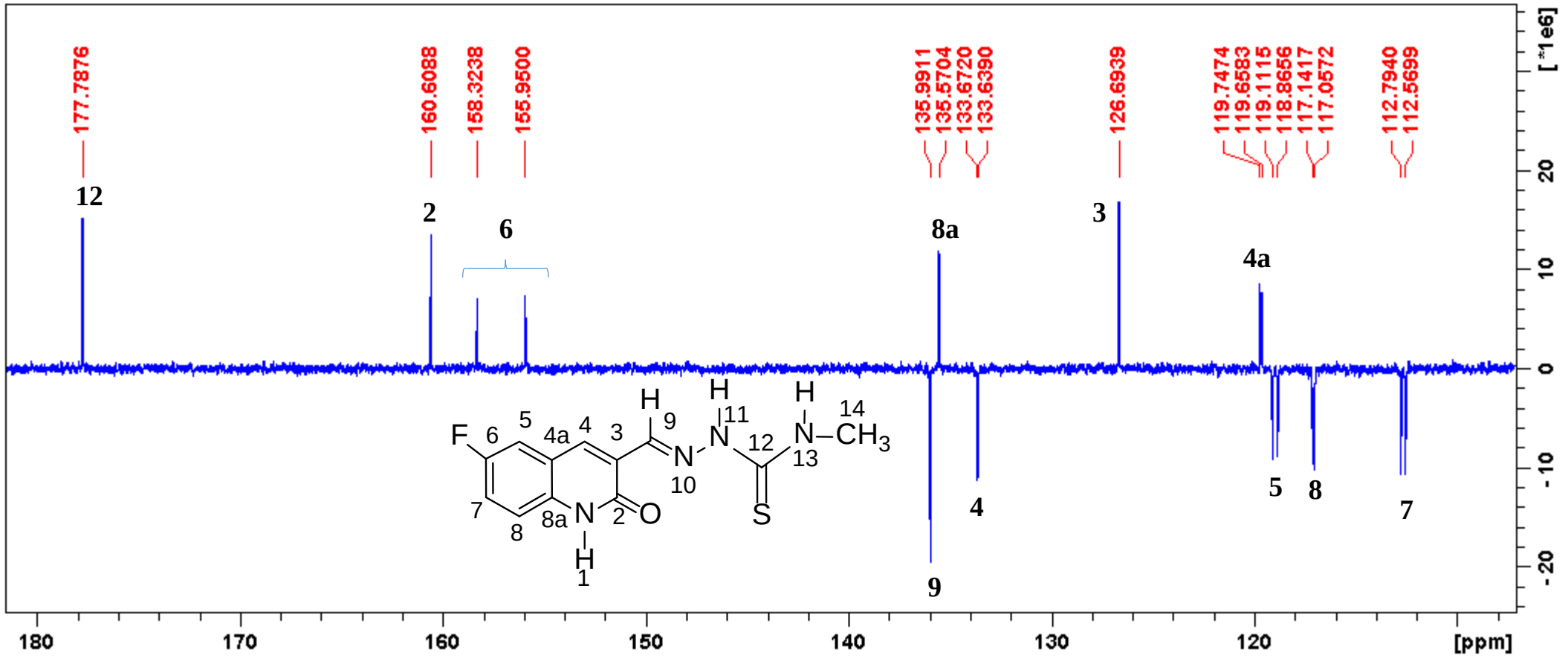
^{1}H NMR spectrum of compound 5j



^{1}H NMR spectrum of compound 5j (expanded)



^{13}C NMR spectrum of compound 5j



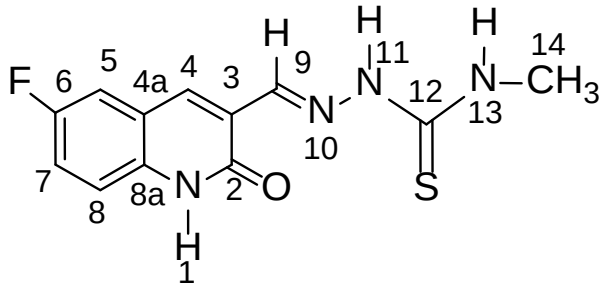
^{13}C NMR spectrum of compound 5j (expanded)

Elemental Composition Report

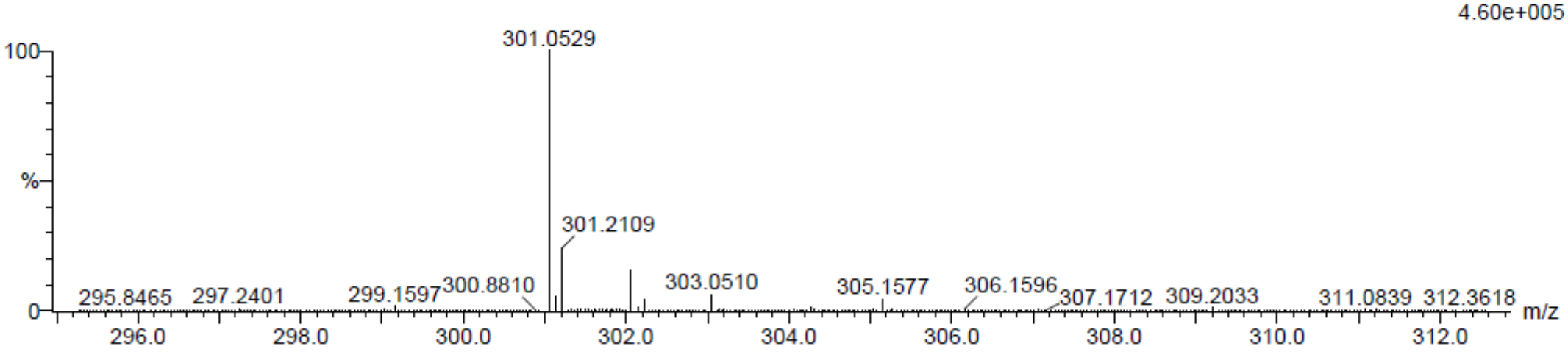
Single Mass Analysis

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
67 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 10-15 N: 1-5 O: 0-3 F: 1-2 Na: 0-1 S: 0-1

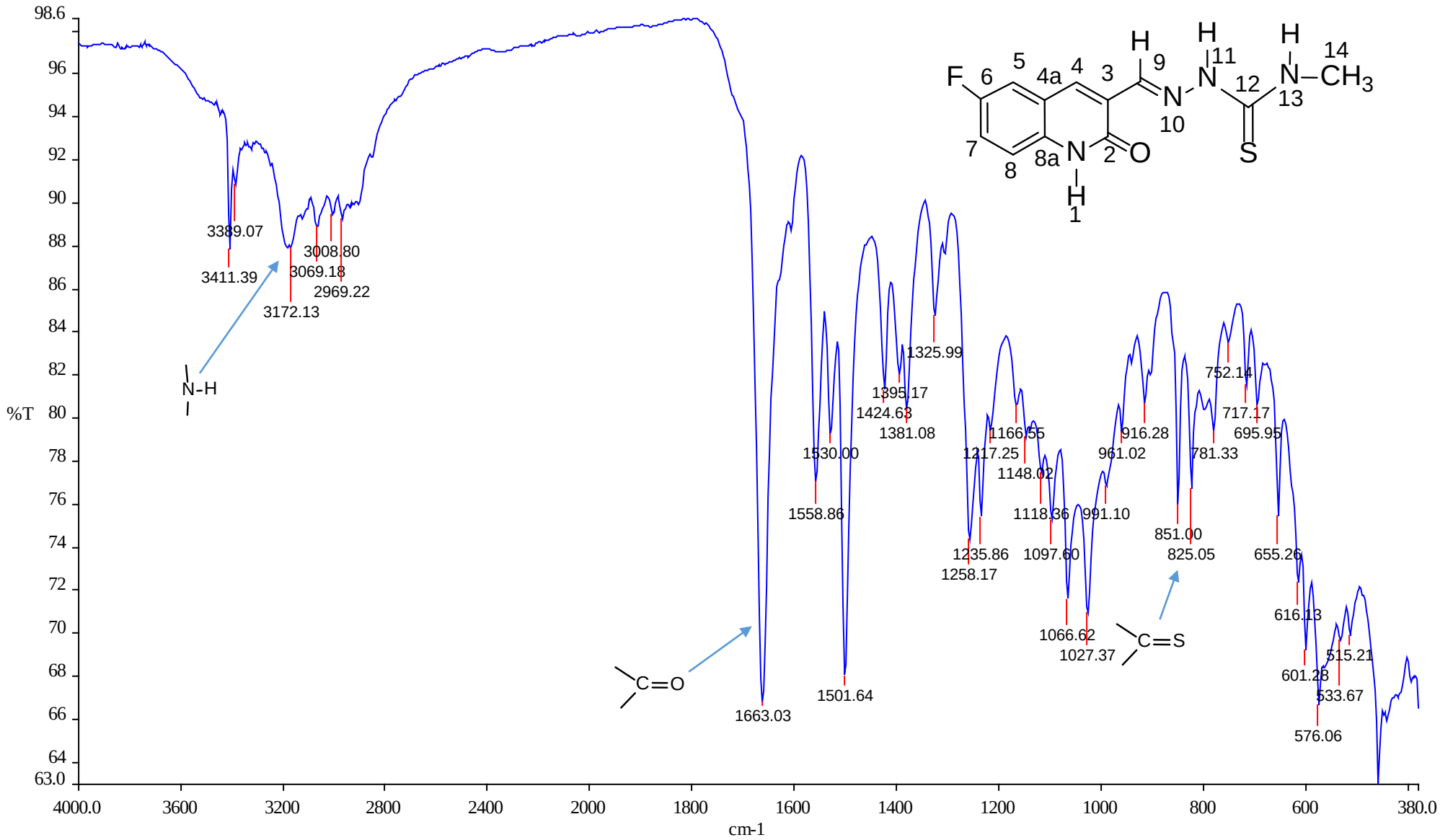


Compound 1e HG 52 (1.751) Cm (1:60)
TOF MS ES+

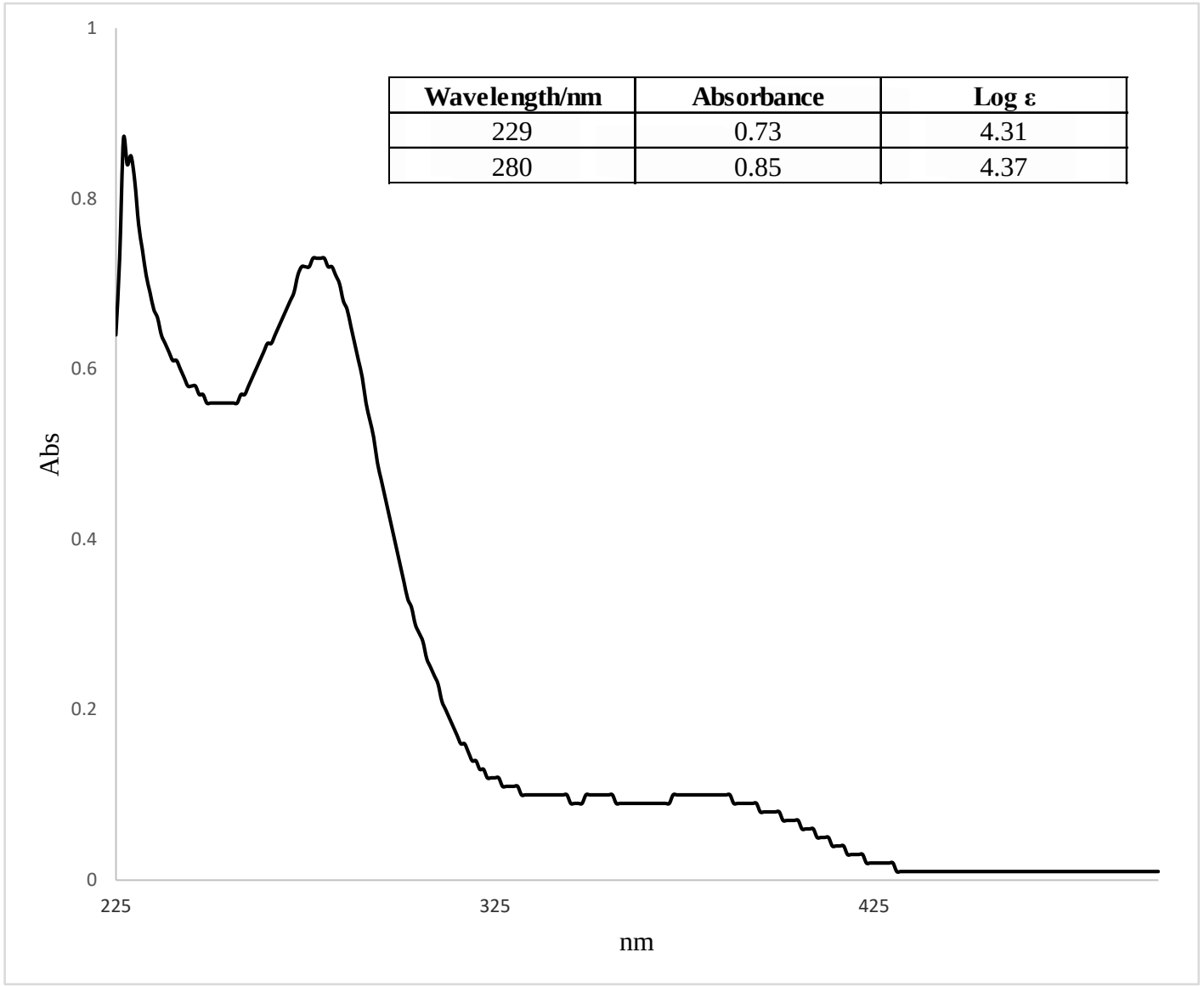


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
301.0529	301.0535	-0.6	-2.0	8.5	723.5	0.0	C12 H11 N4 O F Na S

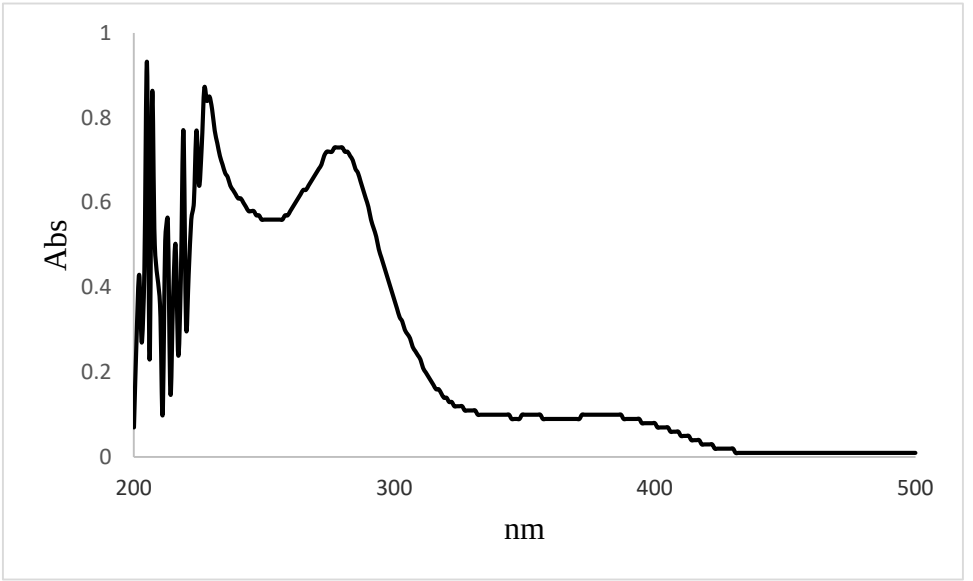
HRMS spectrum of compound 5j

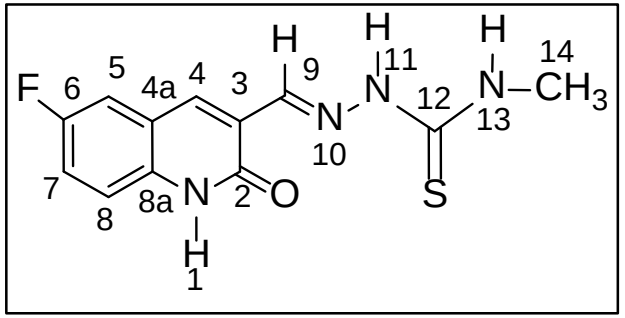


IR spectrum of compound 5j

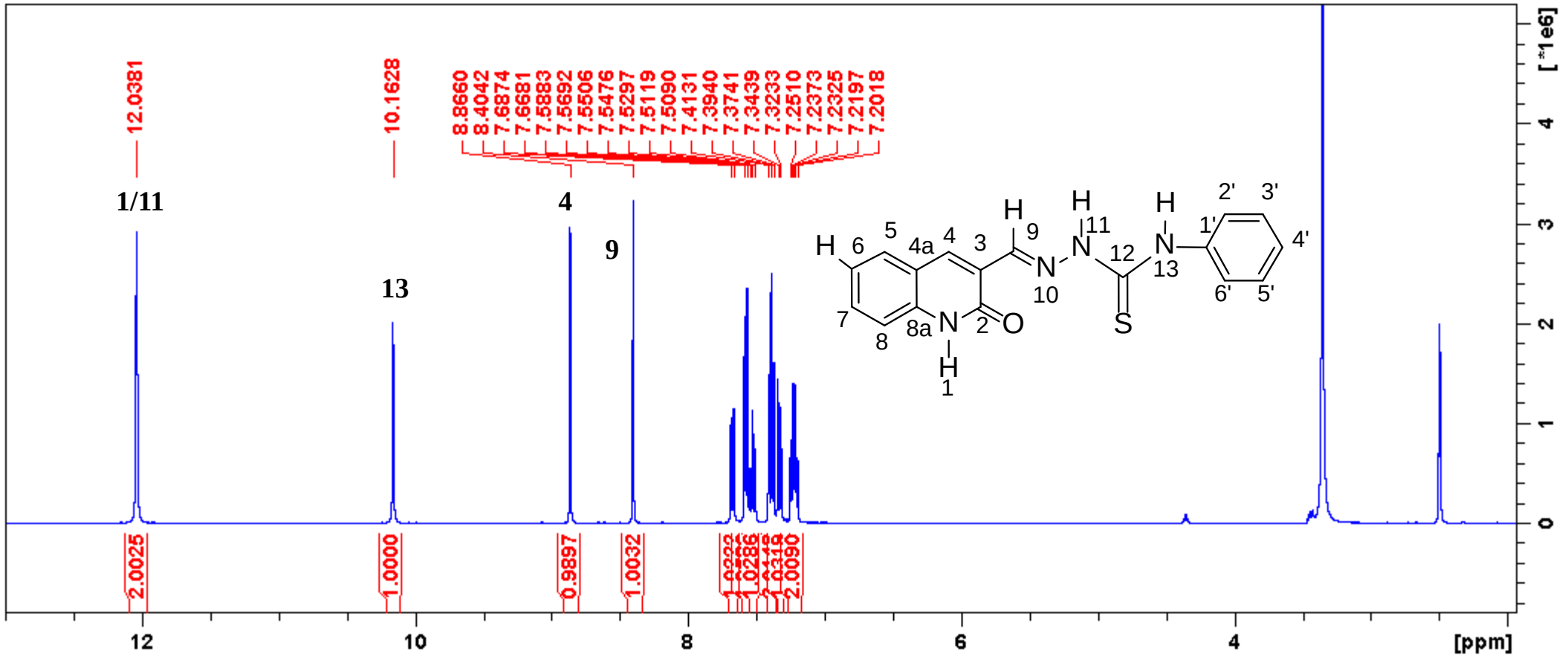


Wavelength/nm	Absorbance	Log ε
229	0.73	4.31
280	0.85	4.37

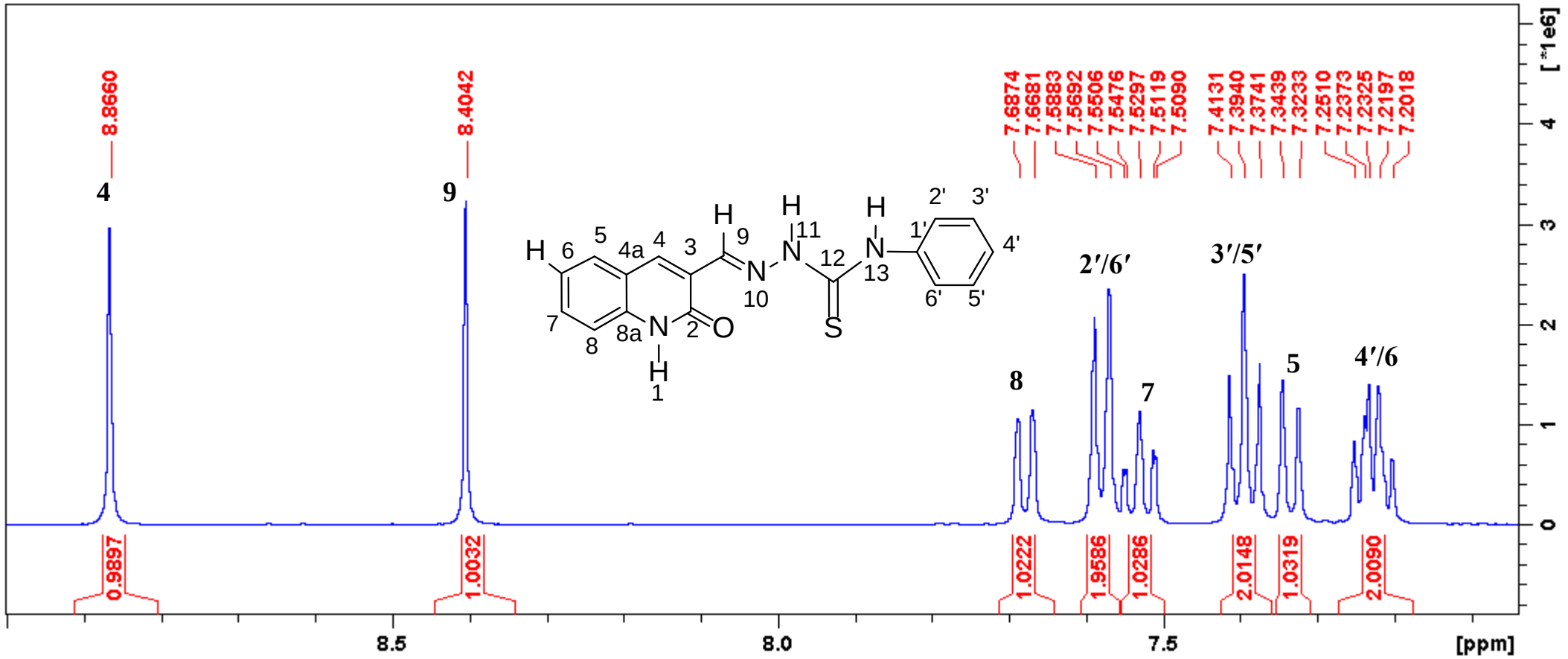




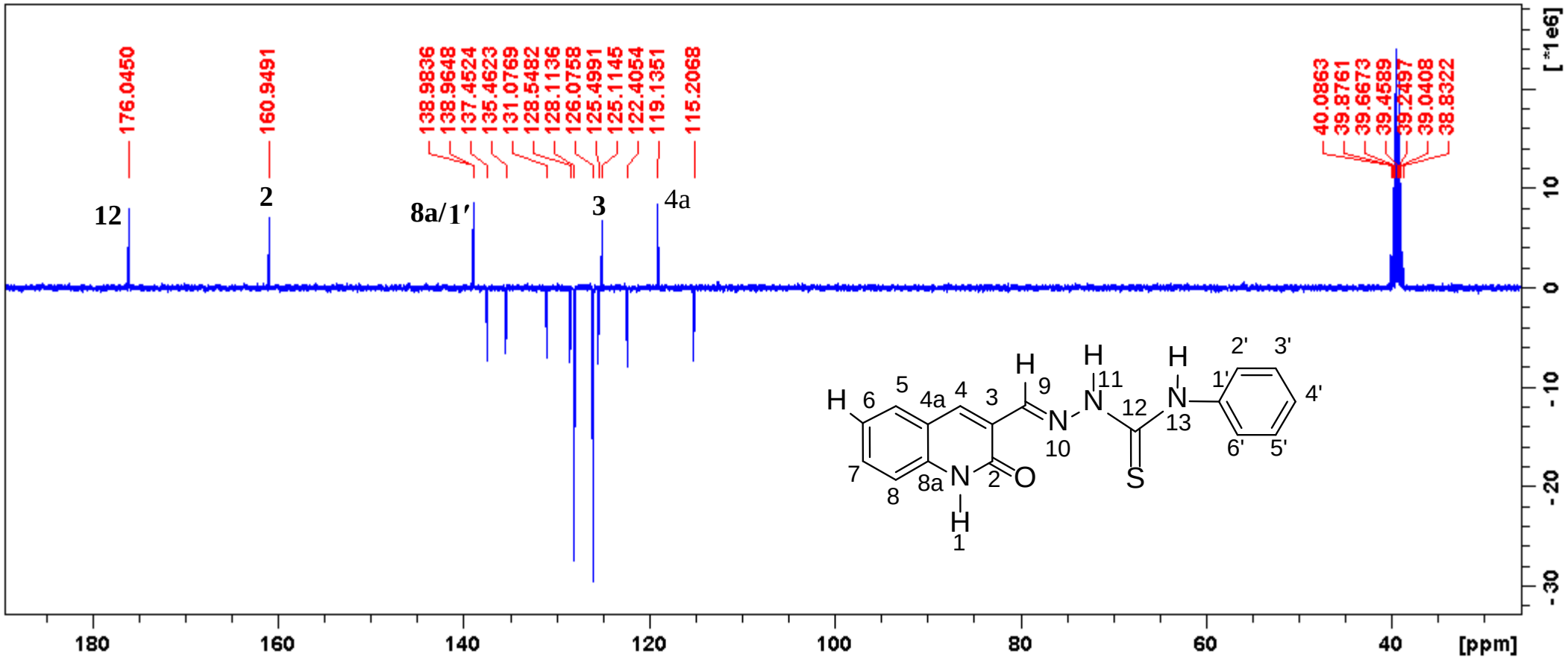
UV-Vis spectrum of compound 5j



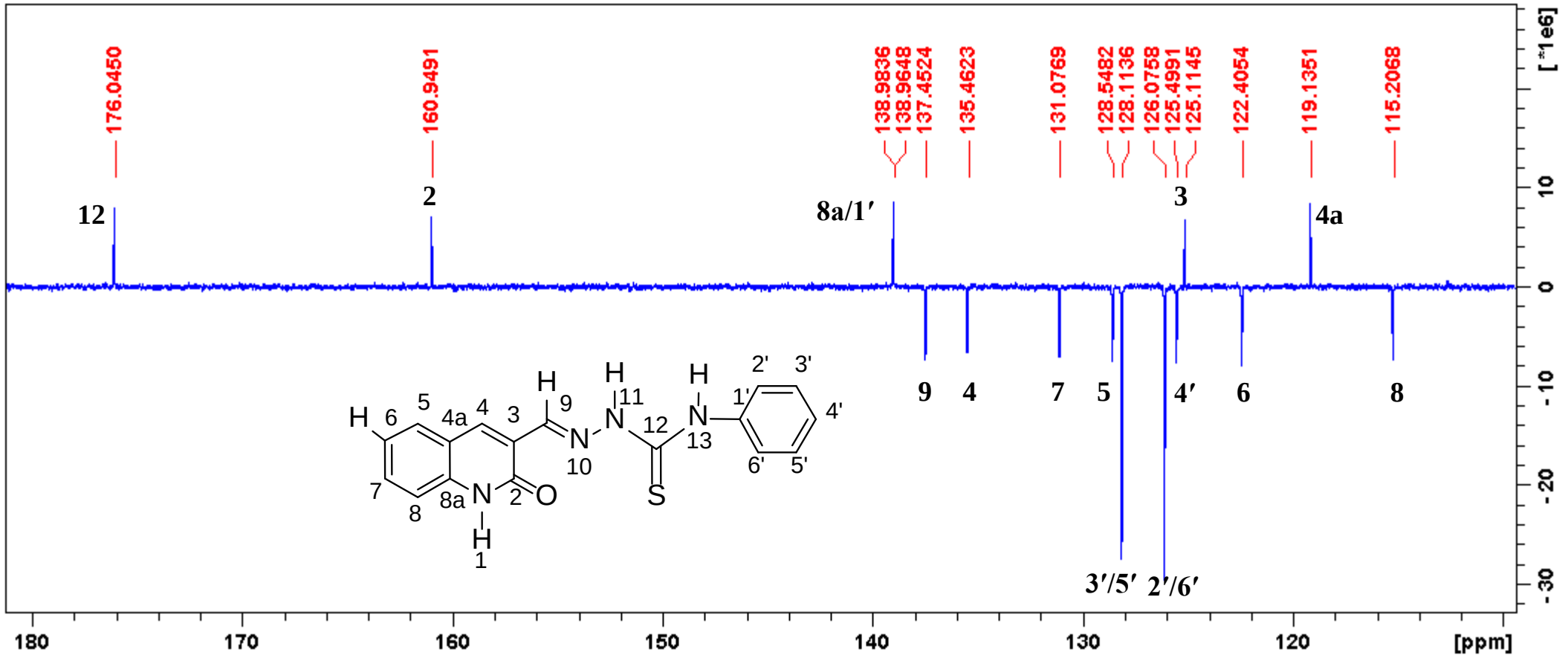
^{1}H NMR spectrum of compound 5k



^{1}H NMR spectrum of compound 5k (expanded)



^{13}C NMR spectrum of compound 5k



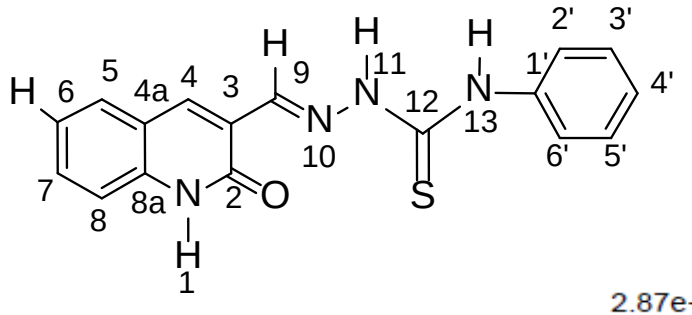
^{13}C NMR spectrum of compound 5k (expanded)

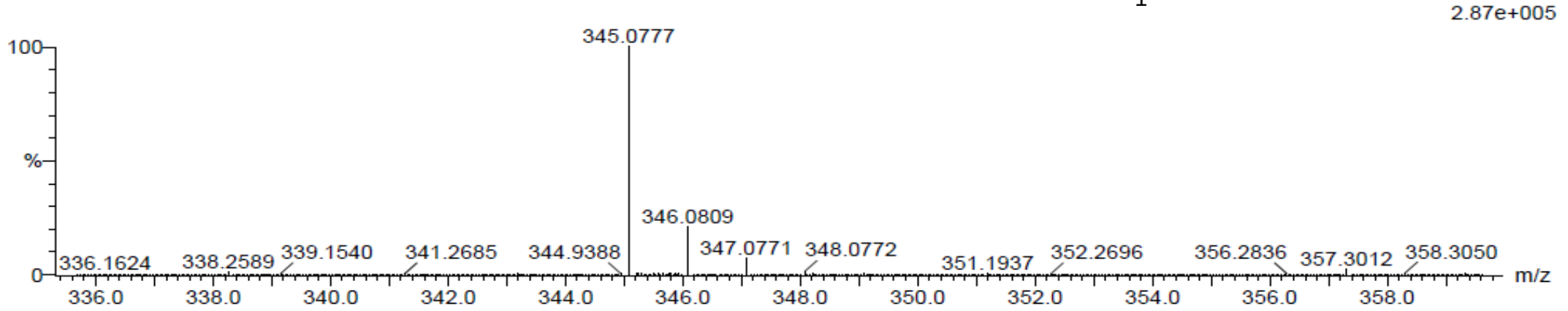
Single Mass Analysis

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
10 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 O: 1-1 Na: 0-1 S: 0-1

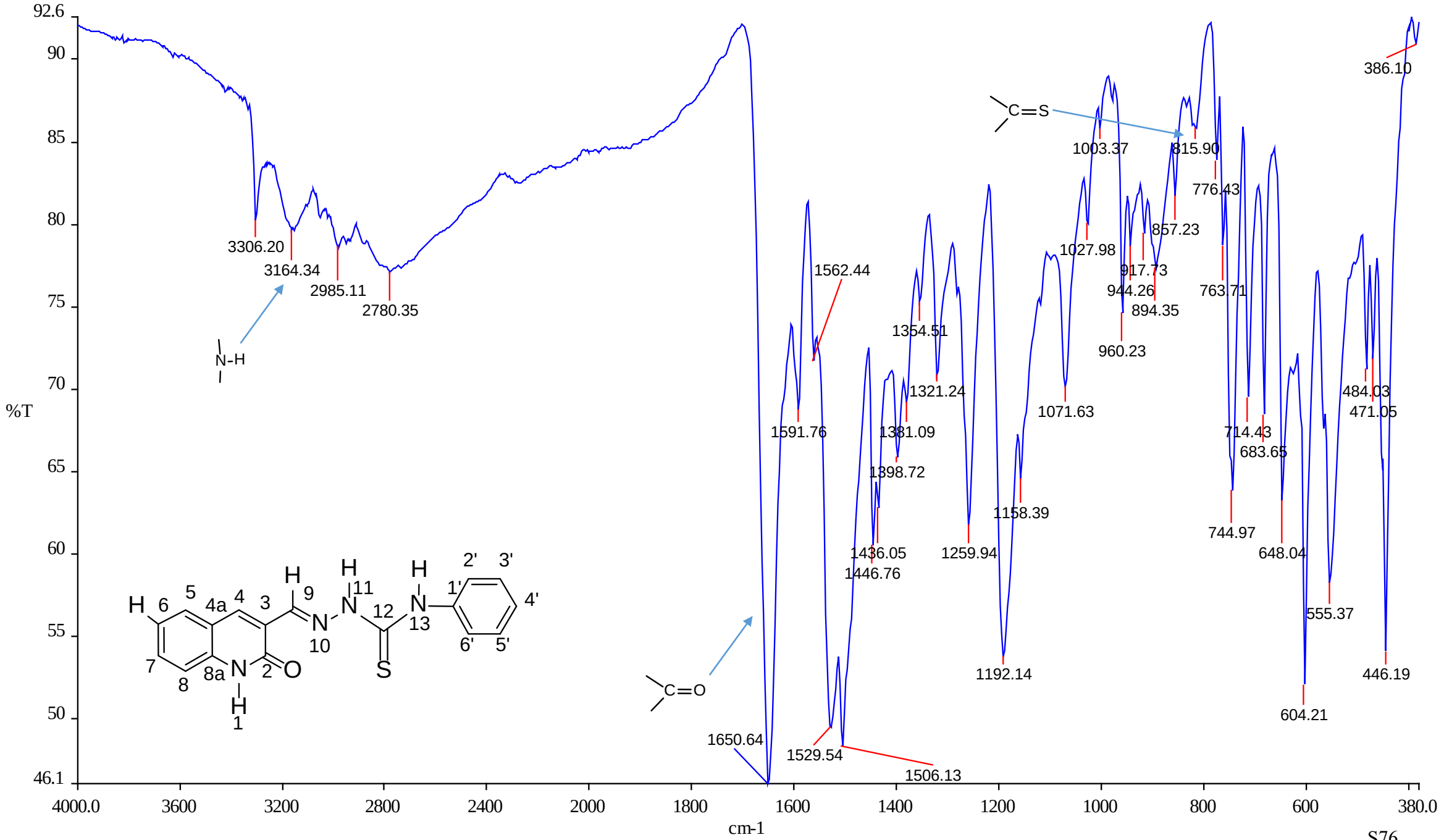
a8 HG 9 (0.270) Cm (1:61)
TOF MS ES+





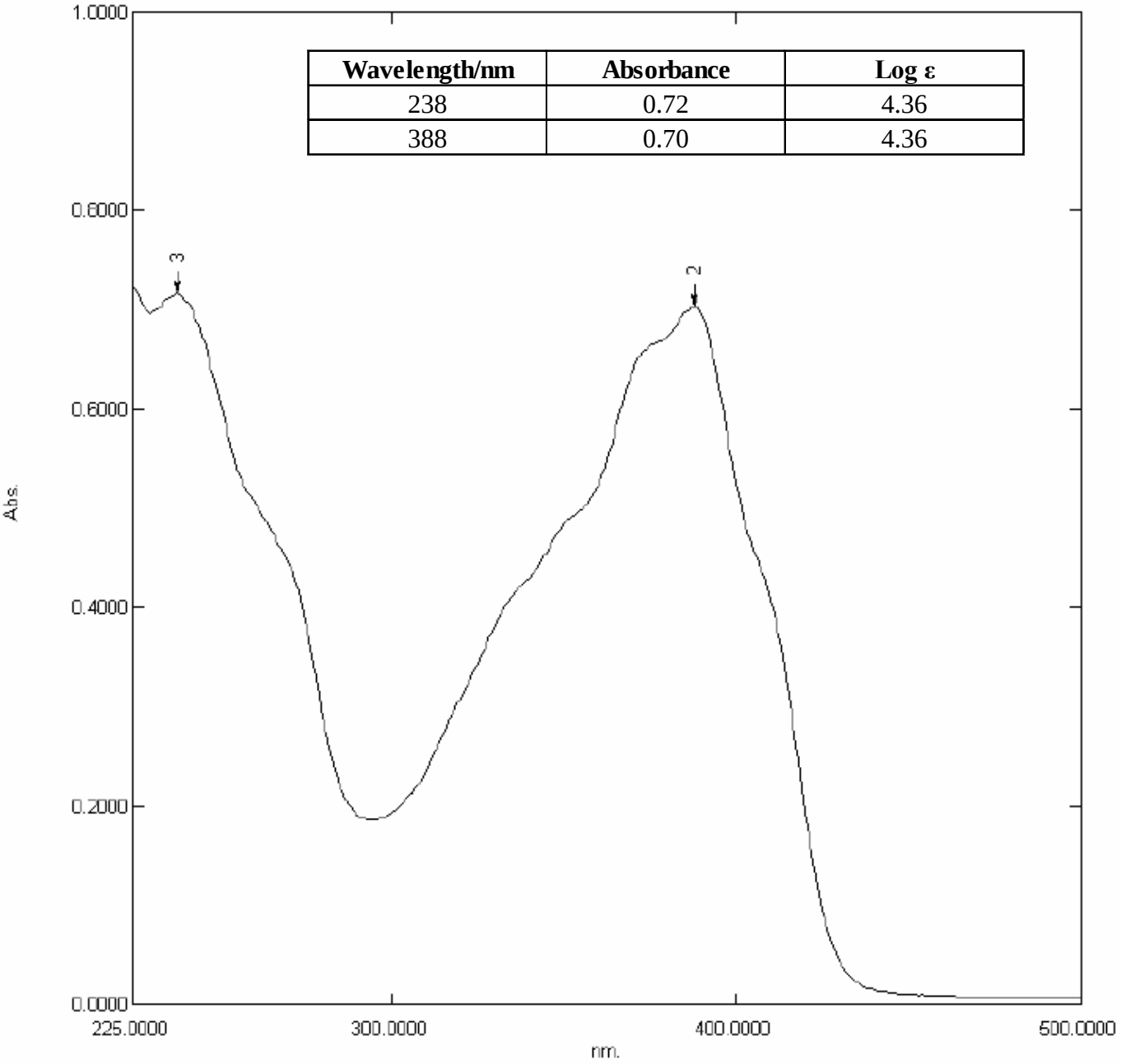
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
345.0777	345.0786	-0.9	-2.6	12.5	615.9	0.0	C17 H14 N4 O Na S

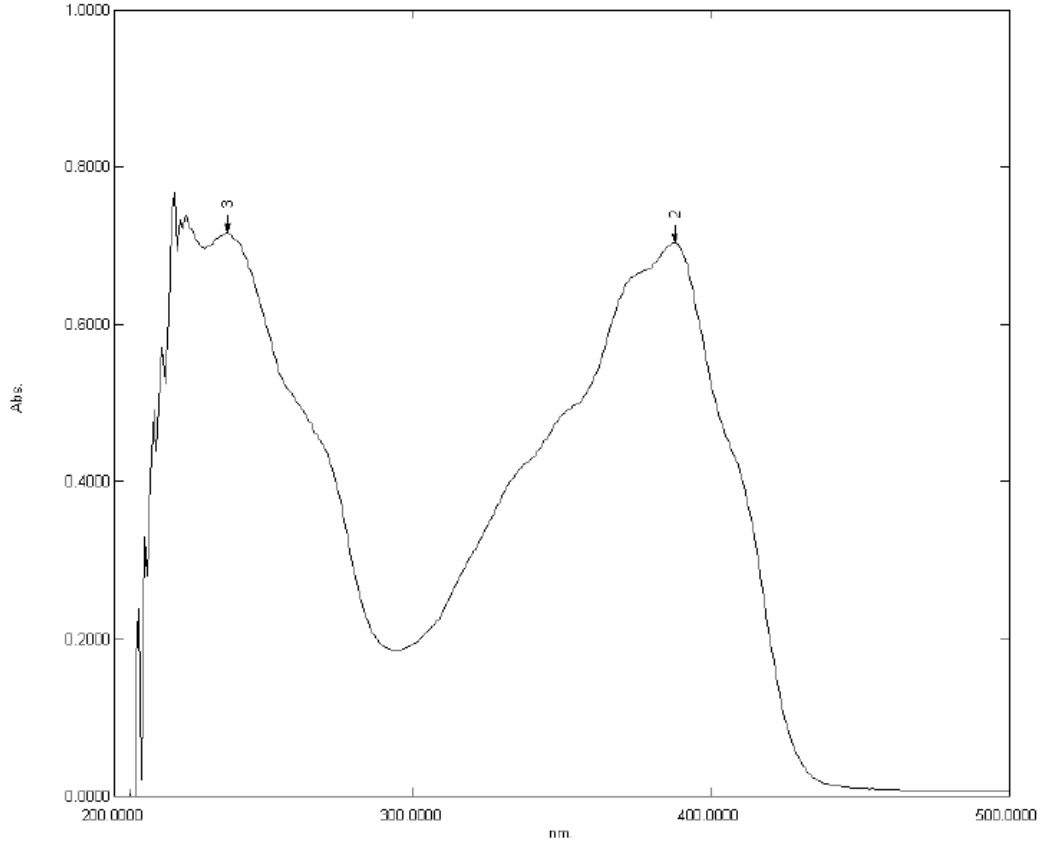
HRMS spectrum of compound 5k

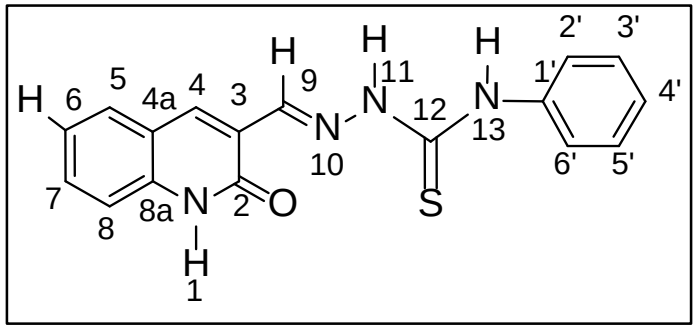


IR spectrum of compound 5k

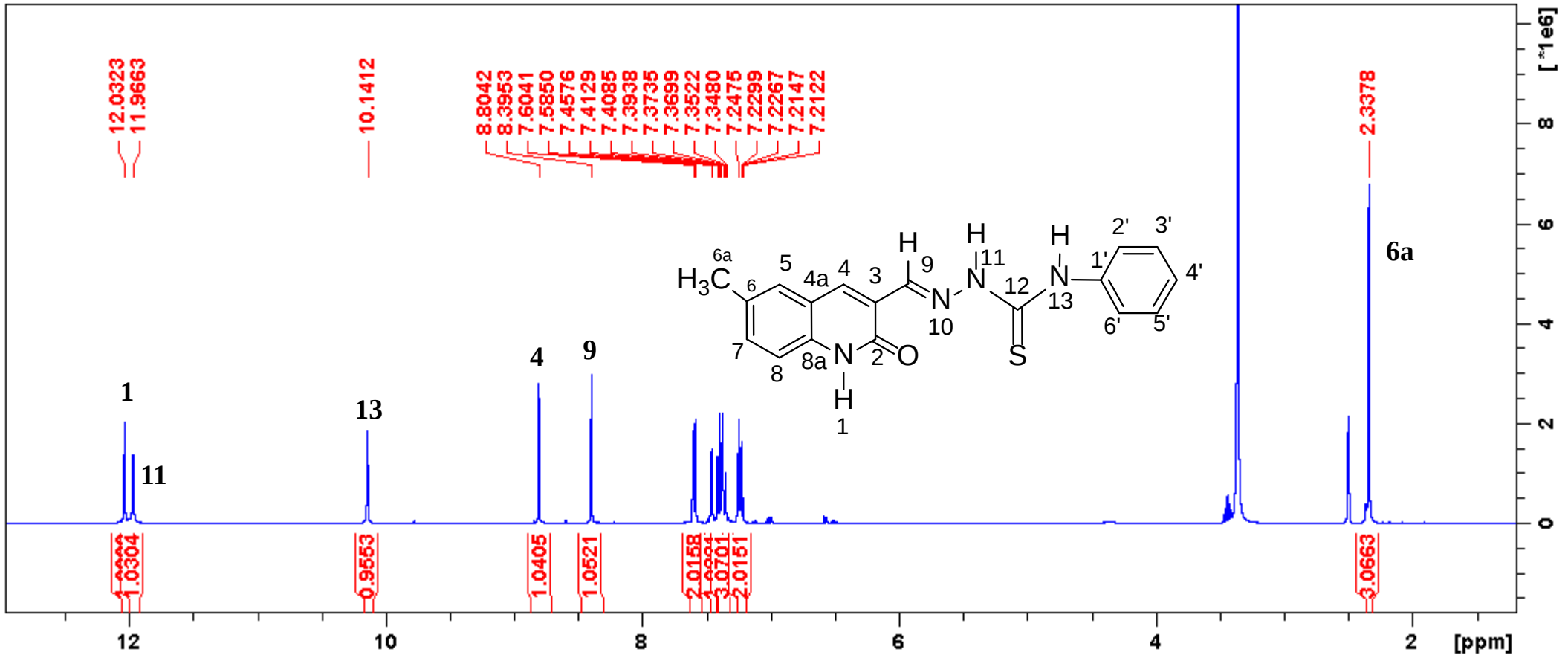
Wavelength/nm	Absorbance	Log ε
238	0.72	4.36
388	0.70	4.36



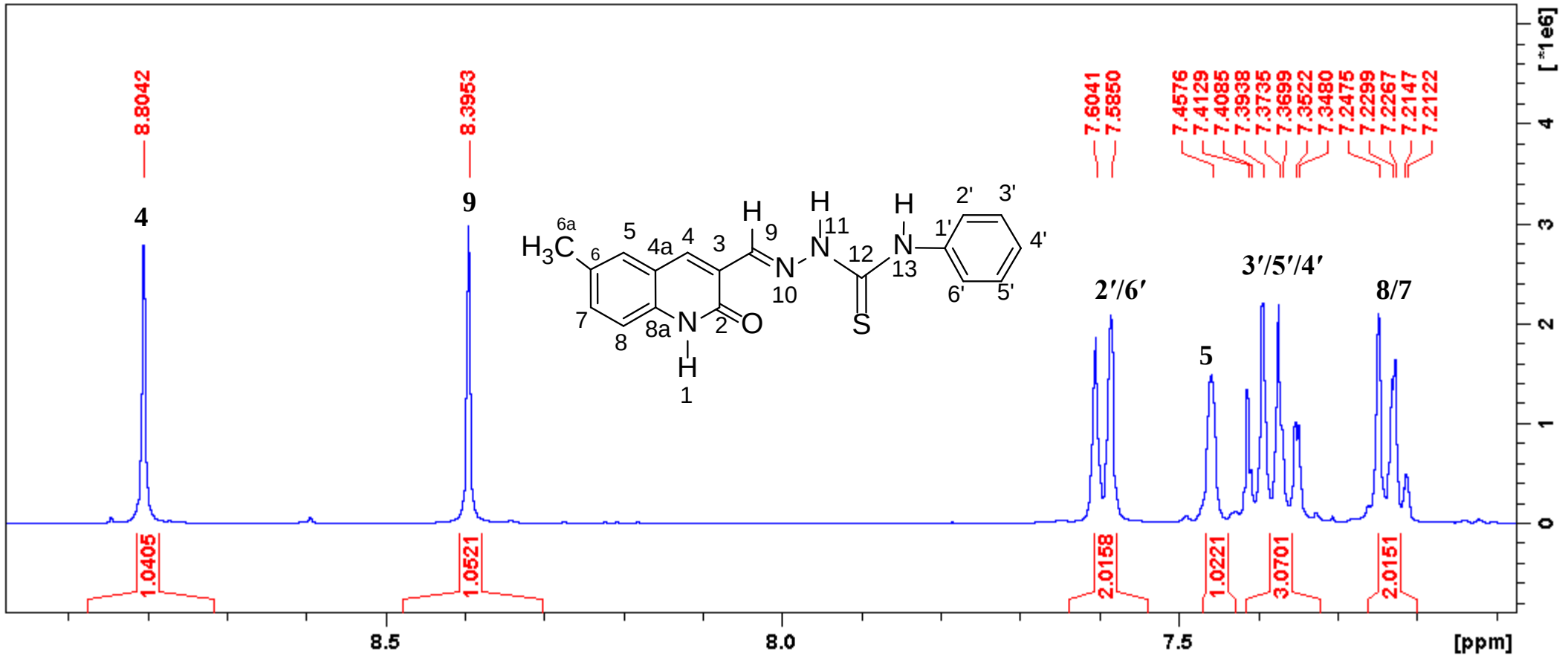




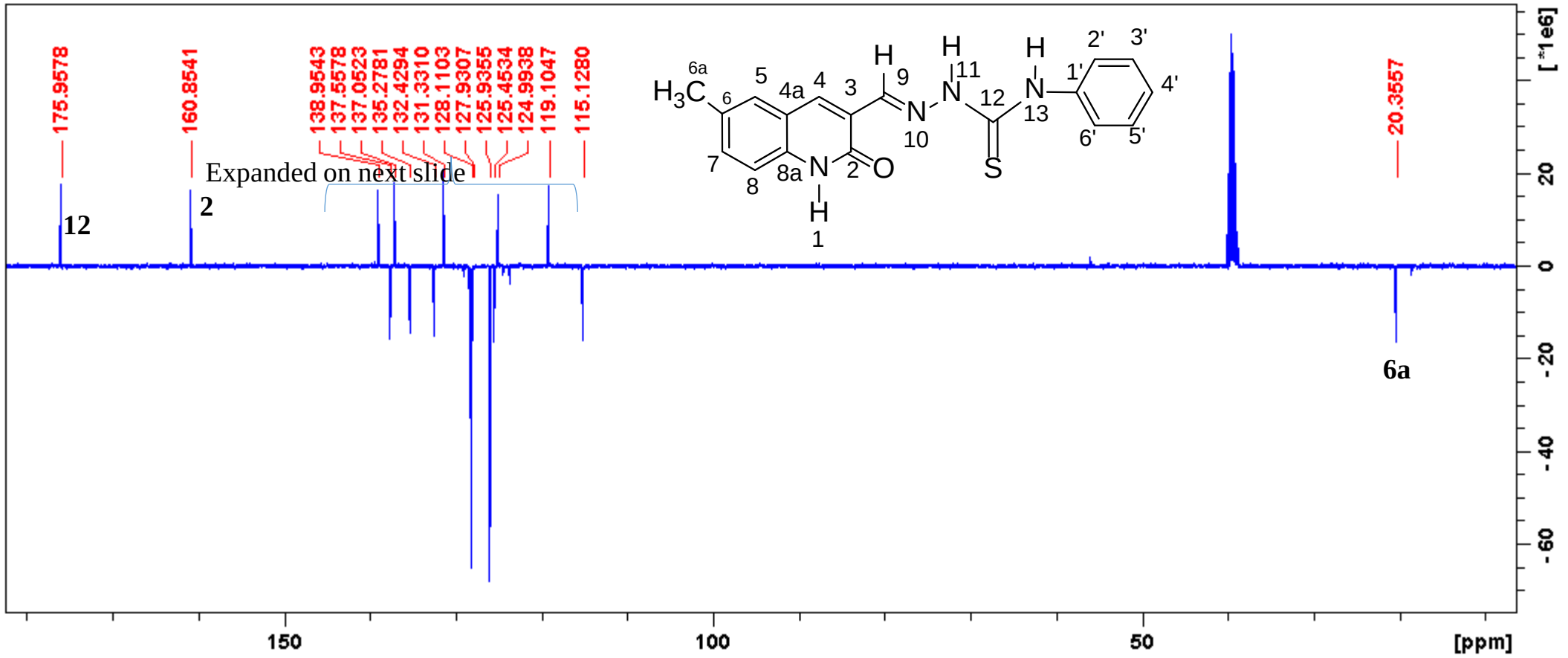
UV-Vis spectrum of compound 5k



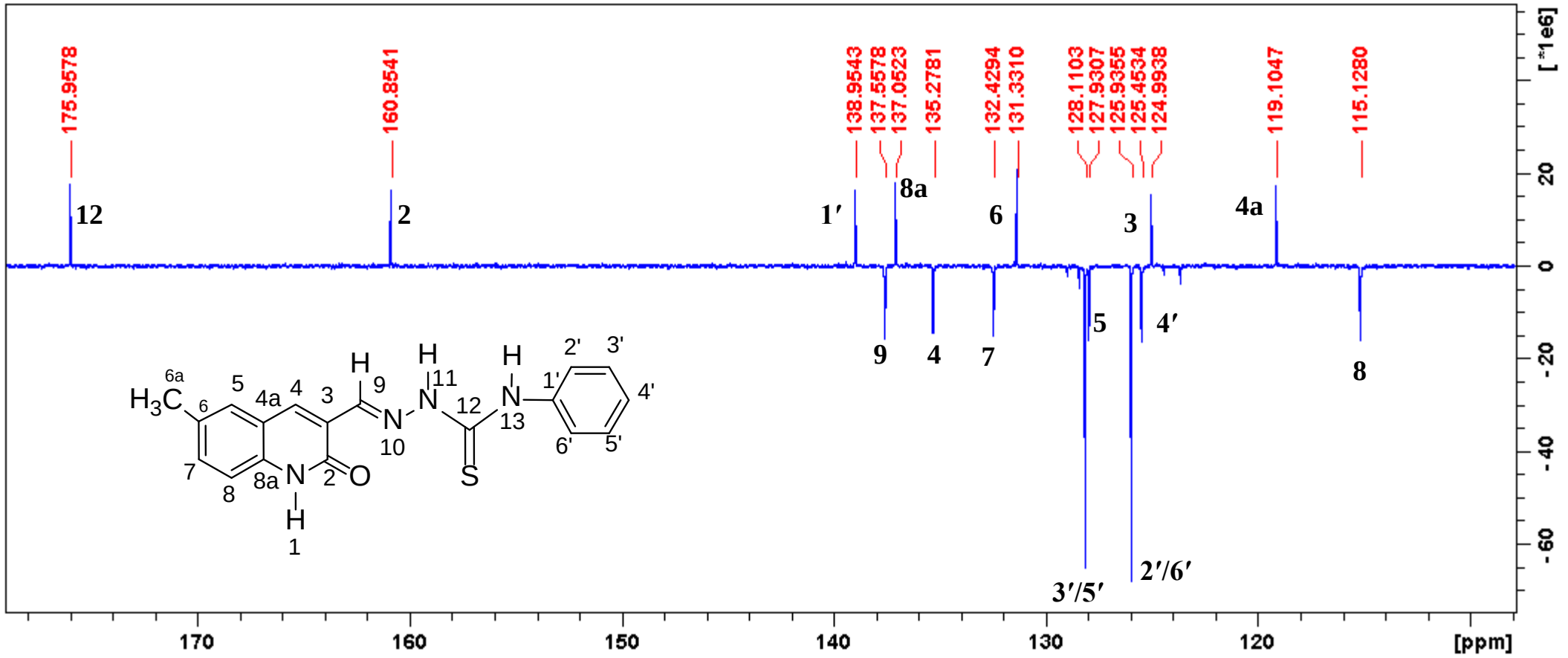
^{1}H NMR spectrum of compound 5l



^{1}H NMR spectrum of compound 5l (expanded)



^{13}C NMR spectrum of compound 5l

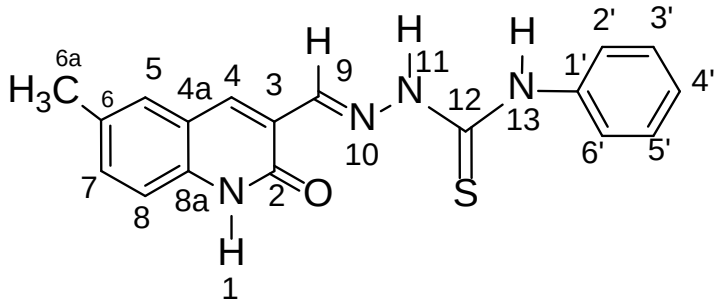


^{13}C NMR spectrum of compound 5l (expanded)

Elemental Composition Report

Single Mass Analysis

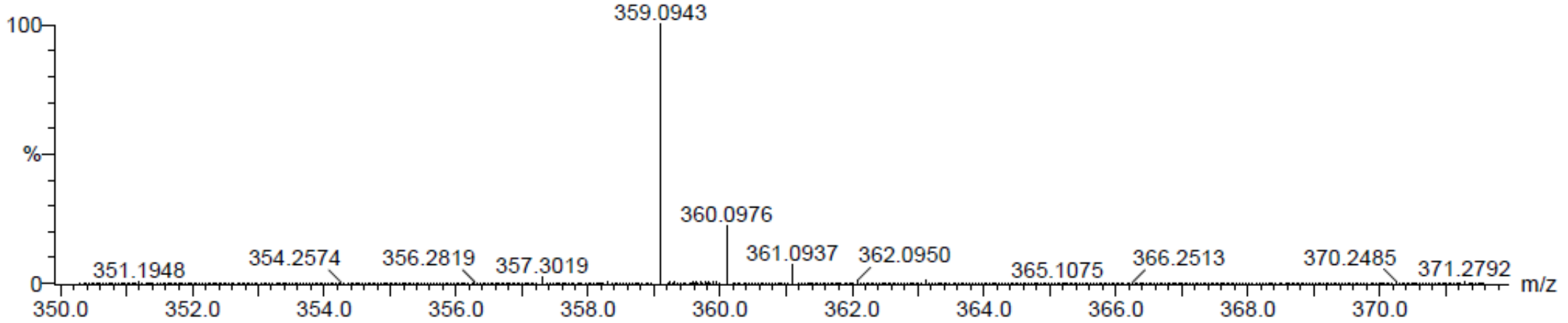
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
10 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 O: 1-1 Na: 0-1 S: 0-1

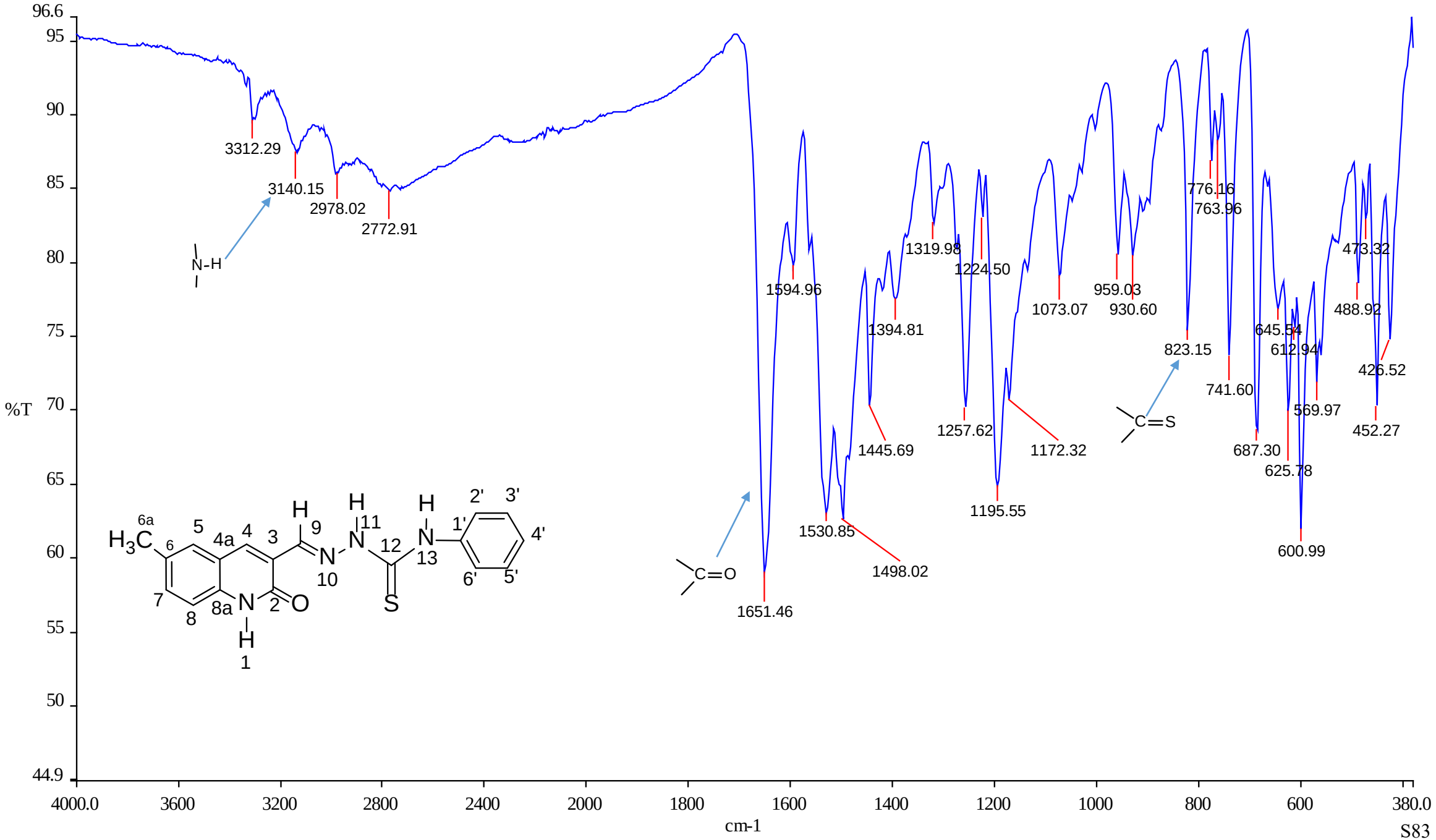
a4 HG 54 (1.790) Cm (1:61)
TOF MS ES+

2.78e+005



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
359.0943	359.0943	0.0	0.0	12.5	619.6	0.0	C18 H16 N4 O Na S

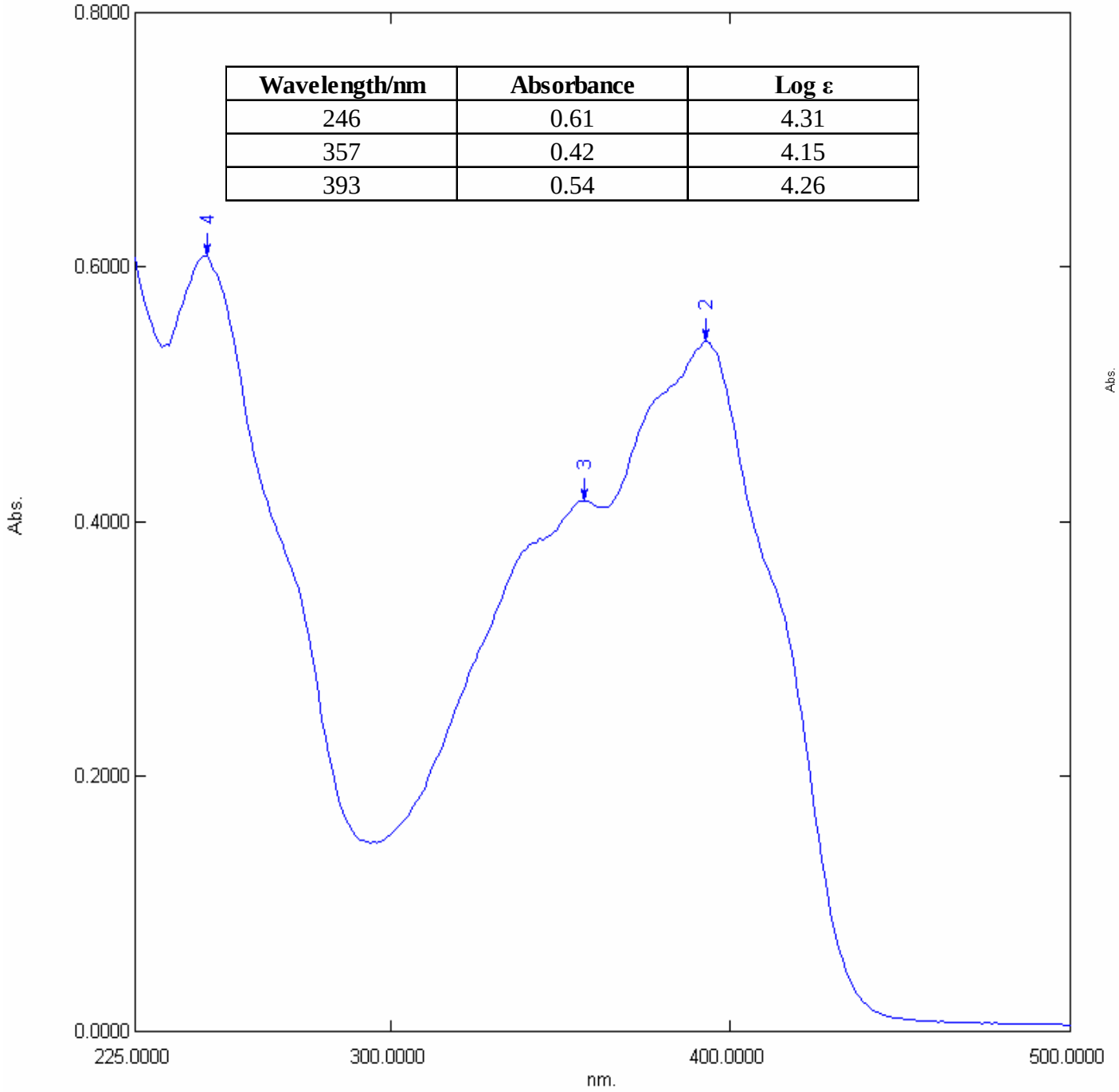
HRMS spectrum of compound 5l

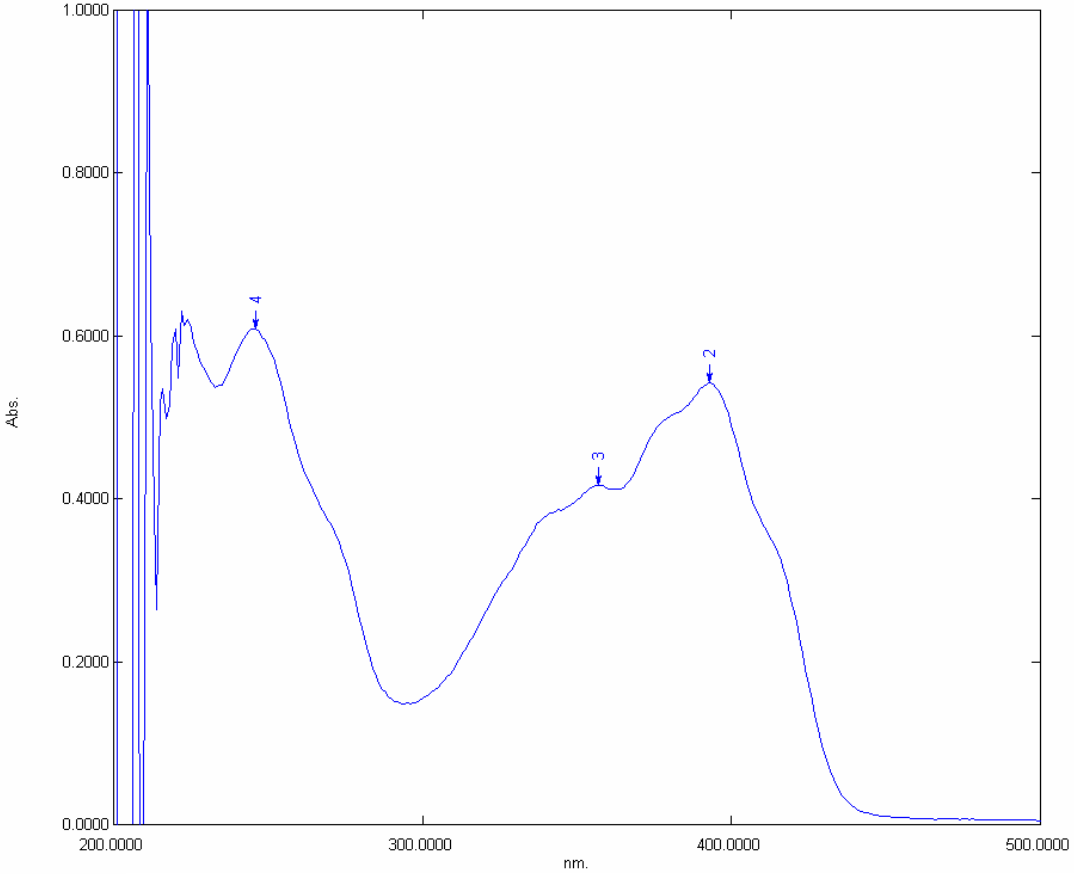


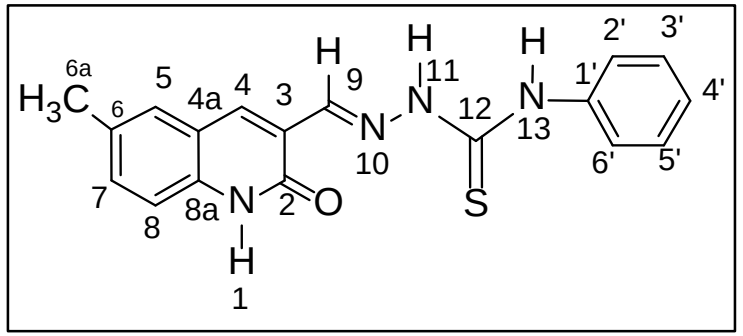
S83

IR spectrum of compound 5l

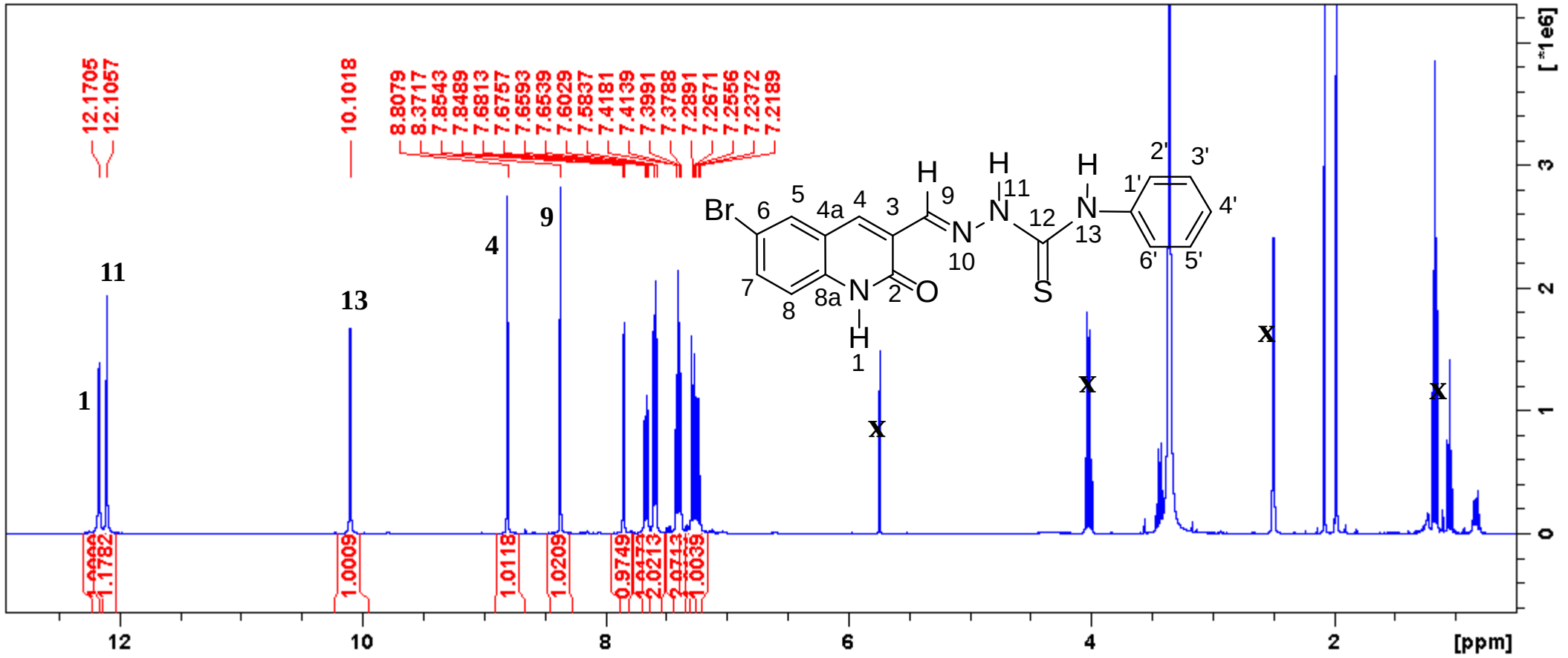
Wavelength/nm	Absorbance	Log ε
246	0.61	4.31
357	0.42	4.15
393	0.54	4.26



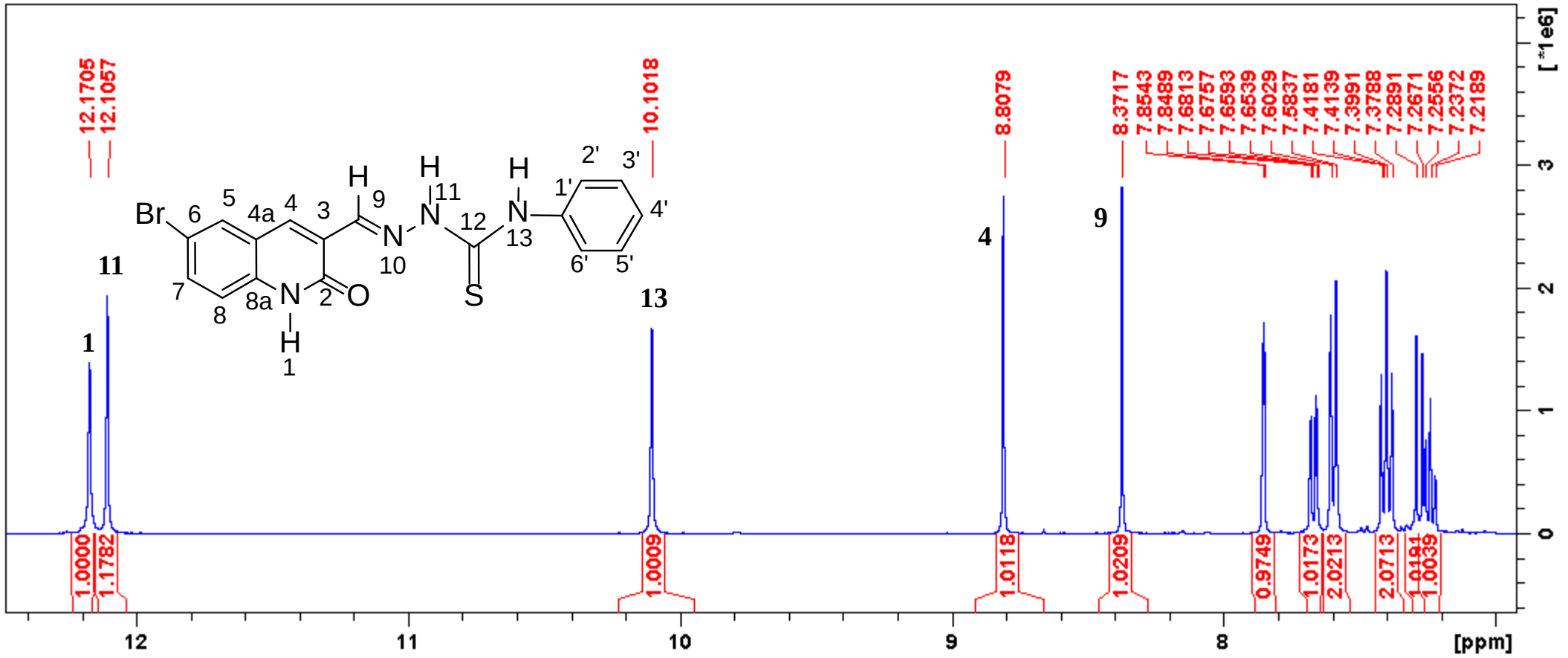




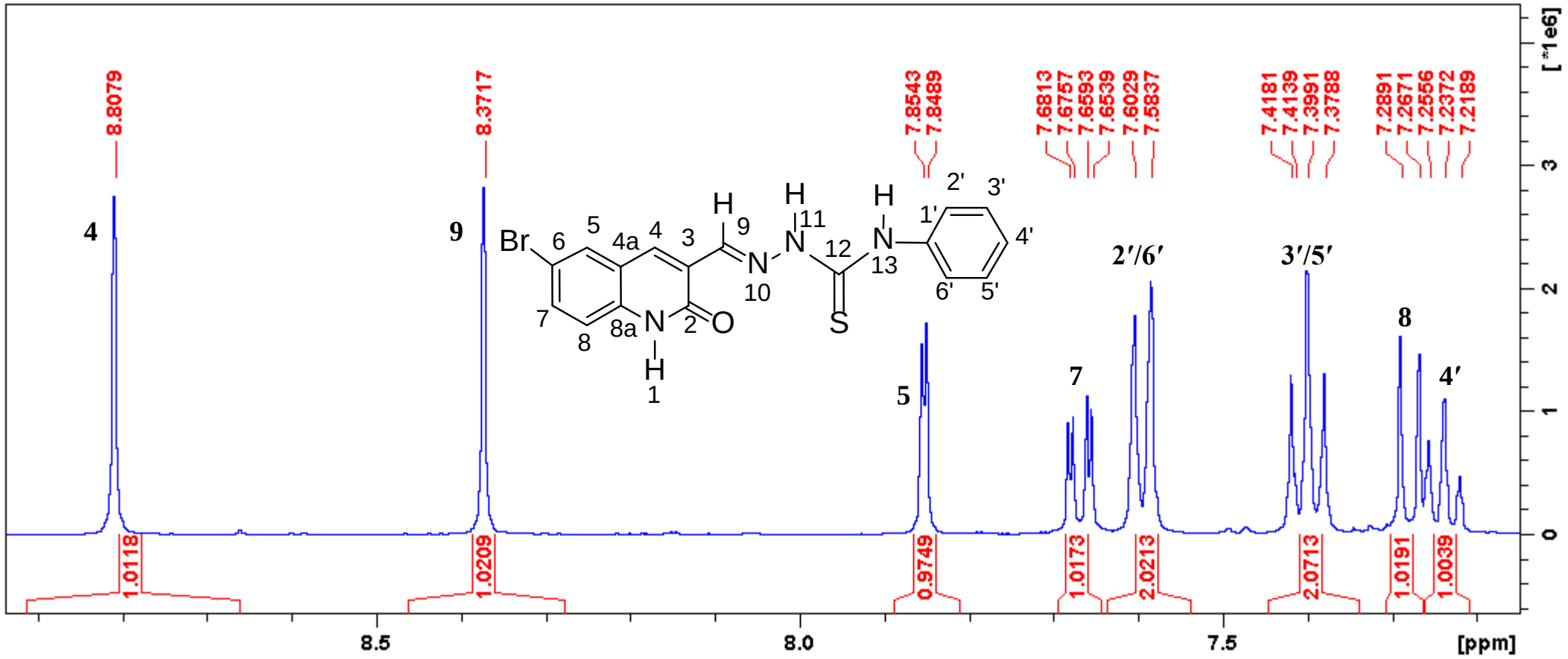
UV-Vis spectrum of compound 5l



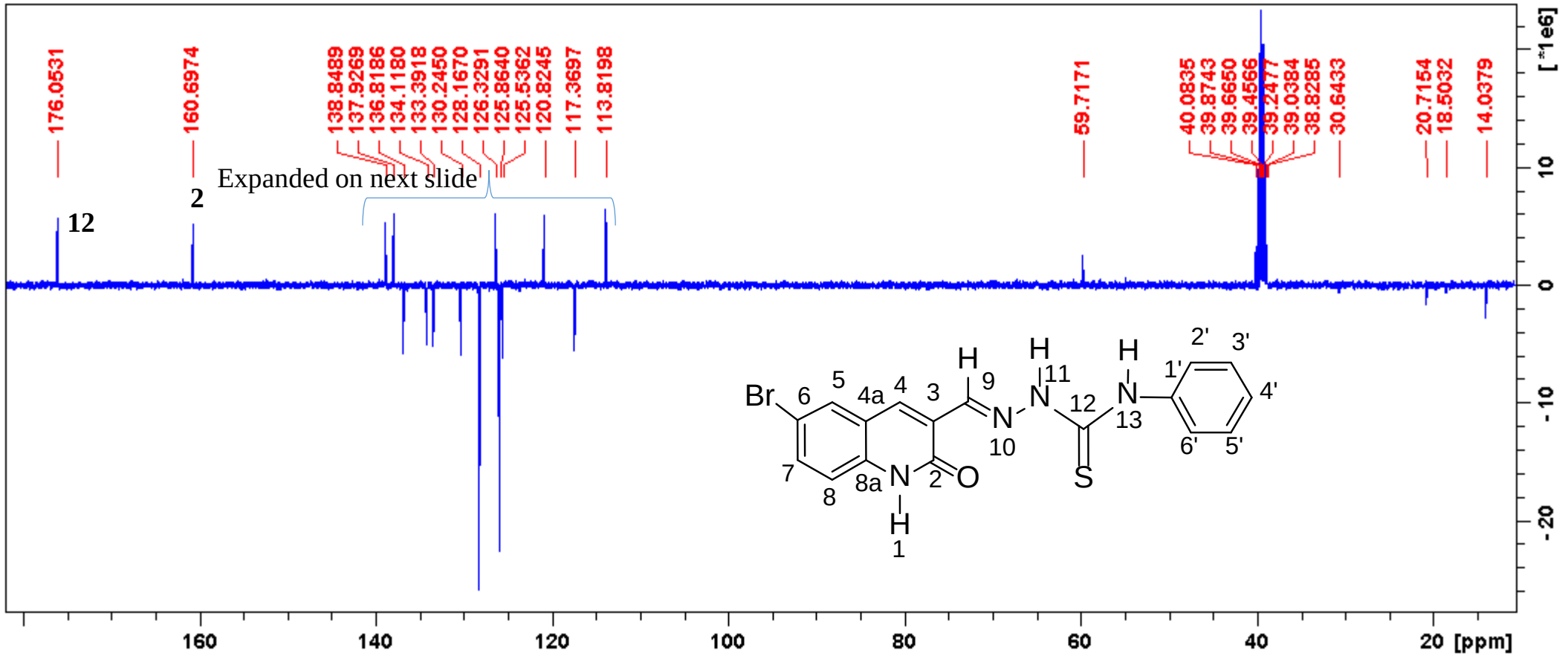
^{1}H NMR spectrum of compound 5m



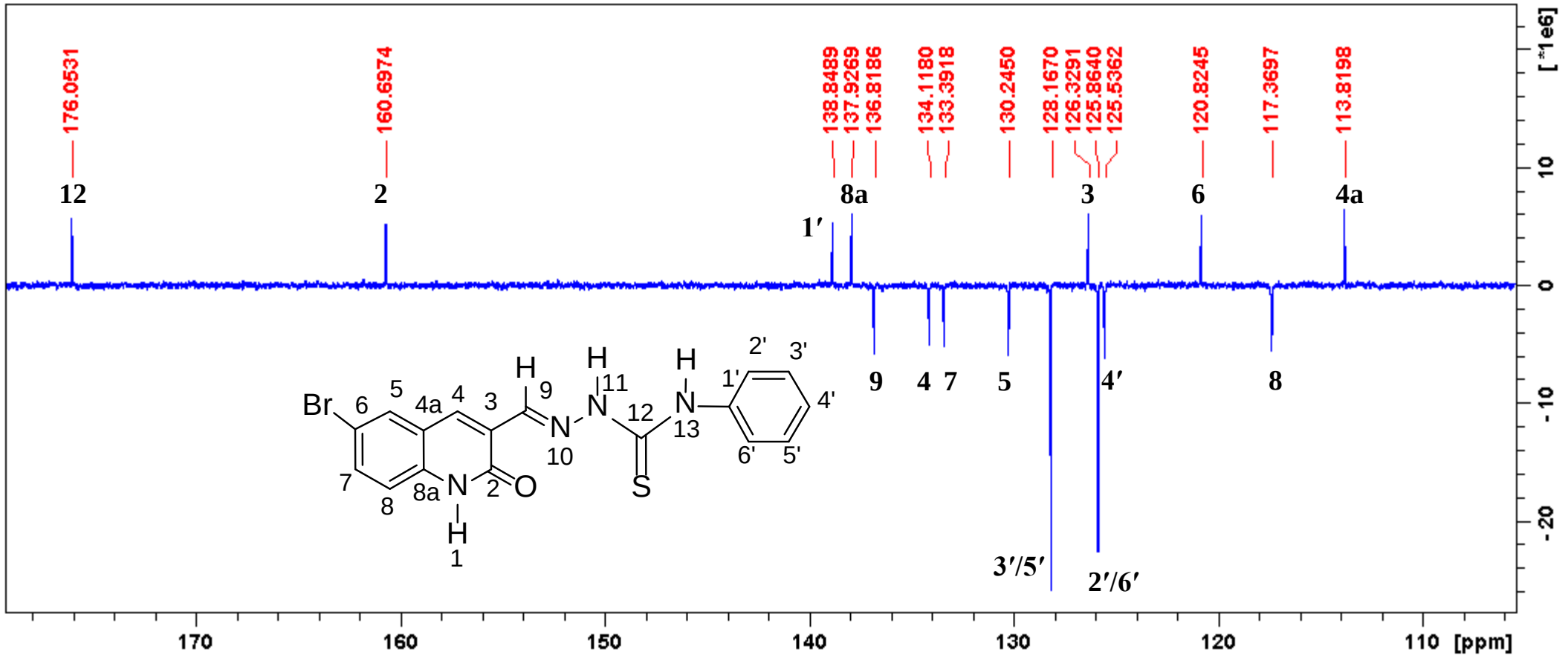
^{1}H NMR spectrum of compound 5m



^{1}H NMR spectrum of compound 5m (expanded)



^{13}C NMR spectrum of compound 5m

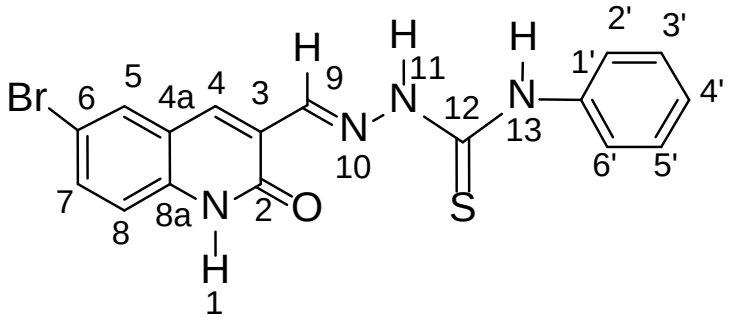


^{13}C NMR spectrum of compound 5m (expanded)

Elemental Composition Report

Single Mass Analysis

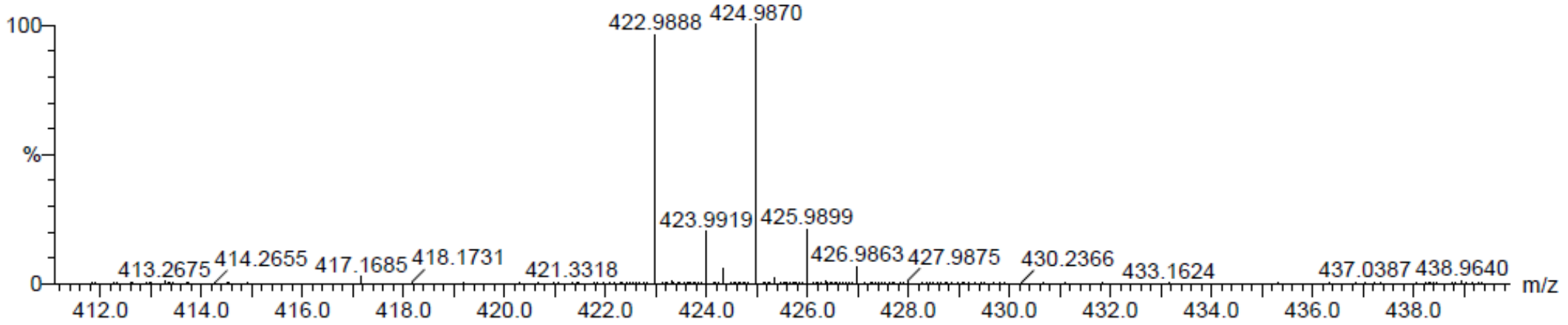
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



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: 10-15 N: 0-5 O: 1-1 Na: 0-1 S: 0-1 Br: 0-1

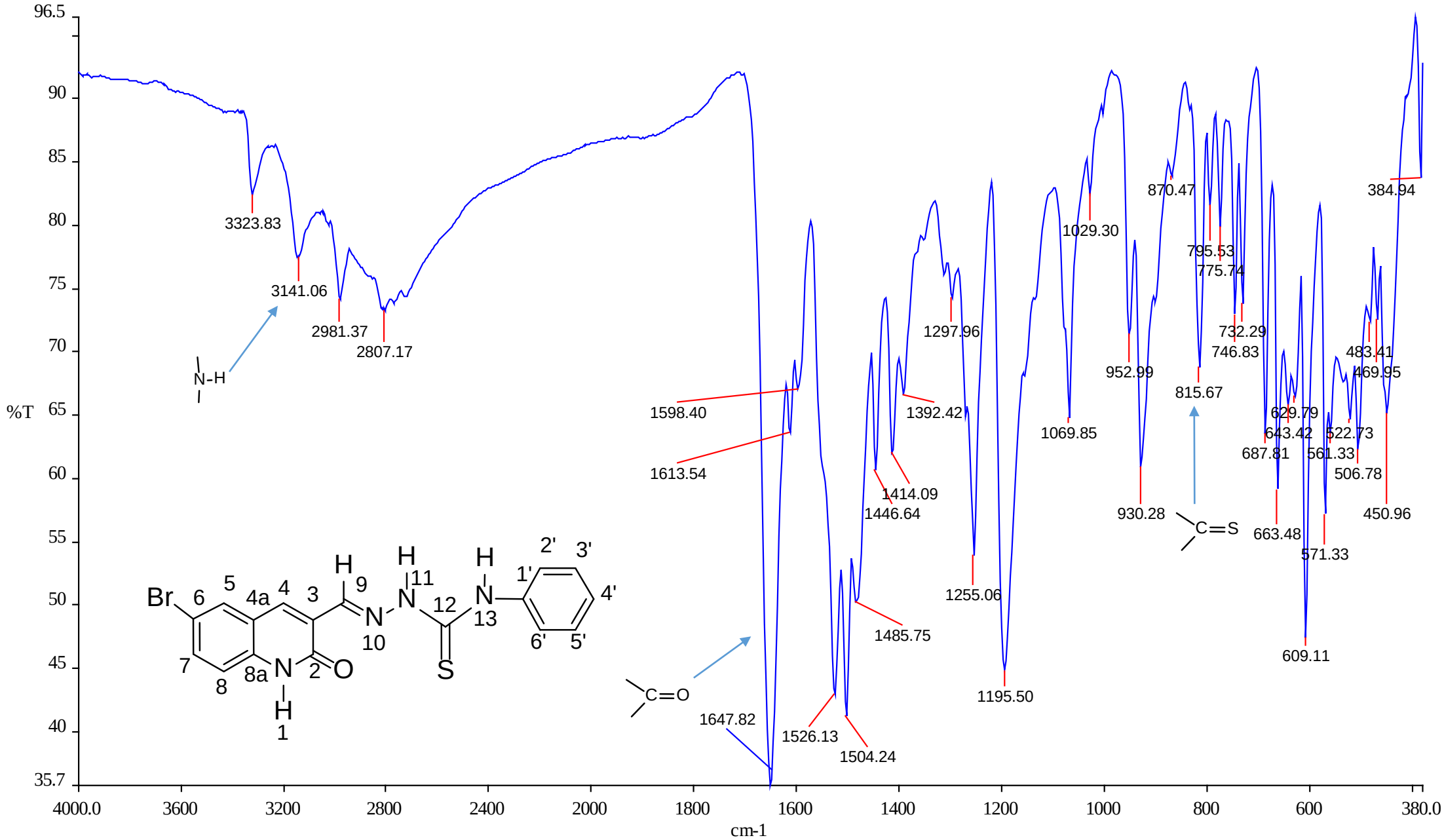
a2 HG 4 (0.102) Cm (1:61)
TOF MS ES+

1.94e+005



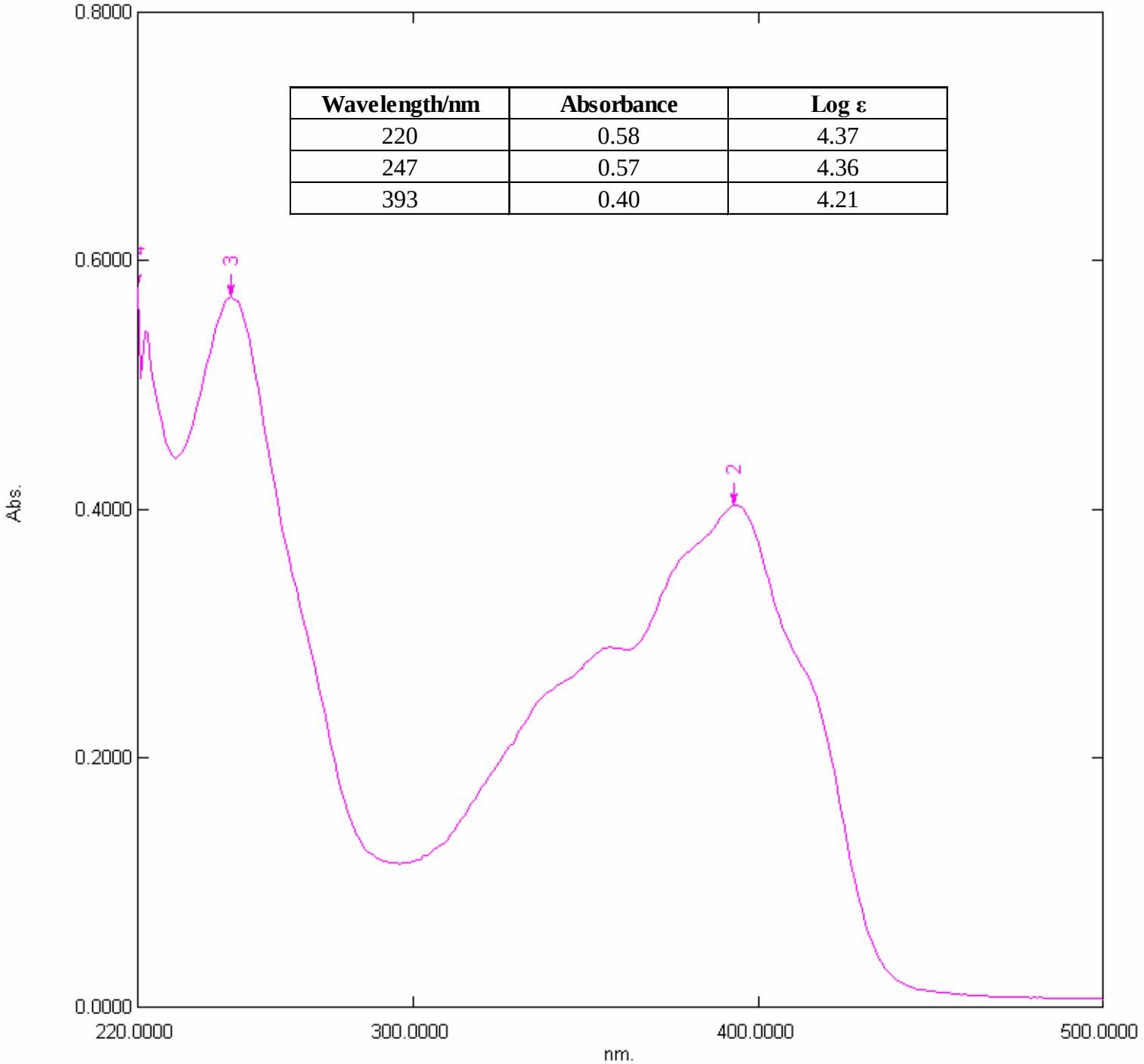
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
422.9888	422.9891	-0.3	-0.7	12.5	494.8	0.0	C17 H13 N4 O Na S Br

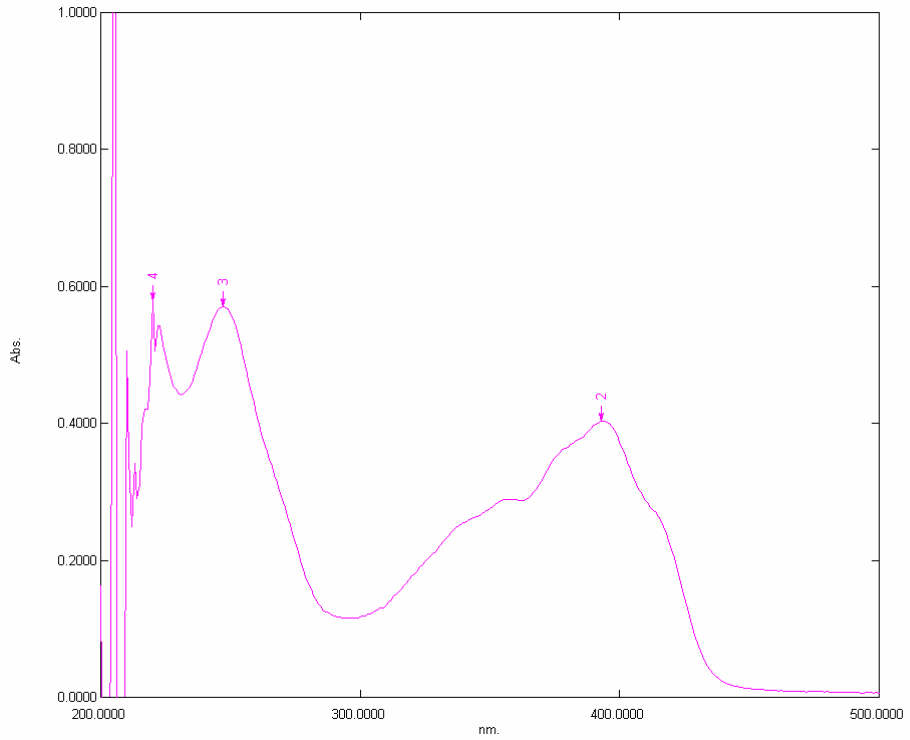
IR spectrum of compound 5m

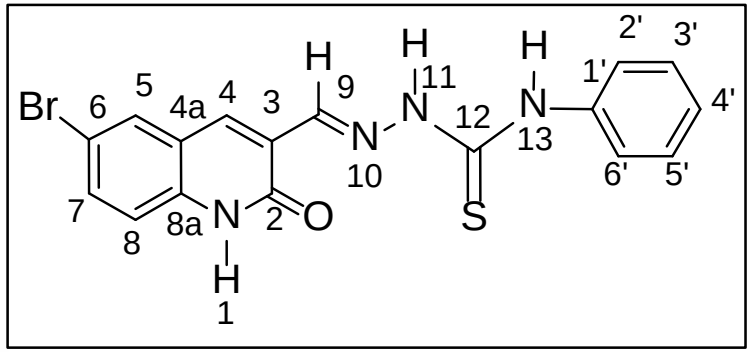


IR spectrum of compound 5m

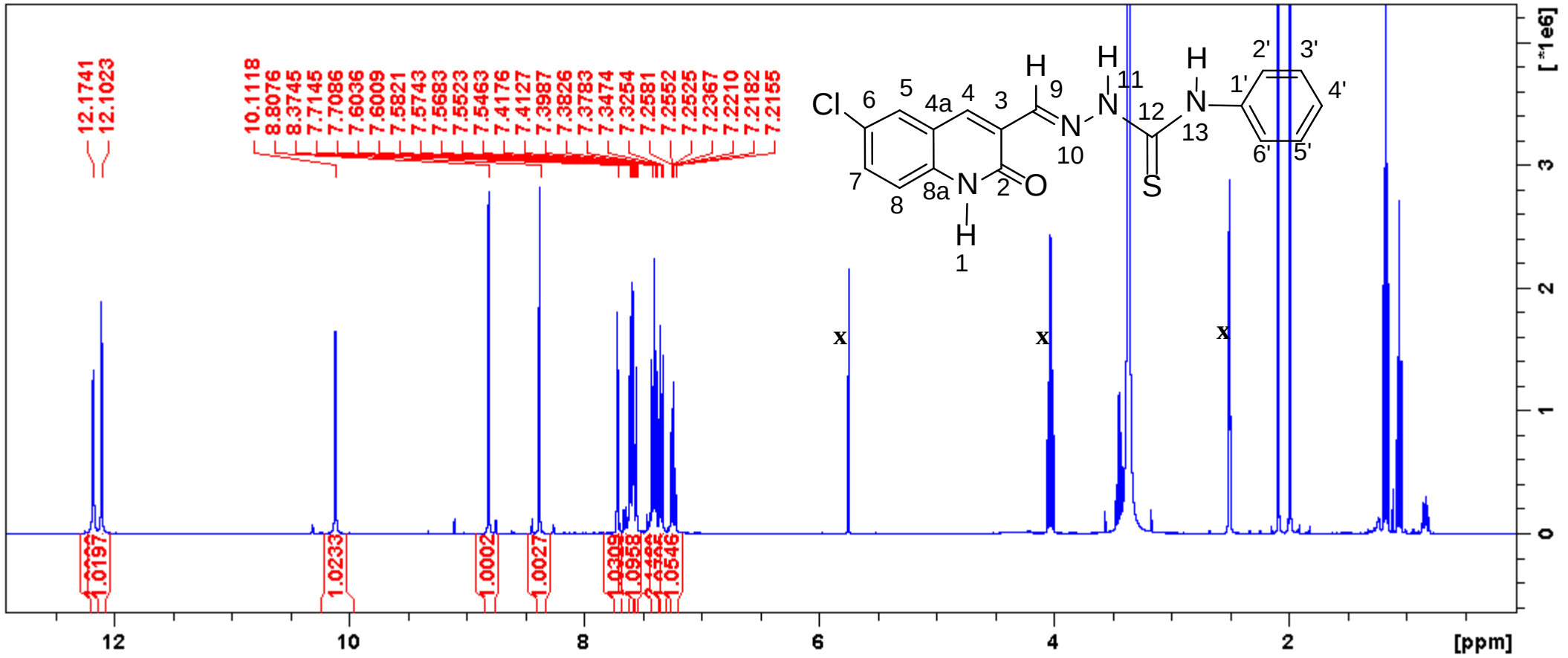
Wavelength/nm	Absorbance	Log ε
220	0.58	4.37
247	0.57	4.36
393	0.40	4.21



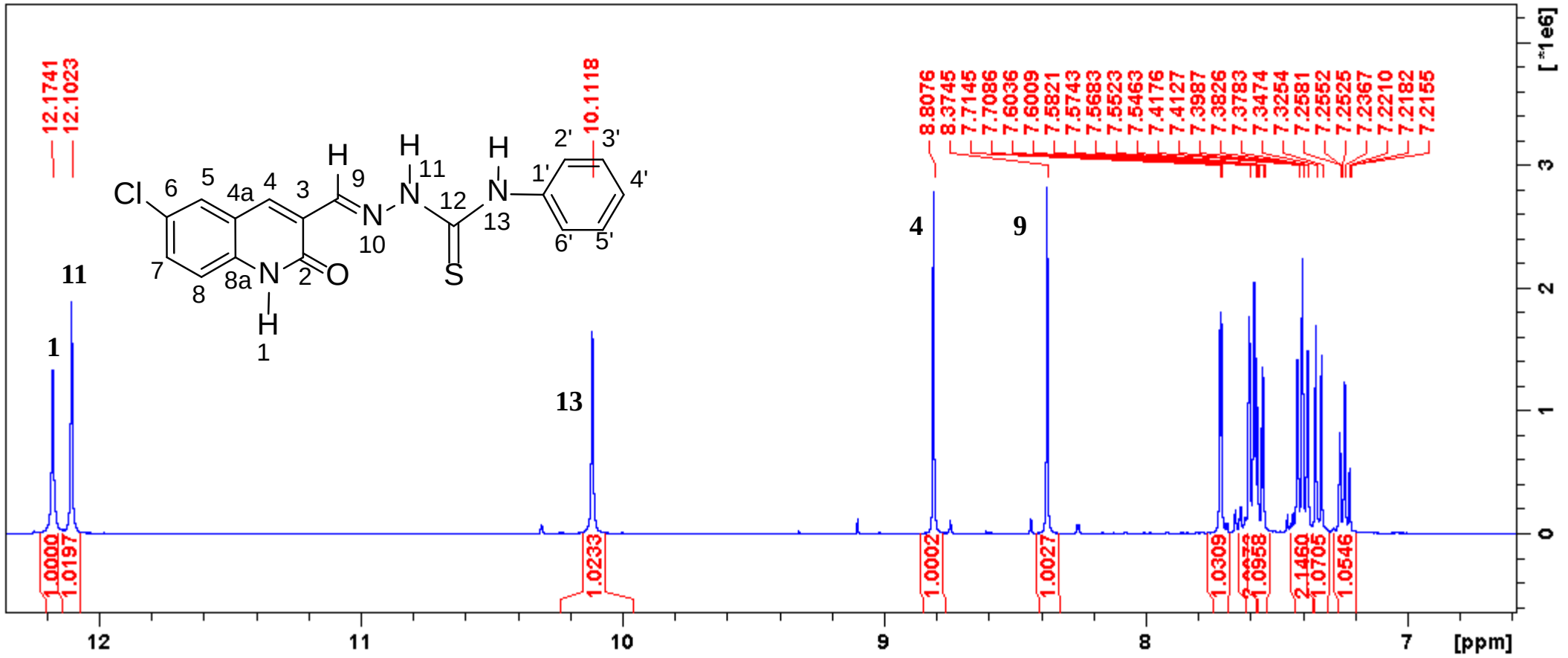




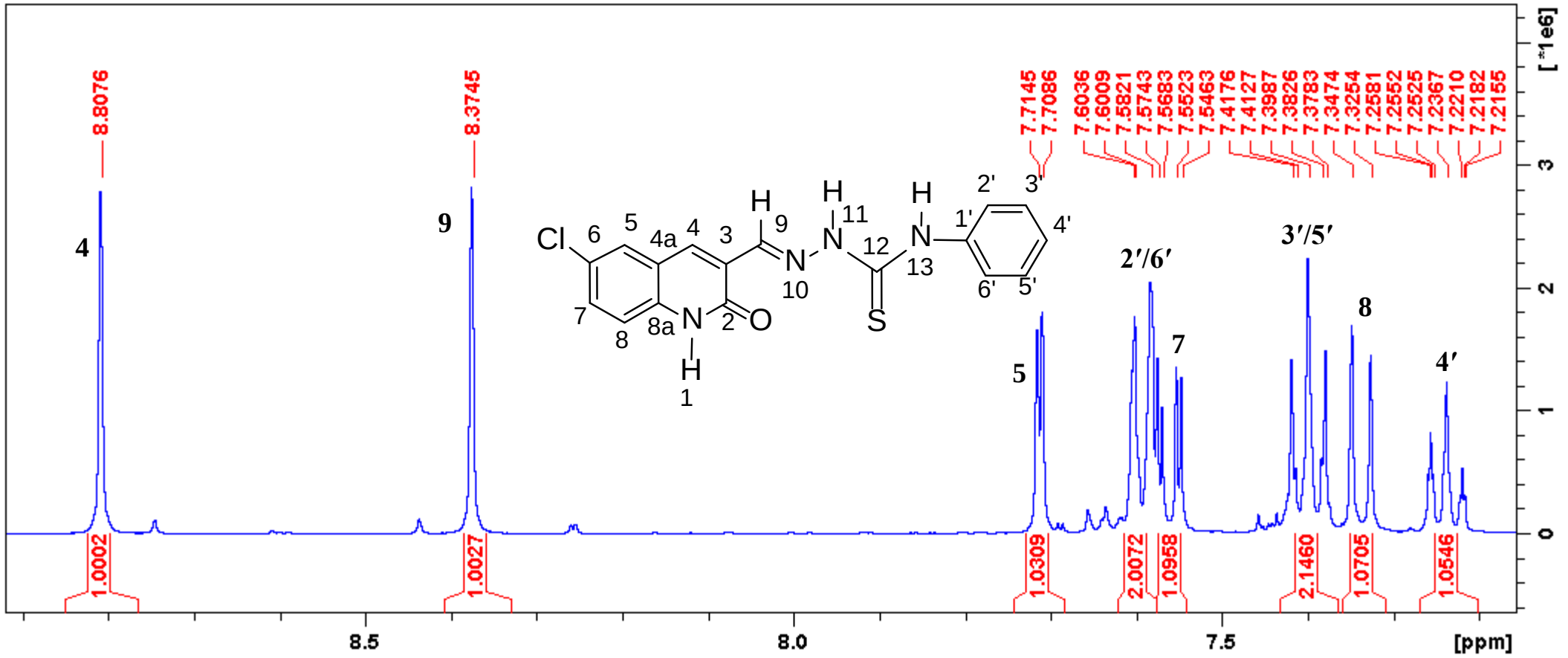
UV-Vis spectrum of compound 5m



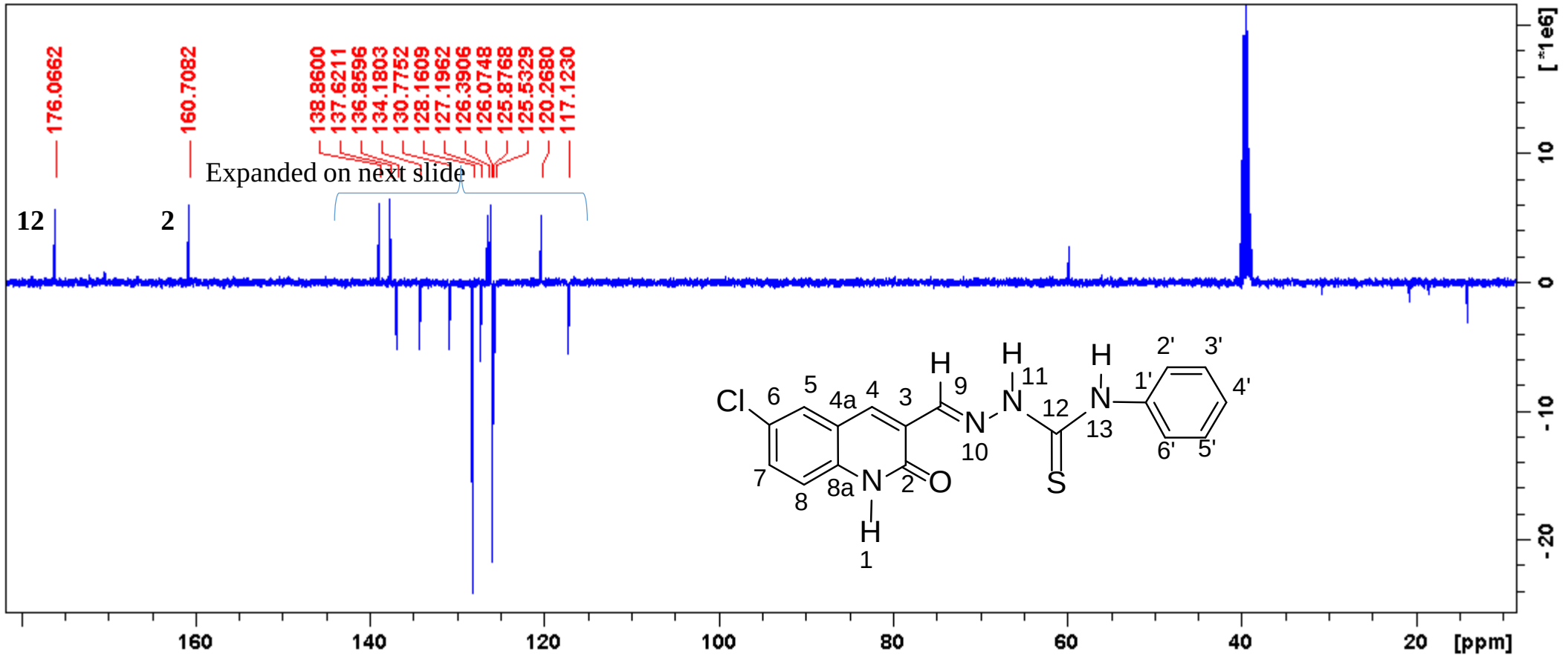
^{1}H NMR spectrum of compound 5n



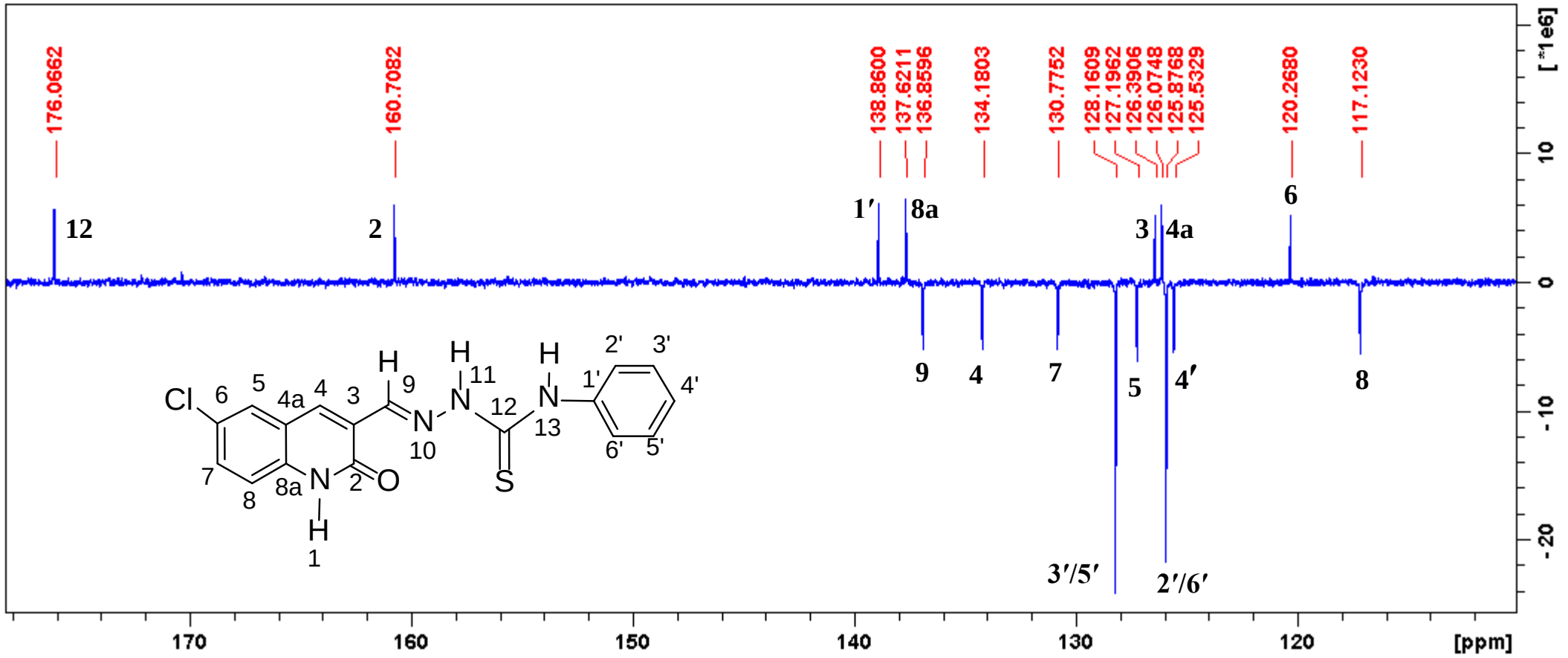
^{1}H NMR spectrum of compound 5n



^{1}H NMR spectrum of compound 5n- expanded



^{13}C NMR spectrum of compound 5n

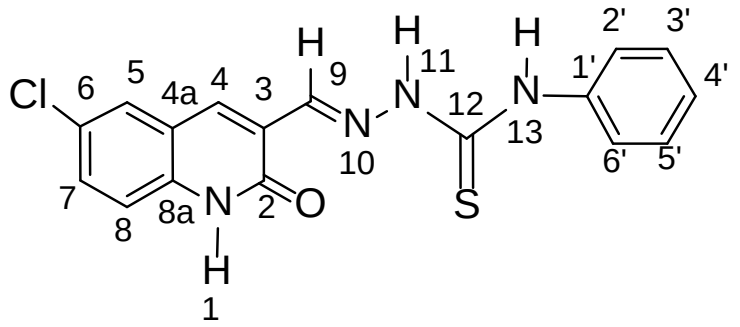


^{13}C NMR spectrum of compound 5n (expanded)

Elemental Composition Report

Single Mass Analysis

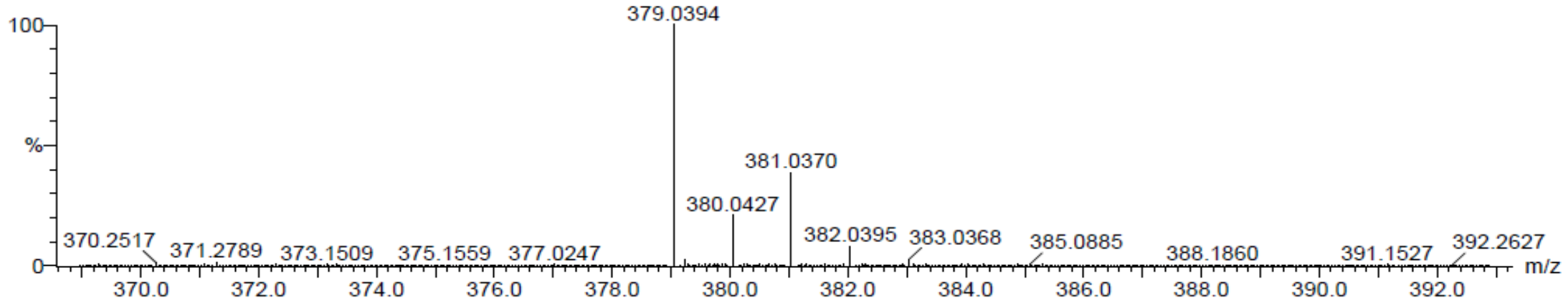
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



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: 10-15 N: 0-5 O: 1-1 Na: 0-1 S: 0-1 Cl: 0-1

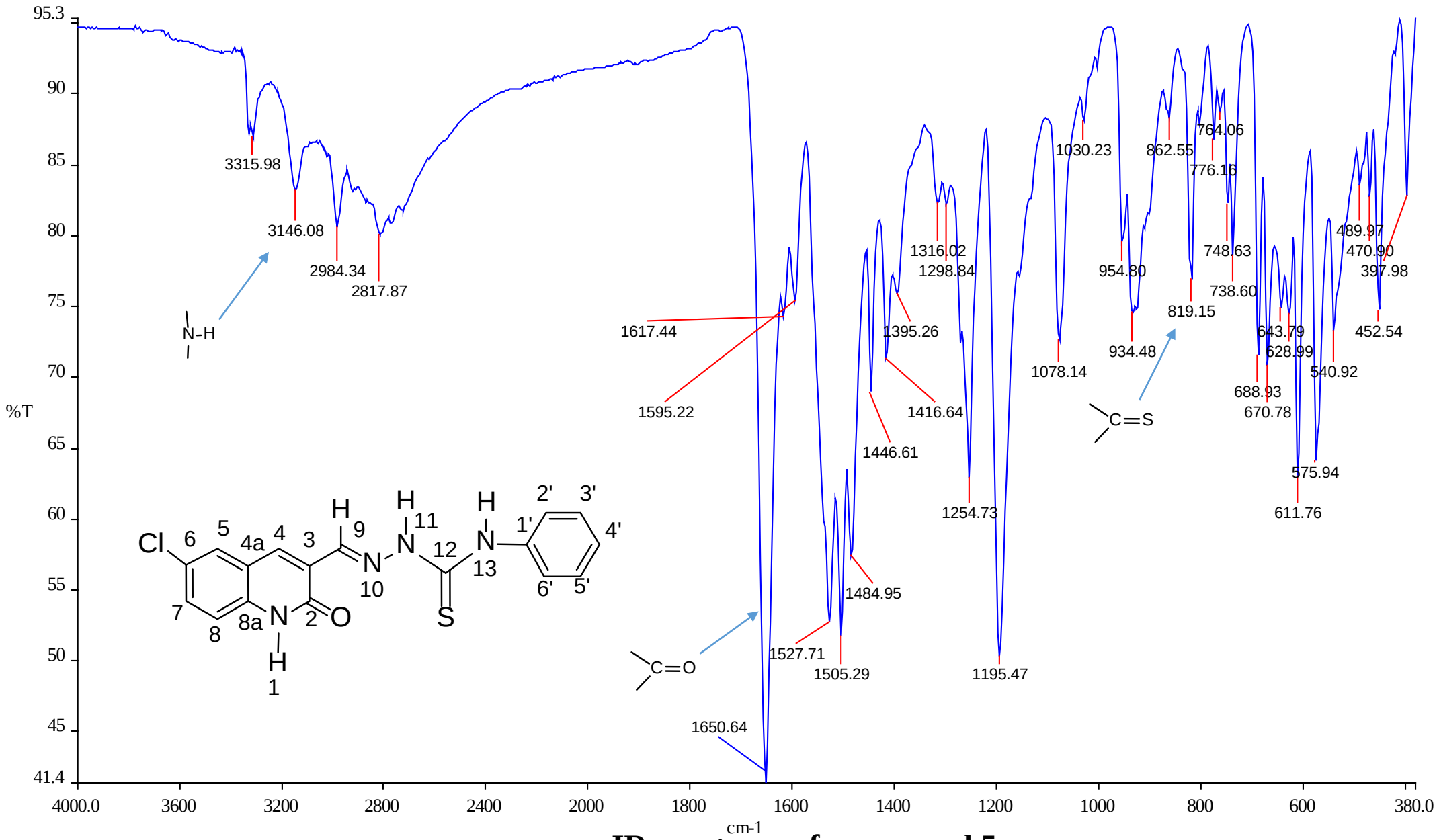
a3 HG 11 (0.338) Cm (1:61)
TOF MS ES+

1.67e+005



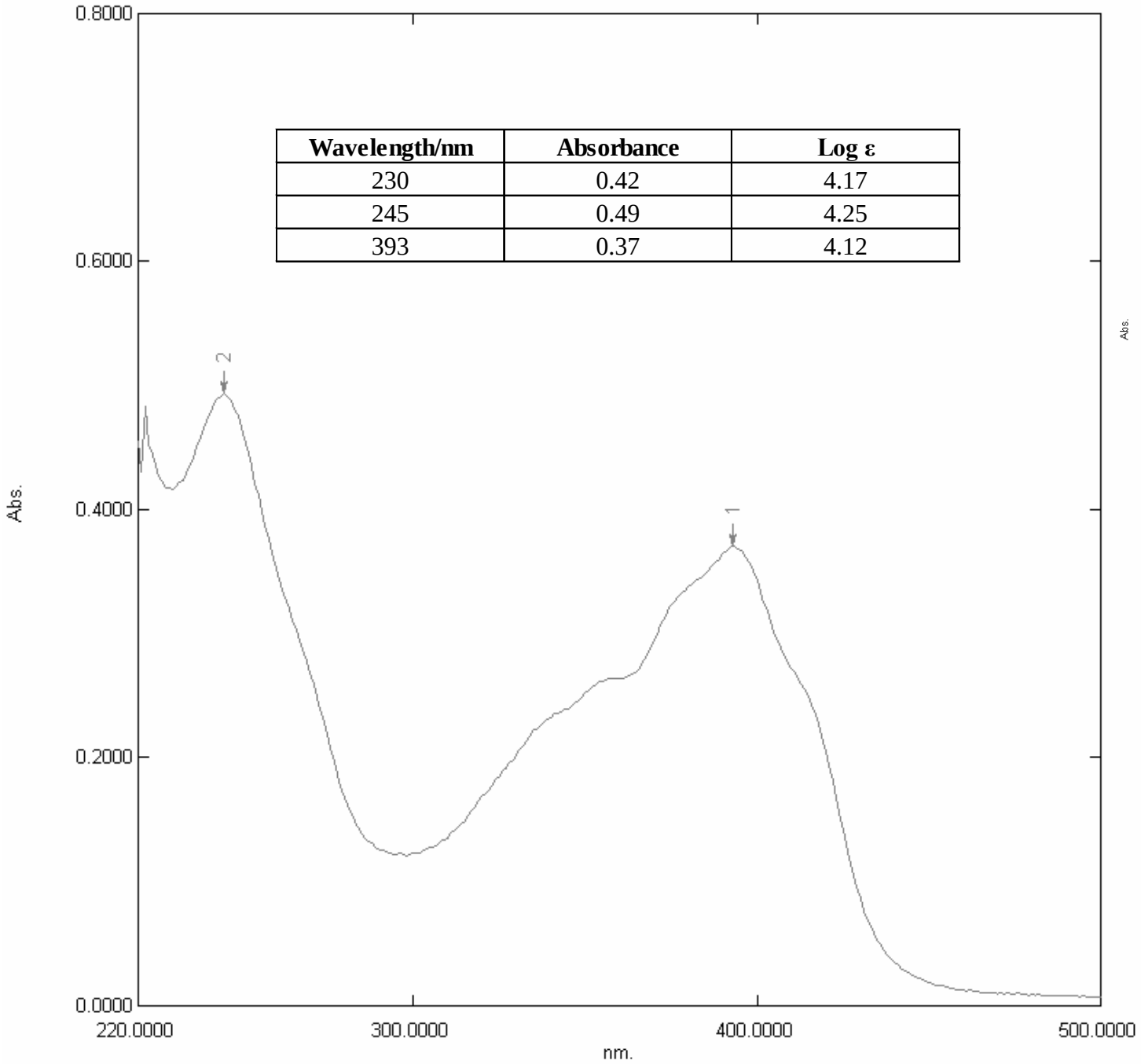
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
379.0394	379.0396	-0.2	-0.5	12.5	560.8	0.0	C17 H13 N4 O Na S Cl

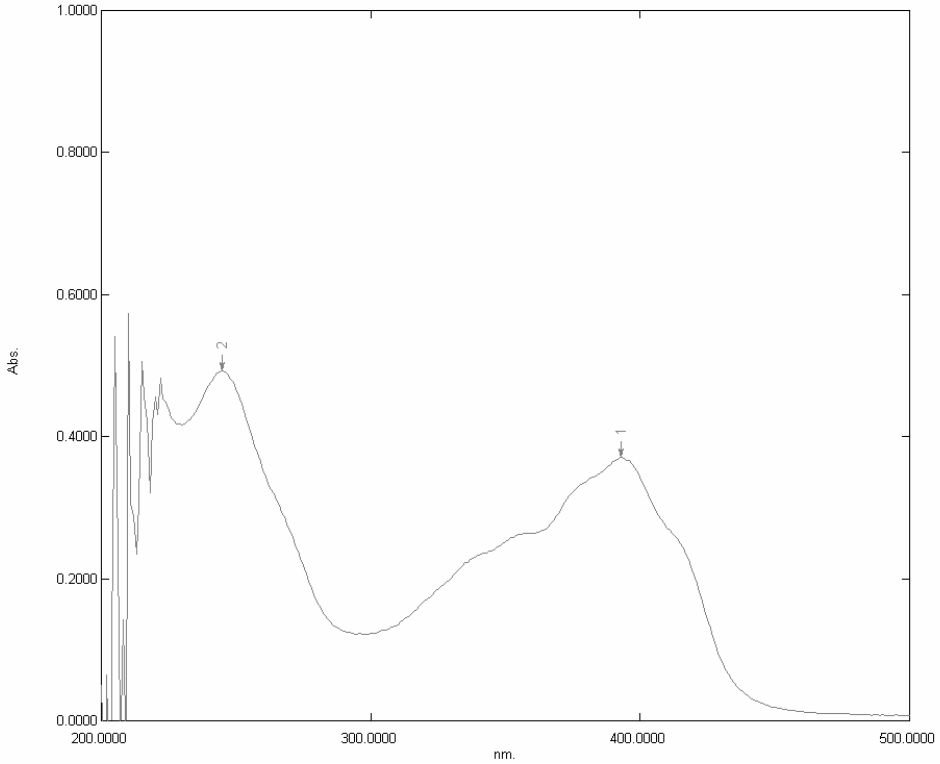
IR spectrum of compound 5n

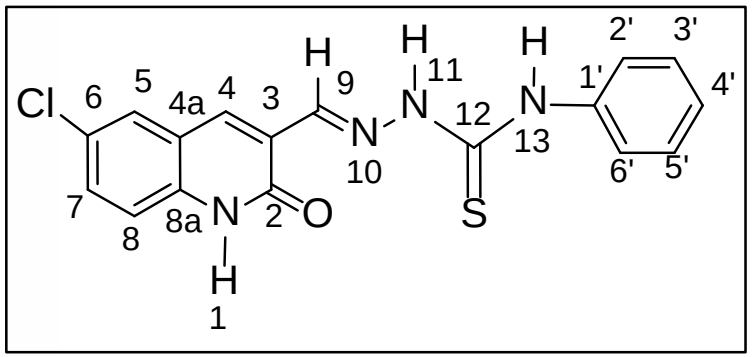


IR spectrum of compound 5n

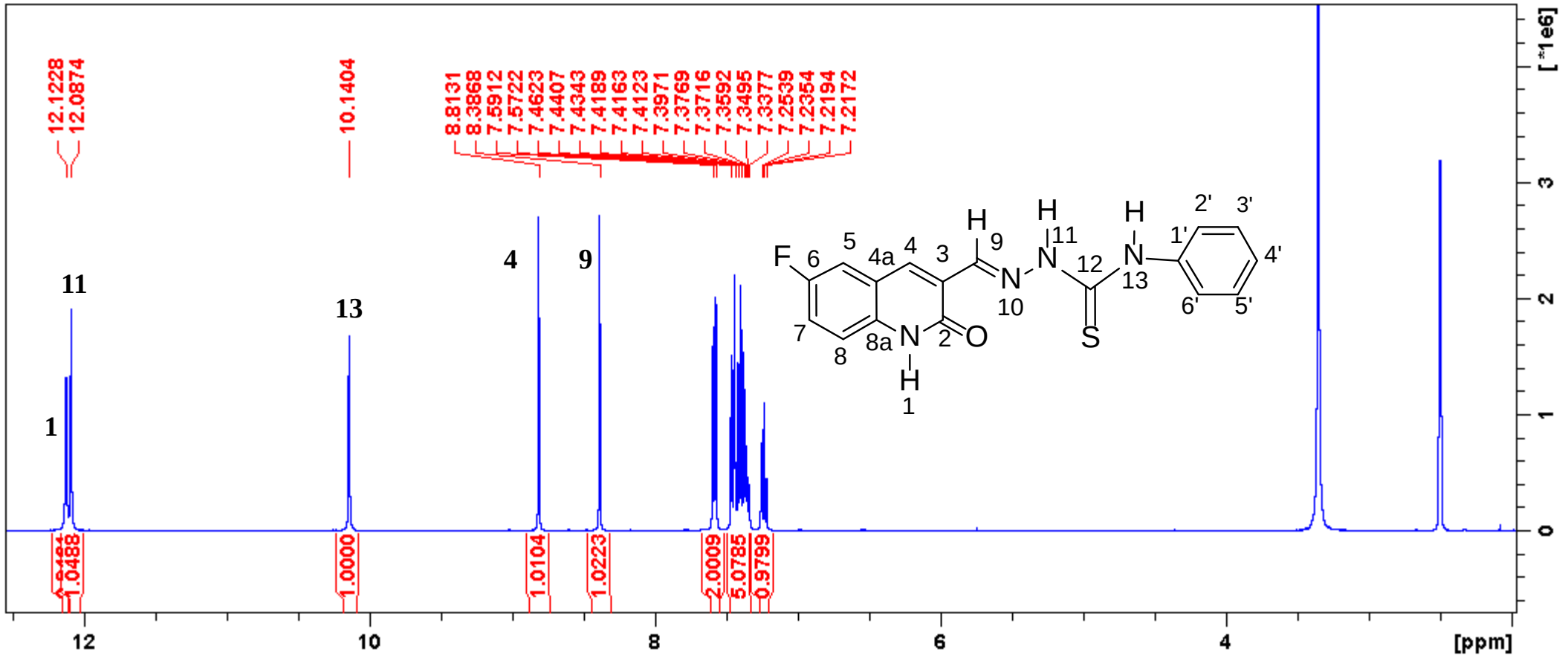
Wavelength/nm	Absorbance	Log ε
230	0.42	4.17
245	0.49	4.25
393	0.37	4.12



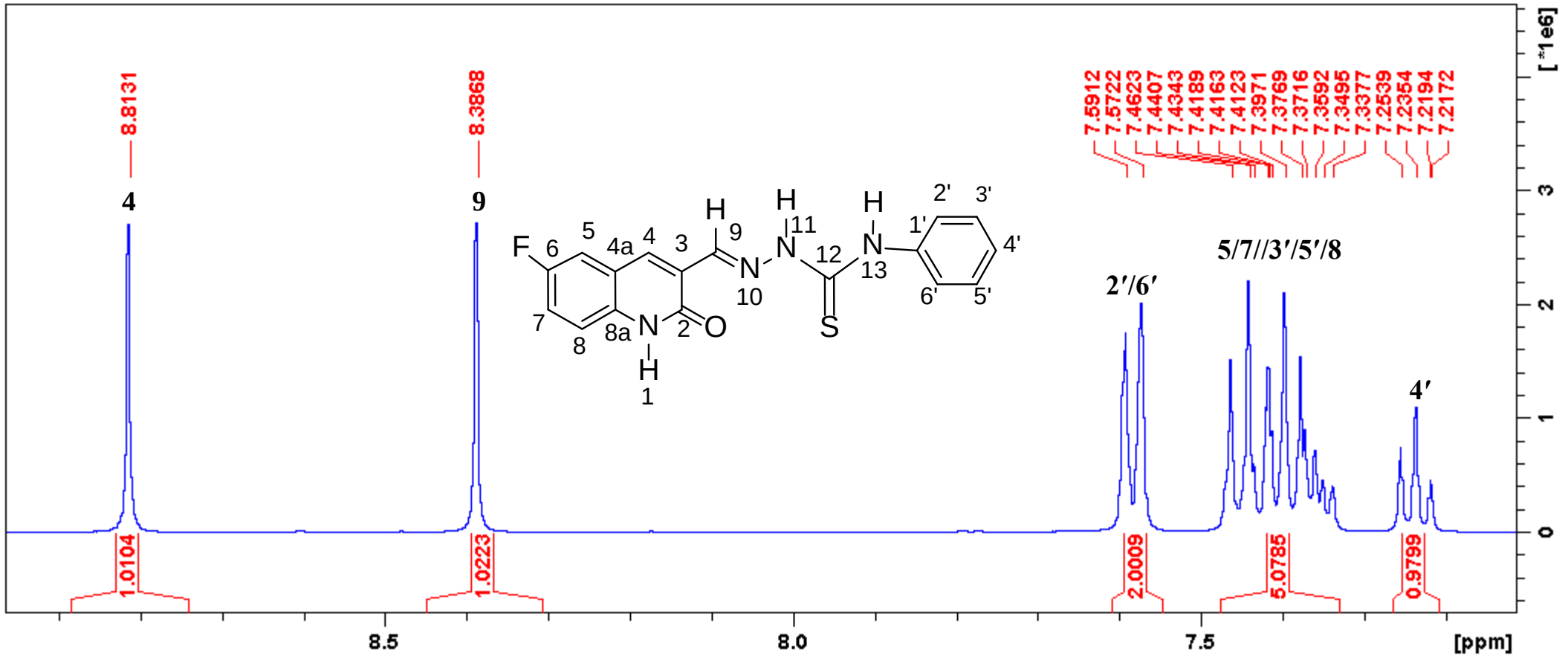




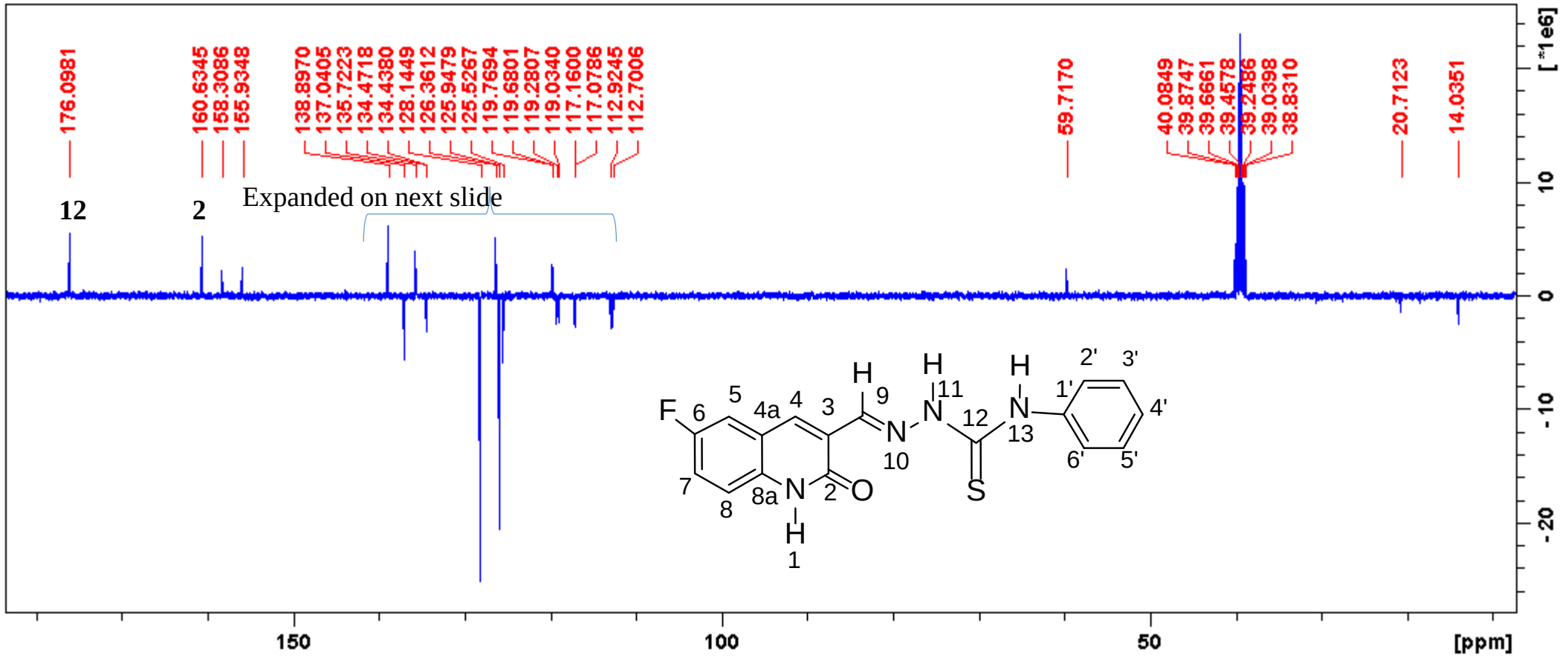
UV-Vis spectrum of compound 5n



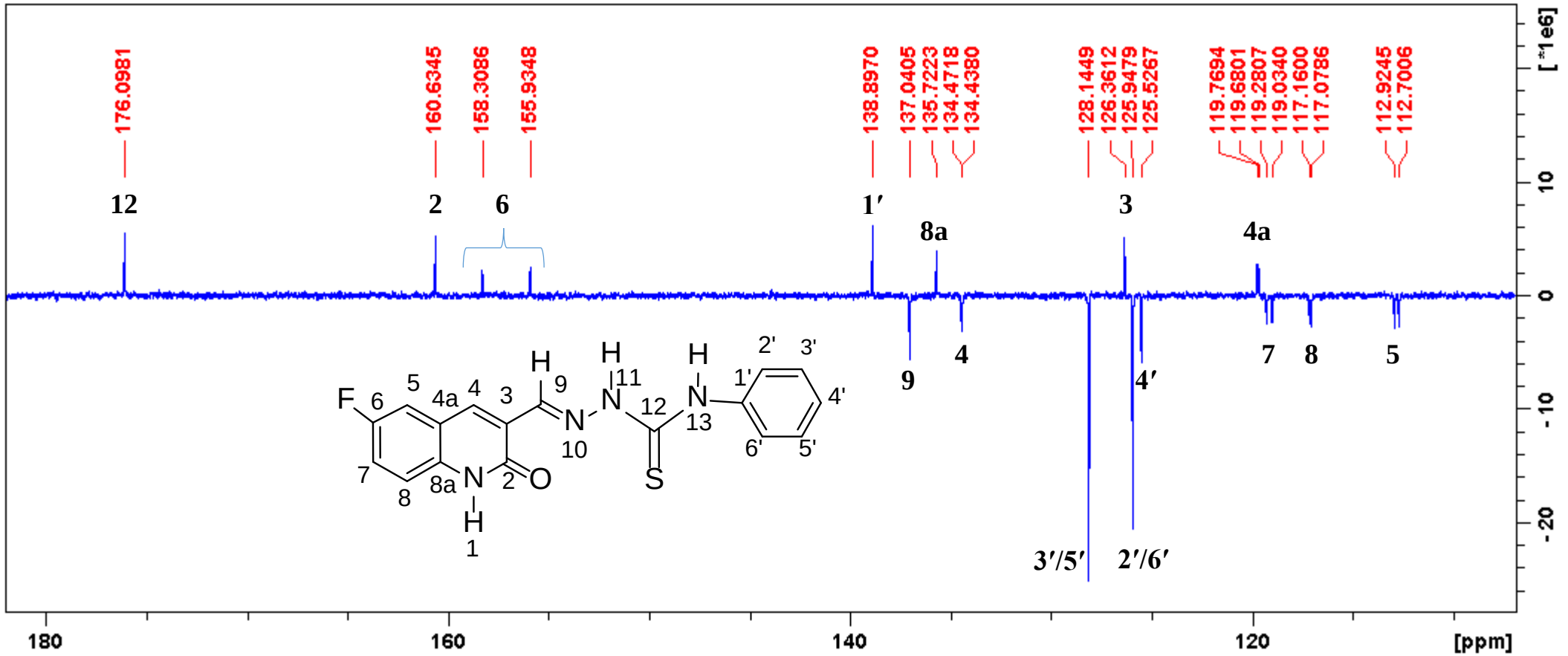
^{1}H NMR spectrum of compound 5o



^{1}H NMR spectrum of compound 5o (expanded)



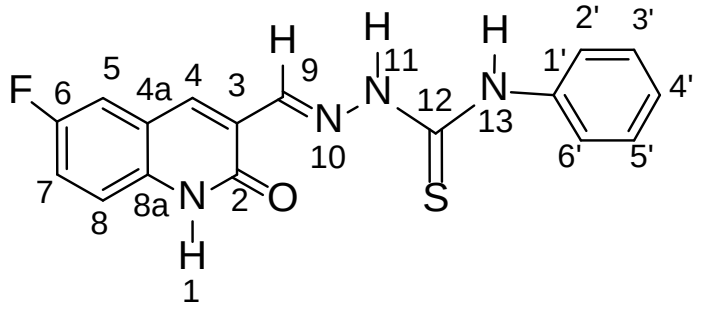
^{13}C NMR spectrum of compound 5o



^{13}C NMR spectrum of compound 5o (expanded)

Single Mass Analysis

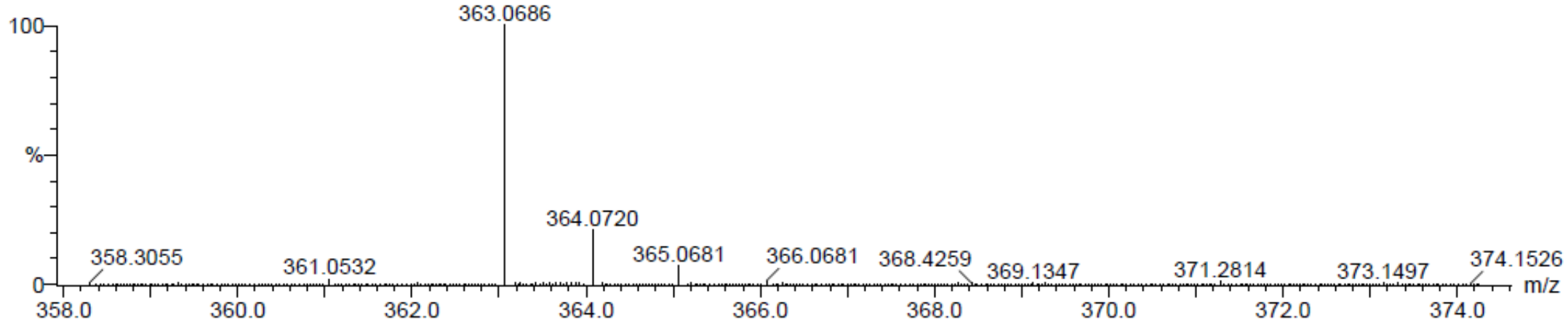
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
20 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 O: 1-1 F: 0-1 Na: 0-1 S: 0-1

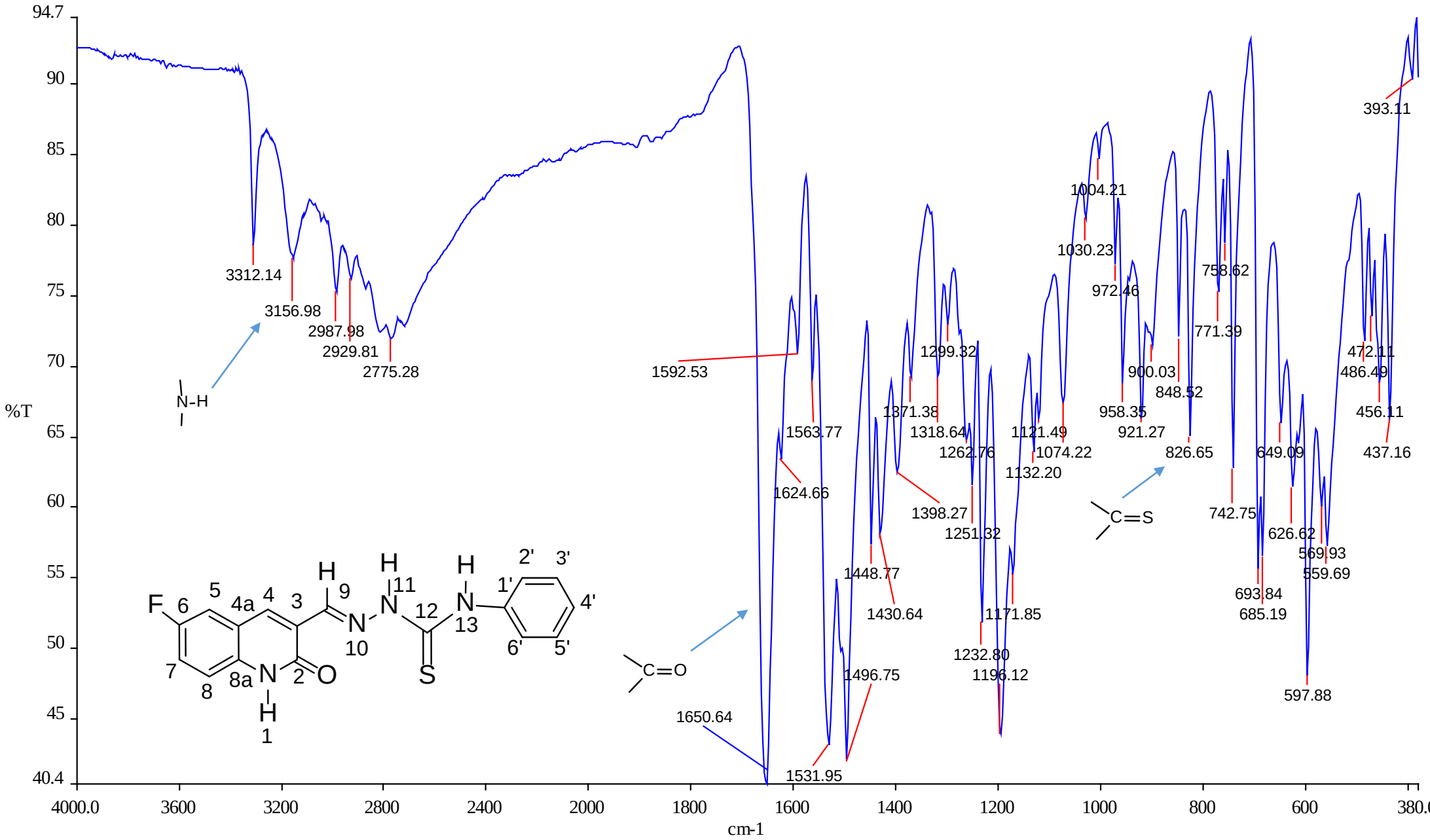
a1 HG 60 (1.992) Cm (1:61)
TOF MS ES+

1.59e+005

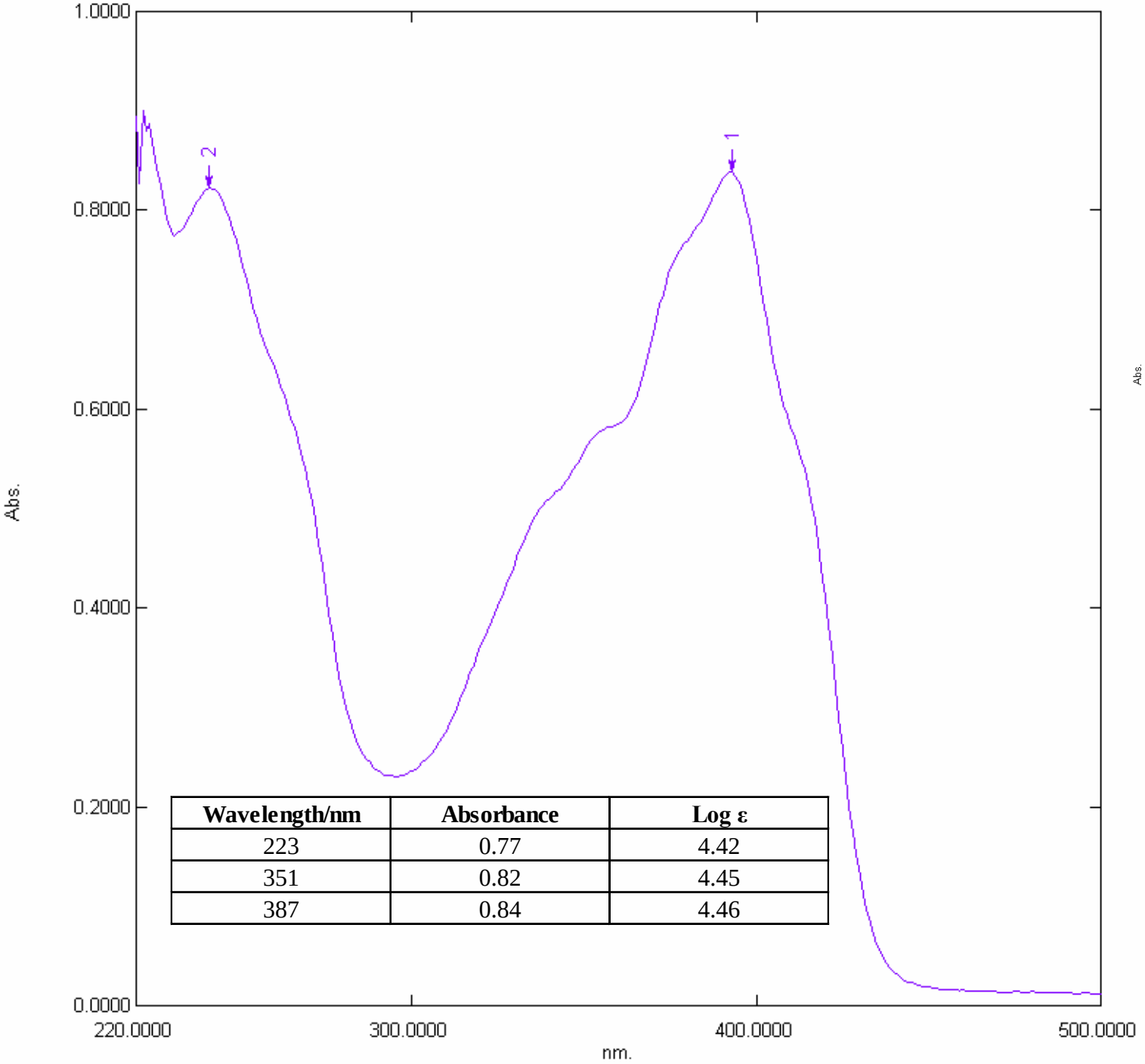


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
363.0686	363.0692	-0.6	-1.7	12.5	546.7	0.0	C17 H13 N4 O F Na S

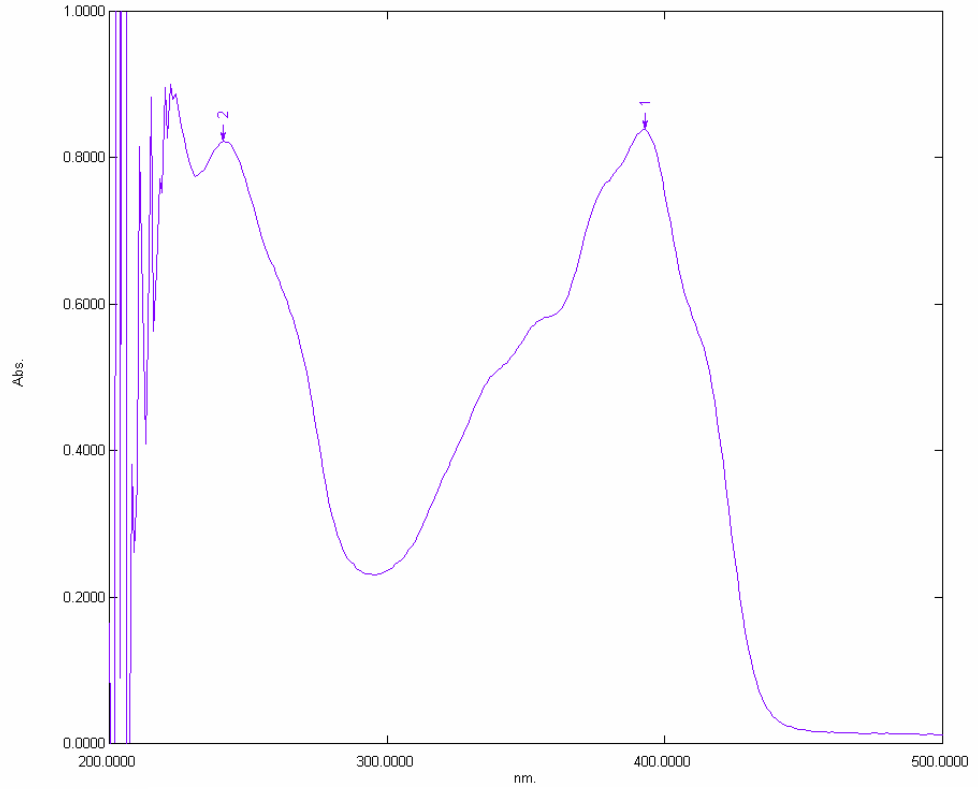
IR spectrum of compound 5o

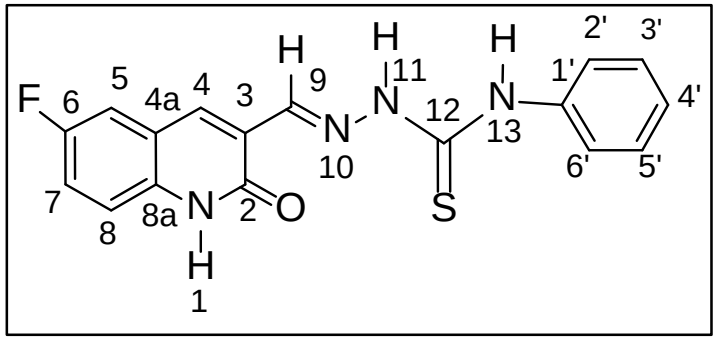


IR spectrum of compound 5o



Wavelength/nm	Absorbance	Log ε
223	0.77	4.42
351	0.82	4.45
387	0.84	4.46



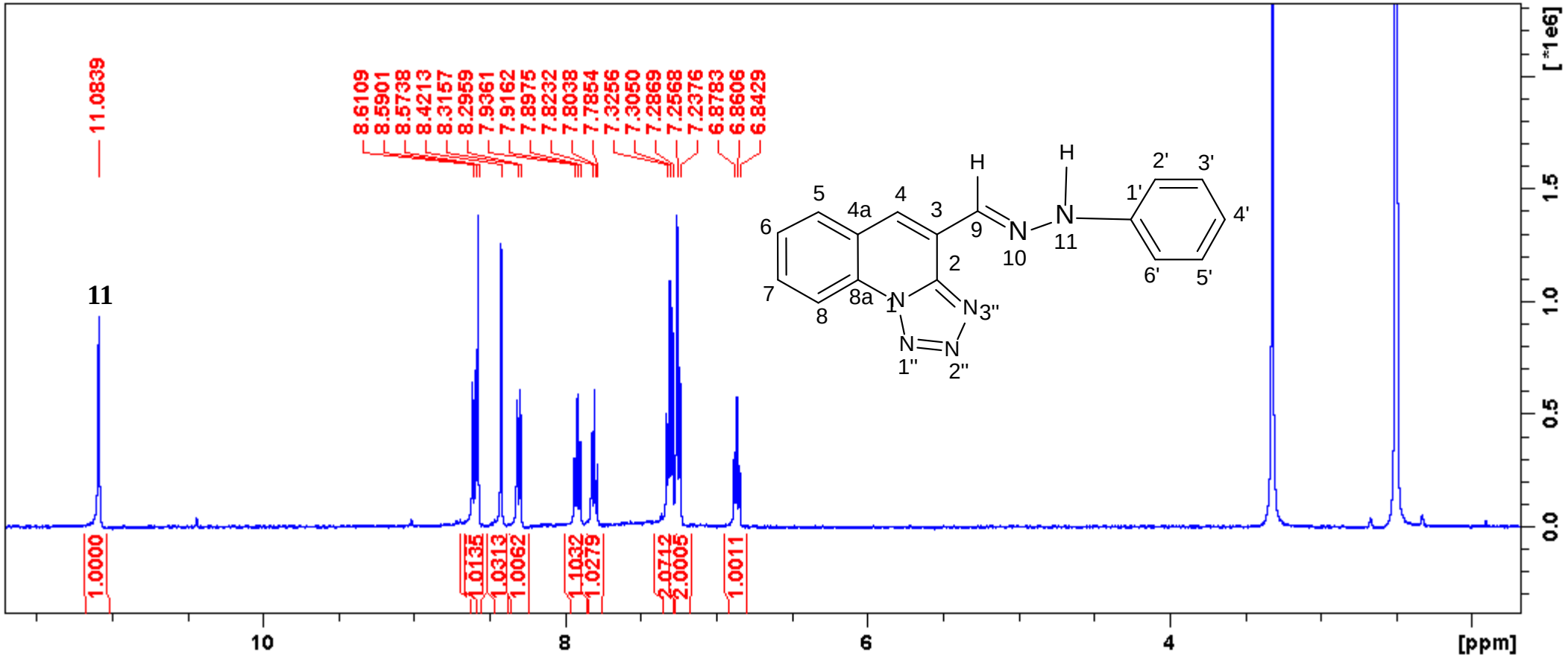


UV-Vis spectrum of compound 5o

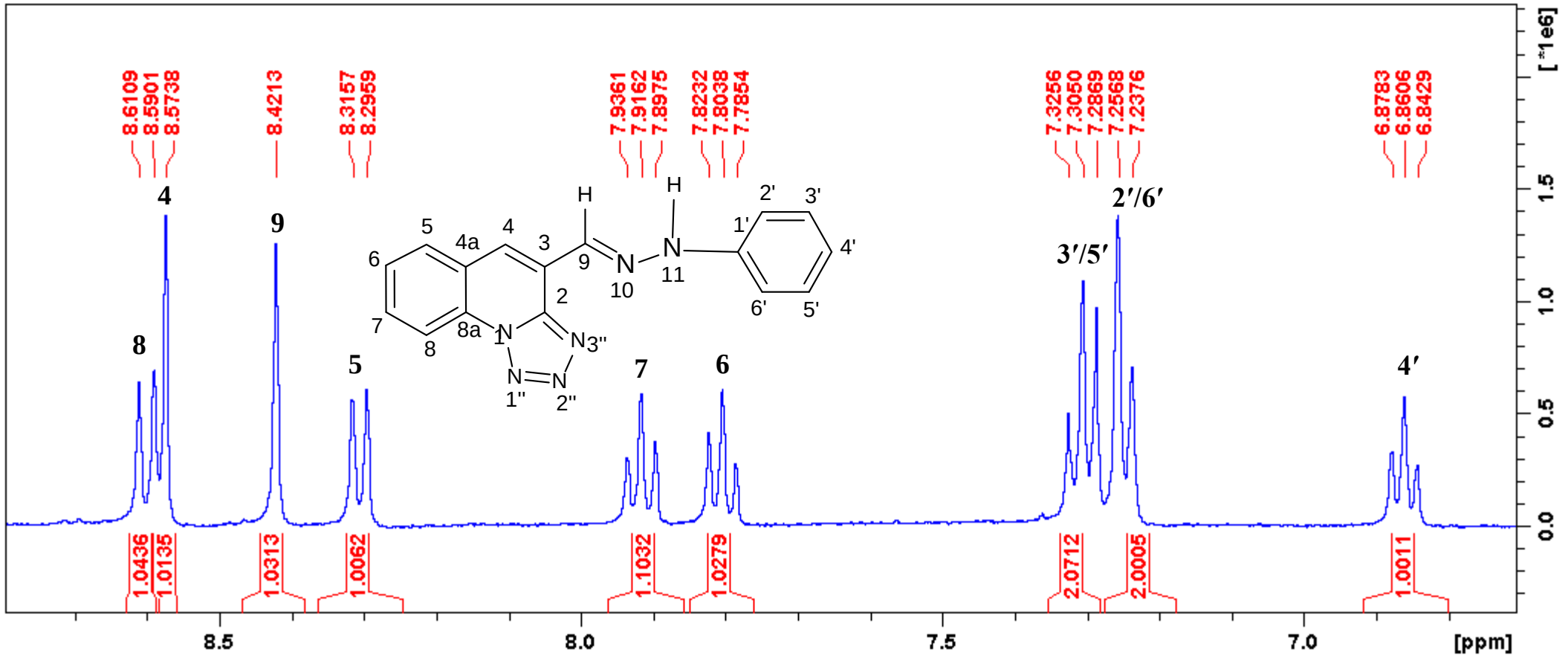
Supplementary Information

Chapter 4. Synthetic Study and Biological Investigation of Quinoline Tetrazolo Phenylhydrazine Hybrids

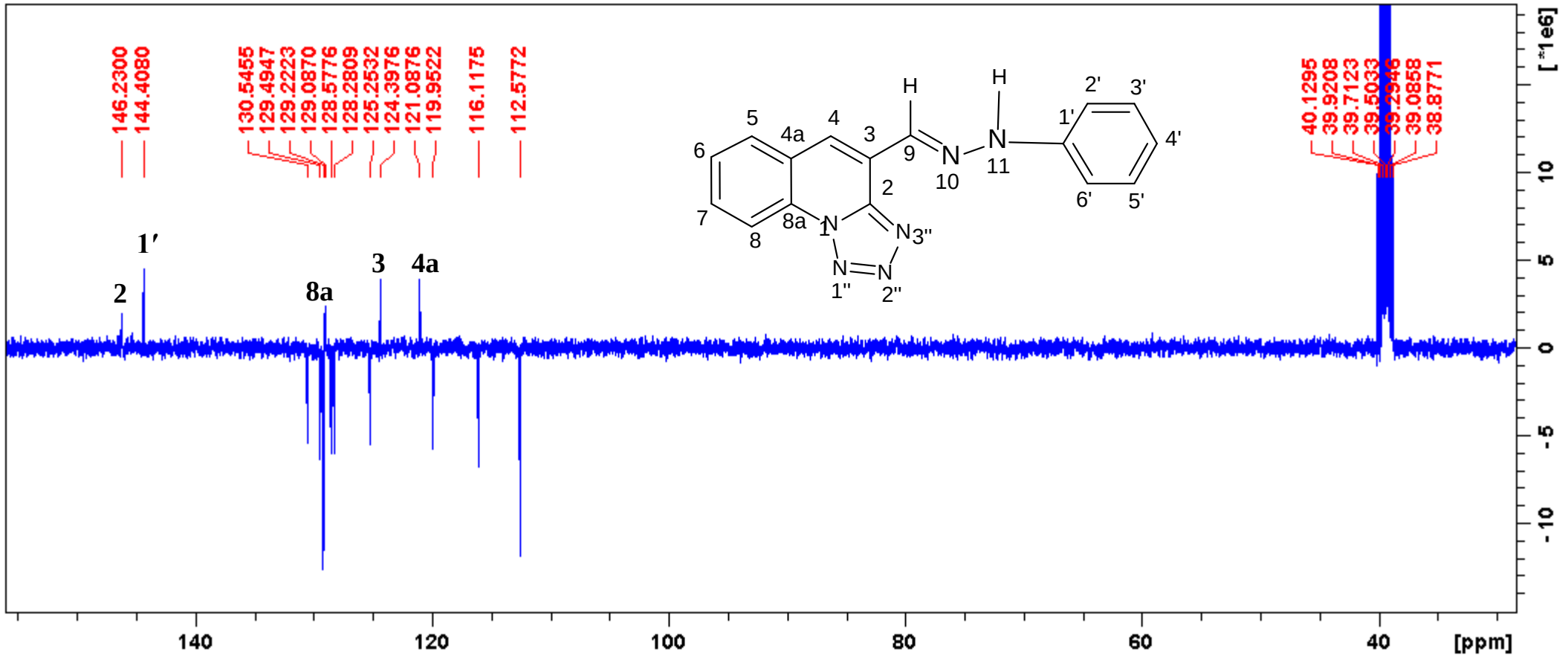
Contents



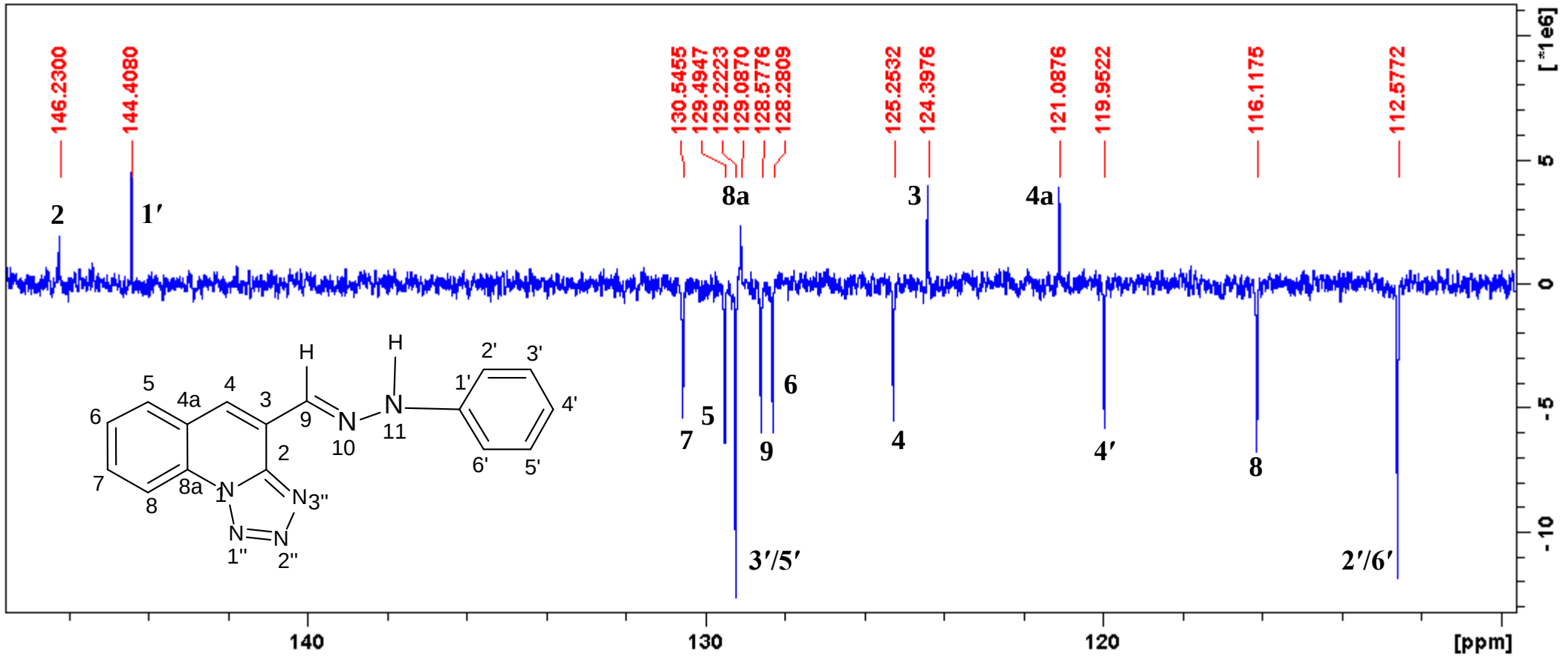
The ^{1}H NMR spectrum of compound 5a



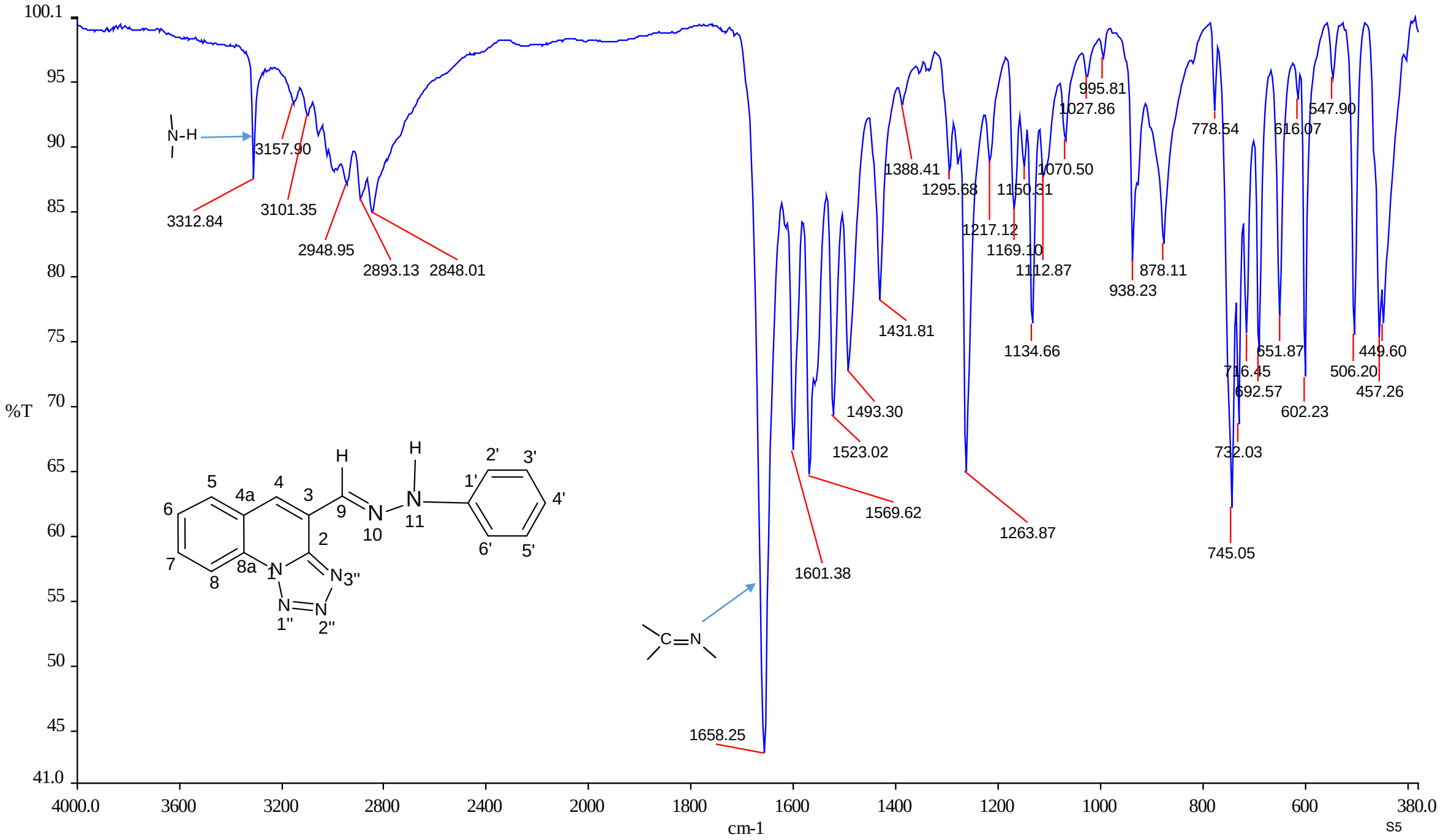
The ^{1}H NMR spectrum of compound 5a (expanded)



The ^{13}C NMR spectrum of compound 5a

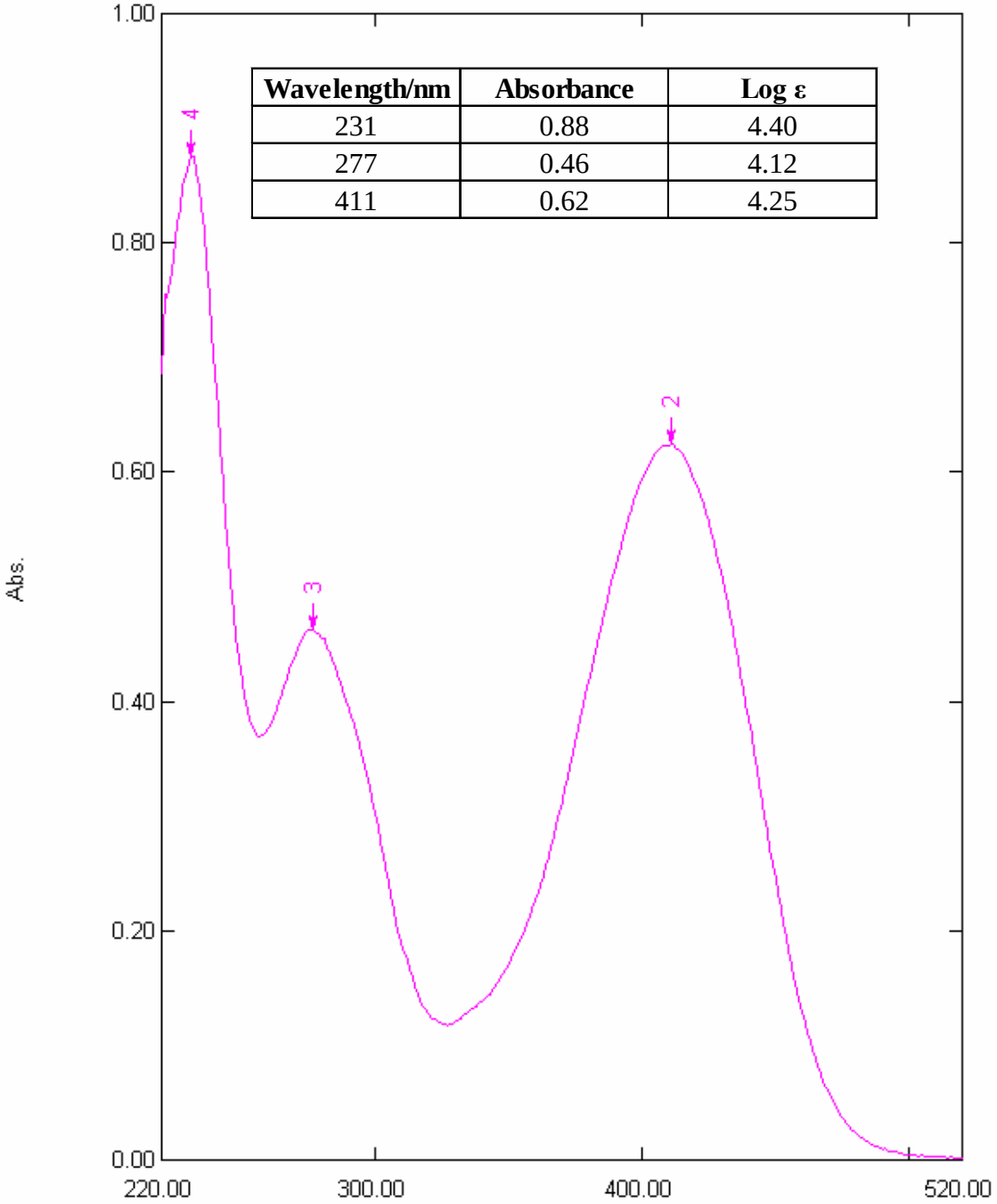


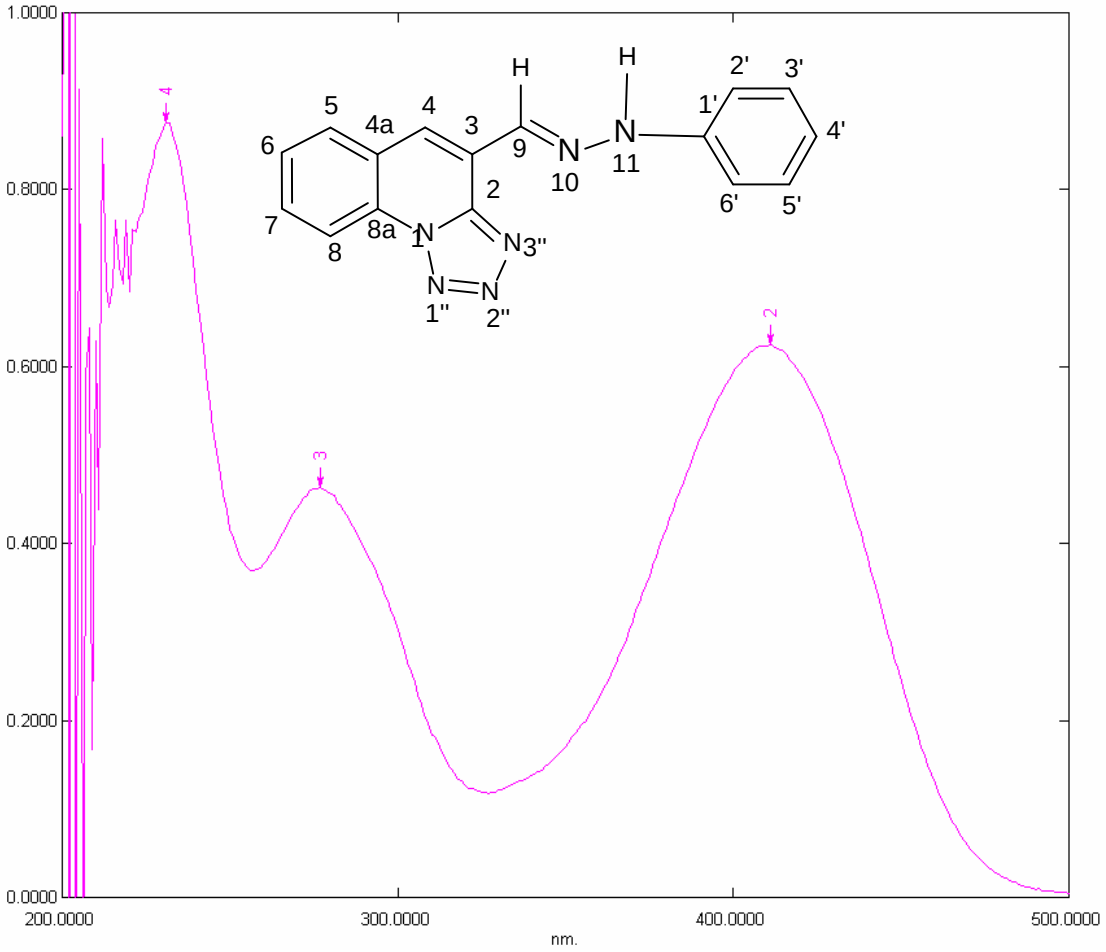
The ^{13}C NMR spectrum of compound 5a (expanded)



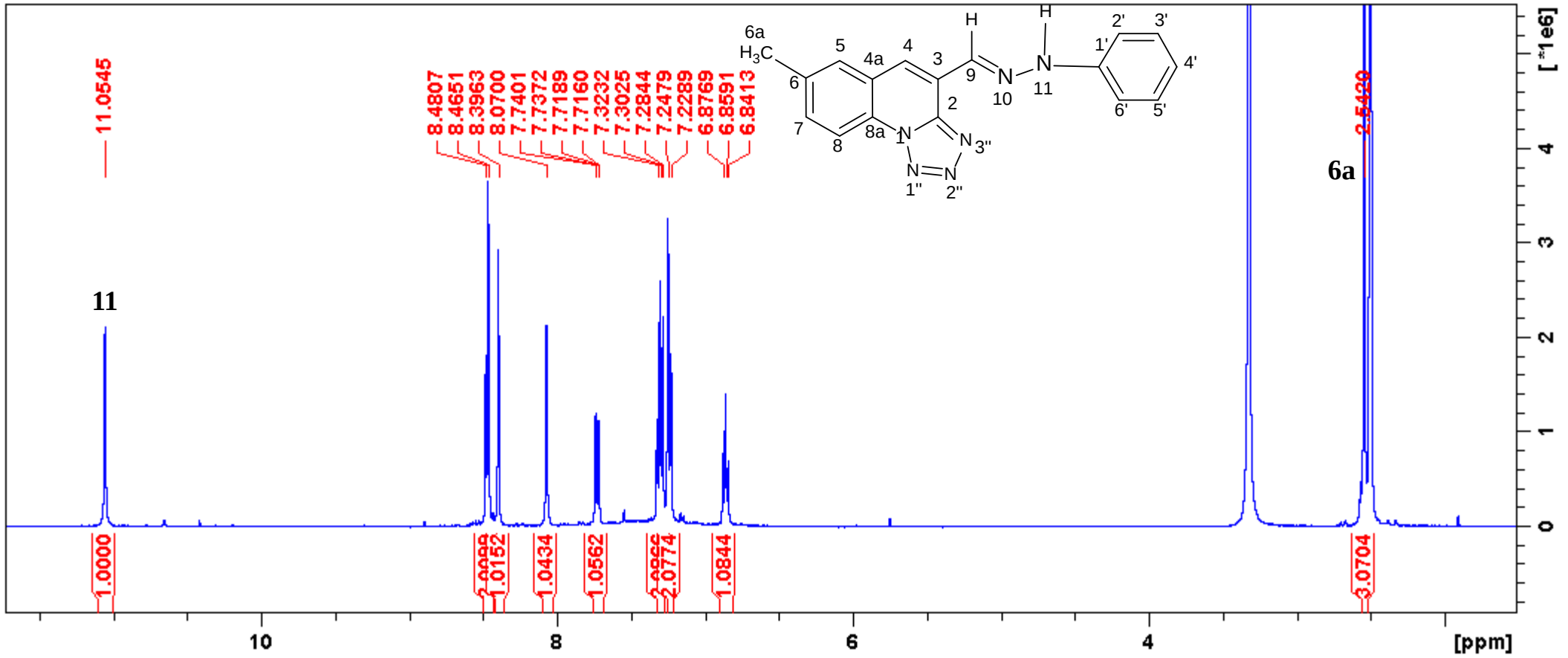
The IR spectrum of compound 5a

Wavelength/nm	Absorbance	Log ε
231	0.88	4.40
277	0.46	4.12
411	0.62	4.25

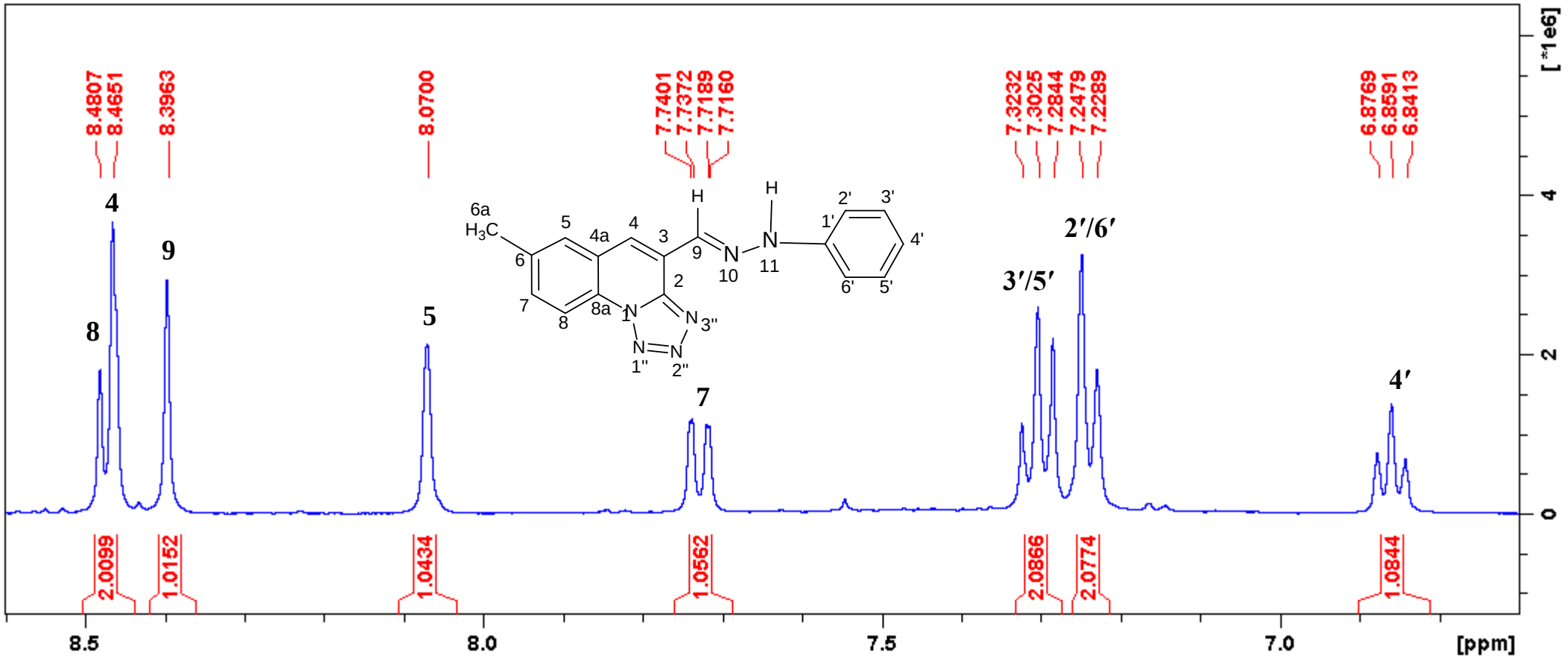




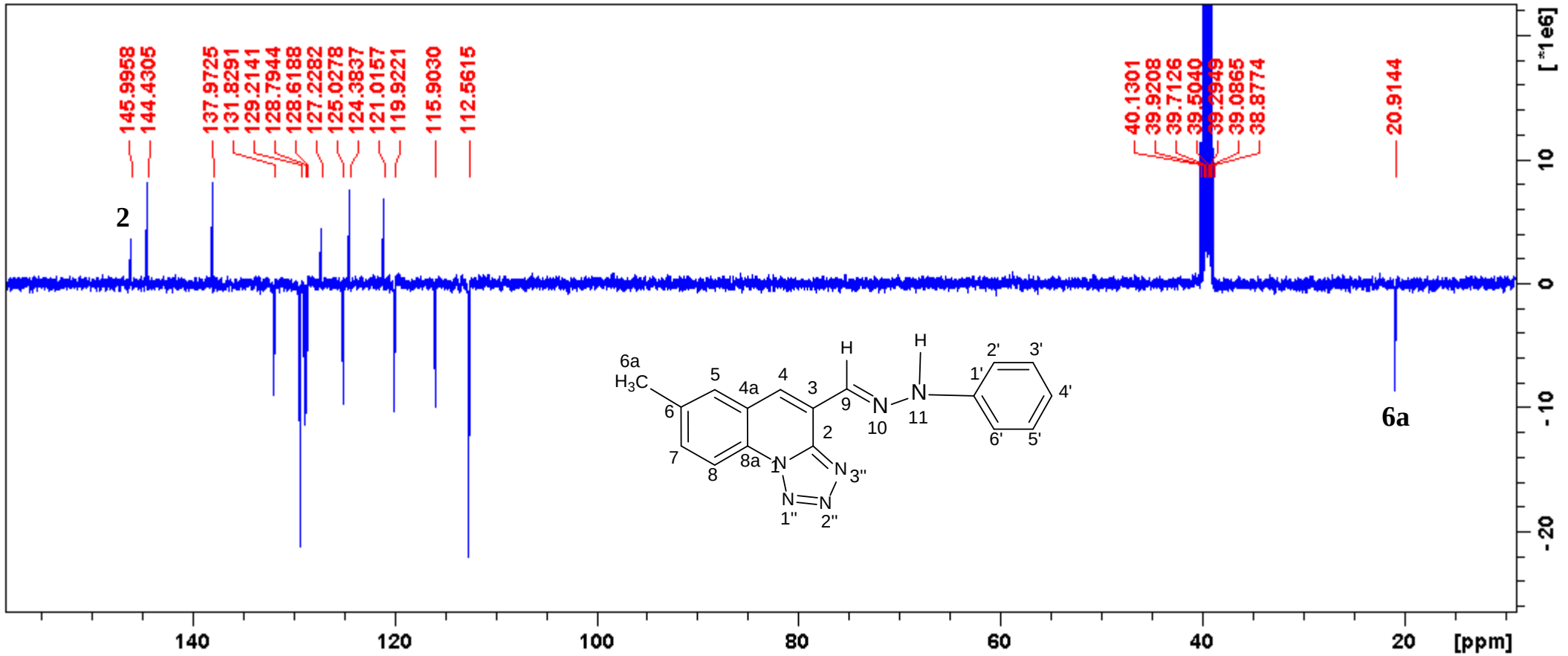
The UV-Vis spectrum of compound 5a



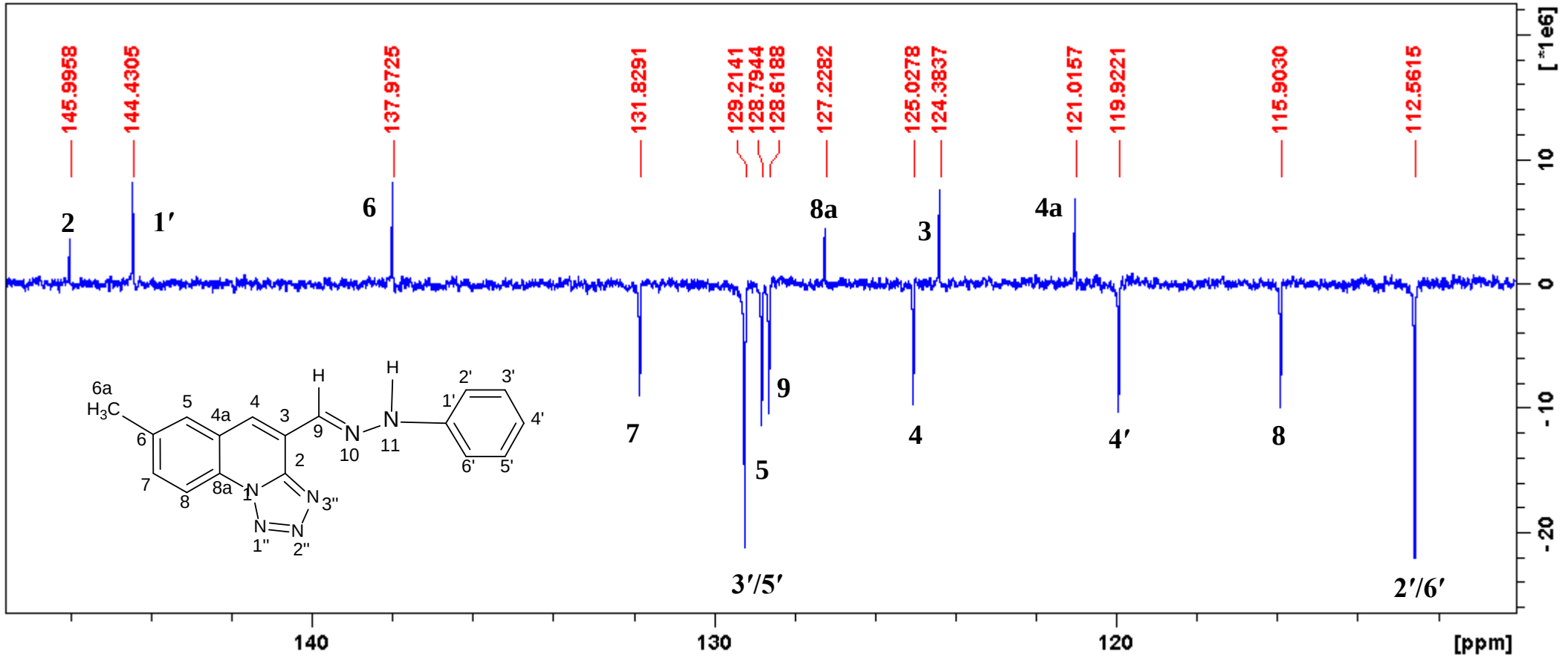
The ^{1}H NMR spectrum of compound 5b



The ^{1}H NMR spectrum of compound 5b (expanded)



The ^{13}C NMR spectrum of compound 5b



The ^{13}C NMR spectrum of compound 5b (expanded)

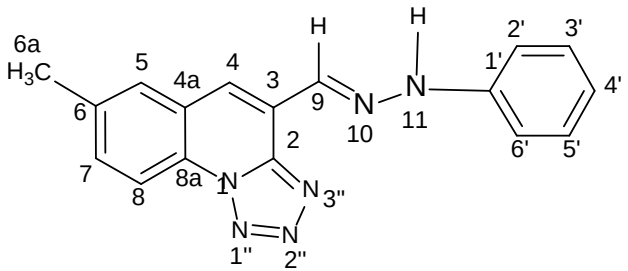
Elemental Composition Report

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
10 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: 0-10 Na: 0-1

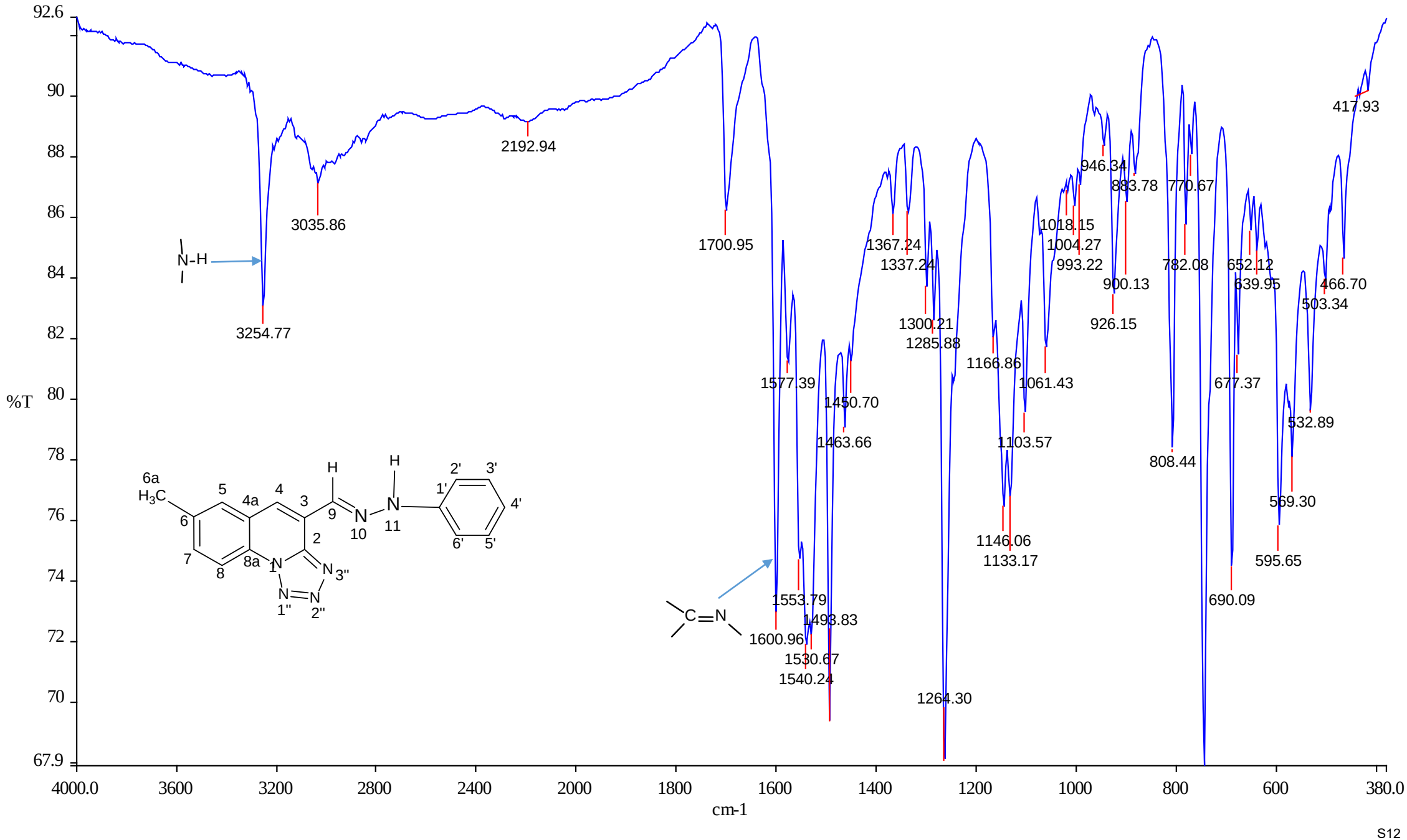
Compound 4 HG 35 (1.147) Cm (1:61)
TOF MS ES+



1.58e+005

Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
325.1180	325.1178	0.2	0.6	13.5	610.7	0.0	C17 H14 N6 Na

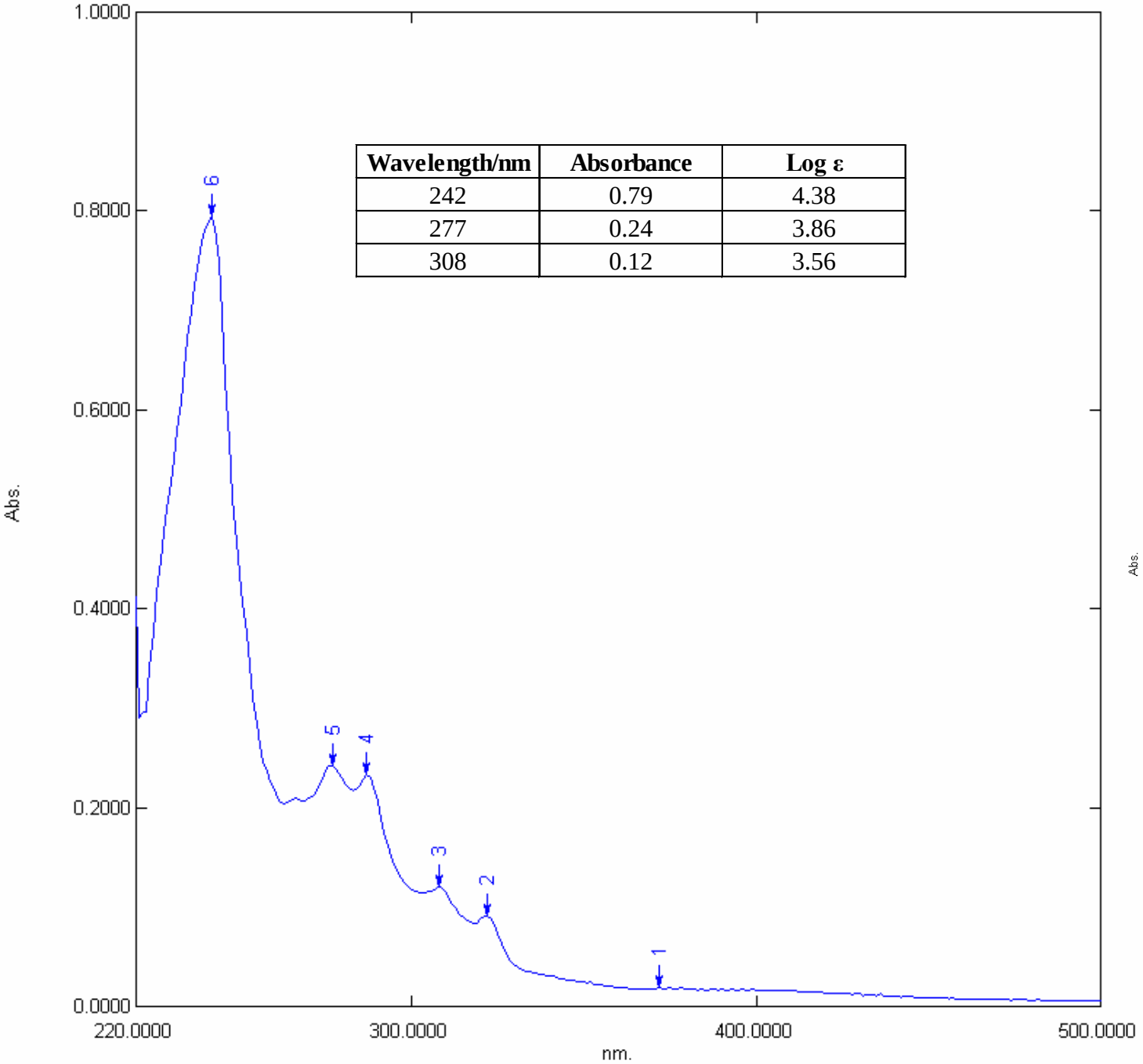
The HRMS spectrum of compound 5b

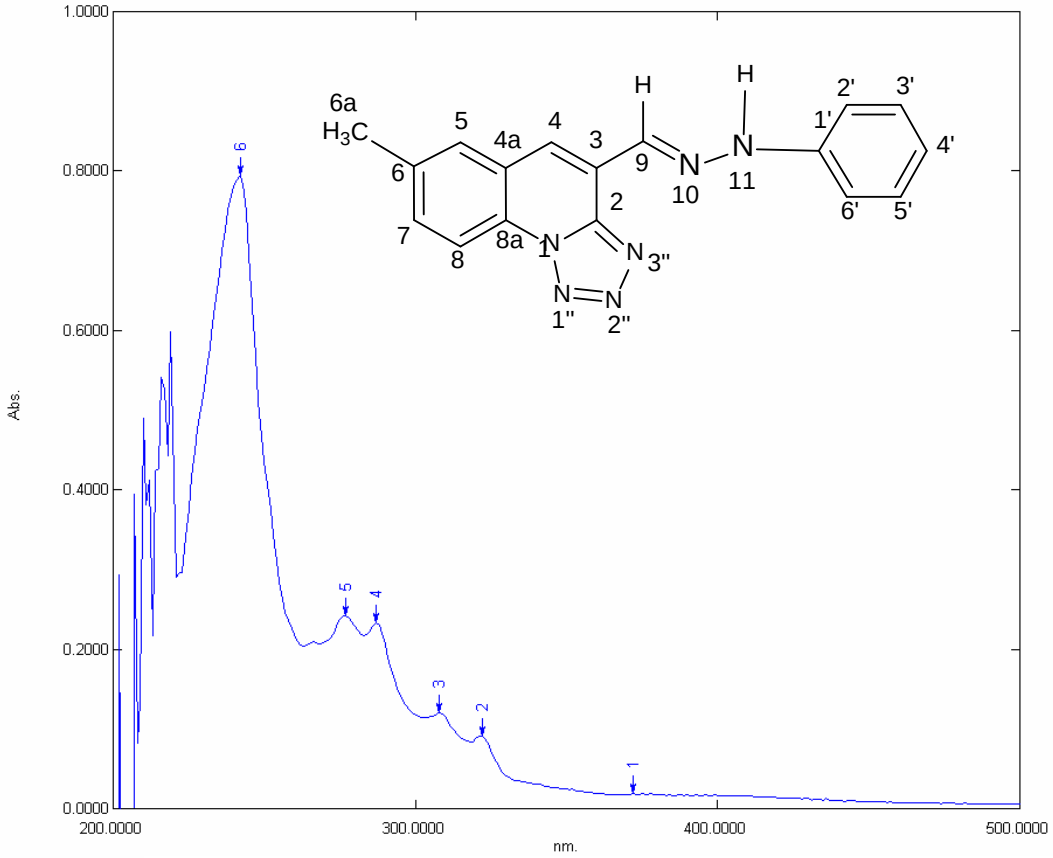


S12

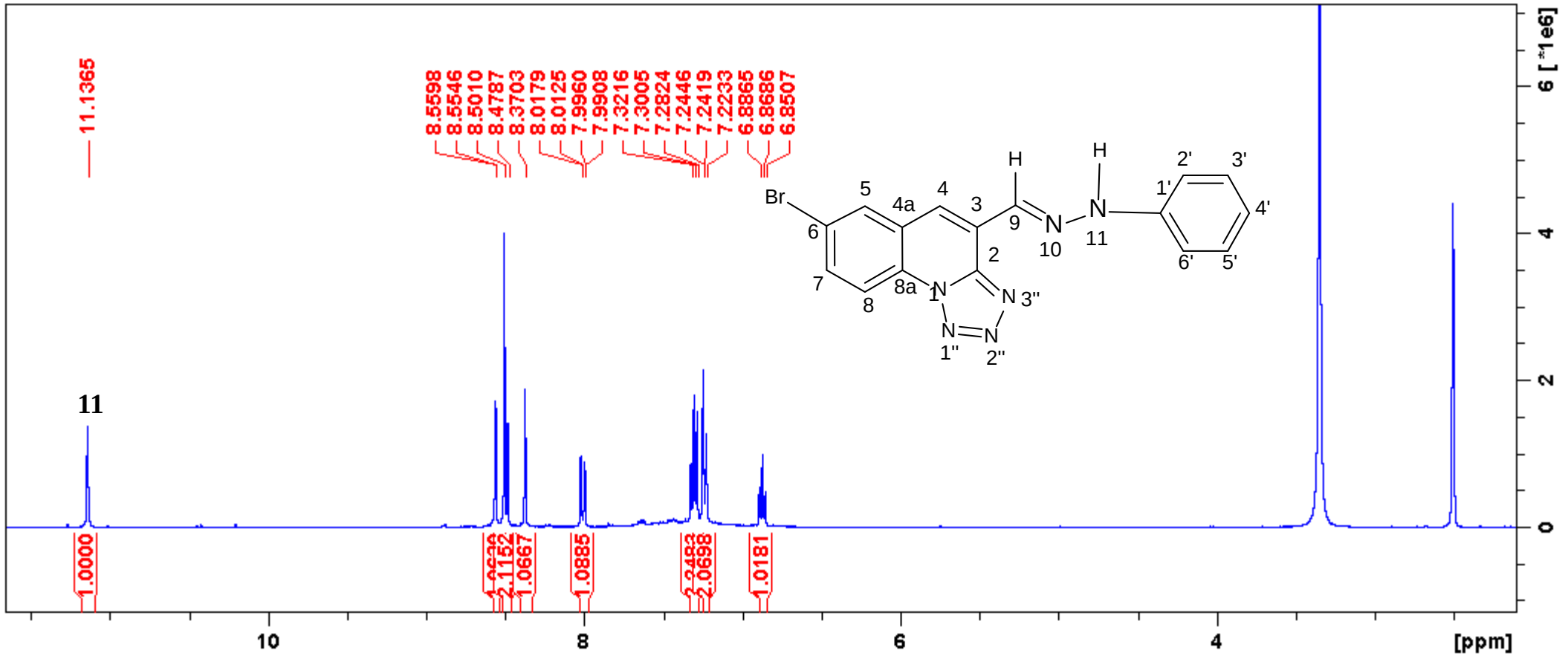
The IR spectrum of compound 5b

Wavelength/nm	Absorbance	Log ε
242	0.79	4.38
277	0.24	3.86
308	0.12	3.56

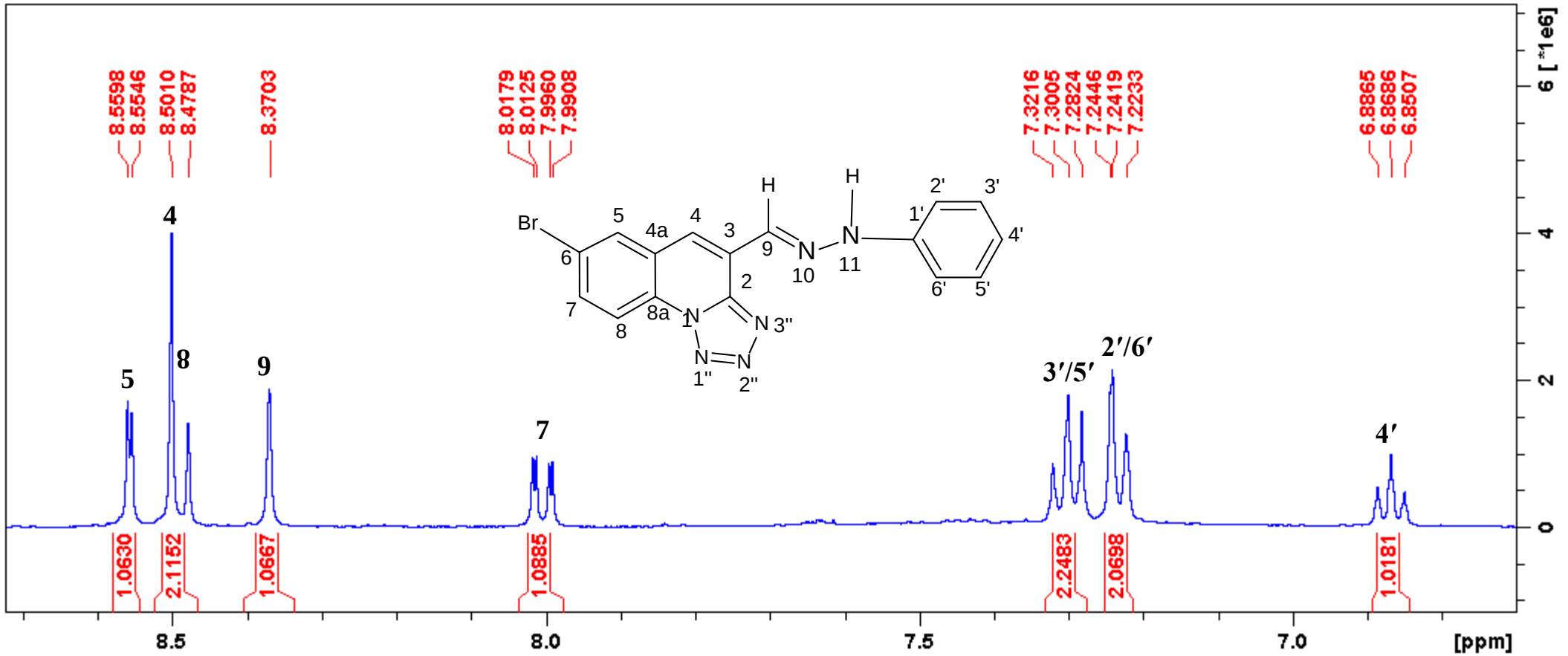




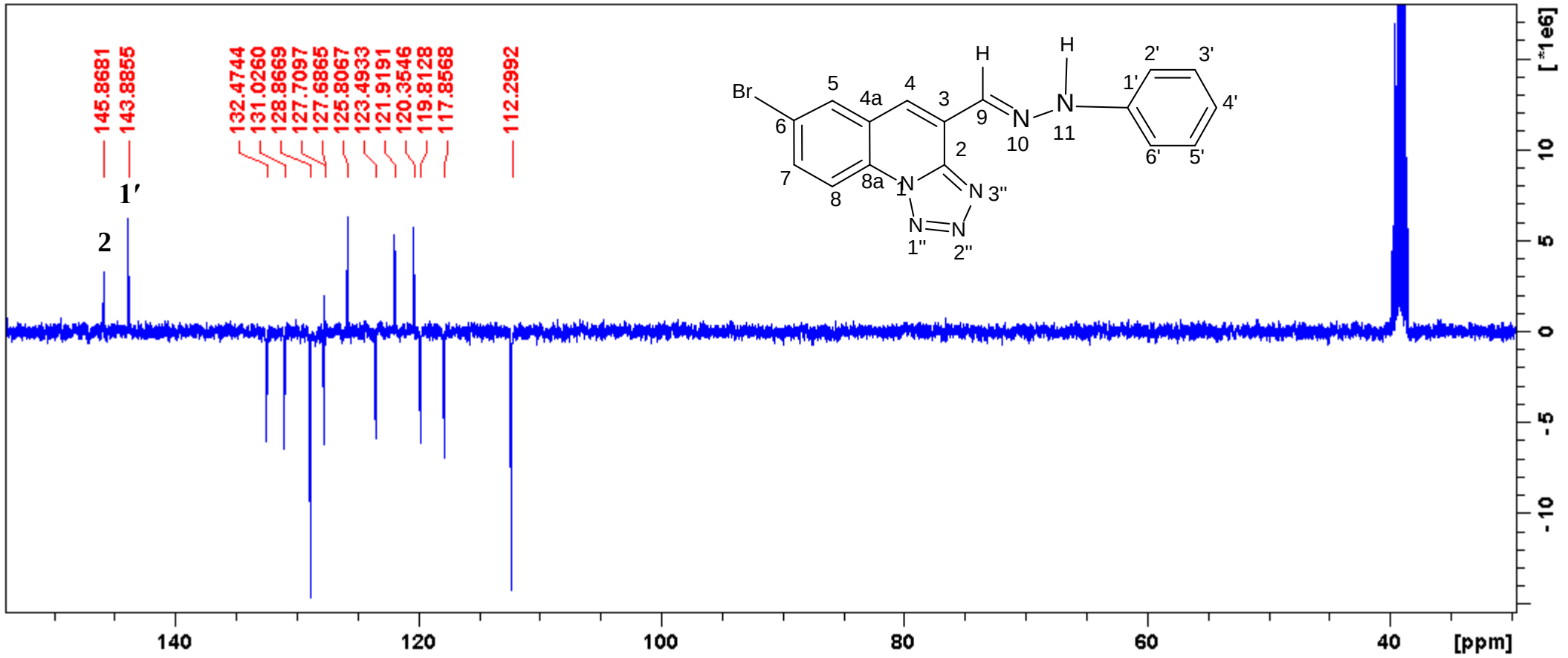
The UV-Vis spectrum of compound 5b



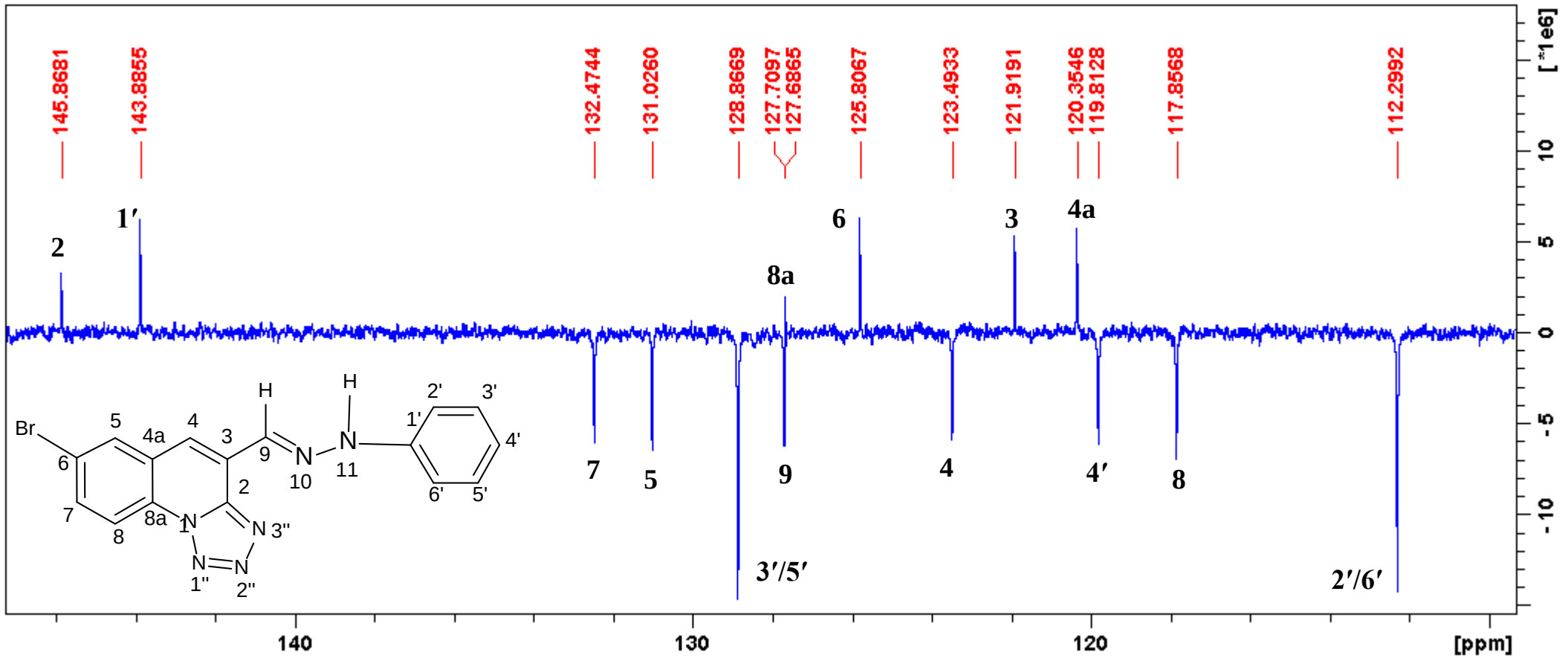
The ^{1}H NMR spectrum of compound 5c



The ^{1}H NMR spectrum of compound 5c (expanded)



The ^{13}C NMR spectrum of compound 5c

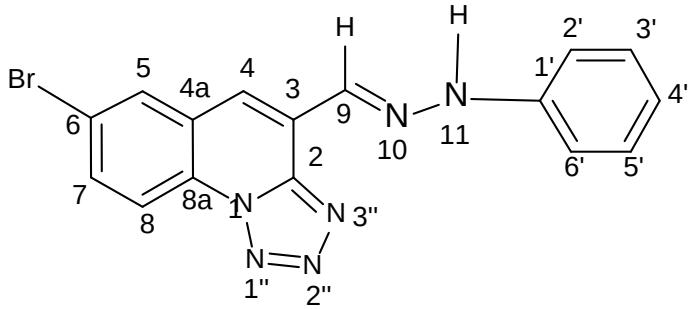


The ^{13}C NMR spectrum of compound 5c-expanded

Elemental Composition Report

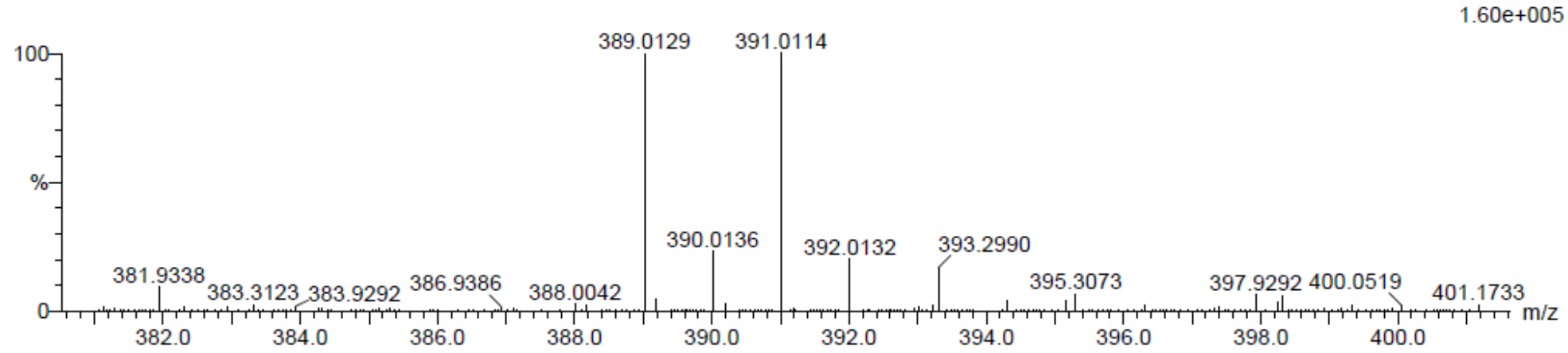
Single Mass Analysis

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



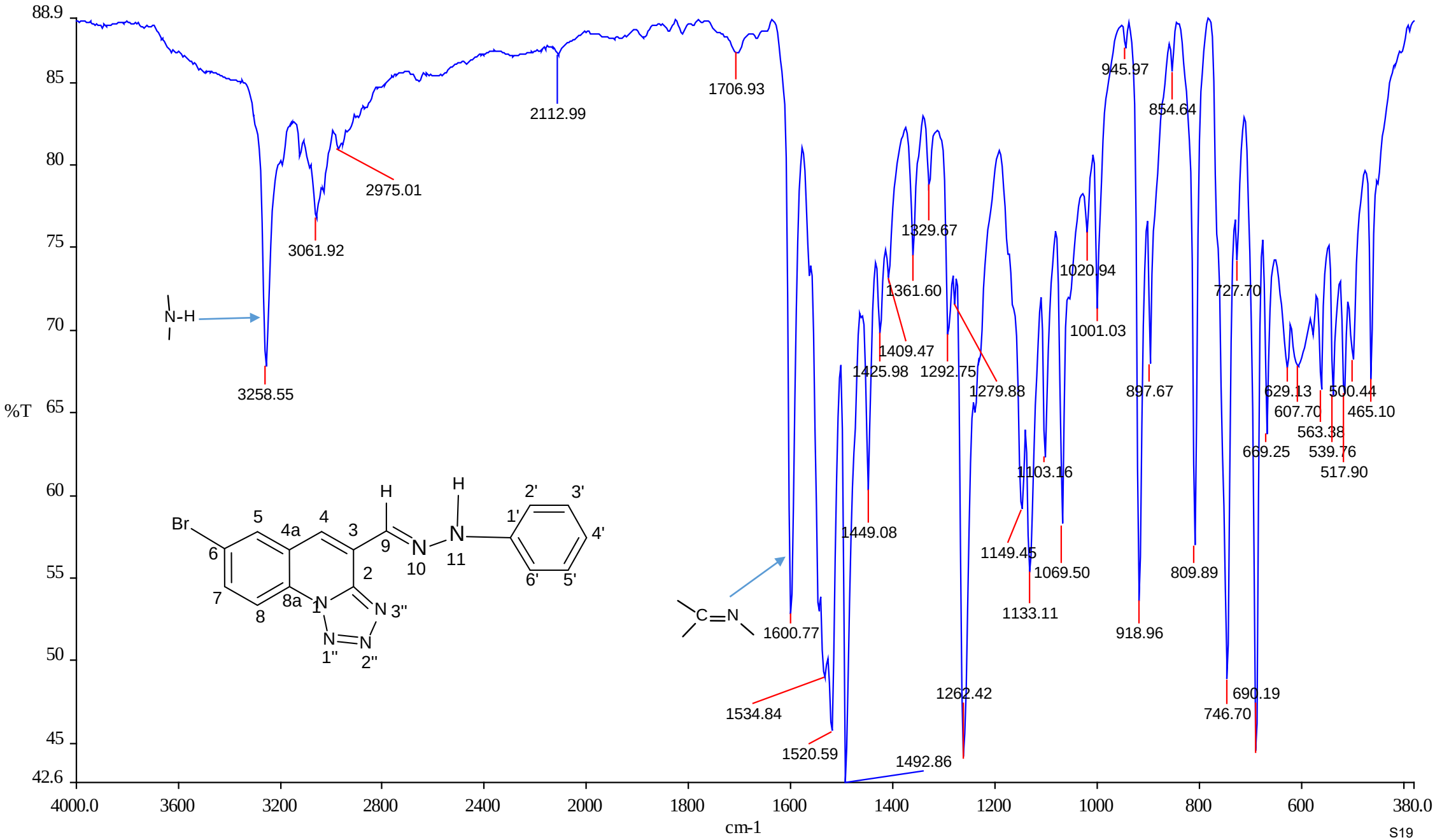
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-10 Na: 0-1 Br: 0-1

Compound 2 HG 31 (1.011) Cm (1:61)
TOF MS ES+

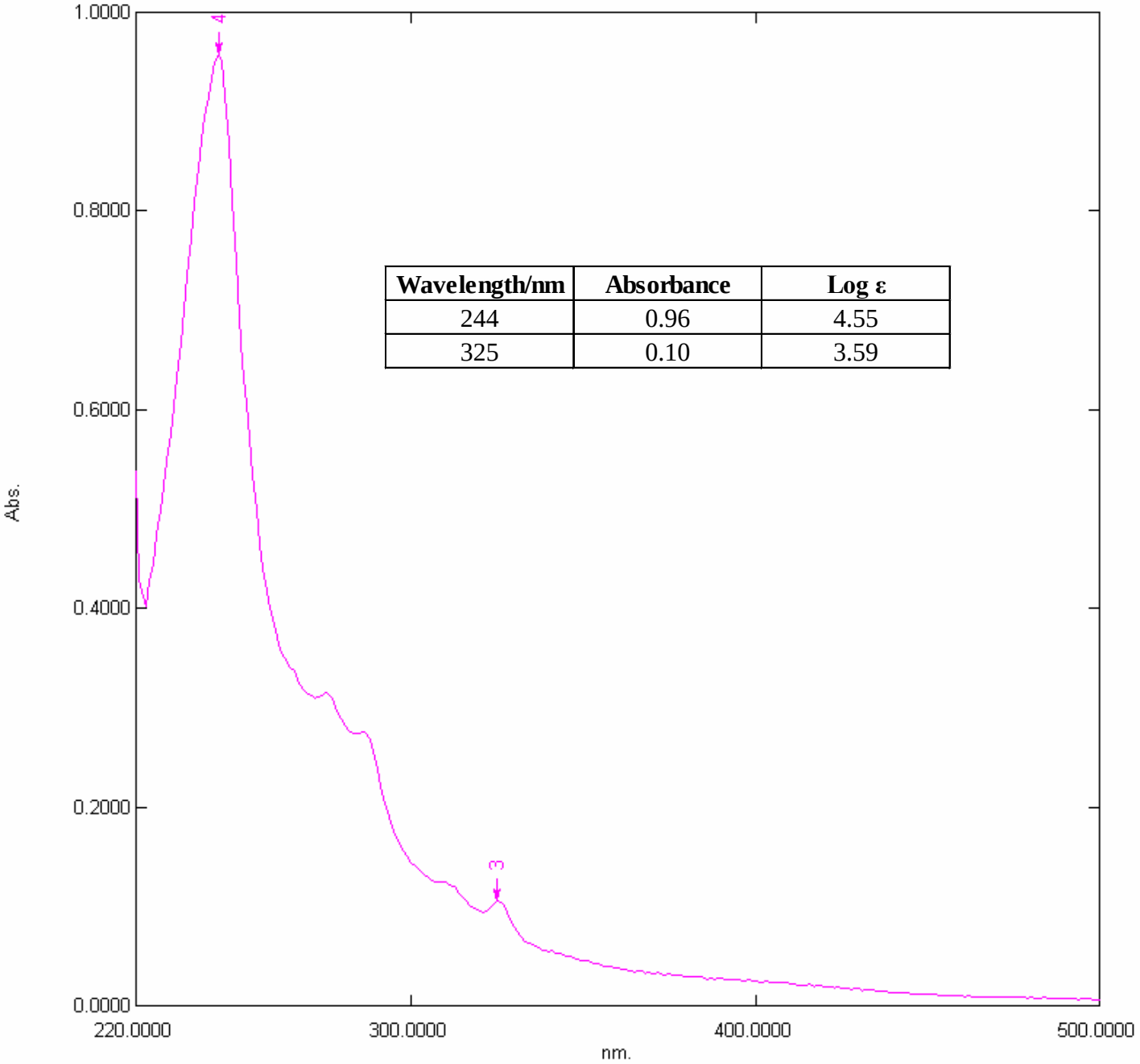


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
389.0129	389.0126	0.3	0.8	13.5	455.1	0.0	C16 H11 N6 Na Br

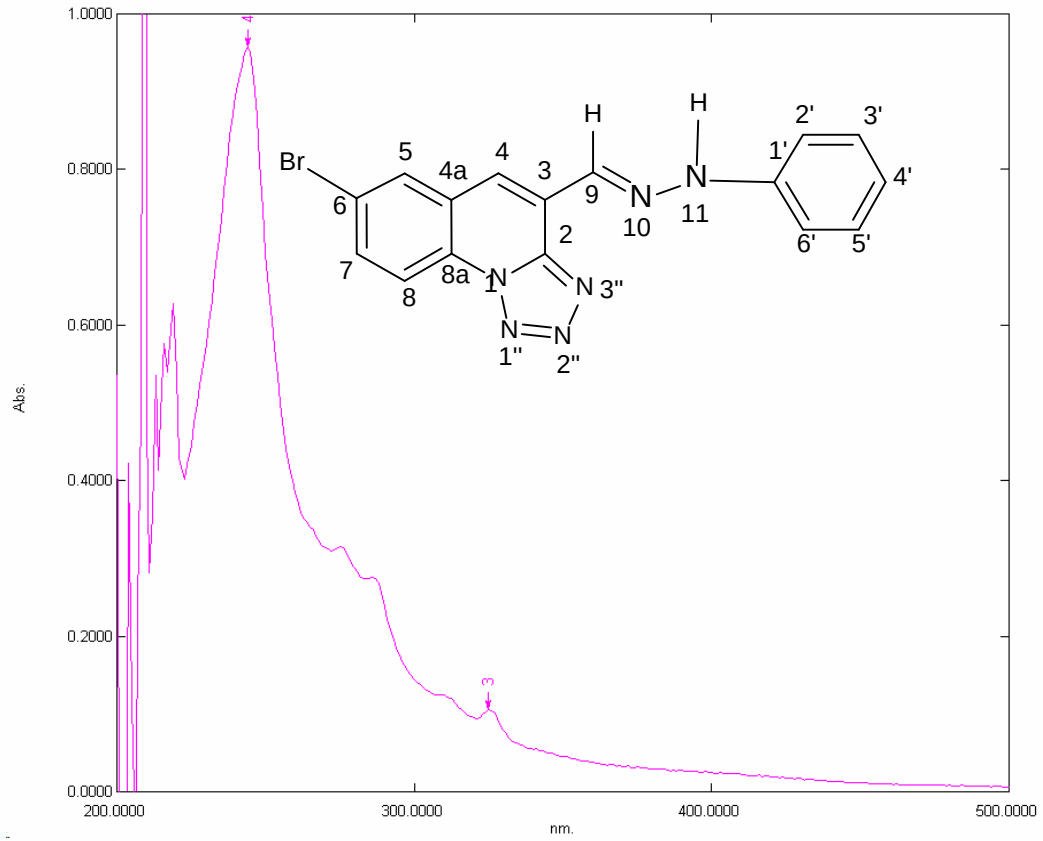
The HRMS spectrum of compound 5c



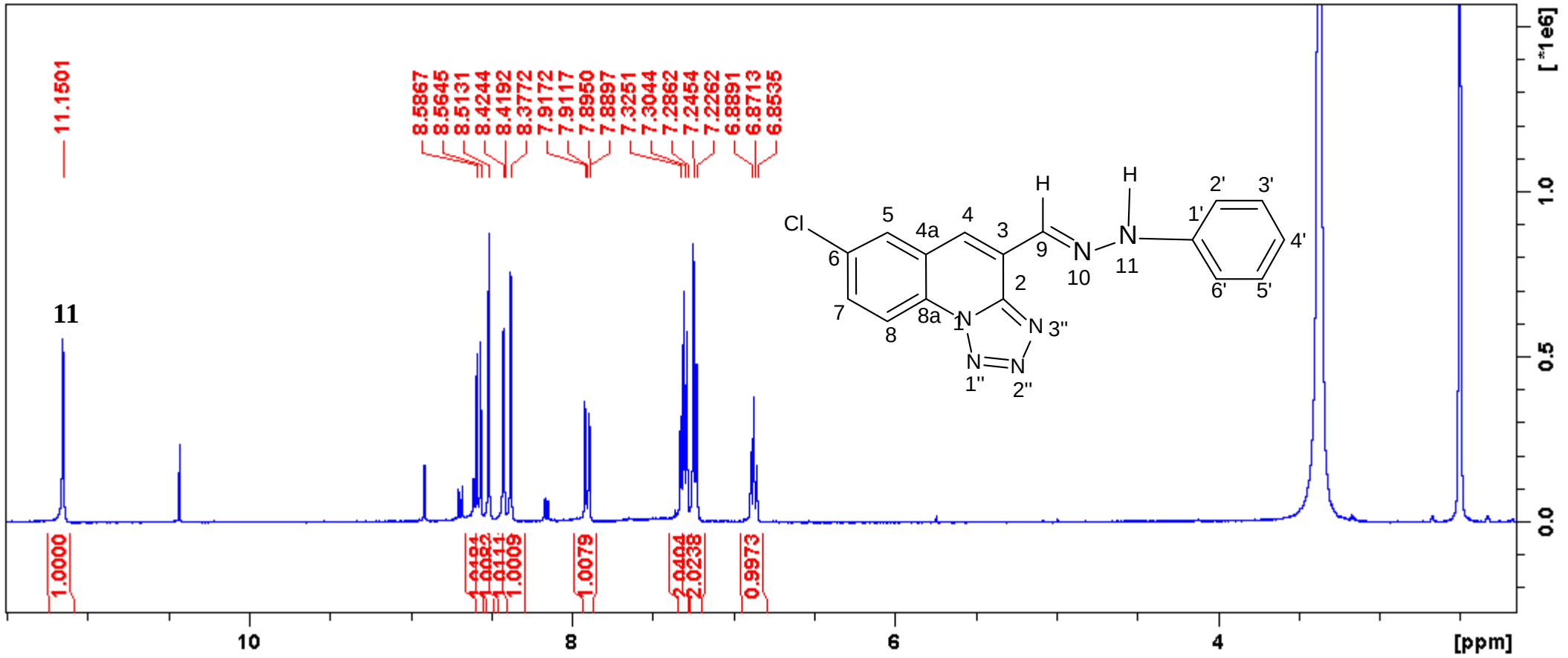
The IR spectrum of compound 5c



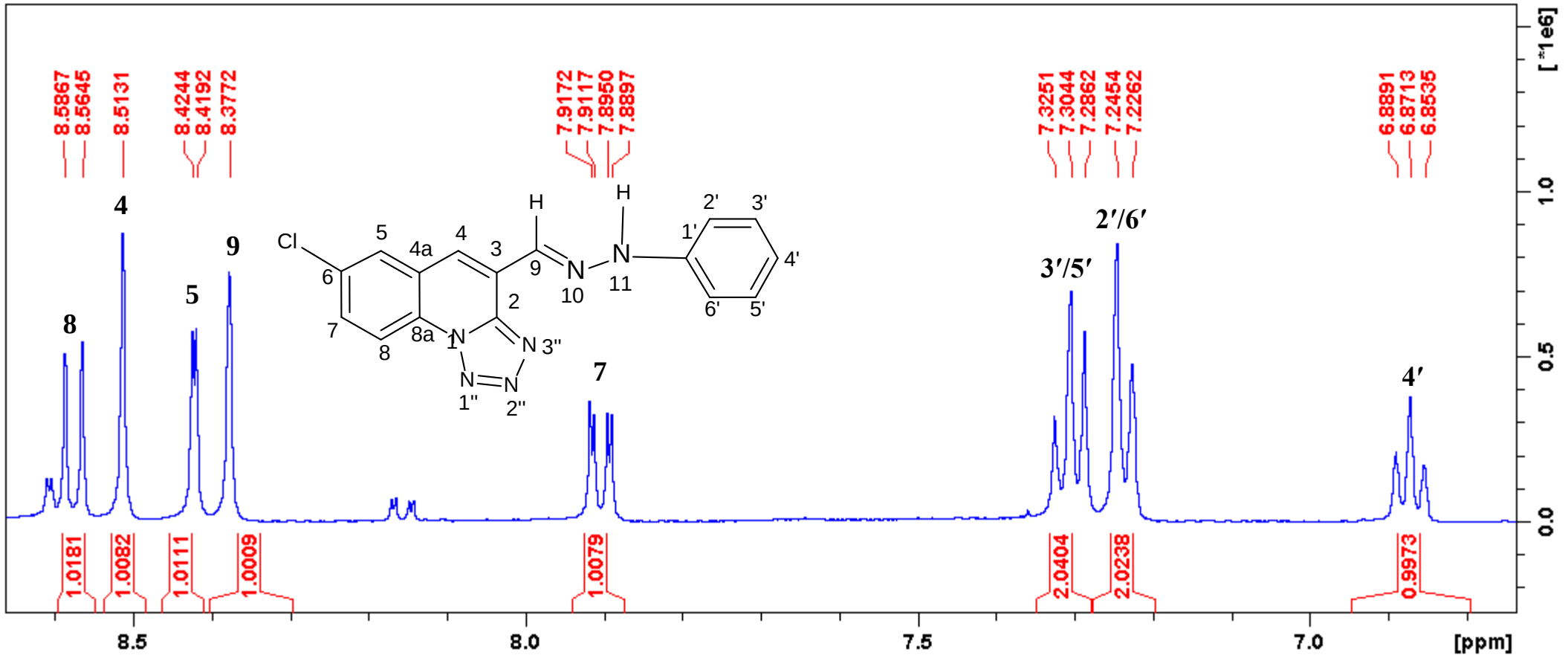
Wavelength/nm	Absorbance	Log ε
244	0.96	4.55
325	0.10	3.59



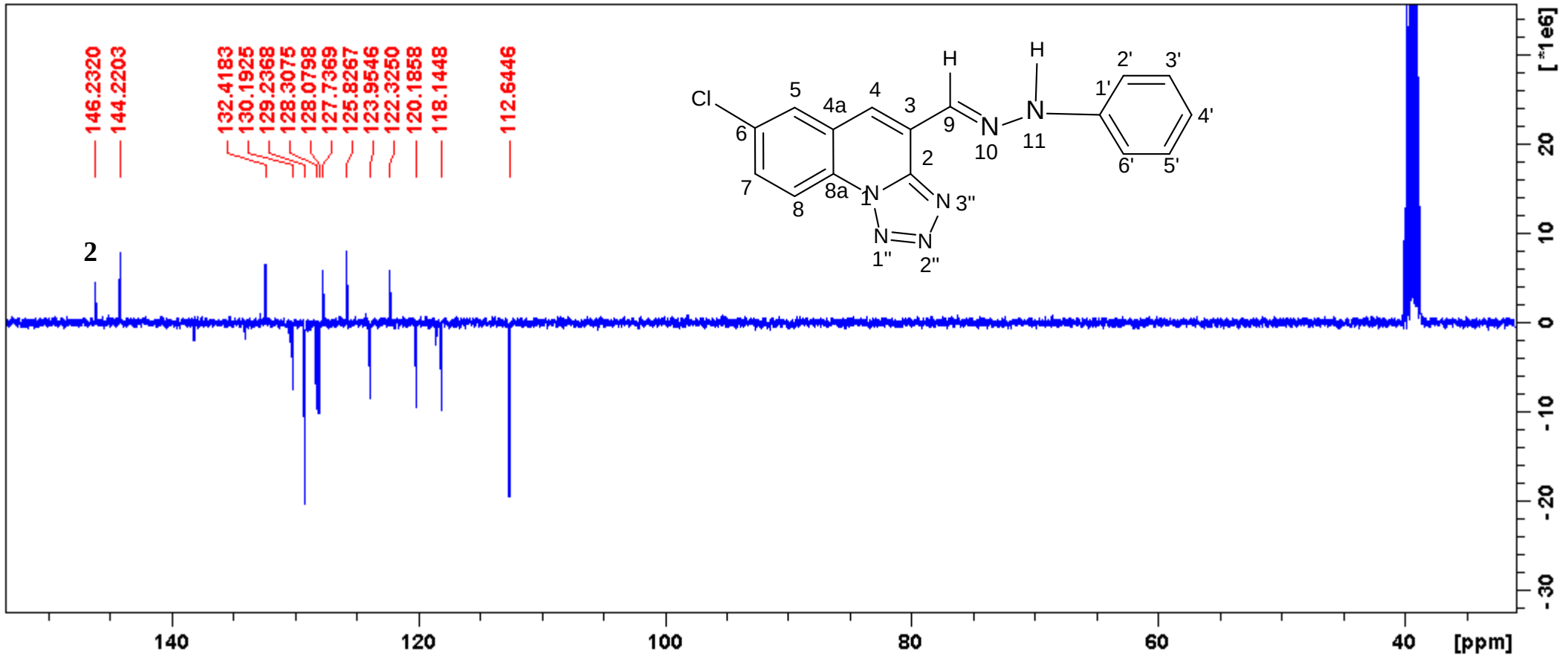
The UV-Vis spectrum of compound 5c



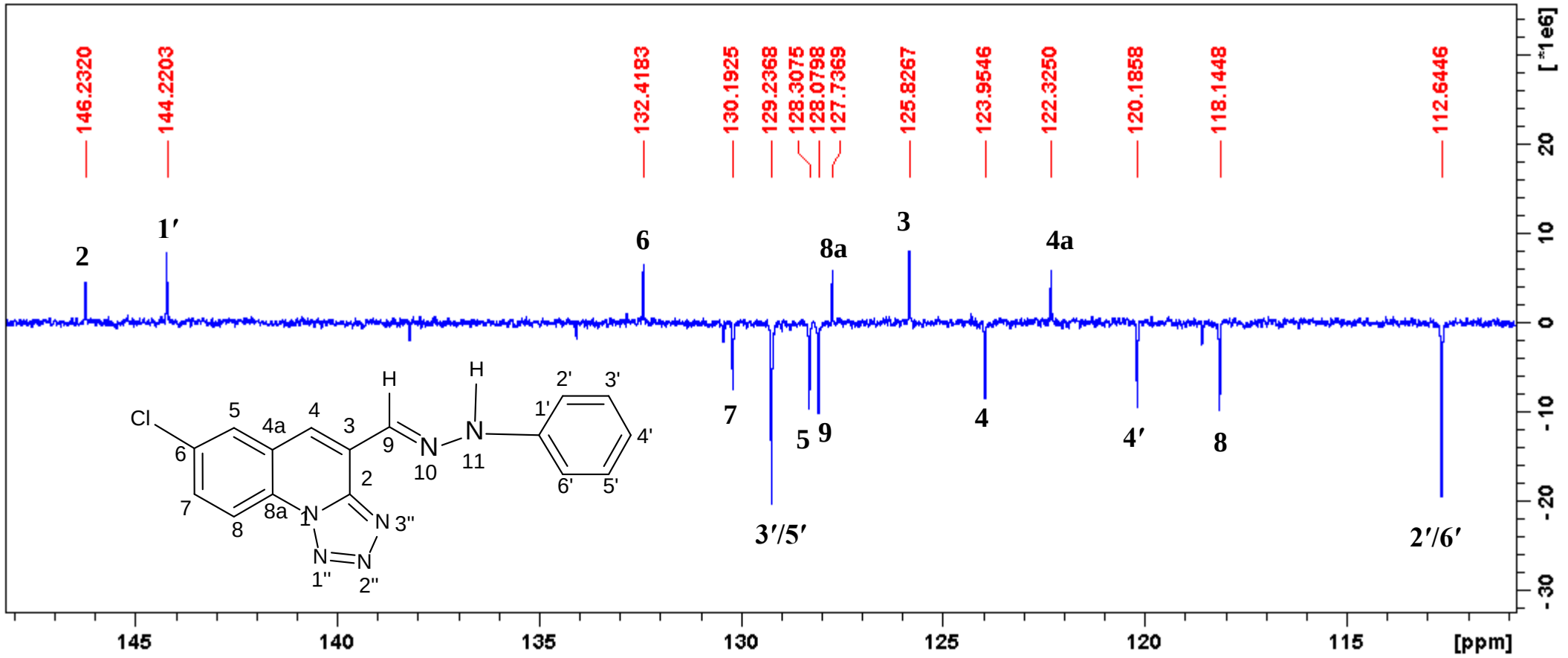
The ^{1}H NMR spectrum of compound 5d



The ^{1}H NMR spectrum of compound 5d (expanded)



The ^{13}C NMR spectrum of compound 5d

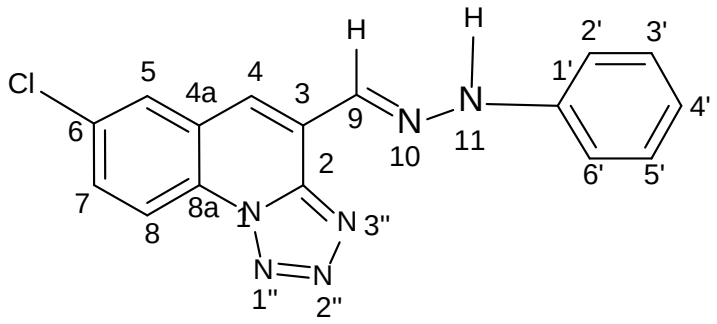


The ^{13}C NMR spectrum of compound 5d (expanded)

Elemental Composition Report

Single Mass Analysis

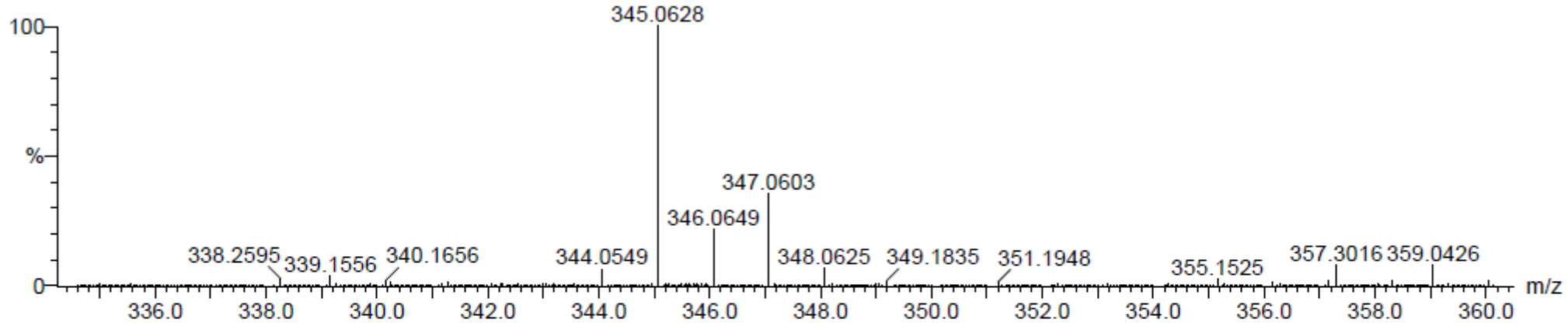
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
11 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: 5-10 Na: 0-1 Cl: 0-1

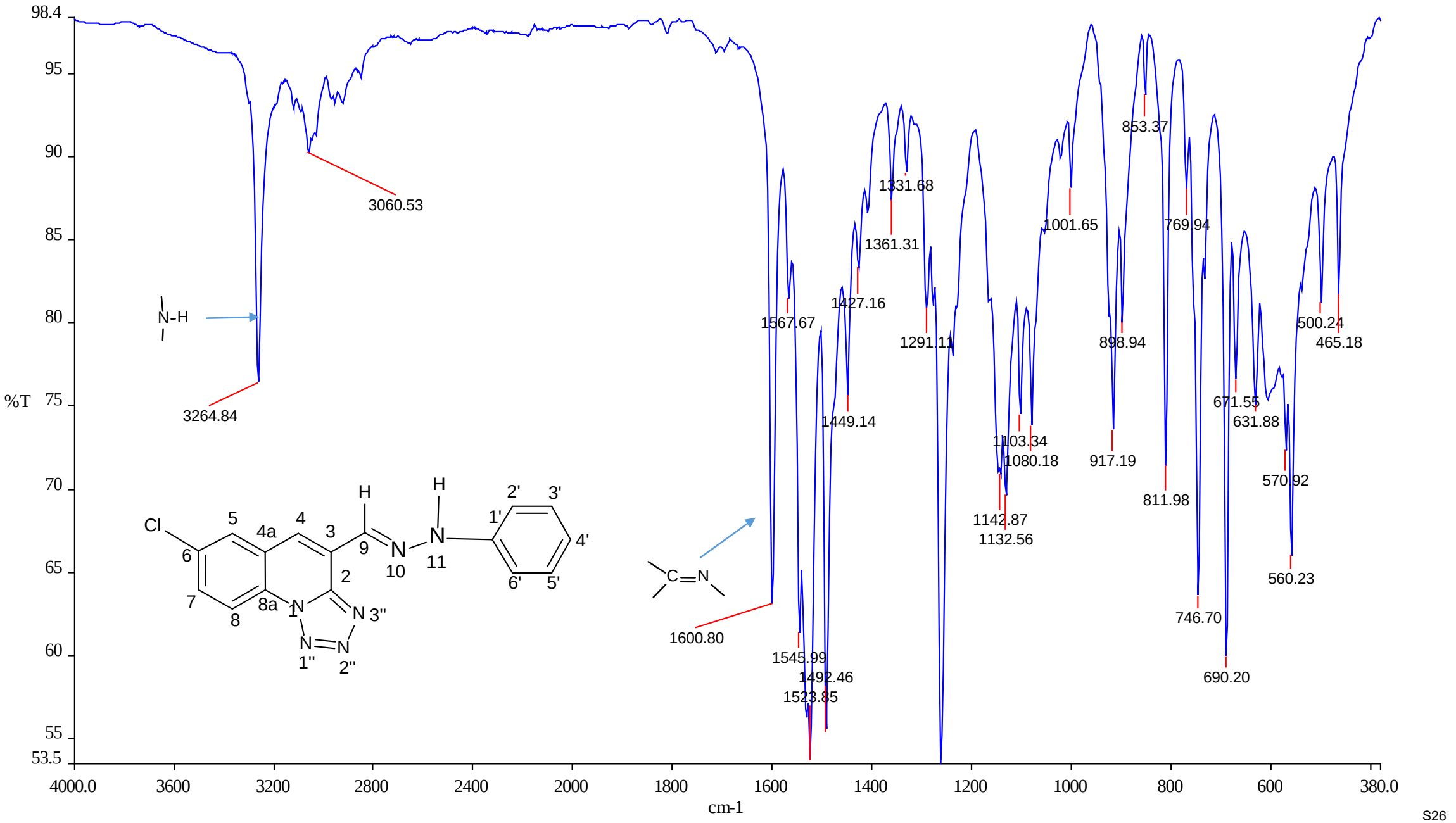
Compound 2e HG 32 (1.045) Cm (1:61)
TOF MS ES+

7.05e+005



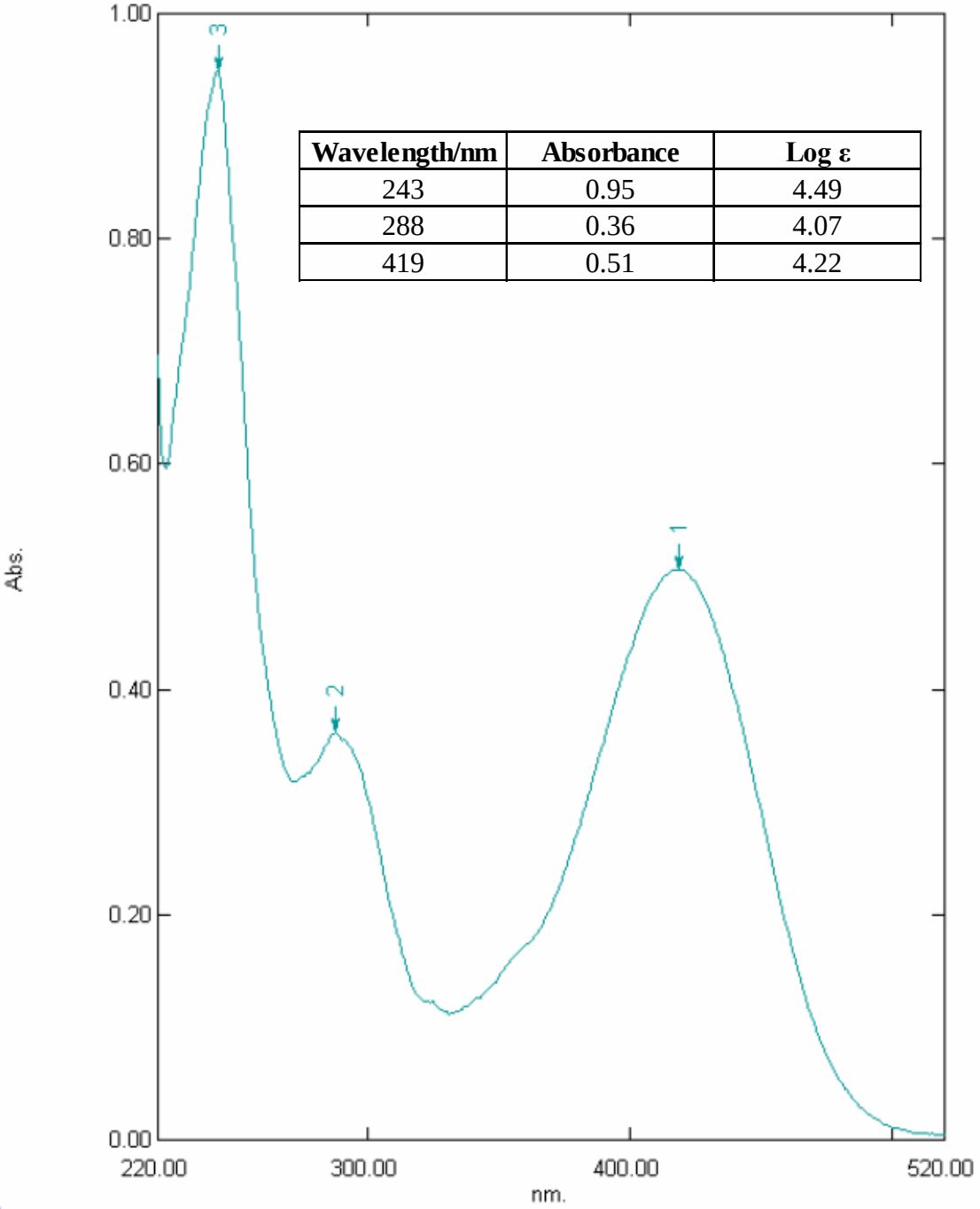
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
345.0628	345.0631	-0.3	-0.9	13.5	679.6	0.0	C16 H11 N6 Na Cl

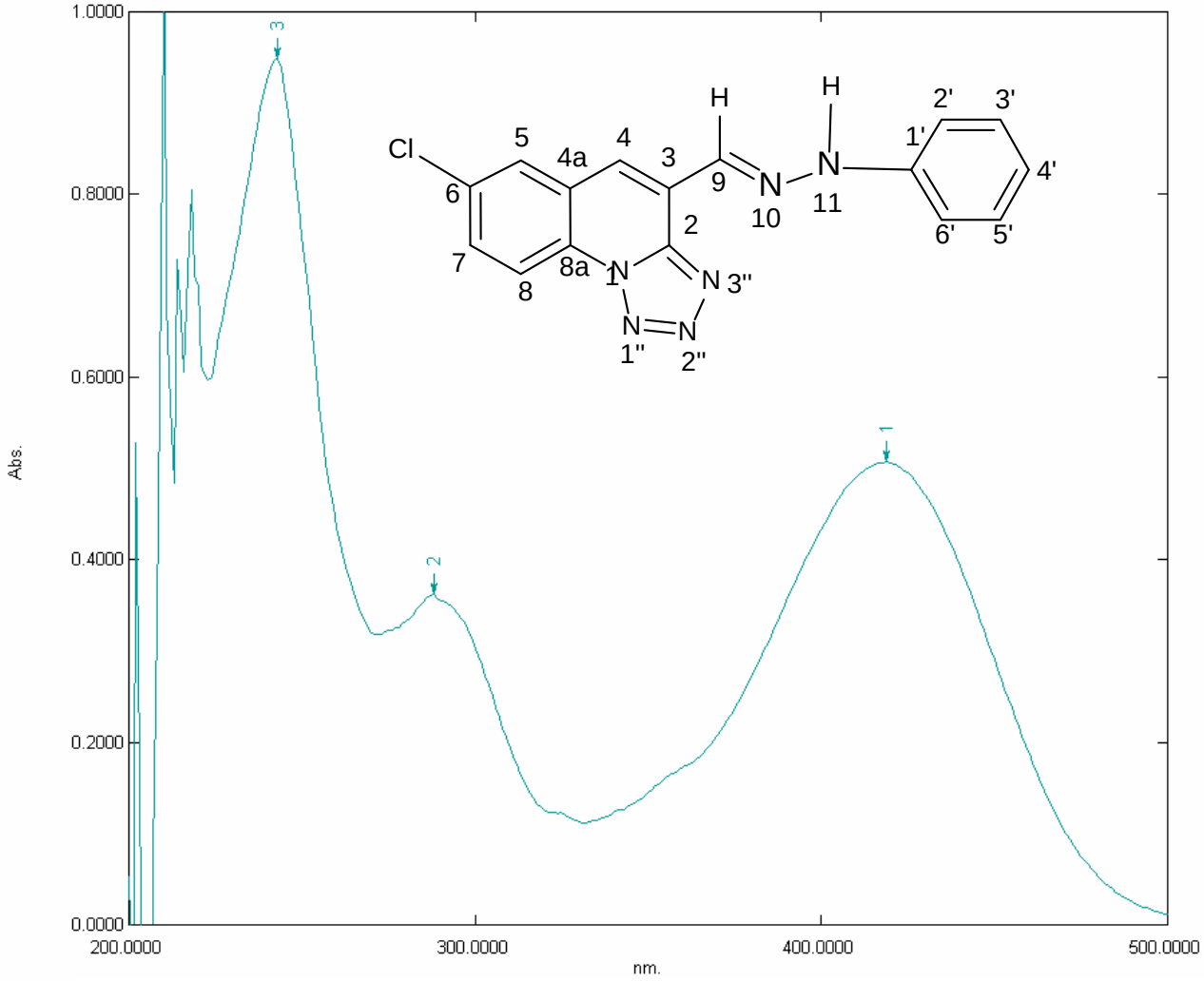
The HRMS spectrum of compound 5d



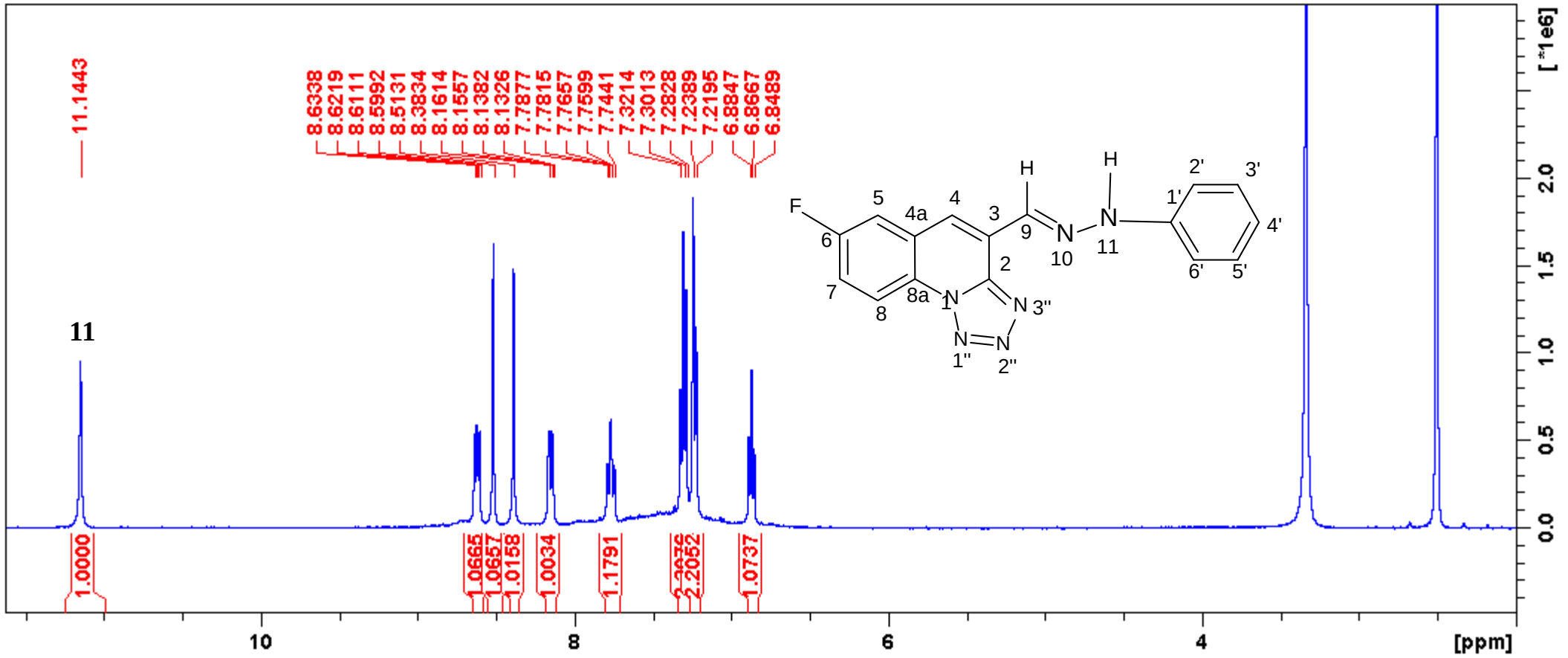
The IR spectrum of compound 5d

Wavelength/nm	Absorbance	Log ε
243	0.95	4.49
288	0.36	4.07
419	0.51	4.22

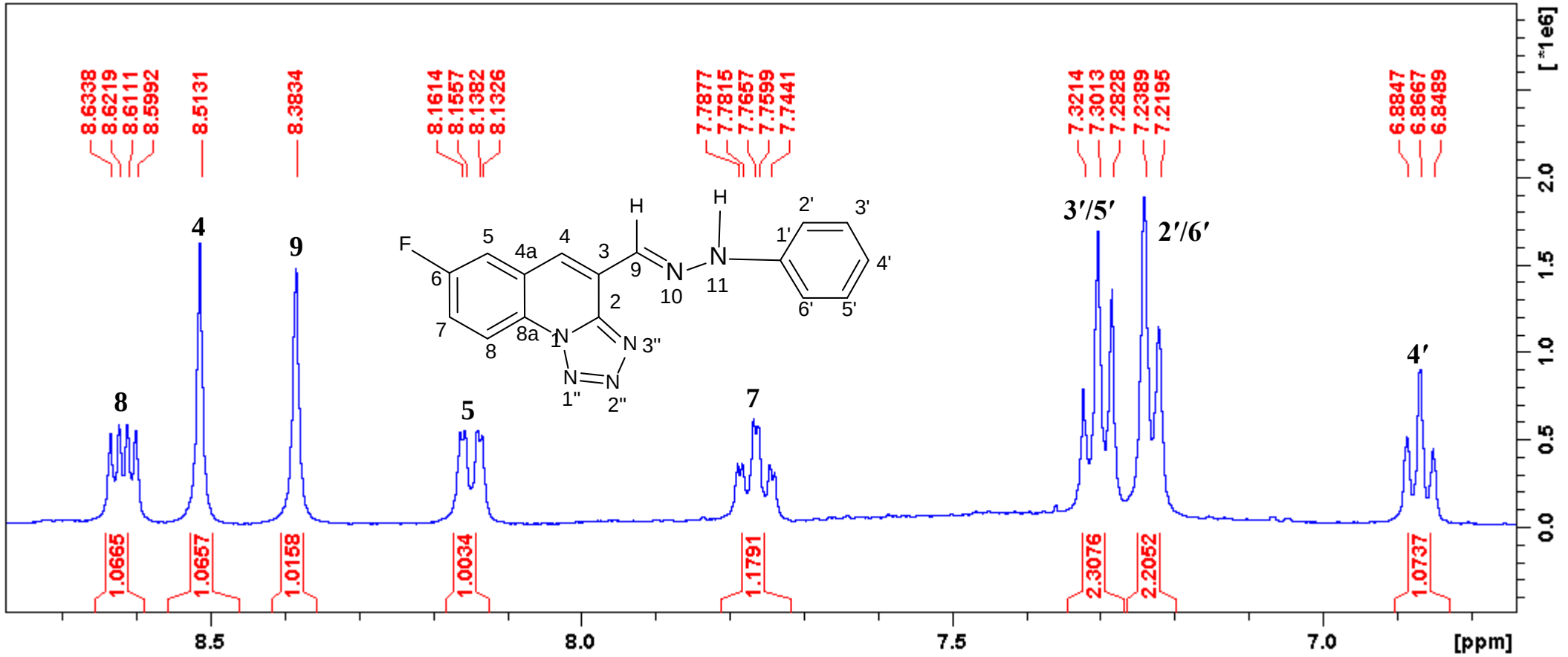




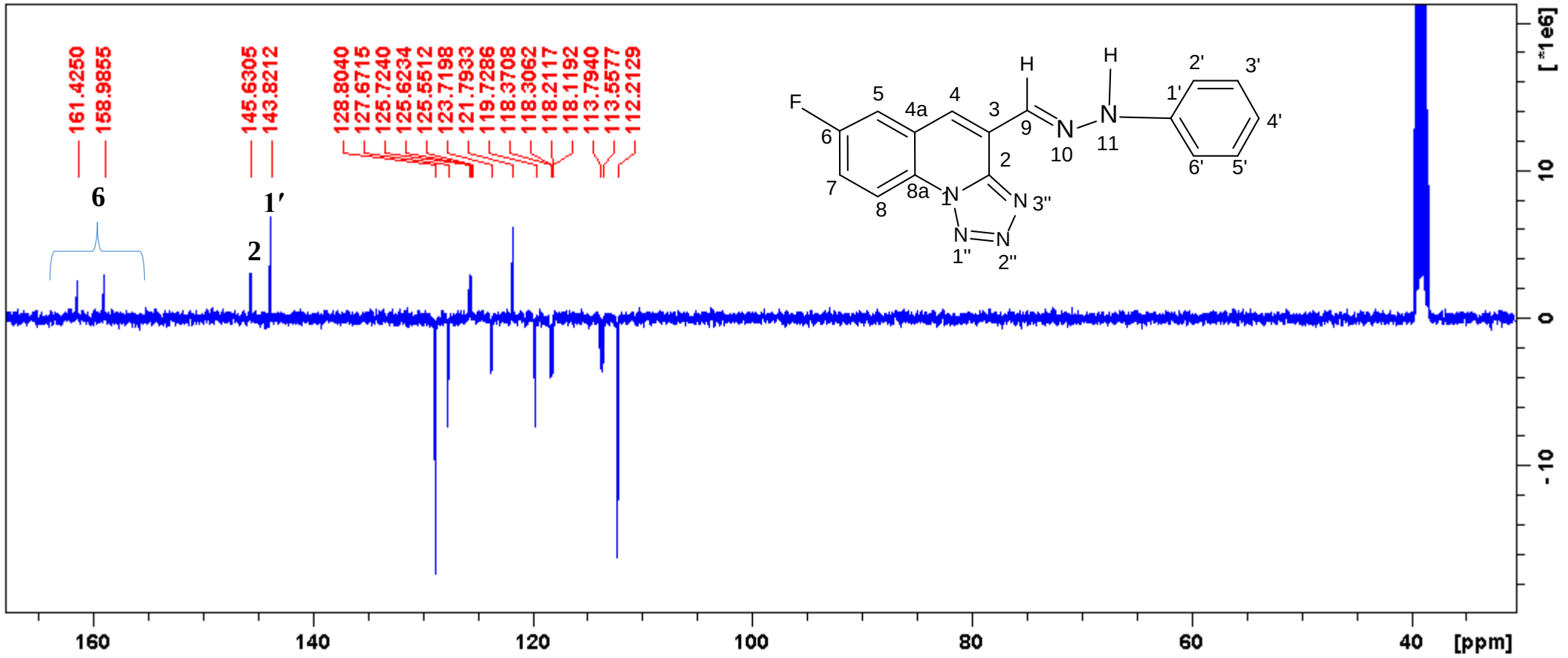
The UV-Vis spectrum of compound 5d



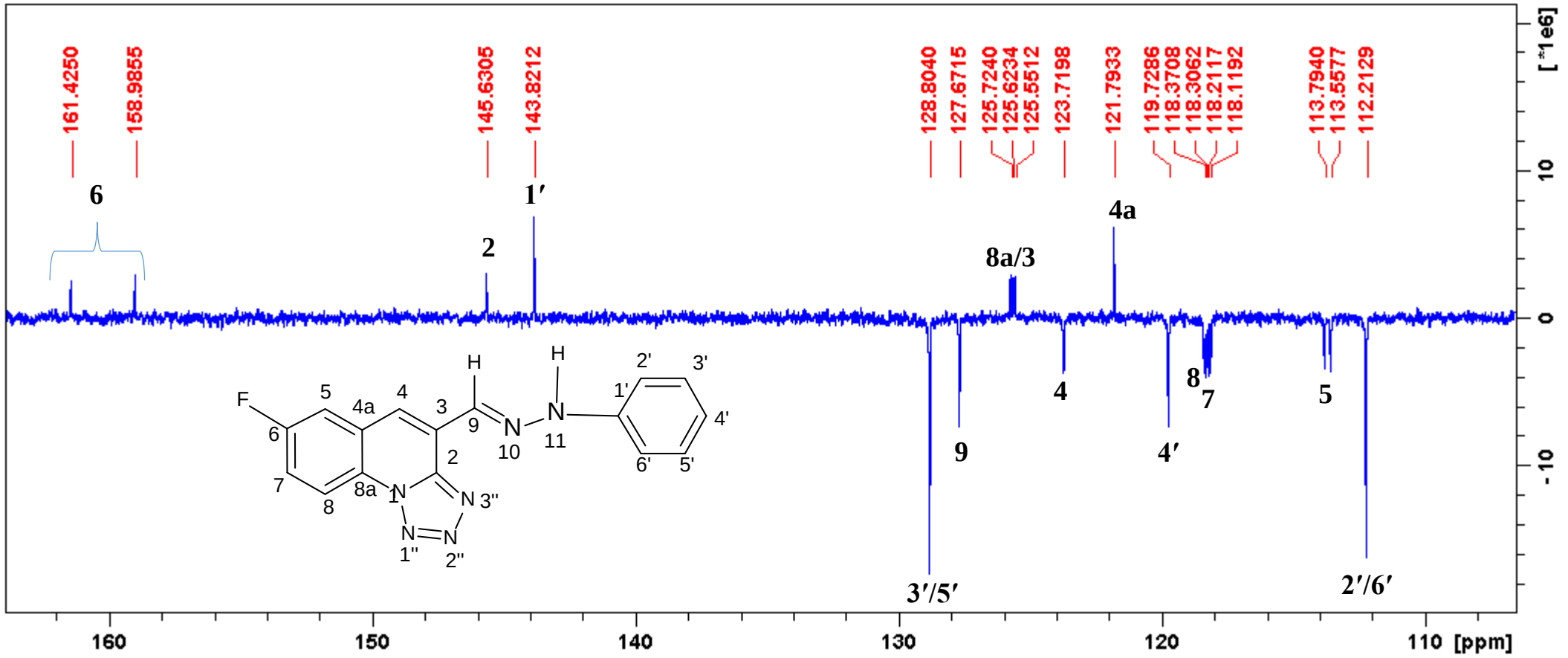
The ^{1}H NMR spectrum of compound 5e



The ^{1}H NMR spectrum of compound 5e (expanded)



The ^{13}C NMR spectrum of compound 5e

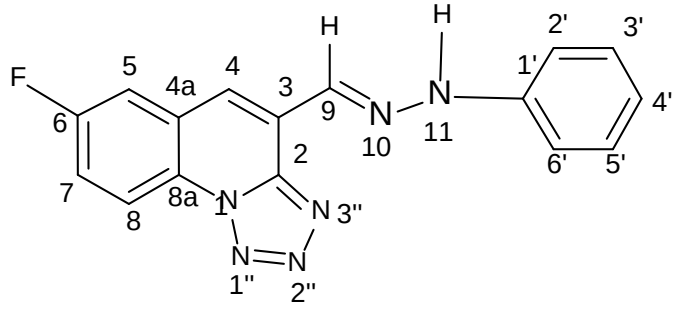


The ^{13}C NMR spectrum of compound 5e (expanded)

Elemental Composition Report

Single Mass Analysis

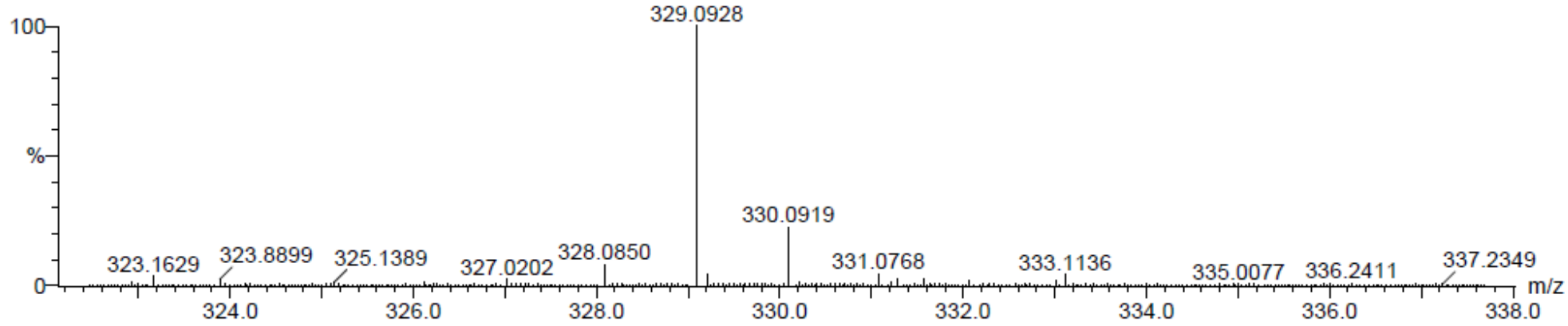
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
20 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: 0-10 F: 0-1 Na: 0-1

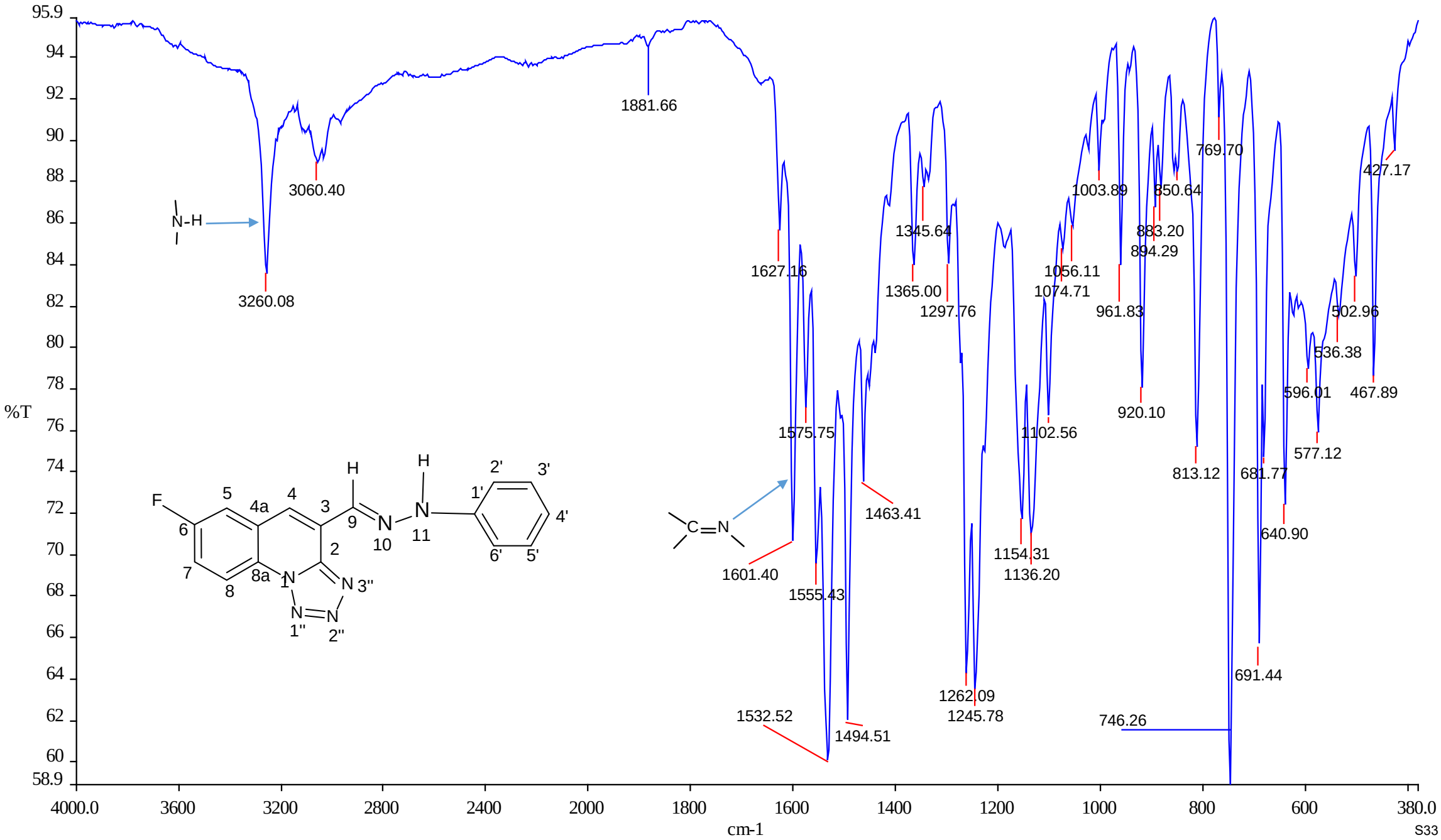
Compound 3 HG 56 (1.855) Cm (1:61)
TOF MS ES+

1.01e+005



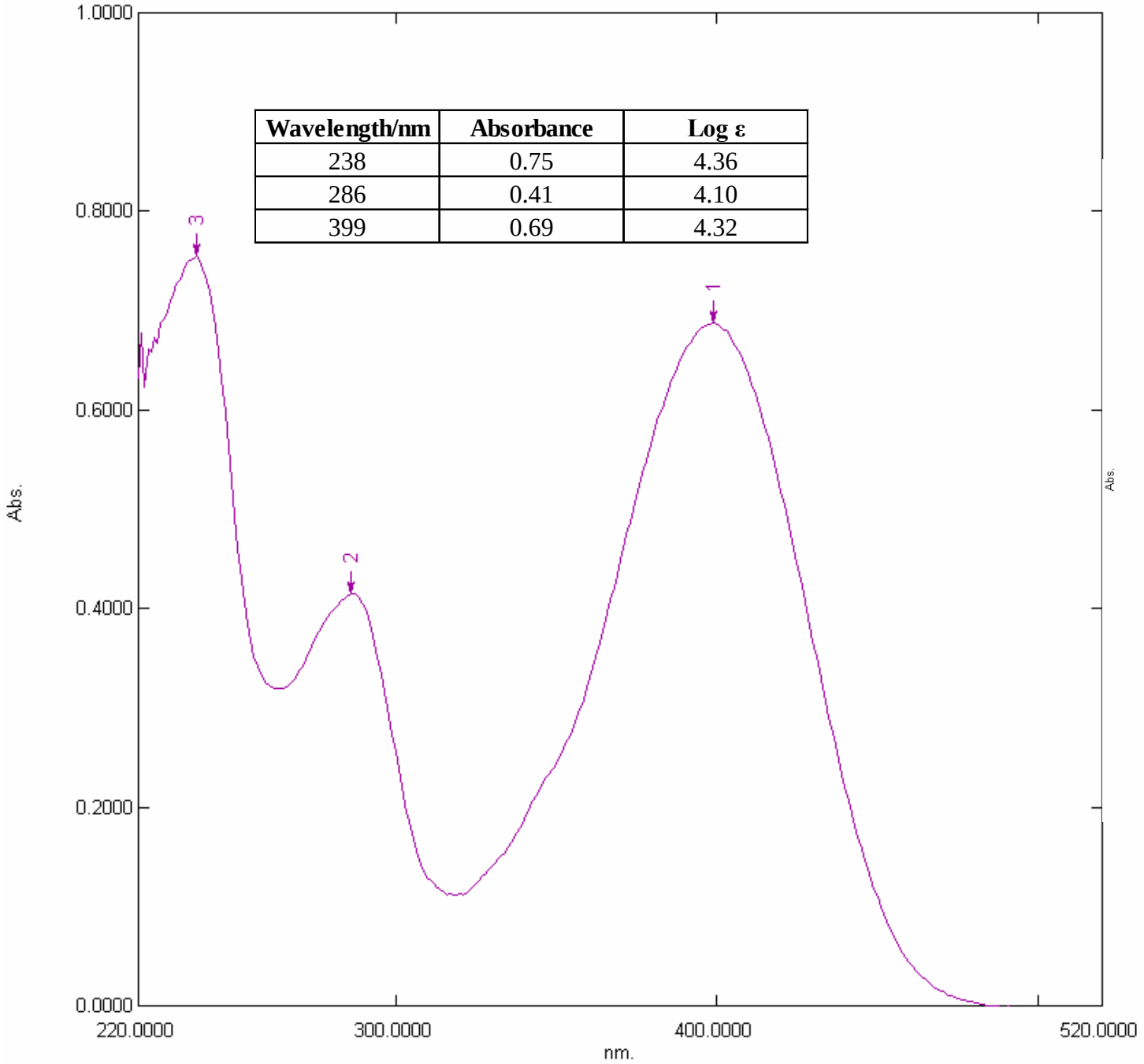
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
329.0928	329.0927	0.1	0.3	13.5	614.8	0.0	C16 H11 N6 F Na

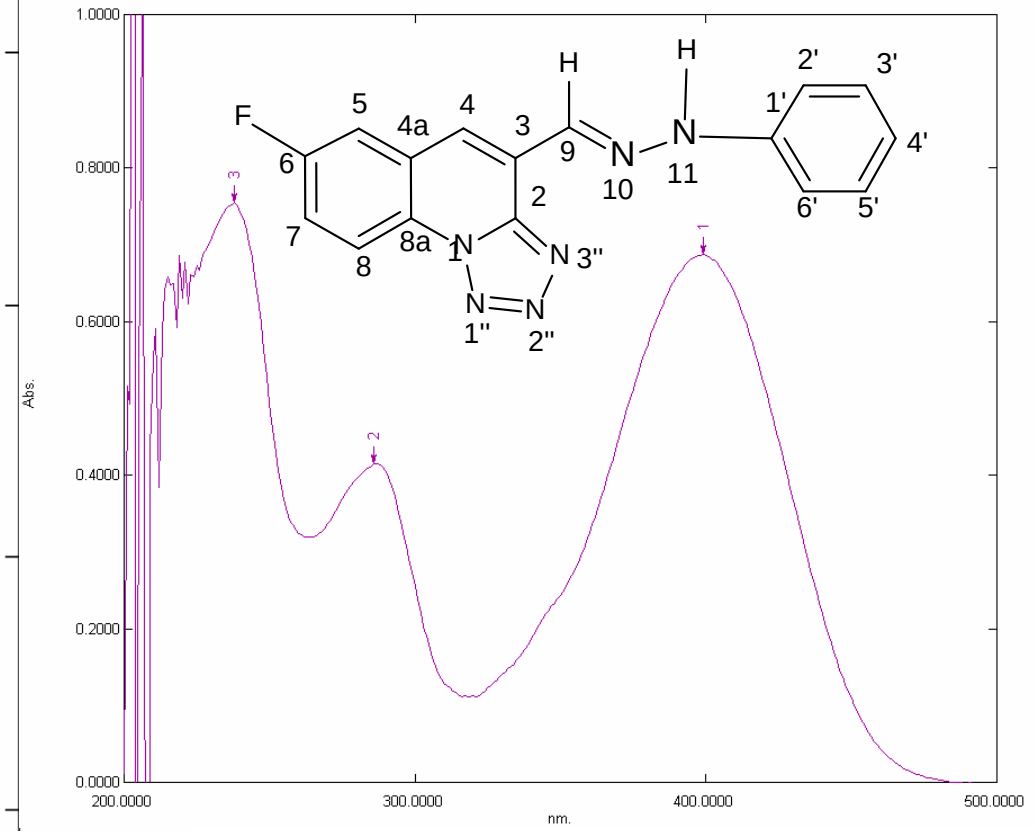
The HRMS spectrum of compound 5e



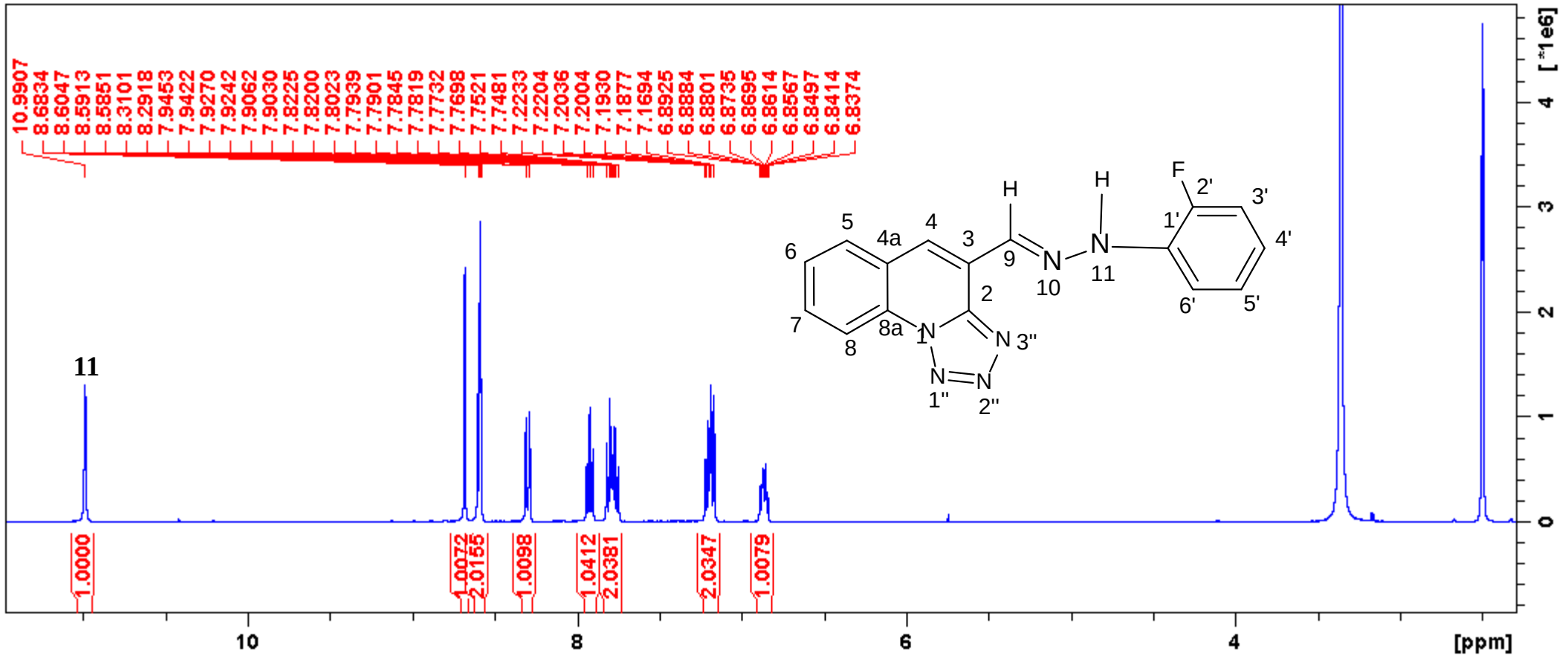
The IR spectrum of compound 5e

Wavelength/nm	Absorbance	Log ε
238	0.75	4.36
286	0.41	4.10
399	0.69	4.32

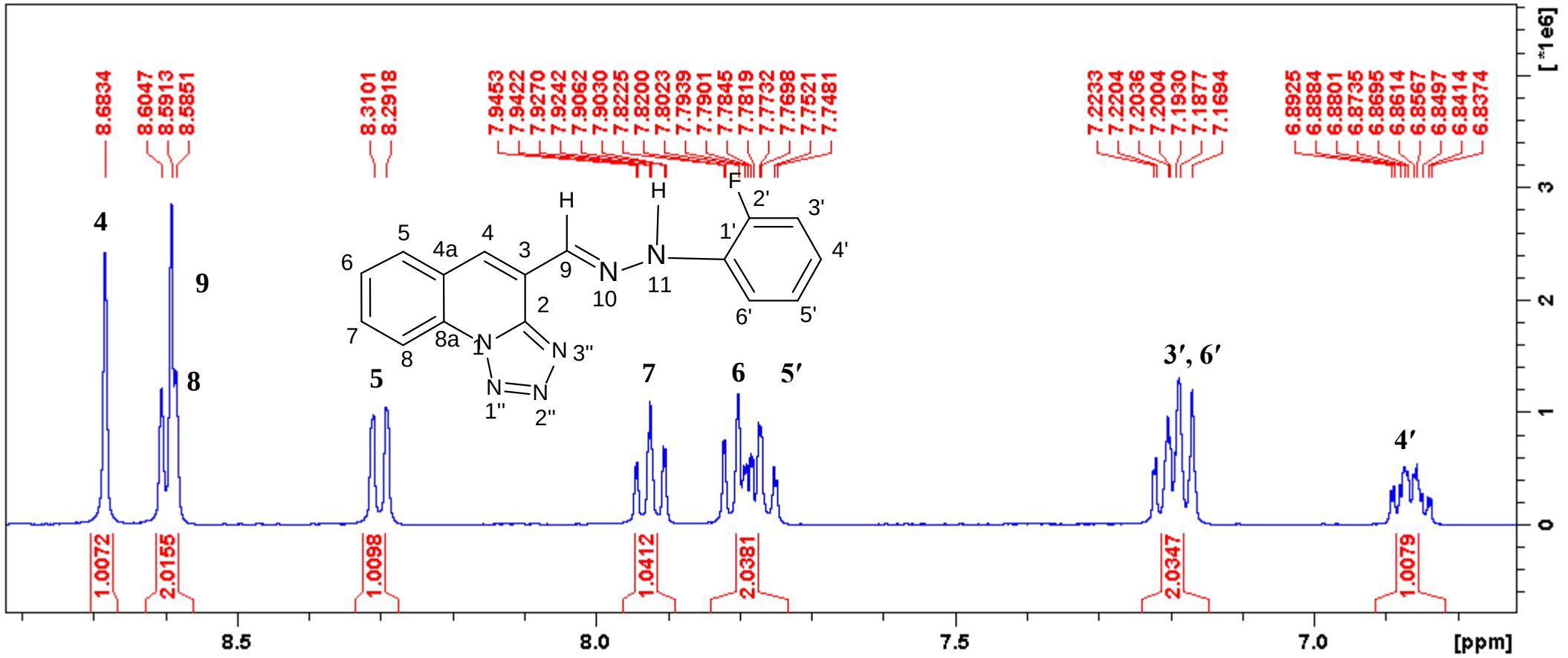




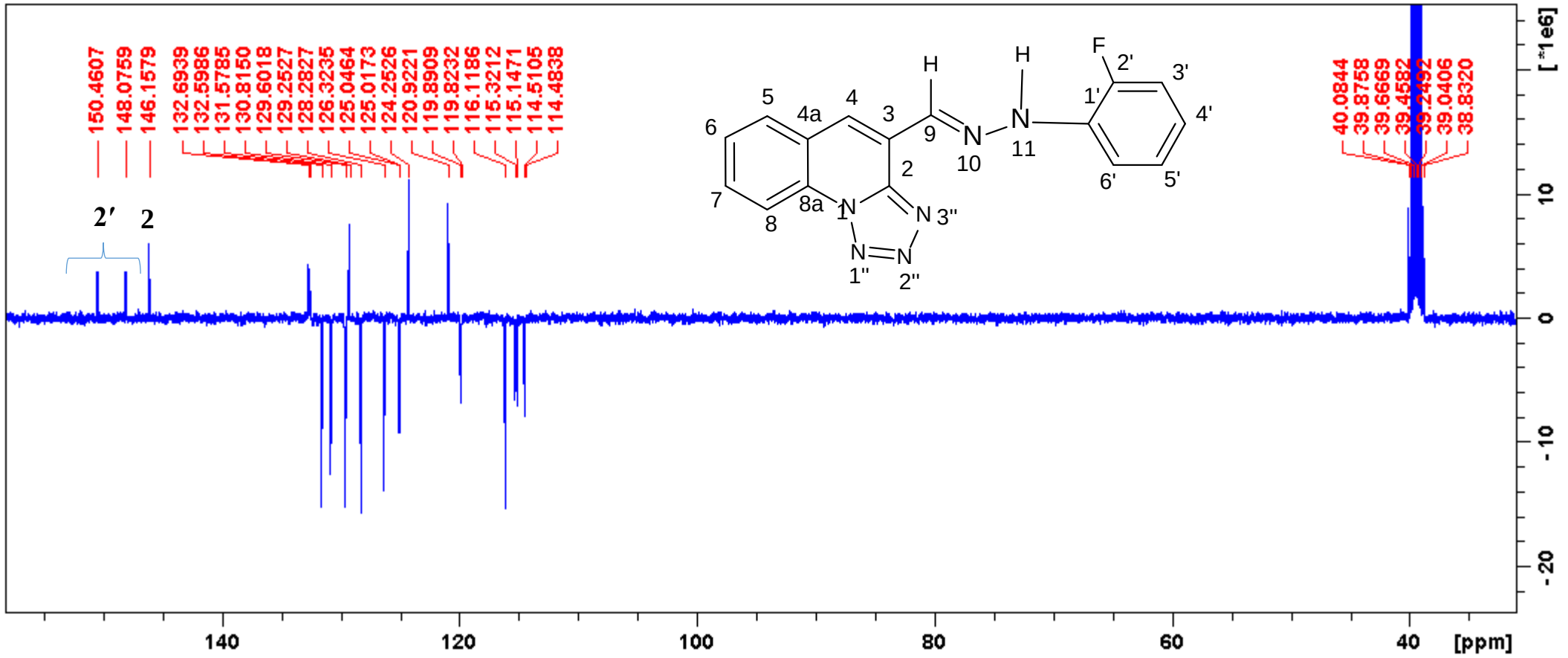
The UV-Vis spectrum of compound 5e



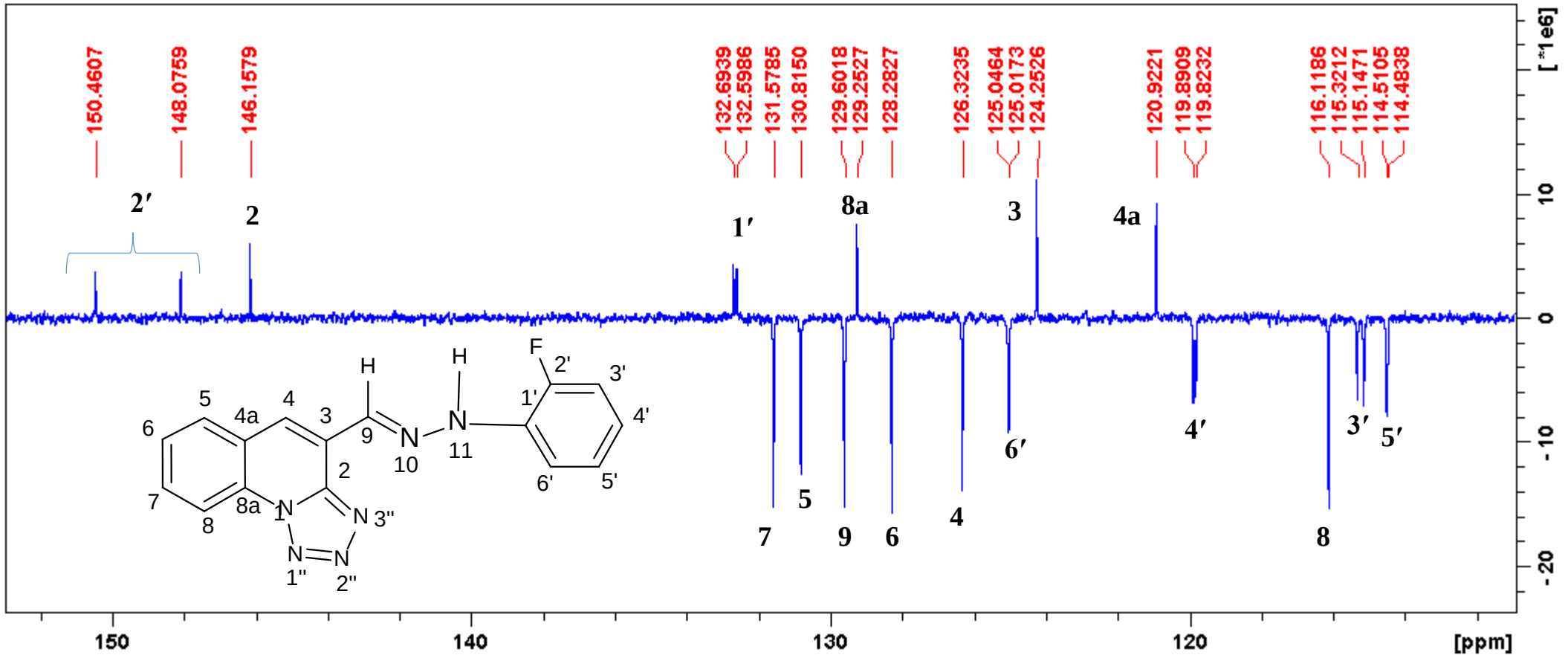
The ^{1}H NMR spectrum of compound 5f



The ^{1}H NMR spectrum of compound 5f (expanded)



The ^{13}C NMR spectrum of compound 5f



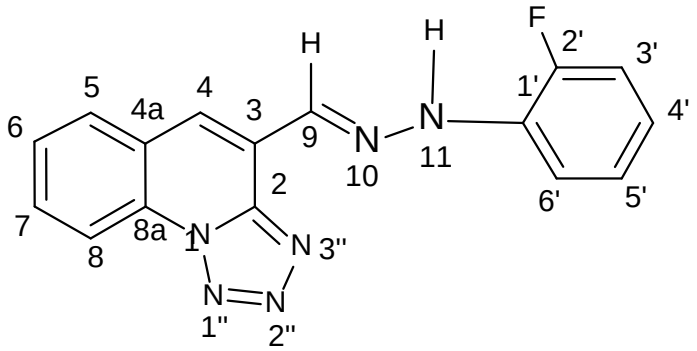
The ^{13}C NMR spectrum of compound 5f- expanded

Elemental Composition Report

Single Mass Analysis

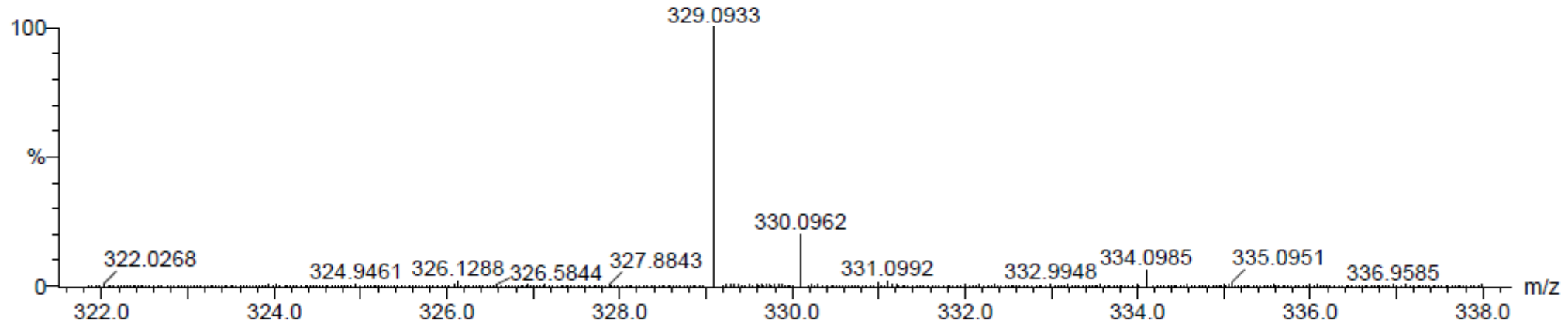
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
12 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: 5-10 F: 0-1 Na: 0-1



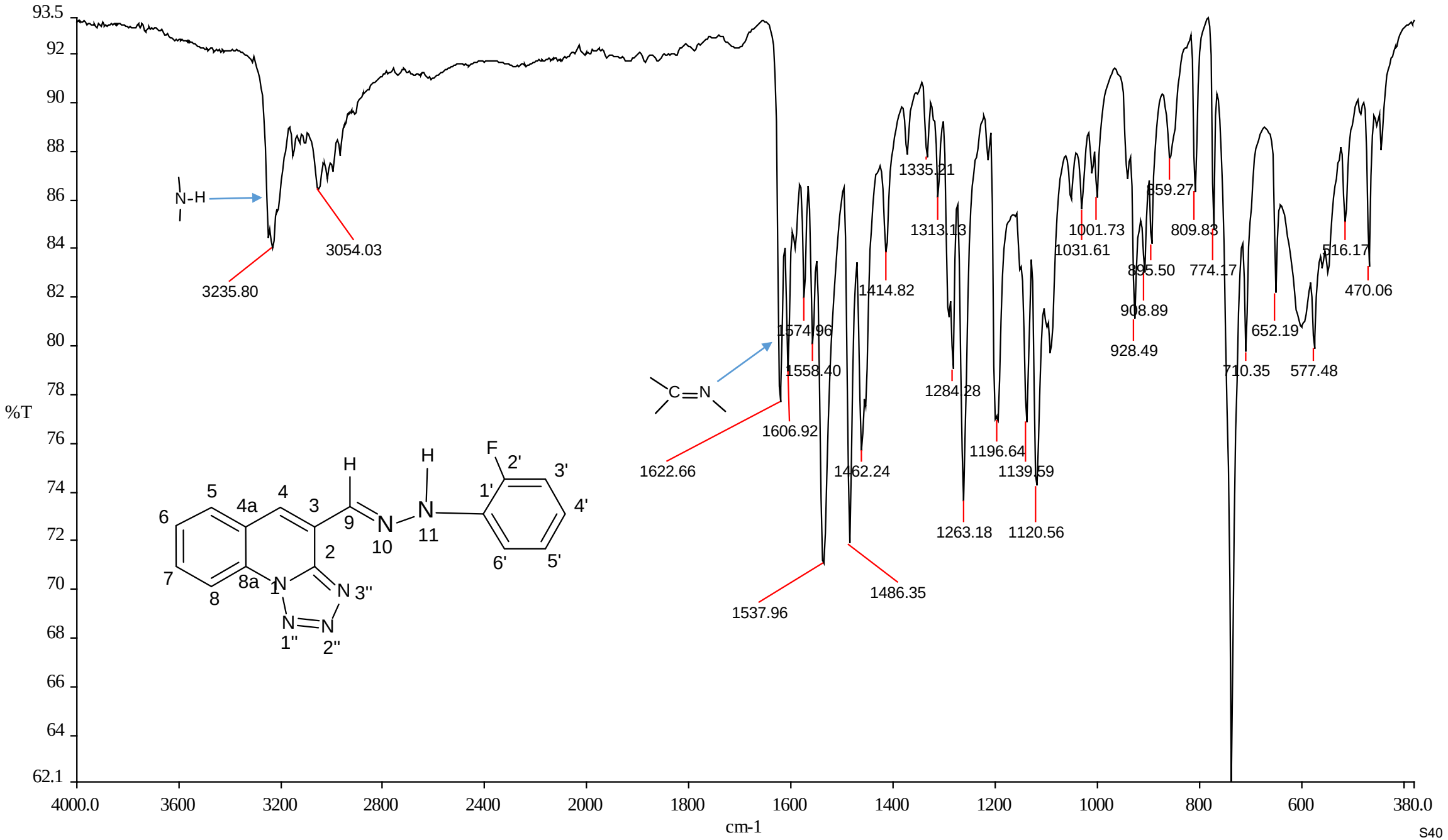
Compound 2H 32 (1.047) Cm (1:61)
TOF MS ES+

1.43e+005



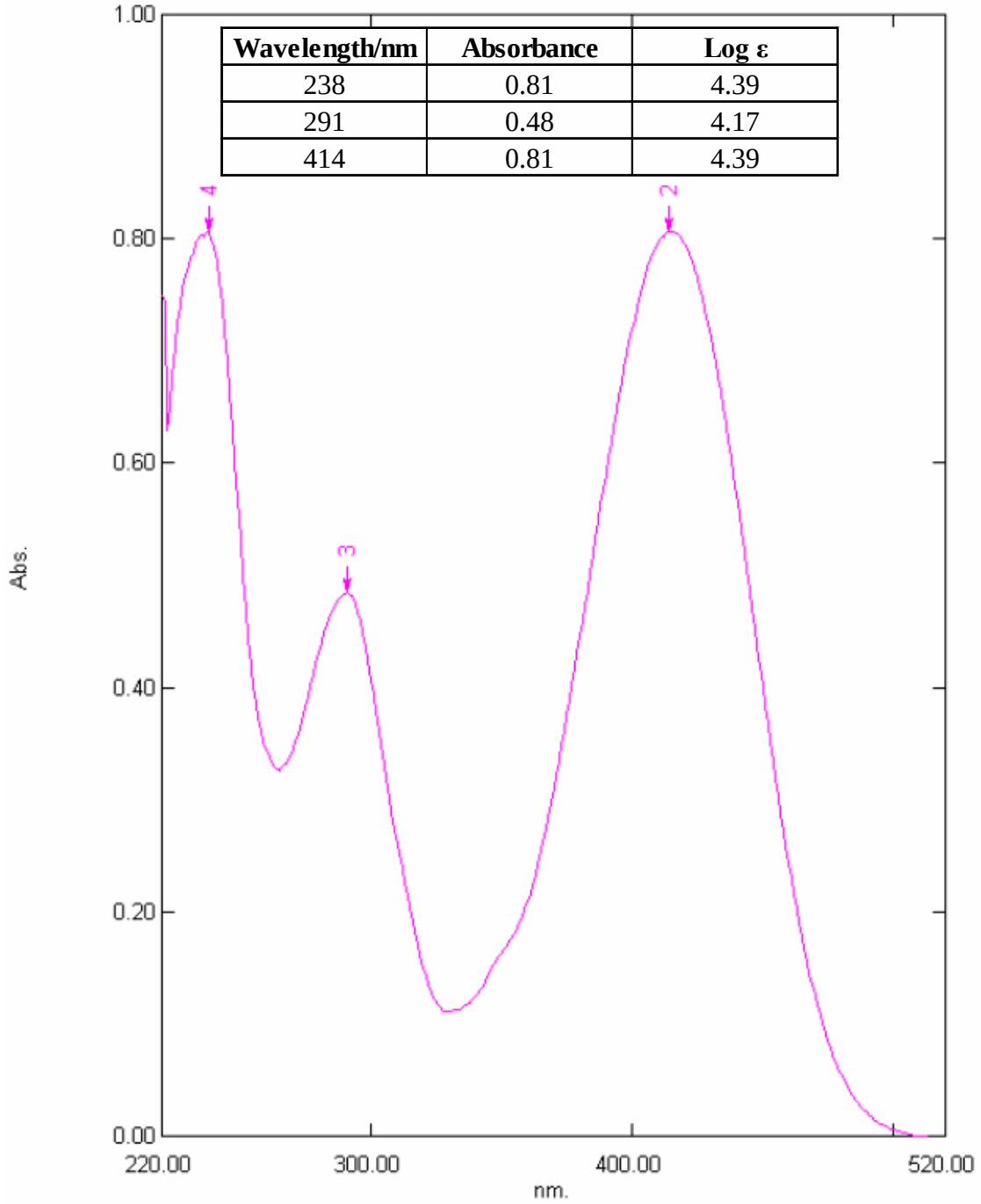
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
329.0933	329.0927	0.6	1.8	13.5	572.9	0.0	C16 H11 N6 F Na

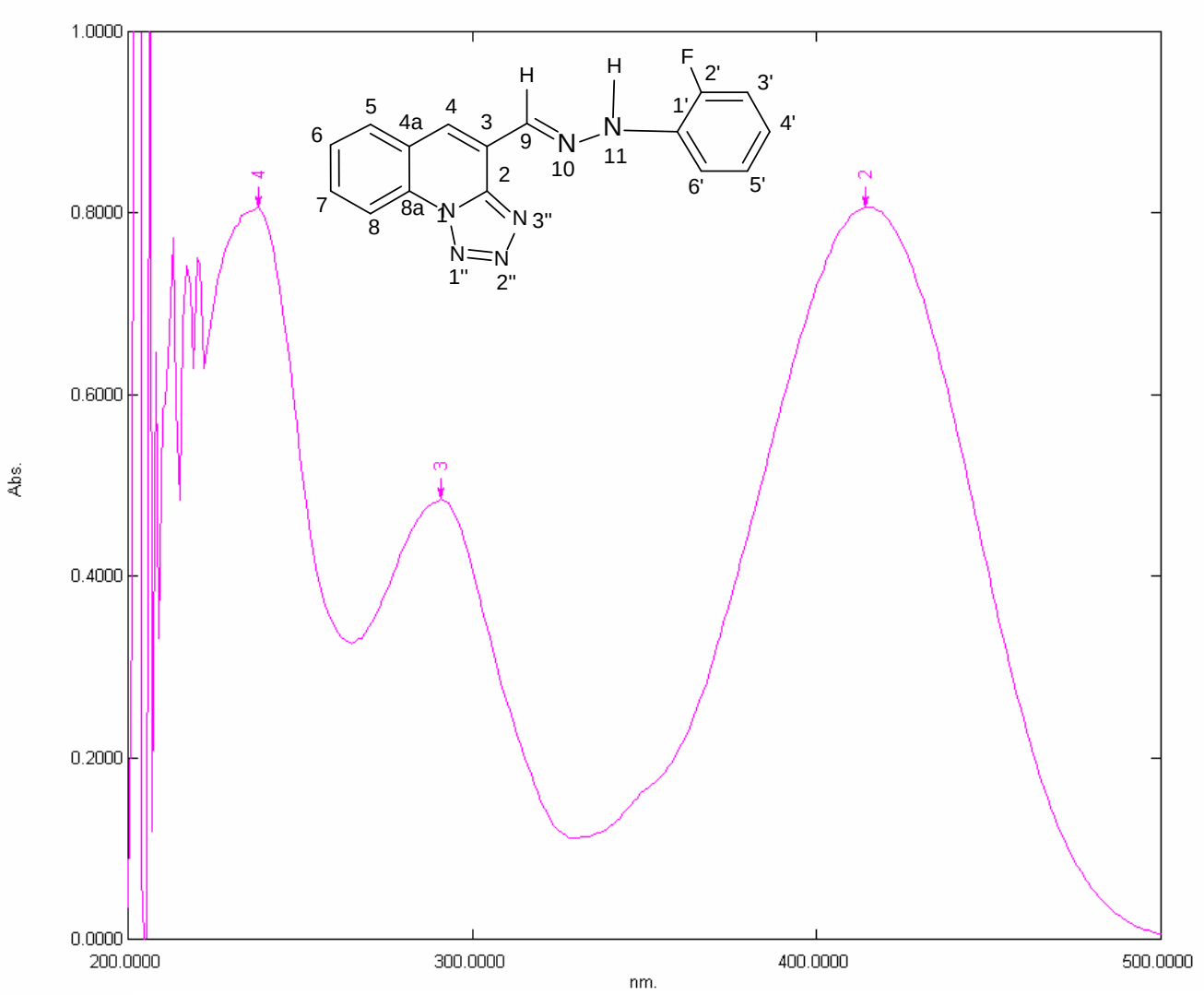
The HRMS spectrum of compound 5f



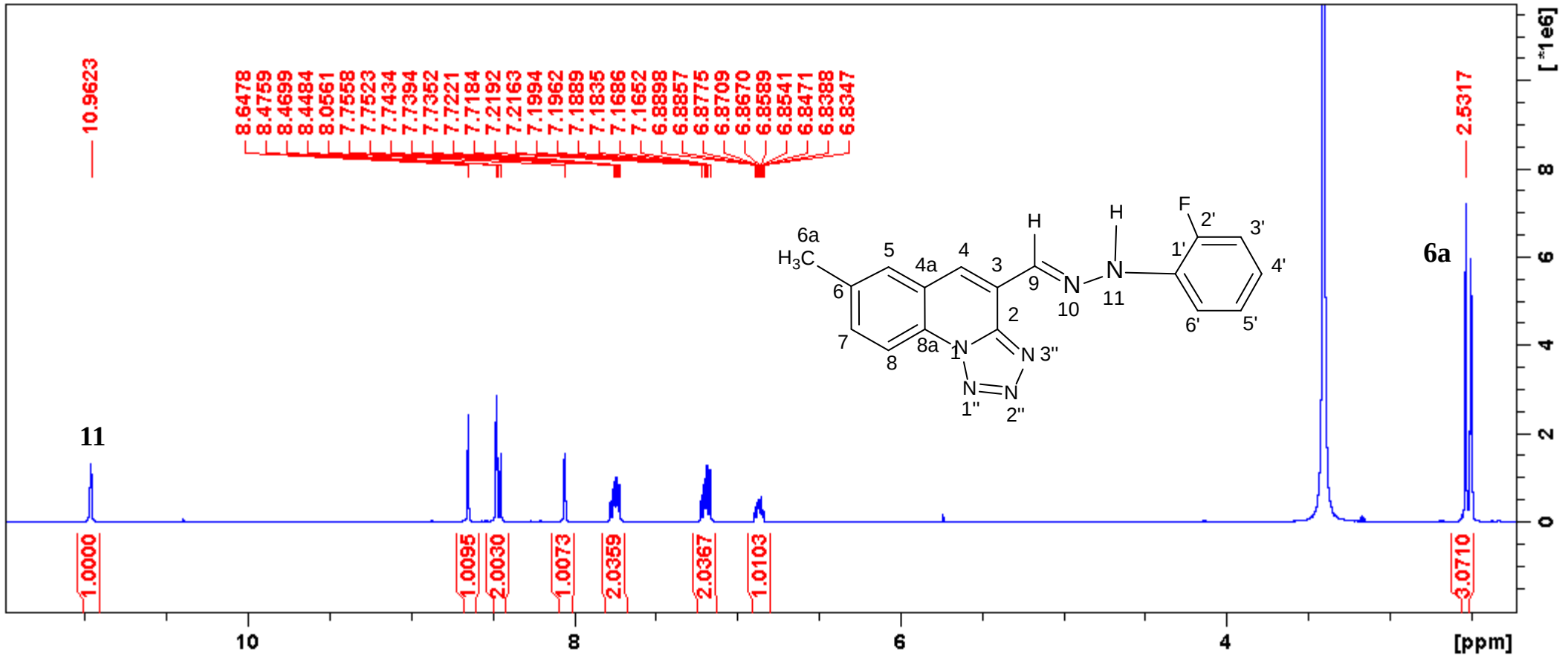
The IR spectrum of compound 5f

Wavelength/nm	Absorbance	Log ε
238	0.81	4.39
291	0.48	4.17
414	0.81	4.39

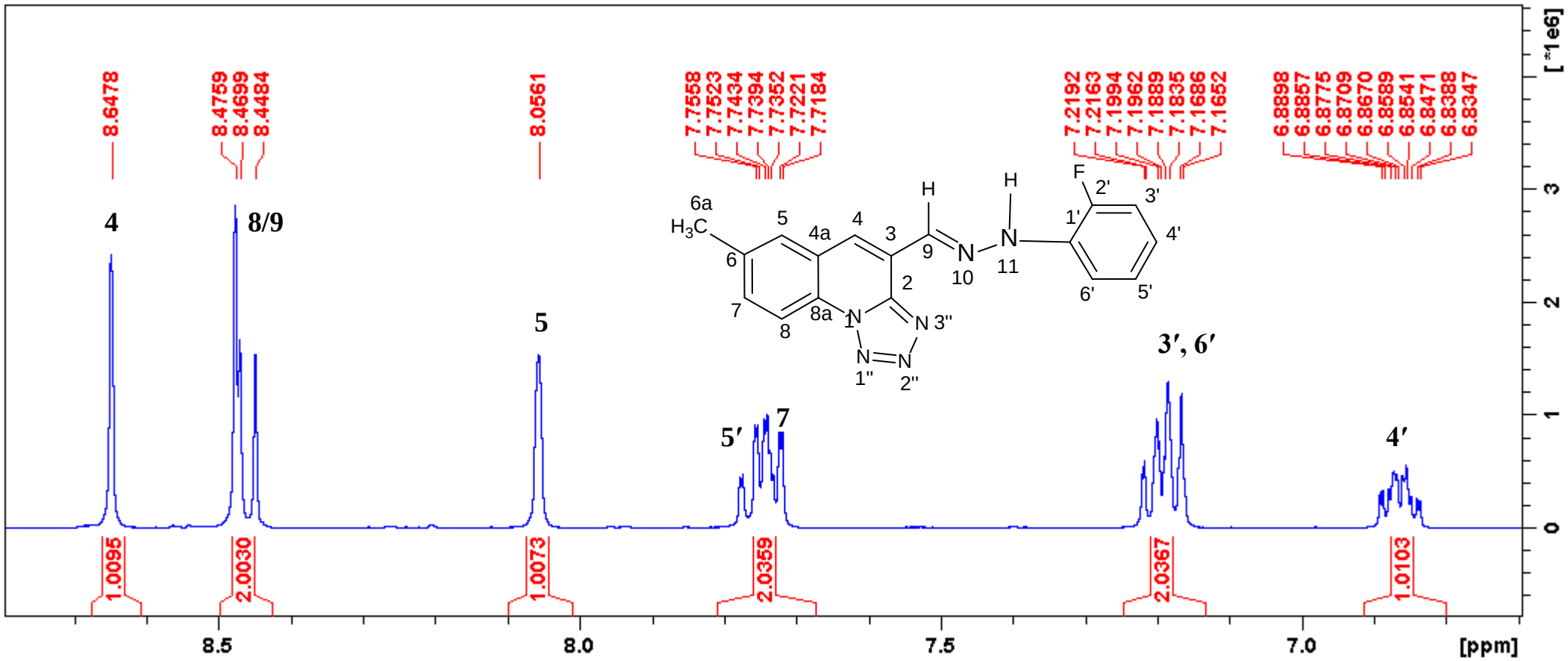




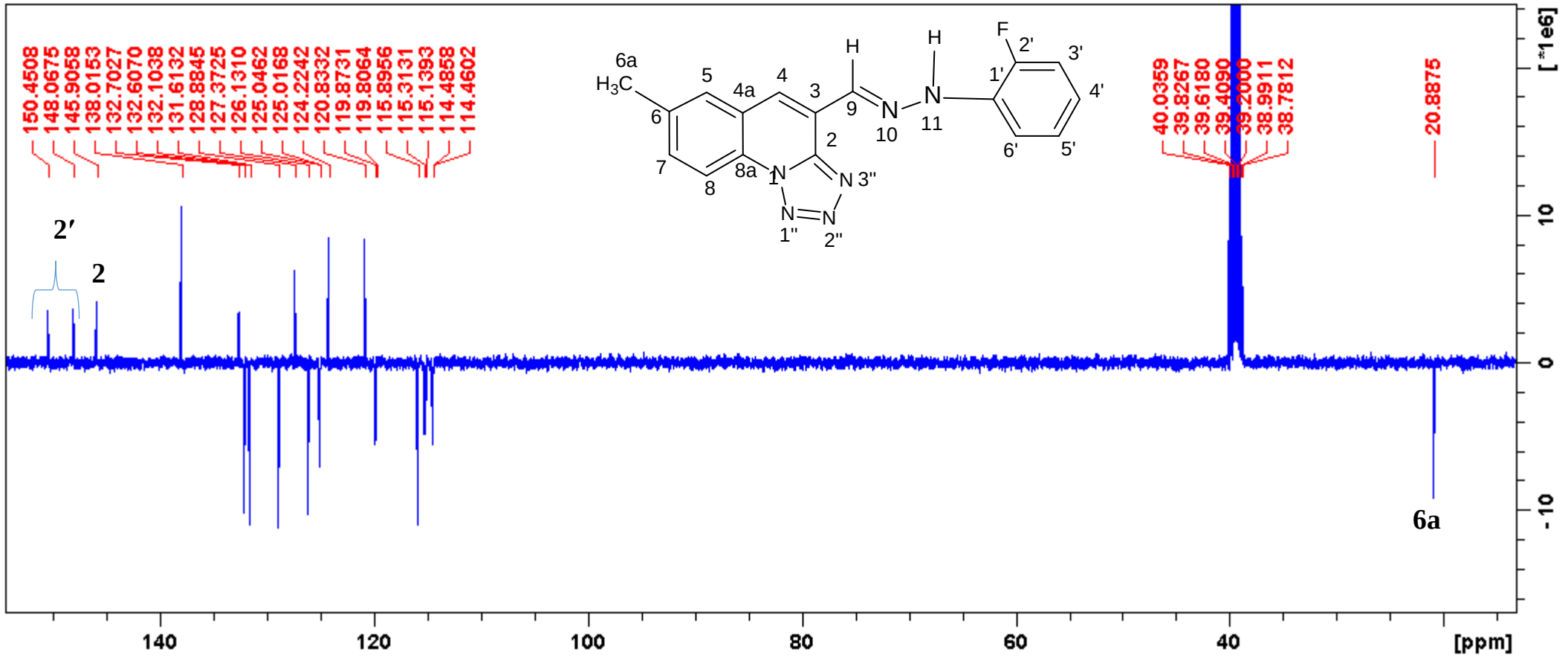
The UV-Vis spectrum of compound 5f



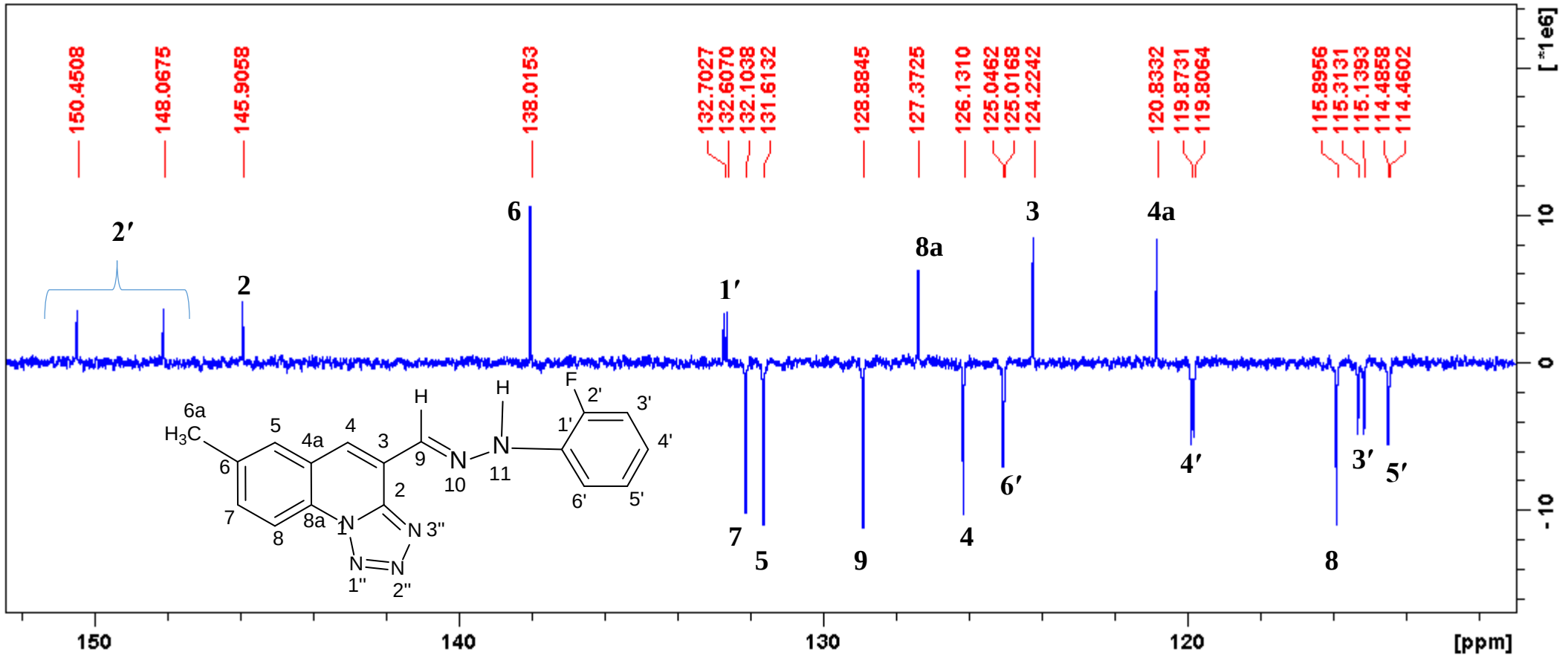
The ^{1}H NMR spectrum of compound 5g



The ^{1}H NMR spectrum of compound 5g (expanded)



The ^{13}C NMR spectrum of compound 5g

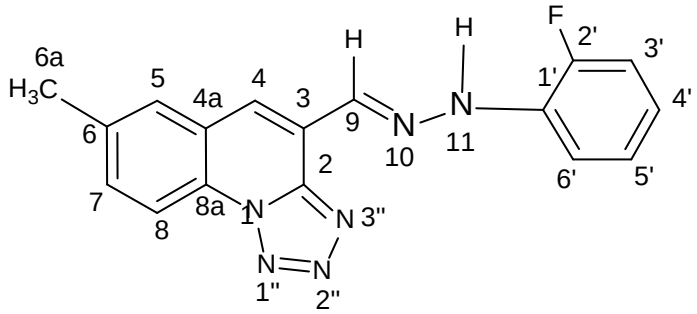


The ^{13}C NMR spectrum of compound 5g (expanded)

Elemental Composition Report

Single Mass Analysis

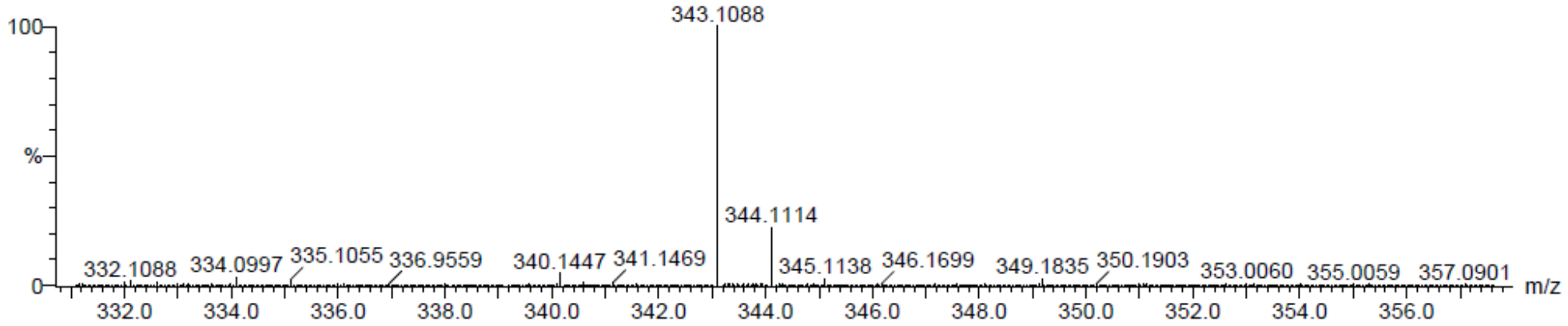
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
12 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: 5-10 F: 0-1 Na: 0-1

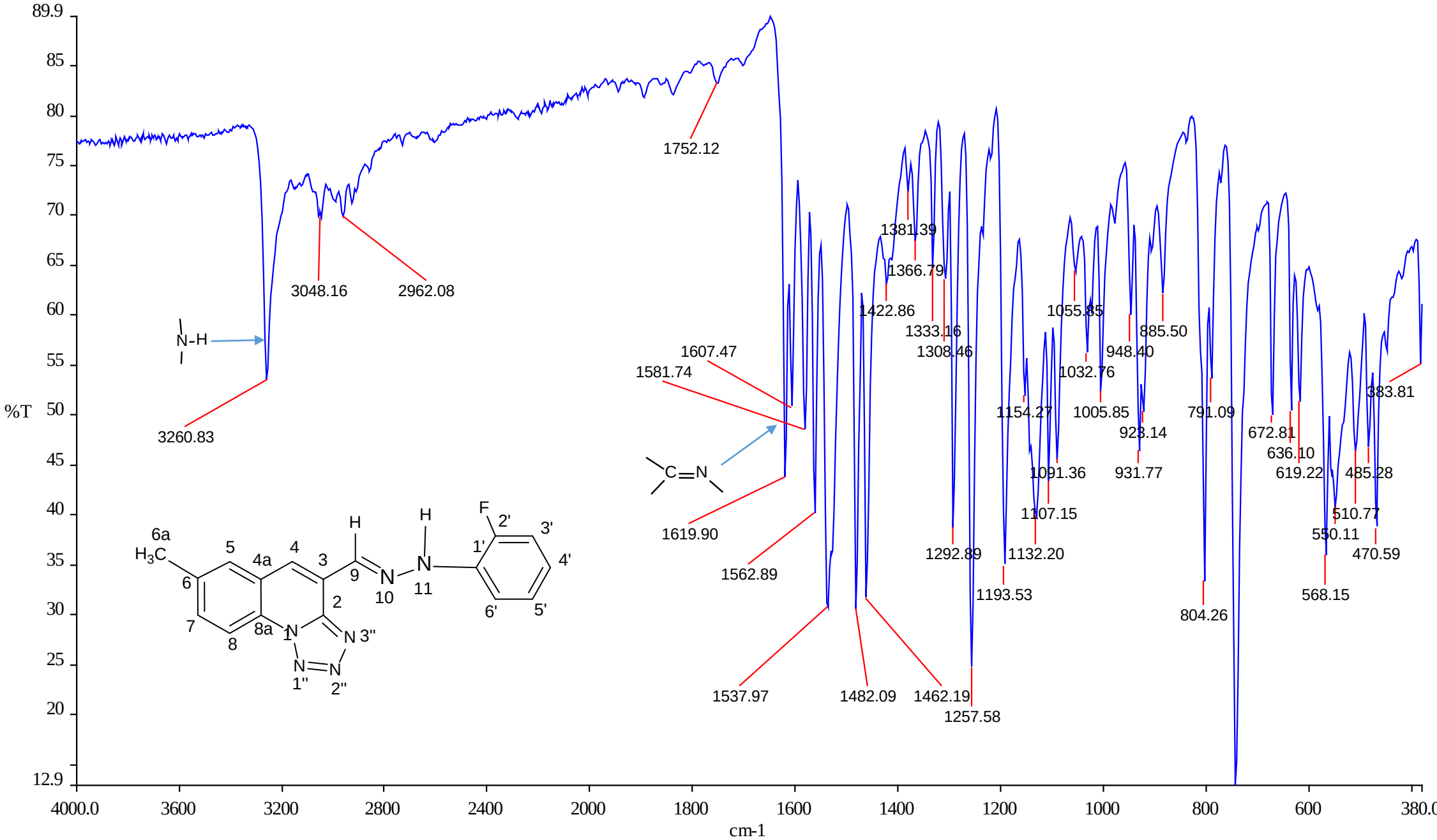
Compound 1H 20 (0.642) Cm (1:61)
TOF MS ES+

1.33e+005



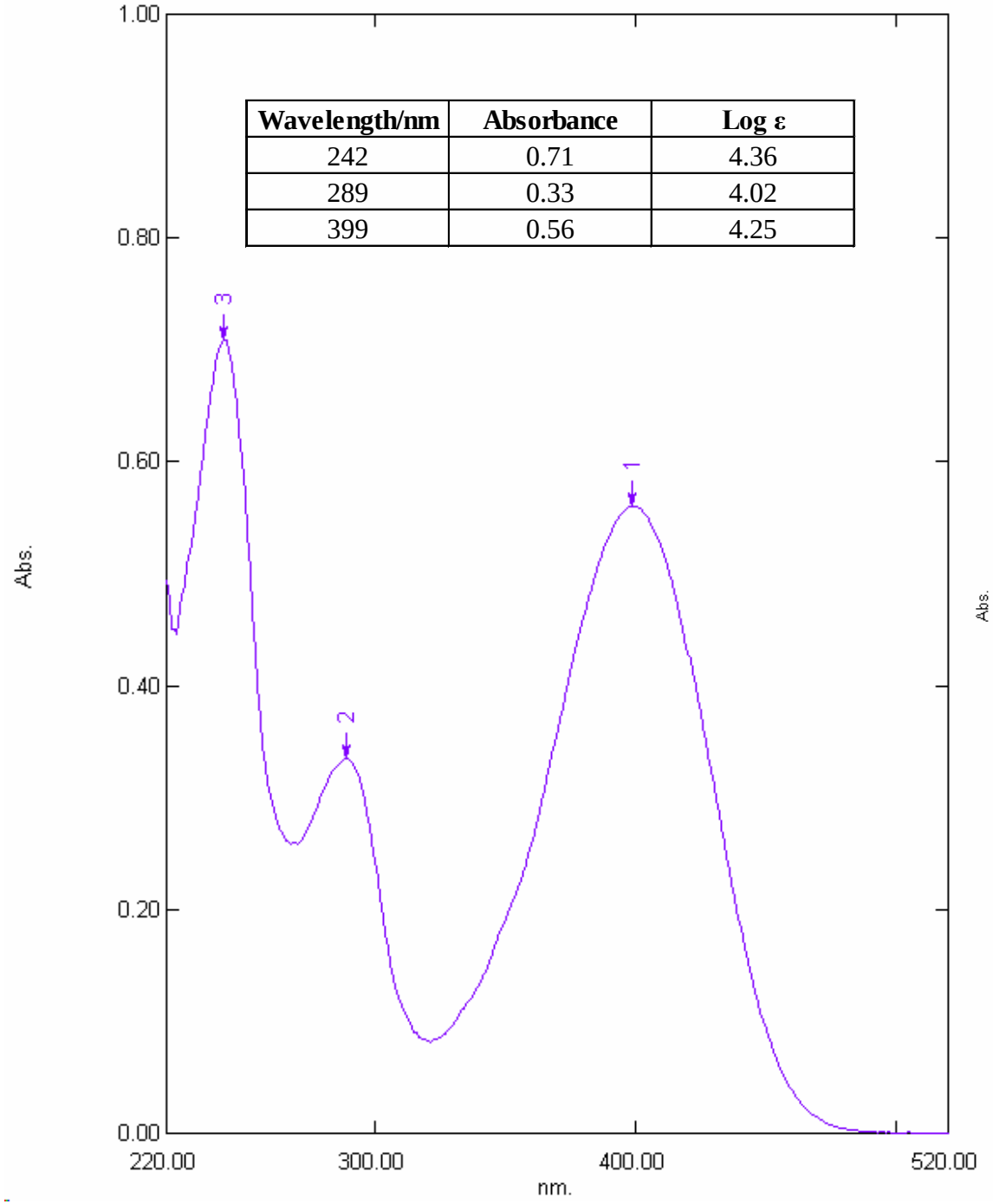
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
343.1088	343.1083	0.5	1.5	13.5	579.1	0.0	C17 H13 N6 F Na

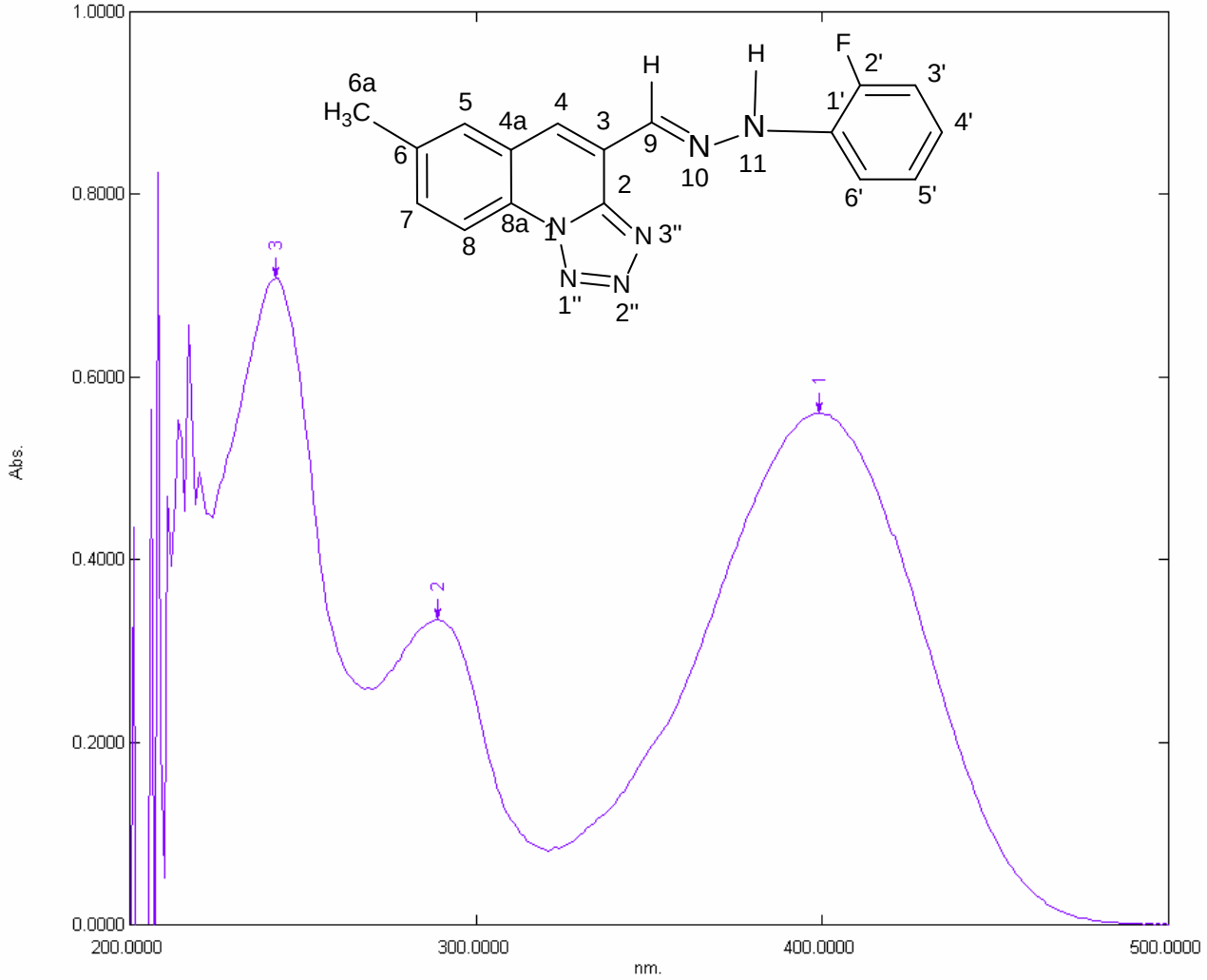
The HRMS spectrum of compound 5g



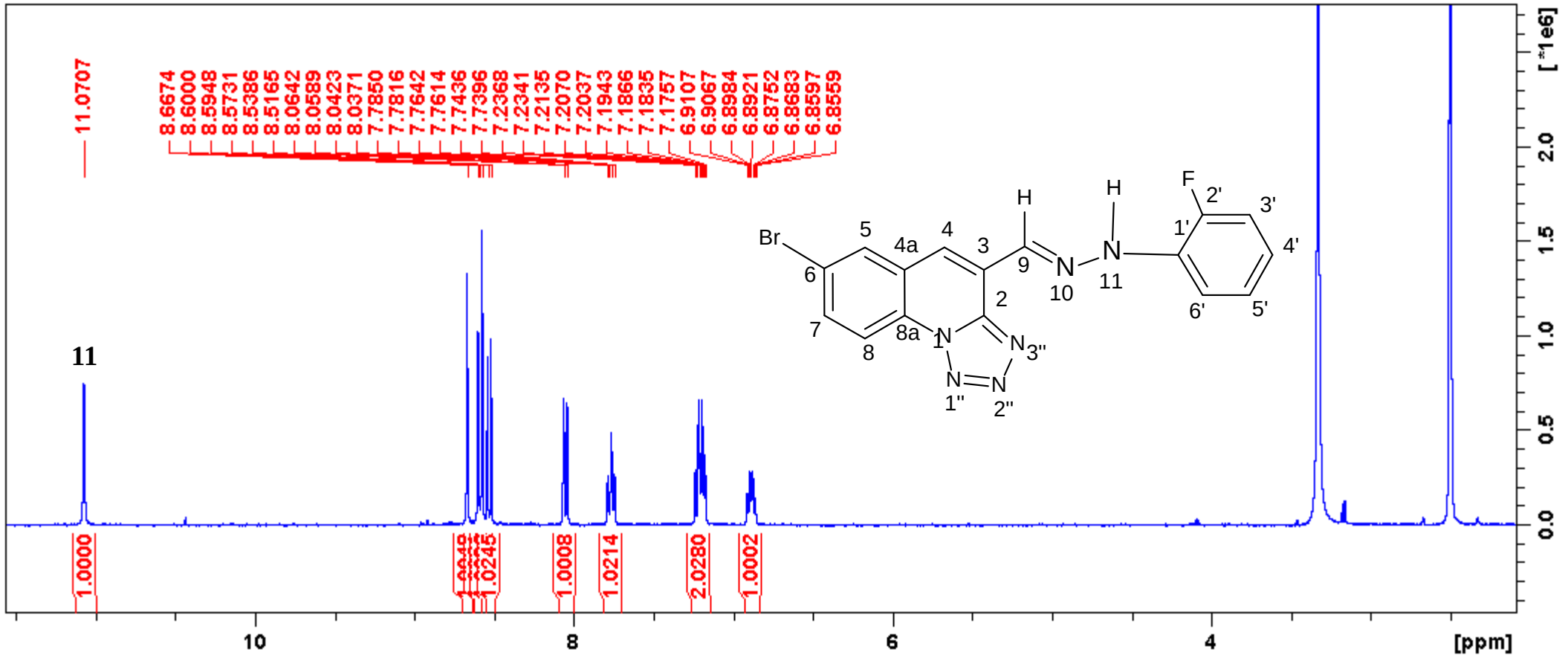
The IR spectrum of compound 5g

Wavelength/nm	Absorbance	Log ε
242	0.71	4.36
289	0.33	4.02
399	0.56	4.25

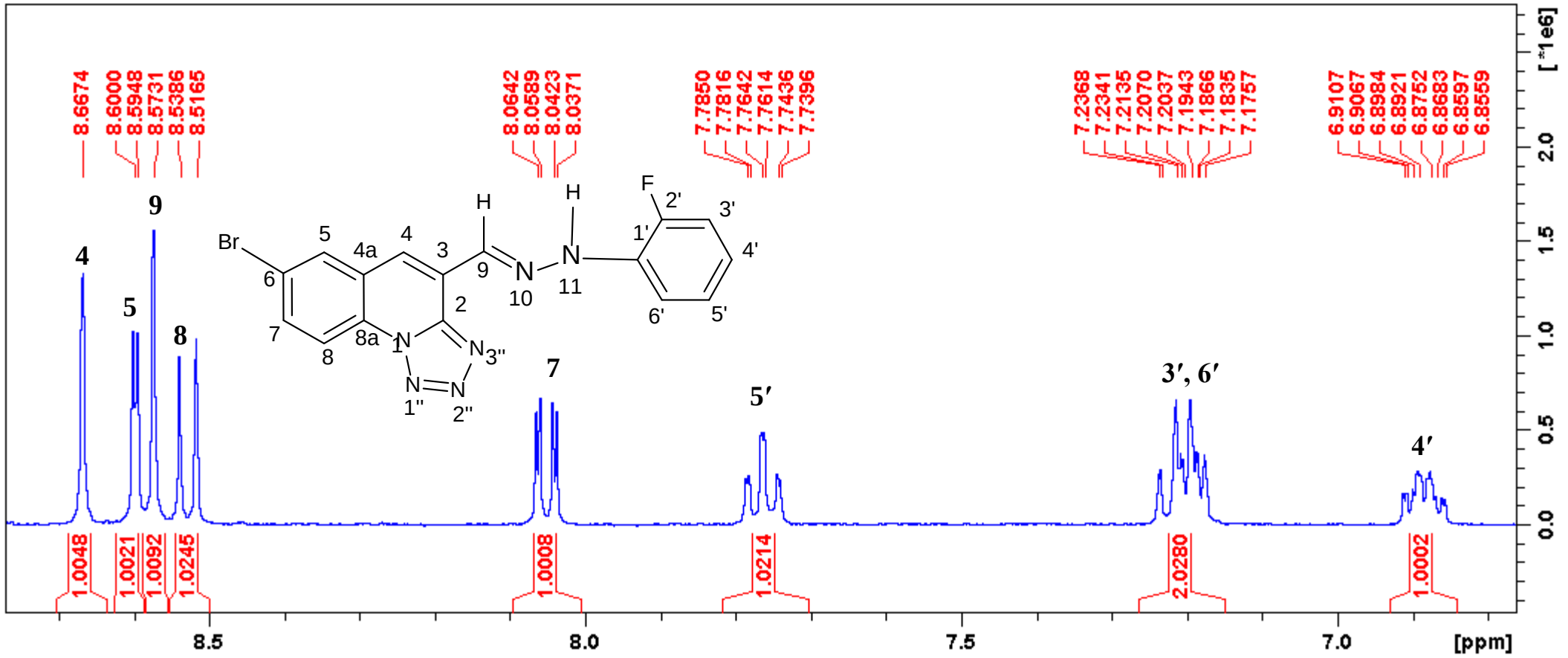




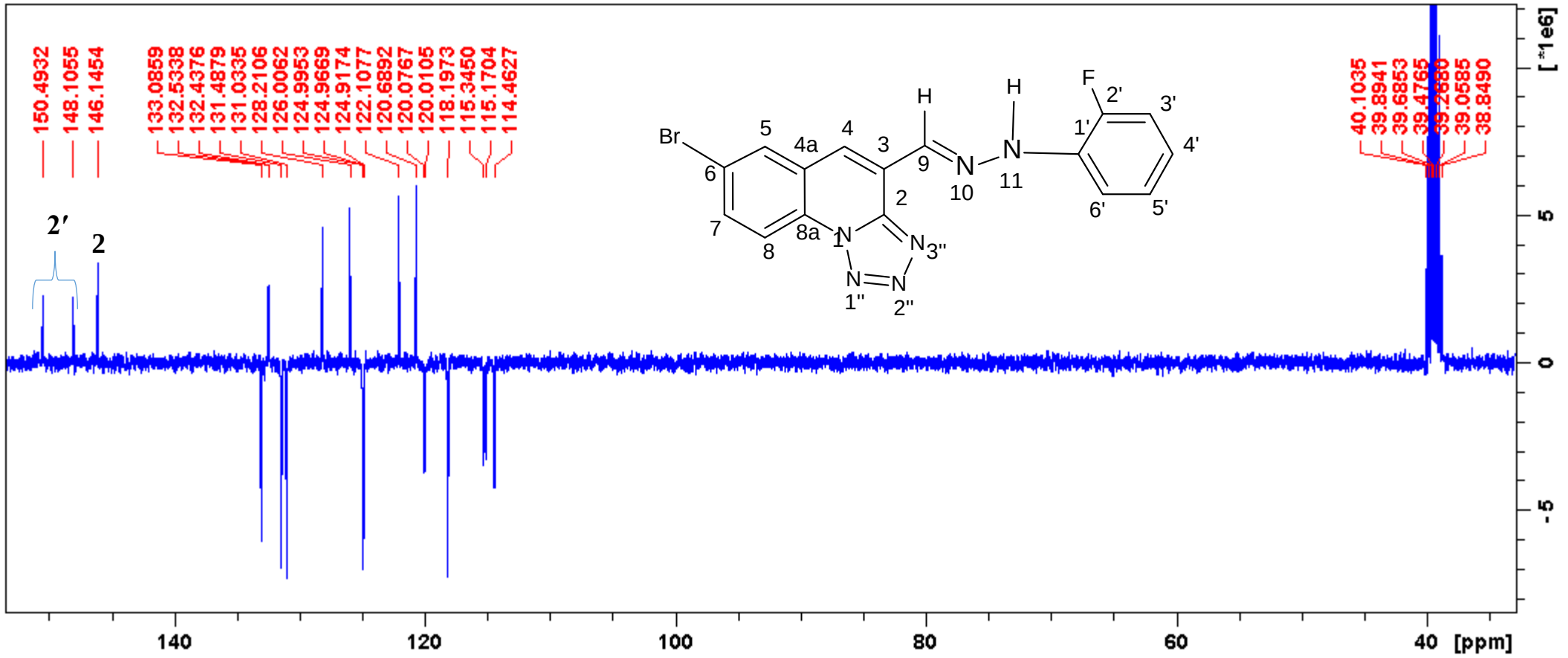
The UV-Vis spectrum of compound 5g



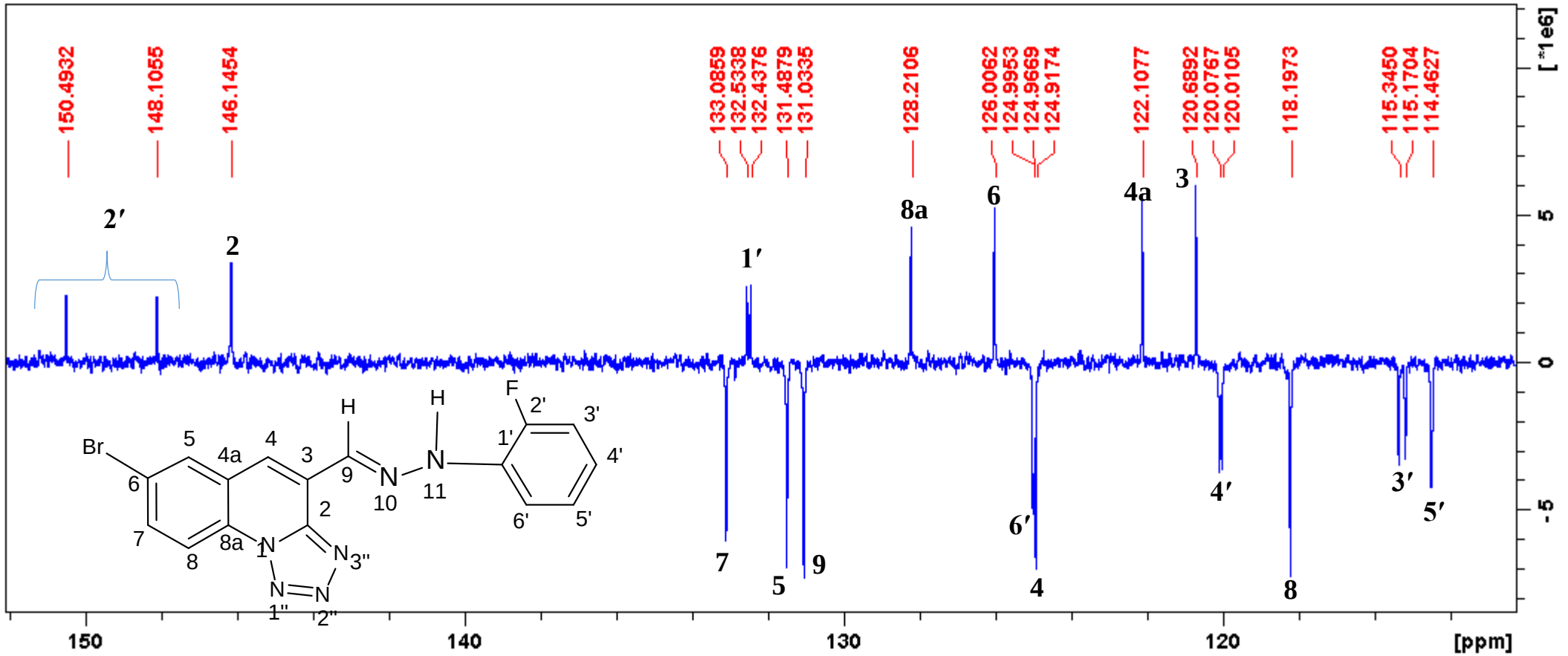
The ^{1}H NMR spectrum of compound 5h



The ^{1}H NMR spectrum of compound 5h (expanded)



The ^{13}C NMR spectrum of compound 5h



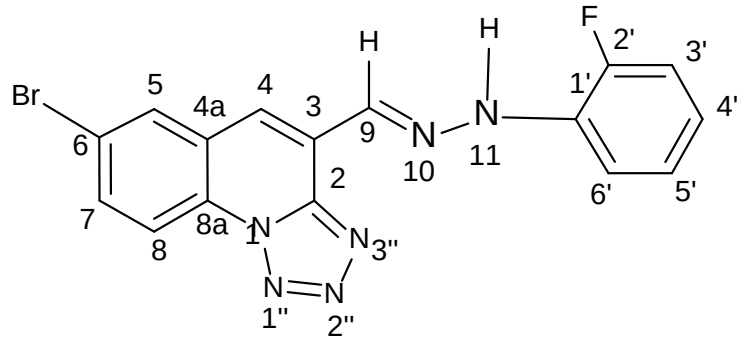
The ^{13}C NMR spectrum of compound 5h (expanded)

Elemental Composition Report

Single Mass Analysis

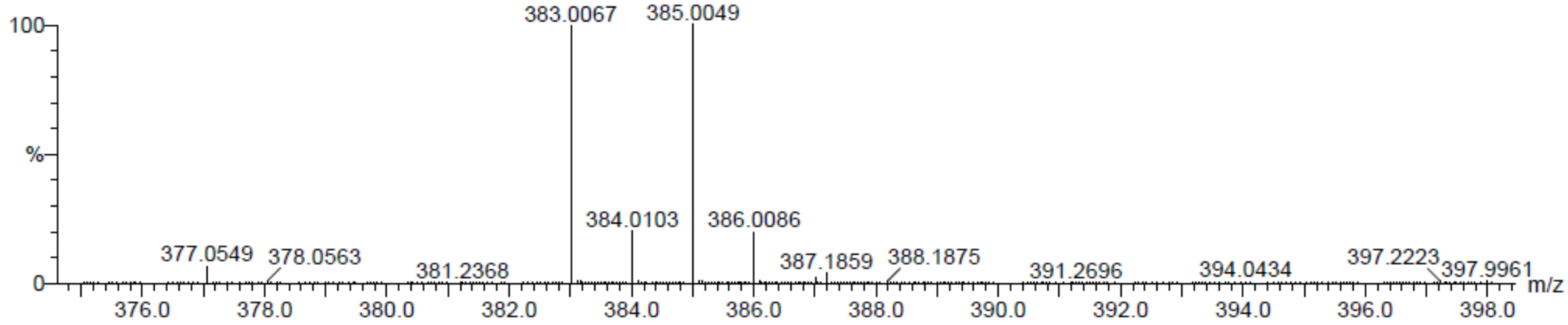
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

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



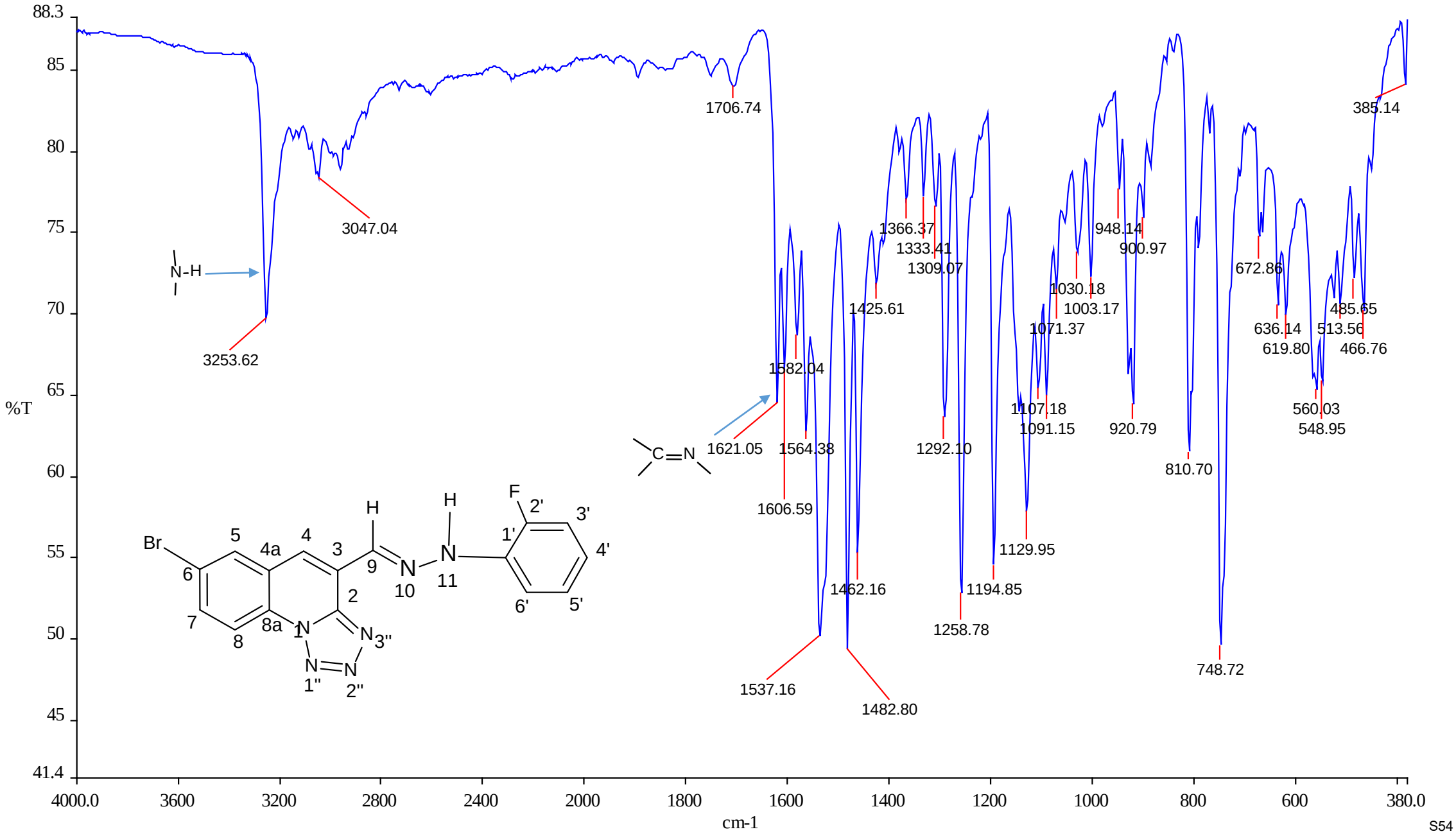
Compound 2g HG 29 (0.945) Cm (1:61)
TOF MS ES-

9.02e+004



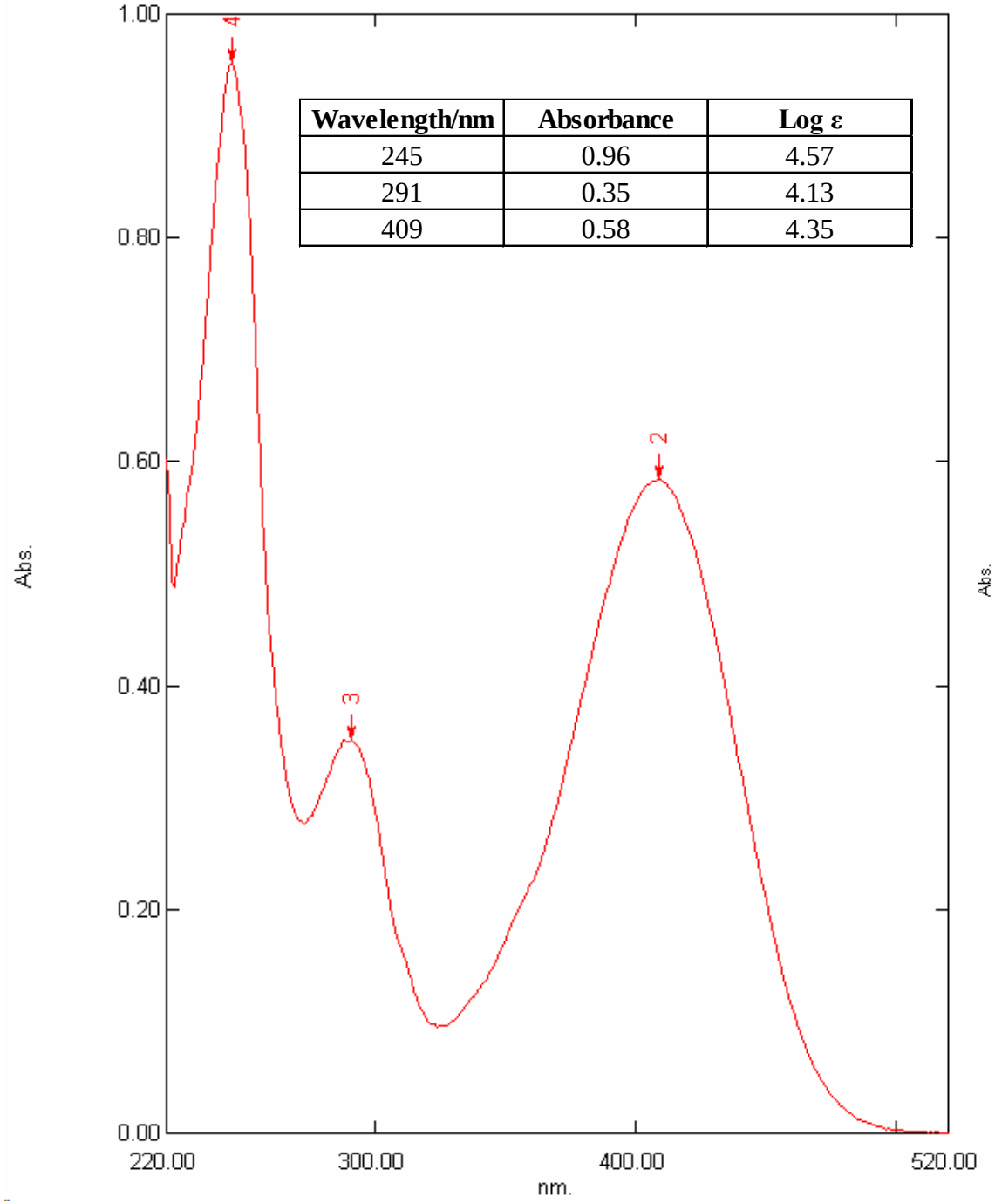
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
383.0067	383.0056	1.1	2.9	14.5	549.1	0.0	C16 H9 N6 F Br

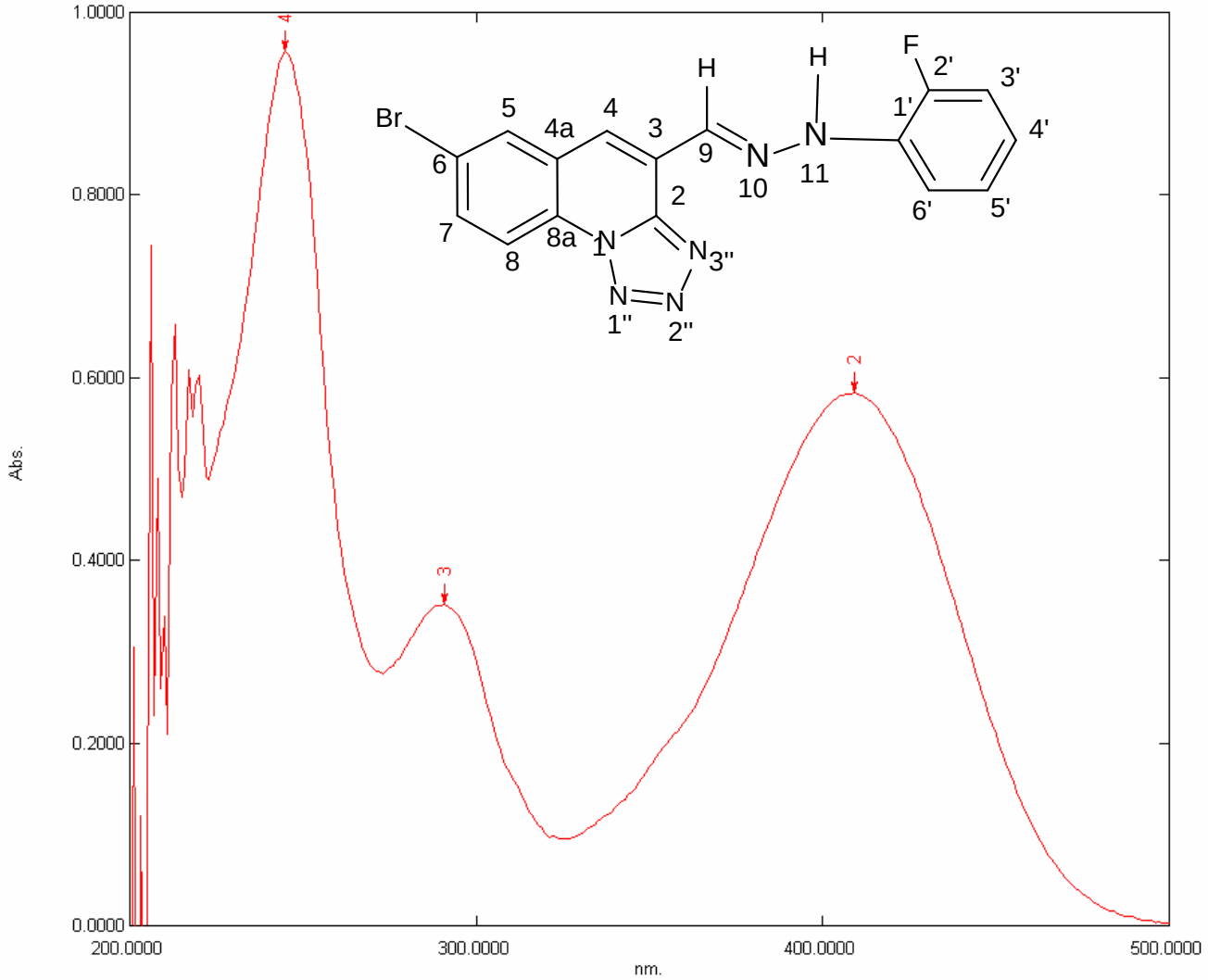
The HRMS spectrum of compound 5h



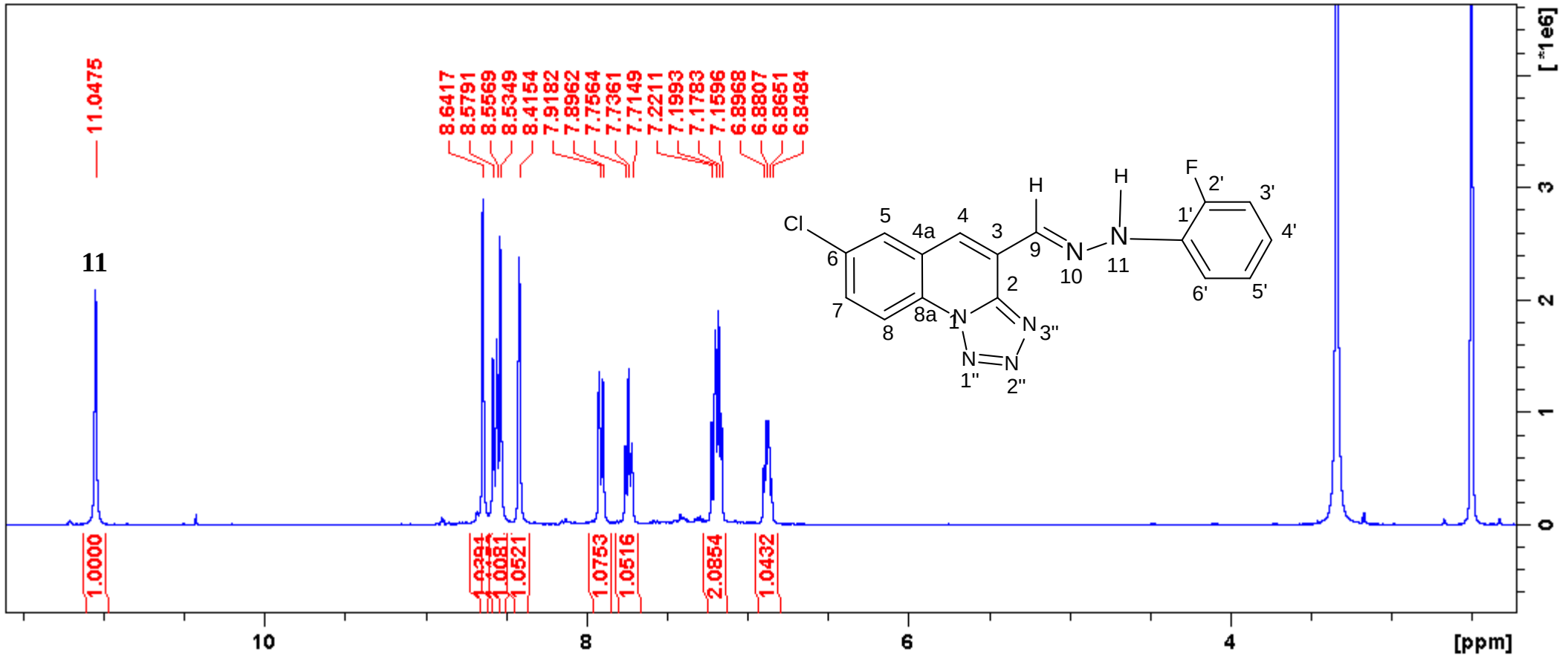
The IR spectrum of compound 5h

Wavelength/nm	Absorbance	Log ε
245	0.96	4.57
291	0.35	4.13
409	0.58	4.35

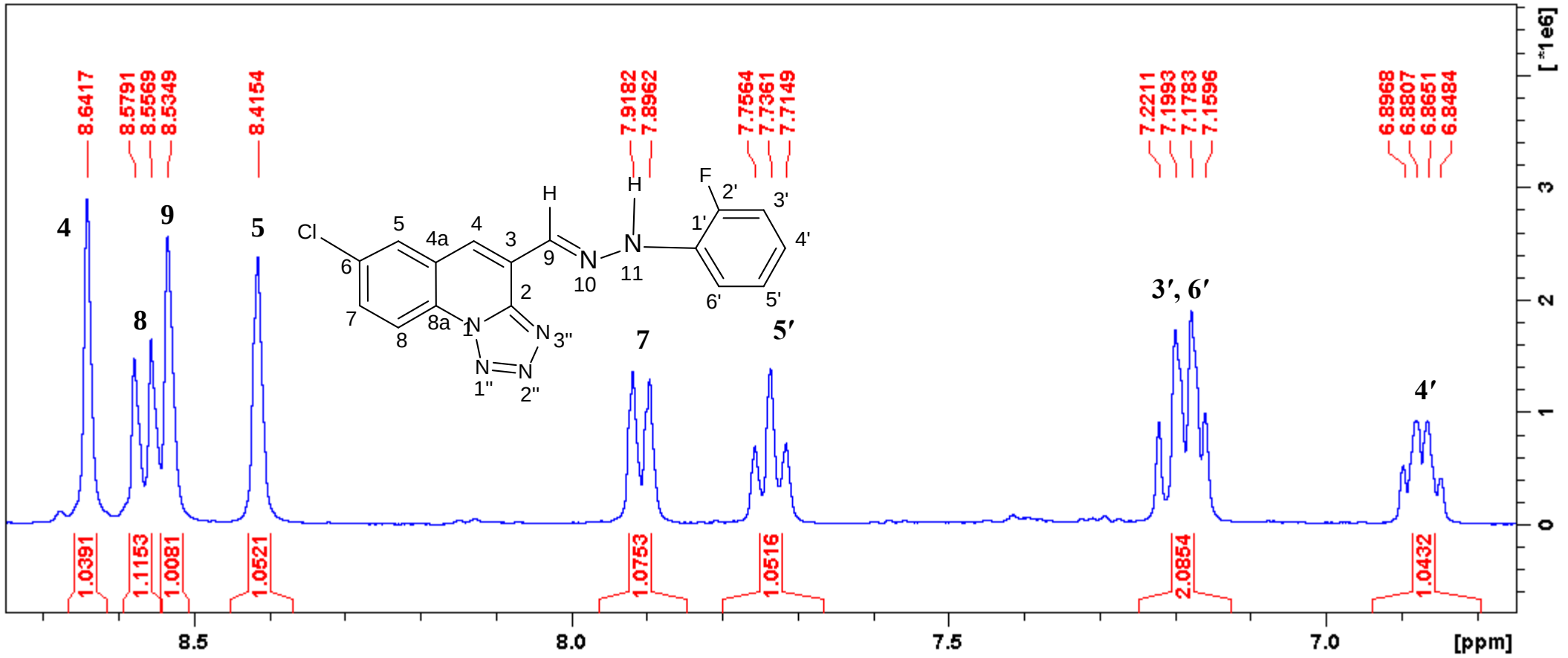




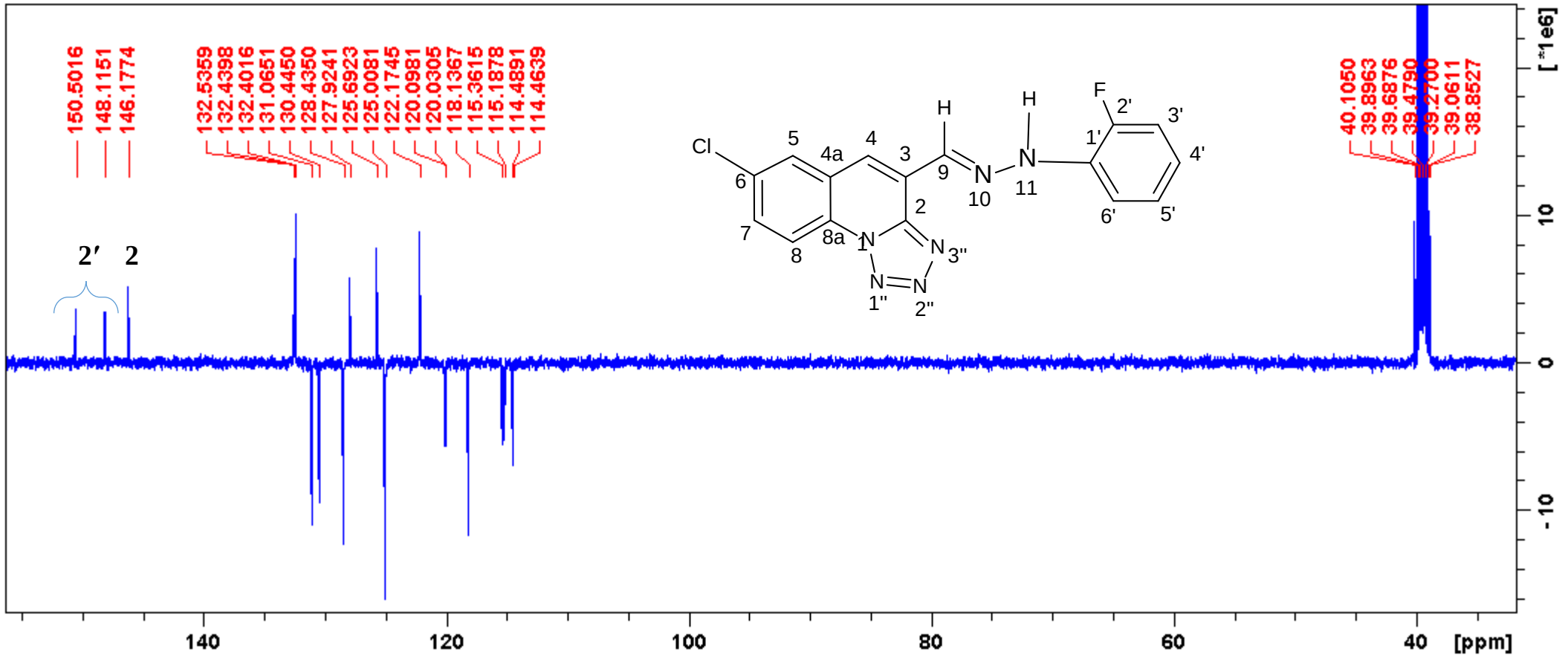
The UV-Vis spectrum of compound 5h



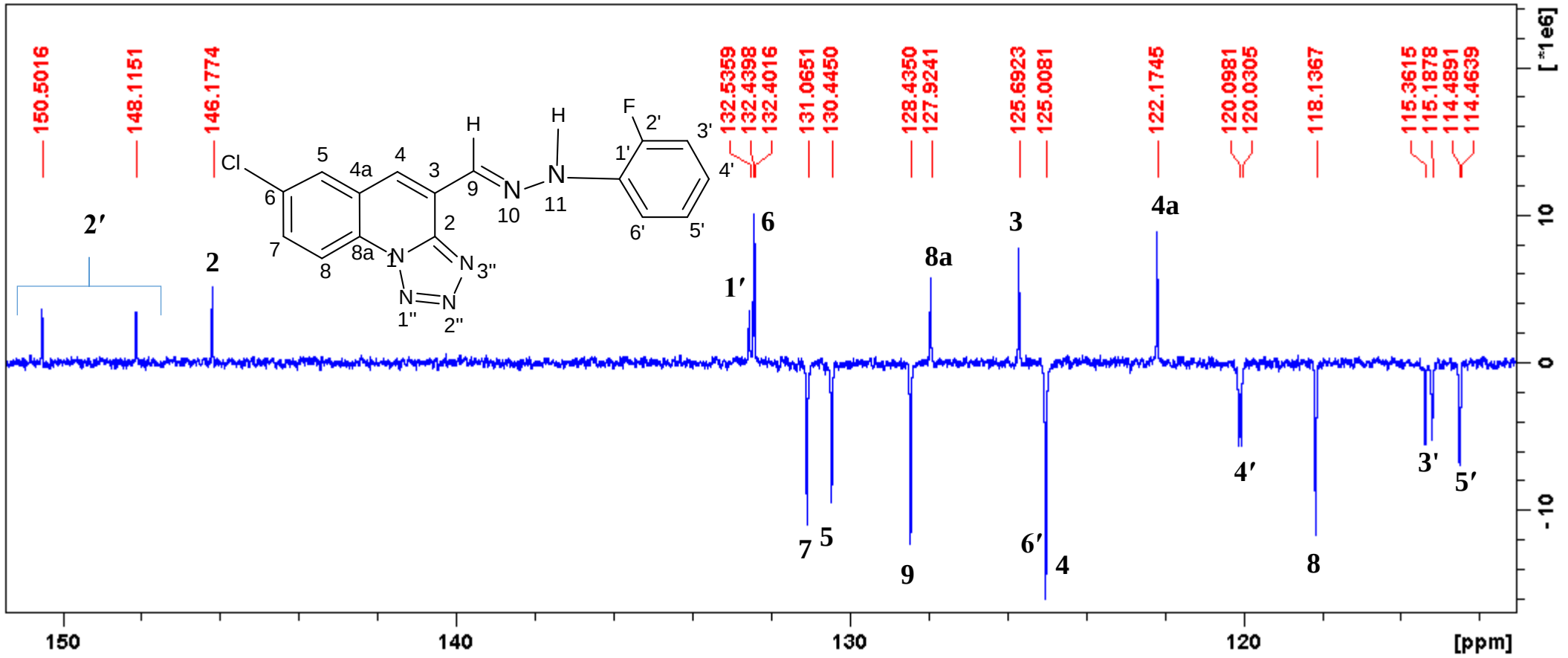
The ^{1}H NMR spectrum of compound 5i



The ^{1}H NMR spectrum of compound 5i (expanded)



The ^{13}C NMR spectrum of compound 5i



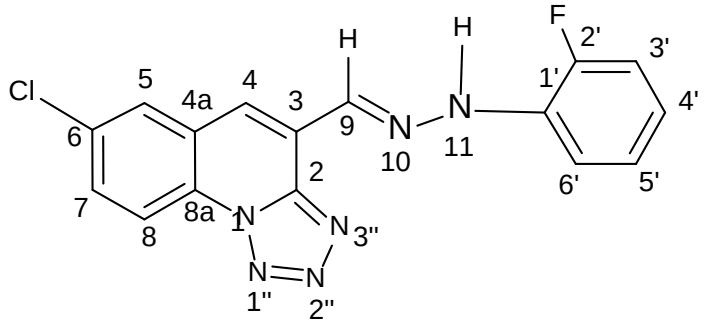
The ^{13}C NMR spectrum of compound 5i-expanded

Elemental Composition Report

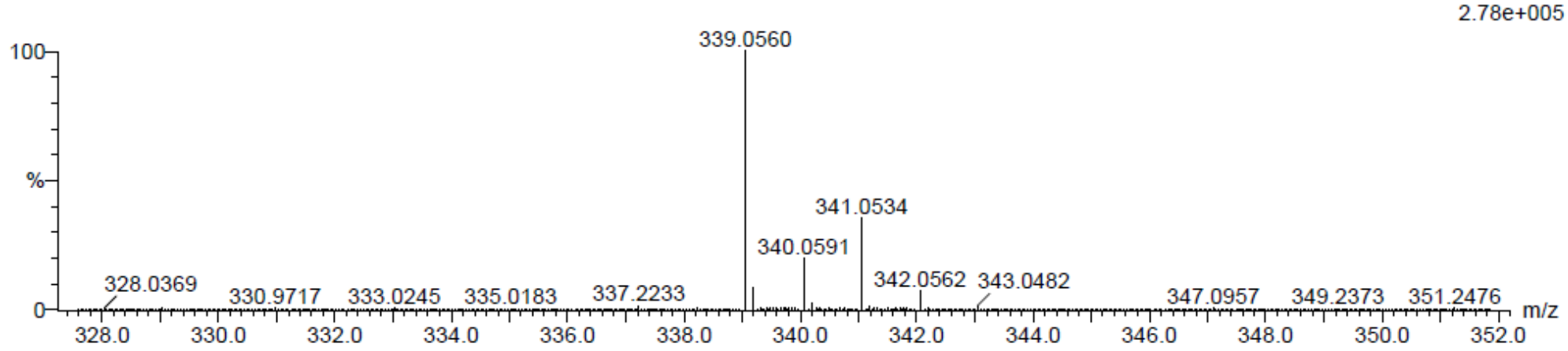
Single Mass Analysis

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
33 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 5-10 F: 0-1 Cl: 0-1 Zn: 0-1

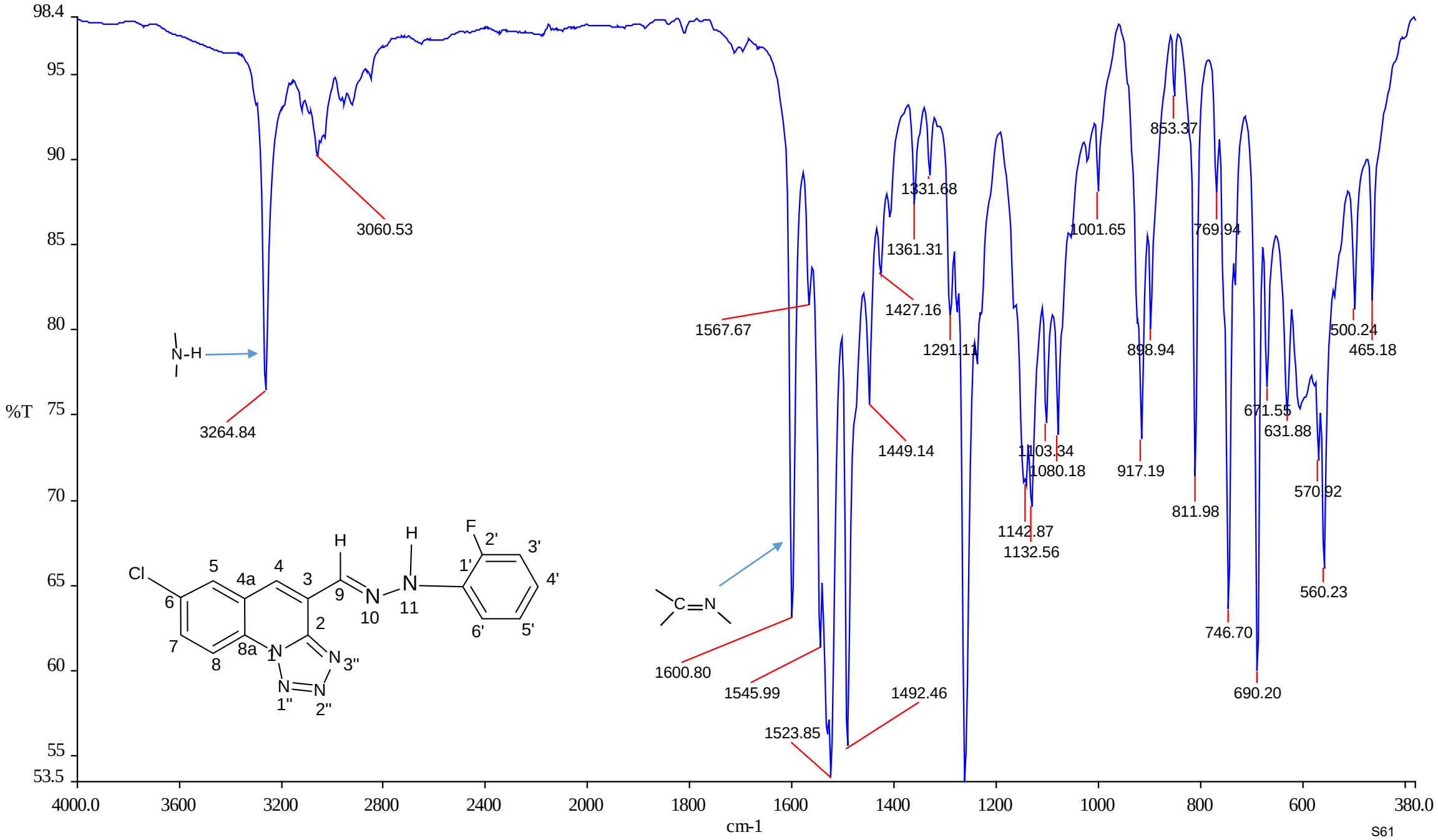


Compound 3g HG 58 (1.923) Cm (1:61)
TOF MS ES-



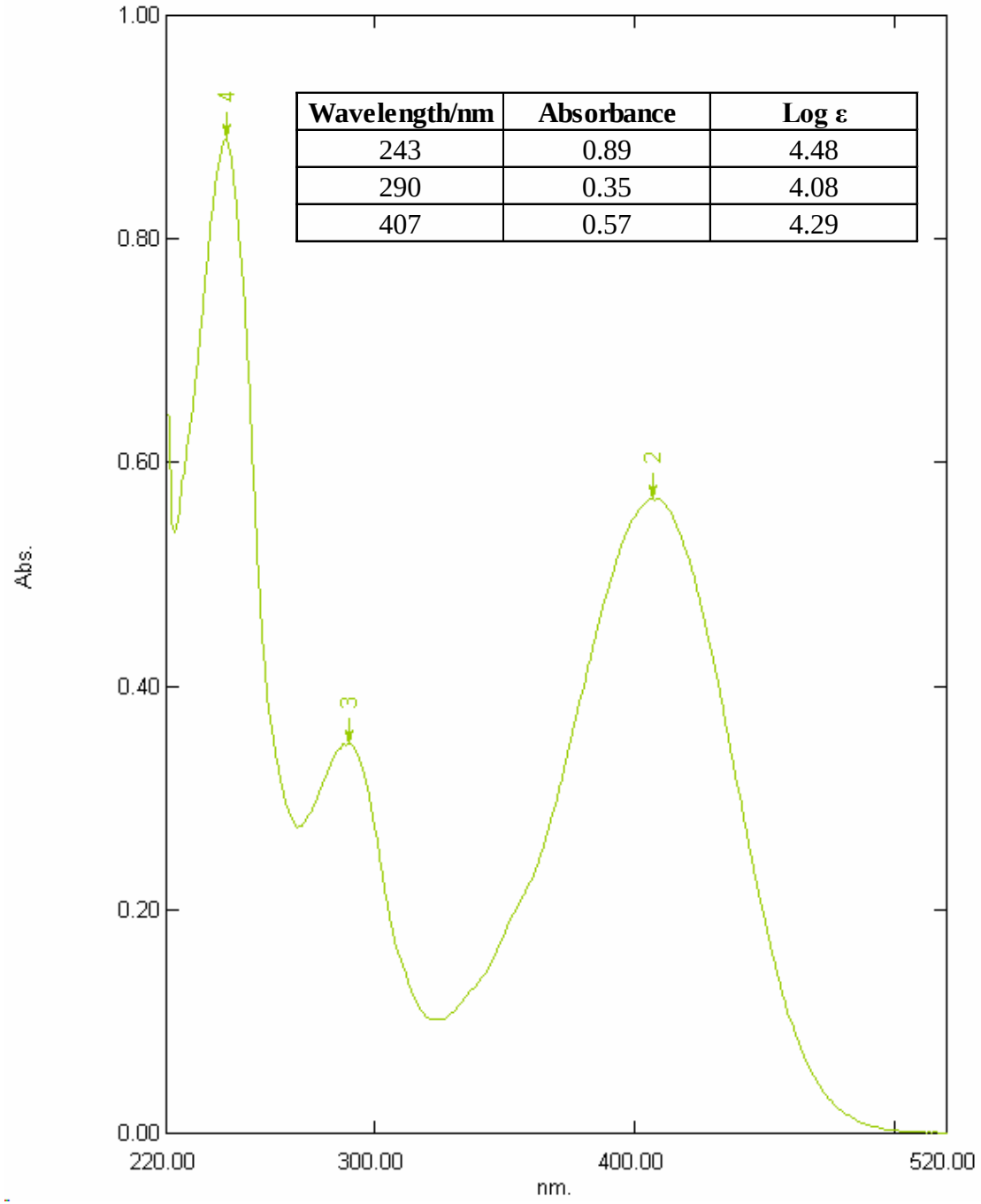
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
339.0560	339.0561	-0.1	-0.3	14.5	647.2	0.0	C16 H9 N6 F Cl

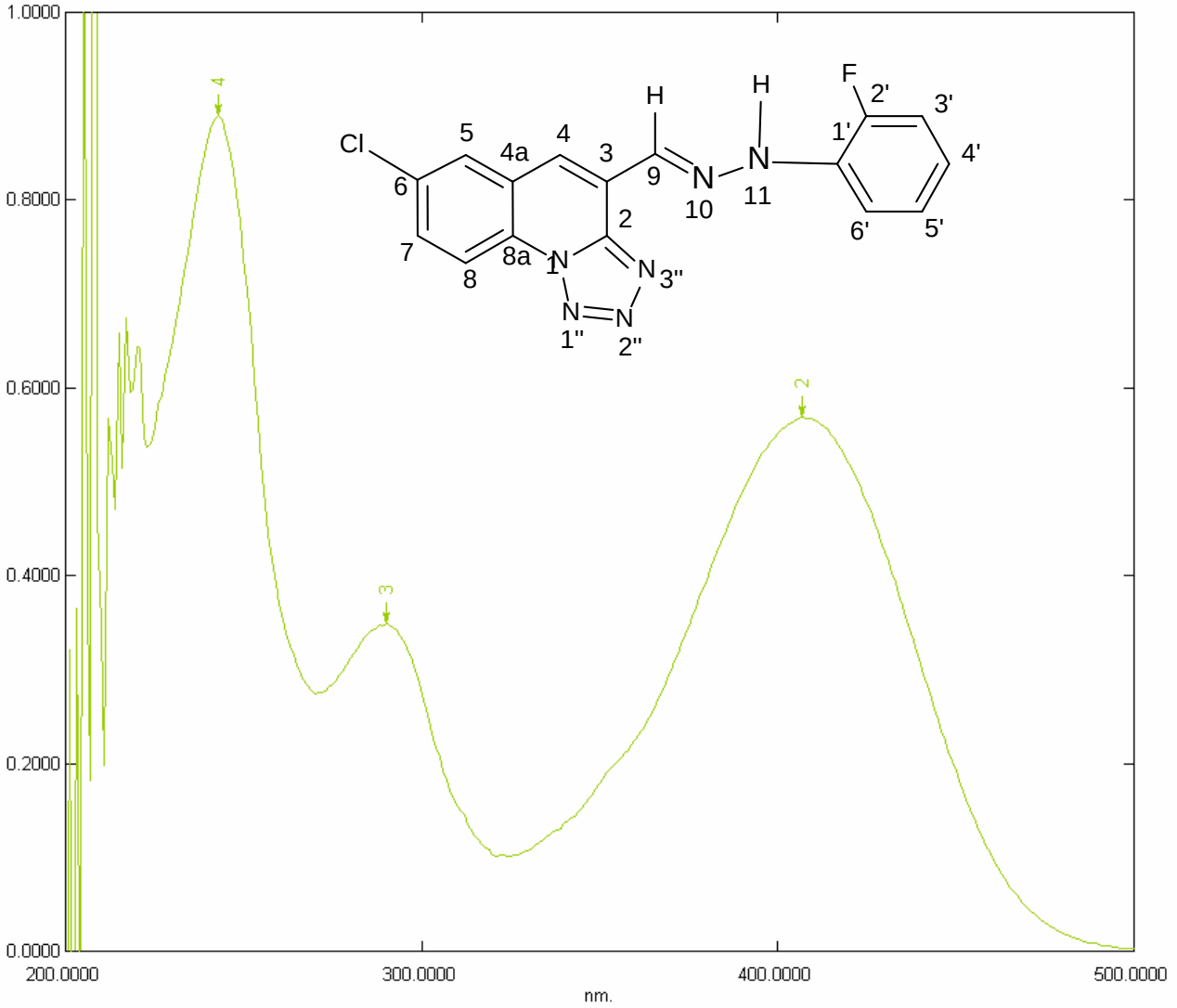
The HRMS spectrum of compound 5i



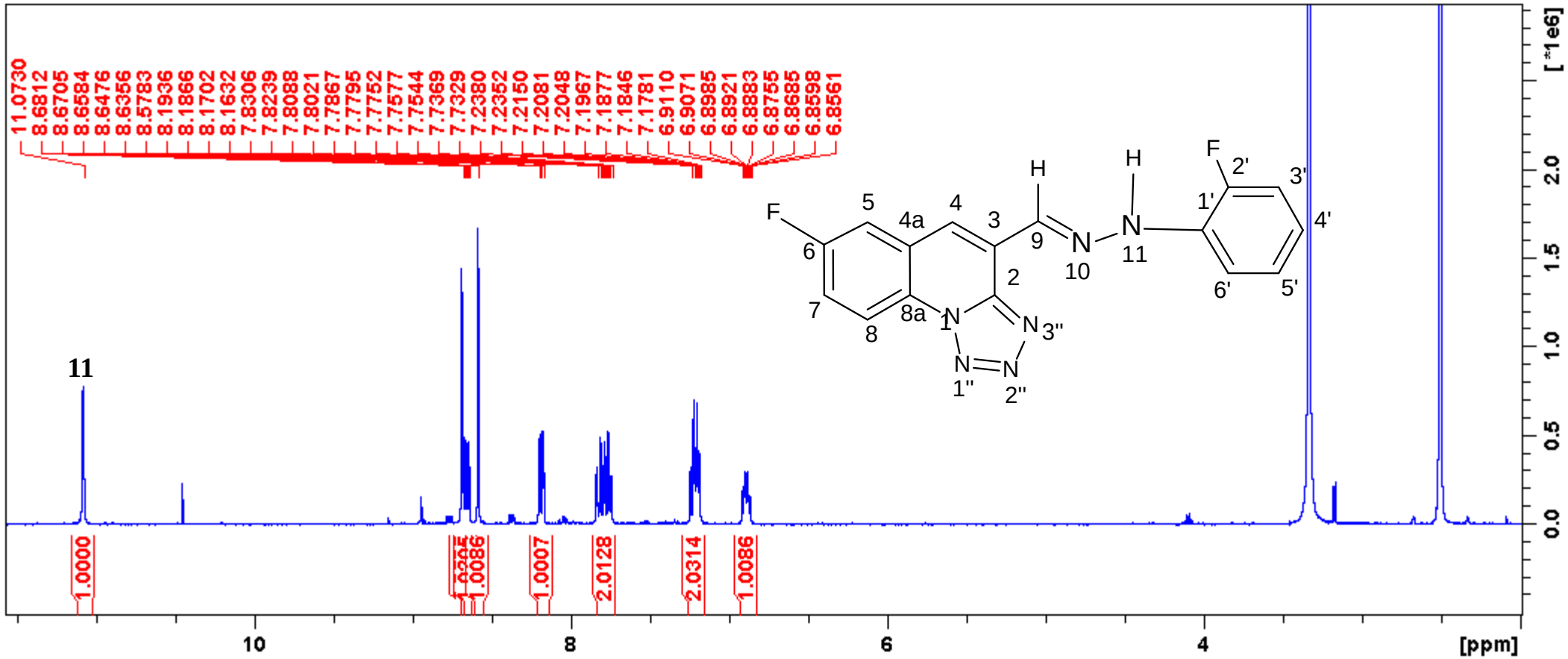
The IR spectrum of compound 5i

Wavelength/nm	Absorbance	Log ε
243	0.89	4.48
290	0.35	4.08
407	0.57	4.29

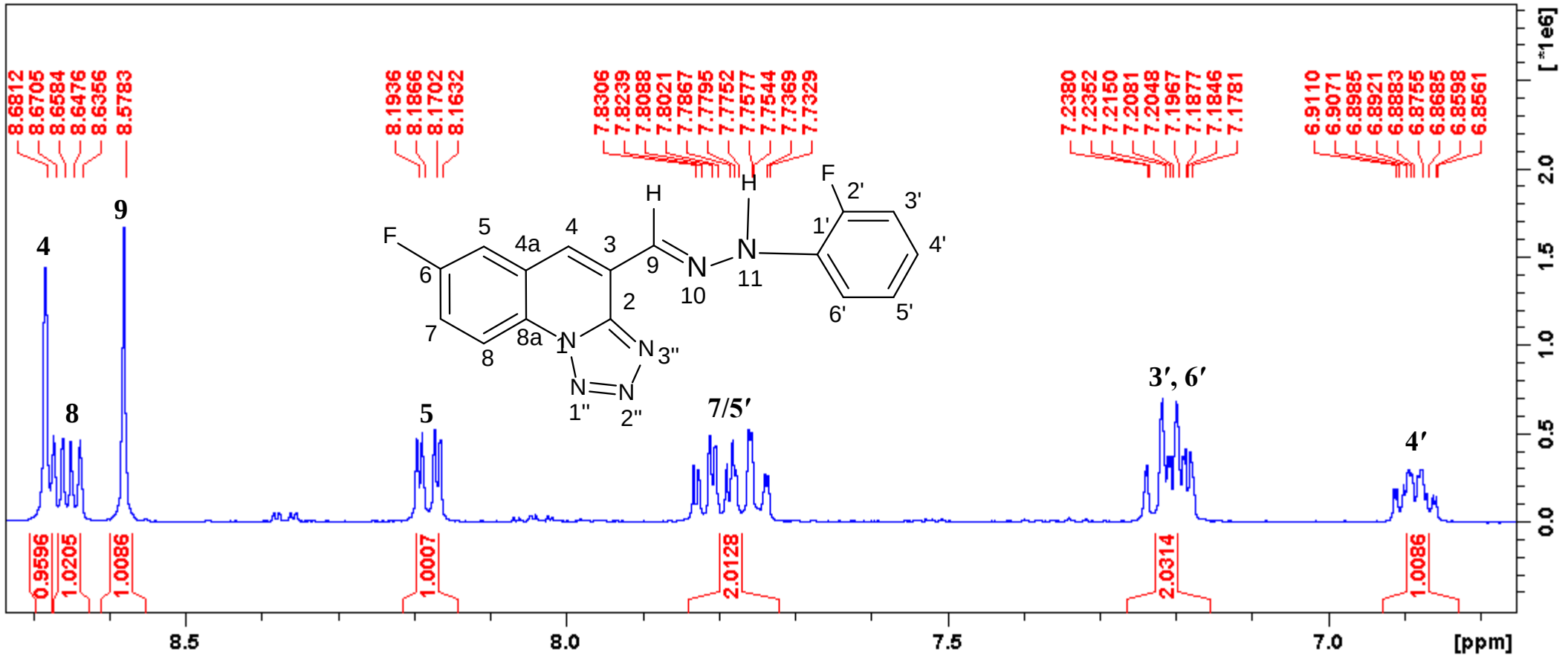




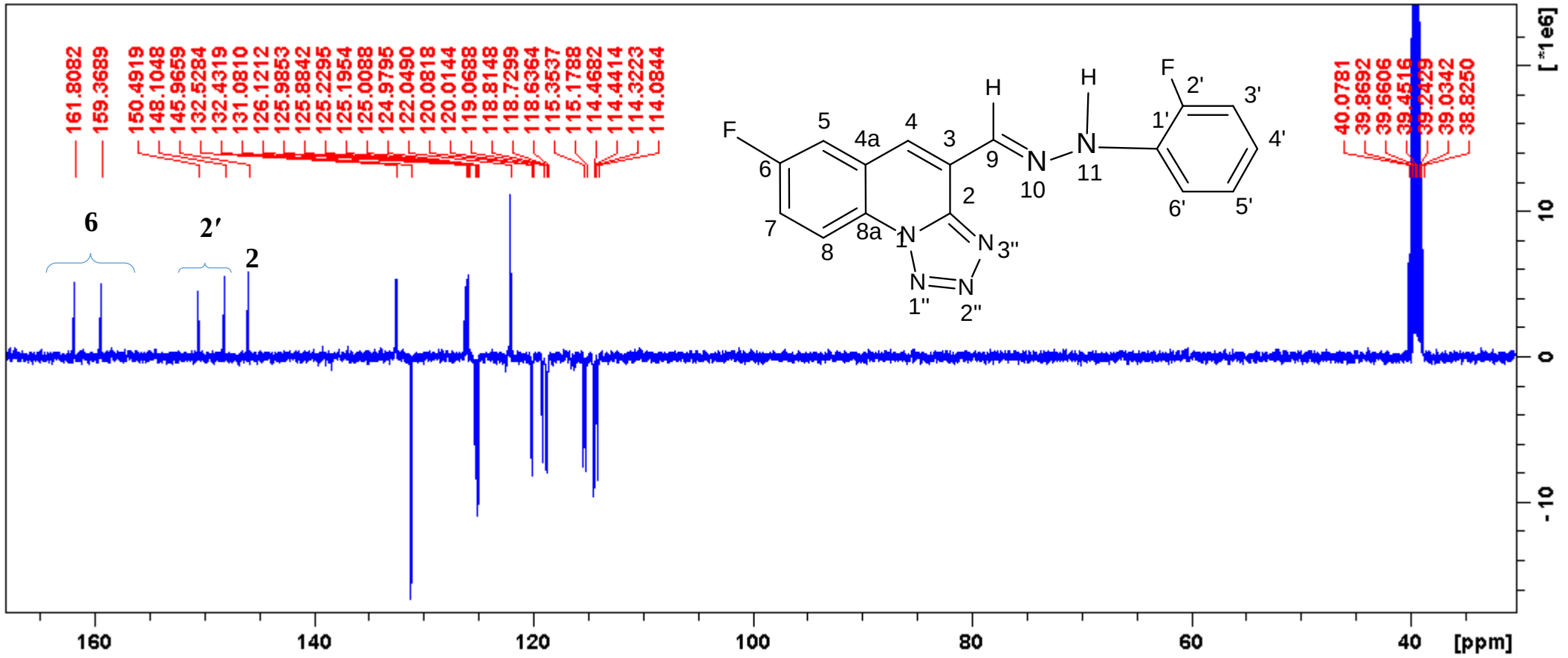
The UV-Vis spectrum of compound 5i



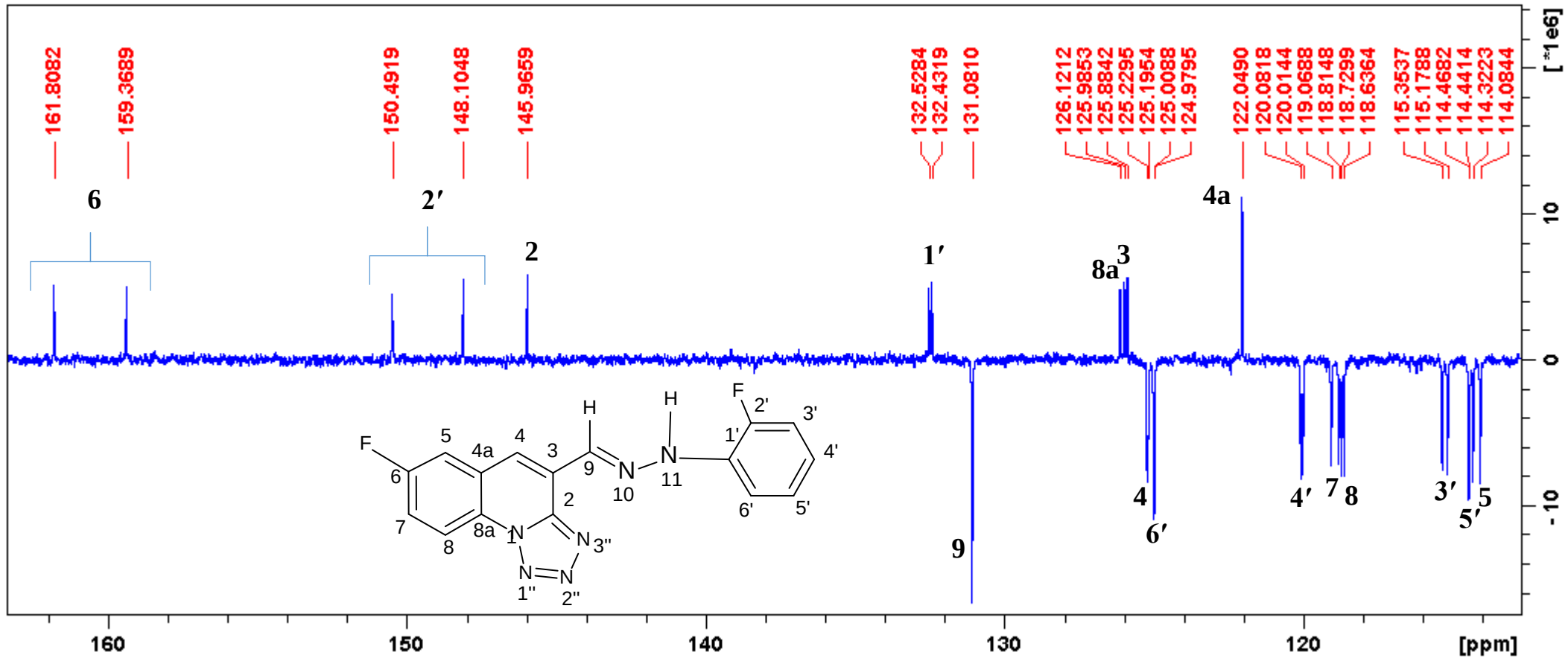
The ^{1}H NMR spectrum of compound 5j



The ^{1}H NMR spectrum of compound 5j (expanded)



The ^{13}C NMR spectrum of compound 5j



The ^{13}C NMR spectrum of compound 5j-expanded

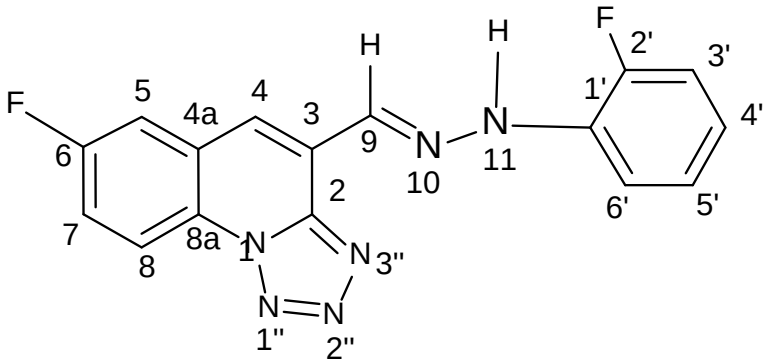
Elemental Composition Report

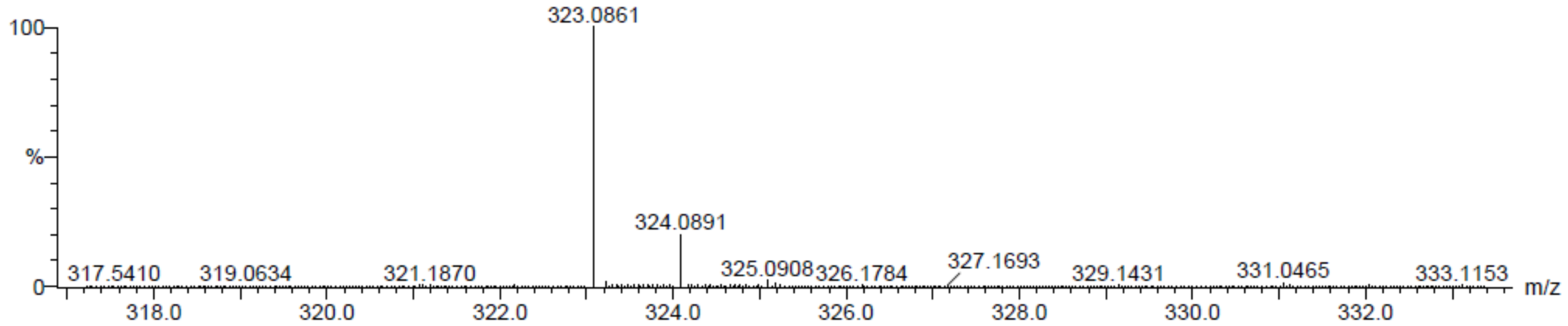
Single Mass Analysis

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
10 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 5-10 F: 0-2

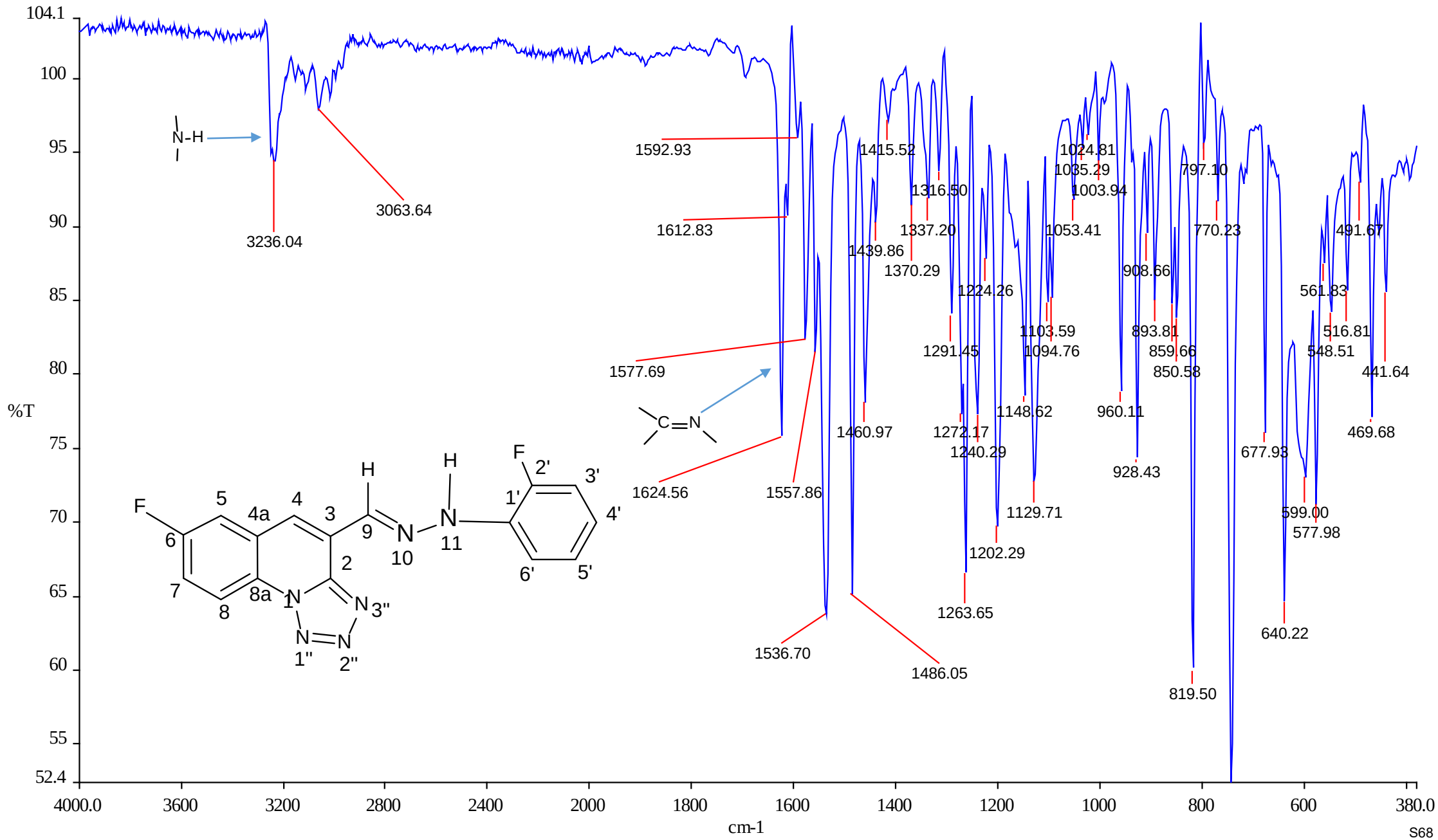
Compound 4g HG 13 (0.405) Cm (1:61)
TOF MS ES-





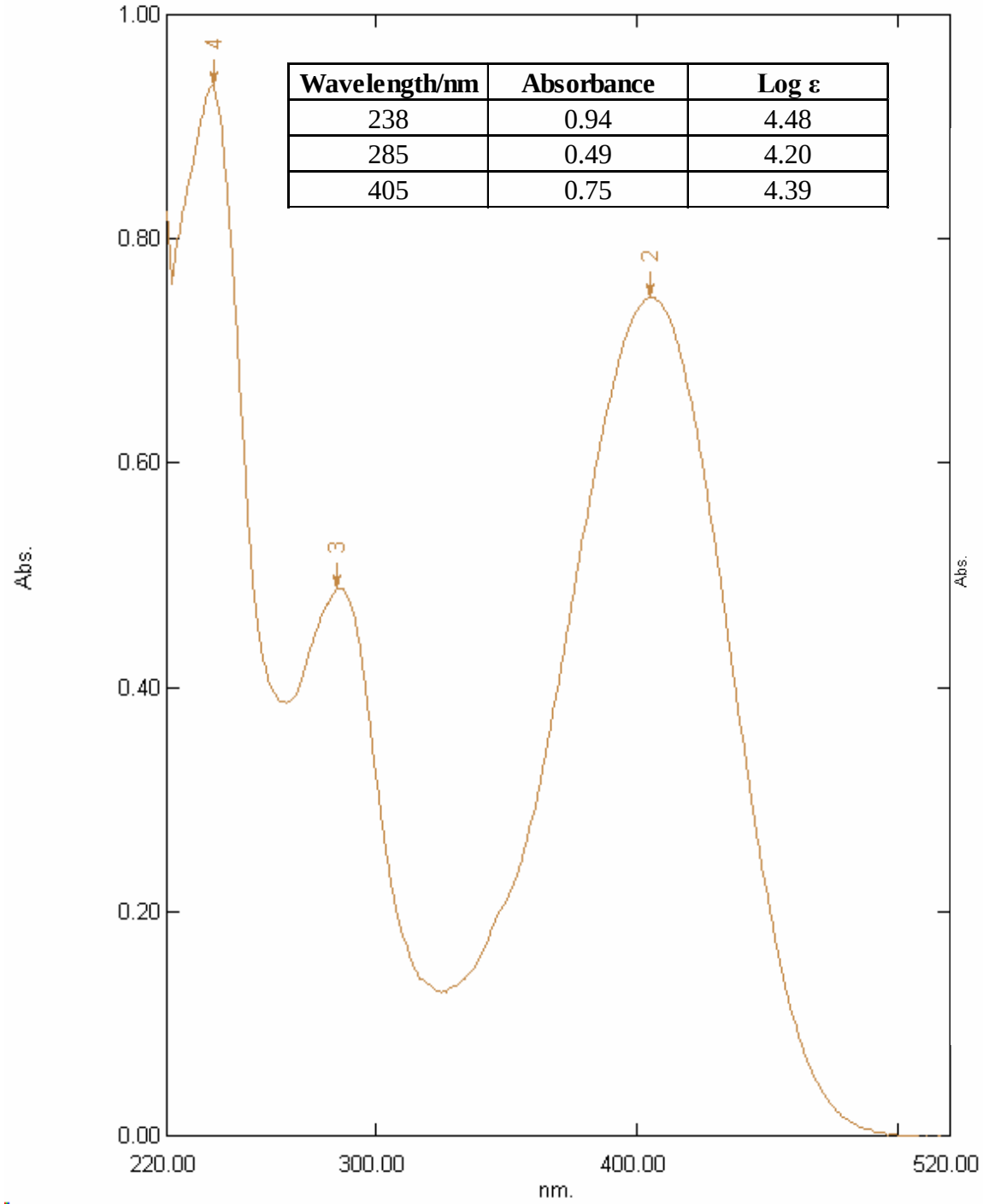
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
323.0861	323.0857	0.4	1.2	14.5	640.8	0.0	C16 H9 N6 F2

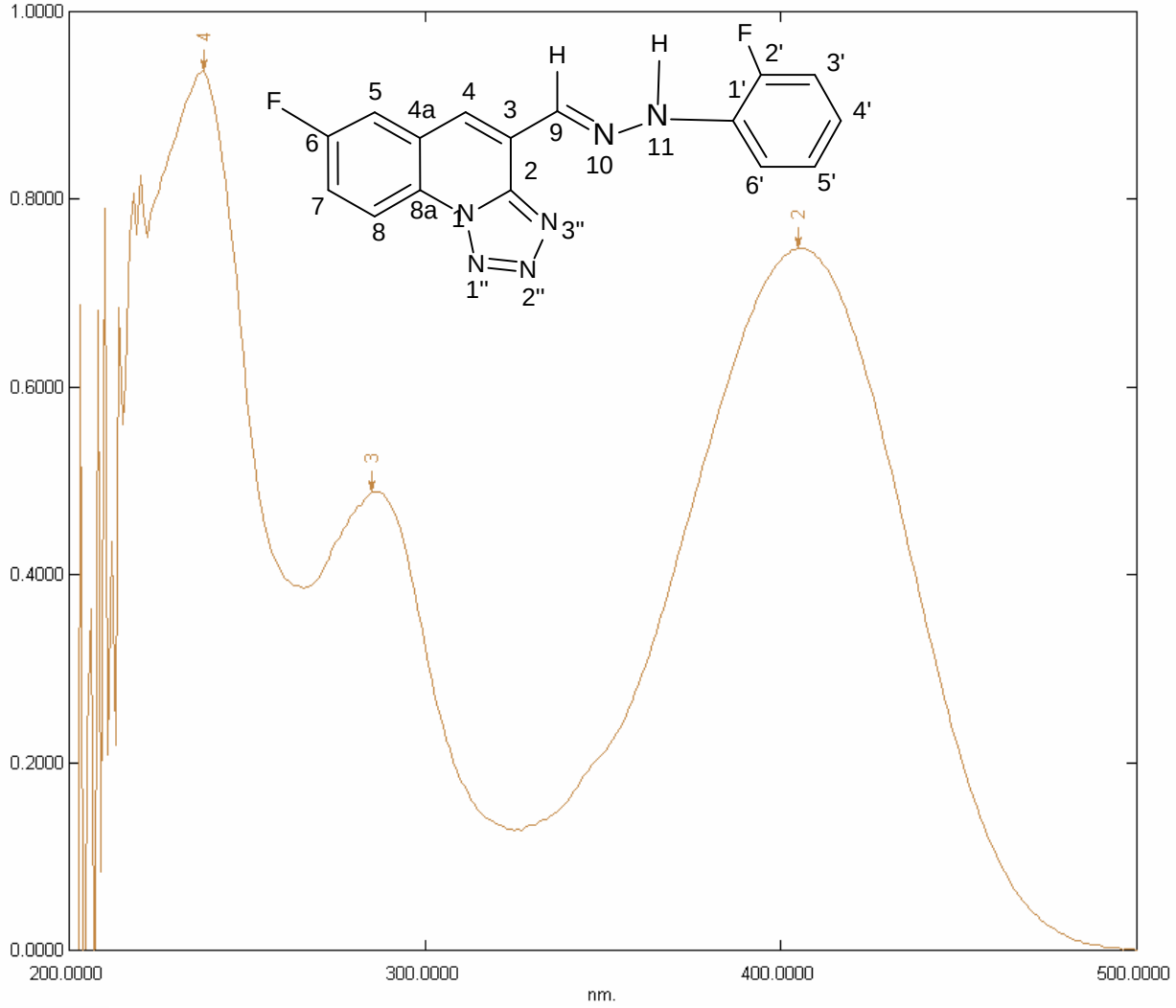
The HRMS spectrum of compound 5j



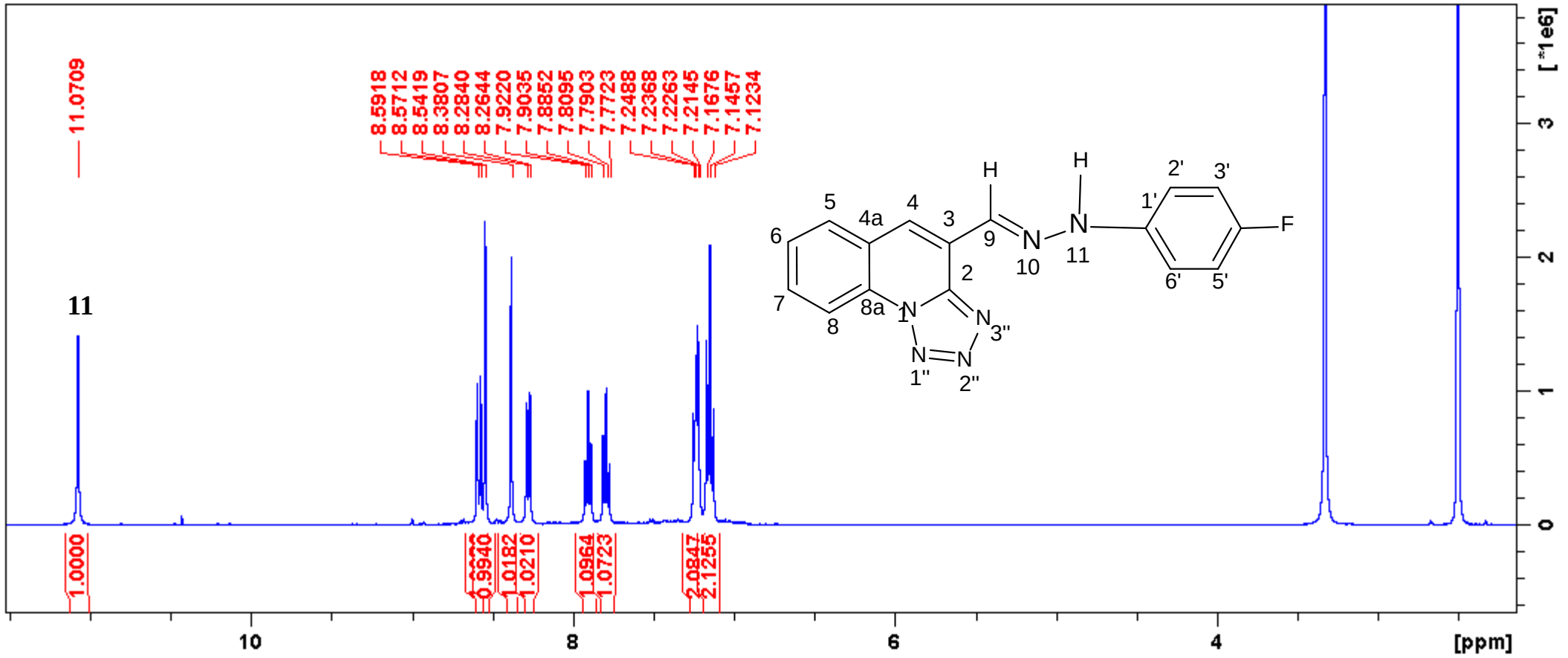
The IR spectrum of compound 5j

Wavelength/nm	Absorbance	Log ε
238	0.94	4.48
285	0.49	4.20
405	0.75	4.39

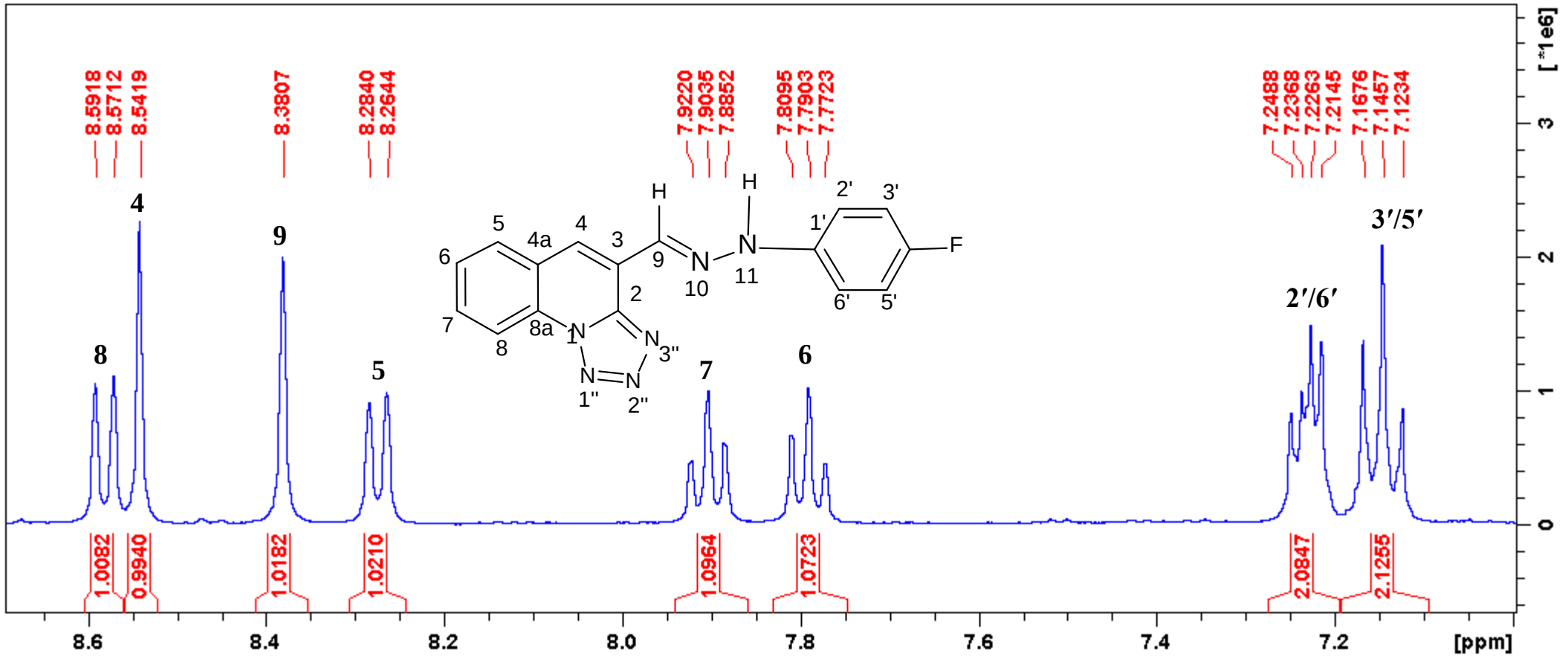




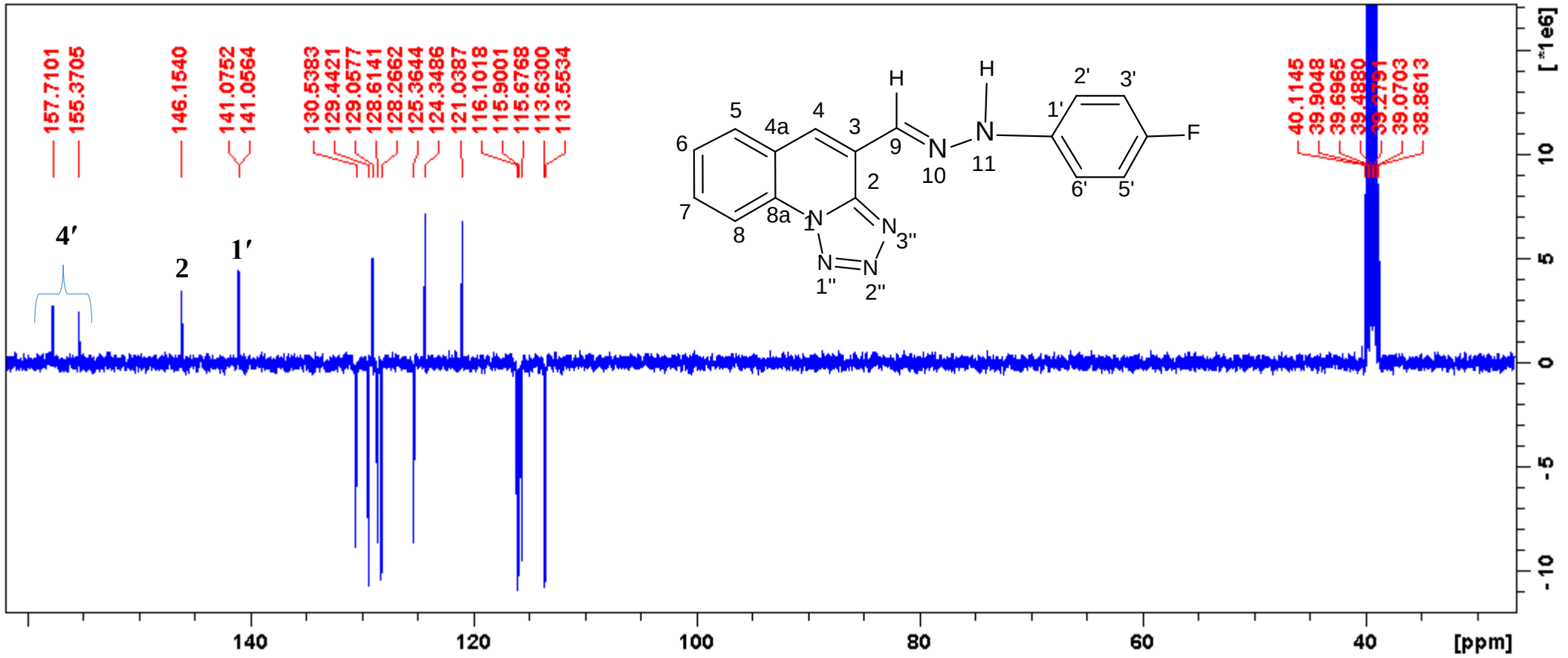
The UV-Vis spectrum of compound 5j



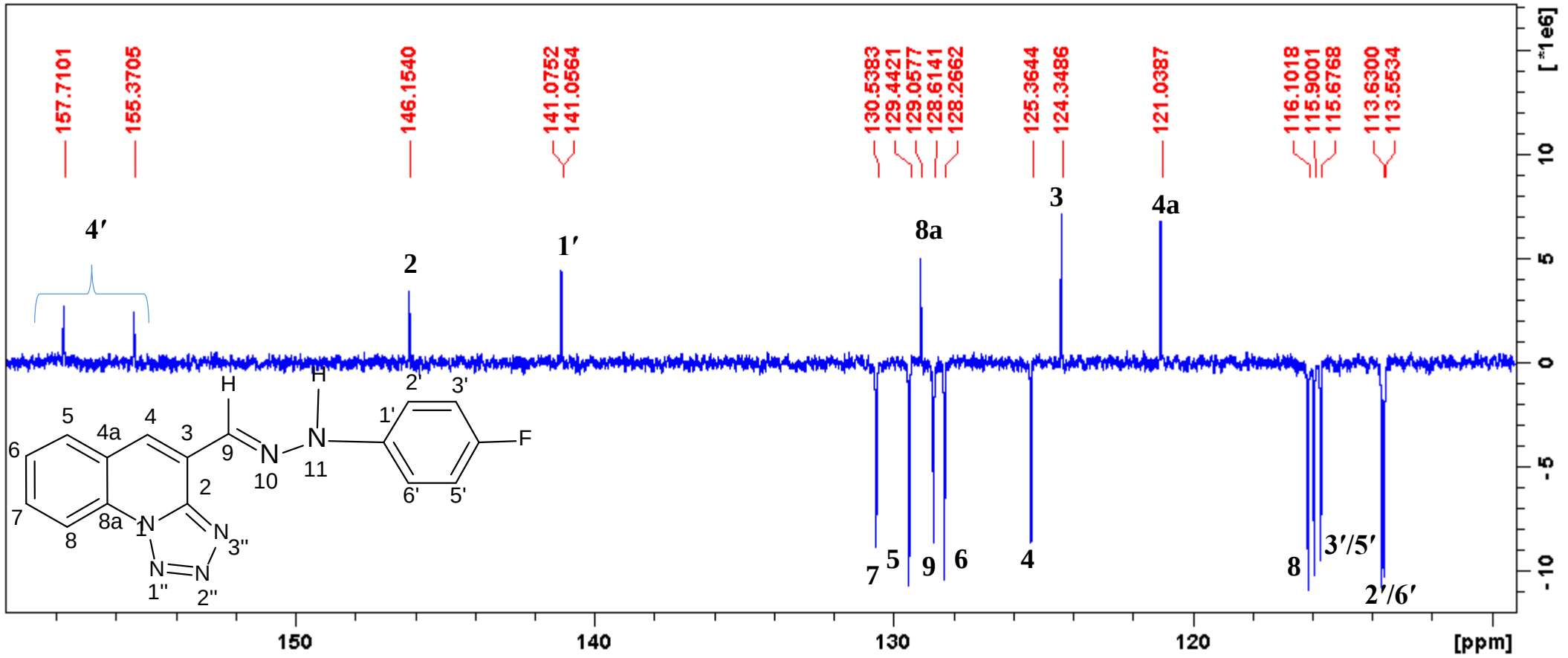
The ^{1}H NMR spectrum of compound 5k



The ^{1}H NMR spectrum of compound 5k (expanded)



The ^{13}C NMR spectrum of compound 5k



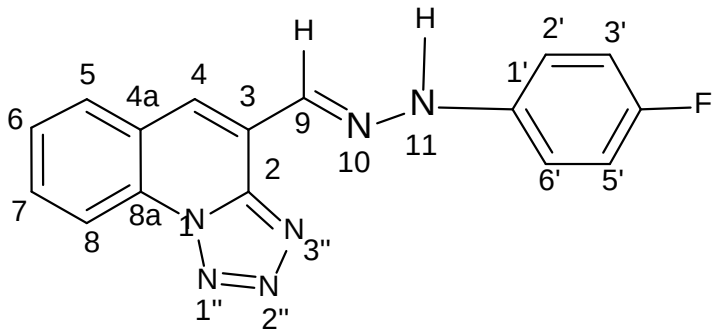
The ^{13}C NMR spectrum of compound 5k (expanded)

Elemental Composition Report

Single Mass Analysis

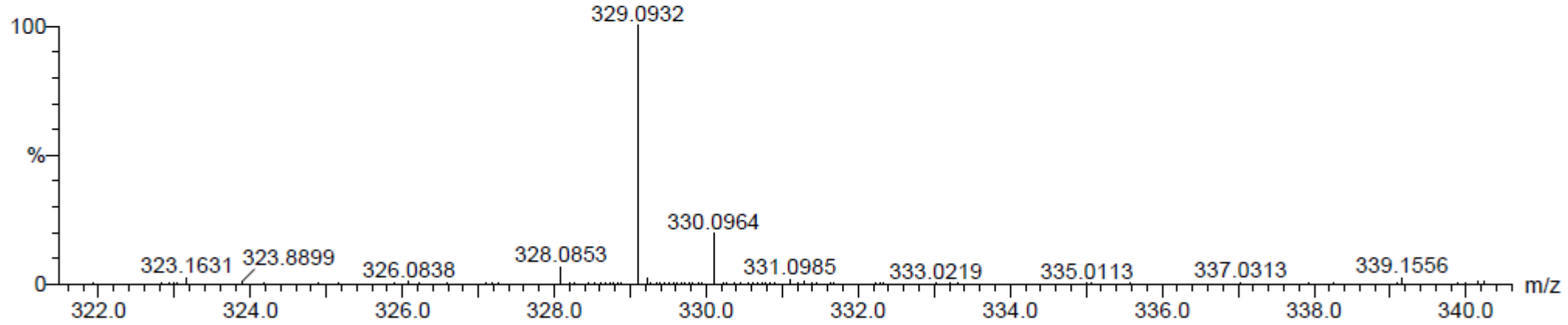
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
12 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: 5-10 Na: 0-1 F: 0-1



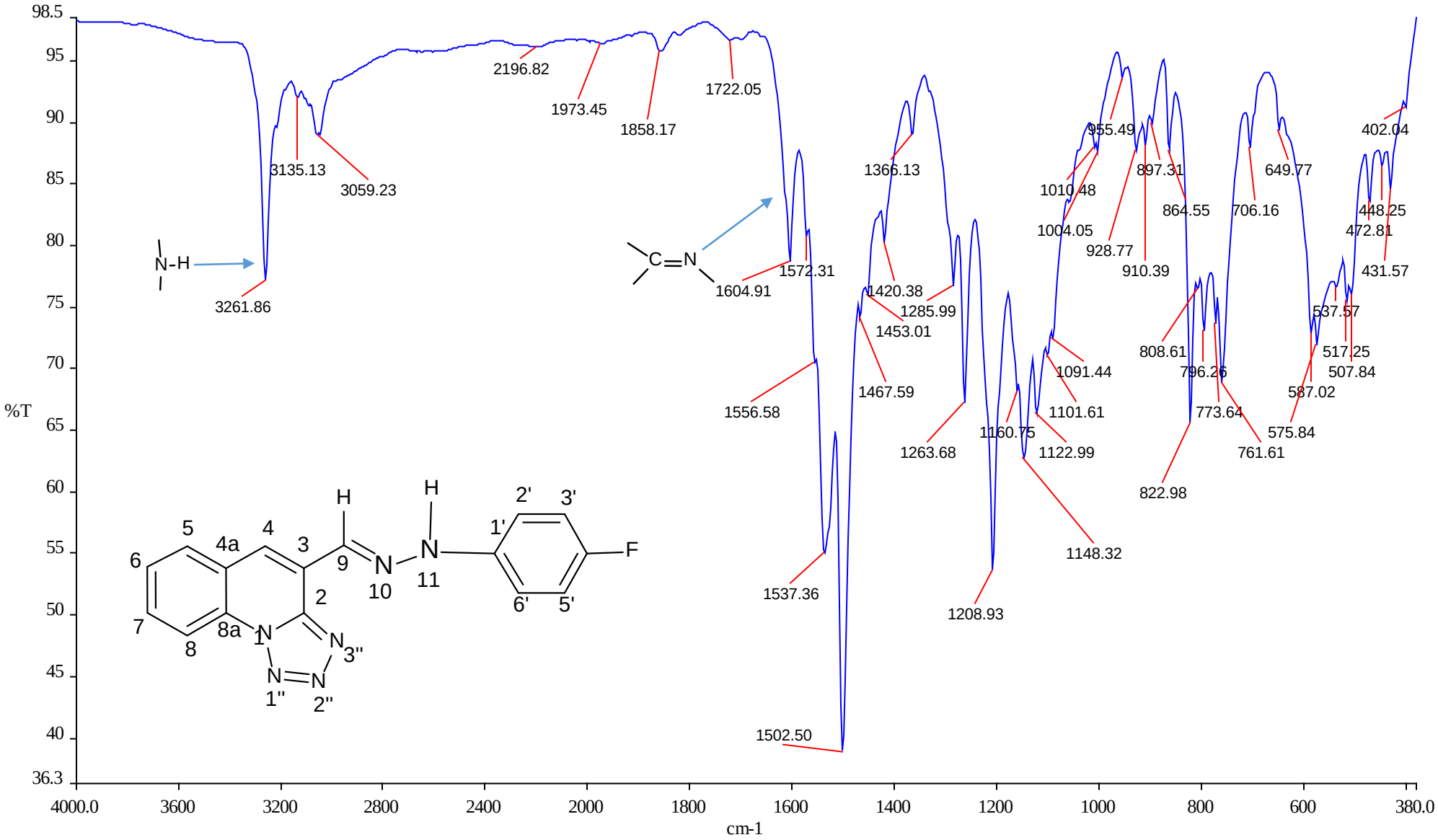
Compound 3e HG 50 (1.651) Cm (1:61)
TOF MS ES+

1.12e+006



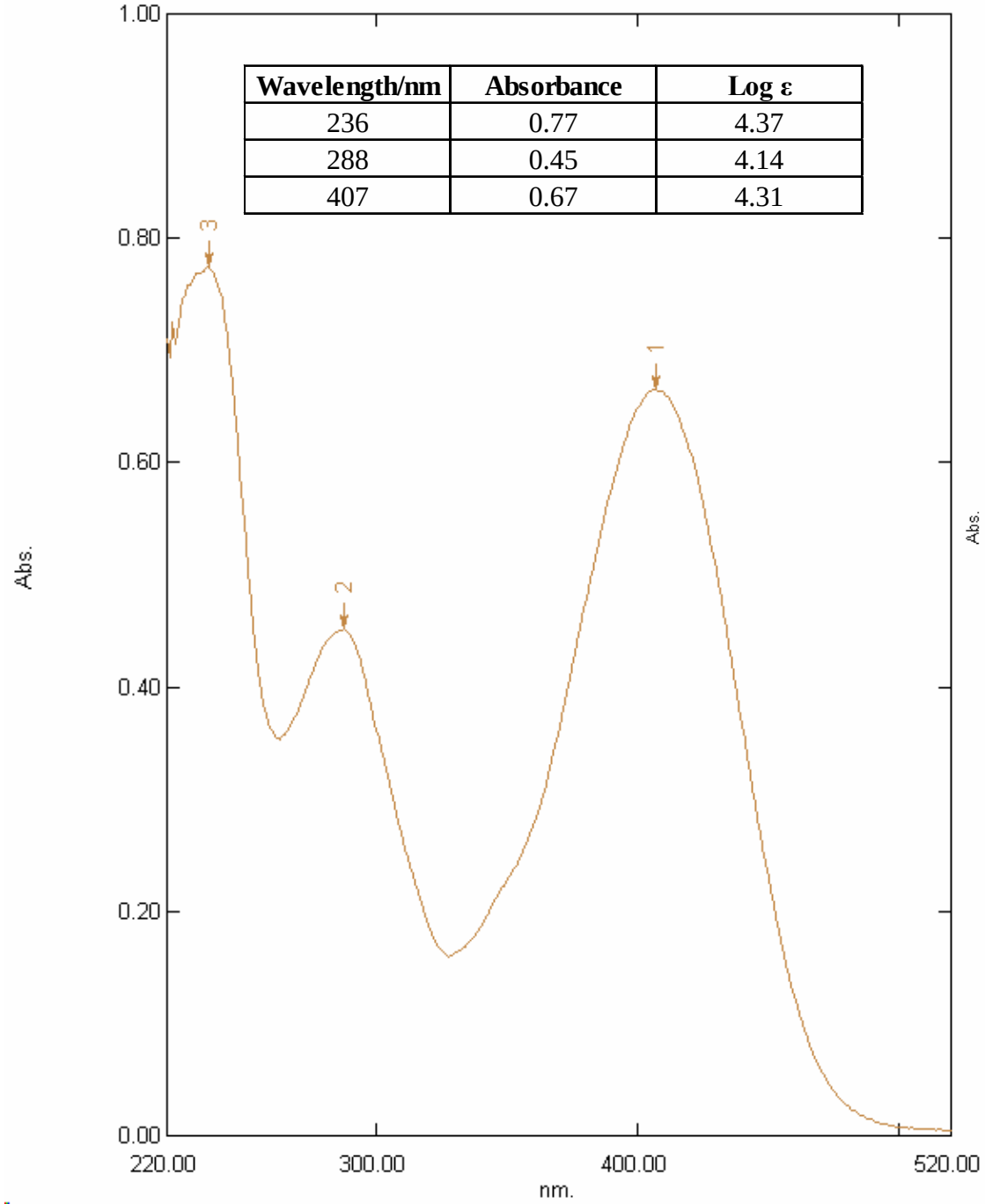
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
329.0932	329.0927	0.5	1.5	13.5	724.9	0.0	C16 H11 N6 Na F

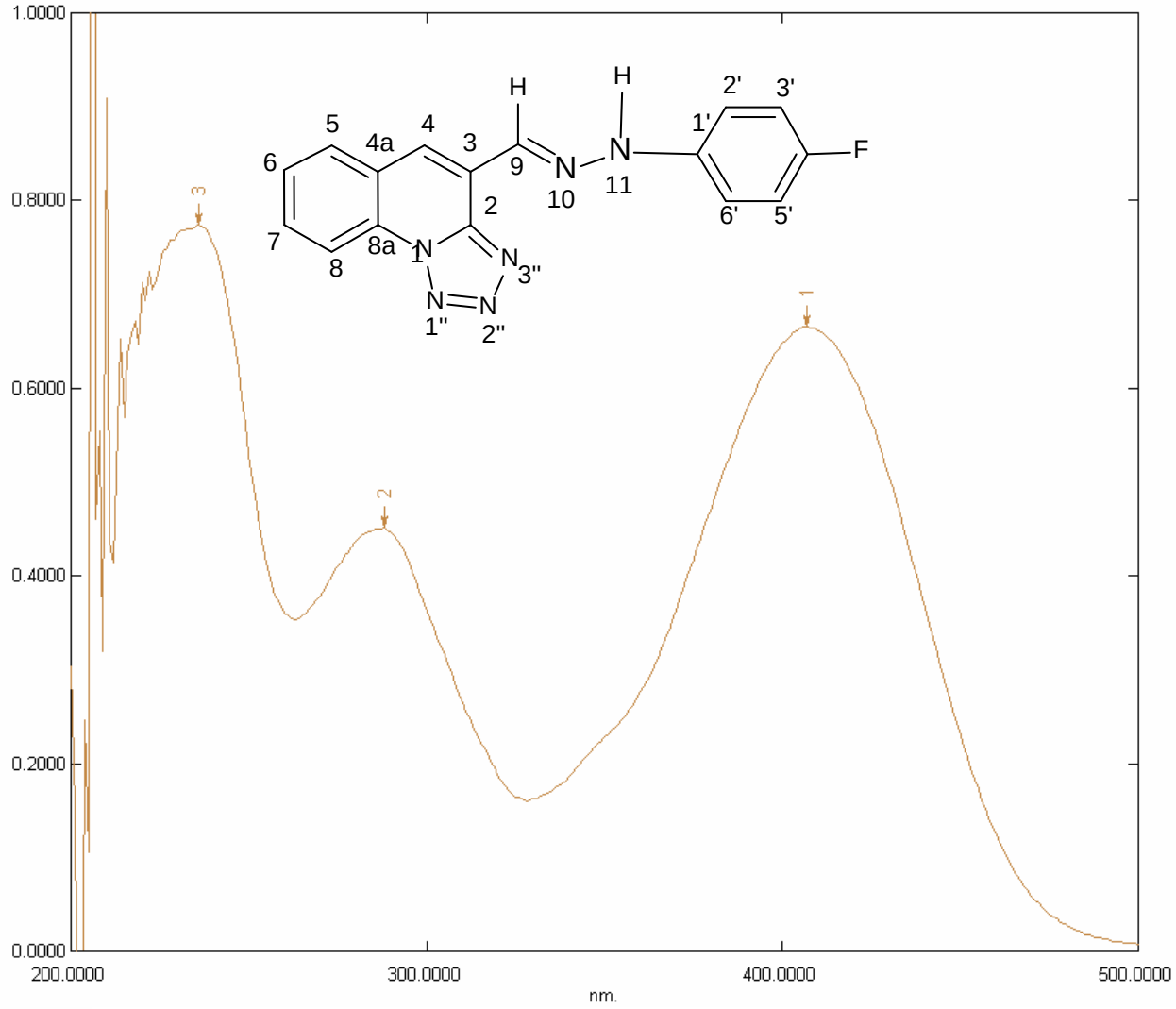
The HRMS spectrum of compound 5k



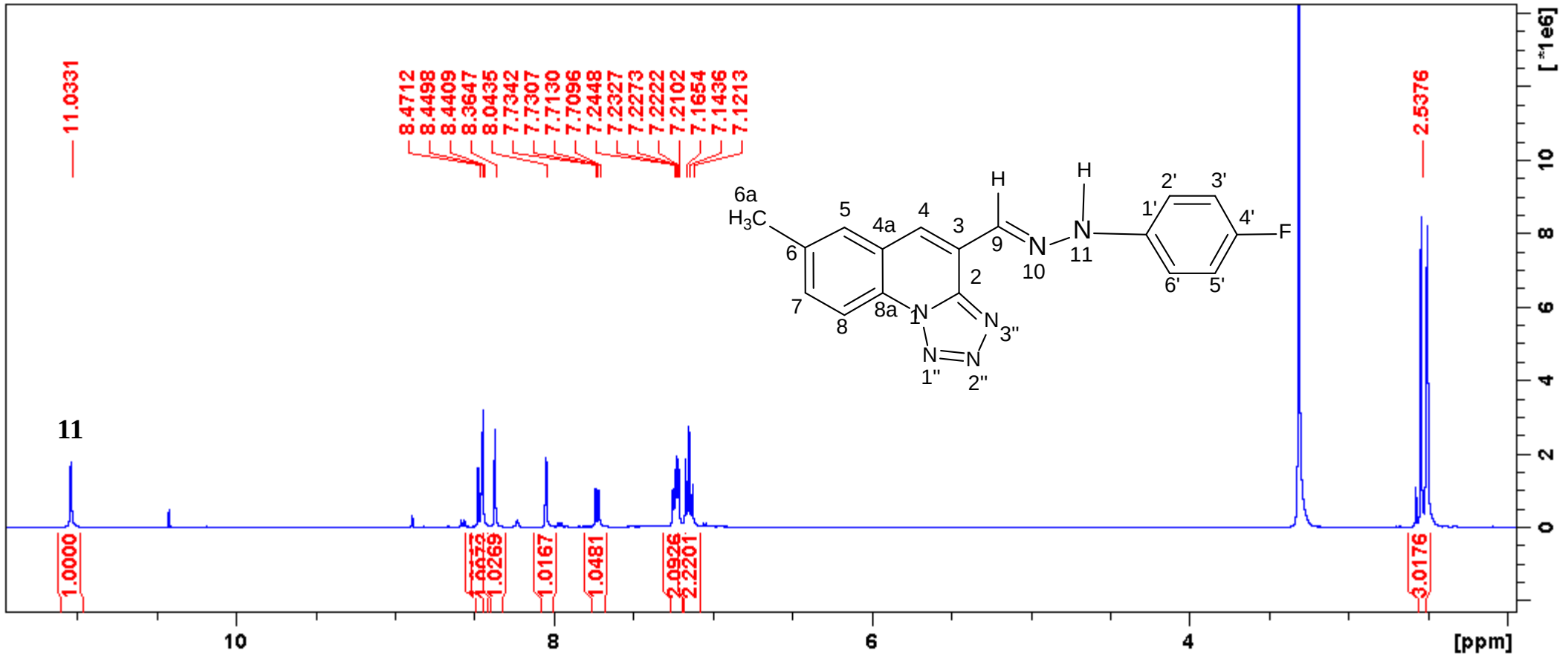
The IR spectrum of compound 5k

Wavelength/nm	Absorbance	Log ε
236	0.77	4.37
288	0.45	4.14
407	0.67	4.31

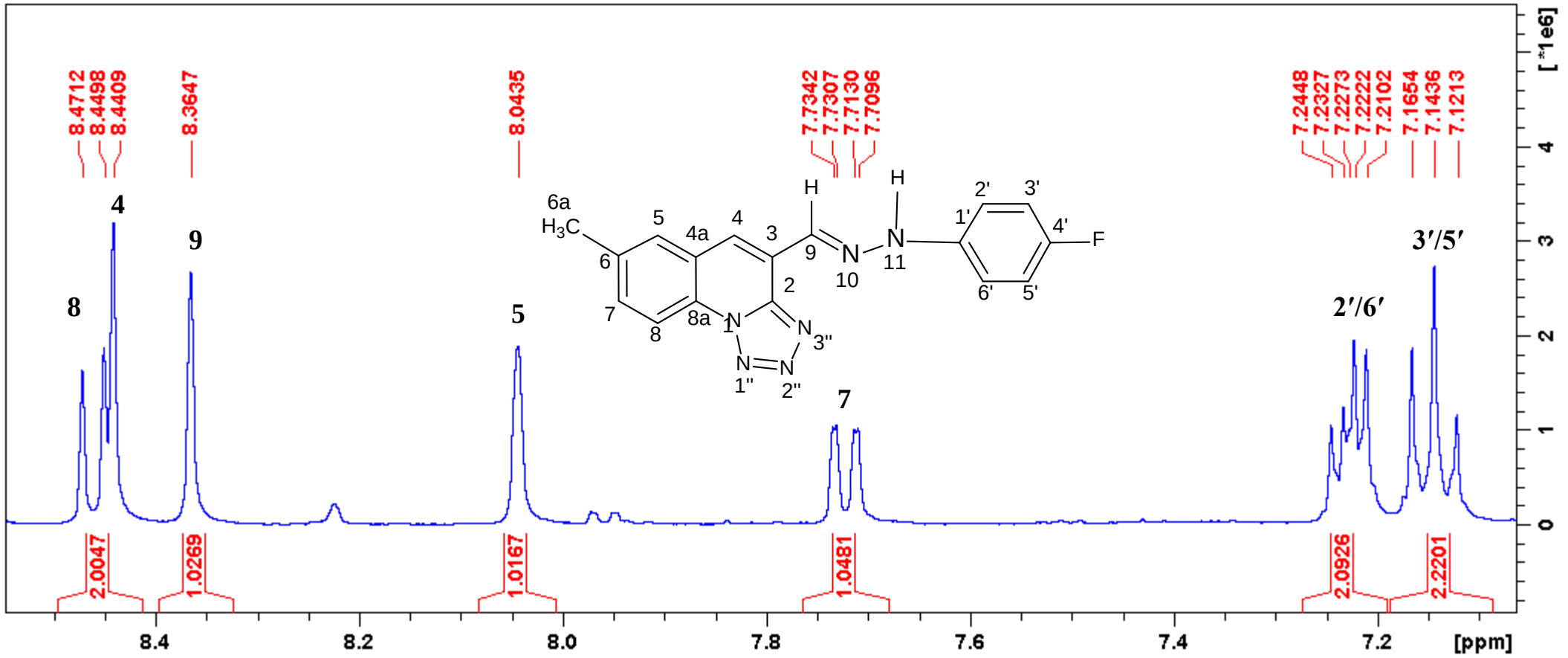




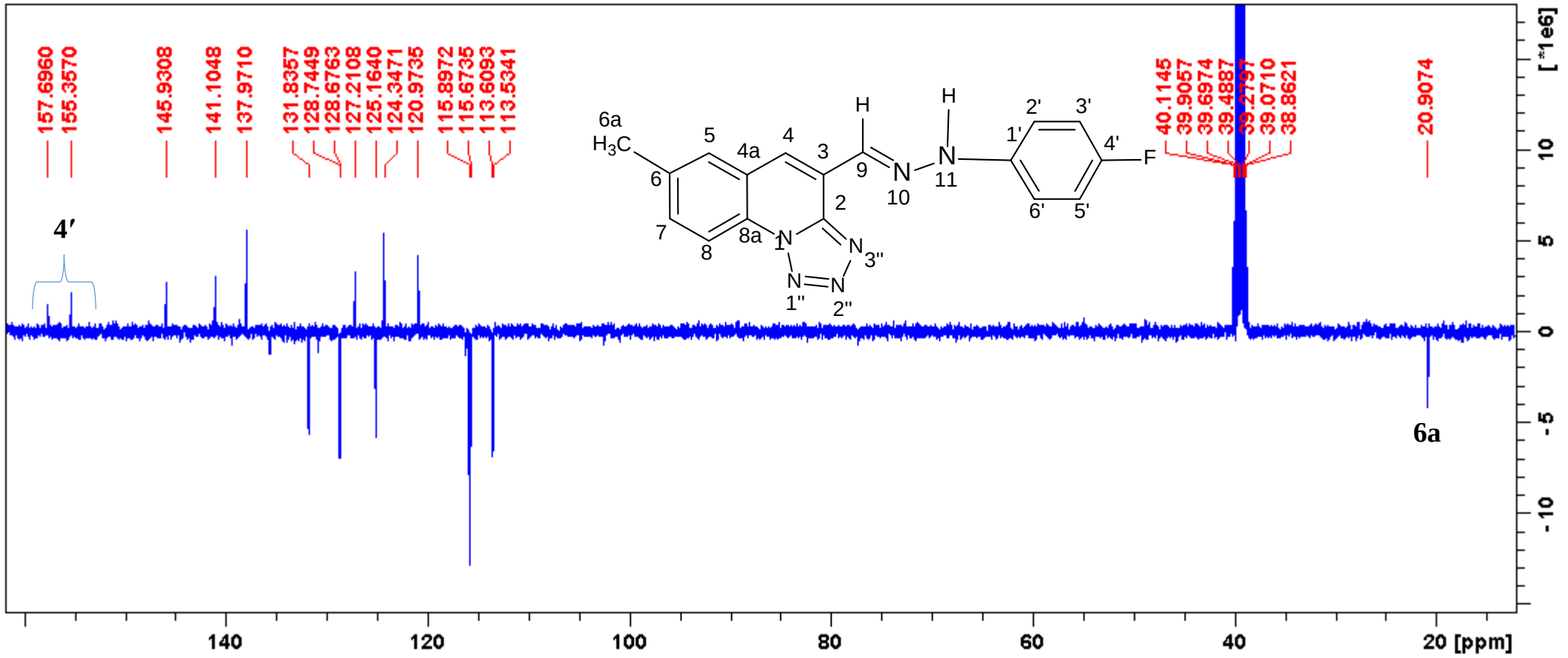
The UV-Vis spectrum of compound 5k



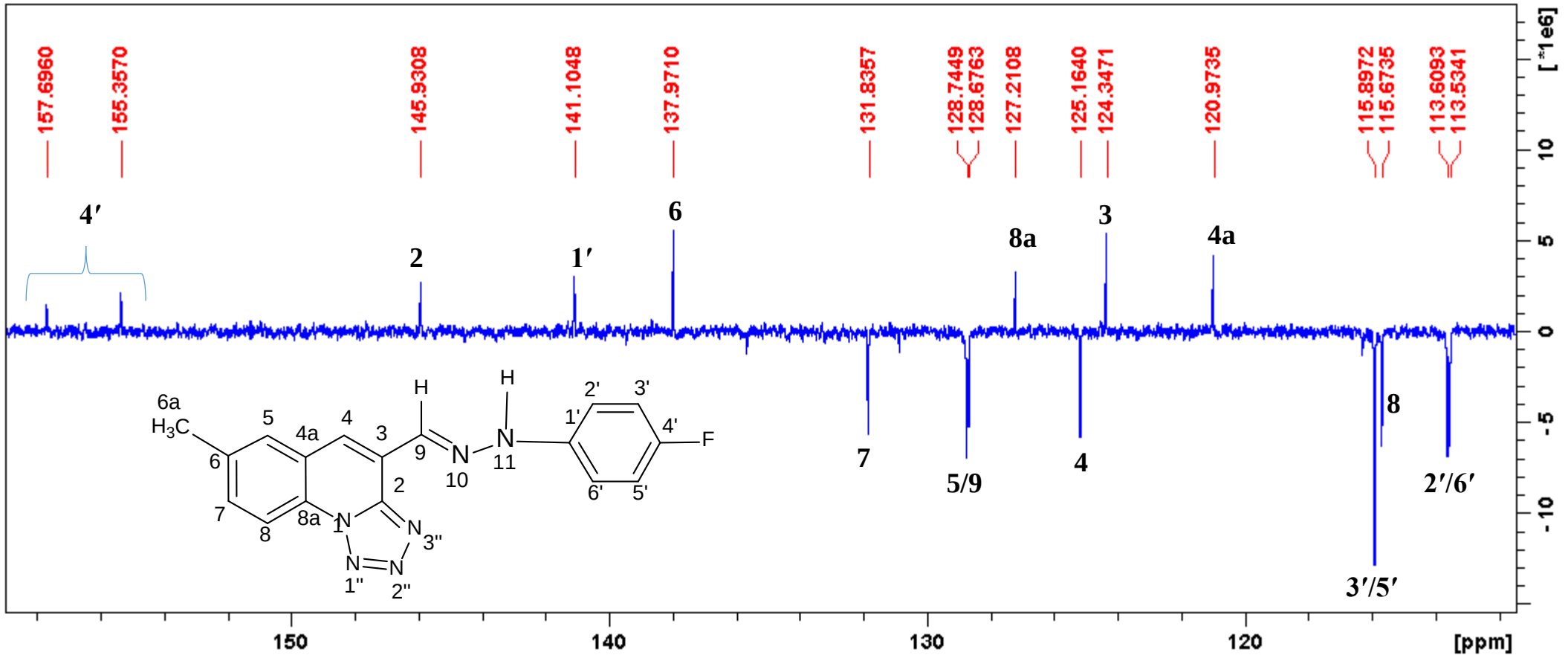
The ^{1}H NMR spectrum of compound 5l



The ^{1}H NMR spectrum of compound 5l (expanded)



The ^{13}C NMR spectrum of compound 5l

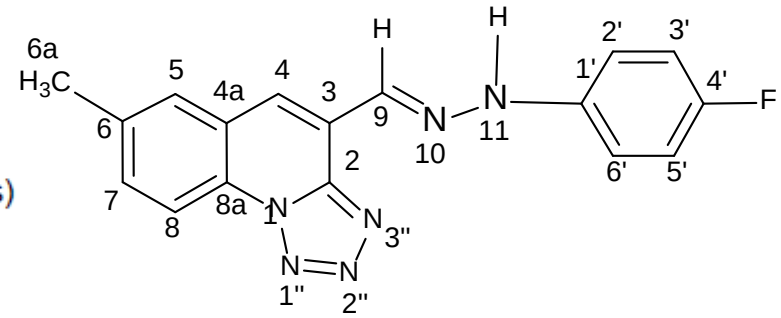


The ^{13}C NMR spectrum of compound 5l-expanded

Elemental Composition Report

Single Mass Analysis

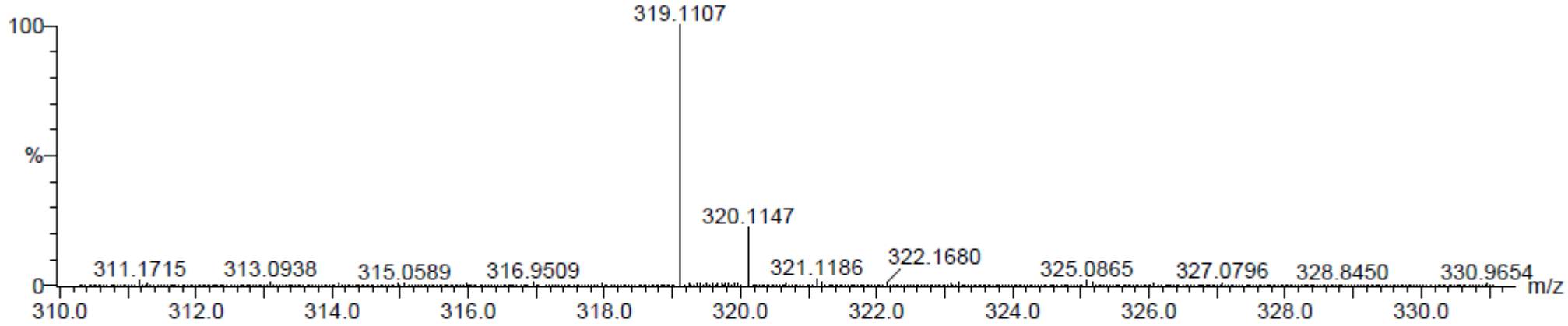
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
6 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: 5-10 F: 0-1

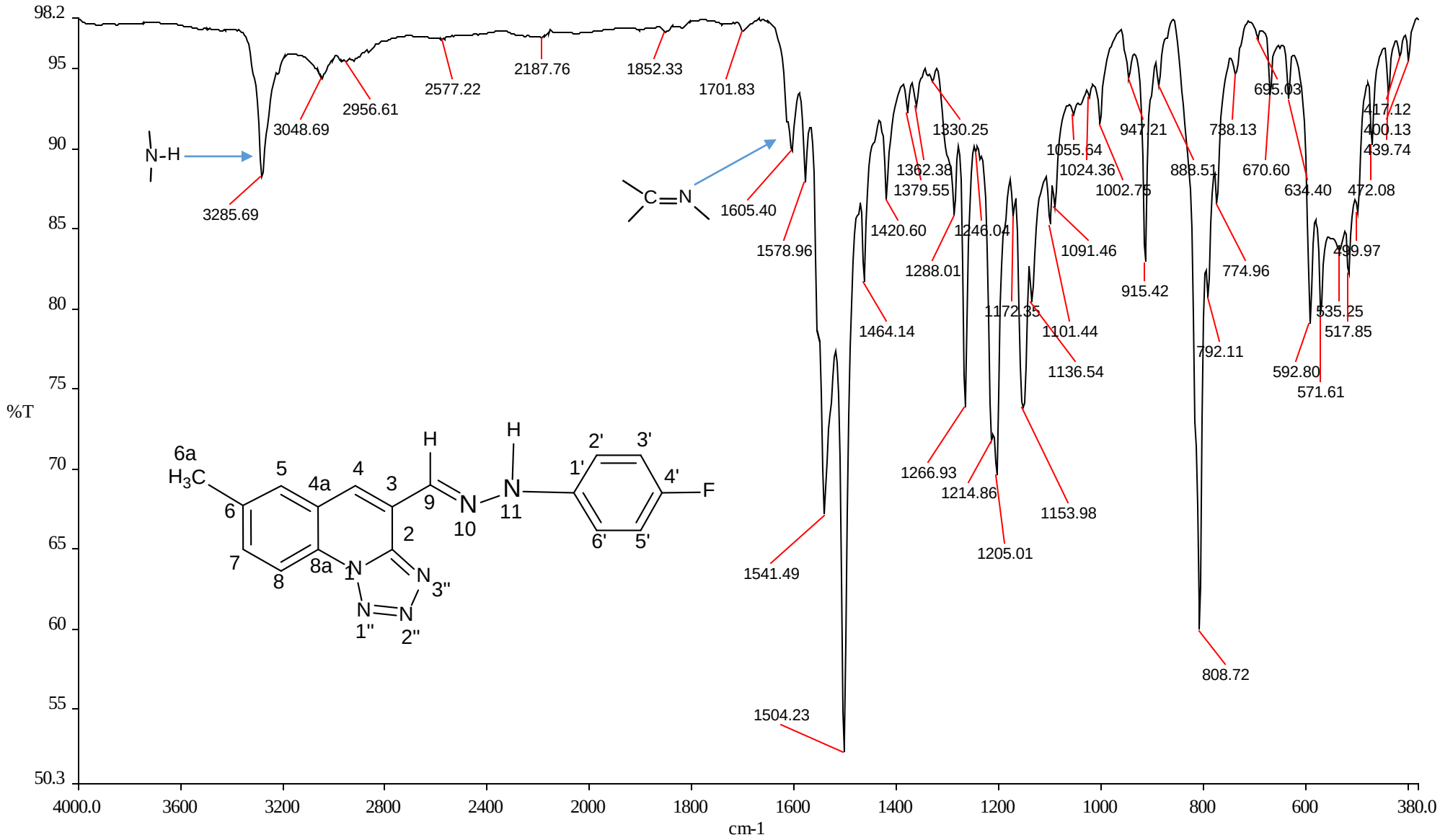
Compound 1f 54 (1.788) Cm (1:61)
TOF MS ES-

4.08e+005



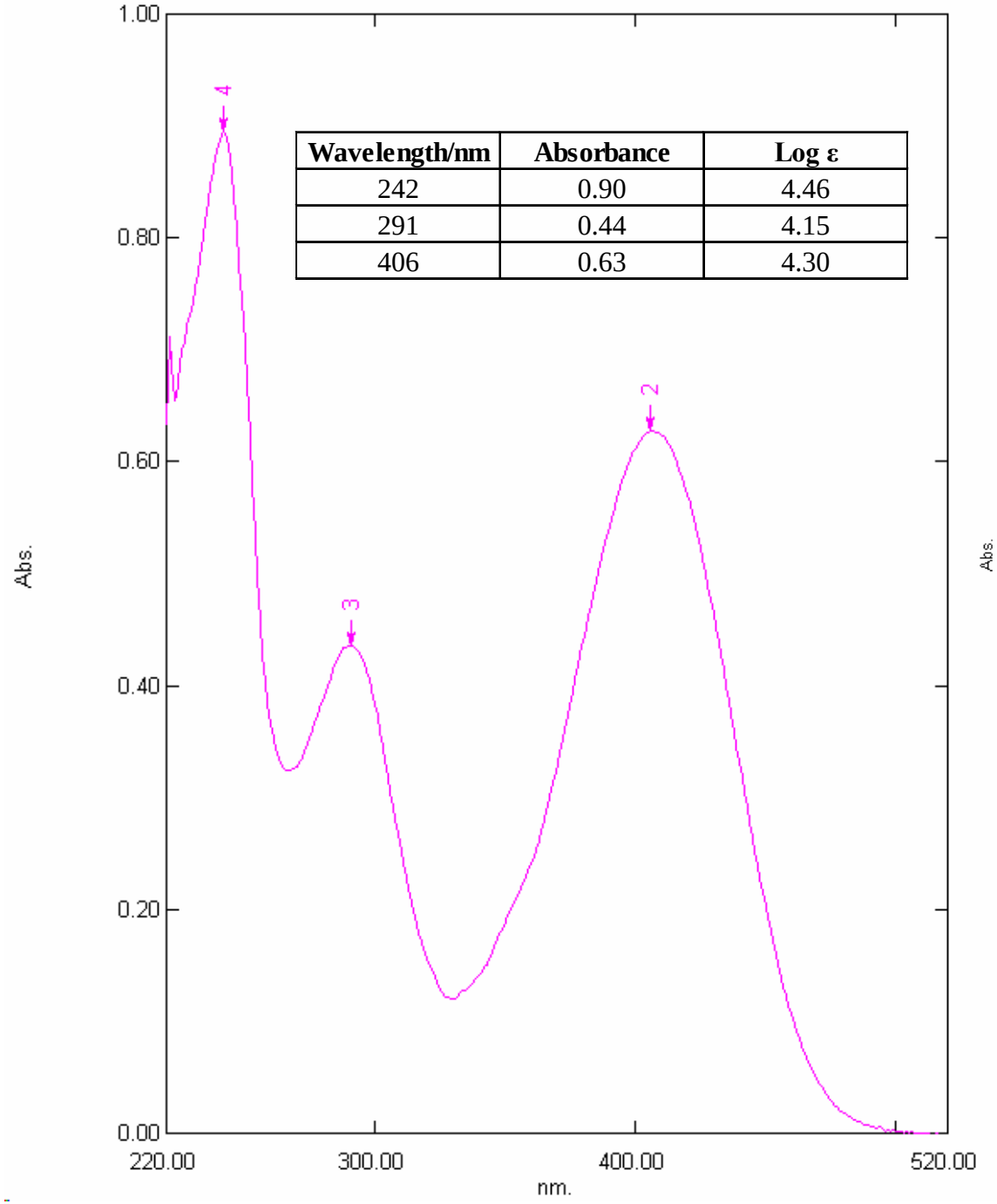
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
319.1107	319.1107	0.0	0.0	14.5	662.0	0.0	C17 H12 N6 F

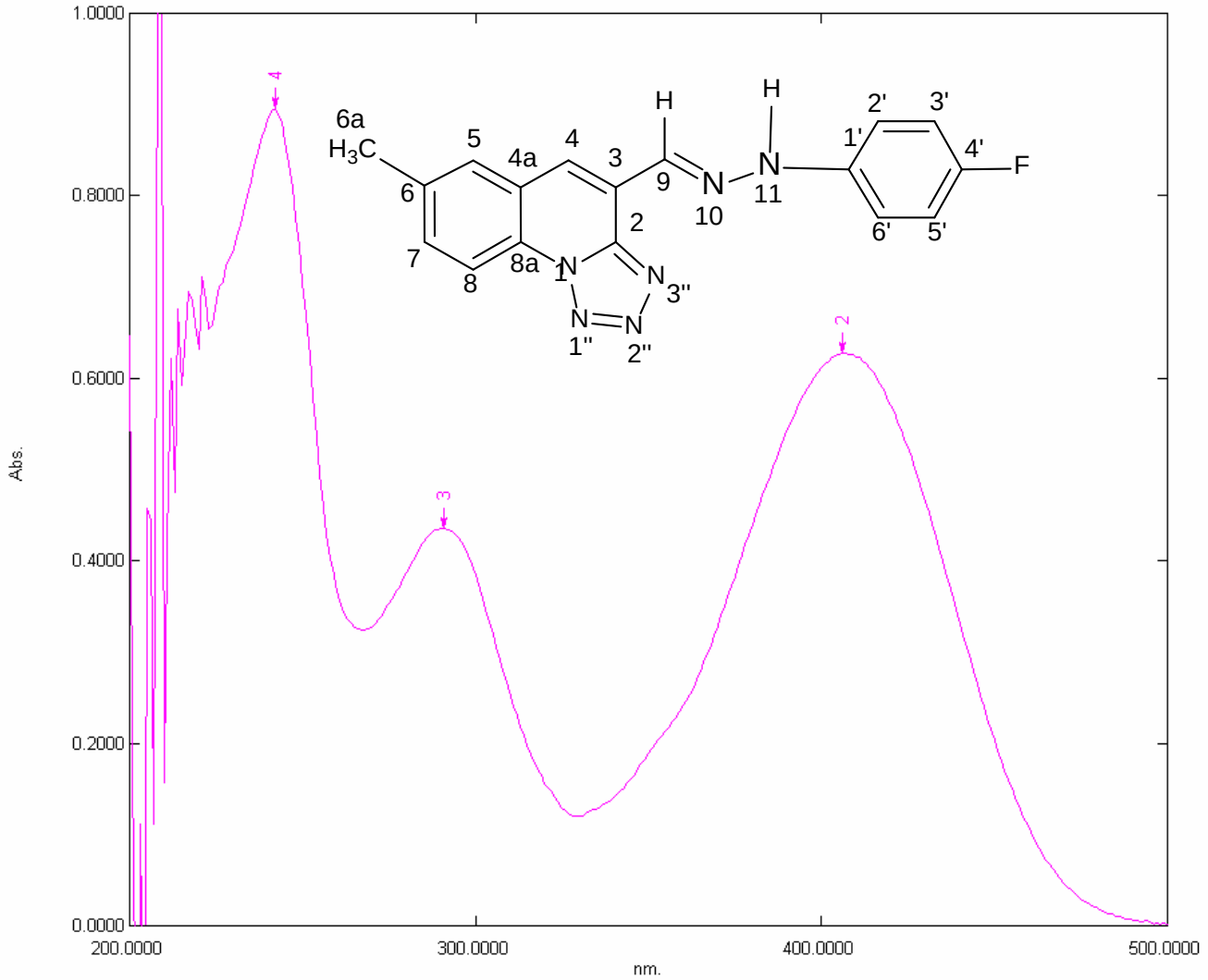
The HRMS spectrum of compound 5l



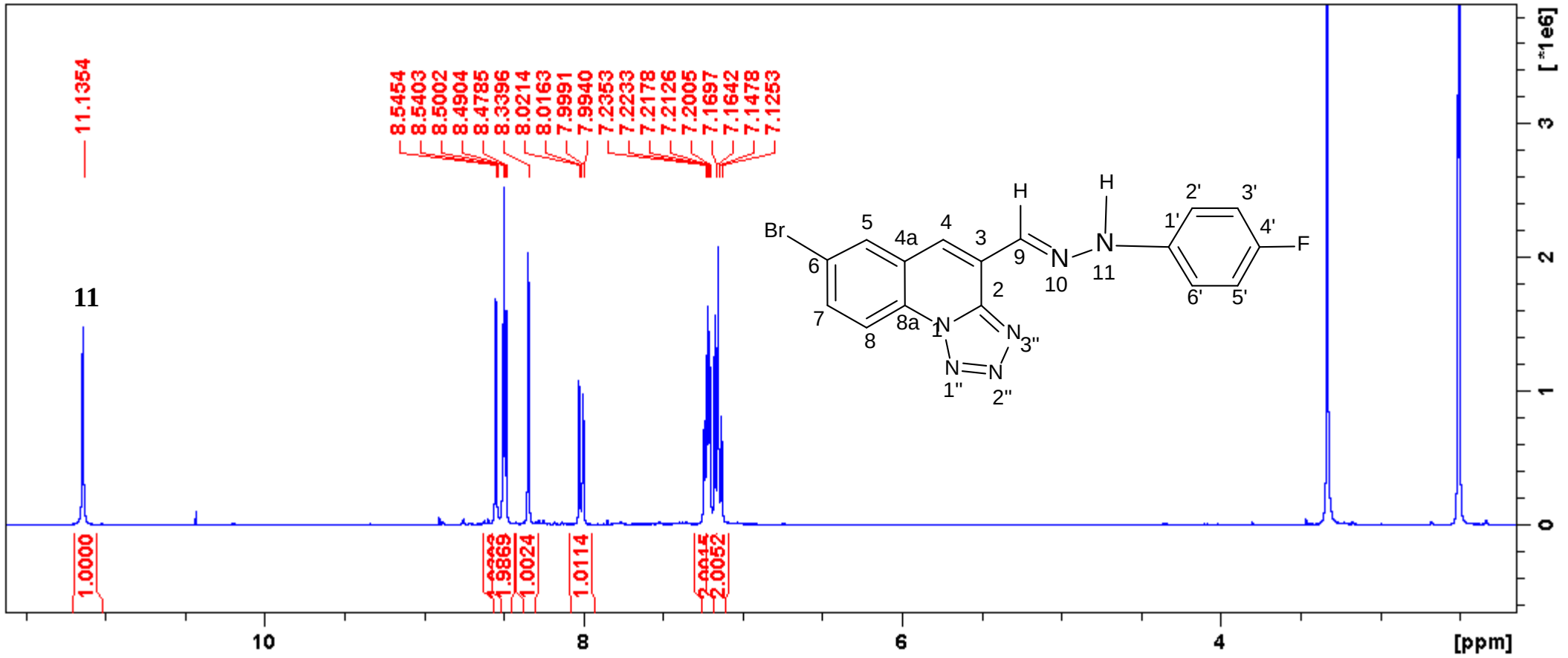
The IR spectrum of compound 5l

Wavelength/nm	Absorbance	Log ε
242	0.90	4.46
291	0.44	4.15
406	0.63	4.30

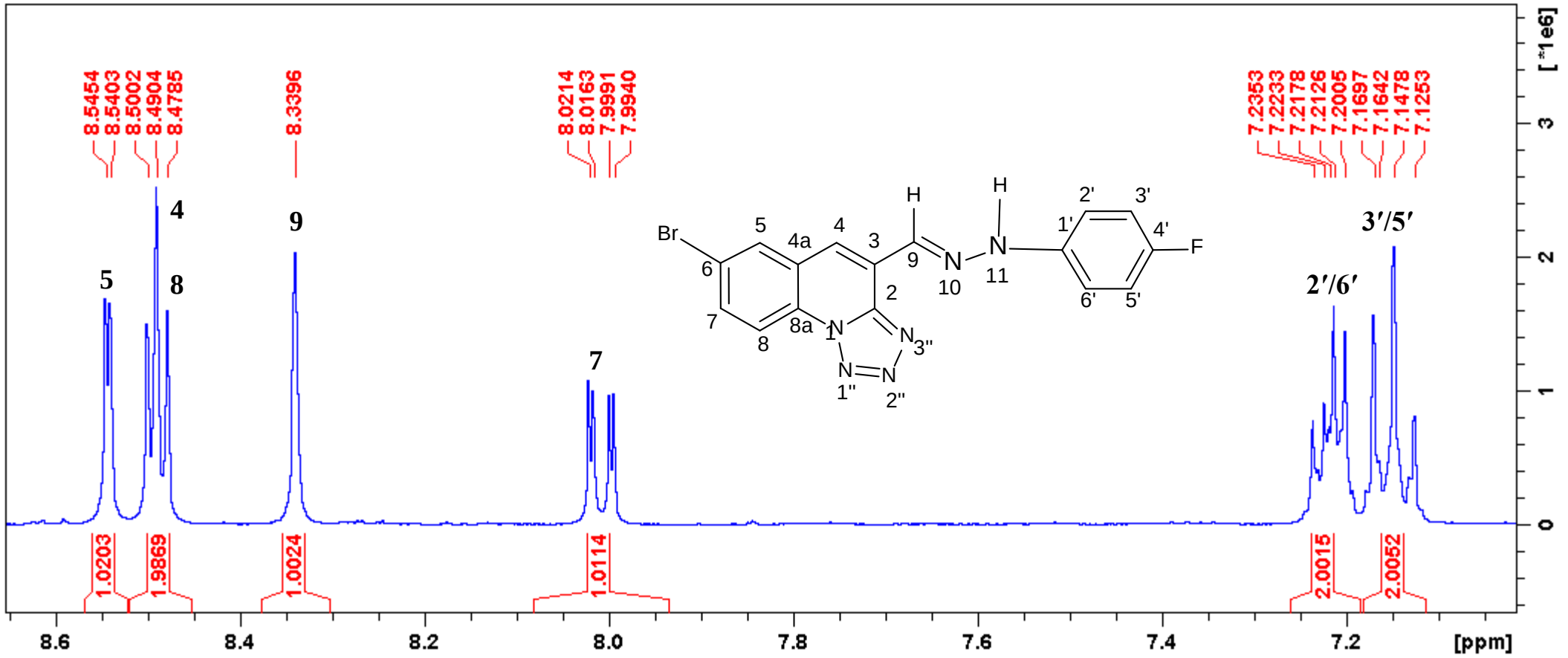




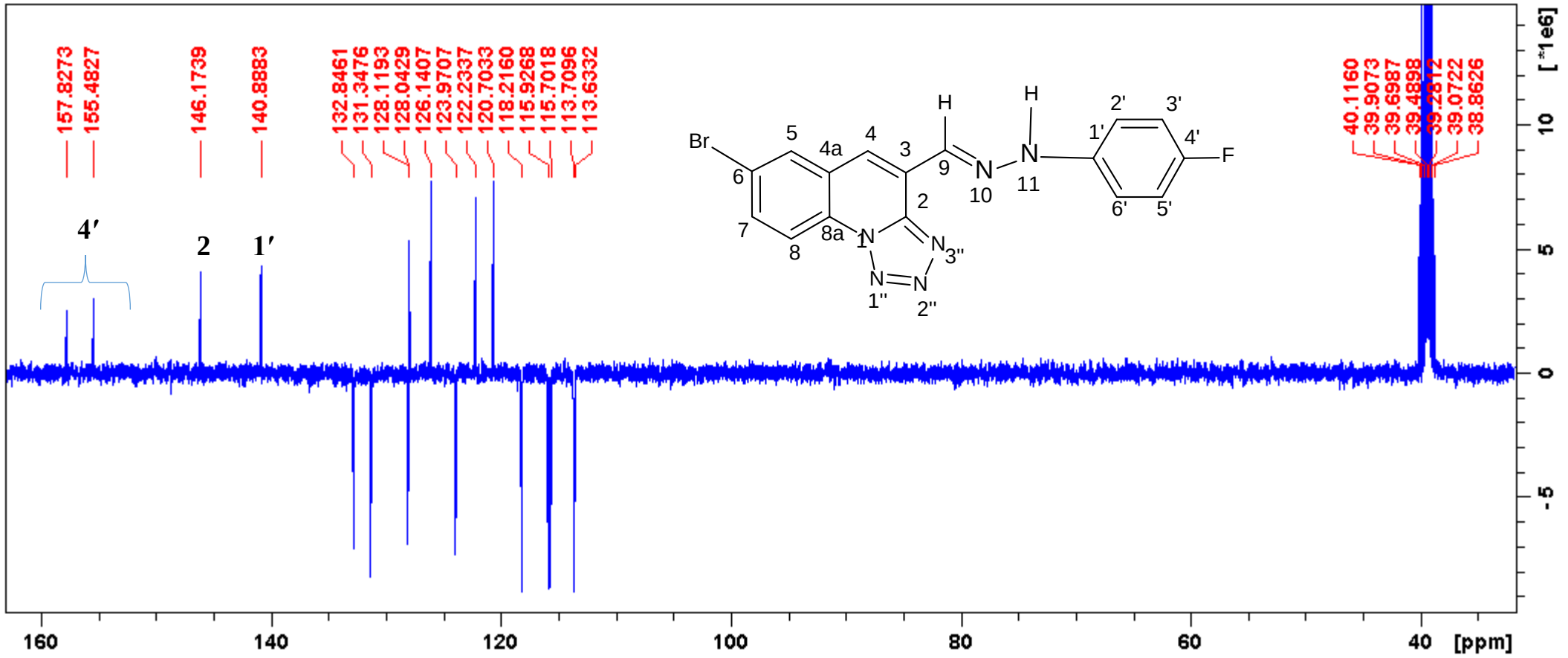
The UV-Vis spectrum of compound 5l



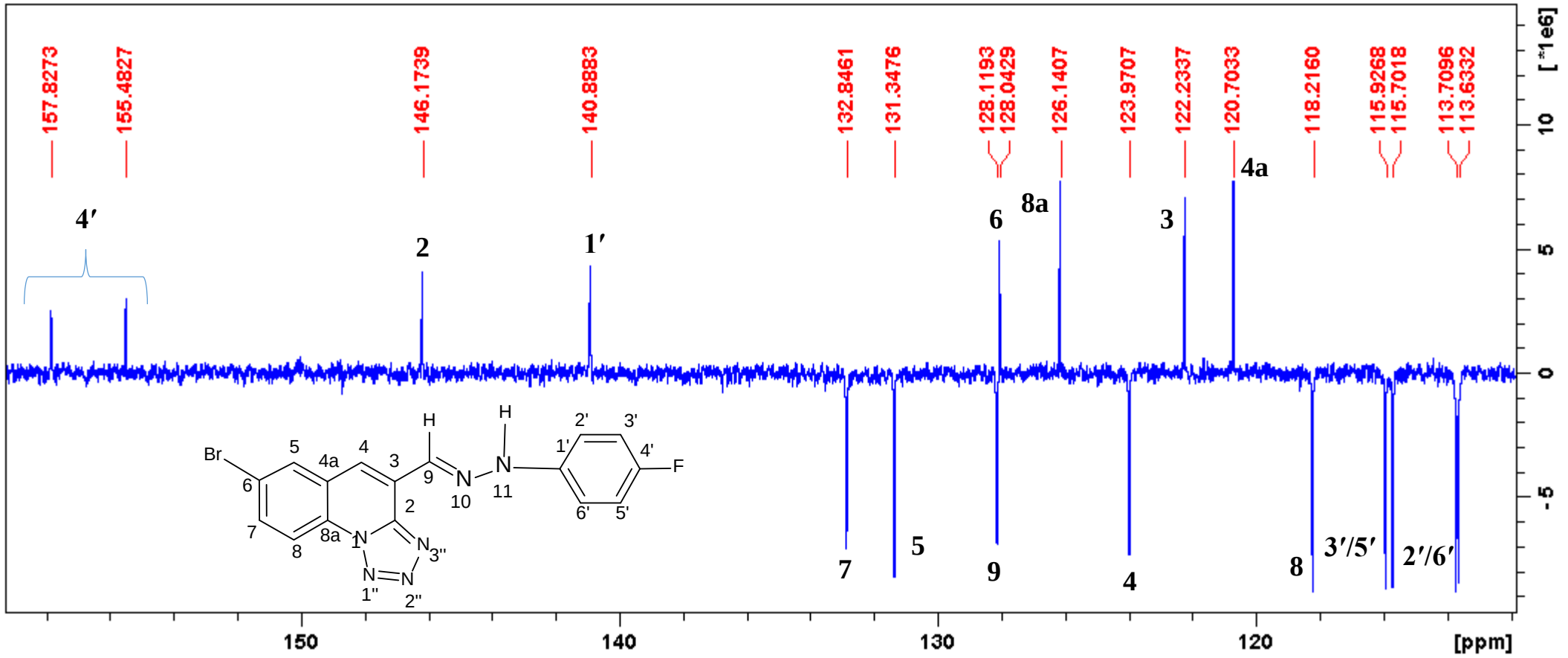
The ^{1}H NMR spectrum of compound 5m



The ^{1}H NMR spectrum of compound 5m-expanded



The ^{13}C NMR spectrum of compound 5m

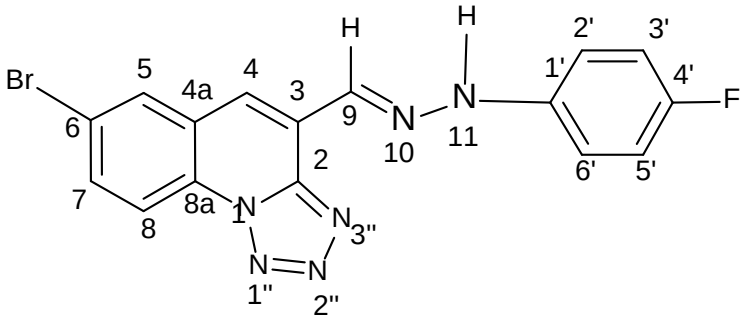


The ^{13}C NMR spectrum of compound 5m-expanded

Elemental Composition Report

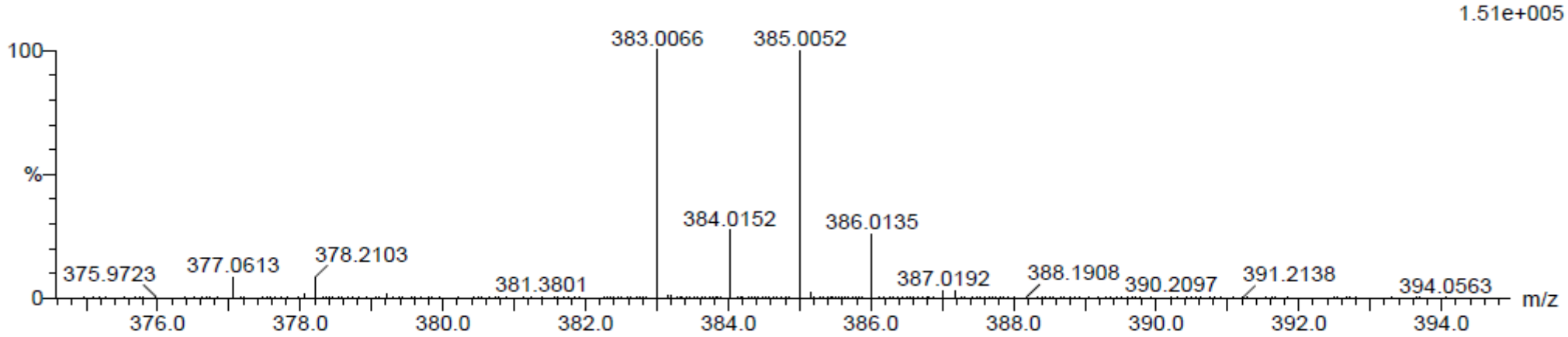
Single Mass Analysis

Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



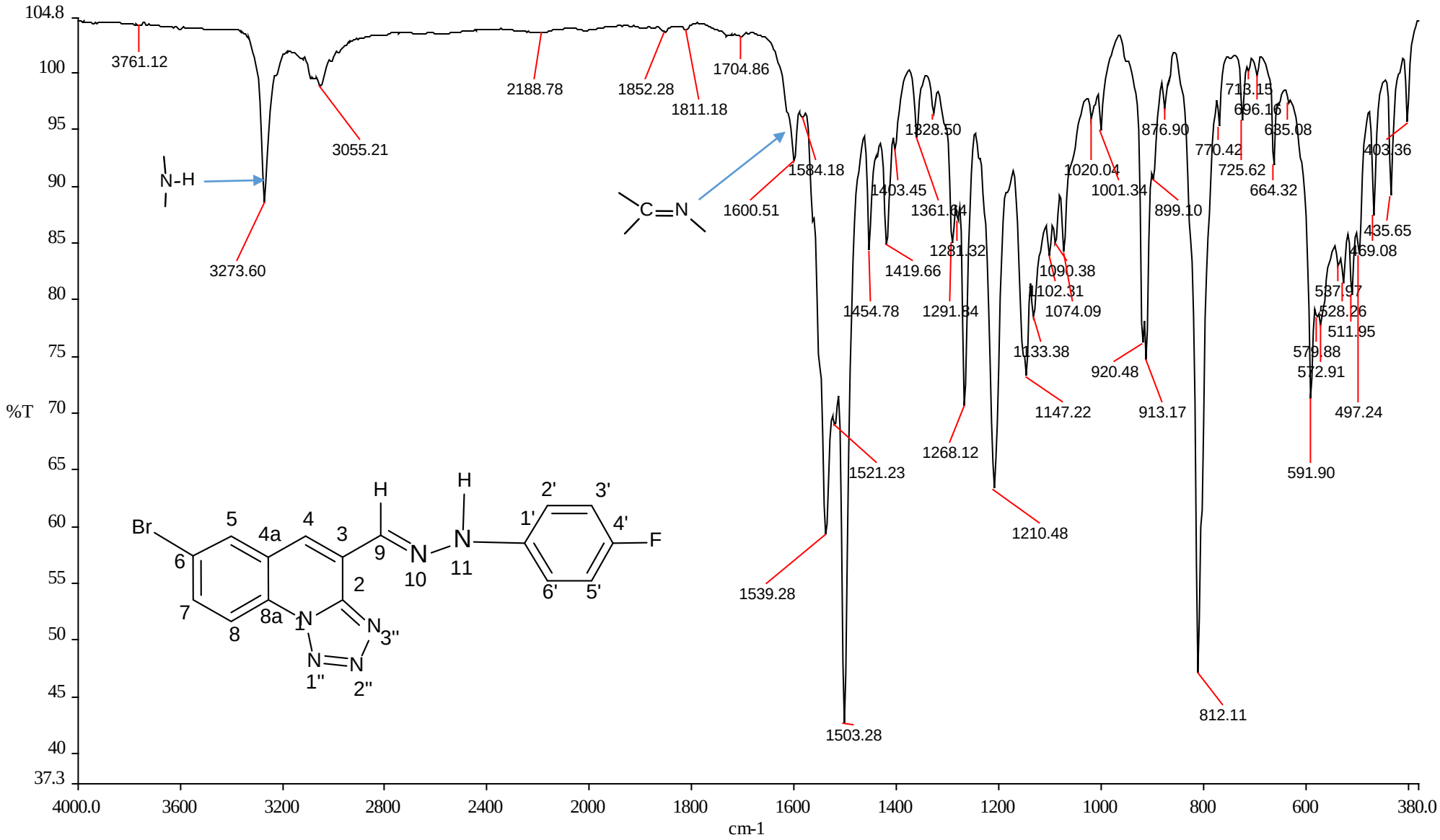
Monoisotopic Mass, Even Electron Ions
11 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 5-10 F: 0-1 Br: 0-1

Compound 2f 43 (1.416) Cm (1:61)
TOF MS ES-



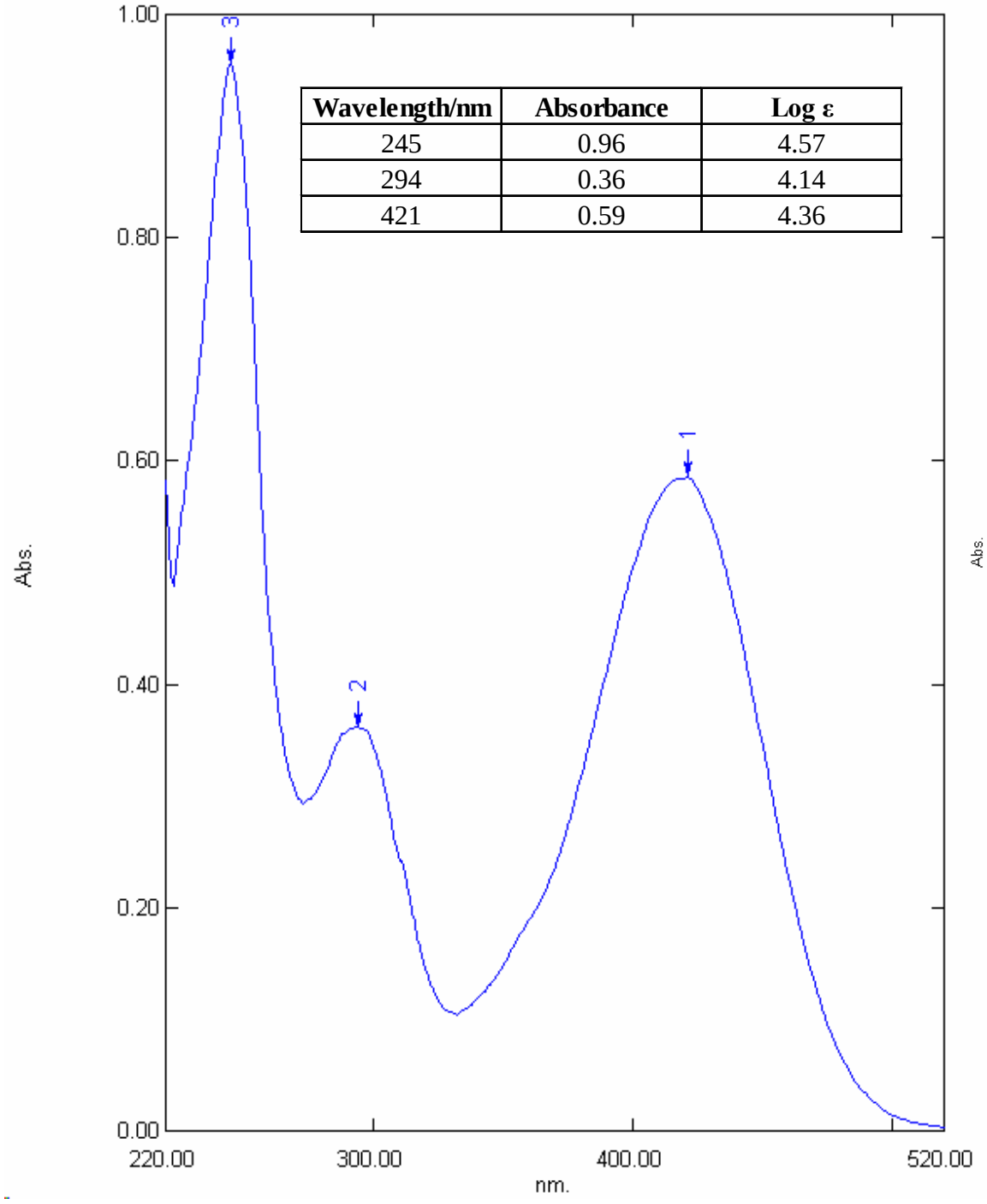
Minimum:				-1.5			
Maximum:		5.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
383.0066	383.0056	1.0	2.6	14.5	590.9	0.0	C16 H9 N6 F Br

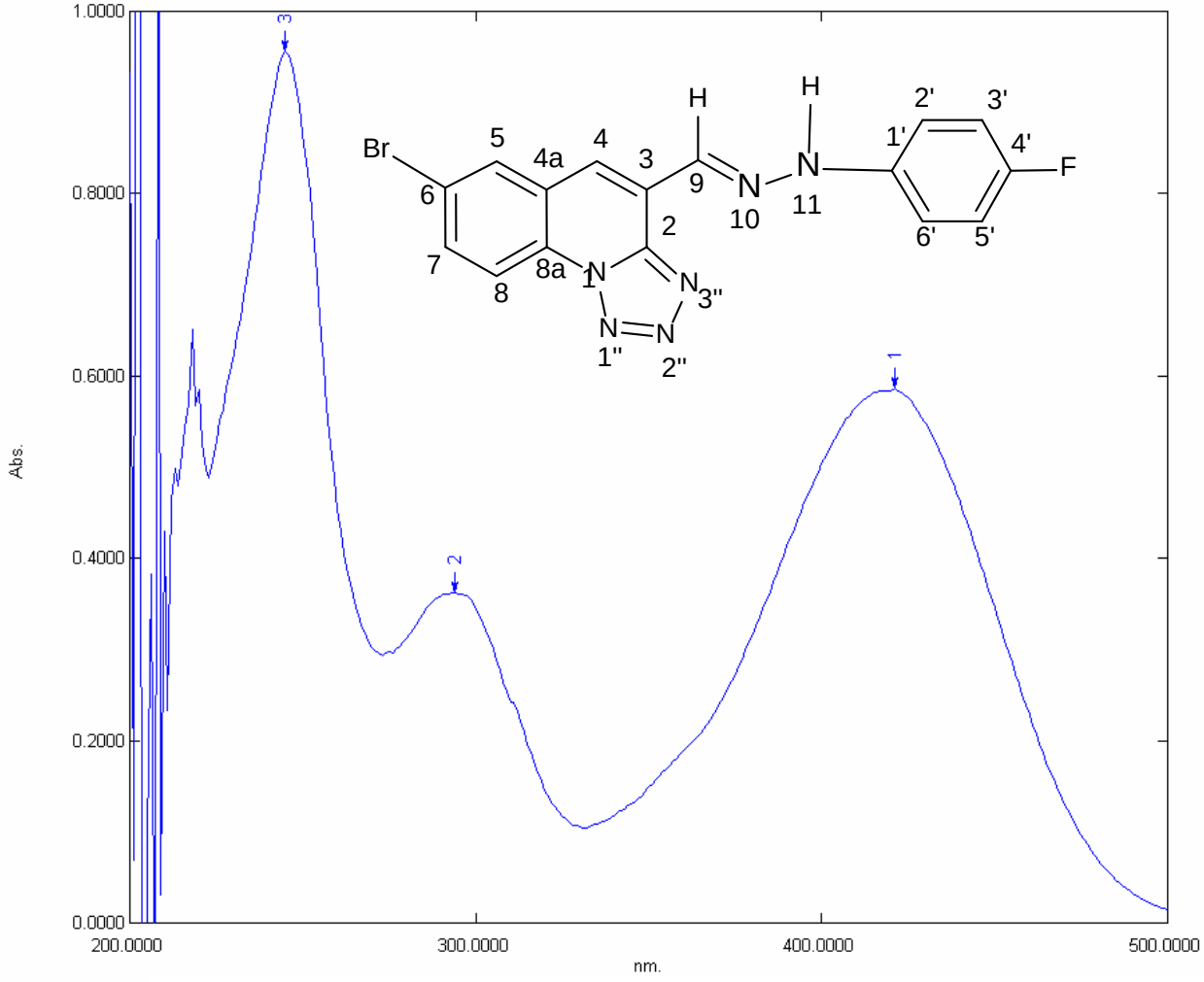
The HRMS spectrum of compound 5m



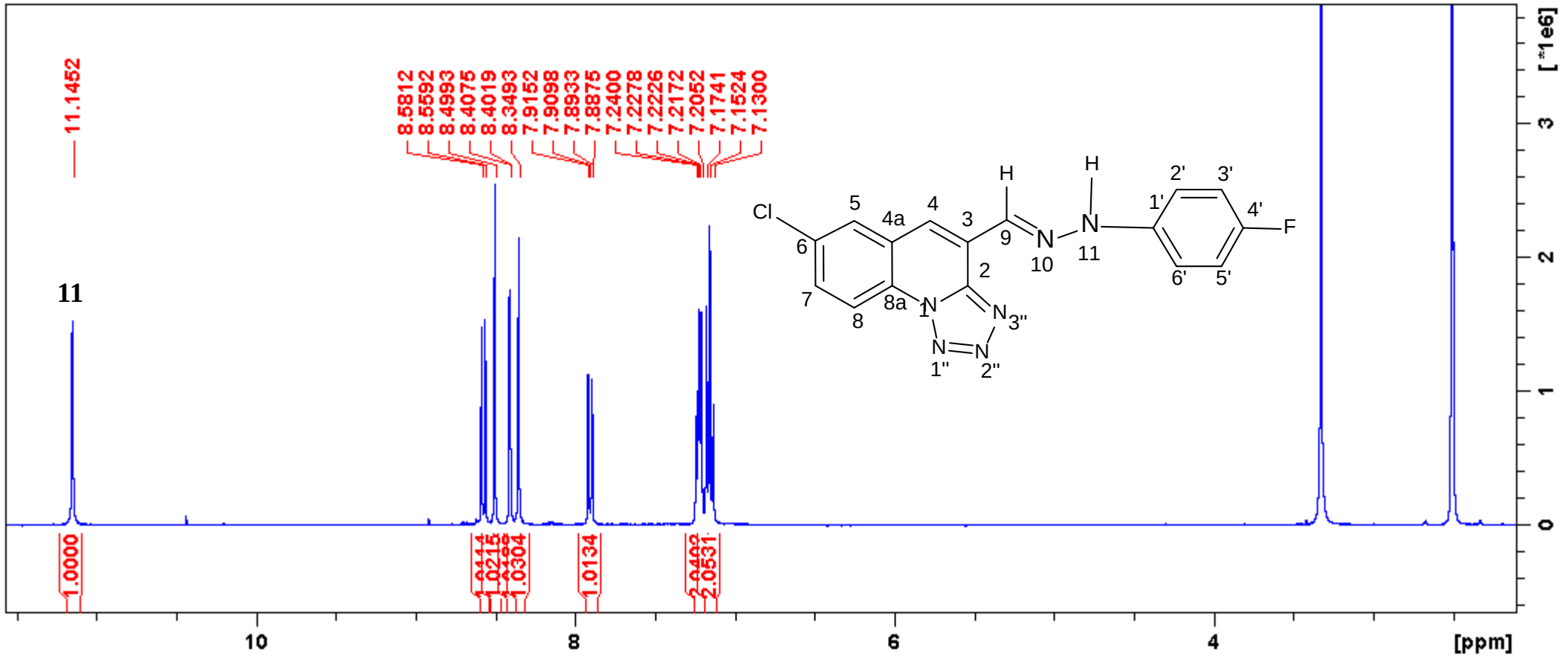
The IR spectrum of compound 5m

Wavelength/nm	Absorbance	Log ε
245	0.96	4.57
294	0.36	4.14
421	0.59	4.36

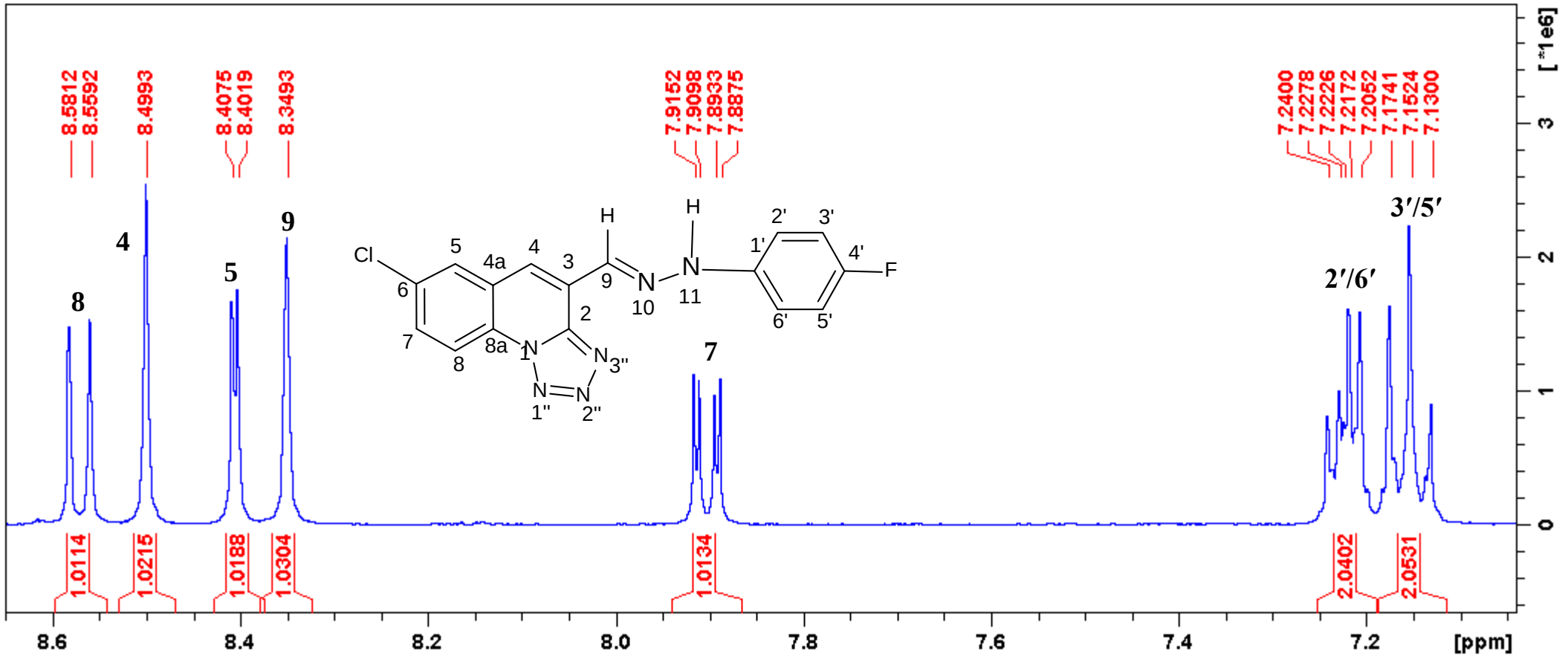




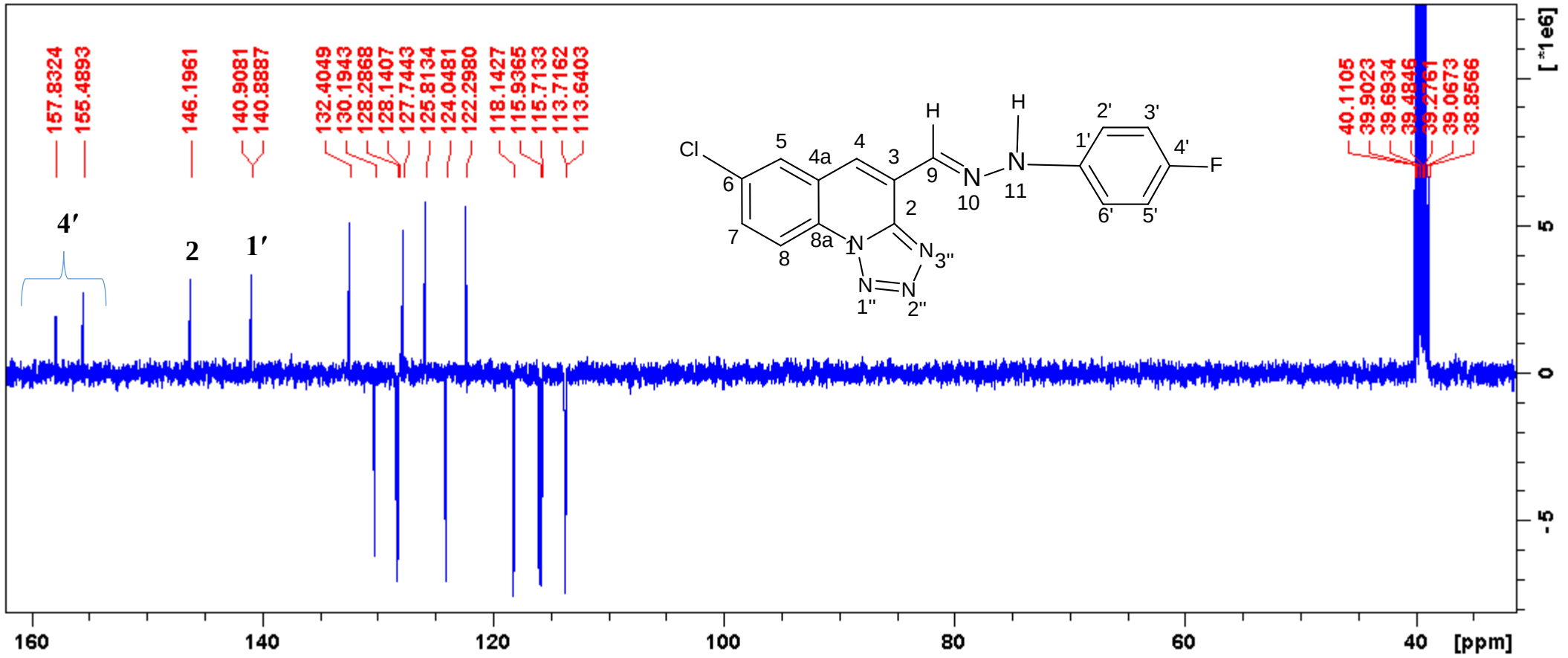
The UV-Vis spectrum of compound 5m



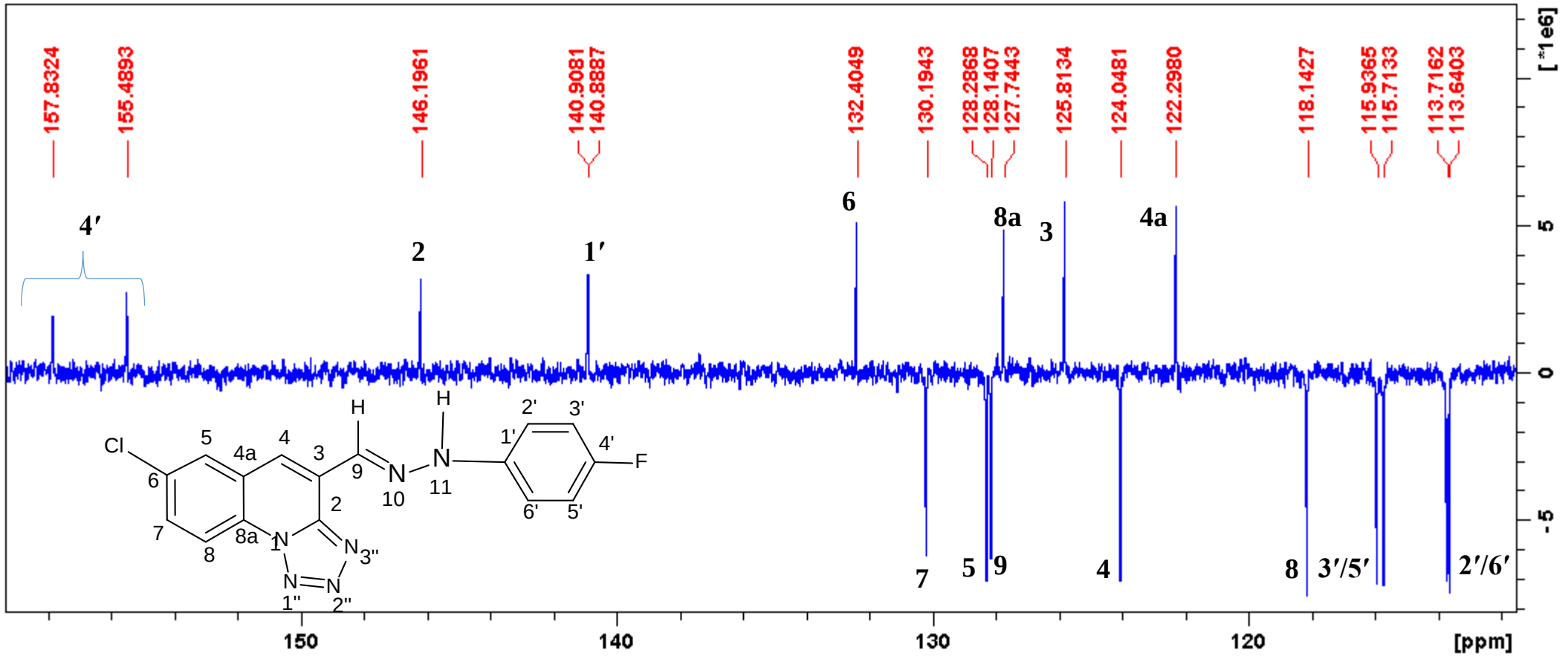
The ^{1}H NMR spectrum of compound 5n



The ^{1}H NMR spectrum of compound 5n-expanded



The ^{13}C NMR spectrum of compound 5n

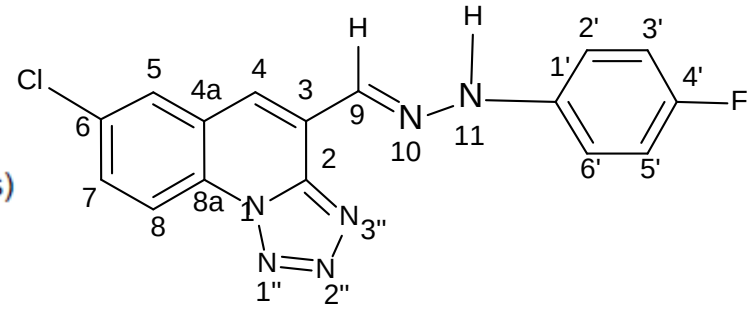


The ^{13}C NMR spectrum of compound 5n-expanded

Elemental Composition Report

Single Mass Analysis

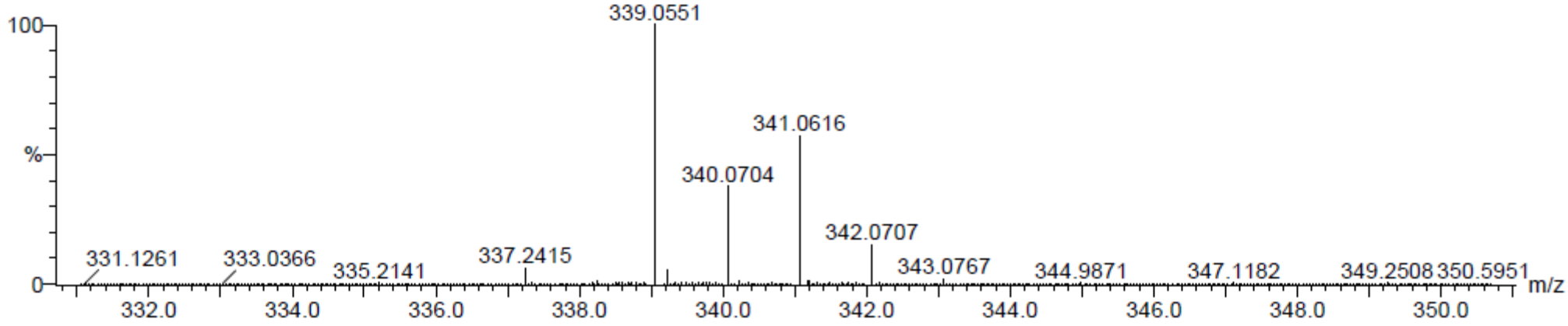
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



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

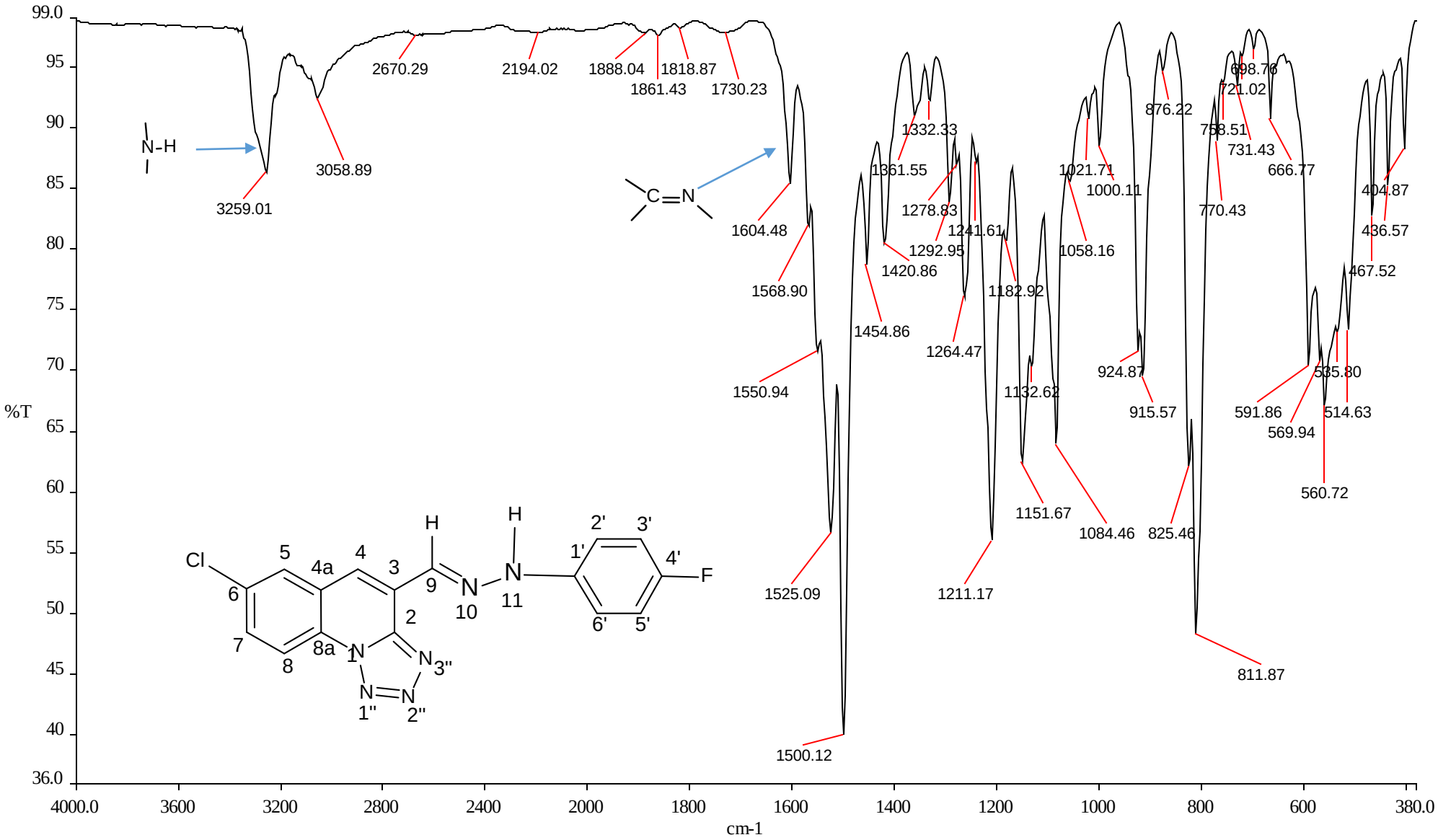
Compound 3f 35 (1.147) Cm (1:61)
TOF MS ES-

1.42e+005



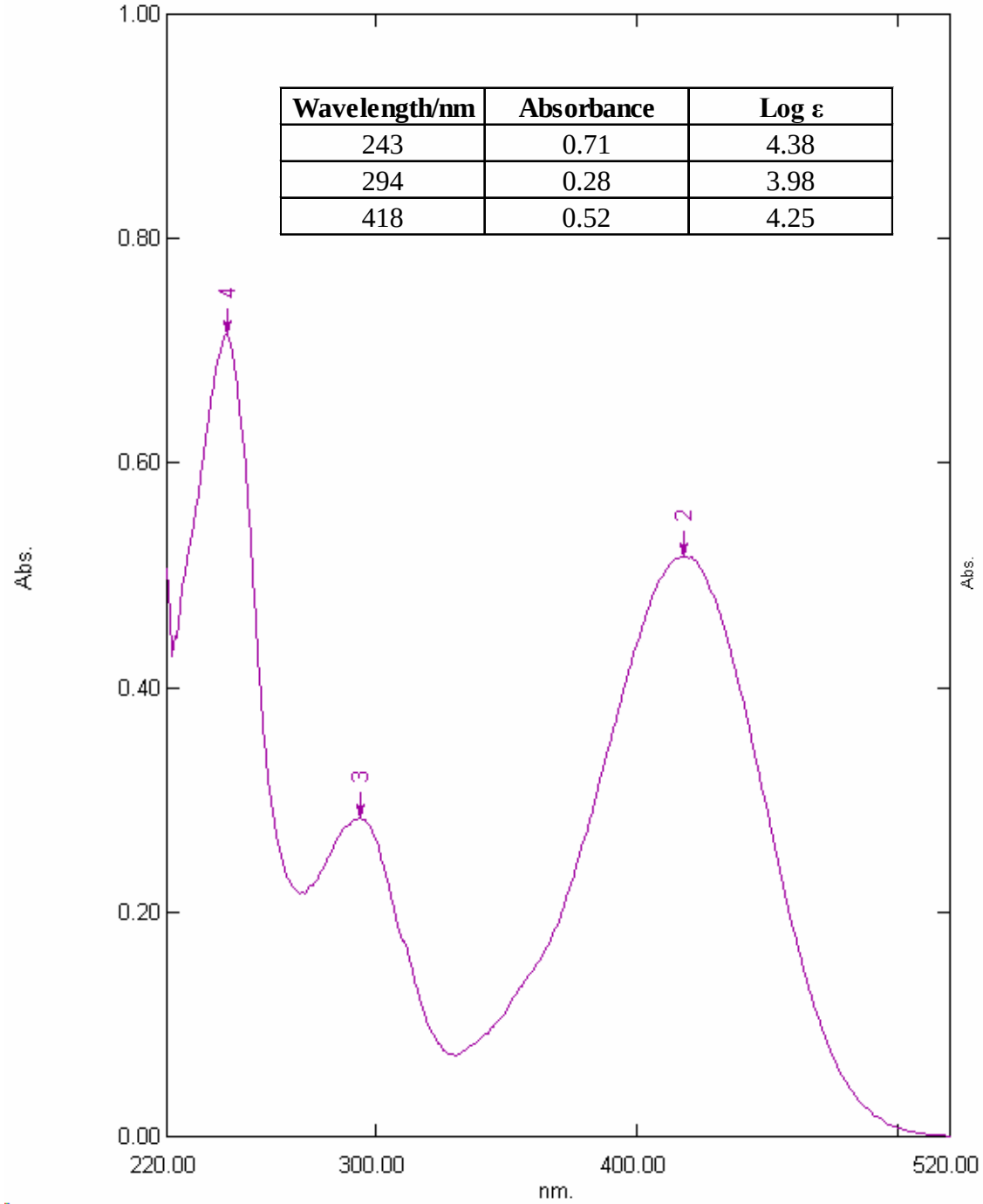
Minimum:				-1.5			
Maximum:		5.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
339.0551	339.0561	-1.0	-2.9	14.5	588.0	0.0	C16 H9 N6 F Cl

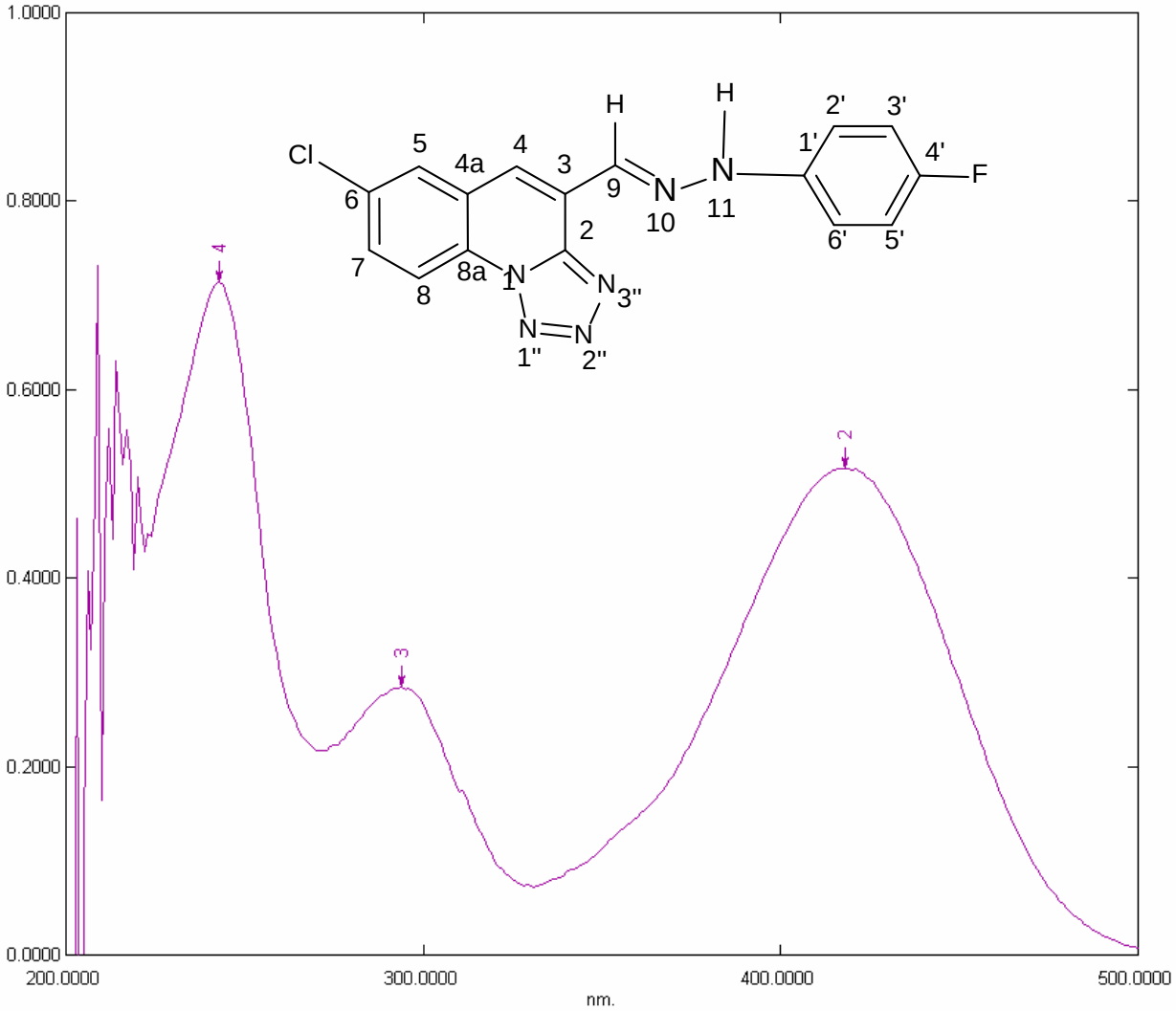
The HRMS spectrum of compound 5n



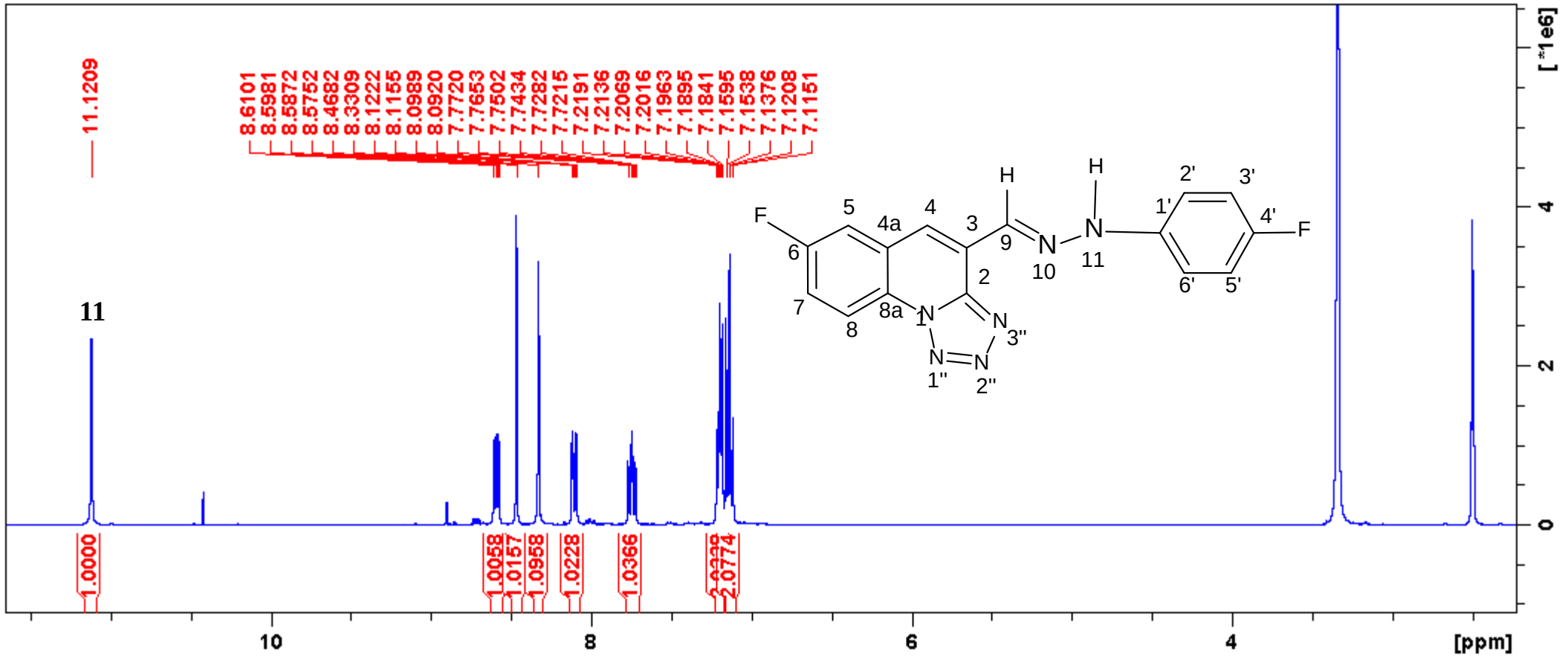
The IR spectrum of compound 5n

Wavelength/nm	Absorbance	Log ε
243	0.71	4.38
294	0.28	3.98
418	0.52	4.25

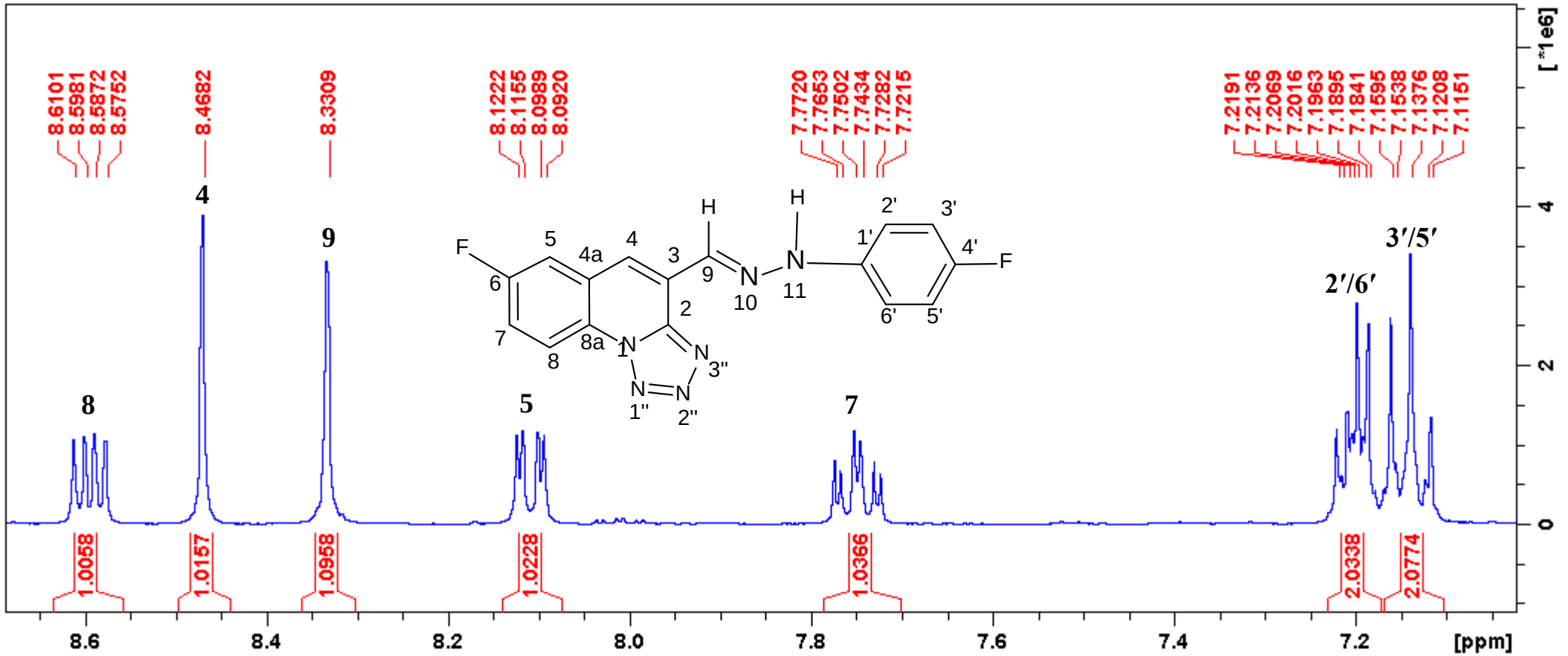




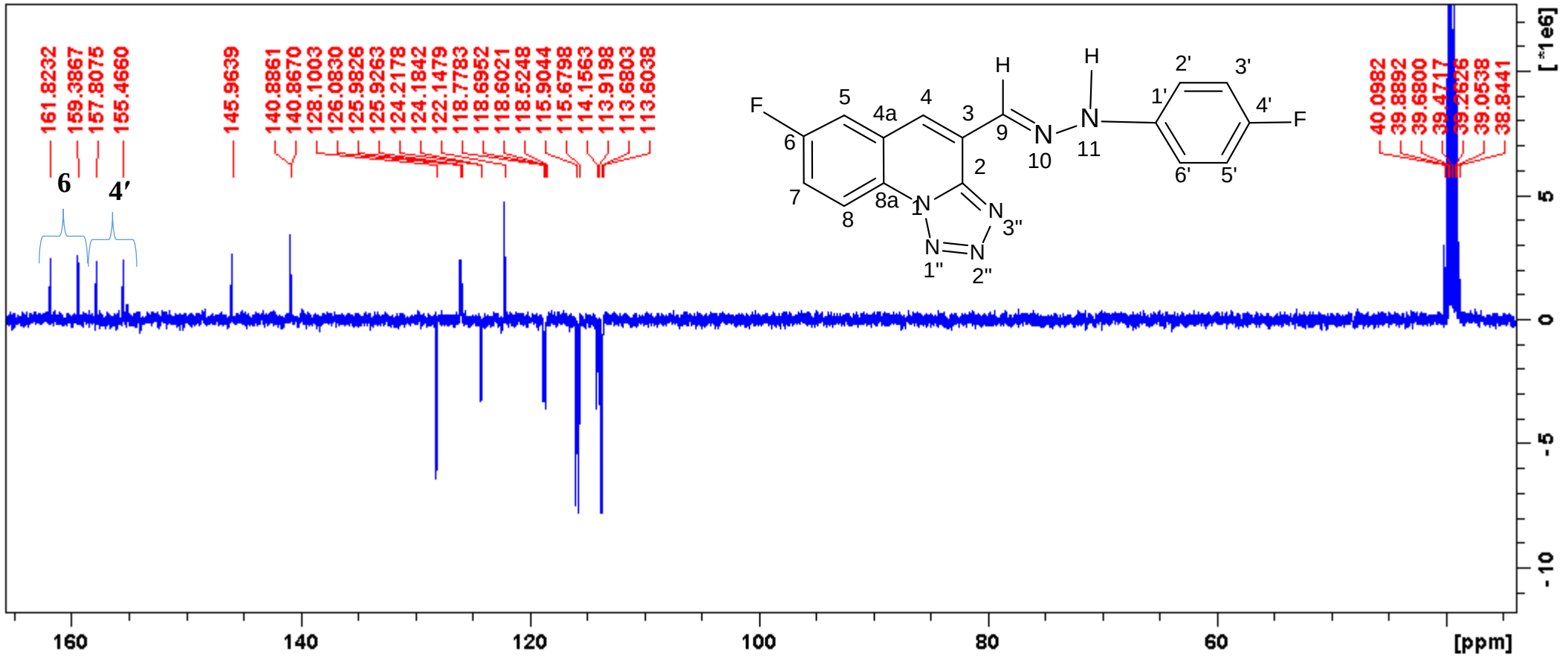
The UV-Vis spectrum of compound 5n



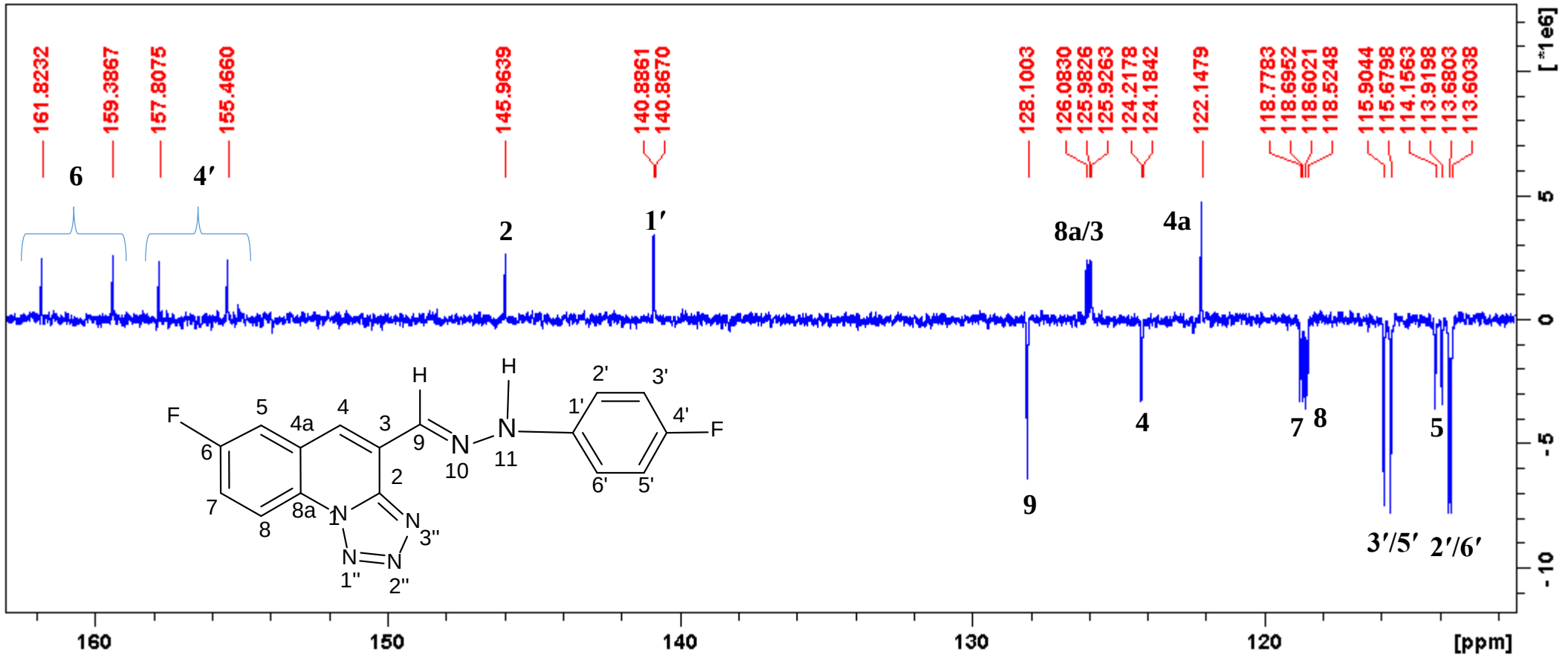
The ^{1}H NMR spectrum of compound 5o



The ^{1}H NMR spectrum of compound 5o-expanded



The ^{13}C NMR spectrum of compound 5o

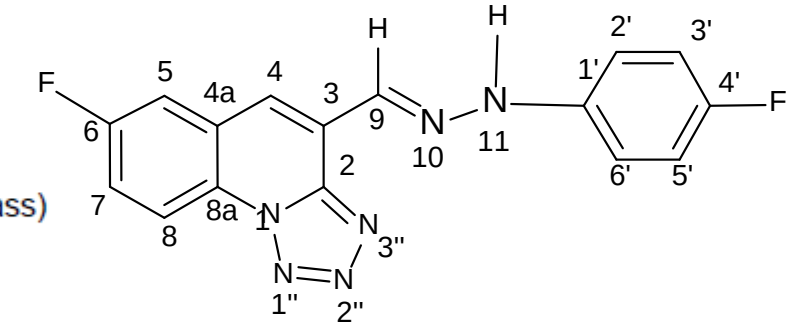


The ^{13}C NMR spectrum of compound 5o-expanded

Elemental Composition Report

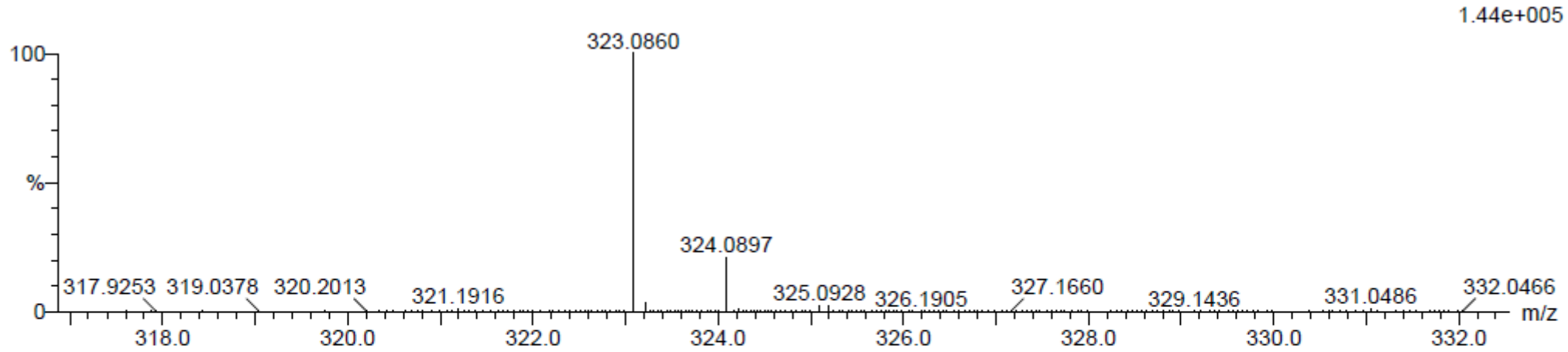
Single Mass Analysis

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



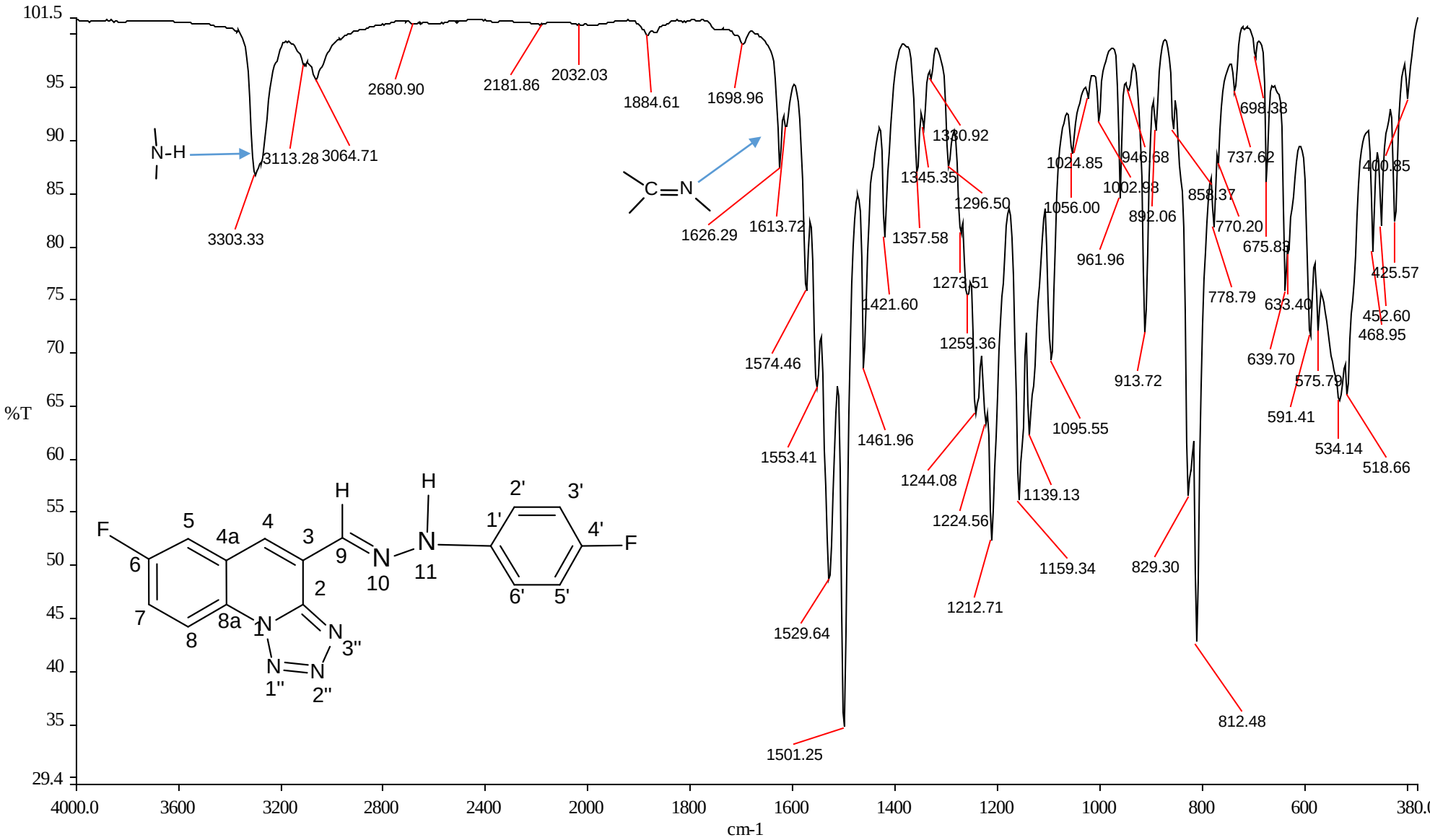
Monoisotopic Mass, Even Electron Ions
27 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 5-10 Zn: 0-1 F: 0-2

Compound 1g HG 36 (1.181) Cm (1:61)
TOF MS ES-



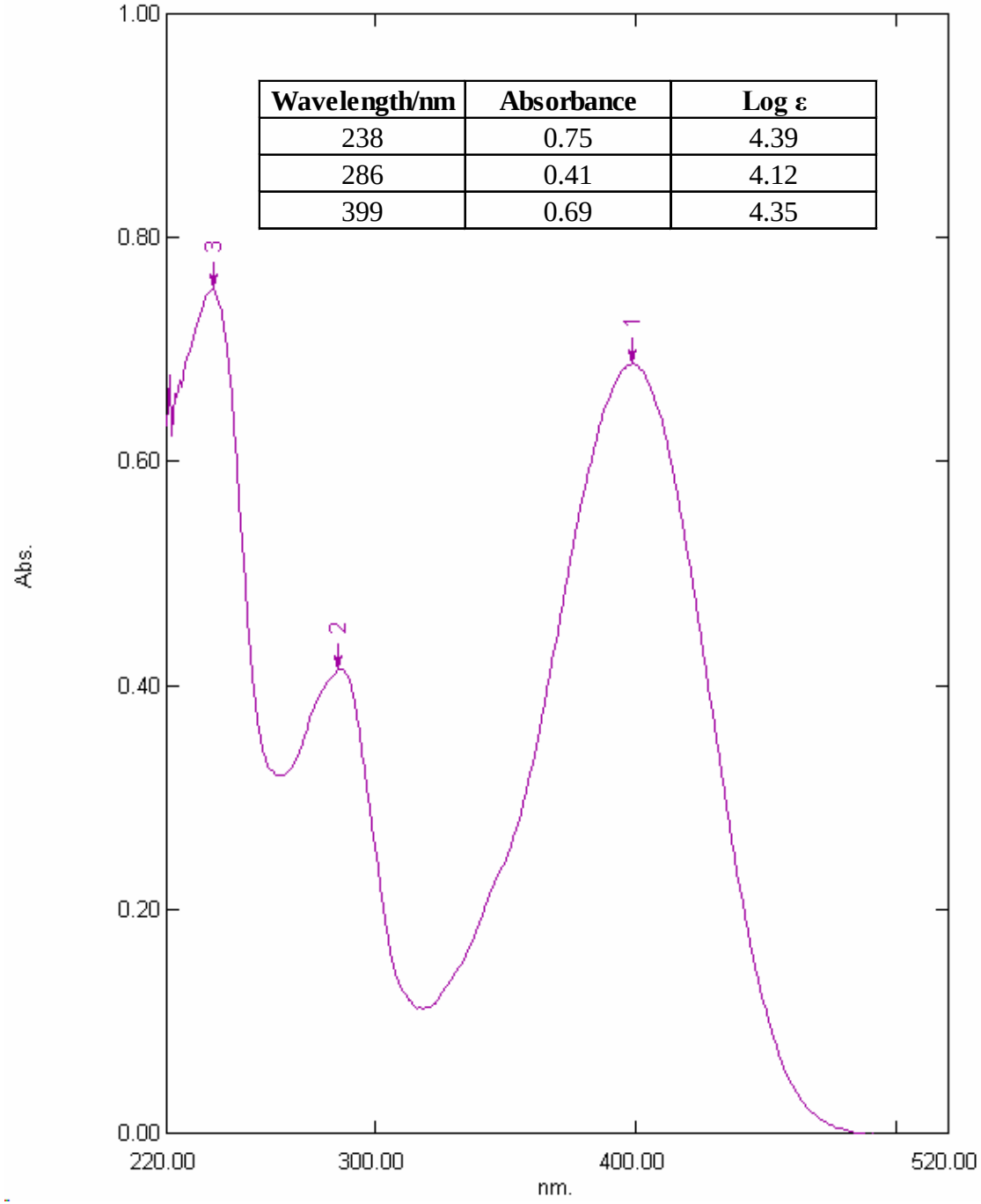
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
323.0860	323.0857	0.3	0.9	14.5	602.5	0.0	C16 H9 N6 F2

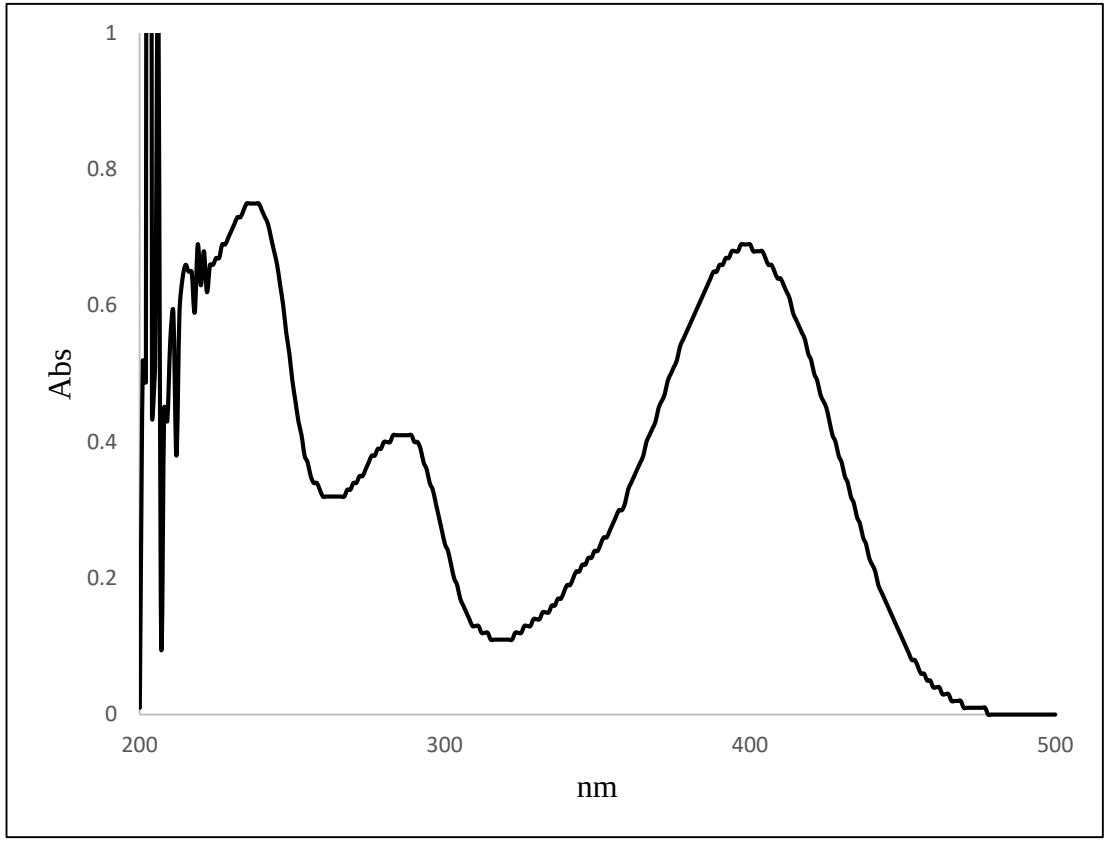
The HRMS spectrum of compound 5o

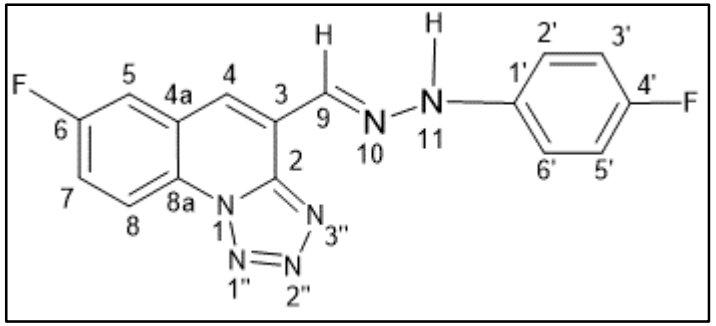


The IR spectrum of compound 5o

Wavelength/nm	Absorbance	Log ε
238	0.75	4.39
286	0.41	4.12
399	0.69	4.35







The UV-Vis spectrum of compound 5o