SYNTHESIS, CHARACTERIZATION OF 1,3-DIARYLPROPENES AND TRICYCLIC INDOLINES AND THE CYTOTOXIC ACTIVITIES OF 1,3-DIARYLPROPENES

TAN AIK SIAN

UNIVERSITI SAINS MALAYSIA

2022

SYNTHESIS, CHARACTERIZATION OF 1,3-DIARYLPROPENES AND TRICYCLIC INDOLINES AND THE CYTOTOXIC ACTIVITIES OF 1,3-DIARYLPROPENES

by

TAN AIK SIAN

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

September 2022

ACKNOWLEDGEMENT

"*Appreciation is the highest form of prayer, for it acknowledges the presence of good wherever you shine the light of your thankful thoughts*.", quoted Alan Cohen. I would like to express my gratitude to the Almighty for the opportunity to march in the hall of academia. The biggest appreciation to my supervisor Dr Mohamad Nurul Azmi (USM), for his countless guidance and support throughout my postgraduate life. It has been an incredible journey to immerse myself in the world of organic chemistry I am longing for. I am genuinely grateful for his enormous efforts to refine my skills as a future researcher, an organic chemist, particularly. Special thanks to Dr Yvan Six, Prof Dr Laurent El-kaim (ENSTA) & LSO team for research support and guidance when I met the research bottlenecks. It was an eye-opening experience to learn from them as I am an amateur in this field. Their meticulous and exquisite work quality is worth the learning process. Thanks to Dr Nik Nur Syazni & Ms Mustahhimah (IPPT) for the biological assay part and Mass Spectroscopy. The success of the research projects never left behind the contributions from the science officers/staff or technicians such as the belated Mr Khairul, Mdm. Arlita, Mdm. Alia, Mr. Ramli, Mr. Megat, Mr. Azhar, Mr. Fahmi. Undeniable, firm financial support is still the core of any research as in material and accesses to specific services. Accordingly, thanks to the PHC Hibiscus grant MyPAIR/1/2019/STG01/USM/2 and the USM Fellowship scheme for financial support. About USM Fellowship, I appreciate the assistance from Mdm Zamreena IPS from the start until the end of the process, indeed, the panel IPS who entrusted me with this scheme. Worth mentioning a big thanks to previous Deputy Vice-Chancellor Prof Dr Aldrin Abdullah for his supporting letter to IPS USM. The NPSO labmates such as Hadi,

Solehin, Amirah, Nurin, Huda, Syifa, Jaymeer, Atikah, Kok Zhuo, Unforgettable Ain, Shira & other NPSO members consistently help each other and ease the research progress.

Non-academic wise, the community of BFPP, MPD, MYDP, BHEPA, IMCC, PS, PHS, and USM functional units, including café owners, cleaners, and friends who made my master postgraduate life lived to the fullest. Everyone plays a crucial role and deserves the same degree of respect. Last but not least, my family believed in me as an independent son and my reason for standing here. To end this section, I would like to quote a meaning phrase from Prof Hamka: "*Manusia itu asalnya dari tanah, makan hasil tanah, berdiri di atas tanah, dan kembali ke tanah. Kenapa masih bersifat langit?*" Perhaps postgraduate students are highly educated but hold no right to possess haughtiness. Every person and resource from the community should be back to the community. During my master's postgraduate journey, I humbly appreciated every single person I met and got assistance, whether directly or indirectly. Although it is insufficient to write all of them in this section, I will remember them. Je vous remercie de tout cœur.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF SCHEMES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

α	Alpha
Å	Angstrom
β	Beta
cm^{-1}	Per centimeter
δ	Chemical shift
δ_C	Chemical shift carbon
δ_H	Chemical shift proton
°C	Degree Celsius
g	Gram
g mol^{-1}	Gram per mol
Hz	Hertz
J	Coupling constants (Hz)
L	Liter
µM	Micro mole/L
M	Mole/L
m-	Meta
mg	Milligram
MHz	MegaHertz
mL	Milliliter
mm	Millimeter
mm^{-1}	Per millimeter
mmol	Millimole
o-	Ortho
p-	Para
ppm	Parts per million
σ	Sigma
θ	Theta
$\mu(M_oK_\alpha)$	Molybdenum monochromic radiation wavelength
%	Percentage
υ	Wavenumber

LIST OF ABBREVIATIONS

2CzPN	4,5-di (9H-carbazol-9-yl) phthalonitrile
Ac	Acetyl
ACR-IMAC	Organic photosensitizer
$AgSbF_6$	Silver hexafluoroantimonate
Ar	Argon gas
ATR-FTIR	Attenuated Total Reflectance – Fourier-transform infrared
$AuCl(PPh_3)$	Chloro(triphenylphosphine)gold(I)
$BF_3.Et_2O$	Boron trifluoride etherate
Bn	Benzyl
BxPC-3	Pancreatic cancer cell line
C_6H_6	Benzene
CA4	Combretastatin A-4
$CDCl_3$	Deuterated chloroform
CF_3	Trifluoromethyl
CH_3COOH	Acetic acid
CHOOH	Formic acid
Cl	Chlorine
CN	Cyano group
CO_2H	Carboxylic acid
COSY-NMR	Correlated spectroscopy – Nuclear Magnetic Resonance
Cp	Cyclopentanyl
CSA	Camphorsulfonic acid
$Cu(PPh_3)_3Br$	Bromotris(triphenylphosphine)copper(I)
CuI	Copper Iodide
Cy	Cyclohexanyl
D_{cal}	Density calculation
DCM	Dichloromethane
DMEM	Dulbecco's modified Eagle's medium
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMU-212	3,4,4′,5-Tetramethoxystilbene
DNA	Deoxyribonucleic acid
DU-145	Prostate cancer cell line
ER	Estrogen receptor
Et	Ethyl
Et_3N	Triethylamine

$Et_3N.HCl$	Triethylamine hydrochloride
F	Fluorine
FBS	Foetal bovine serum
$FeCl_3$	Iron(III) chloride
FTIR	Fourier-transform Infrared
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
hEGF	Human epidermal growth factor
HEP-G2	Liver cell line
HIV	Human immunodeficiency virus
HMBC-NMR	Heteronuclear Multiple Bond Correlation – Nuclear Magnetic Resonance
HNO_3	Nitric acid
HRMS	High-resolution mass spectroscopy
HSQC-NMR	Heteronuclear Single Quantum Coherence – Nuclear Magnetic Resonance
HT-29	Colon cancer cell line
hTERT-HPNE	Normal pancreatic cell line
hv	Irradiation
HWE	Horner-Wadsworth-Emmons reaction
IC_{50}	50% inhibitory concentration
IDO1	Indoleamine 2,3-dioxygenase 1
i-Pr	*iso*-Propyl
$Ir(dFCF_3ppy)_2(dtbbpy)PF_6$	[4,4′-Bis(1,1-dimethylethyl)-2,2′-bipyridine-N1,N1′] bis[3,5-difluoro-2-[5-(trifluoromethyl)-2-pyridinyl-N] phenyl-C]Iridium(III) hexafluorophosphate
K_2CO_3	Potassium carbonate
KOH	Potassium hydroxide
KO^{t}Bu	Potassium tert-butylate
L1210	Murine leukemia cell line
$LiAlH_4$	Lithium aluminum hydride
MCF-10A	Normal breast cell line
MCF-7	Estrogen-sensitive breast cancer cell line
MDA-MB-231	Estrogen-insensitive breast cancer cell line
Me	Methyl
MeCN	Acetonitrile
MOM	Methoxymethyl
MS	Mass spectrometer
MTT	[3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Microwave
N	Nitrogen

N_2	Nitrogen gas
Na_2SO_4	Sodium sulfate
$NaHCO_3$	Sodium bicarbonate
n-Amyl	Pentyl
NaOAc	Sodium acetate
Naph	Naphthalenyl
n-Bu	Butyl
n-BuLi	*n*-Butyllithium
nC_5H_{11}	Pentyl
nC_9H_{19}	Nonyl
NH_4Cl	Ammonium chloride
$NiBr_2$	Nickel bromide
NMe_4Cl	Tetramethylammonium chloride
NMR	Nuclear Magnetic Resonance
NO_2	Nitro group
n-Pr	Propyl
O.D.	Optical density
OAc	Acetoxy
OMe	Methoxyl
P388	Leukemia cell line
Pd	Palladium
$Pd(OAc)_2$	Palladium acetate
$PdCl_2$	Palladium(II) chloride
$PdCl_2(PPh_3)_2$	Bis(triphenylphosphine)palladium(II) dichloride
PenStrep	Penicillin-Streptomycin
Ph	Phenyl
Ph.D	Doctor of Philosophy
PhMe	Toluene
PPh_3	Phosphane
PPh_3MeBr	Methyltriphenylphosphonium bromide
R	Alkyl group
R_{int}	Internal reflection
R_{sigma}	Sigma reflection
r.t.	Room temperature
RNA	Ribonucleic acid
SAR	Structure-Activity-Relationship
SI	Selectivity index
$SnCl_2$	Tin(II) chloride
TAAILs	Tunable aryl alkyl ionic liquids
TADF	Thermally activated delayed fluorescence
t-Bu	*tert*-Butyl

t-$Bu_3P.HBF_4$	Tri-*tert*-butylphosphonium tetrafluoroborate
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TsOH	Tosyl-*p*-toluenesulfonyl acid
UV	Ultra-Violet
Zn	Zinc
$ZnCl_2$	Zinc chloride

LIST OF APPENDICES

SINTESIS, PENCIRIAN 1,3-DIARILPROPENA DAN INDOLINA TRISIKLIK DAN AKTIVITI SITOTOKSIK 1,3-DIARILPROPENA

ABSTRAK

Alkaloid ialah sebatian penting yang mengandungi atom nitrogen dan menunjukkan pelbagai aktiviti biologi dan farmaseutikal, tanpa mengira perancah sebatian. Oleh demikian, ia memacu sintesis amido 1,3-diarilpropena dan 6-5-4 indolina trisiklik bawah kelas Alkaloid dalam kajian ini, berpotensi sebagai agen kemoterapi untuk rawatan kanser. Dalam kajian pertama, empat puluh (40) terbitan 1,3-diarilpropena, direka bentuk sebagai analog stilbenoid dan dihidrostilbenoid, telah disintesis oleh tindak balas bermangkin paladium bagi terbitan 2-amidoiodobenzena dengan estragol atau eugenol. Produk diperoleh dengan stereoselektiviti-(*E*) yang tinggi tetapi sebagai dua regioisomer. Nisbah isomer didapati bergantung kepada sifat pasangan alilbenzena dan dirasionalkan oleh kesan elektronik yang menggunakan pengaruh penentu dalam langkah penyingkiran *β*-hidrida. Di samping itu, kesan sitotoksik semua produk Heck dinilai terhadap beberapa jenis sel kanser payudara manusia. Antaranya, sebatian **117i** menunjukkan aktiviti sitotoksik yang lemah terhadap sel MCF-7 dengan nilai IC_{50} 47.92 μM berbanding tamoxifen dan dianggap mempunyai ketoksikan umum (nilai SI < 2). Dalam kajian kedua, dua puluh (20) terbitan tetrahidro-1*H*-cyclobut[*b*]indol atau siklobutana bercantum indolina trisiklik bertindak balas pemangkin asid Lewis yang ringkas bagi terbitan 2-vinilanilin dengan ester piruvat, menggunakan protokol yang telah dioptimumkan. Produk tersebut bergantung kepada ketegangan cincin siklobutana dan selektif tinggi terhadap satu diastereomer, walaupun ada pemilikan dua pusat stereogenik. Selaras dengan skop yang berbeza bagi kedua-dua kajian,

pendekatan sintesis yang digunakan adalah tersendiri dan mencapai objektif penyelidikan masing-masing.

SYNTHESIS, CHARACTERIZATION OF 1,3-DIARYLPROPENES AND TRICYCLIC INDOLINES AND THE CYTOTOXIC ACTIVITIES OF 1,3-DIARYLPROPENES

ABSTRACT

Alkaloids are crucial nitrogen-containing compounds that exhibit diverse biological and pharmaceutical activity, regardless of the compound scaffold. Accordingly, it drives the synthesis of amido 1,3-diarylpropene and 6-5-4 tricyclic indoline under the alkaloid class, potentially the chemotherapeutic agents for cancer treatment. In the first study, forty (40) 1,3-diarylpropene derivatives, designed analogs of stilbenoids and dihydrostilbenoids, were synthesized by the palladium-catalyzed reactions of 2-amidoiodobenzene derivatives with either estragole or eugenol. The products were obtained with high (*E*) stereoselectivity but as two regioisomers. The ratio of isomers was found to be dependent on the nature of the allylbenzene partner and is rationalized by electronic effects exercising a determining influence in the *β*-hydride elimination step. In addition, the cytotoxic effects of all the Heck reaction products were evaluated against human breast cancer cell lines. Among all, compound **117i** exhibited weak cytotoxic activity towards MCF-7 cell lines with IC_{50} values of 47.92 μM compared with tamoxifen and was considered to have general toxicity (SI value < 2). In the second study, twenty (20) tetrahydro-1*H*-cyclobut[*b*]indoles or cyclobutane-fused tricyclic indoline derivatives were afforded by a simple Lewis acid-catalyzed reaction of 2-vinylaniline derivatives with pyruvate ester, utilized the optimized protocol. The products depend on the ring strain and high selective towards one diastereomer, although the possession of two stereogenic

centers. Along with the different scopes of both studies, the applied synthesis approaches are distinctive and achieved their relative research objectives.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Alkaloid is one of the prominent natural product classes broadly defined as the basic nitrogen-containing organic compounds in any position of the structural framework (Cordell et al., 2001). The plant′s secondary metabolite has a significant role in chemical defense. The defense mechanism mode is dispersed into three domains: against herbivores/predators, antimicrobe/antiviral, and the inhibition of competing organisms (Wink, 1993). In most cases, alkaloids are not only limited to one domain. However, they possess a wide range of biological activity due to the multiple active functional groups in one molecular structure (Wink, 1993).

Berberine (**1**), for example, is an isoquinoline alkaloid isolated from a species of goldthread flowering plant *Coptis Chinensis* (Ma et al., 2017). It has remarkable diverse biological activities such as anti-HIV, antifungal, antimalarial, anti-inflammatory, antioxidant, anti-mutagenic, cardioprotective, cerebroprotective, immunoregulative, vasorelaxant, anxiolytic, and analgesic activities (Zuo et al., 2006). Besides that, amide alkaloids and indole alkaloids such as neoplaether (**2**) (Wang et al., 2006), vinblastine (**3**), and ajmaline (**4**) managed to attract chemist′s interest due to their potent antifungal and antimicrobial activity (Hanafy et al., 2016). On account of its diverse biological activity, the organic synthesis chemists gain motivation and attention to explore the potent alkaloid compounds.

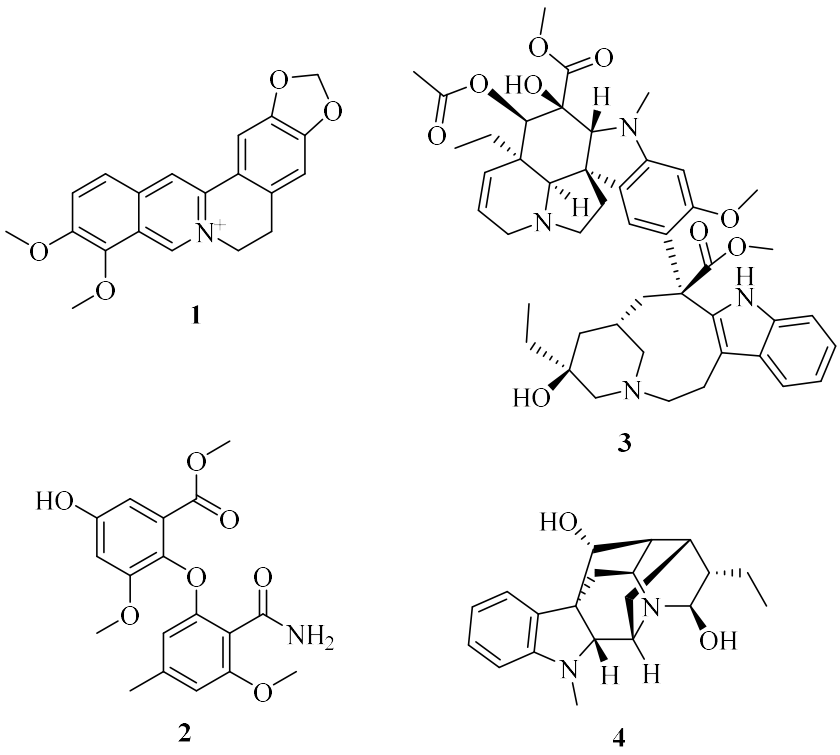


Figure 1.1 Alkaloids with diverse biological activity.

In the early stage of organic synthesis, classical synthetic chemists focused on three major approaches: structural elucidation, the discovery of new substances, and the reaction fundamentals (Hudlicky & Reed, 2007). As modern technology progresses along with the emphasis on application purposes, these three activities are unified into one of the five current organic synthesis approaches, besides the association of biological interest, medicinal chemistry, material sciences, and environmental sciences (Hudlicky & Reed, 2007). Along the synthetic endeavor based on the respective approaches, the reaction methodology used by synthetic chemists can be divided into five domains: classical methods, modern methods, biocatalysis, electrochemistry, and transition-metal catalysis (Hudlicky & Reed, 2007).

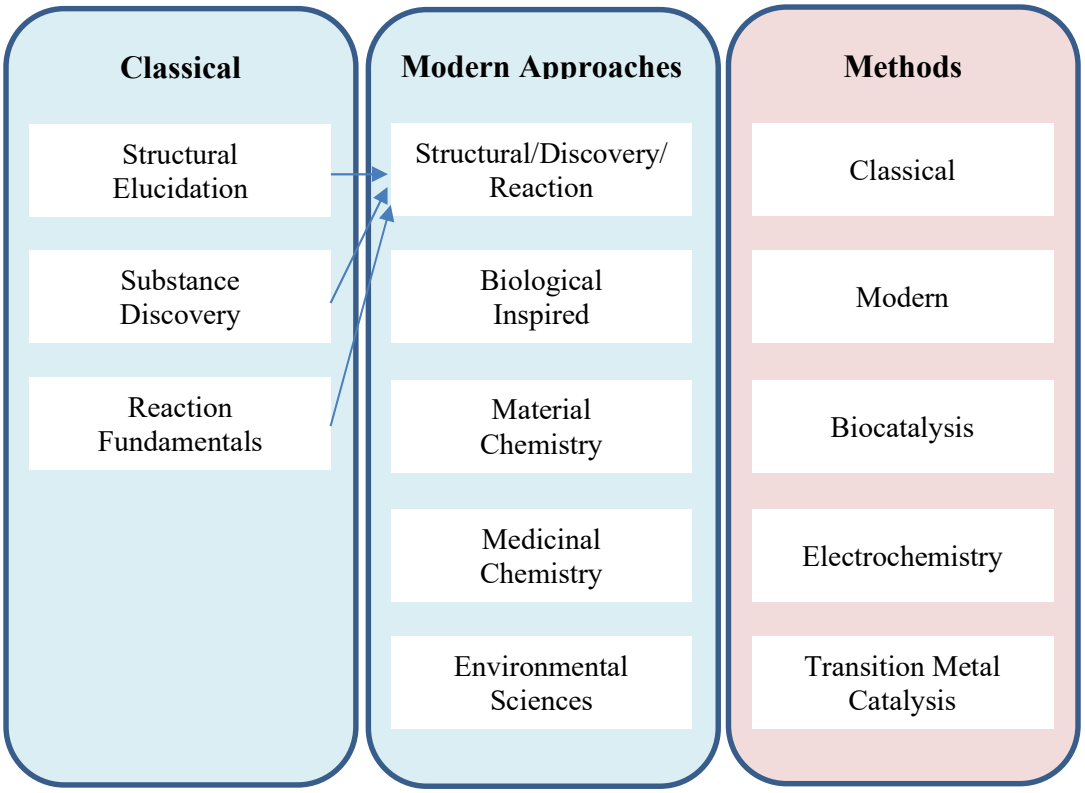


Figure 1.2 The evolution of organic synthesis approaches with its methods.

In this study, both organic synthesis projects towards different classes of alkaloids as in endocyclic or exocyclic nitrogen positions were performed under independent approaches, leading to different research focus. The synthesis of exocyclic nitrogen alkaloids or proto-alkaloids such as 1,3-diarylpropene amide **5** is a biologically inspired approach using the transition metal catalysis method which is the well-established reaction method. At the same time, the synthesized compound structure possessed a high potential to mimic the biological activity of the reference compound structure. Meanwhile, the classical method of synthesizing cyclobutane-fused tricyclic indoline compounds **6** as endocyclic nitrogen alkaloids or *N*-heterocyclic alkaloids are still fragmented; hence, this project implements the

development of the structural/functional/method approach to attempt the investigation of the new structure and its optimized condition for the synthesis.

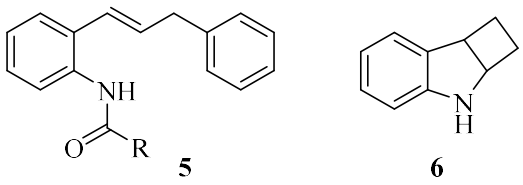


Figure 1.3 Skeleton framework of this study.

1.2 Project A: Synthesis of 1,3-Diarylpropene Amide Compounds and Their Biological Activity

1,3-diarylpropene amide compounds are alkaloids with a nitrogen atom out of the skeleton ring structure. Such compounds can be viewed as high homologs of 1,2-diphenylethylene derivatives (stilbenes) and dihydrostilbenes. With this respect, it is worth pointing out that hydroxylated stilbenes (stilbenoids) and hydroxylated dihydrostilbenes are of particular biological importance. Most of these phytoalexin molecules are derived from *trans*-resveratrol, which in grapevines has been thought to be involved in the "French paradox" (Siemann & Creasy, 1992). (*E*)-Resveratrol (**7**) and other naturally occurring (*E*)-stilbene derivatives, such as piceatannol (**8**), oxyresveratrol (**9**), or isorhapontigenin (**10**), have attracted much attention and have been the subject of extensive studies. Various interesting pharmacological properties have been disclosed, including antioxidantsnt, (Morabito et al., 2014) anticancer, (De Filippis et al., 2017) anti-inflammatory, cardioprotective, antifungal (Teplova et al., 2018,) and antibacterial activities (Babic et al., 2000). Interestingly, dihydrostilbenoids have exhibited a similar range of biological effects (Vitalini et al., 2018). The attempt to prepare the stilbenoid

analogs with an amido substituent at the *ortho* position of one of the two aromatic parts, using the Heck reaction, succeeded (Kee et al., 2010; Azmi et al., 2013). Some of these molecules possessed significant anticancer properties against HT-29, P388, DU-145, MCF-7, and BxPC-3 cancer cell lines or chemopreventive action (Kee et al., 2010; Azmi et al., 2013).

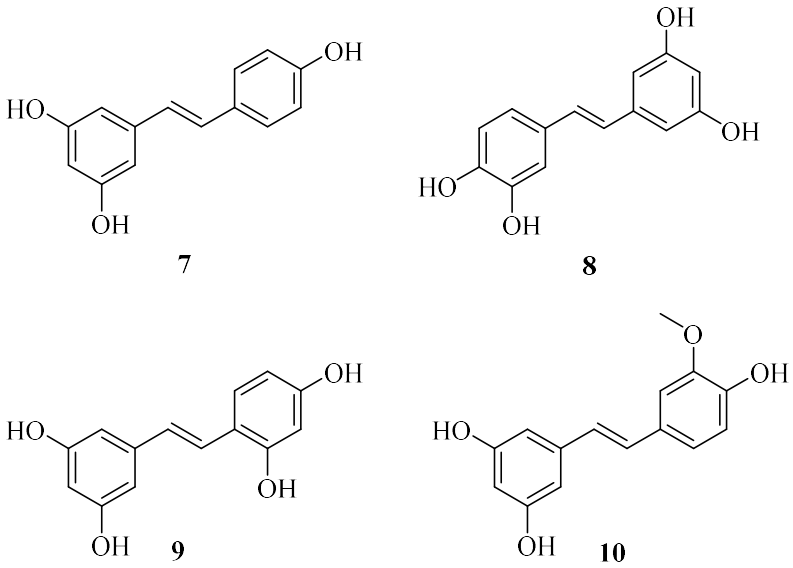


Figure 1.4 (*E*)-Resveratrol and the (*E*)-stilbene derivatives.

With the stability of both aryl groups, the synthesis approach of diarylalkene is focused on forming C-C coupling at the bridged carbon chain of the skeleton structure. Those developed methodologies were Perkin Aldol condensation, Wittig olefination, Horner-Wadsworth-Emmons reaction, and transition metal couplings such as Mizoroki-Heck and Sonogashira and Suzuki-Miyaura (Khan et al., 2017). Among all the well-established methodologies in stilbene synthesis, 1,3-diarylpropene amide compounds adapted the Heck cross-coupling method as it gives more promising results than existing literature with a similar model (Azmi et al., 2013).

1.3 Project B: Synthesis of Cyclobutane Tricyclic Indoline Compounds

Cyclobutane tricyclic indoline or tetrahydro-1*H*-cyclobut[*b*]indoles in specific, is an *N*-heterocyclic alkaloid where *N*-atom is in a 6-5-4 tricyclic-fused ring skeleton. The first discovery of this skeleton framework is by photoaddition of olefins towards *N*-acylindoles, continuing the previous work in photoadditions of ketones to the same substrate (Julian & Foster, 1973). After two decades, the breakthrough of the non-photocatalytic method was founded by using 2-vinylaniline derivatives and piperidone analogs *via* a 1,5-dipole cyclization route with toluene-4-sulfonic acid as catalyst (Walter et al., 1993).

The synthesis difficulty of cyclobutane compounds is driven by the angle and steric strains, leading to an unstable structure and low production yield (Li et al., 2020). As the alternative to the cyclization approach in synthesizing tetrahydro-1*H*-cyclobut[*b*]indoles, radical chemistry such as photocatalytic synthesis in [2+2] cycloaddition is practiced in the mainstream approach. Although several attempts to improve the synthesis yield have been tried, even with transition metal catalysis, photocatalytic reaction still retains its deadliest weakness: prolonged reaction time (Oderinde et al., 2020).

1.4 Problem Statements

1.4.1 Project A: Problem Statement

In the past decades, the intensive study of stilbene has been done in synthetic methods or biological activities (Roat & Saraf, 2015). In contrast, homologs of stilbene, 1,3-diarylpropene gains little attention as a study subject but serves broadly as an intermediate or precursor of *N*-heterocyclic compound′s synthesis (Evoniuk et al., 2015), which is an existing void. As the higher homologs of 1,2-diphenylethylene derivatives (stilbenes) and dihydrostilbenes, the scaffold of amido 1,3-diarylpropene may potentially have characteristics of both types of molecules. Based on the experimental evidence that the amido moiety in diarylalkene is effective against breast cancer cell lines (Azmi et al., 2013), amido 1,3-diarylpropene may mimic the same cytotoxic properties and should be further investigated.

1.4.2 Project B: Problem Statement

From meticulous observation and intensive interpretation, significant discoveries of noble organic synthesis methods bloomed in the latter of the 20th Century. Consequently, discovering new ways is becoming strenuous, while organic chemists focus on method optimization and the study of scopes/limitations as to their methodology development priorities (Hudlicky & Reed, 2007). Acid-catalyzed 1,5-dipolar cyclization is one of the fascinating classical synthetic methods that required further investigation into the best-fit reaction condition in synthesizing tetrahydro-1*H*-cyclobut[*b*]indoles its stereochemistry study (Walter & Schneider, 1995). With the lack-established reaction in the existing literature, synthesizing

tetrahydro-1*H*-cyclobut[*b*]indoles is worth the attention for the methodology development.

1.5 Research Objectives

1.5.1 Project A: Research Objectives

The objectives of this study are as the following:

1. To synthesize 1,3-diarylpropene amide compounds *via* the Heck method.
2. To characterize 1,3-diarylpropene amide compounds using spectroscopic techniques.
3. To propose the mechanistic pathway of 1,3-diarylpropene amide compounds.
4. To determine the cytotoxic activity of 1,3-diarylpropene amide compounds against breast cancer cell lines.

In continuing efforts directed toward the discovery of novel biologically active analogs, it is worth investigating the application of the Heck reaction to the synthesis of new compounds having a 1,3-diarylpropene skeleton, thus moving from a C6-C2-C6 to a C6-C3-C6 structure but retaining the pharmacophores, i.e. an amido substituent on one of the phenyl rings and oxygen-based substituents on the other. It is noteworthy that these new target molecules can be viewed as analogs of stilbenoids and dihydrostilbenoids but, in fact, as a hybrid compound having characteristics of both types of molecules.

1.5.2 Project B: Research Objectives

The objectives of this study are as the following:

1. To optimize the synthesis of cyclobutane tricyclic indoline compounds with and without the presence of acid.
2. To synthesize the cyclobutane tricyclic indoline compounds using the acid-catalyzed cyclization method.
3. To characterize the cyclobutane tricyclic indoline compounds using spectroscopic techniques.
4. To propose the mechanistic pathway of the cyclization method with and without the presence of acid.

In this study, the usage of cheap and active carbonyl species such as glycol ester expects a rapid reaction with an excellent yield. Target compounds of two stereogenic centers potentially provide remarkable insights into stereochemistry and detailed mechanistic interpretations of this synthesis. Improvised acid-catalyzed cyclization method may contribute to the more practical alternative in tricyclic indoline synthesis and economical option than the photocatalytic settings.

1.6 Scope of the Study

In this thesis, the presentation of the five chapters is about the synthesis of endocyclic and exocyclic nitrogen alkaloids in their respective projects: A) Synthesis of 1,3-diarylpropene amide compounds and their biological activity. B) Synthesis of cyclobutane tricyclic indoline compound. Chapter 1 briefs the research background and approaches in general. Chapter 2 will demonstrate the literature sources that contributed to the research concept and framework, where alkaloid is the main domain as the initial point of review. Chapter 3 outlines the

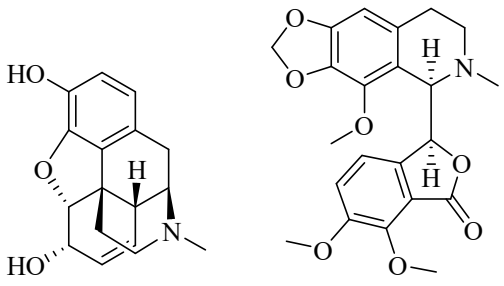
experimental details carried out for both projects. Chapter 4 manifests the findings and discussion in detail of the research outcomes. Lastly, Chapter 5 concludes the thesis with research outcomes in the respective projects.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Alkaloids

A plant is a eukaryotic organism that serves as the primary energy source, crucial in sustaining the whole ecosystem (Wink, 1993). Being the food resource of other organisms, the plant evolves different strategies to defend against herbivores, viruses, fungi, parasites, and even microorganisms (Wink, 1993). It develops both chemical and physical defense mechanisms as a limited mobility organism (Wink, 1993). Worth to mention that for the virtuoso of chemical defense, the plant is placed at the pinnacle among the organisms (Wink, 2010). A plant can produce diverse chemical defense compounds called secondary metabolites regardless of macromolecular or molecular compounds (Wink, 2010). Among all the secondary metabolites, alkaloids are one of the most renowned chemical defense compound classes (Wink, 2010).



11 **12**

Figure 2.1 First isolation of plant-based alkaloid compounds.

Alkaloids-containing plants have contributed their significance and utilized as medicines since the ancient time of humanity (Seigler, 1998). Accompanied by the curiosity of its active ingredients, the attempt for isolation and characterization was

initiated in the 19th century (Seigler, 1998). Opium is the first drug subject to be studied because of its astonishing narcotic and analgesic properties, by dint of morphine (**11**) and narcotine (**12**), first isolated plant-based alkaloids in 1803 (Bribi, 2018). With such pioneering efforts, the field of natural product and organic synthesis bloomed at a fascinating rate, accumulating innumerable and precious literature on the isolated alkaloid compound along with the structural elucidation data until the early 20th century (Bribi, 2018). As the alkaloids compound database was getting entrenched, the alkaloids have been highlighted by the chemist in drug discovery specifically on anticancer activity starting from the late 20th century until now (Bribi, 2018). Paclitaxel (**13**) and vincristine (**14**) are one of the anticancer alkaloids, explicitly targeted on spindle microtubules of the cells (Cormier et al., 2010; Prota et al., 2013).

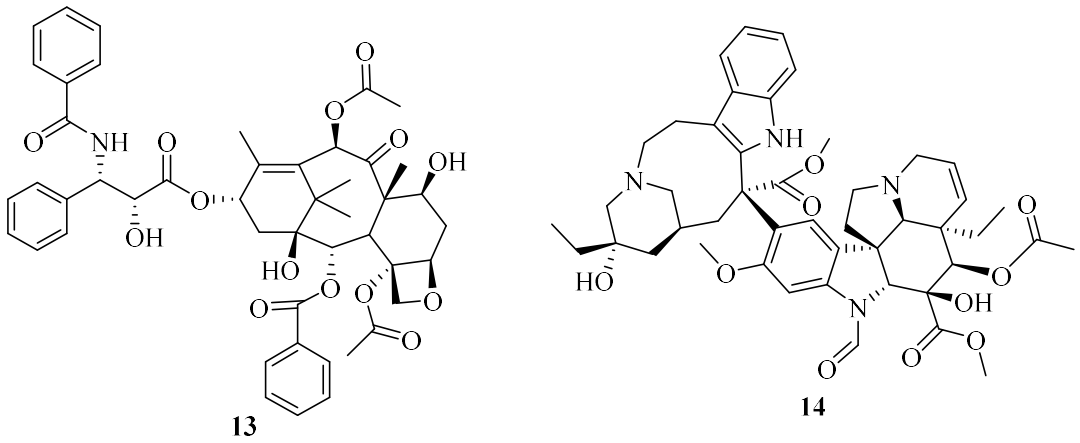


Figure 2.2 Anticancer alkaloids compounds.

Alkaloid defines as an organic compound that is basic and contains one or more nitrogen atoms in any position of its structural framework (Bribi, 2018). As a consequence of such a general definition, alkaloids have indistinctive categories and tremendous variations under this term (Bribi, 2018). Due to the molecular

structure understanding still in bewilderment with scarce spectroscopic evidence, the naming of the alkaloid category was based on the source of natural product in the early stage of alkaloid discovery (Fattorusso & Taglialatela-Scafati, 2007). Along with the refinement of spectroscopic techniques such as Nuclear Magnetic Resonance (NMR) and X-ray Crystallography, alkaloid compounds′ structural elucidation and biogenesis gained a clearer picture than before (Fattorusso & Taglialatela-Scafati, 2007). At the same time, organic chemists started to categorize alkaloids according to the skeleton structure relationship and sometimes based on the spectroscopic properties. After decades until now, there is still no unified taxonomy applicable to the alkaloid, with its enormous structural variations (Fattorusso & Taglialatela-Scafati, 2007). In an attempt to group the alkaloids with the biogenesis and structural basis factor, the three categories of alkaloids: Are true-alkaloids (amino acid origin, *N*-heterocyclic), proto-alkaloids (amino acid origin, *N*-exocyclic position), and pseudo-alkaloids (non-amino acid origin) are widely used and accepted (Eagleson, 1994). Within the organic synthesis scope, the alkaloids category will be emphasized the structural aspect and provide a perspicuous context. Accordingly, the classification of alkaloids will be based on the position of nitrogen atom within the ring skeleton (*N*-heterocyclic alkaloids) and out-of-the-ring skeleton (proto-alkaloids) in this chapter.

2.2 Exocyclic Nitrogen Alkaloid

Exocyclic nitrogen alkaloid is the alkaloid where the nitrogen atom is located out of the skeleton ring framework, which is also known as proto-alkaloids (Eagleson, 1994). Whereas this category of alkaloids is generally not included in the ′true alkaloid′ which defines as the basic nitrogen atom at cyclic skeleton structure

such as *N*-heterocyclic compounds (Badri et al., 2019). The amide alkaloid is one of the common proto-alkaloids, such as phomoenamide (**15**) (Rukachaisirikul et al., 2008), IFB-Lactam-1 (**16**) (Hu et al., 2006), and neoplaether (**17**), isolated from endophytic fungi with diverse pharmacological activity (Wang et al., 2006). As the nitrogen atom or functional group is exempted from skeleton framework construction, the general synthesis approach of proto-alkaloids is the substituents. With the limited literature on proto-alkaloids, the respective section is unable to proceed with depths.

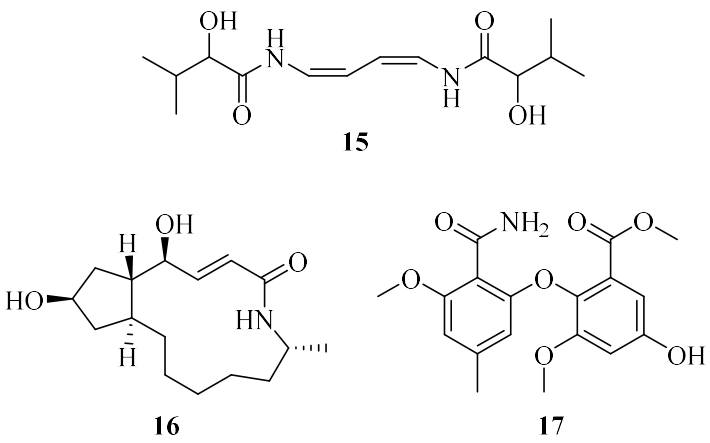


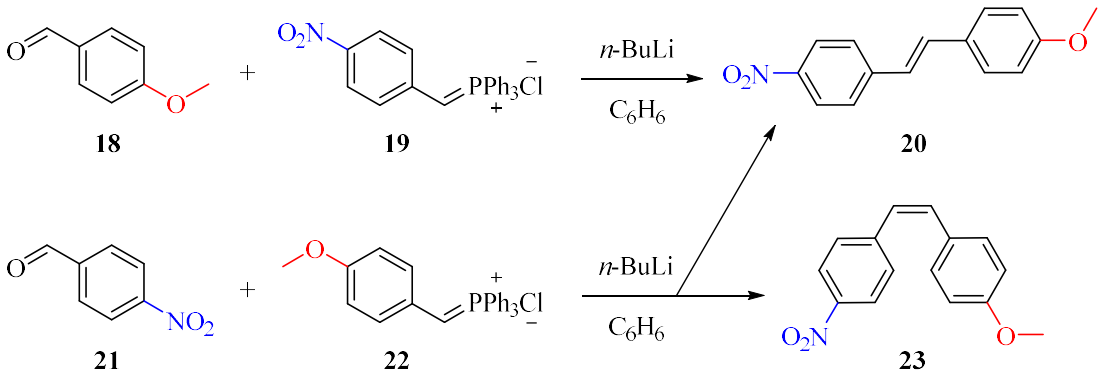
Figure 2.3 Amide alkaloids from endophytic fungi.

2.2.1 Example Synthesis & Application of Stilbenes

As the skeleton framework of 1,3-diarylpropene amide is the homolog of stilbene, a biologically inspired stilbene scaffold will be the main reference of this study. Stilbenes are the secondary metabolites with a C6-C2-C6 skeleton structure, also the phytoalexins in response to pathogens (Langcake & Pryce, 1977). Due to the conjugation of the ethenediyl group excites into p-orbitals, stilbene also exhibits excellent absorption and fluorescence properties, in response to UV radiation in the

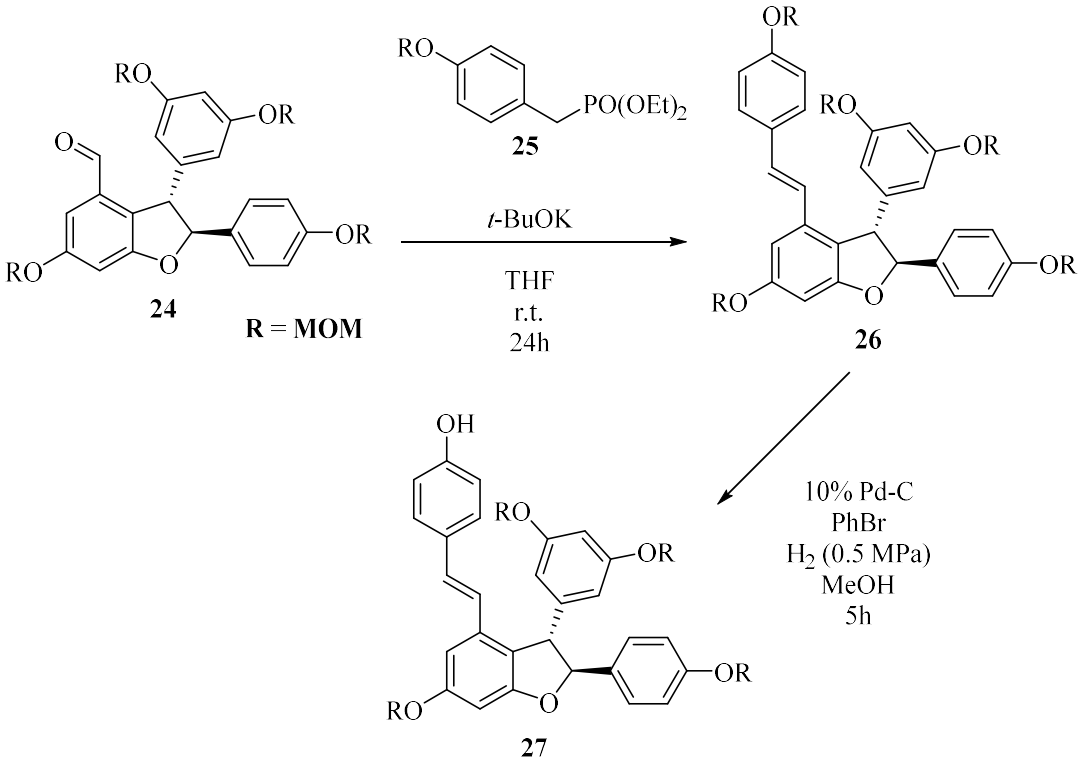
plant (Likhtenshtein, 2009; Rupasinghe, 2015). With the biogenesis and functionality of stilbenes in the plant, it possesses enormous pharmacological activity, such as anticancer, antioxidant, anti-inflammatory, anti-diabetic, cardioprotective, neuroprotective, and anti-aging, which caught the interest of chemists to attempt the synthesis (Błaszczyk et al., 2019; Kataria & Khatkar, 2019; Shaito et al., 2020). Along with the two inactive phenyl groups in the stilbene scaffold, synthetic chemists shifted their attention to the carbon bridge between the phenyl groups by using two approaches: C=C formation and alkene arylation (Giacomini et al., 2016).

The double bond formation approach can be harking back to 1954, an organophosphorus reaction developed by Georg Wittig, using the triphenylphosphonium ylides to cooperate with carbonyls (Wittig & Schöllkopf, 1954). 8 years later, the first attempt from team Ketcham (1962) to synthesize stilbene using Wittig reaction, outlined the electrostatic effects of precursors towards the regioselectivity of *cis*- or *trans*- products. The reaction of *p*-anisaldehyde (**18**) with *p*-nitrobenzyltriphenylphosphonium chloride (**19**) yields 89% (*E*)-4-nitro-4′-methoxystilbene (**20**) solely. Interestingly, in the condition of interchanged substituents for the reactants, the reaction of *p*-nitrobenzaldehyde **21**) with *p*-methoxybenzyltriphenylphosphonium chloride (**22**) yields both *E*/*Z*-isomers of 46% (*E*)-4-nitro-4′-methoxystilbene (**20**) and 43% (*Z*)-4-nitro-4′-methoxystilbene (**23**) respectively, which indicates the unstable anisal phosphonium ylide reacted rapidly towards **21** to form both *threo* and *erthyo* transition states, resulting in both isomers products (Scheme 2.1) (Ketcham et al., 1962).



Scheme 2.1 Wittig reaction between stable and unstable ylide towards aldehyde (Ketcham et al., 1962).

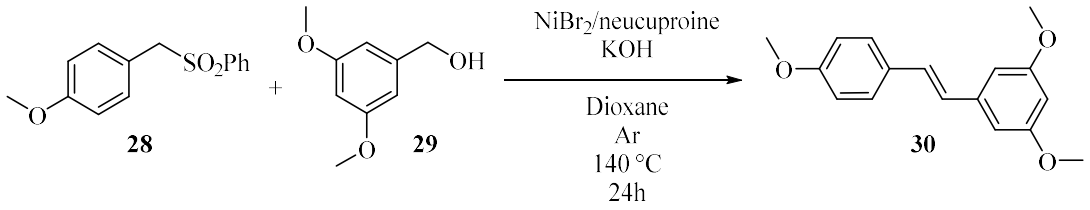
By underlying the foundation of the Wittig reaction, several variants of organophosphorus reactions have been discovered, such as the Horner-Wadsworth-Emmons reaction (HWE) in promoting its high selectivity in forming (*E*)-stilbene. With high alkali tolerance reactant as a precondition, HWE is still one of the popular olefination reactions in the synthesis of (*E*)-stilbene. In the total synthesis of active stilbene dimer (±)-ε-viniferin (**27**), team Zhu (2022) applied HWE olefination towards aldehyde **24** with diethyl 4-methoxy methoxybenzylphosphate (**25**) to yield 93% of Methoxymethyl (MOM) protected ether **26**, under the condition of room temperature for 24 hours, in the presence of *tert*-butylate as a strong base. Along with the deprotection of the MOM group, it yields 97% of (±)-ε-viniferin (**27**) (Scheme 2.2) (Zhu et al., 2022), which has prominent applications in anti-inflammatory, antioxidant, anticancer, anti-obese, vascular protective, and neuroprotective properties (Beaumont et al., 2022).



Scheme 2.2 Horner-Wadsworth-Emmons reaction in the total synthesis of (±)-ε-viniferin (**27**) (Zhu et al., 2022).

Aside from phosphorus-activated olefination, organosulfur reactions such as classical Julia olefination and its variants have been applied to the synthesis of stilbene scaffold as well (Giacomini et al., 2016). Instead of the formation of phosphonium ylide, Julia reaction high dependent on the sulfones in inducing the relatively stable carbanions formation and applied various mechanisms towards such precondition (Giacomini et al., 2016). In implementing the concept of green chemistry, team Waiba (2019) developed a Julia olefination protocol for primary alcohols under mild conditions, without external redox reagent, and with low catalyst loading. By reacting sulfone precursor **28** towards (3,5-dimethoxyphenyl)methanol (**29**) under Nickel salt, ligands, and KOH as the base, under Argon atmosphere at 140 °C in 1,4-dioxane for 24 hours, yields 76% (*E*)-stilbene DMU-212 (**30**)

(Scheme 2.3) (Waiba et al., 2019), a potent stilbene exhibits remarkable antitumor and anticarcinogenic properties (Piotrowska et al., 2014).

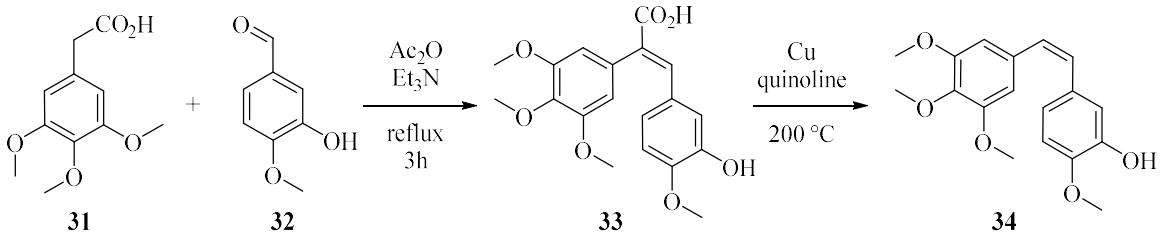


Scheme 2.3 Julia olefination reaction in the synthesis of DMU-212 (**30**) (Waiba et al., 2019).

Although both phosphorus and sulfur-activated olefination show excellent yield production, the excess by-products are the drawbacks of this particular method. The unnecessary by-products make the chemical production in low economic practicality, especially when it applies to the industrial scale (Giacomini et al., 2016).

Other than the olefination method, the alternative of carbon double bond formation can be the Aldol method in the stilbene synthesis, specifically the Perkin reaction. The Perkin reaction involved the condensation of carboxylic acid anhydride and aldehyde under a mild basic condition, to form unsaturated carboxylic acid, particularly for the synthesis of coumarin by Perkin in 1868 (Perkin, 1868). Although Perkin did examine the reaction and unintentionally formed stilbene by the alternative pathway out of his discovered method (Perkin & Hodgkinson, 1880), fortunately with his contribution, the early attempt to synthesize stilbene using the Perkin reaction was initiated after decades, to yield substituted stilbene and diphenylbutadiene (Bergmann & Weinberg, 1941). Worth to mention that the synthesis of stilbene in the high (*Z*)-selectivity manner has been reported by team Gaukroger (2001), starts with the Perkin reaction of 3,4,5-trimethoxyphenyl acetic acid (**31**) with isovanillin (**32**) forms cinnamic acid (**33**), furthered by the

decarboxylation step to yield 70% (*Z*)-combretastatin A-4 (**34**) (Scheme 2.4) (Gaukroger et al., 2001), a powerful inhibitor of tubulin polymerization, antineoplastic and potent anticancer agents towards L1210 murine leukemia cells (Lin et al., 1988).



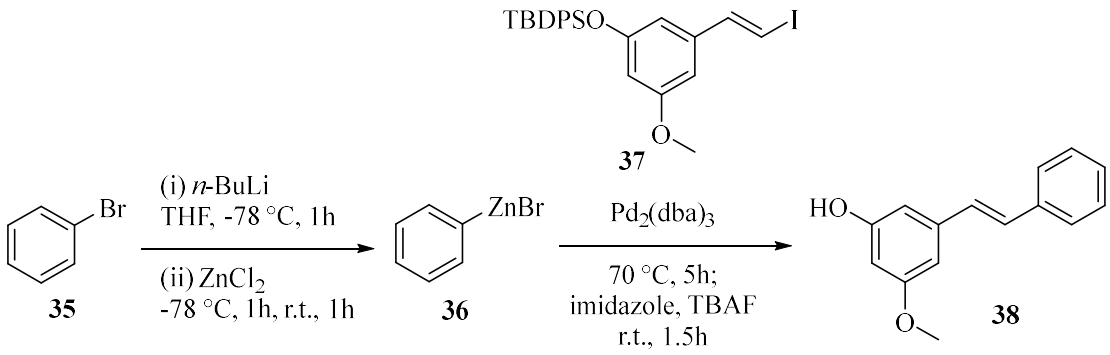
Scheme 2.4 Perkin condensation reaction in the synthesis of *Z*-CA4 (**34**) (Gaukroger et al., 2001).

Apart from the mainstream approaches to forming carbon double bonds in the synthesis of stilbene, under the same hub, there are methods such as olefin cross-metathesis, McMurry coupling, Siegrist reaction, alkyne reduction, and other miscellaneous methods, that are less popular due to their limitations (Giacomini et al., 2016). Concerning the context and focus of this study, these reactions are less relevant for the intensive discussion in this section.

The rise of palladium chemistry revealed another possibility in the carbon-carbon formation through the alkene arylation approach. The Pd-catalyzed methods gained popularity globally because of the efficient and convenient settings. Accordingly, the rapid development in synthesizing stilbene using the Pd-catalyzed cross-coupling reactions such as Negishi, Suzuki, Sonogashira, and Mizoroki-Heck coupling has been actively explored even after decades since the pioneering year (Giacomini et al., 2016; Khan et al., 2017).

In the mid-1970s, along with the discovery of Negishi coupling, intensive studies, and expansion of the domains in application locus and selectivity enhancement has been done. The Negishi coupling becomes more established with the findings such as the zinc salts as reaction promoter, palladium catalyst more favorable than nickel catalyst, and the ligands or additives performance in the reaction (Negishi et al., 2005). In the scope of stilbene synthesis, the general protocol is the reaction between aryl/vinyl zinc- reactants and aryl/vinyl halide to yield stilbenes, with the presence of palladium catalyst.

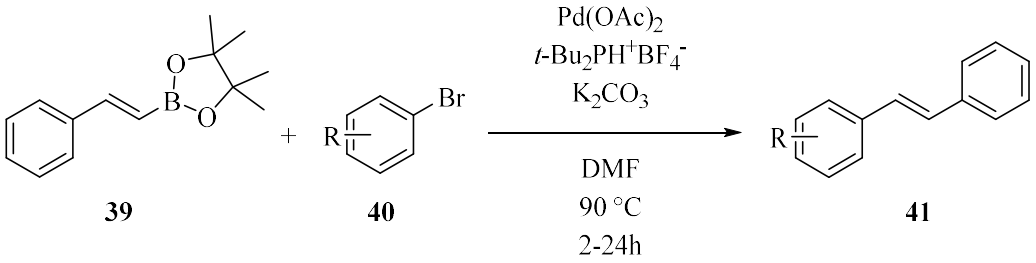
Team Kabir (2007) successfully synthesized eight (*E*)-stilbene analogs with a good yield of 60-78%, using the Negishi coupling method, meanwhile, other six (*E*)-styrylthiophenes yielded in the excellent manner of 81-88% although they reported incorrectly under the term (*E*)-stilbenes. The reaction has been optimized through the proportion of reactants, to maximize the hetero couple′s products and removed the undesirable homocoupled products. The lithiation took place, followed by the transmetallation with $ZnCl_2$ towards aryl halide **35** to form the aryl zinc reagent **36**. Then, the Negishi cross-coupling happened between aryl zinc reagent **36** and arylvinyliodide **37** with palladium catalyst to obtain (*E*)-stilbenes **38** (Scheme 2.5) (Kabir et al., 2007), which have antimicrobial activities (Monte et al., 2007). Perhaps of the complexity in the preparation of this method, no literature on stilbene synthesis using the Negishi method within the recent 5-year reports.



Scheme 2.5 The preparation of aryl zinc reagent and the Negishi coupling reaction (Kabir et al., 2007).

As with the Negishi cross-coupling reaction, the organometallic reagent used in Suzuki coupling is the organoboron reagent as the nucleophile, by transferring the organic moiety to the palladium catalyst via transmetalation reaction under a basic condition (Suzuki, 1982). In comparison with the former aforementioned, Suzuki coupling is more widely used in the stilbene synthesis, on account of its expedient preparation of arylboronic acids or borates from trialkylborates using Grignard or organolithium reagents, apposite to the industrial production besides laboratory scale (Wu et al., 2010). In one of the recent works, the addition of *t*-Bu_3PHBF_4 ligand in the Suzuki reaction enhances the stereoselectivity of (*E*)-stilbene formation; and improves the flexibility in adapting diverse substrates electronically and sterically (Rau & Werner, 2018). The pinacol boronic ester **39** coupled with aryl bromide **40** gained (*E*)-stilbene analogs **41** with a range yield of 44-76% (Scheme 2.6) (Rau & Werner, 2018). However, similar to Negishi coupling, Suzuki coupling is still not the preferred method of this study due to the reactant complexity, the organometallic reagent preparation, time efficiency, or even synthesis yield performance, as aforementioned above in the literature shreds of evidence briefly. Hereby, instead of

using organometallic reagents, another two alternatives involving alkenes and alkynes such as Sonogashira and Heck coupling reactions are taken into consideration.

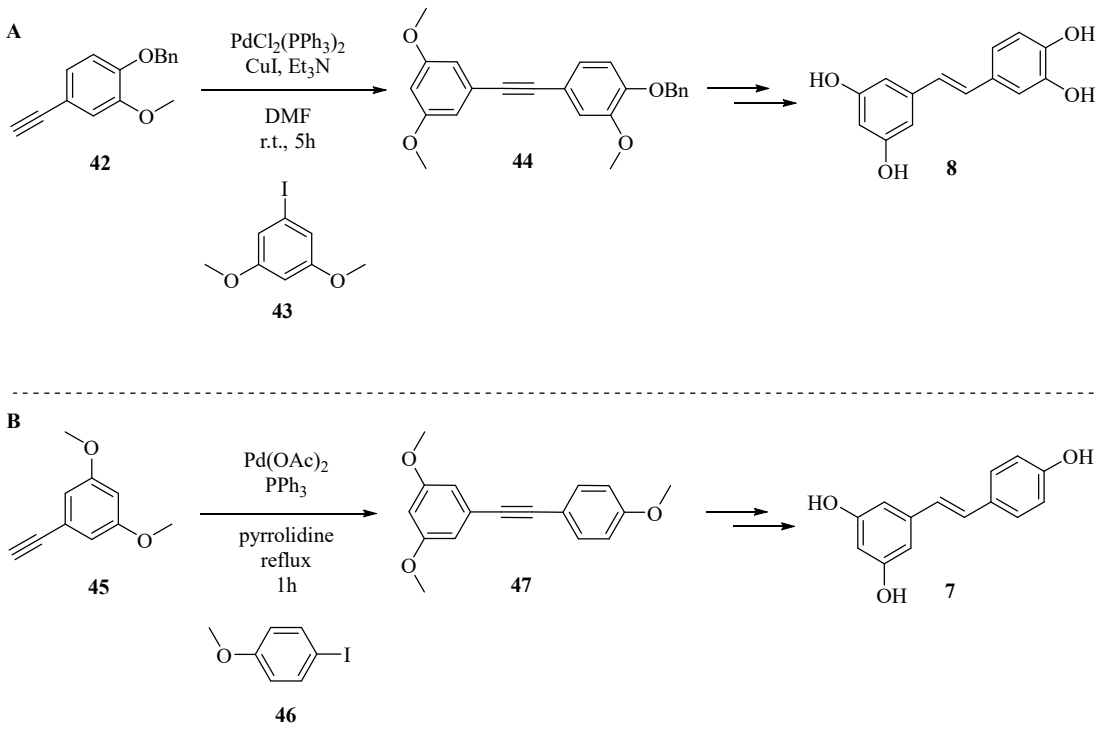


R = H, naph, 4-Me, 2-Me, 4-NMe_2, 4-NO_2, 4-Cl, 2-Cl, 4-CN, 4-Ac, 4-CF_3

Scheme 2.6 Suzuki cross-coupling reaction in the synthesis of stilbene scaffold (Rau & Werner, 2018).

By cooperating with the palladium catalyst and copper salts, typically, the formation of a carbon-carbon bond using Sonogashira coupling reacts acyl halides with alkynes (Sonogashira et al., 1975). The strategy to synthesize stilbene-related scaffolds, generally, involves three phases: alkyne preparation, Sonogashira coupling, and the reduction of alkyne to an alkene. Team Su (2008) published the 6-step synthesis of piceatannol (**8**), starting with the preparation of ethnylbenzene **42** by Colvin rearrangement, followed by Sonogashira coupling with 1-iodo-3,5-dimethoxybenzene (**43**) to gain acethylene **44**, subsequently, three more steps to form the target product piceatannol (**8**) (Scheme 2.7A). In the meantime, Sonogashira coupling retains the use of co-catalysts palladium and copper, particularly the synthesis of stilbene, until team Lara-Ochoa (2015) withdraw the use of copper salt and showed the Sonogashira coupling still performed well under palladium catalyst solely in synthesizing resveratrol (**7**). The 4-step synthesis initiated with the Sonogashira coupling of 3,5-dimethoxy-1-ethynylbenzene (**45**)

with 4-iodoanisole (**46**), afforded acethylene **47** followed by the $LiAlH_4$ reduction, diphenyldisulfide-assisted isomerization, and methoxy deprotection gave resveratrol (**7**) in 70% yield (Scheme 2.7B) (Lara-Ochoa et al., 2015). In contemplating the post-protocols after the Sonogashira coupling in an unsatisfactory manner, especially the extra synthesis steps, and the uncertain fashion of reduction to form *E*/*Z*-isomers mixture, the tools to synthesize stilbene headed to the last option: Mirozoki-Heck cross-coupling reaction.



Scheme 2.7 Sonogashira coupling reaction: **A** The synthesis of piceatannol (**8**) with Pd and Cu co-catalysts (Su et al., 2008); **B** The synthesis of resveratrol (**7**) with Pd catalyst solely (Lara-Ochoa et al., 2015).

The Heck reaction has perpetuated its significance in synthetic organic chemistry for over half a century. Indeed, the introduction of this carbon-carbon bond-making cross-coupling reaction by Heck and Mizoroki has revolutionized synthetic methodology, thanks to its simplicity and effectiveness (Mizoroki et al.,

1971; Heck & Nolley, 1972; De Meijere & Meyer, 1995). Since the first detailed mechanism was proposed by Heck and Nolley in 1972 (Heck & Nolley, 1972), the elementary steps, reaction parameters, and catalytic systems have counted among the major aspects attracting the interest of many researchers (Beletskaya & Cheprakov, 2000; Jagtap, 2017). Unfortunately, with the dawn of Tsutomu Mizoroki because of cancer at a young age, only Richard Heck followed up his work, gradually, the name Heck reaction been commonly used instead of Mizoroki-Heck reaction (Wu et al., 2010). The Heck reaction is performed as the arylation or vinylation of olefins with aryl halides, in the presence of Pd catalyst, base, and solvent. In the case of stilbene synthesis, the commonly reported strategy is the Heck reaction between styrenes and aryl halides (Becker, 1983).

In a close inspection of Richard Heck′s findings, the pioneering move to synthesize stilbene using his own discovered reaction has been carried out in several parameters, obtaining the following crucial fundamentals: 1. The activation temperature of the Heck reaction is in the range of 80-120 °C, pointedly 120 °C more preferable if the reactant is aryl halides; 2. The addition trend of organopalladium intermediate to the olefin is predictable on a steric factor basis and electronic factor, which toward more electron-deficient double bond carbon; 3. The reaction is favorable with the smaller triphenylphosphine as ligands instead of tri-*o*-tolylphosphine due to the insufficient space around the aryl group on the palladium center; 4. The use of palladium acetate and triethylamine is recyclable and usable for another two times of reaction (Heck, 1979). The practicality of the Heck reaction in stilbene synthesis is still widely used by synthetic chemists even in the modern era. For instance, one of the recent Heck applications in the synthesis of stilbene was published with the use of imidazolium-based tunable aryl alkyl ionic