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N˚ of order ……..

Series ….....

THESIS

PRESENTED TO THE CHEMISTRY DEPARTMENT TO OBTAIN THE 3 rd CYCLE DOCTORATE DIPLOMA

OPTION

Organic Chemistry, Bioactive molecule

THEME

Synthesis and anticancer properties of some novel Cyanopyridines-based hybrid molecules bearing pyrazole, oxadiazole, and N-acylhydrazone moieties

By

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I dedicate this work:

To My Parents

Not everything I say will express my gratitude to you, so I'll just be happy to say thank you for making me what I am today.

To my sisters Dounia and lina and my brother Hamza

Thank you for the moral and physical support.

ACKNOWLEDGMENTS

This work was carried out at the " Laboratory of Synthesis of Molecules with Biological Interest " at the University Constantine-1. I would like to thank all the people who took part in the development of this work.

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And finally, thank you to all those who have crossed my path, and whose list would be far too long here, but who have contributed to making these years finally pass so quickly and in the best conditions possible

List of ABBREVIATIONS

A-2780: The human ovarian cancer Cell line

ATCC: American Type Culture Collection

Caco-2: The human colon carcinoma cell line

AcOH: Acetic acid

CAN: Cerium Ammonium Nitrate

°C: degree Celsius

cm^{-1}: per centimeter

DIEA: N, N-Diisopropylethylamine

DBU: 1,8-Diazabicyclo [5.4.0] undec-7-ene

DMAC: Dimethylacetamide

DMF: dimethylformamide

DMSO: dimethyl sulfoxide

DMAP: 4-Dimethylaminopyridine

d: doublet

EeAChE: Electrophorus electricus acetylcholinesterase

g: gram

HeLa: an immortal cell line used in scientific research

HIV: human immunodeficiency virus

HATU: 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxidehexafluorophosphate

Hz, MHz: Hertz, Mega Hertz

h: hour

IBM: International Business Machines Corporation

IR: infrared

IC_{50}: The half maximal inhibitory concentration

***J*:** coupling constant

MCF-7: The human breast cancer Cell line

ml: milliliter,

mol, mmol: mole, milli mole

m: multiplet

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

MCRs: multi-component reactions

MDA-MB-231: an epithelial, human breast cancer cell line.

MDA-MB-468: breast cancer cell line isolated from a 51 years old human female in 1977, commonly used in breast cancer research.

NaPTS: Sodium toluene-4-sulfonate

Nafion NR50: sulfonated tetrafluoroethylene-based fluoropolymer-copolymer

NMR: nuclear magnetic resonance

(OTf): Trifluoromethanesulfonate

PIM-1: Proto-oncogene serine/threonine-protein kinase

PBS: Phosphate-buffered saline

pTsOH: *p*-Toluenesulfonic acid

Ppm: parts per million

RAGE: Receptor for Advanced Glycation End Products

S: singlet

SPSS: Statistical Package for the Social Sciences

TCCA: trichloroisocyanuric acid

TLC: thin layer chromatography

TEA: Triethylamine

THF: Tetrahydrofuran

t: Triplet

ZIKV: Zika virus

δ : chemical shift relative to TMS

General information

- The starting materials and reagents used in the reactions were supplied commercially by Aldrich, Acros, ABCR, and Merck.

- Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm), and the coupling constants (*J*) are expressed in Hertz (Hz). The addition of D_2O confirmed the assignment of exchangeable protons (NH).

- Elemental analyses were performed by LECO CHNS-932 elemental analyzers.

- Infrared spectra were recorded with ATR equipment in the range 4000-650 cm^{-1} on a Perkin Elmer Spectrum One FTIR spectrophotometer.

- Melting points (mp) were measured in open capillary tubes and are uncorrected using a Gallenkamp MPD350.BM3.5 apparatus.

- Thin-layer chromatography (TLC) was performed on silica 60 F254.

- The human breast (MCF-7) cancer cell line and female ovarian (A-2780) cancer cell line, and the human colon carcinoma cell line (Caco-2) were retrieved from the American Type Culture Collection (ATCC).

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Chapter 02: Results and Discussion

General Introduction

General Introduction

Cancer is a serious health problem in all societies, regardless of wealth or social status. In 2018, 18.1 million people worldwide had cancer, and 9.6 million patients died from the disease. By 2040 these figures will almost double and will be the most massive increase in low and middle-income countries where more than two-thirds of world cancers will occur [1].

Consequently, numerous drugs have been developed to cure cancer. Amongst the approved anticancer drugs between 2010 and 2015 by Food and Drug Administration, about 65% of anticancer drugs have one or more cyclic rings bearing oxygen or nitrogen atoms [2,3]. However, good treatable values have not been achieved yet with existing single-target drugs due to a multifactorial basis of cancer that involves both genetic and environmental risk factors. Moreover, most drugs are not only unable to overcome the resistance mechanism involved in primary and secondary cancer cells but also are unable to differentiate normal cells from neoplastic ones [4]. This has led to the general belief that using two different drugs would have a better therapeutic effect and a lowered side effect profile compared with single-target drugs.

Even though this approach seems to disagree with conventional strategies usually used in medicinal chemistry, it is in full agreement with the logic of using "chemotherapeutic cocktails" in clinics that are combining several chemotherapeutic agents exhibiting different mechanisms of action (e.g., 5-Fluorouracil, Pirubicin, Cyclophosphamide for the treatment of breast cancer) that are found to be more effective than the use of the same agents alone [5,6]. However, the use of such a combination of separate active molecules decreases the probability of seeing the molecules arrive at their respective targets at the same time and increases the risk of losing the possible synergy between these molecules. Consequently, in recent years, the polychemotherapeutic approach was translated into the design of hybrid molecules aiming to modulate more than one target and fight different types of cancer at once.

Hybrid molecules, also called "multi-target-directed ligands," are defined as different structural features of two active fragments combined in a single molecule with different biological functions and dual activity, indicating that a hybrid molecule acts as two distinct pharmacophores showing its ability to modulate multiple intracellular pathways simultaneously and higher overall efficacy than a molecule directed only at a single target. Furthermore, hybrid molecules are considered as the most prevalent chemical entities to work upon for developing modified scaffolds with many improved and excellent properties in biology and medicinal science. Nowadays, hybrid drugs containing two or more covalently linked known potential pharmacophores are designed to simultaneously modulate multiple targets of multifactorial diseases to overcome the side effects associated with a single drug.

In this context and owing to the pharmacological data of pyridine, 1,3,4 oxadiazole, and pyrazole ring, we are interested in synthesizing new hybrid molecules types A, B, and C (Figure (a)) and examining their anti-cancer activities against A2780, MCF-7, and Caco-2 cell lines.

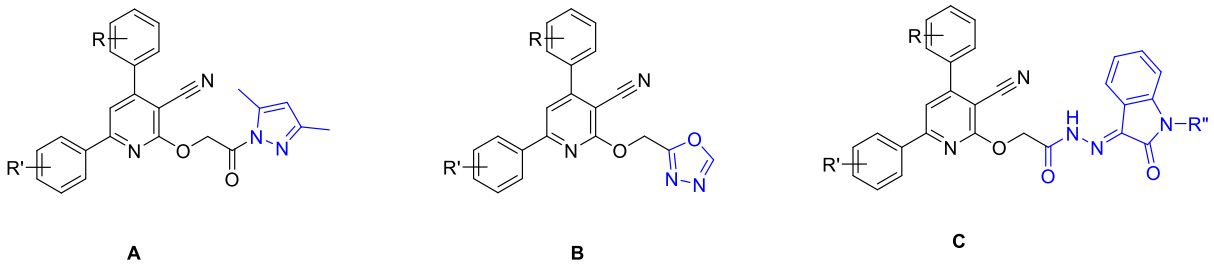


Figure (a).

Thus, this thesis will be presented in three main chapters:

- The first chapter will introduce the different therapeutic interests and the different synthesis methods of cyanopyridine, pyrazole, oxadiazole, and *N*-acylhydrazone moieties. (Figure (b))

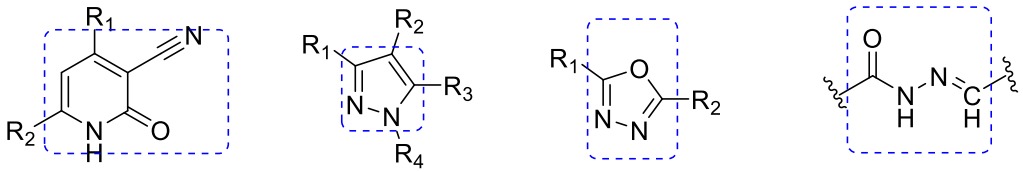


Figure (b).

- The second chapter will be related to the results obtained during the synthesis of the original molecules A, B, C, with the description of the various experimental conditions, followed by the comments on the achieved results. (Figure (c))

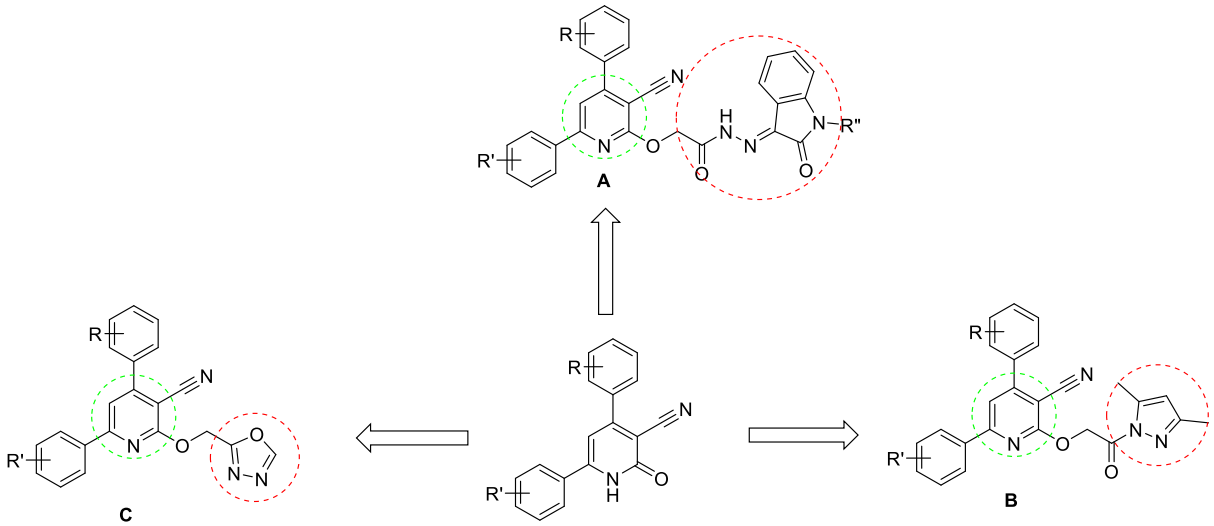


Figure (c).

- In the third chapter, we will evaluate and discuss the anti-cancer results for the three novel coupled molecules, particularly: "pyridine-pyrazoles," "pyridine-oxadiazoles," and "pyridine-N-acylhydrazones ".

Chapter 01

Bibliographic review on the synthesis and biological interests of Cyanopyridine, pyrazole, oxadiazole, and N-acylhydrazone moieties

I.1 Introduction:

Heterocycles represent an essential class in organic chemistry. Indeed, heterocyclic units are present not only in many natural products but also in the skeleton of numerous molecules with useful biological activities [7]. Their structure can engage in a broad range of intermolecular interactions, making them the molecules of choice in pharmaceutical research, food and beverage, and agrochemicals.

Among the heterocycles, pyridine-containing molecules have always fascinated researchers and aroused great interest among molecular biologists and organic chemists due to their wide range of biological activities generated partly by the presence of the pyridine nucleus in the structures of these products. Indeed, the pyridine ring can engage in a broad range of intermolecular interactions due to its properties, including molecular weight less than 500, low log P, not many hydrogen bonds. All this favours a temporary fixation on sites or receptors [8], leading to an agonistic or antagonistic signal response. As a result, more than 7000 [9] of many therapeutic interest molecules have a pyridine ring in their structures through the codex. The present chapter will discuss the chemistry and biological significance of heterocyclic systems containing cyanopyridine, pyrazole, 1,3,4-oxadiazole, and acylhydrazone moieties.

I.2 Cyanopyridine

I.2.1 The biological interest of Cyanopyridine derivatives

The importance of cyanopyridines in organic synthesis and pharmaceutical industries has increased over the past few decades as they display a broad spectrum of potential biological and pharmacological activities such as:

I.2.1.1 Cardiovascular activity

- **Milrinone** [10] is a drug marketed under the brand name **Primacor**. while it has been used to treat heart failure for many years, Milrinone can decrease pulmonary vascular resistance and increase the heart's contractility by inhibiting phosphodiesterase 3 (Figure I. 01).

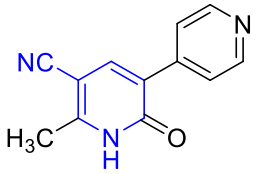


Figure I. 01

- **Amrinone** [11] is a marketed drug under the brand name **Inocor**. it has been introduced to clinical trials to treat heart failure. In comparison, its mechanism of action involves phosphodiesterase 3 inhibitors (Figure I. 02).

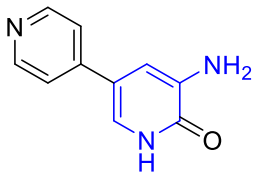


Figure I. 02

I.2.1.2 Anti-Cancer activity

- Khaled M. Abouzid *et al*. [12] developed a novel series of cyanopyridines bearing 2-methyl-3H-benzo[f]chromen-3-one as inhibitors of PIM-1 kinases, a serine family/threonine kinase that plays a fundamental role in cell survival (Figure I. 03).

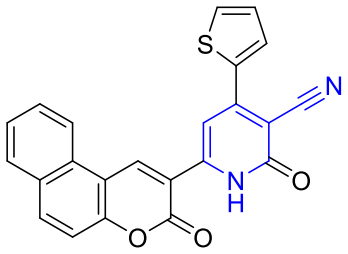


Figure I. 03

- Sabour *et al.* [13] designed and developed several cyanopyridines with higher lipophilic properties and Survivin inhibiting capability, specific tumor genes in the human genome, and represent an attractive target for cancer therapy (Figure I. 04).

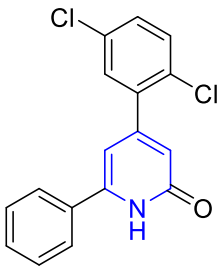


Figure I. 04

I.2.1.3 Anti-viral activity

- INDOPY-1 is an essential class of inhibitors that interfere with Reverse Transcriptase (RT), which is used by certain viruses such as HIV to replicate their genomes by retrotransposon mobile genetic elements to proliferate within the host genome [14] (Figure I. 05).

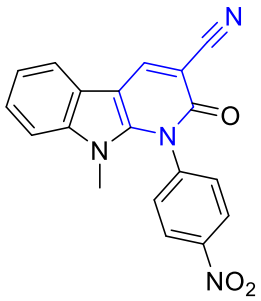


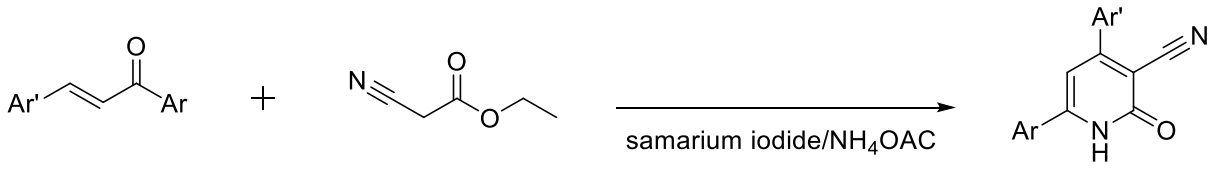
Figure I. 05

I.2.2 Reported synthetic strategies for Cyanopyridines

Numerous synthetic pathways have been reported for 2-oxo-3-cyanopyridine derivatives. We report, herein, only the most effective synthetic pathways:

I.2.2.1 From α,β-unsaturated ketones

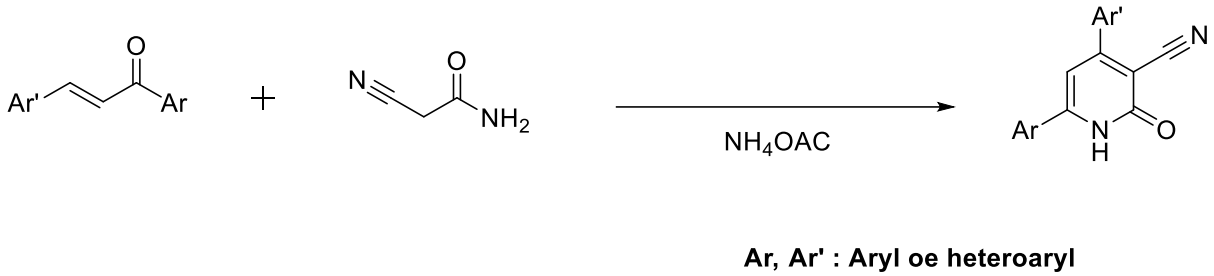
Many synthons have been reported for preparing 2-oxo-3cyanopyridines involving the condensation of α,β-unsaturated ketones with ethyl cyanoacetate, or cyanoacetamide. Thus, Yongmin *et al*.[15] described an environmentally friendly process by using samarium iodide as a catalyst in the reaction of α,β-unsaturated ketones with ethyl cyanoacetate in the presence of excess ammonium acetate (Scheme I. 01).



Ar, Ar' : Aryl or heteroaryl

Scheme I. 01

El-Sayed *et al*. [16] reported a new synthetic strategy of 3-cyanopyridin-2-ones by condensing cyanoacetamide with α, β-unsaturated ketones in the presence of excess ammonium acetate. To date, several research papers were reported following this methodology (Scheme I. 02).



Ar, Ar' : Aryl oe heteroaryl

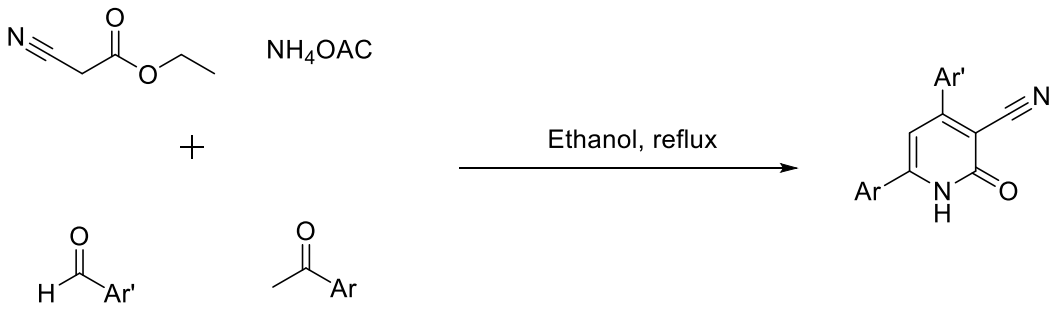
Scheme I. 02

I.2.2.2 One-pot multi-component reaction

Compared to conventional methods, multi-component reactions (MCRs) have several advantages, including short reaction time, good product quality, and high yields. These factors have encouraged researchers to investigate MCRs further. Numerous publications have been reported for synthesizing 3-cyanopyridin-2-ones using the MCRs strategy, among which we can cite:

I.2.2.2.1 Four-component Condensation

Abadi *et al*.[17] reported the synthesis of 2-oxo-3-cyanopyridines by a four-component condensation between an aromatic aldehyde, substituted acetophenone, ethyl cyanoacetate, and ammonium acetate in ethanol at reflux temperature. (Scheme I. 03).

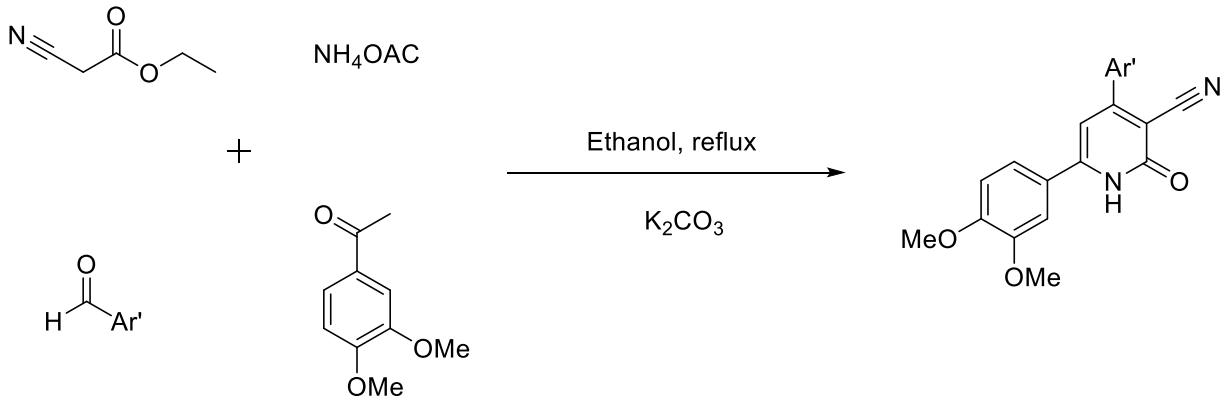


Ar, Ar' : Aryl or heteroaryl

Scheme I. 03

An environmentally friendly method for the synthesis of 2-oxo-3cyanopyridines was reported by Beheshti *et al*.[18] through four-component condensation of 4-dimethoxyacetophenone, aromatic aldehyde, ethyl cyanoacetate, and excess of ammonium acetate in the presence of potassium carbonate

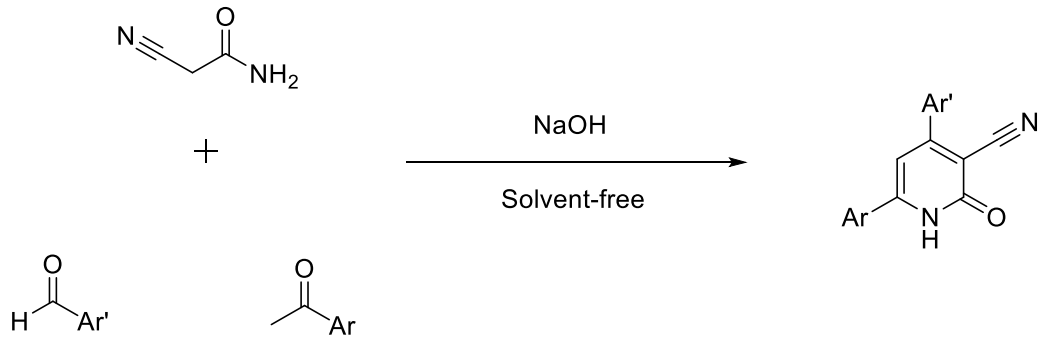
as a catalyst. Several organic solvents were checked in this reaction, such as ethanol, water, DMF, chloroform, dichloromethane, and toluene. The best results, including reaction time, yield, and purity, were obtained in ethanol (Scheme I. 04).



Scheme I. 04

I.2.2.2.2 Three-component Condensation

Rong *et al*.[19] reported three-component reaction for the synthesis of 2-oxo-3cyanopyridines through the reaction of aromatic ketones, aromatic aldehydes, and 2-cyanoacetamide at 75 °C under solvent-free conditions by using NaOH as a catalyst. The reactions were processed in a short time, and the final products were isolated in good yields (Scheme I. 05).



Scheme I. 05

I.3 Pyrazole

Pyrazoles are Heterocyclic Nitrogen-containing compounds representing a valuable class of bioactive compounds and significantly impact the agrochemical and pharmaceutical industries[20].

The pyrazole ring [21], an isomer structural of imidazole, has two atoms of nitrogen with different properties: one has the property of pyridine nitrogen that can be exposed to protonation in an acidic medium, the other one behaving like pyrrole nitrogen. In official nomenclature, the pyrazole unit is called 1,2-diazole. The pyrazole rings contain six delocalized π-electrons in their planar conjugated structures, which make them aromatic (Figure I. 06).

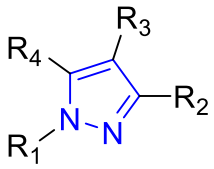


Figure I. 06

Pyrazoles is a natural product that was first isolated in the 1950s on the name "3-n-nonylpyrazole" from the *Houttuynia Cordata* plant, a family of the *Piperaceae* from Asia. Levo- β -(1-pyrazolyl) alanine is also a pyrazole natural product that has been isolated from *Citrullus Vulgaris*, a watermelon seed [22] (Figure I. 07).

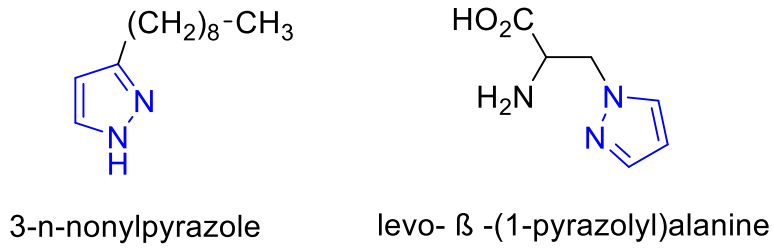


Figure I. 07

I.3.1The biological interest of pyrazole derivatives

Nowadays, numerous clinically useful compounds have pyrazole core in their structures, (Figure I. 08), such as:

- **Celecoxib**[23],[24] (the brand name Celebrex), a Nonsteroidal anti-inflammatory drug used to treat acute pain, osteoarthritis, menstruation, and rheumatoid arthritis. Moreover, Celecoxib has been considered an effective anti-inflammatory agent with less undesirable side effects.
- **Sildenafil citrate** [25](the brand name Viagra) is a drug developed in 1998 by Pfizer (pharmaceutical company) and has been used to increase blood flow and improve erectile function.
- **Betazole** also known as **Ametazole,** is an H_2 receptor agonist. It has been clinically used to test gastric secretory function[26].
- **Rimonabant**[27](the brand name Acomplia, Zimulti) is a selective CB1 receptor blocker developed by Sanofi-Aventis.
- **Zoniporide**[28] (Cardiovasc Drug) is a selective and potent inhibitor of the human Na^+/H^+ exchanger isoform 1 (NHE-1).
- **Lonazolac**[29] (the brand name Irritren, Argun L) is a Nonsteroidal anti-inflammatory used to block prostaglandins formation by inhibiting the enzyme cyclooxygenase.
- **Fezolamine**[30] is an antidepressant drug investigated and developed by Sterling Drug Company in the 1980s.
- **Difenamizole**[31] (brand name Pasalin) is an analgesic drug and Nonsteroidal anti-inflammatory. Difenamizole has monoaminergic properties such as monoamine oxidase inhibition and Dopamine reuptake inhibition capability.

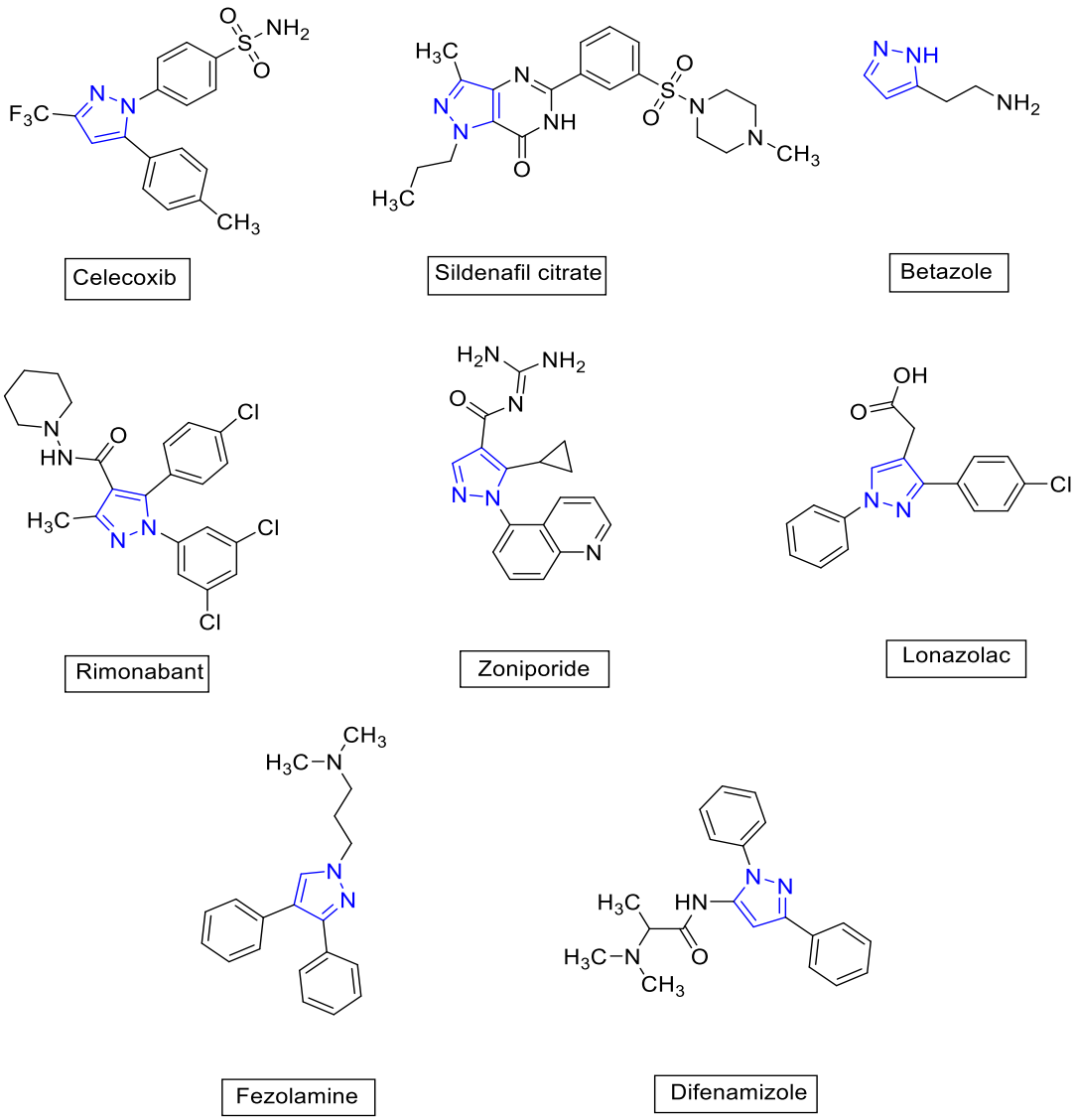

Celecoxib
Sildenafil citrate
Betazole
Rimonabant
Zoniporide
Lonazolac
Fezolamine
Difenamizole

Figure I. 08

Heterocycles containing the pyrazole unit have attracted considerable attention in recent years due to their numerous biological activities reported, such as:

I.3.1.1 Anti-cancer activity

- Ibrahim *et al.*[32] synthesized a new series of hybrids molecules containing isatin-pyrazole and benzenesulfonamide derivatives. The biological activity of the target molecules was performed against transmembrane, tumour-associated enzymes. Compounds **(1)** and **(2)** were reported as more potent than the reference drug acetazolamide (Figure I. 09).

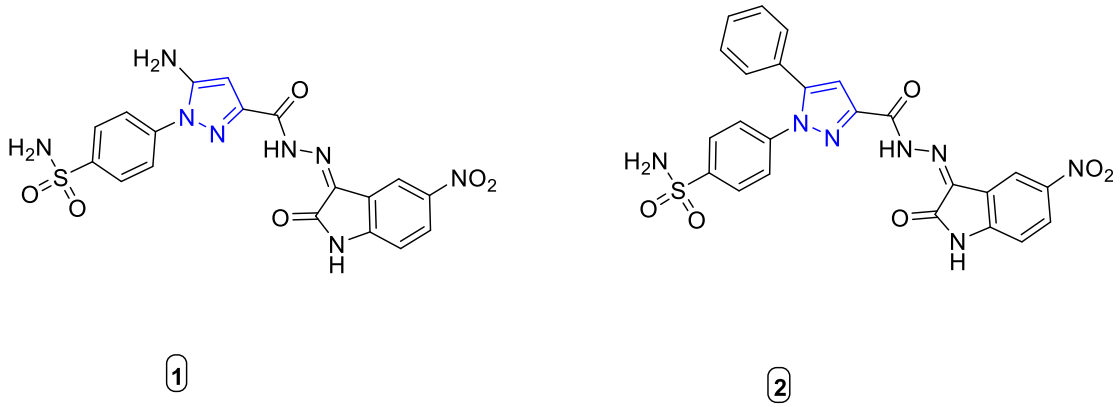


Figure I. 09

- Rai U *et al.*[33] developed a new series of pyrazole-chalcone derivatives. The target compounds screened for anticancer activity using human cervical tumor cells (HeLa) and human breast adenocarcinoma cells (MCF-7). Compound **(3)** was identified as the most active compound of the series (Figure I. 10).

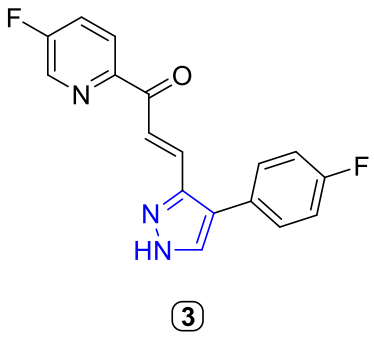


Figure I. 10

I.3.1.2 Anti-inflammatory activity

- Li *et al*.[34] reported a novel series of pyrazole derivatives containing furan-2-carbohydrazide or aminoguanidine moieties and evaluated their anti-inflammatory potential. Among all the compounds tested, compound **(4)** was more effective than the reference drugs Ibuprofen and Indomethacin (Figure I. 11).

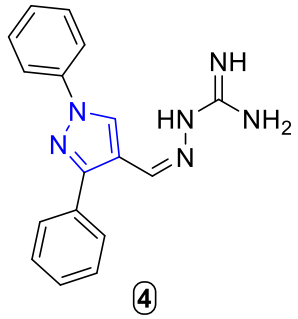


Figure I. 11

- Pelcman *et al*.[35] has prepared a series of 1N-substituted pyrazole-3-carboxanilides and investigated them as 15-lipoxygenase-1 (15-LOX-1) inhibitors. Compounds **(5)** and **(6)** were found as the most effective inhibitors of 15-lipoxygenase-1 (Figure I. 12).

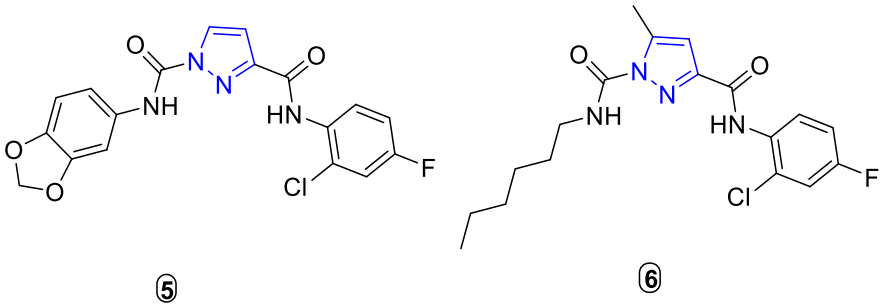


Figure I. 12

I.3.1.3 Antimalarial activity

- A series of hybrid compounds incorporating pyrazole and its bioisosters were developed by Bekhit *et al*[36]. Upon the evaluation of their antimalarial potential. Compounds **(7)** and **(8)** were identified five times higher than the reference drug chloroquine (Figure I. 13).

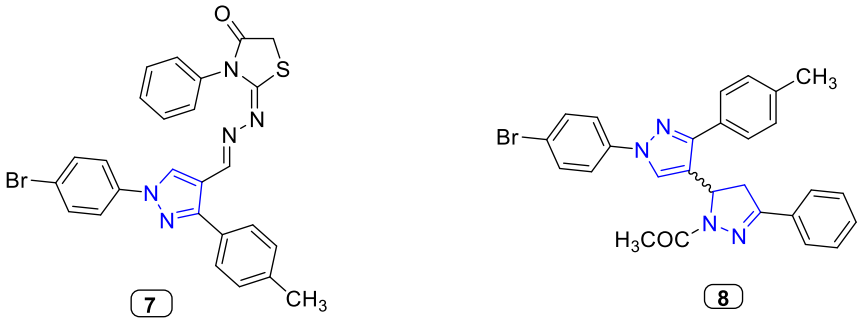


Figure I. 13

- Cabrera *et al*.[37] developed a series of aminomethylthiazole pyrazole compounds. The target molecules were screened for their antimalarial activity. Compound **(9)** was found to have a magnificent *in vitro* antiplasmodial activity with low cytotoxicity. Additionally, compound **(9)** showed promising *in vivo* activity in the mouse model *Plasmodium berghei* by exhibiting an activity of 99.5% (Figure I. 14).

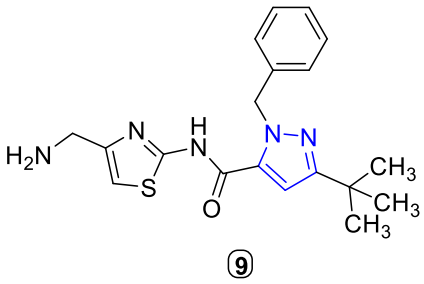


Figure I. 14

I.3.1.4 Anti-hypertensive activity

- Recently, a Japanese team has discovered that molecules derived from pyrazole-benzoxazine analogues exhibited selective nonsteroidal mineralocorticoid receptor (MR) antagonists. Compound **(10)** showed significant anti-hypertensive properties with excellent blood pressure-lowering effect and good pharmacokinetic profiles on oral administration [38] (Figure I. 15).

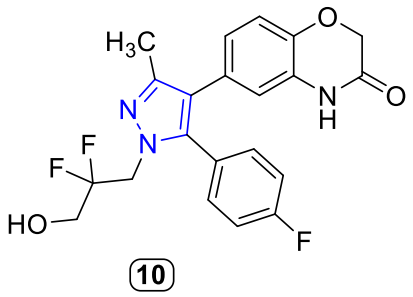


Figure I. 15

- Bonesi *et al.*[39] designed a series of pyrazole derivatives and screened their potential activity as Angiotensin I-converting Enzyme (ACE) inhibitors. Amongst the series, compound **(11)** showed the highest activity with an IC_{50} value of 0.219 mM (Figure I. 16).

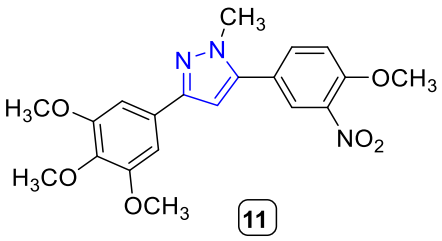


Figure I. 16

I.3.1.5 Anti-Alzheimer's activity

- Han *et al.*[40] designed new series of pyrazole-5-carboxamides and evaluated their anti-Alzheimer's activity. The results revealed that compound **(12)** was identified as the most potent inhibitor of RAGE (Receptor for Advanced Glycation End Products) (Figure I. 17).

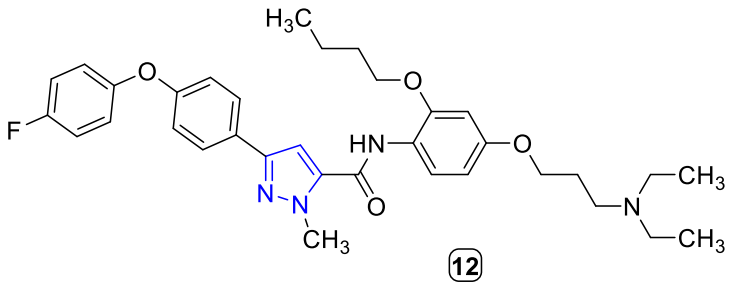
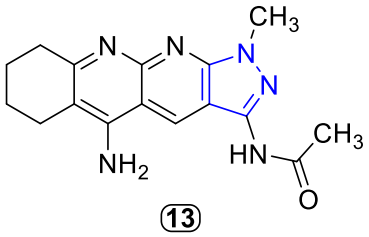


Figure I. 17

- Silva *et al*.[41] synthesized and evaluated the *in vitro* anti-Alzheimer's activity of novel pyrazolotacrines and found that compound **(13)** has potent inhibition of Electrophorus electricus acetylcholinesterase (EeAChE) among all the studied compounds (Figure I. 18).

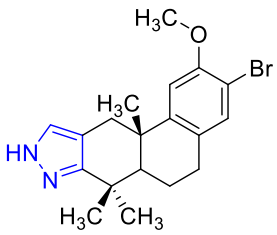


13

Figure I. 18

I.3.1.6 Antimicrobial activity

- The antibacterial activity of a series of pyrazole-fused tricyclic diterpene derivatives synthesized by Yu et *al*. [42] was evaluated against *Staphylococcus aureus* (S. aureus). Compounds **(14)** and **(15)** were identified as the most active compounds of the series (Figure I. 19).



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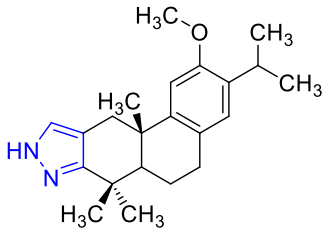


15

Figure I. 19

- Recently, a Chinese team developed a series of multi-pyrazole moieties and screened their antibacterial activity against *Bacillus subtilis* (*B. subtilis*). Compound **(16)** has exhibited maximum grams inhibition[43] (Figure I. 20).

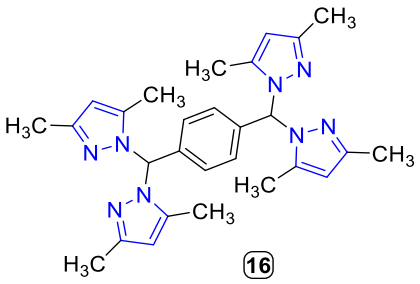


Figure I. 20

I.3.1.7 Anti-viral activity

- Recently another derivative of bis-1,3-thiazole derivatives containing pyrazole has been reported for its particular ability to inhibit the replication of the hepatitis C virus in chronically infected cells. Compound **(17)** was identified as the highest activity in the series [44] (Figure I. 21).

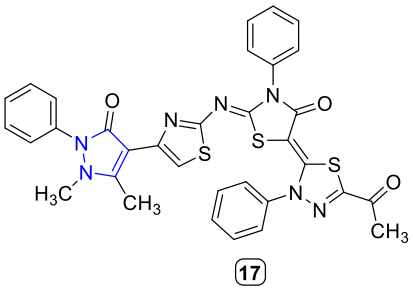


Figure I. 21

- Since the emergence of HIV-associated AIDS (human immunodeficiency virus), the discovery of new anti-viral compounds has become a research priority. In this context, Mizuhara *et al.*[45] has demonstrated that compound **(18)**, shown below, has an excellent capacity to inhibit the replication of the AIDS virus (HIV-1) in chronically infected cells (Figure I. 22).

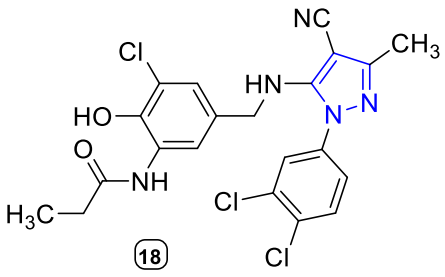


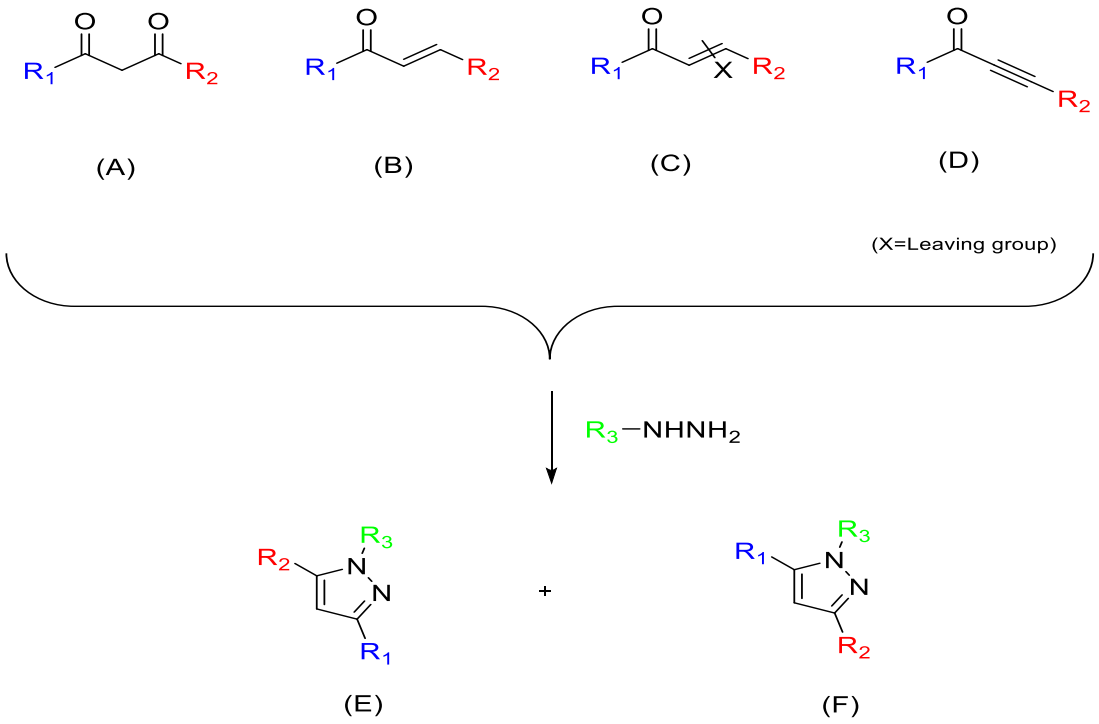
Figure I. 22

I.3.2 Reported synthetic strategies for Pyrazoles

As mentioned previously, substituted pyrazoles possess a broad range of biological activities, which makes them particularly interesting. The different techniques to prepare the pyrazole core has been subjected to many changes since Knorr's first syntheses [46]. The various methods reported, pyrazoles commonly synthesized by (1) The cyclocondensation of hydrazine and its derivatives on carbonyl systems or (2) dipolar cycloadditions.

I.3.2.1 Cyclocondensation of hydrazine and its derivatives on carbonyl systems

The most commonly used synthetic strategy to obtain substituted pyrazoles consists of a cyclocondensation reaction between a carbon unit with two electrophilic carbons in positions 1 and 3, such as 1,3-dicarbonyl derivative **(A)** or an unsaturated α-β-ketone **(B, C, D)** and hydrazine as a nucleophile (Scheme I. 06).

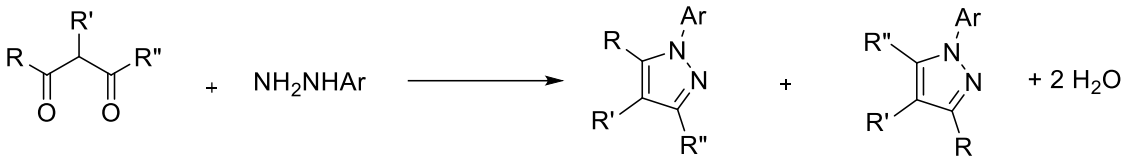


Scheme I. 06

When using non-symmetrical electrophilic substrates ($R_1 \neq R_2$), a mixture of two regioisomers E and F is often obtained if $R_3 \neq H$. When $R_3 = H$, a prototropic rearrangement makes pyrazole E equivalent to F.

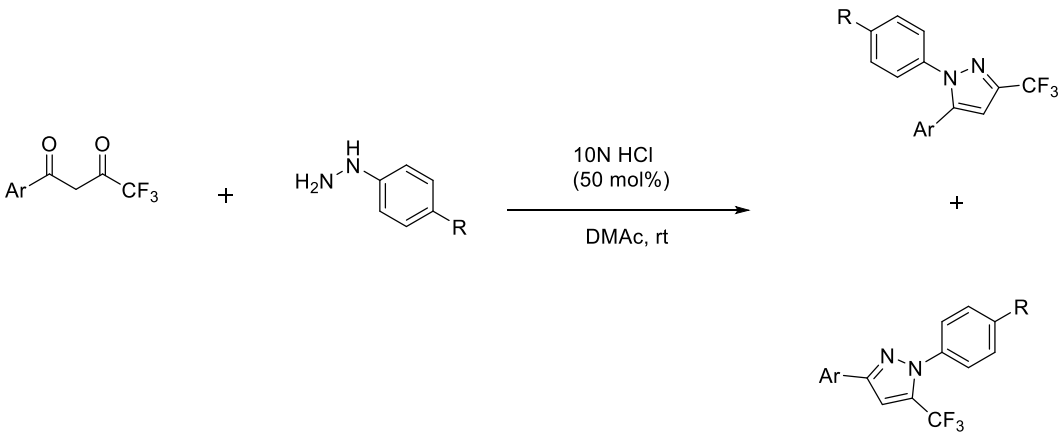
I.3.2.1.1 From 1,3-diketones

The cyclocondensation of hydrazine derivatives with 1,3-dicarbonyl compounds is a fast and straightforward approach to obtain substituted pyrazoles. Knorr developed this method at the end of the 19th century [46]. The presence of non-symmetrical 1,3-dicarbonyl substrates generally produced a mixture of two regioisomers that may be difficult to separate (Scheme I. 07).



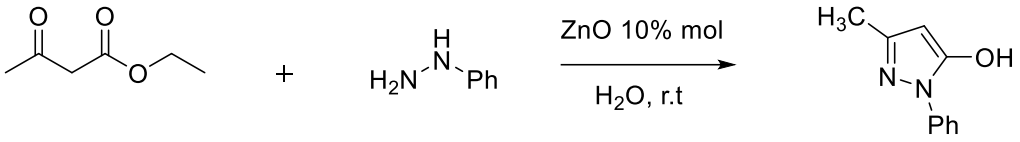
Scheme I. 07

In 2006, Gosselin et *al*.[47] developed new reaction conditions allowing the regioselective synthesis of 3, 4,5-substituted 1-arylpyrazoles from 1,3-dicarbonyl compounds. Indeed, Gosselin and co-workers showed that the cyclocondensation of 1,3-diketones with aryl hydrazine hydrochloride in dipolar aprotic solvents displays better performance than the use of protic polar solvents such as ethanol, which is commonly used for this type of condition. After conditions optimization, it seems that the addition of Hydrochloric acid solution (10N) to the amide-type solvents, mainly Dimethylformamide, N-Methyl-2-pyrrolidone, Dimethylacetamide, can increase the yields by accelerating the stage of dehydration (Scheme I. 08).



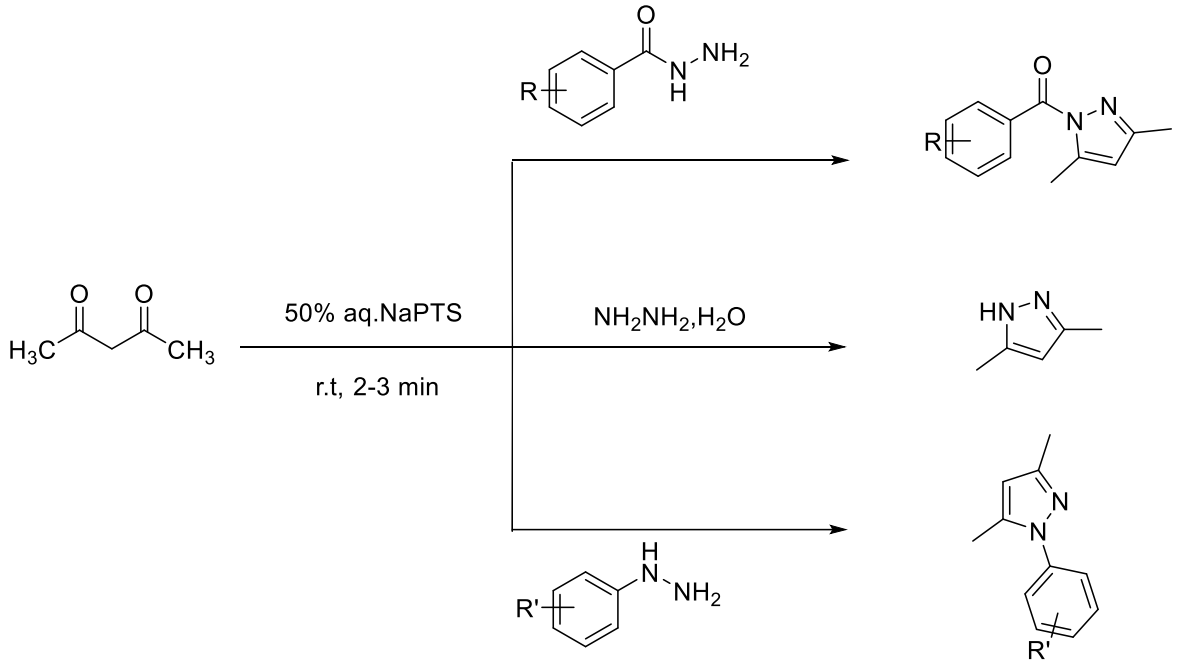
Scheme I. 08

Recently, an efficient green method for the synthesis of 1,3,5-substituted pyarzoles derivatives was reported by Grish et *al*.[48] consists of the condensation of ethyl acetoacetate, phenylhydrazine in the presence of ZnO (10 % mol) as a catalyst. The final products were collected in a short reaction time and excellent yield (95%) (Scheme I. 09)



Scheme I. 09

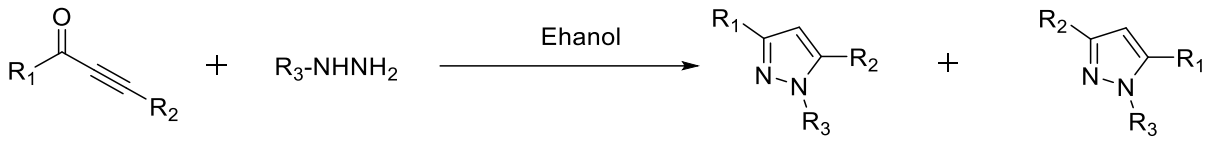
Similarly, Madhuri *et al*.[49] described an efficient catalyst-free synthetic protocol for pyrazole derivative by the condensation between hydrazine/hydrazide and 1,3-diketone in an aqueous hydrotropic solution. This method's main advantage is mild reaction conditions. The substituted pyrazole was afforded in good yield and short reaction time (Scheme I. 10)



Scheme I. 10

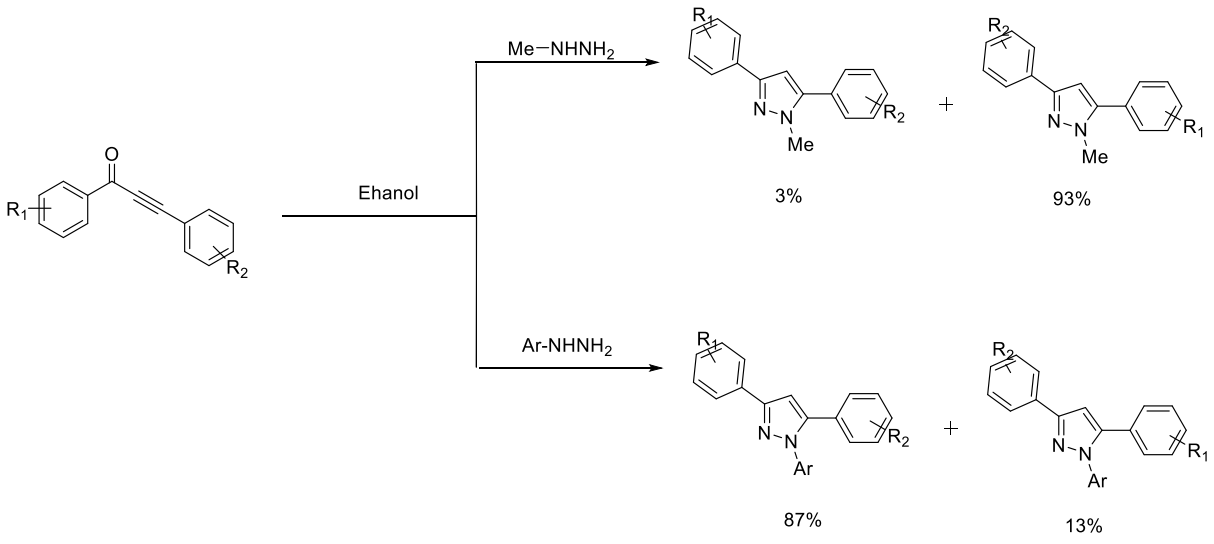
I.3.2.1.2 From Acetylenic ketones

Over the last century, the pyrazole unit has known many synthetic strategies involving the cyclocondensation of hydrazine derivatives on acetylenic ketones. However, the condensation still afforded a regioisomeric mixture (Scheme I. 11).



Scheme I. 11

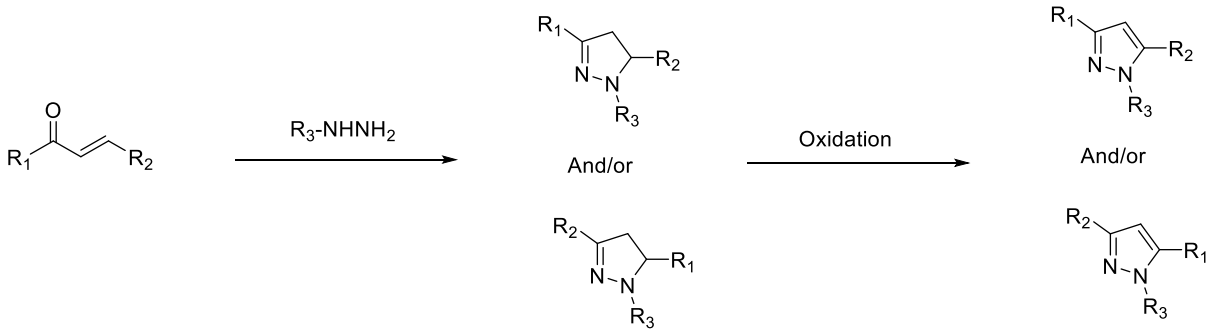
A study Interested in determining the regioselectivity factors of cyclocondensation of hydrazine derivative on acetylenic ketones in ethanol was reported by Bishop *et al*.[50] The reaction afforded two separable regioisomeric pyrazoles. The authors observed the deferent regioselectivity results in using arylic hydrazine (ration 87:13) or methyhlhydrazine (ration 93:3) was explained by nucleophilic power of the nitrogen attached to a methyl group, which make him react first on the triple bond by Michael addition followed by the formation of the intramolecular imine. On the other hand. The primary amine of arylic hydrazine is the most nucleophilic, and his reaction behavior will be the opposite of methylhydrazine (Scheme I. 12).



Scheme (I. 12)

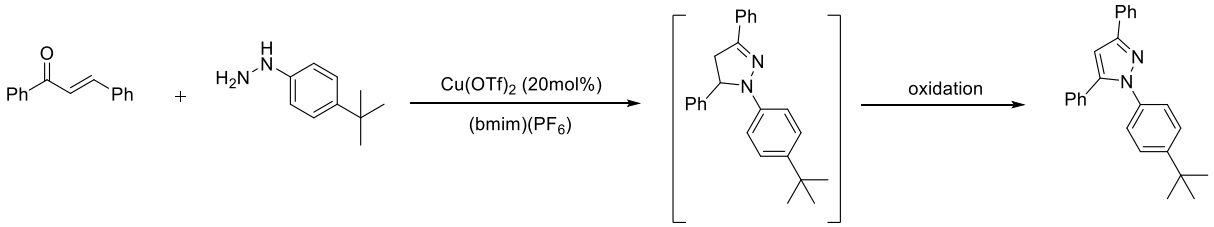
I.3.2.1.3 From α, β-ethylenic ketones

The cyclocondensation reaction of hydrazine derivative on vinyl ketones leads to the synthesis of the pyrazoline ring, which can provide the pyrazole ring after oxidation (Scheme I. 13).



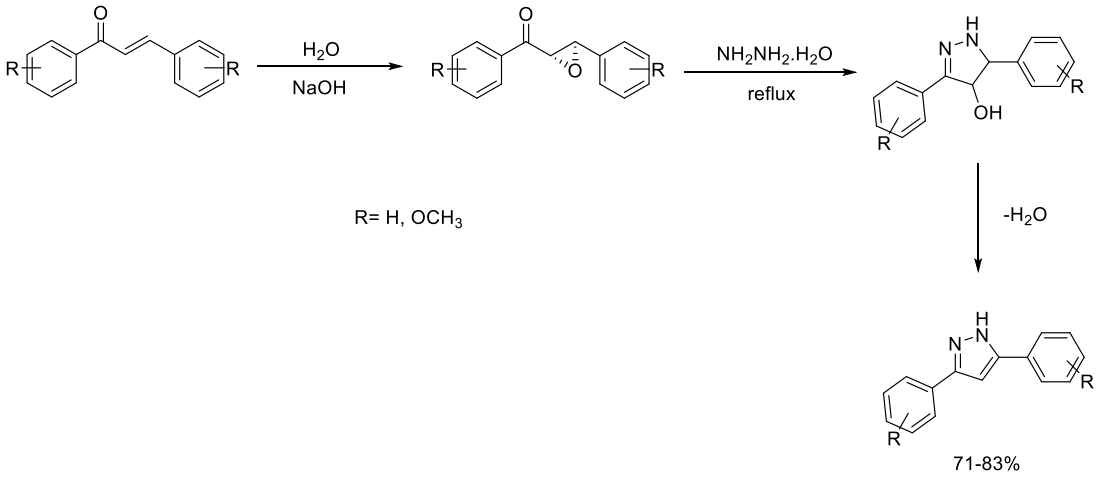
Scheme I. 13

Effective synthetic strategy for 1,3,5-trisubstituted pyrazole has been described by Rao *et al.*[51] through the access to pyrazoline by cyclocondensation of p-(4-(tert-butyl)phenyl) hydrazine with chalcones in the presence of 1-butyl-3methylimidazolium hexafluorophosphate, and copper triflate as catalysts, followed by oxidation *in situ* of the obtained pyrazolines to afforded the corresponding 1,3,5-trisubstituted pyrazoles in good yields (Scheme I. 14).



Scheme I. 14

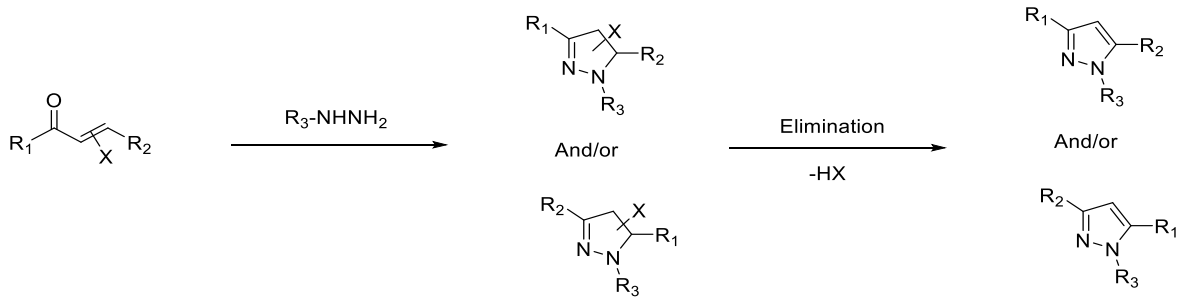
Bhat *et al*. [52]developed an efficient protocol for the preparation of 3,5-diaryl-1H-pyrazoles. Firstly, the reaction of epoxides with hydrazine hydrate results in pyrazoline intermediates. After the dehydration stage, the desired 3,5-diaryl-1H-pyrazoles were afforded in good yields (Scheme I. 15).



Scheme I. 15

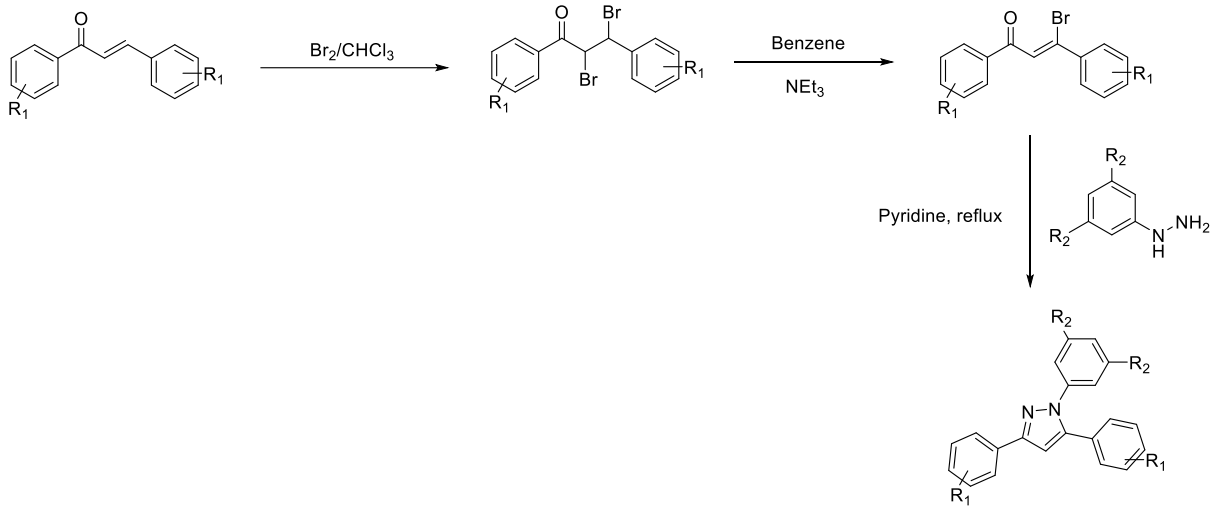
I.3.2.1.4 From α, β-ethylenic ketones Having a Leaving Group

The vinyl ketones containing a leaving group can react with hydrazine and its derivative to form pyrazoline intermediate, which provides the pyrazole ring after the elimination stage (Scheme I. 16).



Scheme I. 16

Adel A *et al*. [53]developed a method for synthesizing pyarzoles from the cyclocondensation reaction of monobromo chalcone with hydrazine derivatives. Firstly, dibromochalcones were prepared by the bromination reaction of chalcones then treated with dry benzene in the presence of triethylamine to give monobromo chalcone derivative, which reacts with hydrazine derivatives to afford the desired pyrazoles (Scheme I. 17).



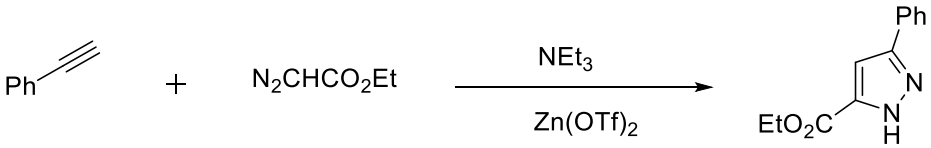
Scheme I. 17

I.3.2.2 From 1,3-dipolar cycloadditions

Alkyne (or olefin) can react with 1,3-dipolar compounds by the [3+2] cycloaddition reactions to allow access to the pyrazole ring.

I.3.2.2.1 From diazocarbonyl compounds

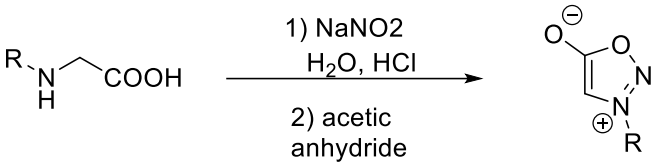
He *et al*.[54] described an efficient pyrazoles synthesis via 1,3-dipolar cycloaddition reaction of phenylpropargyl and ethyl α-diazoacetate in the presence of zinc triflate as a catalyst. The main advantages of this procedure, easy manipulation and good yielding (Scheme I. 18).



Scheme I. 18

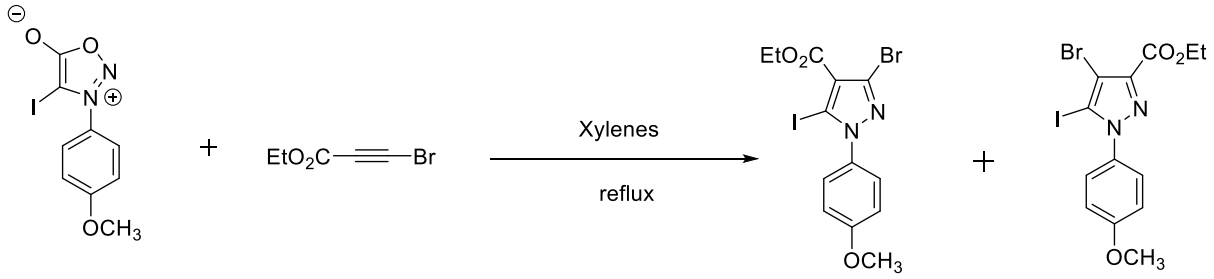
I.3.2.2.2 From Sydnones

Pyrazoles can also be obtained by a cycloaddition reaction of sydnones on a substituted alkyne. Sydnones were usually prepared by nitrosation of N-alkyl or N-arylglycines, followed by cyclization in acetic anhydride (Scheme I. 19).



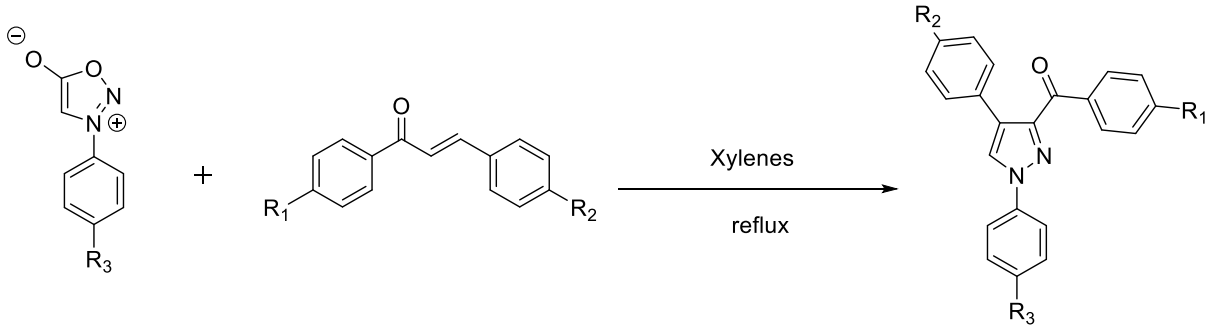
Scheme I. 19

Delaunay *et al*. [55] described a facile procedure for the regioisomeric synthesis of two 1,3,4,5-substituted pyrazoles by 1,3-dipolarcycloaddition of alkyne and sydnones in dry xylene (Scheme I. 20).



Scheme I. 20

In 2013 Fei Chen *et al*. [56] presented an effective protocol for synthesizing 1,3,4-trisubstituted pyrazoles via 1,3-dipolarcycloaddition reaction between 3arylsydnones and α, β -unsaturated ketones (Scheme I. 21).



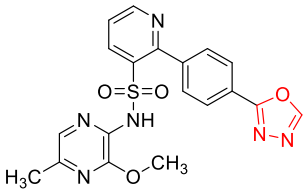
Scheme I. 21

I.4 Oxadiazoles

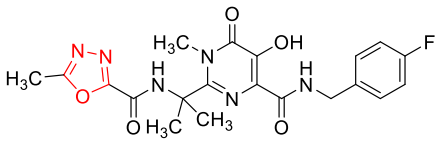
Oxadiazoles are cyclic compounds containing one oxygen and two nitrogen atoms in a five-membered ring. The position of these atoms can be different as 1,2,4-oxadiazole, 1,2,5-oxadiazoles, 1,2,3-oxadiazoles, and 1,3,4-oxadiazoles (Figure I. 23). However, researchers more widely study 1,3,4-oxadiazoles due to their wide range of applications in several areas, such as pesticide chemistry, scintillation of materials, electron-transport materials, polymers, and dyestuffs, herbicides, and corrosion inhibitors.

Figure I. 23

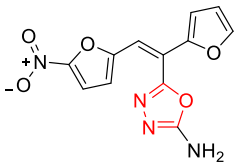
The privileged structure of 1,3,4-oxadiazole can act as a hydrogen bond acceptor, and can engage in a broad range of intermolecular interactions, which make them pose an enormous biological activity. For example.1,3,4-Oxadiazole core present in several drug molecules clinically useful, such as Nesapidil[57] and Tiodazosin [58] as an anti-hypertensive drug, Zibotentan [59,60] used against prostate cancer, Raltegravir [61,62] an antiretroviral, Furamizole [63] as an antibiotic (Figure I. 24).



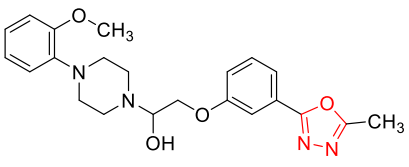
Zibotentan (anticancer)



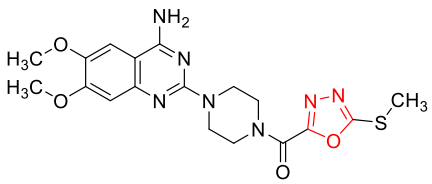
Raltegravir (antiretroviral)



Furamizole (antibacterial)



Nesapidil (antihypertensive)



Tiodazosin (antihypertensive)

Figure I. 24

I.4.1The biological interest of 1,3,4-oxadiazole derivatives

Over the last two decades, molecules bearing 1,3,4-oxadiazole moieties have attracted considerable attention due to their pharmaceutical interest, for example:

I.4.1.1 Anti-cancer activity

- Lee *et al*. [64] designed a series of 2,5-diaryl-1,3,4-oxadiazoline moieties of combretastatin. The biological evaluation of the target compounds showed excellent results against cancer cells. However, compounds **(19-22)** were identified as the most active compounds of the series with potent antiproliferative activities against multiple cancer cell lines (Figure I. 25).

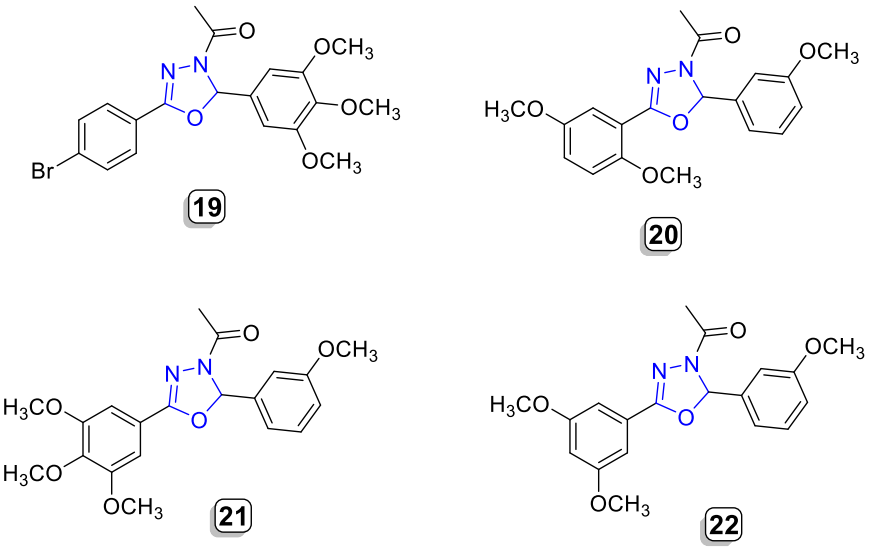


Figure I. 25

- Holla *et al.* [65] designed and synthesized a series of 2-chloro-1,4-bis-(5-substituted-1,3,4-oxadiazol-2-ylmethyleneoxy)phenylene derivative and evaluated their anticancer activity against different cancer types, such as ovarian, leukemia, lung, breast, renal prostate, melanoma, and colon cancer, respectively. The compounds **(23)** and **(24)** were identified as the most active compounds of the series (Figure I. 26).

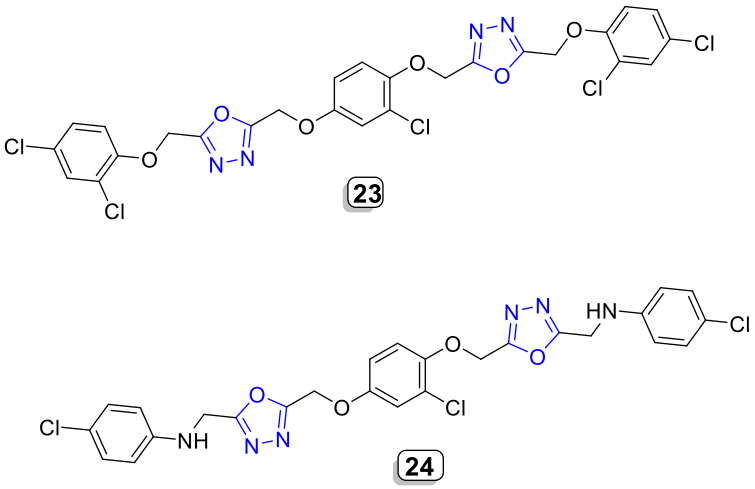


Figure I. 26

I.4.1.2 Antimicrobial activity

- Chandrakantha *et al*. [66] developed a series of 1,3,4-oxadiazole derivatives containing 2-fluoro-4-methoxy and screened their antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. The study revealed that compounds **(25)** and **(26)** were more potent than the reference drug fluconazole (Figure I. 27).

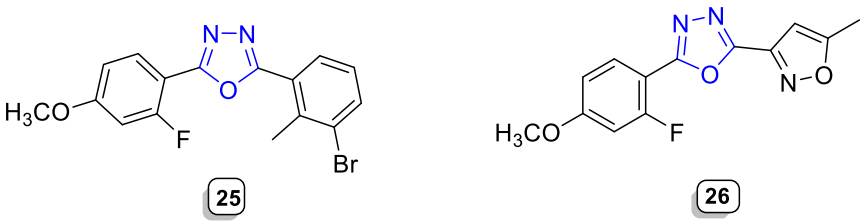


Figure I. 27

- Patel *et al*. [67] designed a series of compounds containing 1,3,4 oxadiazole scaffolds and evaluated their antimicrobial activity against *S.aureus*, *S.pyogenes*, *E.coli*, and *P.aeruginosa* using ampicillin as the drug standard. The compounds **(27)** and **(28)** were identified five times more potent than ampicillin (Figure I. 28).

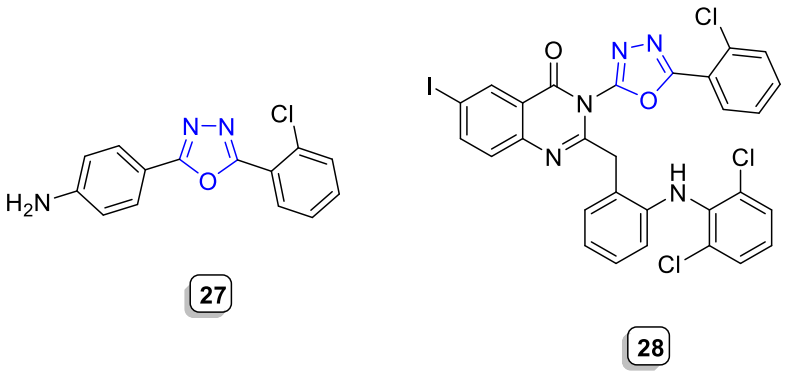


Figure I. 28

I.4.1.3 Anticonvulsant activity

- Kashaw and co-workers [68] synthesized and designed a new quinazoline-4(3H)-one series containing 1,3,4 oxadiazole scaffold and screened them for anticonvulsant activity. Out of all the tested compounds, only **(29)** and (**30)** were showed anticonvulsant activity (Figure I. 29).

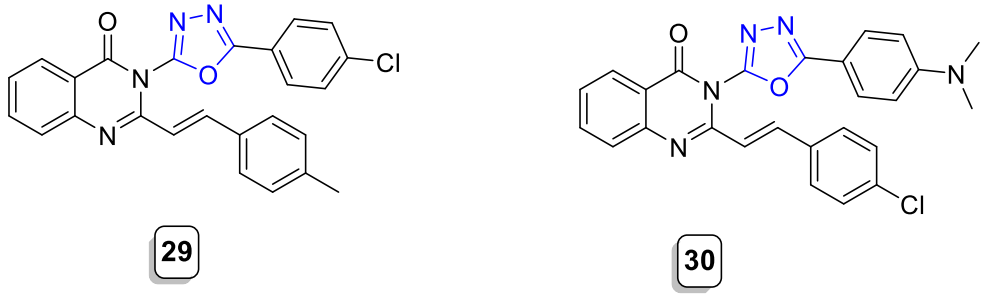
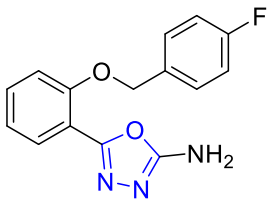


Figure I. 29

- Zarghi *et al*. [69] synthesized a novel series of 2-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazoles derivatives and evaluated their anticonvulsant activity. The authors found that Compound **(31)** shows significant anticonvulsant activity due to fluorine's introduction at the para position of the benzyl group and amino group at position 2 of the 1,3,4-oxadiazole ring can improve the anticonvulsant activity (Figure I. 30).

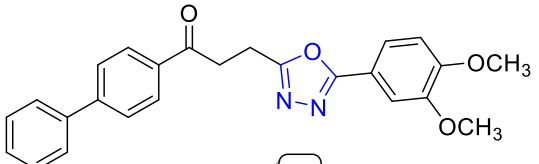


31

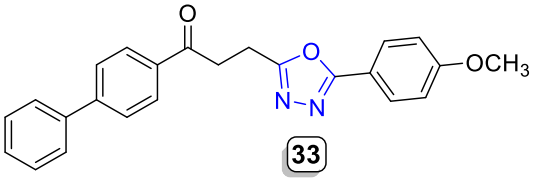
Figure I. 30

I.4.1.4 Anti-inflammatory activity

- Asif Husain *et al*. [70] designed a series of 3-[5-(substituted aryl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-ones derived from the anti-inflammatory drug Fenbufen (4-oxo-4-(biphenyl-4-yl)butanoic acid) and screened their anti-inflammatory potential. Compounds **(32)** and **(33)** were more potent than the Fenbufen and equal to sodium diclofenac (Figure I. 31).



32



33

Figure I. 31

- Kadi *et al*. [71] described the synthesis of a novel -(1-adamantyl)-5-substituted-1,3,4-oxadiazole compounds and evaluated their *In-vivo* anti-inflammatory using the carrageenin-induced paw oedema method in rats. Compounds **(33**, **34**, **35**, and **36)** were more potent than the indomethacin standard (Figure I. 32).

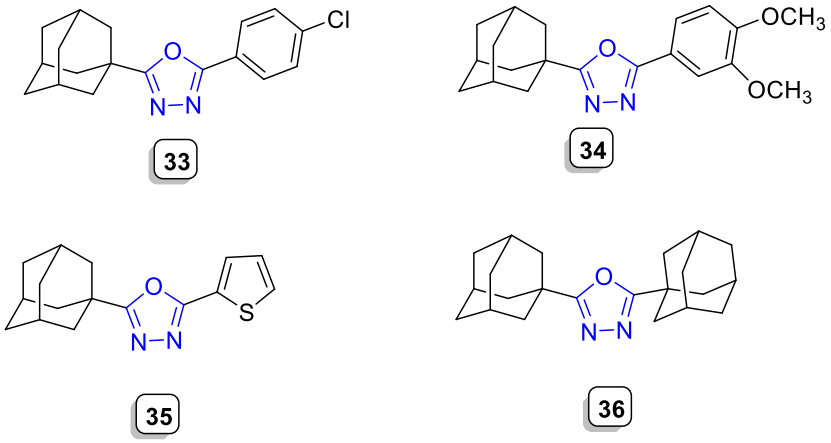


Figure I. 32

I.4.1.5 Analgesic activity

- Compound **(37)** was synthesized by Amir *et al* and evaluated for anti-analgesic activity.The biological studies showed that oxadiazole derivative **(37)** was the lead molecule, with a maximal analgesic activity [72] (81,86%) (Figure I. 33).

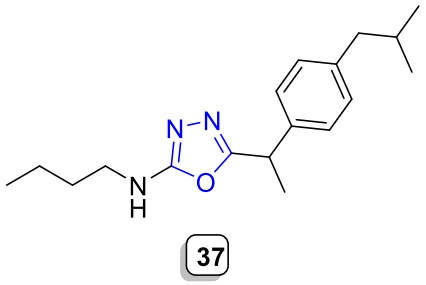


Figure I. 33

- Gilani *et al*. [73] reported the synthesis and analgesic activity of some 1,3,4-oxadiazole derivatives of isoniazid. Compound **(38)** showed a maximal activity of (70.37 ± 1.67%), almost equivalent to the standard Ibuprofen (73.52 ± 1.00%) (Figure I. 34).

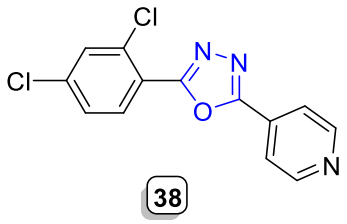
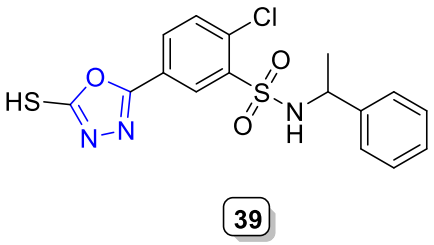


Figure I. 34

I.4.1.6 Anti-viral activity

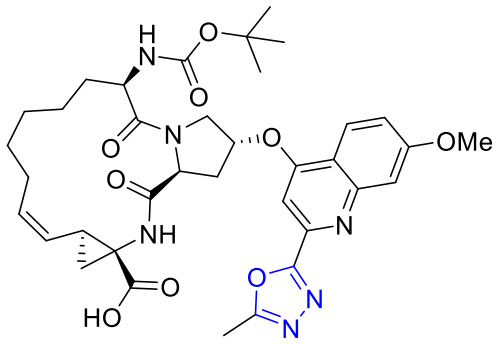
- Iqbal *et al*. [74] designed a novel benzenesulfonamides series bearing 1,3,4-oxadiazole moiety and evaluated their anti-viral potential against the human immunodeficiency virus type 1 (HIV-1), which determined by using the XTT assay on MT-4 cells. Compounds **(39)** showed the highest activity (Figure I. 35).



39

Figure I. 35

- Brian *et al*. [75] reported the synthesis of new derivatives containing 1,3,4-oxadiazole and 8-hydroxy-1,6-naphthyridine ring system and screen their anti-viral activity against the hepatitis C virus NS3 protease. Compound **(40)** showed the maximum inhibitory against the hepatitis C virus (Figure I. 36).



40

Figure I. 36

I.4.1.7 Anti-hypertensive activity

- Bankar *et al*. [76] reported the vasorelaxant activity of compound **(41)**. The study investigates a 1,3,4-oxadiazole derivative effect on vascular smooth muscles in rat aorta by blocking L-type calcium channels (Figure I. 37).

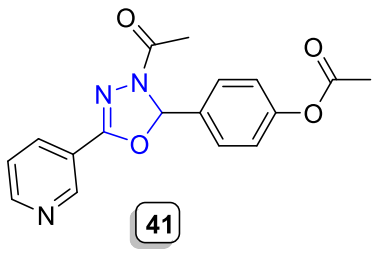


Figure I. 37

I.4.1.8 Enzyme Inhibitors

- Leung and co-workers [77] designed a new series of 1,3,4 oxadiazoles derivatives from oleic acid and screened their capability to inhibit fatty acid amide hydrolase. Compound **(42)** exhibited the most potent against the enzyme (Figure I. 38).

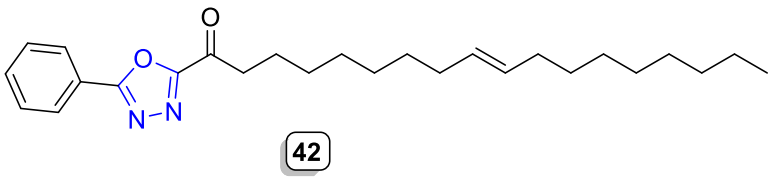
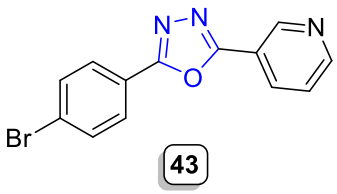


Figure I. 38

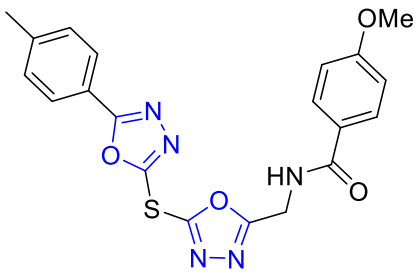
- Khan *et al*. [78] performed studies on tyrosinase inhibition effects of compounds 2,5-disubstituted-1,3,4-oxadiazole derivative. The compound **(43)** was more potent than the standard drug L-mimosine (Figure I. 39).



43

Figure I. 39

- Tomi *et al*. [79] reported the synthesis of novel bis-1,3,4-oxadiazole and performed a study on the transferase activity of enzymes, mainly glutamic oxaloacetic transaminase (GOT), alanine aminotransferase (GPT), and Gamma-glutamyl transferase (γ-GT) in serum. Compound **(44)** showed inhibitory effects on the activity of γ-GT and activation for GOT and GPT (Figure I. 40).



44

Figure I. 40

I.4.2 Reported synthetic strategies for 1,3,4 Oxadiazole

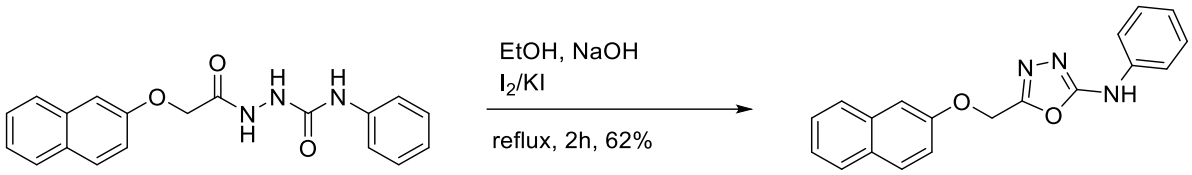
Considering the influence of 1,3,4 Oxadiazole on both heterocyclic and medicinal chemistry, the researchers are rapidly developing new techniques and methodologies to prepare these moieties. A short review of the methods adopted so far for the synthesis of 1,3,4-oxadiazoles is given below.

I.4.2.1 From the compound N,N'-Diacylhydrazines

Common synthetic approaches to oxadiazoles involve the cyclization of diacylhydrazine. A variety of reaction conditions and anhydrous reagents, as mentioned below, have been used to cause the cyclization of N,N'-diacylhydrazines to their respective 1,3,4-oxadiazoles. The following scheme represents the general reaction (Scheme I. 22).

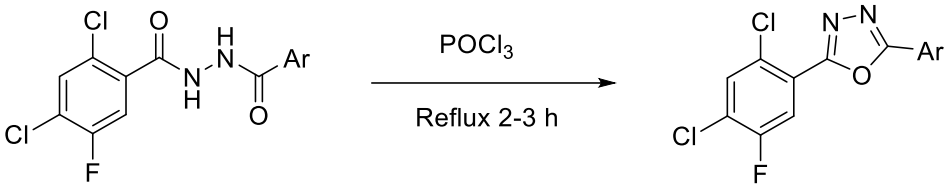
Scheme I. 22

Effective synthetic strategy for the preparation of 5-((naphthalen-2-yloxy)methyl)-N-phenyl-1,3,4-oxadiazol-2-amine was reported by El-Sayed *et al.* [80] the target compounds prepared in good yields by heating diacylhydrazine in ethanol in the presence of sodium hydroxide and iodine in potassium iodide (Scheme I. 23).



Scheme I. 23

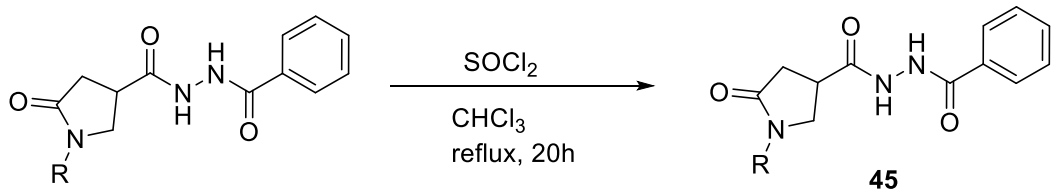
Zheng and co-workers [81] reported the synthesis of 5-(2,4-dichloro-5-flurophenyl)-2-(aryl)-1,3,4-oxadiazole by heating diacylhydrazines with phosphorus oxychloride in excellent yields (93-96%) (Scheme I. 24).



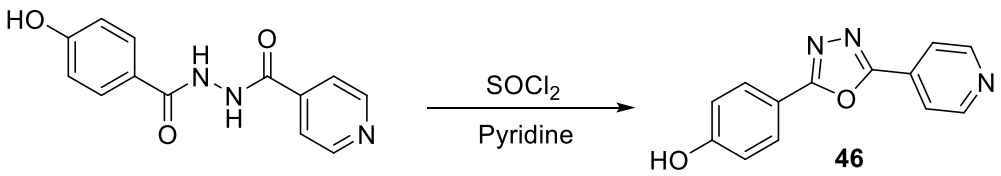
Ar= 2,3,4,5-tetrafluoro (93 %), 2,4,5-trifluoro (94 %), 2,6-difluoro (96 %), 2-chloro (96 %), 2-chloro-4,5-difluoro (93 %)

Scheme I. 24

Thionyl chloride is another anhydrous reagent generally used for the cyclization of diacylhydrazines, (Scheme I. 25), outlines some examples. **(45)** [82] and **(46)** [83].



R = Ph, 4-EtOC6H4, 4-PhOC6H4, 4-ClC6H4 27-33%



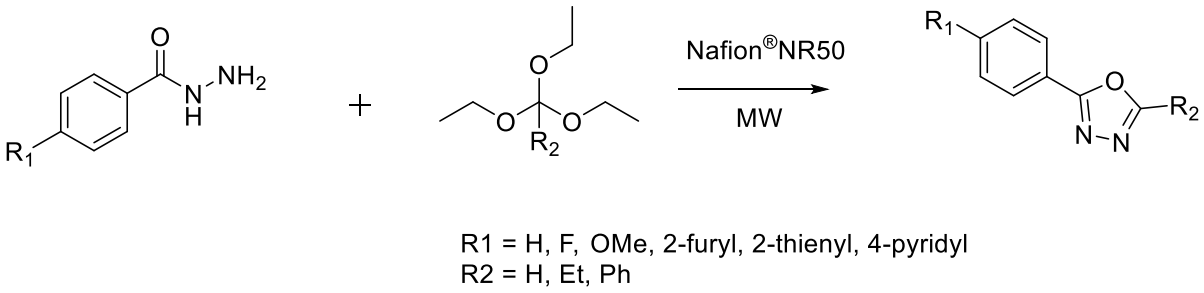
Scheme I. 25

I.4.2.2 From acylhydrazide

Four different pathways are known to synthesize 1,3,4 oxadiazole from acylhydrazide:

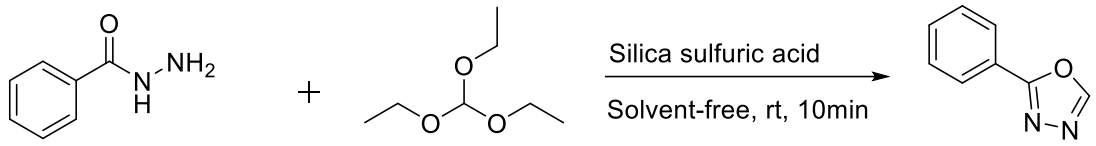
I.4.2.2.1 The reaction of acylhydrazide with acetals

Vivek Polshettiwar *et al*. [84] described the solvent-free condition for the synthesis of 1,3,4 oxadiazoles by condensation of triethyl orthoalkanates and acylhydrazide under microwave irradiations in the presence of solid-supported Nafion NR50 as a catalyst. The main advantages of this procedure are easy manipulation and good yielding (Scheme I. 26).



Scheme I. 26

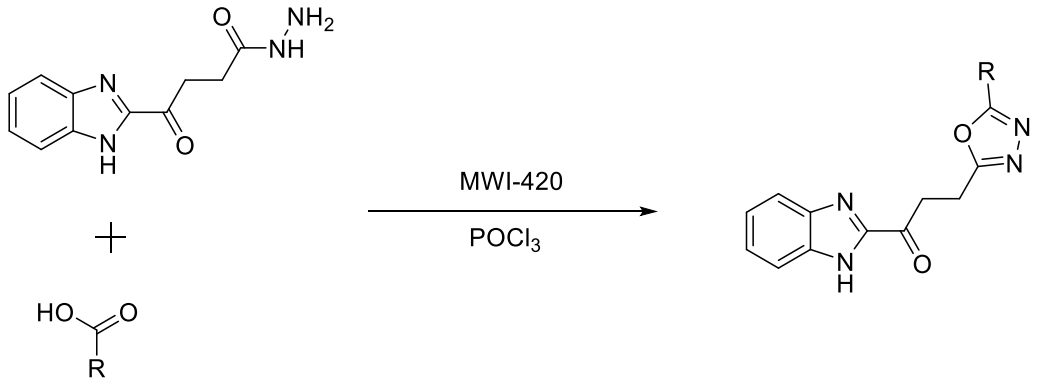
Rapid and green synthesis of 2,5-disubstituted 1,3,4-oxadiazoles has been described by Dabiri *et al.*[85] The target compounds were prepared by the reaction of different acyl hydrazides and orthoesters under a solvent-free condition in the presence of silica sulfuric acid as catalyst (Scheme I. 27).



Scheme I. 27

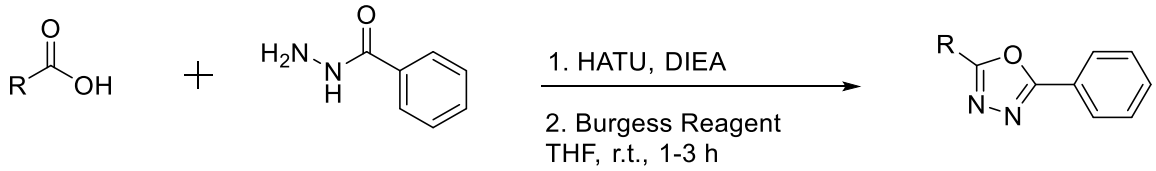
I.4.2.2.2 The reaction of acylhydrazide with carboxylic acid

Rashid *et al.* [86] reported the synthesis of novel imidazole bearing the 1,3,4 oxadiazoles motif by condensation of 4-(1H-Benzo[d]imidazol-2-yl)-4-oxobutane hydrazide and deferent carboxylic acid in the presence of Phosphoryl chloride ($POCl_3$) under microwave irradiation (Scheme I. 28).



Scheme I. 28

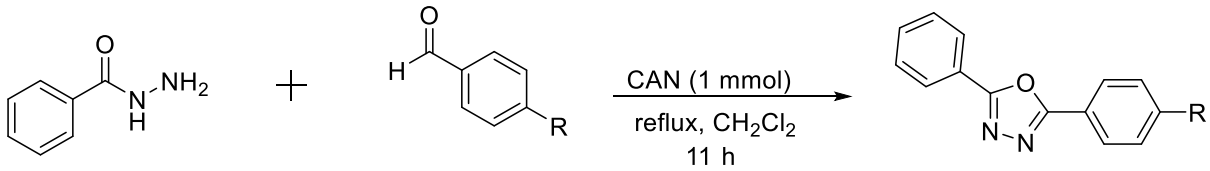
Li *et al.*[87] described a convenient one-pot procedure for the preparation of 1,3,4-oxadiazoles derivative by the reaction of carboxylic acids and acylhydrazides in the presence of HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) and Burgess reagent (Scheme I. 29).



Scheme I. 29

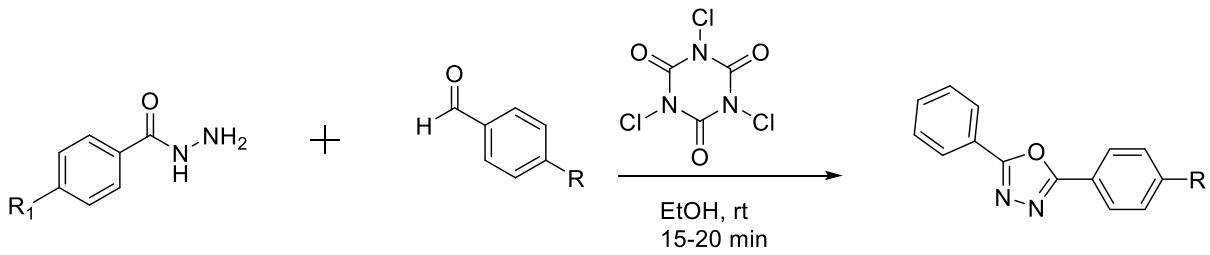
I.4.2.2.3 The reaction of acylhydrazide with aldehydes

Dabiri and co-workers [88] reported a novel protocol for the synthesis of disubstituted 1,3,4 oxadiazoles via a one-pot reaction of acylhydrazide and aromatic aldehydes in dichloromethane at reflux temperature catalysed by cerium ammonium nitrate (CAN) (Scheme I. 30).



Scheme I. 30

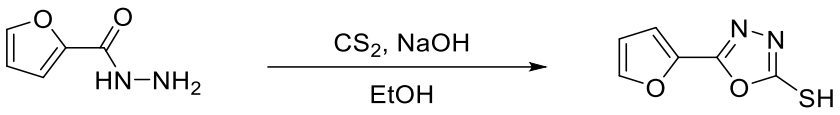
A practical method for one-pot synthesis of unsymmetric 2,5-disubstituted 1,3,4-oxadiazoles was developed by Pore and co-workers [89] through the reaction of acylhydrazides and aromatic aldehydes in the presence of trichloroisocyanuric acid (TCCA) at room temperatures. The main advantages of this method are short reaction time and high yields (Scheme I. 31).



Scheme I. 31

I.4.2.2.4 The reaction of acylhydrazide with carbon disulfide

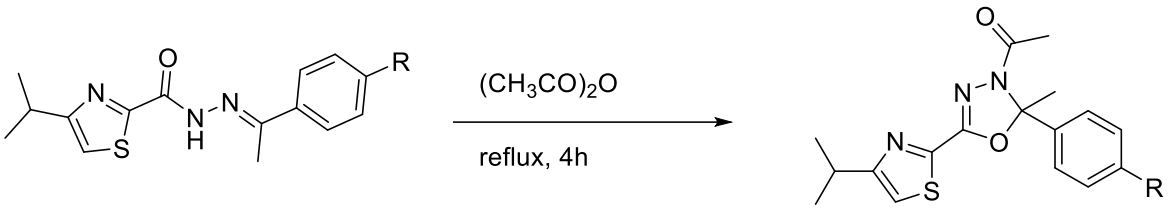
An efficient method for obtaining 2-mercapto-1,3,4-oxadiazole derivatives was described by Koparir *et al.* [90] based on the reaction between carbon disulfide and acylhydrazides by heating the alkali alcohol solution (Scheme I. 32).



Scheme I. 32

I.4.2.3 From *N*-acylhydrazone

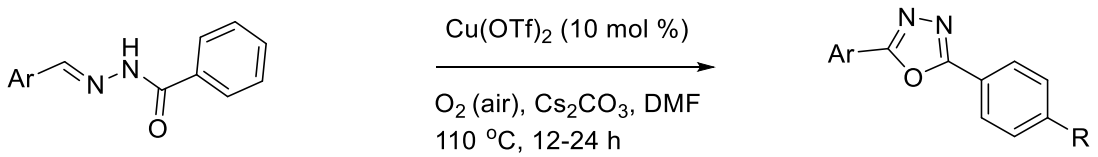
Kumar *et al.* [91] reported the synthetic strategy for preparing 1,3,4-oxadiazole derivatives by the reaction of *N*-acylhydrazones treated with acetic anhydride under reflux conditions to give the final products in good yields (Scheme I. 33).



R = NO_2, Cl, Br, OH, OCH_3, CH_3

Scheme I. 33

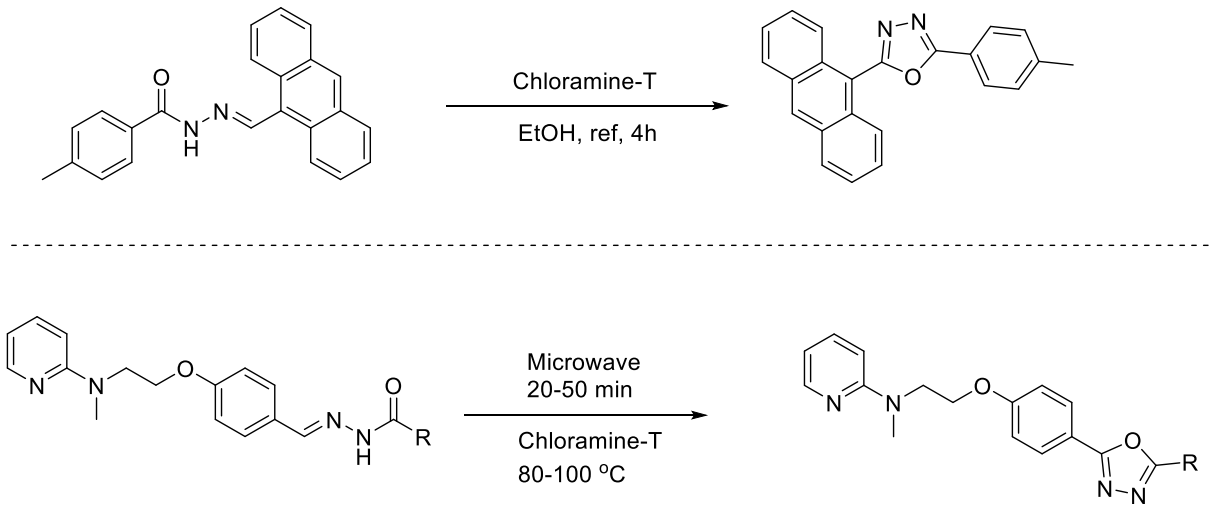
Guin *et al*. [92] reported direct access to 2,5-disubstituted-1,3,4-oxadiazoles through the cyclization of N-acylhydrazones within the presence of Cu $(OTf)_2$ as a catalyst. These reactions can be performed in air atmosphere and moisture, making it exceptionally efficient for organic synthesis (Scheme I. 34).



Ar = Ph, 4-MeC_6H_4, 4-t-BuC_6H_4, 4-$OMeC_6H_4$, 3,4-di$OMeC_6H_3$, 4-$BuOC_6H_4$, 4-FC_6H_4, 3-FC_6H_4, 4-ClC_6H_4, 4-BrC_6H_4, 4-$AcOC_6H_4$, 2-pyridyl, 2-furyl, 2-thienyl

Scheme I. 34

Li *et al.*[93] synthesized a series of 1,3,4-oxadiazole derivatives in good yields from oxidative cyclization of N-acylhydrazone using chloramine-T in reflux ethanol. Similarly, Gaonkar and co-workers [94] described the synthesis of a series of 1,3,4-oxadiazole derivatives from the oxidative cyclization of N-acylhydrazones in the presence of chloramine-T under microwave irradiation (Scheme I. 35).

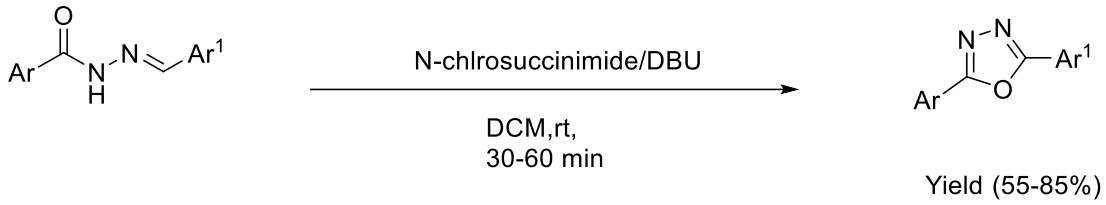


R = phenyl, 2-flurophenyl, 3-flurophenyl, 4-flurophenyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 2-furanyl, 2-thiophene, 3-pyridinyl, 4-pyridinyl, 2-hydroxyphenyl, 4-hydroxyphenyl, 4-bromophenyl, 6-hydroxynaphthalenyl, 2-methyl-1,3-thyazolyl, 4-methoxyphenyl, 2,4-dichlorophenyl, 2,4-diflurophenyl, 4-nitrophenyl

Scheme I. 35

In 2010, a practical method for the synthesis of various 2,5-disubstituted 1,3,4-oxadiazoles had been described by Pardeshi and co-workers[95]. The title compounds were prepared by oxidative cyclization of acylhydrazone with a mixture of N-chlorosuccinimide and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as oxidative agents. This protocol's main advantages are short

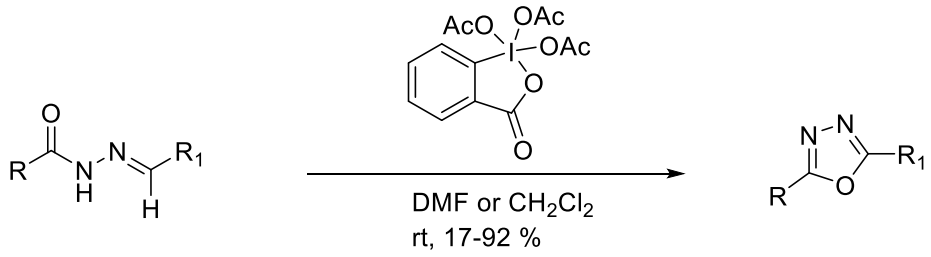
reaction time, mild reaction conditions, excellent yields, and a simple workup procedure (Scheme I. 36).



Ar = Ph, 4-NO_2C_6H4, 4-$OCH_3C_6H_4$, 4-$CH_3C_6H_4$, 3,5-$(CF_3)C_6H_3$
Ar^1 = Ph, 4-$OCH_3C_6H_4$, 4-ClC_6H_4, 4-$CH_3C_6H_4$, 4-pyridyl

Scheme I. 36

Dobrotã *et al*.[96] described the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles, skillfully produced by oxidative cyclization of N-acylhydrazone derivatives in the presence of excess Dess-Martin periodinane under mild conditions (Scheme I. 37).

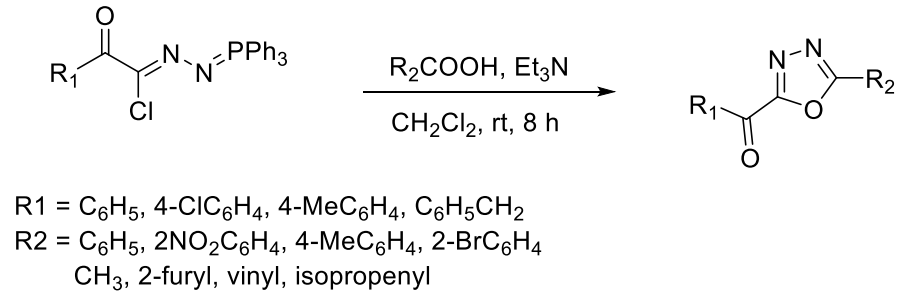


R = Ph, 4-ClC_6H_4, 4-$NO_2C_6H_4$, 2-furyl, 4-pyridyl, 3-chloro-benzo[b]thien-2-yl
R1 = Ph, 4-$MeOC_6H_6$, 4-BrC_6H_4, 2-furyl, 2-thienyl, 4-pyridyl, 3-thienyl, 3-$NO_2C_6H_4$
Pr, i-pr, 2-$NO_2C_6H_4$, 3-MeO-4-$BnOC_6H_3$,

Scheme I. 37

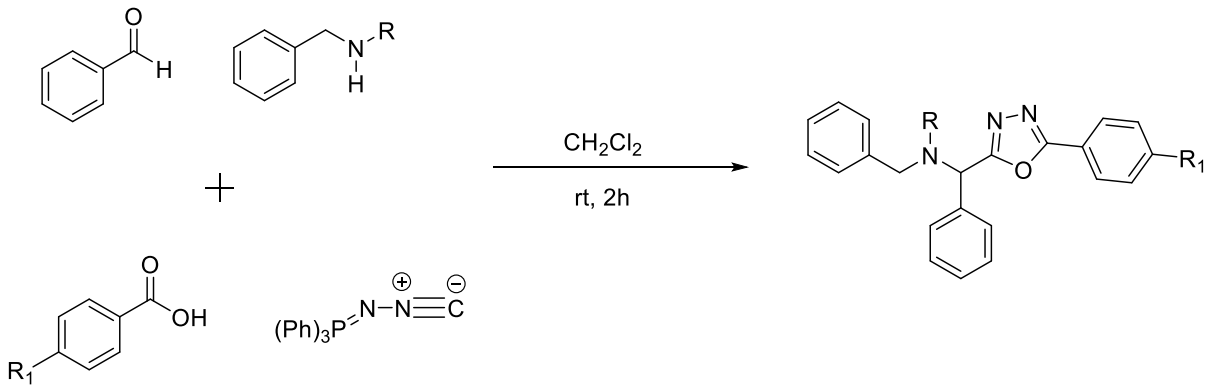
I.4.2.4 Other methods

An efficient protocol for the synthesis of α-keto-1,3,4-oxadiazole derivatives has been reported by Cui *et al* [97]. The target compounds were prepared through the intermolecular aza-Wittig reaction of carboxylic acids and imidoyl chloride in CH_2Cl_2 at room temperature (Scheme I. 38).



Scheme I. 38

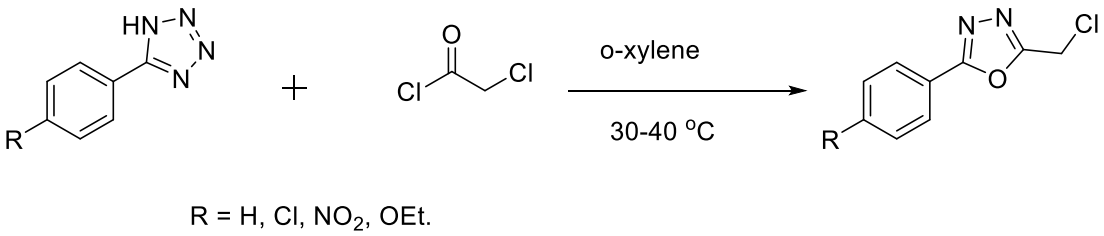
Ramazani *et al*.[98] reported a novel and efficient protocol for preparing 2,5-disubstituted-1,3,4-oxadiazoles in good yields through a one-pot condensation procedure of (N-isocyanimino)triphenylphosphorane, a secondary amine, a carboxylic acid, and an aromatic aldehyde in CH_2Cl_2 at room temperature (Scheme I. 39).



Scheme I. 39

The Huisgen reaction is commonly used to synthesize various 2,5-disubstituted-1,3,4-oxadiazoles through the reaction of 5-aryl/acyltetrazoles with acid chlorides or acid anhydrides. Some interesting examples are mentioned below:

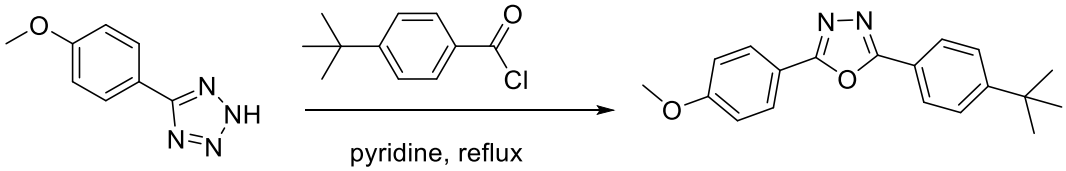
Baranov *et al*.[99] described the synthesis of disubstituted oxadiazole through the reaction of Tetrazole with chloroacetyl chloride in *o*-xylene in 80-93% yields (Scheme I. 40).



R = H, Cl, NO_2, OEt.

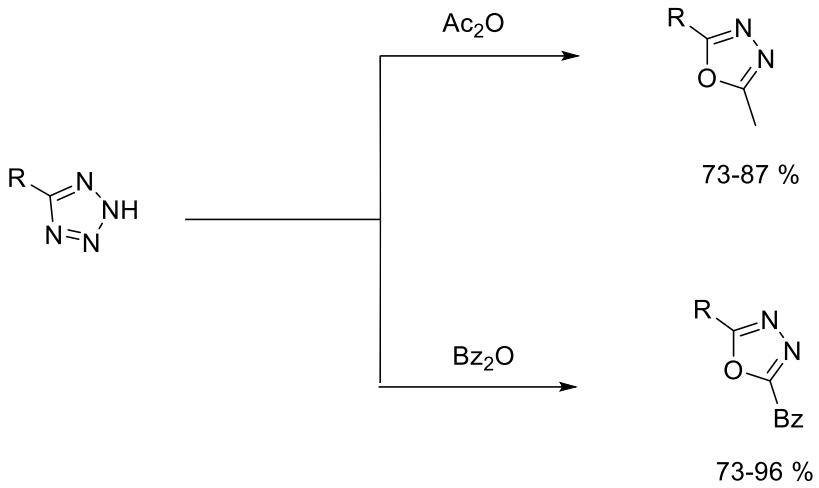
Scheme I. 40

Similarly, Jiang and co-workers [100] described the synthesis of 2-(4-tert-butylphenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole by the reaction of 4-methoxyphenyltetrazole with 4-tert-butylbenzoyl chloride in refluxed pyridine to give the final product in an excellent yield (96%) (Scheme I. 41).



Scheme I. 41

Efimova and co-workers [101] Reported a novel protocol for synthesized 1,3,4-oxadiazole derivative through The Huisgein reaction of 5-(aryl or hetero) tetrazoles with acetic and benzoic anhydrides instead of acid chlorides under microwave irradiation conditions (Scheme I. 42).



R = 4-MeOC$_6$H$_4$, Ph, 4-BrC$_6$H$_4$, 4-O$_2$NC$_4$H$_4$, pyridin-2-yl, pyridin-3-yl

Scheme I. 42

I.5 *N*-acylhydrazone

Over the years, molecules containing N-acylhydrazone (NAH) scaffold have been proven to be a versatile and promising motif in medicinal chemistry and drug design due to their privileged structures that can provide a ligand point for different types of bioreceptor[102,103].

The NAH core is characterized by the combination of amide and imine unit, which provide ligand-receptor interactions comprising both hydrogen-bond acceptor and donor sites, making them perform intermolecular interactions with a broad range of amino-acid residues (Figure I. 41).

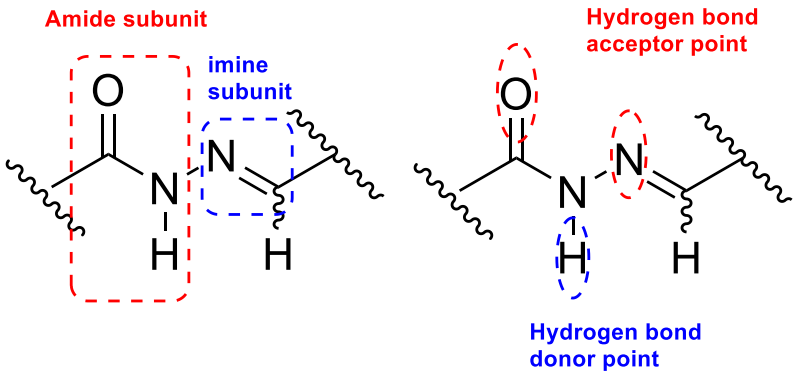


Figure I. 41

I.5.1 The biological interest of N-acylhydrazone derivatives

In the past decade, the research in NAH-based drugs has progressed dramatically, therefore. various compounds containing NAHs scaffold have been approved for clinical use, such as:

- **Nitrofurazone** and **Nitrofurantoin** [104], the primary use of these two drugs is to treat a bacterial infection.
- **Carbazochrome** [105], an antihemorrhagic agent, it was specifically indicated to promote hemostasis (stops bleeding).
- **Testosterone 17-enanthate 3-benzilic acid hydrazone** [106] is an injectable medication combined with estradiol dienanthate, and estradiol benzoate has been used in menopausal hormone therapy under the brand names Climacteron, Lactimex, Lactostat, and Amenose.
- **Nifuroxazide** [107] is a NAH-based antibiotic drug approved for the treatment of diarrhea and colitis in humans and non-humans (Figure I. 42).

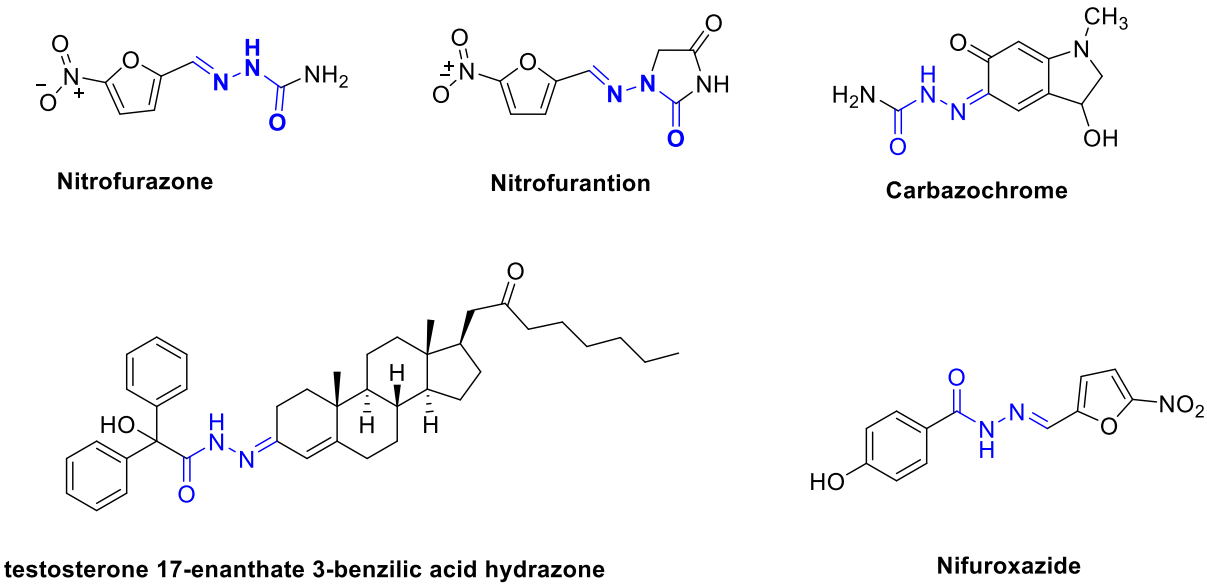


Figure I. 42

Recently. Compounds containing the N-acylhydrazone motif in their structure have been considered extremely important due to their biological and pharmacological properties, such as:

I.5.1.1 Anticancer activity

- Dandawate *et al*. [108] designed novel plumbagin hydrazone derivatives and evaluated them against breast cancer cell lines (MCF-7 and MDA-MB-231 and MDA-MB-468). Compound **(47)** showed the highest activity (Figure I. 43).

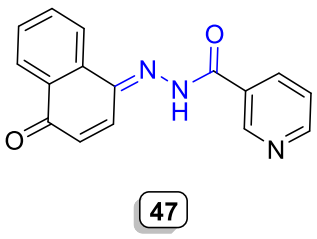
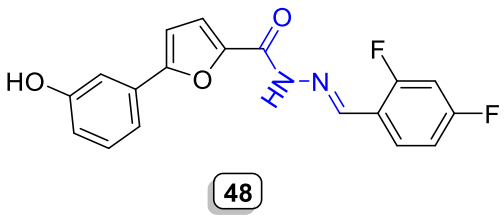


Figure I. 43

- Cui *et al*. [109] reported a novel series of N-acylhydrazone containing furan and screened their anticancer activity against the human promyelocytic leukemic cells. Compound **(48)** was identified as the most potent activity (Figure I. 44).

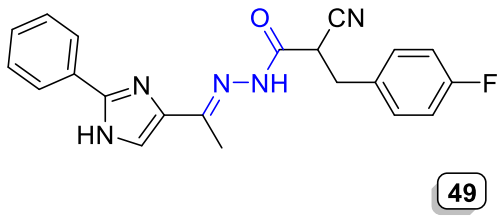


48

Figure I. 44

I.5.1.2 Antimicrobial activity

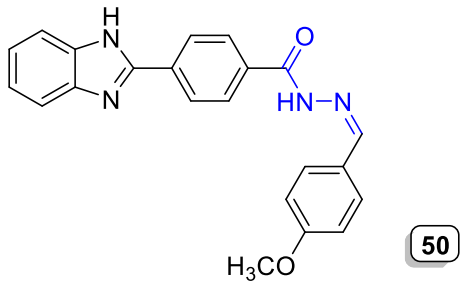
- Abdel-Wahab *et al*. [110] reported the synthesis of novel acylhydrazones bearing imidazoles and screened their antibacterial activity against multiple bacterial strains (*B. megaterium, B. subtilis, S. aureus, K. peneumoniae, P. aeruginosa, E. coli).* Compound **(49)** was identified as effective against all the above-mentioned bacterial strains (Figure I. 45).



49

Figure I. 45

- Özkay *et al*.[111] described new benzimidazole derivatives' synthesis containing the acylhydrazone motif and evaluated their antibacterial potential against different bacterial strains (*Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Candida albicans, Candida globrata, Candida tropicalis).* Compound **(50)** showed the highest activity (Figure I. 46).



50

Figure I. 46

I.5.1.3 Anti-inflammatory activity

- Mohamed Eissa *et al*.[112] developed bis-acylhydrazones derivatives and screened their anti-inflammatory activity against carrageenan-induced paw edema in rats. Compound **(51)** was identified as the most potent anti-inflammatory activity in the series (Figure I. 47).

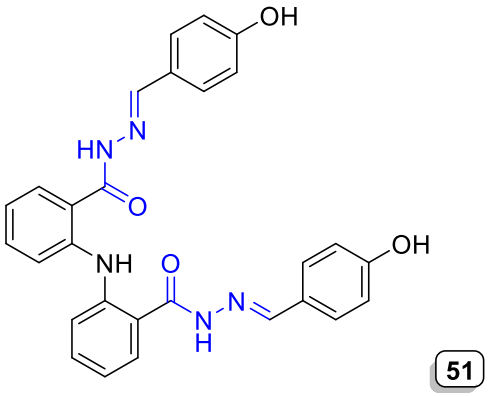


Figure I. 47

- Compound **(52)** was synthesized and evaluated for anti-inflammatory activity by Salgin-Gökşen *et al* [113]. The results of biological studies showed that compound **(52)** was the lead molecule, with a maximal anti-inflammatory activity (Figure I. 48).

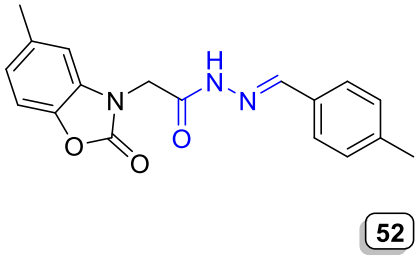


Figure I. 48

I.5.1.4 Analgesic activity

- Benzylidene hydrazides derivative with promising analgesic activity have been synthesized by Bhandari *et al*.[114] compound **(53)** showed potent analgesia in acetic acid-induced writhing tests with the highest percentage inhibition 68.66% (Figure I. 49).

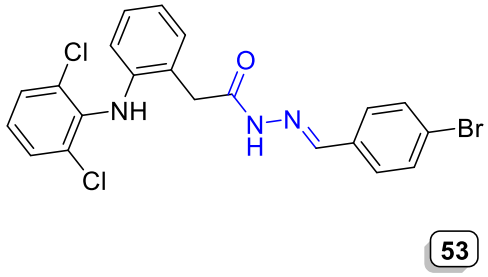


Figure I. 49

- Gökçe *et al*. [115] reported the synthesis of novel N-acylhydrazones derivatives bearing pyridazinone motif and screened their analgesic activity against p-benzoquinone-induced writhings in mice. Compound **(54)** showed the highest inhibition in the series (Figure I. 50).

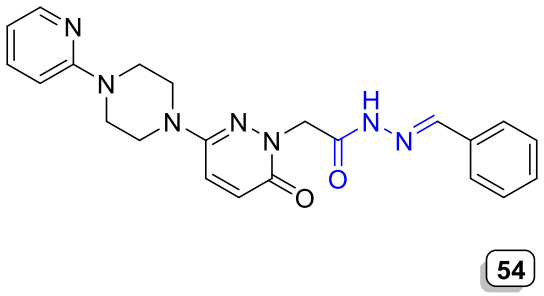


Figure I. 50

I.5.1.5 Anti-hypertensive activity

- Leal *et al*.[116] reported the synthesis of novel acylhydrazone derivatives containing benzodioxole and thienyl ring and evaluated their anti-hypertensive by activating the A_{2A} adenosine receptor. Compound **(55)** was showed the highest activity (Figure I. 51).

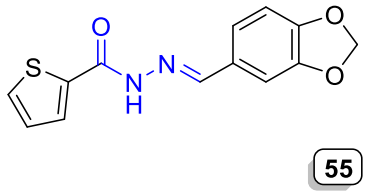


Figure I. 51

I.5.1.6 Anti-viral activity

- Marra *et al.*[117] synthesized a novel quinolone bearing N-acylhydrazone scaffold and screened their anti-viral property towards ZIKV and Chikungunya viruses. Compound **(56**, **57**, and **58)** were identified as more potent than standard Ribavirin (Figure I. 52).

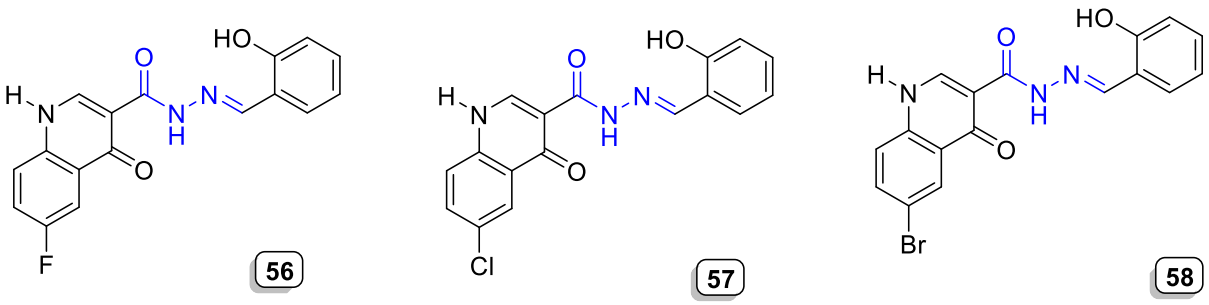


Figure I. 52

- Carcelli *et al.*[118] synthesized a new series of N-acylhydrazone derivative and evaluated their anti-viral activity against the HIV-1 virus. Compound **(59)** is considered a Dual target inhibitor of HIV-1 Integrase and Reverse Transcriptase Ribonuclease H, and it also inhibits viral replication in cell-based anti-viral assays (Figure I. 53).

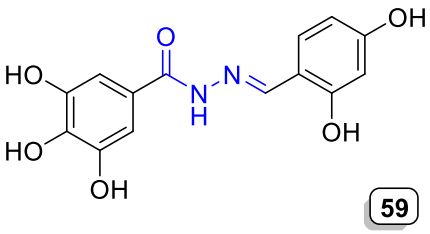
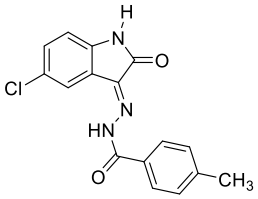
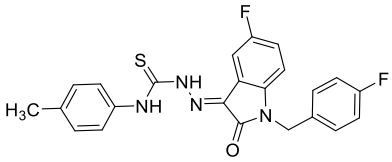
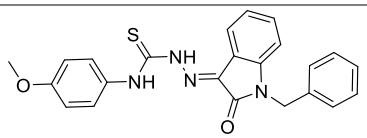
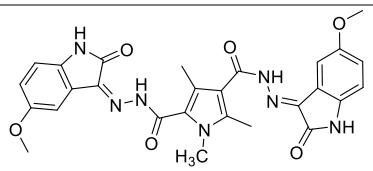
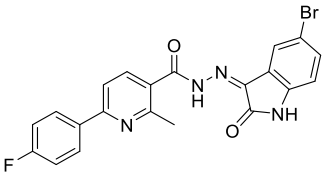
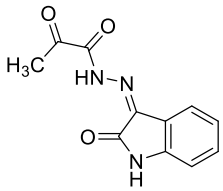


Figure I. 53

I.5.1.7 pharmacological properties of NAHs derived from isatin

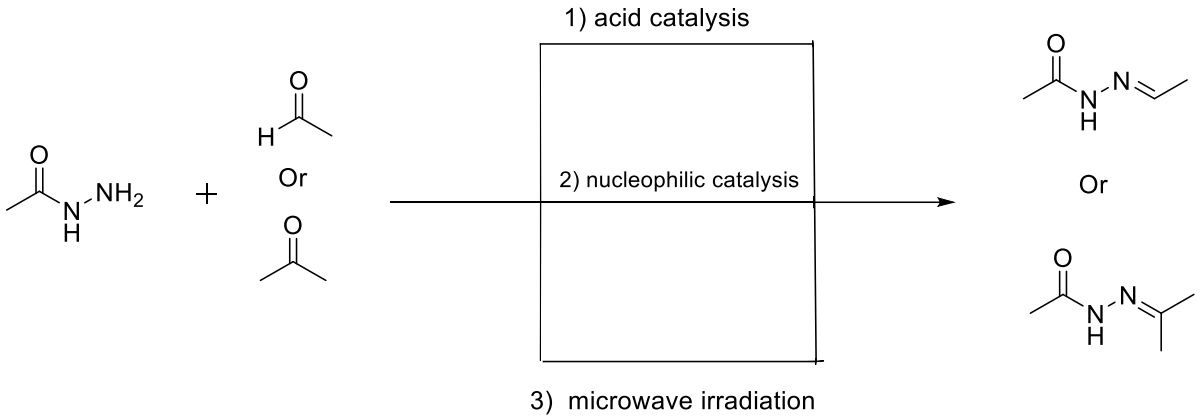
in the past few years, NAHs based on isatin derivatives have been considered significantly important due to their various pharmacological properties. Some of the recently published works are mentioned in the table below.

Table (I. 01)

Molecule	Biological properties
	anti-diabetic[119]
	Anti-tumor[120]
	Anticonvulsant[121]
	Anticancer[122]
	Antitubercular[123]
	Antimicrobial[124]

I.5.2 Reported synthetic strategies for N-acylhydrazone (NAH)

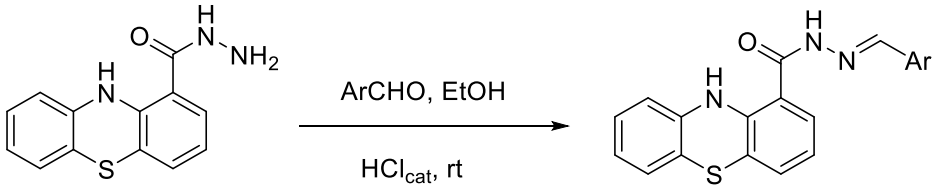
Notably, many methods have been reported for the preparation of N-acylhydrazones. In general, N-acylhydrazones are readily obtained by easy condensation of hydrazides with aldehydes or ketones via acid or nucleophilic catalysis or microwave irradiation (Scheme I. 43).



Scheme I. 43

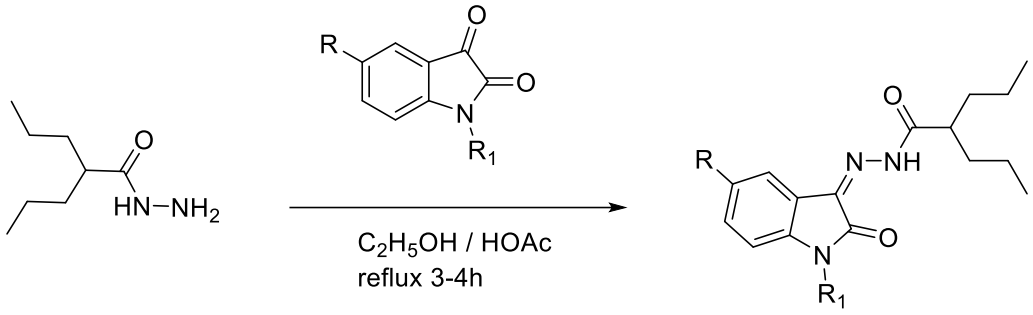
I.5.2.1 Acid catalysis

Silva *et al*.[125] Reported the synthesis of novel H-phenothiazine-1-acylhydrazone derivatives through the reaction of hydrazide with corresponding aromatic aldehyde derivative in the presence of hydrochloric acid as a catalyst (Scheme I. 44).



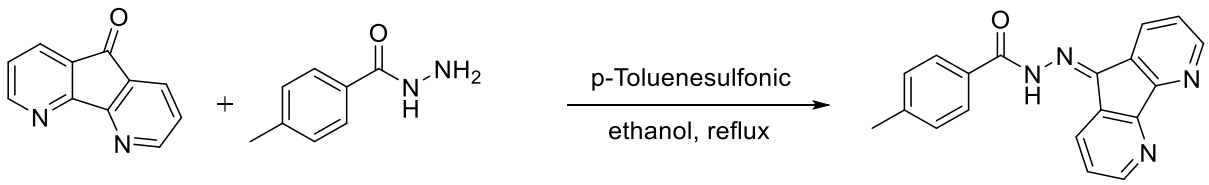
Scheme I. 44

El-Faham *et al*.[126] reported the synthesis of New N′-(2-Oxoindolin-3-ylidene)-2-propylpentane hydrazide-hydrazones derivatives in yields (83-89%) by heating valproic hydrazide with isatin in ethanol in the presence of acetic acid as a catalyst (Scheme I. 45).



Scheme I. 45

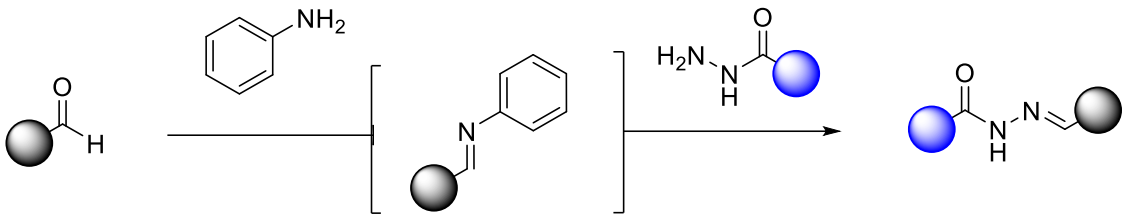
Hou *et al*.[127] have been reported p-Toluenesulfonic acid as an effective catalyst in the condensation of 4-methylbenzoylhydrazine with 4,5-Diazafluoren-9-one in reflux ethanol (Scheme I. 46).



Scheme I. 46

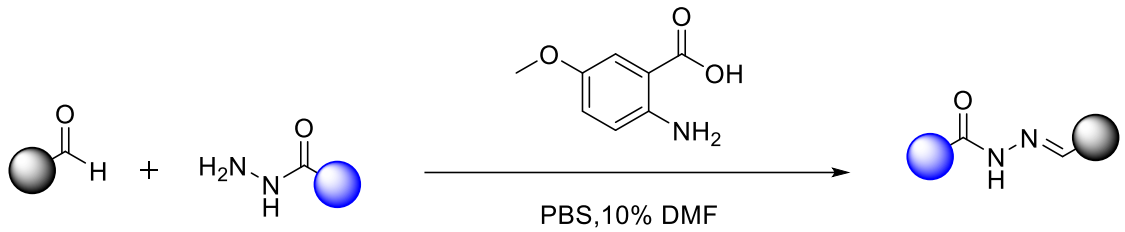
I.5.2.2 Nucleophilic catalyst

In the 1960s, Jencks *et al*.[128] have been reported aniline as a nucleophilic catalyst for synthesizing acylhydrazone via transamination of an imine intermediate in a buffer solution such as phosphate-buffered saline solution commonly used in biological research under neutral pH (Scheme I. 47).



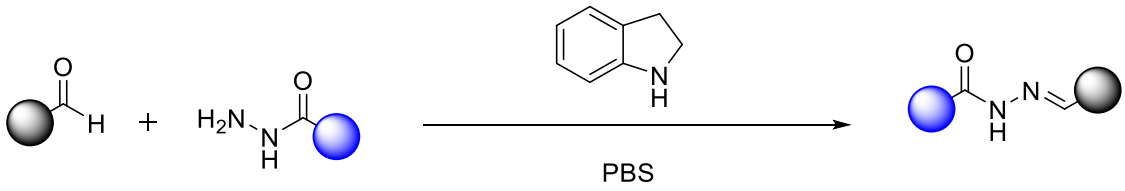
Scheme I. 47

Recently, Pete Crisalli and Eric T. Kool. [129] reported the use of 5-methoxyanthranilic acid as an efficient catalyst in the reaction of aromatic aldehydes with hydrazides in a phosphate-buffered saline solution (pH 7.4) containing 10% DMF (Scheme I. 48).



Scheme I. 48

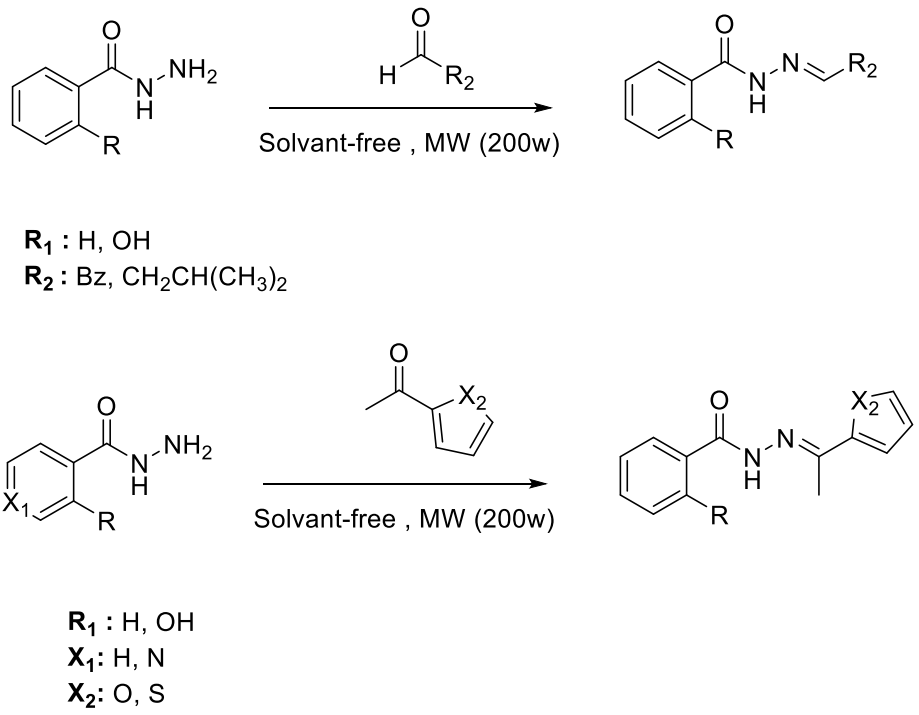
Similarly, Zhou *et al*.[130] have been reported the use of indoline as an efficient catalyst for the formation of acylhydrazones via the condensation of aromatic aldehydes with hydrazides in PBS solution (Scheme I. 49).



Scheme I. 49

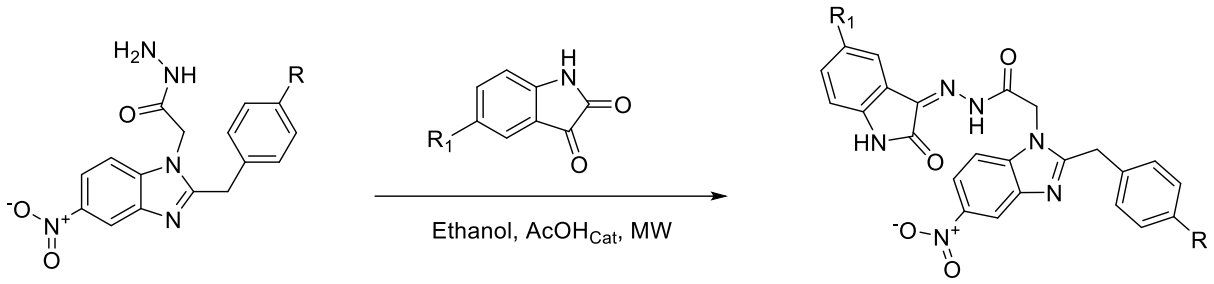
I.5.2.3 Microwave irradiation

Marta *et al*. [131] described an efficient solvent-free method for synthesizing various N-acylhydrazones via the condensation of hydrazides with aldehydes or ketones under microwave irradiation. This protocol's main advantage short reaction time (2.5-10 min) and high yields (70-98%) (Scheme I. 50).



Scheme I. 50

An efficient protocol for the synthesis of novel N-acylhydarzones bearing 5-nitrosubstituted benzimidazole and isatin derivatives has been reported by Yılmaz *et al* [132]. The target compounds were prepared via the condensation of hydrazide with isatin derivatives under microwave irradiation (Scheme I. 51).



Scheme I. 51

I.6 CONCLUSION

Hybrid molecules, obtained by combining two different biologically active moieties, gave promising information for medicinal chemistry, as they act at different targets [133]. Therefore, the synthesis of such molecules has gained much interest in the last decades [134].

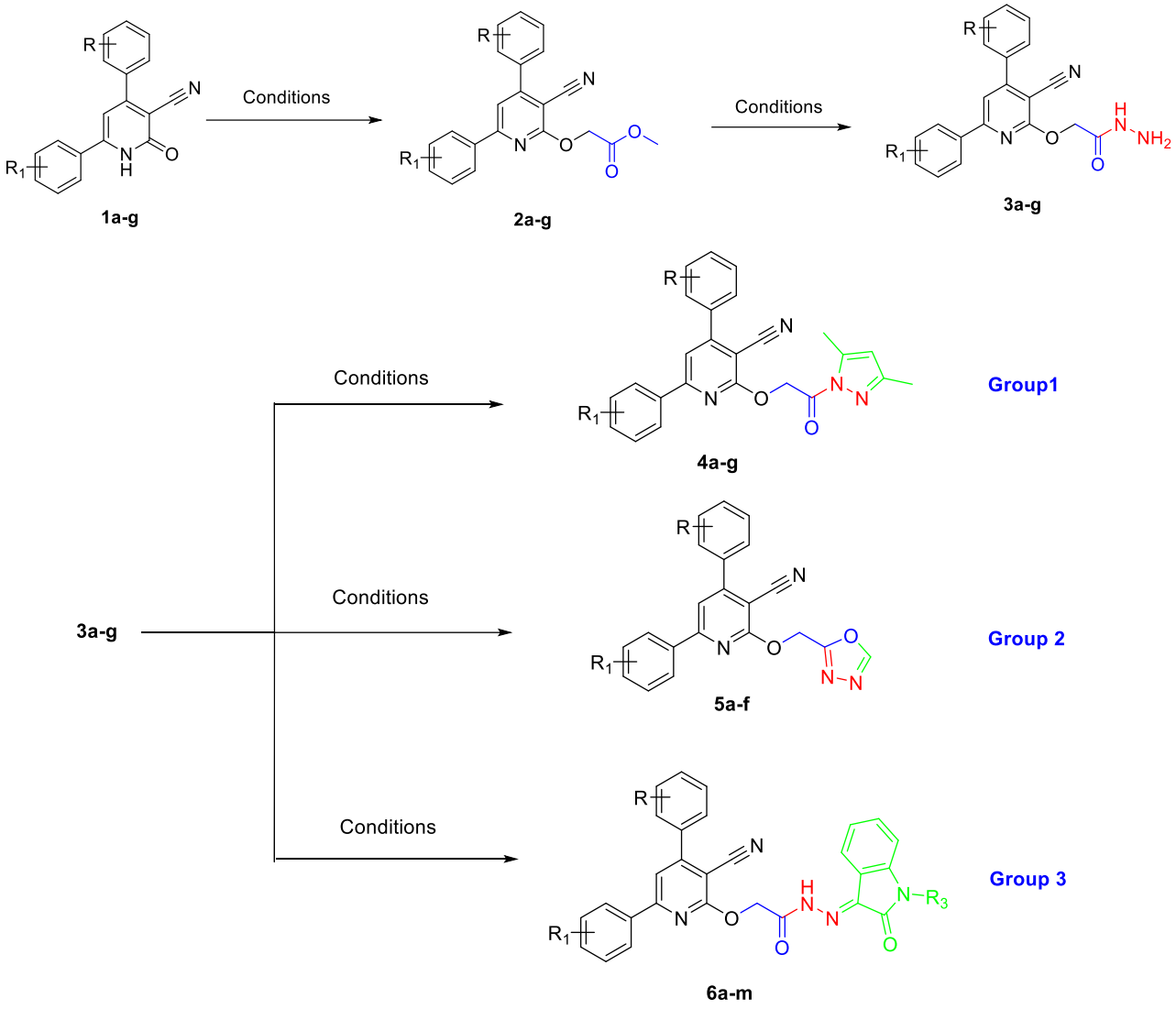
As mentioned in this chapter, heterocycles bearing cyanopyridine, oxadiazole, pyrazole, and N-acylhydrazone moieties are reported to possess a broad spectrum of biological and pharmaceutical activities. Guided by these findings, we planned to prepare novel hybrid molecules containing pyrazole, oxadiazole, or N-acylhydrazone moiety attached to the 3-cyanopyridine as the main scaffold.

Chapter 02

Results and Discussion

II Results and Discussion

To prepare the novel desired hybrid molecules based on cyanopyridines derivatives as the main skeleton, we performed a series of reactions based on the product's reactivity at each step, the synthetic pathways adopted to prepare the target molecules are depicted in (Scheme II. 01).



Scheme II. 01

II.1 Synthesis of 2-oxo-3-cyanopyridones (1a-g)

The starting material Cyanopyridones (**1a-g**) were synthesized via a one-pot four-component reaction, as reported in the literature [17]. The results obtained are shown in Table (II. 01)

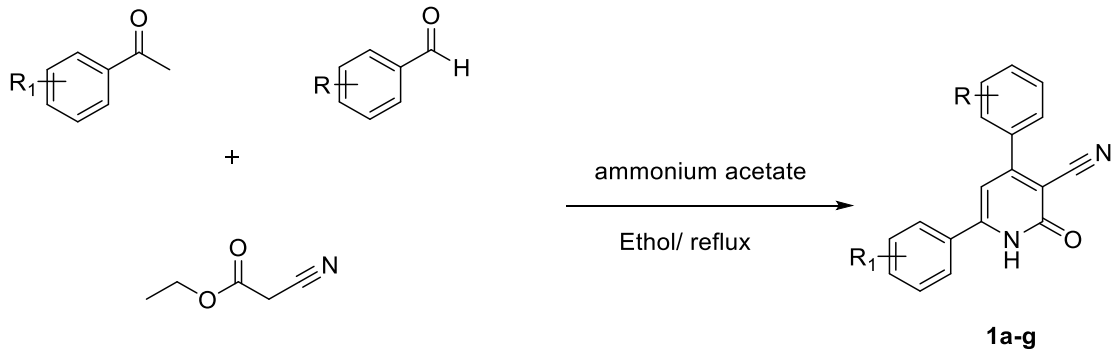
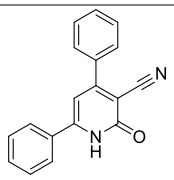
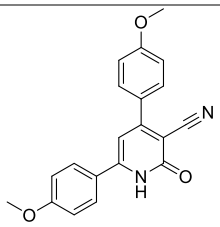
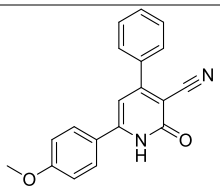
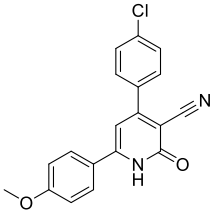
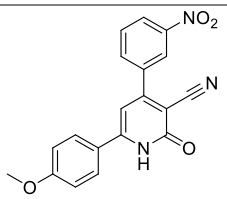
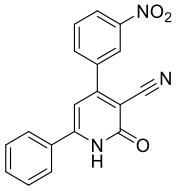
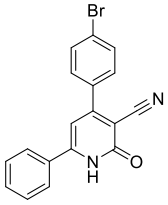


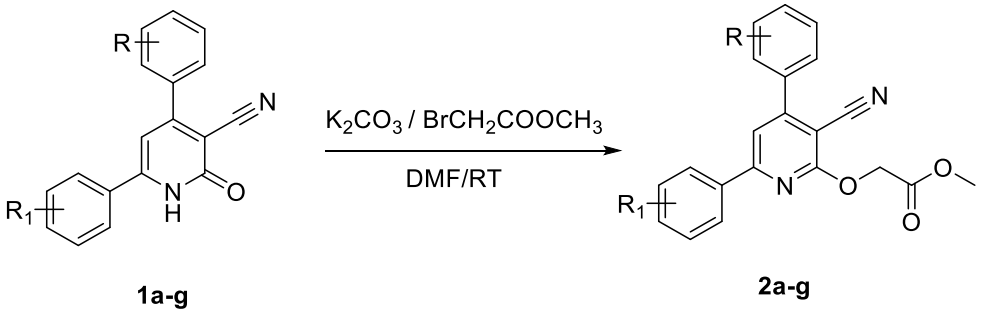
Table (II. 01): The prepared cyanopyridones **(1a-g)**

N°	Product	Yield %	Mp (C°)
1a		57	>300
1b		55	>300
1c		49	>300
1d		59	>300

1e		51	>300
1f		45	>300
1g		61	>300

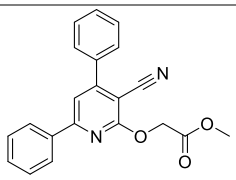
II.2 Synthesis of methyl 2-((3-cyano-4,6-diarylpyridin-2-yl)oxy)acetate (2a-g)

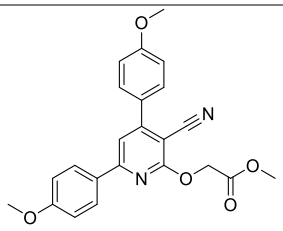
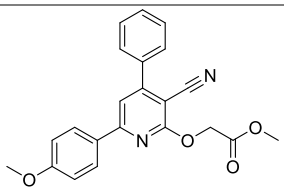
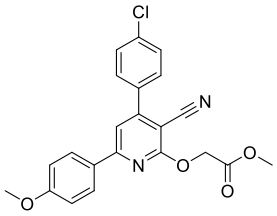
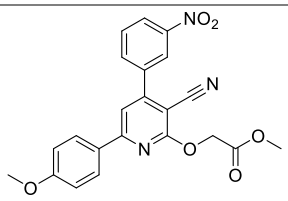
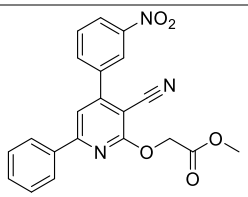
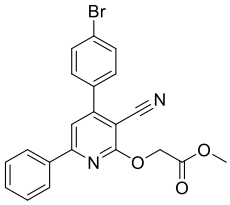
Among the reported procedures, These series were prepared according to the method described by Hassan A. El-Sayed *et al* [135]. The results are summarised in Table (II. 02)



1a-g **2a-g**

Table (II. 02): The Synthesized Methyl 2-((3-cyano-4,6-diarylpyridin-2-yl)oxy)acetate **(2a–g)**

N°	Product	Yield %	Mp (C°)
2a		89	157-159

2b		75	130-132
2c		92	148-150
2d		79	145-147
2e		77	190-192
2f		72	170-172
2g		83	177-179

II.2.1 Spectral study

All the prepared compounds showed in IR spectroscopy a high absorption between 1733.12-1787.33 cm^{-1}which indicates the presence of an ester carbonyl additionally to the disappearance of the amide carbonyl band at 1680 cm^{-1}, which signifies the creation of an *O*-alkylated derivative.

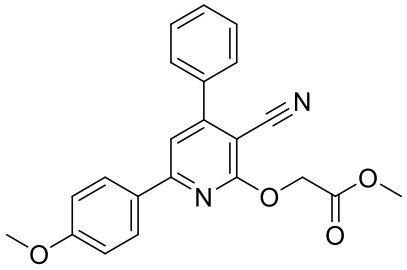
The spectroscopic results in nuclear magnetic resonance (NMR of proton and carbon-13) are consistent with the proposed structures. Indeed, the spectral analysis in ^{1}H NMR of compound **(2c)** (Figure II. 01), for example, shows two singlets at the downfield region 3.79 ppm, 5.08ppm corresponding to methyl protons ($COO\underline{C}H_3$) and CH_2O protons, respectively. Methoxy group OCH_3

showed a singlet peak at 3.87 ppm, the proton of the nicotinonitrile nucleus resonates at 7.45 ppm as a singlet, while the aromatic protons are observed in the range [7.98-6.97] ppm.

The carbon spectrum analysis of the compound **(2c)** shows in particular:

- The presence of an ester carbonyl function signal at 168.99 ppm.

- Two signals around δ 63.58, 52.24 ppm corresponding to (CH_2O), and ($COO\underline{C}H_3$), respectively.



2c

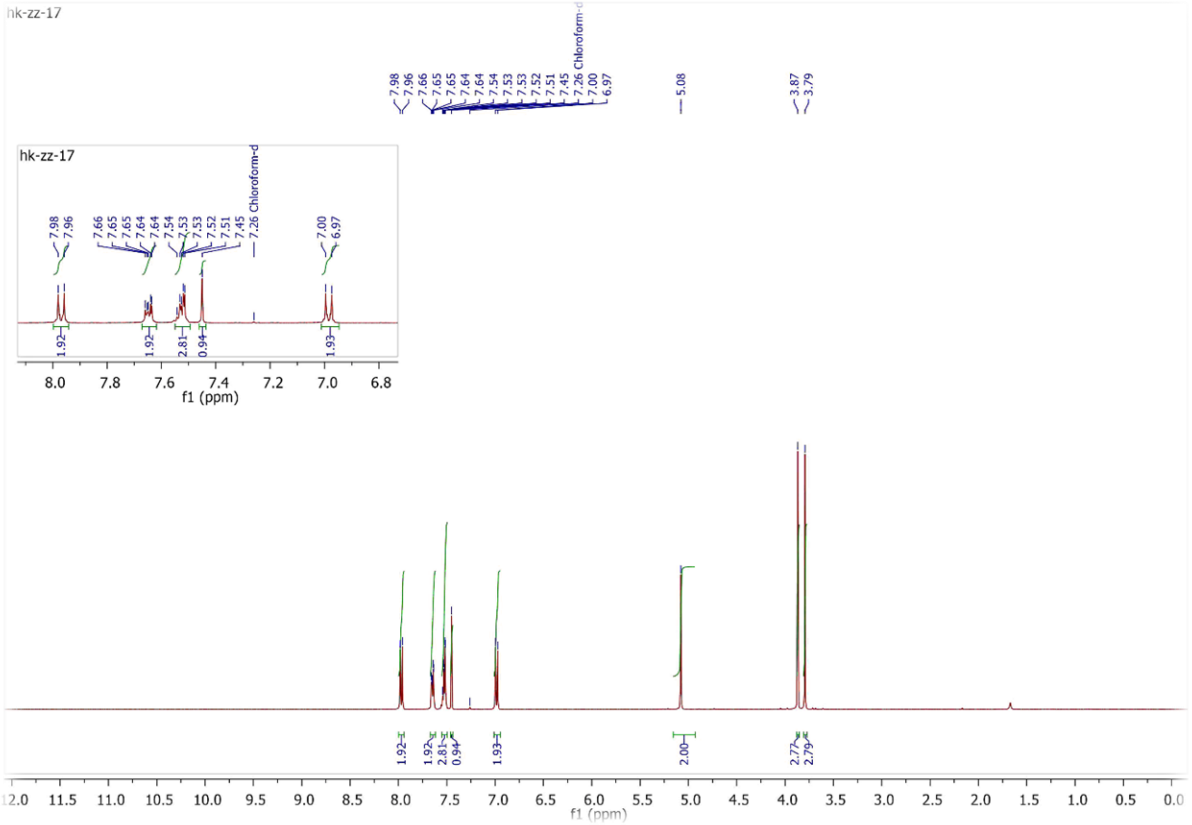
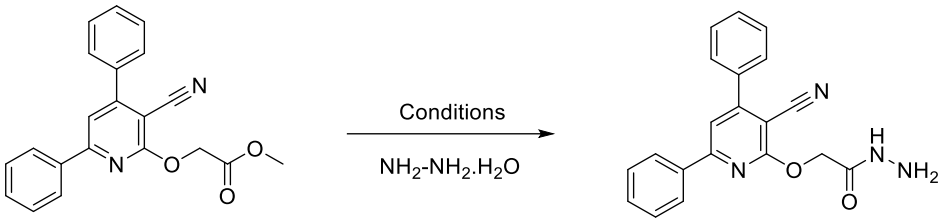


Figure II. 01

II.3 Synthesis of 2-((3-cyano-4,6-diarylpyridin-2-yl)oxy)acetohydrazide (3a-g)

We first investigated the possibility of synthesizing acetohydrazides from a direct condensation between esters **(2a-g)** and hydrazine under different reaction conditions given by literature [135] . However, the desired product was isolated with a low yield. Consequently, we accomplished an optimization study for the current reaction using **(2a)** (1.0eq) and hydrazine monohydrate (4 eq) to improve the reaction performance.



II.3.1 Influence of solvents

Initially, we studied the reaction in an ethanol medium at reflex temperature. However, the reaction was still slow, and yields were also very low. In order to improve the results of these first tests, we have taken up the same reaction under various solvents such as methanol, propanol, dioxane, CH_3CN, benzene, THF. In conclusion, the use of Polar aprotic solvents, particularly THF or dioxane, was proven to be essential to achieve maximum conversion for this reaction. Attempts to use other solvents resulted in lower yields or no reaction (Figure II. 02). The use of protic solvents such as ethanol, methanol, and propanol, produced a side-product (Scheme II. 02).

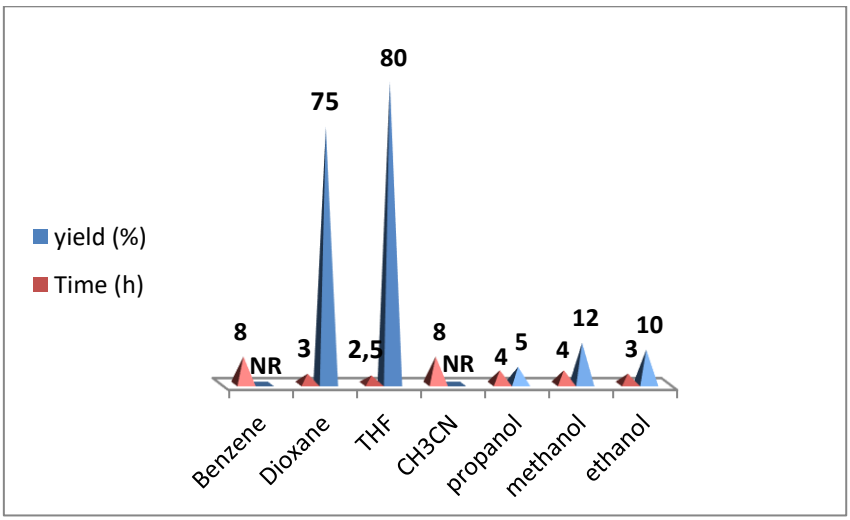
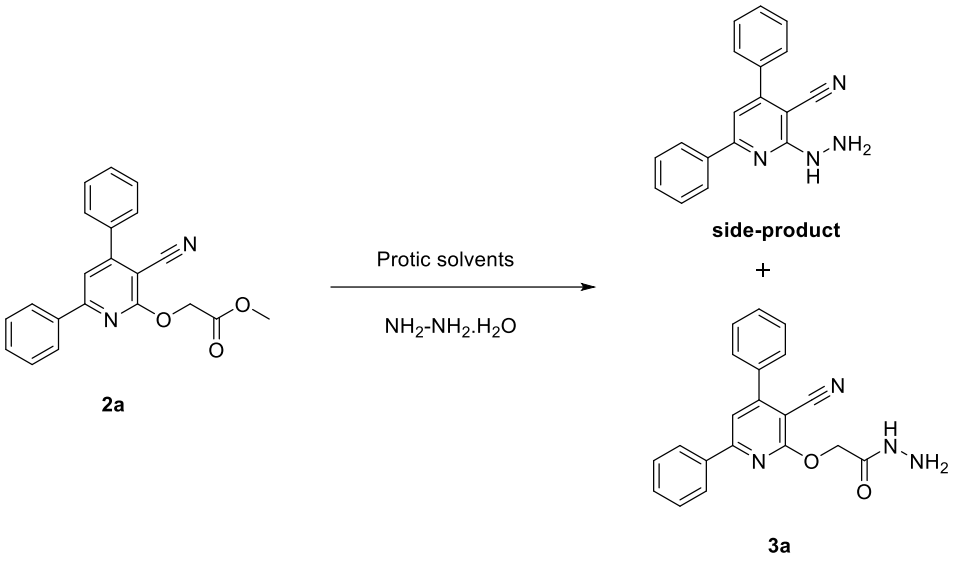


Figure II. 02



Scheme II. 02

II.3.2 The influence of temperature

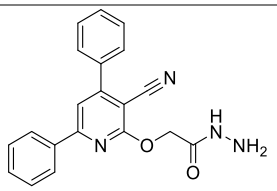
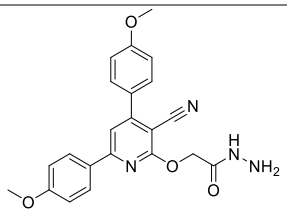
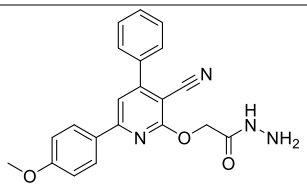
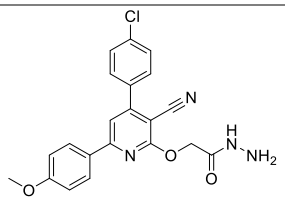
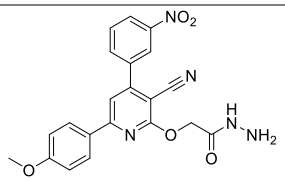
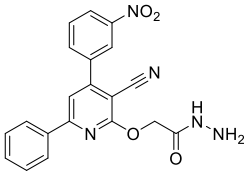
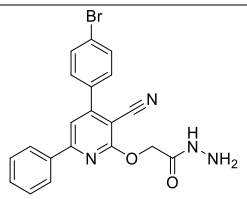
Because of the results previously obtained using polar aprotic solvents, the reaction seems to be effective at reflux temperatures. First, the reaction was tested under different temperatures starting at r.t and 40°C, and then at reflux temperature. According to the results obtained, the reaction in THF at 70°C showed a significant performance improvement compared to the other temperatures tested.

Table (II. 03): Optimisation of the reaction temperature

Entry	Solvent	Temperature (C°)	Time (h)	Yield (%)
1	THF	r.t	24	45
2	THF	40	12	62
3	THF	70 (reflux)	2.5	80
4	1,4 dioxane	r.t	24	21
5	1,4 dioxane	40	24	43
6	1,4 dioxane	70	3.5	71
7	1,4 dioxane	102 (reflux)	3	75

After determining the reaction's optimal conditions, we proceeded to their application to prepare some acetohydrazides, using a variety of differently substituted Methyl 2-((3-cyano-4, 6-diarylpyridin-2-yl)oxy)acetate, hydrazine monohydrate, and THF as polar aprotic solvent. The results obtained are summarized in the Table below (Table II.04).

Table (II. 04): The prepared Acetohydrazides **(3a-g)**

N°	Product	Yield %	Mp (C°)
3a		80	227-228
3b		75	234-236
3c		73	209-211
3d		71	230-232
3e		89	236-238
3f		69	219-221
3g		75	235-237

II.3.3 Spectral study

All acetohydrazides **(3a-g)** products have been identified by the usual spectroscopic analysis, including IR, ^{1}H NMR, ^{13}C NMR spectroscopy.

The IR spectra of **(3a-g)** showed absorption bands around 1663.50-1675.80 cm^{-1}, which indicates the presence of amide carbonyl group, and a second band in the range 3333.42-3260.62 cm^{-1} corresponding to the presence of the NH-NH_2 function.

The analysis of the spectroscopic results in high field nuclear magnetic resonance (NMR of proton and carbon 13) shows that these results are in perfect agreement with the proposed structures. The main results of the spectral analysis of compound **(3c)** in ^{1}H NMR, (Figure II. 03), showed two singlet Peaks at δ 9.44 and δ 4.33 ppm corresponding to NH and NH_2, respectively, besides the disappearance of $COOCH_3$ protons. CH_2O protons showed a singlet peak at 5.01 ppm with 2H integration, while the aromatic protons are observed in the downfield region [8.19-7.06] ppm. Analysis of the ^{13}C NMR spectrum of compound **(3c)** shows the presence of a signal at 167.1 ppm attributed to the carbonyl of acylhydrazide.

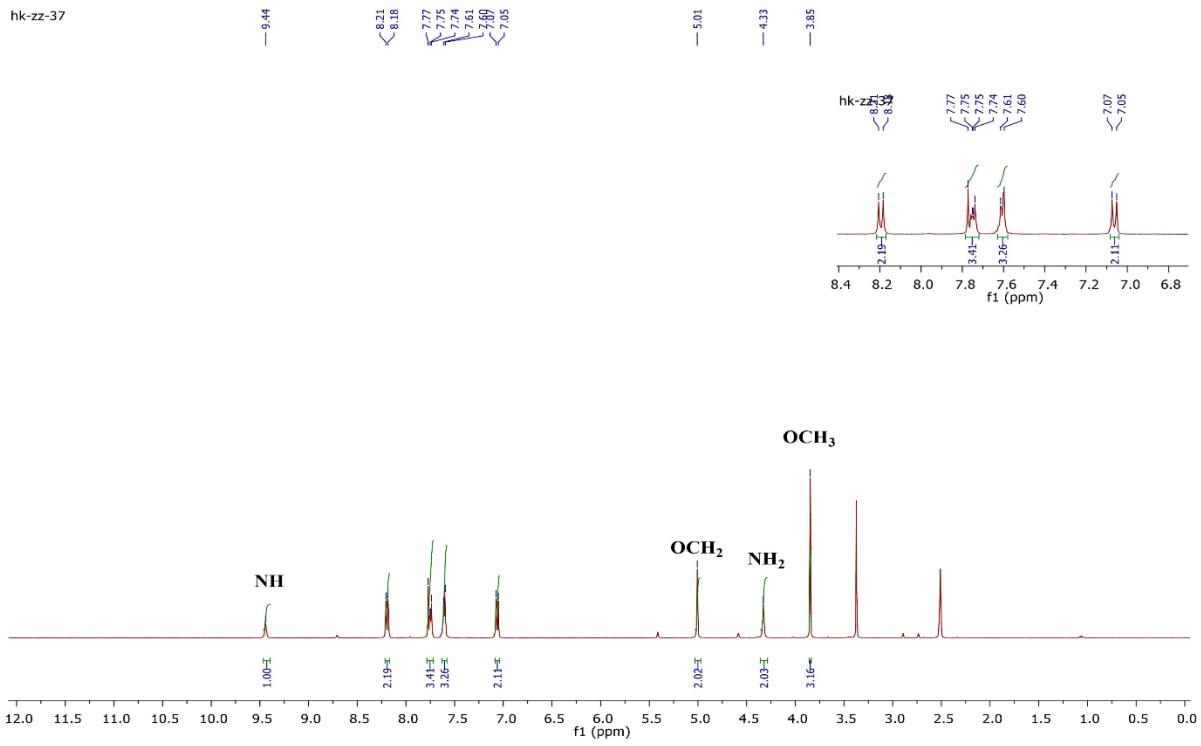
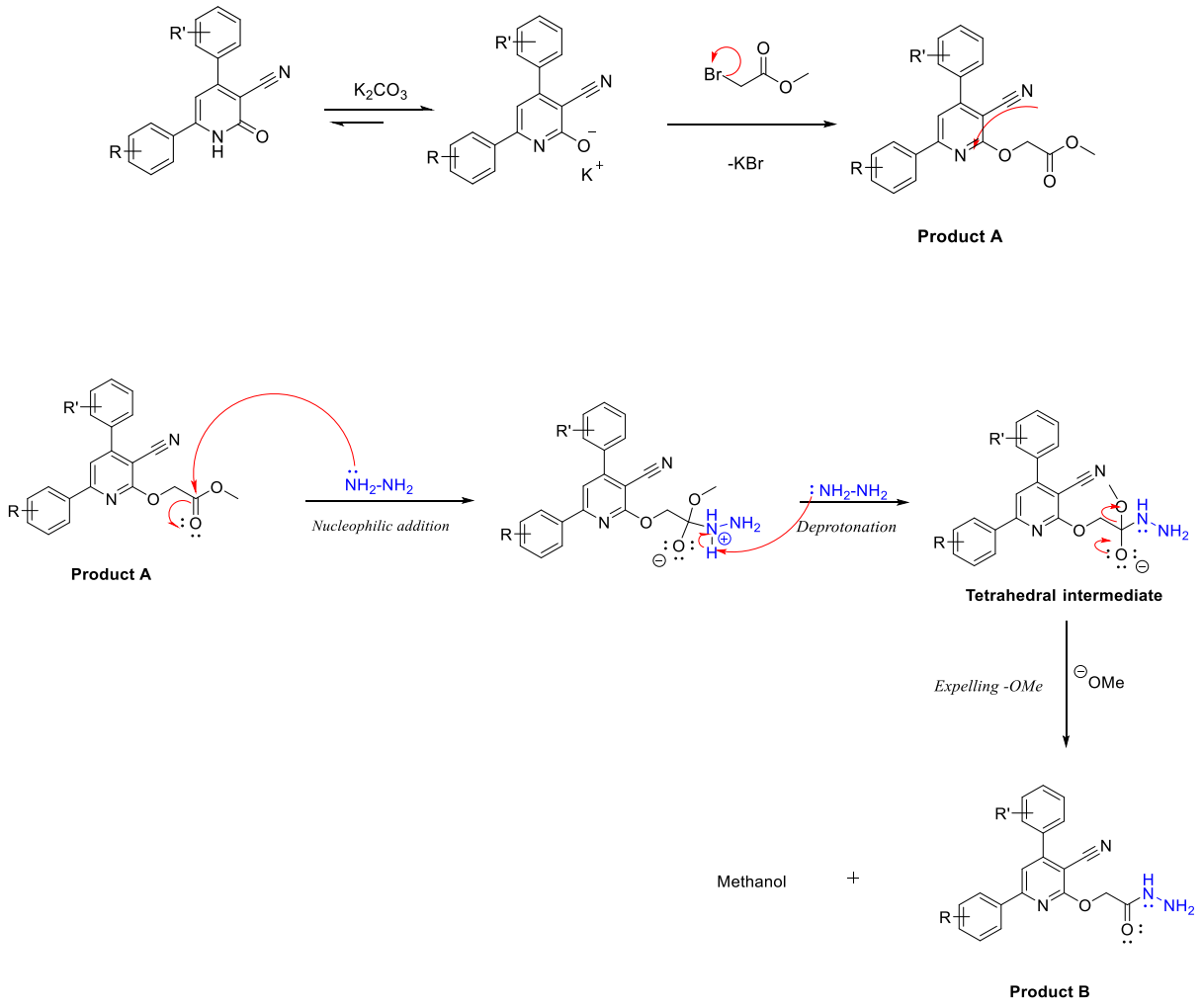


Figure II. 03

II.3.3 Reaction Mechanism

It has been proposed that product **A** is produced by nucleophilic substitution of 2-pyridones with methyl 2-bromoacetate. Subsequently, product **B** was manufactured by a nucleophilic addition-elimination mechanism. In the first, we have a nucleophilic addition of the hydrazine to the carbonyl. With an excess of hydrazine in the reaction mixture, the nitrogen is quickly deprotonated, forming a negatively charged tetrahedral intermediate. In order to restore the C=O double, the methoxy group is kicked out, producing an acetohydrazide (Scheme II. 03).



Scheme II. 03

II.4 Synthesis of Cyanopyridine-based pyrazoles (4a-g)

In order to determine the best conditions, we considered the condensation of acetohydrazide **(3a)** and acetylacetone in the respective proportions of 1/1.5 in reflux ethanol (5ml) as a model reaction in the presence of different catalysts. (The reaction was monitored by TLC). The results are given in Table (II.05).

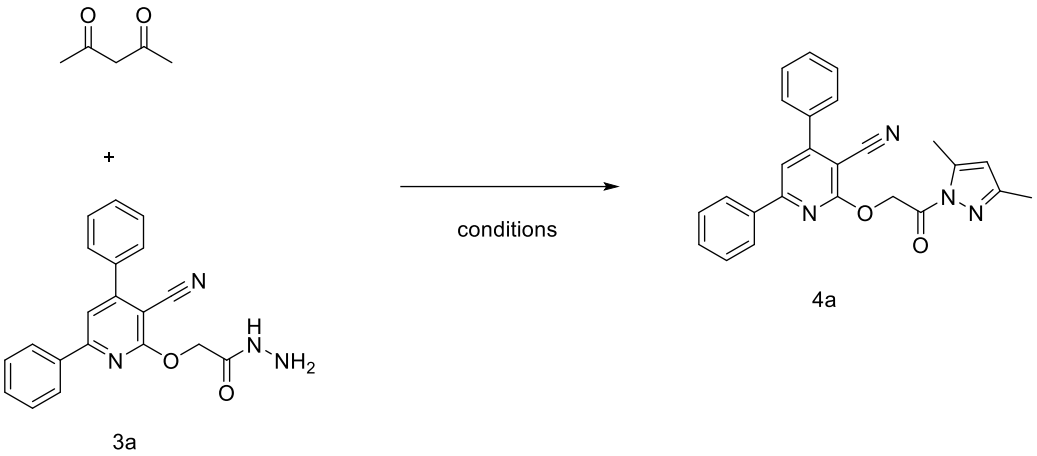


Table (II. 05): Screening of catalyst

Entry	Catalyst (20%)	Time (h)	Yield%
1	---	24	N.R
2	AcOH	12	26
3	TEA	14	10
4	pTsOH	6	51
5	K_2CO_3	24	N.R
6	KOH	24	5
7	DMAP	15	19

N.R: no reaction

The results given in Table (II.05) showed that acidic catalysts gave a higher yield and shorter reaction time compared to basic catalysts. Therefore, our choice was focused on the use of pTsOH as a catalyst in the synthesis of pyrazoles as it gave the best results (Table II.05, entry 4).

II.4.1 Determination of optimal conditions with " pTsOH " as a catalyst

II.4.1.1 Influence of solvents

The choice of the solvent is decisive for a good yield of the reaction. For this purpose, we performed the same model reaction in the presence of pTsOH (20 mol%) in different solvents at reflux temperatures. The yields obtained are shown in Table (II. 06).

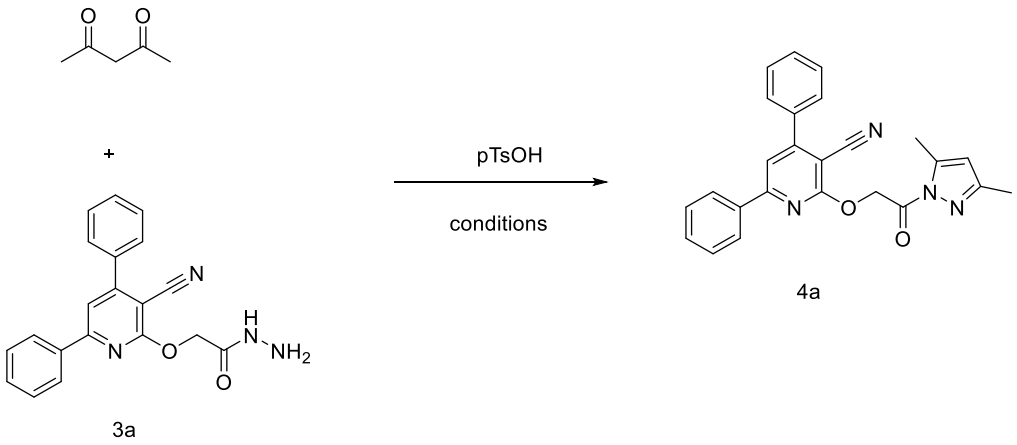


Table (II. 06): Screening of solvents

Entry	Solvent	Temperature (°C)	Time (h)	Yield%
1	---	100	24	N.R
2	Ethanol	reflux	6	51
3	1-propanol	reflux	6	57
4	methanol	reflux	10	20
5	Toluene	reflux	7	55
6	CH3CN	reflux	10	35
7	1,4 dioxane	reflux	2	66
8	H_2O	reflux	24	N.R
9	THF	reflux	15	25

N.R: no reaction

The results gathered in the Table above show that the better yield and the shorter reaction time are obtained by using 1,4 dioxane at reflux temperature due undoubtedly to the solubility of acetohydrazide in these conditions. Based on these results, we consider 1,4 dioxane as the reference solvent for our catalyst pTsOH.

II.4.1.2 Determining the optimal amount of catalyst (pTsOH)

The quantity of catalyst is a determining factor in a chemical reaction, which requires a study to determine the exact quantity with maximum catalytic effect.

The study was performed using the same model reaction in reflux 1,4 dioxane at reflux temperature with a variation of the catalyst quantity from 0 to 30 mol% (the reaction was followed by TLC). The results are collected in the following Table (Table II.07).

Table (II. 07): Effect of concentration of catalyst

Entry	pTsOH (mol (%))	Time (h)	Yield %
1	---	24	N.R
2	5	3	63
3	10	2	69
4	15	1.5	71
5	20	2	66
6	30	3	50

N.R: no reaction

The best result was obtained by using 15 mol% pTsOH (Table II. 07, entry 4). On the other hand, the same reaction carried out in 1,4 dioxane in the absence of a catalyst gave no evolution even after 24 hours of agitation at reflux temperature (Table II. 07. entry 1).

II.4.1.3 The influence of temperature

The same reaction was repeated at different temperatures: reflux, 50 °C, and at room temperature. The best performance is obtained by conducting the reflux reaction (Table II.08, entry 3).

Table (II. 08): Effect of temperature

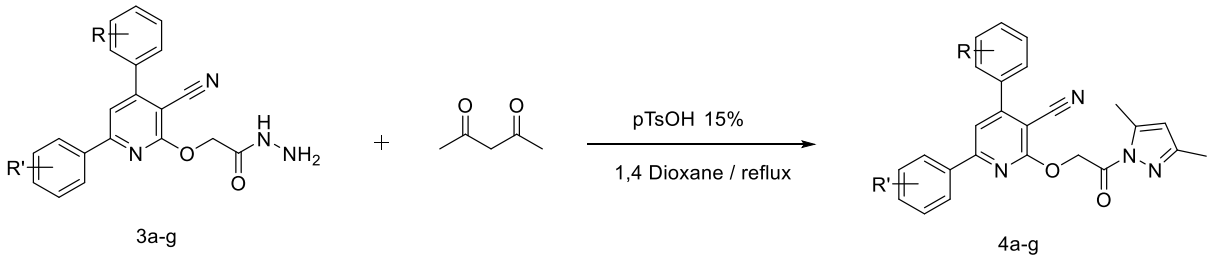
Entry	Temperature (°C)	Time (h)	Yield %
1	r.t	24	N.R
2	50	15	35
3	reflux	1.5	71

N.R: no reaction

Thus, we have determined the optimal conditions: (pTsOH 15 mol% as a catalyst, 1,4 dioxane as a solvent, and heating at reflux temperature) that we will apply to synthesize a series of novel pyrazoles linked to cyanopyridine moiety as the main scaffold.

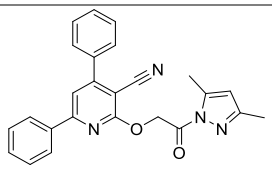
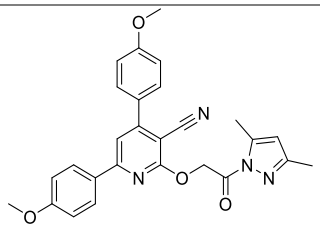
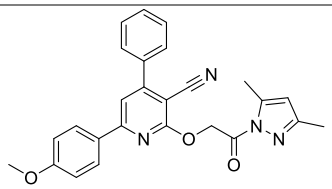
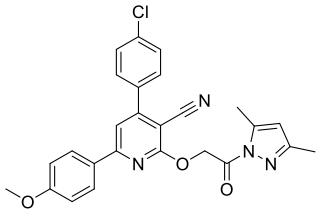
II.4.2 Application of the new optimal condition in the preparation of coupled cyanopyridine-pyrazole analogs

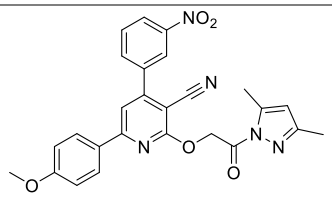
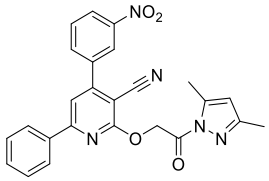
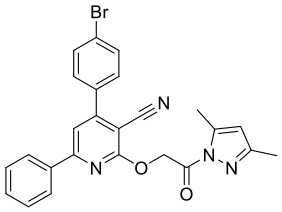
After determining the reaction's optimal conditions, we proceed with their application to prepare some novel coupled pyridine-pyrazole analogs. The results are summarized in Table (II. 09).



4a: R= H, R'= H
4b: R= 4OMe, R'= 4OMe
4c: R= H, R'= 4OMe
4d: R= 4Cl, R'= 4OMe
4e: R= 3NO_2, R'= 4OMe
4f: R= 3NO_2, R'= H
4g: R= 4Br, R'= H

Table (II. 09): Prepared cyanopyridine-pyrazole derivatives **(4a-g)**

N°	Product	Yield %	Mp (C°)
4a		71	229-231
4b		86	157-159
4c		84	176-178
4d		77	167-169

4e		69	247-249
4f		73	238-240
4g		65	231-233

II.4.3 Spectral study

The IR spectra of **(4a-g)** showed absorption bands in the range 1728.14-1786.81 cm^{-1}, 1591.10-1579.29 cm^{-1} characteristics to (C=O) amid group and (C=N) imine group, respectively, in addition to the disappearance of the amine NH bands at 3333.42-3260.62 cm^{-1}.

The ^{1}H-NMR data of **(4a)** showed four singlets at 6.34 ppm, 5.94 ppm, 2.45 ppm, and 2.29 ppm corresponding to pyrazole C4 proton, OCH_2, and $2CH_3$ Protons, respectively (Figure II. 04).

The ^{13}C NMR spectrum of **(4a-g)** has identified the presence (C=O) amide, and pyrazole C3, pyrazole C5, pyrazole C4 signals around 167.9 -168.1 ppm, 153.2 -153.2 ppm, 144.17-144.15 ppm, 112.0-111.9 ppm, respectively.

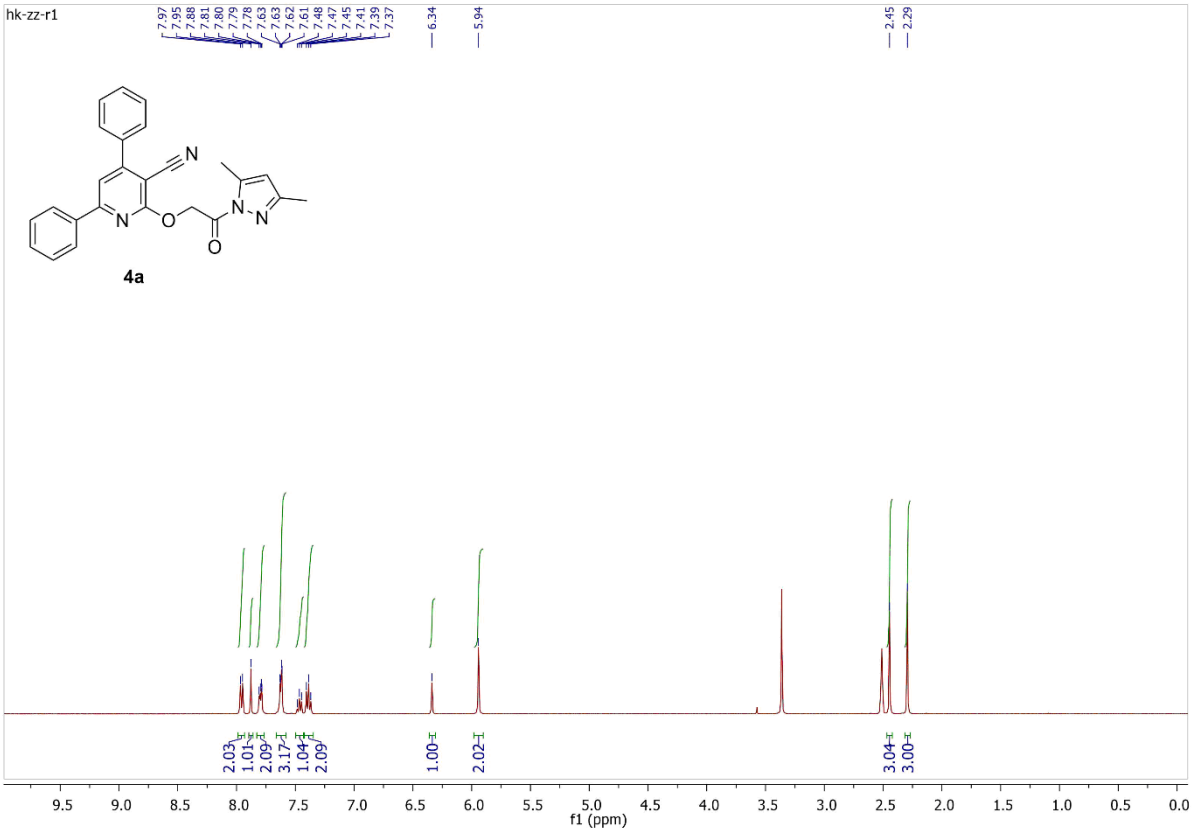
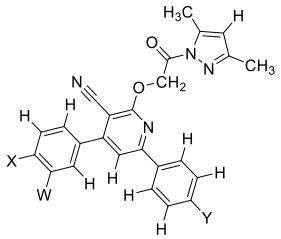

hk-zz-r1
7.97
7.95
7.88
7.81
7.80
7.79
7.78
7.63
7.62
7.61
7.48
7.47
7.45
7.41
7.39
7.37
6.34
5.94
2.45
2.29
N
N
O
N
N
O
4a
2.03
1.01
2.09
3.17
1.04
2.09
1.00
2.02
3.04
3.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Figure II. 04

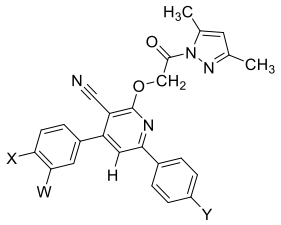
Table (II. 10): ^{1}H NMR data for compounds **(4a-g)**



4a: X=Y=W= H.
4b:X=Y= OMe, W= H.
4c: X= H, Y= OMe, W= H.
4d: X= Cl, Y= OMe, W= H.
4e: X= H, Y= OMe, W= NO_2
4f: X=Y= H, W= NO_2
4g: X= Br, Y=H, W= NO_2

N°	^{1}H NMR (DMSO-d_6) (δ, ppm)
4a	δ 7.96 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.88 (s, 1H, pyridine C5-H), 7.79 (dd, *J* = 6.4, 3.0 Hz, 2H, Ar-H), 7.66 – 7.58 (m, 3H, Ar-H), 7.47 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.39 (t, *J* = 7.5 Hz, 2H, Ar-H), 6.34 (s, 1H, pyrazole C4-H), 5.94 (s, 2H, OCH_2), 2.45 (s, 3H,CH_3), 2.29 (s, 3H, CH_3).
4b	δ 7.90 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.78 – 7.69 (m, 3H, Ar-H, pyridine C5-H), 7.15 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.90 (d, *J* = 8.9 Hz, 2H, Ar-H), 6.33 (s, 1H, pyrazole C4-H), 5.90 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3), 2.29 (s, 3H, CH_3).
4c	δ 7.92 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.81 – 7.69 (m, 3H, Ar-H, pyridine C5-H), 7.62 – 7.55 (m, 3H, Ar-H), 6.91 (d, *J* = 8.9 Hz, 2H, Ar-H), 6.33 (s, 1H, pyrazole C4-H), 5.91 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3), 2.30 (s, 3H, CH_3)
4d	δ 7.92 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.81 – 7.79 (m, 3H, Ar-H, pyridine C5-H), 7.69 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.92 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.34 (s, 1H, pyrazole C4-H), 5.91 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3), 2.30 (s, 3H, CH_3)
4e	δ 8.63 (t, *J* = 1.9 Hz, 1H, Ar-H), 8.47 – 8.41 (m, 1H, Ar-H), 8.27 – 8.20 (m, 1H, Ar-H), 7.98 – 7.88 (m, 4H, Ar-H, pyridine C5-H), 6.94 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.35 (s, 1H, pyrazole C4-H), 5.94 (s, 2H, OCH_2), 3.81 (s, 3H, OCH_3), 2.45 (s, 3H, CH_3), 2.30 (s, 3H, CH_3)
4f	δ 8.66 (t, *J* = 1.9 Hz, 1H, Ar-H), 8.48 – 8.44 (m, 1H, Ar-H), 8.29 – 8.26 (m, 1H, Ar-H), 8.04 (s, 1H, pyridine C5-H), 7.98 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.93 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.49 (d, *J* = 7.3 Hz, 1H, Ar-H), 7.41 (d, *J* = 7.7 Hz, 2H, Ar-H), 6.35 (s, 1H, , pyrazole C4-H), 5.96 (s, 2H, OCH_2), 2.45 (s, 3H, CH_3), 2.30 (s, 3H, CH_3)
4g	δ 7.98 – 7.70 (m, 7H, Ar-H, pyridine C5-H), 7.58 – 7.25 (m, 3H, Ar-H), 6.34 (s, 1H, pyrazole C4-H), 5.94 (s, 2H, OCH_2), 2.44 (s, 3H, CH_3), 2.29 (s, 3H, CH_3)

Table (II. 11): ^{13}C NMR data for compounds **(4a-g)**

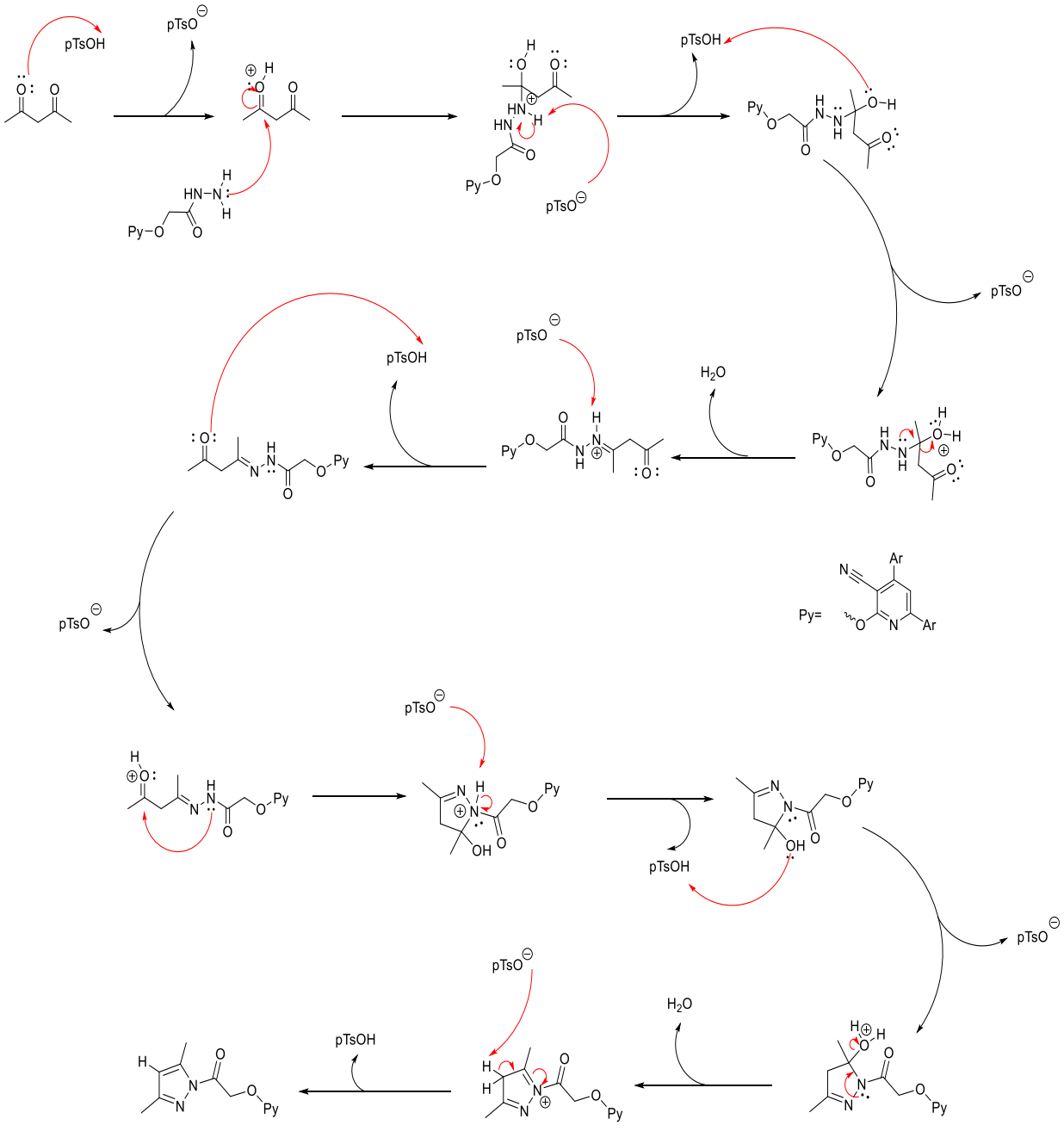


4a: X=Y=W= H.
4b:X=Y= OMe, W= H.
4c: X= H, Y= OMe, W= H.
4d: X= Cl, Y= OMe, W= H.
4e: X= H, Y= OMe, W= NO_2
4f: X=Y= H, W= NO_2
4g: X= Br, Y=H, W= NO_2

N°	^{13}C NMR (DMSO-d_6) (δ, ppm)
4a	*δ* 168.05 (CON), 163.43, 157.28, 157.25 (Ar-C), 153.22 (pyrazole C3), 144.15 (pyrazole C5), 136.63, 136.14, 131.29, 130.69, 129.40, 129.31, 129.17, 127.66, 115.53, 114.99 (Ar-C), 111.99 (pyrazole C4), 92.70 (CN), 65.29 (OCH_2), 14.05 (CH_3), 14.04 (CH_3)
4b	*δ* 168.10 (CON), 163.50, 161.85, 161.32, 156.89, 156.52 (Ar-*C*), 153.20 (pyrazole C3), 144.14 (pyrazole C5), 130.70, 129.30, 129.09, 128.34, 116.01, 114.78, 114.62, 113.64(Ar-*C*), 111.96 (pyrazole C4), 91.03 (CN), 65.06 (OCH_2), 55.89 (OCH_3), 55.83 (OCH_3), 14.05 (CH_3), 14.03 (CH_3)
4c	*δ* 168.06 (CON), 163.38, 161.93, 157.13, 156.96 (Ar-*C*), 153.23 (pyrazole C3), 144.15 (pyrazole C5), 136.28, 130.58 ,129.35 ,129.25, 129.09, 128.98, 115.71, 114.65, 113.95 (Ar-*C*), 111.98 (pyrazole C4), 91.49 (CN), 65.13 (OCH_2), 55.84 (OCH_3), 14.05 (CH_3), 14.04 (CH_3)
4d	*δ* 168.01 (CON), 163.35, 162.01, 157.27, 155.66 (Ar-*C*), 153.24 (pyrazole C3), 144.15 (pyrazole C5), 135.59, 135.06, 131.06, 129.39, 128.95, 128.92, 115.57, 114.69, 113.93 (Ar-*C*), 111.99 (pyrazole C4), 91.44 (CN), 65.17 (OCH_2), 55.87 (OCH_3), 14.11 (CH_3), 14.05 (CH_3).
4e	*δ* 167.98 (CON), 163.31, 162.12, 157.53, 154.56 (Ar-*C*), 153.27 (pyrazole C3), 148.43 (Ar-*C*), 144.17 (pyrazole C5), 137.66, 135.85, 131.01, 129.51, 128.84, 125.24, 124.12, 115.40, 114.72, 114.23 (Ar-*C*), 112.02 (pyrazole C4), 91.71 (CN), 65.25 (OCH_2), 55.90 (OCH_3), 14.06 (CH_3), 14.01 (CH_3).
4f	*δ* 167.96 (CON), 163.33, 157.65, 154.84 (Ar-*C*), 153.26 (pyrazole C3), 148.45 (Ar-*C*), 144.17 (pyrazole C5), 137.51, 136.46, 135.89, 131.49, 131.04, 129.35, 127.75, 125.33, 124.20, 115.24, 115.21 (Ar-*C*), 112.02 (pyrazole C4), 92.99 (CN), 65.39 (OCH_2), 14.06 (CH_3), 14.05 (CH_3).
4g	*δ* 168.00 (CON), 163.39, 157.42, 156.02 (Ar-*C*), 153.24 (pyrazole C3), 144.16 (pyrazole C5), 136.54, 135.27, 132.37, 131.38, 131.32, 129.33, 127.67, 124.50, 115.39, 114.88 (Ar-*C*), 112.00 (pyrazole C4), 92.60 (CN), 65.32 (OCH_2), 14.05 (CH_3), 14.04 (CH_3).

II.4.4 Reaction Mechanism

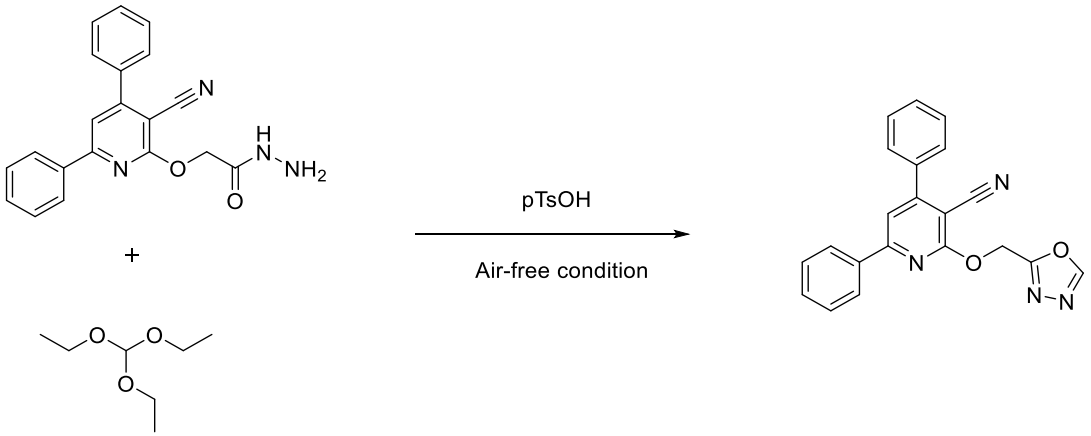
A proposed mechanism for synthesizing pyridine-pyrazole coupled molecules **(4a-g)** is illustrated in (Scheme II. 04). The mechanism begins with an acid-catalyzed imine formation. The second nitrogen of the hydrazine derivative attacks the other carbonyl group, which has also been protonated by the acid and forms a second imine group. This cyclic diimine compound gets deprotonated to regenerate the acid catalyst and provide the final pyrazole product.



Scheme II. 04

II.5 Synthesis of Cyanopyridine-based oxadiazoles (5a–f)

As we have already mentioned, our goal to develop novel cyanopyridines-oxadiazoles hybrid molecules. For this purpose, an Optimized study was accomplished using **(3a)** (1.0eq) and triethyl orthoformate (1ml, 6.0 eq) as a model reaction under air-free conditions.



We undertook the study of the different parameters of the reaction to determine the optimal conditions.

II.5.1 Influence of solvents

To determine the appropriate solvent for the reaction, we studied the same model reaction in the presence of 5% pTsOH using different solvents such as ethanol, acetonitrile, DMF, methanol, and dioxane, at reflux temperature, in addition to solvent-free conditions at 100°C. The results obtained are summarized in Table (II. 12).

Table (II. 12): Screening of solvents

Entry	**Solvent**	**Temperature (°C)**	**Time (h)**	**[a]Yield%**
1	Solvent-Free	100	24	20
2	ethanol	reflux	24	15
3	methanol	reflux	24	18
4	DMF	reflux	10	42
5	acetonitrile	reflux	15	35
6	dioxane	reflux	1	73

[a] purified by column chromatography.

Among the different results obtained, the best yield and a shorter time were obtained using 1,4 dioxane at reflux temperature due certainly to the solubility of acetohydrazide under these conditions.

II.5.2 Determining the optimal amount of catalyst (pTsOH)

The study performed on the model reaction: **3a** (1.0 eq) and triethyl orthoformate (1ml, 6.0 eq) in reflux 1,4 dioxane under air-free condition in the presence of different amounts of pTsOH. The results are gathered in the following table (Table II.13).

Table (II. 13): Effect of concentration of catalyst

Entry	pTsOH (mol (%))	Time (h)	[a]Yield %
1	----	24	15
2	5	1	73
3	10	1.5	70
4	15	1.5	69
5	20	2.5	67
6	30	3	51

[a] purified by column chromatography.

A detailed study of the results obtained shows that 5mol% is solely enough to give the best yield (TableII.13, entry 2).

II.5.3 The influence of temperature

To determine the suitable temperature, we carried out the model reaction in dioxane in the presence of pTsOH (5 mol%) at different temperatures (rt, 50°C, reflux). The results are summarized in Table II.14.

Table (II. 14): Effect of temperature

Entry	Temperature (°C)	Time (h)	Yield %
1	r.t	24	25
2	50	10	57
3	reflux	1	73

[a] purified by column chromatography.

The reaction is very slow at room temperature, and the best result was obtained at reflux temperature.

II.5.4 The influence of atmosphere nature

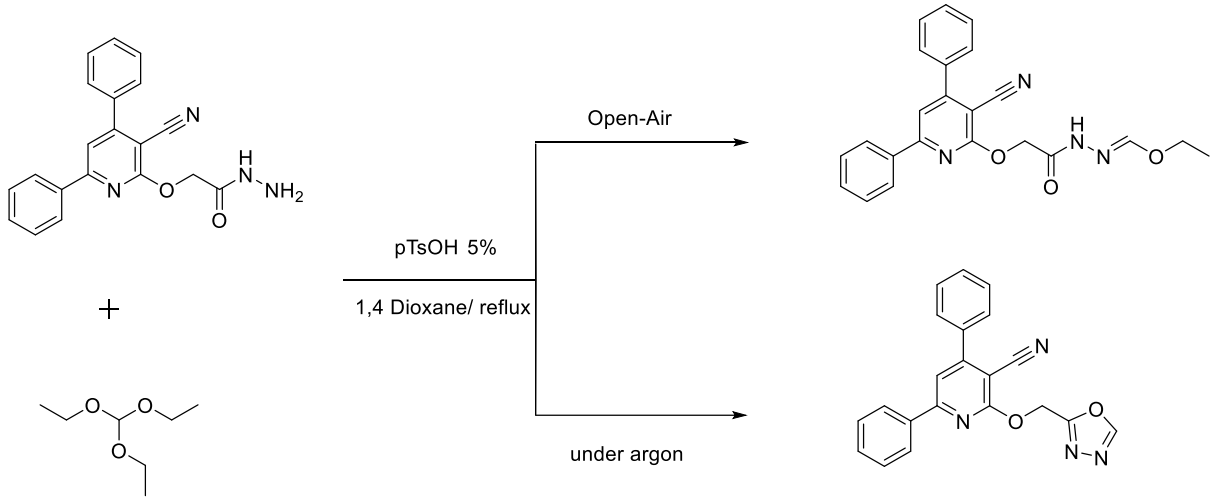
To determine the effect of atmosphere nature on the reaction, we conducted a study using the Open-air and Air-free (inert atmosphere). The results are collected in the following Table (Table II.15).

Table (II. 15): Determination of atmosphere nature

Entry	Atmosphere nature	Time (h)	[a]Yield %
1	Open-air	24	S-p
2	Inert atmosphere (argon)	1	73

[a] purified by column chromatography. S-p: Side-product.

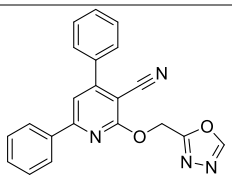
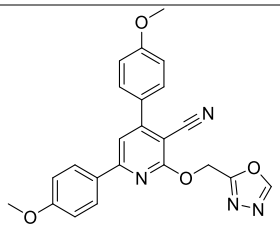
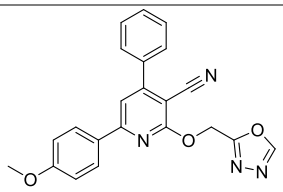
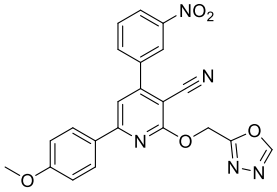
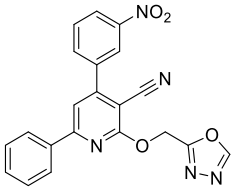
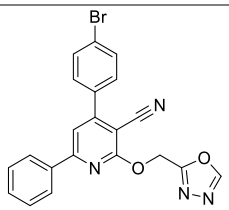
From the results collected in the table above, we assumed the atmosphere's nature could affect the reaction. Consequently, the best reaction performance was obtained under an argon atmosphere. On the other hand, only a side product was formed under an Open-air atmosphere (Scheme II. 05).



Scheme II. 05

After having successfully optimized the reaction conditions using pTsOH as catalyst and dioxane as solvent at reflux temperature, we have generalized these conditions by using various substrates in order to synthesize a rich series of similar products ***(5a-f)*** *in good yields.*

Table (II. 16): The prepared oxadiazole derivatives **(5a-f)**

N°	Product	Yield %	Mp (C°)
5a		73	192-194
5b		80	179-181
5c		71	162-164
5d		69	164-166
5e		67	154-156
5f		76	212-214

II.5.5 Spectral study

IR spectral data obtained here lead to confirm the structures of target compounds. IR spectrum of **(5a-f)** showed the presence of imine C=N absorption bands at the downfield range 1526.97-1595.67 cm^{-1} and the disappearance of both amide C=O and amine -NH-NH_2 bands.

The ^1H Nuclear Magnetic Resonance data obtained for the final products **(5a-f)** were useful in confirming their structure. The ^1H NMR for compound **(5b)** showed a δ value at 8.46 ppm as a singlet proved the presence of oxadiazole C5 proton, CH_2O protons showed a singlet peak at 5.85 ppm, the spectrum revealed the appearance of singlet peak at 3.78 ppm Corresponding to 2(OCH_3) while the aromatic protons are observed in the downfield region [8.01-6.98] ppm (Figure II. 05).

The ^{13}C NMR spectra of **(5b)** showed, in particular, three signals at 162.9 ppm, 153.6 ppm, 57.8 ppm corresponding to oxadiazole C2, oxadiazole C5, and OCH_2, respectively.

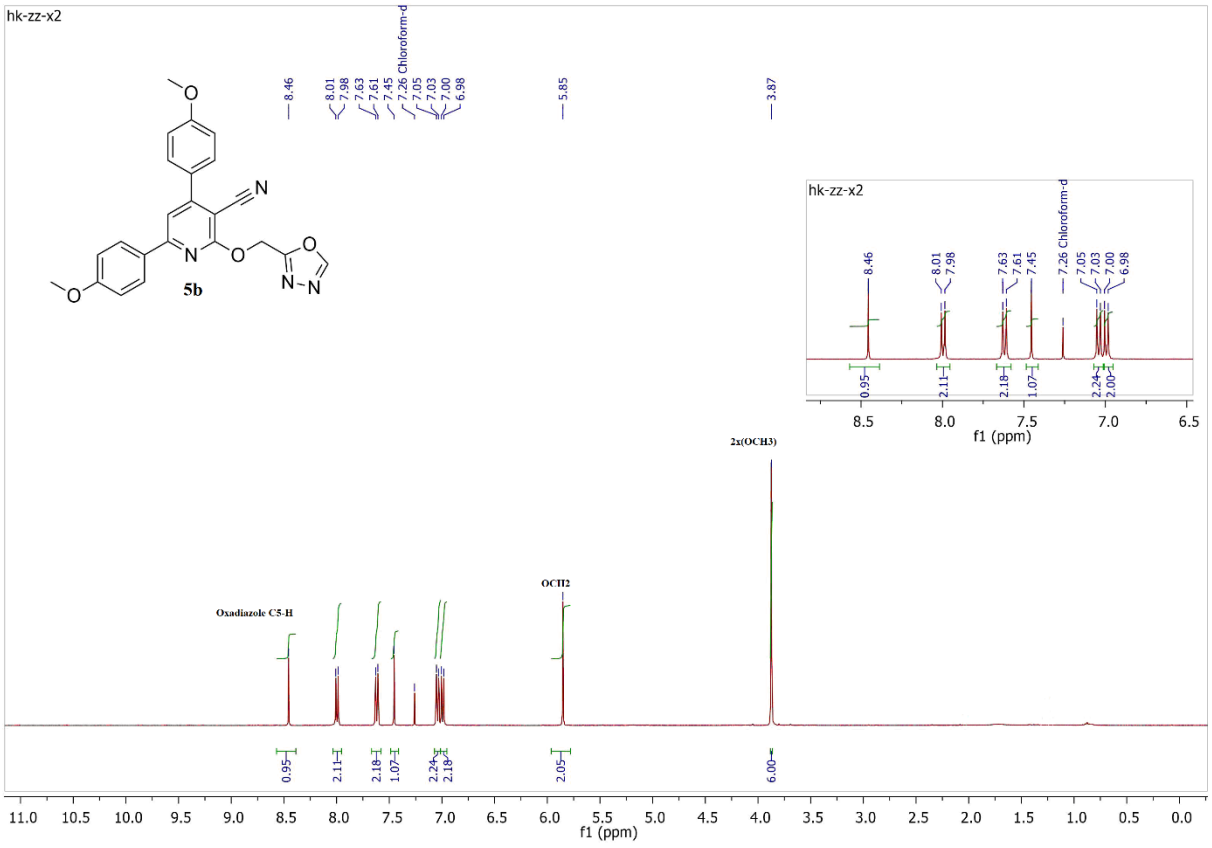
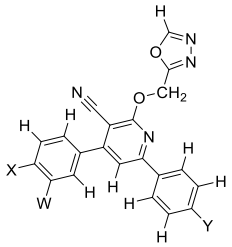


Figure II. 05

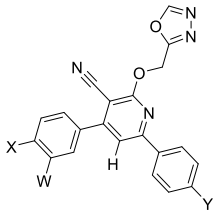
Table (II. 17): ^{1}H NMR data for compounds **(5a-f)**



5a: X=Y=W= H.
5b:X=Y= OMe, W= H.
5c: X= H, Y= OMe, W= H.
5d: X= H, Y= OMe, W= NO_2
5e: X=Y= H, W= NO_2
5f: X= Br, Y=H, W= NO_2

N°	^{1}H NMR ($CDCl_3$) (δ, ppm)
5a	δ 8.47 (s, 1H, oxadiazole C5-H), 8.05 – 8.03 (m, 2H, Ar-H), 7.67 – 7.65 (m, 2H, Ar-H), 7.58 (s, 1H, pyridine C5-H), 7.56 – 7.49 (m, 6H, Ar-H), 5.90 (s, 2H,OCH_2).
5b	δ 8.46 (s, 1H, oxadiazole C5-H), 8.00 (d, *J* = 8.9 Hz, 2H , Ar-H), 7.62 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.45 (s, 1H, pyridine C5-H), 7.04 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.99 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.85 (s, 2H, OCH_2), 3.87 (s, 6H,2OCH_3).
5c	δ 8.46 (s, 1H, oxadiazole C5-H), 8.01 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.65 – 7.63 (m, 2H, Ar-H), 7.54 – 7.52 (m, 3H, Ar-H), 7.49 (s, 1H, pyridine C5-H), 7.00 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.87 (s, 2H, OCH_2), 3.88 (s, 3H, OCH_3).
5d	δ 8.48 (s, 1H, oxadiazole C5-H), 8.47 – 8.46 (m, 1H, Ar-H), 8.40 – 8.38 (m, 1H, Ar-H), 8.04 – 8.00 (m, 3H, Ar-H), 7.75 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.51 (s, 1H, pyridine C5-H), 7.01 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.89 (s, 2H, OCH_2), 3.89 (s, 3H, OCH_3).
5e	δ 8.49 – 8.48 (m, 2H, oxadiazole C5-H, Ar-H), 8.42 – 8.38 (m, 1H, Ar-H), 8.07 – 8.02 (m, 3H, Ar-H), 7.77 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.60 (s, 1H, , pyridine C5-H), 7.53 – 7.51 (m, 3H, Ar-H), 5.92 (s, 2H,OCH_2).
5f	δ 8.47 (s, 1H, oxadiazole C5-H), 8.04 – 8.02 (m, 2H, Ar-H), 7.69 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.54 – 7.50 (m, 6H, Ar-H, pyridine C5-H), 5.89 (s, 2H, OCH_2).

Table (II. 18): ^{13}C NMR data for compounds **(5a-f)**

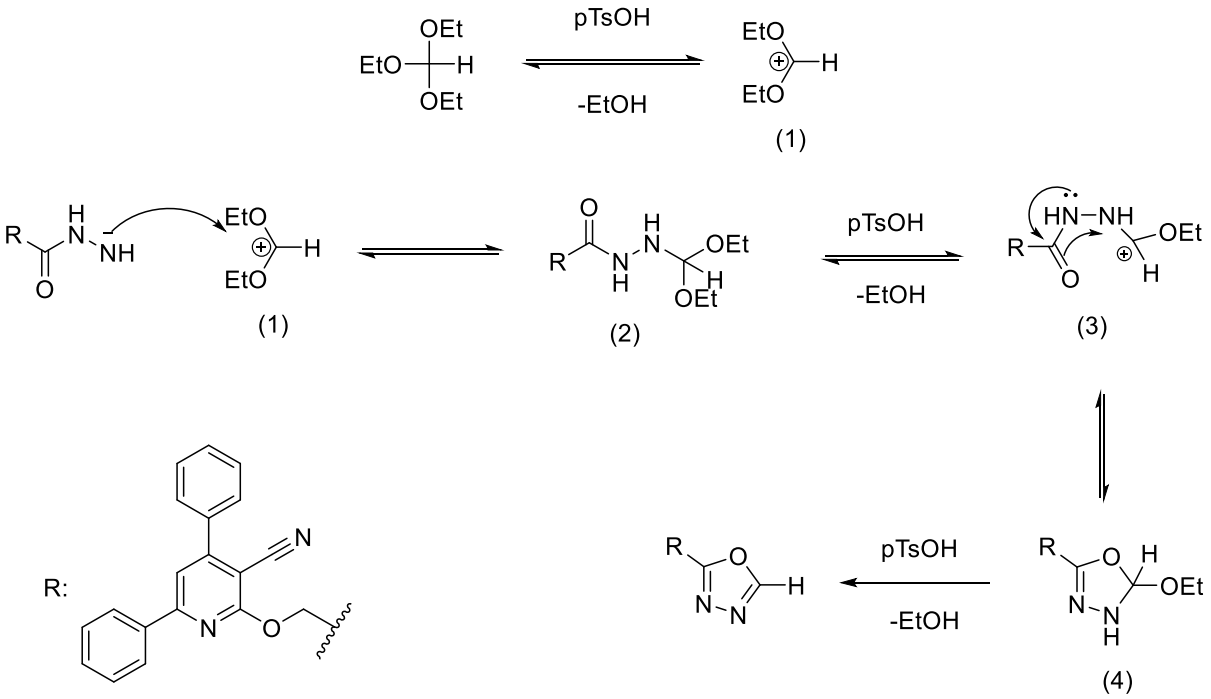


5a: X=Y=W= H.
5b:X=Y= OMe, W= H.
5c: X= H, Y= OMe, W= H.
5d: X= H, Y= OMe, W= NO_2
5e: X=Y= H, W= NO_2
5f: X= Br, Y=H, W= NO_2

N°	^{13}C NMR ($CDCl_3$) (δ, ppm)
5a	*δ* 162.87 (oxadiazole C2), 162.40, 157.97, 157.37 (Ar-*C*), 153.69 (oxadiazole C5), 136.56, 135.90, 130.91, 130.31, 129.11, 129.06, 128.39, 127.37, 114.90, 114.70 (Ar-*C*), 93.32 (CN), 57.95 (OCH_2).
5b	*δ* 162.93 (oxadiazole C2), 162.59, 161.90, 161.26, 157.42, 156.67 (Ar-*C*), 153.63 (oxadiazole C5), 129.89, 129.15, 128.94, 128.28, 115.32, 114.50, 114.39, 113.56 (Ar-*C*), 91.72 (CN), 57.86 (OCH_2), 55.48 ($2OCH_3$).
5c	*δ* 162.82 (oxadiazole C2), 162.53, 161.99, 157.64, 157.12 (Ar-*C*), 153.65 (oxadiazole C5), 136.09, 130.19, 129.06, 129.02, 128.99, 128.36, 114.94, 114.43, 113.87 (Ar-*C*), 92.18 (CN), 57.91 (OCH_2), 55.49 (OCH_3).
5d	*δ* 162.84 (oxadiazole C2), 162.36, 162.28, 158.50, 154.34 (Ar-*C*), 153.70 (oxadiazole C5), 148.58, 137.62, 134.35, 130.30, 129.18, 128.51, 124.83, 123.45, 114.57, 114.29, 113.55 (Ar-*C*), 92.13 (CN), 58.07 (OCH_2), 55.54 (OCH_3).
5e	*δ* 162.89 (oxadiazole C2), 162.16, 158.87, 154.61 (Ar-*C*), 153.74 (oxadiazole C5), 148.60, 137.41, 136.08, 134.36, 131.36, 130.37, 129.20, 127.49, 124.94, 123.49, 114.60, 114.04 (Ar-*C*), 93.37 (CN), 58.13 (OCH_2).
5f	*δ* 162.90 (oxadiazole C2), 162.30, 158.29, 156.09 (Ar-*C*), 153.68 (oxadiazole C5), 136.38, 134.72, 132.41, 131.07, 129.94, 129.11, 127.40, 125.04, 114.55, 114.48 (Ar-*C*), 93.13 (CN), 58.01 (OCH_2).

II.5.6 Reaction Mechanism

According to the reaction mechanisms reported in the literature [136], the reaction firstly goes through protonation and loss of one ethanol from triethyl orthoformate to generate an ether carbocation **(1)**. The Necluphilic attack by hydrazide on this species afforded the Intermediate **(2)** would be followed by protonation of methoxy group, which led to the loss of the second molecule of ethanol to give Intermediate **(3)**. Ring closure by intramolecular cyclization to give **(4)**. which generates the oxadiazole ring after eliminating the last molecule of ethanol (Scheme II. 06).



Scheme II. 06

II.6 Synthesis of the novel Cyanopyridines hydrazones bearing isatin moieties (6a-i)

The novel cyanopyridines-hydrazones bearing isatin moieties **(6a-i)** were prepared via condensation of acetohydrazides **(3a-g)** and isatin derivative using pTsOH as a catalyst, according to the method described by Hou *et al*. [127] The results are summarized in Table (II. 19).

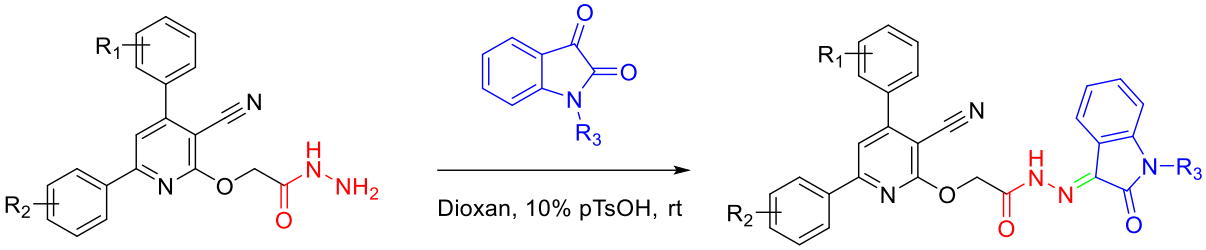
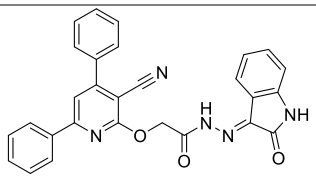
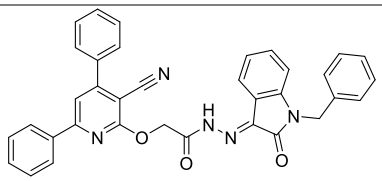
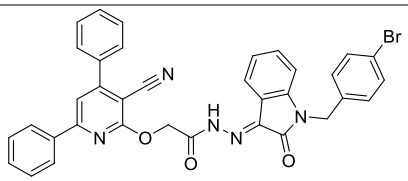
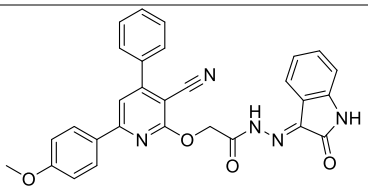
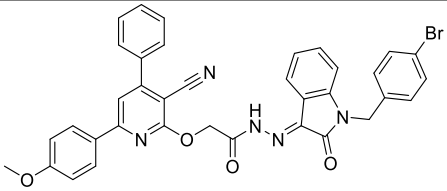
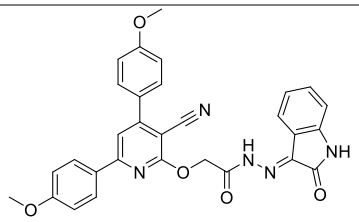
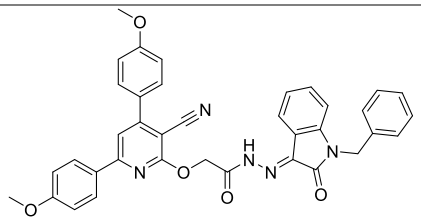
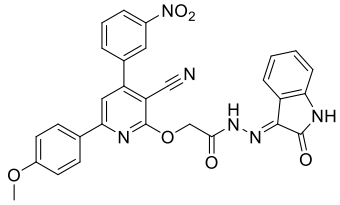
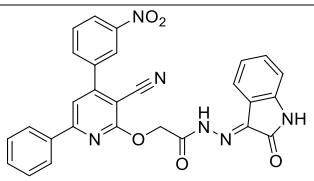


Table (II. 19): The novel cyanopyridine-hydrazones bearing isatin moiety **(6a-i)**

N°	Product	Yield %	Mp (C°)
6a		70	243–245
6b		68	217–219
6c		71	228–230
6d		80	230–232
6e		71	212–214

6f		70	240–242
6g		69	168–170
6h		66	224–226
6i		69	280–282

II.6.1 Spectral study

The analysis of the IR spectroscopic results is in perfect agreement with the proposed structures. The infrared spectra of **(6a-i)** showed NH stretching bands in the range of 3301–3089 cm^{-1}. The absorption bands at 1717.75-1729.42 cm^{-1} and 1675.58-1675.11 cm^{-1} confirm the presence of C=O amide and C=N imine functions, respectively.

The ^{1}H Nuclear Magnetic Resonance results of **(6a-i)** were significantly useful in confirming their structure, for compound **(4a)** showed three singlet peaks at 11.67 ppm, 10.90 ppm, 5.70 ppm assigned to NH of the hydrazide linker, NH group of isatin, and OCH_2 protons, respectively. Simultaneously, the aromatic protons are observed in the downfield region [8.20-6.95] ppm (Figure II. 06).

The ^{13}C NMR spectrum of the compound **(4a)** shows, in particular, the presence of two signals at 164.9 ppm, 163.6 ppm assigned to C=O amide of isatin, and hydrazide linker, respectively. While the carbon of imine C=N was observed at 133.3 ppm.

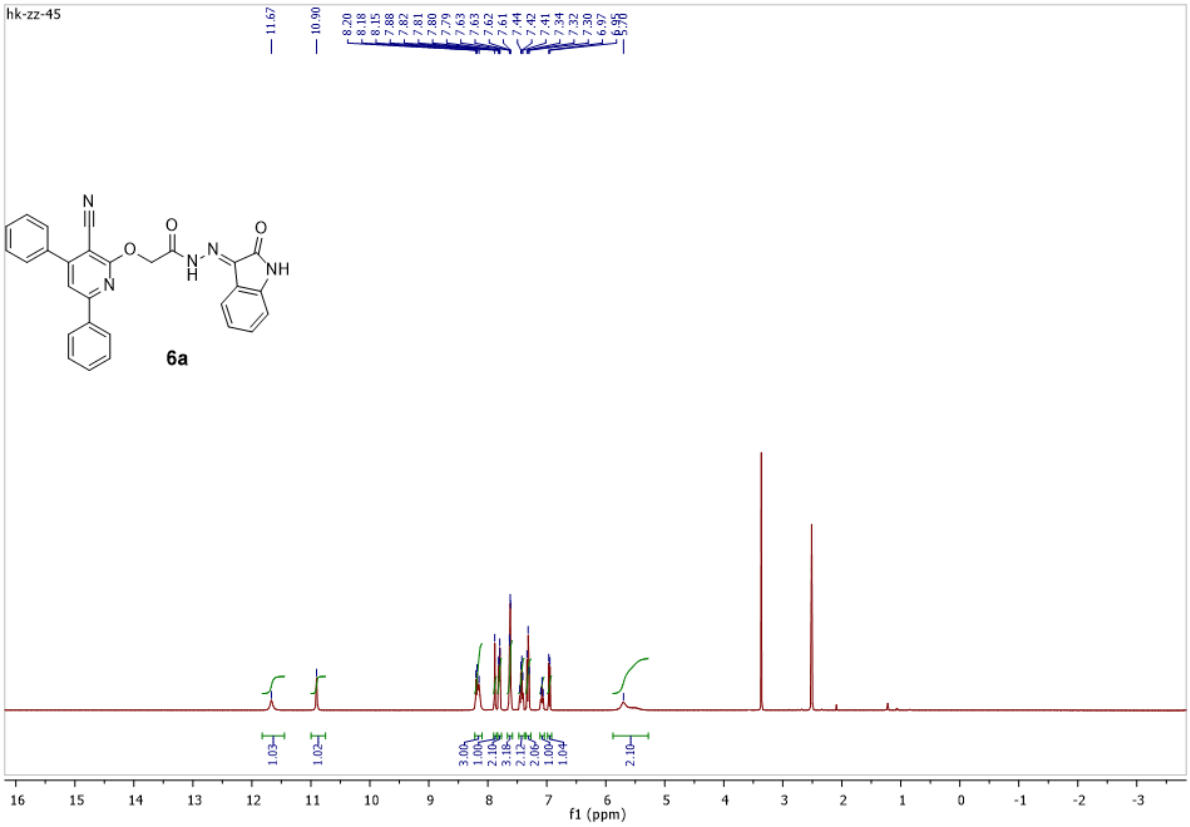
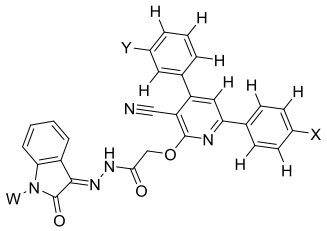

hk-zz-45
11.67
10.90
8.20
8.18
8.15
7.88
7.82
7.81
7.80
7.79
7.63
7.62
7.61
7.44
7.42
7.41
7.34
7.32
7.30
6.97
6.96
N
O
O
N
N
NH
O
6a
1.03
1.02
3.00
1.00
2.10
3.18
2.12
2.06
1.00
1.04
2.10
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)

Figure II. 06



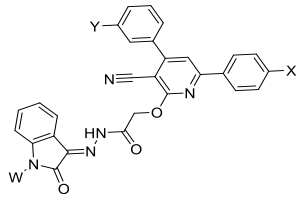
6a: X=Y=W= H.
6b:X=Y= H, W= Bz.
6c: X=Y= H, W= 4-BrBz.
6d: X= OMe, Y= H, W= H.
6e: X= OMe, Y= H, W= 4-BrBz.
6f: X= Y=OMe, W= H.
6g: X= Y=OMe, W= Bz.
6h: X= OMe, Y= NO_2, W=H.
6i: X= H, Y= NO_2, W=H.

Table (II. 20): ^{1}H NMR data for compounds **(6a-i)**

N°	^{1}H NMR (DMSO-d_6) (δ, ppm)
6a	δ 11.67 (s, 1H, NH), 10.90 (s, 1H, NH isatin), 8.22–8.10 (m,3H, Ar–H), 7.88 (s, 1H, pyridine C5–H), 7.83–7.77 (m, 2H, Ar–H),7.67–7.58 (m, 3H, Ar–H), 7.48–7.38 (m, 2H, Ar–H), 7.32 (t, *J* = 7.6 Hz, 2H, Ar–H), 7.08 (t, *J* = 7.5 Hz, 1H, Ar–H), 6.96 (d, *J* = 7.8 Hz, 1H, Ar–H), 5.70 (s, 2H, OCH_2)
6b	δ 11.79 (s, 1H, NH), 8.27 (d, *J* = 7.6 Hz, 1H, Ar–H), 8.16 (d, *J* = 7.2 Hz, 2H, Ar–H), 7.89 (s, 1H, pyridine C5–H), 7.84–7.77 (m, 2H, Ar–H), 7.67–7.60 (m, 3H, Ar–H), 7.48–7.26 (m, 9H, Ar–H), 7.14 (t, *J* = 7.6 Hz, 1H, Ar–H), 7.09 (d, *J* = 8.0 Hz, 1H, Ar–H), 5.70 (s, 2H, OCH_2), 5.01 (s, 2H, NCH_2).
6c	δ 11.81 (s, 1H, NH), 8.27 (d, *J* = 7.6 Hz, 1H, Ar–H), 8.19–8.11 (m, 2H, Ar–H), 7.89 (s, 1H, pyridine C5–H), 7.83–7.77 (m, 2H, Ar–H), 7.65–7.60 (m, 3H, Ar–H), 7.55 (d, *J* = 8.4 Hz, 2H, Ar–H), 7.49–7.27 (m, 6H, Ar–H), 7.15 (t, *J* = 7.6 Hz, 1H, Ar–H), 7.08 (d, *J* = 8.0 Hz, 1H, Ar–H), 5.73 (s, 2H, OCH_2), 4.98 (s, 2H, NCH_2).
6d	δ 11.67 (s, 1H, NH), 10.92 (s, 1H, NH isatin), 8.21 (d, *J* = 7.7 Hz, 1H, Ar–H), 8.15–8.05 (m, 2H, Ar–H), 7.82–7.72 (m, 3H, Ar–H, pyridine C5–H), 7.65–7.57 (m, 3H, Ar–H), 7.44 (t, *J* = 7.7 Hz, 1H, Ar–H), 7.10 (t, *J* = 7.2 Hz, 1H, Ar–H), 6.97 (d, *J* = 7.8 Hz, 1H, Ar–H), 6.79 (d, *J* = 8.8 Hz, 2H, Ar–H), 5.67 (s, 2H, OCH_2), 3.71 (s, 3H, OCH_3).
6e	δ 11.80 (s, 1H, NH), 8.28 (d, *J* = 7.7 Hz, 1H, Ar–H), 8.14–8.06 (m, 2H, Ar–H), 7.82–7.74 (m, 3H, Ar–H, pyridine C5–H), 7.64–7.59 (m, 3H, Ar–H), 7.53 (d, *J* = 8.3 Hz, 2H, Ar–H), 7.46 (t, *J* = 7.8 Hz, 1H, Ar–H), 7.33 (d, *J* = 8.3 Hz, 2H, Ar–H), 7.16 (t, *J* = 7.6 Hz, 1H, Ar–H), 7.09 (d, *J* = 8.0 Hz, 1H, Ar–H), 6.77 (d, *J* = 8.8 Hz, 2H, Ar–H), 5.67 (s, 2H, OCH_2), 4.98 (s, 2H, NCH_2), 3.66 (s, 3H, OCH_3).
6f	δ 11.68 (s, 1H, NH), 10.93 (s, 1H, NH isatin), 8.21 (d, *J* = 7.7 Hz, 1H, Ar–H), 8.14–8.03 (m, 2H, Ar–H), 7.80–7.70 (m, 3H,Ar–H, pyridine C5–H), 7.44 (t, *J* = 7.8 Hz, 1H, Ar–H), 7.16 (d, *J* = 8.8 Hz, 2H, Ar–H), 7.10 (d, *J* = 7.3 Hz, 1H, Ar–H), 6.96 (d, *J* = 7.8 Hz, 1H, Ar–H), 6.78 (d, *J* = 8.9 Hz, 2H, Ar–H), 5.66 (s, 2H,OCH_2), 3.86 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3)
6g	δ 13.38 (s, 1H, NH $_{cis\ conformer}$), 12.61 (s, 1H, NH $_{trans\ conformer}$), 8.20–8.04 (m, 2H, Ar–H), 7.79–7.57 (m, 4H, Ar–H, pyridine C5–H), 7.46–7.24 (m, 6H,

	Ar–H), 7.21–7.02 (m, 4H, Ar–H), 7.02–6.77 (m, 2H, Ar–H), 5.78 (s, 2H, $OCH_{2\ trans\ conformer}$), 5.35 (s, 2H, $OCH_{2\ cis\ conformer}$), 5.00 (s, 2H, NCH_2), 3.86 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3).
6h	δ 11.66 (s, 1H, NH), 10.90 (s, 1H, NH isatin), 8.62 (m, 1H, Ar–H), 8.47–8.40 (m, 1H, Ar–H), 8.29–8.17 (m, 2H, Ar–H), 8.16–8.07 (m, 2H, Ar–H), 7.96–7.86 (m, 2H, Ar–H, pyridine C5–H), 7.44 (t, *J* = 7.7 Hz, 1H, Ar–H), 7.10 (t, *J* = 7.5 Hz, 1H, Ar–H), 6.96 (d, *J* = 7.9 Hz, 1H, Ar–H), 6.81 (d, *J* = 8.8 Hz, 2H, Ar–H), 5.66 (s, 2H, OCH_2), 3.72 (s, 3H, OCH_3).
6i	δ 13.49 (s, 1H, $NH_{cis\ conformer}$), 12.70 (s, 1H, $NH_{trans\ conformer}$), 11.35 (s, 1H, NH isatin), 8.65–8.61 (m, 1H, Ar–H), 8.44 (d, *J* = 8.0 Hz, 1H, Ar–H), 8.26 (d, *J* = 7.8 Hz, 1H, Ar–H), 8.22–7.95 (m, 3H, Ar–H, pyridine C5–H), 7.92 (t, *J* = 7.9 Hz, 1H, Ar–H), 7.64–7.32 (m, 5H, Ar–H), 6.99 (m, 2H, Ar–H), 5.80 (s, 2H, $OCH_{2\ trans\ conformer}$), 5.39 (s, 2H, $OCH_{2\ cis\ conformer}$).

Table (II. 21): ^{1}H NMR data for compounds **(6a-i)**

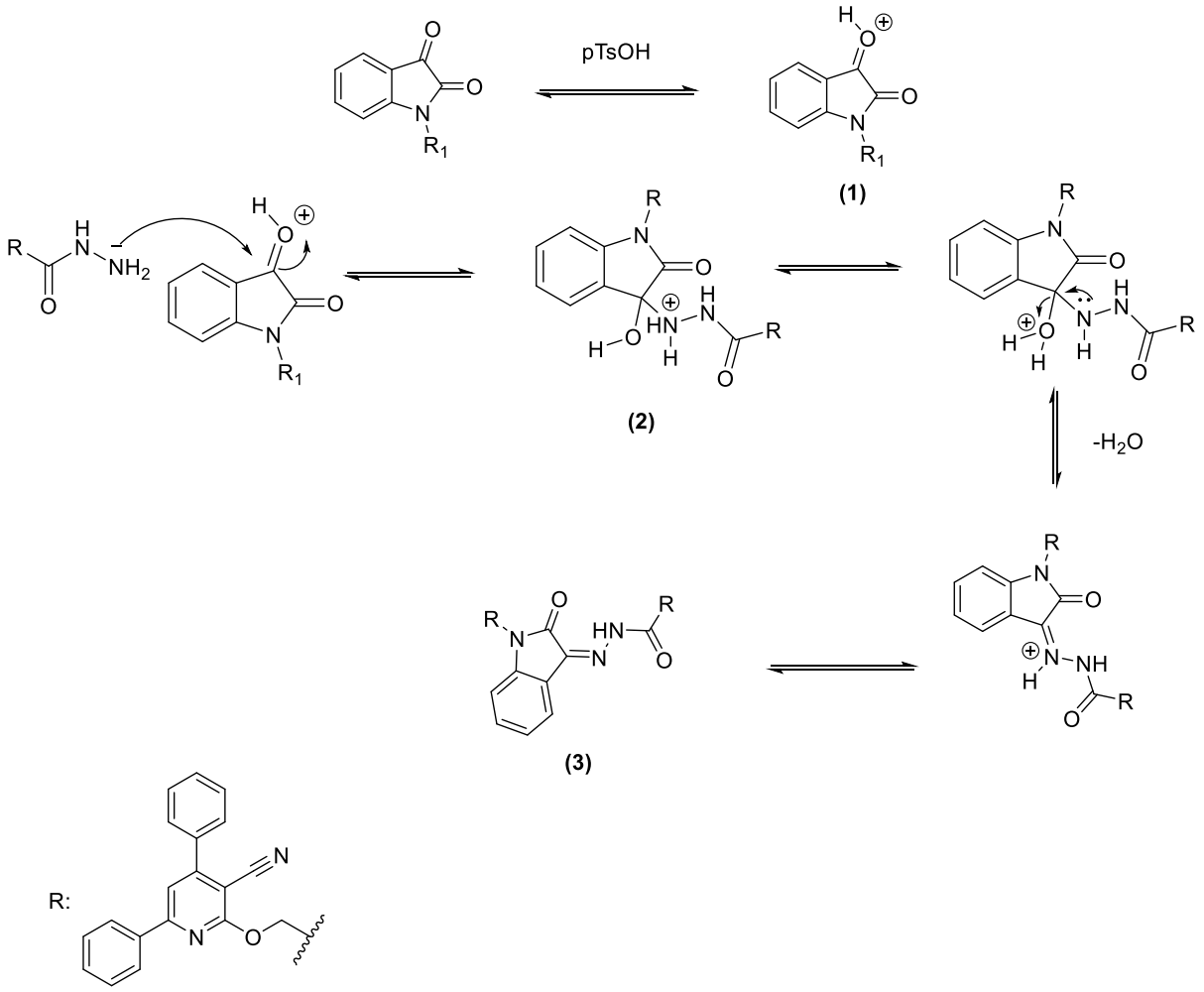


6a: X=Y=W= H.
6b:X=Y= H, W= Bz.
6c: X=Y= H, W= 4-BrBz.
6d: X= OMe, Y= H, W= H.
6e: X= OMe, Y= H, W= 4-BrBz.
6f: X= Y=OMe, W= H.
6g: X= Y=OMe, W= Bz.
6h: X= OMe, Y= NO_2, W=H.
6i: X= H, Y= NO_2, W=H.

N°	^{13}C NMR (DMSO-d_6) (δ, ppm)
6a	δ 164.93 (CONHN), 163.65 (CONH isatin), 157.44, 157.14, 144.45, 136.78, 136.18 (Ar–C), 133.38 (C=N imine), 131.23, 130.67, 129.39, 129.22, 129.17, 127.91, 126.78, 122.25, 115.67, 115.63, 114.83, 111.20 (Ar–C), 92.72 (CN), 66.83 (OCH_2).
6b	δ 163.80 (CONHN), 163.64 ($CONCH_2$ isatin), 157.46, 157.15, 144.40, 136.79, 136.61, 136.18 (Ar–C), 133.18 (C=N imine), 131.21, 130.68, 129.40, 129.22, 129.20, 129.18, 128.02, 127.92, 127.73, 126.65, 122.95, 115.67, 115.21, 114.87, 110.44 (Ar–C), 92.73 (CN), 43.20 (NCH_2).
6d	δ 164.95 (CONHN), 163.56 CONH isatin), 161.85, 157.24, 156.87, 144.47, 136.33 (Ar–C), 133.38(C=N imine), 130.56, 129.59, 129.35, 129.11, 126.80, 122.27, 115.85,115.66, 114.54, 113.80, 111.21 (Ar–C), 91.51 (CN), 66.82 (OCH_2), 55.73 (OCH_3).
4e	δ 163.82 (CONHN), 163.57 ($CONCH_2$ isatin), 161.84, 157.27, 156.91, 144.20, 136.33, 136.09 (Ar–C), 133.20 (C=N imine), 132.09, 130.57, 130.02, 129.60, 129.36, 129.14, 129.11, 126.70, 123.06, 121.15, 115.84, 115.27, 114.55, 113.86, 110.39 (Ar–C), 91.55 (CN), 55.69 (OCH_3), 42.61 (NCH_2).
4f	δ 164.96 (CONHN), 163.67 (CONH isatin), 161.76, 161.30, 157.00, 156.44, 144.46 (Ar–C), 133.36 (C=N imine), 130.72, 129.52, 129.23, 128.53, 128.38, 126.79, 125.98, 122.72, 116.15, 115.65, 114.79, 114.50, 113.49, 111.21 (Ar–C), 91.04 (CN), 67.49 (OCH_2), 55.89 (OCH_3), 55.71 (OCH_3)
4g	δ 161.86 (CONHN), 161.33 ($CONCH_2$ isatin), 161.14, 157.06, 156.50, 143.26, 136.06 (Ar–C), 132.21 (C=N imine), 130.71, 129.57,129.20, 128.33, 128.15, 127.88, 123.85, 121.26, 119.57, 115.96, 114.79, 114.61, 113.75, 111.02 (Ar–C), 91.21 (CN), 66.83 (OCH_2), 55.90 (OCH_3), 55.72 (OCH_3), 43.06 (NCH_2).
4h	δ 164.95 (CONHN), 163.48 (CONH isatin), 162.01, 157.63, 154.42, 148.41, 144.48, 137.69, 135.82 (Ar–C), 133.39 (C=N imine), 130.99, 129.71, 128.95, 126.79, 125.19, 124.09, 122.27, 115.65, 115.51, 114.58, 114.01, 111.21 (Ar–C), 91.70 (CN), 64.33 (OCH_2), 55.76 (OCH_3).
4i	δ 163.41 (CONHN), 163.02 (CONH isatin), 157.83, 154.72, 148.43, 143.08, 137.46, 136.54, 135.85 (Ar–C), 132.42 (C=N imine), 131.48, 131.02, 129.30, 128.00, 125.31, 124.15, 123.16, 121.41,120.00, 115.26, 115.21, 115.18, 111.72 (Ar–C), 93.06 (CN), 63.67(OCH_2).

II.6.2 Reaction Mechanism

The reaction firstly goes through the protonation of isatin carbonyl **(1)**. Then Nucleophilic addition of hydrazide to isatin carbonyl will occur to form intermediate **(2)**. Finally, a water molecule was kicked out after the second protonation to reinstate the double bound of imine **(3)** (Scheme II. 07).



Scheme II. 07

II.7 Conclusion

In this part of the thesis, we have designed and synthesized a series of hybrid heterocyclic compounds by coupling cyanopyridine derivatives with different bioactive molecules such as pyrazole, 1,3,4 oxadiazole, and hydrazone of isatin derivatives. These combinations resulted in new compounds that will be evaluated for their anticancer activity.

II.8 Experimental Section

II.8.1 General procedure for the synthesis of the products (2a-g)

A mixture of 2-pyridones **(1a-g)** (0.01 mol) and anhydrous K_2CO_3 (0.015 mol) was stirred at room temperature in *N*,*N*-Dimethylformamide (10 mL) for one hour, followed by the addition of methyl bromoacetate (0.011 mol). The reaction mixture was stirred for a further 3 hours and poured into ice-cold water. The product obtained was filtered, dried, and recrystallized from ethanol/acetone (2:1) to give the pure product.

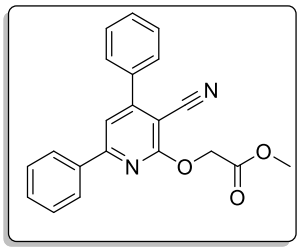
Methyl 2-((3-cyano-4, 6-diphenylpyridin-2-yl)oxy)acetate (2a)

White crystals

M= 344.37 g/mol

Yield = 89%

Mp: 157-159°C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (dd, J = 6.8, 3.0 Hz, 2H, Ar-H), 7.70 –7.64 (m, 2H, Ar-H), 7.58–7.45 (m, 7H, Ar-H, pyridine C5-H), 5.11 (s, 2H, OCH_2), 3.81 (s, 3H, $COOCH_3$).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 164.1 ($\underline{C}OOCH_3$), 158.6, 152.8, 152.3, 132.0, 131.3, 125.9, 125.4, 124.3, 124.2, 123.6, 122.5, 110.2, 109.7 (Ar-*C*), 88.5 (CN), 58.8 (OCH_2), 47.5 ($COO\underline{C}H_3$).

IR (cm-1) ν(C-O-C) aliphatic ether: 1145.43, ν(C=O) ester: 1733.12, ν(CN):2226.56, ν(C-H) aromatic: 2959.37.

Elemental analysis: Anal, Calcd, for $C_{21}H_{16}N_2O_3$ (344.37): % C, 73.24; H, 4.68; N, 8.13; fund: C, 74.10; H, 4.95; N, 8.21.

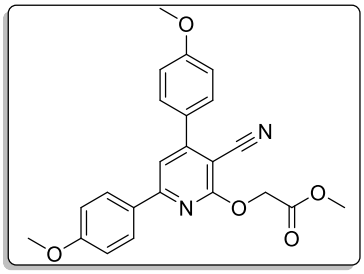
Methyl 2-((3-cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl)oxy)acetate (2b)

White solid

M= 404.42 g/mol

Yield = 75%

Mp: 130-132°C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.63 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.43 (s, 1H, pyridine C5-H), 7.04 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.98 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.07 (s, 2H, OCH_2), 3.87 (s, 6H, $2OCH_3$), 3.79 (s, 3H, $COOCH_3$)

^{13}C NMR ($CDCl_3$, 400 MHz) δ 169.0 (COOCH3), 163.4, 161.7, 161.1, 157.0, 156.4, 129.9, 129.4, 128.7, 128.5, 115.6, 114.4, 114.2, 113.1 (Ar-*C*), 91.7, (CN), 63.5 (OCH_2), 55.4 ($2OCH_3$), 52.2 ($COOCH_3$).

IR (cm-1) $_{v}$(C-O-C) aliphatic ether: 1140.53, 1170.69, 1182.51, $_{v}$(C=O) ester: 1761.85, $_{v}$(CN): 2220.54, $_{v}$(C-H) aromatic: 2838.91, 2949.49

Elemental analysis: Anal, Calcd, for $C_{23}H_{20}N_2O_5$ (404.42): % C, 68.31; H, 4.98; N, 6.93; fund: % C, 68.98; H, 5.02; N, 6.94.

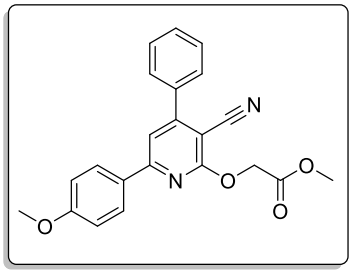
Methyl 2-((3-cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl) oxy) acetate (2c)

White crystals

M= 374.40 g/mol

Yield = 92 %

Mp: 148-150 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (d, *J*= 9.0 Hz, 2H, Ar-H), 7.67-7.62 (m, 2H, Ar-H), 7.55-7.49 (m, 3H, Ar-H), 7.45 (s, 1H, pyridine C5-H), 6.98 (d, *J*= 9.0 Hz, 2H, Ar-H), 5.08 (s, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 3.79 (s, 3H, $COOCH_3$).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 168.9 ($COOCH_3$), 163.3, 161.8, 157.2, 156.8, 136.2, 130.0, 129.2, 129.0, 128.8, 128.4, 115.2, 114.3, 113.4 (Ar-*C*), 92.1 (CN), 63.5 (OCH_2), 55.4 (OCH_3), 52.2 ($COOCH_3$).

IR (cm-1) ν(C-O-C) aliphatic ether:1140.53, 1172.51, ν(C=O) ester: 1760.38, ν(CN): 2223.11, ν(C-H) aromatic: 2842.39, 2950.17.

Elemental analysis: Anal, Calcd, for $C_{22}H_{18}N_2O_4$ (374.40): % C, 70.58; H, 4.85; N, 7.48; fund: % C, 71.25; H, 4.90; N, 7.61

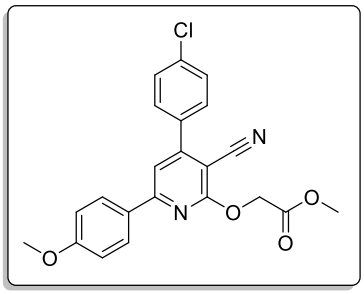
Methyl2-((4-(4-chlorophenyl)-3-cyano-6-(4-methoxyphenyl)pyridin-2-yl)oxy)acetate (2d)

White solid

M= 408.84 g/mol

Yield = 79 %,

Mp: 145-147 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (d, *J*= 9.0 Hz, 2H, Ar-H), 7.58 (d, *J*= 8.6 Hz, 2H, Ar-H), 7.50 (d, *J*= 8.6 Hz, 2H, Ar-H), 7.41 (s, 1H, pyridine C5-H), 6.98 (d, *J*= 8.9 Hz, 2H, Ar-H), 5.07 (s, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 3.79 (s, 3H, $COOCH_3$)

^{13}C NMR ($CDCl_3$, 400 MHz) δ 168.8 ($\underline{C}OOCH_3$), 163.3, 161.9, 157.5, 155.5, 136.4, 134.6, 129.7, 129.3, 129.0, 128.8, 115.0, 114.3, 113.1 (Ar-*C*), 91.9 ($\underline{C}N$), 63.5 ($O\underline{C}H_2$), 55.4 ($O\underline{C}H_3$), 52.2 ($COO\underline{C}H_3$).

IR (cm-1) ν(C-O-C) aliphatic ether: 1144.17, 1171.79, ν(C=O) ester: 1760.80, ν(CN): 2223.85, ν(C-H) aromatic: 2841.07 .

Elemental analysis: Anal, Calcd, for $C_{22}H_{17}ClN_2O_4$ (408.84): % C, 64.63; H, 4.19; N, 6.85; fund: % C, 64.48, H, 4.77, N, 6.53.

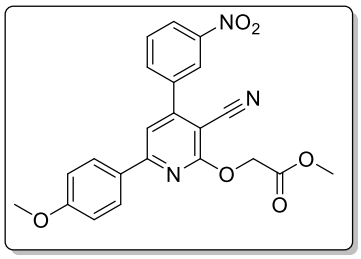
Methyl2-((3-cyano-6-(4-methoxyphenyl)-4-(3-nitrophenyl)pyridin-2-yl)oxy)acetate (2e)

Yellow solid

M= 419.39 g/mol

Yield = 77 %

Mp: 190-192 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.60 (t, *J* = 1.9 Hz, 1H, Ar-H), 8.47 – 8.40 (m, 1H, Ar-H), 8.26 – 8.20 (m, 1H, Ar-H), 8.16 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.94 (s, 1H, pyridine C5-H), 7.90 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.09 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.19 (s, 2H, OCH2), 3.85 (s, 3H, OCH_3), 3.75 (s, 3H, $\underline{C}OOCH_3$)

^{13}C NMR (DMSO-d_6, 400 MHz) δ 169.2 ($\underline{C}OOCH_3$), 163.2, 162.2, 157.6, 154.4, 148.3, 137.5, 135.8, 130.9, 129.6, 128.8, 125.2, 124.0, 115.3, 114.8, 114.1 (Ar-*C*), 91.6 (CN), 64.1 (OCH_2), 55.9 (OCH_3), 52.4 ($COO\underline{C}H_3$)

IR (cm-1) $_{\nu}$(C-O-C) aliphatic ether: 1149.23, 1176.64, $_{\nu}$(N-O) nitro: 1531.41, $_{\nu}$(C=O) ester: 1787.33, $_{\nu}$(CN): 2217.02, $_{\nu}$(C-H) aromatic: 2837.24, 2950.66.

Elemental analysis: Anal, Calcd, for $C_{22}H_{17}N_3O_6$ (419.39): % C, 63.01; H, 4.09; N, 10.02; fund: % C, 63.69; H, 4.09; N, 10.02.

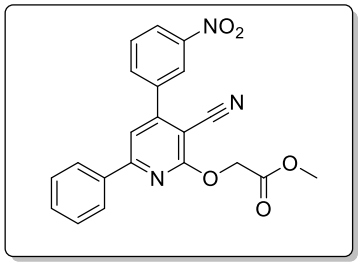
Methyl 2-((3-cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl)oxy)acetate (2f)

Yellow solid

M= 389.37 g/mol

Yield = 72 %

Mp: 170-172 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.64 (t, *J* = 1.9 Hz, 1H, Ar-H), 8.47 – 8.41 (m, 1H, Ar-H), 8.26 (d, *J* = 8.2 Hz, 1H, Ar-H), 8.20 (dd, *J* = 6.7, 3.0 Hz, 2H, Ar-H), 8.05 (s, 1H, pyridine C5-H), 7.92 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.59 – 7.52 (m, 3H, Ar-H), 5.23 (s, 2H, OCH_2), 3.75 (s, 3H, $COOCH_3$).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 169.2 ($\underline{C}OOCH_3$), 163.2, 157.7, 154.7, 148.4, 137.4, 136.5, 135.8, 131.5, 131.0, 129.4, 127.9, 125.3, 124.1, 115.2, 115.1 (Ar-*C*), 92.9 (CN), 64.1 (OCH_2), 52.4 ($COO\underline{C}H_3$).

IR (cm-1) $_{v}$(C-O-C) aliphatic ether: 1155.06, $_{v}$(N-O) nitro: 1528.48, $_{v}$(C=O) ester: 1754.76, $_{v}$(CN): 2225.46, $_{v}$(C-H) aromatic: 3092.87.

Elemental analysis: Anal, Calcd, for $C_{21}H_{15}N_3O_5$ (389.37): % C, 64.78; H, 3.88; N, 10.79; fund: % C, 65.04; H, 4.02; N, 10.69.

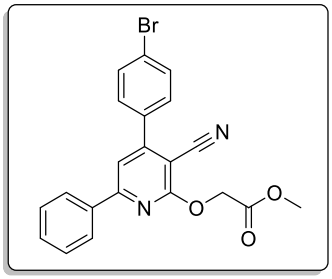
Methyl 2-((4-(4-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)oxy)acetate (2g)

White crystals

M= 423.27 g/mol

Yield = 83 %

Mp: 177-179 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 – 7.96 (m, 2H, Ar-H), 7.68 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.56 – 7.46 (m, 6H, Ar-H, pyridine C5-H), 5.11 (s, 2H, OC$\underline{H}_2$), 3.80 (s, 3H, COOC$\underline{H}_3$).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 168.7 ($\underline{C}OOCH_3$), 163.4, 157.9, 155.8, 136.6, 134.9, 132.3, 130.8, 129.9, 129.0, 127.2, 124.8, 114.8, 114.1 (Ar-*C*), 93.0 (CN), 63.6 (OCH_2), 52.2 (COO$\underline{C}H_3$).

IR (cm-1) $_{v}$(C-O-C) aliphatic ether: 1142.91, $_{v}$(C=O) ester: 1749.70, $_{v}$(CN): 2224.30, $_{v}$(C-H) aromatic: 2960.22.

Elemental analysis: Anal, Calcd, for $C_{21}H_{15}BrN_2O_3$ (423.27): % C, 59.59; H, 3.57; N, 6.62; fund: % C, 60.09, H, 3.67, N, 6.83.

II.8.2 General procedure for the synthesis of the products (3a-g)

A mixture of **(2a-g)** (0.01 mol) and hydrazine monohydrate (0.04 mol,1 mL) in tetrahydrofuran (10 mL) was boiled for 2.5-3 h. After cooling, the formed precipitate was filtered off, dried, and recrystallized from ethanol/DMF (2:1) to give the target compounds **(3a-g)**

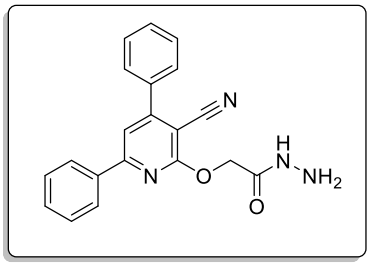
2-((3-cyano-4,6-diphenylpyridin-2-yl)oxy)acetohydrazide (3a)

Yellowish white crystals

M= 344.37 g/mol

Yield = 80 %

Mp: 227-228 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.45 (s, 1H, NH), 8.21 (dd, *J*= 6.7 Hz, 3.3 Hz, 2H, Ar-H), 7.85 (s, 1H, pyridine C5-H), 7.77-7.75 (m, 2H, Ar-H), 7.61-7.52 (m, 6H, Ar-H), 5.04 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.0 (CONH-NH_2), 163.7, 157.4, 156.8, 136.9, 136.2, 131.2, 130.6, 129.4, 129.3, 129.1, 128.0, 115.7, 114.6 (Ar-*C*), 93.1 (CN), 64.8 (OCH_2).

IR (cm-1) $_{\nu}$(C-O-C) aliphatic ether: 1142.28, $_{\nu}$(C=O) amide: 1675.80, $_{\nu}$(CN): 2224.20, $_{\nu}$(C-H) aromatic: 2934.87, 3051.01, $_{\nu}$(N-H): 3282.25, 3333.42.

Elemental analysis: Anal, Calcd, for $C_{20}H_{15}N_5O_4$ (389.37): % C, 61.69; H, 3.88; N, 17.99; fund: % C, 59.83; H, 4.04; N, 17.35.

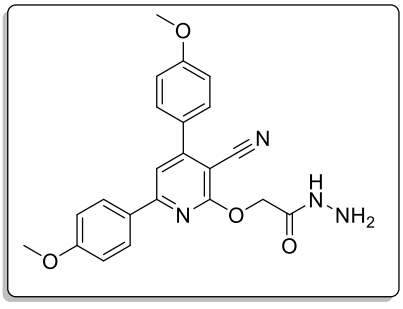
2-((3-cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl)oxy)acetohydrazide (3b)

white solid

M= 344.37 g/mol

Yield = 80 %

Mp: 227-228 °C



^{1}H NMR (DMSO-d6, 400 MHz) δ 9.43 (s, 1H, NH), 8.17 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.79 – 7.66 (m, 3H, Ar-H), 7.15 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.05 (d, *J* = 8.8 Hz, 2H, Ar-H), 4.99 (s, 2H, OCH_2), 4.33 (s, 2H, NH_2), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3).

^{13}C NMR (DMSO-d6, 400 MHz) δ 167.1 (CONH-NH2), 163.8, 161.8, 161.2, 157.0, 156.1, 130.6, 129.6, 129.4, 128.4, 116.2, 114.7, 114.7, 113.3 (Ar-C), 91.4 (CN), 64.6 (OCH_2), 55.8 (OCH_3), 55.8 (OCH_3).

IR (cm-1) $_{\nu}$(C-O-C) aliphatic ether: 1150.79, $_{\nu}$(C=O) amide: 1662.45, $_{\nu}$(CN): 2214.42, $_{\nu}$(C-H) aromatic: 2938.47, $_{\nu}$(N-H): 3282.26

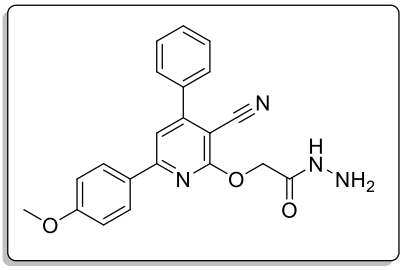
2-((3-cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl)oxy)acetohydrazide (3c)

White solid

M= 374.40 g/mol

Yield = 73 %

Mp: 209-211°C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.44 (s, 1H, NH), 8.19 (d, J = 8.9 Hz, 2H, Ar-H), 7.80 – 7.69 (m, 3H, Ar-H, pyridine C5-H), 7.64 – 7.55 (m, 3H, Ar-H), 7.06 (d, J = 8.9 Hz, 2H, Ar-H), 5.01 (s, 2H, OCH_2), 4.33 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3)

^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.1 (CONH-NH_2), 163.7, 161.9, 157.3, 156.6, 136.4, 130.5, 129.7, 129.3, 129.3, 129.0, 115.9, 114.7, 113.6 (Ar-*C*), 91.8 (CN), 64.7 (OCH_2), 55.8 (OCH_3).

IR (cm-1) $_{\nu}$(C-O-C) aliphatic ether: 1141.80, 1171.45, $_{\nu}$(C=O) amide: 1665.12, $_{\nu}$(CN): 2217.43, $_{\nu}$(N-H): 3281.79.

Elemental analysis: Anal, Calcd, for $C_{21}H_{18}N_4O_3$ (374.40): % C, 67.37; H, 4.85; N, 14.96; fund: % C, 68.15; H, 4.91; N, 14.90.

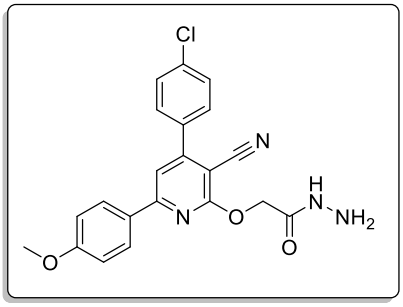
2-((4-(4-chlorophenyl)-3-cyano-6-(4-methoxyphenyl)pyridin-2-yl)oxy)acetohydrazide (3d)

Yellowish white solid

M= 408.84 g/mol

Yield = 71 %

Mp: 230-232 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.43 (s, 1H, NH), 8.18 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.80 – 7.65 (m, 5H, Ar-H, pyridine C5-H), 7.06 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.01 (s, 2H, OCH_2), 4.33 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.0 ($\underline{C}ONH\text{-}NH_2$), 163.6, 162.0, 157.4, 155.3, 135.5, 135.1, 130.9, 129.7, 129.3, 129.2, 115.7, 114.7, 113.5 (Ar-*C*), 91.8 ($\underline{C}N$), 64.7 ($O\underline{C}H_2$), 55.8 ($O\underline{C}H_3$).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether:1144.95, 1170.45, $_{\nu}$(C=O) amide: 1663.50, $_{\nu}$(CN): 2219.92, $_{\nu}$(C-H) aromatic: 2839.32, 2940.13, $_{\nu}$(N-H): 3288.38.

Elemental analysis: Anal, Calcd, for $C_{21}H_{17}ClN_4O_3$ (408.84): % C, 61.69; H, 4.19; N, 13.70; fund: % C, 61.34; H, 4.33; N, 13.49.

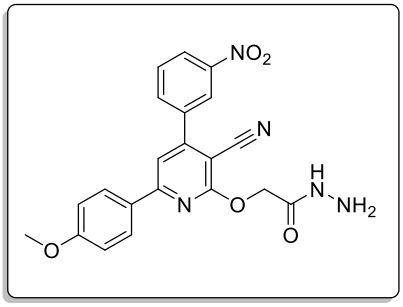
2-((3-cyano-6-(4-methoxyphenyl)-4-(3-nitrophenyl)pyridin-2-yl)oxy)acetohydrazide(3e)

Yellow solid

M= 419.40 g/mol

Yield = 89 %

Mp: 236-238 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.44 (s, 1H, NH), 8.59 (t, *J* = 2 Hz, 1H, Ar-H), 8.46 – 8.39 (m, 1H, Ar-H), 8.23 – 8.18 (m, 3H, Ar-H, pyridine C5-H), 7.92 (t, *J* = 7.9 Hz, 2H, Ar-H), 7.08 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.03 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.0 (CONH-NH_2), 163.6, 162.0, 157.7, 154.1, 148.3, 137.7, 135.7, 131.0, 129.8, 129.1, 125.1, 124.0, 115.6, 114.7, 113.8 (Ar-*C*), 92.0 (CN), 64.7 (OCH_2), 55.9 (OCH_3).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether:1150.79, 1171.99, $_{\nu}$(N-O) nitro: 1516.22, $_{\nu}$(C=O) amide: 1665.56, $_{\nu}$(CN): 2218.28, $_{\nu}$(C-H) aromatic: 2836.60,3016.19, $_{\nu}$(N-H): 3290.90.

Elemental analysis: Anal, Calcd, for $C_{21}H_{17}N_5O_5$ (419.40): % C, 60.14; H, 4.09; N, 16.70; fund: % C, 59.85; H, 4.16; N, 16.18.

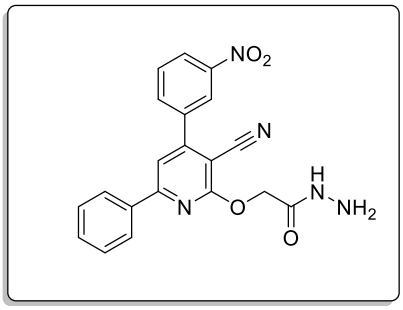
2-((3-cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl)oxy)acetohydrazide (3f)

Yellow solid

M= 389.37 g/mol

Yield = 69 %

Mp: 219-221°C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.45 (s, 1H, NH), 8.61 (t, *J* = 1.8 Hz, 1H, Ar-H), 8.45 (dd, *J* = 8.3, 1.5 Hz, 1H, Ar-H), 8.26 – 8.21 (m, 3H, Ar-H), 8.00 (s, 1H, pyridine C5-H), 7.96 – 7.88 (m, 1H, Ar-H), 7.56 – 7.53 (m, 3H, Ar-H), 5.06 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 166.9 (CONH-NH_2), 163.6, 157.8, 154.4, 148.4, 137.5, 136.7, 135.7, 131.4, 131.0, 129.4, 128.1, 125.2, 124.0, 115.4, 114.8 (Ar-*C*), 93.3 (CN), 64.8 (OCH_2).

IR (cm^{-1}) ν(C-O-C) aliphatic ether: 1158.46, ν(N-O) nitro: 1542.40, ν(C=O) amide: 1665.97, ν(CN): 2219.83, ν(C-H) aromatic: 2954.09, ν(N-H): 3299.03.

Elemental analysis: Anal, Calcd, for $C_{20}H_{15}N_5O_4$ (389.37): % C, 61.69; H, 3.88; N, 17.99; fund: % C, 59.83; H, 4.04; N, 17.35.

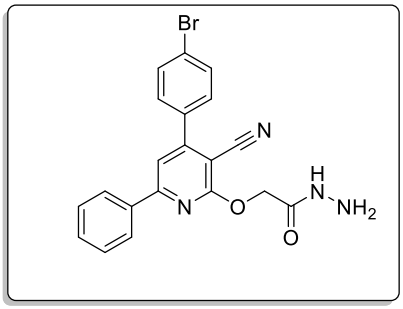
2-((4-(4-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)oxy)acetohydrazide (3g)

White solid

M= 389.37 g/mol

Yield = 75 %

Mp: 235-237 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.43 (s, 1H, NH), 8.24 – 8.18 (m, 2H, Ar-H), 7.88 – 7.80 (m, 3H, Ar-H, pyridine C5-H), 7.73 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.56 – 7.51 (m, 3H, Ar-H), 5.03 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.0 (CONH-NH_2), 163.7, 157.6, 155.6, 136.8, 135.3, 132.3, 131.3, 131.2, 129.4, 128.0, 124.3, 115.6, 114.5 (Ar-*C*), 92.9 (CN), 64.8 (OCH_2).

IR (cm^{-1}) ν(C-O-C) aliphatic ether: 1149.74, ν(C=O) amide: 1667.43, ν(CN): 2214.06, ν(N-H): 3260.62.

Elemental analysis: Anal, Calcd, for $C_{20}H_{15}BrN_4O_2$ (423.27): % C, 56.75; H, 3.57; N, 13.24; fund: % C, 57.14; H, 3.76; N, 13.34.

II.8.3 General procedure for the synthesis of the products (4a-g)

A catalytic amount of PTSOH 15% was added to a mixture of acetylacetone (1.1 mmol) and corresponding hydrazide **(3a-g)** (1 mmol) in 5.0 mL of 1,4 dioxane. The resulting mixture was stirred at reflux temperature from 1 h to 3h. After cooling, the reaction mixture was poured into ice-cold water. The solids obtained were filtered off, washed with ethanol, and recrystallized from ethanol/acetone to afford the pure products **(4a-g)**.

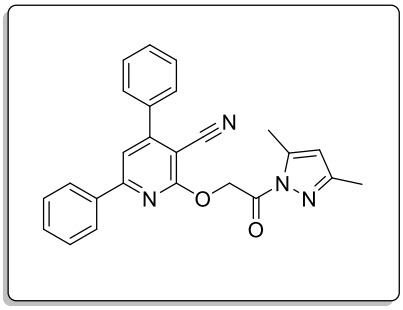
2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)-4,6-diphenylnicotinonitrile (4a)

White solid

M= 408.46 g/mol

Yield = 71 %

Mp = 229-231°C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.96 (d, J = 7.5 Hz, 2H, Ar-H), 7.88 (s, 1H, pyridine C5-H), 7.79 (dd, J = 6.4, 3.0 Hz, 2H, Ar-H), 7.66 – 7.58 (m, 3H, Ar-H), 7.47 (t, J = 7.3 Hz, 1H, Ar-H), 7.39 (t, J = 7.5 Hz, 2H, Ar-H), 6.34 (s, 1H, pyrazole C4-H), 5.94 (s, 2H, OCH_2), 2.45 (s, 3H, CH_3), 2.29 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 168.0 (CON), 163.4, 157.2, 157.2 (Ar-*C*), 153.2 (pyrazole C3), 144.1 (pyrazole C5), 136.6, 136.1, 131.2, 130.6, 129.4, 129.3, 129.1, 127.6, 115.5, 114.9 (Ar-*C*), 111.9 (pyrazole C4), 92.7 (CN), 65.2 (OCH_2), 14.05 (CH_3), 14.04 (CH_3).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether: 1149.09, $_{\nu}$(C=N): 1587.90, $_{\nu}$(C=O) amide: 1734.56, $_{\nu}$(CN): 2222.94, $_{\nu}$(C-H) aromatic: 2957.94.

Elemental analysis: Anal, Calcd, for $C_{25}H_{20}N_4O_2$ (408.46): % C, 73.51; H, 4.94; N, 13.72; fund: % C, 73.86; H, 5.09; N, 13.51.

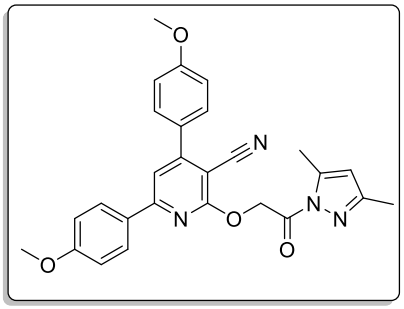
2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)-4,6-bis(4-methoxyphenyl)-nicotinonitrile (4b)

White solid

M= 468.51g/mol

Yield = 86 %

Mp = 157-159 °C



^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.90 (d, J = 8.9 Hz, 2H, Ar-H), 7.78 – 7.69 (m, 3H, Ar-H, pyridine C5-H), 7.15 (d, J = 8.8 Hz, 2H, Ar-H), 6.90 (d, J = 8.9 Hz, 2H, Ar-H), 6.33 (s, 1H, pyrazole C4-H), 5.90 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3), 2.29 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 168.1 (CON), 163.5, 161.8, 161.3, 156.8, 156.5 (Ar-*C*), 153.2 (pyrazole C3), 144.1 (pyrazole C5), 130.7, 129.3, 129.0, 128.3, 116.0, 114.7, 114.6, 113.6 (Ar-*C*), 111.9 (pyrazole C4), 91.0 (CN), 65.0 (OCH_2), 55.8 (OCH_3), 55.8 (OCH_3), 14.05 (CH_3), 14.03 (CH_3).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether:1152.19, 1173.92, $_{\nu}$(C=N):1582.76, $_{\nu}$(C=O) amide:1735.50, $_{\nu}$(CN):2224.14, $_{\nu}$(C-H) aromatic: 2841.11, 2956.85.

Elemental analysis: Anal, Calcd, for $C_{27}H_{24}N_4O_4$ (468.51): % C, 69.22; H, 5.16; N, 11.96; fund: % C, 69.54; H, 5.37; N, 11.84.

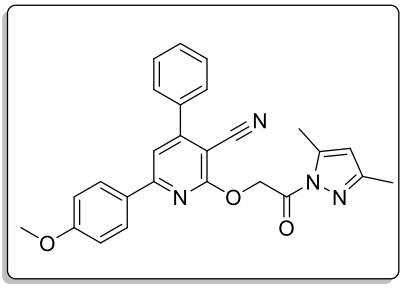
2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)-6-(4-methoxyphenyl)-4-phenylnicotinonitrile (4c)

White solid

M= 438.49 g/mol

Yield = 84 %

Mp = 176-178 °C



^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.92 (d, J = 8.9 Hz, 2H, Ar-H), 7.81 – 7.69 (m, 3H, Ar-H, pyridine C5-H), 7.62 – 7.55 (m, 3H, Ar-H), 6.91 (d, J = 8.9 Hz, 2H, Ar-H), 6.33 (s, 1H, pyrazole C4-H), 5.91 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3), 2.30 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 168.0 (CON), 163.3, 161.9, 157.1, 156.9 (Ar-*C*), 153.2 (pyrazole C3), 144.1 (pyrazole C5), 136.2, 130.5, 129.3, 129.2, 129.0, 128.9, 115.7, 114.6, 113.9 (Ar-*C*), 111.9 (pyrazole C4), 91.4 (CN), 65.1 (OCH_2), 55.8 (OCH_3), 14.05 (CH_3), 14.04 (CH_3).

IR (cm^{-1}) ν(C-O-C) aliphatic ether:1152.74, 1176.74, ν(C=N): 1585.92, ν(C=O) amide: 1786.81, ν(CN): 2222.72, ν(C-H) aromatic: 2842.87, 2956.98.

Elemental analysis: Anal, Calcd, for $C_{26}H_{22}N_4O_3$ (438.49): % C, 71.22; H, 5.06; N, 12.78; fund: % C, 70.98; H, 5.09; N, 12.72.

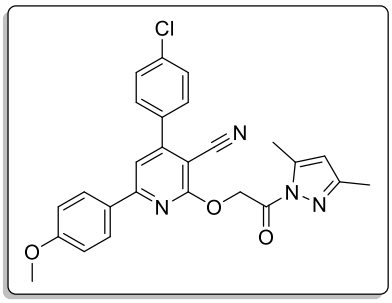
4-(4-chlorophenyl)-2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)-6-(4-methoxyphenyl)nicotinonitrile (4d)

White solid

M= 472.93 g/mol

Yield = 77 %

Mp = 167-169 °C



^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.92 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.81 – 7.79 (m, 3H, Ar-H, pyridine C5-H), 7.69 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.92 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.34 (s, 1H, pyrazole C4-H), 5.91 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3), 2.30 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 168.0 (CON), 163.3, 162.0, 157.2, 155.6 (Ar-*C*), 153.2 (pyrazole C3), 144.1 (pyrazole C5), 135.5, 135.0, 131.0, 129.3, 128.9, 128.9, 115.5, 114.6, 113.9 (Ar-*C*), 111.9 (pyrazole C4), 91.4 (CN), 65.1 (OCH_2), 55.8 (OCH_3), 14.11 (CH_3), 14.05 (CH_3).

IR (cm^{-1}) ν(C-O-C) aliphatic ether:1154.37, 1168.73, ν(C=N): 1579.29, ν(C=O) amide: 1728.14, ν(CN): 2215.87, ν(C-H) aromatic: 2944.75, 3028.59.

Elemental analysis: Anal, Calcd, for $C_{26}H_{21}ClN_4O_3$ (472.93): % C, 66.03; H, 4.48; N, 11.85; fund: % C, 66.14; H, 4.63; N, 11.97.

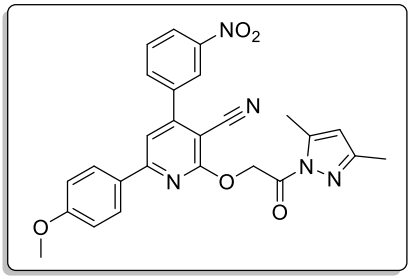
2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)-6-(4-methoxyphenyl)-4-(3-nitrophenyl)nicotinonitrile (4e)

Yellow solid

M= 472.93 g/mol

Yield = 69 %

Mp = 247-249 °C



^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.63 (t, *J* = 1.9 Hz, 1H, Ar-H), 8.47 – 8.41 (m, 1H, Ar-H), 8.27 – 8.20 (m, 1H, Ar-H), 7.98 – 7.88 (m, 4H, Ar-H, pyridine C5-H), 6.94 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.35 (s, 1H, pyrazole C4-H), 5.94 (s, 2H, OCH_2), 3.81 (s, 3H, OCH_3), 2.45 (s, 3H, CH_3), 2.30 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.9 (CON), 163.3, 162.1, 157.5, 154.5 (Ar-*C*), 153.2 (pyrazole C3), 148.4 (Ar-*C*), 144.1 (pyrazole C5), 137.6, 135.8, 131.0, 129.5, 128.8, 125.2, 124.1, 115.4, 114.7, 114.2 (Ar-*C*), 112.0 (pyrazole C4), 91.7 (CN), 65.2 (OCH_2), 55.9 (OCH_3), 14.0 (CH_3), 14.0 (CH_3).

IR (cm^{-1}) ν(C-O-C) aliphatic ether:1156.59, 1177.68, ν(C=N): 1585.53, ν(C=O) amide: 1742.04, ν(CN): 2223.53.

Elemental analysis: Anal, Calcd, for $C_{26}H_{21}N_5O_5$ (483.48): % C, 64.59; H, 4.38; N, 14.49; fund: % C, 64.80; H, 4.66; N, 14.12.

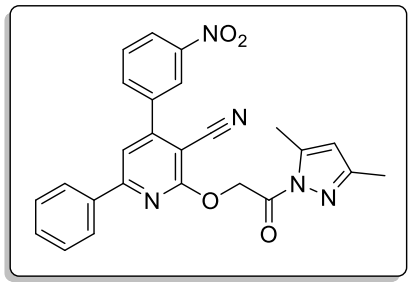
2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)-4-(3-nitrophenyl)-6-phenylnicotinonitrile (4f)

White solid

M= 453.46 g/mol

Yield = 73 %

Mp = 238-240 °C



^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.66 (t, *J* = 1.9 Hz, 1H, Ar-H), 8.48 – 8.44 (m, 1H, Ar-H), 8.29 – 8.26 (m, 1H, Ar-H), 8.04 (s, 1H, pyridine C5-H), 7.98 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.93 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.49 (d, *J* = 7.3 Hz, 1H, Ar-H), 7.41 (d, *J* = 7.7 Hz, 2H, Ar-H), 6.35 (s, 1H, , pyrazole C4-H), 5.96 (s, 2H, OCH_2), 2.45 (s, 3H, CH_3), 2.30 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.9 (CON), 163.3, 157.6, 154.8 (Ar-*C*), 153.2 (pyrazole C3), 148.4 (Ar-*C*), 144.1 (pyrazole C5), 137.5, 136.4, 135.8, 131.4, 131.0, 129.3, 127.7, 125.3, 124.2, 115.2, 115.21 (Ar-*C*), 112.0 (pyrazole C4), 92.9 (CN), 65.3 (OCH_2), 14.0 (CH_3), 14.0 (CH_3).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether: 1152.64, $_{\nu}$(C=N): 1591.10, $_{\nu}$(C=O) amide: 1729.17, $_{\nu}$(CN): 2225.07, $_{\nu}$(C-H) aromatic: 3074.65.

Elemental analysis: Anal, Calcd, for $C_{25}H_{19}N_5O_4$ (453.46): % C, 66.22; H, 4.22; N, 15.44; fund: % C, 65.99; H, 4.46; N, 15.75.

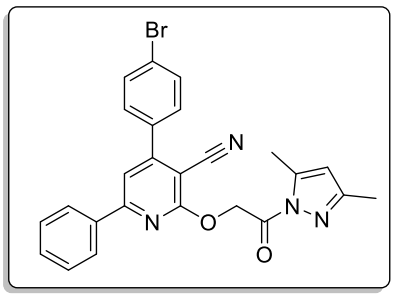
4-(4-bromophenyl)-2-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethoxy)-6-phenylnicotinonitrile (4g)

White solid

M= 487.36 g/mol

Yield = 65 %

Mp = 231-233 °C



^{1}H NMR (600 MHz, DMSO-d_6): 7.98 – 7.70 (m, 7H, Ar-H, pyridine C5-H), 7.58 – 7.25 (m, 3H, Ar-H), 6.34 (s, 1H, pyrazole C4-H), 5.94 (s, 2H, OCH_2), 2.44 (s, 3H, CH_3), 2.29 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 168.0 (CON), 163.3, 157.4, 156.0 (Ar-*C*), 153.2 (pyrazole C3), 144.1 (pyrazole C5), 136.5, 135.2, 132.3, 131.3, 131.3, 129.3, 127.6, 124.5, 115.3, 114.8 (Ar-*C*), 112.0 (pyrazole C4), 92.6 (CN), 65.3 (OCH_2), 14.05 (CH_3), 14.04 (CH_3).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether: 1151.60, $_{\nu}$(C=N): 1582.10, $_{\nu}$(C=O) amide: 1732.91, $_{\nu}$(CN): 2225.54, $_{\nu}$(C-H) aromatic: 2946.33.

Elemental analysis: Anal, Calcd, for $C_{25}H_{19}BrN_4O_2$ (487.36): % C, 61.61; H, 3.93; N, 11.50; fund: % C, 61.82; H, 4.15; N, 11.60.

II.8.4 General procedure for the synthesis of the product (5a-f)

To a mixture of corresponding hydrazide **(3a-f)** (1 mmol) and triethyl orthoformate (1 mL) in dry dioxane (4 mL) was added a catalytic amount of PTSOH 5%. The reaction mixture was refluxed for 1-2 h under an argon atmosphere. The excess of solvent was removed under reduced pressure, the residue was triturated with ethyl ether. The solid formed was filtered off and purified by column chromatography on silica gel (hexane 75% / ethyl acetate 25%).

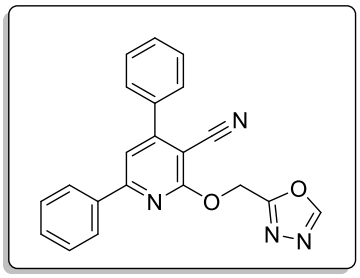
2-((1,3,4-oxadiazol-2-yl)methoxy)-4,6-diphenylnicotinonitrile (5a)

White solid

M= 354.37g/mol

Yield = 73 %

Mp = 192-194 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (s, 1H, oxadiazole C5-H), 8.05 – 8.03 (m, 2H, Ar-H), 7.67 – 7.65 (m, 2H, Ar-H), 7.58 (s, 1H, pyridine C5-H), 7.56 – 7.49 (m, 6H, Ar-H), 5.90 (s, 2H, OCH_2).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 162.8 (oxadiazole C2), 162.4, 157.9, 157.3 (Ar-*C*), 153.6 (oxadiazole C5), 136.5, 135.9, 130.9, 130.3, 129.1, 129.0, 128.3, 127.3, 114.9, 114.7 (Ar-*C*), 93.3 (CN), 57.9 (OCH_2).

IR (cm^{-1}) ν(C-O-C) aliphatic ether: 1139.42, ν(C=N): 1547.27,1590.28, ν(CN): 2221.54, ν(C-H) aromatic: 2923.78.

Elemental analysis: Anal, Calcd, for $C_{21}H_{14}N_4O_2$ (354.37): % C, 71.18; H, 3.98; N, 15.81; fund: % C, 70.96; H, 3.69; N, 15.73.

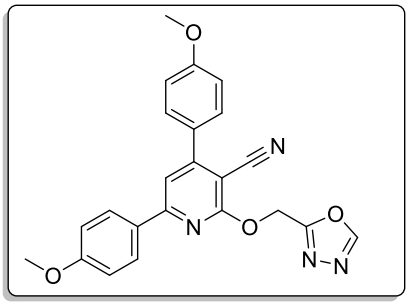
2-((1,3,4-oxadiazol-2-yl)methoxy)-4,6-bis(4-methoxyphenyl)nicotinonitrile (5b)

Yellow solid

M= 414.42 g/mol

Yield = 80 %

Mp =179-181 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.46 (s, 1H, oxadiazole C5-H), 8.00 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.62 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.45 (s, 1H, pyridine C5-H), 7.04 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.99 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.85 (s, 2H, OCH_2), 3.87 (s, 6H,2OCH_3).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 162.9 (oxadiazole C2), 162.5, 161.9, 161.2, 157.4, 156.6 (Ar-*C*), 153.6 (oxadiazole C5), 129.8, 129.1, 128.9, 128.2, 115.3, 114.5, 114.3, 113.5 (Ar-*C*), 91.7 (CN), 57.8 (OCH_2), 55.4 (2OCH_3).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether: 1140.32, 1173.43, $_{\nu}$(C=N): 1538.73, 1585.59, $_{\nu}$(CN): 2214.00, $_{\nu}$(C-H) aromatic: 2851.14, 2923.10.

Elemental analysis: Anal, Calcd, for $C_{23}H_{18}N_4O_4$ (414.42): % C, 66.66; H, 4.38; N, 13.52; fund: % C, 66.41; H, 4.57; N, 13.48.

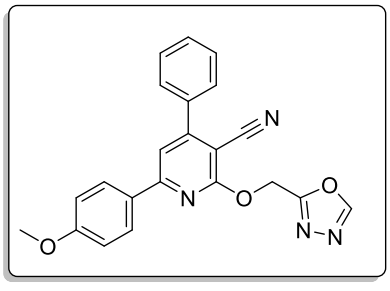
2-((1,3,4-oxadiazol-2-yl)methoxy)-6-(4-methoxyphenyl)-4-phenylnicotinonitrile (5c)

White solid

M= 384.40 g/mol

Yield = 71 %

Mp = 162-164 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.46 (s, 1H, oxadiazole C5-H), 8.01 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.65 – 7.63 (m, 2H, Ar-H), 7.54 – 7.52 (m, 3H, Ar-H), 7.49 (s, 1H, pyridine C5-H), 7.00 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.87 (s, 2H, OCH_2), 3.88 (s, 3H, OCH_3).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 162.8 (oxadiazole C2), 162.5, 161.9, 157.6, 157.1 (Ar-*C*), 153.6 (oxadiazole C5), 136.0, 130.1, 129.0, 129.0, 128.9, 128.3, 114.9, 114.4, 113.8 (Ar-*C*), 92.1 (CN), 57.9 (OCH_2), 55.4 (OCH_3).

IR (cm^{-1}) ν(C-O-C) aliphatic ether: 1135.25, 1177.04, ν(C=N): 1540.43, 1587.87, ν(CN): 2213.65, ν(C-H) aromatic: 3011.34.

Elemental analysis: Anal, Calcd, for $C_{22}H_{16}N_4O_3$ (384.40): % C, 68.74; H, 4.20; N, 14.58; fund: % C, 68.49; H, 4.36; N, 14.31.

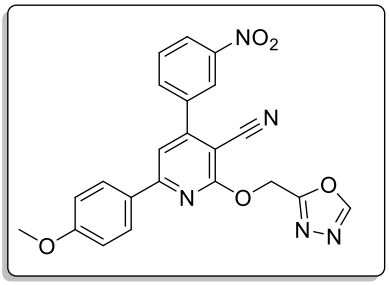
2-((1,3,4-oxadiazol-2-yl)methoxy)-6-(4-methoxyphenyl)-4-(3-nitrophenyl)nicotinonitrile (5d)

Yellow solid

M= 429.39 g/mol

Yield = 69 %

Mp = 164-166 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.48 (s, 1H, oxadiazole C5-H), 8.47 – 8.46 (m, 1H, Ar-H), 8.40 – 8.38 (m, 1H, Ar-H), 8.04 – 8.00 (m, 3H, Ar-H), 7.75 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.51 (s, 1H, pyridine C5-H), 7.01 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.89 (s, 2H, OCH_2), 3.89 (s, 3H, OCH_3).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 162.84 (oxadiazole C2), 162.3, 162.2, 158.5, 154.3 (Ar-*C*), 153.7 (oxadiazole C5), 148.5, 137.6, 134.3, 130.3, 129.1, 128.5, 124.8, 123.4, 114.5, 114.2, 113.5 (Ar-*C*), 92.1 (CN), 58.0 (OCH_2), 55.5 (OCH_3).

IR (cm^{-1}) ν(C-O-C) aliphatic ether: 1138.43, 1174.84, ν(C=N): 1527.01, 1579.65, ν(CN): 2215.52, ν(C-H) aromatic: 3084.97.

Elemental analysis: Anal, Calcd, for $C_{22}H_{15}N_5O_5$ (429.39): % C, 61.54; H, 3.52; 16.31; fund: % C, 61.47; H, 3.71; N, 15.95.

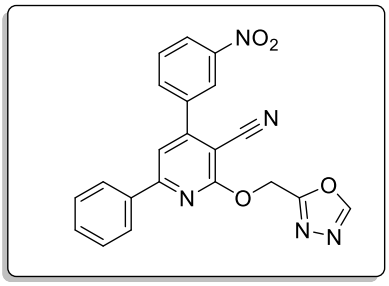
2-((1,3,4-oxadiazol-2-yl)methoxy)-4-(3-nitrophenyl)-6-phenylnicotinonitrile (5e)

Pell Yellow solid

M= 399.37g/mol

Yield = 67 %

Mp = 154-156 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.49 – 8.48 (m, 2H, oxadiazole C5-H, Ar-H), 8.42 – 8.38 (m, 1H, Ar-H), 8.07 – 8.02 (m, 3H, Ar-H), 7.77 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.60 (s, 1H, , pyridine C5-H), 7.53 – 7.51 (m, 3H, Ar-H), 5.92 (s, 2H,OCH_2).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 162.89 (oxadiazole C2), 162.16, 158.87, 154.61 (Ar-*C*), 153.74 (oxadiazole C5), 148.60, 137.41, 136.08, 134.36, 131.36, 130.37, 129.20, 127.49, 124.94, 123.49, 114.60, 114.04 (Ar-*C*), 93.37 (CN), 58.13 (OCH_2).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether: 1148.04 cm^{-1}, $_{\nu}$(C=N): 1526.97, 1583.47 cm^{-1}, $_{\nu}$(CN): 2220.49 cm^{-1}, $_{\nu}$(C-H) aromatic: 2923.24, 3087.14 cm^{-1}.

Elemental analysis: Anal, Calcd, for $C_{21}H_{13}N_5O_4$ (399.37): % C, 63.16; H, 3.28; 17.54; fund: % C, 63.24; H, 3.53; N, 17.32.

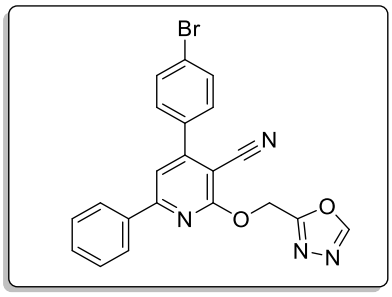
2-((1,3,4-oxadiazol-2-yl)methoxy)-4-(4-bromophenyl)-6-phenylnicotinonitrile (5f)

White solid

M= 433.27 g/mol

Yield = 76 %

Mp = 212-214 °C



^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (s, 1H, oxadiazole C5-H), 8.04 – 8.02 (m, 2H, Ar-H), 7.69 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.54 – 7.50 (m, 6H, Ar-H, pyridine C5-H), 5.89 (s, 2H, OCH_2).

^{13}C NMR ($CDCl_3$, 400 MHz) δ 162.9 (oxadiazole C2), 162.3, 158.2, 156.0 (Ar-*C*), 153.6 (oxadiazole C5), 136.3, 134.7, 132.4, 131.0, 129.9, 129.1, 127.4, 125.0, 114.5, 114.4 (Ar-*C*), 93.1 (CN), 58.0 (OCH_2).

IR (cm^{-1}) $_v$(C-O-C) aliphatic ether: 1142.78, $_v$(C=N): 1541.16, 1595.67, $_v$(CN): 2220.45, $_v$(C-H) aromatic: 3134.48.

Elemental analysis: Anal, Calcd, for $C_{21}H_{13}BrN_4O_2$ (433.27): % C, 58.22; H, 3.02; 12.93; fund: % C, 58.48; H, 3.22; N, 12.79.

II.8.5 General procedure for the synthesis of the products (6 a-i)

A mixture of isatin derivative (0.01 mol) and hydrazides **(3a-g)** (0.01 mol) in dioxane (10 ml) containing 10% PTSOH as catalyst was stirred at room temperature for 3 h. The resulting solid was filtered, washed with ethanol, and recrystallized from ethanol/DMF.

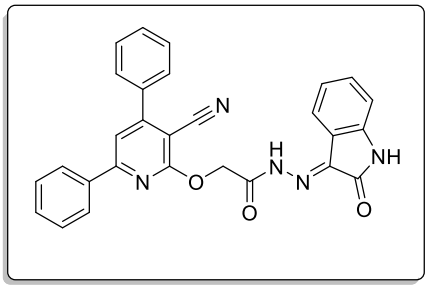
(E)-2-((3-cyano-4,6-diphenylpyridin-2-yl)oxy)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (6a)

Yellow solid

M= 473.49 g/mol

Yield = 70 %

Mp = 243-245 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.67 (s, 1H, NH), 10.90 (s, 1H, NH $_{isatin}$), 8.22 – 8.10 (m, 3H, Ar-H), 7.88 (s, 1H, pyridine C5-H), 7.83 – 7.77 (m, 2H, Ar-H), 7.67 – 7.58 (m, 3H, Ar-H), 7.48 – 7.38 (m, 2H, Ar-H), 7.32 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.08 (t, *J* = 7.5 Hz, 1H, Ar-H), 6.96 (d, *J* = 7.8 Hz, 1H, Ar-H), 5.70 (s, 2H, OCH_2).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.9 (CONHN), 163.6 (CONH $_{isatin}$), 157.4, 157.1, 144.4, 136.7, 136.1 (Ar-*C*), 133.3 (C=N$_{imine}$), 131.2, 130.6, 129.3, 129.2, 129.1, 127.9, 126.7, 122.2, 115.6, 115.6, 114.8, 111.2 (Ar-*C*), 92.7 (CN), 66.8 (OCH_2).

IR (cm^{-1}) $_v$(C-O-C) aliphatic ether: 1146.83 cm^{-1}, $_v$(C=N) imine: 1608.97, $_v$(C=O) amide: 1717.75, $_v$(CN): 2228.78, $_v$(C-H) aromatic: 2837.66, $_v$(N-H): 3147.71.

Elemental analysis: Anal. Calcd. for C_{28} H_{19} N_5 O_3 (473.49): % C, 71.03; H, 4.04; N, 14.79. Found: C, 70.87; H, 4.04; N, 14.82.

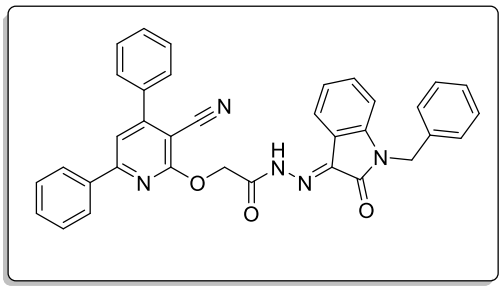
(E)-N'-(1-benzyl-2-oxoindolin-3-ylidene)-2-((3-cyano-4,6-diphenylpyridin-2-yl)oxy)acetohydrazide (6b)

Yellow solid

M= 563.62 g/mol

Yield = 68 %

Mp = 217-219 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.79 (s, 1H, NH), 8.27 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.16 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.89 (s, 1H, pyridine C5-H), 7.84 – 7.77 (m, 2H, Ar-H), 7.67 – 7.60 (m, 3H, Ar-H), 7.48 – 7.26 (m, 9H, Ar-H), 7.14 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.09 (d, *J* = 8.0 Hz, 1H, Ar-H), 5.70 (s, 2H, OCH_2), 5.01 (s, 2H,NCH2).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.8 (CONHN), 163.6 ($CONCH_{2\ isatin}$), 157.4, 157.1, 144.4, 136.7, 136.6, 136.1 (Ar-*C*), 133.1 ($C=N_{imine}$), 131.2, 130.6, 129.4, 129.2, 129.2, 129.1, 128.0, 127.9, 127.7, 126.6, 122.9, 115.6, 115.2, 114.8, 110.4 (Ar-*C*), 92.7 (CN), 43.2 (NCH_2).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether: 1143.54, $_{\nu}$(C=N) imine: 1675.58, $_{\nu}$(C=O) amide: 1694.48, 1724.92, $_{\nu}$(CN): 2225.44, $_{\nu}$(N-H): 3198.56.

Elemental analysis: Anal. Calcd. for $C_{35}H_{25}N_5O_3$ (563.62): % C, 74.59; H, 4.47; N, 12.43. Found: C, 74.47; H, 4.46; N, 12.48.

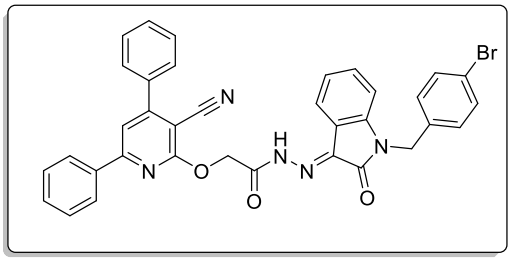
(E)-N'-(1-(4-bromobenzyl)-2-oxoindolin-3-ylidene)-2-((3-cyano-4,6-diphenylpyridin-2-yl)oxy)acetohydrazide (6c)

Yellow solid

M= 642.51g/mol

Yield = 71 %

Mp = 228-230 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.81 (s, 1H, NH), 8.27 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.19 – 8.11 (m, 2H, Ar-H), 7.89 (s, 1H, pyridine C5-H), 7.83 – 7.77 (m, 2H, Ar-H), 7.65 – 7.60 (m, 3H, Ar-H), 7.55 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.49 – 7.27 (m, 6H, Ar-H), 7.15 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.08 (d, *J* = 8.0 Hz, 1H, Ar-H), 5.73 (s, 2H,OCH_2), 4.98 (s, 2H, NCH_2).

IR (cm^{-1}) $_\nu$(C-O-C) aliphatic ether: 1143.67, $_\nu$(C=N) imine: 1672.08, $_\nu$(C=O) amide: 1690.59, 1725.73, $_\nu$(CN): 2226.51, $_\nu$(C-H) aromatic: 2982.40, $_\nu$(N-H): 3220.55.

Elemental analysis: Anal. Calcd. for $C_{35}H_{24}BrN_5O_3$ (642.51): % C, 65.43; H, 3.77; N, 12.44. Found: C, 65.42; H, 4.46; N, 12.48.

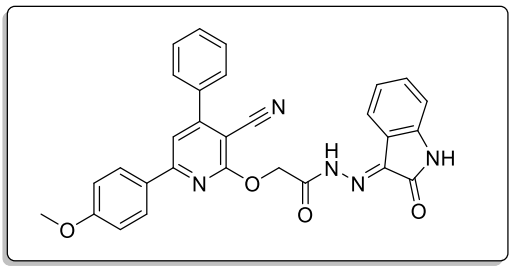
(E)-2-((3-cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl)oxy)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (6d)

Yellow solid

M= 503.52 g/mol

Yield = 80 %

Mp = 230-232°C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.67 (s, 1H, NH), 10.92 (s, 1H,NH_{isatin}), 8.21 (d, *J* = 7.7 Hz, 1H, Ar-H), 8.15 – 8.05 (m, 2H, Ar-H), 7.82 – 7.72 (m, 3H, Ar-H, pyridine C5-H), 7.65 – 7.57 (m, 3H, Ar-H), 7.44 (t, *J* = 7.7 Hz, 1H, Ar-H), 7.10 (t, *J* = 7.2 Hz, 1H, Ar-H), 6.97 (d, *J* = 7.8 Hz, 1H, Ar-H), 6.79 (d, *J* = 8.8 Hz, 2H, Ar-H), 5.67 (s, 2H,OCH_2), 3.71 (s, 3H, OCH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.9 (CONHN), 163.5 (CONH $_{isatin}$), 161.8, 157.2, 156.8, 144.4, 136.3 (Ar-*C*), 133.3 (C=N_{imine}), 130.5, 129.5, 129.3, 129.1, 126.8, 122.2, 115.8, 115.6, 114.5, 113.8, 111.2 (Ar-*C*), 91.5 (CN), 66.8 (OCH_2), 55.7 (OCH_3).

IR (cm^{-1}) $_v$(C-O-C) aliphatic ether: 1146.56, 1170.17, $_v$(C=N) imine: 1584.37, $_v$(C=O) amide: 1715.81, $_v$(CN): 2222.83, $_v$(C-H) aromatic: 2839.13, $_v$(N-H): 3170.35.

Elemental analysis: Anal. Calcd. for $C_{29}H_{21}N_5O_4$ (503.52): % C, 69.18; H, 4.20; N, 13.91. Found: C, 68.95; H, 4.21; N, 14.03.

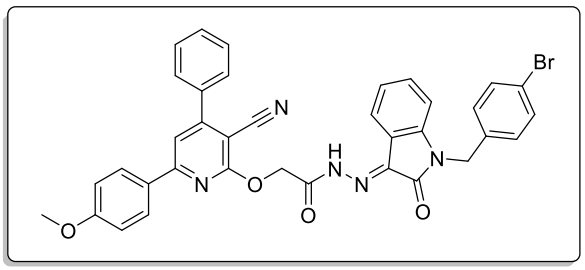
(E)-N'-(1-(4-bromobenzyl)-2-oxoindolin-3-ylidene)-2-((3-cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl)oxy)acetohydrazide (6e)

Yellow solid

M= 672.54 g/mol

Yield = 71 %

Mp = 212-214 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.80 (s, 1H, NH), 8.28 (d, *J* = 7.7 Hz, 1H, Ar-H), 8.14 – 8.06 (m, 2H, Ar-H), 7.82 – 7.74 (m, 3H, Ar-H, pyridine C5-H), 7.64 – 7.59 (m, 3H, Ar-H), 7.53 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.46 (t, *J* = 7.8 Hz, 1H, Ar-H), 7.33 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.16 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.09 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.77 (d, *J* = 8.8 Hz, 2H, Ar-H), 5.67 (s, 2H, OCH_2), 4.98 (s, 2H,NCH_2), 3.66 (s, 3H, OCH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.8 (CONHN), 163.5 ($CONCH_{2isatin}$), 161.8, 157.2, 156.9, 144.2, 136.3, 136.0 (Ar-*C*), 133.2 (C=N_{imine}), 132.0, 130.5, 130.0, 129.6, 129.3, 129.1, 129.1, 126.7, 123.0, 121.1, 115.8, 115.2, 114.5, 113.8, 110.3 (Ar-*C*), 91.5 (CN), 55.6 (OCH_3), 42.6(NCH_2).

IR (cm^{-1}) $_v$(C-O-C) aliphatic ether: 1142.76, 1175.30, $_v$(C=N) imine: 1675.11, $_v$(C=O) amide: 1693.27, 1724.24, $_v$(CN): 2224.31, $_v$(C-H) aromatic: 2974.03, $_v$(N-H): 3143.95.

Elemental analysis: Anal. Calcd. For $C_{36}H_{26}BrN_5O_4$ (672.54): % C, 64.29; H, 3.90; N, 10.41. Found: 64.21; H, 3.89; N, 10.53.

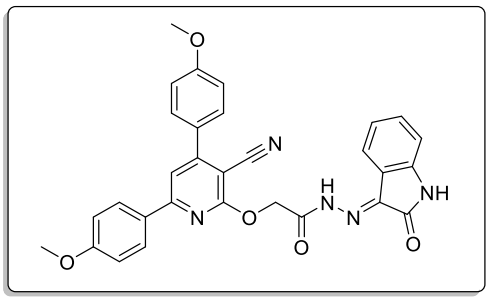
(E)-2-((3-cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl)oxy)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (6f)

Yellow solid

M= 533.54 g/mol

Yield = 70 %

Mp = 240-242 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.68 (s, 1H,NH), 10.93 (s, 1H, NH $_{isatin}$), 8.21 (d, *J* = 7.7 Hz, 1H, Ar-H), 8.14 – 8.03 (m, 2H, Ar-H), 7.80 – 7.70 (m, 3H, Ar-H, pyridine C5-H), 7.44 (t, *J* = 7.8 Hz, 1H, Ar-H), 7.16 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.10 (d, *J* = 7.3 Hz, 1H, Ar-H), 6.96 (d, *J* = 7.8 Hz, 1H, Ar-H), 6.78 (d, *J* = 8.9 Hz, 2H, Ar-H), 5.66 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.9 (CONHN), 163.6 ($\underline{C}ONH_{isatin}$), 161.7, 161.3, 157.0, 156.4, 144.4 (Ar-*C*), 133.3 ($C=N_{imine}$), 130.7, 129.5, 129.2, 128.5, 128.3, 126.7, 125.9, 122.7, 116.1, 115.6, 114.7, 114.5, 113.4, 111.2 (Ar-*C*), 91.0 (CN), 67.4 (OCH_2), 55.8 (OCH_3), 55.7 (OCH_3).

IR (cm^{-1}) $_{v}$(C=N) imine: 1603.46, $_{v}$(C=O) amide: 1721.22, 1750.23, $_{v}$(CN): 2226.39, $_{v}$(C-H) aromatic: 2946.79, $_{v}$(N-H): 3301.03, 3393.82.

Elemental analysis: Anal. Calcd. For $C_{30}H_{23}N_5O_5$ (533.54): % C, 67.54; H, 4.35; N, 13.13. Found: C, 67.47; H, 4.35; N, 13.19.

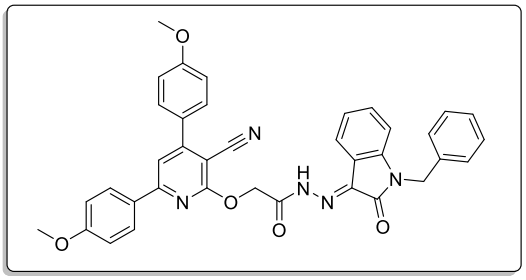
N'-(1-benzyl-2-oxoindolin-3-ylidene)-2-((3-cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl)oxy)acetohydrazide (6g)

Yellow solid

M= 623.67 g/mol

Yield = 68 %

Mp = 168-170 °C



^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.38 (s, 1H,NH *cis conformer*), 12.61 (s, 1H, NH *trans conformer*), 8.20 – 8.04 (m, 2H, Ar-H), 7.79 – 7.57 (m, 4H, Ar-H, pyridine C5-H), 7.46 – 7.24 (m, 6H, Ar-H), 7.21 – 7.02 (m, 4H, Ar-H), 7.02 – 6.77 (m, 2H, Ar-H), 5.78 (s, 2H,OCH_2 *trans conformer*), 5.35 (s, 2H, ,OCH_2 *cis conformer*), 5.00 (s, 2H, NCH_2), 3.86 (s, 3H,OCH_3), 3.57 (s, 3H, OCH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 161.8 (CONHN), 161.3 ($CONCH_2$ isatin), 161.1, 157.0, 156.5, 143.2, 136.0 (Ar-*C*), 132.2 ($C=N_{imine}$), 130.7, 129.5, 129.2, 128.3, 128.1, 127.8, 123.8, 121.2, 119.5, 115.9, 114.7, 114.6, 113.7, 111.0 (Ar-*C*), 91.2 (CN), 66.8 (OCH_2), 55.9 (OCH_3), 55.7 (OCH_3), 43.0 (NCH_2).

IR (cm^{-1}) $_{v}$(C-O-C) aliphatic ether:1142.92, 1170.32, $_{v}$(C=N) imine: 1610.41, $_{v}$(C=O) amide: 1698.07, 1729.42, $_{v}$(CN): 2215.51, $_{v}$(C-H) aromatic: 2963.10, $_{v}$(N-H): 3225.70.

Elemental analysis: Anal. Calcd. For $C_{37}H_{29}N_5O_5$ (623.67): % C, 71.26; H, 4.69; N, 11.23. Found: C, 71.34; H, 4.70; N, 11.39.

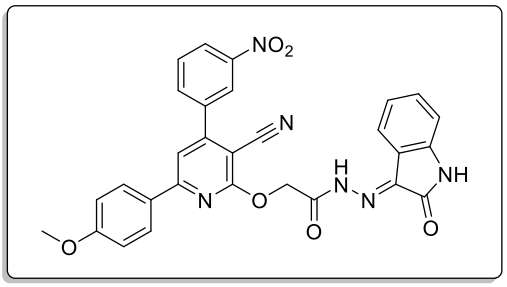
(E)-2-((3-cyano-6-(4-methoxyphenyl)-4-(3-nitrophenyl)pyridin-2-yl)oxy)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (6h)

Yellow solid

M= 548.52 g/mol

Yield = 66 %

Mp = 224-226 °C



^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.66 (s, 1H, NH), 10.90 (s, 1H, NH $_{isatin}$), 8.62 (m, 1H, Ar-H), 8.47 – 8.40 (m, 1H, Ar-H), 8.29 – 8.17 (m, 2H, Ar-H), 8.16 – 8.07 (m, 2H, Ar-H), 7.96 – 7.86 (m, 2H, Ar-H, pyridine C5-H), 7.44 (t, *J* = 7.7 Hz, 1H, Ar-H), 7.10 (t, *J* = 7.5 Hz, 1H, Ar-H), 6.96 (d, *J* = 7.9 Hz, 1H, Ar-H), 6.81 (d, *J* = 8.8 Hz, 2H, Ar-H), 5.66 (s, 2H, OCH_2), 3.72 (s, 3H, OCH_3).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.9 (CONHN), 163.4 (CONH $_{isatin}$), 162.0, 157.6, 154.4, 148.4, 144.4, 137.6, 135.8 (Ar-*C*), 133.3 (C=N $_{imine}$), 130.9, 129.7, 128.9, 126.7, 125.1, 124.0, 122.2, 115.6, 115.5, 114.5, 114.0, 111.2 (Ar-*C*), 91.7 (CN), 64.3 (OCH_2), 55.7 (OCH_3).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether:1148.39, 1173.92, $_{\nu}$(N-O) nitro:1536.99, $_{\nu}$(C=N) imine: 1606.06, $_{\nu}$(C=O) amide:1669.94, 1722.80, $_{\nu}$(CN): 2225.58, $_{\nu}$(C-H) aromatic: 2967.74, $_{\nu}$(N-H): 3089.46.

Elemental analysis: Anal. Calcd. For $C_{29}H_{20}N_6O_6$ (548.52): % C, 63.50; H, 3.68; N, 15.32. Found: C, 63.43; H, 3.67; N, 15.35.

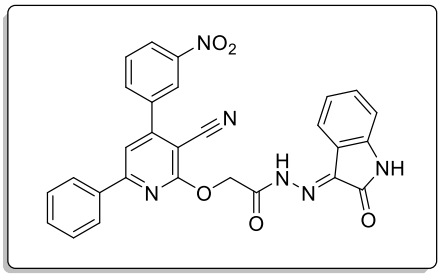
2-((3-cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl)oxy)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (6i)

Yellow solid

M= 518.49 g/mol

Yield = 69 %

Mp = 280-282 °C



^{1}H NMR (DMSO-d_6, 600 MHz) δ 13.49 (s, 1H, NH *cis conformer*), 12.70 (s, 1H, NH *trans conformer*), 11.35 (s, 1H, NH_{isatin}), 8.65 – 8.61 (m, 1H, Ar-H), 8.44 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.26 (d, *J* = 7.8 Hz, 1H, Ar-H), 8.22 – 7.95 (m, 3H,Ar-H, pyridine C5-H), 7.92 (t, *J* = 7.9 Hz, 1H, Ar-H), 7.64 – 7.32 (m, 5H, Ar-H), 6.99 (m, 2H, Ar-H), 5.80 (s, 2H, OCH_2 *trans conformer*), 5.39 (s, 2H OCH_2 *cis conformer*).

^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.4 (CONHN), 163.0 ($CONH_{isatin}$), 157.8, 154.7, 148.4, 143.0, 137.4, 136.5, 135.8 (Ar-*C*), 132.4 ($C=N_{imine}$), 131.4, 131.0, 129.3, 128.0, 125.3, 124.1, 123.1, 121.4, 120.0, 115.26, 115.21, 115.1, 111.7 (Ar-*C*), 93.0 (CN), 63.6 (OCH_2).

IR (cm^{-1}) $_{\nu}$(C-O-C) aliphatic ether:1141.09, $_{\nu}$(N-O) nitro:1526.53, $_{\nu}$(C=N) imine: 1617.90, $_{\nu}$(C=O) amide: 1691.35, 1729.31, $_{\nu}$(CN): 2226.86, $_{\nu}$(N-H): 3204.30.

Elemental analysis: Anal. Calcd. For $C_{28}H_{18}N_6O_5$ (518.49): % C, 64.86; H, 3.50; N, 16.21. Found: C, 64.77; H, 3.48; N, 16.26.

Chapter 03

Biological evaluation

III.1 Material and methods

III.1.1 Cell lines and culture conditions

The human cancer lines, ovarian cancer (A-2780), breast cancer (MCF-7), and the human colon carcinoma cell line (Caco-2) were used for in vitro screening experiments. The cell lines were purchased from the ATCC. All cells were fed in 25- and 75-cm^2 flasks with the RPMI-1640 medium (containing 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin) 2 days apart. In cells with a carbon dioxide (5%CO_2) incubator (Panasonic), the cells maintained at 37°C and in a humid environment were separated from the flasks using a solution of trypsin–EDTA (Sigma-Aldrich) when confluent. The cells' viability was determined using 0.4% trypan blue, and experiments were started when the viability was above 90%.

III.1.2 Statistical analysis

The IBM SPSS Statistics 24.0 (Windows) package program was used in the analysis. The Shapiro–Wilk test evaluated conformity to normal distribution. Intergroup comparisons of quantitative variables were measured by Kruskal–Wallis H test. When significant statistical differences were determined between groups, multiple comparisons were made with the Bonferroni correction Mann–Whitney U test. All p values <0.05 were considered statistically significant. Log IC_{50} values of melatonin and agomelatine were calculated using GraphPad Prism 6 program on a computer based on the MTT results obtained from the experiments.

III.1.3 MTT assay

MTT assay is one of the most frequent cell viability tests, also known as a colorimetric assay, to assess cell metabolic activity in microcapsules. It is based on reducing yellow-colored MTT into a DMSO-soluble purple color formazan due to NAD(P)H-dependent cellular oxidoreductase enzymes in the active cells (Figure III. 01). The Higher intensity of purple color represents higher cell viability, while the purple color intensity's decrease indicates low cell viability.

The antitumor activities of the novel substances were evaluated by MTT assay [137]. Cells were removed using a trypsin–EDTA solution from flasks and counted by a hemocytometer to determine cytotoxic effects. Furthermore, 15×10^3 cells per well were plated in 96-well plates (Figure III. 02), including 200 µl of the RPMI-1640 medium. Cells were incubated at 37°C in a CO_2 incubator for 24 h to adhere to a 96-well plate base. When the incubation ended, concentrations of 0.1, 1, 10, and 100 µM of the novel substances were added to the wells in which the cells were contained. Incubation with cancer cells for 24 h at 37°C in a CO_2 incubator was performed to determine the effects of different concentrations of the novel substances on cell viability for 24 h. When the incubation was over, 0.5 mg/ml of MTT solution in sterile phosphate-buffered solution was prepared and added to 96-well

plates. After the addition of MTT, the plates were incubated again for 3 h. After this time, incubation was stopped by adding DMSO to the wells, and the optical densities of the cells in the plates were read on a spectrophotometer (Synergy HTX) at a wavelength of 550 nm [138]. The cell viability percentage was calculated by proportioning the absorbance values obtained from the novel substances applied wells to that of the control group. MTT trials were performed 10 times in triplicate on different days (Figure III. 03), and the log IC_{50} values of the applied compounds were calculated based on MTT results using the GraphPad Prism 6 program on a computer.

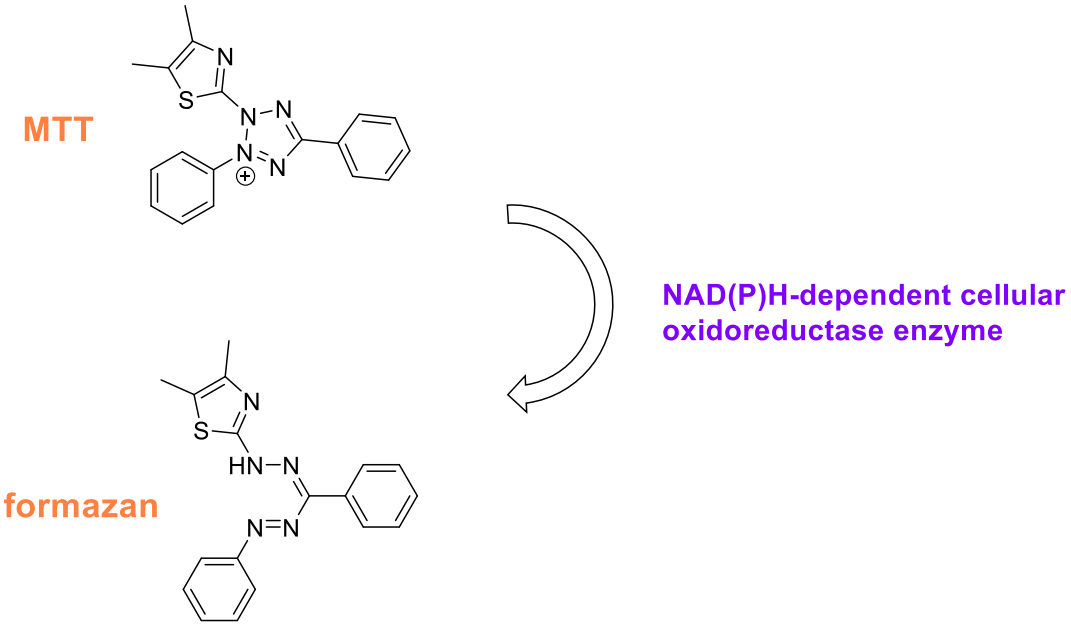


Figure III. 01

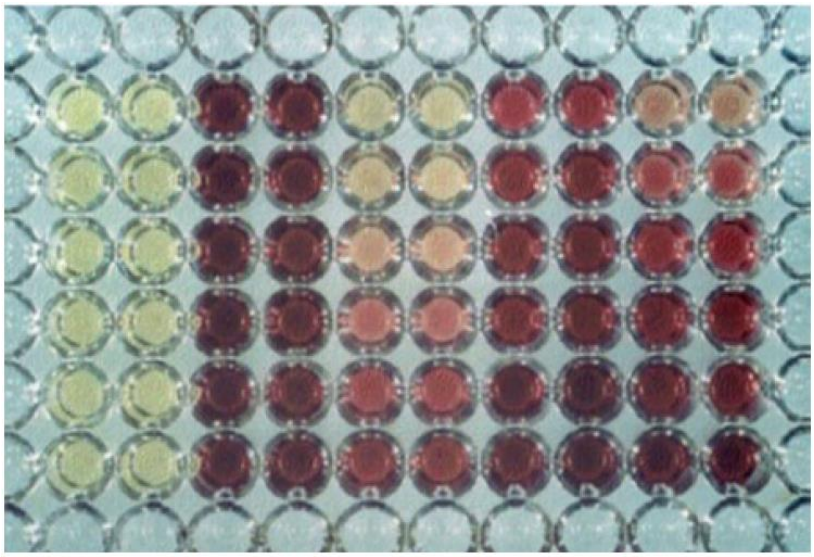


Figure III. 02

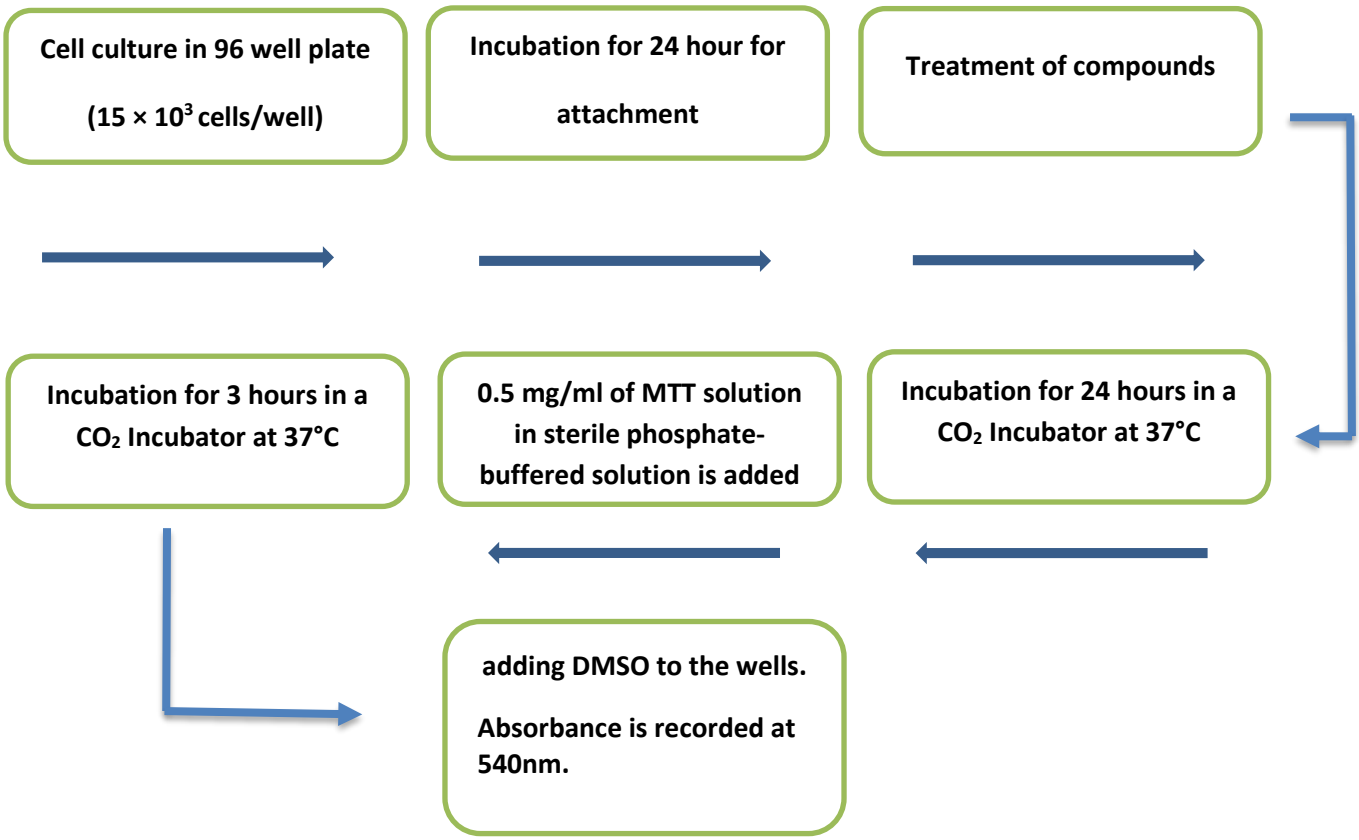
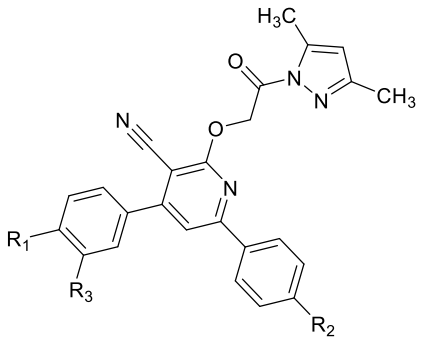


Figure III. 03

III.2 Cytotoxicity study

III.2.1 Results of Cyanopyridines-based pyrazoles (4a-g)



4a: R_1=R_2=R3= H.
4b: R_1=R_2= OMe, R_3= H.
4c: R_1= H, R_2= OMe, R_3= H.
4d: R_1= Cl, R_2= OMe, R_3= H.
4e: R_1= H, R_2= OMe, R_3= NO_2
4f: R_1=R_2= H, R_3= NO_2
4g: R_1= Br, R_2=H, R_3= NO_2

The cyanopyridine-based pyrazoles **(4a-g)** were subjected to cytotoxicity testing to identify the structure-activity relationship. In this context, a cytotoxicity study was carried out for our synthetic cyanopyridine-based pyrazoles **(4a-g)** against the human breast cancer cell line (MCF-7) and the human colon carcinoma cell line (Caco-2) using MTT assay. The results are summarized in the tables (III. 01, 02). The results show that for cyanopyridine-based pyrazoles **(4a-g)**, the compounds bearing 4-methoxy substituent for R and R_1 are generally low active than the other compounds. As shown in Table (III. 01), all the tested compounds showed anticancer activity against the MCF-7 cell line at 0.1, 1, 10, and 100 µM concentrations with $p < 0.05$, except **(4b)** at 0.1, 1, 10, and 100 µM concentrations, and **(4c)**, **(4f)**, and **(4g)** at 0.1, 1 µM concentrations. Among the tested cyanopyridine-based pyrazoles derivatives, compound **(4d)** at 0.1 µM showed better cytotoxicity against MCF-7 cells than the standard drug docetaxel with a log IC_{50} value of -0.6358 µM.

As shown in Table (III. 02). All the synthesized compounds **(4a-g)** were found to exhibit cytotoxicity against the human colon carcinoma cell line (Caco-2), except **(4b)** at 0.1, 1, 10, 100 µM concentrations, and **(4c)**, **(4e)** at 0.1, 1 µM concentrations. Among the tested cyanopyridine-based pyrazoles derivatives, compounds **(4a)**, and **(4f)** at 0.1 µM concentrations. while compounds **(4a)**, **(4d)**, and **(4g)** at 1 µM concentrations showed better cytotoxicity against Caco-2 cells than the standard drug Docetaxel with a log IC_{50} value of -0.6779 µM. Inhibitory concentration Log IC_{50} values calculated for the MCF-7 and Caco-2 cells, based on the results of MTT assays for a 24-h interaction of cyanopyridine-based pyrazoles derivatives, are presented in Table (III. 03).

Table (III. 01)

MCF-7 cell viability (%)						
Compound no.	Control	Solvent (DMSO)	0.1 µM	1 µM	10 µM	100 µM
4a	100.00±6.13	92.41±4.29	64.12±7.84*	66.40±5.15*	55.54±6.19*	34.51±6.26*
4b	100.00±6.13	92.41±4.29	87.04±9.10	78.22±8.59	83.14±10.53	92.37±8.99
4c	100.00±6.13	92.41±4.29	79.06±9.80	66.10±7.48*	55.39±6.036*	39.87±9.15*
4d	100.00±6.13	92.41±4.29	58.54±7.98*	57.40±8.25*	40.96±6.19*	32.33±4.16*
4e	100.00±6.13	92.41±4.29	62.93±6.40*	56.40±7.30*	60.70±4.27*	53.60±10.26*
4f	100.00±6.13	92.41±4.29	76.04±12.62	78.24±8.82	55.24±6.15*	47.71±6.90*
4g	100.00±6.13	92.41±4.29	76.63±8.49	48.61±5.39*	52.67±8.66*	45.08±5.14*
Docetaxel (ref. drug)	100.00±6.13	92.41±4.29	60.21±7.76*	30.22±3.86*	14.82±4.14*	1.22±0.09*

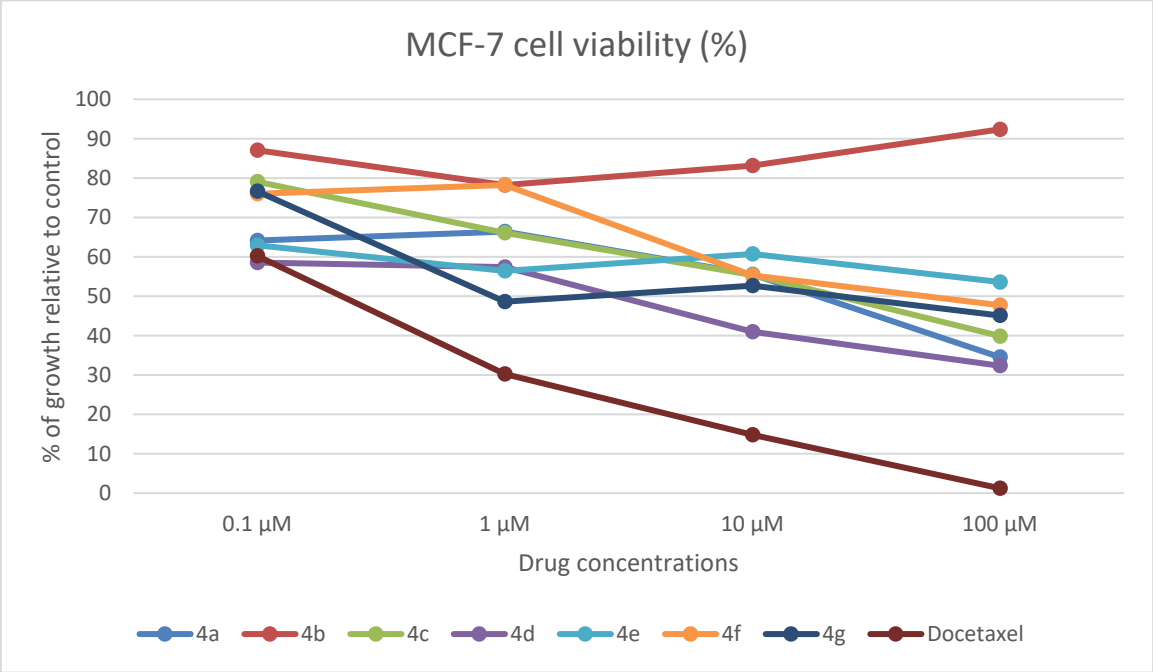


Figure III. 04

Table (III. 02)

Caco-2 cell viability (%)						
Compound no.	Control	Solvent (DMSO)	0.1 µM	1 µM	10 µM	100 µM
4a	100.00±7.81	90.22±5.43	51.97±7.78*	30.48±6.26*	19.42±5.54*	16.67±6.42*
4b	100.00±7.81	90.22±5.43	95.97±10.92	96.60±12.89	98.49±7.96	99.30±9.50
4c	100.00±7.81	90.22±5.43	80.52±7.16	81.65±9.23	62.16±73*	51.33±7.98*
4d	100.00±7.81	90.22±5.43	60.82±5.58*	32.07±6.35*	24.69±4.13*	14.78±3.96*
4e	100.00±7.81	90.22±5.43	74.71±7.10	74.49±8.70	60.13±6.18*	47.87±7.99*
4f	100.00±7.81	90.22±5.43	53.00±5.86*	36.98±6.80*	27.41±3.54*	25.91±4.22*
4g	100.00±7.81	90.22±5.43	66.97±9.47*	28.77±5.65*	27.26±5.39*	26.02±6.39*
Docetaxel (ref. drug)	100.00±7.81	90.22±5.43	55.46±8.21*	33.87±6.25*	17.49±2.61*	1.49±0.81*

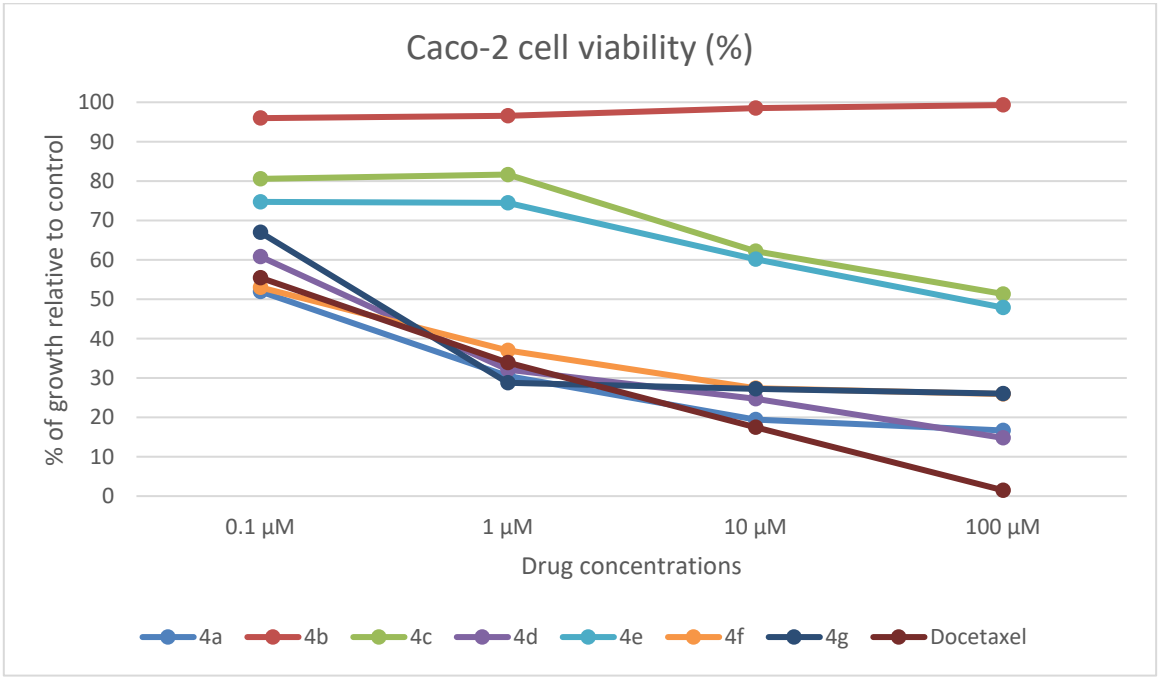
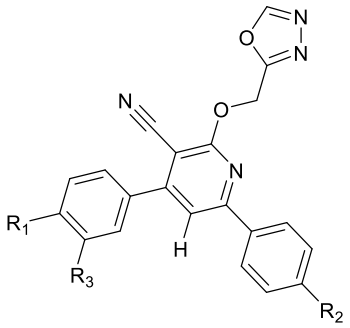


Figure III. 05

Table (III. 03)

log IC_{50} calculation		
Compound	MCF-7	Caco-2
4a	1.123	-0.7938
4b	2.963	3.636
4c	1.2	1.784
4d	0.3522	-0.5669
4e	1.774	1.626
4f	1.48	-0.6569
4g	0.9848	-0.498
Docetaxel	-0.6358	-0.6779

III.2.2 Results of Cyanopyridines-based oxadiazoles (5a-f)



5a: R_1= R_2 = R_3= H.
5b: R_1= R_2 = OMe, R_3= H.
5c: R_1= H, R_2= OMe, R_3= H.
5d: R_1= H, R_2= OMe, R_3= NO_2
5e: R_1= R_2= H, R_3= NO_2
5f: R_1= Br, R_2=H, R_3= NO_2

In vitro cytotoxicity of cyanopyridine-based oxadiazoles **(5a-f)** were evaluated against the human breast cancer cell line (MCF-7) and the human colon carcinoma cell line (Caco-2), also known as colon cancer by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The results are summarized in Tables (III.04, 05). Among the tested compounds, those bearing 4-methoxy substituent for R showed lower cytotoxicity activity than the other compounds. As shown in Table (III.04), all the tested compounds showed anticancer activity against the MCF-7 cell line at 0.1, 1, 10, and 100 µM concentrations with $p < 0.05$, except **(5c)**, **(5e)**, and **(5f)** at 0.1, 1, and 10 µM. Among the tested six cyanopyridine-based oxadiazoles derivatives, compound **(5d)** at 0.1 µM showed better cytotoxicity against MCF-7 cells than the standard drug docetaxel with a log IC_{50} value of -0.6358 µM. and the log IC_{50} values of the compounds were calculated after a 24-h treatment. Log IC_{50} values calculated for the MCF-7 and Caco-2 cells, based on the results of MTT assays for a 24-h interaction of cyanopyridine-based oxadiazoles derivatives, are presented in Table (III. 06). Similar to MCF-7 cell line results as shown in Table (III. 05). All the synthesized compounds (**5a-f)** were found to exhibit cytotoxicity against the human colon carcinoma cell line (Caco-2), except (**5b)**, **(5c)**, (**5d)**, and **(5f)** at 0.1, and 1 µM concentrations compared with the standard drug Docetaxel a log IC_{50} value of -0.6779 µM.

Table (III. 04)

MCF-7 cell viability (%)						
Compound no.	Control	Solvent (DMSO)	0.1 µM	1 µM	10 µM	100 µM
5a	100.00±6.13	92.41±4.29	59.31±7.69*	52.49±4.59*	59.71±8.23*	43.27±7.00*
5b	100.00±6.13	92.41±4.29	63.63±6.47*	57.78±6.03*	64.22±6.79*	45.36±7.18*
5c	100.00±6.13	92.41±4.29	74.15±9.20	82.74±10.16	78.57±9.39	60.26±4.19*
5d	100.00±6.13	92.41±4.29	53.02±9.20*	58.32±7.38*	61.37±9.13*	32.79±5.29*
5e	100.00±6.13	92.41±4.29	83.54±7.27	67.10±4.04*	46.16±7.24*	37.77±5.28*
5f	100.00±6.13	92.41±4.29	79.96±7.91	72.60±13.14	57.25±9.65*	48.04±8.26*
Docetaxel	100.00±6.13	92.41±4.29	60.21±7.76*	30.22±3.86*	14.82±4.14*	1.22±0.09*

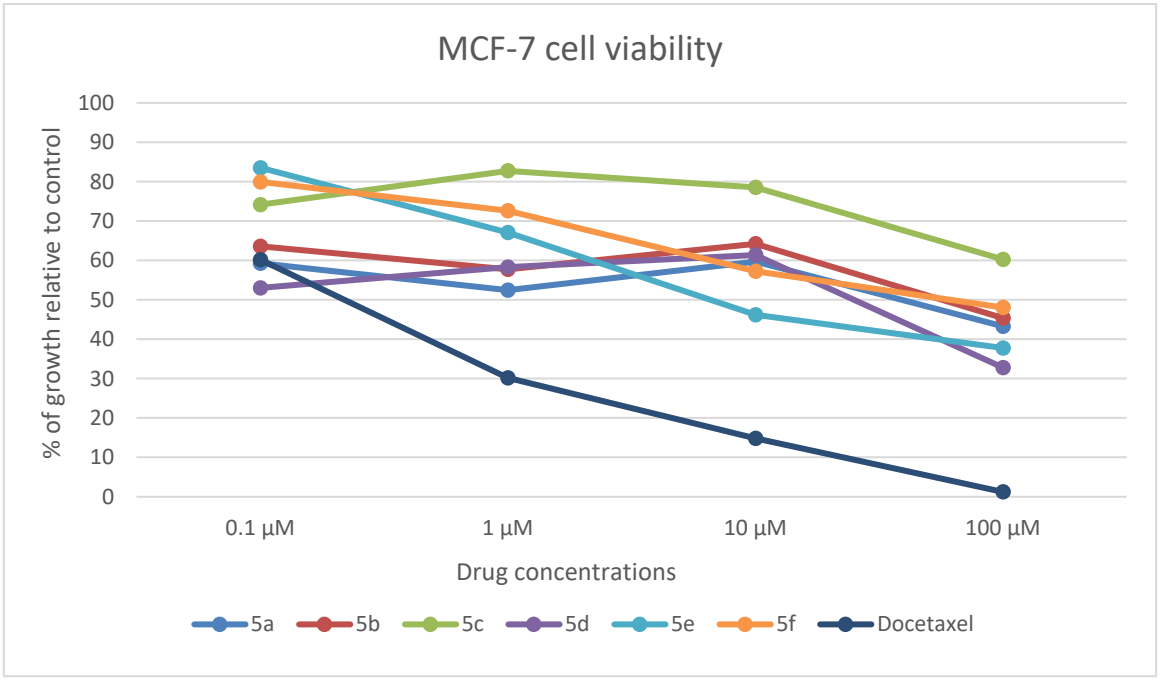


Figure III. 06

Table (III. 05)

Caco-2 cell viability (%)						
Compound no.	Control	Solvent (DMSO)	0.1 µM	1 µM	10 µM	100 µM
5a	100.00±7.81	90.22±5.43	61.70±6.55*	64.73±9.85*	34.78±5.14*	20.18±4.73*
5b	100.00±7.81	90.22±5.43	85.32±7.78	83.27±9.26	30.35±4.90*	19.35±4.83*
5c	100.00±7.81	90.22±5.43	74.03±8.29	75.79±9.63	46.55±5.85*	26.67±4.12*
5d	100.00±7.81	90.22±5.43	84.39±9.13	68.06±13.29	58.95.±7.79*	19.99±4.31*
5e	100.00±7.81	90.22±5.43	67.35±6.19*	62.44±8.34*	41.52±4.5*	25.37±6.41*
5f	100.00±7.81	90.22±5.43	76.50±9.53	57.32±8.36*	46.91±9.50*	27.98±4.68*
Docetaxel	100.00±7.81	90.22±5.43	55.46±8.21*	33.87±6.25*	17.49±2.61*	1.49±0.81*

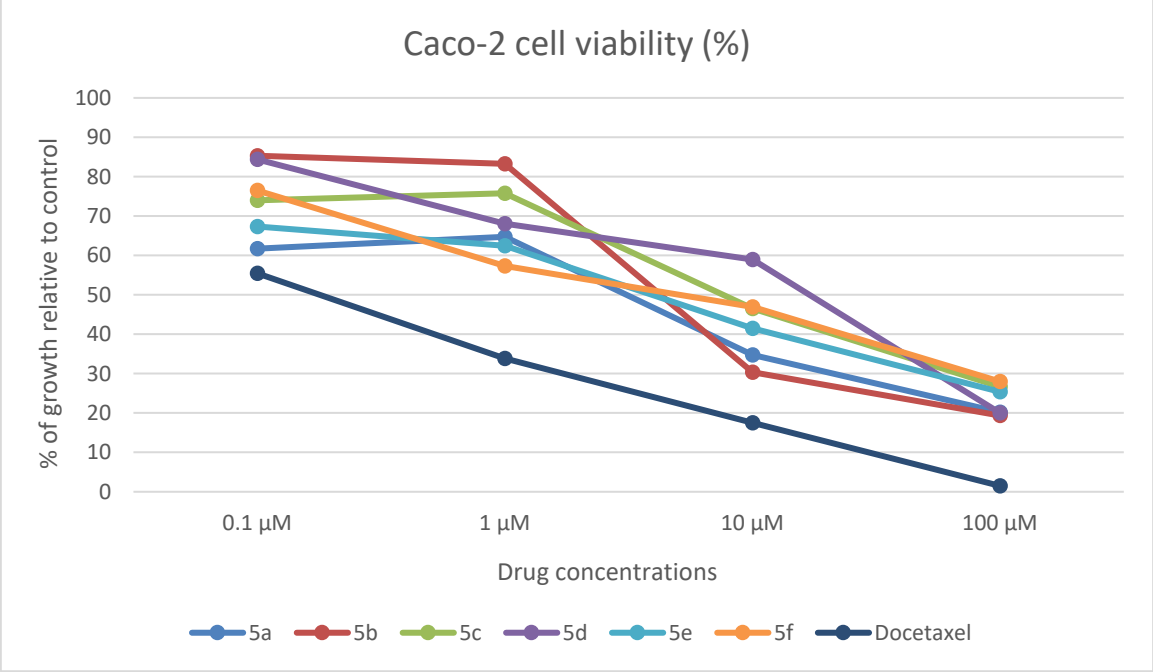
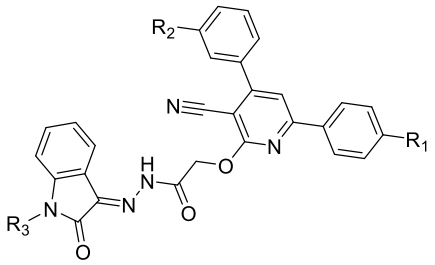


Figure III.07

Table (III. 06)

log IC_{50} calculation		
Compound	MCF-7	Caco-2
5a	1.303	0.417
5b	1.581	0.6819
5c	2.101	0.924
5d	1.198	1.09
5e	0.9218	0.5873
5f	1.539	0.7068
Docetaxel	-0.6358	-0.6779

III.2.3 Results of N-acylhydrazone (6a-i)



6a: R_1=R_2=R_3= H.
6b:R_1=R_2= H, R_3= Bz.
6c: R_1=R_2= H, R_3= 4-BrBz.
6d: R_1= OMe, R_2= H, R_3= H.
6e: R_1= OMe, R_2= H, R_3= 4-BrBz.
6f: R_1= R_2=OMe, R_3= H.
6g: R_1= R_2=OMe, R_3= Bz.
6h: R_1= OMe, R_2= NO_2, R_3=H.
6i: R_1= H, R_2= NO_2, R_3=H.

Cytotoxic activities of nine new N-acylhydrazone derivatives, including pyridine and isatin moieties, were determined using human ovarian (A-2780) and human breast cancer (MCF-7) cell lines. To obtain the cytotoxic properties of the newly synthesized N-acylhydrazone derivatives on A-2780 and MCF-7 cells, the respective cell lines were incubated with increasing concentrations (0–100 µg/ml) of the compounds for 48 h and then subjected to an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cell viability results of A-2780 and MCF-7 cells after a 48h treatment with the nine N-acylhydrazone derivatives are shown in Tables (III.07, 08), respectively. As shown in Table (III.07), all tested hydrazone derivatives demonstrated anticancer activity against the A-2780 cell line at 0.1, 1, 10, and 100 µM concentrations with $p < 0.05$, except compounds **(6a)** and **(6f)** at 0.1 µM concentration. Like A-2780 cell line results, all tested compounds show cytotoxic activity against the MCF-7 cell line at 0.1, 1, 10, and 100 µM concentrations with $p < 0.05$, except compounds **(6a)**, **(6f)**, and **(6h)** at 0.1 µM concentration. Among the tested nine N-acylhydrazone derivatives, compounds **(6b)** and **(6d)** showed better cytotoxicity against MCF-7 cells than the standard drug Docetaxel with an inhibitory logarithmic 50 (log IC_{50}) value of 0.24 µM. A time-dependent cell viability assay for the tested hydrazone derivatives was performed, and the log IC_{50} values of the compounds were calculated after a 24-h treatment. Log IC_{50} values calculated for the A-2780 and MCF-7 cells, based on the results of MTT assays for a 24-h interaction of N-acylhydrazone derivatives, are presented in Table (III.09). As shown in Table (III.08), all tested N-acylhydrazone derivatives showed promising antitumor activity against A-2780 cells compared with the standard drug Docetaxel with a log IC_{50} value of 0.22 µM. Among the tested nine N-acylhydrazone derivatives, compounds **(6b)** and **(6d)** showed better cytotoxicity against MCF-7 cells than the standard drug Docetaxel with a log IC_{50} value of 0.24 µM.

Table (III. 07)

A2780 cell viability (%)						
Compound no.	Control	Solvent (DMSO)	0.1 µM	1 µM	10 µM	100 µM
6a	100.00±3.90	94.72±2.39	83.60±6.70	64.85±5.21*	42.47±3.98*	37.76±5.44*
6b	100.00±3.90	94.72±2.39	55.11±8.36*	23.48±4.43*	18.53±2.70*	17.59±3.08*
6c	100.00±3.90	94.72±2.39	44.80±1.48*	39.28±5.30*	30.10±2.49*	9.74±1.08*
6d	100.00±3.90	94.72±2.39	21.45±5.13*	21.23±1.10*	18.08±5.14*	13.45±1.34*
6e	100.00±3.90	94.72±2.39	50.24±7.65*	38.11±3.64*	19.50±3.29*	12.64±3.05*
6f	100.00±3.90	94.72±2.39	103.44±7.64	67.46±5.78*	27.82±3.84*	11.78±0.79*
6g	100.00±3.90	94.72±2.39	31.21±7.96*	25.77±4.95*	23.87±7.15*	20.97±5.44*
6h	100.00±3.90	94.72±2.39	50.34±9.10*	27.44±3.50*	38.35±2.09*	13.03±3.67*
6i	100.00±3.90	94.72±2.39	58.50±5.06*	61.93±3.23*	25.76±3.30*	12.27±2.00*
Docetaxel (ref. drug)	100.00±3.90	94.72±2.39	60.21±5.97	40.13±6.58	14.12±2.73	0.48±0.02

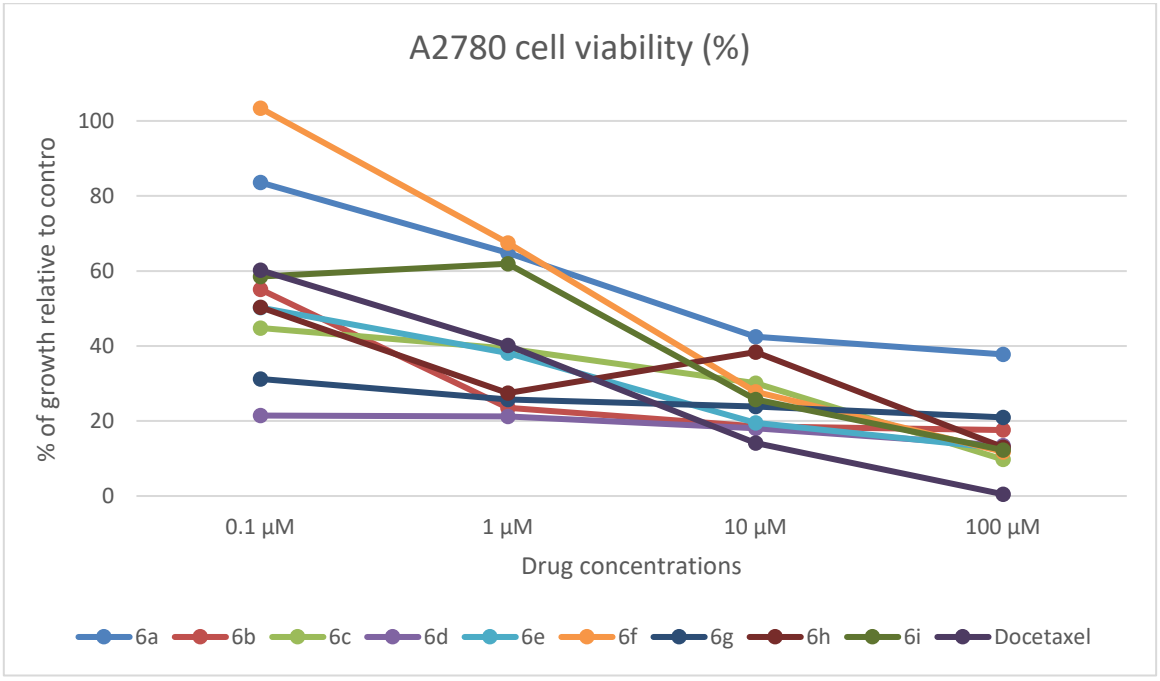


Figure III.08

Table (III. 08)

MCF-7 cell viability (%)						
Compound no.	Control	Solvent (DMSO)	0.1 µM	1 µM	10 µM	100 µM
6a	100.00±5.32	95.79±2.47	76.51±9.13	65.60±6.61*	64.37±3.77*	46.87±2.59*
6b	100.00±5.32	95.79±2.47	53.98±6.15*	44.46±4.47*	39.14±3.09*	34.32±3.30*
6c	100.00±5.32	95.79±2.47	70.19±6.91*	68.06±2.10*	51.57±3.50*	53.38±3.55*
6d	100.00±5.32	95.79±2.47	50.95±2.55*	43.11±4.16*	42.22±1.96*	44.05±3.32*
6e	100.00±5.32	95.79±2.47	69.37±3.95*	68.48±5.68*	57.96±4.97*	49.57±5.74*
6f	100.00±5.32	95.79±2.47	84.42±4.70	71.87±6.84*	53.51±7.44*	51.73±7.16*
6g	100.00±5.32	95.79±2.47	63.21±6.60*	54.36±3.57*	50.44±1.93*	53.13±3.75*
6h	100.00±5.32	95.79±2.47	80.37±2.73	57.02±6.97*	56.52±5.12*	45.03±1.79*
6i	100.00±5.32	95.79±2.47	58.96±4.60*	51.89±4.92*	44.88±2.13*	42.56±2.11*
Docetaxel (ref. drug)	100.00±5.32	95.79±2.47	64.56±6.14	36.85±4.92	20.01±3.09	0.88±0.04

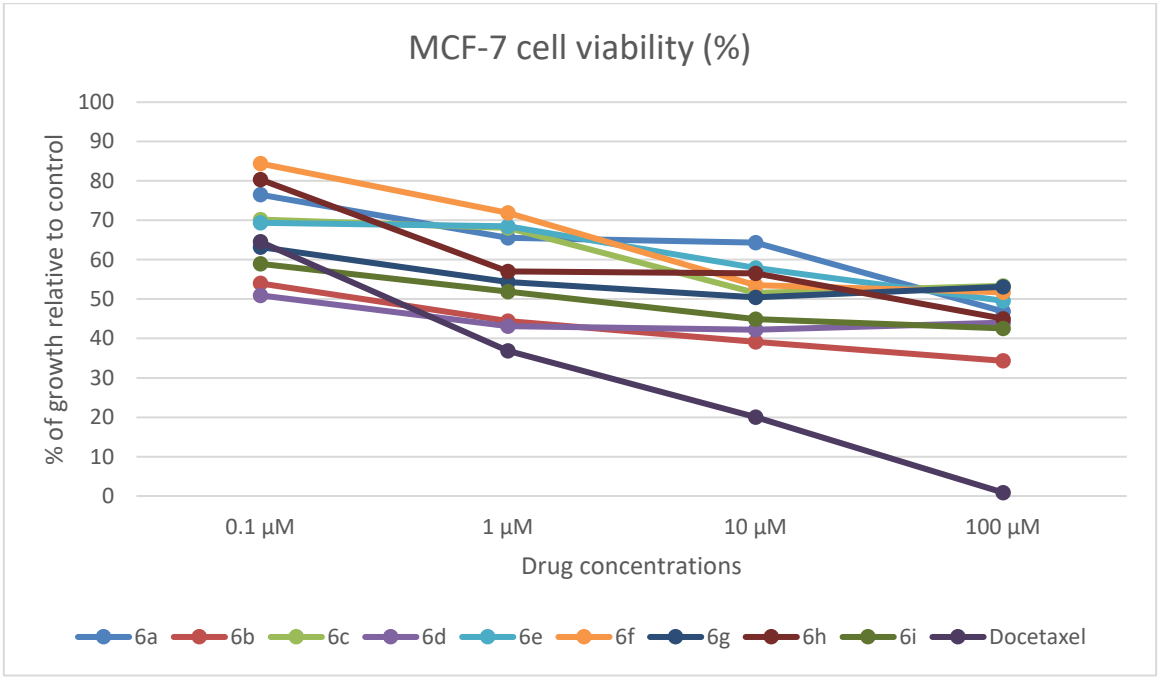


Figure III.09

Table (III. 09)

log IC_{50} calculation		
Compound	A-2780	MCF-7
6a	0.7816	1.660
6b	−0.4987	−0.1293
6c	−0.4044	1.465
6d	−0.8138	−0.1700
6e	−0.3868	1.598
6f	0.4636	1.551
6g	−0.6954	1.085
6h	−0.4751	1.267
6i	0.1809	0.2459
Docetaxel	0.2200	0.2400

III.3 Conclusion

Twenty-seven new Cyanopyridine hybrid molecules bearing pyrazoles, oxadiazoles, N-acylhydrazones derivatives were synthesized successfully, and their anticancer activities were evaluated against human cancer cell lines (A-2780, MCF-7, and Caco-2). Among the tested cyanopyridine-based pyrazole derivatives, four out of the seven compounds **(4a)** and **(4f)** at 0.1 μM concentration, and **(4a)**, **(4d)**, **(4g)** at 1 μM concentration exhibited better anticancer activity than the standard drug Docetaxel against the Caco-2 cell line. The log IC_{50} of Docetaxel was -0.6779 μM for Caco-2 cells at 24 h, whereas the IC_{50} values of compounds **(4a)**, **(4d)**, **(4f)**, and **(4g)** were -0.7938, -0.5669, -0.6569, and -0.498 μM, respectively. While. The results against the MCF-7 cells, only compound **(4d)**, showed better anticancer activity than the standard drug Docetaxel at 0.1 μM concentration. The log IC_{50} of Docetaxel was -0.6358 μM for MCF-7 cells at 24 h, whereas the log IC_{50} value of compound **(4d)** was 0.3522 μM.

Among the tested cyanopyridine-based oxadiazole derivatives, Compound **(5d)** at 0.1 μM concentration showed better anticancer activity against MCF-7 cells than the standard drug Docetaxel. The log IC_{50} of Docetaxel was -0.6358 μM for MCF-7 cells at 24 h, whereas the IC_{50} value of compound **(5d)** was 1.198 μM.

Among the tested Cyanopyridine-based N-acylhydrazones derivatives, seven out of the nine compounds **(6b)**, **(6c)**, **(6d)**, **(6e)**, **(6g)**, **(6h)**, and **(6i)** showed better anticancer activity than the standard drug Docetaxel at a concentration of 0.1 μM against the A-2780 cell line. The log IC_{50} of Docetaxel was 0.2200 μM for A-2780 cells at 24 h, whereas the log IC_{50} values of compounds **(6b)**,

(6c), **(6d)**, **(6e)**, **(6g)**, **(6h)**, and **(6i)** were −0.4987, −0.4044, −0.8138, −0.3868, −0.6954, −0.4751, and 0.1809 μM, respectively. Three out of the nine compounds **(6b)**, **(6d)**, and **(6i)** at a concentration of 0.1 μM showed better anticancer activity against the MCF-7 cancer cell line than the standard drug. The log IC_{50} of Docetaxel was 0.2400 μM for MCF-7 cells at 24 h, whereas the log IC_{50} values of compounds **(6b)**, **(6d)**, and **(6i)** were −0.1293, −0.1700, and 0.2459 μM, respectively. This study's outcomes indicate that the newly studied cyanopyridine hybrid molecules derivatives may act as potential drug candidates for cancer treatment.

General Conclusion

General Conclusion

Cancer is a serious health problem in all societies, regardless of wealth or social status. Although various discoveries have been made worldwide to cure cancer, good treatable values have not been achieved yet with existing single-target drugs as most of them are not only unable to overcome the resistance mechanism involved in primary and secondary cancer cells but also are unable to differentiate normal cells from neoplastic ones.

Nowadays, hybrid molecules containing two or more covalently linked known potential pharmacophores in one molecule are more medically effective to beat this deadliest disease than their individual components due to their improved affinity and efficacy toward the target sites

In this context and owing to the pharmacological data of pyridine, 1,3,4 oxadiazole, and pyrazole ring, we are interested in synthesizing new hybrid molecules and examining their anti-cancer activities against A2780, MCF-7, and Caco-2 cell lines. Therefore, the present thesis was constructed as follow:

In the first chapter, we referred to a literature review that described the biological and medicinal interests of some types of heterocyclic systems, including cyanopyridines, pyrazoles, oxadiazoles, and N-acylhydrazones derivatives, also their reactivities and the significant synthesis methods described in the literature.

In the second chapter, we have developed, in the first part, new conditions to prepare a library of acetohydrazide starting from cyanopyridones, and we have used the reactivity of these derivatives to build three different series all Cyanopyridines-based:

- a series of cyanopyridine-pyrazole hybrid molecules by the reaction of acetohydrazides with acetylacetone.

- a series of cyanopyridine-oxadiazole hybrid molecules by thermal reaction of acetohydrazides with Triethyl orthoformate.

- a series of N-acylhydrazones bearing cyanopyridine and isatin derivatives by the reaction of acetohydrazides with isatin derivatives.

In the third chapter, the synthesized heterocycles have undergone biological tests to highlight their anticancer properties. Indeed, different cancer cell lines were used in bioassays using the MTT assay method, which revealed increased activity for these compounds. Some of the tested compounds exhibited better anticancer activity than the standard drug Docetaxel.

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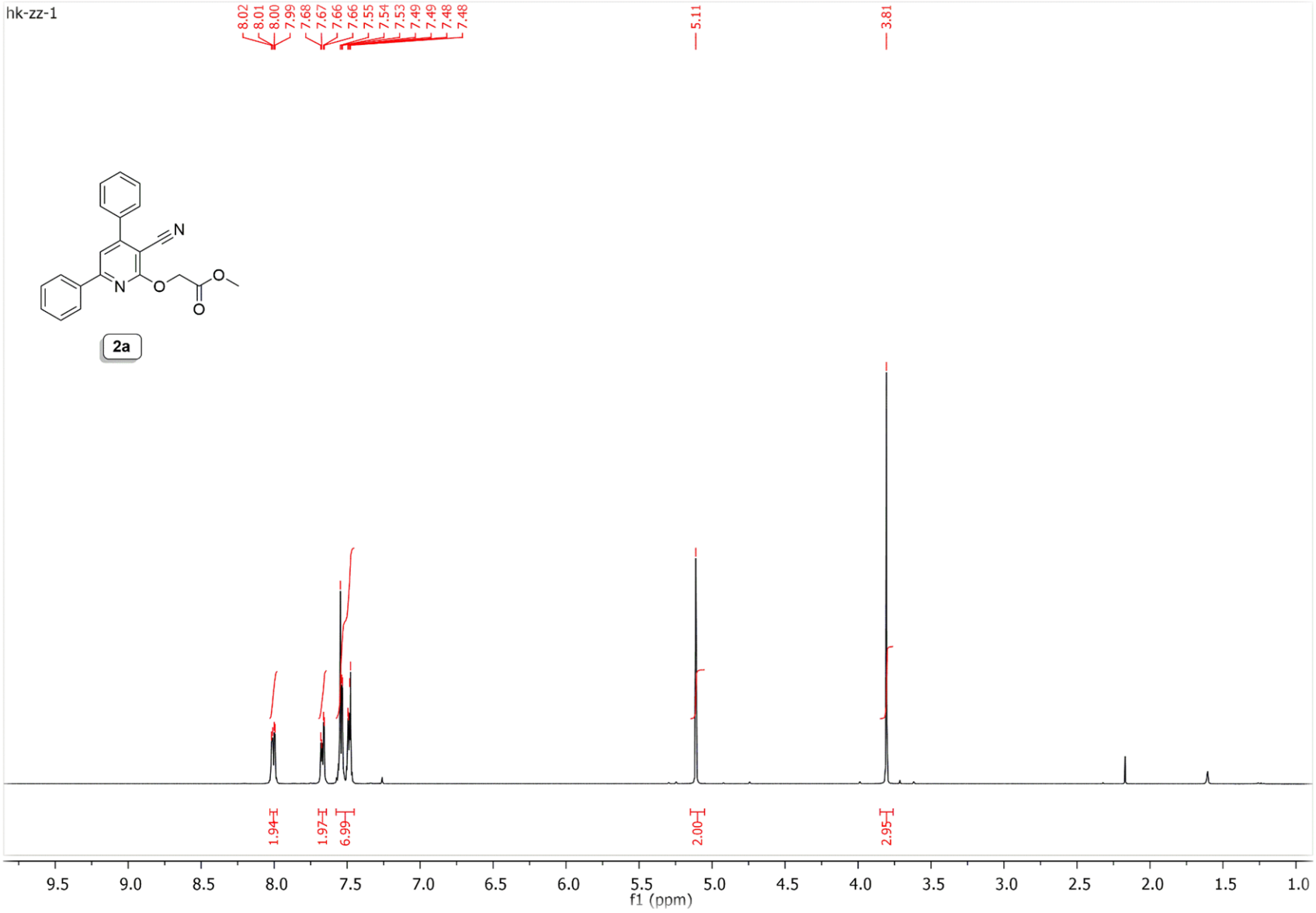
APPENDIX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8.01
8.00
7.99
7.68
7.67
7.66
7.66
7.55
7.54
7.53
7.49
7.49
7.48
7.48
5.11
3.81
N
N
O
O
O
2a
1.94
1.97
6.99
2.00
2.95
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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3.5
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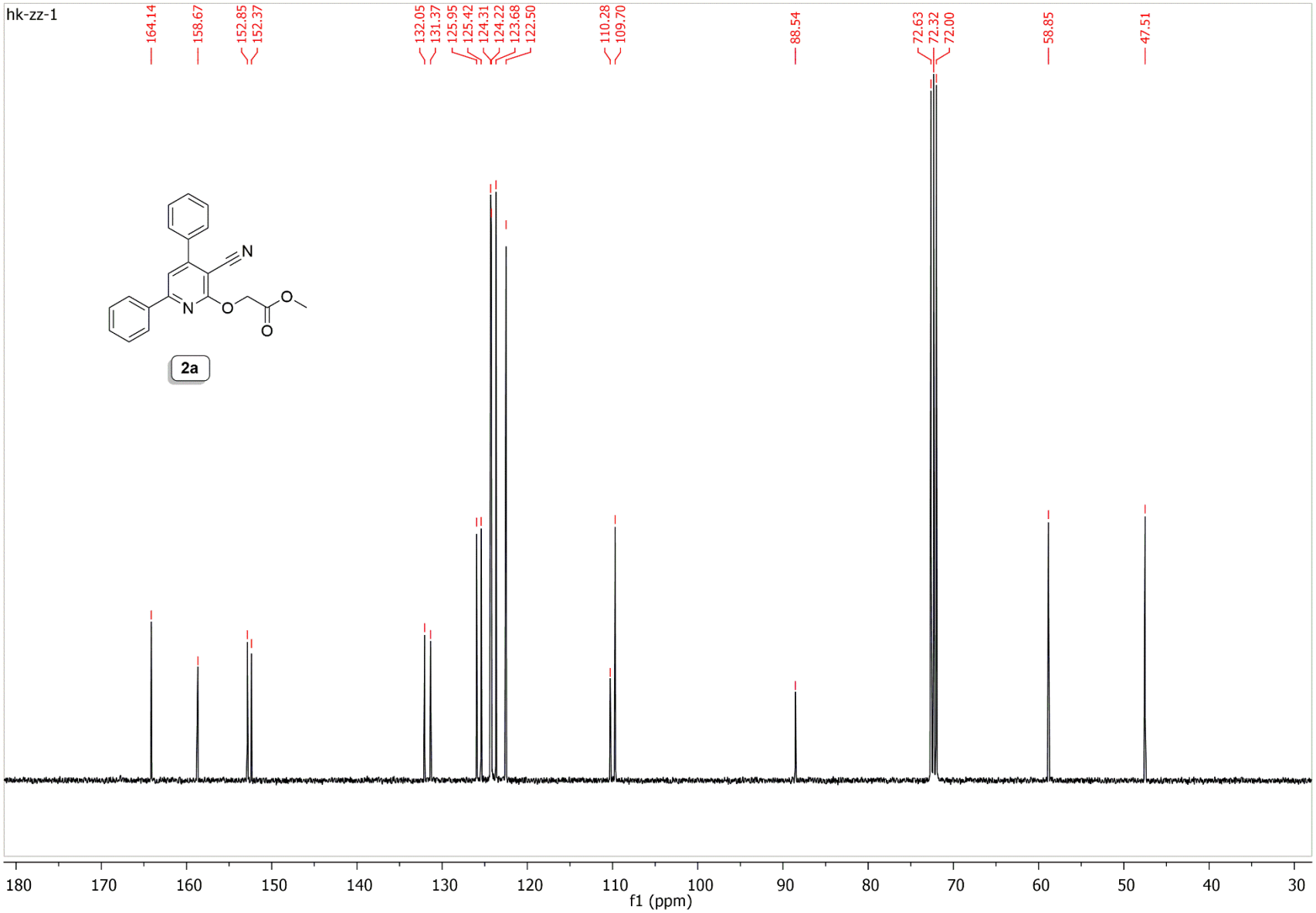
f1 (ppm)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152.37
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131.37
125.95
125.42
124.31
124.22
123.68
122.50
110.28
109.70
88.54
72.63
72.32
72.00
58.85
47.51
N
N
O
O
O
2a
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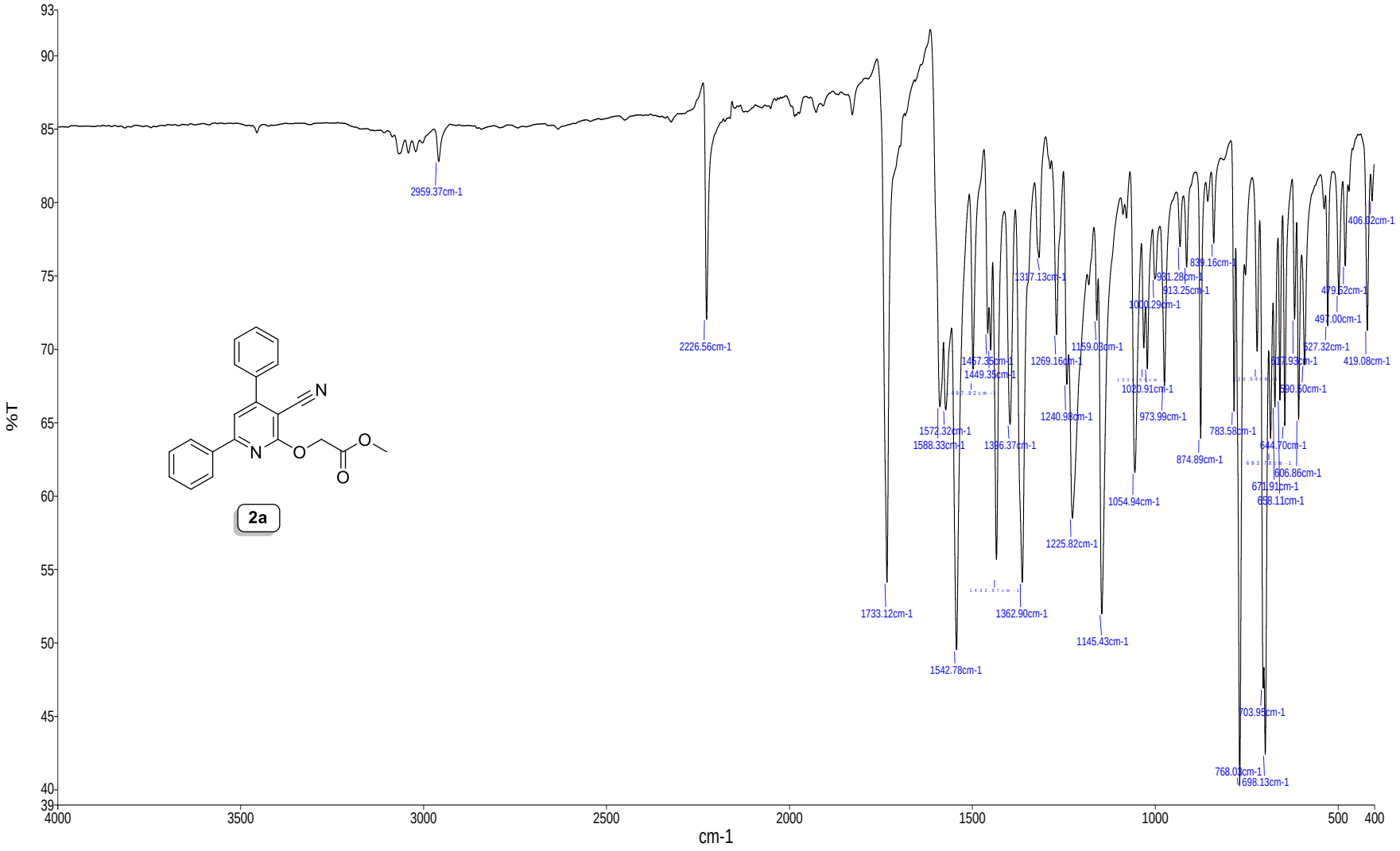
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)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1269.16cm-1
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1225.82cm-1
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1145.43cm-1
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1020.91cm-1
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931.28cm-1
913.25cm-1
874.89cm-1
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783.58cm-1
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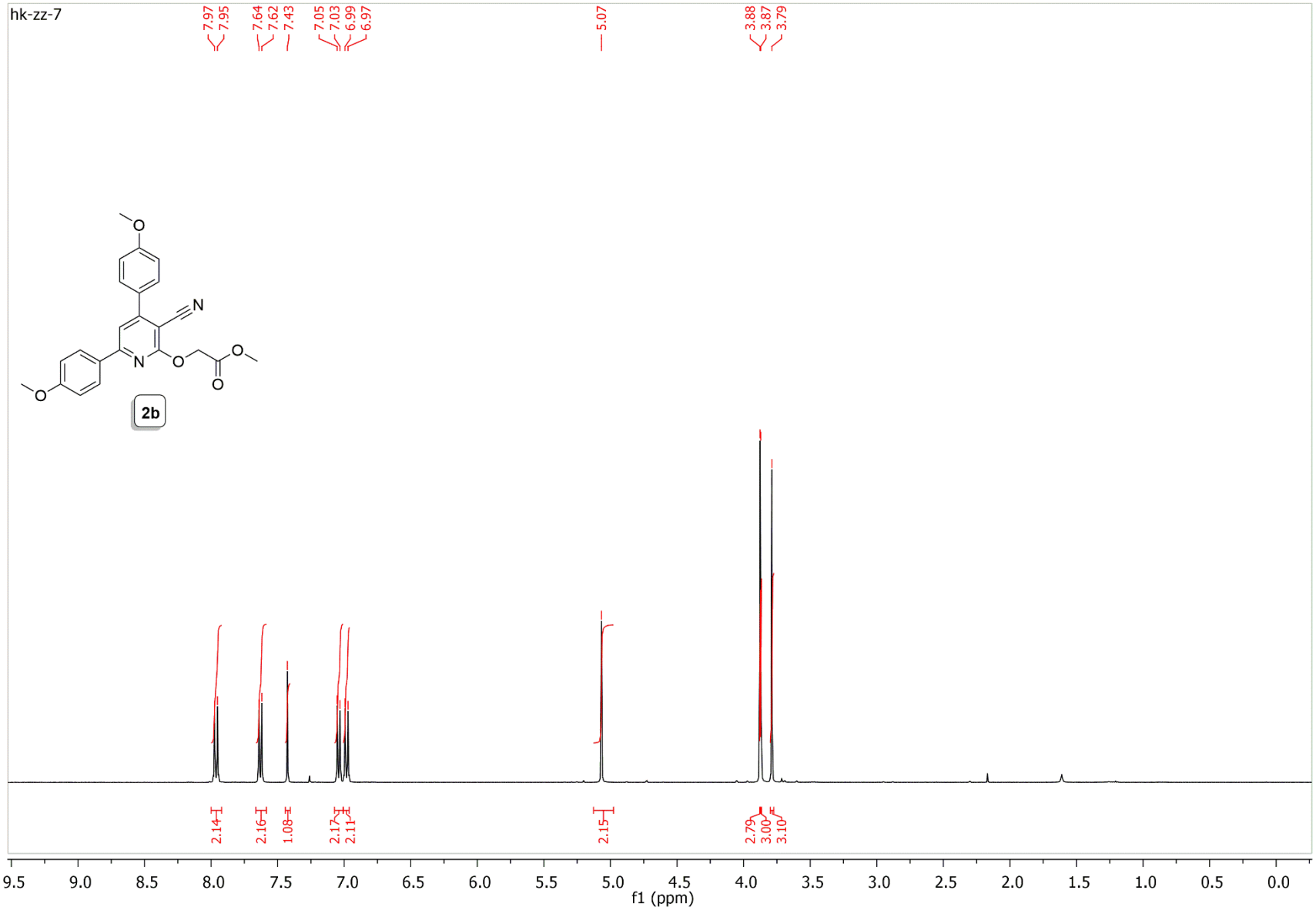
2a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6.97
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3.87
3.79
2b
2.14
2.16
1.08
2.17
2.11
2.15
2.79
3.00
3.10
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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3.0
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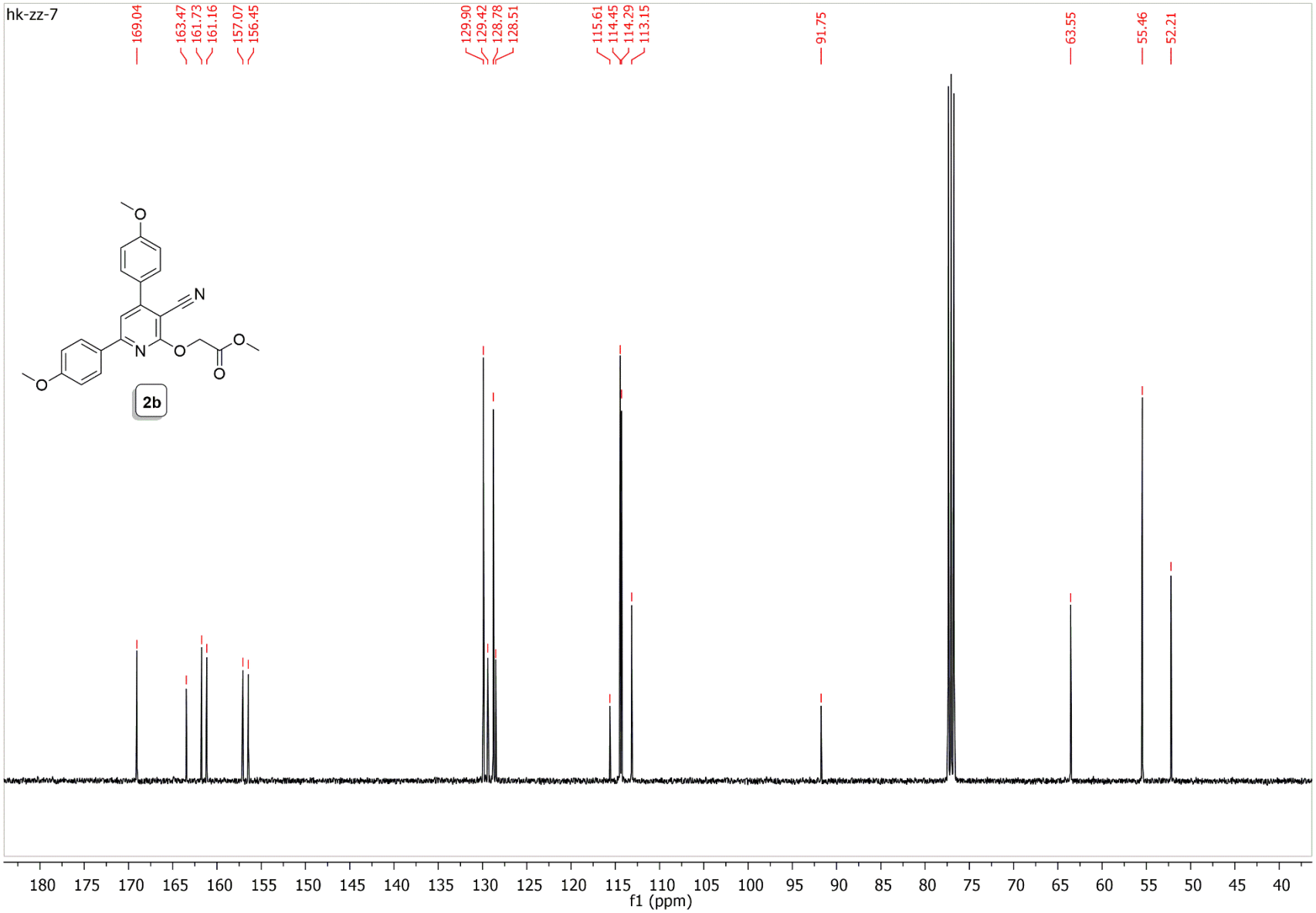
0.0
f1 (ppm)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161.16
157.07
156.45
129.90
129.42
128.78
128.51
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114.45
114.29
113.15
91.75
63.55
55.46
52.21
2b
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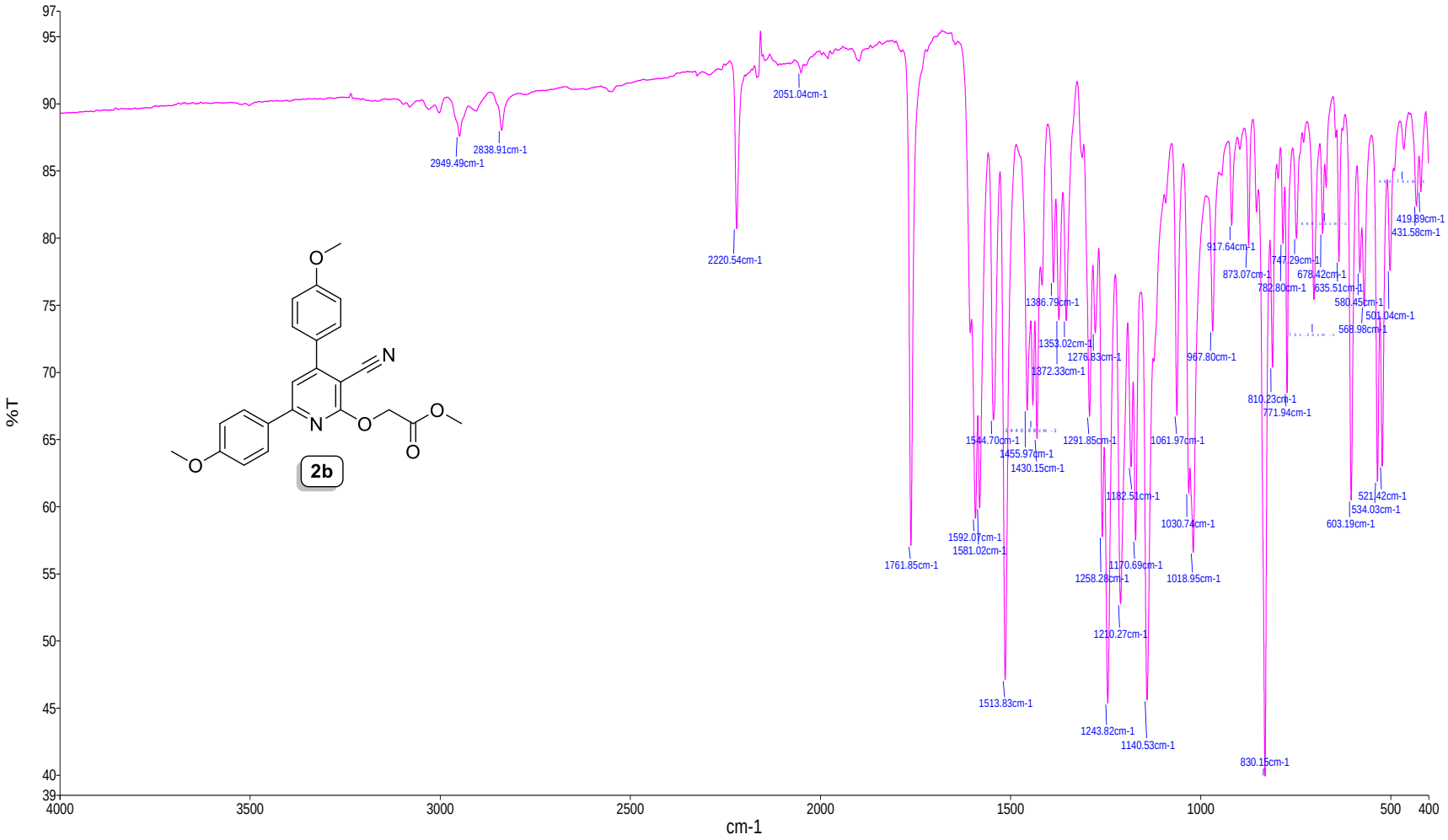
f1 (ppm)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1581.02cm-1
1544.70cm-1
1513.83cm-1
1455.97cm-1
1430.15cm-1
1386.79cm-1
1372.33cm-1
1353.02cm-1
1291.85cm-1
1276.83cm-1
1258.28cm-1
1243.82cm-1
1210.27cm-1
1182.51cm-1
1170.69cm-1
1140.53cm-1
1061.97cm-1
1030.74cm-1
1018.95cm-1
967.80cm-1
917.64cm-1
873.07cm-1
830.15cm-1
810.23cm-1
782.80cm-1
771.94cm-1
747.29cm-1
678.42cm-1
635.51cm-1
603.19cm-1
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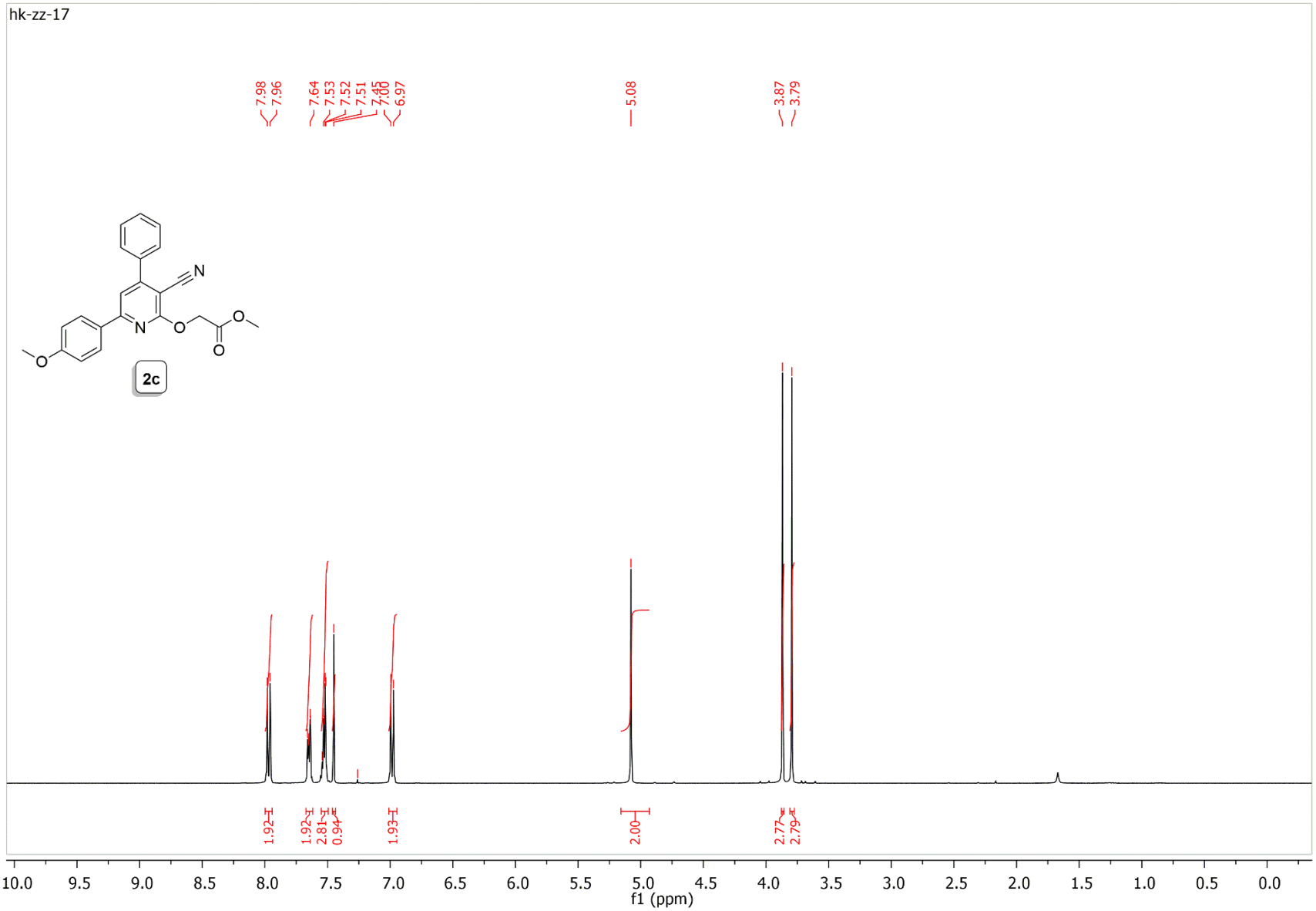
534.03cm-1
521.42cm-1
501.04cm-1
431.58cm-1
419.89cm-1
2b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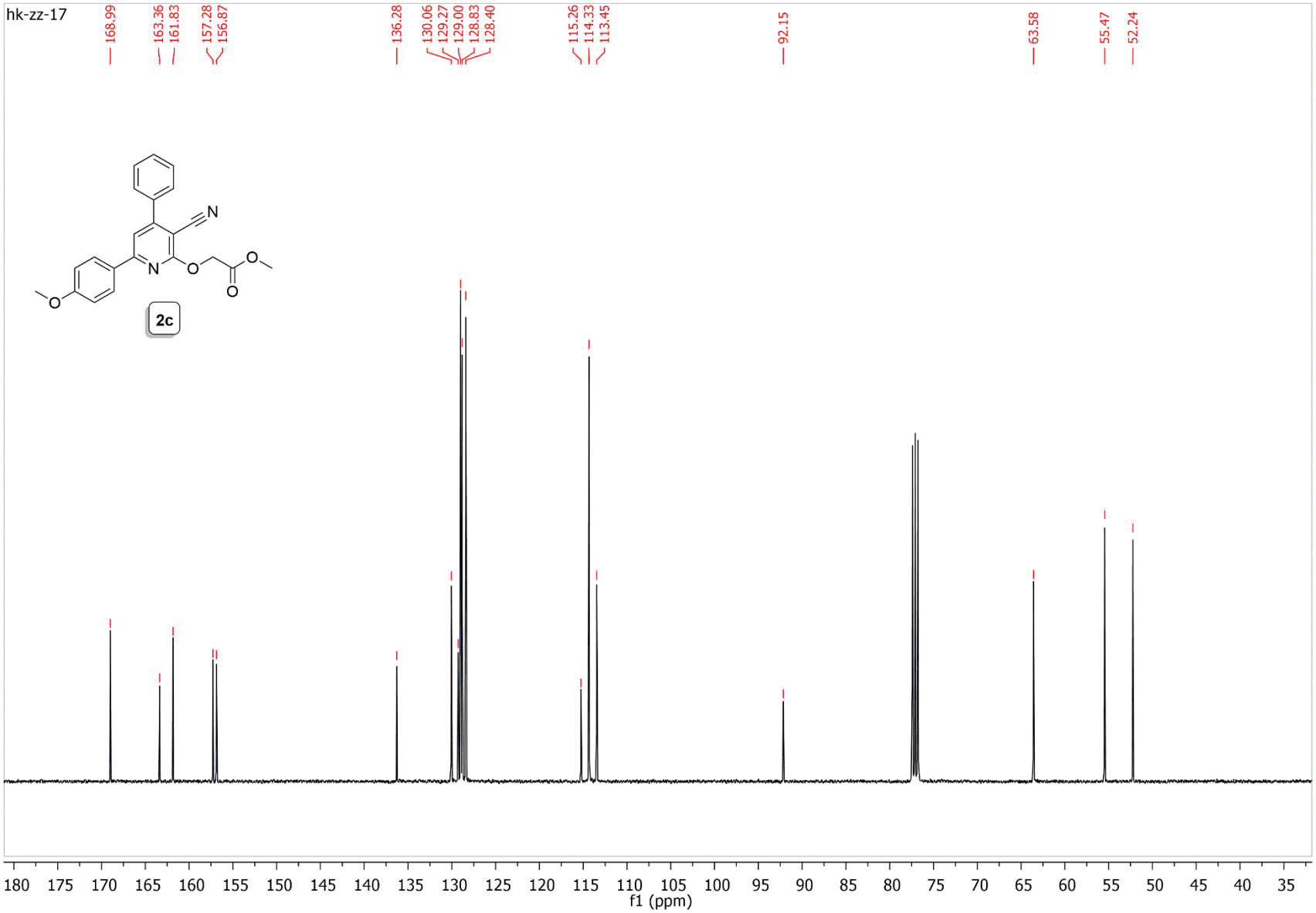
7.53
7.52
7.51
7.45
7.00
6.97
5.08
3.87
3.79
N
O
N
O
O
O
2c
1.92
1.92
2.81
0.94
1.93
2.00
2.77
2.79
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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156.87
136.28
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129.27
129.00
128.83
128.40
115.26
114.33
113.45
92.15
63.58
55.47
52.24
N
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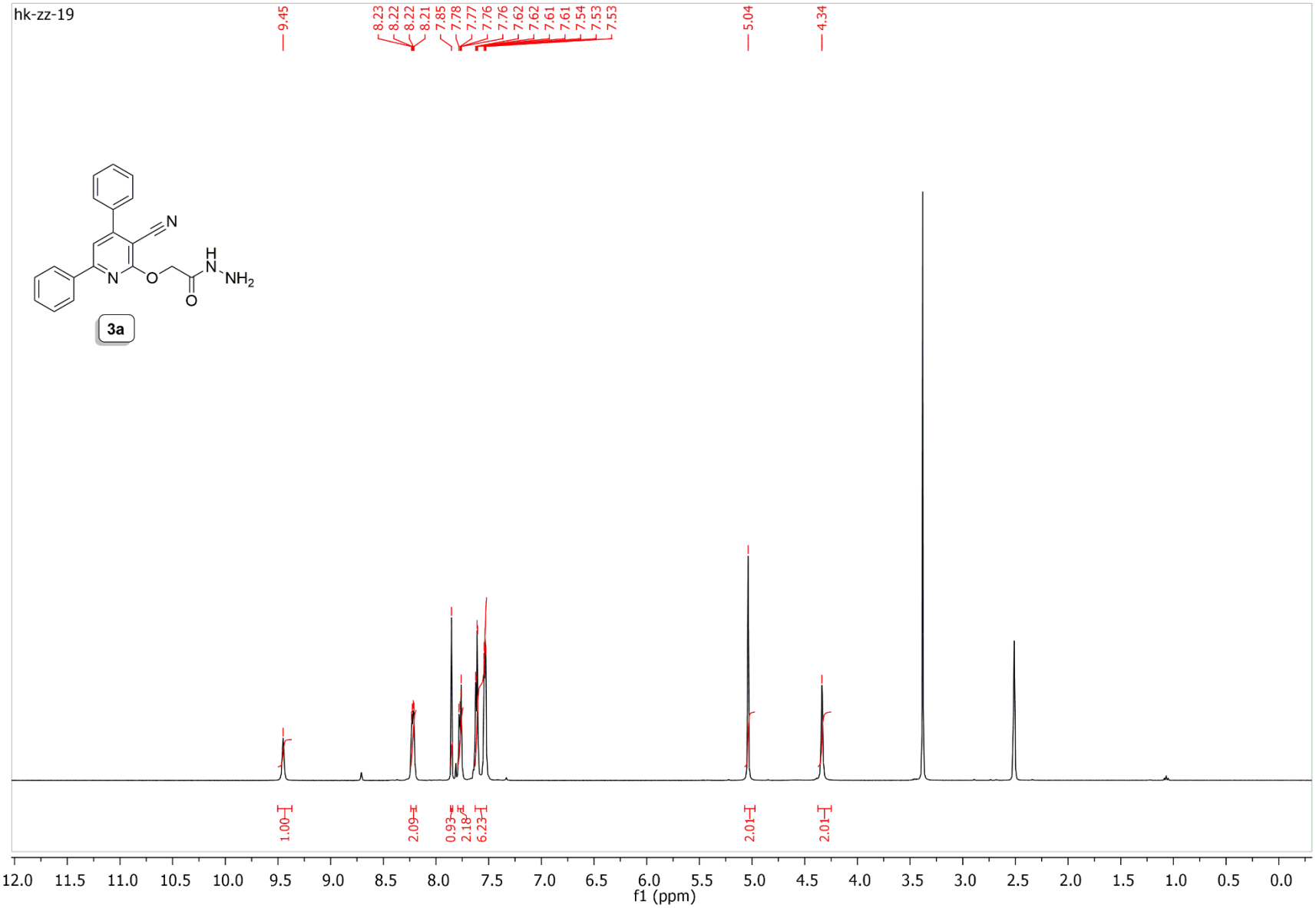
O
O
O
O
2c
180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35
f1 (ppm)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8.22
8.22
8.21
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7.78
7.77
7.76
7.76
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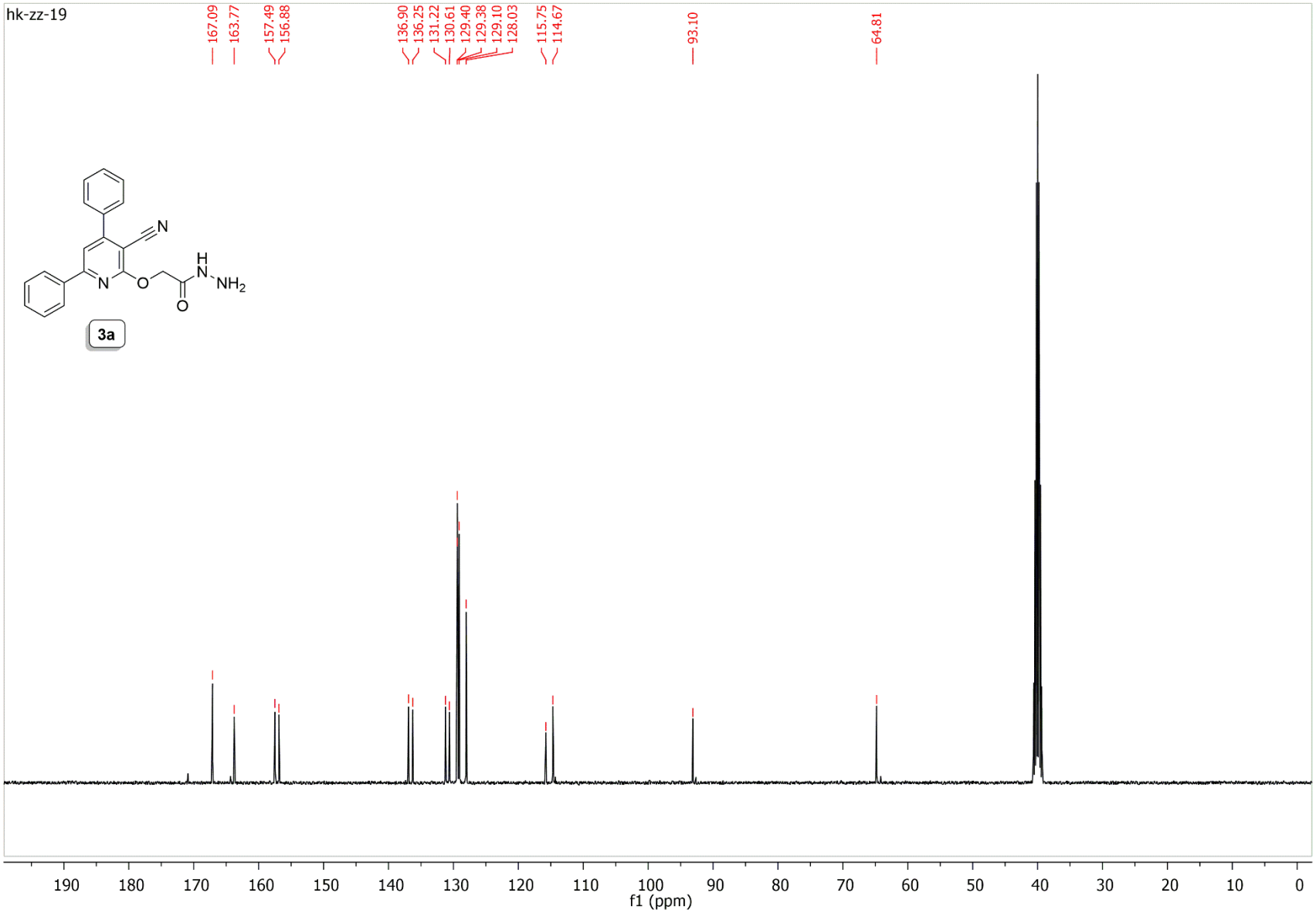
7.62
7.61
7.61
7.54
7.53
7.53
5.04
4.34
N
H
N
NH2
N
O
O
3a
1.00
2.09
0.93
2.18
6.23
2.01
2.01
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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129.40
129.38
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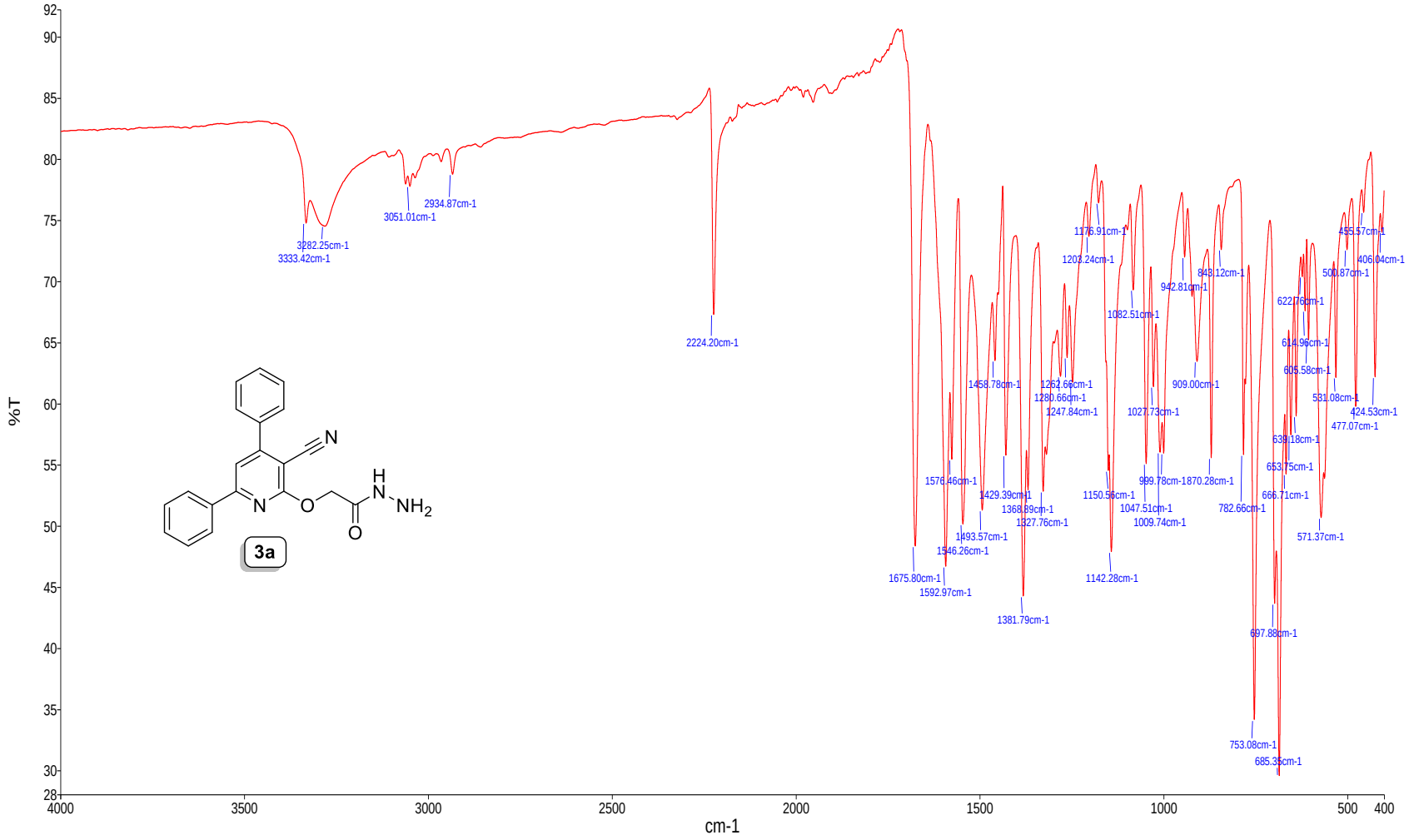
H
N
NH2
N
O
O
3a
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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1576.46cm-1
1546.26cm-1
1493.57cm-1
1458.78cm-1
1429.39cm-1
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1262.66cm-1
1247.84cm-1
1203.24cm-1
1176.91cm-1
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1142.28cm-1
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1009.74cm-1
999.78cm-1
942.81cm-1
909.00cm-1
870.28cm-1
843.12cm-1
782.66cm-1
753.08cm-1
697.88cm-1
685.35cm-1
666.71cm-1
653.75cm-1
639.18cm-1
622.76cm-1
614.96cm-1
605.58cm-1
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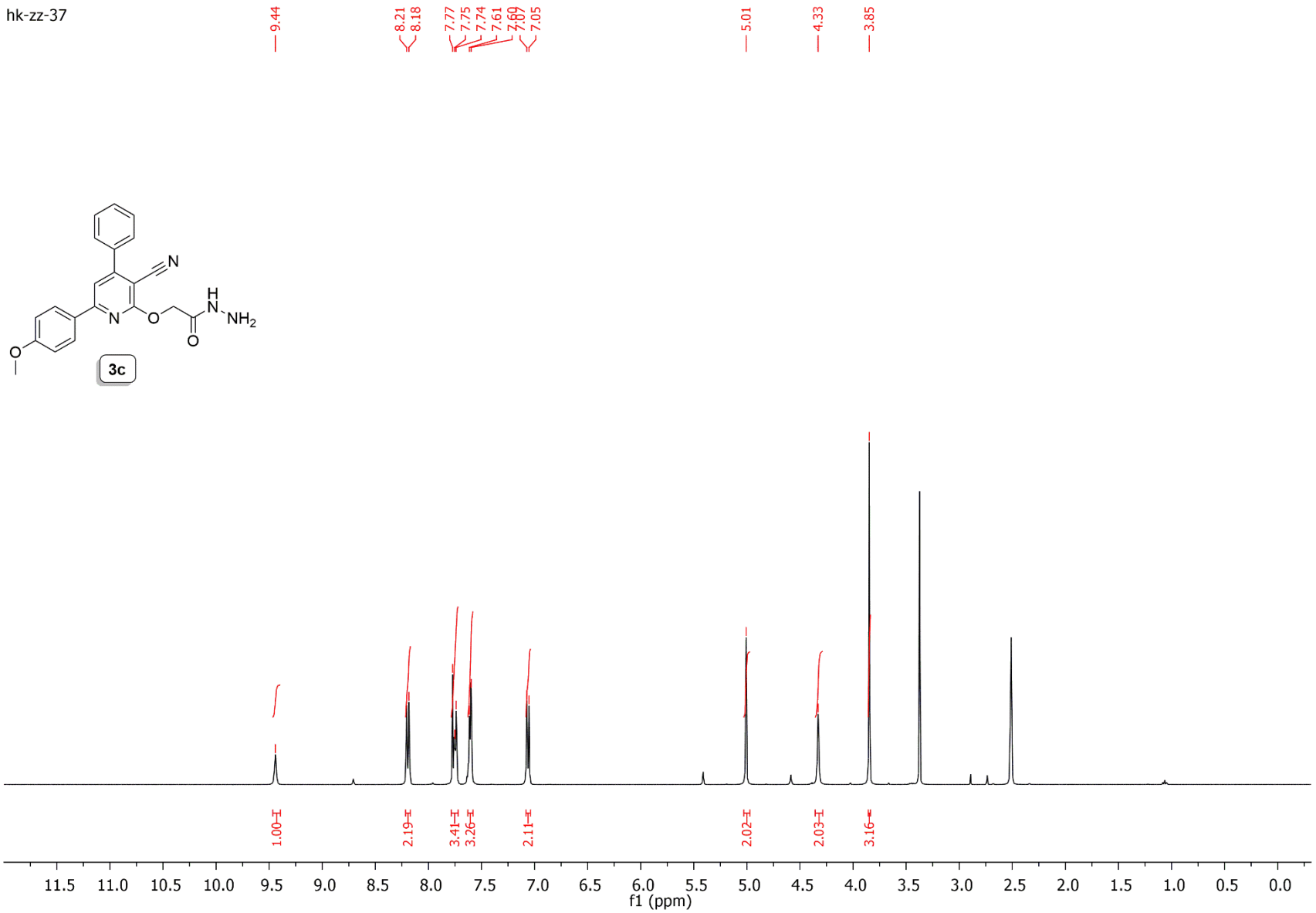
424.53cm-1
406.04cm-1
3a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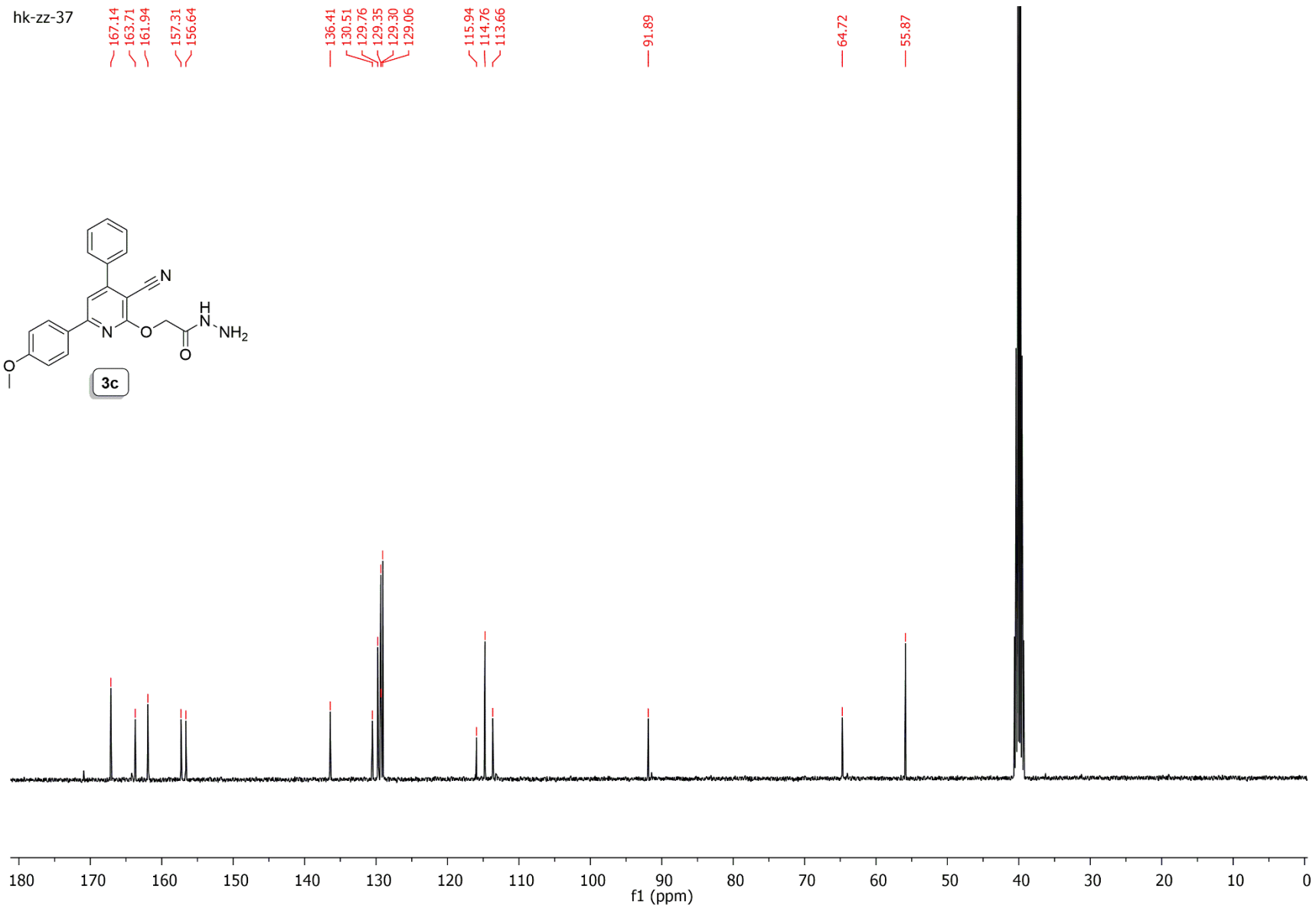
7.77
7.75
7.74
7.61
7.60
7.07
7.05
5.01
4.33
3.85
N
H
N
NH2
O
N
O
O
3c
1.00
2.19
3.41
3.26
2.11
2.02
2.03
3.16
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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130.51
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129.35
129.30
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115.94
114.76
113.66
91.89
64.72
55.87
N
H
N
NH2
O
N
O
O
O
3c
180
170
160
150
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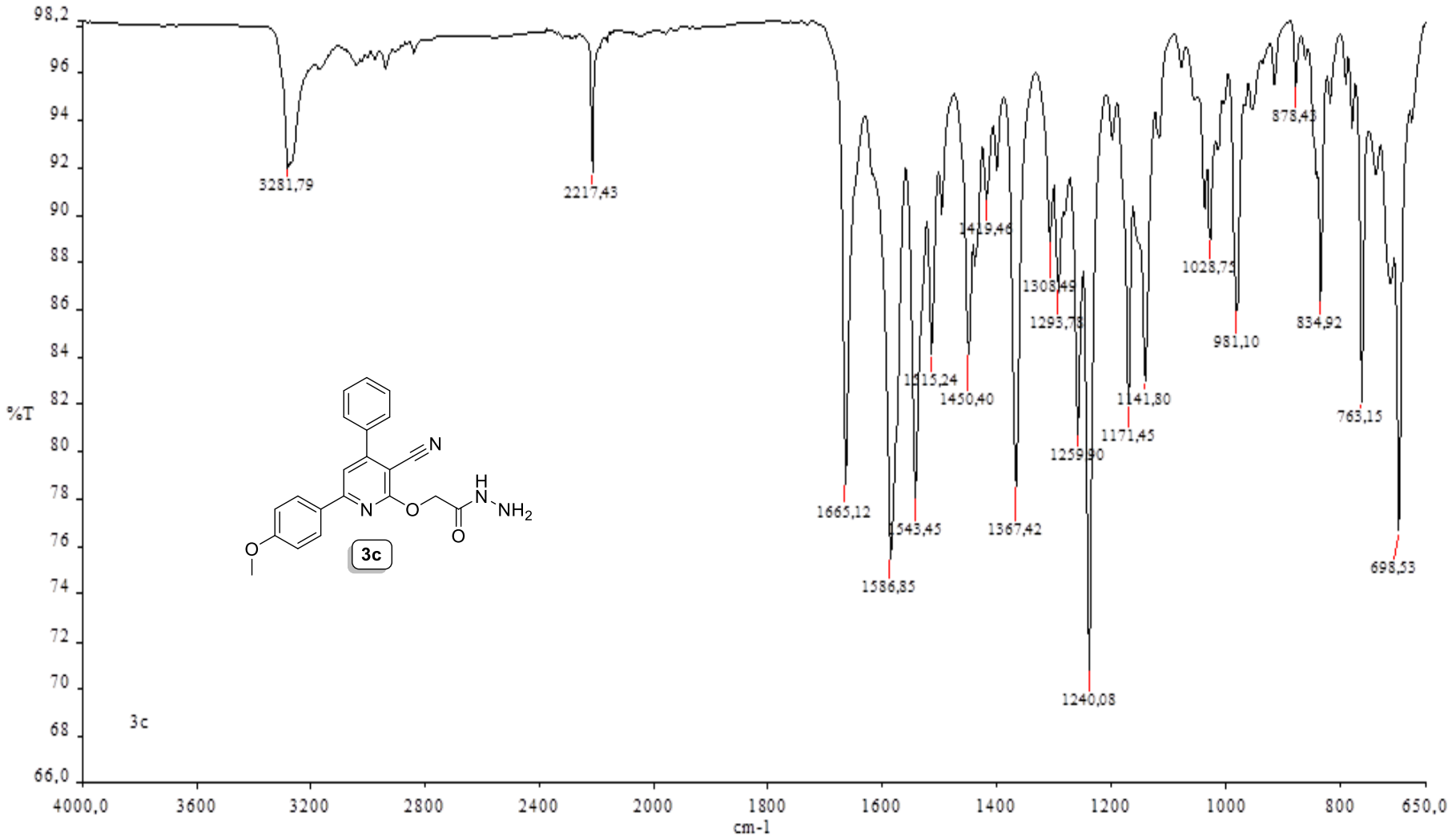
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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96
94
92
90
88
86
84
82
80
78
76
74
72
70
68
66,0
%T
4000,0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650,0
cm-1
3281,79
2217,43
1665,12
1586,85
1543,45
1515,24
1450,40
1419,46
1367,42
1308,49
1293,73
1259,90
1240,08
1171,45
1141,80
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834,92
763,15
698,53
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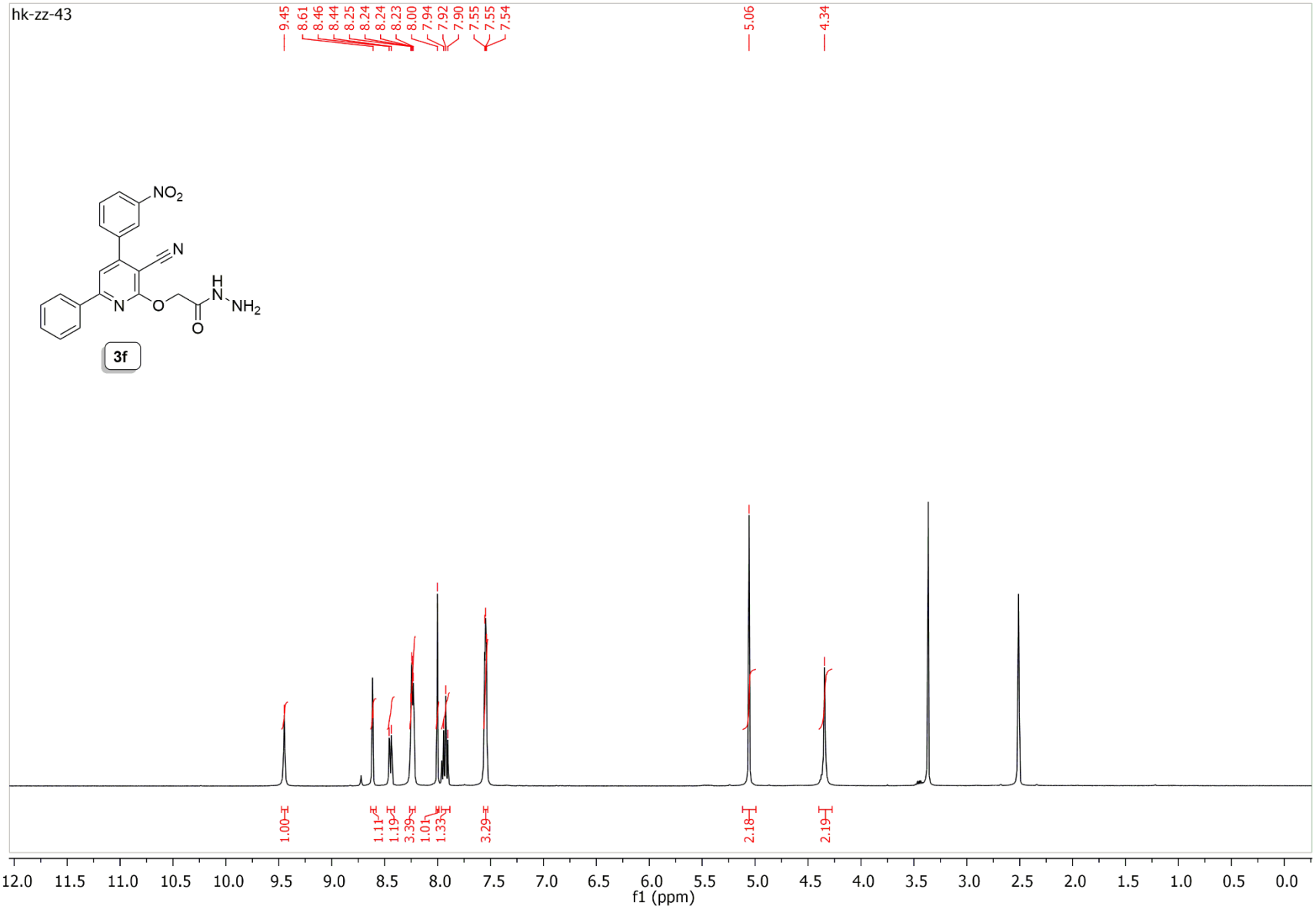
N
H
N
NH2
O
O
O
3c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8.24
8.24
8.23
8.00
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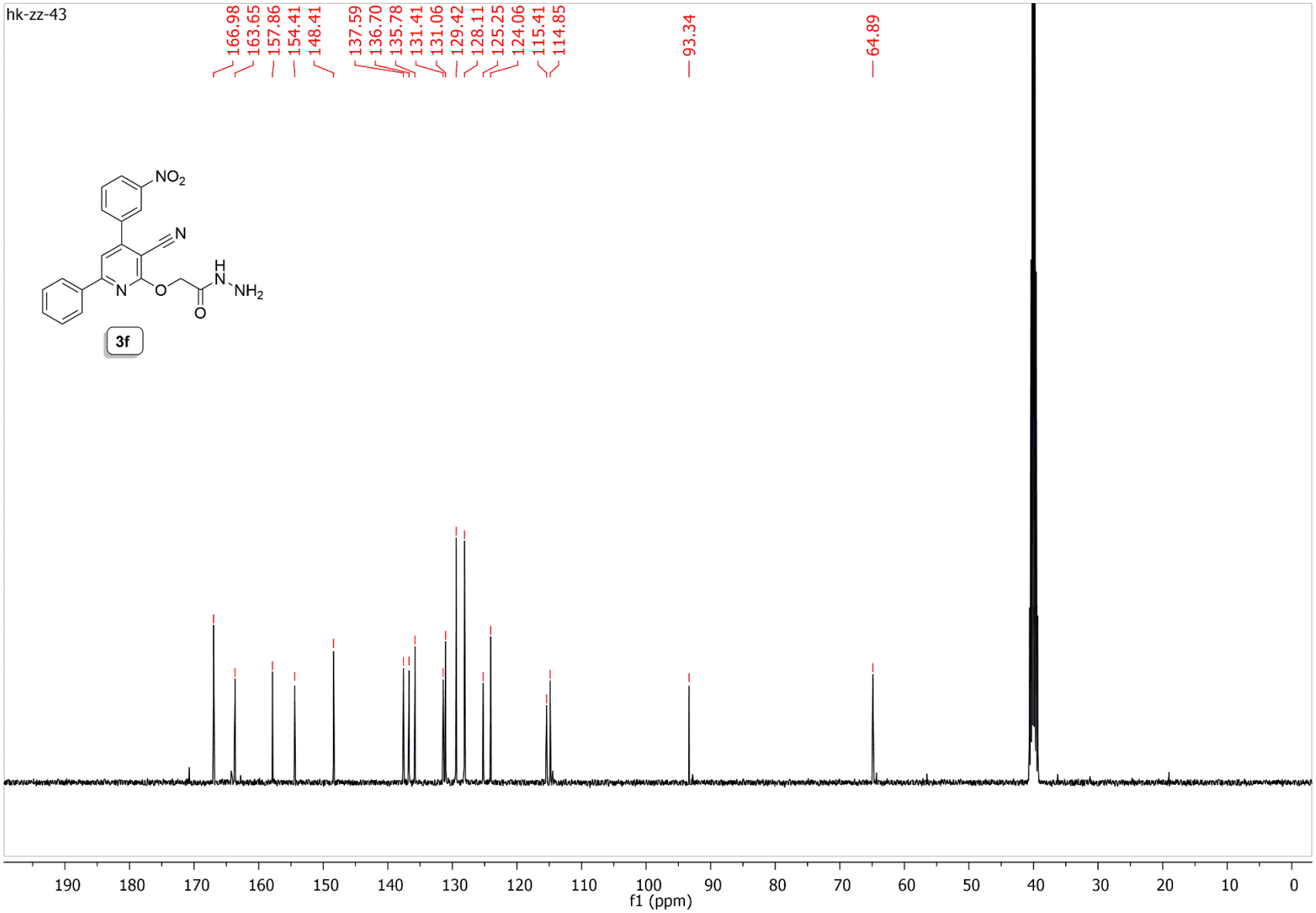
7.92
7.90
7.55
7.55
7.54
5.06
4.34
NO2
N
H
N
NH2
N
O
O
3f
1.00
1.11
1.19
3.39
1.01
1.33
3.29
2.18
2.19
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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137.59
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131.06
129.42
128.11
125.25
124.06
115.41
114.85
93.34
64.89
NO2
N
H
N
NH2
O
N
O
3f
190
180
170
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130
120
110
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90
80
70
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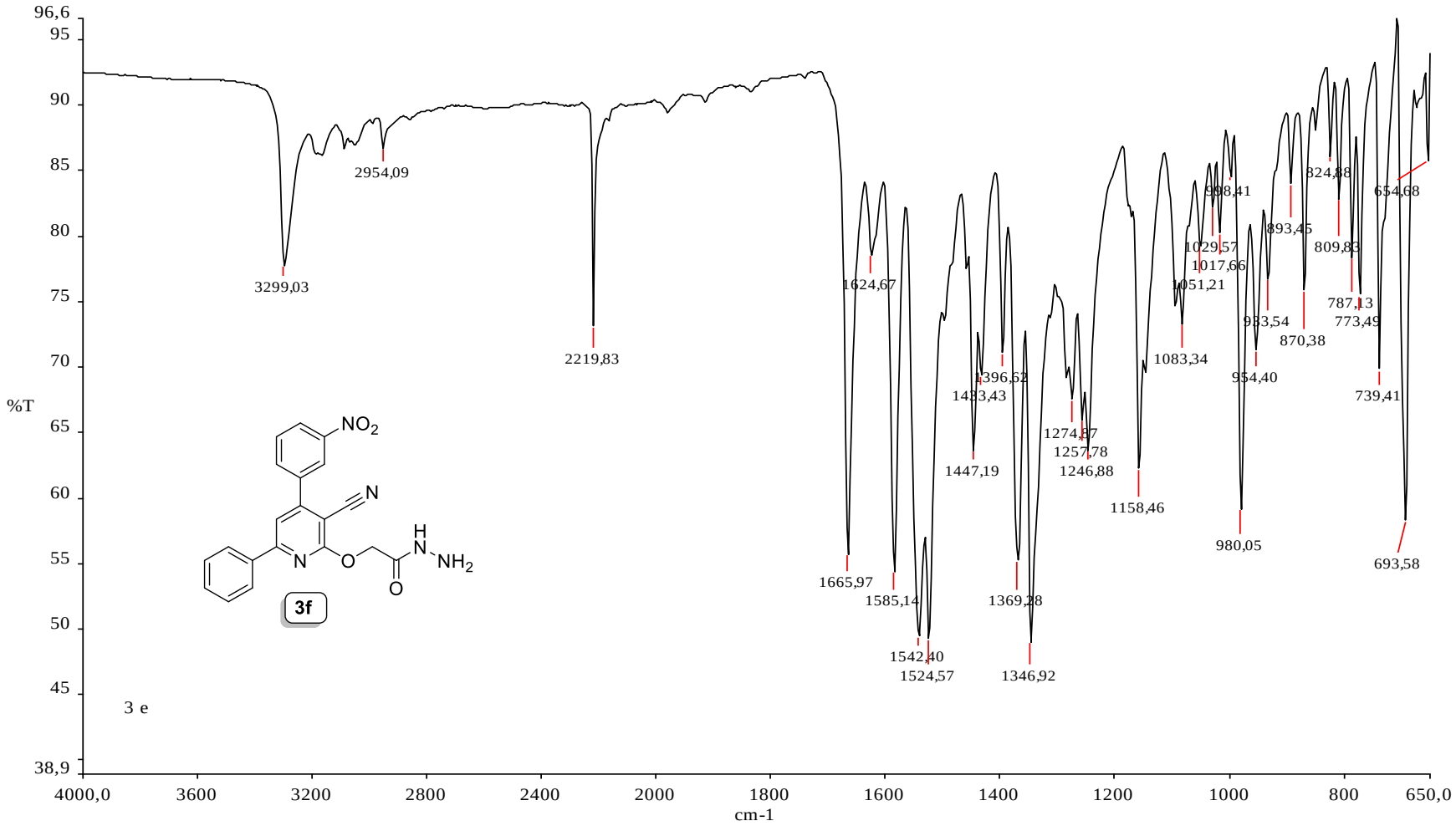
50
40
30
20
10
0
f1 (ppm)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2800
2400
2000
1800
1600
1400
1200
1000
800
650,0
cm-1
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2954,09
2219,83
1624,67
1665,97
1585,14
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1524,57
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1369,28
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1257,78
1246,88
1158,46
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1017,66
998,41
980,05
954,40
933,54
893,45
870,38
824,88
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739,41
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654,68
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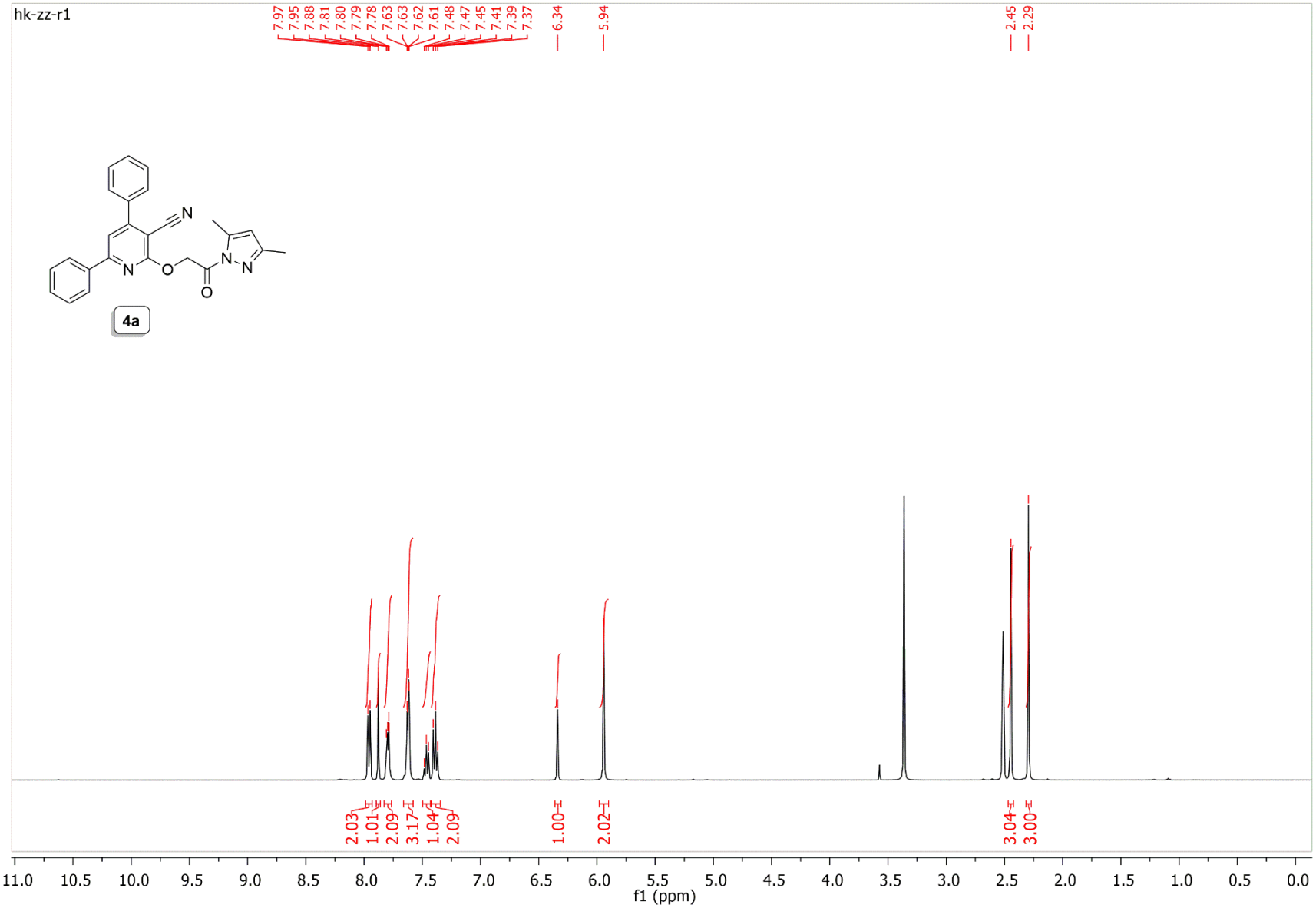
O
H
N
NH2
O
3f
3 e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7.63
7.63
7.62
7.61
7.48
7.47
7.45
7.41
7.39
7.37
6.34
5.94
2.45
2.29
N
N
O
N
N
O
4a
2.03
1.01
2.09
3.17
1.04
2.09
1.00
2.02
3.04
3.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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3.5
3.0
2.5
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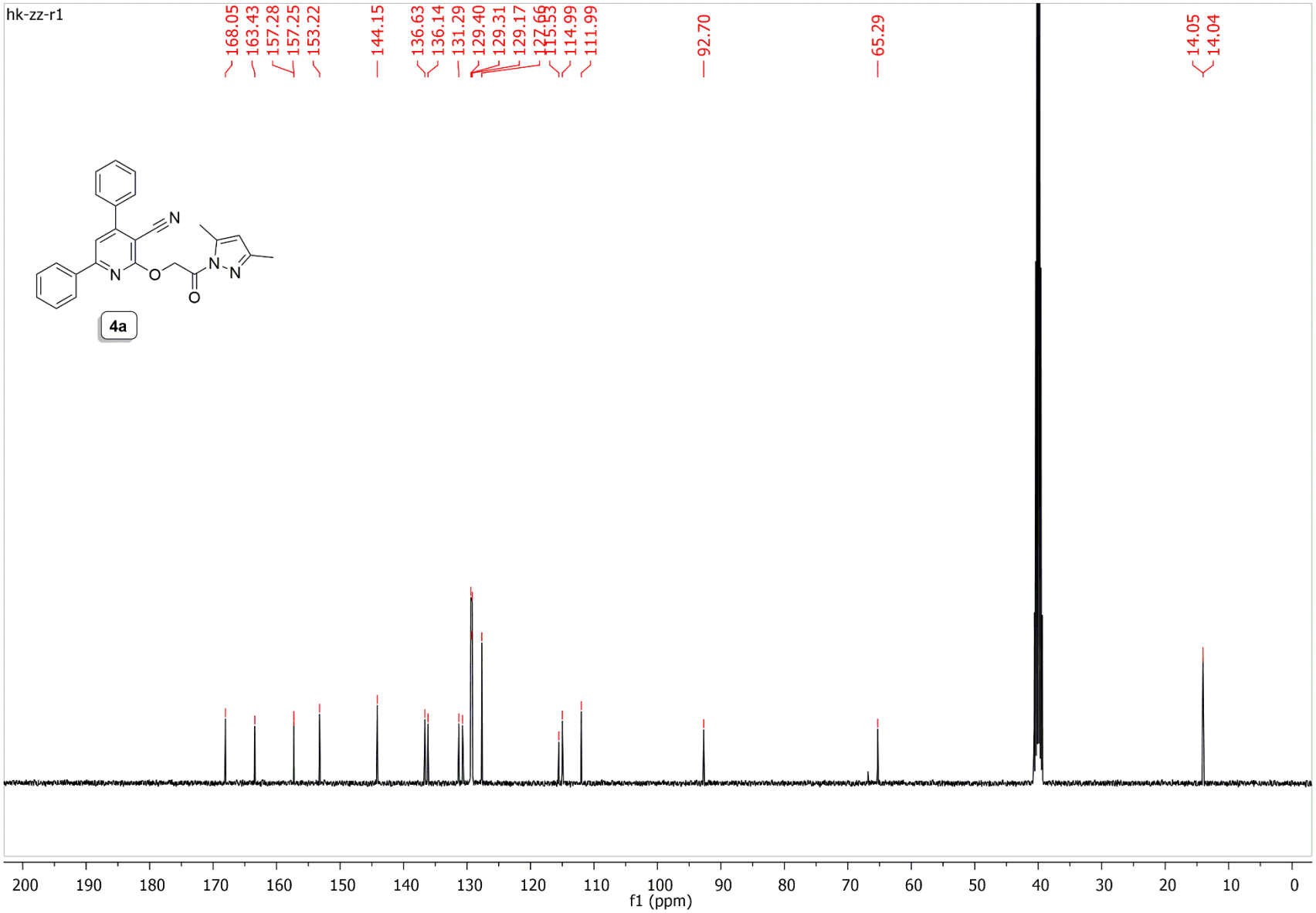
1.0
0.5
0.0
f1 (ppm)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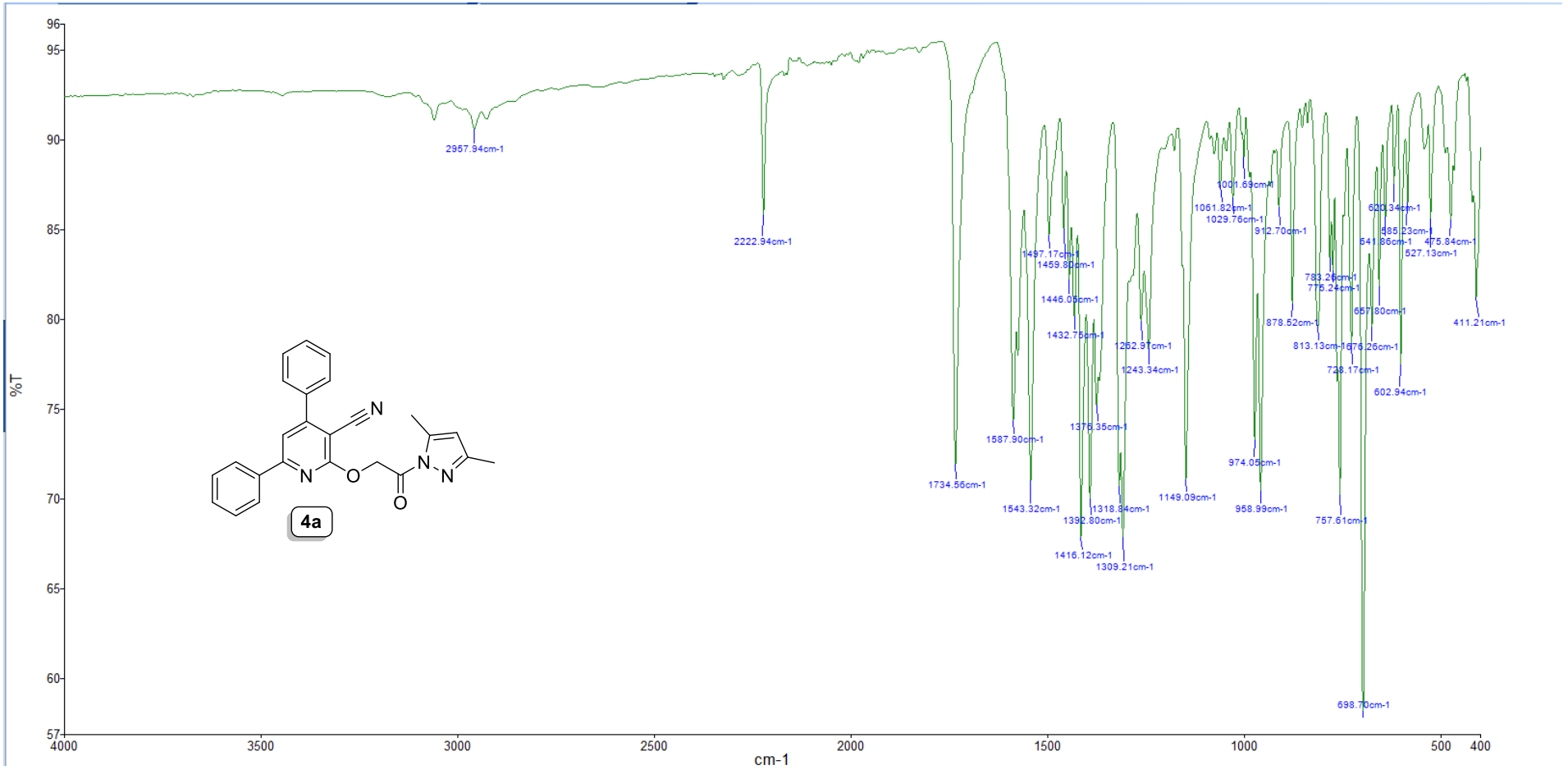
168.05
163.43
157.28
157.25
153.22
144.15
136.63
136.14
131.29
129.40
129.31
129.17
127.66
115.53
114.99
111.99
92.70
65.29
14.05
14.04
N
N
O
N
N
O
4a
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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1318.84cm-1
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1001.69cm-1
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912.70cm-1
878.52cm-1
813.13cm-1
783.26cm-1
775.24cm-1
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698.70cm-1
676.26cm-1
657.80cm-1
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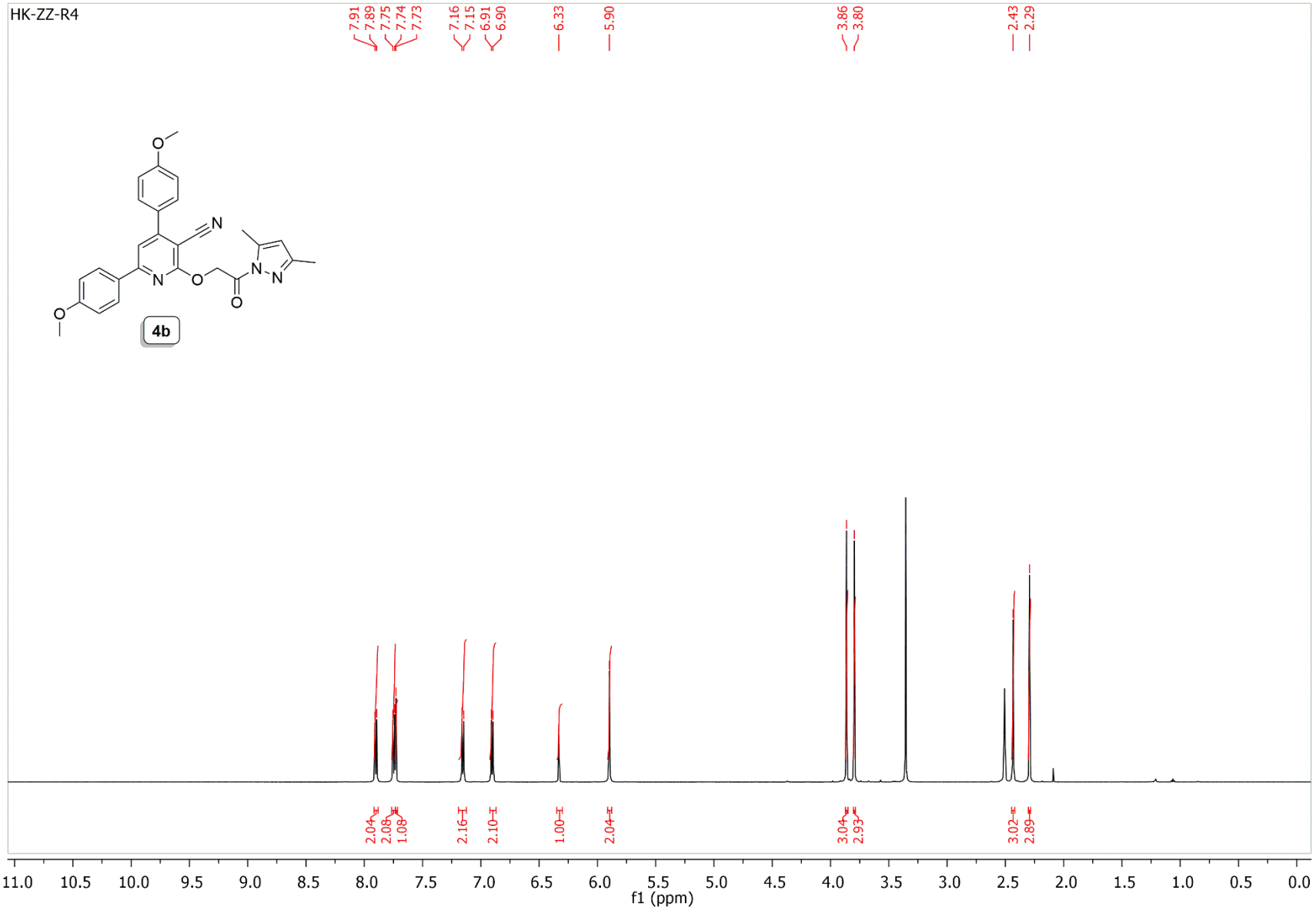
4a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7.75
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6.91
6.90
6.33
5.90
3.86
3.80
2.43
2.29
4b
2.04
2.08
1.08
2.16
2.10
1.00
2.04
3.04
2.93
3.02
2.89
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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3.5
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2.5
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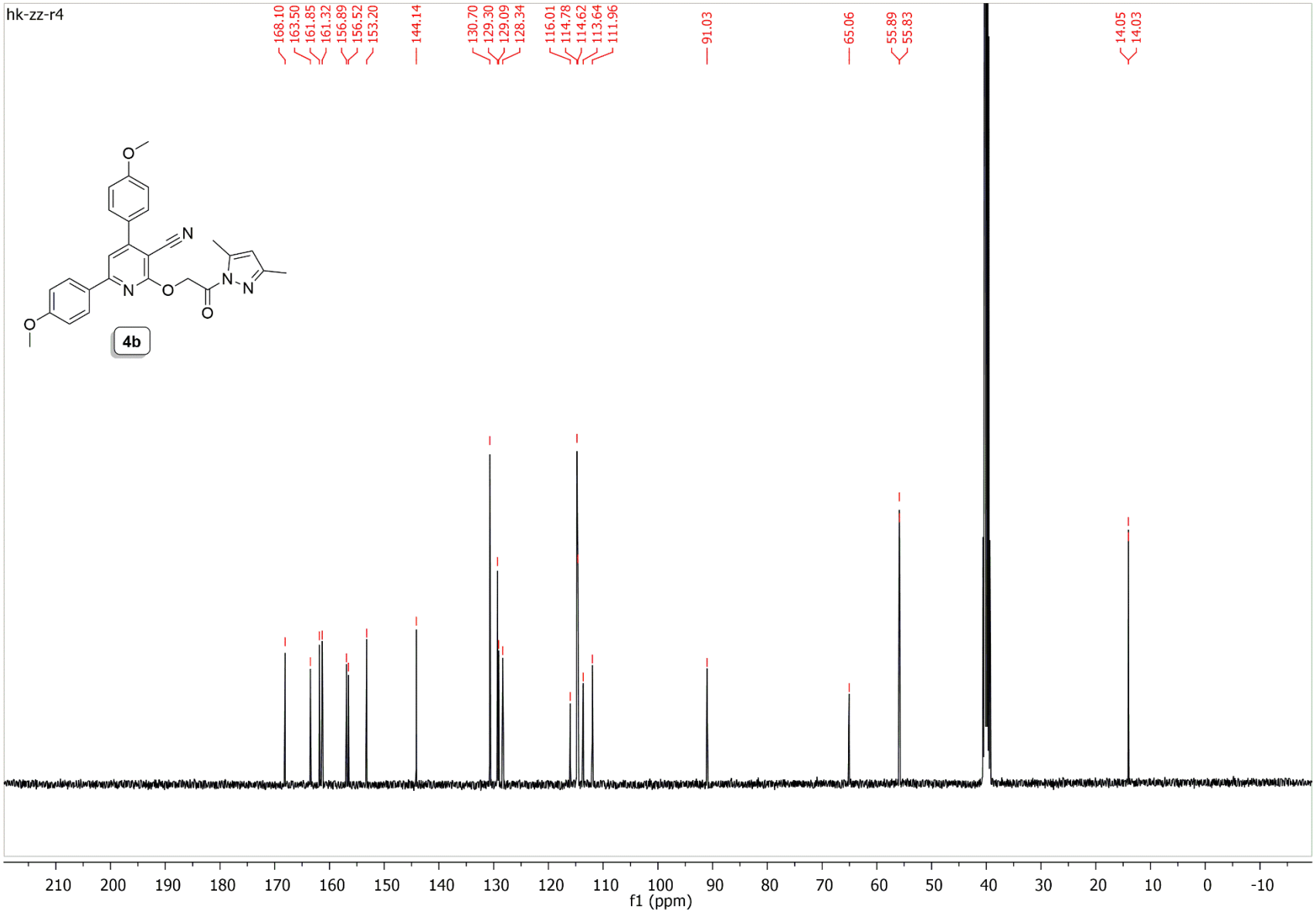
0.0
f1 (ppm)


hk-zz-r4
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161.32
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156.52
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129.30
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128.34
116.01
114.78
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113.64
111.96
91.03
65.06
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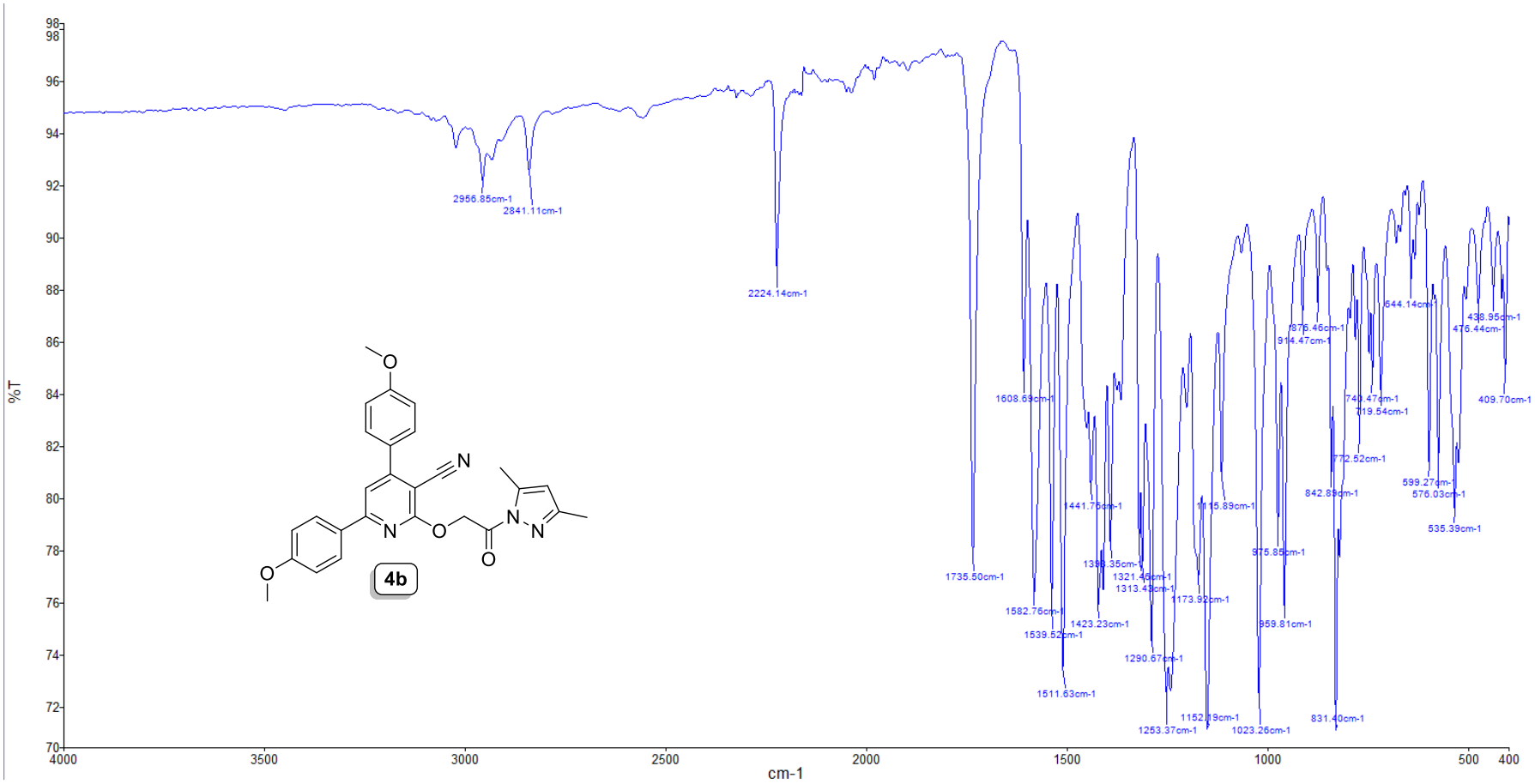
55.83
14.05
14.03
4b
f1 (ppm)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1441.75cm-1
1423.23cm-1
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1173.92cm-1
1152.19cm-1
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1023.26cm-1
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959.81cm-1
914.47cm-1
876.46cm-1
842.89cm-1
831.40cm-1
772.52cm-1
749.47cm-1
719.54cm-1
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476.44cm-1
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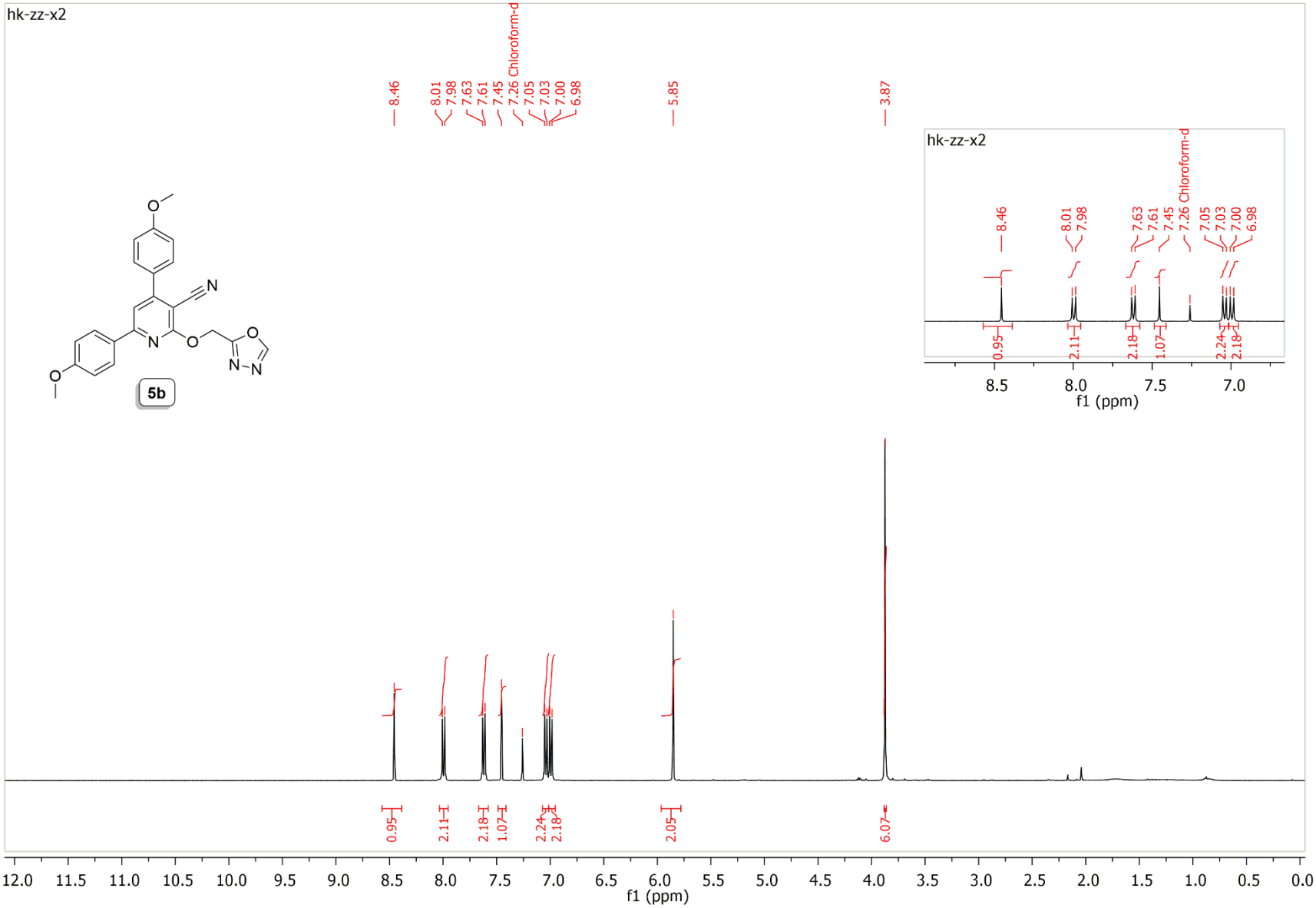
409.70cm-1
4b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8.01
7.98
7.63
7.61
7.45
7.26 Chloroform-d
7.05
7.03
7.00
6.98
5.85
3.87
5b
0.95
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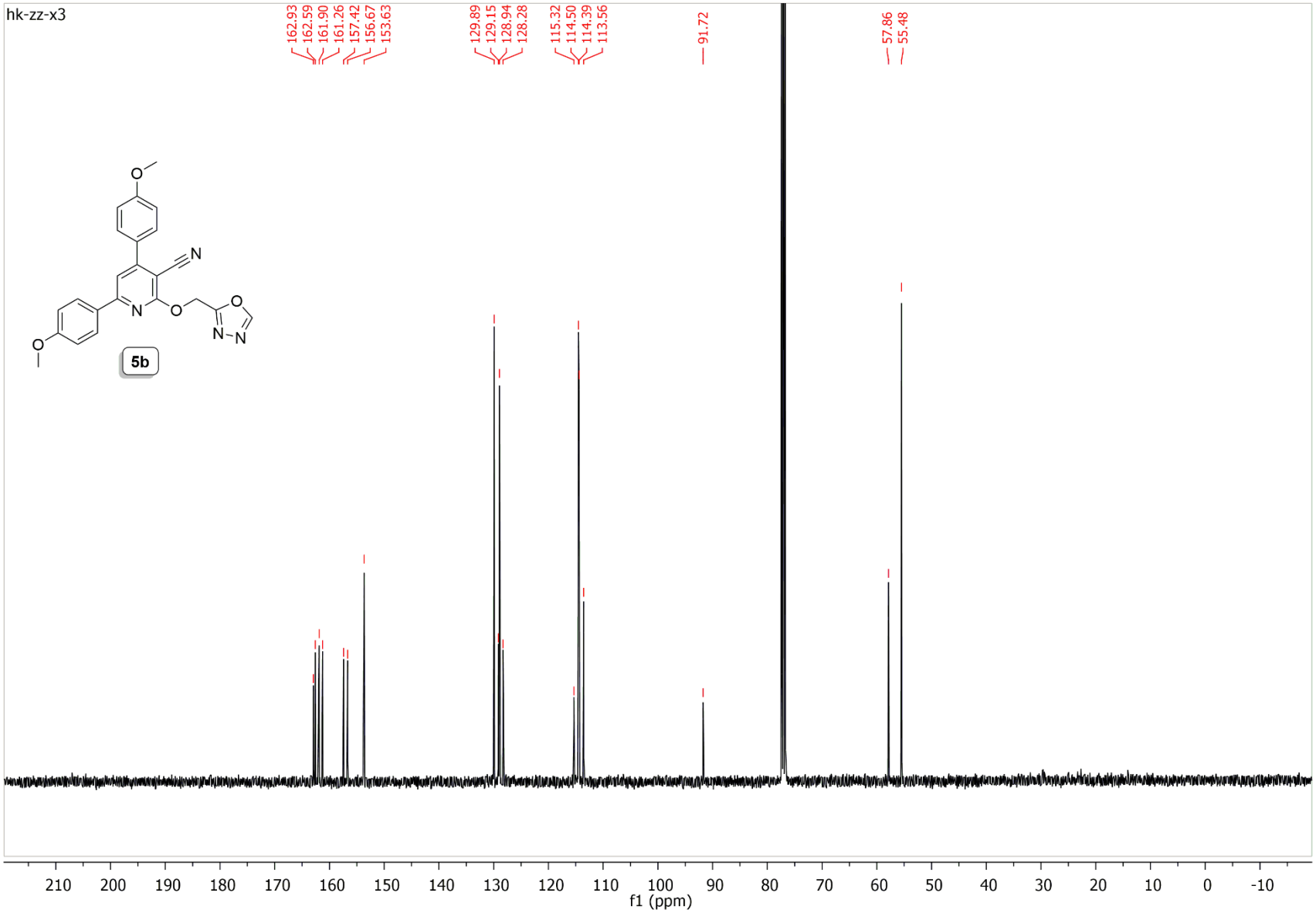
2.18
1.07
2.24
2.18
2.05
6.07
f1 (ppm)
hk-zz-x2
f1 (ppm)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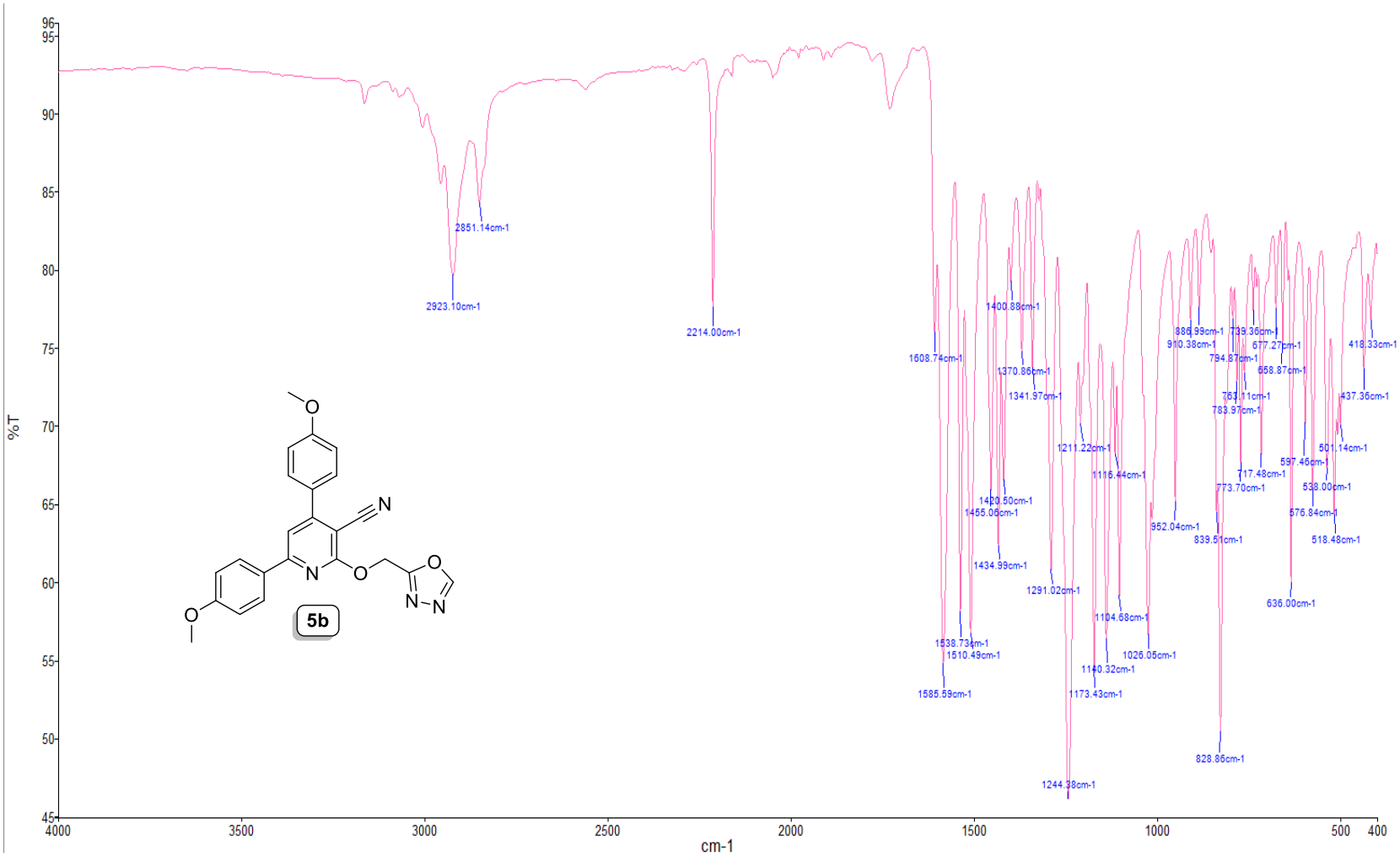
162.59
161.90
161.26
157.42
156.67
153.63
129.89
129.15
128.94
128.28
115.32
114.50
114.39
113.56
91.72
57.86
55.48
5b
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


%T
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1116.44cm-1
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1026.05cm-1
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910.38cm-1
886.99cm-1
839.51cm-1
828.86cm-1
794.87cm-1
783.97cm-1
773.70cm-1
761.11cm-1
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717.48cm-1
677.27cm-1
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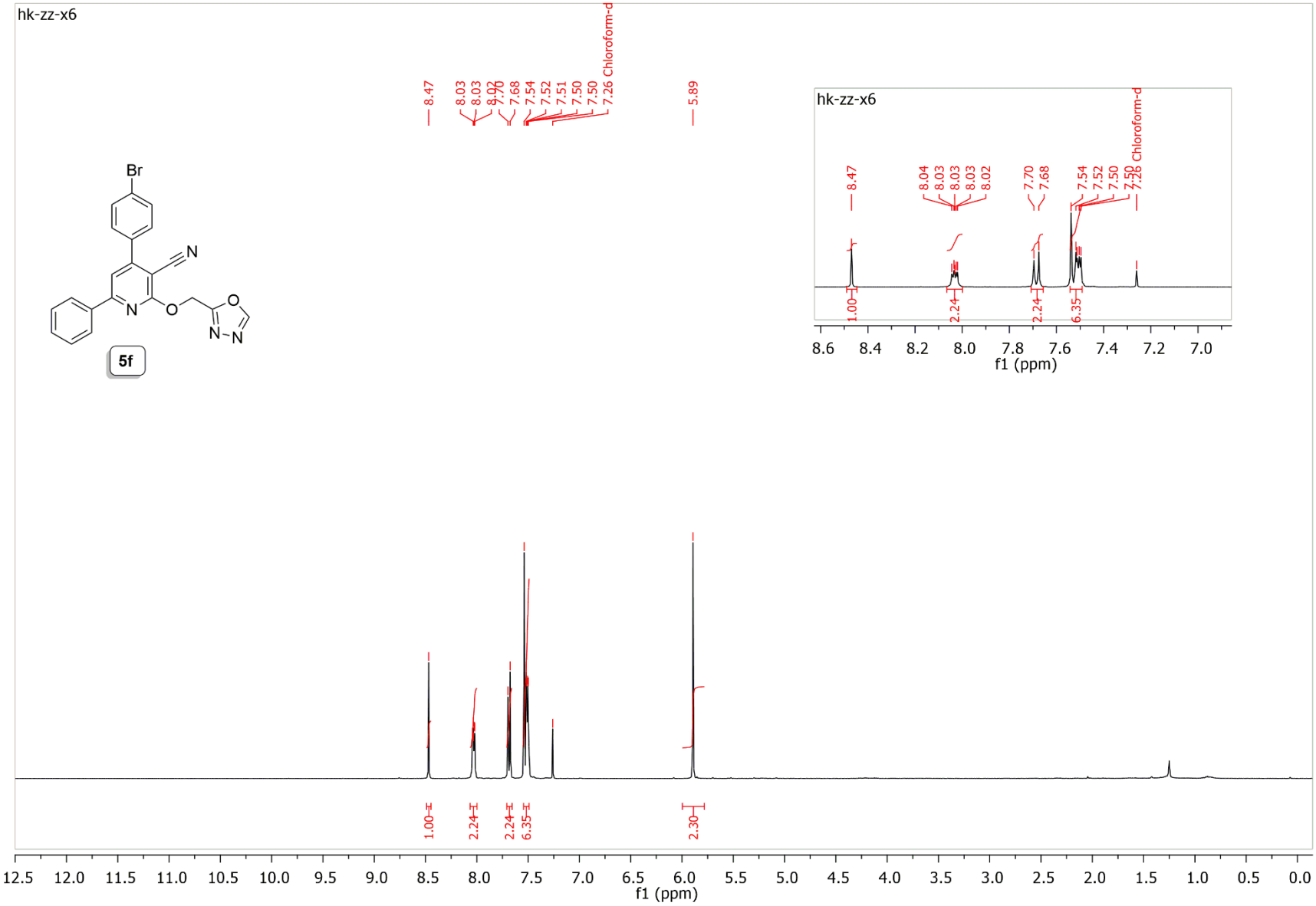
5b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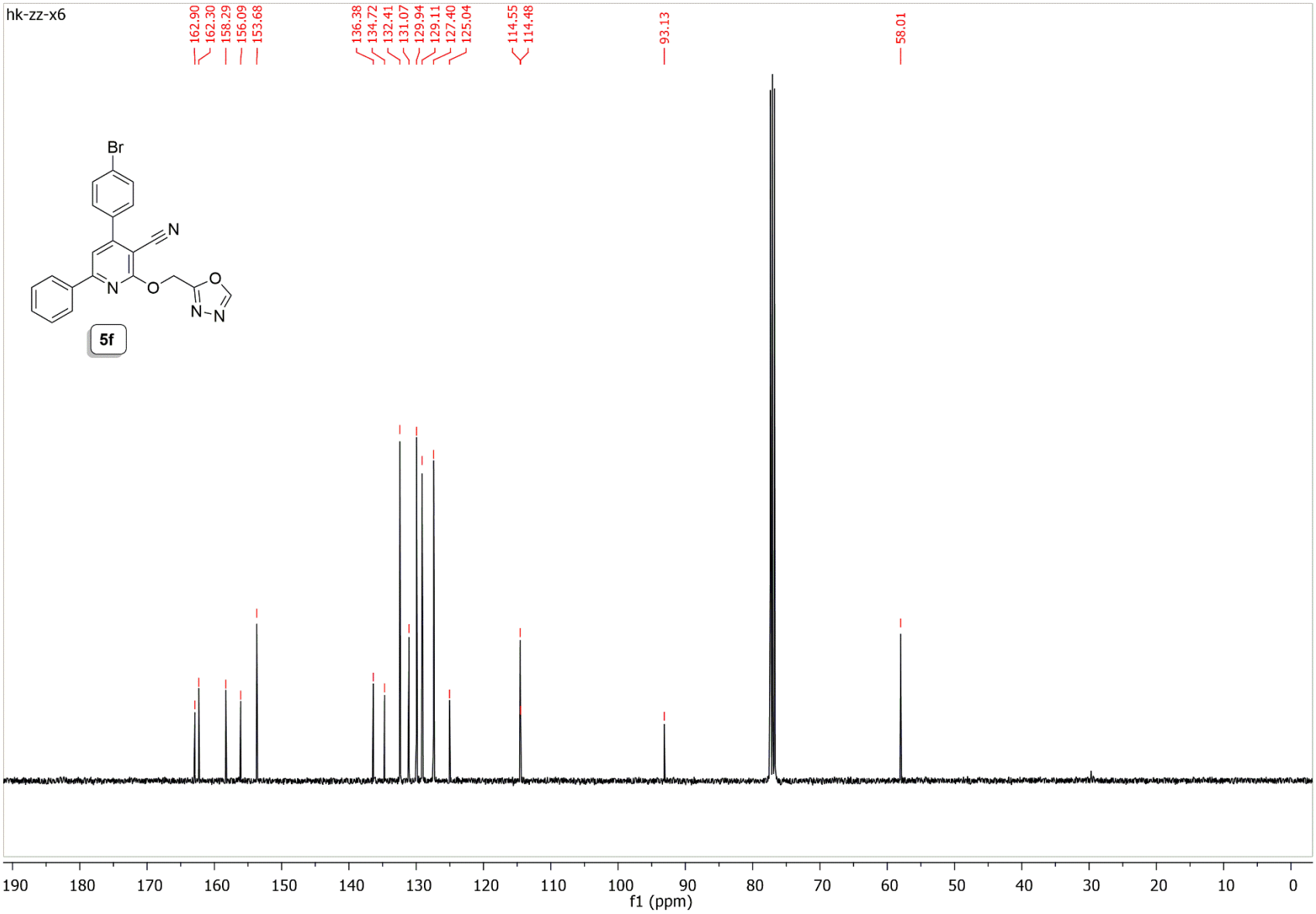
8.03
8.03
8.02
7.70
7.68
7.54
7.52
7.51
7.50
7.50
7.26 Chloroform-d
5.89
Br
N
N
O
O
N-N
5f
1.00
2.24
2.24
6.35
2.30
f1 (ppm)
hk-zz-x6
8.47
8.04
8.03
8.03
8.03
8.02
7.70
7.68
7.54
7.52
7.50
7.50
7.26 Chloroform-d
1.00
2.24
2.24
6.35
f1 (ppm)


hk-zz-x6
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162.30
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153.68
136.38
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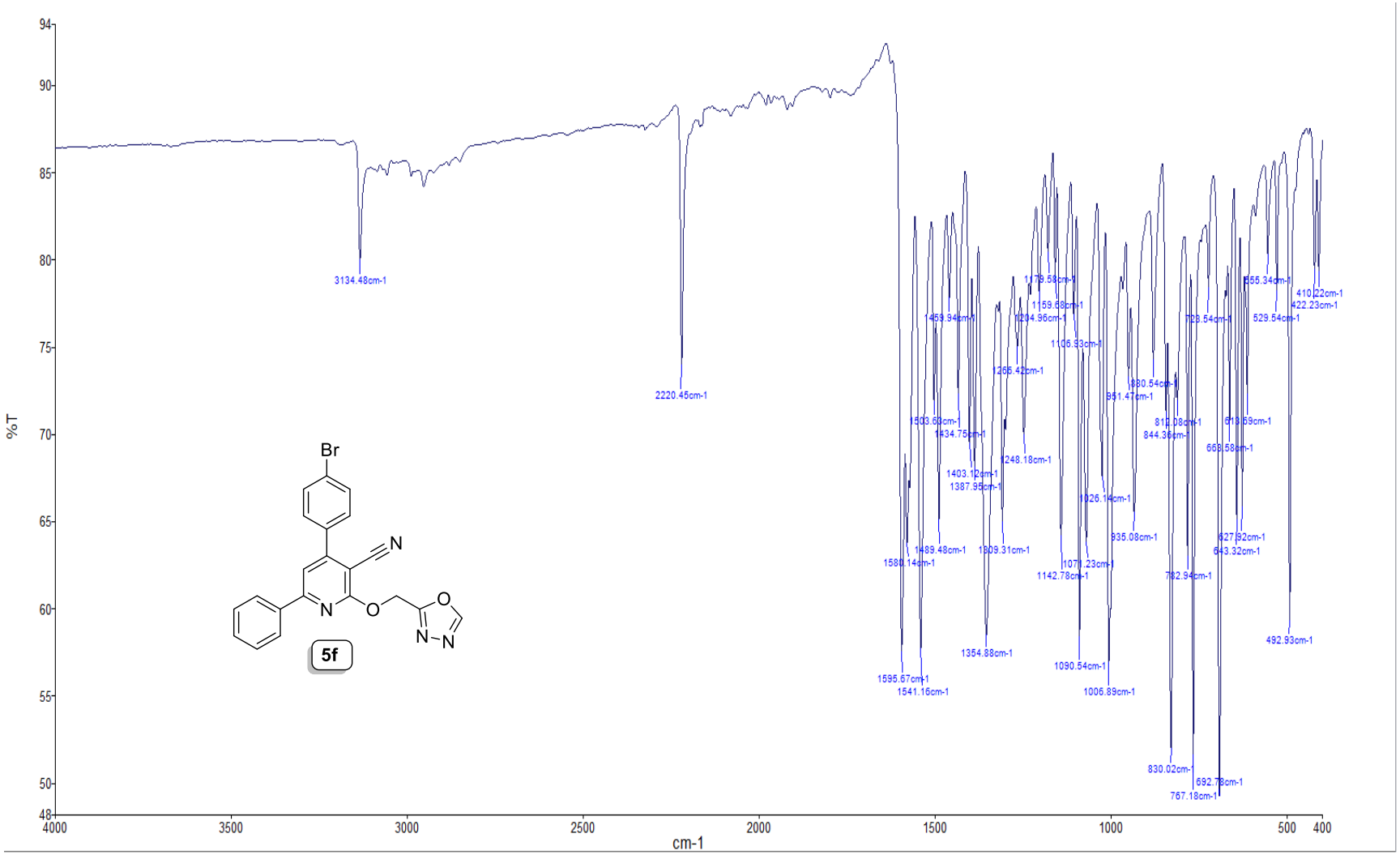
132.41
131.07
129.94
129.11
127.40
125.04
114.55
114.48
93.13
58.01
Br
N
N
O
O
N
N
5f
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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1354.88cm-1
1309.31cm-1
1265.42cm-1
1248.18cm-1
1204.96cm-1
1179.58cm-1
1159.68cm-1
1106.93cm-1
1090.54cm-1
1142.78cm-1
1071.23cm-1
1026.14cm-1
1006.89cm-1
951.47cm-1
935.08cm-1
930.54cm-1
844.36cm-1
830.02cm-1
767.18cm-1
762.94cm-1
729.54cm-1
692.78cm-1
668.58cm-1
643.32cm-1
627.92cm-1
619.59cm-1
565.34cm-1
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492.93cm-1
422.23cm-1
410.22cm-1
Br
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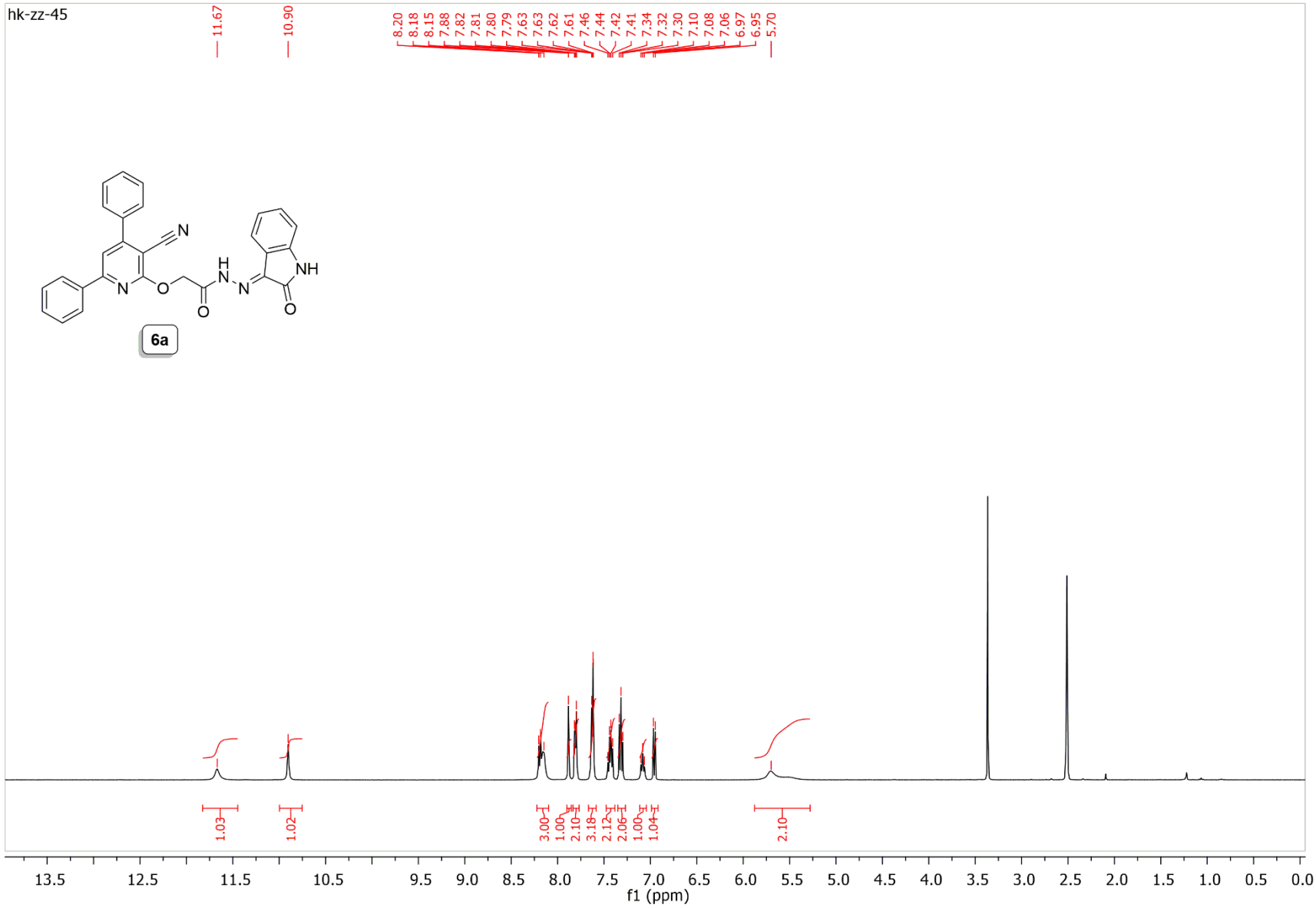
O
N
N
O
5f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7.81
7.80
7.79
7.63
7.63
7.62
7.61
7.46
7.44
7.42
7.41
7.34
7.32
7.30
7.10
7.08
7.06
6.97
6.95
5.70
N
H
N
N
O
O
NH
O
6a
1.03
1.02
3.00
1.00
2.10
3.18
2.12
2.06
1.00
1.04
2.10
13.5
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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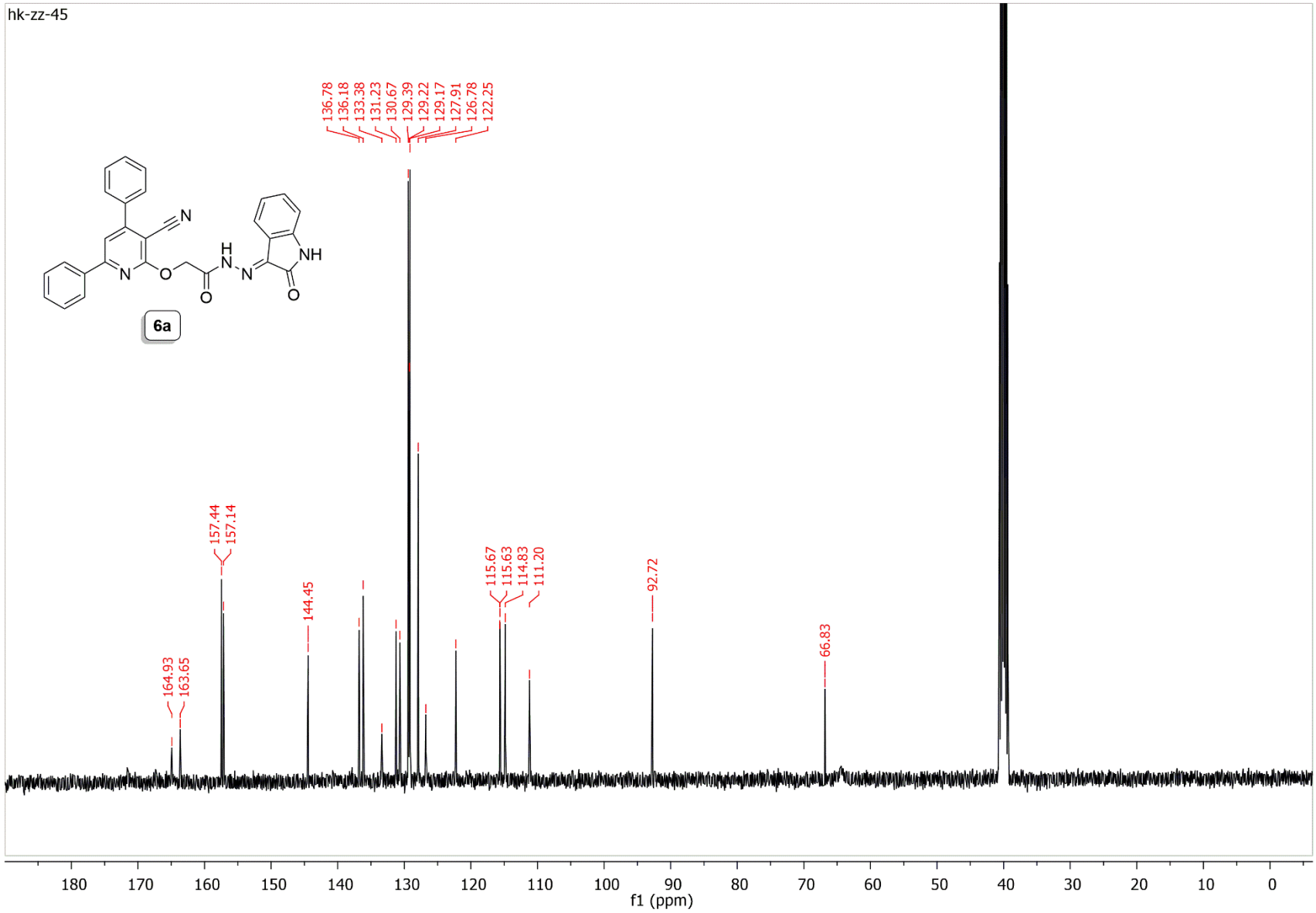
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


hk-zz-45
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131.23
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122.25
N
H
N
N
O
O
NH
O
6a
157.44
157.14
144.45
115.67
115.63
114.83
111.20
92.72
66.83
164.93
163.65
180
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120
110
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70
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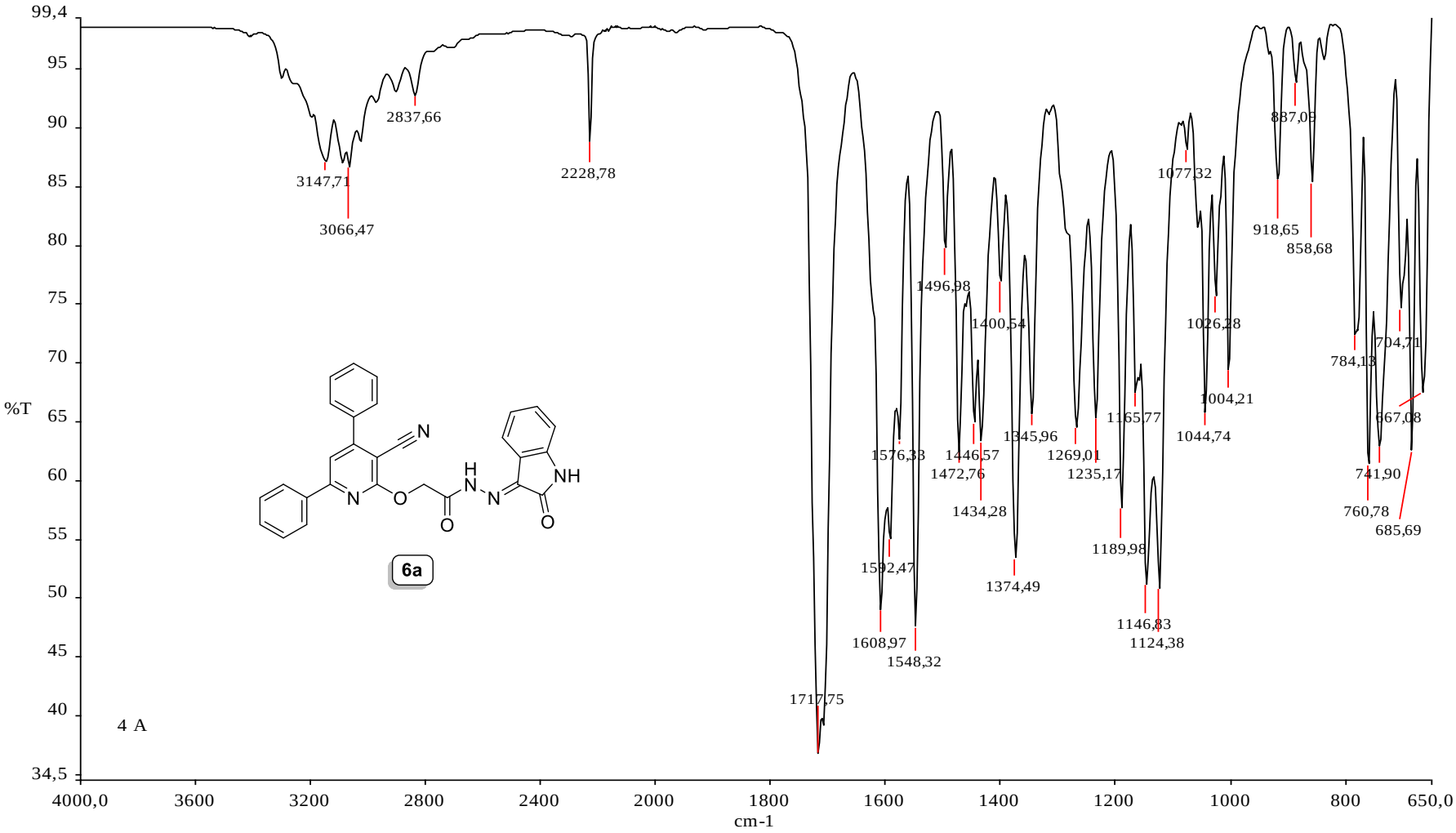
50
40
30
20
10
0
f1 (ppm)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45
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34,5
%T
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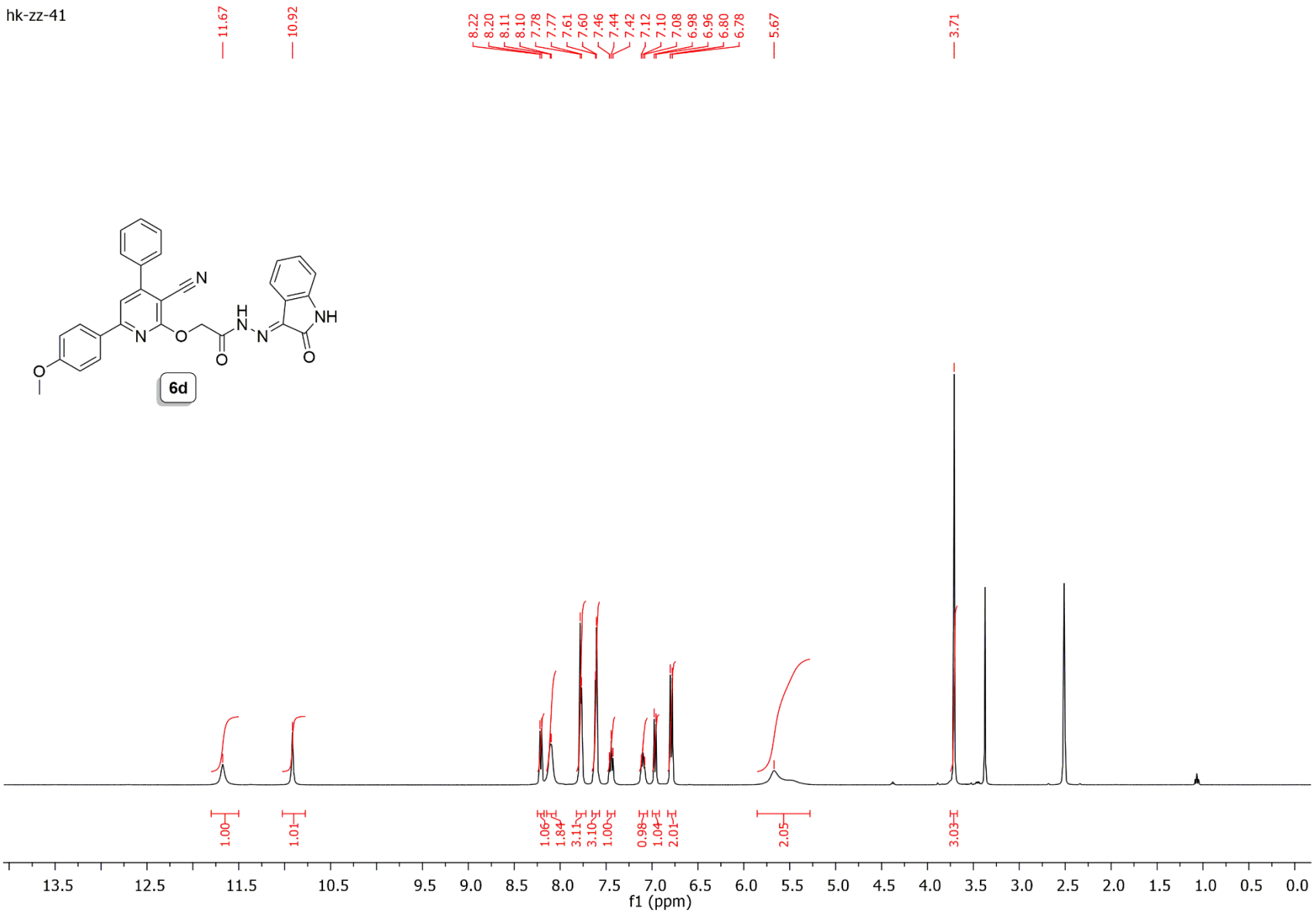
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650,0
cm-1
3147,71
3066,47
2837,66
2228,78
1717,75
1608,97
1592,47
1576,33
1548,32
1496,98
1472,76
1446,57
1434,28
1400,54
1374,49
1345,96
1269,01
1235,17
1189,98
1165,77
1146,83
1124,38
1077,32
1044,74
1026,28
1004,21
918,65
887,09
858,68
784,13
741,90
704,71
685,69
667,08
760,78
4 A
6a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8.11
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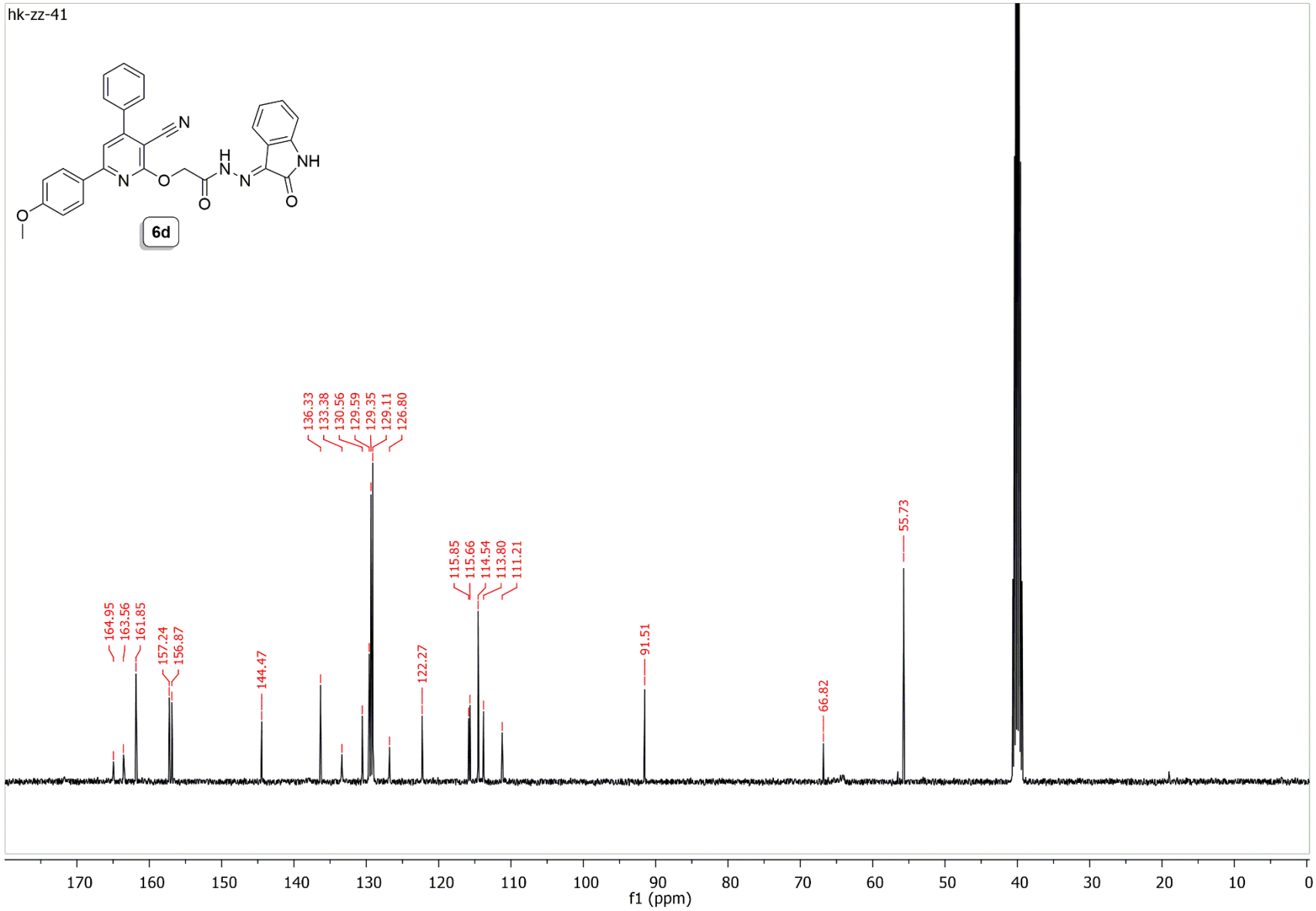
7.78
7.77
7.61
7.60
7.46
7.44
7.42
7.12
7.10
7.08
6.98
6.96
6.80
6.78
5.67
3.71
6d
1.00
1.01
1.06
1.84
3.11
3.10
1.00
0.98
1.04
2.01
2.05
3.03
13.5
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


hk-zz-41
6d
164.95
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161.85
157.24
156.87
144.47
136.33
133.38
130.56
129.59
129.35
129.11
126.80
122.27
115.85
115.66
114.54
113.80
111.21
91.51
66.82
55.73
170
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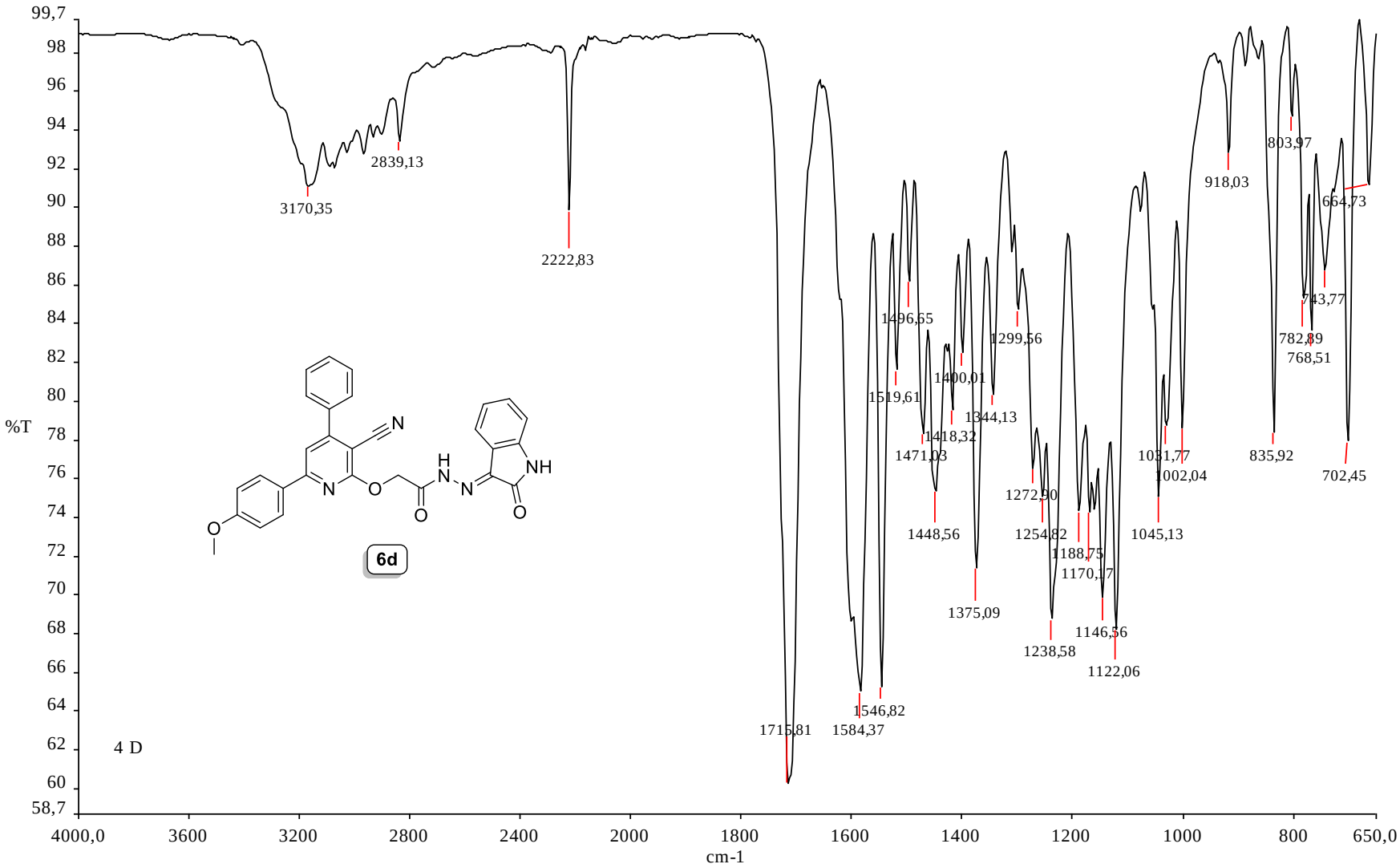
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


99,7
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94
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86
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82
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76
74
72
70
68
66
64
62
60
58,7
%T
4000,0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650,0
cm-1
3170,35
2839,13
2222,83
1715,81
1584,37
1546,82
1519,61
1496,65
1471,03
1448,56
1418,32
1400,01
1375,09
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1002,04
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803,97
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768,51
743,77
702,45
664,73
N
H
N
N
O
O
NH
O
6d
4 D

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FULL PAPER

ARCH PHARM DPhG Archiv der Pharmazie

Synthesis and anticancer properties of novel hydrazone derivatives incorporating pyridine and isatin moieties

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Abstract

Nine novel hydrazone derivatives (**4a–i**) incorporating pyridine and isatin moieties were synthesized through one-pot, four-component heterocyclic condensation reactions. The structures of all new compounds (**2a–e**, **3a**, **3c–e**, and **4a–e**) were identified by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and Fourier-transform infrared spectroscopic techniques and elemental analysis. Cell viability assays for the tested hydrazone derivatives were performed and the log IC_{50} values of the compounds were calculated after a 24-h treatment. All hydrazide derivatives tested showed a promising antitumor activity against A-2780 cells as compared with the standard drug docetaxel with a log IC_{50} value of 0.2200 μM ($p < .05$). Seven of the examined compounds (**4b–e**, **4g–i**) showed high cytotoxic activity against A-2780 cells as compared with the standard drug docetaxel. Whereas the log IC_{50} of docetaxel was 0.2200 μM for A-2780 cells at 24 h, the IC_{50} values of these compounds were −0.4987, −0.4044, −0.8138, −0.3868, −0.6954, −0.4751, and 0.1809 μM, respectively. Three of the compounds, **4b**, **4d**, and **4i**, showed high cytotoxic activity against MCF-7 cells as compared with docetaxel ($p < .05$). Whereas the log IC_{50} of docetaxel was 0.2400 μM for MCF-7 cells at 24 h, the log IC_{50} values of compounds **4b**, **4d**, and **4i** were −0.1293, −0.1700, and 0.2459 μM, respectively.

KEYWORDS

anticancer activity, hydrazone derivatives, isatin derivatives, pyridine derivatives

1 | INTRODUCTION

Due to the increase in cancer incidence, scientific research on cancer morbidity and mortality and improvement in the quality of life of cancer patients is increasing rapidly. Cancer is a serious health concern in all societies, regardless of wealth or social status.

In 2018, 18.1 million people worldwide suffered from cancer and 9.6 million patients died from the disease. By 2040, these figures will almost double, and the largest increase will be noticed in low- and middle-income countries where more than two-thirds of world cancer cases occur.[1] Over the last two decades, hydrazide–hydrazone derivatives have been known as one of the most important groups in medicinal chemistry,[2–5] and they are present in a large number of compounds due to their diverse biological properties such as anti-inflammatory,[6] antimicrobial,[7,8] analgesic,[9] anti-HIV,[10] anticonvulsant,[11,12] antileishmanial,[13,14] antimalarial,[15] antitumor,[16–19] and antitubercular activities.[20] Furthermore, hydrazide–hydrazones are increasingly considered to be a valuable core in medicinal chemistry.[21–24] However, isatin is a natural product discovered in the early 19th century in the genus *Isatis* and in *Couroupita guianensis* Aubl. plants,[25,26] and it is known as oxidized indole that attracts much attention as a building block in the design of countless compounds. Isatin has a wide variety of biological and pharmacological activities such as antifungal and

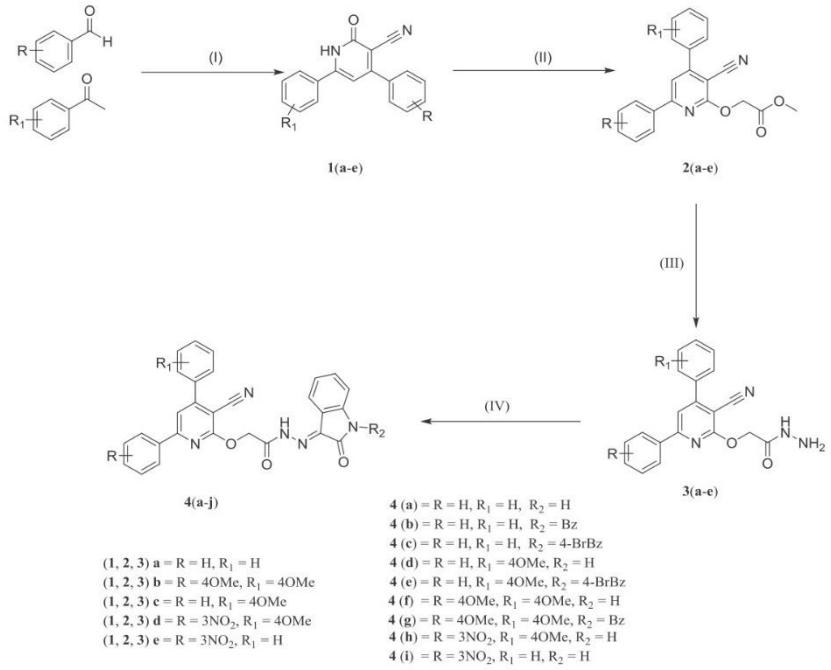
antibacterial properties[27] and potent caspase inhibitory[28,29] and anticancer activities.[30] Schiff bases of isatin have a wide range of pharmacological activities including antismallpox,[31] anti-inflammatory,[32] antibacterial,[33] antiviral,[34] and antitubercular activities[35]; they also act as a GAL3 receptor antagonist.[36] However, 3-cyano-2-pyridones show remarkable biological and pharmacological properties, particularly antidepressant,[37] anticancer,[38,39] antimicrobial activity,[40] and p38 MAP kinase inhibitory capabilities.[41] Although the developments in chemotherapy increase the fight against cancer, numerous side effects of the drugs used pose serious problems in cancer treatment. A great number of drugs have been used to treat cancer, such as *cis*-platinum and ruthenium compounds, but good treatable values have not been achieved with existing drugs. To eliminate these disadvantages, increasing numbers of studies are continuously being carried out in this area. We have also synthesized and tested anticancer activities of many heterocyclic compounds against several cell lines such as A-2780 (human ovarian),[42,43] PC-3 (human prostate),[43] DU-145 (human prostate),[42] A549 (human lung cancer),[44] and BEAS-2B (human lung cancer).[44] As a part of these research works, we planned to synthesize a number of new hydrazone derivatives incorporating pyridine and isatin moieties and examine their anticancer activities against A-2780 and MCF-7 cell lines.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthetic pathways utilized to prepare the new hydrazone derivatives are depicted in Scheme 1.

Different cyanopyridinones **1a–e** were prepared as a starting material via one-pot, four-component heterocyclic condensation process, as reported in the literature.[39] The reaction of **1a–e** with methyl bromoacetate in the presence of anhydrous K_2CO_3 in dimethylformamide (DMF) yielded the corresponding methyl ester derivatives **2a–e**. In infrared data, the presence of $\upsilon_{(COOR)}$ at around 1750 cm^{-1} and the disappearance of the amide carbonyl band at position 2 of the pyridine core prove that the desired ester derivatives (**2a–e**) are synthesized. The singlet peaks around 5.00 ppm for compounds **2a–e** were assigned to the OCH_2 protons of the acetyl group, whereas the singlet signals around 3.70 ppm were assigned to the methyl protons of the ester group for compounds **2a–e**. The ^{13}C nuclear magnetic resonance (NMR) spectra of **2a–e** showed three signals around δ 168.9, 55.47, and 52.24 ppm, corresponding to (C=O, ester), (OCH_3), and CH_2O, respectively. The reaction of esters (**2a–e**) with hydrazine monohydrate in boiling THF yielded the corresponding acetohydrazides **3a–e**. The optimization studies were



SCHEME 1 Synthetic pathway for compounds **1–4**. Reagents and conditions: (I) $CNCH_2COOEt/CH_3COONH_4$/EtOH/reflux; (II) methyl bromoacetate/anhydrous K_2CO_3/dimethylformamide/RT; (III) N_2H_4 100%/tetrahydrofuran/reflux; (IV) appropriate isatin derivative/1,4-dioxane/10% *p*-toluenesulfonic acid/RT

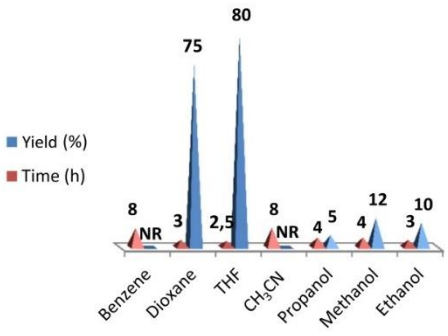


FIGURE 1 The effect of solvent with time in the reaction. THF, tetrahydrofuran

accomplished for the current reaction using **2a** (1.0 eq) and hydrazine monohydrate (4 eq). The use of THF has proven to be essential to achieve maximum conversion for this reaction. Attempts to use other solvents (such as ethanol, methanol, propanol, dioxane, CH_3CN, and benzene) resulted in lower yields or no reaction (Figure 1). The use of protic solvents such as ethanol, methanol, and propanol generated a side product, as indicated in Scheme 2. The infrared spectra of **3a–e** show an absorption peak at around 3333.42 and 1675.80 cm^{-1} for NH/NH_2 and the amide carbonyl groups, respectively, which confirms the presence of the hydrazide group. The ^1H NMR data confirm the presence of the NH_2 and NH peaks in the regions 4.33–4.37 and 9.43–9.45 ppm, respectively, and the disappearance of OCH_3 protons. The ^{13}C NMR spectra of compounds **3a–e** confirmed the absence of the methoxy carbon atom.

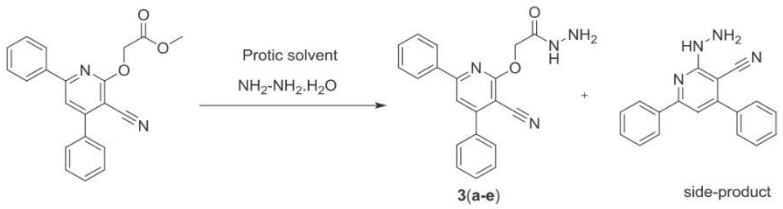
The condensation of hydrazides **3a–e** with different isatins in dioxane containing 10% PTSOH (*p*-toluenesulfonic acid) at room temperature afforded the corresponding isatin Schiff base derivatives **4a–i**. The infrared spectra of these compounds showed the appearance of NH stretching bands in the range of 3301–3089 cm^{-1}. The ^1H NMR spectra of **4a–i** also showed two D_2O exchangeable signals due to the NH group of isatin (**4a**, **4d**, **4f**, **4h**, and **4i**) in the range of 10.90–10.93 ppm and NH of the hydrazide linker in the range of 11.66–11.80 ppm. All other spectral data were in accordance with the assumed structures.

According to the literature,[45] the *N*-substituted hydrazones may exist as *Z*/*E* geometrical isomers about C=N double bonds and as *cis*/*trans* amide conformers (Scheme 3). In this respect, the ^1H NMR spectra of **4a–i** in dimethyl sulfoxide (DMSO)-d_6 showed only the presence of *E* conformers; however, the spectra for compounds **4i** and **4g** revealed two sets of protons at 13.49–12.61 and 5.80–5.61 ppm, which are attributed to CO–NH amide and OCH_2 due to *cis* and *trans* conformers. According to the literature, the *cis* conformers of amide were assigned to upfield signals of CO–NH and OCH_2 protons, whereas the downfield peaks were caused by the *trans* conformer.

The *N*-benzylisatin derivatives used in this study were prepared from isatin and an appropriate benzyl halide in the presence of anhydrous K_2CO_3 and KI in DMF, according to the literature.[46,47] The structures of all isatin derivatives were confirmed by ^1H NMR and ^{13}C NMR, and melting point data and all spectral and analytical data were in accordance with the literature values.[46,47]

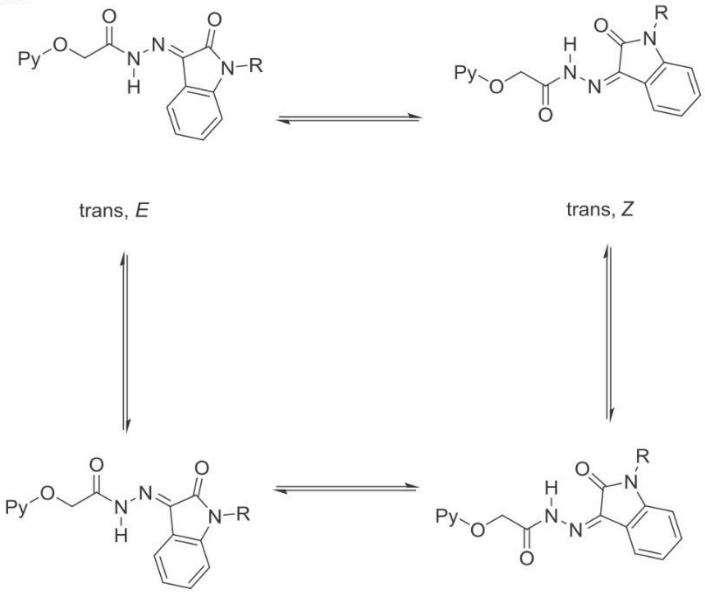
2.2 | Cytotoxicity study

Cytotoxic activities of nine new hydrazone derivatives including pyridine and isatin moieties were determined using human ovarian (A-2780) and human breast cancer (MCF-7) cell lines. To obtain the cytotoxic properties of the newly synthesized hydrazone derivatives on A-2780 and MCF-7 cells, the respective cell lines were incubated with increasing concentrations (0–100 μg/ml) of the compounds for 48 h and then subjected to an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Among the tested nine hydrazone derivatives, those bearing hydrogen atoms for R, R_1, and R_2 substituents and with 4-methoxy substituent for R, R_1, and hydrogen for R_2 at position 1 of the isatin moiety showed a lower cytotoxicity on A-2780 cells than the other compounds and the standard drug docetaxel at 0.1 μM. A nearly similar result was obtained at 1 μM concentration for the A-2780 cell line. The cell viability results of A-2780 and MCF-7 cells after a 48-h treatment with the nine hydrazone derivatives are shown in Tables 1 and 2, respectively. As shown in Table 1, all tested hydrazone derivatives demonstrated an anticancer activity against the A-2780 cell line at 0.1, 1, 10, and 100 μM concentrations with $p < .05$, except compounds **4a** and **4f** at 0.1 μM concentration. Similar to A-2780 cell line results, all tested compounds show a cytotoxic activity against MCF-7 cell line at 0.1, 1, 10, and 100 μM concentrations with $p < .05$, except compounds **4a**, **4f**, and **4h** at 0.1 μM concentration. Among the tested nine hydrazone derivatives,



SCHEME 2 The condensation of ester with hydrazine under protic solvents conditions

SCHEME 3 *E/Z* geometrical isomers about the C=N double bond and *cis/trans* amide conformers of the *N*-substituted hydrazones



compounds **4b** and **4d** showed a better cytotoxicity against MCF-7 cells as compared with the standard drug docetaxel with an inhibitory logarithmic 50 (log IC_{50}) value of 0.24 μM.

A time-dependent cell viability assay for the tested hydrazone derivatives was performed, and the log IC_{50} values of the compounds were calculated after a 24-h treatment. Log IC_{50} values calculated for the A-2780 and MCF-7 cells, based on the results of MTT assays for a 24-h interaction of hydrazone derivatives, are presented in Table 3. As can be seen in Table 3, all tested hydrazone derivatives showed promising antitumor activity against A-2780 cells as compared with the standard drug docetaxel with a log IC_{50} value of 0.22 μM. Among the tested nine hydrazone derivatives, compounds **4b** and **4d** showed better cytotoxicity against MCF-7 cells as compared with the standard drug docetaxel with a log IC_{50} value of 0.24 μM.

3 | CONCLUSIONS

Nine new hydrazone derivatives were synthesized successfully and their anticancer activities were evaluated against human cancer cell lines (A-2780 and MCF-7). Seven out of the nine compounds (**4b**, **4c**, **4d**, **4e**, **4g**, **4h**, and **4i**) showed better anticancer activity than the

TABLE 1 The cell viability results of A-2780 cells after a 48-h treatment with nine hydrazone derivatives[a]

A2780 cell viability (%)						
Compound no.	Control	Solvent (DMSO)	0.1 μM	1 μM	10 μM	100 μM
4a	100.00 ± 3.90	94.72 ± 2.39	83.60 ± 6.70	64.85 ± 5.21*	42.47 ± 3.98*	37.76 ± 5.44*
4b	100.00 ± 3.90	94.72 ± 2.39	55.11 ± 8.36*	23.48 ± 4.43*	18.53 ± 2.70*	17.59 ± 3.08*
4c	100.00 ± 3.90	94.72 ± 2.39	44.80 ± 1.48*	39.28 ± 5.30*	30.10 ± 2.49*	9.74 ± 1.08*
4d	100.00 ± 3.90	94.72 ± 2.39	21.45 ± 5.13*	21.23 ± 1.10*	18.08 ± 5.14*	13.45 ± 1.34*
4e	100.00 ± 3.90	94.72 ± 2.39	50.24 ± 7.65*	38.11 ± 3.64*	19.50 ± 3.29*	12.64 ± 3.05*
4f	100.00 ± 3.90	94.72 ± 2.39	103.44 ± 7.64*	67.46 ± 5.78*	27.82 ± 3.84*	11.78 ± 0.79*
4g	100.00 ± 3.90	94.72 ± 2.39	31.21 ± 7.96*	25.77 ± 4.95*	23.87 ± 7.15	20.97 ± 5.44
4h	100.00 ± 3.90	94.72 ± 2.39	50.34 ± 9.10*	27.44 ± 3.50*	38.35 ± 2.09*	13.03 ± 3.67*
4i	100.00 ± 3.90	94.72 ± 2.39	58.50 ± 5.06*	61.93 ± 3.23*	25.76 ± 3.30*	12.27 ± 2.00*
Docetaxel (ref. drug)	100.00 ± 3.90	94.72 ± 2.39	60.21 ± 5.97	40.13 ± 6.58	14.12 ± 2.73	0.48 ± 0.02

Abbreviation: DMSO, dimethyl sulfoxide.

[a]The changes in cell viability caused by hydrazone derivatives are compared with the control data. Each data point is an average of eight viability measurements.

*$p < .05$.

TABLE 2 The cell viability results of MCF-7 cells after a 48-h treatment with nine hydrazone derivatives[a]

MCF-7 cell viability (%)						
Compound no.	Control	Solvent (DMSO)	0.1 μM	1 μM	10 μM	100 μM
4a	100.00 ± 5.32	95.79 ± 2.47	76.51 ± 9.13	65.60 ± 6.61*	64.37 ± 3.77*	46.87 ± 2.59*
4b	100.00 ± 5.32	95.79 ± 2.47	53.98 ± 6.15*	44.46 ± 4.47*	39.14 ± 3.09*	34.32 ± 3.30*
4c	100.00 ± 5.32	95.79 ± 2.47	70.19 ± 6.91*	68.06 ± 2.10*	51.57 ± 3.50*	53.38 ± 3.55*
4d	100.00 ± 5.32	95.79 ± 2.47	50.95 ± 2.55*	43.11 ± 4.16*	42.22 ± 1.96*	44.05 ± 3.32*
4e	100.00 ± 5.32	95.79 ± 2.47	69.37 ± 3.95*	68.48 ± 5.68*	57.96 ± 4.97*	49.57 ± 5.74*
4f	100.00 ± 5.32	95.79 ± 2.47	84.42 ± 4.70	71.87 ± 6.84*	53.51 ± 7.44*	51.73 ± 7.16*
4g	100.00 ± 5.32	95.79 ± 2.47	63.21 ± 6.60*	54.36 ± 3.57*	50.44 ± 1.93*	53.13 ± 3.75*
4h	100.00 ± 5.32	95.79 ± 2.47	80.37 ± 2.73	57.02 ± 6.97*	56.52 ± 5.12*	45.03 ± 1.79*
4i	100.00 ± 5.32	95.79 ± 2.47	58.96 ± 4.60*	51.89 ± 4.92*	44.88 ± 2.13*	42.56 ± 2.11*
Docetaxel (ref. drug)	100.00 ± 5.32	95.79 ± 2.47	64.56 ± 6.14	36.85 ± 4.92	20.01 ± 3.09	0.88 ± 0.04

Abbreviation: DMSO, dimethyl sulfoxide.

[a]The changes in cell viability caused by hydrazone derivatives are compared with the control data. Each data point is an average of eight viability measurements.

*$p < .05$.

standard drug docetaxel at a concentration of 0.1 μM against the A-2780 cell line. The log IC_{50} of docetaxel was 0.2200 μM for A-2780 cells at 24 h, whereas the IC_{50} values of compounds **4b**, **4c**, **4d**, **4e**, **4g**, **4h**, and **4i** were −0.4987, −0.4044, −0.8138, −0.3868, −0.6954, −0.4751, and 0.1809 μM, respectively. Three out of the nine compounds (**4b**, **4d**, and **4i**) at a concentration of 0.1 μM showed better anticancer activity against the MCF-7 cancer cell line than the standard drug. The log IC_{50} of docetaxel was 0.2400 μM for MCF-7 cells at 24 h, whereas the log IC_{50} values of compounds **4b**, **4d**, and **4i** were −0.1293, −0.1700, and 0.2459 μM, respectively. The outcomes of this study indicate that the newly studied hydrazone derivatives may act as potential drug candidates for cancer treatment, and the results of the present study are encouraging us to continue our anticancer activity screening with further modification in their structure in the future.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

The starting materials and reagents used in the reactions were supplied commercially by Aldrich, Acros, ABCR, and Merck. The human breast (MCF-7) cancer cell line and female ovarian (A-2780) cancer cell line were retrieved from the American Type Culture Collection (ATCC). The nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra (see the Supporting Information) were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). The assignment of exchangeable protons (NH) was confirmed by the addition of D_2O. Elemental analyses were performed by a LECO CHNS 932 Elemental Analyzer. The infrared spectra were recorded with ATR equipment in the range of 4000–650 cm^{-1} on a Perkin Elmer Spectrum One Fourier-transform infrared spectrophotometer. Melting points (mp) were measured in open capillary tubes and were uncorrected, using a Gallenkamp MPD350.BM3.5 apparatus. 1-Benzylindoline-2,3-dione,[46,47] 1-(4-bromobenzyl)indoline-2,3-dione,[46,47] and compound **3b**[48] were prepared according to the published procedures.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

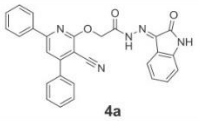
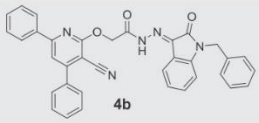
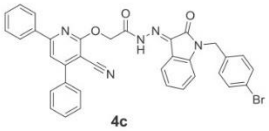
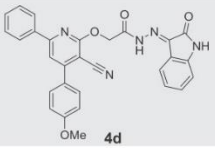
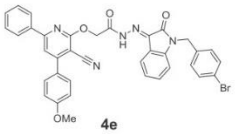
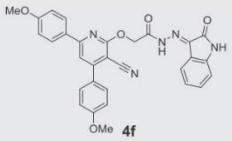
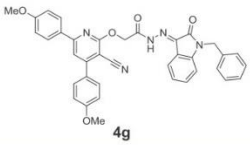
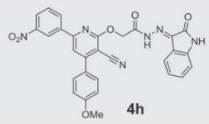
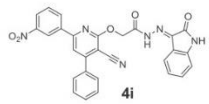
4.1.2 | General procedure for the synthesis of the products 2a–e

A mixture of an appropriate pyridin-2(1*H*)-one (**1a–e**; 0.01 mol) and anhydrous K_2CO_3 (0.015 mol) was stirred at room temperature in DMF (10 ml) for 1 h, and then 0.011 mol of methyl bromoacetate was added. The reaction mixture was stirred for an additional 3 h and poured into ice-cooled water. The obtained product was filtered off, dried, and crystallized from ethanol/acetone (2:1).

*Methyl 2-[(3-cyano-4,6-diphenylpyridin-2-yl)oxy]acetate (**2a**)*

White crystals, yield 89%, mp: 157–159°C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (dd, J = 6.8, 3.0 Hz, 2H, Ar–H), 7.70–7.64 (m, 2H, Ar–H), 7.58–7.45 (m, 7H, Ar–H, pyridine C5–H), 5.11 (s, 2H, OCH_2), 3.81 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 164.14 ($\underline{C}OOCH_3$), 158.67, 152.85, 152.37, 132.05, 131.37, 125.95, 125.42, 124.31, 124.22, 123.68, 122.50, 110.28, 109.70 (Ar–*C*), 88.54 (CN), 58.85 (OCH_2), 47.51 ($COO\underline{C}H_3$). ν(C–O–C) aliphatic ether: 1145.43 cm^{-1}, ν(C═O) ester: 1733.12 cm^{-1}, ν(CN): 2226.56 cm^{-1},

TABLE 3 Log IC_{50} (μM) concentrations calculated for A-2780 and MCF-7 cells in the GraphPad Prism 6 program of hydrazone derivatives

	log IC_{50} calculation		
	Compound	A-2780	MCF-7
 4a	4a	0.7816	1.660
 4b	4b	−0.4987	−0.1293
 4c	4c	−0.4044	1.465
 4d	4d	−0.8138	−0.1700
 4e	4e	−0.3868	1.598
 4f	4f	0.4636	1.551
 4g	4g	−0.6954	1.085
 4h	4h	−0.4751	1.267
 4i	4i	0.1809	0.2459
	Docetaxel	0.2200	0.2400

$_{\nu}$(C–H) aromatic: 2959.37 cm^{-1}. Anal. calcd. for $C_{21}H_{16}N_2O_3$: C, 73.24; H, 4.68; N, 8.13. Found: C, 74.10; H, 4.95; N, 8.21.

*Methyl 2-[(3-cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl)oxy]-acetate (**2b**)*

White solid, yield 75%, mp: 130–132°C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (d, J = 9.0 Hz, 2H, Ar–H), 7.63 (d, J = 8.8 Hz, 2H, Ar–H), 7.43 (s, 1H, pyridine C5–H), 7.04 (d, J = 8.8 Hz, 2H, Ar–H), 6.98 (d, J = 9.0 Hz, 2H, Ar–H), 5.07 (s, 2H, OCH_2), 3.87 (s, 6H, $2OCH_3$), 3.79 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 169.04 (COOCH3), 163.47, 161.73, 161.16, 157.07, 156.45, 129.90, 129.42, 128.78, 128.51, 115.61, 114.45, 114.29, 113.15 (Ar–C), 91.75 (CN), 63.55 (OCH_2), 55.46 ($2OCH_3$), 52.21 ($COOCH_3$). $_{\nu}$(C–O–C) aliphatic ether: 1140.53, 1170.69, 1182.51 cm^{-1}, $_{\nu}$(C=O) ester: 1761.85 cm^{-1}, $_{\nu}$(CN): 2220.54 cm^{-1}, $_{\nu}$(C–H) aromatic: 2838.91, 2949.49 cm^{-1}. Anal. calcd. for $C_{23}H_{20}N_2O_5$: C, 68.31; H, 4.98; N, 6.93. Found: C, 68.98; H, 5.02; N, 6.94.

*Methyl 2-{[3-cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl]oxy}-acetate (**2c**)*

White crystals, yield 92%, mp: 148–150°C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (d, J = 9.0 Hz, 2H, Ar–H), 7.67–7.62 (m, 2H, Ar–H), 7.55–7.49 (m, 3H, Ar–H), 7.45 (s, 1H, pyridine C5–H), 6.98 (d, J = 9.0 Hz, 2H, Ar–H), 5.08 (s, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 3.79 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 168.99 ($COOCH_3$), 163.36, 161.83, 157.28, 156.87, 136.28, 130.06, 129.27, 129.00, 128.83, 128.40, 115.26, 114.33, 113.45 (Ar–C), 92.15 (CN), 63.58 (OCH_2), 55.47 (OCH_3), 52.24 ($COOCH_3$). $_{\nu}$(C–O–C) aliphatic ether: 1140.53, 1172.51 cm^{-1}, $_{\nu}$(C=O) ester: 1760.38 cm^{-1}, $_{\nu}$(CN): 2223.11 cm^{-1}, $_{\nu}$(C–H) aromatic: 2842.39, 2950.17 cm^{-1}. Anal. calcd. for $C_{22}H_{18}N_2O_4$: C, 70.58; H, 4.85; N, 7.48. Found: C, 71.25; H, 4.90; N, 7.61.

*Methyl 2-{[3-cyano-6-(4-methoxyphenyl)-4-(3-nitrophenyl)pyridin-2-yl]oxy}acetate (**2d**)*

Yellow solid, yield 77%, mp: 190–192°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.60 (t, J = 1.9 Hz, 1H, Ar–H), 8.47–8.40 (m, 1H, Ar–H), 8.26–8.20 (m, 1H, Ar–H), 8.16 (d, J = 8.9 Hz, 2H, Ar–H), 7.94 (s, 1H, pyridine C5–H), 7.90 (t, J = 8.0 Hz, 1H, Ar–H), 7.09 (d, J = 9.0 Hz, 2H, Ar–H), 5.19 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.75 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 169.28 ($COOCH_3$), 163.20, 162.23, 157.61, 154.45, 148.39, 137.58, 135.81, 130.97, 129.65, 128.85, 125.21, 124.09, 115.31, 114.86, 114.17 (Ar–C), 91.63 (CN), 64.10 (OCH_2), 55.91 (OCH_3), 52.42 ($COOCH_3$). $_{\nu}$(C–O–C) aliphatic ether: 1149.23, 1176.64 cm^{-1}, $_{\nu}$(N–O) nitro: 1531.41 cm^{-1}, $_{\nu}$(C=O) ester: 1787.33 cm^{-1}, $_{\nu}$(CN): 2217.02 cm^{-1}, $_{\nu}$(C–H) aromatic: 2837.24, 2950.66 cm^{-1}. Anal. calcd. for $C_{22}H_{17}N_3O_6$: C, 63.01; H, 4.09; N, 10.02. Found: C, 63.69; H, 4.09; N, 10.02.

*Methyl 2-{[3-cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl]oxy}-acetate (**2e**)*

Yellow solid, yield 72%, mp: 170–172°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.64 (t, J = 1.9 Hz, 1H, Ar–H), 8.47–8.41 (m, 1H, Ar–H), 8.26 (d, J = 8.2 Hz, 1H, Ar–H), 8.20 (dd, J = 6.7, 3.0 Hz, 2H, Ar–H), 8.05 (s, 1H, pyridine C5–H), 7.92 (t, J = 8.0 Hz, 1H, Ar–H), 7.59–7.52 (m, 3H, Ar–H), 5.23 (s, 2H, OCH_2), 3.75 (s, 3H, $COOCH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 169.23 ($COOCH_3$), 163.25, 157.75, 154.76, 148.42, 137.44, 136.50, 135.86, 131.56, 131.00, 129.47, 127.90, 125.31, 124.18, 115.23, 115.12 (Ar–C), 92.96 (CN), 64.18 (OCH_2), 52.44 ($COOCH_3$). $_{\nu}$(C–O–C) aliphatic ether: 1155.06 cm^{-1}, $_{\nu}$(N–O) nitro: 1528.48 cm^{-1}, $_{\nu}$(C=O) ester: 1754.76 cm^{-1}, $_{\nu}$(CN): 2225.46 cm^{-1}, $_{\nu}$(C–H) aromatic: 3092.87 cm^{-1}. Anal. calcd. for $C_{21}H_{15}N_3O_5$: C, 64.78; H, 3.88; N, 10.79. Found: C, 65.04; H, 4.02; N, 10.69.

4.1.3 | General procedure for the synthesis of the products 3a–e

A mixture of **2a–e** (0.01 mol) and hydrazine monohydrate (0.04 mol, 1 ml) was boiled in tetrahydrofuran (10 ml) for 2.5–3 h. After cooling, the formed precipitate was filtered off, dried, and crystallized from ethanol/DMF (2:1) to give the target compounds **3a–e**.

*2-[(3-Cyano-4,6-diphenylpyridin-2-yl)oxy]acetohydrazide (**3a**)*

Yellowish white crystals, yield 80%, mp: 227–228°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.45 (s, 1H, NH), 8.21 (dd, J = 6.7 Hz, 3.3 Hz, 2H, Ar–H), 7.85 (s, 1H, pyridine C5–H), 7.77–7.75 (m, 2H, Ar–H), 7.61–7.52 (m, 6H, Ar–H), 5.04 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.06 ($CONH–NH_2$), 163.77, 157.49, 156.88, 136.90, 136.25, 131.22, 130.61, 129.40, 129.38, 129.10, 128.03, 115.75, 114.67 (Ar–C), 93.10 (CN), 64.81 (OCH_2). $_{\nu}$(C–O–C) aliphatic ether: 1142.28 cm^{-1}, $_{\nu}$(C=O) amide: 1675.80 cm^{-1}, $_{\nu}$(CN): 2224.20 cm^{-1}, $_{\nu}$(C–H) aromatic: 2934.87, 3051.01 cm^{-1}, $_{\nu}$(N–H): 3282.25, 3333.42 cm^{-1}. Anal. calcd. for $C_{20}H_{16}N_4O_2$: C, 69.76; H, 4.68; N, 16.27. Found: C, 70.29; H, 4.91; N, 16.31.

*2-{[3-Cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl]oxy}-acetohydrazide (**3c**)*

White solid, yield 73%, mp: 209–211°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.44 (s, 1H, NH), 8.19 (d, J = 8.9 Hz, 2H, Ar–H), 7.80–7.69 (m, 3H, Ar–H, pyridine C5–H), 7.64–7.55 (m, 3H, Ar–H), 7.06 (d, J = 8.9 Hz, 2H, Ar–H), 5.01 (s, 2H, OCH_2), 4.33 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.14 ($CONH–NH_2$), 163.71, 161.94, 157.31, 156.64, 136.41, 130.51, 129.76, 129.35, 129.30, 129.06, 115.94, 114.76, 113.66 (Ar–C), 91.89 (CN), 64.72 (OCH_2), 55.87 (OCH_3). $_{\nu}$(C–O–C) aliphatic ether: 1141.80, 1171.45 cm^{-1}, $_{\nu}$(C=O) amide: 1665.12 cm^{-1}, $_{\nu}$(CN): 2217.43 cm^{-1}, $_{\nu}$(N–H): 3281.79 cm^{-1}. Anal. calcd. for $C_{21}H_{18}N_4O_3$: C, 67.37; H, 4.85; N, 14.96. Found: C, 68.15; H, 4.91; N, 14.90.

*2-{[3-Cyano-6-(4-methoxyphenyl)-4-(3-nitrophenyl)pyridin-2-yl]oxy} acetohydrazide (**3d**)*

Yellow solid, yield 89%, mp: 236–238°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.44 (s, 1H, NH), 8.59 (t, J = 2 Hz, 1H, Ar–H), 8.46–8.39 (m, 1H, Ar–H), 8.23–8.18 (m, 3H, Ar–H, pyridine C5–H), 7.92

(t, J = 7.9 Hz, 2H, Ar–H), 7.08 (d, J = 9.0 Hz, 2H, Ar–H), 5.03 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 167.03 (CONH–NH_2), 163.60, 162.09, 157.70, 154.15, 148.38, 137.74, 135.76, 131.03, 129.87, 129.10, 125.17, 124.00, 115.61, 114.78, 113.84 (Ar–C), 92.05 (CN), 64.79 (OCH_2), 55.90 (OCH_3). $_{\nu}$(C–O–C) aliphatic ether: 1150.79, 1171.99 cm^{-1}, $_{\nu}$(N–O) nitro: 1516.22 cm^{-1}, $_{\nu}$(C=O) amide: 1665.56 cm^{-1}, $_{\nu}$(CN): 2218.28 cm^{-1}, $_{\nu}$(C–H) aromatic: 2836.60, 3016.19 cm^{-1}, $_{\nu}$(N–H): 3290.90 cm^{-1}. Anal. calcd. for $C_{21}H_{17}N_5O_5$: C, 60.14; H, 4.09; N, 16.70. Found: C, 59.85; H, 4.16; N, 16.18.

*2-[[3-Cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl]oxy}-acetohydrazide (**3e**)*

Yellow solid, yield 69%, mp: 219–221°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.45 (s, 1H, NH), 8.61 (t, J = 1.8 Hz, 1H, Ar–H), 8.45 (dd, J = 8.3, 1.5 Hz, 1H, Ar–H), 8.26–8.21 (m, 3H, Ar–H), 8.00 (s, 1H, pyridine C5–H), 7.96–7.88 (m, 1H, Ar–H), 7.56–7.53 (m, 3H, Ar–H), 5.06 (s, 2H, OCH_2), 4.34 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 166.98 (CONH–NH_2), 163.65, 157.86, 154.41, 148.41, 137.59, 136.70, 135.78, 131.41, 131.06, 129.42, 128.11, 125.25, 124.06, 115.41, 114.85 (Ar–C), 93.34 (CN), 64.89 (OCH_2). $_{\nu}$(C–O–C) aliphatic ether: 1158.46 cm^{-1}, $_{\nu}$(N–O) nitro: 1542.40 cm^{-1}, $_{\nu}$(C=O) amide: 1665.97 cm^{-1}, $_{\nu}$(CN): 2219.83 cm^{-1}, $_{\nu}$(C–H) aromatic: 2954.09 cm^{-1}, $_{\nu}$(N–H): 3299.03 cm^{-1}. Anal. calcd. for $C_{20}H_{15}N_5O_4$: C, 61.69; H, 3.88; N, 17.99. Found: C, 59.83; H, 4.04; N, 17.35.

4.1.4 | General procedure for the synthesis of the products 4a–j

A mixture of isatin derivative (0.01 mol) and hydrazides 3**a-e** (0.01 mol) in dioxane (10 ml) containing 10% PTSOH as catalyst was stirred at room temperature for 3 h. Then the resulting solid was filtered, washed with ethanol, filtered and recrystallized from ethanol/DMF.

*(E)-2-[(3-Cyano-4,6-diphenylpyridin-2-yl)oxy]-N′-[(2-oxoindolin-3-ylidene)acetohydrazide] (**4a**)*

Yellow solid, yield 70%, mp: 243–245°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.67 (s, 1H, NH), 10.90 (s, 1H, NH_{isatin}), 8.22–8.10 (m, 3H, Ar–H), 7.88 (s, 1H, pyridine C5–H), 7.83–7.77 (m, 2H, Ar–H), 7.67–7.58 (m, 3H, Ar–H), 7.48–7.38 (m, 2H, Ar–H), 7.32 (t, J = 7.6 Hz, 2H, Ar–H), 7.08 (t, J = 7.5 Hz, 1H, Ar–H), 6.96 (d, J = 7.8 Hz, 1H, Ar–H), 5.70 (s, 2H, OCH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.93 (CONHN), 163.65 ($CONH_{isatin}$), 157.44, 157.14, 144.45, 136.78, 136.18 (Ar–C), 133.38 (C=N_{imine}), 131.23, 130.67, 129.39, 129.22, 129.17, 127.91, 126.78, 122.25, 115.67, 115.63, 114.83, 111.20 (Ar–C), 92.72 (CN), 66.83 (OCH_2). $_{\nu}$(C–O–C) aliphatic ether: 1146.83 cm^{-1}, $_{\nu}$(C=N) imine: 1608.97 cm^{-1}, $_{\nu}$(C=O) amide: 1717.75 cm^{-1}, $_{\nu}$(CN): 2228.78 cm^{-1}, $_{\nu}$(C–H) aromatic: 2837.66 cm^{-1}, $_{\nu}$(N–H): 3147.71 cm^{-1}. Anal. calcd. for $C_{28}H_{19}N_5O_3$: C, 71.03; H, 4.04; N, 14.79. Found: C, 70.87; H, 4.04; N, 14.82.

*(E)-N′-(1-Benzyl-2-oxoindolin-3-ylidene)-2-[(3-cyano-4,6-diphenylpyridin-2-yl)oxy]acetohydrazide (**4b**)*

Yellow solid, yield 68%, mp: 217–219°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.79 (s, 1H, NH), 8.27 (d, J = 7.6 Hz, 1H, Ar–H), 8.16 (d, J = 7.2 Hz, 2H, Ar–H), 7.89 (s, 1H, pyridine C5–H), 7.84–7.77 (m, 2H, Ar–H), 7.67–7.60 (m, 3H, Ar–H), 7.48–7.26 (m, 9H, Ar–H), 7.14 (t, J = 7.6 Hz, 1H, Ar–H), 7.09 (d, J = 8.0 Hz, 1H, Ar–H), 5.70 (s, 2H, OCH_2), 5.01 (s, 2H, NCH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.80 (CONHN), 163.64 ($CONCH_{2\ isatin}$), 157.46, 157.15, 144.40, 136.79, 136.61, 136.18 (Ar–C), 133.18 (C=N_{imine}), 131.21, 130.68, 129.40, 129.22, 129.20, 129.18, 128.02, 127.92, 127.73, 126.65, 122.95, 115.67, 115.21, 114.87, 110.44 (Ar–C), 92.73 (CN), 43.20 (NCH_2). $_{\nu}$(C–O–C) aliphatic ether: 1143.54 cm^{-1}, $_{\nu}$(C=N) imine: 1675.58 cm^{-1}, $_{\nu}$(C=O) amide: 1694.48, 1724.92 cm^{-1}, $_{\nu}$(CN): 2225.44 cm^{-1}, $_{\nu}$(N–H): 3198.56 cm^{-1}. Anal. calcd. for $C_{35}H_{25}N_5O_3$: C, 74.59; H, 4.47; N, 12.43. Found: C, 74.47; H, 4.46; N, 12.48.

*(E)-N′-[1-(4-Bromobenzyl)-2-oxoindolin-3-ylidene]-2-[(3-cyano-4,6-diphenylpyridin-2-yl)oxy]acetohydrazide (**4c**)*

Yellow solid, yield 71%, mp: 228–230°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.81 (s, 1H, NH), 8.27 (d, J = 7.6 Hz, 1H, Ar–H), 8.19–8.11 (m, 2H, Ar–H), 7.89 (s, 1H, pyridine C5–H), 7.83–7.77 (m, 2H, Ar–H), 7.65–7.60 (m, 3H, Ar–H), 7.55 (d, J = 8.4 Hz, 2H, Ar–H), 7.49–7.27 (m, 6H, Ar–H), 7.15 (t, J = 7.6 Hz, 1H, Ar–H), 7.08 (d, J = 8.0 Hz, 1H, Ar–H), 5.73 (s, 2H, OCH_2), 4.98 (s, 2H, NCH_2). $_{\nu}$(C–O–C) aliphatic ether: 1143.67 cm^{-1}, $_{\nu}$(C=N) imine: 1672.08 cm^{-1}, $_{\nu}$(C=O) amide: 1690.59, 1725.73 cm^{-1}, $_{\nu}$(CN): 2226.51 cm^{-1}, $_{\nu}$(C–H) aromatic: 2982.40 cm^{-1}, $_{\nu}$(N–H): 3220.55 cm^{-1}. Anal. calcd. for $C_{35}H_{24}BrN_5O_3$: C, 65.43; H, 3.77; N, 12.44. Found: C, 65.42; H, 4.46; N, 12.48.

*(E)-2-[[3-Cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl]oxy}-N′-[(2-oxoindolin-3-ylidene)acetohydrazide] (**4d**)*

Yellow solid, yield 80%, mp: 230–232°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.67 (s, 1H, NH), 10.92 (s, 1H, NH_{isatin}), 8.21 (d, J = 7.7 Hz, 1H, Ar–H), 8.15–8.05 (m, 2H, Ar–H), 7.82–7.72 (m, 3H, Ar–H, pyridine C5–H), 7.65–7.57 (m, 3H, Ar–H), 7.44 (t, J = 7.7 Hz, 1H, Ar–H), 7.10 (t, J = 7.2 Hz, 1H, Ar–H), 6.97 (d, J = 7.8 Hz, 1H, Ar–H), 6.79 (d, J = 8.8 Hz, 2H, Ar–H), 5.67 (s, 2H, OCH_2), 3.71 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.95 (CONHN), 163.56 ($CONH_{isatin}$), 161.85, 157.24, 156.87, 144.47, 136.33 (Ar–C), 133.38 (C=N_{imine}), 130.56, 129.59, 129.35, 129.11, 126.80, 122.27, 115.85, 115.66, 114.54, 113.80, 111.21 (Ar–C), 91.51 (CN), 66.82 (OCH_2), 55.73 (OCH_3). $_{\nu}$(C–O–C) aliphatic ether: 1146.56, 1170.17 cm^{-1}, $_{\nu}$(C=N) imine: 1584.37 cm^{-1}, $_{\nu}$(C=O) amide: 1715.81 cm^{-1}, $_{\nu}$(CN): 2222.83 cm^{-1}, $_{\nu}$(C–H) aromatic: 2839.13 cm^{-1}, $_{\nu}$(N–H): 3170.35 cm^{-1}. Anal. calcd. for $C_{29}H_{21}N_5O_4$: C, 69.18; H, 4.20; N, 13.91. Found: C, 68.95; H, 4.21; N, 14.03.

*(E)-N′-[1-(4-Bromobenzyl)-2-oxoindolin-3-ylidene]-2-[[3-cyano-6-(4-methoxyphenyl)-4-phenylpyridin-2-yl]oxy}acetohydrazide (**4e**)*

Yellow solid, yield 71%, mp: 212–214°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.80 (s, 1H, NH), 8.28 (d, J = 7.7 Hz, 1H, Ar–H),

8.14–8.06 (m, 2H, Ar–H), 7.82–7.74 (m, 3H, Ar–H, pyridine C5–H), 7.64–7.59 (m, 3H, Ar–H), 7.53 (d, J = 8.3 Hz, 2H, Ar–H), 7.46 (t, J = 7.8 Hz, 1H, Ar–H), 7.33 (d, J = 8.3 Hz, 2H, Ar–H), 7.16 (t, J = 7.6 Hz, 1H, Ar–H), 7.09 (d, J = 8.0 Hz, 1H, Ar–H), 6.77 (d, J = 8.8 Hz, 2H, Ar–H), 5.67 (s, 2H, OCH_2), 4.98 (s, 2H, NCH_2), 3.66 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.82 (CONHN), 163.57 ($CONCH_{2\ isatin}$), 161.84, 157.27, 156.91, 144.20, 136.33, 136.09 (Ar–C), 133.20 ($C{=}N_{imine}$), 132.09, 130.57, 130.02, 129.60, 129.36, 129.14, 129.11, 126.70, 123.06, 121.15, 115.84, 115.27, 114.55, 113.86, 110.39 (Ar–C), 91.55 (CN), 55.69 (OCH_3), 42.61 (NCH_2). ν(C–O–C) aliphatic ether: 1142.76, 1175.30 cm^{-1}, ν(C=N) imine: 1675.11 cm^{-1}, ν(C=O) amide: 1693.27, 1724.24 cm^{-1}, ν(CN): 2224.31 cm^{-1}, ν(C–H) aromatic: 2974.03 cm^{-1}, ν(N–H): 3143.95 cm^{-1}. Anal. calcd. for $C_{36}H_{26}BrN_5O_4$: C, 64.29; H, 3.90; N, 10.41. Found: C, 64.21; H, 3.89; N, 10.53.

*(E)-2-[[3-Cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl]oxy}-N′-[(2-oxoindolin-3-ylidene)acetohydrazide] (**4f**)*

Yellow solid, yield 70%, mp: 240–242°C, ^1H NMR (DMSO-d_6, 400 MHz) δ 11.68 (s, 1H, NH), 10.93 (s, 1H, NH_{isatin}), 8.21 (d, J = 7.7 Hz, 1H, Ar–H), 8.14–8.03 (m, 2H, Ar–H), 7.80–7.70 (m, 3H, Ar–H, pyridine C5–H), 7.44 (t, J = 7.8 Hz, 1H, Ar–H), 7.16 (d, J = 8.8 Hz, 2H, Ar–H), 7.10 (d, J = 7.3 Hz, 1H, Ar–H), 6.96 (d, J = 7.8 Hz, 1H, Ar–H), 6.78 (d, J = 8.9 Hz, 2H, Ar–H), 5.66 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.96 (CONHN), 163.67 ($CONH_{isatin}$), 161.76, 161.30, 157.00, 156.44, 144.46 (Ar–C), 133.36 ($C{=}N_{imine}$), 130.72, 129.52, 129.23, 128.53, 128.38, 126.79, 125.98, 122.72, 116.15, 115.65, 114.79, 114.50, 113.49, 111.21 (Ar–C), 91.04 (CN), 67.49 (OCH_2), 55.89 (OCH_3), 55.71 (OCH_3). ν(C–O–C) aliphatic ether: 1141.86, 1171.08, 1189.61 cm^{-1}, ν(C=N) imine: 1603.46 cm^{-1}, ν(C=O) amide: 1721.22, 1750.23 cm^{-1}, ν(CN): 2226.39 cm^{-1}, ν(C–H) aromatic: 2946.79 cm^{-1}, ν(N–H): 3301.03, 3393.82 cm^{-1}. Anal. calcd. for $C_{30}H_{23}N_5O_5$: C, 67.54; H, 4.35; N, 13.13. Found: C, 67.47; H, 4.35; N, 13.19.

*N′-(1-Benzyl-2-oxoindolin-3-ylidene)-2-[[3-cyano-4,6-bis(4-methoxyphenyl)pyridin-2-yl]oxy}acetohydrazide (**4g**)*

Yellow solid, yield, mp: 168–170°C, ^1H NMR (DMSO-d_6, 600 MHz) δ 13.38 (s, 1H, $NH_{cis\ conformer}$), 12.61 (s, 1H, $NH_{trans\ conformer}$), 8.20–8.04 (m, 2H, Ar–H), 7.79–7.57 (m, 4H, Ar–H, pyridine C5–H), 7.46–7.24 (m, 6H, Ar–H), 7.21–7.02 (m, 4H, Ar–H), 7.02–6.77 (m, 2H, Ar–H), 5.78 (s, 2H, $OCH_{2\ trans\ conformer}$), 5.35 (s, 2H, $OCH_{2\ cis\ conformer}$), 5.00 (s, 2H, NCH_2), 3.86 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 161.86 (CONHN), 161.33 ($CONCH_{2\ isatin}$), 161.14, 157.06, 156.50, 143.26, 136.06 (Ar–C), 132.21 ($C{=}N_{imine}$), 130.71, 129.57, 129.20, 128.33, 128.15, 127.88, 123.85, 121.26, 119.57, 115.96, 114.79, 114.61, 113.75, 111.02 (Ar–C), 91.21 (CN), 66.83 (OCH_2), 55.90 (OCH_3), 55.72 (OCH_3), 43.06 (NCH_2). ν(C–O–C) aliphatic ether: 1142.92, 1170.32 cm^{-1}, ν(C=N) imine: 1610.41 cm^{-1}, ν(C=O) amide: 1698.07, 1729.42 cm^{-1}, ν(CN): 2215.51 cm^{-1}, ν(C–H) aromatic: 2963.10 cm^{-1}, ν(N–H): 3225.70 cm^{-1}. Anal. calcd. for $C_{37}H_{29}N_5O_5$: C, 71.26; H, 4.69; N, 11.23. Found: C, 71.34; H, 4.70; N, 11.39.

*(E)-2-{[(3-Cyano-6-(4-methoxyphenyl)]-4-[(3-nitrophenyl)pyridin-2-yl]oxy}-N′-(2-oxoindolin-3-ylidene)acetohydrazide (**4h**)*

Yellow solid, yield 66%, mp: 224–226°C, ^1H NMR (DMSO-d_6, 400 MHz) δ 11.66 (s, 1H, NH), 10.90 (s, 1H, NH_{isatin}), 8.62 (m, 1H, Ar–H), 8.47–8.40 (m, 1H, Ar–H), 8.29–8.17 (m, 2H, Ar–H), 8.16–8.07 (m, 2H, Ar–H), 7.96–7.86 (m, 2H, Ar–H, pyridine C5–H), 7.44 (t, J = 7.7 Hz, 1H, Ar–H), 7.10 (t, J = 7.5 Hz, 1H, Ar–H), 6.96 (d, J = 7.9 Hz, 1H, Ar–H), 6.81 (d, J = 8.8 Hz, 2H, Ar–H), 5.66 (s, 2H, OCH_2), 3.72 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 164.95 (CONHN), 163.48 ($CONH_{isatin}$), 162.01, 157.63, 154.42, 148.41, 144.48, 137.69, 135.82 (Ar–C), 133.39 ($C{=}N_{imine}$), 130.99, 129.71, 128.95, 126.79, 125.19, 124.09, 122.27, 115.65, 115.51, 114.58, 114.01, 111.21 (Ar–C), 91.70 (CN), 64.33 (OCH_2), 55.76 (OCH_3). ν(C–O–C) aliphatic ether: 1148.39, 1173.92 cm^{-1}, ν(N–O) nitro: 1536.99 cm^{-1}, ν(C=N) imine: 1606.06 cm^{-1}, ν(C=O) amide: 1669.94, 1722.80 cm^{-1}, ν(CN): 2225.58 cm^{-1}, ν(C–H) aromatic: 2967.74 cm^{-1}, ν(N–H): 3089.46 cm^{-1}. Anal. calcd. for $C_{29}H_{20}N_6O_6$: C, 63.50; H, 3.68; N, 15.32. Found: C, 63.43; H, 3.67; N, 15.35.

*2-{[3-Cyano-4-(3-nitrophenyl)-6-phenylpyridin-2-yl]oxy}-N′-(2-oxoindolin-3-ylidene)acetohydrazide (**4i**)*

Yellow solid, yield 69%, mp: 280–282°C, ^1H NMR (DMSO-d_6, 600 MHz) δ 13.49 (s, 1H, $NH_{cis\ conformer}$), 12.70 (s, 1H, $NH_{trans\ conformer}$), 11.35 (s, 1H, NH_{isatin}), 8.65–8.61 (m, 1H, Ar–H), 8.44 (d, J = 8.0 Hz, 1H, Ar–H), 8.26 (d, J = 7.8 Hz, 1H, Ar–H), 8.22–7.95 (m, 3H, Ar–H, pyridine C5–H), 7.92 (t, J = 7.9 Hz, 1H, Ar–H), 7.64–7.32 (m, 5H, Ar–H), 6.99 (m, 2H, Ar–H), 5.80 (s, 2H, $OCH_{2\ trans\ conformer}$), 5.39 (s, 2H, $OCH_{2\ cis\ conformer}$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 163.41 (CONHN), 163.02 ($CONH_{isatin}$), 157.83, 154.72, 148.43, 143.08, 137.46, 136.54, 135.85 (Ar–C), 132.42 ($C{=}N_{imine}$), 131.48, 131.02, 129.30, 128.00, 125.31, 124.15, 123.16, 121.41, 120.00, 115.26, 115.21, 115.18, 111.72 (Ar–C), 93.06 (CN), 63.67 (OCH_2). ν(C–O–C) aliphatic ether: 1141.09 cm^{-1}, ν(N–O) nitro: 1526.53 cm^{-1}, ν(C=N) imine: 1617.90 cm^{-1}, ν(C=O) amide: 1691.35, 1729.31 cm^{-1}, ν(CN): 2226.86 cm^{-1}, ν(N–H): 3204.30 cm^{-1}. Anal. calcd. for $C_{28}H_{18}N_6O_5$: C, 64.86; H, 3.50; N, 16.21. Found: C, 64.77; H, 3.48; N, 16.26.

4.2 | Cytotoxicity study

4.2.1 | Cell lines and culture conditions

The human cancer lines, ovarian cancer (A-2780) and prostate cancer (MCF-7), were used for in vitro screening experiments. Both cell lines were both purchased from the ATCC. All cells were fed in 25- and 75-cm^2 flasks with the RPMI-1640 medium (containing 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin) 2 days apart. In cells with a carbon dioxide (5% CO_2) incubator (Panasonic), the cells maintained at 37°C and in a humid environment were separated from the flasks using a solution of trypsin–EDTA (Sigma-Aldrich) when confluent. The viability of the cells was determined using 0.4% trypan blue and experiments were started when the viability was above 90%.

4.2.2 | MTT assay

Antitumor activities of these substances were evaluated by MTT assay.[30] Cells were removed using a trypsin–EDTA solution from flasks and counted by a hemocytometer to determine cytotoxic effects. Furthermore, 15×10^3 cells per well were plated in 96-well plates including 200 µl of the RPMI-1640 medium. Cells were incubated at 37°C in a CO_2 incubator for 24 h to adhere to a 96-well plate base. When the incubation ended, concentrations of 0.1, 1, 10, and 100 µM of the hydrazone derivatives were added to the wells in which the cells were contained. Incubation with cancer cells for 24 h at 37°C in a CO_2 incubator was performed to determine the effects of different concentrations of hydrazone derivatives on cell viability for 24 h. When the incubation was over, 0.5 mg/ml of MTT solution in sterile phosphate-buffered solution was prepared and added to 96-well plates. After the addition of MTT, the plates were incubated again for 3 h. After this time, incubation was stopped by adding DMSO to the wells, and the optical densities of the cells in the plates were read on a spectrophotometer (Synergy HTX) at a wavelength of 550 nm.[31] The cell viability percentage was calculated by proportioning the absorbance values obtained from hydrazone derivative-applied wells to that of control group. MTT trials were performed 10 times in triplicate on different days, and the log IC_{50} values of the applied compounds were calculated on the basis of MTT results using the GraphPad Prism 6 program on a computer.

4.3 | Statistical analysis

The IBM SPSS Statistics 24.0 (Windows) package program was used in the analysis. Conformity to normal distribution was evaluated by the Shapiro–Wilk test. Intergroup comparisons of quantitative variables were measured by Kruskal–Wallis *H* test. When significant statistical differences were determined between groups, multiple comparisons were made with Bonferroni correction Mann–Whitney *U* test. All p values <.05 were considered statistically significant. Log IC_{50} values of melatonin and agomelatine were calculated using GraphPad Prism 6 program on a computer based on the MTT results obtained from the experiments.

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CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests.

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Abstract

In order to decrease the level of toxicity and improve the selectivity of drugs toward cancer targets, hybrid drugs are designed to simultaneously modulate multiple targets of multifactorial diseases to overcome the side effects associated with a single target-drugs. As a result, the development of hybrid molecules has become the centre of research.

Owing to the pharmacological data of pyridine, 1,3,4 oxadiazole, and pyrazole rings, we are interested in synthesizing new hybrid molecules and examining their anti-cancer activities.

In the first chapter of the manuscript, a bibliographical study described the biological interests and synthesis methods of four heterocyclic systems, namely cyanopridines, pyrazoles, oxadiazoles, and N-acylhydrazones.

In the second chapter, we developed novel conditions to prepare a well-furnished library of acetohydrazides from cyanopyridones as starting materials. The reactivity of the formers was used to synthesize three novel series bearing pyarzoles, 1,3,4 oxadiazoles, and hydrazones containing isatin moieties.

in the final chapter of the thesis, as the main objective, was evaluating and discussing the synthesized molecules' anticancer properties against different cancer cell lines (A-2780, MCF-7, and Caco-2) using MTT assay.

Keywords: Cyanopyridine, pyrazole, oxadiazole, N-acylhydrazone, anticancer, MTT assay.

Résumé

Afin de réduire le niveau de toxicité et d'améliorer la sélectivité des médicaments vis-à-vis des cibles cancéreuses, les molécules hybrides sont conçues pour moduler simultanément plusieurs cibles de maladies multifactorielles afin de surmonter les effets secondaires associés à l'utilisation d'un médicament à cible unique, ainsi, le développement de molécules hybrides est devenu le centre de la recherche.

Dans le premier chapitre de ce manuscrit, une étude bibliographique a été décrite portant sur les intérêts biologiques et les méthodes de synthèse de quatre systèmes hétérocycliques, à savoir le cyanopridone, le pyrazole, l'oxadiazole et les N-acylhydrazones.

Dans le deuxième chapitre, nous avons développé de nouvelles conditions pour préparer une bibliothèque d'acétohydrazides à partir de cyanopyridones, puis la réactivité de l'acétohydrazide a été utilisée pour synthétiser trois nouvelles séries portant des pyarzoles, des 1,3,4 oxadiazoles, et des hydrazones contenant des fragments d'isatine.

Le dernier chapitre de la thèse a pour objectif principal d'évaluer et de discuter les propriétés anticancéreuses des molécules synthétisées contre différentes lignées cellulaires cancéreuses (A-2780, MCF-7 et Caco-2) en utilisant le test MTT.

Mots-clés : Cyanopyridine, pyrazole, Oxadiazole, N-acylhydrazone, anticancéreuses, test MTT.

ملخص

من أجل تقليل مستوى السمية وتحسين انتقائية الأدوية كاهداف لمرض السرطان ، تم تصميم الأدوية الهجينة لاستهداف امراض متعددة في نفس الوقت و للتغلب على الآثار الجانبية المرتبطة بدواء مستهدف واحد. نتيجة لذلك ، أصبح تطوير الجزيئات الهجينة مركزًا للبحث.

في الفصل الأول من هذه الاطروحة ، تم وصف دراسة ببليوغرافية تتعلق بالاهتمامات البيولوجية وطرق اصطناع لأربعة أنظمة حلقية غير متجانسة ، وهي "سيانوبريدون" ، و"بيرازول" ، و"أوكساديازول" ، و" N-acylhydarazones .

في الفصل الثاني ، قمنا بتطوير شروط جديدة لإعداد سلسلة أسيتوهيدرازيدات جديدة من السيانوبيريدون ، ثم تم استخدام تفاعل أسيتوهيدرازيد لأصطناع ثلاث سلاسل جديدة تحمل البيرزولات ، 1،3،4 أوكساديازول ، والهيدرازونات التي تحتوي على إيزاتين.

الفصل الأخير من الأطروحة يهدف بشكل رئيسي إلى تقييم ومناقشة الخصائص المضادة للسرطان للجزيئات المركبة ضد سلالات الخلايا السرطانية المختلفة (A-2780 و MCF-7 و Caco-2) باستخدام اختبارMTT

مفاتيح اللفظ : سيانوبيريدين ، بيرازول ، أوكساديازول ، إن-أسيل هيدرازون ، مضادات السرطان ، اختبار MTT.