ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ONE-POT SYNTHESIS OF HIGHLY FUNCTIONALIZED THIAZOLO[3,2-a]PYRIDIN-8-YL-PHOSPHONATE DERIVATIVES

MASTER OF SCIENCE

LANGE YAKUBU SALEH

BOLU, JANUARY 2016

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APPROVAL OF THE THESIS

ONE-POT SYNTHESIS OF HIGHLY FUNCTIONALIZED THIAZOLO[3,2-a]PYRIDIN-8-YL-PHOSPHONATE DERIVATIVES. submitted by **LANGE YAKUBU SALEH** in partial fulfillment of the requirements for the degree of Master of Science in **Department of Chemistry, Abant Izzet Baysal University** by,

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January 21, 2016

Prof. Dr. Duran KARAKAS ……

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

To my family

DECLARATION

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

Lange Yakubu SALEH

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ABSTRACT

ONE-POT SYNTHESIS OF HIGHLY FUNCTIONALIZED THIAZOLO[3,2-a]PYRIDIN-8-YL-PHOSPHONATE DERIVATIVES

MSC THESIS
LANGE YAKUBU SALEH
ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF CHEMISTRY
(SUPERVISOR: ASSOC. PROF. DR CEVHER ALTUĞ)

BOLU, JANUARY 2016

In this study, highly effecient synthesis of thiazolo[3,2-a]pyridin-8-yl-phosphonate containing heterocycles were succeeded *via* multicomponent reactions (MCRs) which proceeds through a domino-Knoevenagel condensation/nucleophlic addition/ condensation sequence to afford titled products. Moreover, the mechanism of formation leading to the obtained product was anticipated. As the major course of this study, our starting material diethyl phosphonate containing heterocyclic ketene aminal (HKA), diethyl ((4-oxothiazolidin-2-ylidene)methyl)phosphonate, was synthesized for the first time. Then, it was reacted with malononitrile and two equivalents of various aromatic aldehydes to give thiazolo[3,2-a]pyridin-8-yl-phosphonate derivatives. Precipitated products were filtered and purified by recrystallization with good to excellent yields. All compounds were screened for anticancer properties against mcf7 and t98g cancer cells. Diethyl-(Z)-(5-amino-6-cyano-2-(4-nitrobenzylidene)-7-(4-nitrophenyl)-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-yl)phosphonate showed significant cytotoxic activity against breast cancer cell line. The structures of all multi-component reaction products were identified by means of spectroscopic methods IR, ^{1}H NMR, ^{13}C NMR, HRMS and physical characteristics (melting point and R_f values).

KEYWORDS: Multi-component Reactions, Knoevenagel Reaction, Alkylidene Thiazolidin-4-one, Thiazolopyridine, Phosphonate.

ÖZET

OLDUKÇA İŞLEVSELLEŞTİRİLEN TİYAZOLO[3,2-a]PİRİDİN-8-İL-FOSFONAT TÜREVLERİNİN TEK BASAMAKTA SENTEZİ
YÜKSEK LISANS TEZI
LANGE YAKUBU SALEH
ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
KIMYA ANABILIM DALI
(TEZ DANIŞMANI: DOÇ. DR. CEVHER ALTUĞ)

BOLU, OCAK - 2016

Bu çalışmada, oldukça etkin bir sentez yöntemi kullanılarak heterohalkalar ihtiva eden tiyazolo [3,2-a]piridin-8-il–fosfonat bileşikleri, çok-bileşenli reaksiyonlar yoluyla sıralı bir şekilde domino-Knoevenagel kondensasyonu-nükleofilik katılma-kondensasyon reaksiyonlarıyla başlıkta verilen bileşikler elde edildi. Dahası, oluşum reaksiyonu önerildi. Bu çalışmanın en önemli kısmını oluşturan fosfat içeren heterohalkalı keten aminal (HKA), dietil (4-oksotiyazolidin-2-iliden) metil) fosfonat, bileşiği ilk kez sentezlendi. Daha sonra, tiyazolo [3,2-a] piridin-8-il-fosfonat türevleri planlanan ürünü verecek şekilde malononitril ve iki eşdeğerli çeşitli aromatik aldehitler ile reaksiyona sokuldu. Reaksiyon ortamında çöken ürün süzüldü ve mükemmele yakın verimlerle yeniden kristalleştirme yöntemiyle ile saflaştırıldı. Bütün bileşikler mcf7 ve t98g kanser hücrelerine karşı anti-kanser özellikleri için çalışıldı. Dietil-(Z)-(5-amino-6-siyano-2-(4-nitrobenzylidin)-7-(4-nitrofenil)-3-oxo-3,7-dihidro-2H-tiyazolo[3,2-a]piridin-8-yl)fosfonat bileşiği göğüs kanseri hücre hatlarına karşı önemli sitotoksik aktivite göstermiştir. Bileşiklerin yapıları spektroskopik yöntemler IR, ^{1}H, ^{13}C NMR, HRMS ve fiziksel özellikler (erime noktası ve R_f değerleri) ile tespit edilmiştir.

ANAHTAR KELİMELER: Çok Bileşenli Reaksiyonlar, Knoevenagel Reaksiyonu, Alkiliden Tiyazolidin-4-on, Tiyazolopiridin, Fosfat.

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LIST OF ABBREVIATIONS AND SYMBOLS

$AlCl_3$	: Aluminium chloride
1,4-DPH	: 1,4-dihydropyridines
$C_2H_5O(C=S)SK$	: Potassium ethylxanthate
CH_3CO_2H	: Acetic acid
$CDCl_3$	: Deuterated Chloroform
CN	: Nitrile
Cs_2CO_3	: Cesium Carbonate
d	: Doublet (NMR)
dd	: Doublet of doublets (NMR)
Decomp.	: Decomposed
$(CH_2)_2Cl_2$	: Dichloroethane
DME	: Dimethoxyethane
DMF	: Dimethylformamide
EtOH	: Ethanol
Et_3N	: Triethylamine
eq.	: Equivalent
FT-IR	: Fourier Transform Infrared Spectroscopy
GHz	: Gigahertz
h	: Hour
H-4CR	: Hantzsch four component reaction
H_2O	: Water

HCl : Hydrochloric acid

HCN : Hydrogen cyanide

HIV : Human Immunodeficiency Virus

HRMS : High-resolution mass spectrometry

Hz : Hertz

IMCRs : Isocyanide Multi Component Reactions

IR : Infrared

J : Coupling constant (NMR)

KBr : Potassium bromide

KSCN : Potassium thiocyanide

LDA : Lithium Di-isopropyl-Amide

MAO-B : Monoamide oxidase beta inhibitor

Mid : Middle

MW : Microwave

min : Minute

MeCN : Acetonitrile

M^+ : Molecular ion

$[M+H]^+$: Protonated molecular ion

MCR : Multi-component reaction

MHz : Megahertz

mp : Melting point

mL : Milliliter

mmol : Millimolar

m : Multiplet (NMR)

NH_3 : Ammonia

$NH_2NH_2{\cdot}2H_2O$: Hydrazine dihydrate

$NH_2OH{\cdot}HCl$: Hydroxylamine hydrochloride

NMP : *N*-Methyl-2-pyrrolidone

NMR : Nuclear magnetic resonance

P-3CR : Passerini three component reaction

rt : Room temperature

R : Any group (Alkyl or Aryl)

R_f : Retardation factor

SAR : Structure Activity Relationship

s : Singlet

TBAI : Tetrabutylammonium Iodide

TBM : Tert-butylmercaptan

THF : Tetrahydrofuran

TLC : Thin layer chromatography

t : Triplet

Ugi-4CR : Ugi four component reaction

W : Watt

X : Any halogen atom

ν_{max} : Maximum frequency

δ : Chemical shift

Δ : Heat

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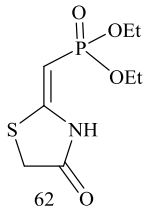
FORMULAE



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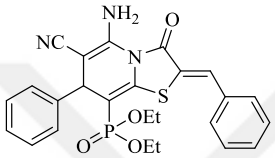

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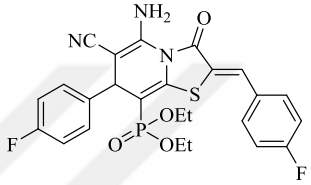

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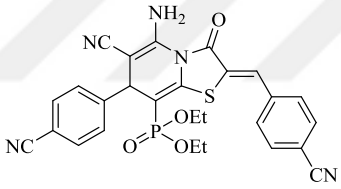

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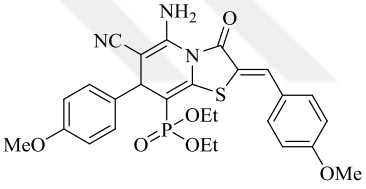

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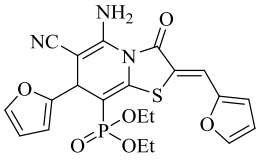

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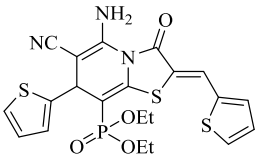

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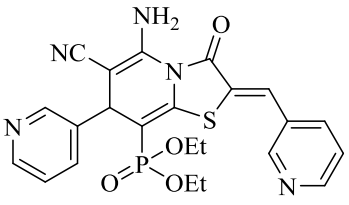

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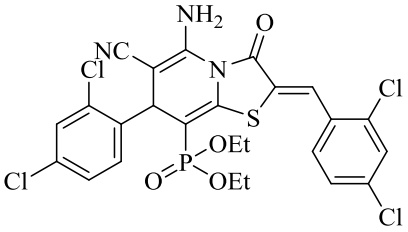

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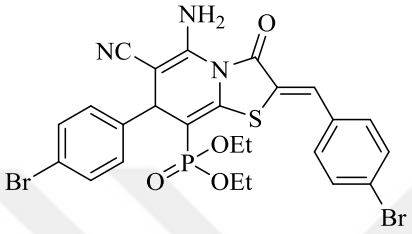

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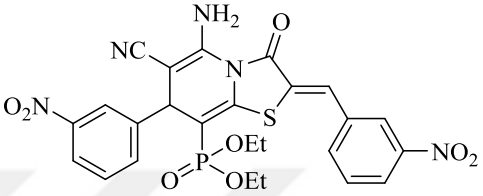

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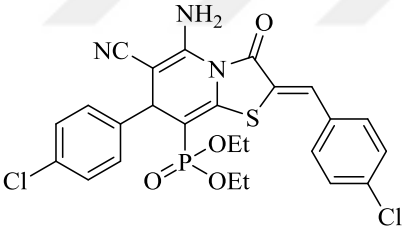

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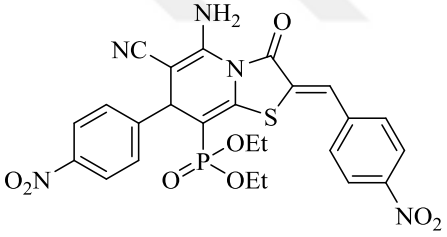

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1. INTRODUCTION

1.1 Definition of Multi-Component Reactions (MCRs)

MCRs has become one of the most useful methods giving its applicability and eco-friendly adding to the investigation of new synthetic methods to advance the preparation of organic compounds is a vital point of research activity in bioorganic, modern organic and medicinal chemistry (Sunderhaus and Martin, 2009).

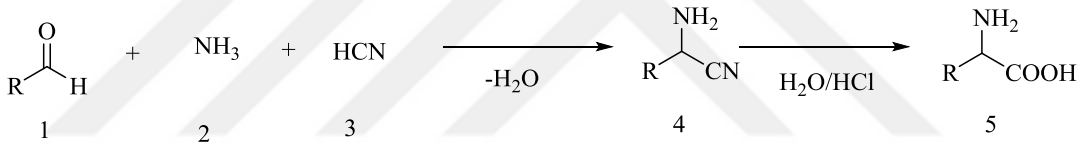
MCRs (Mingji, 2006; Ugi et al., 1994) are process whereby at least three or more accessible reactants are joined together in a reaction vessel to obtain a final product showing pieces of the whole contributions of the starting materials without adding additional reagents or catalysts. Consequently, this kind of reactions supplies great opportunities to design molecules with various functional groups and prevents synthesis time and energy consumption.

The importance of MCRs for drugs discovery were acknowledged and significant labours from not only academic but also industrial researchers have been concentrating deeply on the strategy and expansion of multi-component practices for the group of archives of heterocyclic compounds. Even MCRs came to light around 166 years ago, in the last decade, the importance of MCRs for drug discovery has been considerably recognized. MCRs are specifically active at assembling functionalised drug-like structures (Domling and Ugi, 2000). MCRs having one-pot reaction properties has generate many advantages. Thanks to this attributes, numerous libraries of different products can be synthesize in a shorter time and least energy by using a minimal set of starting materials (Domling, 2000). Also the function of the firm well-defined structures of heterocycles were shown in Structure Activity Relationship (SAR)-reports (Weber, 2002; Hulme and Gore, 2003). These studied structures also consist of thiazolopyridine containing compounds due to their unique chemical and biological activities (Bakulev et al., 2003; Shi et al., 2009; El-Hag et al., 2005). Such as antibacterial, antimicrobial, α-glucosidase inhibitor (Park et al., 2008) and cholestrol controlling agent (Furrer et al., 1994).

The chemistry of 2-alkylidenethiazolidin-4-one compounds were widely studied throughout the years. These compounds can be used for the synthesis of diverse functionalized molecules depending on the substituent of the alkylidene part (Krause and Duburs, 1998) and the preparation of scaffolds using these compounds have demonstrated to exhibit various biological properties (Shi et al., 2009).

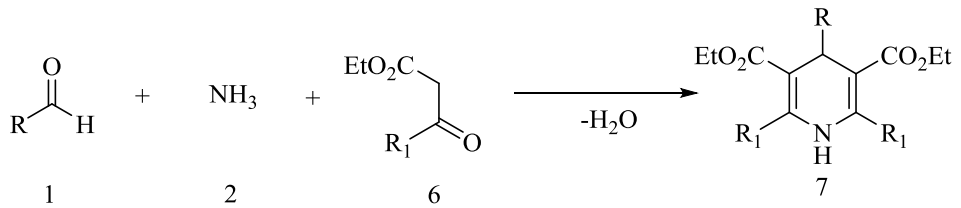
1.2 Some Important MCRs

In 1850, the Chemistry of MCRs had begun with synthesis of α-amino acid from α-aminocyanates by Adolph Strecker (Strecker et al., 1850) known as the first MCR reaction. Which involve three component reaction between aldehyde **(1)**, an amine (ammonia) **(2)** and hydrogen cyanide **(3)** to form an α-amino nitrile **(4)**. Then, research shows that an α-amino nitrile is sequentially hydrolyzed to give the desired amino-acid **(5)** (Scheme 1.1).



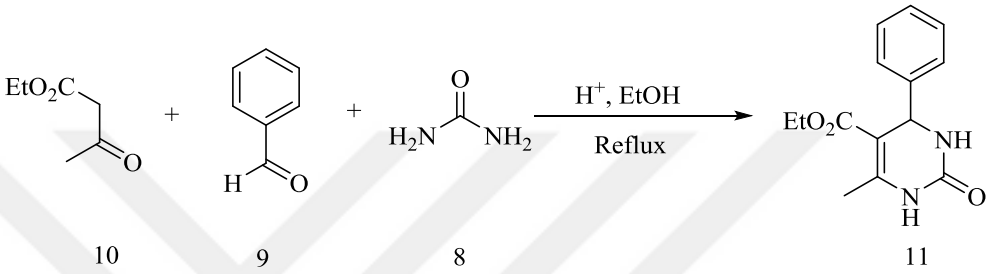
Scheme 1.1. Amino-acid synthesis from The Stecker three component reaction

In 1882, Arthur Rudolf Hantzsch first synthesized 1,4-dihydropyridines (DHPs) (Hantzsch et al., 1882) that comprises of many important heterocycle system *via* MCRs. The synthesized product is known to be 1,4-DHP compound or a Hantzsch compound. Hantzsch synthesized symmetrically substituted dihydropyridines **(7)** from aldehyde **(1)**, ammonia **(2)** and two equivalents of β-ketoesters **(6)** (Scheme 1.2).



Scheme 1.2. 1,4-dihydropyridine synthesis by Arthur Rudolf Hantzsch

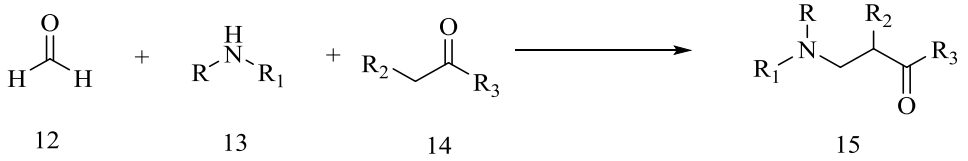
In 1893, an Italian chemist Pietro Biginelli explained the acid-catalyzed cyclo-condensation reaction of ethyl β-ketoester **(10)**, benzaldehyde **(9)** and urea **(8)**. The reaction was achieved at refluxed temperature with three components dissolved in ethanol using a catalytic amount of HCl. Dihydropyrimidinones **(11)** the products of this novel one-pot Biginelli reaction are widely used in the pharmaceutical industries (Horton et al., 2003) because pyrimidine derivatives have important biological properties as calcium channel blockers and antihypertensive agents (Kappe et al., 1993; Kappe et al., 2000) (Scheme 1.3).



10 9 8 11

Scheme 1.3. Dihydropyrimidinone synthesis of one-pot Biginelli reaction

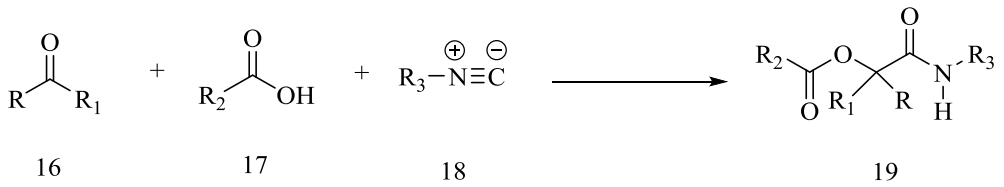
In 1912, as a result of Carl Mannich reaction, an aminomethylated product **(15)** was obtained essentially by using a non-enolizable aldehyde **(12)** that contains active hydrogen, a primary or secondary amine **(13)**, and an enolizable carbonyl compound **(14)**. (Mannich and Krosche, 1912) (Scheme 1.4).



12 13 14 15

Scheme 1.4. β-Amino-carbonyl synthesis that is also known as a Mannich base

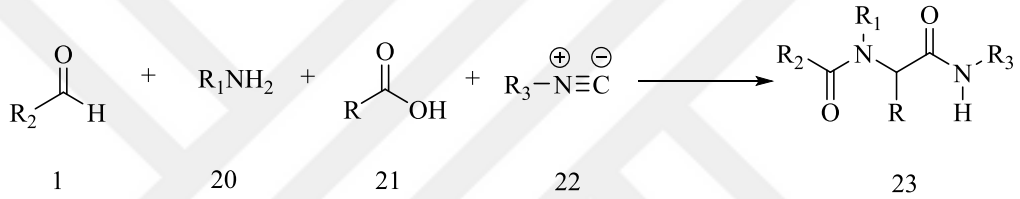
Passerini three-component reaction (P-3CR)(Park et al., 2008) was first expressed in 1921 (Papeo and Pulici, 2013). An enormous and important class of MCRs which is based on the chemistry of isocyanide multi component reactions (IMCRs) and also one of the most important of Passerini three-component reaction (P-3CR). Result of Passerini reaction shows a way to obtaining α-acyloxycarboxamides **(19)** with treatment of an isonitrile **(18)** with a carboxylic acid **(17)** and an aldehyde or ketone **(16)** (Scheme 1.5).



16 17 18 19

Scheme 1.5. α- Acyloxycarboxamides synthesis by Passerini three component reaction

Ugi reactions also known as Ugi four-component condensation (U-4CC) (Ugi et al., 1996) exposes α-aminoacyl amide derivatives **(23)** using an aldehyde **(1)**, an amine **(20)**, a carboxylic acid **(21)**, and an isocyanide **(22)** which was discovered in 1959 by Ivan Carl Ugi (Scheme 1.6).



1 20 21 22 23

Scheme 1.6. α -Acylamino amide synthesis by using Ugi reaction

Thanks to these advancement and improvement from Passerini and Ugi, many new biological active natural products IMCRs were explained (Zhu et al., 2003). In addition, they are widely used in the building of biologically applicable heterocycles such as oxazoles (Bossio et al., 1991) oxazolines, thiazoles, thiazolines, pyrroles, imidazoles and imidazolines (Marcaccini et al., 1993). As a result, the isocyanides create a broadly valued compound class in preparative and drug researches in medicinal chemistry (Rossen et al., 1998).

1.3 Some Important Drug-Like Structure Synthesized by MCRs

By incorporating Ugi−4CR, scientists working at Merck company improved the original synthesis of the anti−HIV drug Crixivan(Indinavir) hence making the period shorter, easier and with a better yielding (Breslow et al., 1958) (Figure 1.1).

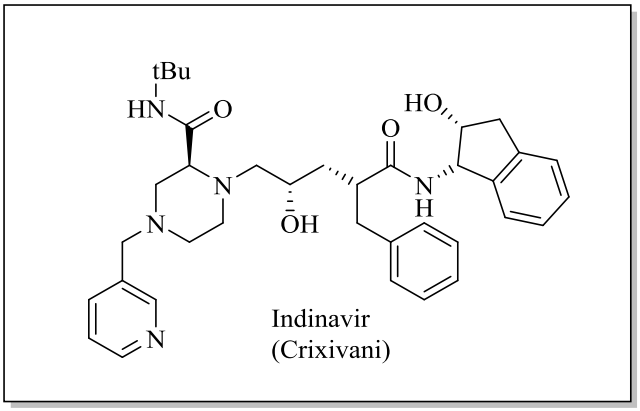


Figure 1.1. The anti-HIV drug Crixivan by incorporating a Ugi-4CR

Bayer AG company effectively made Nifedipine (Adalat^{R}) (Marcaccini et al., 1991) which is potentially used for the treatment of cardiovascular disorder and calcium channel blocker (Figure 1.2).

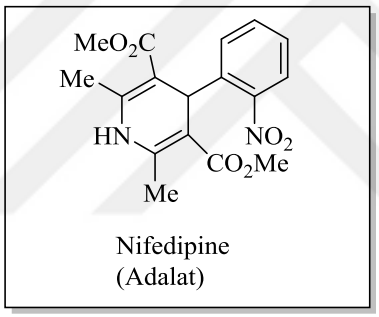


Figure 1.2. The one-step synthesis of Nifedipine, by Bayer AG Company

Some major pharmaceutical companies such as Pfizer, Sandoz, Bayer have synthesized their original molecules like Nifedipine based on the biological activity discovered in Nifedipine (Figure 1.3).

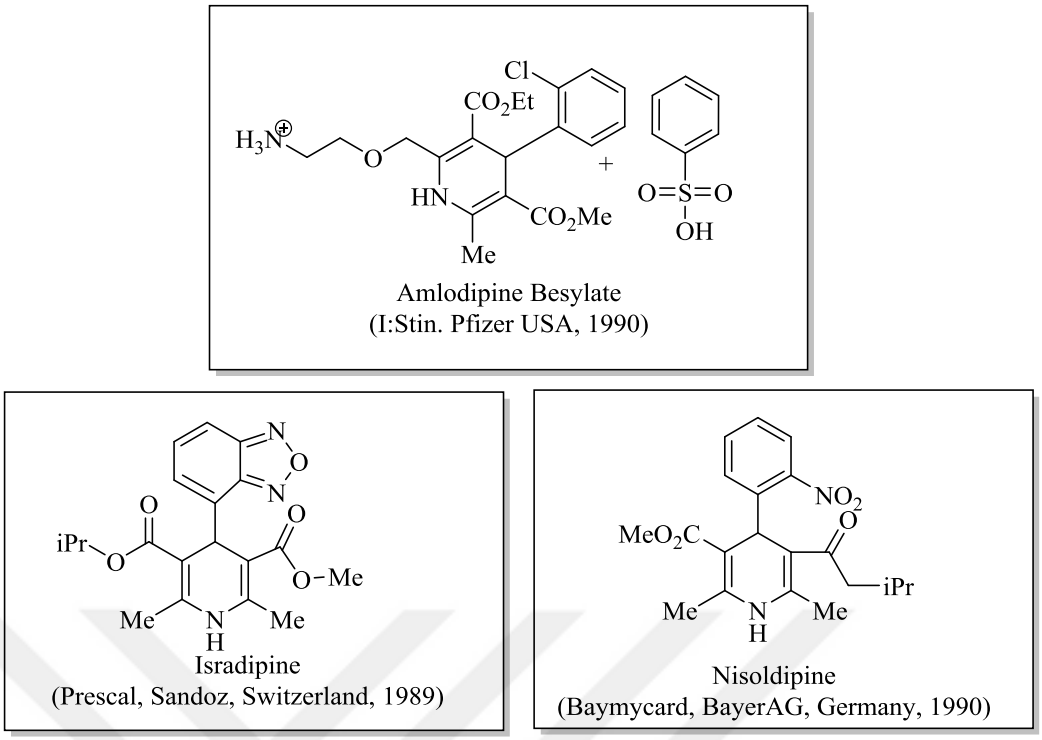


Figure 1.3. Some original molecules which they act like Nifedipine

In 1943, Lidocaine the first amino-amide type local anesthetic and antiarrhythmic drug prepared from the three component reaction of formaldehyde, piperidine and 2,6-dimethylphenylisocyanide, was first synthesized under the name Xylocain® by a Swedish chemist Nils Löfgren (Hulme and Gore et al., 2003) (Figure 1.4).

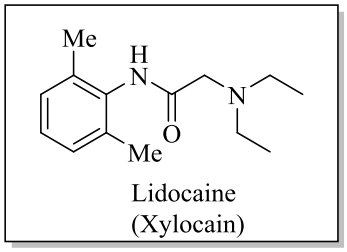


Figure 1.4. Lidocaine the first amino-amide type local anesthetics and anti-arrhythmic drug

1.4 Thiazolopyridines

Thiazolopyridine ring is a constructed system that comprises of two fused heterocylic structure and there are six isomeric thiazolopyridine systems. Thiazolopyridines can be classified into the following classes relying on the fusion of the thiazole moiety to the pyridine ring; thiazolo[3,2-a]pyridine (**I**), thiazolo[3,4-a]pyridine (**II**), thiazolo[5,4-b]pyridine (**III**), thiazolo[4,5-b]pyridine (**IV**), thiazolo[4,5-c]pyridine (**V**) and thiazolo[5,4-c]pyridine (**VI**) (El-Gaby et al., 2006) (Figure 1.5).

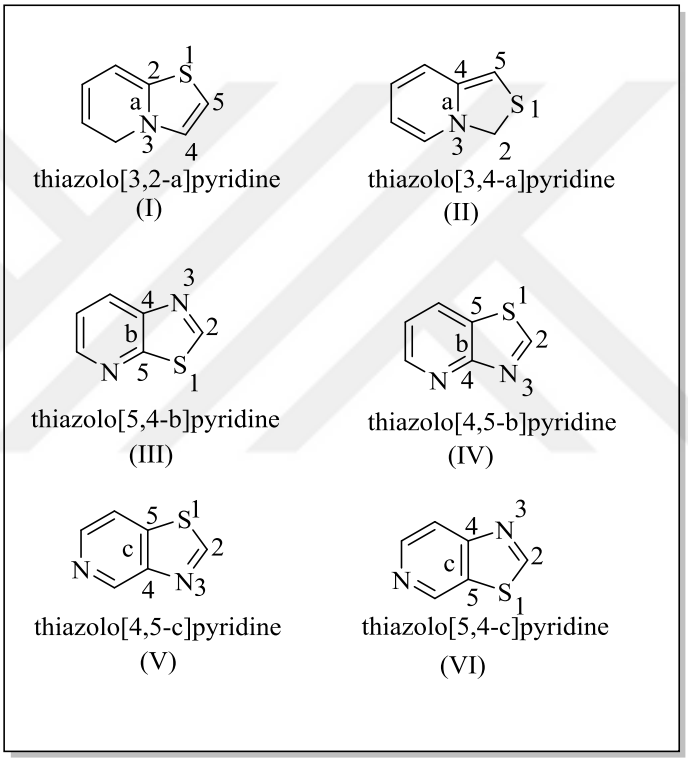


Figure 1.5. Thiazolopyridines can be classified into six isomeric pyridine rings

Substituted thiazolopyridines reveals an important classes of heterocycles according to their diverse kinds of pharmaceutical (Komoriya et al., 2006) and pesticide (Mabkhot et al., 2007) activity. As a biological active compounds thiazolopyridine ring system has a wide range (Gawish and El-Gaby, 2014). Thiazolo[3,2-a]pyridine derivatives also possess α-glucosidase inhibitor (used as an anti-diabetic drug) (Park et al., 2008), antimicrobial activity (El-Hag et al., 2002), anti-imflammatory (used in treatment of a number of arthritic diseases) (Demirdamar

et al., 1999) and analgesic (Khalifa et al., 2015) properties. Specific examples of thiazolopyridines with biological and pharmacological activities (Figure 1.6).

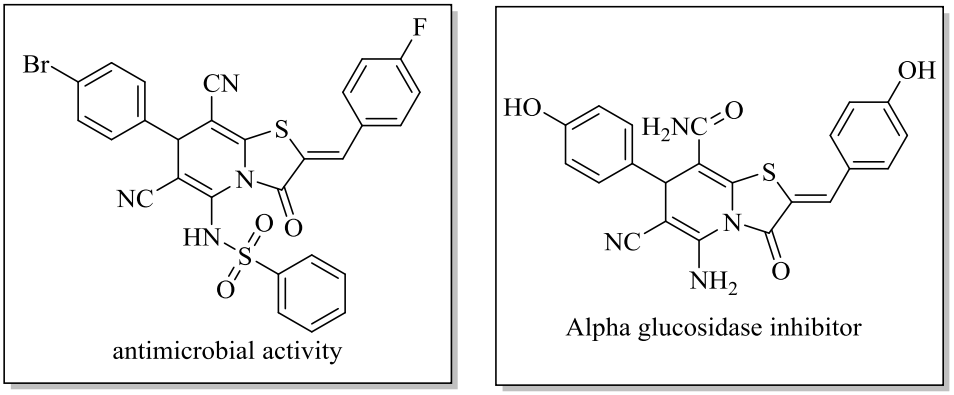


Figure 1.6. Above are two examples of thiazolopyridines with biological and pharmacological activities

Lots of biologically active drugs have been synthesized from thiazole derivatives such as anti-infective drug like sulfathiazole (Nichols and Guay, 1989), fanetizole that possesses anti-inflammatory effect (Lin et al., 2009), an anthelmintic agent niridazole (Blumer et al., 1980), thiamine identified as vitamin B_1 an antineuritic vitamin (Jurgenson et al., 2009) and wide spectrum antibiotic penicillin group which isolated from *Penicillium* fungi (Garrod et al., 1960) (Figure 1.7).

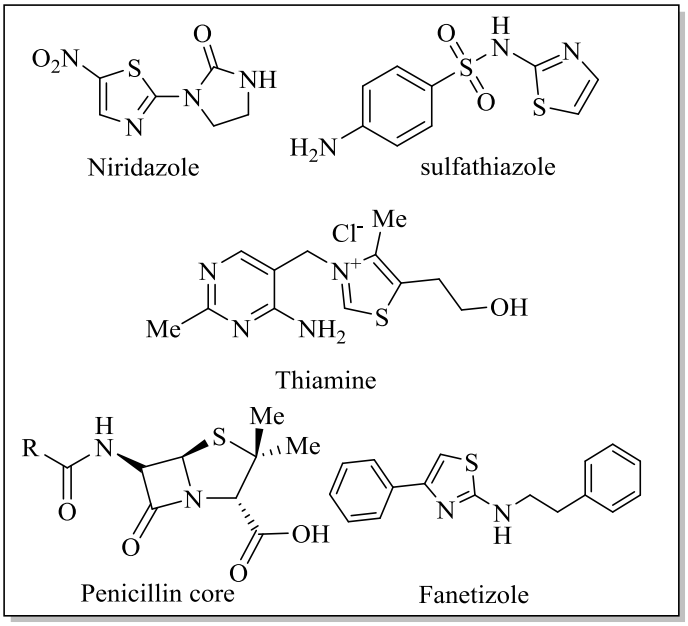
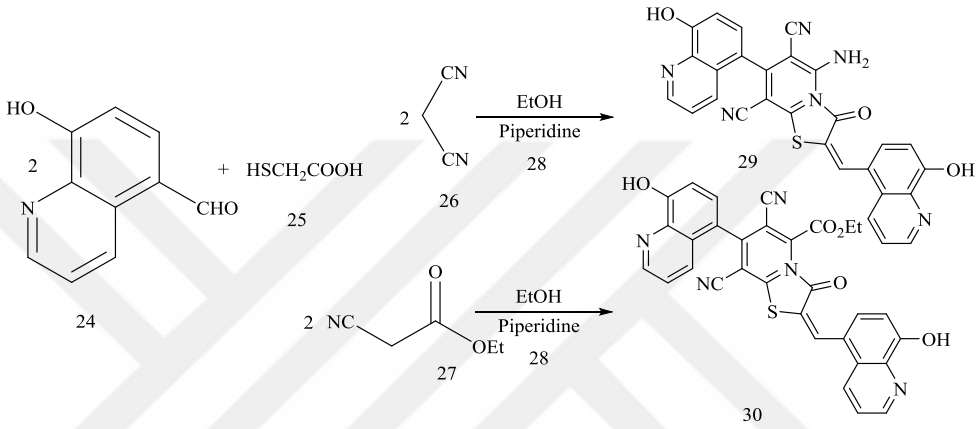


Figure 1.7. Clinically active some thiazole derivatives

1.5 Some Reactions for Thiazolopyridine Synthesis

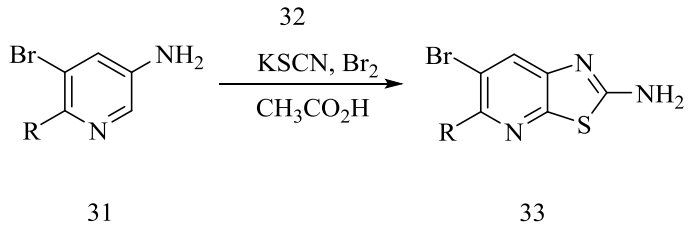
The synthesis of thiazolo[3,2-a]pyridine **(29,30)** was basically obtained from the reaction of 2-mercaptoacetic acid **(25)**, an active methylene (e.g malononitrile **(26)** or ethyl cyanoacetate **(27)** and aromatic aldehyde **(24)** in molar ratio of 1:2:2 in absolute ethanol in the presence of a catalytic amount of piperidine **(28)**. Screening of these thiazolopyridine compounds showed Anti-fungal trials for quinoline substitute with active against four species of fungi (Marzoog, 2000) (Scheme 1.7).



Scheme 1.7. Synthesis of thiazolopyridine derivatives by Marzoog S. Al-Thebeiti.

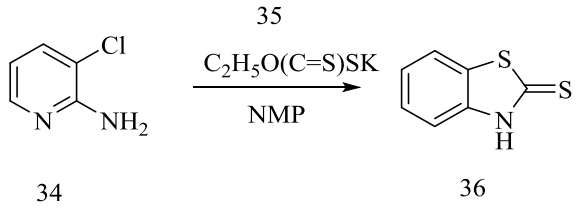
Thiazolopyridines under different conditions

Substituted thiazolopyridine ureas **(31)** had been synthesized by (Kale et al., 2013) by using potassium thiocyanide **(32)** in acetic acid medium (Scheme 1.8),



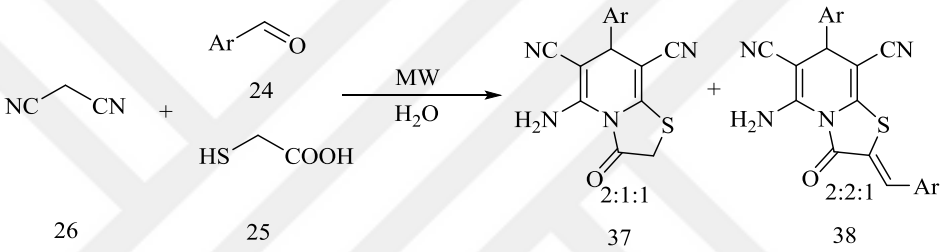
Scheme 1.8. Thiazolopyridine urea synthesis

The key intermediate thiazolo[4,5-b]pyridine-2(3*H*)-thione **(35)** was synthesized by (Rao et al., 2009) starting with the reaction of 2-amino-3-chloropyridine **(34)** with potassium ethyl xanthate in refluxing NMP (Scheme 1.9).



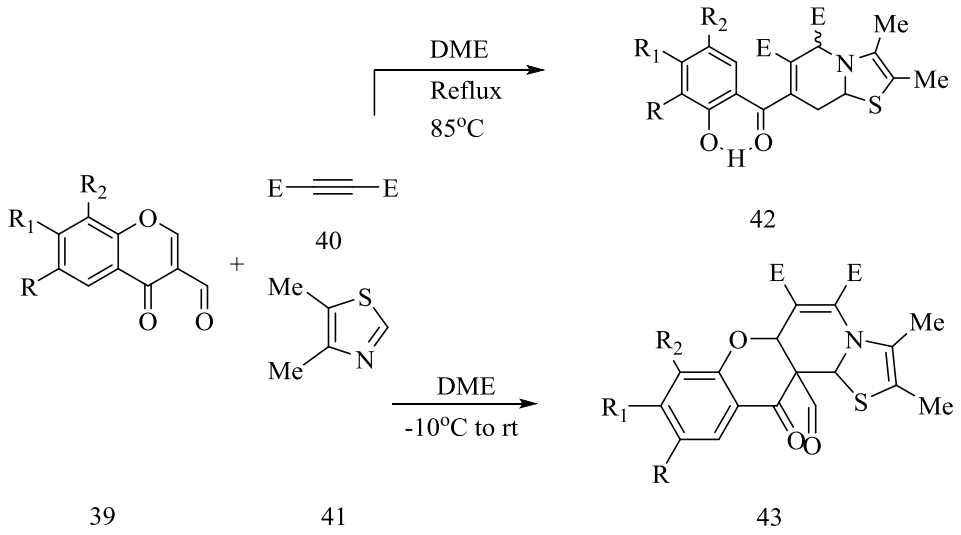
Scheme 1.9. Potassium ethyl xanthate in refluxing NMP to afford thiazolo[4,5-b]pyridine-2(3*H*)-thione

The synthesis of thiazolo[3,2-a]pyridine derivatives **(37-38)** was initiated by MCR of malononitrile **(26)**, an aromatic aldehyde **(24)**, and 2-mercaptoacetic acid **(25)** under MW irradiation in water by (Shi et al., 2009) (Scheme 1.10).



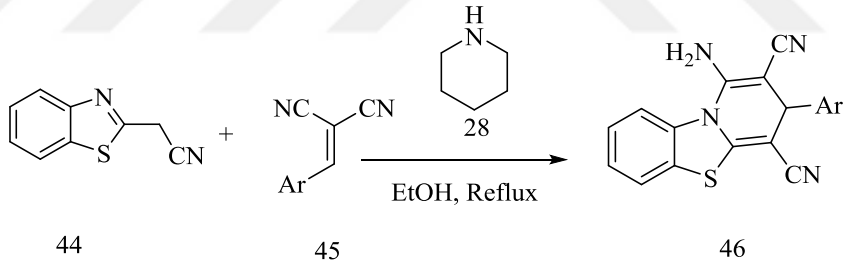
Scheme 1.10. Thiazolo[3,2-a]pyridine synthesis under MW irradiation in water

Terzidis et al. (2010) have identified different products of thiazolopyridines at different conditions in dimethoxyethane. Example is the reaction of 3-formylchromones **(39)** with acetylenedicarboxylates **(40)** and thiazole **(41)** to afford thiazolopyridine derivatives **(42-43)** (Scheme 1.11).



Scheme 1.11. Synthesis of thiazolopyridine derivatives by Terzidis

The synthesis of benzothiazole derivatives was achieved by threating 2-(benzo[d]thiazol-2-yl)acetonitrile **(44)** and with appropriate cinnamonitrile **(45)** with a catalytic amount of piperidine **(28)** in ethanol by Hassan et al. (2009) (Scheme 1.12).



Scheme 1.12. 1-Amino-3-aryl-4-cyano-3*H*-pyrido[2,1-b]benzothiazole-2-carbonitrile **(46)** synthesis

1.6 Phosphonates

Phosphonates are organophosphorus compounds, which contains carbon, phosphorus and oxygen. Phosphonate and its derivatives happen to draw substantial attention due to their biologically vital possessions and synthetic features (Cohen et al., 2003). Phosphorus is a vital structural component of numerous biomolecules and also plays an essential role in energy preservation and metabolic order (Guo et al., 2009). Organophosphorus compounds are important substrates in the study of biochemical processes having a tetra-organized pentavalent phosphorus compounds, having at least a phosphonic acid **(I)**, phosphinic acid **(II)** (R= H, alkyl or aryl) and phosphonate group **(III)** are broadly used as biologically active compounds (Engel, 1977; Palacios et al., 2005) (Figure 1.8).

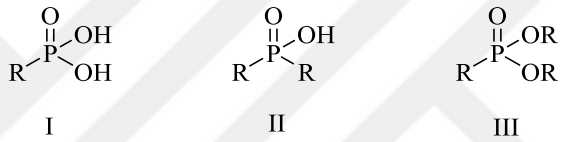


Figure 1.8. Classes of phosphorus

Phosphonates in spite of being scarce in nature they have drawn a substantial synthetic and pharmacological interest due to their diverse bioactivities. Which are widely use as antibacterial agents, plant growth regulators, enzyme inhibitors, drugs to treat bone disorders and anti-HIV agents (Peng et al., 2008).

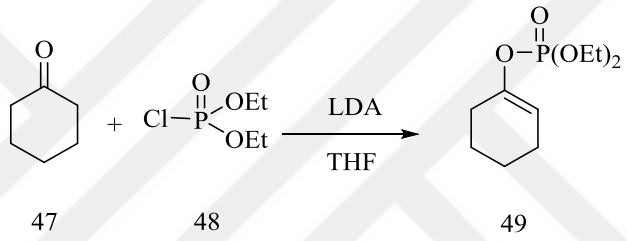
1.6.1 Synthesis of some Phosphonate Analogues

Synthesis of phosphonate analogues is a developing area in organic chemistry because of their wide using areas for both biological activities and industrial applications. They happen to be tons of diverse reactions for the synthesis of phosphonate analogues. The most often used reactions are the carbon-phosphorus (C-P), carbon-carbon (C-C), carbon-nitrogen (C-N) and carbon-oxygen (C-O) bond formation processes (Palacios et al., 2005).

1.6.2 Carbon-Phosphorus (C-P) Bond Formation

Michaelis-Becker reaction was the first for the generation of a carbon-phosphorus (C-P) bond. In this reaction, dialkylphosphite and a strong base were reacted to form the salts of dialkylphosphites (Eymery et al., 1999; Rodrigues et al., 2002). This technique involves the nucleophilic phosphorylation of a saturated carbon by the salts of dialkylphosphites. The Michaelis-Becker reaction is a famous approach for the synthesis of phosphonates however the yields of the achieved phosphono esters are low.

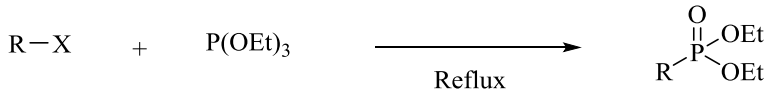
Wiemer et al. (1989) synthesize phosphono esters **(49)** from cyclohexanone **(47)** and diethyl phosphorochloridate **(48)** (Scheme 1.13).



47 48 49

Scheme 1.13. phosphono ester reaction

Michaelis-Arbuzov reaction is another method for the generation of a carbon-phosphorus (C-P) bond formation. The synthetic route involves reaction of aryl/alkyl halides **(50)** with triethyl phosphite **(51)** at reflux temperature and the formation of new aryl/alkyl phosphonates **(52)** involved Michaelis-Arbuzov rearrangement (Bhattacharya et al., 1981; Eymery et al., 1999) (Scheme 1.14).

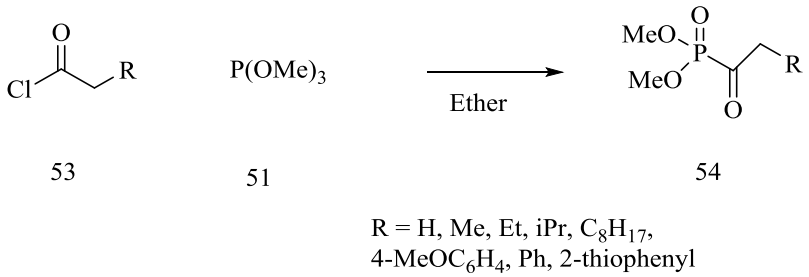


50 51 52

R = alkyl, aryl, etc.
R1 = alkyl, acyl, etc.
X = Cl, Br, I

Scheme 1.14. General reaction pathway for the Michaelis-Arbuzov reaction

Application of Michaelis-Arbuzov reaction can be seen to synthesis α-ketophosphonates **(54)** via reacting acyl halides **(53)** with phosphite **(51)** under relatively mild conditions (Afarinkia et al., 1997) (Scheme 1.15).



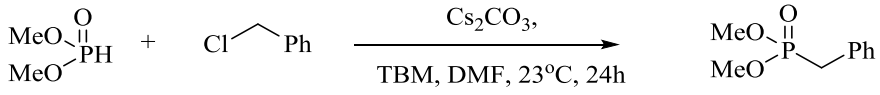
53 51 54

R = H, Me, Et, iPr, C_8H_{17}, 4-$MeOC_6H_4$, Ph, 2-thiophenyl

Scheme 1.15. Synthesis of α- ketophosphonates with Michaelis-Arbuzov reaction

Although the Michaelis-Becker and Michaelis-Arbuzov reactions are the usual techniques for obtaining organophosphonate esters, there are some major drawbacks. In Michaelis-Arbuzov reaction; the condition of higher temperature restricts the opportunity of the expected product for the reaction besides having a very long reaction time which can also lead to a complex combination of getting side products. The use of strong anhydrous base and also longer reaction times in Michaelis-Becker reaction results in lowly yield of phosphonates.

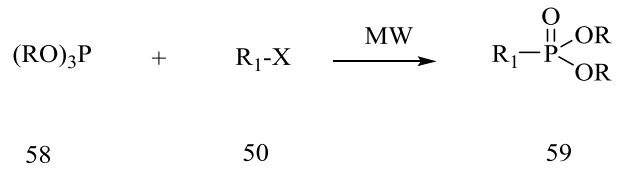
Cohen et al. (2003) used many alkali metal carbonates in order to improve new methods for the procurement of phosphonates. Cesium carbonate (Cs_2CO_3) was found to be the most successful base. The reaction was performed with dimethylphosphite **(55)** and benzyl chloride **(56)** in the presence of TBAI at 23°C with Cs_2CO_3. Dimethyl benzylphosphonate **(57)** was attained in 97 % yield. Same procedure was applied to various dialkylphosphites **(58)** and alkyl halides **(50)** and the products were obtained in good yields (Scheme 1.16).



55 56 57

Scheme 1.16. Synthesis of dimethyl benzylphosphonate **(57)** in the presence of Cs_2CO_3

The synthesis of phosphonates was tried in microwave irradiation an applicable technique. This technique permits the synthesis of phosphonates in a short period of time at higher temperatures. Kiddle et al. (2000) used microwave irradiation for the synthesis of alkylphosphonates **(59)** from alkylphosphites **(58)** and alkyl halides **(50)** were achieved in good yields (Scheme 1.17).



Scheme 1.17. Microwave irradiated Michaelis-Arbuzov reaction

2. AIM AND SCOPE OF THE STUDY

Thiazolo[3,2-a]pyridin-8-yl **(60)** derivatives being one of the bioactive compounds that have been extensively studied in the field of organic and medicinal chemistry due to their biochemical and biological activities. (Altug et al. 2011; Ganpat and Pemawat, 2011) They have been synthesized by numerous ways for the past century. The bioactivities and the challenge related with the formation of heterocyclic and carbocyclic compounds make these synthetic targets very attractive.

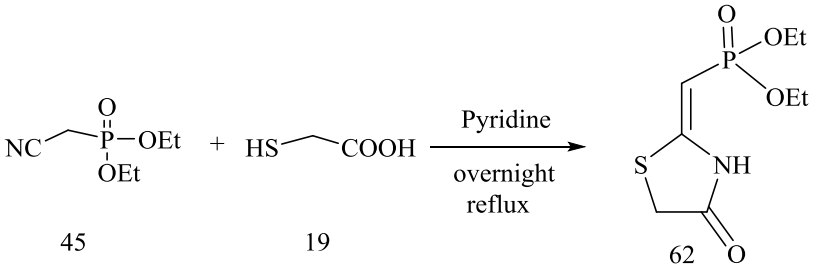
In this study, the aim is to develop a new synthetic method with the help of MCR approaches to synthesize thiazolo[3,2-a]pyridin-8-yl-phosphonates **(63)**. In the course of this work, the diethyl ((4-oxothiazolidin-2-ylidene)methyl)phosphonate **(62)** was first synthesized by reacting diethyl (cyanomethyl)phosphonate **(61)** and 2-mercaptoacetic acid **(25)** in pyridine. Then, diethyl ((4-oxothiazolidin-2-ylidene)methyl)phosphonate, malononitrile **(26)** and two equivalents of various aromatic aldehydes **(24)** reacted to give desired compounds.

The prime objective of this study is to identify safe and effective synthesis of phosphorous containing new compounds and to observe their anti-cancer activities.

3. MATERIALS AND METHODS

^{1}H and ^{13}C NMR spectra were recorded on a BRUKER AVANCE spectrometer (400 MHz for proton, 100 MHz for carbon). Infrared spectra were recorded on a SHIMADZU FTIR-8400S instrument (KBr pellet and neat). Mass spectra were recorded on a Fisons VG Platform II spectrometer and/or on a Micromass Q-TOF Micro spectrometer. Flash Column chromatography was performed on Silica Gel (Merck, 230-400 mesh). Melting points were determined on a MELTEMP apparatus and were found to be correct. TLC was done using precoated plates with fluorescent indicator (Merck 5735). The stain solution of permanganate was used for visualization of the TLC spots. Synthesis of diethyl (thiazolidin-2-ylidenemethyl) phosphonate was synthesized according to the methods described in the literature.

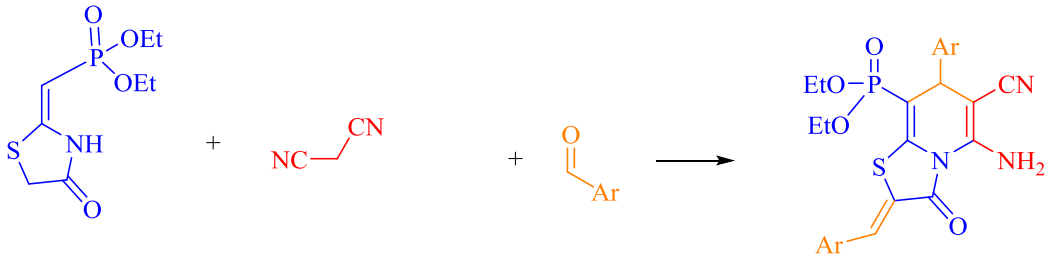
3.1 Synthesis of diethyl-((4-oxothiazolidin-2-ylidene)methyl) phosphonate (62)



45 19 62

Diethyl (cyanomethyl)phosphonate (15 mmol, 2.657 g) and mercaptoacetic acid (15 mmol, 1.382 g) were mixed in pyridine (7.5 mL) in a round-bottomed flask and refluxed at 120˚C for overnight. Open side of condenser was closed using stopper to prevent the vaporising of pyridine under reflux. After cooling, excess pyridine was removed by rotary evaporator. Then, it was subjected to column chromatography. White solid 1.79 g (47%) R_f (EtOAc: Hex = 1:1): 0.12 $\nu_{max.}$ (KBr): 1714 (C=O), 3400 (N-H), 684 (C-S), 1182 (P=O), 1697 (C=C), 1051 (P-O). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.33 (s, 1H), 4.76 (d, *J* = 8.9 Hz, 1H), 3.92 – 3.81 (m, 4H), 3.76 (s, 2H), 1.18 (t, *J* = 7.0, 3H), 1.17 (t, *J* = 7.1, 3H).

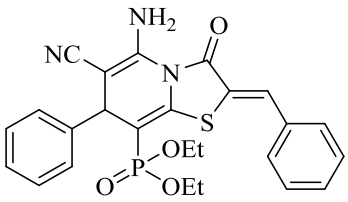
3.2 General reaction procedure for preparation of thiazolo[3,2-a] pyridin-8-yl-phosphonate derivatives: (63a-l)



62 25 24 63a-l

Diethyl ((4-oxothiazolidin-2-ylidene)methyl)phosphonate (1.0 eq, 0.5 mmol), malononitrile (1.2 eq, 0.6 mmol) and an aromatic aldehyde (2.2 eq, 1.1 mmol) were set in a round-bottomed flask. Then, the mixture was dissolved using MeCN then a catalytic amount of triethylamine was added, after which it was heated under reflux. The reaction were completed within 2 hours, while were controlled by TLC. The solid formed during the reaction were filtered and then recrystallized from ethyl acetate/hexane.

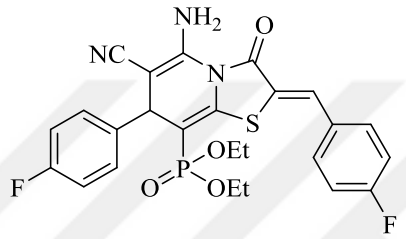
3.2.1 Diethyl (Z)-(5-amino-2-benzylidene-6-cyano-3-oxo-7-phenyl-2,3-dihydro-7H-thiazolo[3,2-a]pyridin-8-yl)phosphonate 63a



Orange solid obtained (177 mg, 72%) m.p: 238-239 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.50 $\nu_{max.}$ (KBr): 3383 (NH_2), 2195 (C≡N), 1712 (C=O), 1149 (P=O), 1022 (P-O). *m/z* (TOF ES$^+$) 494.13 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.60 – 7.57 (m, 2H), 7.46 (t, *J* = 7.3 Hz, 2H), 7.41 (d, *J* = 7.2 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 2H), 7.26 – 7.24 (m, 1H), 7.21 – 7.18 (m, 2H), 6.56 (s, NH_2, 2H) 4.31 (d, *J* = 8.4 Hz, 1H), 4.14 – 4.01 (m, 2H), 3.72 (p, *J* = 9.8, 7.0 Hz, 1H), 3.17 (p, *J* = 9.8, 7.1 Hz, 1H), 1.35 (t, *J* = 7.1 Hz, 3H), 0.80 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 147.7, 142.84, 141.2 (d, *J* = 21.7 Hz), 133.4, 132.1, 130.5, 130.2,

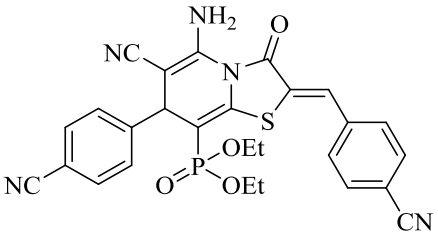
129.2, 128.7, 127.7 (d, *J* = 2.3 Hz), 120.8, 118.6, 103.2, 101.2, 68.4 (d, *J* = 13.0 Hz), 62.4 (d, *J* = 5.2 Hz), 62.1 (d, *J* = 4.9 Hz), 41.9 (d, *J* = 7.0 Hz), 16.4 (d, *J* = 6.4 Hz), 15.6 (d, *J* = 7.2 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 16.26. HRMS: required for $C_{25}H_{25}N_3O_4PS$ 494.1303 and found 494.1314.

3.2.2 Diethyl-(Z)-5-amino-6-cyano-2-(4-fluorobenzylidene)-7-(4-fluorophenyl)-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-ylphosphonate 63b



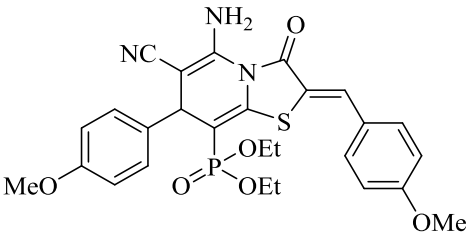
Light yellow solid obtained (133 mg, 50%) m.p: 228 °C decomp. R_f (EtOAc: Hex = 1:1): 0.56 $\nu_{max.}$ (KBr): 3379 (NH_2), 2195 (C≡N), 1712 (C=O), 1149 (P=O), 1049 (P-O). *m/z* (TOF ES+) 530.11 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.57 (dd, *J* = 8.6, 5.3 Hz, 2H), 7.20 – 7.12 (m, 4H), 7.03 – 6.97 (m, 2H), 6.58 (s, NH_2, 2H), 4.33 (d, *J* = 8.2 Hz, 1H), 4.13 – 4.02 (m, 2H), 3.76 (p, *J* = 9.9, 7.1 Hz, 1H), 3.29 (p, *J* = 14.2, 9.9, 7.1 Hz, 1H), 1.35 (t, *J* = 7.1, 0.4 Hz, 3H), 0.87 (t, *J* = 7.1, 0.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 164.2 (d, *J* = 129.7 Hz), 161.7 (d, *J* = 122.4 Hz), 147.8 (d, *J* = 1.5 Hz), 141.1 (d, *J* = 21.6 Hz), 138.8 (d, *J* = 3.2 Hz), 132.7 (d, *J* = 8.7 Hz), 131.1, 129.8 (d, *J* = 3.4 Hz), 129.5 (d, *J* = 8.3 Hz), 120.3 (d, *J* = 2.7 Hz), 118.6 (d, *J* = 2.4 Hz), 116.7 (d, *J* = 22.1 Hz), 115.7 (d, *J* = 21.7 Hz), 103.2, 101.2, 68.1 (d, *J* = 14.2 Hz), 62.6 (d, *J* = 5.4 Hz), 62.2 (d, *J* = 5.1 Hz), 41.3 (d, *J* = 7.1 Hz), 16.4 (d, *J* = 6.3 Hz), 15.8 (d, *J* = 7.2 Hz). HRMS: required for $C_{25}H_{23}N_3O_4PSF_2$ 530.1115 and found 530.1116.

3.2.3 Diethyl-(Z)-5-amino-6-cyano-2-(4-cyanobenzylidene)-7-(4-cyanophenyl)-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-ylphosphonate 63c



Dark yellow solid obtained (185 mg, 68%) m.p: 205-207 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.64 $\nu_{max.}$ (KBr): 3433 (NH_2), 2187 (C≡N), 2233 (Ar-C≡N), 1701 (C=O), 1149 (P=O), 1045 (P-O). *m/z* (TOF ES$^+$) 544.12 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (apparently d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.66 (s, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.3 Hz, 2H), 6.63 (s, 2H), 4.43 (d, *J* = 7.9 Hz, 1H), 4.16 – 4.05 (m, 2H), 3.79 (p, *J* = 9.9, 7.2 Hz, 1H), 3.45 – 3.34 (p, 1H), 1.37 (t, *J* = 7.1 Hz, 3H), 0.86 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ165,6, 147.7 (dd, *J* = 6.6, 1.2 Hz), 140.9 (d, *J* = 21.5 Hz), 137.3, 132.8, 132.6, 130.6, 129.6, 128.6, 124.4, 118.2 (d, *J* = 31.3 Hz), 117.8 (d, *J* = 2.5 Hz), 113.3, 111.7, 103.3, 101.3, 67.1 (d, *J* = 12.9 Hz), 62.8 (d, *J* = 5.5 Hz), 62.4 (d, *J* = 5.4 Hz), 42.0 (d, *J* = 6.5 Hz), 16.3 (d, *J* = 6.2 Hz), 15.7 (d, *J* = 6.9 Hz). HRMS: required for $C_{27}H_{23}N_5O_4PS$ 544.1208 and found 544.1218.

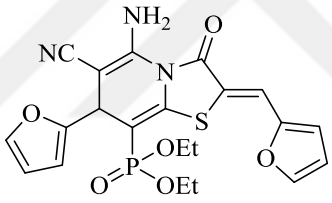
3.2.4 Diethyl-(Z)-5-amino-6-cyano-2-(4-methoxybenzylidene)-7-(4-methoxyphenyl)-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-ylphosphonate 63d



Orange solid obtained (210 mg, 76%) m.p: 218-219 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.61 νmax. (KBr): 3383 (NH_2), 2195 (C≡N), 1708 (C=O), 1149 (P=O), 1026

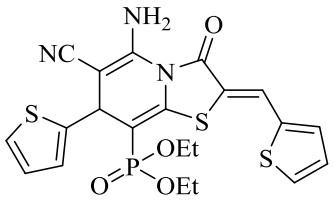
(P-O), 1103 (O-CH_3). *m/z* (TOF ES^+) 554.15 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.55 (d, *J* = 8.7 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.55 (broad s, 2H), 4.28 (d, *J* = 8.3 Hz, 2H), 4.13 – 4.01 (m, 1H), 3.86 (s, 3H), 3.82 – 3.71 (m, 4H), 3.25 (p, *J* = 9.9, 7.1 Hz, 1H), 1.36 (t, *J* = 7.1 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 161.2, 159.1, 147.7, 141.2 (d, *J* = 21.6 Hz), 135.3, 132.6, 132.0, 128.8, 126.2, 118.8 (d, *J* = 2.4 Hz), 117.6, 114.7, 114.1, 102.7, 100.8, 68.4 (d, *J* = 13.2 Hz), 62.3 (d, *J* = 5.3 Hz), 62.0 (d, *J* = 4.8 Hz), 55.4 (d, *J* = 13.5 Hz)., 41.1 (d, *J* = 7.0 Hz), 16.4 (d, *J* = 6.5 Hz), 15.7 (d, *J* = 7.2 Hz). HRMS: required for $C_{27}H_{29}N_3O_6PS$ 554.1515 and found 554.1522.

3.2.5 Diethyl-(Z)-5-amino-6-cyano-7-(furan-2-yl)-2-(furan-2-ylmethylene)-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-ylphosphonate 63e



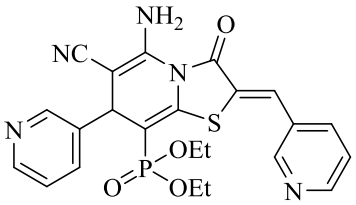
Dark brown solid obtained (178 mg, 75%) m.p: 191-192 ^oC. R_f (EtOAc: Hex = 1:1): 0.38 $\nu_{max.}$ (KBr): 3379 (NH_2), 2195 (C≡N), 1708 (C=O), 1153 (P=O), 1053 (P-O). *m/z* (TOF ES^+) 474.09 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 1.7 Hz, 1H), 7.44 (s, 1H), 7.29 (dd, *J* = 1.7, 0.5 Hz, 1H), 6.78 (d, *J* = 3.6 Hz, 1H), 6.59 (s, 2H), 6.55 (dd, *J* = 3.5, 1.8 Hz, 1H), 6.30 (dd, *J* = 3.2, 1.9 Hz, 1H), 6.20 (dd, *J* = 3.2, 0.7 Hz, 1H), 4.47 (d, *J* = 8.3 Hz, 1H), 4.13 – 4.01 (m, 2H), 3.91 (p, *J* = 9.9, 7.1 Hz, 1H), 3.53 (p, *J* = 9.9, 7.1 Hz, 1H), 1.34 (t, *J* = 7.1, 3.7 Hz, 3H), 1.07 (t, *J* = 7.1, 0.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 153.5, 150.3, 146.5, 142.4, 118.8, 118.0, 117.4, 113.3, 110.9, 110.8, 107.2, 101.9, 97.7, 74.4, 63.6, 62.5 (d, *J* = 5.1 Hz), 62.4 (d, *J* = 4.8 Hz), 35.2 (d, *J* = 6.7 Hz), 16.4 (d, *J* = 6.3 Hz), 16.0 (d, *J* = 7.3 Hz. HRMS: required for $C_{21}H_{21}N_3O_6PS$ 474.0889 and found 474.0898.

3.2.6 Diethyl-(Z)-5-amino-6-cyano-3-oxo-7-(thiophen-2-yl)-2-(thiophen-2-ylmethylene)-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-ylphosphonate 63f



Yellow solid obtained (200 mg, 79%) m.p: 188-189 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.35 $\nu_{max.}$ (KBr): 3375 (NH_2), 2198 (C≡N), 1708 (C=O), 1149 (P=O), 1049 (P-O). m/z (TOF ES+) 506.04 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.64 (d, *J* = 5.0 Hz, 1H), 7.43 (d, *J* = 3.6 Hz, 1H), 7.21 – 7.16 (m, 2H), 6.95 (d, *J* = 3.3 Hz, 1H), 6.90 (dd, *J* = 5.0, 3.6 Hz, 1H), 6.58 (s, 2H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.13 – 4.01 (m, 2H), 3.91 (p, *J* = 9.9, 7.0 Hz, 1H), 3.49 (p, *J* = 9.8, 7.2 Hz, 1H), 1.34 (t, *J* = 7.1 Hz, 3H), 1.00 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.1, 147.5, 138.0, 132.8, 131.8, 128.9, 127.0, 125.4 (d, *J* = 8.0 Hz), 124.8, 118.7, 103.1, 67.7 (d, *J* = 1.3 Hz), 62.6 (d, *J* = 6.8 Hz), 36.8 (d, *J* = 6.3 Hz), 16.4 (d, *J* = 6.6 Hz), 15.9 (d, *J* = 7.3 Hz). HRMS: required for $C_{21}H_{21}N_3O_4PS_3$ 506.0432 and found 506.0411.

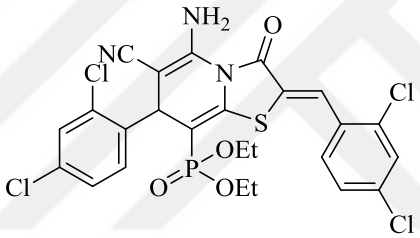
3.2.7 Diethyl-(Z)-5-amino-6-cyano-3-oxo-7-(pyridin-3-yl)-2-(pyridin-3-ylmethylene)-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-ylphosphonate 63g



Yellow solid obtained (195 mg, 79%) m.p: 202-203 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.32 $\nu_{max.}$ (KBr,): 3371 (NH_2), 2187 (C≡N), 1701 (C=O), 1149 (P=O), 1018 (P-O), 1643 (C=N). m/z (TOF ES+) 496.12 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.3 Hz, 1H), 8.60 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.52 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.48 (dd, *J* = 2.3, 0.6 Hz, 1H), 7.92 (ddd, *J* = 4.2, 2.2, 1.0 Hz, 1H), 7.56 – 7.50

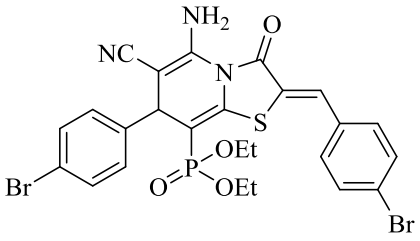
(m, 1H), 7.40 (dd, *J* = 8.1, 4.8 Hz, 1H), 7.27 (dd, *J* = 7.9, 4.8, 0.7 Hz, 2H), 6.67 (s, 2H), 4.39 (d, *J* = 7.9 Hz, 1H), 4.15 – 4.03 (m, 2H), 3.84 – 3.71 (p, 1H), 3.43 – 3.32 (p, 1H), 1.36 (t, *J* = 7.1 Hz, 3H), 0.84 (t, *J* = 7.1, 0.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 152.2, 150.6, 149.2 (d, *J* = 32.9 Hz)., 148.1, 140.9, 138.4, 136.0, 135.5, 129.5, 128.4, 124.0, 123.8, 123.2, 118.2, 103.1, 101.2, 67.1 (d, *J* = 12.9 Hz), 62.8 (d, *J* = 5.6 Hz), 62.4 (d, *J* = 5.4 Hz), 39.8 (d, *J* = 6.6 Hz), 16.4 (d, *J* = 6.3 Hz), 15.8 (d, *J* = 7.3 Hz). HRMS: required for $C_{23}H_{23}N_5O_4PS$ 496.1208 and found 496.1205.

3.2.8 Diethyl-(Z)-5-amino-6-cyano-2-(2,4-dichlorobenzylidene)-7-(2,4-dichlorophenyl)-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-ylphosphonate 63h



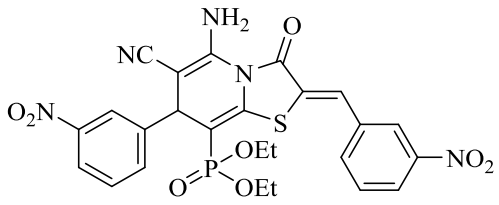
Yellow solid obtained (215 mg, 68%) m.p: 250-251 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.41 $\nu_{max.}$ (KBr): 3371 (NH_2), 2198 (C≡N), 1716 (C=O), 1149 (P=O), 1049 (P-O). m/z (TOF ES+) 629.97 M-H (72%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H, C=CH), 7.59 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.50 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 1H), 6.65 (broad s, 2H, NH_2), 5.10 (s, 1H, ArCH), 4.21 – 3.95 (m, 2H), 3.86 – 3.62 (m, 1H), 3.38 (s, 1H), 1.37 (t, *J* = 7.1 Hz, 3H), 0.85 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 134.4, 133.8, 133.6, 131.6, 129.7, 127.9, 127.8, 127.5, 127.4, 126.7, 124.8, 120.39, 120.35, 117.9, 100.8, 62.7 (d, *J* = 4.8 Hz), 62.2 (d, *J* = 5.4 Hz), 16.3 (d, *J* = 6.3 Hz), 15.5 (d, *J* = 7.4 Hz). HRMS: required for $C_{25}H_{21}N_3O_4PSCl_4$ 629.9745 and found 629.9737.

3.2.9 Diethyl-(Z)-5-amino-2-(4-bromobenzylidene)-7-(4-bromophenyl)-6-cyano-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-ylphosphonate 63i



Dark Yellow solid obtained (257 mg, 79%) m.p: 217-219 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.48 $\nu_{max.}$ (KBr): 3375 (NH_2), 2195 (C≡N), 1720 (C=O), 1149 (P=O), 1006 (P-O) m/z (TOF ES+) 649.95 M-H (56%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 1.3 Hz, 2H), 7.58 (s, 1H), 7.45 (d, *J* = 3.1 Hz, 2H), 7.43 (d, *J* = 3.2 Hz, 2H), 7.10 – 7.06 (m, 2H), 6.58 (s, 2H), 4.31 (d, *J* = 8.1 Hz, 1H), 4.15 – 4.01 (m, 2H), 3.77 (p, *J* = 9.9, 7.1 Hz, 1H), 3.32 (p, *J* = 14.2, 9.9, 7.1 Hz, 1H), 1.36 (t, *J* = 7.1 Hz, 3H), 0.88 (t, *J* = 7.0, 0.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 147.8, 141.9, 132.7, 132.6, 132.3, 132.0, 131.9, 131.8, 131.6, 130.9, 129.5, 125.0, 121.7, 121.4, 67.8 (d, *J* = 3.1 Hz), 62.7 (d, *J* = 1.3 Hz), 59.3 (d, *J* = 4.3 Hz), 41.5 (d, *J* = 6.8 Hz), 16.4 (d, *J* = 6.4 Hz), 15.8 (d, *J* = 7.4 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 15.9. HRMS: required for $C_{25}H_{23}N_3O_4PSBr_2$ 649.9514 and found 649.9511.

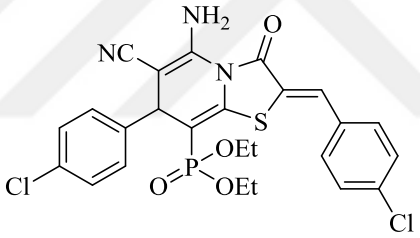
3.2.10 Diethyl-(Z)-(5-amino-6-cyano-2-(3-nitrobenzylidene)-7-(3-nitrophenyl)-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-yl)phosphonate 63j



Yellow solid obtained (207 mg, 71%) m.p: 209-210 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.24 $\nu_{max.}$ (KBr): 3379 (NH_2), 2195 (C≡N), 1716 (C=O), 1153 (P=O), 1045 (P-O), 1350 and 1323 (symmetric NO_2), 1562 and 1520 (asymmetric NO_2). m/z (TOF ES+) 584.10 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (t, *J* = 2.0 Hz, 1H),

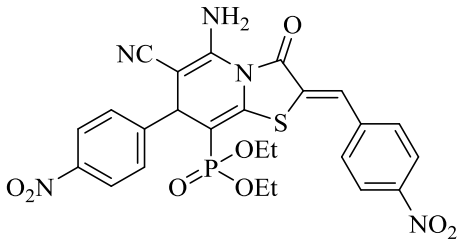
8.27 – 8.22 (m, 1H), 8.17 – 8.13 (m, 1H), 8.06 (t, *J* = 1.9 Hz, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.73 (s, 1H), 7.68 (t, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.53 (t, *J* = 7.8 Hz, 1H), 6.71 (s, *J* = 15.1 Hz, 2H), 4.54 (d, *J* = 7.6 Hz, 1H), 4.18 – 3.99 (m, 2H), 3.89 – 3.74 (m, 1H), 3.57 – 3.37 (m, 1H), 1.35 (t, *J* = 7.1 Hz, 3H), 0.85 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 148.7 (d, *J* = 19.7 Hz), 148.0, 147.8, 144.8 (d, *J* = 1.4 Hz), 135.0, 134.7, 134.3, 130.5, 129.8, 129.4, 125.6, 124.5, 124.0, 123. (d, *J* = 7.8 Hz), 103.2, 101.3, 66.9, 66.8 (d, *J* = 12.9 Hz), 66.7, 63.0, 62.5 (d, *J* = 5.7 Hz), 41.9 (d, *J* = 6.7 Hz), 16.4 (d, *J* = 6.4 Hz), 15.8 (d, *J* = 7.2 Hz) ^{31}P NMR (162 MHz, $CDCl_3$) δ 15.8. HRMS: required for $C_{25}H_{23}N_5O_8PS$ 584.1005 and found 584.1008.

3.2.11 Diethyl-(Z)-(5-amino-2-(4-chlorobenzylidene)-7-(4-chlorophenyl)-6-cyano-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-yl)phosphonate 63k



Orange solid obtained (206 mg, 73%) m.p: 233-234 ^{o}C. R_f (EtOAc: Hex = 1:1): 0.53 $\nu_{max.}$ (KBr): 3390 (NH_2), 2195 (C≡N), 1705 (C=O), 1153 (P=O), 1010 (P-O). m/z (TOF ES+) 562.05 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 6.8 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.58 (s, 2H), 4.32 (d, *J* = 8.1 Hz, 1H), 4.14 – 3.99 (m, 2H), 3.85 – 3.68 (m, 1H), 3.39 – 3.24 (m, 1H), 1.36 (t, *J* = 7.1 Hz, 3H), 0.87 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 165.7 (d, *J* = 104.9 Hz), 147.5, 138.3, 137.5, 136.5, 134.8, 133.6, 131.7 (d, *J* = 16.8 Hz), 132.0, 129.7 (d, *J* = 6.4 Hz), 129.2, 121.2, 117.9, 103.1, 89.2, 65.7, 62.7 (d, *J* = 5.3 Hz), 62.5 (dd, *J* = 33.0, 5.2 Hz), 41.5, 16.1 (dd, *J* = 69.7, 6.5 Hz). ^{31}P NMR (162 MHz, $CDCl_3$) δ 15.8. HRMS: required for $C_{25}H_{23}N_3O_4PSCl_2$ 562.0524 and found 562.0518.

3.2.12 Diethyl-(Z)-(5-amino-6-cyano-2-(4-nitrobenzylidene)-7-(4-nitrophenyl)-3-oxo-3,7-dihydro-2H-thiazolo[3,2-a]pyridin-8-yl)phosphonate 63l

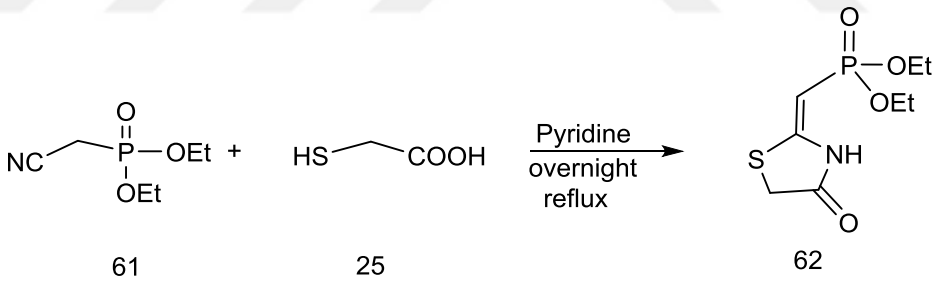


Brown solid obtained (216 mg, 74%) m.p: 211-212 ^{o}C. R_f (EtOAc: Hex = 1:1) 0.35 $\nu_{max.}$ (KBr): 3406 (NH_2), 2195 (C≡N), 1705 (C=O), 1149 (P=O), 1049 (P-O). m/z (TOF ES+) 584.09 M-H (100%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 8.8 Hz, 2H), 8.18 (d, *J* = 8.8 Hz, 2H), 7.72 (d, *J* = 1.9 Hz, 1H), 7.70 (d, *J* = 3.8 Hz, 2H), 7.38 (d, *J* = 8.8 Hz, 2H), 6.73 (s, *J* = 15.0 Hz, 2H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.20 – 4.02 (m, 2H), 3.83 – 3.60 (m, 1H), 3.49 – 3.26 (m, 1H), 1.35 (t, *J* = 7.1 Hz, 3H), 0.84 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 149.7, 149.6, 147.8, 147.4, 139.3, 131.4, 131.0, 129.2, 128.8, 125.1, 124.7, 124.5, 124.1, 66.8 (d, *J* = 12.9 Hz), 63.0 (d, *J* = 5.7 Hz), 62.6, 62.5 (d, *J* = 5.6 Hz), 41.9 (d, *J* = 6.6 Hz), 16.5 (d, *J* = 6.3 Hz), 15.8 (d, *J* = 6.9 Hz).^{31}P NMR (162 MHz, $CDCl_3$) δ 15.1. HRMS: required for $C_{25}H_{23}N_5O_8PS$ 584.1005 and found 584.0996.

4. RESULTS AND DISCUSSIONS

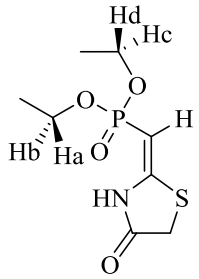
Multi-component reaction has been used broadly in synthetic organic chemistry, because of its time and energy saving, simplicity, low-cost, environmental safety and also favorable for drug synthesis and industrial usage (Akbarzadeh et al., 2014). According to this scientific knowledge, we aimed to synthesis of thiazolo[3,2-a]pyridin-8-yl-phosphonate derivatives **(63a-l)** *via* multicomponent reactions in a solvent under reflux conditions.

In the first part of this study, diethyl ((4-oxothiazolidin-2-ylidene)methyl) phosphonate **(62)** was prepared by mixing diethyl (cyanomethyl)phosphonate **(61)** and mercaptoacetic acid **(25)** in pyridine in a reaction vessel, then reactants were heated under refluxed at 120 ^{o}C for overnight. TLC was used to decide the completion of the reaction. Then, the reaction was cooled, pyridine removed under reduce presure and the observed yellowish liquid was subjected to column chromatography (EtOAc/Hexane: 3/1) (Scheme 4.1).



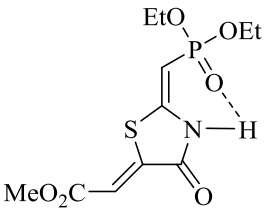
Scheme 4.1. Synthesis of diethyl-((4-oxothiazolidin-2-ylidene)methyl)phosphonate

Structure of the compound (**62)** was determined on the basis of IR and ^{1}H NMR. The IR spectrum of compound (**62)** showed amide carbonyl stretch at 1708 cm^{-1}, NH stretching at around 3362 cm^{-1}, and P=O stretch at 1176 and 1049 cm^{-1}.

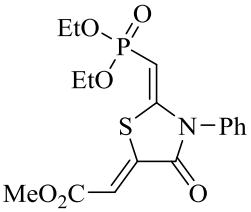


^{1}H NMR spectrum of (**62)** in DMSO-*d6* displays interesting characteristics because of phosphorous-hydrogen interaction and magnetic non equivalence of atoms within geminal groups attached to the prochiral phosphorous atom. In compound (**62)** the phosphorous atom is prochiral and the geminal ethoxy groups are enantiotopic and hence the chemical shifts are equivalent in an achiral environment. Nevertheless, the methylene carbon atoms within each ethoxy group are also prochiral and the methylene protons are diastereotopic (Byrne et al., 1994). For compound **(62)** ethoxy groups methylene protons showed a signal in the region of 3.96-4.10 ppm and 4.18-4.32 ppm region is ascribed for methyl proton. Methylene protons on the heterocyclic ring showed a signal at 3.76 ppm.

The stereochemistry of the similar analogues of compound **(62)** was investigated by Kozlov et al., 2004. They confirmed the structure of the compound (**64)** by the X-ray spectrometry. The *E,Z* isomers of (**64)** are stabilized in a solid state by strong intramolecular hydrogen bonds P=O···HN. In addition to this, when they introduced phenyl group to nitrogen atom, they confirmed the stereochemistry of the compound (**65)** goes to *Z* isomer rather than the *E* isomer.



64



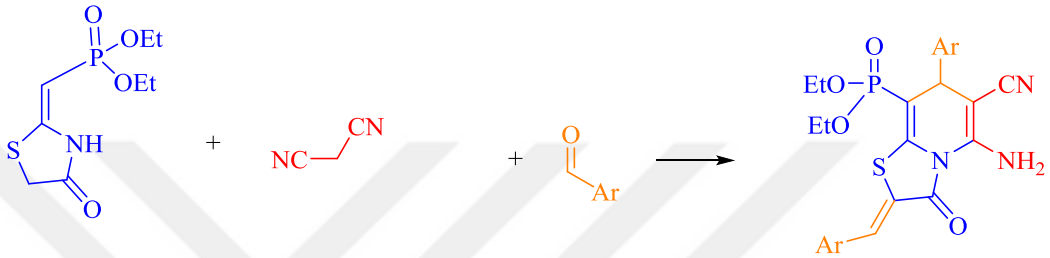
65

Thiazolo[3,2-a]pyridin-8-yl-phosphonates **(63a-l)** were simply prepared through multicomponent reaction by adding diethyl ((4-oxothiazolidin-2-ylidene)methyl)phosphonate **(62)**, malononitrile **(25)** and two equivalent of aromatic aldehydes **(24)** in the presence of a catalytic amount of Et_3N in a reaction vessel at 80 ^{0}C in dry acetonitrile and the progress of the reactions were monitored by TLC. The reactions were completed within 2 h without starting materials found after filtration. When we used aliphatic aldehydes like propanal or hexanal. we couldn't obtain the desired product with acceptable yields which might be attributed to the electronic releasing property of alkyl group rather than electron withdrawing feature of phenyl

ring on Knoevenagel intermediate which may not allow nucleophilic attack of thiazolinone ring.

The scope and generality of the reaction with respect to various aromatic or hetero aromatic aldehydes were investigated. The reaction continues rapidly and affords the corresponding thiazolo[3,2-a]pyridin-8-yl-phosphonate in high yields. The results shown in Table 4.1.

Table 4.1. Substituents, reaction yields and time of compound **63a-l** and IR spectra



62 25 24 63a-l

Entry	Compounds	Ar	%Yield	Time	$\nu_{max.\ C\equiv N}$	$\nu_{max.\ NH}$
1	63a	C_6H_5	72	2h	2195	3383
2	63b	4-F-C_6H_4	50	2h	2195	3379
3	63c	4-CN-C_6H_4	68	2h	2187	3433
4	63d	4-MeO-C_6H_4	76	2h	2195	3383
5	63e	2-furyl	75	2h	2195	3379
6	63f	2-thienyl	79	2h	2198	3375
7	63g	3-pyridyl	79	2h	2187	3371
8	63h	2,4-diCl_2-C_6H_3	68	2h	2198	3371
9	63i	4-Br-C_6H_4	79	2h	2195	3375
10	63j	3-NO_2-C_6H_4	71	2h	2195	3379
11	63k	4-Cl-C_6H_4	73	2h	2195	3390
12	63l	4-NO_2-C_6H_4	74	2h	2195	3406

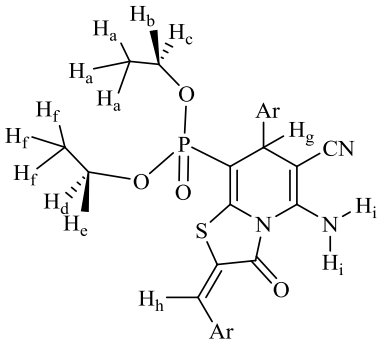


Table 4.2. Selected proton with their chemical shifts in ppm of compounds from **63a-l** in $CDCl_3$

	Ha	**Hb**	**Hc**	**Hf**	**Hd-e**	**Hg**	**Hh**	**Hi**
63a	0.80	3.17	3.72	1.35	4.14-4.01	4.31	7.68	6.52
63b	0.87	3.29	3.76	1.35	4.13-4.02	4.33	7.64	6.58
63c	0.86	3.45-3.34	3.79	1.37	4.16-4.05	4.43	7.66	6.63
63d	0.88	3.25	3.71-3.82	1.36	4.13-4.01	4.28	7.64	6.5
63e	1.07	3.53	3.91	1.34	4.13-4.01	4.47	7.44	6.59
63f	1.00	3.49	3.91	1.34	4.13-4.01	4.70	7.86	6.58
63g	0.84	3.43-3.32	3.84-3.71	1.36	4.15-4.03	4.39	8.80	6.67
63h	0.85	3.38	3.86-3.62	1.37	4.21-3.95	5.10	8.03	6.65
63i	0.88	3.32	3.77	1.36	4.15-4.01	4.31	7.58	6.58
63j	0.85	3.57-3.37	3.89-3.74	1.35	4.18-3.99	4.54	7.73	6.71
63k	0.87	3.39-3.24	3.85-3.68	1.36	4.14-3.99	4.32	7.38	6.58
63l	0.84	3.49-3.26	3.83-3.60	1.35	4.20-4.02	4.49	7.72	6.73

In the IR spectra of compound **(63a-l)**, distinctive absorption band for NH_2 stretching frequencies appeared at around 3371 – 3433 cm^{-1}. The C=O stretching bands can be seen at around 1700 – 1718 cm^{-1}, a strong sharp peak for C≡N at about 2195 cm^{-1}, P=O at 1153cm^{-1}, and for P – O at about 1010 cm^{-1}.

The results obtained from ^{1}H NMR compounds **(63a-l)** seem to possess similar protons (H_a-H_h) with similar chemical shifts as seen in Table 4.2. The NH_2 (H_i) shows a signal around 6.52 – 6.73 ppm with a broad singlet, the C=C-H (H_g) on the hydropyridine ring shows a peak around 4.28 – 5.10 ppm, with a doublet, C=CHR (H_h) shows also a doublet in most cases around 7.38 – 8.80 ppm. Additionally, the CH_2 on the diethoxy phosphonate show diastereotopic property due to P-H couplings. It shows one H split from the other as distinct single proton and the readings of each are as follows one of the proton (H_b) shows around 3.17 – 3.57 ppm and the other at (H_c) 3.60 – 3.91 ppm with both showing a quintet on the ^{1}H NMR spectra.

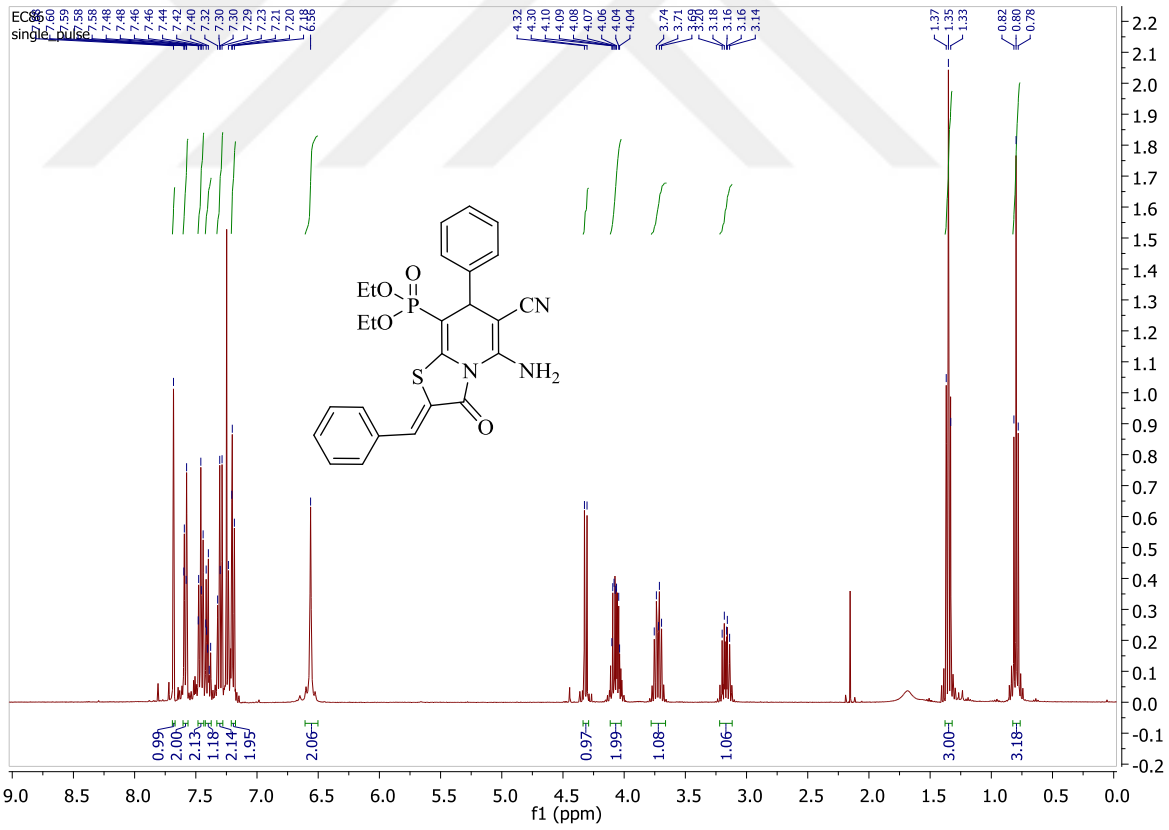


Figure 4.1. ^{1}H NMR of compound **63a**

On the ^{13}C NMR part similar shifts from **(63a-l)** were also observed with almost precise values as they were read on the spectra (C=O) peaks shows around 146–148 ppm, (C=C) peaks shows around 127–132 ppm and (C≡N) peaks shows around 146 – 148 ppm and methyl resonate at regions between 15–17 ppm. Peaks that appeared at 15.9, 15.1 and 15.8 ppm on ^{31}P NMR are for 63i, 63j and 63l respectively. At 16.4 and 15.6 ppm there is a carbon-phosphorus with a coupling constant at 6.4 and 7.2 Hz.

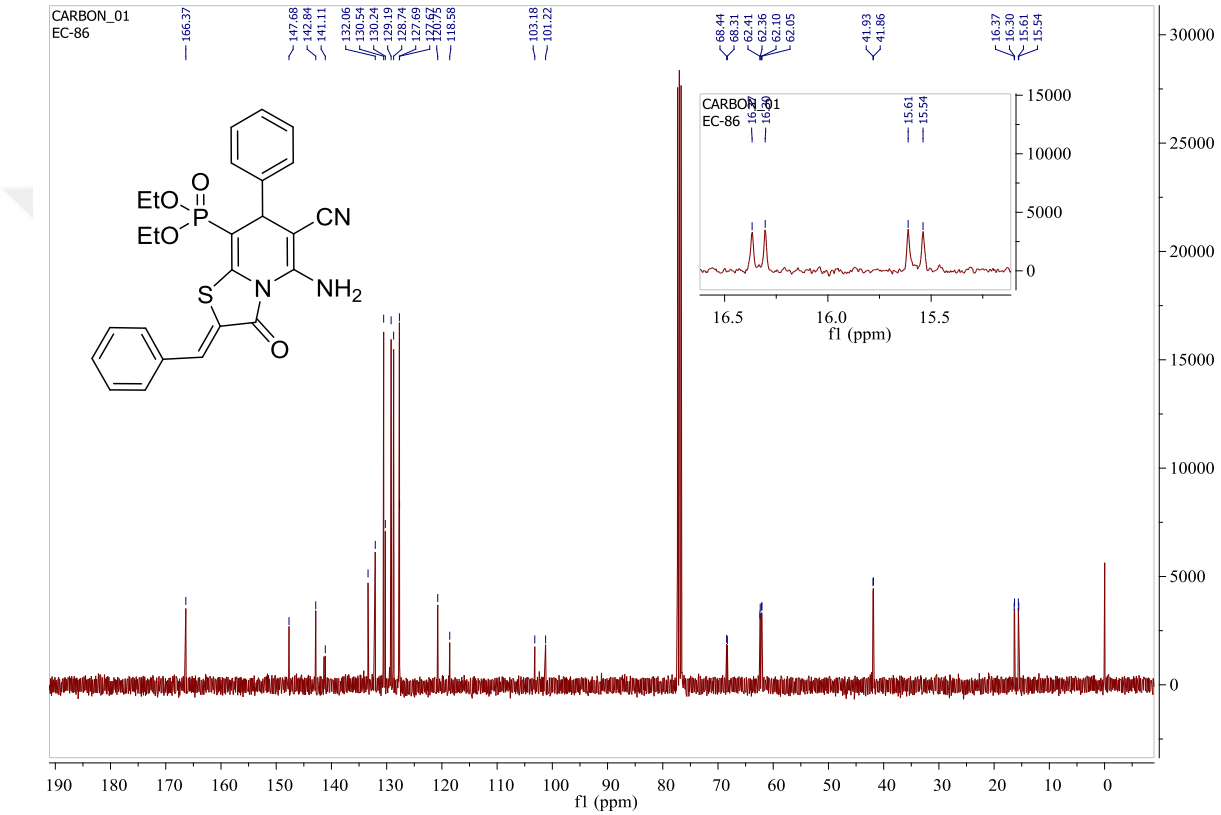


Figure 4.2. ^{13}C NMR of compound **63a**

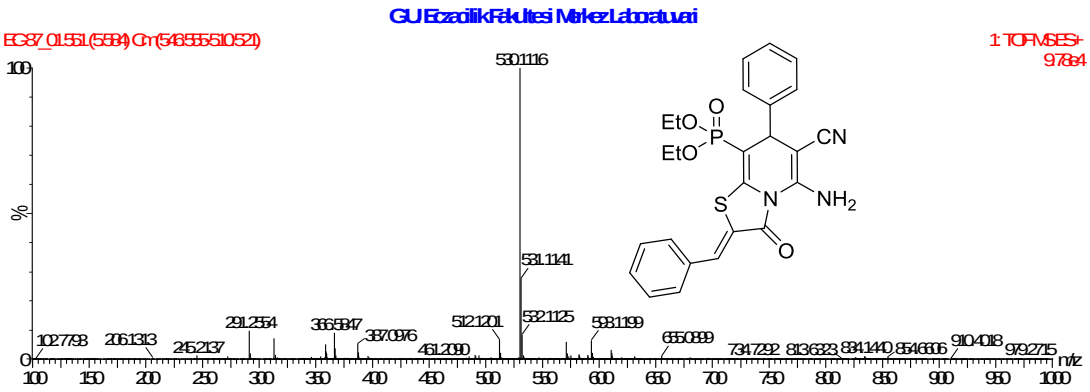
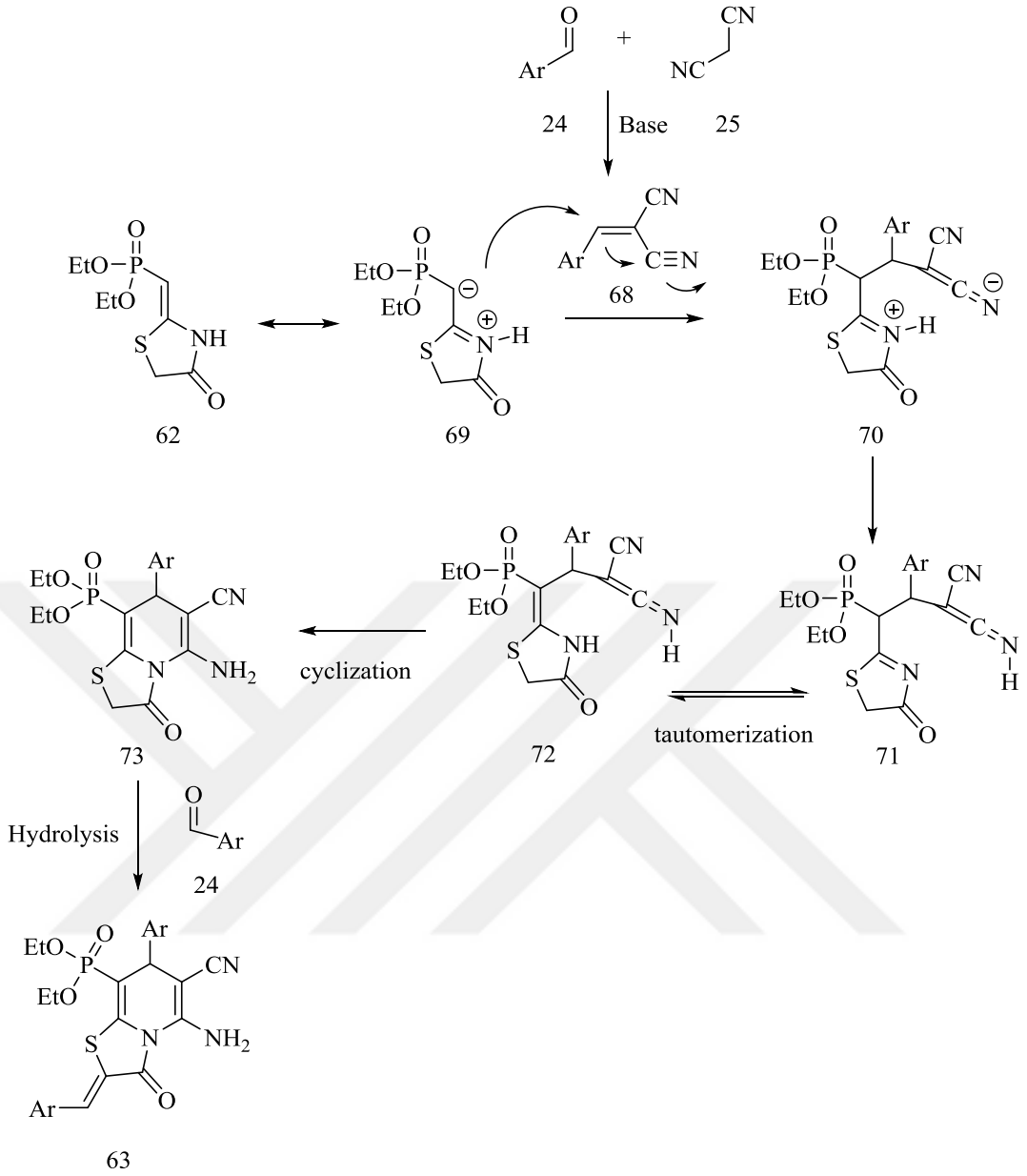


Figure 4.3. HRMS of compound **63a**

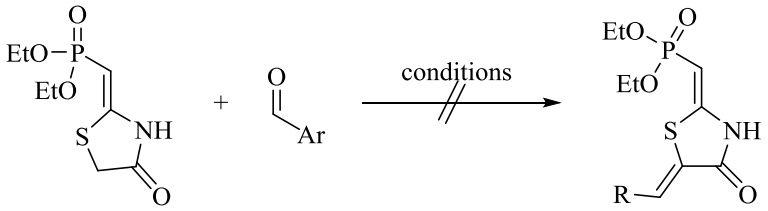
In all cases, the desired products obtained are highly stable and easy to handle, the reaction were very clean and no side reaction products were formed and also the complete consumption of the reactants were observed with good to excellent yields achieved. All compounds were characterized by spectroscopic (IR, ^{1}H, ^{13}C, MS and ^{31}P for 63i, 63j and 63l) and physical methods.

The annulation reaction starts with nucleophilic attack of aza–ene reaction of heterocyclic ketene aminal **(69)** to initially formed Knoevenagel intermediate **(68)** in the presence of a triethylamine. Deprotonation of **(70),** results **(71)**. Then, imine-enamine tautomerization formed **(72)** which were intramolecular cyclization gave **(73)**, which is subsequently hydrolyzed with another aldehyde to give the expected product **(63)** (Scheme 4.2) (Altug et al., 2011).



Scheme 4.2. Plausable reaction mechanisms for the obtained compounds

When we tried to obtain condensation of compound 62 with aromatic aldehydes in the presence of various bases (Et_3N, NaOH, NaH, Cs_2CO_3) or without using a base at elevated temperature, desired product for further reaction couldn't be obtained. These attempts support our proposed reaction mechanism which shows condensation of aromatic aldehyde with methylene might occur at the final step.

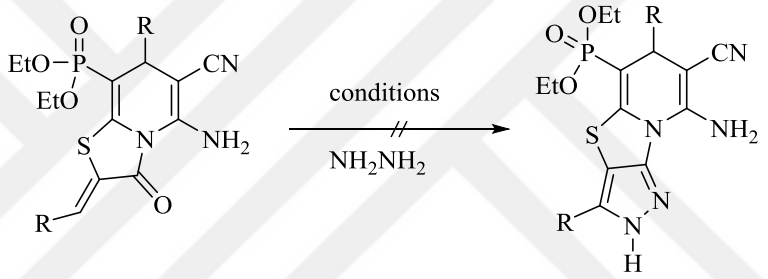


62 24 66

Scheme 4.3. Attempted reactions of compound **(62)** with an aromatic aldehyde

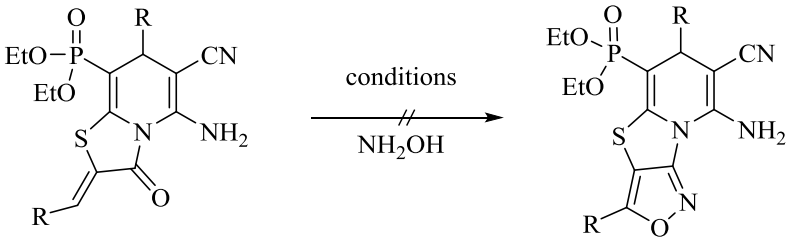
Synthesized compounds was reacted with hydrazine hydrate to obtain pyrrazole fused. But, even if we have used diffrent reaction conditions and catalyst we couldn't observe desired products.

Table 4.3. Attempted reactions of **63a** with hydrazine hydrate



63 74

R	Heat Source	Catalyst	Time
Ph	Conventional	-	18 h
Ph	Conventional	DBU	18 h
Ph	Conventional	Et_3N	18 h
Ph	Conventional	Al_2O_3-KF	18 h
Ph	MW	Al_2O_3	1 h
Ph	MW	KF	3 h
Ph	MW	piperidine	1 h
Ph	MW	NaOH	1 h



63 67

Scheme 4.4. Attempted reactions of **63a** with hydroxylamine

Instead of hydrazine similar reaction was also performed with hydroxylamine to obtain isoxazole fused thiazolopyridine. Different reaction conditions were also tried for this batch but we couldn't obtain desired compounds.

Biological Assay

The synthesized compounds have been assayed against four cancer cell lines. Since compounds having low GI values are more toxic on cells and they cause cell death at lower doses. However, such compounds have higher activities in the treatment of diseases such as cancer (Carmichael et al., 1988b). Compound 63l showed cytotoxic activities against Mcf-7 cell line.

Table 4.4. Cytotoxic activities of compounds against cancer cell lines [a]

		GI values (μM)[b] in cell lines[c]	
Entry	**Compounds**	**T98g**	**MCF-7**
1	**63a**	>100	>100
2	**63b**	>100	>100
3	**63c**	>100	>100
4	**63d**	>100	>100
5	**63e**	>100	>100
6	**63f**	>100	>100
7	**63g**	>100	>100
8	**63h**	>100	>100
9	**63i**	>100	>100
10	**63J**	>100	>100
11	**63k**	>100	>100
12	**63l**	>100	90

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

10% FBS (fetal bovine serum) and 1% antibiotic-antimycotic appropriate medium containing cells were used as a control measurement. 20,000 units MCF-7

(human breast adenocarcinoma cell) and T98G (human glioblastoma cells) cell were plated on 96 well plates. After 24 h of incubation, substances were given in different doses. Cells were then treated with substances and incubated for 24 h, then, MTT was measured (Mortimer et al., 2006).

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

5. CONCLUSIONS

An efficient three-component domino reaction between aromatic aldehydes **(24)**, malononitrile **(25)**, and the novel diethyl ((4-oxothiazolidin-2-ylidene)methyl) phosphonate **(62)** reagent is reported that enables a general, mild, and straightforward access to 12 new thiazolo[3,2-a]pyridin-8-yl-phosphonate derivatives **(63a-l)**. Moreover, synthesized compounds were subjected for anti-cancer studies and one of the compounds showed cytotoxic activities against Mcf7 cancer cell lines. Products were identified by physical constants (e.g. TLC, Melting points, R_f values) and by means of spectral (IR, ^{1}H NMR, ^{13}C NMR and HRMS) measurements.

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APPENDICES

7. APPENDICES

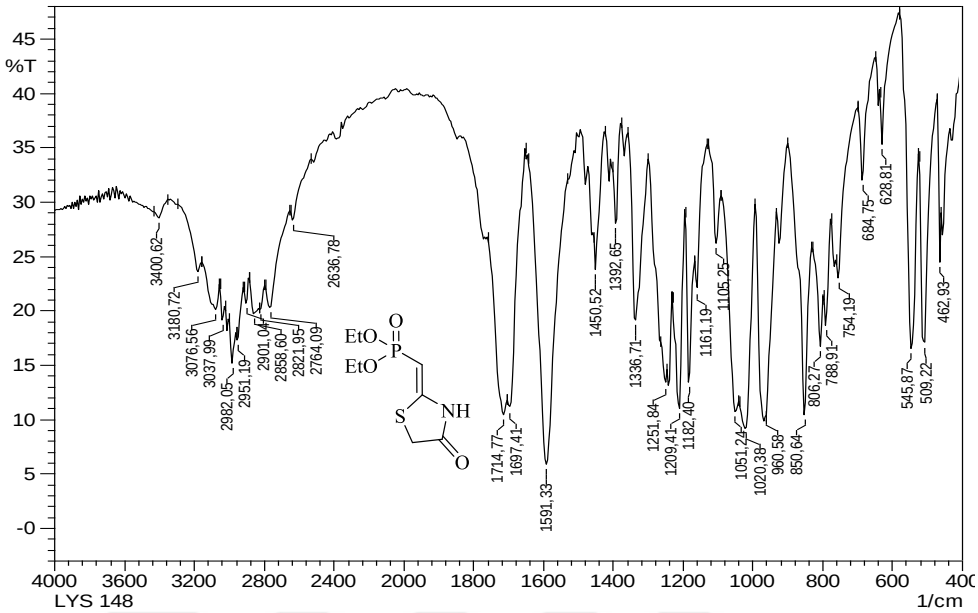

Figure 7.1. IR spectrum of compound **62**

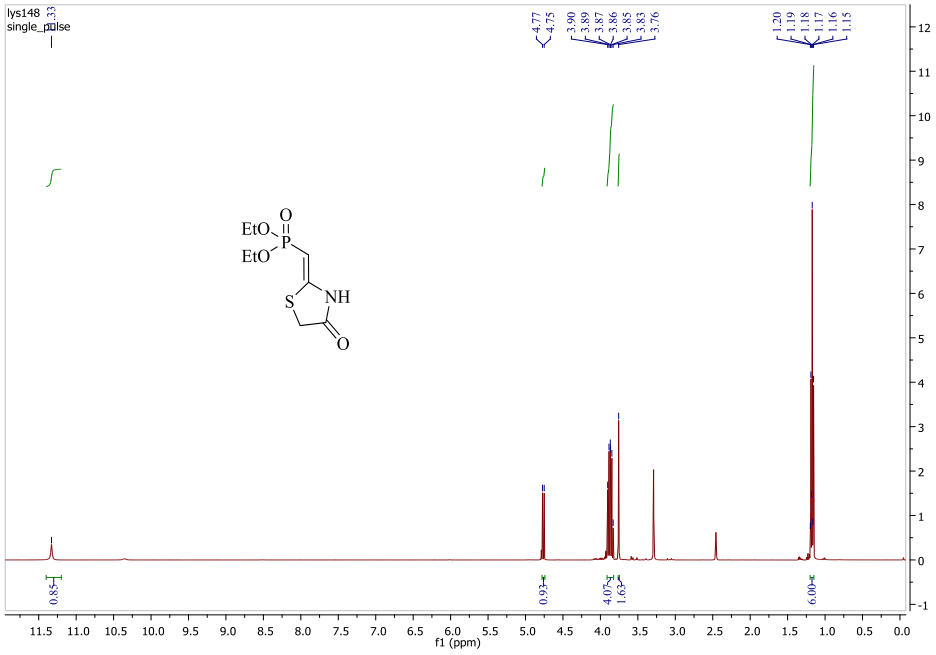

Figure 7.2. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **62**

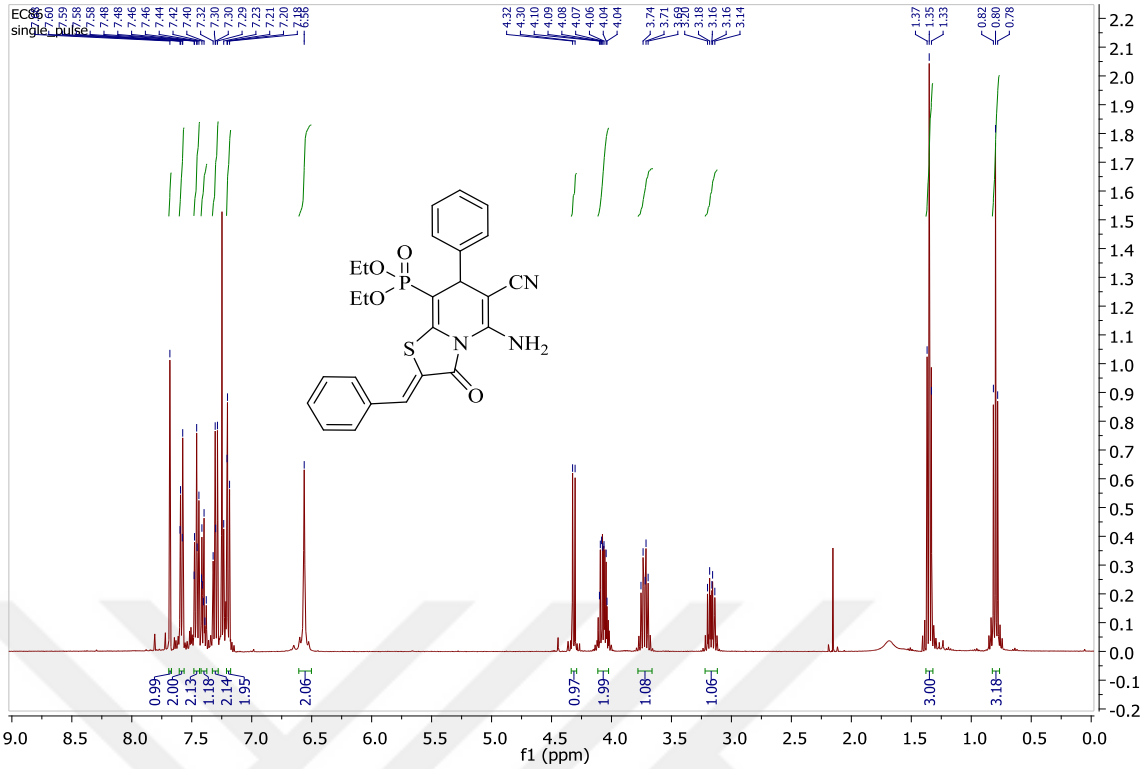

Figure 7.3. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63a**

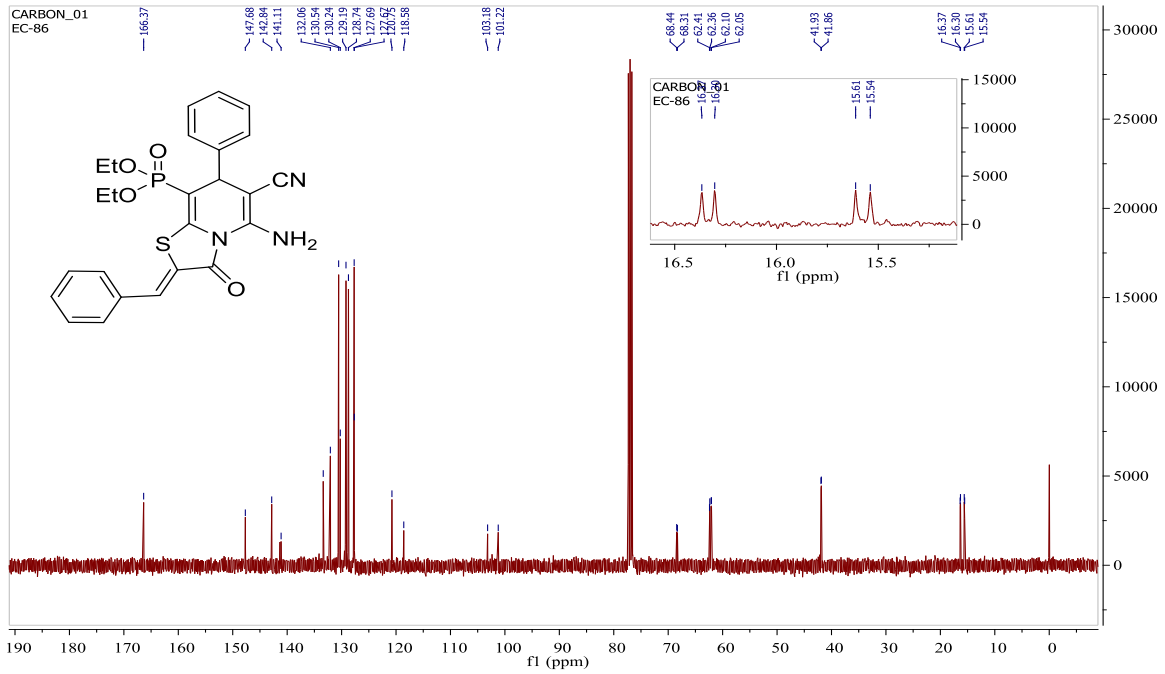

Figure 7.4. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63a**

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

278 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O	P	S
494.1314	494.1321	-0.7	-1.4	15.5	C24 H24 N5 O3 S2	161.2	8.2	24	24	5	3		2
	494.1303	1.1	2.2	15.5	C25 H25 N3 O4 P S	153.0	0.0	25	25	3	4	1	1

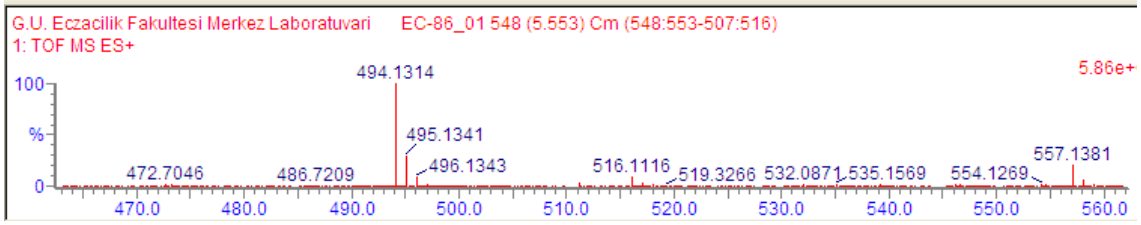

Figure 7.5. HRMS of compound **63a**

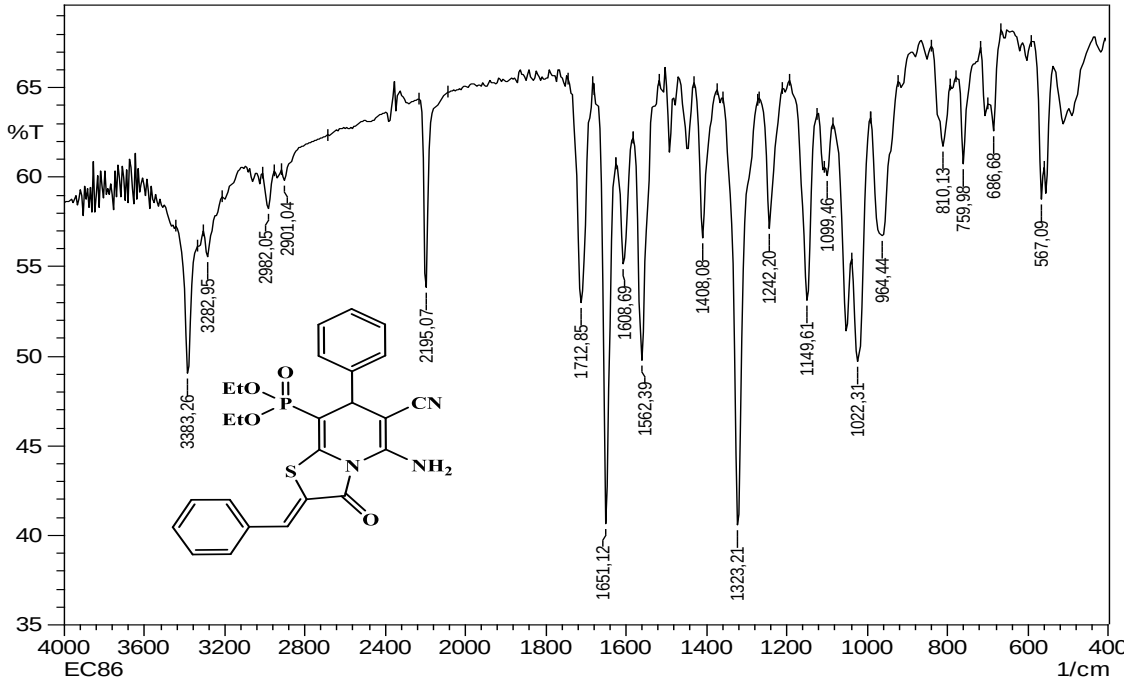

Figure 7.6. IR spectrum of compound **63a**

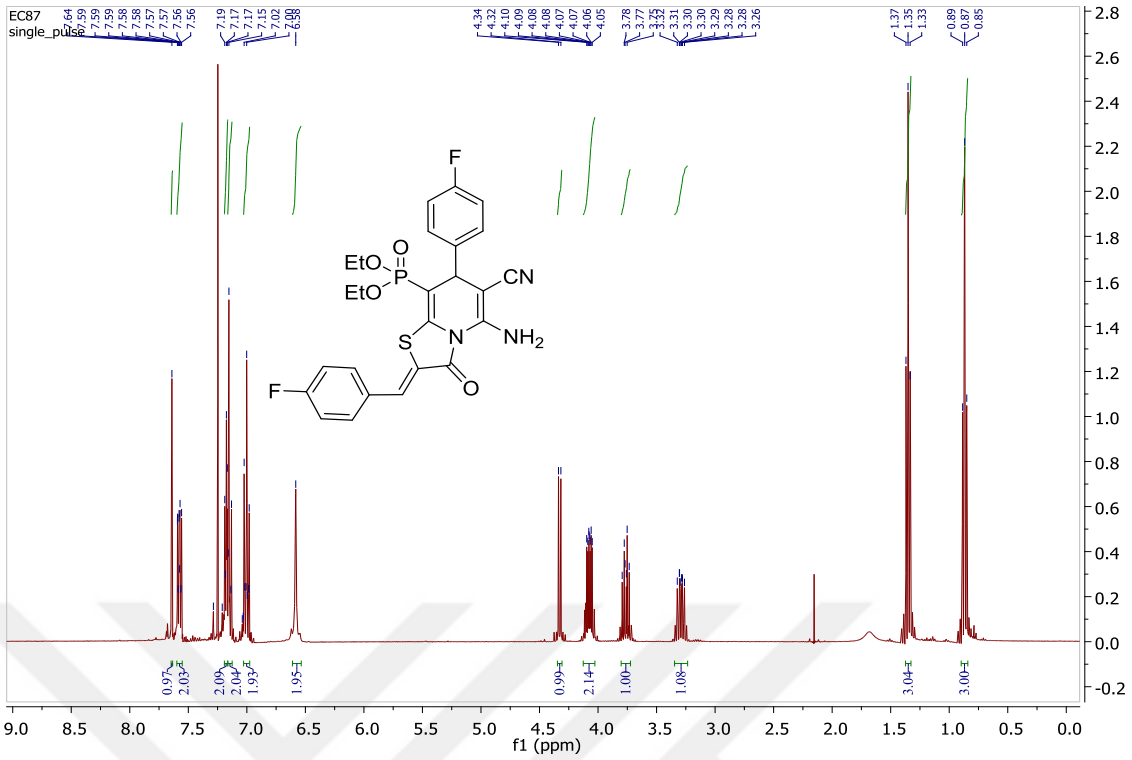

Figure 7.7. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63b**

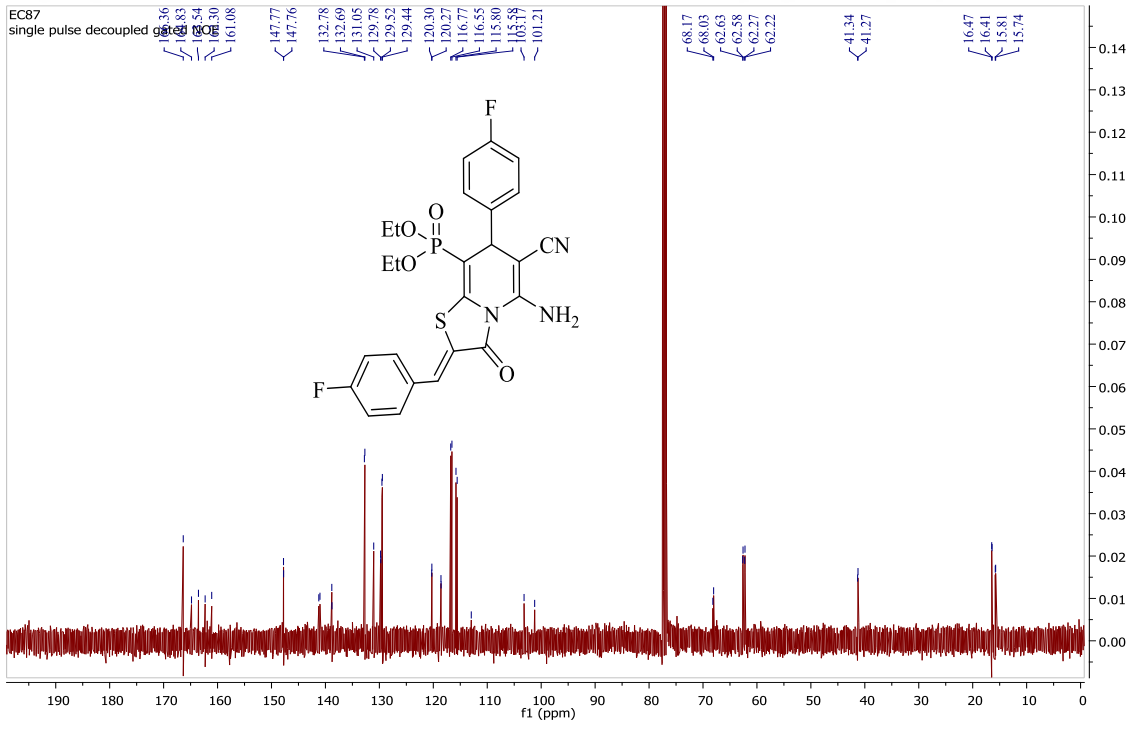

Figure 7.8. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63b**

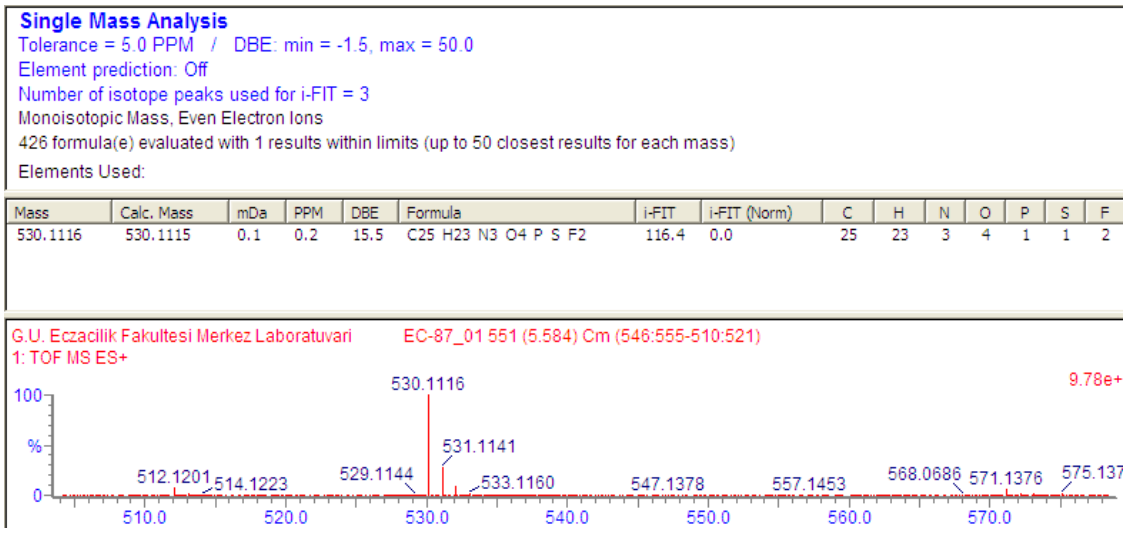

Figure 7.9. HRMS of compound **63b**

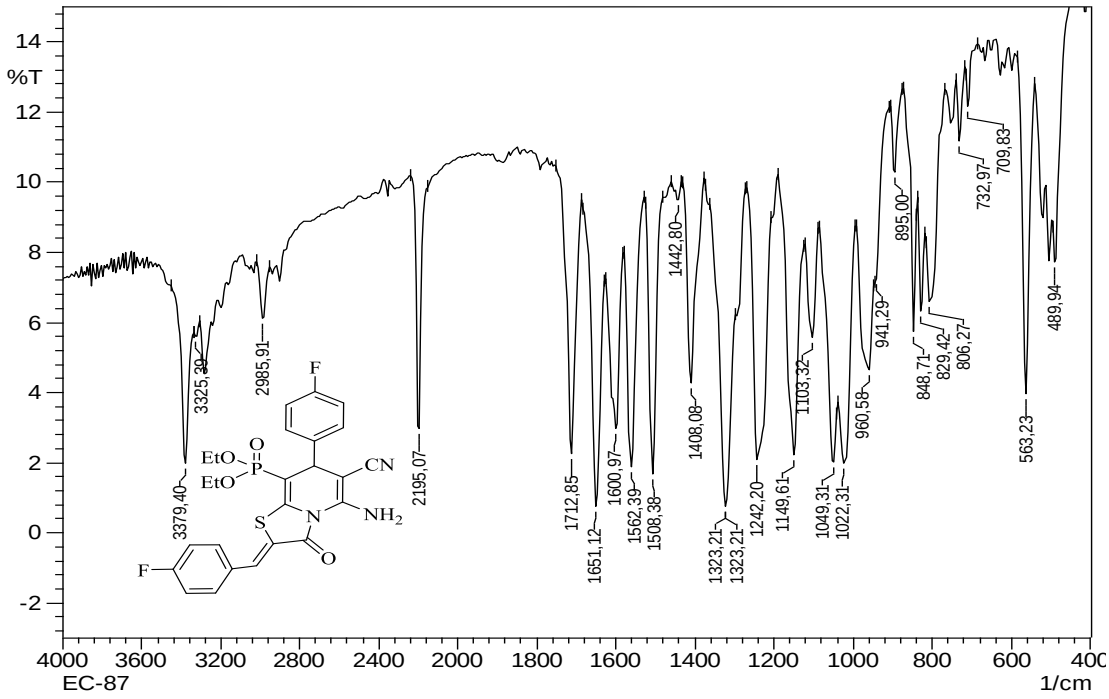

Figure 7.10. IR spectrum of compound **63b**

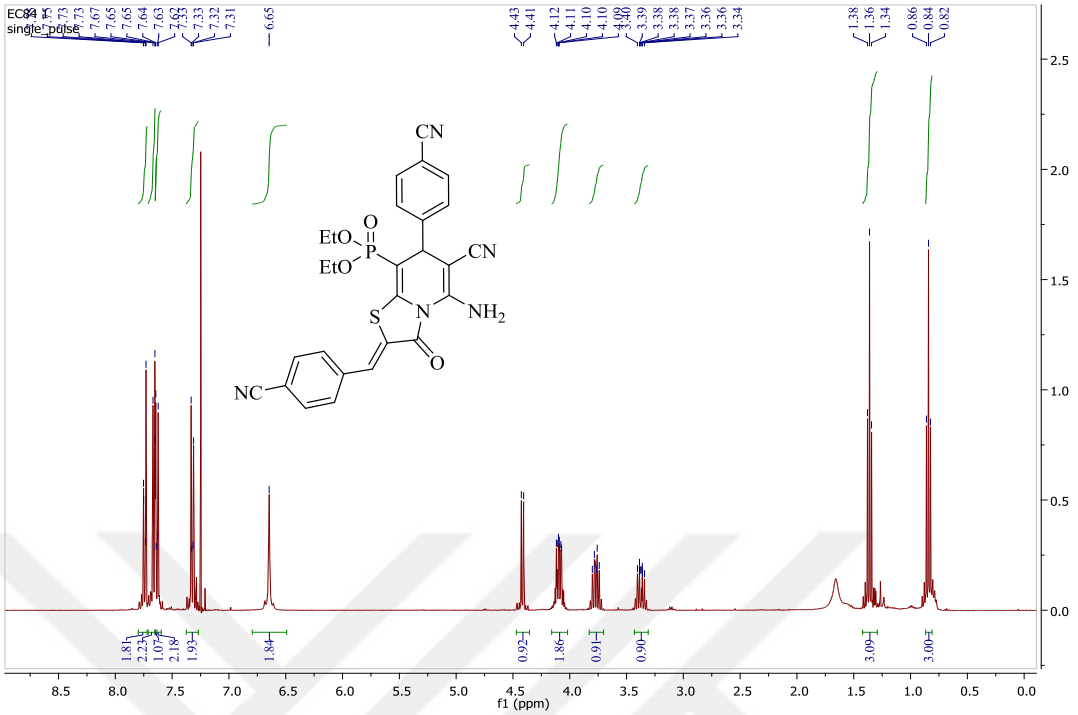

Figure 7.11. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63c**

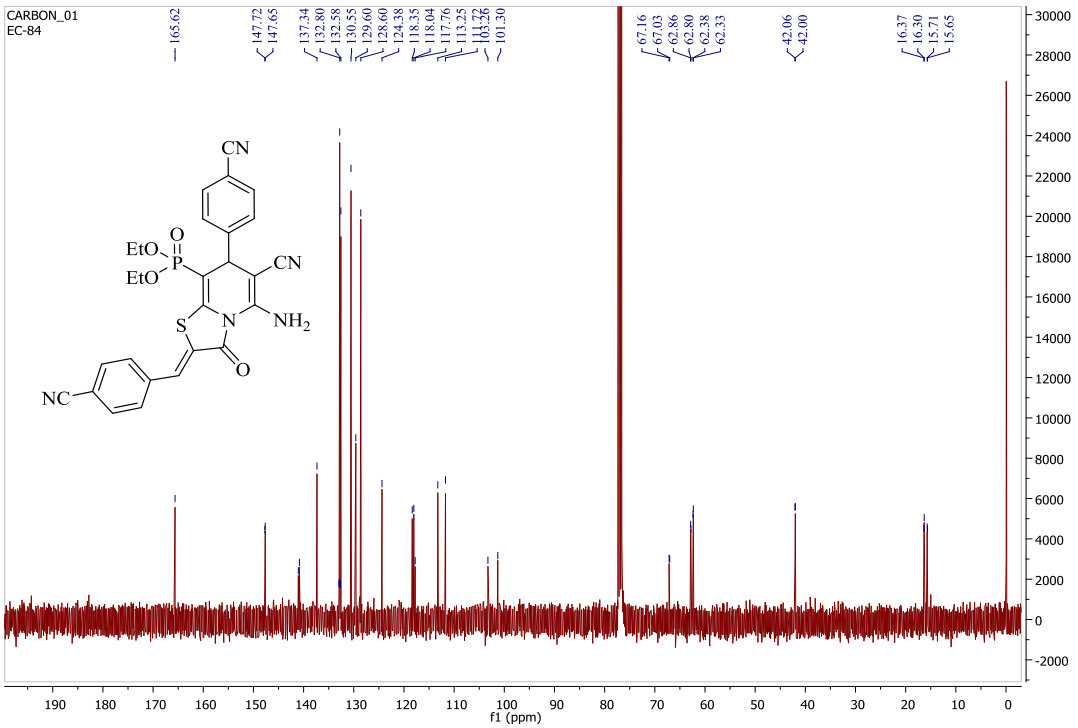

Figure 7.12. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63c**

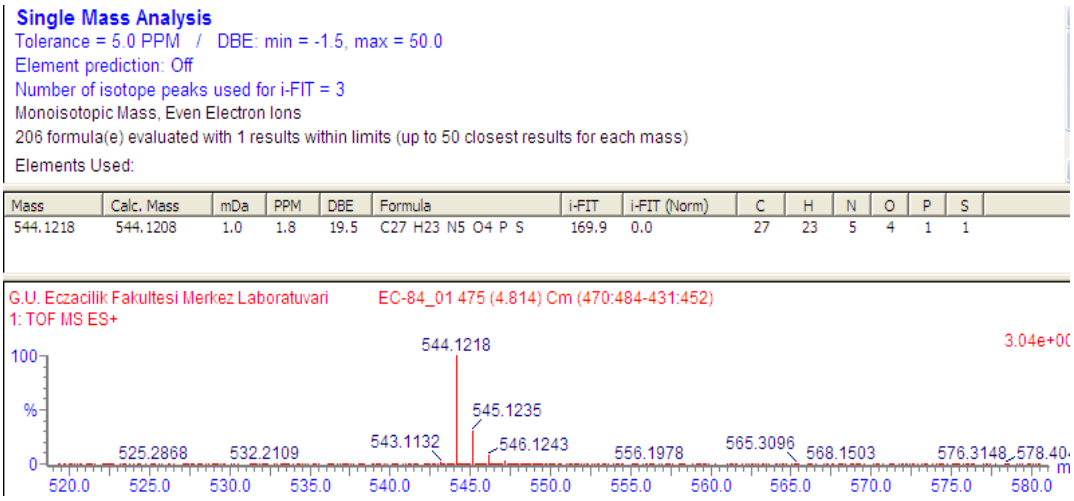

Figure 7.13. HRMS of compound **63c**

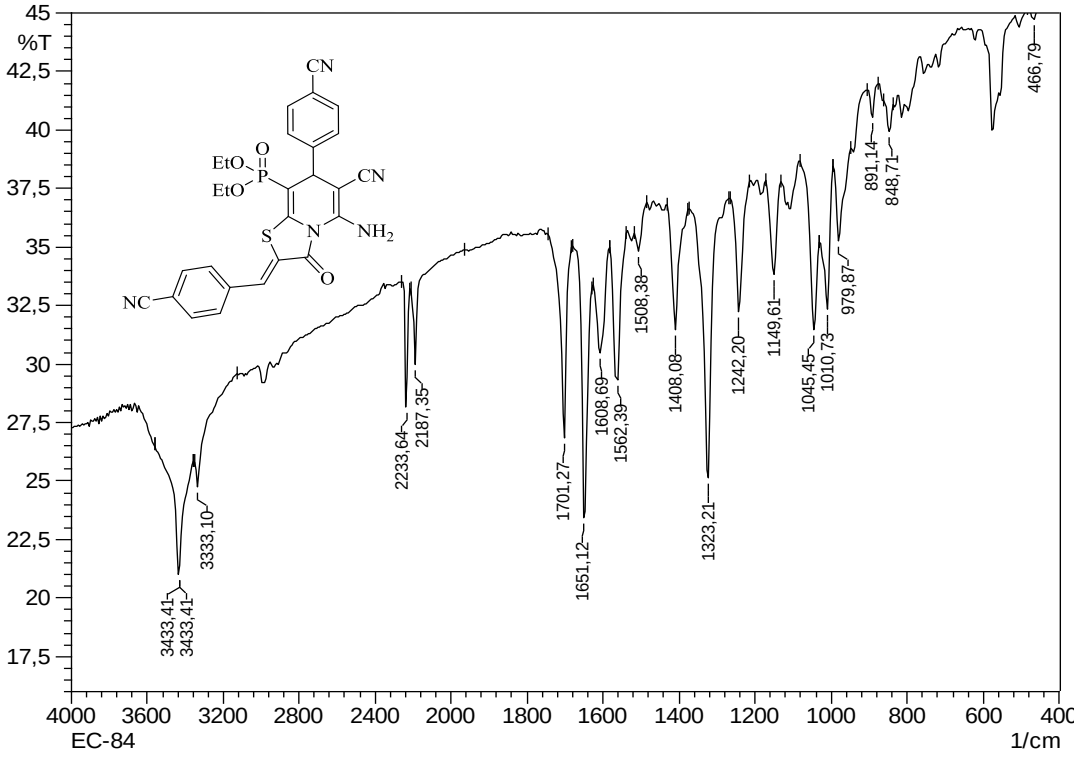

Figure 7.14. IR spectrum of compound **63c**

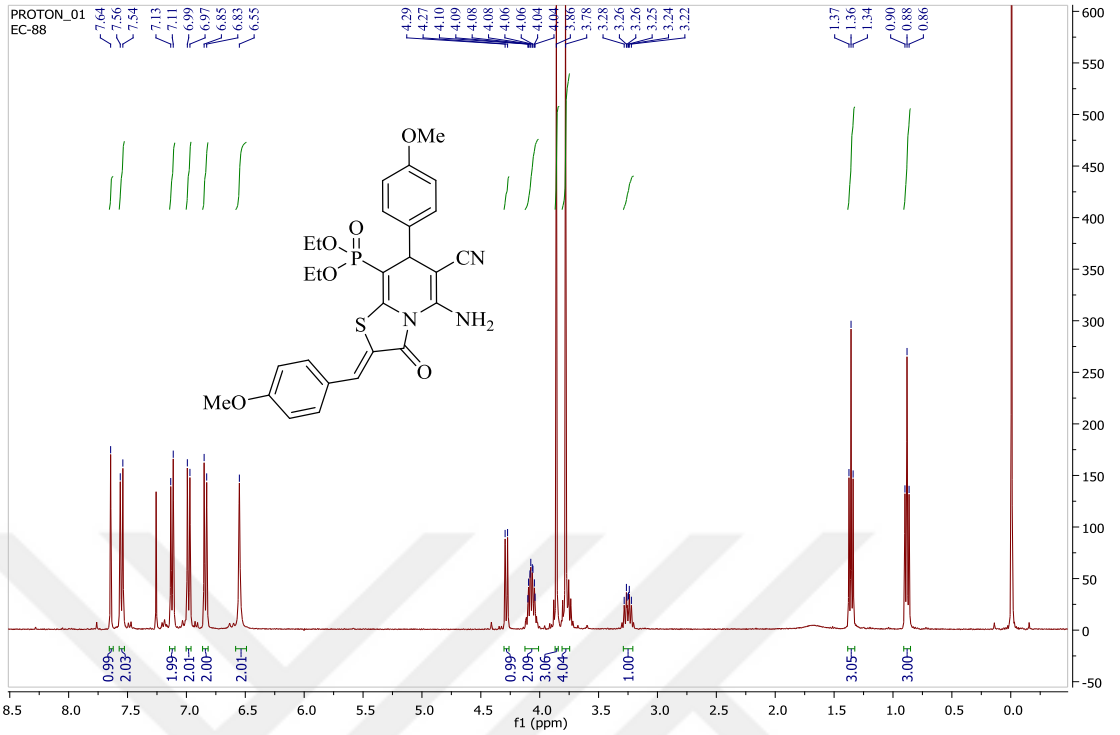

Figure 7.15. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63d**

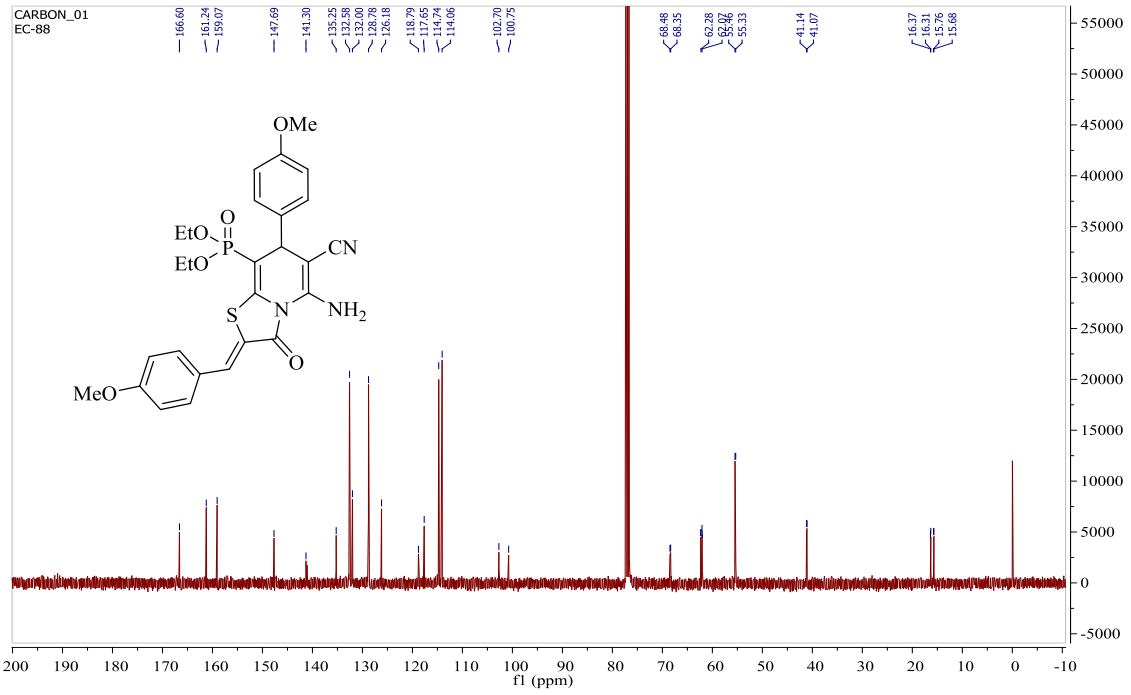

Figure 7.16. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63d**

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

139 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O	P	S
554.1522	554.1515	0.7	1.3	15.5	C27 H29 N3 O6 P S	126.5	0.0	27	29	3	6	1	1

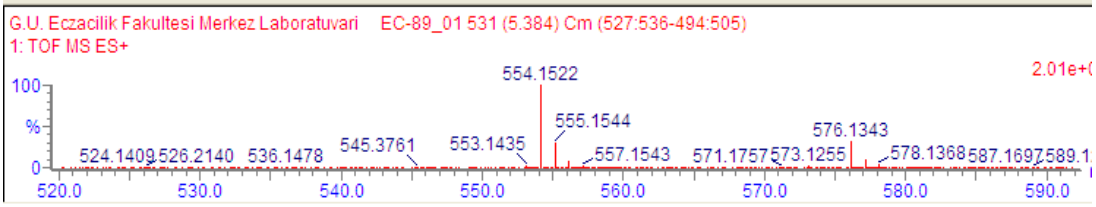

Figure 7.17. HRMS of compound **63d**

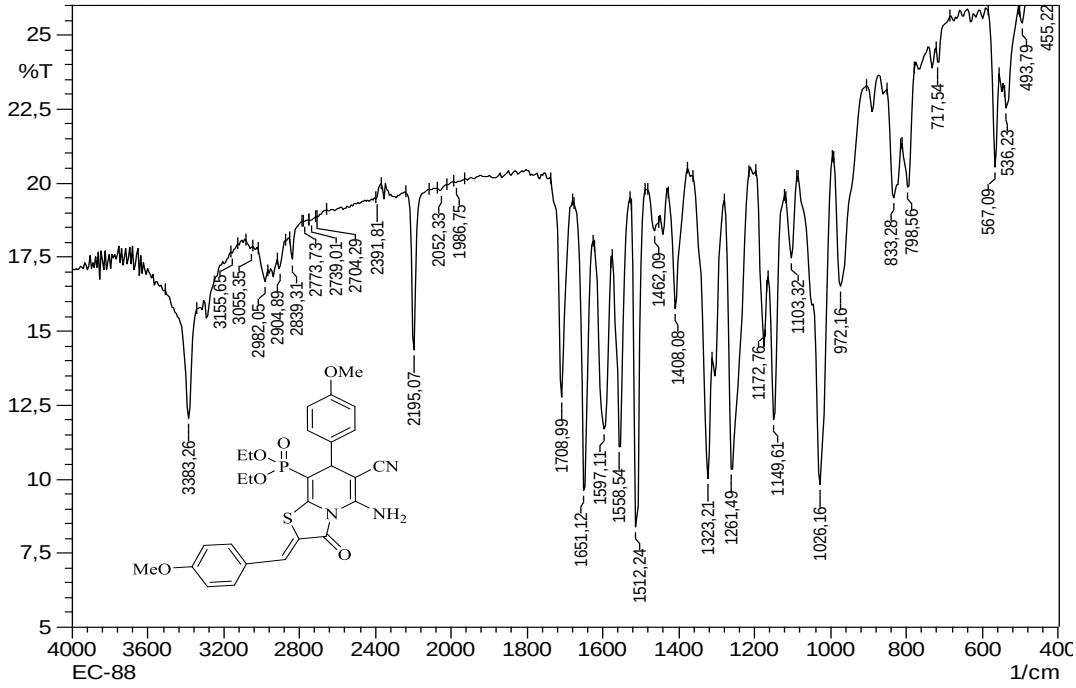

Figure 7.18. IR spectrum of compound **63d**

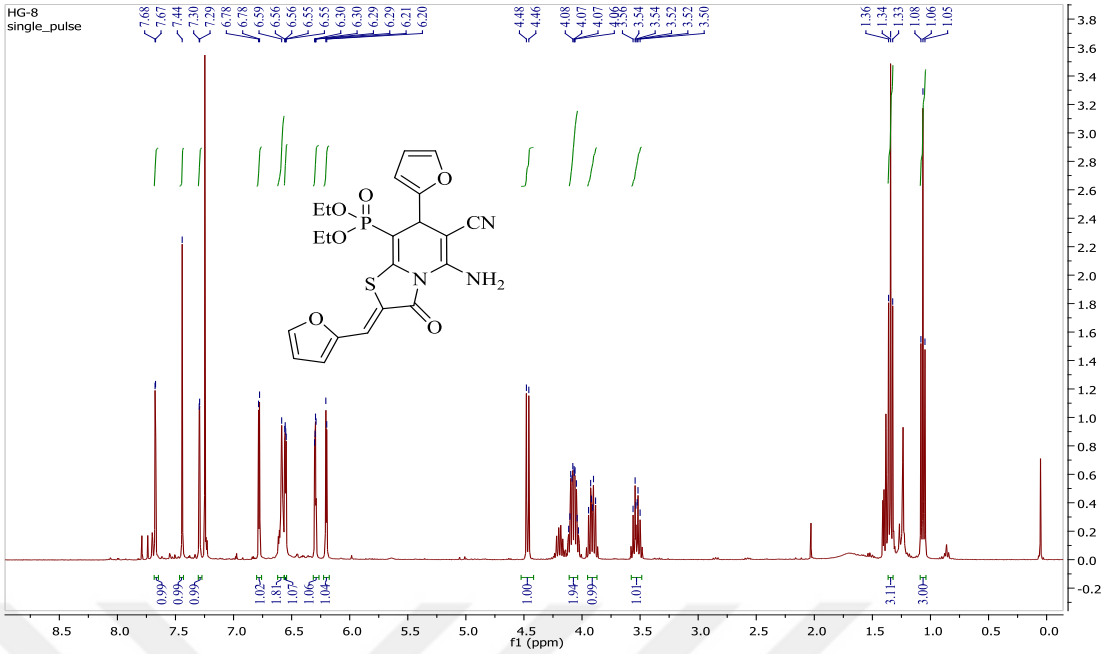

Figure 7.19. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63e**

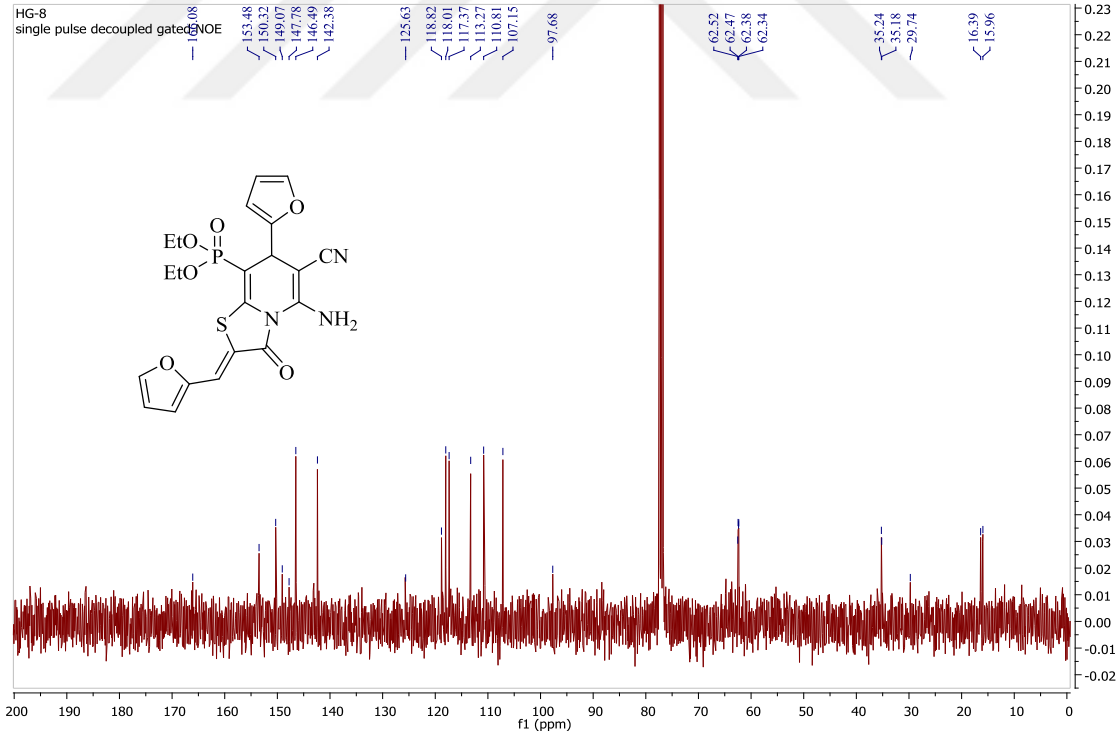

Figure 7.20. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63e**

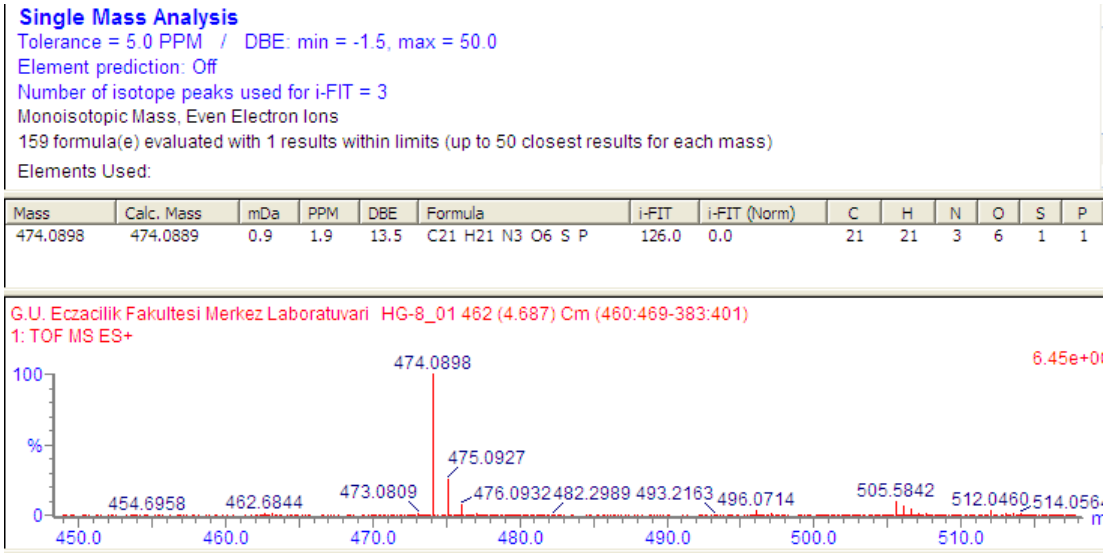

Figure 7.21. HRMS of compound **63e**

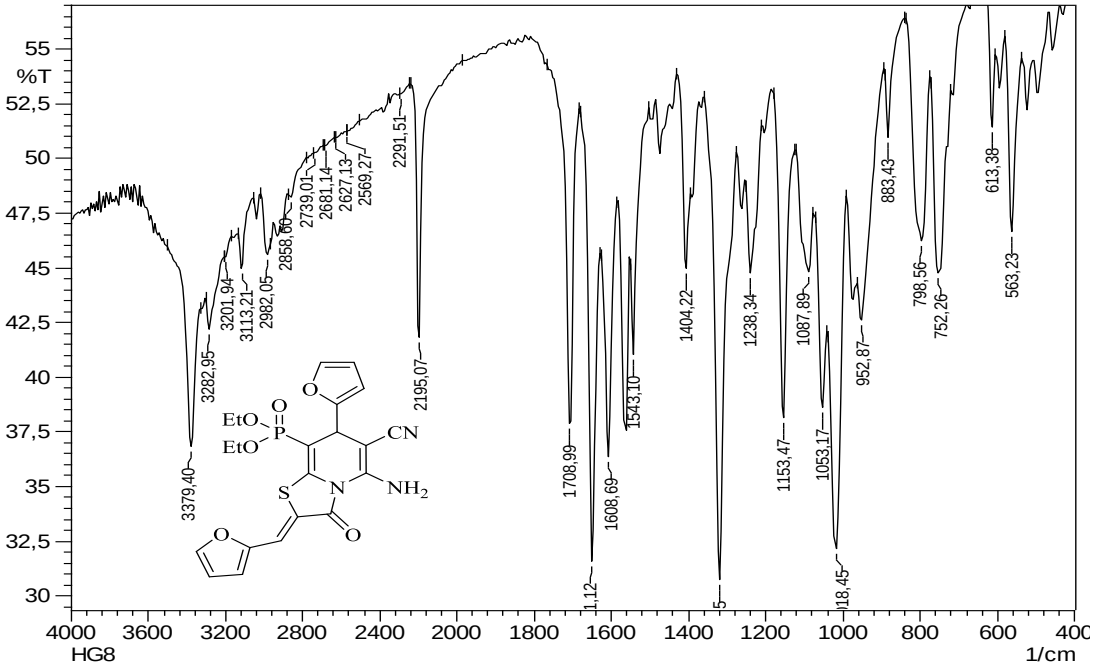

Figure 7.22. IR spectrum of compound **63e**

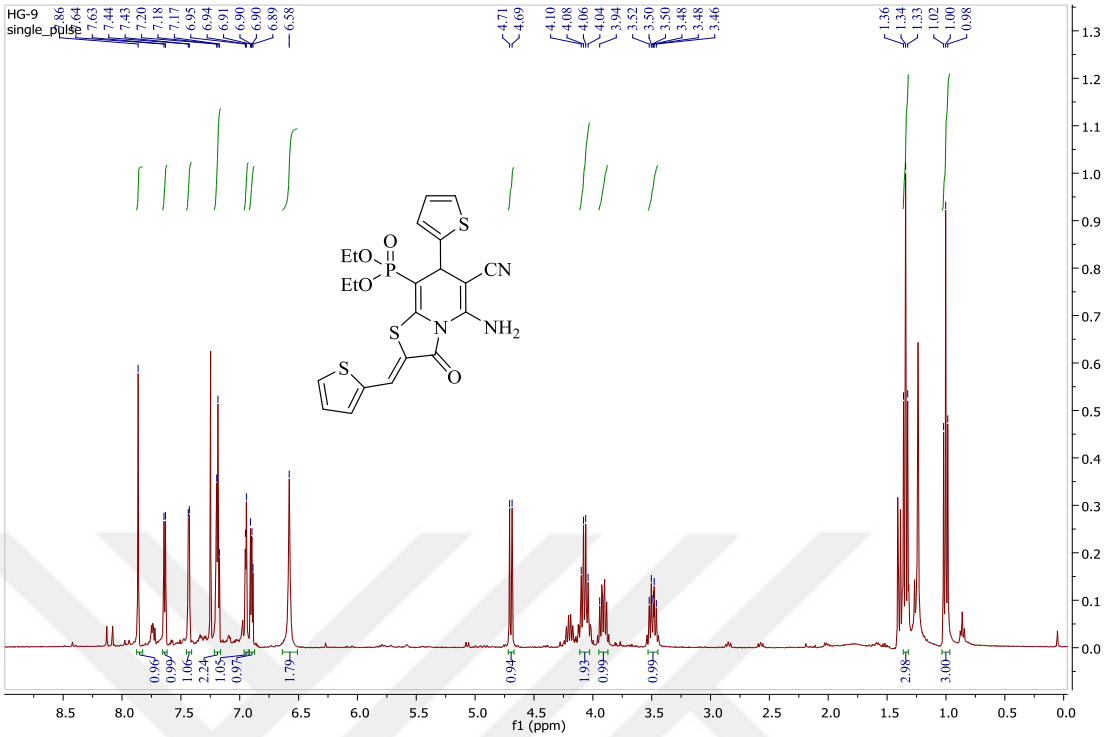

Figure 7.23. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63f**

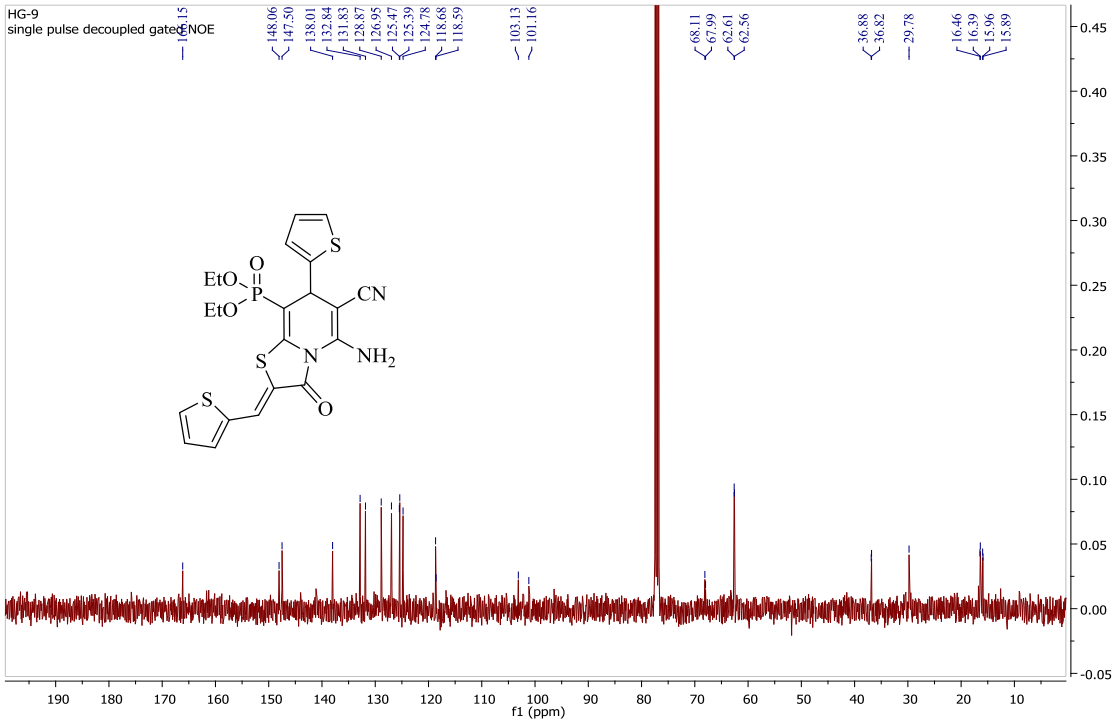

Figure 7.24. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63f**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
154 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O	S
506.0411	506.0432	-2.1	-4.1	13.5	C21 H21 N3 O4 S3 P	89.6	0.0	21	21	3	4	3

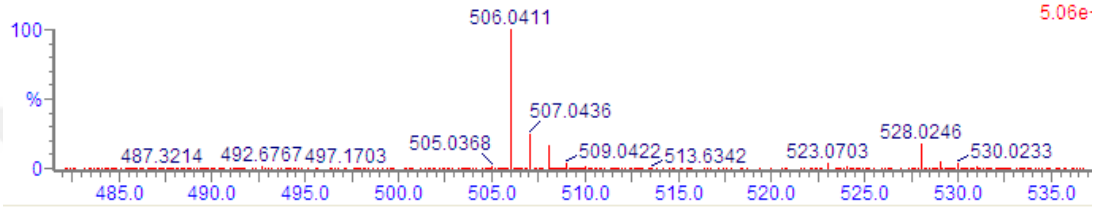

Figure 7.25. HRMS of compound **63f**

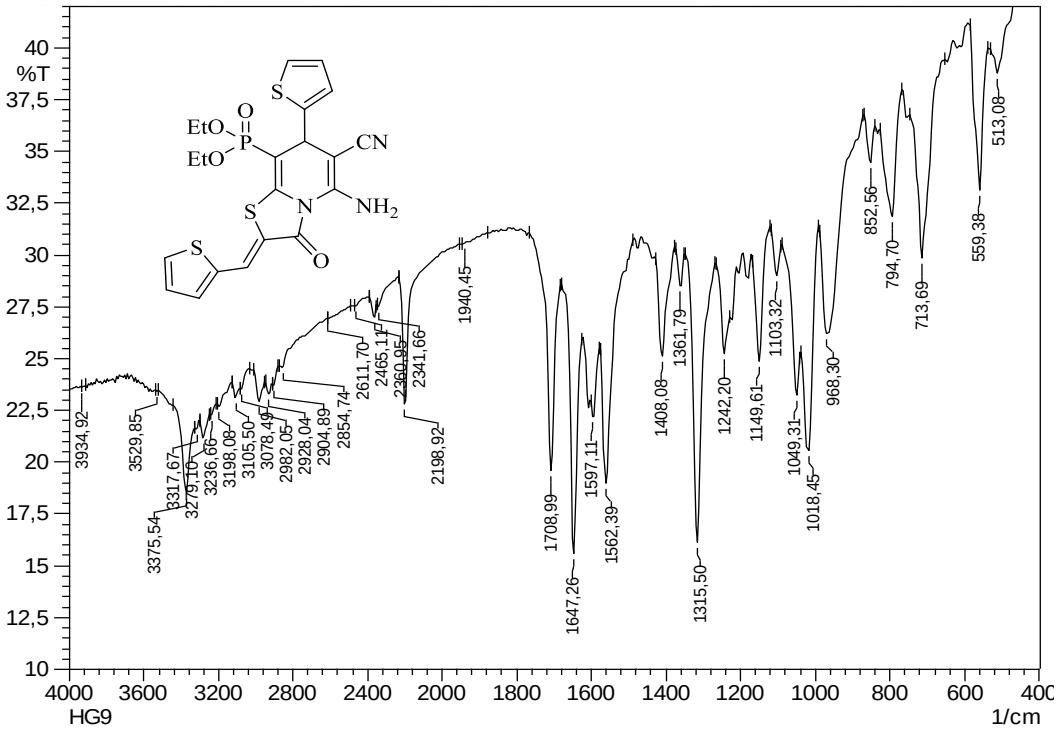

Figure 7.26. IR spectrum of compound **63f**

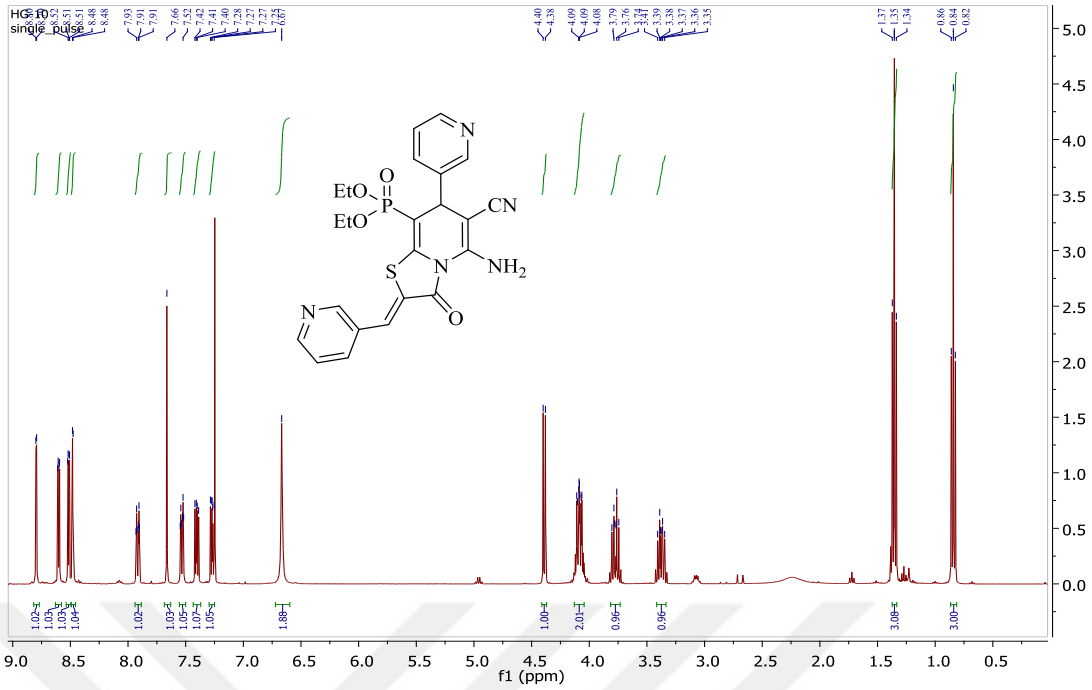

Figure 7.27. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63g**

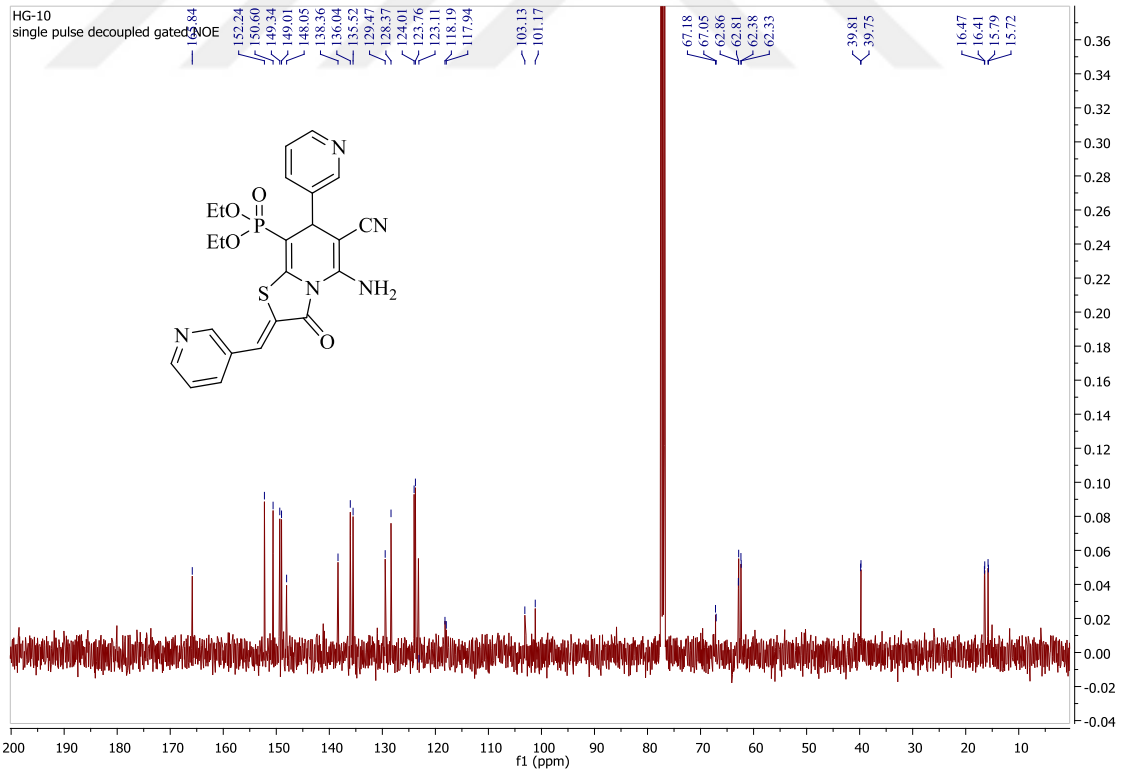

Figure 7.28. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63g**

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

156 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O	S	P
496.1205	496.1208	-0.3	-0.6	15.5	C23 H23 N5 O4 S P	111.3	0.0	23	23	5	4	1	1

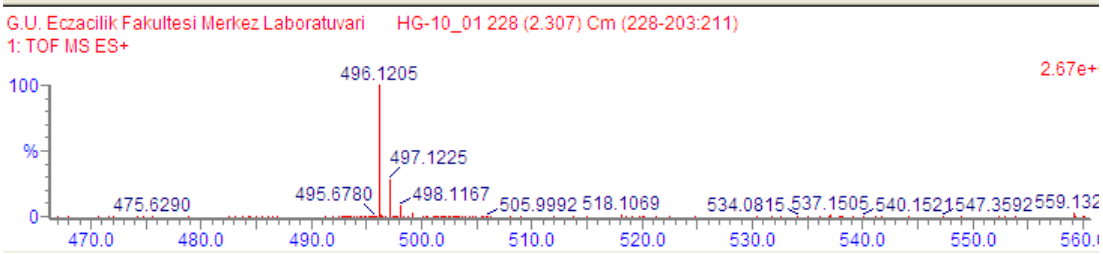

Figure 7.29. HRMS of compound **63g**

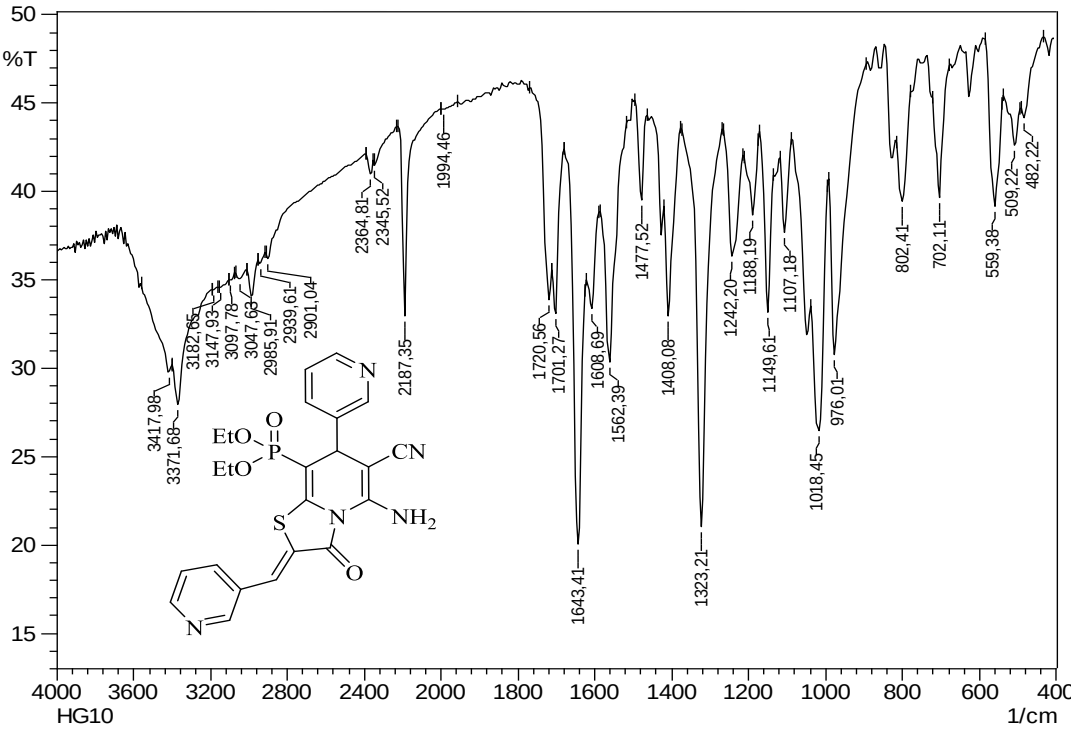

Figure 7.30. IR spectrum of compound **63g**

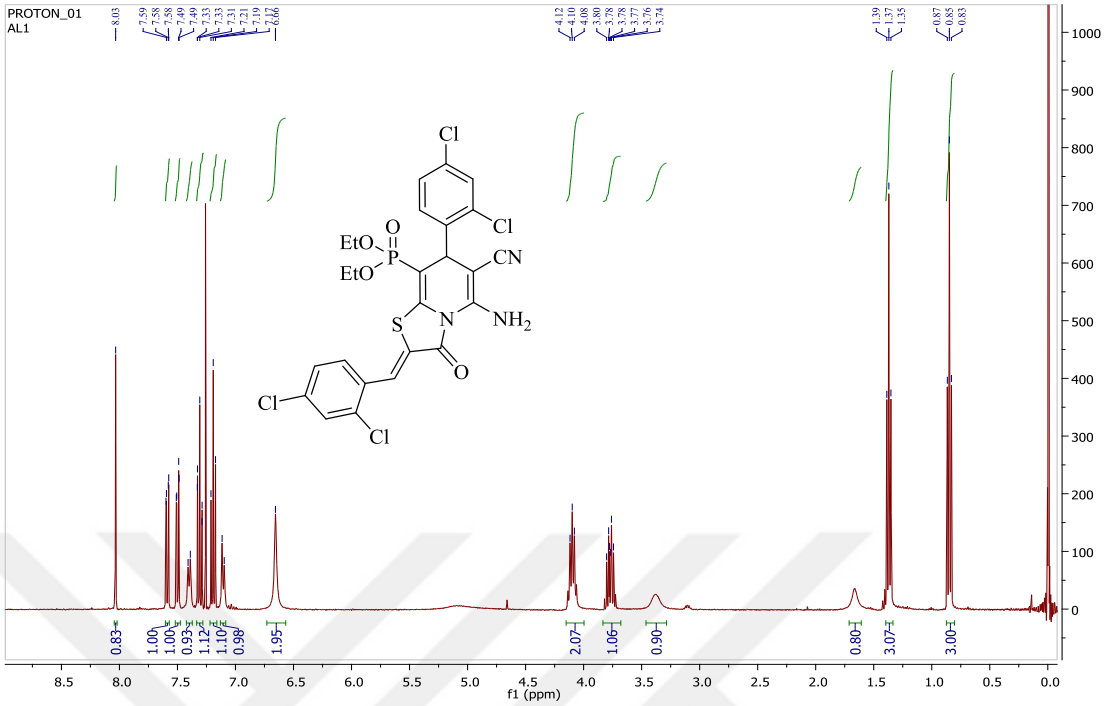

Figure 7.31. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63h**

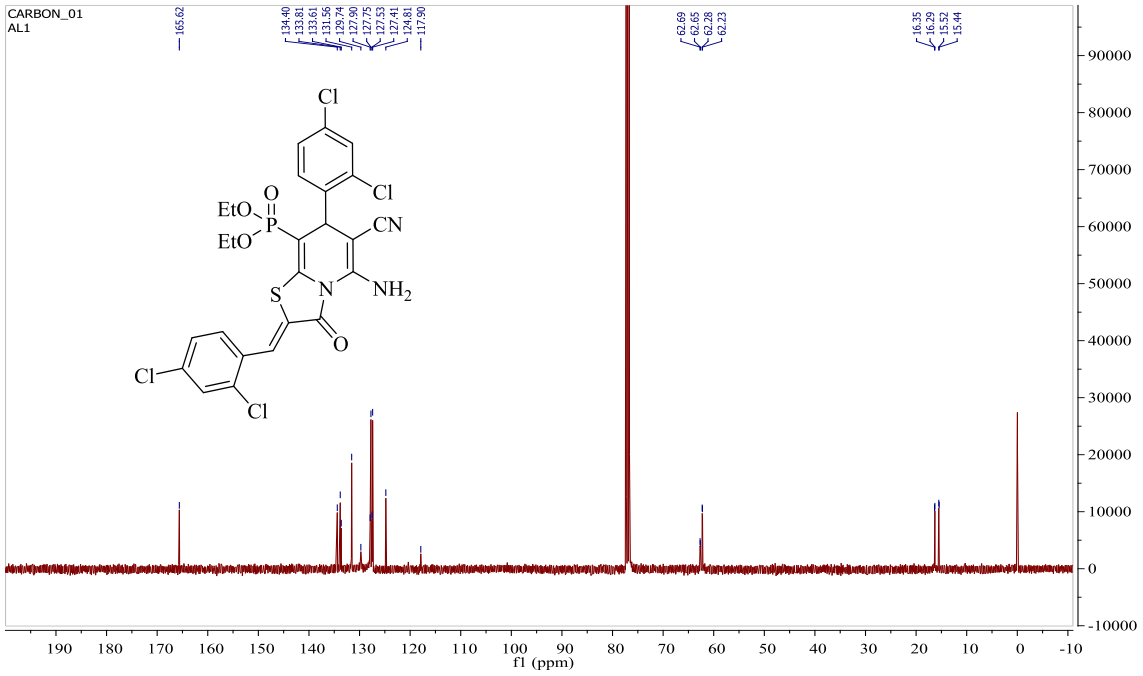

Figure 7.32. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63h**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
648 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O	P	S	Cl
629.9737	629.9745	-0.8	-1.3	15.5	C25 H21 N3 O4 P S Cl4	163.2	0.0	25	21	3	4	1	1	4
	629.9712	2.5	4.0	20.5	C25 H14 N5 O7 S2 Cl2	178.2	15.0	25	14	5	7		2	2

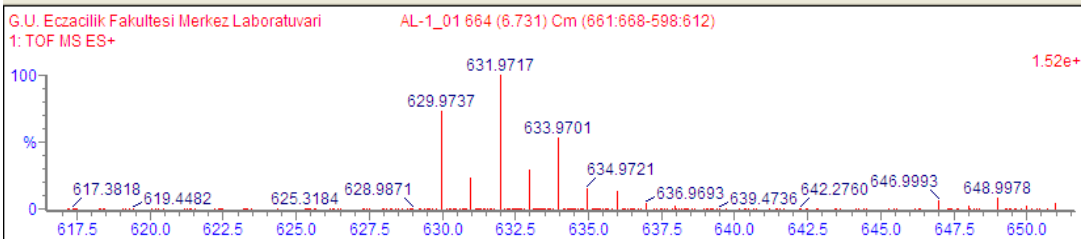

Figure 7.33. HRMS of compound **63h**

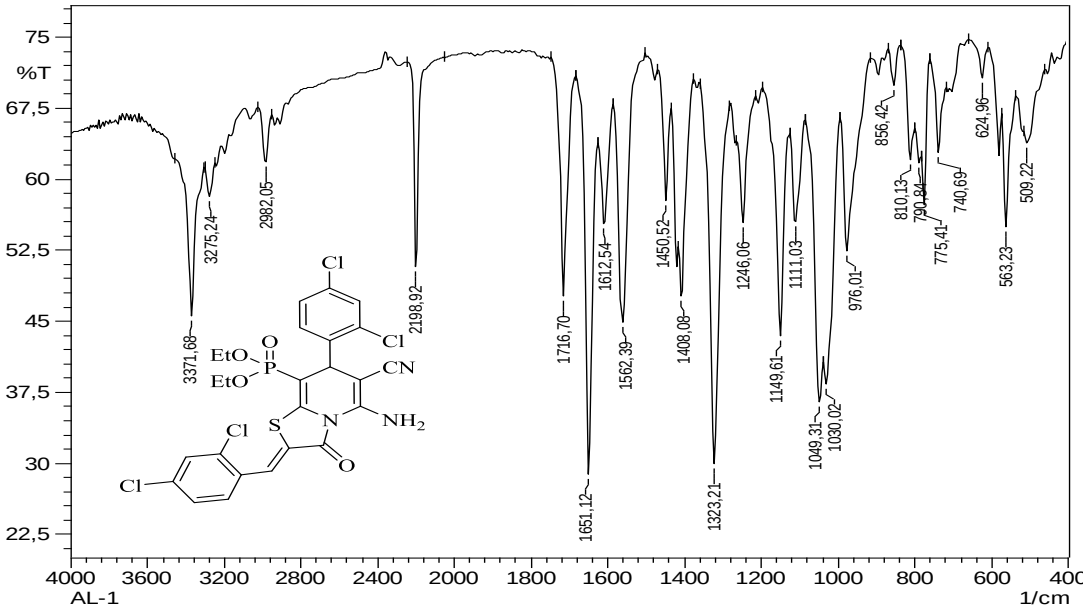

Figure 7.34. IR spectrum of compound **63h**

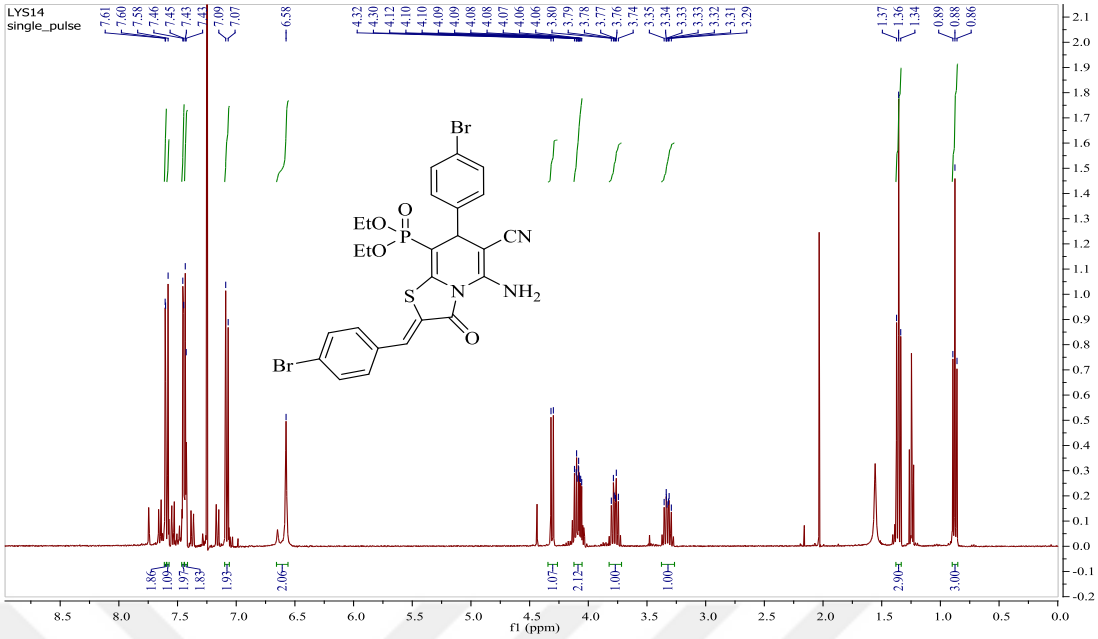

Figure 7.35. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63i**

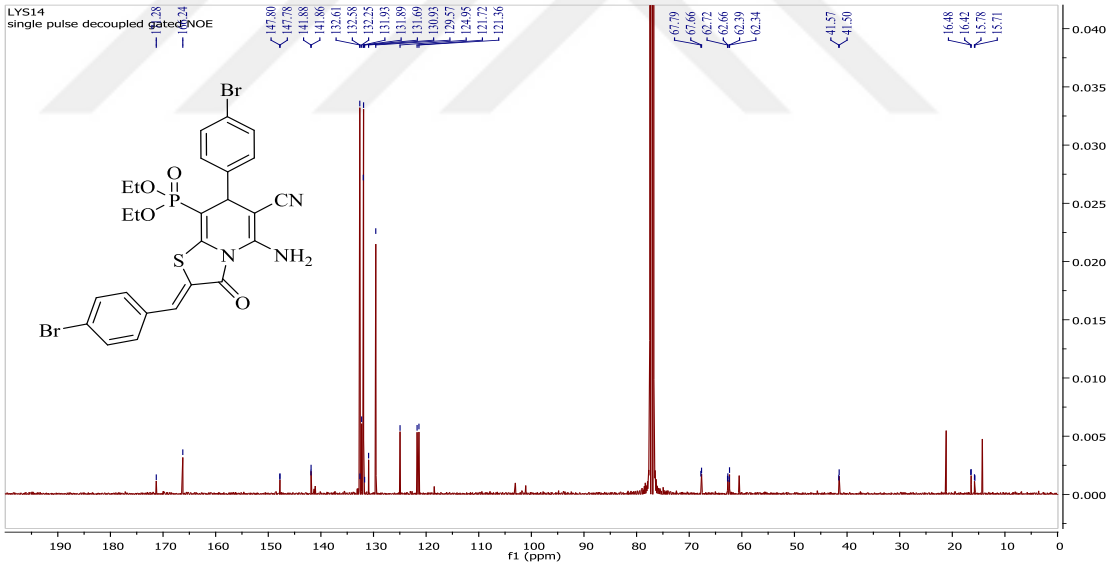

Figure 7.36. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63i**

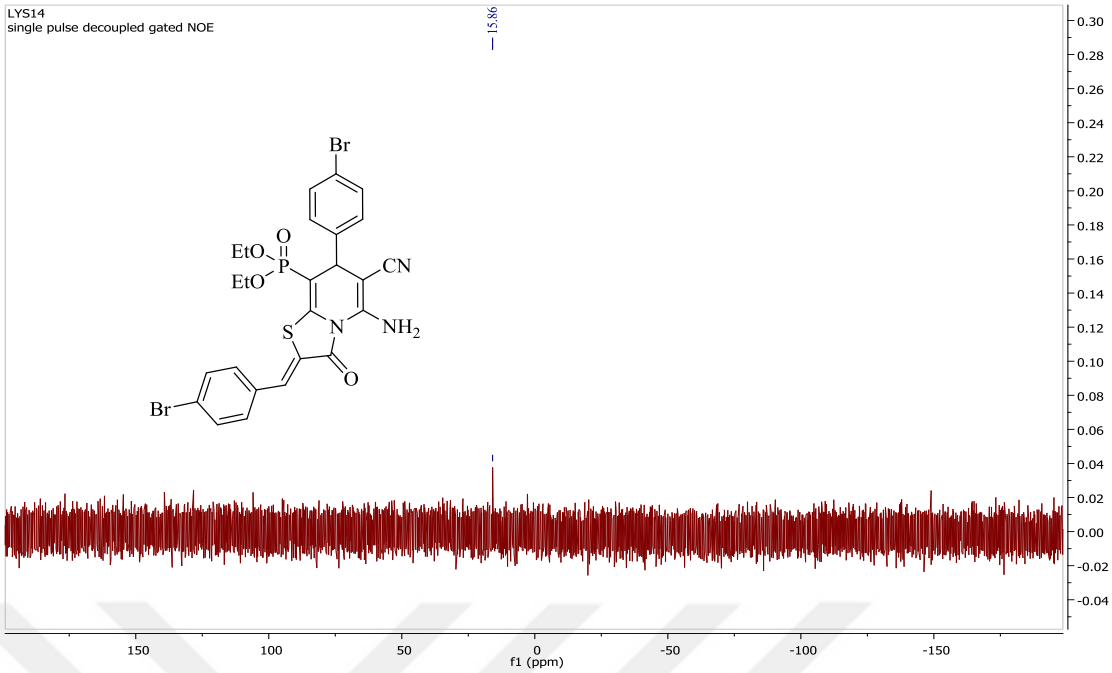

Figure 7.37. ^{31}P NMR for compound **63i**

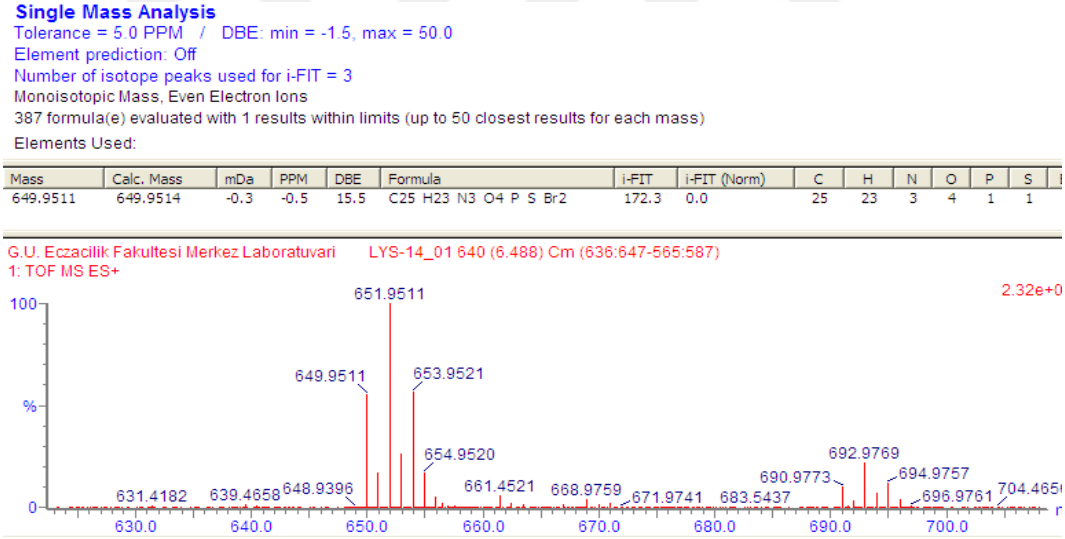

Figure 7.38. HRMS of compound **63i**

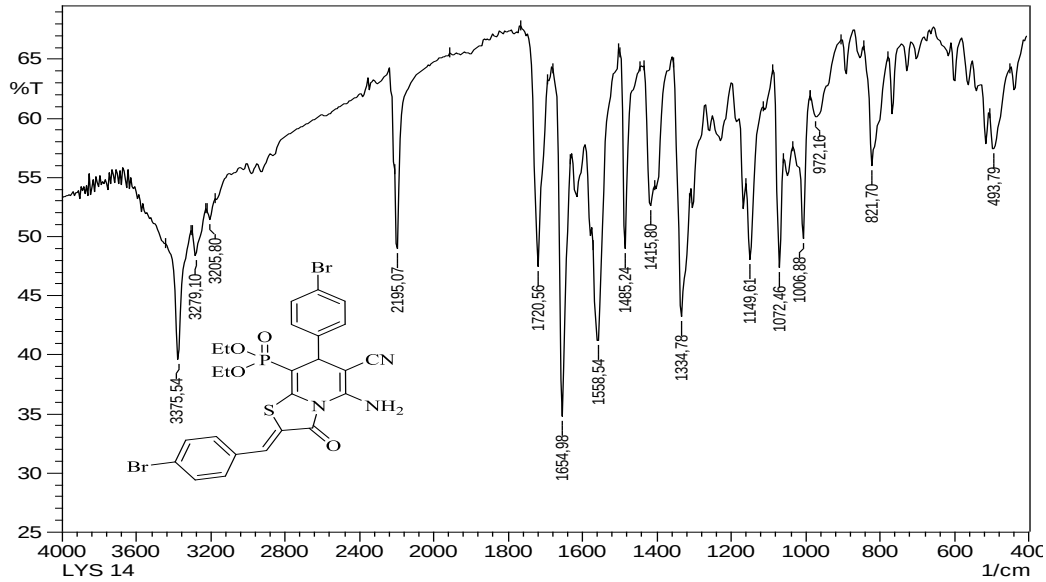

Figure 7.39. IR spectrum of compound **63i**

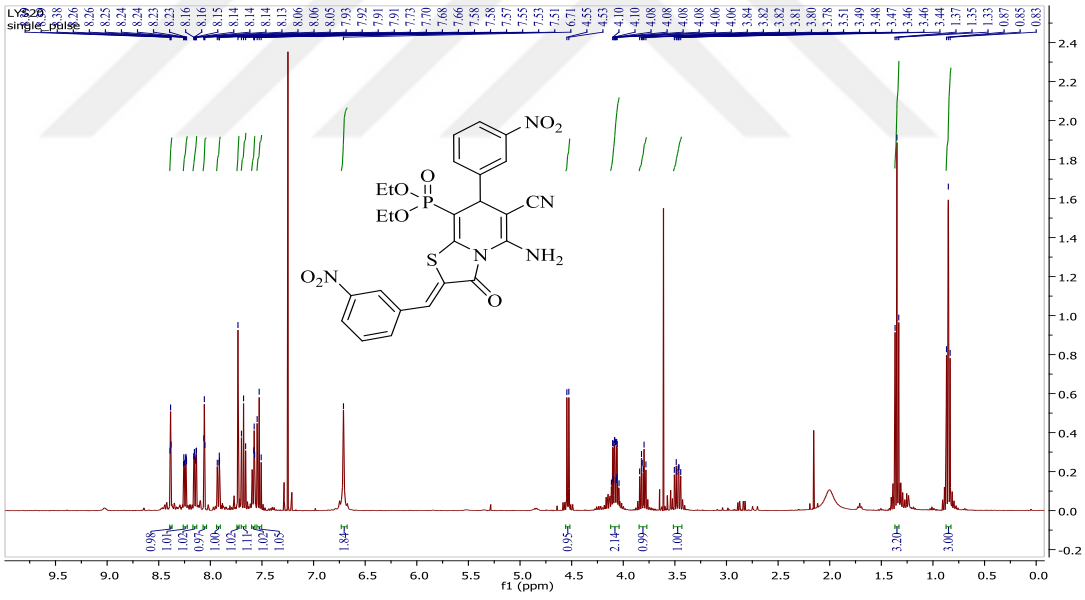

Figure 7.40. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63j**

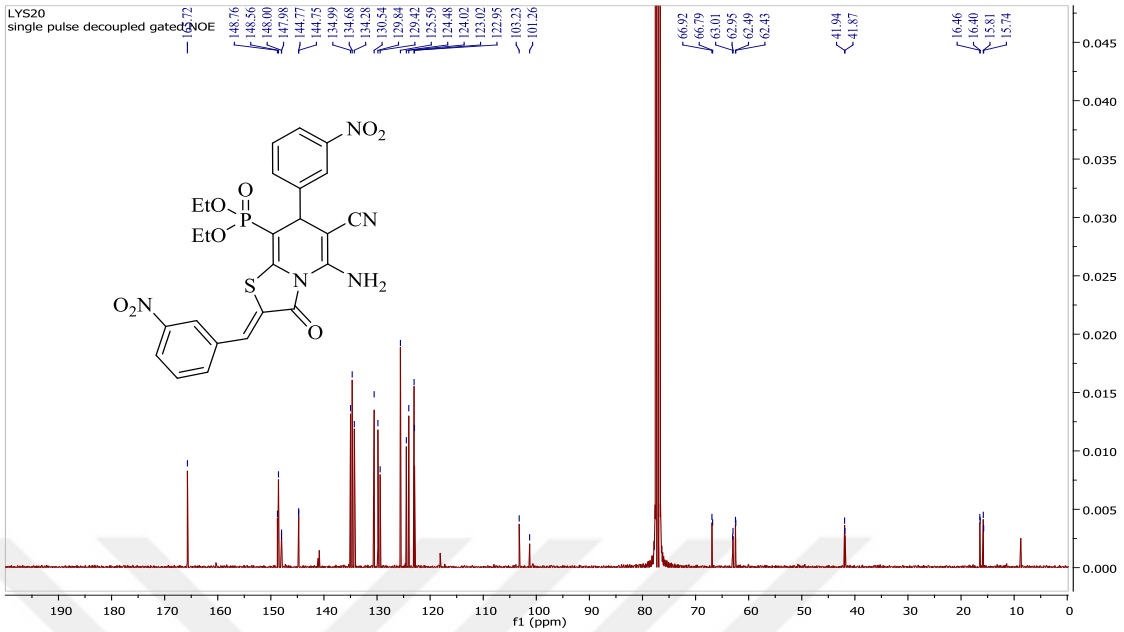

Figure 7.41. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63j**

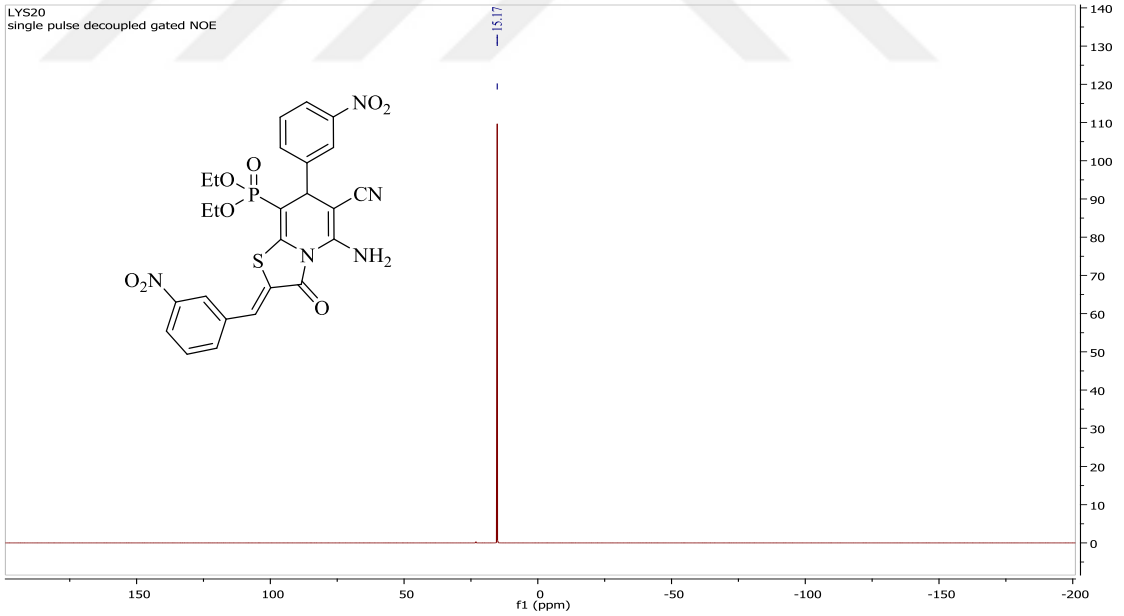

Figure 7.42. ^{31}P NMR for compound **63j**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
193 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O	P	S
584.1008	584.1005	0.3	0.5	17.5	C25 H23 N5 O8 P S	110.5	0.0	25	23	5	8	1	1

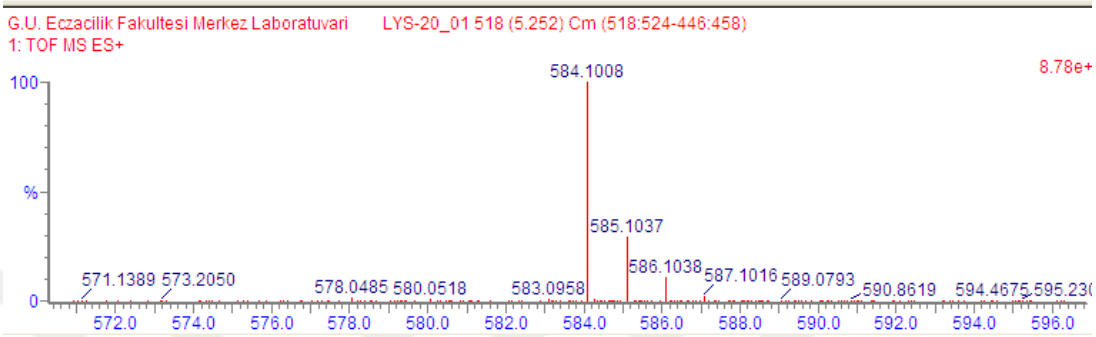

Figure 7.43. HRMS of compound **63j**

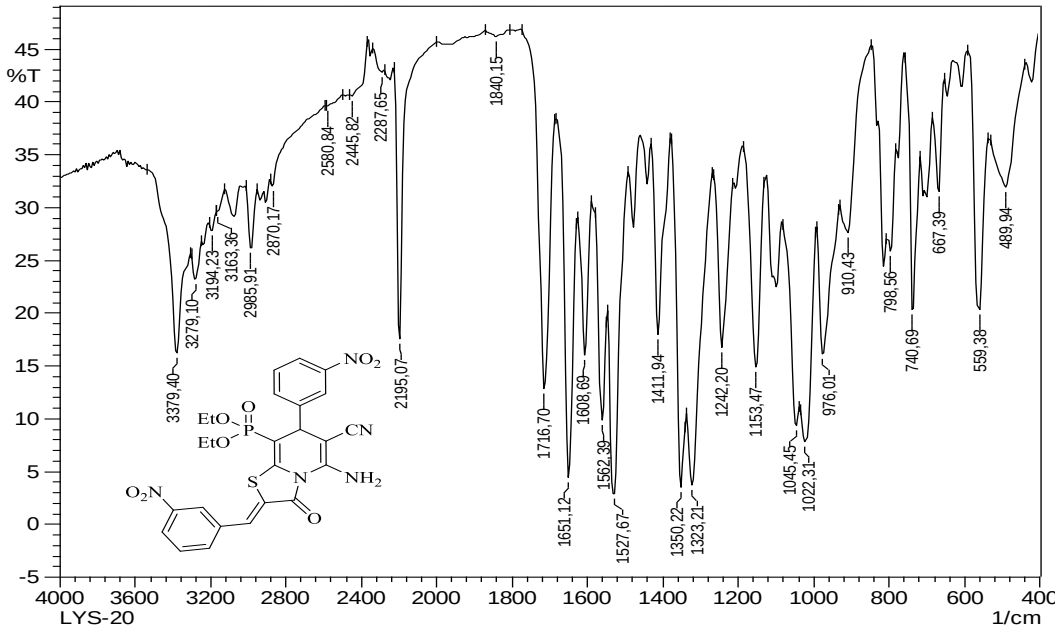

Figure 7.44. IR spectrum of compound **63j**

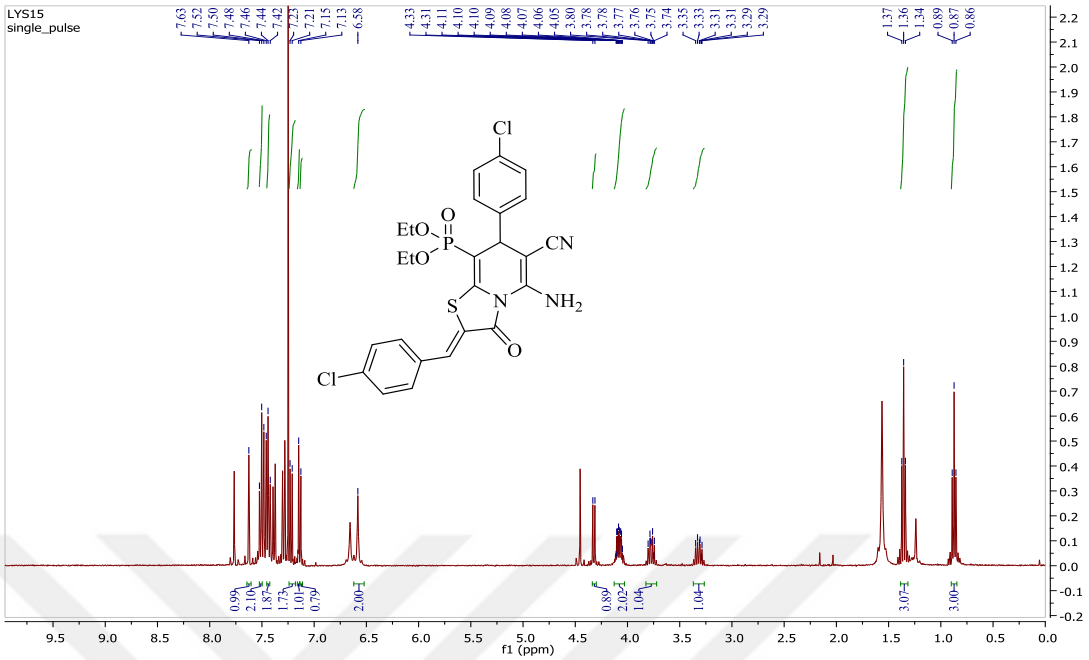

Figure 7.45. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63k**

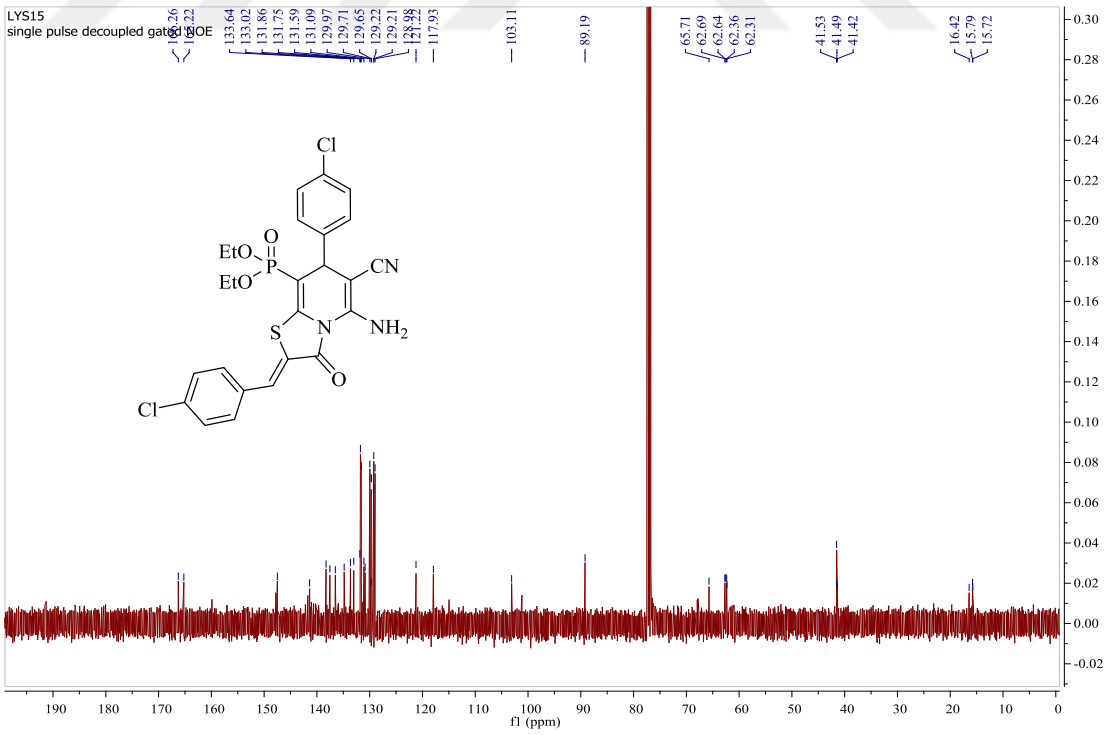

Figure 7.46. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63k**

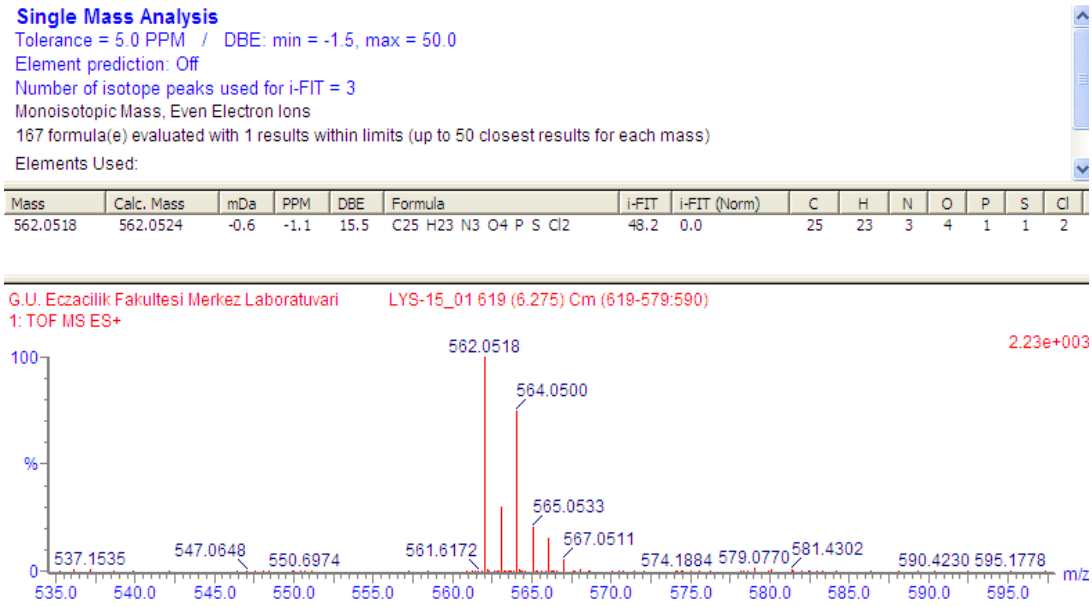

Figure 7.47. HRMS of compound **63k**

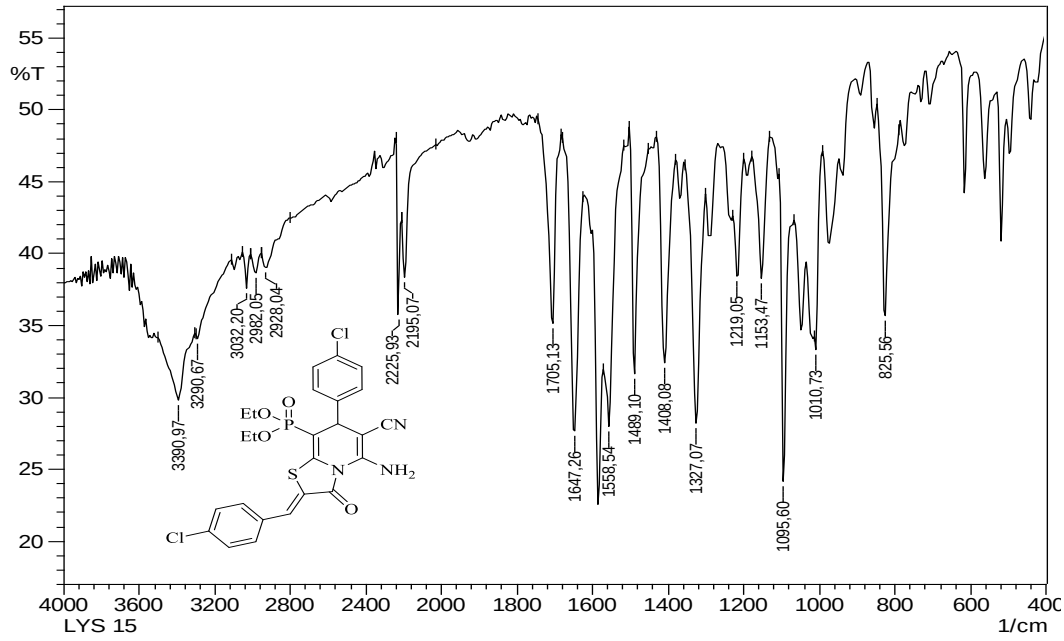

Figure 7.48. IR spectrum of compound **63k**

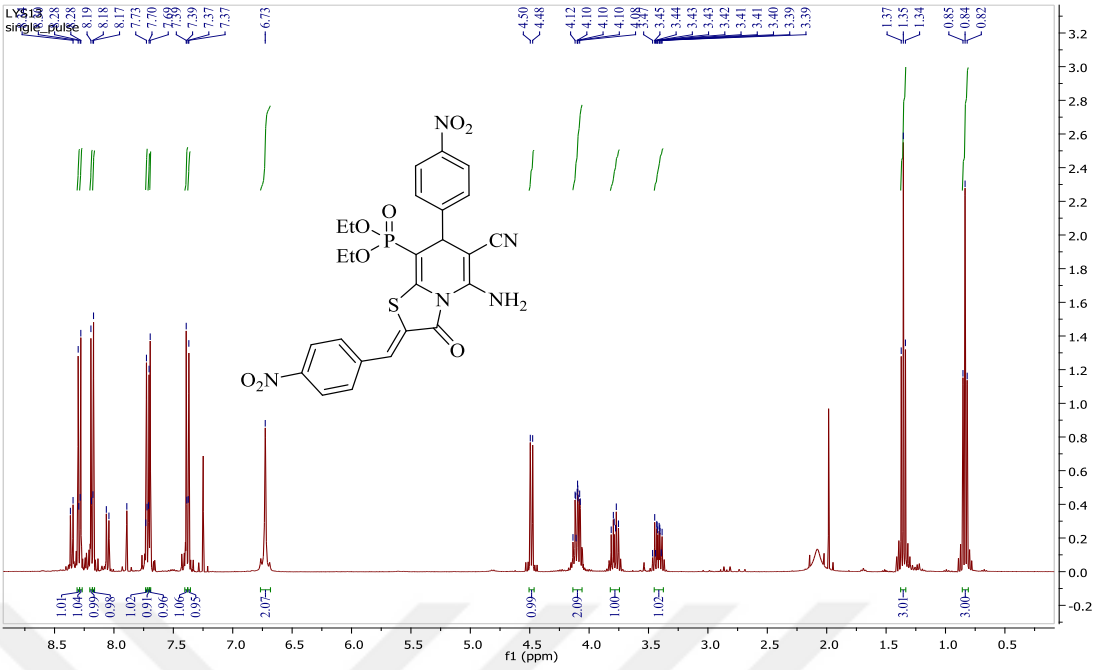

Figure 7.49. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **63l**

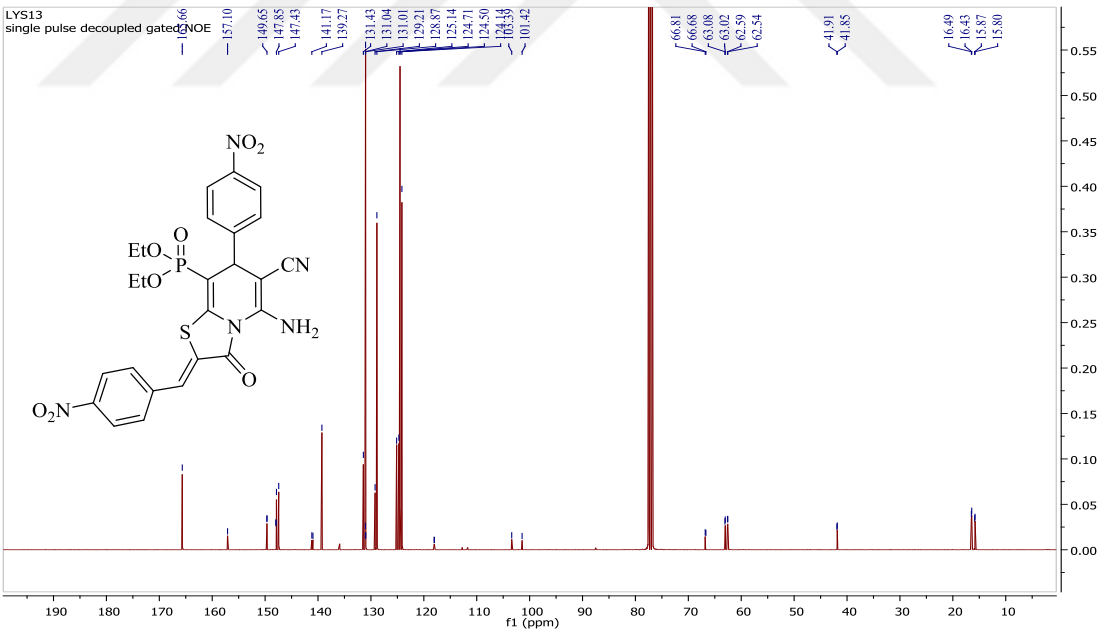

Figure 7.50. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **63l**

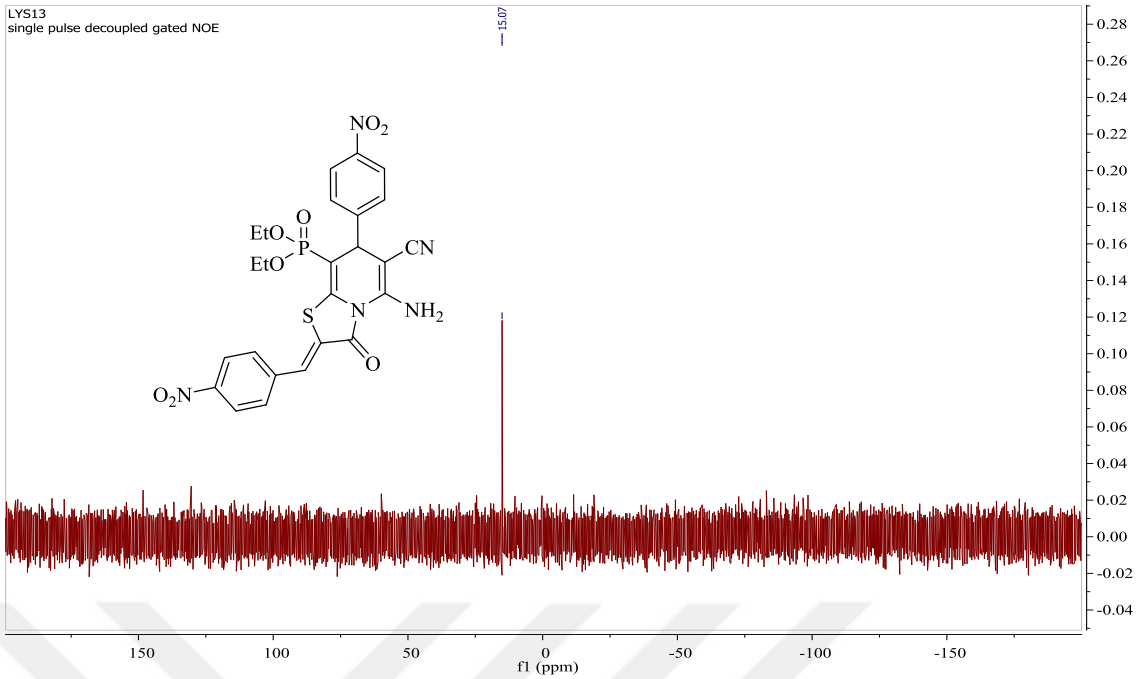

Figure 7.51. ^{31}P NMR for compound **63l**

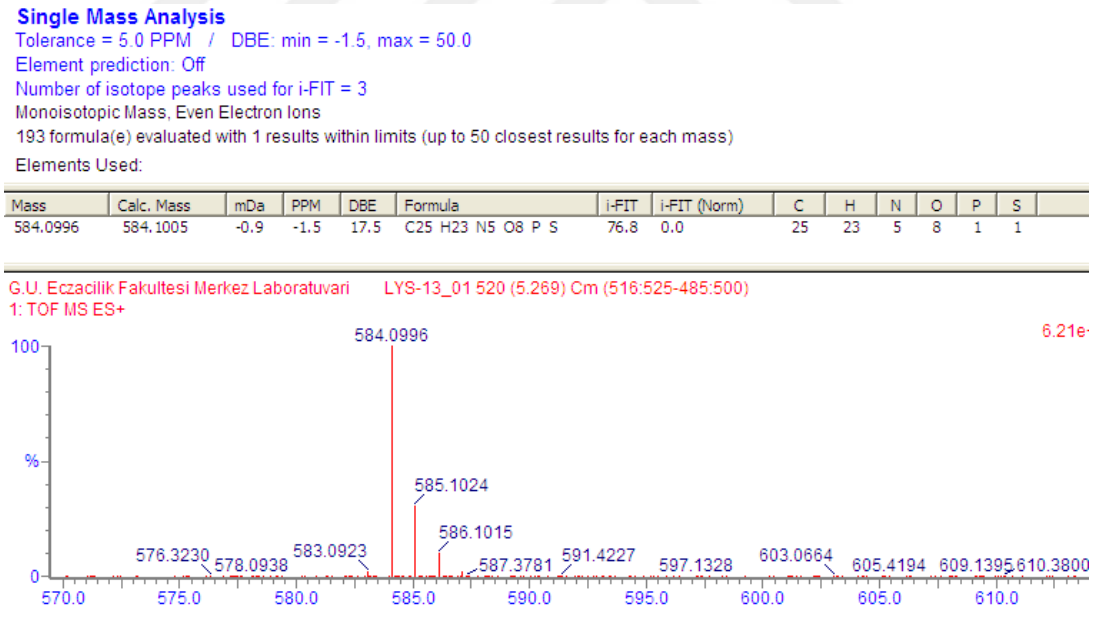

Figure 7.52. HRMS of compound **63l**

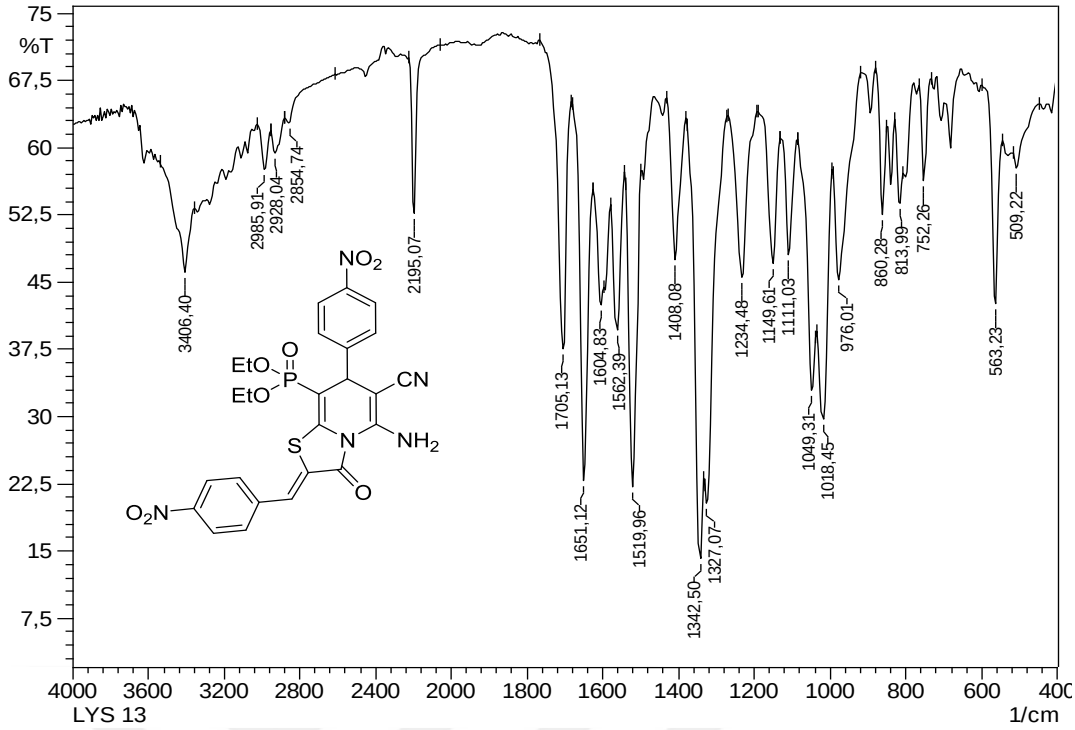

Figure 7.53. IR spectrum of compound **63l**

8. CURRICULUM VITAE

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List of puplications **:**

1. Lange YS, Altuğ C, Caner E, Güneş H "Multicomponent Synthesis of Thiazolo[3,2-a]pyridin-8-yl phosphonates" Trans Mediterranean Colloquium on Heterocyclic Chemistry, TRAMECH *VIII, 11-15 November, 2015, Beldibi, ANTALYA.*