بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Post Graduate Studies

كلية الدراسات العليا

Modeling QSAR, Docking and Grinding Preparation of Some 3.5 Pyrazoline Derivatives, Against AsPC-1 Human Pancreatic Cell Line and U251 Human Glioblastoma Cell Line

النمذجة والعلاقة الكمية بين البنية الجزئية والفعالية والإرساء والتحضير الطحني لبعض مشتقات البايرازولين ضد سرطان البنكرياس والدماغ

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A thesis submitted for the partial fulfillment of the requirement of the M.Sc. degree in chemistry

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March 2019

الآيــــة

قال تعالى :

﴿ إِنَّا عَرَضْنَا الْأَمَانَةَ عَلَى السَّمَاوَاتِ وَالْأَرْضِ وَالْجِبَالِ فَأَبَيْنَ أَن يَحْمِلْنَهَا وَأَشْفَقْنَ مِنْهَا وَحَمَلَهَا الْإِنسَانُ ۖ إِنَّهُ كَانَ ظَلُومًا جَهُولًا ﴾.

صدق الله العظيم

سورة الأحزاب الآية (72)

Dedication

I Dedicate this work with deep love and respect to

My Family

And to

My friends

Acknowledgement

Thanks to **ALLAH** the most gracious, the compassionate for given me strength and health to complete this work.

I would like to specially thank my supervisor, Prof. Dr. Ahmed Alsadig, for the patient guidance, encouragement and advice he has provided throughout my time as his student. I have been extremely lucky to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly.

I would like also to acknowledge everyone who played role in my academic accomplishment. First of all, my family who supported me with love and understanding. Secondly, I wish to express my sincere thanks to all faculty members of the department of Chemistry, for providing me with all necessary facilities.

Abbreviations

AM_1	Austin Model 1
ASA_H	Total hydrophobic surface area
F.Wt	Formula Weight
HOMO	Highest Occupied Molecular Orbital
IR	Infra-Red
Log p	log octanol/water partition coefficient
LUMO	Lowest unoccupied Molecular Orbital
M.F	Molecular Formula
M.P	Melting point
M.Wt	Molecular Weight
MLR	Multi Linear Regression
MR	Molar Refractivity
N.O	Number
P-Value	Probability Value
QSAR	Quantitative Structure Activity Relationship
RF	Retention Factor
S	Sample standard deviation
TLC	Thin Layer Chromatography
U.V	Ultra Violet
VSA	Van Der Waals Surface Area

Abstract

The QSAR model were successfully developed to calculate biological activity of 3, 5 di-substituted Pyrazoline derivatives as anticancer agents against AsPC-1 human pancreatic cell line and U251 human glioblastoma cell line, a good correlation models were obtained with R^2=74.30% and R^2=75.92% respectively, compounds (5-(4-bromophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole) and (N, N-dimethyl-4-[3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl] aniline) were found to be the most effective anticancer agent against AsPC-1 and U251 cell lines, with IC_{50} values 0.31mM and 0.062mM respectively.

Series of 3, 5 di-substituted Pyrazoline derivatives were successfully synthesized in two steps under solvent free condition, which verify one of goals of green chemistry is the use of less hazardous solvents. Step one was synthesized enone by condensing aldehyde with ketone via sodium hydroxide according to Claisen-Schmidt condensation. Step two was a nucleophilic addition of hydrazine hydrate to enon. The structures of these compounds have been elucidated by infra red, ultra violet, thin lyer chromatography and Melting Point.

الخلاصة

تم حساب النشاط البيولوجي لمشتقات مركبات 3,5 بايرازولين ضد سرطان البنكرياس و سرطان الدماغ عن طريق ايجاد العلاقة بين النشاط البيولوجي والخصائص الفيزيائية او الفيزيوكيميائية لمركبات لها نفس التركيبة البنائية. كما تم ايضا تقيم فعاليتها من خلال دراسة ارتباط هذة المركبات مع بعض البروتينات المسببة للمرض . حيث وجد ان المركب (5-(4-برمو فينيل)-3-(4-نايترو فينيل)-4,5 ثنائي الهيدروجين- بايرازولين) و مركب (ثنائي الميثيل-4-[3،(4-نايتروفينيل)-4,5-ثنائي الهيدروجين بايرازوا-5-يل] انيلين هما الاكثر فاعلية ضد سرطان البنكرياس و الدماغ بقيمة فاعلية تبلغ0.31 ملي مول و 0.062 ملي مول علي الترتيب.

سبعة من مشتقات مركبات البايرازولين تمت تخليقها بواسطة تكاثف الالدهيد مع الكيتون في وسط قاعدي لتكون مركبات الكاربونيل الغير مشبعة.ومن ثم الاضافة النيوكليفية لسلفات الهيدرازين الي مركبات الكاربونيل غير المشبعة في وجود الصوديوم هيدروكسيد كعامل مساعد. كلا التفاعلين تم بواسطة الطحن في عدم وجود المذيبات كواحدة من طرق الكيمياء الخضراء في تقليل مخاطر المخلفات الكيميائية علي البيئة ، حيث اثبتت هذة الطريقة فعاليتها في اعطاء نسب عالية من النواتج. تم التاكد من البنية التركيبة للمركبات عن طريق مطياف الاشعة تحت الحمراء، التحليل الطيفي للاشعة فوق البنفسجية، كروماتوغرافيا الطبقة الرقيقة و درجة الانصهار .

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Chapter One

1. Introduction

1.1 Computational Chemistry:

The term theoretical chemistry may be defined as the mathematical description of chemistry. The term computational chemistry is generally used when a mathematical method is sufficiently well developed that it can be automated for implementation on a computer. Note that the words ``exact'' and ``perfect'' do not appear in these definitions. Very few aspects of chemistry can be computed exactly, but almost every aspect of chemistry has been described in a qualitative or approximately quantitative computational scheme. The biggest mistake a computational chemist can make is to assume that any computed number is exact. However, just as not all spectra are perfectly resolved, often a qualitative or approximate computation can give useful insight into chemistry if the researcher understands what it does and does not predict(Young, D.C. 2001).

Computational chemistry (also called molecular modeling; the two terms mean about the same thing) is a set of techniques for investigating chemical problems on a computer. Questions commonly investigated computationally are:

Molecular geometry: the shapes of molecules – bond lengths, angles and dihedrals. Energies of molecules and transition states: this tells us which isomer is favored at equilibrium, and (from transition state and reactant energies) how fast a reaction should go. Chemical reactivity: for example, knowing where the electrons are concentrated (nucleophilic sites) and where they want to go (electrophilic sites) enables us to predict where various kinds of reagents will attack a molecule.

IR, UV and NMR spectra: these can be calculated, and if the molecule is unknown, someone trying to make it knows what to look for.

The interaction of a substrate with an enzyme: Seeing how a molecule fits into the activesite of an enzyme is one approach to designing better drugs.

The physical properties of substances: These depend on the properties of individual molecules and on how the molecules interact in the bulk material. For example, the strength and melting point of a polymer (e.g. a plastic) depend on how well the molecules fit together and on how strong the forces between them are they (Lewars, E. 2004).

1.2 Computational Tool:

The main tools available belong to five broad classes as described below:

1. Molecular mechanics (MM) is based on a model of a molecule as a collection of balls (atoms) held together by springs (bonds). By known of the normal spring lengths and the angles between them, and how much energy it takes to stretch and bend the springs, the energy can calculated of a given collection of balls and springs, i.e. of a given molecule; changing the geometry until the lowest energy is found enables us to do a geometry optimization, i.e. to calculate a geometry for the molecule. Molecular mechanics is fast: a fairly large molecule can be optimized in seconds on a powerful desktop computer (a workstation); on a personal computer the job might also take only a few seconds.

2. Ab initio calculations (is from the Latin: "from first principles") are based on the Schrodinger equation. This is a one of the fundamental equations of modern physics and describes, among other things, how the electrons in a molecule behave.The ab initio method solves the Schrodinger equation for a molecule and gives us the molecule's energy and wavefunction. The wavefunction is a mathematical function that can be used to calculate the electron distribution (and, in theory at least, anything else about the molecule). From the electron distribution we can tell things like how polar the molecule is, and which parts of it are likely to be attacked by nucleophiles or electrophiles.

3. Semi empirical (SE) calculations are, like ab initio, based on the Schrodinger equation.

4. Density functional calculations (often called density functional theory (DFT) calculations)are, like ab initio and SE calculations, based on the Schrodinger equation. However, unlike the other two methods, DFT does not calculate a wavefunction, butrather derives the electron distribution (electron density function) directly. A functionalis a mathematical entity related to a function.

5. Molecular dynamics calculations apply the laws of motion to molecules. Thus onecan simulate the motion of an enzyme as it changes shape on binding to a substrate, orthe motion of a swarm of water molecules around a molecule of solute (Lewars, E. 2004).

At one time, computational chemistry techniques were used only by experts extremely experienced in using tools that were for the most part difficult to understand and apply. Today, advances in software have produced programs that are easily used by any chemist. Along with new software comes new literature on the subject. There are now books that describe the fundamental principles of computational chemistry at almost any level of detail. A number of

books also exist that explain how to apply computational chemistry techniques to simple calculations appropriate for student assignments. There are, in addition, many detailed research papers on advanced topics that are intended to be read only by professional theorists (Lewars, E. 2004).

1.3 Quantitative Structure Activity Relationships (QSAR):

To search for the existence of any correlation among different objects or observation is a natural human tendency.Structure activity relationship (QSAR) modeling was evolved from the idea of possible existence of a mathematical correlation between the nature of a chemicals and their behavioral manifestation. Following the initial observation, the "chemical nature" was conceptualized and explored in terms of information extractable from the structure and property of chemicals.

Properties elicited by chemicals are significantly influenced by the nature of their chemical structure (branching, functional groups, electronegative behavior, stero chemical features, etc.), as well as different physicochemical properties (hydrophobicity, electronic nature, steric influence, etc.). The development of suitable descriptors (predictor variables) possessing a potential diagnostic nature in extracting the chemical attributes can help in exploring the mechanistic basis involved in a chemical biological process following mathematical model development. Thus, QSAR studies allow a rational explanation for the behavioral manifestation of chemicals accompanied with providing knowledge on the mechanism involved, thereby creating an opportunity to tune the behavior of a chemical. As it provides a good alternative method to laboratory experimentation, at least for initial screening, QSAR is utilized in costly research like the design drug molecules and assessment of chemical hazards (Roy, K.; *etal* 2015).

Quantitative structure activity relationship (QSAR) is one of the most widely used tools to design newer candidates for several therapeutic areas. It provides useful insights into the structural features which are responsible for the biological activity and help to generate a mathematical model which can predict activity of untested compounds quantitatively. QSAR study usually leads to a predictive formula by correlation of physicchemical properties of a congeneric series with the biological activity (Soni, H., *et al* 2015).

1.4 Molecular Docking:

Molecular docking has become an increasingly important tool in drug discovery. The molecular docking also can be used to model the interaction between a small molecule and a protein at the atomic level. This which allows us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes (Jasril, J. *et al* 2017).

1.5 Solvent Free method:

Though it is a common practice to run the organic reactions in solvent media, the chemists' concern to minimize the environmental pollution caused by solvents and also their academic interest in solid-solid reactions have led them in recent times to develop methodologies for solvent free reactions with considerable success.

A general assumption with regard to organic reactions is that they are performed in a solvent medium. The rationale behind this concept is simple. That is, the reactants can interact effectively if they are in a homogeneous solution, which facilitates the stirring, shaking or other ways of agitation, where by the reactant molecules come together rapidly and continuously.A solvent-free or solid state reaction may be carried out using the reactants alone or incorporating them in clays, zeolites, silica, alumina or other matrices.

Thermal process or irradiation with UV, microwave or ultrasound can be employed to bring about the reaction. Solvent-free reactions obviously reduce pollution, and bring down handling costs due to simplification of experimental procedure, work up technique and saving in laboratory. These would be especially important during industrial production. Often, the products of solid state reactions turn out to be different from those obtained in solution phase reactions. This is because of specific spatial orientation or packing of the reacting molecules in the crystalline state. This is true not only of the crystals of single compounds, but also of co-crystallized solids of two or even more reactant molecules. The host-guest interaction complexes obtained by simply mixing the components intimately also adopt ordered structure. The orientationrequirements of the substrate molecules in the crystalline state have provided excellent opportunities to achieve high degree of stereo selectivity in the products. This has made it possible to synthesize chiral molecules from prochiral ones either by complexation with chiral hosts or formation of intermediates with chiral partners(Nagendrappa, G. 2002).

1.6 Enone (α, β –carbonyl) compounds:

α, β-unsaturated enones are important intermediates in many addition reactions of nucleophiles due to inductive polarization of carbonyl group at the β-position. Several strategies for the synthesis of these systems based on the formation of carbon-carbon bond have been reported, and among them the direct aldol condensation and Claisen-Schimdt condensation still occupy prominent positions (Bora, *et al* 2005).
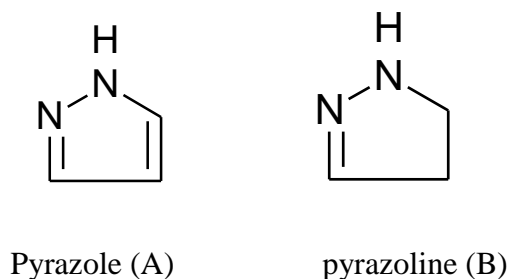
1.7Pyrazolines:

α,β -Unsaturated ketones constitute an important class of naturally occurring flavonoid compounds that exhibit a wide spectrum of biological activities such as anticancer, anti-inflammatory, antiviral and antibacterial activities etc. These are well-known intermediates for the synthesis of a large number of bioactive molecules, such as pyrazolines, pyrimidines, isoxazolines and thiazolines.

Amongst nitrogen containing five membered hetero cycles, pyrazolines have proved to be the most useful framework for biological activities. The dihydropyrazoles are called pyrazolines. Pyrazolines have attracted attention of medicinal chemists for both with regard to heterocyclic chemistry and the pharmacological activities associated with them and various methods have been carried out for their synthesis. Considerable attention has been focused on pyrazolines and substituted pyrazolines due to their interesting biological activities. The pharmaceutical importance of these compounds lies in the fact that they can be effectively utilized as antibacterial, antifungal, antiviral, antiparasitic, antitubercular, herbicidal, fungicidal, analgesic, antioxidant, antipyretic, insecticidal, anticancer, antitumor, antidiabetic, anticonvulsant, antidepressant and anti-inflammatory agents. Moreover, many selectively fluoro-substituted pyrazolines show promising agrochemical properties (Jayaroopa, P. 2013).
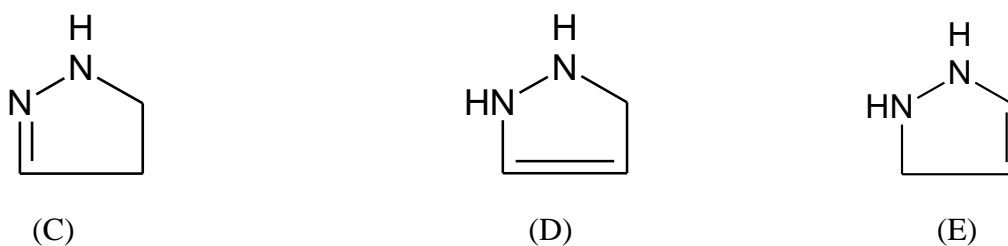
1.7.1 Structure of pyrazolines:

A five membered ring containing adjacent nitrogen atoms was termed as pyrazole (A) by Knorr in 1883. The dihydropyrazole is called as pyrazoline (B)(figure 1.1).

Pyrazole (A)   pyrazoline (B)

Figure (1.1)Prazoline Structure

If the nitrogen atom in pyrazoline is substituted, tautomerism is exhibited by the structures C. D and E. The taulomers C and D are more stable than E and are known as Δ^2-pyrazolines and Δ^3-pyrazolines respectively. No evidence for the stability of tauiomer E has been reported (Junghare, N. 2001).

(C)   (D)   (E)

Figure (1.2) Tautomeric Structure of Pyrazoline

1.7.2 Synthesis of Pyrazolines:

The most convenient method for the synthesis of pyrazoline is the action of hydrazine hydrate or hydrazine derivatives on α, β -carbonyl compounds. The intermediate formed is a hydrazone or phenylhydrazone respectively. This was subsequently cyclised to pyrazoline, in the presence of a suitable cyclizing reagent.

The most widely used reagent for cyclization of hydrazone is acetic acid/ Alcohol containing a little HCl also serves this purpose, but gives poor yield. The rate of cyclization of hydrazane to pyrazoline also depends upon the nature of R. It is in the order R=Ph>Me>H (Junghare, N. 2001).

The mechanism of the formation of pyrazoline was suggested by Aubagnac et al" From α, β-unsaturated ketones by 1, 4 addition of phenyl hydrazine through an adduct intermediate. The rate determining step is the dehydration giving hydrazone which has been supported from the activation energy calculation of l, 3, 5-tripyrazoline. The cyclization of pyrazoline is shown to be spontaneous (Junghare, N. 2001).

1.8 Aim and objectives:

- Modeling some of 3, 5 di-substituted pyrazoline derivatives using ACD lab program.
- To develop QSAR model to predict the biological activity of the modeling pyrazoline derivatives as potent AsPC-1 human pancreatic cell line and U251 human glioblastoma cell lines.
- To elucidate the interaction between the inhibitor molecules through molecular docking.
- To prepare seven compounds of some of 3, 5 di-substituted pyrazoline derivatives under solvent-free condition (grinding technique).

Chapter Two

2. Materials and Methods

2.1 Materials:

2.1.1Chemicals:

N, N DimethylaminoBenzaldehyde, Acetaldehyde Assay (20-30%) ALPH CHEMIKA India, P-BromoBezaldehyde, Benzaldehyde Minimum Assay 98% LOBA Chemica India, P-nitro AcetophenoneAssay G.C (min 98%) LOBA Chemie India, Acetone99% Assay (GC) (min 99%) LOBA Chemie, Sodium hydroxide Assay (95.0-100.5%) J.T Baker Sweden, Hydrazinesulphate Alpha chemika India,Ethyl alcohol Assay (94.8-95.8 vol %) REAGENT DUKSAN.

2.1.2 Instruments:

IR300, Spectrometer (Thermo Nicolet), 7205 UV/Visible Spectrophotometer (JENWAY), Melting Point.

2.1.3 Apparatus:

A mortar, pistol, spatula, beakers 50ml, glass rod and funnel, (all glass ware were Pyrex type).

2.1.4 Thin layer chromatography (TLC):

Thin layer chromatography was carried out using pre-coated LK5DF silica gel from WhatmanInc, by using n-hexane and ethyl acetate (1:3) as mobile phase.

2.1.5 Programs software:

- **2.1.5.1** ACD lab/chemsketck free ware 2015 downloaded fromwww.acd labs.com:

ChemSketch (we tested free ware 2015) is a graphic interface that can be used as the front end for a host of programs sold by Advanced Chemistry Development.
Both free and commercial versions are available. It is a two-dimensional structure drawing program primarily designed for organic molecules. Although the drawing mode is essentially a two-dimensional drawing routine, it is also possible to rotate the molecule in three dimensions. The program automatically keeps track of the number of hydrogens bonded to each atom. The reviewer felt that the molecule sketch mode was convenient to use.

- **2.1.5.2 Moe 2009.10:**

MOE (Version 2009.02) stands for molecular operating environment. The developers of this package took the unique approach of creating a programming language for writing molecular modeling software. The package currently includes molecular mechanics, dynamics, periodic boundary conditions, QSAR, a combinatorial builder, and many functions ideal for protein modeling, including multiple-sequence alignment and homology model building. Functions are also available for computing polymer properties and direction patterns. Several conformation search routines are included. Other property calculations include log P and molar refractivity calculation. The graphic interface is a multitasking environment that works well. The protein and carbohydrate builders are particularly convenient to use. The small-molecule builder has a selection of common organic functional groups as well as individual atoms for organics and common hetero atoms. There are a number of rendering modes to create fairly nice graphic images. However, the program does not have a way to save the display as an image file. MOE can be accessed through a Web browser also.

- **2.1.5.3 Minitab 17:**

Throughout Getting Started with Minitab 17, you analyze data from the shipping centers as you learn to use Minitab.

You create graphs and perform statistical analyses to identify the shipping center that has the most efficient computer system. You then concentrate on the data from this shipping center. First, you create control charts to test whether the shipping center's process is in control. Then, you perform a capability analysis to test whether the process is operating within specification limits. Finally, you perform a designed experiment to determine ways to improve those processes.
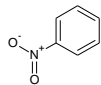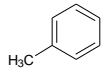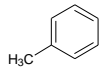
2.2 Methods:
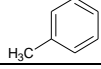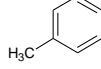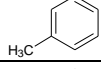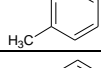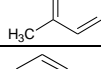
2.2.1 Modeling:

28Pyrzoline derivative compounds were modeled by using two-dimensional structure drawing mode (table 2.1).Then some of their properties were generated by using ACD lab and MOE 2009 software.

Table (2.1) Designed Pyrazoline compounds descriptors
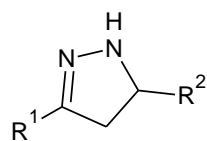
| N.O	R^1	R^2	M.F	F.Wt	S log P	S log_VSA4	AM1_dipole	AM1_HOMO
VIII	nitrophenyl	CH_3	$C_{10}H_{11}N_3O_2$	205.213	1.6807	3.1856	6.7783	- 9.2473
IX	nitrophenyl	N,N-dimethylaniline	$C_{17}H_{18}N_4O_2$	310.350	3.195	6.3712	8.1496	- 8.4847
X	nitrophenyl	bromophenyl	$C_{15}H_{12}BrN_3O_2$	346.178	3.8915	6.3712	5.5877	- 9.4216
XI	nitrophenyl	phenyl	$C_{15}H_{13}N_3O_2$	267.282	3.1290	6.3712	6.6009	- 9.2872
XII	CH_3	N,N-dimethylaniline	$C_{12}H_{17}N_3$	203.283	2.9549	3.1856	2.8672	- 9.2779
XIII	CH_3	bromophenyl	$C_{10}H_{11}BrN_2$	239.111	2.2584	3.1856	2.5694	- 8.2653
XIV	CH_3	phenyl	$C_{10}H_{12}N_2$	160.215	23.861	3.1856	2.4444	- 9.1275
XV	CH_3	CH_3	$C_5H_{10}N_2$	98.146	0.7441	0.0000	2.6843	-9.0872
XVI	CH_3	H	$C_4H_8N_2$	84.120	0.3556	0.0000	2.7302	-9.1239
XVII	CH_3	furyl	$C_8H_{10}N_2O$	150.178	1.7854	5.4488	2.1379	-9.1515
XVIII	CH_3	hydroxyphenyl	$C_{10}H_{12}N_2O$	176.215	1.8980	3.1856	2.8382	-9.0732
XIX	nitrophenyl	H	$C_9H_9N_3O_2$	191.187	1.2922	3.1856	6.5800	-9.3003
XX	nitrophenyl	furyl	$C_{13}H_{11}N_2O_3$	257.245	2.7220	8.6344	6.385	-9.4031

N.O	R¹	R²	M.F	F.Wt	S log P	S log_VSA4	AM1_dipole	AM1_HOMO
XXI	$O^-\text{N}^+$ nitrophenyl	phenol (OH)	$C_{15}H_{13}N_3O_3$	283.282	2.8346	6.3712	6.2701	-9.2432
XXII	H_3C-phenyl	CH_3	$C_{11}H_{14}N_2$	174.242	2.0809	6.3712	2.3963	-8.5856
XXIII	H_3C-phenyl	H_3C-N-CH$_3$ phenyl	$C_{18}H_{21}N_3$	279.379	3.5952	9.5567	2.3023	-8.2778
XXIV	H_3C-phenyl	Br-phenyl	$C_{10}H_{15}BrN_2$	315.208	4.2917	9.5567	2.8346	-8.7457
XXV	H_3C-phenyl	phenyl	$C_{16}H_{16}N_2$	236.312	3.5292	9.5567	2.2942	-8.6446
XXVI	H_3C-phenyl	H	$C_{10}H_{12}N_2$	160.216	1.6924	6.3712	2.4503	-8.6206
XXVII	H_3C-phenyl	furan (O)	$C_{14}H_{14}N_2O$	226.274	3.1222	11.8200	1.9828	-8.7375
XXVIII	H_3C-phenyl	phenol (OH)	$C_{16}H_{16}N_2O$	253.311	3.2348	9.5567	2.7362	-8.8869
XXIX	phenyl	CH_3	$C_{10}H_{12}N_2$	160.216	1.7725	3.1856	2.4071	-8.6969
XXX	phenyl	H_3C-N-CH$_3$ phenyl	$C_{17}H_{19}N_3$	265.353	3.2868	6.3712	2.4817	-8.3047
XXXI	phenyl	Br-phenyl	$C_{15}H_{11}BrN_2$	299.165	4.0462	6.3712	4.0419	-8.9773
XXXII	phenyl	phenyl	$C_{15}H_{14}N_2$	222.285	3.2208	6.3712	2.2687	-8.7610
XXXIII	phenyl	H	$C_9H_{10}N_2$	146.189	1.3840	3.1856	2.4662	-8.7379
XXXIV	phenyl	furan (O)	$C_{13}H_{12}N_2O$	212.247	2.8138	8.6344	2.7008	-8.8708
XXXV	phenyl	phenol (OH)	$C_{15}H_{14}N_2O$	238.284	2.9264	6.3712	2.6659	-8.9742

- M.F and F.wt were calculated using ACD lab.

- Slog p, Slog_VSA4, AM1_dipole and AM1_Homo were calculated using MOE2009.

2.2.2 Quantitative Structure Activity Relationship Experimental:

2.2.2.1Data set:

From literature (e.i.Karabacak, M. *etal* 2015) twelve compounds of 3, 5 di-substituted pyrazoline derivatives reported and evaluated for their biological activity effects as potential anticancer agents against AsPC-1 human pancreatic and U251 human glioblastoma cell lines respectively was selected. The twelve compounds were randomly divided into ten compounds as a training set and two compounds as test set. Structures of all the compounds were used for 2D-QSAR analysis and their anticanceractivity (PIC_{50}mM) are given inTable (2.2). For all these compounds, the values of biological activity ($IC_{50}\mu_{.M}$) were converted to (mM) then they were used in the negative logarithmic scale (PIC_{50}) to achieve normal distribution. Structures of all compounds were sketched by ACD Lab and then some descriptors were calculated using ACD/lab and MOE2009 software.

2.2.2.2 Multi Linear Regression Analysis (MLR):

It is statistic technique used to study the relation between one dependent variable and several independent variables to minimize the differences between actual and predicted values(Samira, M.; *etal*, 2011).

Minitab 17 software was used to check the correlation between the dependent variables (PCI_{50}) and the independent variables (descriptors) and also to check the inter-correlation between the descriptors themselves which have a correlated with the dependent variable to avoid the multicollinearity. Eight descriptorswere calculated by ACD lab (Formula weight, Molar refractivity, Molar volume, Index of refraction, Surface tension, Density, Polarizability and log P) plus twenty two descriptors (energy, slog p, slog p_VSA0, slog p_VSA1 …… slog p_VSA9, SMR_VSA0, SMR_VSA1, SMR_VSA2 ……. SMR_VSA6 and SMR_VSA7) were calculated by MOE2009.1. Then were subjected to correlation analysis to generate MLR for model (1). Beside this another four descriptors (AM1_dipole, AM1_Homo, AM1_lumo and ASA_H) were calculated by Moe 2009.01 to generate MLR for model (2)

Inter data >> stat >> basic statistics>> correlation.

Table (2.2) Observed PIC$_{50}$ for AsPC-1 and U251 cell line
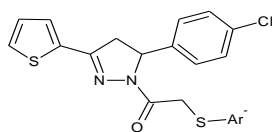
	Compound N.O	Ar	Observed PIC50mM	
			AsPC-1 cell line	U251 cell line
Training set	1		0.301029996	0.30103
	2		0.301029996	0.30103
	3		1.187086643	0.30103
	4		0.666149855	0.30103
	5		0.700492701	0.30103
	6		0.626720107	0.30103
	7		0.856048884	0.4055
	8		0.966174306	0.30103
	9		0.956637722	0.77833
	10		1.774690718	1.92445
Test set	11		0.945	0.88639
	12		1.207	0.1.15428

2.2.3 Docking:

2.2.3.1 Protein preparation:

Molecular docking was conducted using MOE 2009.10 software. The (5kk5) human pancreatic and (1NQU) human glioblastoma (brain cancer) protein inhibitors structures were downloaded from the protein data bank and they were used as the macromolecules for molecular docking. These proteins were kept in rigid,for then, all hydrogen atoms were added.

2.2.3.2 Preparation of the ligand:

The Pyrazoline derivatives which were designed (table 2.1) were used as the ligands and drawn using ACD/Lab software. These ligands were required to minimize the energies for then the minimized structures were subsequently prepared with the detected root of torsion and number of torsions for flexible-ligand docking using MOE 2009.10 software.

2.2.4 General Synthetic Methods:

2.2.4.1 Enone Synthesis (step1):

(2.5m mole) of the required ketone with (2.5m mole) of the required aldehyde and (0.1g, 2.5m mole) of sodium hydroxide were weighted and then transferred to the mortar and grinded by a pistol at room temperature until the desired product was obtained. Then the product was transferred by water to beaker (50ml) and was kept for 24 hours. After that it was filtered and the percentage yield was calculated. The solid formed was recrystallized with ethanol (95%).

2.6.4.2 Pyrazoline Synthesis (step2):

(1.25m mole) of the required enone (which prepared in step 1), with (0.163g, 1.25m mole) of hydrazine sulphate and (0.05g, 1.25m mole) of sodium hydroxide were weighted and then transferred to the mortar and grinded by a pistol at room temperature until the desired product was obtained. Then the product was transferred by water to beaker (50ml) and was kept for 24 hours. After that it was filtered and the percentage yield was calculated. The solid formed was recrystallized with ethanol (95%).
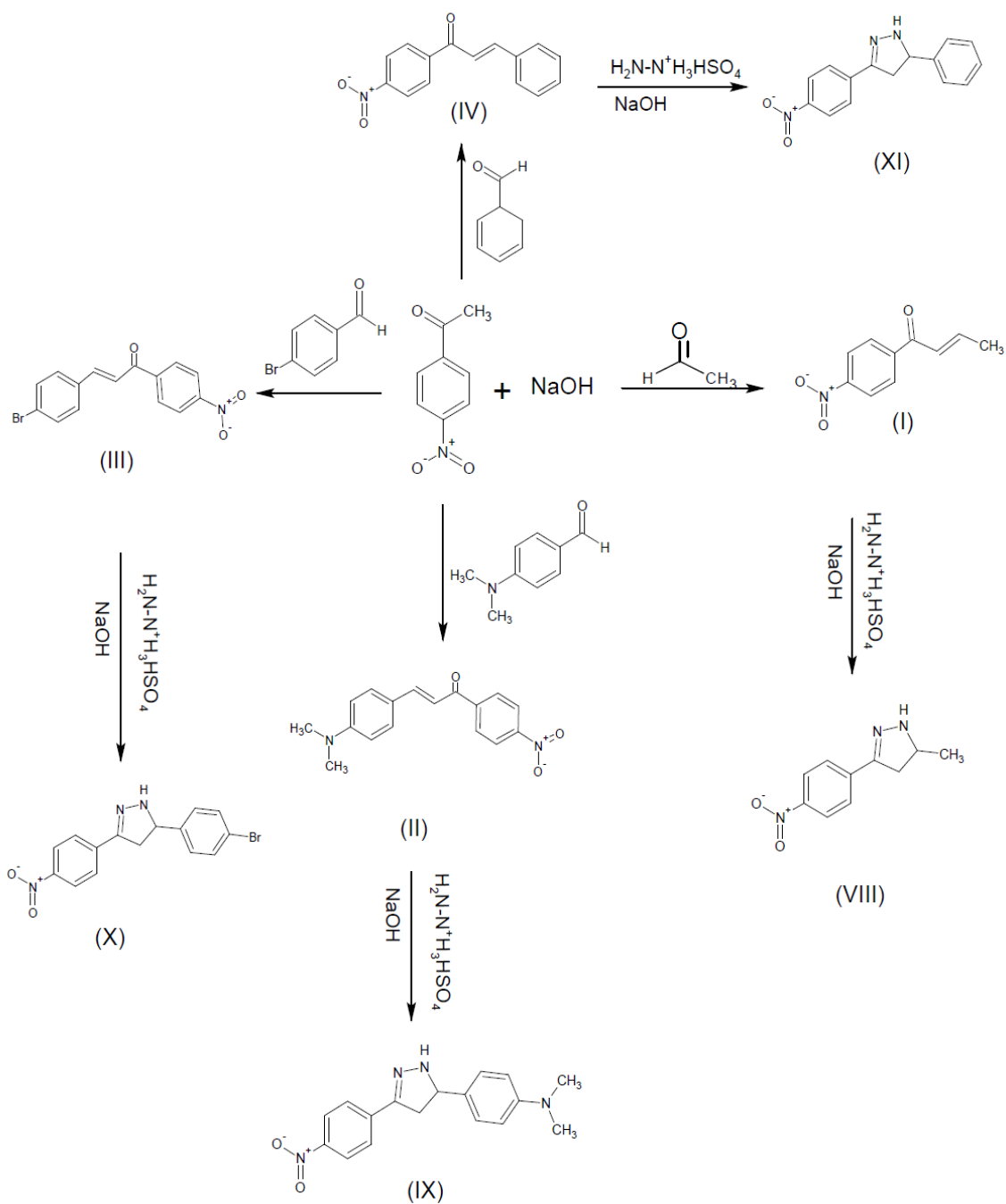
**Scheme (2.1) synthesis of the pyrazoline compounds via p-nitro acetophenone as a precursor**
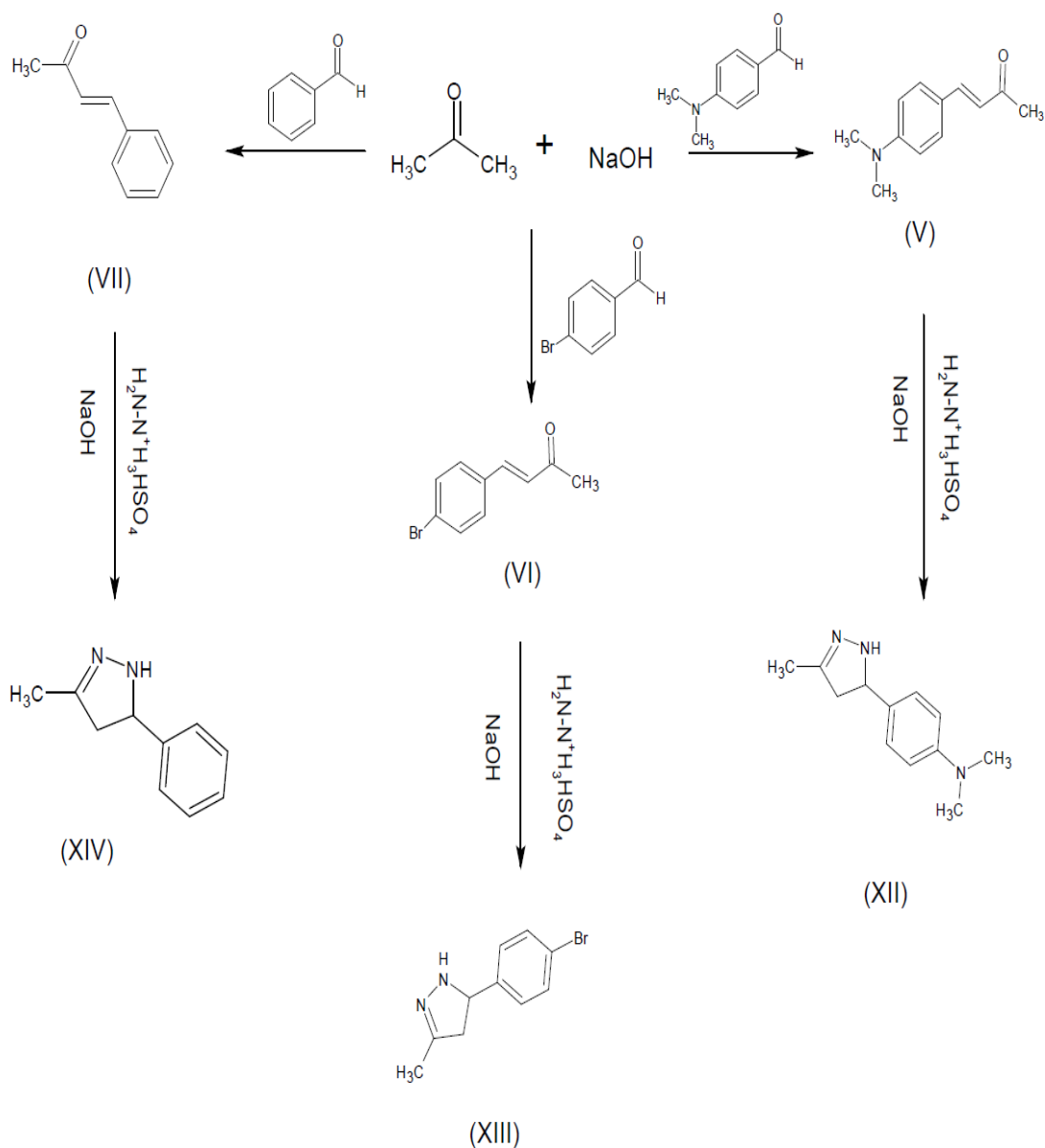
Scheme (2.2) synthesis of the pyrazoline compounds via acetone as a precursor

The synthesis of new pyrazoline derivatives (**VIII -XIV**) was carried out according to the steps shown in Scheme (2.1and 2.2). In the initial step, α, β unsaturated compounds (enone) was synthesized via the base-catalyzed Claisen-Schmidt condensation of p-nitro acetophenone and aceton with the required aldehayde, the ring closure reaction of the enone with hydrazine sulphate via the base-catalyzed afforded 3,5 di-substituted pyrazoline, all compounds obtained under solvent free condition methodology.

Table (2.3) chemical name of the synthesized enone compounds:
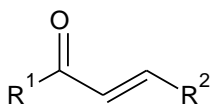
Compound N.O	R^1	R^2	Chemical Name
I	(4-nitrophenyl)	CH$_3$	(2E)-1-(4-nitrophenyl)but-2-en-1-one
II	(4-nitrophenyl)	(4-dimethylamino)phenyl	(2E)-3-[4-(dimethylamino)phenyl]-1-(4-nitrophenyl)prop-2-en-1-one
III	(4-nitrophenyl)	(4-bromophenyl)	(2E)-3-(4-bromophenyl)-1-(4-nitrophenyl)prop-2-en-1-one
IV	(4-nitrophenyl)	phenyl	(2E)-1-(4-nitrophenyl)-3-phenylprop-2-en-1-one
V	CH$_3$	(4-dimethylamino)phenyl	(3E)-4-[4-(dimethylamino)phenyl]but-3-en-2-one
VI	CH$_3$	(4-bromophenyl)	(3E)-4-(4-bromophenyl)but-3-en-2-one
VII	CH$_3$	phenyl	(3E)-4-phenylbut-3-en-2-one

- The chemical name was generated by using ACD/lab.

Table (2.4) Chemical Name of the synthesized Pyrazoline Compounds:
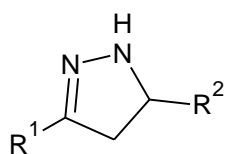
Compound NO.	R^1	R^2	Chemical Name
VIII		CH$_3$	5-methyl-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole
IX			N, N-dimethyl-4-[3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl] aniline
X			5-(4-bromophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole
XI			3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole
XII	CH$_3$		N,N-dimethyl-4-(3-methyl-4,5-dihydro-1H-pyrazol-5-yl)aniline
XIII	CH$_3$		5-(4-bromophenyl)-3-methyl-4,5-dihydro-1H-pyrazole/
XIV	CH$_3$		3-methyl-5-phenyl-4,5-dihydro-1H-pyrazole

- The chemical name was generated by using ACD/lab.

Chapter Three

3. Results and Discussion

3.1 Quantitative Activity Relationship:

From the MLR analysis results found that:

The PCI_{50} has a correlated with (log p), formula weight and slogp_VSA4. But also found that (log p) and (slog p) has a high correlated to each other so (slog p) was used instead of (log p) for AsPC-1 cell line data regression analysis. And PCI_{50} has a correlated with AM1_dipole and AM1_Humofor U251 cell line data regression analysis.

The statistical analysis or correlation analysis results based on the value of Pearson correlation (is a measure of the linear correlation between two variables X and Y, it has a value between +1 and −1, where 1 is total positive linear correlation, 0 is no linear correlation, and −1 is total negative linear correlation) and the P-value.

3.1.1 Fit regression model:

Minitab 17 software was used to generate the fit regression model (Stat >> regression >> regression >> fit regression model).

The QSAR model (with three descriptors) built due to P IC_{50} value for AsPC-1 cell line using multiple linear regressions (MLR) method is represented by the following equation:

pCI_{50} = -3.16 + 0.046 slog P + 0.00524 Formula weight + 0.2629 Slog_VSA4 **model (1)**

The QSAR model (with two descriptors) built due to IC_{50} value for U251 cell line using multiple linear regressions (MLR) method is represented by the following equation:

PCI_{50}= 0.0011 + 0.1706 AM1_dipole + 0.0219 AM1_humo **model (2)**

3.1.2 Validation of the Models:

Table (3.1) validation models

	S	**R^2**	**R^2 (adj)**	**R^2 (predi)**
Model (1)	0.269838	74.30%	61.44%	41.49%
Model (2)	0.193578	75.92%	74.13%	42.73%

R-squared (R^2) is a statistical measure of how close the data are to the fitted regression line.

For a statistically significant model, it is inevitable that the descriptors evolved in the equation should be least inter-correlated with each other, so the variance inflation factor (VIF) is the statistical factor detects multicolinearity in regression analysis. So the VIF values of these descriptors were found to be 1.81 for slog p and Slog_VS4, 1.54 for formula weight and 1.23 for Model (1), and 3.32 for each of the AM1_dipole and for AM1_Humo in model (2) . All the VIF values were found to be less than 10, which suggest very less multicollinearity within descriptors.

Table (3.2) Predictive value (PIC$_{50}$) of the data set through QSAR mod
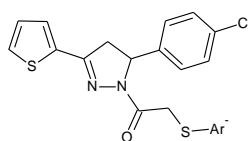
	Compound N.O	Ar	Predicted PIC50 (mM)	
			AsPC-1 cell line	U251 cell line
Training set	1		0.407	0.181
	2		0.782	0.359
	3		0.853	0.532
	4		0.703	0.699
	5		0.794	0.871
	6		0.428	1..034
	7		0.832	1.214
	8		0.809	1.384
	9		1.042	1.559
	10		1.757	1.746
Test set	11		0.430	0.181
	12		0.990	1.082
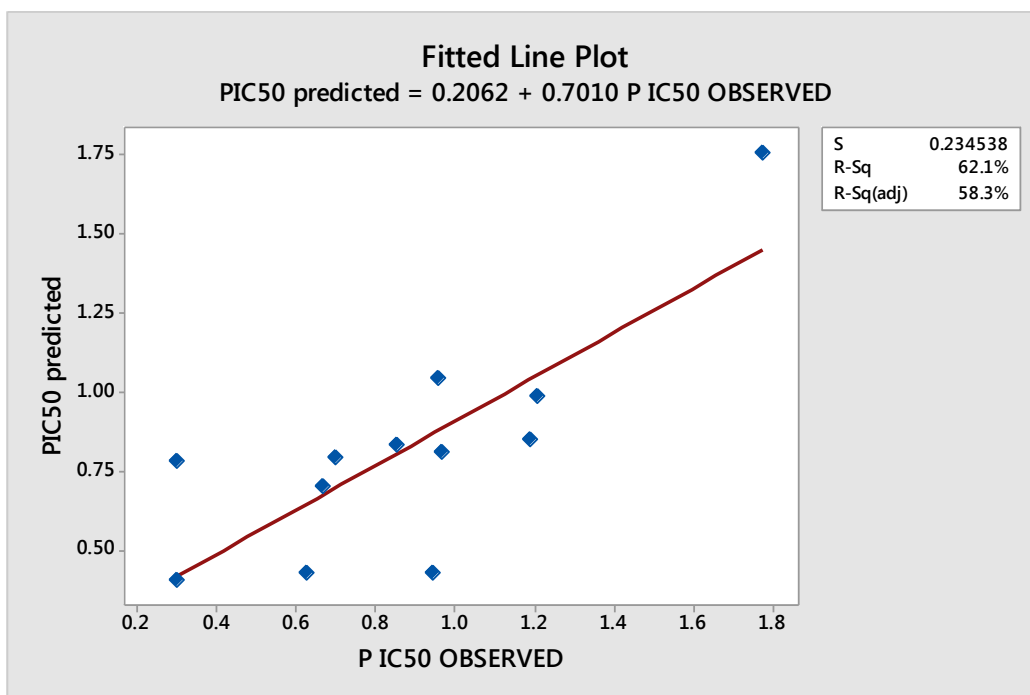
Figure (3.1) Predicted pancreatic anticancer activities by (MLR) in comparison with experimental values
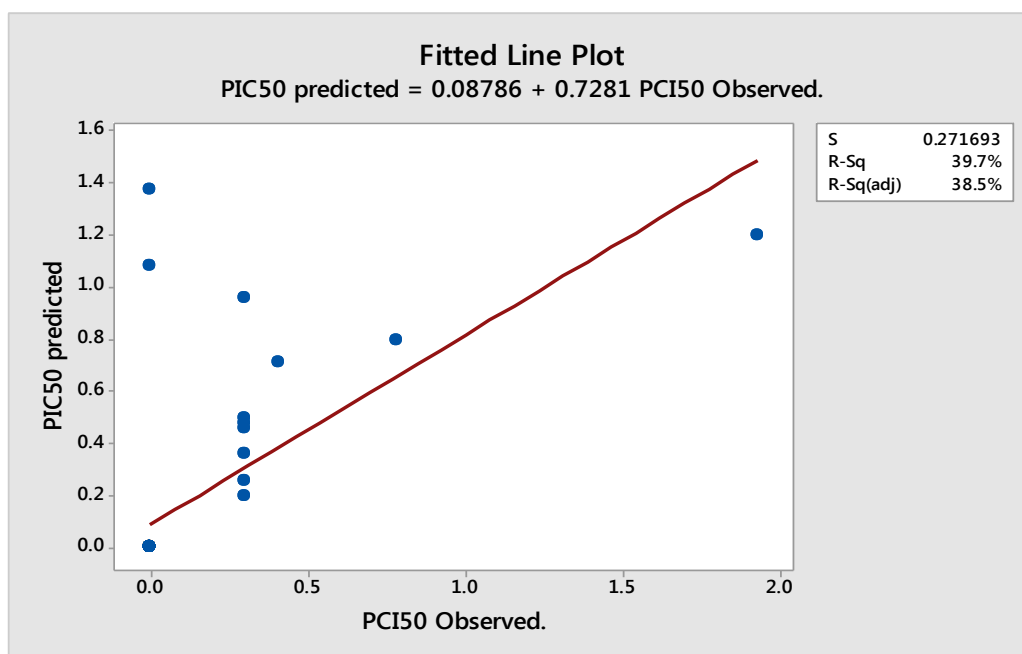
Figure (3.2) Predicted glioblastoma anticancer activities by (MLR) in comparison with experimental values
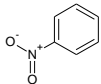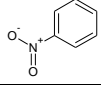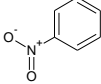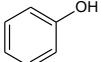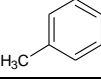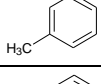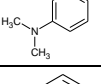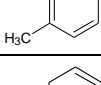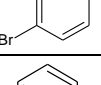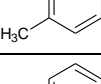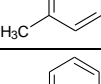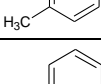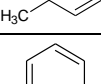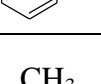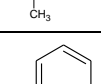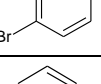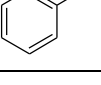
A good correlation was obtained with cross validation R^2=74.3 and 75.92% for model 1 (AsPC-1 cell line) and model 2 (U251 human glioblastoma cell lines) respectively. So the predictive power of this model is significantly good.

The most important result of this investigation is that the anticancer activity could be predicted using QSAR methods. So, the model proposed in this study shows moderate predictive power, and the compounds which were designed show have abiological activity against AsPC-1 human pancreatic cell line and U251 human glioblastoma cell lines (table 3.3).

**Table (3.3) Predicated biological activity for the designed pyrazoline compounds**
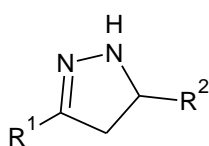
| Compound N.O	R$_1$	R$_2$	AsPc_1 cell line		U251	
			PIC$_{50}$	IC$_{50}$ (mM)	PIC50	IC$_{50}$ (mM)
VIII		CH$_3$	-1.1698774	14.791	0.9549621	0.111
IX			0.28819248	0.515	1.2056068	0.062
X			0.5079702	0.31	0.7480286	0.179
XI			0.05948016	0.872	0.9238239	0.119
XII	CH$_3$		-1.1213774	13.213	0.2870583	0.516
XIII	CH$_3$		-0.9656777	9.247	0.2584296	0.552
XIV	CH$_3$		-0.3853732	2.427	0.2182224	0.605
XV	CH$_3$	CH$_3$	-2.6114864	408.319	0.2600319	0.55
XVI	CH$_3$	H	-2.7028536	504.661	0.2670587	0.541
XVII	CH$_3$		-0.8584494	7.211	0.1654079	0.683
XVIII	CH$_3$		-1.3118312	20.512	0.2865938	0.516

Compound N.O	R₁	R₂	AsPc_1 cell line		U251	
			PIC_{50}	IC_{50} (mM)	PIC50	IC_{50} (mM)
XIX	nitrobenzene	H	-1.2612447	18.239	0.9199714	0.12
XX	nitrobenzene	furan	0.58315956	0.261	0.8844531	0.131
XXI	nitrobenzene	phenol	0.12977776	0.742	0.868353	0.136
XXII	toluene	CH_3	-0.476262	2.992	0.2218841	0.599
XXIII	toluene	N,N-dimethylaniline	0.98178159	0.104	0.2125886	0.612
XXIV	toluene	bromobenzene	1.20156455	0.0623	0.2931519	0.509
XXV	toluene	benzene	0.75307451	0.177	0.2031738	0.627
XXVI	toluene	H	-0.5676293	3.698	0.23033	0.589
XXVII	toluene	furan	1.27677496	0.053	0.1480144	0.711
XXVIII	toluene	phenol	0.82860687	0.148	0.2732726	0.533
XXIX	benzene	CH_3	-1.4014389	25.176	0.2212892	0.601
XXX	benzene	N,N-dimethylaniline	0.056631	0.878	0.2426051	0.571
XXXI	benzene	bromobenzene	0.26873828	0.538	0.4940453	0.321
XXXII	benzene	benzene	-0.1720813	1.486	0.1962743	0.637
XXXIII	benzene	H	-1.4928114	31.117	0.2304737	0.588
XXXIV	benzene	furan	0.35159284	0.445	0.267586	0.54
XXXV	benzene	phenol	-0.101789	1.265	0.2593676	0.551

3.2 Docking output:
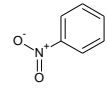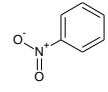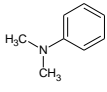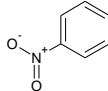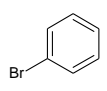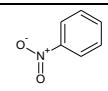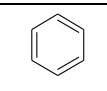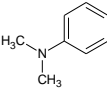
Table (3.4) Output of the Interaction between the ligand with5kk5 protein active sides:

Compound N.O	R₁	R₂	Estimated free energy of binding (kcal/mol)	Interactions	
				H-bond	Induced dipole interaction
VIII	O⁻–N⁺(=O)–phenyl	CH₃	-10.67	—	—
IX	O⁻–N⁺(=O)–phenyl	H₃C–N(CH₃)–phenyl	-18.57	—	Lys 570
X	O⁻–N⁺(=O)–phenyl	Br–phenyl	-17.445	Ser A542	—
XI	O⁻–N⁺(=O)–phenyl	phenyl	-16.864	Ser A542	—
XII	CH₃	H₃C–N(CH₃)–phenyl	-12.94	Lys A177	—
XIII	CH₃	Br–phenyl	-14.402	—	—
XIV	CH₃	phenyl	-12.947	—	—
XXIII	H₃C–phenyl	H₃C–N(CH₃)–phenyl	-16.66	Ser A542	—

From the interaction of the ligand with 5kk5 protein active side results (table 3.4) showed that compounds X, XI and XIII were observed to have hydrogen bonding between nitrogen atom of the ligand with 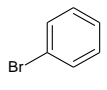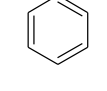residue Ser A542 (figures 3.5 and 3.6 respectively). Residue LysA177 showed also hydrogen bond interaction with the nitrogen atom of the ligand with compound XIII (figure 3.8). Compound IX was observed to display induced dipole interaction with residue Lys 570.

The strength of a single hydrogen bond usually varies from 2 to 5kcal/mol. Hence formation of single hydrogen bond is relatively weak. However, a significant amount of bonding stability can be achieved when there is formation of multiple hydrogen bonds between two such systems (Roy, K.; *et al* 2015).
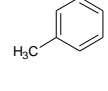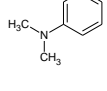
Table (3.5) Output of the Interaction of the ligand with 1NQU protein active side:

Compound N.O	R₁	R₂	Estimated free energy of binding (kcal/mol)	Interactions	
				pi-pi (π-π) stacking	Induced dipole interaction
VIII	(nitrophenyl)	CH₃	-17.0292	Phe 22 (arene-arene)	—
IX	(nitrophenyl)	(N,N-dimethylaminophenyl)	-20.81	Phe 22;Trp 57 (arene-arene)	Hiss 88
X	(nitrophenyl)	(bromophenyl)	-19.3455	Phe 22; Trp 57 (arene-arene)	—
XI	(nitrophenyl)	(phenyl)	-18.1315	Phe 22; Trp 57 (arene-arene)	—
XII	CH₃	(N,N-dimethylaminophenyl)	-15.5401	Phe 22 (arene-arene)	—
XIII	CH₃	(bromophenyl)	-15.8141	—	Hiss 88
XIV	CH₃	(phenyl)	-14.8892	Phe 22 (arene-arene)	—
XXIII	(methylphenyl)	(N,N-dimethylaminophenyl)	-18.39	Phe 22;Trp 57 (arene-arene)	Hiss 88

From the interaction of the ligand with 1NQU protein active side results (table 3.5), both compound (IX) and (XXIII) were observed displayed pi-pi stacking interaction with residue Phe 22 and Trp 57(figures 3.32 and 3.56).This refers to a special type of non-covalent attractive force due to overlapping of the orbital occurring between compounds possessing un-saturation; that is pi electrons. This interaction provides stability to proteins containing aromatic amino acid (Roy, K.; *etal* 2015).On another hand compounds (IX) and (XXIII) observed to have induce dipole interaction between nitrogen atoms of the lignd with residue Hiss 88. Comparing between compound IX and XXIII showed the same interaction clearly can observed the existence of withdrawing group (nitro) in compound IX shows high binding score (-20.81 kcal/mol) while the existence of donating group (methyl) shows less binding score(-18.39 kcal/mol).

Compounds X and IX were found to be the most effective anticancer agent against AsPC-1 and U251 cell lines, with IC_{50} values 0.31mM and 0.062mM respectively.

3.3 Synthesis of Pyrazoline Compounds:

Table (3.6) Reaction conditions of synthesized enone compounds:
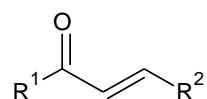
Compound N.O	R1	R2	Reaction time	Yield (g)	Yield (%)	Color	M.wt	M.P(C°)
I	nitrophenyl	CH_3	10 min	0.28	58.58	brown	191.2	64-69
II	nitrophenyl	N,N-dimethylaminophenyl	1 hour	0.67	90.4	Dark red	296.34	190-194
III	nitrophenyl	bromophenyl	1 hour	0.78	93.8	Yellow	332.17	155-158
IV	nitrophenyl	phenyl	5 min	0.53	79.9	brown	253.27	118-123
V	CH_3	N,N-dimethylaminophenyl	2 mint	0.37	84.09	Orange	189.27	75-77
VI	CH_3	bromophenyl	10 min	0.46	84.1	Light yellow	255.1	190-195
VII	CH_3	phenyl	15 min	0.15	41.0	Beige	146.2	97-100

- All the structures of the compounds were elucidated by IR, TLC, UV and melting point.

Table (3.7) Reaction conditions of pyrazolines compounds
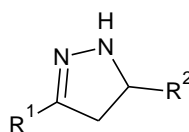
Compound N.O	R1	R2	Reaction time	Yield (g)	Yield (%)	Color	M.wt	M.P(C°)
VIII	$O^-\,N^+\!\!=\!\!O$ phenyl	CH$_3$	20 min	0.13	100.78	brown	205.21	145-150
IX	$O^-\,N^+\!\!=\!\!O$ phenyl	H$_3$C–N(CH$_3$)–phenyl	30 min	0.38	98.7	Dark red	310.35	159-161
X	$O^-\,N^+\!\!=\!\!O$ phenyl	Br–phenyl	30 min	0.74	88.76	Orange	346.18	137-145
XI	$O^-\,N^+\!\!=\!\!O$ phenyl	phenyl	10 min	0.05	93.6	Dark yellow	267.28	83-85
XII	CH$_3$	H$_3$C–N(CH$_3$)–phenyl	15 min	0.16	124.03	brown	203.28	189-192
XIII	CH$_3$	Br–phenyl	30 min	0.11	73.83	Light yellow	239.11	84-93
XIV	CH$_3$	phenyl	30 min	0.04	73.43	Yellow	160.22	125-130

- All the structures of the compounds were elucidated by IR, TLC, UV and melting point.

A series of some enone and 3, 5 di-substituted pyrazoline derivatives were successfully synthesized by using grinding as one of the solvent free methodologies. The results was obtained show that this method has given a high yields (Tables 3.6 and 3.7), all the compounds was successfully syntheized which were elucidated by TLC, IR and U.V.

3.3.1 Retro synthetic analysis (RSA) of Pyrazoline:
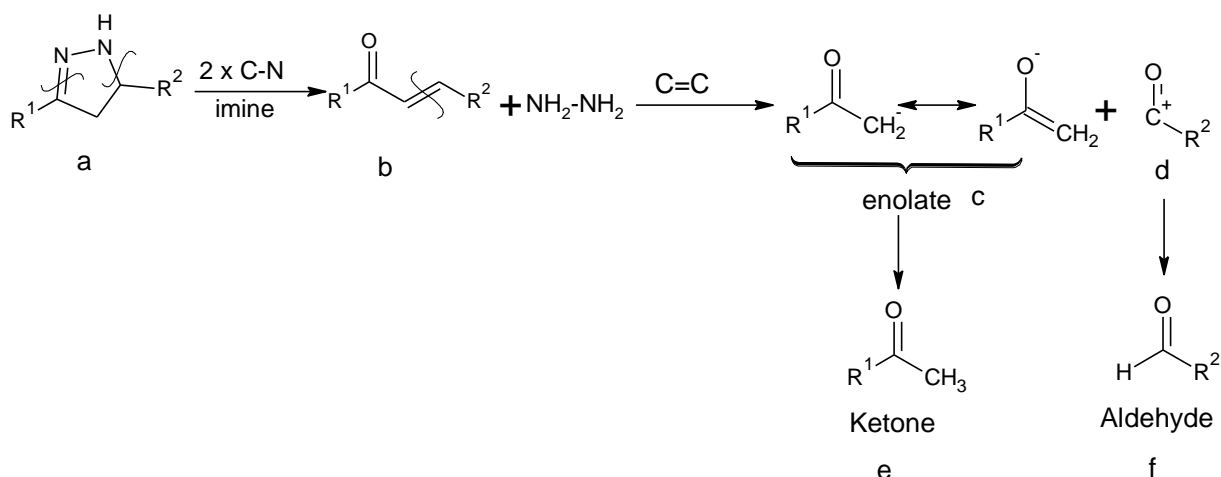
Scheme (3.1) Pyrazoline retro synthetic analysis

By the disconnected of tow C-N bonds of imine (a) we get back of enone (b), the disconnected of C=C of the enone (b) gives the nucleophile enolate (c) and electrophlie (d) which are the synthones of the reagents kenton (e) and aldehyde (f) respectively (Warren, S. Wyatt, P., 2008).
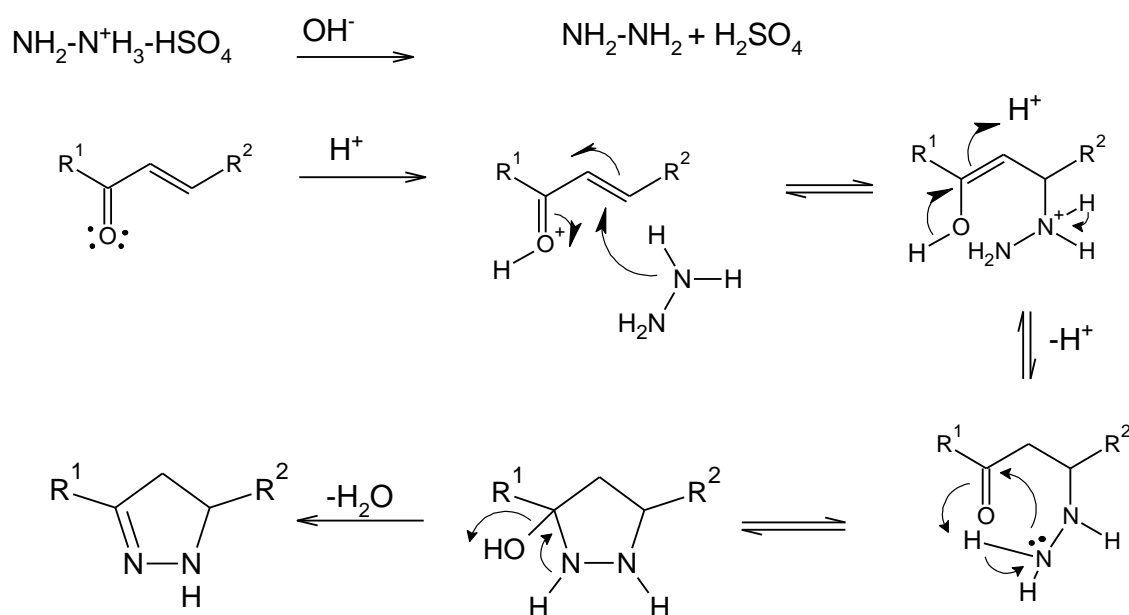
3.3.2 Mechanism:

3.3.2.1 Mechanism of Enone Synthesis:
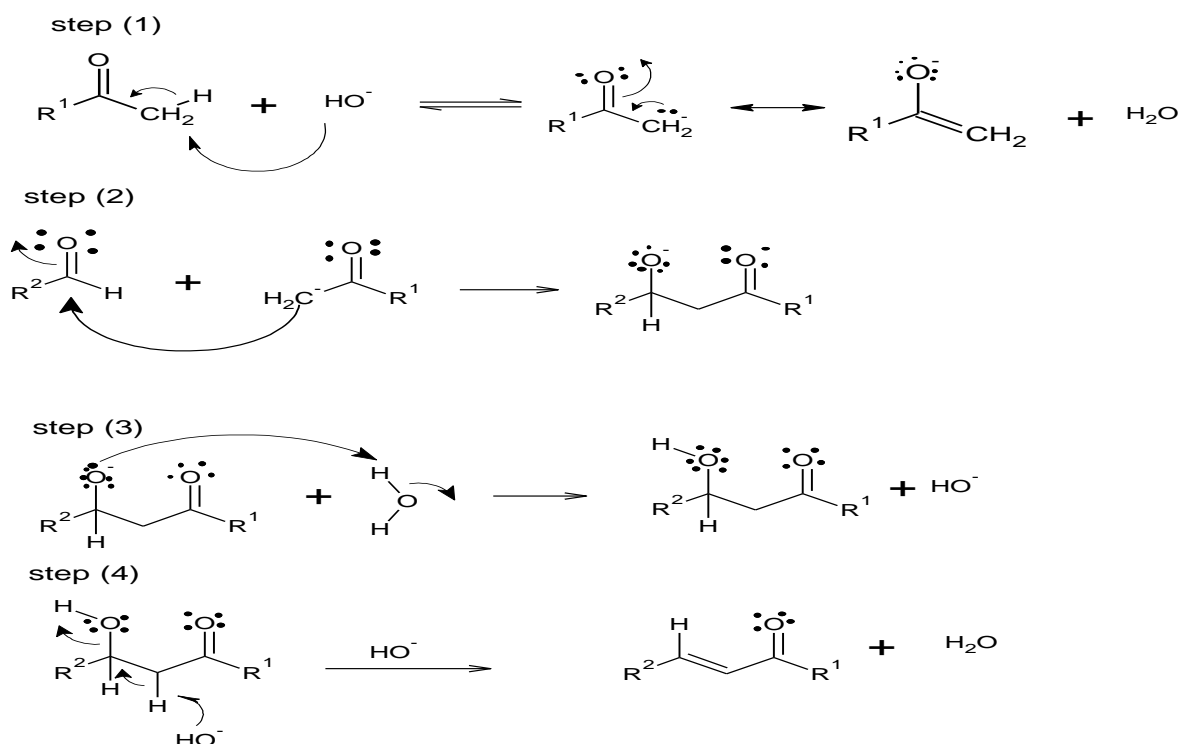
Scheme (3.2) Claisen-Schimdt condensation mechanism

The first step involves the acid-base reaction between a strong base (hydroxide ion) and a hydrogen located alpha to aketon carbonyl group to generate enolate form. The step (2) the enolate attacks the carbonyl group of the aldehyde (nucloephlic addition). Then the reaction undergoes an acid-base reaction with the remaining acidic alpha-hydrogen, followed by the loss of OH^- as a leaving group to give an enone, which has the alkene and the keton functional groups (step3 and 4). The net loss of H^+ and OH^- represents the loss of water .

3.3.2.2 Mechanism of Pyrazoline Synthesis:

Scheme (3.3) Pyrazoline synthesis mechanism

The first stop involves the protonated of carbonyl group ($H^+$is hard cation prefer 1, 2 addition than 1, 4 addition), according to the nature of the electrophile (enone) and particularly steric hindrance around the carbonyl group the 1, 4 conjugate addition is favorable followed by loss of H^+ . The ring closure reaction obtain by the 1, 2 direct addition followed by loss of water molecule (Patai, S., Rappoport, Z. 1989).

3.3.3 Characterizations of the Compounds:

Table (3.8) Physical parameters of synthesized enone compounds
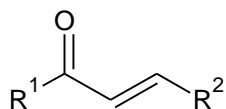

Compound N.O	R1	R2	R.F Value	Solvent system (n-hexane: ethyl acetate)	U.V (Ethanol 95%) λ max (nm)	Melting point C°
I	O⁻-N⁺(=O)-phenyl	CH₃	0.95	1:3	288	64-69
II	O⁻-N⁺(=O)-phenyl	H₃C-N(CH₃)-phenyl	0.45	3:1	458	190-194
III	O⁻-N⁺(=O)-phenyl	Br-phenyl	0.78	3:1	333	155-158
IV	O⁻-N⁺(=O)-phenyl	phenyl	0.90	1:3	310	118-123
V	CH₃	H₃C-N(CH₃)-phenyl	0.88	1:3	349	75-77
VI	CH₃	Br-phenyl	0.72	3:1	336	190-195
VII	CH₃	phenyl	0.70	3:1	326	97-100

32

Table (3.9) shows Physical parameters of synthesized Pyrazolines:
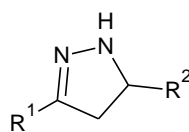
Compound N.O	R₁	R₂	R.F Value	Solvent system (n-hexane: ethyl acetate)	U.V (Ethanol 75%) λmax (nm)	Melting point C°
VIII	(4-nitrophenyl)	CH_3	0.93	1:3	309	145-150
IX	(4-nitrophenyl)	(N,N-dimethylaminophenyl)	0.22	3:1	294	159-161
X	(4-nitrophenyl)	(bromophenyl)	0.55	3:1	324	137-145
XI	(4-nitrophenyl)	(phenyl)	0.85	1:3	331	83-85
XII	CH_3	(N,N-dimethylaminophenyl)	0.90	1:3	398	189-192
XIII	CH_3	(bromophenyl)	0.70	1:3	337	84-93
XIV	CH_3	(phenyl)	0.61	1:3	302	125-130

Table (3.10) shows the IR spectrum data of enone compounds

IR (KBr) ν max (cm^{-1})
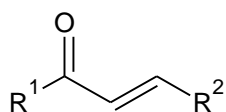
Compound N.O	R$_1$	R$_2$	C=O St.vib	C=C St.vib	C-H St.vib	C-H bending	O-N=O asym	O-N=O sym
I	(nitrophenyl)	CH$_3$	1690	1600	3104 (SP^2)	1399	1522	1347
II	(nitrophenyl)	(N,N-dimethylaminophenyl)	1648	1564	2906	1440	1523	1336
III	(nitrophenyl)	(bromophenyl)	1662	1593	_____	_____	1517	1327
IV	(nitrophenyl)	(phenyl)	1663	1599	___	____	1516	1335
V	CH$_3$	(N,N-dimethylaminophenyl)	1666	1599	2909	1438	____	___
VI	CH$_3$	(bromophenyl)	1649	1549	2918	1486	_____	____
VII	CH$_3$	(phenyl)	1706	1660	3028 (SP^2), 2916	1449	____	_____

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Table (3.11) shows the IR spectrum data of synthesized Pyrazolines

IR (KBr) ν max (cm^{-1})
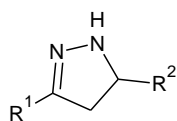
Compound N.O	R$_1$	R$_2$	C=N St.vib	N-H St.vib	C-H St.vib	C-H Bending	O-N=O asym	O-N=O sym	C-N St.vib
VIII	nitrophenyl	CH$_3$	1588	3396	____	1403	1515	1342	____
IX	nitrophenyl	N,N-dimethylaminophenyl	1566	3425	2917	1434	1524	1314	1179
X	nitrophenyl	bromophenyl	1657	3425	____	___	1518	1335	____
XI	nitrophenyl	phenyl	1599	3423	___	___	1517	1340	1140
XII	CH$_3$	N,N-dimethylaminophenyl	1500	3430	2916	____	___	____	___
XIII	CH$_3$	bromophenyl	1591	___	2915	1484	____	___	1185
XIV	CH$_3$	phenyl	1602	3437	___	1406	___	____	1134

3.3.3.1 Thin Layer Chromatography:

According to the experiment, the solvent system is employed in order to dissolve and assist the compounds to move along the silica plate by capillary action.In solvent system mixture of 70% of n-hexane and 30% of ethyl acetate showed less polarity compared with solvent system mixture of 30% n-hexane and 70% ethyl acetate, due to the presence of 70 part of non-polar n-hexane in 30part of polar ethyl acetate which slightly decreases the polarity of the overall solvent.This is also decrease the movement of the sample by capillary action through the stationary phase (moisture adsorbed by silica gel).

From the results, tables (3.8) and (3.9) the differences on the R.f value verify the formation of Pyrazoline compound.

3.3.3.2 Ultra Violate/Visible Spectroscopy:

Light in the ultraviolet-visible (UV-Vis) region the electromagnetic spectrum (200 – 800 nm) can affect the electronic transitions.

Absorbed UV-Vis energy is strong enough to actually excite an electron from a low-lying bonding/nonbonding orbital to a higher-lying antibonding orbital. The energy of the transition (or the difference in energy between the ground state and excited state energy levels) is measured by the wavelength of the absorbed light (λmax).

From the result obtained both enone and pyrazolines compounds show the absorption at visible region form (288 – 458 nm) and (298-398nm) tables (3.8) and (3.9) respectively, as transition from π-π*. Due to the conjugated systems the π electrons of the double bond are delocalized, the HOMO-LUMO gap decreases as conjugation increases, shifting λmax to longer wavelengths.

For molecules having conjugated systems of electrons, the ground states and excited states of the electrons are closer in energy than for non-conjugated systems. This means that lower energy light is needed to excite electrons in conjugated systems, which means that lower energy light is absorbed by conjugated systems. So a highly conjugated system then might absorb the lower energy portions of the light and reflect what is not absorbed. It is this reflected portion that the eye will perceive as the color of that object. A less highly conjugated system will require the absorption of the higher energy part of the spectrum, allowing the lower energy parts to be reflected to the eye. So this could explain the colors of the synthesized compounds this is clearly observed in compound (II).

3.3.3.3 Infra-Red:

Table (3.10) IR spectrum of enone shows the absorption of the carbonyl group st.vib between $(1648-1706cm^{-1})$and the alkene group (C=C) st.vib absorption in the range $(1600-1546cm^{-1})$it proved of successfully synthesized enone. It clearly observed that the absorption of carbonyl group (C=O)st.vib was present in range that is less than thecharacteristicabsorption of aldehyde $(1725 \ cm^{-1})$,thisdue to the presence of the conjugated system. Table (3.11) IR spectrum of Pyrazoline showed mainly the absence of the carbonyl st.vib absorption with the present of imines (C=N) $1500-1657cm^{-1}$absorption. This proved that the Pyrazolines is successfully synthesized.

Conclusion:

Some of Pyrazoline derivatives were modeled and investigated for their anticacer activity on AsPC-1 human pancreatic, and U251 human glioblastoma cell lines through QSAR and molecular docking or protein ligand interaction.Compounds X and IXshowed the best result against AsPC-1 and U251 cell lines, with IC_{50} values 0.31mM and 0.062mM respectively.

Recommendations:

In the view of this study we recommended H^1-NMR, C^{13}-NMR and mass spectroscopy as further characterization assay to the synthesized compounds. Moreover research can be carried out on the development of strong effective anticancer agents bymodifyto the structures of these compounds.

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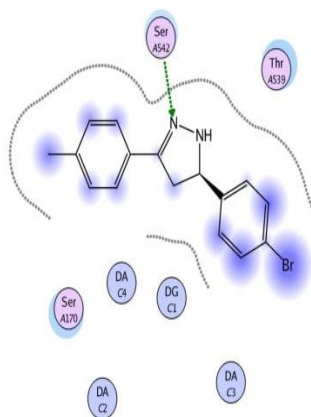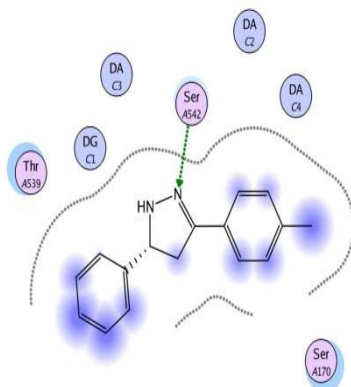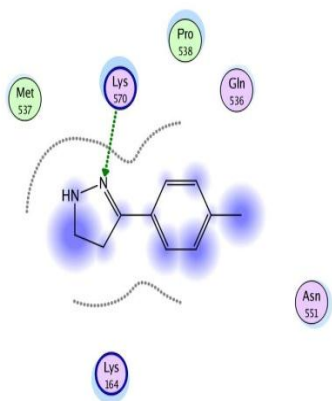
Appendixes
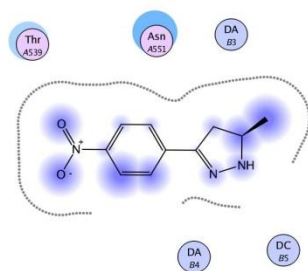
Figure (3.3) show the interactions between the ligand (compound VIII) and the 5kk5 protein active side
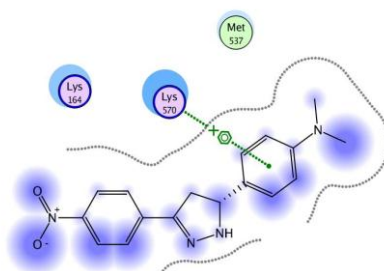
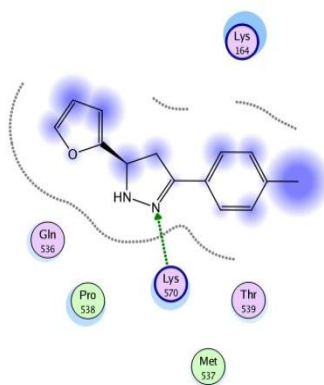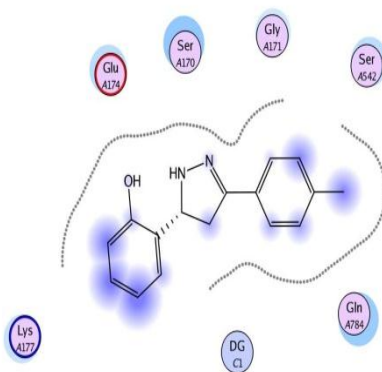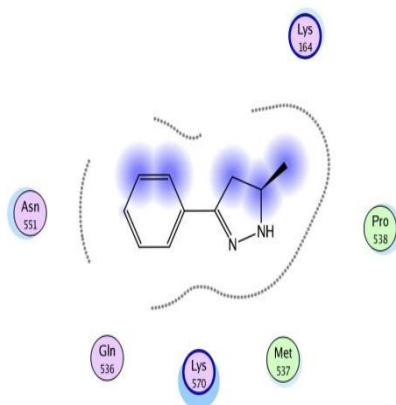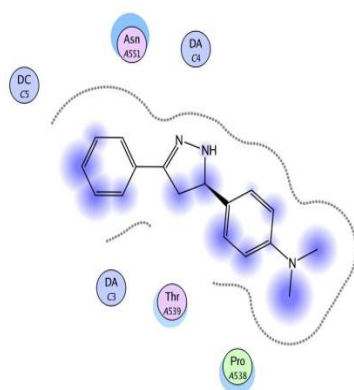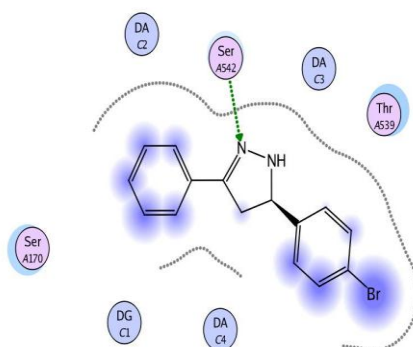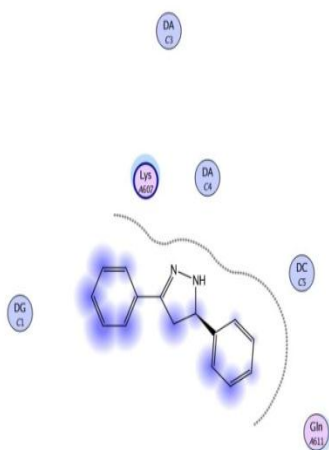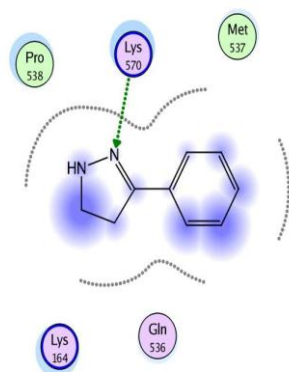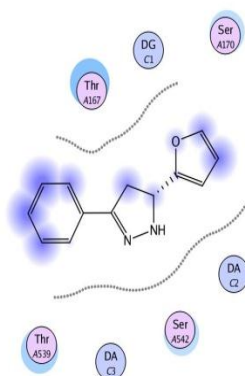**Figure (3.4) show the interactions between the ligand (compound IX) and the 5kk5 protein active side**
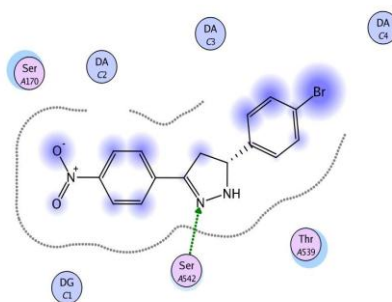
Figure (3.5) show the interactions between the ligand (compound X) and the 5kk5 protein active side
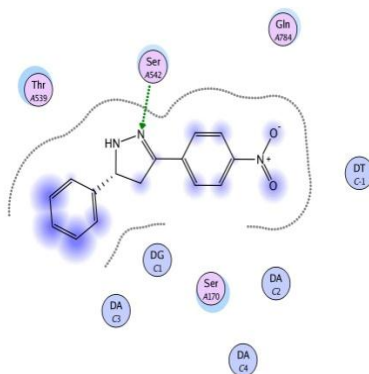
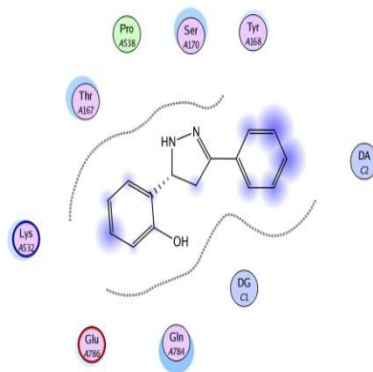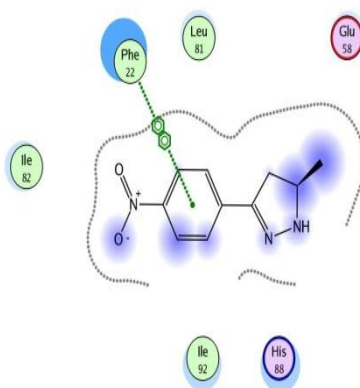**Figure (3.6) show the interactions between the ligand (compound XI) and the 5kk5 protein active side**
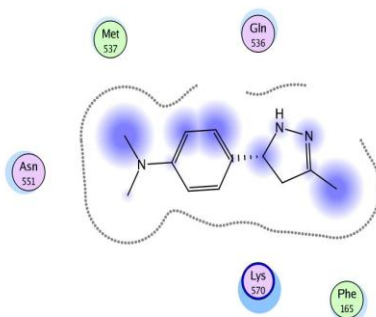
Figure (3.7) show the interactions between the ligand (compound XII) and the 5kk5 protein active side
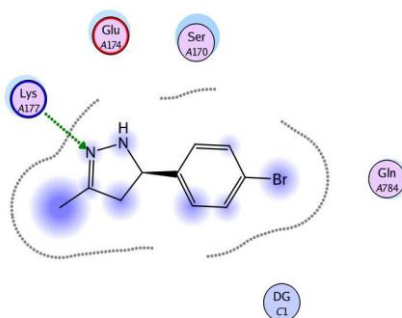
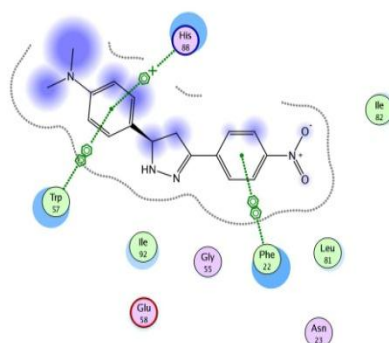**Figure (3.8) show the interactions between the ligand (compound XIII) and the 5kk5 protein active side**
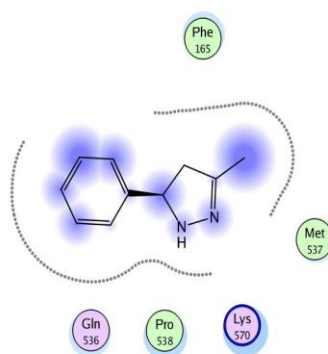
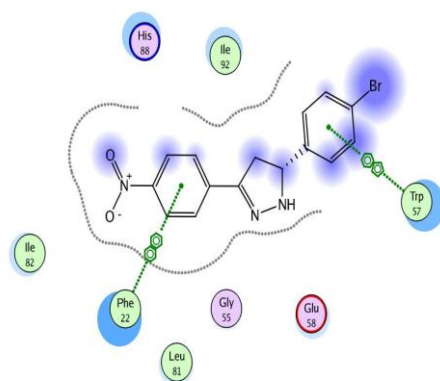**Figure (3.9) show the interactions between the ligand (compound XIV) and the 5kk5 protein active side**
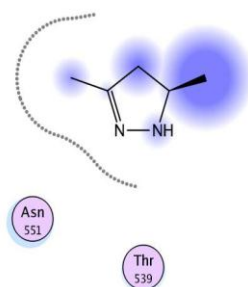
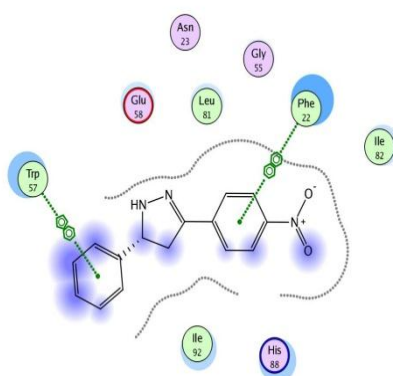**Figure (3.10) show the interactions between the ligand (compound XV) and the 5kk5 protein active side**
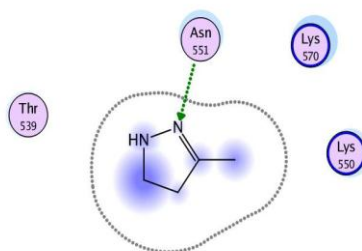
Figure (3.11) show the interactions between the ligand (compound XVI) and the 5kk5 protein active side
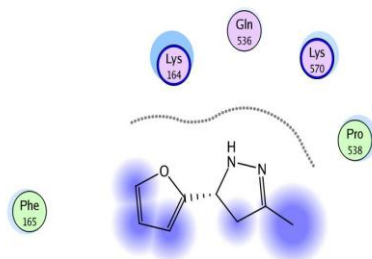
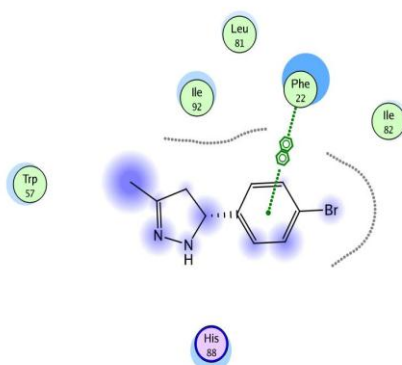**Figure (3.12) show the interactions between the ligand (compound XVII) and the 5kk5 protein active side**
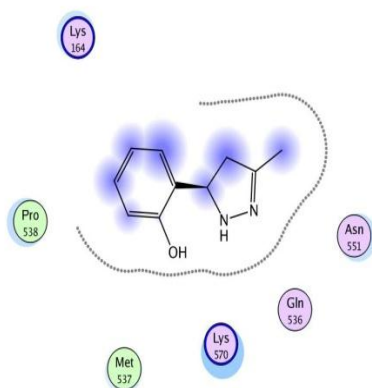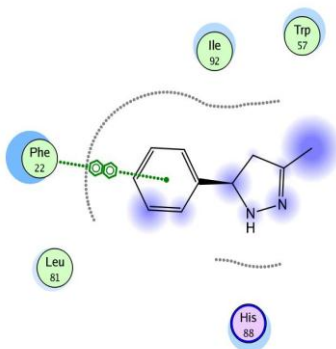

Figure (3.13) show the interactions between the ligand (compound XVIII) and the 5kk5 protein active side
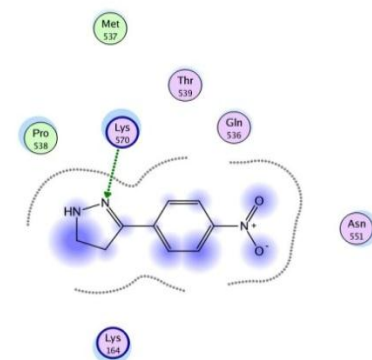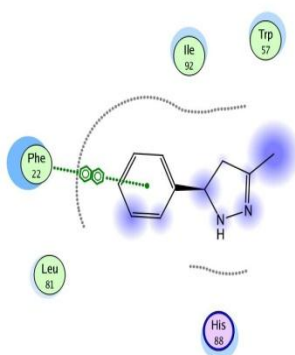

Figure (3.14) show the interactions between the ligand (compound XIX) and the 5kk5 protein active side
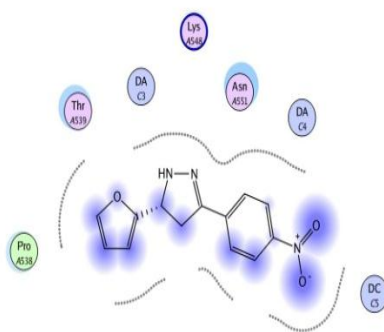
Figure (3.15) show the interactions between the ligand (compound XX) and the 5kk5 protein active side
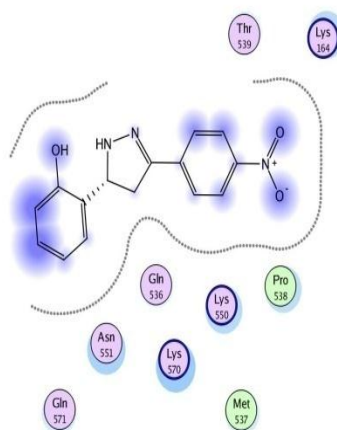
Figure (3.16) show the interactions between the ligand (compound XXI) and the 5kk5 protein active side
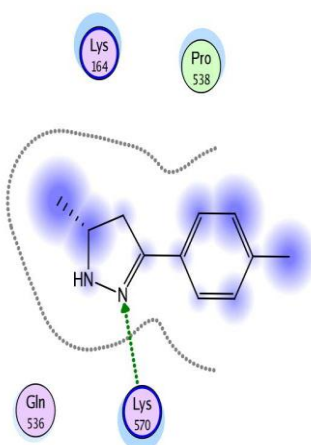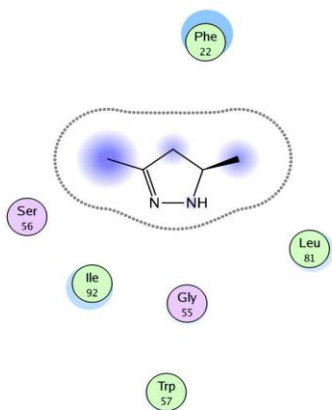

Figure (3.17) show the interactions between the ligand (compound XXII) and the 5kk5 protein active side
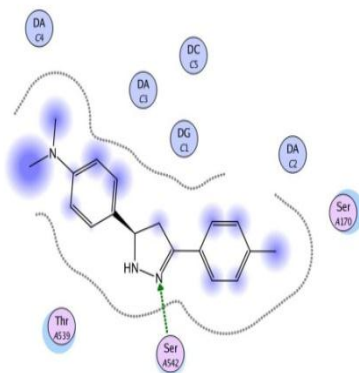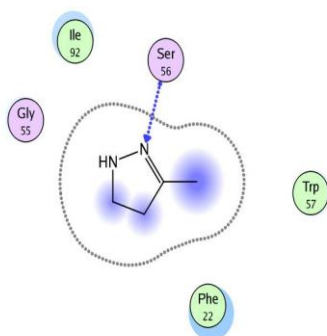
Figure (3.18) show the interactions between the ligand (compound XXIII) and the 5kk5 protein active side
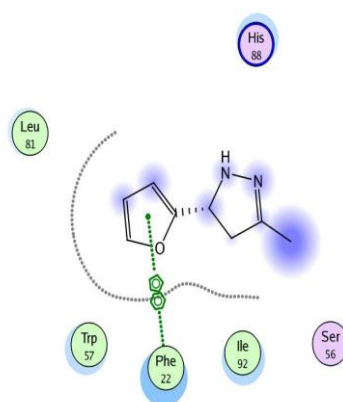

Figure (3.19) show the interactions between the ligand (compound XXIV) and the 5kk5 protein active side

Figure (3.20) show the interactions between the ligand (compound XXV) and the 5kk5 protein active side

Figure (3.21) show the interactions between the ligand (compound XXVI) and the 5kk5 protein active side
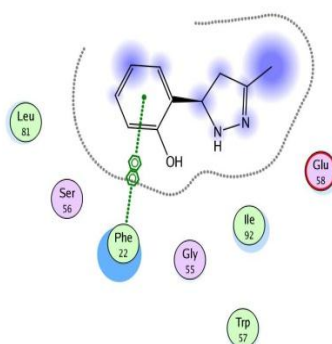

Figure (3.22) show the interactions between the ligand (compound XXVII) and the 5kk5 protein active side
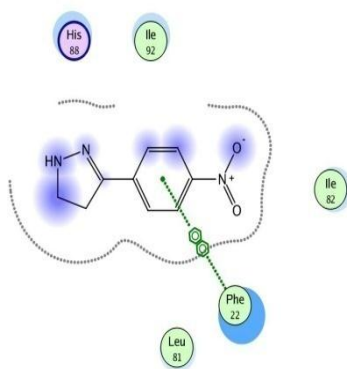

Figure (3.23) show the interactions between the ligand (compound XXVIII) and the 5kk5 protein active side

Figure (3.24) show the interactions between the ligand (compound XXIX) and the 5kk5 protein active side

Figure (3.25) show the interactions between the ligand (compound XXX) and the 5kk5 protein active side
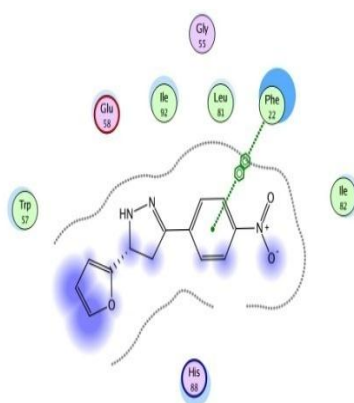

Figure (3.26) show the interactions between the ligand (compound XXXI) and the 5kk5 protein active side
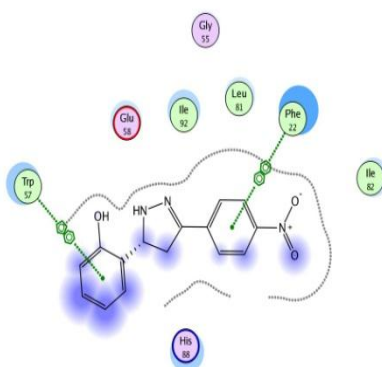
Figure (3.27) show the interactions between the ligand (compound XXXII) and the 5kk5 protein active side

Figure (3.28) show the interactions between the ligand (compound XXXIII) and the 5kk5 protein active side

Figure (3.29) show the interactions between the ligand (compound XXXIV) and the 5kk5 protein active side

Figure (3.30) show the interactions between the ligand (compound XXXV) and the 5kk5 protein active side

Figure (3.31) show the interactions between the ligand (compound VIII) and the 1NQU protein active side

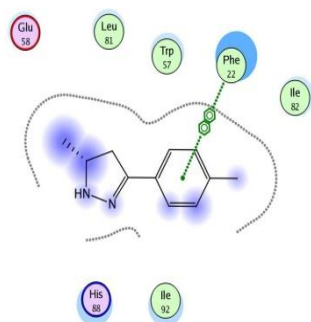**Figure (3.32) show the interactions between the ligand (compound IX) and the 1NQU protein active side**

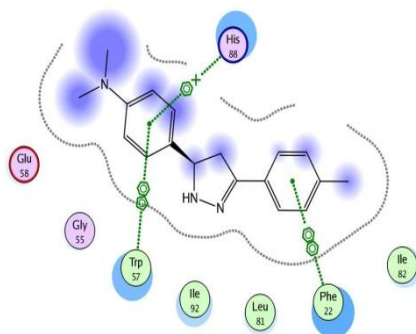**Figure (3.33) show the interactions between the ligand (compound X) and the 1NQU protein active side**

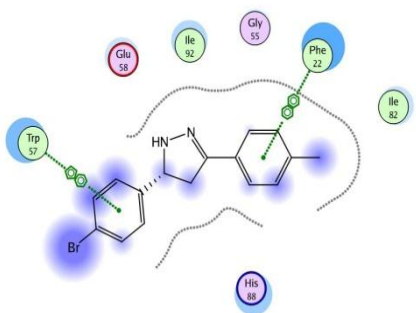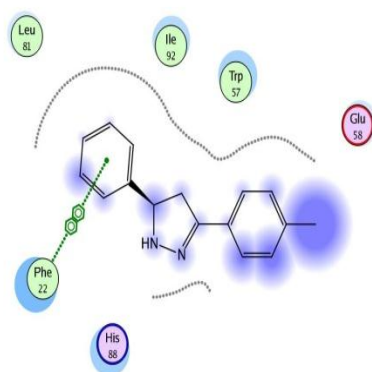**Figure (3.34) show the interactions between the ligand (compound XI) and the 1NQU protein active side**

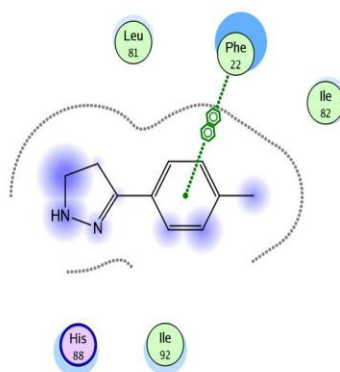**Figure (3.35) show the interactions between the ligand (compound XII) and the 1NQU protein active side**

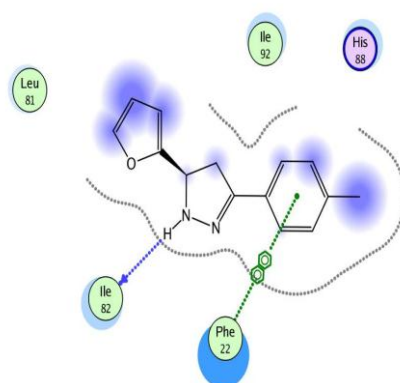**Figure (3.36) show the interactions between the ligand (compound XIII) and the 1NQU protein active side**

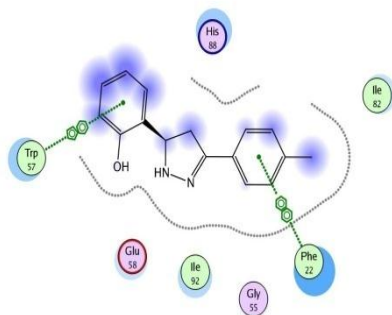**Figure (3.37) show the interactions between the ligand (compound XIV) and the 1NQU protein active side**

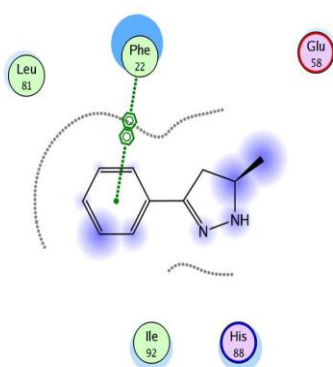**Figure (3.38) show the interactions between the ligand (compound XV) and the 1NQU protein active side**

Figure (3.39) show the interactions between the ligand (compound XVI) and the 1NQU protein active side

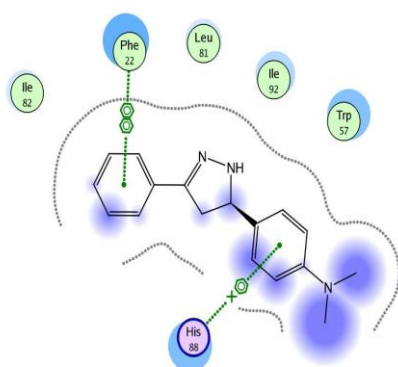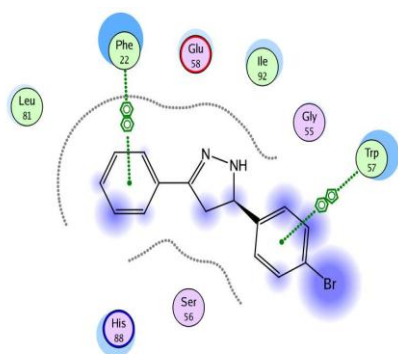**Figure (3.40) show the interactions between the ligand (compound XVII) and the 1NQU protein active side**

Figure (3.41) show the interactions between the ligand (compound XVIII) and the 1NQU protein active side

Figure (3.42) show the interactions between the ligand (compound XIX) and the 1NQU protein active side

Figure (3.43) show the interactions between the ligand (compound XX) and the 1NQU protein active side

Figure (3.44) show the interactions between the ligand (compound XXI) and the 1NQU protein active side

Figure (3.45) show the interactions between the ligand (compound XXII) and the 1NQU protein active side

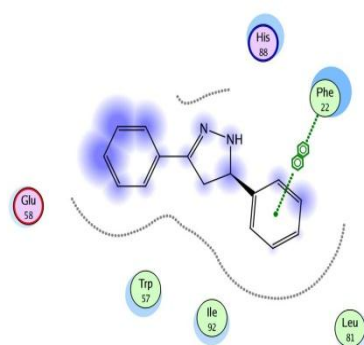**Figure (3.46) show the interactions between the ligand (compound XXIII) and the 1NQU protein active side**

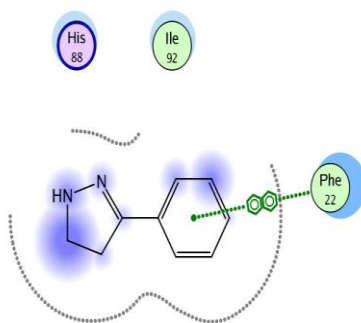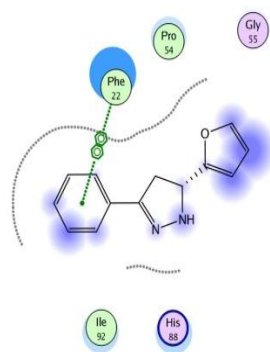**Figure (3.47) show the interactions between the ligand (compound XXIV) and the 1NQU protein active side**

Figure (3.48) show the interactions between the ligand (compound XXV) and the 1NQU protein active side

Figure (3.49) show the interactions between the ligand (compound XXVI) and the 1NQU protein active side
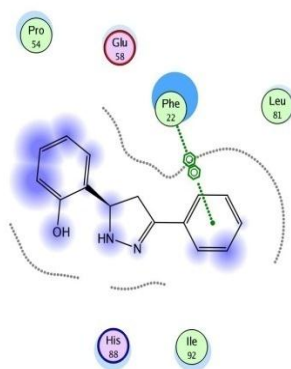

Figure (3.50) show the interactions between the ligand (compound XXVII) and the 1NQU protein active side

Figure (3.51) show the interactions between the ligand (compound XXVIII) and the 1NQU protein active side

Figure (3.52) show the interactions between the ligand (compound XXIX) and the 1NQU protein active side

Figure (3.53) show the interactions between the ligand (compound XXX) and the 1NQU protein active side

Figure (3.54) show the interactions between the ligand (compound XXXI) and the 1NQU protein active side

Figure (3.55) show the interactions between the ligand (compound XXXII) and the 1NQU protein active side
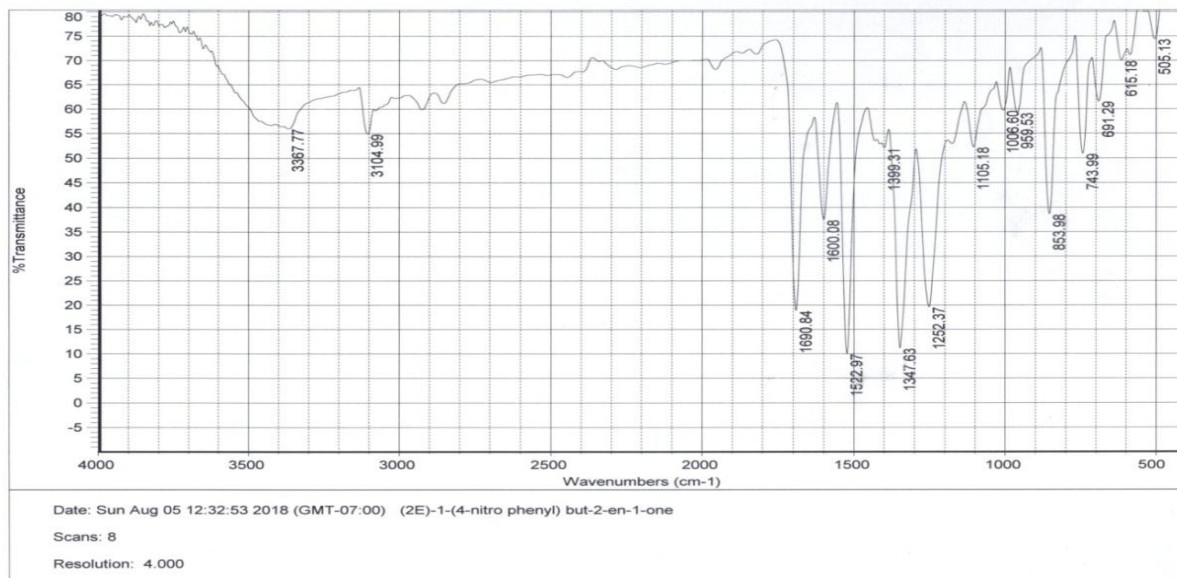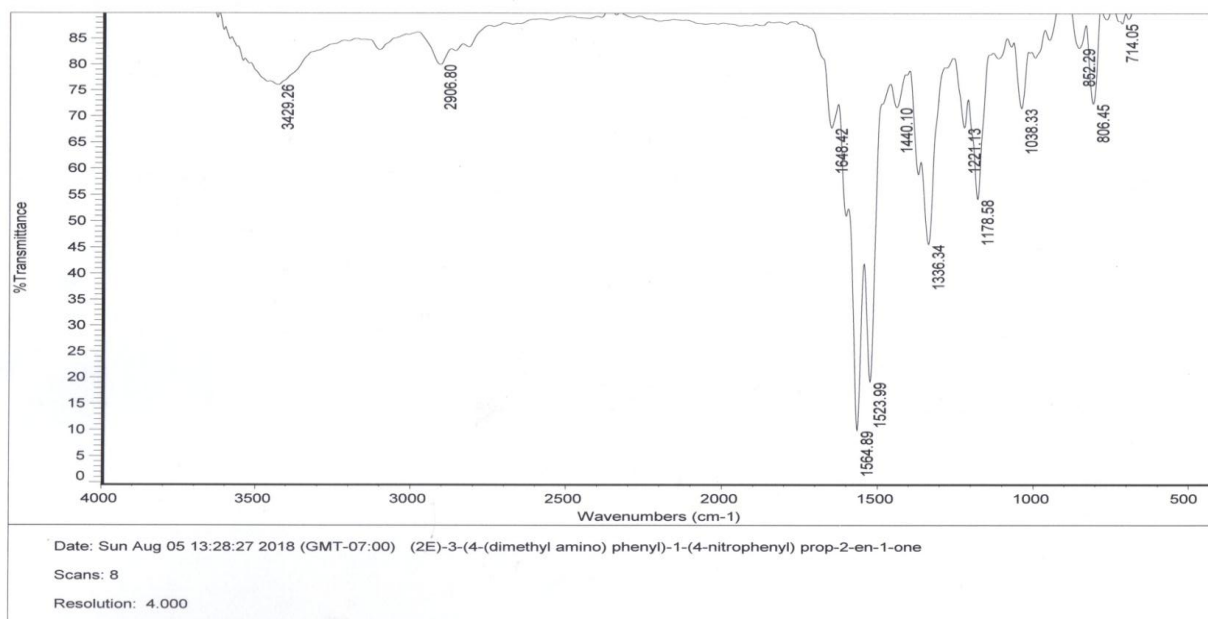

Figure (3.56) show the interactions between the ligand (compound XXXIII) and the 1NQU protein active side

Figure (3.57) show the interactions between the ligand (compound XXXIV) and the 1NQU protein active side

Figure (3.58) show the interactions between the ligand (compound XXXV) and the 1NQU protein active side

Date: Sun Aug 05 12:32:53 2018 (GMT-07:00)   (2E)-1-(4-nitro phenyl) but-2-en-1-one

Scans: 8

Resolution: 4.000

Figure (3.59) show the IR spectrum of compound I

Date: Sun Aug 05 13:28:27 2018 (GMT-07:00)   (2E)-3-(4-(dimethyl amino) phenyl)-1-(4-nitrophenyl) prop-2-en-1-one

Scans: 8

Resolution: 4.000

Figure (3.60) show the IR spectrum of compound II
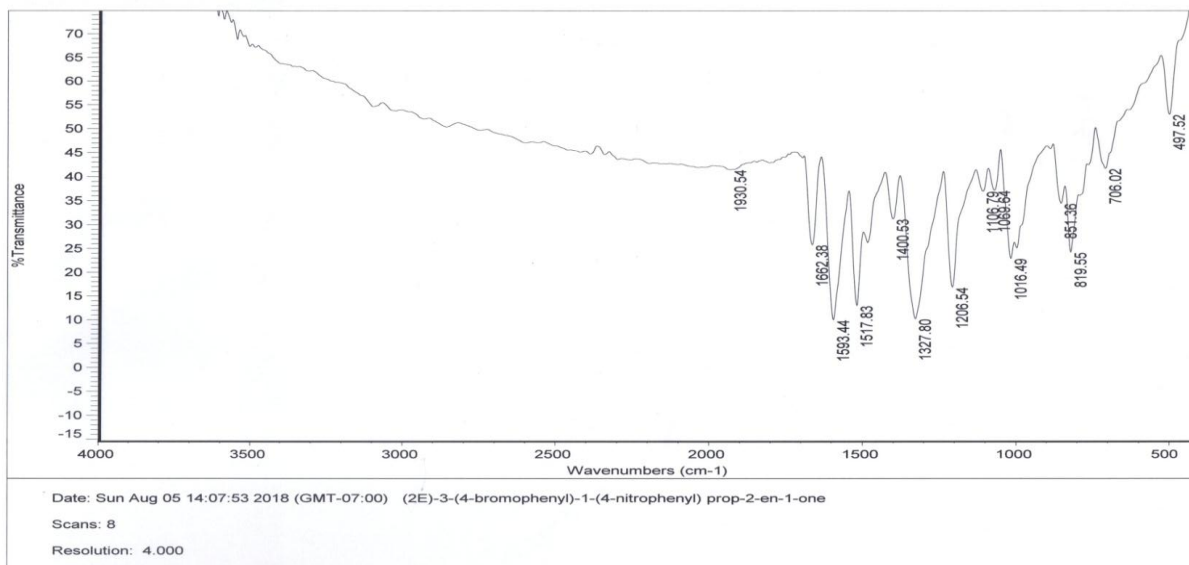
Figure (3.61) show the IR spectrum of compound III
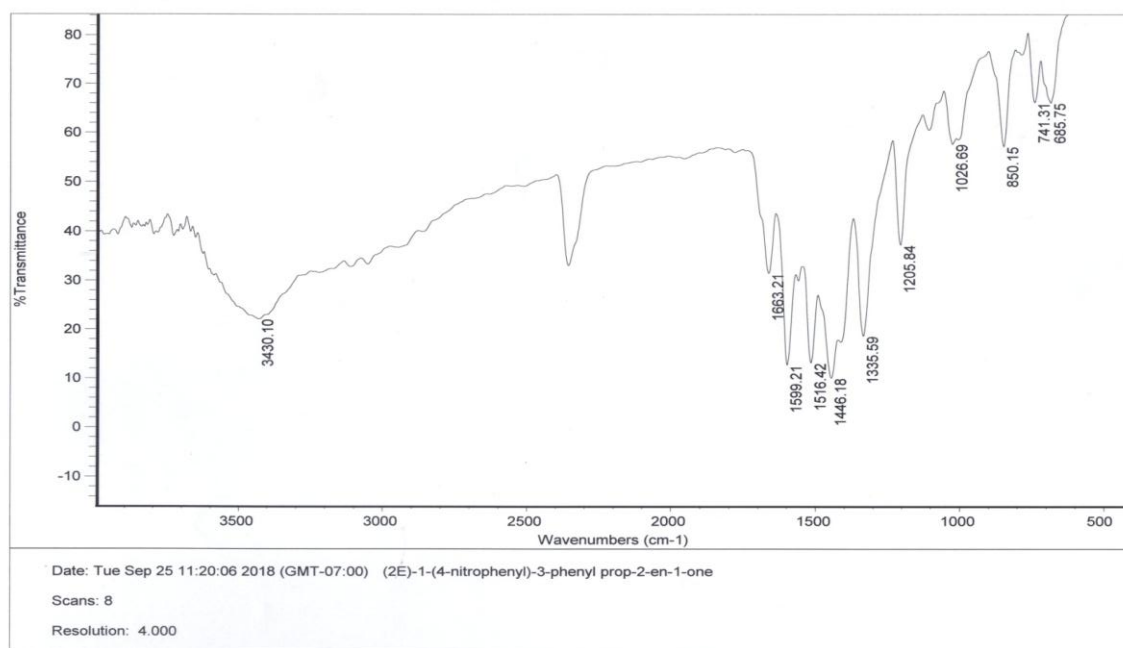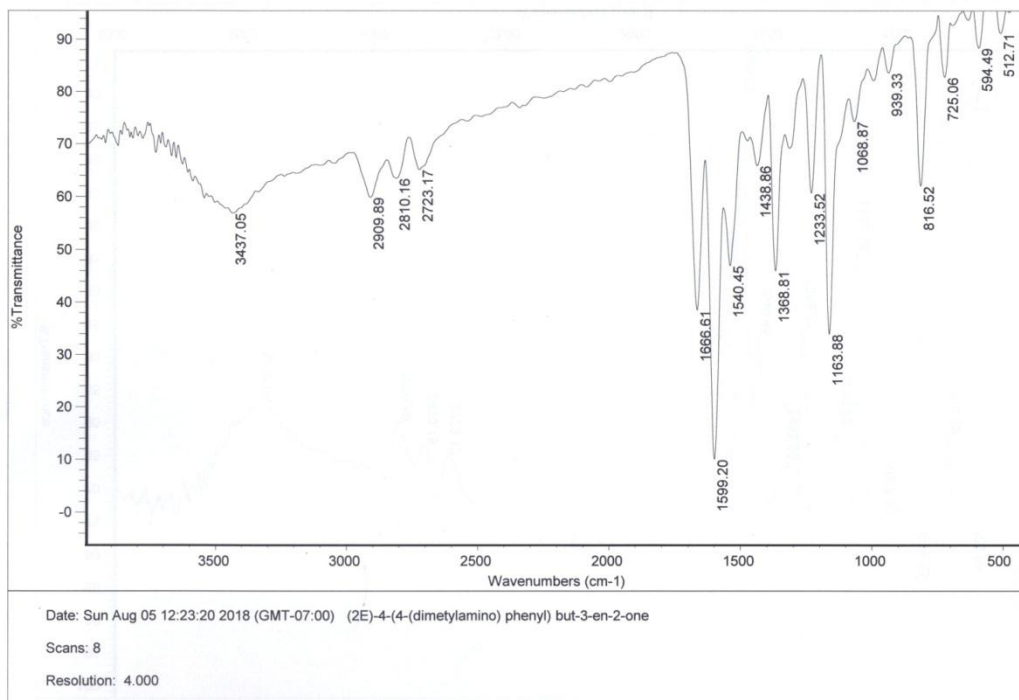

Figure (3.62) show the IR spectrum of compound IV

60

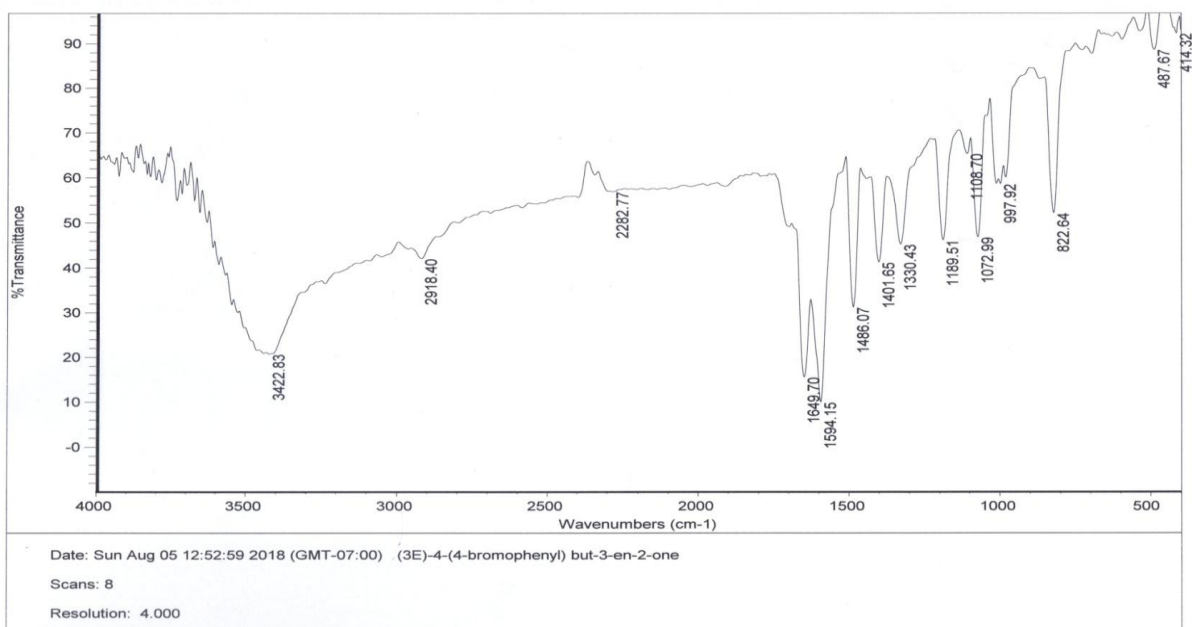**Figure (3.63) show the IR spectrum of compound V**
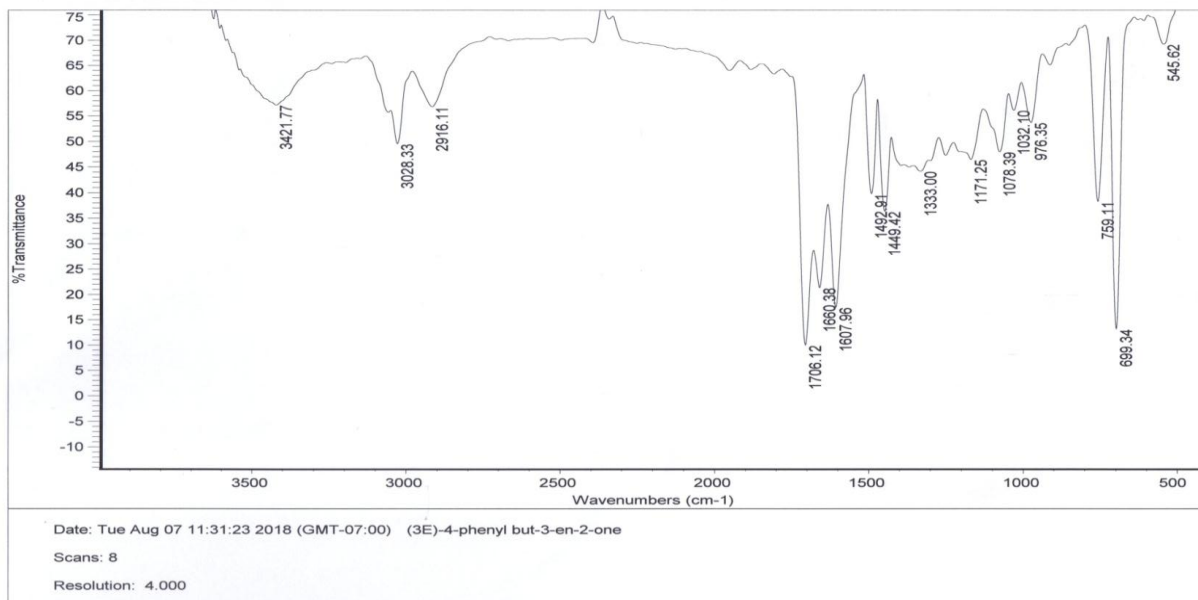

Figure (3.64) show the IR spectrum of compound VI
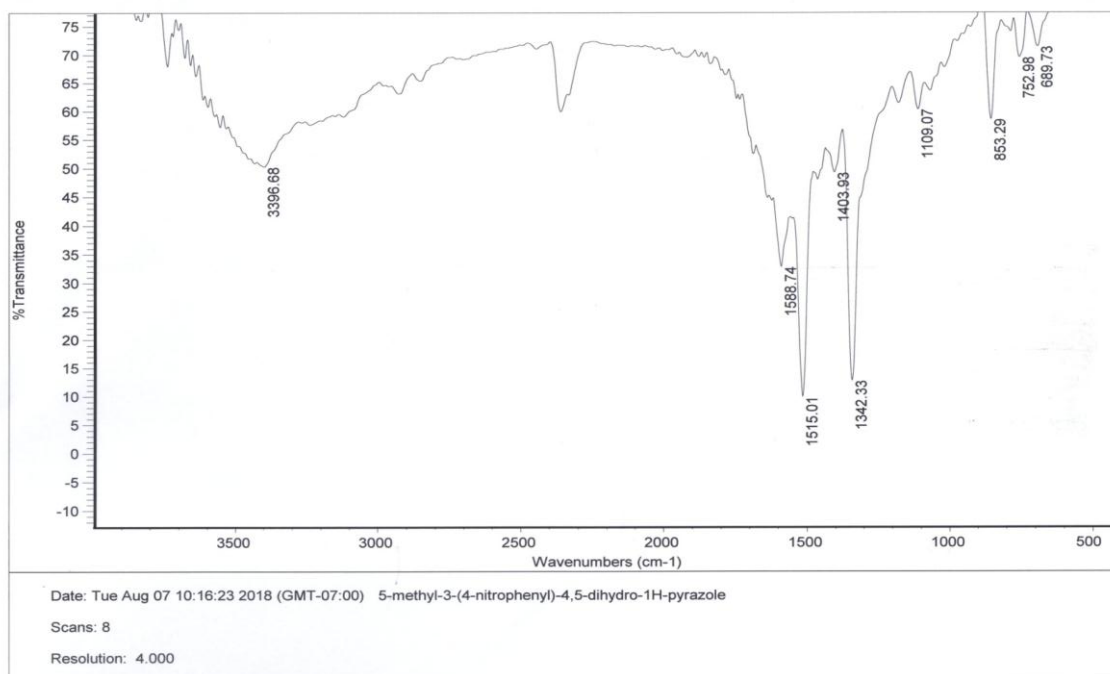
Figure (3.65) show the IR spectrum of compound VII

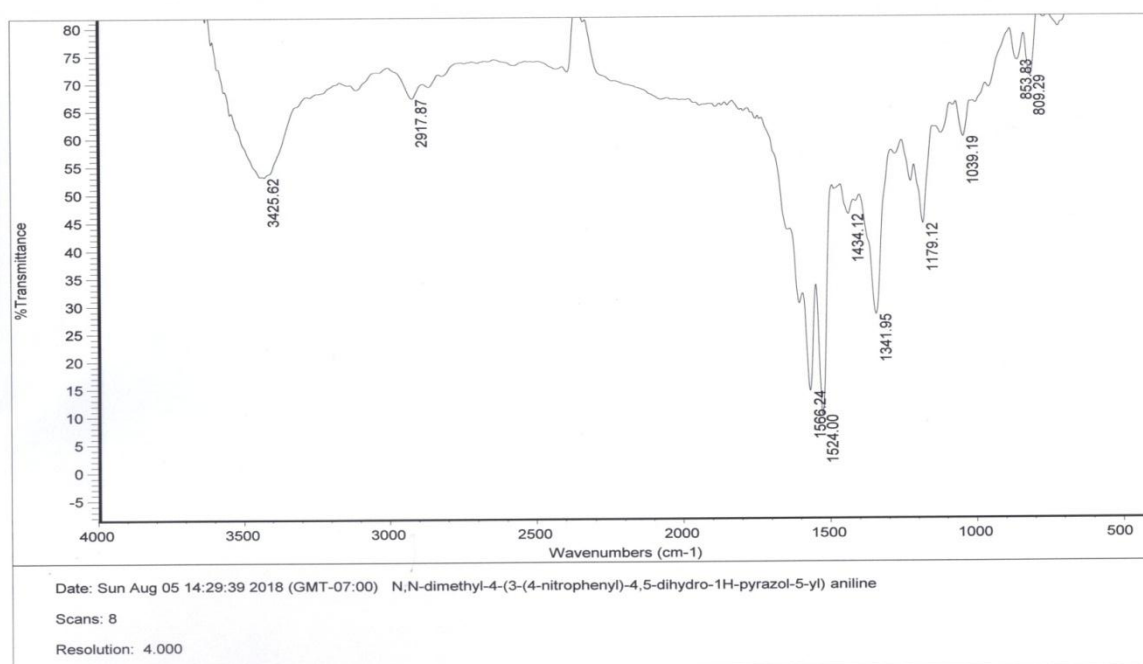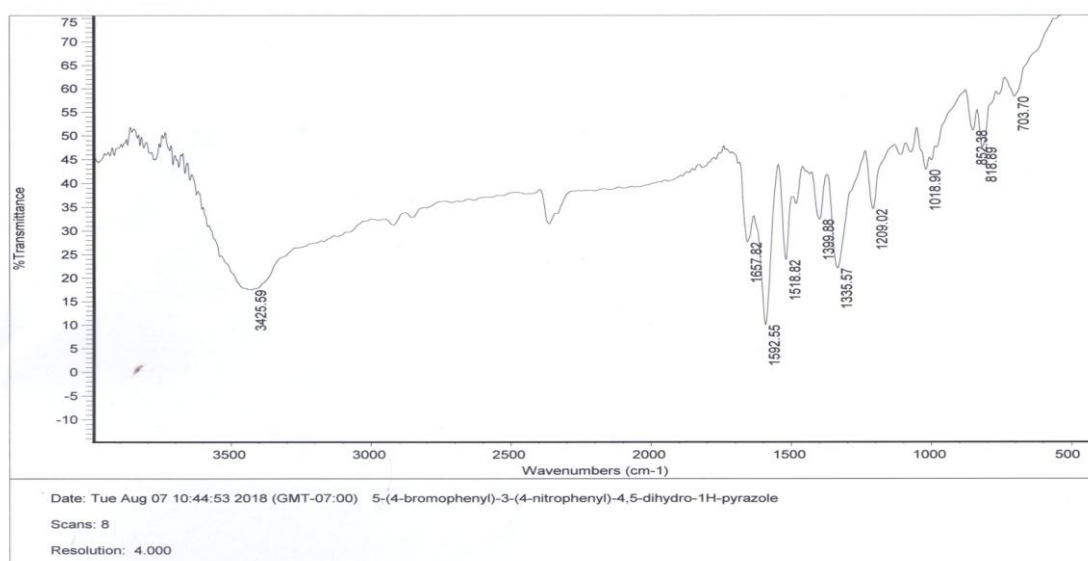**Figure (3.66) show the IR spectrum of compound VIII**

Date: Sun Aug 05 14:29:39 2018 (GMT-07:00)   N,N-dimethyl-4-(3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl) aniline

Scans: 8

Resolution: 4.000

Figure (3.67) show the IR spectrum of compound IX

Date: Tue Aug 07 10:44:53 2018 (GMT-07:00)   5-(4-bromophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole

Scans: 8

Resolution: 4.000

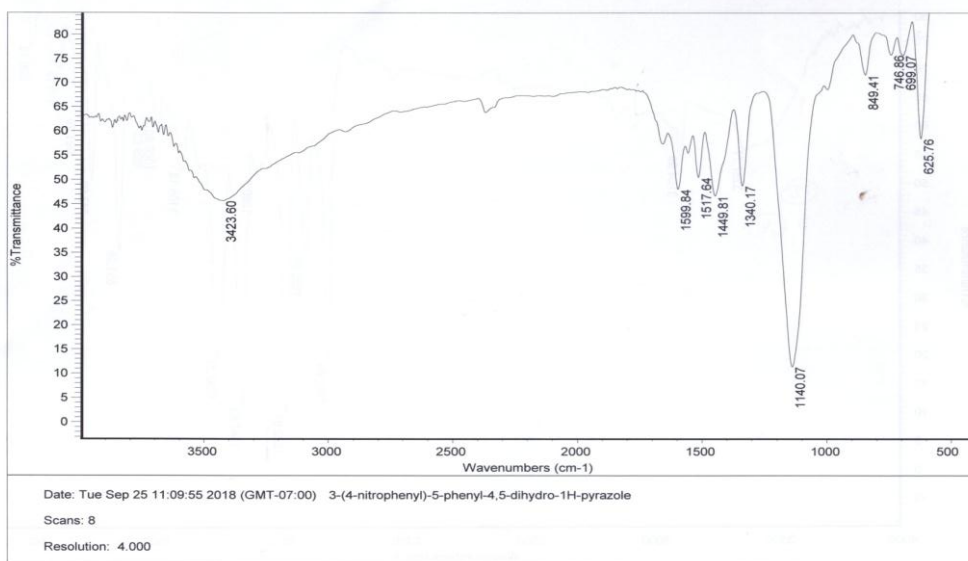**Figure (3.68) show the IR spectrum of compound X**

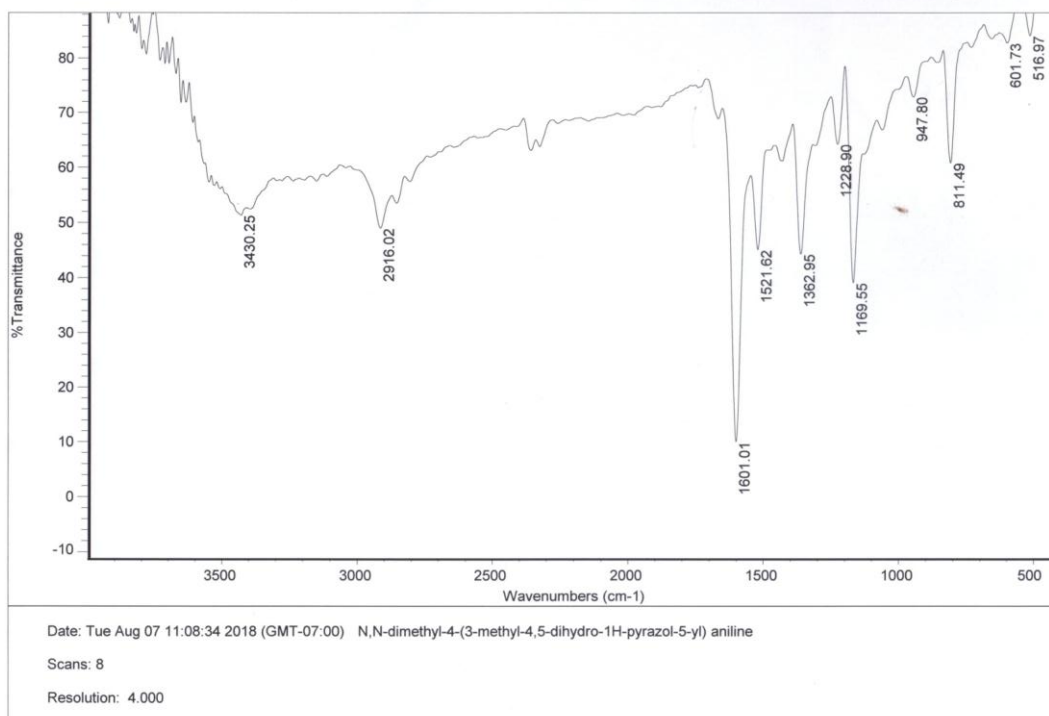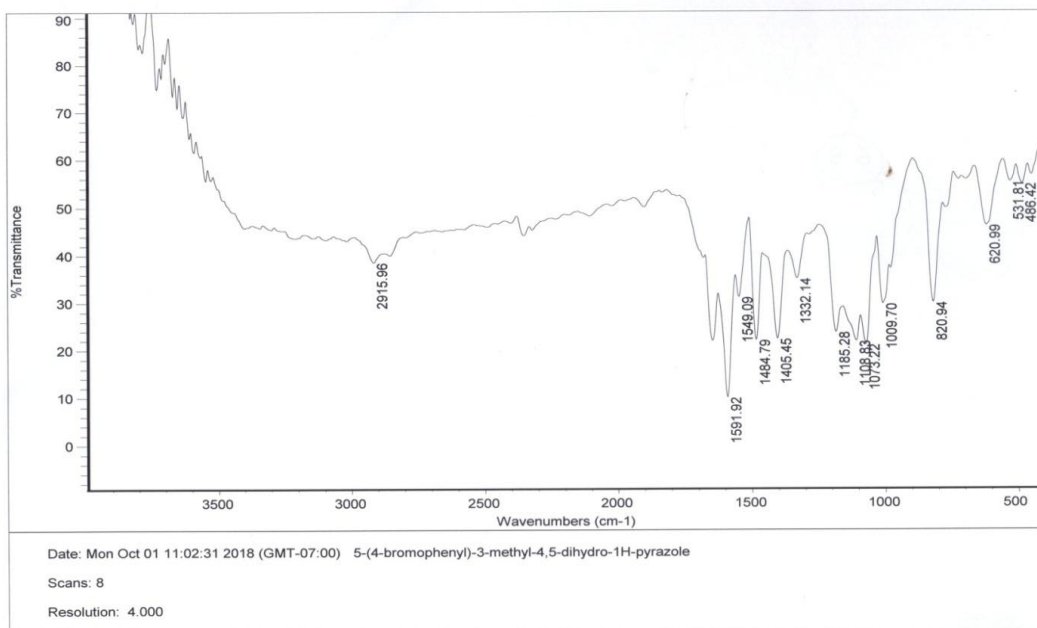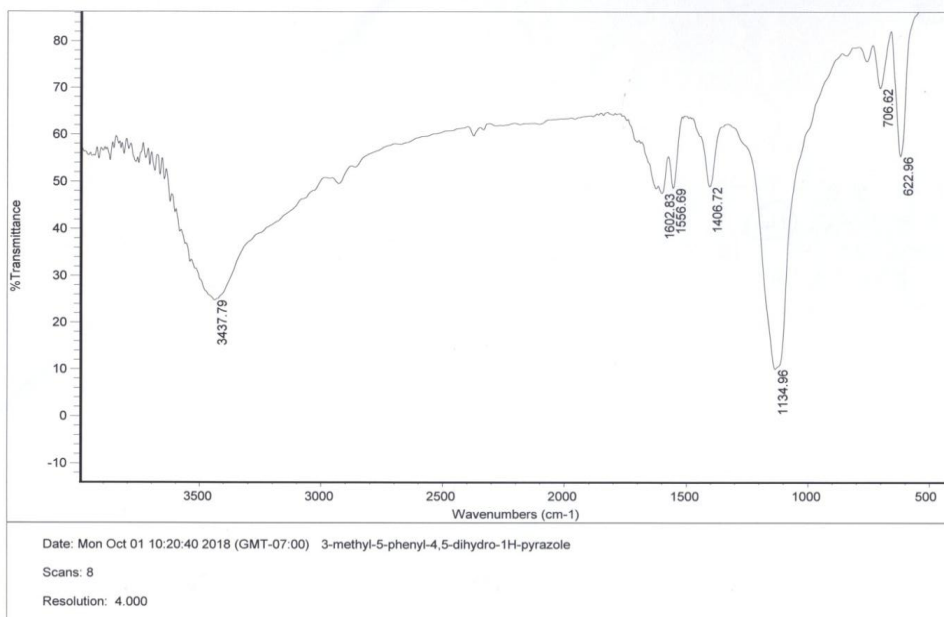**Figure (3.69) show the IR spectrum of compound XI**

Figure (3.70) show the IR spectrum of compound XII

Figure (3.71) show the IR spectrum of compound XIII

Figure (3.72) show the IR spectrum of compound XIV