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University of Diyala
College of Science
Department of Chemistry

Synthesis New Schiff bases From Indole Derivatives and Studying Their Anti-Cancer Activity

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Fulfillment of the Requirements for the Degree
of Master of science in Chemistry
by

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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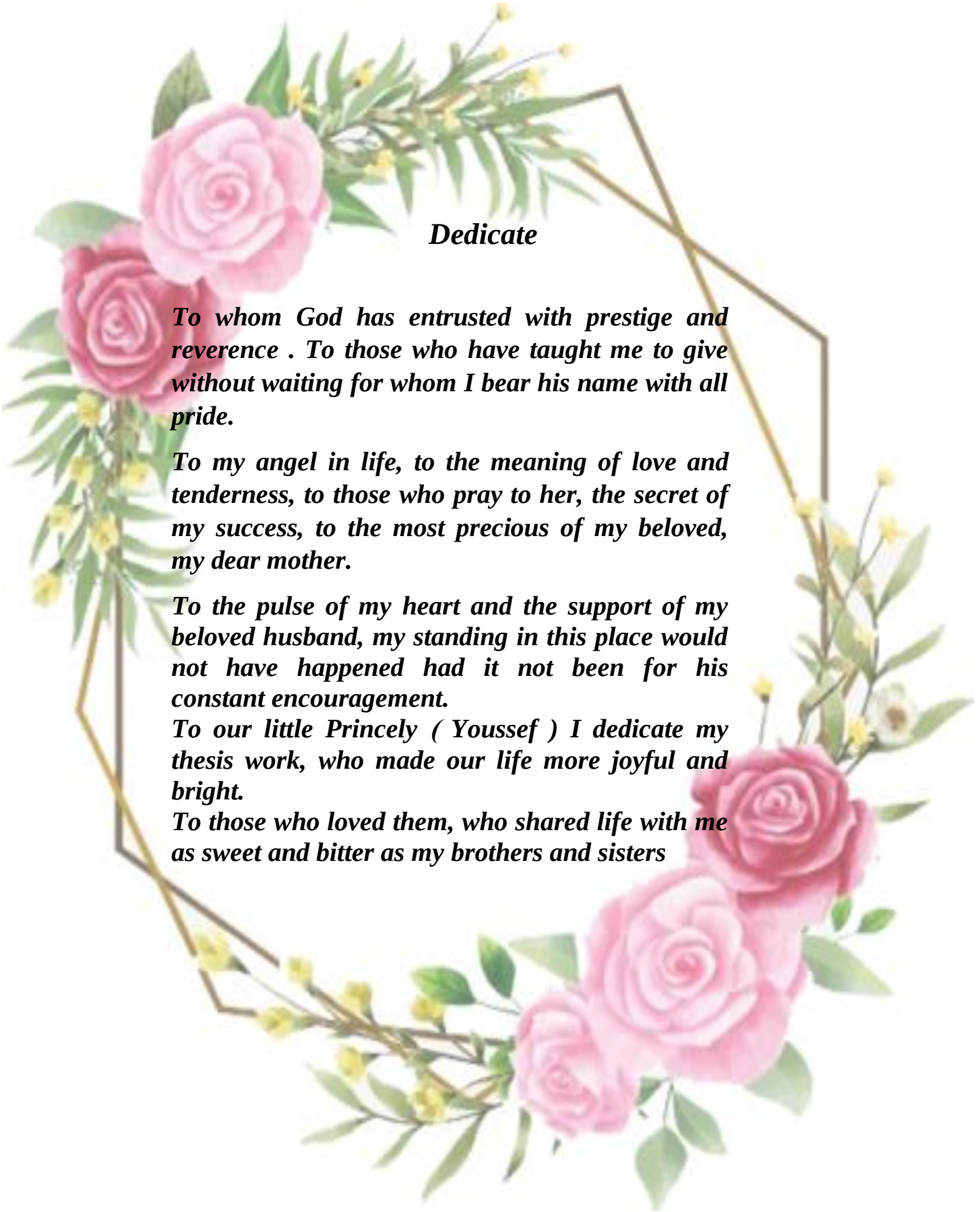

Dedicate

To whom God has entrusted with prestige and reverence . To those who have taught me to give without waiting for whom I bear his name with all pride.

To my angel in life, to the meaning of love and tenderness, to those who pray to her, the secret of my success, to the most precious of my beloved, my dear mother.

To the pulse of my heart and the support of my beloved husband, my standing in this place would not have happened had it not been for his constant encouragement.

To our little Princely (Youssef) I dedicate my thesis work, who made our life more joyful and bright.

To those who loved them, who shared life with me as sweet and bitter as my brothers and sisters

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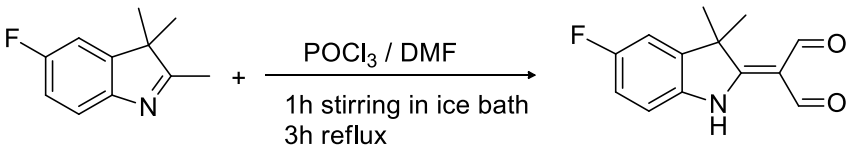
Abstract

In the present research, new indole schiff bases have been successful synthesized from one of indole derivatives.

The chemical structures of all new compounds have been characterized and confirmed by some spectroscopic techniques such as, (FT-IR, ^{1}H-NMR, and APT ^{13}C-NMR), their purities tested by thin layer chromatography (TLC). four new synthesized compounds evaluated for their cytotoxicity activity against HepG2 and SK-GT2 cell line of cancer and revealed good results compare to the control.

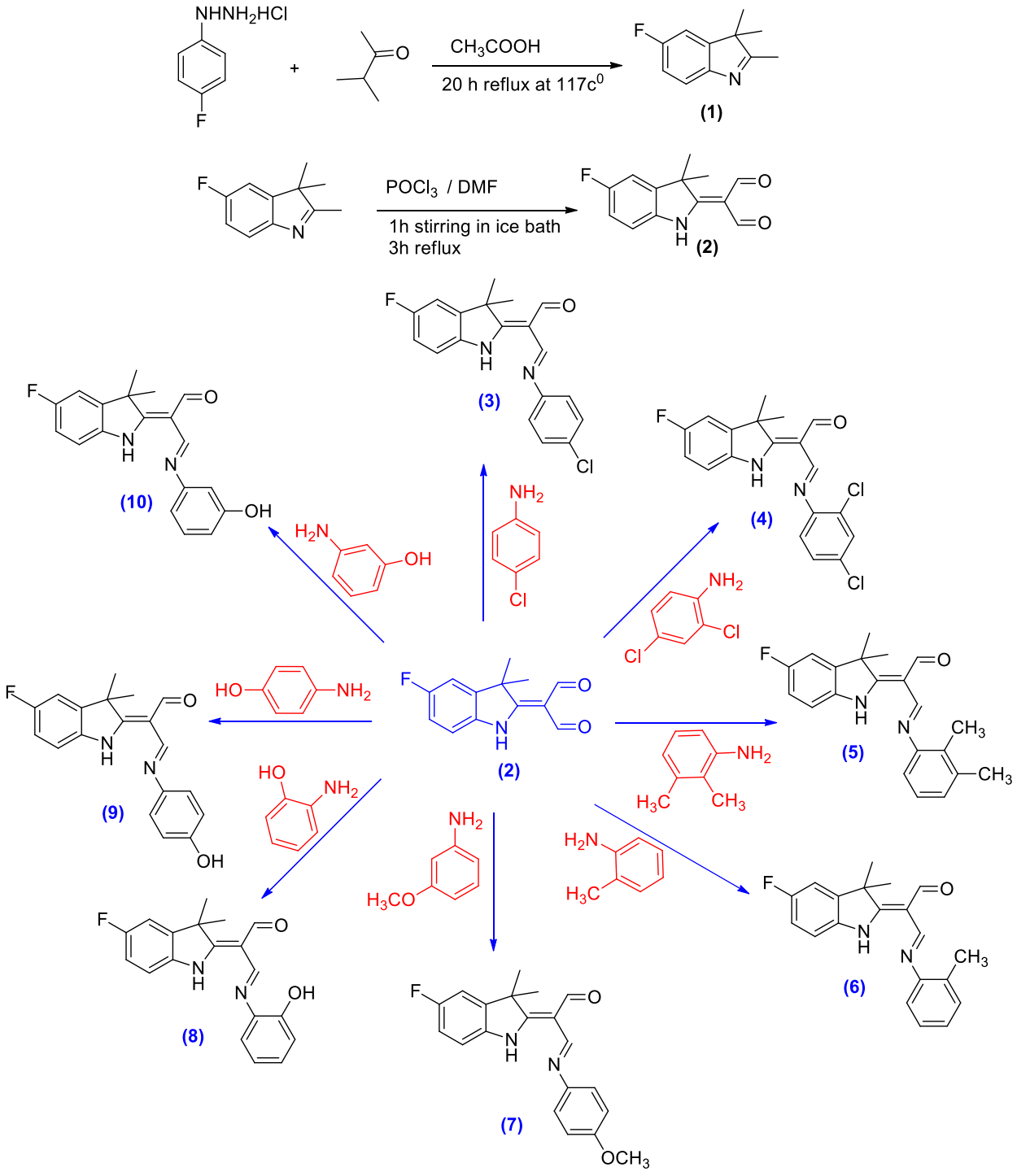
The indolic aldehyde 2-(5-Fluror -3,3-dimethyl-1,3-dihydro-indol-2 ylidene)-malonaldehyde (**2**) was prepared by Vilsmier- Haack reaction through the reaction of 5-Fluoro-2,3,3-trimethyl-3H-indole (**1**) with Phosphoryl chloride ($POCl_3$) in the present of dimethyl formamide (DMF) as a reactant and solvent as shown below.



(**1**) (**2**)

Compound (**2**) was considered as a precursor to synthesis various kinds of imines by reaction with different substituted anilines as shown in the scheme below

The cytotoxicity of the synthesized derivatives were screened against HepG2 and SK-GT2 cancer cell line with seven different concentrations 15.1, 31.2, 62.5, 125, 250, 400 and 500) µg\ml .studying the effect of the tested compounds in inhibitory of the HepG2 and SK-GT2 cell line with 24 h exposure time. Most of the tested derivatives exhibited acceptable inhibition rates against used cell line.



The general scheme of the new synthesized compounds

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°C	Degree Celsius
EAS	Electrophilic Aromatic Substitution
Pym	Pyrimidine
A	*Alfa*
B	*Beta*
DMF	Dimethyle formamide
TLC	Thin Layer Chromatography

FTIR	Fourier-Transform Infrared
^{1}H-NMR	Proton Nuclear Magnetic Resonance Spectrometer
^{13}CNMR	Carbon Nuclear Magnetic Resonance Spectrometer
APT ^{13}C NMR	Attached Proton Test ^{13}C- Nuclear Magnetic Resonance Spectrometer
ml	Milliliters
mmol	Millimole
h, hrs	Hour, Hours
m.p.	Melting point
Cm	Centimeter
MHz	Megahertz
DMSO	Dimethyl Sulfoxide
Γ	Chemical shift
ppm	Part per million
s	Singlet
d	Doublet
t	Triplet
m	Multiplet
TMS	Tetramethylsilane
Ar	Aromatic ring
Rf	Retetion factor
ICCMGR	Iraqi Center for Cancer and Medical Genetics Research
HEPES	4(2-hydroxy ethyle)-1-piperazine ethanole sulfonic acid

CHAPTER ONE

INTRODUCTION

Heterocyclic compounds are organic compounds that contain at least one carbon atom and at least one difference element carbon, such as sulfur, oxygen and nitrogen isinside the ring structure. While cyclic organic compounds all atoms in the ring are same kind. The most common heterocyclic loops are those that have five- or six-membered rings . It contains heterocyclic atoms of nitrogen (N), oxygen (O), or sulfur (S) in their chemical structures [(1)]. Heterocyclic compounds have a role at plurality fields of sciences such as medicinal chemistry, biochemistry as well as another fieled of sciences[(2)]**.** Among the various clinical applications, heterocyclic compounds contain significant active role as anti-bacterial, antiviral, anti-fungal, anti-inflammatory and anti-tumor drug [(4)]**.** Heterocyclic compounds considered one of the biological classes of organic compounds, which are used in many biological fields, given that it is of activity in multiple diseases.

Heterocyclic nitrogen is of special importance because it is an important type of natural and unnatural products, Many exhibit beneficial biological activities, and some heterocyclic derivatives, It may allow the development of new therapies for epilepsy, pain and other neurological disorders [(5)].

Indole is a significant class of organic heterocyclic compounds which it has a two-ring structures, consisting of a six-membered benzene ring embedded in a five-pyrrole rings containing nitrogenIndole is a common ingredient in perfumes and a precursor to many drugs.The Compounds that contain an indole ring are called indoles. While, a tryptophan is amino acid which contains indole ring in its structure is the precursor of the neurotransmitter serotonin[(6)]. Tryptophan plays a substantial role as a building synthesis in protein biosynthesis. Proteins containing tryptophan have a reducing effect on hormone-related depression and insomnia [(7)].

In addition, the indole ring is found in many marines or terrestrial natural compounds, which has beneficial biological properties. In anotherfew years it was reported that indole.

Vilsmeier Reaction:

The use of Vilsmeier-Haack reagent ($PoCl_3$ / DMF) has been documented to form a variety of aromatic and heterocyclic substrates well [(8)] . It can be applied to enter an aldehyde group to Aromatic compounds, but many other transformations can be achieved using this technique [(9)]. The reagent has also been widely used to induce various chemical conversions of another classes of compounds. Many of these reactions lead to new and convenient methods of synthesizing various heterocyclic compounds [(10)].

Schiff Base:

Schiff bases also called imines are characterized by the azomethine (-C=N-) group and are It usually consists of condensation of an aldehyde or ketone with a primary amine (Graham and Fryhle, 2004). This condensation reaction between a carbonyl compound and primary amine leading to the configure of schiff base. Schiff's rules are generally excellent chelating agents especially when they are a functional groups such as –OH or –SH . It is located near the isomethene groups to form a five or six-membered rings with the metal ion [(11).]

Ghaidan, A. F *et al* synthesized 2-(5-Chloro-3,3-dimethyl- 1,3-dihydro - indol - 2- ylidene)- 3 - (2,4 - disubstituted phenylimino)-propionaldehyde derivatives figure 1.1 and were appraisal for their in vitro against– AMJ breast cancer cell line. The appeared data showed that compounds have promising anticancer activity toward AMJ13 cell line at low concentrations[(12)].

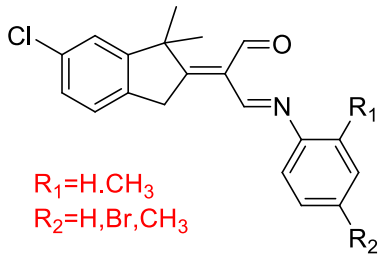
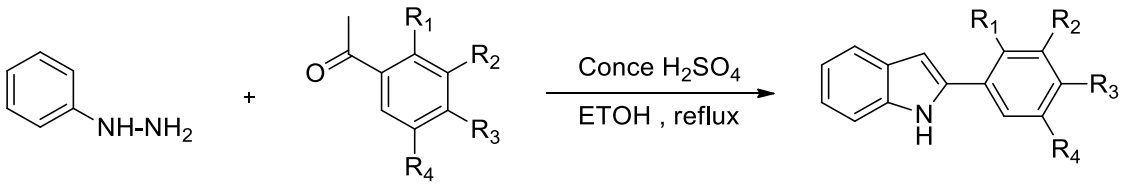


Figure 1. 1: 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4- disubstituted phenylimino)-propionaldehyde derivatives

Mohammad Zamir *et al* reponted A series of new -1 H-indole aryl derivatives were synthesized 2-replaced by the Phenylhydrazine reaction with different acetophenones derivatives in the presence of sulfuric acid from good to excellent yield Eqution1.1. This transformation is dependent on the Fisher-indole reaction [13].



Eqution1. 1: Synthesis of substituted 2-aryl-1*H*-indoles

Also some of an important heterocyclic compounds such as tryptophan and serotonin contain indole rings have been synthesized by Nagendra Kumar Kaushik *at el.* Figure 1. 3 [14].

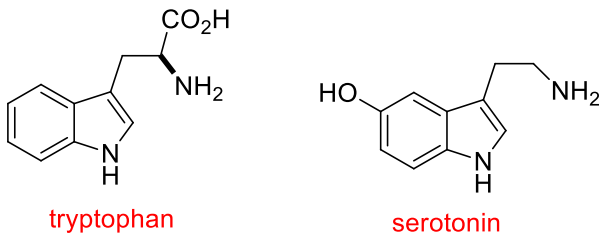


Figure 1. 2. Structures of some naturally occurring indoles.

Mohd Javed Naim *at el.* Extensive research has been carried out on indole and its derivatives, which led to the presence of many indole-containing drugs approved in the global market in addition to many of them in the preparation stages. They were synthesized many heterocyclic compounds contain indole rings which have biological activity against cancer, some examples of them as shown in **figure 1. 4**[15].

2-(6-bromo-2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde

methyl 6-amino-4-cyclohexylmethoxy-1Hindole-2-carboxylate

Figure 1. 3. Some examples of anti-cancer compounds

This review focused on recent developments of indole derivatives that have different pharmacological characteristics as well as different perspectives on how to use this indole fraction as a franchise structures in the near future. Its also covers some related and recent achievements in biological, chemical and pharmacological activity of indole derivatives that are important in the fields of drug discovery and analysis. For the above reasons related to the importance of the heterocyclic compounds especially containing the indole ring in synthesizing and biological activities , and continuing with the efforts of the previous researchers, we focused on synthesizing a series of heterocyclic compounds containing the indole ring and conducting biological applications on them.

Aim of the work

The main objectives of the present study are :

1. Synthesis new derivatives of indole based Schiff bases.
2. Characterization the chemical structure of the synthsized compounds by spectral techniques (FT-IR, ^{1}H, APT ^{13}C NMR).
3. Evaluating anticancer activity of the synthesized derivatives against HepG2 and Sk-GT2 cancer cell line.

CHAPTER TWO

Theoretical part

2. INTRODUCTION

2.1. Review on Indole

Indole ring is a typical example of the aromatic heterocyclic compounds which has a two-ring Structure, consisting of a five-pointed pyrrole ring embedded in a hexagonal benzene ring figure 2.1

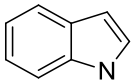
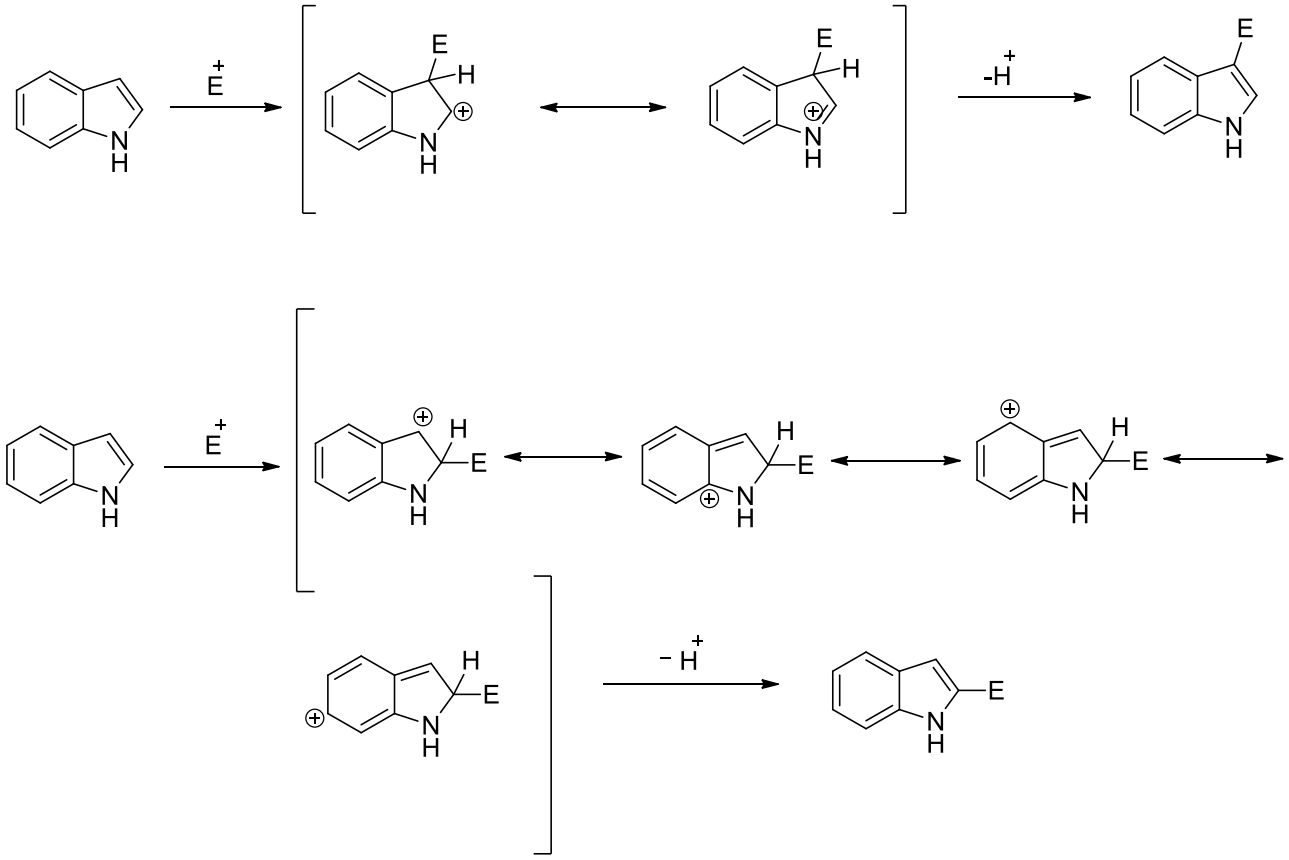


Figure 2. 1: the chemical structure of Indole ring

In fact, the most effective anticancer and anti-HIV drugs are derived from the indole basic structure, with substituents in both the aromatic and heterocyclic rings [(16)]**.** It has been proven that indole alkaloids .Clinically important natural compounds. Indole compounds include the phyto-hormone auxin, the anti-inflammatory drug indomethacin, beta-pendulum blockers, and the natural hallucinogen dimethyltryptamine [(17)]. Derivatives of 2-phenyl-1-H- Indole has been found to inhibit the growth of human breast cancer cells through various mechanisms depending on the type and location of the alternatives . Substituted methane with two units of indole is ommonly known as di (indolyl) methane (BIM) or diindolyl methane (DIM), it is found in many natural products that have predictable carcinogenic activity. DIMs induction of apoptosis in many cancer cells by sending signals to different precursor genes and proteins [(18)]**.** Because of the de-locating of the excessive electrons, the indole readily undergoes electrophysiological substitution reactions similar to a benzene ring [(19)] **.** Indole shows a high reactivity to EAS, which is estimated in greater numbers than benzene. This is due to the electron-rich nature of the indole, and the high electron density in its third position is responsible for the selectivity of the indole for EAS reactions. Scheme 2. 1



Scheme (2. 1): Possible regioisomers in the electrophilic attack on the indole ring

2.1.1 Physical Properties of Indole

Indole is a white color solid that melts at 52-54 ° C, boils at 253-254 ° C, 0.19 g of insoluble indole in 100 ml of hot water . Indole is soluble in alcohol, ethyl acetate etc. Indole has a planar molecular shape, a density of 1.22 g / cm3 and a dipole moment of 2.11 D in gasoline. All common indole derivatives, such as Indole, form crystal clear bikes, from yellow to red Cylinder molding is usually a convenient procedure for identificaton and purificaton [(20)]**.** Indole is a non-polar isotope of purine found in some important biochemical molecules such as tryptophan, serotonin and melatonin. [(21)]**.**

2. 1. 2. Indole Derivatives

Indole derivatives represent one of the most important heterogeneous nitrogen cycles due to their widespread and interesting biological activities. [(22)]. Tryptophan is one of the most important indole derivatives and is an essential amino acid, and it is one of the 22 natural

amino acids. This amino acid cannot be synthesized by living organisms but must be in their daily diet.

Tryptophan is a biochemical precursor to the tryptamine family, as is serotonin (5-hydroxytryptamine) and melatonin figure 2.2. Serotonin it is a major a neurotransmitter in a central nervous system, and melatonin is a hormone that regulates smooth muscle function in the cardiovascular and digestive systems. [23]

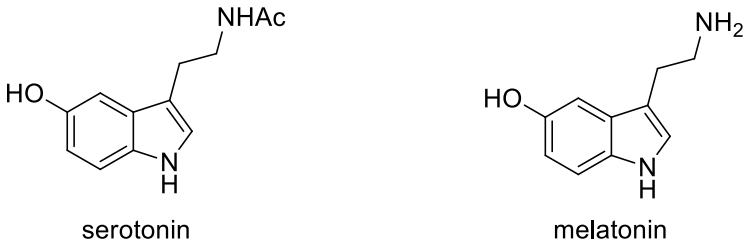
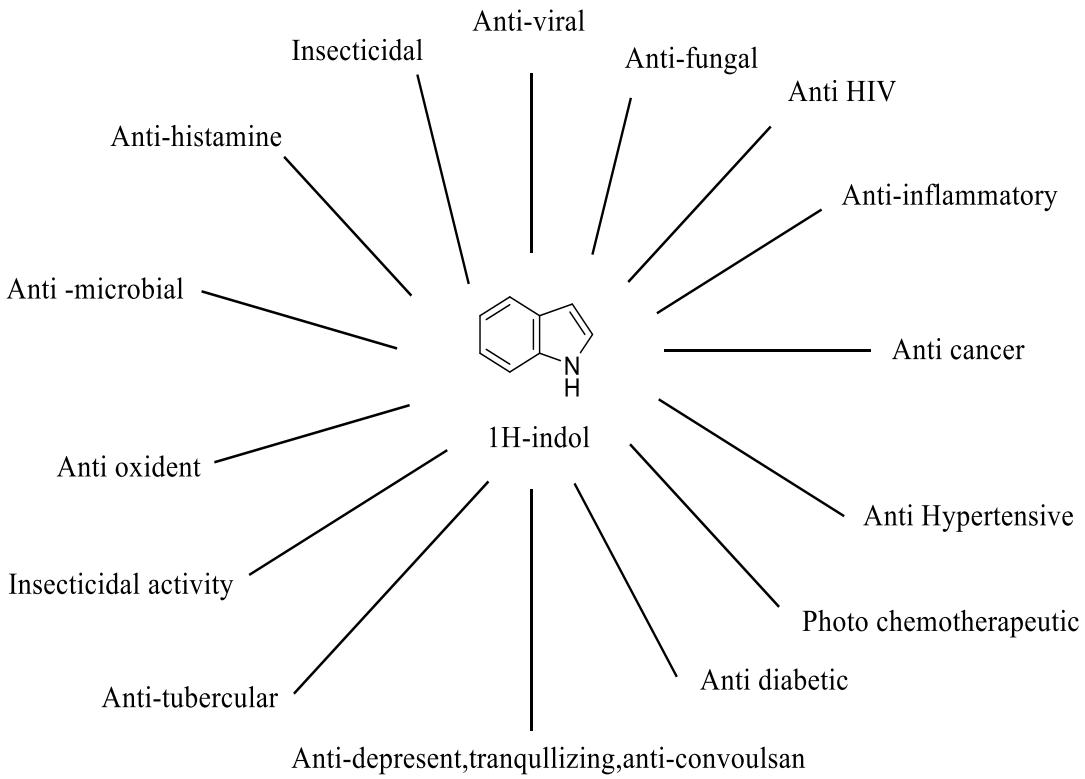


Figure 2. 2: the chemical structures of serotonin and melatonin

2. 1. 3. Pharmacological importance of Indole

Indole possesses the following activities that are shown in Eqation 2. 1 **(24).**



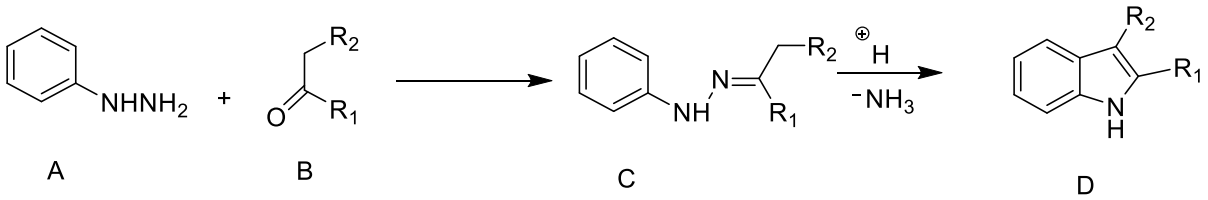
Eaqtion 2. 1: the biological activities of indole ring

2. 1. 4. Synthesis of Indole Derivatives

2. 1. 4. 1. Fischer Indole Synthesis

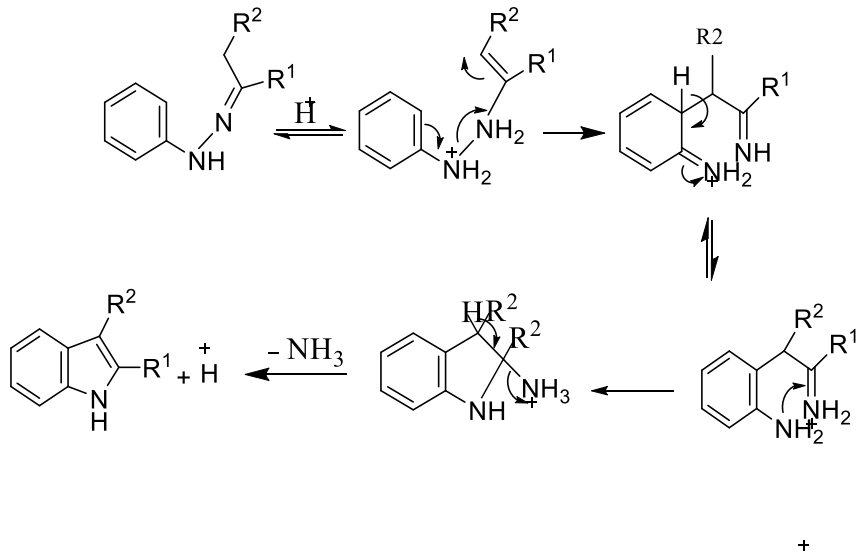
Indole derivatives can be synthesized by many pathways, the main methods are reported by (Kapti, 2013). Some of indole synthesis methods were illustrated below:

The Fischer-indole synthesis, first discovered by German chemist Hermann Emil Fischer in 1883, is the most widely used method among all other indole synthesis the basic principle of the Fisher indole circuit reaction is that, under acidic conditions, aryl hydrazonesate (C), which can be easily synthesized by ketone condensation (B) or an aldehyde with phenylhydrazine (A), converted to substituted indoles (D) with ammonia loss Scheme2. 2.



Scheme 2. 2: Fischer indole synthesis

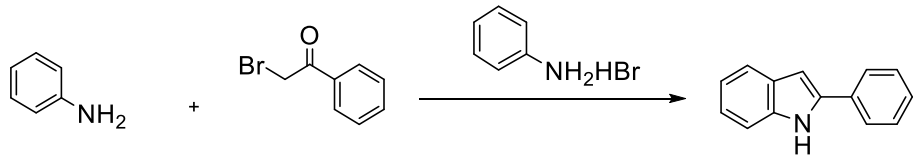
The mechanism includes the (3,3)-sigmatropic rearrangment of the ene-hydrazine isomers of aryl hydrazone, with the NN bond split and the CC bond formed . After aromatization, imine form converts into the intermediate, Then the results from the completion of cyclic aminoacetal rings. The aroma provides by loss of ammonia due to the substituted indoles Scheme 2. 3 [(25)].



Scheme 2. 3: Mechanism of Fischer Indole Synthesis

2. 1. 4. 2. Bischler Synthesis

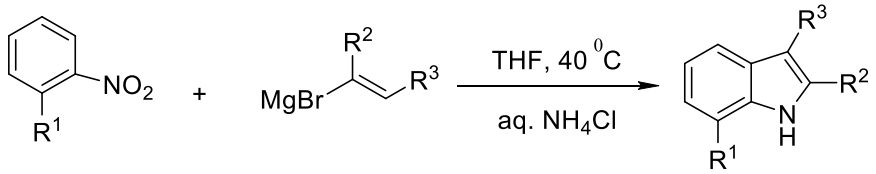
In 1892, Bischler et al, put forwarded a simple method affording a 2-aryl-indole froms an -bromo-acetophenone and excess aniline equation 2. 2.



Equation 2. 2: Synthesis of 2-aryl indole from -bromo acetophenone and excess aniline

2. 1. 4. 3. Bartoli indole synthesis

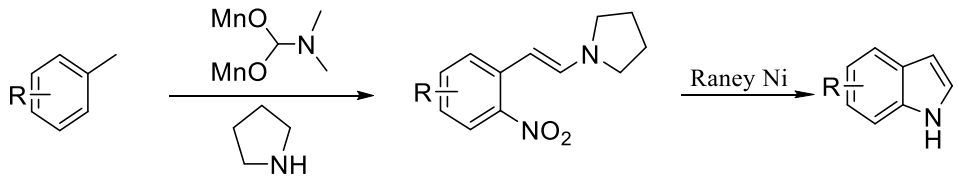
In Bartoli, andole, the indole synthesis of indoles substitution (13) are obtained by the reaction of *o*-substituted nitroarenes with Grignard vinyl reagents. In absence of *o*- substitution on nitro arene, reaction fails equation **2. 3**



Equation 2. 3: Bartoli indole synthesis

2. 1. 4. 4. Leimgruber-Batcho Indole Synthesis

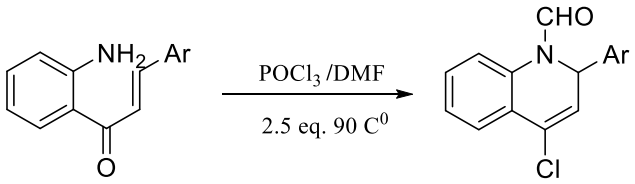
Leimgruber et al, investigated the synthesis of indole from *o*-nitrotoluenes. In the first step, enamine is generated using N,N-dimethyl formamide dimethyl acetal and pyrrolidine which upon reductive cyclisation afforded indole in the subsequent step Scheme 2. 4 [(26)].



Scheme 2. 4: Leimgruber–Batchosynthesis of indole

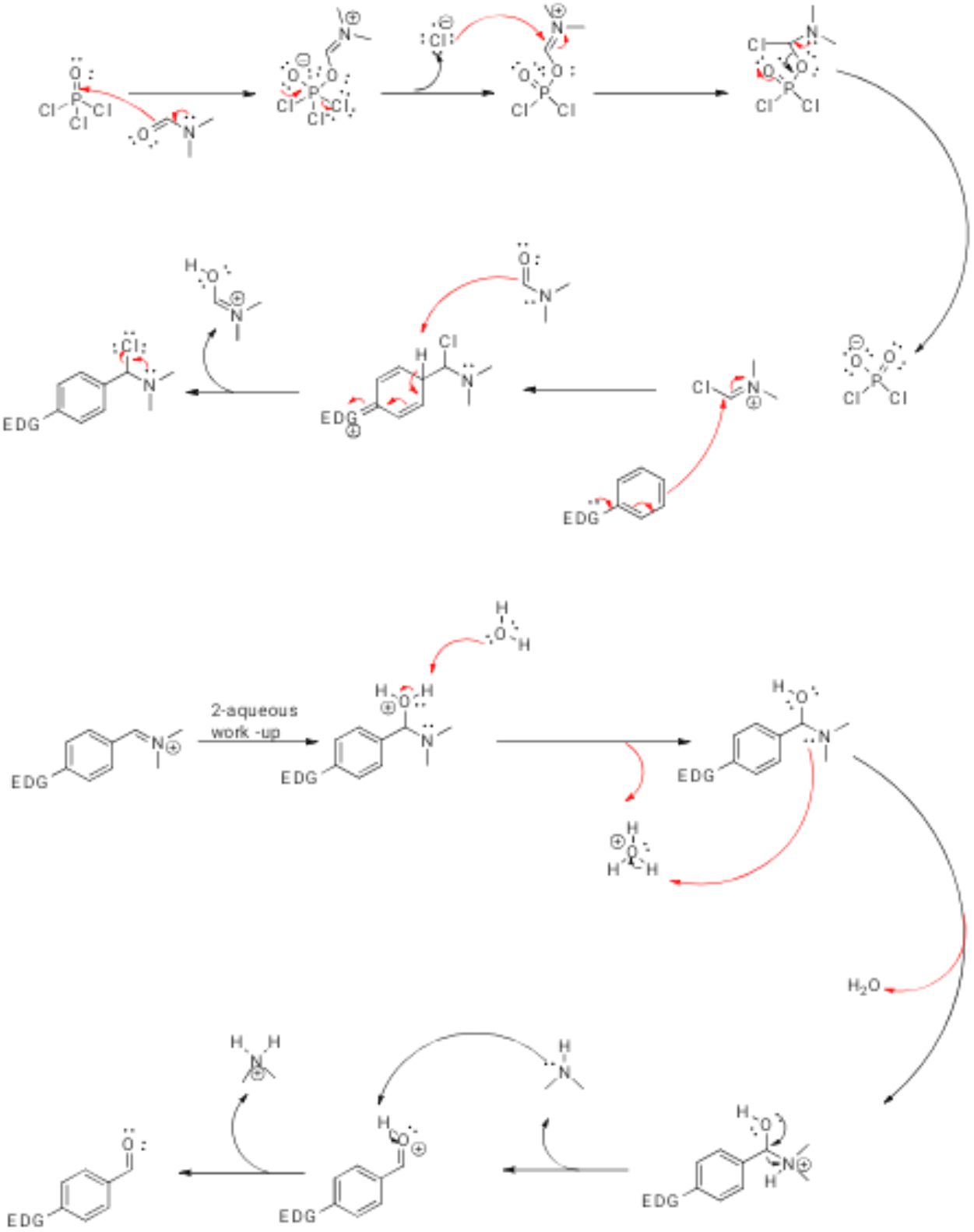
2. 2. Review on Vilsmeier Reaction:

Vilsmeier's reagent is not limited to the interaction of the aromatic formula alone. A varety of alkene derivatives, activated methyl and methylene groups demonstrate a reaction with Vilsmeier's reagent.
. In addition to the carbon core, some oxygen and nitrogen nuclei also react with vilsmeier's reagent (([27)]. Vilsmeier-Haack reaction procedure for seven derivatves of 2'-aminochalcones (Ar = phenyl, p-chlorophenyl, ptolyl, p-anesyl, o-chlorophnyl, o-tolyl, m-chlorophenyl). The reactions proceeded similarly to the observations reported providing the equation 2-aryl / hetryl-4-chloro-N-formyl-1,2-dihydroquinolnes. 2. 4 [(28)] .



Equation 2. 4: 2-aryl/hetryl-4-chloro-N-formyl-1,2-dihydroquinolines

Generally speaking, N, N-dimthylformamide (DMF), and POCl3 phosphorus oxychloride . It is used to generate the halomethyleniminium salt used in the synthesis of a large number of heterocyclic compounds [(29)]. The reaction of an N,N-disubstituted formamide, like di methylformamide (DMF) or N-methylformanilide, with acid chlorides, such as phosphoryl chloride or phosgene, leads to the formation of adducts. These adducts which are usually referred to as the Vilsmeier-Haack reagent findimportant applications in synthetic organic chemistry specially in the formylation of electron rich aromatic compounds or alkenes. In the present time it is well established that the reaction proceeds by the attack of the carbonyl oxygen of the amide to form the adduct at first which reacts further to give chloromethylene iminium salt scheme (1.5)**[(30)]**.



Scheme 2.5: Vilsmeier Haack mechanism

2. 3. Review on Schiff Base:

Schiff bases also called imines are characterized by the azomethine (-C= N-) group and are it is usually formed through condensation of an aldehyde or ketone with a primary amine (Graham and Fryhle, 2004). This condensation reaction between a carbonyl compound and an amine leads to the formaton of a Schiff base. It is an easy reaction due to good electrophilicity. **(31)**. The first researcher who reported Schiff bases is Hugo Schiff in 1864 (1). The common synthetic characteristic of these compounds is the azomethane group with the general formula R1R2C = N-R3, where R1 and R2 are alkyl, aryl, or alkyl cyclo groups or heterocyclic groups that can be substituted differently. R3 is any alkyl, aryl group but not hydrogen atom figure 2.3 .

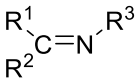
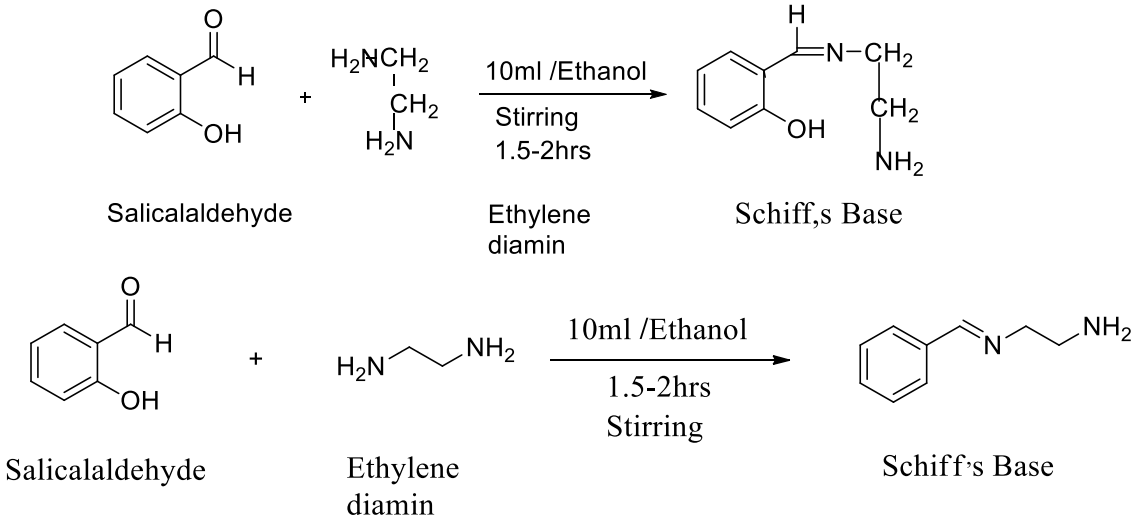


Figure 2.3: General structure of Schiff bases

Schiff's bases are in general deem excellent chelating agents especially when a functional group like –OH or –SH is in close proximity to the isomethene group to form a five or six-membered ring with the metal ion. **(32)** Schiff base may obtained by either aliphatic or aromatic aldehyde **(33)** Schiff's bases are generally double or triple-ligandsbonds capable of forming highly stable complexes with transition metals **(34)** . chelation tends to make the ligand act as a more potent and effective germicidal agent .**(35)** Schiff's bases have a multiple using; Some are the basic units in specific pigments, while others are used as liquid crystals. In organic synthesis, the reactions of Schiff's bases are useful in making the carbon-nitrogen bond equation **2. 5** (36).



Equation 2. 5: Synthetic pathway of Schiff bse

Azomethene or imine groups are found in many natural derivatives and unnatural compounds. The azomethene group presence in such compounds has been shown to be important for their biological activities 2.4[37]

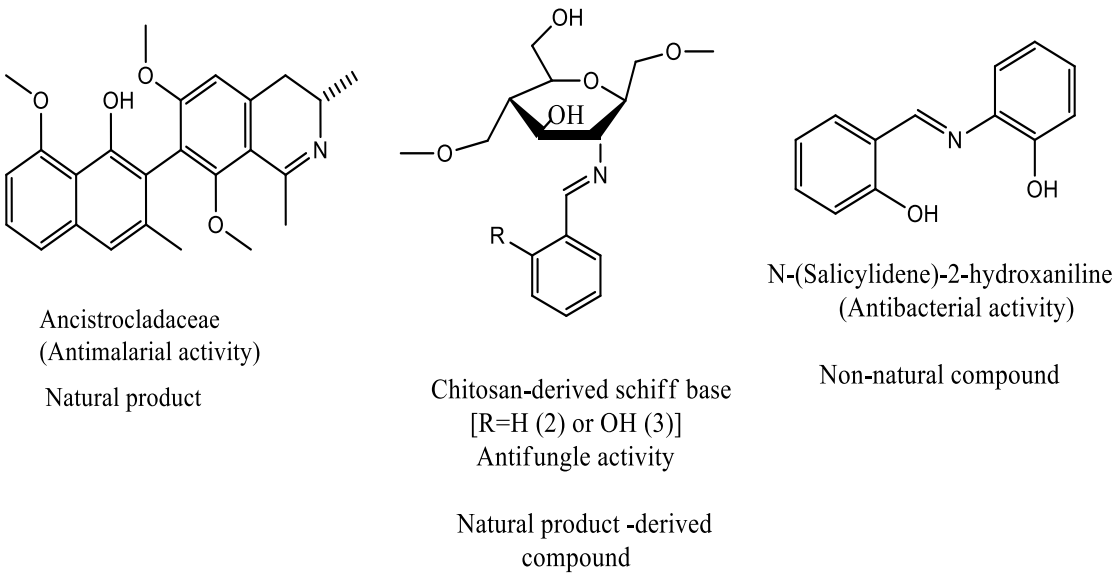


figure (2. 4): Example of bioactive Schiff bases

Imine complexes of 2-(2-ydroxybenzylidene) amino phenol as ligand figure 2.5 has active against mycobacterium tuberculosis as well as have a broad range of biological properties: antitumor, antiviral, antifungal, antibacterial [38].

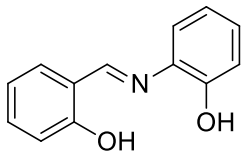
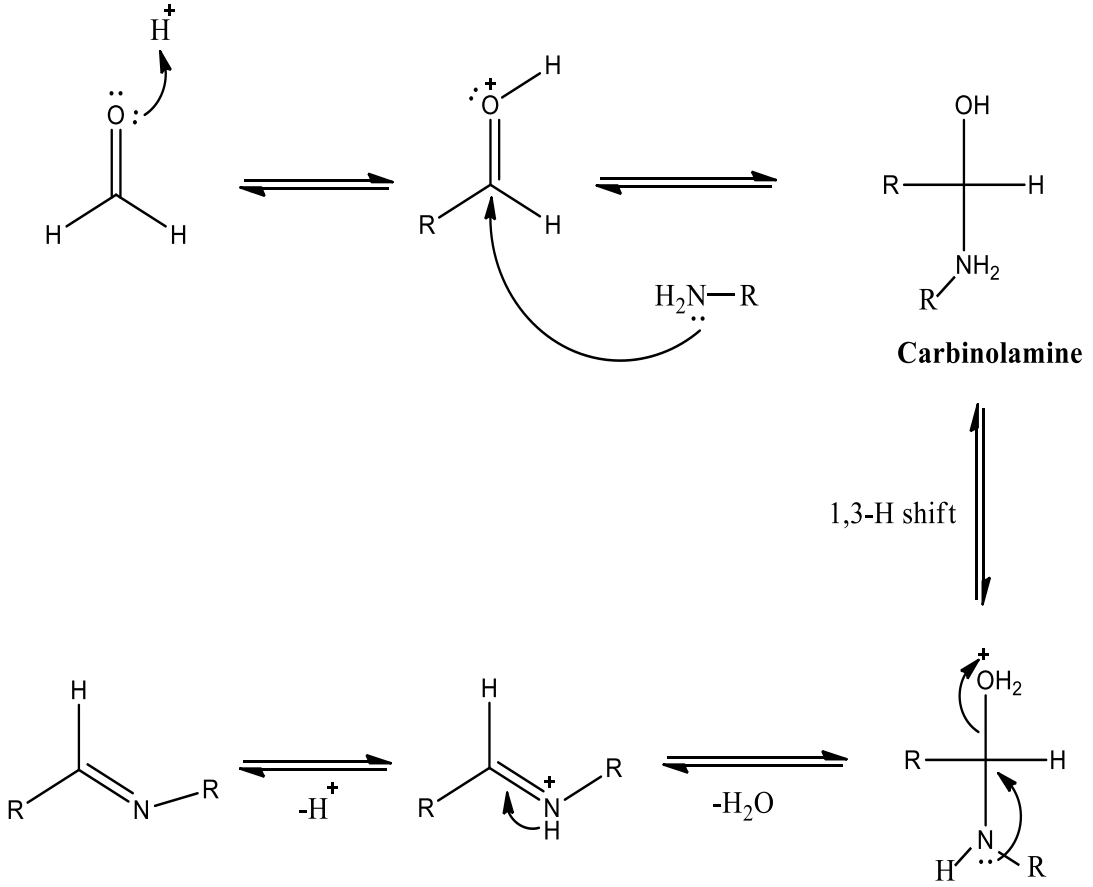


Figure 2. 5: 2- (2-hydroxybenzylidene) amino phenol as the example of bioactive Schiff base

Schiff's bases were found to have more inhibition efficiency than the carbonyl compounds and their constituent amines. Some authors have attributed the stronger inhibition efficiency largely to the existens of unoccupied p * -orbitals in Schiff base molecules, which enables the electron to be donated again from the transition metal d-orbitals thus stabilizing the current metal inhibitor bond.This is not possible with the component amines [39] . Schiff's bases belong to a widely used group of organic intermediates used in the manufacture of pharmaceutical or rubber additives. In organic synthesis, the amino protective group, different formulations occupy an important role in medicinal chemistry [40].

2. 3. 1. Mechanism of Schiff bases formation

He general concept of the Schiff base formation mechanism, as demonstrated in scheme (2.6) is the addition of nucleophilic to the carbonyl group. During formation of the Schiff base, the primary amine is the nucleophileIn the first part of the mechanism, a single pair of electrons, a nitrogen amine, attacks an aldehyde or a ketone to give an unstable addition compound called (carbenolamine). [41]



scheme (2. 6): Mechanism of Schiff base formation

General preparation of Schiff base

The Schiff base is commonly prepared via condensation reaction of aldehyde with amine in alcohol. The reaction is straight forward and usually can be conducted under open air condition. Condensation between aldehydes and amines was found in dissimilar reaction conditions and solvents Since the water is the by-product from the reaction, the formation of Schiff base is favored at the prsence of $MgSO_4$, which is a dehydrating agent for the reaction [(42).]

Green methods

Green methods in organic synthesis have several advantages: reduced pollution, low costs, simplicity in handling. Mechanical chemistry can be as simple as grinding two reactants in a mortar and mortar pestle or more complicated , as with the use of commercially available ball mills.

Some new azo Schiff bases have been previously reported in organic solvents . According to the facts mentioned above and in adopting the principles of green chemistry, we here mention two clean, simple and versatile methods for mono and bis azo Schiff in water or by grinding [43].

2. 4. Cancer

Abnormal and disorganized proliferation (growth) of cells, Stems from the cells of a particular organ the general name of a group of more than 100 diseases despite many types, it all starts because abnormal cells grow (proliferate). Uncontrolled cancer cells have the ability to make their own blood supply, break away from the original organ, and travel., Also it spreads to other parts of the body [44] In order for the cells in our bodies to have certain functions to perform normal cells that divide in an orderly manner, and die when they are shabby or damaged, and are replaced by new cells. In cancer, cells continue to grow and make new cells. They push off normal cells and this causes problems in the part of the body where the cancer started and it can spread to other parts of the body [45] There are several causes of cancer: family history, age, bacterial infection, viral infection, smoking, contact to radiation and chemicals, alcohol use and drinking, eating, touching or breathing harmful materials, these cancer causing are called ―carcinogens‖ and contact to these carcinogen does not mean you will get cancer but depends on how many times you are exposed to it [46]Cancer considered to be one of the major reasons of deaths in the 20st century and increasing occurrence in 21st century. This represents main world health problem. Almost 7.6 million deaths are usually caused by cancer which represents (13%) of all deaths)[47]

2. 4. 1. Liver Cancer

Hepatocellular carcinoma (HCC) is the most common malignancy tumor of the liver. and among the world's top five causes of cancer death. Almost all cases of chronic viral liver cancer associated with hepatitis B virus (HBV) or hepatitis C virus infection (Beasley, 1988; Hasan et al., 1990), But the molecular nature of this relationship is not well understood, and HCC treatment cucumberare still finite. Only surgical excision is considered "curative treatment" (Lynn et al. 1987), but 80% of patients have extensive Liver cancer at the time of diagnosis and are not nominee for surgical treatment.. Among patients with localized HCC who underwent surgery, 50% had a repetition (Okuda et al., 1984) [48]. Liver cancer is the sixth most propagation cancer worldwide, with 1,2 cancers accounting for 5.7% of all cancers. here is wide geographical variation in incidence with the majority of cases occurring in developing countries (82%, 366,000 newcondition were estimated in males in 2002, 147,000 in women) comparison to developed countries (74,000 new cases in men and 36,000 women). [49] Since 1980, HCC surgery Dramatically changed with the establishment of methods for early detection of small HCC, and many of these cancers are now surgically treated. Among the various surgical techniques, intraoperative ultrasound (IOUS), which was first introduced to liver surgery by Makuuchi et al. New surgical procedures are made possible using IOUS guidelines, and vaso-occlusive techniques are now routinely implementation for patients with cirrhosis **[50]** . The metabolism of the drug in the liver can be divided tome two stages (Williams, 1971). The first stage is charactrized by oxidative, reductive and hydrolytic pathways that add a functional group (for examples, OH, SH, or NH_2) to the substrate. In the second stage, the recently introduced functional group was amendment into O- and N-glucuronides, sulfate esters, various carboxyamides, and glutathioneil nodes, all with

increasing polarity relative to unpaired molecules (Parkinson, 1996). This two-step transformtion should make the substrates more soluble in water, so that they can be excreted more easily, it should lead to detoxification.. However, many promotagens are inactive as mutagen prior to this biological transformation.

2. 4. 2. Esophageal cancer

Esophageal cancer (Esc), including squamous cell carcinoma (SCC) and adenocarcinoma, is a serious malignancy with regard to diagnosis and its result fatal in the vast majority of cases.. Esophageal cancerI Inffects more than 450,000 people worldwide and the incidence is fast increasing. presently, EsC is the eighth most common type of cancer in the world insmuch to its extremely aggressive nature and poor permanence rate.. EsC shows an epidemiological pattern distinct from all other types of cancer [(51)] · Cancers that emerge from the esophagus, including the gastrosophageal junction, are relatively uncommon in the United States, with 13,900 new condition and 13,000 expected deaths in 2003. he lifetime risk of developing this cancer is 0.8 percent about men and 0.3 percent about women. The danger increases with age, and the average age at diagnosis is 67 years. Esophageal cancer is the seventh leading cause of death from cancer with in American men, mostly black men, who have a higher incidence of the disease (13 cases per 100,000 people) compared to men in other racial or ethnic groups.. Esophageal cancer is the sixth main reason of cancer death worldwide[(52).]

CHAPTER THREE

EXPERIMENTAL PART

3. EXPERIMENTAL SECTION

3. 1. Chemistry part:

3. 1. 1. Materials

Table (**3. 1**). Chemicals and solvents used in the chemistry part

Materials and chemicals	Molecular formula	Company
4-Florophenylhydrazinhydrochloride	$C_6H_8FN_2$	Sigma-Aldrich
Dimethyl formamide	C_3H_7NO	Romil
Methyl isopropyl ketone	$C_5H_{10}O$	Merck
Phosphoryl chloride	$POCl_3$	Merck
4-Chloroaniline	C_6H_6ClN	BDH
2,4-dichloroaniline	$C_6H_5Cl_2N$	Fulka
2,3- dimethyl aniline	$C_8H_{11}N$	Merck
Sodium hydroxide	NaOH	Thomas Baker
Sodium sulfate	Na_2SO_4	Loba chemie
4-Hydroxy aniline	C_6H_9NO	Merck
Ethanol	CH_3CH_2OH	Scharlaw
Ethyl acetate	$C_4H_8O_2$	Himedia
Glacial acetic acid	CH_3COOH	BDH
Hexane	C_6H_{14}	Sigma Aldrich
Methyl isobutyl ketone	$C_5H_{10}O$	Aldrich
4-Methoxy aniline	C_7H_9NO	Hopkin and Williams
2-Methyl aniline	C_7H_9N	Merck
2-Hydroxy aniline	C_6H_9NO	Merck
3-Hydroxy aniline	C_6H_9NO	Merck
Phosphoryl chloride	$POCl_3$	Merck
Sulfuric acid	H_2S0_4	Scharlaw

3. 1. 2. Instruments

3. 1. 2. 1. Fourier Transform Infrared Spectroscope (FTIR):

IR spectra, registered on a Perkin-Elmer.Spectrum version 10.02 by using a disk of KBr for solid material in the Department of Chemistry, College of Science, University of Diyala.

3. 1. 2. 2. Nuclear Magnetic Resonance Spectrometer (NMR):

^{1}H and APT ^{13}C NMR spectra offered to a Bruker 400 MHz Spectroscope in College of science University of Science and technology, Irbid city Jordan.

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

The purification of the new synthesized compounds was obtained by using silica gel sheets and the spots of the starting materials and the products were detected by using a fluorescence analysis cabinet model CM-10. In the Department of Chemistry, College of Science, University of Diyala.

3. 1. 2. 4. Melting Point:

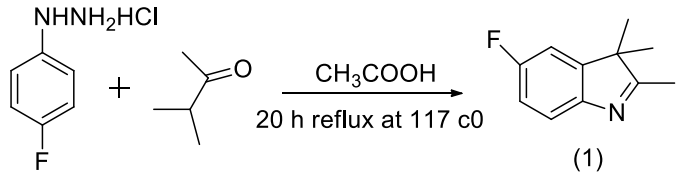
The melting points of the compounds prepared in with open capillary tubes were determined using the Stuart SMP10 UK Melting Point Instrument in the Department of Chemistry, College of Science, University of Diyala.

3. 1. 2. 5. Rotary Evaporator:

The solvents were evaporated by using Heldove apparatus, HeiVAP, Germany in the Department of Chemistry, College of Science, University of Diyala.

3. 1. 3. Synthetic methods:

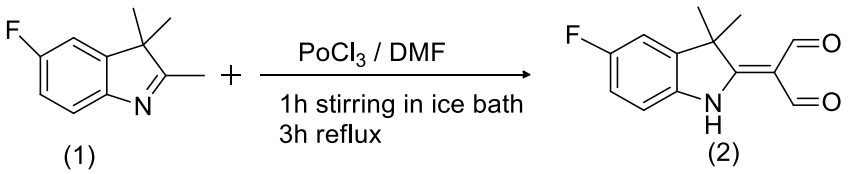
3. 1. 3. 1. Synthesis of 5-Fluror -2,3,3-trimethyl-3H-indole (1) as elucidated in Equation (3. 1)



Equation (3. 1): the synthetic pathway of 5-Fluoro-2,3,3-trimethyl-3H-indole (1)

A mixture of (3g, 17 mmol) of (4-Fluoro-phenylhydrazin hydrochloride and (1.76 g, 25.5 mmol) of isopropyl methyl ketone as dissolved with in (40 ml) of glacial acetic acid and the mixture was refluxed in oil path at 117 ºC for 20h. Then the product was cooled by adding it in the icy distilled water, and neutralized with aqueous solution of 25 % NaOH, then it was extract with ethyl acetate and water (3×25ml). The organic layer was dry over Na_2SO_4 and the solvent was vaporize. The product is red oily of 5-Fluoro-2,3,3-trimethyl-3H-indole (1)[(53)]. Yield: (2.57g, 87%) ^{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).

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

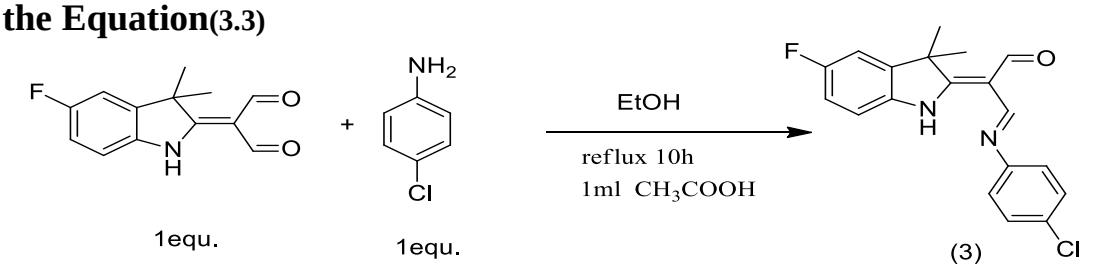


Equation

(3. 2): the synthetic pathway of 2-(5-Fluror -3,3-dimethyl-1,3-dihydro-indol-2- ylidene)-malonaldehyde (2)

8.6ml of N,N-dimethyl formamide (DMF)it was cooled with in an ice bath then added dropwise from (4.4ml, 48 mmol) of Phosphoryl chloride ($POCl_3$) with stirring for awhile 10 minutes at 5°C, then a solution (3g, 16mmol) of 5-Fluoro-2,3,3-trimethyl-3H-indole (**1**) in DMF (8.6ml) was added drop by drop for 10 minutes at 5°C , the reaction mixture was stirred in ice path for 1h 5°C. Then reflux for 3h at 85-90ºC. The resulting solution was added to icy distill water and neutralized with aqueous solution of 25 % NaOH, the brown precipitate is formed filtrated off, wash through hot distillation water and dry up in oven at 78 ºC 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 was determined by using TLC (4:1) hexane: ethyl acetate as an particular with pre-coated silica gel, which gave one spot. Yield: (2.8g, 71%), m.p.178-180 °C. FT-IR data (cm1): 3201, 3042, 2983, 2854, 1657.4, 1616.3,1534, 1469.4, 1372, 1275.5, 1178, 814. 1H-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).

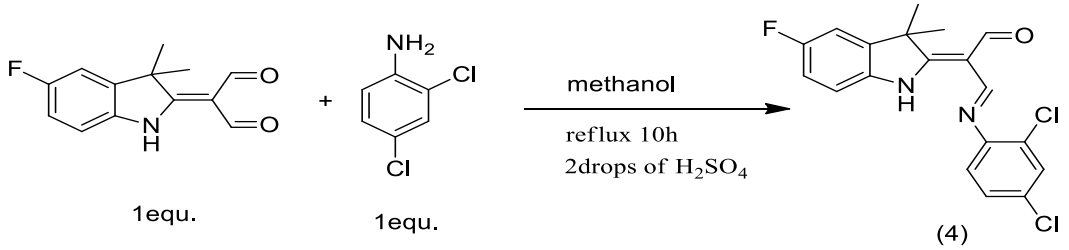
3. 1. 3. 3. Synthesis of 2-(5-Fluoro -3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-chloro-phenylimino)-propionaldehyde (3) as shown in the Equation(3.3)



Equation (3. 3): The synthetic pathway of 2-(5- Fluoro -3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-chloro-phenylimino)-propionaldehyde (3)

A solution of (0.25g, 1mmol) of 2-(4-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde as dissolved in ethanol 10ml and (0.136g, 1mmol) of 4-Chloro-aniline was dissolved in ethanol 5ml and then added glacial acetic acid 1ml to the solution. The mixture was refluxed with in water bath at 78ºC during 10h. A solvent was reduced to one quarter; yellow precipitate is formed, filtrated off, washed with ethanol and dry up in an oven with a 78 ºC. The purity of this compound was determined by using TLC (4:2) hexane: ethyl acetate with pre-coated silica gel, which gave one spot. Yield (0.25g 75.9%), m.p.189-190^{0}C. IR data in (cm^{-1}): 3069 υ(CH aromatic), 2967 υ(CH aliphatic), 2725 υ(CHaldehyde), 1663 υ(CH=O), 1652 υ(CHN), 1579 υ(C=C), 1396 υ(CH_3), 1267 υ(C-N), 813 υ(C-Cl), 773 υ(C-H bending), $_1$H NMR (400 MHz, DMSO, δ in ppm): δ = 13.96 (s, 1H, N*H*), 9.38 (s, 1H, *H*CO), 8.62 (s, 1H, *H*CN), 8.59-7.08 (7H, Ar-*H*), and 2.49 (s, 3H, CH_3), 1.56 (s, 3H, CH_3). APT $_{13}$C NMR shown signals for CH and CH_3 appear in the negative part (below fundamental streak of the spectrum) 188.25, 156.52, 129.45, 119.44, 113.93, 109.08, 108.41 and 21.51. Whereas quaternary carbons, CH_2 carbons and carbons deuterated DMSO solvent were observed at positive side (above fundamental line of the spectrum) 182.73, 161.67, 159.28, 147.53, 147.44, 138.70, 129.00, 108.83, 54.29 and 38.71.

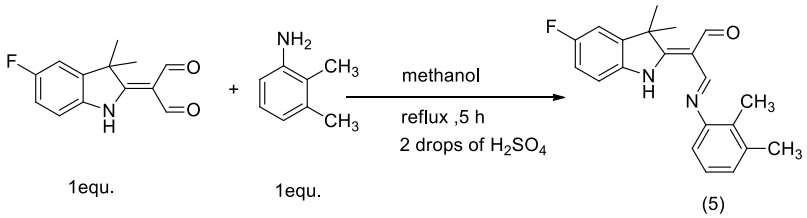
3. 1. 3. 4. Synthesis of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dichloro-phenylimino)-propionaldehyde (4) as elucidated in Equation (3. 4)



Equation (3. 4): Synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dichloro-phenylimino)-propionaldehyde (4)

A solution of (0.25g, 1mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde as dissolved in methanol 10ml and (0.173g, 1mmol) of 1,4-dichloro aniline was dissolved in methanol 5 mL and then added 3 drops from 20% H2SO4 to the solution. The mixture was refluxed with in water bath at 65 ºC during 5h. Then stirring within room temperature for 3h. Yellow precipitate is formed, filtrated off, washed with methanol and dry up in oven at 65 ºC. The purity of this compound was determined by using TLC (4:2) hexane: ethyl acetate with pre-coated silica gel, which gave one spot. Yield (0,3g 91%), m.p.189-190^{0}C. IR data in (cm^{-1}): 3055 υ (CH aromatic), 2923 υ (CH aliphatic), 2725 υ (CH aldehyde), 1663υ(CH=O), 1626 υ(CHN), 1579 υ(C=C), 1396 υ(CH_3), 1286 υ(C-N), 813 υ(C-Cl) and 766 υ(C-H bending), $_1$HNMR(400MHz, DMSO, δppm): 13.96(s,1H,N*H*), 9.38(s,1H,*H*CO), 8.63(s,1H,*H*CN), 8.60-7.08 (6H,Ar-*H*), and 1.57(s,6H,2x CH_3);. APT $_{13}$C NMR shown signals for CH and CH_3 appear in the negative part (below fundamental streak of the spectrum) 188.20, 156.49, 129.45, 119.72, 119.41, 113.92, 109.07, 108.42 and 21.56. Whereas quaternary carbons, CH_2 carbons and carbons deuterated DMSO solvent were observed at positive side (above fundamental line of the spectrum) 182.72, 161.69, 159.29, 147.53, 146.34, 138.77, 129.01, 108.83, 54.28 and 40.21.

3. 1. 3. 5. Synthesis of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,3-dimethyl-phenylimino)-propionaldehyde (5) ashown in the Equation (3. 5)

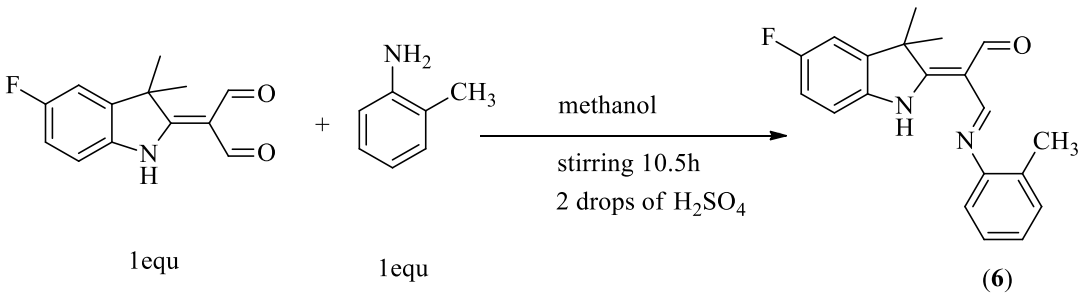


Equation (3. 5): the synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-2,3-dimethyl-phenylimino -propionaldehyde (5)

A solution of (0.25g, 1mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde as dissolved in methanol 10ml and (0.130g, 1mmol) of 2,3-dimethyl aniline was dissolved in methanol 5ml and then added 2 drops of 20% H_2SO_4 to the solution. The mixture was stirring with in water bath at 65 ºC during 5h. A solvent was reduced to one quarter, orange precipitate is formed, filtrated off, washed with methanol and dry up in oven at 65 ºC. The purity of this compound was determined by using TLC (4:1) hexane: ethyl acetate with pre-coated silica gel, which gave one spot. Yield (0,29g 80%), m.p.287-288 ^{0}C. IR data in (cm^{-1}): 3069 υ(CH aromatic), 2960 υ(CH aliphatic), 2769 υ(CH aldehyde), 1633 υ(CH=O), 1608 υ(CHN), 1582 υ(C=C), 1396 υ(CH_3), 1267 υ(C-N), and 769 υ(C-H bending), $_1$H NMR (400 MHz, DMSO, δ *in* ppm): 14.17 (1H,s,N*H*), δ = 9.40 (s, 1H, *H*CO), 8.71 (1H, s, *H*CN), 7.73-7.12(7H Ar-*H*), and 1.58 (6H, s, 2x CH_3).. APT $_{13}$CNMR shown signals for CH and CH_3 appear in the negative part (below fundamental streak of the spectrum) 128.15, 124.89, 120.18, 113.82, 21.42, 17.66, and 17.04. Whereas quaternary carbons, CH_2 carbons and carbons deuterated DMSO solvent were observed at positive side (above fundamental line of the

spectrum) 136.70, 135.28, 134.93, 128.15, 124.66, 39.37, 40.00, and 38.75ppm.

3. 1. 3. 6. Synthesis of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2-methyl anilin)-propionaldehyde (6) as demonstrated in Equation (3. 6)

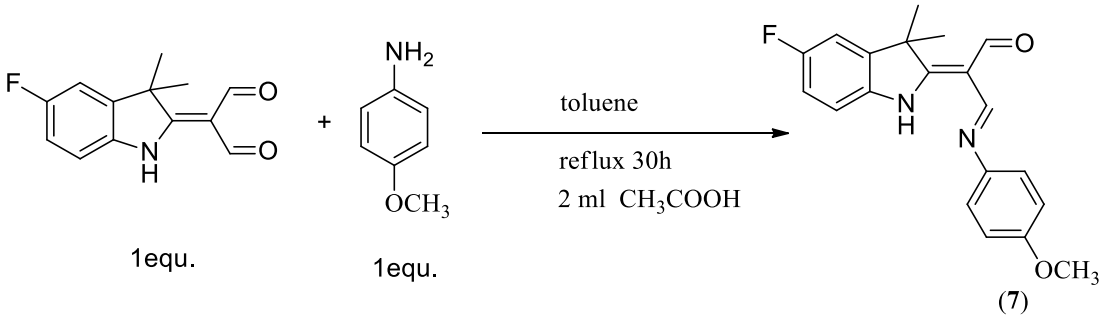


Equation (3. 6): the synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-2-methyl anilin -propionaldehyde (6)

A solution of (0.25g, 1mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde as dissolved in methanol 30ml and (0.114g, 1mmol) of 2-methyl anilin was dissolved in methanol 5ml and then added 2 drops of 20% H2SO4 to the solution. The mixture was stirring with in water bath at 65 ºC during 5h. A solvent was reduced to one quarter; orange precipitate isformed, filtrated off, washed with methanol and dry up in oven at 65 ºC **(54)**. The purity of this compound was determined by using TLC (4:1) hexane: ethyl acetate with pre-coated silica gel, which gave one spot. Yield (0,29g 84%), m.p.130-131ºC. IR data in (cm^{-1}): 3062 υ(CH aromatic), 2967 υ(CH aliphatic), 2703 υ(CH aldehyde), 1656 υ(CH=O), 1630 υ(CHN), 1586 υ(C=C), 1374 υ(CH_3), 1271 υ(C-N), and 733 υ(C-H bending), $_1$HNMR(400MHz, DMSO, δ in ppm): δ = 14.15 (s,1H,N*H*), 9.37 (s,1H,*H*CO), 8.62(s,1H,*H*CN), 8.59-7.03(6H,Ar-*H*), 2.49 and 2.26(s, *6H*, 2x CH_3) and 1.58(s,6H, 2x CH_3). APT $_{13}$CNMR shown signals for CH and CH_3 appear in the negative part (below fundamental streak of the spectrum) 188.08, 156.49,126.45, 118.81, 113.75, 109.20 and108.96 whereas quaternary carbons, CH_2

carbons and carbons deuterated DMSO solvent were observed at positive side (above fundamental line of the spectrum) 183.29, 161.67, 159.27, 147.74, 137.91, 125.88,108.96,54.49 and 40.03.

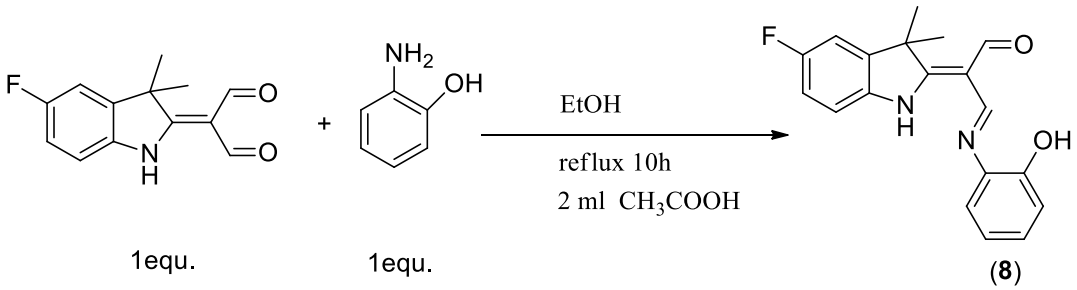
3. 1. 3. 7. Synthesis of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-methoxy-phenylimino)-propionaldehyde (7) as demonstrated in Equation (3. 7)



Equation (3. 7): The synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-methoxy-phenylimino)-propionaldehyde (7)

A solution of (0.25g, 1mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde as dissolved in toluene 10ml and (0.098g, 1mmol) of 4-methoxy aniline was dissolved in toluene 30ml and then added glacial acetic acid 2ml to the solution. The mixture was refluxed in water a bath at 111ºC during 30h. Solvent was reduced to one quarter; yellow precipitate is formed, filtrated off, washed with toluene and dry up in oven at 111ºC. The purity of compound was determined by using TLC (4:2) hexane : ethyl acetate with pre-coated silica gel, which gave one spot. Yield (0,3g 82.8%), m.p.145-146 ^{0}C. IR data in (cm^{-1}): 3113 (CH aromatic) 2974υ (CH aliphatic), 2747υ (CH aldehyde), 1648υ (CH=O), 1626 υ(CHN), 1597 υ(C=C), 1366 υ(CH_3), 1271 υ(C-N), and 733 υ(C-H bending). $_1$H NMR (400 MHz, DMSO, δ in ppm): δ = 13.23 (s, 1H, N*H*), 9.80s, 1H, *H*CO), 7. 65 (s, 1H, *H*CN), 7.56-6.60 (7H, Ar-*H*), 4.32 (s, 3H, OCH_3), and 1.63 (6H, s, 2x CH_3.

3. 1. 3. 8. Synthesis of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2-hydroxy-phenylimino)-propionaldehyde (8) as shown in the Equation (3. 8)

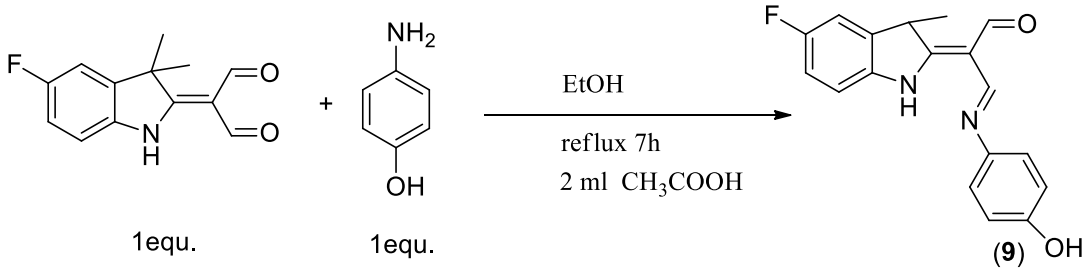


Equation (3. 8): The synthetic pathway of: 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2-hydroxy-phenylimino)-propionaldehyde (8)

A solution of (0.25g, 1mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 10ml with heating and (0.167g, 1mmol) of 2-hydroxy aniline was dissolved in ethanol 10ml and then added glacial acetic acid 2ml to the solution. The mixture was refluxed with in water bath at 78ºC during 10h. A solvent was reduced to one quarter; brown precipitate is formed, filtrated off, washed with ethanol and dry up in oven at 78ºC. The purity of this compound was determined by using TLC(4:1) hexane : ethyl acetate with, which gave one spot. Yield (0.3g, 86%), m.p 210-211 °C. IR data in (cm-1): 3633 υ(O-H), 3062 (CH aromatic) 2982 υ(CH aliphatic), 2703υ (CH aldehyde), 1619 υ(CHN), 1506 υ(C=C), , 1348 υ(CH_3), 1228 υ(C-N), and 751 υ(C-H bending). ^{1}H NMR (400 MHz, DMSO, δ in ppm): δ = 14.04 (s, 1H, N*H*), 9.80 (s, 1H, *H*CO), 8.69 (s, 1H, *H*C=N), 7.87-6.96 (7H, Ar-*H*), and 1.63 (6H, s, 2x CH_3); APT 13CNMR shown signals for CH and CH_3 appear in the negative part (below fundamental streak of the spectrum) 187.95, 147.80,125.38, 119.57, 118.73, 115.38,113.80,109.03 and 21.22 whereas quaternary carbons, CH_2 carbons and carbons deuterated DMSO solvent were observed at positive side (above fundamental line of the spectrum) 182.95, 161.41, 159.02, 147.72,

127.06, ,108.78,54.41,39.87 and 40.03

3. 1. 3. 9. Synthesis of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-hydroxy-phenylimino)-propionaldehyde (9) as shown in the Equation (3. 9)

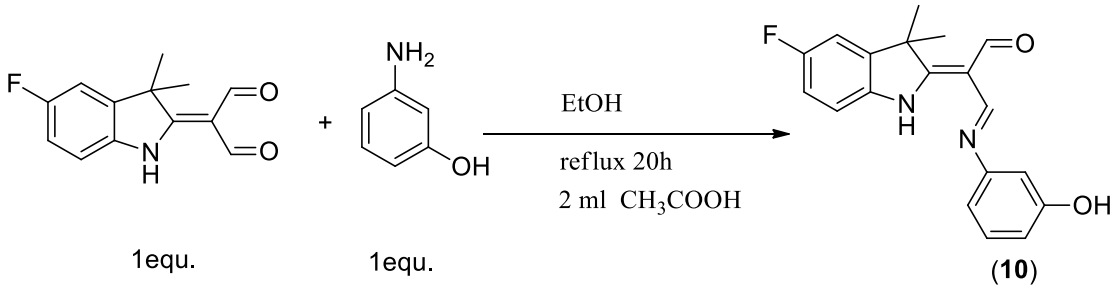


Equation (3. 9): The synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro indol-2-ylidene)-3-(4-hydroxy-phenylimino)-propionaldehyde (9)

A solution of (0.25g, 1mmol) of 2-(5-Fluror-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde as dissolved in ethanol 10ml with heating and (0.167g, 1mmol) of 4-hydroxy-aniline was dissolved in ethanol 5ml and then added glacial acetic acid 2ml to the solution. The mixture was refluxed in a water bath at 78ºC during7h. A solvent was reduced to one quarter; dark brown precipitate is formed, filtrated off, washed with water and dry up in oven at 78 ºC. The purity of this compound was determined by using TLC (3:1) hexane: ethyl acetate, which gave one spot. Yield (0,28g 80,6%), m.p 209-210°C. IR data in (cm-1): 3524 υ(O-H), 2967 (CH aromatic) 2755 υ(CH aliphatic), 1641 υ(C=O), 1601 υ(CH=N), 1557 υ(C=C), 1374 υ(CH3), 1282 υ(C-N), and 714 υ(C-H bending). 1H NMR (400 MHz, DMSO, δ in ppm): δ = 13.98 (s, 1H, N*H*),9.39 (s, 1H, *H*C=O), 8.73 (s, 1H, *H*C=N), 7.59-6.88 (7H, Ar-*H*), and 1.62 (6H, s, 2x CH_3); APT 13C NMR shown signals for CH and CH3 appear in the negative part (below fundamental streak of the spectrum) 120.18, 118.61, 116.11, 113.90, 109.00,25.24 and 21.00 whereas quaternary carbons, CH_2 carbons and carbons deuterated DMSO solvent were observed at positive side (above fundamental line of the

spectrum) 182.44, 172.03, 160.90, 158.52, 155.02, 101.73,52,59 and 40.05 .

3. 1. 3. 10. Synthesis of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2ylidene)-3-(3-hydroxy-phenylimino)-propionaldehyde (10) as shown in the Equation (3. 10)



Equation (3. 10): The synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro indol-2-ylidene)-3-(3-hydroxy-phenylimino)-propionaldehyde (10)

A solution of (0.25g, 1mmol) of 2-(5-Fluoro-3,3-dimethyl-1,3- dihydro-indol-2-ylidene)-malonaldehyde as dissolved in ethanol 10ml and (0.116g, 1.2mmol) of 3-hydroxy aniline was dissolved in ethanol 10ml and then added glacial acetic acid 2ml to the solution. The mixture was refluxed in a water bath at 78ºC during 20h. A solvent was reduced to one quarter; dark brown precipitate is formed, filtrated off, washed with water and dry up in oven in 78 ºC. The purity of this compound was determined by using TLC (3:1) hexane: ethyl acetate, which gave one spot. Yield (0,32g 92%), m.p 242-243°C. IR data in (cm-1): 3049 υ(N-H), 3049 (CH aromatic) 2974 υ(CH aliphatic), 2718 υ(CH aldehyde), 1625 υ(C=O), 1601 υ(CH=N), 1524M υ(C=C), 1410 υ(C-C), 1337 υ(CH_3), 1267 υ(C-N), , and 751 υ(C-H bending). ^{1}H NMR (400 MHz, DMSO, δ in ppm): δ = 9.67 (s, 1H, *H*C=O), 8.95 (s, 1H, *H*C=N), 8.16-7.09 (7H, Ar-*H*), and 1.67 (6H, s, 2x CH_3.

Table (3. 2): Physical properties of the synthesized compounds (1-10)

Comp. No.	Molecular formula	Molecular weight	Percentage Yield	Melting Point ºC	Rf
1	$C_{11}H_{12}FN$	177.22	87%	-	-
2	$C_{13}H_{12}FNO_2$	233.24	71%	178-180	0.46
3	$C_{19}H_{16}ClFN_2O$	342.79	75.9%	189-190	0.47
4	$C_{19}H_{15}Cl_2FNO$	377.24	91%	189-190	0.44
5	$C_{21}H_{21}FN_2O$	336.41	80%	130-131	0.48
6	$C_{20}H_{19}FN_2O$	322.38	84%	287-288	0.31
7	$C_{20}H_{19}FN_2O_2$	338.38	82.8%	145-146	0.46
8	$C_{19}H_{17}FN_2O_2$	324.35	86%	210-211	0.45
9	$C_{19}H_{17}FN_2O_2$	324.35	80.6%	209-210	0.37
10	$C_{19}H_{17}FN_2O_2$	324.35	92%	242-243	0.49

3. 2. Biological part:

3. 2. 1. Materials

All chemicals used in the biological part were obtained from different company suppliers as registered in table (3. 3).

Table (3. 3): Biological and Chemical Materials are used in the biological part.

Materials and chemicals	Company	Manufacturer
RPMI-1640	Sigma	U.S.A
Trypsin\Versine	Sigma	U.S.A
Ascorbic acid	Sigma	U.S.A
MTT Dye Sigma Aldrich	Sluka	Germany
Crystal violate	BDH	England
DPPH	Sigma	U.S.A
DMSO	Sigma	U.S.A
Fetal Bovine Serum	Sigma	U.S.A
Giemsa stain	Thomas Baker	India

3. 2. 2. Instruments:-

The instruments used on this study are found in Iraqi Center for Cancer and Medical Genetics Research (ICCMGR) / University of Mustansiriya, with their Manufacturers are listed in table (**3. 4**).

Table (3. 4) : Materials Equipment's and Apparatuses used in the biological part.

Equipment'	Company	Manufacturer
96 – well Microtiter Plate	SantaCruz	U.S.A
ELISA	Quik Fit	Germany
Micro Centrifuge	Hermle	Germany
Incubator	Memert	Germany
CO2 Incubator	Gallenkamp	England
Shaker Incubator	Selecta	Spain
Water Bath	Gallenkamp	England
Oven	Memaret	Germany

Plastic Flasic For Tissue Culture	Falcon	U.S.A
Laminar Flow Hood	K & K	Korea
Vortex	Griffin	England
Micropipette	Volac	England
Deep Freezer	The Electro Cotporation	U.S.A
Inverted Microscope	Oltmpus	Japan
pH-Meter	Radiometer	Denmark
Autoclave	Gallenkamp	U.K
Sensitive Balance	Sartorius	Germany
Filter unit 0.22 µm	Millipore	Spain

3. 2. 3. Preparation methods

Media and solutions for tissue culture were prepared according to the method(Freshney, 2012) and as explained in the following paragraphs mentioned:

3. 2. 3. 1. Antibiotics

Penicillin, Benzyl Pencillin

The contents of a package containing one million international units were dissolved in (5) ml of sterile distilled water, and a storage solution of (200,000) IU / ml was prepared, kept at a temperature of (-20) ° C.

Streptomycin

The contents of a package containing one gram were dissolved in (5) ml of sterile distilled water, and a storage solution of (200,000)

micrograms / ml was prepared at a temperature of (-20) ° C.

3. 2. 3. 2. Phosphate buffered saline (PBS) (pH 7.2)

NaCl	8 gm
KCl	0.2 gm
Na_2HPO_4	0.15
KH_2PO_4	0.2

The materials were dissolved in (1000) ml of distilled water and sterilized by autoclaving at a temperature of (121) C for 15 minutes, then it was kept at a temperature of (4) Celsius.

3. 2. 3. 3. Trypsin solution

Dissolve one gram of trypsin powder in (100) mL of PBS solution, sterilize with a filter (0.23 □m) and keep at (4) c.

3. 2. 3. 4. Versine solution

It was prepared by dissolving one gram of (EDTA) powder in (100) ml of distilled water and sterilizing it at a temperature of (121) for (10) minutes and storing it at a temperature of (4) C.

3. 2. 3. 5.Trypsin-Versine solution

Prepare by mixing (20) ml of trypsin solution, (10) ml of alfresin solution, and (370) ml of (PBS) solution before use in aseptic conditions and kept at (4) C.

3. 2. 3. 6. Hanks balanced salt solution (HBSS)

10X off-the-shelf solution from Flow laboratories, Irvine, Scotland.

3. 2. 3. 7. Trypan blue stain solution

One gram of dye was dissolved in (100) ml of Hanks' solution (HBSS), then filtered using (Whatman No. 1) filter paper and kept at a temperature of (4) C, and upon use it was diluted simultaneously with a

ratio of (10: 1) with the solution Himself.

3. 2. 3. 8. Crystal violet stain solution

was prepared by dissolving five grams of dye powder in (200) ml of methyl alcohol absolute and filtered using (Whatman No. 1) filter paper and then adding (50) ml of formalin at a concentration of (40%) and completed the volume To (1000) ml with distilled water and keep in a sterile vial at room temperature.

RPMI-1640 median (Roswell park memorial institute)

This median has been used in the cultivation and growth of cancer cell lines and is called Complete Growth Media.

RPMI-1640 with hepes buffer, L-glutamin	10.4 gm
NaHCO3 (4.4%)	15ml
Fetal Calf serum (% 10)	100ml
Benzyl penicillin	0.5ml
Streptomycin	0.5ml

Complete to a volume of (1) liter by adding distilled water and adjust the pH to (pH 7.2), then sterilize using membrane filters (0.22 □m) and distribute in sterile containers and keep at a temperature
(4) M until use.

3. 2. 3. 9. DMSO solution

Prepare by mixing 0.2 ml of DMSO with 100 ml of distilled water, then sterilize using a perforated filter with a diameter of 0.22 microns, and keep at 4 C until use. Use to dissolve the crude alkaline extract.

using the crystal violate stain the cytotoxic assay was measured (Freshney, 2012). at brief, the extract was dissolved at DMSO then mixed by serum free media (SFM) and (15.1, 31.2, 62.5, 125, 250, 400 and 500) µg\ml concentrations, seeded in 96-well microplate than planted cells were and incubated for 24 hours at 37˚C, then changed old media was alteration with new media SFM containing the serial concentrations of each extract, and then incubated plate at 37˚C for 24 hours containining 5%CO2 of all cell lines and moisturizer incubator at 37˚C. After finshing the exposur time were completed, the media was eluted and the wells treated with 100 µl\well of crystal violate dye and incubated the plate for 20 min, at 37˚C, the wells were washed with phosphate-buffered saline (PBS), and the plates left for 15 min in room temperature and then the absorbenc (O.D.) of wells measured by ELISA reader at 492 nm wave length [(55)].

CHAPTER FOUR

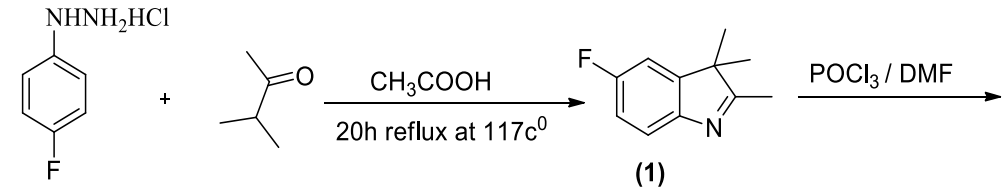
RESULT AND DISCUSSION

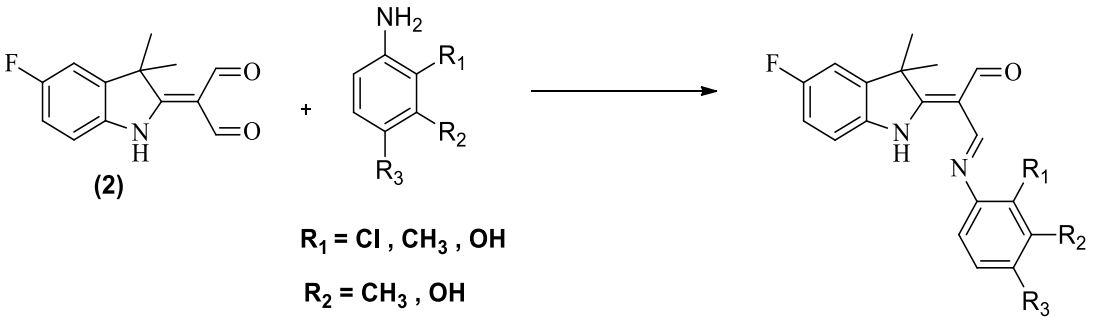
4. RESULTS AND DISCUSSION

4. 1. Chemistry part

4. 1. 1. Methodology

A series of new compounds have been synthesized from 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde **(2)** through the condensation reaction of this compound with substited aniline according to synthetic pathway as shown in Scheme (4. 1).





R_1 = Cl , CH_3 , OH

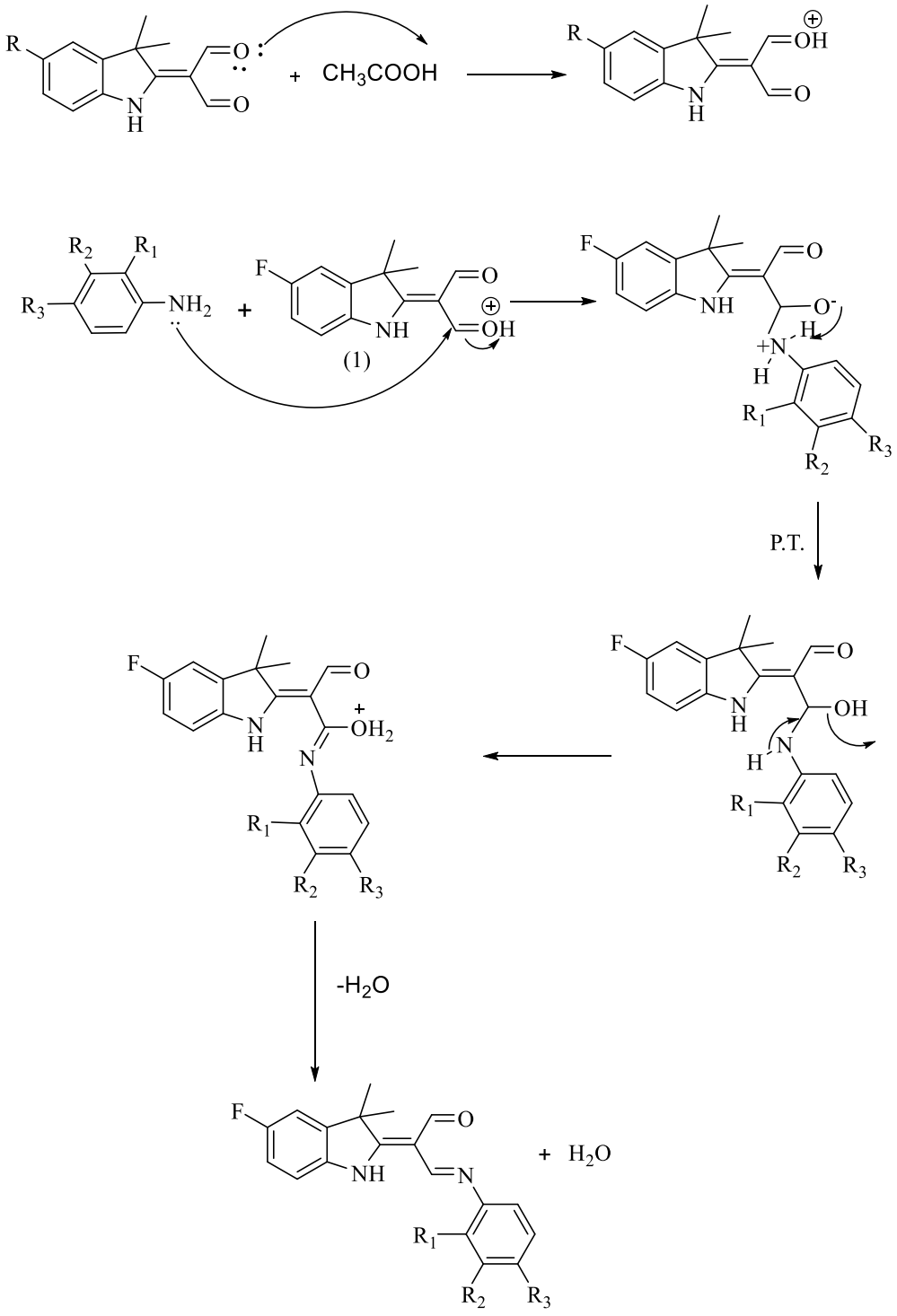
R_2 = CH_3 , OH

R_3 = Cl , CH_3 ,OH ,OCH_3

Compound (3) R1=H, R2=H, R3=Cl
Compound (4) R1=Cl R2=H, R3=Cl
Compound (5) R1=CH_2 R2=CH_2, R3=H
Compound (6) R1=CH_2 R2=H R3=H
Compound (7) R1=H R2=H, R3=OCH_3
Compound (8) R1=OH R2=H, R3=H
Compound (9) R1=H R2=H R3=OH
Compound (10) R1=H R2=OH R3=H

Scheme (4. 1): synthetic pathway of compounds (3-10)

The mechanism for the formation of the synthesized compounds are illustrated in the scheme (4. 2).[56].

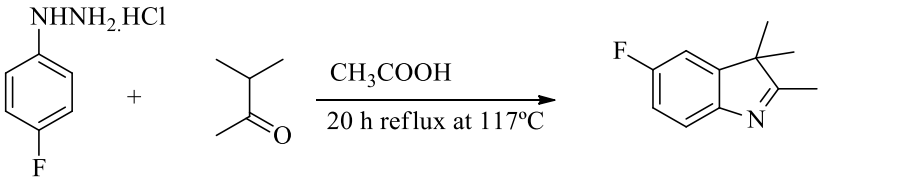


P.T. = proton transfer

scheme (4. 2): Mechanism of the synthesized compounds

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

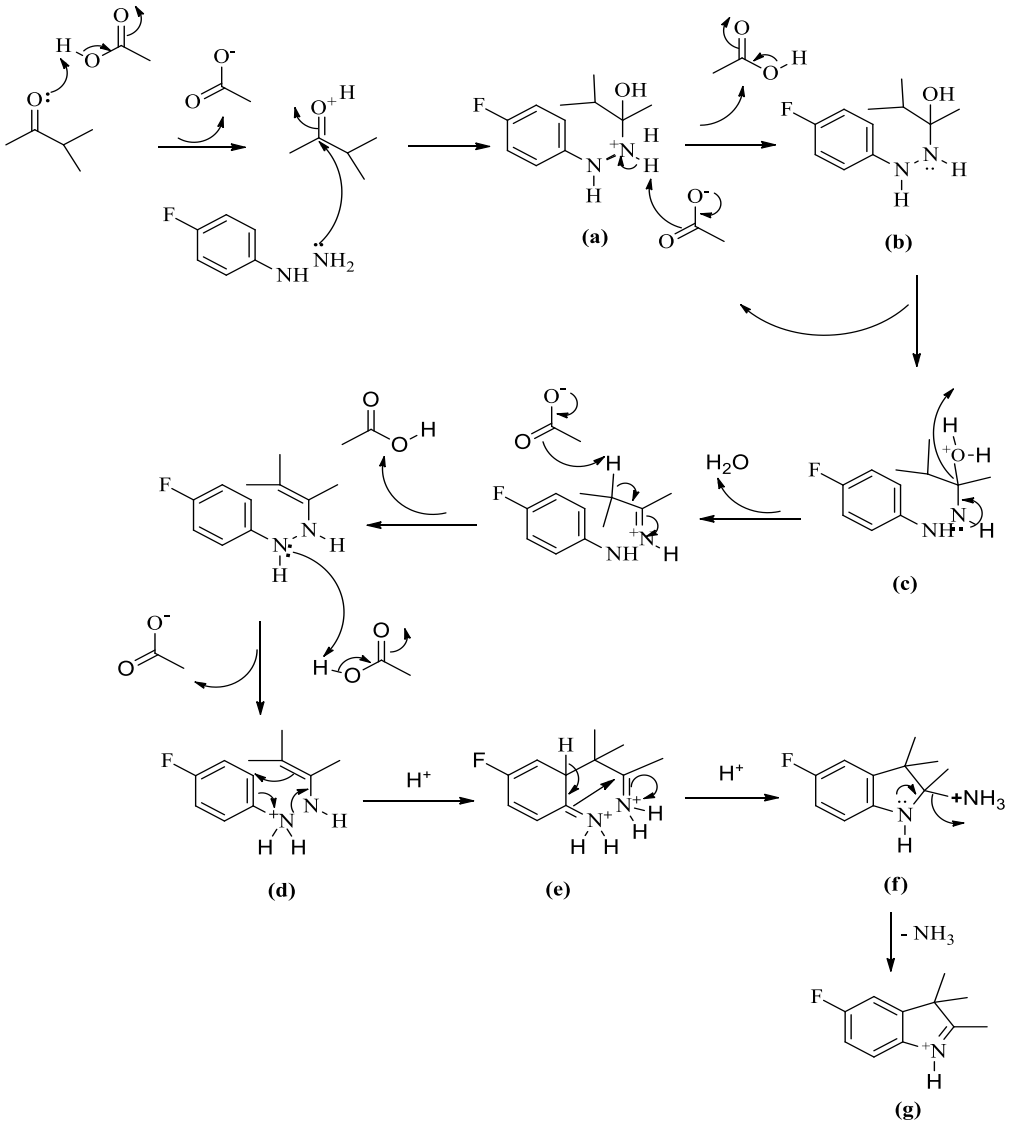
First step: synthesis of 2,3,3-trimethyl-3H-indole (indoline) at a perfect yield by Fischer indole synthesis as shown in Equation (4. 1)



4-Fluoro-phenyl hydrazinehydrochloride 3-Methyl-butan-2-one 5-Fluoro- 2,3,3-trimethyl-3H-indole (1)

Equation (4. 1): Synthetic pathway of compound (1)

The suggested mechanism to the formation of 5- Fluoro -2,3,3-trimethyl-3H-indole as suggested by [57] as shown in Scheme (4. 3)

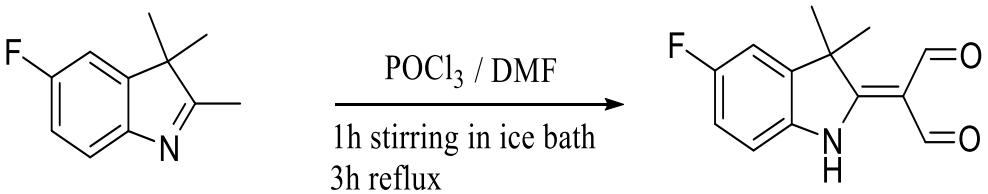


Scheme (4. 3): Mechanism of Fischer reaction to form 2,3,3-trimethyl-3H-indole(1)

The ayrlhydrazone (c) as formed from condensation reaction of 4-Fluoro-phenyl hydrazine hydrochloride and 3-Methyl-butan-2-one in presence of acid, then the arylhydrazone rearranges to form (d) compound, and this intermediate was rearrange again to form a new C-C bond to form compound (e).

A final rearrangement takes place to repair the benzene ring and at the same time close the five member ring with loss of ammonia to give the final indole product (g).

Second step: Vilsmier Haack reaction, the reaction of indoline (1) with Phosphoryl chloride ($PoCl_3$) in a presence of N,N-dimethyl formamide (DMF) to form the starting material 2-(5-Fluoro-3,3- dimethyl-1,3-dihydro-indol-2-ylidene)malonaldehyde (2) in a good yield, [58] as shown in Equation(4.2), which was reacted with different Substituted Aniline in ratio (1:1) to form our new compound (3- 10) by the condensation reaction..



5-Fluoro-2,3,3-trimethyl-3H-indole compound (2)

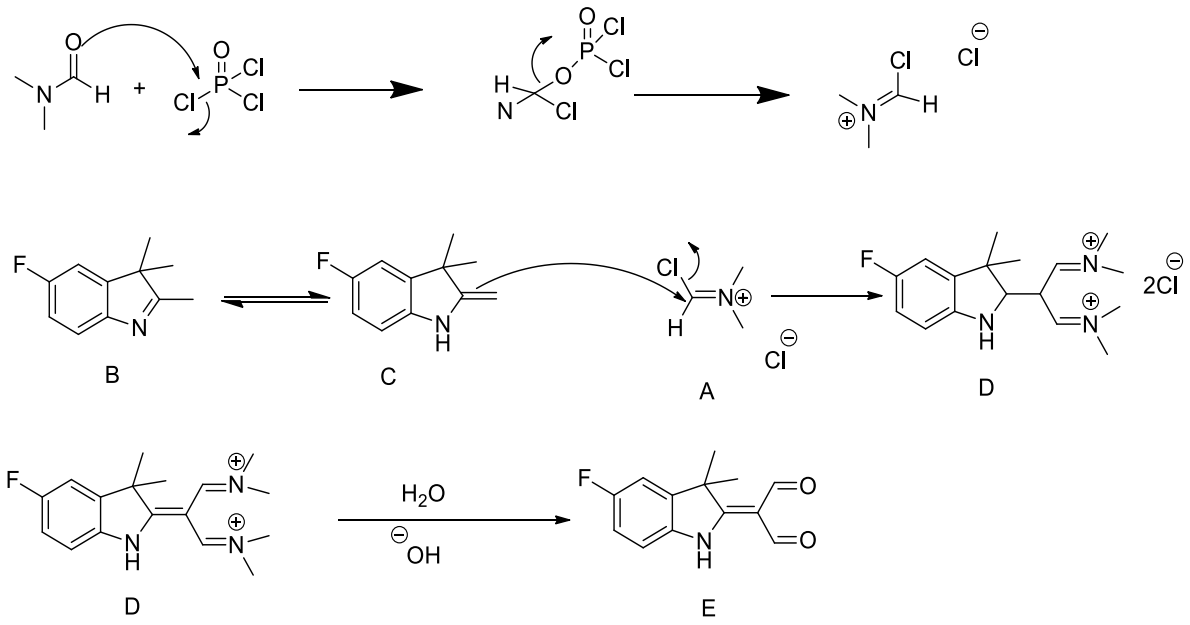
Equation (4. 2): Synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)malonaldehyde (2)

suggested mechanism of the formation of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)malonaldehyde **(2)** involves three steps a proposed by [59] as shown in Scheme (4. 4).

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

In the second step: the reaction of chloroiminium ion A with 5-Fluoro-2,3,3-trimethyl-3H-indole (indoline) B, that going to be in the equilibrium with enamine tautomer C. latter the chloroiminium ion from the first step was attacks, to create the intermediate D.

In the third step hydrolysis of intermediate D, to produce E, 2-(5-Fluoro -3,3-dimethyl-1,3-dihydro-indol-2-ylidene)malonaldehyde **(2).**



Scheme (4. 4): Mechanism of Vilsmier reaction to form the compound (2)

Compound (2) was found as ketoamine-enolimine tautomer forms [60] as shown in Figure (4.1)

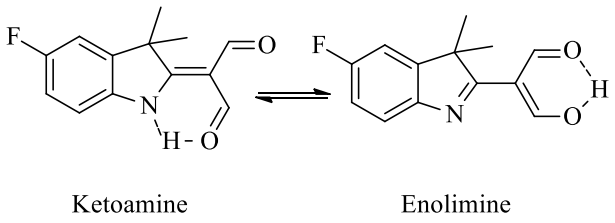


Figure (4.1): Tautomer forms of, 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)malonaldehyde (2)

last new synthesized compound **(1)** was used to synthesize new compounds, all new synthesized compounds are colored, stable in the air and not soluble in water and hexane. Their purities have been tested by TLC and their chemical structures have been confirmed by spectroscopic technique such as FT-IR and ^{1}HNMR, APT ^{13}C NMR as well as their physical properties like, melting points were determenined.

4. 1. 2. Spectral study of the new synthesized compounds (2-10)

4. 1. 2. 1. FT-IR Study

The results of the FTIR Spectrum for the new synthesized compounds displayed absorption bands in range between 400 - 4000 cm^{-1}.

The new compounds (**2-10**) showed disappeared the absorption bands of (NH_2) group which belonged to substituted anilines and showed new absorption bands of imine groups (CH=N) so that approved to formation of these new compounds. Also some of these new compounds (2-10) don‘t showed absorption bands was attributed to (NH) group for indole ring because of these new compounds are stabilize through intramolcular N-H…N and N-H…O hydrogen bonds.

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

This compound was synthesized by reaction of compound 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde and 2,3-dimethylaniline in ethanol with glacial acetic acid as a cataleptic .

FT-IR spectra to this synthesized compound, figure (4. 2) display absorption band into 3165 cm^{-1} was belonged within (N-H), 3069 cm^{-1} was belonged aromatic (C-H), 2960 and 2923 cm^{-1} were belonged and aliphatic (C-H) [61] ,also absorption bands at 2864and 2696 cm^{-1} were belonged to (C-H) aldehyde, the strong absorption band at 1663cm^{-1} was belonged at the carbonyl group (C=O). [62] and at 1608 cm^{-1} for azomethine group (CH=N), the new functional group which was indicated and approved to the formation of this compound.[63] As well as stretching frequency at 1582cm^{-1} was referred to(C=C) group [64], at the same time the absorption bands was appeared at 1392cm^{-1} was belonged to the CH_3 group [65] , also The absorptione band at 1267cm^{-1} which 63belonged to (C-N) groups [66] , and finely A sharp peak at 769cm^{-1} is attributed to out -of-plane group (C-H) . [67]

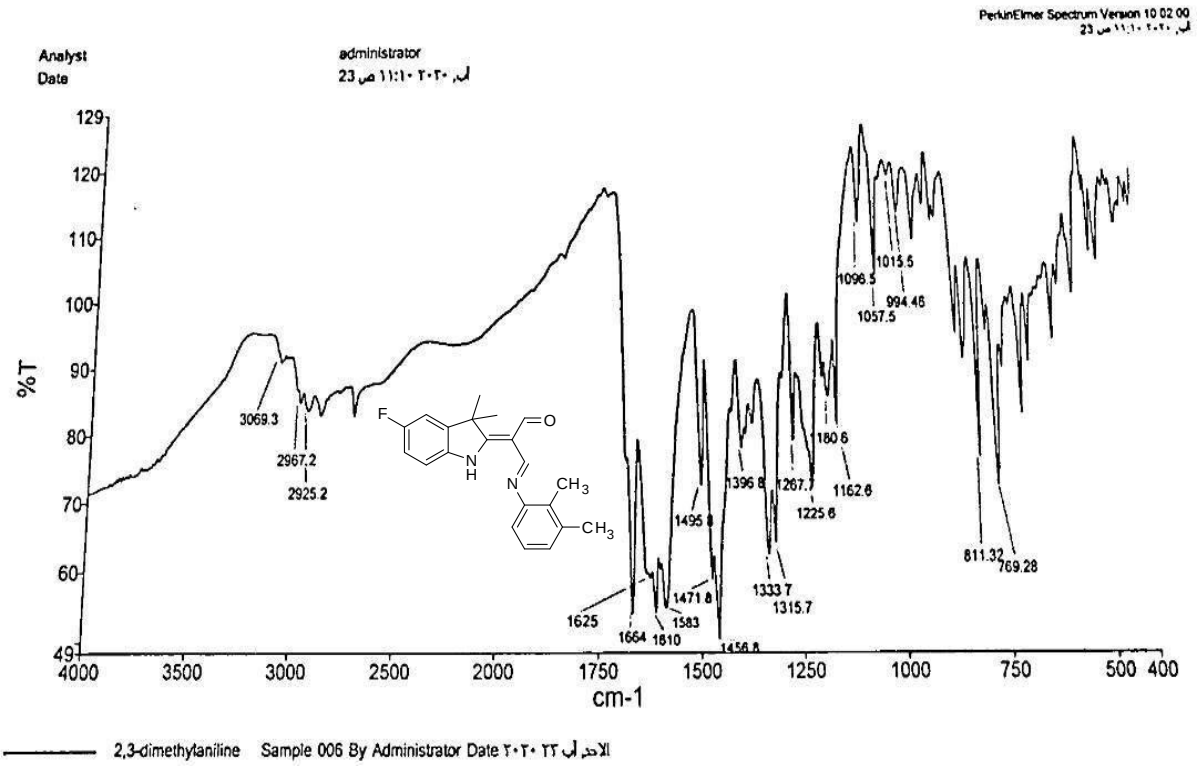


Figure (4. 2): The FTIR spectra of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,3-dimethyl-phenylimino)-propionaldehyde (5)

FT-IR for the compound 2-(5-Fluoro -3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-chloro-phenylimino)-propionaldehyde (3)

This compound was synthesized by reaction of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde and 4-Chloro-phenylamine at ethanol with glacial acetic acid as a cataleptic. The FT-IR spectra to this synthesized compound, figure (4. 3) display absorption band into 3318 cm^{-1} was belonged within (N-H), 3069 cm^{-1} was assigned for aromatic (C-H), 2967 and 2930 cm^{-1} for aliphatic (C-H), also absorption bands into 2857 and 2725 cm^{-1} were belonged to (C-H) aldehyde, the strong absorption band at 1663cm^{-1} was belonged at the carbonyl group (C=O) and at 1652 cm^{-1} for azomethine group (CH=N), the new functional group which was indicated and approved to the formation of this compound. As well as stretching frequency at 1579 cm^{-1} was referred to(C=C) group, at the same time the absorption bands was

appeared at 1396 cm^{-1} was belonged to the CH_3 group, also The absorption band into 1267 cm^{-1} which belonged to (C-N) group. , a peak at 813 cm^{-1} was attributed for (C-Cl) group. And finally, a sharp peak at 773 cm^{-1} is attributed onto out -of-plane group (C-H) of aromatic ring.

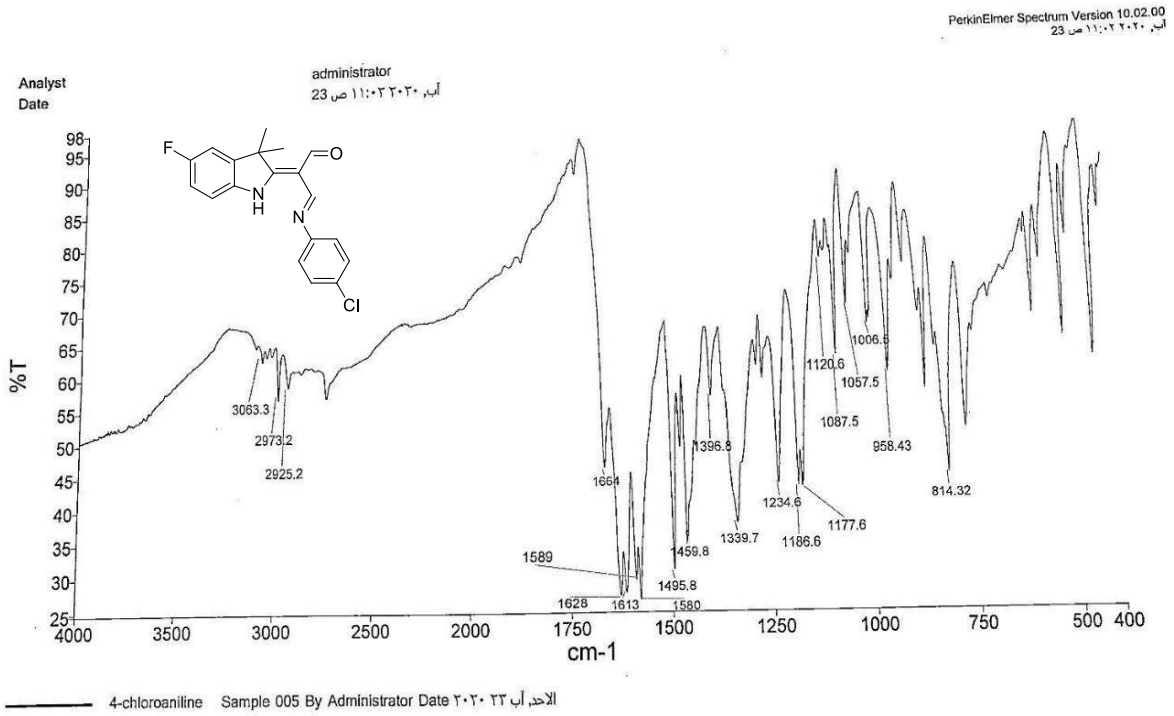


Figure (4. 3): The FTIR spectra of 2-(5-Fluoro -3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-chloro-phenylimino)-propionaldehyde (3)

FT-IR for the compound 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dichloro-phenylimino)-propionaldehyde (6)

This compound was synthesized by reaction of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde and 2,4-diChloro-phenylamine in ethanol with glacial acetic acid as a cataleptic.

FT-IR spectra to this synthesized compound, figure (4. 4) display absorption band into 3091 cm^{-1} was belonged to (N-H), 3055 cm^{-1} was assigned aromatic (C-H), 2982 and 2923 cm^{-1} for aliphatic (C-H), also absorption bands into 2864 and 2725 cm^{-1} were belonged to (C-H) aldehyde, the strong absorption band at 1663cm^{-1} was belonged at the carbonyl group (C=O) and at 1626 cm^{-1} for azomethine group (CH=N),

the new functional group which was indicated and approved to the formation of this compound. As well as stretching frequency at 1579 cm^{-1} was referred to(C=C) group, at the same time the absorption bands was appeared at 1396 cm^{-1} was belonged to the CH_3 group, also The absorption band into 1286 cm^{-1} which attributed for (C-N) group. , a peak at 813 cm^{-1} was attributed to (C-Cl) group. And finely A sharp peak at 766cm^{-1} is attributed to out -of-plane group (C-H) of aromatic ring.

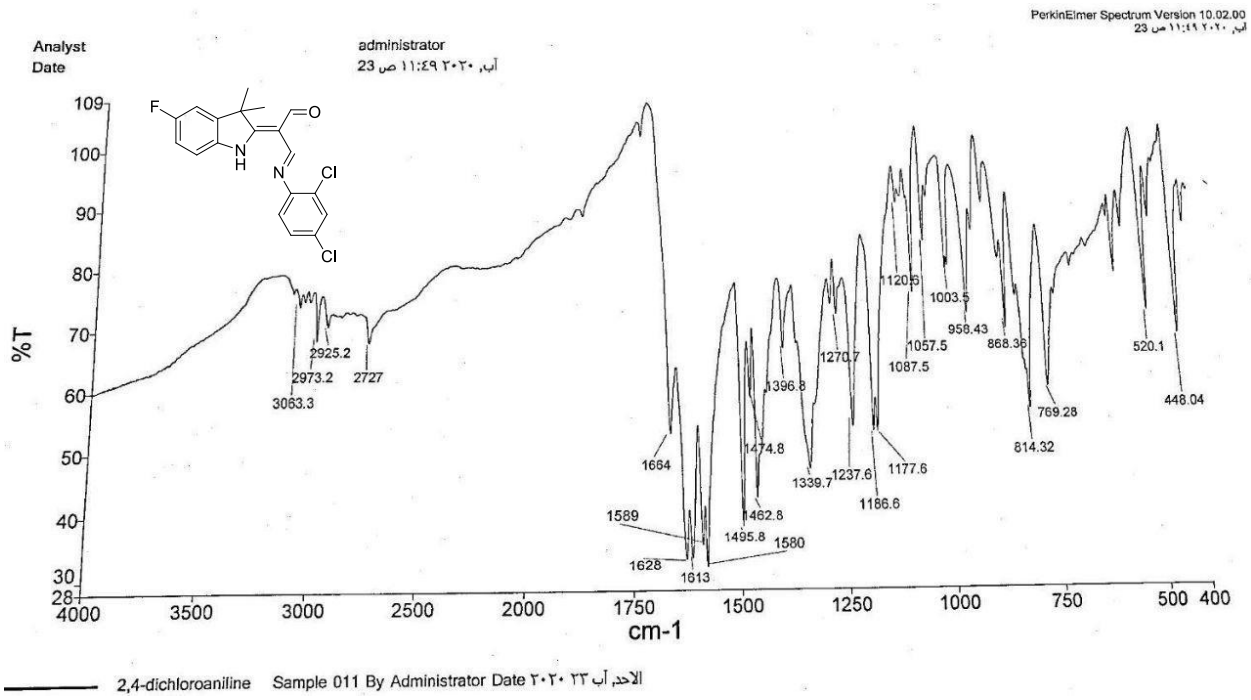


Figure (4. 4): The FTIR spectra of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dichloro-phenylimino)-propionaldehyde (6)

The FTIR values of compounds (2-10) are listed in table (4. 1) and their figures are attached as appendixes frome 4. 1 to 4. 5

Table (4. 1).The FTIR values of compounds (2-10)

Com. No.	4Characteristic band of(FT- IR) spectra (cm^{-1})										
	N-H	C-H Ar.	C-H Alph.	C-H Ald.	C=O	C=N	C=C	CH_3	C-N	C-H	Others
2	3201	3042	2983 and 2854	2859 and 2728	1657	1616	1534	1372	1275	750	-
3	3318	3069	2930 and 2967	2857 and 2725	1663	1652	1579	1396	1267	773	C-Cl 813
4	3091	3055	2923 and 2982	2864 and 2725	1663	1626	1579	1396	1286	766	C-Cl 813
5	3165	3069	2960	2864	1663	1608	1582	1392	1267	769	-
6	3450	3062	2967	2703	1656	1630	1586	1374	1271	736	-
7	3326	3113	2974	2747	1648	1626	1597	1366	1271	733	-
8	3487	3062	2982	2703	1619	-	1506	1348	1238	751	O-H 3633
9	3443	3182	2967	2755	1641	1601	1557	1374	1282	714	O-H 3524
10	3524	3049	2974	2718	1625	1601	1524	1337	1267	751	O-H 3743

4. 1. 2. 2. NMR Study

^{1}H-NMR and APT ^{13}C-NMR spectra reported in duterated dimethyl sulfuxide ($DMSOd_6$) together with chemical shifts in ppm and using TMS (tetramethylsilane) as standard.

^{1}H-NMR results of the newly synthesized compounds (3-10) showed disappearance signals and appearance of new signals. Such as disappearance of one proton atom of the carbonyl group and disappearance a signal of NH_2 group on spectrum of substituted anilines

and appearance new signal of one proton atom of imine group. This is approval to the formation of the new compounds. Also the signals of protons of aromatic ring were represented the number of protons that belong for each new compound.

4. 1. 2. 2. 1. ^{1}H-NMR and APT ^{13}C-NMR results of the compound 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,3-dimethyl-phenylimino)-propionaldehyde (5).

The ^{1}H-NMR results for compound **(5)** figure (4. 5) displayed single signal at 14.15 ppm was belonged to proton of (NH) of indole ring **(68)** A singlet signal at 9.37 ppm was referred to the one proton atom of carbonyl group (C=O) and single signal at 8.62 ppm was attributed to proton of Schiff base group (CH=N) (69). A signals were appeared in the region between (8.59-7.03) ppm were belonged to six protons of aromatic ring for compound **(5)** **(70 and71).** A signal at (2.49 and 2.26) ppm which attributed to two CH_3 group. And finally signal at 1.58 ppm was belonged to six protons of two methyl groups **(72).**

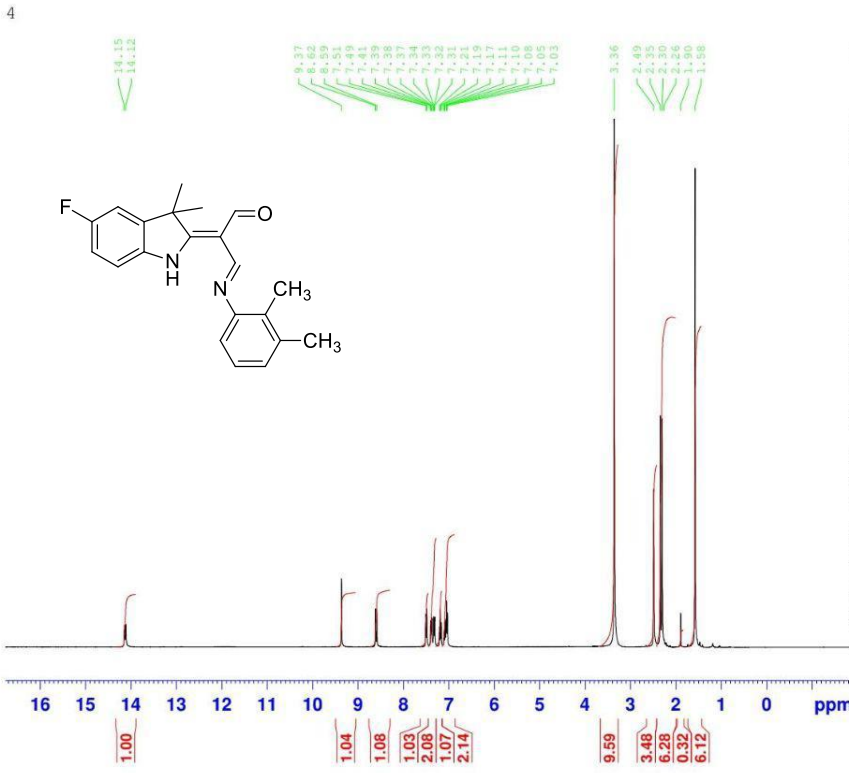


Figure (4. 5): ^{1}H-NMR spectrum of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,3-dimethyl-phenylimino)-propionaldehyde (5).

APT^{13}C-NMR results were used to characterize this new compound and support the results of ^{1}H-NMR, figure (4. 6) displayed signals for the quaternary carbons and methylene CH_2 group appeared at a positive side (above of the spectrum). While carbons of CH and CH_3 groups observed at a negative side (below of the spectrum). A signal at 188.08 ppm and 182.73 ppm were assigned to the carbonyl group C=O and to NH-C=C group respectively **(73)** while a signal of CH=N group detected at 161.67 ppm. **(74)** The signals were appear in the range between 159.27-108.96 ppm were belonged to the carbon atoms of aromatic rings. **(75)** In addition, two signals appeared at 108.47 ppm and 54.49 ppm were assigned to O=C-C and CH_3-C-CH_3 groups respectively. **(76)**Signals at (21.41 and 20.02) ppm belongs to two methyl groups at positions ortho and meta of benzene ring. Finally, signal at 13.28 ppm was belongs to the two methyl groups **(77)**

All these results found 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.

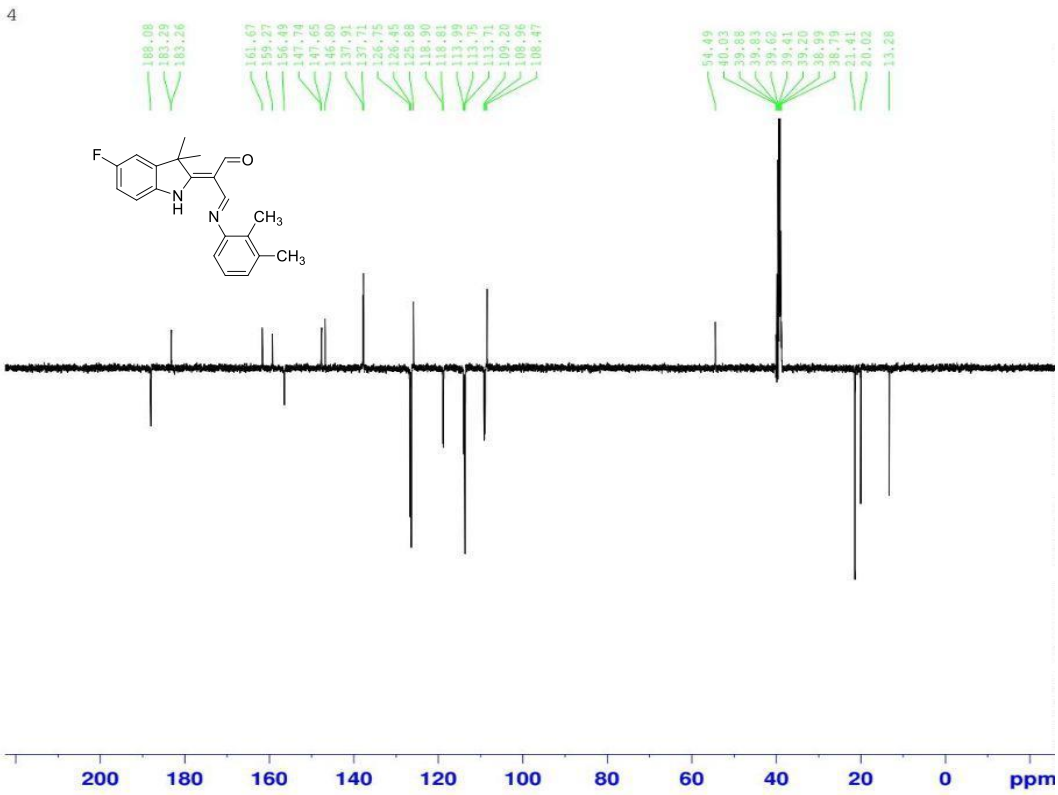


Figure (4. 6): APT ^{13}C-NMR spectrum of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,3-dimethyl-phenylimino)-propionaldehyde (5).

4. 1. 2. 2. 2. ^{1}H-NMR and APT ^{13}C-NMR results of the compound 2-(5-Fluoro -3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-chloro-phenylimino)-propionaldehyde (3)

The ^{1}H-NMR results for compound **(3)** figure (4. 7) displayed single signal at 13.96 ppm was belonged to proton of (NH) of indole ring. A singlet signal at 9.38 ppm was referred to the one proton atom of carbonyl group (C=O) and single signal at 8.62 ppm was attributed to proton of Schiff base group (CH=N). A signals were appeared in the region between (8.59-7.08) ppm were belonged to nine protons of aromatic ring for compound **(3)** [(78).] And finally signal at 1.56 ppm was belonged to six protons of two methyl groups.

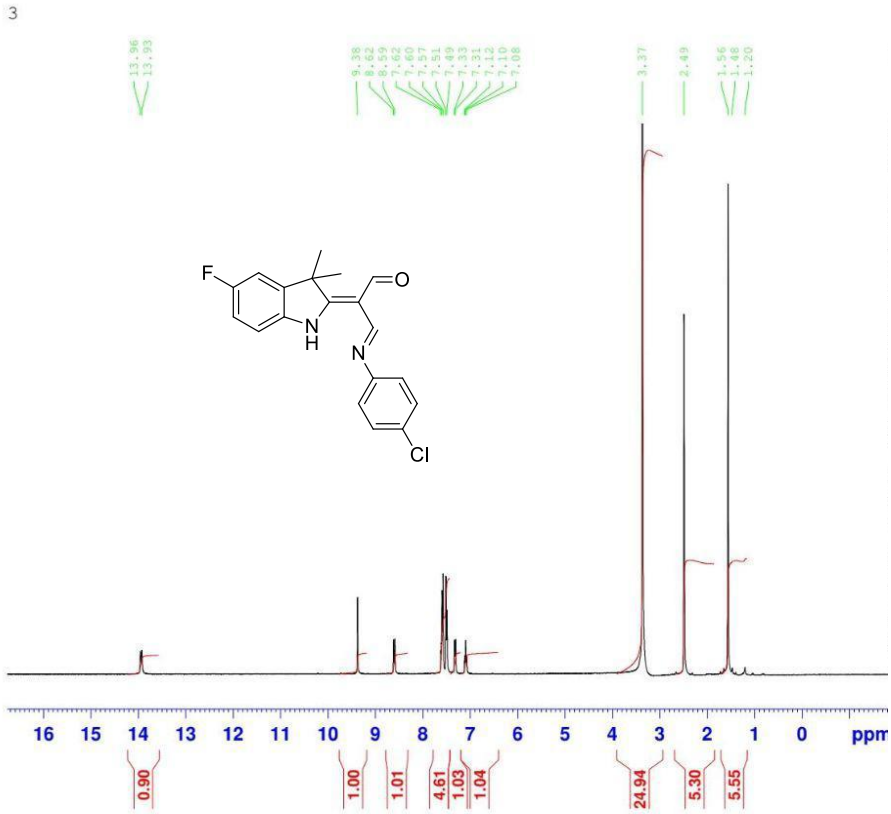


Figure (4. 7): ^{1}H-NMR spectrum of 2-(5-Fluoro -3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-chloro-phenylimino)-propionaldehyde (3)

APT^{13}C-NMR results were supported the ^{1}H NMR results figure (4. 8). This compound show a signal at 188.25 ppm and 182.73 ppm were assigned to the carbonyl group C=O and to NH-C=C group respectively, while a signal of CH=N group` detected at 161.67 ppm. The signals were appear in the range between 147.53-109.02 ppm were belonged to the carbon atoms of aromatic rings. In addition, two signals appeared at 108.47ppm and 54.29 ppm were assigned to O=C-C and CH_3-C-CH_3 groups respectively. Also a signal at 21.51 ppm was belongs to the two methyl groups. **(79)**

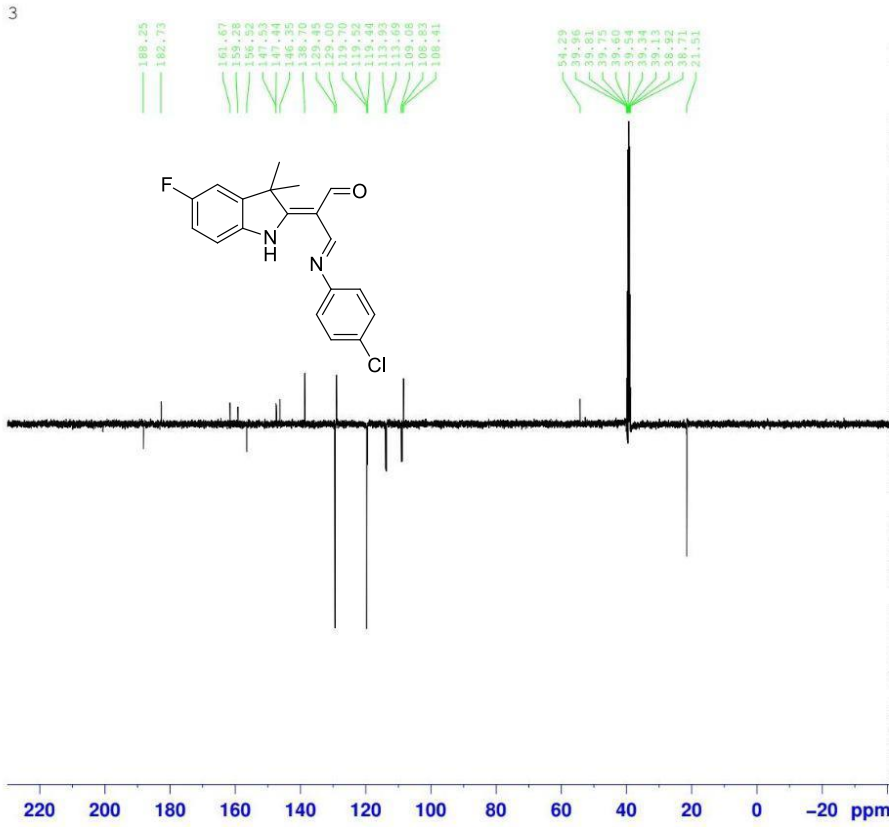


Figure (4. 8): APT ^{13}C NMR spectrum of 2-(5-Fluoro -3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-chloro-phenylimino)-propionaldehyde (3)

4. 1. 2. 2. 3. 1H-NMR and APT 13C-NMR results of the compound 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dichloro-phenylimino)-propionaldehyde (4)

The ^{1}H-NMR results for compound **(4)** figure (4. 9) displayed single signal at 13.96 ppm was belonged to proton of (NH) of indole ring.A singlet signal at 9.38 ppm was referred to the one proton atom of carbonyl group (C=O) and single signal at 8.63 ppm was attributed to proton of Schiff base group (CH=N).A signals were appeared in the region between (8.60-7.08) ppm were belonged to nine protons of aromatic ring for compound **(4).** And finally signal at 1.57 ppm was belonged to six protons of two methyl groups.

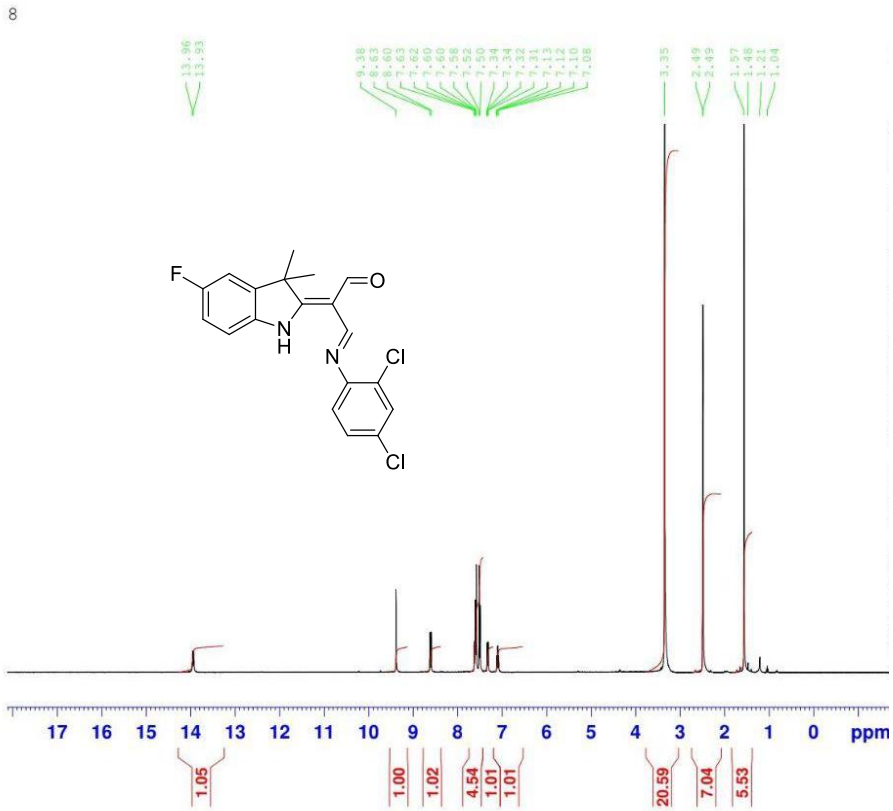


Figure (4. 9): ^{1}H-NMR spectrum of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dichloro-phenylimino)-propionaldehyde (4)

APT^{13}C-NMR results were used to characterize this new compound and support the results of ^{1}H-NMR . figure (4. 10) displayed signals for the quaternary carbons and methylene CH^{2} group appeared at a positive side (above of the spectrum). While carbons of CH and CH_3 groups observed at a negative side (below of the spectrum). A signal at 188.20 ppm and 182.72 ppm were assigned to the carbonyl group C=O and to NH-C=C group respectively, while a signal of CH=N group detected at 161.69 ppm. The signals were appear in the range between 159.29-108.83 ppm were belonged to the carbon atoms of aromatic rings.In addition, two signals appeared at 108.42 ppm and 54.28 ppm were assigned to O=C-C and CH_3-C-CH_3 groups respectively.. Finally, signal at 21.56 ppm was belongs to the two methyl groups. All these results found 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.

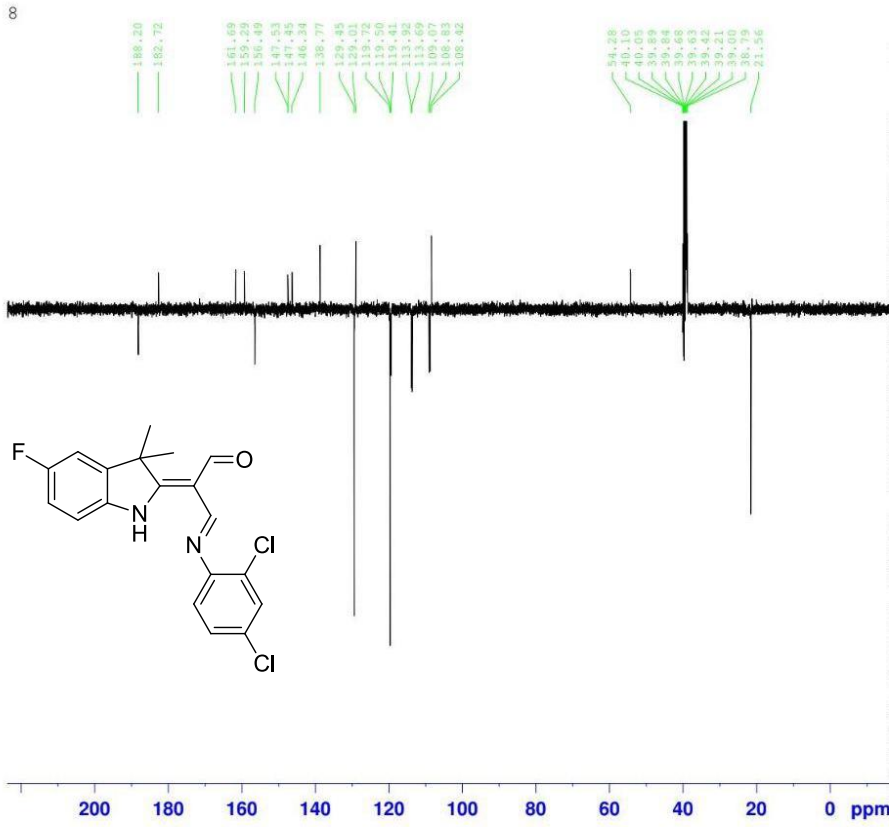


Figure (4. 10): APT ^{13}C-NMR spectrum of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dichloro-phenylimino)-propionaldehyde (4)

The ^{1}H-NMR results of the synthetic other new compounds are listed in table (4. 2) and their figures attached as appendixes from 4.6 to 4. 15

Table (4. 2): The chemical shift in ppm to ^{1}H NMR results for the synthesized compounds

Com.No.	NH-	C=O	CH=N	Ar-H	6H,2xCH_3	other
1	-	-	-	6.71-7.57	1.40	Ortho CH_3 2.17
2	13.14	9.75	-	7.12-7.62	1.67	-
3	13.96	9.38	8.62	8.59-7.08	1.56	C$\underline{H}_3$ 2.49
4	13.96	9.38	8.63	8.63-7.08	1.54	CH_3 2.49
5	14.15	9.37	8.62	8.59-7.03	1.58	Ortho and meta CH_3 2.49,2.26
6	-	9.40	8.71	7. 73-7.12	1.58	
7	13.23	9.80	7.65	7.56-6.60	1.63	OCH_3 4.32
8	14.04	9.80	8.69	7.87-6.96	1.63	OH 11.15
9	13.98	9.39	8.73	7.59-6.88	1.62	OH 9.68
10	14.1	9.67	8.95	8.16-7.09	1.67	OH 10.56

The APT ^{13}C-NMR results of the others new synthesized compounds are discussed and recorded in table (4. 3).

Table (4. 3): chemicl shift in ppm to theAPT^{13}CNMR for the other synthetic Compounds

Com. No.	above of the spectrum			below of the spectrum					
	NH-<u>*C*</u>H=C	O=C-<u>*C*</u>=C	CH_3-<u>*C*</u>-CH_3	<u>*C*</u>H=O	<u>C</u>H=N	*o*-<u>*C*</u>H_3	*M*-<u>*C*</u>H_3	*P*-<u>*C*</u>H_3	2x <u>*C*</u>H_3
3	182.73	108.47	54.29	188.25	161.67	-	-	-	21.51
4	182.72	108.42	54.28	188.20	161.69	-	-	-	21,56
5	182.73	108.47	54.49	188.08	161.67	21.41	20.02	-	13.28
6	182.94	108.17	53.99	188.0	156.58	-	-	-	12.19
8	183,29	108.73	54.08	187.9	157.17	-	-	-	21.25
9	183.51	108.48	53.35	187.0	152.22	18.14	-	-	23.39
10	183.74	108.26	54.42	187.0	150.05	20.59	13.9	-	23.36

4. 2. Biological part

4. 2. 1. Cytotoxicity assay

In this study was investigated the effect of compound of compound 1234 for cytotoxicity and anti-cancer effect in different concentrations for HepG2, SK_GT2 compounds with normal WRL68 cell line concent. The all four investigated compounds were revealed anti-cancer activity in dose dependent manner for both HepG2, SK_GT2 cell line, while low toxicity effect for WRL68 cell line. The result for this study was conclude the anti-cancer activity for new Synthesis Schiff bases compounds that may he usual as anti-cancer dray in the future

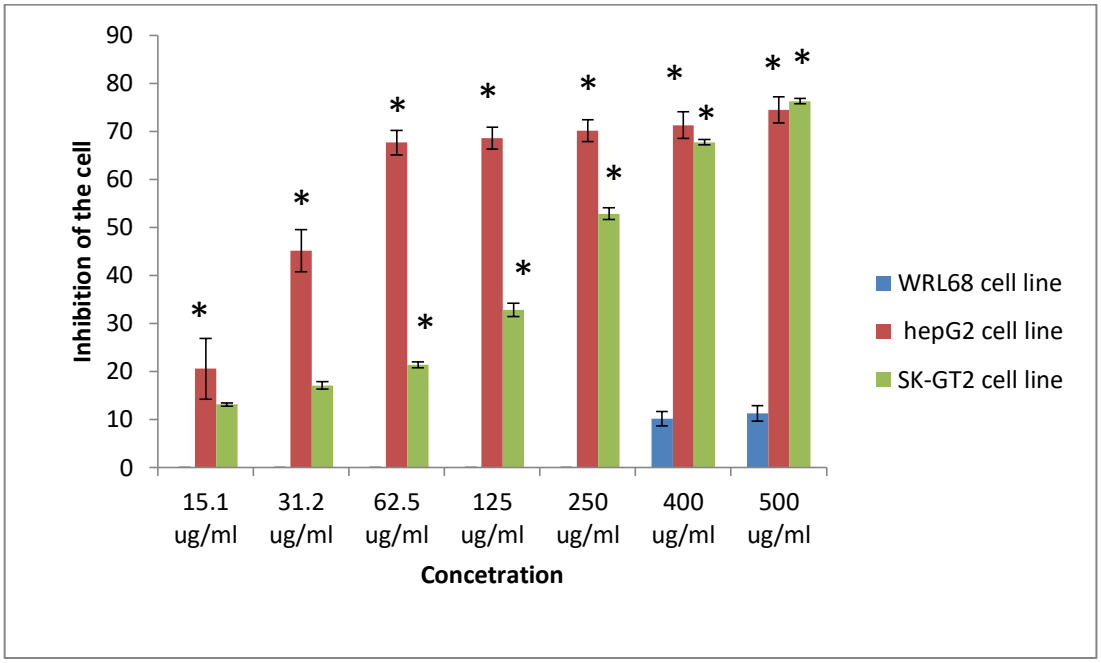


Figure 4. 11(: Showed the inhibition of HepG2 cell line and SK-GT2 cell line compared with WRL68 cell line when treated with different dose from compound 1

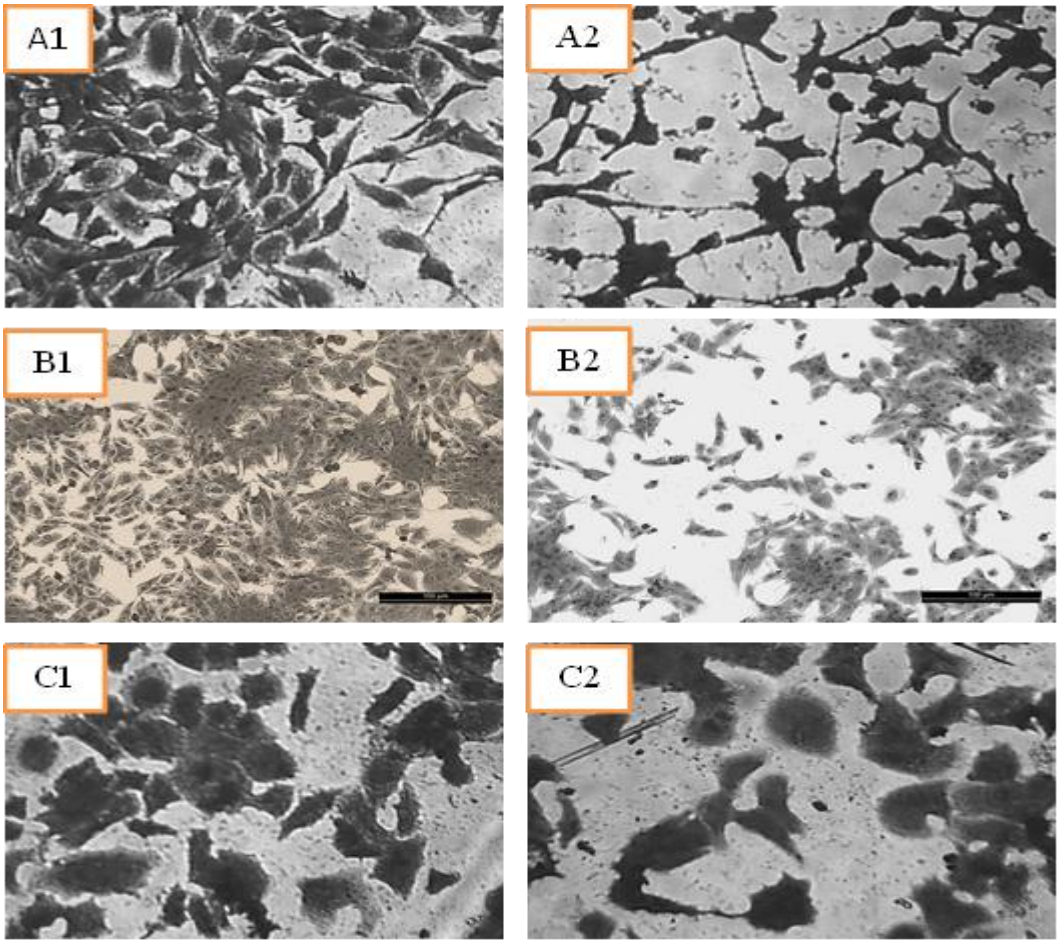


Figure 4. 12: Showed the morphology of cell after treated with compound 1 in 24 hours as presented: (A1) WRL68 cell exposed to composite 1 in concentration 15.1 µg/mL, (A2) WRL68 cell exposed to compound 1 in concentration 500 µg/mL (B1) HepG2 cell exposed to compound 1 in concentration 15.1 µg/mL, (B2) HepG2 cell exposed to compound 1 in concentration 500 µg/mL . (C1) SK-GT2 cell exposed to compound 1 in concentration 15.1 µg/mL, (C2) SK-GT2 cell exposed to compound 1 in concentration 500 µg/mL

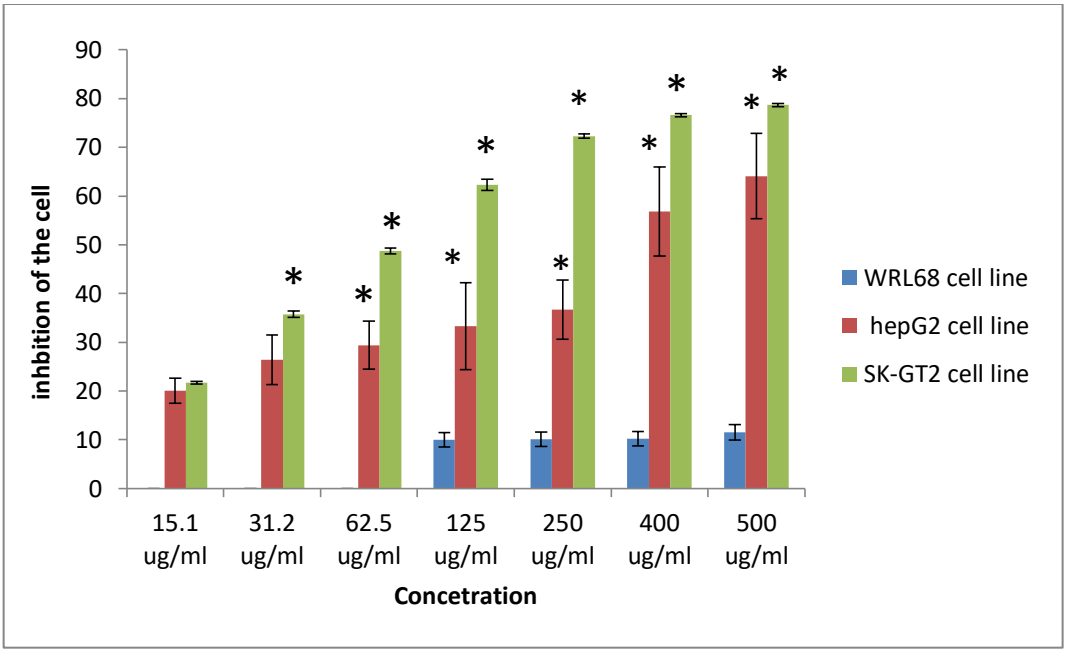


Figure 4. 13: Showed the inhibition of HepG2 cell line and SK-GT2 cell line compared with WRL68 cell line when treated with different dose from compound 2

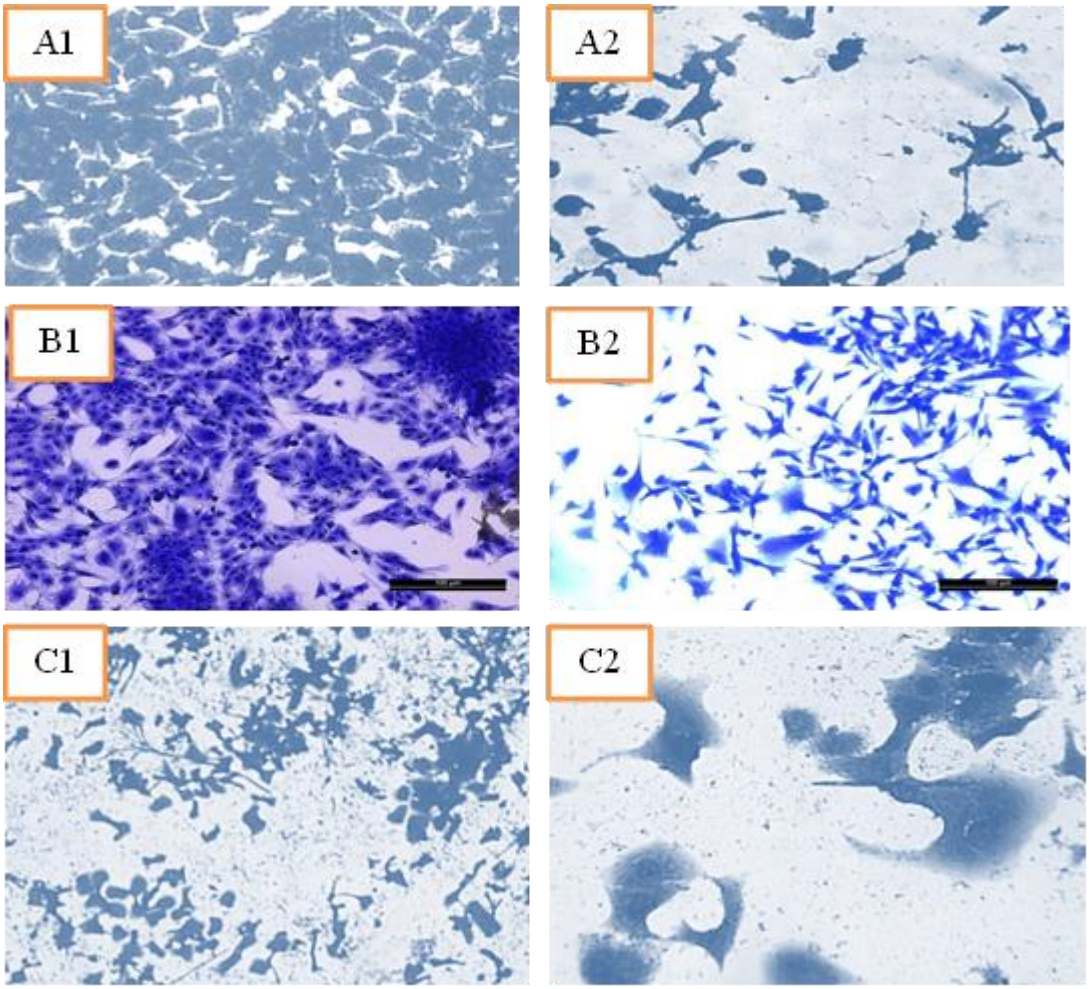


Figure 4. 14Showed the morphology of cell after treated with compound 2 in 24 hours as presented: (A1) WRL68 cell exposed to compound 2 at concentration 15.1 µg/mL, (A2) WRL68 cell exposed to compound 2 in concentration 500 µg/mL . (B1) HepG2 cell exposed to compound 2 in concentration 15.1 µg/mL, (B2) HepG2 cell exposed to compound 3 in concentration 500 µg/mL . (C1) SK-GT2 cell exposed to compound 2 in concentration 15.1 µg/mL, (C2) SK-GT2 cell exposed to compound 2 in concentration 500 µg/mL

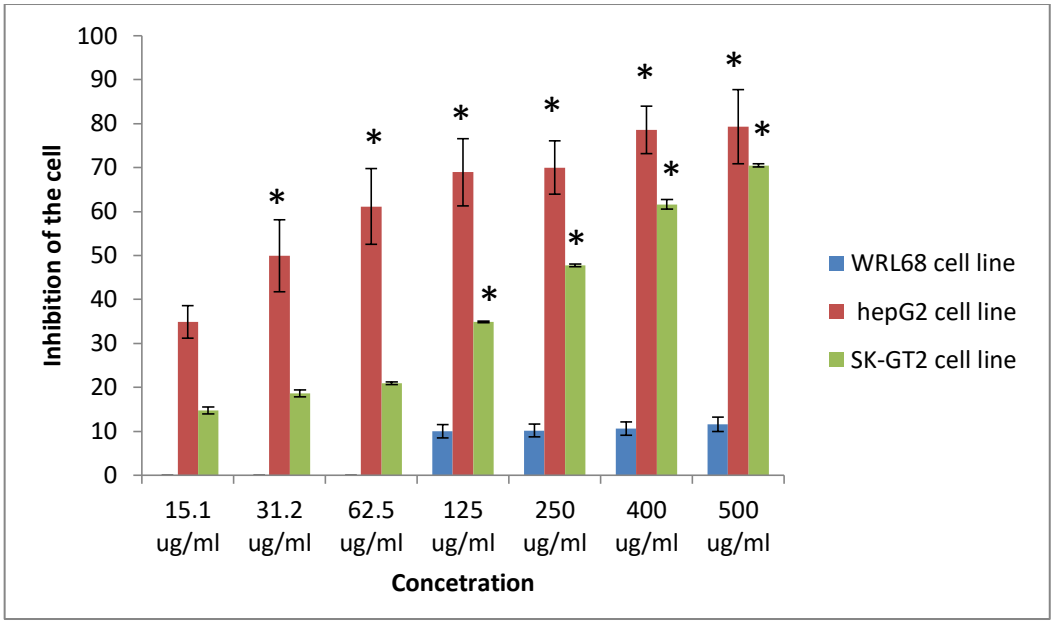


Figure 4. 15): Showed the inhibition of HepG2 cell line and SK-GT2 cell line compared with WRL68 cell line when treated with different dose from compound 3

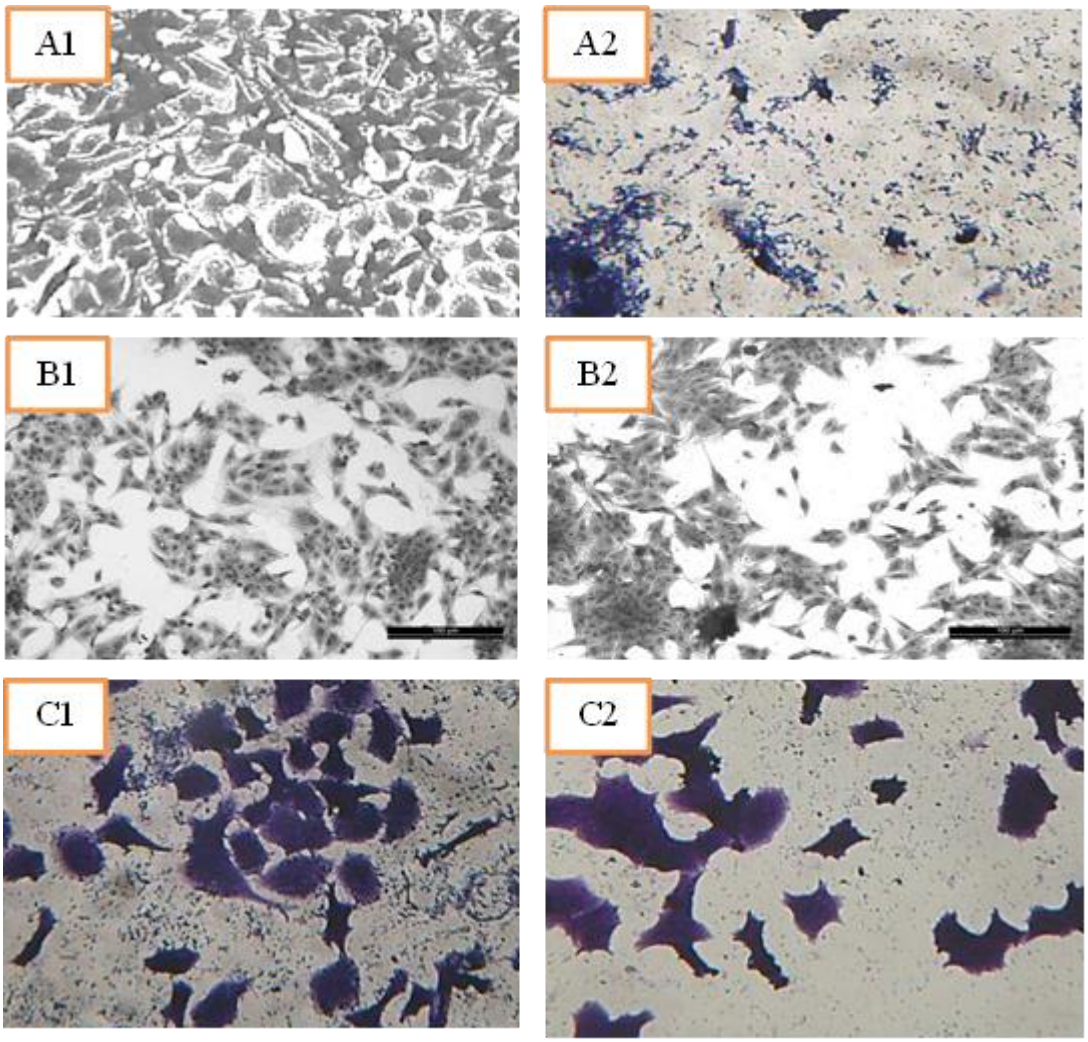


Figure 4. 16: Showed the morphology of cell after treated with compound 3 in 24 hours as presented: (A1) WRL68 cell exposed to compound 3 in concentration 15.1 µg/mL, (A2) WRL68 cell exposed to compound 3 at concentration 500 µg/mL . (B1) HepG2 cell exposed to compound 3 in concentration 15.1 µg/mL, (B2) HepG2 cell exposed to compound 3 in concentration 500 µg/mL . (C1) SK-GT2 cell exposed to compound 3 in concentration 15.1 µg/mL, (C2) SK-GT2 cell exposed to compound 3 in concentration 500 µg/mL .

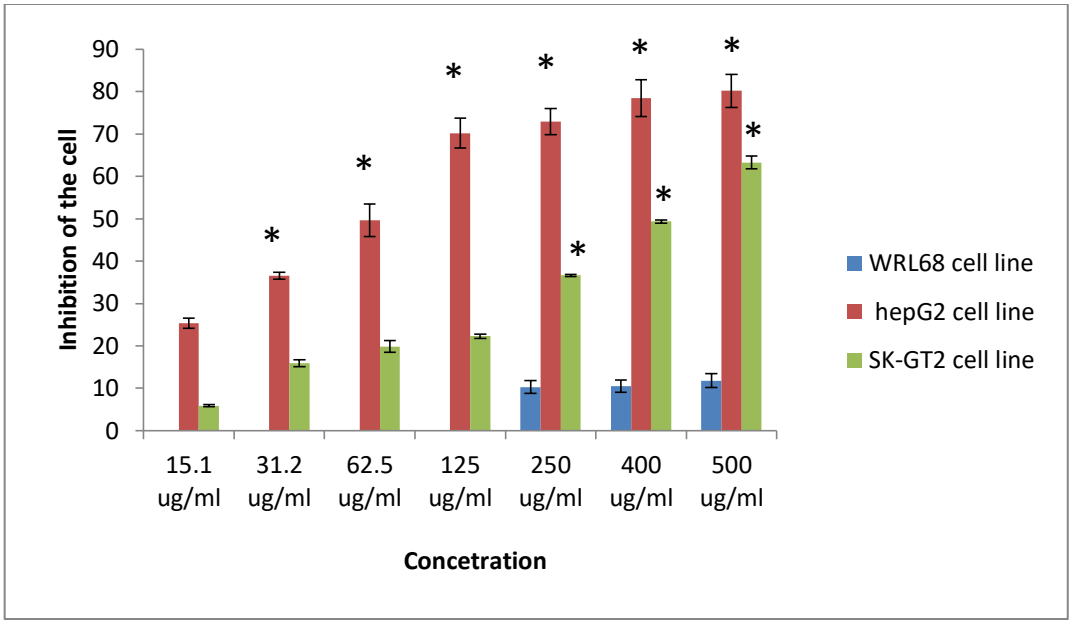


Figure 4. 17: Showed the inhibition of HepG2 cell line and SK-GT2 cell line compared with WRL68 cell line when treated with different dose from compound 4

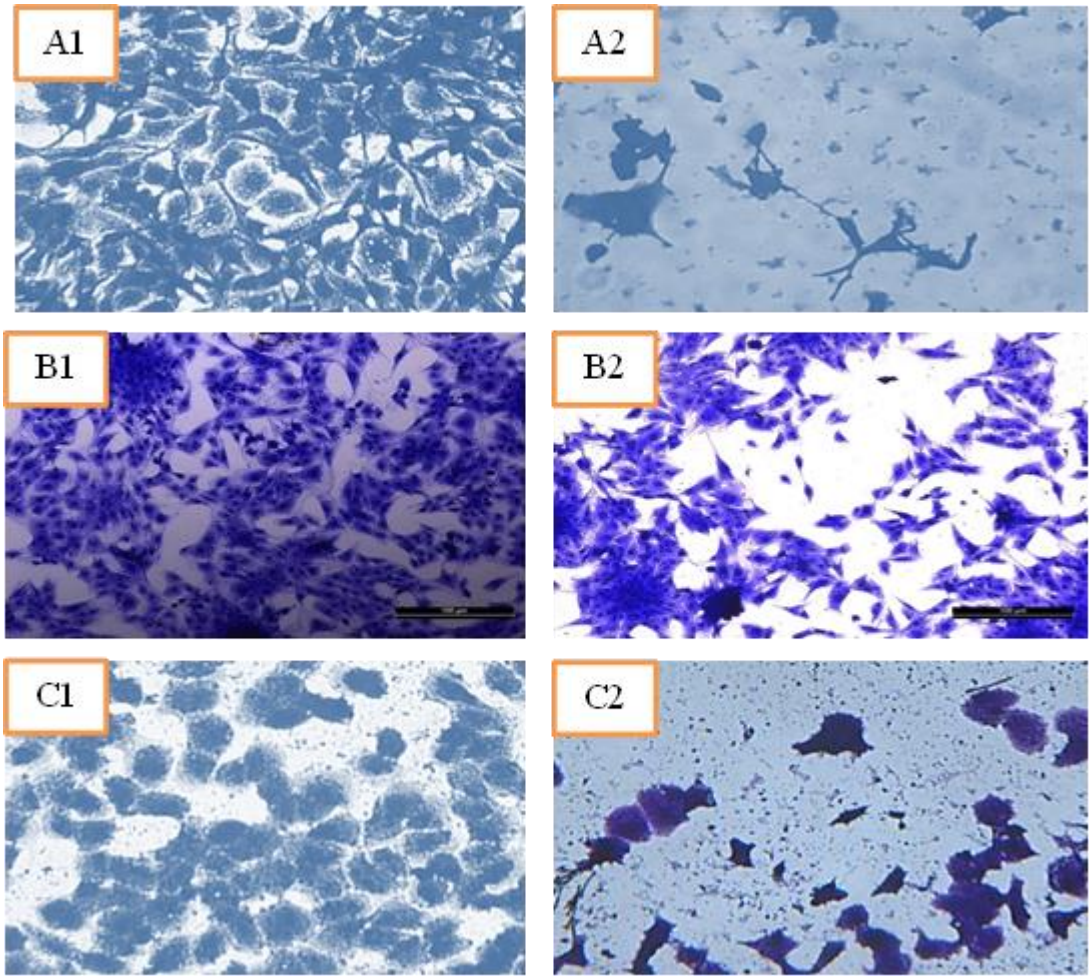


Figure 4. 18: Showed the morphology of cell after treated with compound 4 in 24 hours as presented: (A1) WRL68 cell exposed to compound 4 at concentration 15.1 µg/mL, (A2) WRL68 cell exposed to compound 4 in concentration 500 µg/mL . (B1) HepG2 cell exposed to compound 4 in concentration 15.1 µg/mL, (B2) HepG2 cell exposed to compound 4 in concentration 500 µg/mL . (C1) SK-GT2 cell exposed to compound 4 in concentration 15.1 µg/mL, (C2) SK-GT2 cell exposed to compound 4 in concentration 500 µg/mL

Conclusion

In the present work. New derivatives indole based Schiff bases were synthesized and identified by some spectral methods like, (FT-IR, ^{1}H, APT ^{13}C-NMR). The synthesized compounds are:

1- 5-Fluoro-2,3,3-trimethyl-3H-indole

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

3-2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-chloro-phenylimino)-propionaldehyde

4- 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dichloro-phenylimino)-propionaldehyde

5- 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-o-tolylimino-propionaldehyde

6- 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-o-tolylimino-propionaldehyde

7- 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-methoxy-phenylimino)-propionaldehyde

8- 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2-hydroxy-phenylimino)-propionaldehyde

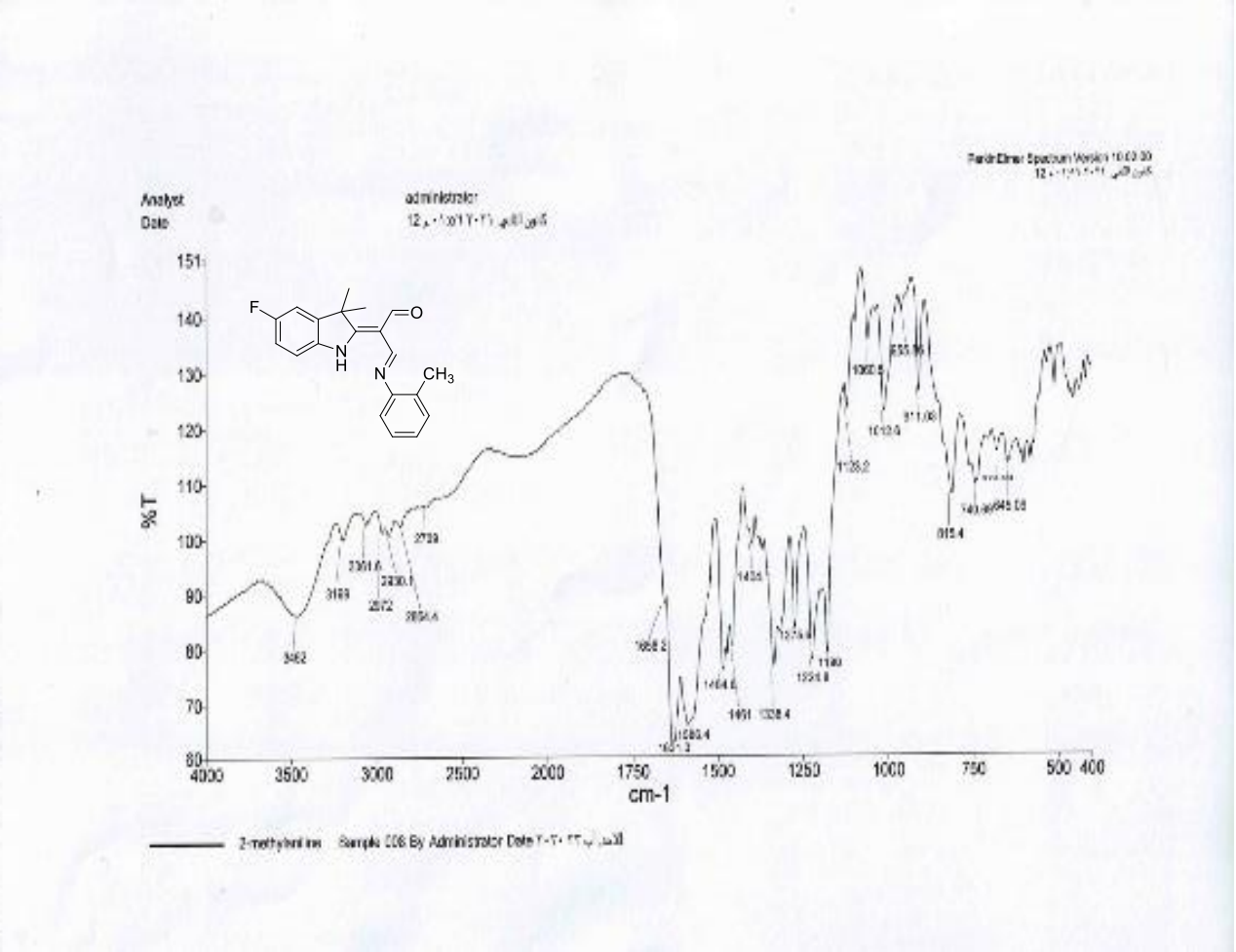
9- 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro indol-2-ylidene)-3-(4-hydroxy-phenylimino)-propionaldehyde

Four of the synthesized compounds with different concentrations 15.1, 31.2, 62.5, 125, 250, 400 and 500) µg\ml were evaluated for their anticancer activity against HepG2 and SK-GT2 cancer cell line. Most of the tested showed good results

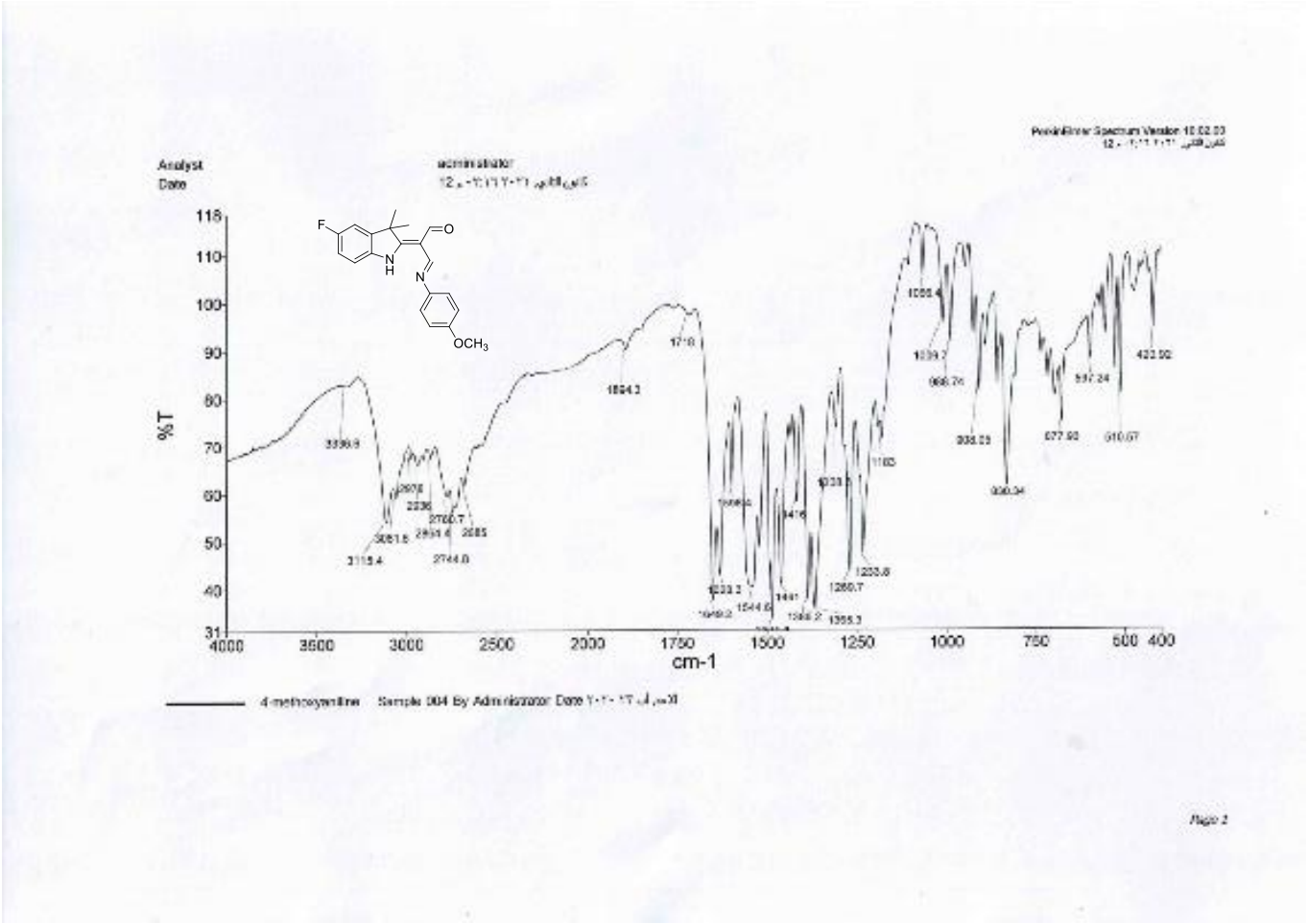
2-Recommendations and future work:

1. Synthesizing a series of new complexes from the new synthesized compounds (**3-10**) by their reaction with different transition metal ions and evaluate their biological activities.
2. Evaluating the biological activities for all the new synthesized compounds (**3-10**) against other cancer cell line such as, breast cancer, colon cancer and cervical cancer.

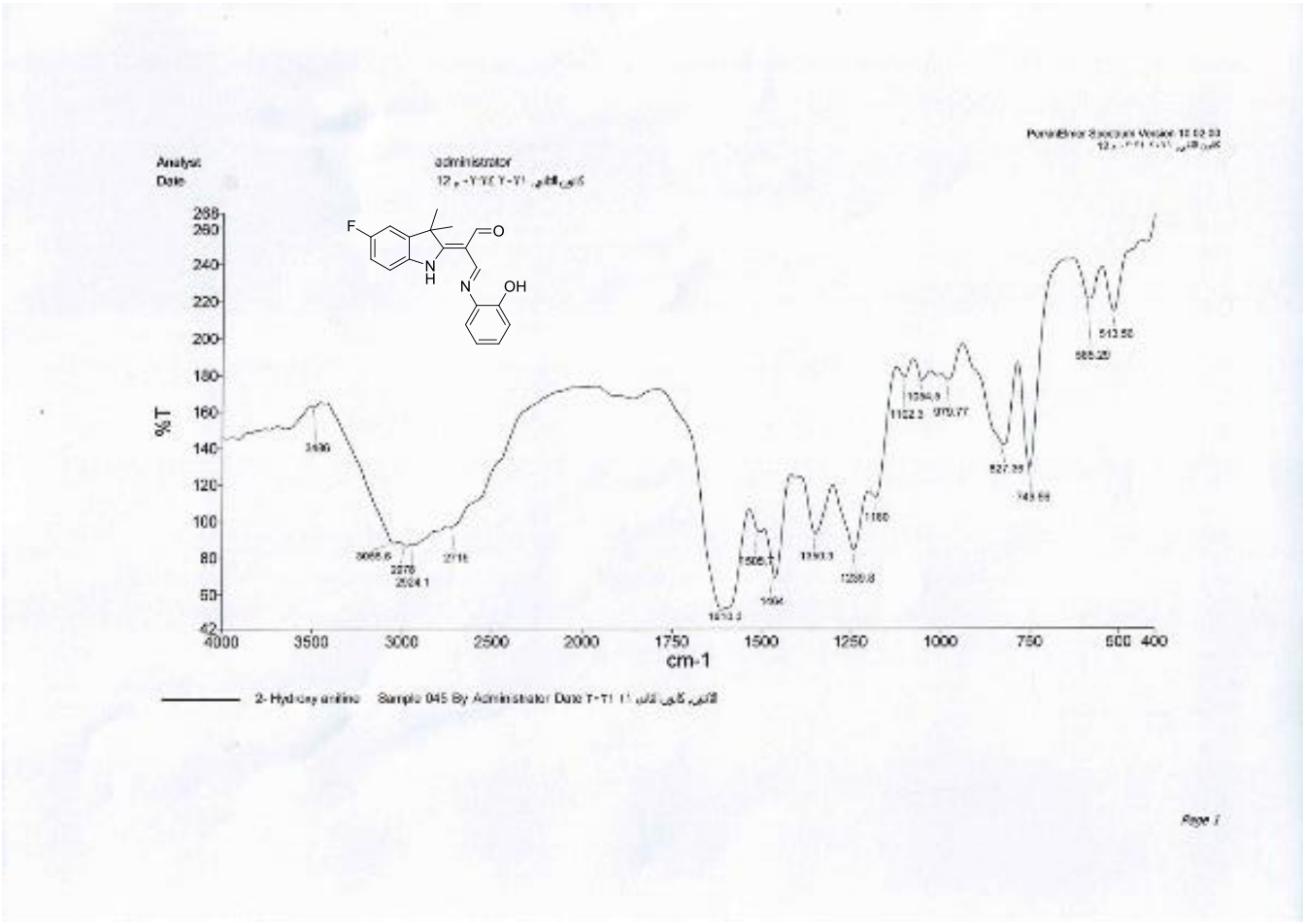
APPENDIXE



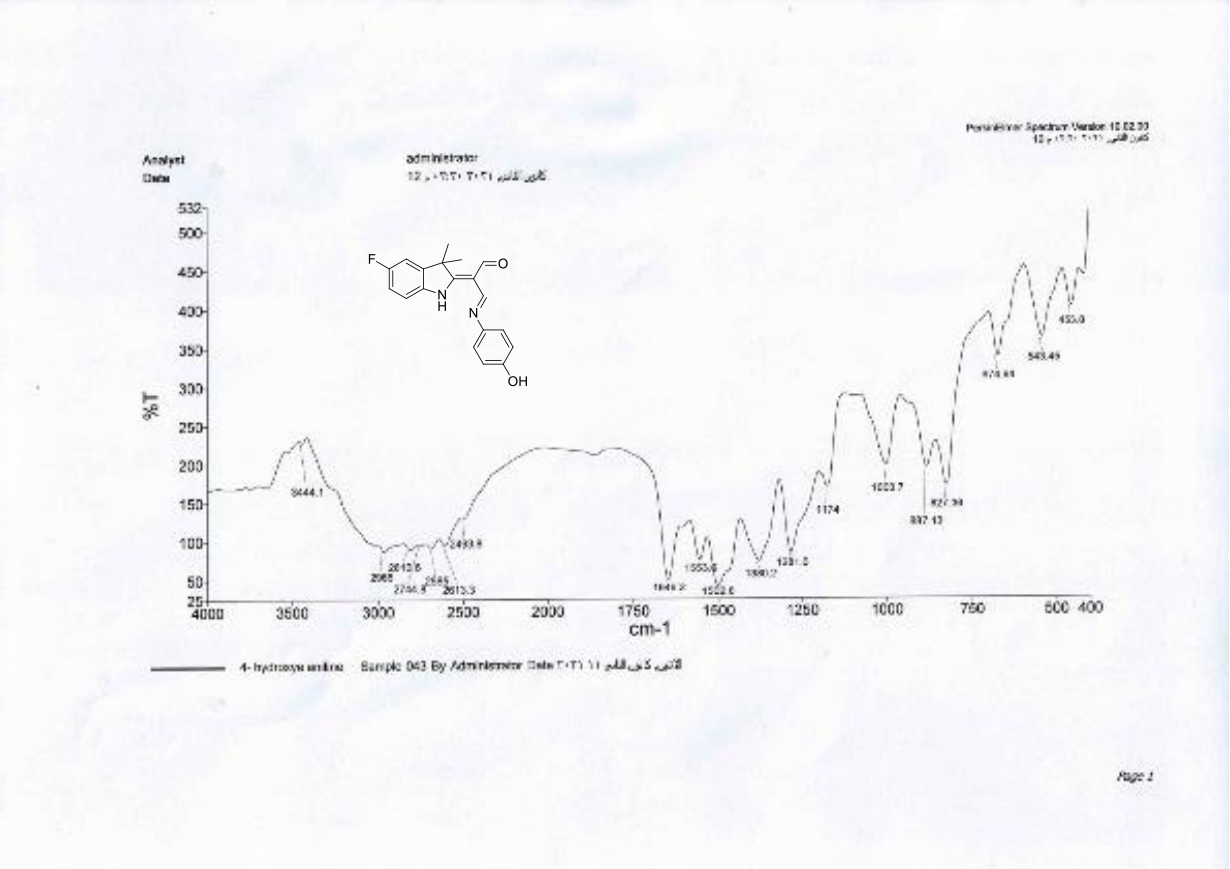
Appendix (4. 1): The FTIR spectra of the compound (6)



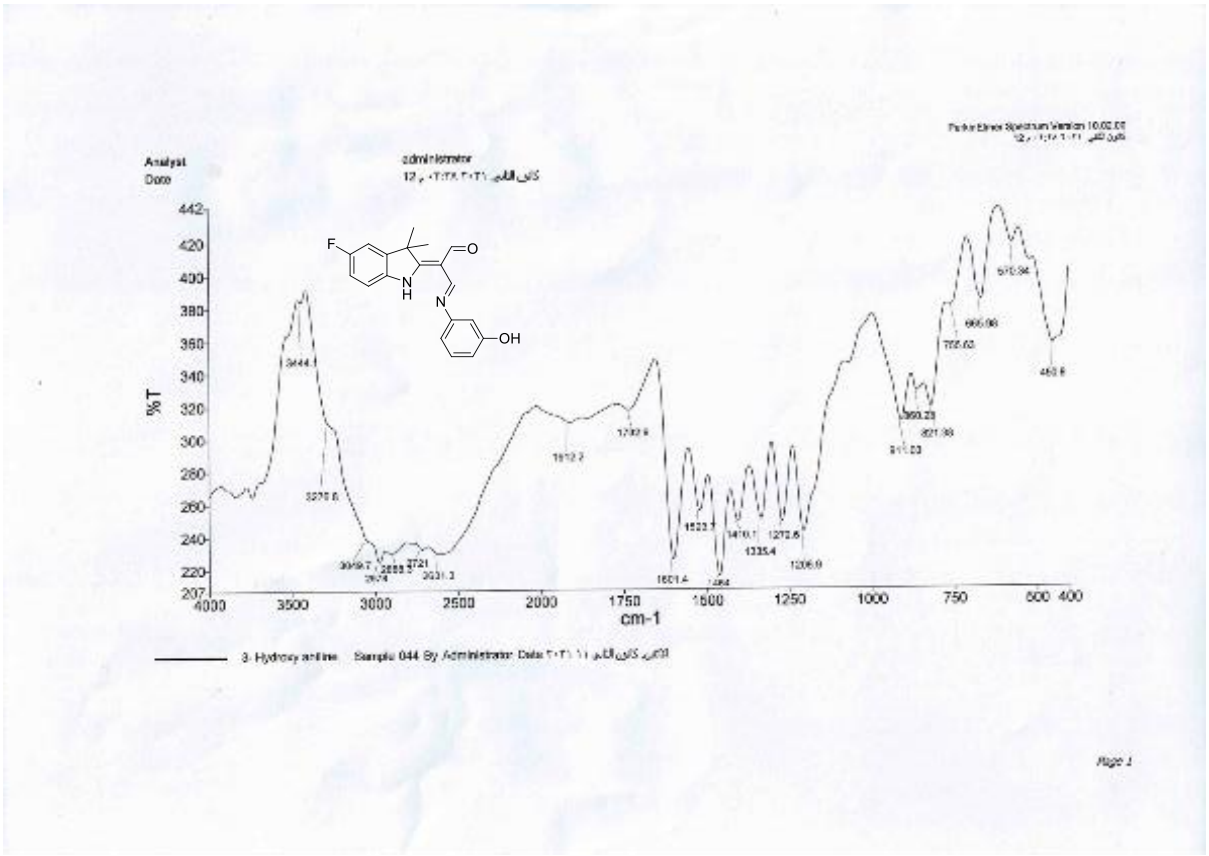
Appendix (4. 2): The FTIR spectra of the compound (7)



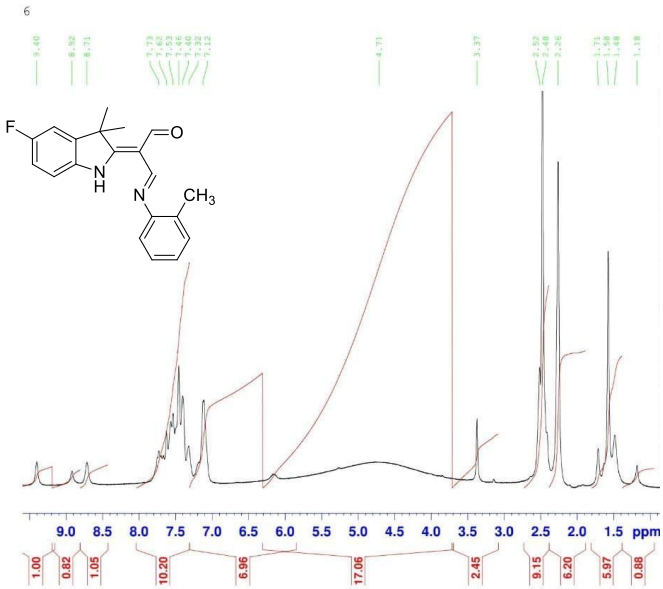
Appendix (4. 3): The FTIR spectra of the compound (8)



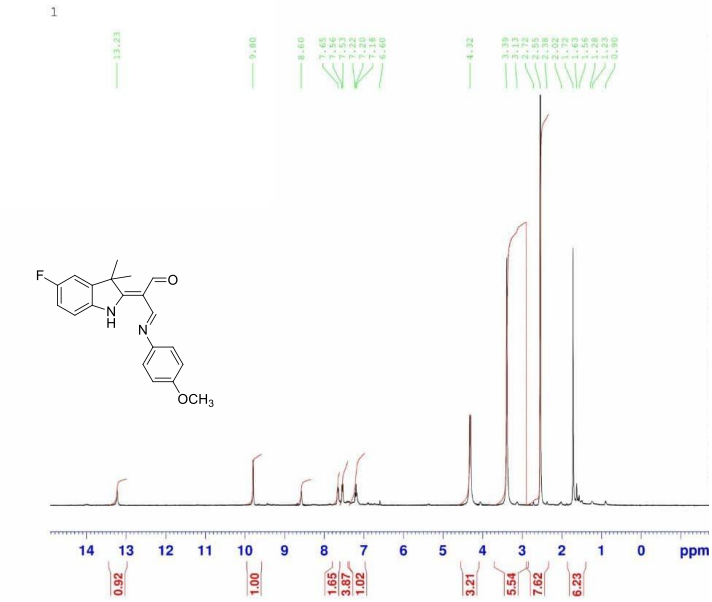
Appendix (4. 4): The FTIR spectra of the compound (9)



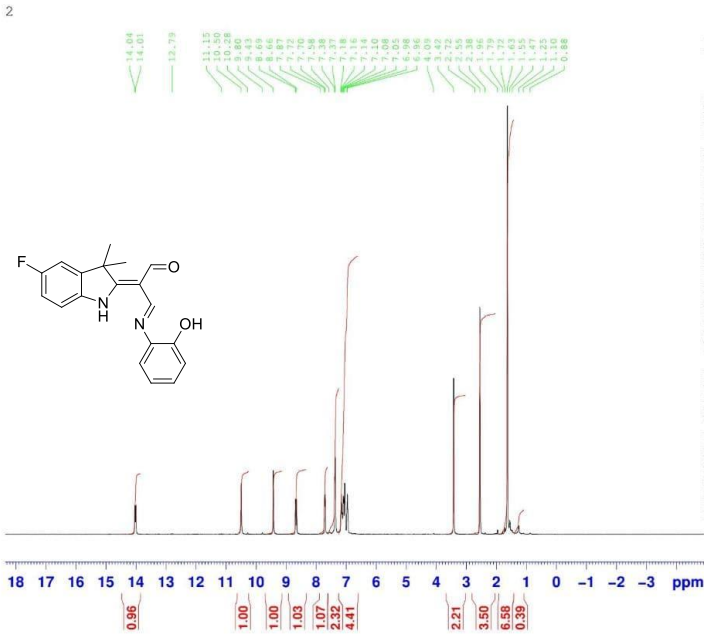
Appendix (4. 5): The FTIR spectra of the compound (10)



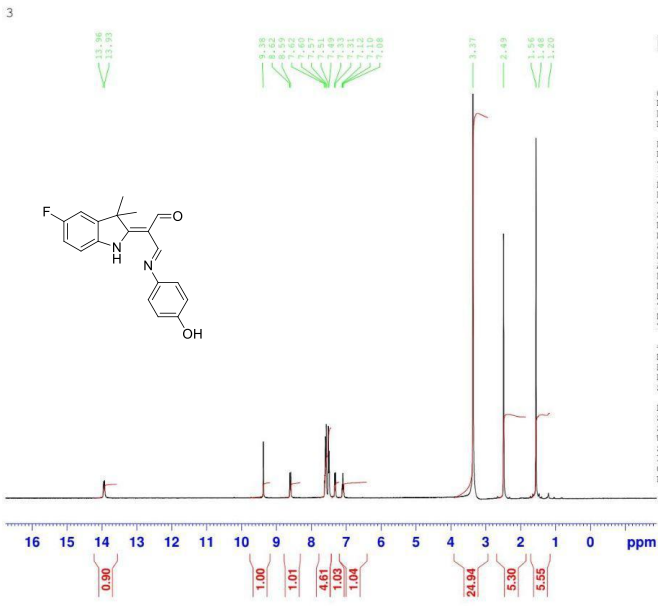
Appendix (4. 6): ^{1}H-NMR spectrum of the compound (6)



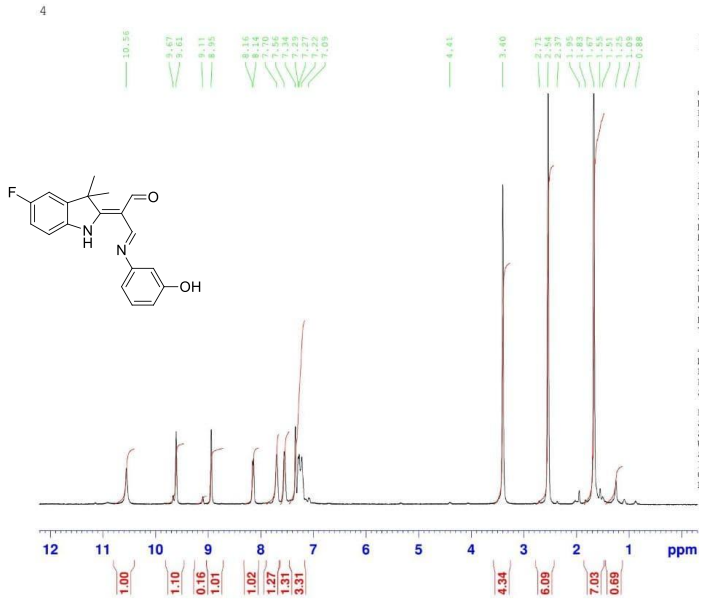
Appendix (4. 7): ^{1}H-NMR spectrum of the compound (7)



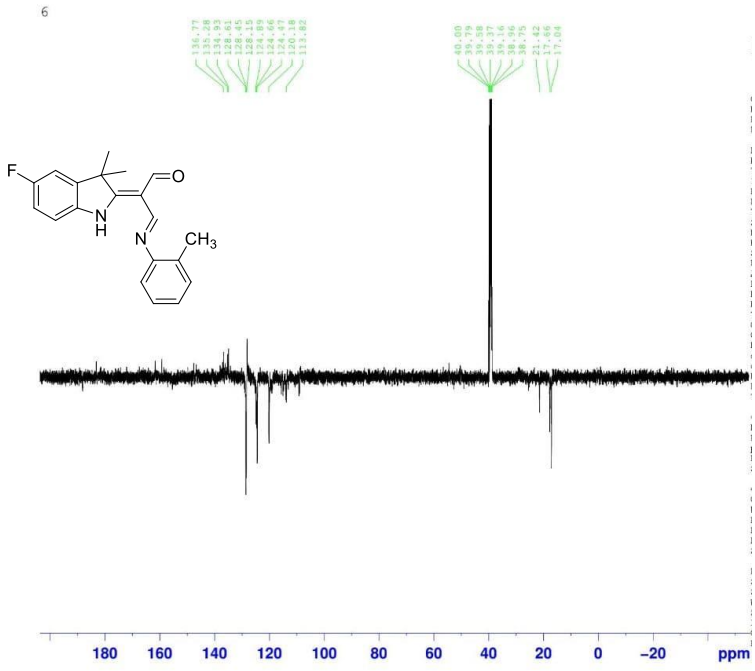
Appendix (4. 8): ^{1}H-NMR spectrum of the compound (8)



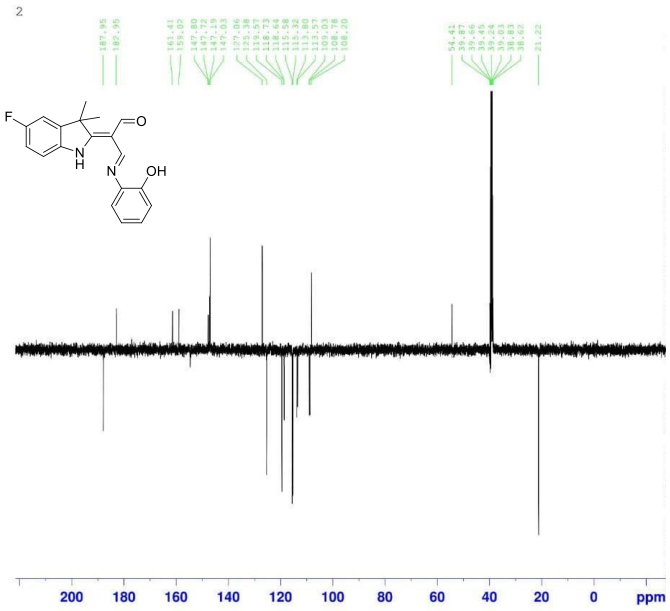
Appendix (4. 9): ^{1}H-NMR spectrum of the compound (9)



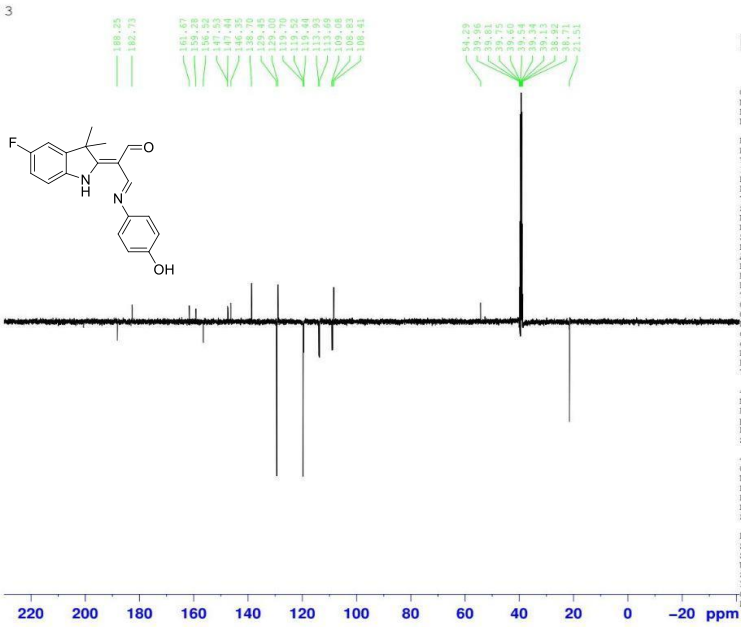
Appendix (4. 10): ^{1}H-NMR spectrum of the compound (10)



Appendix (4. 11): APT ^{13}C NMR spectrum of the compound (6)



Appendix (4. 13): APT ^{13}C NMR spectrum of the compound (8)



Appendix (4. 14): APT ^{13}C NMR spectrum of the compound (9)

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REFERENCES

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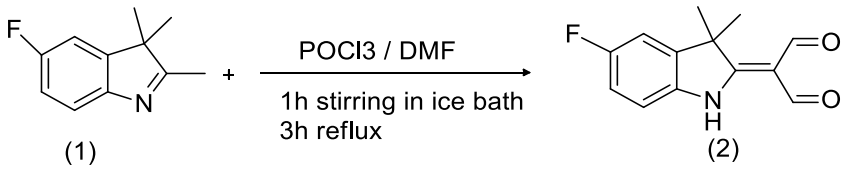
الخلاصة

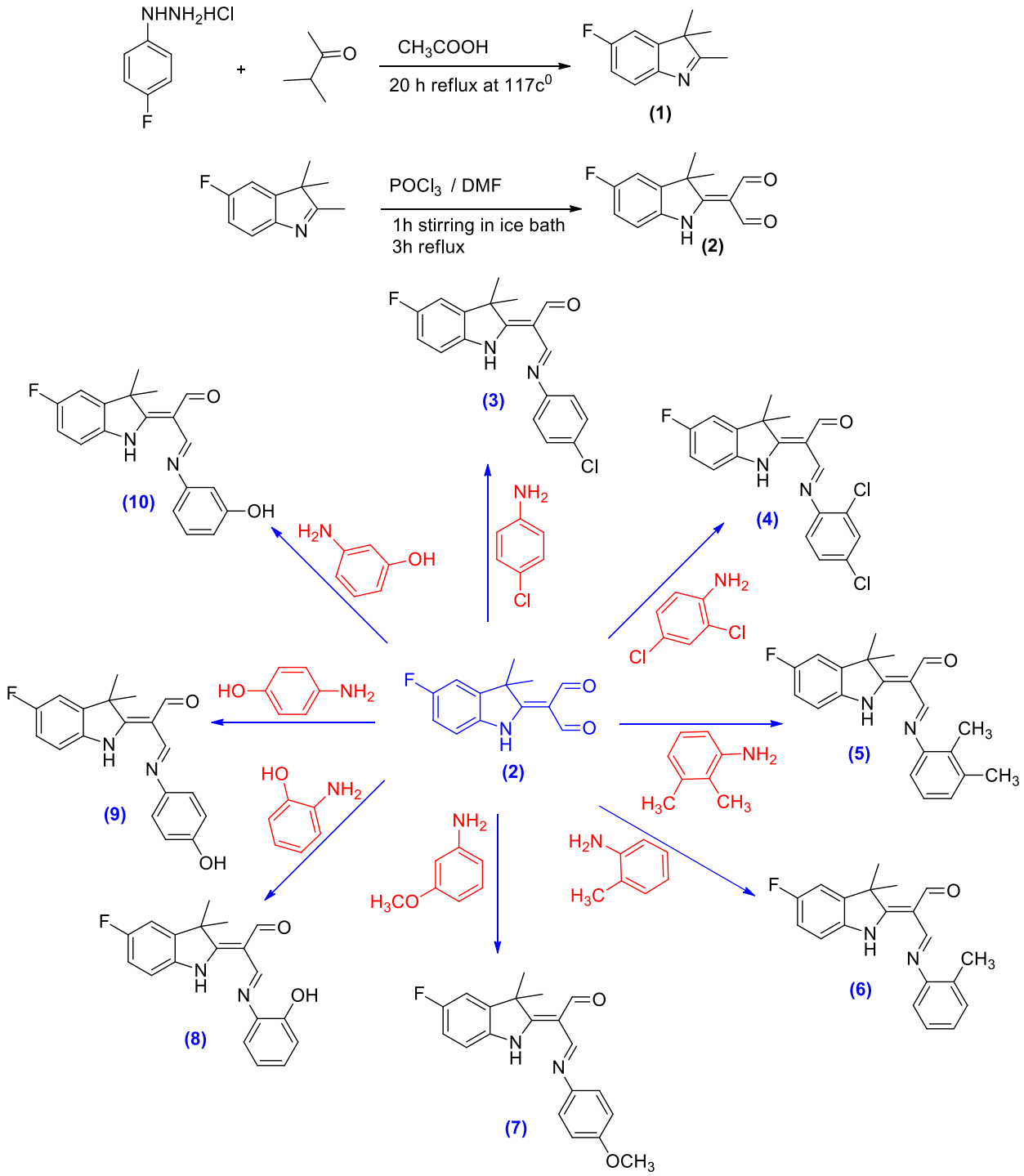
في هذه الدراسة تم تحضير مركبات جديدة لقواعد شيف بنجاح من احد مشتقات الاندول .

التركيب الكيمياوي لجميع المركبات المحضرة الجديدة تم تشخيصه واثباته بعض التقنيات الطيفية مثل طيف الاشعة تحت الحمراء FTIR وطيف الرنين النووي المغناطيسي للبروتون والكاربون (^{1}H–NMR and ^{13}C– NMR APT) ونقاوتها اثبتت بواسطة كروماتوعرافيا الطبقة الرقيقة (TLC). معظم المركبات المحضرة الجديدة تم دراسة تأثيرها البيولوجي ضد خلايا سرطان العنق وسرطان الكبد **HepG2 and SK–GT2** وتم مقارنته مع الخلايا الطبيعية واظهرت نتائج جيدة مقارنة المركب 5- 2-(5-Fluror -3,3-dimethyl-1,3-dihydro-indol-2- ylidene)-Fluoro-2,3,3-trimethyl-3H-indole (**1**)

حضر بطريقة تفاعل فليسيمير من تفاعل مع ثلاثي كلوريد الفسفور وثنائي مثيل فورمايد كما موضح ادناه:



المركب (2) هنا اعتبر كمادة اساس لتحضير أنواع مختلفة من الايمينات (قواعد شيف) من خلال التفاعل مع الانيلين وبعض من مشتقاتها كما مبين في الشكل ادناه: تمت دراسة تأثير اربعه من المشتقات المحضرة ضد خط الخلية **HepG2 and SK–GT2** السرطان باستخدام تراكيز مختلفة 15.1, 31.2, 62.5, 125, 250, 400 and 500) مايكروغم / مل خلال فترة تعرض 24 ساعة. أظهرت هذه المركبات نسب تثبيط جيدة وواضحة ضد الخط المستخدم.



مخطط عام للمركبات الجديدة التي تم تحضيرها

وزارة التعليم العالي والبحث العلمي
جامعة ديالى/ كلية العلوم
قسم الكيمياء

تحضير قواعد شف جديدة من مشتقات الأندول ودراسة فعاليتها البايلوجية

رسالة مقدمة إلى
مجلس كلية العلوم / جامعة ديالى
وهي جزء من متطلبات الحصول على شهادة الماجستير في علوم الكيمياء

من قبل الطالب
عطارد صالح حسن
بكالوريوس علوم كيمياء / جامعة ديالى ٢٠١٧

بإشراف
أ.م.د. فاضل لفته فرج

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