Hebron University

Faculty of Graduate Studies

Chemistry Department

Synthesis and Biological Evaluation of Novel Adamantylated Heterocyclic Rings

By

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Faulty of Graduate Studies

Synthesis and Biological Evaluation of Novel Adamantylated Heterocyclic Rings

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Dedication

This work is dedicated to my dear parents, brothers, and sisters who supported and encouraged me during my study.

I also dedicate this dissertation to my teachers, supervisors and friends at Hebron University and Arab American University-Jenin, who have supported me throughout the master thesis work. I will always appreciate all they have done.

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First and foremost, I am deeply thankful to Almighty Allah from whom I always receive help and protection.

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Abbreviations	Definition
Chalcone (3)	(E)-1-((3r, 5r, 7r)-adamantan-1-yl)-3-(pyridin-2-yl) prop-2-en-1-one
DMEM	Dulbecco's modified Eagle's medium
FT-IR	Fourier Transform Infra- Red
HCl	Hydrochloric acid
MCF-7	Breast carcinoma cell line
ml	Milliliter
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
OD	Optical density
pH	Power of hydrogen
PPM	Part per million
pyran (5a)	6-((3s)-adamantan-1-yl)-2-amino-4-(pyridin-2-yl)-4H-pyran-3-carbonitrile
pyran (5b)	6-((3s)-adamantan-1-yl)-2-amino-4-(pyridin-2-yl)-4H-pyran-3-carbonitrile
pyridine (6)	6'-((3r,5r,7r)-adamantan-1-yl)-2'-amino-[2, 4'-bipyridine]-3'-carbonitrile
pyrimidine(4)	4-((3r, 5r, 7r)-adamantan-1-yl)-6-(pyridin-2-yl) pyrimidin-2-amine
R_f	Retention factor
rpm	Round per minutes
TLC	Thin layer chromatography
UV	Ultraviolet
µg	Microgram

Abstract

Throughout the world, cancer is one of the major diseases that devastate the lives of people. Enormous efforts are expended in dealing with this disease, but only limited success has ever been achieved with the therapeutic strategies available. These efforts are usually complicated by the lack of specificity of available drugs, high cost and a wide range of undesirable side effects from existing drugs. Chalcones are among growing lists of compounds with promising anti-cancer activity. Several studies have revealed that chalcone based structure derivatives inhibit cancer cell proliferation, as well as induce apoptosis in a different variety of cell lines. Moreover, heterocyclic chalcones exhibit many pharmacological activities, for instance, anti-cancer, anti-oxidant and the cytotoxic agent. These promising topics stimulate the researchers worldwide to make a novel compound heterocyclic membered ring with chalcone based structure and test biological activities.

Four adamantylated heterocyclic compounds (pyrimidine **(4)**, pyran **(5a)**, pyran **(5b)**, and pyridine **(6)**) were synthesized and characterized by ^{1}H-NMR, FT-IR, and UV-Vis spectroscopy. All the compounds were investigated for their anti-cancer effect against MCF-7 cells (human breast adenocarcinoma cell line). The cells were treated in a dose-dependent manner (0, 4, 16, 32, 64, 128 and 256 µg/ml) for 24 h. The MTT assay was used to test cytotoxicity of the compounds. Results indicated that pyrimidine **(4)**, pyran **(5a)**, and pyridine **(6)** have cytotoxicity effects against MCF-7 cell line at concentration 64-256 µg/ml. However, pyran **(5b)** has little effect on MCF-7 cell line at 128 µg/mL.

Chapter one

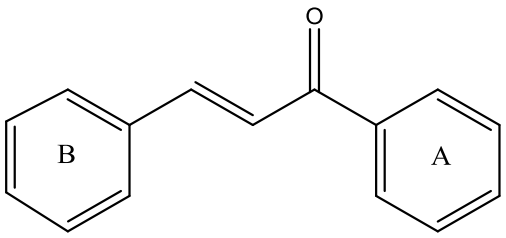
1. Introduction

1.1 Chalcone: structure and terminology

The chemical structure of chalcone (Scheme 1.1) possesses two aromatic rings connected by a three carbon α,β-unsaturated carbonyl group (–CO-CH=CH-) (Firoozpour *et al*., 2012). The term ”chalcone” was firstly called by Kostanecki and Tambor, 1899, it is among a family of bicyclic flavonoids. More interestingly, chalcones have the ability to form a variety of novel heterocyclic rings such as pyridine, pyrimidine, and pyran in its backbone.

Chalcones (1,3-diphenylprop-2-en-1-one) are very important compounds due to the presence of reactive α,β-unsaturated ketone moiety. The presence of chromophore (-CO-CH=CH-) and other auxochromes, make chalcone compounds highly active in the visible range (colored compounds) (Townsend, 2010).

Chalcones are unique templates that are important active intermediate in organic synthesis (Bergman *et al*., 1959; Sandler, Karo, 1972; Straub, 1995), and considered as starting material for the synthesis of various heterocyclic ring systems such as pyrazoles, isoxazoles, pyran, cyanopyran, and pyrimidine derivatives.



Scheme 1.1: Simple chalcone structure

1.2 Naturally occurring chalcones

Chalcones are abundantly present in plants. However, isolation of chalcone derivatives from plants needs a complicated procedure, time-consuming and gives a low yield. Moreover, natural sources of heterocyclic chalcones are limited even though they have been documented a diverse range of biological activities, essentially cytotoxic activity

(Shin *et al*., 2013). Therefore, researchers develop an efficient new heterocyclic chalcone derivative with anti-cancer activity against different types of cancer cells.

1.3 Nomenclature of chalcones

Chalcone nomenclature is based on IUPAC system in which carbonyl group has priority over the double bond when numbering the longest carbon chain, the substituent group, preceded by the number designating its location on the chain. Moreover, the chalcones have two nomenclatures systems according to prime numbering. The first method is used by American Chemical Society that uses a pattern upon " Chemical Abstracts" as illustrated below in scheme 1.2. Based on the prime numbers of phenyl group at the side of carbonyl group.

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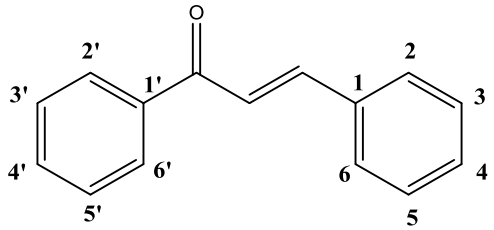
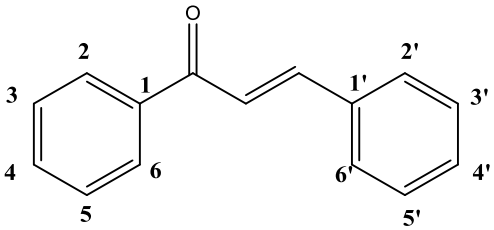


Scheme 1.2: Nomenclature of chalcones based on American Society

The second method is adapted by British Chemical Society, the journals have followed inversion system, the prime numbers are originated closed to the double bond as shown in scheme 1.3

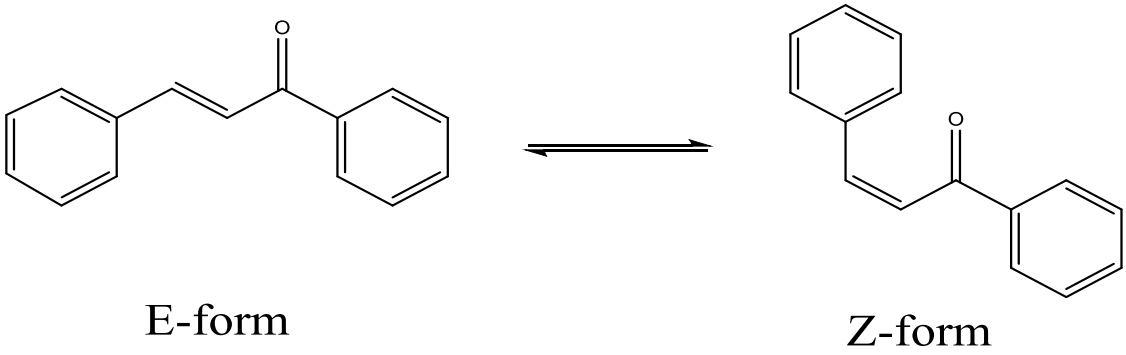


Scheme 1.3: Nomenclature of chalcones based on British Society

The American system of nomenclature of chalcones is the most practiced system as per "chemical abstract".

1.4 Isomerism of chalcones

Chalcones are interesting compounds that can be able to exist in equilibrium ratio conformation (E-Z form), depending on the position of hydrogen atoms on the active α,β-unsaturated as indicated in Scheme 1.4.



Scheme 1.4: E- and Z-isomerism of chalcones

The two forms of chalcones exist in the solution in different yield. However, mostly E-form occupied the highest yield because it is thermodynamically more stable. Z-form is unstable due to high steric hindrance between the carbonyl group and aromatic B-ring (Larsen *et* al., 2005). To isolate Z-isomer, the product must be re-crystallized with suitable solvents (Larsen *et al.*, 2005; Xue & Gong, 2009). The E and the Z forms can extremely impact the therapeutic activity of chalcones.

1.5 Synthesis of chalcones

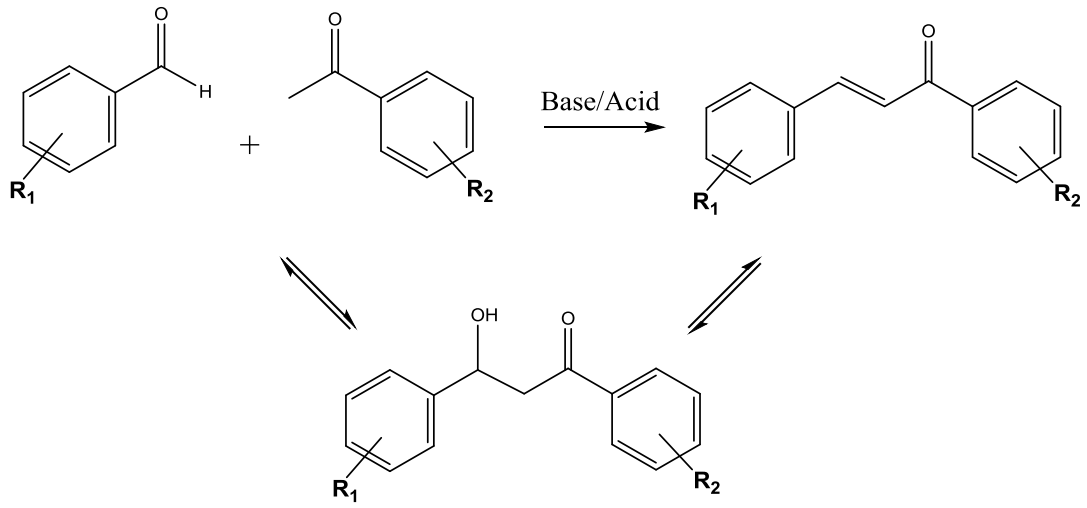
Chalcones have been synthesized by a large number of routes which can be classified as classical and non-classical routes (Matos *et al*., 2014). A major classical route is via the Claisen--Schmidt condensation in an alkaline or acidic media. In contrast, Suzuki, Julia—Kocienski, direct crossed-coupling, Heck, and Friedel-Crafts reaction are categorized as a non-classical route (Matos *et al*., 2014).

1.5.1 Synthesis methods of chalcones

1.5.1.1 The Claisen-Schmidt condensation reaction

The Claisen-Schmidt condensation reaction is the most common method used as simple and efficient for double bond formation, based on the structural complexity of the reactants.

Chalcones are relatively facile and simple in preparation based on the base or acid-catalyzed Claisen-Schmidt condensation reactions, which are carried out by reaction between benzaldehyde derivatives and active methylene ketones under a homogenous condition in an aqueous/ethanolic solution. The resultant is an active α,β-unsaturated ketone as illustrated in Scheme 1.5.

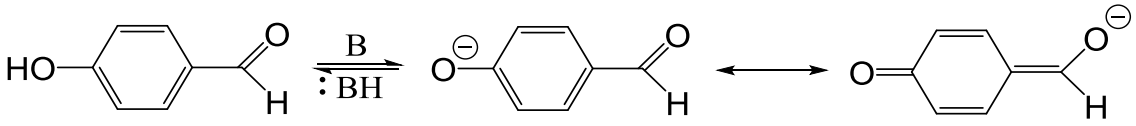


Scheme 1.5: Synthesis of chalcone through the intermediate β-hydroxy ketone via Claisen-Schmidt condensation

Among strong basic catalysts that can be used are natural phosphates, $Ba(OH)_2$, KOH, NaOH, LiHMDS etc. (Bhagat *et al.*, 2006). On the other hand, many acid catalysts have been employed in the synthesis of chalcones, including p-toluene sulfonic acid (Petrov *et al.*, 2008), B_2O_3, $RuCl_3$, $AlCl_3$, BF_3, dry HCl etc. (Bhagat *et al.*, 2006; Patil, Mahajan *et al.*, 2009).

Acidic catalysts play a major role in the preparation of hydroxychalcones instead of basic catalysts. In a particular condition; a hydroxyl substitution in the aromatic aldehyde restricts the function of the base as a deprotonation agent of ketones, affecting

the activity of aldehyde by delocalization of the anion (scheme 1.6) (Patil *et al*., 2009). Using of a basic catalyst in this reaction may require protection of the hydroxy group of aldehyde.

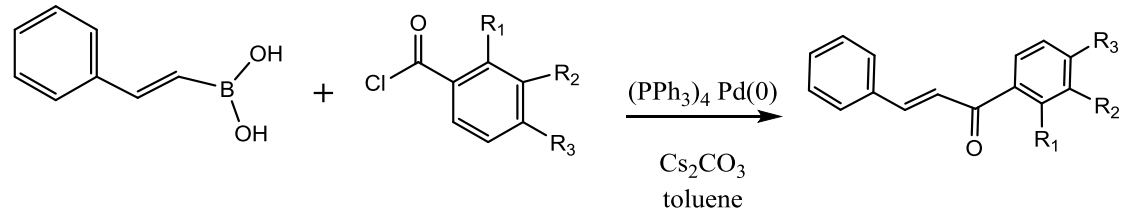


Scheme 1.6: Anion delocalization of hydroxy-aldehyde molecule

1.5.1.2 Suzuki coupling reaction

Suzuki coupling reaction or Suzuki–Miyaura reaction is an organic reaction occurs between a boronic acid and an organohalide catalyzed by a palladium complex. Chemists improve the reaction condition in order to produce chalcones with new characteristics.

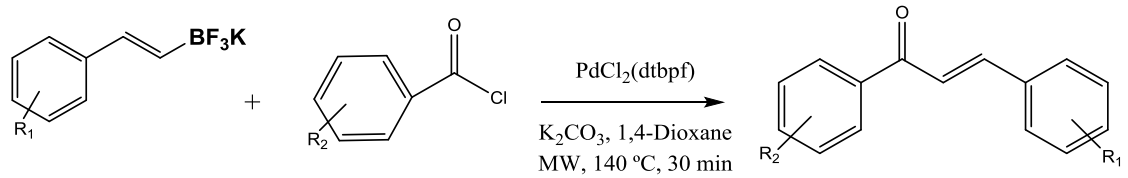
Chalcones can be produced by the reaction between benzoyl chlorides and phenylvinylboronic acids, or cinnamoyl chlorides and phenylboronic acids, catalyzed by tetrakis (triphenylphosphine) palladium in an anhydrous toluene as a solvent and using cesium carbonate as a base (Eddarir *et al*., 2003) (Scheme 1.7).



Scheme 1.7: Synthesis of chalcones via Suzuki coupling reaction

1.5.1.3 Direct-crossed coupling reaction

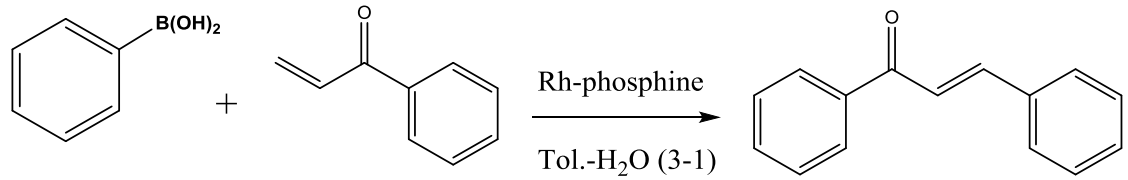
In a direct-crossed coupling reaction, benzoyl chlorides and potassium styryltrifluoroborates were reacted giving α,β-unsaturated ketones catalyzed by $PdCl_2$ (dtbpf) in dry 1,4 dioxane, and K_2CO_3 as a base under microwave irradiation. The microwave irradiation catalyzes palladium metal in direct cross-coupling reaction of benzoyl chlorides and potassium styryltrifluoroborates. The final product is α,β-unsaturated aromatic ketones (Al-Masum *et al.*, 2011) as shown in Scheme 1.8.



Scheme 1.8: Synthesis of chalcones via direct-crossed coupling reaction

1.5.1.4 Heck-type coupling reaction

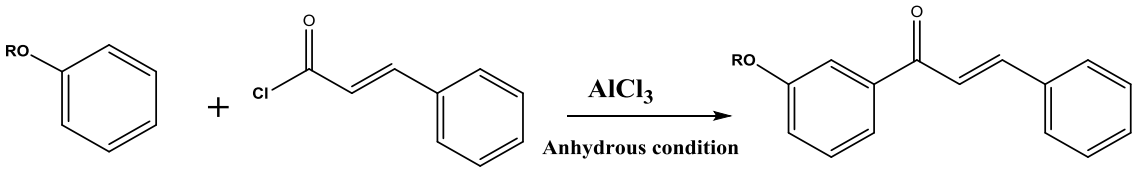
The Heck-type coupling reaction is used mainly to form a substituted alkene (Geoghegan, 2014). The coupling reaction was carried out between aryl boronic acids with α,β -unsaturated carbonyls, catalyzed with phosphine–rhodium by using a toluene–H_2O biphasic system as a solvent (Zou, Guo *et al*., 2007) (Scheme 1.9).



Scheme 1.9: Synthesis of chalcones via Heck-type coupling reaction

1.5.1.5 Friedel- Crafts acylation

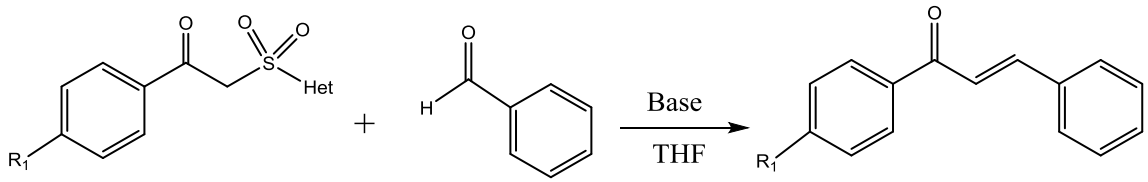
Chalcones can be achieved by acylation reaction involves adding an acyl chloride to the phenol in the presence of Lewis acid ($AlCl_3$) as a strong catalyst. The mechanism of this reaction is an electrophilic aromatic substitution. Acylation agent (acyl chloride) represents B-ring, and α,β-unsaturated carbon, while phenol displays an A-ring in chalcone (Bohm, 1998) (Scheme 1.10).



Scheme 1.10: Synthesis of chalcones via Friedel-Crafts acylation reaction

1.5.1.6 Julia-Kocienski olefination reaction

Julia-Kocienski olefination is a powerful method for synthesis of chalcones and flavanones (Kumar *et al*,. 2010), which involves direct coupling of 2-(benzo[d]thiazol-2-ylsulfonyl)-1-phenylethanones with aromatic aldehyde in presence of a base such as DBU, LiHMDS, P4-t-Bu, t-BuOK, and DABCO (Scheme 1.11). High yields of chalcone were produced in good to excellent percents. On the other hand, 2-(benzo[d]thiazol-2-ylsulfonyl)-1-(2-hydroxyphenyl) ethanone reacted with the aromatic aldehydes yielding flavanones instead of chalcone, through an intra-molecular cyclization.



Scheme 1.11: Synthesis of chalcones via Julia-Kocienski olefination

1.6 Mechanisms of chalcone formation

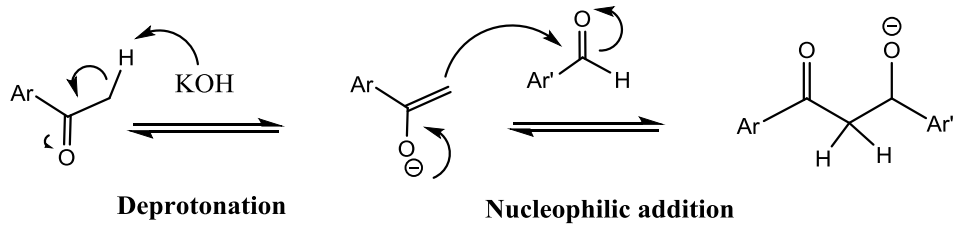
There are many mechanisms for chalcones formation; and the only mechanism that will be discussed is related to Claisen-Schmidt condensation which is the method used to prepare chalcone in this work. This mechanism has the following steps (scheme 1.12):

Step 1: Acidic α-hydrogen of the carbonyl compound deprotonates using KOH base giving an enolate. The enolate attacks the carbonyl group of aryl aldehyde giving alkoxide intermediate.

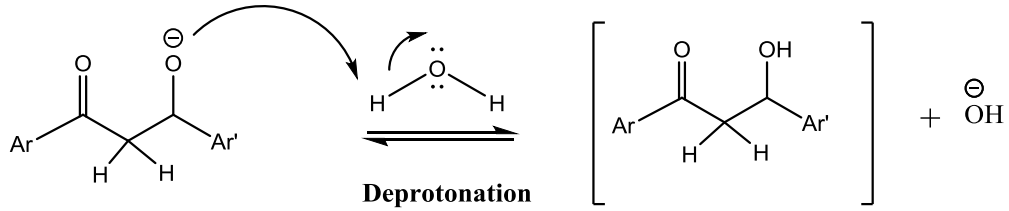
Step 2: The alkoxide then abstracts a proton from the water molecule forming β-hydroxy ketone intermediate.

Step 3: The β-hydroxy ketone undergoes dehydration via base-catalyzed to form α,β-unsaturated ketone.

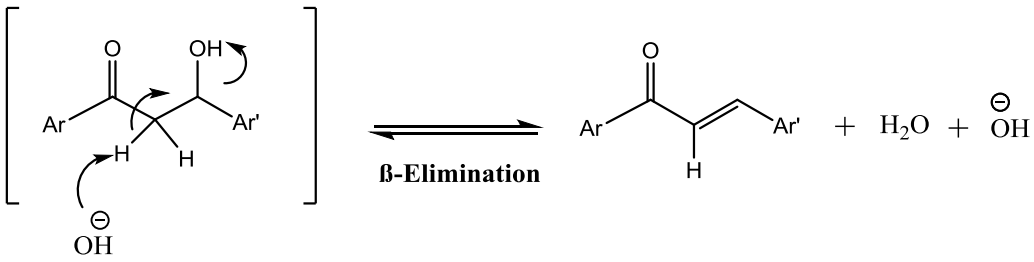
STEP 1 :



STEP 2 :



STEP 3 :



Scheme 1.12: Mechanism of chalcone formation via Claisen-Schmidt condensation

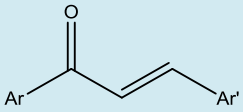
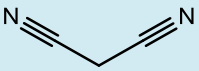
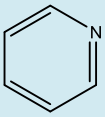
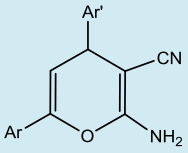
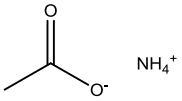
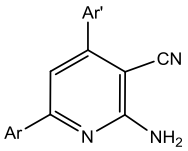
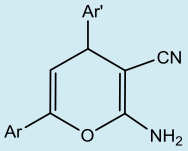
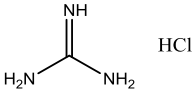
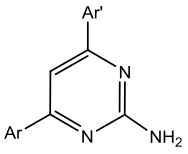
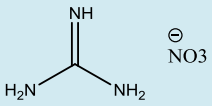
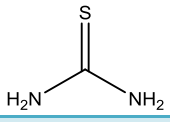
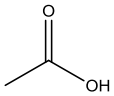
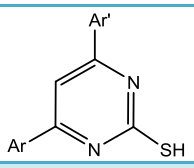
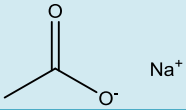
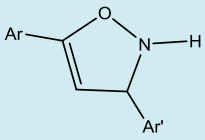
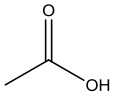
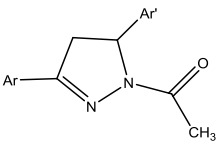
1.7 Reactions of chalcones

Different methods are available in the literature for the reactions of chalcones. The reactions are affected by the sort of reagent and the reaction conditions as well. Some of the important methods are listed below in Table 1.1.

1. Vyas *et al*. have reported that 2-amino-3-cyano-4,6-disubstituted pyrans are obtained by the condensation of chalcones with malononitrile $CH_2(CN)_2$ in the presence of pyridine.
2. Vyas *et al*. prepared 2-amino-3-cyano-4,6-disubstituted pyridines by the reaction of chalcones with malononitrile $CH_2(CN)_2$ in the presence of ammonium acetate (CH_3COONH_4) in ethanol.

3. Gouhar and Youns synthesized 2-amino-3-cyano pyrans by the cyclo condensation of chalcones with malononitrile $CH_2(CN)_2$ in presence of alkali sodium hydroxide (NaOH).
4. Padarthi *et al*. have prepared 2-amino pyrimidine derivatives by using guanidine hydrochloride and/or guanidine nitrate in the presence of potassium hydroxide (KOH).
5. Thriveni *et al*. have synthesized thiopyrimidine derivatives by the cyclo-condensation of chalcones with thiourea in the presence of small amount of catalyzed glacial acetic acid (CH_3COOH).
6. Fanshawe and co-workers have prepared isoxazole derivatives by the condensation of chalcones with hydroxyl amine hydrochloride ($NH_2OH.HCl$) in the presence of sodium acetate (CH_3COONa).
7. Al-abdullah synthesized pyrazoline derivatives by the condensation of chalcones with hydrazine hydrate ($NH_2NH_2.H_2O$) in the presence of acetic acid (CH_3COOH).

Table 1.1: Reactions of chalcones with different reagents

Chalcone	Reagent	Condition	Heterocyclic ring (produce)	Ref.
	Malononitrile 	Pyridine 		(Vyas *et al.*, 2009)
		Ammonium acetate 		
		Sodium hydroxide NaOH		(Gouhar & Youns, 2013)
	Guanidine hydrochloride 	Potassium hydroxide KOH		(Padarthi *et al.*, 2013)
	Guanidine nitrate 			(Padarthi *et al.* 2013)
	Thiourea 	Acetic acid 		Thriveni *et al.*, (2014)
	Hydroxylamine $H_2N—OH$	Sodium acetate 		(Fanshawe, 1977)
	Hydrazine hydrate $H_2N—NH_2$ $.H_2O$	Acetic acid 		(Al-abdullah, 2011)

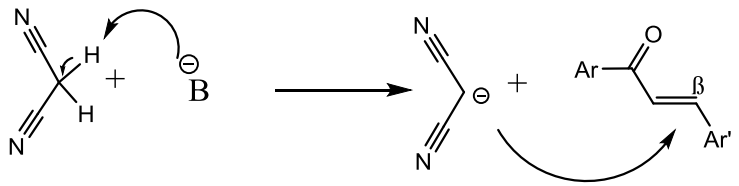
1.8 Mechanism of heterocyclic compounds formation

1.8.1 Mechanism of pyran ring formation

Pyran formation is carried out through conjugate addition reaction using active methylene group (malononitrile) catalyzed by a strong base producing carbanion.

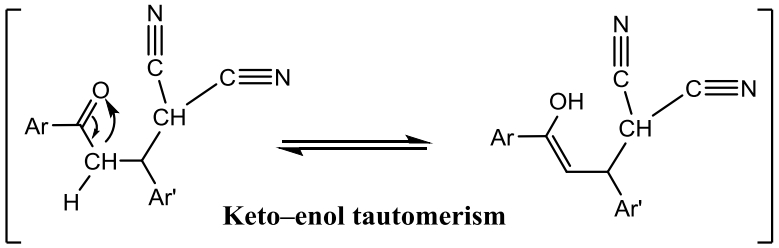
The carbanion reacts with electrophilic alkene forming a carbon-carbon bond via conjugate addition reaction. Acidic α-hydrogen which is bonded to the carbon atom near carbonyl group undergoes keto-enol tautomerism. The hydroxyl group attacks nitrile carbon yielding a cyclic ring. Finally, the proton deprotonates by a base generating the pyran ring (Scheme1.13).

STEP 1 :

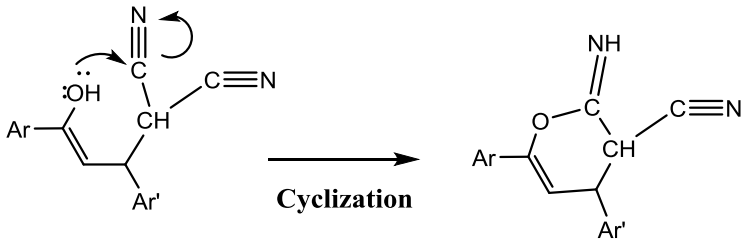


Deprotonation **Conjugate addition**

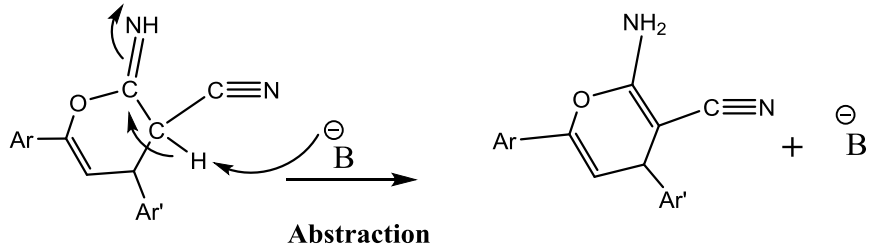
STEP 2:



STEP 3:



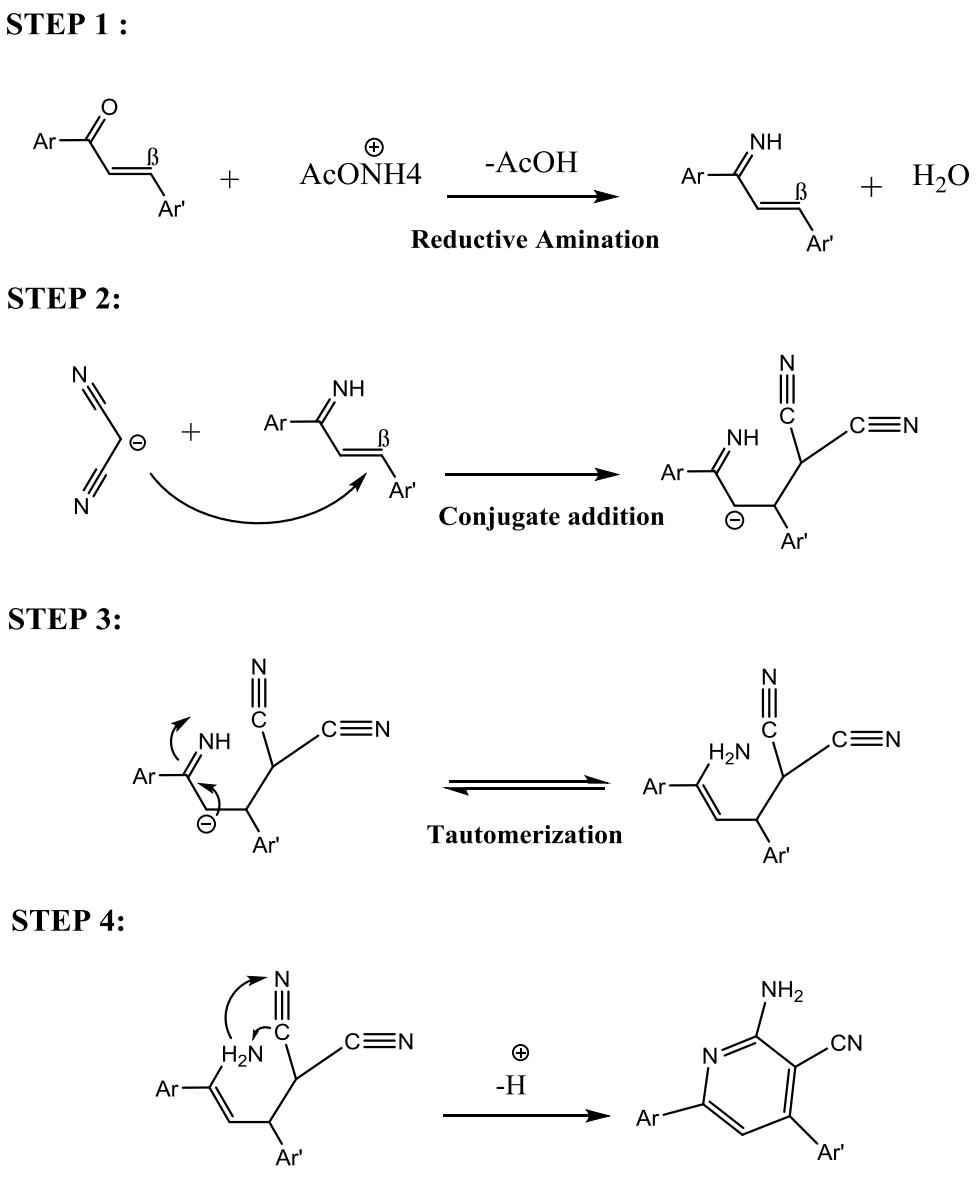
STEP 4:



Scheme 1.13: Mechanism of pyran formation via conjugate addition reaction

1.8.2 Mechanism of pyridine ring formation

Pyridine ring is mostly formed by using two reagents; ammonium acetate and active methylene group (Malononitrile). The first step represents a reductive amination of the carbonyl group to imine by basic ammonia. The second step demonstrates a conjugate addition of nucleophilic methylene group to electrophilic alkene yielding a carbon-carbon bond. The third step exhibits a tautomerization of imine to enamine. In the final step, a cyclic ring is produced through attacks the lone pair of amine for the nitrile carbon and then a proton is abstracted giving heterocyclic pyridine ring (scheme 1.14).

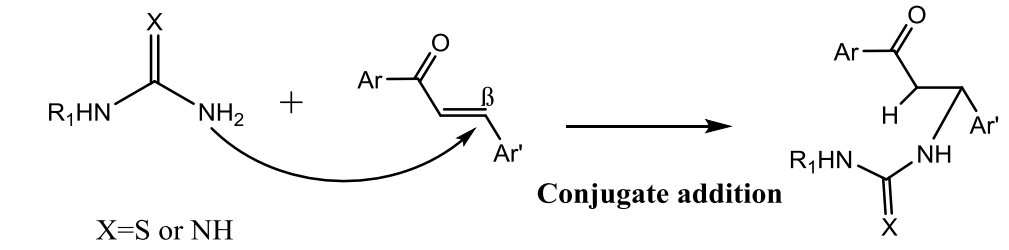


Scheme 1.14: Mechanism of pyridine formation via conjugate addition reaction

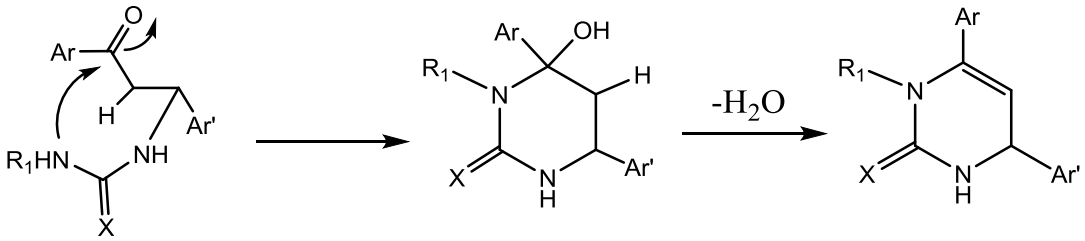
1.8.3 Mechanism of pyrimidine formation

Formation of pyrimidine heterocyclic ring is performed by the conjugate addition reaction, it involves the reaction of guanidine and/or thiourea derivatives with the α,β-unsaturated compound forming carbon-carbon bond, and then the free lone pair of nitrogen attacks the electrophilic carbonyl group, converts it to a hydroxyl group and forms a closed ring. After that, a water molecule is eliminated from the compound producing a cyclized pyrimidine product (scheme 1.15).

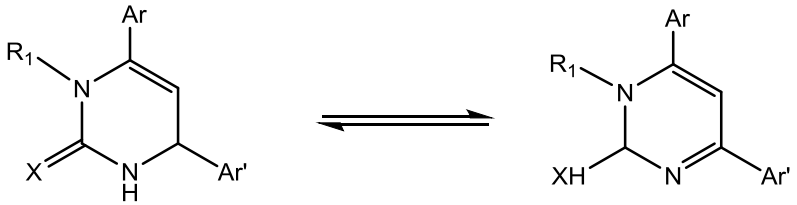
STEP 1 :



STEP 2:



STEP 3:



Scheme 1.15: Mechanism of pyrimidine formation via conjugate addition reaction

1.9 Cancer

Cancer is a major public health impediment in both developed and developing countries. According to research statistic, more than one-third of the world's population has been affected by cancer (Ibrahim et al., 2010). Future predictions indicate there will be an increase in the cancer cases worldwide around 26 million new cancer cases and 17 million cancer deaths per year in 2030 (Bray, Moller, & Møller, 2006).

In Palestine, cancer is considered as a third chronic disease cause of death after cardiovascular and cerebrovascular diseases (Abdeen, 2006). Among the death cancer types in Palestine is breast cancer, which occupied highest-reported cancer among women that represent 35.8% of all cancer in West Bank mid-year 2012 followed by colon cancer, these numbers obtained from statistics released by the Palestinian Ministry of Health as shown in Figure 1.1.

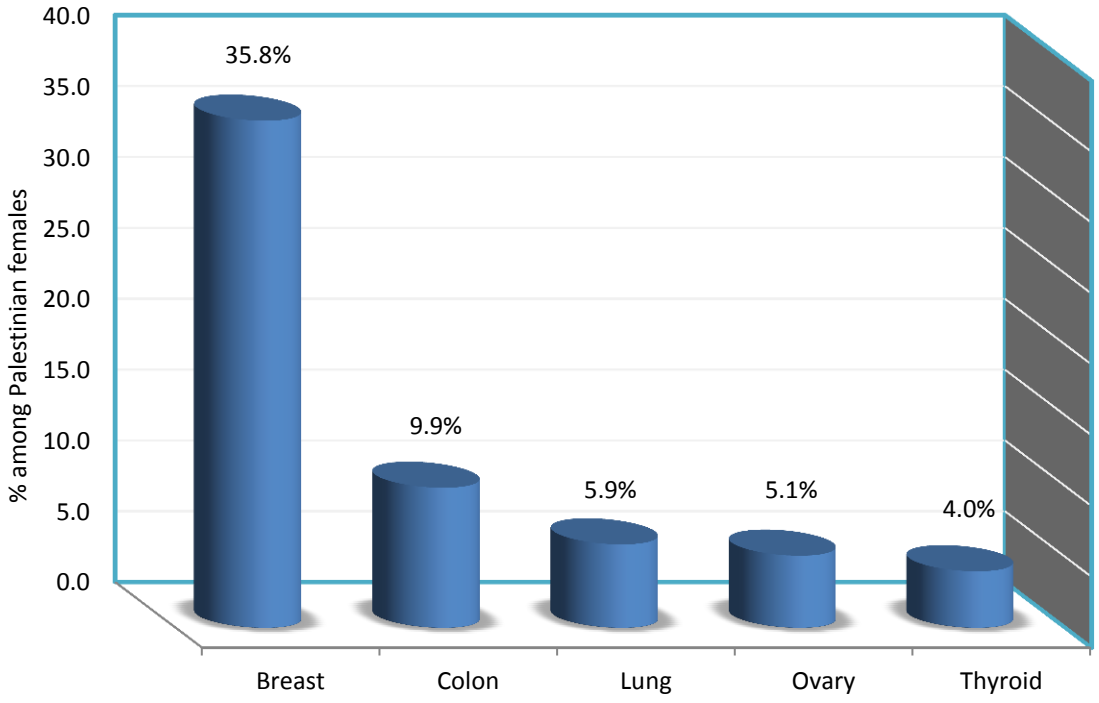


Figure 1.1: Distribution of percentage of top reported cancer among females, West Bank, Palestine, Mid-Year 2012

1.10 Molecular mechanism of carcinogenesis

The cell is the basic building blocks of all living organisms. In normal tissue, cells are growing and divide to produce new cells; this process is called "cell proliferation".

When cells become harmful, damaged or unwanted, they undergo to death, the term death is described as apoptosis (programmed cell death). Apoptosis plays a significant role in our bodies; it's maintained of tissue balances by controlling cell growth and cell death, and defense against mutation by removed damaged cells.

Internal factors (genetics and hormones) and/or external factors such as tobacco, chemicals, radiation, infection by organisms, nutritional factors, the lack of physical activities and alcohol consumption contribute in carcinogenesis by causing mutations in deoxyribonucleic acid (DNA) of the cells (Rodriguez, 1995). These mutations (especially if occur in the oncogene proteins (Chial *et al.*, 2015) causes an accelerated and unregulated cellular proliferation in the body and loses the ability to undergo low rate of apoptosis (programmed cell death) which leading to increase mass of cells and a cancer formation (Cooper & Hausman, 2007).

Cancer is not a localized disease; it can be spread to other parts of the body by two mechanisms: invasion and metastasis. The Invasion takes place when cancer cells migrated and penetrated into neighboring tissues. On the other hand, metastasis refers to the ability of cancer cells to invade normal tissues and other organs such as liver, bone, and brain via the circulatory or lymphatic systems, creating secondary cancer regions (Fidler, 1978).

Cancers, and also called tumors are classified as being either benign or malignant according to whether or not they can spread by invasion and metastasis. Malignant tumors are tumors that are able to spread by invasion and metastasis but benign do not. In fact, the term ’cancer’ usually refer to malignant tumors, and this causes a dangerous problem since its ability to invade and metastasize makes them resistant to such localized treatment, while benign tumors usually removed by surgery (Cooper & Hausman, 2007).

Since cancer is a fatal disease, this stimulated the scientists to developing new and effective anti-cancer agents to make cancer under control.

1.11 Treatment of breast cancer

Most of the current conventional approaches to treating breast cancer include surgery, radiation, and chemotherapy. Surgery and radiation are only used before cancer progress has occurred. On the other hand, Chemotherapy can be used complementary with radiation therapy but the side effect will be worse. Chemotherapy used chemical compounds to treat cancer including antineoplastic drugs (also called anticancer drugs) such as alkylating agents, antimetabolites, anti-tumor antibiotics, plant alkaloids, mitotic inhibitors and topoisomerase inhibitors (Payne & Miles, 2008).

Chemotherapeutic agents can be classified according to the mechanism of action into drugs that prevents reproducing of cancer cells and others cause sometimes DNA damage that leads to apoptosis (Payne & Miles, 2008). However, conventional approaches sometimes are inconvenient and unsatisfactory. Drugs resistance (Gottesman, 2016), negative side effects and poor selectivity which kills cancer and normal cells (Pisha *et al.*, 1995). Therefore, the treatment of breast cancer requires novel drug compounds with a high-potential treatment ability as well as low resistance, minimum side effects and high selective to its target mutated cells which are the starting point of cancer. For these reasons, synthetic modification of novel drugs may improve potency as an anti-cancer agent. These drugs can be natural or synthetic are called chemopreventive agents which were first described by Sporn (Sporn *et al.*, 1976). Among the chemopreventive agents are chalcones and its derivative.

1.12 Therapeutic importance of chalcones

During studies on the structure of chalcones, scientists found that their biological activity could be attributed to the presence of the α,β-unsaturated keto (en-one) functional group. Recent studies have shown that chalcones exhibit multifarious biological activities against cancers (Echeverria *et al.*, 2009; Forejtníková *et al.*, 2005; Kamal *et al.*, 2013; Modzelewska *et al.*, 2006; Vogel & Heilmann, 2008; Wang *et al.*, 2015), microbes (Prasad *et al.*, 2008), parasites (Roussaki *et al.*, 2013), and viruses (Wan *et al.*, 2015). They also have shown anti-analgesic (Chamakuri *et al.*, 2016), anti-mitotic (Edwards *et al.*, 1990), anti-inflammatory (Bandgar *et al.*, 2010; Vogel *et al.*, 2010), and anti-oxidant activities *(Doan, 2011; Kim et al., 2008; Sivakumar et al., 2011; Vogel et al.*, 2008).

1.13Anti-cancer Activity of Chalcones

Table (1.2) below represented some chalcones that demonstrated anti-cancer activity against different types of cell lines.

De Vasconcelos *et al* recently reported six chalcone derivatives based thiophene rings were tested on human colon adenocarcinoma cells HT-29. Two of them, 3-(2-nitrophenyl)-1-(thiophen-2-yl) prop-2-en-1-one **(I)**, and 3-(4-bromophenyl)-1-(thiophen-2-yl) prop-2-en-1-one **(II)** exhibited higher cytotoxicity, analyzed by using cell morphology, live/dead and MTT assays. Moreover, Chalcone **(II)** induced apoptosis on flow cytometry annexin V assay. The assays were performed using a different concentration of compounds (0.1-40 µM) which were incubated for three different times (24, 48, 72 hours).

A recent report by Kong *et al*. studied inhibition of 16 human carcinoma cell lines including breast cancer cell line MCF-7 over a range of concentration (10–200 nM) of methoxylated chalcones ((E)-(2-methoxy-5-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)boronic acid **(III)** , which are very similar to combretastatin structure. The anti-cancer activity demonstrated by cell proliferation assay and inhibits tubulin polymerization. In addition, the compound has important anti-angiogenesis effects evaluated by HUVEC tube formation and aortic ring assay.

A novel benzofuran substituted chalcones was found to exhibit anti-tumor activity on two human cancer cell lines; breast (MCF-7) and prostate (PC-3). In vitro anti-cancer activity of two compounds **(IV)** and **(V)** was determined using colorimetric MTT assay after 24-hour treatment. The results indicated that the compounds have anti-tumor activity (Coskun *et al*., 2016).

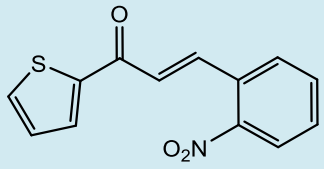
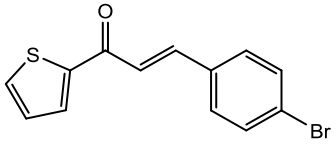
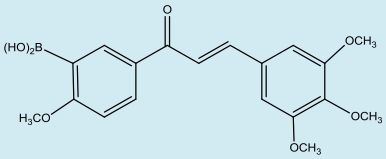
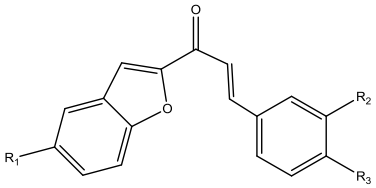
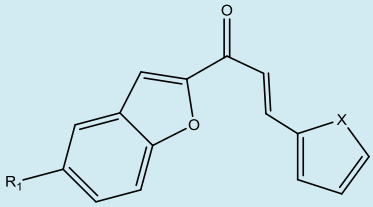
A series of heterocyclic chalcones has evaluated for the cytotoxicity against rhabdomyosarcoma (RMS) and noncancerous cell line (LLC-PK1) (Do *et al.*, 2016). The cytotoxicity assay was tested using MTT assay over a concentration of drugs (100 µM 50 µM 10 µM). They studied the influence of replacement different heterocyclic groups such as thiophene, furan, phenothiazine, and pyridine on cytotoxicity of chalcones. The results were very interesting, indicated that the cytotoxicity of phenothiazinyl chalcone derivative **(VI)** increased extremely when electronegative

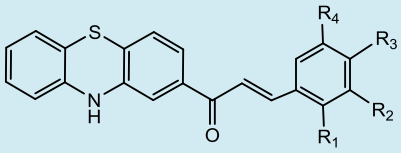
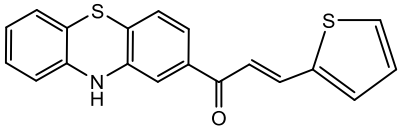
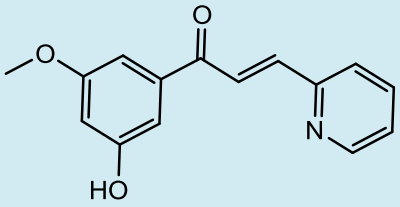
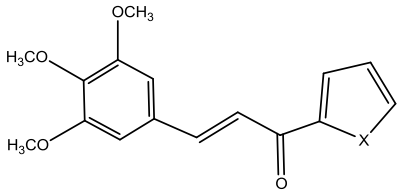
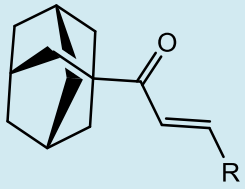
groups (halogen) occur in position 2 on ring B (R_1=Cl). In contrast, the presence of the halogen in position 3 and 4 show limited activity compared to the lack of halogen (R_2=Br), (R_3=Cl). Besides, they demonstrated that the most tumor-selective cytotoxic compounds when containing both phenothiazine and thiophene rings **(VII)**. For the chalcone with a pyridine rings instead of ring B in chalcone structure, substituted a methoxy group on ring A at position 4' promotes cytotoxicity effect **(VIII)**.

Additionally, cytotoxicity effect of the presence of three methoxy groups on position 3,4 and 5 on ring B, replacement of ring A by thiophene, furan and pyridine moiety. Pyridine based compound has the highest cytotoxicity followed by thiophene, then furan (X=N,S,O) (**IX**).

A patent (Anderson & Kaimari, 2005) described a series of novel 1-adamantly chalcones **(X)** as potential anticancer agents against breast cancer. The compounds were tested on two 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 did not affect the normal cell line. (R=pyrid-2-yl, pyrid-3-yl, pyrid-4-yl, 6-methylpyrid-2-yl, quinol-4-yl).

Table 1.2: Anti-cancer activity of chalcones

Chalcone structure	Conditions	Assay	Cell line	Ref.
 **(I)**	Five different concentration (0.1-1-10-20-40 µM)	-MTT -cell morphology -live/dead	Human colon cancer cells (HT-29)	(De Vasconcelos et al., 2013)
 **(II)**	Five different concentration (0.1-1-10-20-40 µM)	-MTT -cell morphology -live/dead - flow cytometry annexin V	-Colon (HT-29)	(De Vasconcelos et al., 2013)
 **(III)**	10–200 nM for proliferation assay	-Tubulin polymerization -MCF-7 Cell proliferation -[^{3}H]Colchicine binding -NCI Human cancer cell proliferation -Indirect immunofluorescence -Cell cycle -Angiogenesis	- Leukemia -Lung -Colon -Breast -Renal -Prostate -Ovarian	(Kong et al., 2010)
 **(IV)**	Five different concentration (1-5-25-50-100µM)	MTT	- Breast (MCF-7) - Prostate (PC-3)	(Coskun et al., 2016)
 **(V)**				

Chalcone structure	conditions	Assay	Cell line	Ref.
 **(VI)**	(10- 50-100 µM)	MTT	Rhabdomyosarcoma (RMS) Noncancerous (LLC-PK1)	(Do et al., 2016)
 **(VII)**				
 **(VIII)**				
 **(IX)**				
 **(X)**	(50-40-5-0.5-0.1-0.01µM)	MTT	-breast MCf-7/MDA-MB435 -MCF-10	(Anderson & Kaimari, 2005)

1.14 Biological screening of heterocyclic compounds

Pyrimidine derivatives are well-known as an important nitrogen-containing six-membered heterocyclic compounds due to their therapeutic activities that include anti-microbial, anti-tubercular (Patel *et al*., 2006) analgesic, anti-inflammatory(Sondhi *et al*., 2005), anti-HIV (Wilson *et al*., 1993)and anti-tumor(He *et al*., 2011).

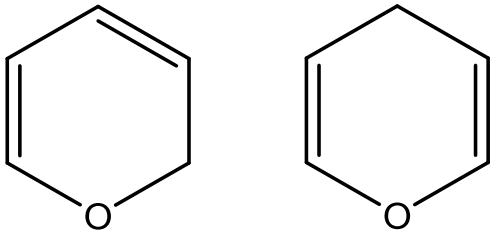
Pyrimidines found in three bases Uracil, Thymine, and Cytosine, which represent an integral structural feature of nucleic acids, DNA and RNA (Smith & Joule,1979; Mishr & Mishra,2008) and its possible that is the reason for their activity.

Recently, different thienopyrimidine derivatives have significant attention due to their anticancer activity (scheme 1.17). Verma *et al*., (2014) reported a series of novel N-(sugar pyranosyl) thienopyrimidine 4-amine derivatives **(XI)**. These derivatives showed anticancer activity in different cell lines. Additionally, a series of thieno[2,3-]pyrimidines derivatives **(XII)** were found to have effective anticancer activity against human colon carcinoma (HCT 116) cell line (Kandeel *et al*., 2012).

The pyridine cyclic rings are of particular interest due to their importance in the pharmaceutical field. About more than 700 drugs found to have a pyridine ring in its structure (Li *et al*., 1999; Vacher *et al*., 1999). They display various biological activities such as anti-oxidant (Worachartcheewan *et al*., 2012), anti-viral (El-Hawash *et al*., 2006) and anti-diabetic. Some of these compounds also possess anti-microbial (Chavan *et al.*, 2006), anti-Inflammatory (Márquez-Flores *et al*., 2012) and anti-malarial agents (Narayan Acharya *et al*., 2008) Further, pyridine compounds are also used as anti-cancer agents (Abadi *et al*., 2009).

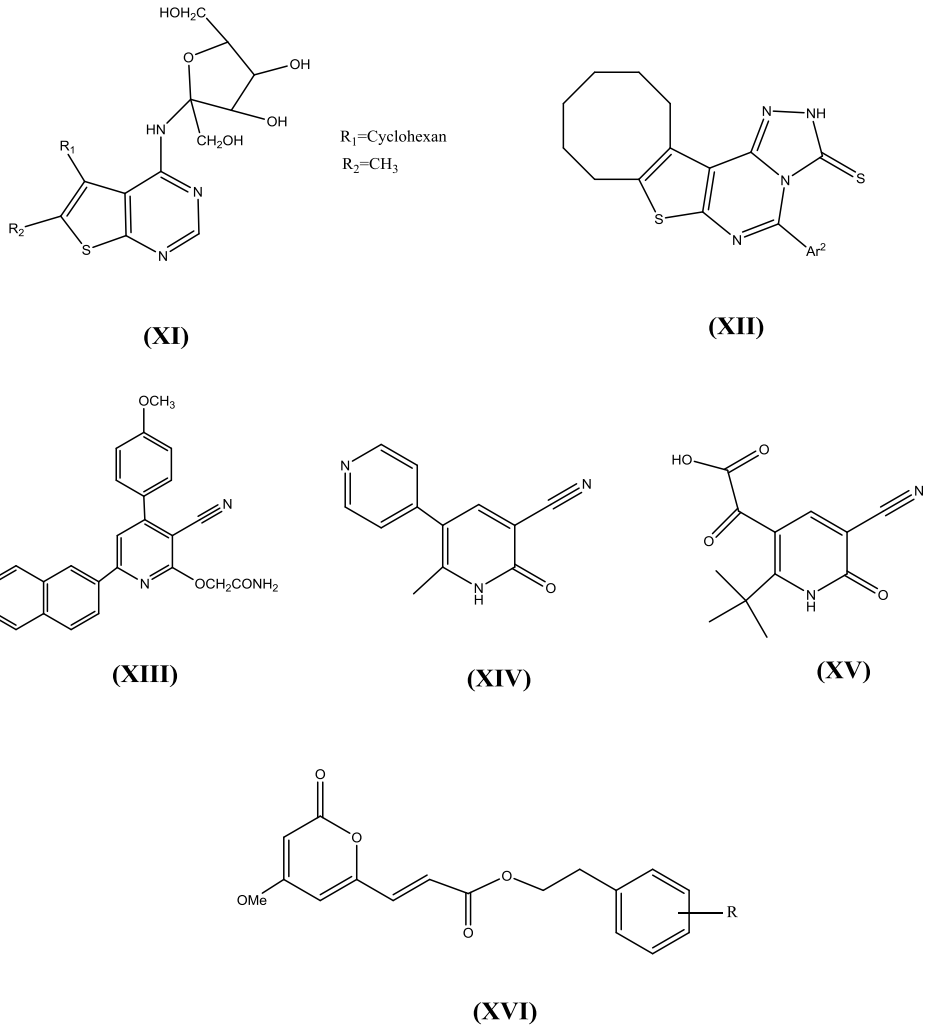
Additionally, cyanopyridine derivatives of pyridine have played a crucial part in the production of nicotinamide (Nagasawa *et al.*, 1988) and also extensively in organic synthesis as an intermediate. A recent review that published in 2014 (Sciences, 2014) focused on the most reactive compounds, 2-oxo-3-cyanopyridine derivatives, which have been reported to possess biological activities. Compounds **(XIII)**, **(XIV),** and **(XV)** (scheme 1.17) were found to be biologically active such as anti-microbial, anti-cancer (Cheney *et al*., 2007), and cardiovascular activities, respectively. Besides, they were used for the treatment of diabetes, metabolic syndrome, and obesity (Sciences, 2014).

Pyrans derivatives are important six-membered heterocyclic compounds containing oxygen in their ring. They are classified according to the position of the two double bonds; they may be isolated or conjugated recognized as 2H-pyran and 4H-pyran respectively (scheme 1.16).



Scheme 1.16: Heterocyclic 2H and/4H- pyran respectively

Pyran heterocyclic compounds are found to have an interesting biological activity such as anti-microbial, anti-fungal (Delitheos, 1992), anti-inflammatory and anti-ulcer agents (Mohareb *et al*., 2015). Moreover, they display varied anti-tumor activity. For instance, 6-acrylic phenethyl ester-2-pyranone derivatives **(XVI)** (scheme 1.17) exhibit a potent cytotoxicity against different cell lines (Fang *et al.*, 2015).

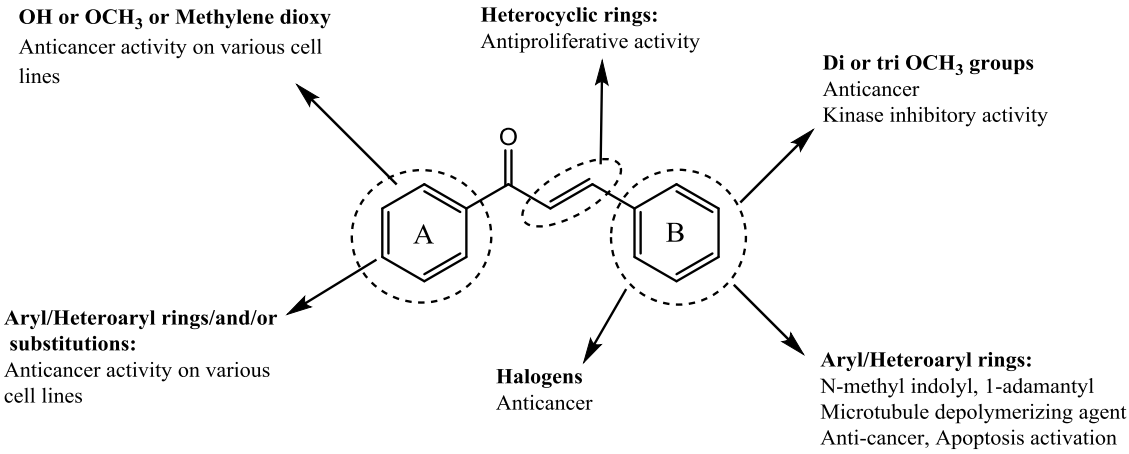


(XI) **(XII)** **(XIII)** **(XIV)** **(XV)** **(XVI)**

Scheme 1.17: Structures of some compounds with heterocyclic moieties

1.15 Development of chalcones

Recently, great efforts have been made by the researchers to develop chalcones as an anticancer agent. Most of the research publications concentrate on the structural activity of chalcone based compounds as shown in scheme 1.18. The anticancer activity of chalcones are extremely affected by the replacement of the two aryl rings with heteroaryl moiety and their substitution groups such as methoxy and halogen groups on rings A and B for improvement of anticancer properties (Karthikeyan *et al.*, 2014).



Scheme 1.18: Structure- anticancer activity relationship of chalcone compounds

Dr. Kaimari and others previously reported the structural modulation of chalcone (Anderson & Kaimari, 2005) in both aryl rings, including the replacement of aryl ring A with hydrocarbon moiety (adamantyl group) and aryl ring B with heteroaryl moieties, as potential anti-cancer agents. The importance of the steric bulk adamantyl group is due to its ability to increase drug stability by restricting intramolecular reactivity and hinder the access of hydrolytic enzymes and prevent degradation (Liu et al., 2011).

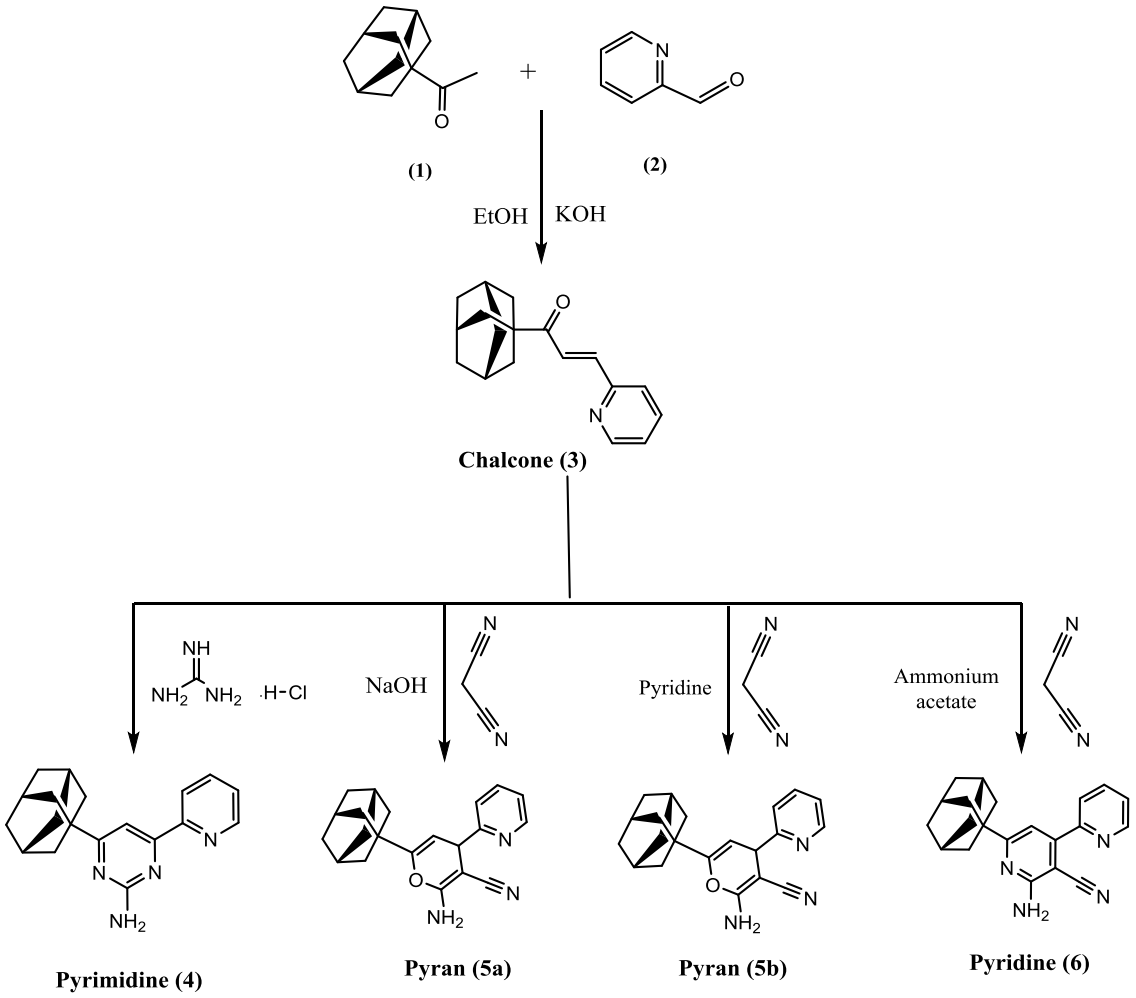
Moreover, compounds must contain a lipophilic characteristic to cross cell membrane to increase the rate of absorption and avoid rapid excretion (Peck, Hill, & Williams, 2008).

Many adamantly-based structures such as a class of atypical retinoids have been reported for potential anti-cancer agents against lung, prostate, ovarian, breast, melanoma and leukaemia cancers (L Altucci & Gronemeyer, 2001; Lucia et al., 2007; Charpentier & Bernardon, 1995).

1.16 Thesis objectives

The objectives of the thesis are listed as follow:

I. Synthesize number of adamantylated heterocyclic membered rings with chalcone-based structure (Scheme 1.19):



Scheme 1.19: Synthesis of the chalcone **(3)** and its cyclized products

- Chalcone **(3)**: (E)-1-((3r, 5r, 7r)-adamantan-1-yl)-3-(pyridin-2-yl) prop-2-en-1-one.
- Pyrimidine **(4)**: 4-((3r, 5r, 7r)-adamantan-1-yl)-6-(pyridin-2-yl) pyrimidin-2-amine.
- Pyran **(5a)**:6-((3s)-adamantan-1-yl)-2-amino-4-(pyridin-2-yl)-4H-pyran-3-carbonitrile.
- Pyran **(5b)**:6-((3s)-adamantan-1-yl)-2-amino-4-(pyridin-2-yl)-4H-pyran-3-carbonitrile.
- Pyridine **(6)**: 6'-((3r,5r,7r)-adamantan-1-yl)-2'-amino-[2, 4'-bipyridine]-3'-carbonitrile.

II. Analysis of adamantylated compounds by using spectroscopic methods:

1) Fourier Transform Infra-Red Spectroscopic (FT-IR).
2) Ultraviolet-Visible (UV/Vis) Spectroscopic.
3) Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR).

A chromatography method is used to investigate chalcone based structures, Thin Layer Chromatography (TLC).

III. Evaluate the effect of adamantylated heterocyclic compounds on cells viability and toxicity using the breast carcinoma cell line (MCF-7).

Chapter Two

2. Materials &Methods

This chapter consists of three parts. The first one concerns with the reagents and instruments that used to achieve the synthesis reactions and the biological tests. The second one describes the specific preparations of heterocyclic chalcone **(3)** based structure. And the Third one demonstrates the anti-cancer evolution of the compounds using MTT colorimetric assay.

2.1 Materials and instrumentation

2.1.1 Materials:

- **For synthesis part**: 2-Pyridinecarboxaldehyde (99%), 1-Adamantyl methyl ketone (99%), Sodium hydroxide, potassium hydroxide (90%), malononitrile (99%), guanidine hydrochloride (99.55%), ammonium acetate, pyridine (99.5%) and dilute hydrochloric acid (HCl 20%) were commercially obtained from Sigma-Aldrich. High purity ethyl acetate, hexane, acetic acid, methanol and ethanol (> 99%) were purchased from Biolab.

- **For biological part**: MCF 7 cell line (AAUJ laboratory stock) with Dulbecco's modified Eagle's growth medium (DMEM) was used to culture cells, supplemented with fetal calf serum, penicillin streptomycin, amphotericin B, L-glutamine, and non-essential amino acids All chemicals were obtained from Sigma-Aldrich company.

2.1.2 Instrumentation:

-**For synthesis part**: the melting points were determined in open capillaries on electrothermal Stuart SMP3 advanced melting point apparatus, IR spectra were obtained from a KBr matrix (4000–400 cm^{-1}) using a Perkin-Elmer Precisely, Spectrum Two, FT-IR spectrometer.

^{1}H-NMR spectra of compounds pyrimidine **(4)**, pyran **(5a)**, and pyridine **(6)** were recorded in DMSO-d6 while compound pyran **(5b)** was recorded in D_2O on a Bruker

DPX 300 Spectrometer (1H: 300.1 MHz) Spectrometer at 295 K. Chemical shifts (δ) are given in parts per million (ppm) downfield relative to TMS.

The progress of reaction was monitored by TLC (Ethyl acetate: Hexane (2:5)). Thin-layer chromatography (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 (3:5); the spots were detected by UV light.

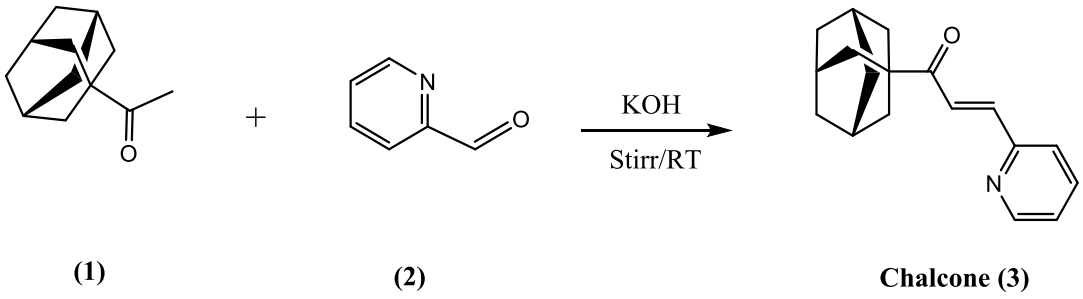
The absorption spectra were obtained by using a NanoDrop ND-100 spectrophotometer.

-**For biological part**: Olympus optical microscope CKX53 was purchased from Italy tailored the cell culture process. Cell counting chamber Improved Neubauer Hemocytometer was obtained from Marienfeld-Superior, Germany. 96-well cell culture plates were obtained 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.

2.2 Chemical synthesis

2.2.1 General procedure of the synthesis of chalcone (3)

A 1-adamantyl methyl ketone **(1)** (0.0028 mol) was added to an ethanolic solution of KOH (0.0028 mol in 40 mL ethanol 96%) and stirred for 10 min, then 2-pyridinecarboxaldehyde **(2)** (0.0028 mol) was added dropwise to the solution and stirred at room temperature for 48 hrs. The reaction mixture was poured into ice. The yellow solid was formed, filtered, washed several times with water and dried. The chalcone **(3)** was obtained in a good yield according to published method (Anderson & Kaimari, 2005). The progress of the reaction was monitored by TLC (scheme 2.1).

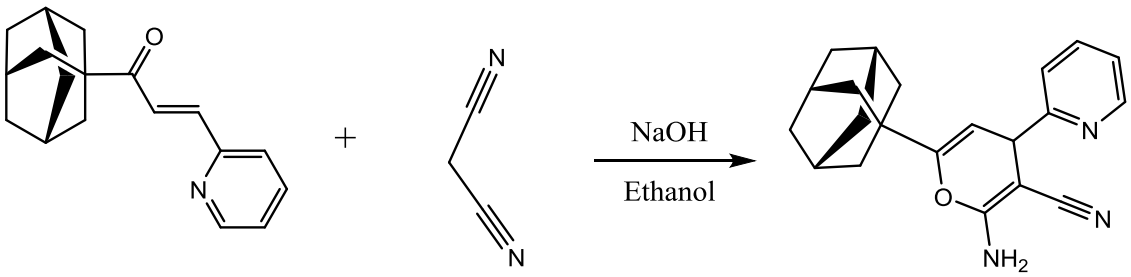


(1) **(2)** **Chalcone (3)**

Scheme 2.1: Synthesis of chalcone **(3)**

2.2.2 Synthesis of pyran (5a) from malononitrile and chalcone using NaOH in ethanol (method a):

A mixture of chalcone **(3)** (0.000187 mol) and malononitrile (0.000187 mol) in 1% ethanolic sodium hydroxide (15 mL) was refluxed for 10 hrs. The formed precipitate on hot was filtered off, washed several times with water and dried to give pyran as white solid according to the work of Gouhar & Youns, 2014. The progress of the reaction was monitored by TLC (scheme 2.2).



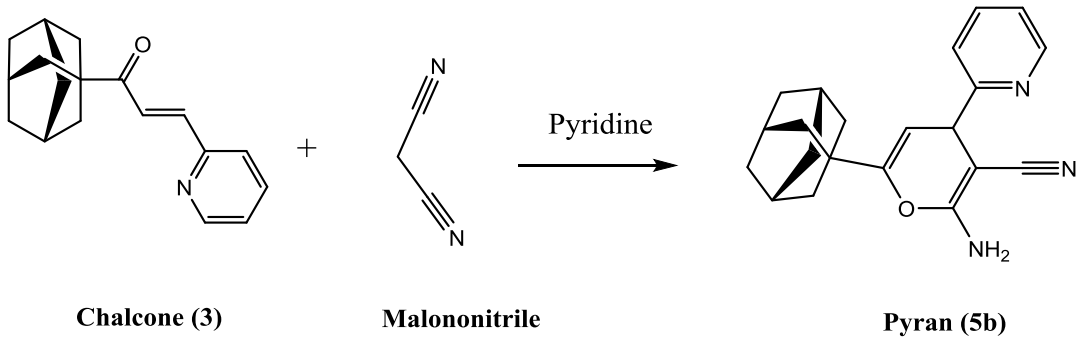
Chalcone(3) **Malononitrile** **Pyran (5a)**

Scheme 2.2: Synthesis of pyran **(5a)** from malononitrile and chalcone using NaOH in ethanol.

2.2.3 Synthesis of pyran (5b) from malononitrile and chalcone using pyridine (method b):

A mixture of chalcone **(3)** (0.00019 mol), and malononitrile (0.0003 mol) in pyridine (5ml) was refluxed for 15 h. The progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mixture was cooled and poured into ice. The residue was neutralized with 10% HCL. The reaction mixture was filtered through

Buchner funnel, the filtrate was obtained and the solvent was removed by evaporation. The residue was washed several times with methanol to get a white solid (Vyas *et al*., 2009) (scheme 2.3).

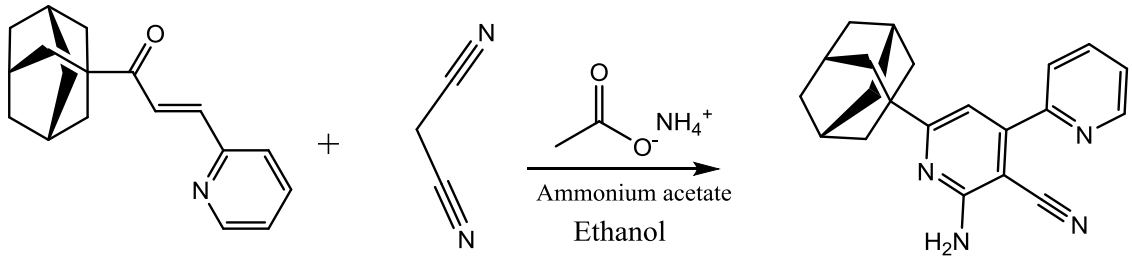


Chalcone (3) **Malononitrile** **Pyran (5b)**

Scheme 2.3: Synthesis of pyran **(5b)** from malononitrile and chalcone using pyridine

2.2.4 Synthesis of pyridine (6) from malononitrile and chalcone

A mixture of chalcone **(3)** (0.000374 mol), malononitrile (0.000374 mol) and ammonium acetate (0.000299 mol) was grinded together in a mortar. Then the reaction mixture was transferred into a 100 mL round bottom flask with the addition of ethanol (30 ml). The reaction mixture was heated under reflux for 20 hrs. on boiling bath. After the completion of reaction, the reaction mixture was cooled and poured into ice to give a yellow precipitated solid that was filtered off according to the work of Gupta *et al*., 2010. The progress of reaction was monitored by TLC. (scheme 2.4).

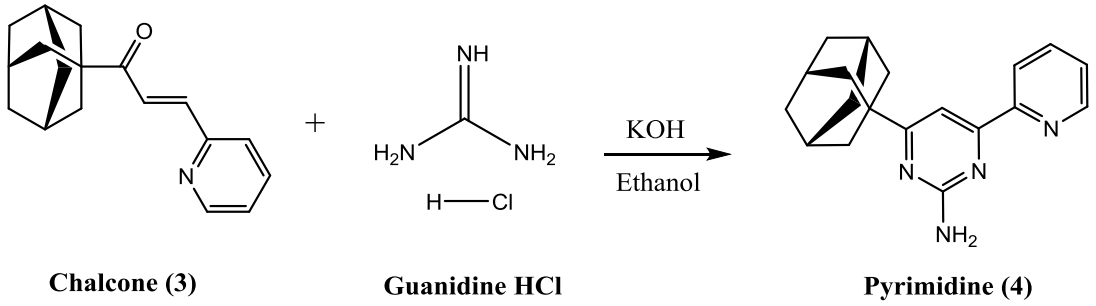


Chalcone (3) **Malononitrile** **Pyridine (6)**

Scheme 2.4: synthesis of pyridine **(6)** from malononitrile and chalcone

2.2.5 Synthesis of pyrimidine (4) from guanidine hydrochloride and chalcone

A mixture of chalcone **(3)** (0.00035 mol) and guanidine hydrochloride (0.00097 mol) was stirred in ethanol 96% (20ml) and then potassium hydroxide (0.0014 mol) was added to it . The reaction mixture was heated under refluxed for 24 hrs. on boiling water bath. The mixture was poured into crushed ice to give a precipitated solid that was filtered off. The solid obtained was crystallized from ethanol according to the work of Padarthi *et al.*, 2013. The progress of reaction was monitored by TLC (scheme 2.5).



Scheme 2.5: Synthesis of pyrimidine (4) from guanidine HCl and chalcone

2.3 Anti-cancer evaluation

2.3.1 Preparation of compounds concentration

For chalcone **(3)**, pyrimidine **(4)**, pyran **(5a)**, and pyridine **(6)**, the stock concentration was 10mg/ml in DMSO, then it was diluted serially with growth medium (DMEM) to a different concentration (256, 128, 64, 32, 16, 4 µg/ml). While pyran **(5b)** concentration (10 mg/ml) was prepared immediately in growth medium because it's water-soluble, then diluted with growth medium (DMEM) at the same concentrations of compounds as mention above.

2.3.2 Cell culture

2.3.2.1 Cell line MCF7

Human breast adenocarcinoma cell line (MCF7) is stored in at the animal cell culture unit, Arab American University of Jenin- at −80°C freezer, Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS), 1% penicillin streptomycin, 1% amphotericin B, 1% L-glutamine, and 1 % non-essential amino acids (sigma) in a humidified atmosphere of 5% CO_2, 95% air at 37 °C. The cells must be prepared by certain process called thawing before cultured in the flasks.

2.3.2.2 Thaw procedure

The Procedure was performed swiftly, in order to get rid of DMSO that was used as freezing media for the cells.

1. Cells were thawed by putting the vial in 37° water bath until just a small bit of the ice remains.
2. Using sterile 5 ml pipette, 2ml warm fresh media with serum was transferred to 15 ml falcon tube.
3. Slowly added 1 ml of the cells to the media, the tube was shaken gently while adding the cells, dilute DMSO media at least 1 in 20.
4. Cells were centrifuged at an appropriate speed (5 minutes at 2000 rpm).
5. The supernatant media was aspirated carefully, using sterile 5 ml pipette.
6. The Cell pellet was resuspended gently in 4 ml of warm growth media.
7. The mixture was transferred (media + cells) to cell culture flask ($25cm^2$).
8. Flask was placed in the CO_2 incubator.
9. The cells were checked every day under the microscope.

2.3.2.3 MTT cell viability assay:

MTT assay is a colorimetric method allows quantitative determination of proliferation, viability, and cytotoxicity of the viable cells that are growing in the absence or presence of drugs by using MTT tetrazolium dye (sigma). The mechanism of the assay based on the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide

(MTT) by mitochondrial succinate dehydrogenase into purple formazan crystals of living cells (Gerlier & Thomasset, 1986), dead cells will not make any change.

A. Trypsinization was performed for 2 confluent flasks.
B. 100 µl (2.0×10^4cell/well) of detached cells were seeded/well in a 96-well plate and incubated for 24 h.
C. Cell growth medium was prepared serially diluted to reach concentrations of drugs of 256, 128, 64, 32, 16, 4 µg/ml, and incubated at 37^0C in a 5% CO_2 for 24 hours.
D. After 24 hour of incubation, A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma) was prepared using media without serum (0.5 mg/ml).
E. 100 µl of MTT solution was added to each well and incubated at 37 °C for 4 hours.
F. Then MTT solution was removed, 100 µl of mixture isopropanol+ formic acid (9:1) was added to solubilize with acidified of the formazan product in order to measure the absorbance because MTT formazan is insoluble in water.
G. The 96-well plate was placed on an orbital shaker and covered with tinfoil for 15-20 min.
H. Measured the optical density (OD) of the MTT formazan at 570nm by using enzyme-linked immunosorbent assay (ELISA) reader. Viability was calculated by expressing the absorbance of exposed compound test media and DMSO control media as a percentage of the absorbance of the positive control cells (Prinsloo, Pieters, & Bezuidenhout, 2013).

Trypsinization procedure for step A

1. The old media was removed from the flasks.
2. 2ml of (Trypsin – EDTA) was added to each flask ($25cm^2$) and placed the flask in the incubator CO_2.
3. Allow 2-3 minutes for trypsin to work,
   - Trypsinization time varies depending on the cell type.
   - The cells were monitoring by microscope.
4. If the cells are detachment, 1:1 media without serum is added to stop trypsinization.

5. The media was pipetted to detach all cells from the cell culture flasks.
6. The suspension was transferred to a 15ml falcon tube.
7. Centrifugation at 2000 rpm for 5 minutes.
8. Removed the supernatant.
9. The cell pellet was re-suspended in 3 ml of appropriate warm fresh media.
10. 10µl was taken for counting cells by hemocytometer.

Chapter Three

3. Results & Discussion

3.1 Characterizations:

All compounds were fully characterized by UV/Vis, FT-IR, and ^{1}H-NMR spectroscopic methods. Also, the compounds chalcone **(3)**, pyrimidine **(4)**, pyran **(5a)**, pyran **(5b)**, and pyridine **(6)** were characterized by TLC and melting point.

The progress of the reaction was monitored by TLC (Ethyl acetate: Hexane (2:5)). ^{1}H-NMR spectra of chalcone **(3)**, pyrimidine **(4)**, pyran **(5a)** and pyridine **(6)** were recorded in DMSO-d_6 while pyran **(5b)** was recorded in D_2O on a Bruker DPX 300 Spectrometer (1H: 300.1 MHz) Spectrometer at 295 K. Chemical shifts (δ) are given in parts per million (ppm) downfield relative to TMS.

3.1.1 Thin layer chromatography (TLC):

TLC was performed to ensure the product purity. The Mobile phase was Ethyl acetate: Hexane (2:5) for all compounds except pyrimidine **(4)** (used the same eluent + 2 drops of ammonia). TLC shows that a pure product was obtained; the retention factor (R_f) values of products were shown in Table 3.1.

Table 3.1: Retention factor (R_f) of synthetic compounds

Compounds	**R_f (cm)**
2-Pyridinecarboxaldehyde	0.465
1-adamantyl methyl ketone	-
Chalcone **(3)**	0.534
Pyrimidine **(4)**	0.733
Pyran **(5a)**	0.686
Pyran **(5b)**	0.288
Pyridine **(6)**	0.431

3.1.2 Melting point:

The melting point measurements were performed for compounds chalcone **(3)**, pyrimidine **(4)**, pyran **(5 a)**, and pyridine **(6)** as a preliminary analysis of these compounds, while the melting point for pyran **(5b)** was not able to detect because it's in the form of salts ؛melting point was determined by using SMP3 advanced melting point apparatus. It was given that the melting point of the compounds as following (Table 3.2):

Table 3.2 : Melting point of synthetic compounds

Entry	Compound	Melting point (C^o)	Yield (%)
1	Chalcone **(3)**	97.5-98	71.86
2	Pyrimidine **(4)**	72-75	63
3	Pyran **(5a)**	81-84	71
4	Pyran **(5b)**	N.A	99
5	Pyridine **(6)**	102-104	75

As shown, products melting points were different from that of chalcone **(3)**. This gives a preliminary indication that these compounds are not chalcone **(3)**.

3.1.3 UV-Vis absorption

For the absorption behavior, a general observation is that, by compared the starting materials chalcone **(3)**, malononitrile, and guanidine hydrochloride with the adamantylated heterocyclic compounds (figure 3.1), the maximum absorption band of chalcone **(3)** is at a value of 330 nm in the visible region. Therefore, chalcone **(3)** is yellow compound due to the presence of chromophore conjugation (-CO-CH=CH-). Additionally, the presence of pyridine ring (π–$\pi*$ transition) affects the absorption of the radiation in the visible region. Guanidine hydrochloride has no visible sign of any light being absorbed (low intensity). Furthermore, malononitrile has two absorption peaks at 360 and 430 nm.

In panel A, the UV spectra of the pyrimidine **(4)** show maximum secondary absorption bands around 330 and 360 nm due to the presence of an electron-donating group

(NH_2/adamantyl) which attributes to increase electron shift through π-bond of pyrimidine ring, thus pyrimidine **(4)** has an orange color. Panel B displays pyran **(5a)** which has maximum absorption band around 320 nm, it has a white color, pyran **(5a)** contains an electron-releasing (NH_2/adamantyl), electron-withdrawing (CN) group, and pyridine ring.

In panel C, Pyran **(5b)** has very low intensity in UV-Vis spectra, due to the low concentration of the compound, and it has a white color. In the final panel D, UV spectra of pyridine (6) show broad absorption maxima at 320-330 nm. Pyridine **(6)** contains NH_2, adamantly, pyridine, and CN groups. Therefore, it has yellow-orange color.

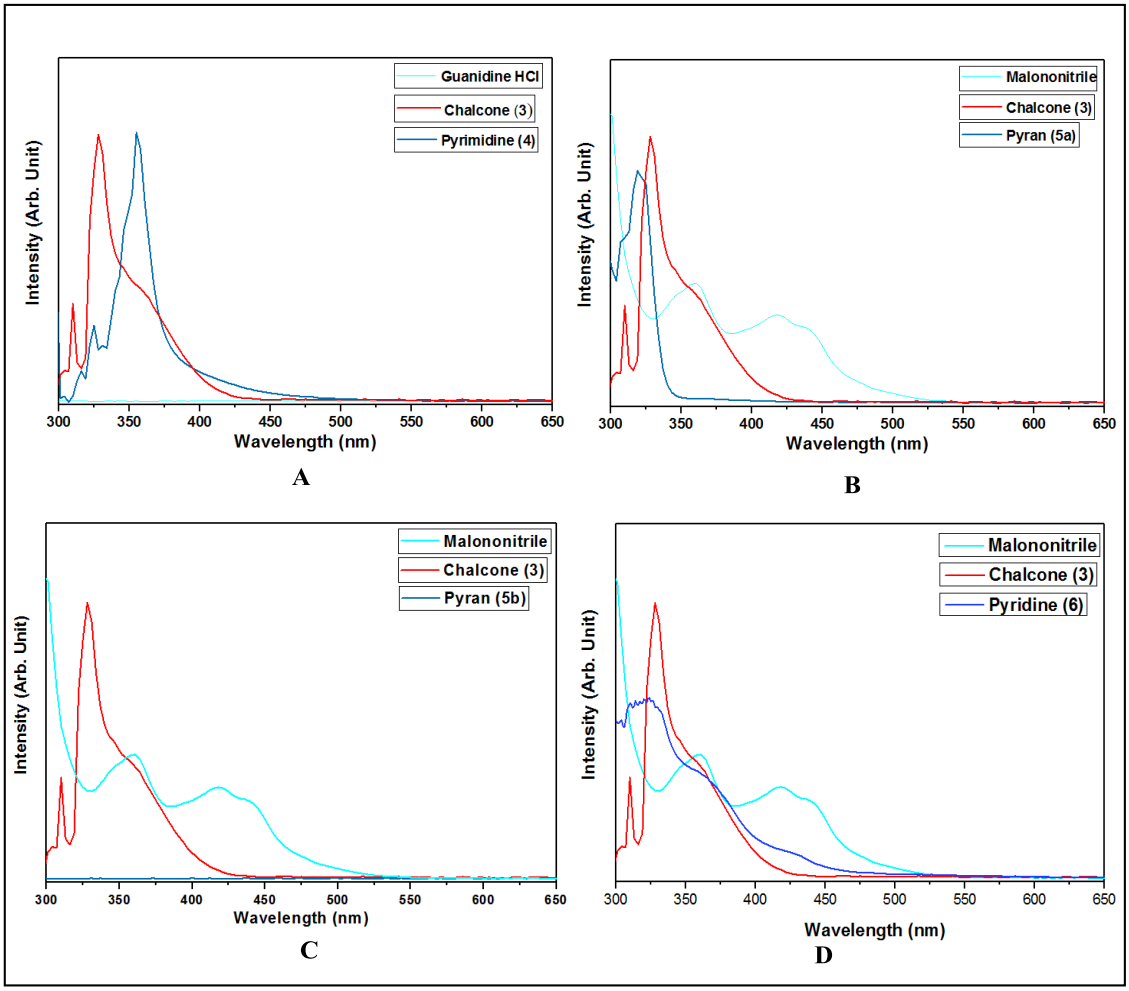


Figure 3. 1 : UV-Vis spectra of chalcone **(3)** and its cyclized products (A-D)

3.1.4 FT-IR analysis:

3.1.4.1 FT-IR of pyrimidine (4)

The following figures show the FT-IR spectra of starting materials chalcone **(3)**, and guanidine hydrochloride, and for the product pyrimidine **(4)**. The spectrum of the chalcone **(3)** shows that α,β-unsaturated stretching of C=O group at 1699, C-N (pyridine ring) group at 1446 is present (Yadav *et al*., 2007). C=C in the aromatic ring have one band at 1607 cm-^{1} in IR-spectrum, that indicated that chalcone **(3)** already existed. The guanidine hydrochloride spectrum shows three peaks were existed corresponding to primary amine (NH_2), C=NH and NH_2 (bending) bands.

The product pyrimidine **(4)** spectrum (figure 3.2) demonstrates a three new fundamental absorption at 3496, 3210, 1686, and 1358 cm^{-1} as a result of presence novel functional group in its structure, NH_2 (primary amine), C=N (pyrimidine ring), and C-N (aromatic amine) (Lambert *et al*., 1987; Register *et al*., 2016).

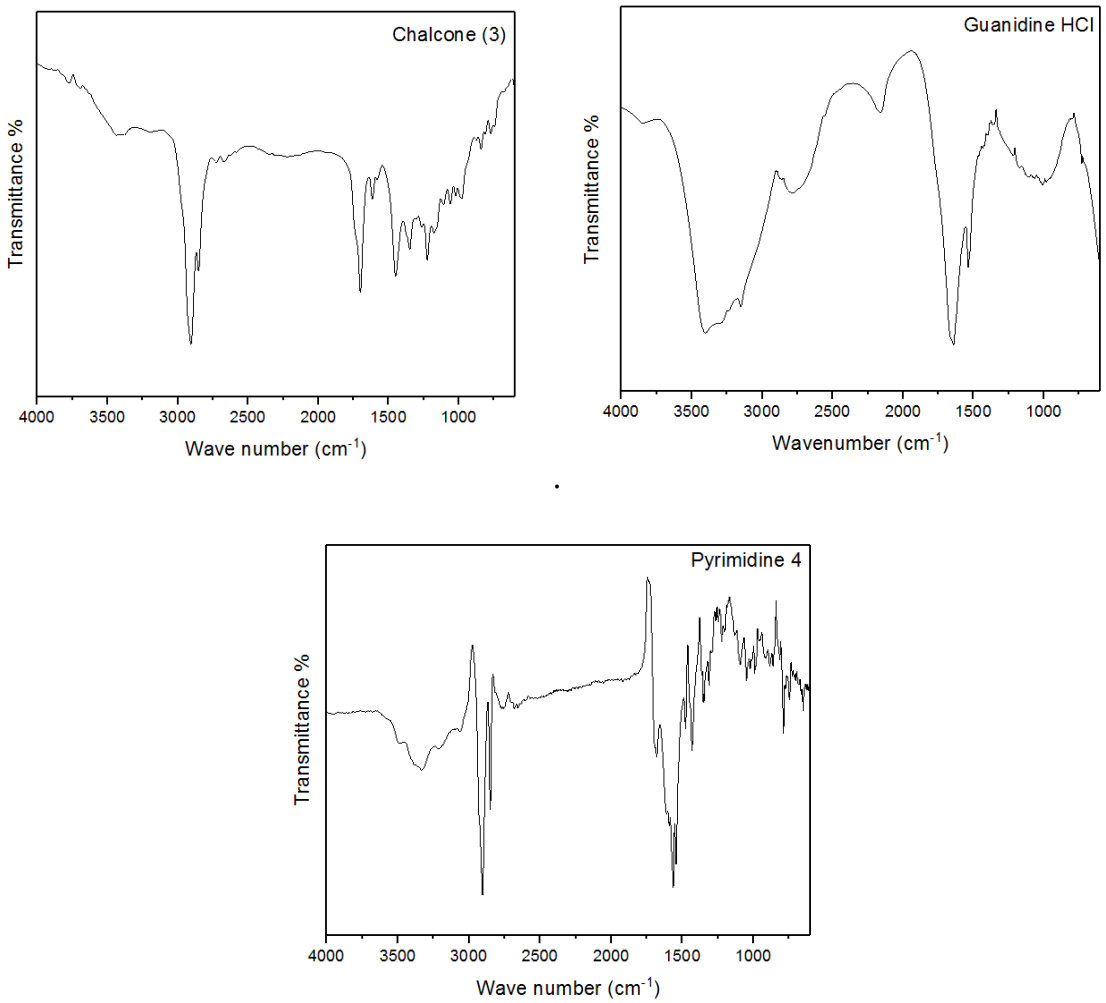


Figure 3.2: FT-IR spectrum of Chalcone **(3)**, guanidine HCl, and pyrimidine **(4)**

Table 3.3: FT-IR spectrum analysis of chalcone **(3)**

IR frequency band (cm^{-1})	**Group responsible**
2904, 2843	C-H str.
1699	C=O str. (α,β-unsaturated)
1607	Conjugated C=C (aromatic)
1446.5	C-N (pyridine ring)

Table 3.4: FT-IR spectrum analysis of guanidine hydrochloride

IR frequency band (cm^{-1})	**Group responsible**
3400, 3200	NH_2 str.
1645	C=NH
1550	NH_2 (bending)

Table 3.5: FT-IR spectrum analysis of pyrimidine **(4)**

IR frequency band (cm^{-1})	**Group responsible**
3496, 3210	N-H (str./primary amine)
2913, 2850	C-H
1686	C=N (aromatic/pyrimidine ring)
1563	C-N (pyridine ring)
1438	Conjugated C=C (aromatic)
1358	C-N (aromatic amine)

3.1.4.2 FT-IR of pyran (5a)

IR-spectrum of the starting material (chalcone **(3)**) is mention above in section 3.1.4.1 Pyran **(5a)** is synthesized using malononitrile and sodium hydroxide serves as a base. IR-Malononitrile spectrum has a CH-stretch at 2963 cm-^{1}, and a sharp peak responsible for $(C \equiv N)$ functional group at 2267 cm-^{1}, and also another peak at 1389 cm^{-1} for CH_2 bending.

For the product pyran **(5a)**, IR spectrum (Figure 3.3) shows an additional signal with absorbance 3379, 3338cm^{-1}, 2209 cm^{-1}, 1345 cm^{-1}, and 1196 cm^{-1}, corresponds to

primary amine (NH_2), nitrile ($C \equiv N$), aromatic amine (C-N) and C-O-C groups respectively. And disappearance of α,β-unsaturated stretching of C=O group (Lambert *et al.*, 1987; Register *et al*., 2016).

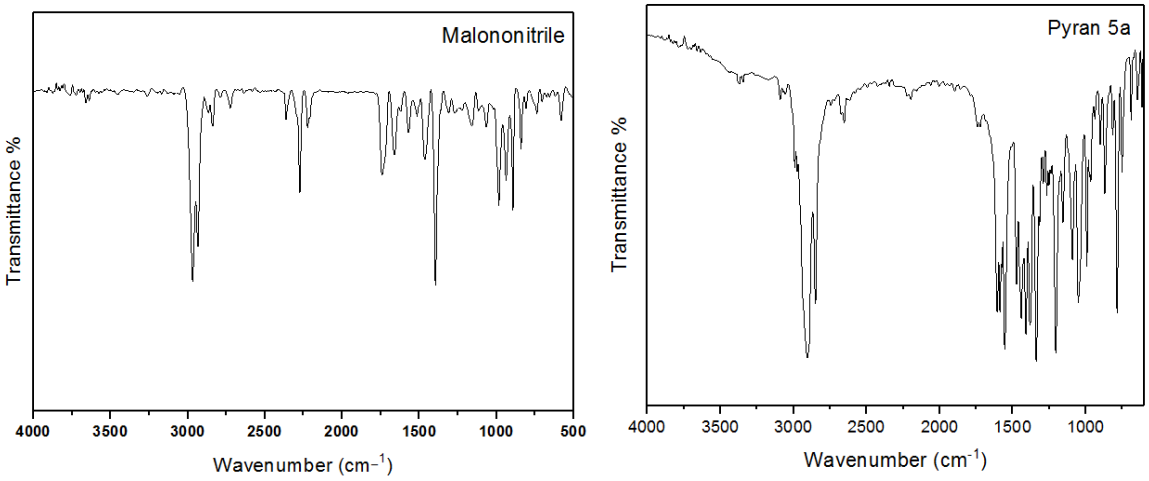


Figure 3.3: FT-IR spectrum of malononitrile and pyran **(5a)**

Table 3.6: FT-IR spectrum analysis of malononitrile

IR frequency band (cm^{-1})	**Group responsible**
2964-2928	C-H str.
2267	$C \equiv N$
1389	CH_2 (bending)

Table 3.7: FT-IR spectrum analysis of pyran **(5a)**

IR frequency band (cm^{-1})	**Group responsible**
3379, 3348	NH_2 (str./ primary amine)
2906, 2845	C-H str.
2209	$C \equiv N$
1605, 1587	N-H (bending/primary amine)
1470	Conjugated C=C (aromatic)
1345	C-N (aromatic amine)
1196	C-O-C str.

3.1.4.3 FT-IR of pyran (5b)

The starting materials for this reaction are the same as mention above in section 3.1.4.2 but in different condition (using pyridine acts as a base and solvent). The data below illustrates the functional groups that represent in the product pyran **(5b)** as shown in Figure (3.4).

Pyran **(5b)** contains extra functional groups differ from the starting materials, amine salt (RNH_3^+), and C-O-C groups which were appeared at 3440, 1150 cm^{-1} respectively. Moreover, a weak nitrile $(C \equiv N)$ group appears at 2339 cm^{-1} differ from a sharp peak of the starting material malononitrile, that means nitrile $(C \equiv N)$ group undergoes to chemical shift due to change of environmental moiety(Lambert *et al*., 1987; Register *et al.*, 2016).

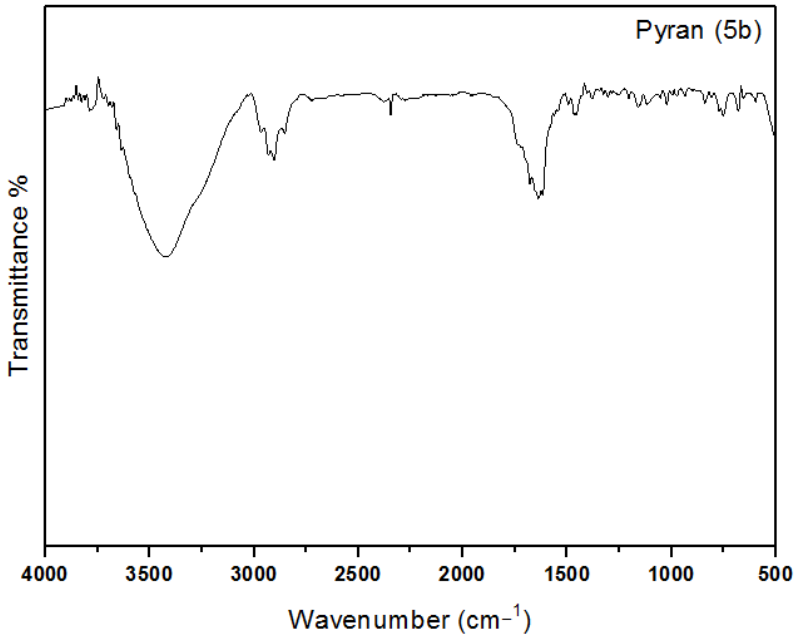


Figure 3.4: FT-IR spectrum of pyran **(5b)**

Table 3.8: FT-IR spectrum analysis of pyran **(5b)**

IR frequency band (cm^{-1})	**Group responsible**
3440	N-H (broad/amine salt)
2905, 2854	C-H str.
2339	$C \equiv N$
1634	Conjugated C=C (aromatic/ring)
1380	C-N (aromatic amine)
1150	C-O-C str.

3.1.4.4 FT-IR of pyridine (6)

The starting materials for this reaction are the same as mention above in section 3.1.4.2 but in different condition (by used ammonium acetate acts as a base). The IR spectrum (figure 3.5) for the product pyridine **(6)** exhibits a new functional groups appear in the compound which is indicated a preliminary production of pyridine **(6)** as following, primary amine stretching wavenumber appear at 3350, 3282 cm^{-1}, and a nitrile group at 2213 cm^{-1}. Furthermore, a new band observed at 1426 cm^{-1} corresponded to (C-N) in substituted pyridine ring (Lambert *et al*., 1987; Register *et al*., 2016).

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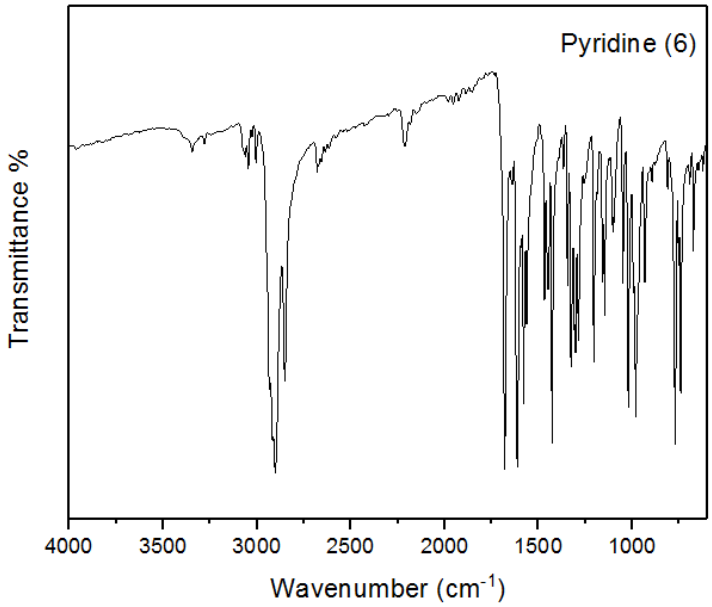

Figure 3.5: FT-IR spectrum of pyridine (6)

Table 3.9: FT-IR spectrum analysis of pyridine **(6)**

IR frequency band (cm^{-1})	**Group responsible**
3350, 3282	N-H (str. /primary amine)
2902, 2851	C-H str.
2213	$C \equiv N$
1679	Conjugated C=C (aromatic)
1577, 1561	N-H (bending/ primary amine)
1426	C-N (pyridine ring)
1328	C-N (aromatic amine)

3.1.5 ^{1}H-NMR Analysis:

3.1.5 .1 ^{1}H-NMR of pyrimidine (4):

The structure of pyrimidine (4) is confirmed by ^{1}H-NMR spectrum shown in Figure 3.7a. This spectrum exhibits a multiplet signals at 8.65-7.46 ppm, which is due to the aromatic protons of C10, C11, C12, and C13. Whereas the singlet signal due to H7 is observed at 6.53 ppm. In addition, residual protons of adamantly group are observed as a multiplet signals at 2.02-1.89 ppm, and for the proton of C18 at 1.69-1.19 ppm. A singlet signal of proton of C4 can be observed in this spectrum at 2.867 ppm.

- Pyrimidine **(4)**, ^{1}H-NMR (300 MHz, DMSO-d6) δ 8.65-7.46 (m, 4H, CH=CH-CH=CH), 6.53 (s, 2H, NH_2), 2.86 (s, 1H, CH=C), 2.02-1.89 (m, 13H, 15-23 aliphatic carbon exp. 18), 1.69-1.19 (m, 2H, 18 CH).

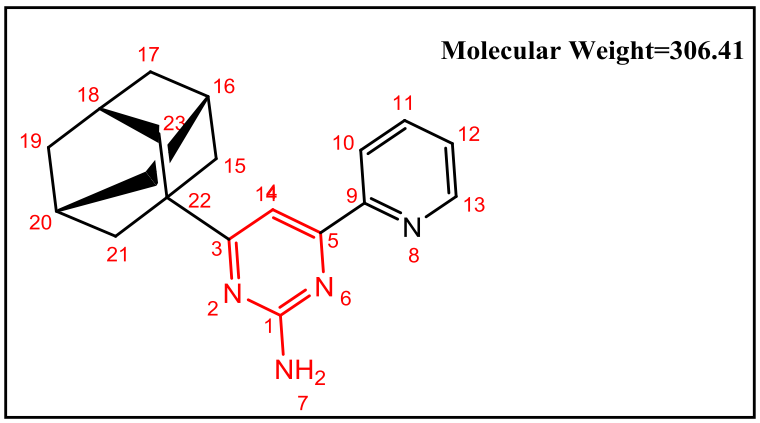

Figure 3.6: Chemical structure of pyrimidine **(4)**

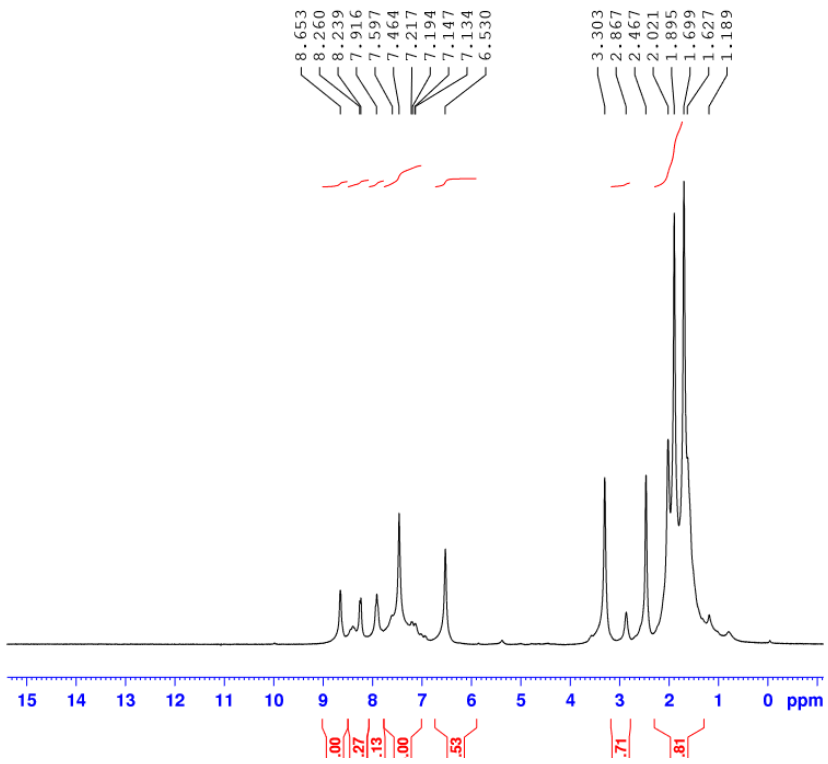

Figure 3.7 a: Pyrimidine **(4)** ^{1}H-NMR spectrum

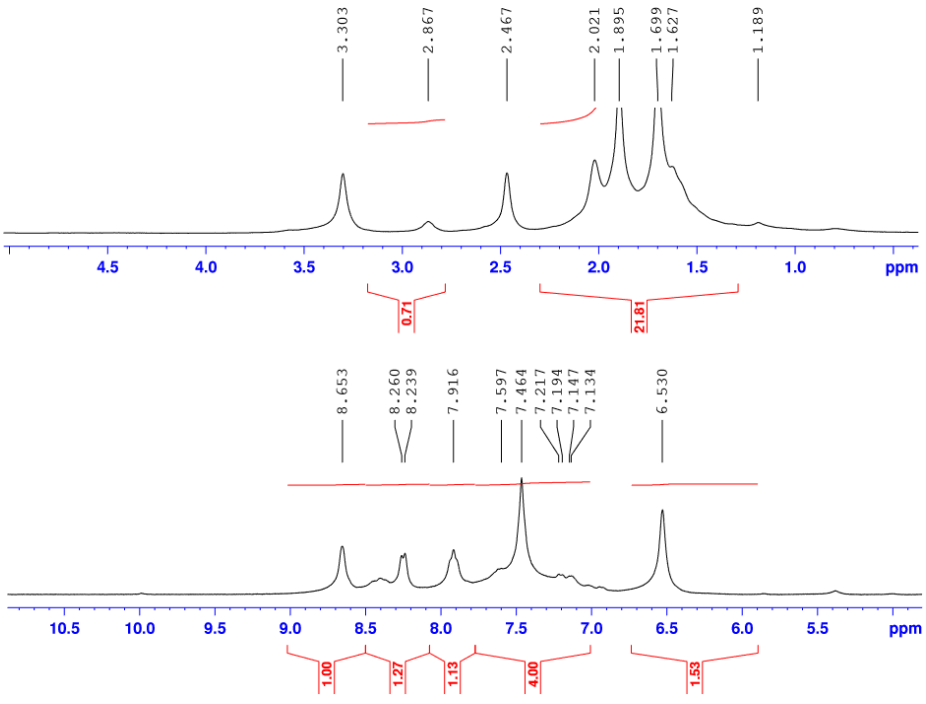

Figure 3.7 b: Pyrimidine **(4)** ^{1}H-NMR spectrum; with zooming

3.1.5 .2 ^{1}H-NMR of pyran (5a):

Figure 3.9a shows ^{1}H-NMR analysis of pyran **(5a)**. The peaks at 2.238-1.404 ppm correspond to the hydrogen in the adamantly group. The protons attached to carbon 20 were featured at 1.38-1.23 ppm. The peaks at 4.43-4.31 ppm are related to the protons of carbon 3 and 4 in the pyran ring. Moreover, the amine protons (NH_2) that attached to the pyran ring were appeared at 7.17 ppm. The aromatic protons of pyridine ring can be observed at 8.7-7.31 ppm. 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.

- Pyran **(5a)**, 1H NMR δ (ppm) (300 MHz, DMSO-d6) δ 8.7-7.31 (m, 4H, CH=CH-CH=CH), 7.17 (s, 2H, NH_2), 4.43-4.31 (m, 2H, CH-CH), 2.24-1.41 (m, 13H, 17-25 aliphatic carbon exp. 20), 1.38-1.23 (m, 2H, 20 CH).

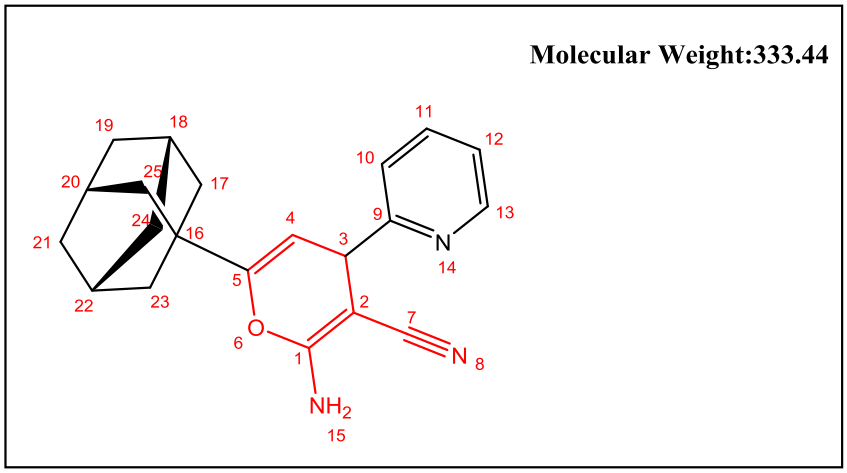

Figure 3.8: Chemical structure of pyran **(5a)**

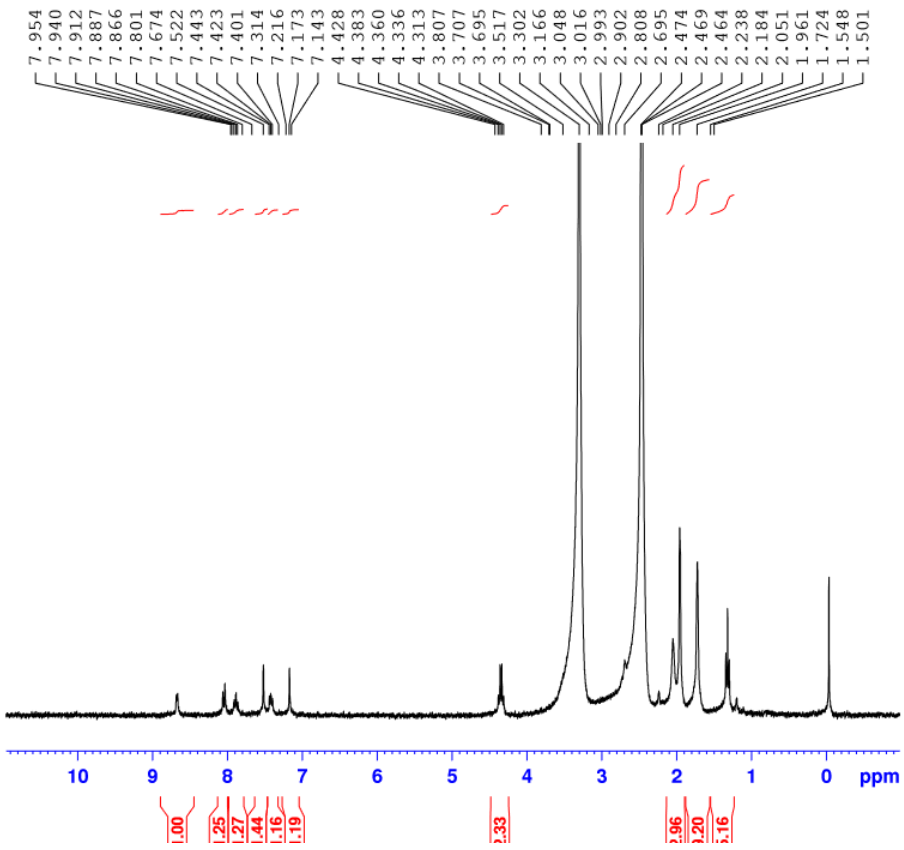

Figure 3.9 a: Pyran **(5a)** 1H-NMR specturm

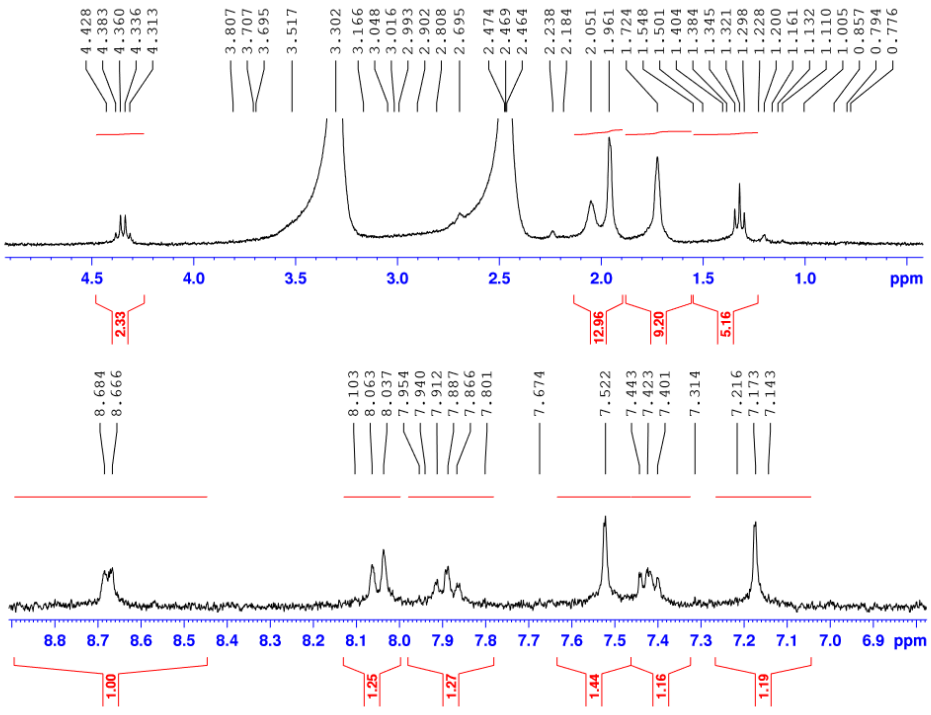

Figure 3.9 b: Pyran **(5a)** 1H-NMR spectrum; with zooming.

3.1.5 .3 ^{1}H-NMR of pyran (5b):

The ^{1}H-NMR spectrum of pyran **(5b)** is not detected due to the low concentration of compound in the salt form.

3.1.5 .4 ^{1}H-NMR of pyridine (6):

The ^{1}H-NMR spectrum (Figure 3.11) suggests a presence of aromatic protons linked to pyridine ring appeared at 8.13-7.21 ppm. The amine protons (NH_2) were prominent around 7.08 ppm. The peaks at 4.71 ppm are related to the protons of carbon 10 in the new pyridine ring. Also, noted that the solvent peaks appeared around 2.47 ppm for DMSO and 3.31 ppm for water that absorbed from DMSO. Unfortunately, the protons peaks that responses of adamantly group did not appear maybe the solvent is not suitable for use.

- Pyridine (6), ^{1}H NMR (300 MHz, DMSO-d6) δ 8.13-7.21 (m, 4H, CH=CH-CH=CH), 4.71 (s, 1H, 10, CH=C), 7.08 (s, 2H, NH_2).

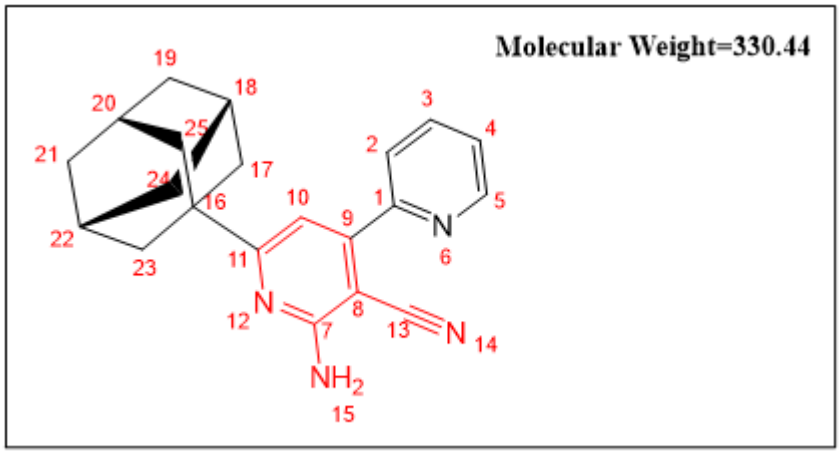

Figure 3.10: Chemical structure of pyridine **(6)**

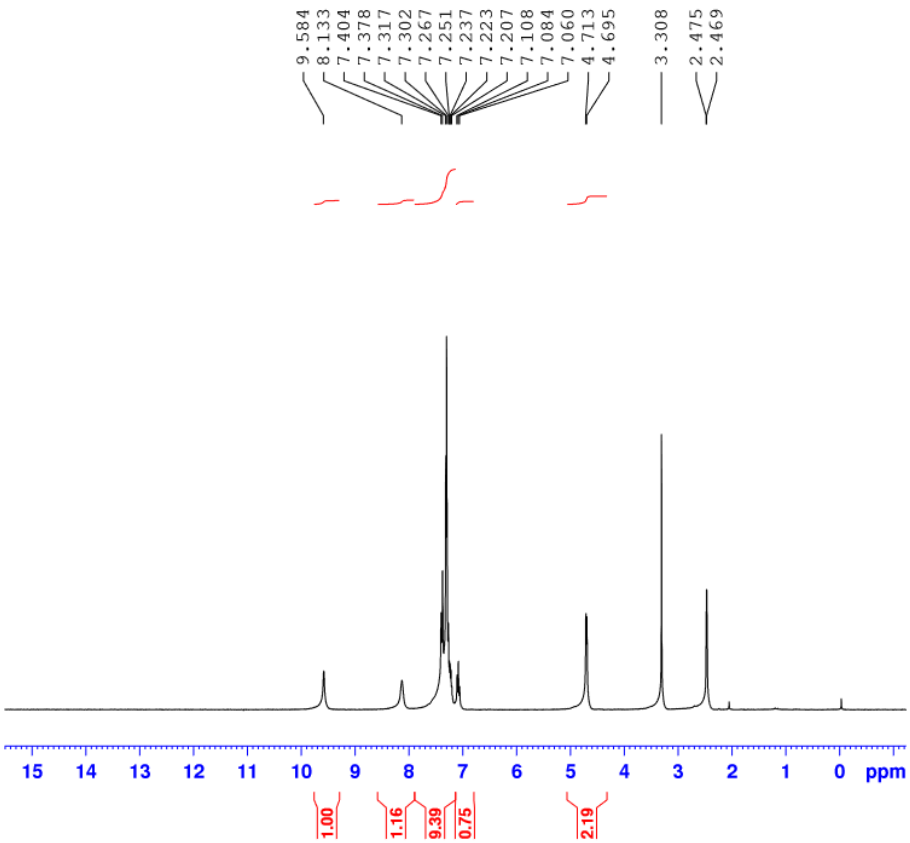

Figure 3.11: Pyridine **(6)** ^{1}H-NMR spectrum

3.2 Cytotoxic effect of the compounds, using MTT assay:

Cells were seeded on 96 well plate, at a density of 20000 cells per well and incubated for 24 hours to stick the bottom of the plate. Then the compounds were added in a dose-dependent manner (0, 4, 16, 32, 64, 128 and 256 µg/ml). On each plate 12 wells received freshly prepared DMEM media, these represented positive control cells (maximum viability). Another 12 wells were used as DMSO control and DMEM media resulted that DMSO at low concentration didn't affect the cancer cells; therefore its presence was neglected.

After 24 hours of incubation, cytotoxicity of the compounds was detected using MTT assay. After 4 hours of adding MTT, isopropanol and formic acid were added for 15-20 minutes in dark to dissolve the formazan crystals, then absorbance was measured at 570 nm using ELISA reader.

Chalcone **(3)** which was used as starting material for the synthesis heterocyclic chalcone based compounds showed significant anti-cancer activity against human breast cancer cell line MCF-7. The compound demonstrated great activity at a concentration of 32-

256 µg/mL. According to MTT assay 75%, 60%, and 40% of cells were dead at 256, 128, and 32µg/mL, respectively as shown in Figure 3.12

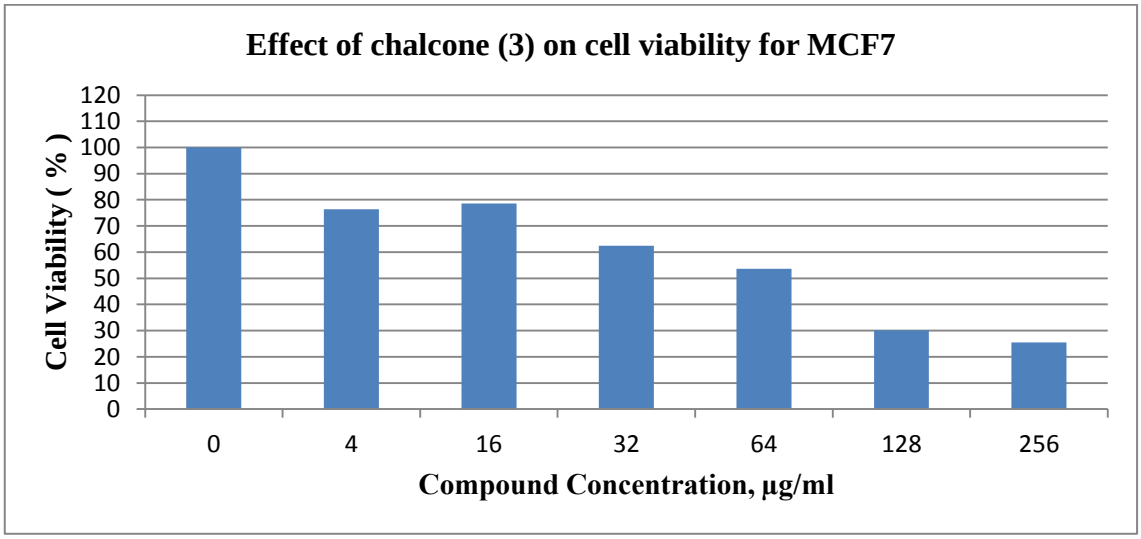

Figure 3.12: The effect of different concentration of chalcone **(3)** on cell survival of human breast adenocarcinoma cell line (MCF7) obtained by MTT colorimetric assay. Results are expressed as the percentage of surviving cells.

Pyran **(5b)**, pyrimidine **(4)**, pyridine **(6)**, and pyran **(5a)** were synthesized from chalcone **(3)**. All of them were screened for their in vitro cytotoxic against MCF-7 cell line, and compared with chalcone **(3)** at a concentration between (0.003-256 µg/mL). All the compounds were ineffective at a lower concentration; these compounds had activity only at 64-256µg/mL.

For pyrimidine **(4)**, at the highest concentrations, the compound demonstrated a significant decreasing of cell viability by 75, 40 and 30% for the concentrations of 256, 128, and 64µg/mL, respectively (Figure 3.13).

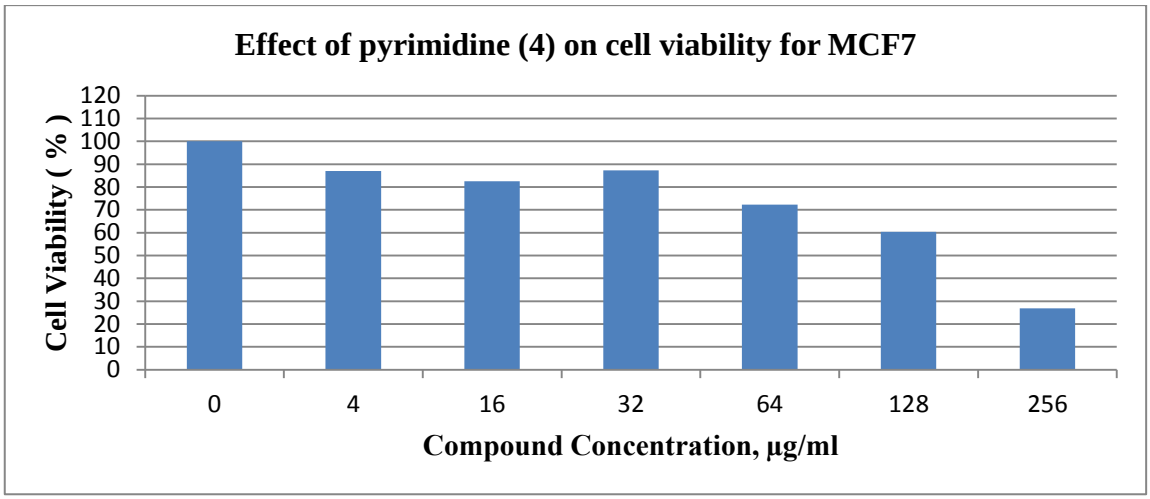

Figure 3.13: The effect of different concentration of pyrimidine **(4)** on cell survival of human breast adenocarcinoma cell line (MCF7) obtained by MTT colorimetric assay. Results are expressed as the percentage of surviving cells.

Note:

Interestingly, pyrimidine **(4)** attacks breast cancer cell lines (MCF-7) in a different way from other compounds at only two concentrations 64 and 128.µg/mL. We cannot judge that this compound may convert these cells into fatty cells (oily). So we have to do many experiments to know the mechanism of the interaction of the compound with the cells (Figure 3.14).

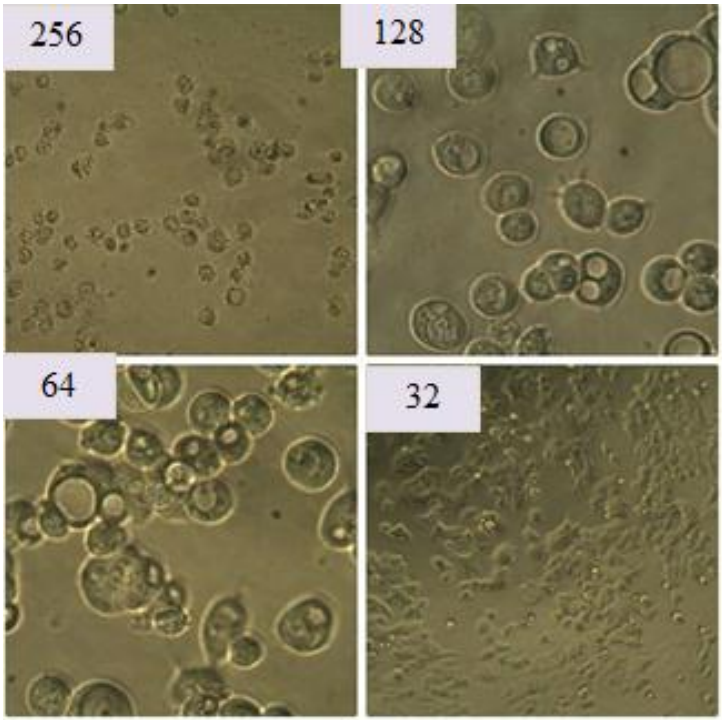

Figure 3.14: Pyrimidine (4) behaviors against breast cancer cell line (MCF-7) at 256, 128, 64, and 32 µg/mL

Pyridine **(6)** shows a greater potency at a higher concentration (128µg/mL) compared to pyrimidine **(4)**, and about 65% of the cells were dead. Pyridine **(6)** as well as pyrimidine **(4)** exhibited a decreased in %cell viability of MCF-7 by 40% at concentration 64 µg/mL and 77% at 256 µg/mL as illustrated in Figure 3.15.

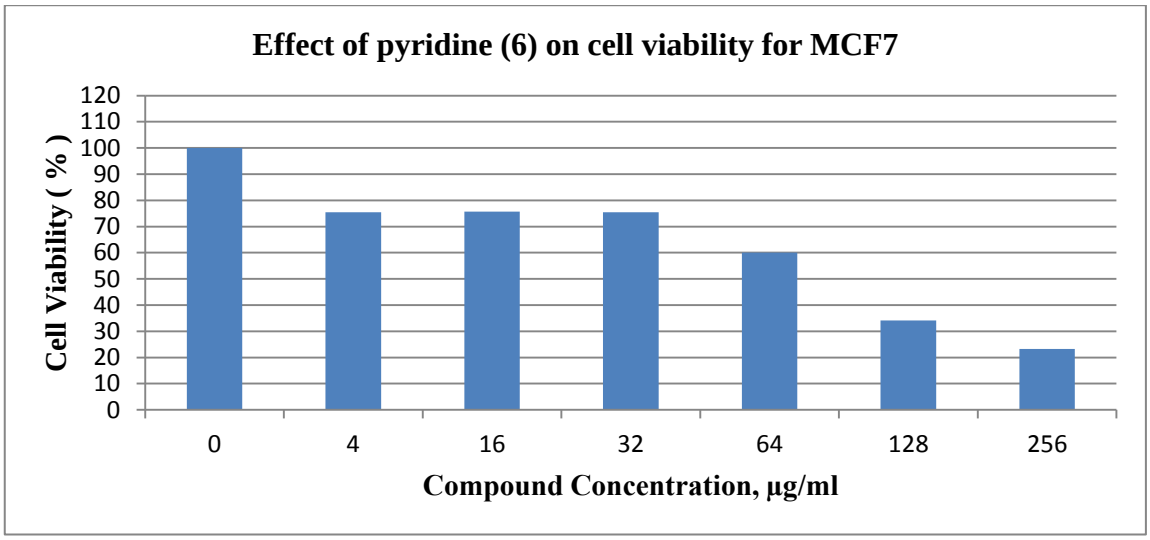

Figure 3. 15: The effect of different concentration of pyridine **(6)** on cell survival of human breast adenocarcinoma cell line (MCF7) obtained by MTT colorimetric assay. Results are expressed as the percentage of surviving cells.

The two pyran compounds **(5a, 5b)** showed diverse activities against MCF-7 viability. Pyran **(5b)** showed anti-cancer activity (40% decreased in cell viability) at 128µg/mL higher than pyran (5a) (figure 3.16). However, Pyran **(5a)** showed better activity (reduced cell viability by 40%) at concentration 64µg/mL, and decreased cell viability by 50% at 256µg/mL (Figure 3.17).

The variation in an anti-cancer activity of the two compounds pyran **(5a, 5b)** can be attributed to a number of reasons.

- Pyran **(5b)** exists in a salt form, the hydrogen atom is attached to amine group and form amine salt, and this may affect the compound reactivity.
- The concentration of the compound is too low in the salt form, so it must be increased to be more effective

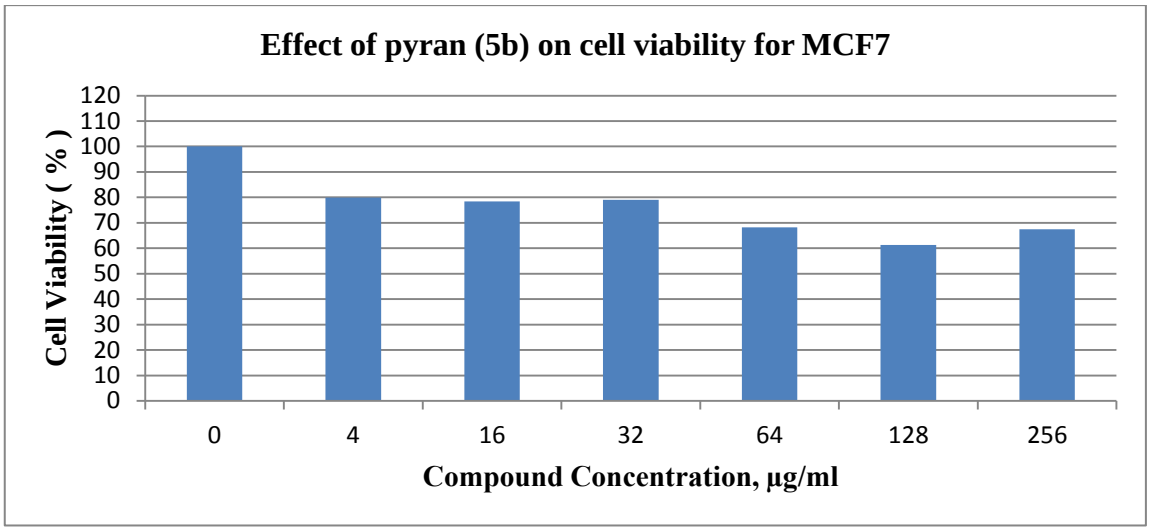

Figure 3.16: The effect of different concentration of pyran **(5b)** on cell survival of human breast adenocarcinoma cell line (MCF7) obtained by MTT colorimetric assay. Results are expressed as the percentage of surviving cells

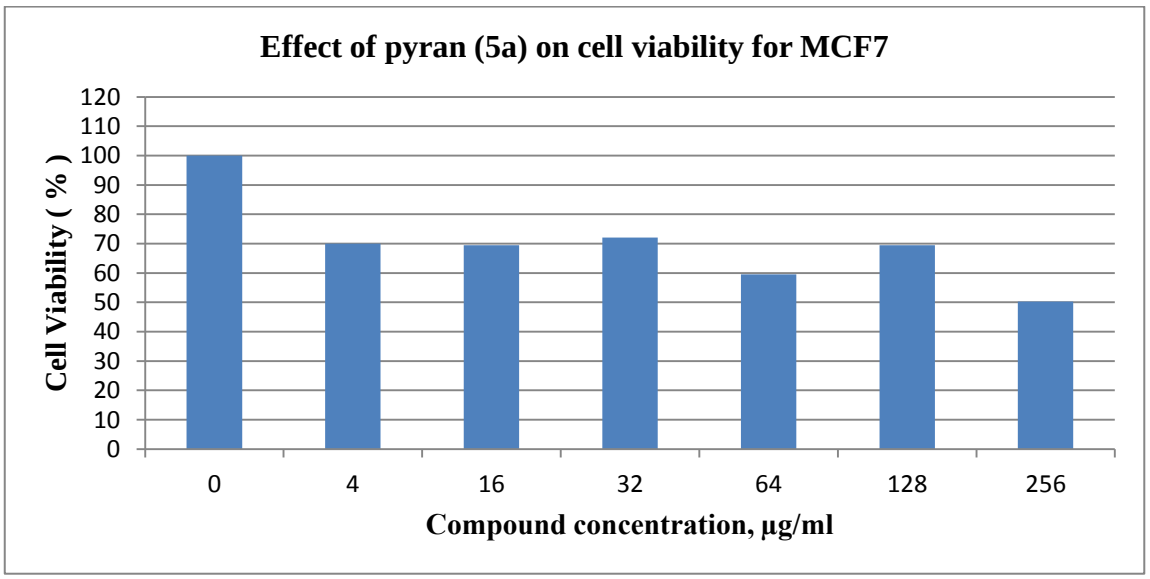

Figure 3.17: The effect of different concentration of pyran **(5a)** on cell survival of human breast adenocarcinoma cell line (MCF7) obtained by MTT colorimetric assay. Results are expressed as the percentage of surviving cells.

Chapter Four

4. Conclusions and future directions

4.1 Conclusion:

In conclusion, the present study showed that the compounds (pyrimidine **(4)**, pyran **(5a)** and pyridine **(6)** have an anti-cancer property in the in vitro MCF-7 cell line. These compounds reduced cells viability at the concentration 64-256 µg/mL. We suggest that these compounds may have potential therapeutic properties and further studies especially in vivo need to be done.

4.2 Future directions:

I. To investigate chalcone based structures using extra-analysis including Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR), and X-Ray crystallography.
II. To evaluate the adamantylated heterocyclic compounds in other *in vitro* screening tests such as apoptosis assay.
III. To test these compounds on various carcinoma cell lines.
IV. To evaluate the DNA binding potential of these newly synthesized agents and test their anti-cancer activity in vivo by utilizing animal modules.

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الملخص

السرطان– في جميع أنحاء العالم– واحد من الأمراض الرئيسية التي تدمر حياة الناس. تبذل جهود ضخمة في التعامل مع هذا المرض، ولكن لم يتحقق سوى نجاح محدود مع الاستراتيجيات العلاجية المتاحة وعادة ما تكون هذه الجهود معقدة بسبب الافتقار إلى خصوصية الأدوية المتاحة، والتكلفة العالية ومجموعة واسعة من الآثار الجانبية غير المرغوب فيها من الأدوية الموجودة. وتعد Chalcones من بين قوائم النمو المتزايد للمركبات ذات النشاط الواعد لمكافحة السرطان (Orlikova *et al.*, 2011)(Karthikeyan *et al.*, 2014). وقد كشفت العديد من الدراسات أن chalcone based structure derivatives تمنع انتشار الخلايا السرطانية، وكذلك تحفيز موت الخلايا المبرمج في مختلف أنواع الخلايا السرطانية (Calliste et al., 2001). وعلاوة على ذلك، تظهر chalcones غير المتجانسة العديد من الأنشطة الدوائية، مثل مكافحة السرطان , (Sharma *et al.*, 2013)ومضادات أكسدة (Chidan Kumar *et al.*, 2013), وعامل السمية للخلايا (Reddy *et al.*, 2008). هذه المواضيع الواعدة تحفز الباحثين في جميع أنحاء العالم لتصنيع مركبات جديدة من chalcones تحوي حلقات غير متجانسة واختبار أنشطتها البيولوجية.

وقد تم تصنيع أربعة مركبات غير متجانسة تحوي الادمنتل (**4**) pyrimidine، (**5a**) pyran، (**5b**) pyran ، و(**6**) pyridine وقد تم دراسة خصائصها بإستخدام أجهزة ^{1}HNMR, FT–IR, UV/Visومن ثم دراسة فعاليتها كمضادات لسرطان الثدي(MCF–7). تم معالجة الخلايا بتراكيز متزايدة من هذه المركبات (0, 4 ,16 32, 64, 128، 256 ميكروغرام / مل) لمدة 24 ساعة . تم استخدام صبغة (MTT) لفحص سمية المركبات. أظهرت النتائج أن (**4**) pyrimidine، (**5a**) pyran)، و(**6**) pyridine لهم آثار سمي على خلايا سرطان الثدي بتركيز 64 ميكروغرام / مل فما فوق، أما (**5a**) pyran) له تأثير سمي قليل على خلايا سرطان الثدي (MCF7).

جامعة الخليل

كلية الدراسات العليا

التصنيع والتقييم البيولوجي لمركبات حلقية غير متجانسة مستحدثة تحوي مجموعة الادمانتيل

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