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& Scientific Research

University of Diyala – College of Science

Department of Chemistry

Synthesis and Characterization of New Indole Derivatives from 4-flouro Phenylhydrazine Hydrochloride and Study Their Biological Activity

A Thesis Submitted to the Council of the College of Science, University of Diyala

In Partial Fulfillment of the Requirements for the Degree of Master in Chemistry Sciences

By:

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

نَرْفَعُ دَرَجَاتٍ مَّن نَّشَاءُ ۗ

وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ

صَدَقَ اللَّهُ الْعَظِيمُ

سورة يوسف (٧٦)

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Researcher

Dedication

Thanks to Allah (SWT) for his help in completing this thesis.

To the one who gave me everything he had in order to fulfill his hopes, to the one who was pushing me forward to get the target that I am looking for. To the man who taught me with great sacrifices, translated in his appreciation of science, to my first school of life, **my dear father**, may Allah extend his life.

To the Lady who was and still the permanent source of giving and affection, to the one who showed her patience for everything, who gave me the right care and helped me during the hardships, and her supplication for my success. She followed me step by step during my work, to whom I relaxed whenever I remembered her smile at me, the spring of tenderness, **my dear mother**, may Allah reward her in both the life and hereafter.

To them I dedicate this humble work so that I may grant them some happiness to my brothers and sisters who have shared with me the burden of life.

I also dedicate the fruit of my effort to my **dear husband**, who whenever the road closes before me, I go to him and he lighten it and whenever there is despair in me, he gives me a hope in order to go forward.

Finally, to the professors of the Department of Chemistry and to the College of Science at our dear University and to everyone who believes that the seeds of success are in ourselves rather than in other things.

Sumaya

CONTENS

List of Figures

List of Schemes

List of Equations

List of Tables

Symbols and Abbreviations

α	Alfa
B	Beta
&	And
δ	Chemical shifts
%	Percent (per cent)
μm	Micrometer
^{1}H-NMR	Proton Nuclear Magnetic Resonance Spectrometer
APT ^{13}C-NMR	Attached Proton Test 13C- Nuclear Magnetic Resonance Spectrometer
Ar	Aromatic ring
B	Base
cm	Centimeter
cm^3	Cubic centimeter
CMC	Comprehensive Medicinal Chemistry
$CoCl_2$	Cobalt (II) chloride (phosgene)
$CoX-_2$	Cyclooxygenase type 2
DMA	N-N-Dimethylacetamide
DMF	N, N-Dimethyl Formamide
DNA	Deoxyribonucleic acid
DMSO	Dimethyl Sulfoxide
E. arvense	Equisetum arvense
FT-IR	Fourier-Transform Infrared
g	Gram
h	Hour(s)
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
IBD	Iodobenzene Diacetate
IU	International Units
min	Minute
mg	Milligram
ml	Milliliters
mmol	Millimoles
m.p	Melting point
MHz	Megahertz
PH	potential of hydrogen
ppm	Parts per million
PBS	Phosphate Buffer Saline
$POCl_3$	Phosphorus Oxychloride
RSV	Respiratory Syncytial Virus
RPMI	Roswell Park Memorial Institute medium

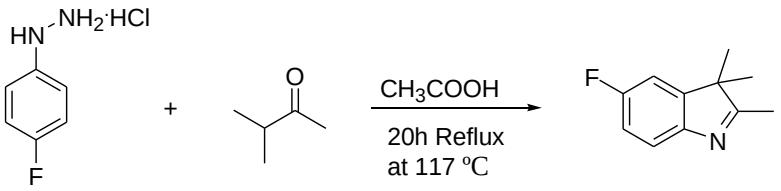
s	Singlet
$SOCl_2$	Thionyl chloride
Bu_3SnH	Tributyltin hydride
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
VH	Vilsmeier Haack

ABSTRACT

In this thesis, A series of new pyrazole derivatives have been successfully synthesized, their purity confirmed by thin layer chromatography, The chemical structures of the synthesized compounds identified by some spectroscopic techniques like, ^{1}H, APT ^{13}C-NMR, and FT-IR, as well as some their physical properties were determined such as, melting points and colors. The synthesized compounds were divided into two sections:

The first section; includes synthesis of new starting material, 5-Fluoro-2,3,3-trimethyl -3*H*-indole **(1)** and 2-(5-Fluoro-3,3- dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde **(2).**

The first compound, 5-Fluoro-2,3,3-trimethyl -3*H*-indole **(1)** has been synthesized by Fischer indole synthesis via reaction of 4-Fluoro-phenylhydrazine hydrochloride with methyl isopropyl ketone in the presence of glacial acetic acid as a catalyst. The **second compound**, 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde **(2)** has been synthesized by Vilsmeier Haack reaction via reaction of 5-Fluoro-2,3,3-trimethyl-3*H*-indole **(1)** with Phosphoryl chloride ($POCl_3$) in a presence of N, N-dimethyl formamide (DMF).

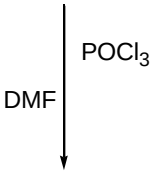


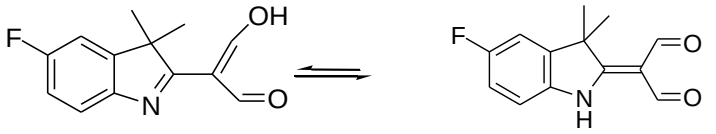
(4-fluorophenyl)hydrazine hydrochloride

3-methylbutan-2-one

5-fluoro-2,3,3-trimethyl-3*H*-indole

(1)

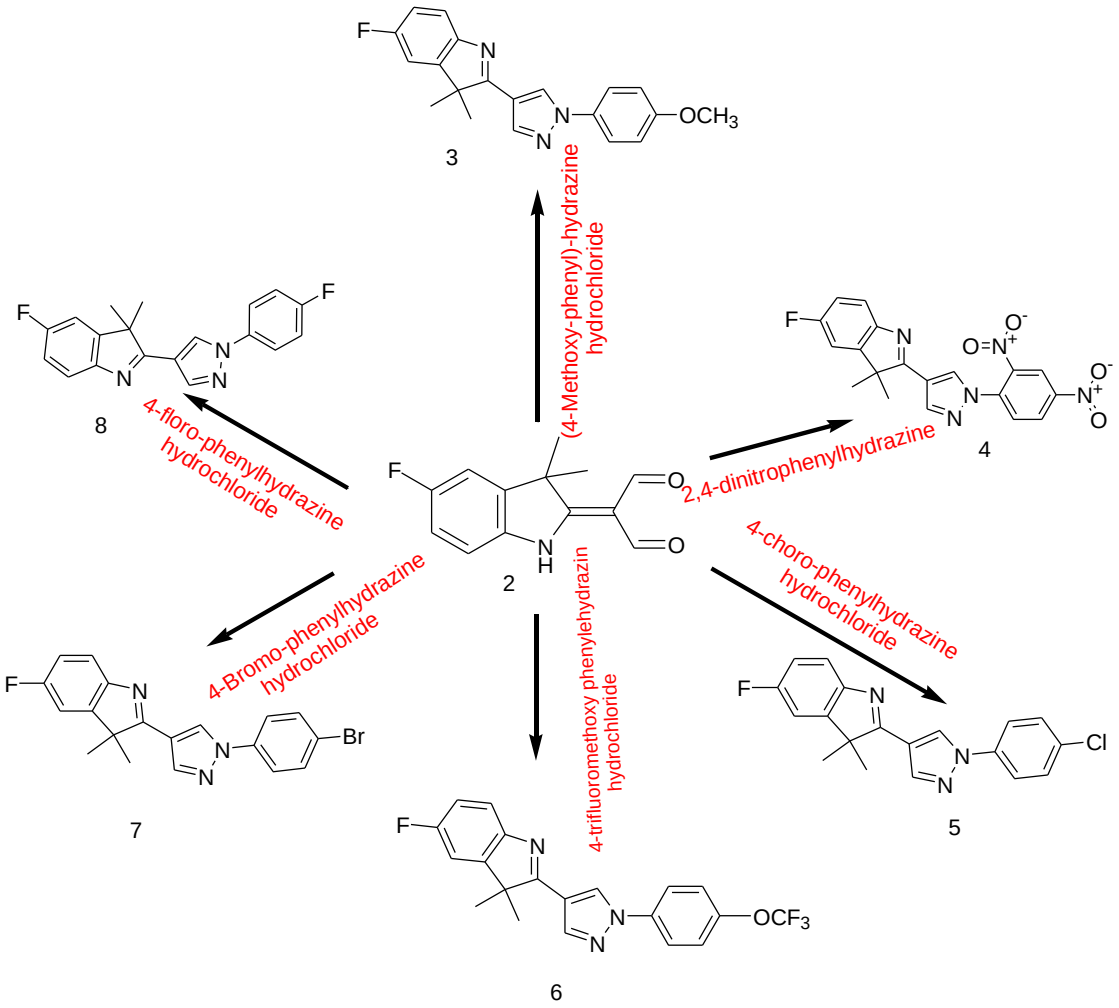




2-(5-Fluoro-3,3- dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

(2)

The second section; involves the synthesis of number of new pyrazole derivatives resulting from the reaction 2-(5-Fluoro-3,3-dimethyl-1,3- dihydro-indol-2-ylidene)-malonaldehyde (**2**) with different substituted phenyl hydrazine, in ethanol as a solvent as shown in scheme below:



The biological activity of the new synthesized compounds tested against breast cancer cells AMJ13 and determined significant antiproliferative.

CHAPTER ONE

INTRODUCTION

1. 1. Heterocyclic

Heterocyclic compounds are organic compounds that contain heteroatoms in their ring structure in addition to carbon atoms, such as sulfur, oxygen or nitrogen, as the heteroatom. The ring may be aromatic or non-aromatic.

Organic compounds contain ring made up of carbon atoms and another kind of atoms like five membered heterocyclic compounds containing one heteroatom in their structures (1*H*-pyrrole, furan and thiophene Figure 1. 1 which contain atoms most commonly N, O, S atoms in their structures. In these cases, oxygen, nitrogen and sulfur with non-bonding lone pair of electrons contribute to conjugated pi bond system in 5 membered ring system and are therefore have stable aromatic structures, but other atoms such as boron, phosphorus, or silicon can also be members of heterocyclic rings. [1]

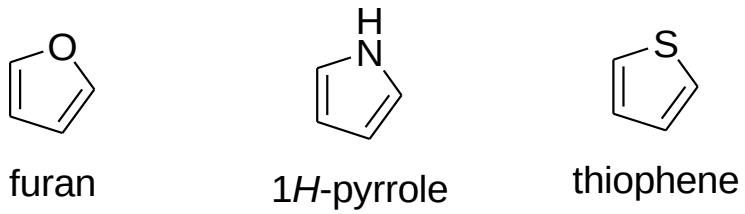


Figure 1. 1: the chemical structures of pyrrole, furan and thiophene

Heterocyclic compounds have a wide range of many applications, but are of particular interest in medicinal chemistry and industrial application [2] [3]

Nitrogen atom as well as oxygen or sulfur atoms is also found in many 5-membered heterocyclic compounds bearing more than one heteroatom like **oxazole and thiozole** as shown in the Figure 1. 2

Figure 1. 2: the chemical structures **thiozole and oxazole**

5-membered heterocyclic compounds bearing multiple nitrogen atoms. like**, 1*H*-imidazole, 1*H*-Pyrazole, 1H-1,2,3-triazole** and **1*H*-tetrazole** respectively, Figure 1. 3

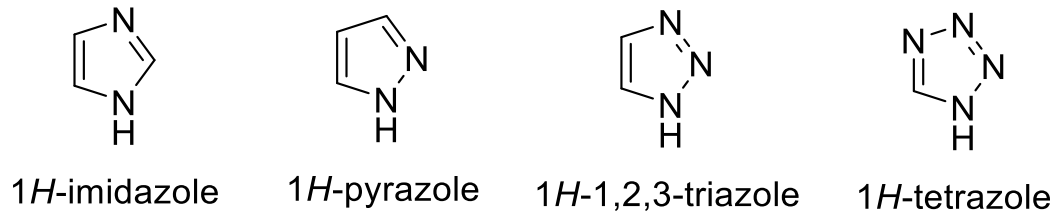


Figure 1. 3: the chemical structures of 1*H*-imidazole, 1*H*-Pyrazole, 1*H*-1,2,3-triazole and 1*H*-tetrazole respectively.

Six-membered heterocyclic compounds bearing one hetero atom (nitrogen, oxygen and sulfur) in their chemical structures in addition to carbon atoms such as Pyridine or Azine, 4*H*-pyran, Oxane and thiopyran or Thiane bear [4][5] Figure 1. 4

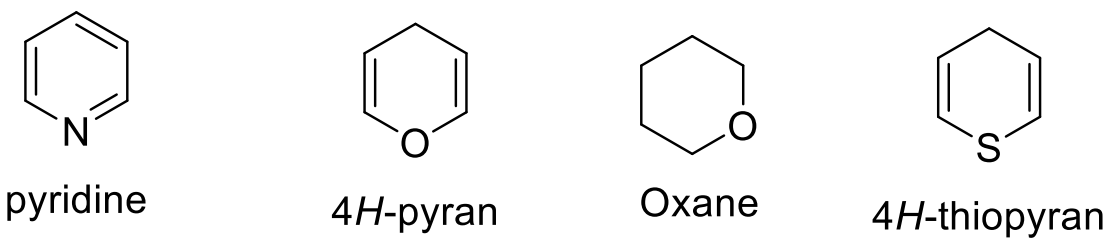


Figure 1. 4: the chemical structures of Pyridine, 4*H*-pyran, Oxane and thiopyran

Six-membered heterocyclic compounds bearing two hetero atoms (same kind of atoms) like pyridazine or 1,2-Diazine, pyrimidine or 1,3-Diazine and pyrazine or 1,4-Diazine Figure 1. 5

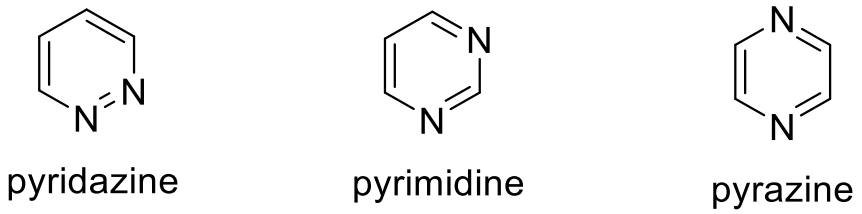


Figure 1. 5: the chemical structures of pyridazine, pyrimidine and pyrazine

Heterocyclic compounds are the largest type in organic chemistry which have a great importance in biologically and industrially field. Most pharmaceuticals and biologically active agrochemicals are heterocyclic .[6]

Organic heterocyclic compounds have cyclic structure with more than one type of dissimilar atoms besides carbon atoms such as, 6*H*-1,3-oxazine and 4*H*-1,3-thiazine [7] Figure 1. 6

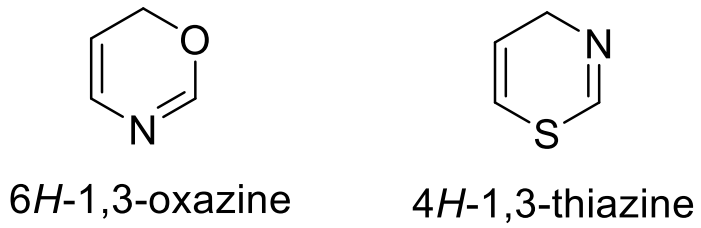


Figure 1. 6: the chemical structures of 6*H*-1,3-oxazine and 4*H*-1,3-thiazine

Heterocyclic compounds are occupying a significant place in heterocyclic chemistry due to their valuable properties as therapeutic agents, drugs, dyestuffs etc. These compounds have so many biological activities such as, antimicrobial, anti-inflammatory, blood platelet aggregation inhibiting property, anti-diabetic, heamoregulatory as well as pesticidal properties.[8]

The majority of pharmaceutical products are heterocycles which have biological activities [9]

The type and size of ring structures, together with the substituent groups of the core scaffold, impact strongly on the physicochemical properties.[10].

1. 2. Indole

Indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a benzene ring and a pyrrole nucleus are fused in 2, 3 positions of the pyrrole ring Figure 1. 7

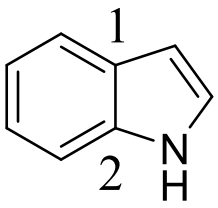
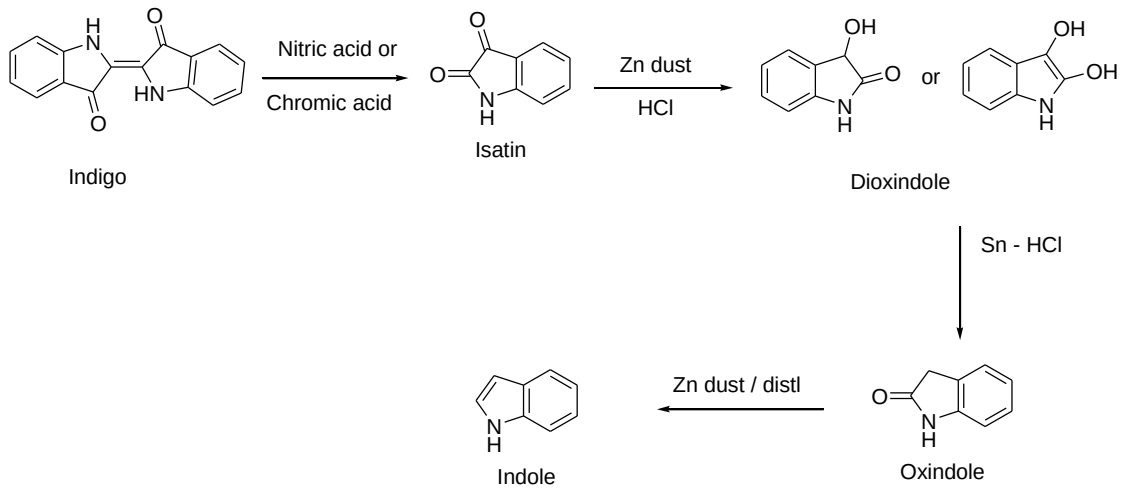


Figure 1. 7: the chemical structures of indole ring

The name *indole* is composed of the words *indigo* and *oleum.* Indole is non-basic nitrogenous compound. Indole chemistry began to develop with the study of the dye indigo. The word Indole is coined from the word India, a blue dye imported from India known as Indigo. In 1866, Adolf von Baeyer reduced oxindole to indole by using zinc dust Indigo can be converted to isatin and then to oxindole [11]

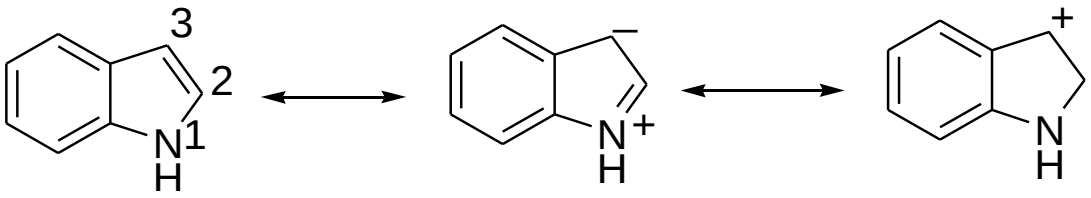
in 1869; he proposed a formula for indole.



Scheme 1. 1 Adolf von Baeyer indole synthesis

Indole ring is an aromatic heterocyclic compound, and it is a white solid compound at room temperature. The indole chemical formula is C_8H_7N. [12]

Indole is called as a π-excessive heterocycle. because of the π-excessive property, indole shows enhanced reactivity in electrophilic aromatic substitution, compared to benzene Scheme (1. 2) [13]



Scheme 1. 2: Resonance structures of indole

1. 2. 1. Previous synthetic methods of indole ring.

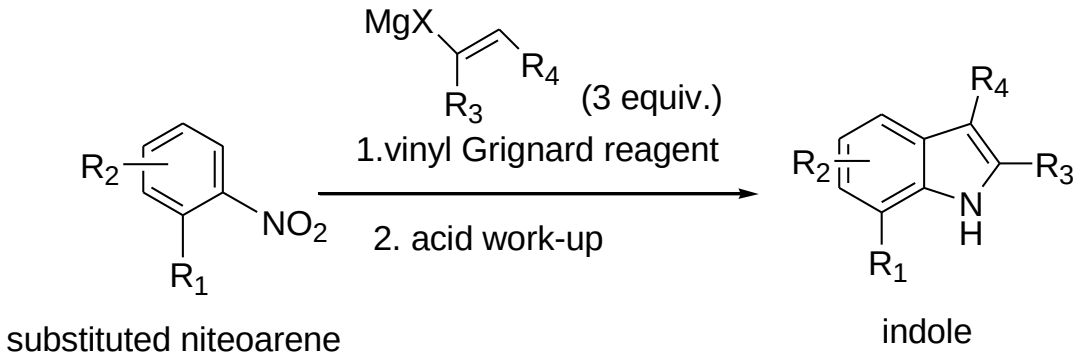
The indole nucleus is an important substrate of many natural and synthetic molecules with significant biological activity. This review summurized some of the relevant and recent achievements in the biological, chemical and pharmacological activity of important indole derivatives in the areas of drug discovery and analysis.[14] Indole compounds and their derivatives were found to numerous pharmacological activities like anti-tumor[15], anti-convulsant[16], anti-microbial[17], anthelmintic[18], anti-leishmanial[19], anti-tubercular[20] , anti-oxidant[21], anti-fungal[22], anti-inflammatory[23] and anti-psychotic[24] activities. The present review focuses on the Indole moiety with potential activities that are now in development [25] Indole is a typical fecal odor compound and has negative impact on water quality at low concentrations.[26]

The preparation of indole ring considered to be one of the most exciting reactions in organic chemistry. The following reactions are the most famous synthesizing of indole ring [27]

1. 2. 2. Bartoli indole synthesis.

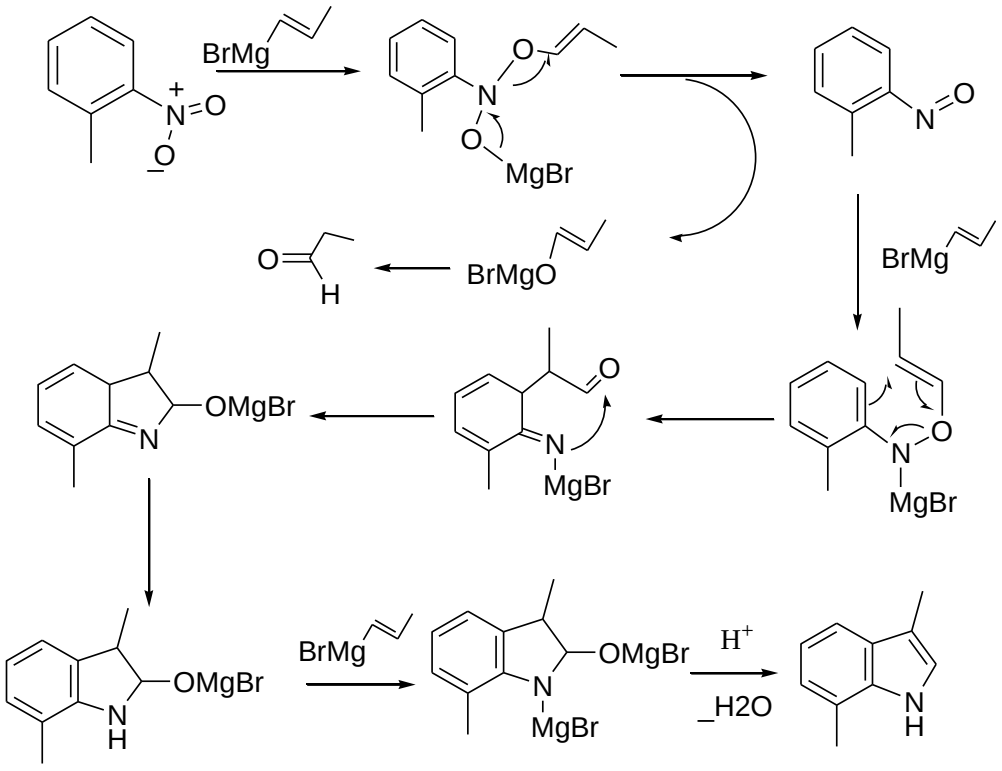
The Bartoli indole is an organic reaction where a substituted nitroarene is turned to an indole using an excess of a vinyl Grignard reagent succeeded by an acid workup. The yield of this reaction has affected by the substituents on the nitroarene, and the highest yield observed with ortho substituted reagents and the bulky groups equation (1. 1). The mechanism of Bartoli indole synthesis began with a series of attacks on the nitroarene regent by the Grignard reagents and followed by a sigmatropic rearrangement (Claisen) step which results in an aldehyde intermediate. Then the aldehyde is quickly attacked by the nearby nitrogen

intramolecular, and a subsequent attack by the third equivalent of the Grignard reagent restored aromaticity. A final acid workup step affords the indole product. As illustrated in the scheme (1-3) [28]



Equation (1. 1): Bartoli indole synthesis

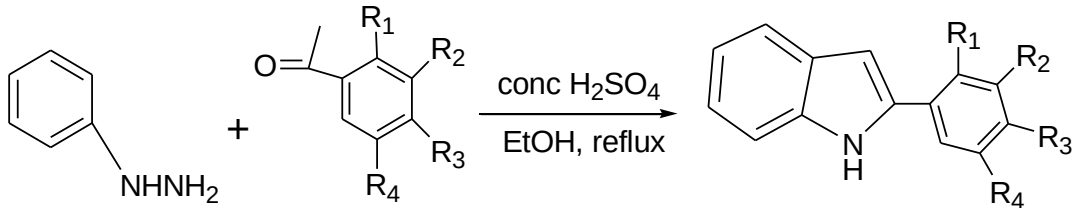
Reaction mechanism



Scheme (1-3): Mechanism of Bartoli indole synthesis.

1. 2. 3. Fischer-Indole Synthesis.

The Fischer indole synthesis is considered one of the best methods for preparing indoles. Named after the famous chemist Emil Fischer, it converts arylhydrazones into indoles in the presence of an acid catalyst.[29] The most method widely used in chemistry field to synthesis of heterocyclic organic compounds is Fischer indole synthesis. The Fischer indole synthesis converts ayrlhydrazones of aldehydes or ketones into indoles in the presence of an acid catalyst[30][31]. Below is the overall reaction equation (1. 2).



Equation (1. 2): Synthesis of substituted 2-aryl-1*H*-indoles

The accepted mechanism for Fischer synthesis has three steps **Figure (1. 8)** (a) toutomeric conversion of phenyl equation to enehydrazine (b) carbon-carbon double bond formation (c) cyclization with ammonia elimination and finellly indole synthesis.[31]

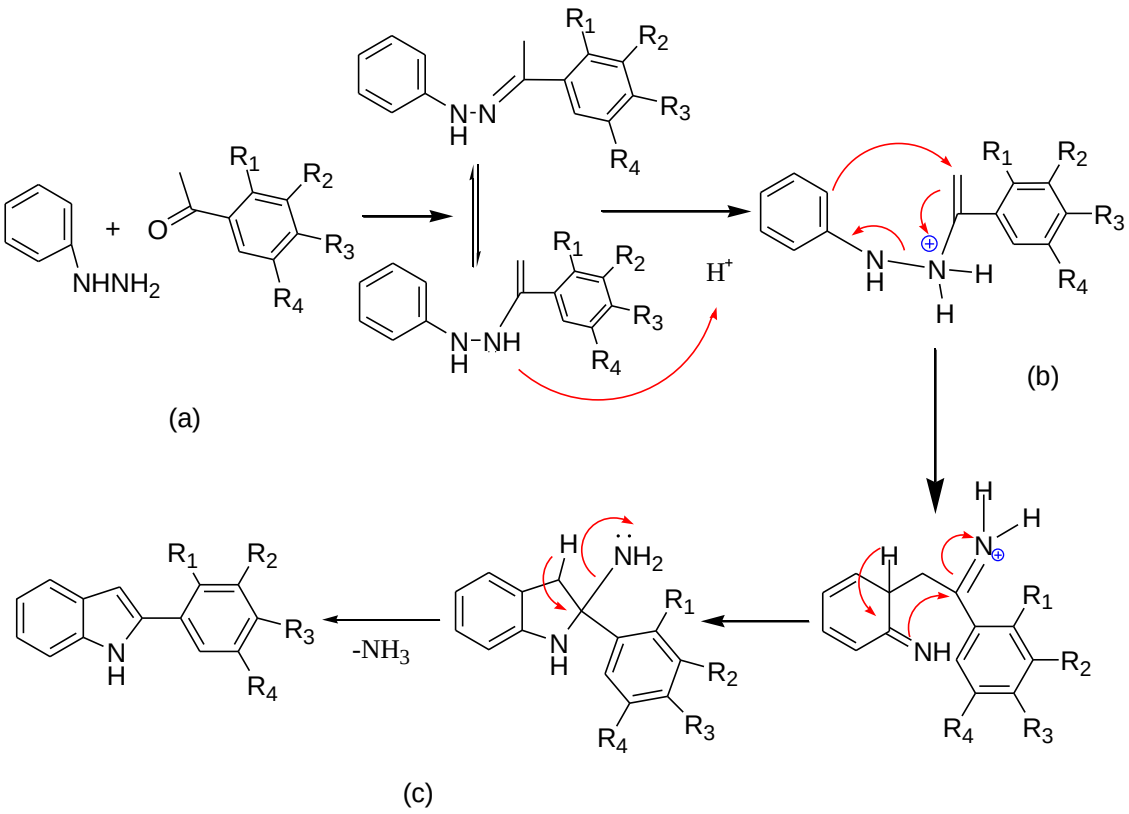
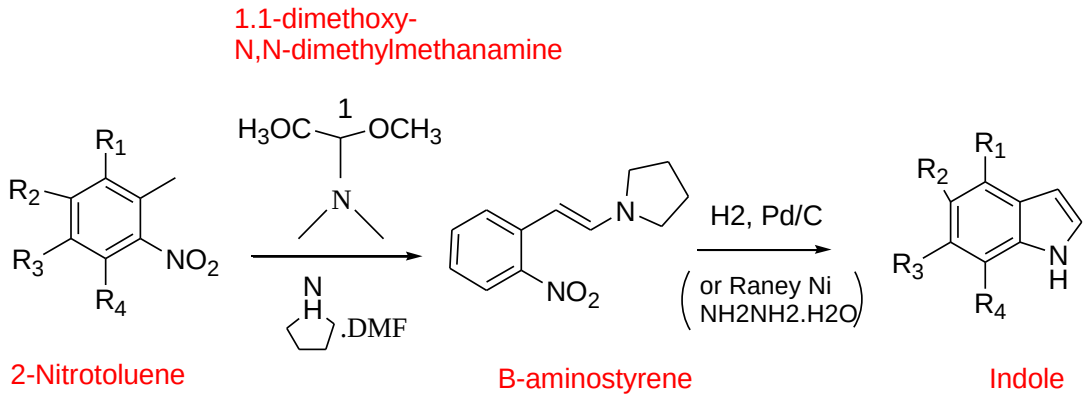


Figure 1. 8 : reaction mechanism for Fischer indole synthesis

1. 2. 4. Leimgruber-Batcho indole synthesis.

It is an efficient method of synthesizing indole and substituted indoles. Originally disclosed in a patent in 1976, this method is high-yielding and can generate substituted indole.[32] Leimgruber and Batcho synthesized a large number of indoles and employed several reduction conditions with and without the isolation of the nitro enamine[33]



R1= H ,OBn ,Cl ,CN ,Me ,CO2Me ,CO2Et
R2= H ,OBn ,Cl ,OMe ,F ,CO2Et
R3= H ,OBn ,Cl ,CN ,Me ,CO2Me ,CO2Et ,OMe ,NH2 ,F, CH-Pr2 ,CH(OMe)2 ,Br
R4= H ,Me ,CO2Me

Scheme (1. 4) : Leimgruber-Batcho indole synthesis

1. 2. 5. Indole activity.

Indole compound is aromatic heterocyclic, but exhibit very distinctive reactivity. Here are some general rules:

1. The nitrogen is not basic. (pKa -3.6)

2. Indole can readily undergo aromatic equation substitution. The C-3position is the most nucleophillic, followed by the N and C-2 positions.

3. The C-2 – C-3 bond can often react like alkenes.

4. Indole can be deprotonated at nitrogen. The resulting salts can be good nucleophiles.

5. Highly ionic salts (e.g. Li+, K+) favours N substitution.

6. Softer counter ions favours C-3 substitution.

7. When N is substituted, C-2 can be deprotonated [34]

Indole is an important heterocyclic system because it has built into protein in the form of the amino acid tryptophan, indole derivatives remain to be a fascinated subject for studying and it has found that indole derivatives exhibit antimicrobial and antiviral activities, against several types of virus including Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV) types 1, 2. Flavivirus, Respiratory Syncytial Virus (RSV), and Coxsackie B virus. Besides, some indole derivatives have shown significant protect properties for red blood cells and DNA against radical-induced oxidation [35]

Electrophilic Substitution of Indole: The electron density of carbons in heterocyclic ring of indole is higher due to contribution from nitrogen as in case of pyrrole. Therefore, the heterocyclic ring of indole is more reactive towards electrophiles compared to its benzene ring. The electrophilic substitution in indole takes place at C-3 and not at C-2 as in pyrrole. This can be explained from the following observations. Electrophilic attack C-2 and C-3 of indole which gives different intermediates as shown below [36]

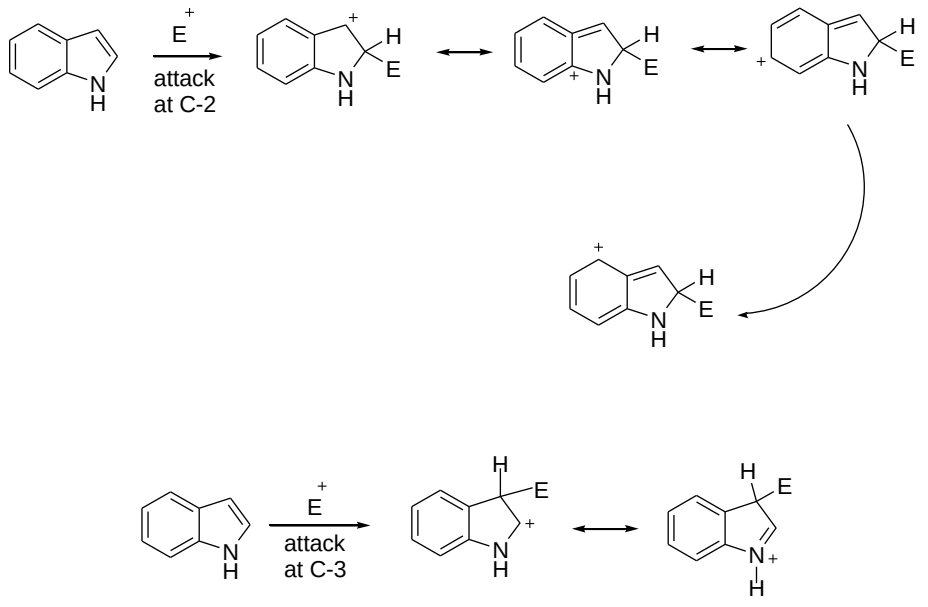


Figure 1. 9: The electrophilic substitution in indole

1. 3. Vilsmeier– Haack.

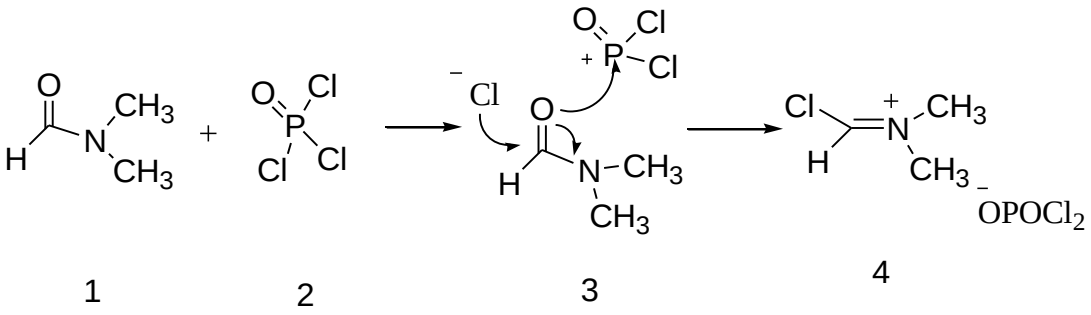
The Vilsmeier-Haack reagent ($POCl_3$+DMF) has attracted the attention of synthetic organic chemists since its discovery in 1927. 1 It is most commonly used for the introduction of CHO group into aromatic rings, since it is one of the most common functional group for carbon-carbon bond formation. In Vilsmeier-Haack reaction, DMF/$POCl_3$ has a dual role of reagent as well as solvent. 2 $POCl_3$ is a highly toxic solvent and its use is hazardous to health and is also pollutant of the environment.(37)

Vilsmeier reaction was initially used for the formylation of activated aromatic substrates and carbonyl compounds;4 it is now used as a powerful synthetic tool for the construction of many heterocyclic compounds 5–10 such as equations, indoles, quinozolines, and pyridines. The synthesis of various substituted chloronicotinaldehydes using Vilsmeier reaction have been much less reported in the literature. 11,12 Meth-Cohn and Westwood reported the synthesis of 2-chloropyridines, equation , and equation s using enamides under Vilsmeier reaction conditions.(38)

1. 3. 1. The Vilsmeier– Haack constituting.

The Vilsmeier-Haack reaction can also be applied to introduce an acetyl group on activated aromatic or hetero aromatic compounds, many other conversions can be achieved with this technology. The reaction is named after Anton Vilsmeier and Albrecht Haack. In general, *N*,*N* dimethylformamide (DMF) (1) and phosphorus oxychloride ($POCl_3$) (2) are used to generate a halomethyleniminium salt (4) used in the synthesis of a large number of heterocyclic compounds. When Vilsmeier reagent was

used in the reaction, the product of Vilsmeier reaction is an aldehyde. Thus, Vilsmeier reaction is often called as Vilsmeier reagent formylation. **(39)**

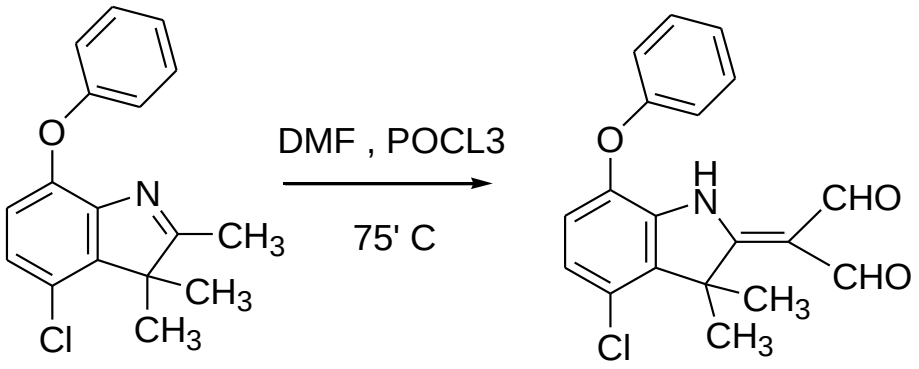


1 2 3 4

Equation (1. 3): The Vilsmeier– Haack constituting.

1. 3. 2. Some applications of Vilsmeier Haack reagent.

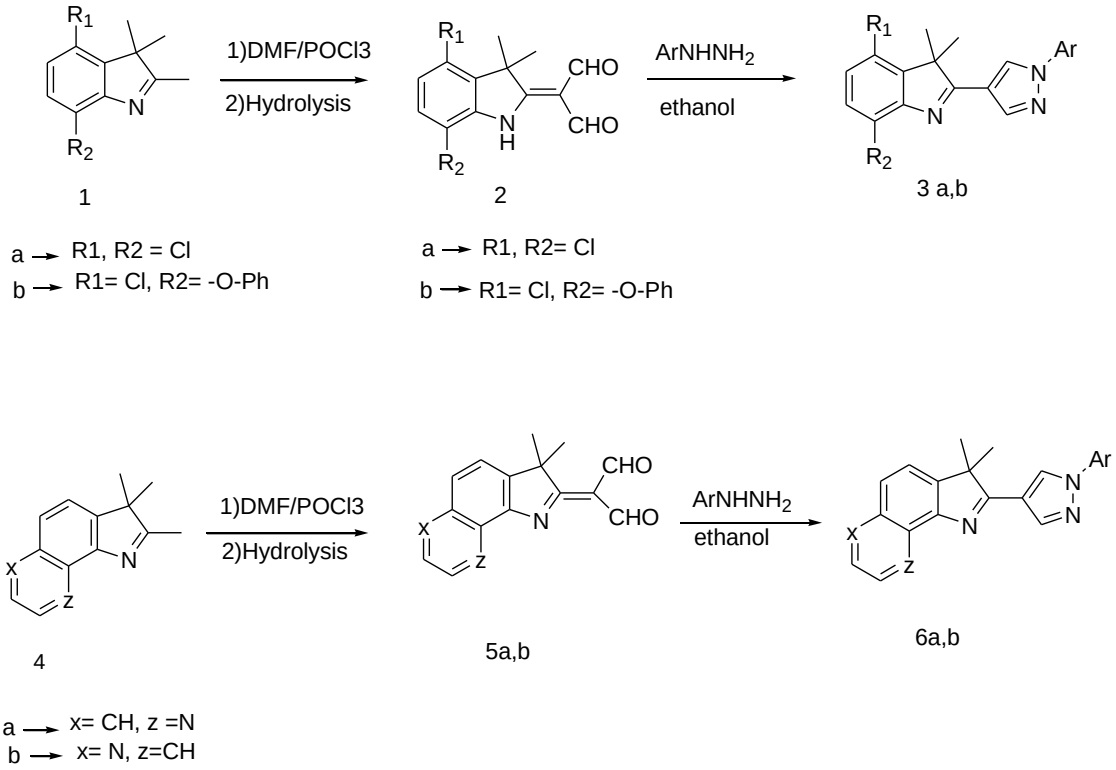
The reaction of compound **5** with Vilsmeier reagent at 75°C, led to diformylation of imine-methyl group in excellent yield Scheme (1. 5). The structure of malonaldehyde **6** was confirmed by its spectral data. The IR absorptions at 3159 and 1675 ,1639 cm^{-1} support a presence of N-H and two carbonyl groups, thus in ^{1}H-NMR spectrum signal for the *N*-hydrogen appearing at δ 13.55 ppm and two aldehyde hydrogens at δ 9.75 ppm. The ^{13}C-NMR spectrum of **6** showed the presence of two carbon signals at 187.66 and 192.64 ppm corresponding to CHO groups **(40)**



5 6

Scheme (1. 5) synthesis of 2-(4-Chloro-3,3-dimethyl-7-phenoxy-1,3-dihydro-indol-2-ylidene)-malonaldehyde

We described the reactions of several 2,3,3-trimethylindolenines (2,3,3-trimethyl-3*H*indoles) **1a,b** with the Vilsmeier reagent formed from *N*,*N*-dimethylformamide and phosphoryl trichloride to produce aminomethylene malondialdehydes **2a,b** (indol-2- ylidene malondialdehydes). Additionally we showed that the pyridoindolenines **4a,b** behave similarly, forming aminomethylene malondialdehydes **5a,b**. The condensation of hydrazine or aryl hydrazines with the aminomethylene malondialdehydes **2a,b** and **5a,b** afforded the corresponding 4-substituted pyrazoles **3a,b** and **6a,b**[41]



Scheme (1. 6) synthesis of compuned 3a,b and 6a,b

1. 4. Pyrazole

Pyrazole is a chemical compound that has a five-membered heterocycle with two nitrogen atoms and three adjacent carbons. Pyrazole derivatives have shown good pharmacological effects or have the potential biological activities, such as, anti-inflammatory [42], antiviral [43],

antimicrobial [44], anticonvulsant [45], antitumor [46], fungicidal activities [47] and antihistaminic [48]

1. 4. 1. The important of pyrazole ring.

The pyrazoles are an interesting class of heterocyclic compounds and important building blocks in organic synthesis and more potent biologically active molecules in pharmaceutical and medicinal chemistry. [49]

1. 4. 2. Some important Reactions of pyrazole rings.

1. 4. 2. 1. Synthesis of 3, 5-dimethyl-1*H*-pyrazole and some derivatives

3,5-dimethyl-1*H*-pyrazole was prepare using acetyl acetone and hydrazine hydrate in tetrahydrofuran as solvent figure (1. 10)

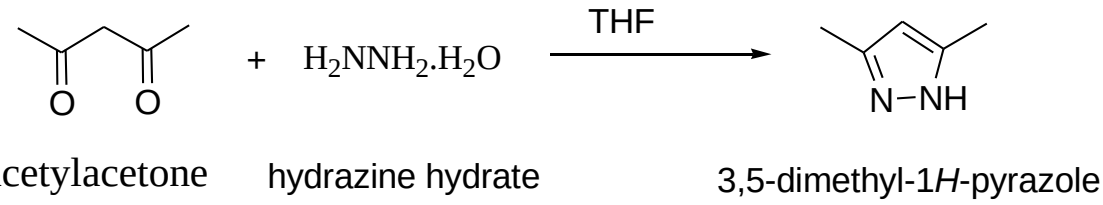


Figure (1. 10): Chemical structure of 3,5-dimethyl-1*H*-pyrazole.

4-chloro-1-(2-chloroethyl)-3.5-dimethyl-1*H*-pyrazole was prepare by N-alkylation of 4-chloro-3,5-dimethyl-1*H*-pyrazole with 1,2 dichloroethane (DCE), a phase transfer catalyst tetrabromoammonium chloride (TBAC) in aqueous NaOH and the latter compound was prepare from reaction 3,5-dimethyl-1*H*-pyrazole with N-chlorosuccinamide or N-bromosuccinamide as illustrated in figure (1. 11)

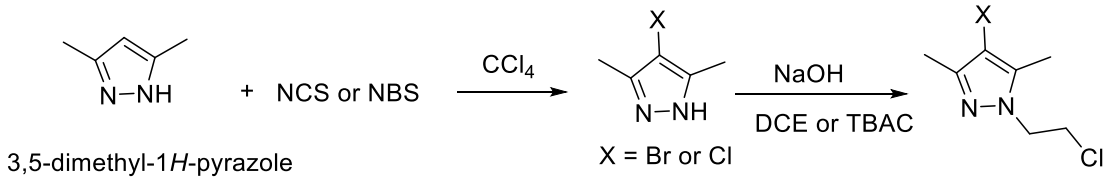


Figure (1. 11): Chemical structure of 4-chloro-3,5-dimethyl-1*H*-pyrazole and 4-chloro-1-(2-chloroethyl)-3.5-dimethyl-1*H*-pyrazole

N-chloroethyl-3,5-dimethyl pyrazole-4-carbaldehyde was carried out by the formylation of at C-4 position of 4-chloro-1-(2-chloroethyl)-3,5-dimethyl-1*H*-pyrazole by Vilsmeier Haack reaction [(50)] figure (1. 12)

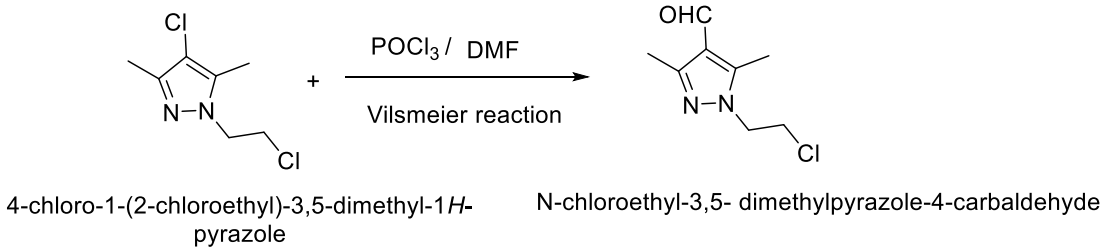


Figure (1. 12): Chemical structure of N-chloroethyl-3,5-dimethyl pyrazole-4-carbaldehyde

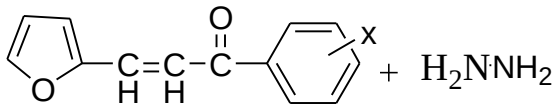
1. 4. 3. Pyrazole synthesis

Pyrazole derivatives are preparing in many synthetic pathways which represent an interesting topic since these compounds have various applications in the pharmaceutical and agrochemical industry[(51)]

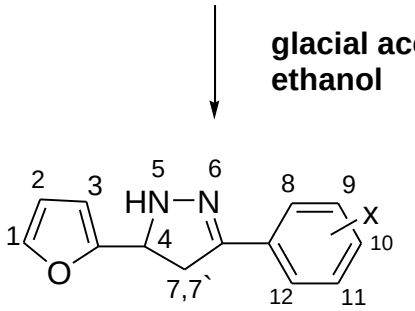
1. 4. 3. 1. From chalcones

Treatment of chalcones derivatives with hydrazine hydrate in boiling ethanol gave pyrazoline derivatives compounds, after purification by

recrystallization from ethanol, pure pyrazoline derivatives compounds as shown in scheme (1.7). The structures of these products were established from their elemental analysis, FT-IR, C.H.N and ^{1}H NMR spectra.[52]



X= H , 4-OCH_3 , 4-Br ,4-NO_2 , 3-NH_2 , 3-NO_2

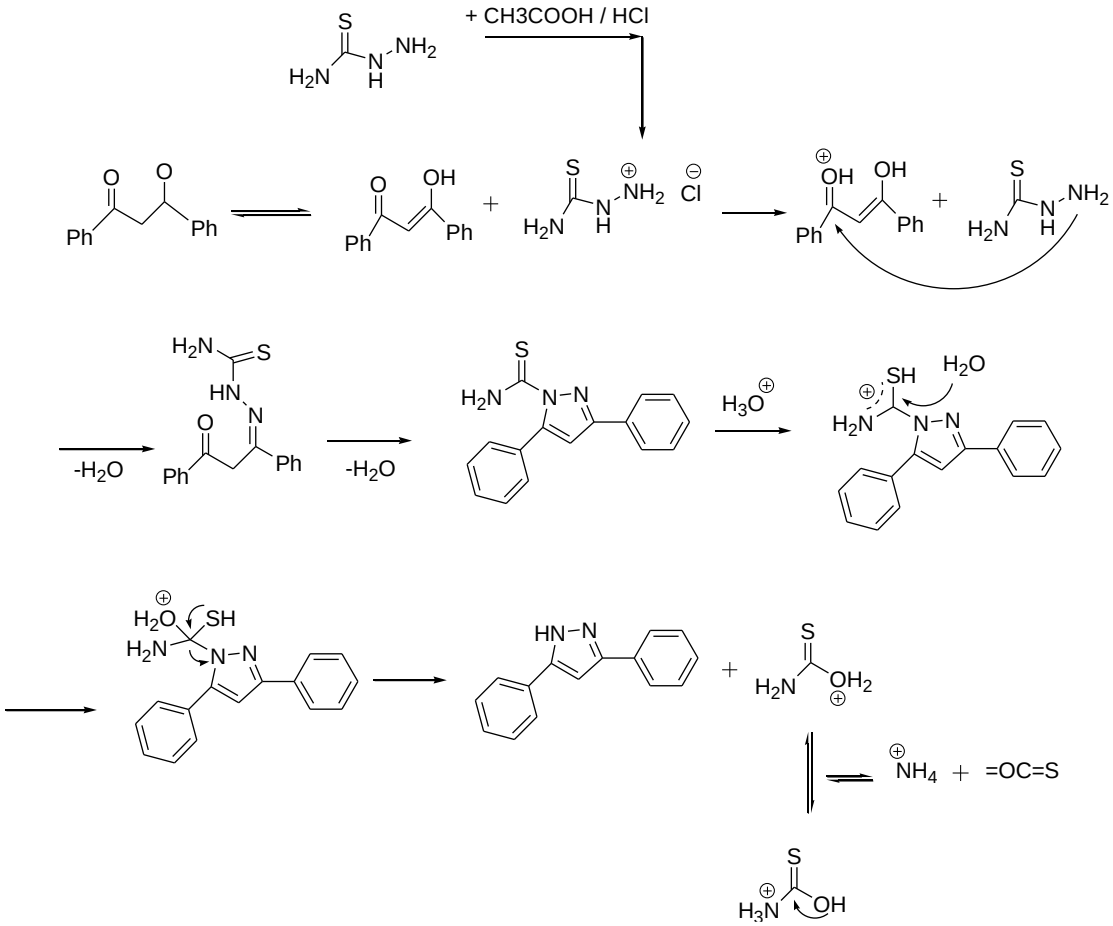


X= H , 4-OCH_3 , 4-Br ,4-NO_2 , 3-NH_2 , 3-NO_2

Scheme (1. 7) some of pure pyrazoline derivatives compounds

1. 4. 3. 2. From dibenzoylmethane and thiosemicarbazide.

The 3,5-diphenyl-1*H*-pyrazole prepared reaction of dibenzoylmethane and thiosemicarbazide in acetic acid Scheme (1.8). This reaction was catalyzed by HCl and resulted in good yield. The heating was carried out by both conventional and microwave irradiation. The rates and percentage yields were compared by both methods. The results show that the microwave irradiation is rapid and pure more than conventional heating..[53]

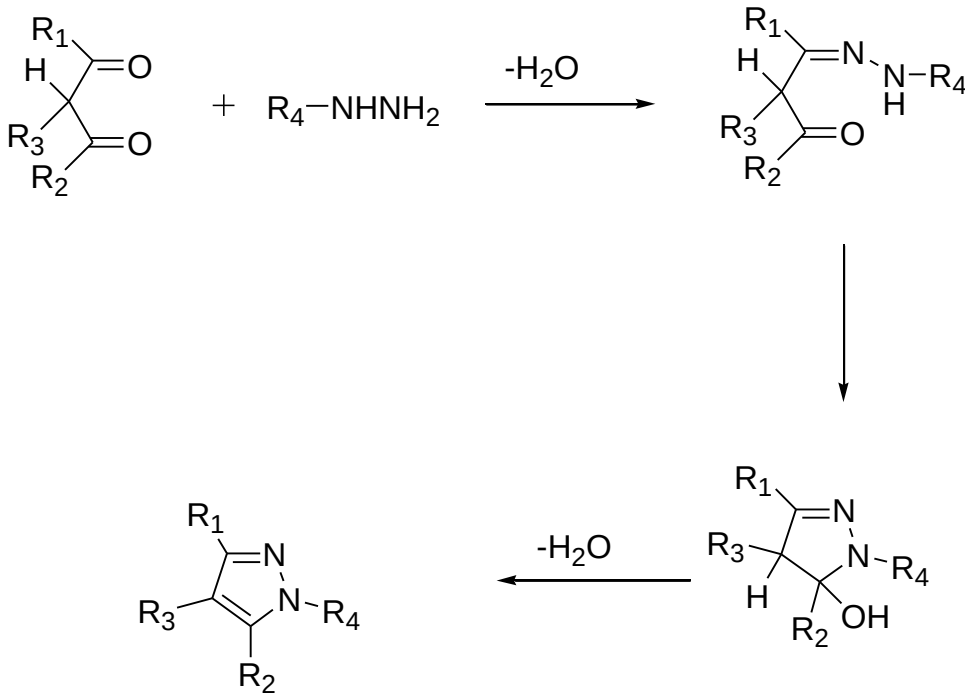


Scheme (1. 8) prepared 3,5-diphenyl-1*H*-pyrazole

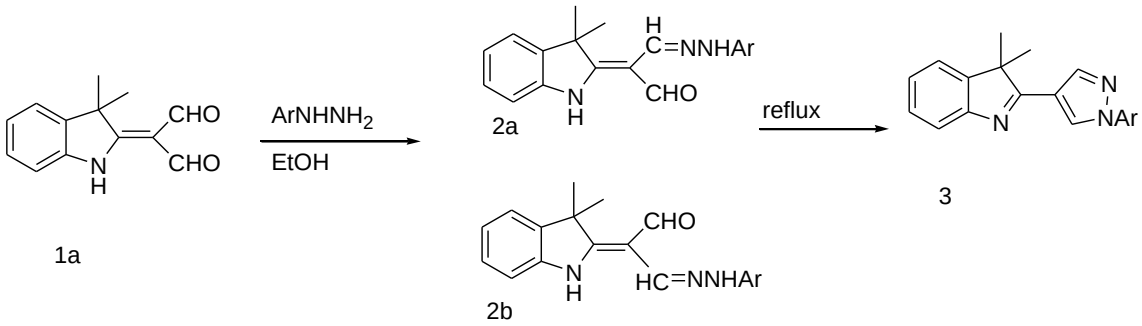
1. 4. 3. 3. Knorr pyrazole synthesis.

It's one of the essential methods for synthesis involving the reaction of 1,3- dicarbonyls with hydrazine derivatives. The reaction proceeds by the initial composition of the mono hydrazine that converted to the pyrazoles by the action of acids and heat. Previous studies, have described the reaction of 2- (diformylmethylidene)-3,3 dimethylindole (1a) with arylhydrazines to produce the related pyrazolylindolenines . As they reported, the initial products of the reaction, separated at room temperature, were mono-hydrazones (**2a** or **2b**) although it was not possible to ascertain which carbonyl group had reacted. Heating the mono-hydrazones in refluxing ethanol produced 4-(3,3-dimethylindol-2-yl)-substituted

pyrazoles **3**, with migration of the double bond into the dihydropyrrole ring.(54)



Scheme (1. 9) Knorr pyrazole synthesis.

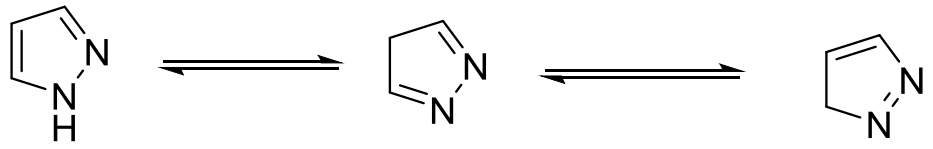


Scheme (1. 10) synthesis of 4-(3,3-dimethylindol-2-yl)-substituted pyrazoles.

1. 4. 4. Pyrazole bioactivity.

Pyrazoles are aromatic molecules due to their planar conjugated ring structures with six delocalized π-electrons. Therefore, many important properties of these molecules were analyzed by comparing with the properties of benzene derivatives. Like other nitrogen involving

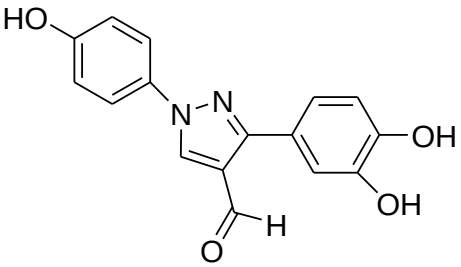
heterocycles, different tautomeric structures can be written for pyrazoles. Unsubstituted pyrazole can be represented in three tautomeric forms [55] scheme (1. 11).



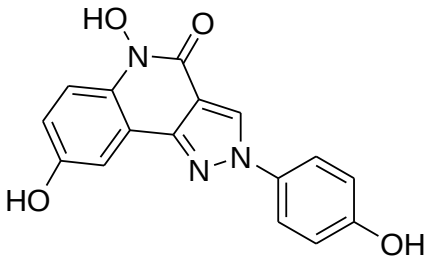
scheme (1. 11) : Tautomeric forms of substituted pyrazole

1. 4. 5. Examples to Pyrazole Derivatives

Christodoulou *et al*. in (2010) synthesized a series of tri substituted pyrazole derivatives (**7**) and PIFA-mediated conversion of molecules bearing the fused pyrazolo [4,3-c]quinolone ring system and evaluated them for anti- Md. Jahangir Alam *et* .1437 angiogenic activity by using *in vitro* assays for endothelial cell proliferation and migration, in the chicken chorioallantoic membrane (CAM) assay. Compounds having fused pyrazolo [4, 3-c]quinoline motifs emerged as potent anti-angiogenic compounds, and also inhibit the growth of human breast (MCF-7) and cervical (Hela) ncarcinoma cells *in vitro*.2[56]



7a

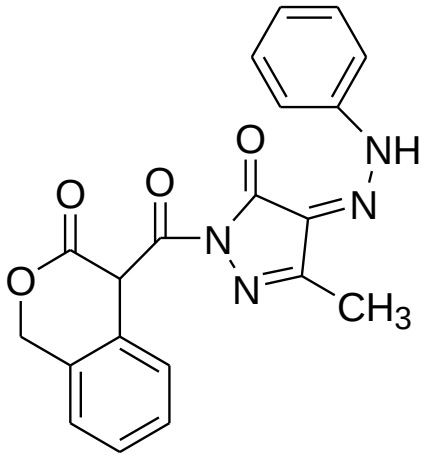


7b

Scheme (1.12)

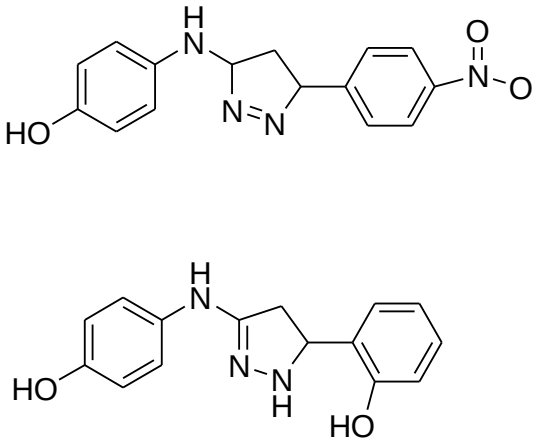
Pyrazole-3-carboxylic acid derivatives represent important building blucks in organic and medicinal chemistry because their pharmacological properties. For example, pyrazole-3- carboxylic acids and pyrazolo[1,5-c]quinazoline-2-caboxylates are nicotinic acid receptor agonists, bis (benzo[*g*]indazol-3-carboxamides) exhibit antiproli-ferative activity against various cancer cell lines. Ethyl- 5-propyl-1H-pyrazol-3-carboxylate is a key intermediate for the synthesis of Viagra ***Tung T. Dang et al., 2008*** [57]

A series of (4Z)- 3 methyl-1-[2 oxo-2H- chromen-4yl) carbonyl] – 1H pyrazole -4, 5-dione4-[(4- substituted phenyl) hydra zone). The entire compounds were screened for their anti-inflammatory and analgesic activities Sivakumar, K. K., Rajasekaran *et al*.[58]



Scheme (1. 13)

Jamwal, A., Javed, A., & Bhardwaj, V. synthesized a series of Pyrazole derivative and screened for their analgesic activity.[59]



Scheme (1.14)

1. 5. Biological Activities.

1. 5. 1. Cancer

Cancer is a group of diseases that cause the body's cells to divide uncontrollably destroying nearby tissue. Cancer is the second-leading cause of death in the United States following heart disease. cancer in the United States was responsible for 582,623 deaths in accounting for 22.9% of all mortality in 2012[(60)]

1. 5. 2. Breast cancer

Breast cancer is the most common cancer among women in Arab countries[(61)] It has the highest occurrence forming of about 31.1% of newly diagnosed cancer cases.[(62)] One million women in the worldwide develop breast cancer every year and almost 600,000 die from it [(63)] Numerous factors are known to increase the disease's occurrence, including many that are modifiable (e.g., tobacco use and excess body weight) and those that are not (e.g., inherited genetic mutations and immune conditions). [(64)]

1. 6. Research Outline.

1. 6. 1. Aims of this thesis

The objectives of this study are: -

1. synthesize a series of new pyrazole derivatives.
2. Identify the chemical purity and structures of the synthesized compounds; by various spectroscopic techniques like nuclear magnetic resonance spectroscopy ^{1}H, APT ^{13}C-NMR and Fourier Transform infrared spectroscopy (FT-IR) as well as to confirm their physical properties.
3. Evaluating the biological activity of the newly synthesized compounds to inhibit breash cancer cell AMJ13.

CHAPTER TWO

EXPERIMENTAL SECTION

2. 1. Chemistry part

2. 1. 1. Materials

All substances and solvents used in this research were purchased from various companies as listed in the Table (2-1). They used as received without additional purification. (4-Fluoro-phenyl)-hydrazine hydrochloride was synthesized with a qualification of a procedure described by reference [65].

The purity of the synthesized compounds was checked it by TLC plates, and the melting points determined by the open capillary melting point device.

Table (2-1): Chemicals and solvents used in the chemistry part.

No.	Chemicals	Molecular formula	Company
1	4-Florophenylhydrazinhydrochloride	$C_6H_8FN_2Cl$	Sigma-Aldrich
2	4-Chlorophenylhydrazinhydrochloride	$C_6H_8Cl_2N_2$	Merck
3	4-Bromophenylhydrazinhydrochlorid	$C_6H_8BrN_2Cl$	Merck
4	4-Methoxyphenylhydrazine hydrochloride	$C_7H_8ClN_2O$	TCI & Merck
5	2,4-Dinitrophenylhydrazine	$C_6H_6N_4O_4$	TCI
6	4-Trifluoromethoxyphenylhydrazine	$C_7H_7F_3N_2O$	Merck

7	Dimethyl formamide	C_3H_7NO	Romil
8	Ethanol	CH_3CH_2OH	Scharlaw
9	Ethyl acetate	$C_4H_8O_2$	Romil
10	Glacial acetic acid	CH_3COOH	Chem lab
11	Hexane	C_6H_{14}	Sigma Aldrich
12	Methyl isopropyl ketone	$C_5H_{10}O$	Merck
13	Phosphoryl chloride	$POCl_3$	Merck
14	Sodium hydroxide	NaOH	Thomas Baker
15	Sodium sulfate	Na_2SO_4	**Loba chemie**
16	Magnesium sulfate	Mg_2SO_4	**Loba chemie**

2. 1. 2. Instruments.

2. 1. 2. 1. Fourier Transform Infrared Spectrometer (FT-IR):

IR spectra achieved on a Perkin-Elmer Spectrum version 10.02 by using a disk of KBr for solid substance in Department of Chemistry, College of Sciences, University of Diyala

2. 1. 2. 2. Nuclear Magnetic Resonance Spectrometer (NMR):

^{1}H and APT ^{13}C-NMR spectra measured on a Bruker 400 MHz spectrometer in Jordan, College of science, University of Science and technology, Irbid city.

2. 1. 2. 3. Thin Layer Chromatography (TLC):

The new compounds have taken to purification by using Silica gel plates , and the spots have detected by using a fluorescence analysis cabinet model CM10 In Department of Chemistry, College of Sciences, University of Diyala

2. 1. 2. 4. Melting Point:

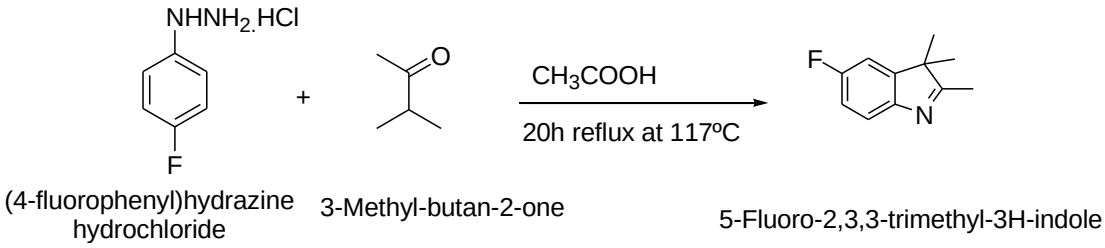
The melting points of synthesis compounds were estimated by open capillary tubs in the Stuart melting point apparatus SMP10 UK, in the Department of Chemistry, College of Sciences, University of Diyala

2. 1. 2. 5. Rotary Evaporator:

Rotary evaporator used to evaporate the solvent from solutions made by Heldove apparatus, HeiVAP, Germany in Department of Chemistry, College of Sciences, University of Diyala

2. 1. 3. Synthetic methods.

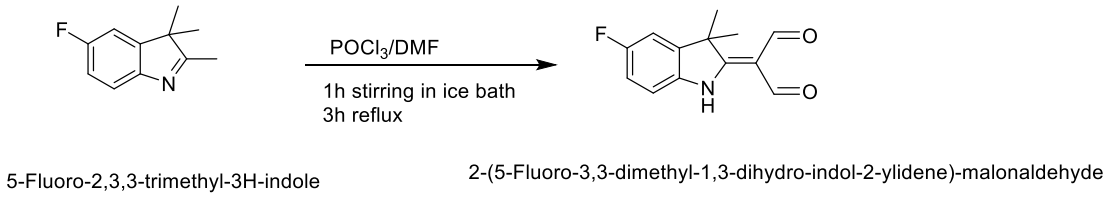
2. 1. 3. 1. Synthesis of 5-Fluoro-2,3,3-trimethyl-3H-indole, (Indolenine) 1 as elucidated in equation (2. 1)



Equation (2. 1): The synthetic pathway of 5-fluoro -2,3,3-trimethyl-3H-indole. (**1**)

A mixture of (2g, 12 mmol) of 4-Fluoro-phenylhydrazine hydrochloride and methyl isopropyl ketone (1.58g, 18mmol) have dissolved in (35 mL) of glacial acetic acid, and the mixture refluxed in oil path at 117 ^{0}C for 20h. Then the red product was quenched with ice distilled water, and neutralized with aqueous 25% NaOH, then extracted with ethyl acetate and water three times (3×25mL). The organic layer dried over Na_2SO_4, and the solvent was concentrated in vacuum to afford the oily red liquid of indolenine. (**1**) Yield: (1.99g, 91%) ^{1}H-NMR (400MHz, DMSO, δ in ppm): 6.71-7.57 (m, 3H, *Ar-H*), 2.17 (s, 3H, CH_3), 1.40 (s, 6H, 2xCH_3).

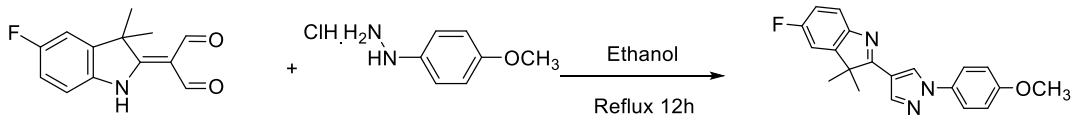
2. 1. 3. 2. Synthesis of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (2) as illustrated in equation (2. 2)



Equation (2. 2): The synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**).

5 mL of N, N-dimethyl formamide (DMF) cooled in an ice bath then (3 mL, 30 mol) of Phosphoryl chloride ($POCl_3$) added dropwise with stirring under 7°C for 10 minutes , Then a solution of (1.99g, 10mmol) indolenine (**1**) in DMF (5 mL) was added dropwise for 10 minutes at 7 °C , The reaction mixture stirred in ice bath for 1h. Then reflux for 3h, at 85-90ºC. The resulting solution was poured on icy distill water and neutralized with aqueous 25% NaOH. The resulting is a brown precipitate was filtered off, washed with hot distill water and dried in oven at 78ºC then recrystallized from ethanol to give Pure of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**). The purity of this compound determined by using TLC (3:1) hexane: ethyl acetate as an eluent, with pre-coated silica gel, which gave one spot on polar area. Yield: (2g, 81%), m.p. 178-180 °C. FT-IR (cm^{-1}): 3201, 3042, 2983, 2854, 1657.4, 1616.3, 1534, 1469.4, 1372, 1275.5, 1178, 814. ^{1}H-NMR (400MHz, DMSO, δ in ppm): δ= 13,13 (s, 1H, N*H* indole ring), 9.75 (s, 2H,*CHO*) 7.12-7.62 (m, 3H, *Ar-H*), and 1.67 (s, 6H, 2xCH_3).

2. 1. 3. 3. Synthesis of 5-Fluoro-2-[1-(4-methoxy-phenyl)-1*H*-pyrazol-4-yl]-3,3-dimethyl-3*H*-indole (3) as illustrated in equation (2. 3)



2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

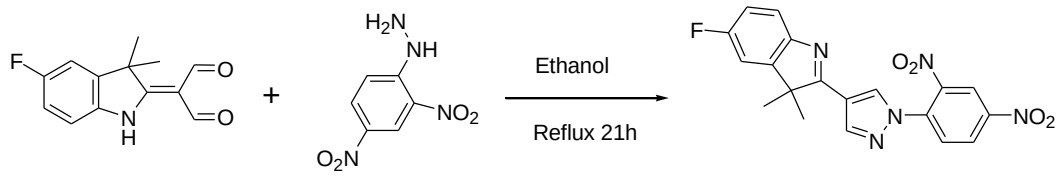
4-methoxy-phenylhydrazine hydrochloride

5-Fluoro-2-[1-(4-methoxy-phenyl)-1H-pyrazol-4- yl]-3,3-dimethyl-3H-indole

Equation (2. 3): The synthetic pathway of 5-Fluoro-2-[1-(4-methoxy-phenyl)-1*H*pyrazol- 4-yl]-3,3-dimethyl-3*H*-indole. (**3**)

A mixture solution of (0.2g, 8mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) dissolved in 10 ml ethanol and (0.14g, 8mmol) of 4-methoxy-phenylhydrazine hydrochloride dissolved in 15mL ethanol, the mixture was left refluxing at 78^{o}C for 12 h in waterbath. The solvent evaporated under the reduced pressure, and the brown residue was filtered off, washed with hexane and dried in the oven. The purity of this compound determined by using TLC (3:1) hexane: ethyl acetate with pre-coated silica gel, which gave one spot on the polar area. Yield: (0.28g, 96%), m.p.240-242 ^{o}C. FT-IR in (cm^{-1}): 3065, 2942, 2836, 2443,1880.7, 1587, 1349, 1243, 1172, 1075, 743. ^{1}H-NMR (400 MHz, DMSO, δ ppm): 10.31 (s, 1H, pyrazole ring), 9.60 (s, 1H, pyrazole ring), 7.95-8.77 (m, 7H, *Ar-H*), 4.66 (s. 3H, OCH_3), 1.67 (s, 6H, 2xCH_3). APT^{13}C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH_3 appeared at negative value (below baseline of the spectrum) δ = 142.18, 121.46, 119.05, 115.54 , 115.30,115.12. (carbon atoms of the aromatic and pyrazole ring), 24.51 (2xCH_3). Whereas quaternary carbons, CH_2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ= 179.10, 162.73, 160.31, 159.11, 130.65 (carbon atoms of the aromatic and pyrazole ring) and 53.71 (CH_3-*C*-CH_3).

2. 1. 3. 4. Synthesis of 2-[1-(2,4-Dinitro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3*H*-indole (4) as illustrated in equation (2. 4)



2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

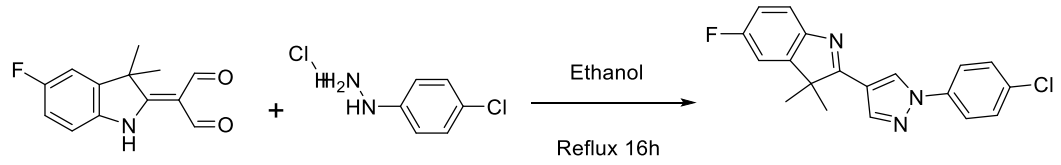
2,4-dinitrophenylhydrazine

2-[1-(2,4-Dinitro-phenyl)-1H-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3H-indole

Equation (2. 4): The synthetic pathway of 2-[1-(2,4-Dinitro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3*H*-indole. (**4**)

A mixture solution of (0.2g, 6mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3- dihydro-indol-2-ylidene)-malonaldehyde (**2**) dissolved in 10 ml ethanol and (0.16g, 6mmol) of 2,4-dinitrophenylhydrazine dissolved in 40mL ethanol, the mixture was left refluxing at 78℃ for 21h in water a bath. The solvent evaporated under the reduced pressure, and the red residue was filtered off, washed with hexane and dried in the oven. The purity of this compound determined by using a recrystallization process by hot water and TLC (3:1) hexane: ethyl acetate, with pre-coated silica gel, which gave one spot. Yield: (0.27g, 90%), m.p. 198-199 ^{o}C FT-IR in (cm^{-1}): 3259, 2977, 1642, 1607, 1540,1487,1381, 1331, 1272, 1181, 737. ^{1}H-NMR (400 MHz, DMSO, δ ppm): 10.35 (s, 1H, pyrazole ring), 9.56 (s, 1H, pyrazole ring), 8.12-8.96 (m, 6H, *Ar-H*), 1.73 (s, 6H, 2xCH_3).

2. 1. 3. 5. Synthesis of 2-[1-(4-Chloro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro- 3,3-dimethyl-3*H*-indole (5) as demonstrated in equation (2. 5)



2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

4-chloro-phenylhydrazine hydrochloride

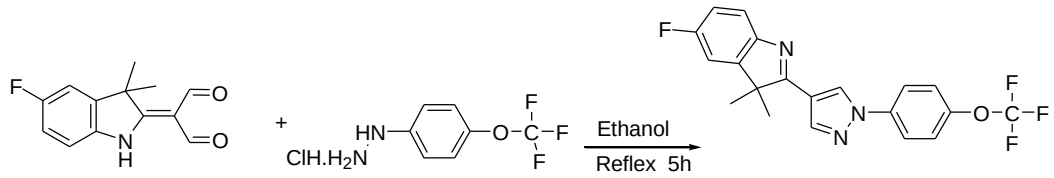
2-[1-(4-Chloro-phenyl)-1H-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3H-indole.

Equation (2. 5): The synthetic pathway of 2-[1-(4-Chloro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3*H*-indole. (**5**)

A mixture solution of (0.2 g, 6 mmol) 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) dissolved in 10ml ethanol and (0.15g, 6mmol) of 4-choro-phenylhydrazine hydrochloride dissolved in 25mL ethanol; the mixture was left refluxing at 78^{o}C for 16h in waterbath. The solvent evaporated under the reduced pressure, and the brown residue was filtered off, washed with hexane and dried in an oven. The purity of the compound determined by using TLC (3:1) hexane: ethyl acetate, with pre-coated silica gel, which gave one spot. Yield: (0.25g, 86%), m.p. 223-224^{o}C. FT-IR in (cm^{-1}): 3077, 2983, 2425, 1883, 1607, 1584, 1352, 1260, 1087, 826,743 and 552. 1H-NMR (400 MHz, DMSO, δ ppm): 9.50 (s, 1H, pyrazole ring), 8.74 (s, 1H, pyrazole ring), 7.25-8.06 (m, 7H, *Ar-H*), 1.62 (s, 6H, 2x C*H*$_3$). APT^{13}C-NMR (100MHz, DMSO,δ in ppm): shown signals for CH and CH_3 appeared at negative side (below baseline of the spectrum) δ= 138.03, 129.93, 119.73, 115.97, 115.25, 115.01 (carbon atoms of the aromatic and pyrazole ring), 24.24 (2x*C*H$_3$).Whereas quaternary carbons, CH_2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ = 178.73, 162.57,

160.15, 148.25, 138.03,130.22, 115.97 (carbon atoms of the aromatic and pyrazole ring) and 53.77 (CH_3-*C*-CH_3)

2. 1. 3. 6. Synthesis of 5-Fluoro-3,3-dimethyl-2-[1-(4-trifluoro methoxy-phenyl)-1H-pyrazol-4-yl]-3H-indole.(6) as illustrated in equation (2. 6)



2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

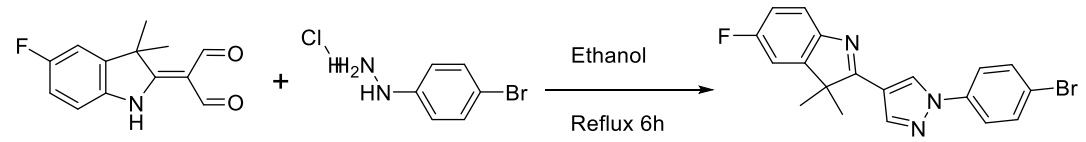
5-Fluoro-3,3-dimethyl-2-[1-(4-trifluoromethoxy-phenyl)-1*H*-pyrazol-4-yl]-3*H*-indole

Equation (2. 6): The synthetic pathway 5-Fluoro-3,3-dimethyl-2-[1-(4-trifluoro methoxy-phenyl)-1H-pyrazol-4-yl]-3H-indole.(**6**)

A mixture solution of (0.1g, 8mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) dissolved in 10 ml ethanol and (0.09g, 8mmol) of 4-trifluoromethoxy phenylehydrazin hydrochloride dissolved in 10 ml ethanol; the mixture was left refluxing at 78^{o}C for 5h in waterbath. The solvent evaporated under the reduced pressure, and the brown residue was filtered off, washed with hexane and dried in the oven. The purity of this compound determined by using TLC (3:1) hexane: ethyl acetate, with pre-coated silica gel, which gave one spot. Yield: (0.16g, 96%), m.p. 225-226 ^{o}C. FT-IR in (cm^{-1}) : 3065, 2977, 2290,1895, 1592, 1475, 1357, 1260, 1166,1075,743 ^{1}H-NMR (400 MHz, DMSO, δ ppm): ^{1}H-NMR (400 MHz, DMSO, δ ppm): 10.66 (s, 1H, pyrazole ring), 9.89 (s, 1H, pyrazole ring), 8.30-9.20 (m,7H, *Ar-H*), 1.85 (s, 6H, 2x CH_3) APT^{13}C-NMR (100MHz, DMSO,δin ppm): shown signals for CH and CH_3 appeared at negative side (below baseline of the spectrum) δ= 143.32, 131.45,

122.77, 121.72, 119.20, 115.57,115.33 (carbon atoms of the aromatic and pyrazole ring), 24.37 (2x*C*H_3).Whereas quaternary carbons, CH_2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ = 178.90, 162.82, 160.40, 147.71, 147.57, 137.49, 115.57 (carbon atoms of the aromatic and pyrazole ring) and 53.76(CH_3-*C*-CH_3)

2. 1. 3. 7. Synthesis of 2-[1-(4-Bromo-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3*H*-indole (7) as illustrated in equation (2. 7)



2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

4-bromo-phenylhydrazine hydrochloride

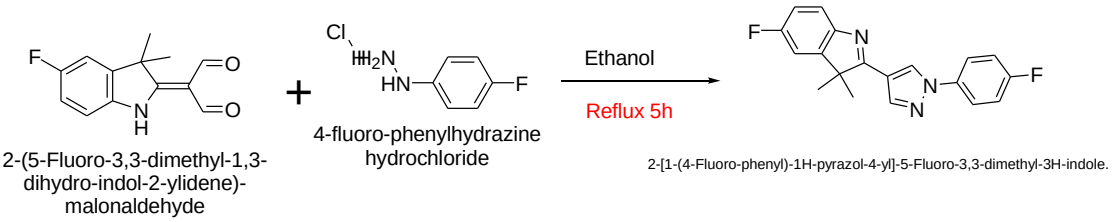
2-[1-(4-Bromo-phenyl)-1H-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3H-indole

Equation (2. 7): The synthetic pathway 2-[1-(4-Bromo-phenyl)-1*H*-pyrazol-4-yl]-5-FLuoro-3,3-dimethyl-3*H*-indole. (**7**)

A mixture solution of (0.2g, 8mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) dissolved in 10 ml ethanol and (0.16g, 8mmol) of 4-Bromo-phenylhydrazine hydrochloride dissolved in 20 mL ethanol; the mixture was left refluxing at 78^{o}C for 6h in water a bath. The solvent evaporated under the reduced pressure, and the brown residue was filtered off, washed with hexane and dried in the oven. The purity of this compound determined by using TLC (3:1) hexane: ethyl acetate, with pre-coated silica gel, which gave one spot. Yield: (0.23g, 95%), m.p. 245-246 ^{o}C. FT-IR in (cm^{-1}): 3065, 3030, 2325, 1901, 1598, 1354, 1269, 1072,743, 685. ^{1}H-NMR (400 MHz, DMSO, δ ppm): 10.52 (s,

1H, pyrazole ring), 9.78 (s, 1H, pyrazole ring), 8.12-9.84 (m,7H, *Ar-H*), 1.67 (s, 6H, 2xCH_3) APT^{13}C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH_3 appeared at negative side (below baseline of the spectrum) δ = 132.81 , 121.59 , 118.93, 115.31, 110.68 , 110.68 (carbon atoms of the aromatic and pyrazole ring), 24.26 (2xCH_3) Whereas quaternary carbons, CH_2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ=178.78 ,139.12 (carbon atoms of the aromatic and pyrazole ring) and 53.69 (CH_3-*C*-CH_3)

2. 1. 3. 8. Synthesis of 2-[1-(4-Fluoro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro- 3,3-dimethyl-3*H*-indole (8) as demonstrated in equation (2-8)



Equation (2-8): The synthetic pathway of 2-[1-(4-Fluoro-phenyl)-1*H*-pyrazol-4-yl]-5-fluoro -3,3-dimethyl-3*H*-indole. (8)

A mixture solution of (0.15g, 8mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) dissolved in 10 ml ethanol and (0.13g, 8mmol) of 4-floro-phenylhydrazine hydrochloride dissolved in 25ml ethanol, the mixture was left refluxing at 78°C for 5h in water a bath. The solvent evaporated under the reduced pressure, and the red residue was filtered off, washed with hexane and dried in the oven. The purity of this compound determined by using TLC (3:1) hexane: ethyl acetate, with

precoated silica gel, which gave one spot on the polar area. Yield: (0.19g, 95%), m.p. 200-202 °C. FT-IR data in (cm^{-1}): 3083, 2971, 2360,1895, 1587, 1331, 1231, 1184, 1081,740. ^{1}H-NMR (400 MHz, DMSO, δ ppm): 10.42 (s, 1H, pyrazole ring), 9.69 (s, 1H, pyrazole ring), 8.08-8.91 (m,7H, *Ar-H*),1.66 (s, 6H, 2xC*H*3). APT^{13}C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH_3 appeared at negative side (below baseline of the spectrum) δ = 141.75, 121.34, 118.53, 116.21,115.98, 114.77, 114.53 (carbon atoms of the aromatic and pyrazole ring), 23.66 (2x*C*H_3).Whereas quaternary carbons, CH_2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ= 178.26, 162.01, 159.59, 147.15, 130.30, 115.98 (carbon atoms of the aromatic and pyrazole ring), and 53.02 (CH_3-*C*-CH_3).

2. 2. Biological part

2. 2. 1. Materials

All chemicals used in the biological part were obtained from different company suppliers as listed in Table (2.2)

Table (2. 2). Chemicals and solvents used in the biological part

Materials and chemicals	Molecular formula	Company
Benzatin penicillin	-	SDI
Crystal Violate stain	-	BDH
Dimethyl sulfoxide	C_2H_6OS	Aldrich
Ethanol	CH_3CH_2OH	Scharlaw
Fetal Galf serum	-	Flow lap(Uk)
potassium chloride	KCl	Merck
Potassium dihydrogen phosphate	KH_2PO_4	BDH
Rosswell Park Memorial Institute-1640 medium (RPMI)	-	Gibco
Sodium Bicarbonate	$NaHCO_3$	BDH
Sodium chloride	NaCl	BDH
Sodium hydrogen phosphate	Na_2HPO_4	Merck
Sodium hydroxide	NaOH	Thomas Baker
Sulfuric acid	H_2S0_4	Scharlaw
Streptomycin	-	Ajanta
Tripsin/Versene	-	US biological
Trypan Blue stain	-	Pharma

2. 2. 2. Instruments

The instruments used in this study are founded in Iraqi Center for Cancer and Medical Genetics Research (ICCMGR) and their Manufacturers are listed in Table (2.3)

Table (2. 3): Instruments and Manufacturers are used in the biological part

Instrument	Manufacturer
Autoclave	Hospital management (Germany)
CO_2 incubator	Gallenkamp (UK)
Enzyme linked immune absorbent assay reader (Elisa)	Biotek Winooski Elisa (USA)
Hot plate with magnetic stirrer:	Gallenkamp (UK)
Inverted phase contrast microscope CK 40	Olympus (japan)
PH meter	Orient Research (USA)
Vortex	Buchi (Germany)
Water path	précis term (Germany)

2. 2. 3. Preparation methods

2. 2. 3. 1. Solutions preparation for cell culture

2. 2. 3. 1. 1. Antibiotics solution

Two types of antibiotics were added to the culture media, first one was benzathin penicillin (1000000 IU) was dissolved in 10ml of triple distilled water to get a stock solution with 100000 IU. Second antibiotic used was streptomycin and 1gm was dissolved in 5ml triple distilled water to get a stock solution with 200mg/ml. To each 500ml of cell culture media 0.5ml of benzathin penicillin (100000 IU) and 0.25ml of streptomycin (200mg/ml) were added. The final concentration of benzathin penicillin and streptomycin were 100 IU/ml and 100μg/ml of culture media respectively (Mrdanovic, *et al*., 2012 and Alves,*et al*., 2000).[66 and 67]

2. 2. 3. 1. 2. Sodium Bicarbonate

This solution was prepared according to (Freshney, 2000) [68] by dissolving 2.2g of sodium bicarbonate in one liter of culture media.

2. 2. 3. 1. 3. Phosphate buffer saline (PBS)

This buffer was prepared according to (Freshney, 1994) [69] by dissolving 8g of NaCl, 0.2g of KCl, 0.92g of Na_2HPO_4 and 0.2g of KH_2PO_4 in one litter of triple distilled water. Stirred constantly on a magnetic stirrer at room temperature then adjust the PH to 7.2. This solution was autoclaved at 121 ºC for 15 min. and stored at 4 ºC until used.

2. 2. 3. 1. 4. Fetal Calf Serum

Bottle that contains serum was water bathed for an hour at 56 ºC, then sterilize it and use directly for culture media.

2. 2. 3. 1. 5. Trypsin/ Versene solution

This solution was prepared according to the manufacturer recommendations by dissolving 10.1g of trypsin/ versene powder in 900ml triple distilled water plus 1g of sodium bicarbonate and stirred constantly on a magnetic stirrer at room temperature and the volume was completed to one liter. The solution then sterilized by a Nalgene filter unit (0.22μm) and divided into several batches and stored at 4 ºC.

2. 2. 3. 1. 6. Crystal violate stain

Five mg of crystal violate dissolved in 200ml of methanol and 50ml of 37% formaldehyde was prepared and stored at room temperature.

2. 2. 3. 1. 7. Trypan Blue Stain

One g of Trypan Blue was dissolved in 100mL of Phosphate buffer saline (PBS) then filtered with filter paper and the solution was diluted before use in ratio 1:10 with PBS.

2. 2. 3. 2. Tissue Culture Media

2. 2. 3. 2. 1. Rosswell Park Memorial Institute1640 medium (RPMI)

The culture media was prepared as follows:

About 10.4 g of powder medium that contains (HEPES buffer and L-glutamine) was dissolved in approximately 600ml of triple distilled water then the other contains were added gradually:

A) Sodium bicarbonate powder 2.2g

B) Benzyl penicillin 100mg/ml

C) Streptomycin 100 IU/ml

D) Fetal Calf Serum 100ml

The volume was completed to one litter of triple distilled water and then sterilized using Nalgene filter (0.2μm).

2. 2. 3. 3. Compounds stock and diluted concentrations preparations

Solubility assessment of the new synthesized compounds were carried out according to standard test method protocol (The National Toxicology Program). The new chemical compounds (3, 5, 6, 8, 9 and 10) were dissolved in 1ml of Dimethyl sulfoxide (DMSO) and filtered with Millipore filter 0.2 µm, this will represent as stock solutions. From stock solution then prepare the next dilutions by diluted the stock with free serum media to the chosen concentrations (25µg/ml, 50µg/ml and 100µg/ml) for each compounds.

2. 2. 4. Cell lines that used in this project

One type of cell lines were provided by (ICCMGR).

2. 2. 4. 1. Infiltrating Ductal Carcinoma AMJ 13

This cell line was established from primary tumor of 70 years old Iraqi woman was diagnosed histologically with infiltrating ductal carcinoma and is named as AMJ13 according to (Ahmed Majed Jabrea 2013, and the year in which the cell line was established). The cells were morphologically characterized by light and scanning electron microscopy and shown to be elongated multipolar epithelial-like cells with a population doubling time of 22h. (Al-Shammari *et al*., 2015). [(70)]

2. 2. 5. Cell line maintenance:

Cell lines were maintained to grow continuously; to do this confluent cell lines were sub-cultured by decanting the medium off and cells washed with 1ml PBS. From trypsin/ versin solution 0.5ml was added to the last washed cells and waiting 3-5 min. until the cells started detach from the falcon. Cells were dispensed in growth medium and incubated with 5% CO_2 at 37 ºC (Al-Shammari, 2003). [71]

2. 2. 6. Cytotoxicity assay on tumor cell lines

The effect of the six chemical compounds and their dilutions on ductal carcinoma AMJ13 cell line was measured by cytotoxicity assay using 96-well micro titration plat as described by the method of (Freshney, 2010). [72]

2. 2. 6. 1. Cells seeding

cell line (AMJ13) was treated as described in (**2. 2. 6**) to get cell suspension. Each well of 96- well plate was seeded with 100μl (10^4 cell/ well) from the cell suspension, then the cover of the plate was placed on and contoured with parafilm and placed in the incubator 5% CO_2 for 24h. at 37 ºC.

2. 2. 6. 2. Exposure

The 96- well plate was divided to seven groups, six of the wells were including six compounds with their three concentrations in triplicate, the seventh one was for control untreated cells. Before exposure of the compounds, media was decanted from the seeded cells by micropipette. Each row represent a chemical compounds with its concentration in three replicates, 200μl of each concentration of specific chemical compounds were added to the seeded cells' well. The control cells untreated with the chemical compounds were treated with free serum media. The plate was covered again and sealed with parafilm and placed in the incubator at 37 ºC with 5% CO_2. [73]

2. 2. 6. 3. Cytotoxicity evaluation

The effect of the tested chemical compounds on was measured after 48 h. of exposure. Cell viability was measured by removing the media from the plate and adding 200μl of crystal violate and replace it in the incubator for 20 min at 37 ºC. After extensive washes with water, plates were air-dried and absorbance measured at 560–600nm on a spectrophotometric micro plate reader. Cell adhesion or growth inhibition was expressed as percentage of control cells as the following equation:

$$\text{Inhibition Rate \%} = \frac{\text{mean abs.of control wells} - \text{mean abs.of treated concentration}}{\text{mean abs.of control wells}} \times 100$$

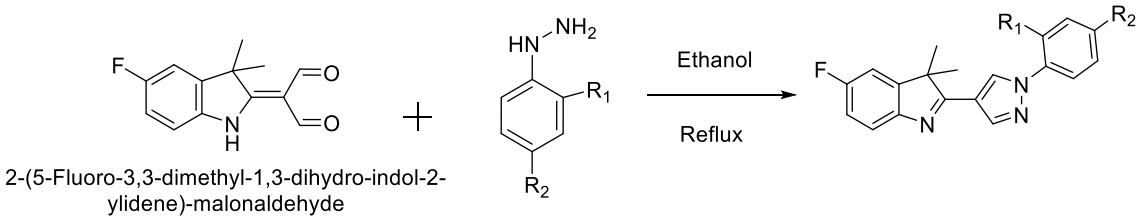
CHAPTER THREE

RESULTS AND DISCUSSION

3. 1. Chemistry part.

3. 1. 1. Methodology.

A series of new pyrazole compounds have been synthesized from new 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2ylidene)-malonaldehyde (**2**) with virouse Substituted phenylhydrazinehydrochloride according to the synthetic pathway, as shown in the reaction equation (**3. 1**)



2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

1equiv.

1equiv.

R_1 = H, R_2 = OCH_3 Compound (**3**) R_1 = NO_2, R_2= NO_2 Compound(**4**)

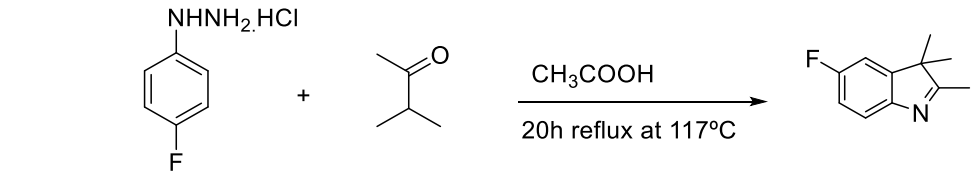
R_1 = H, R_2 = Cl Compound(**5**) R_1 = H, R_2 = OCF_3 Compound(**6**)

R_1 = H, R_2 = Br Compound(**7**) R_1 = H, R_2 = F Compound(**8**)

Reaction equation (**3. 1**): The synthetic pathway of the synthesized compounds. (**3-8**)

The compound 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)- malonaldehyde (**2**) was synthesized by two steps:

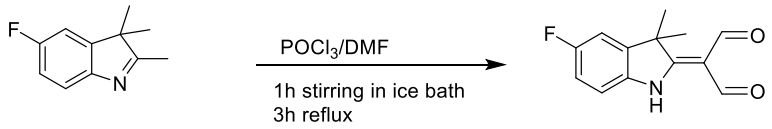
The first step: by Fischer indole synthesis from reaction of 4-Fluoro phenylhydrazine hydrochloride with methyl isopropyl ketone in glacial acetic acid to give 5-Fluoro-2,3,3-trimethyl-3*H*-indole (indoline) (**1)** in a good yield, as shown in reaction equation (**3. 2**)



(4-fluorophenyl)hydrazine hydrochloride — 3-Methyl-butan-2-one — 5-Fluoro-2,3,3-trimethyl-3H-indole

Reaction quation (**3. 2**): The synthetic pathway of 5-Fluoro-2,3,3-trimethyl-3*H*-indole (**1**)

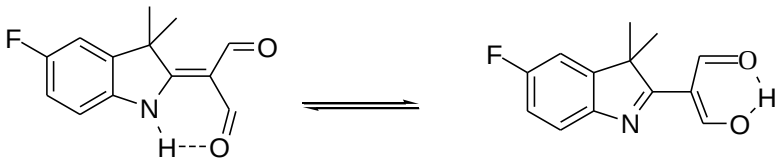
Second step: with Vilsmeier Haack reaction by reaction of indoline (**1**) with Phosphoryl chloride ($POCl_3$) in the presence of N, N-dimethyl formamide (DMF) to form 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) in a good yield, as shown in reaction equation (**3. 3**)



5-Fluoro-2,3,3-trimethyl-3H-indole — 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

Reaction equation (**3. 3**): The synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydroindol- 2-ylidene)-malonaldehyde (**2**)

The compound (**2**) found as keto-amine, enol-imine tautomer forms[74]. As shown in figure (**3. 1**).

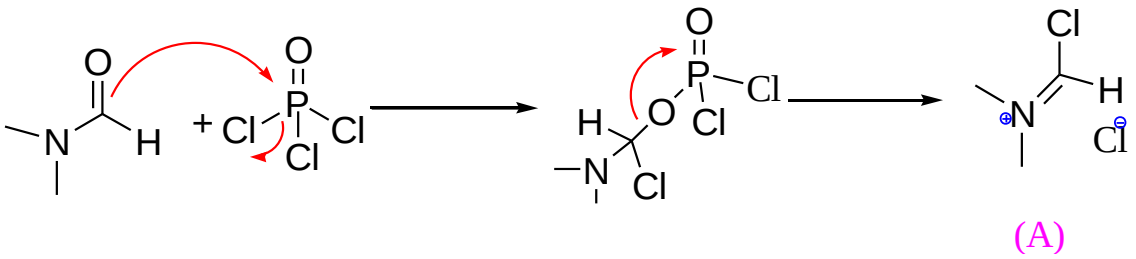


Ketoamine — Enolimine

Figure (**3. 1**): Tautomer forms of, 2-(5-Fluoro3,3-dimethyl-1,3-dihydro-indol- 2-ylidene) malonaldehyde. (**2**)

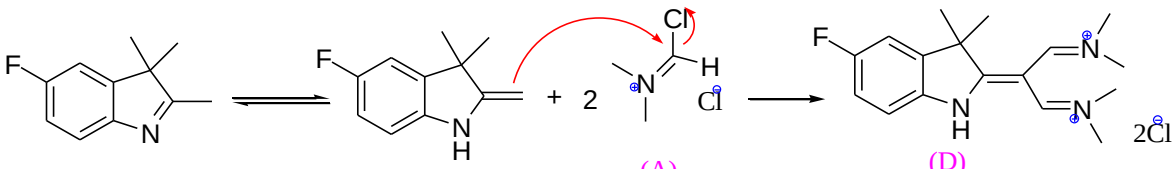
The mechanism of the formation of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) involves three steps as proposed , as shown in Scheme (**3. 1**)

In the first step: combination of DMF with $POCl_3$ to formation of chloroiminium ion (**A**)



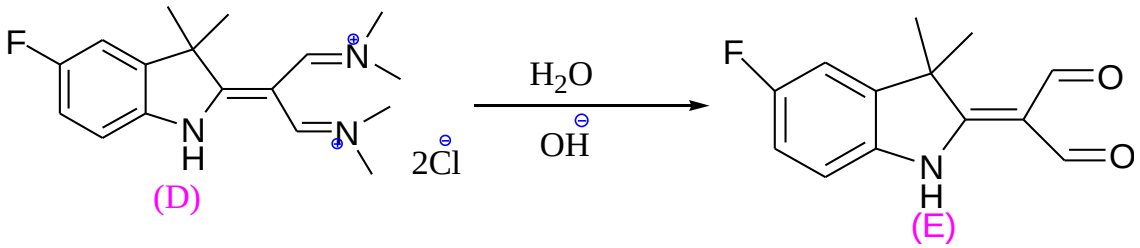
(A)

In the second step: the reaction of chloroiminium ion (**A**) with 5-Fluoro- 2,3,3-trimethyl-3*H*-indole (indoline) (**B**), but the indoline in the equilibrium with enamine tautomer (**C**). So, (**C**) will reacts with. (**A**) Later the chloro-iminium ion forms the first step attacked to create the intermediate. (**D**)



(B) (C) (A) (D)

In the third step: hydrolysis of intermediating (**D**), to produce (**E**), which it's 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene) malonaldehyde (**2**)

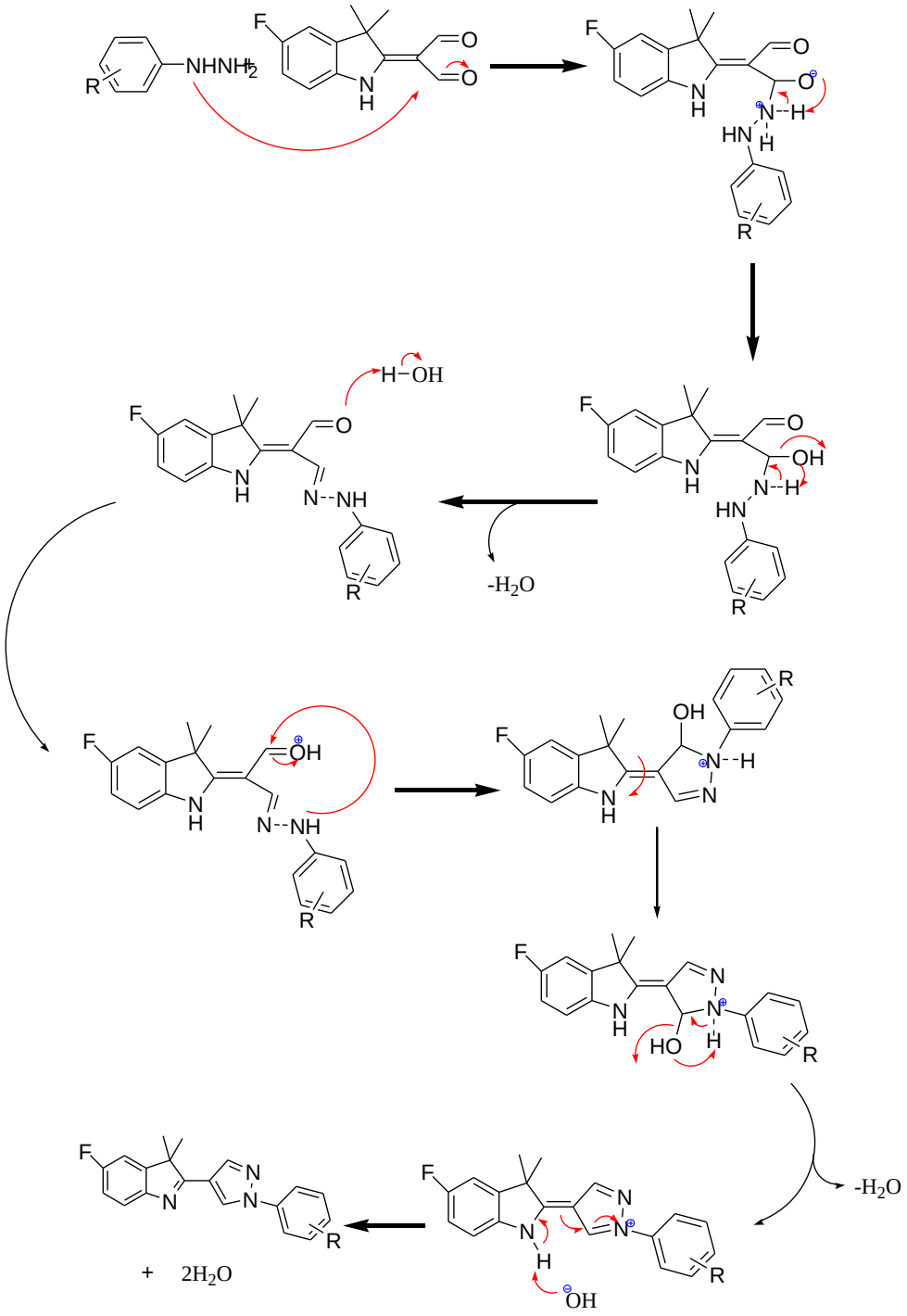


(D) (E)

Scheme (**3. 1**): Mechanism of Vilsmeier Haack reaction to form the compound **(2)**

Since compound **(2)** use as starting material to synthesize the new synthesized pyrazole compounds by the condensation reaction with different Substituted phenylhydrazinehydrochloride in the ratio (1:1) to form our new compounds .

The proposed mechanism of the new synthesized compounds (**3-8**), as illustrate in scheme (**3. 2**)



Scheme (**3. 2**): Proposal mechanism of the synthesized compounds (**3-8**)

The newly synthesized compounds are colored, stable in air and have tested by TLC, FT-IR, ^{1}H-NMR and APT^{13}C-NMR. The physical properties such as the melting point and percentage yield of the new compounds represented in the table (**3. 1**)

Table (**3. 1**): Physical properties of the synthesized compounds (**1-8**)

Com.No.	Molecular formula	Molecular weight	Percentage Yield	Melting Point ؛C	Color
1.	$C_{11}H_{12}FN$	177.22	91%	_	Red
2.	$C_{13}H_{12}FNO_2$	233.24	81%	178-180 °C	Yellow
3.	$C_{20}H_{18}FN_3O$	335.37	96%	240-242 °C	Yellow
4.	$C_{19}H_{14}FN_5O_4$	395.34	90%	198-199 °C	Brown
5.	$C_{19}H_{15}ClFN_3$	339.79	86%	223-224 °C	Orange
6.	$C_{20}H_{15}F_4N_3O$	389.35	96%	225-226 °C	Yellow
7.	$C_{19}H_{15}BrFN_3$	384.24	95%	245-246 °C	Yellow
8.	$C_{19}H_{15}F_2N_3$	323.34	95%	200-202°C	Yellow

3. 1. 2. Spectral studies of newly synthesized compounds.

3. 1. 2. 1. FT-IR Study.

The results of the FT-IR for the newly synthesized compounds displayed absorption bands in the range between 400-4000 cm^{-1}. IR spectra achieved on a Perkin-Elmer Spectrum version 10.02 by using a disk of KBr for solid substance in Department of Chemistry, College of Sciences, University of Diyala.

3. 1. 2. 1. 1. FT-IR for compound 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (2)

This compound was synthesized by reaction of 5-Fluoro-2,3,3-trimethyl-3H-indole, (Indolenine**)** **(1**) and N, N-dimethyl formamide (DMF) and Phosphoryl chloride ($POCl_3$). The yellow precipitate was formed, filtered off, washed with hexane and dried in an oven. The purity of this compound determined by using TLC in a ratio (3:1) hexane: ethyl acetate which gave one spot.

The FT-IR spectra of the synthesized compound (**2**) Figure (**3. 2**) displayed absorption bands. Absorption at 3201 cm^{-1} for (N-H) group[75]. The absorption at 3042 cm^{-1} for (C-H $_{\text{aromatic ring}}$)[76]. The band at 2983 cm^{-1} was assigned to alphatic (C-H)[77] . Also, the absorption band at 1657 cm^{-1} belonged to (C=O)[78]. as will as the absorption band at 1616 cm^{-1} belonged to (C=N). As well as the stretching frequency at 1534 cm^{-1} was referred to (C=C) group. At the same time, the absorption bands have appeared at 1372 cm^{-1} belonging to bending vibration of CH_3 group. the absorption band at 1275 cm^{-1} which attributed to (C-N). Also absorption band at 1178 cm^{-1} for (C-O).

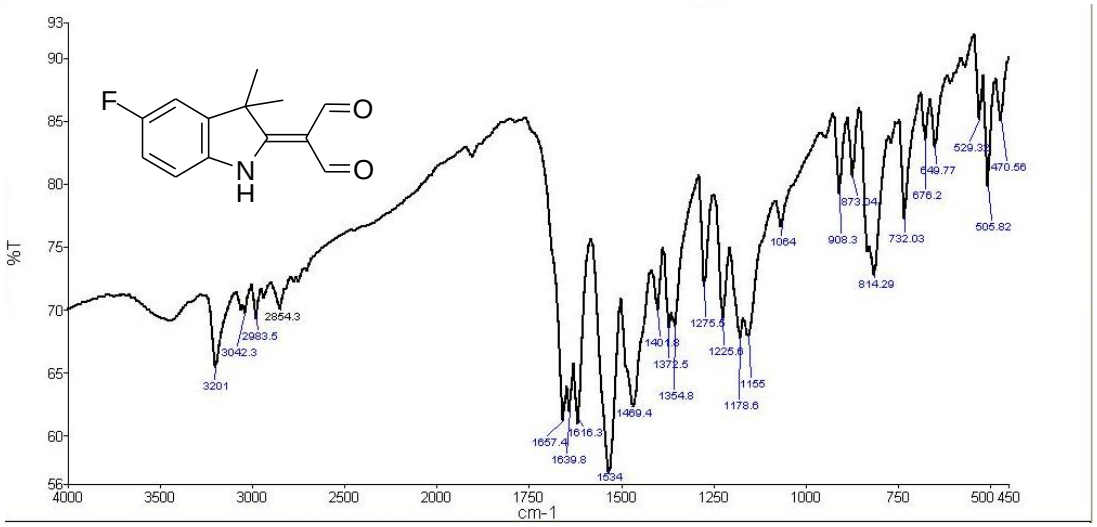


Figure (3. 2): The FT-IR spectra of the compound. (**2**)

3. 1. 2. 1. 2. FT-IR for compound 5-Fluoro-2-[1-(4-methoxy-phenyl)-1*H*pyrazol- 4-yl]-3,3-dimethyl-3*H*-indole (3)

This compound was synthesized by reaction of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) and 4-methoxy-phenylhydrazine hydrochloride in ethanol; the yellow precipitate was formed, filtered off, washed with hexane and dried in an oven. The purity of this compound determined by using TLC in a ratio (3:1) hexane: ethyl acetate which gave one spot .The FT-IR spectra of the synthesized compound (**3**) figure (**3. 3**) displayed absorption bands, absorption at 3065 cm^{-1} for (C-H $_{aromatic\ ring}$). 2942 cm^{-1} was assigned to alphatic (C-H). And 2443 cm^{-1} overtones of (C=N) of pyrazole ring[79]. Also, the absorption band at 1880cm^{-1}belonged to (C=N)[80] . the stretching frequency at 1587 $cm^{-1}$was referred to (C=C) group[81] . the absorption bands have appeared at 1349 cm^{-1} was belonged to bending vibration of CH_3 group[82]. moreover the absorption band at 1243 cm^{-1} which attributed to (C-N)[83]. Also absorption band at 1172 cm^{-1} for (C-O)[84] . and absorption band at 1075 cm^{-1} which attributed to (C-F). Finally, a sharp band at 743 cm^{-1} attributed to out-of-plane (C-H)[85].

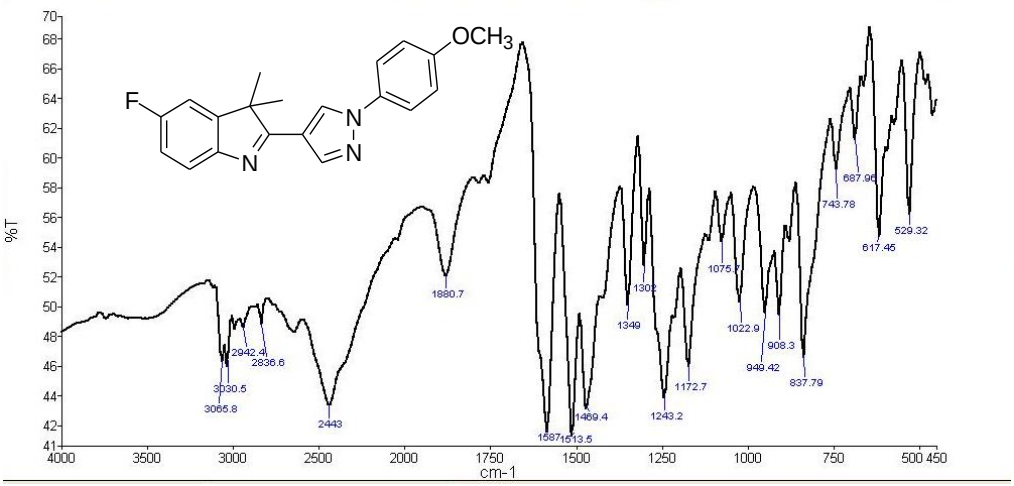


Figure (3. 3): The FT-IR spectra of the compound. (**3**)

3. 1. 2. 1. 3. FT-IR for the 2-[1-(4-Fluoro-phenyl)-1*H*-pyrazol-4-yl]-5-fluoro -3,3-dimethyl-3*H*-indole (8)

This compound was synthesized by reaction of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (**2**) and 4-floro-phenylhydrazine hydrochloride in ethanol, the yellow precipitate was formed, filtered off, washed with hexane and dried in an oven. The purity of this compound determined by using TLC in a ratio (3:1) hexane: ethyl acetate which gave one spot.

The FT-IR spectra of the synthesized compound (**8**) Figure (**3. 4**) displayed absorption bands, the first band at 3083 cm^{-1} attributed to (C-H $_{aromatic\ ring}$)[86] and at 2971 cm^{-1} was assigned to alphatic (C-H)[87]. Absorption band at 2360 cm^{-1} belonged to overtones of (C=N) of pyrazole ring. Also, the absorption band at 1895 cm^{-1} belonged to (C=N)[88] . As well as the stretching frequency at 1587 cm^{-1} referred to (C=C) group[89] and absorption band appeared at 1331 cm^{-1} was belonged to bending vibration of CH_3 group[90]. Plus the absorption band at 1231 cm^{-1} for (C-N)[91] and absorption band at 1184 cm^{-1} which attributed to (C-F)[92]. Finally, a sharp band at 740 cm^{-1} attributed to out-of-plane (C-H)[93]

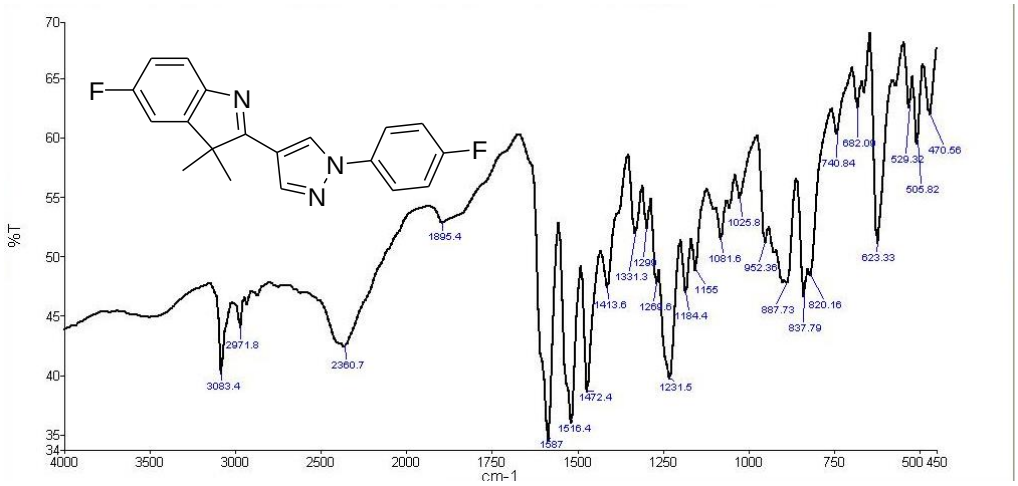


Figure (3. 4): The FT-IR spectra of the compound. (**8**)

3. 1. 2. 1. 4. FT-IR for the compound 2-[1-(4-Chloro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3*H*-indole. (5)

This compound was synthesized by reaction of 2-(5-Fluoro-3,3-dimethyl- 1,3 dihydro-indol-2-ylidene)-malonaldehyde (**2**) and 4-choro-phenyl hydrazine hydrochloride in ethanol, the brown precipitate was formed, filtered off, washed with hexane and dried in an oven. The purity of this compound determined by using TLC in a ratio (3:1) hexane which gave one spot.

The FT-IR spectra of the synthesized compound **(5)** as shown in Figure (3. 5) displayed absorption bands, at the 3077 cm^{-1} aromatics (C-H)[94]. And 2425cm^{-1} overtones of (C=N) in pyrazole. Also, the absorption band at 1883 cm^{-1} belonged to (+C=N)[95]. As well as the stretching frequency at 1584 cm^{-1} referred to (C=C) groups[96] . At the same time, the absorption bands have appeared at 1352cm^{-1} was belonged to bending vibration of CH_3 group [97] Plus the absorption band at 1260 cm^{-1} which attributed to (C-N) group[98]. And absorption band at 1087 cm^{-1} which attributed to (C-F). Finally, a band at 826 cm^{-1} attributed to (C-Cl) group [99] and a sharp band at 743 cm^{-1} assigned to out-of-plane (C-H) group[100].

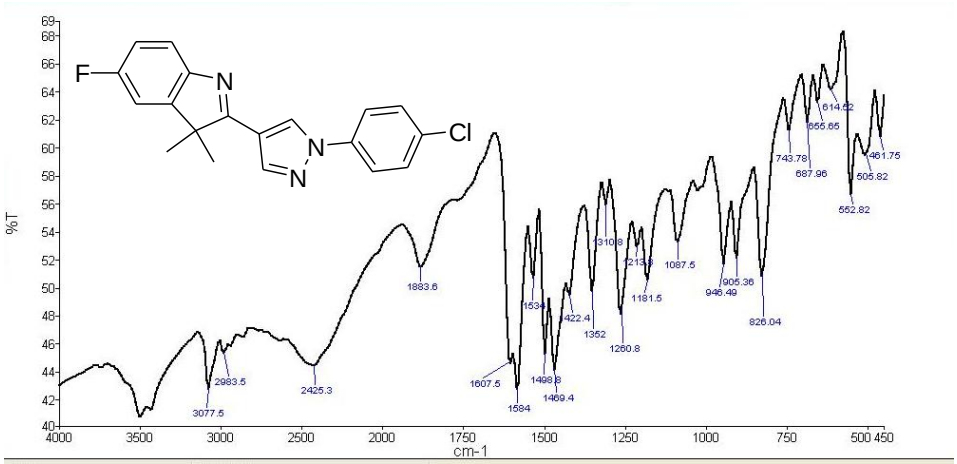


Figure (3. 5): The IR spectra for compound (**5**)

The IR results for compounds **4, 6, 7** are listed as appendix (3. 1), (3. 2) and (3. 3).

Table (3. 2): FT- IR spectra for the compounds (2-8)

Com. No.	N-H	C-H Asym.	C-H Alph.	overtones (C=N) of pyrazole	C=O	C=N	C=C	CH_3	C-N	C-O	Others
2	3201	3042	2983	-	1657	1616	1534	1372	1275	1178	-
3	-	3065	2942	2443	-	1880	1587	1349	1243	1172	-
4	-	3259	2977	2352	-	1871	1540	1331	1272	-	1381 and 1487 (N-O)
5	-	3077	2983	2425	-	1883	1584	1352	1260	-	826 (C-Cl)
6	-	3065	2977	2290	-	1895	1592	1357	1260	1166	1075 (C-F)
7	-	3065	2980	2325	-	1901	1598	1354	1269	-	685 (C-Br)
8	-	3083	2971	2360	-	1895	1587	1331	1231	-	1081 (C-F)

3. 1. 2. 2. NMR Study

^{1}H-NMR and APT^{13}C-NMR spectra were reported in (deuterated dimethyl sulfoxide) DMSO with chemical shifts in ppm. ^{1}H-NMR results of the new starting materials (5-Fluoro-2,3,3-trimethyl-3*H*-indole (**1)** and 2-(5-Fluoro-3,3-dimethyl-1,3-dihydroindol-2-ylidene)-malonaldehyde (**2**) shown and confimred the cyclation of 5-Fluoro-2,3,3-trimethyl-3*H*-indole (**1)** and formation of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydroindol-2-ylidene)-malonaldehyde (**2**) through the new signals on their spectrums. As well as ^{1}H-NMR results of the newly synthesized compounds (**3-8**) showed the disappearance signals of starting materials and appearance of new signals of new synthesized compounds, Such as the disappeared of two protons atoms from two carbonyl groups and proton atoms of amines of 4-methoxy-phenylhydrazine hydrochloride, 2,4-dinitrophenylhydrazine,

4-choro-phenylhydrazine hydrochloride, 4-trifluoromethoxy phenylehydrazin, 4-Bromo-phenylhydrazine hydrochloride, 4-floro-phenylhydrazine hydrochloride appearance of new signals of two protons atoms of pyrazole ring. As well as appearance of a new signals of protons pyrazole ring. All these signals (aisapperance and appearance) are a good an evidence to form newly synthesized compounds.

3. 1. 2. 2. 1. ^{1}H-NMR results of 5-Fluoro-2,3,3-trimethyl-3*H*-indole (**1)**

The ^{1}H-NMR spectra of compound (**1**) Figure (**3. 6**) displayed a signals have appeared in the region between (6.71-7.57) ppm which belonged to three proton atoms of aromatic ring for this compound[(101)] . A single signal at 2.17 ppm assinged to three protons of methyl group CH_3 . Finally a single signal at 1.40 ppm belonged to six protons of two methyl groups CH_3[(102)]

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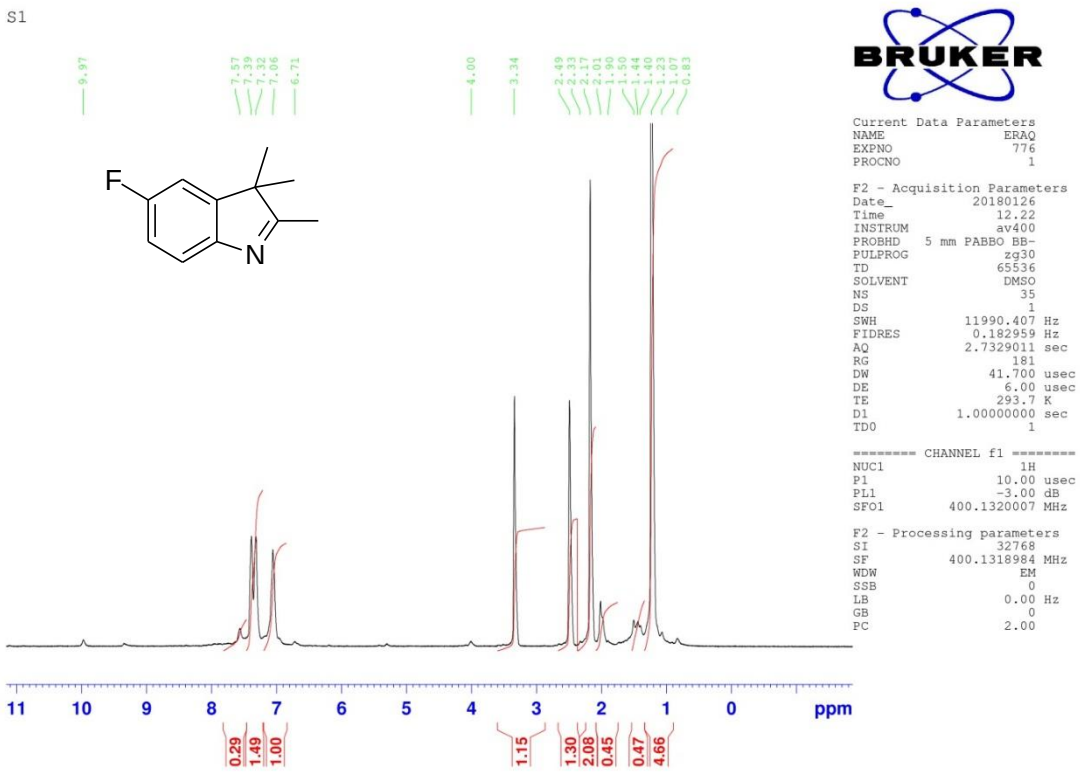

Figure (3. 6) : ^{1}H NMR spectrum of 5-fluoro-2,3,3-trimethyl-3H-indole

3. 1. 2. 2. 2. ^{1}H-NMR and APT ^{13}C-NMR results of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2ylidene)-malonaldehyde (2)

The ^{1}H-NMR spectra for compound (**2**) Figure (**3. 7**) displayed a single signal at 13.13 ppm which belonged to one proton atom of of indole ring (N*H*)[103]. A long single signal at 9.75 ppm which belonged to two protons of two aldehyde groups (2 x C*H*O). Signals were appeared in the region between (7.12-7.62) ppm which belonged to three proton atoms of aromatic of an aromatic ring . Also a single signal at 1.67 ppm belonged to six protons of two methyl groups (2 x CH_3) .

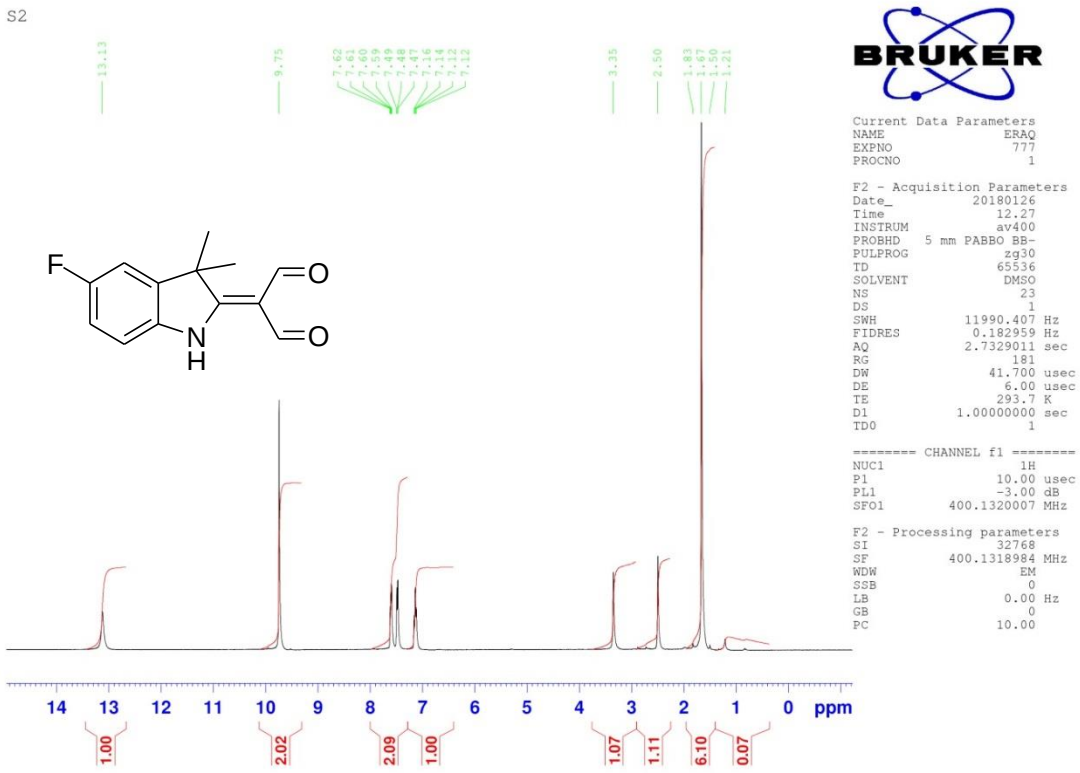

Figure (3. 7) : ^{1}H NMR spectrum of 2-(5-fluoro-3,3-dimethylindolin-2 ylidene)malonaldehyde

3. 1. 2. 2. 3. ^{1}H-NMR and APT ^{13}C-NMR results of the compound 5-Fluoro-2-[1-(4-methoxy-phenyl)-1*H*pyrazol- 4-yl]-3,3-dimethyl-3*H*-indole (3)

The ^{1}H-NMR spectra for compound (**3**) Figure (**3. 8**) displayed a single signal at 10.31 and 9.60 ppm belonged to the two proton atoms of the pyrazole ring[104]. As well as, signals have appeared in the region between (7.95-8.77) ppm which belonged to seven proton atoms of an aromatic ring for this compound[105]. Also a single signal at 4.66 ppm attributed to three protons of methoxy group OCH_3. Finally, a single signal at 1.67 ppm belonged to six protons of two methyl groups CH_3[106] .

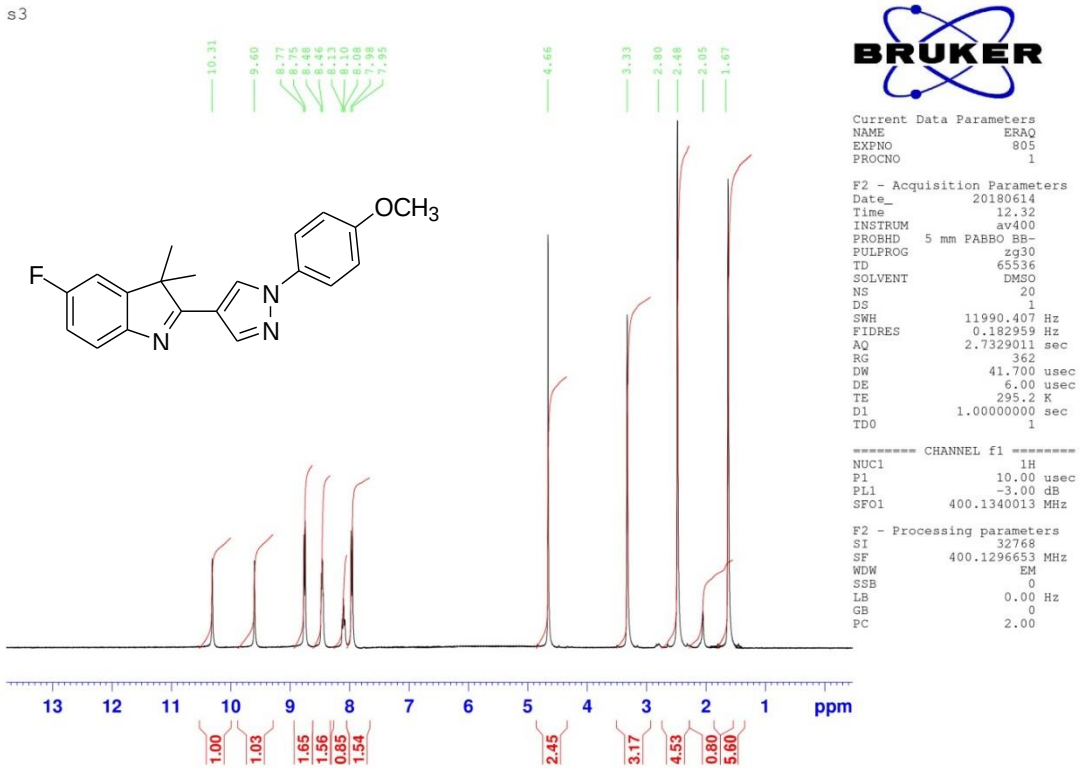

Figure (**3. 8**): ^{1}H NMR spectrum of 5-fluoro-2-(1-(4-methoxyphenyl)-1H-pyrazol-4-yl)-3,3-dimethyl-3H-indole (**3**)

APT ^{13}C-NMR spectra was used to characterize this new compound. Figure (**3. 9**) displayed signals for CH and CH_3 which observed at a negative side (below of the spectrum) (110.54- 132.61) ppm for (pyrazole and Ar-*C*H)[107] 55.97 ppm assigned to carbon atom of methoxy group OCH_3 and 24.51ppm for the two methyl groups (2 x *C*H_3)[108]. While the quaternary carbons, and carbon atoms of DMSO solvent which appeared at a positive side (above of the spectrum) (179.10-115.12)ppm for (pyrazole and *Ar*-H)[109-110] and 55.97ppm for CH_3-*C*-CH_3[111]. All ^{1}H-NMR and APT^{13}C-NMR results were matched well with the expected signals and was regular with the formation of this new synthesized compound.

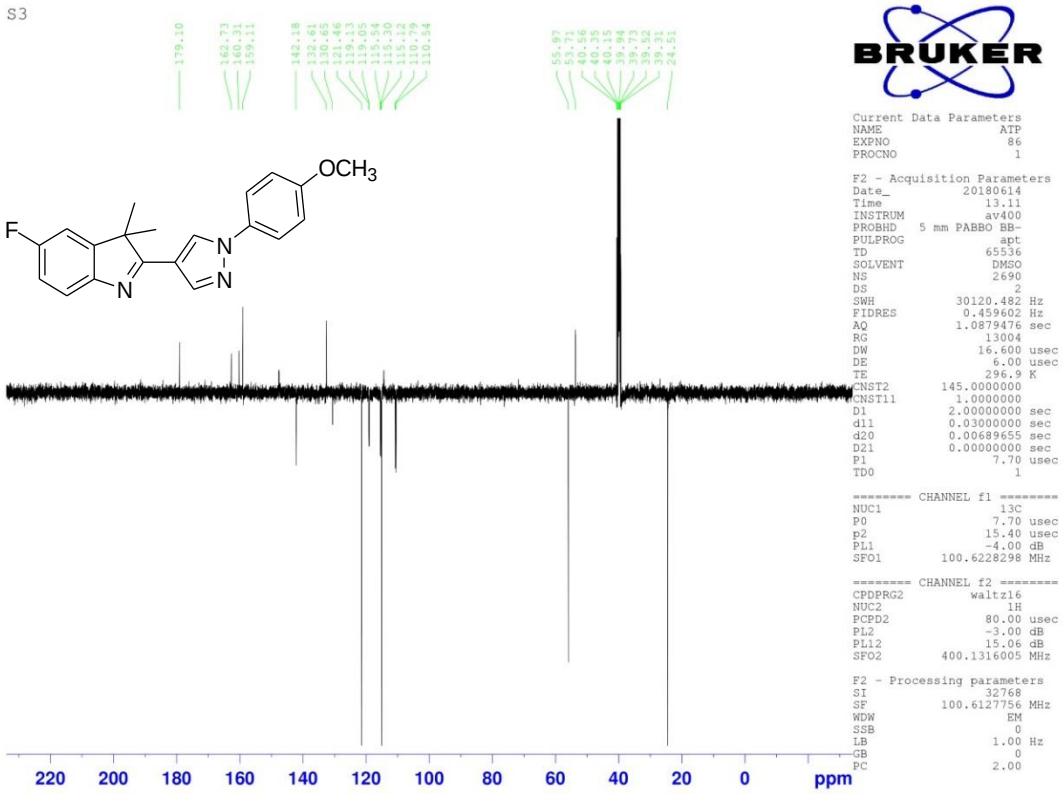

Figure (**3. 9**): APT ^{13}C NMR spectrum of 5-fluoro-2-(1-(4-methoxyphenyl)-1H-pyrazol-4-yl)-3,3-dimethyl-3H-indole

3. 1. 2. 2. 4. ^{1}H-NMR and APT ^{13}C-NMR results for compound 2-[1-(4-Fluoro-phenyl)-1*H*-pyrazol-4-yl]-5-fluoro -3,3-dimethyl-3*H*-indole. (8)

The ^{1}H-NMR results for compound (**8**), figure (3. 10) displayed single signals at 10.42 ppm and 9.69 ppm belonged to proton atoms of the pyrazole ring[112]. Signals appeared in the region between (8.08-8.91) ppm were belonged to seven protons of an aromatic ring[113]. Finally, a single signal has appeared at 1.66 ppm belonged to six proton atoms of two methyl groups (C*H*$_3$)$_2$ [114].

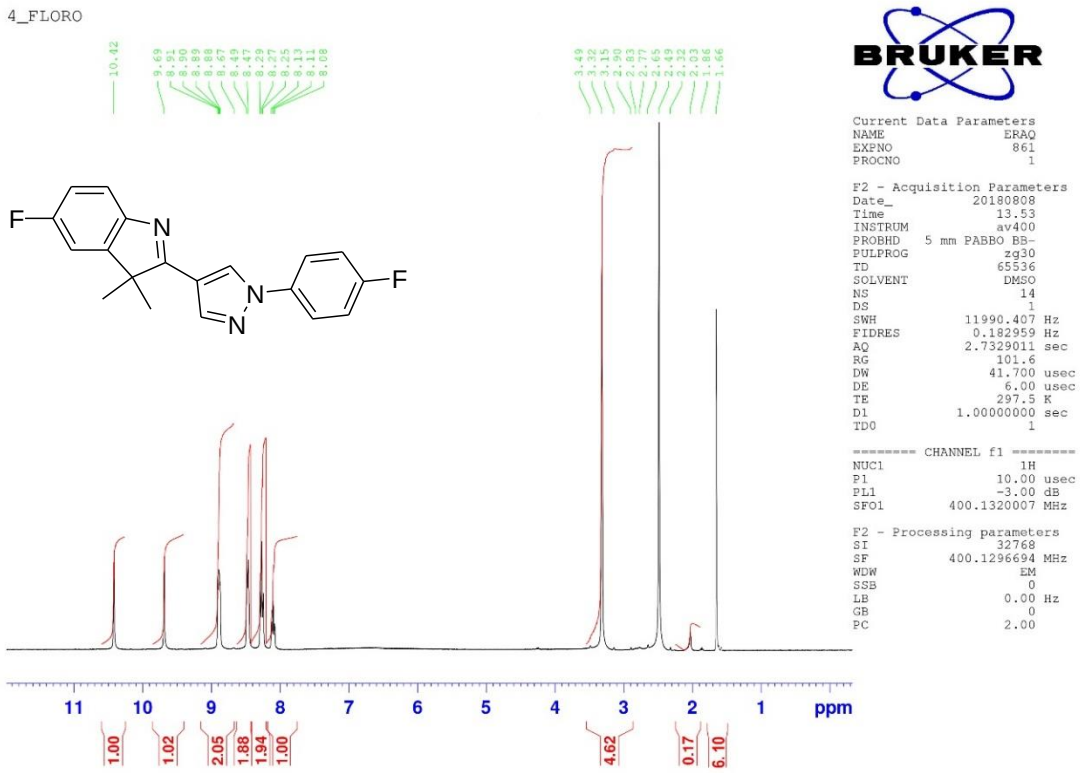

Figure (3. 10) : ^{1}H NMR spectrum of of 2-[1-(4-Fluoro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro- 3,3-dimethyl-3*H*-indole.

APT ^{13}C-NMR results were used to characterize the newly synthesized compound Figure (3. 11). Which displayed signals for CH and CH_3 observed at a negative side (below of the spectrum) (109.75-141.75)ppm for (pyrazole and Ar- *C*H)[115-116] A signal at 23.66 for the two methyl groups CH_3 [117] .While, the quaternary carbons, methylene CH_2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) (178.26- 115.98) ppm for (pyrazole and *Ar-C*)[118-119]. A signal at 53.02 ppm which belonged to the carbon atom bearing two methyl group CH_3-*C*-CH_3 [120]. All these results founded the ^{1}H-NMR, and APT^{13}C-NMR spectrum wae matched well with the expected signals and were regular with the formation of this new compound.

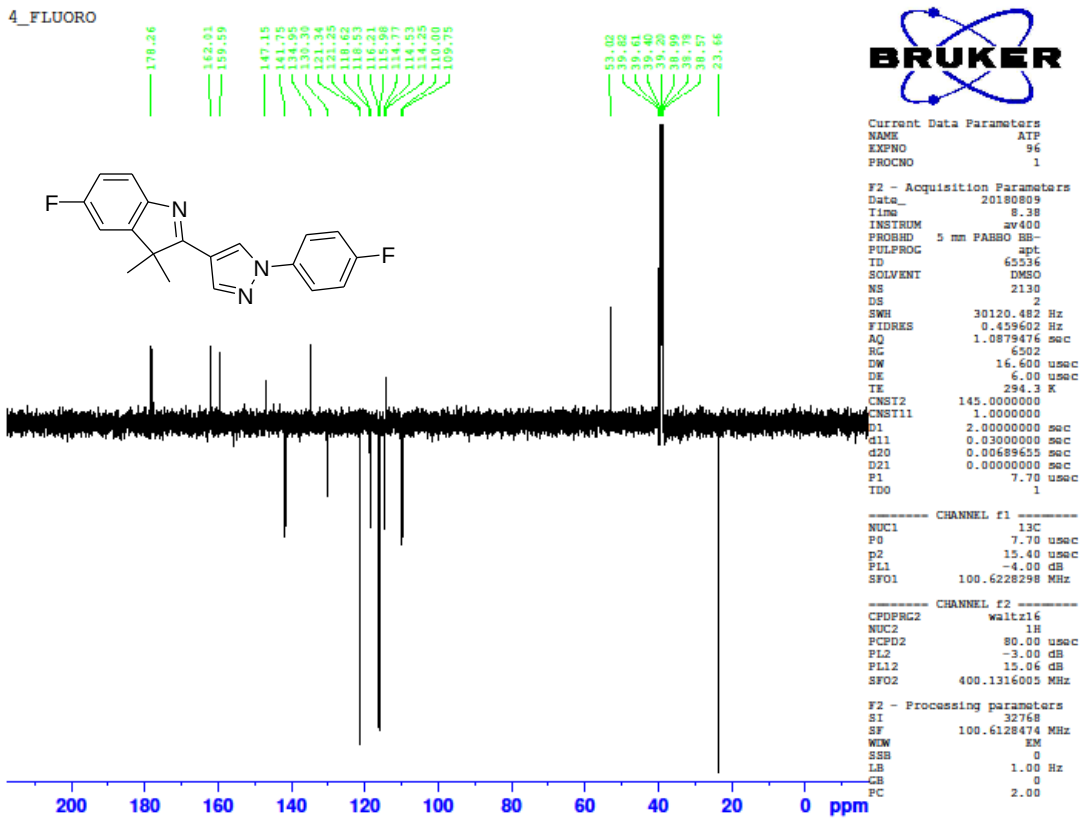

Figure (3. 11) : APT ^{13}C NMR spectrum of 2-[1-(4-Fluoro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro- 3,3-dimethyl-3*H*-indole.

3. 1. 2. 2. 5. ^{1}H-NMR and APT ^{13}C-NMR results of the compound 2-[1-(4-Chloro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3*H*-indole. (5)

The ^{1}H-NMR results for compound (**5**) Figure (3. 12) displayed two signals at 9.50 ppm and 8.74 ppm belonged to two protons of pyrazole ring [(121)].

As will as signals were appeared in the region between (7.25-8.06) ppm which belonged to seven protons of an aromatic ring for this compound[(122)]. Finally, a single peak at 1.62 ppm attributed to six protons of two methyl groups CH_3[(123)].

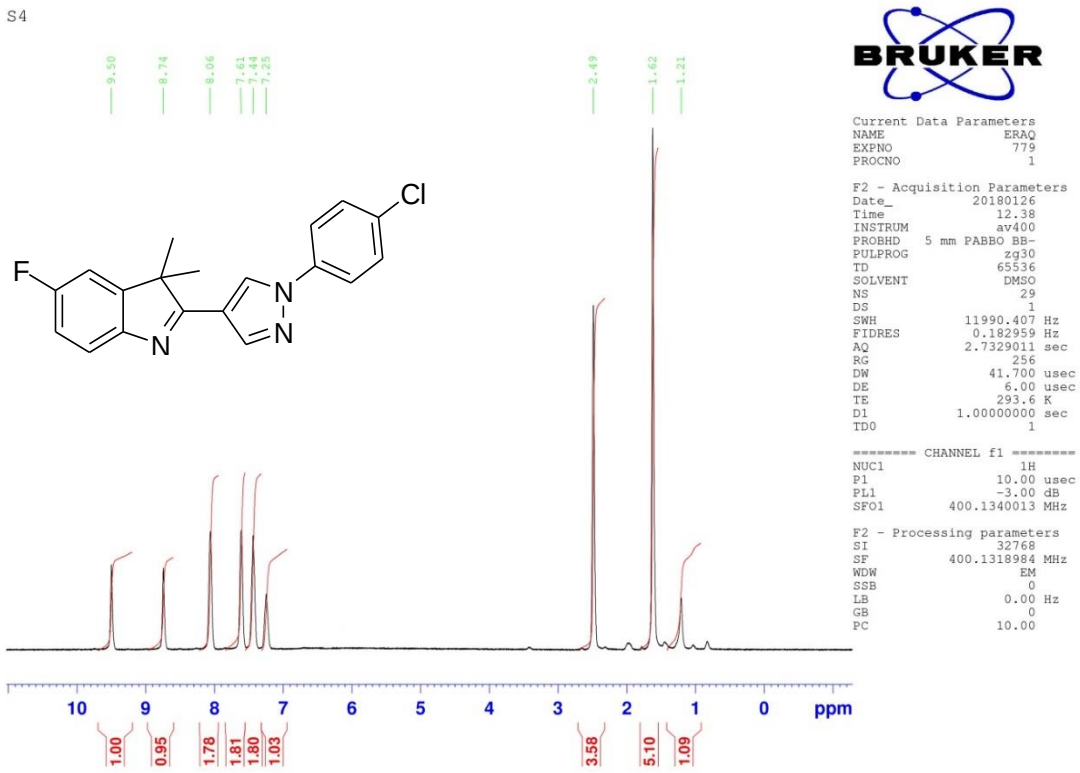

Figure (3. 12) : ^{1}H NMR spectrum of 2-(1-(4-chlorophenyl)-1H-pyrazol-4-yl)-5-fluoro-3,3-dimethyl-3H-indole

APT^{13}C-NMR results were used to characterize the newly synthesized compound, Figure (3. 13) displayed signals for CH and CH_3 which observed at a negative side (below of the spectrum) (142.33-115.25) for carbon atoms of (pyrazole and *Ar-C*H)[124-125] and the signal at 24.24 for the two methyl groups *C*H_3[126] . While the quaternary carbons, methylene CH_2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) (178.73-115.97) for carbon atoms of pyrazole and *Ar-C.* [127-128]. The signal at 53.77ppm assigned to one carbon atom of CH_3-*C*-CH_3[128] . From all these results, founded the ^{1}H-NMR, and APT^{13}C-NMR spectrum were matched well with the expected signals and was regular with the formation of this new compound.

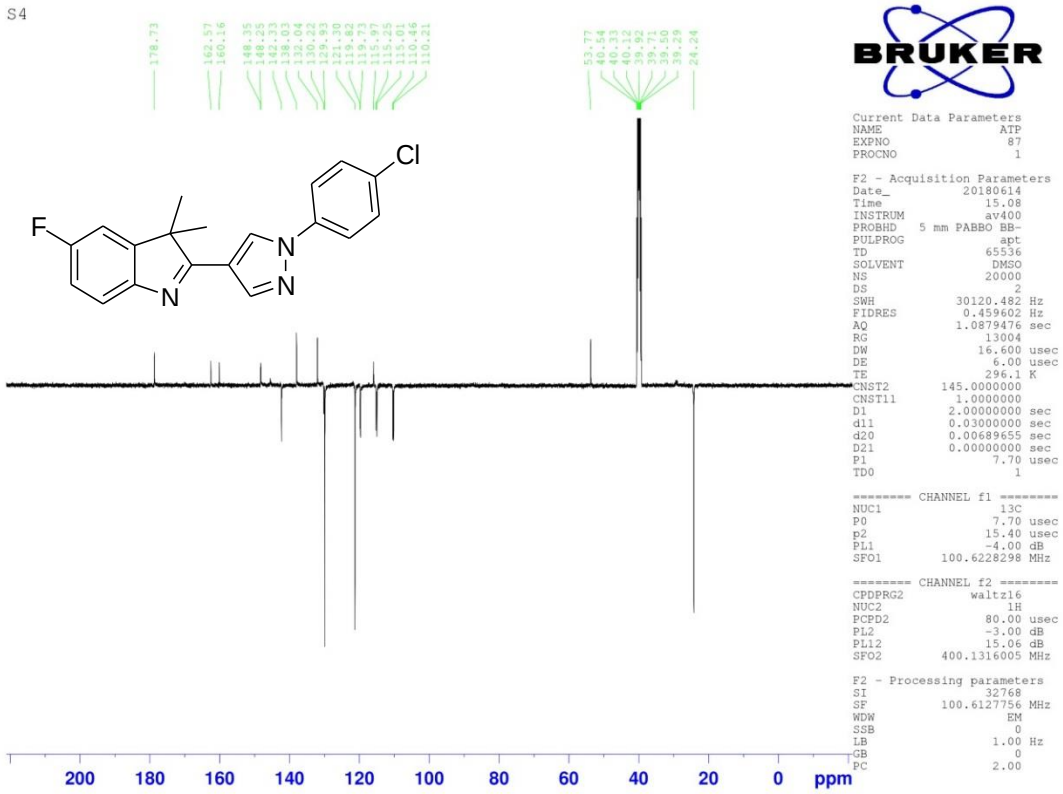

Figure (3. 13): APT ^{13}C NMR spectrum of 2-(1-(4-chlorophenyl)-1H-pyrazol-4-yl)-5-fluoro-3,3-dimethyl-3H-indole

3. 1. 2. 2. 6. ^{1}H-NMR and APT ^{13}C-NMR results for compound 2-[1-(2,4-Dinitro-phenyl)-1*H*-pyrazol-4-yl]-5- Fluoro-3,3-dimethyl-3*H*-indole (4)

The ^{1}H-NMR results for compound (**4**) Figure (3. 14) was displayed two signals at 10.35 ppm and 9.56 ppm belonged to protons of pyrazole ring.Signals were appeared in the region between (8.12-8.96) ppm which belonged to six proton atoms of an aromatic ring for this compound .Finally, a single signal at 1.73 ppm belonged to six protons of two methyl

C*H*3 groups.

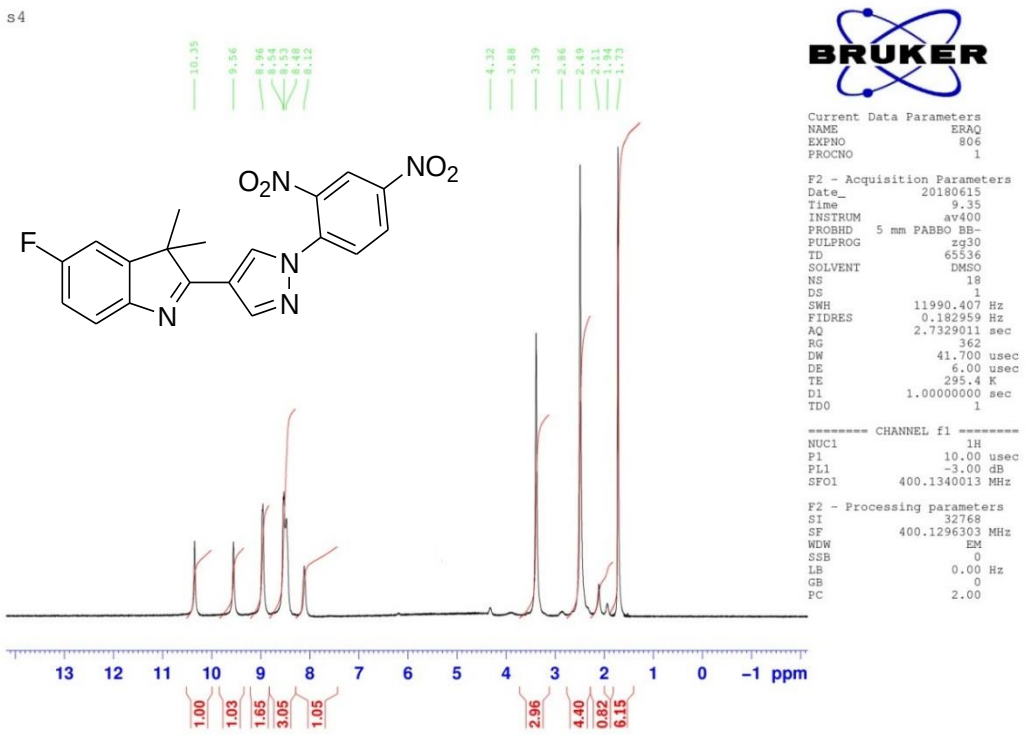

Figure (3. 14): APT ^{13}C NMR spectrum of 2-[1-(2,4-Dinitro-phenyl)-1*H*-pyrazol-4-yl]-5- Fluoro-3,3-dimethyl-3*H*-indole

3. 1. 2. 2. 7. ^{1}H-NMR and APT ^{13}C-NMR results for compound 5-Fluoro-3,3-dimethyl-2-[1-(4-trifluoro methoxy-phenyl)-1H-pyrazol-4-yl]-3H-indole (6)

The ^{1}H-NMR results for compound (**6**) Figure (3. 15) displayed a single signal at 10.66 ppm and 9.89 ppm belonged to the two proton atoms of the pyrazole ring. As well as signals have appeared in the region between (8.30-9.20) ppm which belonged to seven proton atoms of aromatic ring of indole ring for this compound. Finally, a single peak at 1.85 ppm presented to six protons of two methyl groups C*H*$_3$.

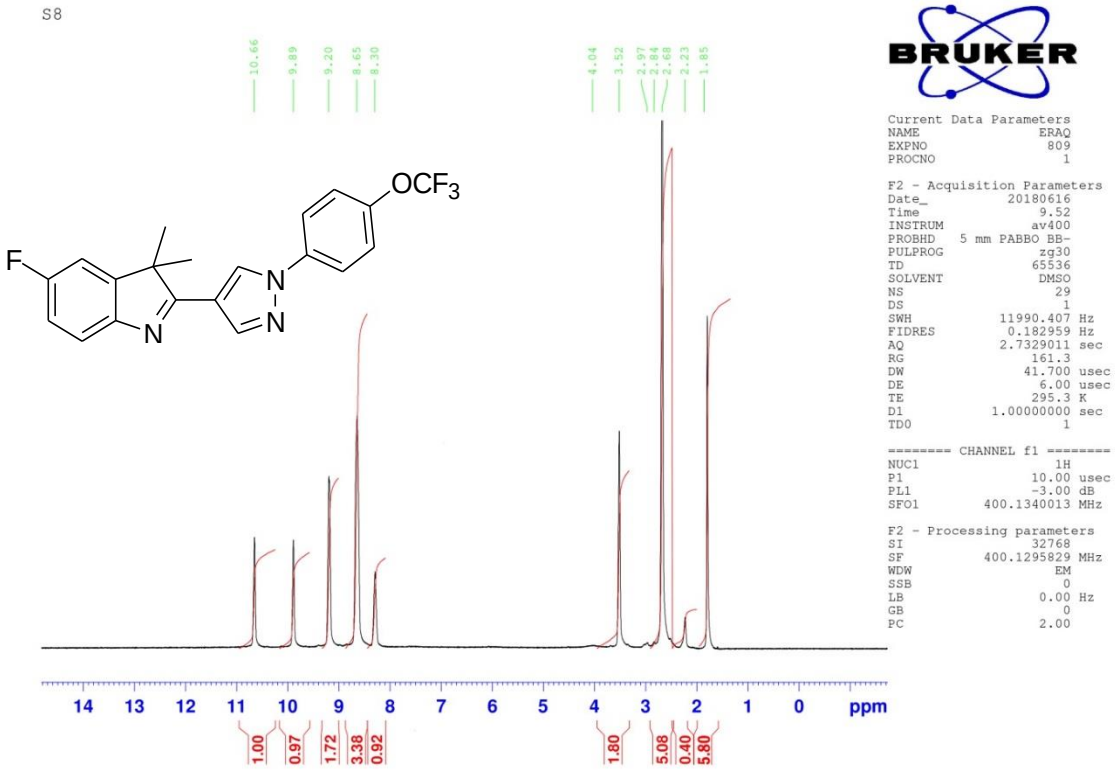

Figure (3. 15) : ^{1}H NMR spectrum of 5-fluoro-3,3-dimethyl-2-(1-(4-(trifluoromethoxy)phenyl)-1H-pyrazol-4-yl)-3H-indole

APT^{13}C-NMR results were used to characterize the newly synthesized compound Figure (3. 16) displayed signals for CH, and CH_3 observed at a negative side (below of the spectrum) (143.32-115.33) for (pyrazole and Ar- *C*H). A signal at 24.37 for the two methyl groups CH_3. While, the quaternary carbons, methylene CH_2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) (178.90-115.57) for (pyrazole and *Ar-C*H). A signal at 53.76 ppm which belonged to the carbon atom bearing two methyl groups CH_3-*C*-CH_3. All these data founded the ^{1}H-NMR and APT^{13}C-NMR spectrum matched well with the expected signals and was regular with the formation of this new compound.

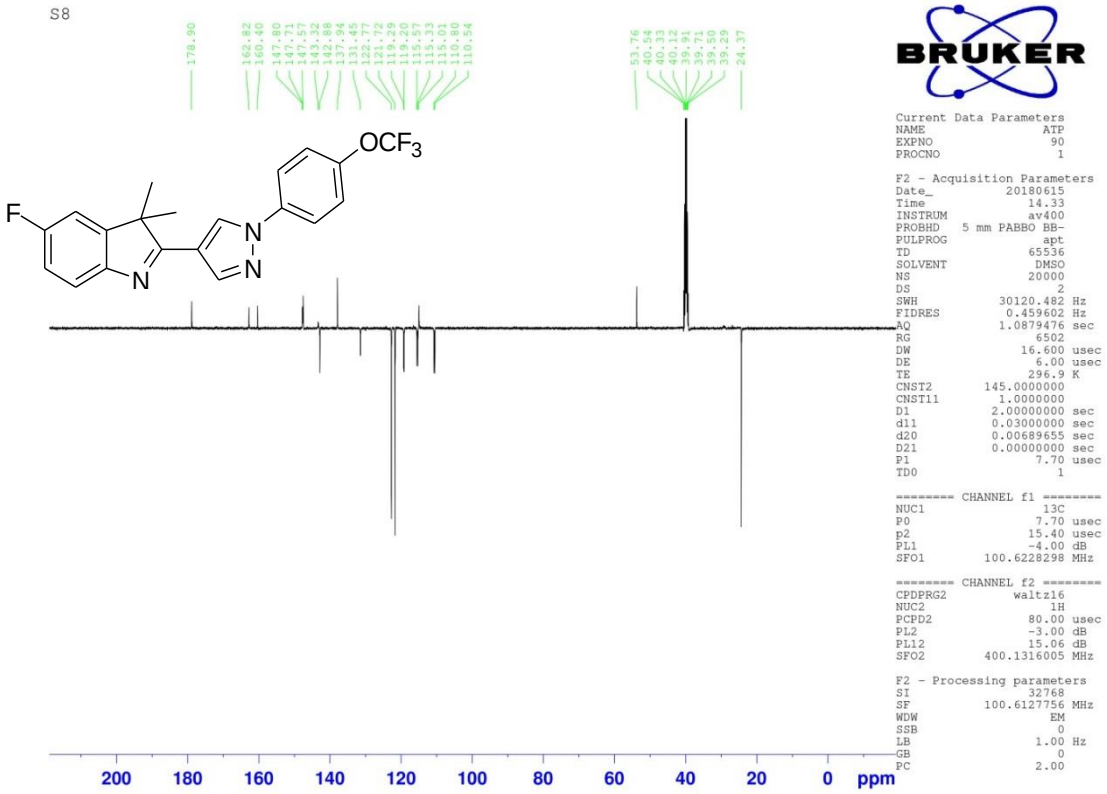

Figure (3. 16) : APT ^{13}C NMR spectrum of 5-fluoro-3,3-dimethyl-2-(1-(4-(trifluoromethoxy)phenyl)-1H-pyrazol-4-yl)-3H-indole.

3. 1. 2. 2. 8. ^{1}H-NMR and APT ^{13}C-NMR results for compound 2-[1-(4-Bromo-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3*H*-indole (7)

The ^{1}H-NMR results for compound (**7**) Figure (3. 17) displayed single signals at 10.52 ppm and 9.78 ppm belonged to proton atoms of the pyrazole ring. Signals appeared in the region between (8.12-9.35) ppm were belonged to seven protons of an aromatic ring. Finally, a single signal has appeared at 1.67 ppm belonged to six proton atoms of two methyl groups (C*H*$_3$)$_2$.

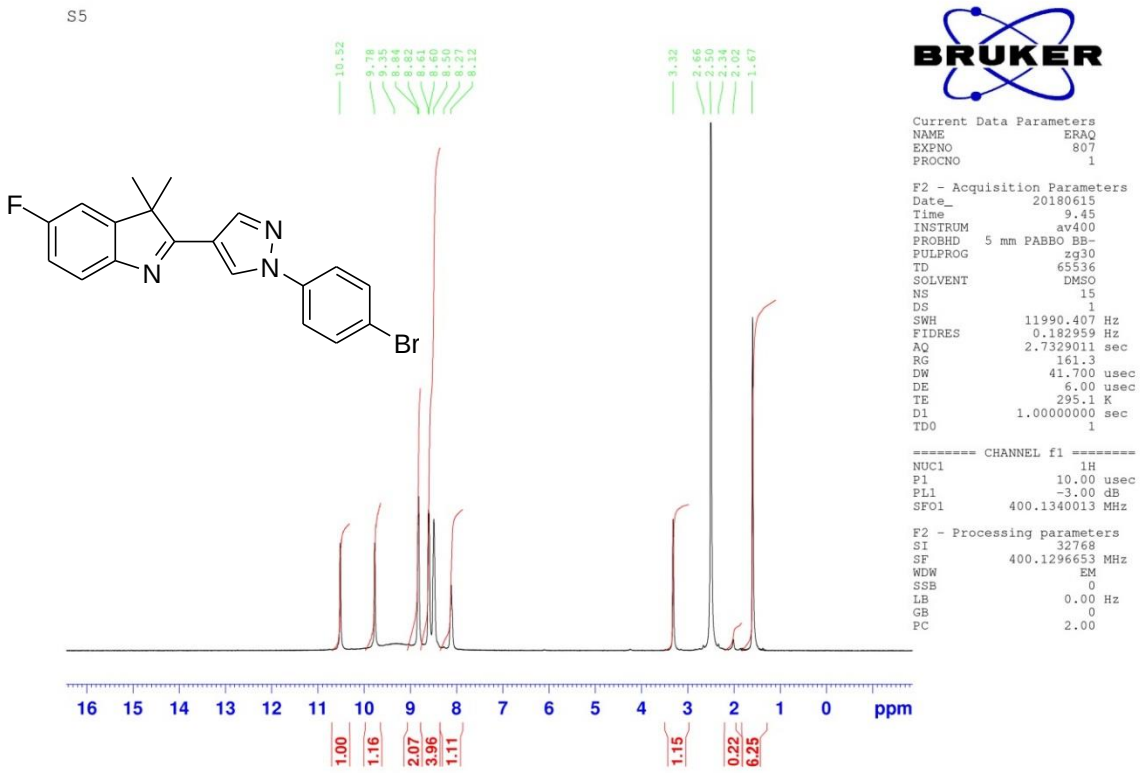

Figure (3. 17) : ^{1}H NMR spectrum of 2-(1-(4-bromophenyl)-1H-pyrazol-4-yl)-5-fluoro-3,3-dimethyl-3H-indole

APT ^{13}C-NMR results were used to characterize the newly synthesized compound Figure (3. 18) displayed signals for CH, and CH3 observed at a negative side (below of the spectrum) (132.81-110.68) ppm for (pyrazole and Ar-*C*H). A signal at 24.26 ppm for the two methyl groups CH_3. While, the quaternary carbons, methylene CH2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) (178.78-118.93) ppm for (pyrazole and *Ar-C*). A signal at 53.69 ppm which belonged to the carbon atom bearing two methyl group CH_3-*C*-CH_3. All these results founded the ^{1}H-NMR, and APT^{13}C-NMR spectrum matched well with the expected signals and was regular with the formation of this new compound.

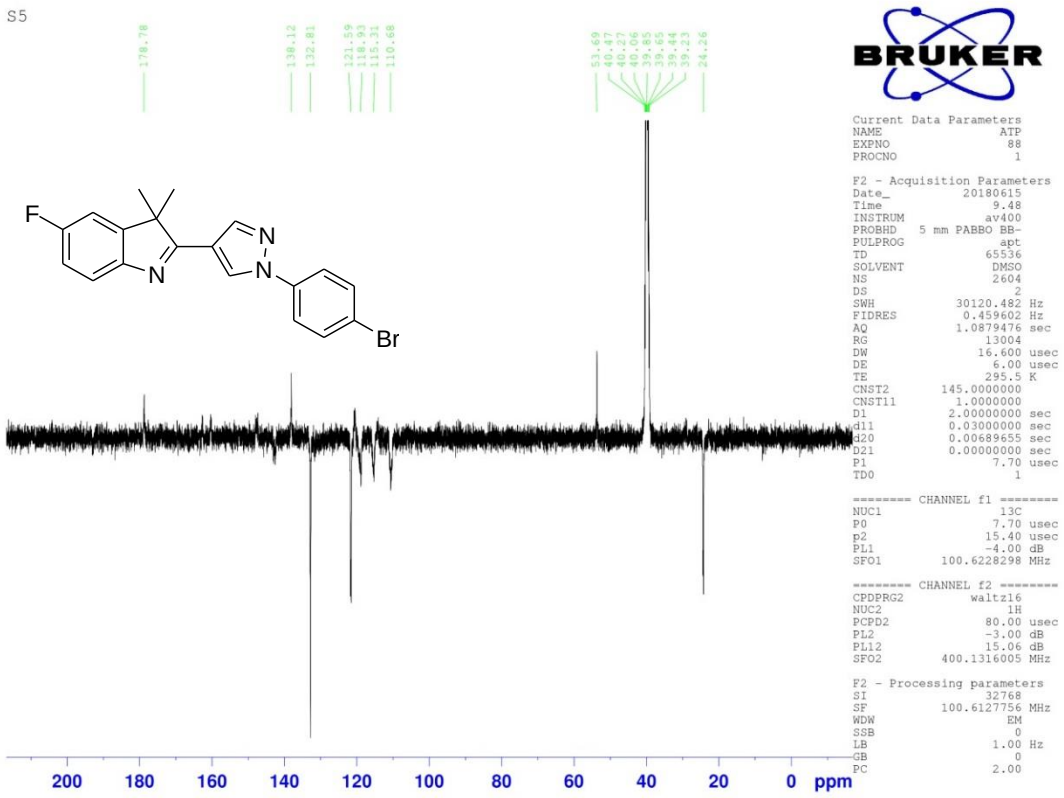

Figure (3. 18) : APT ^{13}C NMR spectrum of 2-(1-(4-bromophenyl)-1H-pyrazol-4-yl)-5-fluoro-3,3-dimethyl-3H-indole

3. 2. Biological part

3. 2. 1. Cytotoxicity assay

concentrations of six new compound were stable in the air for extended periods and they soluble in dimethyl formamide and DMSO. For this reason we added amount of DMSO and then added RPMI medium slightly to prepare the concentrations (25, 50 and 100µg/ml)

3. 2. 1. 1. Cytotoxicity toward AMJ13 cell line

Cancer cell line AMJ13 were seeded as $2*10^4$ cells / well in 96 well plats and after 24 h. when the cells become confluent monolayer, they were exposed to the compound's concentrations at 25, 50 and 100 µg/ml and incubated in 37℃ for 48 h, then stained with crystal violate dye and calculated the inhibition rate (%) for each compound.

3. 2. 1. 1. 1. The cytotoxicity of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (2)

This compound showed a good cytotoxic inhibition rate after 48 h. of exposure to AMJ13 cancer cell line at concentrations 25, 50 and 100 µg/ml were 63.6, 76.60 and 75.90 % respectively without any significant correlations between them. Concentration 50 µg/ml represent the ideal concentration prepared from compound (2) that inhibited 50.3% of AMJ13 cell line after 48 h. The result has been summerized in Figure (3. 19).

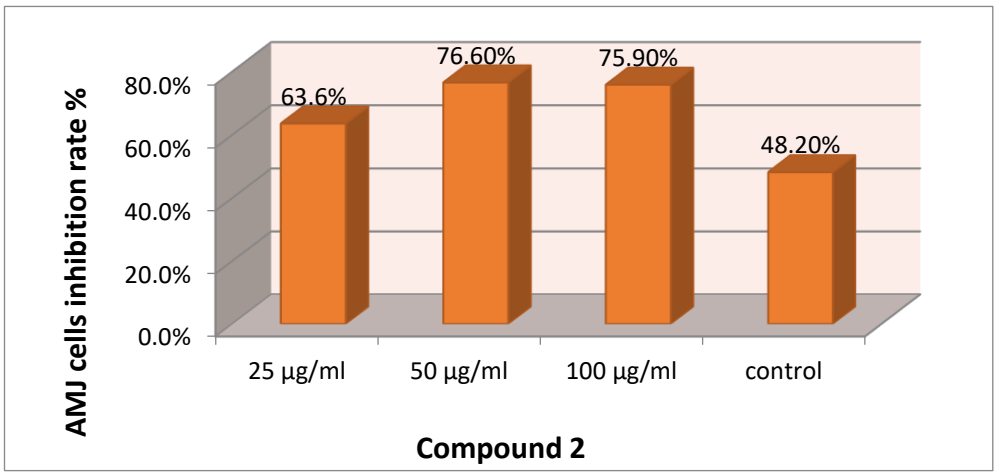

Figure (3. 19): AMJ13 cell line treated with compound (**2**) concentrations (25, 50 and 100) µg/ml for 48 hours

3. 2. 1. 1. 2. Cytotoxic activity of 5-Fluoro-2-[1-(4-methoxy-phenyl)-1*H*-pyrazol-4- yl]-3,3-dimethyl-3*H*-indole (3)

The resultes of compound (3) and its concetrations 25, 50 and 100 µg/ml were illustrated in Figure (3. 20) that showed their dependence on concentration at 48h. The inhibition rates were 63.9, 74.00 and 81.50 % for 48 h.

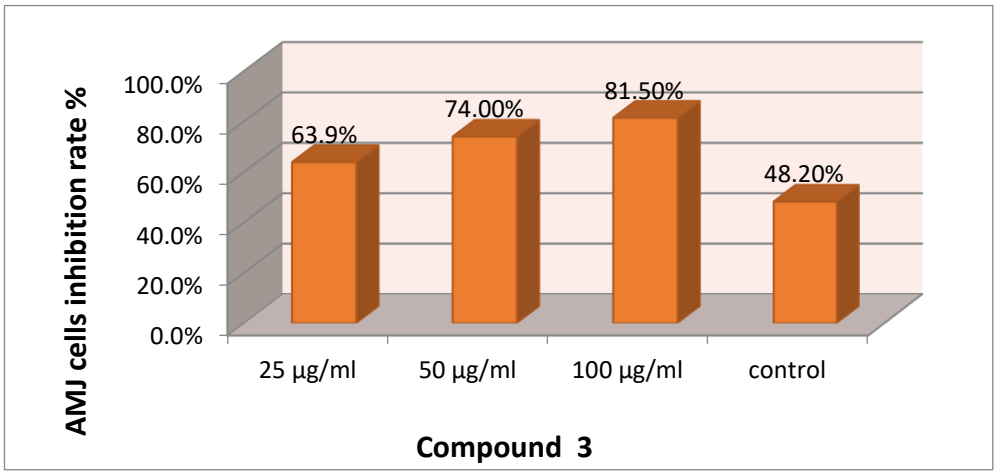

Figure (3. 20): AMJ13 cell line treated with compound (**3**) concentrations (25, 50 and 100) µg/ml for 48 hours

3. 2. 1. 1. 3. Cytotoxic activity of 2-[1-(4-Chloro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro- 3,3-dimethyl-3*H*-indole (5)

The results of this compound and its concentrations According to Figure (3. 21). The concentration 25 and 50 µg/ml have 43.9 and 50.40 % inhibition rates respectively after 48h to AMJ13 cells exposure. While the concentration 100 µg/ml has 63.90 % inhibition rates respectively after 48h to AMJ13 cells exposure.

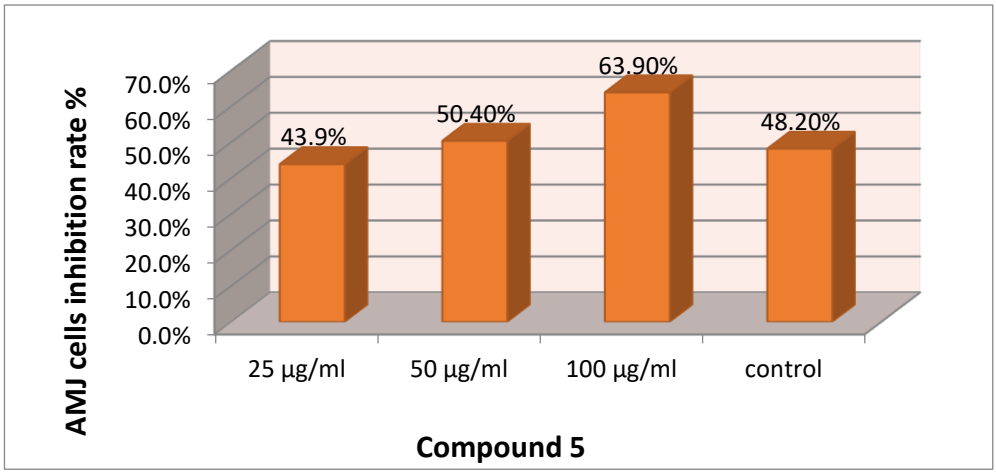

Figure (3. 21): AMJ13 cell line treated with compound (**5**) concentrations (25, 50 and 100) µg/ml for 48 hours

3. 2. 1. 1. 4. Cytotoxic activity of 5-Fluoro-3,3-dimethyl-2-[1-(4-trifluoro methoxy-phenyl)-1H-pyrazol-4-yl]-3H-indole.(6)

Figure (3. 22) determines the higher concentrations represented the ideal concentrations that prepared from this compound. This compound showed a higher cytotoxic inhibition rate after 48 h of exposure to AMJ13 cancer cell line at concentrations 25, 50 and 100 µg/ml were 66.8, 60.70, 44.10% respectively. The concentration 100 µg/ml of this compound showed the highest inhibition rate among rest of the tested compounds.

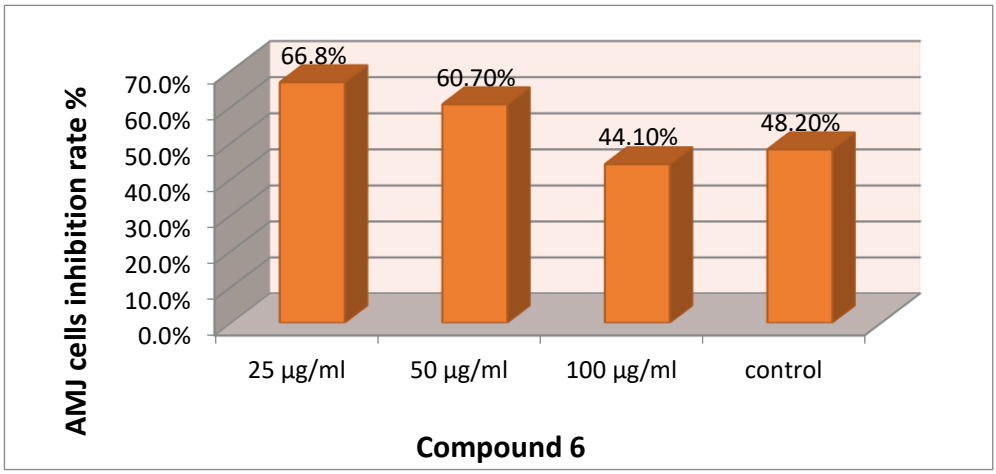

Figure (3. 22): AMJ13 cell line treated with compound (**6**) concentrations (25, 50 and 100) µg/ml for 48 hours

3. 2. 1. 1. 5. Cytotoxic activity of 2-[1-(4-Bromo-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3*H*-indole (7)

The resultes of compound (7) and its concetrations 25, 50 and 100 µg/ml were illustrated in Figure (3. 23) that showed their dependence on concentration at 48h. The inhibition rates were 22.8, 29.40 and 58.00 % for the concentrations 25, 50 and 100 µg/ml respectively.

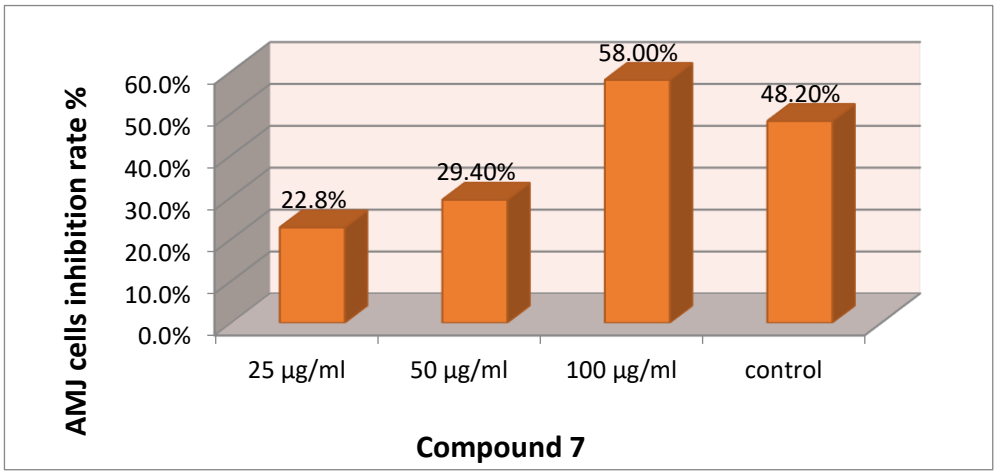

Figure (3. 23): AMJ13 cell line treated with compound (**7**) concentrations (25, 50 and 100) µg/ml for 48 hours

3. 2. 1. 1. 6. Cytotoxic activity of 2-[1-(4-Fluoro-phenyl)-1*H*-pyrazol-4-yl]-5-Fluoro- 3,3-dimethyl-3*H*-indole (8)

The inhibition of this compound and its dilutions was showed in Figure (3. 24). The concentrations 25, 50 and 100 μg/ml in 48 h. of exposure time that inhibited AMJ13 cell lines growth with inhibition rates 31.5, 42.90 and 18.00%The inhibition of this compound and its dilutions was showed in Figure (3. 24). The concentrations 25, 50 and 100 μg/ml in 48 h. of exposure time that inhibited AMJ13 cell lines growth with inhibition rates 31.5, 42.90 and 18.00%.

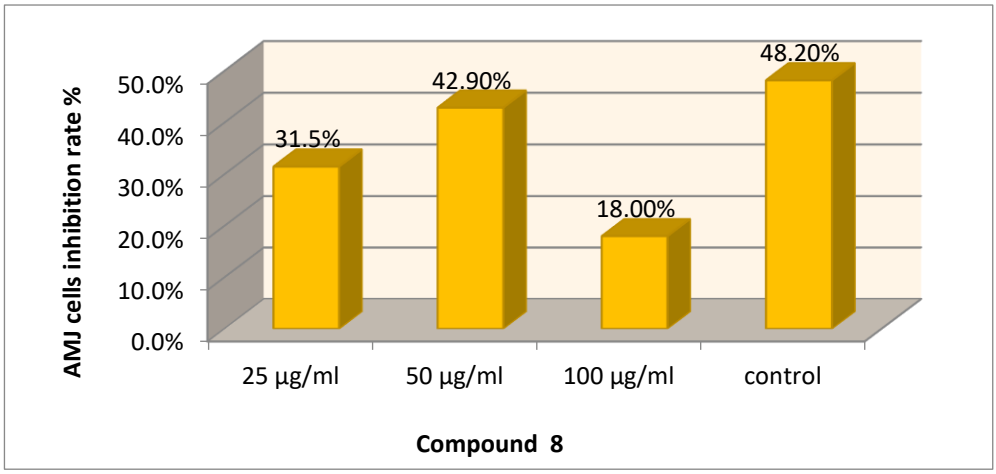

Figure (3. 24): AMJ13 cell line treated with compound (**8**) concentrations (25, 50 and 100) µg/ml for 48 hours

Biological efficacy conclusions

The compound 5-Fluoro-2-[1-(4-methoxy-phenyl)-1H-pyrazol-4-yl]-3,3-dimethyl-3H-indole (3) presented the highest inhibitory compared with other compounds; that showed their dependence on concentration at 48h. The inhibition rates were 63.9, 74.00 and 81.50 % for 48 h.

The compound 2-[1-(4-Fluoro-phenyl)-1H-pyrazol-4-yl]-5 Fluoro-3,3-dimethyl-3H-indole (8) showed less inhibitory percent among all other compounds, The concentrations 25, 50 and 100 µg/ml in 48 h. of exposure time that inhibited AMJ13 cell lines growth with inhibition rates 31.5, 42.90 and 18.00%.

CHAPTER FOUR

CONCLUSIONS AND RECOMMENDATIONS

4. 1. Conclusions

1. Eight new pyrazole derivatives have been synthesized from the reaction of hydrazine, semicarbazide hydrochloride and variously substituted phenyl hydrazine with 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (2) which prepared by Fischer indole synthesis and Vilsmeier Hack reaction. The chemical structures of newly synthesized compounds have been examined and affirmed by the spectroscopic techniques (FT-IR, ^{1}H, and APT^{13}C-NMR), evaluating the biological activities has been done for some of the synthesized compounds (3-8),

2. New compounds and their concentrations were air-stable for extended periods and soluble in DMSO.

3. 5-Fluoro-2-[1-(4-methoxy-phenyl)-1H-pyrazol-4- yl]-3,3-dimethyl-3H-indole (3) was the best compound which gives highest inhibitory compare to with other compounds; that showed their dependence on concentration at 48h. The inhibition rates were 63.9, 74.00 and 81.50 % for 48 h.
4. The compound 2-[1-(4-Fluoro-phenyl)-1*H*-pyrazol-4-yl]-5 Fluoro- 3,3-dimethyl-3*H*-indole (8) showed less inhibitory percent among all other compounds, The concentrations 25, 50 and 100 μg/ml in 48 h. of exposure time that inhibited AMJ cell lines growth with inhibition rates 31.5, 42.90 and 18.00%

4. 2. Recommendations

1. Synthesis new complexes from some newly synthesized compounds by the reaction with various transition metal ions and evaluate their biological activities.

2. Evaluating the other biological activities, like Anti-inflammatory, Antimicrobial, lung cancer cells, colon cancer cells and Antivirus, etc. on the newly synthesized compounds.

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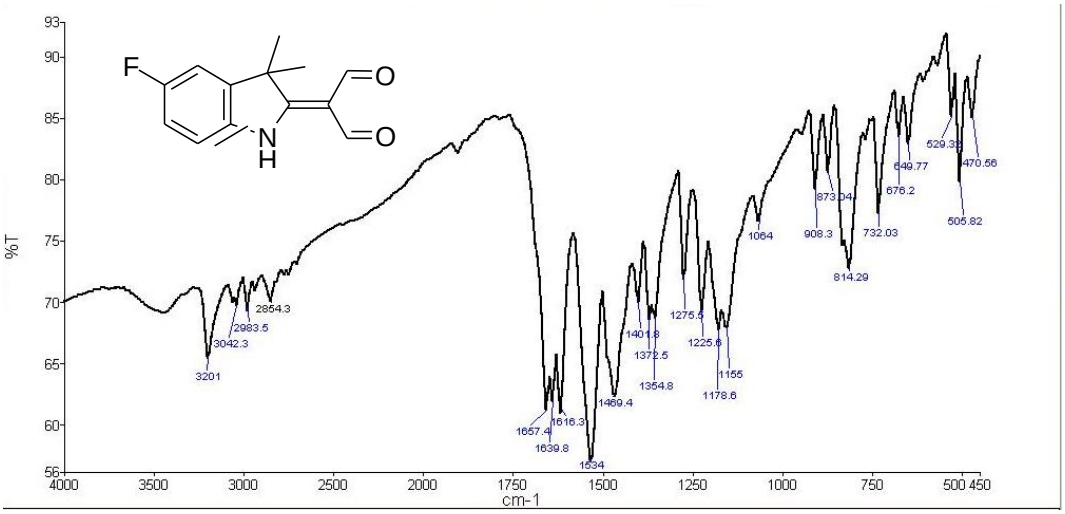
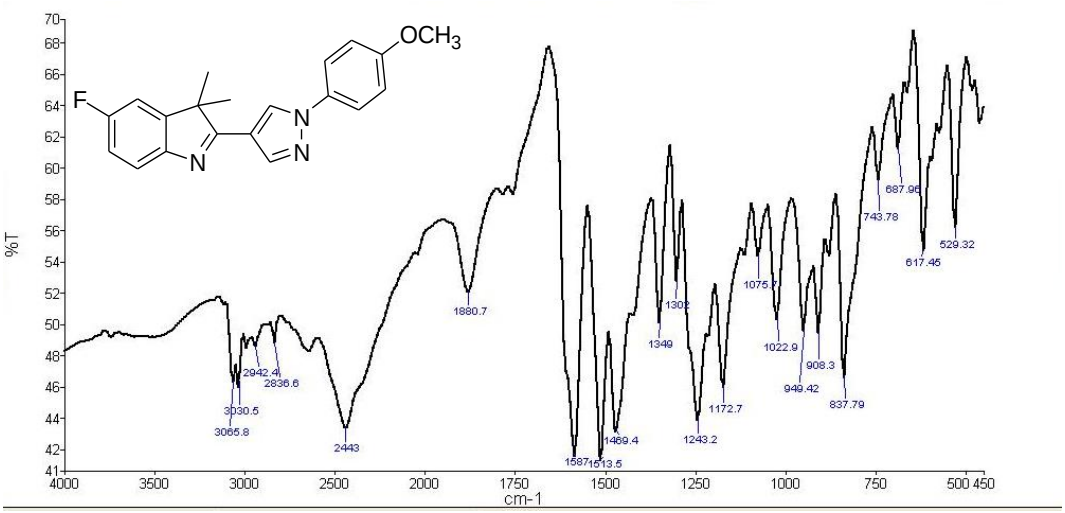
APPENDIX

Appendix:

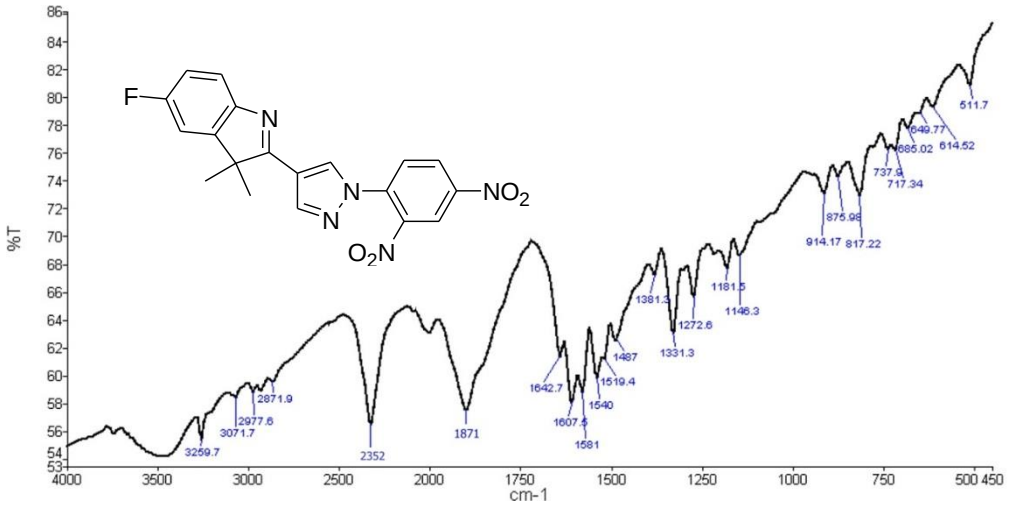
1. IR APPENDIX



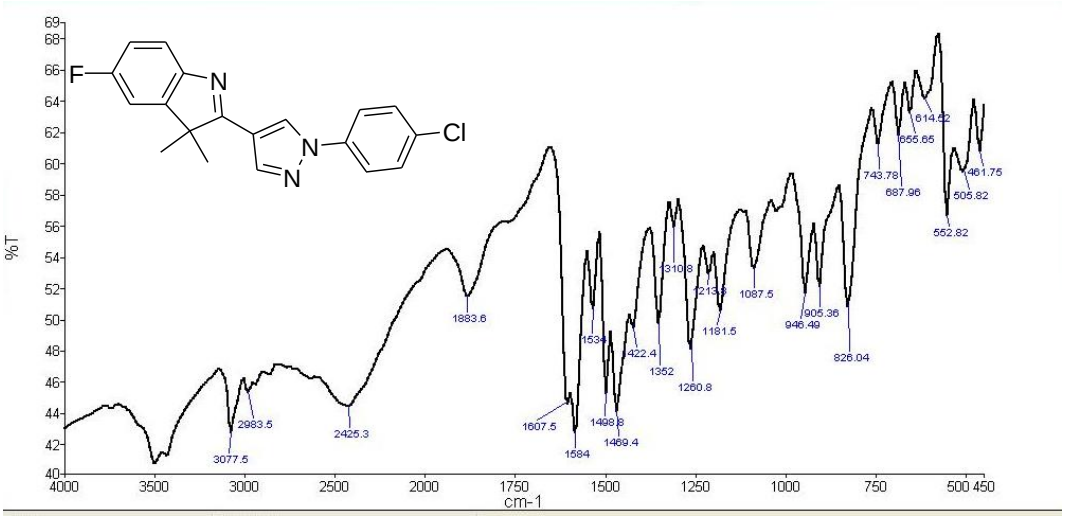
Appendix 1: IR spectra in cm -1 for compound (2)



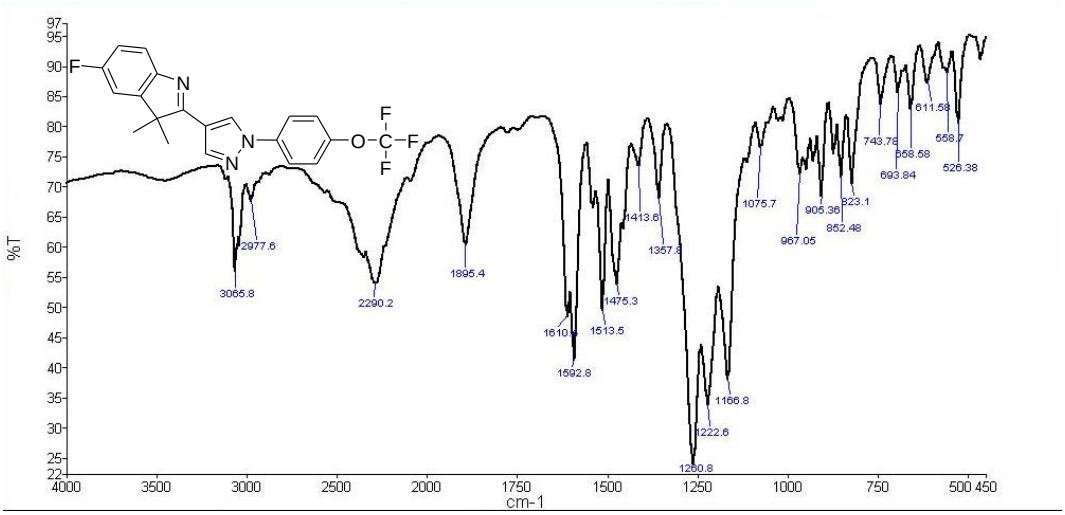
Appendix 2 : IR spectra in cm -1 for compound (3)



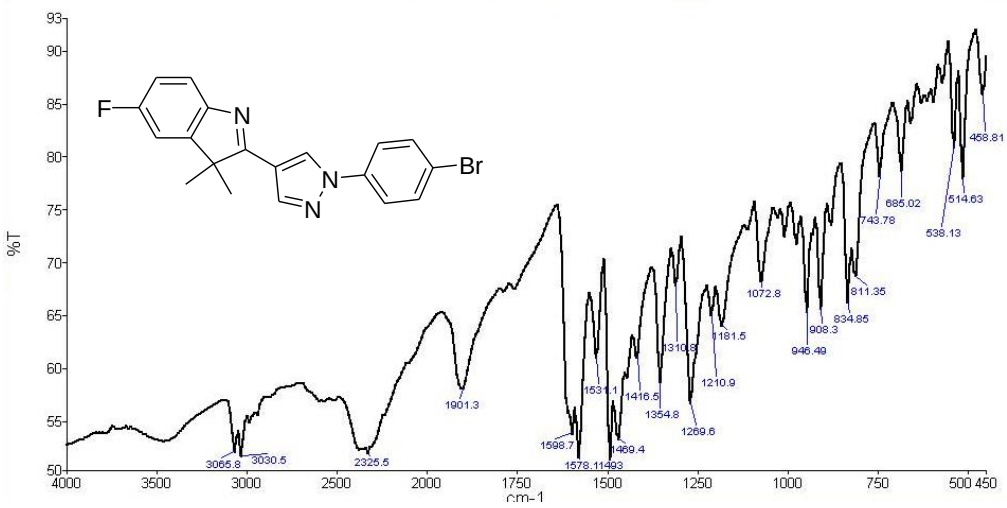
Appendix 3 : IR spectra in cm -1 for compound (4)



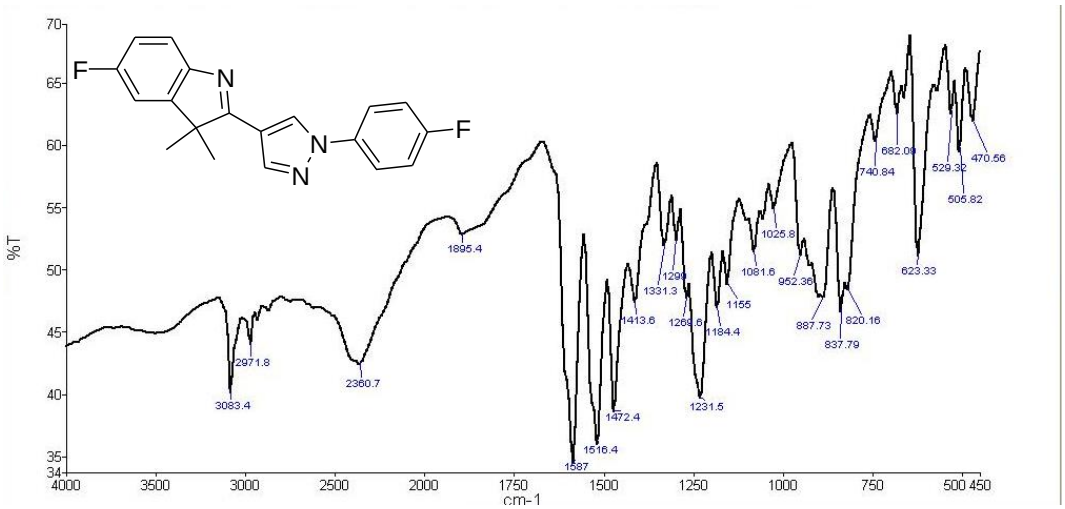
Appendix 4 : IR spectra in cm -1 for compound (5)



Appendix 5 : IR spectra in cm -1 for compound (6)

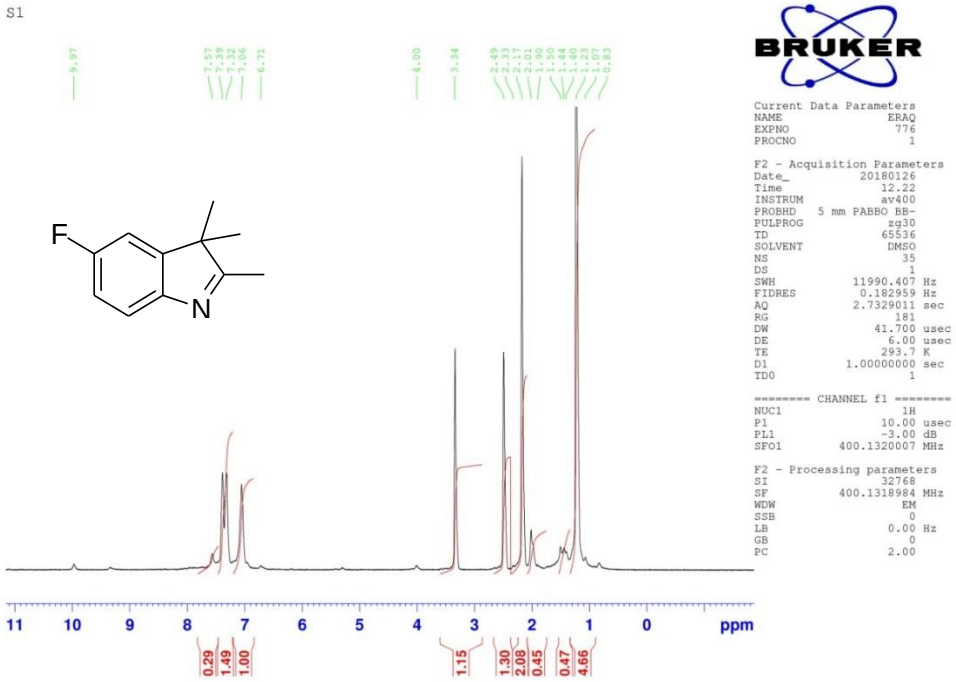


Appendix 6 : IR spectra in cm -1 for compound (7)

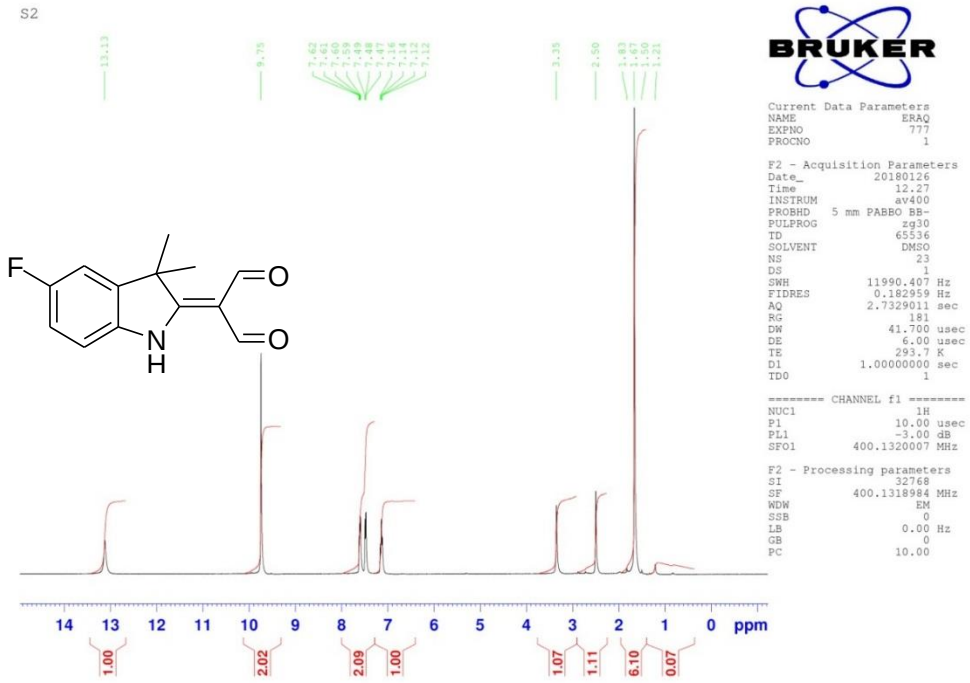


Appendix 7 : IR spectra in cm -1 for compound (8)

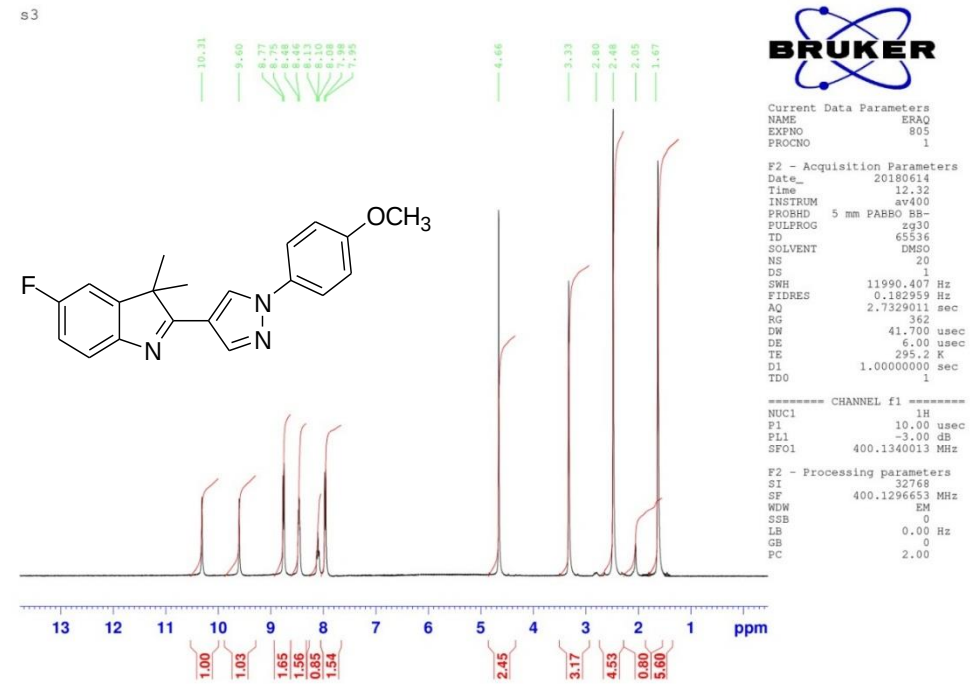
2. ^{1}H-NMR APPENDIX



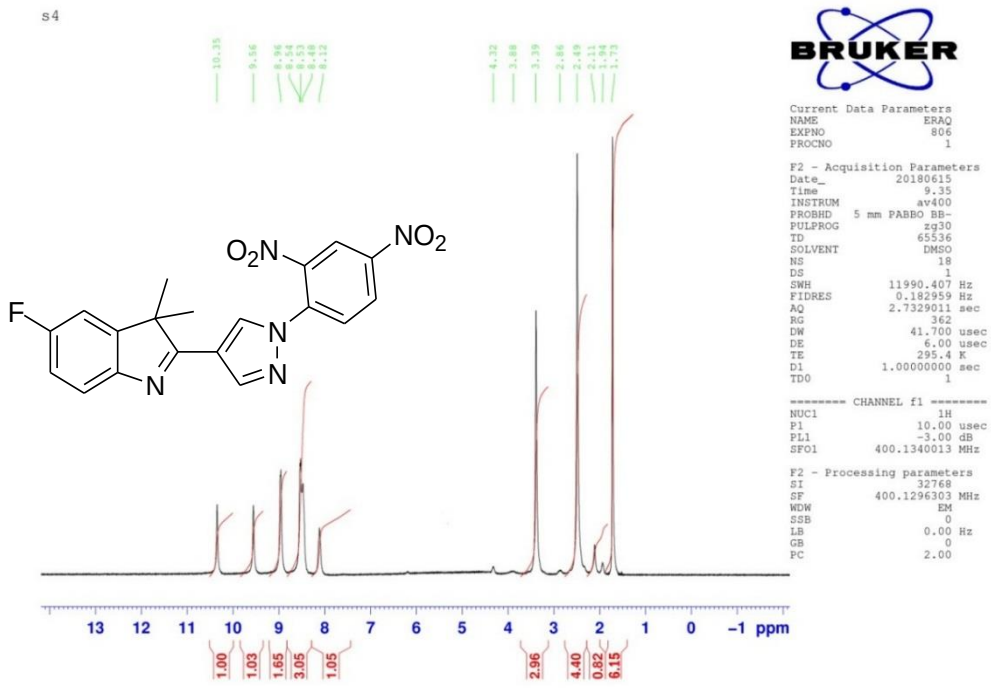
Appendix 8 : ^{1}H-NMR for compound (**1**)



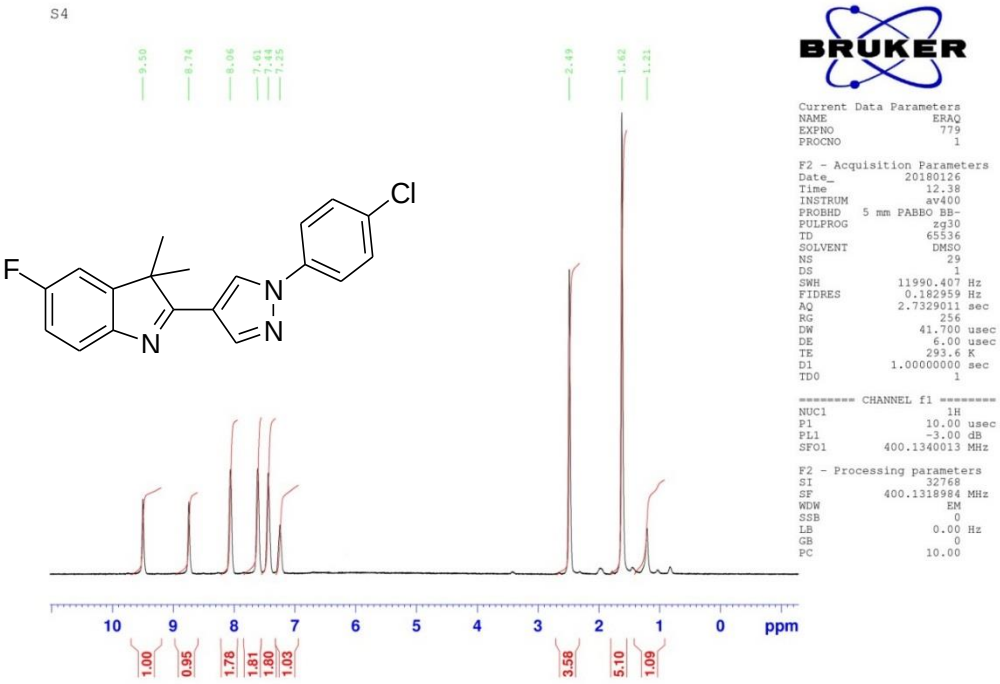
Appendix 9 : ^{1}H-NMR for compound (**2**)



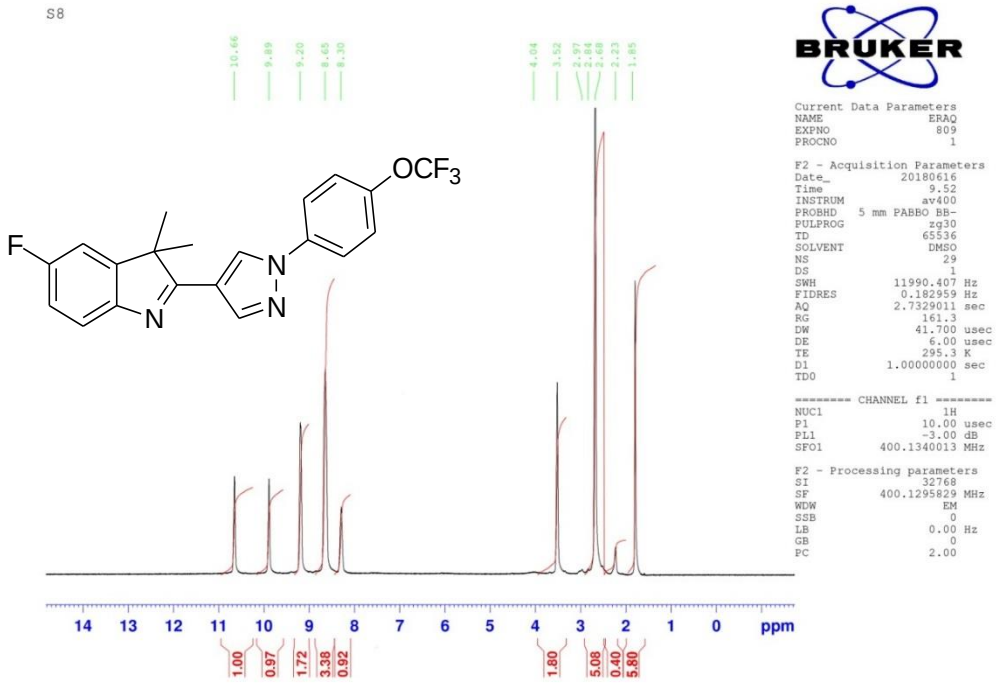
Appendix 10 : ^{1}H-NMR for compound (**3**)



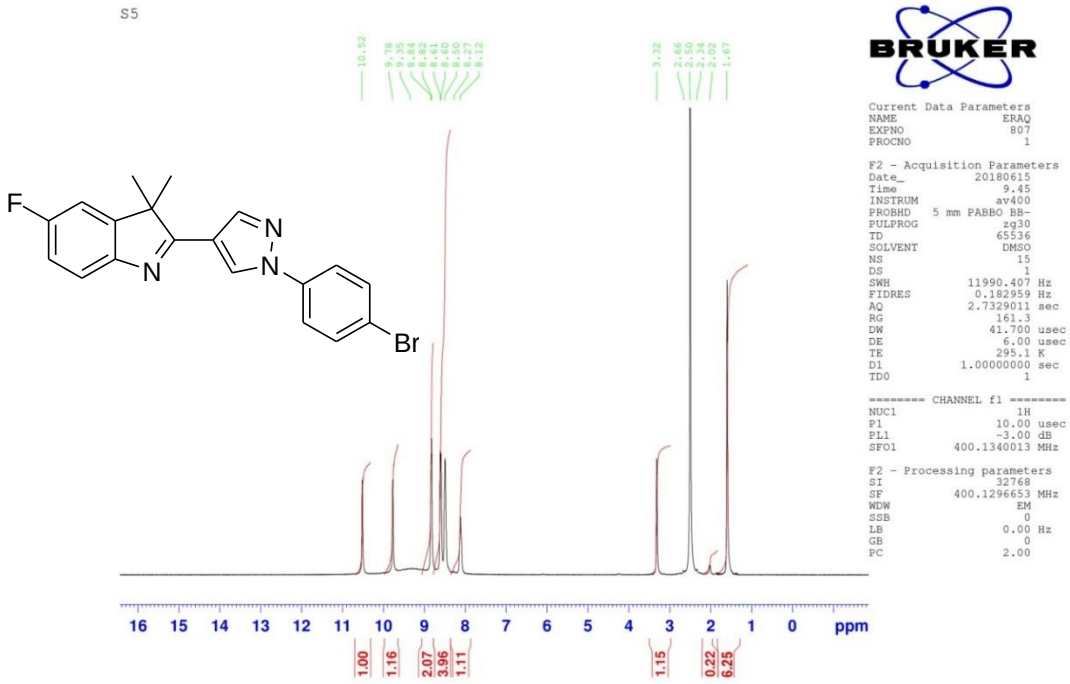
Appendix 11 : ^{1}H-NMR for compound (**4**)



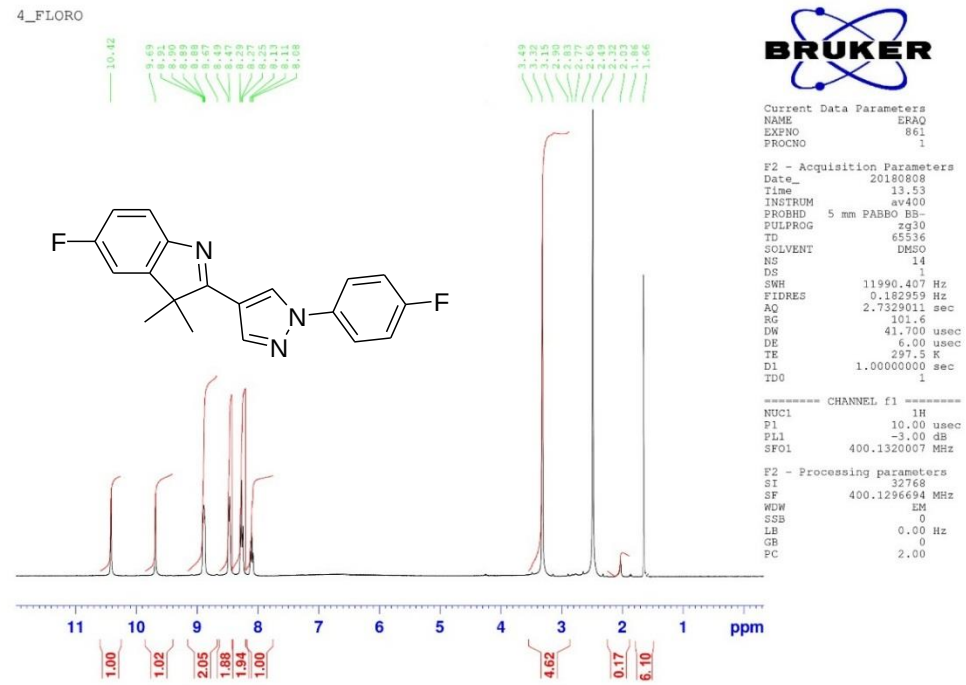
Appendix 12 : ^{1}H-NMR for compound (**5**)



Appendix 13 : ^{1}H-NMR for compound (**6**)

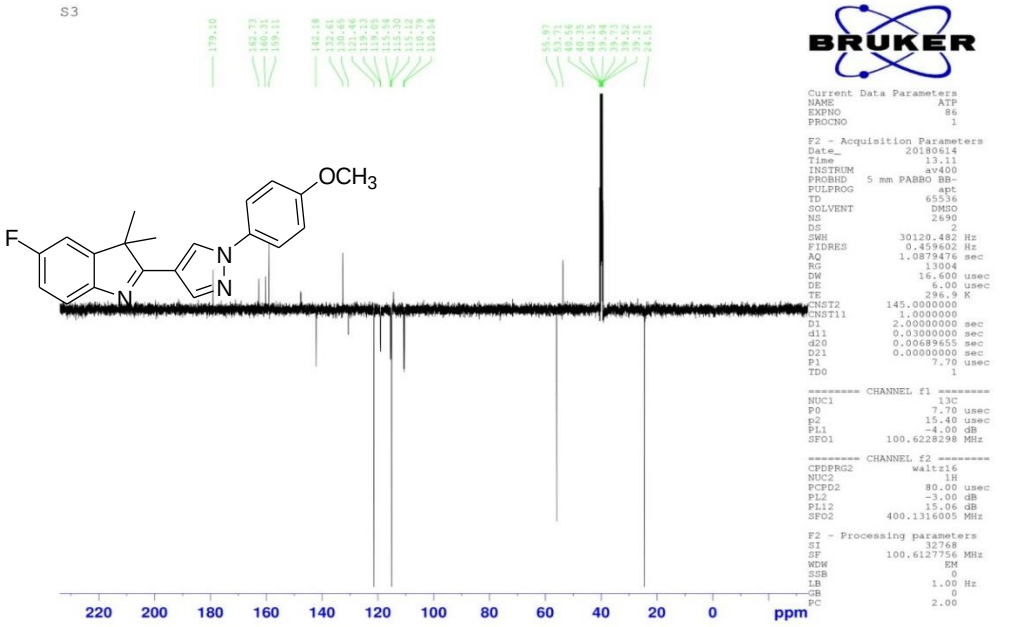


Appendix 14 : ^{1}H-NMR for compound (**7**)

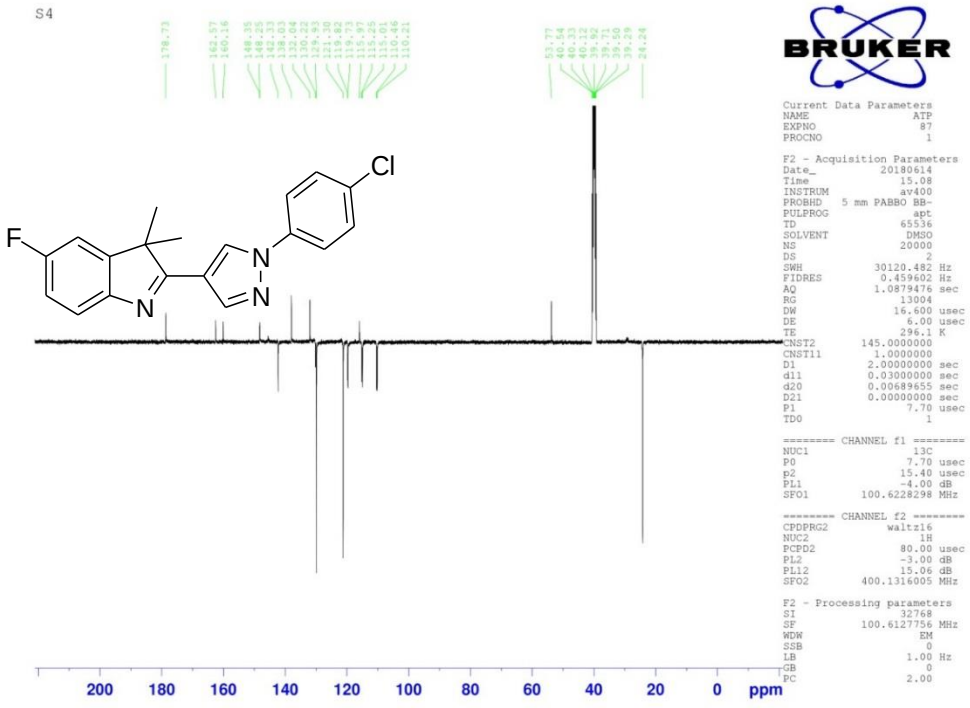


Appendix 15 : ^{1}H-NMR for compound (**8**)

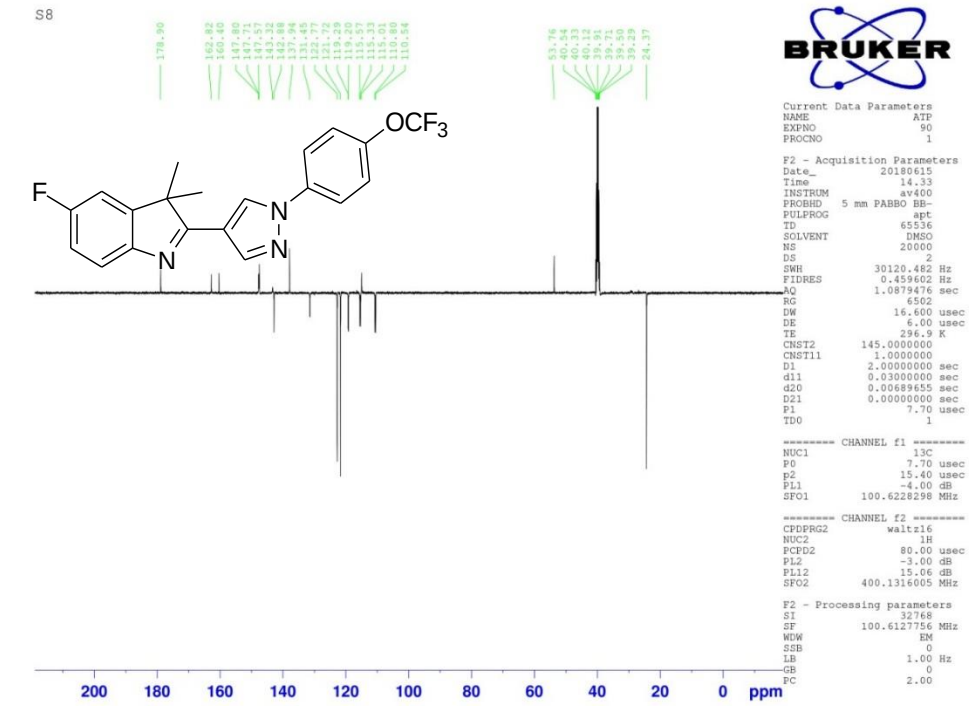
3. APT^{13}C-NMR APPANDIX



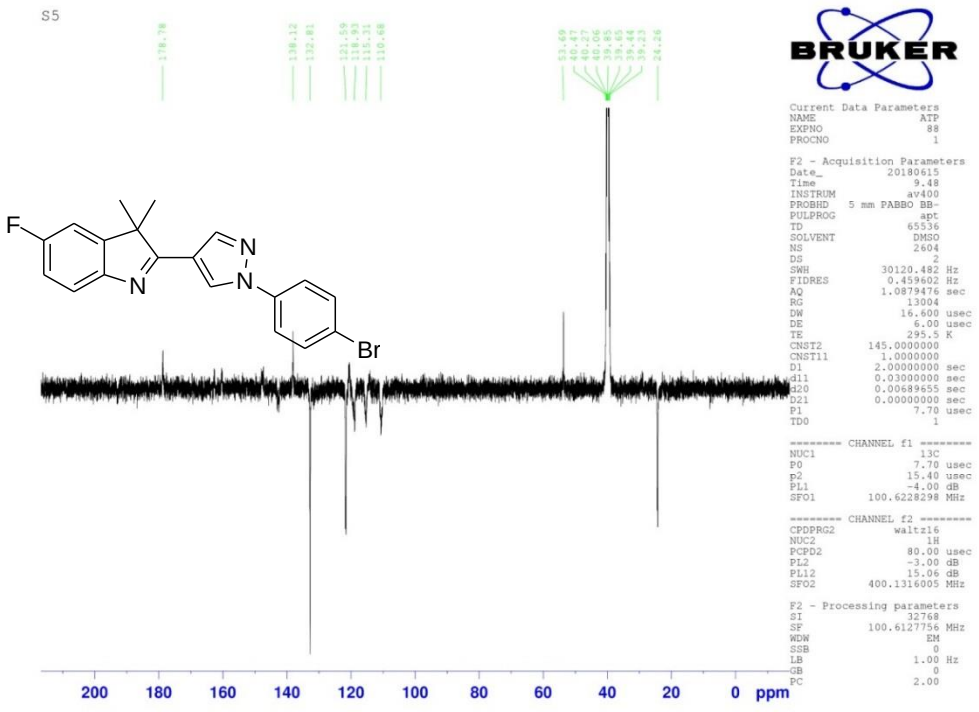
Appendix 16 : APT^{13}C-NMR for compound (**3)**



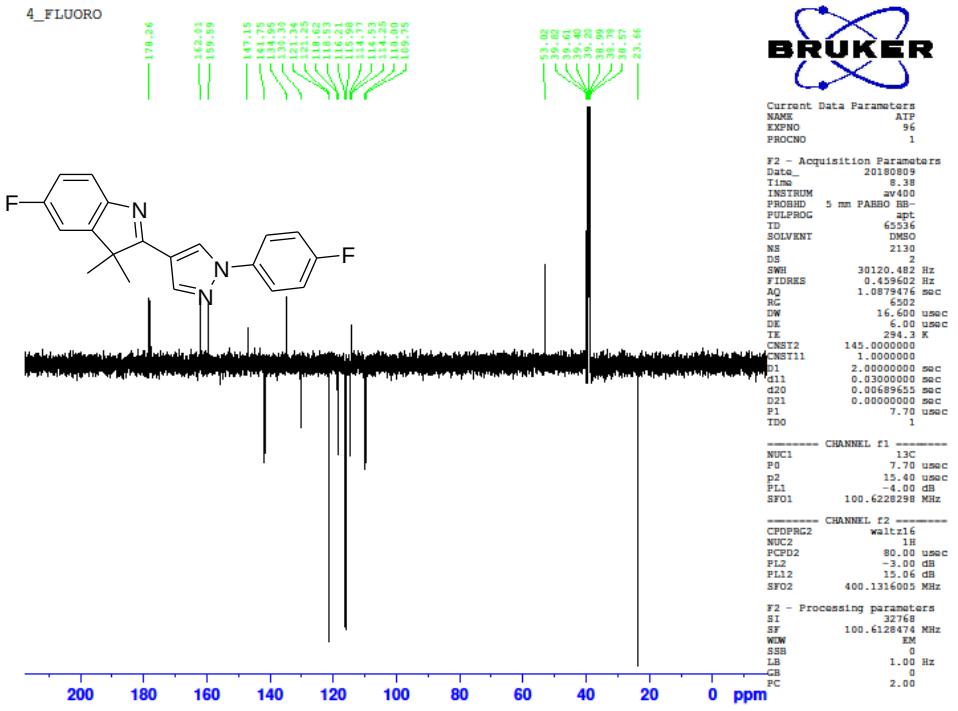
Appendix 17 : APT^{13}C-NMR for compound (**5)**



Appendix 18 : APT^{13}C-NMR for compound (**6)**



Appendix 19 : APT^{13}C-NMR for compound (**7**)



Appendix 20 : APT^{13}C-NMR for compound (**8**)

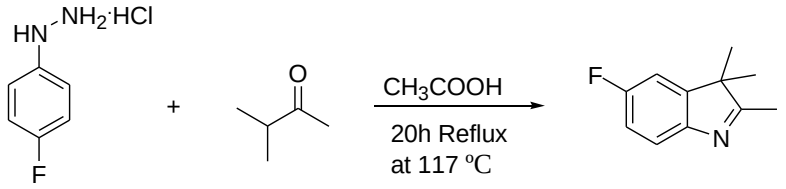
الخلاصة

في هذه الرسالة تم تحضير سلسلة من مشتقات البايرازول بنجاح وتم التأكد من نقاوتها عن طريق كروموتغرافيا الطبقة الرقيقة وعن طريق التركيب الكيميائي للمركبات التي تم تحديدها عن طريق بعض التقنيات الطيفية مثل ^{1}H NMR و APT $^{13}CNMR$ وFT-IR وتم تحديد بعض خصائصها الفيزيائية مثل نقاط الانصهار والالوان وتم تقسيم المركبات إلى قسمين:

القسم الاول:

يتضمن تحضير المادة الأولية الجديدة وهي ٥-فلورو-٢-٣-٣-ميثيل-3H-الأندول (١) و ٢(٥-فلورو-٣-٣-ثنائي ميثيل-٣, ١-ثنائي هيدرو-أندول-٢-يليدين)-المالديهايد (٢).

وتم تحضير المركب الأول ٥,فلورو٢-٣-٣-تراي ميثيل-3H-اندول(١) بوساطة تفاعل فيشر أندول عن طريق تفاعل ٤-فلورو-فنيل هيدرازين هيدروكلورايد مع ايزوبروبيل ميثيل كيتون بوجود حامض الخليك الثلجي كمادة محفزة. وتم تحضير المركب الثاني ٢-(٥-فلورو-٣-٣-ثنائي مثيل-٣-١-ثنائي هيدرو-اندول-٢-يليدين)-المونالديهايد (٢) بوساطة تفاعل فليسميرهاك عن طريق تفاعل ٥-فلورو-٢-٣-٣,تراي مثيل -3H –اندول (١) مع ثلاثي كلورو اوكسيد فسفور ($POCl_3$) في وجود N,N-ثنائي ميثيل فرومايد DMF .

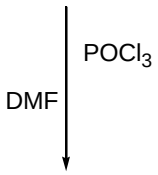


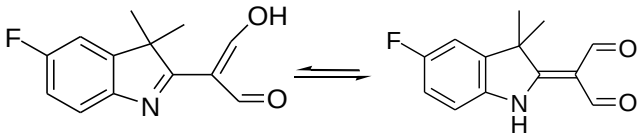
(4-fluorophenyl)hydrazine hydrochloride

3-methylbutan-2-one

5-fluoro-2,3,3-trimethyl-3*H*-indole

(1)



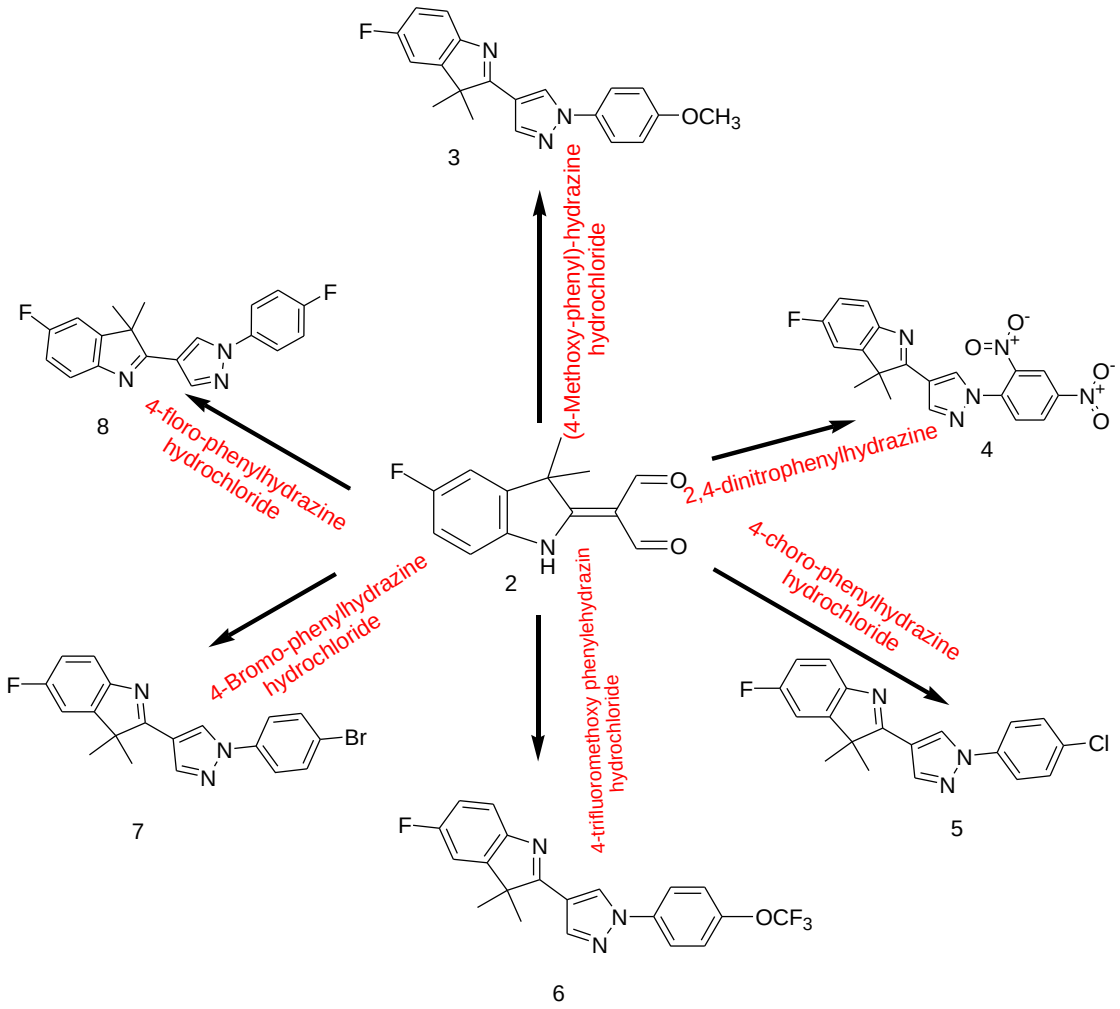


2-(5-Fluoro-3,3- dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

(2)

القسم الثاني:

هذا القسم ينطوي على تحضير عدد من مشتقات البايرازول الجديدة الناتجة من تفاعل ٢-(٥-فلورو-٣-٣-ثنائي ميثيل -٣-١-ثنائي هيدرو-اندول-٢-يليدين) المونالديهايد (٢) مع معوضات الفنيل هيدرازين في مذيب الايثانول كما مو ضح في المخطط أدناه:



وتم قياس الفعالية البايولوجية لبعض المركبات التي تم تحضيرها على خلايا سرطان الثدي واظهرت النتائج انه تم قتل هذه الخلايا السرطانية.

وزارة التعليم العالي والبحث العلمي

جـامـعــة ديــالــى

كـلـيــة الـعـلــوم

قسم علوم الكيمياء

تخليق وتشخيص مشتقات اندول جديدة من ٤-فلوروفنيل هايدرازين هايدروكلورايد ودراســة فعاليتها البايولوجية

رسالة مقدمة إلى مجلس كلية العلوم – جامعة ديالى

كجزء من متطلبات نيل شهادة الماجستير في علوم الكيمياء

من قبل الطالبة:

سـمـيــه مـهـدي صــالـح خـمــاس

بكالوريوس علوم الكيمياء – جامعة ديالى ٢٠١٧

بإشراف

أ. م. د. فــاضل لفــته فــرج

أ. م. د. حميد مدلول محمد

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