SYNTHESIS, CYTOTOXICITY STUDY, AND MOLECULAR DOCKING OF NEW IMIDAZOLE-BASED CHALCONE AND PYRAZOLINE HYBRIDS

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SYNTHESIS, CYTOTOXICITY STUDY, AND MOLECULAR DOCKING OF NEW IMIDAZOLE-BASED CHALCONE AND PYRAZOLINE HYBRIDS

by

BAZRI IZWAN BIN BAKAR

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LIST OF SYMBOLS

δ	Chemical shift
J	Coupling constant
^{o}C	Degree Celsius
d	Doublet
dd	Double doublet
ΔG	Free binding energy
h	hour
K_i	Inhibition constant
MHz	Megahertz
mg	milligram
mL	milliliter
μL	Microliter
mmol	millimole
mins	minutes
m	Multiple
nm	nanometer
mol	Number of moles
ppm	Parts per million
cm^{-1}	Per centimeter
s	Singlet
IC_{50}	The half-maximal inhibitory concentration

LIST OF ABBREVIATIONS

^{1}H-NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
ADME	Absorption, Distribution, Metabolism, Excretion
ALA	Alanine
ASP	Aspartic acid
ATR-IR	Attenuated Total Reflectance Infrared
BBB	Blood-brain barrier
$CDCl_3$	Deuterated Chloroform
DMSO-d_6	Deuterated Dimethyl sulfoxide
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
GLY	Glycine
HBA	Hydrogen bond donor
HBD	Hydrogen bond acceptor
HIS	Histidine
ILE	Isoleucine
LEU	Leucine
MET	Methionine
PDB	Protein data bank
TAM	Tamoxifen
THR	Threonine
TLC	Thin layer chromatography
TPSA	Topological surface area

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SINTESIS, KAJIAN SITOTOKSIK, DAN PENDOKKAN MOLEKUL BAGI SEBATIAN HIBRID KALKON DAN PIRAZOLINA BAHARU BERASASKAN IMIDAZOL

ABSTRAK

Kanser adalah salah satu penyakit utama di seluruh dunia, dan kanser payudara merupakan penyakit yang umum di kalangan wanita. Terdapat penyelidikan berterusan untuk mencari agen kemoterapi baharu yang lebih berkesan akibat keterbatasan ubat antikanser sedia ada seperti kesan sampingan dan kesan rintangan. Sebatian yang mempunyai perancah produk semulajadi seperti kalkon dan pirazolina telah menunjukkan pelbagai aktiviti farmakologi, terutamanya sebagai agen antikanser. Sebatian hibrid yang mempunyai lebih daripada satu farmakofor aktif telah terbukti mensasarkan pelbagai aksi mekanisme. Oleh yang demikian, dalam kajian ini, sebatian hibrid imidazol-kalkon, **1-3** telah disintesis melalui tidak balas kondensasi Claisen-Schmidt. Pensiklikan sebatian ini kemudiannya membentuk terbitan imidazol-pirazolina, **1-3(i-iii)**. Spektroskopi Transformasi Fourier Inframerah (FT-IR) dan Resonan Nuklear Magnetik (NMR) telah digunakan untuk mencirikan semua sebatian ini. Pengedokkan molekul menggunakan perisian AutoDock4.2 telah dilakukan terhadap kesemua sebatian (ligan) dengan protein penerima estrogen alfa (3ERT) bagi mengkaji interaksi mereka. Kajian *in silico* seterusnya menggunakan alatan sesawang SwissADME telah dijalankan untuk menganalisa sifat fizikokimia dan farmakokinetik serta sifat keserupaan ubat-ubatan. Aktiviti ketoksikan *in vitro* ditentukan secara asai MTT terhadap sel kanser payudara, MCF-7 dengan Tamoxifen sebagai rujukan positif. Keputusan mendapati semua sebatian tidak menunjukkan aksi perencatan yang signifikan. Sebatian imidazol-pirazolina **1ii** yang mempunyai penukargantian

karbotioamida pada gelang pirazolina menunjukkan nilai IC_{50} 47.33 ± 2.45 µM, berbanding Tamoxifen dengan nilai IC_{50} 21.87 ± 2.18 µM. Tambahan pula, pengedokkan molekul bagi imidazol-pirazolina **1ii** ini menunjukkan interaksi yang paling stabil dengan nilai tenaga pengikatan -7.79 kcal/mol serta pemalar perencatan (K_i) 1.96 µM, berbanding dengan Tamoxifen (ΔG = -10.46 kcal/mol dan K_i 0.02155 µM). Walaupun aktiviti sitotoksik sebatian ini tidak sebaik yang dijangka, namun sebatian ini diramalkan mempunyai sifat fizikokimia dan farmakokinetik yang baik serta mempunyai sifat keserupaan ubat-ubatan. Kesemua sebatian ini juga didapati tidak melanggar mana-mana tapisan peraturan ubat-ubatan.

SYNTHESIS, CYTOTOXICITY STUDY, AND MOLECULAR DOCKING OF NEW IMIDAZOLE-BASED CHALCONE AND PYRAZOLINE HYBRIDS

ABSTRACT

Cancer is one of the leading diseases across the globe, and breast cancer has become the most common disease among women. There is an ongoing search for a new chemotherapeutic agent to control this condition due to the limitations of existing anticancer drugs, such as the adverse effects and drug resistance. Compounds that consist of scaffolds from natural products such as chalcone and pyrazoline have shown diverse pharmacological activity, particularly as an anticancer agent. Hybrid compounds consisting of more than one active pharmacophore have proven to target various mechanisms of action. In this study, hybrid imidazole-chalcones, **1–3** were synthesised *via* a Claisen-Schmidt condensation reaction. The cyclisation of these compounds formed imidazole-pyrazoline derivatives, **1-3 (i-iii)**. Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopies were utilised to characterise all these compounds. The molecular docking of these compounds (ligands) with estrogen receptor alpha protein (3ERT) was performed using AutoDock 4.2 software to investigate their interactions. Further *in silico* study using SwissADME web tools was performed to analyse their physicochemical and pharmacokinetic properties and drug-likeness properties. The *in vitro* cytotoxicity activity was assessed by utilising an MTT assay against the breast cancer cell line, MCF-7 with Tamoxifen as a positive control. The result emphasised that all compounds did not show significant inhibitory action. Among all compounds, imidazole-pyrazoline **1ii** with the presence of carbothioamide substituent on the pyrazoline ring showed a moderate IC_{50}

value of 47.33 ± 2.45 µM, compared to Tamoxifen with an IC_{50} value of 21.87 ± 2.18 µM. Additionally, the molecular docking of imidazole-pyrazoline **1ii** also showed the most stable interaction among all synthesised compounds with a binding energy value of -7.79 kcal/mol and an inhibition constant (K_i) of 1.96 µM, compared to Tamoxifen (ΔG = -10.46 kcal/mol and K_i of 0.02155 µM). Although their activity was not as promising, their attribute as drug-like compounds was predicted to have good physicochemical and pharmacokinetic properties with well-behaved drug-like compounds since there is no violation of any of the filtering rules of drug-likeness.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cancer is a group of diseases involving the uncontrollable multiplication of abnormal cells that can invade and destroy normal human tissue. Cancer cells can be invasive, aggressive, and metastatic, spreading throughout the body (Hanna & Shevde, 2016). In economically developed nations, cancer is the prominent cause of death while cardiovascular disease is the dominant cause of death in developing nations. (Fitzmaurice et al., 2017). Cancer cells respond differently to treatment reliant on the type of cancer and the phase of diagnosis. Breast cancer treatment shows more than 90% of survival probability, especially if treated in the early stage (World Health Organization- Breast Cancer, 2021).

Systemic therapy is used to treat and/or reduce the risk of cancer spreading and consists of chemotherapy drug which is administered orally or intravenously (metastasis). The multiplication of breast carcinoma cells was stimulated by the attachment of hormones which is estrogen and progesterone to its receptor. Hormone therapy or endocrine therapy corresponds to forms of treatments that inhibit hormones from binding to their receptors thereby reducing the body's hormone production. This helps to slow or stop the growth of tumours (Hormone Therapy, 2020). Almost all cancer cells in the body are accessible to hormone therapy (Hormone Therapy for Breast Cancer, 2013). Estrogen is a crucial hormone for the development of the breast and reproductive tissues, as well as the regulation of a woman's menstrual cycle and the maintenance of healthy bones and the heart. However, a long time of exposure to estrogen can increase the risk of breast cancer. Ovaries release estrogen into the bloodstream during the menstrual cycle. The estrogen travels through the blood and

exerts numerous impacts on the proliferation and progression of cells in its target tissues, which include the uterus, ovaries, breast, bone marrow and brain. Estrogen and estrogen receptors combine to form a unit that enters the cell nucleus. The estrogen receptors ERα and Erβ, which have variable estrogen attraction are notable prognostic indicators to identify breast cancer tumours (Pagano et al., 2020). ERα is accountable for cell proliferation and is the most prevalent cause of breast cancer (Liu et al., 2020). A huge number of drugs (ligands) have been investigated on their binding with ERα which is important in the genesis and evolution of hormone-dependent breast cancer (Bafna et al., 2020).

Selective estrogen receptor modulators (SERM) are a diverse group of nonsteroidal compounds that function as ligands for estrogen receptors (Herfindo et al., 2020). Tamoxifen is an example of a SERM that most readily used adjuvant hormonal therapy for breast cancer, which can minimize the risk of breast cancer reappearance and death (Yao et al., 2020). Tamoxifen is both antagonist and agonist of the estrogen receptor and plays a crucial role in breast cancer therapy in which it can reduce breast cancer relapse significantly. Tamoxifen is an anti-estrogen that binds to estrogen receptors and modifies its activity. Tamoxifen has cytotoxic activity against MCF-7 breast cancer cells (Huang et al., 2015). The estrogen-responsive MCF-7 cells are frequently used *in vitro* to study estrogen receptor-positive breast cancers, serving as a valuable model for understanding estrogen receptors and breast cancer biology (Vantagouli et al., 2015). Since tamoxifen also has adverse effects (Fisher et al., 2005), alternative treatment is needed and research in the area has been continuously developed. Tamoxifen's efficacy is also restricted by the possibility of resistance.

Some cancer patients respond better to chemotherapeutic medications and radiation therapy, which are used to prevent the expansion of cancer cells, rather than to surgery (Kutova et al., 2019). However, a combination of chemotherapy and radiotherapy treatments is commonly practised after a cancer patient undergoes surgery. Chemotherapeutic drugs can impede cancer cell division and finally destroy them (Mansoori et al., 2017). However, these chemotherapeutic drugs have negative side effects with medical complications, damaging the regular cells which lowers cancer patients' quality of life (Nurgali et al., 2018). Despite the advancement in chemotherapy, there are no drugs that specifically target cancer cells (Saini et al., 2012). Common chemotherapeutic drugs kill the cancer cell and also the normal cells which usually give side effects such as nausea, hypertension, dizziness, and nail or hair loss (Liu et al., 2015). Therefore, it is important to continually discover new drugs that have high selectivity toward cancer cells.

In contemporary drug discovery, molecular docking is the most recent strategy for understanding the drug-receptor correlation. It is a computational approach to determining the intermolecular interactions of the ligand-protein complex (Meng et al., 2011). Molecular docking gives the optimized conformation of a ligand's interactions when docked into a receptor's binding sites either protein or enzyme through the minimized binding energy (Rizvi et al., 2013). New molecules with potential anticancer activities can be designed by using molecular docking. New molecules with natural product scaffolds have attracted considerable interest. Small molecules without unnecessary functional groups are preferred because large-size molecules have solubility and absorption limitations (Liew et al., 2020). Many important constituents of the secondary metabolites have been used and most of them exhibited anticancer activities against various types of cancers with fewer side effects.

These elements are continuously changed to generate novel compounds with increased anticancer activity (Guo, 2017).

Current research on the therapeutic molecule for cancer therapy, particularly breast cancer has prompted us to develop a new series of compounds. In this work, secondary metabolite scaffolds with simple structures such as chalcone and pyrazoline have been of great interest. Chalcone is a class of flavonoids, a naturally occurring compound in plants. It has an open-chained molecule, with two benzene rings linked by an α,β-unsaturated carbonyl system (Gomes et al., 2017; Zhuang et al., 2017). Chalcone adopts *trans* or *cis* configurations, with the *trans* isomer being much more relatively stable and predominant compared to the *cis* isomer caused by the steric impacts of the carbonyl group and the A-ring (Figure 1.1). Research works on chalcone scaffolds have shown a great deal of interest in the synthetic, biosynthetic and biological activities perspectives (Sapra et al., 2016; Basappa et al., 2022). Chalcone hybrids have a considerable anti-cancer effect *via* binding to a variety of biological targets linked to breast cancer. Due to their minimal toxicity profile across several target organs, chalcone hybrids have shown their therapeutic potential against breast cancer cells (Alman et al., 2020).

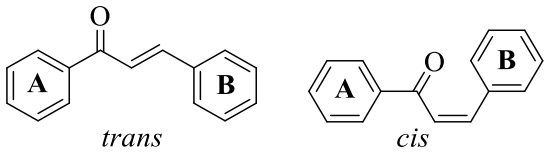


Figure 1.1 Chalcone in *trans* and *cis* configurations.

Heterocyclic compounds, in particular, the nitrogen atom rings have been of great interest due to their remarkable pharmaceutical activities, especially their anticancer properties. Molecular docking studies have shown that nitrogen-containing

heterocyclic scaffolds in the molecules exhibited better metabolic profiles with the ability to form hydrogen bonds (Irannejad, 2018). Nitrogen-based heterocyclic ring in a wide variety of drugs has a broad range of pharmacological applications for the treatment of cancer. Due to the inability of natural sources to meet the high demand for these compounds, they are synthesised by various means. Most medicinal compounds contain a lot of these nitrogen heterocycles, particularly imidazole and pyrazoline, which have become important in the creation of novel, efficient cancer therapies (Kerru et al., 2020).

Imidazole is a five-membered ring that has aromaticity due to the presence of a sextet of π-electrons and possesses three carbons, two nitrogens, and two double bonds with a molecular formula of $C_3H_4N_2$ (Siwach & Verma, 2021). It is also known as 1,3-diazole with two nitrogen atoms, one of which contains a hydrogen atom and the other of which is of the pyrrole type. Due to its amphoteric makeup, imidazole behaves like both an acid and a base and is vulnerable to both electrophilic and nucleophilic attacks (Kumar et al., 2017). The imidazole ring exhibits two equivalent tautomeric forms with the hydrogen atom located on either of the two nitrogen atoms due to its distinctive structural features and electron-rich surroundings (Figure 1.3). Apart from that, because of the lone pair of electrons, the nitrogen atom (N-3) in the ring is more reactive to the electrophilic compound (Bhatnagar et al., 2011).

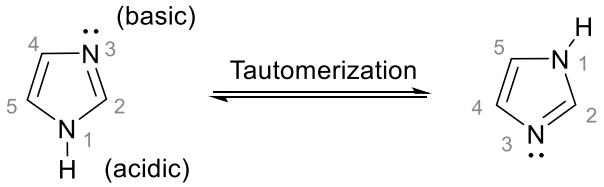


Figure 1.2 Tautomers of imidazole ring.

The chalcone compounds undergo a cyclisation reaction to form pyrazoline molecules. The five-membered ring compound pyrazoline, which has two adjacent nitrogen atoms and one endocyclic double bond, is one of the most well-known heterocyclic compounds with significant biological activities. Interestingly, pyrazoline occurs naturally as alkaloids, vitamins and pigments of plant and animal cells (Varghese et al., 2017). Due to their intriguing biological activity, pyrazolines and substituted pyrazolines have garnered considerable study (Matiadis & Sagnou, 2020). The position of the endocyclic double bond differs between the three pyrazoline kinds, 1-pyrazoline, 2-pyrazoline, and 3-pyrazoline, as shown in Figure 1.4. Among its various derivatives, the 2-pyrazoline scaffold is the most frequently studied and is considered a cyclic hydrazine moiety, used as important synthons in organic synthesis (Bhutani et al., 2015). Compounds containing a 2-pyrazoline ring have a wide range of possible pharmacological effects since many techniques for their production have been documented (Praceka et al., 2021).

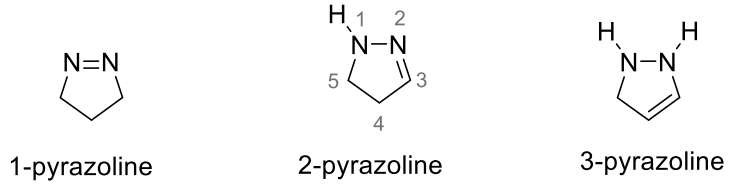


Figure 1.3 Types of pyrazolines.

1.2 Problem Statement

Current cancer treatment using the chemotherapeutic drug has challenges ranging from lack of selectivity, toxicity, resistance, and the development of secondary malignancy in cancer patients. Chemotherapeutic drugs prevent the proliferation of malignant cells, but their lack of selectivity makes them toxic to normal cells as they

cannot distinguish between cancerous and healthy cells. Resistance to the existing drugs also might lead to the development of secondary malignancy. Given these limitations, there has been a growing interest in finding more potent and selective chemotherapeutic drugs that have fewer side effects and are better tolerated by cancer patients. One area of exploration is the use of herbal medicines and natural products scaffold, such as chalcones. Chalcones, a class of compounds commonly found in plants, have demonstrated promising effects and multiple mechanisms of action as anticancer agents (Zhuang et al., 2017). In chalcones, the anticancer activity displayed by these compounds appears to be influenced by the manipulation of both aryl rings and the substitution of aryl rings with heteroaryl scaffolds as well as the substituent attached to them. Additionally, the ring-closing of these chalcones resulted in compounds with heterocyclic scaffolds, like pyrazoline, which is widely used in chemotherapy treatments to treat cancer. Apart from that, the combination of more than one scaffold in a compound creates hybrid compounds whereby these diverse pharmacophores exhibit great biological processes because different pharmacophores in a compound show different modes of action and selectivity with better efficiency (Barreiro, 2016; Rudrapal et al., 2021). In this research work, a series of hybrid imidazole-chalcone compounds and their corresponding imidazole-pyrazoline derivatives (Figure 1.4) have been synthesised and characterized.

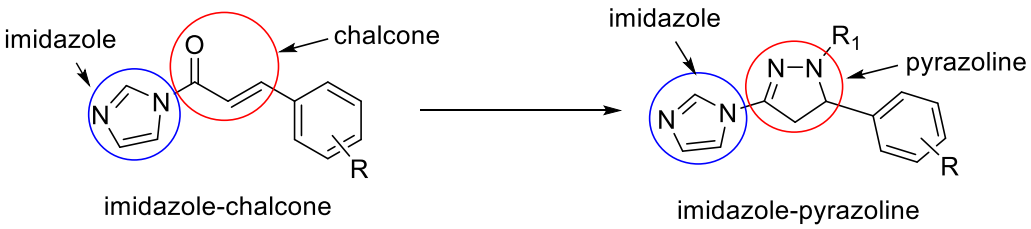


Figure 1.4 New compounds with imidazole, chalcone and pyrazoline scaffolds.

1.3 Objectives

This research work focuses on four main objectives:

1. To synthesise and characterize hybrid compounds with imidazole-chalcone and imidazole-pyrazoline scaffolds using Fourier transform infrared (FT-IR) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy.
2. To determine the binding interaction of the synthesised compounds (ligands) with the estrogen receptor alpha, 3ERT protein *via* a molecular docking technique.
3. To analyze the physicochemical and pharmacokinetic properties and the drug-likeness of the synthesised compounds using the SwissADME web tool method.
4. To determine the cytotoxicity activity of the synthesised compounds against the breast cancer cells (MCF-7).

1.4 Scope of Study

The purpose of this study is to accomplish the aforementioned research objectives. It involves the synthesis of hybrid compounds that consist of imidazole-chalcone and imidazole-pyrazoline derivatives that aim to make potential anticancer agents. Structure elucidation of all synthesised compounds was carried out using several spectroscopic methods using the equipment located at the School of Chemical Sciences, USM, Penang. In this study, all the imidazole-chalcone and imidazole-pyrazoline compounds were undergo molecular docking study and their binding affinities towards common protein targets for breast cancer were evaluated. Apart from that, their physicochemical, pharmacokinetic and drug-likeness properties were also evaluated by using the Swiss ADME web-tool. Finally, all of the compounds were sent for cytotoxicity assay against breast cancer cell line, MCF-7 that had been performed

at the School of Pharmaceutical Sciences, USM resulting in the IC_{50} value of all compounds is compared to the positive control drugs, Tamoxifen.

CHAPTER 2

LITERATURE REVIEW

2.1 Imidazole Compounds

Since the discovery of imidazole in the 1840s, research and development of imidazole-based compounds has been a quickly evolving and growing topic due to its broad prospective applications as pharmaceuticals. The use of imidazole derivatives in medicinal chemistry has made significant progress. Many imidazole-based therapeutic medications have proven to be effective in the treatment of a wide range of disorders, and new imidazole derivatives with medicinal potential are being actively researched around the world. Imidazole is a secondary metabolite scaffold of an alkaloid which is present in various natural and synthetic bioactive compounds. Research has revealed that compounds with imidazole moieties have been designed and developed for different types of cancer treatment. The first anticancer agent, Dacarbazine has triggered interest in the development of imidazole anticancer compounds (Gao et al., 2021). Several drugs with imidazole moiety are shown in Figure 2.1.

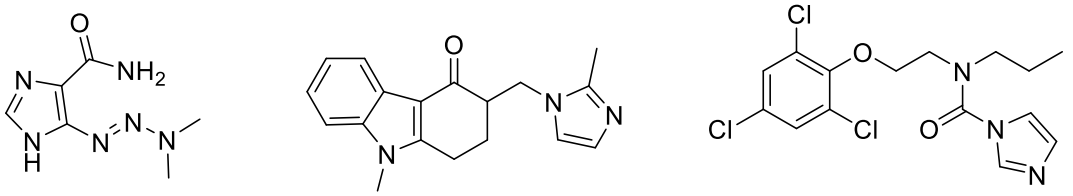


Figure 2.1 Several anticancer drugs with imidazole moiety.

2.1.1 Synthesis of imidazole compounds

Imidazole can be synthesised through various methods. One approach is through divergent cyclisation reaction (Figure 2.2). Abbiati et al. (2001) reported this method by reacting 4-alkoxycarbonyl-3-methyl-1,2-diaza-1,3-butadienes and amines in MeOH/THF at room temperature. Further reaction in EtOH/THF, NaH at room temperature produced 1-amino-carbonyl-1H-pyrazol-5(2H) (Abbiati et al., 2001).

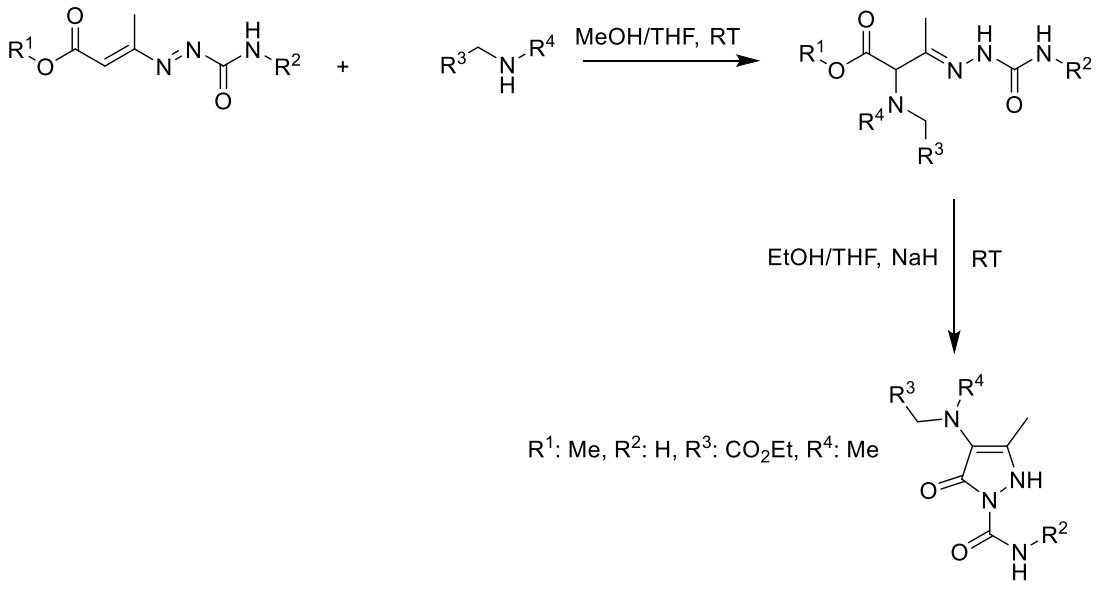


Figure 2.2 Synthesis of imidazole from amines.

Apart from that, an acid, metal, and peroxide-free synthesis of 2,4,5-trisubstituted imidazoles have been reported using an iodine/DMSO system, providing moderate to good yields under mild reaction conditions as shown in Figure 2.3 (Jayram & Jeena, 2018).

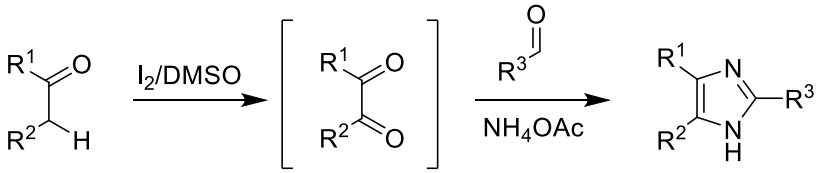


Figure 2.3 Synthesis of 2,4,5-trisubstituted imidazoles.

Besides in 2021, Zhan et al. (2021) reported the synthesis of trisubstituted imidazoles from iodine-catalysed 3+2 cycloaddition (Zhan et al., 2021). This involves the reaction between amidines with enaminones in the presence of $FeCl_3.6H_2O$ in 1,2-DCB and stirred at 110°C for 10 hours as shown in Figure 2.4.

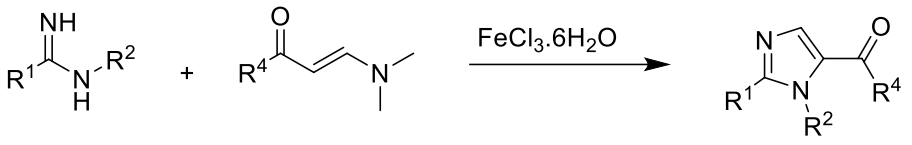
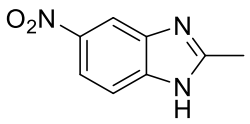


Figure 2.4 Synthesis of imidazole from amidines.

2.1.2 Anticancer activity of imidazole-containing derivatives

Several studies have investigated the anticancer activity of imidazole compounds, particularly in breast cancer. Imidazole-based heterocyclic compounds have shown potent anti-proliferative and antitumor effects in vitro and in vivo in various malignancies, including breast cancer. Ramla and colleagues synthesised benzimidazole derivatives and studied them against breast cancer cell, MCF-7 (Ramla et al., 2006). Compounds **1** and **2** showed anticancer activity against breast cancer cell line, MCF-7 with the IC_{50} value of 4.52 μg and 8.29 μg, respectively.



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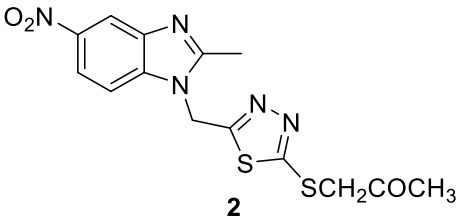
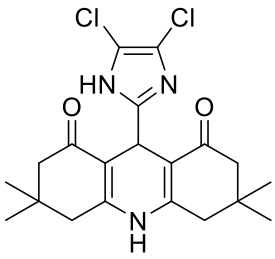


2

Jamalian and coworkers have studied the potential of imidazolyl derivatives of 1,8-acridinedione as an anticancer agent by enhancing the activity by incorporating imidazoles moieties bearing electron-withdrawing groups, as shown in compounds **3** and **4** (Jamalian et al., 2011).



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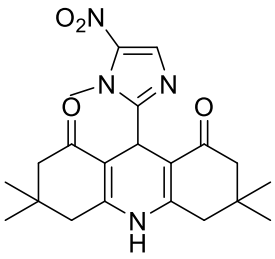
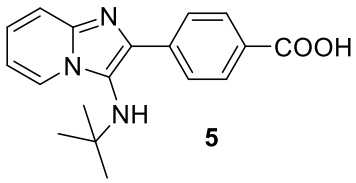


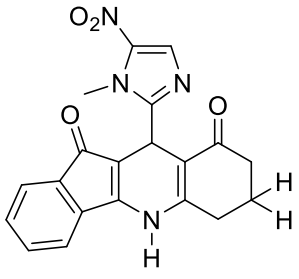
4

Baviskar et al. developed a series of N-fused imidazoles and evaluated them against a breast cancer cell line, MCF-7 and a normal cell, MCF-10A by using an MTT assay. Compound **5** caused 50% cell death at 15 μM in MCF-7, whereas 75% cell survival was noted at 100 μM in MCF-10A (Baviskar et al., 2011).



5

In 2013, Sarkarzadeh et al. synthesised a series of new imidazole-substituted indeno[1,2-b]quinoline-9,11-dione derivatives and evaluated them against the few types of cancer cell lines, including breast cancer cell, MCF-7. The MTT assay with compounds **6** and **7** showed good anticancer activity with IC_{50} value of 25 μM (Sarkarzadeh et al., 2013).



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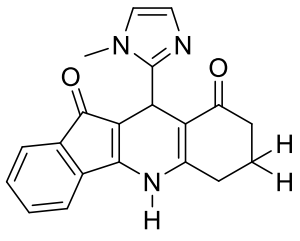


7

2.2 Chalcone Compounds

The word "chalcone" originated from the Greek word "chalcos", meaning bronze, referring to the colours of the natural chalcones (Zhuang et al., 2017). Chalcone is a secondary metabolite of flavonoids and isoflavonoids from plants that can act on a wide range of pharmacological targets (Prashar et al., 2012). Chalcone can be easily obtained, affordable, and able to target important chemical processes which contribute to cancer treatment (Mahapatra et al., 2015). Some of the examples of anticancer drugs with chalcone scaffold are shown in Figure 2.5.

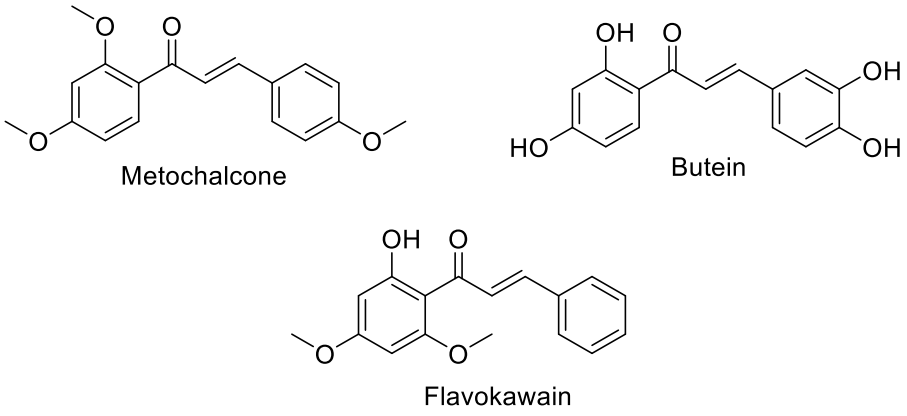


Figure 2.5 Some examples of anticancer drugs with chalcone scaffold

2.2.1 Synthesis of chalcone compounds

Due to the simple structures and various pharmacological effects, chalcones are used as intermediates in the synthesis of valuable medicinal compounds. Numerous strategies and procedures which have been created in recent years to synthesise chalcone are discussed. Generally, the most common method to synthesise chalcone is the Claisen-Schmidt condensation reaction. This reaction is named after J. G. Schmidt and Rainer Ludwig Claisen, two pioneering chemists. **Claisen-Schmidt condensation** is one of the most popular techniques to synthesise chalcones from benzaldehyde and

acetophenone. Some of the catalysts were either a strong base or an acid (Anam et al., 2017). Zainuri et al. (2017) narrated a base-catalysed aldol condensation which involved a reaction of 4-bromoacetophenone and 4-iodobenzaldehyde in methanol with a dropwise addition of NaOH (Figure 2.6). The reaction was stirred for 5 to 6 hours at room temperature. The crude product was filtered and recrystallised to give a pure compound (Zainuri et al., 2017).

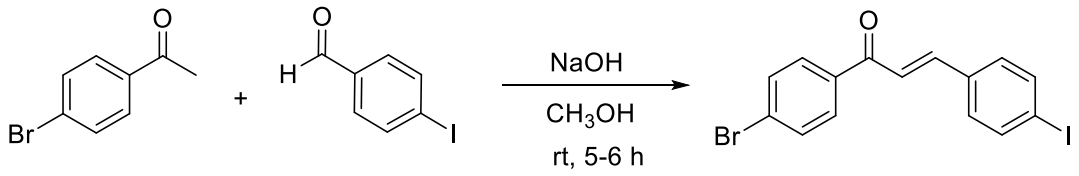


Figure 2.6 NaOH-catalysed Claisen-Schmidt condensation.

Rawat et al. (2017) described a base-catalysed Claisen-Schmidt condensation (Figure 2.7) involving the reaction of ethyl-4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylate and 2-chlorobenzaldehyde in the presence of potassium hydroxide and methanol (Rawat et al., 2017).

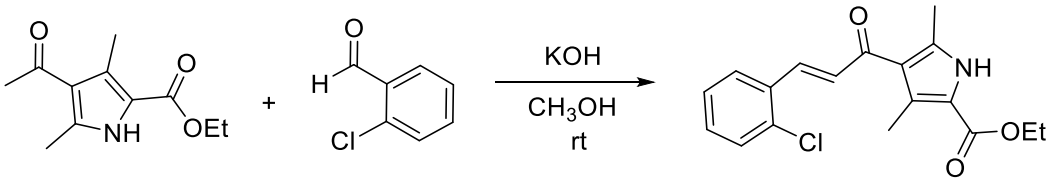


Figure 2.7 Claisen-Schmidt reaction with KOH as a catalyst

Acid-catalysed Claisen-Schmidt condensation has also been reported for the reaction of cyclohexanone with substituted aromatic aldehyde, in the presence of 0.02 molar equivalent of anhydrous $RuCl_3$ in a sealed tube at 120°C for 4-24 hours (Figure 2.8) (Iranpoor & Kazemi, 1998).

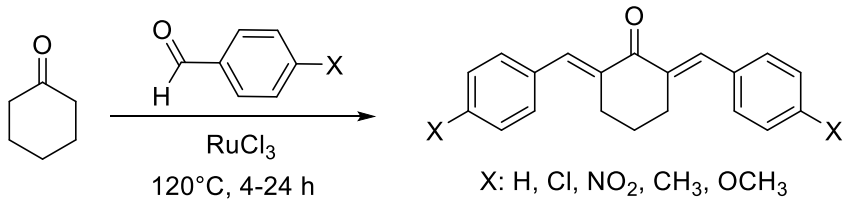


Figure 2.8 Acid-catalysed Claisen-Schmidt condensation.

Narender & Papi Reddy (2007) used BF_3-Et_2O as an acid catalyst in the reaction of substituted acetophenone and substituted benzaldehyde. The product was formed within 90 minutes (Figure 2.9). The reaction mixture was washed with water to remove the BF_3 complex and recrystallized to give a pure compound (Narender & Papi Reddy, 2007).

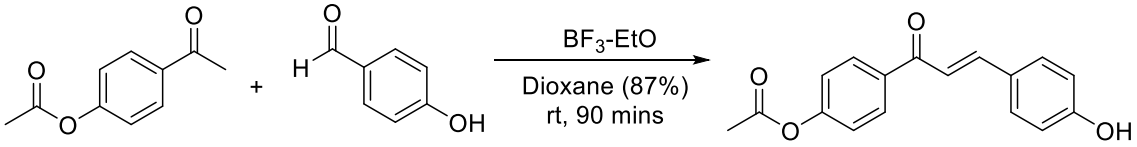


Figure 2.9 Claisen-Schmidt condensation with the presence of BF_3-Et_2O catalyst

The **Suzuki-Miyaura coupling** reaction also has been used for synthesised chalcone, for example in the presence of $(PPh_3)_4Pd$ and Cs_2CO_3 in anhydrous toluene, benzoyl chloride and phenylvinylboronic acid were coupled to create the chalcone compound (Figure 2.10) (Eddarir et al., 2003).

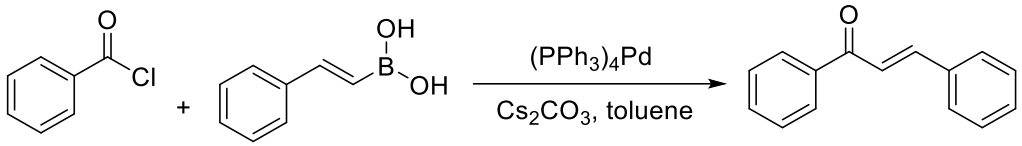


Figure 2.10 Suzuki-Miyaura Coupling Reaction

Furthermore, Guo et al. (2015) reported the synthesis of chalcone *via* **Heck coupling reaction** (Figure 2.11). The reaction is carbonylative vinylation of aryl halide, iodobenzene and 3-chloropropiophenone in DMF solution, in the presence of palladium (II) acetate as a catalyst, triphenylphosphine (PPh_3) as a ligand and also K_2CO_3 as a base, which gave the chalcone product (Guo et al., 2015).

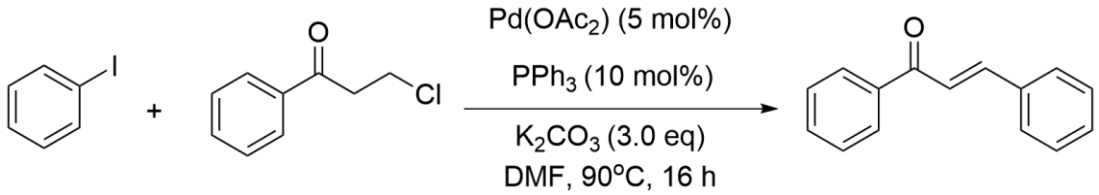


Figure 2.11 Heck coupling reaction of the chalcone compound

In 2017, Gomes et al., also synthesised chalcone compounds *via* the carbonylative Heck coupling reaction (Figure 2.12). In the presence of carbon monoxide, the carbonylative vinylation of phenyl halide with styrene was catalysed by palladium (Gomes et al., 2017). It involves the coordination of the catalyst with the phenyl halide, insertion of the vinylating agent into the metal-halide bond, and subsequent migration of the carbonyl group (C=O) from the CO ligand to the metal center. This results in the formation of a new carbon-carbon double bond (C=C) between the phenyl group and the vinyl group.

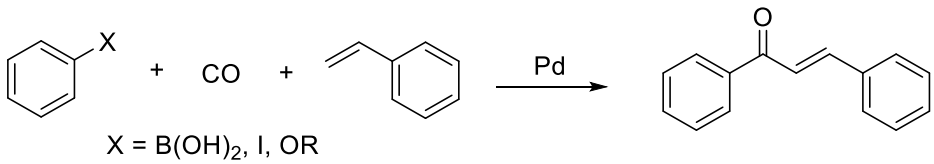
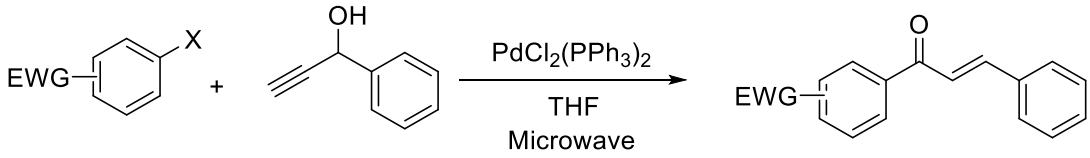


Figure 2.12 Carbonylative Heck Coupling with Pd catalyst

Another method for producing chalcone was **Sonogashira isomerisation coupling** with the use of a $PdCl_2(PPh_3)_2$ catalyst in THF in an equal amount of electron-deficient phenyl-halide and propargyl alcohol. This reaction uses microwave irradiation to form a chalcone (Figure 2.13) (Braun et al., 2006).



X = halogen; EWG = electron-withdrawing group

Figure 2.13 Sonogashira isomerization coupling

Chalcone can also be synthesised by direct **Fridel-Crafts acylation** of phenol (Figure 2.14). The reaction between 3-phenylpropenoyl chloride and 2,4-dimethoxybenzene-1,3,5-triol aluminium trichloride formed a chalcone compound (Bohm & Stuessy, 2001).

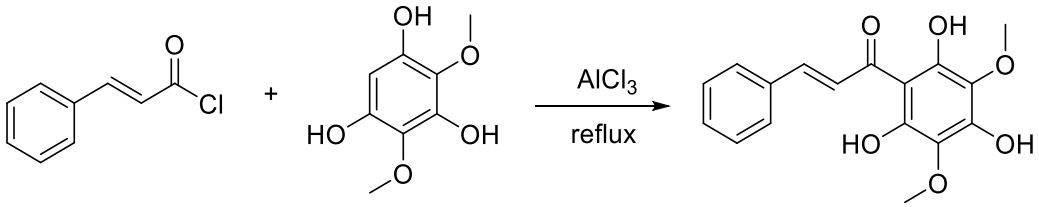
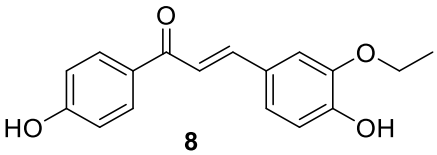


Figure 2.14 Friedel-Craft acylation of a chalcone

2.2.2 Anticancer activity of chalcone derivatives

Chalcone contains an α,β-unsaturated ketones moiety that is reactive towards several reagents. Over the years, chalcone compounds remained fascinating with a broad range of biological actions, including anticancer (Mahapatra et al., 2015; Maioral et al., 2017; Gupta et al., 2018). Devi et al. (2017) reported the Claisen-Schmidt condensation reaction of the formation of two chalcone compounds, **8** and **9**.

Using an MTT assay, their anticancer efficacy against MCF-7 cell lines was evaluated. Both chalcones showed promising anticancer activity with IC_{50} values of 2.754 μM and 2.349 μM, respectively (Devi et al., 2017).



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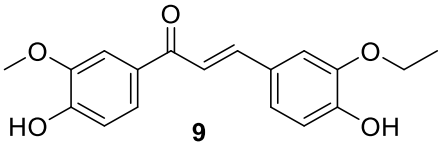
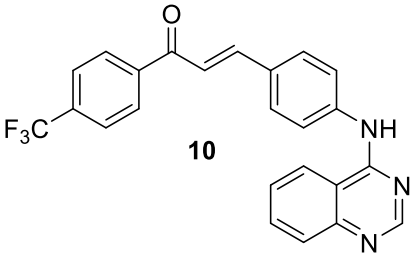


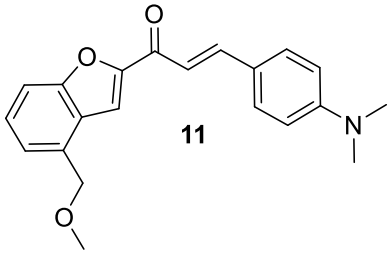
9

Madhavi and coworkers (2017) have synthesised chalcone that incorporated quinazoline derivatives *via* Claisen-Schmidt condensation in the presence of piperidine. Their anticancer activities against four types of human cancer cell lines which are A549 (alveolar), HT-29 (colorectal), MCF-7 (breast) and A375 (melanoma) were evaluated *via* MTT assay. Compound **10** showed potent inhibition towards the MCF-7 cell line with the IC_{50} of 0.17 μM, which is better than a controlled drug, Combrestatin-A4 (IC_{50} of 0.18 μM).



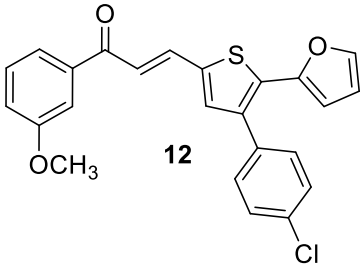
10

Coskun et al. (2017) synthesised a set of benzofuran-substituted chalcone moieties *via* the base-catalysed Claisen-Schmidt reaction (Coskun et al., 2017). The compounds were tested against lung cancer cells (A549), prostate cancer cells (PC-3) and breast cancer cells (MCF-7). Compound **11** showed a strong inhibitory effect against the breast cancer cells with an IC_{50} of 9.2 μM.



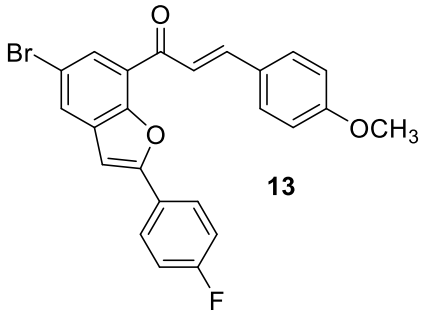
11

Duddukuri and coworkers (2018) have synthesised a series of new thiophene-chalcones. The *in vitro* cytotoxicity test showed that compound **5** exhibited moderate to robust cytotoxicity activity towards breast cancer cells (MCF-7 **)** with an IC_{50} of 22.0 ± 1.7 µM while Cisplatin (standard compound) showed an IC_{50} of 24.5 ± 0.4 µM. It was concluded that compound **12** can be a potential drug used to treat breast cancer (Duddukuri et al., 2018).



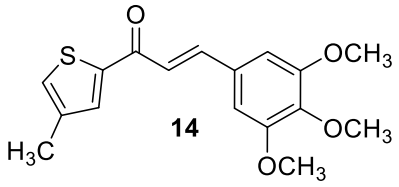
12

In the presence of NaOH, Mphahlele et al. (2018) synthesised a series of benzo(c)furan-chalcones *via* an Aldol condensation. Actinomycin D was used as a positive control to assess the antiproliferative effect of these compounds against the human breast cancer cells (MCF-7) *in vitro*. Compound **13** demonstrated significant anticancer activity with an IC_{50} value of 3.55×10^{-4} ± 0.07 µM, compared to Actinomycin D (IC_{50} = 37.82 ± 1.30 µM).



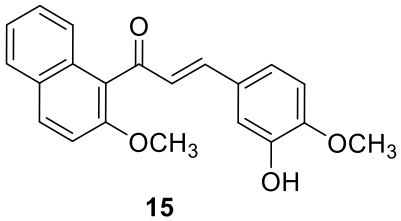
13

Pinto et al. (2019) synthesised different analogues of chalcone *via* Claisen-Schmidt condensation using microwave (MW) irradiation. Different cell lines of A375-C5 (melanoma), MCF-7 (breast), and NCI-H460 (lung) were used to evaluate the anticancer activity of these compounds *in vitro*. Compound **14** showed a beneficial growth-inhibiting effect on A375-C5, MCF-7, and NCI-H460 cell lines with IC_{50} of 3.21 ± 0.45 μM, 3.26 ± 0.11 μM and 3.02 ± 0.01 μM, respectively (Pinto et al., 2019).



14

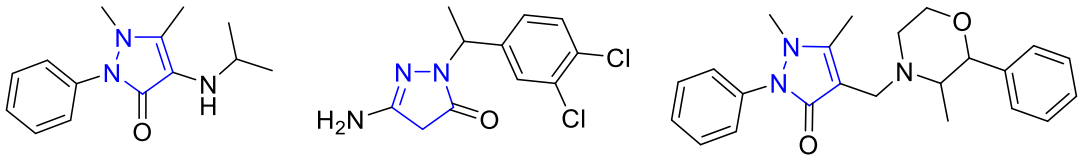
A series of naphthalene-chalcone compounds were synthesised by Wang et al. (2020) which were then tested against the MCF-7 cell line. Compound **15** displayed the best potent antiproliferative activity (IC_{50}: 1.42 ± 0.15 μM), which exhibited better activity compared to the reference drug, cisplatin (IC_{50}: 15.24 ± 1.27 μM) (Wang et al., 2020).



15

2.3 Pyrazoline Compounds

Heterocyclic rings in a variety of drugs have a broad range of pharmacological applications. Compounds containing a 5-membered heterocyclic ring, in particular, pyrazoline compounds, have become crucial to the development of new, effective cancer treatments (Desh & Karim, 2021). Pyrazoline and its derivatives are heterocycles containing nitrogen as it consists of two adjacent nitrogen atoms in the ring (Marella et al., 2013). The nitrogen in the pyrazoline ring can accept a proton whenever the lone pairs on the N atom act as Lewis base forms a coordinate bond with the proton, while it donates from one of its bonded hydrogens when an appropriate base is present to establish the interactions in this case, the N atom acts as an acid. Nitrogen compounds can bind to a wide range of enzymes and receptors in biological targets due to intermolecular forces including hydrogen bonds, dipole-dipole interactions, hydrophobic effects, van der Waals forces, and stacking interactions (Kerru et al., 2020). The unique molecular structure of pyrazoline scaffolds has been extensively studied in medicinal chemistry due to their ease of preparation and wide application in the pharmaceutical industry (Ardiansah, 2017). Some examples include Ramifenazone, Muzolimine and Morazone (Figure 2.15).



Ramifenazone Muzolimine Morazone

Figure 2.15 Pyrazoline consisting of marketed drugs

2.3.1 Synthesis of pyrazoline compounds

Chalcone scaffold is a prominent intermediate in synthesising heterocyclic compounds such as pyrazoline *via* a cyclisation reaction. Recently, the interest in synthesising new compounds that consist of pyrazoline moiety has increased significantly. Thus, various synthesis methods to form pyrazoline rings will be discussed.

2.3.1(a) One-pot reaction

Traven and Ivanov (2008) have reported the synthesis of pyrazoline compound using a one-pot reaction that involved a direct mixture of aryl aldehyde, acetophenone and phenylhydrazine in the presence of the base, NaOH. The reaction mixture was refluxed in ethanol for over 40 minutes to obtain a high yield (78%) of the desired product (Figure 2.16) (Traven & Ivanov, 2008).

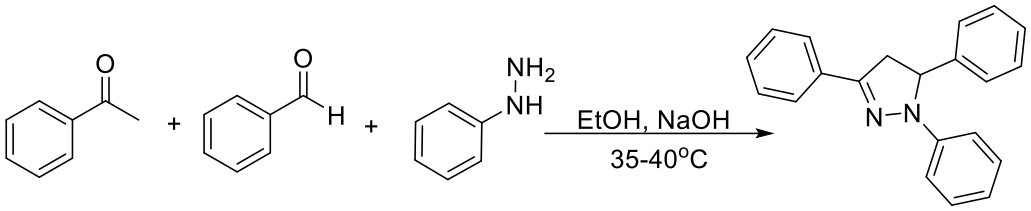


Figure 2.16 Synthesis of 1,3,5-triaryl-2-pyrazoline compound

Another one-pot reaction method was reported by Hawaiz and coworkers (2014). The reaction involved a mixture of azobenzyloxy acetophenone, substituted benzaldehyde and phenylhydrazine in alcoholic NaOH which was refluxed for 3-6 hours to afford the targeted products in good yield (77-95%) (Figure 2.17) (Hawaiz et al., 2014).

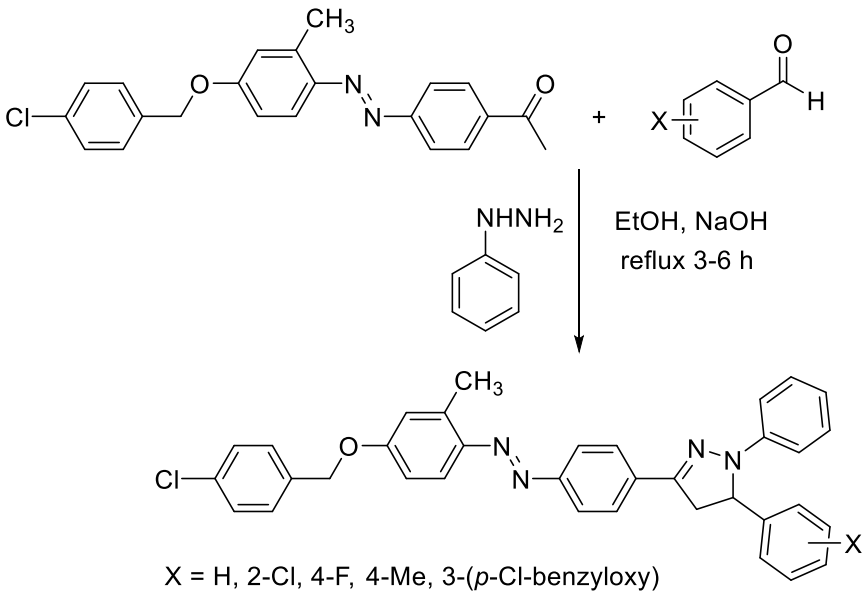


Figure 2.17 Synthesis of azo-pyrazoline compounds

2.3.1(b) Two-pot reaction

The two-pot reaction is a common synthesis method of pyrazoline. Although cyclisation can occur during the one-pot reaction, in some cases, isolation or purification of the intermediate was necessary to control the side reaction, formation of by-products as well as instability of intermediates. Such a two-pot approach can be employed. This method involves the preparation of a chalcone compound, followed by the cyclisation reaction with hydrazine hydrate derivative under suitable reaction conditions to form pyrazoline compounds. Wahyuningsih et al. (2019) reported three *N*-acetyl pyrazoline derivatives. The reaction of veraltraldehyde and acetophenone in NaOH formed the chalcone compound (Wahyuningsih et al., 2019). The cyclisation of the chalcone compound with hydrazine hydrate and glacial acetic acid gave the pyrazoline compounds in good yields, between 83-98% (Figure 2.18).