T.R.
BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Chemistry

SYNTHESIS, CHARACTERIZATION AND CARBONIC ANHYDRASE STUDIES OF SOME NEW SULFONAMIDO THIAZOLE COMPOUNDS

DOCTOR OF PHILOSOPHY

ESRA CANER ERİGÜR

ACADEMIC SUPERVISOR
Prof. Dr. Cevher ALTUĞ

BOLU, JULY-2023

APPROVAL OF THE THESIS

SYNTHESIS, CHARACTERIZATION AND CARBONIC ANHYDRASE STUDIES OF SOME NEW SULFONAMIDO THIAZOLE COMPOUNDS submitted by **Esra CANER ERİGÜR** and defended before the Examining Committe Members listed below in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Department of Chemistry, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 28.07.2023** by

Examining Committee Members	**Signature**
Supervisor Prof. Dr. Cevher ALTUĞ Bolu Abant İzzet Baysal University	.....
Member Prof. Dr. Öznur DEMİR ORDU Bolu Abant İzzet Baysal University	.....
Member Assoc. Prof. Dr. Derya DAVARCI Gebze Technical University	.....
Member Assoc. Prof. Dr. Mustafa DURGUN Harran University	.....
Member Assist. Prof. Dr. Cem GÖL Bolu Abant İzzet Baysal University	.....

Prof. Dr. İbrahim KÜRTÜL

Director of Instıtute of Graduate Studies

ETHICAL DECLARATION

In this thesis dissertation that was properly prepared according to the Thesis Writing Rules of Bolu Abant İzzet Baysal University of the Institute of Graduates Studies, I hereby declare that;

- All data, information, and documents presented in the thesis were obtained in accordance with the academic and ethical rules,
- All data, documents, assessments, and results were presented in accordance with the scientific ethical and moral rules,
- All works that were benefitted in the thesis were appropriately cited,
- No alteration was made in the data used,
- Study presented in this thesis is original,

Otherwise, I declare that I accept the loss of all my rights in case any contradiction that may arise against me.

The similarity rate obtained by using the Turnitin program, a plagiarism detection software, is within the limits approved by the University Senate.

_____

Esra CANER ERİGÜR

To my big Family

ABSTRACT

SYNTHESIS, CHARACTERIZATION AND CARBONIC ANHYDRASE STUDIES OF SOME NEW SULFONAMIDO THIAZOLE COMPOUNDS

PHD THESIS
ESRA CANER ERİGÜR
BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY
(SUPERVISOR: PROF.DR. CEVHER ALTUĞ)

BOLU, JULY 2023
(XXII+ 128)

Thiazoles have an important place in the class of heterocyclic compounds since they have a wide biological activity due to their ring structure and are widely used in medicinal chemistry and pharmaceutical research. Sulfonamides are also known as sulfa-drugs with important biological activities and their carbonic anhydrase inhibitory properties have been extensively studied in recent years. In this work, we have accomplished a synthesis of thirty different novel molecules containing thiazole based benzenesulfonamide molecules. In the first study, we obtained the desired molecules by the reaction of 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylic acid and corresponding alcohols (as a solvent) at reflux conditions. In the second study, we sythesized the novel molecules by the reaction of 4-((4-hydrazinecarbonyl)thiazol-2-yl)amino)benzenesulfonamide and cyclic aldehydes in ethanol and reflux condition. The structures of the newly synthesized compounds were confirmed using spectroscopic methods, including IR, ^{1}H NMR, ^{13}C NMR, MALDI TOF-MS and HRMS. In search of discovering novel biologically active compounds, we designed and synthesized 1,3-thiazole sulfonamide molecules, which exhibit potential as carbonic anhydrase inhibitors. All compounds underwent testing to evaluate their ability to inhibit hCAI, hCAII, hCAIX, and hCAXII isoforms in comparison to the standard carbonic anhydrase inhibitor (AAZ).

.**KEYWORDS:** Heterocyclic compounds, Thiazole, Sulfonamide, Hydrazide-Hydrazone, Carbonic Anhydrase

ÖZET

BAZI YENİ SÜLFONAMİDO TİYAZOL BİLEŞİKLERİNİN SENTEZİ, KARAKTERİZASYONU VE KARBONİK ANHİDRAZ ÇALIŞMALARI

DOKTORA TEZİ
ESRA CANER ERİGÜR
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
KİMYA ANABİLİM DALI

(TEZ DANIŞMANI: PROF.DR. CEVHER ALTUĞ)

BOLU, TEMMUZ - 2023
(XXII+ 128)

Tiyazoller halka yapısı gereği geniş bir biyolojik aktiviteye sahip olduklarından heterosiklik bileşikler sınıfında önemli bir yere sahiptir ve tıbbi kimya ve farmasötik araştırmalarda yaygın olarak kullanılırlar. Sülfonamidler de önemli biyolojik aktivitelere sahip sülfa-ilaçları olarak bilirinler ve son yıllarda karbonik anhidraz inhibitör özellikleri yoğun şekilde araştırılmaktadır. Bu çalışmada tiyazol bazlı benzensülfonamid moleküllerini içeren otuz farklı yeni molekülün sentezini gerçekleştirdik. İlk çalışmada 2-((4-sülfamoilfenil)amino)tiyazol-4-karboksilik asit ve karşılık gelen alkolleri (çözücü olarak) reflüx koşullarında reaksiyona sokulması ile istenilen moleküller elde edildi. İkinci çalışmada, 4-((4-hidrazinkarbonil)tiyazol-2-il)amino)benzensülfonamid ve siklik aldehitlerin etanol ve reflüx koşullarında reaksiyona girmesiyle yeni molekülleri sentezledik. Spektroskopik yöntemlerle (IR, ^{1}H, ^{13}C NMR, MALDI-TOF MS ve HRMS) yeni bileşiklerin yapıları doğrulandı. Biyolojik olarak aktif yeni bileşikler keşfetme arayışında, 1,3-tiyazol sülfonamid molekülleri, potansiyel karbonik anhidraz inhibitörleri özellikleri sergileyecek şekilde tasarlandı ve sentezlendi. Tüm bileşikler, standart karbonik anhidraz inhibitörüne (AAZ) karşı hCAI, hCAII, hCAIX ve hCAXII izoformlarını inhibe etme yetenekleri açısından test edildi.

ANAHTAR KELİMELER: Heterosiklik bileşikler, Tiyazol, Sülfonamid, Hidrazid-Hidrazon, Karbonik Anhidraz

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF SCHEME

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS AND SYMBOLS

α : Alpha

AAZ **:** Acetazolamide

AcOH **:** Acetic acid

app d **:** Apparently doublet (NMR)

app q **:** Apparently quarted (NMR)

app s **:** Apparently singlet (NMR)

app t **:** Apparently triplet (NMR)

Ar **:** Aryl or aromatic

ArOH **:** Phenol or aromatic alcohol

asym **:** Asymmetric

broad s **:** Broad singlet (NMR)

^{13}C NMR **:** Carbon nuclear magnetic resonance

CA **:** Carbonic anhydrase

CAs **:** Carbonic anhydrase isoforms

CO_2 **:** Carbon dioxide

CS_2 **:** Carbon disulfide

d **:** Doublet (NMR)

dd **:** Doublets of doublet (NMR)

decomp. **:** Decomposed

DMSO **:** Dimethyl sulfoxide

DMSO-d_6 **:** Deuterated dimethyl sulfoxide

EtOAc **:** Ethyl acetate

EtOH **:** Ethanol

eq. **:** Equivalent

FT-IR **:** Fourier transform infrared

FDA **:** U.S. Food and Drug Administration

g **:** Gram

Glu **:** Glutamic acid

^{1}H NMR **:** Proton nuclear magnetic resonance

hCA **:** Human carbonic anhydrase

HCl **:** Hydrochloric acid

HCO_3 **:** Sodium bicarbonate

Hex : Hexane

His : Histidine

Hz : Hertz

H_2O : Water

HRMS : High resolution mass spectrometry

H_2SO_4 : Sulfuric acid

I_2 : Iodine

J : Coupling constants

K_2CO_3 : Potassium carbonate

KIs : Inhibition constants

m : Multiplet (NMR)

MeCN : Acetonitrile

MeOH : Methanol

mg : Milligram

MHz : Megahertz

min. : Minute

mL : Milliliter

m.p. : Melting point

NaOH : Sodium hydroxide

NH_4SCN : Ammonium thiocyanate

NH_2NH_2 : Hydrazine

NMR : Nuclear magnetic resonance

nM : Nanomolar

ppm : Parts per million

q : Quartet (NMR)

Rf : Retardation factor

rt : Room temperature

s : Singlet (NMR)

SAR : Structure–activity relationship

sym. : Symmetric

t : Triplet (NMR)

THF : Tetrahydrofuran

Thr : Threonine

TLC : Thin-layer chromatography

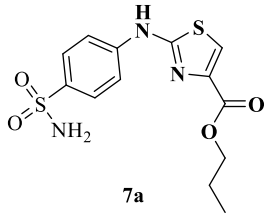
TOF **:** Time-of-flight

δ **:** Chemical shift

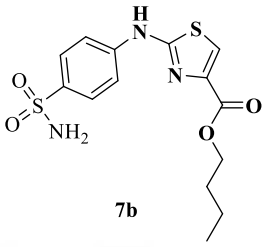
ν_{max} **:** Maximum frequency

Δ **:** Heat

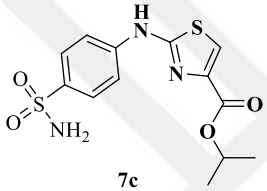
FORMULAE



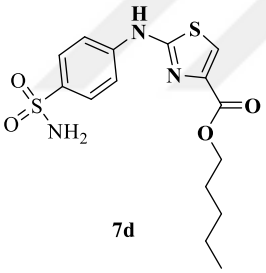
7a



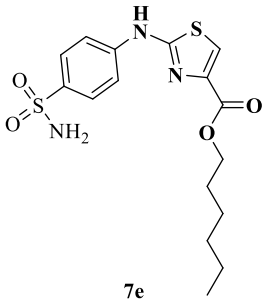
7b



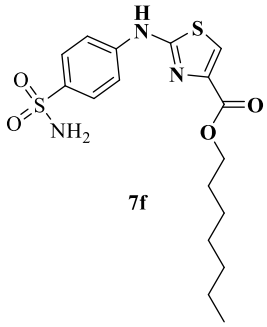
7c



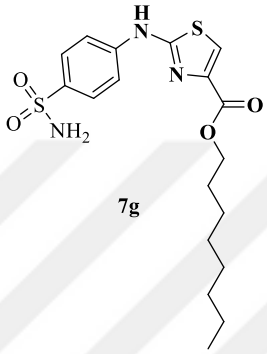
7d



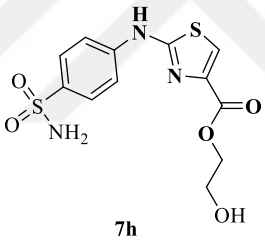
7e



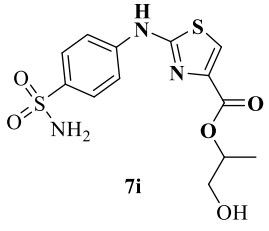
7f



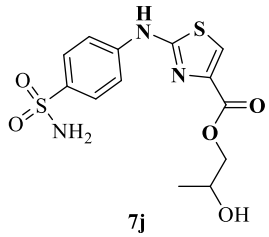
7g



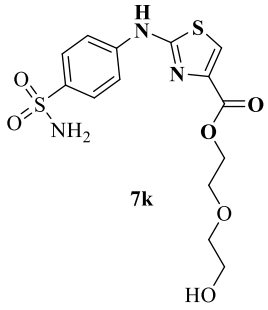
7h



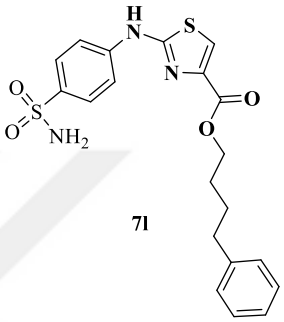
7i



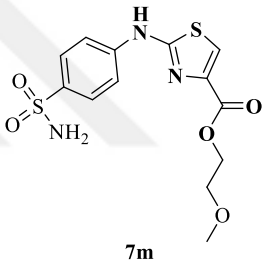
7j



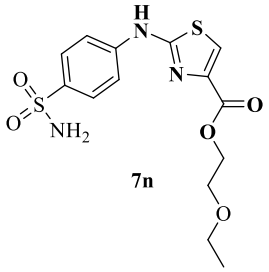
7k



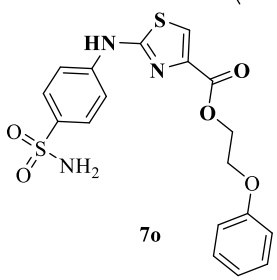
7l



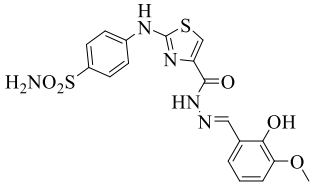
7m



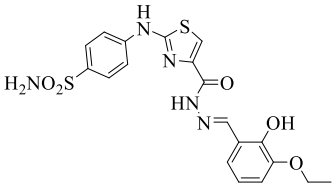
7n



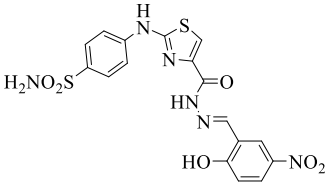
7o



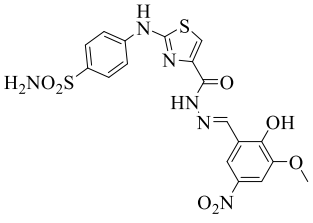
10a



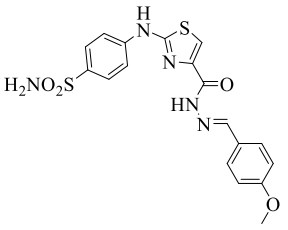
10b



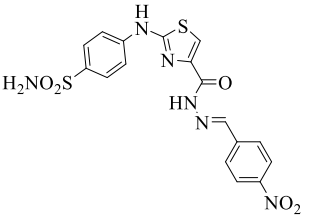
10c



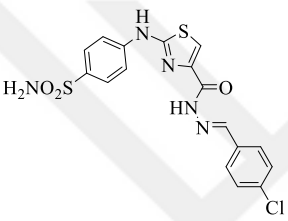
10d



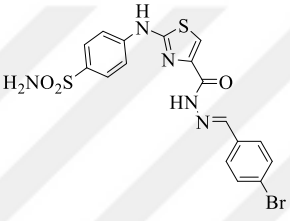
10e



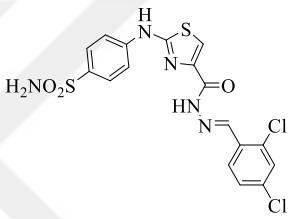
10f



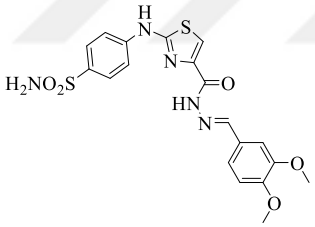
10g



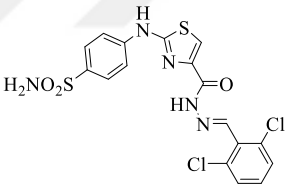
10h



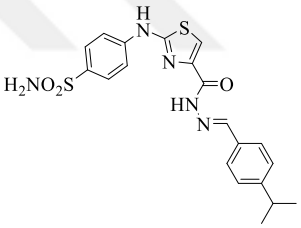
10i



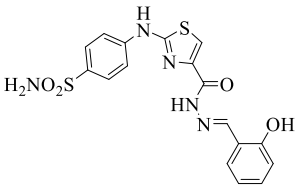
10j



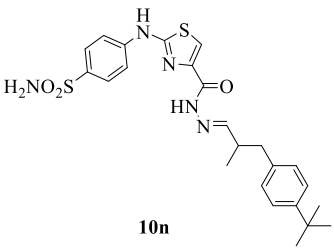
10k



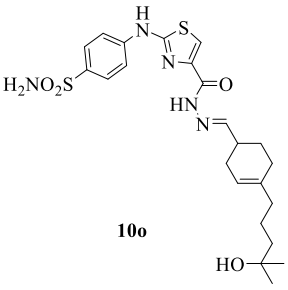
10l



10m



10n



10o

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my supervisor Prof. Dr. Cevher ALTUĞ for giving me the opportunity to work on my doctoral thesis under his expert supervision and the continuous support of my thesis study, for his patience, understanding and motivation. It was an honor and pleasure to work with him.

Furthermore, I would also like to thank Claudiu T. SUPURAN and his team. (University of Florence, NEUROFARBA dept.) for carbonic anhydrase study. I would also like to thank Hayat Kimya San AŞ, where I am still working, for enabling and supporting me to work on this thesis. I would like to special thank to Prof. Dr. Öznur DEMİR ORDU and Assist. Prof. Dr. Cem GÖL for fruitful discussions and for guiding me.

Finally, I want to express my very profound gratitude to my big family and my friends for providing me with continuous encouragement throughout my years of study.

1. INTRODUCTION

Heterocyclic compounds are members of the most comprehensive and largest family of organic compounds containing one or more ring structures, where at least one atom of the ring contains a common heteroatom other than carbon, such as sulfur (S), oxygen (O), nitrogen. (N).[1]

They play a key role in medicinal chemistry, have wide biological activities due to the heteroatoms in their structure, especially their antibacterial, antifungal, anti-inflammatory, anticancer, antiallergic properties are known, so they are closely followed by departments of biochemistry, medicinal chemistry and organic chemistry.[2]

Heterocyclic compounds are classified according to the types of heteroatoms present and the number of atoms in the ring. Some common examples are pyridine, thiophene, furan, pyrole, imidazole, thiazole etc.

The diversity of atoms in the thiazole structure makes it one of the extensively studied heterocyclic compounds due to its unique properties and reactivity. Thiazole rings can be found in various natural products and have diverse biological activities and widely used in medicinal chemistry application and pharmaceutical research for the development of drugs.[3]

1.1. Thiazole

Thiazole is a five-membered ring containing three carbon atoms, one nitrogen and one sulfur atoms. The 1,3-thiazole ring is a heteroaromatic structure with a sulfur atom in the first position and a nitrogen atom in the third position. [4]

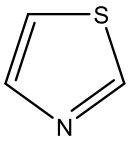


Figure 1.1. Structure of 1,3-Thiazole Ring

Due to the position of carbon, sulfur and nitrogen atoms on it; the aromaticity of thiazole is high because; the pi (π) electrons circulate freely between the bonds and sulfur lone pair electrons delocalize around the ring.[5] Thiazoles have a wide range of biological activities, thanks to the ability of the thiazole ring to both electron

donor and electron acceptor. Therefore, medicinal and organic chemists have focused their efforts on thiazole-containing compounds to develop new therapeutic agents.

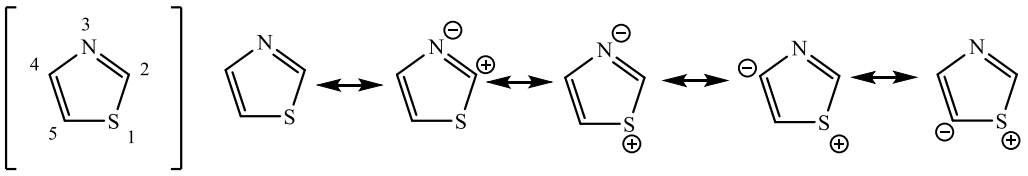


Figure 1.2. Resonating structure of thiazole

Thiazole compounds can be found in many natural products (Vitamin B1, thymine), penicillin, amino acids etc. Due to their extensive biological activities, they are widely used in medicinal chemistry and pharmaceutical research for the development of drugs.

In medicinal chemistry, thiazole structures have therapeutic potential as human drugs so far they present in more than 18 FDA-approved drugs[6] as antimicrobial drug; sulfathiazole (I)[7], antifungal drug; abafungin(II)[8], anti-inflammatory drug; meloxicam(III)[9], vitamine B1; thiamine(IV)[10], anticancer drug; dasatinip(V)[11] etc. In recent years, cancer is one of the most common and serious diseases worldwide so one of the main goal of organic and medicinal chemistry is development of new drug like molecules having wide range of biological activities especially those that can be used as anticancer agents. Thizaole scaffold is a fundamental part of anticancer drug discovery.

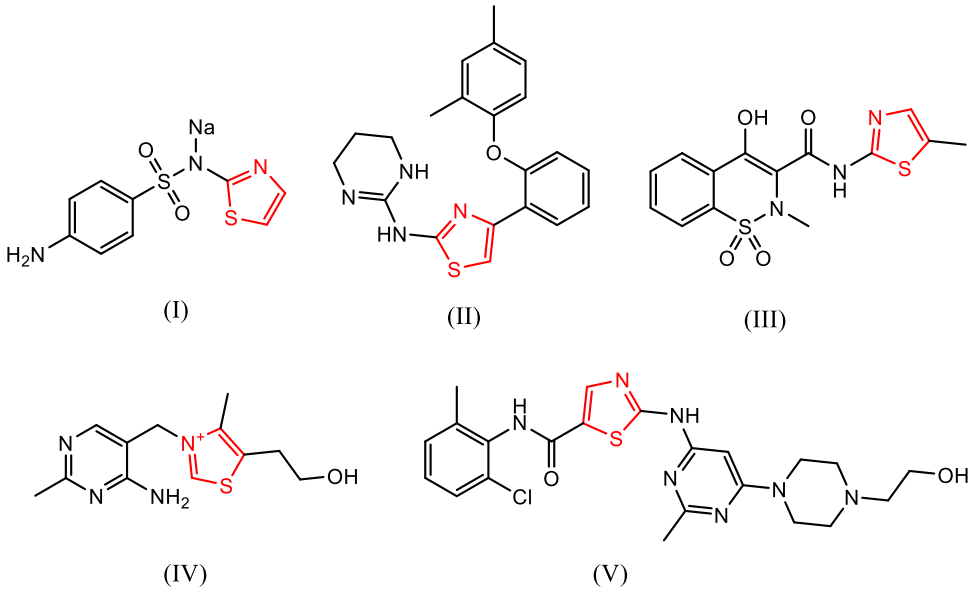


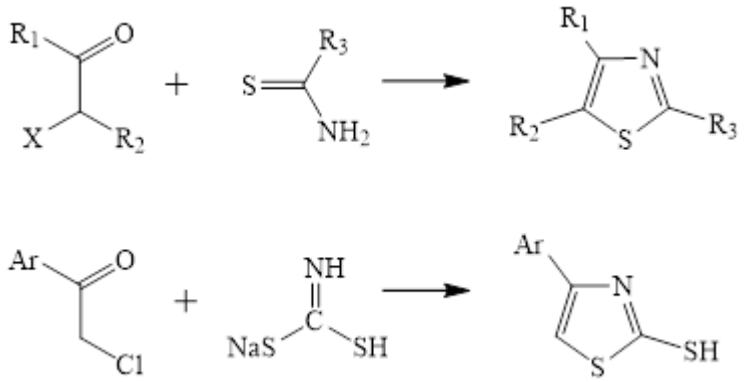
Figure 1.3. Structure of some drug molecules containing thiazole groups.

1.1.1. Synthesis Methods of Thiazole

There are different types of methods for synthesizing thiazole derivatives, there are a few important synthesis examples as follow.

1.1.1.1. Hantzsch Thiazole Synthesis

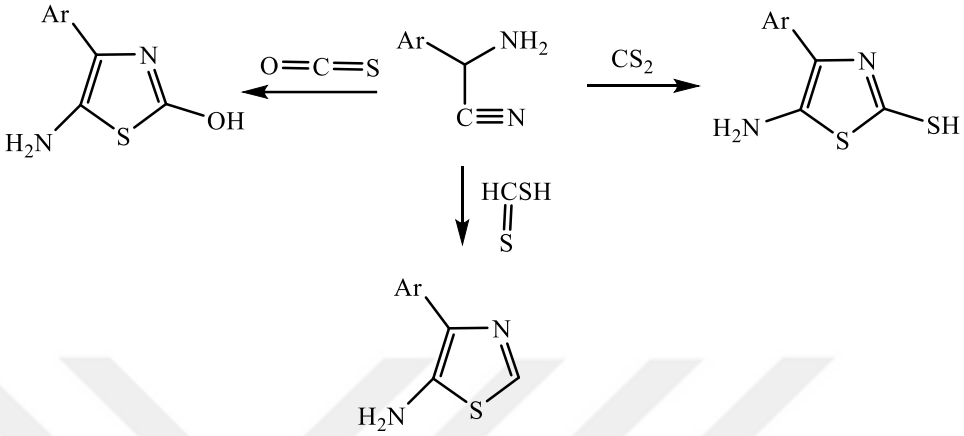
The Hantzsch thiazole synthesis reported first in 1889[12], using condensation reaction of α-haloketones and thioamides, after cyclization steps, resulting in the synthesis of thiazole derivatives. The reaction mechanism can be summarized as follows:



Scheme 1.1. Example of Hantzch Thiazole Synthesis

1.1.1.2. Cook–Heilbron Thiazole Synthesis

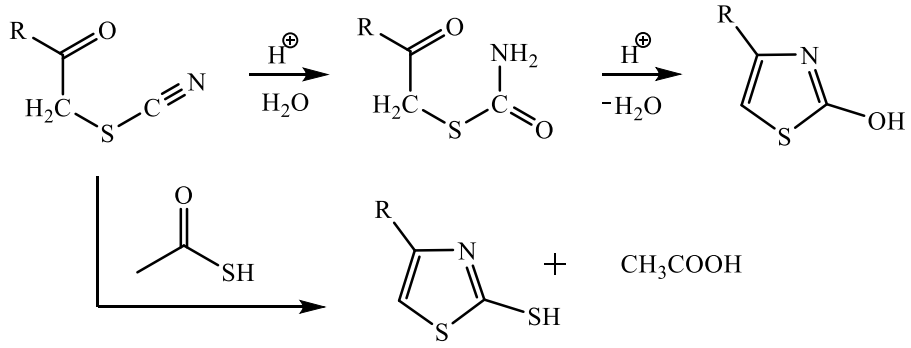
Used the Synthesis of 5-aminothiazoles from the reaction of α-aminonitriles or aminocyanoacetates with carbon disulphide, dithioacids, isothiocyanates or carbon oxysulfide under aqueous conditios.[13]



Scheme 1.2. Example of Cook–Heilbron Thiazole Synthesis

1.1.1.3. Tcherniac's Thiazole Synthesis

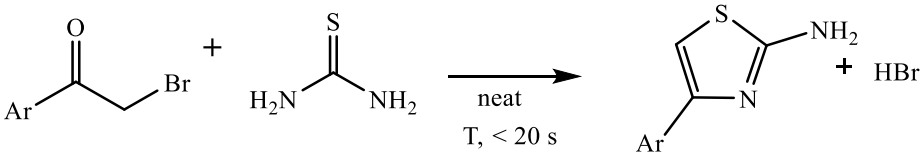
Used to Synthesis of 2-substituted thiazoles from the hydrolysis of α-thiocyanic ketones in acidic condition or its reaction with sulfur compounds.[14]



Scheme 1.3. Example of Tcherniac's Thiazole Synthesis

1.1.1.4. Thiazole Synthesis using Thiourea and α-haloketones

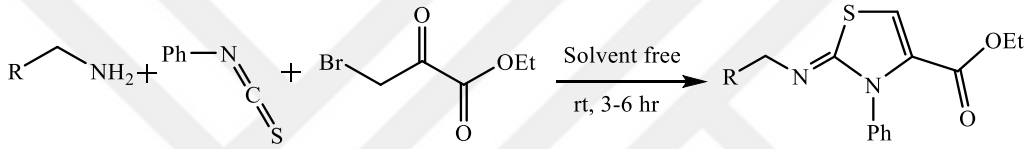
2-aminothiazoles are synthesized from the reaction of 2-bromoacetophenones with thiourea in the solvent and uncatalyzed condition. The reaction is completed in high yield and in a short time.[15]



Scheme 1.4. Example of Thiazole Synthesis using Thiourea and α-haloketones

1.1.1.5. Thiazole Synthesis using Primary Alkylamines, Phenyl Isothiocyanate and Ethyl Bromopyruvate

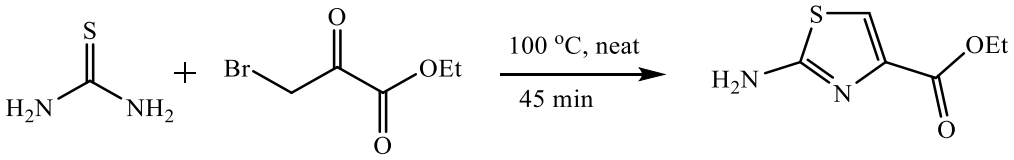
Three-component reaction were performed under mild, solvent and catalyst free conditions at room temperature using ethyl bromopyruvate, phenyl isothiocyanate and primary alkylamines.[16]



Scheme 1.5. Example of Thiazole synthesis using primary alkylamines, phenyl isothiocyanate and ethyl bromopyruvate.

1.1.1.6. Thiazole Synthesis using Thiourea and Ethyl bromopyruvate

Synthesis of thiazole with condensation reaction of thiourea and ethyl bromopyruvate under neat condition. [17]



Scheme 1.6. Example of Thiazole Synthesis using thiourea and ethyl bromopyruvate.

1.2. Sulfonamide

The sulfonamides with the general formula $-SO_2NHR$ are in the class of antibacterial drugs used to treat various bacterial infections, also called sulfa drugs.[18] Sulfonamides inhibit the growth of bacteria in the body and help the immune system to eliminate the infection from the body. Since the first discovery of sulfonamides, sulfa drugs form an important part of medical and pharmacological chemistry. The first antibiotic discovered by German chemist Gerhard Domagk in 1932 is Sulfamidochrysoidin (Prontosil).[19] As a result of the researches, it was determined that prontosil is active in the "in vivo" environment, thus many new sulfonamide molecules were synthesized and clinical studies of sulfonamides began. Especially sulfonamides were used in the treatment of infections during the Second World War. In the 1940s, their popularity declined somewhat due to both the discovery of the antibacterial properties of penicillin and the toxicity of sulfonamides in some patients.[20]

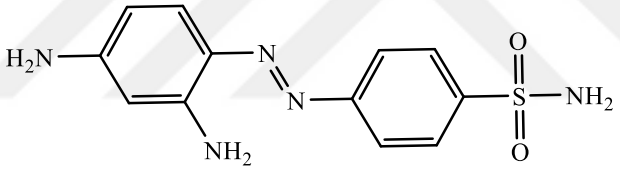


Figure 1.4 Molecular Structure of Sulfamidochrysoidin (Prontosil)

Over time, bacteria developed resistance to sulfonamides, so medical and organic chemists continued to synthesize and research for new sulfonamide molecules that were more resistant to bacteria. As a result of researches, Over 30 drug molecules containing sulfonamide structures are used in pharmacology, including antibacterial[21], antifungal[22], anti-inflammatory[23], anticancer[24], antiepileptic[25], antitumor[26], carbonic anhydrase inhibitors[27] etc. Because sulfonamides have wide-spectrum biological activities, they inspired the synthesis of many new molecules in the sulfonamide structure.

1.2.1. Some Drug Molecules Containing Sulfonamide Groups and Their Uses

Sulfamethoxazole

Sulfamethoxazole; It is an antibiotic used in combination with trimethoprim to treat respiratory and urinary tract infections. Its trade name is known as Bactrim.[28]

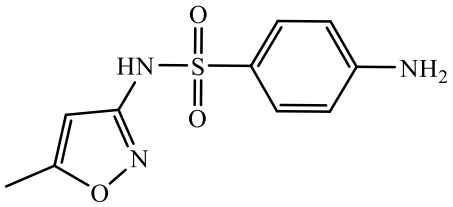


Figure 1.5. Molecular Structure of Sulfamethoxazole

Sulfathiazole

Sulfathiazole; It is used in the treatment of diseases caused by bacterial infections, especially gram positive and gram negative bacteria. It inhibits the synthesis of folic acid by bacteria, and in this way, bacteria are expelled from the body before they can multiply. [29]

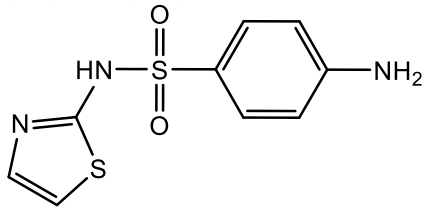


Figure 1.6. Molecular Structure of Sulfathiazole

Nimesulid

Nimesulide is a nonsteroidal anti-inflammatory drug used to reduce inflammation, pain and fever, especially in rheumatoid arthritis, musculoskeletal pain, menstrual pain.[30]

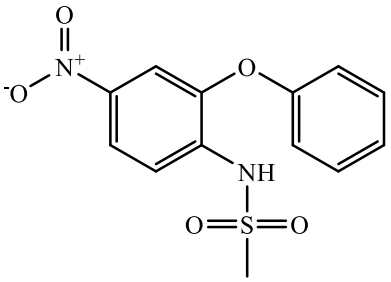


Figure 1.7. Molecular Structure of Nimesulide

Hydrochlorothiazide

Hydrochlorothiazide is in a class of diuretic drugs used to treat high blood pressure (hypertension). It is also used in the treatment of kidney disorders, congestive heart failure and edema caused by fluid retention in people.[31]

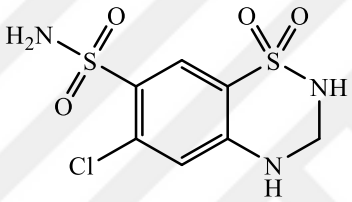


Figure 1.8. Molecular Structure of Hydrochlorothiazide

Mafenide

Mafenide is an antimicrobial drug used for the treatment of severe burns and skin infections. It helps to heal deep burns by removing the bacteria in the burn tissue. [32]

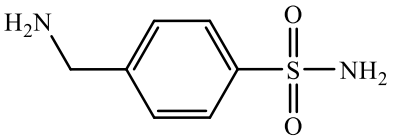
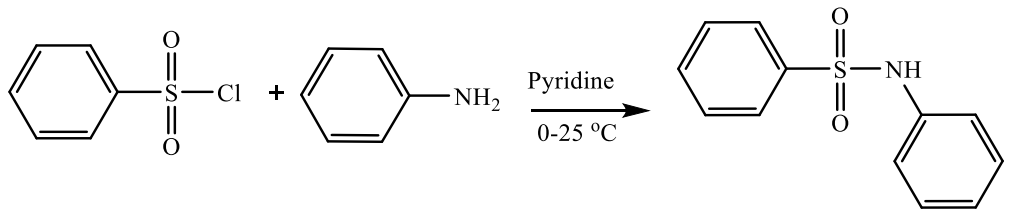


Figure 1.9. Molecular Structure of Mafenide

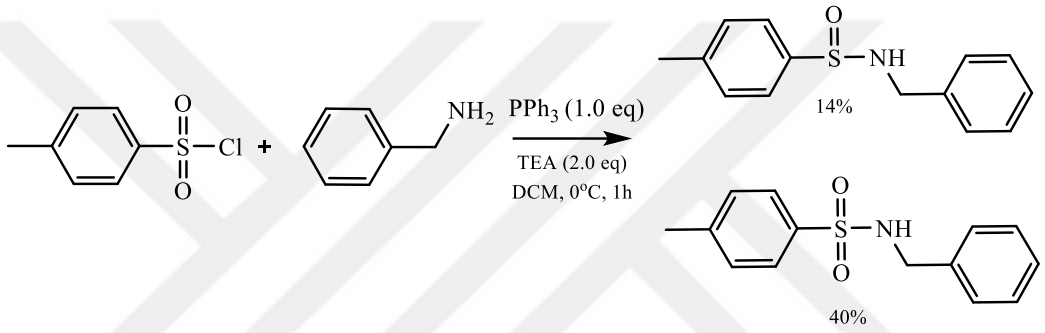
1.2.2. General Synthesis Methods of Sulfonamides

Basic methods involve the reaction of aryl primary amine as aniline, with aryl sulfonyl chloride as benzenesulfonyl chloride in the presence of a pyridine as base at 0-25 ^{o}C.[33]



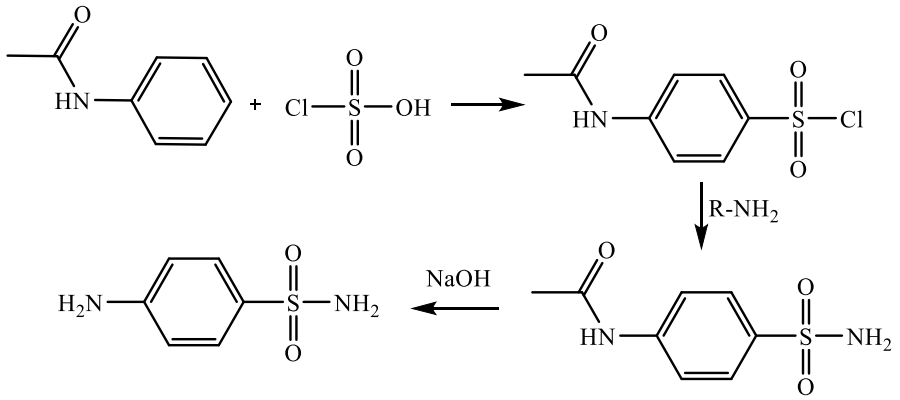
Scheme 1.7. Sulfonamide synthesis using aniline and benzenesulfonyl chloride

Synthesized by the reaction of a phenylmethanamine with a 4-methyl benzenesulfonyl chloride in the presence of a triphenylphosphine, in dichloromethane and triethanolamine at 0°C, 1 hr obtaining 40% N-benzyl-4-methylbenzenesulfonamide.[34]



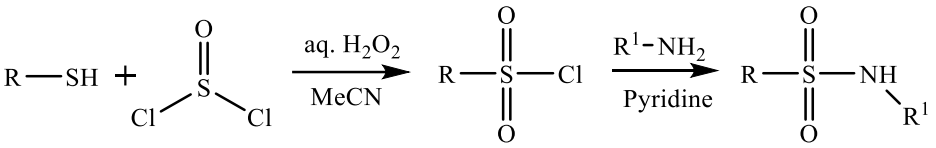
Scheme 1.8. Sulfonamide synthesis using phenylmethanamine and 4-methyl benzenesulfonyl chloride

Synthesized by reaction of acetanilide with chlorosulfonic acid was then converted to acetylsulfonamide by hydrolysis of the acetyl group in alkaline medium using the corresponding amine.[35]



Scheme 1.9. Sulfonamide synthesis using acetanilide and chlorosulfonic acid

Synthesized by reaction of thiols with thionyl chloride was then converted to sulfonamide by in alkaline medium using the corresponding amine.[36]



Scheme 1.10. Sulfonamide synthesis using thiols and thionyl chloride

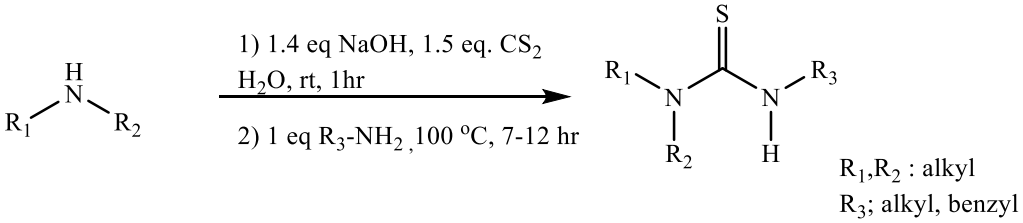
1.3. Thiourea

Thiourea is a sulfur-containing compound with the chemical formula $SC(NH_2)_2$. Thiourea has various applications in different industries that are often used as raw materials for the manufacture of pharmaceutical and dye molecules.

1.3.1. General Synthesis Methods of Thioueas

1.3.1.1. Synthesis from Amines and Carbon Disulfide Reaction

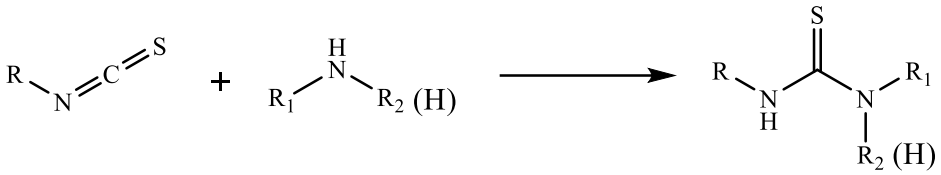
Condensation reaction between amines and carbon disulfide in aq. medium gives efficient synthesis of thiourea derivatives. Di- and tri-substituted thiourea derivatives can be sythesized using aliphatic primary amines.[37]



Scheme 1.11. Thiourea synthesis using amines and carbon disulfide

1.3.1.2. Synthesis from Isothiocyanate and Amine Reaction

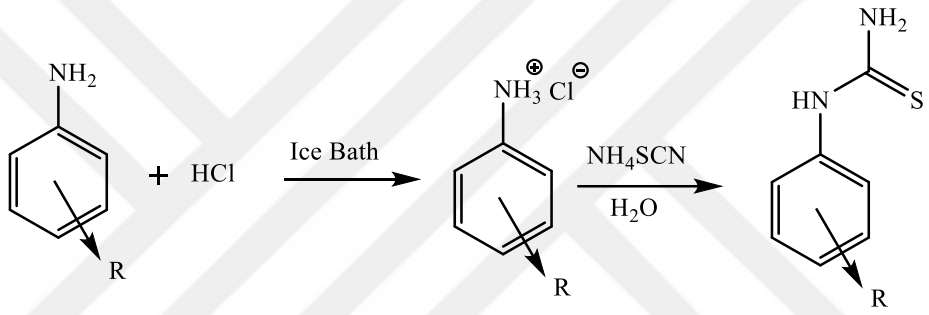
The reaction typically takes place in the presence of a suitable catalyst or under specific reaction conditions at an elevated temperature and may require a solvent or a reaction medium. [38]



Scheme 1.12. Thiourea synthesis using isothiocyanate and amine

1.3.1.3. Synthesis from Ammonium thiocyanate and Phenyl amine Reaction

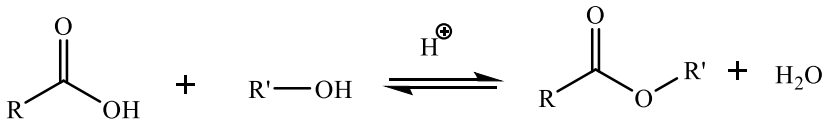
Reaction of phenyl amine and ammonium thiocyanate in water and presence of HCl.[39]



Scheme 1.13. Thiourea synthesis using ammonium thiocyanate and phenyl amine reaction.

1.4. Esterification Reactions

Esterification is a chemical reaction involving the formation of an ester derived from carboxylic acids and alcohols. Esters are characterized by the (-COO-) functional group. Typical Fisher esterification reactions occur when a carboxylic acid reacts with an alcohol in the presence of an acid catalyst, resulting in the formation of an ester and water. In this reaction, the carbon is protonated by the acid and thus the alcohol attacks the carbonyl with nucleophilic attack, forming ester and water. Esters are widely used in perfumes, cosmetics, soaps, detergents or personal care products, especially as essential ingredients, as they have a unique scent. Esters also have an important place in the pharmaceutical industry.[40]



Scheme 1.14. General reaction of esterification

Esterification is a reversible reaction and they can be hydrolyze by an acid or a base. The esterification reaction rate and yield depend on the structure of the reactants. Esterification reactions of primary alcohols are fast and highly efficient. In other words, the simplest alcohol, methanol, gives the fastest reaction. On the other hand, as it starts to branch (in secondary and tertiary alcohols), reaction rates and yields decrease due to stearic hindrance.[41] Since the reactions are generally reversible and have a low rate, esterification reaction realized by Esterification-assist methods and substances.

1.4.1. Examples of Esterification-assist Methods and Substances

Acid Catalysts:

Strong acids such as sulfuric acid (H_2SO_4), hydrochloric acid (HCl), or p-toluenesulfonic acid (PTSA) are commonly used as acid catalysts in esterification reactions. They help activate the carboxylic acid, making it more reactive to the nucleophilic attack of the alcohol. In addition, commercial solid catalysts have been developed to aid esterification reactions, such as Amberlyst 15. These materials are composed of porous polymers and have ion exchange properties. Amberlyst 15 has been specially designed with sulfonic acid functional groups attached to a styrene-divinylbenzene copolymer matrix. The sulfonic acid group in this material catalyzes ester reactions.

Removel of water:

According to Le Chatelier's Principle, reducing the water concentration of a product shifts the balance towards more ester formation. Since esterification reactions are reversible, water is removed to increase the efficiency of the reaction. For this, molecular sieves, dessicants (e.g., anhydrous magnesium sulfate), zeolites or a Dean-Stark apparatus can be used.

Using Coupling Agents:

It is used to activate the carboxylic acid in esterification reactions and to facilitate the binding of the alcohol to the acid. Dicyclohexylcarbodiimide (DCC) and N,N'-diisopropylcarbodiimide (DIC) are examples of these agents. In addition, Mitsunobu reagents[42]; Phosphine-based reagent (PPh3), triphenylphosphine and azodicarboxylate (DEAD) reagents can be used. In this method, alcohol becomes more nucleophile and reacts more easily with carboxylic acid.

Usage of Lewis Acid

The use of Lewis acids such as aluminum chloride ($AlCl_3$) or zinc chloride ($ZnCl_2$) in esterification reactions increases the reactivity of the carboxylic acid and becomes more electrophilic by forming an intermediate (acyl cation) with the carbonyl oxygen of the carboxylic acid. Thus, it allows the alcohol to attack the carbonyl carbon of the carboxylic acid and promote ester formation. In addition, rapid esterification reactions are carried out by using acyl chlorides ((Phosphorus trichloride (PCl_3), thionyl chloride ($SOCl_2$)) in esterification reactions.

1.5. Acid Hydrazides

Acid hydrazides are organic compounds that contain both hydrazine and carboxylic acid groups. Their general formula is $RCONHNH_2$.

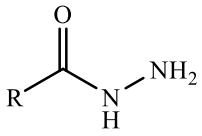
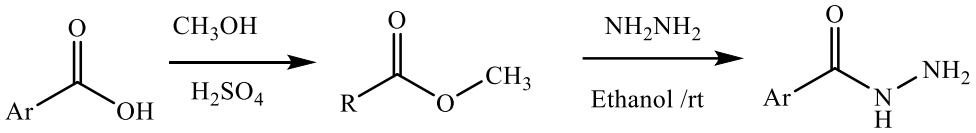


Figure 1.10. Structure of acid hydrazides

Acid hydrazides are generally synthesized by the reaction of hydrazine (N_2H_4) with a carboxylic acid, ester or an acyl chloride. Carbonyl hydrazide structures are versatile building blocks for organic synthesizers or pharmaceutical field. There are some methods for Acid hydrazides synthesis.

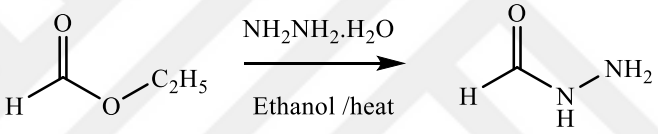
1.5.1. Synthesis Methods Examples of Acid Hydrazides

Acid hydrazides are synthesized from ester and hydrazine hydrate using ethanol as solvent and room temperature conditions. Esters to be used can be synthesized using H_2SO_4 and the required alcohol. [43]



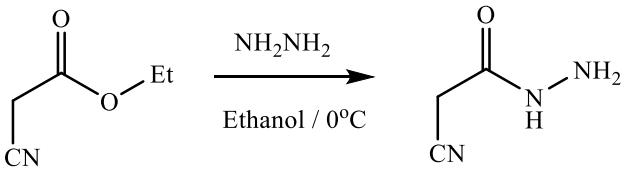
Scheme 1.15. Synthesize of Acid hydrazides using carboxylic acid then ester and hydrazine hydrate.

Another example, Acid hydrazides are synthesized with ethyl ester and hydrazine hydrate in ethanol and by heating. [44]



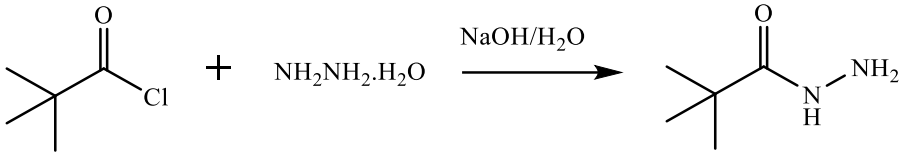
Scheme 1.16. Synthesize of Acid hydrazides using ethyl ester and hydrazine hydrate.

At this study, Cyano aceteic acid hydrazides are synthesized with ethylcyano acetate and hydrazine hydrate in ethanol and at 0 ^oC.[45]



Scheme 1.17. Synthesize of Cyano aceteic acid hydrazides using ethylcyano acetate and hydrazine hydrate.

Synthesize of Acid hydrazide (2,2-dimethylpropanohydrazide) from acid chlorides and hyrdazine hydrate using sodium hydroxide and water as solvent. [46]



Scheme 1.18. Synthesize of acid hydrazides using acid chlorides and hydrazine hydrate

1.6. Hydrazide – Hydrazone Derivatives

Hydrazide-hydrazone derivatives are a class of organic compounds that contain both a hydrazide functional group and a hydrazone functional group in their structure. As a result of the reaction of acid hydrazine (-$CONHNH_2$) with an aldehyde or a ketone, the hydrazone (-$C=N-NH_2$) group is formed. General structure of a hydrazide-hydrazone derivative RCONHNHN=CR'R" and R, R' and R" represent various organic groups or hydrogen atoms. By replacing these groups, hydrazide-hydrazone derivatives with very different structures are obtained.

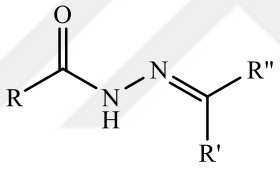


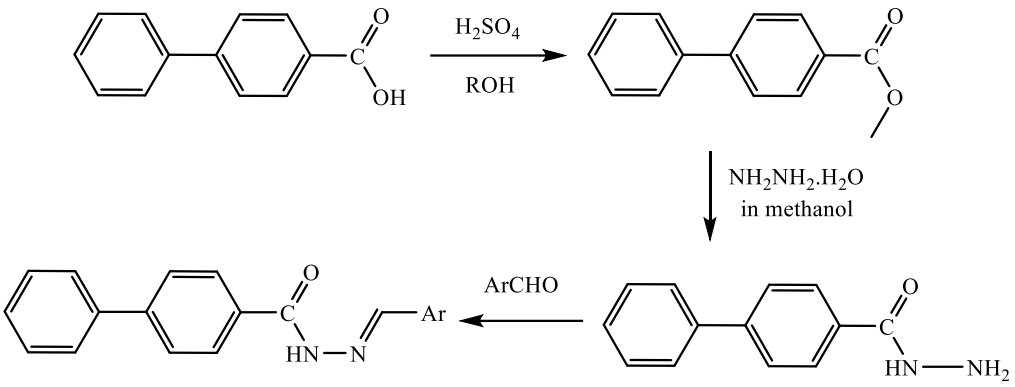
Figure 1.11. Structure of hydrazide-hydrazone derivative

Hydrazide-hydrazones are of interest to medicinal chemists because of their pharmaceutical applications, as they contain a carbonyl-linked azomethine group (–NH–N=CH–) in their structure.[47]

1.6.1. Synthesis Method Examples of Hydrazide-Hydrazone Derivatives

Synthesis of biphenyl-4-carboxylic acid hydrazide-hydrazone[48]

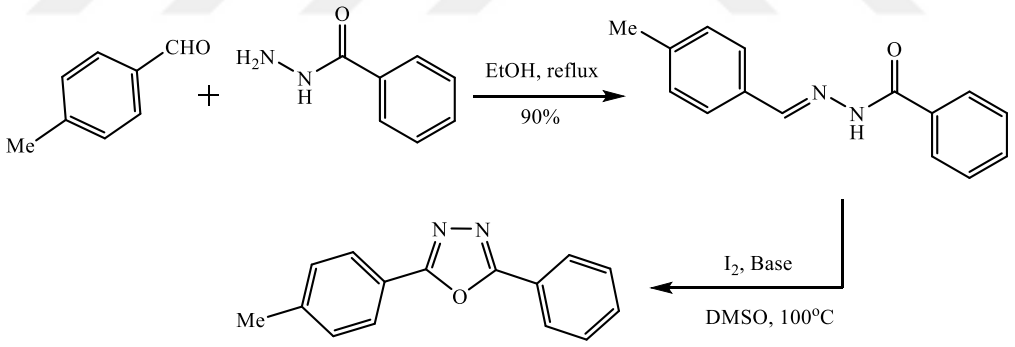
At first part of this rxn, biphenyl carboxylic acid is converted to its methyl ester using methanol and H_2SO_4. Then ester is reacted with hydrazine hydrate in methanol as solvent. At final acid hydrazide is treated with aromatic aldehyde using methanol as solvent and catalytic amount of acetic acid under reflux conditions and obtained hydrazide-hydrazone derivatives.



Scheme 1.19. Preparation of biphenyl-4-carboxylic acid hydrazide-hydrazone

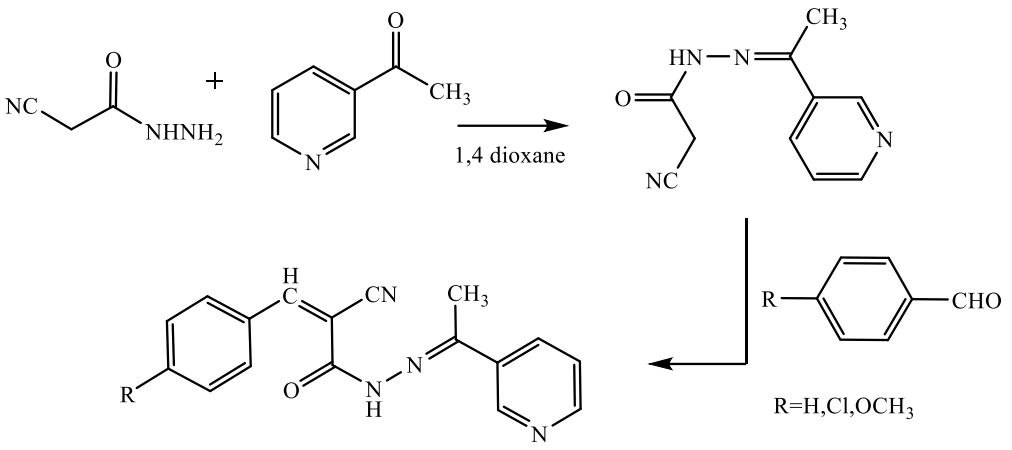
Synthesis of Benzoyl Hydrazone then oxadiazole derivatives.[49]

Hydrazide-hydrazone derivatives are prepared from condensation rxn of 4- methyl benzaldehyde and benzohydrazide using ethanol as solvent and reflux condition. For second rxn the hydrazide-hydrazone derivatives redissolved in DMSO, then the treated with molecular iodine and potassium carbonate to obtain oxadiazole derivatives.



Scheme 1.20. Benzoyl Hydrazone then oxadiazole derivatives

Synthesis of Hydrazide-hydrazone derivatives using the reaction of cyanoacetyl hydrazine and 3-acetylpyridine using 1,4-dioxane as solvent.[50]



Scheme 1.21. Synthesis of Hydrazide-hydrazone derivatives using the reaction of cyanoacetyl hydrazine and 3-acetylpyridine

1.7. Carbonic Anhydrase

The human carbonic anhydrases (hCAs) are a group of metalloenzymes in almost all living systems that have sixteen different isoforms. [51],[52] They are (CA-I, CA-II, CA-III, CA-VII and CA-XIII) as cytosolic isoform, (CA-IV, CA-IX, CA-XII, CA-XIV and CA-XV) as membrane-associated isoform, (CA-VA and CA-VB) as mitochondrial isoform, (CA-VI) as secreted isoform and (CA-VIII, CA-X, and CA-XI) as inactive proteins.[53]

These enzymes catalyze many fundamental reactions, especially hydration reaction of carbon dioxide to proton and bicarbonate ions is critical.

$$HCO_3^{\ominus} + H^{\oplus} \underset{}{\overset{CAs}{\rightleftharpoons}} CO_2 + H_2O$$

The interconversion reaction between CO_2 and HCO_3 is realised by the active site of the enzyme that contains a tightly bond Zn^{2+} ion.[54],[55] By means of a metal hydroxide nucleophilic mechanism, The Zn^{2+} ion interact with water molecule and catalyze the revesible hydration reaction. This reaction helps to acid- base balance, regulation of cellular pH and transportation of CO_2 in metabolism.[56], [57]

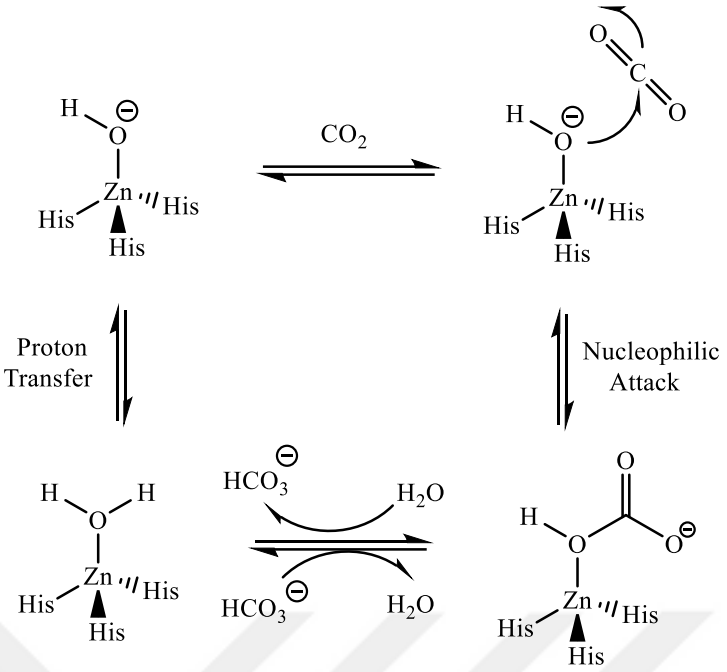


Figure 1.12. Cyclization reaction mechanism of carbonic anhydrase

Carbonic anhydrase inhibitor is a molecule that binds to the active site of the enzyme that contains Zn^{2+} ion and decreases its catalyzing activity and prevent excessive reversible hydration reaction.[58]

These enzymes are found in many tissues and organs in the body and catalyze the conversion of carbon dioxide and bicarbonate. If this conversion is more than desired, the pH balance in some parts of the body is disturbed, the internal body pressure increases. At this stage, carbonic anhydrase inhibitors come into play and inhibit carbonic anhydrase and prevent excess conversion.

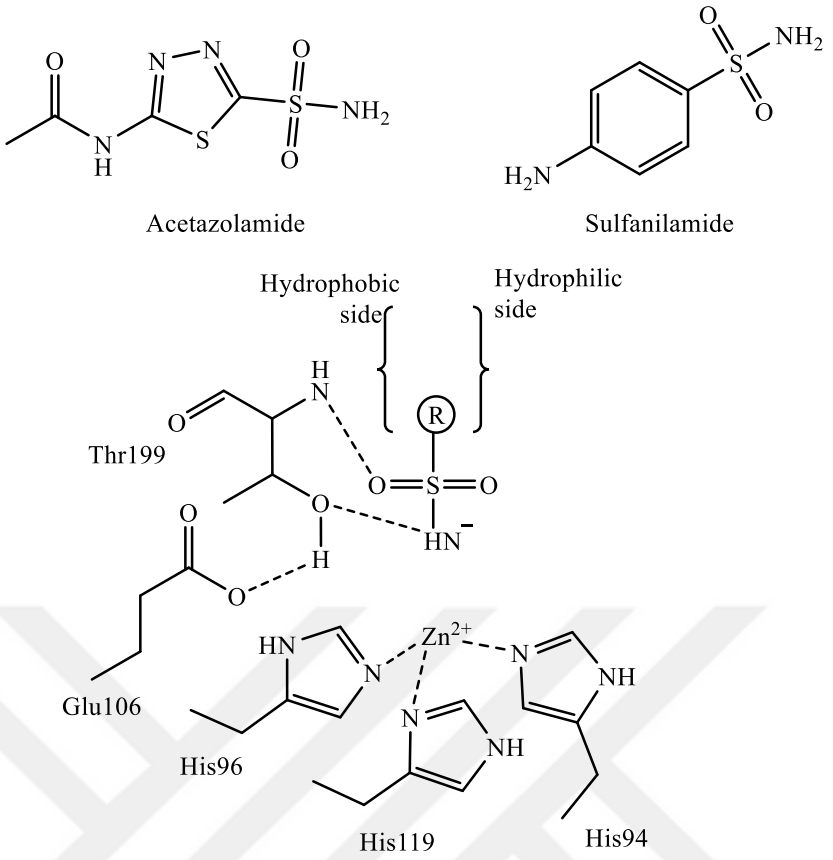


Figure 1.13. Binding of Carbonic anhydrase inhibitors (sulfonamide derivatives) to catalytic side of CAs

Carbonic anhydrase inhibitors are involved in physiologic or pathologic processes[59] so they are important targets for pharmacological applications such as diuretics, anticancer agents, obesity, epilepsy, antiglaucoma etc.[60], [61],[62]

Acetazolamide (AAZ), methazolamide (MZA), ethoxzolamide (EZA), dorzolamide (DZA), dichlorphenamide (DCP) are a membered of sulfonamide family were clinically used as Carbonic anhydrase inhibitors[63]. Acetazolamide is the first aromatic and heterocyclic sulfonamide drug molecule that was synthesized as diuretic agents[64] and used to medicinal treatment of obesity, glaucoma, epileptic seizure etc.[65]

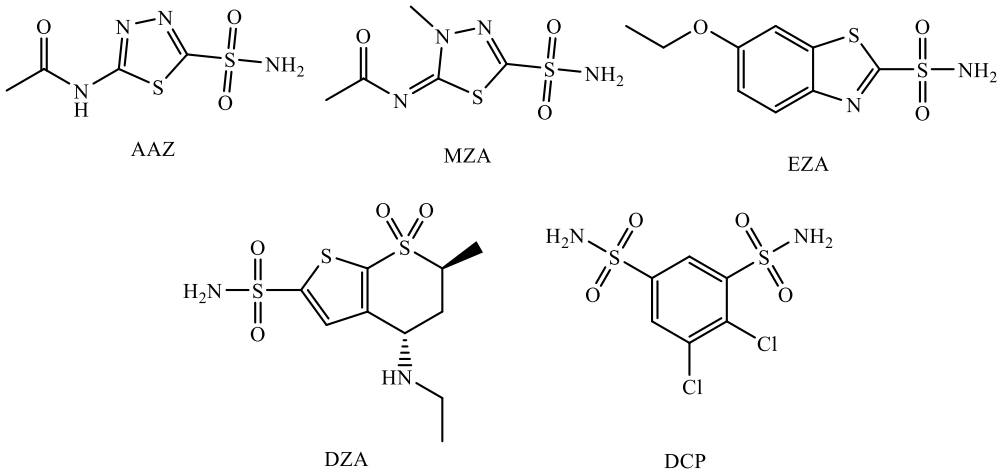


Figure 1.14. Examples of Carbonic anhydrase inhibitor molecules

2. AIM AND SCOPE OF THE STUDY

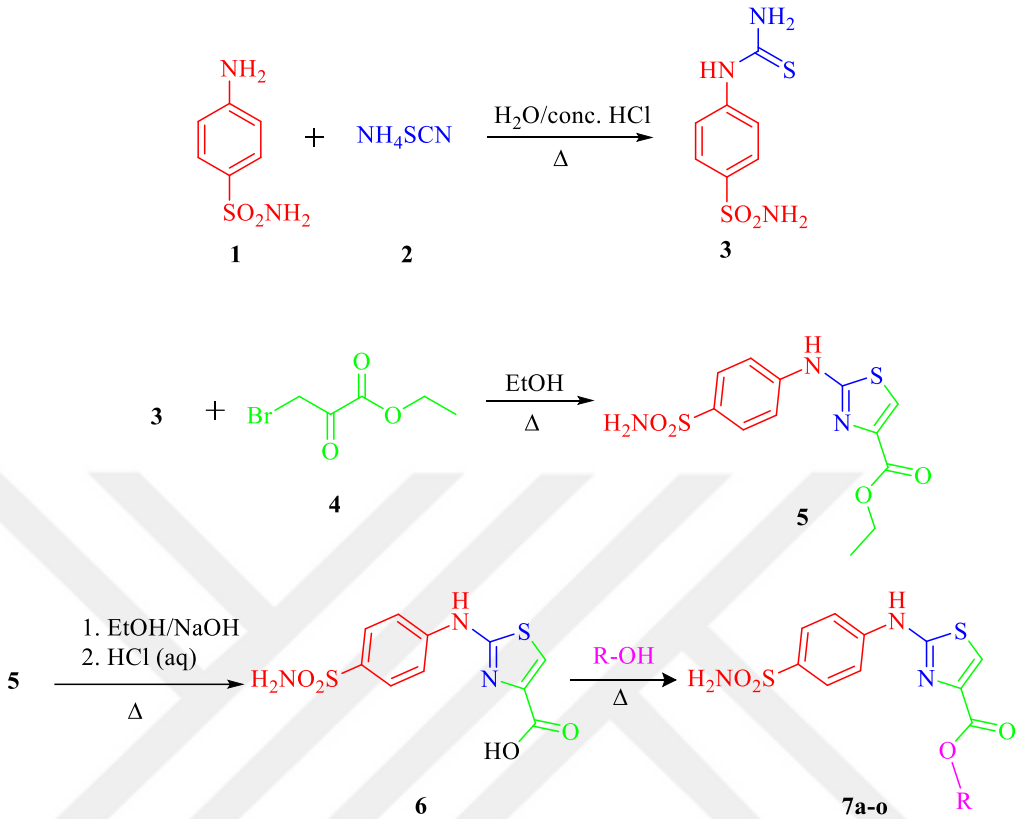
During the last five decades the design, synthesis, and biological investigations on thiazole and sulfonamide derivatives have grown exponentially due to their biological and pharmacological features as anti-inflammatory, antibacterial, anticancer, antifungal, antimicrobial, carbonic anhydrase inhibitory etc. In this study, sulfonamide containing thiazole and their cyclization reaction were examined. Totally, two sets of 30 novel thiazole based benzenesulfonamide molecules were designed, synthesized and their carbonic anhydrase inhibitor properties over hCAI, hCAII, hCAIX, hCAXII enzymes were studied. The obtained compounds were synthesized with easy purification methods and medium to high yields.

3. MATERIALS AND METHODS

3.1. Materials and Apparatus

All reagents and organic solvents were bought from commercial sources (Sigma–Aldrich and Merck) and used as without further purifications. Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a BRUKER AVANCE spectrometer (400MHz for proton, 100MHz for carbon) and Varian Inova (500 MHz for proton, 125 MHz for carbon) in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s (singlet); d (doublet); t (triplet); q (quarted); m (multiplet); broad s (broad singlet); dd (doublets of doublet); ddd (a dublet of doublets of doublets); td (triplet of doublets); app s (apparently singlet); app d (apparently doublet); app t (apparently triplet); app sx (apparently sextet). Fourier Transform Infrared (FT-IR) spectra were recorded on SHIMADZU IR Affinity-1 (ATR). The mass analyses were performed on Agilent ZQ instrument (HRMS) and BrukerMicroflex LT MALDI-TOF MS. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates and Merck Reverse Phase (RP) F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (70–230 mesh ASTM) as the stationary phase and ethyl acetate/n-hexane and ethyl acetate/ether were used as eluents and the spots were detected by ultraviolet light. Melting points (mp) were measured in open capillary tubes with a Barnstead Electrothermal BI IA9100 apparatus and are uncorrected. The formation of new compounds during synthesis was checked by benchtop NMR (The Magritek Spinsolve 60) and GC-MS (Agilent 6890N).

3.2. Experimental Part-1



Scheme 3.1. Reaction pathway to obtain sulfonamide-based thiazole-4-carboxylate **7a-o** derivatives

3.2.1. General Procedures of Part-1

3.2.1.1. Synthesis of 4-thioureidobenzene sulfonamide 3

4-Aminobenzenesulfonamide (40 mmol, 6.888 g) was dissolved in 20 ml of water and 3.6 ml of conc. HCl. The solution was warmed to dissolve and cooled to room temperature. Then ammonium thiocyanate (40 mmol, 3.045 g) was added to the solution and heated overnight at 90 ºC. After TLC control, the solution was cooled and waited in the refrigerator for 3 hr. The precipitates were filtered and washed with cold water and dried.

Obtained as beige-white solid (7.03 g, 76%), m.p. 208–210˚C, Rf: (EtOAc: Hex = 1:1): 0.17, Vmax (ATR, cm^{-1}) 3387 (NH), 3310 (NH_2), 3217 (NH_2), 1636 (NH bent), 1519 (C=C), 1312 (asym. SO_2), 1150 (sym. SO_2). ^1H NMR (400 MHz, DMSO-d_6) δ 9.95 (broad s, 1H, N*H*), 7.70 (app. d, J = 8.7 Hz, 2H, Aromatic C*H*), 7.62 (app. d, J = 8.8 Hz, 2H, Aromatic C*H*), 7.25 (broad s, 2H, SO_2-NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.2, 142.4, 138.8, 126.3, 121.8. Calculated $C_7H_9N_3O_2S_2$ $[M+H]^+$ 232.0136, found 232.0213.

3.2.1.2. Synthesis of ethyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate 5

4-Thioureidobenzene sulfonamide (30 mmol, 6.939 g) and ethyl 3-bromopyruvate (33.3 mmol, 6.494 g) were dissolved in 25 ml ethanol and reflux for 1-2 hr, after TLC control, the reaction was finished and the solvent was evaporated and crystallized from ethanol.

Obtained as beige-white solid (8.15 g, 83%), m.p. 228–230˚C, Rf: (EtOAc: Hex = 2:1): 0.57, Vmax (ATR, cm^{-1}) 3317 (NH), 3217 (NH_2), 1721 (C=O), 1551 (C=C), 1304 (asym. SO_2), 1219 (C-S), 1142 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (broad s, 1H, N*H*), 7.87 (s, 1H, thiazo C*H*), 7.80 – 7.73 (app. m, 4H, aromatic C*H*), 7.21 (broad s, 2H, SO_2-NH_2), 4.27 (q, J = 7.1 Hz, 2H, CH_2), 1.29 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 160.7, 143.4, 142.3, 136.5, 127.1, 119.7, 116.2, 60.6, 14.2. Calculated $C_{12}H_{13}N_3O_4S_2$ $[M+H]^+$ 328.0348, found 328.0436.

3.2.1.3. Synthesis of 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylic acid 6

Ethyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (20 mmol, 6.547 g) was dissolved in 20 ml ethanol and heated to reflux temperature then 10 ml of 8N sodium hydroxide was added dropwise during 10 min. After 0.5 hr, yellow precipitates were obtained and then the ethanol was evaporated. All precipitates were dissolved in water and acidified with conc. HCl until pH=1. The solution was placed in the refrigerator 2-3 hr then the precipitates were collected with cold filtration and washed with cold water.

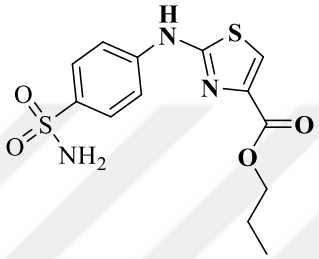
Obtained as beige-white solid (4.55 g, 76%), m.p. 285–287˚C decomp. Rf: RP (EtOAc: Hex = 3:2): 0.48, Vmax (ATR, cm^{-1}) 3348 (NH), 3264 (NH_2), 1713 (C=O), 1559 (C=C), 1327 (asym. SO_2), 1219 (C-S), 1103 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (s, 1H, O*H*), 10.74 (s, 1H, N*H*), 7.80 (s, 1H, thiazo C*H*), 7.78 (app. d, J = 9.1 Hz, 2H, aromatic C*H*), 7.75 (app. d, J = 9.1 Hz, 2H, aromatic C*H*), 7.21 (broad s, 2H, SO_2-NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.2, 162.1, 143.5, 143.4, 136.4, 127.1, 119.4, 116.2. Calculated $C_{10}H_9N_3O_4S_2$ $[M+H]^+$ 300.0034, found 300.0102.

3.2.1.4. General Reaction Procedure for Preparation of Thiazole Sulfonamides 7a – g

2-((4-Sulfamoylphenyl)amino)thiazole-4-carboxylic acid (1.0 eq, 1.0 mmol) was dissolved in corresponding alcohol as a solvent (3-4 ml) with heating then added 2-3 drops of conc. HCl to the mixture and refluxed at the boiling point of the corresponding alcohol. The progress of the reactions was monitored by TLC. For

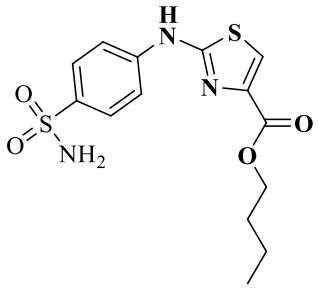
compounds **7a, 7b, 7c**; the solvents were removed under reduced pressure and the obtained residues purified by silica gel column chromatography eluting with EtOAc- Hexane (1:1). For compounds **7d, 7e, 7f, 7g**; the reaction flasks were placed in the refrigerator for 1-2 days, the formed precipitates were filtered and washed with hexane. Filtrates may also contain desired molecules so the reaction mixtures were concentrated in vacuo and the remaining residues purified by silica gel column chromatography eluting with EtOAc- Hexane (1:3).

3.2.1.4.1. Propyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7a):



Obtained as beige-white solid (321 mg, 94 %), m.p. 166–168˚C, Rf: (EtOAc: Hex = 1:1): 0.35, Vmax (ATR, cm^{-1}) 3325 (NH), 3233 (NH_2), 1705 (C=O), 1528 (C=C), 1319 (asym. SO_2), 1227 (C-S), 1150 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H, N*H*), 7.88 (s, 1H, thiazo C*H*), 7.80 – 7.73 (m, 4H, aromatic C*H*), 7.21 (broad s, 2H, SO_2-N*H*$_2$), 4.18 (t, J = 6.6 Hz, 2H, C*H*$_2$$CH_2CH_3$), 1.70 (app. sx, J =21.2, 14.1, 7.1 Hz, 2H, CH_2C*H*$_2$$CH_3$), 0.96 (t, J = 7.4 Hz, 3H, CH_2CH_2C*H*$_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 160.7, 143.4, 142.3, 136.5, 127.1, 119.7, 116.2, 65.9, 21.7, 10.3. Calculated $C_{13}H_{15}N_3O_4S_2$ $[M+H]^+$ 342.0504, found 342.0591.

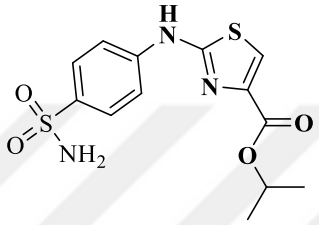
3.2.1.4.2. Butyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7b):



Obtained as beige-white solid (323 mg, 91 %), m.p. 188–190˚C, Rf: (EtOAc: Hex = 1:1): 0.42, Vmax (ATR, cm^{-1}) 3341 (NH), 3287 (NH_2), 1697 (C=O), 1520 (C=C), 1319 (asym. SO_2), 1219 (C-S), 1157 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ

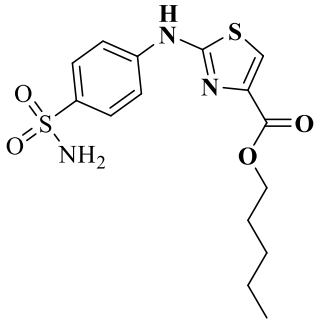
10.81 (broad s, 1H, N*H*), 7.88 (s, 1H, thiazo C*H*), 7.79 (app. d, J = 9.2 Hz, 2H aromatic C*H*), 7.76 (app. d, J = 9.2 Hz, 2H aromatic C*H*), 7.22 (s, 2H, SO_2-N*H*$_2$), 4.24 (t, J = 6.5 Hz, 2H, C*H*$_2$$CH_2$ CH_2CH_3), 1.72 – 1.62 (m, 2H CH_2C*H*$_2$$CH_2CH_3$), 1.48 – 1.36 (m, 2H CH_2CH_2C*H*$_2$$CH_3$), 0.94 (t, J = 7.4 Hz, 3H, $CH_2CH_2CH_2$C*H*$_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 160.7, 143.4, 142.3, 136.5, 127.0, 119.7, 116.2, 64.2, 30.2, 18.7, 13.6. Calculated $C_{14}H_{17}N_3O_4S_2$ $[M+H]^+$ 356.0660, found 356.0749.

3.2.1.4.3. Isopropyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7c):



Obtained as beige-white solid (181 mg, 53 %), m.p. 232–234˚C, Rf: (EtOAc: Hex = 1:1): 0.35, Vmax (ATR, cm^{-1}) 3318 (NH), 3263 (NH_2), 1705 (C=O), 1543 (C=C), 1304 (asym. SO_2), 1227 (C-S), 1157 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H, N*H*), 7.84 (s, 1H, thiazo C*H*), 7.80 – 7.65 (app. m, 4H, aromatic C*H*), 7.22 (broad s, 2H, SO_2-N*H*$_2$), 5.13 – 5.04 (m, 1H, CH_3C*H*CH_3), 1.31 (d, *J* = 6.2 Hz, 6H, C*H*$_3$$CHCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 160.1, 143.4, 142.7, 136.5, 127.1, 119.4, 116.2, 68.0, 21.7. Calculated $C_{13}H_{15}N_3O_4S_2$ $[M+H]^+$ 342.0504, found 342.0580.

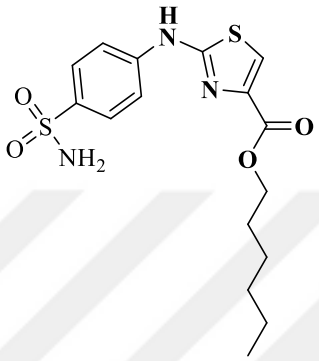
3.2.1.4.4. Pentyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7d):



Obtained as beige-white solid (325 mg, 88 %), m.p. 168–170˚C, Rf: (EtOAc: Hex = 1:1): 0.48, Vmax (ATR, cm^{-1}) 3341 (NH), 3287 (NH_2), 1705 (C=O), 1520 (C=C), 1319 (asym. SO_2), 1219 (C-S), 1157 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H, N*H*), 7.88 (s, 1H, thiazo C*H*), 7.83 – 7.74 (m, 4H, aromatic C*H*), 7.22

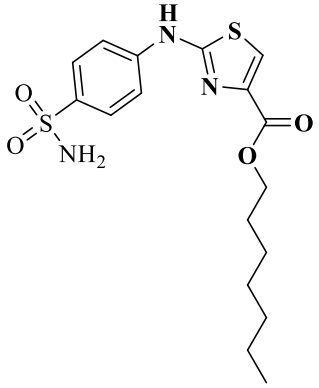
(broad s, 2H, SO_2-N*H*$_2$), 4.23 (t, *J* = 6.4 Hz, 2H, C*H*$_2$(CH_2)$_3$$CH_3$) 1.74 – 1.63 (m, 2H, CH_2C*H*$_2$(CH_2)$_2$$CH_3$), 1.44 – 1.28 (m, 4H, CH_2CH_2C*H*$_2$C*H*$_2$$CH_3$), 0.90 (t, *J* = 6.2 Hz, 3H, (CH_2)$_4$C*H*$_3$) ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 162.4, 160.7, 143.4, 142.3, 136.5, 127.0, 119.7, 116.2, 64.5, 27.9, 27.7, 21.8, 13.9. Calculated $C_{15}H_{19}N_3O_4S_2$ $[M+H]^+$ 370.0817 found 370.0910.

3.2.1.4.5. Hexyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7e):



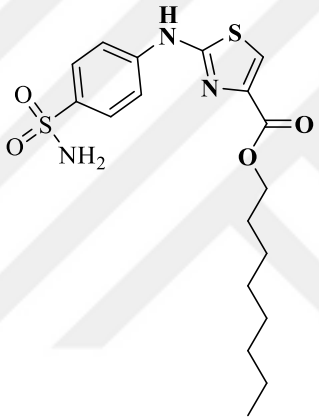
Obtained as beige-white solid (310 mg, 81 %), m.p. 153–155˚C, Rf: (EtOAc: Hex = 1:1): 0.52, V̄max (ATR, cm^{-1}) 3341 (NH), 3287 (NH_2), 1705 (C=O), 1520 (C=C), 1319 (asym. SO_2), 1219 (C-S), 1157 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.80 (s, 1H, N*H*), 7.87 (s, 1H, thiazo C*H*), 7.78 (app. d, J = 9.2 Hz, 2H aromatic C*H*), 7.75 (app. d, J = 9.1 Hz, 2H aromatic C*H*), 7.22 (broad s, 2H, SO_2-N*H*$_2$), 4.22 (t, J = 6.6 Hz, 2H, C*H*$_2$(CH_2)$_4$C*H*$_3$), 1.73 – 1.63 (m, 2H, CH_2C*H*$_2$(CH_2)$_3$C*H*$_3$), 1.44 – 1.35 (m, 2H (CH_2)$_2$C*H*$_2$(CH_2)$_2$$CH_3$), 1.34 – 1.26 (m, 4H, ($CH_2$)$_3$C*H*$_2$C*H*$_2$$CH_3$), 0.87 (t, J = 7.0 Hz, 3H, (CH_2)$_5$C*H*$_3$).^{13}C NMR (100 MHz, DMSO-d$_6$) δ 162.4, 160.7, 143.4, 142.3, 136.5, 127.1, 119.7, 116.2, 64.5, 30.9, 28.1, 25.1, 22.1, 13.9. Calculated $C_{16}H_{21}N_3O_4S_2$ $[M+H]^+$ 384.0973 found 384.1068.

3.2.1.4.6. Heptyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7f):



Obtained as beige-white solid (318 mg, 80 %), m.p. 150–152˚C, Rf: (EtOAc: Hex = 1:1): 0.61, Ѵmax (ATR, cm^{-1}) 3341 (NH), 3287 (NH_2), 1705 (C=O), 1520 (C=C), 1319 (asym. SO_2), 1219 (C-S), 1157 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H, N*H*), 7.88 (s, 1H, thiazo C*H*), 7.85 (app. d, J = 9.2 Hz, 2H aromatic C*H*), 7.82 (app. d, J = 9.2 Hz, 2H, aromatic C*H*), 7.27 (broad s, 2H, SO_2-NH_2), 4.24 (t, J = 6.6 Hz, 2H, CH_2(CH_2)$_5$$CH_3$), 1.74 – 1.64 (m, 2H, CH_2CH_2(CH_2)$_4$$CH_3$), 1.44 – 1.35 (m, 2H ($CH_2$)$_2CH_2$($CH_2$)$_3$$CH_3$), 1.34 – 1.22 (m, 6H, ($CH_2$)$_3$(C$H_2$)$_3$$CH_3$), 0.86 (t, J = 6.8 Hz, 3H, (CH_2)$_6$CH_3).^{13}C NMR (100 MHz, DMSO-d_6) δ 162.6, 160.8, 143.6, 142.5, 136.5, 127.2, 119.7, 116.4, 64.6, 31.4, 28.5, 28.3, 25.6, 22.2, 14.1. Calculated $C_{17}H_{23}N_3O_4S_2$ $[M+H]^+$ 398.1130 found 398.1238.

3.2.1.4.7. Octyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7g):



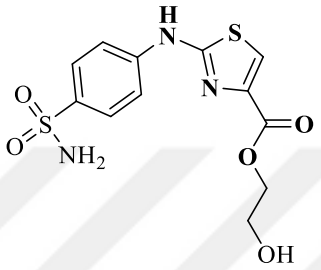
Obtained as beige-white solid (337 mg, 82 %), m.p. 161–163˚C, Rf: (EtOAc: Hex = 1:1): 0.62, Ѵmax (ATR, cm^{-1}) 3341 (NH), 3287 (NH_2), 1705 (C=O), 1520 (C=C), 1319 (asym. SO_2), 1219 (C-S), 1157 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H, N*H*), 7.87 (s, 1H, thiazo C*H*), 7.78 (app. d, J = 9.2 Hz, 2H aromatic C*H*), 7.75 (app. d, J = 9.2 Hz, 2H, aromatic C*H*), 7.21 (broad s, 2H, SO_2-NH_2), 4.22 (t, J = 6.6 Hz, 2H, CH_2(CH_2)$_6$$CH_3$), 1.73 – 1.61 (m, 2H, CH_2CH_2(CH_2)$_5$$CH_3$), 1.44 – 1.34 (m, 2H ($CH_2$)$_2CH_2$($CH_2$)$_4$$CH_3$), 1.34 – 1.21 (m, 8H, ($CH_2$)$_3$(C$H_2$)$_4$$CH_3$), 0.84 (app.t, J = 6.8 Hz, 3H, (CH_2)$_7$CH_3).^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 160.7, 143.4, 142.3, 136.5, 127.1, 119.7, 116.2, 64.5, 31.2, 28.7, 28.6, 28.2, 25.5, 22.1, 14.0. Calculated $C_{18}H_{25}N_3O_4S_2$ $[M+H]^+$ 412.1286 found 412.1388.

3.2.1.5. General Reaction Procedure for Preparation of Thiazole Sulfonamides 7h – o

2-((4-Sulfamoylphenyl)amino)thiazole-4-carboxylic acid (1.0 eq, 1.0 mmol) was dissolved in corresponding alcohol as a solvent (3-4 ml) with heating then added 2-3 drops of conc. HCl to the mixture and refluxed at the boiling point of the

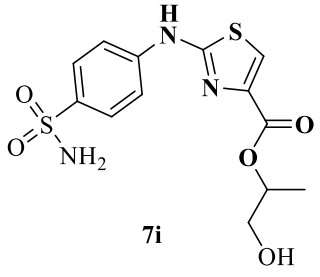
corresponding alcohol. The progress of the reactions was monitored by TLC. The reaction mixtures were extracted with EtOAc/H_2O mixture, the organic layer was dried on sodium sulfate and evaporated under reduced pressure to obtain desired molecules. If necessary, desired molecules were purified by silica gel column chromatography eluting with EtOAc/ n-hexane mixture and for compound **7n** eluting solvent was EtOAc/ ether mixture.

3.2.1.5.1. 2-Hydroxyethyl 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylate (7h):

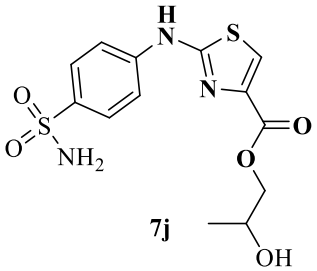


Obtained as pale yellow solid (213 mg, 62 %), m.p. 204–206˚C, Rf: (EtOAc: Hex = 4:1): 0.39, Ѵmax (ATR, cm^{-1}) 3471 (NH), 3256 (NH_2), 1705 (C=O), 1543 (C=C), 1319 (asym. SO_2), 1226 (C-S), 1142 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H, N*H*), 7.93 (s, 1H, thiazo C*H*), 7.86 – 7.78 (m, 4H aromatic C*H*), 7.27 (broad s, 2H, SO_2-N*H*$_2$), 4.31 – 4.27 (m, 2H, C*H*$_2$CH$_2$OH), 3.75 – 3.70 (m, 2H, CH$_2$C*H*$_2$OH).^{13}C NMR (100 MHz, DMSO-d_6) δ 162.6, 160.9, 143.6, 142.4, 136.5, 127.3, 120.0, 116.4, 66.5, 59.2. Calculated $C_{12}H_{13}N_3O_5S_2$ $[M+H]^+$ 344.0297 found 344.0352.

3.2.1.5.2. 1-Hydroxypropan-2-yl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7i) and 2-Hydroxypropyl 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylate (7j):



7i

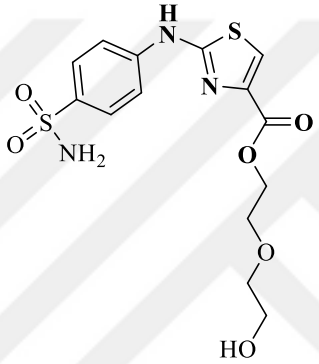


7j

Obtained as yellow solid (93 mg, 27 %, 1:1.21 mixture of 7i/7j according to ^{1}H NMR spectroscopic data), m.p. 163–165˚C, Rf: (EtOAc: Hex = 4:1): 0.34, Ѵmax (ATR, cm^{-1}) 3333 (NH), 3294 (NH_2), 1705 (C=O), 1520 (C=C), 1319 (asym. SO_2), 1211 (C-S), 1157 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (broad s,

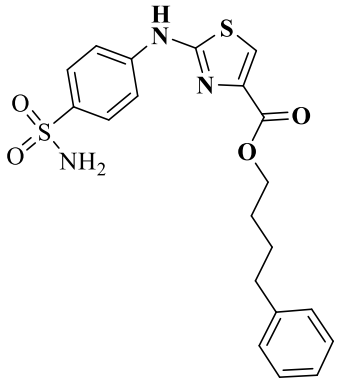
1H, N*H* of 7_i), 10.87 (s, 1H, N*H* of 7_j), 7.95 (s, 1H, thiazo C*H* of 7_i), 7.89 (s, 1H, thiazo C*H* of 7_j), 7.85 – 7.75 (m, 8H aromatic C*H* of 7_i,7_j), 7.24 (broad s, 4H, SO_2-N*H*$_2$ of 7_i,7_j), 5.23 (td, J = 6.2, 3.9 Hz, 1H, C*H*CH_3CH_2OH of 7_i), 4.50 (td, J = 6.6, 4.4 Hz, 1H, CH_2CHCH_3OH of 7_j), 4.45 – 4.33 (m, 2H, $CHCH_3CH_2OH$ of 7_i), 3.88 (ddd, J = 17.8, 11.7, 4.9 Hz, 2H, CH_2CHCH_3OH of 7_j) 1.56 (d, J = 6.6 Hz, 3H, of $CHCH_3CH_2OH$ 7_i), 1.37 (d, J = 6.4 Hz, 3H, CH_2CHCH_3OH of 7_j).^{13}C NMR (100 MHz, DMSO-d_6) δ 162.6, 162.6, 160.2, 159.9, 143.4, 142.0, 141.7, 136.5, 127.1, 127.1, 120.5, 120.3, 116.3, 70.2, 68.4, 55.4, 47.4, 21.3, 17.5. Calculated $C_{13}H_{15}N_3O_5S_2$ $[M+18]^+$ 358.0453 found 376.0249.

3.2.1.5.3. 2-(2-Hydroxyethoxy)ethyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (7k):



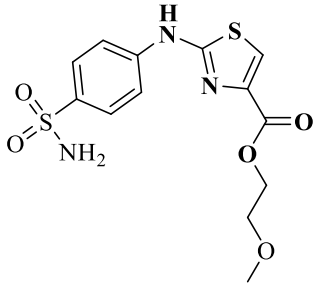
Obtained as yellow solid (190 mg, 49 %), m.p. 188–190˚C, Rf: (EtOAc: Hex = 4:1): 0.39, Vmax (ATR, cm^{-1}) 3302 (NH), 3194 (NH_2), 1705 (C=O), 1543 (C=C), 1319 (asym. SO_2), 1219 (C-S), 1150 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H, N*H*), 7.88 (s, 1H, thiazo C*H*), 7.80 – 7.74 (m, 4H aromatic C*H*), 7.22 (broad s, 2H, SO_2-N*H*$_2$), 4.37 – 4.32 (m, 2H, $CH_2CH_2OCH_2CH_2OH$), 3.74 – 3.70 (m, 2H, $CH_2CH_2OCH_2CH_2OH$). 3.52 – 3.48 (m, 4H, $CH_2CH_2OCH_2CH_2OH$).^{13}C NMR (100 MHz, DMSO-d_6) δ 162.5, 160.6, 143.4, 142.1, 136.5, 127.1, 120.0, 116.3, 72.4, 68.3, 64.1, 60.3. Calculated $C_{14}H_{17}N_3O_6S_2$ $[M+H]^+$ 388.0559 found 388.0660.

3.2.1.5.4. 4-Phenylbutyl 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylate (7l):



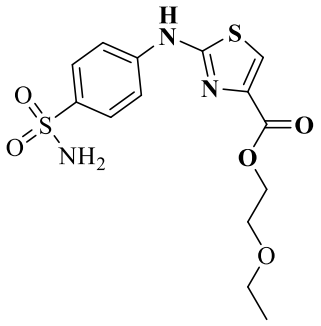
Obtained as pale yellow solid (397 mg, 92 %), m.p. 169–171˚C, Rf: (EtOAc: Hex = 1:1): 0.77, Vmax (ATR, cm^{-1}) 3341 (NH), 3294 (NH_2), 1705 (C=O), 1520 (C=C), 1319 (asym. SO_2), 1211 (C-S), 1157 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H, N*H*), 7.87 (s, 1H, thiazo C*H*), 7.78 (app. d, J = 9.4 Hz, 2H aromatic C*H*), 7.75 (app. d, J = 9.4 Hz, 2H, aromatic C*H*), 7.31 – 7.24 (m, 2H phenyl C*H*), 7.23 – 7.13 (m, 5H, two of SO_2-N*H*$_2$, three of phenyl C*H*), 4.25 (t, J = 5.4 Hz, 2H, C*H*$_2$$CH_2$ CH_2CH_2Ph), 2.64 (t, J = 6.8 Hz, 2H, CH_2CH_2 CH_2C*H*$_2$Ph), 1.74 – 1.65 (m, 4H, CH_2C*H*$_2$C*H*$_2$$CH_2CH_2$Ph).^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 160.7, 143.4, 142.3, 141.9, 136.5, 128.3, 127.1, 125.8, 119.8, 116.2, 64.3, 34.7, 27.8, 27.5. Calculated $C_{20}H_{21}N_3O_4S_2$ $[M+H]^+$ 432.0973 found 432.1080.

3.2.1.5.5. 2-Methoxyethyl 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylate (7m):



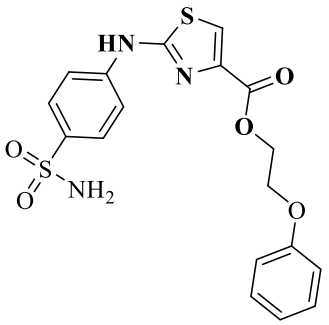
Obtained as beige-white solid (211 mg, 59 %), m.p. 187–189˚C, Rf: (EtOAc: Hex = 2:1): 0.54, Vmax (ATR, cm^{-1}) 3302 (NH), 3194 (NH_2), 1705 (C=O), 1536 (C=C), 1319 (asym. SO_2), 1219 (C-S), 1142 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H, N*H*), 7.91 (s, 1H, thiazo C*H*), 7.86 – 7.78 (m, 4H aromatic C*H*), 7.31(broad s, 2H, SO_2-N*H*$_2$), 4.41 (app. t, J = 4.1 Hz, 2H, C*H*$_2$$CH_2OCH_3$), 3.70 – 3.66 (m, 2H, CH_2C*H*$_2$$OCH_3$). 3.35 (s, 3H, CH_2CH_2OC*H*$_3$), ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 161.0, 143.7, 142.4, 136.6, 127.4, 120.3, 116.6, 70.1, 64.1, 58.5. Calculated $C_{13}H_{15}N_3O_5S_2$ $[M+H]^+$ 358.0453 found 358.0521.

3.2.1.5.6. 2-Ethoxyethyl 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylate (7n):



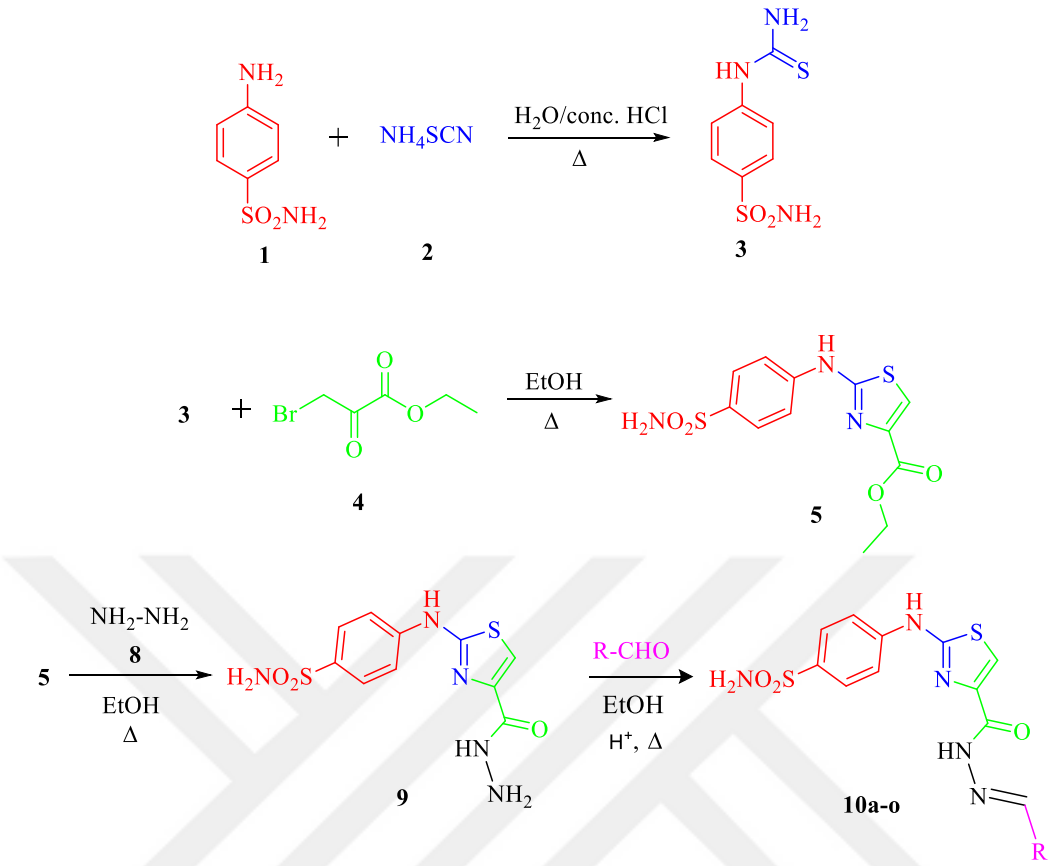
Obtained as pale yellow solid (235 mg, 65 %), m.p. 143–145˚C, Rf: (EtOAc: Hex = 2:1): 0.77, Vmax (ATR, cm^{-1}) 3294 (NH), 3248 (NH_2), 1713(C=O), 1536 (C=C), 1319 (asym. SO_2), 1219 (C-S), 1142 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H, N*H*), 7.90 (s, 1H, thiazo C*H*), 7.81 (app. d, J = 9.2 Hz, 2H aromatic C*H*), 7.78 (app. d, J = 9.2 Hz, 2H, aromatic C*H*), 7.25 (broad s, 2H, SO_2-NH_2), 4.36 (app. t, J = 4.6 Hz, 2H, C$H_2$$CH_2OCH_2CH_3$), 3.69 (app. t, J = 4.6 Hz, 2H, CH_2C$H_2$$OCH_2CH_3$), 3.52 (q, J = 7.0 Hz, 2H, $CH_2CH_2OCH_2$$CH_3$), 1.14 (t, J = 7.0 Hz, 3H, $CH_2CH_2OCH_2C$$H_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.5, 160.7, 143.4, 142.1, 136.5, 127.1, 120.1, 116.3, 67.7, 65.7, 64.1, 15.2. Calculated $C_{14}H_{17}N_3O_5S_2$ $[M+H]^+$ 372.0610 found 372.0683.

3.2.1.5.7. 2-Phenoxyethyl 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylate (7o):



Obtained as pale yellow solid (257 mg, 61 %), m.p. 214–216˚C, Rf: (EtOAc: Hex = 2:1): 0.33, Vmax (ATR, cm^{-1}) 3287 (NH), 3217 (NH_2), 1721 (C=O), 1551(C=C), 1312 (asym. SO_2), 1204 (C-S), 1150 (sym. SO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H, N*H*), 7.90 (s, 1H, thiazo C*H*), 7.82 – 7.76 (m, 4H, aromatic C*H*), 7.31 (dd, J = 8.6, 7.4 Hz, 2H, two of phenyl C*H*), 7.26 (broad s, 2H, SO_2-NH_2), 7.03 – 6.92 (m, 3H, three of phenyl C*H*), 4.60 (app t, J = 4.5 Hz, 2H, C$H_2$$CH_2$OPh), 4.32 (app t, J = 4.5 Hz, 2H, CH_2CH_2OPh). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.6, 160.7, 158.3, 143.5, 142.0, 136.5, 129.7, 127.2, 121.0, 120.3, 116.3, 114.7, 65.8, 63.3. Calculated $C_{18}H_{17}N_3O_5S_2$ $[M+H]^+$ 420.0610 found 420.0678.

3.3. Experimental Part-2



Scheme 3.2. Reaction pathway to obtain thiazole and hydrazine-1-carbonyl-based benzenesulfonamide **10a-o** derivatives

3.3.1. General procedures of part-2

3.3.1.1. 4-((4-(hydrazinecarbonyl)thiazol-2-yl)amino) benzenesulfonamide 9

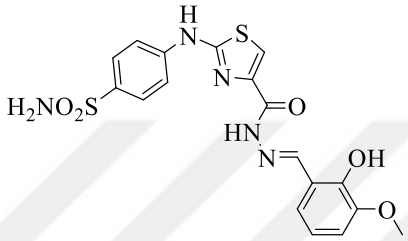
Ethyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate (20 mmol, 6.547 g) and hydrazine hydrate 50-60% (80 mmol, 4.005 g) was dissolved in 20 ml ethanol and reflux 1-2 hr, after TLC control, white precipitates were filtered and washed with cold ethanol.

Obtained as beige-white solid (5.36 g, 86%), m.p. 256–258˚C, Rf: (EtOAc: AcOH = 25:1): 0.43, V̄max (ATR, cm^{-1}) 3385-3294 (NH_2–NH), 1620 (C=O, amide), 1539 (C=C), 1327 (asym. SO_2), 1155 (sym. SO_2). ^1H NMR (500 MHz, DMSO-d_6) δ 10.69 (broad s, 1H, N*H*), 9.53 (s, 1H, N*H*), 7.91 (d, J = 8.7 Hz, 2H, aromatic CH), 7.73 (d, J = 8.8 Hz, 2H, aromatic CH), 7.56 (s, 1H, thiazo CH), 7.19 (s, 2H, SO_2-N*H*$_2$), 4.51 (broad s, 2H, NH-N*H*$_2$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.7, 160.2, 145.0, 143.5, 136.3, 127.3, 116.9, 114.1. $C_{10}H_{11}N_5O_3S_2$. m/z (TOF ES^-) 298.4 (9%), 313.7 (M+, 100%), 335.6 (M + Na, 10%).

3.3.1.2. General reaction procedure for preparation of thiazole and hydrazine-1-carbonyl-based benzenesulfonamide derivatives 10a-o

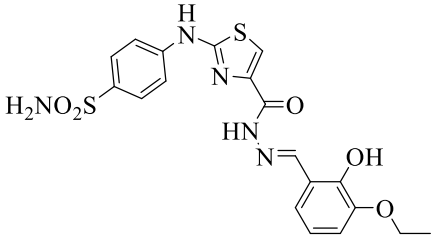
1 mmol 4-((4-(hydrazinecarbonyl)thiazol-2-yl)amino)benzenesulfonamide (9) and 1 mmol corresponding aldehydes were dissolved in 10 ml ethanol then reflux 1-8 hr. . The progress of the reactions was monitored by TLC. After cooling, the formed precipitates were filtered and washed with cold ethanol.

3.3.1.2.1. (E)-4-((4-(2-(2-hydroxy-3-methoxybenzylidene)hydrazine-1-carbonyl)thiazol-2-yl)amino)benzenesulfonamide (10a):



Obtained as beige-white solid (412 mg, 92 %), m.p. 284-286˚C, Rf: (EtOAc: AcOH = 25:1): 0.57. V̇max (ATR, cm^{-1}) 3385-3111 (NH, NH_2, OH), 1645(C=O, amide), 1537(C=C), 1328 (asym. SO_2), 1150 (sym. SO_2).^{1}H NMR (500 MHz, DMSO-d_6) δ 11.64 (s, 1H), 10.98 (s, 1H), 10.78 (s, 1H), 8.80 (s, 1H), 7.92 (d, *J* = 8.5 Hz, 2H), 7.82 (s, 1H), 7.78 (d, J = 8.7 Hz, 2H), 7.21 (s, 2H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.86 (app. t, *J* = 7.8 Hz, 1H), 3.80 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.8, 156.9, 149.3, 148.0, 147.3, 144.4, 143.3, 136.5, 127.1, 119.2, 119.1, 119.0, 116.8, 116.7, 113.9, 23.1. $C_{18}H_{17}N_5O_5S_2$, m/z (TOF ES^-) 447.7 (M+, 100%), 470.2 (M + Na, 70%).

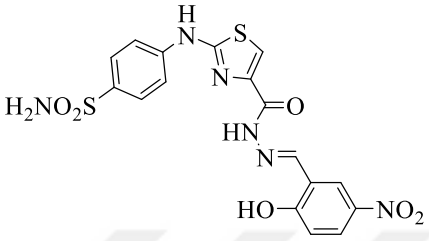
3.3.1.2.2. (E)-4-((4-(2-(3-ethoxy-2-hydroxybenzylidene)hydrazine-1-carbonyl)thiazol-2-yl)amino)benzenesulfonamide (10b):



Obtained as beige-white solid (372 mg, 80.6 %), m.p. 277–279˚C, Rf: (EtOAc: AcOH = 25:1): 0.71. V̇max (ATR, cm-1) 3371-3111 (NH, NH_2, OH), 1651(C=O, amide), 1537(C=C), 1328 (asym. SO_2), 1150 (sym. SO_2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.68 (s, 1H), 11.04 (s, 1H), 10.80 (s, 1H), 8.82 (broad s, 1H), 7.94 (d, *J* = 8.1 Hz, 2H), 7.85 (s, 1H), 7.81 (d, J = 8.3 Hz, 2H), 7.23 (s, 2H), 7.14 (d, *J* = 7.70 Hz, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 6.86 (app. t, *J* = 7.5 Hz, 1H), 4.08 (app. q, *J*

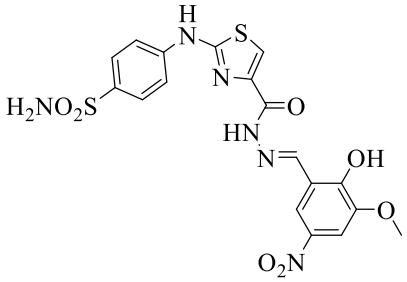
= 6.9 Hz, 2H), 1.36 (app.t, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.8, 156.9, 149.5, 147.6, 147.1, 144.4, 143.3, 136.5, 127.1, 121.2, 119.1, 119.0, 116.7, 116.6, 115.3, 64.2, 14.8. $C_{19}H_{19}N_5O_5S_2$, m/z (TOF ES$^-$) 328.1 (53%), 461.3 (M+, 100%), 483.5 (M + Na, 30%).

3.3.1.2.3. (E)-4-((4-(2-(2-hydroxy-5-nitrobenzylidene)hydrazine-1-carbonyl)thiazol-2-yl)amino)benzenesulfonamide (10c):



Obtained as light yellow solid (440 mg, 95.1 %), m.p. 342-344˚C decomp. Rf: (EtOAc: AcOH = 25:1): 0.49. Vmax (ATR, cm^{-1}) 3350-3115 (NH, NH_2, OH), 1643(C=O, amide), 1537(C=C), 1328 (asym. SO_2), 1136 (sym. SO_2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.29 (s, 1H), 11.85 (s, 1H), 10.79 (s, 1H), 8.91 (s, 1H), 8.58 (d, J = 2.9 Hz, 1H), 8.18 (dd, *J* = 9.1, 2.9 Hz, 1H) , 7.93 (d, *J* = 8.8 Hz, 2H), 7.86 (s, 1H), 7.80 (d, J = 8.8 Hz, 2H), 7.23 (s, 2H), 7.13 (d, J = 9.1 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.8, 162.6, 157.2, 145.3, 144.3, 143.2, 140.0, 136.5, 127.1, 126.6, 120.2, 117.2, 117.2, 116.9, 116.7.$C_{17}H_{14}N_6O_6S_2$, m/z (TOF ES$^-$) 298.4 (30%), 356.3 (27%), 446.1 (42%), 461.9 (M+, 100%).

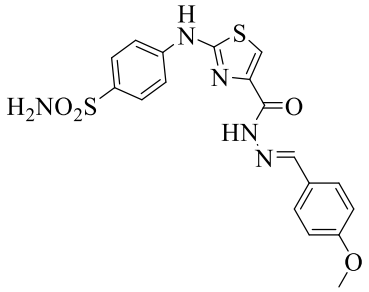
3.3.1.2.4. (E)-4-((4-(2-(2-hydroxy-3-methoxy-5-nitrobenzylidene) hydrazine-1-carbonyl)thiazol-2-yl)amino)benzenesulfonamide (10d):



Obtained as beige-white solid (382 mg, 77.6 %), m.p. 334–336˚C decomp. Rf: (EtOAc: AcOH = 25:1): 0.23. Vmax (ATR, cm^{-1}) 3279-3100 (NH, NH_2, OH), 1645 (C=O, amide), 1548 (C=C), 1328 (asym. SO_2), 1147 (sym. SO_2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.94 (s, 1H), 11.83 (s, 1H), 10.79 (s, 1H), 8.93 (s, 1H), 8.25 (app. d, *J* = 2.4 Hz, 1H), 7.93 (d, *J* = 8.9 Hz, 2H), 7.86 (s, 1H), 7.82 – 7.77 (m, 3H), 7.23 (s, 2H), 3.97 (s, 3H).^{13}C NMR (125 MHz, DMSO-d_6) δ 162.8, 157.2, 152.8,

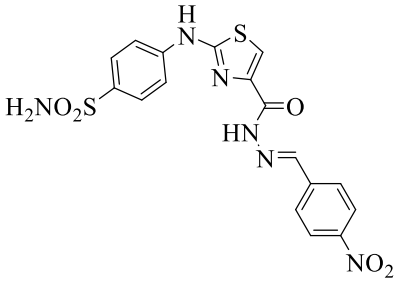
148.2, 145.5, 144.3, 143.2, 139.6, 136.5, 127.1, 119.3, 116.8, 116.7, 115.8, 107.2, 17.7. $C_{18}H_{16}N_6O_7S_2$, m/z (TOF ES$^-$) 168.8 (36%), 390.8 (100%), 493.0 (M+, 99%).

3.3.1.2.5. (E)-4-((4-(2-(4-methoxybenzylidene)hydrazine-1-carbonyl)thiazol-2-yl)amino)benzenesulfonamide (10e):



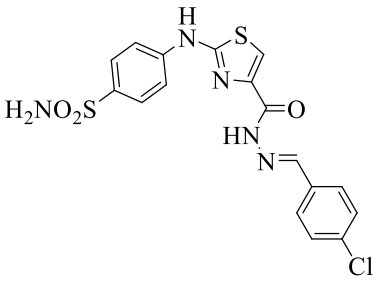
Obtained as beige-white solid (310 mg, 71.9 %), m.p. 277–279˚C, Rf: (EtOAc: AcOH = 25:1): 0.80. Vmax (ATR, cm^{-1}) 3243-3224 (NH, NH_2), 1657 (C=O, amide), 1589 (C=C), 1342 (asym. SO_2), 1152 (sym. SO_2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.23 (s, 1H), 10.77 (s, 1H), 8.54 (s, 1H), 7.92 (d, J = 7.3 Hz, 2H), 7.81 (s, 1H), 7.78 (app. d, J = 6.9 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.22 (s, 2H), 7.03 (app. d, J = 8.5 Hz, 2H), 3.81 (s, J = 4.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.6, 160.9, 156.9, 148.8, 144.9, 143.3, 136.4, 128.8, 128.8, 127.2, 126.9, 116.7, 114.4, 55.3. $C_{18}H_{17}N_5O_4S_2$, m/z (TOF ES$^-$) 226.0 (20%), 268.1 (37%), 356.4 (20%), 431.5 (M+, 100%), 483.5 (M + K, 10%).

3.3.1.2.6. (E)-4-((4-(2-(4-nitrobenzylidene)hydrazine-1-carbonyl)thiazol-2-yl)amino)benzenesulfonamide (10f):



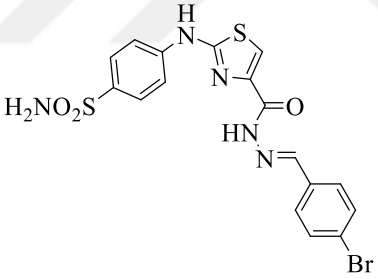
Obtained as bright orange solid (403 mg, 90.1 %), m.p. 282–284˚C, Rf: (EtOAc: Hex: AcOH = 25:5:1): 0.31. Vmax (ATR, cm^{-1}) 3340-3327 (NH, NH_2), 1650 (C=O, amide), 1529 (C=C), 1336 (asym. SO_2), 1155 (sym. SO_2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.64 (s, 1H), 10.78 (s, 1H), 8.70 (s, 1H), 8.29 (d, J = 8.5 Hz, 2H), 7.98 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.84 (s, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.21 (s, 2H).^{13}C NMR (125 MHz, DMSO-d_6) δ 162.8, 157.3, 147.9, 146.3, 144.4, 143.2, 140.6, 136.5, 128.1, 127.1, 124.1, 116.9, 116.7. $C_{17}H_{14}N_6O_5S_2$ m/z (TOF ES$^-$) 431.0 (18%), 446.2 (M+, 100%), 468.2 (M + Na, 69%).

3.3.1.2.7. (E)-4-((4-(2-(4-chlorobenzylidene)hydrazine-1-carbonyl) thiazol-2-yl)amino)benzenesulfonamide (10g):



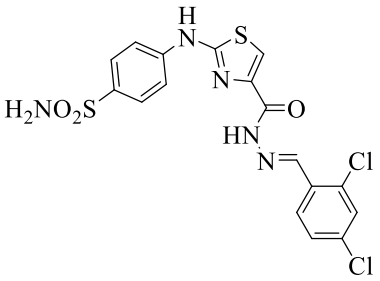
Obtained as beige-white solid (381 mg, 87.4 %), m.p. 278–280˚C, Rf: (EtOAc: Hex: AcOH = 25:5:1): 0.37. Vmax (ATR, cm^{-1}) 3343-3258 (NH, NH_2), 1682 (C=O, amide), 1537 (C=C), 1317 (asym. SO_2), 1155 (sym. SO_2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.42 (s, 1H), 10.77 (s, 1H), 8.59 (s, 1H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.80 (app. d, *J* = 6.1 Hz, 2H), 7.77 (app. d, *J* = 5.1 Hz, 2H), 7.75 (s, 1H), 7.52 (d, *J* = 7.5 Hz, 2H), 7.21 (s, 2H), ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.7, 157.1, 147.6, 144.7, 143.3, 136.4, 134.6, 133.3, 129.0, 128.8, 127.2, 116.7, 116.4. $C_{17}H_{14}ClN_5O_3S_2$ m/z (TOF ES^-) 435.2 (M+, 100%).

3.3.1.2.8. (E)-4-((4-(2-(4-bromobenzylidene)hydrazine-1-carbonyl) thiazol-2-yl)amino)benzenesulfonamide (10h):



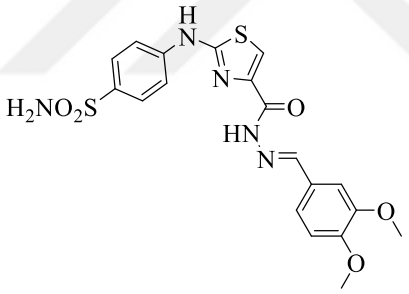
Obtained as dark beige-white solid (346 mg, 72.1 %), m.p. 261–263˚C, Rf: (EtOAc: Hex: AcOH = 25:5:1): 0.34. Vmax (ATR, cm^{-1}) 3310-3254 (NH, NH_2), 1682 (C=O, amide), 1530 (C=C), 1315 (asym. SO_2), 1153 (sym. SO_2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.44 (s, 1H), 10.79 (s, 1H), 8.59 (s, 1H), 7.92 (d, *J* = 8.5 Hz, 2H), 7.81 (app. dd, *J* = 15.0, 7.6 Hz, 3H), 7.74 – 7.66 (m, 4H), 7.23 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.7, 157.1, 147.6, 144.7, 143.3, 136.4, 133.6, 131.9, 129.0, 127.2, 123.4, 116.7, 116.4. $C_{17}H_{14}BrN_5O_3S_2$ m/z (TOF ES^-) 366.1 (100%), 481.5 ($M+H^+$, 27%).

3.3.1.2.9. (E)-4-((4-(2-(2,4-dichlorobenzylidene)hydrazine-1-carbonyl) thiazol-2-yl)amino)benzenesulfonamide (10i)



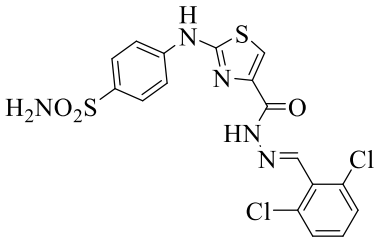
Obtained as beige-white solid (410 mg, 87.2%), m.p. 299–301˚C, Rf: (EtOAc: Hex: AcOH = 25:5:1): 0.46. Vmax (ATR, cm^{-1}) 3370-3223 (NH, NH_2), 1651 (C=O, amide), 1537 (C=C), 1319 (asym. SO_2), 1155 (sym. SO_2). ^1H NMR (500 MHz, DMSO-d_6) δ 11.77 (s, 1H), 10.76 (s, 1H), 8.95 (s, 1H), 8.02 (app. d, *J* = 8.4 Hz, 1H), 7.89 (app. d, *J* = 8.7 Hz, 2H), 7.82 (s, 1H), 7.76 (app. d, *J* = 8.8 Hz, 2H), 7.70 (app. d, *J* = 2.1 Hz, 1H), 7.50 (app. d, *J* = 6.5 Hz, 1H), 7.19 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.1, 157.4, 144.6, 143.6, 143.3, 136.5, 135.2, 134.0, 130.9, 129.4, 128.2, 127.1, 116.8, 116.7. $C_{17}H_{13}Cl_2N_5O_3S_2$ m/z (TOF ES^-) 470.3 (M+, 100%), 492.7 (M + Na, 52%), 510.4 (M + MeCN, 23%).

3.3.1.2.10. (E)-4-((4-(2-(3,4-dimethoxybenzylidene)hydrazine-1-carbonyl)thiazol-2-yl)amino)benzenesulfonamide (10j)



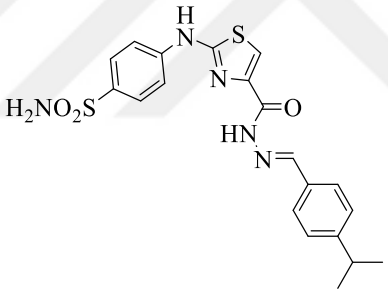
Obtained as white solid (410 mg, 88.9%), m.p. 283–285˚C, Rf: (EtOAc: Hex: AcOH = 25:5:1): 0.26. Vmax (ATR, cm^{-1}) 3366-3275 (NH, NH_2), 1630 (C=O, amide), 1527 (C=C), 1267 (asym. SO_2), 1138 (sym. SO_2). ^1H NMR (500 MHz, DMSO-d_6) δ 11.22 (s, 1H), 10.77 (s, 1H), 8.52 (s, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.83 – 7.73 (m, 3H), 7.36 (s, 1H), 7.26 – 7.18 (m, 3H), 7.04 (d, J = 8.3 Hz, 1H), 3.82 (app. s, 3H), 3.81 (app. s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.7, 156.9, 150.8, 149.3, 149.1, 144.9, 143.3, 136.4, 127.2, 127.0, 122.0, 116.7, 115.9, 111.6, 108.4, 55.6, 55.5. $C_{19}H_{19}N_5O_5S_2$ m/z (TOF ES^-) 461.9 (M+, 57%), 484.8 (M + Na, 100%).

3.3.1.2.11. (E)-4-((4-(2-(2,6-dichlorobenzylidene)hydrazine-1-carbonyl) thiazol-2-yl)amino)benzenesulfonamide (10k):



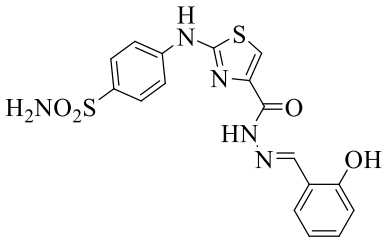
Obtained as beige-white solid (400 mg, 85.1%), m.p. 290–292˚C, Rf: (EtOAc: Hex: AcOH = 25:5:1): 0.37. Vmax (ATR, cm^{-1}) 3412-3115 (NH, NH_2), 1668 (C=O, amide), 1537 (C=C), 1317 (asym. SO_2), 1141 (sym. SO_2). ^1H NMR (500 MHz, DMSO-d_6) δ 11.77 (s, 1H), 10.79 (s, 1H), 8.80 (s, 1H), 7.92 (d, *J* = 8.3 Hz, 2H), 7.83 (s, 1H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 7.9 Hz, 2H), 7.45 (app. t, *J* = 7.9 Hz, 1H), 7.21 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.8, 157.3, 144.5, 144.0, 143.2, 136.5, 134.0, 130.9, 129.1, 129.0, 127.1, 116.8, 116.7. $C_{17}H_{13}Cl_2N_5O_3S_2$ m/z (TOF ES^-) 470.3 (M+, 32%), 494.1 (M + Na^+, 100%), 510.1 (M + MeCN, 20%).

3.3.1.2.12. (E)-4-((4-(2-(4-ısopropylbenzylidene)hydrazine-1-carbonyl) thiazol-2-yl)amino)benzenesulfonamide (10l):



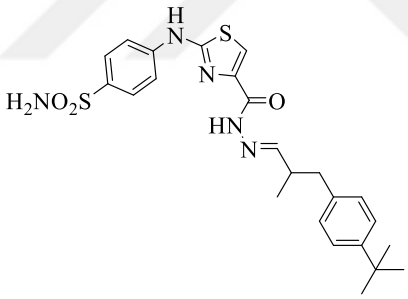
Obtained as beige-white solid (313 mg, 70.8 %), m.p. 232–234˚C Rf: (EtOAc: Hex: AcOH = 25:5:1): 0.40. Vmax (ATR, cm^{-1}) 3264-33076 (NH, NH_2), 1674 (C=O, amide), 1537 (C=C), 1327 (asym. SO_2), 1151 (sym. SO_2). ^1H NMR (500 MHz, DMSO-d_6) δ 11.30 (s, 1H), 10.78 (s, 1H), 8.58 (s, 1H), 7.92 (d, *J* = 7.3 Hz, 2H), 7.80 (d, *J* = 8.2 Hz, 3H), 7.67 (d, *J* = 7.5 Hz, 2H), 7.34 (d, *J* = 6.7 Hz, 2H), 7.22 (s, 2H), 3.00 – 2.85 (m, 1H), 1.22 (app. d, *J* = 6.9 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.7, 157.0, 150.8, 148.9, 144.8, 143.3, 136.4, 132.0, 127.3, 127.2, 126.9, 116.7, 116.1, 33.4, 23.7. $C_{20}H_{21}N_5O_3S_2$ m/z (TOF ES^-) 304.5 (8%), 444.0 (M+, 100%), 467.2 (M + Na^+, 40%).

3.3.1.2.13. (E)-4-((4-(2-(2-hydroxybenzylidene)hydrazine-1-carbonyl) thiazol-2-yl)amino)benzenesulfonamide (10m):



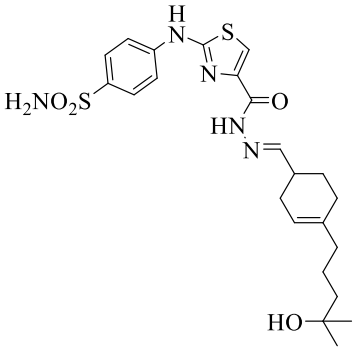
Obtained as beige-white solid (320 mg, 76.7%), m.p. 296–298˚C, Rf: (EtOAc: Hex: AcOH = 25:5:1): 0.34. Ѵmax (ATR, cm^{-1}) 3342-3105 (NH, NH_2, OH), 1651 (C=O, amide), 1522 (C=C), 1317 (asym. SO_2), 1150 (sym. SO_2). ^1H NMR (500 MHz, DMSO-d_6) δ 11.67 (s, 1H), 11.31 (s, 1H), 10.78 (s, 1H), 8.80 (s, 1H), 7.93 (d, *J* = 8.8 Hz, 2H), 7.83 (s, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.30 (app. t, *J* = 7.5 Hz, 1H), 7.22 (s, 2H), 6.97 – 6.88 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.8, 157.6, 156.9, 149.5, 144.3, 143.3, 136.5, 131.5, 129.7, 127.0, 119.4, 118.7, 116.7, 116.5, 116.5. $C_{17}H_{15}N_5O_4S_2$ m/z (TOF ES^-) 417.8 (M+, 15%), 440.8 ($M + Na^+$, 100%).

3.3.1.2.14. (E)-4-((4-(2-(3-(4-(tert-butyl)phenyl)-2-methylpropylidene) hydrazine-1-carbonyl)thiazol-2-yl)amino)benzenesulfonamide (10n):



Obtained as white solid (291 mg, 78.3 %), m.p. 219–221˚C, , Rf (EtOAc: Hex: AcOH = 25:5:1): 0.37. Ѵmax (ATR, cm^{-1}) 3292-2962 (NH, NH_2), 1667 (C=O, amide), 1537 (C=C), 1342 (asym. SO_2), 1153 (sym. SO_2). ^1H NMR (500 MHz, DMSO-d_6) δ 10.97 (s, 1H), 10.74 (s, 1H), 7.90 – 7.85 (m, 3H), 7.77 (app. d, *J* = 6.9 Hz, 2H), 7.72 (s, 1H), 7.29 (d, *J* = 8.3 Hz, 2H), 7.20 (s, 2H), 7.15 (d, *J* = 7.8 Hz, 2H), 2.85 (app dd, *J* = 13.4, 6.5 Hz, 1H), 2.71 (app. dt, *J* = 15.3, 7.8 Hz, 1H), 2.58 (app. dd, *J* = 13.5, 7.4 Hz, 1H), 1.24 (s, 9H), 1.04 (d, *J* = 6.8 Hz, 3H).^{13}C NMR (125 MHz, DMSO-d_6) δ 162.6, 156.8, 156.8, 148.2, 144.9, 143.3, 136.5, 136.4, 128.8, 127.1, 125.0, 116.7, 115.6, one peak under DMSO, 38.13, 34.09, 31.19, 17.44. $C_{24}H_{29}N_5O_3S_2$ m/z (TOF ES^-) 487.8 (94%), 499.6 (M+, 100%).

3.3.1.2.15. (E)-4-((4-(2-((4-(4-hydroxy-4-methylpentyl)cyclohex-3-en-1-yl)methylene)hydrazine-1-carbonyl)thiazol-2-yl)amino) benzenesulfonamide (10o):



Obtained as white solid (390 mg, 77.2 %), m.p. 214–216˚C, Rf (EtOAc: AcOH = 25:1): 0.71 Vmax (ATR, cm^{-1}) 3261-2932 (NH, NH_2, OH), 1668 (C=O, amide), 1537 (C=C), 1327 (asym. SO_2), 1151 (sym. SO_2). ^1H NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 1H), 10.73 (s, 1H), 7.93 – 7.84 (m, 3H), 7.80 – 7.70 (m, 3H), 7.20 (s, 2H), 5.40 (s, 1H), 4.10 – 4.03 (m, 1H), 2.21 – 1.80 (m, 7H), 1.57 – 1.24 (m, 6H), 1.05 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.6, 156.8, 156.4, 144.9, 143.3, 137.5, 136.4, 127.1, 118.9, 116.7, 115.7, 68.8, 43.3, one peak under DMSO, 37.7, 29.4, 29.3, 27.6, 26.2, 22.0. $C_{23}H_{31}N_5O_4S_2$ m/z (TOF ES^-) 486.9 (100%), 505.1 (M+, 25%), 527.9 (M + Na, 10%).

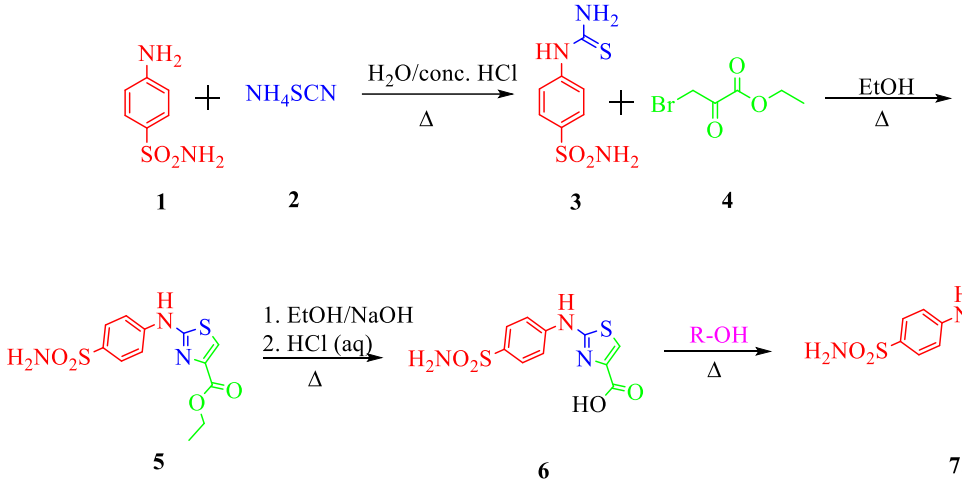
3.4. Carbonic Anhydrase Inhibition

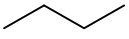
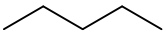
The Carbonic anhydrase catalyzed CO_2 hydration activity was measured using an Applied Photophysics stopped-flow instrument.[66] 20 mM Hepes (pH 7.4) as a buffer, 20 mM Na_2SO_4 as maintain constant ionic strength, phenol red (at a concentration of 0.2 mM) as an indicator were used as solutions. Studied at the maximum absorbance of 557 nm and initial rates of the CA-catalyzed CO2 hydration reaction for a period of 10-100s. The CO_2 concentrations used to calculate the kinetic parameters and inhibition constants ranged from 1.7 to 17 mM.[56] The assay system's enzyme concentrations are between 5 to 12 nM. The initial velocity was calculated for each inhibitor using at least six traces of the first 5–10% of the reaction. The total observed rates were calculated after subtracting the uncatalyzed rates, which were determined in the same way. The inhibitor stock solutions (0.1 nM) were prepared using deionised-distilled water and diluted the solutions with assay buffer to 0.01nM. Enzyme and inhibitor solutions were pre-incubated at room temperature for 15 minutes to allow the E–I complex formation prior to the assay. The inhibition constants, which reflect the mean of at least three separate measurements, were derived by non-linear least-squares approaches using PRISM 3 and the Cheng-Prusoff equation as previously published. As previously stated, all CA isoforms were recombinant proteins obtained in house.[67,68,69]

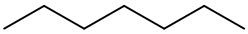
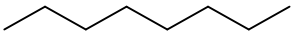
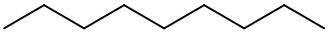
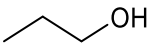
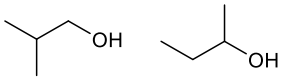
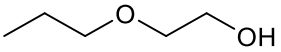
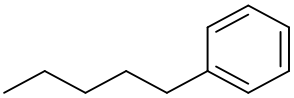
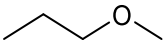
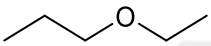
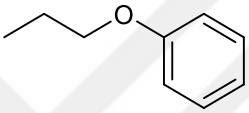
4. RESULTS AND DISCUSSION

4.1. The Results and Discussion of Sulfonamide-Based Thiazole-4-Carboxylate 7a-o Derivatives

In this study, two sets of thiazole based heterocyclic molecules were synthesized. To achieve this, 4-thioureidobenzene sulfonamide **3** was obtained using a literature method. [70] Compound **3** was then subjected to a reaction with ethyl 3-bromopyruvate **4** in ethanol under reflux conditions, resulting in the formation of the intermediate thiazole-4-carboxylate compound **5**. Subsequently, it was converted into its carboxylic acid form. The synthesis of target sulfonamide-based thiazole-4-carboxylate **7 a-o** derivatives was achieved through the reaction of 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylic acid **6** and corresponding alcohols, employing an acid catalyst, in moderate to high yields. For the esterification reactions, attempts were made to react equimolar amounts of alcohols and carboxylic acid in different solvents such as THF and DMSO; however, the reaction yield remained low. Subsequently, the mole ratio of alcohol in the reaction was increased, but similar low yields were obtained. Consequently, the decision was made to employ alcohols as solvents in the reaction process.



Compound	R	Yield (%)*	Melting point (˚C)
7a		94	166-168
7b		91	188-190
7c		53	232-234

7d		88	168-170
7e		81	153-155
7f		80	150-152
7g		82	161-163
7h		62	204-206
7i/7j		27	163-165
7k		49	188-190
7l		92	169-171
7m		59	187-189
7n		65	143-145
7o		61	214-216

* The yields were calculated from compound **6**.

Scheme 4.1. Reaction pathway to obtain sulfonamide-based thiazole-4-carboxylate **7a-o** derivatives

Short-chain alcohols, ranging from 1-propanol, a primary alcohol with a boiling point of 97°C, to 1-octanol, a fatty alcohol with a boiling point of 195°C, were employed for synthesizing compounds **7a-g**. These compounds were obtained with high yields, as illustrated in **Scheme 4.1**.

For compounds **7a**, **7b**, and **7c**, the corresponding solvents (alcohols) were removed under reduced pressure and subsequently purified using silica gel column chromatography, eluted with an EtOAc:hexane mixture in a 1:1 ratio. Regarding compounds **7d**, **7e**, **7f** and **7g**, the solvents had higher boiling points, necessitating evaporation. To achieve this, the mixture was refrigerated for 1-2 days, resulting in the formation of precipitates that were subsequently filtered and washed with hexane. If necessary, further purification was performed using column chromatography.

Following the purification steps, the purity of the desired samples was evaluated using a GC-MS headspace device to detect the presence of any residual reaction

solvents. In the initial phase of the study, it was observed that short-chain alcohols exhibited quicker reactions with carboxylic acids, resulting in higher yields, except for 2-propanol. This disparity can be attributed to the branched and secondary nature of 2-propanol, which renders ester formation more challenging compared to primary alcohols.

Figure 4.1 illustrates that the target molecules (carboxylic acid to ester) were successfully obtained, evident from the presence of -CH_2CH_2- hydrogens within the range of 1 to 5 ppm on the ^{1}H NMR spectrum.

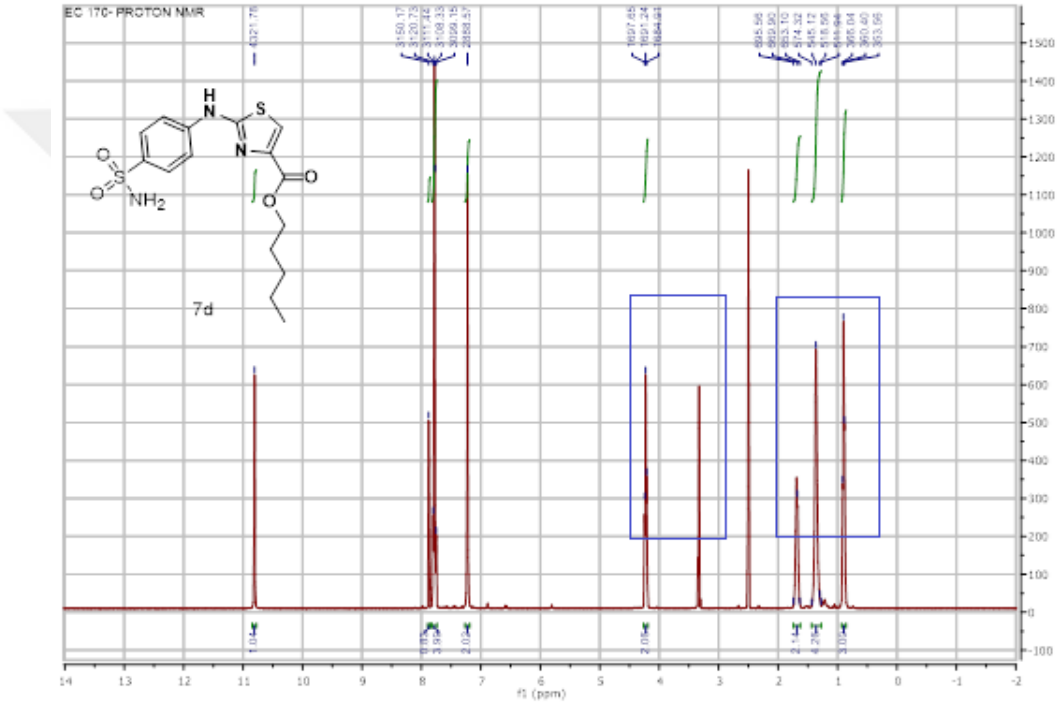


Figure 4.1. ^{1}H NMR spectrum of **7d** compound.

Symmetric and asymmetric diols, glycol ethers and cyclic alcohols were used for synthesis of **7 h-o** derivatives and the compounds were obtained in moderate yields. Ethylene glycol, propylene glycol, diethylene glycol were used as diols for the synthesis of **7h, 7i/7j** and **7k.** They were purified by extraction with EtOAc/H_2O mixture, the organic layer was dried over sodium sulfate and evaporated under reduced pressure.

The ^{1}H NMR spectrum revealed the presence of aliphatic hydrogens within the alcohol groups, appearing at approximately 5.0 ppm and 1.0 ppm. Nonetheless,

observation of the OH protons in compounds **7h**, **7i/7j** and **7k** proved elusive due to obscuring by water originating from DMSO.

From the reaction involving compound **6** with propylene glycol (an asymmetric diol), two isomeric mixtures, namely **7i** and **7j** emerged. The closely aligned polarity and solubility of these isomers rendered them indistinguishable through column chromatography or alternative separation techniques. The mixture's ratio of 1:1.2 (**7i/7j**) was determined by evaluating the proportion of the CH_3 group of **7i** at 1.56 ppm and the CH_3 group of **7j** at 1.37 ppm within the ^1H NMR spectra, as depicted in Figure 4.2.

Furthermore, the HRMS spectrum demonstrated the presence of a molecular ion peak, M+18, corresponding to compounds **7i/7j** as depicted in **Figure 4.3**.

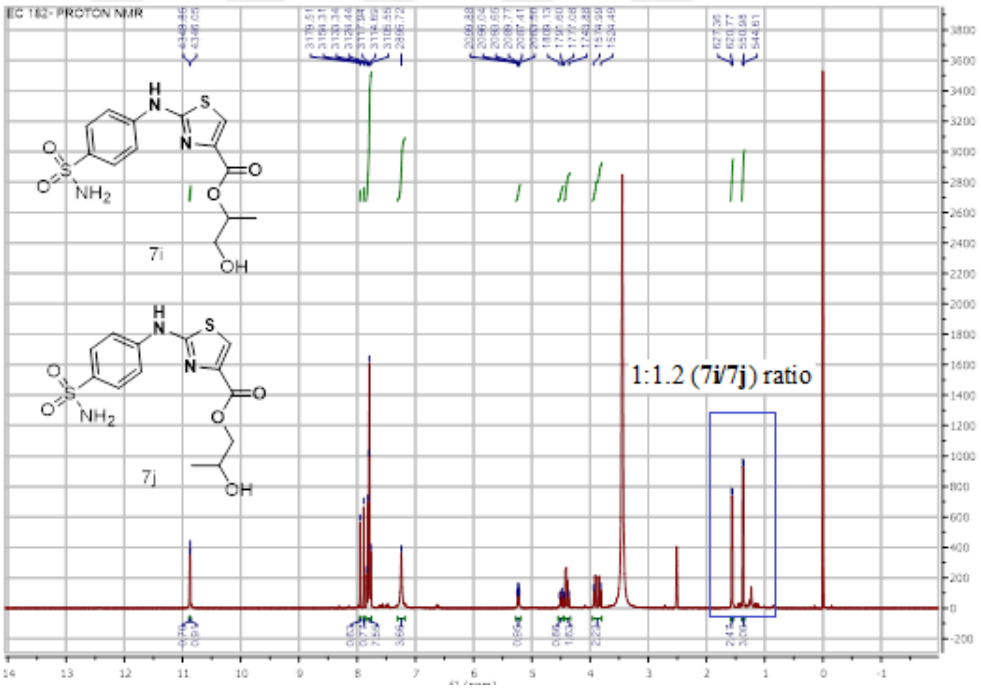


Figure 4.2. ^1H NMR spectrum of **7i**/**7j** compounds.

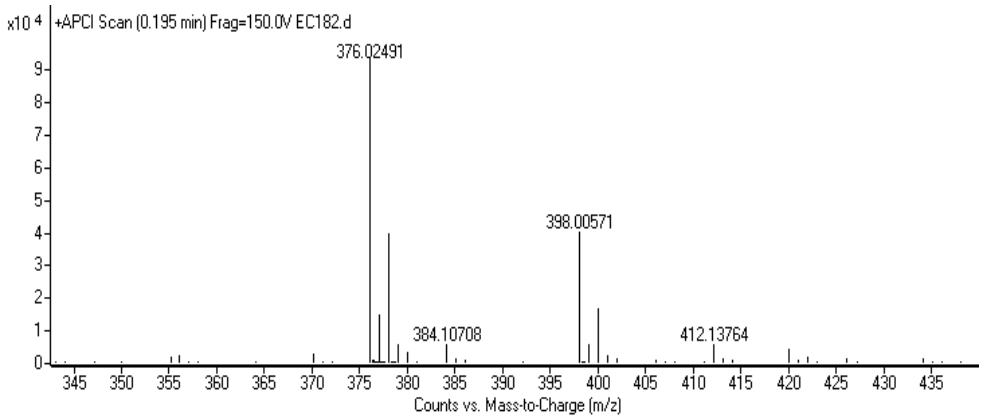


Figure 4.3. HRMS spectrum of **7i**/**7j** compounds

Longer chain fatty alcohols such as 1-decanol and 1-dodecanol were also used during the synthesis of sulfonamide-based thiazol-4-carboxylate **7a-o**, but the reactions could not be obtained in high yield, and purification processes were difficult. At the same time, reactions with triol alcohol such as glycerol and cyclic alcohols such as phenol, benzyl alcohol, anise alcohol were tried, but in some reactions the yield was low, and some reactions did not occur at all. The reason may be that the OH group is not far enough from the ring and the ring effect on it.

Target sulfonamide-based thiazole-4-carboxylate **7a-o** derivatives were purified and characterized with spectroscopic measurements (IR, ^{1}H NMR, ^{13}C NMR, HRMS) and physical data (m.p. and Rf values). IR spectrum of compounds **7a-o** showed characteristic peaks for the NH between benzene sulfonamide and thiazole ring at around 3340 cm^{-1} and NH_2 group connected to benzensulfonamide at 3280 cm^{-1}, whereas were observed at 1320 cm^{-1} asymmetric and at 1150 cm^{-1} symmetric vibrational frequencies for sulfonamides. Moreover ester carbonyl (C=O) was observed at around 1700 cm^{-1} as shown in **Figure 4.4.** At ^{1}H NMR spectrum, chemical shift of protons of N*H* group were identified at around 10.80 ppm, and thiazo C*H* group at around 7.88 ppm. And also aromatic protons of benzensulfonamide group appeared at around 7.80 and 7.70 ppm respectively as apparently doublet. Sulfonamide (NH_2) group protons showed at around 7.20 ppm as broad singlets as shown in **Figure 4.5.** At ^{13}C NMR spectrum, thiazole connected amine carbon chemical shift is at around 162 ppm, carbonyl carbon is 160 ppm, both equal hydrogens of aromatic ring is at around 127 and 116 ppm respectively.

Other carbon chemical shifts in the skeletal structure are shown in detail in **Figure 4.6.**

The molecule **7b** was chosen to show the major hydrogens, carbons and shifts in the IR spectra in the skeletal structure.

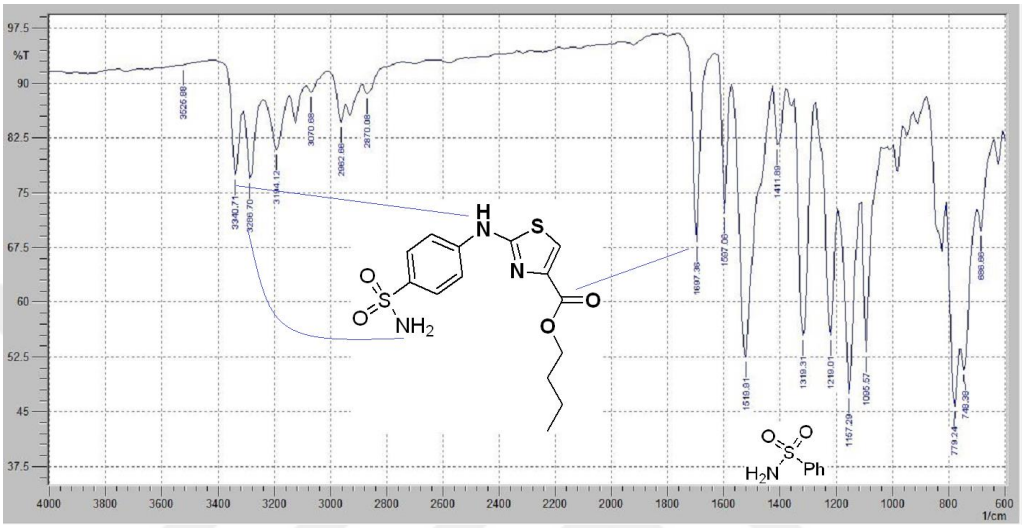


Figure 4.4. IR spectrum of **7b**

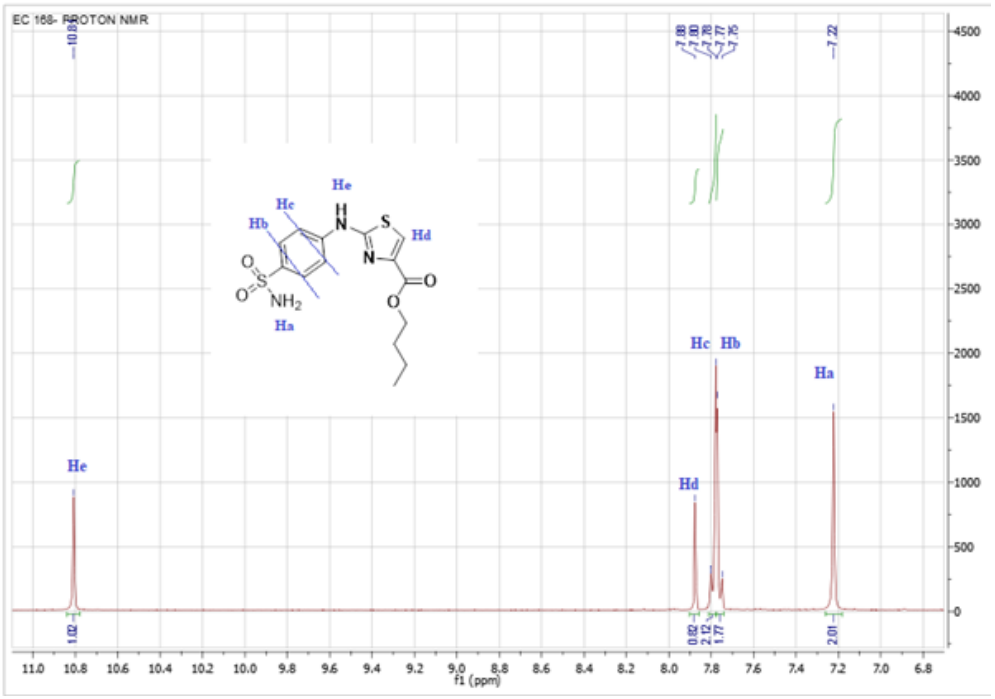


Figure 4.5. Expanded ^{1}H NMR spectrum of **7b**

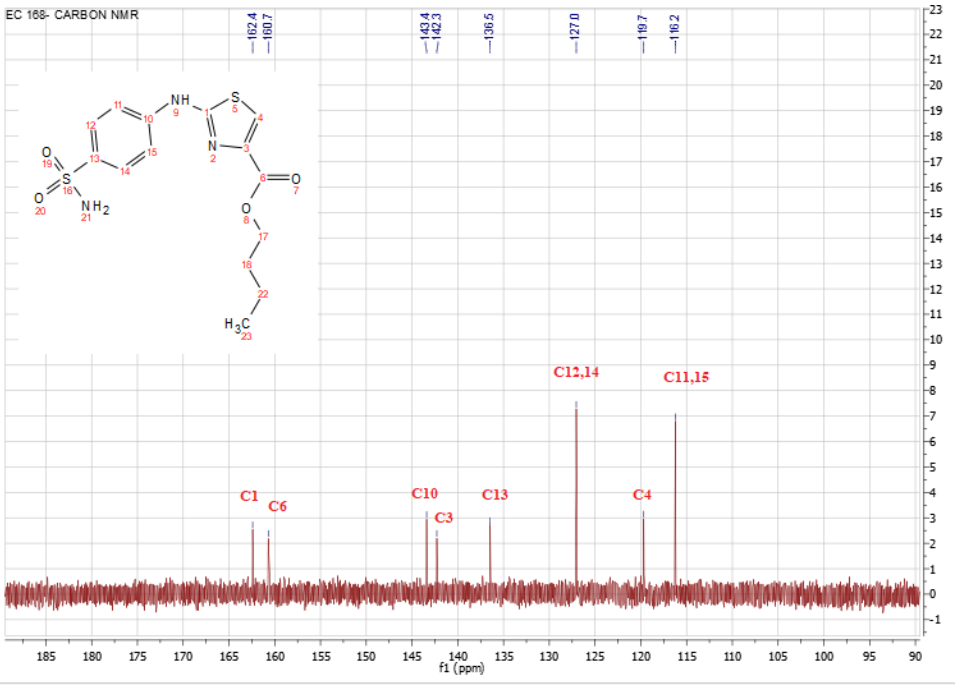
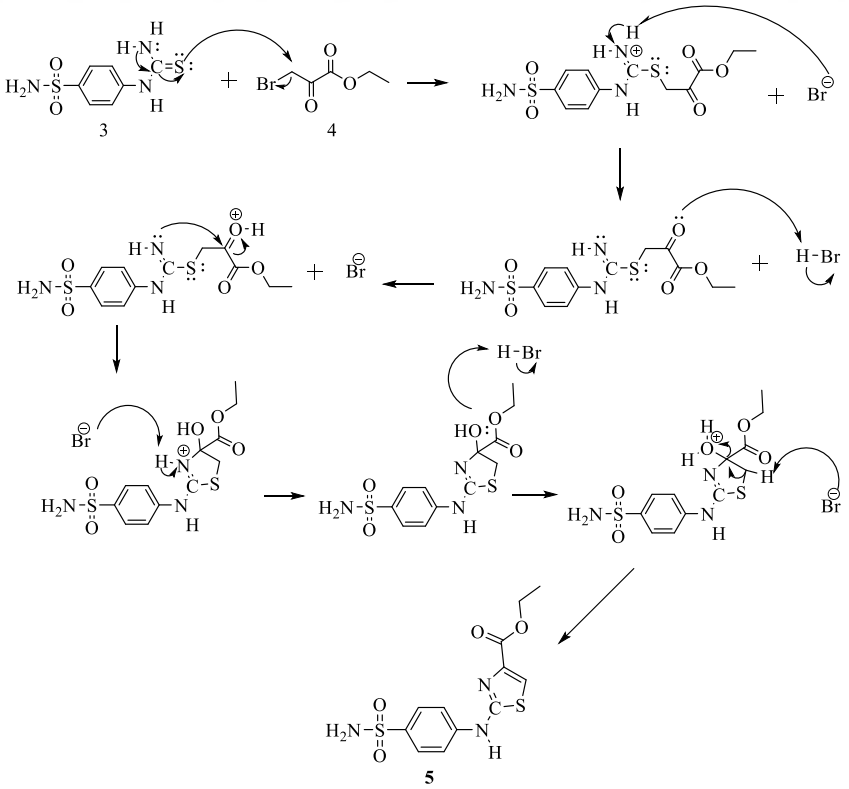


Figure 4.6. Expanded ^{13}C NMR spectrum of **7b**

The proposed mechanism for the thiazole ring cyclization reaction in the synthesis of ethyl 2-((4-sulfamoylphenyl)amino) thiazole-4-carboxylate 5, using thioureidobenzene sulfonamide 3 and ethyl 3-bromopyruvate 4, is depicted in **Scheme 4.2**.



Scheme 4.2. Proposed thiazole ring cyclization reaction mechanism

4.1.1. Carbonic Anhydrase Inhibition of Novel 7a-o Compounds

CA inhibitory activities of compounds **7a-o** were investigated by applying the stopped flow carbon dioxide hydrase assay, in comparison to standard drug acetazolamide (AAZ) against a panel of human CA (hCA) isozymes such as hCA I, II, IX and XII isoforms as shown in **Table 4.1.**

Table 4.1. Inhibition data of human CA isoforms, standard inhibitor Acetazolamide (AAZ) and **7a-o** derivatives.

K_I (nM)*				
Compound	**hCA I**	**hCA II**	**hCA IX**	**hCA XII**
7a	72.5	4.6	49.9	24.1
7b	524.3	9.0	71.1	297.1
7c	75.3	5.4	90.3	729.6
7d	2081	88.8	72.0	403.2
7e	7235	502.2	70.6	551.1
7g	6545	801.3	78.9	470.6
7i/7j	92.6	5.5	37.9	84.4
7l	5697	695.9	86.8	372.5
7k	255.3	32.3	26.8	57.8
7f	6390	345.3	74.3	319.2
7h	283.5	32.3	43.2	28.1
7m	73.0	5.8	35.1	9.3
7n	192.8	5.3	51.2	9.7
7o	809.6	33.3	149.6	522.8
AAZ	250	12.1	25.7	5.7

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

On the basis of the kinetic data reported in Table 4.1., thiazole derivatives 7a-o showed remarkable variation among the different CA isoforms.

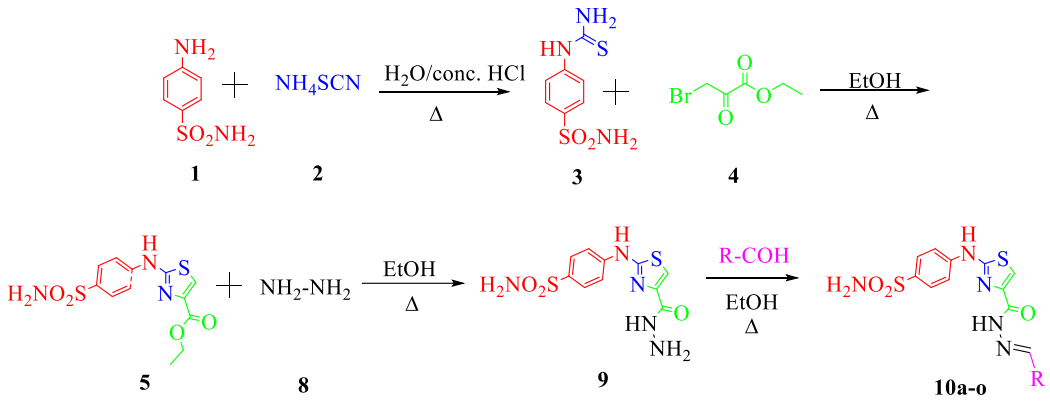
hCA I (cytosolic widespread enzyme) was inhibited by compounds **7a-o** and showed a wide range of potency with inhibition constant (KIs) spanning from 72.5 to 7235 nM. Significant potency decreases were observed from the propyl (**7a**) to the octyl moieties (**7g**), indicating that the length of the alkyl chain greatly influences strength. The same activity decrease was obtained for alkoxy moieties from **7m** to **7n**. The hCA II (second abundant human cytosolic isoform), was strongly inhibited in the low nanomolar range by most of the compounds.

Interestingly, the length of the alkyl group also plays an important role in the inhibitory effect of hCA II isoform. Indeed, we observed the potency decreased almost 170 fold, inhibition constant (KI) is 4.6 nM for the propyl chain (7a) and is 801.3 nM for the octyl chain (7g). Conversely, the hCA IX (the tumor membrane-associated isoform) was inhibited by **7a-o** compounds in the medium nanomolar range. As with the other isoforms mentioned above, alkyl chain length did not play a significant role in modulating inhibition for hCA IX isoform.This property makes compounds **7e** and **7g** more selective than hCA IX. Compound **7e** showed a selectivity index of almost 7-fold and **7g** over 10-fold compared to hCA II. The hCA XII (second tumor membrane-associated isoform), exhibited broad potency with KIs ranging from 9.3 to 729.6 nM.The most potent compounds against this isoform contain alkoxy chains (**7m** with 9.3 nM KI and **7n** with 9.7 nM KI) that proved to be key moieties for generating potent inhibitors against hCA XII.

4.2. The Results and Discussion of Novel Thiazole and Hydrazine-1-Carbonyl-Based Benzenesulfonamide 10a-o Derivatives

In the second part of the study, ethyl 2-((4-sulfamoylphenyl)amino)thiazole-4-carboxylate **5** was converted into a hydrazine carbonyl derivative using an excess of hydrazine hydrate in ethanol under reflux conditions. Subsequently, the resulting compound, 4-((4-(hydrazinecarbonyl)thiazol-2-yl)amino)benzenesulfonamide **9**, was subjected to various aromatic aldehydes to generate novel thiazole and hydrazine-1-carbonyl-based benzenesulfonamides **10 a-o**.

During this investigation, it was observed that benzaldehyde and salicylaldehyde derivatives exhibited rapid reactivity with hydrazine, leading to the formation of the desired products. Notably, the reaction involving p-NO_2 benzaldehyde was completed within approximately 0.5 hours, yielding 90%. Generally, novel compounds **10 a-o** were synthesized with high yields and easily achievable purification procedures. The synthesis process is presented in **Scheme 4.3**.



Comp.	R	Yield (%)*	Melting point (°C)
10a	-2-OH,3-OCH_3-C_6H_3	92,0	284-286
10b	-2-OH,3-OCH_2CH_3-C_6H_3	80,6	277–279
10c	-2-OH,5-NO_2-C_6H_3	95,1	342-344 decomp.
10d	-2-OH,3-OCH_2CH_3; 5-NO_2-C_6H_2	77,6	334–336 decomp
10e	-4-OCH_3-C_6H_4	71,9	277–279
10f	-4-NO_2-C_6H_4	90,2	282–284
10g	-4-Cl-C_6H_4	87,4	278–280
10h	-4-Br-C_6H_4	72,1	261–263
10i	-2,4-diCl-C_6H_3	87,2	299–301
10j	-3,4-diOCH_3-C_6H_3	88,9	283–285
10k	-2,6-diCl-C_6H_3	85,1	290–292
10l	-4-$CH(CH_3)_2$-C_6H_4	70,5	232–234
10m	-2-OH-C_6H_4	76,7	296–298
10n	*-4-$C(CH_3)_3$-C_6H_4-CH_2-CH-$(CH_3)_2$*	78,3	219–221
10o	*-4-$(CH_2)_3$-C-$(CH_3)_2$-OH-C_6H_8*	77,2	214–216

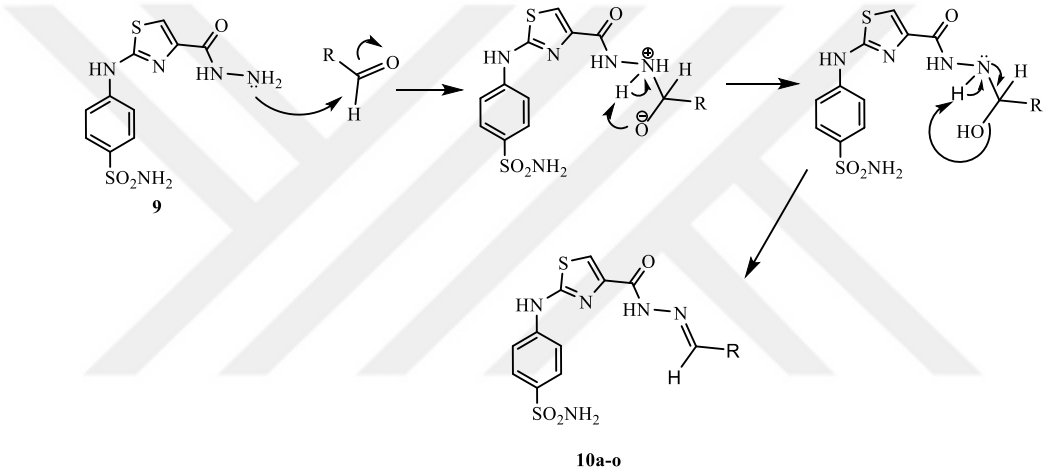
*The yields were calculated from compound **9**

Scheme 4.3. Reaction pathway to obtain novel thiazole and hydrazine-1-carbonyl-based benzenesulfonamide **10a-o** derivatives

In addition to the reactions involving aromatic aldehydes, we also subjected straight-chain aldehydes such as hexanal, octanal and nonanal to react with compound **9** under identical conditions. Within the initial thirty minutes, a transformation of the intended product was evident on TLC analysis. However, by the end of the first hour, TLC analysis revealed multiple transformations.

Furthermore, we attempted the reactions of these straight-chain aldehydes using various solvents, including methanol, acetonitrile and hexane. Despite the solvent variations, the outcome remained consistent. Interestingly, an investigation into these reactions highlighted that hexanal undergoes decomposition within the reaction medium, attributed to its instability arising from the absence of resonance stabilization.[71]

In addition, in the continuation of this study an efficient synthesis was conducted involving lilial and lyral molecules, recognized as essential aldehydes. The proposed mechanism for the hydrazide-hydrazone cyclization reaction responsible for the formation of the synthesized molecules can be seen below **Scheme 4.4**.



Scheme 4.4. Proposed hydrazide-hydrazone cyclization reaction mechanism

Target thiazole and hydrazine-1-carbonyl-based benzenesulfonamide **10a-o** derivatives were purified and characterized with spectroscopic measurements (IR, ^{1}H NMR, ^{13}C NMR, MALDI-TOF MS) and physical data (m.p. and Rf values)

In the IR spectrum of compounds **10a-o**, unlike the novel compounds **7a-o** in part 1, vibrational frequencies were observed at around 3300 cm^{-1} of amidic NH bond, at 1520 cm^{-1} for C=N bond and at 1650 cm^{-1} for amidic C=O bond. At ^{1}H NMR spectrum, the chemical shift of new proton of amidic N*H* group was identified at around 11.0 ppm, and N=C*H* group at around 8.6 ppm. As shown in **Figure 4.7.** At ^{13}C NMR spectrum, chemical shifts of new amidic carbonyl carbon was 157 ppm and new hyrazone carbon N=*C*HR was at around 147 ppm, however, the chemical

shift of the new hyrazone carbon N=*C*HR can be in a wide range relative to the R group to which it is attached, as shown in **Figure 4.8.** The molecule **10h** was chosen to show the chemical shifts of new protons and carbons on spectra.

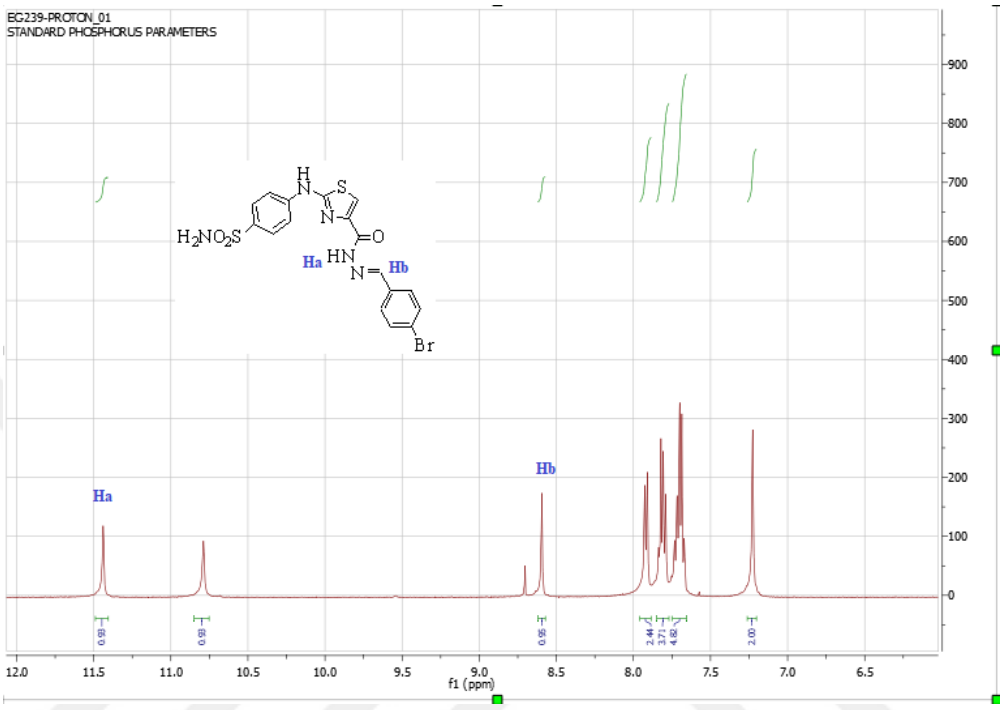


Figure 4.7. Expanded ^{1}H NMR spectrum of **10h**

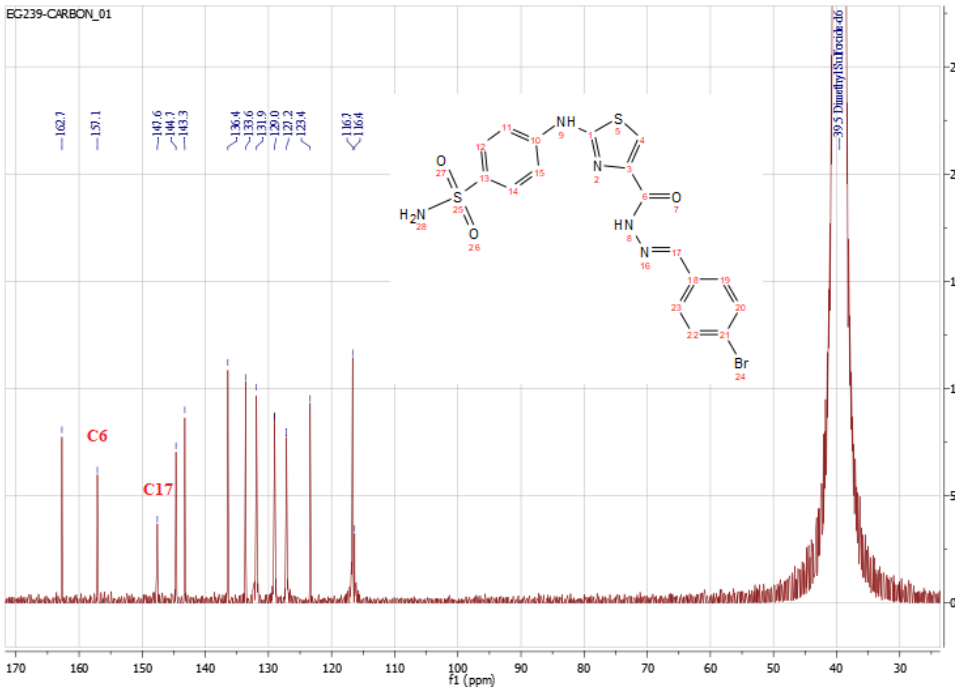
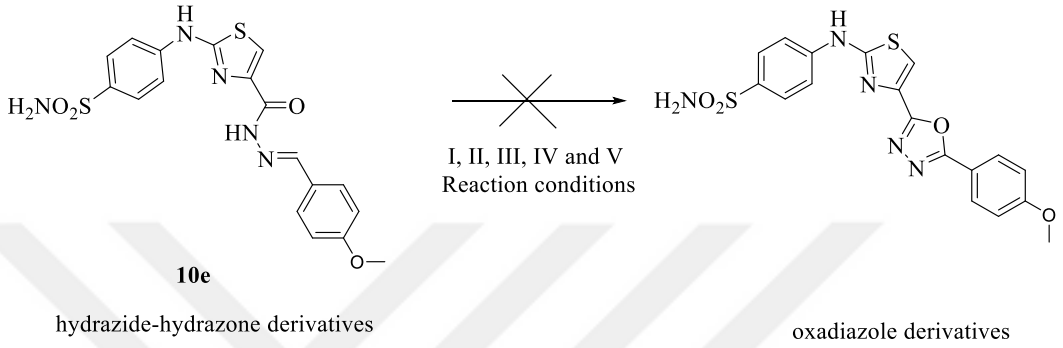


Figure 4.8. Expanded ^{13}C NMR spectrum of **10h**

In the following of the above study, we tried several methods to convert obtained hydrazide-hydrazone derivatives to oxadiazole derivatives. 1 eq of (E)-4-((4-(2-(4-methoxybenzylidene)hydrazine-1-carbonyl)thiazol-2-yl)amino) benzenesulfonamide **10e** compound was reacted with different reaction conditions, reagents and solvents to obtain oxadiazole form but no conversion was observed.



10e

hydrazide-hydrazone derivatives

oxadiazole derivatives

	Reagents	**Solvents**	**Temp. /Time**
I	1.2 eq. I_2 3.0 eq. K_2CO_3	5 ml DMSO	100ºC / 1-2 hr
II	1.2 eq. I_2 3.0 eq. K_2CO_3	5 ml DMSO	rt / 24 hr
III	1.25 ml Pyridine	2.5 ml Acetic Anhydride	rt / 24 hr
IV	2.0 eq. Sodium acetate anhydrous 2.0 eq. I_2	3 ml Glacial Acetic acid	rt / 24 hr
V	2.0 eq. K_2CO_3 2.0 eq. I_2 4.0 eq. 30% H_2O_2 in aq.	5 ml DMSO	rt / 24 hr

Scheme 4.5. Attempted rections of sulfonamide thiazoles to obtain oxadiazole derivatives

4.2.1. Carbonic Anhydrase Inhibition of Novel 10a – o Compounds

CA inhibitory activities of compounds **3, 5, 9** and **10a-o** were investigated by applying the stopped flow carbon dioxide hydrase assay, in comparison to standard drug acetazolamide (AAZ) against a panel of human CA (hCA) isozymes such as hCA I, II, IX and XII isoforms as shown in **Table 4.2.**

Table 4.2. Inhibition data of human CA isoforms, standard inhibitor Acetazolamide (AAZ) and **3, 5, 9** and **10a-o** derivatives.

K_I (nM)*				
Compound	**hCA I**	**hCA II**	**hCA IX**	**hCA XII**
3	470.7	70.0	32.9	37.3
5	132.2	61.5	58.6	86.7
9	87.9	58.4	92.3	74.4
10a	254.1	33.4	63.5	32.9
10b	310.2	46.9	58.7	44.5
10c	540.9	81.0	88.3	55.8
10d	807.5	125.8	90.0	64.3
10e	180.0	26.8	97.3	56.6
10f	556.3	93.2	86.8	66.6
10e	643.9	63.9	77.3	88.7
10h	766.6	98.8	62.3	73.6
10i	1002	237.0	40.1	54.4
10j	230.2	91.9	76.6	34.4
10k	798.8	314.1	69.5	83.2
10l	702.9	171.2	47.7	74.2
10m	78.2	39.3	53.7	31.8
10n	282.9	121.6	36.5	52.9
10o	193.4	164.8	78.7	43.6
AAZ	250.0	12.5	26.0	5.7

*Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

The cytosolic isoform hCA I was inhibited by compounds **3, 5, 9 and 10a-o** with a wide range of potency, inhibition constants (Kıs) spanned from 78.2 to 1002 nM. Especially, intermediate compounds **5**, **9** and aryl groups containing OH moiety, **10m** and methoxy moieties, **10e, j** and **10o** molecules have better inhibitor potency then standard AAZ.

The second abundant human cytosolic isoform, hCA II, was strongly inhibited by almost no compounds reported here in the low nanomolar range. K_Is is ranging between the medium-high nanomolar range (26.8-314.1 nM). Although they are far from the standard AAZ inhibition value, the closest value is 26.8, which is **10e** compound.

On the other hand, the tumor membrane-associated isoform hCA IX was inhibited by compounds in the low nanomolar range, but not as much as the standard AAZ. The inhibition values of compounds **3, 10i** and **10n** are closest to the standard AAZ for hCA IX.

For the second tumor membrane-associated isoform, hCA XII, the synthesized compounds were observed to act as weak inhibitors of hCA XII, with K_{IS} ranging from 31.8 to 88.7 nM, although they were inhibited at low nanomolars.

5. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, a total of 15 novel compounds of sulfonamide-based thiazole-4-carboxylate **7a-o** were synthesized in first part using thiazole cyclization reaction of 4-thioureidobenzene sulfonamide **3** and ethyl 3-bromopyruvate **4**. Subsequently, the intermediate compound thiazole-4-carboxylate **5** converted to its carboxylic acid form **6** and target sulfonamide-based thiazole-4-carboxylate **7a-o** derivatives were synthesized through esterification reaction of corresponding alcohols, yielding moderate to high yields. In the second part, a total of 15 novel compounds of thiazole and hydrazine-1-carbonyl-based benzenesulfonamide **10 a-o** were synthesized. This was achieved by initiating the carbonyl hydrazide formation reaction between the intermediate compound thiazole-4-carboxylate **5** and hydrazine hydrate. Furthermore, the second target compounds, thiazole and hydrazine-1-carbonyl-based benzenesulfonamide **10a-o** derivatives, were successfully synthesized through a hydrazide-hydrazone formation reaction involving 4-((4-(hydrazinecarbonyl)thiazol-2-yl)amino)benzenesulfonamide **9** and corresponding aldehydes, yielding high yields for the majority of cases. The structure characterizations of all synthesized compounds were illuminated by physical (m.p, Rf) and spectral data (IR, ^{1}H NMR, ^{13}C NMR, MALDI-TOF MS and HRMS). Furthermore, carbonic anhydrase inhibition activities of novel synthesized compounds were investigated using human isoforms hCA I, II, IX and XII to comparing standard carbonic anhydrase inhibitor drug Acetazolamide (AAZ). Compounds **7a-o** and **10a-o** showed a wide range of inhibiton potency to hCA I. Especially, **7a**, **7c**, **7i**/**7j**, **7m, 7n** and **10e, 10j, 10m, 10o** have shown higher inhibition potential as compared to Acetazolamide (K_I = 250 nM. For hCA II, it was strongly inhibited by most **7a-o** derivatives in the low nanomolar range (KI < 12.5 nM) so **7a**, **7b**, **7c**, **7i**/**7j** , **7m** and **7n** molecules were observed act as strong inhibitors of hCA II but **10a-o** derivatives not inhibited at sufficiently low nanomolar levels. On the other hand, hCA IX and hCA XII were weakly inhibited by almost all newly synthesized compounds compared to AAZ.

6. REFERENCES

"Vancouver citation system was used in this thesis."

[1] Katritzky AR, Ramsden CA, Joule JA, Zhdankin VV. Handbook of Heterocyclic Chemistry; 2010. 3rd edition.

[2] Al-Mulla A. Biological Importance of Heterocyclic Compounds. Der Pharma Chemica. 2017;9(13):141-147.

[3] El-Desoky SI, Bondock SB, Etman HA, Fadda AA, Metwall MA. Synthesis of some new thiazole derivatives of pharmaceutical interest. sulfur letters. 2003; 26(3):127-135. doi:10.1080/0278611031000095331

[4] Schaumann E. Hetarenes and Related Ring Systems (Five-Membered Hetarenes with One Chalcogen and One Additional Heteroatom). Product Class 17:Thiazoles. 2002;Category 2 doi:10.1055/sos-SD-011-00783

[5] Ayati A, Emami S, Asadipour A, Shafiee A, Foroumadi A. Recentapplications of 1,3-thiazole core structure in the identification of new lead compounds and drugdiscovery. *European Journal of Medicinal Chemistry.* 2015;97:699–718. doi:10.1016/j.ejmech.2015.04.015.

[6] Petrou A, Fesatidou M, Geronikaki A. Thiazole Ring—A Biologically Active Scaffold. Molecules. 2021;26:3166. doi:10.3390/molecules26113166

[7] Lehr D. Inhibition of Drug Precipitation in the Urinary Tract by the Use of Sulfonamide Mixtures. I. Sulfathiazole-Sulfadiazine Mixture. *Exp Biol Med*. 1945;58: 11–14. doi:10.3181/00379727-58-14820.

[8] Borelli C, Schaller M, Niewerth M, et al. Modes of Action of the New Arylguanidine Abafungin beyond Interference with Ergosterol Biosynthesis and in vitro Activity against Medically Important Fungi. *Chemotherapy*. 2008;54:245–259. doi:10.1159/000142334.

[9] Karthikeyan MS. Synthesis, analgesic, anti-inflammatory and antimicrobial studies of 2,4-dichloro-5-fluorophenyl containing thiazolotriazoles. *Eur J Med Chem*. 2009 Feb;44(2):827-33. doi:10.1016/j.ejmech.2008.04.022.

[10] Tylicki A, Lotowski Z, Siemieniuk M, Ratkiewicz A. Thiamine and selected thiamine antivitamins – biological activity and methods of synthesis. *Biosci Rep*. 2018;38. doi:10.1042/BSR20171148.

[11] Li X, He Y, Ruiz CH, Koenig M, Cameron MD, Vojkovsky T. Characterization of dasatinib and its structural analogs as CYP3A4 mechanism-based inactivators and the proposed bioactivation pathways. *Drug Metab Dispos*. 2009 Jun;37(6):1242-50. doi:10.1124/dmd.108.025932.

[12] Dawane BS, Konda SG, Kamble VT, Chavan SA, Bhosale RB, & M SB. Multicomponent One-Pot Synthesis of Substituted Hantzsch Thiazole Derivatives Under Solvent Free Conditions. *E-Journal of Chemistry.* 2009;6(s1):358–362. doi:10.1155/2009/752580

[13] Li J. Cook-Heilbron thiazole synthesis. Name Reactions. January 2003: pp.82-82.doi: 10.1007/978-3-662-05336-2_66

[14] Ram VJ, Sethi A, Nath M, Pratapi R. The Chemistry of Heterocycle, Nomenclature and Chemistry of Three-to-Five Membered Heterocycles. *Elsevier.* 2017.

[15] Facchinetti V, Avellar M, Nery A, Gomes C, Vasconcelos T, de Souza M. An Eco-friendly, Hantzsch-Based, Solvent-Free Approach to 2-Aminothiazoles and 2-Aminoselenazoles. *Synthesis.* 2015;48(3):437–440. doi:10.1055/s-0035-1560534

[16] Iravania N, Keshavarz M, Ghaedia A, Sabzalia T. A facile and one-pot synthesis of Dihydrothiazole-4-carboxylates under mild, solvent-and catalyst-free conditions. *Iran J Organic Chem.* 2013;5:1071-1077.

[17] De Souza M. V. N. Synthesis and biological activity of natural thiazoles: An important class of heterocyclic compounds. Journal of Sulfur Chemistry. 2005;26(4-5):429–449. doi:10.1080/17415990500322792

[18] Supuran CT. Special Issue: Sulfonamides. *Molecules.* 2017 Sep 29;22(10):1642. doi:10.3390/molecules22101642.

[19] Bhat MA. et al. Biological activities of sulfonamides. *Indian journal of pharmaceutical sciences*. 2005;67(2): 151-159

[20] Davenport D. The war against bacteria: how were sulphonamide drugs used by Britain during World War II. *Med Humanit.* 2012 Jun;38(1):55-8. doi:10.1136/medhum-2011-010024.

[21] Stokes SS, Albert R, Buurman ET, et al. Inhibitors of the acetyltransferase domain of N-acetylglucosamine-1-phosphate-uridylyltransferase/glucosamine-1-phosphate-acetyltransferase (GlmU). Part 2: Optimization of physical properties leading to antibacterial aryl sulfonamides. *Bioorg Med Chem Lett*. 2012;22:7019–7023. doi:10.1016/j.bmcl.2012.10.003.

[22] Ezabadi IR, Camoutsis C, Zoumpoulakis P, Geronikaki A, Soković M, Glamocilija J, Cirić A. Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: synthesis, biological evaluation, lipophilicity, and conformational studies. *Bioorg Med Chem*. 2008 Feb 1;16(3):1150-61. doi:10.1016/j.bmc.2007.10.082

[23] Ghate M, Manohar D, Kulkarni V, Shobha R, Kattimani SY. Synthesis of vanillin ethers from 4-(bromomethyl) coumarins as anti-inflammatory agents. *Eur J Med Chem.* 2003 Mar;38(3):297-302. doi:10.1016/s0223-5234(03)00016-3.

[24] Ghorab MM, Ragab FA, Heiba HI, El-Hazek RM. Anticancer and radio-sensitizing evaluation of some new thiazolopyrane and thiazolopyranopyrimidine derivatives bearing a sulfonamide moiety. Eur J Med Chem. 2011 Oct;46(10):5120-6. doi:10.1016/j.ejmech.2011.08.026.

[25] Thiry A, Dogné JM, Supuran CT, Masereel B. Anticonvulsant sulfonamides/sulfamates/sulfamides with carbonic anhydrase inhibitory activity: drug design and mechanism of action. Curr Pharm Des. 2008;14(7):661-71. doi:10.2174/138161208783877956.

[26] Akurathi V, Dubois L, Celen S, Lieuwes NG, Chitneni SK, Cleynhens BJ, Innocenti A, Supuran CT, Verbruggen AM, Lambin P, Bormans GM. Development and biological evaluation of ^{99}mTc-sulfonamide derivatives for in vivo visualization of CA IX as surrogate tumor hypoxia markers. *Eur J Med Chem.* 2014 Jan;71:374-84. doi:10.1016/j.ejmech.2013.10.027.

[27] Sławiński J, Szafrański K, Vullo D, Supuran CT. Carbonic anhydrase inhibitors. Synthesis of heterocyclic 4-substituted pyridine-3-sulfonamide derivatives and their inhibition of the human cytosolic isozymes I and II and transmembrane tumor-associated isozymes IX and XII. *Eur J Med Chem.* 2013 Nov;69:701-10. doi:10.1016/j.ejmech.2013.09.027

[28] Masters PA, O'Bryan TA, Zurlo J, Miller DQ, Joshi N. Trimethoprim-Sulfamethoxazole Revisited. *Arch Intern Med.* 2003;163(4):402–410. doi:10.1001/archinte.163.4.402

[29] Kapoor VK. Sulfathiazole. *Analytical Profiles of Drug Substances and Excipients*. 1993;22: 389–430. doi:10.1016/s0099-5428(08)60247-6

[30] Karateyev AE. Celecoxib, etoricoxib, meloxicam, and nimesulide: comparison of their merits and demerits. *Modern Rheumatology Journal*. 2011;5(2):9-19. doi:10.14412/1996-7012-2011-662

[31] Brest AN, Lıkoff W. Hydrochlorothiazide in the treatment of congestive heart failure. *Am J Cardiol.* 1959 Feb;3(2):144-7. doi:10.1016/0002-9149(59)90281-4.

[32] McKenna SR, Latenser BA, Jones LM, Barrette RR, Sherman HF, Varcelotti JR. Serious silver sulphadiazine and mafenide acetate dermatitis. *Burns.* 1995 Jun;21(4):310-2. doi: 10.1016/0305-4179(94)00023-q.

[33] El-Gaby M.S.A., Ammar Y.A., El-Qaliei M.I.H., Ali A.M., Hussein M.F., Faraghally F.A. Sulfonamides: Synthesis and the recent applications in medicinal chemistry, *Egyptian Journal of Chemistry*, 2020, 63 (12), 5289-5327.doi: 10.21608/EJCHEM.2020.33860.2707

[34] Harmata M, Zheng P, Huang C, Gomes MG, Ying W, Rayanil KO, Balan G, Calkins NL. Expedient synthesis of sulfinamides from sulfonyl chlorides. *J Org Chem.* 2007 Jan 19;72(2):683-5. doi: 10.1021/jo062296i.

[35] Keerthi DS. Chlorosulfonation of Acetanilide to Obtain an Intermediate for the Preparation of a Sulfa Drug. *Asian Journal of Pharmaceutics.* 2017;11(01). doi:10.22377/ajp.v11i01.1099

[36] Bahrami K, Khodaei MM, Soheilizad M. Direct conversion of thiols to sulfonyl chlorides and sulfonamides. *J Org Chem.* 2009 Dec 18;74(24):9287-91. doi: 10.1021/jo901924m.

[37] Maddani MR, Prabhu KR. A concise synthesis of substituted thiourea derivatives in aqueous medium. J Org Chem. 2010 Apr 2;75(7):2327-32. doi: 10.1021/jo1001593.

[38] Koketsu M, Ishihara H. Thiourea and Selenourea and Their Applications, Current Organic Synthesis 2006; 3(4): 439-455. doi:10.2174/157017906778699521

[39] Patil JC, Patil MC, Patil MC. Studies on Aminobenzothiazole and Derivatives: Part-2. Synthesis of Intermediates -Substituted Phenylthiourea using Ammonium Thiocyanate. *International Journal of Pharmaceutical & Biological Archive.* 2019; 10(3):226-231

[40] SÁ AGA. de Meneses AC, de Araújo PHH, de Oliveira D. A review on enzymatic synthesis of aromatic esters used as flavor ingredients for food, cosmetics and pharmaceuticals industries. *Trends in Food Science & Technology*. 2017;69: 95-105. doi:10.1016/j.tifs.2017.09.004

[41] Khan Z, Javed F, Shamair Z, Hafeez A, Fazal T, Aslam A, Zimmerman WB; Rehman F. Current developments in esterification reaction: A review on process and parameters. *Journal of Industrial and Engineering Chemistry*. 2021;103: 80-101. doi:10.1016/j.jiec.2021.07.018

[42] Hughes DL, Reamer RA, Bergan JJ, Grabowski EJJ. A mechanistic study of the Mitsunobu esterification reaction. *Journal of the American Chemical Society*. 1988;110(19):6487–6491. doi:10.1021/ja00227a032

[43] Koopaei M, Assarzadeh M, Almasirad A, Ghasemi F, Amini M, Kebriaeezadeh A, Nassiri N, Ghadimi M, Tabei A. SYNTHESIS AND ANALGESIC ACTIVITY OF NEW PHENOXYBENZYLIDENE AROYLHYDRAZINE DERIVATIVES. *Journal of Pharmaceutical and Health Sciences.* 2013;2:57-63.

[44] Allen CFH, Alan B. A Publication of Reliable Methods for the Preparation of Organic Compounds. *Org. Synth.* 1944;24(12). doi: 10.15227/orgsyn.024.0012

[45] Bondock S, Tarhoni AEG, Fadda AA. Utility of cyanoacetic acid hydrazide in heterocyclic synthesis. *Arkivoc.* 2006;(9):113–156. doi:10.3998/ark.5550190.0007.905

[46] Li B, Bemish RJ, Bill DR, Brenek S, Buzon RA, Chiu CKF, Newell L. Preparation of Pivaloyl hydrazide in water. *Org. Synth.* 2005;81:254. doi: 10.15227/orgsyn.081.0254

[47] Rollas S, Küçükgüzel SG. Biological activities of hydrazone derivatives. *Molecules.* 2007 Aug 17;12(8):1910-39. doi: 10.3390/12081910.

[48] Deep A, Jain S, Sharma PC, Verma P, Kumar M, Dora CP. Design and biological evaluation of biphenyl-4-carboxylic acid hydrazide-hydrazone for antimicrobial activity. *Acta Pol Pharm.* 2010 May-Jun;67(3):255-9.

[49] Yu W, Huang G, Zhang Y, Liu H, Dong L, Yu X, Li Y, Chang J. I2-mediated oxidative C-O bond formation for the synthesis of 1,3,4-oxadiazoles from aldehydes and hydrazides. *J Org Chem*. 2013 Oct 18;78(20):10337-43. doi: 10.1021/jo401751h.

[50] Mohareb RM, Fleita DH, Sakka OK. Novel Synthesis of Hydrazide-Hydrazone Derivatives and Their Utilization in the Synthesis of Coumarin, Pyridine, Thiazole and Thiophene Derivatives with Antitumor Activity. *Molecules.* 2010;16(1):16–27. doi:10.3390/molecules16010016

[51] Kumar R, Vats L, Bua S, Supuran CT, Sharma PK. Design and synthesis of novel benzenesulfonamide containing 1,2,3-triazoles as potent human carbonic anhydrase isoforms I, II, IV and IX inhibitors. *Eur J Med Chem.* 2018 Jul 15;155:545-551.doi: 10.1016/j.ejmech.2018.06.021

[52] Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, De Simone G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? *Chem Rev.* 2012 Aug 8;112(8):4421-68. doi: 10.1021/cr200176r.

[53] Supuran CT. Carbonic anhydrases as drug targets--an overview. *Curr Top Med Chem.* 2007;7(9):825-33. doi: 10.2174/156802607780636690.

[54] Supuran CT, Scozzafava A. Carbonic anhydrase inhibitors and their therapeutic potential. *Expert Opinion on Therapeutic Patents.* 2000;10(5): 575-600. doi: 10.1517/13543776.10.5.575

[55] (a) Supuran CT, Scozzafava. Carbonic Anhydrase Inhibitors. *Current Medicinal Chemistry - Immunology, Endocrine & Metabolic Agents*. 2001;1(1):61-97
(b) Supuran CT, Scozzafava A, Casini A. Carbonic anhydrase inhibitors. *Med Res Rev.* 2003 Mar;23(2):146-89. doi: 10.1002/med.10025.

[56] Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov. 2008 Feb;7(2):168-81. doi: 10.1038/nrd2467.

[57] Supuran CT. Carbonic anhydrase inhibitors. *Bioorg Med Chem Lett*. 2010 Jun 15;20(12):3467-74. doi: 10.1016/j.bmcl.2010.05.009

[58] Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov.* 2008 Feb;7(2):168-81. doi: 10.1038/nrd2467

[59] Supuran CT. Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism, *Puscas*, I. Ed.; Helicon, Timisoara (Romania), 1994;pp. 29.

[60] Kumar S, Rulhania S, Jaswal S, Monga V. Recent advances in the medicinal chemistry of carbonic anhydrase inhibitors. *Eur J Med Chem.* 2021 Jan 1;209:112923. doi: 10.1016/j.ejmech.2020.112923

[61] Singh S, Lomelino CL, Mboge MY, Frost SC, McKenna R. Cancer Drug Development of Carbonic Anhydrase Inhibitors beyond the Active Site. *Molecules.* 2018 Apr 30;23(5):1045. doi: 10.3390/molecules23051045.

[62] Supuran CT. Indisulam. Eisai. Idrugs. the Investigational Drugs Journal. 2002 Nov;5(11):1075-1079.

[63] Franchi M, Vullo D, Gallori E, Antel J, Wurl M, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors: inhibition of human and murine mitochondrial isozymes V with anions. Bioorg Med Chem Lett. 2003 Sep 1;13(17):2857-61. doi: 10.1016/s0960-894x(03)00581-x.

[64] Supuran CT, Scozzafava A. Carbonic anhydrases as targets for medicinal chemistry. Bioorg Med Chem. 2007 Jul 1;15(13):4336-50. doi: 10.1016/j.bmc.2007.04.020.
[65] Masini E, Carta F, Scozzafava A, Supuran CT. Antiglaucoma carbonic anhydrase inhibitors: a patent review. Expert Opin Ther Pat. 2013;23(6):705-16. doi: 10.1517/13543776.2013.794788.

[66] Khalifah RG. The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C. *J Biol Chem*. 1971 Apr 25;246(8):2561-73. doi: 10.1016/S0021-9258(18)62326-9

[67] Supuran CT. Inhibition of carbonic anhydrase IX as a novel anticancer mechanism. World J Clin Oncol. 2012 Jul 10;3(7):98-103. doi: 10.5306/wjco.v3.i7.98.

[68] Saczewski F, Innocenti A, Brzozowski Z, Sławiński J, Pomarnacka E, Kornicka A, Scozzafava A & Supuran CT. Carbonic anhydrase inhibitors. Selective inhibition of human tumor-associated isozymes IX and XII and cytosolic isozymes I and II with some substituted-2-mercapto-benzenesulfonamides, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2006;21(5):563-568. doi: 10.1080/14756360600648146.

[69] Figaj D, Ambroziak P, Przepiora T, Skorko-Glonek J. The Role of Proteases in the Virulence of Plant Pathogenic Bacteria. *Int J Mol Sci*. 2019 Feb 4;20(3):672. doi: 10.3390/ijms20030672.

[70] Abdoli M, Angeli A, Bozdag M, Carta F, Kakanejadifard A, Saeidian H, Supuran CT. Synthesis and carbonic anhydrase I, II, VII, and IX inhibition studies with a series of benzo[d]thiazole-5- and 6-sulfonamides. *J Enzyme Inhib Med Chem*. 2017 Dec;32(1):1071-1078. doi: 10.1080/14756366.2017.1356295.

[71] Suzuki I, Iwata Y, Takeda K. Biginelli reactions catalyzed by hydrazine type organocatalyst. *Tetrahedron Letters*. 2008;49(20): 3238–3241. doi:10.1016/j.tetlet.2008.03.080

7. APPENDICES

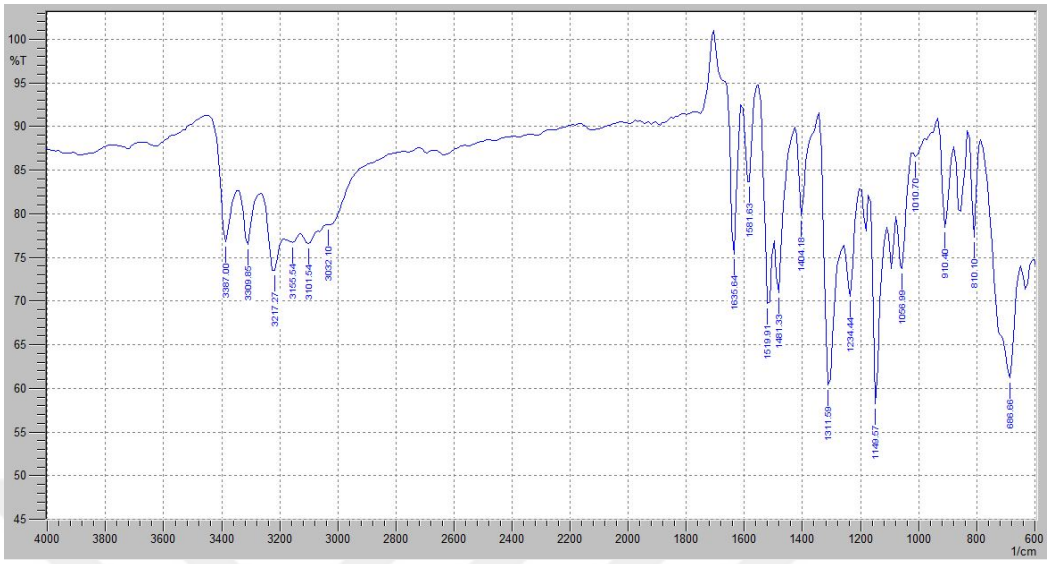


Figure 7.1. IR spectrum of compound **3**

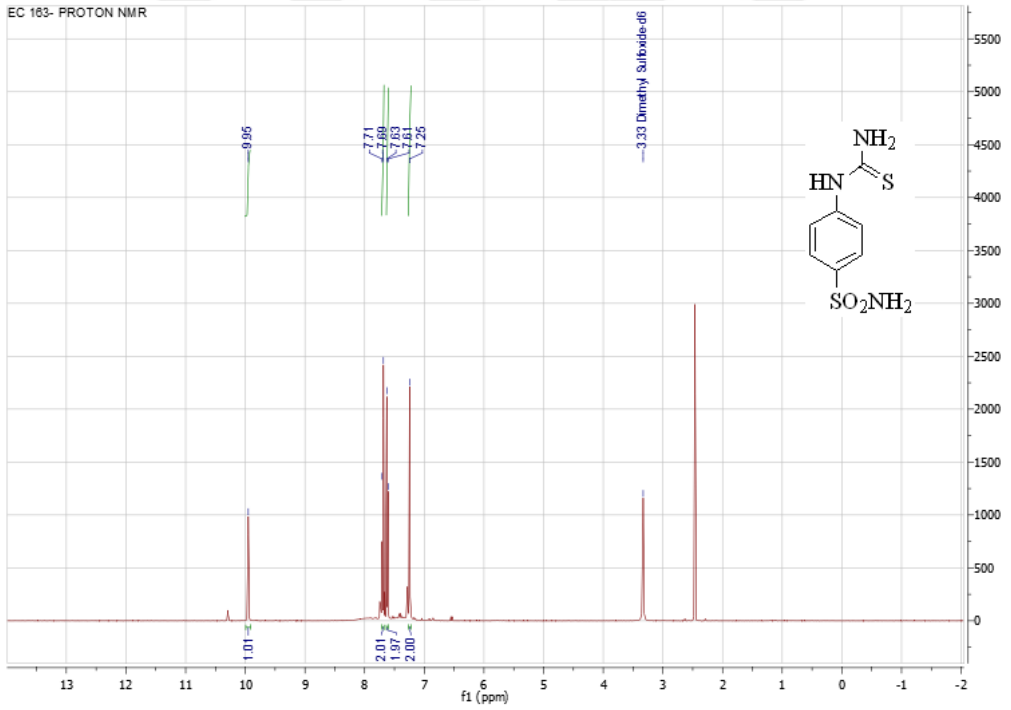


Figure 7.2. ^{1}H NMR spectrum of compound **3**

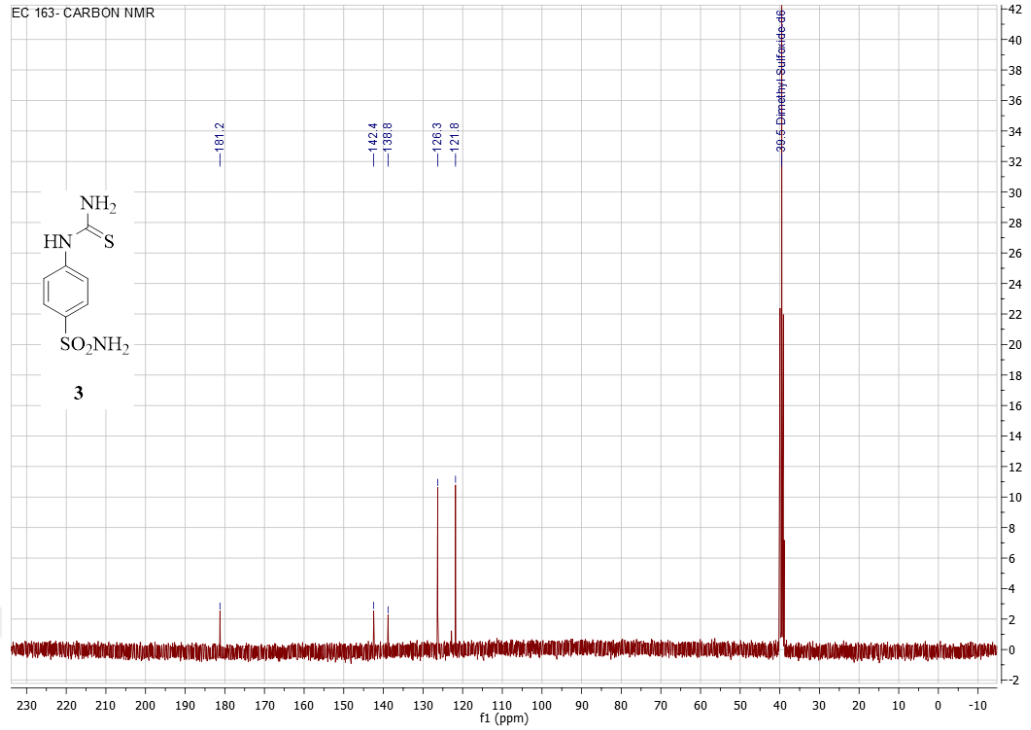


Figure 7.3. ^{13}C NMR spectrum of compound **3**

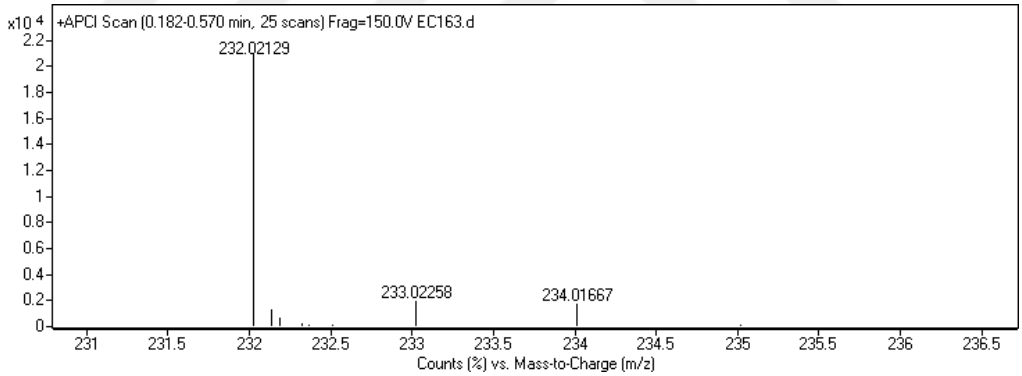


Figure 7.4. HRMS spectrum of compound **3**

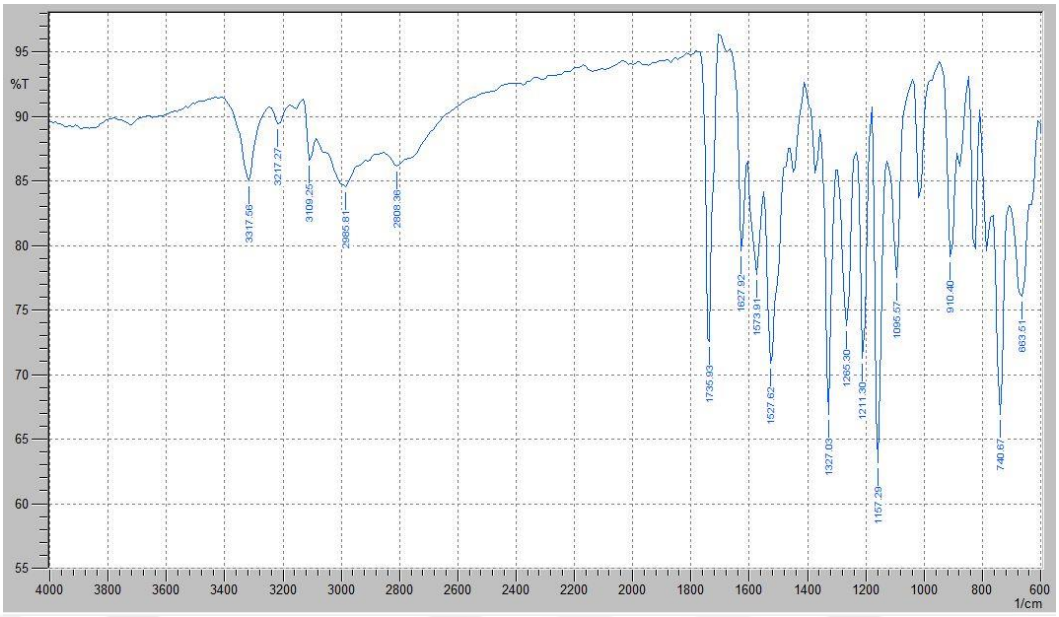


Figure 7.5. IR spectrum of compound **5**

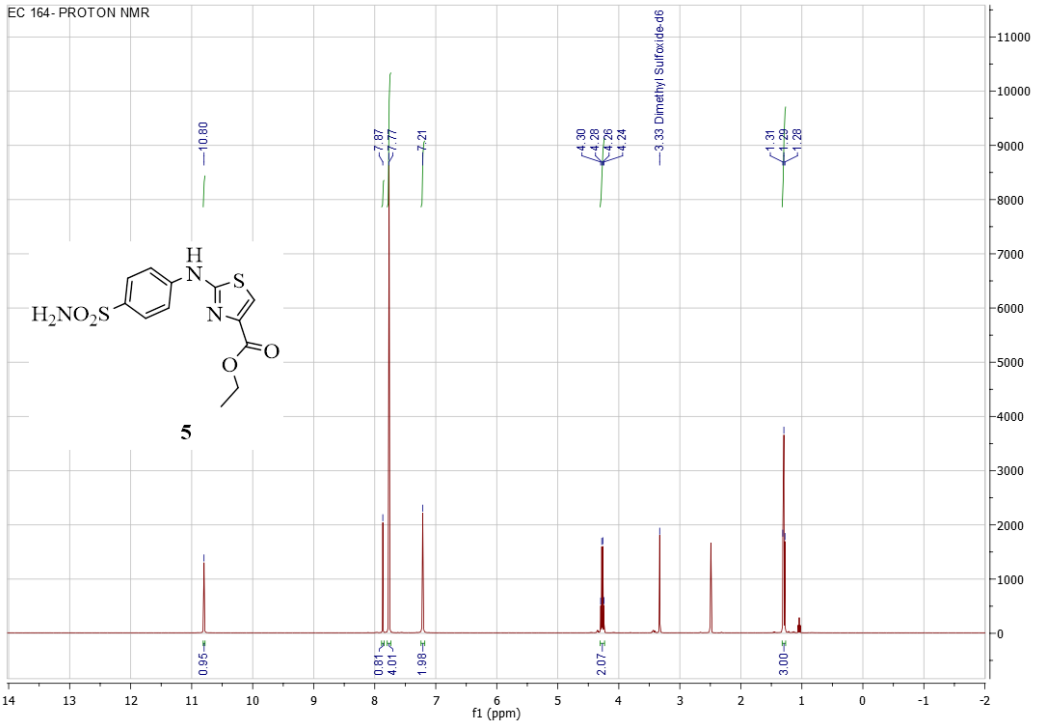


Figure 7.6. 1H NMR spectrum of compound **5**

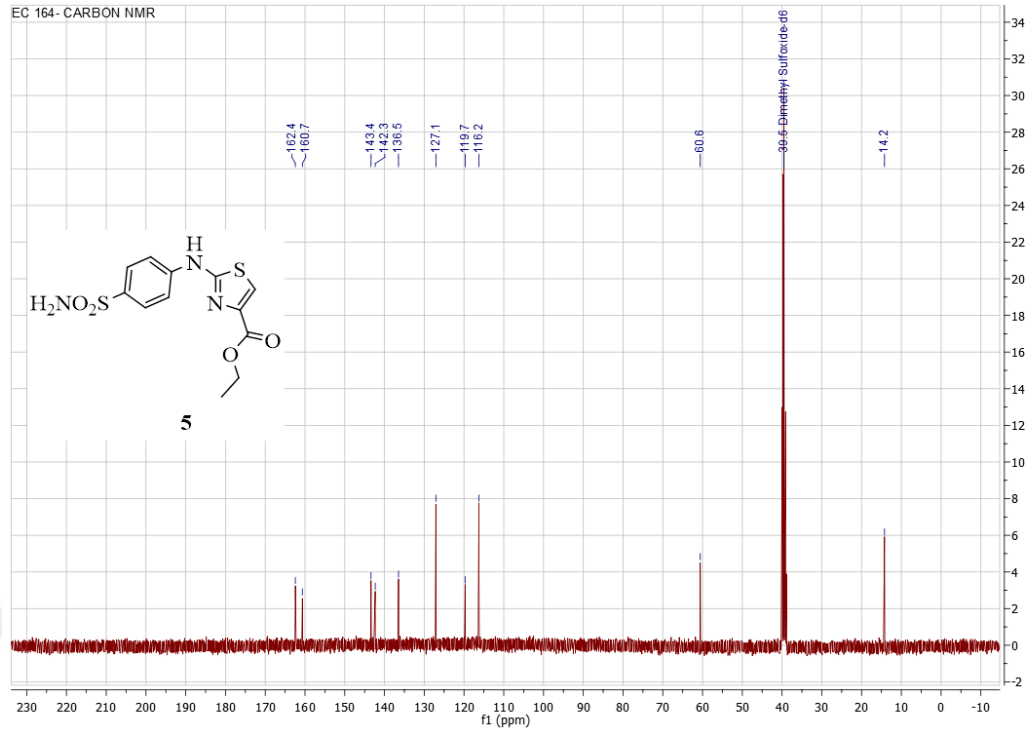


Figure 7.7. ^{13}C NMR spectrum of compound **5**

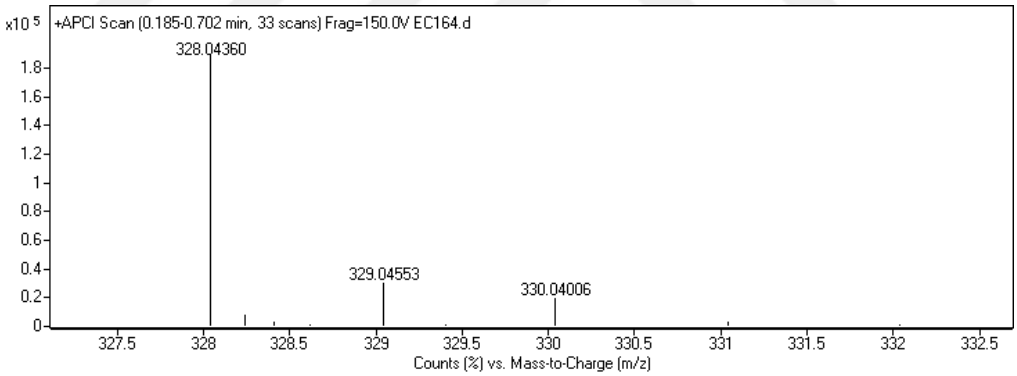


Figure 7.8. HRMS spectrum of compound **5**

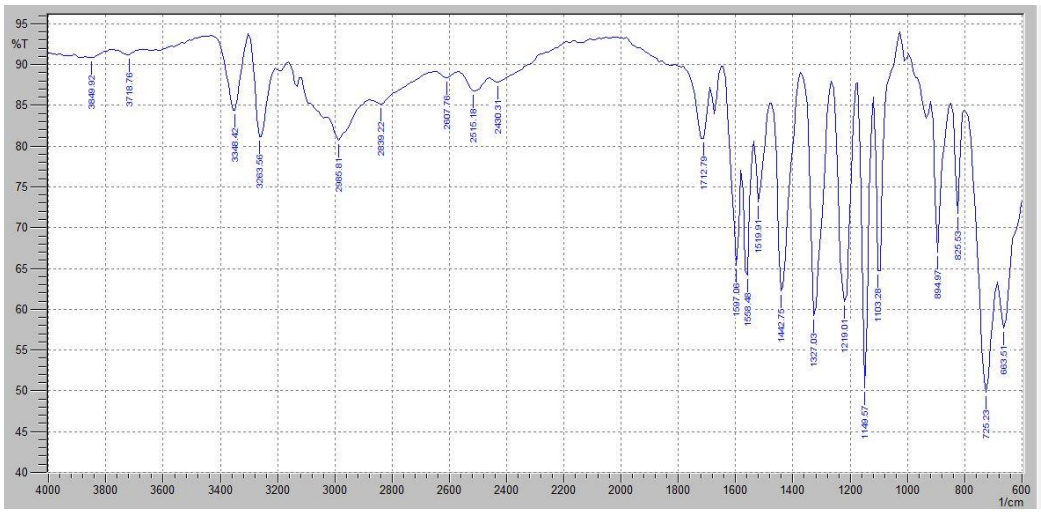


Figure 7.9. IR spectrum of compound **6**

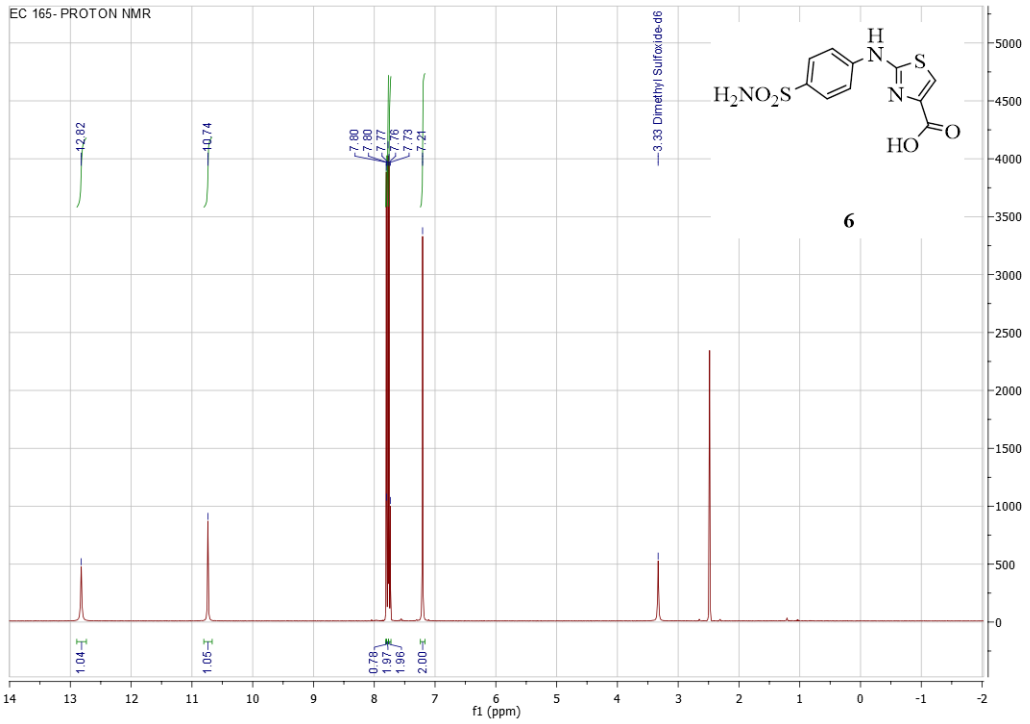


Figure 7.10. ^{1}H NMR spectrum of compound **6**

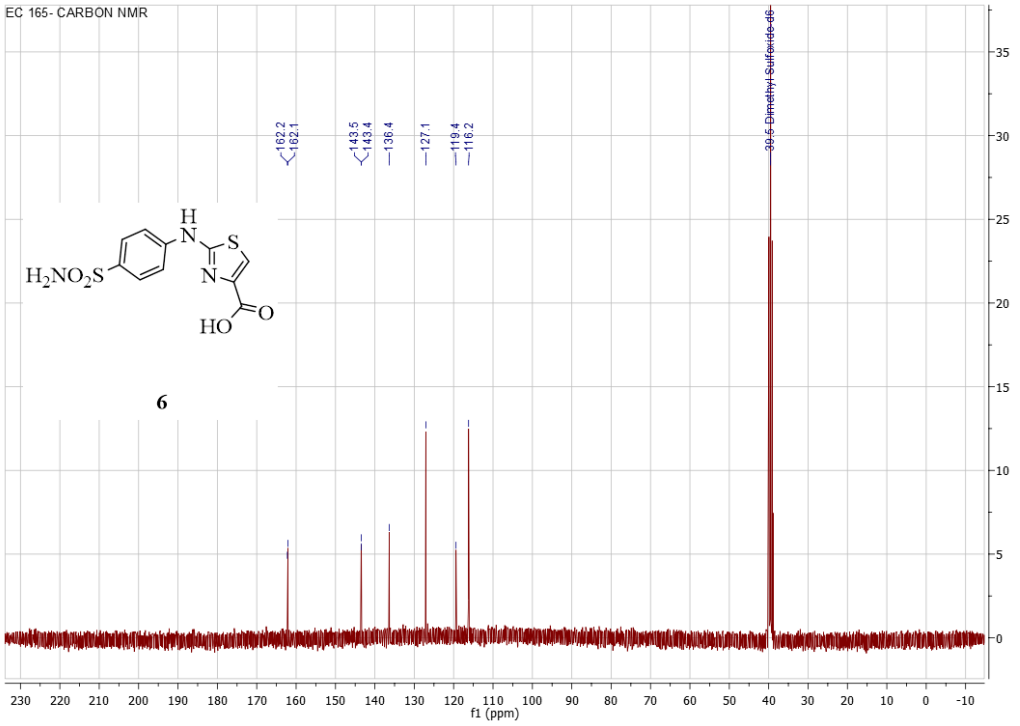


Figure 7.11. ^{13}C NMR spectrum of compound **6**

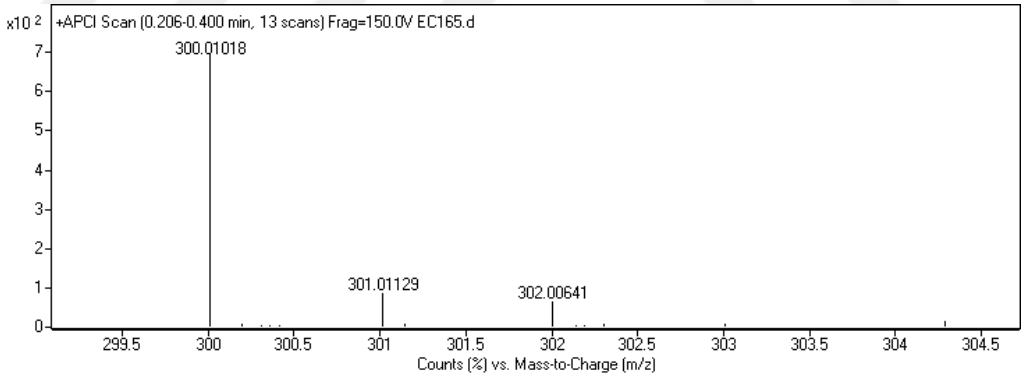


Figure 7.12. HRMS spectrum of compound **6**

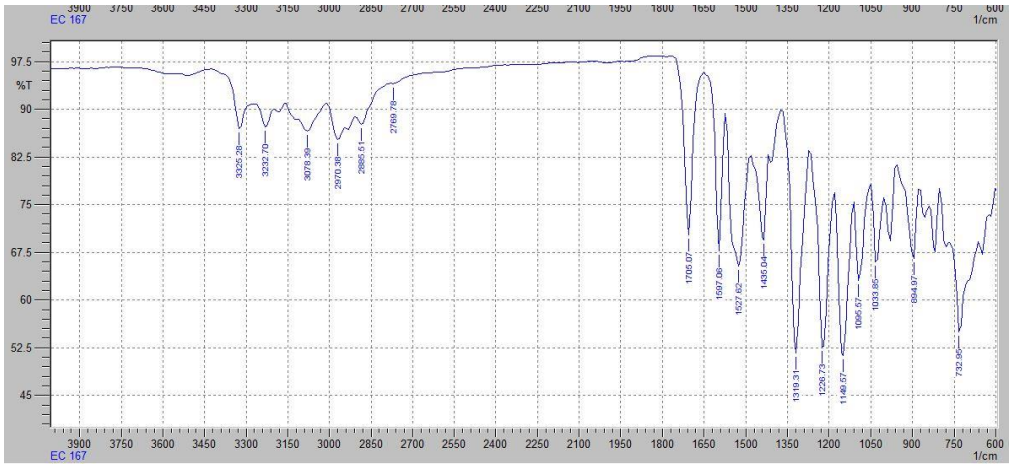


Figure 7.13. IR spectrum of compound **7a**

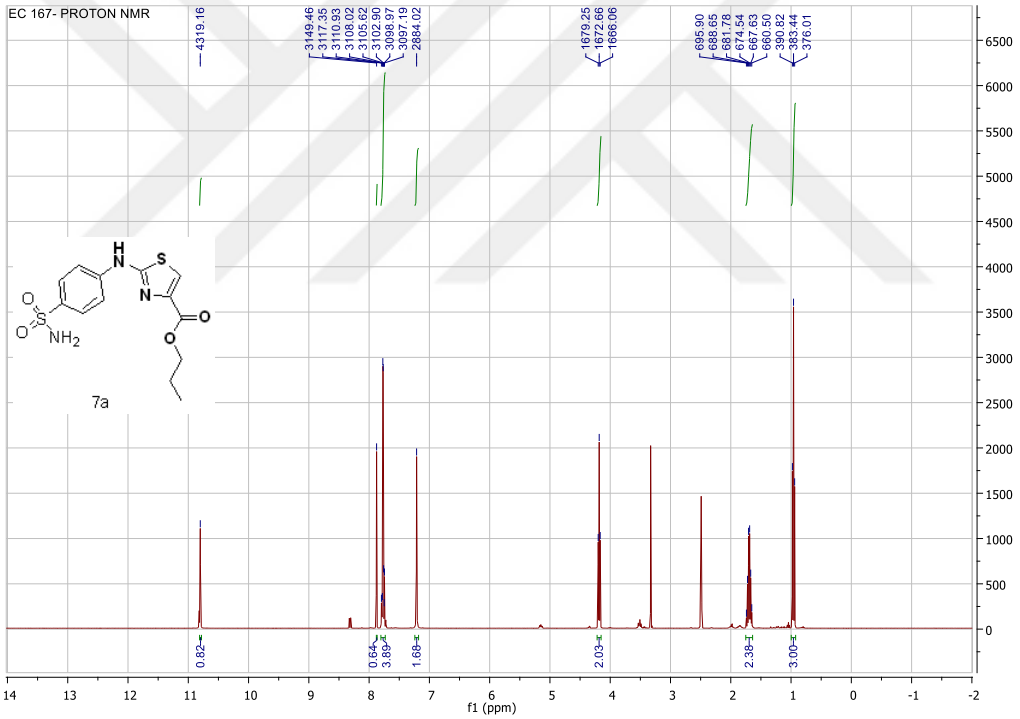


Figure 7.14. ^{1}H NMR spectrum of compound **7a**

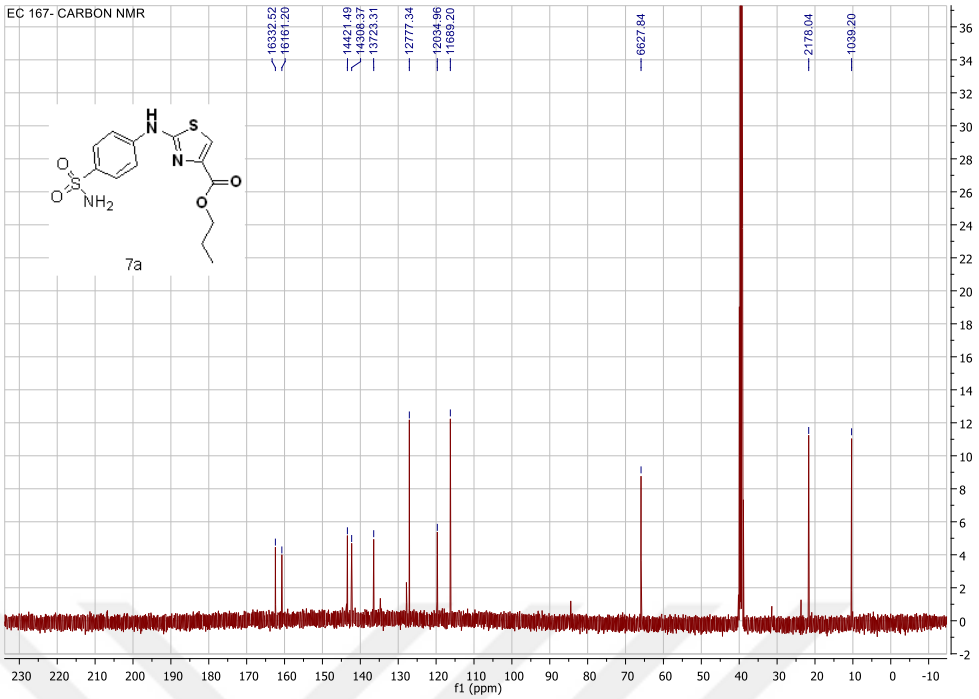


Figure 7.15. ^{13}C NMR spectrum of compound **7a**

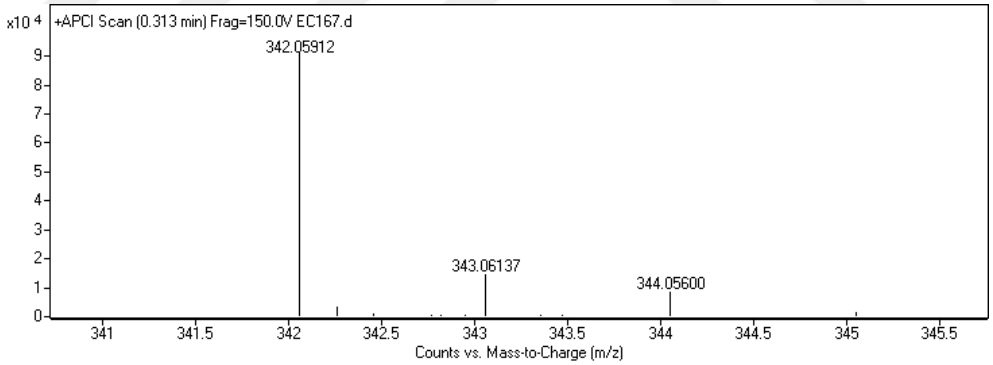


Figure 7.16. HRMS spectrum of compound **7a**

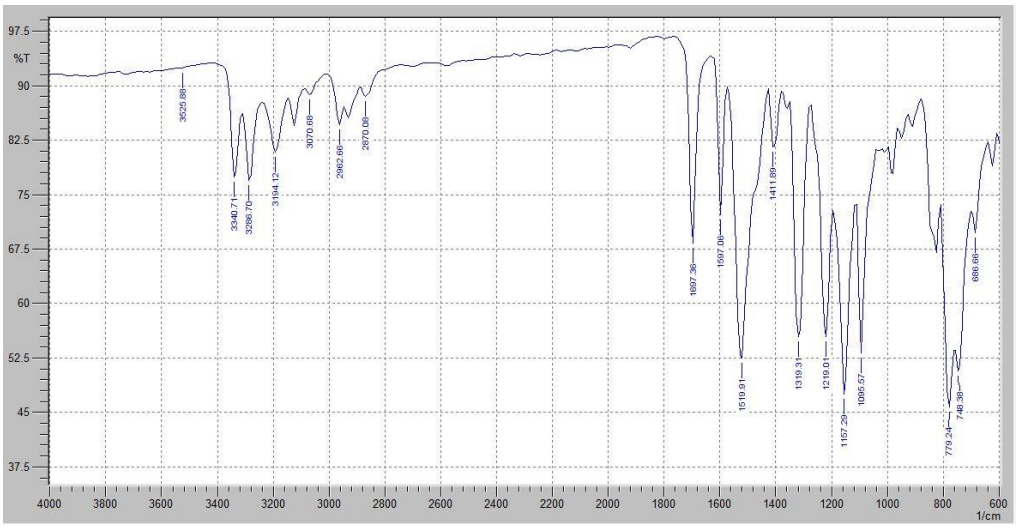


Figure 7.17. IR spectrum of compound **7b**

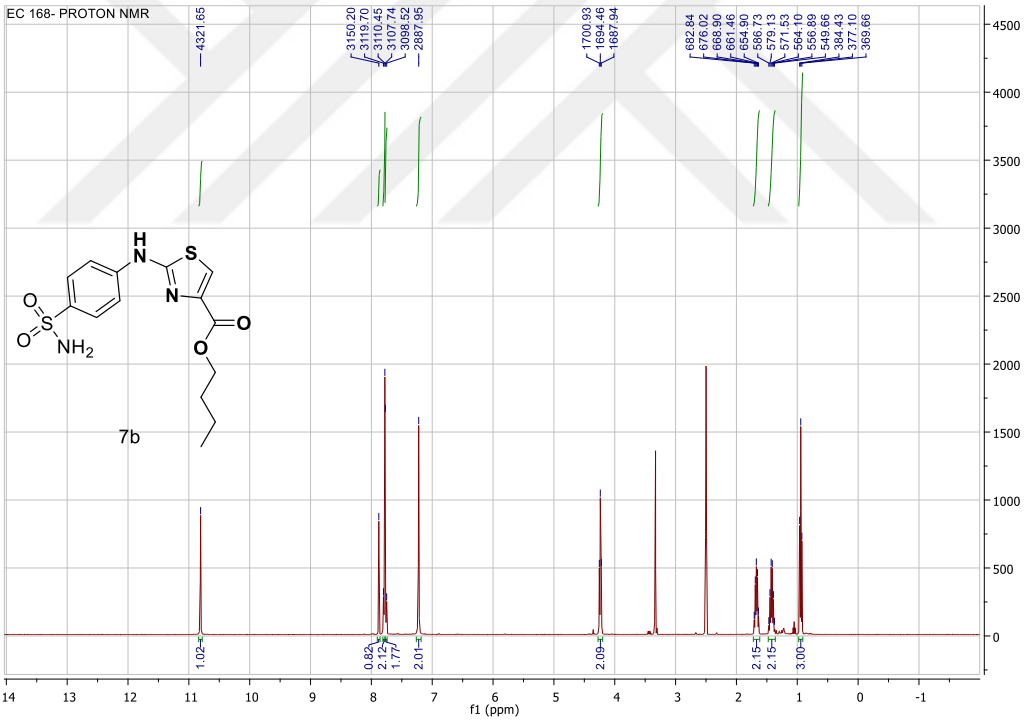


Figure 7.18. ^{1}H NMR spectrum of compound **7b**

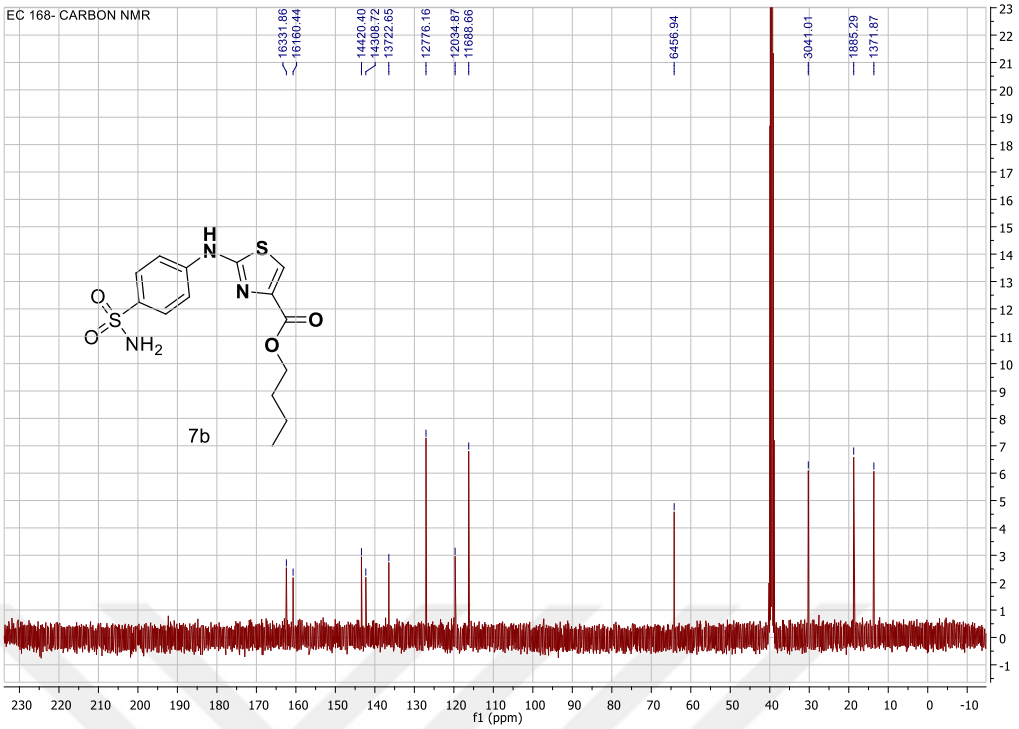


Figure 7.19. ^{13}C NMR spectrum of compound **7b**

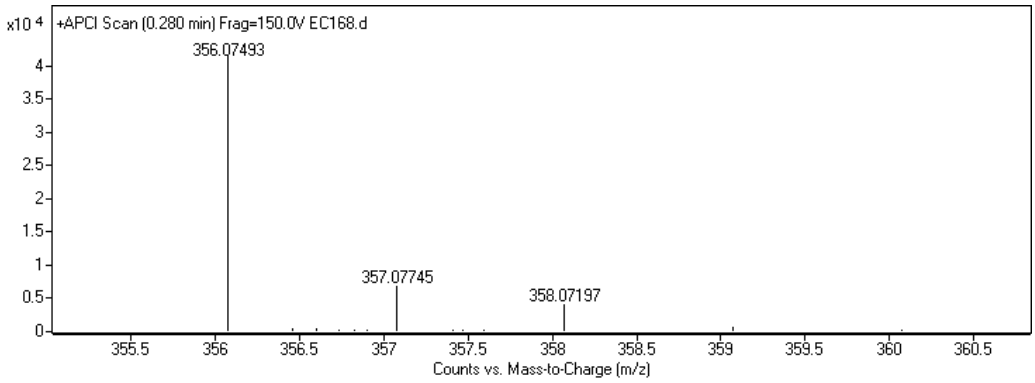


Figure 7.20. HRMS spectrum of compound **7b**

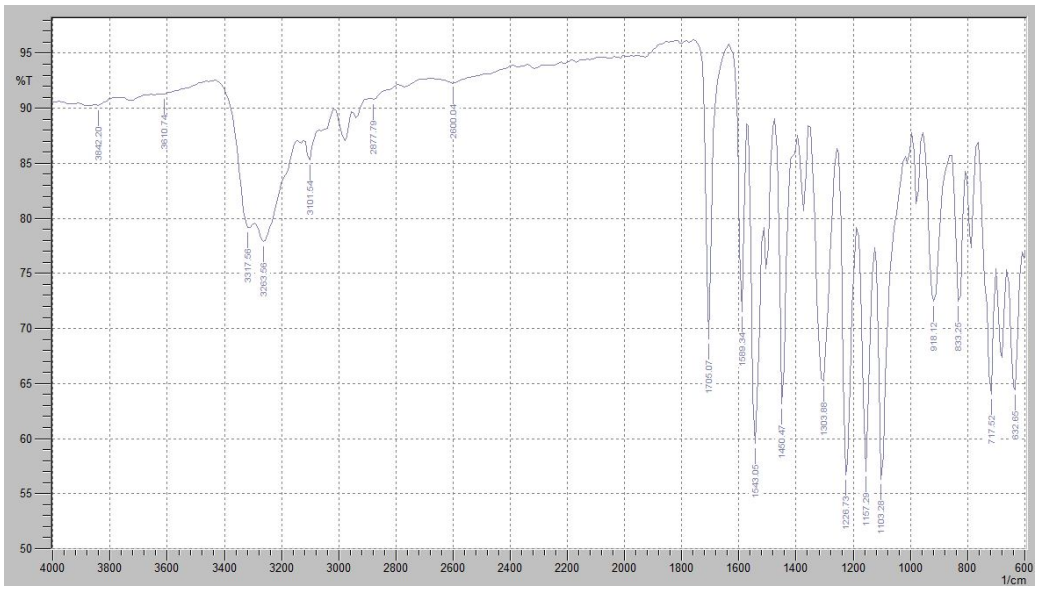


Figure 7.21. IR spectrum of compound **7c**

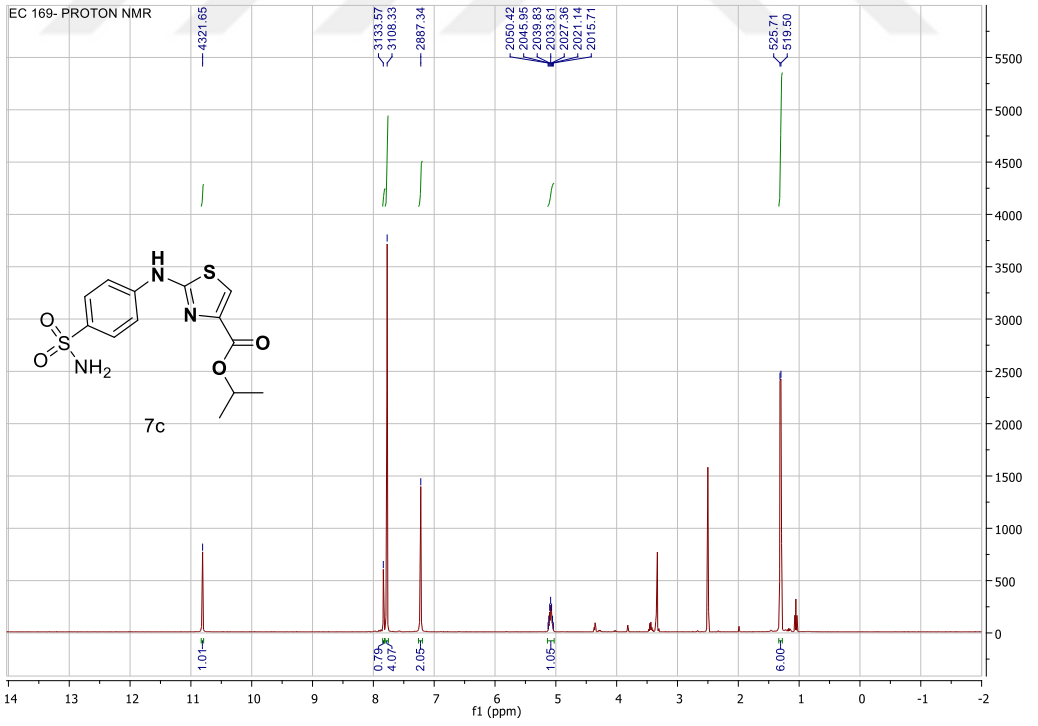


Figure 7.22. ^{1}H NMR spectrum of compound **7c**

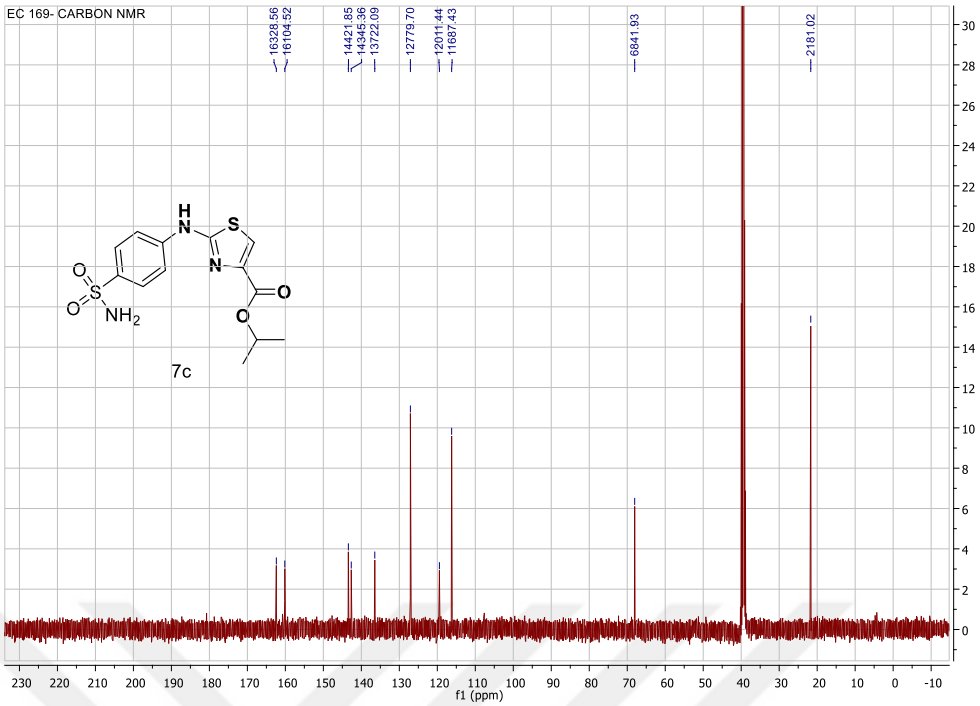


Figure 7.23. ^{13}C NMR spectrum of compound **7c**

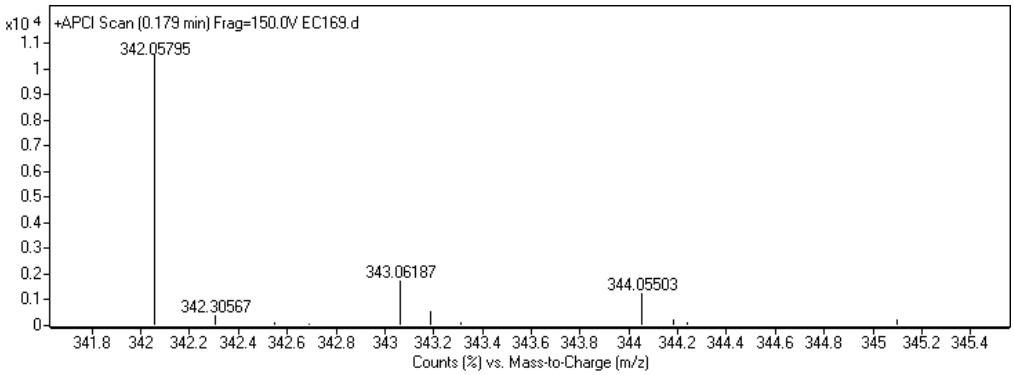


Figure 7.24. HRMS spectrum of compound **7c**

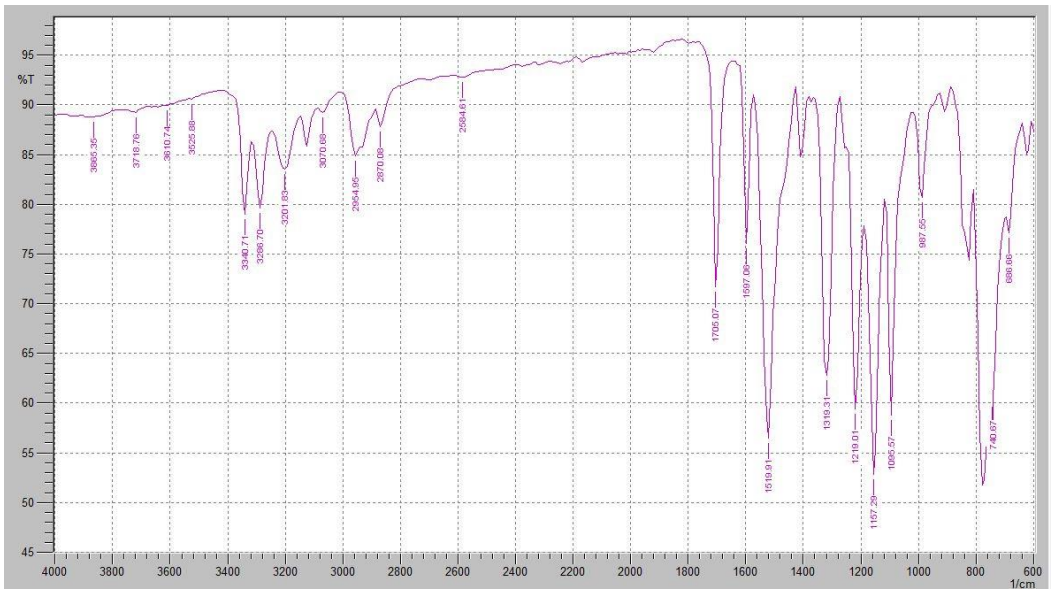


Figure 7.25. IR spectrum of compound **7d**

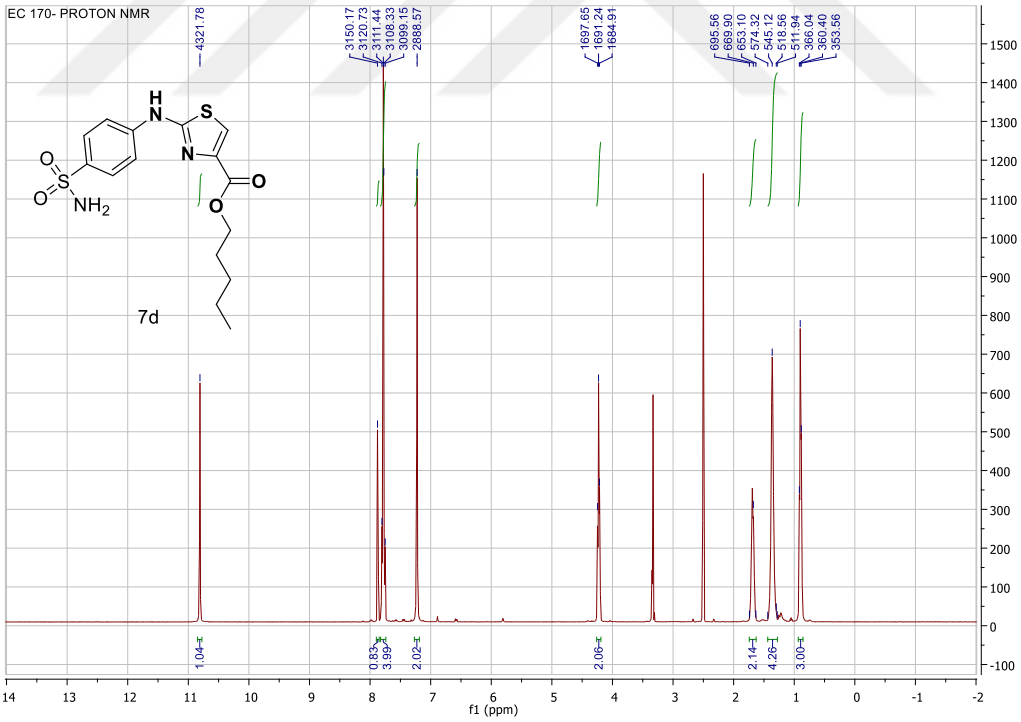


Figure 7.26. ^{1}H NMR spectrum of compound **7d**

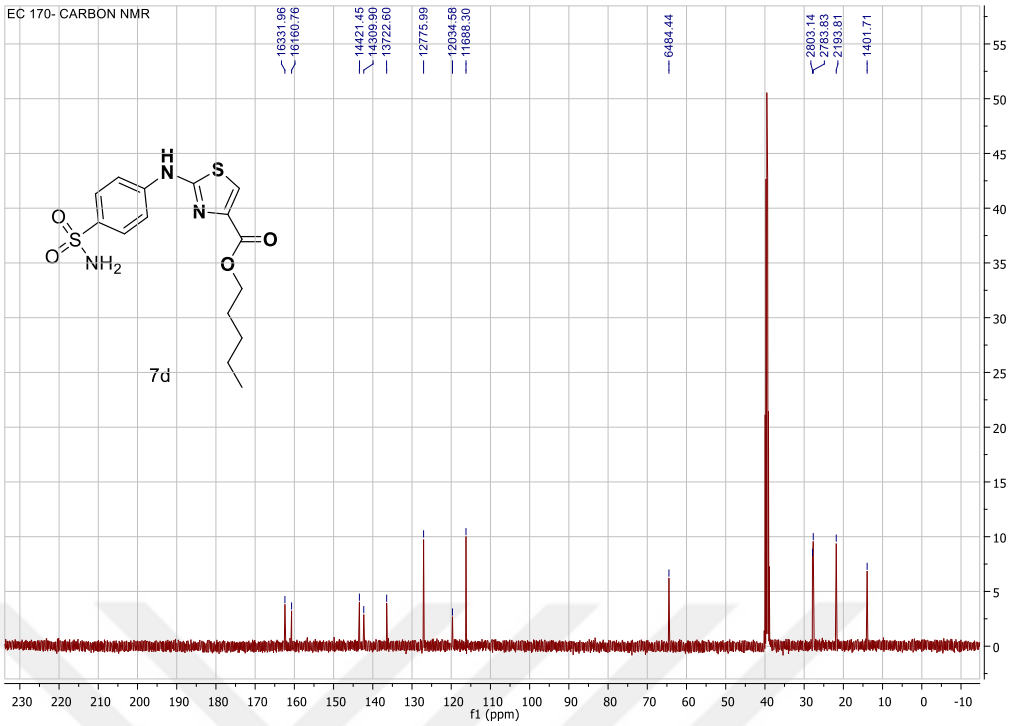


Figure 7.27. ^{13}C NMR spectrum of compound **7d**

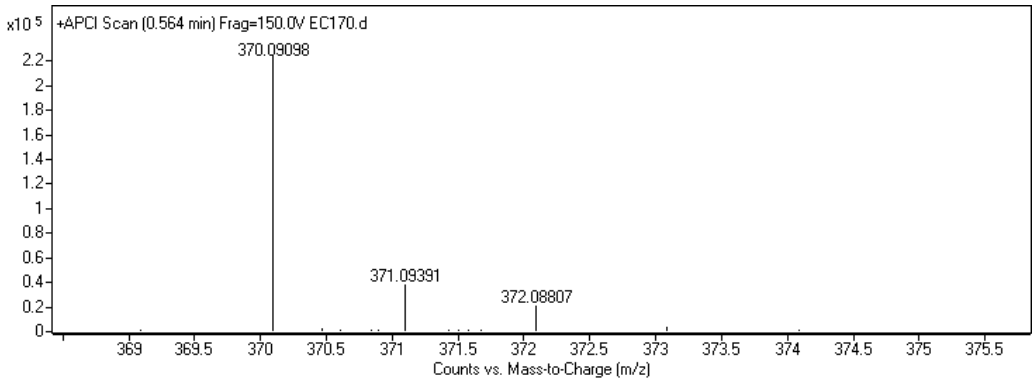


Figure 7.28. HRMS spectrum of compound **7d**

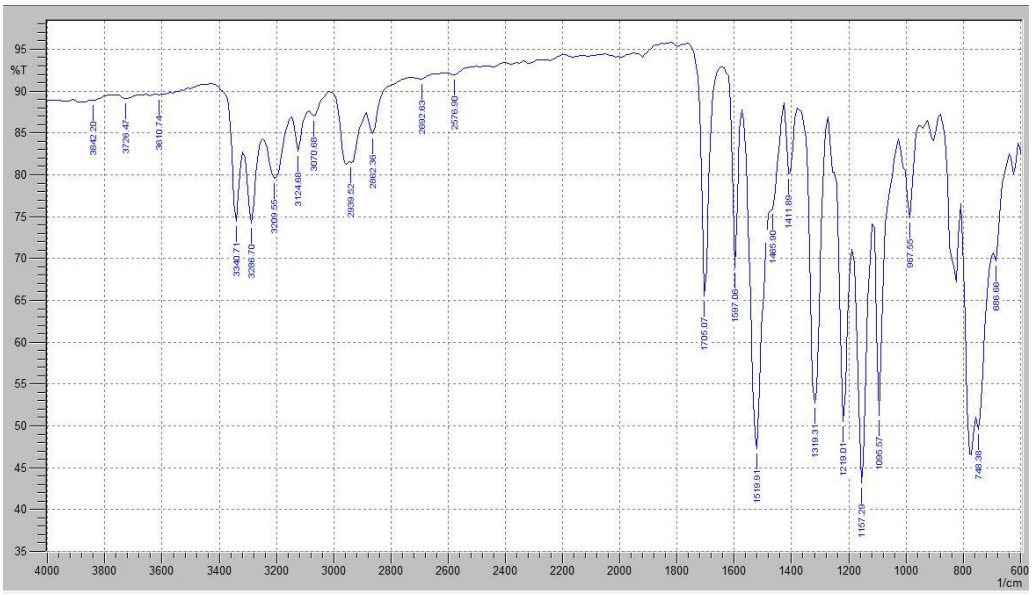


Figure 7.29. IR spectrum of compound **7e**

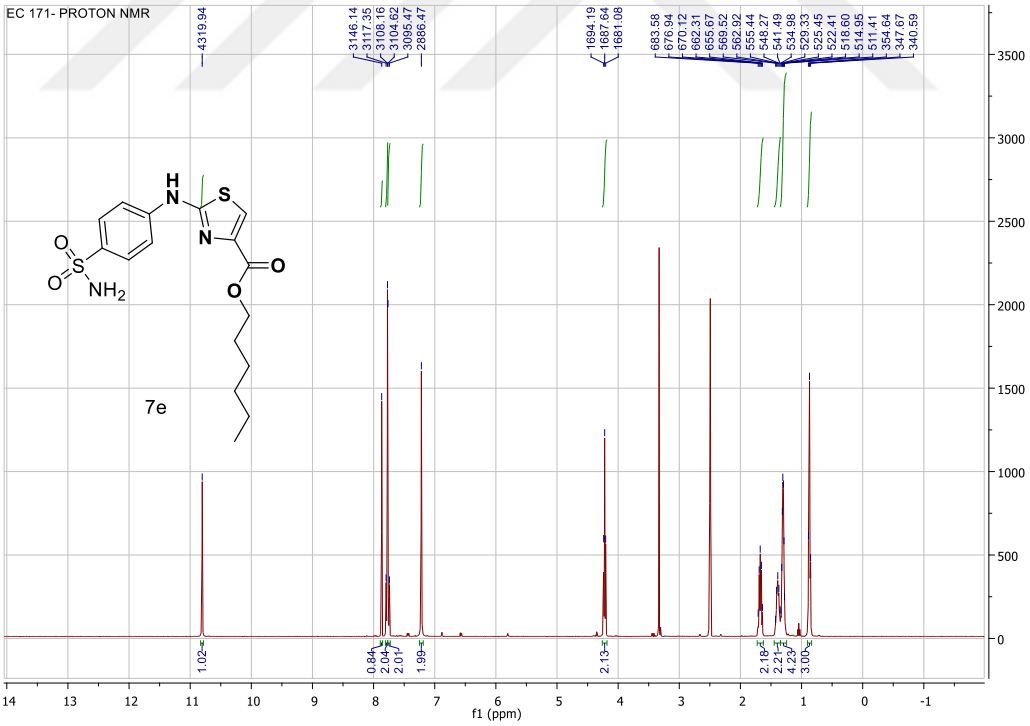


Figure 7.30. ^{1}H NMR spectrum of compound **7e**

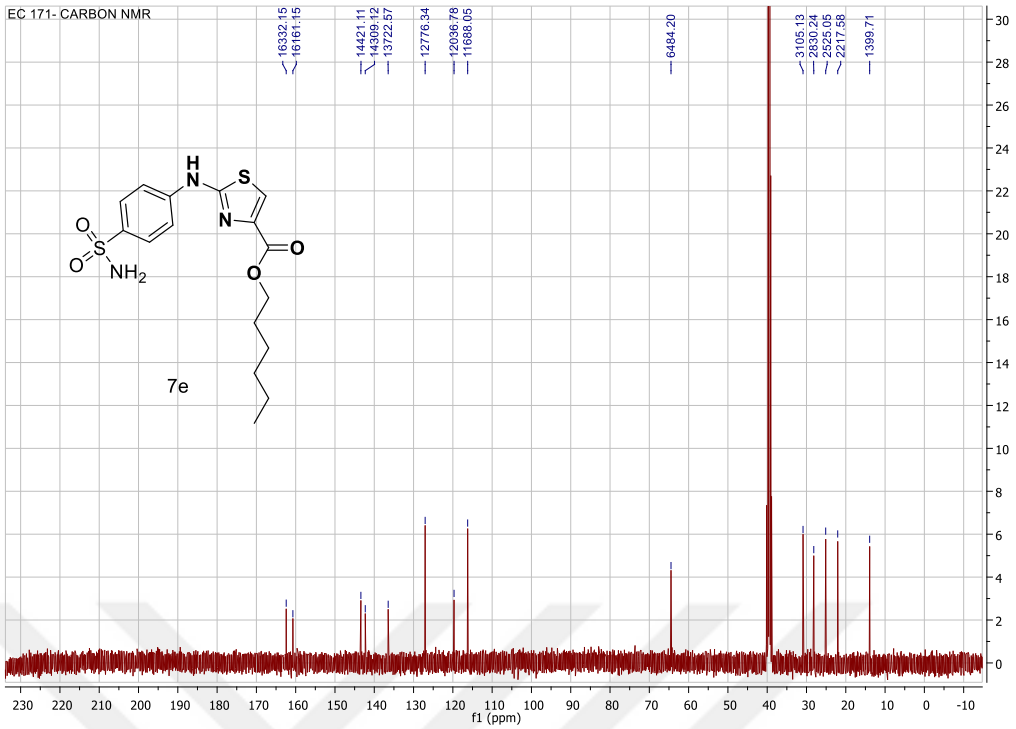


Figure 7.31. ^{13}C NMR spectrum of compound **7e**

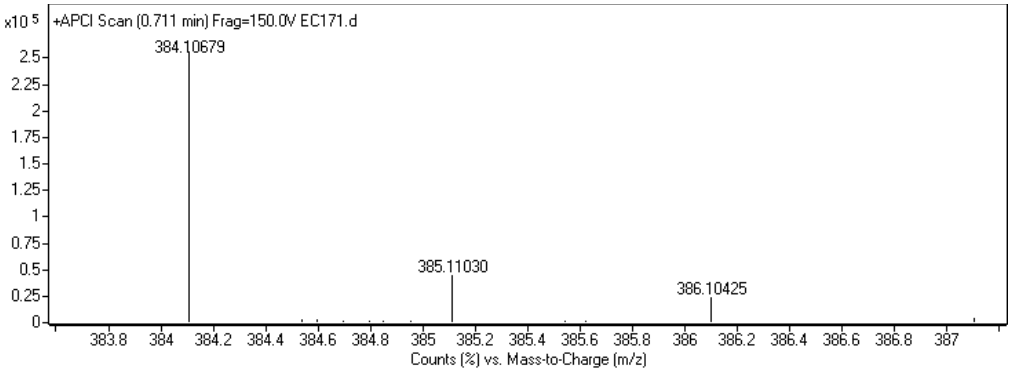


Figure 7.32. HRMS spectrum of compound **7e**

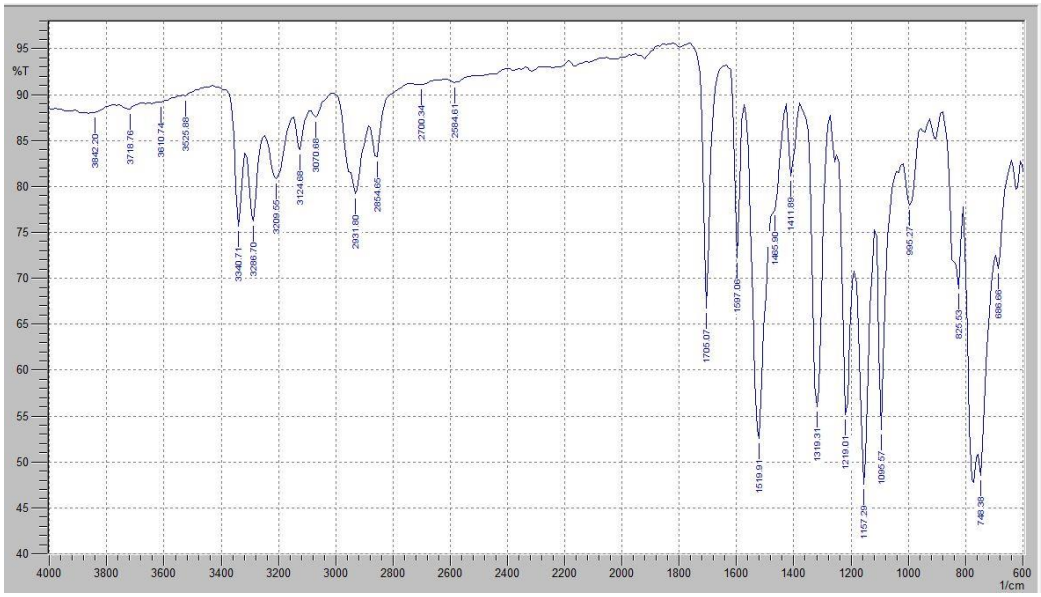


Figure 7.33. IR spectrum of compound **7f**

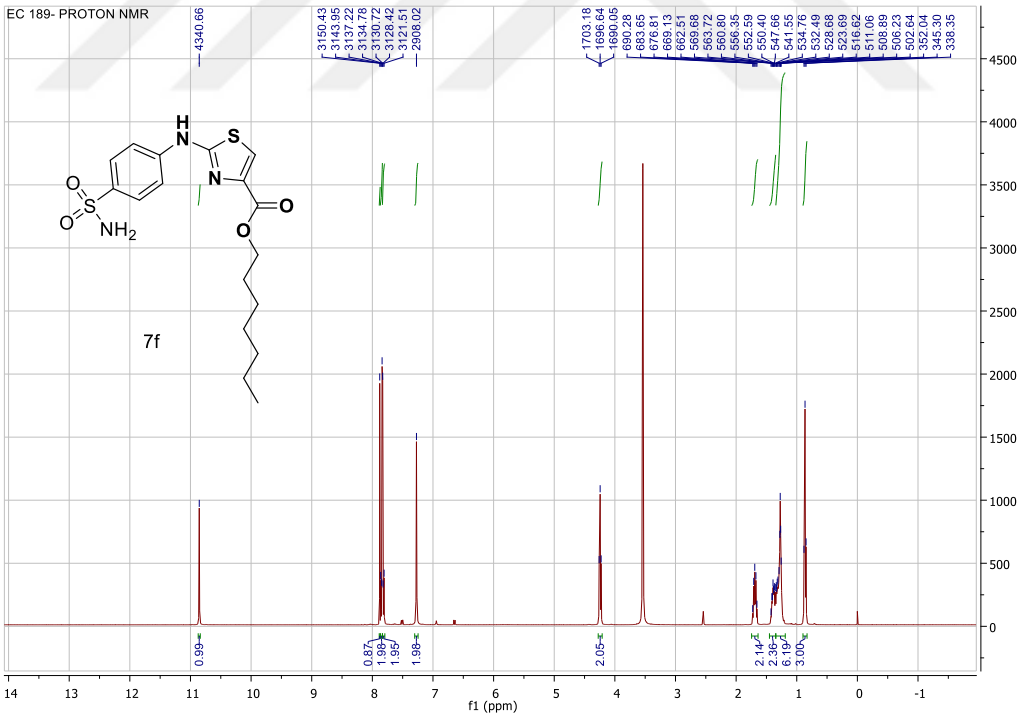


Figure 7.34. ^{1}H NMR spectrum of compound **7f**

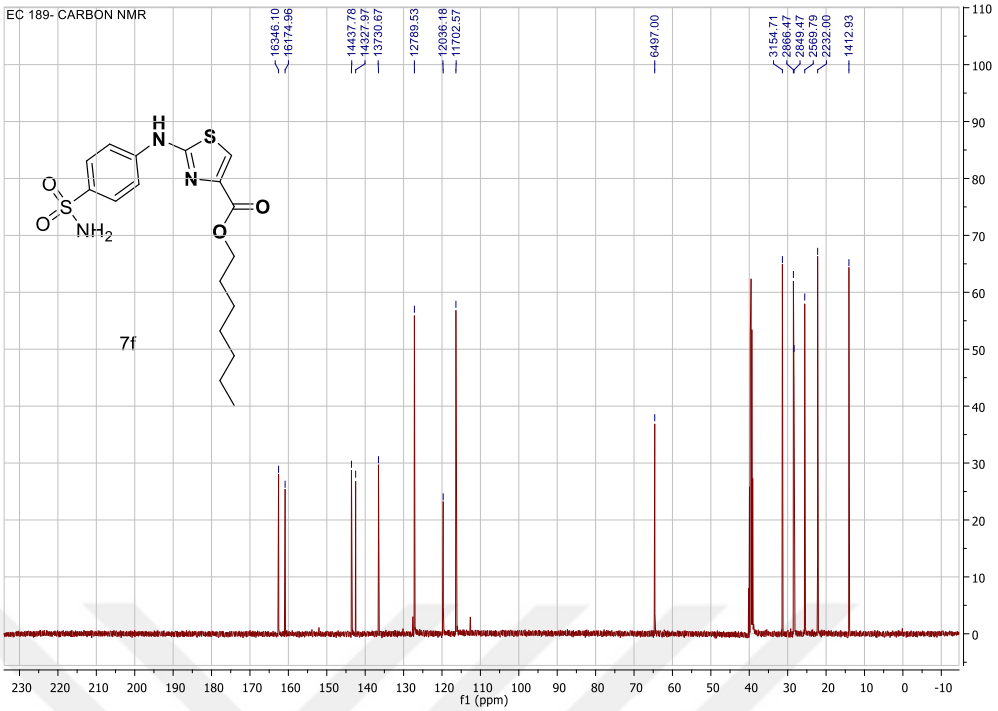


Figure 7.35. ^{13}C NMR spectrum of compound **7f**

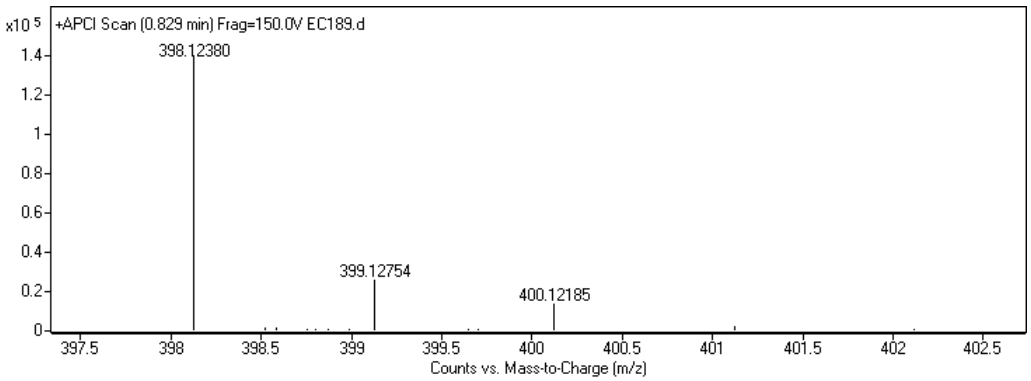


Figure 7.36. HRMS spectrum of compound **7f**

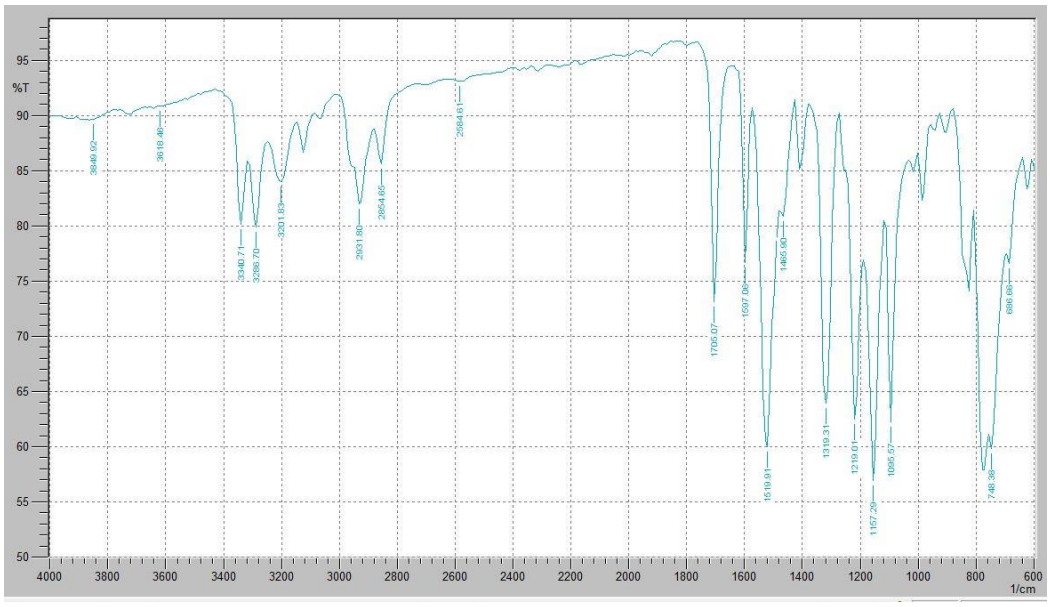


Figure 7.37. IR spectrum of compound **7g**

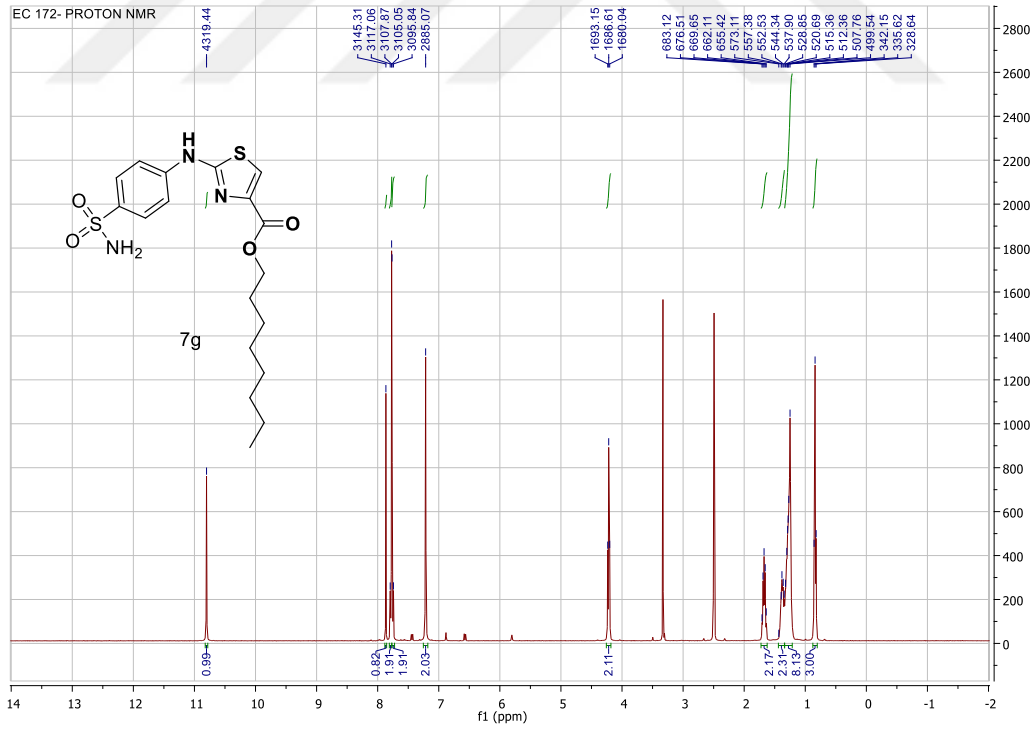


Figure 7.38. ^{1}H NMR spectrum of compound **7g**

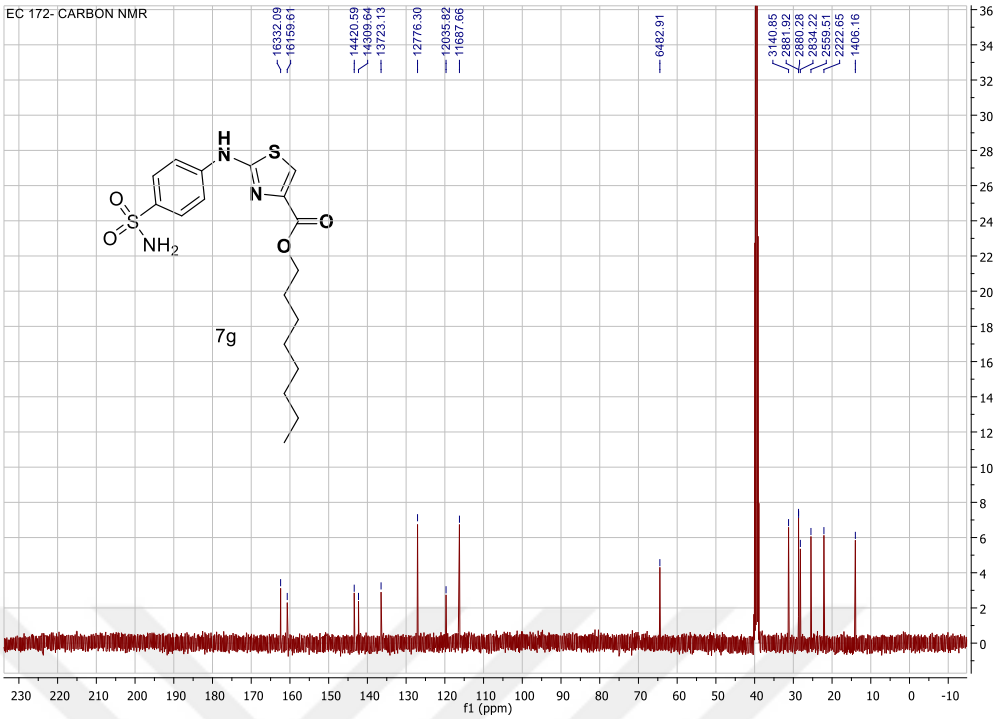


Figure 7.39. ^{13}C NMR spectrum of compound **7g**

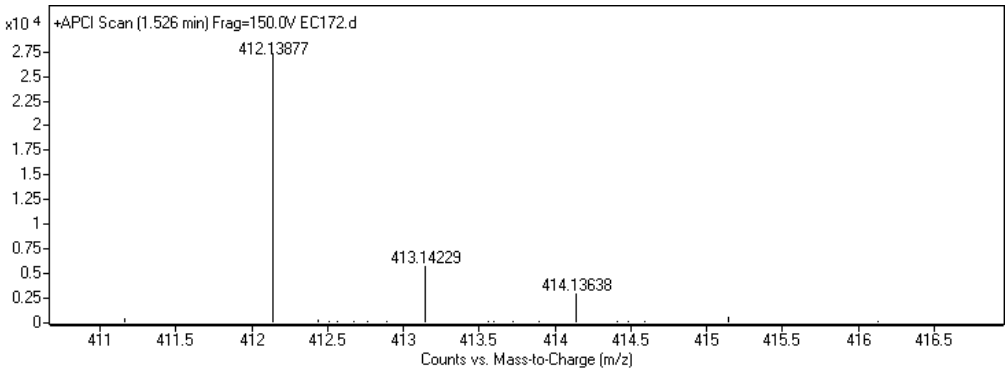


Figure 7.40. HRMS spectrum of compound **7g**

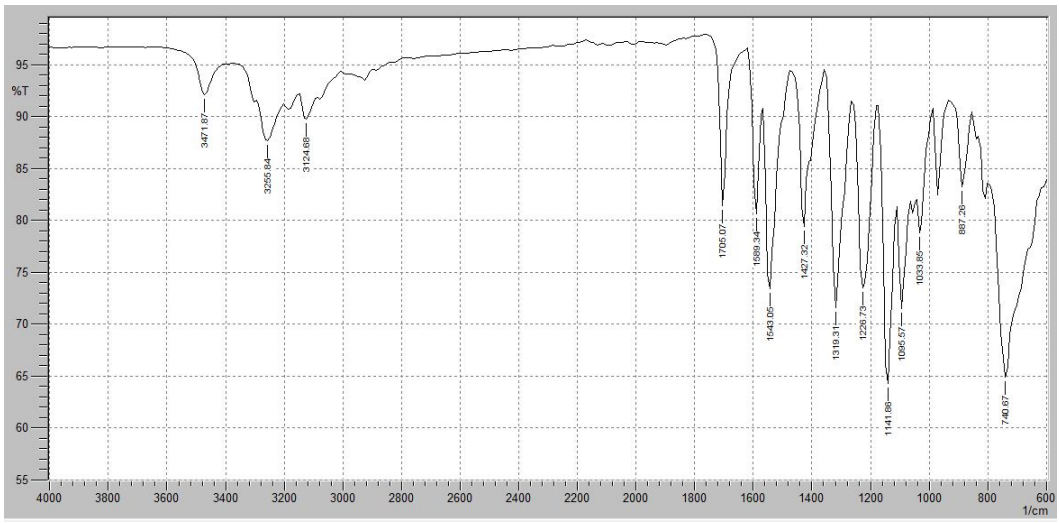


Figure 7.41. IR spectrum of compound **7h**

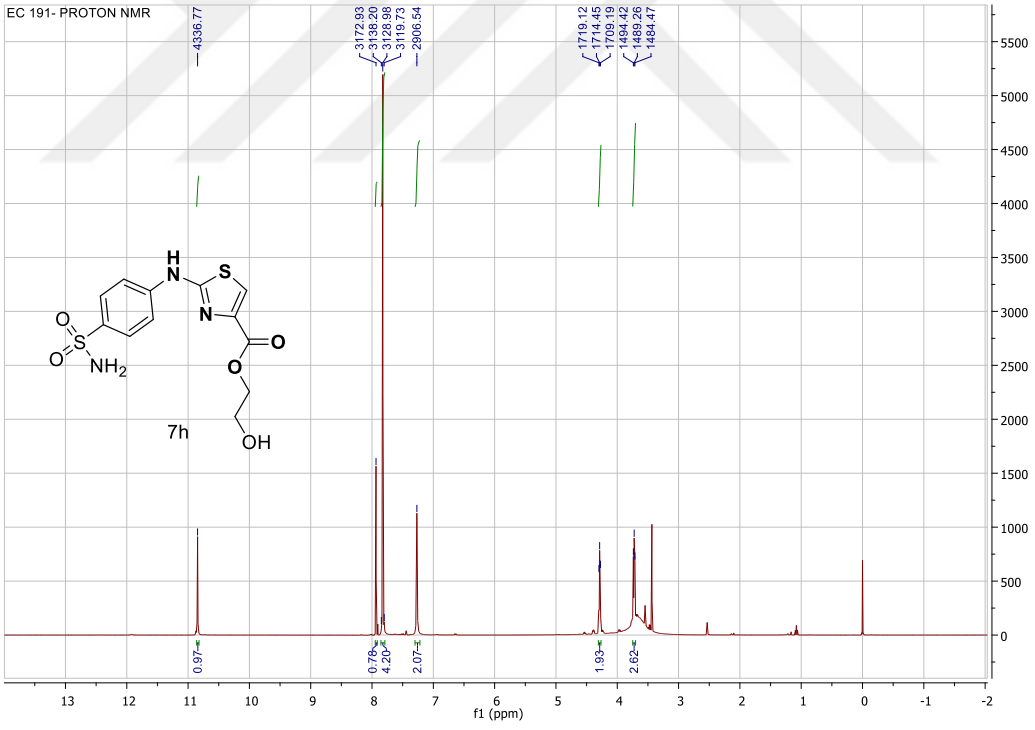


Figure 7.42. ^{1}H NMR spectrum of compound **7h**

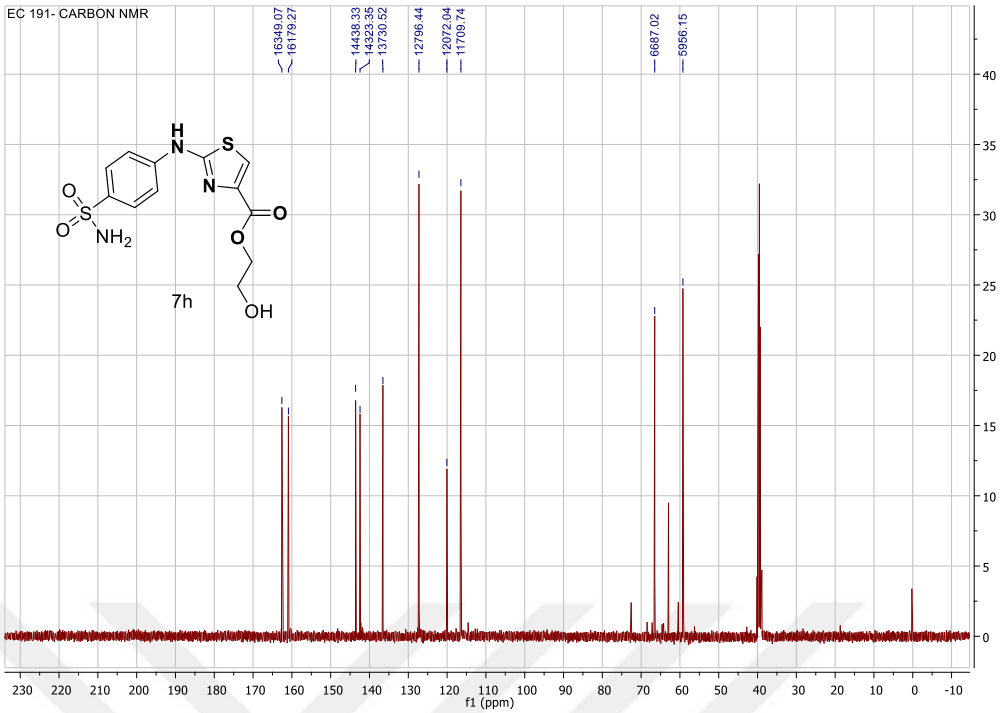


Figure 7.43. ^{13}C NMR spectrum of compound **7h**

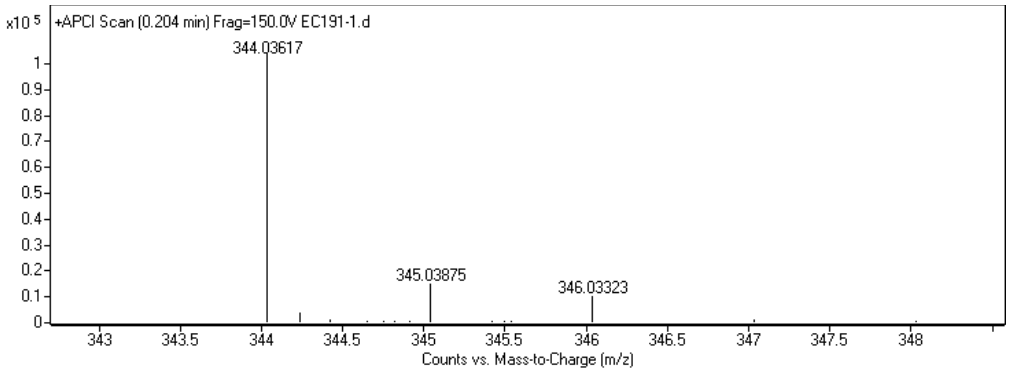


Figure 7.44. HRMS spectrum of compound **7h**

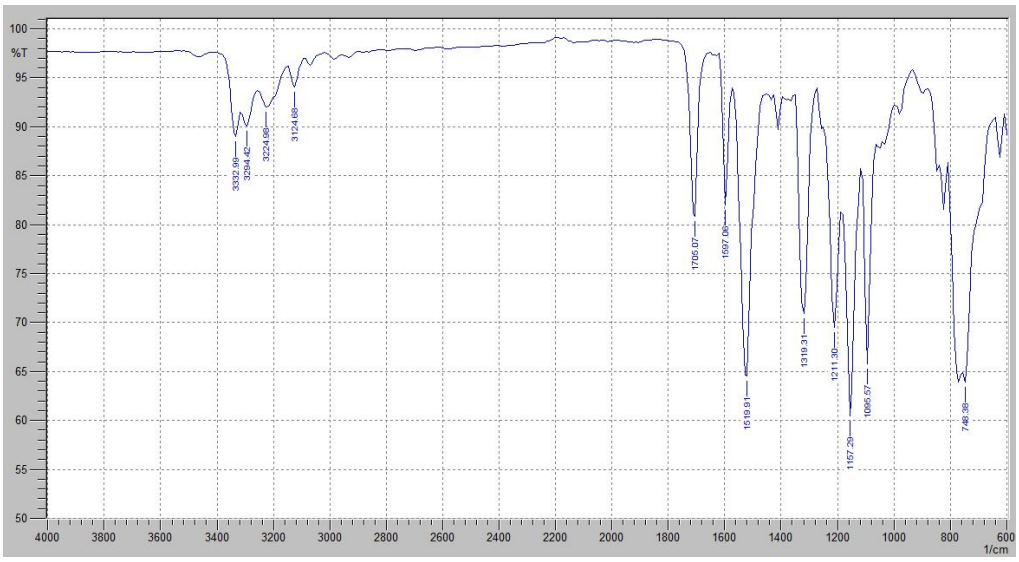


Figure 7.45. IR spectrum of compound **7i/j**

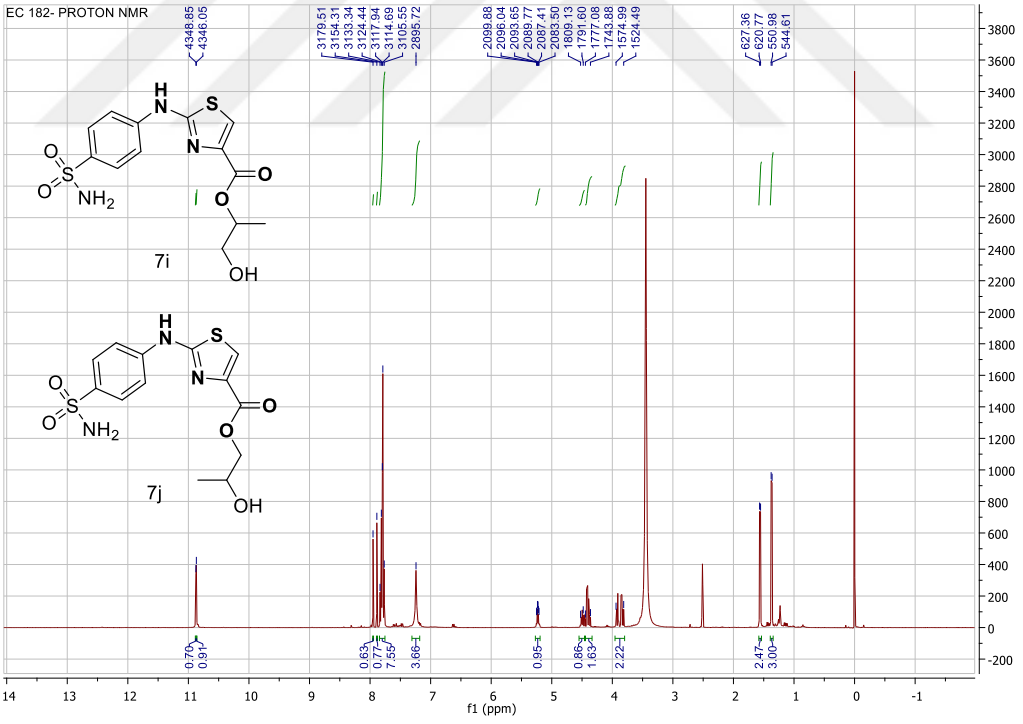


Figure 7.46. ^{1}H NMR spectrum of compound **7i/j**

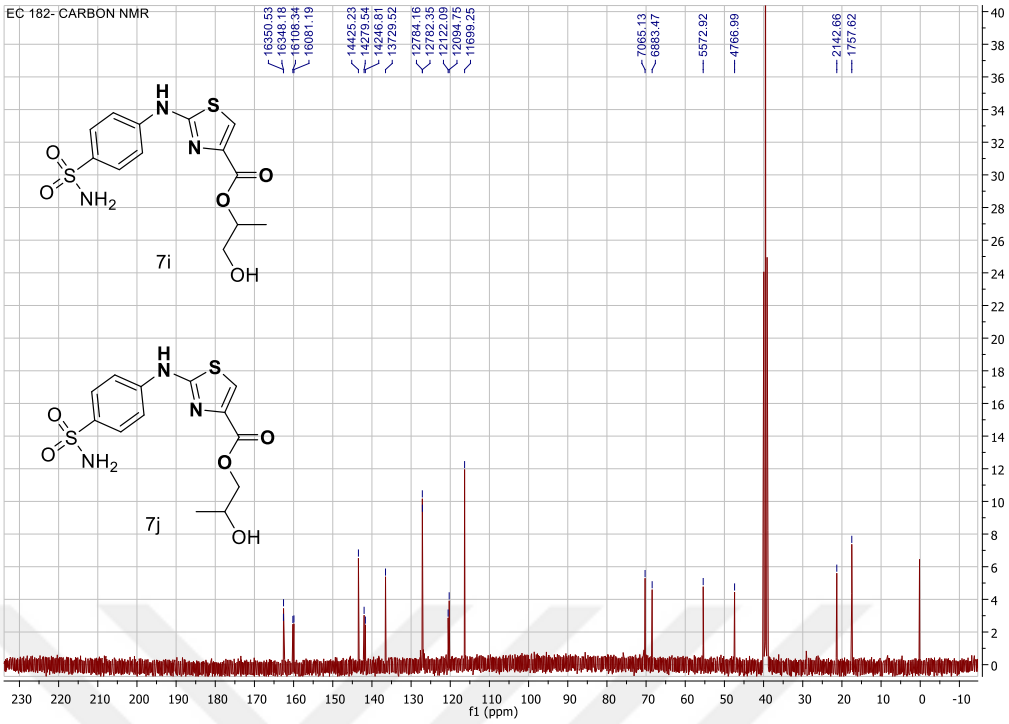


Figure 7.47. ^{13}C NMR spectrum of compound **7i/j**

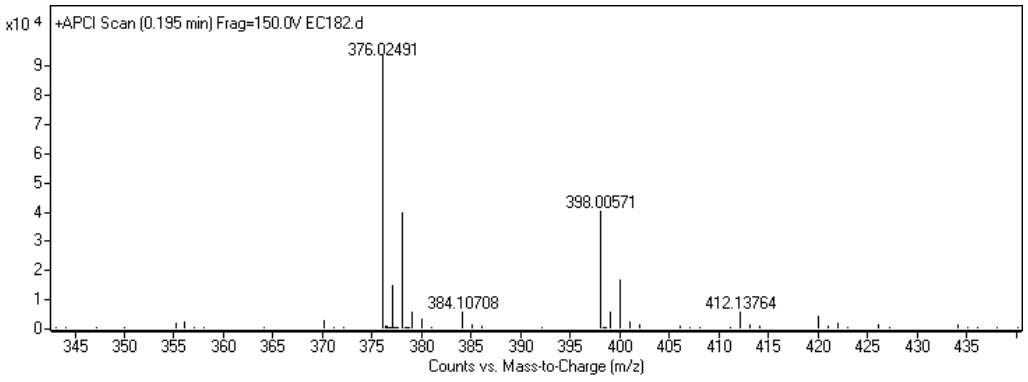


Figure 7.48. HRMS spectrum of compound **7i/j**

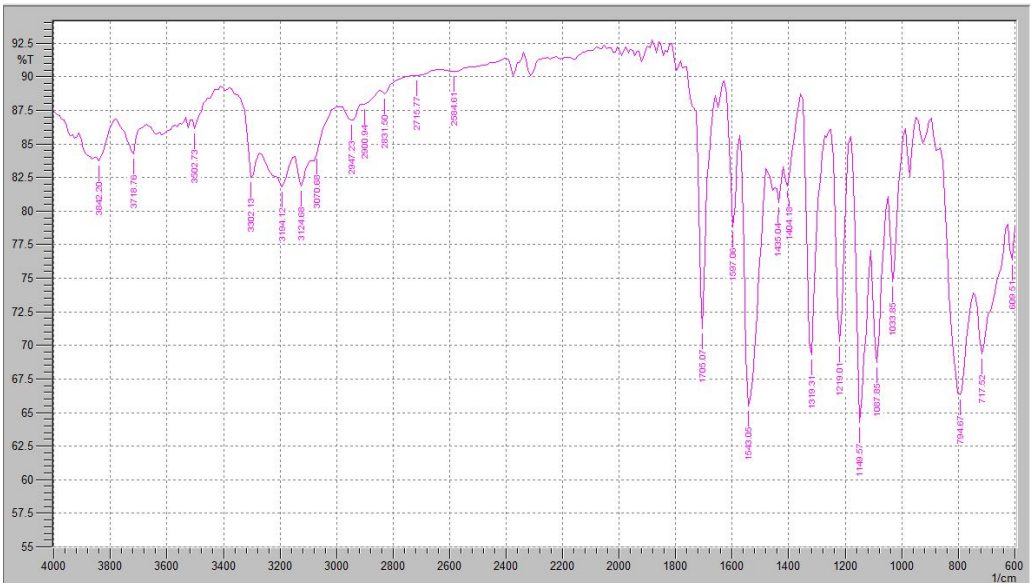


Figure 7.49. IR spectrum of compound **7k**

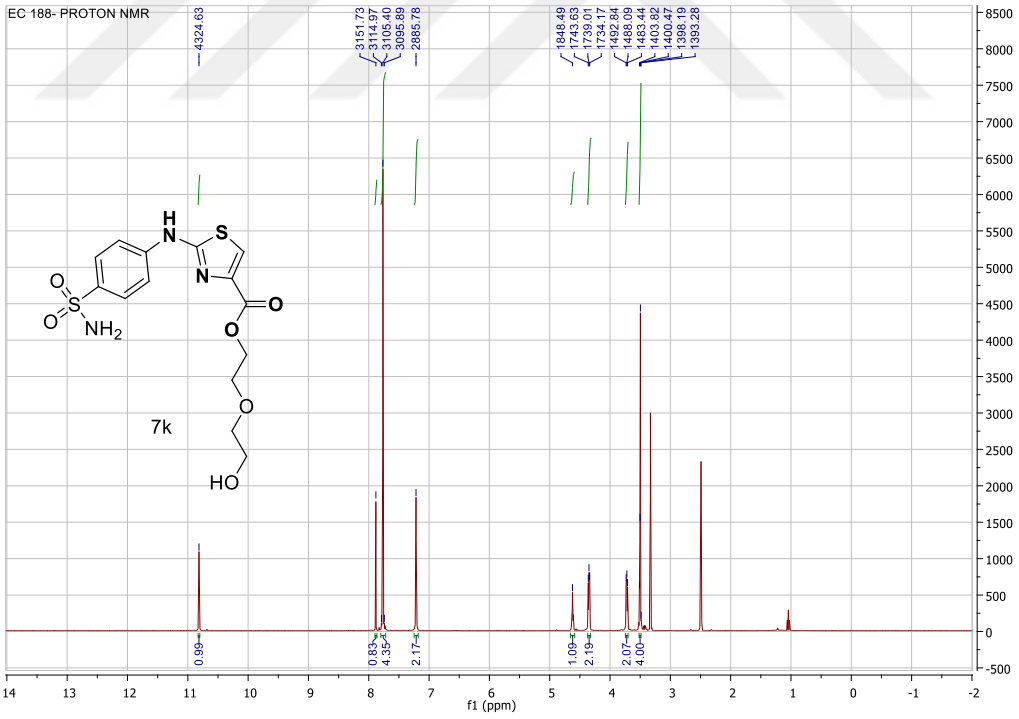


Figure 7.50. ^{1}H NMR spectrum of compound **7k**

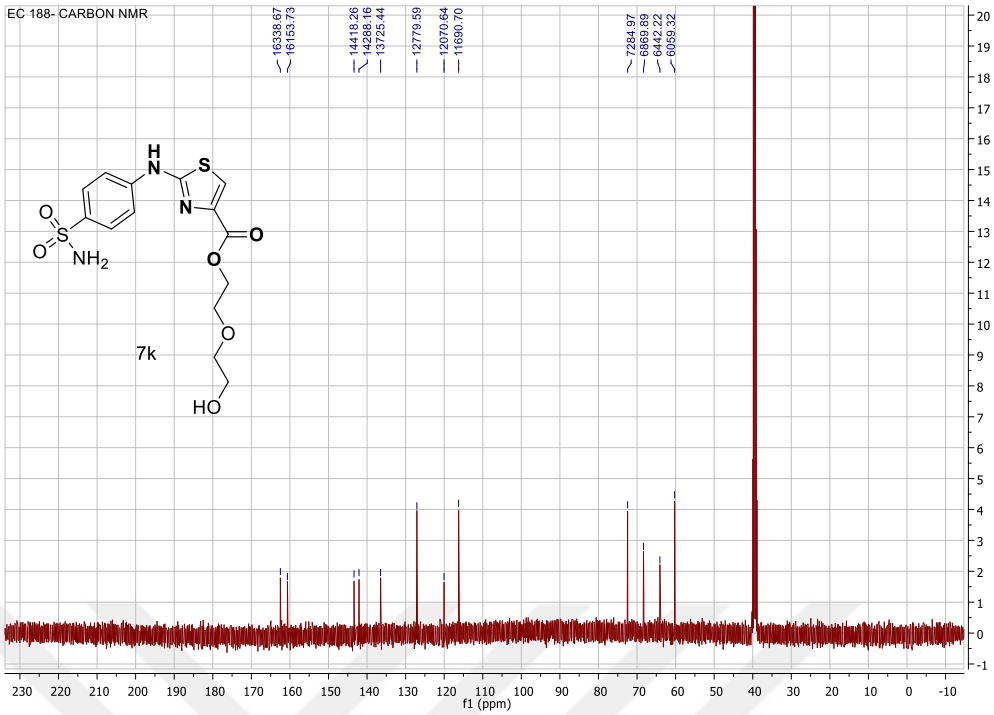


Figure 7.51. ^{13}C NMR spectrum of compound **7k**

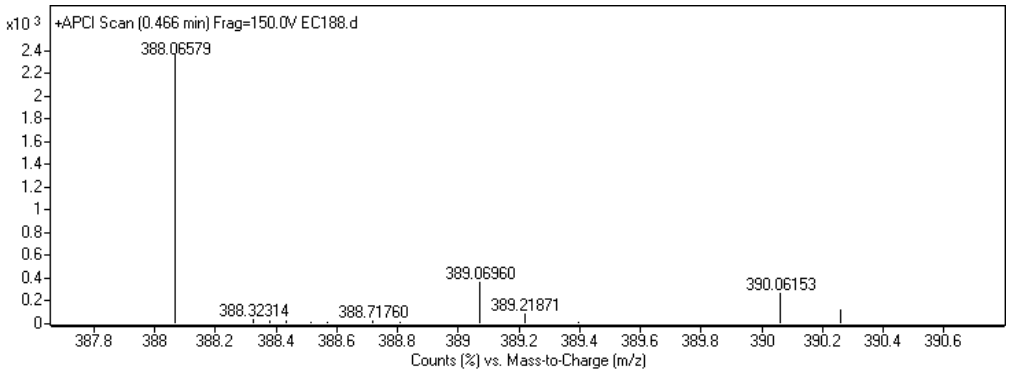


Figure 7.52. HRMS spectrum of compound **7k**

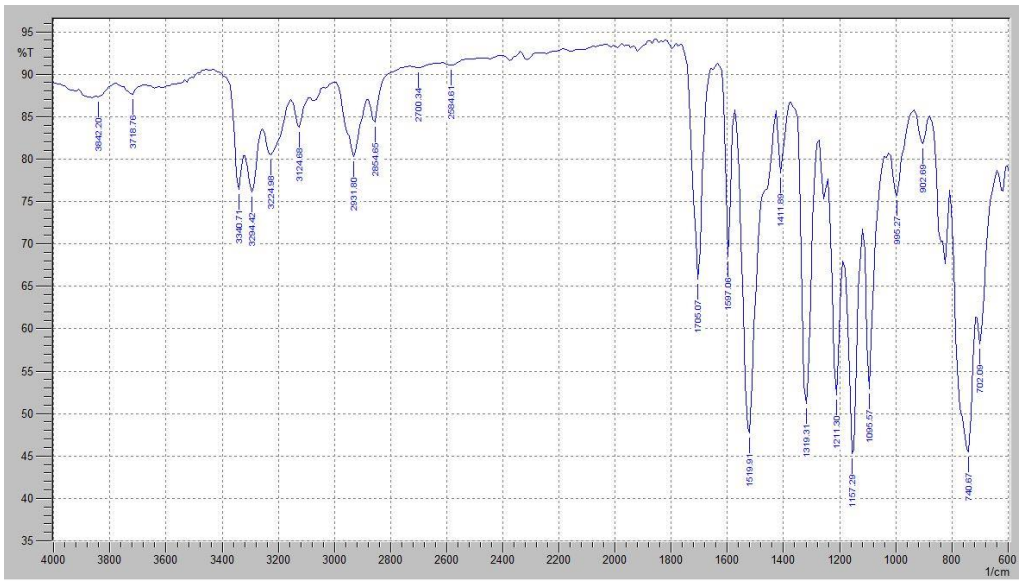


Figure 7.53. IR spectrum of compound **7l**

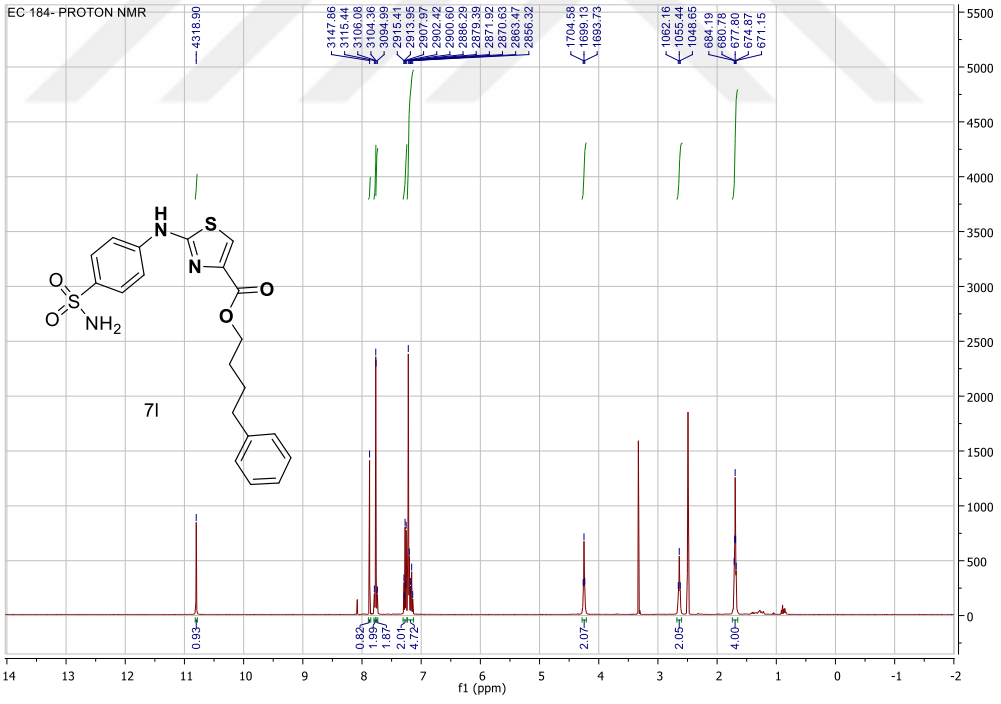


Figure 7.54. ^{1}H NMR spectrum of compound **7l**

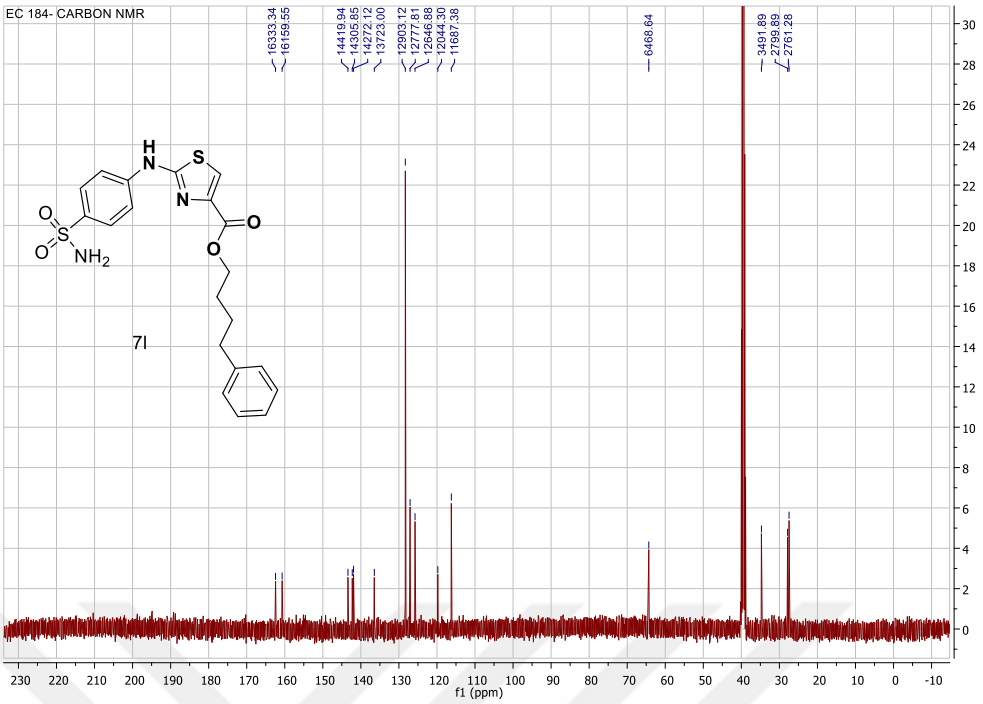


Figure 7.55. ^{13}C NMR spectrum of compound **7l**

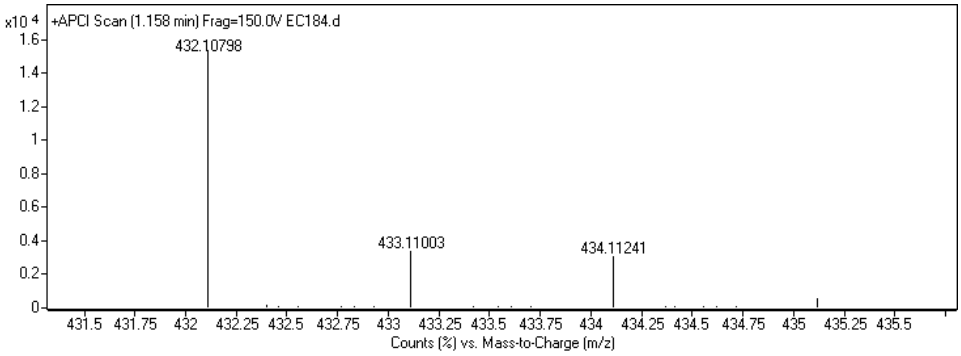


Figure 7.56. HRMS spectrum of compound **7l**

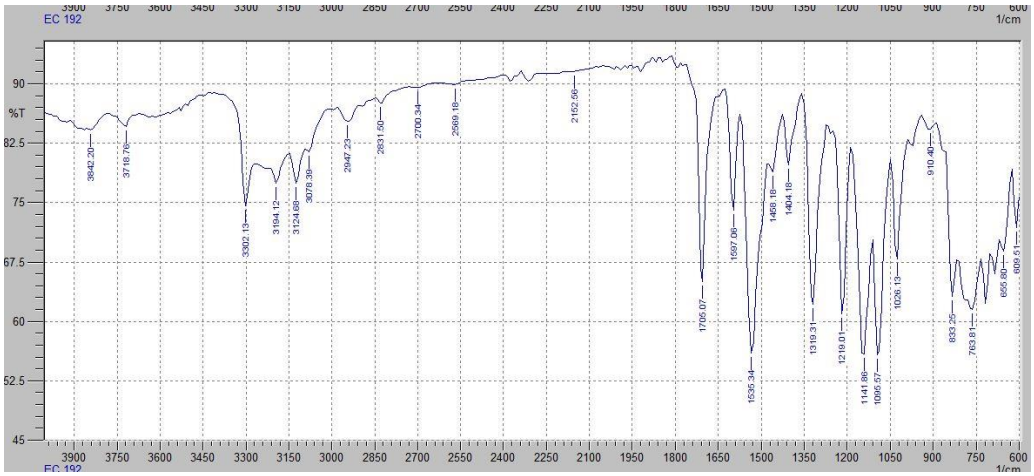


Figure 7.57. IR spectrum of compound **7m**

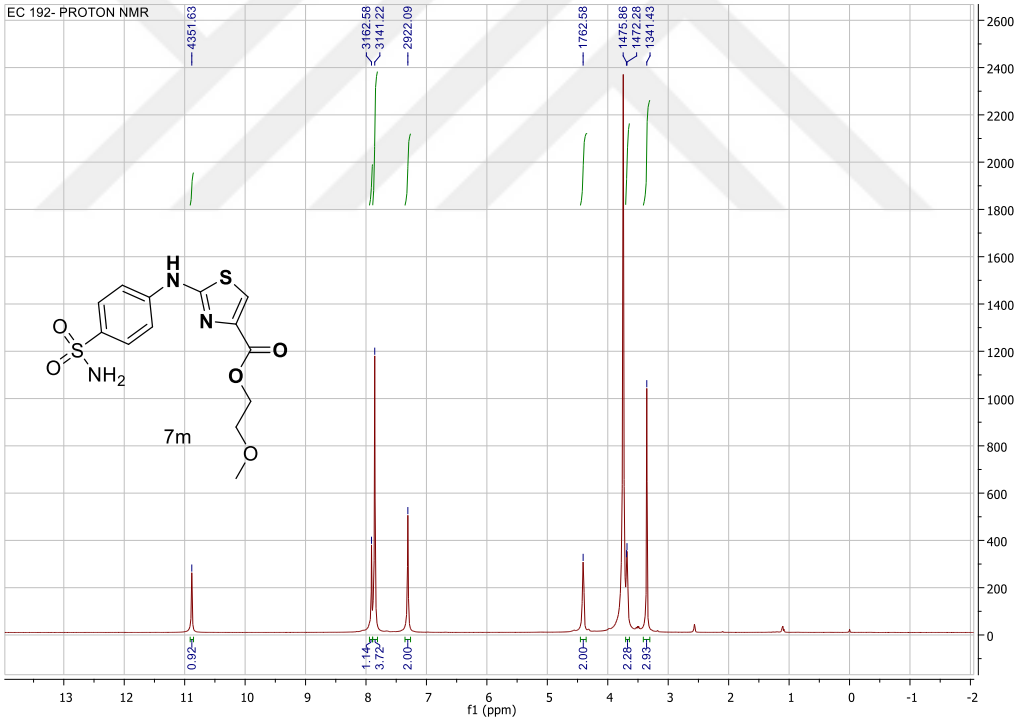


Figure 7.58. ^{1}H NMR spectrum of compound **7m**

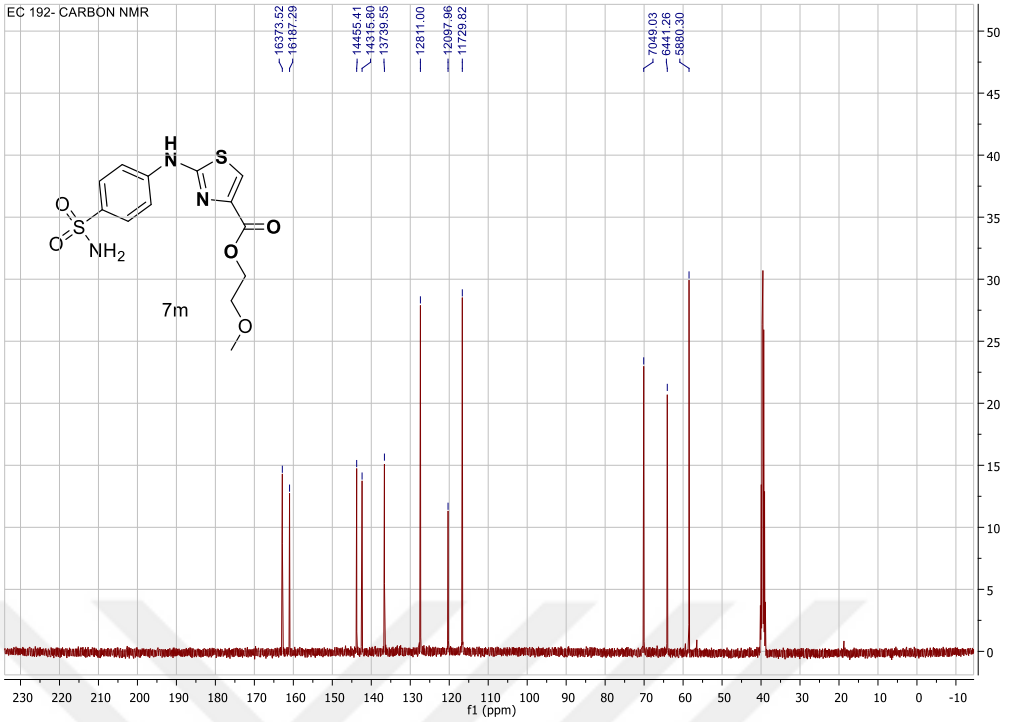


Figure 7.59. ^{13}C NMR spectrum of compound **7m**

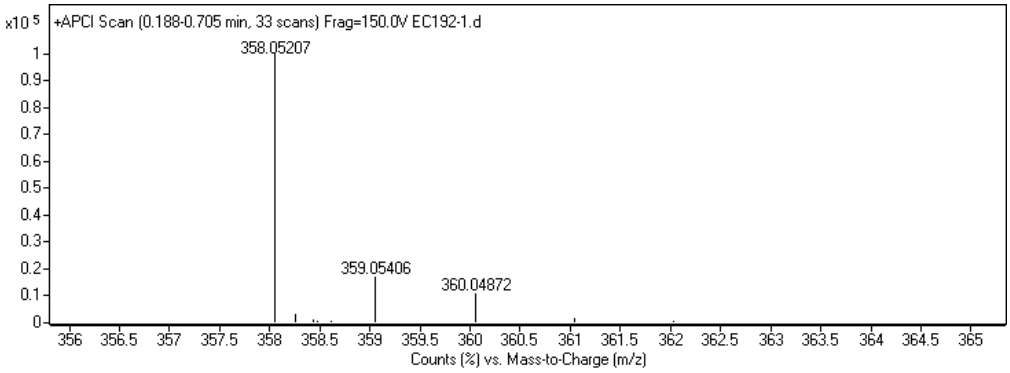


Figure 7.60. HRMS spectrum of compound **7m**

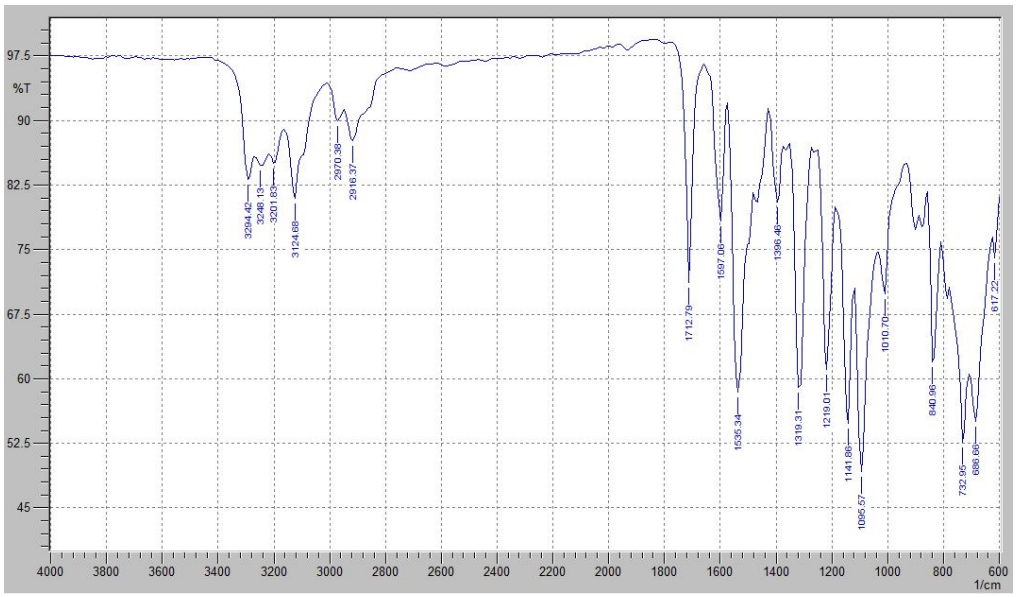


Figure 7.61. IR spectrum of compound **7n**

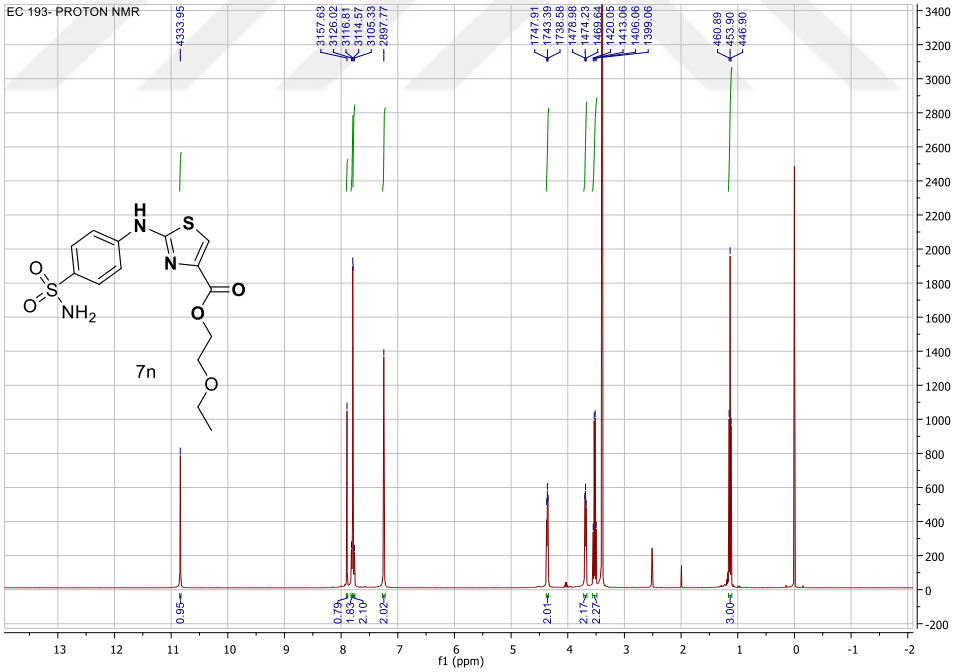


Figure 7.62. ^{1}H NMR spectrum of compound **7n**

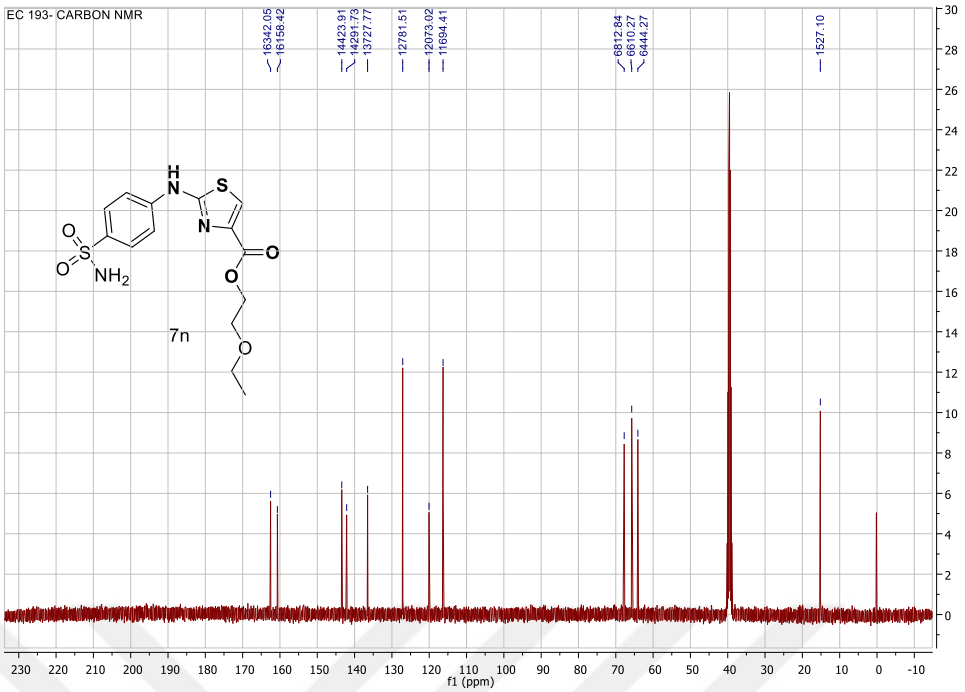


Figure 7.63. ^{13}C NMR spectrum of compound **7n**

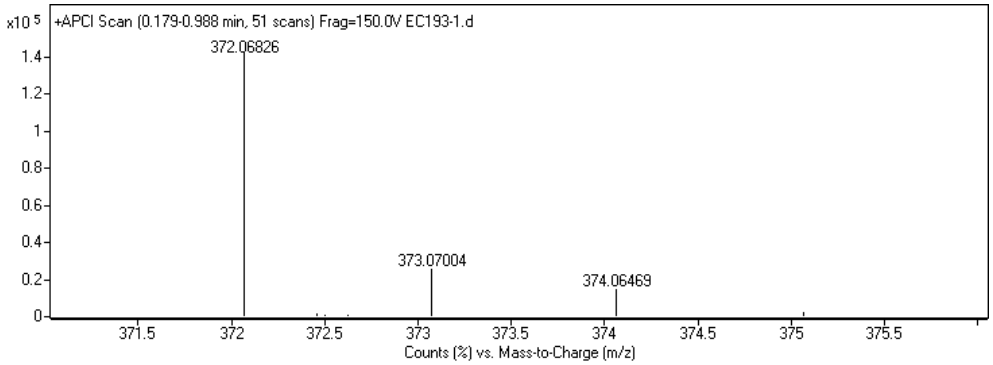


Figure 7.64. HRMS spectrum of compound **7n**

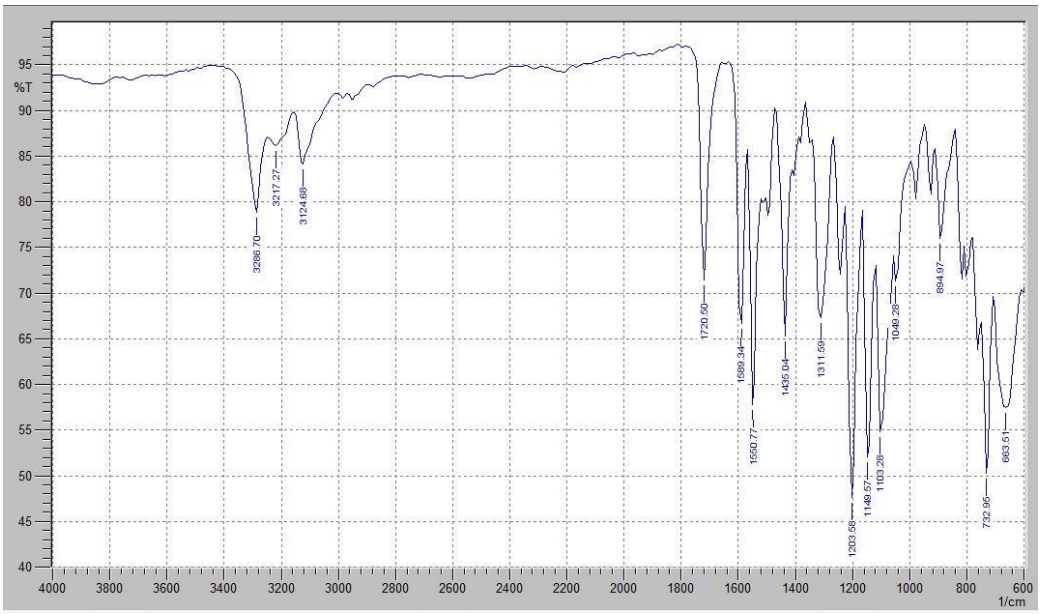


Figure 7.65. IR spectrum of compound **7o**

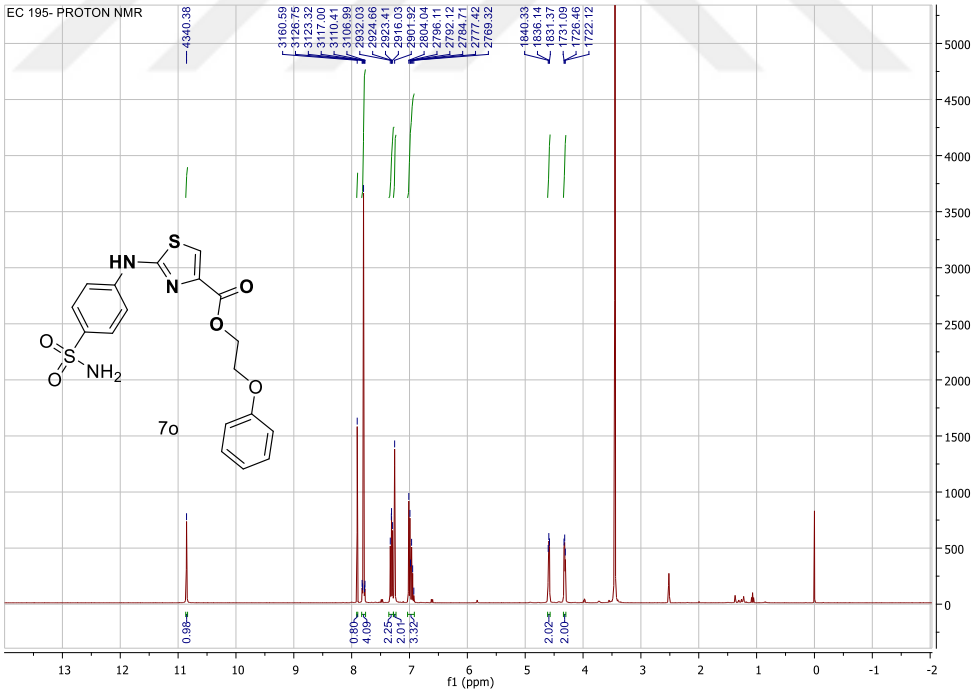


Figure 7.66. ^{1}H NMR spectrum of compound **7o**

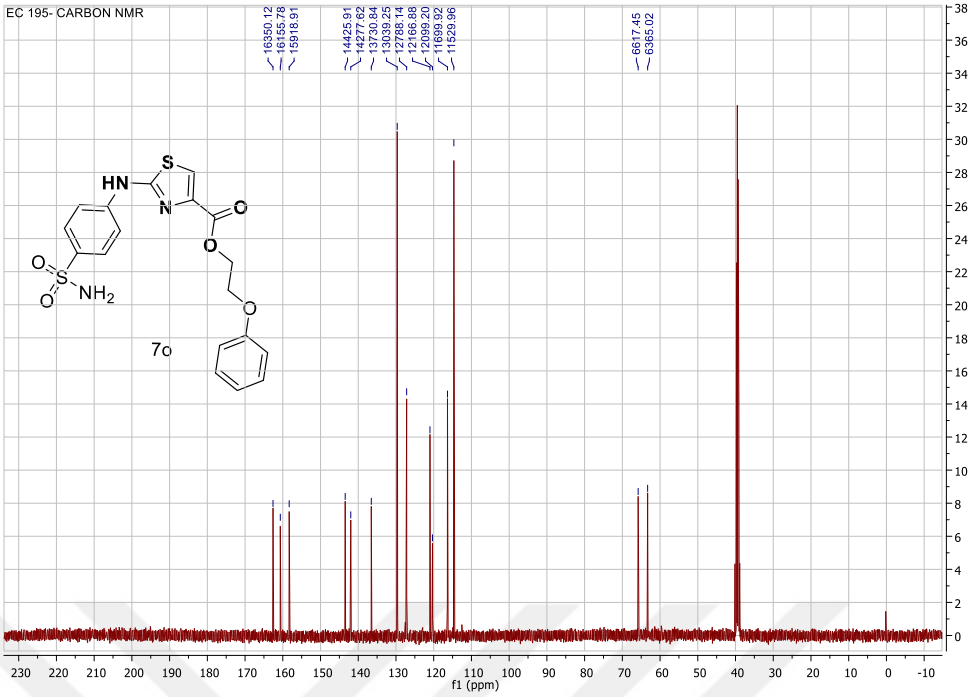


Figure 7.67. ^{13}C NMR spectrum of compound **7o**

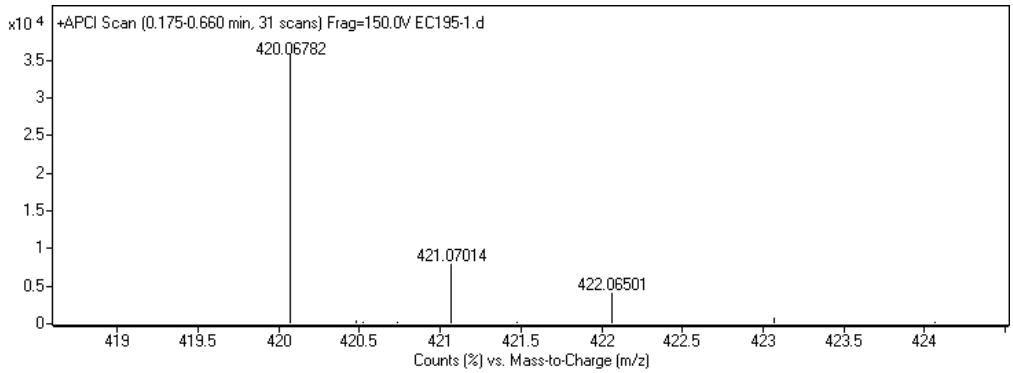


Figure 7.68. HRMS spectrum of compound **7o**

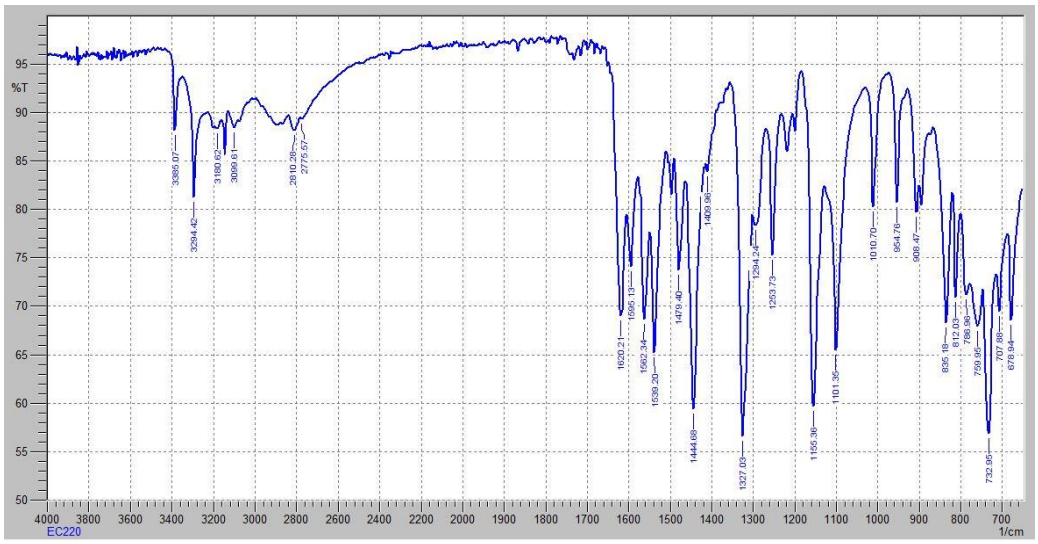


Figure 7.69. IR spectrum of compound **9**

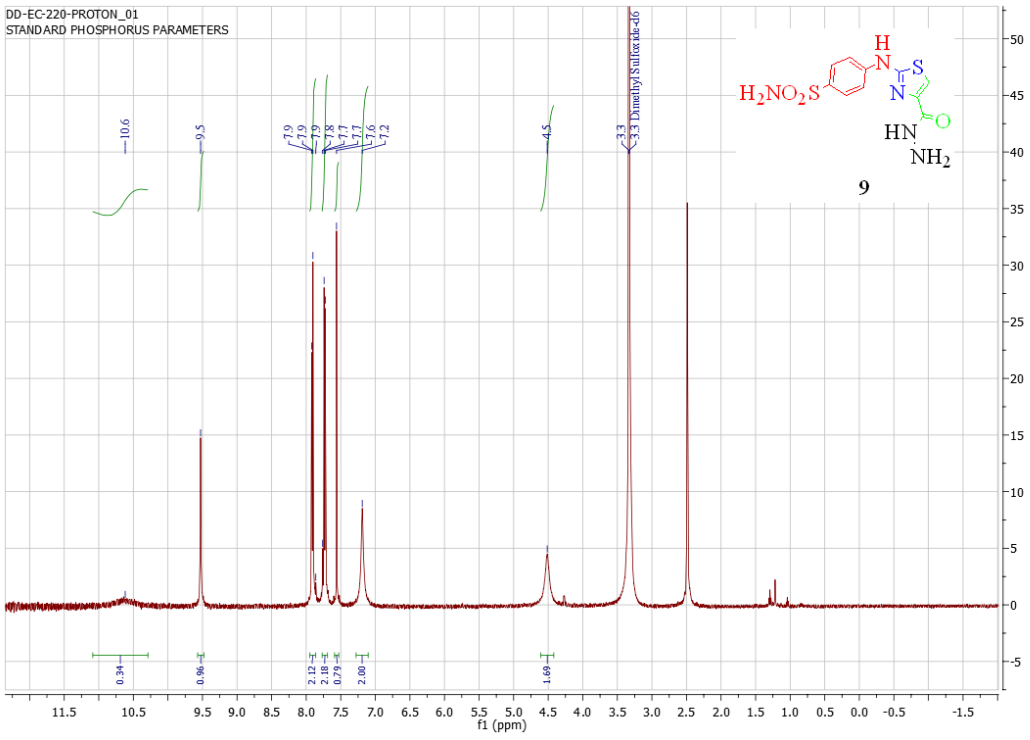


Figure 7.70. ^{1}H NMR spectrum of compound **9**

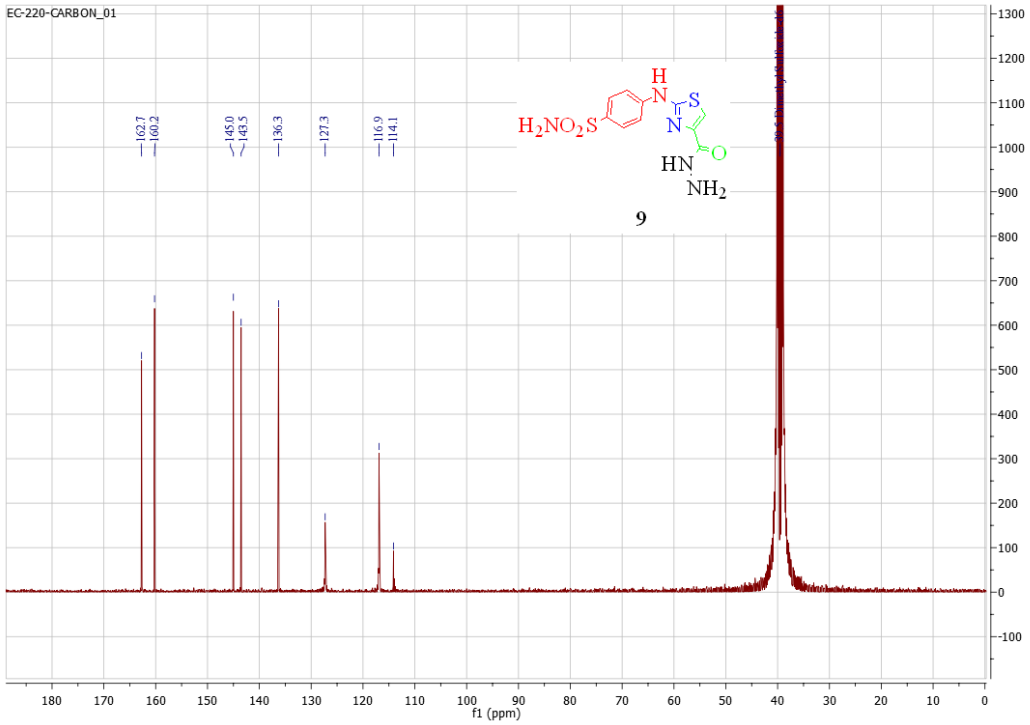


Figure 7.71. ^{13}C NMR spectrum of compound **9**

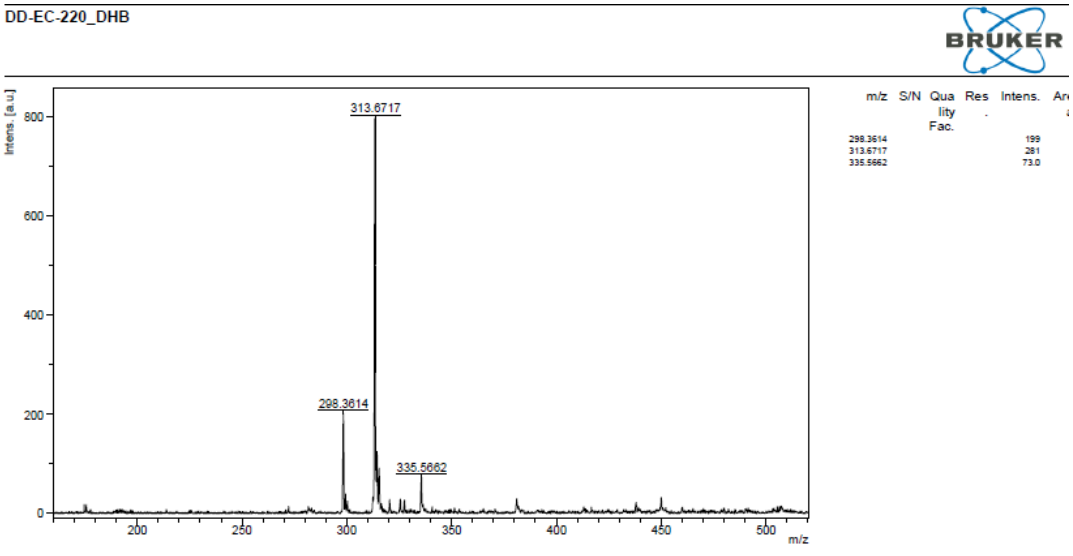


Figure 7.72. MALDI-TOF MS spectrum of compound **9**

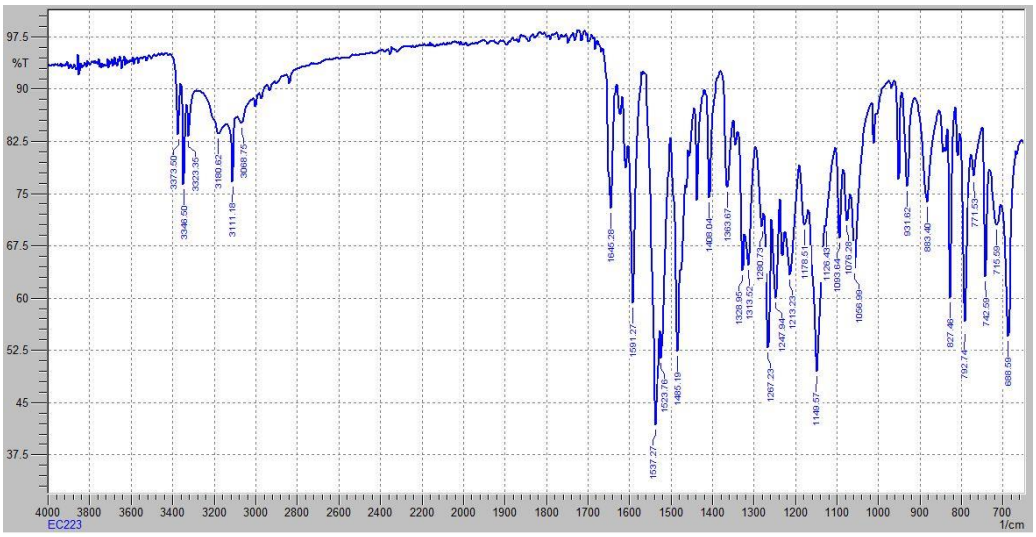


Figure 7.73. IR spectrum of compound **10a**

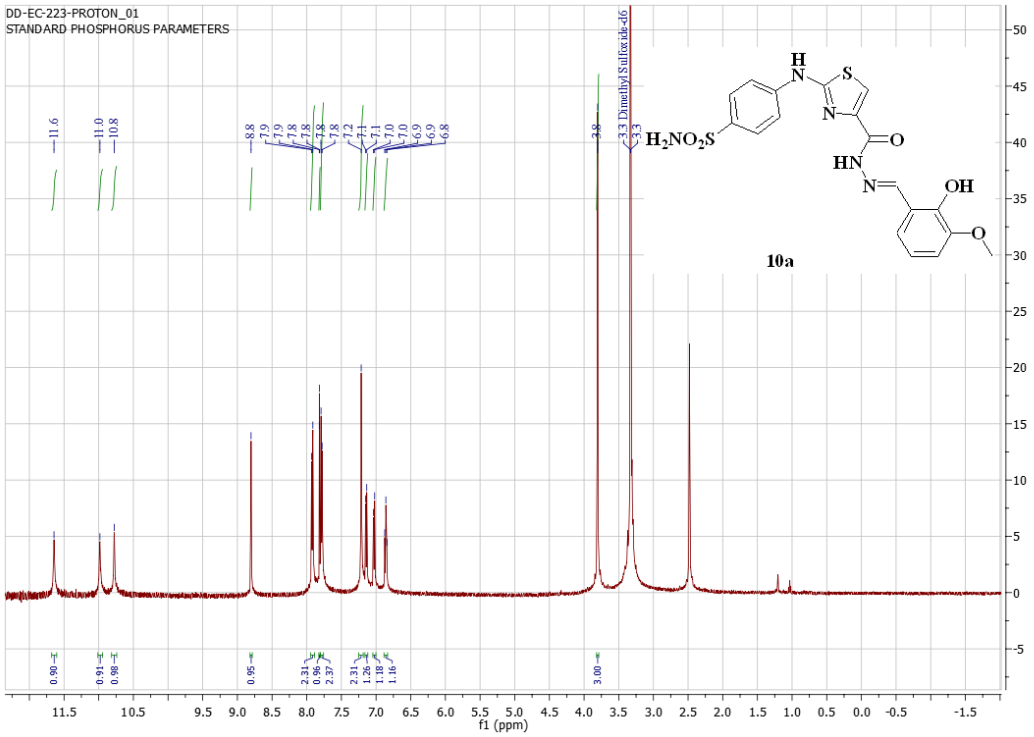


Figure 7.74. ^{1}H NMR spectrum of compound **10a**

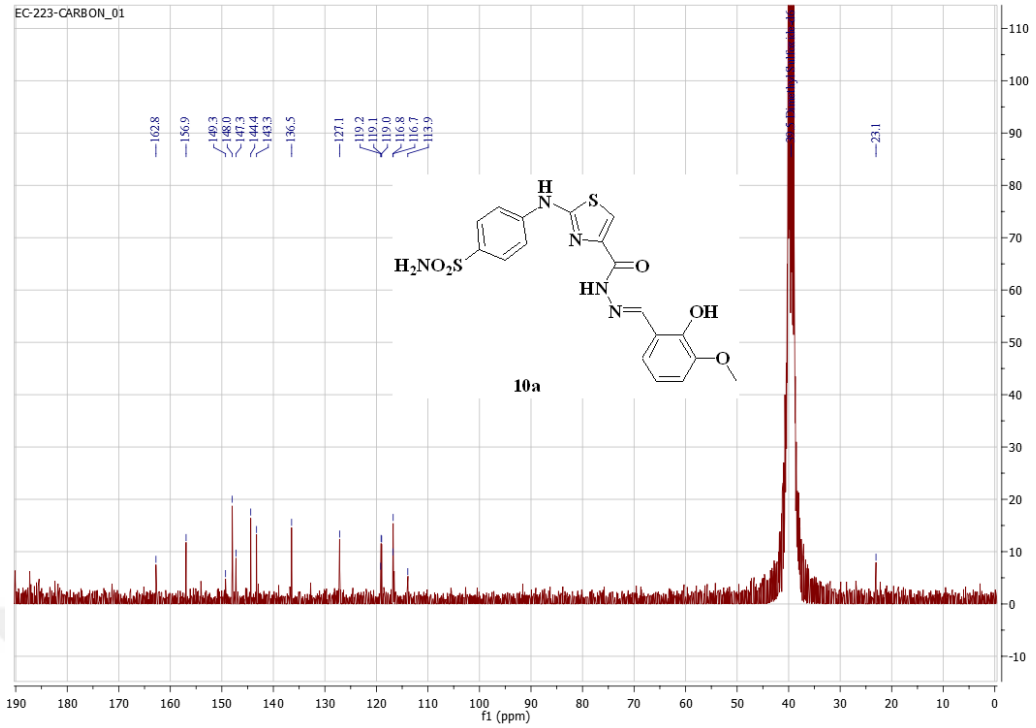


Figure 7.75. ^{13}C NMR spectrum of compound **10a**

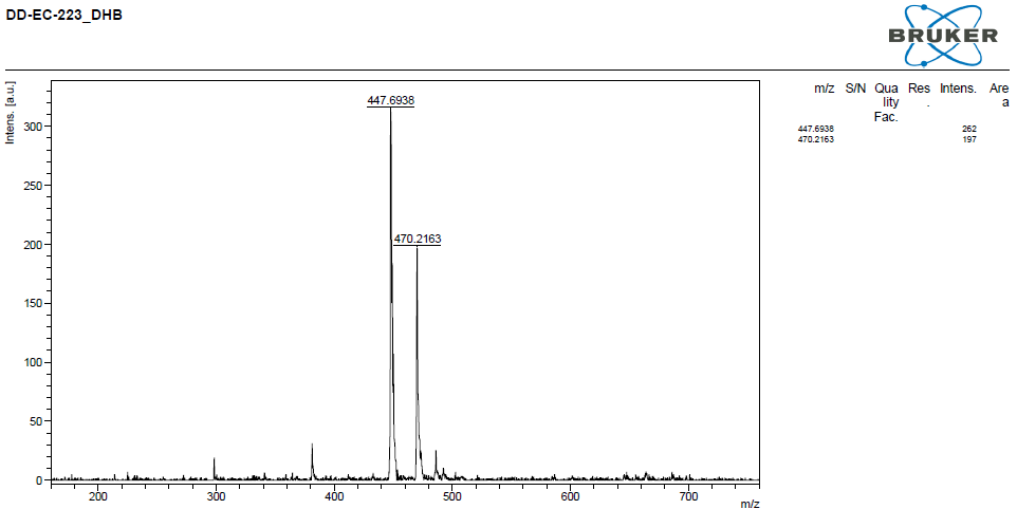


Figure 7.76. MALDI-TOF MS spectrum of compound **10a**

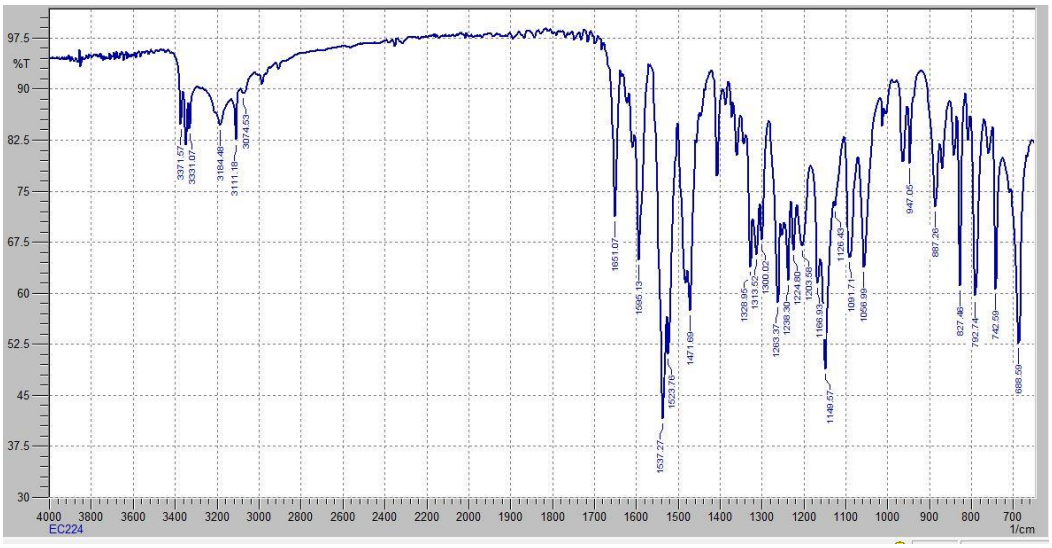


Figure 7.77. IR spectrum of compound **10b**

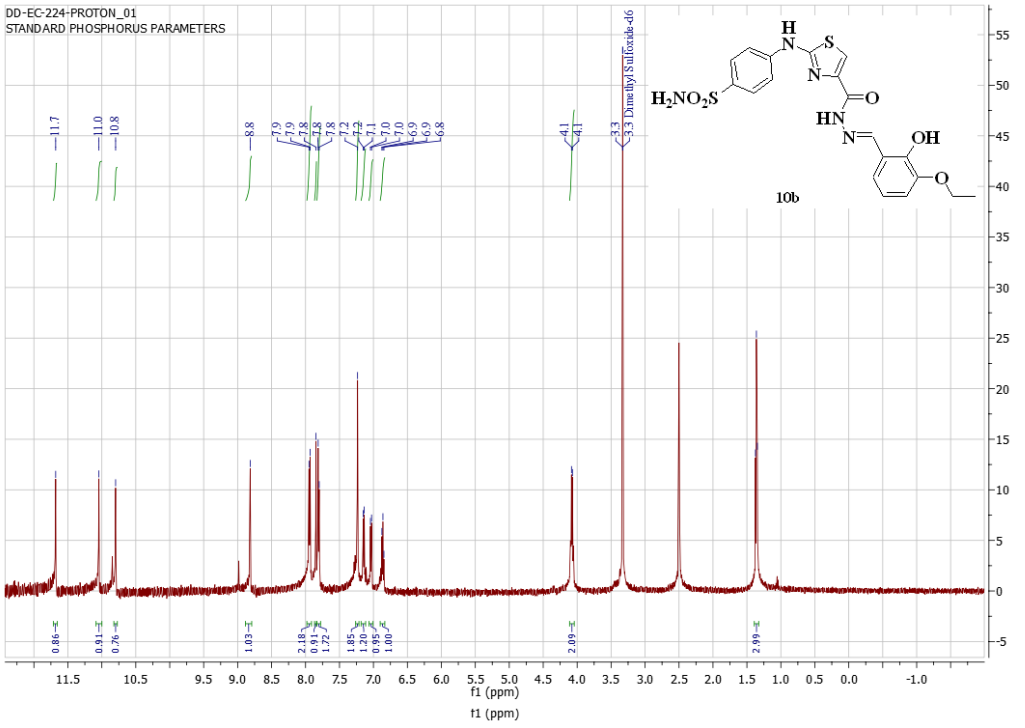


Figure 7.78. ^{1}H NMR spectrum of compound **10b**

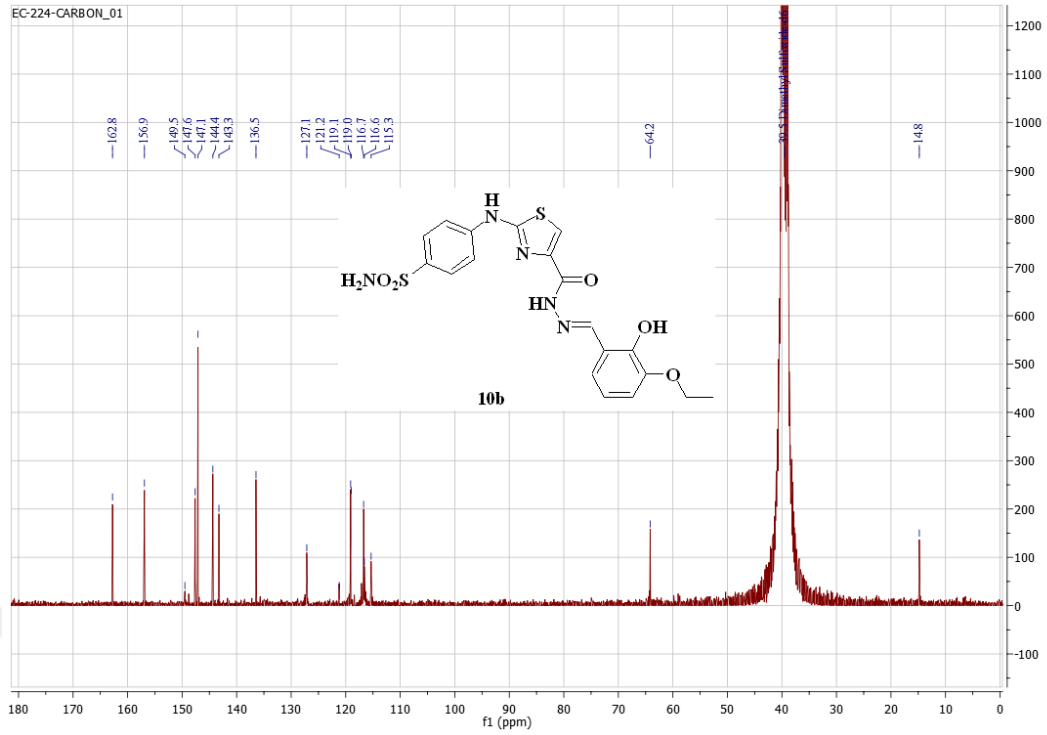


Figure 7.79. ^{13}C NMR spectrum of compound **10b**

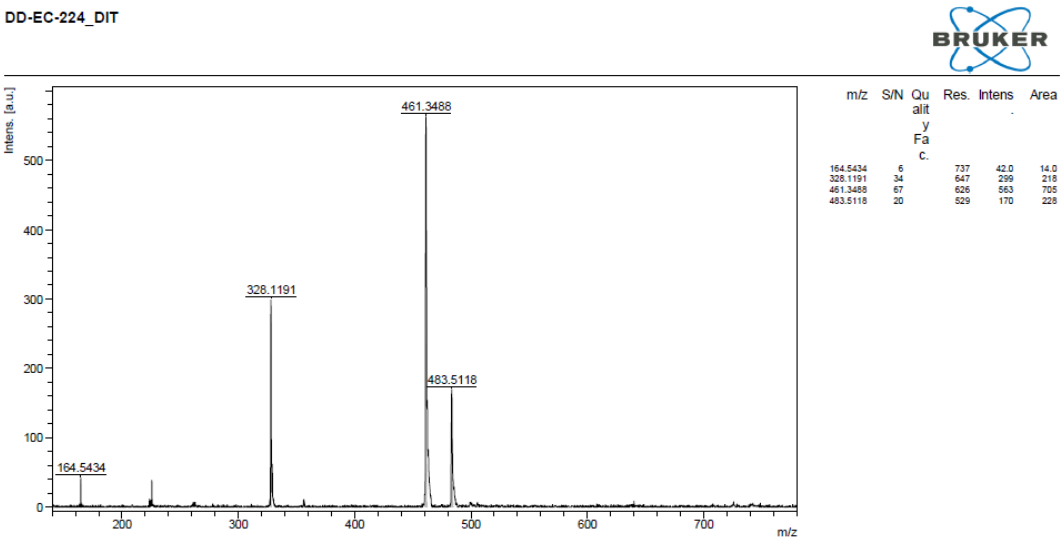


Figure 7.80. MALDI-TOF MS spectrum of compound **10b**

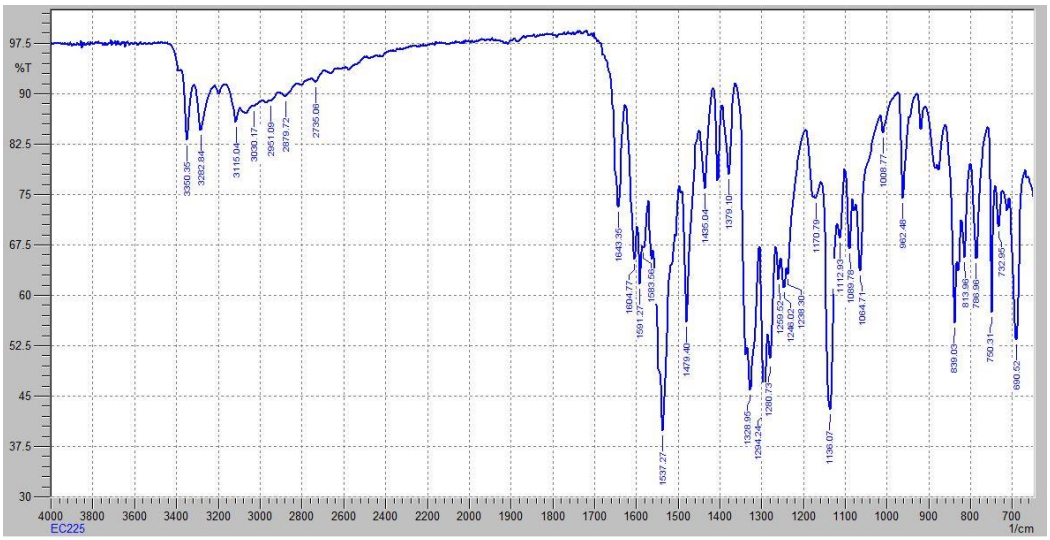


Figure 7.81. IR spectrum of compound **10c**

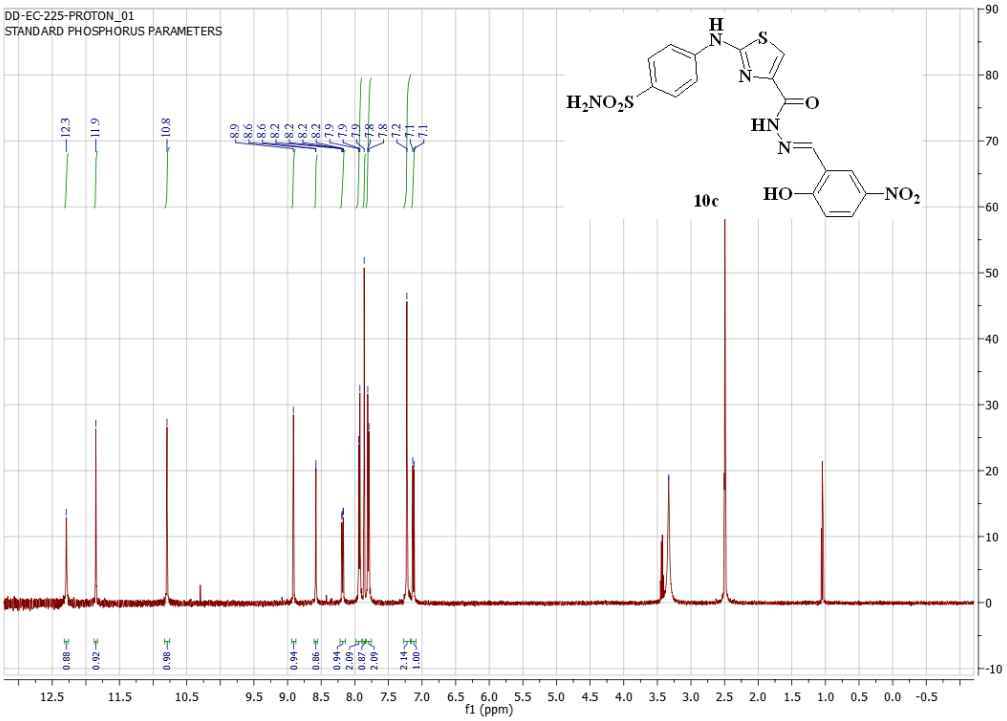


Figure 7.82. ^{1}H NMR spectrum of compound **10c**

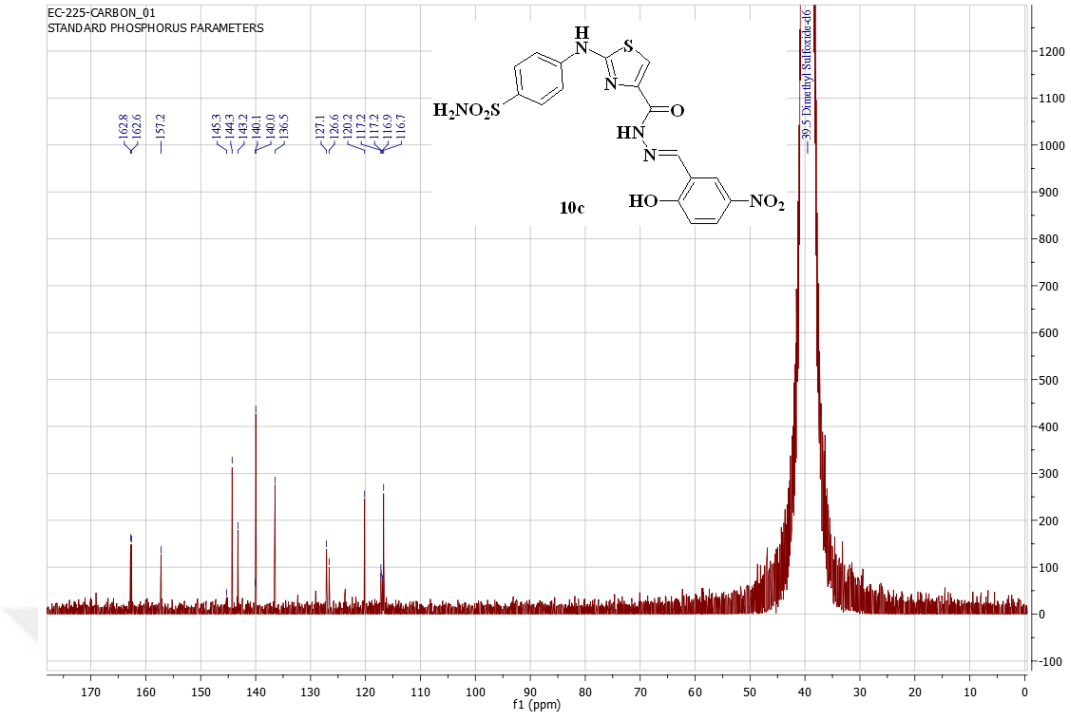


Figure 7.83. ^{13}C NMR spectrum of compound **10c**

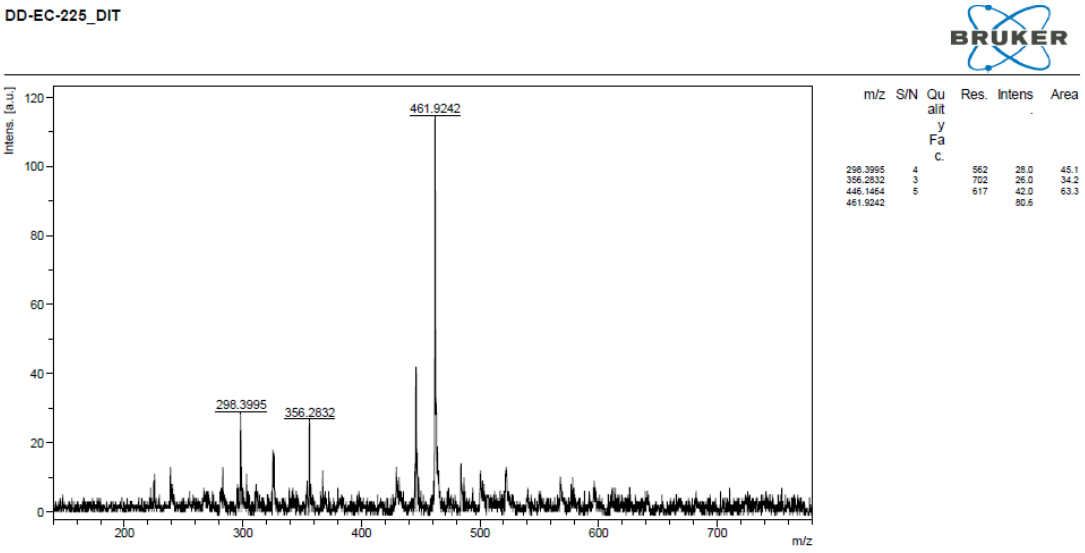


Figure 7.84. MALDI-TOF MS spectrum of compound **10c**

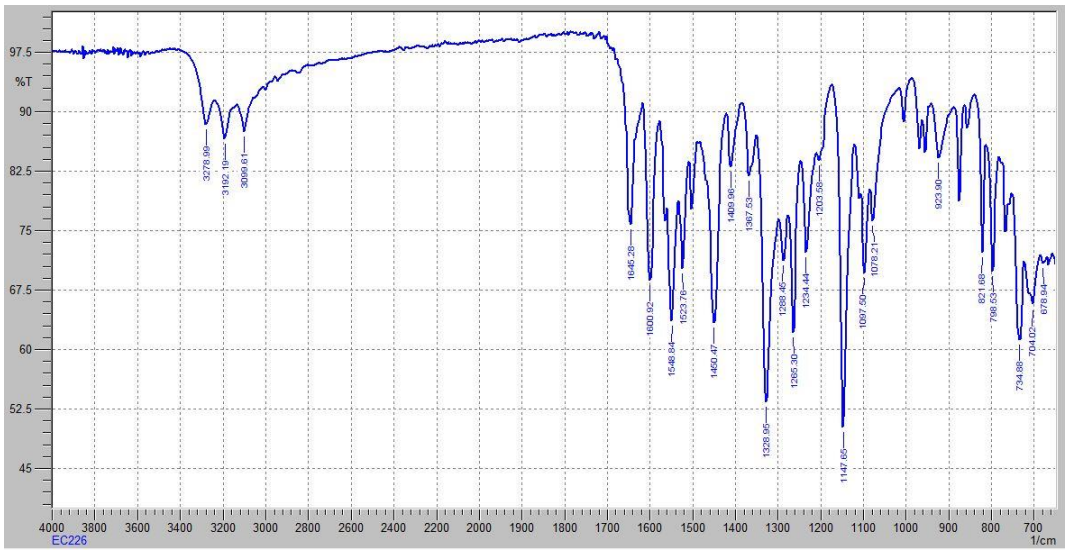


Figure 7.85. IR spectrum of compound **10d**

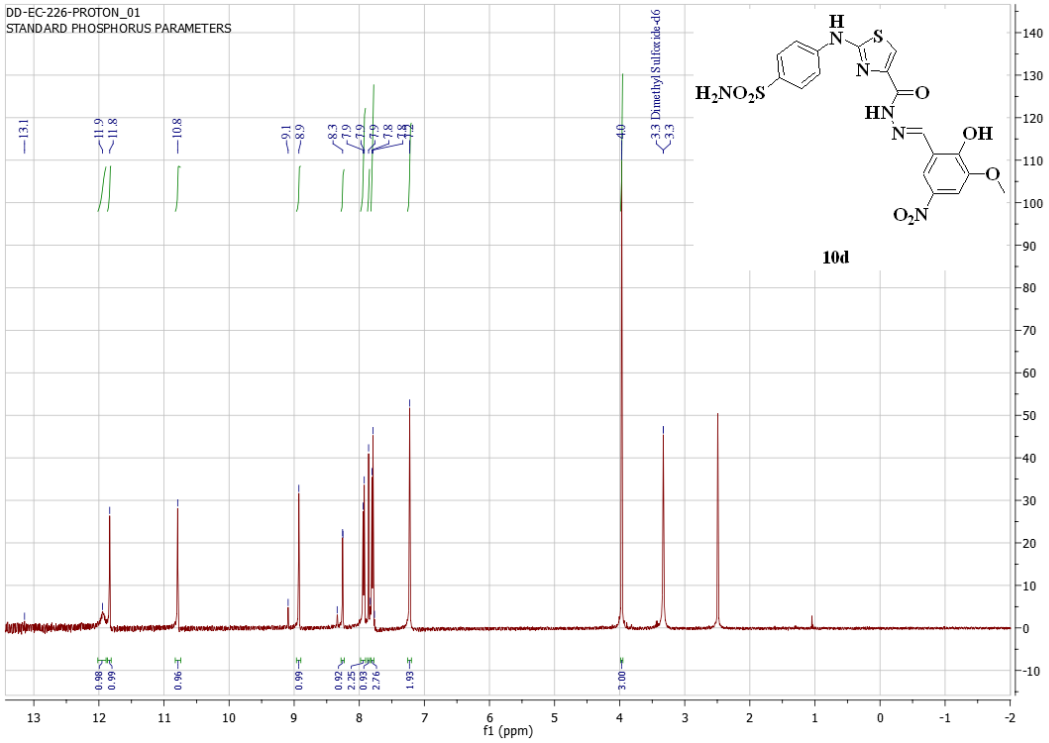


Figure 7.86. ^{1}H NMR spectrum of compound **10d**

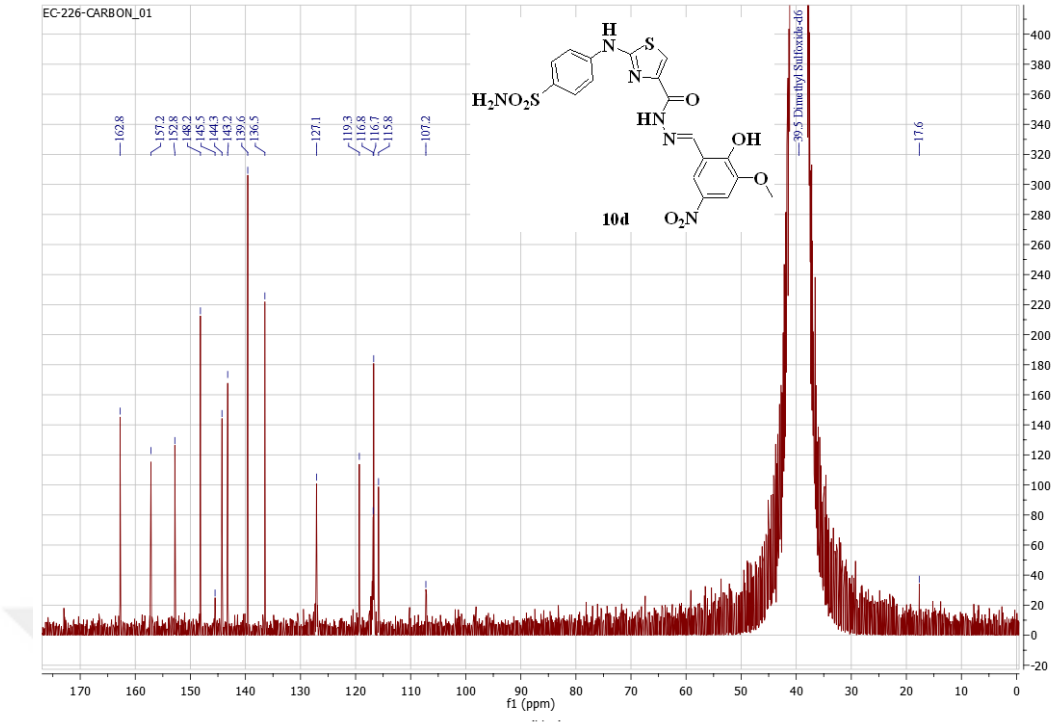


Figure 7.87. ^{13}C NMR spectrum of compound **10d**

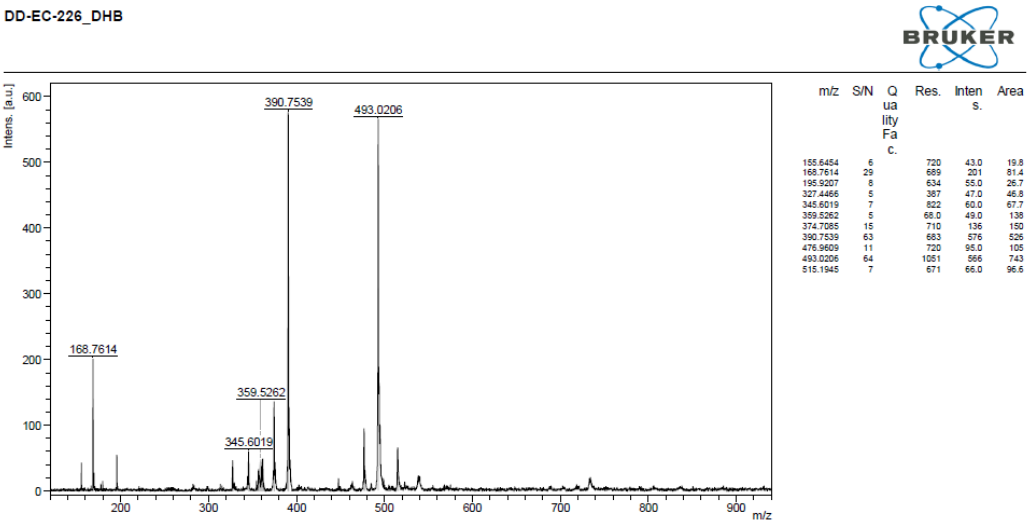


Figure 7.88. MALDI-TOF MS spectrum of compound **10d**

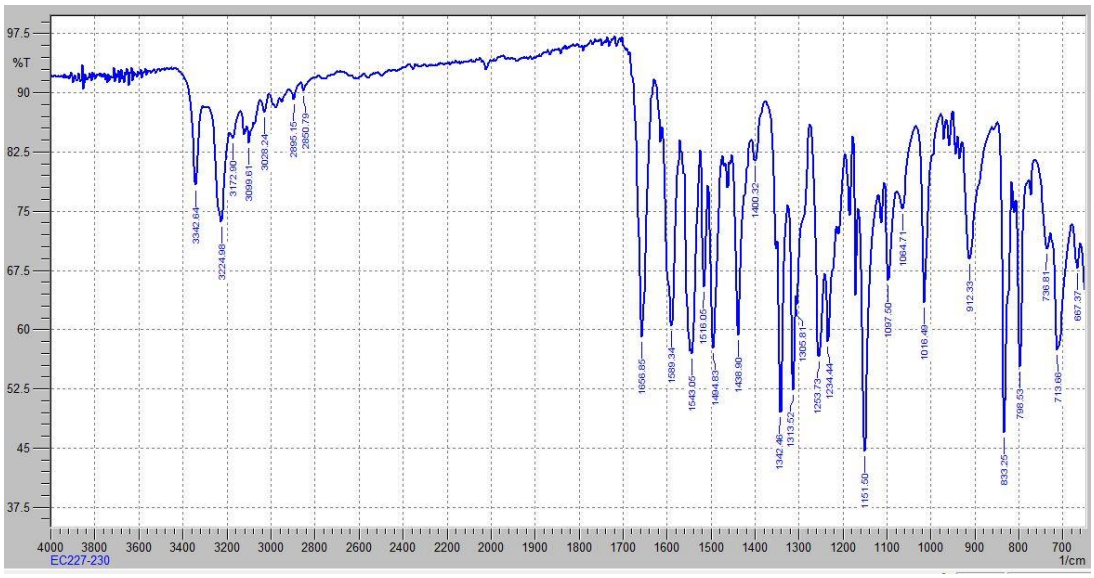


Figure 7.89. IR spectrum of compound **10e**

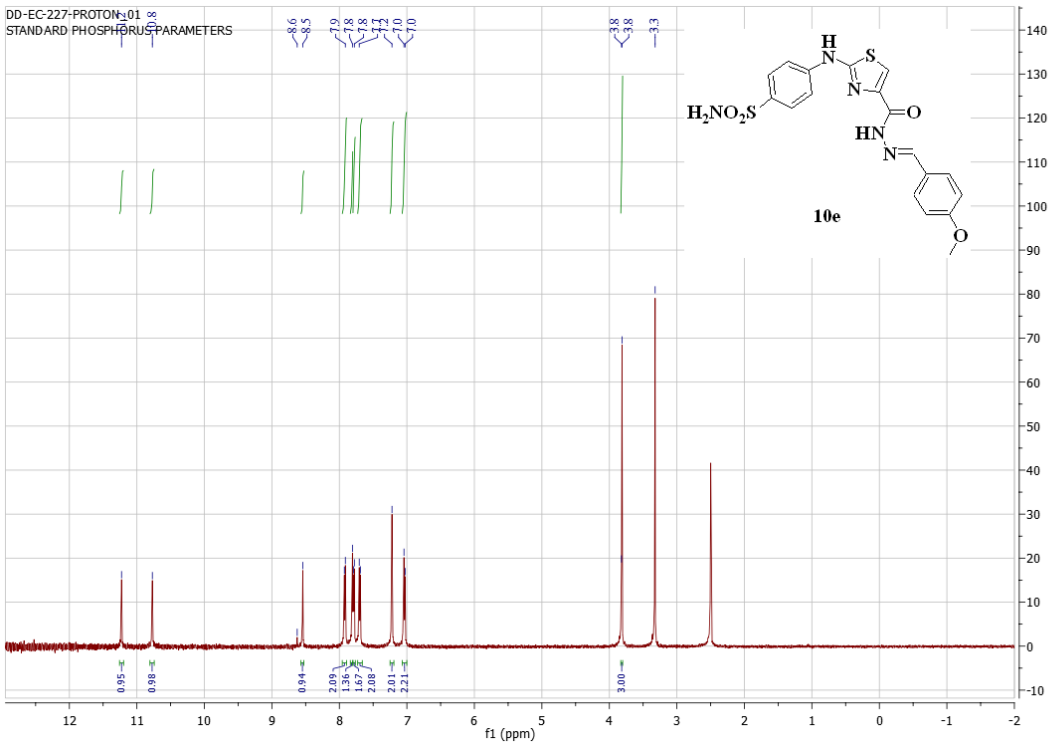


Figure 7.90. ^{1}H NMR spectrum of compound **10e**

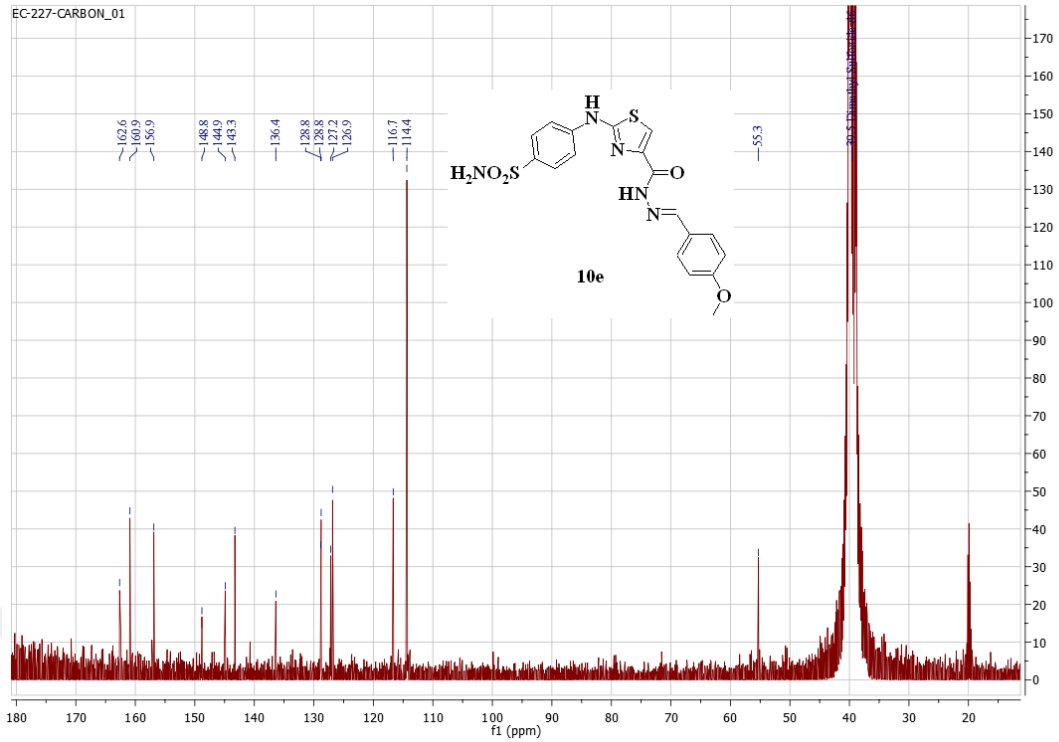


Figure 7.91. ^{13}C NMR spectrum of compound **10e**

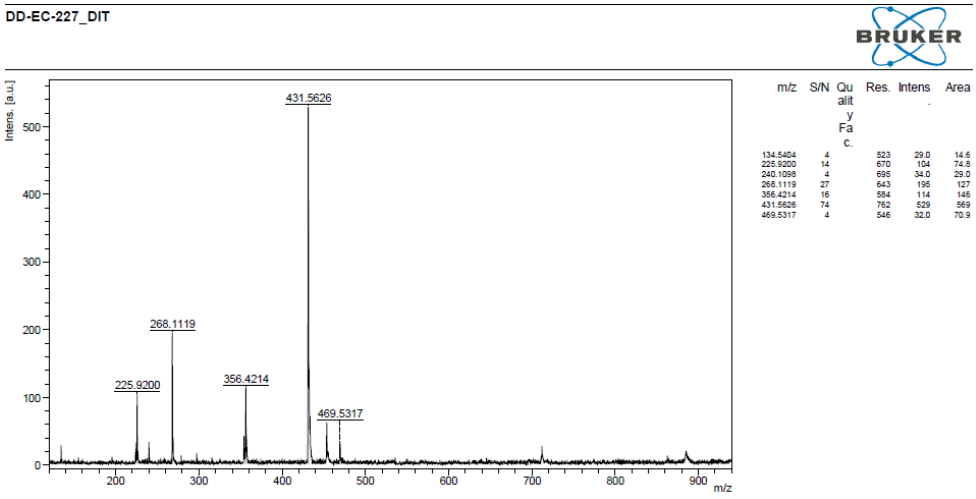


Figure 7.92. MALDI-TOF MS spectrum of compound **10e**

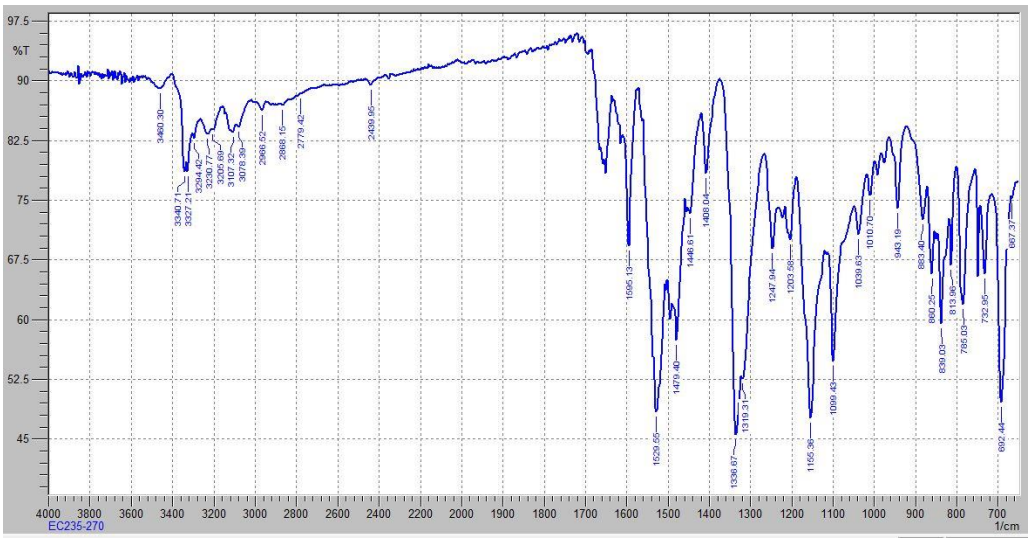


Figure 7.93. IR spectrum of compound **10f**

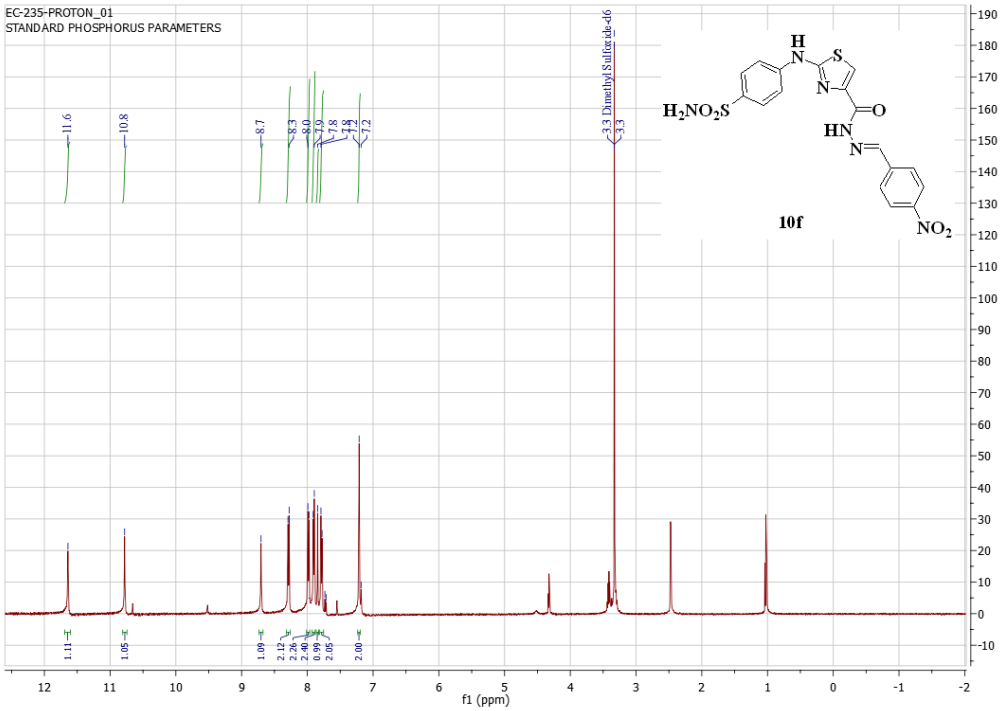


Figure 7.94. ^{1}H NMR spectrum of compound **10f**

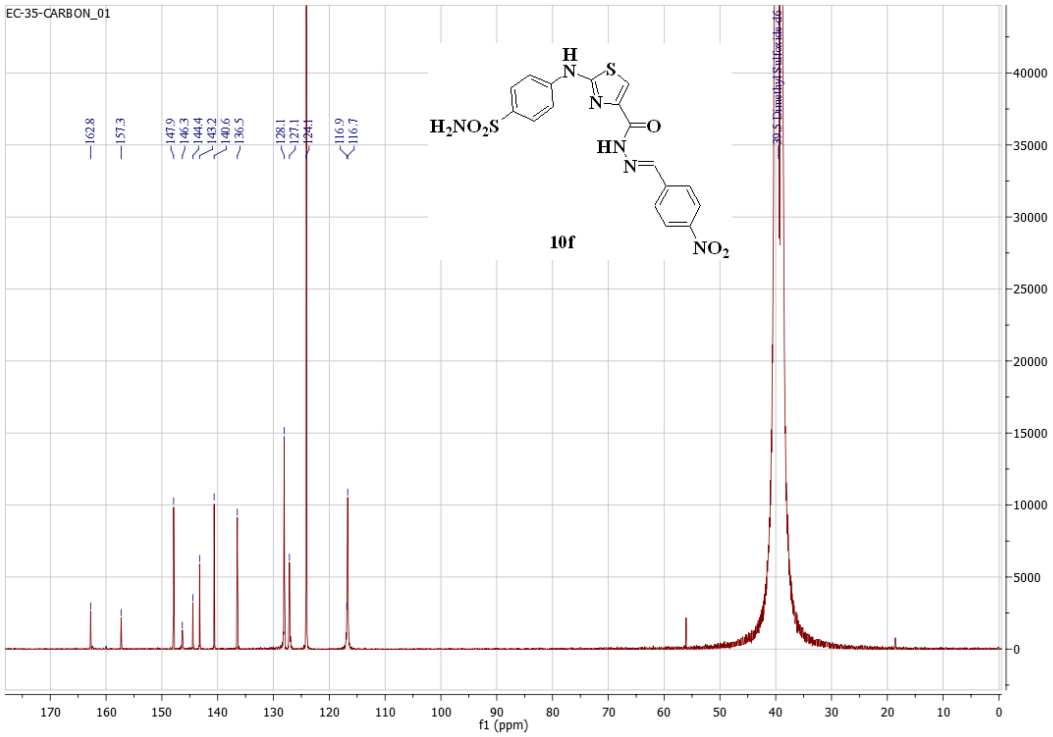


Figure 7.95. ^{13}C NMR spectrum of compound **10f**

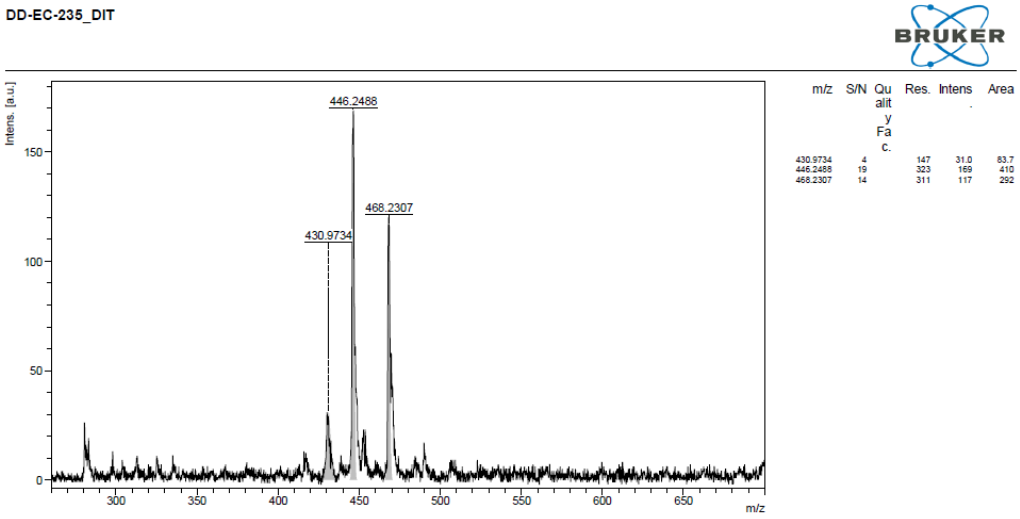


Figure 7.96. MALDI-TOF MS spectrum of compound **10f**

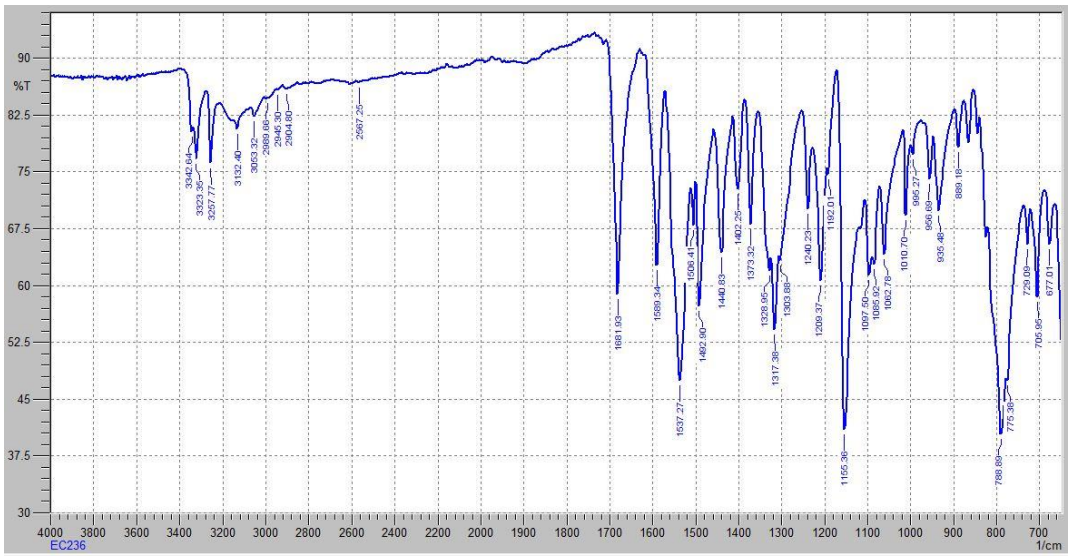


Figure 7.97. IR spectrum of compound **10g**

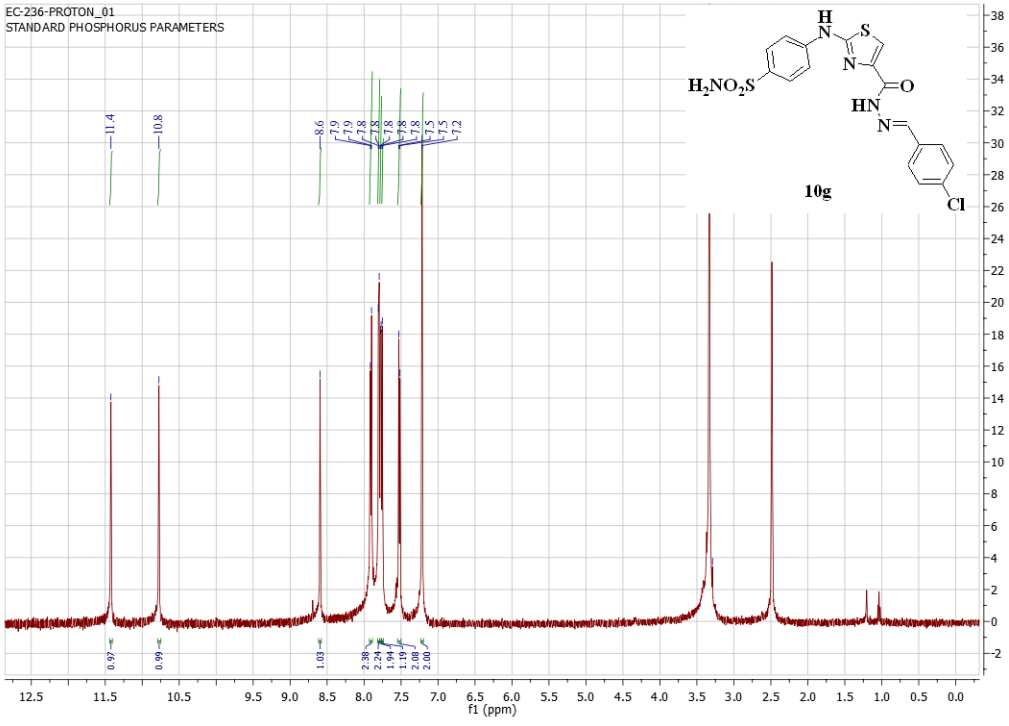


Figure 7.98. ^{1}H NMR spectrum of compound **10g**

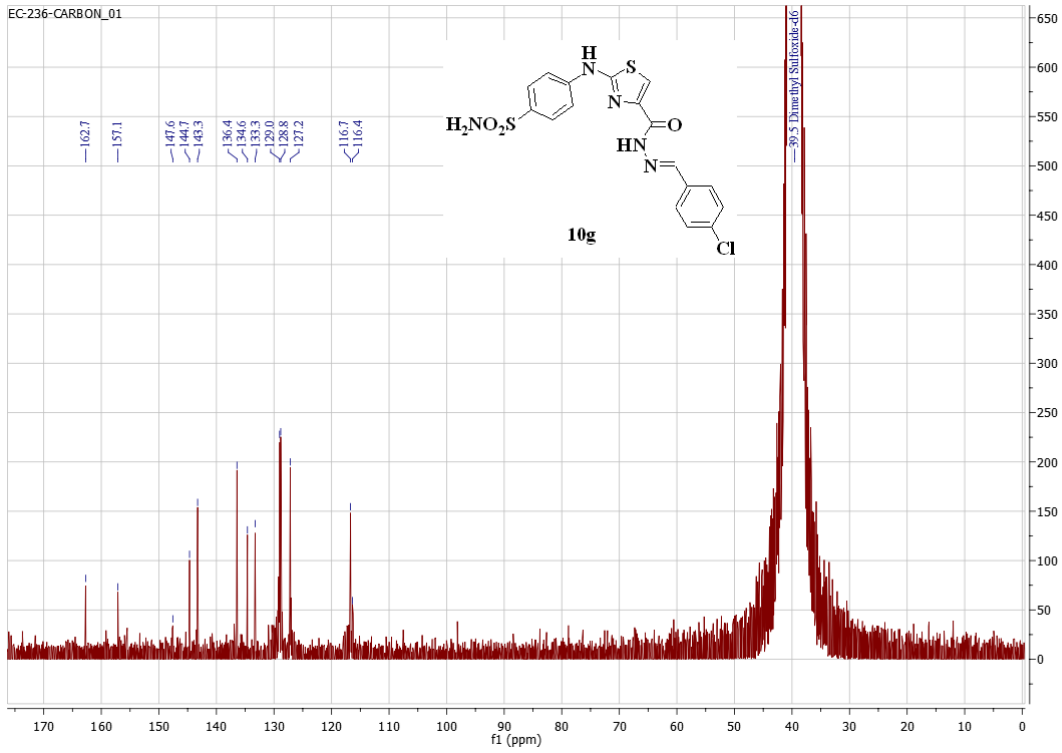


Figure 7.99. ^{13}C NMR spectrum of compound **10g**

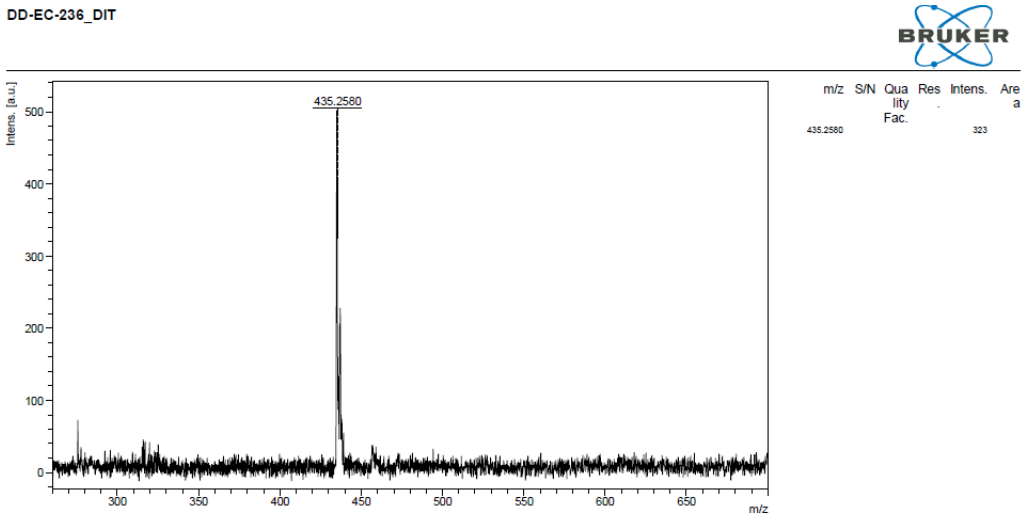


Figure 7.100. MALDI-TOF MS spectrum of compound **10g**

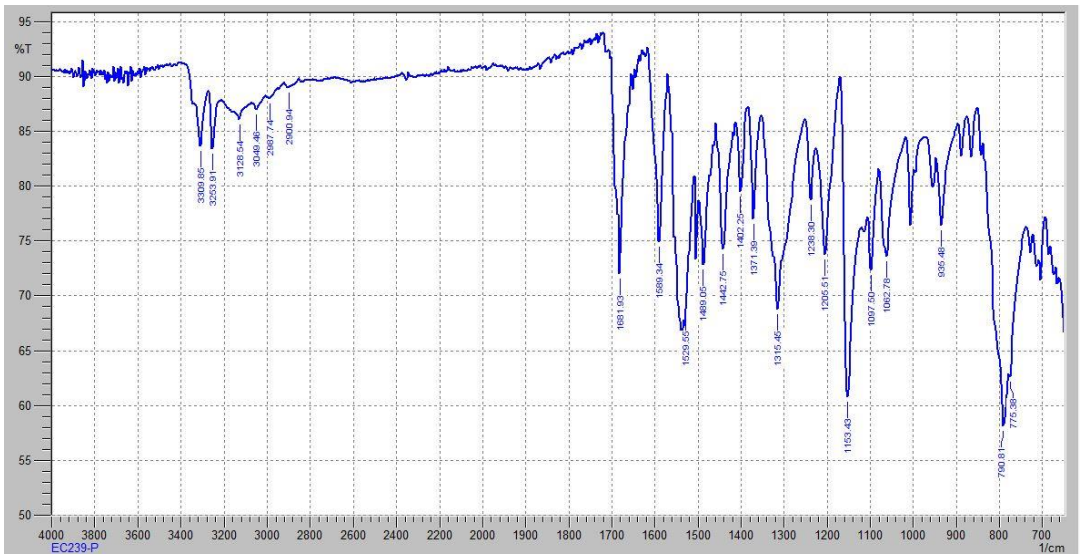


Figure 7.101. IR spectrum of compound **10h**

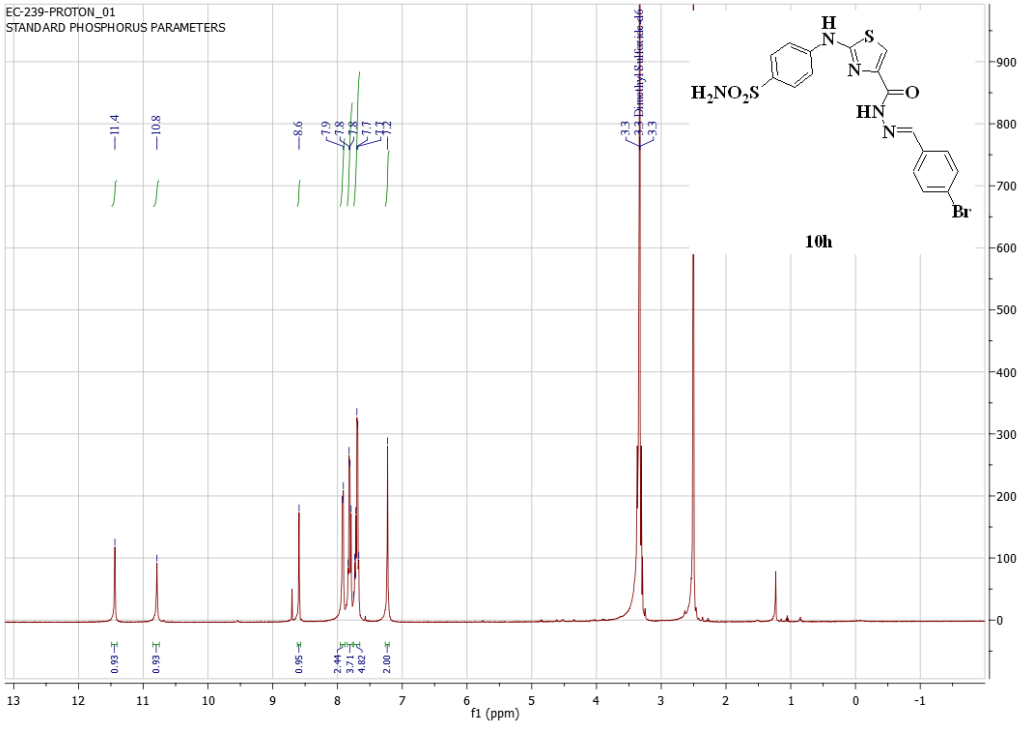


Figure 7.102. ^{1}H NMR spectrum of compound **10h**

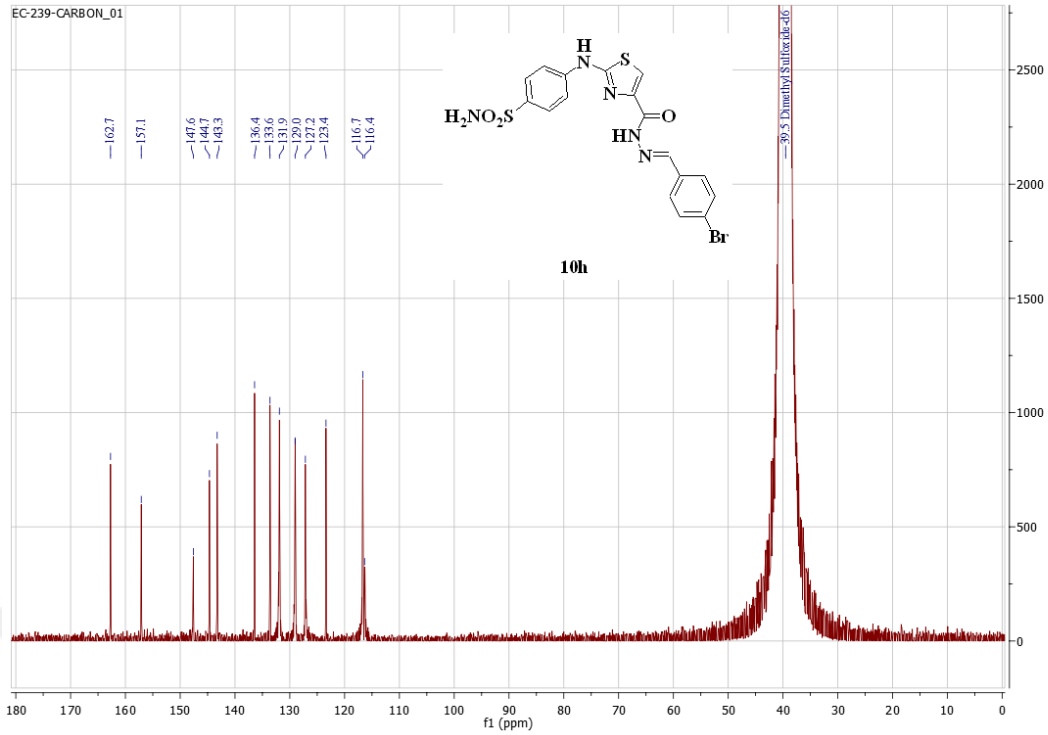


Figure 7.103. ^{13}C NMR spectrum of compound **10h**

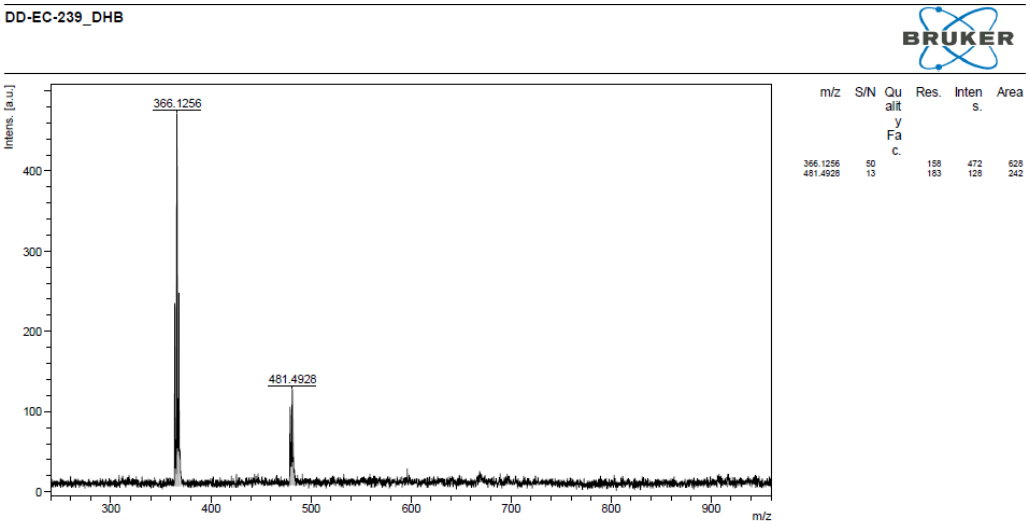


Figure 7.104. MALDI-TOF MS spectrum of compound **10h**

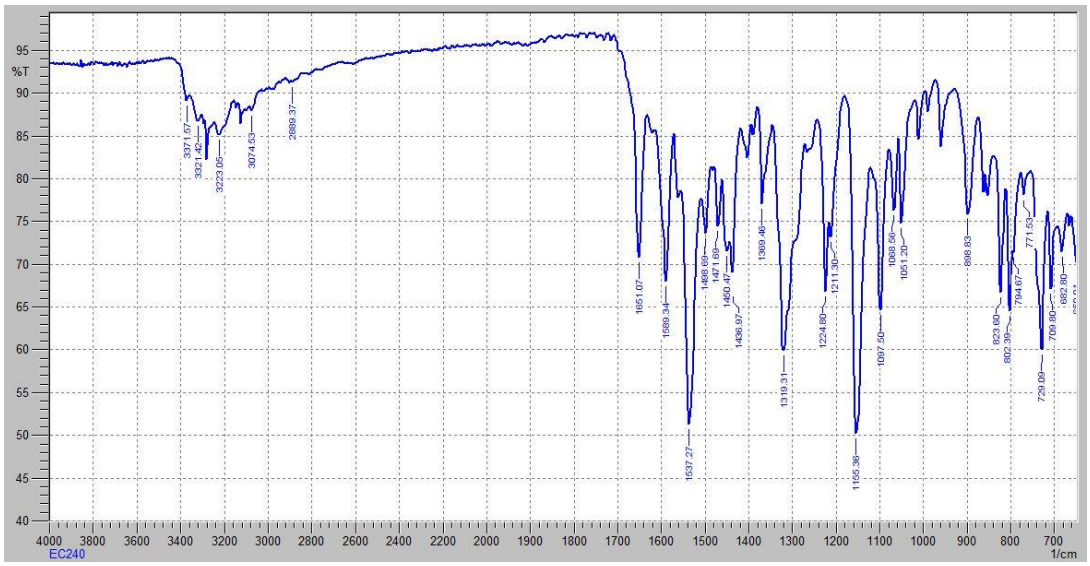


Figure 7.105. IR spectrum of compound **10i**

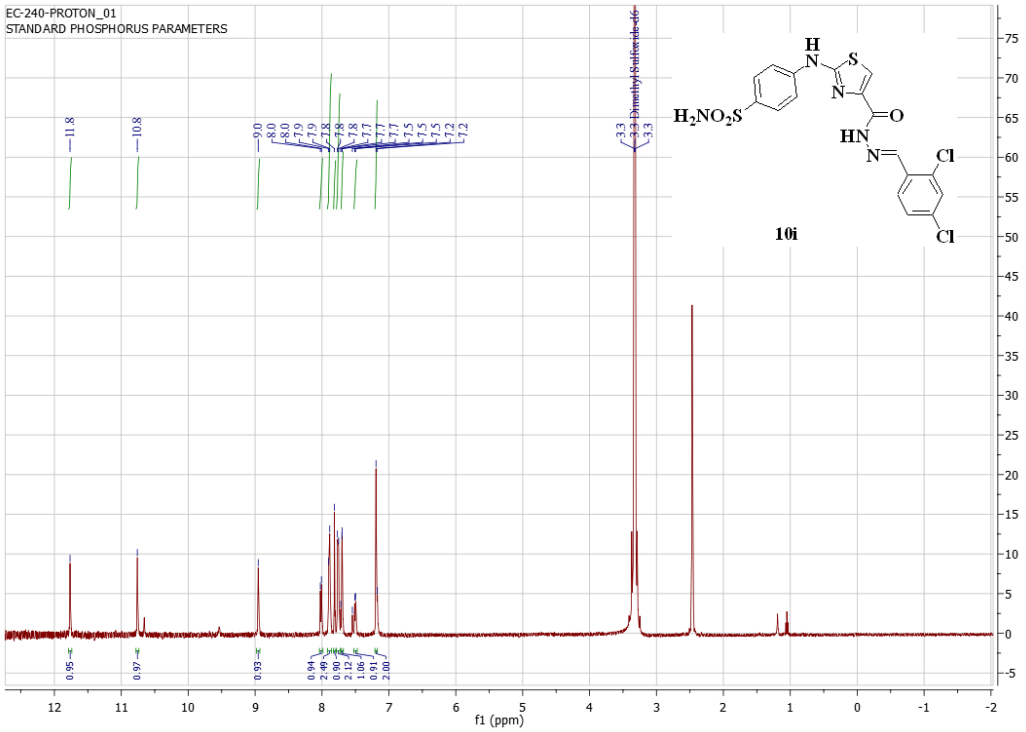


Figure 7.106. ^{1}H NMR spectrum of compound **10i**

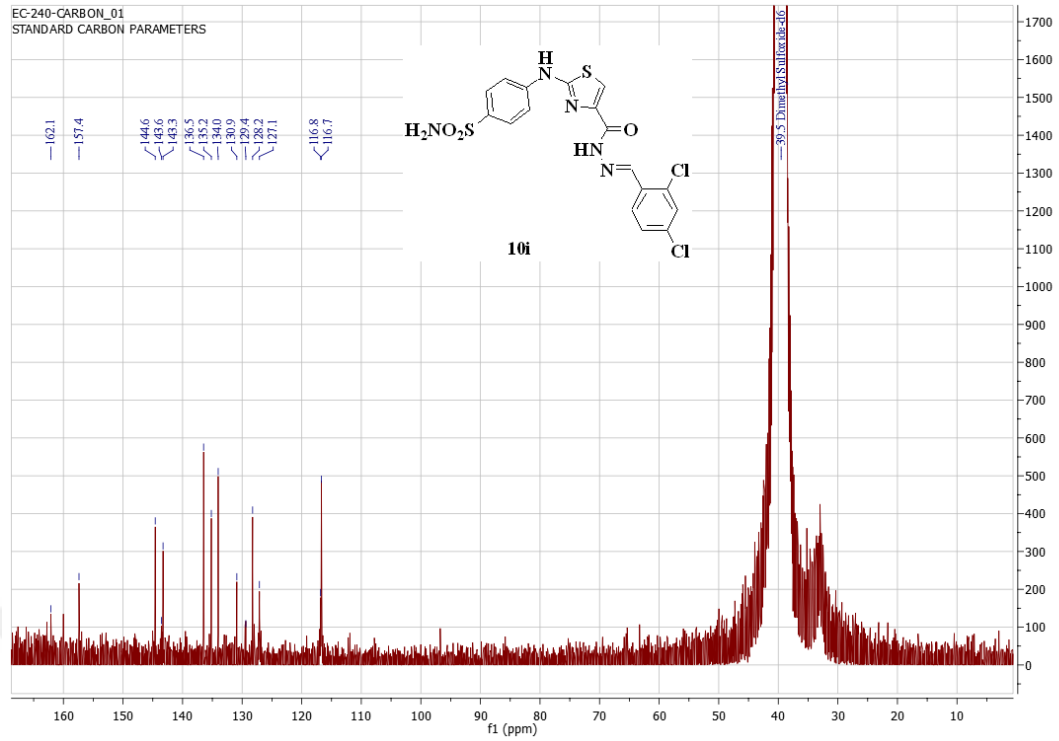


Figure 7.107. ^{13}C NMR spectrum of compound **10i**

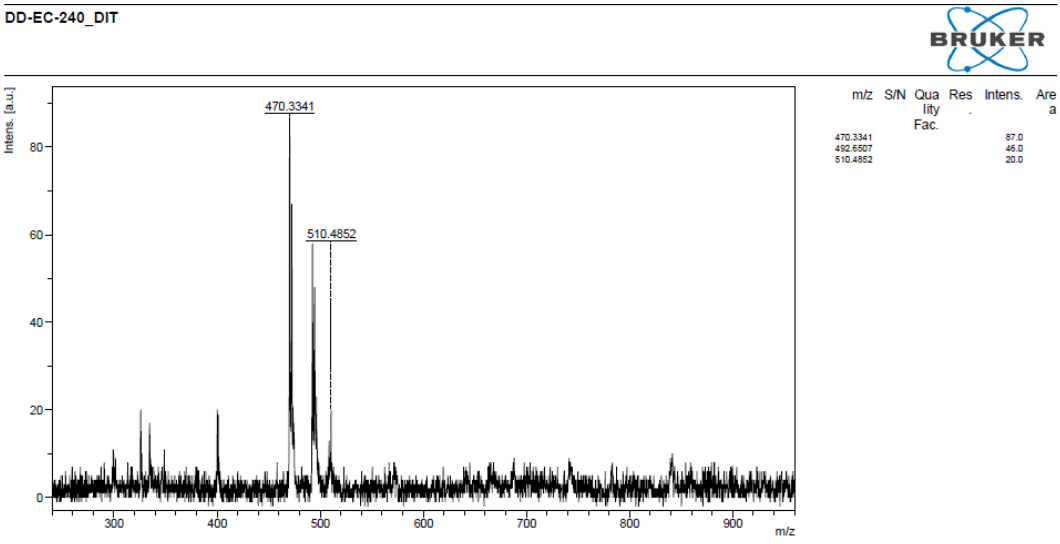


Figure 7.108. MALDI-TOF MS spectrum of compound **10i**

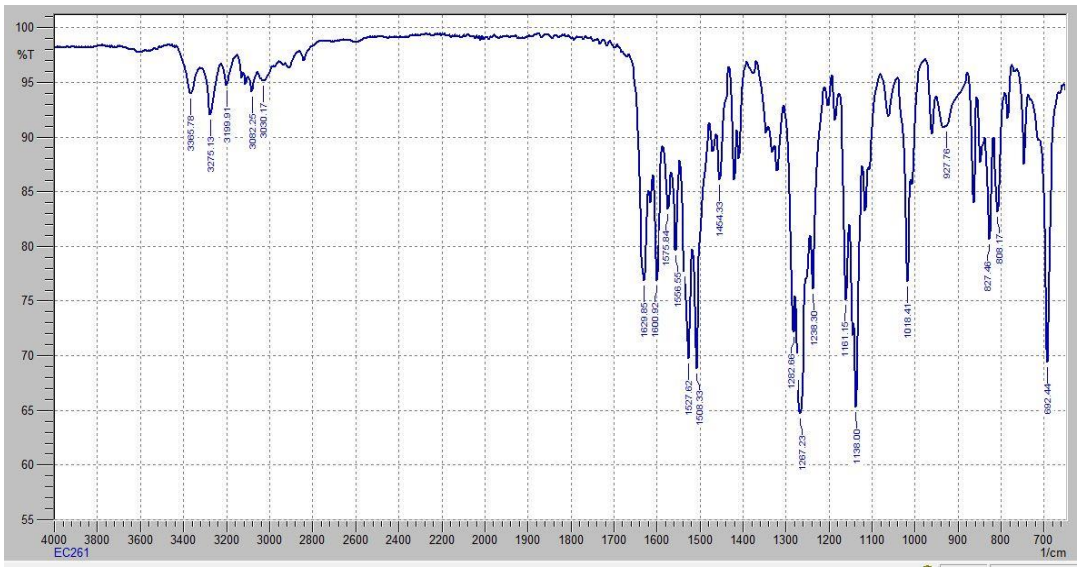


Figure 7.109. IR spectrum of compound **10j**

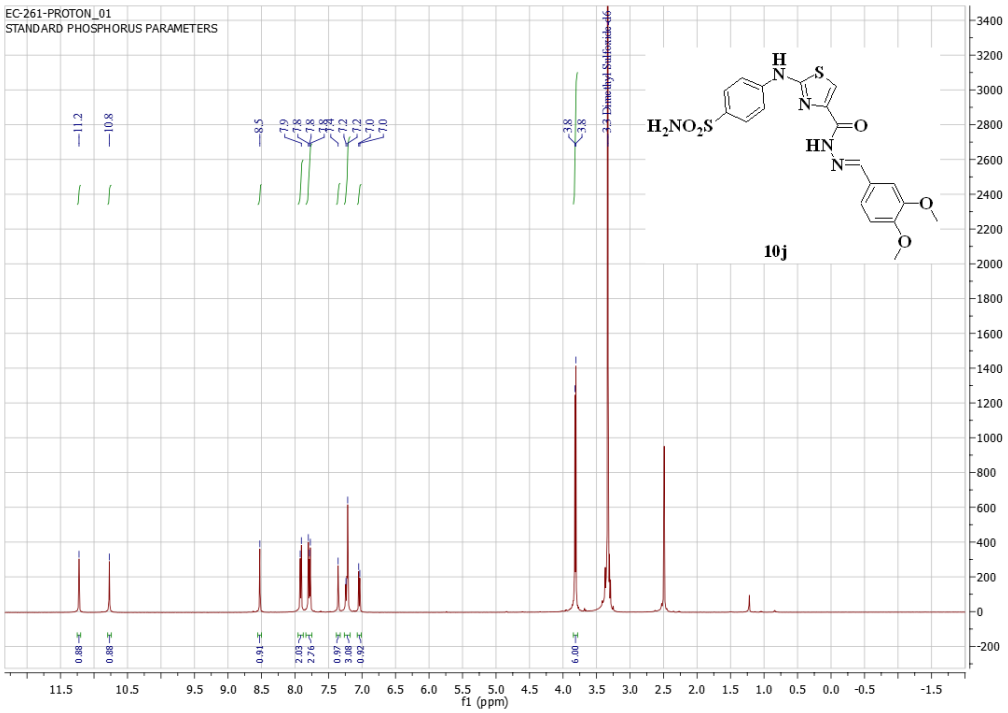


Figure 7.110. ^{1}H NMR spectrum of compound **10j**

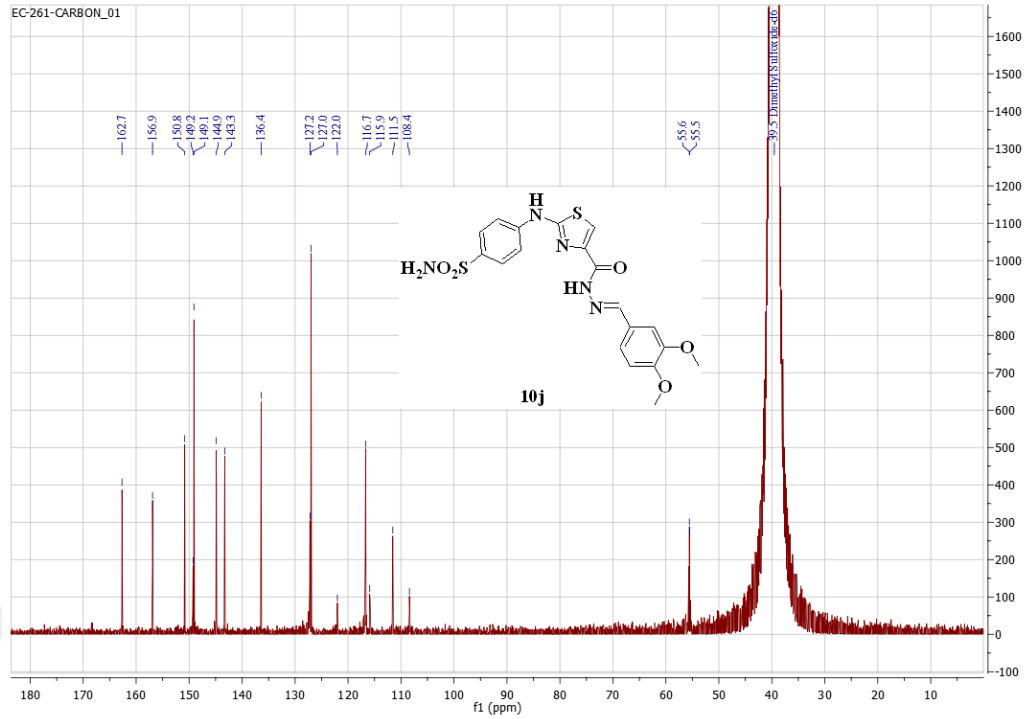


Figure 7.111. ^{13}C NMR spectrum of compound **10j**

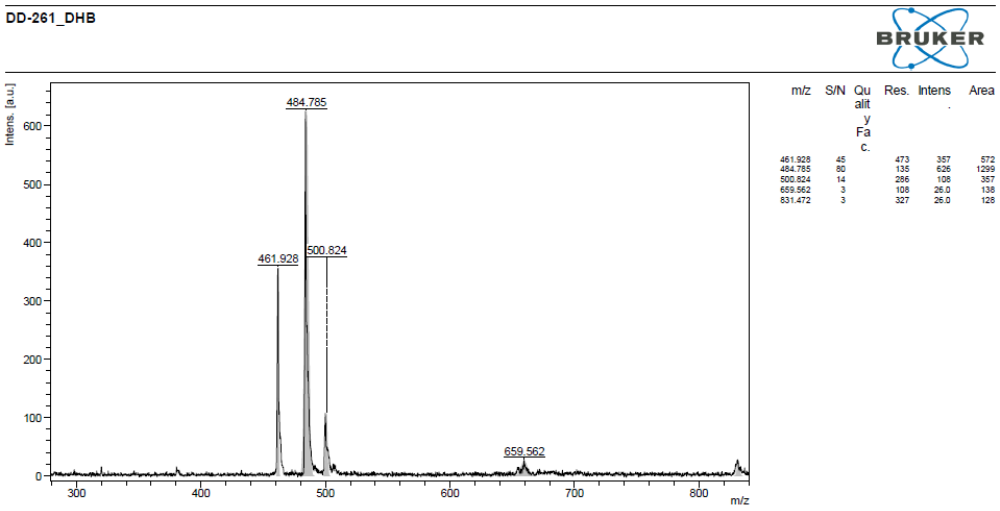


Figure 7.112. MALDI-TOF MS spectrum of compound **10j**

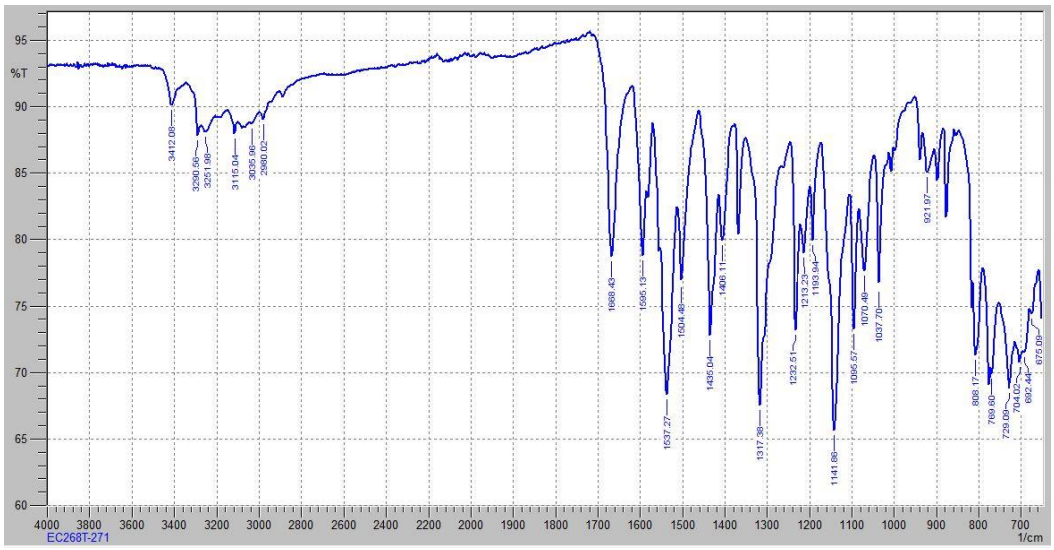


Figure 7.113. IR spectrum of compound **10k**

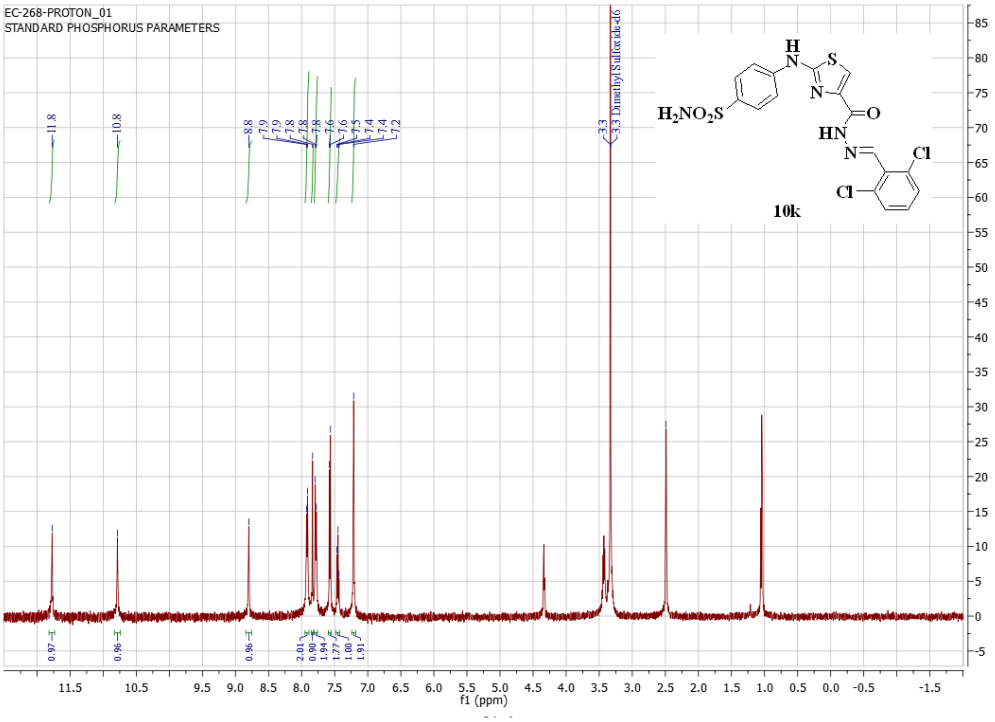


Figure 7.114. ^{1}H NMR spectrum of compound **10k**

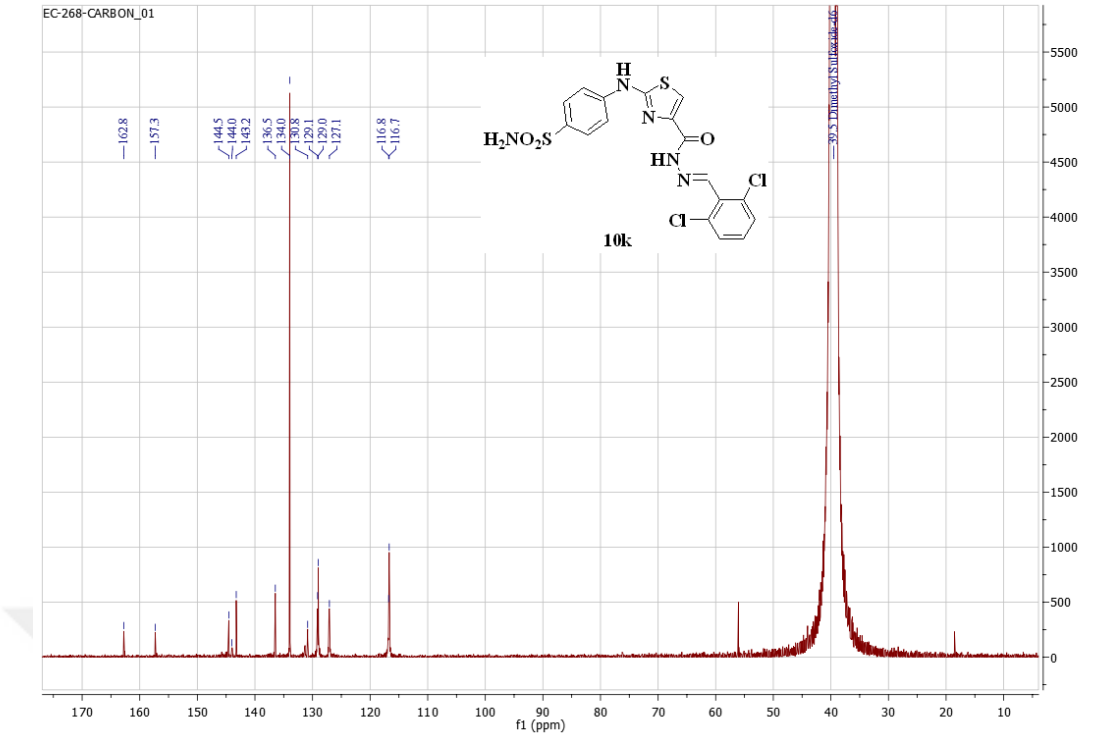


Figure 7.115. ^{13}C NMR spectrum of compound **10k**

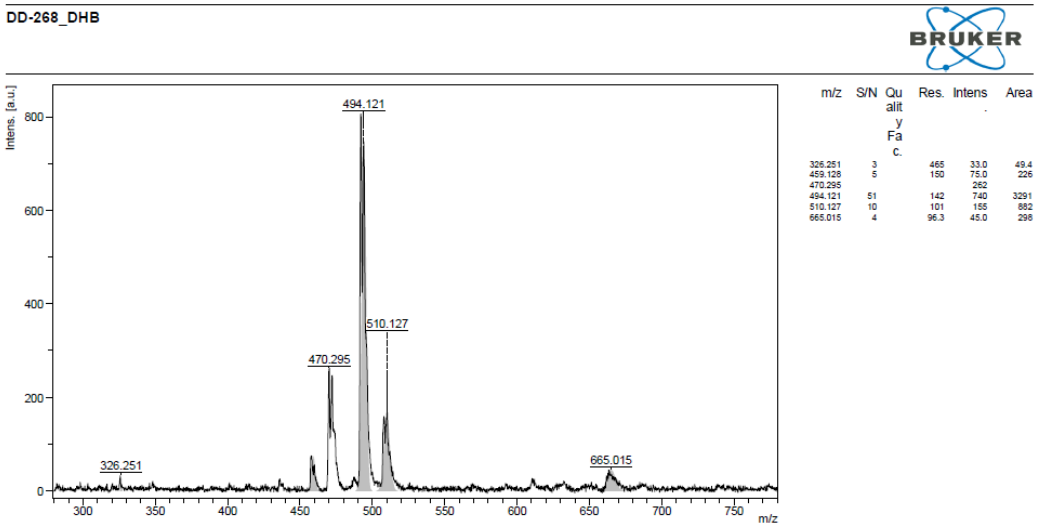


Figure 7.116. MALDI-TOF MS spectrum of compound **10k**

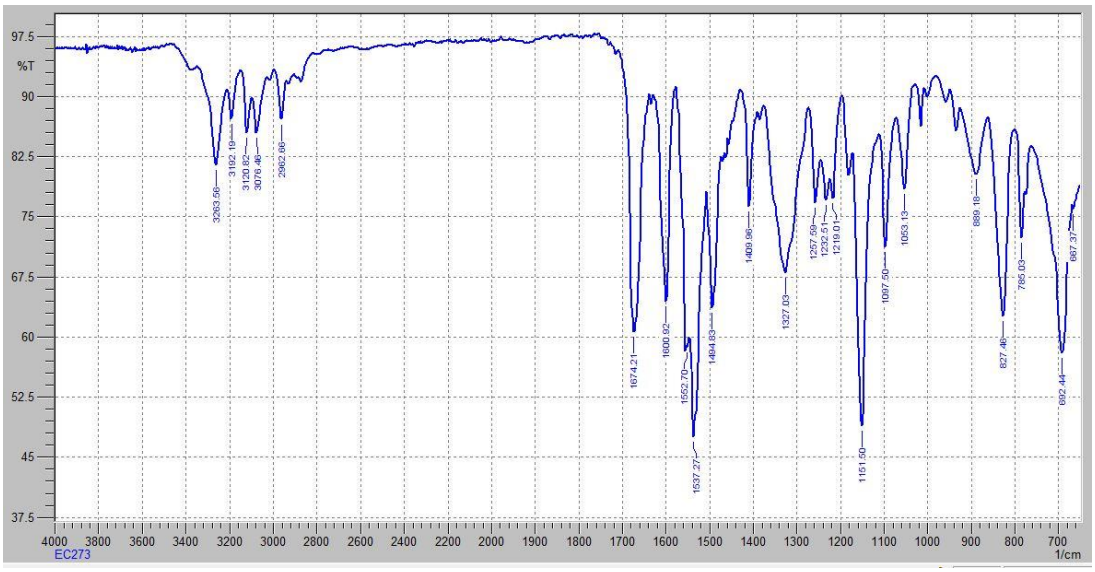


Figure 7.117. IR spectrum of compound **10l**

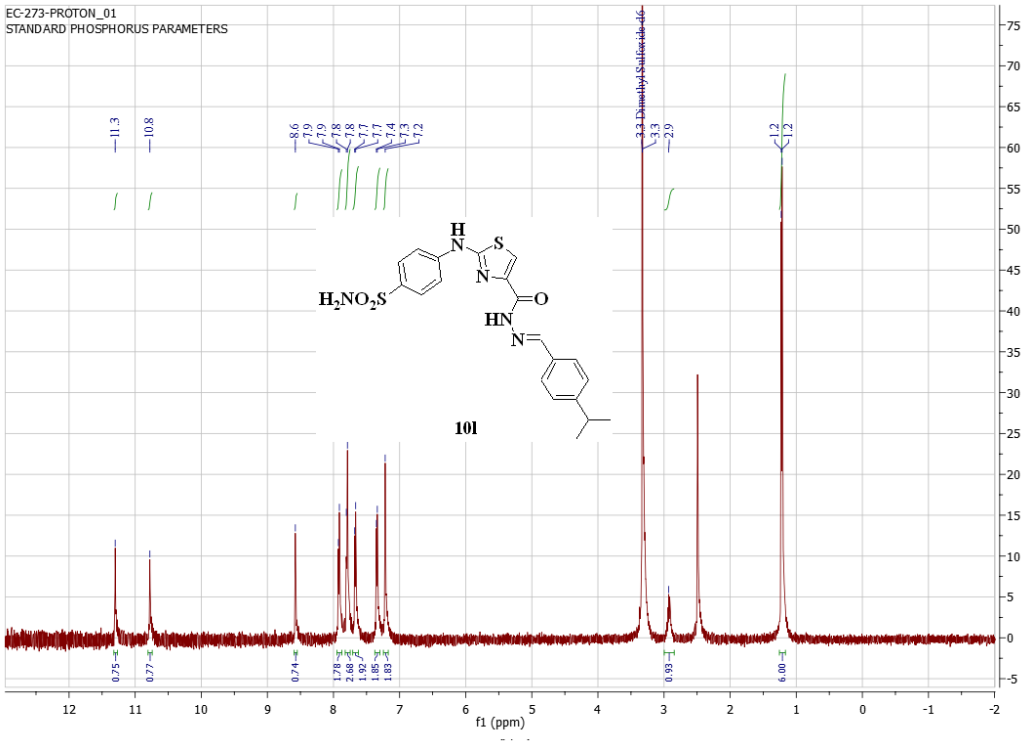


Figure 7.118. ^{1}H NMR spectrum of compound **10l**

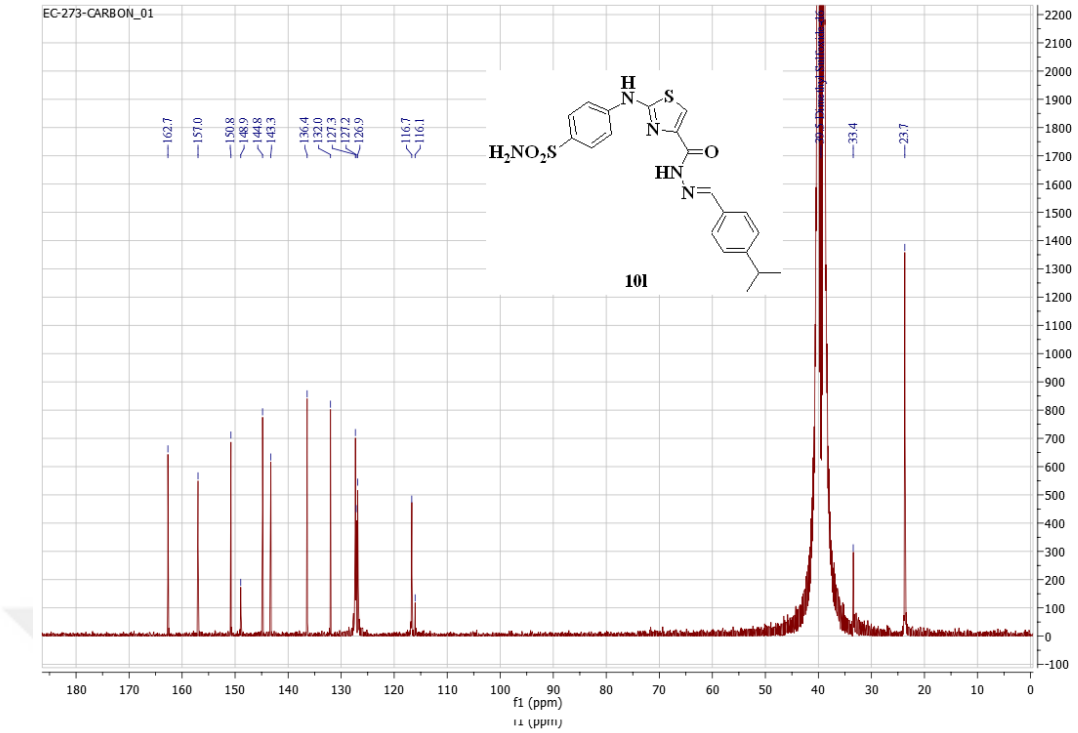


Figure 7.119. ^{13}C NMR spectrum of compound **10l**

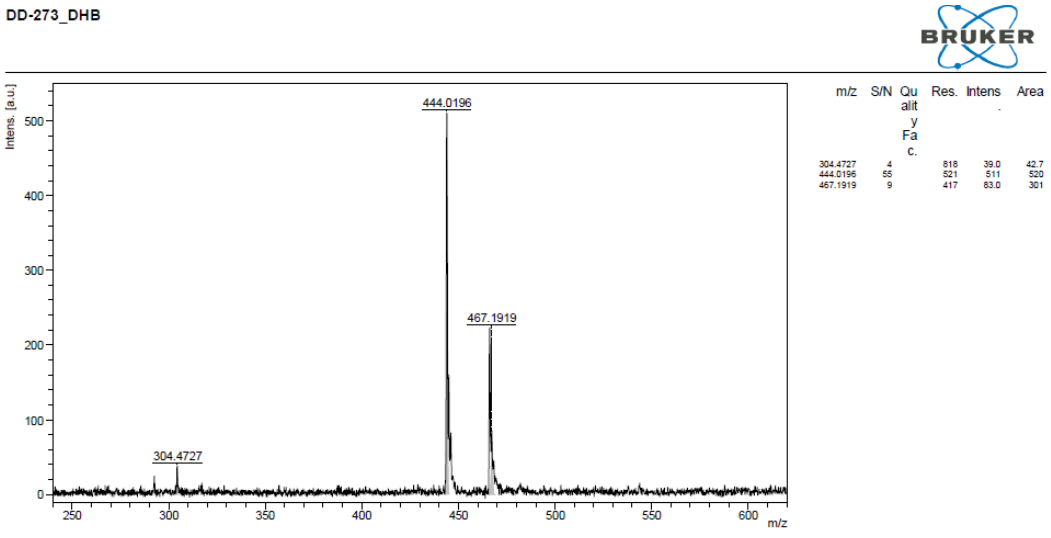


Figure 7.120. MALDI-TOF MS spectrum of compound **10l**

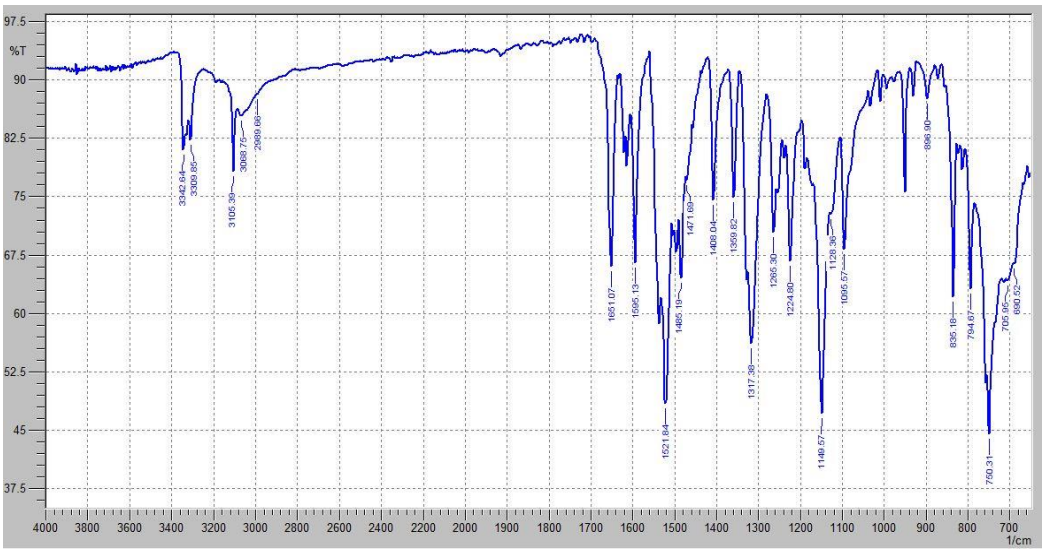


Figure 7.121. IR spectrum of compound **10m**

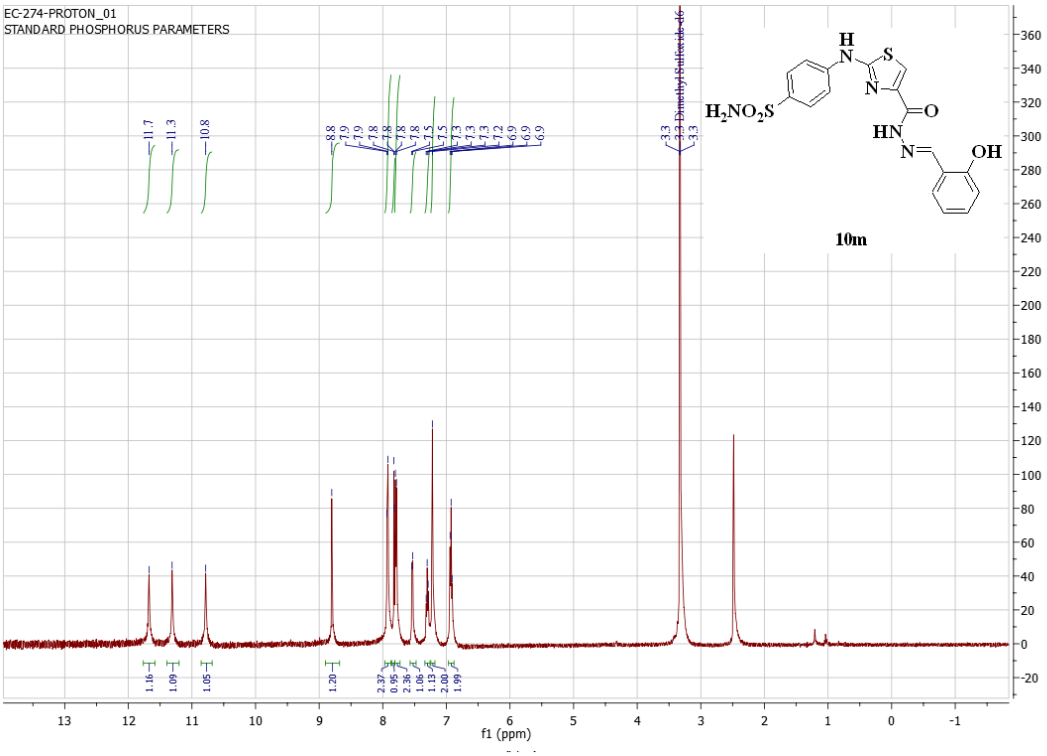


Figure 7.122. ^{1}H NMR spectrum of compound **10m**

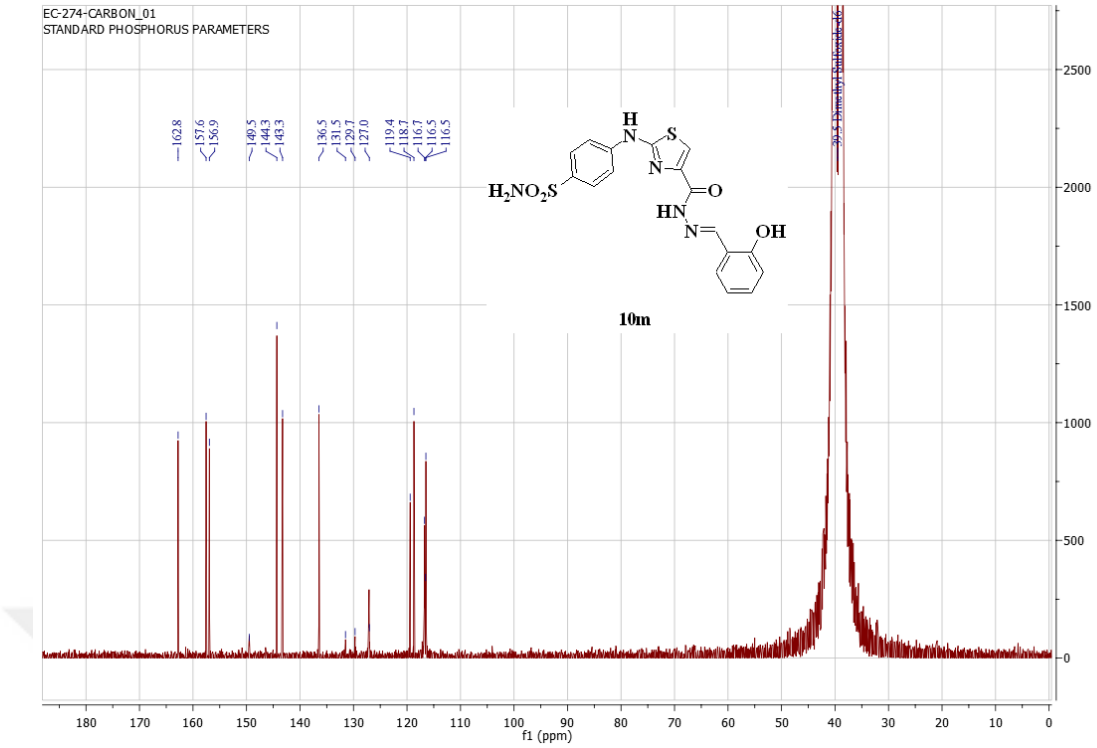


Figure 7.123. ^{13}C NMR spectrum of compound **10m**

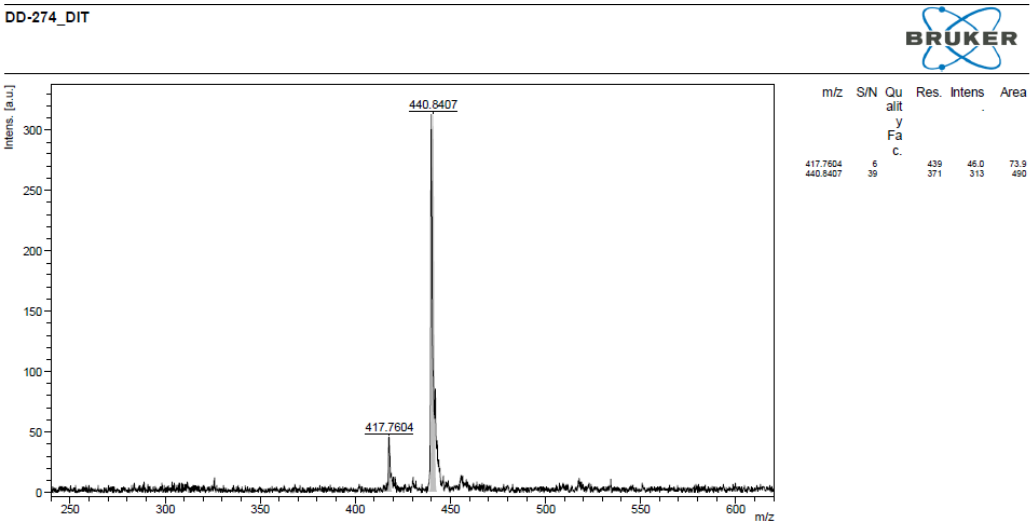


Figure 7.124. MALDI-TOF MS spectrum of compound **10m**

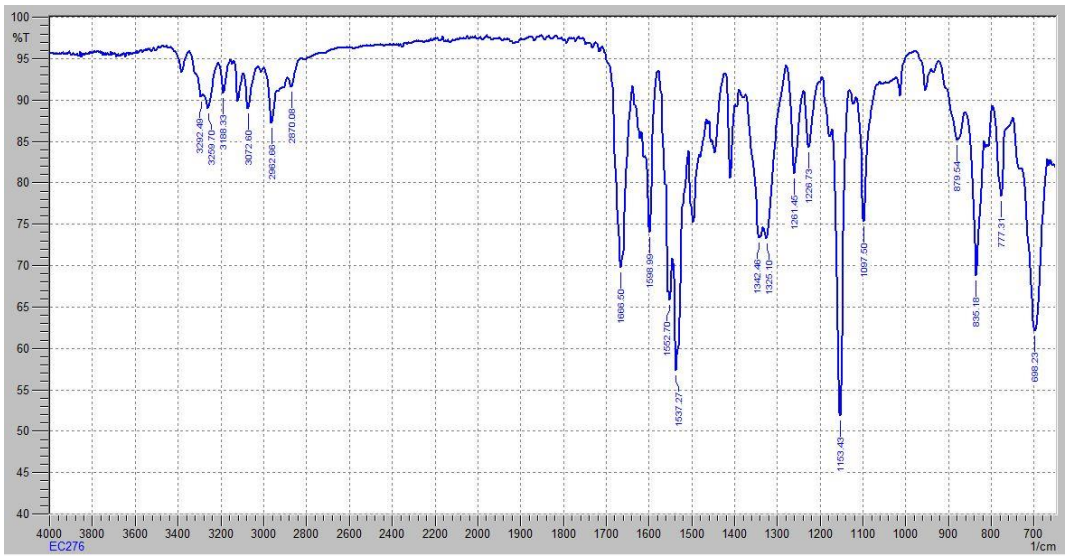


Figure 7.125. IR spectrum of compound **10n**

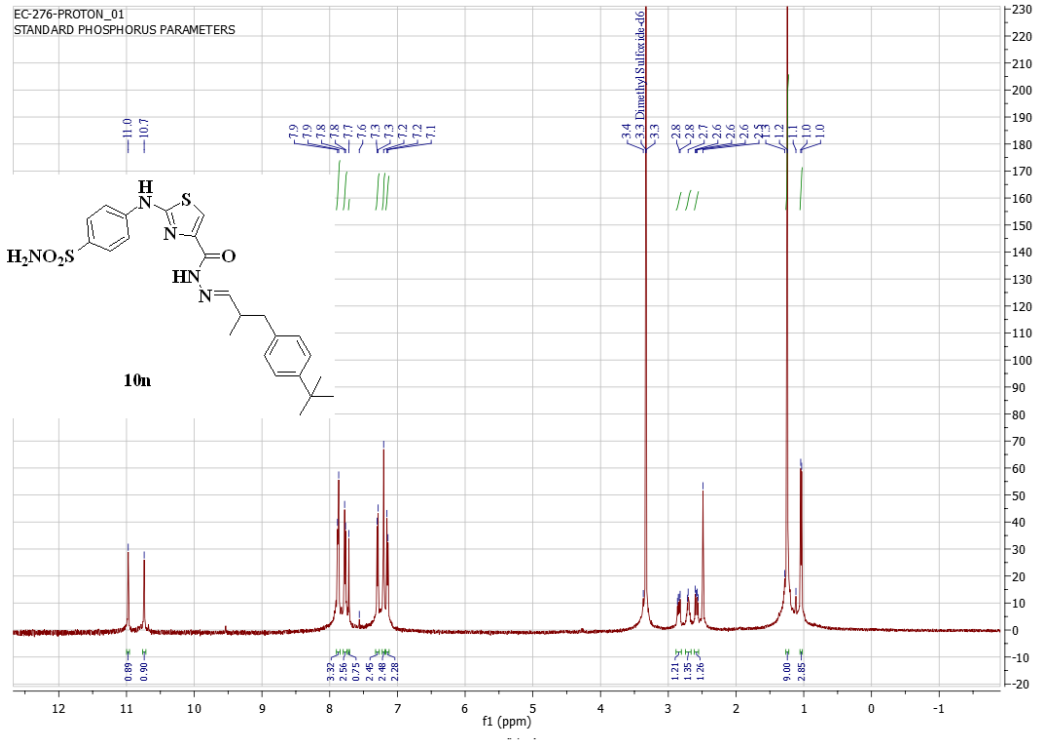


Figure 7.126. ^{1}H NMR spectrum of compound **10n**

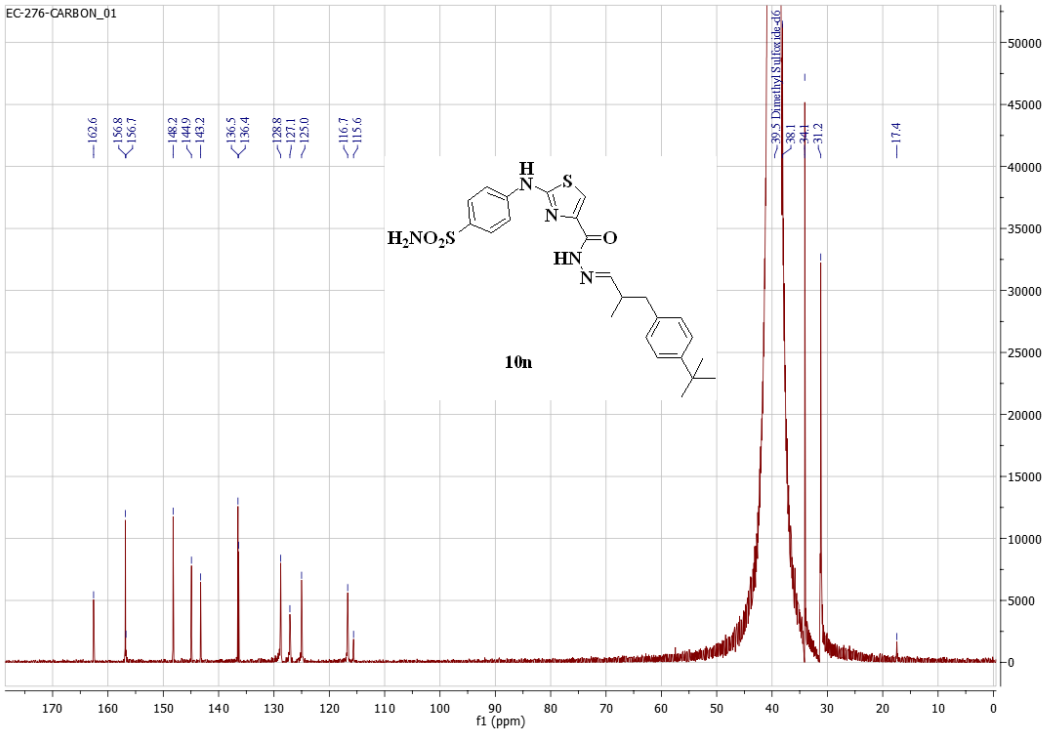


Figure 7.127. ^{13}C NMR spectrum of compound **10n**

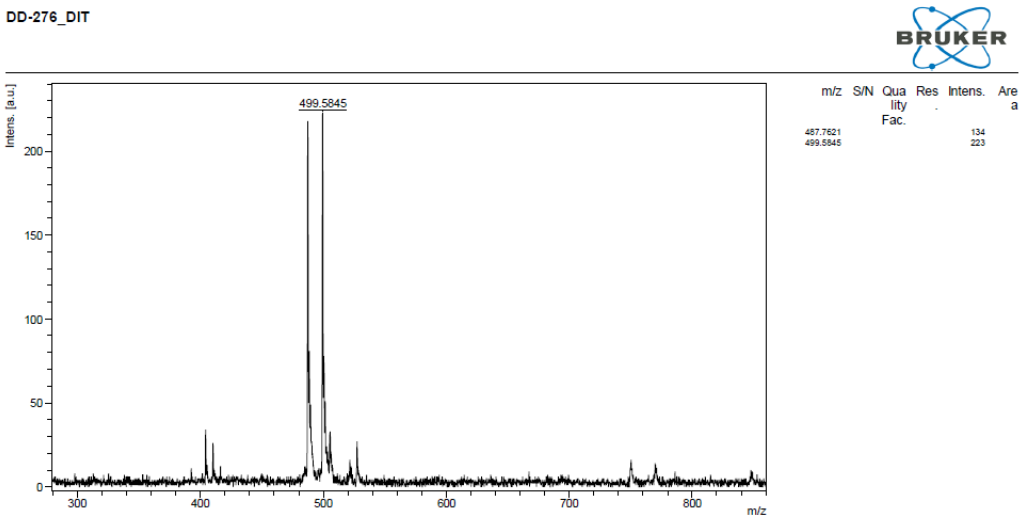


Figure 7.128. MALDI-TOF MS spectrum of compound **10n**

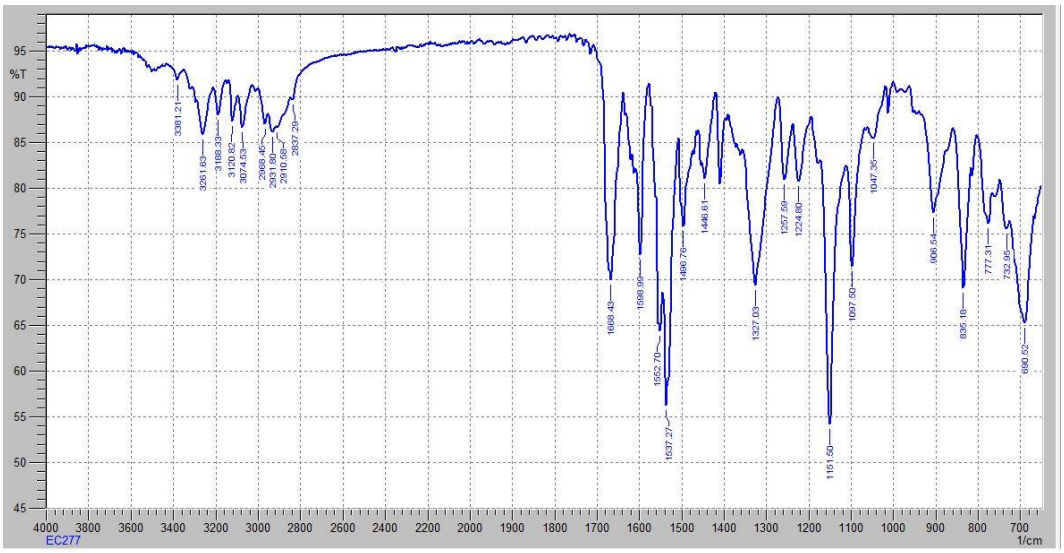


Figure 7.129. IR spectrum of compound **10o**

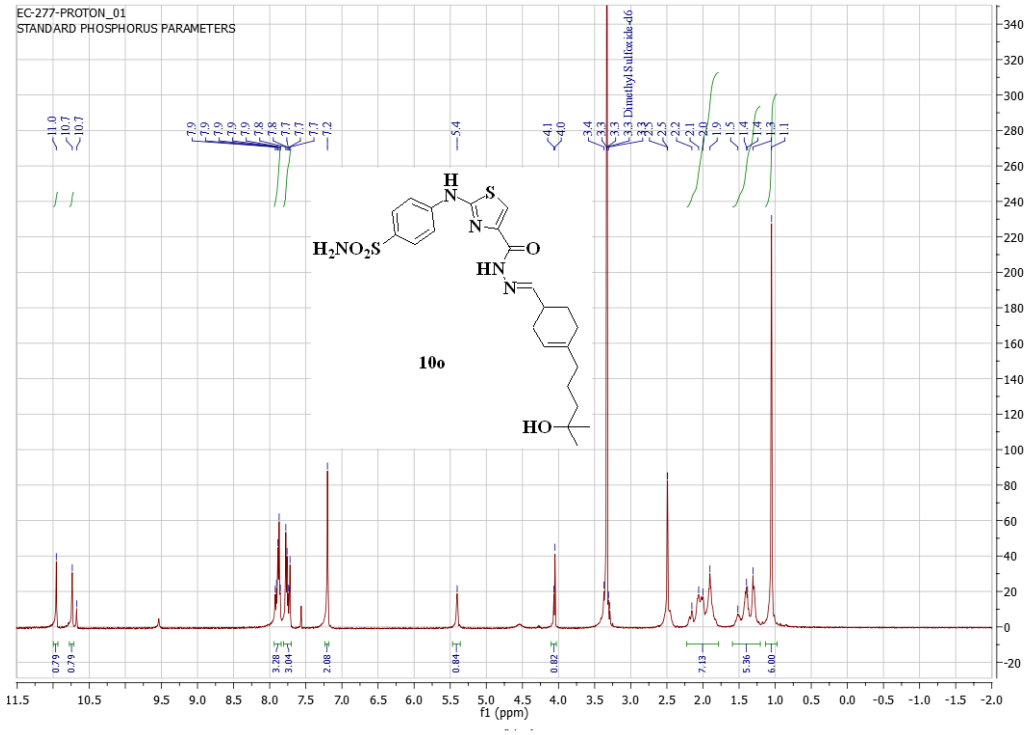


Figure 7.130. ^{1}H NMR spectrum of compound **10o**

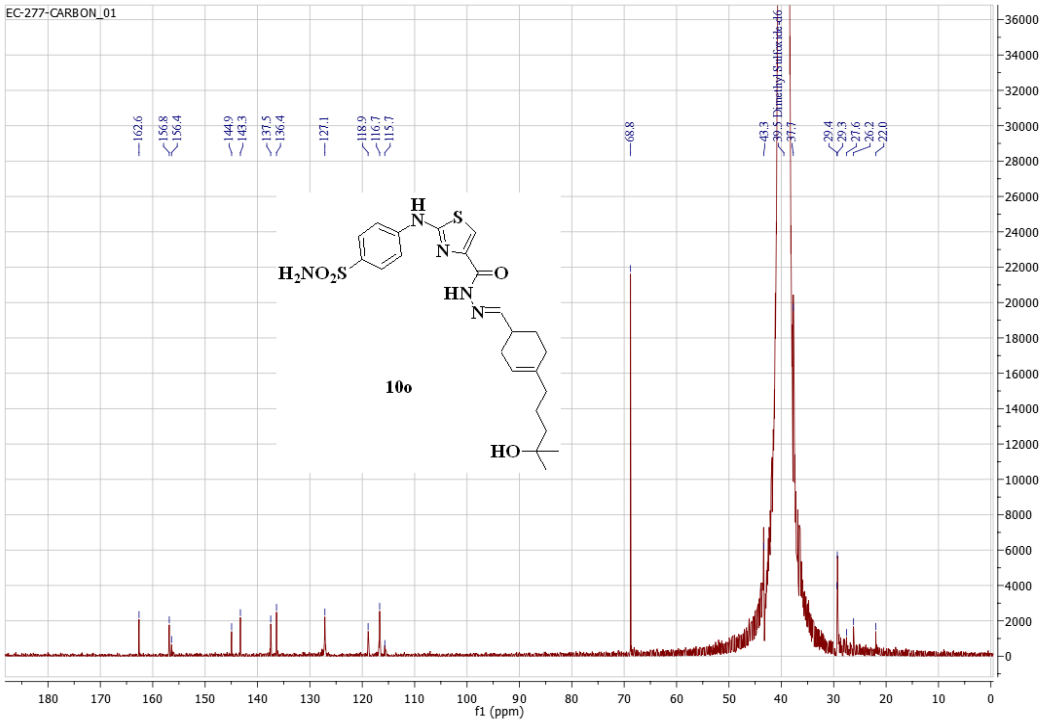


Figure 7.131. ^{13}C NMR spectrum of compound **10o**

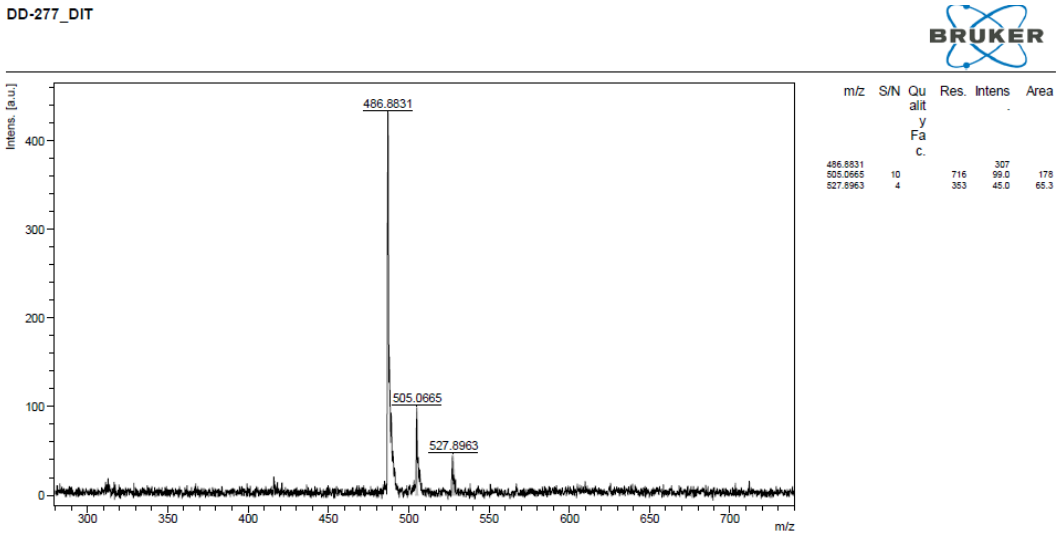


Figure 7.132. MALDI-TOF MS spectrum of compound **10o**