SYNTHESIS OF CARBOXAMIDOSTILBENE ANALOGUES VIA HECK-CROSS COUPLING REACTION, *IN SILICO* AND ANTIDIABETES EVALUATION

MAHDI BABAI

UNIVERSITI SAINS MALAYSIA

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by

MAHDI BABAI

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LIST OF SYMBOLS

α	Alpha
β	Beta
δ	Chemical shift
λ_{max}	Maximum wavelength
nm	Nanometer
mL	Millimolar
mg/mL	Milligram per Milliliter
µg/mL	Microgram per Milliliter
µM	Micromolar
ºC	Degree Celsius
Å	Angstrom
J	Coupling constant
s	Singlet
d	Doublet
dd	Doublet of doublets
dt	Doublet of triplets
t	Triplet
Hz	Hertz
$gmol^{-1}$	Gram per mol
g	Gram
M	Molar
mg	Milligram
MHz	Mega Hertz
cm	Centimeter
cm^{-1}	Per centimeter

δc	Chemical shift carbon
δ_H	Chemical shift proton
nM	Nano molar
o-	*Ortho*
p-	*Para*
ppm	Parts per million
ppb	Parts per billion
%	Percentage
%T	Percentage transmittance
% Yield	Percentage yield
^{1}H	Proton
R	Substituent group

LIST OF ABBREVIATIONS

ADME	Adsorption, distribution, metabolism, and Excretion
ATR	Attenuated Total Reflectance
BBB	Blood-Brain Barrier
b. p.	Boiling Point
cat	Catalyst
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
CH	Methine
CH_2	Methylene
CH_3	Methyl
$CHCl_3$	Trichloromethane
COSY	Correlation Spectroscopy
CVC	Cardiovascular disease
CYP1A2	Cytochrome P450 family 1 subfamily A member 2
CYP2C19	Cytochrome P450 family 2 subfamily C member 19
CYP2C9	Cytochrome P450 family 2 subfamily C member 9
CYP3A4	Cytochrome P450 family 3 subfamily A member 4
DEPT	Distortionless Enhancement by Polarization Transfer
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic acid
DMU-212	*trans*-3,4,5,4′-tetramethoxystilbene

Equiv	Equivalent
Et_3N	Triethylamine
EtOAc	Ethyl acetate
FTIR	Fourier transform infrared
F6P	Fructose-6-phosphate
GFAT	Glutamine fructose-6-phosphate amidotransferase
GlcN6P	Glucosamine-6-phosphate
GIA	Gastrointestinal absorption
HDL	High Density lipoprotein
HMBC	Heteronuclear Multiple Bond Correlation
HSD	11β-hydroxysteroid dehydrogenase
HSQC	Heteronuclear Single Quantum Correlation
HRMS	High resolution mass spectrometry
IC_{50}	Inhibition concentration at 50%
IL-1β	Interleukin 1 beta
IR	Infrared
KOH	Potassium hydroxide
LDL	Low Density lipoprotein
Log P	Logarithm of partition coefficient of compound between n-octane and water
MeCN	Methyl cyanide
MeOH	Methanol
m. p.	Melting point
MR	Molar Refractivity
MW	Molecular weight

NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate oxidase
NH_4Cl	Ammonium chloride
Na_2CO_3	Sodium carbonate
NMR	Nuclear magnetic resonance
nHBD	Number of Hydrogen bond donor
nHBA	Number of Hydrogen bond acceptor
nrot B	Number of rotatable bonds
OCH_3	Methoxy
OH	Hydroxyl
PDB	Protein data bank
$Pd(OAc)_2$	Palladium(II) acetate
Ph	Phenyl
Rf	Retention factor/ Retardation factor
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen specie
RVS	Resveratrol
s	singlet
SAR	Structure-activity relationship
SDR	Short-chain dehydrogenase/reductase
TEA	triethylamine
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethyl silane

TNF-α	Tumor Necrosis Factor Alpha
TOF	Time of flight
TSPA	Topological polar surface area
UV	Ultraviolet
UDP-GlcNAc	Uridine diphosphate *N*-acetylglucosamine

LIST OF APPENDICES

SINTESIS ANALOG KARBOKSAMIDOSTILBENA MELALUI TINDAK BALAS GANDINGAN SILANG HECK, PENILAIAN *IN SILICO* DAN ANTIDIABETIK

ABSTRAK

Satu siri baru sebatian terbitan *orto*-karboksamidostilbena dan *para*-karboksamidostilbena telah disintesis melalui tindak balas gandingan Heck. Struktur sebatian yang disintesis telah disahkan oleh pelbagai analisis spektroskopi, termasuk FTIR, HRESIMS, 1D-NMR (^{1}H NMR dan ^{13}C NMR) serta 2D-NMR (COSY dan HMBC). Karboksamidostilbena telah disintesis dengan tindak balas iodofenilasilamida dalam DMF kering bersama stirena dan Et_3N sebagai bes dengan kehadiran paladium(II) asetat ($Pd(OAc)_2$). Kesemua karboksamidostilbena telah disaring secara *in vitro* untuk sifat perencatan *α*-amilase menggunakan resveratrol dan acarbose sebagai drug rujukan. Sebatian-sebatian ini menunjukkan aktiviti perencatan dari sederhana hingga baik, dengan nilai IC_{50} berjulat antara 13.3 dan 28.2 μM, berbanding drug rujukan resveratrol dan acarbose (masing-masing, IC_{50} = 35.0 ± 3.5, 30.2 ± 0.1 μM). Antaranya, sebatian **186e**, **186f**, **187e**, **191d**, **191e**, **191f**, **192c**, **192d**, **192e** dan **192f** menunjukkan aktiviti perencatan *α*-amilase yang ketara, dengan nilai IC_{50} antara 15 hingga 38 μM. Analisis hubungan struktur-aktiviti (SAR) telah dijalankan untuk memastikan hubungan antara struktur kimia dan aktiviti perencatan *α*-amilase. Pengedokan molekul *in siliko* digunakan untuk menunjukkan interaksi pengikatan *orto*- dan *para*-karboksamidostilbena dengan *α*-amilase. Sifat farmakokinetik (ADME) dan keserupaan drug turut dikaji. Kajian pengedokan molekul menunjukkan interaksi yang kuat antara molekul yang dikaji dengan pengikat *α*-amilase. Dapatan tenaga pengikat adalah berjulat dari −8.3 hingga −9.1 kcal/mol, berbanding akarbosa, yang menunjukkan −7.8 kcal/mol. Hasil ramalan keserupaan drug menunjukkan bahawa sebatian-sebatian yang

disintesis mengikut aturan lima Lipinski, mencadangkan potensinya sebagai molekul seperti drug. Tambahan pula, penilaian sifat ADME menunjukkan profil penyerapan optimum, terutamanya berkenaan dengan penyerapan usus manusia (HIA). Sebatian **186e**, **191f**, **192f** dan **192e** menunjukkan potensi aktiviti perencatan yang paling tinggi dan interaksi yang signifikan dengan poket pengikat α-amilase, dengan nilai IC_{50} berjulat antara 13.3 hingga 21.2 µM. Dapatan ini, berserta sifat farmakokinetik, keserupaan drug dan profil pengedokan molekul yang baik, menunjukkan potensi sebatian-sebatian ini berpotensi sebagai agen terapeutik yang berkesan dalam menguruskan diabetes.

SYNTHESIS OF CARBOXAMIDOSTILBENE ANALOGUES VIA HECK-CROSS COUPLING REACTION, *IN SILICO* AND ANTIDIABETES EVALUATION

ABSTRACT

A new series of *ortho*-carboxamidostilbene and *para*-carboxamidostilbene derivatives were synthesised via the Heck coupling reaction. The structures of the synthesised compounds were confirmed by various spectroscopic analyses, including FTIR, HRESIMS, 1D-NMR (^{1}H NMR and ^{13}C NMR), 2D-NMR (COSY and HMBC). Carboxamidostilbenes were synthesised by reacting iodophenyl acylamide in dry DMF with styrene and Et_3N as the base in the presence of palladium (II) acetate ($Pd(OAc)_2$). All carboxamidostilbenes were screened *in vitro* for their *α*-amylase inhibition properties, using resveratrol and acarbose as referenced drugs. The compounds exhibited moderate to good inhibitory activity, with IC_{50} values ranging from 13.3 to 28.2 μM, compared to resveratrol and acarbose (IC_{50} = 35.0 ± 3.5, 30.2 ± 0.1 μM, respectively). Among them, compounds **186e**, **186f**, **187e**, **191d**, **191e**, **191f**, **192c**, **192d**, **192e** and **192f** showed significant *α*-amylase inhibitory activity, with IC_{50} values between 13.3 and 27.9 μM. Structure-activity relationship (SAR) analysis was performed to establish the relationship between chemical structure and *α*-amylase inhibitory activity. *In silico* molecular docking was used to model the binding interactions of the *ortho*- and *para*-carboxamidostilbenes with *α*-amylase. Pharmacokinetic properties (ADME) and drug-likeness were also investigated. Molecular docking studies showed strong interactions between the investigated molecules and the *α*-amylase binding pocket. The binding energy results ranged from −8.3 to −9.1 kcal/mol, compared to that of acarbose, which is −7.8 kcal/mol. The drug-likeness prediction indicated that the synthesised compounds followed Lipinski's rule

of five, suggesting their potential as drug-like molecules. Additionally, the evaluation of ADME properties demonstrated optimal absorption profiles, particularly with respect to human intestinal absorption (HIA). Compounds **186e**, **191f**, **192f**, and **192e** showed the most promising inhibitory activity and significant interactions with the α-amylase binding pocket, with IC_{50} values ranging from 13.3 to 21.2 μM. These results, in conjunction with favourable pharmacokinetic properties, drug-likeness, and molecular docking profiles, suggested the potential of these compounds as effective therapeutic agents in managing diabetes.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

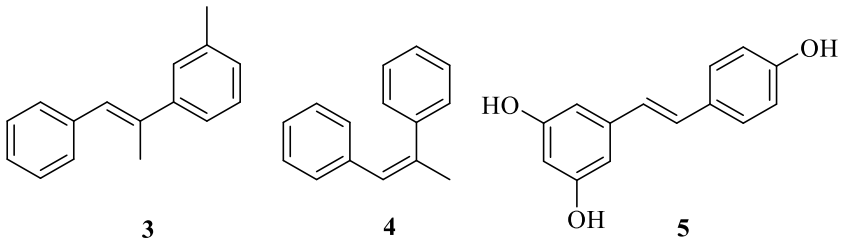
In medicinal chemistry, the selection of a suitable "drug-like" scaffold serves as the first step in the development of a structure tailored to specific molecular targets (Hann *et al*., 2001; Lipinski, 2004). For many years, the chemical peculiarity of stilbene structure has been a source of inspiration to scientists from various fields, including chemistry, biology, pharmacy, and medicine (Dewick, 2009; Madsen & Clausen, 2011). The study of polyphenols, an important group of secondary metabolites that are abundant in plants and plant foods such as grapes, berries, and nuts, is a major focus of research (Kumar & Goel, 2019; Shahidi & Ambigaipalan, 2022; Rodríguez-Gutiérrez *et al*., 2024). Stilbenes exhibit a non-uniform distribution across different plant species with excellent photophysical and photochemical properties (Ams & Wilcos, 2007; Abbey *et al*., 2013). Therefore, stilbene derivatives have been identified in 275 species from 105 genera and 55 families. To date, a total of 1038 stilbene derivatives have been documented, mainly distributed in 11 families, namely Stemonaceae, Dipterocarpaceae, Polygonaceae, Euphorbiaceae, Gnetaceae, Orchidaceae, Cyperaceae, Moraceae, Asparagaceae, Vitaceae and Leguminosae, accounting for 84% of the total stilbene distribution, with fewer occurrences reported in other families (More & Singh, 2015; Teka *et al*., 2022; Xin *et al*, 2022). Extensive research has focused on the functions of stilbenes, investigating their efficacy as antibacterial agents, antioxidants, anti-inflammatory agents, anticancer agents, and cancer prevention agents, and more recently their involvement in the regulation of various degenerative diseases in humans (Ghazarian *et al*., 2011; Kumar *et al*., 2011).

Drug design is an inventive approach that aims to develop new biologically active substances that match the shape and charge of their biological targets so that they can interact and bind effectively to achieve the desired biological effects (Zhou & Zhong, 2017). Stilbene, a structural motif known for its diverse pharmacological activities, serve as a scaffold for these endeavors. They consist two phenyl rings connected by an olefinic bridge, often present in two isomers *trans*-stilbene (**1**) and *cis*-stilbene (**2**) with a planar shape (Mohd Fadzli, 2016). Different conformations exhibit unique properties, exemplified in stilbenes by the remarkable variance in melting points. Studies have shown that the *trans* isomer has higher stability due to steric considerations (Latva-Mäenpää *et al*., 2021). In contrast, the *cis*-stilbene (**2**), which is sterically hindered, exhibits lower stability, as evidenced by its lower melting point of 6ºC. The *trans*-stilbene (**1**), which is more stable, has a significantly higher melting point of around 125 ºC (Likhtenshtein, 2000).

1 **2**

Figure 1.1 Structure of *trans*- and *cis*-stilbenes

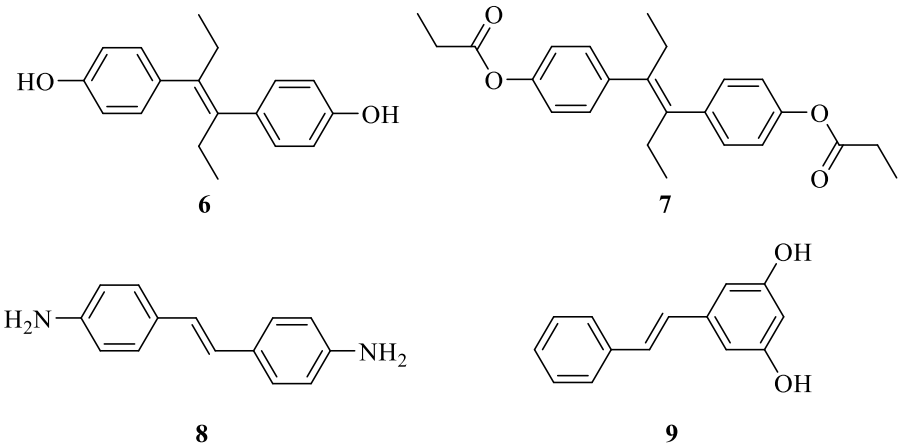
The stilbenes are synthesised in response to UV radiation, toxins, and infections. Stilbenes comprise various subclasses, including oligostilbenes, substituted *trans*-stilbene (**3**), substituted *cis*-stilbene (**4**). Stilbenoids, on the other hand, are hydroxylated forms of stilbenes derived from resveratrol (**5**) (*E*)-5-(4-hydroxystyryl)benzene-1,3-diol) (Kluska *et al*., 2023).



3 **4** **5**

Figure 1.2 Structures of substituted stilbenes and resveratrol

Stilbene derivatives are naturally present in various plant species, while others are synthetically produced (Kluska *et al*., 2023). It has been reported that the derivatives of stilbene such as diethylstilbestrol (**6**), diethylstilbestrol dipropionate (**7**), 4,4'-diaminostilbene (**8**) and 5-[(1*E*)-2-phenylethen-1-yl]benzene-1,3-diol (**9**) have shown impressive pharmacological effects in the treatment of hypocholesterolaemia, sympathomimetic, antifungal, antiallergic, antibacterial, antimalarial, and anticancer properties (Kluska *et al*., 2023; Navarro-Orcajada *et al*., 2022).



6 **7** **8** **9**

Figure 1.3 Chemical structures of various (*E*)-stilbene derivatives

Besides, other stilbenoids including pterostilbene (**10**) (*trans*-3,5-dimethoxy-4-hydroxytoluene), piceatannol (**11**) (*trans*-3,3′,4′,5-tetrahydroxystilbene), exhibit a range of biological activities, such as antioxidant, anti-inflammatory, and potential anticancer effects (Piotrowska *et al*., 2012; Hsieh *et al*., 2013).

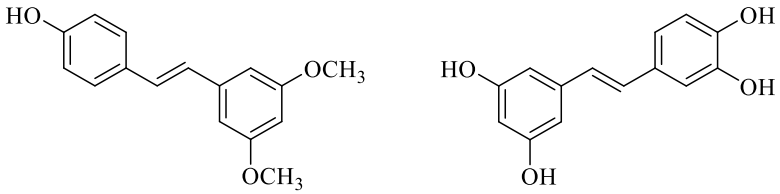


Figure 1.4 Chemical structures of *trans*-3,5-dimethoxy-4-hydroxytoluene and *trans*-3,3,4,5-tetrahydroxystilbene

Stilbenoids can occur in both oligomeric and monomeric form. They can also be present as conjugated glucosides or as free phenol derivatives, also known as aglycones (Kumar *et al*., 2011). Based on their chemical structure, stilbenoids can be categorised into five main groups: simple stilbenes, prenylated and geranylated stilbenes, 2-phenyl-benzofuran derivatives, carbon-substituted stilbenes that do not belong to the group of prenylated and geranylated stilbenes, and various other structures. Examples include puwhuskin A (**12**), rumexoid (**13**) (antidiabetic activity), ω-viniferin (**14**) (antiangiogenic) and latifolol (**15**) (anti-inflammatory) (Mattio *et al*., 2020).

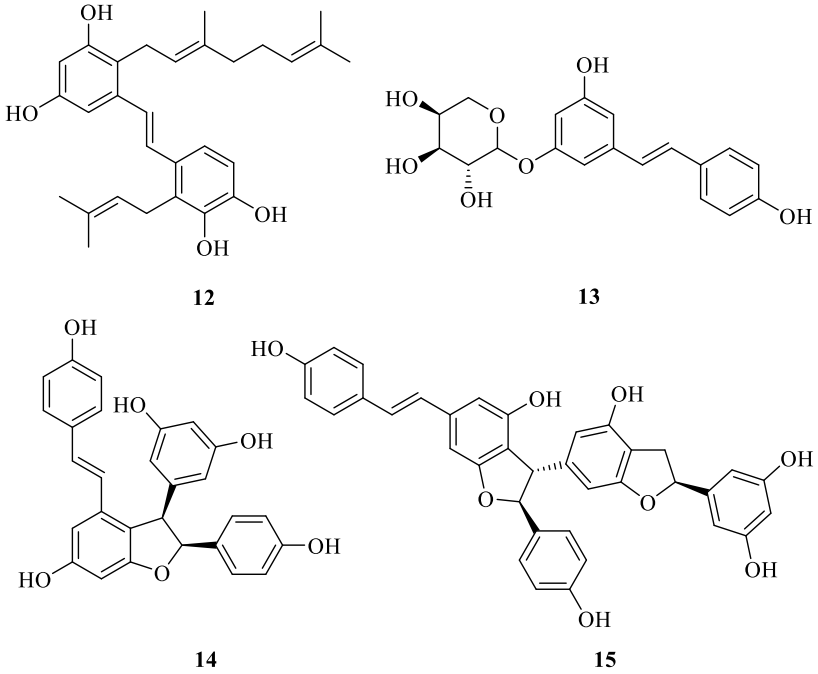


Figure 1.5 Chemical structures of notable polyphenolic compounds

The synthesis of stilbene derivatives is aims to modify the mode of action of drugs, to minimise side effects while increasing efficacy (Singh *et al*., 2022; Goda *et al*., 2023). Therefore, their potential applications have been evaluated either as herbal supplements or as active ingredients in medicinal and cosmetic preparations (Kasiotis *et al*., 2013). Consequently, one of the best-known palladium-catalysed processes for the chemical synthesis of methoxylated stilbenes is the Mizoroki-Heck reaction, often referred to as the arylation of olefins (Mino *et al*., 2012).

As part of our ongoing research, we plan to synthesise a series of carboxamidostilbene derivatives using the Heck coupling reaction, as described by Kee *et al*. (2010); Azmi *et al*. (2013); Mohammad *et al*. (2021). This reaction is a simple and efficient method for the synthesis of carboxamidostilbene compounds. This will involve combining styrene with different iodocarboxamido compounds, which will be our starting materials. The Heck coupling reaction is known for its ability to form carbon-carbon bonds in just one step, allowing us to efficiently produce these derivatives. By using this approach, our goal is to develop a wide range of *ortho*- and *para*-carboxamidostilbene derivatives, which we will then use to evaluate for their potential biological activities and therapeutic applications. The ultimate goal is to contribute to the development of novel therapeutic agents for the treatment of diabetes through the synthesis and characterisation of innovative carboxamidostilbene derivatives (Su *et al*., 2022).

1.2 Problem statement

The rising global prevalence of diabetes, a chronic metabolic disorder characterised by elevated blood glucose levels that cause severe damage to the heart, blood vessels, eyes, kidneys, and nerves (Rendi *et al*., 2021), remains a significant public health challenge in Malaysia and across Asia. This underscores the urgent need for novel and effective therapeutic agents. Each year, an estimated 29.1 million individuals are affected by this condition. According to the National Health and Morbidity Survey (NHMS) conducted by the Ministry of Health Malaysia, the prevalence of diabetes increased from 11.6% in 2006 to 17.5% in 2019 (Ministry of Health Malaysia, 2019). This means that approximately 3.9 million Malaysians are currently living with diabetes (Ministry of Health Malaysia, 2019). Furthermore, the World Health Organization (WHO) predicts that diabetes will become a leading cause of death by 2030, with the death rate expected to double from 2005 to 2030 (Das *et al*., 2018). Despite advancements in antidiabetic medications, many existing treatments have limitations, such as adverse side effects, limited efficacy, and drug resistance. Therefore, there is a critical need to develop new compounds with improved safety profiles and therapeutic efficacy.

The rational design of carboxamidostilbene derivatives as antidiabetic agents is driven by the need for effective, safe, and multi-functional drugs. Their ability to interact with key metabolic targets, improve insulin sensitivity, and mitigate oxidative stress makes them promising candidates for further development in diabetes management, particularly in Malaysia and Asia, where diabetes prevalence continues to rise.

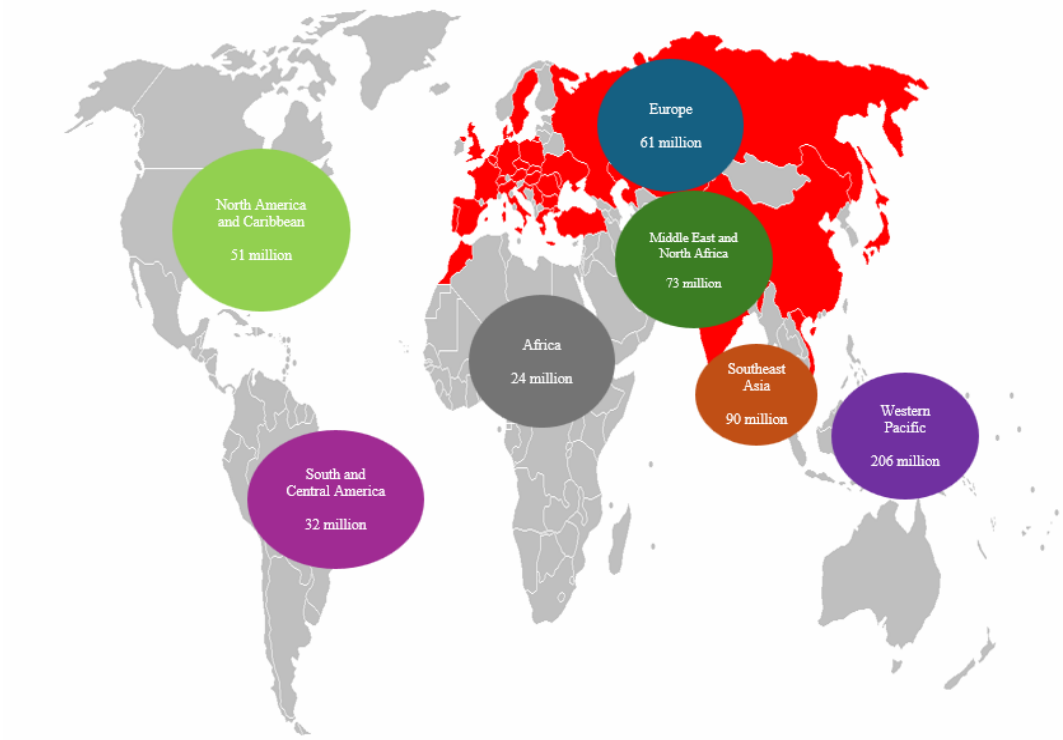


Figure 1.6 Diabetes around the world in 2021

Olefins play a crucial role as essential structural components in natural products, pharmaceuticals, bioactive compounds, and organic materials (Shaikh & Hong, 2013). Olefinic compounds are recognised as a significant metabolically active polyphenol that is currently the focus of research interest as a potential agent to combat insulin resistance (IR) (Shazmeen *et al*., 2021). As these metabolised polyphenols serve as bioavailable end products of metabolism, it is therefore crucial to develop analytical methods to assess the potential effects of these compounds.

1.3 Research objectives

1. To synthesise and characterise new series of *ortho*-carboxamido and *para*-carboxamidostilbene derivatives.
2. To evaluate the inhibitory effects of carboxamidostilbenes on the activities of the α-amylase enzyme.

3. To evaluate the *in silico* drug-like properties, ADME and molecular docking of the active compounds.

1.4 Scope of research

Carboxamidostilbene derivatives were synthesised via Heck coupling, with the methoxy group strategically positioned at the 3,4,5-, 2,4- and 2,5-positions of the aromatic rings. The chemical structures of these synthesised compounds were thoroughly characterised using FTIR, HRESIMS and 1D- and 2D-NMR spectroscopy.

In vitro evaluation on α-amylase enzyme performed at the School of Industrial Technology, Universiti Sains Malaysia, to evaluate the antidiabetic potential of the synthesised compounds. The α-amylase inhibition data were determined in triplicate (n = 3) and expressed as half maximum inhibitory concentration (IC_{50}), allowing quantitative comparison with the reference compound acarbose. In addition, *in silico* molecular docking screening, drug-likeness assessments and analysis of ADME properties (absorption, distribution, metabolism and excretion) were performed at the School of Chemical Sciences using the required software tools.

CHAPTER 2

LITERATURE REVIEW

2.1 Stilbene and its derivatives

Stilbene and its derivatives are categorised as biologically active compounds. Certain derivatives occur naturally in various plant species, while others are produced by synthetic methods (Kluska *et al*., 2023). The structure of stilbene molecule was first elucidated by A. Laurent in 1829, marking a significant advancement in the field of organic chemistry (Kapoor, 1969). His pioneering work laid the groundwork for understanding aromatic compounds and their behaviour, which has continued to influence the synthesis and applications of stilbene derivatives in modern research. Laurent's elucidation demonstrated the potential of molecular structure analysis, emphasizing the importance of selectivity and functional group tolerance in both synthetic and natural systems (Jensen, 2008). Subsequently, numerous stilbene derivatives were synthesised, of which certain compounds exhibited different activities. For many years, the stilbene scaffold has been recognised as an outstanding structure with significant biological potential (Pecyna *et al*., 2020). Therefore, compounds based on the stilbene scaffold, whether natural or synthetic, have shown a wide range of biological activities, including anticancer (De Fillippis *et al*., 2017), antifungal (De Filippis *et al*., 2019), antimicrobial (Singh *et al*., 2019), anti-inflammatory (Dvorakova & Landa, 2017) and neuroprotective (Chen *et al*., 2017) properties. They are also promising potential agents for the treatment of diabetes (Chou *et al*., 2018) and obesity (Li *et al*., 2006).

This popularity has led scientists to synthesise resveratrol and its analogues to test their bioactivity. Resveratrol (**5**) indeed has a low bioavailability as it is metabolised in the liver by sulphation and glucuronidation. The bioavailability can be increase by

the development of resveratrol analogues, which would reduce the activity of the hydroxyl group of resveratrol (Baur & Sinclair 2006). Methoxylation of the hydroxyl groups should prevent the metabolism of polyphenols and increase the bioactivity of stilbenes. Methoxylated analogues of resveratrol have an increased lipophilicity, which would increase their bioavailability as they remain in the body longer instead of being excreted from the body (Zeka *et al*., 2018). Methoxylated analogues of resveratrol such as *trans* 3,4,5,4′-tetramethoxystilbene (DMU 212) (**16**) were synthesised by the Wittig reaction. Other analogues are synthesised by the Horner-Wittig reaction, such as 2,4,4′-trimethoxystilbene (**17**) and 3,4′,5-trimethoxystilbene (**18**). The mechanism of the Horner-Wittig reaction is like that of the Wittig reaction, it is a modification developed by Leopold Horner. The only difference is that the Horner-Wittig reaction selectively generates the *trans* formation of stilbene, while the Wittig reaction generates both *trans* and *cis* products (Zeka *et al*., 2018).

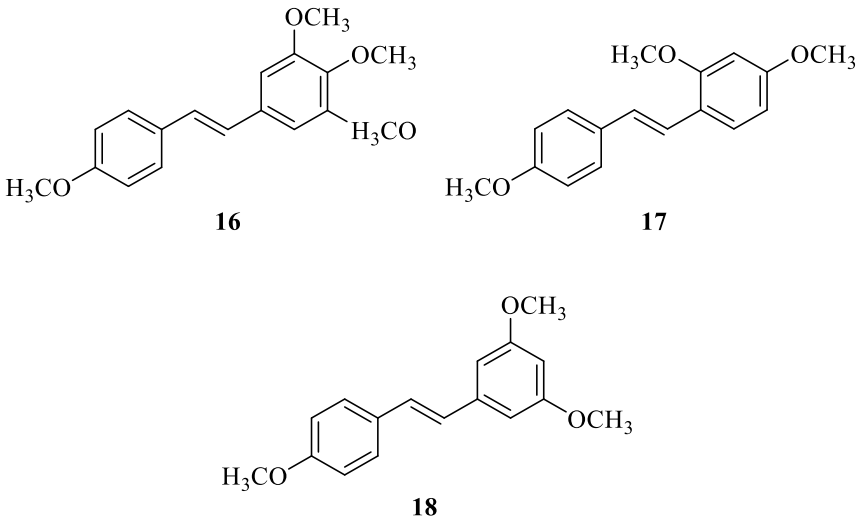


Figure 2.1 Structures of methoxylated stilbene derivatives

2.2 Synthesis of stilbene and its derivatives

Numerous synthetic routes to stilbene derivatives are known, especially those used in total synthesis. The key step in the construction of the stilbene backbone is the formation of the carbon-carbon double bond (Giacomini *et al*., 2016). Stilbene derivatives can be synthesised by using two techniques:

1. Palladium catalysed synthesis.
2. Non palladium catalysed synthesis.

2.2.1 Palladium catalysed synthesis of stilbenes

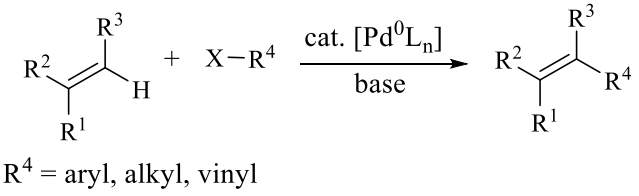
Palladium-catalysed synthesis of stilbenes is a prominent method in organic chemistry, particularly for the formation of carbon-carbon double bonds. The palladium-catalysed reactions, such as the Heck reaction and Suzuki coupling, are widely utilized for their efficiency and selectivity in synthesising these compounds (Jagtap, 2017). In these reactions, palladium acts as a catalyst to facilitate the coupling of aryl halides with alkenes or organoboron, forming the stilbene framework. The process typically occurs under mild conditions and offers a high degree of functional group tolerance, making it versatile for various stilbene derivatives (Gligorijevic *et al*., 2021). There are a variety of reported synthetic routes to stilbene derivatives. However, in this project, the most crucial techniques that have been used in total synthesis were considered:

2.2.1(a) Heck coupling reaction

The Heck reaction, also known as the Mizoroki-Heck reaction, is a widely recognised technique for catalysing the reaction of aryl halides with alkenes to form a substituted alkene (Xue & Lin, 2010; Ruiz-Castillo & Buchwald, 2016; Roy & Uozumi, 2018; Yang *et al*., 2018). This method, facilitated by palladium, is known for its exceptional chemo-selectivity and adaptability to a wide range of functional group

(Surry & Buchwald, 2008; Yang *et al*., 2018). As a result, it has become an important part of modern organic synthesis and was praised for its efficiency and versatility (Karine *et al*., 2004). Since their discovery in the early 1970s, palladium-catalysed cross-coupling reactions have been extensively studied (Xue & Lin 2010; Ruiz & Buchwald 2016; Roy & Uozumi, 2018; Biffis *et al*., 2018; Christoffel & Ward 2018; Gonzalez-sebastian & Morales-Morales, 2019). In the course of their development, these reactions have evolved into new and improved methods for the selective coupling of carbon atoms, not only with other carbon atoms, but also with heteroatoms such as nitrogen, oxygen, and phosphorus (Astruc, 2011).

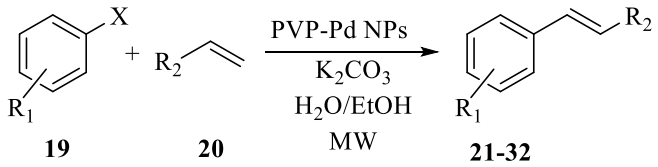
The importance of these reactions was recognised in 2010 when Richard Heck, Ei-ichi Negishi and Akira Suzuki were awarded the Nobel Prize in Chemistry for their fundamental work, which has paved the way for numerous invaluable processes used daily in both academic research and industry (Astruc, 2011). The most common palladium-catalysed cross-coupling reactions using palladium(0) as the catalytic species include the Heck reactions, Stille, Suzuki and Sonogashira (Scheme 2.1) (Nicolaou *et al*., 2005).



Scheme 2.1 Schematic representation of palladium catalysed synthesis of stilbenes

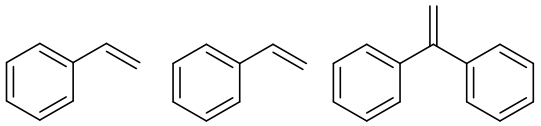
Mizoroki–Heck coupling reactions under microwave irradiation were performed with a colloidal Pd nano catalyst stabilised with polyvinylpyrrolidone (Gonzalez & Morale, 2019). Using this method, numerous stilbenes (**21-32** shown in the scheme below) and innovative heterostilbenes were successfully produced in good to excellent yields using aryl bromides (**19**) and various olefins (**20a-c**) as starting

materials (García *et al*, 2017). The reaction was distinguished by short reaction times and low catalyst loading, leading to high turnover frequencies (TOFs). The protocol offers advantages such as simplicity of operation, high robustness, efficiency, and conversion frequency (García *et al*, 2017). The use of aqueous media and the straightforward product work-up increase the attractiveness of this approach for stilbene syntheses by the Mizoroki–Heck reaction (García *et al*, 2017).



19 **20** **21-32**

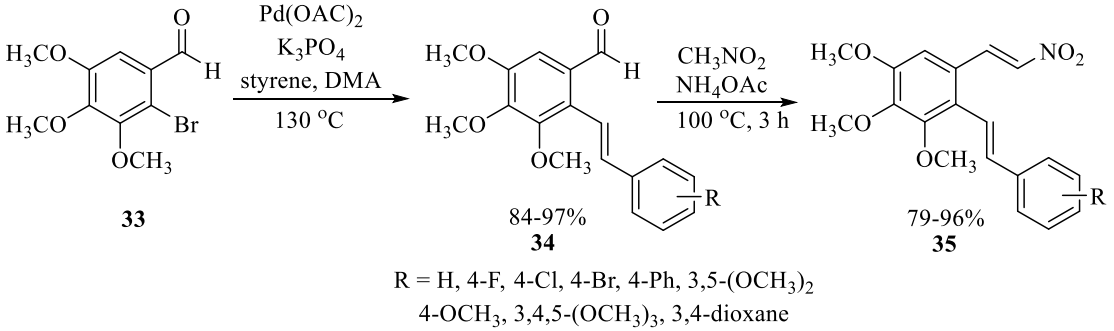
19: X = Br, R_1 = 4-COMe (**19a**); X = Br, R_1 = 4-COPh (**19b**); X = Br, $C_6H_4R_1$ = 3-quinoline (**19c**); X = Br, R_1 = 3,5-diOMe (**19d**); X = Br, R_1 = 4-OMe (**19e**); X = Br, R_1 = 4-Me (**19f**); X = Br, R_1 = 2-Me (**19g**); X = I, R_1 = 4-OH (**19h**)



20a **20b** **20c**

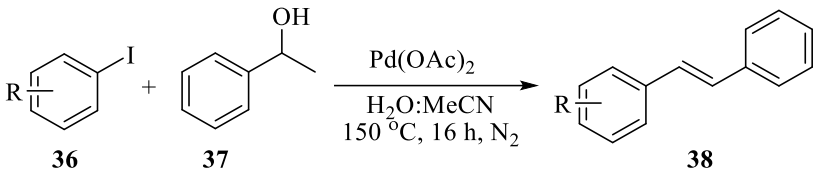
Scheme 2.2 Mizoroki-Heck reaction of aryl halides with olefins catalysed by PVP-Pd NPs (García *et al*, 2017)

Reddy *et al*. successfully synthesised nitrovinyl stilbenes **35** using a Mizoroki-Heck coupling reaction. The synthesis began with intermediate **33**, which was converted into stilbene **34** through a palladium-catalysed Mizoroki–Heck reaction with styrene, achieving yields ranging from 84% to 97%. Subsequently, compound **34** reacted with nitromethane to produce nitrovinyl stilbenes **35**, with yields of 79% to 96%, as illustrated in Scheme 2.3. (Reddy *et al*., 2011).



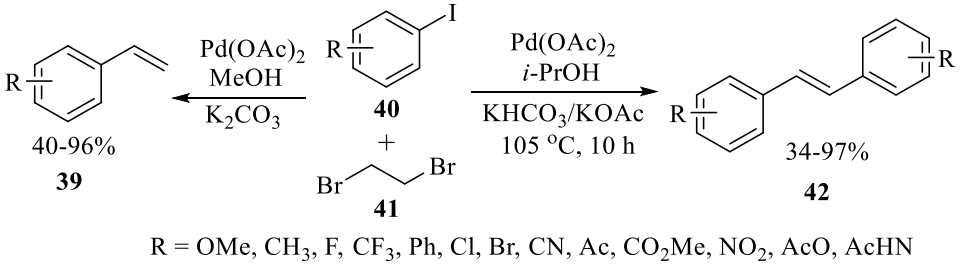
Scheme 2.3 Synthesis of nitrovinyl stilbenes **35**

Similarly, Camp and his co-workers have developed a novel method for the *in situ* formation of palladium nanoparticles. They have also successfully optimised a base-free Mizoroki-Heck cross-coupling method for the synthesis of substituted stilbenes **38** (Bousfield, 2020). In this innovative approach, glucose plays a dual role of stabilising the palladium (0) nanoparticles, and regenerating the active catalyst species. In contrast to conventional protocols, this method does not require the use of a base, resulting in an acidic reaction mixture. It departs from traditional Mizoroki-Heck reactions, where typically alkene substrates react with aryl or alkyl halides to form C-C bonds (Bousfield, 2020). The dehydrating approach was chosen to generate styrene *in situ,* and allow a tandem cross-coupling reaction in a base-free H_2O/MeCN solvent system. This method utilises the acidic conditions of the reaction mixture. After optimisation, the steric, electronic, and substrate-specific possibilities of the reaction were thoroughly investigated. The molar efficiency (mol. E%) was calculated and compared with those obtained from similar methods for the synthesis of substituted stilbenes **38** (Bousfield, 2020).



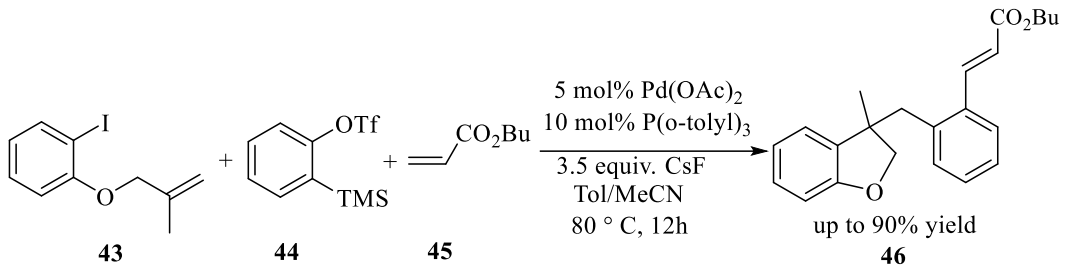
Scheme 2.4 Base-free Mizoroki-Heck cross-coupling for the synthesis of substituted stilbene

In 2024, Guangfa *et al*. conducted a study on the synthesis of styrene **39** and stilbenes **42** using Pd-catalysed vinylation reactions of aryl iodides **40** with 1,2-dibromoethane (**41**). In these reactions, 1,2-dibromoethane (**41**) generates vinyl bromide, which then undergoes reductive cross-coupling with aryl iodides **34** to generate styrene products **39**. By slightly changing the reaction conditions, the styrene **39** can further react with vinyl bromide to form *E*-stilbenes, which is a convenient one-pot method for the synthesis of stilbenes **42**. In the reductive cross-coupling reactions, alcohols such as isopropanol and methanol are used as reducing agents (Guangfa *et al*. 2024).



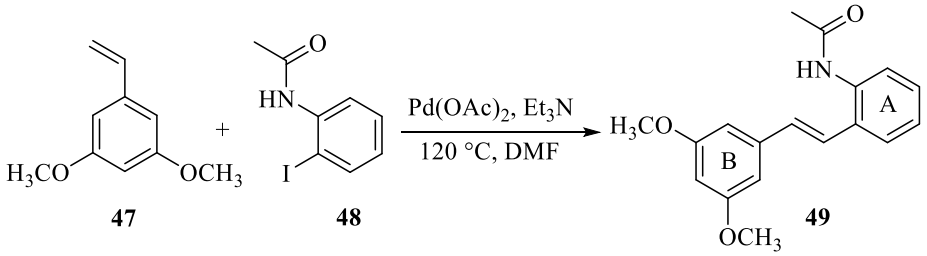
Scheme 2.5 A recent one-pot synthesis of stilbene using Heck reaction with solvent modification

Tuanli *et al*. reported a novel palladium-catalysed multicomponent reaction involving olefin-linked aryl iodides **43**, aryls **44** and electrophilic alkenes **45**. This innovative approach enables the direct synthesis of *o*-alkylated aryl acrylates and stilbenes **46** by an intramolecular tandem process involving Heck cyclisation and dicarbofunctionalisation of the aryne (Tuanli *et al*., 2021).



Scheme 2.6 Synthesis of stilbene scaffold

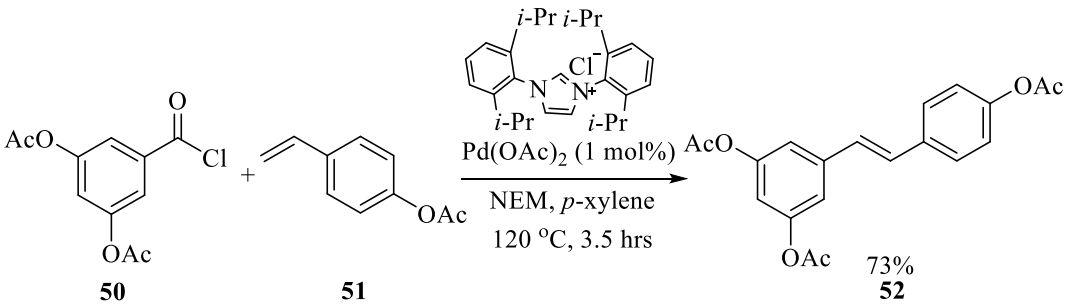
In 2013, Azmi *et al.*, synthesised nineteen *o*-carboxamidostilbenes **49** by Heck coupling by reacting 2-iodoamide **48** with styrene **47**. These compounds had various substituted groups linked to the amide group on ring A, as well as electron-withdrawing groups (Cl, Br and F) and electron-donating groups (methoxy) at different positions on ring B. Among these, three compounds showed remarkable cytotoxicity against three cancer cell lines: Colon cancer, prostate cancer, and breast cancer, with IC_{50} values of 7.51 µM, 16.68 µM and 21.24 µM, respectively (Azmi *et al*., 2013).



Scheme 2.7 Heck reaction of *N*-(2-iodophenyl)acylamide with styrene

The decarbonylative Heck reaction is a frequently used method to produce resveratrol (**5**) and its derivatives **52**. This variant of the Heck reaction has been known since the early 1980s. The pioneering work of Spencer *et al*. demonstrated the palladium-catalysed coupling of alkenes to aroyl chlorides **50** in the presence of a tertiary amine (Spencer, 1983; Moran *et al*., 2009). Spencer also described the arylation of ethylene leading to the formation of styrene and stilbene derivatives **52**. This reaction involves the efficient decarbonylation of the aroyl chloride **50**. It is not particularly

influenced by substituents on the aroyl chloride **50**, although strongly electron-donating groups have been shown to be favourable (Moran *et al*., 2009). Furthermore, when monosubstituted alkenes **51** are used, the *trans* isomer is formed with complete specificity (Spencer, 1983).



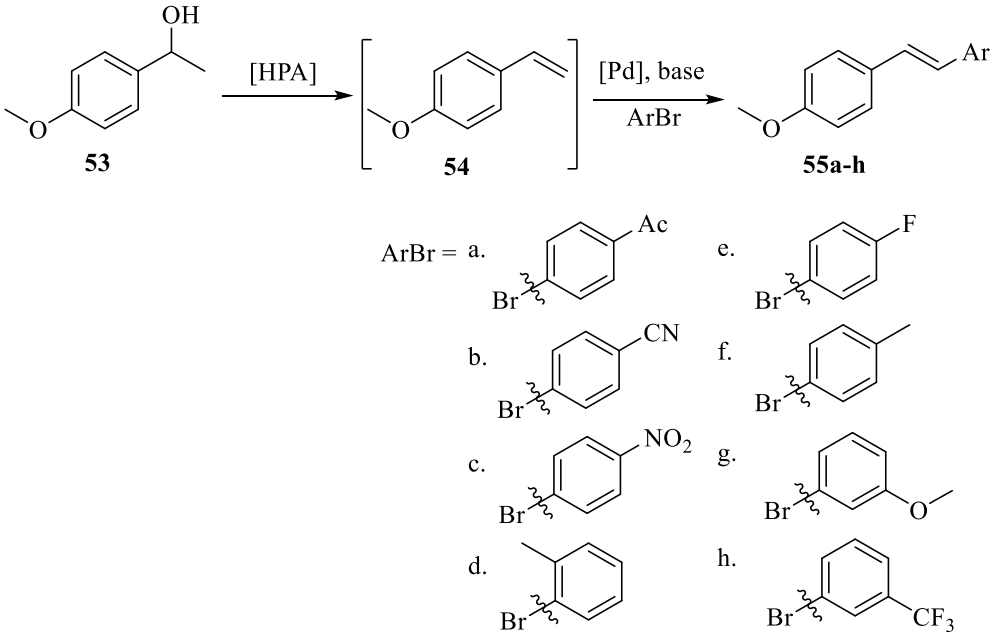
50 **51** **52**

Scheme 2.8 Decarbonylative Heck reaction

The decarbonylative Heck reaction shows optimal performance when tertiary bases with pKa values between 7.5 and 11 are used. It was observed that weaker bases resulted in slower reaction rates, while stronger bases interacted undesirably with the aroyl chloride **50**, leading to side reactions or reduced efficacy (Spencer, 1983). *N*-ethyl morpholine emerged as the most effective base because it did not react with aroyl chlorides **50** and maintained the integrity of bonds between the aroyl chloride **50** and strong electron-withdrawing groups. To optimise the reaction, *p*-xylene was used as the solvent, and the reaction temperature was maintained between 120 and 130 °C, which produced the most favourable outcomes (Spencer, 1983).

In 2012, Paul John Joseph Colbon developed a two-step one-pot reaction. First, the secondary aryl alcohol **53** was dehydrated in the presence of heteropoly acid (HPA) to facilitate the reaction. A mixture of secondary aryl alcohol **53** and HPA in DMSO was stirred at 100°C for 1 hour. A mixture containing aryl bromide, Et_3N, $Pd(dba)_2$ and $P(t\text{-}Bu)_3.HBF_4$ in DMF was then added and the resulting mixture was stirred for a further 4 hours, leading to the efficient formation of the desired stilbene product **55** with

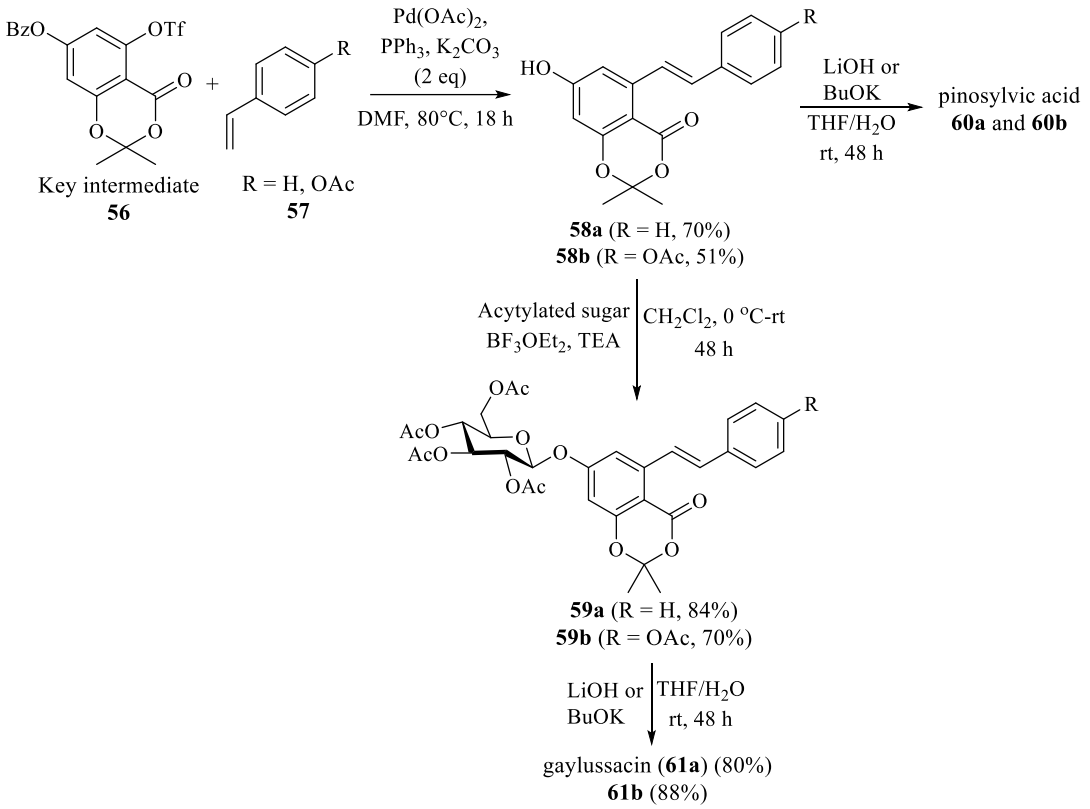
an isolated yield of 82%. The reaction cascade was performed in a sequential manner, exhibiting noteworthy tolerance towards various functionalities that are present on the aryl bromide. As a result, functionalised stilbenes were obtained in favourable yields. Notably, both electron-rich and electron-poor aryl bromides were effectively utilised, with substitutions at the *ortho*-, *meta*-, and *para*-positions presenting no significant hindrance. The demonstrated versatility underscores the robust nature of this method, making it highly suitable for the synthesis of a wide range of stilbene derivatives **55** (Colbon, 2012).



Scheme 2.9 Dehydration Heck arylation of benzylic alcohol **55** with aryl bromides

Song and colleagues have successfully performed the first total synthesis of gaylussacin (**61a**), pinosylvikinic acid (**60a**) and novel stilbene derivatives **61b** and **60b** (Song *et al.*, 2021). An important intermediate **56**, was synthesised in three steps by selective protection and functionalisation of commercially available 2,4,6-trihydroxybenzoic acid. The stilbene segment was efficiently constructed via the Heck reaction while simultaneously removing the benzoyl group. Gaylussacin **61a** and **61b**

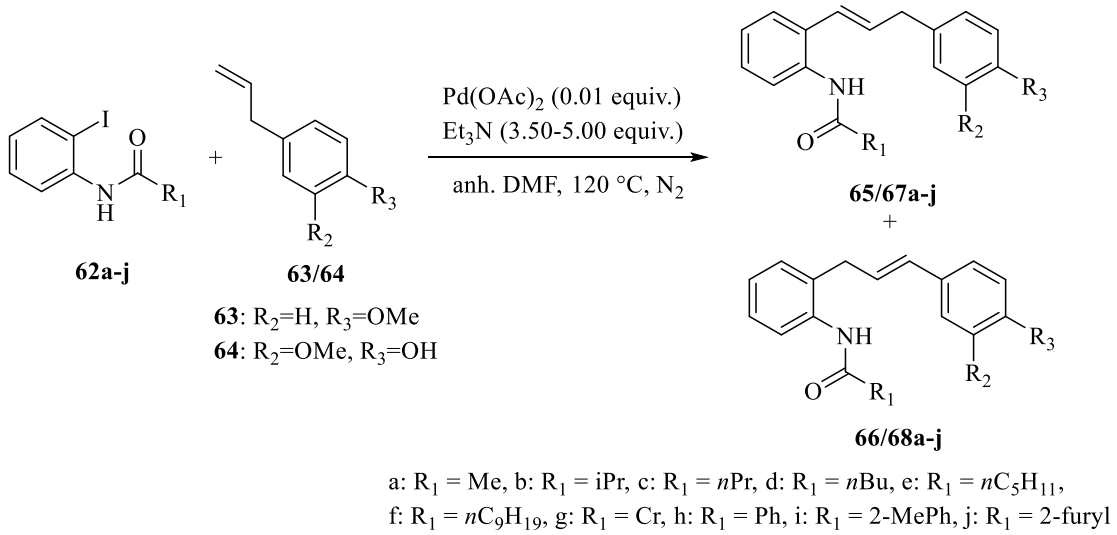
were obtained with an overall yield of 23.8% and 15.9%, respectively. In addition, pinosylvinic acid (**60a**) and resveratronic acid (**60b**) were synthesised via a modified route with an overall yield of 25.3% and 26.4%, respectively (Song *et al*., 2021). Furthermore, the developed synthetic route was utilised to produce a phthalide compound. This investigation provides a concise and effective synthetic pathway for 2-carboxyl stilbene compounds, which is important for the future design and synthesis of stilbene glycoside derivatives (Song *et al*., 2021).



Scheme 2.10 Synthesis of Gaylussacin **(61a)** and its derivative **(61b)**

Recently, Sian conducted research in which forty (40) 1,3-diarylpropene derivatives **65-68** were synthesised and reported. These analogues were designed based on stilbenoids and dihydrostilbenoids which were synthesised using palladium-catalysed reactions with 2-amidoiodobenzene derivatives **62** and either estragole (**63**) or eugenol (**64**). The products showed high (*E*)-stereoselectivity but were present as two

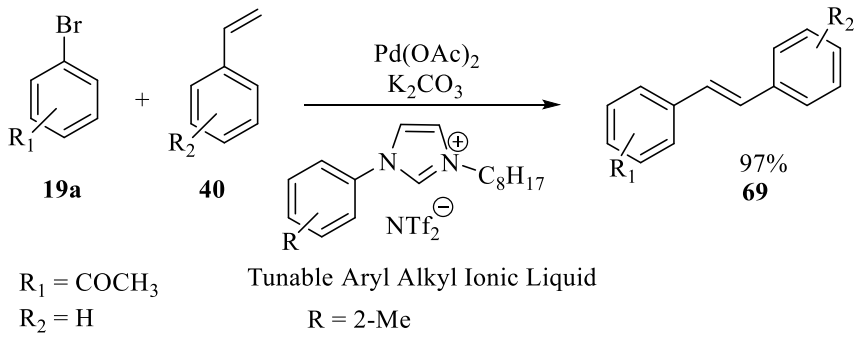
regioisomers. The ratio of these isomers was found to be dependent on the nature of the allylbenzene partner, with electronic effects playing a crucial role in the β-hydride elimination step. In addition, the cytotoxic effects of all Heck reaction products were investigated against human breast cancer cell lines. Compound **65i** showed mild cytotoxic activity against MCF-7 cell lines with IC_{50} values of 47.92 μM compared to tamoxifen (Tan, 2022).



Scheme 2.11 Synthesis of amido 1,3-diarylpropene compounds

Lerch *et al*. reported a study in which imidazolium-based tunable arylalkyl-ionic liquids (TAAILs) were used as solvents in the Mizoroki–Heck reaction. Thus, commercially available palladium (II) acetate ($Pb(OAc)_2$), inorganic bases (K_2CO_3), TAAILs and reaction conditions were investigated for the synthesis of *trans*-stilbene from bromobenzene and styrene (Lerch *et al*., 2022). They successfully synthesised a series of stilbene derivatives yielding exclusively (*E*)-isomers **69** with isolated yields of up to 97%. Optimisation of the reaction conditions showed that the use of only 0.25 mol% $Pd(OAc)_2$ as a catalyst with a reaction time of 4 hours gave the best results. No additional ligands or additives was required. The catalytic system using TAAILs showed higher yields compared to commercially available imidazolium and

phosphonium ionic liquids, highlighting the potential of customised ionic liquids as a reaction medium for the Mizoroki–Heck reaction. The electronic properties of TAAILs can be customised by changing the substitution on the aryl moiety, and varying the length of the alkyl group (Ahrens *et al*., 2009). The usefulness of TAAILs as a reaction medium for the palladium-catalysed Mizoroki–Heck reaction with various aryl bromides **19a** and styrene derivatives **40** has been successfully demonstrated (Lerch *et al*., 2022).

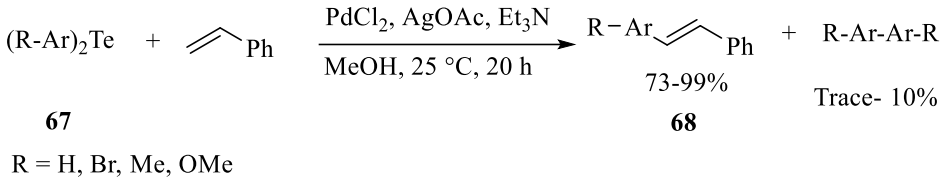


Scheme 2.12 Synthesis of stilbene utilising TAAILs in the Mizoroki–Heck reaction

2.2.1(b) Stille coupling reaction

Among the various Pd-catalysed cross-coupling reactions, the Stille reaction is one of the most efficient and selective methods for building carbon-carbon bonds in organic synthesis. The palladium-catalysed coupling of organotin reagents with organic electrophiles, such as aryl and vinyl halides or triflates, is referred to as the Stille reaction (Yoshiaki *et al*., 1996). Unlike the Suzuki coupling, the Stille reaction does not need a base to be added and is applicable to a broader range of electrophiles, including saturated aliphatic halides and certain alcohol derivatives. While organostannanes are highly valuable synthetic intermediates due to their compatibility with most functional groups and under mild conditions (Yoshiaki *et al*., 1996).

Organic tellurides (**67**) showed more efficient reactivity with a range of alkenes in the presence of Pd(II) salts, yielding the corresponding substituted stilbenes **68**. Initially, the reaction was performed using a stoichiometric amount of palladium acetate or palladium chloride alongside triethylamine, but it became catalytic when an oxidant, such as silver acetate, was introduced into the system (Scheme 2.13) (Yoshiaki *et al*., 1996).



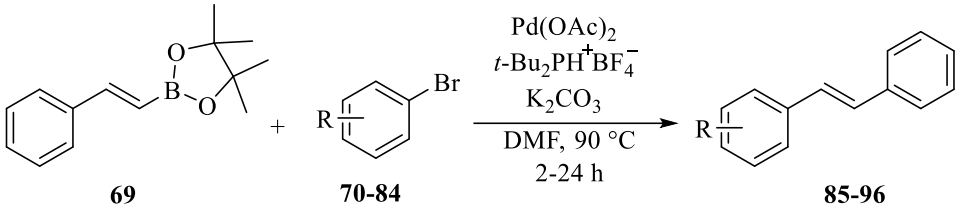
Scheme 2.13 Synthesis of substituted stilbenes via the Stille coupling reaction

2.2.1(c) Suzuki coupling reaction

The Suzuki coupling reaction is frequently used in stilbene synthesis, as it enables efficient production of arylboronic acids or borates from trialkyl borates using Grignard or organolithium reagents. This method is well suited for both industrial production and laboratory scale applications (Wu *et al*., 2010). In a recent study, the incorporation of the *t*-Bu_3PHBF_4 ligand into the Suzuki reaction was found to increase the stereoselectivity of (*E*)-stilbene formation. In addition, it improved the adaptability to a wide range of substrates, both electronically and sterically (Rau & Werner, 2018). The pinacol boronic acid ester (**69**), when coupled with aryl bromide **70-84**, gave (*E*)-stilbene analogues **85-96** with yields between 44% and 76% (Scheme 2.14) (Rau & Werner, 2018).

However, in contrast to Negishi coupling, Suzuki coupling is not the favoured method in the synthesis of stilbene scaffold **85-96**. This is due to several factors, including the complexity of the reactants, preparation of the organometallic reagents, time efficiency and performance of the synthesis yield, as briefly mentioned above in

the references (Rau & Werner, 2018). Instead of using organometallic reagents, two alternative methods using alkenes and alkynes, namely Sonogashira and Heck coupling reactions, are considered here.



69 **70-84** **85-96**

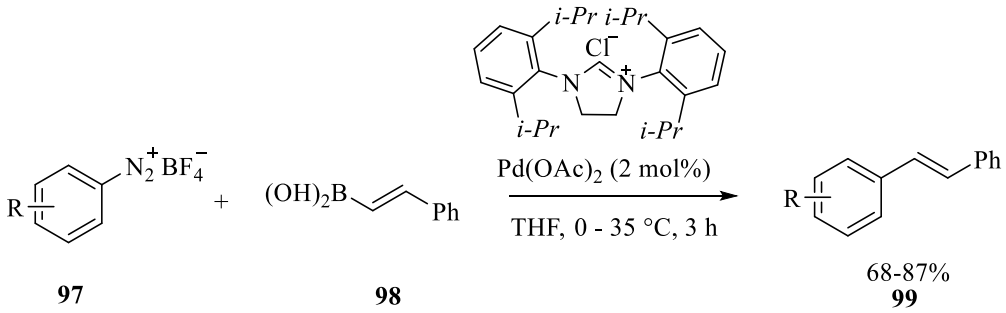
R = 4-Me, 4-NO_2, 2-Me, 4-NMe_2, 4-CN, 4-CF_3, 4-Cl, 4-Ac, 2-Cl, H, naph

Scheme 2.14 The Suzuki cross-coupling synthesis of stilbene scaffold

Besides, Heck coupling reactions involving aryldiazonium salts or organic halides as electrophiles, the utilisation of nucleophilic organoboron species has emerged as a subject of considerable interest for synthesising stilbene derivatives. The Suzuki reaction, a palladium-catalysed cross-coupling reaction between boranes or boronic acids and esters with organic halides or diazonium salts, has proven to be highly selective and compatible with a wide range of functional groups present on either coupling reagent. Moreover, the reagents employed in this reaction are easily prepared and generally exhibit robustness in the presence of water (Eddarir *et al*., 2001).

The Suzuki coupling reaction between 1,1-bromofluorostyrene and phenylboronic acid is commonly utilised as a versatile method for the synthesis of polyhydroxylated stilbenes that are monofluorinated at the central double bond (Eddarir *et al*., 2001). Cross-couplings between aryldiazonium salts **97** and styrylboronic acid (**98**), employing a catalytic system consisting of palladium(II) acetate and an imidazolinium ligand precursor, led to the formation of various stilbene derivatives **99**. These reactions were carried out under mild conditions, without the need for any additional base, and achieved high yields ranging from 68% to 87% (Scheme 2.15). It

was observed that *in situ* generation of diazonium ions did not have any significant adverse effects if the reaction times is extended (Merritt & Song 2001).

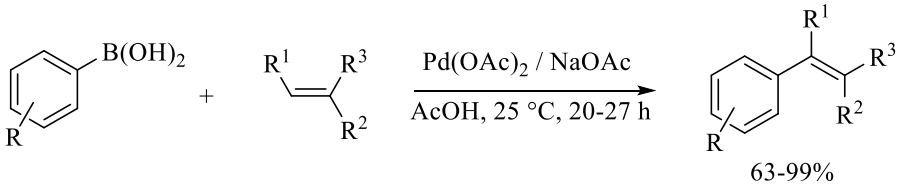


97 **98** **99**

R = H, 2-Me, 3-Me, 4-Me, 2-OMe, 4-OMe, 4-COPh

Scheme 2.15 Synthesis of stilbene derivatives via Suzuki coupling

Du and co-workers reported palladium-catalysed cross-coupling reactions of arylboronic acids **100** with alkenes **101** in acetic acid at 25°C yield stilbene derivatives **102** with satisfactory to excellent yields (63–99%) (Scheme 2.16). Experimental evidence suggests that the reaction most likely occurs through the oxidative addition of Pd(0) to the carbon–boron bond. However, Pd(II)-promoted catalytic pathways become prominent when an appropriate oxidant, such as copper(II) acetate or molecular oxygen, is introduced to the reaction medium (Du *et al*., 2001).



100 **101** **102**

R = H, 4-Me, 4-OMe, 4-Cl, 3-NO_2, 1-naphthyl

R^1 = H, Ph; R^2 = H, Ph; R^3 = H, Ph, 4-MeC_6H_4

Scheme 2.16 Synthesis of stilbene derivatives

2.2.1(d) Sonogashira reaction

Lara-Ochoa *et al*. have reported a novel method for synthesising resveratrol (**5**), an analogue of stilbene, using a Sonogashira coupling strategy (Lara-Ochoa *et al*.,