Faculty of Graduate Studies

Chemistry Department

Synthesis and Biological Evaluation of Chalcones, Isoxazole and Pyrazole Derivatives Attached with Adamantyl Substituent

By

Sara Al Hammouri

Supervisor:

D. Tawfeq Kaimari

Co-supervisor:

Prof. Dr. Rajab Abu-El-Halawa

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Faulty of Graduate Studies

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Prepared By:

Sara Al Hammouri

This thesis was successfully defended on April 27, 2017 and approved by:

Committee members:	Signature
Dr. Tawfeq Kaimari	Supervisor : …….
Prof. Dr. Rajab Abu-El-Halawa	Co-supervisor: …….
Dr. Feras Al Battah	External Examiner: …….
Dr. Sami Makharza	Internal Examiner: .....

Dedication

To my incredible parents, who constantly sacrifice themselves in countless ways, to my dearest husband, who leads me through the valley of darkness with light of hope and support, to my beloved brothers, sister and my beloved kids Omar and Abd AlAzeez , whom I can't force myself to stop loving. I dedicate this research.

Acknowledgment

First and foremost, I express my profound thanks to teachers in chemistry department, supervisors and friends at Hebron University.

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Above all, I thank my god, for giving me strength to complete this research.

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Abbreviations	Definition
Chalcone (C)	(E)-1-((3r, 5r, 7r)-adamantan-1-yl)-3-(pyridin-2-yl) prop-2-en-1-one
SH	1-((1R,3R,5S)-adamantan-1-yl)-2-(aminooxy)-2-(pyridin-4-yl)ethan-1-one
SH-1	1-((1R,3R,5S)-adamantan-1-yl)-2-(aminooxy)-2-(pyridin-4-yl)ethan-1-one
SH-2	5-((1R,3R,5S)-adamantan-1-yl)-3-(pyridin-4-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide
RPMI	Roswell Park Memorial Institute medium
FT-IR	Fourier Transform Infra- Red
^{1}HNMR	Proton nuclear magnetic resonance
TLC	Thin Layer Chromatography
MDA-MB-231/GFP and MCF-7	Breast adenocarcinoma cell line
RT	Room temperature
R_f	Retention factor
XTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide
DMSO	Dimethyl sulfoxide
ppm	Part per million
FBS	Fetal bovine serum
PMS	N-methyl dibenzopyrazine methyl sulfate
ELISA	The enzyme-linked immunosorbent assay
ER	Estrogen receptors

Abstract

In this study, a series of new adamantly chalcone containing isoxazole and pyrazole rings were synthesized and evaluated for anticancer activity against human breast cancer MDA-MB-231(Estrogen Receptor Negative) and MCF7(Estrogen Receptor Positive) cell lines. The effect of new compounds for two cell lines were tested by using XTT assay and total cell count, cell death assay.

XTT results on MCF-7 and MDA-MB-231 cell lines indicate that SH-2 compound has effect on ER+ cell line (MCF-7) more than ER- cell line (MDA-mb231GFP) . Where SH-2 decreased the cells proliferation of MCF-7 and MDA-MB-231 cell line by 72.5% and 51% respectively at 5µM . On the contrary, SH-1 increased the proliferation rate of the human breast cancer cell lines at the same concentration. Cell counting assay was confirmed the XTT results.

The structures of chalcone derivatives (SH-1 and SH-2) were established on the basis of infrared (IR) and ^{1}HNMR spectral data.

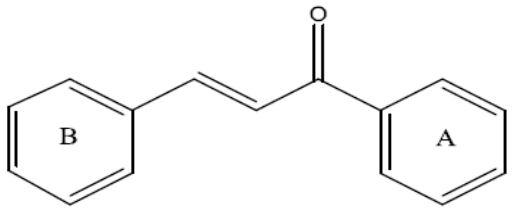
Chapter One
Introduction

1. Introduction

1.1Chemistry of Chalcone: Structure and Nomenclature

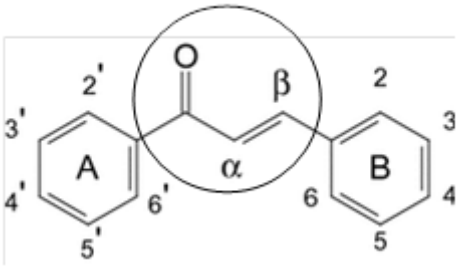
The term chalcones was given by scientists Kostanecki and Tambor in 1899,Chalcones are natural or synthetic compounds which belong to the bicyclic Flavonoid family[1].They are both intermediates and end products in Flavonide biosynthesis [2]. Chalcones are abundant in plants such as Angelica, Glycyrrhiza, Humulus and Scutellaria. Ashitaba (Angelica Keiskei Koidzmi) has been used as a supplement to replenish energy by supplying the blood with vital nutrients and promoting circulation in traditional Chinese Medicine, The presence of chalcones in this herb is what makes it Unique[3].

Chalcones has been targeted by several researchers in recent years due to their wide biological potential [4],as well as their ability to serve as important intermediates for the synthesis of a large number of heterocyclic systems[5]. The structural skeleton of chalcones especially 1,3 – diarylprop –2-en- 1 – ones are constituted by two aromatic rings linked through the open chain three-carbon unit α, β – unsaturated carbonyl system[5] (Scheme 1.1) .



Scheme1. 1: Chemical structure of chalcone

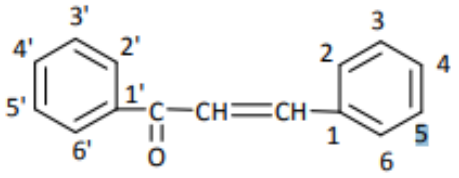
The wide biological activity of chalcone and related compounds such as “chalconoids”, is due to presence of aromatic ketone and an enone functional group [6] (scheme 1.2).



Scheme1. 2: Enone functional group

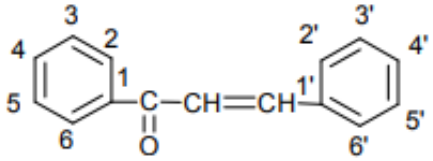
The presence of chromophore (-CO-CH=CH-) and auxochromes give these compounds the colour, hence their ecological role in relation to plant colour. These bright yellow compounds are found in many plant organs[7] [8].

In the United States system, nomenclature of chalcone depends on giving prime numbers to the phenyl ring, which is the nearest to carbonyl group[9] (scheme 1.3).



Scheme1. 3: Nomenclature of chalcones based on American Chemical Society

However, the British Chemical Abstracts and Journal of Chemical Society use a different method depends on giving prime numbers to the phenyl ring, which is the nearest to double bond [9] (scheme 1.4).

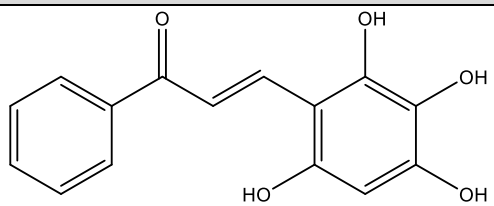


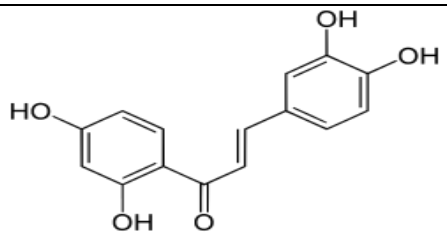
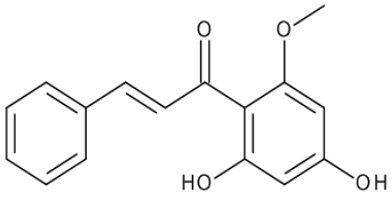
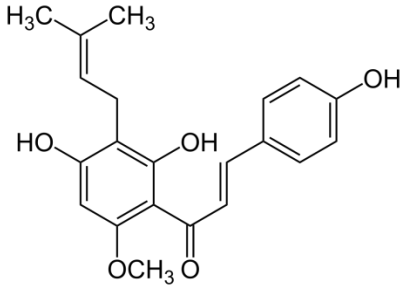
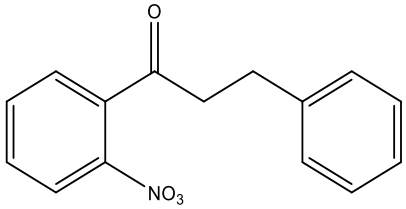
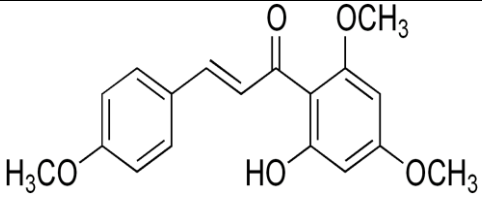
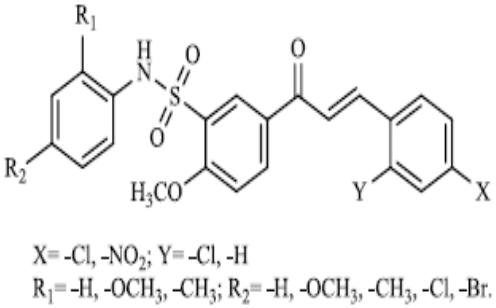
Scheme1. 4: Nomenclature of chalcones based on British Chemical Society

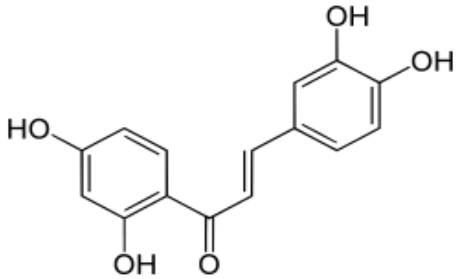
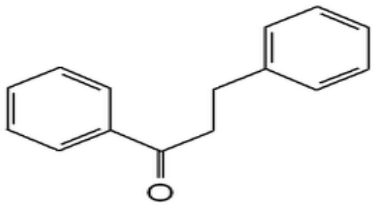
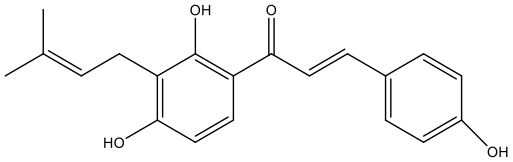
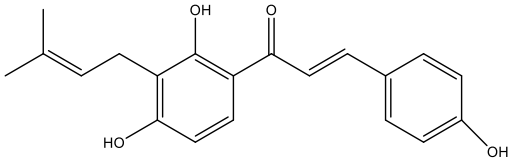
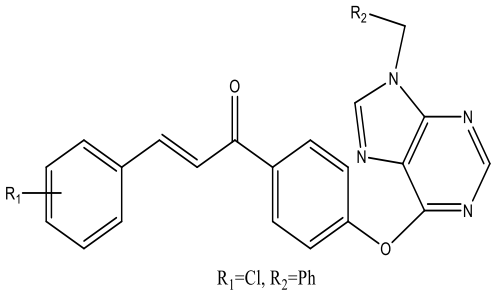
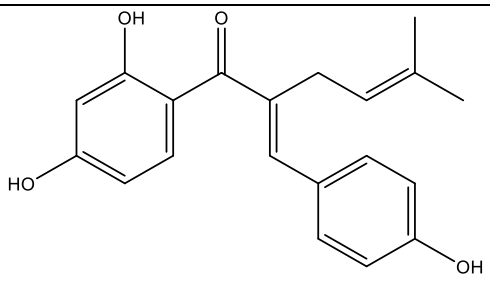
1.2 Medicinal Significance of Chalcones:

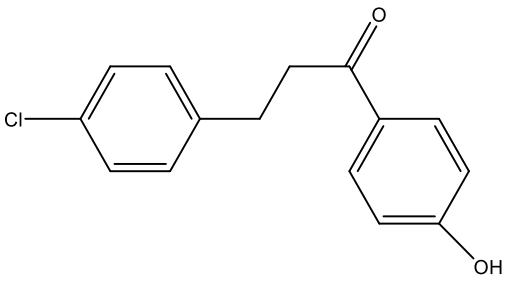
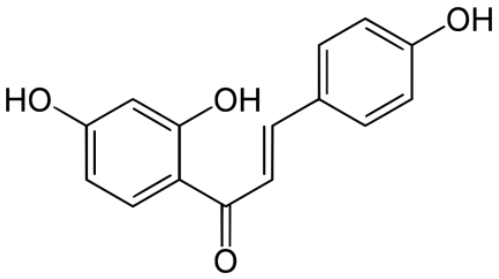
Chalcones are used as a starting material to synthesize many of the heterocyclic ring systems like pyrazolines, isoxazoles, pyrimidines and cyanopyridines. this compound forms a central core for a variety of important biological compounds[2], which are used as an antioxidant[2][10], anticancer[11][12], antibacterial[13][14], antifungal[14], anti-inflammatory[15][16][17], and Antiviral[18] depending on the substitution made on them[3](Table 1.1).

Table 1. 1 : Biological activity of some chalcones

Biological activity	Chalcone name	Structure of chalcone	Modle	Ref.
Antioxidant activity	2,3,4,6-Tetrahydroxychalcone		In vitro	[2]

	3,4,2',4'-tetrahydroxychalcone (butein)		In vitro	[10]
Anti-inflammatory Activity	2',4'-dihydroxy-6'-methoxychalcone (Cardamomin)		In vitro	[15]
	Xanthohumol (natural product)		In vitro	[16]
	(2”-nitrochalcone) synthesized		In vitro	[17]
Anticancer activity	Flavokawain (natural product)		In vivo	[11]
	(E)–2–methoxy–N–(4–methoxyphenyl)–5–(3–(4–nitrophenyl) acryloyl) benzene sulfonamide	 X= -Cl, -NO_2; Y= -Cl, -H R_1= -H, -OCH_3, -CH_3; R_2= -H, -OCH_3, -CH_3, -Cl, -Br.	In vitro	[12]

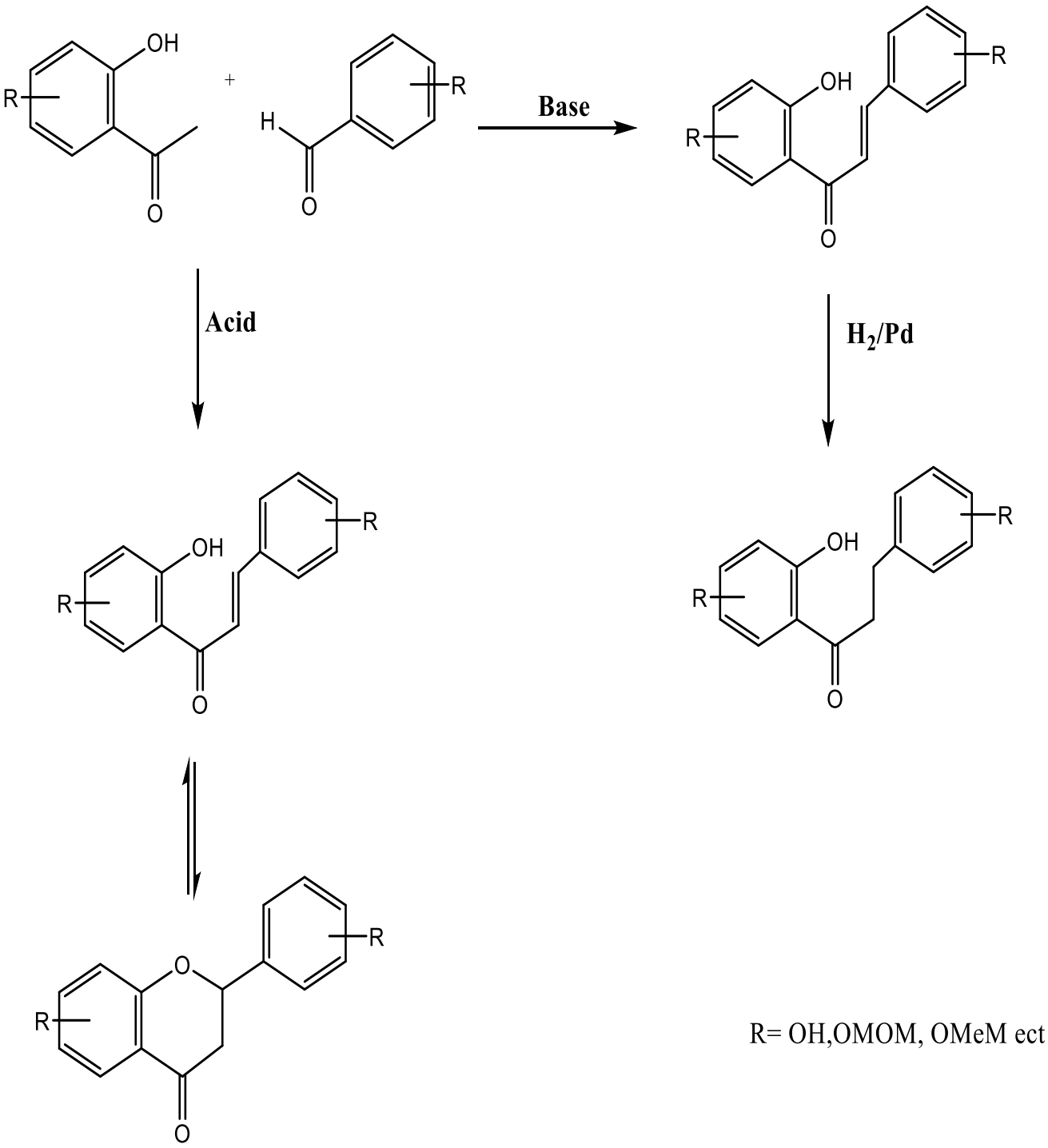
	3,4,2',4'-tetrahydroxychalcone (butein)		In vitro	[2]
Antibacterial activity	Dihydrochalcones synthesized		In vitro	[13]
	Isobavachalcone natural product		In vitro	[14]
Antifungal activity	Isobavachalcone natural product		In vitro	[14]
Antiviral activity	purinechalcone synthesis		In vitro	[18]
	3-prenyl-4, 2', 4'-trihydroxychalcone (licoagrochalcone)		In vitro	[2]

Cytotoxic activity	3-(p-chlorophenyl)-1-(4'-hydroxyphenyl)-2-propen-1-one		In vitro	[19]
	2',4',4-Trihydroxychalcone (Isoliquiritigenin)		In vitro	[20]

1.3 Synthetic Methods of Chalcones

In literature, a series of reactions are used to prepare chalcones, such as Suzuki coupling reaction[21], Friedel- Crafts Acylation reaction[22], and Julia-Kocienski Olefination reaction[23].However, Claisen-Schmidt condensation reaction (aldol condensation) is the simplest method for enone functional group formation[1].

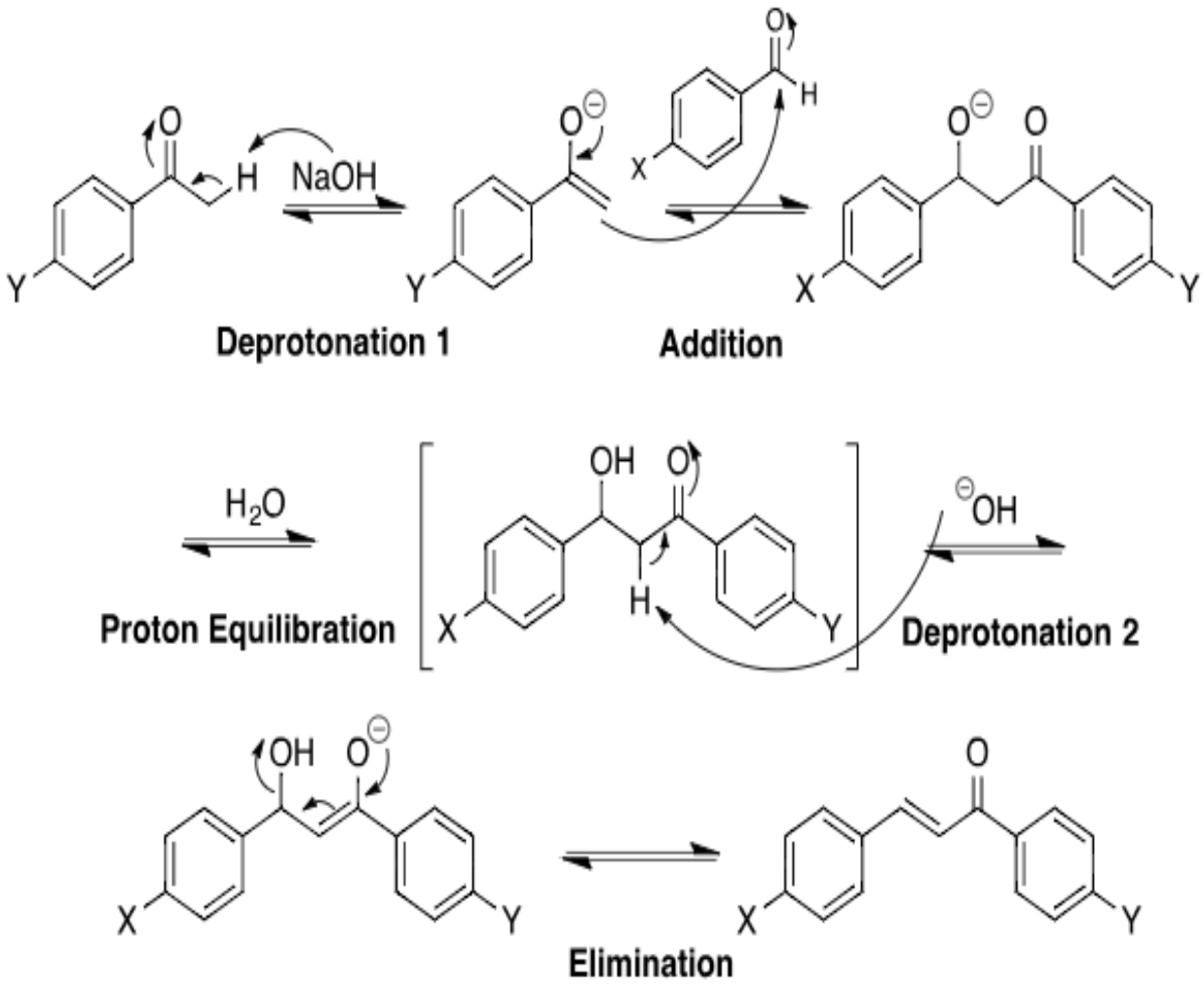
In this reaction, condensation of acetophenones with aromatic aldehydes occurs in the presence of basic catalyst such as aq. NaOH, KOH, and $Ba(OH)_2$,or in acidic condition includes the use of $AlCl_3$, dry HCl, $TiCl_4$, $RuCL_3$ and BF_3-OEt_2 to form α, β –unsaturated carbonyl system[24]. The base catalyzed Aldol condensation is usually the preferred route toward chalone formation[25], since under acidic condition if the chalcone having 2-hydroxy group, protective group like methoxymethyl ether or tetrahydropyrane are used to prevent cyclization of Chalcones which leads to the formation of flavanones as illustrated in scheme1.5 [24].



Scheme1. 5: Acid and base catalyzed synthesis of chalcone [24]

1.4 Mechanism of Chalcone Formation

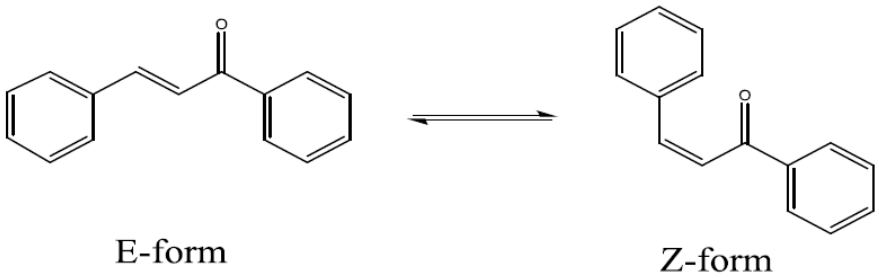
To synthesize chalcone by aldol condensation, there are many steps occur. For the first step, a base removes a proton (deprotonation1) from α -carbon of acetophenone, which creates an enolate ion. the enolate acts as a nucleophile and attacks the carbonyl group carbon of aromatic aldehyde to form alkoxide anion. In the next step, the alkoxide removes a proton from water to give β-hydroxy ketone, then hydroxide deprotonates the α -carbon to the β-hydroxy ketone to give another alkoxide attached to an alkene. Finally, a dehydration process occurs (Deprotonation 2 and Elimination) (Scheme 1.6) [26].



Scheme1.6: Mechanism of chalcone formation via aldol condensation by base catalyst [26]

1.5 Conformational Properties of Chalcone

Chalcones are flexible molecules that can exist as Z(cis)- or E(trans)-isomers as shown in scheme 1.7. Thermodynamically E isomer is most stable and have the lowest heats of formation[27] [28], while Z configuration is unstable because of the strong steric effects between the carbonyl group and B-ring, So recrystallization of an E–Z mixture yields E isomer as the only stereoisomer[29] .

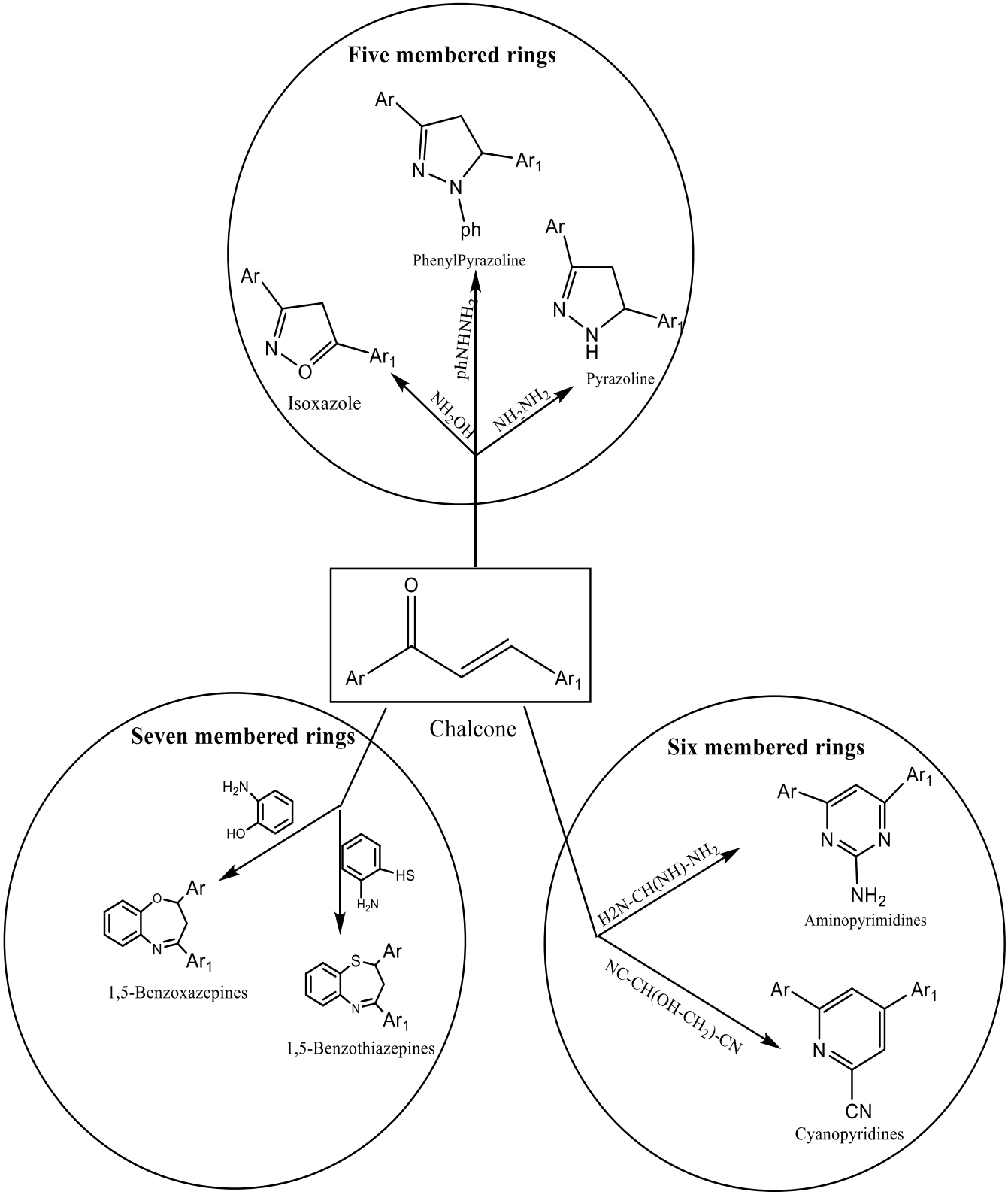


Scheme1. 7: E- and Z-isomerism of chalcones

1.6 N-Heterocyclic Carbenes from Chalcone

Chalcones are considered as one of the most useful resources of heterocyclic compound like isoxazoles and pyrazoles. These compounds and their derivatives are considered as one of the best compounds in medicinal chemistry due to their wide biological activity[2].

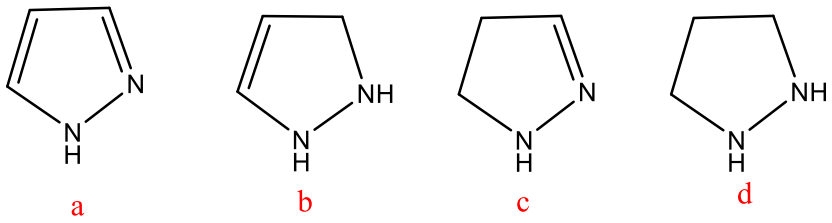
To obtain these heterocyclic rings which contain nitrogen and oxygen with five, six and seven membered ring, chalcone undergoes a cyclization reaction in a certain condition (scheme1.8) [30].



Scheme1. 8: N-Heterocyclic derivatives of chalcones [30]

1.6.1 Overview of Pyrazole and Isoxazole Ring

Pyrazole refers to five-member aromatic ring compound, these are organic nitrogen heterocyclic system consist of two adjacent nitrogen atmos and three carbon atoms with $C_3H_4N_2$ formula. The partially reduced forms of pyrazole are named as pyrazolines (b,c), while completely reduced form is pyrazolidine (d) [31] (scheme 1.9).

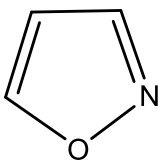


Scheme1. 9: Pyrazole structure and there partially reduced forms

Among the two nitrogen atoms one is basic and the other is neutral in nature. Pyrazole derivative are important targets in medicinal chemistry and drug designing due to their useful biological activities, they have been known to exhibit antimicrobial, analgesic, anticancer, anti-tubercular, anti-inflammatory, antidepressant, anticonvulsant and antioxidant[31].

Scheme 1.10 shown isoxazole ring (1,2-oxazole) which represent five membered heterocyclic compound that contaning nitrogen and oxygen atoms in the 1, 2 positions, N-O bond is the weakest bond in this molecule and is cleaved in all ring-opening reactions [32].

This compound has various pharmacological actions such as antibacterial,antibiotic, antifungal, anticancer and anti-inflammatory [32].

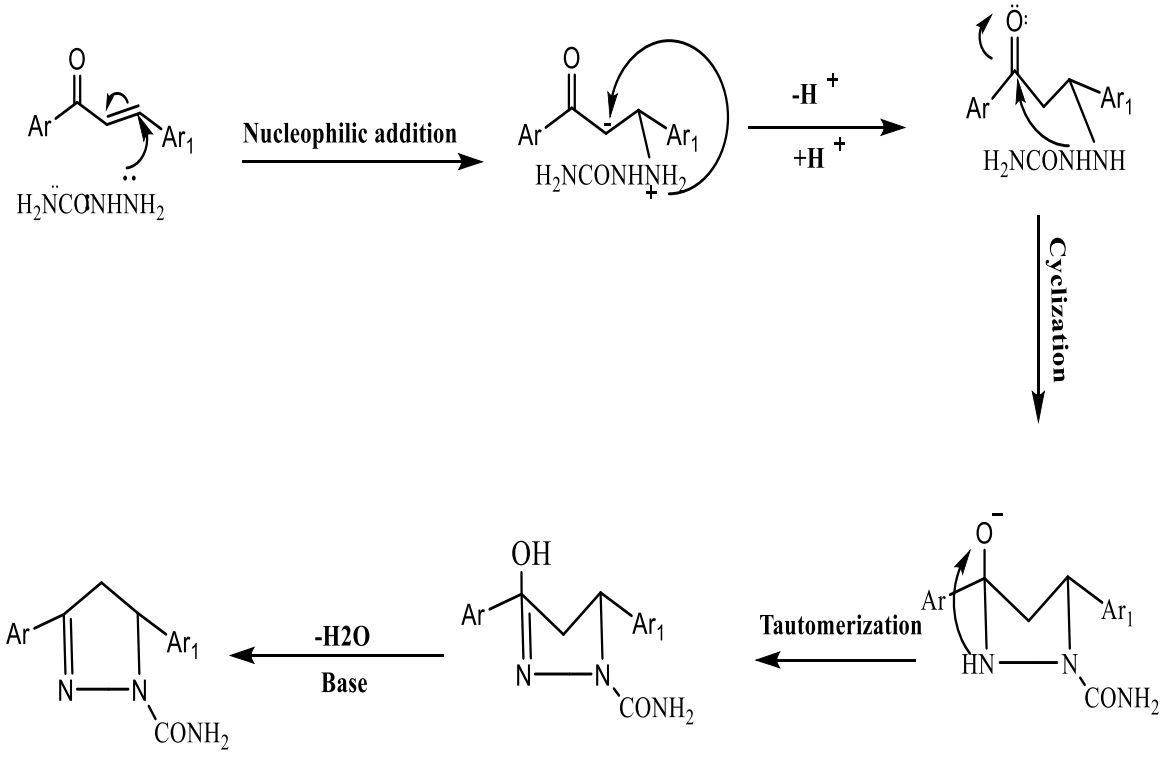


Scheme1. 10: isoxazole structure

1.6.2 Mechanism of Pyrazole and Isoxazole Rings Formation

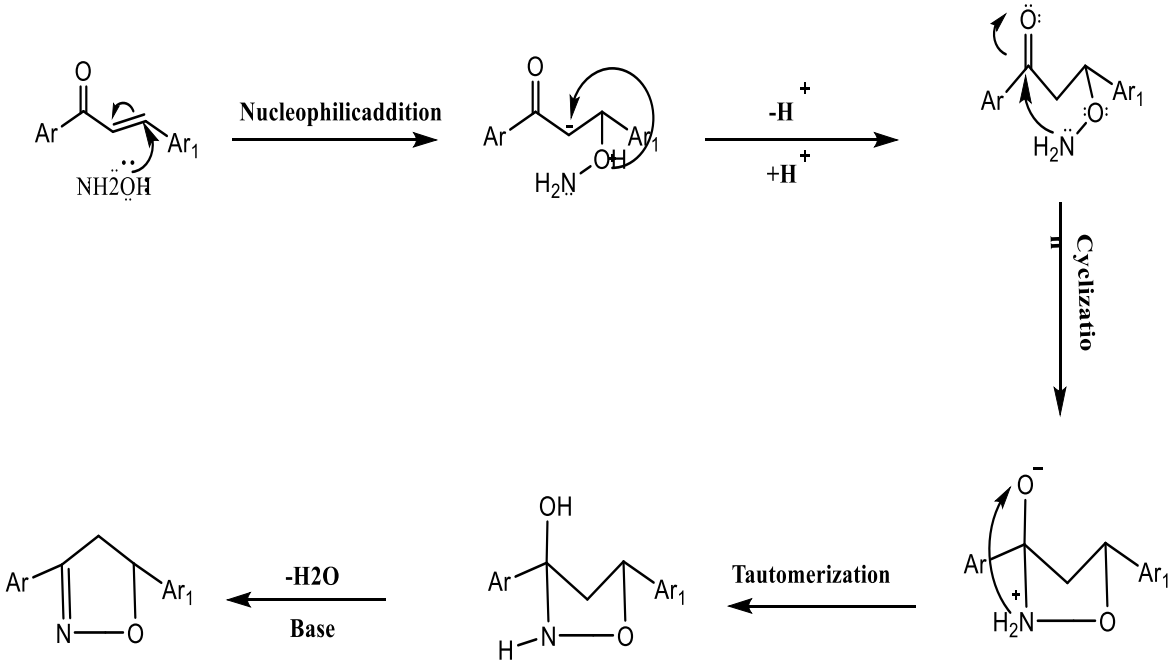
The formation of pyrazole and isoxazole rings is accomplished by nucleophilic conjugate addition reaction. The key reactions involve the formation of carbon-carbon bond in the first step, cyclization reaction occurs by free lone pair of nitrogen attacks the electrophilic carbonyl group, converts it to a hydroxyl group and forms a closed ring. Finally, dehydration reaction takes place to eliminate a water molecule to produce the ring closed final products.

Chalcone further reacted with semicarbazide hydrochloride in alkaline medium to yield the corresponding pyrazole ring by the following reaction mechanism (Scheme 1.11).



Scheme1. 11: Mechanism of formation of pyrazole ring via conjugate addition

While chalcone reacted with hydroxylamine hydrochloride in alkaline medium to yield the corresponding isoxazole ring by the following reaction mechanism (scheme 1.12).



Scheme1. 12: Mechanism of formation of isoxazole ring via conjugate addition

1.7 Cancer Disease: Definition and Biology

According to the world health organization, cancer is the second leading cause of death globally and accounted for 9.6 million deaths in 2018. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men. While breast, colorectal, lung, cervix and stomach cancer are the most common among women [33].

Today cancer remains the third cause of death in Palestine, after heart and vascular diseases, according to Palestinian ministry of health, based on data from the Palestinian cancer registry in the west bank. Crude cancer incidence in Palestine reached 83.8 per 100 thousand persons, which was lower than cancer incidence in neighboring countries like Egypt [34].

Nowadays, the proportion of deaths due to cancer in Palestine reaches 13.8% from the total number of deaths reported per year [34].

Cancer is a genetic disease characterized by abnormal and uncontrolled proliferation of cells. In normal tissues, the rate of new cells growth (cell proliferation) and old cell death are kept in balance, but in cancer this balance is disrupted and this is due to uncontrolled cell growth or to loss of cell ability to undergo apoptosis. Apoptosis process is the process of programmed cell death [35].

Cancer cells are not a localized and behave as independent cells, growing without control to form tumors. Tumors grow in a series of steps as shown in figure1.1 and the type of tumor that forms depends on the type of cell that was initially altered.

The first step is **hyperplasia**, in this step uncontrolled cell division results in producing many cells. These cells appear normal, but changes have occurred that result in some loss of control of growth. While the second step is **dysplasia**, the cells with uncontrolled growth which are formed in the first step are accompanied by abnormality of cells. In the third step **anaplastic** these abnormal cells begin to lose their original function and can now spread over a wider area of tissue but the tumor is still in the original location and is not considered a malignant tumor. In the final step which is **tumor metastasize** the cells invade surrounding tissue and spread to other locations, in this stage cancer is called malignant tumor that leads to the death of most cancer patients [36].

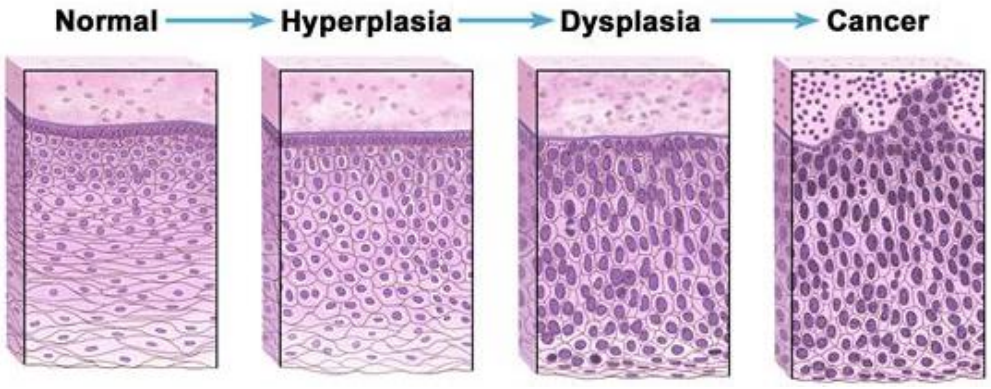


Figure 1. 1: Steps of tumor growth

1.7.1 Breast Cancer: Definition and Treatment Options

1.7.1.1 Breast Cancer

Breast cancer is a malignant tumor that starts in the cells of the breast. The disease occurs almost entirely in women, but men can get it too . The early detection of this type of cancer is the best way to treat it [37]. According to ministry of health in Palestine, "Breast cancer came first for cancers that affect women in Palestine, which reached 33.7% with a rate of 33.1 new cases per 100 thousand females in Palestine annually. Colon cancer comes second regarding cancer cases with the rate of 9.4% and leukemia cancer comes third with the rate of 8.7%" this statically number represented in figure 1.2 [34].

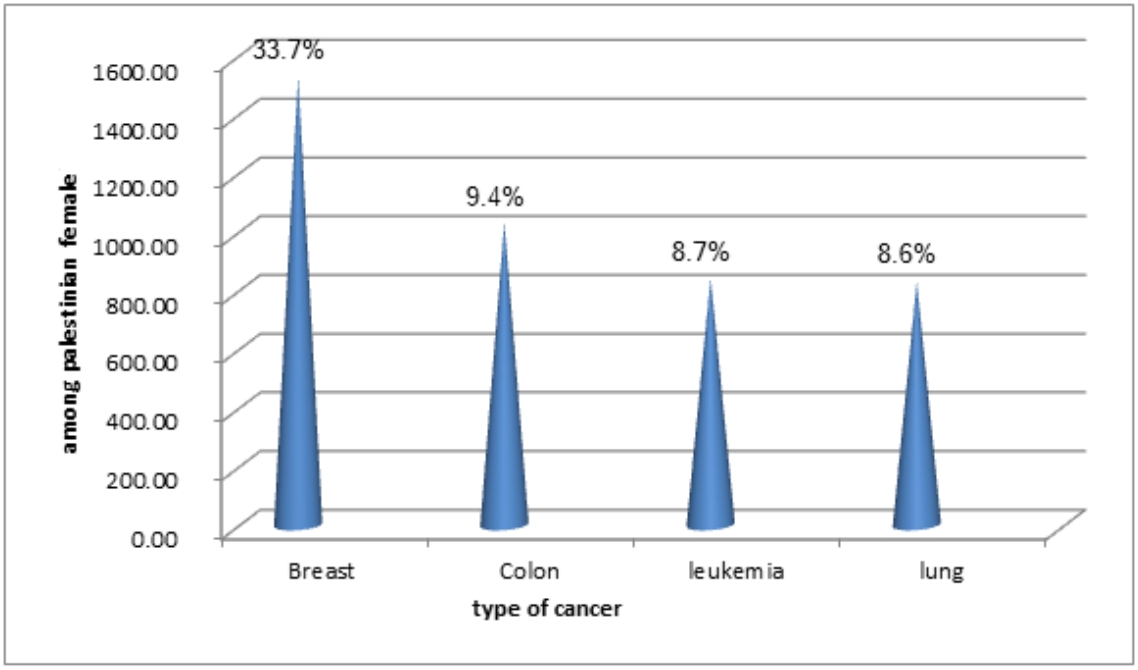


Figure 1. 2: Distribution of percentage of top reported cancer among females, West Bank, Palestine, 2015 [34]

Several different types of breast cancer exist, but depending on the presence of estrogen receptors, there are two types of breast cancer: positive estrogen receptor (ER+) and negative estrogen receptor (ER-). Estrogen is a hormone which is responsible for growth and function of the female body. ER+ has receptors for estrogen, so this type of cancer

takes signals from estrogen to grow and develop. In contrast, ER- does not have receptors for estrogen and this makes it not respond to conventional cytotoxic chemotherapy treatment, thus reducing survival compared with other types of breast cancer [38][39].

1.7.1.2 Treatment Options

To treat breast cancer there are several conventional ways like surgery, radiation and chemotherapy. The treatment option depends on several factors, such as the tumor's subtype and its degree of development [40][41].

Surgery is the first option to treat the tumor if it is amenable ,where the tumor and some normal tissues surrounding it is removed. After surgery, adjuvant therapy is the second option for treatment which includes radiation therapy, chemotherapy, and/or hormonal therapy. Radiation therapy kills cancer and normal cells by using high energy rays. many side effects occur by this type of treatment, such as swelling of the breast, redness, skin discoloration and pain or burning in the skin where the radiation was directed[42].

Chemotherapy drugs are toxic compounds in general, all these drugs work to inhibit the growth of cancer cells by using different ways. This causes some of the side effects of chemotherapy, including gastrointestinal distress, low white blood cell count, and hair loss [41].

Hormonal therapy can be used before or after surgery. This type of treatment depends on the presence of estrogen receptors or progesterone in cancer cells(hormone-receptor-positive). When this hormone is blocked, cancer cells lose the ability to grow [43].

1.7.2 Chalcones as Anticancer Drug

Chalcones have been targeted by several researchers in recent years as anticancer, so several chalcones were synthesized and tested against various human cell lines. Some of these chalcones are represented below in table1.2.

L. J. Michelini1 recently reported a novel nitroaminochalcone (I), this compound showed high cytotoxicity at a single concentration of 93,2 μmol/L against breast (OVCAR), colon (HCT-116) and leukemia(HL-60) human tumor cells, by using MTT assay [4].

Dr. Alexander Ciupa's synthesized many analogue of urocanic-chalcone and investigated it in three cancer cell lines, which are human colon carcinoma (HT29), human breast carcinoma (MDA-MB-231) and non-cancerous human skin fibroblast cell line (FEK-4).All activities are reported as an average IC50 of at least three independent experiments and noted the increased of antiproliferative activity with addition of methoxy groups, where IC50 of MDA-MB-23 in compound (II) was 102.4μM and IC50 of MDA-MB-231 in compound (III) after addition of methoxy group was 49.9 μM, and Nitrogen methylation increased antiproliferative activity (IV)[44].

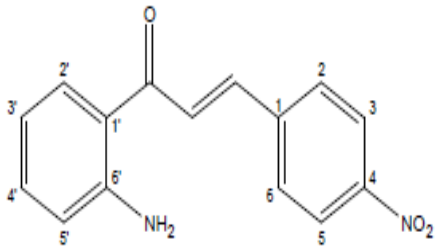
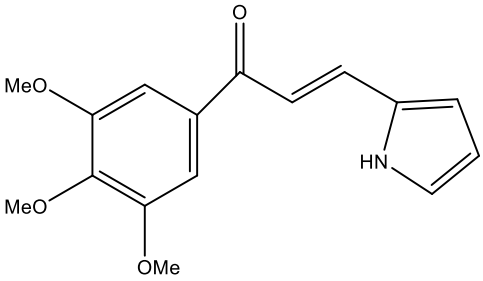
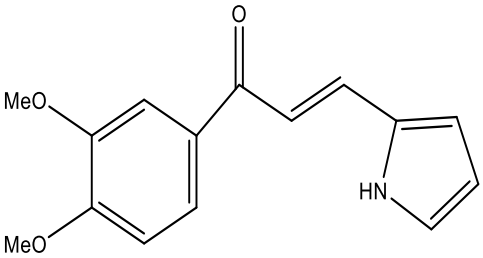
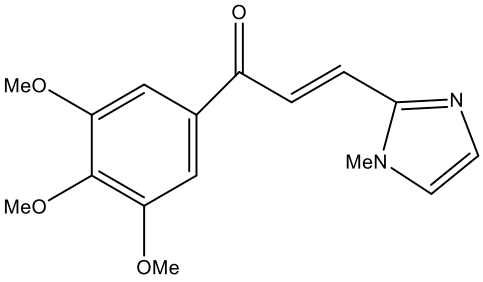
Two types of chalcones containing pyrazole ring were prepared and their cytotoxicity against different human cell lines, including breast (MCF-7), colon (HCT-116) liver (HEPG2) cell lines, as well as normal melanocyte(HFB4) was evaluated using SRB, apoptosis and cell cycle assay. Two chalcones (V) and (VI) have promising antitumor activity on human breast adenocarcinoma cell line MCF-7 and they were less toxic on the normal cell line HFB4 [45].

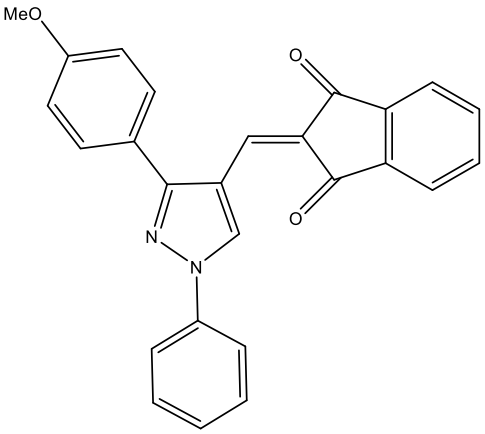
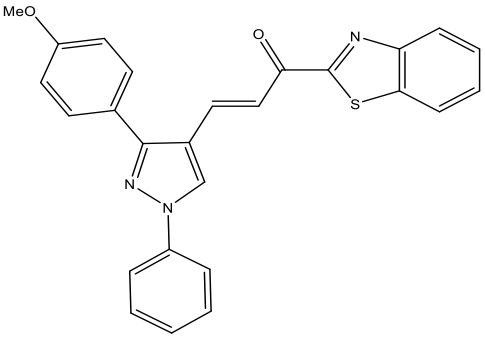
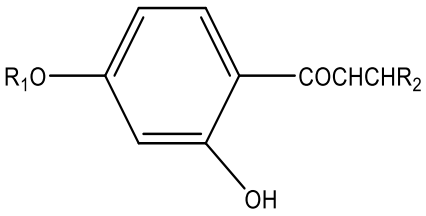
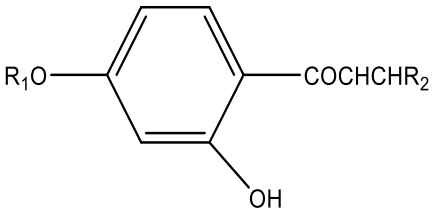
Anticancer activity of newly developed chalcone analogues was studied by using MDA-MB 231 and MCF7 breast cancer cells and the T-leukmia cell line by using cell counts performed, apoptosis assay and cell cycle assay. Compounds (VII) and (VIII) are the most active compounds [46].

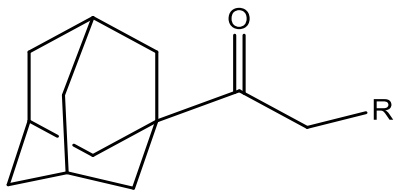
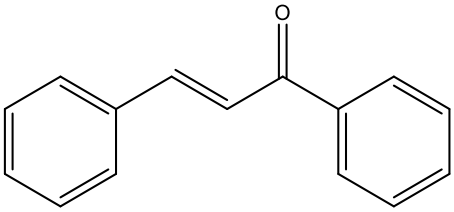
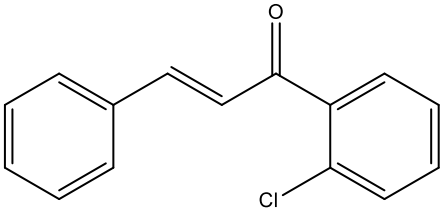
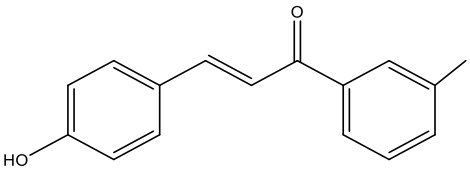
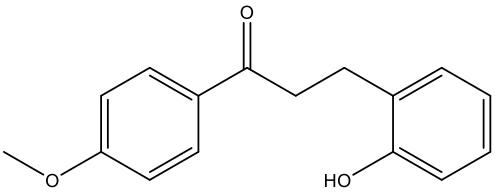
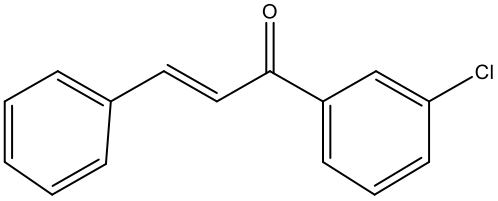
Patent (Anderson & Kaimari,2005) described a series of novel 1-adamantly chalcones (IX) as potential anticancer agents against breast cancer. The compounds were tested on two breast cancer cell lines (MCF-7 and MCF-MB435) and for normal epithelial cell line (MCF-10).The results indicated that these compounds decrease the cell viability for both cancer cell lines, and didn't affect on the normal cell line [47].

Also 25 different chalcones were synthesized and tested as anticancer against various human cell lines, including human breast adenocarcinoma cell line MCF-7, human lung adenocarcinoma cell line A549, human prostate cancer cell line PC3, human adenocarcinoma cell line HT-29 and human normal liver cell line WRL-68 by using the MTT assay. Compounds X(a), X(b), X(c), X(d),and X(f) showed high cytotoxicity in all cell lines, except for the A549 cell line [48].

Table 1. 2: Anticancer activity of chalcones

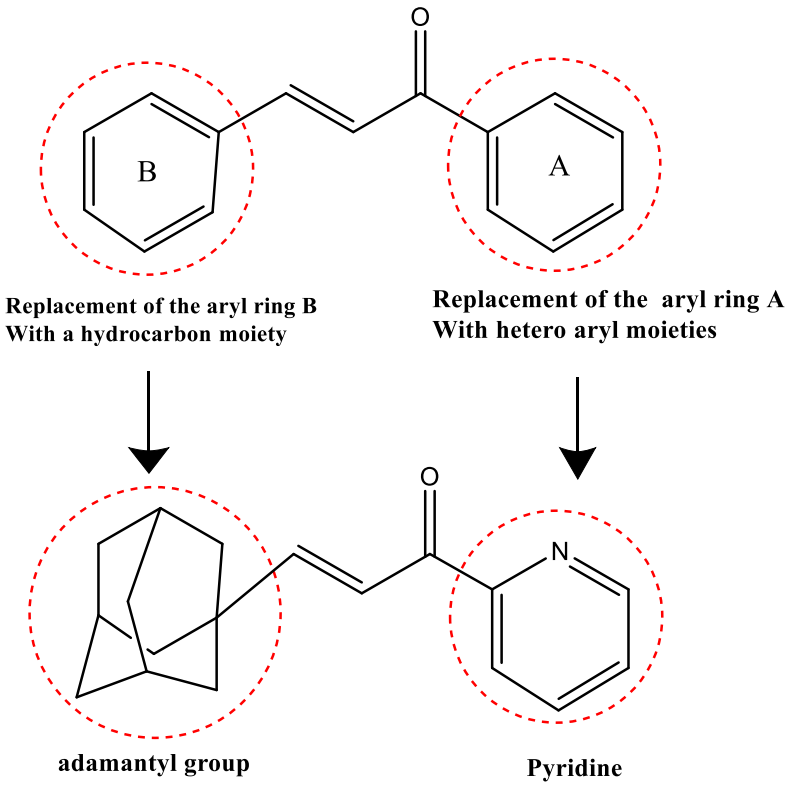
NO.	Chalcone structure	Assay	Cell line	Ref
I		MMT	OVCAR-8 HCT-116 HL-60	[4]
II		MMT	MDA-MB-231 HT29 LNCaP FEK-4	[44]
III				
IV				

V		-SRB -Cell cycle -Apoptosis	HCT116 MCF7 HEPG2 HFB4	[45]
VI				
VII	 R_1: 3-methyl-2-buten-1-yl , R_2 pyrid-4-yl	cell counts Cell cycle Apoptosis	MCF-7 MDA-MB-231	[46]
VIII	 R_1: 3-methyl-2-buten-1-yl , R_2 pyrid-3-yl			

IX		MTT	MCf-7 MDAMB435 MCF-10	[47]
X(a)		MTT	MCF-7 A549 PC3 HT-29 WRL-68	[48]
X (b)				
X(c)				
X (d)				
X(f)				

1.8 Development of Chalcones

In general, chalcone contains two aromatic rings(A) and (B) linked by an olefinic bond and a keto group (B). The anticancer activity of chalcones are extremely affected by the replacement of the two aryl rings. Patent (Anderson & Kaimari,2005) modulated the general chalcone structure by replacement of the aryl ring A with heteroaryl moieties (pyridine) and aryl ring B with a hydrocarbon moiety (adamantyl group) by Aldol condensation in basic medium (44) *(scheme 1.13).*

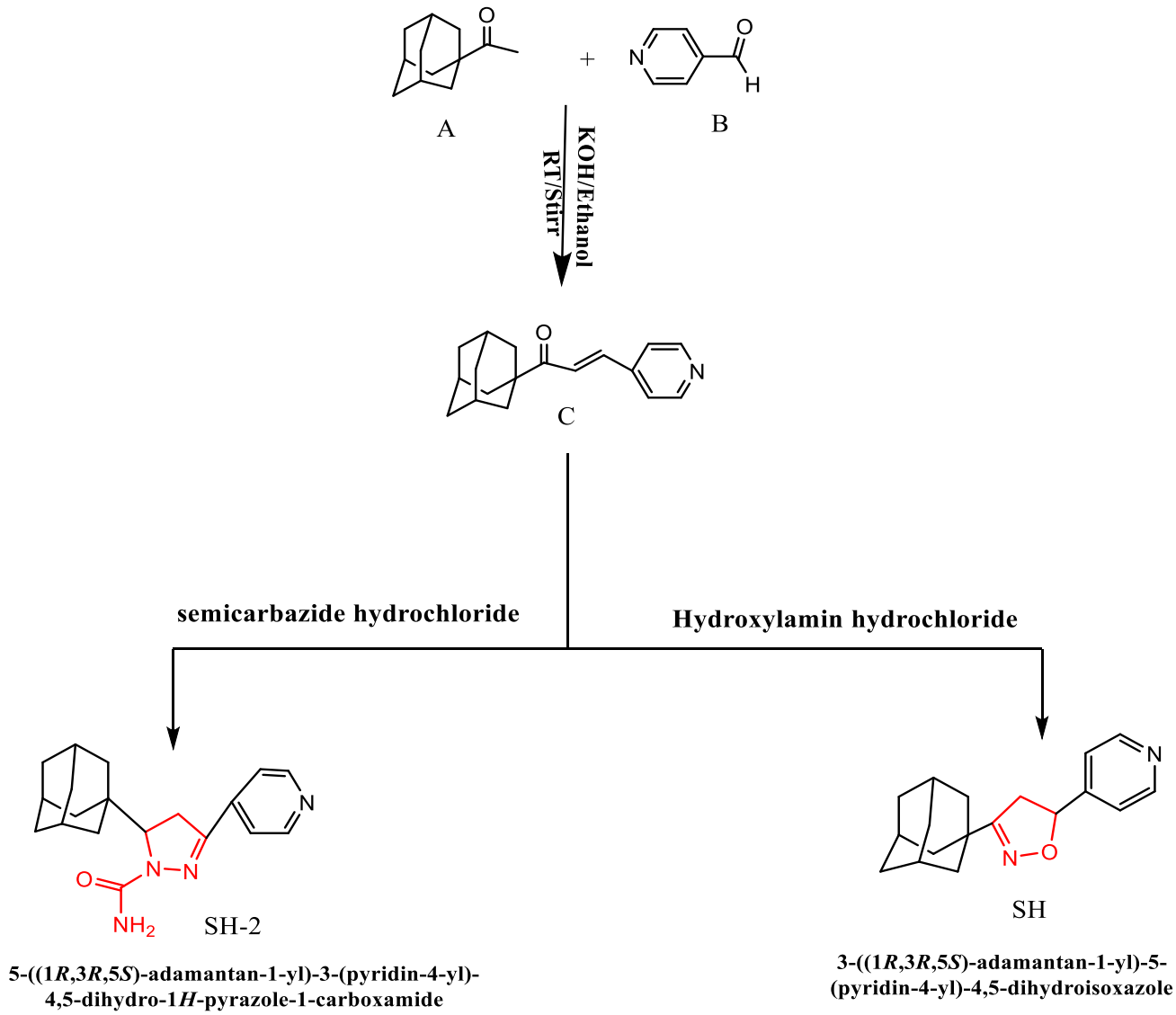


Scheme1. 13: general structure of development chalcone

Addition of adamantyl group to chalcone increases the hydrophobicity and lipophilicity of it and makes favorable conditions for its transport through biological membranes and promotes the biological activities of compounds. Adamantly structures compounds showed anticancer activity against different tumor cell lines [47].

1.9 Aim of this Thesis

The aim of this present work is to synthesize new pyrazole and isoxazole heterocyclic ring by using adamantly chalcone (C) (Scheme 1.14).



Scheme1. 14: Synthesis of the chalcone (C) and its cyclized products

These compounds were synthesized and characterized by ^{1}HNMR and FT-IR. Their effect on cell viability and toxicity was evaluated by using breast carcinoma cell lines (MDA-MB-231/GFP and MCF-7).

Chapter Two
Materials &Methods

2.Materials And Methods

2.1 Materials

For synthesis part: All chemicals were in analytical standard and bought from Sigma-Aldrich 4-Pyridinecarboxaldehyde (99%), 1-Adamantyl methyl ketone (99%), Sodium hydroxide, hydroxylamine hydrochloride, sodium acetate, potassium hydroxide (90%), semicarbazide hydrochloride and potassium bromide. High purity ethyl acetate, hexane, acetic acid and ethanol (> 96%) were purchased from Biolab.

For biological part: A sterile solution containing the XTT reagent and N-methyl dibenzopyrazine methyl sulfate (PMS), DMSO and Cell lines (MCF-7 and MDA-mb231/Gfp). Roswell Park Memorial Institute medium (RPMI) was used to culture cells, supplemented with Fetal bovine serum (FBS), penicillin streptomycin, L-glutamine(L-Glu) and non-essential amino acids, all chemicals were obtained from Al-Quds University laboratory stock.

2.2 Instrumentations

For synthesis part: All chemical reactions were monitored with thin layer chromatography (TLC). TLC was carried out on TLC plastic sheets silica gel, 20*20 cm, layer thickness 0.2 mm eluted with an ethyl acetate/hexane mixture (2:5); the spots were detected by UV light.

Melting points were determined by open capillaries on electro thermal Stuart SMP3 advanced melting point apparatus.

IR spectra were determined with KBr matrix (4000–400 cm^{-1}) using a Perkin-Elmer, FT-IR spectrometer.

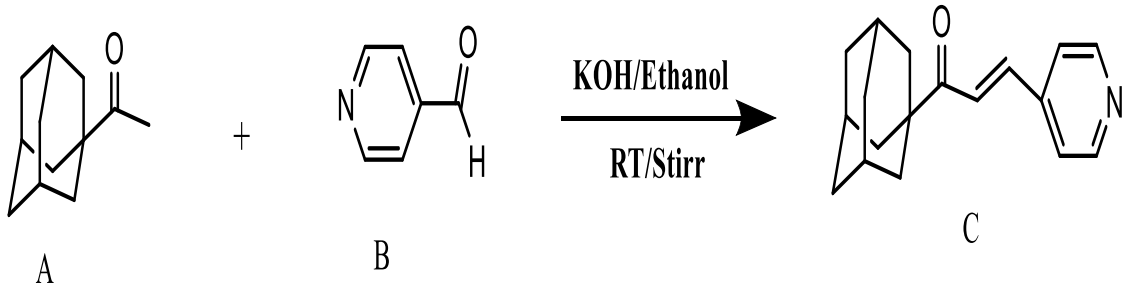
^{1}HNMR spectra of compounds were recorded in DMSO-d6 and recorded in D_2O on a Bruker 29 DPX 300 Spectrometer (1H: 300.1 MHz) at 295 K. The chemical shift values are given in ppm.

For biological part: Olympus optical microscope CK40 was purchased from Italy tailored for the cell culture process. Cell counting chamber Improved Neubauer and Hemocytometer were obtained from Marienfeld-Superior, Germany. 96-well cell culture plates, 6-well plates and 6cm plates from JET Bio-Filtration Company. Cell culture CO_2 incubator humidity control was purchased from Memmert Company, Germany. The enzyme-linked immunosorbent assay (ELISA) reader was obtained from Bio-Rad Company

2.3 Synthesis Protocol

2.3.1 General Procedure for Synthesis of Adamantly Chalcone

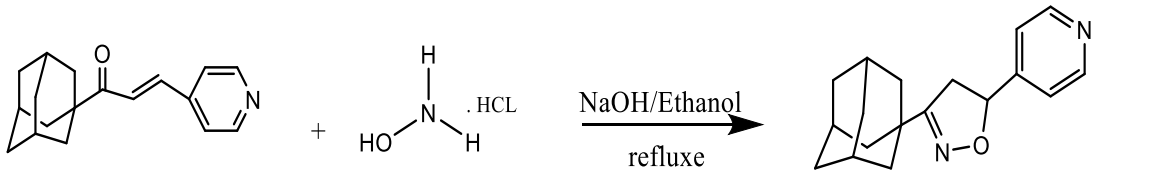
A (0.0028mol) of potassium hydroxide was dissolved in 40ml ethanol and 96%, (0.0028 mol) of 1-adamantyl methyl ketone (A) was added to an ethanolic solution and stirred for 20 minutes. Then (0.0028 mol) of 4-pyridinecarboxaldehyde (B) was added dropwise to the solution and stirred at room temperature for 72 hours. Finally, the reaction mixture was poured into crushed ice, the resulting precipitate was filtrated and washed several times with water, dried in room temperature overnight. (scheme 2.1).



Scheme 2. 1: Synthesis of adamantly chalcone (C)[47]

2.3.2 Synthesis of 3-((1R,3R,5S)-adamantan-1-yl)-5-(pyridin-4-yl)-4,5-dihydroisoxazole (SH)

A mixture of chalcone(C) (0.0002mol), hydroxylamine hydrochloride solution (0.025g in 0.25 mL of water) in 5 mL ethanol 99% was added to sodium hydroxide solution (0.05g NaOH in 0.5mL of water). The mixture was refluxed in water bath for 8hr, cooling to room tempreture then freezing overnight, the mixture was concentrated by destilating out the solvent under reduced pressure and then poured into crushed ice. The precipitate was filtrated, dried and assigned one spot in TLC (Scheme 2.2).

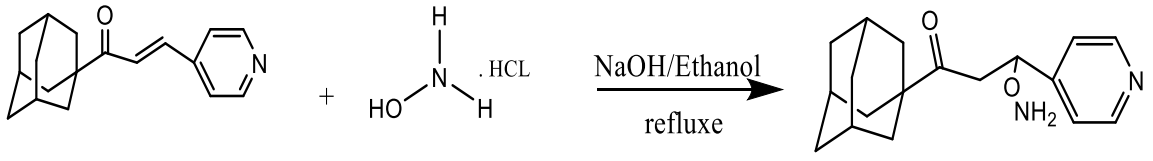


chalcone (C) Hydroxylamin hydrochloride 3-((1*R*,3*R*,5*S*)-adamantan-1-yl)-5-(pyridin-4-yl)-4,5-dihydroisoxazole SH

Scheme 2. 2: synthesis of SH compound

2.3.2.1 Real Structure of SH Compound

According to theoretical data, when hydroxylamine hydrochloride reacted with any type of chalcone, the product must contain isoxazole ring like compound (SH). However, FT-IR and ^{1}HNMR data for compound (SH) indicates that the actual compound was amino (SH-1) (Scheme2.3).

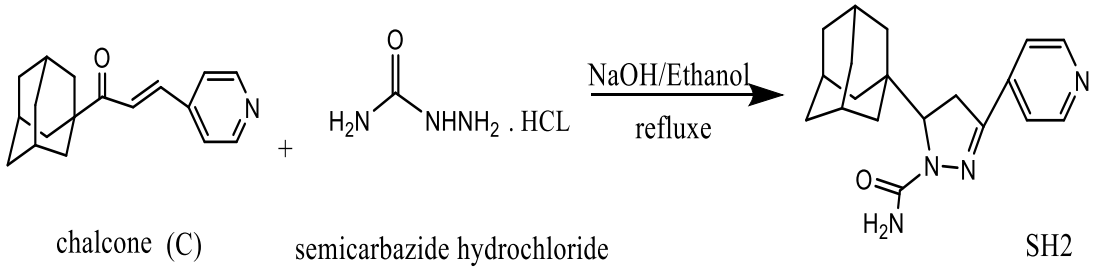


chalcone(C) Hydroxylamin hydrochloride 1-((3*r*,5*r*,7*r*)-adamantan-1-yl)-3-(aminooxy)-3-(pyridin-4-yl)propan-1-one SH-1

Scheme 2. 3: synthesis of SH-1 compound

2.3.3 Synthesis of 5-((1R,3R,5S)-adamantan-1-yl)-3-(pyridin-4-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (SH-2)

A mixture of chalcone(C) (0.0004mol), semicarbazide hydrochloride (0.0006mol) and sodium hydroxide (0.051g) in 15ml ethanol 96% were refluxed for 72hours in water bath. TLC monitoring was extensively done, the mixture was concentrated by destilating out the solvent under reduced pressure and then poured into crushed ice. The precipitate was filtrated and dried in room temperature overnight (Scheme 2.4).



chalcone (C) semicarbazide hydrochloride SH2

5-((1*R*,3*R*,5*S*)-adamantan-1-yl)-3-(pyridin-4-yl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide

Scheme 2. 4: Synthesis of SH-2 compound

2.4 Cell Culture

Two breast cancer cell lines were used for biological test of SH-1 and SH-2 compound which is Michigan Cancer Foundation-7 (MCF-7) (ER+) and MDA-MB-231/GFP (ER-) cell lines. Cells were grown in RPMI medium supplemented with 10% (v/v) Fetal bovine serum(FBS), 1%L-Glu and 1%pen/strep at 37 °C and 5% CO_2.

2.4.1 Thawing Cells and Trypsinization Process

Cells must be prepared by thawing process, the cells were thawed by transfer from -80°C to 37°C water bath until just a small bit of the ice remains. Where upon 2ml warm fresh media with serum was added to the suspended cells. After centrifugation takes place at 1000 rpm for (5-10) minutes, the supernatant was removed and the pellet was re-suspended with 4ml of RPMI media, the cell suspension was plated on 25cm^2 culture

flask and cultured in humidified, 5%CO2 incubator at 37°C. After 24 hours the cells in the flask were examined (healthy and sub confluent). The old media was removed from the flask and cells were washed two times with 4ml of PBS.

Trypsinization process took place when 3ml of trypsin was added after removing of PBS, cells were examined after 2-3 minutes by a microscope to ensure that all cells were detached and 4ml of RPMI media was added to flask to stop trypsinization. Suspension was transferred into a 15ml falcon tube and centrifugation at 1000 rpm for 7 minutes then removed the supernatant from falcon and resuspended the pellet in 3ml fresh media to become homogeneous suspension. 10µl was taken by hemocytometer to count the total number of thawing cells.

2.5 **Biological Assays**

The terms proliferation and viability are used to quantify cell growth and estimate the efficiency of molecules in screening, or evaluating the cytotoxicity of compounds for treating cancer. The viability of cells and cytotoxicity of compound can be assessed by measuring the amount of viable and dead cells in a total cell sample. The total cell count, cell death, cell proliferation and XTT assay are most widely used assays for preliminary results in vitro anticancer test of synthetic compounds. This type of assays is based on a color change caused by the structural differences or metabolic impairment between viable and nonviable cells. It is called colorimetric assays [49].

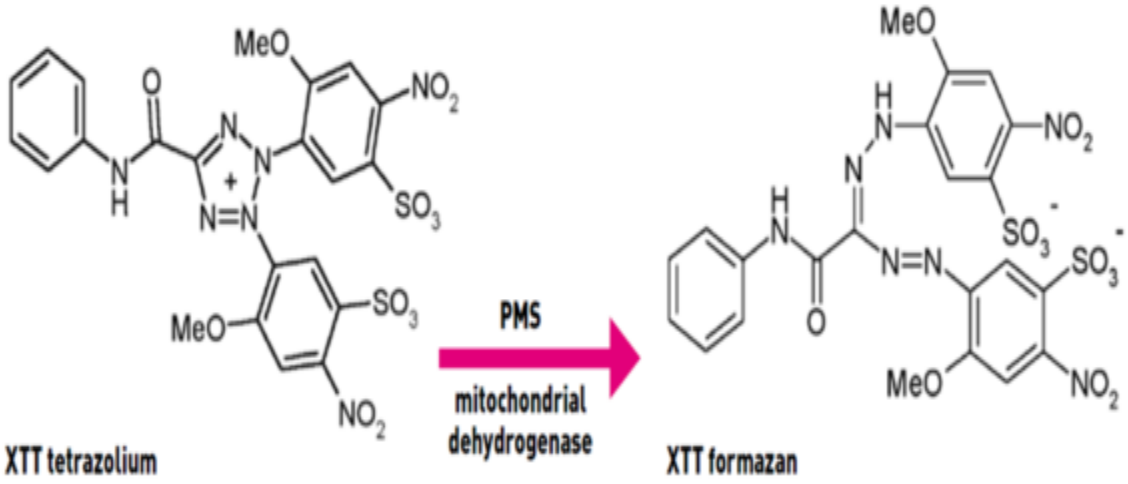
2.5.1 Total cell Count, Cell Death Assay for MDA-MB-231/GFP Cell Line

This assay studied the effect of prepared compounds as anticancer by counting the number of viable and nonviable cells in a sample or by study the morphology of cells. Thawing cells could be suspended to use when it reach 80-90% confluence by removed it from incubator and old media was took away, cutluer media and trypsin were pre-warmed in water bath to 37°C. 1ml of trypsin were added to sufficient cover surface area, and cells were rinsed in trypsin by gently swirling the flask then the flask was incubated in incubator for 2 minutes, cells were examined by microscope to ensure that all cells are detached, then 5ml of fresh medium were added and mixed well in order to transfer 20µl

of suspension cells to eppendorf and dilution with 20µl of trypan blue and 10 µl trypan blue- cells mixture were transferred to both sides of the hemocytometer to calculate the number of cells in original sample. 100,000 cells / well in 6 cm plates were transferred to be treated with three concentrations 1µM.10µM and 50µM of prepared compounds, after 24 and 48 hours a picture was taken for the cells under the microscope then trypsinization the cells, 10µM of suspension cells were transferred to both sides of the hemocytometer to calculate the number of viable and nonviable cells.

2.5.2 Cell Proliferation XTT

Cell proliferation XTT is a technique used in the 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H tetrazolium-5-carboxanilide Salt (XTT). Mitochondria in the live cells can reduce this salt to orange colored water soluble dye formazan (scheme 2.4) and because the mitochondrial enzymes are inactivated in death cells make this a reliable method for detecting live cells [50].



Scheme 2. 5: reduce of XTT tetrazolium salt to formazan dye

1. After trypsinization of MCF7 and MDA-MB-231/GFP Cell Lines, each well in 96-well plate were plated by 2000cell/well(200µl of detached cells) and incubated for 24 hours.
2. old media was changed then the cells were treated with two different concentration (5 µmolar and 25 µmolar) for each drug and incubated at 37^0C in a 5% CO2 for 24 hours.
3. The XTT reagent solution and N-methyl dibenzopyrazine methyl sulfate(activation reagent) were pre-warmed in water bath to 37°C .
4. 0.1ml of activation reagent was added to 5ml XTT reagent to prepare a reaction solution for one 96-plate.
5. After 24 hour of incubation, 50µl of the reaction solution was added for each well in plate and incubate the plate in an incubator for 3 hrs. and 30 min
6. Absorbance was measured at 450nm using ELISA reader from Thermo scientific Multiskan FC device.
7. After 48 hours of incubation, steps 5 and 6 were repeated.

Chapter Three
Results & Discussion

3. Results & Discussion

3.1 Characterization

A number of analytical techniques such as TLC, melting point (mp), FT-IR spectroscopic, and ^{1}H NMR spectroscopic were used to identify the structural and purity of prepared compounds SH-1 and SH-2.

3.1.1 Thin Layer Chromatography (TLC)

TLC is one of the most popular methods used for checking the purity of compounds, it can be used to monitor the progress of a reaction. Silica gel coated on aluminum plate, used as a solid phase and ethyl acetate / hexane mixture (2:5) used as an eluent for all compounds ,except SH1 (3 drop of trimethylamine added to the same eluent).
All products were pure as TLC shows. Retention factor (R_f) of compounds included in table 3.1

Table 3. 1: Retention factor (R_f) of prepared compounds

Compounds	Rf (cm)
Chalcone (C)	0.35
SH-1	0.34
SH-2	0.72

3.1.2 Melting Point

Melting point is one of the physical properties that can provide initial information about purity of solid compound. melting points and percentage yield of prepared compounds appear in table3.2.

Table 3. 2: Melting points and percentage yield of prepared compounds

compound	Melting point	% Yield	Molecular formula(Mol.wt)
Chalcone(C)	98-99	70.5%	$C_{18}H_{21}N$ (267.37)
SH-1	20-22	40%	$C_{18}H_{22}N_2O$ (282.39)
SH-2	123-124	37.5%	$C_{19}H_{24}N_4O$ (324.43)

As shown, narrow melting point range of new compounds and different from chalcone, this values proves the purity of these compounds and gives a primary indication that these compounds are not chalcone (C) [51].

3.1.3 FT-IR Analysis

3.1.3.1 FT-IR of Chalcone (C)

Figure 3.1 shows the spectrum of chalcone (C), where α,β-unsaturated stretching of C=O group appeared at 1699 cm^{-1}, while C-N group at 1446cm^{-1} and aromatic ring have one band refer to C=C at 1607 cm^{-1} [51].

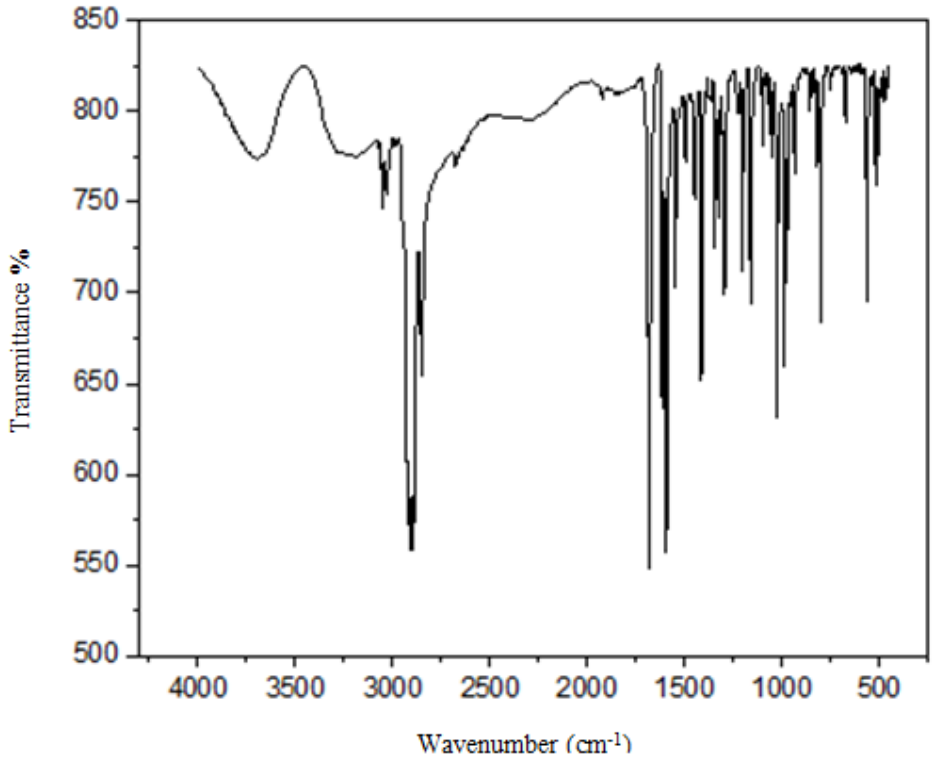


Figure 3. 1: FT-IR spectrum of Chalcone (C)

Table 3. 3: FT-IR spectrum analysis of chalcone (C)

IR frequency band (cm-1)	**Group responsible**
2904, 2843	C-H str. (sp3)
1684	C=O str. (α,β-unsaturated)
1615	Conjugated C=C (aromatic)
1583	C-N (pyridine ring)
1592, 1320	C-H str. (pyridine ring)

3.1.3.2 FT-IR of 3-((1R,3R,5S)-adamantan-1-yl)-5-(pyridin-4-yl)-4,5-dihydroisoxazole (SH-1)

Chalcone (C) and hydroxylamine hydrochloride were used as starting materials to synthesis SH-1**.**

3.1.3.2.1 *FT-IR of* hydroxylamine hydrochloride

IR-spectrum of chalcone (C) is mentioned above in section 3.1.3.1 where Figure 3.2 showes IR-spectrum of hydroxyl amine hydrochloride, which has a NH_2-stretch band at 3244 cm-1 and bending band at 1583, and another peak for (OH) functional group at 3442 cm^{-1} as shown in table3.4.

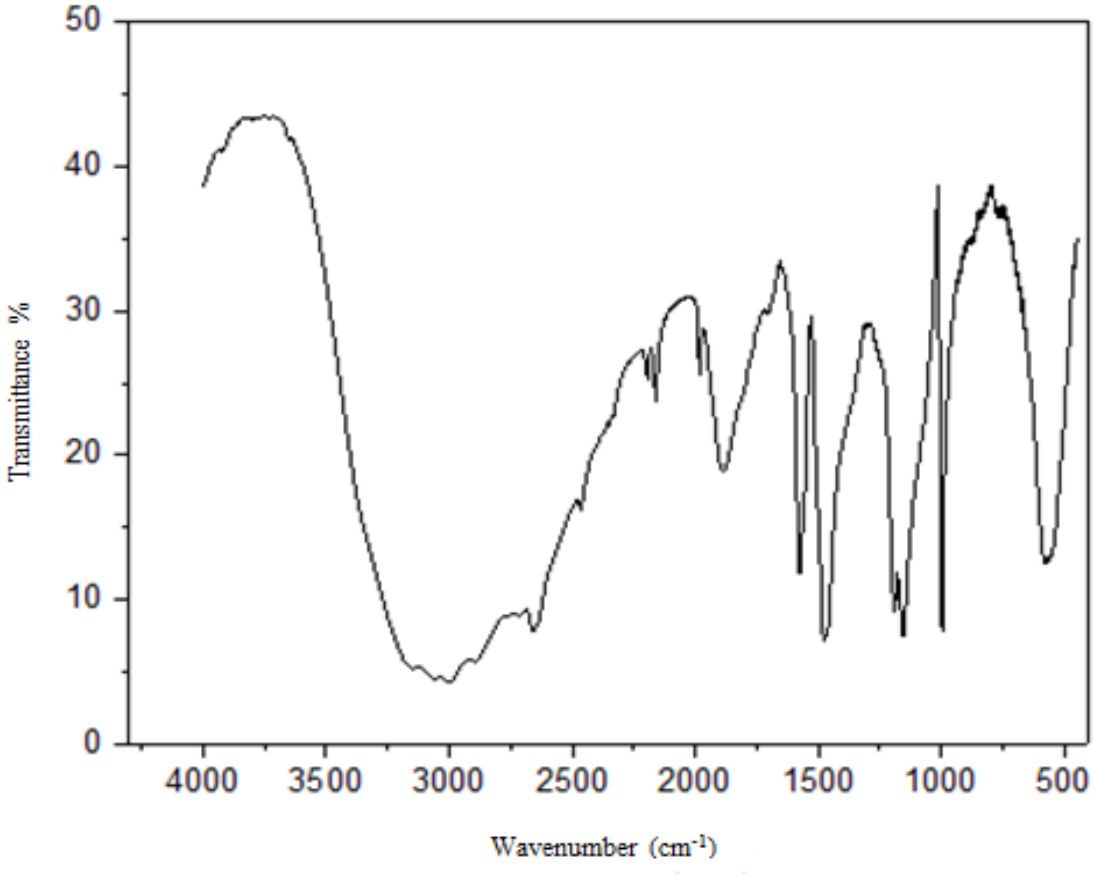


Figure 3. 2: FT-IR spectrum of hydroxylamine hydrochloride

Table 3. 4: FT-IR spectrum analysis of hydroxylamine hydrochloride

IR frequency band (cm-1)	Group responsible
3442	O-H Stretch
3244	NH_2 Stretch (amine salt)(3100-3350)
1583	NH_3+ bending

3.1.3.2.2 FT-IR of 3-((1R,3R,5S)-adamantan-1-yl)-5-(pyridin-4-yl)-4,5-dihydroisoxazole (SH-1)

IR spectrum in figure 3.3 for SH-1 compound, exhibits a new functional group appearing in this compound, however, alkyl hydroxylamine function group appeared in this spectrum rather than isoxazole ring. a new stretching band of alkyl hydroxylamine appear at 3250 cm^{-1} and bending band at 855 cm^{-1} as shown in the table 3.5.

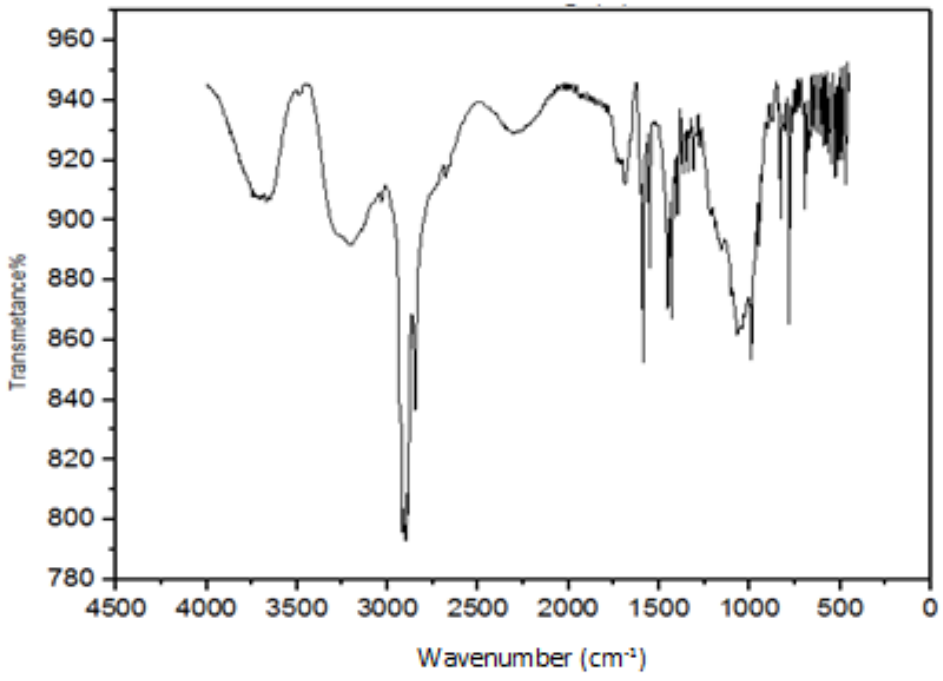


Figure 3. 3: FT-IR spectrum of compound SH-1

Table 3. 5: FT-IR spectrum analysis of SH-1

IR frequency band (cm-1)		Group responsible
Pyridine ring	1430	pyridine ring str.
	1589	C=C
	1452	C=N
	2917	C-H str.
3250 855		alkyl hydroxylamine str. alkyl hydroxylamine bending
2847		C-H str. (sp3)

3.1.3.3 FT-IR of 5-((1R,3R,5S)-adamantan-1-yl)-3-(pyridin-4-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (SH-2)

To synthesis SH-2 Chalcone (C) and semicarbazide hydrochloride were used as starting materials.

3.1.3.3.1 FT-IR of semicarbazide hydrochloride

IR-spectrum of chalcone (C) is mentioned above in section 3.1.3.1 and IR-spectrum (Figure 3.4) of semicarbazide hydrochloride has a C=O stretch band at 1691,other peak at 3250 and 3423 for NH and NH_2 respectively, and C-N band at 1092 as shown in the table 3.6.

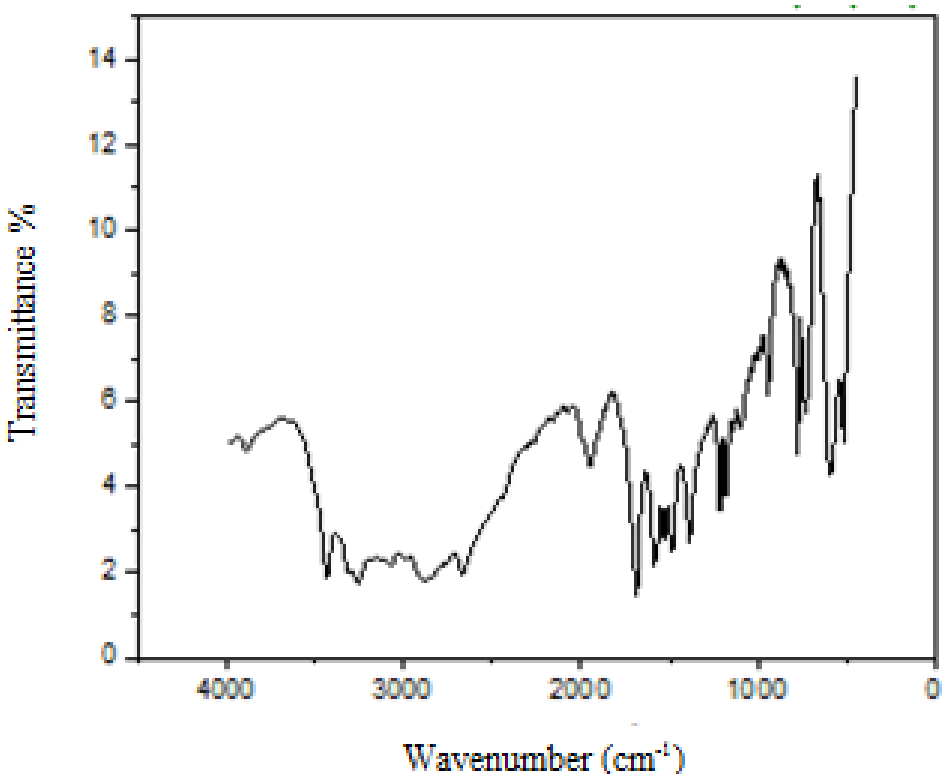


Figure 3. 4: FT-IR spectrum of semicarbazide hydrochloride

Table 3. 6: FT-IR spectrum analysis of semicarbazide hydrochloride

IR frequency band (cm^{-1})	Group responsible
1691	C=O
3250,3423	NH,NH_2
1092	C-N

3.1.3.3.2 FT-IR of 5-((1R,3R,5S)-adamantan-1-yl)-3-(pyridin-4-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (SH-2)

The IR spectra in figure3.5 showed characterisization of SH-2 product ,which band at 3055 cm^{-1} due to stretsting C-H in pyrazole ring and 1414 $cm^{-1}$and 1026 cm^{-1} due to bending pyrazole ring ,and stretching band of Carboxamide group appaer at 3273 cm^{-1} while bending band appaer at 563 cm^{-1} as shown in table3.7.

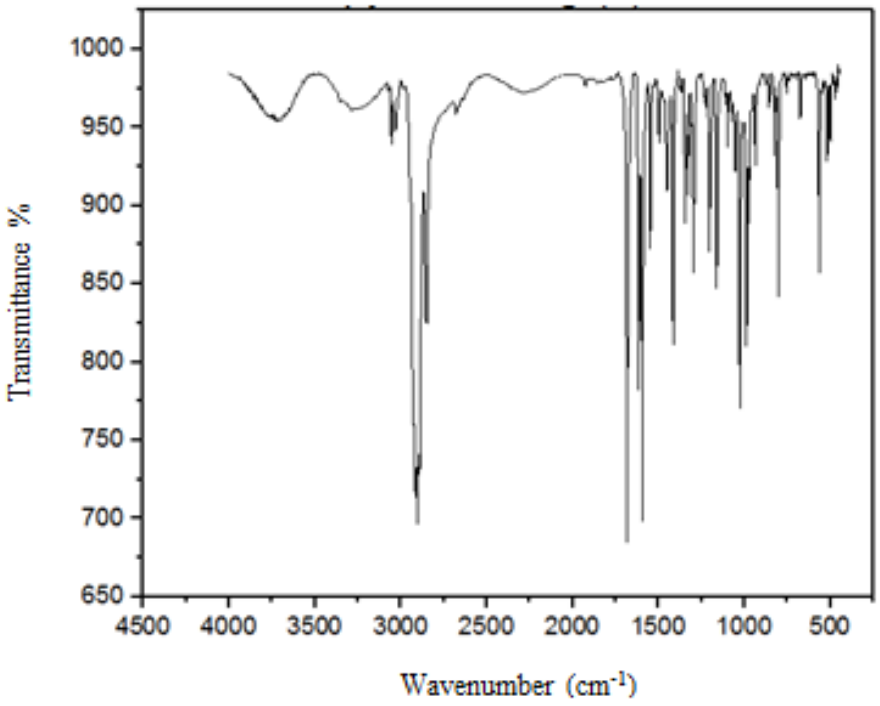


Figure 3. 5: FT-IR spectrum of SH-2 compound

Table 3. 7: FT-IR spectrum analysis of SH-2

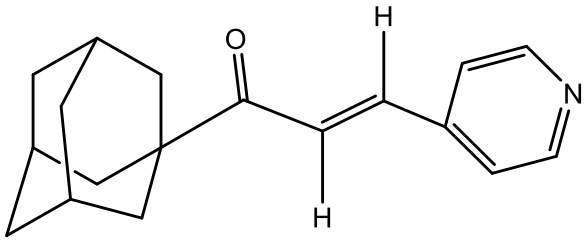
IR frequency band (cm-1)		**Group responsible**
Pyrazole ring	3055	CH str.
	1414,1026	Ring vib.
Pyridine ring	3030	CH str.
	1615	pyridine ring str.
Carboxamide group	3273	Amide str.
	563	Amide bending
	1683	carbonyl str.
2904, 2843		C-H str. (sp3)

3.1.4 ^{1}H-NMR Analysis

The compounds SH-1 and SH-2 were analyzed by using proton nuclear magnetic resonance spectroscopy (^{1}HNMR).

3.1.4 .1 ^{1}H-NMR of Chalcone (C)

The structure of chalcone (C) is confirmed by ^{1}HNMR spectrum shown in figure 3.6. The peak appeared at δ 7.43 ppm attributed to α proton and at δ 7.58 ppm to β proton. A collection of signal observed in the aromatic region δ 8.72-7.53 ppm is due to aromatic protons and the peaks at δ 1.87-1.76 ppm correspond to adamantyl group and the two peaks were shown in the spectrum correspond to DMSO solvent and water absorbed by DMSO at 2.47 and 3.3 ppm respectively. Position of the chemical shift is elucidated in scheme 3.1 [51].



Scheme 3. 1: Chemical structure of chalcone (C) compound

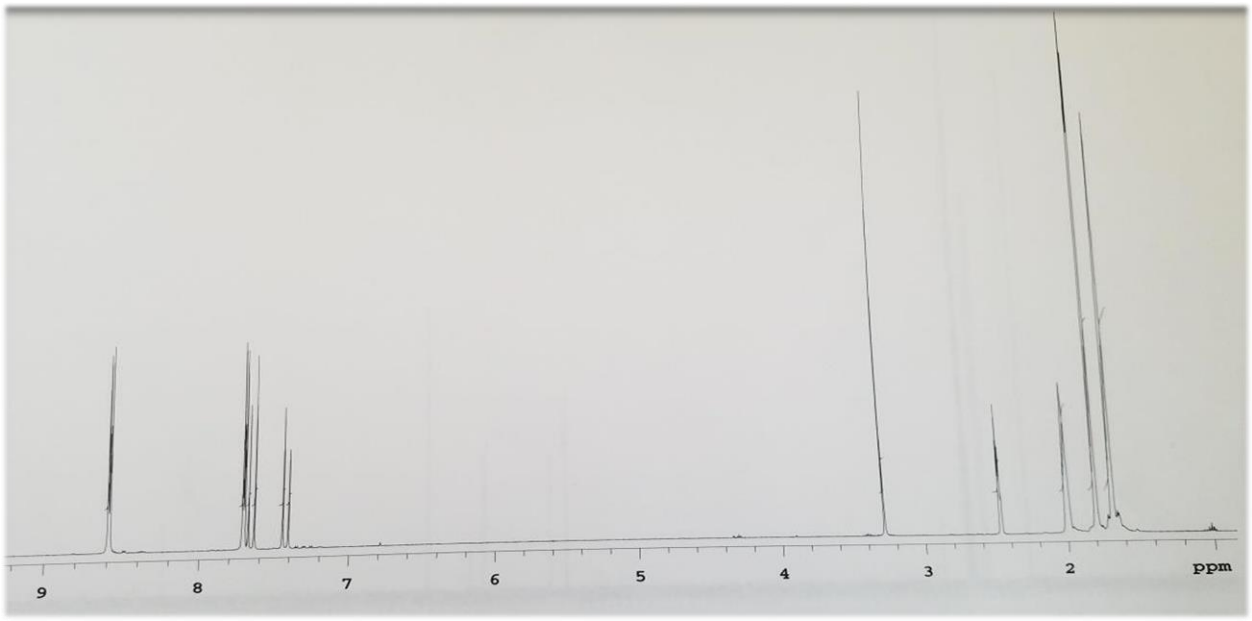
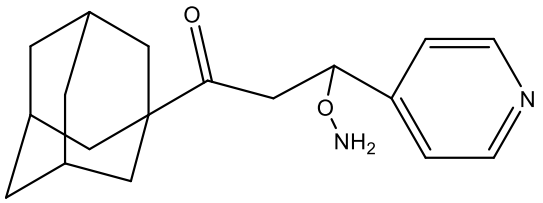


Figure 3. 6: ^{1}HNMR of Chalcone (C)

3.1.4 .2 ^{1}H-NMR of SH-1 compound

^{1}H-NMR spectrum shown in Figure 3.7 refers to SH-1 product. The peak appeared at δ 2.81 and 3.06 ppm due to methylene. Protons in amino group also displayed a peak at 1.5 ppm and peak appeared on 6.91 refer to proton connected to hydroxylamine group.

A collection of signal observed in the aromatic region δ 8.61-7.46 ppm is due to aromatic protons and the peaks at δ 2.01-1.71 ppm correspond to adamantyl group. In addition, two peaks were shown in the spectrum correspond to DMSO solvent and water absorbed by DMSO at 2.47 and 3.3 ppm respectively. Position of the chemical shift is elucidated in scheme 3.2.



Scheme 3. 2: chemical structure SH-1 compound

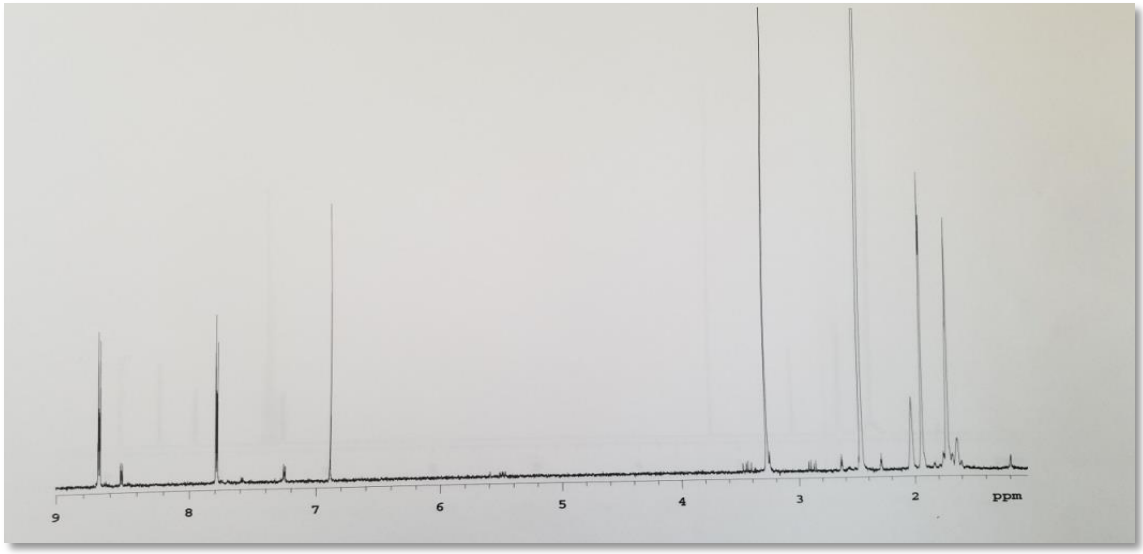
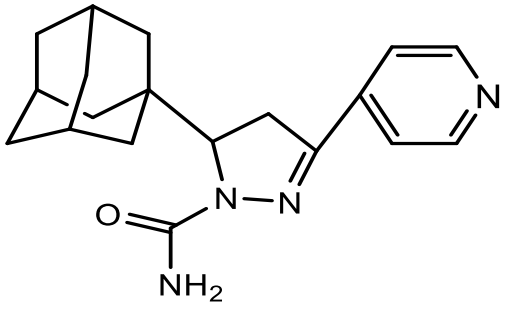


Figure 3. 7: 1HNMR of SH-1 product

3.1.4 .3 ^{1}HNMR of SH-2

Figure 3.6 shows the ^{1}HNMR of SH-2 compound shows the chemical shifts and splitting peaks. The methylene protons of Pyrazole ring appeared in the region δ 2.21 - 2.46 ppm also peak at δ 3.43ppm refer to α proton connected with nitrogen. The peaks are observed for these three protons which supported the formation of compound SH-2. Moreover, the amine protons (NH_2) that attached to the Pyrazole ring appeared at 7.72 ppm. A signal at δ 2.02-1.76 ppm is assigned to adamantyl protons attached to Pyrazole ring at C-3. Moreover, a collection of signal observed in the aromatic region δ 8.69-7.06 ppm is due to aromatic protons of the 4-pyridine ring. Position of the chemical shift elucidated in scheme 3.3.



Scheme 3. 3: chemical structure of SH-2 compound

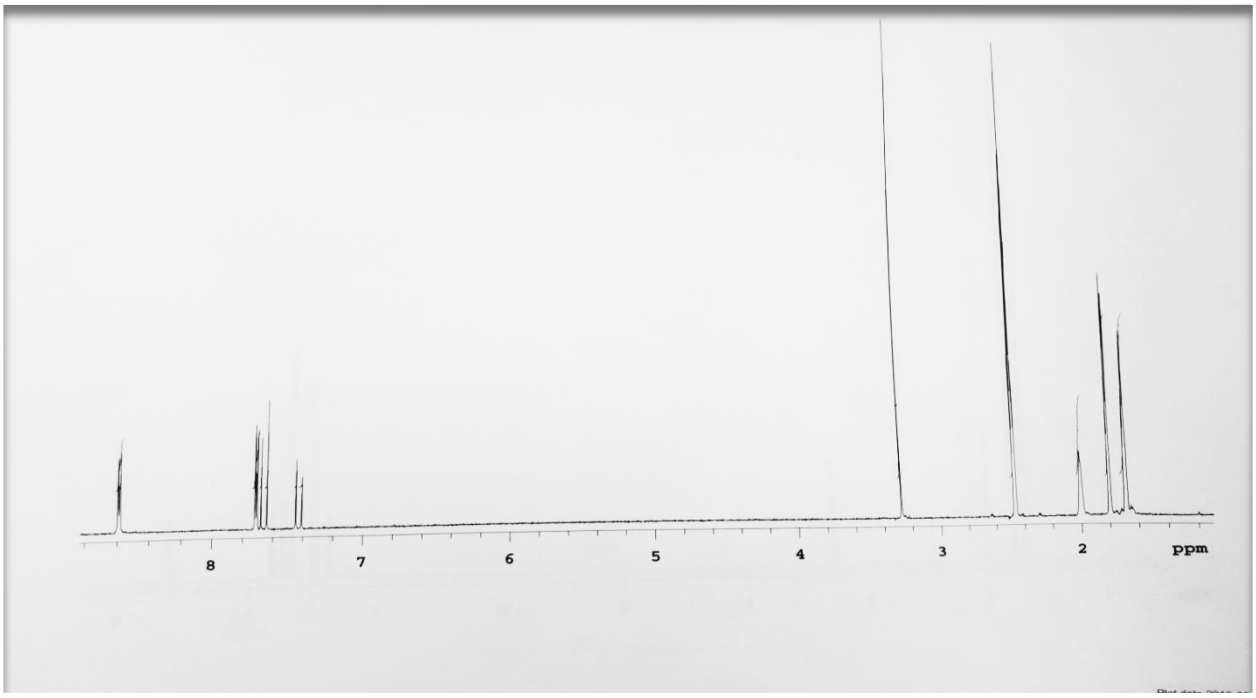


Figure 3. 8: proton NMR of SH-2 product

3.2 Cytotoxic Effect of SH-1 and SH-2 Compounds

MDA-mb231GFP cell line was cultured in 6cm plates for counting assay then was treated with chalcone (C), SH-1 and SH-2 by three different concentrations (1, 10 and 50 µM) for each drug.

Where MCF-7 and MDA-mb231GFP cell lines were cultured in 96 well plates for XTT assay by using two different concentrations (5 and 25 µM) for each drug. RPMI medium (10%FBS, 1%L-Glu and 1%pen/strep) was used for both techniques, both assay were made at the same time.

Cells were treated with new fresh medium after 24 hours of culture and media changed on untreated cells (control)

3.2.1 XTT Assay

The initial value of incubation time was 3 hours and half.

3.2.1.1 XTT Results for MDA-mb231GFP Cell line

Chalcone (C) showed significant anti-cancer activity against MDA-mb231GFP cell line as shown in (Figure 3.9). The activity of compound was dose dependent, because the fold of cell proliferation decreased by 50% at 5µM and 60 % at 25µM. The effect was clear after 48 hours

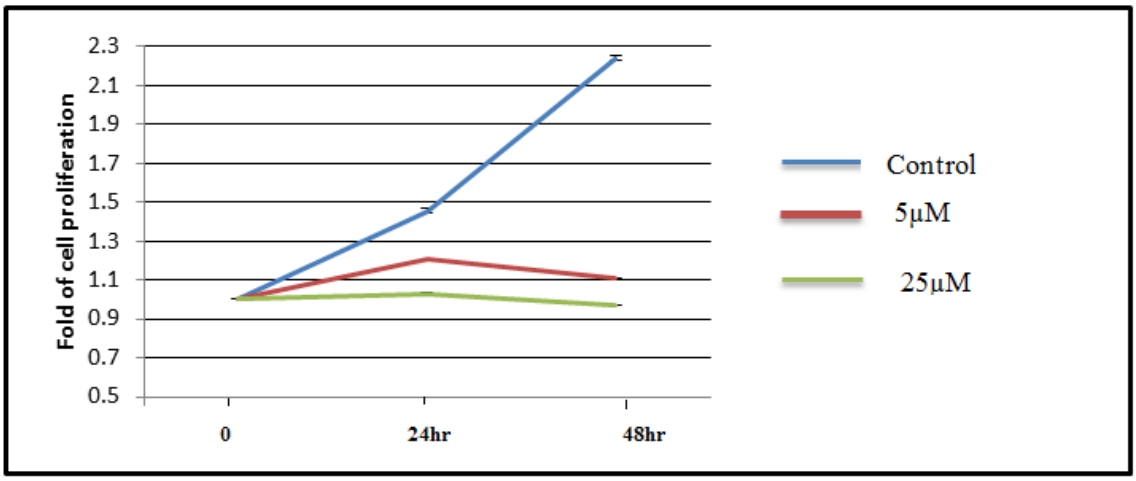


Figure 3. 9: The effect of different concentration of Chalcone(C) on MDA-mb231Gfp cell line by XTT assay

SH-1 was inactive as anticancer on MDA-mb231GFP cell line. On the contrary, it increased the proliferation of cells as shown in figure 3.10, due to structural change (absence of enone functional group) of SH compound which acts as anti-proliferation [51]. After 24 hours, the lowest concentration 5µM increased the cell proliferation more than control and 25µM so the effect of this compound was dose independent. The fold of cell proliferation for control, 5µM and 25µM was almost the same after 48 hours.

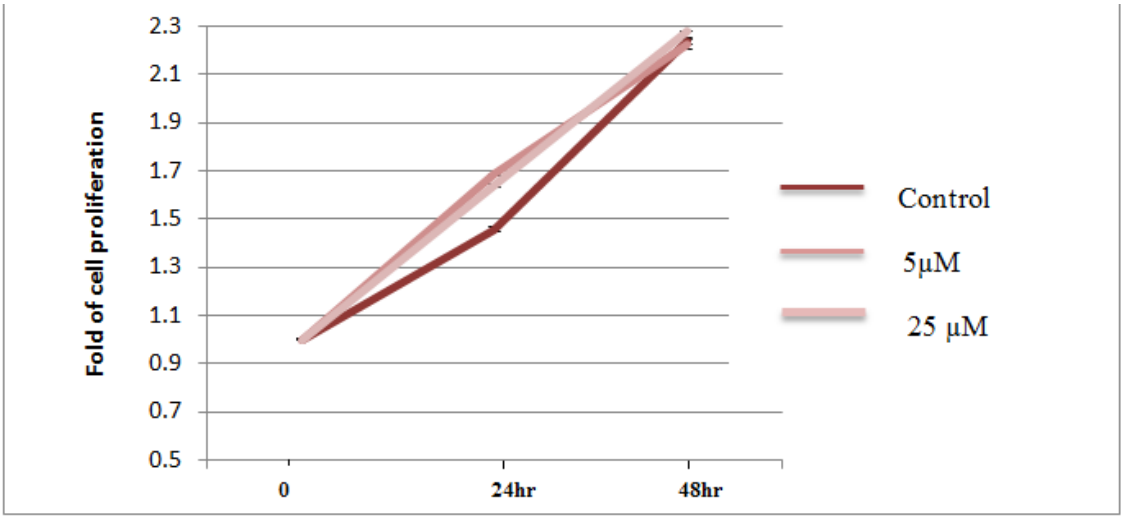


Figure 3. 10: The effect of different concentration of SH-1 compound on MDA-mb231Gfp cell line by XTT assay

On the other hand, SH-2 product acted as anticancer for MDA-mb231Gfp cell line as shown in Figure 3.11. The two concentrations decreased the proliferation of cells by 51% and 55% at 5µM and 25µM respectively. But the addition of Pyrazole ring didn't increase the effectivity of the adamantyl chalcone (C) as anticancer on MDA-mb231Gfp cell line only slightly.

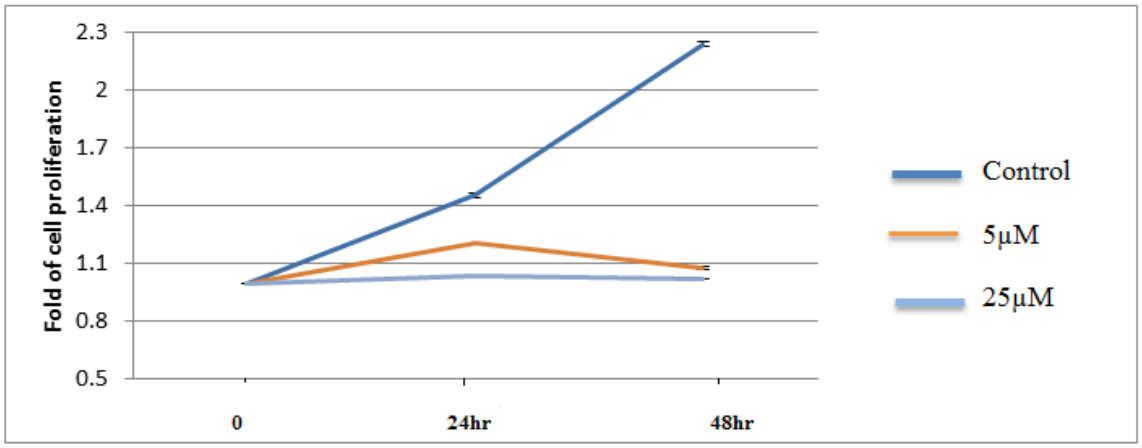


Figure 3. 11: The effect of different concentration of SH-2 product on MDA-mb231GFP cell line by XTT assay

3.2.1.2 XTT Results for MCF-7 Cell line

Chalcone (C) showed significant anti-cancer activity against MCF-7 cell line as shown in figure 3.12. The activity of compound was dose dependent because the fold of cells proliferation of 25µM was lower than 5µM. The effect was clear after 48 hours where cells proliferation decreases more than 24% and 51% after 24 hours while 72% and 76% in day two at 5 µM and 25 µM respectively.

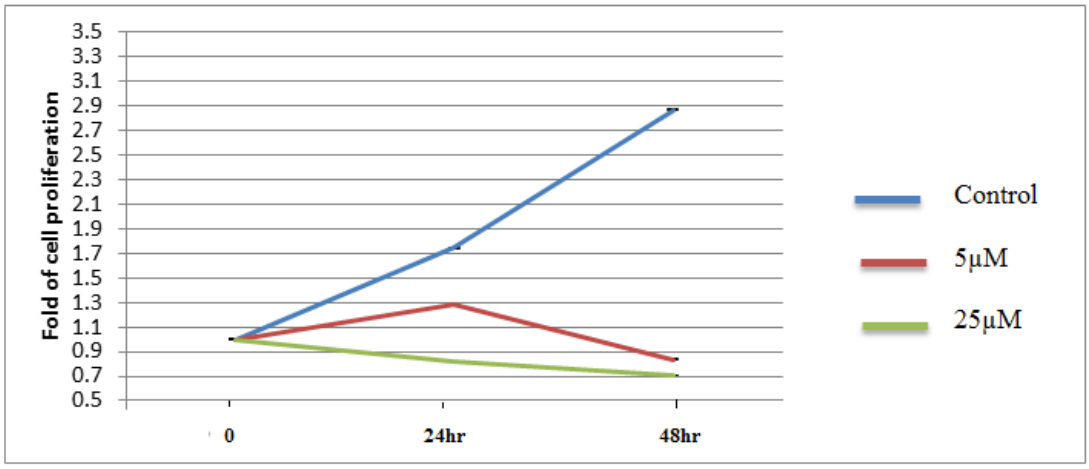


Figure 3. 12: The effect of different concentration of chalcone (C) on MCF-7 cell line by XTT assay

SH-1 compound gave different results from Chalcone (C), it didn't stop the cells growth and increase the cells proliferation but in lower rate than a control at concentrations 5µM and 25µM as shown in figure3.13.

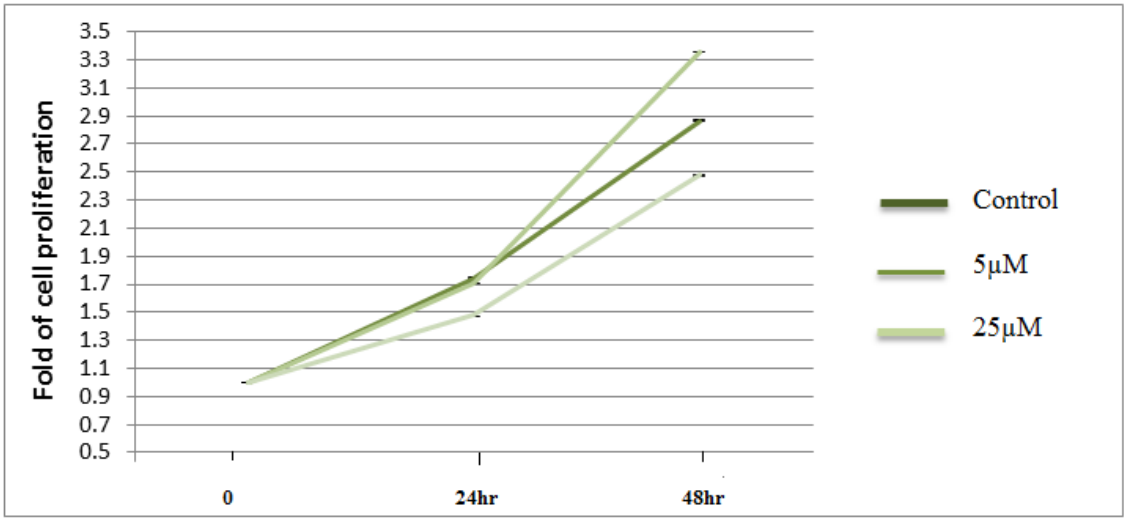


Figure 3. 13: The effect of different concentration of SH-1 on MCF-7 cell line by XTT assay

SH-2 compound acted as an anticancer at two concentrations 5 and 25µM as shown in figure 3.14. Cell proliferation decrease by 44% and 61% after 24hours, while decreased by 72.5% and 76% after 48hours at 5µM and 25µM respectively.

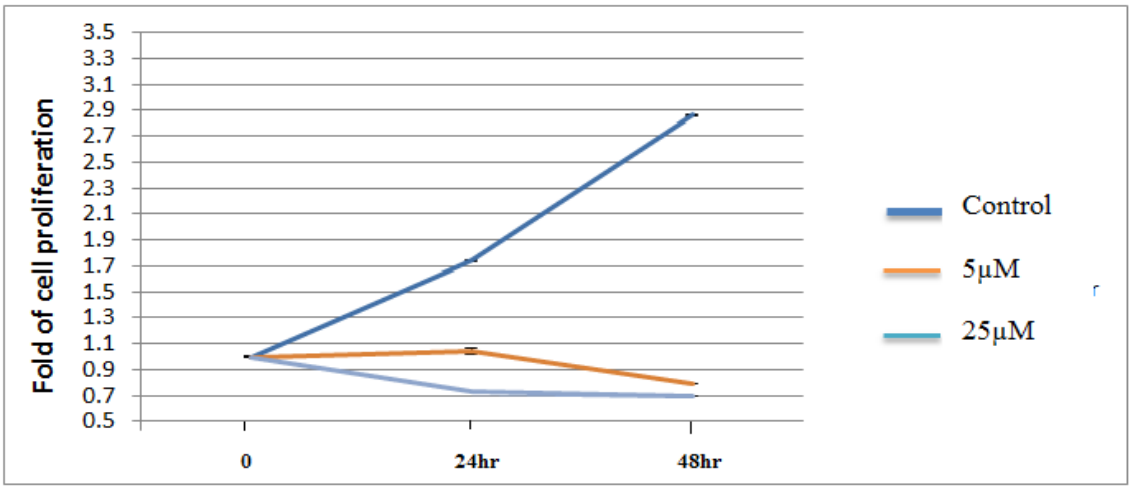


Figure 3. 14: The effect of different concentration of SH-2 on MCF-7 cell line by XTT assay

Comparison of SH-2 and Chalcone (C) results on MCF-7 cell line shows more activity of SH-2 than chalcone (C) as shown in figure 3.15. XTT results on MCF-7 cell line indicate that Chalcone (C) and SH-2 have effects on ER+ cell line (MCF-7) more than ER- cell

line (MDA-mb231Gfp). This is because the ER+ cells have proteins (receptors) that consist of many amino acids which helps them grow when estrogen attaches to these receptors, the structure of these compounds help block estrogen receptors and prevent breast cancer cells to grow.

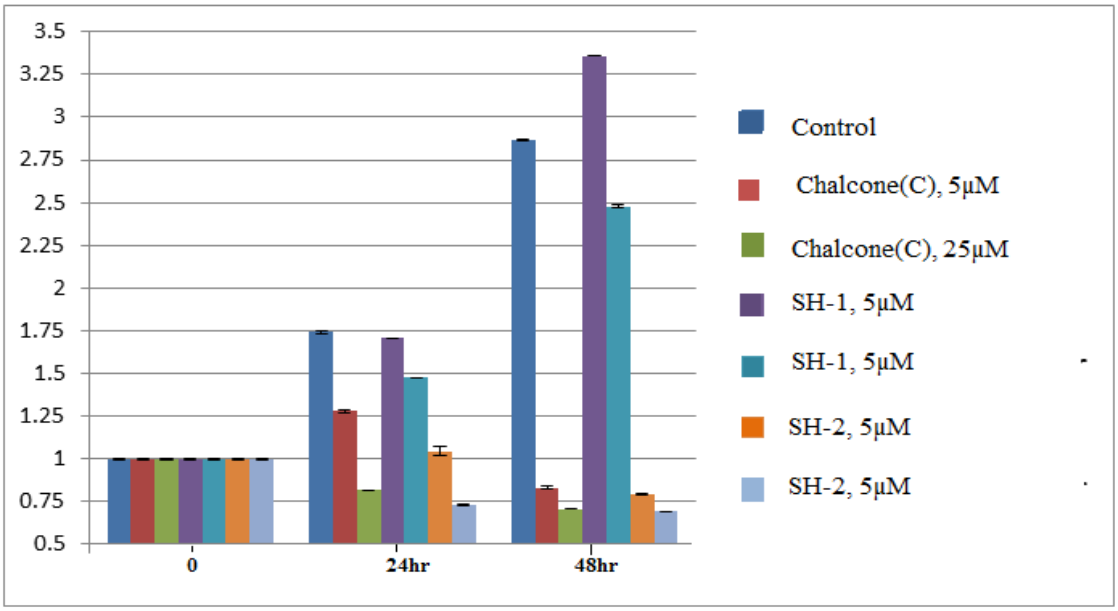


Figure 3. 15: The effect of different concentration of chalcone(C), SH-1 and SH-2 compounds on MCF-7 cell line by XTT assay

3.2.2 Counting Assay

Counting is another assay that was used to study cytotoxic effect of prepared compounds. Before starting counting assay, a picture was taken under a microscope to show the general effects of compounds on MDA-mb231Gfp cell line. Cell proliferation was arrested when cells were treated with chalcone (C) by three different concentration, figure3.16 illustrated killed cells after 24 hours (A) and 48 hours (B). Data of counting assay supported the picture results, where the total number of cells in figure 3.17 (A) decreased by 85% at low concentration (1µM) and 96% at high concentration (50 µM).

In addition, the number of dead cells (B) reached to 15%, 30% and 40% on day two at concentrations 1 µM, 10 µM and 50 µM respectively.

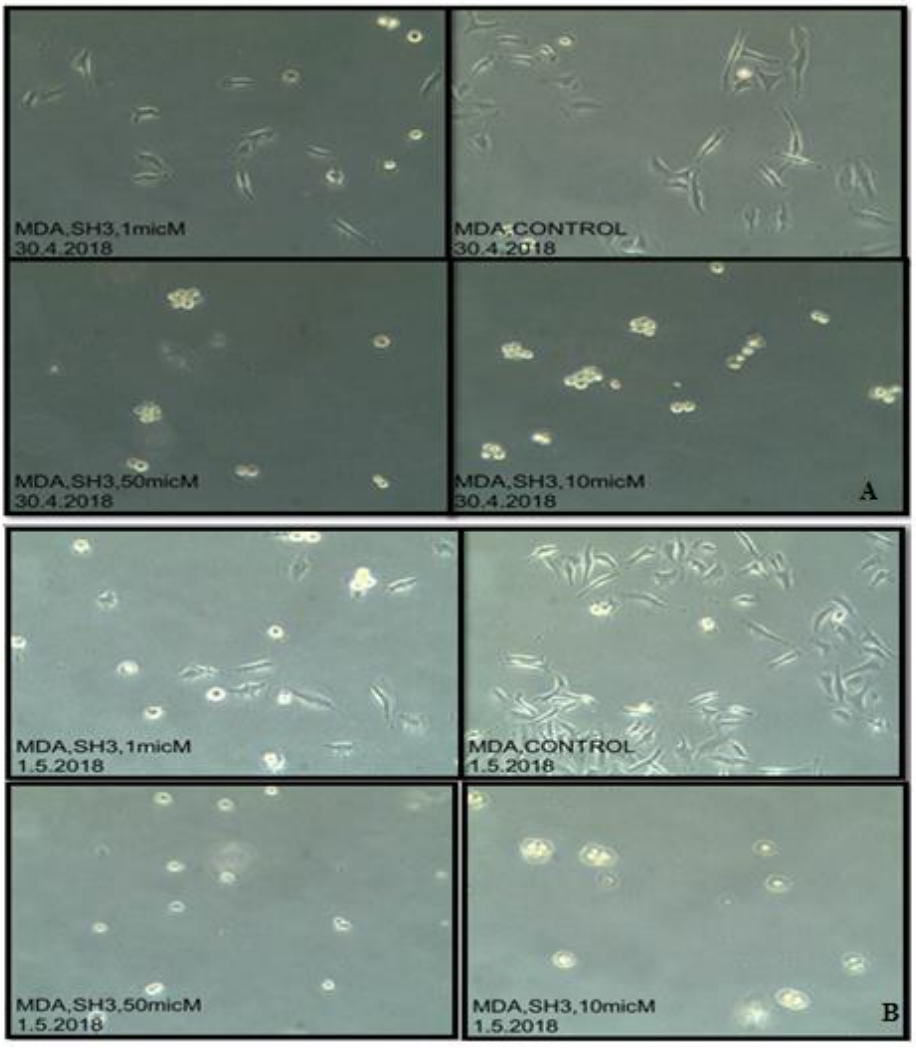


Figure 3. 16: The effect of different of chalcone (C) on MDA-mb231GFP cell line, (A) and (B)indicated to the effect of chalcone (C) after 24hr and 48hr respectively.

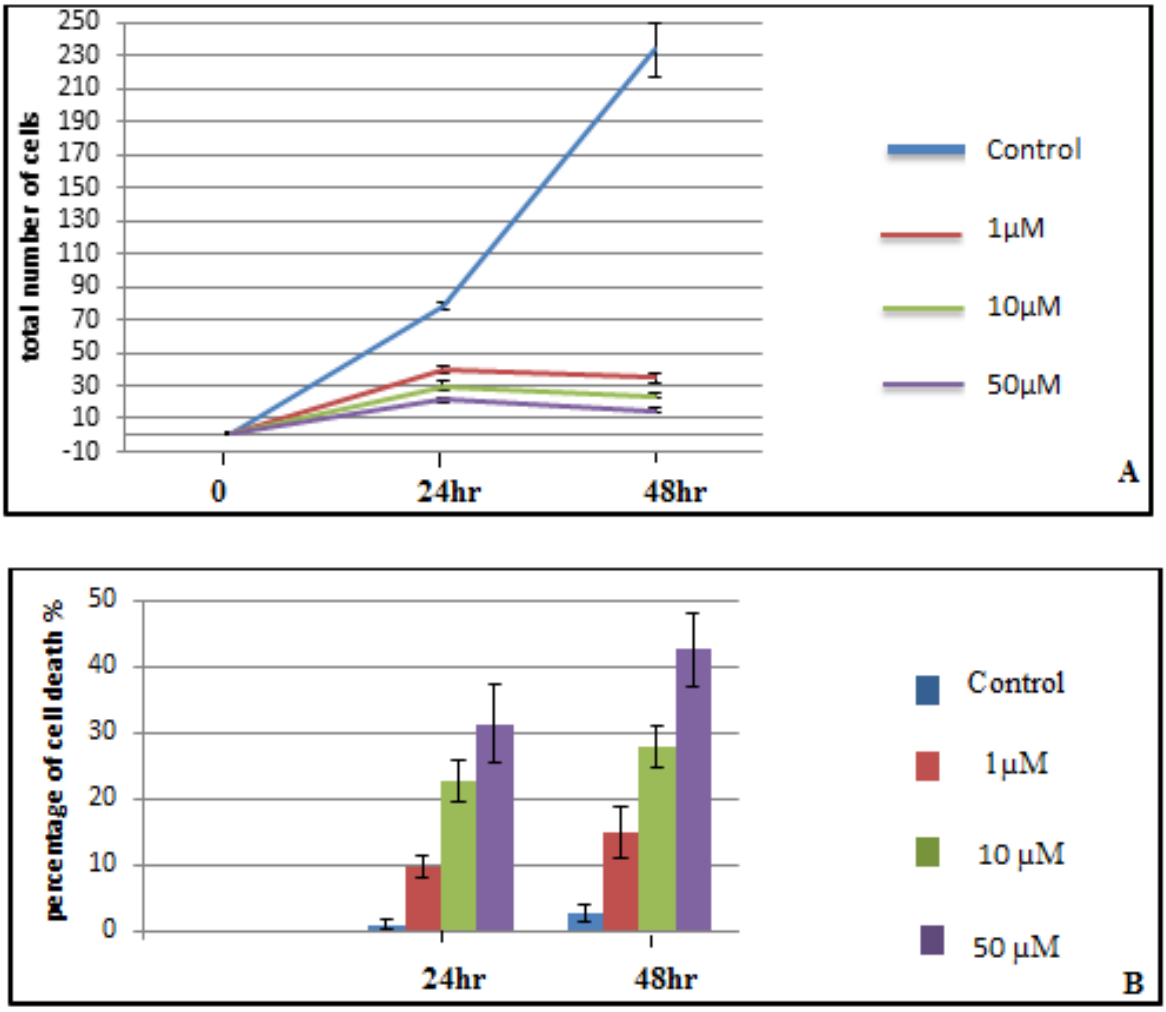


Figure 3. 17: The effect of different concentration of chalcone (C) on MDA-mb231GFP cell line by counting assay, where (A) is the total number of cells and (B) is the percentage dead cells.

When MDA-mb231Gfp cell line are treated with SH-1 compound, cells didn't stop growth and morphology didn't change at a different concentration (1µM,10 µM and 50 µM) after 24hr (A) and 48hr (B) as shown in figure 3.18. The total number of cells (Figure3.19,A) were increased but at a lower rate than control and at different concentrations1µM, 10 µM and 50 µM on day two. But, the lowest number of cells appeared at l µM. This is a reversal expected to be the effects of 50 µM most influential so this drug is dose independent. The highest cell death rate was observed at 1µM, not at 50 µM, and reached only 10%. (Figure3.19,B)

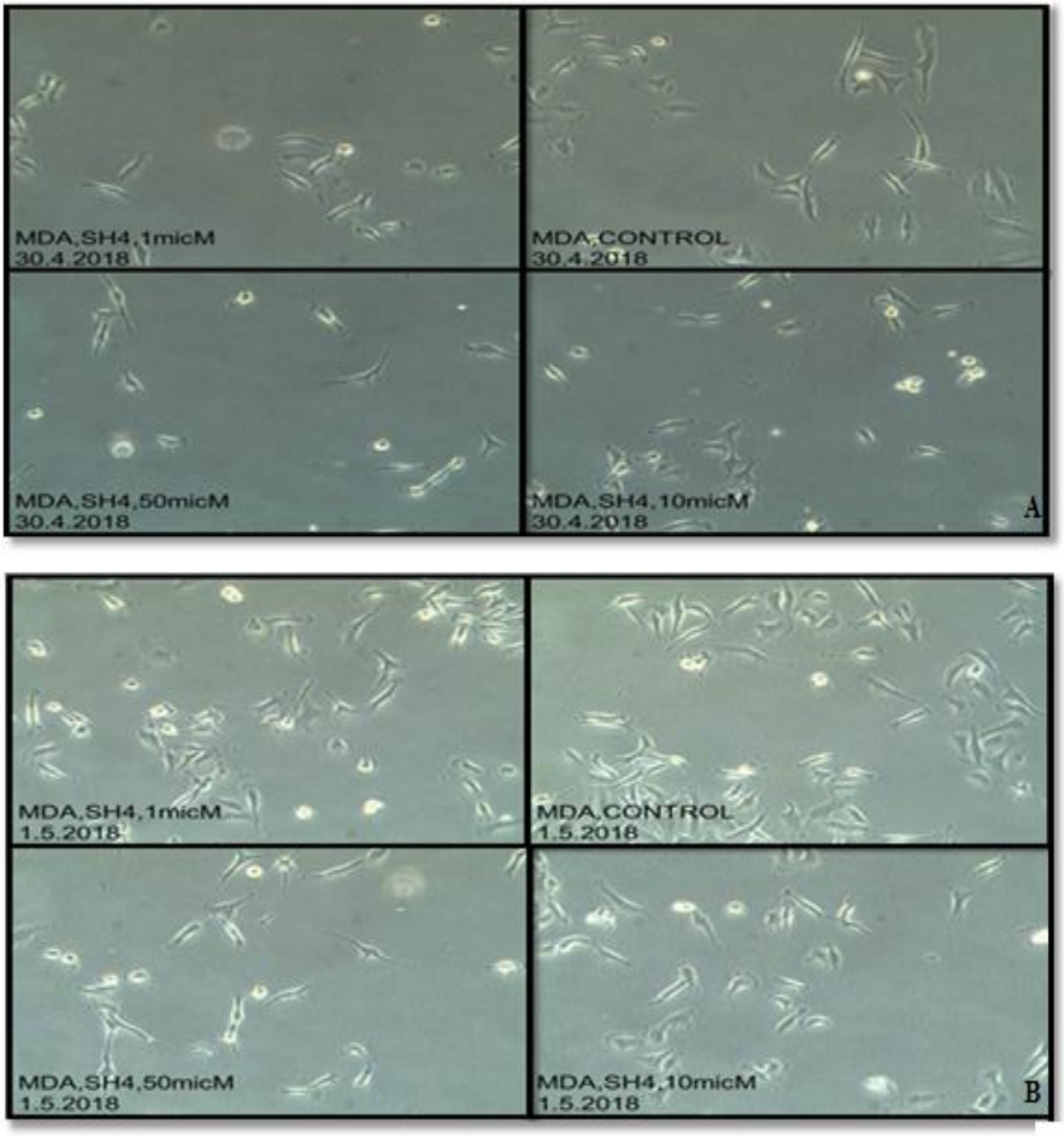


Figure 3. 18: The effect of different concentration of SH-1 product on MDA-mb231GFP cell line, (A) and (B) is the effect of SH-1 after 24hr and 48hr respectively .

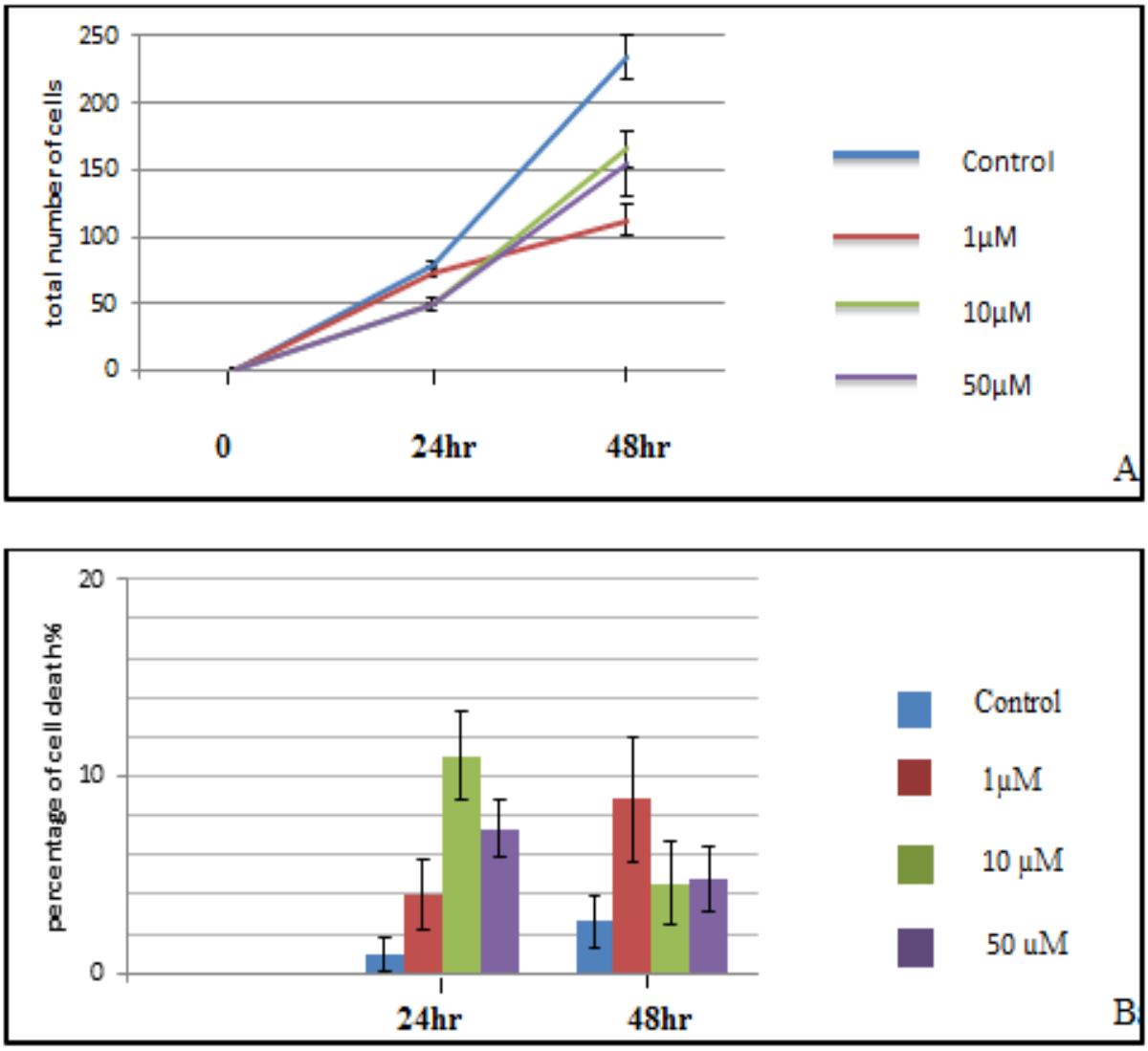


Figure 3. 19: The effect of different concentration of SH-1 on MDA-mb231GFP cell line by counting assay, where (A) the total number of cells and (B) is the percentage of cell death.

SH-2 showed anticancer activity and killed most of the cells after 24 hours and 48 hours when MDA-mb231Gfp cell line was treated with it, as shown in figure 3.20. The total number of cells decreased by 48%,80% and 96% at 1µM,10 µM, and 50 µM respectively (figure3.21, A), the percentage of dead cells were 15% at low concentration, 28% at 10 µM and 26% at high concentration 50 µM as shown in figure3.21, B.

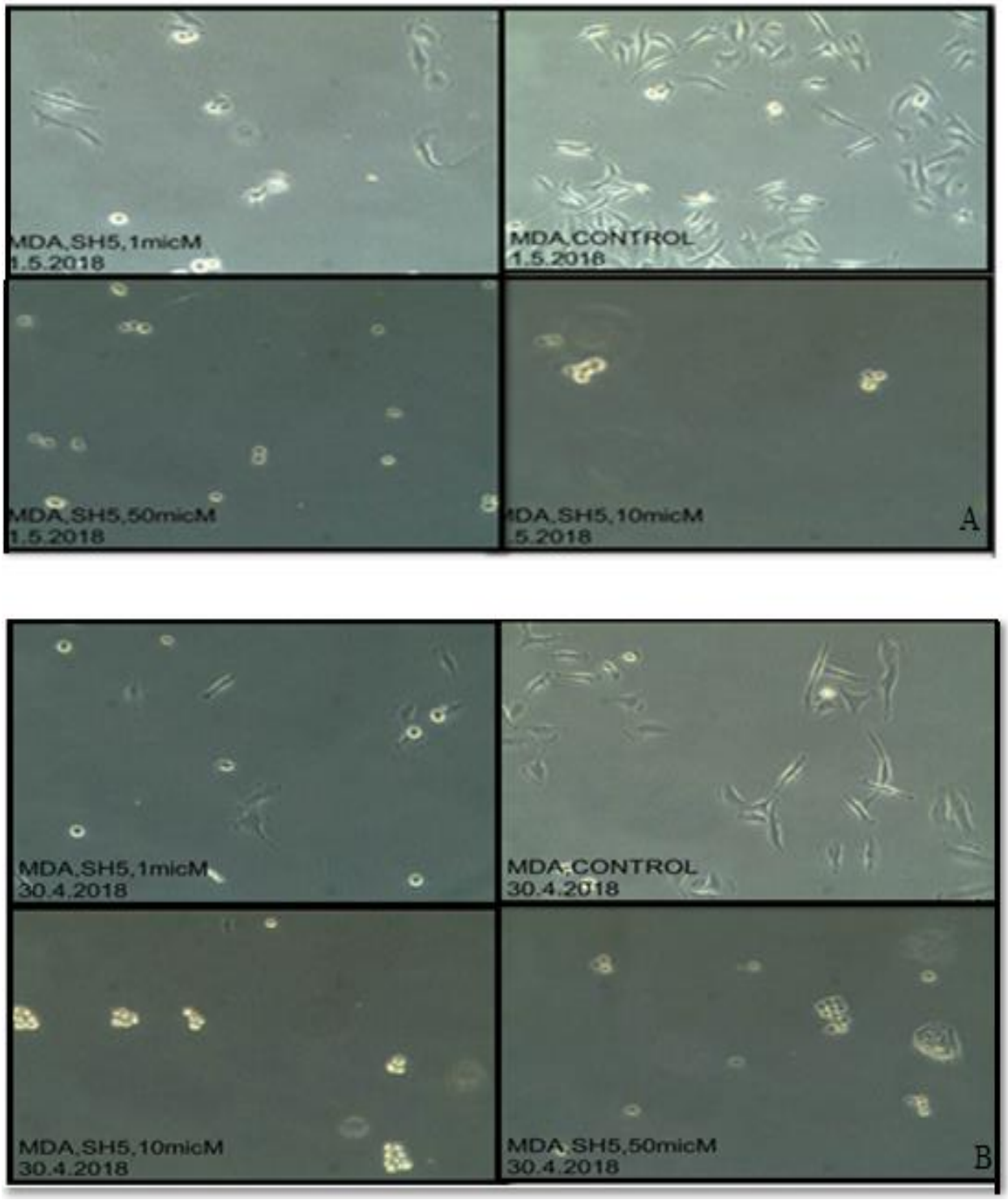


Figure 3. 20: The effect of different concentration of SH-2 on MDA-mb231GFP cell line,(A) and (B) is the effect of SH-2 after 24hr and 48hr respectively

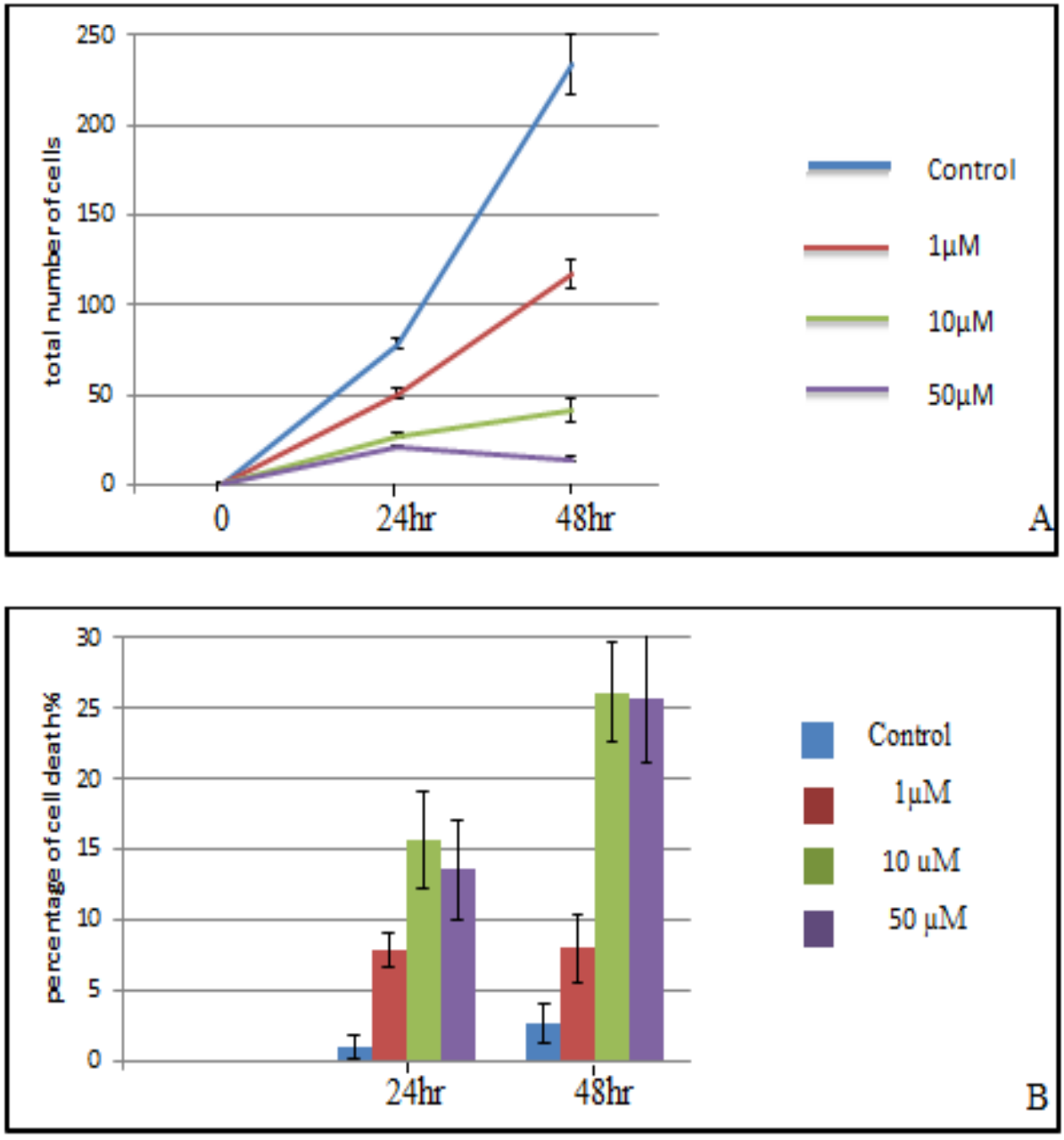


Figure 3. 21: The effect of different concentration of SH-2 on MDA-mb231GFP cell line by counting assay, where (A) the total number of cells and (B) is the percentage of cell death.

Depending on counting assays results, SH-1 didn't act as an anticancer drug so the results of the two assays were identical.

Chapter Four

Conclusions and Future Directions

4. Conclusions and Future Directions

4.1 Conclusion:

In conclusion, the present study showed that the SH-2 compound has an anti-cancer property in the in vitro MCF-7 cell line (ER+) more than the effect it has on MDA-mb231Gfp cell line (ER-). This compound reduced cells viability at the concentration 1-50 µM. We suggest that this compound may have anticancer potential on other type of cell lines, as well as breast cancer.

4.2 Future Directions:

I. Using X-ray to investigate the crystals structure of prepared compound and Carbon-13 Nuclear Magnetic Resonance (13C-NMR) analysis.
II. Using another in vitro Bioassays to evaluate the adamantyl heterocyclic compounds such as the half maximal inhibitory concentration (IC50) and apoptosis assay.
III. Test these compounds on normal cells such as MCF-10 cell line.
IV. Test these compounds on other type of cell lines.

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الخلاصة

تتضمن هذه الدراسة تصنيع سلسلة جديدة من شالكون الادمنتل تحتوي على حلقة الايزوكسازول والبيرازول، وتقيم نشاطها كمضاد للسرطان الثدي لخطين من الخلايا وهما MCF7 وهو من نوع مستقبل الاستروجين الايجابي و MDA-MB-23 من نوع مستقبل الاستروجين السلبي. وتمت الدراسة باستخدام اختبار XTT لكلا الخطين واختبار العدد الكلي للخلايا الحية منها والميته لخط الخلايا MDA-MB-23 فقط.

تشير نتائج XTT أن مركب SH-2 أظهر تأثيرا واضح على كلا الخطين من الخلايا، وكان تأثرهم على خلايا خط MCF7 أكثر من خط MDA-MB-23 ، فانخفضت نسبة نمو خلايا خط MCF7 بنسبة 72.5% ونسبة خلايا خط MDA-MB-23 بنسبة 51% عندما عولجت الخلايا بتركيز منخفض من مركب (SH-2) 5 ميكرومول/لتر. وعلى العكس من ذلك فان مركب SH-1 لم يكن له تأثير كمضاد للسرطان على اي من خطوط الخلايا عند نفس التركيز. وكانت نتائج اختبار العدد الكلي للخلايا متوافقة مع نتائج اختبار XTT .

شخصت جميع المركبات المحضرة في هذا البحث من خلال قياس درجات أنصهارها بالاضافة الى الطرق الطيفية المتمثلة بطيف الأشعة تحت الحمراء وطيف الرنين النووي المغناطيسي البروتوني للجميع المركبات.