ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SYNTHESIS OF 5-AMINOISOXAZOLE DERIVATIVES VIA GREEN CHEMISTRY ROUTES

MASTER OF SCIENCE

ÖZGE KAVAS

BOLU, JANUARY 2016

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APPROVAL OF THE THESIS

SYNTHESIS OF 5-AMINOISOXAZOLE DERIVATIVES VIA GREEN CHEMISTRY ROUTES submitted by **ÖZGE KAVAS** in partial fulfillment of the requirements for the degree of Master of Science in **Department of Chemistry, Abant Izzet Baysal University** by,

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January 08, 2016

Prof. Dr. Duran KARAKAS ……

Director, **Graduate School of Natural and Applied Sciences**

To my parents

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Özge KAVAS

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ABSTRACT

SYNTHESIS OF 5-AMINOISOXAZOLE DERIVATIVES VIA GREEN CHEMISTRY ROUTES
MSC THESIS
ÖZGE KAVAS
ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF CHEMISTRY
(SUPERVISOR: ASSOC. PROF. DR. CEVHER ALTUĞ)

BOLU, JANUARY 2016

Isoxazoles are five membered heterocyclic compounds that show many various biological activities. The aim of this work is to synthesize biologically active 5-amino and 5-amido isoxazoles with green chemistry routes which are microwaves and solvent-free synthesis methods. These synthetic routes compared according to reaction yield, time efficiency and used solvent. In the first part of the study, 5-aminoisoxazoles were synthesized from the reaction of α-chlorooximes with 2-phenylsulfonyl acetonitrile in the presence of triethylamine by grinding the reactants in a mortar without using a solvent. Separately, 5-aminoisoxazoles were also obtained with the same starting materials in microwave system but in this case using methanol as a solvent. α-Chlorooximes that are used in the formation of 5-aminoisoxazoles were synthesized from aldoximes by chlorination with N-chlorosuccinimide according to the literature. In the second part of the study, synthesized 5-aminoisoxazole derivatives were reacted with *p*-nitrobenzoyl chloride in dichloromethane to form 5-amidoisoxazoles. Purification for 5-aminoisoxazoles was succeeded easily by recrystallization. However, 5-amidoisoxazole derivatives were purified by column chromatography. The structure of all products has been characterized by means of spectroscopic methods (IR, ^{1}H, ^{13}C NMR, HRMS and physical characteristics, mp and R_f values).

.

KEYWORDS: Green Chemistry, 5-Aminoisoxazoles, α-Chlorooximes, Microwave, Solvent-Free Synthesis

ÖZET

5-AMİNO İZOKSAZOL TÜREVLERİNİN YEŞİL KİMYA YÖNTEMLERİYLE SENTEZLENMESİ
YÜKSEK LİSANS TEZİ
ÖZGE KAVAS
ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
KİMYA ANABİLİM DALI
(TEZ DANIŞMANI: DOÇ. DR. CEVHER ALTUĞ)

BOLU, OCAK - 2016

İzoksazoller çok sayıda çeşitli biyolojik aktivite gösteren 5 üyeli hetero halkalı bileşiklerdir. Bu çalışmanın amacı yeşil kimya yollarından mikrodalga ve çözgensiz yöntemler kullanarak biyolojik açıdan aktif 5-amino ve 5-amido izoksazolleri sentezlemektir. Bu sentez yolları reaksiyon verimi, zaman tasarrufu ve çözgen kullanımına göre karşılaştırılmıştır. Çalışmanın birinci bölümünde, 5-aminoizoksazoller α-klorooksimlerin 2-fenilsülfonil asetonitril bileşiğiyle trietilamin varlığında çözgensiz ortamda havanda ezerek elde edilmiştir. Ayrı olarak, aynı başlangıç maddeleri kullanılarak 5-aminoizoksazoller bu sefer matanol çözgeni içerisinde mikrodalga sistemi kullanılarak sentezlenmiştir. 5-aminoizoksazollerin oluşumunda kullanılan α-klorooksimler, aldoksimlerin *N*-klor süksinimid ile klorlanmasıyla literatüre göre sentezlenmiştir. Çalışmanın ikinci kısmında, 5-amidoizoksazolleri elde etmek için, sentezlenen 5-aminoizoksazol türevleriyle p-nitrobenzil klorür diklorometan içerisinde karıştırıldı. 5-Aminoizoksazoller yeniden kristallendirme ile kolayca saflaştırıldı. Bununla birlikte 5-amidoizoksazol türevleri kolon kromatografisiyle saflaştırıldı. Tüm ürünlerin yapıları IR, ^{1}H NMR, ^{13}C NMR, HRMS ve fiziksel sabitler (erime noktası, Rf değerleri) ile tanımlanmıştır.

ANAHTAR KELİMELER: Yeşil Kimya, 5-Aminoizoksazoller, α-Klorooksimler, Mikrodalga, Çözgensiz Sentez

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LIST OF ABBREVIATIONS AND SYMBOLS

AcOH	Acetic acid
Al_2O_3	Aluminium oxide
Bu_4NBr	Tetrabutylammonium bromide
C3a	Human hepatocellular carcinoma cell
cat	Catalyst
CCl_4	Carbon tetrachloride
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
CN	Nitrile
conc	Concentrated
Cu	Copper
$CuSO_4.5H_2O$	Copper(II) sulfate pentahydrate
d	Doublet (NMR)
DABCO	1,2-Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicycloundec-7-ene
δ	Chemical shift
DCM	Dichloromethane
dd	Doublet of doublet (NMR)
ddd	Doublet of doublet of doublets (NMR)
decomp.	Decomposed
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
EtOAc	Ethyl acetate
EtOH	Ethyl alcohol
Et_3N	Triethylamine
EtONa	Sodium ethoxide
eq.	Equivalent
ESI^+	Electrospray ionisation (positive)
ESI^-	Electrospray ionisation (negative)
$FeCl_3$	Iron(III) chloride
$FeCl_2.{}_4H_2O$	Iron(II) chloride tetrahydrate

FT-IR	Fourier Transform Infrared Spectroscopy
GHz	Gigahertz
h	Hour
HBF_4.OEt	Tetrafluoroboric acid diethyl ether
HCI	Hydrochloric acid
Hex	Hexane
H_2NSO_3H	Sulfamic acid
H_2O	Water
H_2SO_4	Sulfuric acid
Hz	Hertz
IUPAC	International Union of Pure and Applied Chemistry
J	Coupling constant (NMR)
KBr	Potassium bromide
K_2CO_3	Potassium carbonate
kHz	Kilohertz
KI	Potassium iodide
L929	Mouse fibroblast cell
LiBr	Lithium bromide
LiOH	Lithium hydroxide
m	Multiplet (NMR)
M^+	Molecular ion
MCF-7	Human breast adenocarcinoma cell
MeCN	Acetonitrile
MeOH	Methyl alcohol
mg	Milligram
M-H^+	Protonated molecular ion
MH_Z	Megahertz
min	Minutes
mL	Milliliter
mmol	Millimolar
m.p	Melting point
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Microwave
Na_2CO_3	Sodium carbonate

NaH	Sodium hydride
$NaHCO_3$	Sodium bicarbonate
NaN_3	Sodium azide
NaOAc	Sodium acetate
NaOBu	Sodium butoxide
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
***n*-BuLi**	*n*-Butyl lithium
NCS	*N*-Chlorosuccinamide
NH_3	Ammonia
NH_2OH	Hydroxylamine
$NH_2OH.HCl$	Hydroxylamine hydrochloride
$NH_2OH.H_2SO_4$	Hydroxylammonium sulfate
$NiCl_{2.6}H_2O$	Nickel(II) chloride hexahydrate
NMP	*N*-Methyl-2-Pyrrollidone
NMR	Nuclear magnetic resonance
$RdCl_2$	Palladium(II) chloride
$Pd(OAc)_2$	Palladium(II) acetate
PEG	Polyethylene glycol
$PhI(OAc)_2$	Iodobenzene diacetate
P_2O_5	Phosphorus pentoxide
ppm	Parts per million (NMR)
R	Any group
R_f	Retardation factor
rt	Room temperature
s	Singlet
t	Triplet
T98g	Human glioblastoma cells
***t*-BuLi**	Tert-Butyllithium
***t*-BuOH**	Tertiary butanol
TEBA	Benzyltriethylammonium chloride
TLC	Thin layer chromatography
THF	Tetrahydrofuran
TOF	Time of Flight Instrument

$TsNClNa.3H_2O$	Chloroamine-T trihydrate
TsOH	Tosylic acid
US	Ultrasound
X	Any halogen atom
ν_{max}	Maximum frequency
Δ	Heat

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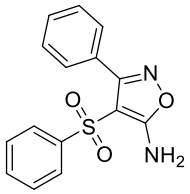
FORMULAE



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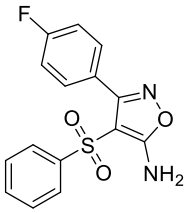

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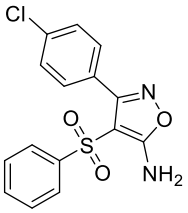

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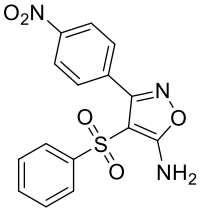

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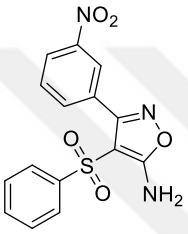

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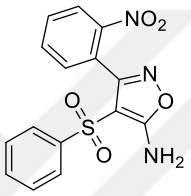

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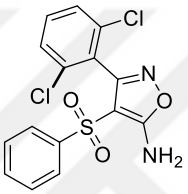

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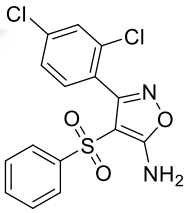

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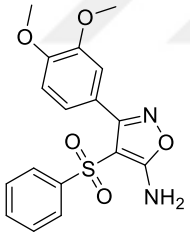

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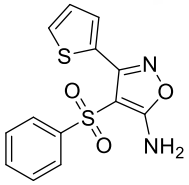

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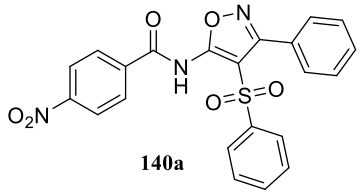

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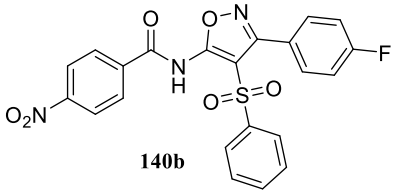

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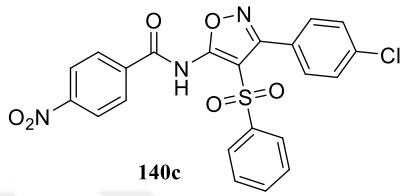

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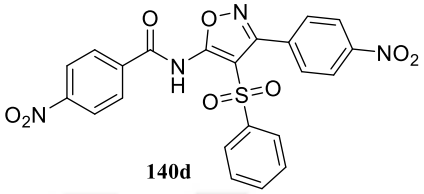

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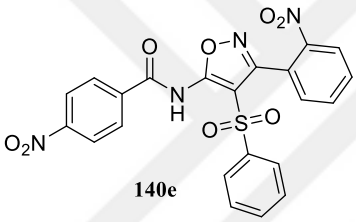

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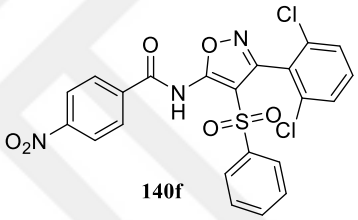

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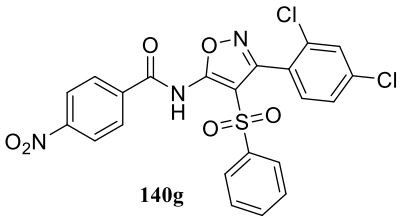

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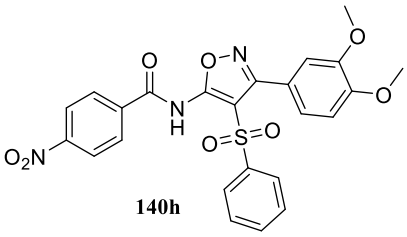

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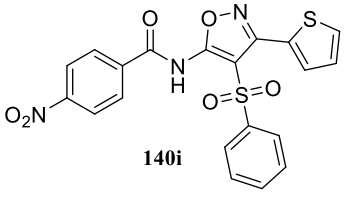

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1. INTRODUCTION

1.1 Green Chemistry

Planet and humans are caused damage unintentionally to develop produce protection, commercial products, and medicines. The idea of green chemistry is a relatively novel idea to prevent natural evolution of pollution. In the ultimate will be an arrangement of a number of environmental, health, safety and economic targets with green chemistry in chemical synthesis (Figure 1.1) (Clark, 1999).

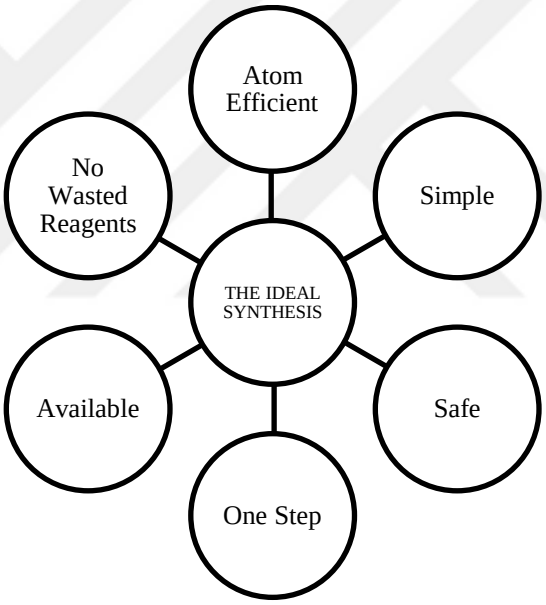


Figure 1.1. The ideal synthesis

12 principles of Green Chemistry allow to find original and advanced methods to reduce waste, conserve energy and discover alternatives for hazardous substances. By this way economy, people and the planet can be protected (Anastas and Warner, 1998).

These principles are;

- Prevention
- Atom economy
- Less hazardous chemical synthesis
- Designing safer chemicals
- Safer solvents and auxiliaries
- Design for energy efficiency
- Use of renewable feed stocks
- Reduce derivatives
- Catalysis
- Design for degradation
- Real-time analysis for pollution prevention
- Inherently safer chemistry for accident prevention.

There are many methods, which are often used in synthetic organic chemistry laboratories for their advantageous of time, solvent, yield and energy. Among many others, some of the synthetic routes for "Green Synthesis" are given below.

1.1.1 Microwave

Awareness in microwave assisted synthesis has been gradually growing after the publication about on microwave assisted synthesis in household microwave ovens (Gedye et al., 1986).

A novel trend is microwave-assisted organic synthesis due to several advantages compared to the conventional heating reactions which requirement of very high temperatures. MW assisted reactions are eco-friendly, occur only in few min, have high yield and reduce waste. These reactions are growing in synthetic routes and new publications are full with MW reactions and applications due to greener method (Roberts and Strauss, 2005).

The microwave irradiation frequency range is 0.3 to 300 GHz. and microwave instrument reactors for chemical synthesis operate at a frequency of 2.45 GHz. Microwave-enhanced chemistry is related with the efficient heating of materials by "microwave dielectric heating" effects and based on the ability of absorption of microwave energy then conversion to heat by a solvent or reagent. This aptitude is called loss factor (tanδ). When tanδ of a reaction medium increases, efficient absorption increases and heating takes place rapidly. While solvents such as water, methanol, DMF, ethyl acetate, acetone, chloroform, acetic acid and dichloromethane are all heated when irradiated with microwaves, solvents like hexane, toluene, diethyl ether, CCl_4, do not heat with microwave irradiation. However these microwave inactive solvents can be mix with microwave active solvents. Loss factors of several common organic solvents can be seen in (Table 1.1) (Kappe, 2004).

Table 1.1. Loss factors of some solvents

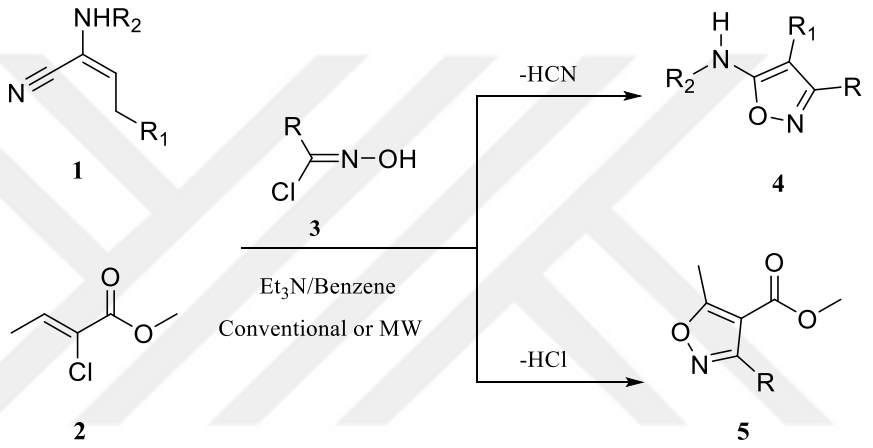
Solvent	Tanδ	Solvent	Tanδ
Ethylene glycol	1.350	DMF	0.161
Ethanol	0.941	1,2-Dichloroethane	0.127
DMSO	0.825	Water	0.123
2-Propanol	0.799	Chlorobenzene	0.101
Formic acid	0.722	Chloroform	0.091
Methanol	0.659	Acetonitrile	0.062
Nitrobenzene	0.589	Ethyl acetate	0.059
1-Butanol	0.571	Acetone	0.054
2-Butanol	0.447	Tetrahydrofuran	0.047
1,2-Dichlorobenzene	0.280	Dichloromethane	0.042
NMP	0.275	Toluene	0.040
Acetic acid	0.174	Hexane	0.020

Oil baths and heating jackets are traditional heating equipment that are used for heating in overall most organic reactions. Though they are somewhat slow and a temperature rise can develop within the sample. Furthermore, product, substrate and reagent decomposition can be caused as a result of local overheating.

In contrast, if the microwave heating apparatus is accurately designed, the temperature increase will be unchanging throughout the sample and formation of by-products and/or decomposition products will be low. Since in MW dielectric heating the MW radiation spreads through the walls of the container and only the reactants

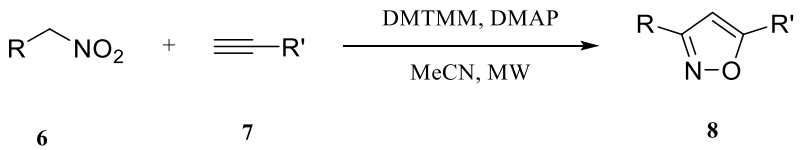
and solvent are heated. In pressurized systems, it is possible to rapidly increase the temperature far above the conventional boiling point of the solvent used. (Lidström et al., 2001).

For example, the [3+2] cycloaddition reactions between olefins **(1 or 2)** and nitrile oxides **(3)** can take several days depending on the nature of the olefin used under stirring at room temperature or refluxing conditions. Lasri et al. (2008) have compared the same synthetic method to obtain isoxazoles *via* thermal and microwave conditions and observed microwave synthesis results higher yield with less time without effecting regioselectivity compared to conventional heating (Scheme 1.1).



Scheme 1.1. Synthesis of substituted isoxazoles

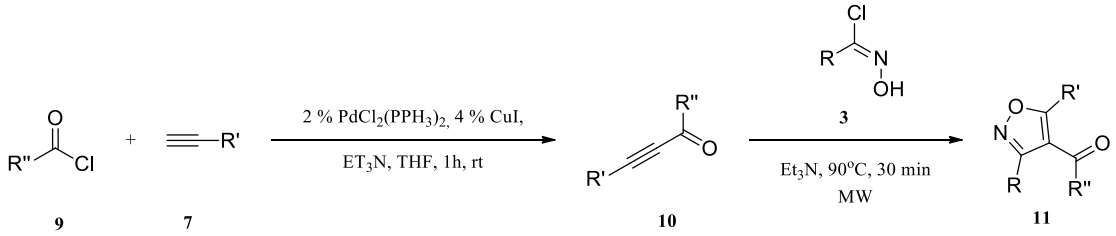
Giacomelli et al. (2003) have obtained isoxazoles **(8)** from the reaction of nitroalkanes **(6)** and alkynes **(7)** using 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) and 4-dimethylaminopyridine (DMAP) under MW irradiation in good yields compared to conventional heating (Scheme 1.2).



Scheme 1.2. Synthesis of isoxazoles from nitroalkanes

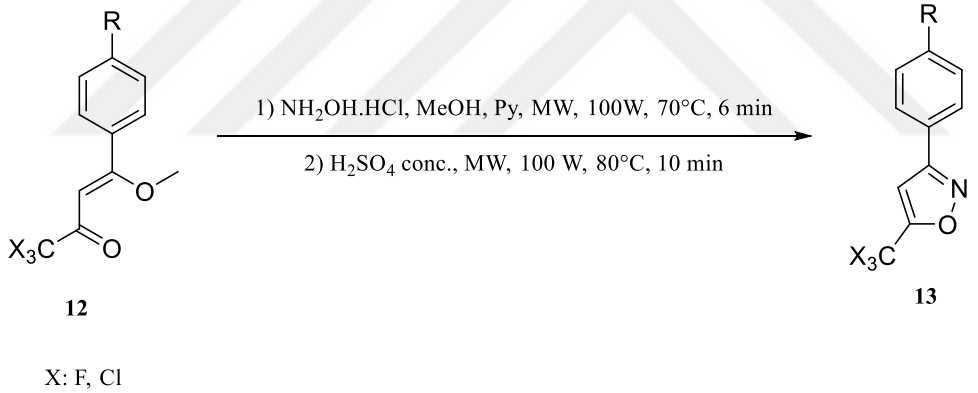
Willya et al. (2008) have developed one pot, three-component synthesis of 3,4,5-substituted isoxazole derivatives **(11)** from the coupling of acid chlorides **(9)**

with terminal alkynes **(7)**, followed by 1,3-dipolar cycloaddition of *in situ* generated nitrile oxides from hydroximinoyl chlorides **(3)** using MW irradiation (Scheme 1.3).



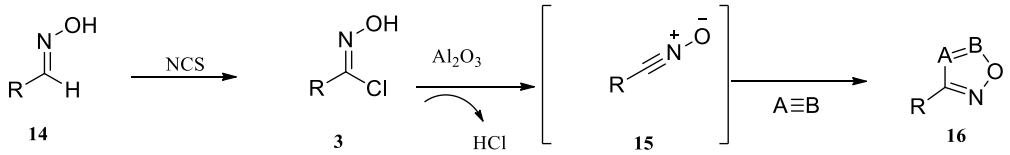
Scheme 1.3. One pot synthesis of 3,4,5-substituted isoxazoles

The 3-arylisoxazoles **(13)** were observed in two steps, using a one-pot process. Firstly, enones **(12)** were reacted with hydroxylamine hydrochloride in the presence of pyridine using methanol and then conc. sulphuric acid was added to cooled mixture. Two steps were occurred in MW system in shorter time (Scheme 1.4) (Martins et al., 2008).



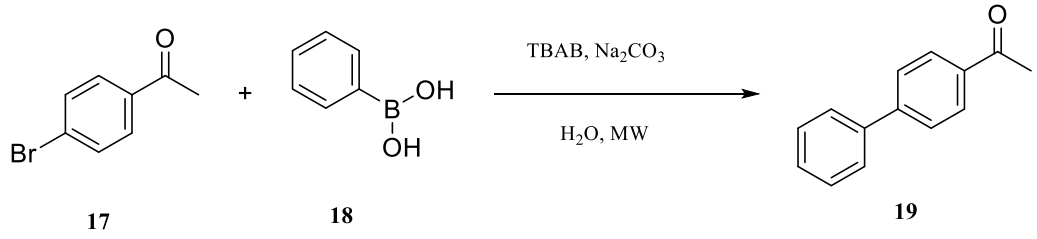
Scheme 1.4. One pot synthesis of 3-arylisoxazole derivatives

Touaux (1998) were mixed aldoximes **(14)** with NCS and alkynes in a one-pot reaction over alumina using MW irradiation to form isoxazoles **(16)** (Scheme 1.5).



Scheme 1.5. MW assisted one-pot synthesis of isoxazoles

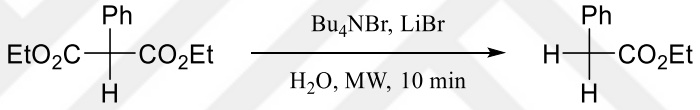
Leadbeater (2005) showed the formation of biaryl product **(19)** from the coupling of 4-bromoacetophenone **(17)** with phenylboronic acid **(18)** in the presence tetrabutylammonium bromide (TBAB) to increase the reaction mixture but without the addition of any transition-metal catalyst in aqueous medium using MW irradiation (Scheme 1.6).



17 **18** **19**

Scheme 1.6. The Suzuki coupling by using water and MW heating

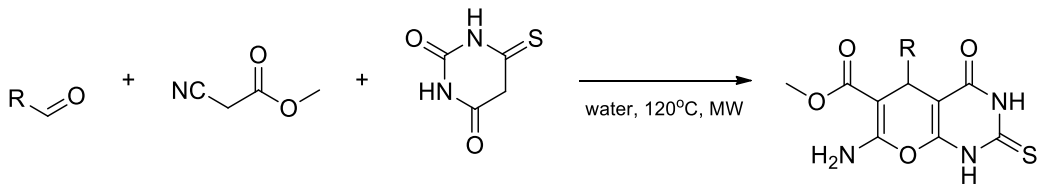
Phase transfer catalysed dealkoxycarboxylations were studied using MW system by Loupy et al. (1993) (Scheme 1.7).



20 **21**

Scheme 1.7. Phase transfer catalysed dealkoxycarboxylations

Bhat et al. (2014) described the catalyst free, efficient, simple and fast green pathway synthesis of highly functionalized pyrano [2,3-d] pyrimidine derivatives **(25)** via one-pot three-component domino Knoevenagel-Michael addition reaction under microwave irradiation (Scheme 1.8).

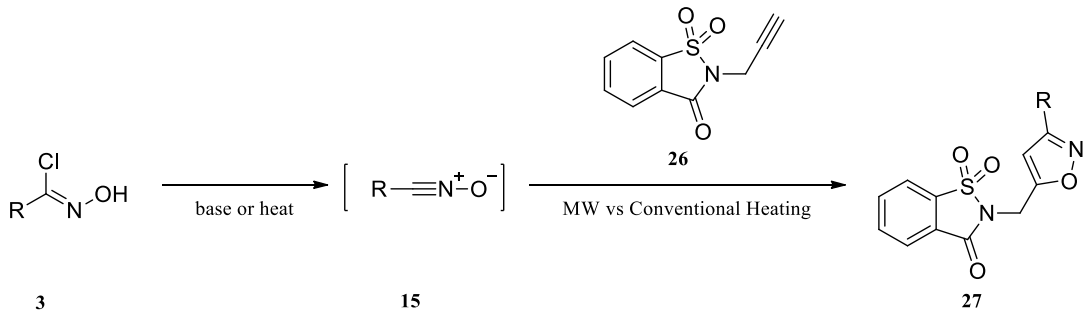


22 **23** **24** **25**

Scheme 1.8. MW assisted Knoevenagel-Michael addition

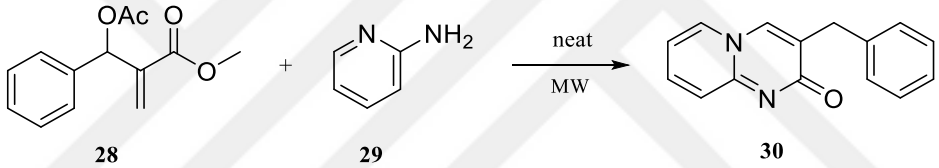
Novel isoxazole *N*-substituted saccharin derivatives **(27)** were synthesized via a one-pot 1,3-dipolar cycloaddition reaction, by using MW activation under solvent-free conditions. Reaction yields were increased comparing to conventional heating

and regio- stereo- selectivity do not effected by using MW (Scheme 1.9) (Mabrour et al., 2007).



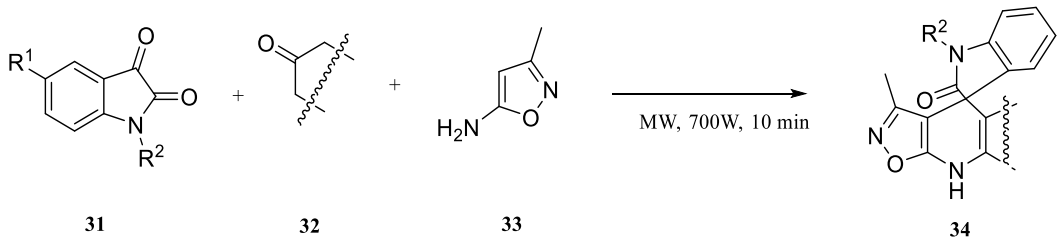
Scheme 1.9. Formation of isoxazoles under solvent-free conditions

2-Heteroaryl amines **(29)** and alkyl-2-(acetoxy(phenyl)methyl)acrylate **(28)** were reacted under microwave irradiation without any catalyst and solvent-free conditions to obtain 3-substituted-2H-pyrido[1,2-a]pyrimidin-2-ones with a simple and efficient method (Scheme 1.10) (Satyanarayana et al., 2013).



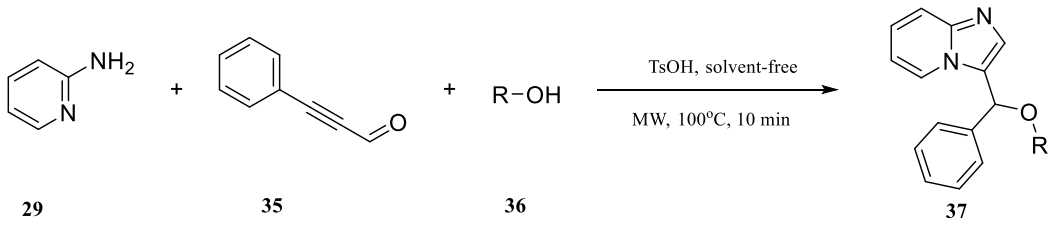
Scheme 1.10. Reaction of Baylis–Hillman acetate with 2-aminopyridine

Formation of oxazolo[5,4-b]quinoline-fused spirooxindole derivatives **(34)** via one-pot three-component reaction of 5-amino-3-methylisoxazole **(33)**, β-diketones **(31)** and isatin **(32)** was observed under neat condition in MW system absence of any catalyst and solvent efficiently (Scheme 1.11) (Yuvaraj et al., 2015).



Scheme 1.11. Synthesis of spirooxindole derivatives

Recently, Zhang and Jiang (2015) studied the reaction of 2-aminopyridine **(29)**, 3-phenylpropiolaldehyde **(35)**, and an alcohol **(36)** to form imidazo[1,2-a]pyridines **(37)** TsOH-catalyzed under MW irradiation and solvent-free conditions in good yields with shorter time (Scheme 1.12).



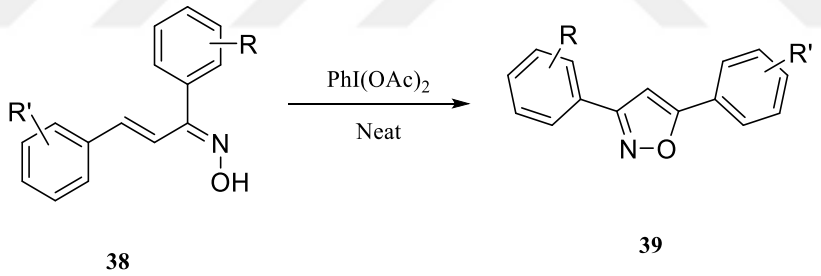
29 35 36 37

Scheme 1.12. Solvent free synthesis under MW irradiation

1.1.2 Solvent-Free Method

Solvent-free method provides high safety, simple workup, decreased cost, environmental friendly reaction conditions. It also furnishes greater amounts of reactants with same equipment, reactivity and selectivity by keeping away from using organic solvents (Loupy et al., 1998).

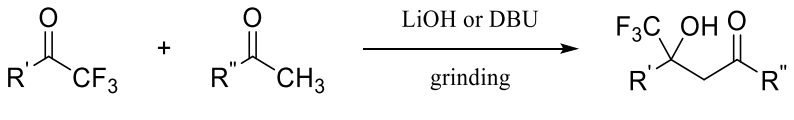
Chandra Sekhar et al. (2013) were used a simple and efficient method to synthesize 3,5-diaryl isoxazole derivatives **(39)** with conversion of chalcone oximes **(38)** under solvent-free conditions in good yields (Scheme 1.13).



38 39

Scheme 1.13. Solvent-free synthesis of 3,5-diaryl isoxazole

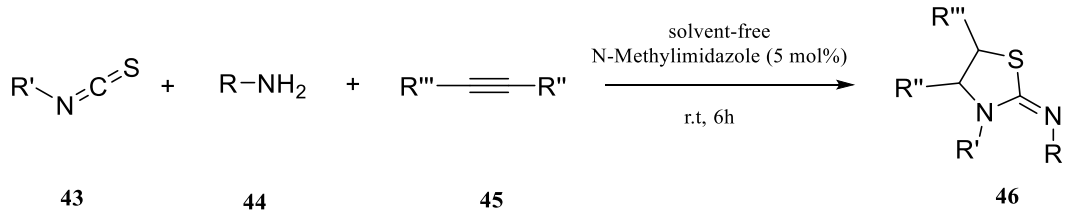
Tao et al. (2015) were grinded methyl ketones **(41)** and trifluoromethyl ketones **(40)** in mortar to get β-trifluoromethyl- β-hydroxyl ketones **(42)** under solvent free conditions (Scheme 1.14).



40 41 42

Scheme 1.14. Solvent free synthesis of β-trifluoromethyl- β-hydroxyl ketones

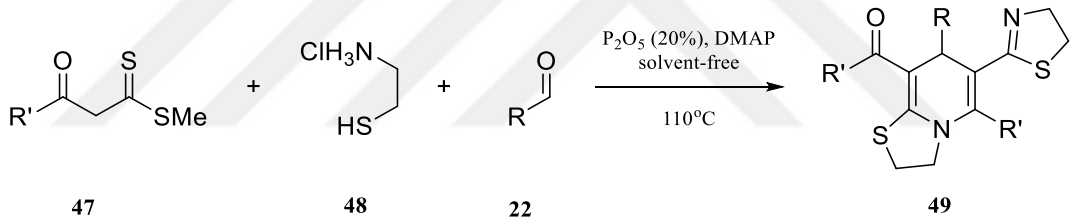
Various functionalized 1,3-thiazolane derivatives **(46)** were synthesized from the reaction of activated acetylenic compounds **(45)** with isothiocyanates **(43)** and primary amines **(44)** in the presence of a catalytic amount of *N*-methylimidazole under solvent-free conditions without using any additional catalyst (Scheme 1.15) (Hossaini et al., 2014).



43 44 45 46

Scheme 1.15. Synthesis of 1,3-thiazolane derivatives

Nagaraju et al. (2015) were obtained dihydrothiazolo[3,2-a]pyridines **(49)** from the one-pot multicomponent coupling (MCC) reaction of dithioesters **(47)**, cysteamine hydrochloride **(48)** and aromatic aldehydes **(22)** under metal-free and solvent-free conditions (Scheme 1.16).



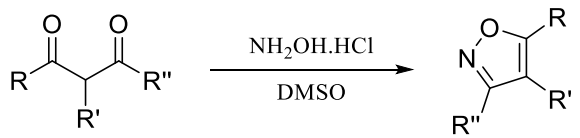
47 48 22 49

Scheme 1.16. Synthesis of dihydrothiazolo[3,2-a]pyridines via MCC

1.1.3 Flow Method

Another green and sustainable chemical synthesis is continuous flow micro reactors which provide in terms of fast mixing based on short diffusion paths in micro reactors, ambient temperatures, the exact residence time control and less energy for transportation and easy recycling of substances (Yoshida et al., 2011).

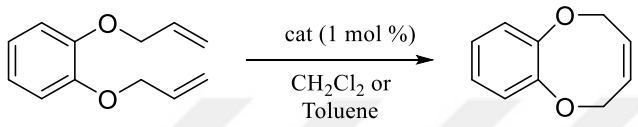
Rodriguez et al. (2012) have studied the effects of the temperature, continuous-flow regime, and microwave irradiation by using optimization reactions for the synthesis of 3,4,5-trisubstituted isoxazoles **(51)**. This work showed that continuous-flow microwave-heated microreactors have significant effect for less reactive diketones (Scheme 1.17).



50 51

Scheme 1.17. Synthesis of 3,4,5-trisubstituted isoxazoles

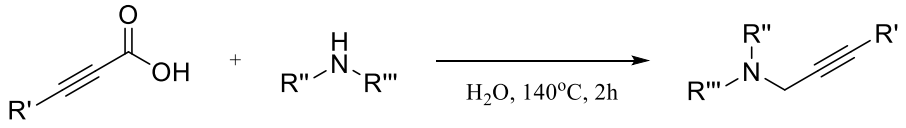
Comer and Organ (2005) studied the Suzuki-Miyaura couplings by novel method of flowing a reaction through a short, straight capillary that sits in a microwave synthesizer (Scheme 1.18).



52 53

Scheme 1.18. Ring-Closing using the Flow MW microreactor

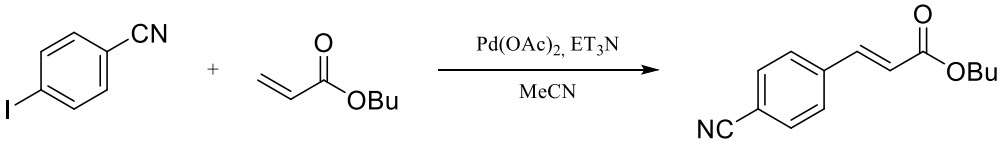
Environmentally friendly and economical continuous flow reaction system was used for the producing of propargylamines **(56)** via a metal-free decarboxylative coupling in aqueous medium in the absence metal catalysts or other additives (Scheme 1.19) (Jung et al., 2015).



54 55 56

Scheme 1.19. Synthesis of propargylamine using the flow process

Glasnov et al. (2009) have developed Mizoroki–Heck couplings of aryl iodide **(57)** with butyl acrylate **(58)** to perform transition-metal catalysed transformations in continuous-flow mode (Scheme 1.20).



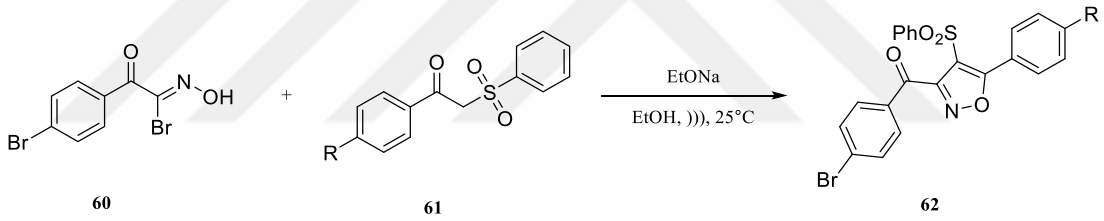
57 58 59

Scheme 1.20. Mizoroki–Heck coupling under flow system using $Pd(OAc)_2$

1.1.4 Ultrasonic Method

Ultrasound-assisted organic synthesis is our last investigation in the concept of "green" synthesis method. Ultrasound (US) synthesis also known as sonochemistry (in the region of 20 to 50 kHz) is a kind of non-conventional chemistry and has many advantages for high efficiency, reduction of wastes, low energy requirements, the design of non-polluting products and processes (Cintas and Luche 1999). Various applications are used for its high energy and the capacity to scatter reagent in small particles and accelerate reactions. Synthesis of pharmaceuticals, drugs, food additives and fine chemicals is easy by using ultrasonic method and this method provides better rates, yields and chemo-, regio- and stereoselectivities (Kardos and Luche, 2001).

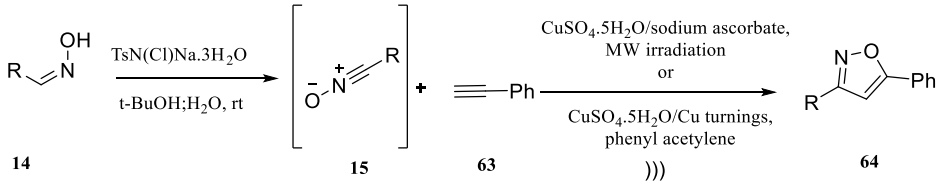
α-Sulfonyl carbanion **(61)** was reacted with hydroxomoyl bromide **(60)** to give novel isoxazoles **(62)** in ethanol under US irradiation (Scheme 1.21) (Saleh and Abd EL-Rahman, 2009).



60 61 62

Scheme 1.21. Reaction of α-sulfonyl carbanion with hydroxymoyl bromide

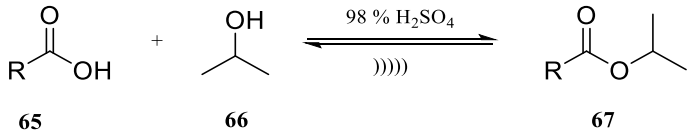
Koufaki et al. (2014a), Koufaki et al. (2014b) have developed a one-pot three-step process for the synthesis of 3,5-disubstituted isoxazole derivatives in the presence of Cu catalyst from the cycloaddition reaction between *in situ* generated nitrile oxides and alkynes, using ultrasound irradiation and microwave-assisted synthesis avoiding toxic reagents and solvents. They compared both methods and MW asisted synthesis gave higher results (Scheme 1.22).



14 15 63 64

Scheme 1.22. One-pot isoxazole synthesis under MW and US irradiations

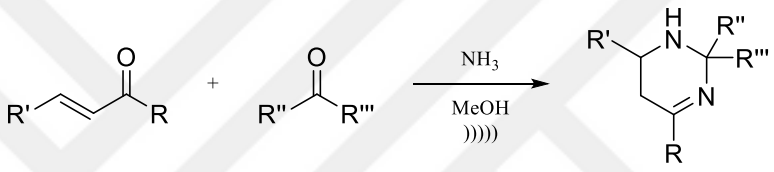
Deshmane et al. (2009) developed novel method for the synthesis of isopropyl esters **(67)** with acid catalysed synthesis from palm fatty acid distillate (PFAD) **(65)** in the presence of ultrasonic irradiations (Scheme 1.23).



65 **66** **67**

Scheme 1.23. Esterification of (PFAD) with isopropyl alcohol

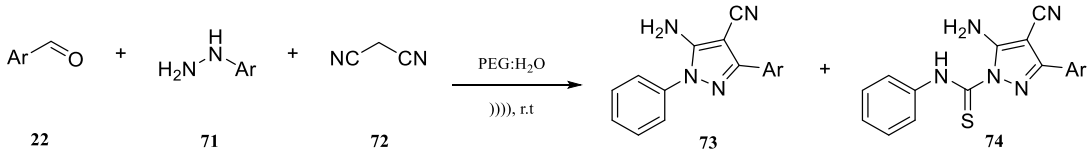
Tetrahydropyrimidine derivatives **(70)** were synthesized by the three-component condensation of 1,3-diarylprop-2-en-1-one **(68)** with ammonia and aldehydes **(69)** under ultrasonic irradiation wit high yields (Scheme 1.24) (Muravyova et al., 2007).



68 **69** **70**

Scheme 1.24. Formation of tetrahydropyrimidines under US irradiation

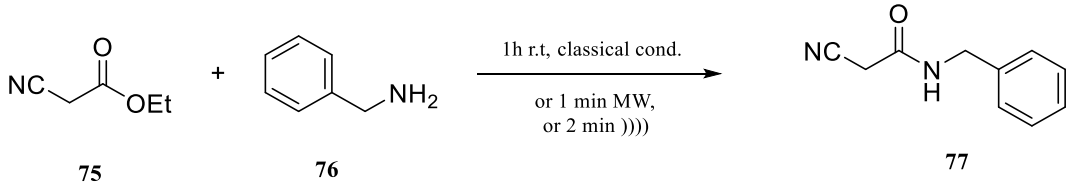
One-pot reactions of various aldehydes **(22)**, malononitrile **(72)** and hydrazine **(71)** gave substituted pyrazoles **(73, 74)** in green solvents (PEG-400 and water) at r.t under ultrasound irradiation (Scheme 1.25) (Nemati et al., 2015).



22 **71** **72** **73** **74**

Scheme 1.25. Synthesis of pyrazoles in green solvents under US irradiation

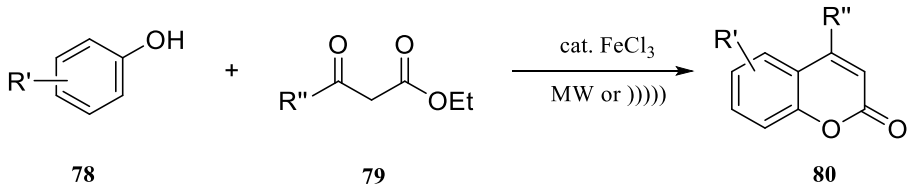
Al-Zaydi (2009) synthesized cyanoacetamides **(77)** from the reaction of ethyl cyanoacetate **(75)** with benzylamine **(76)** under microwave, sonication and classical conditions. When yields of products and reaction times were compared, MW and ultrasound irradiations increased the yields and reduced the time (Scheme 1.26).



75 **76** **77**

Scheme 1.26. Reaction of ethyl cyanoacetate with benzylamine

4-Substituted coumarin derivatives were obtained by high energy techniques like US and MW irradiation in the presence of $FeCl_3$ as catalyst. Pechmann reaction provided coumarins with good yields under US conditions in short time (Scheme 1.27) (Prousis et al., 2014).



78 **79** **80**

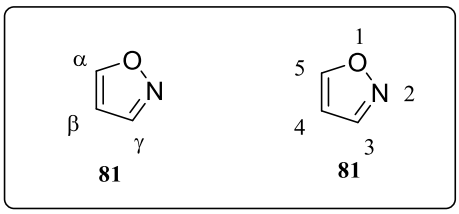
Scheme 1.27. Synthesis of coumarins under MW and US irradiation

1.2 Isoxazoles

Heterocyclic compounds are cyclic compounds that have at least one element other than carbon, like oxygen, nitrogen or sulfur etc. They are very important for life since they are found in natural products also they can be synthesized in laboratories.

Among many other heterocyclic compounds isoxazoles have been attracted chemist's attention since Claisen (1888) discovered the accurate structure (3-methyl-5-phenylisoxazole) from the reaction of hydroxylamine on benzoylacetone and proposed the name monoazole for the five membered rings however Hantsch (1888) changed to isoxazole. The trivial name isoxazole has been accepted by IUPAC and is used in Chemical Abstracts, though some authors use the more systematic name 1,2-azole.

The Greek letters α, β and γ were used starting from the position next to the oxygen atom for the nomenclature until 1950s, but now numbering beginning at oxygen atom is used (Figure 1.2).

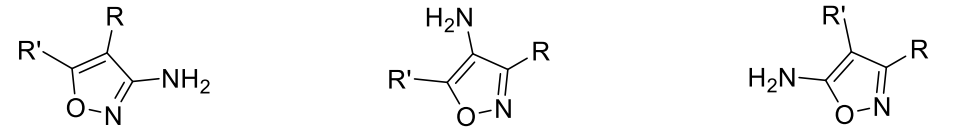


81 **81**

Figure 1.2. Nomenclature of isoxazole

1.2.1 Aminoisoxazoles

Aminoisoxazoles are named according to connection of amino group to 3, 4 and 5-positions of isoxazoles (Figure 1.3).



82 3-aminoisoxazole

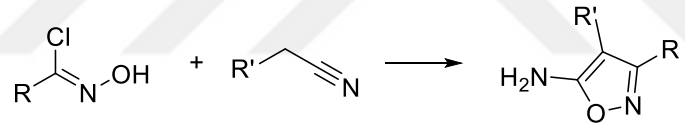
83 4-aminoisoxazole

84 5-aminoisoxazole

Figure 1.3. Structures of aminoisoxazoles

1.2.2 Synthesis of 5-Aminoisoxazoles

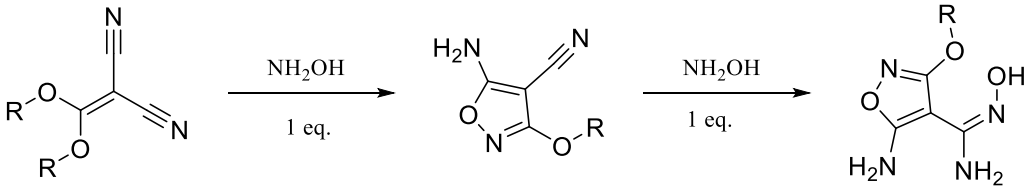
Becalli et al. (1985) mixed hydroxymoyl chloride **(3)** and a substituted acetonitrile **(85)** to form three-substituted isoxazole derivatives **(84)** in the presence of a base (Scheme 1.28).



3 **85** **84**

Scheme 1.28. Synthesis of isoxazoles from substituted acetonitriles

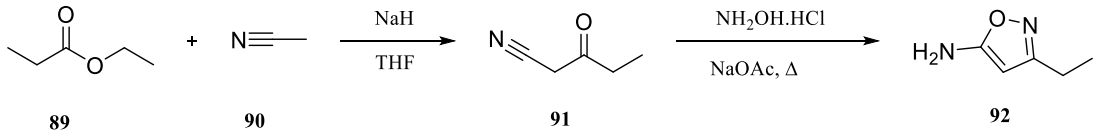
Dicyanoketene acetals **(86)** were reacted with equal amounts of hydroxylamine to form substituted 5-aminoisoxazoles **(87)**. When two equivalents of hydroxylamine were used, first cyanoisoxazole **(87)** was produced and further reaction with hydroxylamine to yield 5-amino-3-(2-hydroxyethoxy)-4-isoxazolecarboxamide oxime **(88)** (Scheme 1.29) (Middleton and Engelhardt, 1958).



86 **87** **88**

Scheme 1.29. Conversion of isoxazole to isoxazolecarboxamide oxime

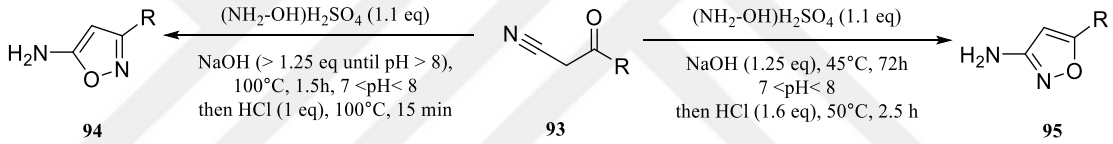
Cyanoketones **(91)** can be used to obtain 5-aminoisoxazoles. Eddington et al. (2002) was condensed ethyl propionate **(89)** with acetonitrile **(90)** to produce cyanoketone **(91)** then, obtained cyanoketone reacted with hydroxylamine to get 3-ethyl-5-aminoisoxazole **(92)** in rational yields (Scheme 1.30).



89 90 91 92

Scheme 1.30. Synthesis of 3-ethyl-5-aminoisoxazole

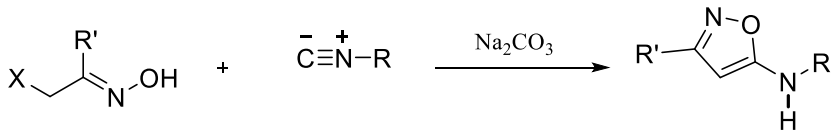
Johnson et al. (2013) developed a pathway that can be used to reach 3-amino **(95)** or 5-amino isoxazoles **(94)**. Altering the reaction temperature and pH for the regioselectivity of the two reactions and these parameters were responsible for the addition of the hydroxylamine either towards the ketone (pH > 8, giving 5-amino-isoxazole) or the nitrile (7 < pH < 8, giving 3-amino-isoxazole) (Scheme 1.31).



94 93 95

Scheme 1.31. Synthesis of 3- or 5-amino isoxazole depending on the pH

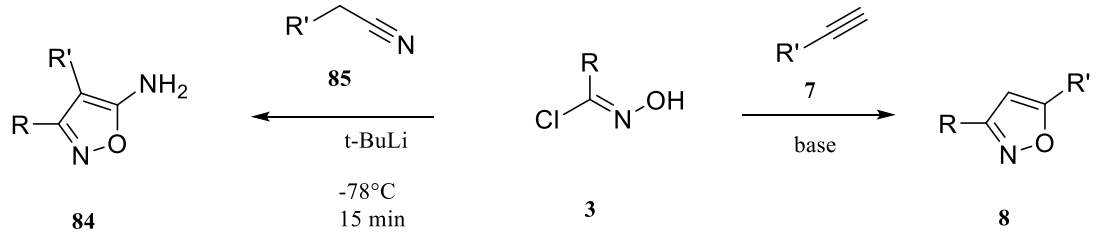
Another synthetic approach to *N*-substituted 5-aminoisoxazoles **(98)** involved reactions of α-haloketone oximes **(96)** with isocyanides **(97)** and sodium carbonate (Scheme 1.32) (Buron et al., 1997).



96 97 98

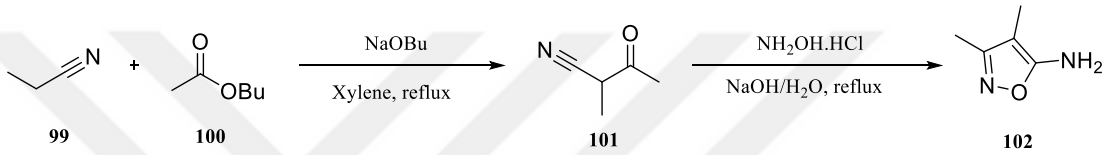
Scheme 1.32. Formation of isoxazole in the presence of Na_2CO_3

The reaction of lithiated nitriles with α-chlorooximes **(3)** led to a novel formation of 5-aminoisoxazoles **(84)**, mainly ones with alkyl functionality at the 4 position. Additionally α-chlorooximes have been demonstrated to be valuable substrates for the synthesis of 5-alkylisoxazole derivatives **(8)** (Scheme 1.33) (Bourbeau and Rider, 2006).



Scheme 1.33. Synthesis of 5-amino and 5-alkylisoxazoles

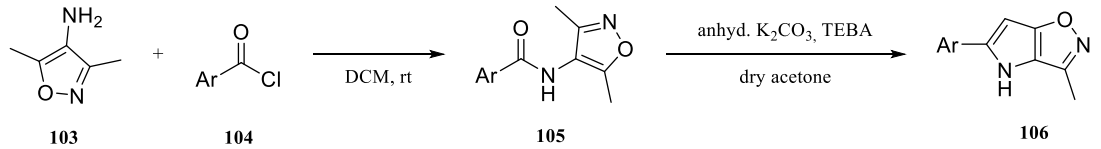
2-Methyl-3-oxobutanenitrile **(97)** obtained from reaction of ethyl cyanide **(95)** with butyl acetate **(97)** in xylene was reacted with oxammonium hydrochloride in sodium hydroxide solution to produce 4-dimethylisoxazol-5-amine **(98)** (Scheme 1.34) (Bai et al., 2012).



Scheme 1.34. The synthetic route for the synthesis of 5-aminoisoxazoles

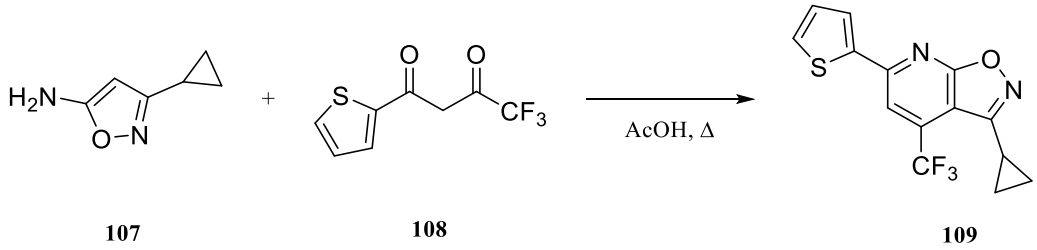
1.2.3 Reactions of Isoxazoles

Rajanarendar et al. (2006) have developed novel method for the synthesis of pyrrolo[2,3-d]isoxazoles **(106)** from the benzoylation reaction of 4-aminoisoxazole **(103)** with aroyl chloride **(104)** in DCM then obtained benzamide **(105)** gave cyclization reaction in the presence of anhydrous K_2CO_3 in dry acetone containing triethylbenzyl ammonium chloride (TEBA) (Scheme 1.35).



Scheme 1.35. Synthesis of pyrrolo[2,3-d]isoxazole derivatives

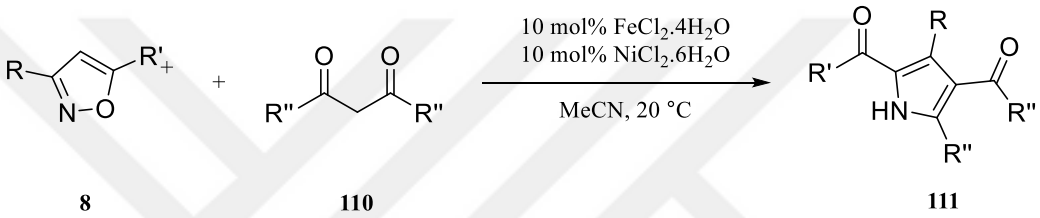
Isoxazolopyrimidines **(109)** were obtained from the reaction of 5-aminoisoxazoles **(107)** with 1,3-diketones **(108)** in glacial AcOH regioselectively (Scheme 1.36) (Petrosyan et al., 2007).



107 108 109

Scheme 1.36. Formation of isoxazolopyrimidine derivatives

Galenko et al. (2015) have studied the domino reaction of 5-alkoxy- or 5-aminoisoxazoles **(8)** with 1,3-dicarbonyl compounds **(110)** to obtain pyrrole-2-carboxylic acids **(111)** with using multicatalysis of $FeCl_2$ and $NiCl_2$ (Scheme 1.37).



8 110 111

Scheme 1.37. Domino reaction of isoxazoles with dicarbonyl compounds

1.2.4 Biological Activities of Isoxazoles

Isoxazoles are 5-membered heterocyclic compounds that have distinctive chemical properties because of adjacent nitrogen-oxygen bond. Isoxazole rings can be found in natural products like ibotenic acid **(112)** and muscimol **(113)** isolated from Amanita muscaria (Figure 1.4) (Gagneux et al., 1965; Eugster et al., 1965). Moreover isoxazole containing heterocycles have many biological activities such as antioxidant, anti-inflammatory, antibacterial, antiprotozoal, cytotoxic, therapeutic and pharmaco therapeutic activities (Selvam et al. 2005; Bjorkman et al., 1996; Kang et al., 2000; Sperandeo and Brun, 2003; Liu et al. 2009) and are forms of drugs like COX-1, COX-2 inhibitor, nitric oxide donor etc. (Figure 1.5) (Kanda et al., 2000; Adib et al., 2009; Vitale et al., 2014; Balsamo et al., 2003; Maksimovic-Ivanic et al., 2008; Nantermet et al., 2002; Kamal et al., 2011). Recently, lots of modifications have been done on isoxazole nucleus and substituted isoxazoles have been discovered in the literature with different types of potent biological activities (Joshna and Lakshmi, 2013).

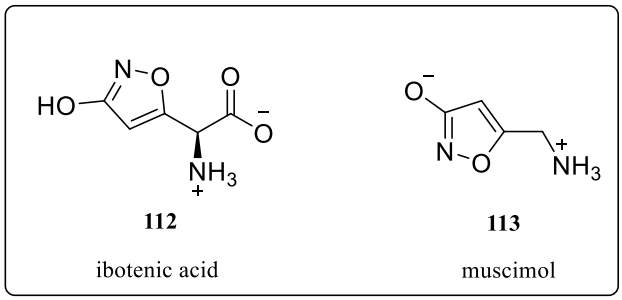


Figure 1.4. Structures of ibotenic acid and muscimol

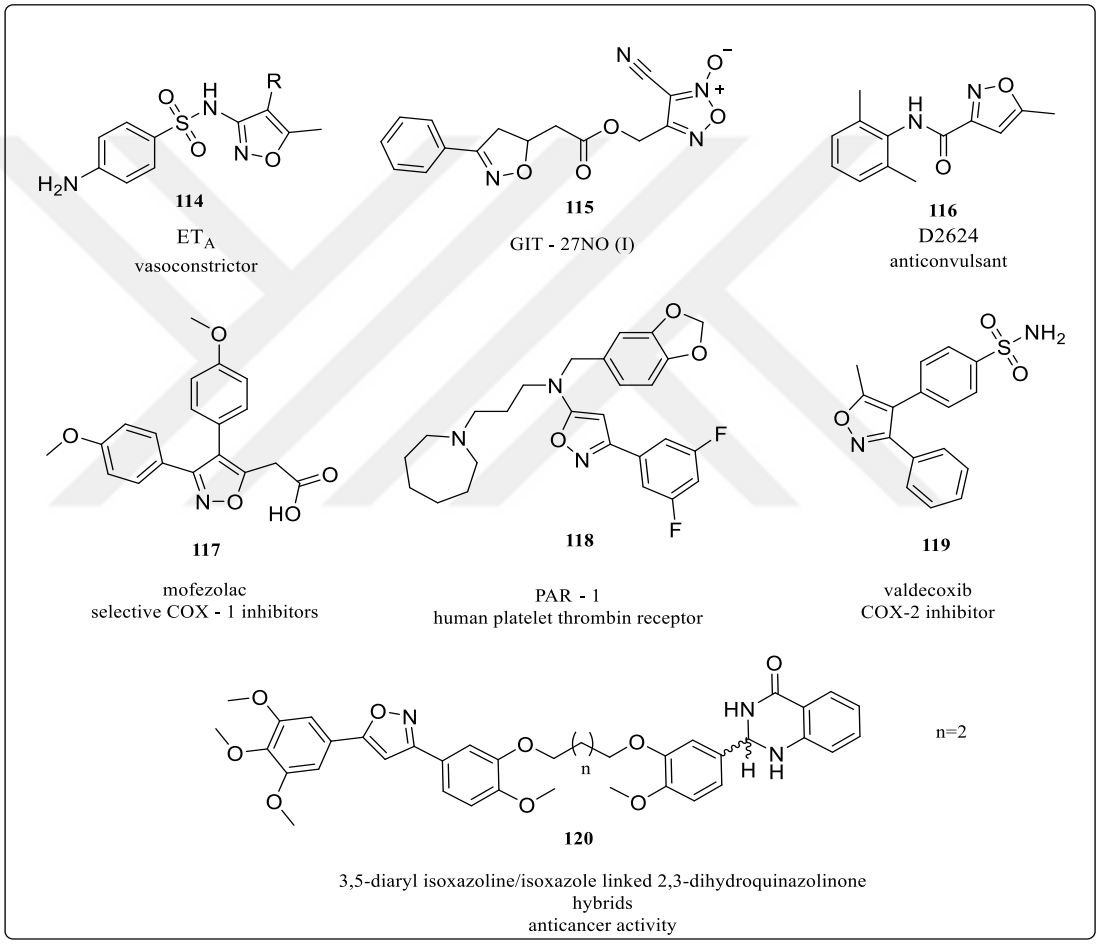


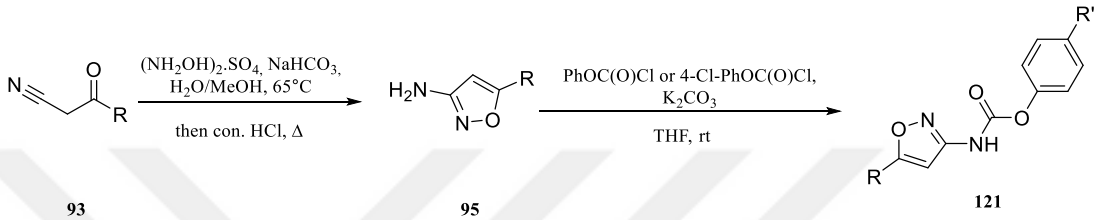
Figure 1.5. Some biologically active isoxazole containing heterocycles

1.3 Amide Synthesis

Among all carbon–heteroatom bonds, the amide bonds are found in many natural products and allow a significant synthetic method in organic chemistry. Amides are also important for medicinal chemists (Montalbetti and Falque, 2005).

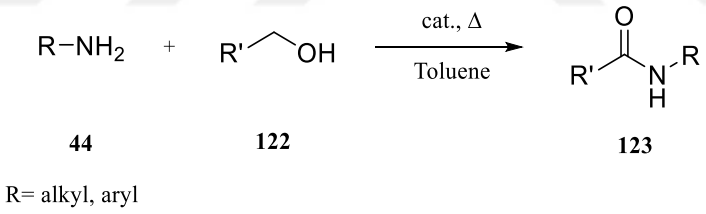
Synthesis of amides is mainly based on the condensation of amines with carboxylic acid derivatives (acid chlorides and anhydrides) (Scheme 1.38) or an alcohol (Scheme 1.39), Beckmann rearrangement from oximes (Scheme 1.40), Schmidt reaction (Scheme 1.41) and carbonylation (Scheme 1.42).

Rowbottom et al. (2012) have converted the aminoisoxazoles **(95)** to the corresponding isoxazole carbamates **(121)** *via* reaction with either phenyl chloroformate or 4-chlorophenyl chloroformate (Scheme 1.38).



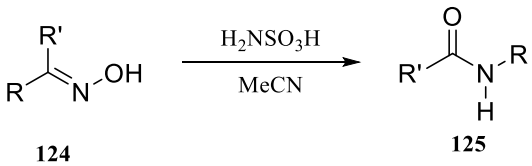
Scheme 1.38. Formation of isoxazole carbamates

Gunanathan et al. (2007) studied the direct catalytic acylation of primary amines **(44)** with alcohols **(122)** to form amides **(123)** in high yields (Scheme 1.39).



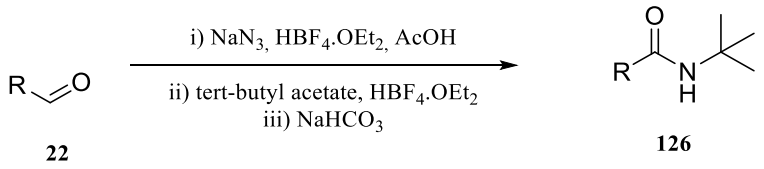
Scheme 1.39. Catalytic acylation of amines

Wang et al. (2004) have developed a green route for the synthesis of amide **(125)** from Beckmann rearrangement of ketoxime **(124)** using green cat. sulfamic acid (H_2NSO_3H) without producing any waste and catalyst are recyclable (Scheme 1.40).



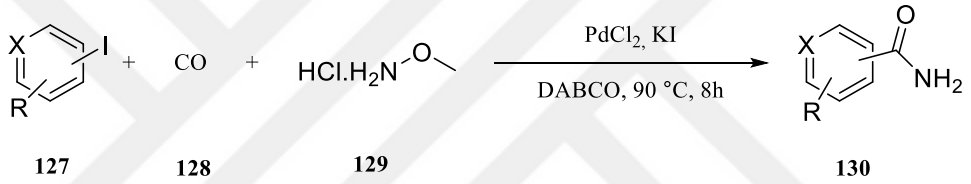
Scheme 1.40. Beckmann rearrangement of ketoximes

Hazarika et al. (2015) studied the Schmidt and Ritter reaction sequence to obtain amide derivatives **(126)** in a one-pot operation with good yields (Scheme 1.41).



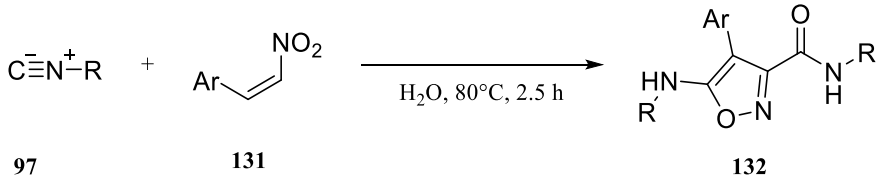
Scheme 1.41. Synthesis of amides via Schmidt and Ritter reaction sequence

Gadge and Bhanage (2013) have developed palladium-catalyzed, phosphine-free carbonylation procedure to form amide derivatives **(130)** at lower temperatures with shorter reaction times (Scheme 1.42).



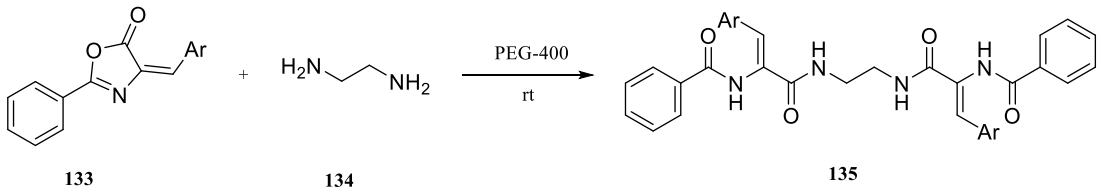
Scheme 1.42. Synthesis of primary amides by aminocarbonylation

Additionaly, Adib et al. (2009) were refluxed an isocyanide **(97)** and a nitrostyrene **(131)** in water to obtain isoxazolecarboxamide derivatives **(132)** (Scheme 1.43).



Scheme 1.43. Synthesis of isoxazolecarboxamide in water

Taki et al. (2015) studied a simple, green, fast and catalyst-free method for the synthesis of benzamide **(135)** by ring opening of azlactones **(133)** with ethylene diamine **(134)** without using catalyst in PEG-400 at rt (Scheme 1.44).



Scheme 1.44. Green synthesis of benzamide derivatives

2. AIM AND SCOPE OF THE STUDY

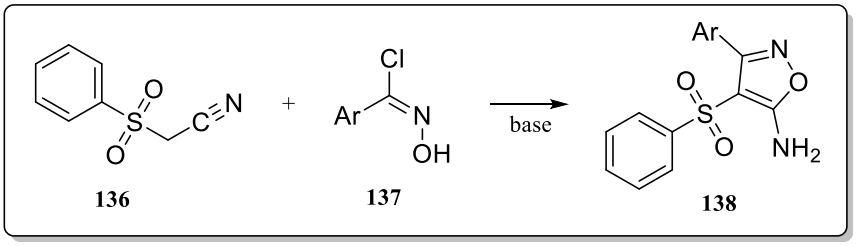
There are many various ways to synthesis isoxazoles. In this study we have proposed two synthetic routes microwave and solvent-free synthesis in a mortar. Those routes are accepted as branch of Green Chemistry. We compared these methods according to their yields, reaction times, operational simplicity, environmental safety. Finally, observed nineteen molecules were tested *in vitro* MTT study to investigate inhibitive abilities to some cancer cell lines (C3a, L929, T98g and MCF-7) and 4 compounds showed noticeable cytotoxic property against four cancer cell lines.

3. MATERIALS AND METHODS

3.1 General

CEM DISCOVERY SP Microwave synthesis device model was used for the sythesis of isoxazole derivatives. The IR spectra were recorded on a SHIMADZU FTIR-8400S instrument with KBr discs. The NMR spectra were recorded on a Bruker Avance III (400 and 500 MHz for ^{1}H NMR and 100 and 125 MHz for ^{13}C) either using DMSO-d_6 or $CDCl_3$. All chemical shifts were reported in parts per million downfield from TMS. Coupling constants (*J*) were reported in Hertz. 2-Phenylsulfonyl acetonitrile was purchased from Sigma–Aldrich. Melting points were determined on a MELTEMP apparatus and uncorrected. The reactions were monitored by TLC on Merck (5735) plates. Mass spectra measurements were performed using a Micromass LCT Premier XE (Waters MS Technologies) orthogonal acceleration Time-of-Flight mass spectrometer operation in both positive and negative ion mode with electrospray ionization.

3.2 Typical procedure for preparation of 3-aryl-4-phenylsulfonyl)isoxazol-5-amines 138



136 137 138

General Procedure with Solvent Free Method (138 a-j)

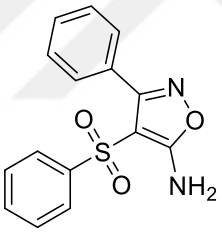
2-Phenylsulfonylacetonitrile (1 mmol, 181 mg, 1 eq), the appropriate α-chlorooxime (1 mmol, 1 eq) and Et_3N (1,1 mmol, 222 mg, 2.2 eq) were mixed in a mortar and grinded for 5 min. After completion of the reaction, as judged by TLC,

H_2O (5 mL) was added and the resulting precipitate was filtered. Filtrated solids were dried and recrystallized from EtOAc/Hexane.

General Procedure for Microwave Method (138 a'-j')

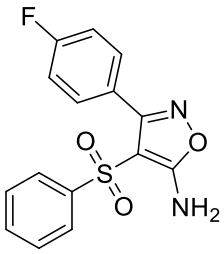
2-Phenylsulfonylacetonitrile (1 mmol, 181 mg, 1 eq), the appropriate α-chlorooxime (1 mmol, 1 eq) and NaOH (1 mmol, 120 mg, 3.0 eq) were dissolved in MeOH (3 ml). Then the reaction mixture was stirred in microwave at 200 Watt, at 25 °C in the dynamic mode for 5 min. After completion of the reaction, as judged by TLC, the solvent was removed under reduced pressure. Extraction was done with EtOAc/H_2O and then brine was added to the mixture. Organic solvent was dried with Na_2SO_4. After evaporation of solvent, the crude sample was recrystallized from EtOAc/Hexane.

3.2.1 3-Phenyl-4-(phenylsulfonyl)isoxazol-5-amine 138a



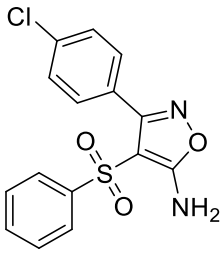
Obtained as brown crystals (180 mg, 60% with solvent-free; 225 mg, 75% with MW); m.p. 123–124 °C; R_f (EtOAc 1:1 Hex) 0.75; $\nu_{max.}$ (KBr) 3392 (NH_2), 1300-1145 (SO_2) and 1639 (C=N) cm^{-1}; HRMS: requires for $C_{15}H_{13}N_2O_3S$ $[M+H]^+$ 301.0647, found, 301.0647. ^1H NMR (400 MHz, DMSO-d_6) δ 8.23 (broad s, 2H, NH_2), 7.64–7.59 (m, 2H), 7.58 (t, *J* 1.8, 1H), 7.54–7.49 (m, 3H), 7.49–7.43 (m, 2H), 7.43–7.38 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 160.9, 143.4, 133.7, 130.7, 129.8, 129.4, 128.7, 127.8, 126.2, 91.5; *m/z* (TOF EI^+) 301 (M+H, 100%), 252 (8).

3.2.2 3-(4-Fluorophenyl)-4-(phenylsulfonyl)isoxazol-5-amine 138b



Obtained as brown crystals (170 mg, 54% with solvent-free; 195 mg, 61% with MW); m.p. 147–148 °C; R_f (EtOAc 1:1 Hex) 0.75; $\nu_{max.}$ (KBr) 3410 (NH_2), 3300 (NH_2), 1640 (C=N), 1300-1140 (SO_2) cm^{-1}; HRMS: requires for $C_{15}H_{10}N_2O_3FS$ $[M-H]^-$ 317.0396, found, 317.0384; ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* 2.2, 1H), 7.56 (dd, *J* 2.2, 1.4, 1H), 7.56–7.54 (m, 1H), 7.53 (d, *J* 1.2, 1H), 7.51 (t, *J* 4.6, 1H), 7.36 (dd, *J* 8.1, 7.4, 2H), 7.10 (t, *J* 8.7, 2H), 6.24 (br s, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 164.1 (d, $^1J_{C-F}$ 249.4, C–F), 160.2, 142.1, 133.3, 131.4 (d, $^3J_{C-F}$ 8.6, CH *meta* to F), 128.9, 126.5, 123.0 (d, $^4J_{C-F}$ 3.3, C *para* to F), 115.5 (d, $^2J_{C-F}$ 21.7, CH *ortho* to F), 93.7; *m/z* (TOF ES^-) 317 (M−H, 100%).

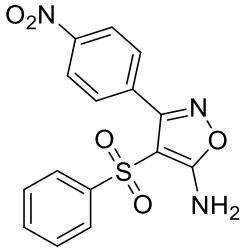
3.2.3 3-(4-Chlorophenyl)-4-(phenylsulfonyl)isoxazol-5-amine 138c



Obtained as pale brown solid (151 mg, 45% with solvent-free; 278, mg 83% with MW); m.p. 142–143 °C; R_f (EtOAc 1:1 Hex) 0.75; $\nu_{max.}$ (KBr) 3450 (NH_2), 3345 (NH_2), 1640 (C=N) and 1300–1140 (SO_2) cm^{-1}; HRMS: requires for $C_{15}H_{10}N_2O_3SCl$ $[M-H]^-$ 333.0101, found, 333.0103; ^1H NMR (400 MHz, DMSO-d_6) δ 8.28 (br s, 2H, NH_2), 7.68–7.62 (m, 2H), 7.59–7.50 (m, 5H), 7.46–7.40 (m, 1H), 7.28–7.23 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 158.9, 143.1,

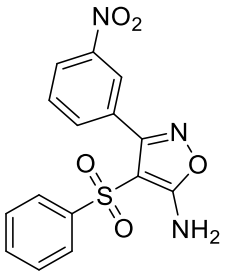
133.8, 133.6, 132.2, 129.7, 127.2, 127.1, 126.4, 92.7; *m*/*z* (TOF ES$^-$) 333 (M–H, 100%).

3.2.4 3-(4-Nitrophenyl)-4-(phenylsulfonyl)isoxazol-5-amine 138d



Obtained as yellow solid (218 mg, 62% with solvent-free; 306 mg, 89% with MW); m.p. 212–214 °C; R_f (EtOAc 1:1 Hex) 0.67; $\nu_{max.}$ (KBr) 3440 (NH_2), 3330 (NH_2), 1640 (C=N) and 1300–1140 (SO_2) cm^{-1}; HRMS: requires for $C_{15}H_{10}N_3O_5S$ [M–H]$^-$, 344.0341 found, 344.0347; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (br s, 2H), 8.31 (d, *J* 8.8, 2H), 7.72 (d, *J* 4.4, 2H), 7.69–7.59 (m, 3H), 7.54 (d, *J* 8.8, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 159.5, 149.0, 143.2, 134.1, 133.9, 131.0, 129.9, 126.3, 123.8, 91.9; *m*/*z* (TOF ES$^-$) 344 (M–H, 100%).

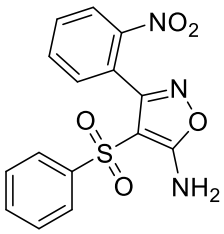
3.2.5 3-(3-Nitrophenyl)-4-(phenylsulfonyl)isoxazol-5-amine 138e



Obtained as yellow solid (117 mg, 34% with solvent-free, 148 mg, 43% with MW); m.p. 173–174 °C; R_f (EtOAc 1:1 Hex) 0.65; $\nu_{max.}$ (KBr) 3430 (NH_2), 3330 (NH_2), 1640 (C=N) and 1300–1135 (SO_2) cm^{-1}; HRMS: requires for $C_{15}H_{10}N_3O_5S$ [M–H]$^-$ 344.0341, found, 344.0329; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40–8.38 (m, NH_2 and aromatic *CH* 3H), 8.23 (s, 1H), 7.90 (d, *J* 6.7, 1H), 7.83–7.74 (m, 1H), 7.72–7.62 (m, 3H), 7.58–7.50 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ 170.0, 158.5,

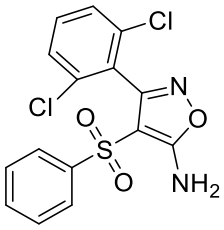
147.2, 142.6, 135.1, 133.3, 130.0, 129.4, 128.2, 125.6, 124.9, 123.5, 91.0; *m*/*z* (TOF ES^-) 344 (M–H, 100%).

3.2.6 3-(2-Nitrophenyl)-4-(phenylsulfonyl)isoxazol-5-amine 138f



Obtained as yellow solid (180 mg, 52% with solvent-free; 277 mg, 80% with MW); m.p. 177–178 °C; R_f (EtOAc 1:1 Hex) 0.42; $\nu_{max.}$ (KBr) 3430 (NH_2), 3330 (NH_2), 1640 (C=N) and 1300–1135 (SO_2) cm^{-1}; HRMS: requires for $C_{15}H_{10}N_3O_5S$ $[M–H]^-$ 344.0341, found, 344.0334; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40–8.37 (m, NH_2 and aromatic *CH*, 3H), 8.22 (d, *J* 1.5, 1H), 7.90 (d, *J* 7.7, 1H), 7.77 (t, *J* 8.0, 1H), 7.69–7.62 (m, 3H), 7.53 (t, *J* 7.7, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 158.5, 147.3, 142.6, 135.2, 133.4, 130.1, 129.4, 128.6, 125.7, 125.0, 123.6, 91.0; *m*/*z* (TOF ES^-) 344 (M–H, 100%).

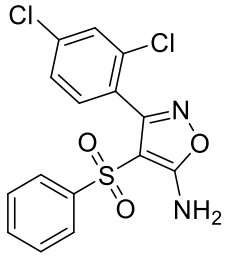
3.2.7 3-(2,6-Dichlorophenyl)-4-(phenylsulfonyl)isoxazol-5-amine 138g



Obtained as greyish brown crystals (310 mg, 84% with solvent-free; 314 mg, 85% with MW); m.p. 185–187 °C; R_f (EtOAc 1:1 Hex) 0.58; $\nu_{max.}$ (KBr) 3430 (NH_2), 3315 (NH_2), 1640 (C=N) and 1300-1145 (SO_2) cm^{-1}; HRMS: requires for $C_{15}H_9N_2O_3SCl_2$ $[M–H]^-$ 366.9711, found, 366.9722; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (s, NH_2, 2H), 7.69–7.62 (m, 3H), 7.60–7.58 (m, 3H), 7.55 (t, *J* 7.6,

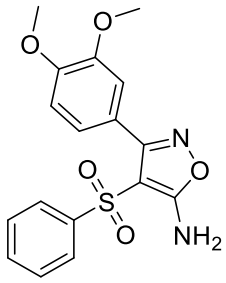
2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 156.4, 142.1, 134.7, 133.2, 132.5, 129.1, 128.0, 126.0, 125.9, 91.7; *m*/*z* (TOF ES$^-$) 366 (M–H, 100%), 180(5).

3.2.8 3-(2,4-Dichlorophenyl)-4-(phenylsulfonyl)isoxazol-5-amine 138h



Obtained as grey crystals (292 mg, 79% wit solvent-free; 234 mg, 64% with MW); m.p. 195–196 °C; R_f (EtOAc 1:1 Hex) 0.58; $\nu_{max.}$ (KBr) 3440–3335 (NH_2), 1640 (C=N) and 1300–1140 (SO_2) cm^{-1}; HRMS: requires for $C_{15}H_9N_2O_3SCl_2$ [M–H]$^-$366.9711, found, 366.9728; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (br s, NH_2, 2H), 7.76 (app. d, *J* 2.0, 1H), 7.71–7.64 (m, 2H), 7.63–7.59 (m, 2H), 7.57–7.53 (m, 2H), 7.32 (d, *J* 8.3, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1, 157.5, 142.4, 135.5, 134.1, 133.2, 132.7, 129.2, 128.8, 127.0, 125.7, 125.6, 92.1; *m*/*z* (TOF ES$^-$) 366 (M–H, 100%).

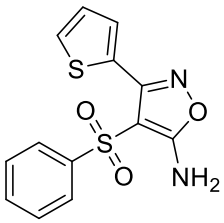
3.2.9 3-(3,4-Dimethoxyphenyl)-4-(phenylsulfonyl)isoxazol-5-amine 138i



Obtained as white solid (238 mg, 66% with solvent-free; 286 mg, 79% with MW); m.p. 166–167 °C; R_f (EtOAc 1:1 Hex) 0.39; $\nu_{max.}$ (KBr) 3400–3320 (NH_2), 1640 (C=N) and 1300–1140 (SO_2) cm^{-1}; HRMS: requires for $C_{17}H_{17}N_2O_5S$ [M+H]$^+$ 361.0858, found, 361.0852; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59–7.54 (m, 2H), 7.52–7.47 (m, 1H), 7.40–7.34 (m, 2H), 7.21 (d, *J* 2.0, 1H), 7.19 (s, 1H), 6.89 (d, *J* 8.6,

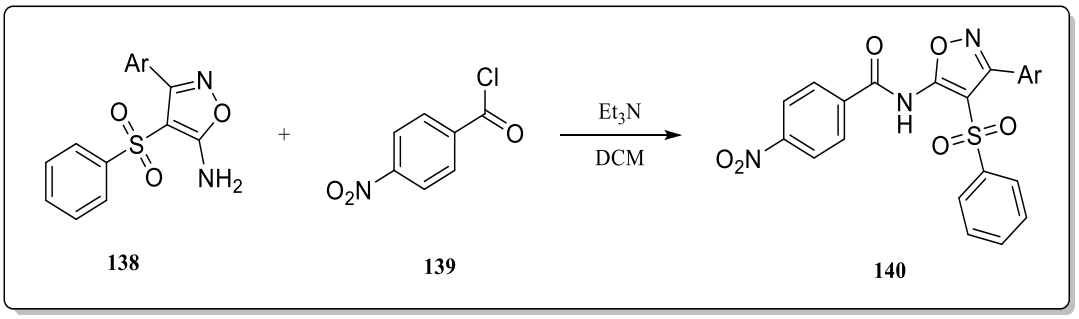
1H), 6.25 (br s, NH_2, 2H), 3.94 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 160.6, 150.6, 148.5, 142.2, 133.1, 128.8, 126.3, 122.1, 119.2, 112.1, 110.6, 93.2, 55.9, 55.8; *m*/*z* (TOF EI^+) 361 (M+H, 100%).

3.2.10 4-(Phenylsulfonyl)-3-(thiophen-2-yl)isoxazol-5-amine 138j



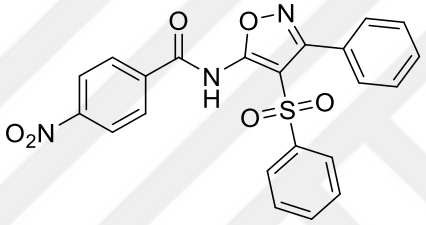
Obtained as grey solid (153 mg, 50% with solvent-free; 159 mg, 52% with MW); m.p. 155–157 °C; R_f (EtOAc 1:1 Hex) 0,58; $\nu_{max.}$ (KBr) 3450–3320 (NH_2), 1640 (C=N) and 1300–1140 (SO_2) cm^{-1}; HRMS: requires for $C_{13}H_{11}N_2O_3S_2$ $[M+H]^+$ 307.0211, found M^+, 307.0203; ^1H NMR (500 MHz, $CDCl_3$) δ 7.87 (dd, *J* 3.7, 1.1, 1H), 7.72 (dd, *J* 8.5, 1.2, 2H), 7.52 (ddd, *J* 8.6, 3.0, 1.8, 1H), 7.42–7.39 (m, 3H), 7.11 (dd, *J* 5.1, 3.8, 1H), 6.29 (br s, NH_2, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 155.1, 142.1, 133.5, 131.4, 129.2, 129.0, 128.1, 127.6, 126.4, 92.4; *m*/*z* (TOF EI^+) 361 (M+H, 100%), 255 (10), 214 (20).

3.3 Typical reaction procedure for preparation of 5-benzamidoisoxazoles 140



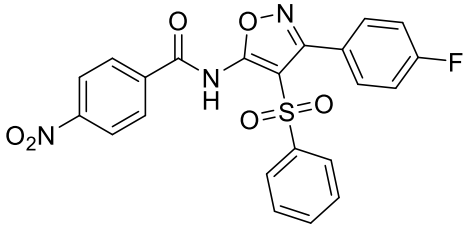
4-Nitrobenzoyl chloride (1 mmol, 1.0 equiv) and 5-aminoisoxazole (1 mmol, 1 equiv) were mixed in DCM (25 mL) in a round-bottomed flask and the reaction mixture was stirred at room temperature until all reactants dissolved. Triethylamine (3 mmol, 3.0 equiv) was then added dropwise and the reaction stirred room temperature for 1-3 h. The reaction mixture was washed with water (3 15 mL), dried (Na_2SO4) and the solvent evaporated. The resulting solid was recrystallized from hexane/benzene to give the pure products with data given below.

3.3.1 N-(3-Phenyl-4-(phenylsulfonyl)isoxazol-5-yl)-4-nitrobenzamide 140a



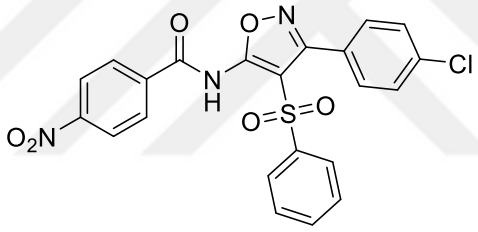
Obtained as light yellow powder (270 mg, 60%); m.p. 169–170 °C; ν_{max}. (KBr) 1647 (C=O), 1530 (C=N) and 1344–1145 (SO_2) cm^{-1}; HRMS: requires for $C_{22}H_{14}N_3O_6S$ $[M–H]^-$ 448.0603, found M^- , 448.0590; ^1H NMR (500 MHz, DMSO-d_6) δ 12.95 (br s, NH, 1H), 8.28 (d, J 9.0, 2H), 8.2–8.22 (m, 2H), 8.20 (d, J 7.4, 2H), 7.62–7.54 (m, 3H), 7.53–7.51 (m, 2H), 7.50–7.45 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.7, 166.4, 160.1, 156.6, 148.1, 145.5, 143.4, 139.2, 132.1, 129.6, 129.1, 128.5, 127.5, 126.5, 122.7, 106.7; *m/z* (TOF ES^-) 448 (M–H, 100%).

3.3.2 N-(3-(4-Fluorophenyl)-4-(phenylsulfonyl)isoxazol-5-yl)-4-nitrobenzamide 140b



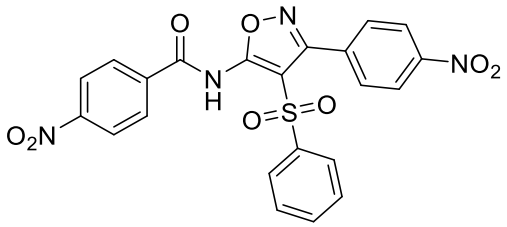
Obtained as yellow solid (262 mg, 56%); m.p. 191–192 C°; ν_{max}. (KBr) 1647 (C=O), 1518 (C=N) and 1342–1147 (SO_2) cm^{-1}; HRMS: requires for $C_{22}H_{13}N_3O_6FS$ $[M–H]^-$ 466.0509, found M^- , 466.0528; ^1H NMR (500 MHz, DMSO-d_6) δ 12.78 (br s, NH, 1H), 8.34 (d, J 8.8, 2H), 8.2–8.19 (m, 3H), 7.60 (d, J 7.4, 1H), 7.58–7.50 (m, 4H), 7.32 (t, J 8.9, 1H), 6.97 (d, J 7.6, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.8, 161.9, 160.87 (d, $^1J_{C-F}$ 281.6, C–F), 156.8, 148.9, 139.0, 133.0, 131.6 (d, $^3J_{C-F}$ 8.8, CH *meta* to F), 131.0, 129.8, 128.8, 126.8, 123.2, 115.1 (d, $^2J_{C-F}$ 21.9, CH *ortho* to F), 114.8, 106.9; *m/z* (TOF ES^-) 466 (M^-H, 100%).

3.3.3 N-(3-(4-Chlorophenyl)-4-(phenylsulfonyl)isoxazol-5-yl)-4-nitrobenzamide 140c



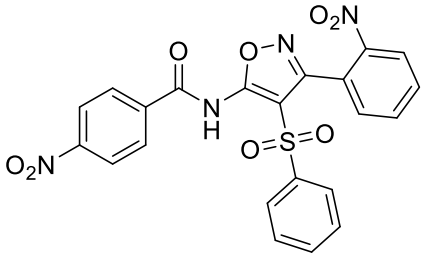
Obtained as yellow solid (260 mg, 54%); m.p. 149–151 °C; ν_{max}. (KBr) 1649 (C=O), 1535 (C=N) and 1344–1149 (SO_2) cm^{-1}; HRMS: requires for $C_{22}H_{13}N_3O_6SCl$ $[M–H]^-$ 482.0214, found M^- , 482.0214; ^1H NMR (500 MHz, DMSO-d_6) δ 10.76 (br s, NH, 1H), 8.44 (d, J 8.7, 2H), 8.23 (d, J 8.7, 2H), 7.60 (t, J 7.4, 1H), 7.47–7.40 (m, 3H), 7.37 (d, J 7.9, 2H), 7.34 (d, J 7.9, 2H), 7.27 (t, J 3.3, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.5, 158.1, 148.2, 145.4, 143.1, 139.1, 133.1, 132.3, 131.3, 130.6, 129.7, 128.8, 128.4, 126.8, 122.8, 106.8; *m/z* (TOF ES^-) 482 (M–H, 100%).

3.3.4 N-(3-(4-Nitrophenyl)-4-(phenylsulfonyl)isoxazol-5-yl)-4-nitrobenzamide 140d



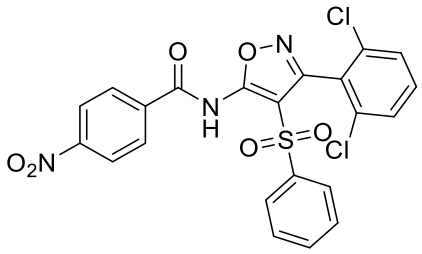
Obtained as yellow solid (272 mg, 55%); m.p. 215–216 °C; ν_{max}. (KBr) 1647 (C=O), 1525 (C=N) and 1340-1145 (SO_2) cm^{-1}; HRMS: requires for $C_{22}H_{13}N_4O_8S$ $[M–H]^-$ 493.0454, found M^- , 493.0447; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.02 (br s, NH, 1H), 8.35 (d, J 8.8, 2H), 8.28 (d, J 8.8, 2H), 8.24–8.18 (m, 4H), 7.84 (d, J 8.8, 2H), 7.67–7.52 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172,3, 166.8, 158.8, 148.3, 143.7, 139.9, 136.6, 133.0, 132.5, 130.8, 131.3, 129.8, 128.7, 126.8, 122.9, 106.9; *m/z* (TOF ES^-) 493 (M–H, 100%).

3.3.5 N-(3-(2-Nitrophenyl)-4-(phenylsulfonyl)isoxazol-5-yl)-4-nitrobenzamide 140e



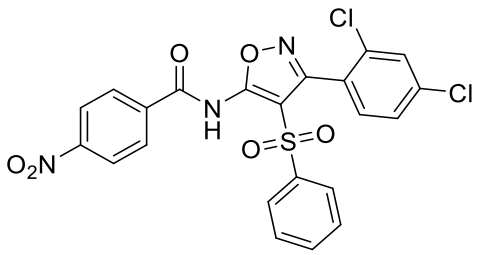
Obtained as yellow solid (292 mg, 59%); m.p. 154–155 °C; ν_{max}. (KBr) 1649 (C=O), 1535 (C=N) and 1334–1157 (SO_2) cm^{-1}; HRMS: requires for $C_{22}H_{13}N_4O_8S$ $[M–H]^-$ 493.0454, found M^- , 493.0430; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (broad s, NH, 1H), 8.40 (d, J 8.8, 2H), 8.36 (ddd, J 8.3, 2.1, 0.9, 1H), 8.19-8.17 (m, 3H), 7.86 (d, J 7.8, 1H), 7.73 (t, J 8.0, 1H), 7.63 (d, J 7.5, 2H), 7.56 (t, J 7.5, 1H), 7.41 (t, J 7.8, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.5, 159.4, 149.9, 147.4, 139.9, 138.4, 135.4, 134.3, 130.4, 129.9, 129.3, 127.8, 127.1, 125.5, 123.9,124.4, 123.8, 106.9; *m/z* (TOF ES^-) 493 (M–H, 100%).

3.3.6 N-(3-(2,6-Dichlorophenyl)-4-(phenylsulfonyl)isoxazol-5-yl)-4-nitrobenzamide 140f



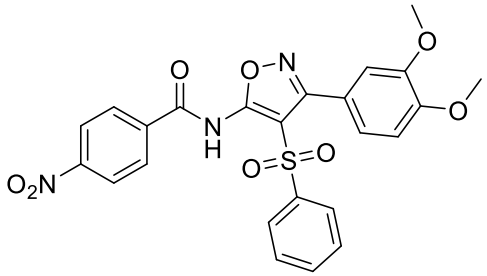
Obtained as yellow solid (218 mg, 42%); m.p. 197–198 °C; ν_{max}. (KBr) 1647 (C=O), 1529 (C=N) and 1344-1145 (SO_2) cm^{-1}; HRMS: requires for $C_{22}H_{12}N_3O_6SCl_2$ $[M–H]^-$ 515.9824, found M^- , 515.9807; ^1H NMR (500 MHz, DMSO-d_6) δ 10.79 (broad s, NH, 1H), 8.46 (d, J 8.7, 2H), 8.25 (d, J 8.8, 2H), 7.64 (t, J 7.4, 2H), 7.49 (d, J 7.6, 1H), 7.41 (t, J 7.5, 3H), 7.34 (d, J 7.7, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.7, 156.9, 156.0, 148.4, 145.6, 142.9, 139.2, 134.7, 132.5, 131.6, 129.8, 128.4, 127.9, 127.1, 122.9, 106.9; *m/z* (TOF ES^-) 515 (M–H, 100%).

3.3.7 N-(3-(2,4-Dichlorophenyl)-4-(phenylsulfonyl)isoxazol-5-yl)-4-nitrobenzamide 140g



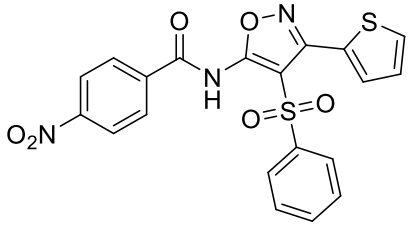
Obtained as dark yellow solid (270 mg, 52%); m.p. 90 °C (decomp.); ν_{max}. (KBr) 1641 (C=O), 1535 (C=N) and 1344–1147 (SO_2) cm^{-1}; HRMS: requires for $C_{22}H_{12}N_3O_6SCl_2$ $[M–H]^-$ 515.9824, found M^- , 515.9808; ^1H NMR (500 MHz, DMSO-d_6) δ 13.11 (broad s, NH, 1H), 8.32-8.23 (m, 4H), 7.81-7.73 (m, 1H), 7.65-7.52 (m, 5H), 7.48 (d, J 7.5, 1H), 6.95 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.8, 157.4, 148.3, 145.6, 143.1, 134.6, 134.4, 132.6, 132.4, 129.8, 128.9, 128.7, 128.6, 128.5, 126.9, 125.9, 122.9, 106.9; *m/z* (TOF ES^-) 515 (M–H, 100%).

3.3.8 N-(3-(3,4-Dimethoxyphenyl)-4-(phenylsulfonyl)isoxazol-5-yl)-4-nitrobenzamide 140h



Obtained as yellow solid (245 mg, 50%); mp 178–180 °C; ν_{max}. (KBr) 1647 (C=O), 1521 (C=N) and 1340-1138 (SO_2) cm^{-1}; HRMS: requires for $C_{24}H_{18}N_3O_8S$ $[M–H]^-$ 508.0815, found M^-, 508.0814; ^1H NMR (500 MHz, DMSO-d_6) δ 11.96 (broad s, NH, 1H), 8.44 (d, J 8.9, 2H), 8.23 (d, J 8.9, 2H), 7.70 (d, J 8.4, 2H), 7.62 (t, J 6.4, 1H), 7.49 (t, J 7.9 Hz, 2H), 7.09–6.95 (m, 3H), 3.82 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.7, 160.8, 150.5, 149.8, 148.2, 140.6, 139.1, 133.9, 129.8, 129.1, 126.9, 123.8, 121.9, 118.7, 114.0, 112.2, 111.4, 106.9, 55.6, 55.4; *m/z* (TOF ES^-) 508 (M–H, 100%).

3.3.9 N-(4-(Phenylsulfonyl)-3-(thiophen-2-yl)isoxazol-5-yl)-4-nitrobenzamide 140i



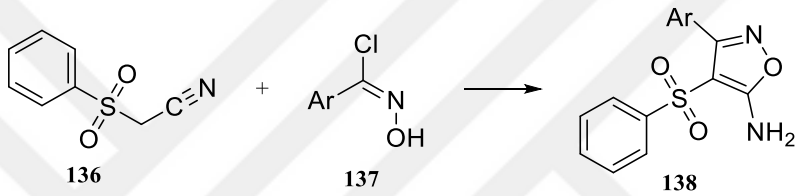
Obtained as dark yellow solid (205 mg, 45%); m.p. 164–165 °C; ν_{max}. (KBr) 1649 (C=O), 1558 (NH) and 1340–1141 (SO_2) cm^{-1}; HRMS: requires for $C_{20}H_{12}N_3O_6S_2$ $[M–H]^-$ 454.0168, found M^- , 454.0155; ^1H NMR (500 MHz, $CDCl_3$) δ 10.93 (br s, 1H), 8.43 (d, J 8.9, 2H), 8.22 (d, J 8.9, 2H), 7.83 (dd, J 3.7, 1.1, 1H), 7.71 (dd, J 8.5, 1.1, 2H), 7.58 (t, J 7.5, 1H), 7.48 (dd, J 5.1, 1.1, 1H), 7.43 (t, J 7.9, 2H), 7.14 (dd, J 5.1, 3.7, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8, 160.2, 154.4,

150.8, 140.0, 136.8, 134.4, 131.8, 129.9, 129.3, 129.1, 128.2, 127.7, 126.9, 125.4, 99.6; *m/z* (TOF ES^-) 455 (M, 100%).

4. RESULTS AND DISCUSSIONS

Two methods were used in the synthesis of 5-aminoisoxazole derivatives **(138)**. One of the method is using mortar by grinding the reagents without use of any solvent and another way is MW radiation. In order to compare the yields, we also performed some of the reactions in a solvent. When these reactions are compared, yields are increased with using MW (Table 4.1). The benefit of reactions was the simplicity of the isolation of products by recrystallization.

Table 4.1. Comparison of methods for synthesis of 138 according to yields



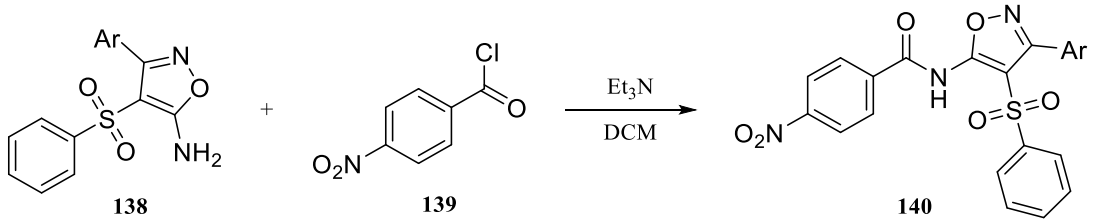
Compound	Ar	Time (min)	Yield[a]	Time (min)	Yield[b]	Time (h)	Yield[c]
138a	Ph	15	60	5	75	1	66
138b	4-FC_6H_4	15	54	5	61		
138c	4-ClC_6H_4	15	45	5	83		
138d	4-$NO_2C_6H_4$	15	62	5	89	1	78
138e	3-$NO_2C_6H_4$	15	34	5	38	1	40
138f	2-$NO_2C_6H_4$	15	52	5	80		
138g	2,6-$DiClC_6H_3$	15	84	5	85		
138h	2,4-$DiClC_6H_3$	15	79	5	64		
138i	3,4-$DiOMeC_6H_3$	15	66	5	79		
138j	2-Thienyl	15	50	5	52		

[a] reaction with mortar
[b] reaction with microwave (25°C, 200W, 300 PSI)
[c] reaction in solvent (DCM)

5-Aminoisoxazoles **(138)** were produced from the reaction of 2-phenylsulfonyl acetonitrile **(136)** with α-chlorooximes **(137)**. Both electron donating and withdrawing substituent on aryl and heteroaryl substitute α-chlorooximes were examined in this study. A strong base was necessary if electron neutral alkyl groups were substituted at the position of the nitriles (Bourbeau and Rider, 2006). Nevertheless, a weak base, Et_3N, was adequate when the electron withdrawing substituted of the nitriles were used under solvent free conditions.

5-Aminoisoxazole derivatives **(138)** were reacted with p-nitrobenzoyl chloride **(139)** to synthesize 5-amido-4-phenylsulfonyl isoxazoles **(140)** in the presence of Et_3N in DCM with moderate yields (Table 4.2).

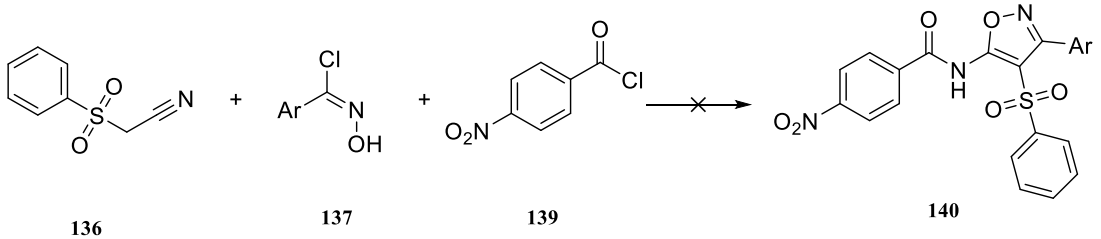
Table 4.2. Synthesis of 5-amidoisoxazole derivatives



138 **139** **140**

Compound	Ar	Yield %	Melting Point °C
140a	Ph	60	169-170
140b	4-FC_6H_4	56	191-192
140c	4-ClC_6H_4	54	149-150
140d	4-$NO_2C_6H_4$	55	215-216
140e	2-$NO_2C_6H_4$	59	154-155
140f	2,6-$DiClC_6H_3$	42	197-198
140g	2,4-$DiClC_6H_3$	52	90 decomp.
140h	3,4-$DiOMeC_6H_3$	50	178-180
140i	2-Thienyl	45	164-165

An attempt to a one-pot reaction of α-chlorooximes **(137)**, 2-phenylsulfonyl acetonitrile **(136)** and benzoyl chloride **(139)** to form 5-amido-4-phenylsulfonyl isoxazoles **(126)** was not effective because inseparable mixtures were obtained (monitored by TLC) (Scheme 4.1). This might be described by the attack of in situ formed nitrile oxide to carbonyl center of benzoyl chloride (Bhat et al., 2009).



136 **137** **139** **140**

Scheme 4.1. An attempt to obtain 5-amido-4-phenylsulfonyl isoxazoles

The structures of the compounds were examined by utilizing IR, ^1H and ^{13}C NMR and HRMS. Distinctive SO_2 stretching frequencies appeared around 1350 cm^{-1} and 1145 cm^{-1} for both 5-amino and 5-amido isoxazoles in the IR spectra. The

vibrational frequency of the C=O group of the 5-amido isoxazoles was detected around 1645 cm $^{-1}$ (Figure 4.1).

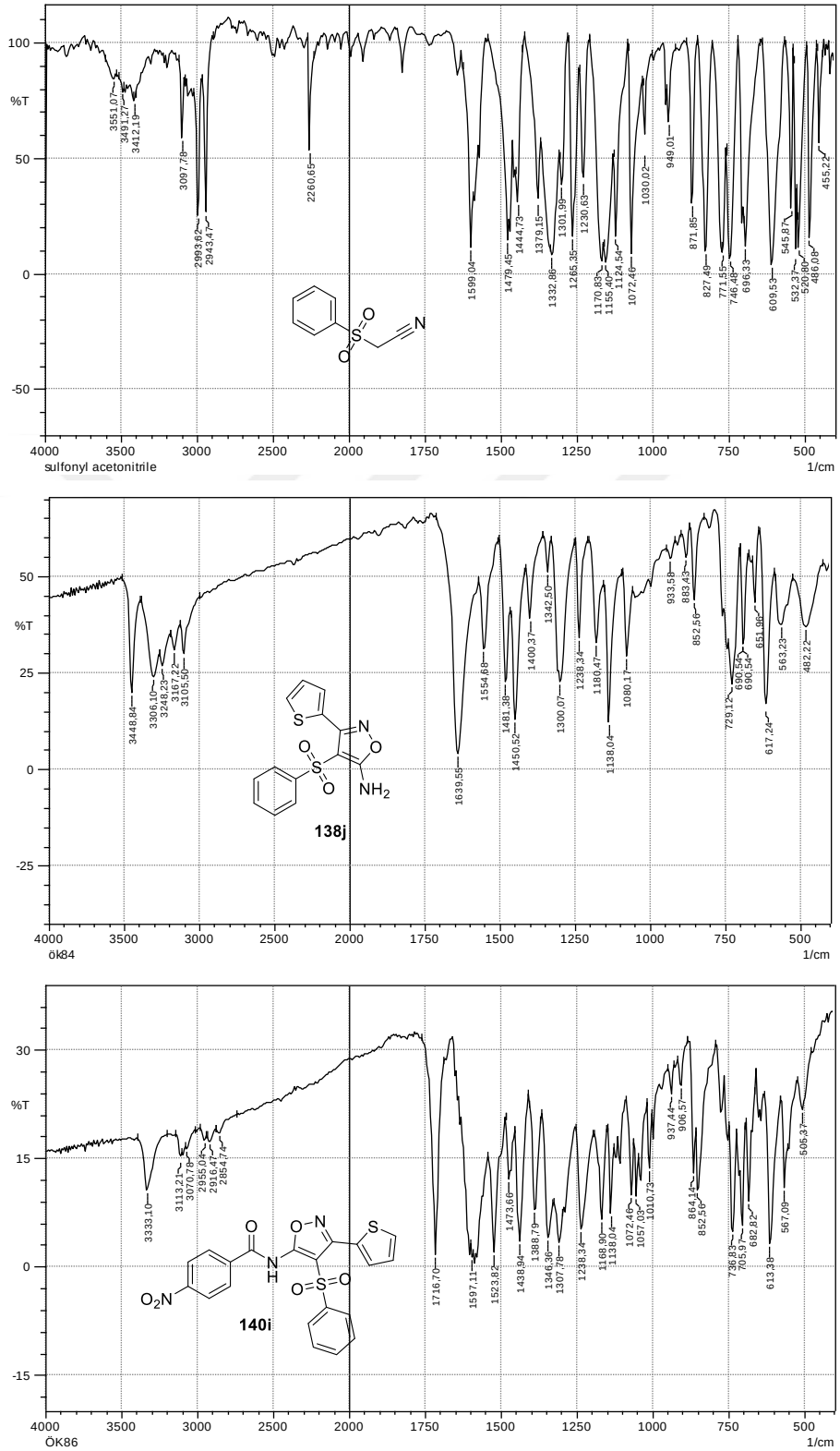
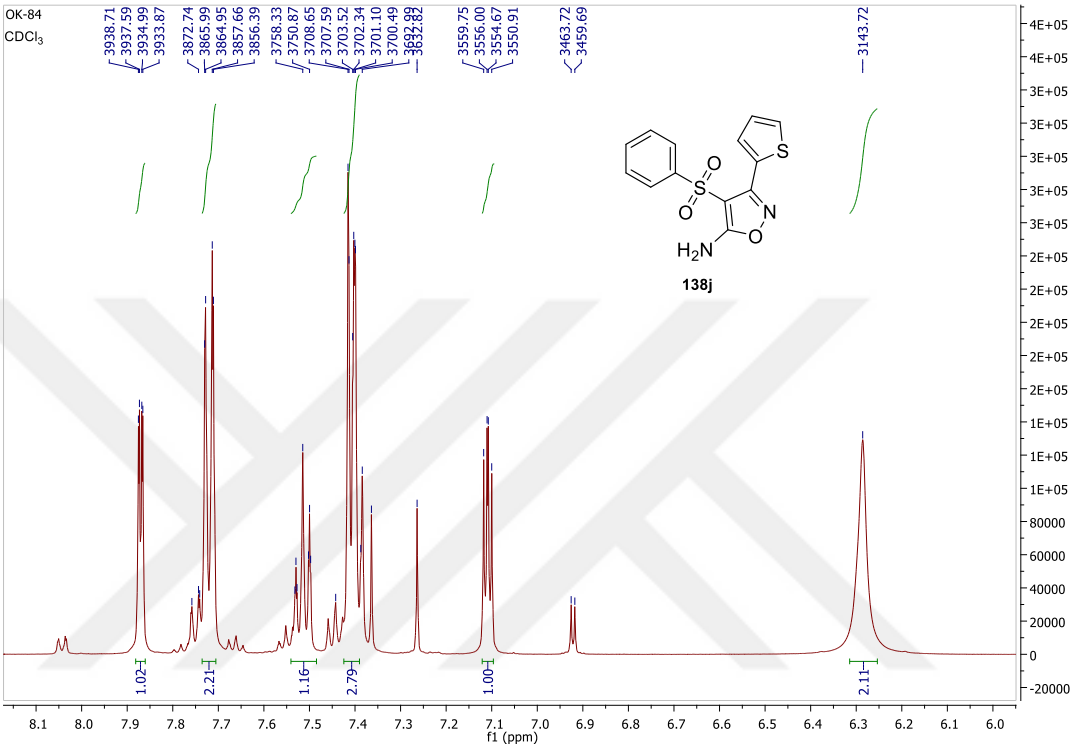


Figure 4.1. IR spectrums of 136, 138 and 140

The ^{1}H NMR spectra of the 5-aminoisoxazoles **(138)** presented a broad singlet for the NH_2 protons between 8.23-8.40 ppm in DMSO-d_6 due to hydrogen bonding. When $CDCl_3$ was used as a solvent, for **138i** and **138j** the NH_2 protons resonated at 6.25 and 6.29 ppm, individually. NH protons gave resonances between 10.79-13.11 ppm for the 5-amido isoxazoles **(140)** in DMSO-d_6 (Figure 4.2).



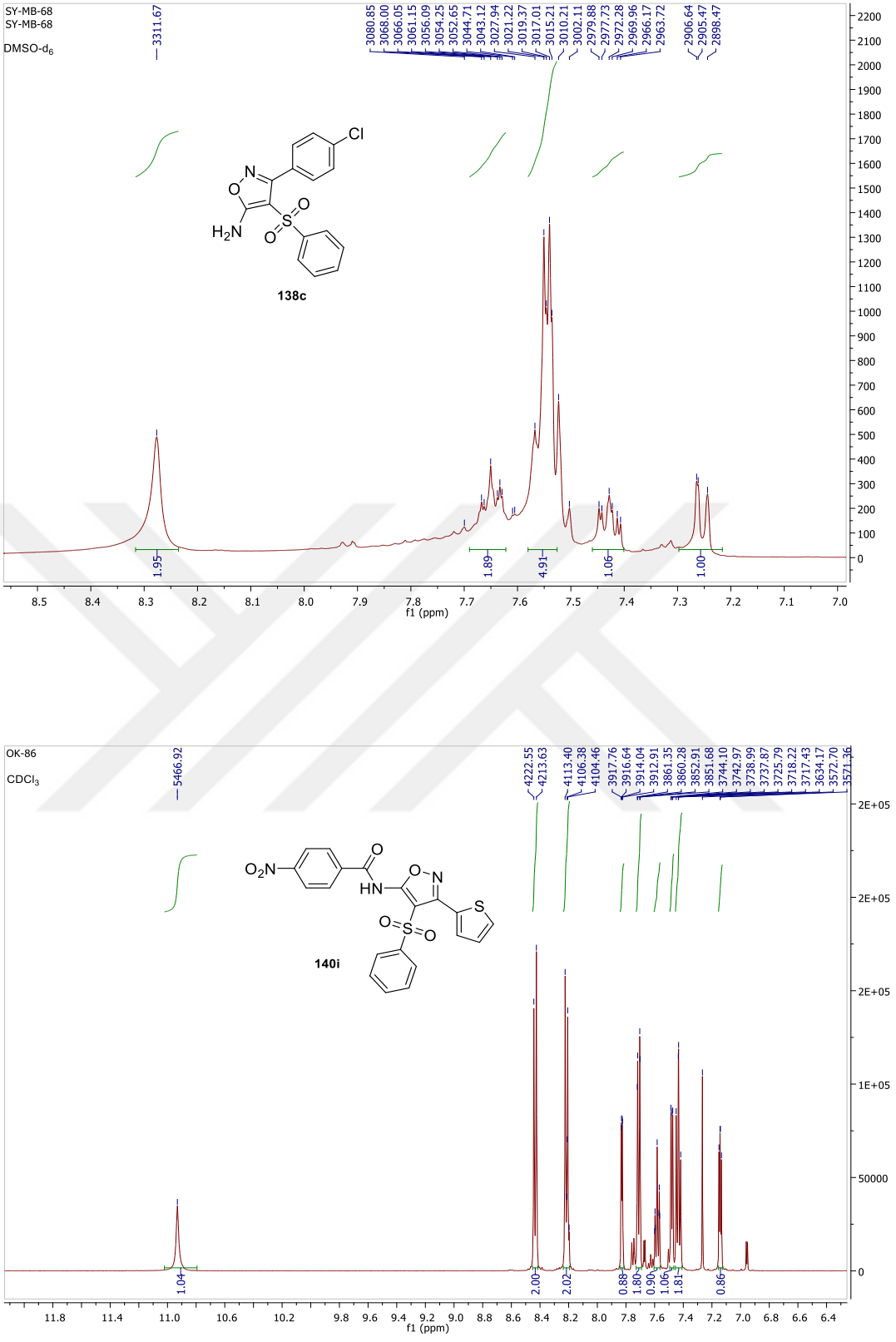


Figure 4.2. ^{1}H NMR spectrums of 138 in $CDCl_3$ and DMSO-d_6 and 140

The ^{13}C NMR spectra of 5-aminoisoxazoles **(138)** displayed the C-5 carbons at 169-170 ppm, iminic carbon resonances at 158-164 ppm, and the C-4 carbons

resonance at 91-93 ppm. 5-Amido isoxazoles **(140)** showed carbonyl carbons at 164-171 ppm, C-5 carbons at 158-161 ppm, iminic carbons at around 148-151 ppm, and C-4 carbons at 106 ppm (Figure 4.3).

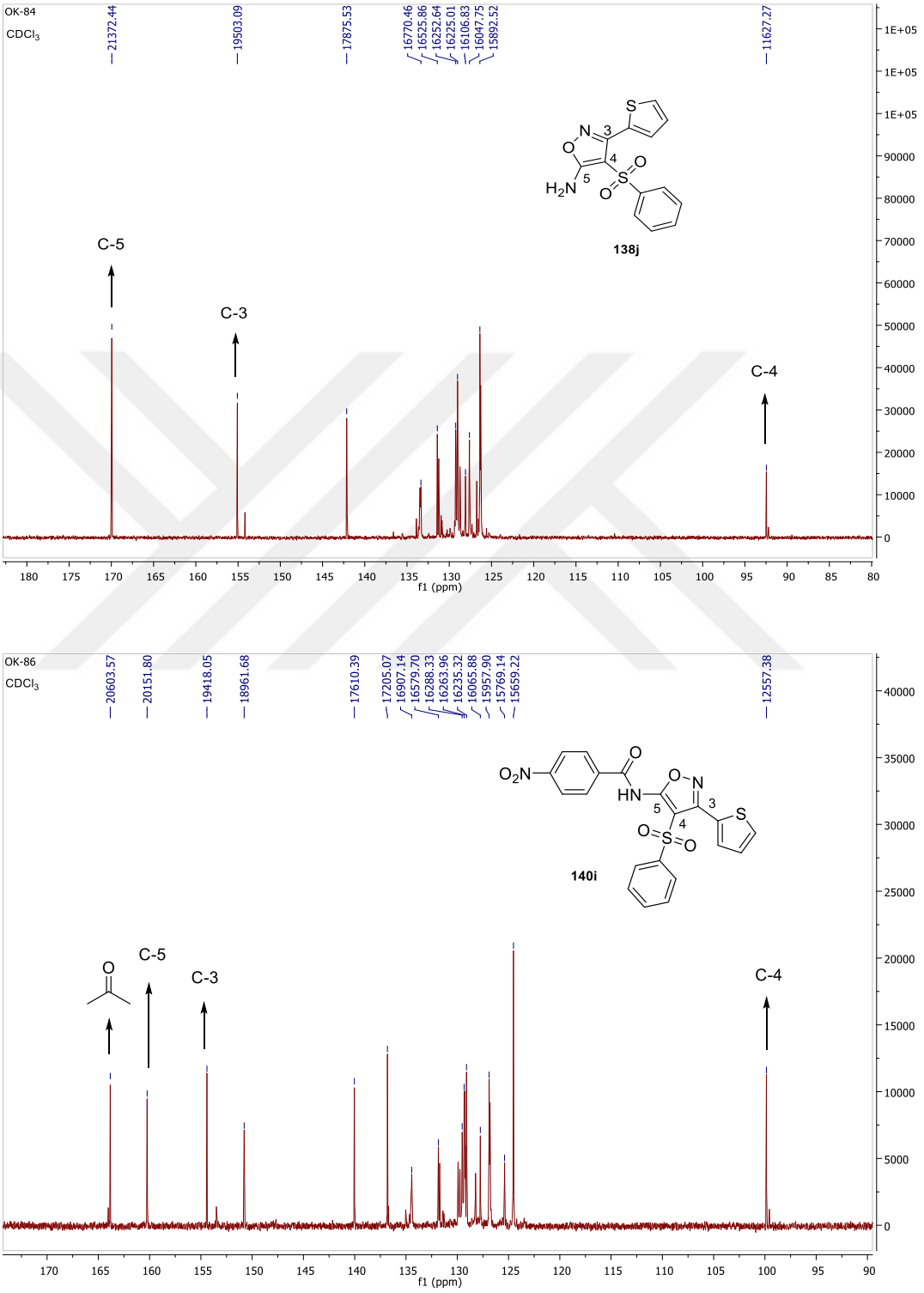
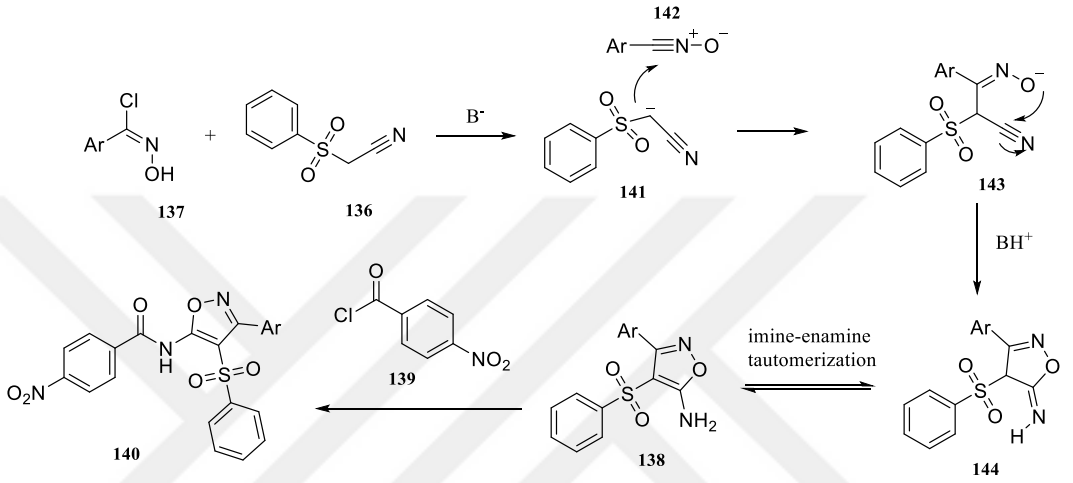


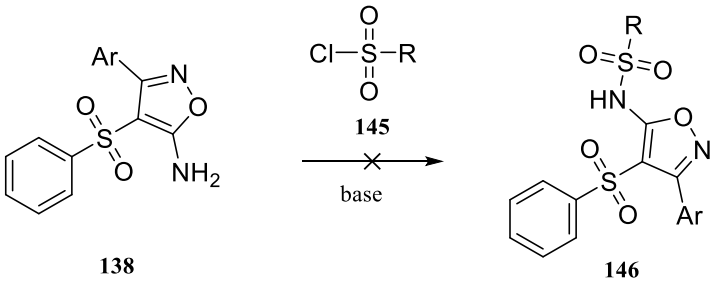
Figure 4.3. ^{13}C NMR spectrums of 138 and 140

A proposed reaction mechanism for the formation of the 5-aminoisoxazoles **(138)** is shown below. The active methylene proton is firstly deprotonated by the base. Subsequently, intermolecular nucleophilic attack of resulting nucleophile to in situ formed nitrile oxide **(142)** produces compound **(143)**. Furthermore, intramolecular attack of oxygen to nitrile results in cyclization compound **(144)**. Finally, tautomerization of this compound gives 5-aminoisoxazole **(138) (**Scheme 4.2**)**.



Scheme 4.2. Proposed reaction mechanism

Although there are examples of reactions between sulfonyl chlorides **(145)** and 5-aminoisoxazoles , our attempts to synthesize 5-sulfonamido isoxazoles **(146)** from 3-aryl-4-benzenesulfonyl-5-aminoisoxazoles **(138)** in the presence of various bases have been unsuccessful (Wu et al., 1997). 5-Aminoisoxazoles **(138)** were reacted with benzenesulfonyl chloride derivatives **(145)** or aliphatic sulfonyl chlorides **(145)**, i.e., methanesulfonyl chloride in the presence of Et_3N, pyridine, NaOH, NaH, n-BuLi or K_2CO_3 to provide sulfonamide substituted isoxazoles **(146)**, but no product was found (Scheme 4.3). The difference in reactivity might be ascribed to the presence of the sterically hindered 4-benzenesulfonyl substituent or lower nucleophlicity of the amine by the effect of electron with drawing property of phenylsulfonyl substituent from the fourth position on the isoxazole.



Base: Et_3N, pyridine, NaOH, NaH, n-BuLi or K_2CO_3
Solvent: DCM or THF

Scheme 4.3. An attempt to synthesis of 5-sulfonamido isoxazole derivatives

Anticancer (Cytotoxic) Studies

The synthesized compounds have been assayed against four cancer cell lines. Since compounds having low GI values are more toxic on cells and they cause cell death at lower doses. However, such compounds have higher activities in the treatment of diseases such as cancer (Carmichael et al., 1988). Compounds **138a, 138c, 138e** and **138h** showed cytotoxic activities against C3a, L929, T98g and Mcf-7 cell lines (Table 4.3). The structure activity relationship revealed that free amino containing 5-aminoisoxazoles **(138)** showed higher activities than the 5-amido isoxazoles **(140)**.

Table 4.3. Cytotoxic activities of compounds against cancer cell lines [a]

GI values (μM)[b] in cell lines[c]				
	C3a	**L929**	**T98g**	**MCF-7**
138a	31	22	29	16
138b	32	35	>100	10
138c	52	35	25	17
138d	71	>100	>100	>100
138e	50	80	34	98
138f	74	30	>100	>100
138g	>100	>100	>100	14
138h	30	29	75	14
138i	85	>100	>100	>100
138j	>100	>100	>100	>100
140a	>100	>100	>100	>100
140b	>100	>100	>100	>100
140c	41	>100	>100	>100

140d	64	>100	>100	>100
140e	>100	>100	>100	>100
140f	>100	>100	>100	>100
140g	>100	>100	>100	>100
140h	69	>100	>100	>100
140i	>100	>100	>100	>100

[a] Determined by MTT assay (24 h drug exposure)
[b] Compounds tested in triplicate, data expressed as mean values.
[c] Cancer cell line origin: MCF-7 (human breast adenocarcinoma cell), T98g (human glioblastoma cells), L929 (mouse fibroblast cell), C3a (human hepatocellular carcinoma cell).

10% FBS (fetal bovine serum) and 1% antibiotic-antimycotic appropriate medium containing cells were used as a control measurement. 20,000 units MCF-7 (human breast adenocarcinoma cell), T98G (human glioblastoma cells) and L929 (mouse fi-broblast cell), 30,000 units c3a (human hepatocellular carcinoma cells) cell were plated on 96 well plates. After 24 h of incubation, substances were given in different doses. Cells were then treated with substances and incubated for 24 h, then, MTT was measured (Mortimer et al., 2006).

MTT solution as a final concentration of 1/10 was added to all samples. Formazan (dissolved by DMSO (1/1: v/v)), which was formed after 24 h incubation at 37 °C in the oven, was added to the final product and kept in an oven at 37 °C for overnight. The optical densities of the wells were measured at a wavelength of 570 nm and compared to control measurements and expressed by means of percent.

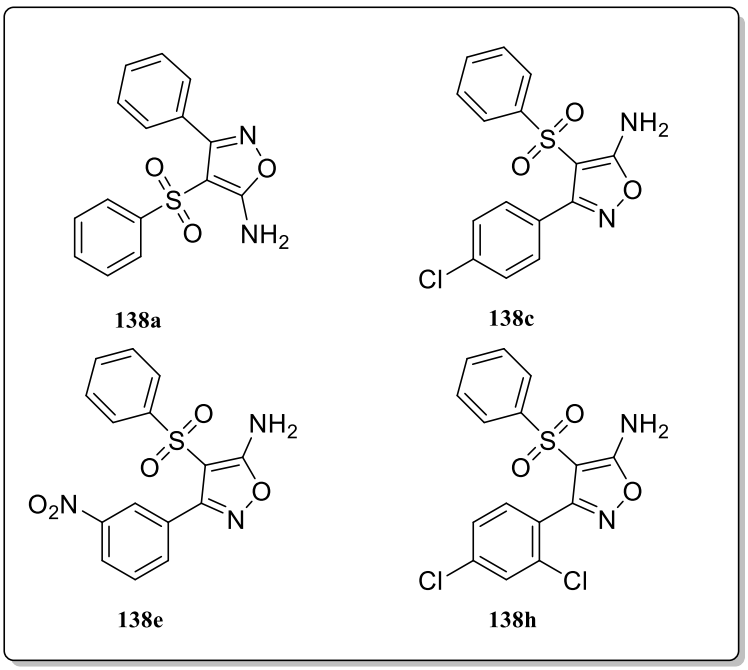


Figure 4.4. 138 derivatives having cytotoxic activities

5. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, we have developed a method for the synthesis of 3-aryl-4-phenylsulfonyl 5-aminoisoxazoles **(138)** with complete regioselectivity in a solvent free medium with moderate yields and via MW radiation with good yields. Transformation of these 5-aminoisoxazoles **(138)** to novel 5-benzamidoisoxazoles **(140)** was also investigated. Structure of compounds was investigated by means of IR, ^{1}H, ^{13}C NMR and HRMS. A reaction mechanism was proposed. Four of nineteen compounds showed high activity against human cancer cell lines. We can say that microwave irradiation technique is more efficient comparing the yields and time with solvent-free method. However, using very little amount of solvent in MW and no solvent with mortar and easy purification methods for both techniques make them good candidate for Green Synthetic Chemistry.

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APPENDICES

7. APPENDICES

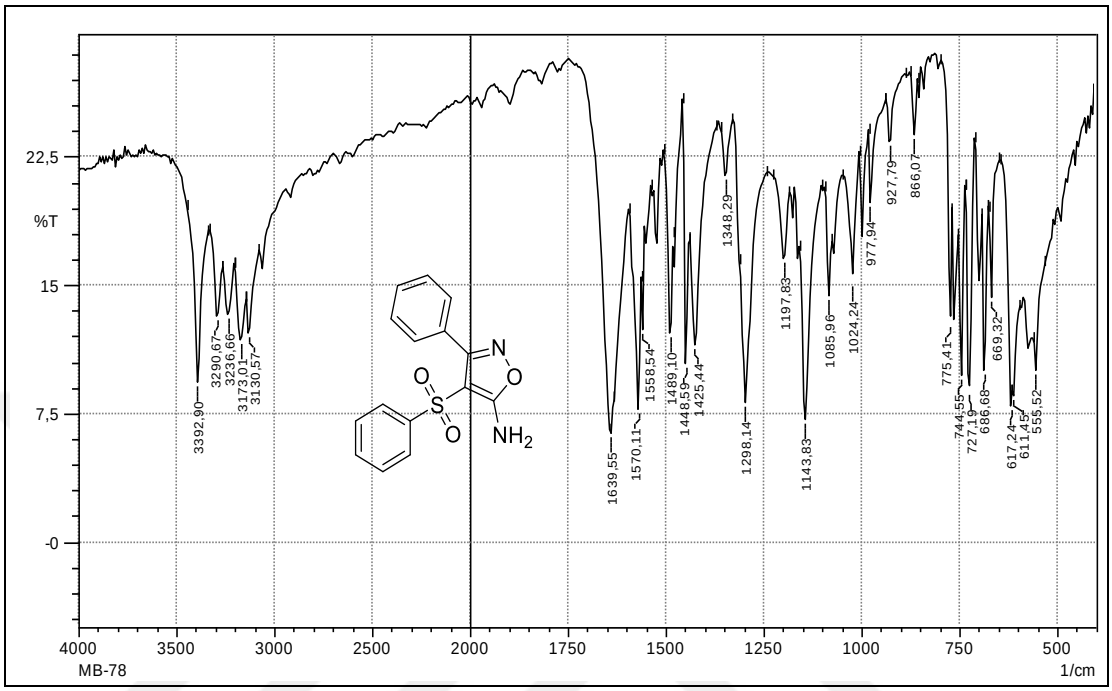


Figure 7.1: IR spectrum of compound 138a

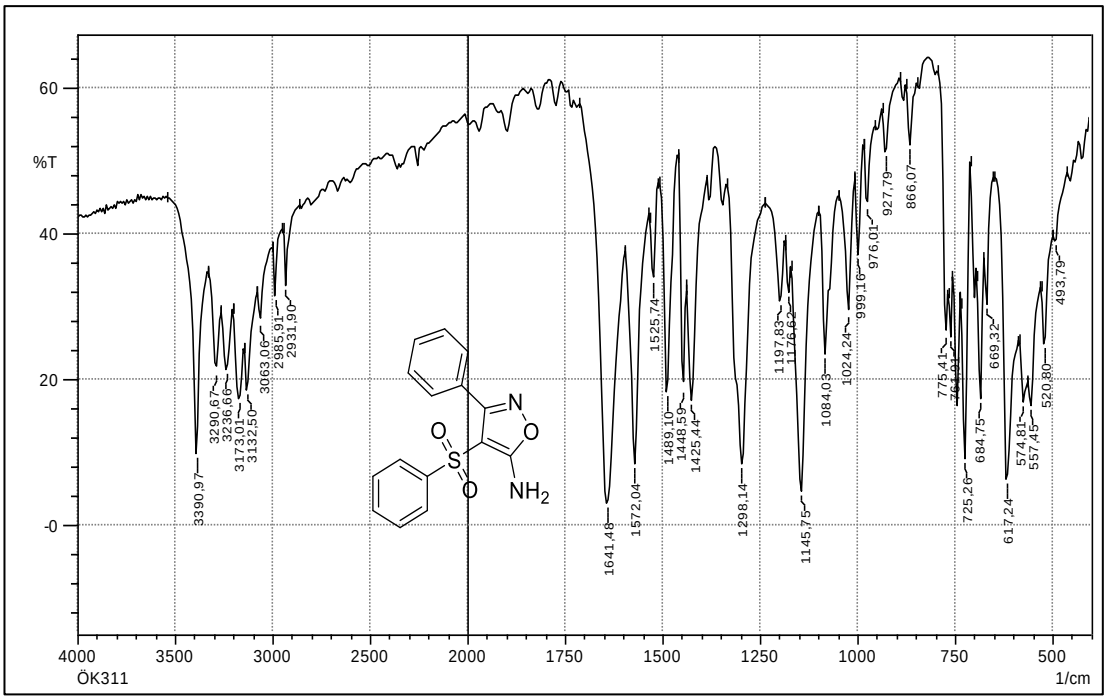


Figure 7.2. IR spectrum of compound 138a’

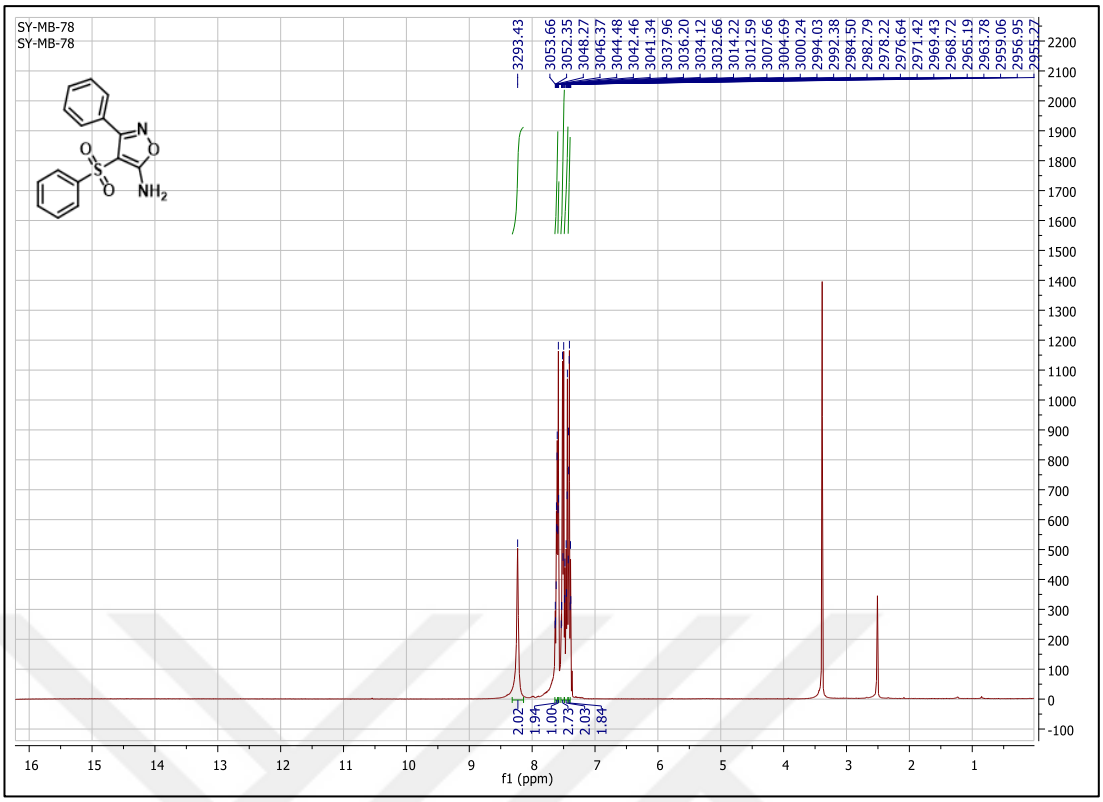


Figure 7.3. ^{1}H NMR spectrum of compound 138a

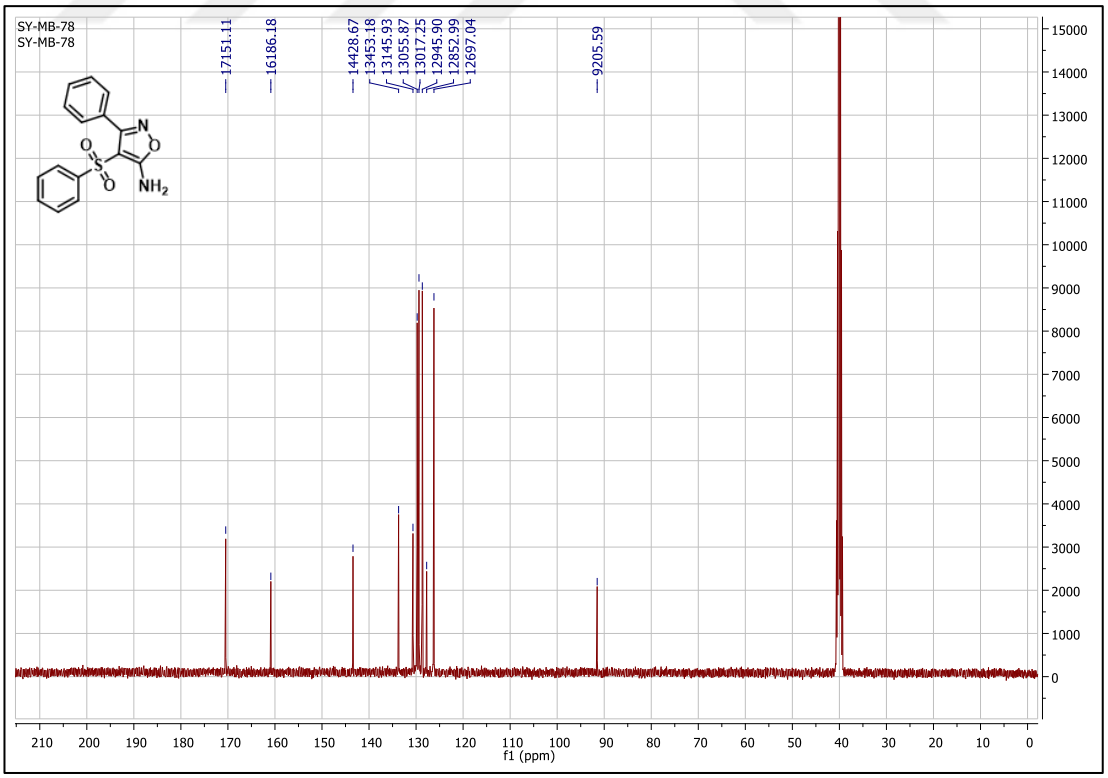


Figure 7.4. ^{13}C NMR spectrum of compound 138a

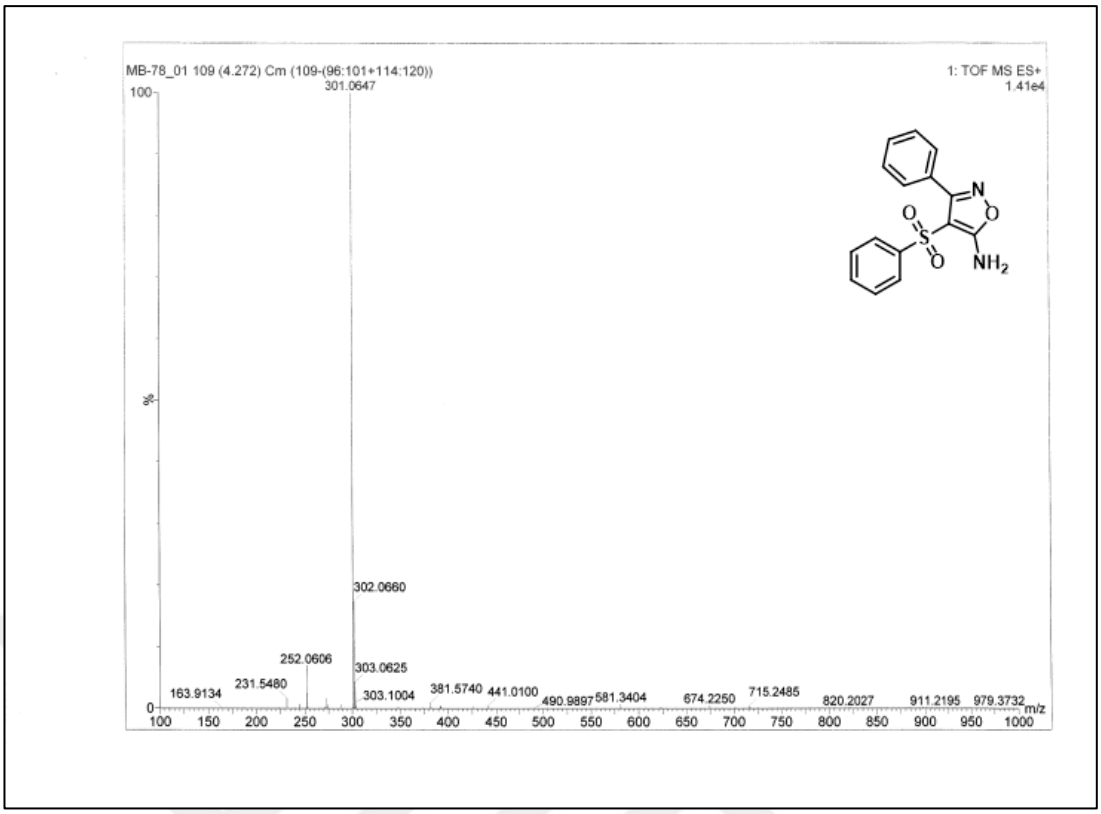


Figure 7.5. HRMS spectrum of compound 138a

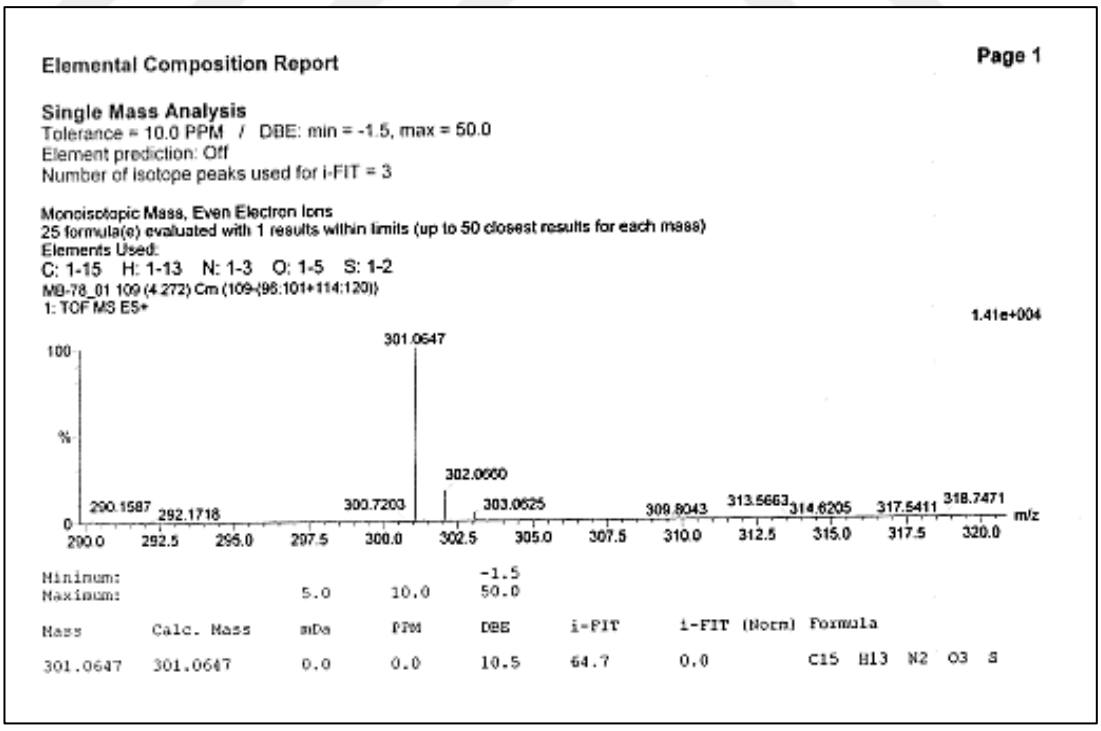


Figure 7.6. Elemental composition of compound 138a

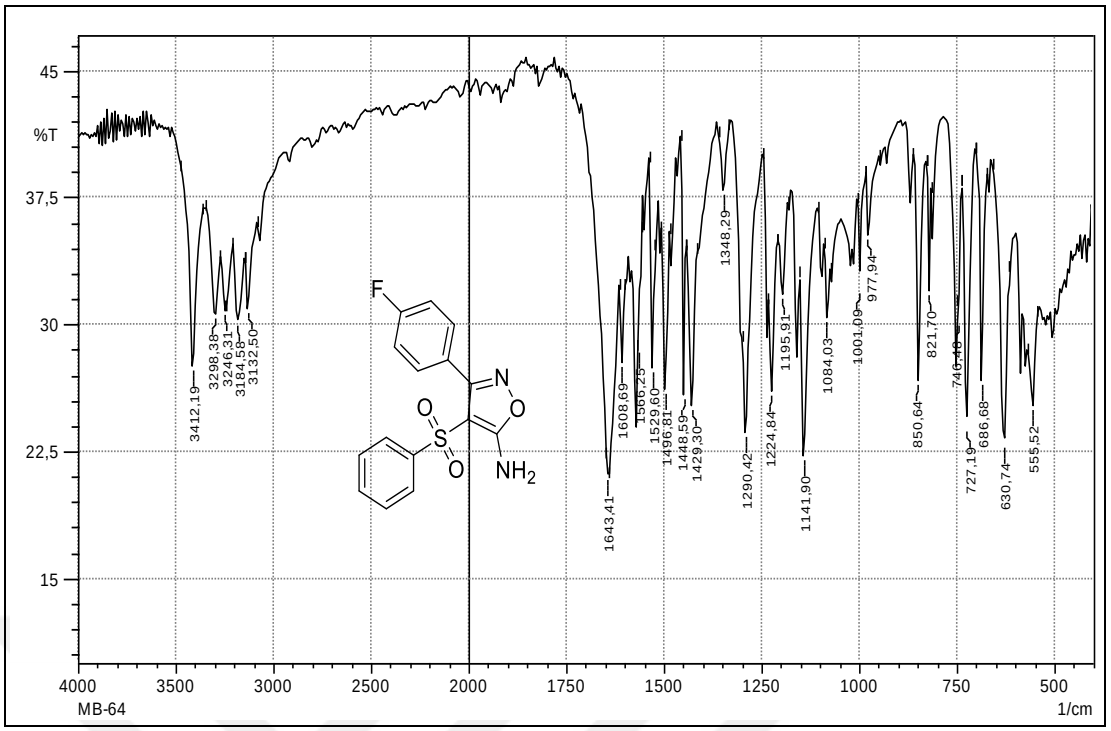


Figure 7.7. IR spectrum of compound 138b

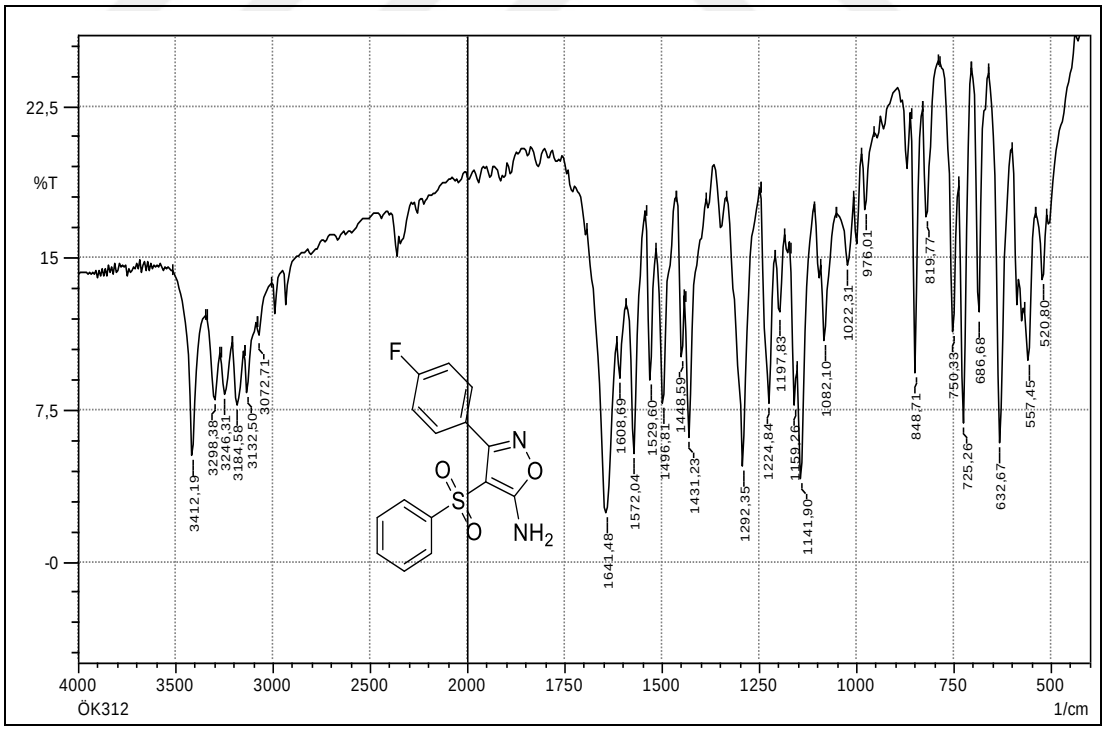


Figure 7.8. IR spectrum of compound 138b’

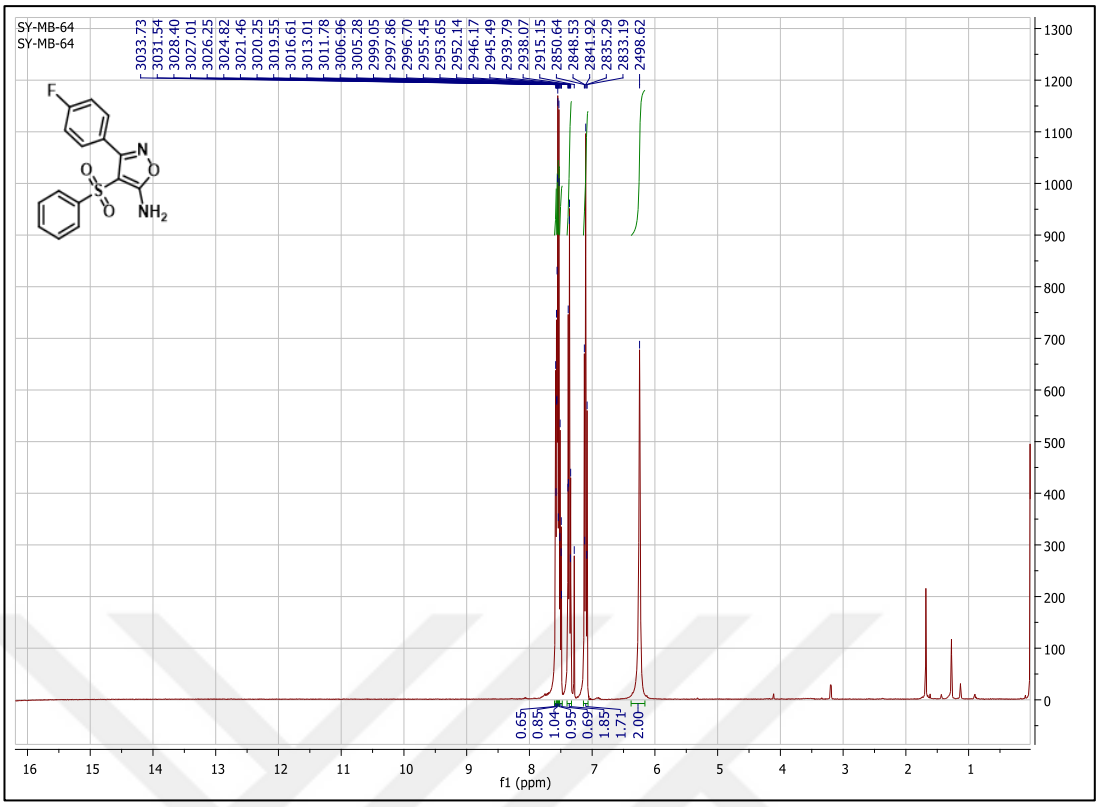


Figure 7.9. ^{1}H NMR spectrum of compound 138b

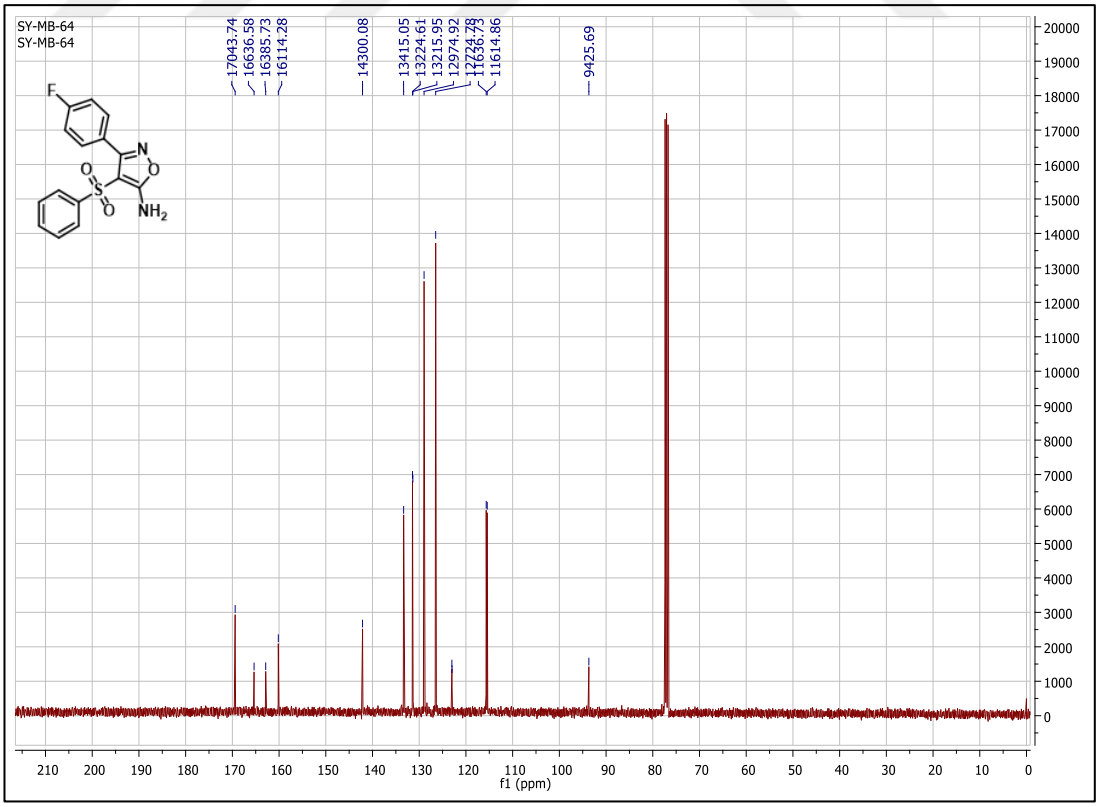


Figure 7.10. ^{13}C NMR spectrum of compound 138b

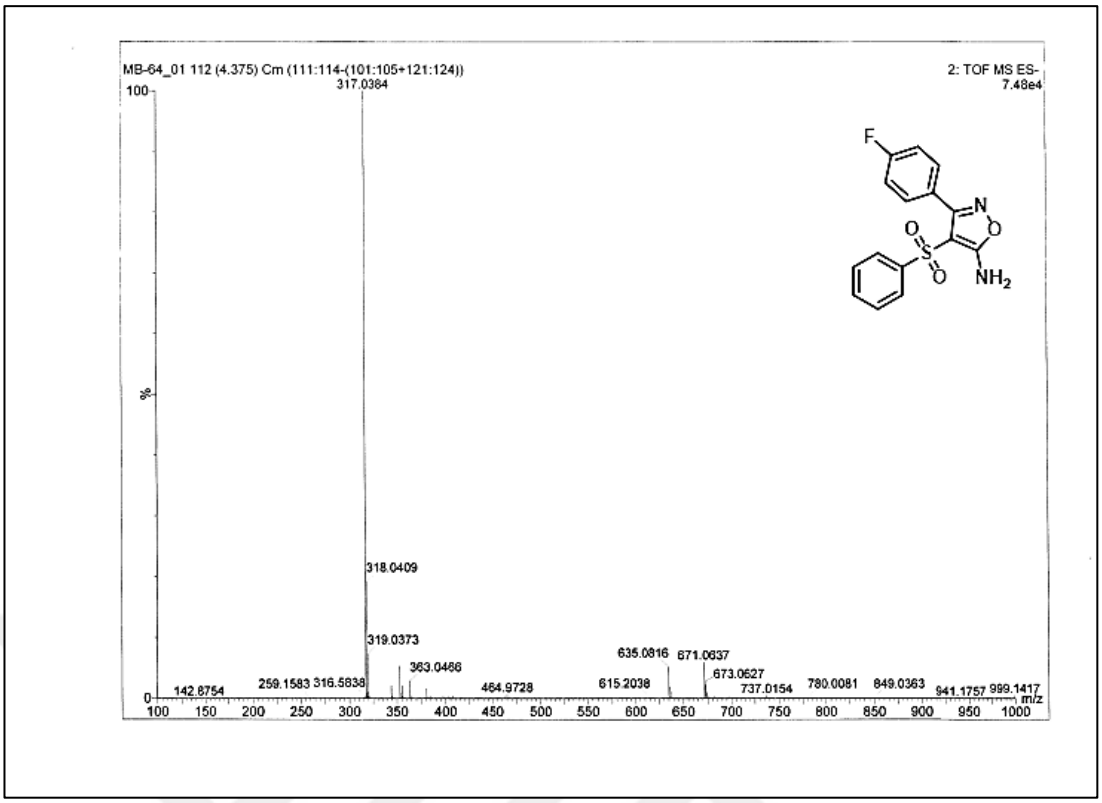


Figure 7.11. HRMS spectrum of compound 138b

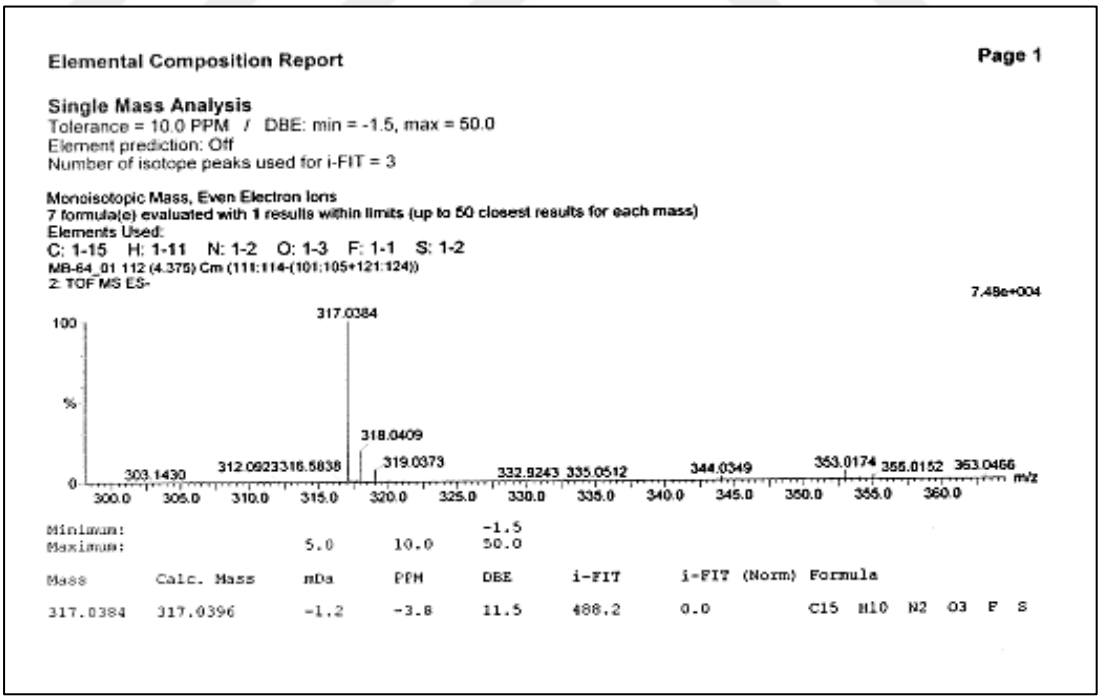


Figure 7.12. Elemental composition of compound 138b

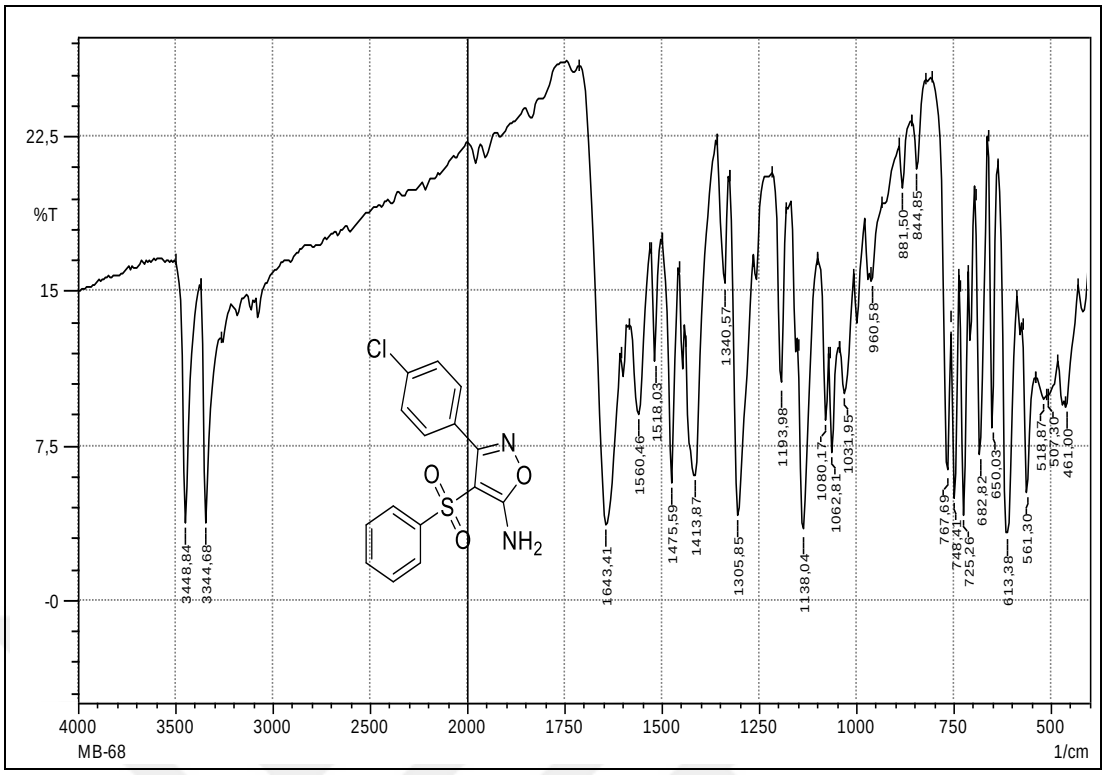


Figure 7.13. IR spectrum of compound 138c

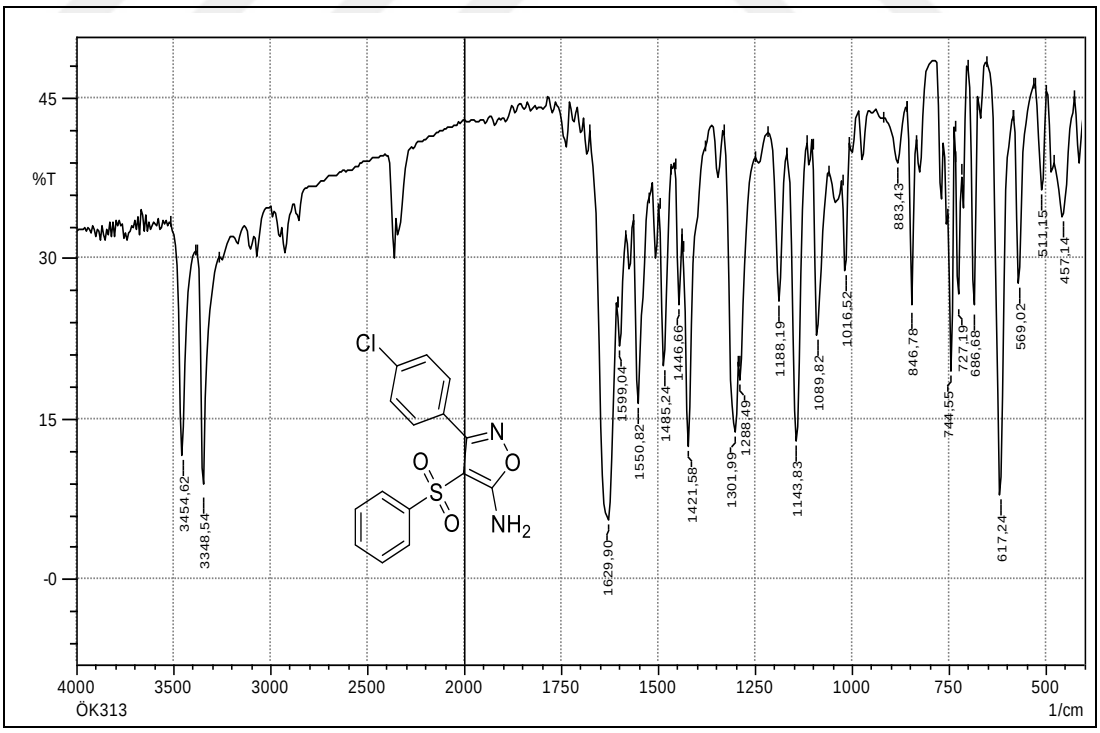


Figure 7.14. IR spectrum of compound 138c’

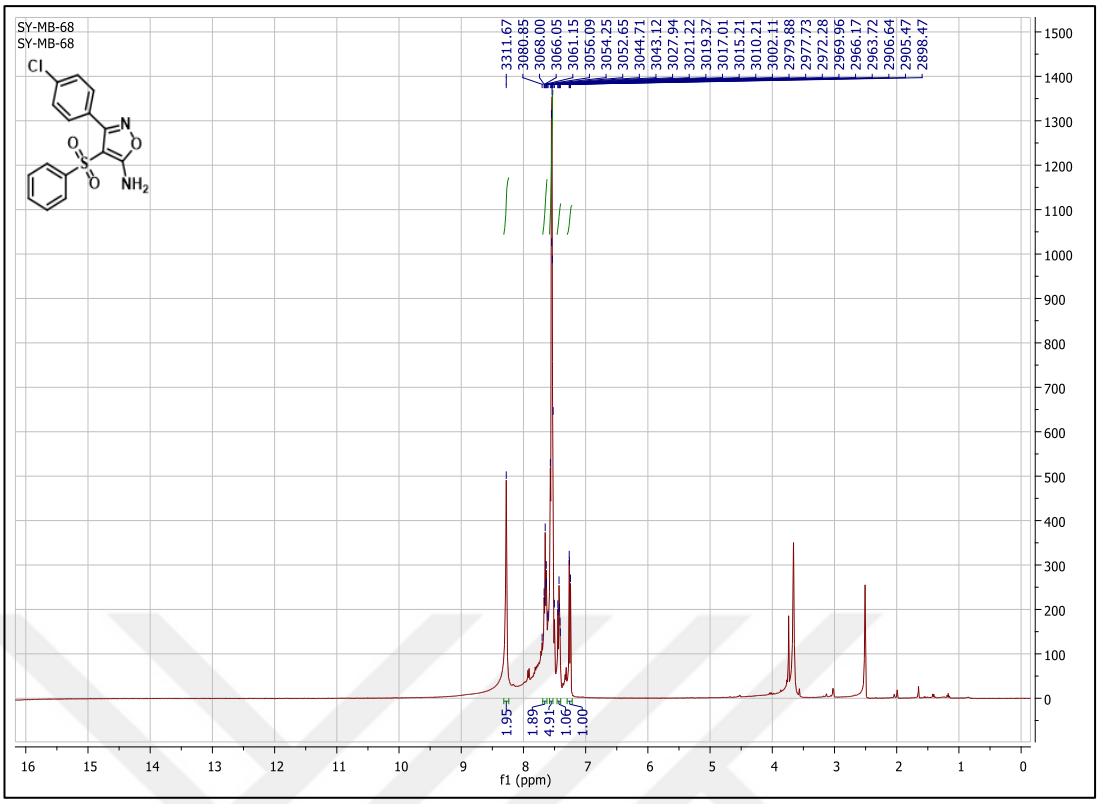


Figure 7.15. ^{1}H NMR spectrum of compound 138c

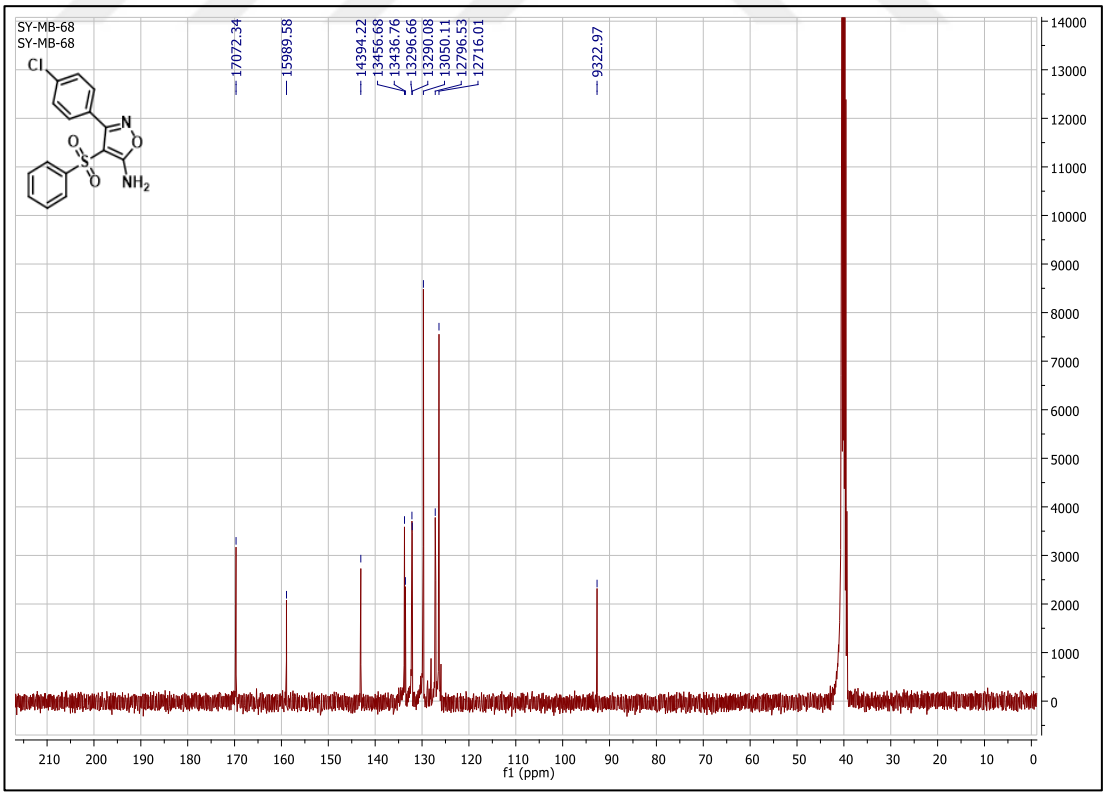


Figure 7.16. ^{13}C NMR spectrum of compound 138c

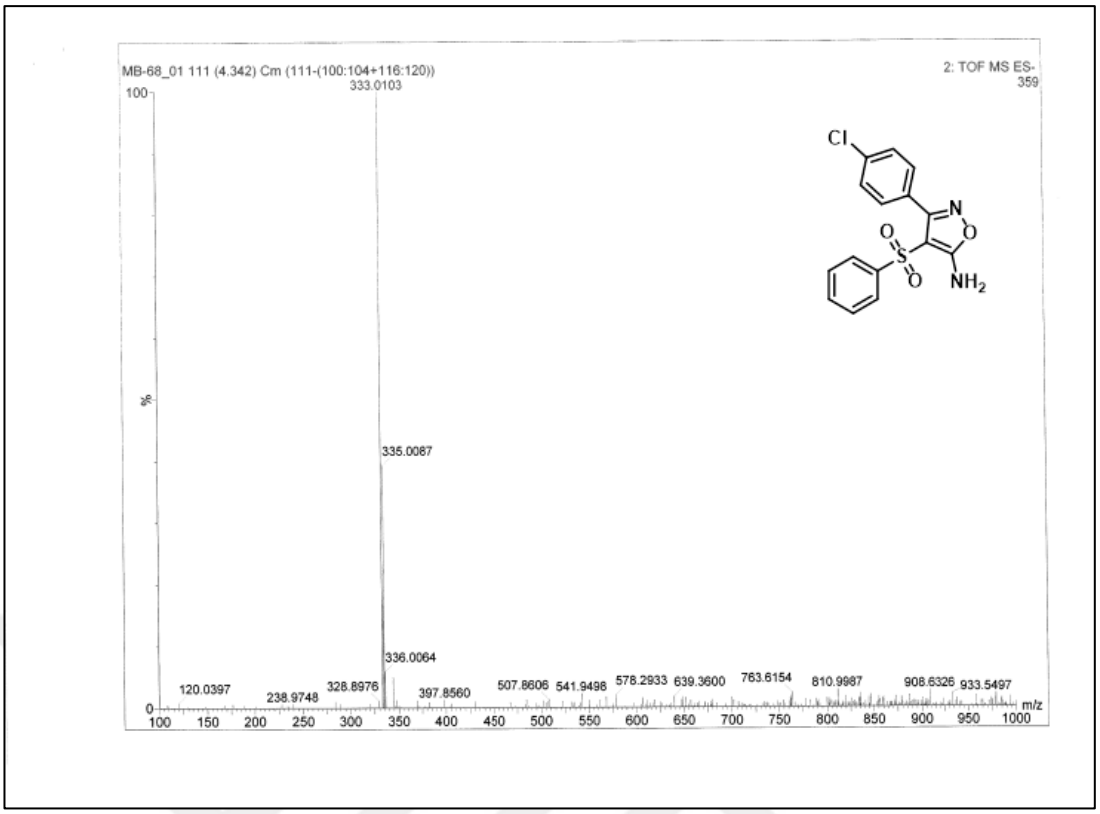
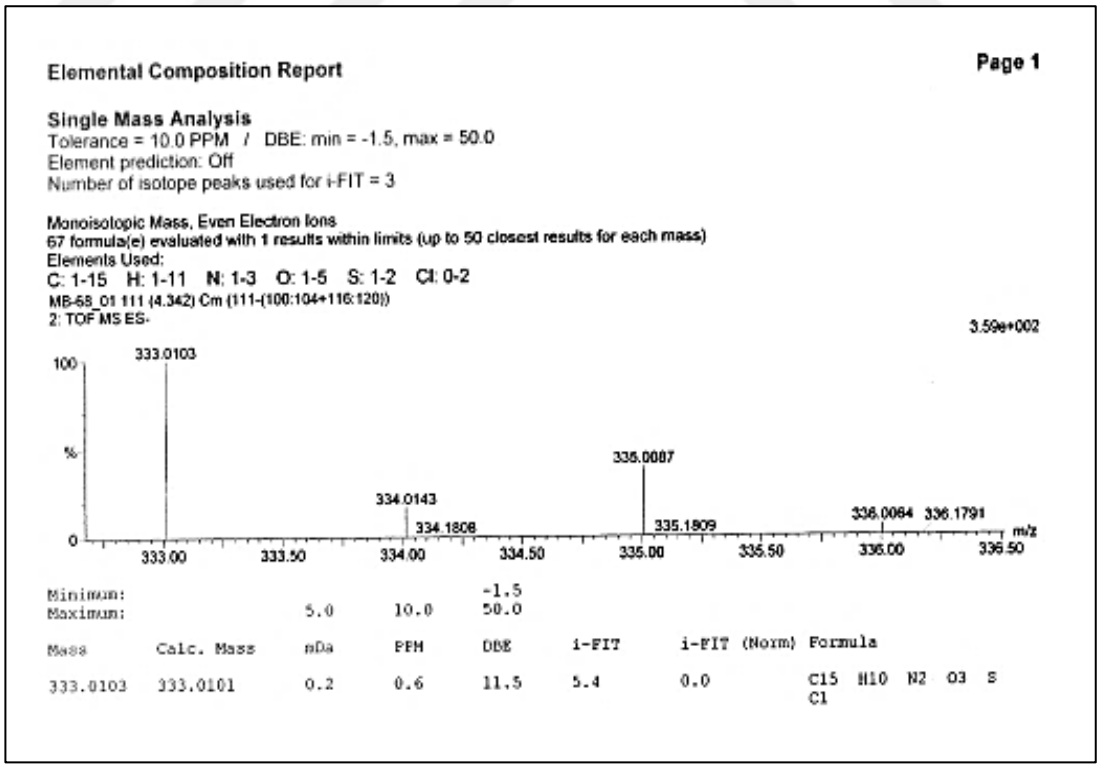


Figure 7.17. HRMS spectrum of compound 138c



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
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 50 closest results for each mass)
Elements Used:
C: 1-15 H: 1-11 N: 1-3 O: 1-5 S: 1-2 Cl: 0-2
MB-68_01 111 (4.342) Cm (111-(100:104+116:120))
2: TOF MS ES-
3.59e+002

Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
333.0103	333.0101	0.2	0.6	11.5	5.4	0.0	C15 H10 N2 O3 S Cl

Figure 7.18. Elemental composition of compound 138c

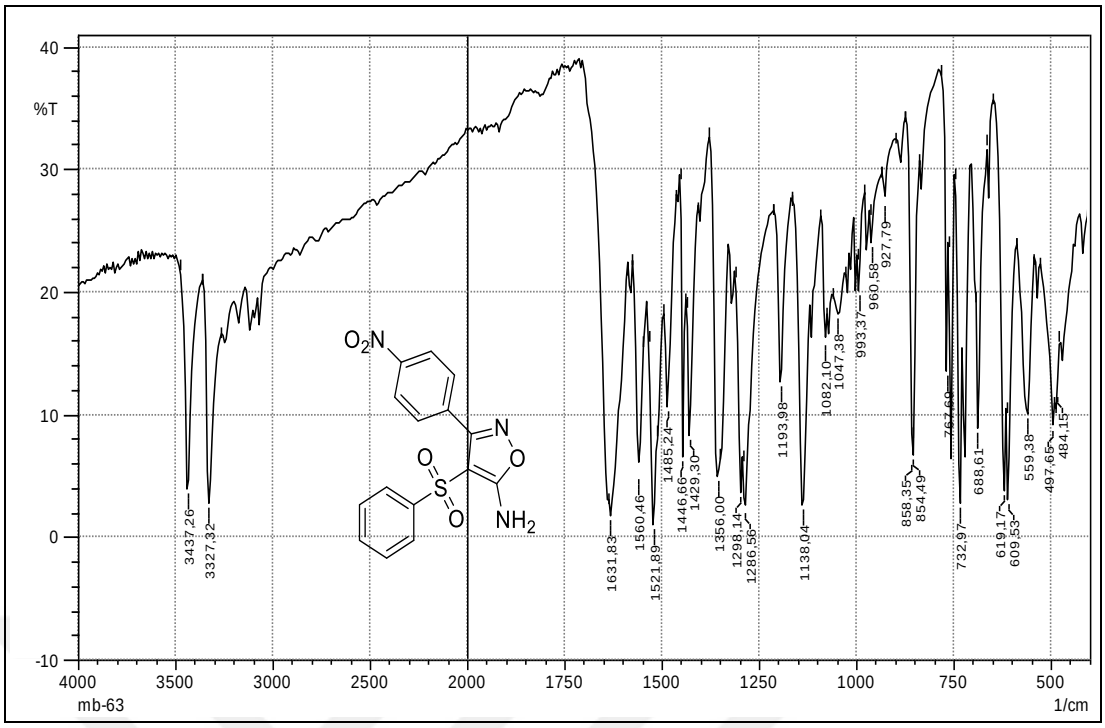


Figure 7.19. IR spectrum of compound 138d

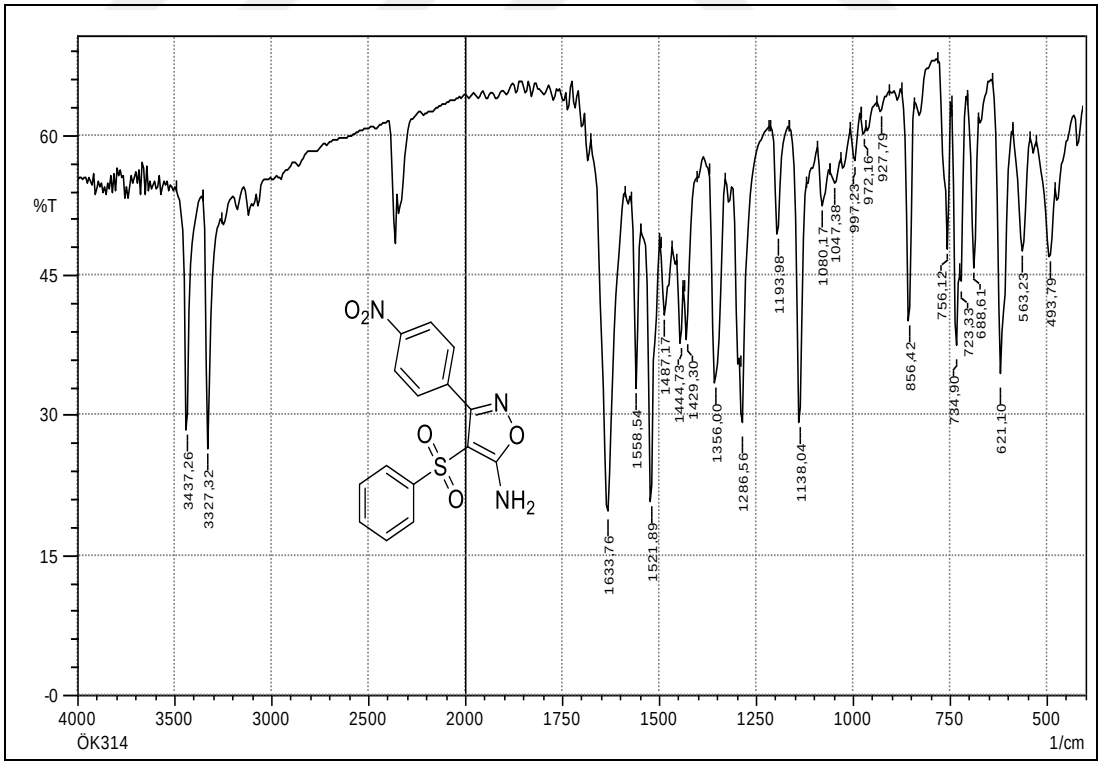


Figure 7.20. IR spectrum of compound 138d'

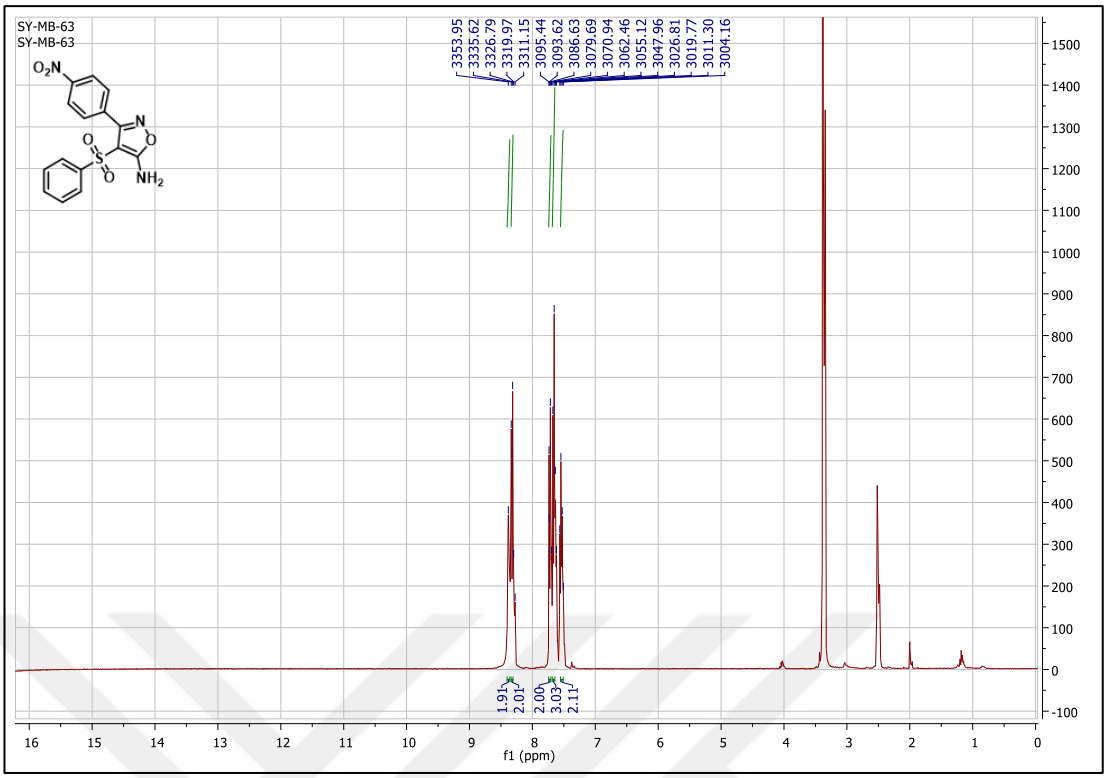


Figure 7.21. ^{1}H NMR spectrum of compound 138d

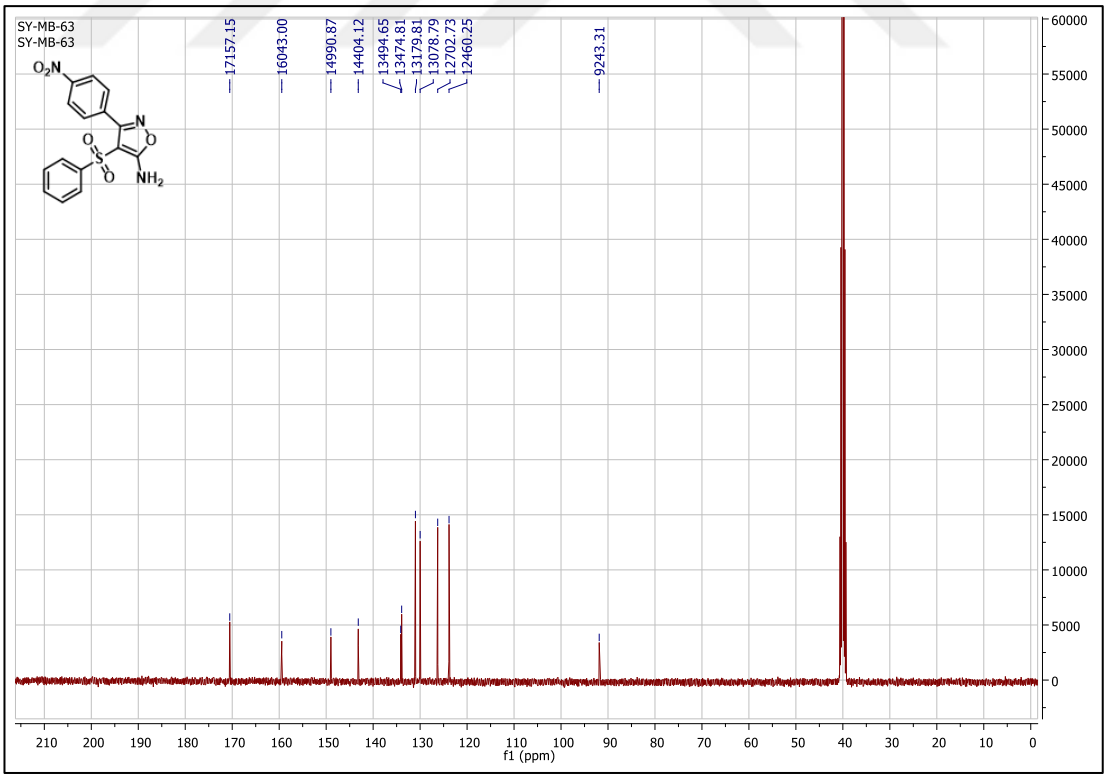


Figure 7.22. ^{13}C NMR spectrum of compound 138d

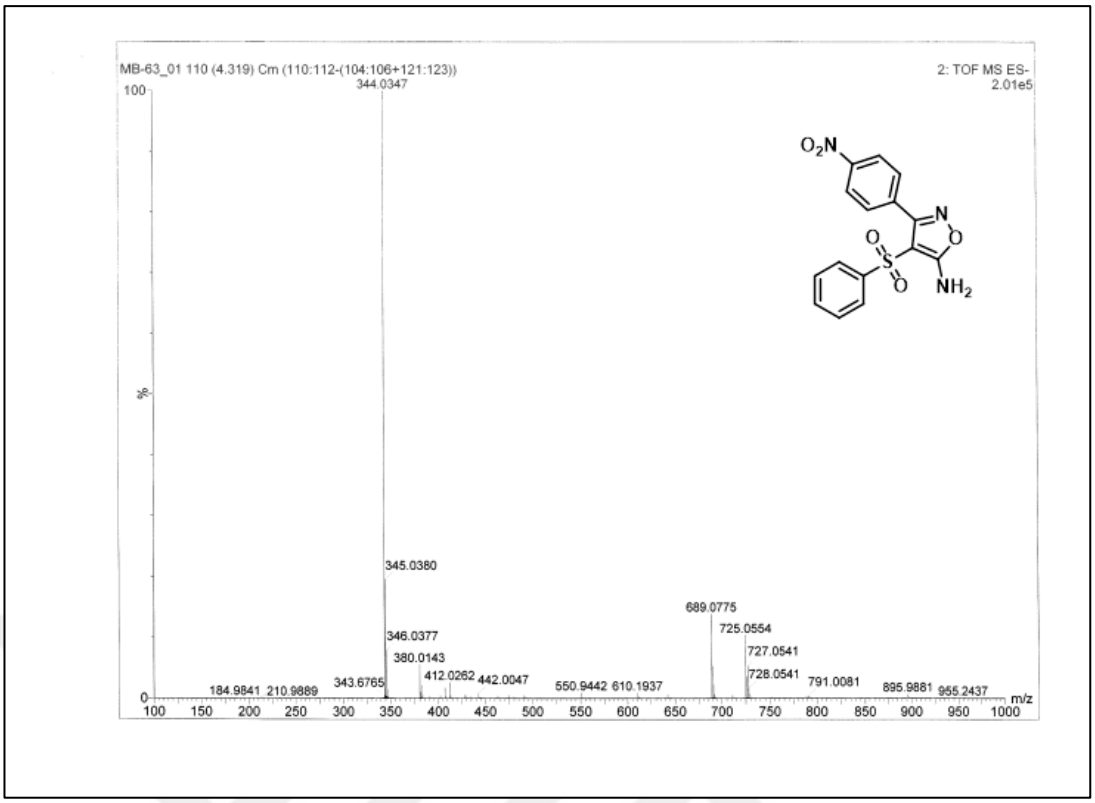
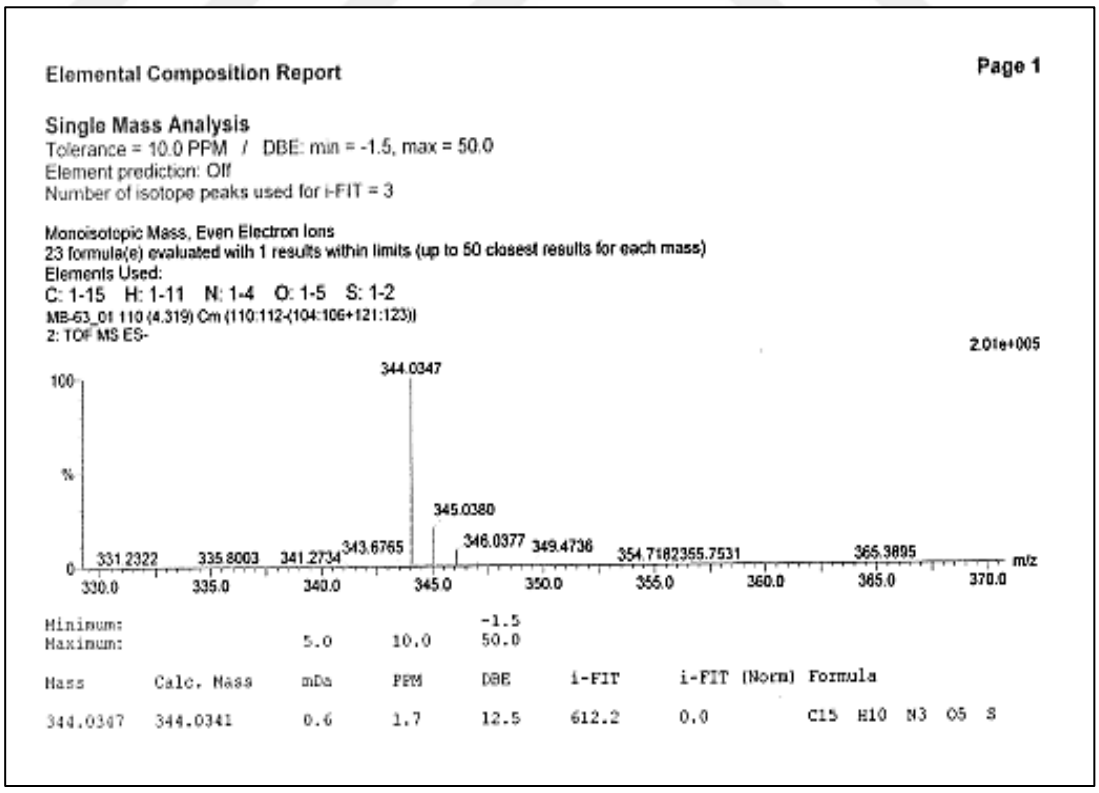


Figure 7.23. HRMS spectrum of compound 138d



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 1-15 H: 1-11 N: 1-4 O: 1-5 S: 1-2
MB-63_01 110 (4.319) Cm (110:112-(104:106+121:123))
2: TOF MS ES-
2.01e+005

Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
344.0347	344.0341	0.6	1.7	12.5	612.2	0.0	C15 H10 N3 O5 S

Figure 7.24. Elemental composition of compound 138d

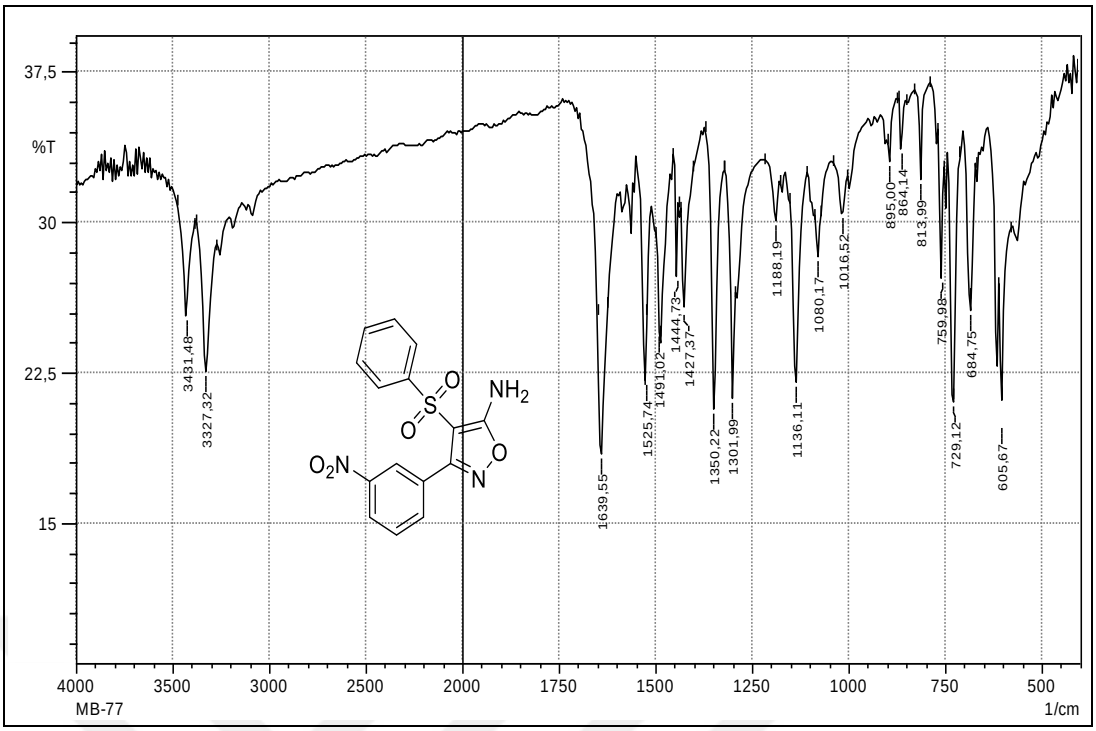


Figure 7.25. IR spectrum of compound 138e

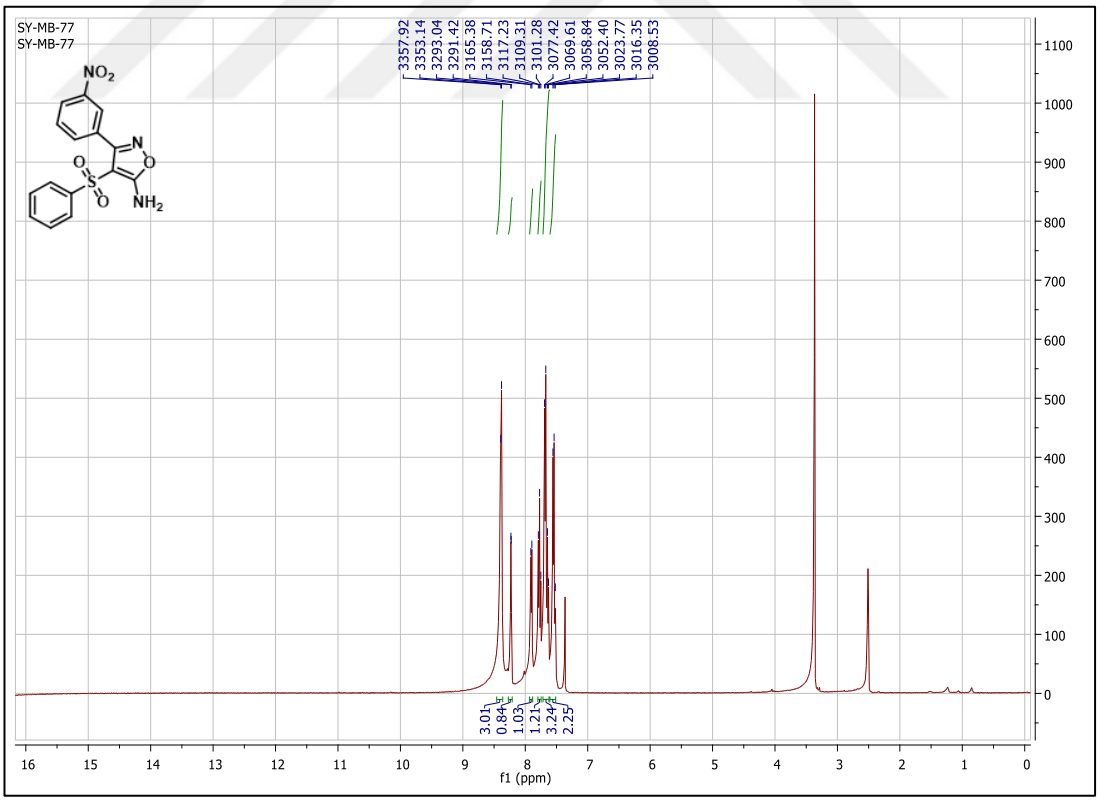


Figure 7.26. ^{1}H NMR spectrum of compound 138e

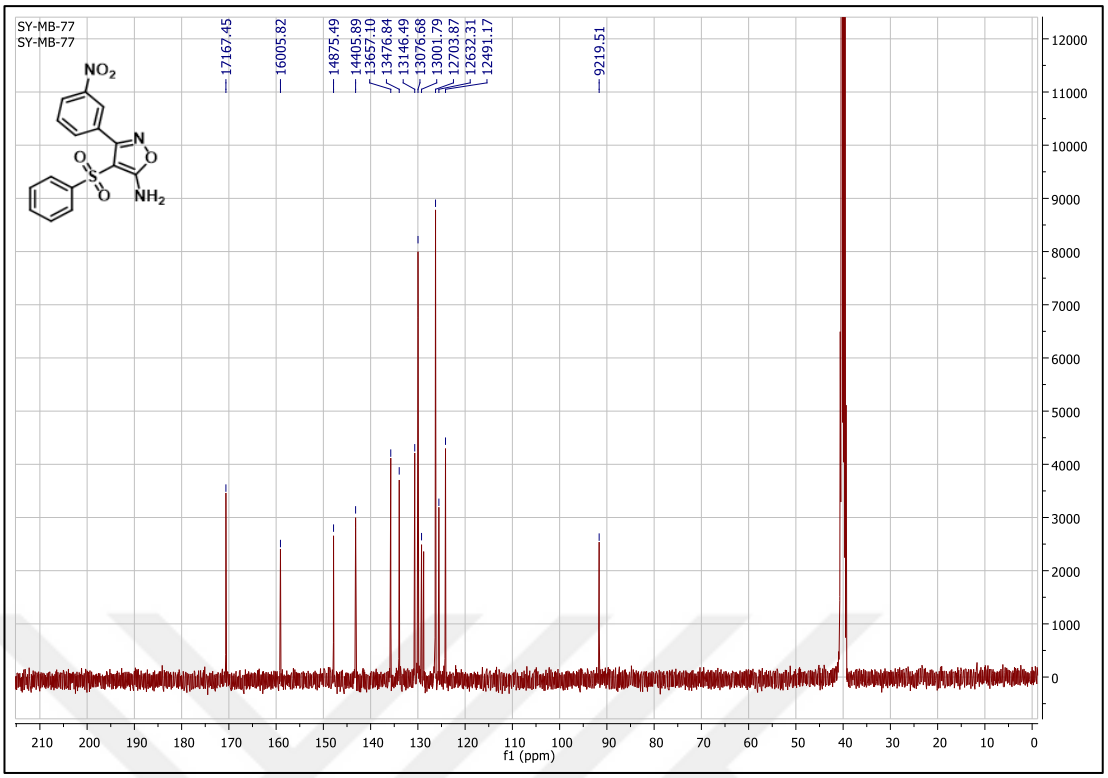


Figure 7.27. ^{13}C NMR spectrum of compound 138e

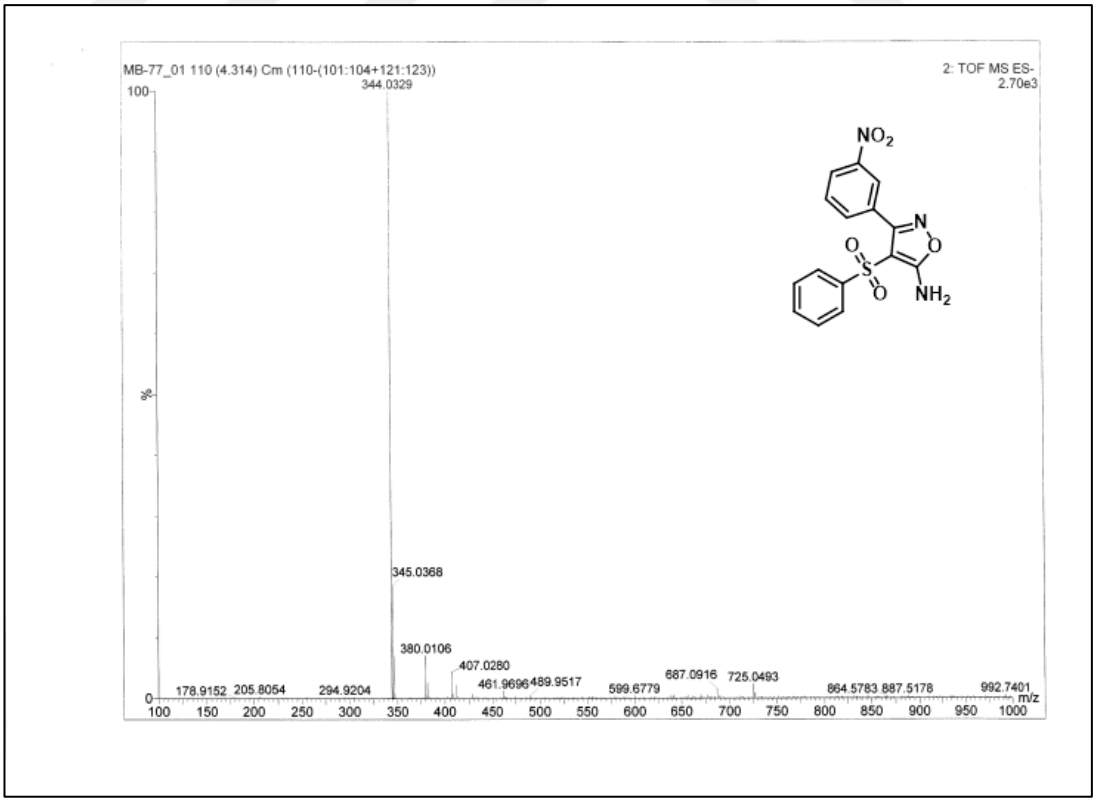


Figure 7.28. HRMS spectrum of compound 138e

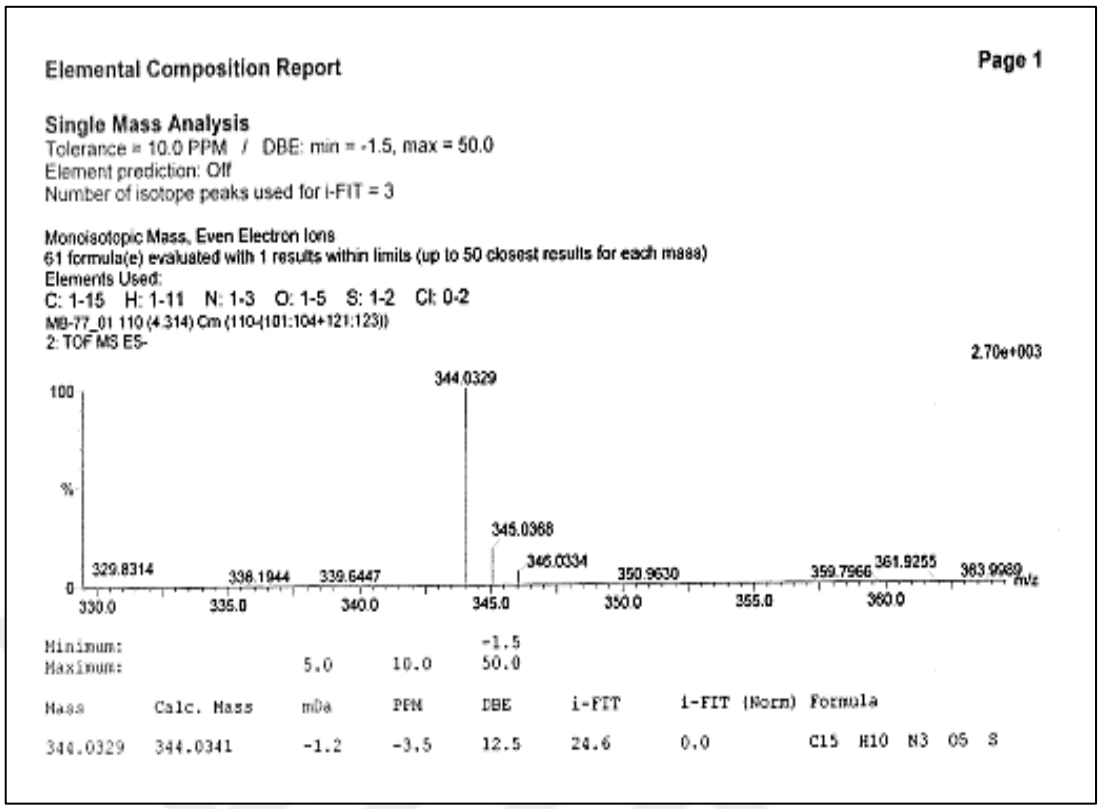


Figure 7.29. Elemental composition of compound 138e

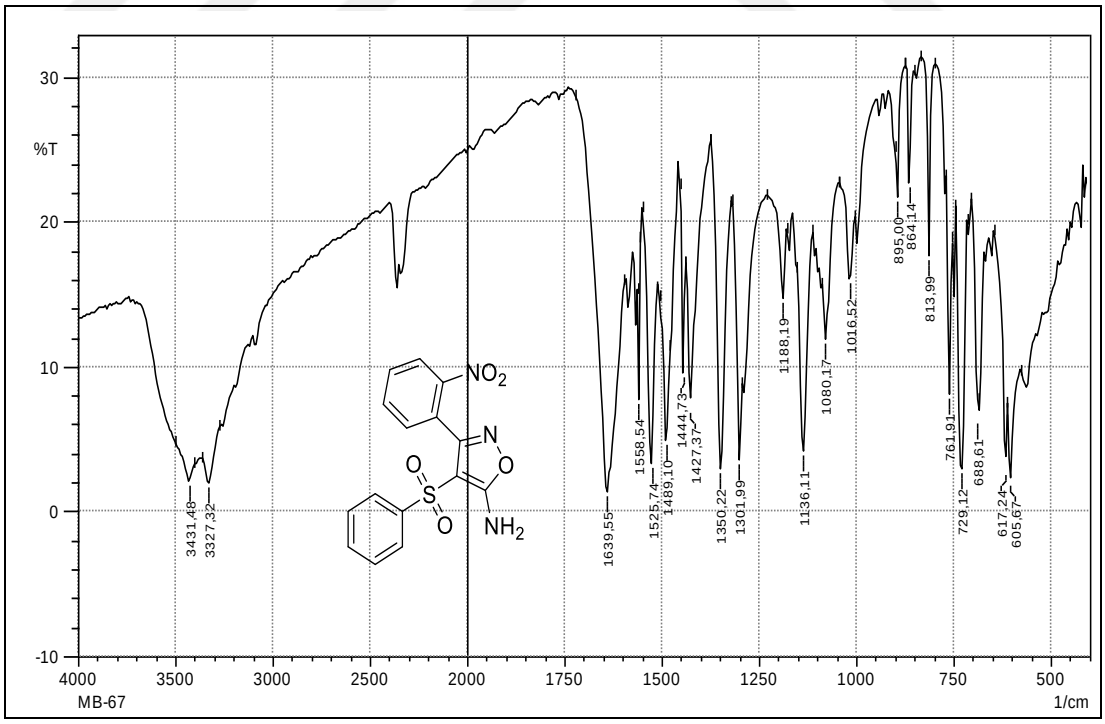


Figure 7.30. IR spectrum of compound 138f

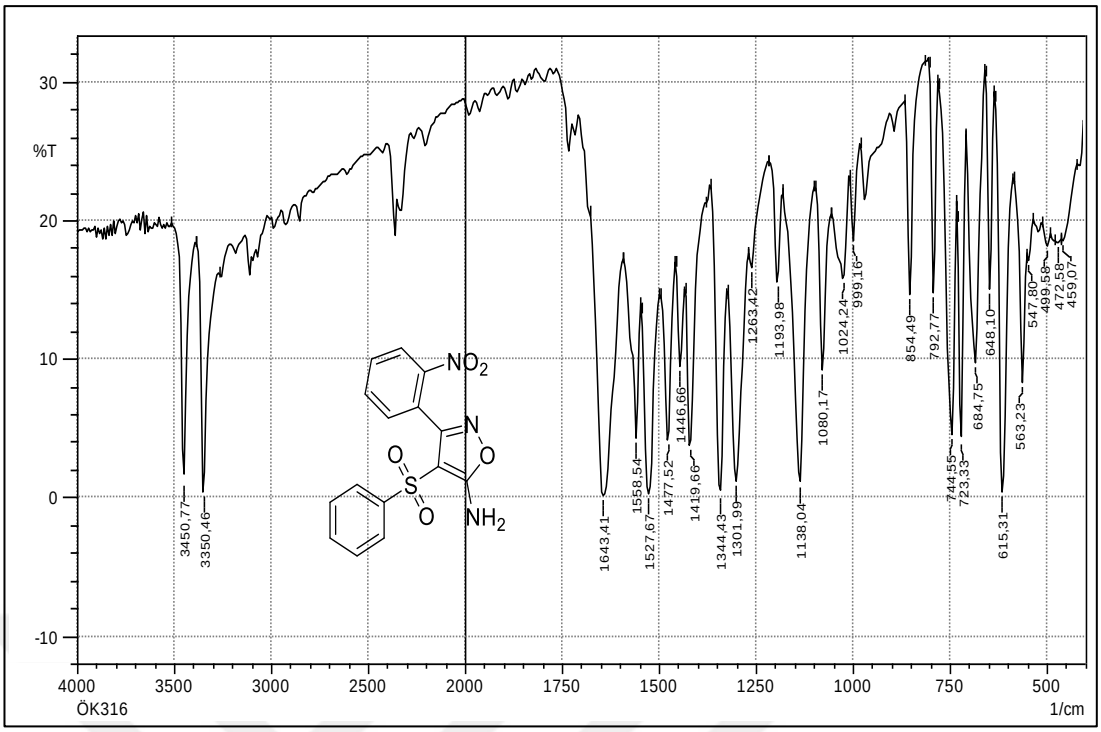


Figure 7.31. IR spectrum of compound 138f'

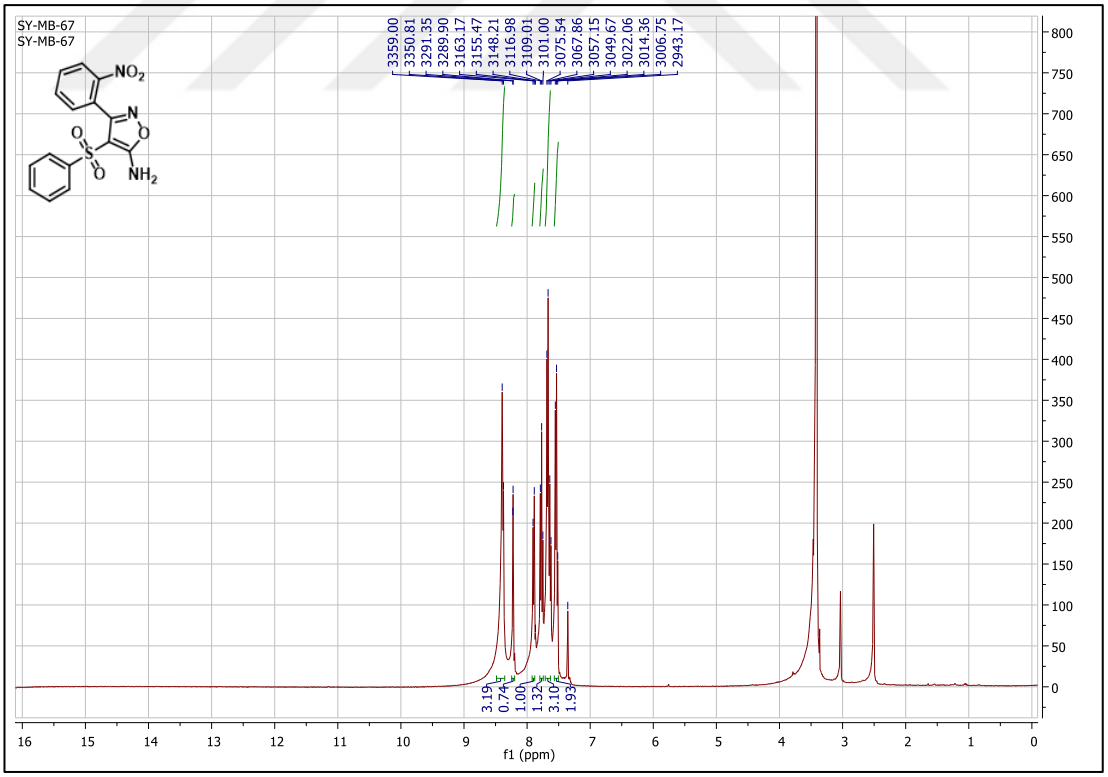


Figure 7.32. ^{1}H NMR spectrum of compound 138f

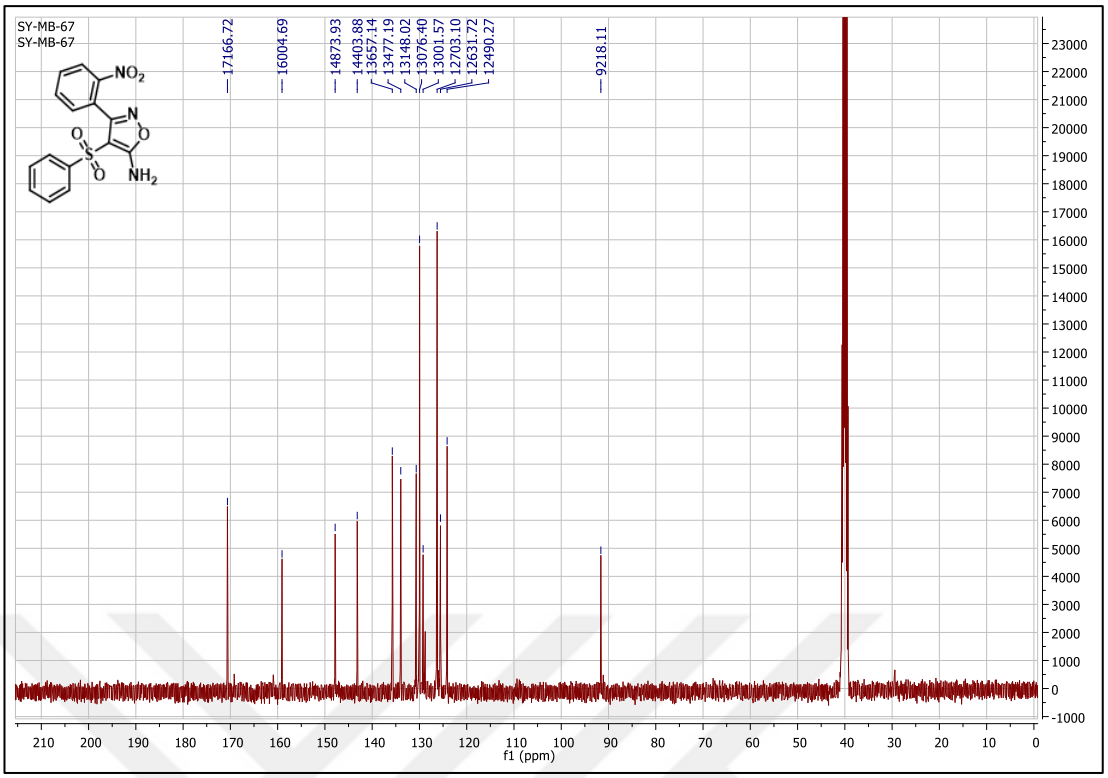


Figure 7.33. ^{13}C NMR spectrum of compound 138f

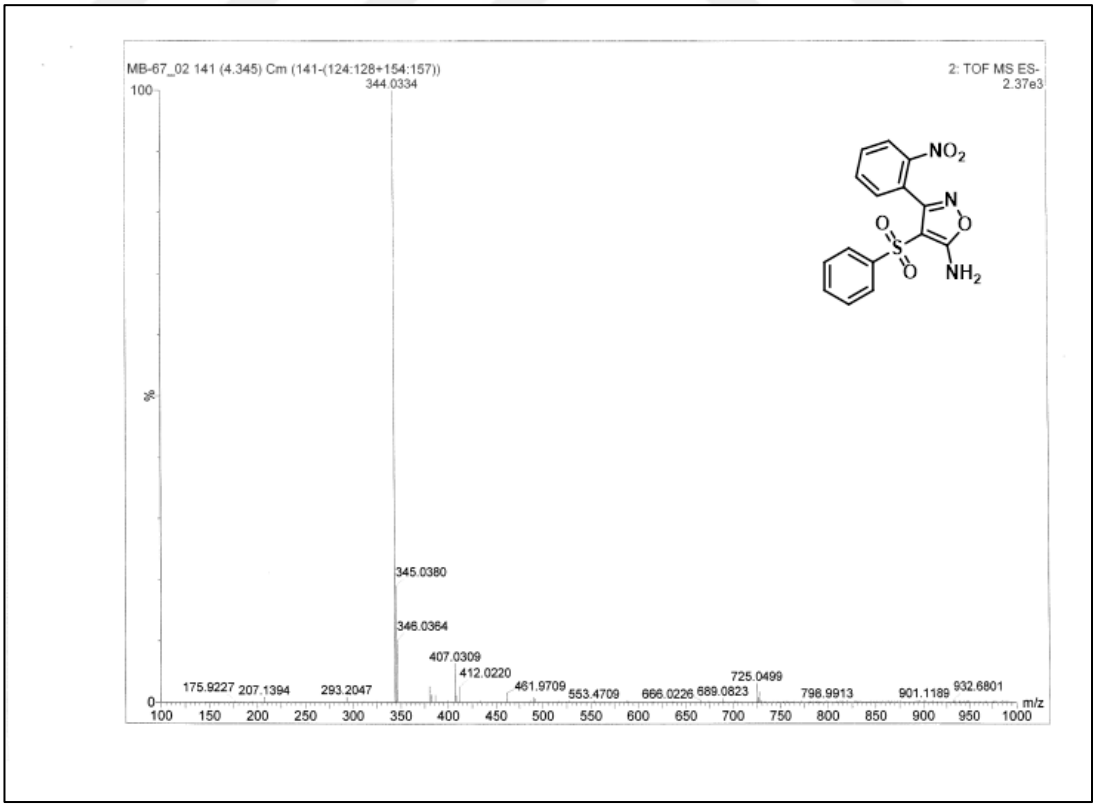


Figure 7.34. HRMS spectrum of compound 138f

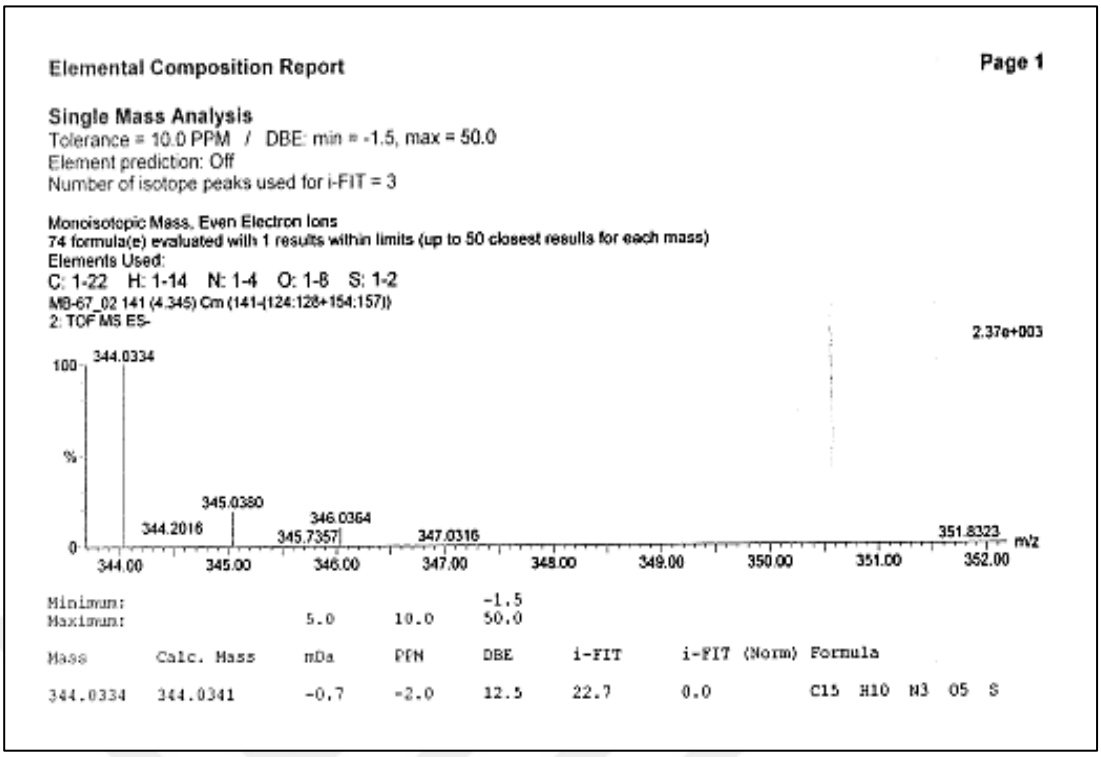


Figure 7.35. Elemental composition of compound 138f

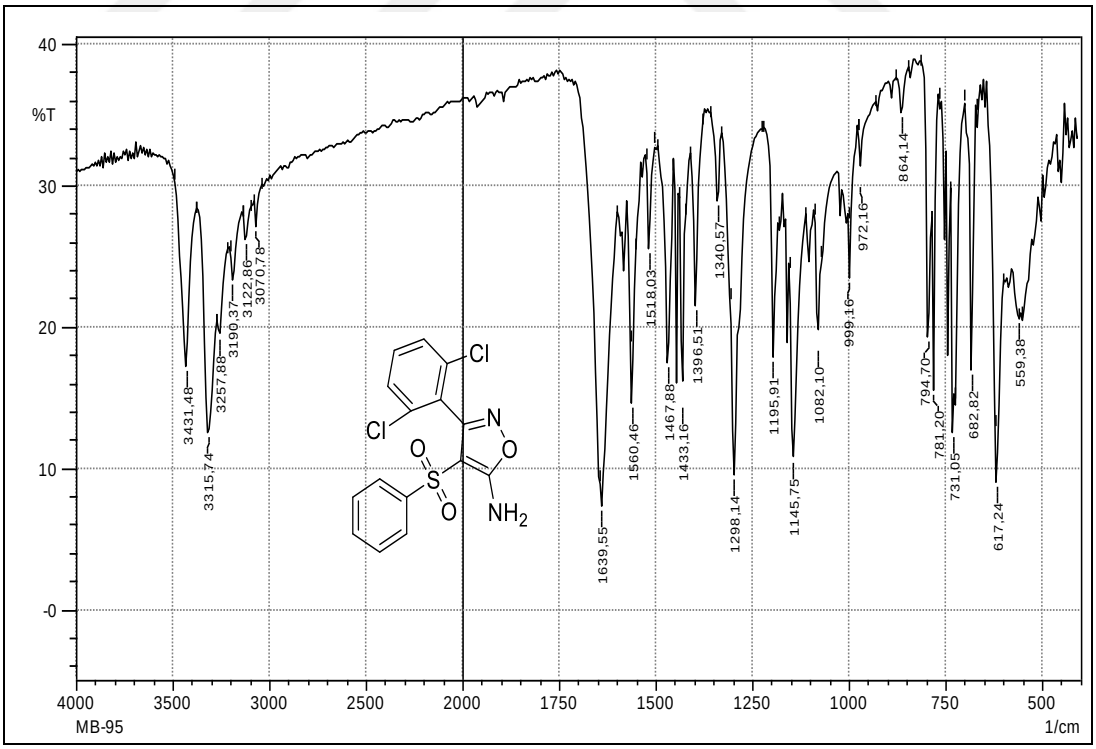


Figure 7.36. IR spectrum of compound 138g

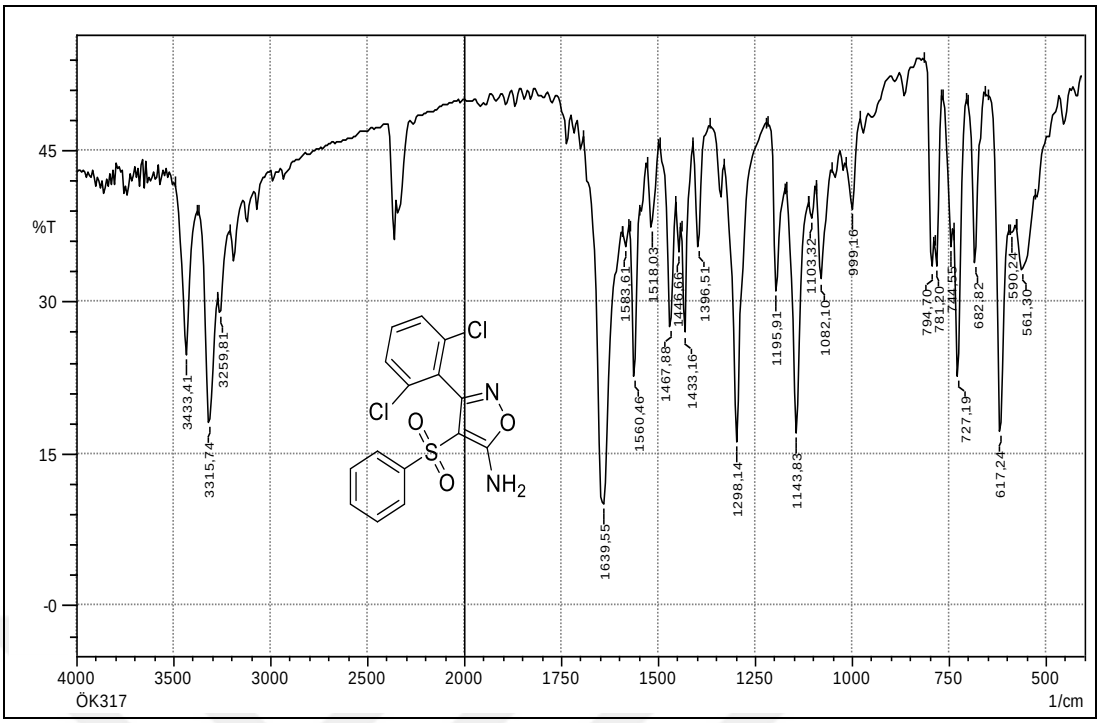


Figure 7.37. IR spectrum of compound 138g'

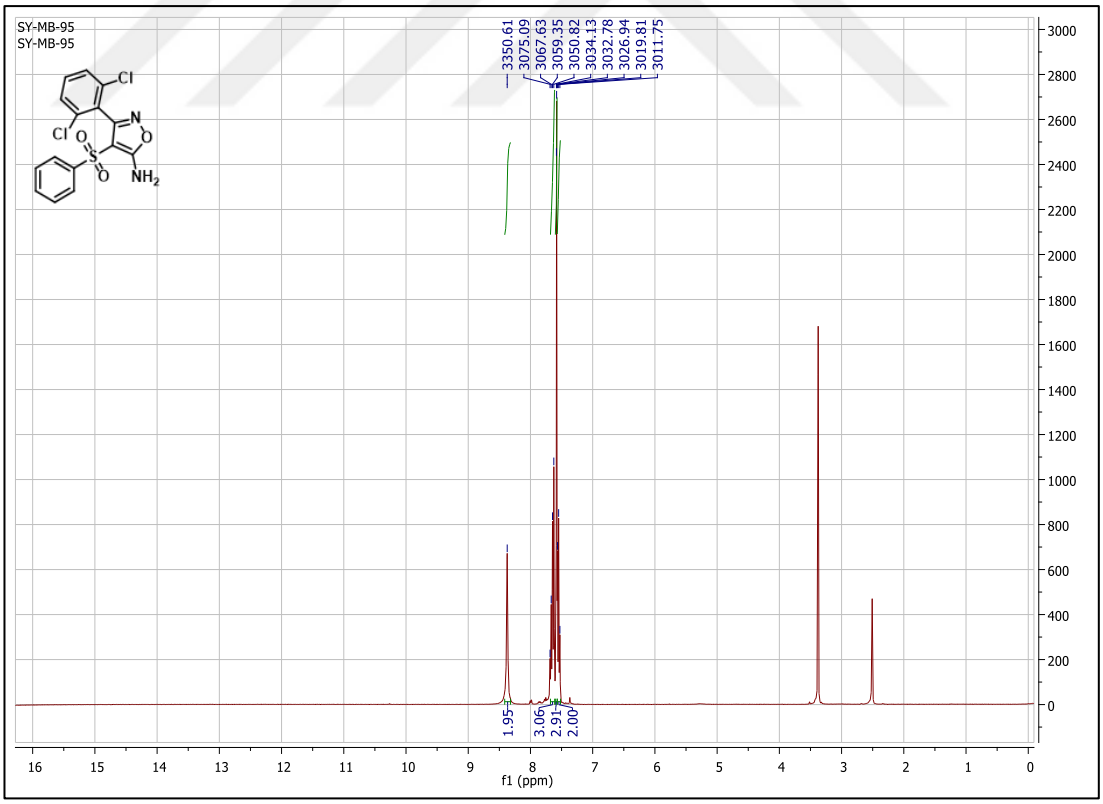


Figure 7.38. ^{1}H NMR spectrum of compound 138g

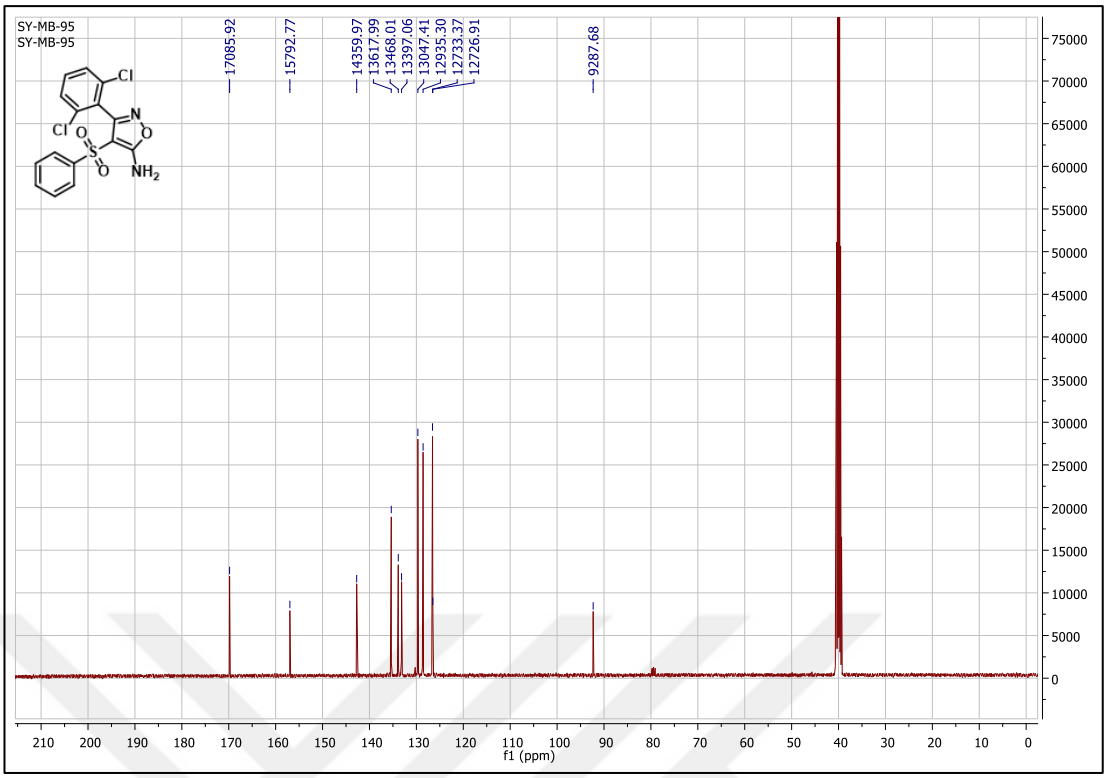


Figure 7.39. ^{13}C NMR spectrum of compound 138g

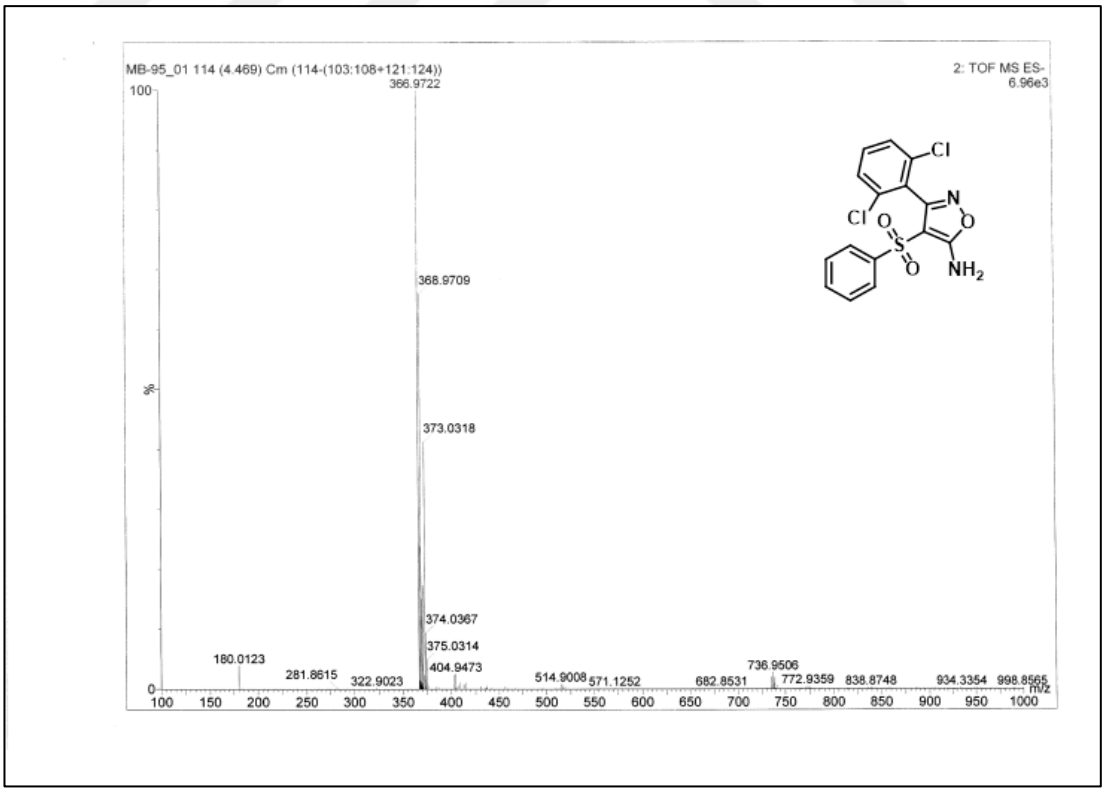


Figure 7.40. HRMS spectrum of compound 138g

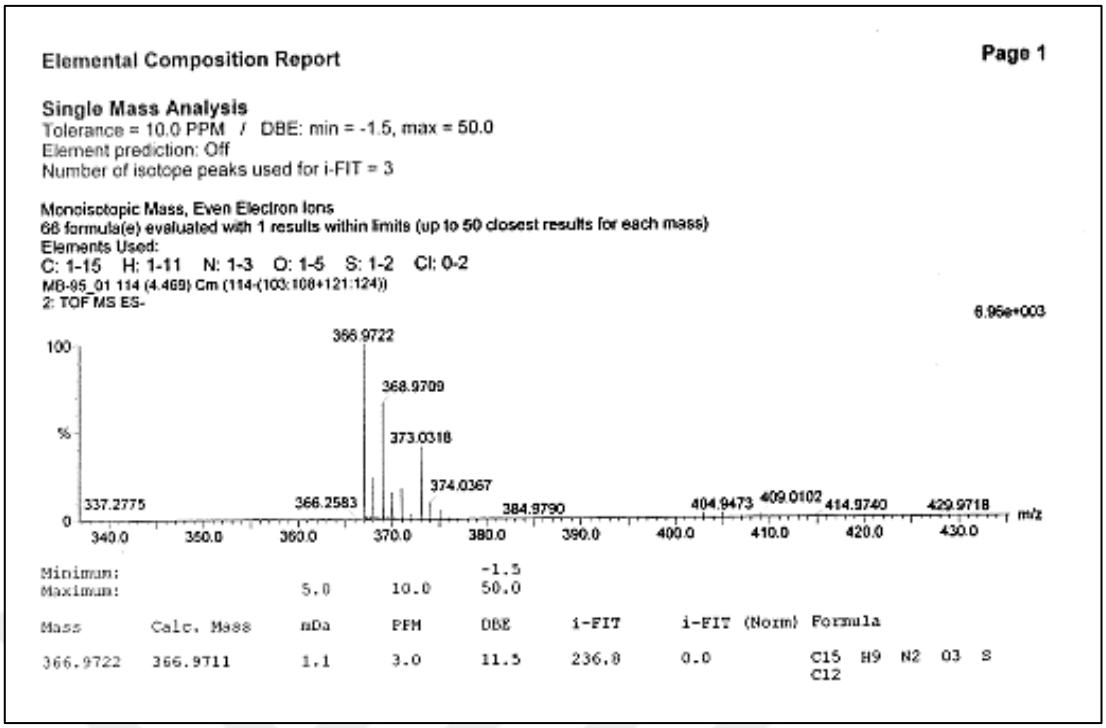


Figure 7.41. Elemental composition of compound 138g

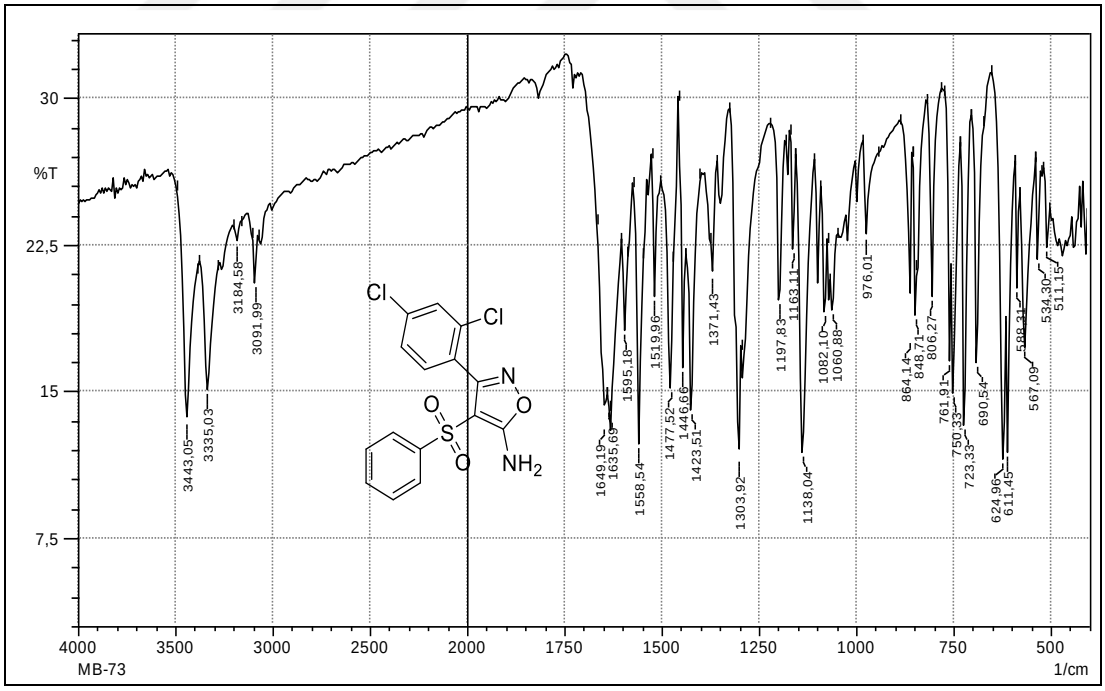


Figure 7.42. IR spectrum of compound 138h

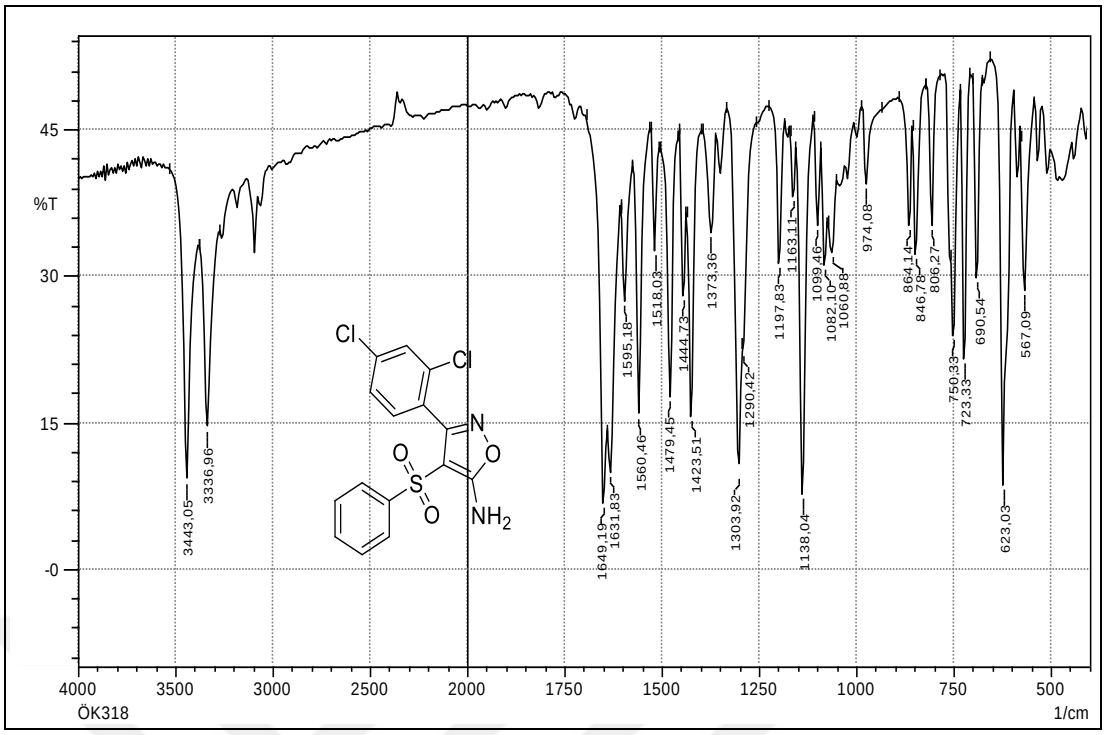


Figure 7.43. IR spectrum of compound 138h'

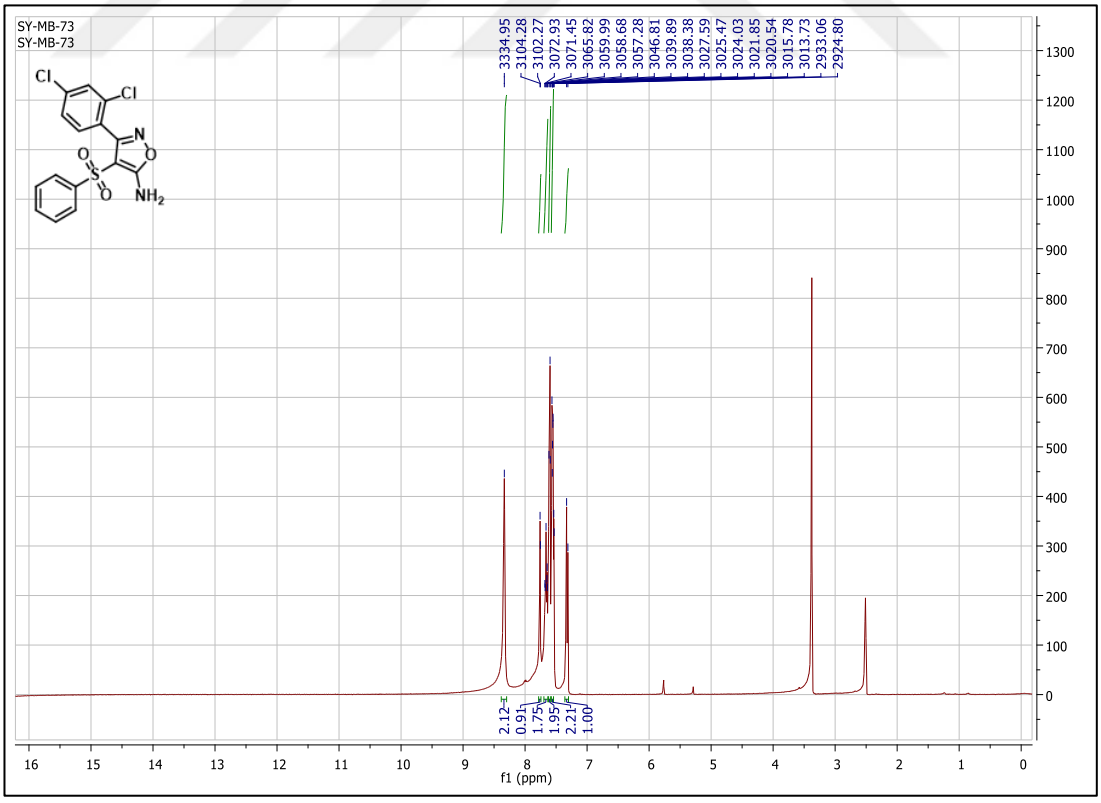


Figure 7.44. ^{1}H NMR spectrum of compound 138h

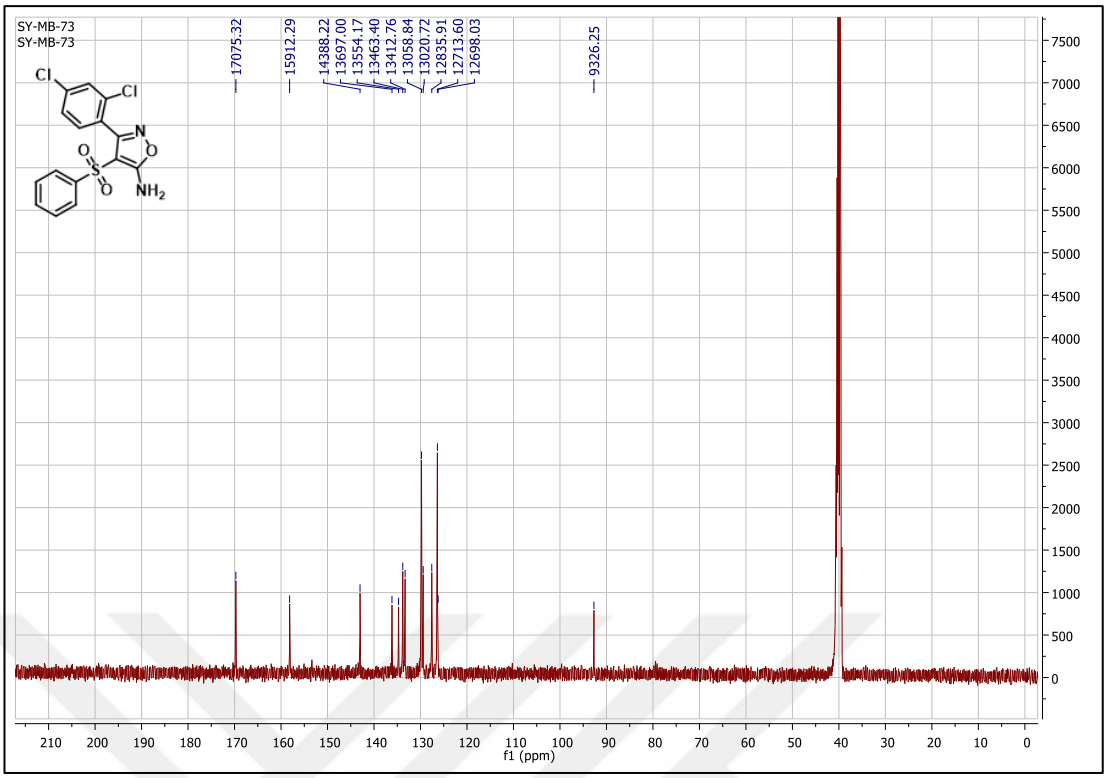


Figure 7.45. ^{13}C NMR spectrum of compound 138h

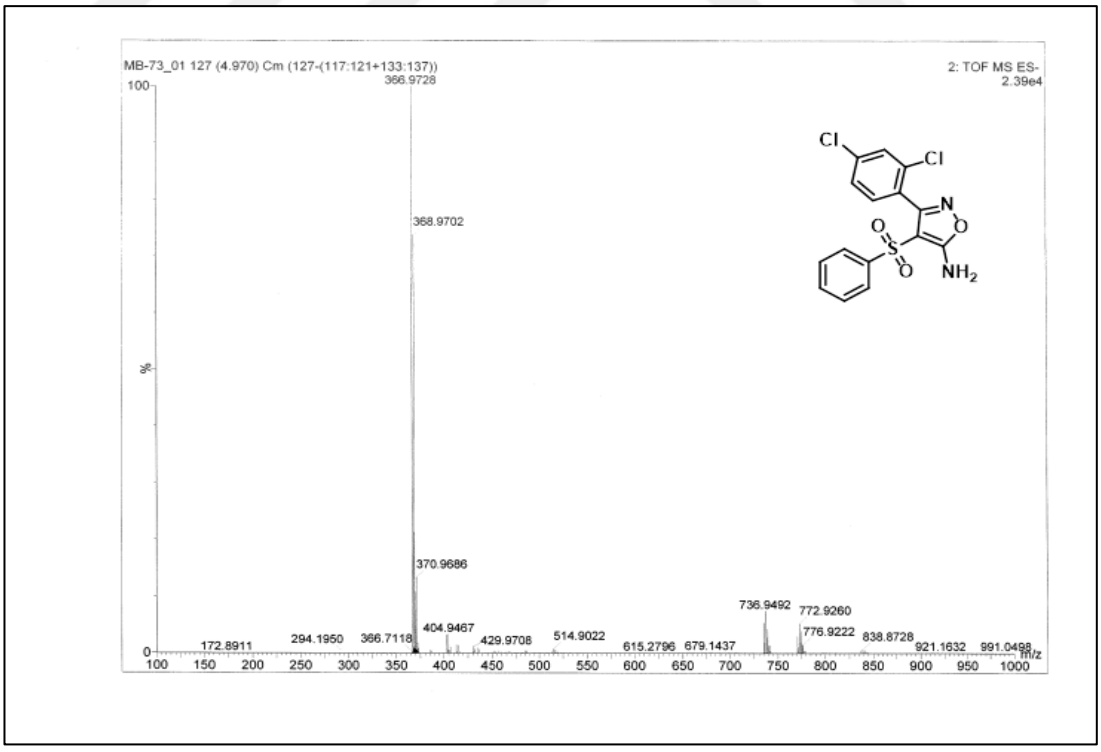


Figure 7.46. HRMS spectrum of compound 138h

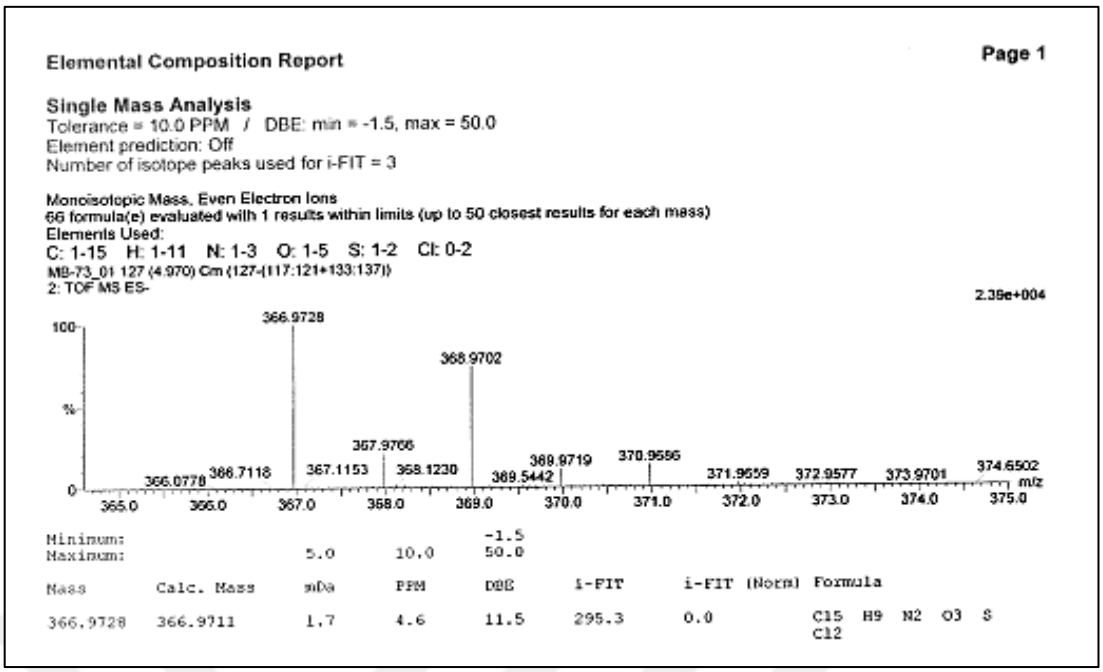


Figure 7.47. Elemental composition of compound 138h

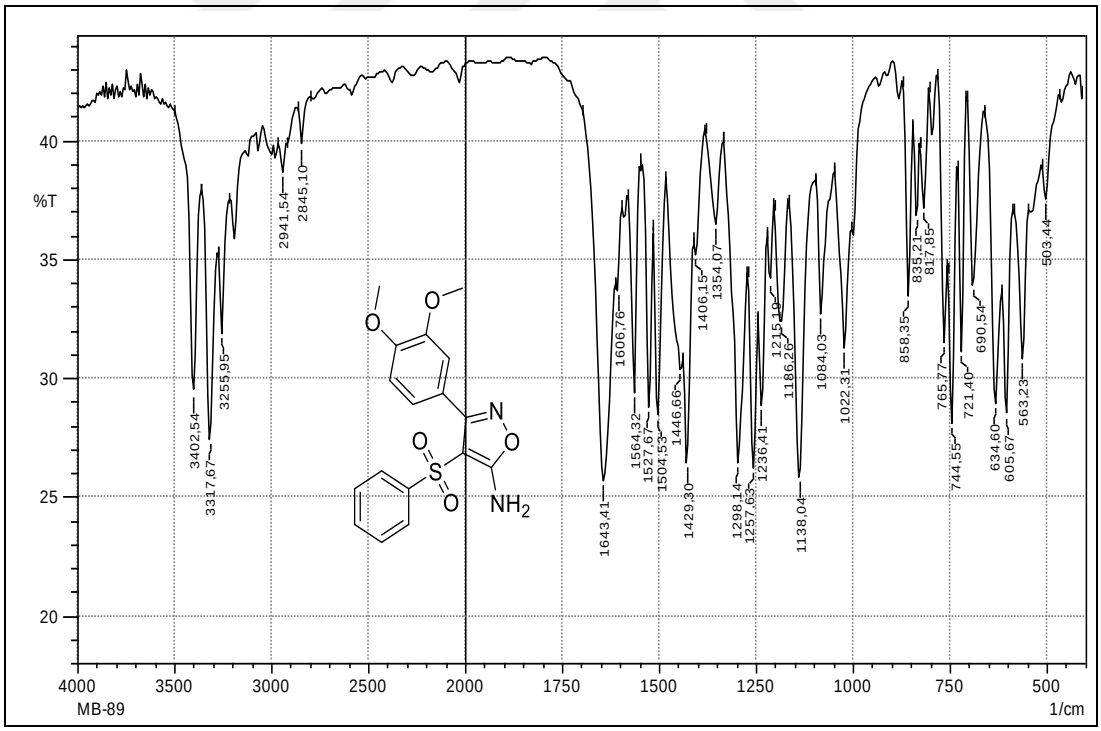


Figure 7.48. IR spectrum of compound 138i

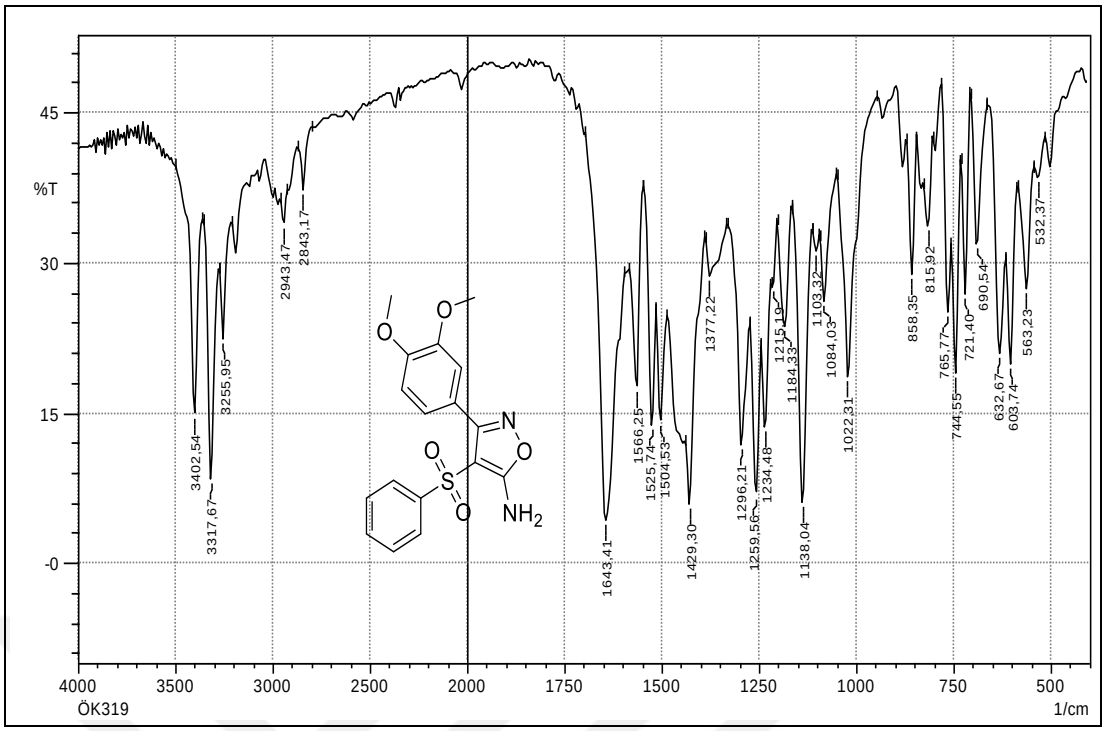


Figure 7.49. IR spectrum of compound 138i'

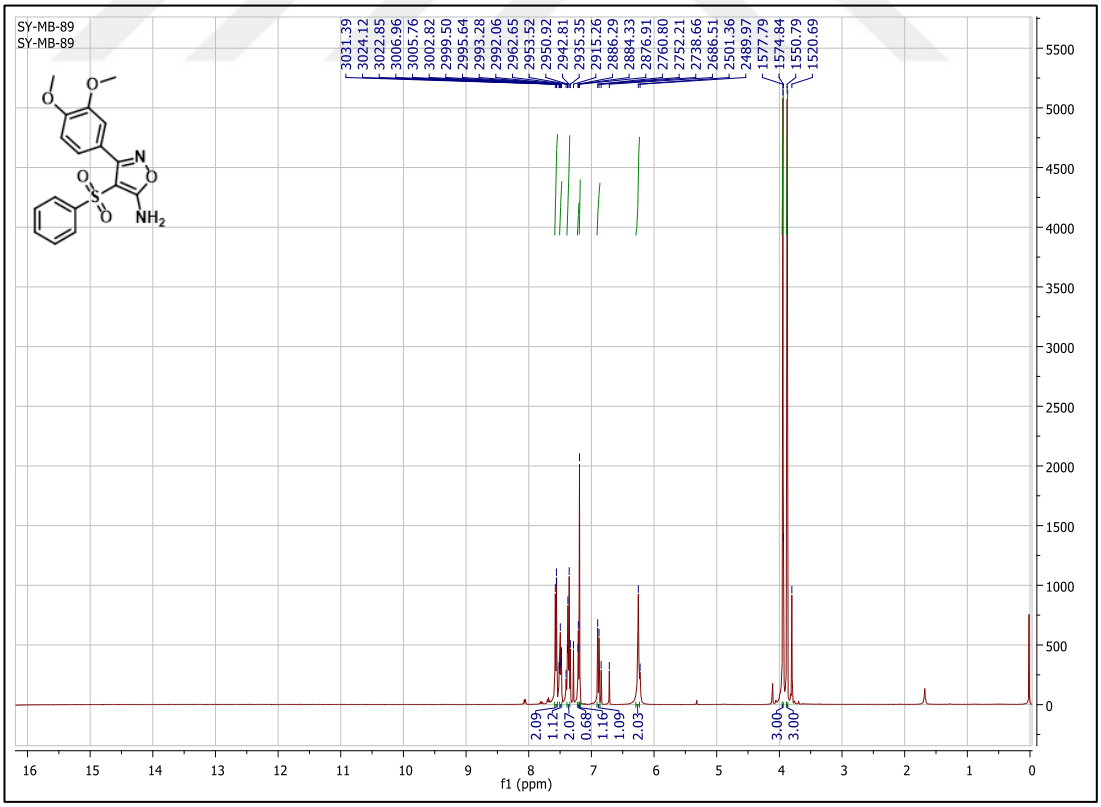


Figure 7.50. ^{1}H NMR spectrum of compound 138i

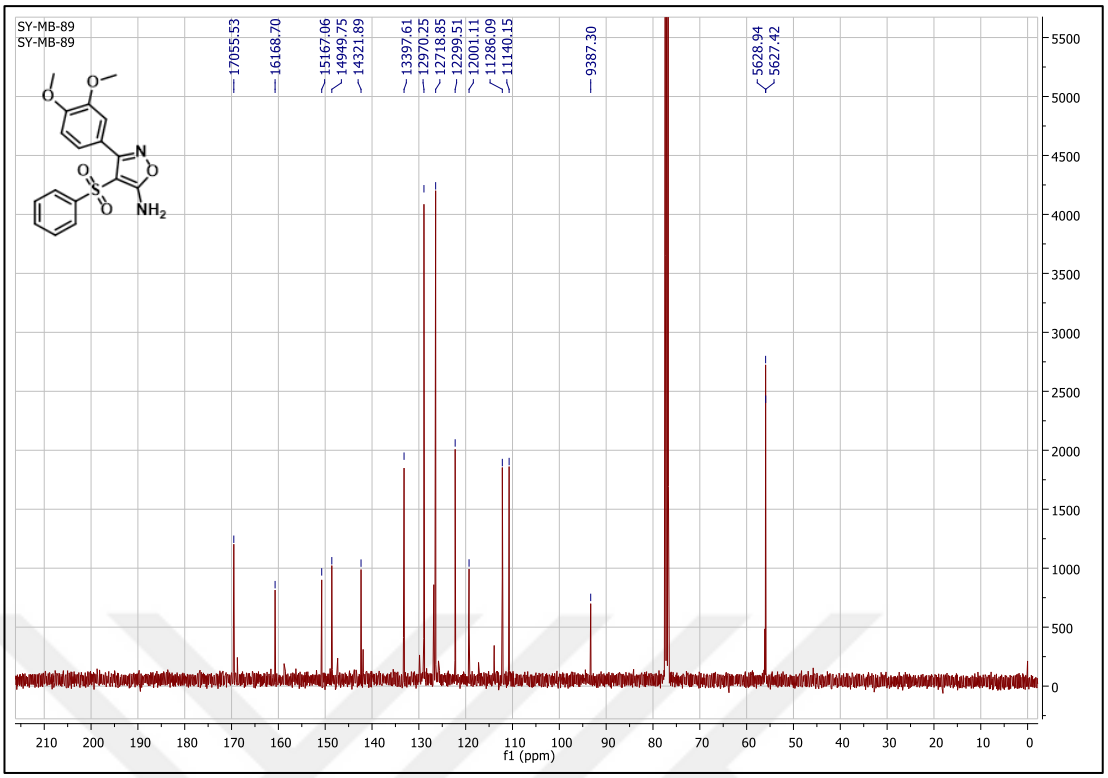


Figure 7.51. ^{13}C NMR spectrum of compound 138i

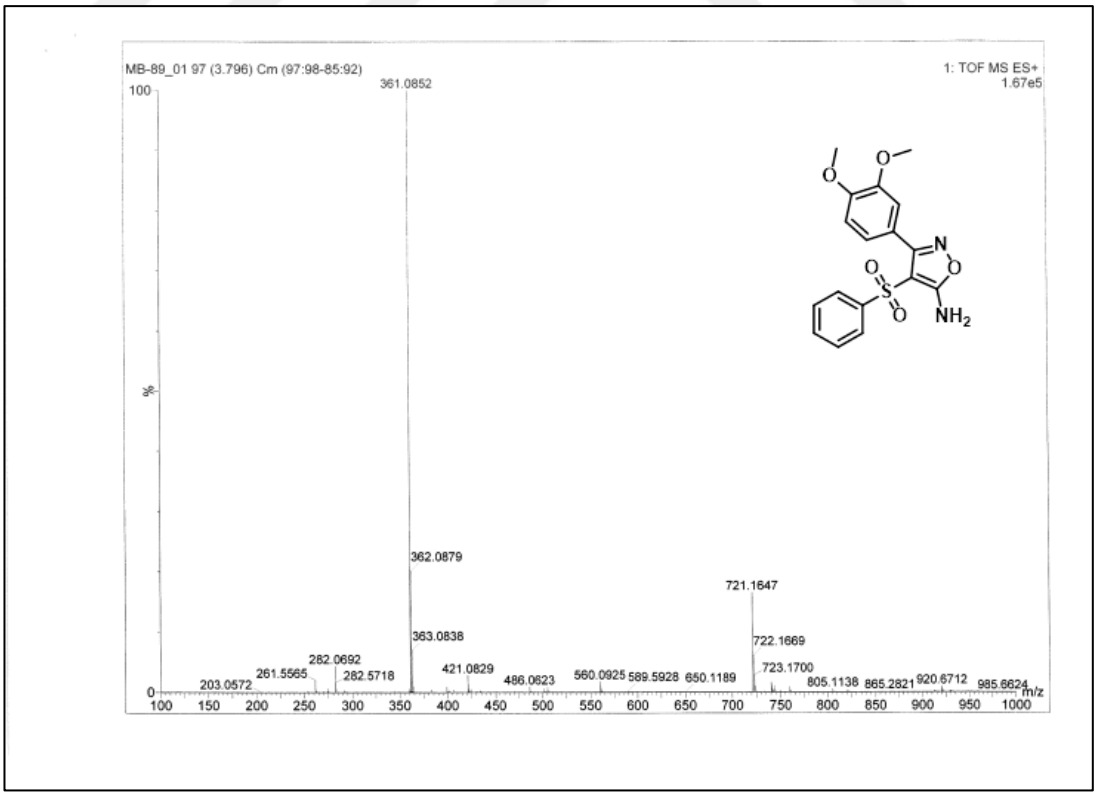


Figure 7.52. HRMS spectrum of compound 138i

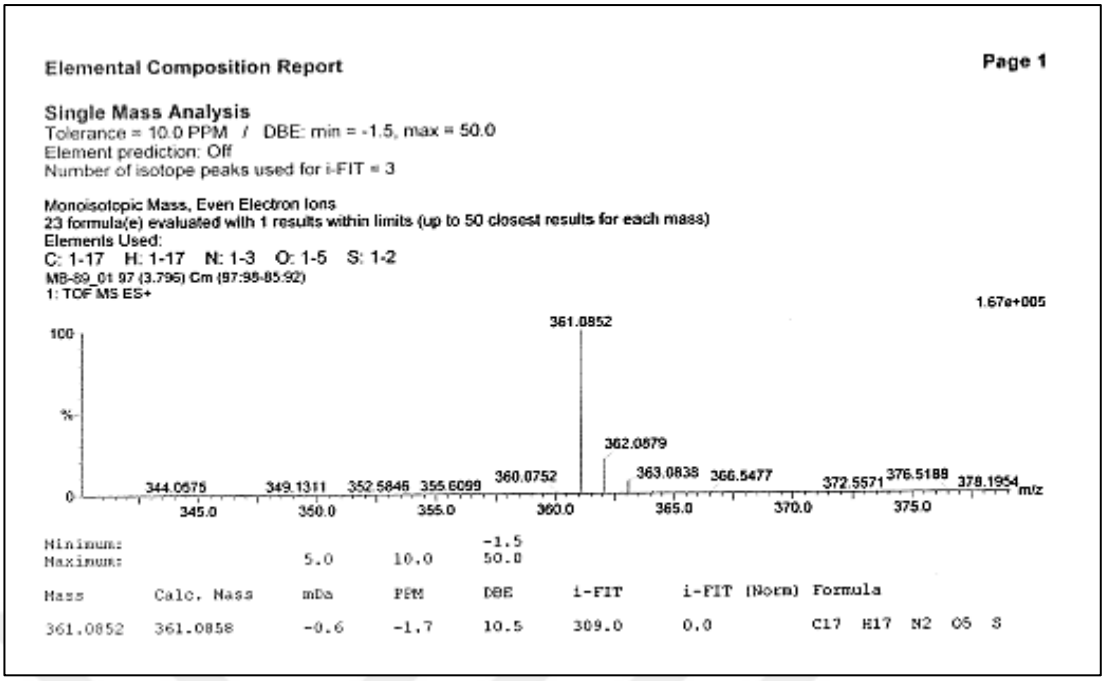


Figure 7.53. Elemental composition of compound 138i

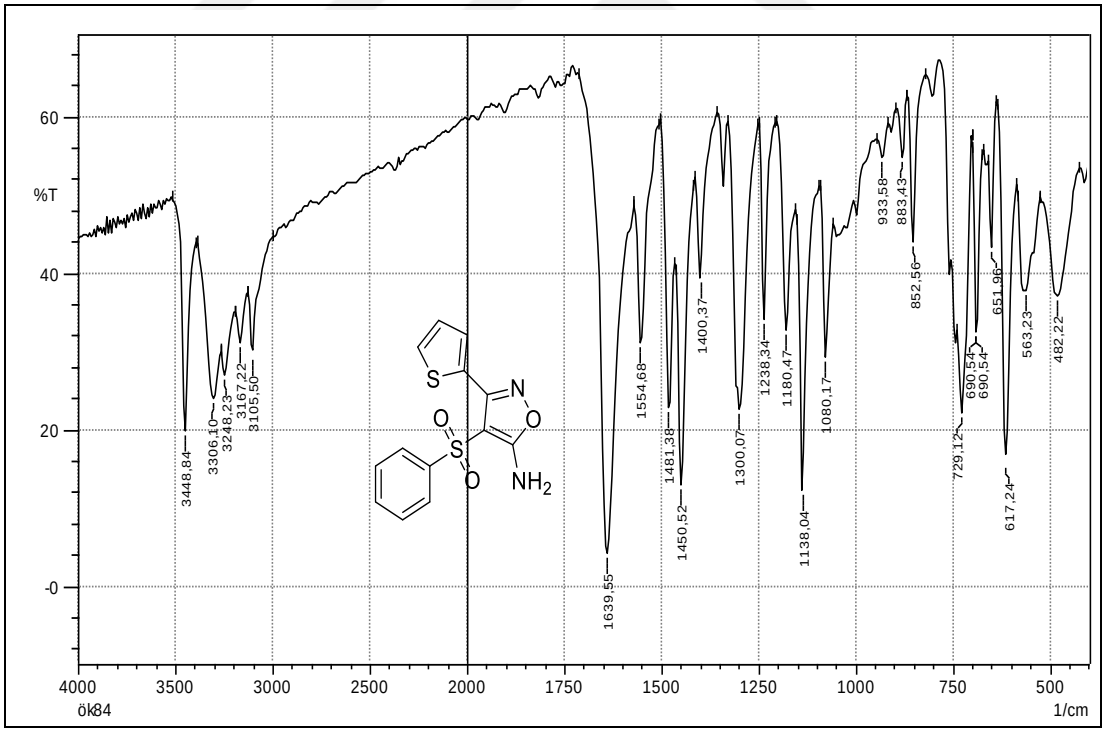


Figure 7.54. IR spectrum of compound 138j

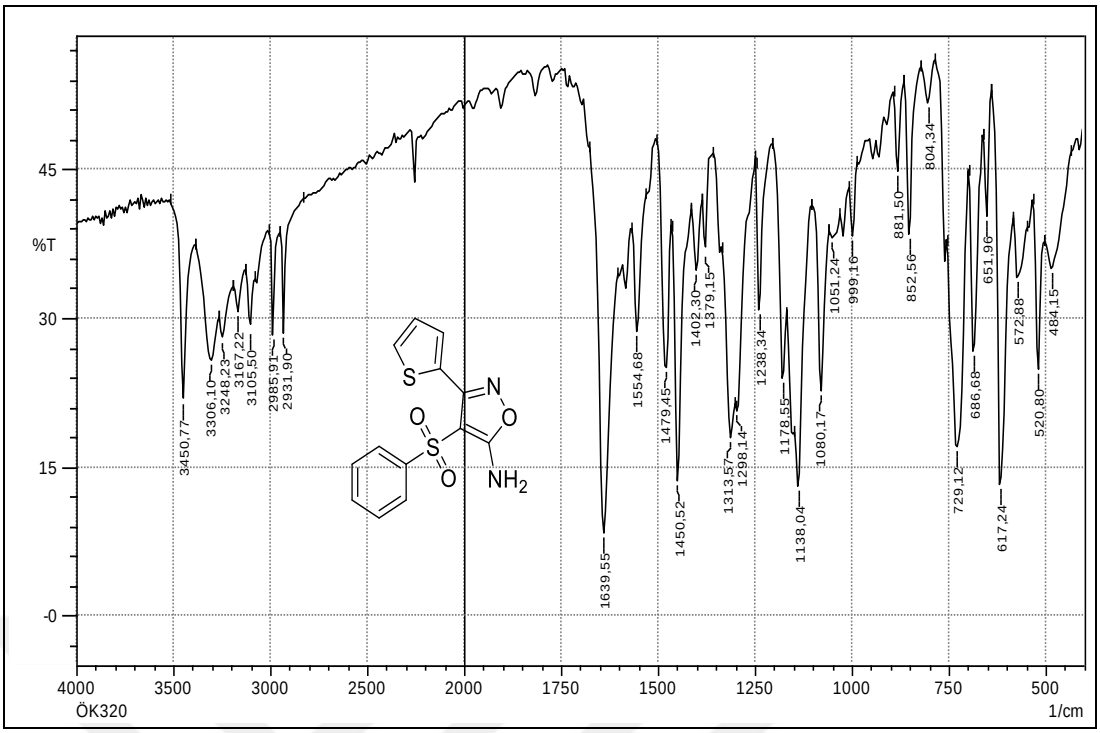


Figure 7.55. IR spectrum of compound 138j'

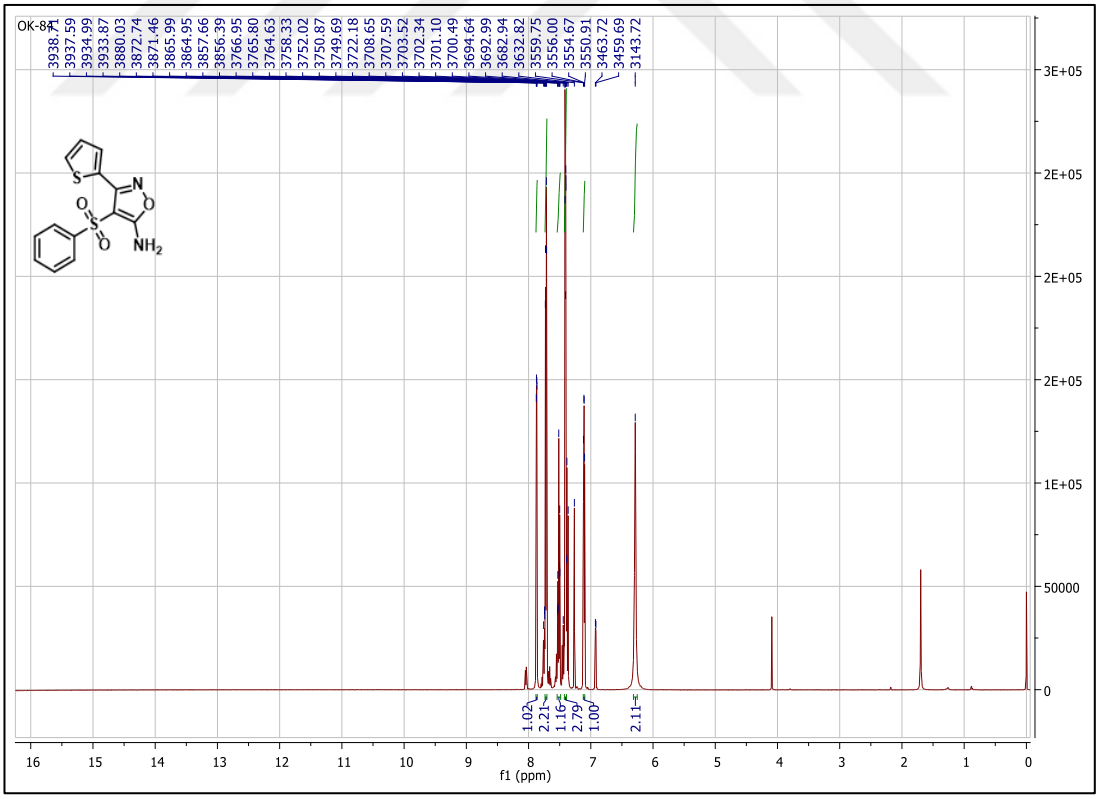


Figure 7.56. ^{1}H NMR spectrum of compound 138j

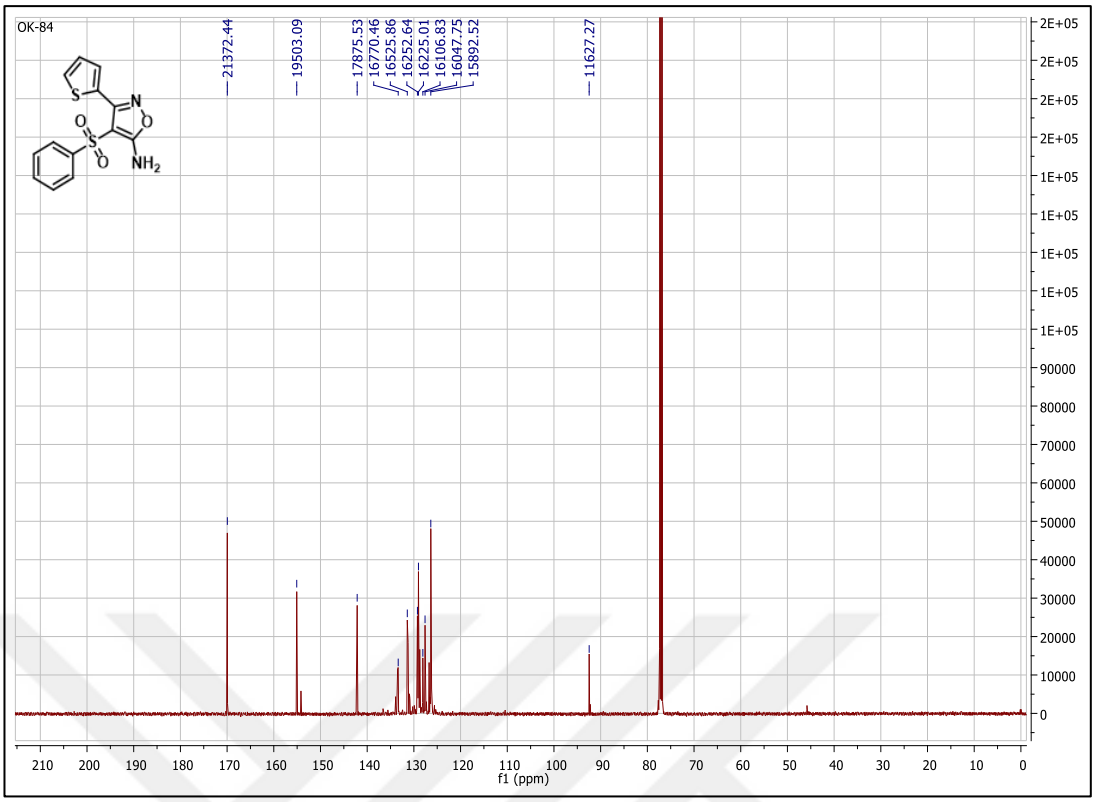


Figure 7.57. ^{13}C NMR spectrum of compound 138j

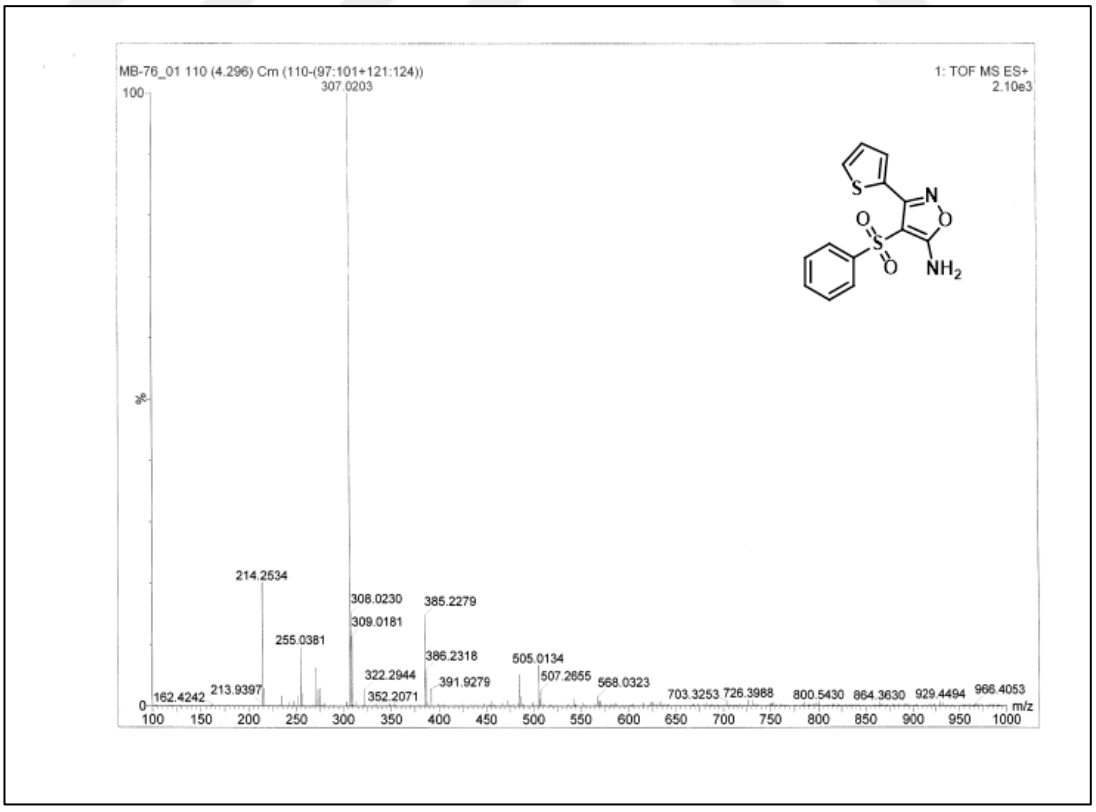


Figure 7.58. HRMS spectrum of compound 138j

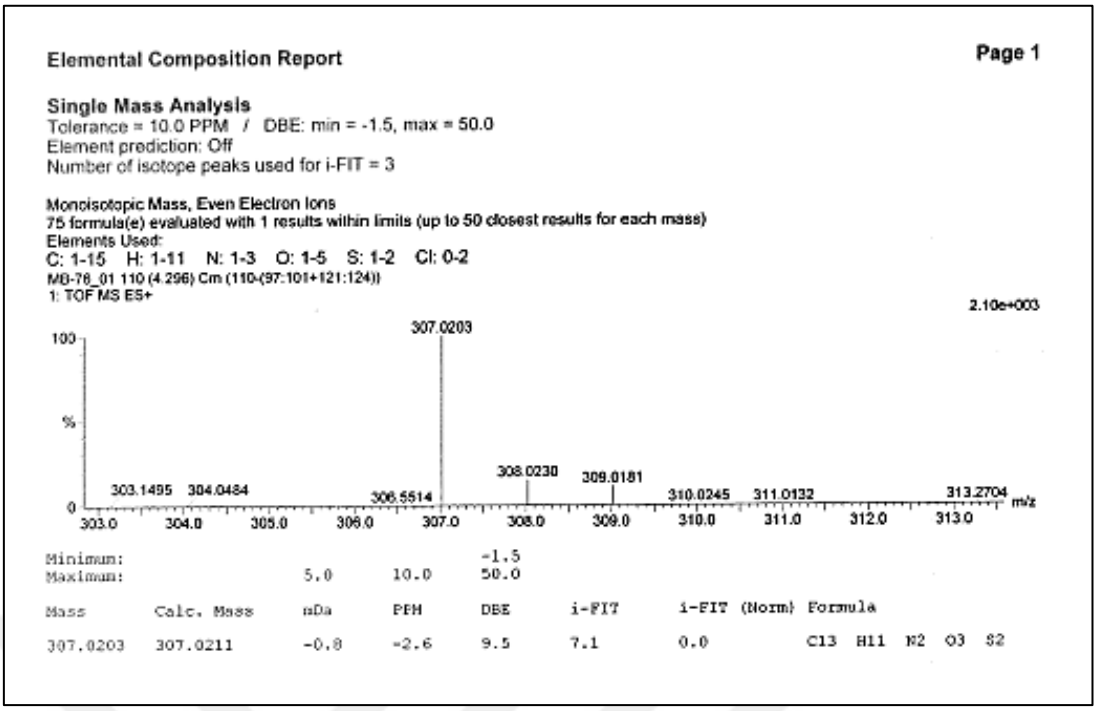


Figure 7.59. Elemental composition of compound 138j

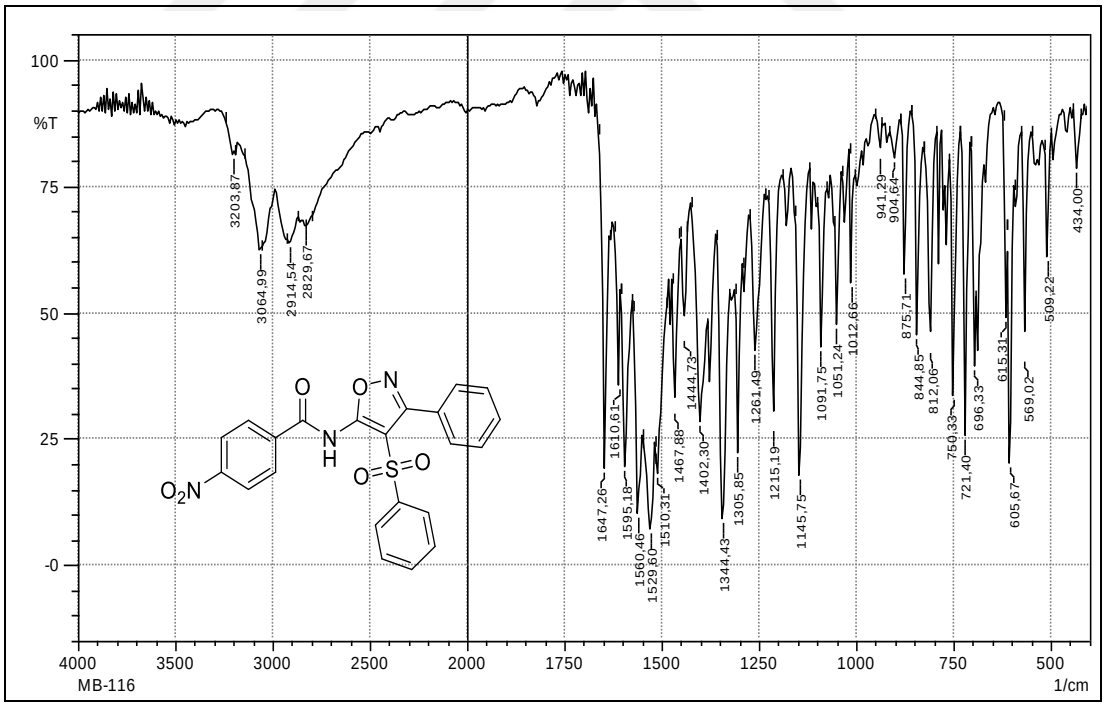


Figure 7.60. IR spectrum of compound 140a

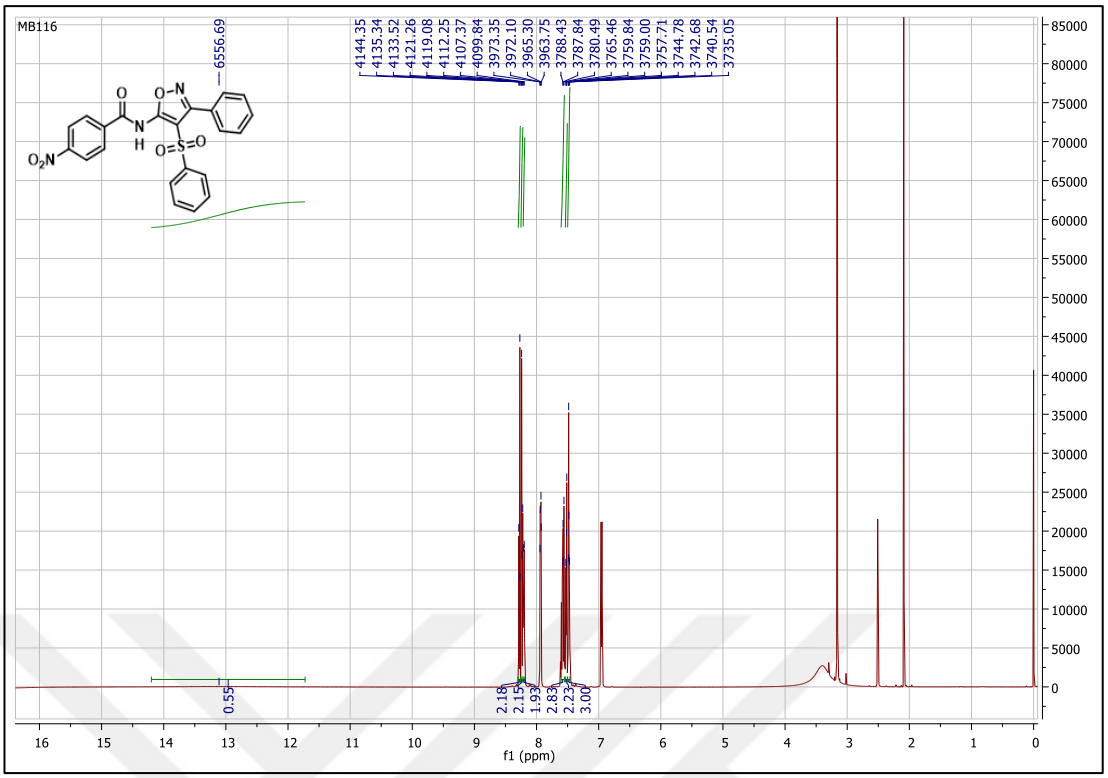


Figure 7.61. ^{1}H NMR spectrum of compound 140a

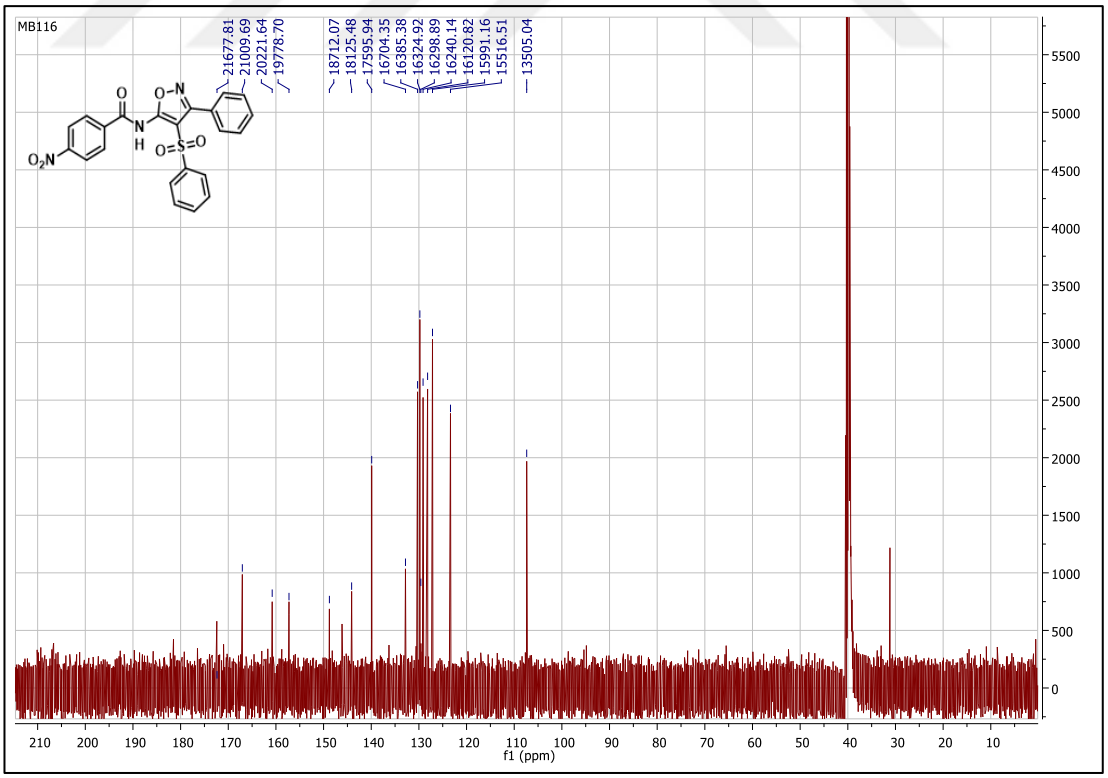


Figure 7.62. ^{13}C NMR spectrum of compound 140a

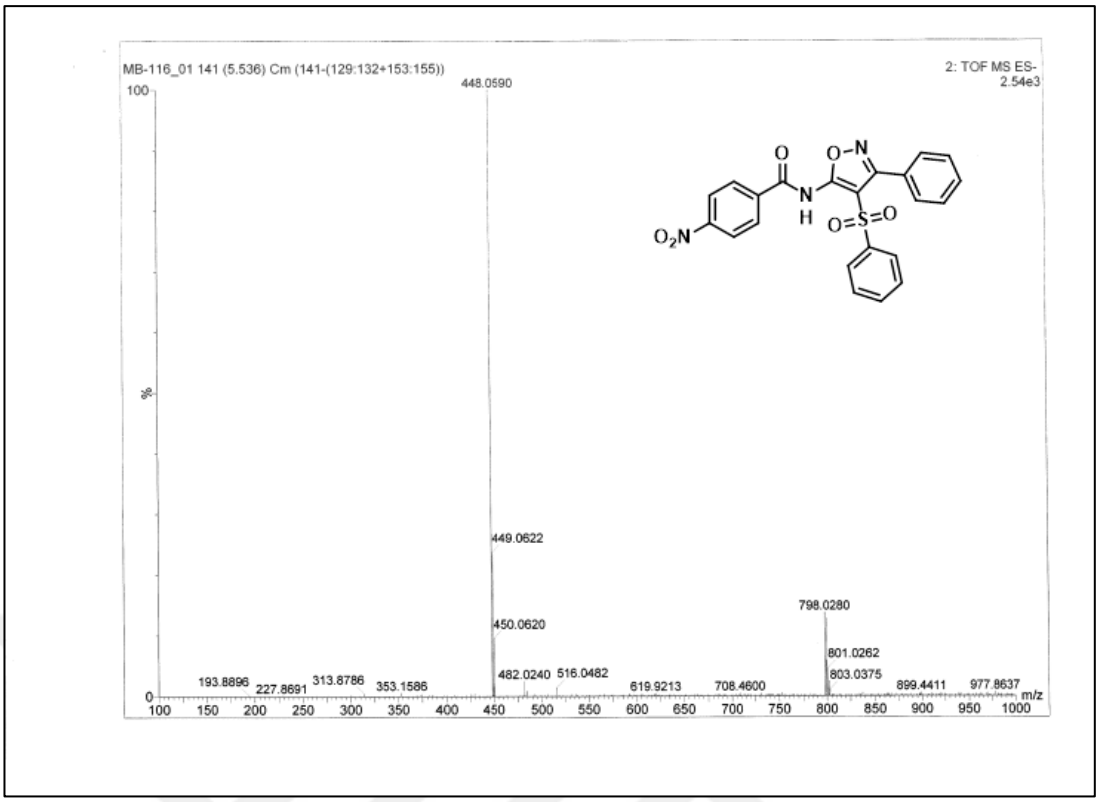
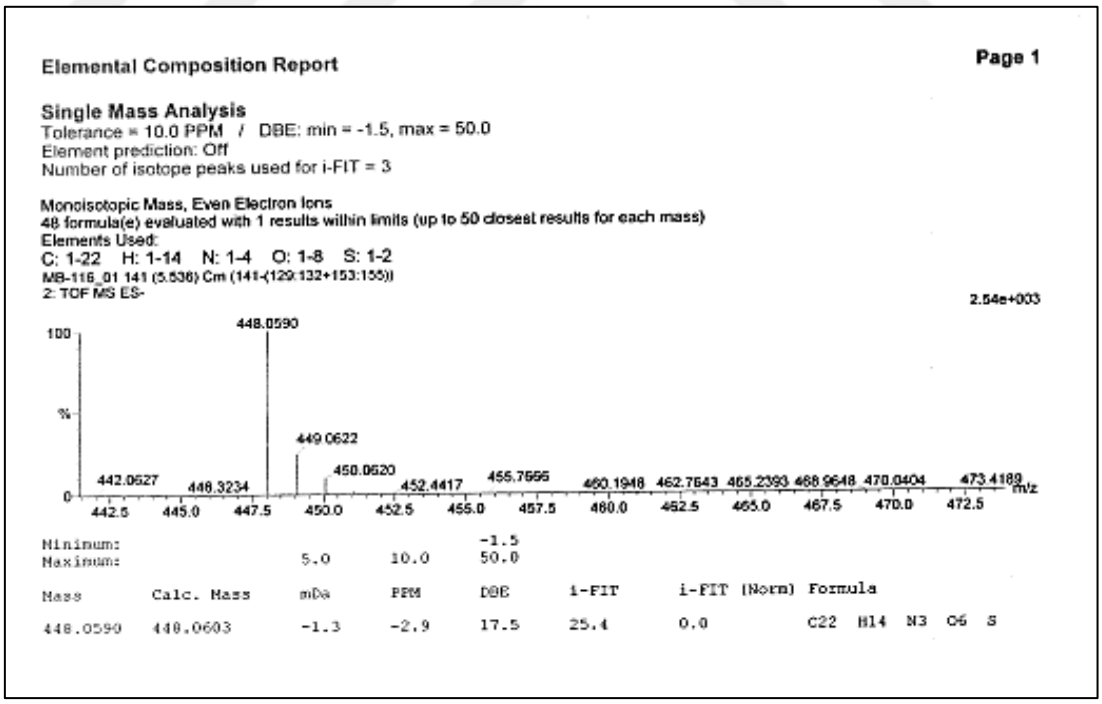


Figure 7.63. HRMS spectrum of compound 140a



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
48 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 1-22 H: 1-14 N: 1-4 O: 1-8 S: 1-2
MB-116_01 141 (5.536) Cm (141-(129:132+153:155))
2: TOF MS ES- 2.54e+003

448.0590
449.0622
450.0620
442.0527
446.3234
452.4417
455.7695
460.1948
462.7643
465.2393
468.9648
470.0404
473.4189
m/z

Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
448.0590	448.0603	-1.3	-2.9	17.5	25.4	0.0	C22 H14 N3 O6 S

Figure 7.64. Elemental composition of compound 140a

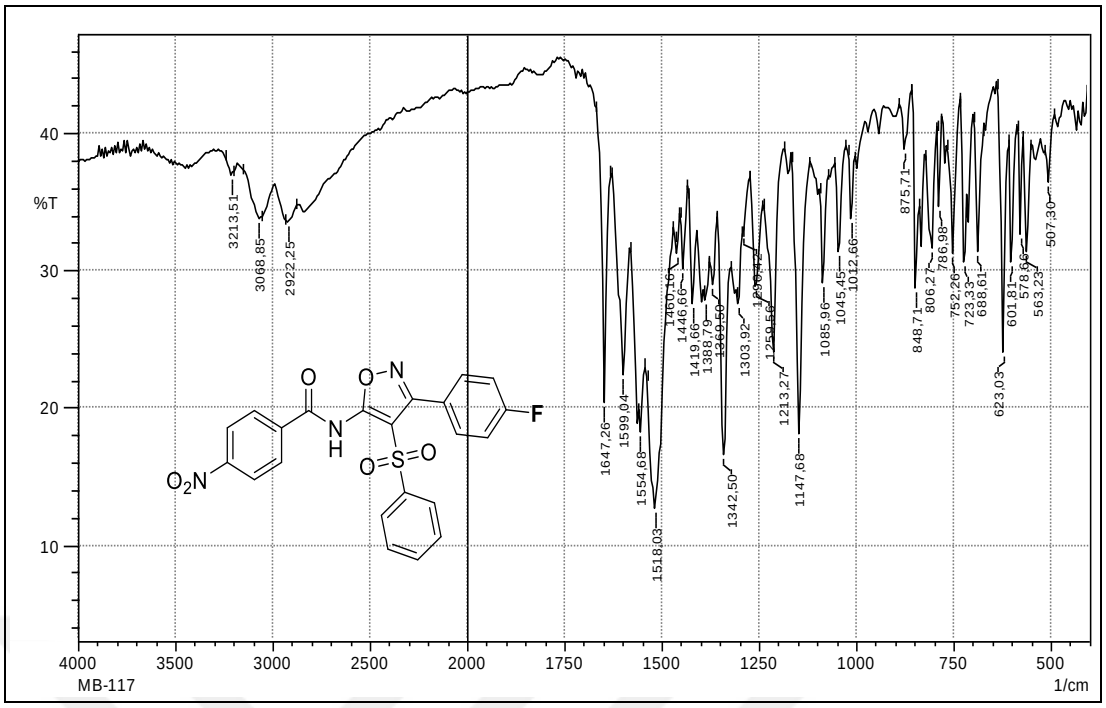


Figure 7.65. IR spectrum of compound 140b

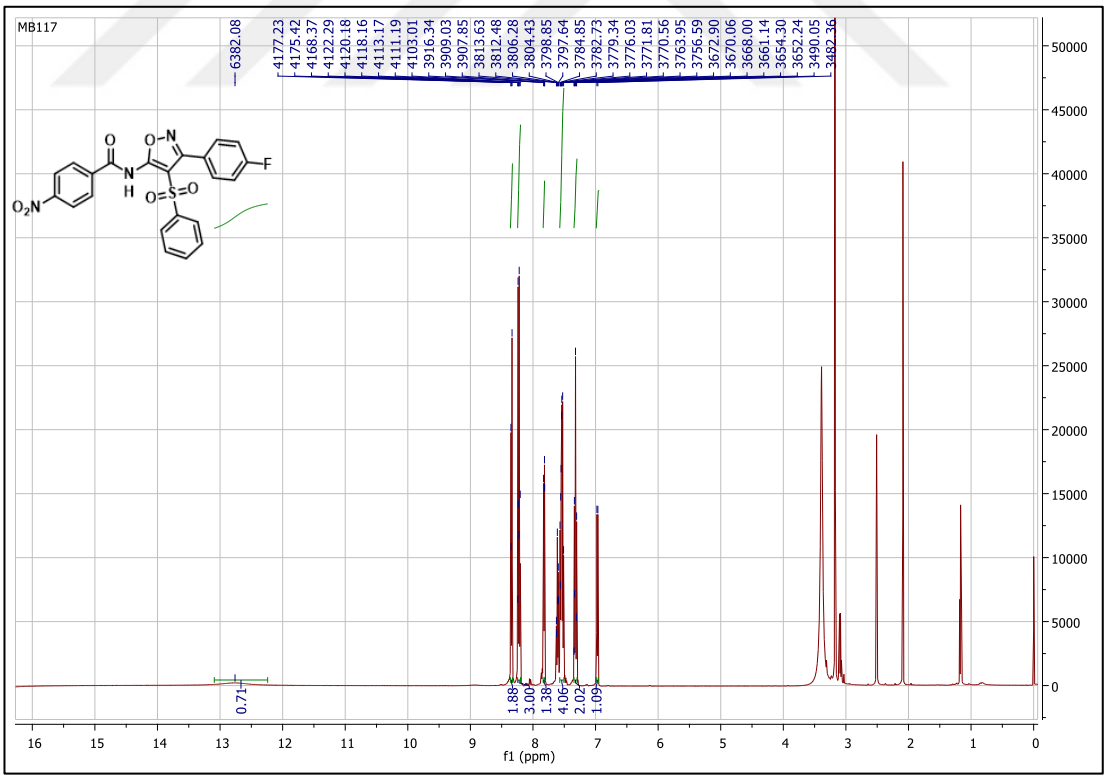


Figure 7.66. ^{1}H NMR spectrum of compound 140b

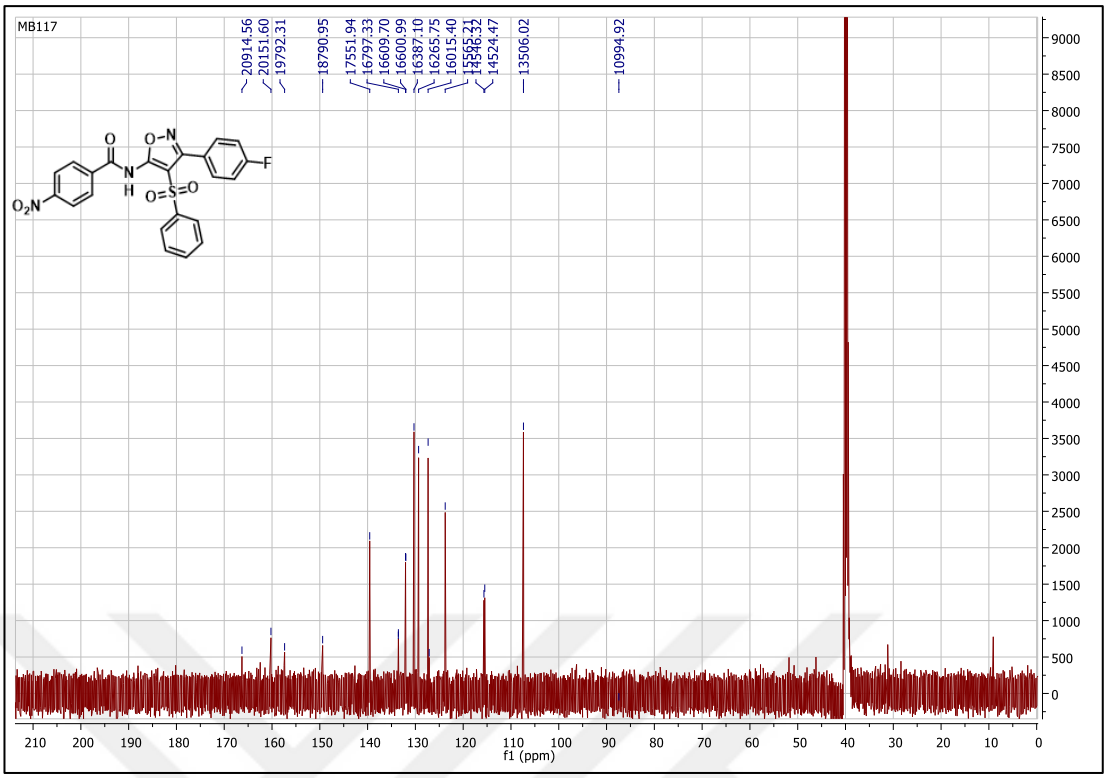


Figure 7.67. ^{13}C NMR spectrum of compound 140b

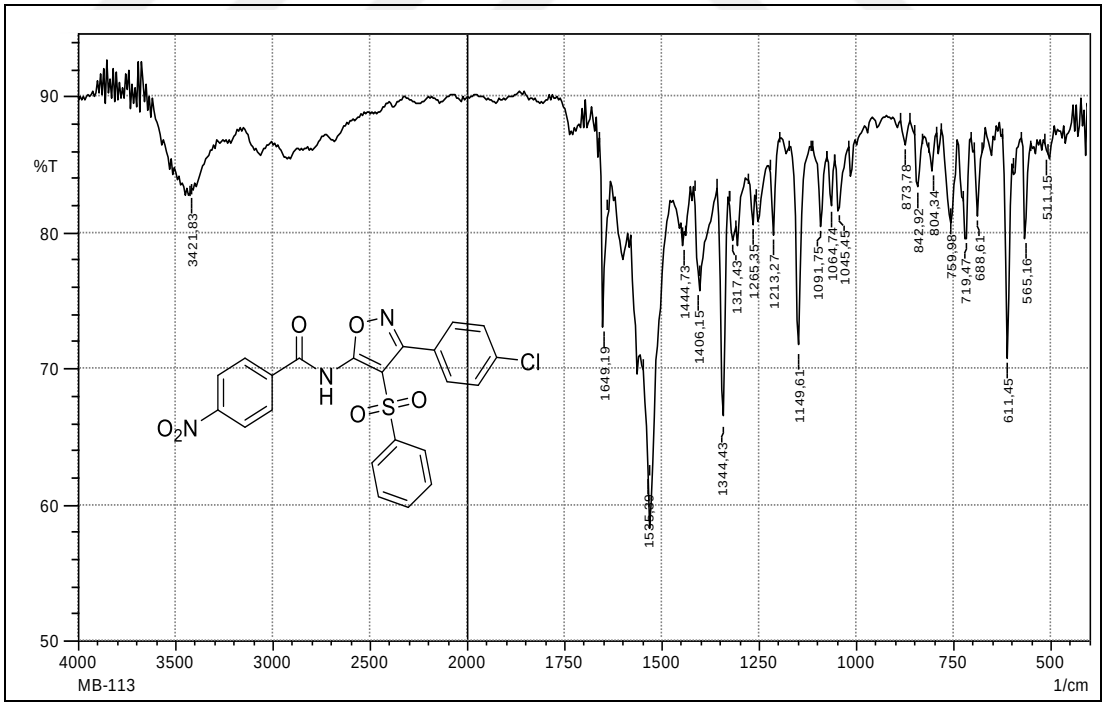


Figure 7.68. IR spectrum of compound 140c

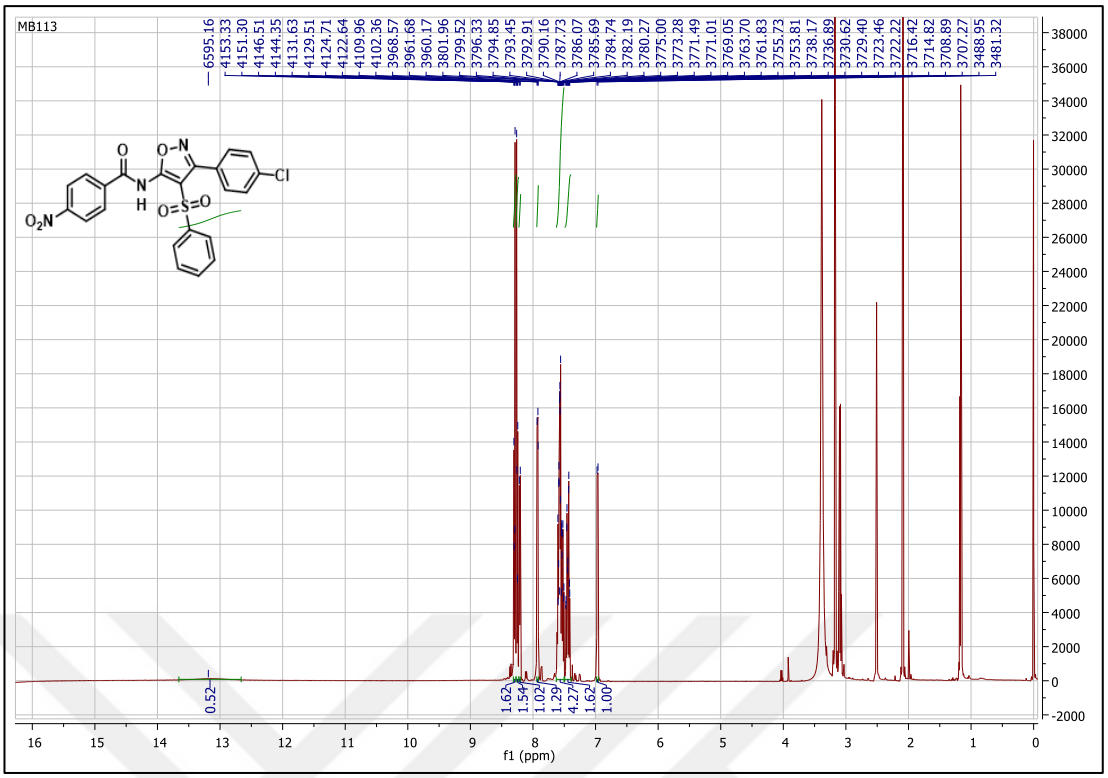


Figure 7.69. ^{1}H NMR spectrum of compound 140c

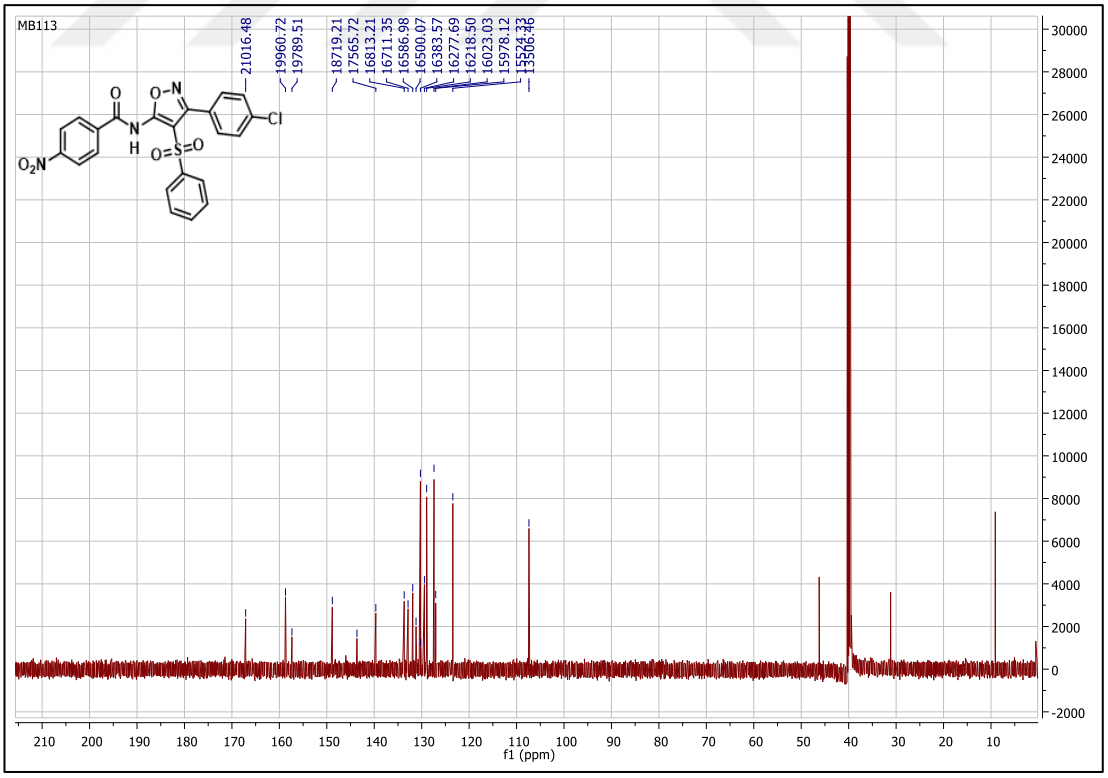


Figure 7.70. ^{13}C NMR spectrum of compound 140c

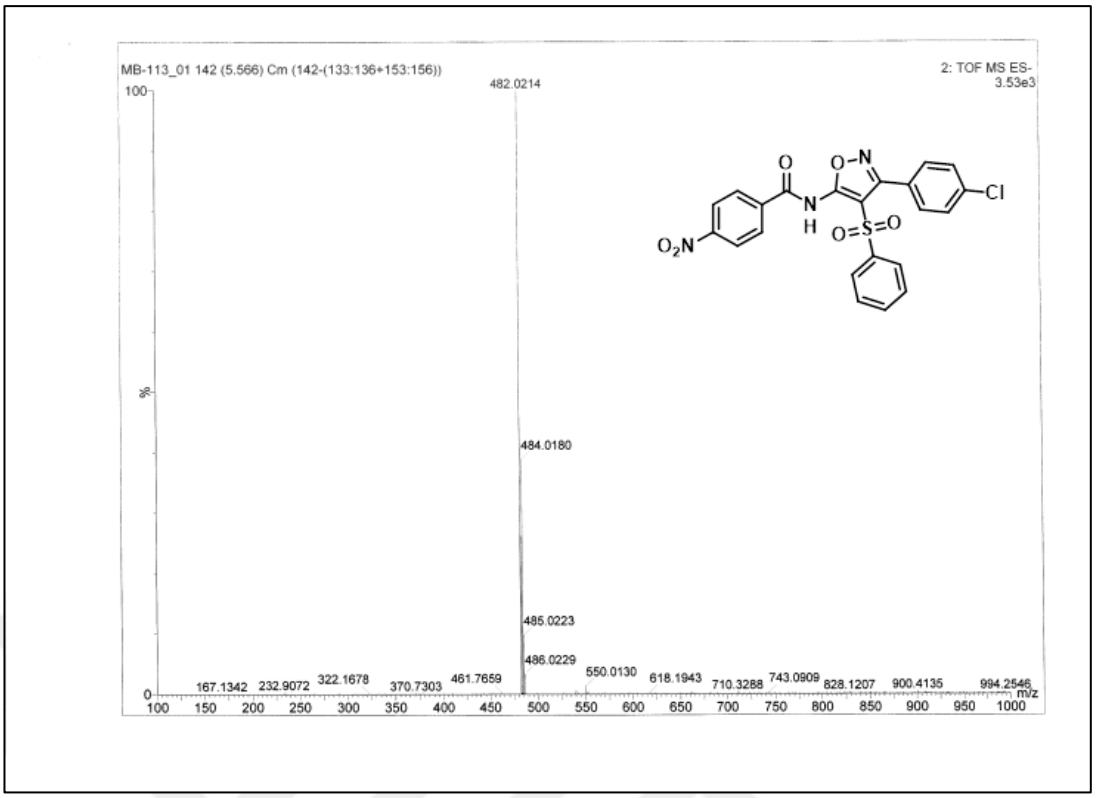
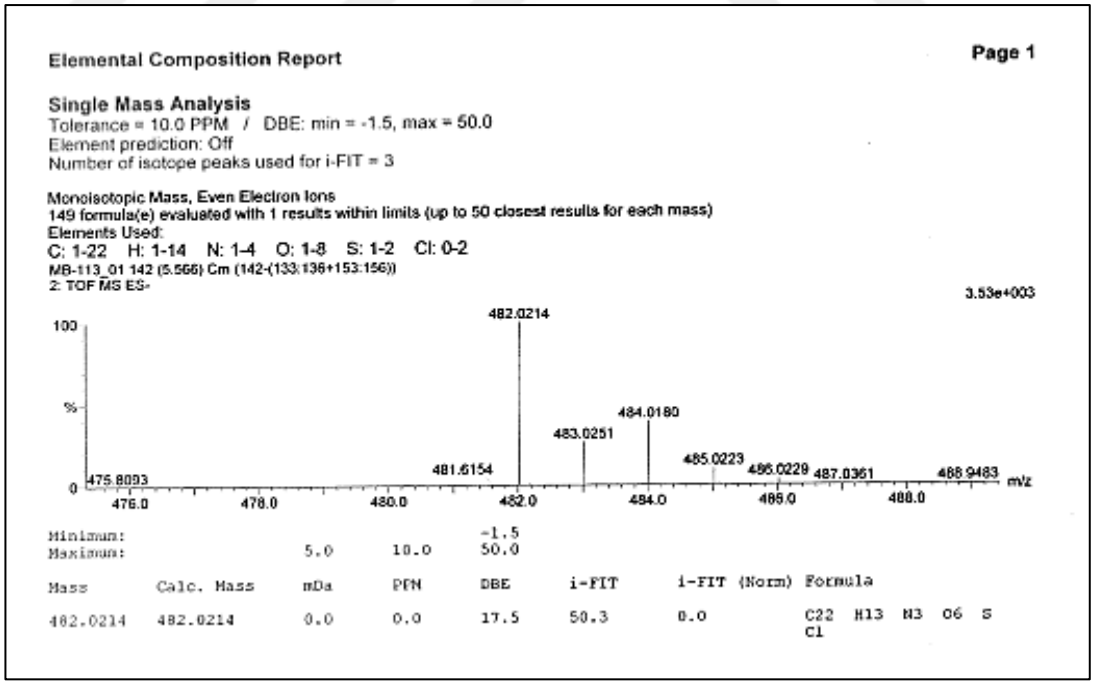


Figure 7.71. HRMS spectrum of compound 140c



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
149 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 1-22 H: 1-14 N: 1-4 O: 1-8 S: 1-2 Cl: 0-2
MB-113_01 142 (5.566) Cm (142-(133:136+153:156))
2: TOF MS ES-
3.53e+003

Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
482.0214	482.0214	0.0	0.0	17.5	50.3	0.0	C22 H13 N3 O6 S Cl

Figure 7.72. Elemental composition of compound 140c

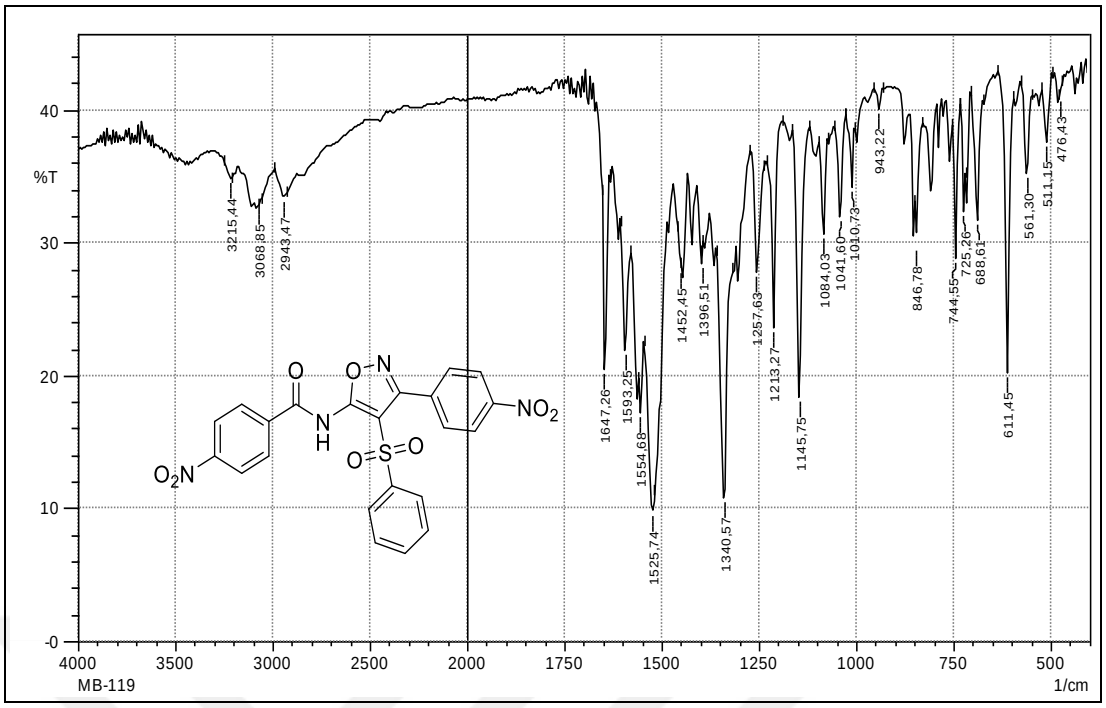


Figure 7.73. IR spectrum of compound 140d

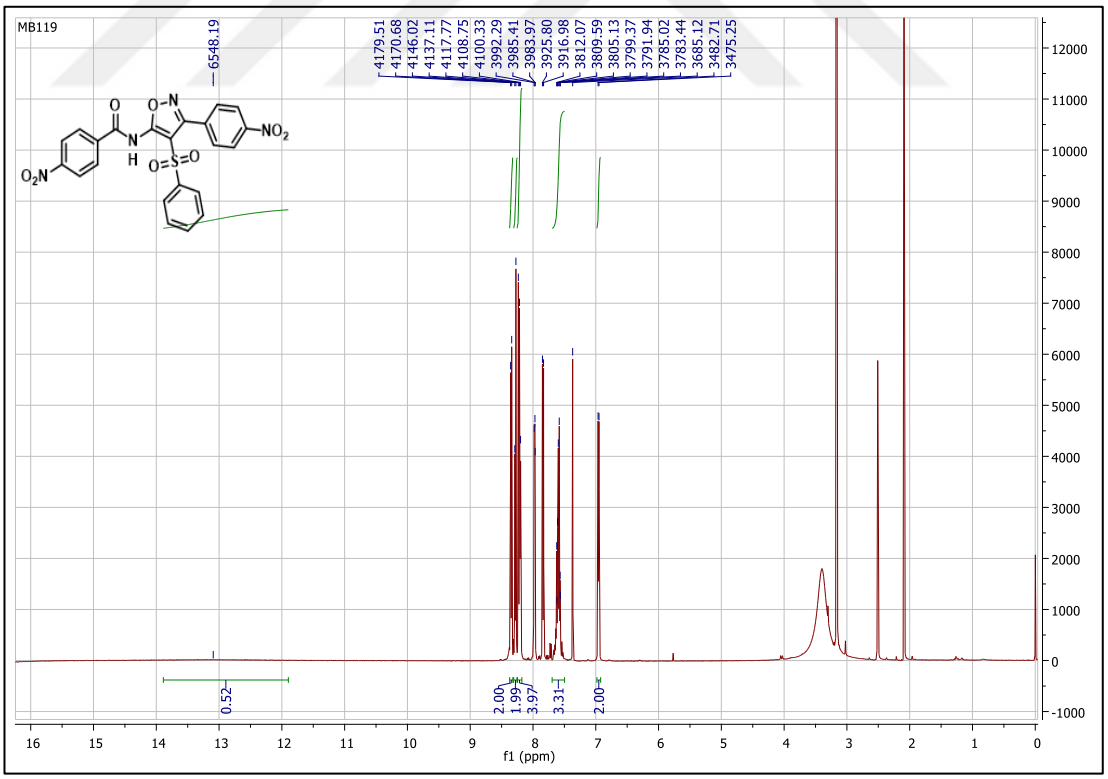


Figure 7.74. ^{1}H NMR spectrum of compound 140d

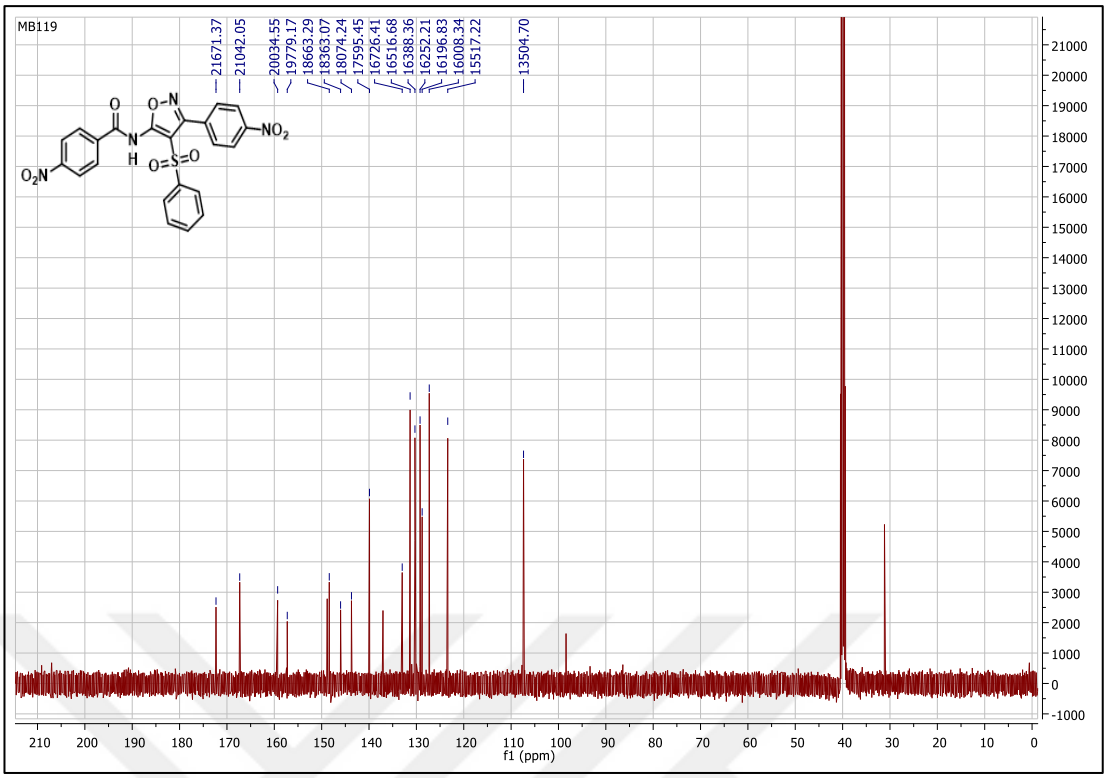


Figure 7.75. ^{13}C NMR spectrum of compound 140d

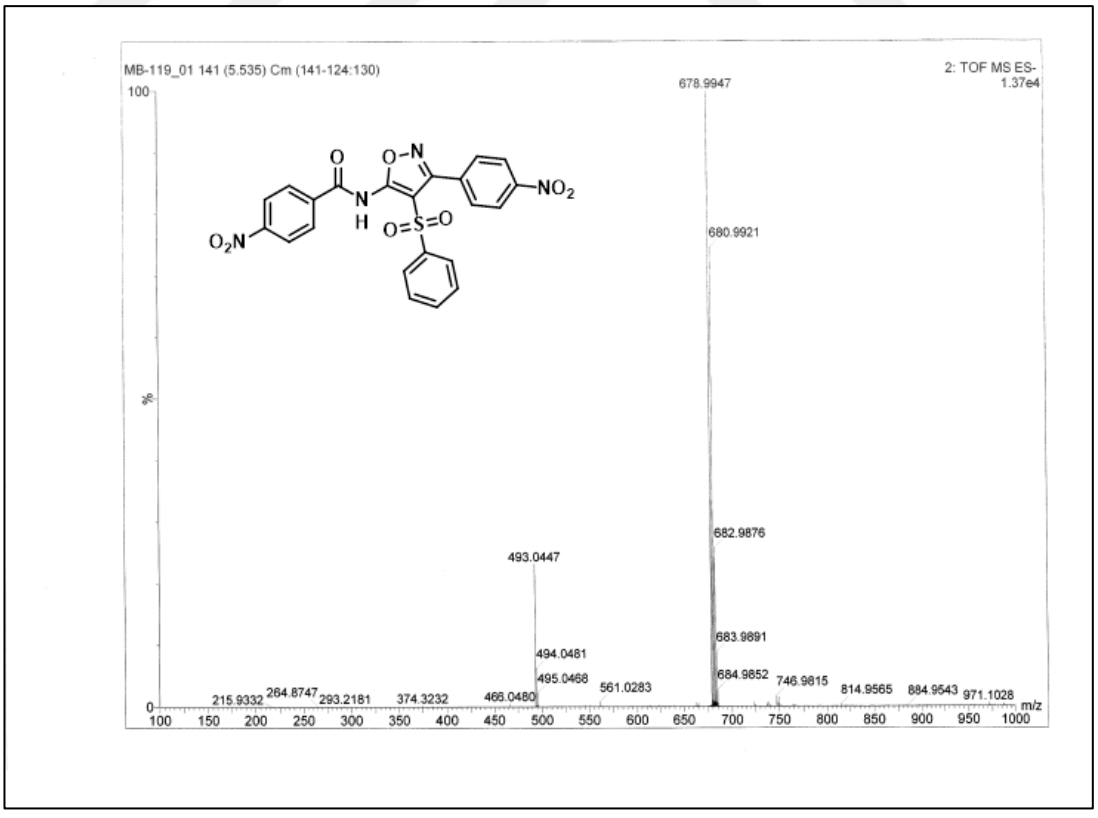


Figure 7.76. HRMS spectrum of compound 140d

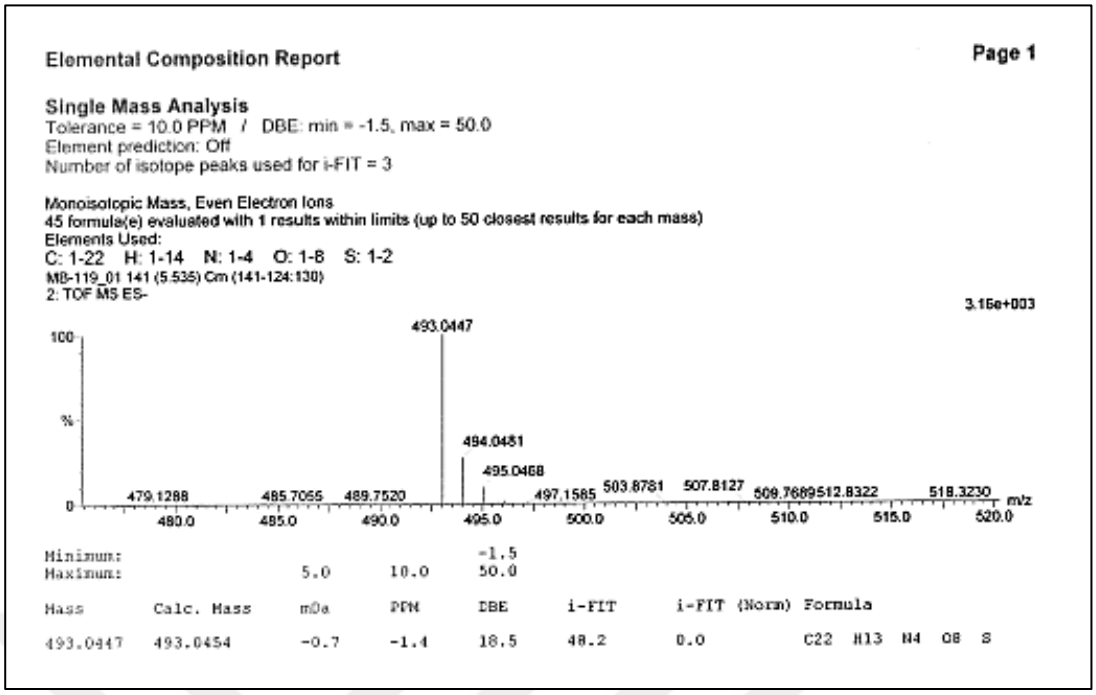


Figure 7.77. Elemental composition of compound 140d

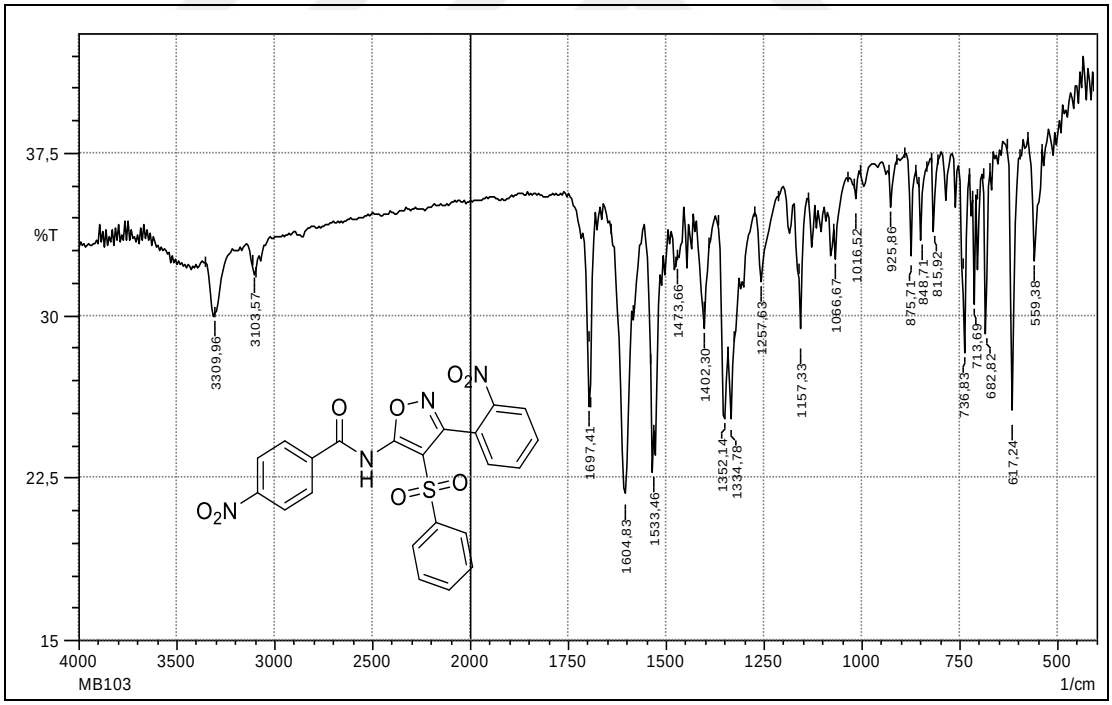


Figure 7.78. IR spectrum of compound 140e

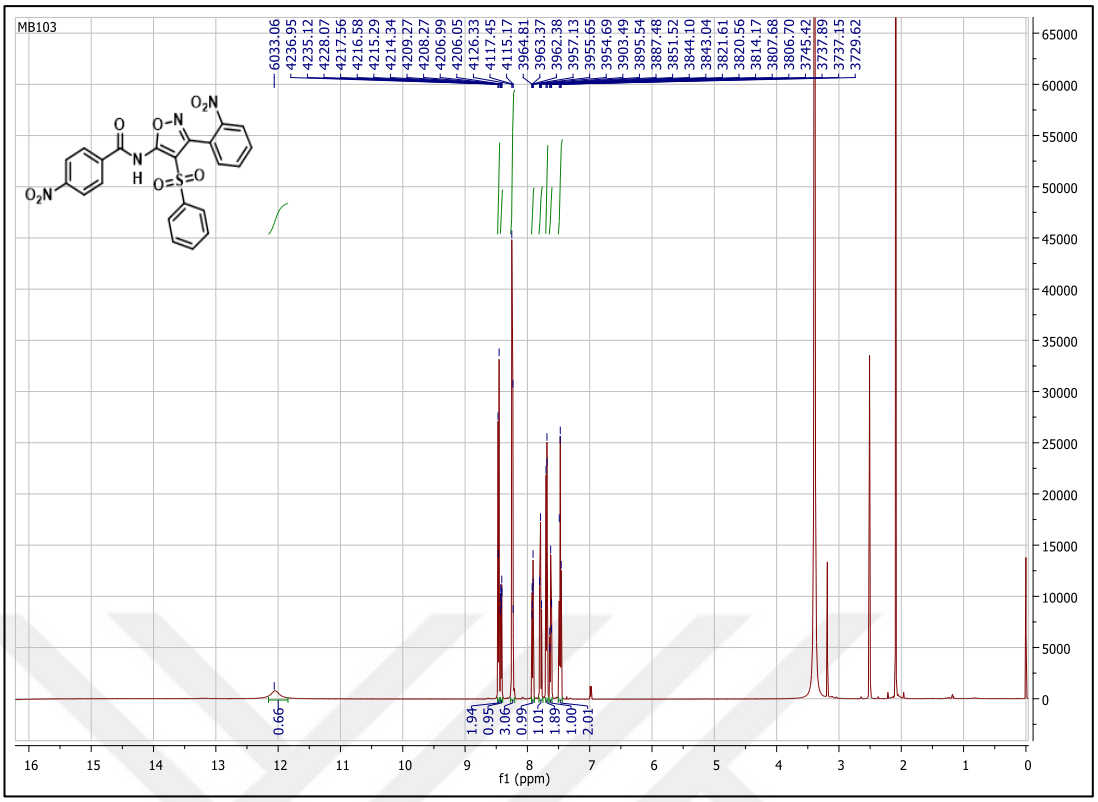


Figure 7.79. ^{1}H NMR spectrum of compound 140e

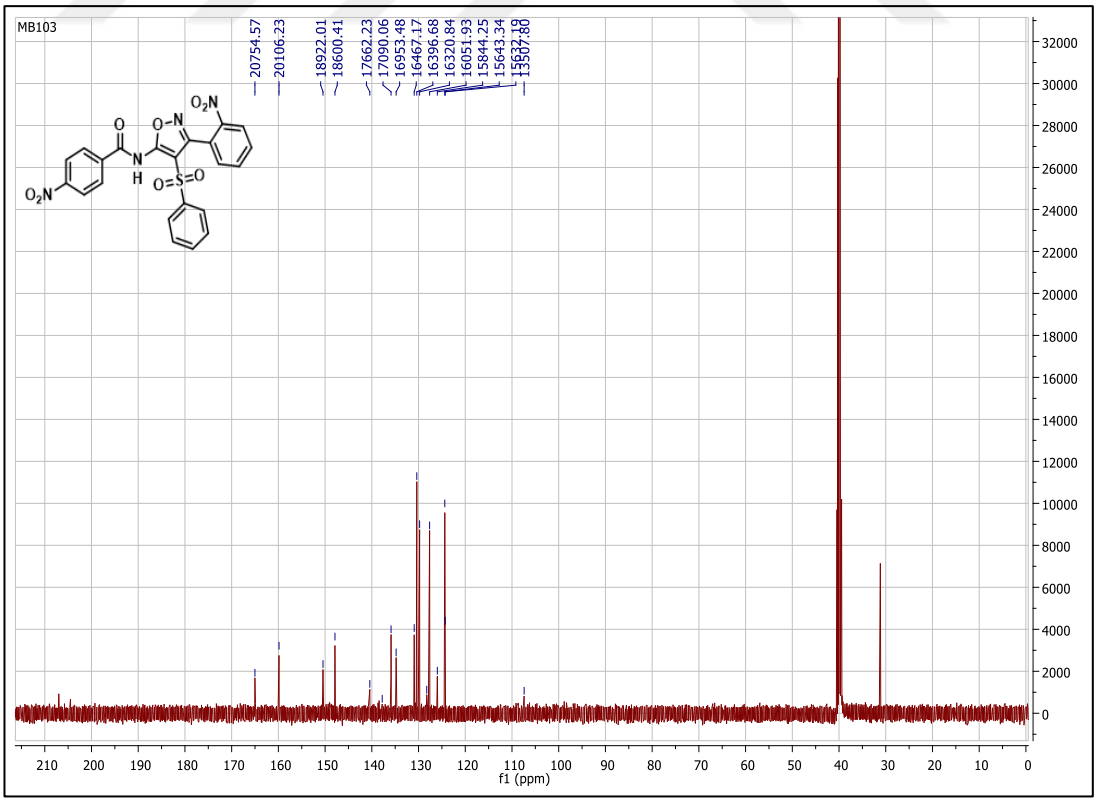


Figure 7.80. ^{13}C NMR spectrum of compound 140e

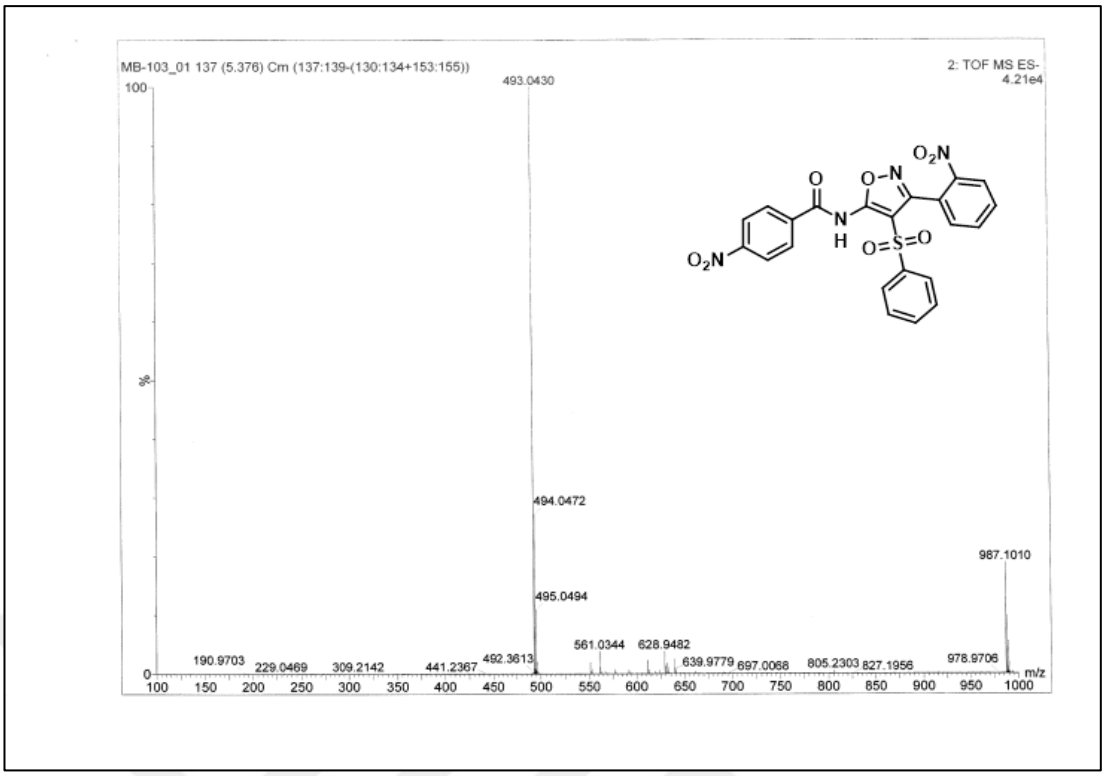


Figure 7.81. HRMS spectrum of compound 140e

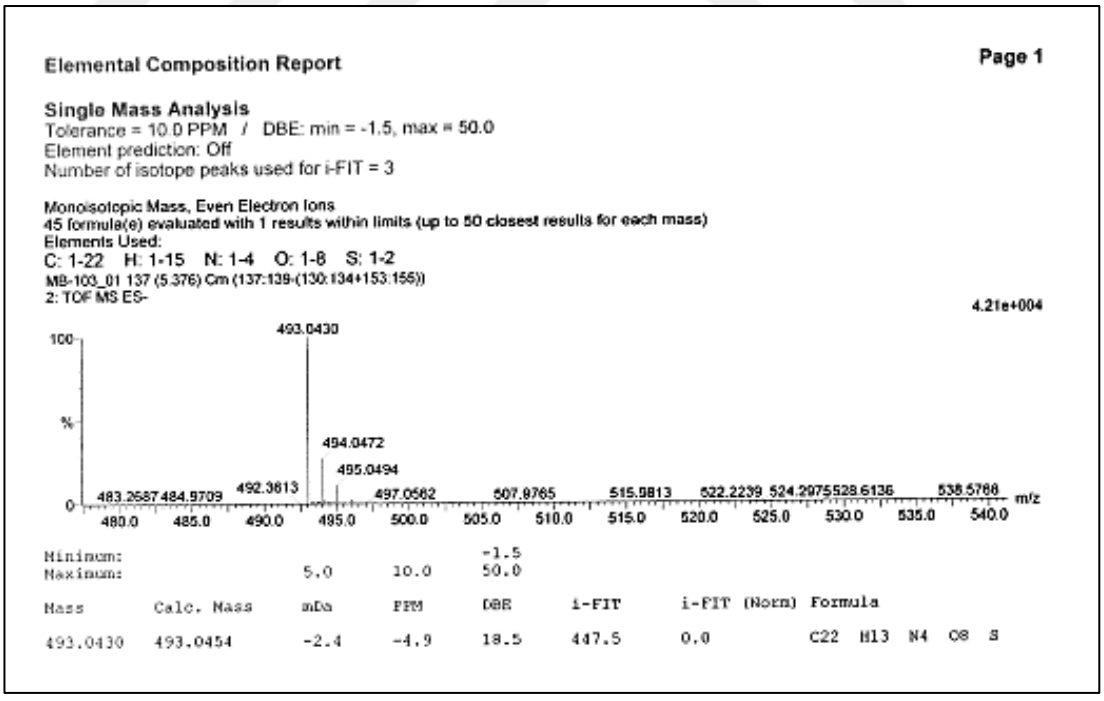


Figure 7.82. Elemental composition of compound 140e

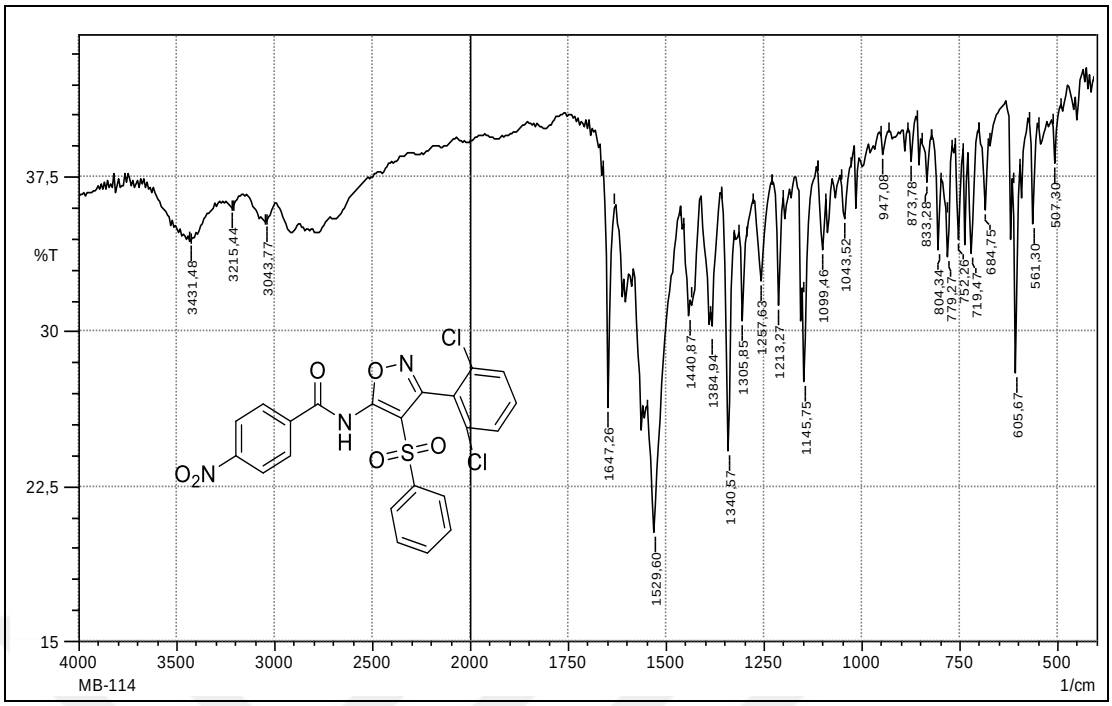


Figure 7.83. IR spectrum of compound 140f

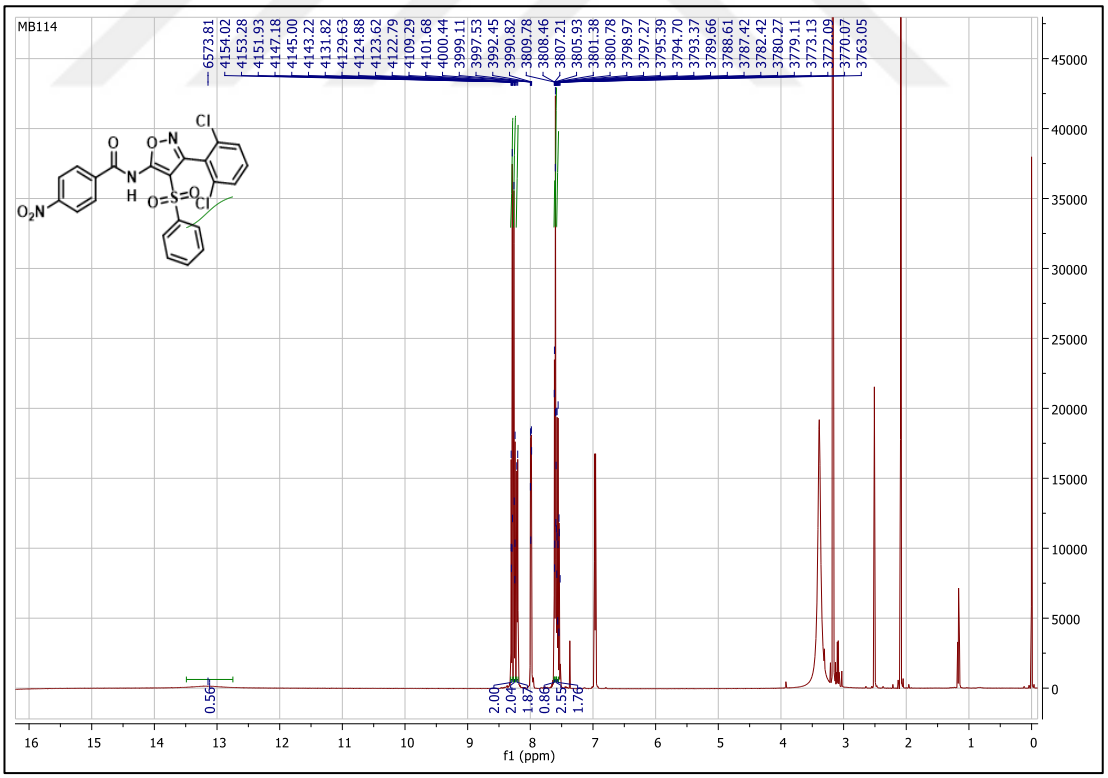


Figure 7.84. ^{1}H NMR spectrum of compound 140f

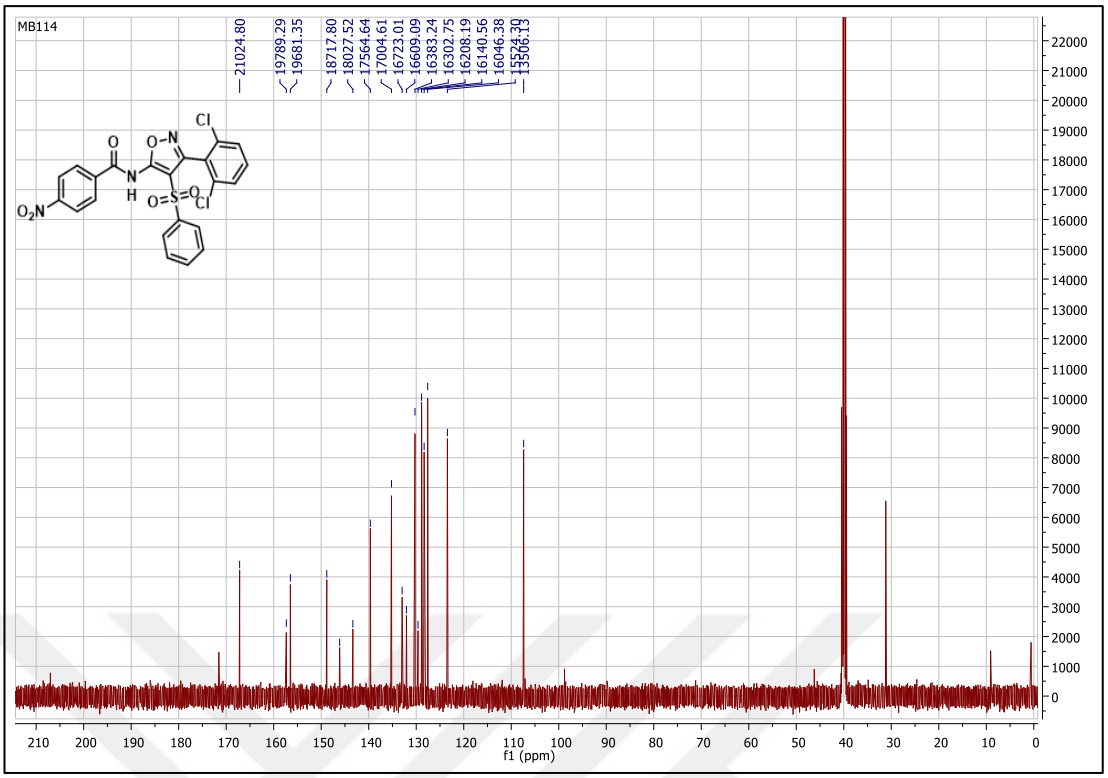


Figure 7.85. ^{13}C NMR spectrum of compound 140f

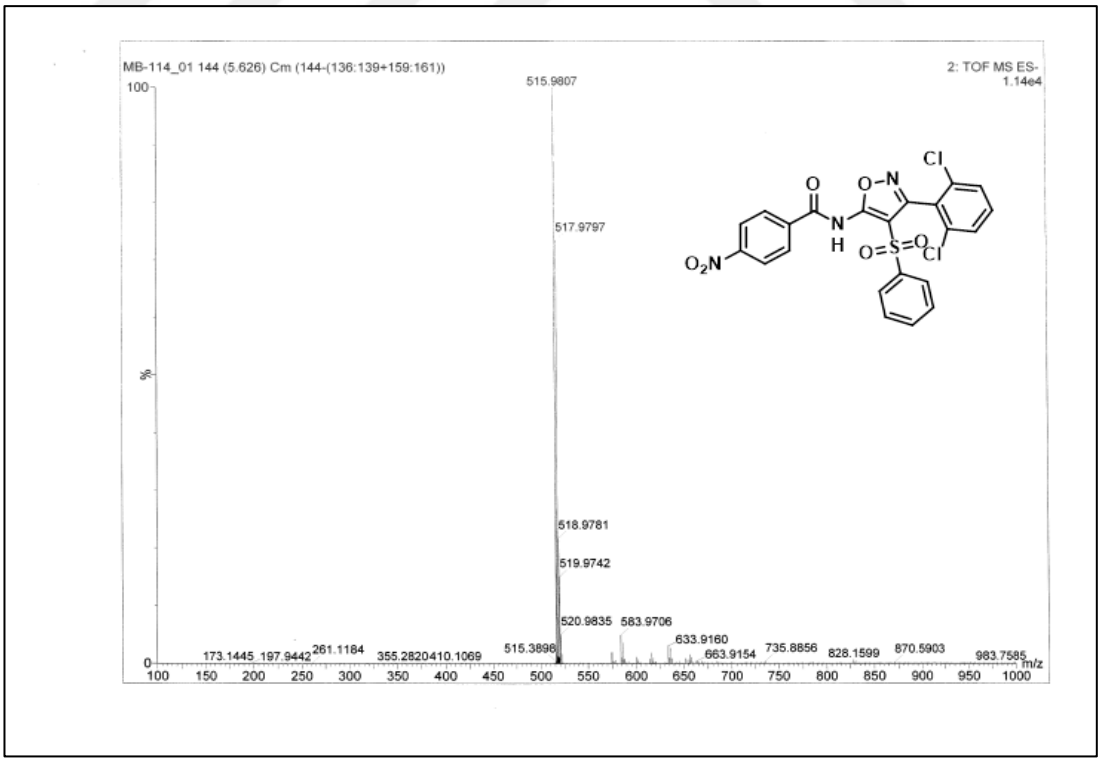


Figure 7.86. HRMS spectrum of compound 140f

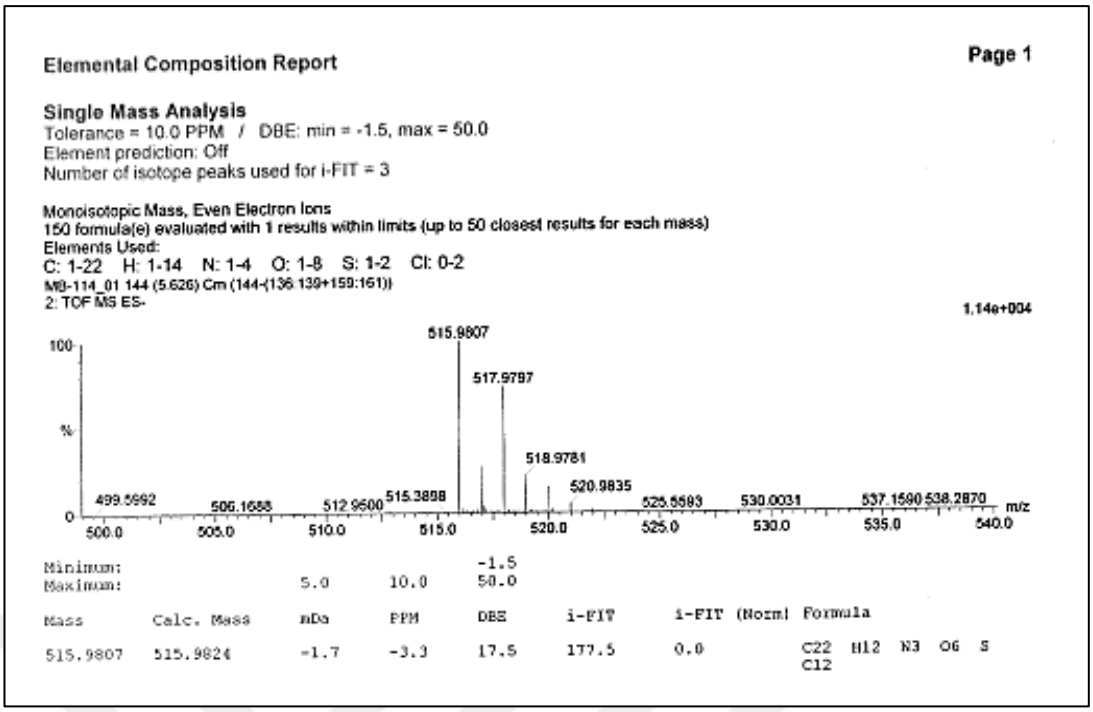


Figure 7.87. Elemental composition of compound 140f

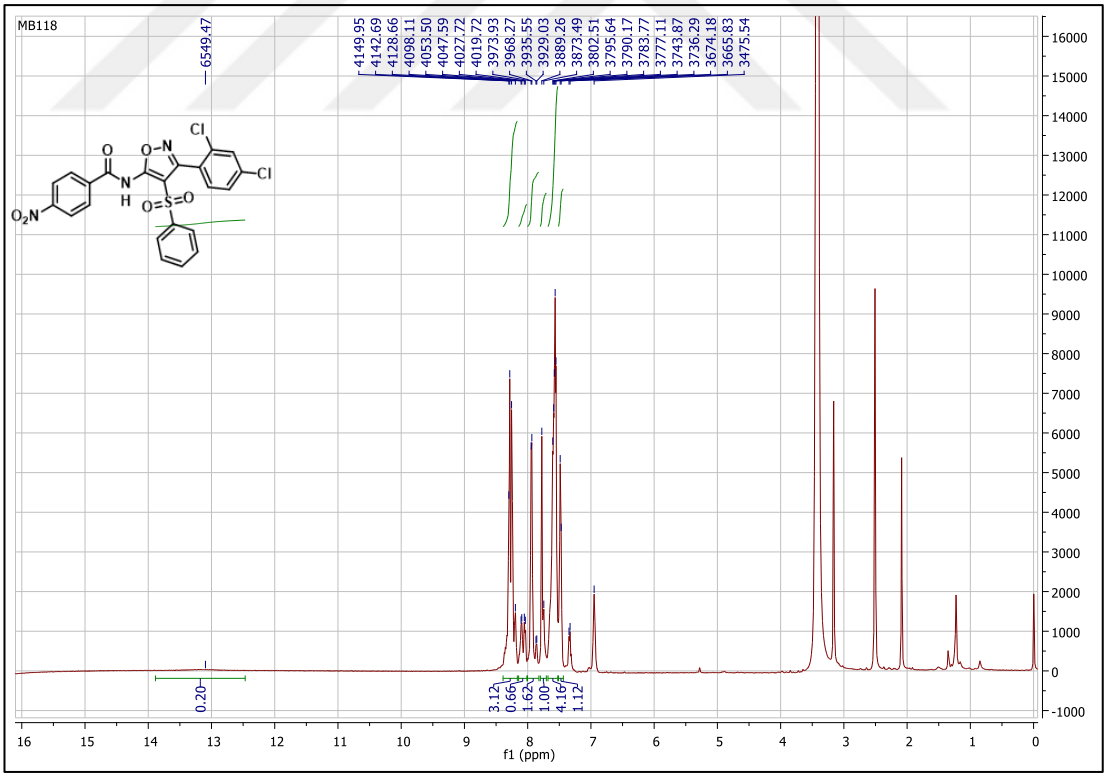


Figure 7.88. ^{1}H NMR spectrum of compound 140g

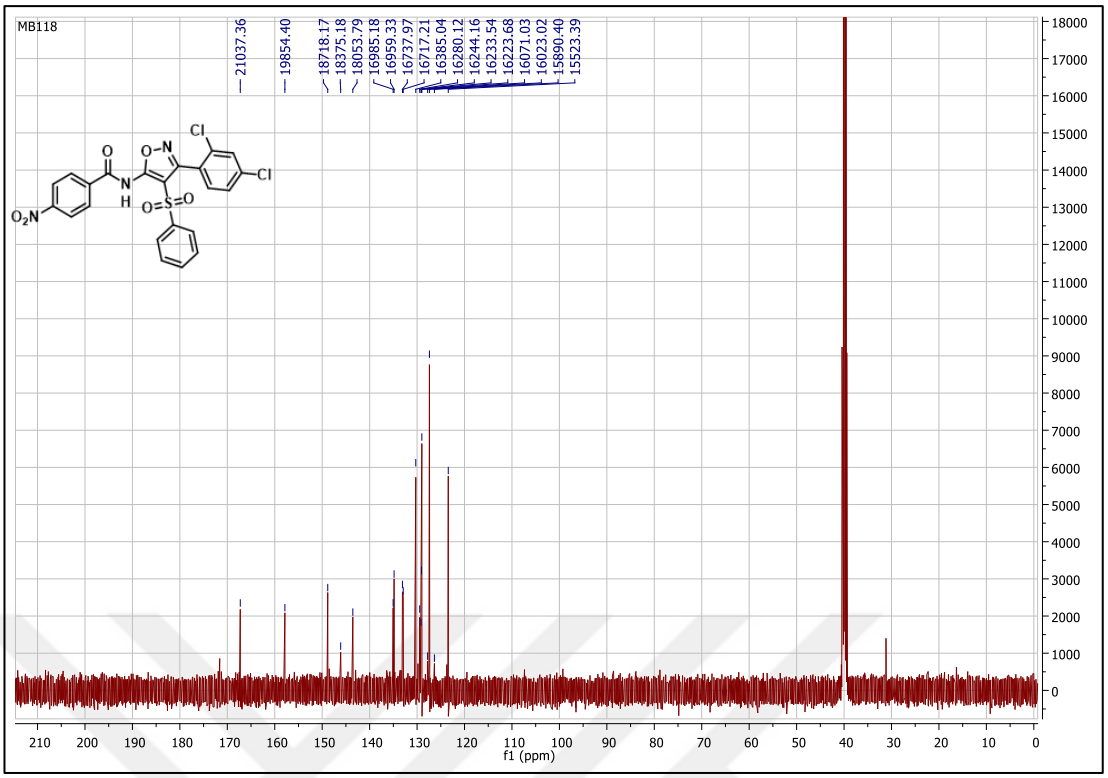


Figure 7.89. ^{13}C NMR spectrum of compound 140g

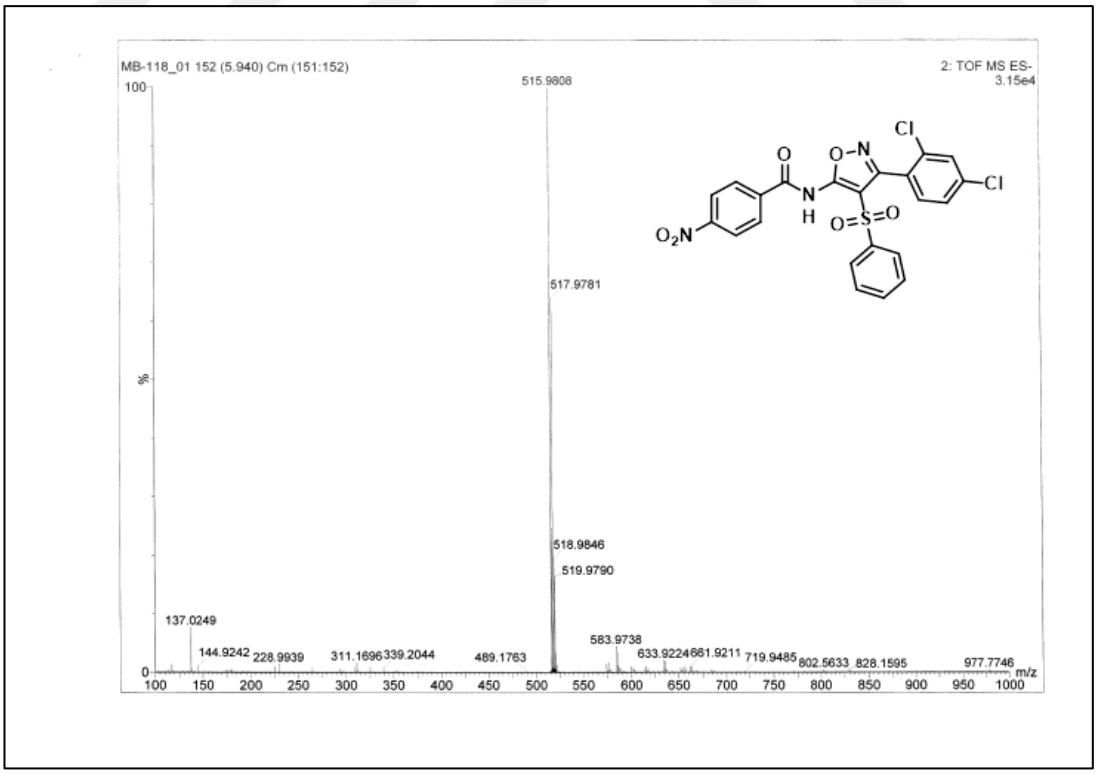


Figure 7.90. HRMS spectrum of compound 140g

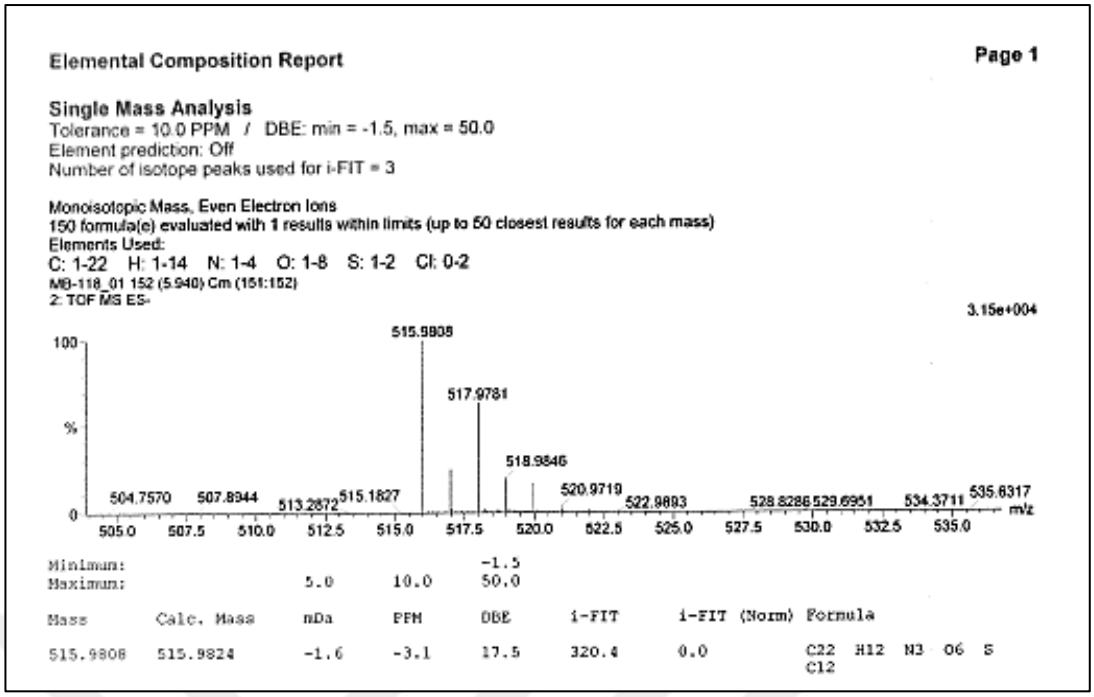


Figure 7.91. Elemental composition of compound 140g

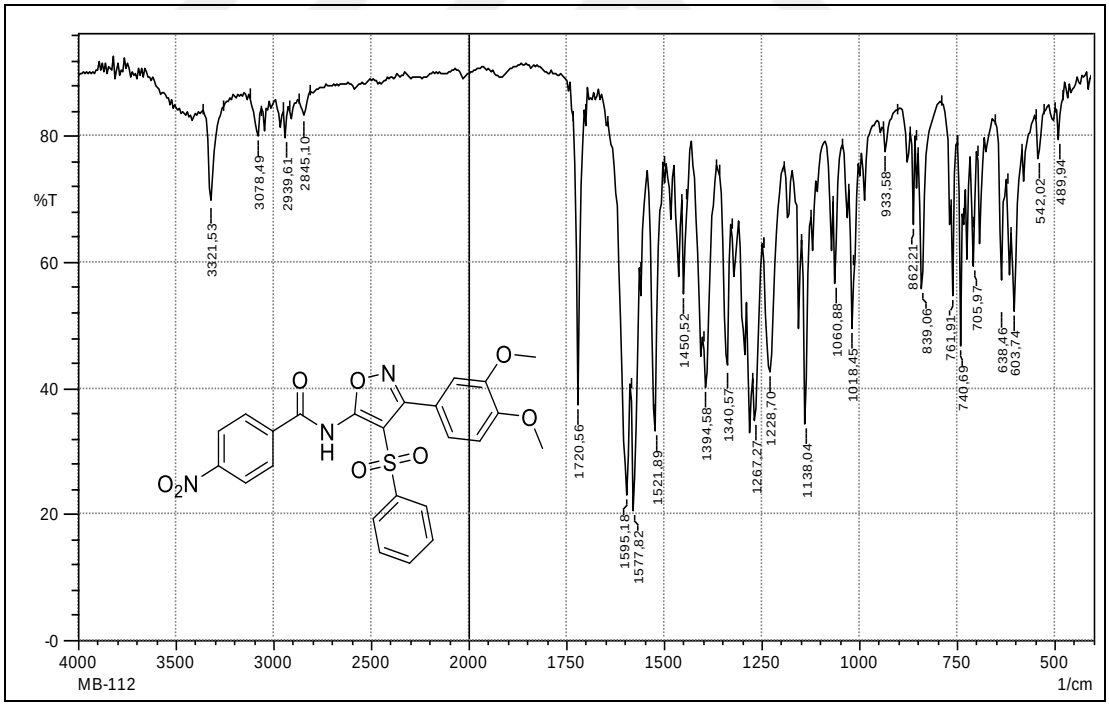


Figure 7.92. IR spectrum of compound 140h

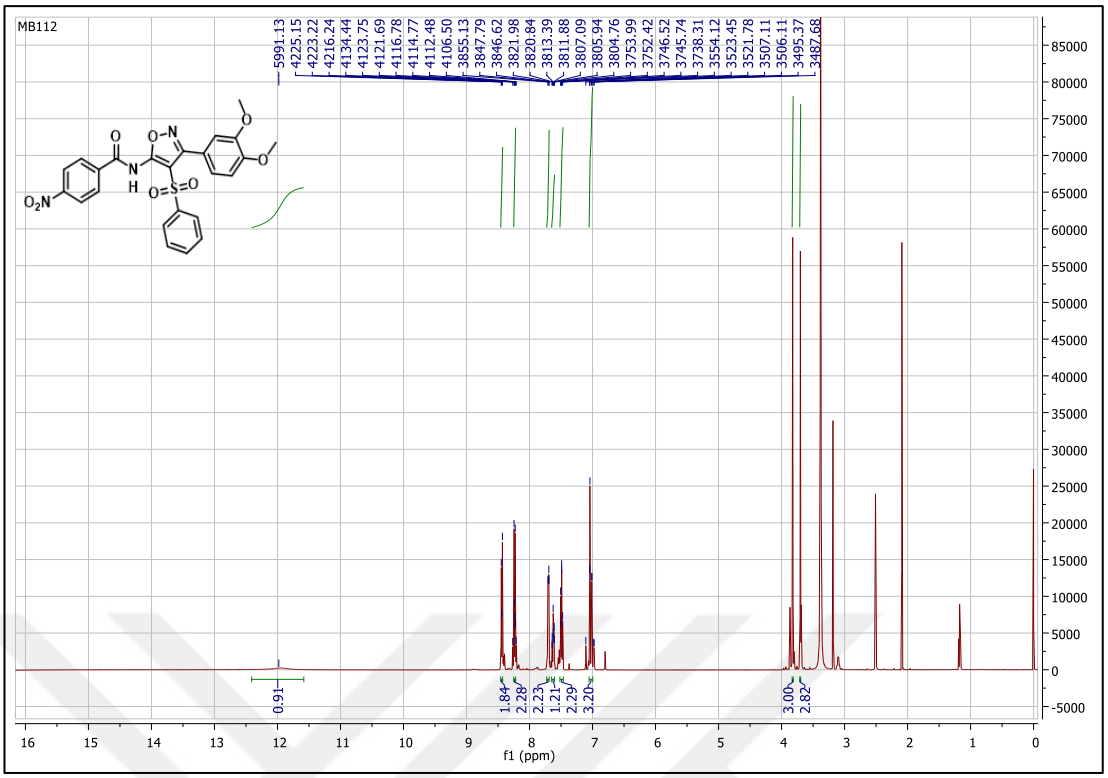


Figure 7.93. ^{1}H NMR spectrum of compound 140h

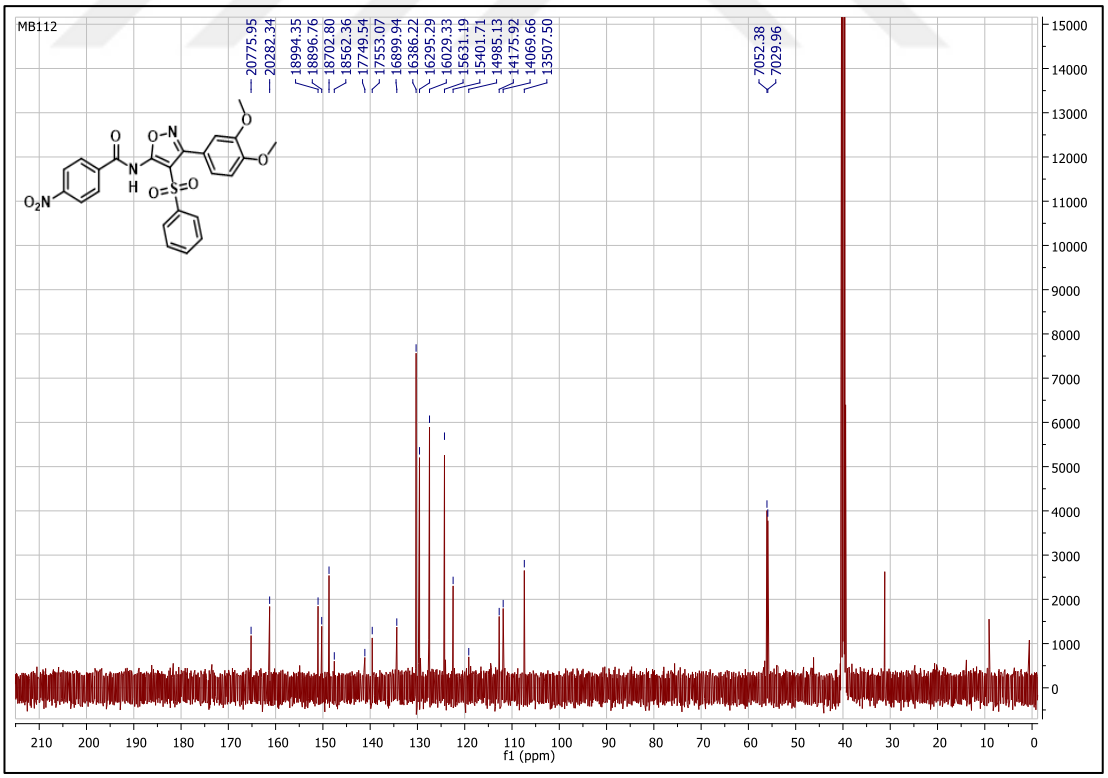


Figure 7.94. ^{13}C NMR spectrum of compound 140h

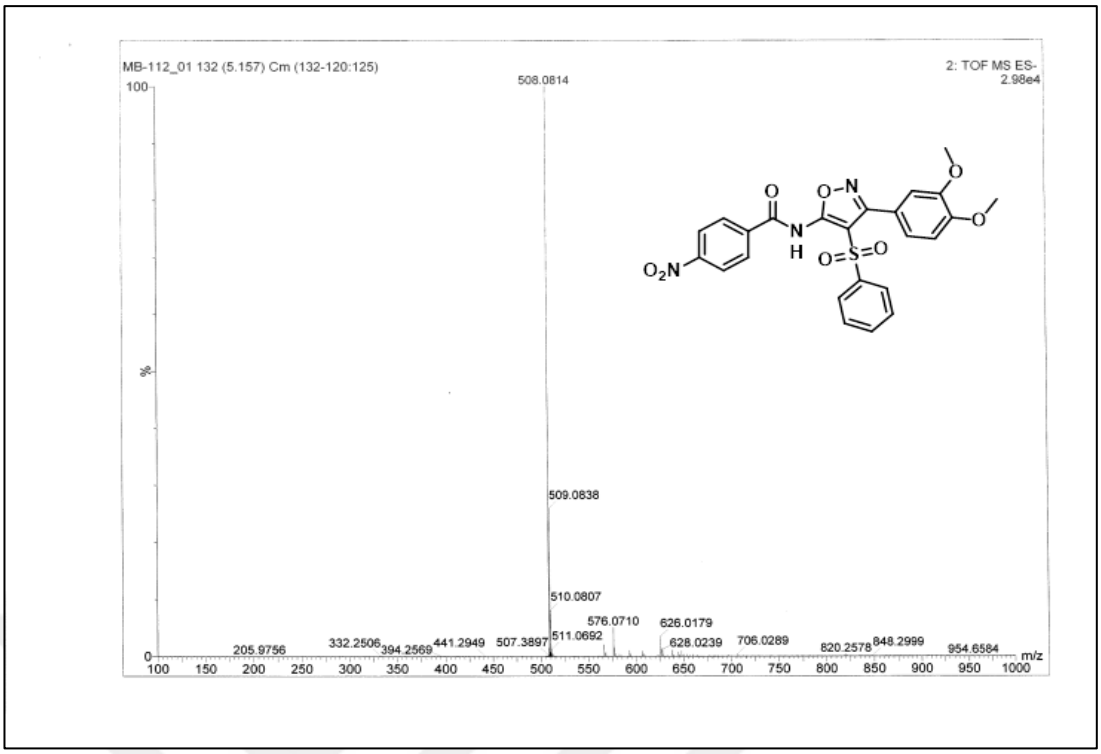
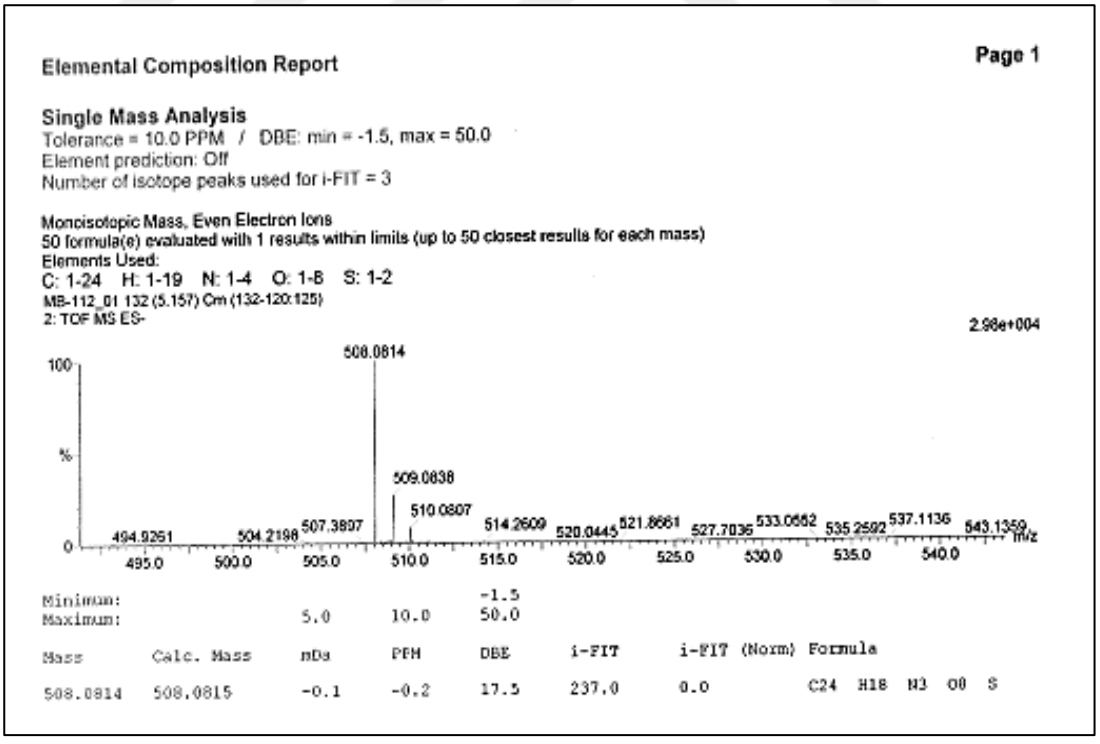


Figure 7.95. HRMS spectrum of compound 140h



Elemental Composition Report

Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
50 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 1-24 H: 1-19 N: 1-4 O: 1-8 S: 1-2
MB-112_01 132 (5.157) Cm (132-120:125)
2: TOF MS ES-
2.98e+004

Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
508.0814	508.0815	-0.1	-0.2	17.5	237.0	0.0	C24 H18 N3 O8 S

Figure 7.96. Elemental composition of compound 140h

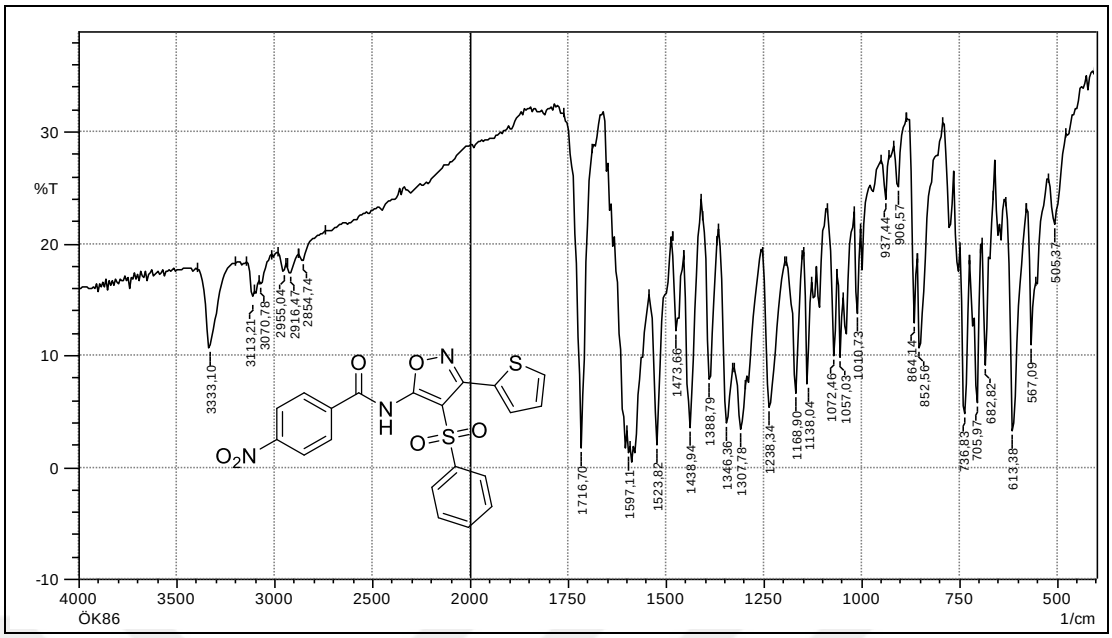


Figure 7.97. IR spectrum of compound 140i

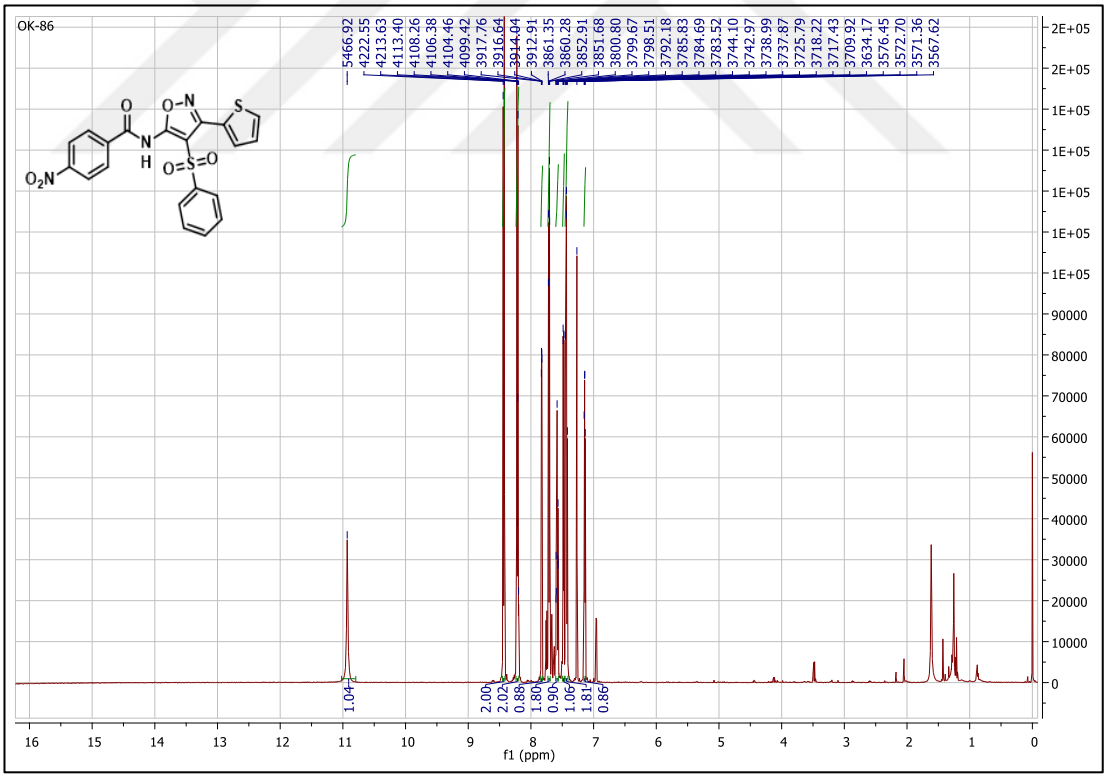


Figure 7.98. ^{1}H NMR spectrum of compound 140i

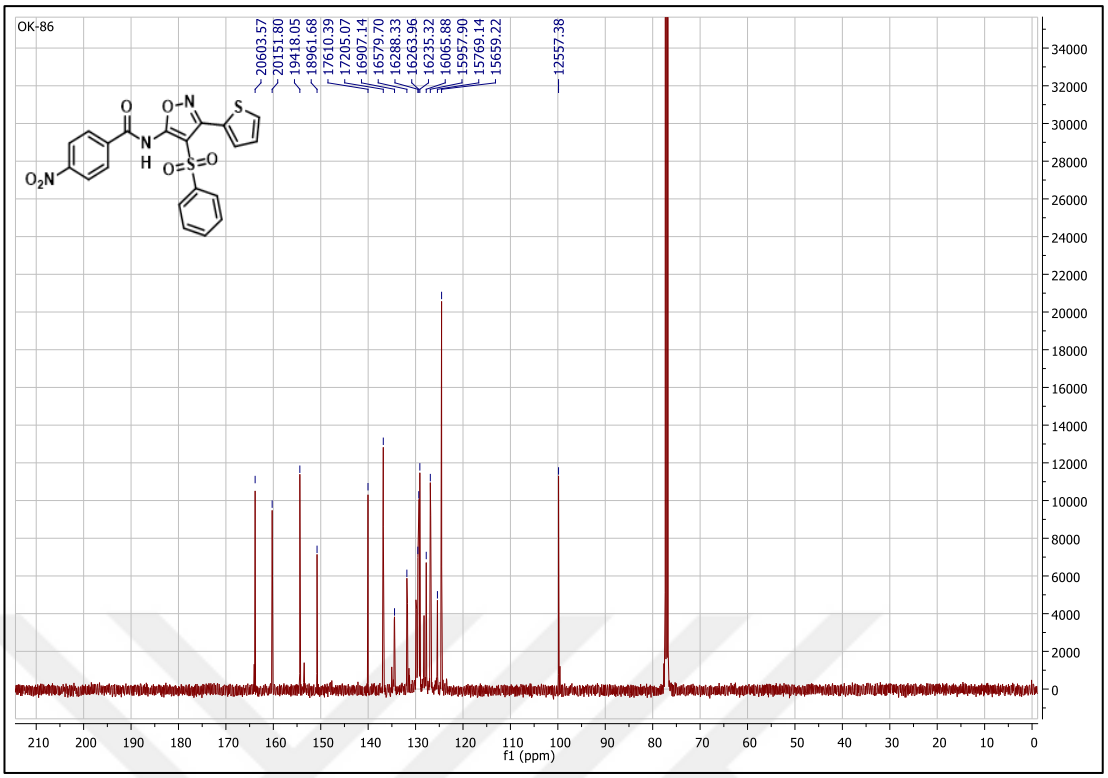


Figure 7.99. ^{13}C NMR spectrum of compound 140i

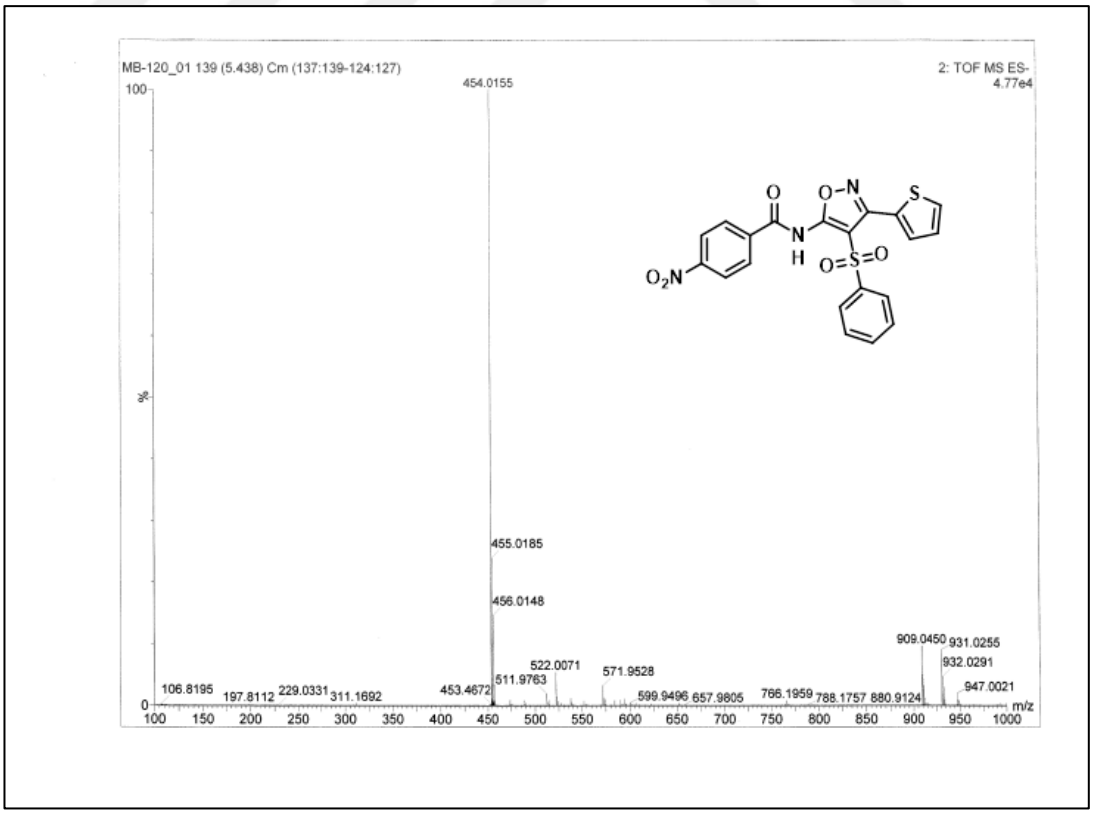


Figure 7.100. HRMS spectrum of compound 140i

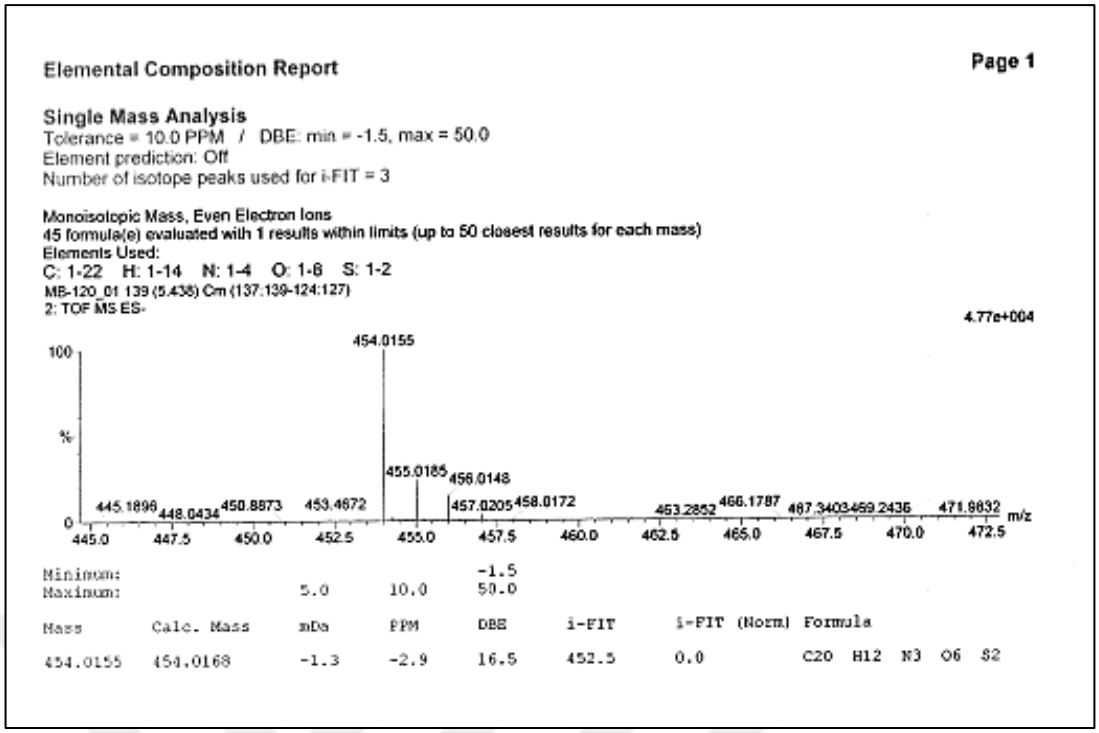


Figure 7.101. Elemental composition of compound 140i

8. CURRICULUM VITAE

Name SURNAME **:** Özge KAVAS

Place and Date of Birth **:** Kadıköy / 05.02.1990

Bachelor's Degree **:** Abant İzzet Baysal University 2013

e-mail **:** ozgekavas@hotmail.com

Address **:** Sümer Mah. Ayhan Sok. Yongacı Apt. No:3 BOLU

List of Publications **:**

- Yücesan B., Mohammed A., Büyükgöçmen R., Altuğ C., Kavas Ö., Gürel S., Gürel E., (2016) "In vitro and ex vitro propagation of Stevia rebaudiana with high Rebaudioside- A content – A commercial scale application", Scientia Horticulturae, *accepted with minor revision.*
- Altuğ C., Büyükbayram M., Kavas Ö., Yavuz M.Z. (2014) "A green synthesis of new 3-aryl-4-phenylsulfonyl-5-aminoisoxazoles", Tetrahedron, 70(22): 3590–3594.
- YÜCESAN B. B., Mohammed, A., Büyükgöçmen R., Kavas Ö., Kılıçsaymaz B., Arslan M., Cihangir C., İlal A.T., Mohammed S., Sağırlı A., Altuğ C., Eker İ., Gürel S., Gürel E. (2015). Comparison of concentional and Biotechnological approaches for Stevia rebaudiana production with elevated Rebaudiosid Acontent. 8th EUSTAS Stevia Symposium, 18, 183-196. Book Chapter. Euprint Ed. ISBN: 978-90-742-53291
- Özge Kavas, Cevher Altuğ. Synthesis of 1,4-Benzothiazines *via* S_ni Reaction of 5-Aminoisoxazoles. Trans Mediterranean Colloquium on Heterocyclic Chemistry TRAMECH VIII, 11-15 November 2015, Antalya, Turkey. Poster
- Cevher Altuğ, Muhammet Büyükbayram, Özge Kavas, Zeynep M.Yavuz. A Green Synthesis and Anti-Cancer Studies of New 3-Aryl-4-Phenylsulfonyl-5-

Amino/5-Amido Isoxazoles, 2.İlaç Kimyası Kongresi, 21-23 March 2014, Antalya, Turkey (Oral Presentation)

Awards **:** Honour Degree in Chemistry, 2013, Abant İzzet Baysal University.