SYNTHESIS, CHARACTERISATION OF STILBENE–ARYLCINNAMIDE HYBRIDS AND CYTOTOXIC STUDIES AGAINST LUNG CANCER CELL A549

NURAIN SYAZWANI BINTI MOHD ZAKI

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by

NURAIN SYAZWANI BINTI MOHD ZAKI

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LIST OF SYMBOLS

α	Alpha
Å	Angstrom
β	Beta
^{1}H	Proton NMR
^{13}C	Carbon NMR
cm	Centimetre
cm^{-1}	Per centimetre
δ	Chemical shift
δ_H	Chemical shift proton
δ_C	Chemical shift carbon
°C	Degree Celsius
g	Gram
g mol^{-1}	Gram per mol
H	Hydrogen atom
Hz	Hertz
J	Coupling constant
mL	Millilitre
mM	Millimole/L
µM	Micromole/L
M	Mole/L
µg	Microgram
v/v	Volume per volume
m-	*Meta*
p-	*Para*

MHz	Megahertz
ppm	Part per million
mmol	Millimole
cm^3	Cubic centimetre
%	Percentage
%T	Percentage of transmittance
% yield	Percentage of yield
R	Substituent group
nm	Nanometre

LIST OF ABBREVIATIONS

A549	Human lung cancer
ARC	Animal Research Complex
$(Boc)_2O$	Di-*tert*-butyl dicarbonate
BEAS-2B	Human normal lung
Boc	*Tert*-butyloxycarbonyl group
BOP	Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
Br	Bromine
CA	Cinnamic acid
Cat.	Catalyst
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
CH_3COOH	Acetic acid
CH_3COONa	Sodium acetate
CH_3PPh_3Br	Methyltriphenylphosphonium bromide
CN	Cyanide
COSY	Homonuclear correlation spectroscopy NMR
d	Doublet
DEPT-90	Distortionless Enhancement by Polarization Transfer
DMAP	4-Dimethylaminopyridine
DMEM	Dulbecco's Modified Eagle's Medium
DMEM/F-12	1:1 mixture of DMEM with Ham's F-12
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DTBNpP	Di-*tert*-butylneopentylphosphine
DU-145	Human prostate cancer
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
Equiv.	Equivalent
Et_3N	Triethylamine
$Et_3N.HCl$	Triethylamine hydrochloride

FBS	Fetal bovine serum
FT-IR	Fourier transform infrared
H_2O	Water
HATU	Hexafluoro-phosphate azabenzo-triazole tetramethyl uronium
HCl	Hydrochloride acid
HMBC	Heteronuclear multiple-bond coherence
HOBt	Hydroxybenzotriazole
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence spectroscopy
Hünig base	*N*,*N*-diisopropylethylamine
I	Iodine
IC_{50}	50% inhibitory concentration
IPPT	Advanced medical and dental institute, USM
K_2CO_3	Potassium carbonate
m	*Meta*
MCF-7	Human breast cancer
Me	Methyl group
MMP-9	Matrix metallopeptidase-9
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
MW	Microwave
$NaHCO_3$	Sodium bicarbonate
NaOEt	Sodium ethoxide
NaOPh	Sodium phenoxide
Na_2SO_4	Sodium sulphate
NH_4HCO_3	Ammonium bicarbonate
NH_4Cl	Ammonium chloride
NHR	Secondary amine group
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
NPs	Nanoparticles
O.D.	Optical density
OH	Hydroxy
OMe	Methoxy

P(*o*-tolyl)$_3$	Tris(*o*-tolyl)phosphine
PBS	Phosphate-buffered saline
Pd	Palladium
Pd(OAc)$_2$	Palladium (II) acetate
PdNP	Palladium nanoparticles
PenStrep	Penicillin-streptomycin
PVP	Poly(*N*-vinylpirrolidone)
r.t.	Room temperature
s	Singlet
SAR	Structure activity relationship
SD	Standard deviation
SI	Selectivity index
$SOCl_2$	Thionyl chloride
STAT3	Signal transducer and activator of transcription 3
t	Triplet
TBS-O	*Tert*-butyldimethylsilyl ethers
t-BuOK	Potassium *tert*-butoxide
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TsOH	*p*-Toluenesulfonic acid
UV	Ultra-violet

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SINTESIS, PENCIRIAN HIBRID STILBENA-ARILSINAMIDA DAN KAJIAN SITOTOKSIK TERHADAP SEL KANSER PEPARU A549

ABSTRAK

Kebanyakan ubatan antikanser direkabentuk daripada sebatian hasilan semula jadi. Beberapa sebatian diperoleh daripada alam semula jadi telah dilaporkan seperti alkaloid, flavonoid, asid sinamik dan stilbena, yang mempunyai pelbagai sifat biologi khususnya sebagai agen antikanser. Dalam kajian ini, sejumlah 24 sebatian dari satu siri hibrid yang baharu telah direkabentuk dan disintesis, hasilan daripada penggandingan perancah stilbena dan arilsinamida. Kesemua hibrid stilbena-arilsinamida yang disintesis telah diperoleh dalam hasil 42-80%. Pencirian dan penentuan struktur hibrid yang terhasil telah disahkan menggunakan jelmaan fourier infra merah (FTIR), spektroskopi resonans nuklear magnetik (NMR), dan spektrometri jisim resolusi tinggi (HRMS). Kajian sitotoksiti terhadap kanser paru-paru manusia A549 dinilai melalui asai 3-(4,5-dimetil-2-thiazolil)-2,5-difeniltetrazolium bromida (MTT), menggunakan sisplatina sebagai kawalan positif. Hasil ini adalah setanding dengan sisplatina yang memperoleh nilai IC_{50} 19.9 μM selepas 72 jam rawatan. Khususnya, sebatian **66b** dan **74b** telah menunjukkan aktiviti yang tinggi terhadap sel A549 dengan nilai IC_{50} masing-masing 20.0 μM dan 19.9 μM. Analisis hubungan aktiviti struktur (SAR) mendedahkan bahawa kehadiran kumpulan isopropil pada posisi para cincin fenil stilbena (cincin A) dalam sebatian **66b** dan **74b** adalah penting untuk peroleh kesan sitotoksik yang baik ke atas sel A549. Selain itu, kumpulan 4-metil sebagai penukarganti dalam cincin fenil arilsinamida (cincin C), telah menyumbang kepada begitu banyak kematian sel kanser. Kesemua sebatian menunjukkan ketidakberkesanan melawan sel normal BEAS-2B kecuali sebatian **66b**

dan **66c**, yang menunjukkan aktiviti sederhana dengan nilai 71.9 dan 81.9 μM**.** Kesimpulannya, keputusan ini menyimpulkan bahawa hibrid stilbena-arilsinamida dapat menjadi petunjuk yang berpotensi dalam rawatan kanser dan penemuan ubat antikanser yang baharu.

SYNTHESIS, CHARACTERISATION OF STILBENE– ARYLCINNAMIDE HYBRIDS AND CYTOTOXIC STUDIES AGAINST LUNG CANCER CELL A549

ABSTRACT

Numerous anticancer drugs have been designed from natural products. Several compounds derived from nature such as alkaloid, flavonoid, cinnamic acid and stilbene, were reported to possess various biological properties primarily as an anticancer agent. In this study, a total of 24 compounds of a new hybrid series has been designed and synthesised, resulting from the coupling of stilbene and arylcinnamide scaffolds. All synthesised stilbene-arylcinnamide hybrids were obtained in 42-80% yield. Elucidation and characterisation of these hybrids have been validated using Fourier Transform Infrared (FT-IR), Nuclear Magnetic Resonance (NMR) spectroscopy and High-Resolution Mass Spectrometry (HRMS). The cytotoxicity against human lung cancer A549 cell line was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay, using cisplatin as the positive control. Particularly, compounds **66b** and **74b** have displayed potent activities on A549 cancer cells with IC_{50} value of 20.0 µM and 19.9 µM, respectively. This result is comparable to cisplatin with IC_{50} value of 19.9 µM after 72 hrs post-treatment. The structural activity relationship (SAR) analysis revealed that the presence of an isopropyl group attached to *para*-position of stilbene phenyl ring (ring A) of compounds **66b** and **74b** was crucial for a good cytotoxic effect on A549 cells. Additionally, 4-methyl group as the substituents on cinnamide phenyl ring (ring C) contributed to an innumerable cancer cell death. All compounds displayed inactivity against BEAS-2B normal cells except compound **66b** and **66c** which exhibit moderate activity with IC_{50} value of 71.9

and 81.9 μM. To conclude, these results inferred that stilbene-arylcinnamide hybrids could be served as a lead molecule for cancer treatment and new drug discovery.

CHAPTER 1

INTRODUCTION

1.1 General

At the dawn of 19th centuries, the state of the art and science of organic synthesis is vigorous as ever. Wohler's synthesis on urea in 1828 marks the beginning of the evolution in organic chemistry history (Wohler et al., 1828). Significantly, this event also marks the first instance, where an inorganic substance (ammonium cyanate) was converted into an organic compound. The history of organic chemistry and the terms "organic" and "inorganic" which have taken on slightly different meanings over time, are traced chronologically (Wentrup et al., 2022). Kolbe introduced the term "synthesis" in his publication back in 1845 to describe the process of assembling a chemical component from other substances, which was noteworthy historically.

Alizarin (**2**), a prominent red dye used for dyeing fabrics in today eras, was first synthesized by Graebe and Liebermann in 1869 (Fieser et al., 1930). A couple of years after that, an indigo dye was successfully synthesized by Baeyer in 1878 (Streb et al., 2007). These two events spurred the legendary German dye and represent ground-breaking achievements in the organic synthesis field. However, the second highly spectacular total organic synthesis in the 19th centuries after the synthesis of urea, was that of glucose by Fischer (Edström et al., 1951). The intricacy of this total synthesis is noteworthy not only for the target's complexity, which includes stereochemical elements, but also for its considerable stereochemical control. At the end of 19th centuries, glucose, with its oxygen-containing monocyclic structure (pyranose), represent the newest product in terms of target molecules among those synthesized compounds in that era (Chen et al., 1991).

At the early age of 20th centuries, Paul Gelmo, the first Austrian chemist has dicovered sulfanilamide, a synthetic sulfonamide with antibacterial properties (Miert et al., 1994). However, it was Gerhard Domagk, who discovered the sulfanilamide, also known as protonsil (**3**), which exhibit biological properties as a chemotherapeutic agent in 1932 (Domagk et al., 1970; Mohammed et al., 2022). In early 1970s, the cross-coupling carbon-carbon bond reaction catalysed by palladium (Heck reaction), was introduced by Tsutomu Mizoroki and Richard F. Heck (Bankar et al., 2020; Liu et al., 2019). An anticancer agent, pterostilbene analog **4**, was synthesized *via* Mizoroki-Heck reaction in 2019 (Laudadio et al., 2019).

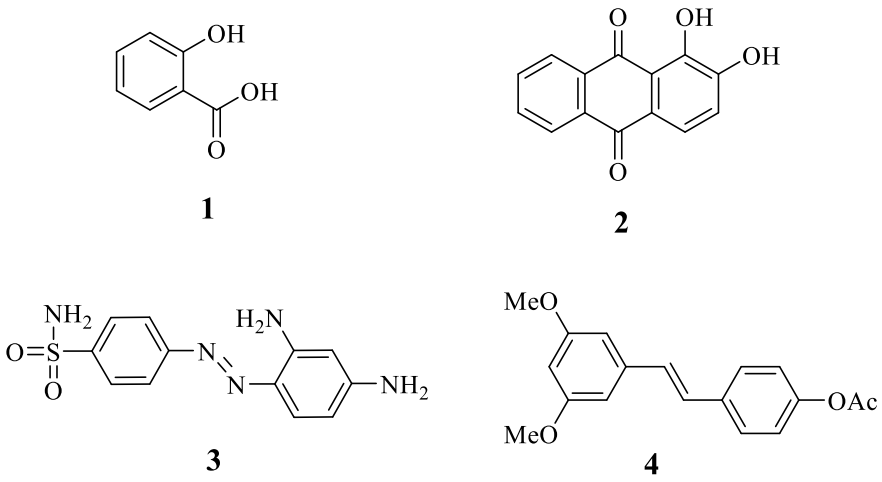


Figure 1.1 The synthesis products in 19th and 20th centuries (Fieser et al., 1930; Domagk et al., 1970; Laudadio et al., 2019).

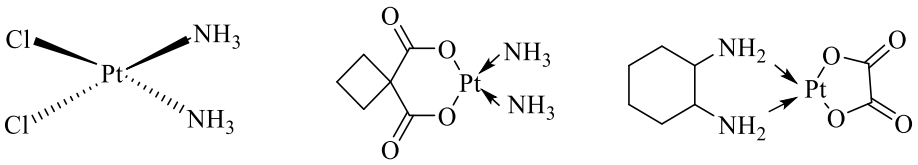
Over the past centuries, advances in synthetic chemistry have transformed the approaches in design and construction of molecules, giving access to a wider range of chemical possibilities and to molecules which possess the essential biological activity for prospective future therapeutic drugs. This fact continues to fuel the drug discovery and development with a variety of methods for new biomedical breakthroughs and applications (Yeh et al., 2007). Advances in the isolation and characterisation of novel

molecular targets from nature, that include plants and fruits, the synthetic methods and technologies are likely to facilitate future advancements in chemistry field. These findings contribute to the evolution of new reactions and new types of heterocyclic compounds (Nicolaou et al., 2013; Nicolaou et al., 2008; Erdmann et al., 2000).

Moving forward with time, the attention from synthetic chemist were focused on development drugs on chemoprevention or chemotherapy against cancer diseases. Collectively, an estimated 19.3 million cases of cancer were diagnosed in 2020 globally (Sung et al., 2021). Cancer implies to a group of diseases which have the potential to surpass all other causes of death as one of the main killers around the world, despite causing abnormal cell growth in human bodies (Banik et al., 2020). Although the educational initiatives to lower the risk of cancer disease, which is currently leading to death, are showing success, the identified risk factors for many cancers are less pronounced, making prevention more challenging. Therefore, cancer therapies played a major role in managing cancer diseases, either directly or as an adjuvant in surgeries (Jones et al., 2014).

Chemotherapy is the treatment of cancer with drugs (known as anti-cancer drugs) that can kill cancer cells. The term "chemotherapy" typically refers to cytotoxic drugs which affect or inhibit rapidly dividing cells in current usage (Banik et al., 2020). Most domestical chemotherapeutic drugs do not specifically target cancer cells, but focus on inhibiting the cells from dividing faster (Cheng et al., 2021). The common chemotherapeutic agents used for the cancer treatment are cisplatin (**5**), carboplatin (**6**), and oxaliplatin (**7**) (Zhang et al., 2022). These drugs either kill or modify the growth of cancer cells to exert their anticancer effects (Bellamy et al., 1996; Olzewski et al., 2010; Johnstone et al., 2016). However, the urgent need for new anticancer drugs

have drawn attention among chemists due to the current conventional drug delivery system's limitations, which are lack of specificity, high toxicity, and induction of drug resistance (Din et al., 2017).



5 **6** **7**

Figure 1.2 Chemotherapy drugs: cisplatin (**5**), carboplatin (**6**), and oxaliplatin (**7**).

Resveratrol, is one of the components which is widely known for their anticancer properties (Aluyen et al., 2012). Over the past decades, stilbene has been reported with their effectiveness in cancer treatment against various type of cancer cells especially lung and cancer cells. Besides stilbene, cinnamic acid derivatives is also known for their biological properties as an antimicrobial, anticancer, and antioxidant agent (Ruwizhi et al., 2020). Thus, apart from discovering new compounds through synthetic techniques, we synthesise a new hybrid series by combining stilbene and arylcinnamide scaffolds and investigate their cytotoxicity against A549 lung cancer cells and BEAS-2B normal lung cells.

1.2 Problem statement

Cancer is a chronic disease responsible for an estimated 10 million cancer patient deaths in 2020 (Sung et al., 2021). However, the conventional anticancer drugs employed for cancer treatment possess several unique problems, including adverse side effects, poor selectivity and high toxicity (Din et al., 2017). Thus, there is a clear

and urgent need for new anticancer drugs with high efficacy for the treatment of cancer disease.

Numerous studies have displayed that resveratrol derivatives have a diverse biological significance effect in the medicinal world, specifically as an anticancer agent. However, resveratrol therapeutic advantages haven't been thoroughly explicitly explored, nor have their beneficial benefits in the medicinal world. As resveratrol and cinnamic acid were reported with their functionalities and capabilities as anticancer drugs, this research focused on generating new hybrid compounds by combining stilbene and arylcinnamide moieties. The resulting products were then evaluated for their cytotoxic activities focusing on the lung cancer cells (A549). Additionally, we modified the structure of stilbene and arylcinnamide with various substituents on ring A and ring C to obtain analogs of active compounds against A549 lung cancer cells.

1.3 Objectives

The main objectives of this research are :

1. To synthesise a new series of stilbene-arylcinnamide hybrids with different amide moieties *via* the acylation method.
2. To characterise a series of stilbene-arylcinnamide hybrids using various spectroscopic techniques.
3. To evaluate the cytotoxicity of the synthesised compounds on A549 lung cancer cells.

1.4 Scope of the study

This study presents the synthesis work of new stilbene-arylcinnamide scaffolds by combining the stilbene and cinnamic acid moieties. The characterisation work on the synthesised compound has been performed using NMR, FT-IR, and HRMS analysis. These instruments are located at the School of Chemical Sciences, USM, while the cytotoxic activities work has been tested at Animal Research Complex (ARC) located at IPPT, USM.

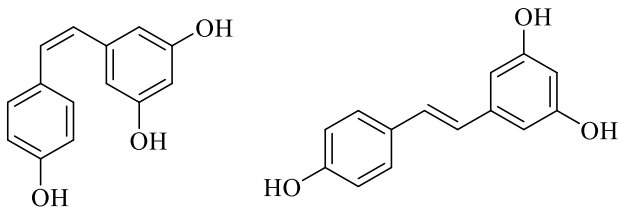
In this thesis, Chapter 1 briefs on the history and development of Organic Chemistry over the last decades. Chapter 2 demonstrates the literature sources that reported findings associated with stilbene and cinnamic acid with their cytotoxicity on lung cancer. Chapter 3 revealed the experimental procedure and research details while Chapter 4 discussed the elucidation products obtained with their cytotoxicity result. SAR analysis has been performed to provide further understanding related to the structural features that enhanced the target compound's cytotoxic activities. Eventually, Chapter 5 concludes the research outcome and the research's significance in the future.

CHAPTER 2

LITERATURE REVIEW

2.1 Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) was discovered in 1940, with two phenol rings linked by an ethylene bridge. It was first isolated from the roots of *Veratrum grandiflorum*, also known as white hellebore (Akinwumi et al., 2018; Khawand et al., 2018). Resveratrol has one hydroxyl (−OH) group at C-4' with two other hydroxyls (−OH) groups at C-3 and C-5 on another ring (Chan et al., 2019). Due to the presence of a central ethylene group, the resveratrol structure contains two possible geometrical isomers, i.e., *cis*- (**8**) and *trans*- (**9**) (Figure 2.1). However, resveratrol from natural resources is usually found in *trans*-form (*E*-configuration). The resveratrol *trans*-isomer can transform into a less stable *cis*-form after long UV radiation exposure (Chan et al., 2019). Additionally, resveratrol is present in many food sources, including cranberries, blueberries, pistachios, and peanuts. The skins and seeds of grapes are the most notable dietary sources of resveratrol, with red wine being the most popular form (Baur et al., 2006; Fernandez-Mar et al., 2011).



8 **9**

Figure 2.1 *cis*- and *trans*-isomer of resveratrol.

Resveratrol has a long history of usage in Asian traditional medicine and red wine to treat cardiovascular disorders (Bommagani et al., 2018; De Filippis et al., 2019). In 1963, resveratrol was isolated from *Polygonum cuspidatum* roots and utilized as antiplatelet and anti-inflammatory agents in traditional Chinese and Japanese medicine (Nawaz et al., 2017). As epidemiological studies revealed an inverse relationship between red wine consumption and the prevalence of cardiovascular diseases, interest in resveratrol's beneficial properties arose in the western world (Langcake et al., 1977). In the early 1990s, most research centred on stilbenes and phenolic compounds in general, and an incident called the “French paradox” was reported (Kopp et al., 1998). This incidence corresponds to the case where the rate of heart infarction among the people in France declined to about 40% lower than in the rest of Europe and the USA, despite having a historically high-saturated-fat diet (Sun et al., 2002). In 1992, resveratrol revealed its functionalities to cause the cardioprotective effect in red wine consumption. Since then, multiple studies have shown that resveratrol can prevent or slow the development of several diseases, including defense against ischemic-reperfusion injury, protect and maintain intact endothelium, promote vasorelaxation, etc. (Pace-asciak et al, 1995; Hao et al, 2004).

As time passes, numerous studies have validated resveratrol's diverse pharmacological effects, primarily brought on by the abundance and variety of its molecular targets (Tsai et al., 2017). For instance, it can act as an antioxidant (Xia et al., 2017), anti-inflammatory (Meng et al., 2021), anti-cancer (De Filippis, 2017), anti-aging (Kasiotis et al., 2013), anti-diabetic (Ahmad & Gani, 2021), anti-osteoporosis, and anti-obesity agents (Carter et al., 2014). However, the adverse pharmacodynamics/pharmacokinetics profile of resveratrol such as low aqueous solubility, poor bioavailability, chemical instability and rapid clearance from the

systemic circulation, has become a significant issue (Bommagani et al., 2018; Hassan et al., 2018; Fu et al., 2019). Despite these limitations, resveratrol still received much attention as a prominent and multitarget anticancer agent. Its potential use and capabilities were displayed in chemoprevention and chemotherapy of various tumors, including breast, colon, lung, ovarian, pancreatic, and prostate. Its capacity is mediated by affecting cell growth, apoptosis, angiogenesis, and metastasis process (De Filippis et al., 2019; Horgan et al., 2019). Dimethoxystilbene **10** noted with anticancer properties against ovarian (Pei et al., 2017), lung (Yang et al., 2017), breast (Nikhil et al., 2014), and prostate (Kumar et al., 2019). Trimethoxystilbene derivatives **11** and **12** were reported as the tubulin-polymerization inhibitor, specifically inducing multipolar spindles coupled with mitotic arrest causing cancer cell death (Traversi et al., 2019).

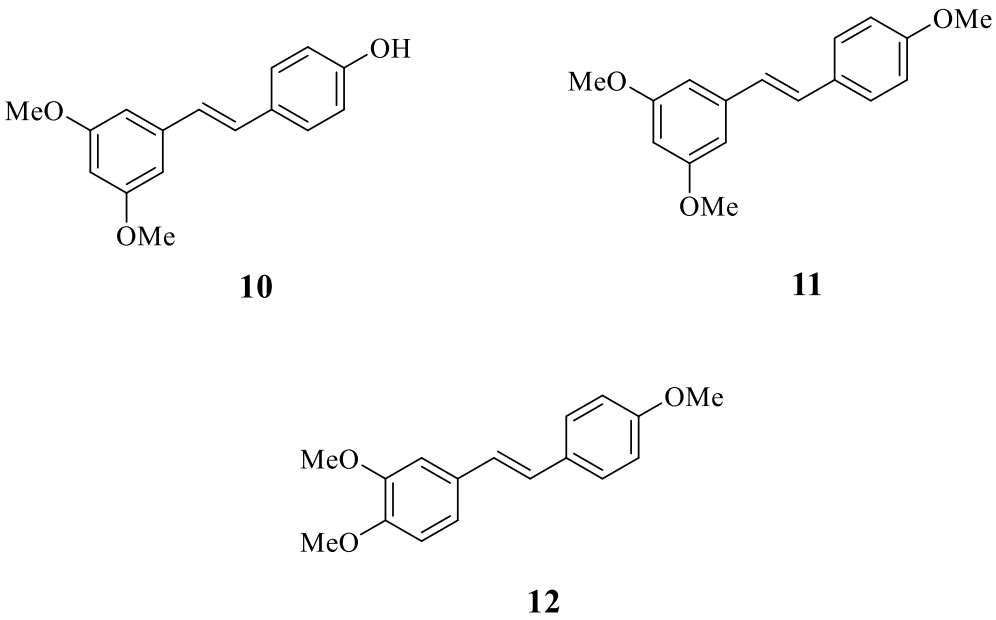
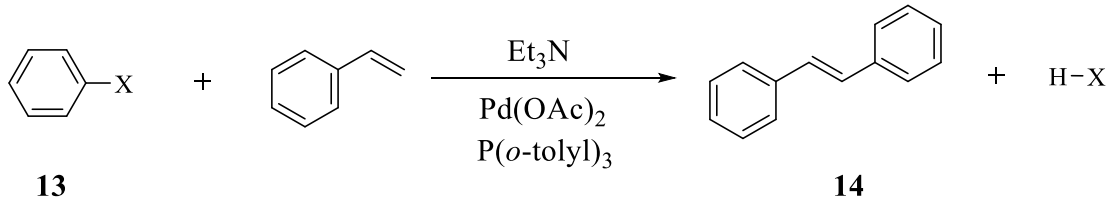


Figure 2.2 Resveratrol derivatives as anticancer agents.

2.2 Heck reaction in the synthesis of stilbene

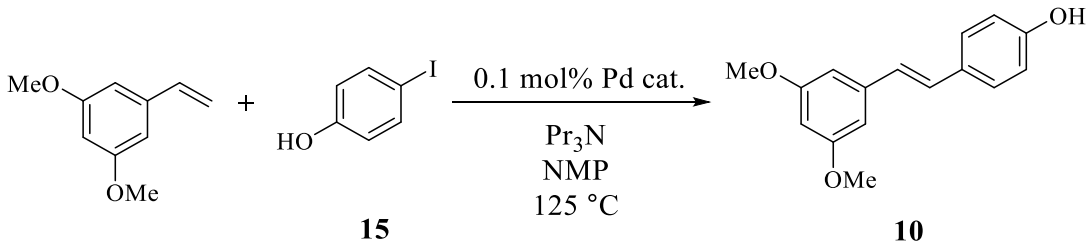
The formation of carbon-carbon double bonds is a crucial step in the production of *(Z)*- and *(E)*-stilbenes (Pérez-Lozano et al., 2021). Over the past few decades, synthetic pathways to stilbene derivatives have been developed and produced, including the Negishi-Stille, Perkin, and Aldol-type condensations (Krepski et al., 1978; Ephritikhine et al., 1998; Gao et al., 2006; Kabir et al., 2007). Heck and Suzuki's reactions were particularly well-known for their great synthetic adaptability and efficiency. However, it appears most promising to use Mizoroki-Heck reactions to synthesize *(E)*-configurated stilbenes **14** (Albert et al., 2011; Xu et al., 2013). The Mizoroki-Heck reaction implies the cross-coupling reaction of an aryl or vinyl halide **13** with an activated olefin (Mori et al., 1973; Wang et al., 2017). The reaction is catalyzed by either Pd(0) or Pd(II) complexes in solution, followed by the addition of a coordinating ligand to facilitate the reaction system (Yang & Zhou, 2012).



Scheme 2.1 General scheme for Mizoroki-Heck reaction.

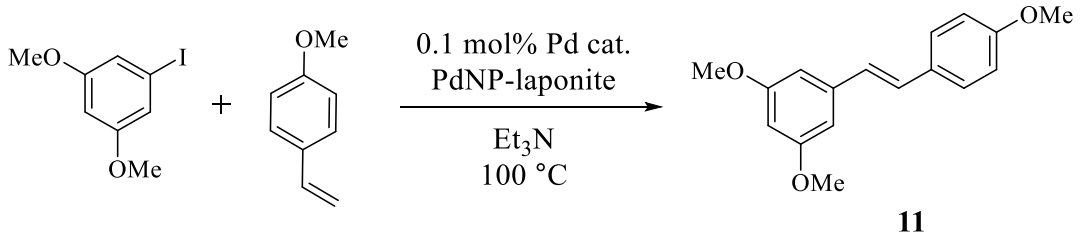
The simple and affordable synthesis of substituted stilbenes, mostly of *(E)* configuration, is confirmed using triethanolamine, which functions simultaneously as a base, ligand, and solvent (Li et al., 2006). In 2019, Laudadio et al. synthesized pterostilbene (**10**) using different Pd catalysts immobilized onto heterogeneous supports in the reaction system (Laudadio et al., 2019) (Scheme 2.2). The palladium

complex employed in the Mizoroki-Heck reaction was crucial to obtain high selectivity of *trans*-form stilbene. Martínez et al. synthesized methoxy-substituted resveratrol **11** by using palladium nanoparticles (PdNP) immobilized on laponite (a synthetic clay) as the catalyst (Martinez et al., 2016) (Scheme 2.3).



15 **10**

Scheme 2.2 Pterostilbene synthesized by Laudadio et al. in 2019.



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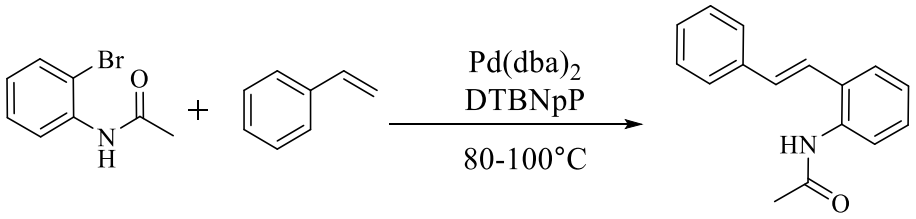
Scheme 2.3 Resveratrol synthesized by Martinez et al. in 2016.

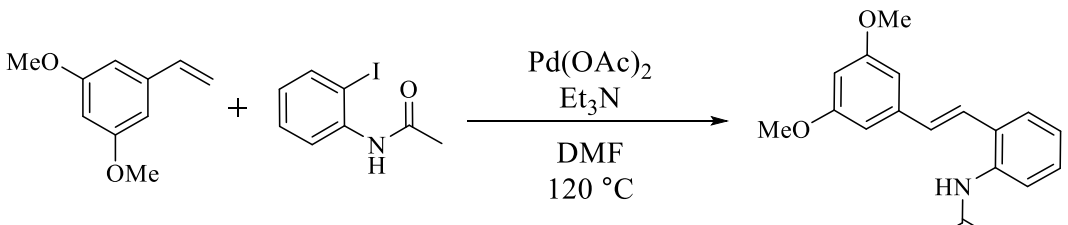
Modification on substituted stilbene with acetamide moiety has been reported to enhance the biological properties of the parent compound. For example, Lauer et al. reported the synthesis of *N*-phenyl substituted stilbene **18** from the coupling reaction of 2-bromoacetinilide **16** and styrene (**17**) with the use of di-*tert*-butylneopentylphosphine (DTBNpP) as the catalyst as shown in Scheme 2.4 (Lauer et al., 2014). These conditions required elevated temperatures (80−100 °C) for the coupling to happen whenever DTBNpP was utilized as a ligand for palladium to obtain a high-yield product (Hill et al., 2008). In 2021, Azmi and his group synthesized 3,5-dimethoxystilbene **21** *via* Mizoroki-Heck reaction with a good yield. The Mizoroki-Heck reaction7 was conducted by heating at 120 °C to the corresponding styrene **19**

and *N*-(2-iodophenyl)acetamide (**20**) with $Pd(OAc)_2$ as the catalyst in dry DMF (Azmi et al., 2021). The cross-coupling Heck reaction under an aprotic solvent such as DMF provides the best product conversion up to 98% (Lauer et al., 2014). In the Heck-reaction catalytic pathway, the solvent played a major role in easily dissociating the triflate group from the oxidative addition complex and forming a cationic complex (Olofsson et al., 1998).



16 **17** **18**

Scheme 2.4 Heck reaction with 2-bromoacetinilide using DTBNpP as the catalyst.

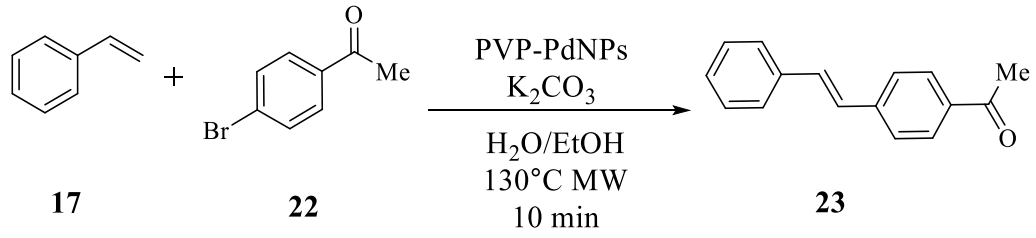


19 **20** **21**

Scheme 2.5 Resveratrol synthesized by Azmi et al. in 2021.

Apart from thermal or conventional heating methods, microwave-assisted synthesis of functional metal nanoparticles (NPs) has gained considerable interest for various reasons, including shorter reaction periods and lower environmental risks (Xin et al., 2022). Furthermore, this method can reduce the agglomeration of nanoparticle supports used as heterogenous catalyst to enhance selectivity and reactivity in reaction system. In 2017, Garcia and her co-workers developed a catalytic application of the

electrochemical synthesised PVP-PdNps (poly(*N*-vinylpirrolidone)-palladium nanoparticles) for Mizoroki-Heck coupling reaction with aryl bromides. In order to provide convenient and efficient coupling reaction with aryl bromides, different stilbene and novel heterostilbenes were synthesised by employing palladium nanoparticles (PdNPs) in aqueous medium under microwave (MW) irradiation (Garcia et al., 2017). *(E)*-1-(4-Styrylphenyl)ethanone (**23**) were synthesized in 10 minutes of reaction between styrene (**17**) and aryl bromide **22** under MW irradiation (Scheme 2.6).



Scheme 2.6 MW-assisted Heck reaction with PdNPs catalyst.

2.3 Cinnamic acid

Natural sources, especially plants, are the major source of compounds with various biological activities (Chinembiri et al., 2014; Albuquerque et al., 2019; Almeida et al., 2020). Cinnamic acid (CA), also known as phenylacrylic acid (3-phenyl-2-acrylic acid), is a member of the auxin family of plant hormones that control cell growth and differentiation (Baltas et al., 2011). It is a crucial component in several plants, including *Cinnamomum cassia* (Chinese cinnamon), *Panax ginseng*, fruits, whole grains, vegetables, honey, cinnamon bark, and benzoin (Chandra et al., 2019). Indeed, the presence of an ethylene group attached to the phenyl ring resulted in two cinnamic acid isomers, either *cis* or *trans*; however, the most common isolated was reported to be the latter case (Yilmaz et al., 2018; Rodrigues et al., 2019).

In plants, the hydroxyl cinnamic acids are byproducts from phenyl alanine deamination (Ahmad et al., 2022). It is the key intermediate in the metabolic of shikimate and phenylpropanoid leading to the formation of flavonoids, phenyl-propanoids, coumarins, tryptamine and the plant structural component lignin (Ruwizhi et al., 2020). Chemically, cinnamides are reported to be commonly synthesised *via* condensation reaction between cinnamic acid with amine (Hu et al., 2021). Studies have reported that cinnamic acids possess an α,β-unsaturated carbonyl, which can be considered as a Michael acceptor, an active moiety that is extensively corporated in the development of anticancer drugs (Zou et al., 2006; Chen et al., 2020).

Five common cinnamic acid derivatives that have been reported in studies included *p*-coumaric acid (**24**), caffeic acid (**25**), ferulic acid (**26**), sinapic acid (**27**) and cinnamic acid (**28**) (Figure 2.3). The presence of either free or methoxylated hydroxyl groups on the phenyl ring distinguishes these cinnamic acid analogs. Apart

from being the intermediates in synthesizing curcumin and stilbene analog, cinnamic acid possess a wide range of biological benefits, including anti-diabetic (Hafizur et al., 2015), anticancer (Zenta et al., 2022), anti-inflammatory (Pontiki et al., 2015), antioxidant (Boz et al., 2015), neuroprotective (Zhang et al., 2019), and aging-related effects (Hseu et al., 2018). Cinnamic acid was reportedly used as a fragrant ingredient in toiletries, detergents, and flavorings cosmetics (Yilmaz et al., 2018). Octinoxate (**29**), known as ethylhexyl methoxycinnamate, is commonly used as a UVB filter in sunscreens and various personal care products (Zambrano et al., 2023). Belong to the same group, isoamyl *p*-methoxycinnamate (amiloxiate) (**30**), is used in cosmetics worldwide as sunscreen, anti-aging agents, and UV absorbers (Gunia-Krzyżak et al., 2018).

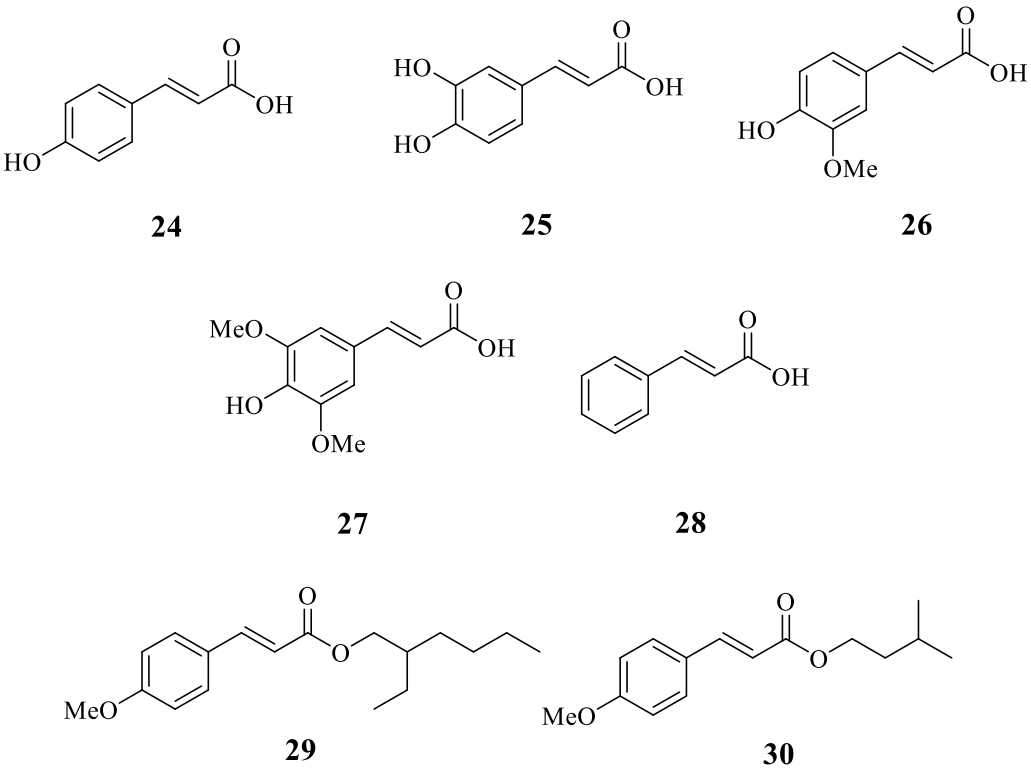
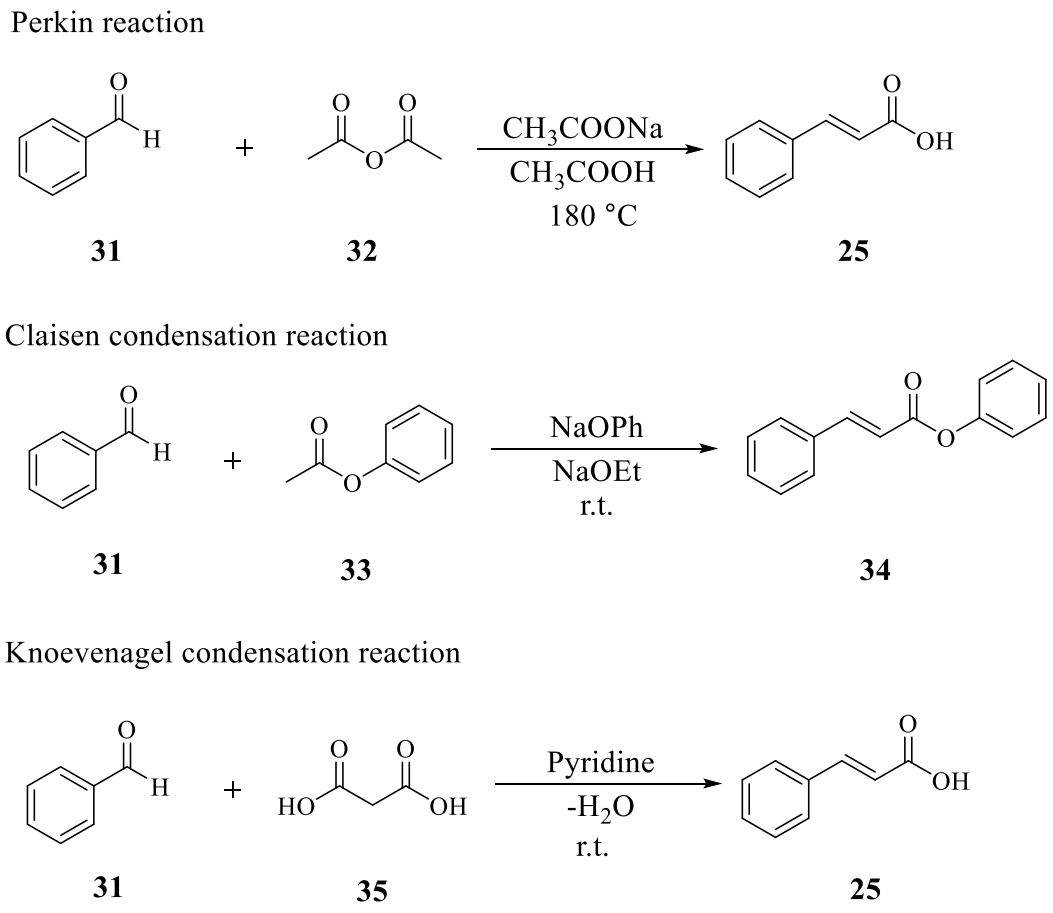


Figure 2.3 Chemical structure of cinnamic acid derivatives.

Cinnamic acid is a privileged and significant pharmacophore in medicinal chemistry due to excellent pharmacological and biological properties as therapeutic agents (Zhu et al., 2015; Guzman et al., 2014; Gaikwad et al., 2019). Over the years, researchers have reported the structural modification of cinnamic acid resulting in more potent derivatives than the parent compound (Rodrigues et al., 2019; Baltas et al., 2011). Herein, this thesis aims to generate a framework by corporating arylcinnamide and resveratrol moieties in the chemical structure of new hybrids as new anticancer drugs designed.

2.4 The synthesis of cinnamic acid analogue

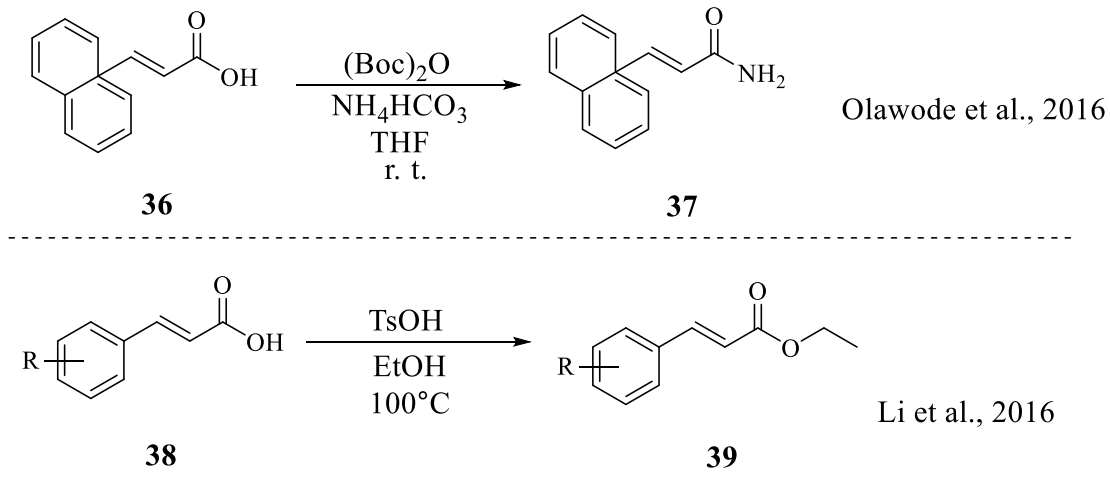
The beginning of synthetic pathway of cinnamic acid was developed in the 19th century and was named the Perkin reaction by William Henry Perkin (Szwaczko et al., 2022). Historically, the procedure involved a condensation reaction between benzaldehyde (**31**) with acetic anhydride (**32**) in the presence of a weak base (such as triethylamine) or alkali salt of acid (such as sodium acetate) as the catalyst. In 1890, cinnamate esters **34** have been synthesized by Rainer Ludwig Claisen *via* Claisen condensation, where benzaldehyde (**31**) was treated with acetic acid esters **33** in the presence of a strong base (Fernandes et al., 2014). In the same year, another method to synthesize cinnamic acid was reported, known as Knoevenagel condensation, involving the condensation of benzaldehyde (**31**) and malonic acid (**35**) in a weak base (such as pyridine) (Scheme 2.7).



Scheme 2.7 Synthetic pathway of cinnamic acid in the 19th centuries.

With time, various strategies and method were discovered and reported by researchers around the world to develop new cinnamic acid derivatives with potential biological properties. In 2016, Olawode and Li et al., reported independently on production of cinnamic acid derivatives as intermediates using the Knoevenagel condensation method (Scheme 2.8). Olawode et al. and his co-workers reported a series of cinnamyl-based compound with high yield and evaluated their proliferation activities (Olawode et al., 2016). The intermediate **36** was converted to cinnamide **37** by introducing *tert*-butyloxycarbonyl (Boc) protecting group into the treatment with ammonium hydrogen carbonate and pyridine. Li et al. disclosed the synthesis of cinnamate derivatives **39** resulted from the esterification of cinnamic acid analog **38**

using *p*-toluenesulfonic acid (TsOH) as the catalyst and alcohol to promote the nucleophilic attack in the mechanistic system. The Knoevenagel condensation method was used to obtain the intermediate cinnamic acid analog **38** (Li et al., 2016).

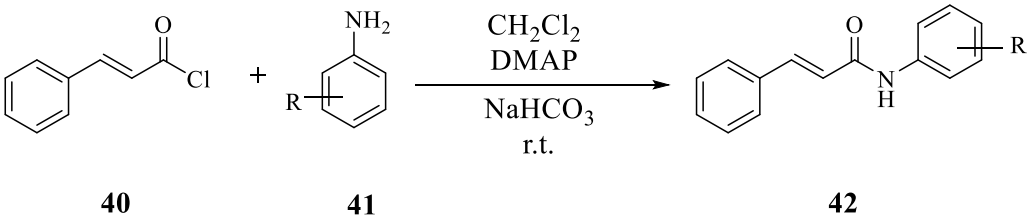


R = H, 2-Cl, 4-CH_3, 3,5-OMe

Scheme 2.8 Synthesis of cinnamic acid analog by Olawode and Li et al. in 2016.

In 2013, Zhang et al. developed a variety of cinnamide derivatives by introducing different substituents to the *N*-phenyl ring, while other ring remain constant. As shown in Scheme 2.9, different substituted phenylcinnamide **42** was obtained from the coupling of cinnamoyl chloride **40** with aniline **41**, in the presence of 4-dimethylaminopyridine (DMAP) and dry dichloromethane (CH_2Cl_2). DMAP played a major role in this reaction, serving as an acyl transfer agent and often utilized in coupling reaction involving carboxylic acid and amines. This report described 2-methoxyphenyl cinnamide as the most potent EGFR kinase inhibitor. The structure-activity relationship analysis reported by Zhang and his group concluded that the

methoxy group attached on *N*-phenyl rings are required to enhance the antitumor activities (Zhang et al., 2013).

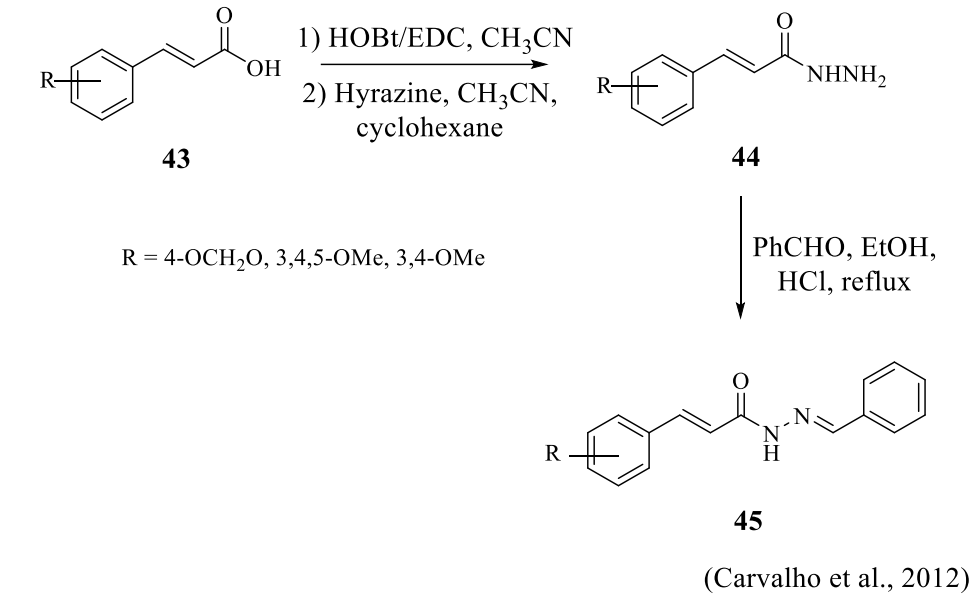


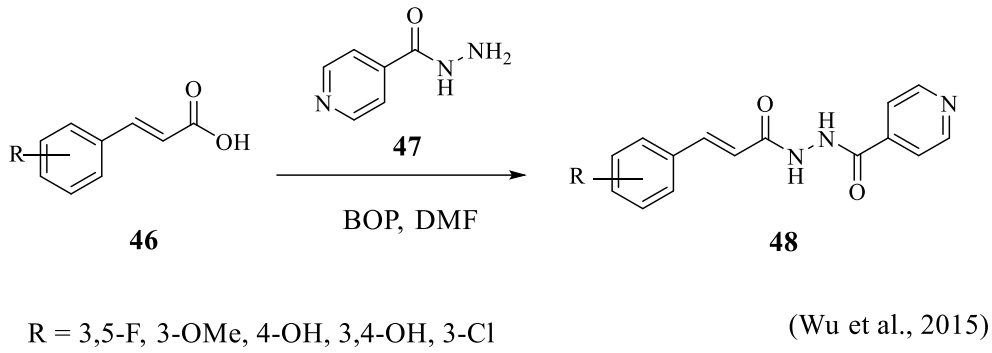
40 **41** **42**

R = 2-OMe, 3-OMe, 3-F, 3-Cl, 4-Cl,
2-Me, 3-Me

Scheme 2.9 Synthesis of cinnamide with DMAP and $NaHCO_3$ by Zhang et al. in 2013.

Carvalho and Wu et al. reported the cinnamic acid containing hydrazide group in 2012 and 2015, respectively (Scheme 2.10). Carvalho and his team reported a new series of cinnamic *N*-acylhydrazide analog **44** which served as an intermediate, generated using the coupling reagent hydroxybenzotriazole (HOBt)/1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in acetonitrile. The collected hydrazides were then undergo acid-catalyzed condensation reaction with aromatic aldehyde in ethanol to produce the desired hydrazone derivatives **45** (Carvalho et al., 2014). On the other hand, Wu et al. reported the synthesis of a bifunctional cinnamic scaffold **48** *via* molecular hybridization of cinnamic acid **46** and isoniazid (**47**) to obtain compounds with better anti-tubercular properties (Wu et al., 2015). The coupling reaction was conducted in the presence of triethylamine and BOP as the coupling additive in DMF (Vishnol et al., 2009).

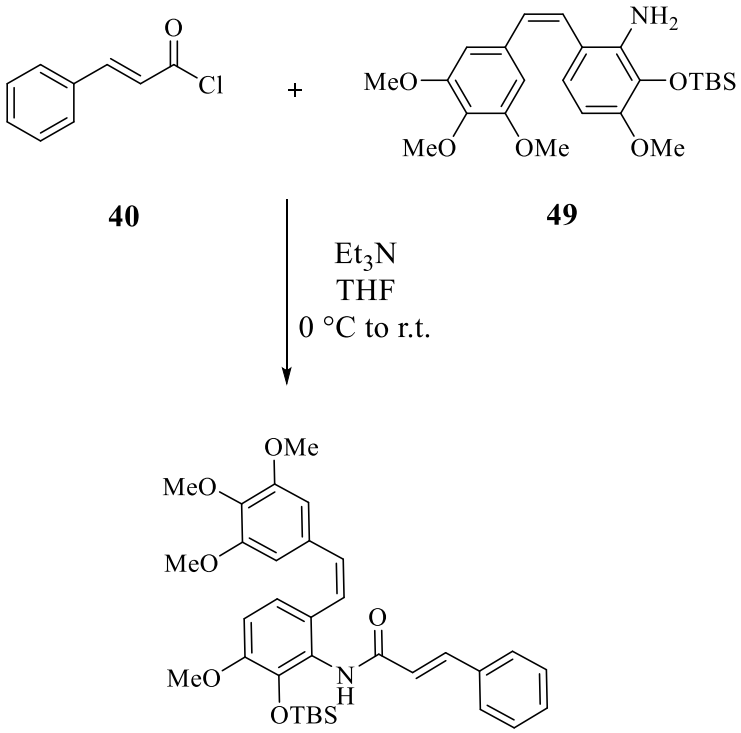




Scheme 2.10 The synthetic pathway of cinnamic acid bearing hydrazide group (Carvalho et al., 2012; Wu et al., 2015)

Coupling reaction between two pharmocophores with the purpose to create molecules with better biological properties than the parent compound was the target of many synthetic chemists nowadays. In 2016, Kamal et al. developed a new series of molecules by combining the *cis*-configuration of arylcinnamide and combretastatin pharmacophores based on a hybridization approach (Kamal et al., 2016). The synthesized cinnamide **50** were successfully obtained from the treatment of *tert*-butyldimethylsilyl ether (TBS-O) substituted aminostilbene **49** with cinnamoyl

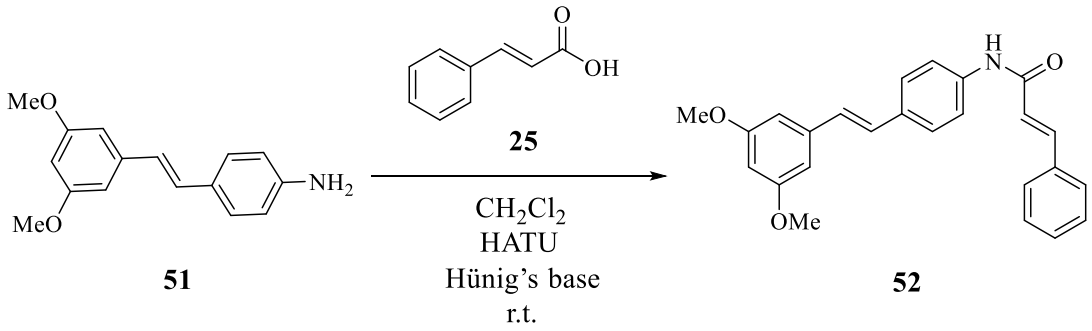
chlorides (**40**) (Scheme 2.11). The presence of a weak base (triethylamine) accommodates the nucleophilic attack in the reaction system.



Scheme 2.11 Synthetic pathway of arylcinnamide-combrestatin conjugate **50**.

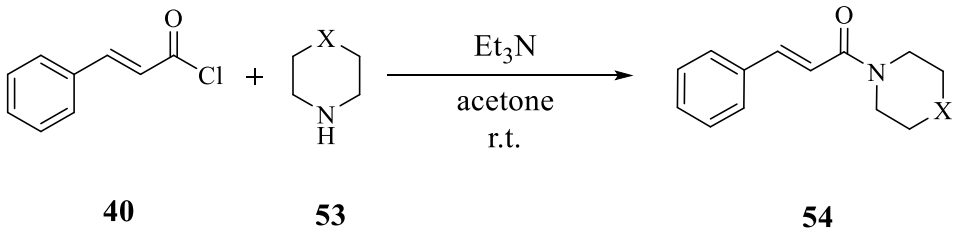
Li et al. has reported on the synthesis of resveratrol-cinnamic acid hybrids in 2016 with the use of coupling reagent HATU (Hexafluoro-phosphate azabenzotriazole tetramethyl uronium). The reagent was utilized along with Hünig's base (*N*,*N*-diisopropylethylamine) to produce molecules with combination of stilbene and cinnamoyl moieites. The high coupling efficiencies and faster reaction rate were associated with HATU coupling, which is commonly used for intramolecular amidation reactions. In that report, the resulted hybrids were validated for their efficiencies in blocking the acetylation and phosphorylation process which highly involves in anticancer activities of STAT3 (Li et al., 2016). As shown in Scheme 2.12,

the cinnamic acid (**25**) coupled with aminostilbene **51** in dichloromethane at room temperature to yield the desired resveratrol-cinnamic acid hybrid **52**.



Scheme 2.12 The synthesis of hybrid **52** by using HATU/Hünig's base.

Apart from resveratrol, secondary amines-heterocyclic **53** such as piperazine and morpholine also were reported to be coupled with cinnamoyl chloride (**40**). This fact was evidenced by Prasanthi et al., who developed a facile and fast method to achieve a new series of cinnamoyl-piperazine analog **54** in 2018 (Prasanthi et al., 2018). The method involves a basic-catalyzed acylation reaction with the presence of triethylamine and acetone at room temperature (Scheme 2.13). The synthesized hybrids were evaluated as potential anticonvulsive and antinociceptive agents.



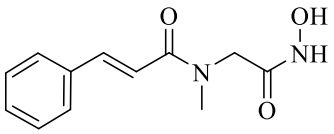
X = NH/CH_2/O/N-Ar

Scheme 2.13 The synthetic route for the synthesis of cinnamoyl-piperazine **54**.

2.5 Cinnamic acid and its derivatives as anticancer agents

At present, attention from synthetic chemist were focused on development drugs on chemoprevention or chemotherapy against cancer diseases. Cancer is a group of diseases that have the potential to surpass all other causes of death as one of the leading killers in the western world, despite causing abnormal cell growth to the human bodies (Banik et al., 2020). Thus, new cinnamic acid derivatives were designed, synthesised, and evaluated for their cytotoxic activities for developing new anti-cancer drugs.

Kaur et al. in 2022, has synthesized a series of phenylcinnamide derivatives as potential anticancer agents and evaluated its cytotoxocities on A549 cancer cells (Kaur et al., 2022). Matrix metalloproteinases (MMPs) served significant part in biological and pathological in nearly all kind of malignancies and human tumours (Hsiao et al., 2019). Hence, Kaur and his team provide novel frameworks that inhibit MMPs to obtain potent anticancer drugs. The *in vitro* cytotoxicity and *in silico* docking results on MMP-9 displayed that compound **55** was selected to be the most potent inhibitor with IC_{50} values of 10.36 µM against A549 cells (Figure 2.4).

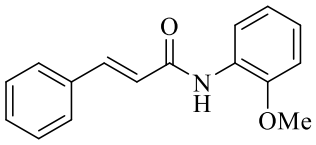


55

Figure 2.4 Chemical structure of phenylcinnamide **55** as the most potent against A549 cell and strong MMP-9 inhibitor.

Modification on *N*-phenyl ring of cinnamic amides have been done by Zhang et al. in 2013, where he and his group reported the synthesis of cinnamide derivatives

and evaluated for antiproliferative activities against human breast cancer cell line MCF-7 and EGFR-inhibitory activities (Zhang et al., 2013). Based on the SAR analysis result, there is a weak fluctuation in the inhibitory activities, which demonstrates that the addition of *o*-> *p*- > *m*- substituents and replacement of Me groups by alkoxy groups (RO) enhance the activities in general. Zhang et al. concluded that the alkoxy group is favorable for increasing the activity rate based on the SAR study and molecular docking result. *N*-(2-methoxyphenyl)cinnamamide **56**, was found to be the most potent with IC_{50} values 2.01 µM and 5.16 µM against MCF-7 cell and EGFR-inhibitory activities (Zhang et al., 2013).



56

Figure 2.5 Cinnamide **56** as the most potent against MCF-7 and EGFR-inhibitory activities.

In recent years, the concept of hybrid molecules or the incorporation of active pharmacophores into an active natural compound has emerged in drug discoveries of new therapeutic agents. A new series of hybrids with the combination of the arylcinnamide and combrestatin pharmacophores have been conducted by Kamal et al. in 2016. These hybrids were evaluated for their effect on the inhibition of tubulin polymerization, apoptosis-inducing ability, and cytotoxic activity. Hybrid **57** showed high cytotoxicity with GI_{50} values of 31.0 µM against the human breast cancer cell line (MCF-7). The substituent of 3,4-difluorine group on the cinnamide aryl ring in conjugate **57**, may contribute to interact and bind at the colchicine binding site of the tubulin (Kamal et al., 2016).