SYNTHESIS, CHARACTERISATION AND MOLECULAR DOCKING OF QUINOXALINE-ISOXAZOLE HYBRIDS AS POTENTIAL ANTI-HYPERGLYCEMIC AGENT

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by

LACKSANY PHONGPHANE

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"It takes a village to raise a child" is a well-known proverb. It resonates with me so much, as I approach to the end of my embarkment on my PhD journey, its profound meaning and experiences have taken on new significance in my life. Therefore, I would like to express my sincere gratitude to the people who played irreplaceable, essential roles in leading me towards this remarkable milestone of my life.

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LIST OF SYMBOLS

α	Alpha
Å	Angstrom
β	Beta
Δ	Chemical shift
δ_C	Chemical shift carbon
δ_H	Chemical shift proton
J	Coupling constant
°C	Degree Celsius
γ	Gamma
g	Grams
$gmol^{-1}$	Grams per mol
Hz	Hertz
µg	Microgram
µM	Micromolar
µL	Microliter
M	Molarity
Mg	Milligram
Mm	Millimeter
mL	Milliliter
mmol	Millimole
Nm	Nanometer
nM	Nanomolar
o	ortho
cm^{-1}	Per centimeter

%	Percentage
tert	Tertiary

LIST OF ABBREVIATIONS

ADMET	Absorption, distribution, metabolism, elimination and toxicity
aq.	aqueous
BBB	blood-brain barrier
calc.	Calculated
cat.	Catalyst
$CDCl_3$	Deuterated chloroform
C57BL/6	C57 black 6 inbred strain of laboratory mouse
COVID-19	Coronavirus disease 2019
DCM	Dichloromethane
d	Doublet
dd	Doublet of doublet
DM	Diabetes Mellitus
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
DNS	3,5-Dinitrosalicylic acid
DPPH	α,α-diphenyl-β-picrylhydrazyl
DPP-4	dipeptidyl peptidase-IV
EC_{50}	Half maximal effective concentration
Et_3N	Triethylamine
EtOH	Ethanol
FDA	Food and Drug Administration
FTIR	Fourie-transform infrared
FXR	Farnesoid X receptor
GBD	Global Burden of Disease
GI	Gastrointestinal
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-Like Peptide-1
GPR40	G-protein coupled receptor 40
GW4064	3-(2,6-dichlorophenyl)-4-(3'-carboxy-2-chlorostilben-4-yl)oxymethyl-5-isopropylisoxazole
HCl	Hydrochloric acid

HCV	Hepatitis C virus
Hep G2	Hepatoblastoma cell line
HDAC	Histone deacetylase
HMBC	Heteronuclear multiple bond correlation
HRMS	High-resolution mass spectroscopy
h	Hour(s)
HIV	Human immunodeficiency virus
IC_{50}	Half-maximal inhibitory concentration
ICR	Institute of Cancer Research
IDF	International Diabetes Federation
IHME	Institute for Health Metrics and Evaluation
IPH	Institute for Public Health
IR	Insulin resistant
LED	Light emitting diode
M	Multiplet
MERS	Middle East respiratory syndrome coronavirus
N_2	Nitrogen gas
NaOCl	Sodium hypochlorite
Na_2CO_3	Sodium carbonate
NCD	Non-communicable disease
NMR	Nuclear magnetic resonance
NOAC	Nitrile oxide-alkyne cycloaddition
NPOQA	*N*-(4-Methyl-2-nitrophenyl)-2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide
OGTT	Oral glucose tolerance test
PEG-400	Polyethylene glycol 400
PDB	Protein data bank
PK	Pharmacokinetic profile
pKa	Acid disassociation constant
pNPG	4-Nitrophenyl α-D-glucopyranoside
$POCl_3$	Phosphoryl chloride
PPARγ	Peroxisome proliferator-activated receptor gamma isotype
PTP1B	Protein tyrosine phosphatase 1B
RCSB	Research Collaboratory for Structural Bioinformatics
RIN5F	Rat insulinoma

Ro5	Lipinski's Rule of Five
r.t	Room temperature
RTG	Radioisotope thermoelectric generator
s	Singlet
SAR	Structure-activity relationship
SGLT-2	Sodium-glucose cotransporter-2
SUR	Sulfonylurea receptors
T	Triplet
t-BuOK	Potassium *tert*- butoxide
T1DM	Type-1 diabetes mellitus
T2MD	Type-2 diabetes mellitus
TBHP	*tert*-Butyl hydroperoxide
TLC	Thin layer chromatography
TMS	Tetramethyl silane
TZD	Thiazolidinediones
UCSF	University of California, San Francisco
UV	Ultraviolet
WHO	World Health Organization

LIST OF APPENDICES

SINTESIS, PENCIRIAN DAN PENGEDOKAN MOLEKUL TERHADAP HIBRID KUINOKSALINA-ISOKSAZOLA SEBAGAI AGEN ANTI-HIPERGLISEMIK YANG BERPOTENSI

ABSTRAK

Diabetes melitus adalah gangguan metabolisme kronik yang menjejaskan berjuta-juta orang di seluruh dunia, dan mendatangkan komplikasi serius seperti penyakit kardiovaskular, obesiti, dan retinopati. Walaupun terdapat kemajuan dalam rawatan, pengurusan diabetes yang berkesan kekal sebagai cabaran yang ketara disebabkan oleh keterbatasan pilihan terapeutik semasa, termasuk kesan sampingan dan keberkesanan terhad dalam mengawal paras glukosa darah. Dalam usaha untuk membangunkan agen anti-hiperglisemik yang berpotensi merencat enzim α-amilase dan α-glukosidase, satu siri hibrid kuinoksalina-isoksazola telah berjaya disintesis melalui pensiklikan lima langkah. Sebatian terbitan kuinoksalina-isoksazola (**84a**-**84v**) telah berjaya disintesis dan diperoleh dengan hasil yang baik dalam julat 54% hingga 95%. Seterusnya, sebatian-sebatian yang disintesiskan ini diciri dan ditentukan melalui kaedah spektroskopi terdiri daripada inframerah-penjelmaan Fourier (FTIR) dan resonans magnet nuklear (NMR) serta spektrometri jisim resolusi tinggi (HRMS). Sebatian yang disintesis telah dinilai secara lanjut untuk aktiviti rencatan α-amilase dan α-glukosidase *in vitro* menggunakan akarbos sebagai kawalan positif. Antara dua puluh dua sebatian yang disintesis, keputusan mendapati sebatian **84j** menunjukkan dwi-aktiviti terhadap α-amilase dan α-glukosidase dengan nilai IC_{50} masing-masing yang baik iaitu 17.0 and 40.1 μM. Tambahan pula, sebatian **84c** juga menunjukkan dwi-aktiviti yang baik dalam perencatan α-amilase (IC_{50} = 24.0 μM) dan α-glukosidase (IC_{50} = 41.7 μM). Dalam meneruskan penilaian perencatan enzim ini, sebatian **84p**

menunjukkan kekuatan mono-perencatan yang ketara melawan α-amilase dengan IC_{50} bernilai 17.3 μM, manakala sebatian **84m** dan **84o** juga memaparkan potensi yang baik dan setanding dengan kawalan positif dengan IC_{50} bernilai 22.3 dan 27.9 μM, mengikut tertib. Tambahan pula, hasil pemerhatian menunjukkan sebatian **84e** memaparkan nilai IC_{50} mono-perencatan terendah melawan α-glukosidase dengan nilai IC_{50} sebanyak 16.6 μM, diikuti sebatian **84f** yang terbukti mempunyai kekuatan rencatan dengan IC_{50} bernilai 18.6 μM. Untuk memahami secara lanjut mengenai potensi hibrid kuinoksalina-isoksazola, kajian pengedokan molekul *in siliko* dijalankan bersama analisis ciri-ciri persamaan ubat turut disiasat dalam kajian ini. Simulasi pengedokan telah dijalankan untuk mengkaji interaksi pengikatan antara sebatian sasaran (ligan) dengan tapak aktif bagi protein terpilih (reseptor) 2QV4 untuk α-amilase dan 3TOP untuk α-glukosidase. Sebatian **84j** mempamerkan interaksi dengan α-amilase bersama dengan skor tenaga pengikat sebanyak –8.5 kcal/mol melalui sisa-sisa asid amino penting, manakala sebatian **84e** menunjukkan skor tenaga pengikat terendah pada nilai –9.1 kcal/mol melalui sisa-sisa asid amino penting pada tapak aktif α-glukosidase. Penilaian persamaan ubat mencadangkan bahawa kebanyakan sebatian yang disintesis mematuhi penilaian peraturan 5 Lipinski, terutamanya sebatian **84j** dengan jisim molekul kurang daripada 500 g/mol. Selain itu, bilangan penerima dan penderma ikatan hidrogen diperoleh masing-masing pada bilangan 6 dan 1. Tambahan pula, pekali sekatan oktanol-air (miLogP) adalah pada nilai 4.10 tanpa memiliki pelanggaran. Oleh itu, melalui dapatan kajian ini, boleh disimpulkan bahawa sebatian **84j** bertindak sebagai ubat aktif yang mematuhi peraturan 5 Lipinski dengan sepenuhnya.

SYNTHESIS, CHARACTERISATION AND MOLECULAR DOCKING OF QUINOXALINE-ISOXAZOLE HYBRIDS AS POTENTIAL ANTI-HYPERGLYCEMIC AGENT

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder that affects millions of people worldwide, leading to severe complications such as cardiovascular disease, obesity, and retinopathy. Despite advances in treatment, effective management of diabetes remains a significant challenge due to the limitations of current therapeutic options, including side effects and limited efficacy in controlling blood glucose levels. Hence, in our effort to develop anti-hyperglycemic agents that potentially inhibiting *α*-amylase and *α*-glucosidase enzymes, a series of new quinoxaline-isoxazole hybrids were successfully synthesised *via* five-step cyclisation. The new quinoxaline-isoxazole derivatives (**84a-84v**) were successful synthesised and obtained twenty-two compounds in a good yield within the range of 54% to 95%. Subsequently, these synthesised compounds were characterised and elucidated by several spectroscopies methods consisting of Fourier transform-infrared (FT-IR), and nuclear magnetic resonance (1D and 2D-NMR) including high-resolution mass spectrometry (HRMS). The synthesised compounds were further evaluated for the *in vitro* α-amylase and α-glucosidase inhibitory activities using acarbose as a positive control. Among 22 synthesised compounds, the results revealed that compound **84j** showed dual inhibitory towards α-amylase and α-glucosidase with promising IC_{50} values of 17.0 and 40.1 μM, respectively. Moreover, compound **84c** was also displayed the good dual activity for the inhibition of α-amylase (IC_{50} = 24.0 μM) and α-glucosidase (IC_{50} = 41.7 μM). In the continuation of these enzyme inhibitory evaluation, **84p** exhibited a

significant mono-inhibition strength against α-amylase with the IC_{50} of 17.3 μM, while compound **84m** and **84o** also revealed good potency which are comparable to the positive control with the IC_{50} of 22.3 and 27.9 μM, in that order. Furthermore, the observed result showed that compound **84e** displayed the lowest IC_{50} value of mono-inhibition against α-glucosidase with the IC_{50} of 16.6 μM, followed by compound **84f** has proved to have inhibitory strength with IC_{50} 18.6 μM. To understand further on the potential of quinoxaline-isoxazole hybrids, *in silico* molecular docking study together with drug-likeness properties analysis were investigated at present study. The docking simulation was carried out to study the binding interactions between the targeted compound (ligand) with the active site of selected protein (receptor) of 2QV4 for α-amylase and 3TOP for α-glucosidase. Compound **84j** exhibited interaction with α-amylase along with the binding energy score of –8.5 kcal/mol *via* important residue amino acids, while compound **84e** showed the lowest binding energy score at –9.1 kcal/mol *via* the important residue amino acids at the α-glucosidase active site. The evaluation of drug-likeness suggested that most of the synthesised compounds followed Lipinski's rule of five (Ro5) assessments, especially compound **84j** with the molecular weight less than 500 g/mol. Besides, the number of hydrogen bond acceptors and bond donors obtained at 6 and 1, respectively. Moreover, the octanol-water partition coefficient (miLogP) showed at 4.10 with no violation. Therefore, from these findings, it can be concluded that compound **84j** acted as an active drug which completely obeyed Lipinski's rule of five.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Homeostasis is defined as a dynamic, adaptive, self-regulating body mechanism by which a living organism can maintain internal stability; or a state of relative constancy by adjusting to changing external conditions (Mas-Bargues *et al*., 2023). Conversely, it can be said that the health and vitality of any organism, including human being is the result of a homeostatic regulation of the internal environment. This natural survival mechanism is enabled by a series of control mechanism that function at the organ, tissue or cellular level through the modulation of hormones and neurotransmitters, to maintain key homeostatic variables or system stock within a desirable range (Kotas & Medzhitov, 2015).

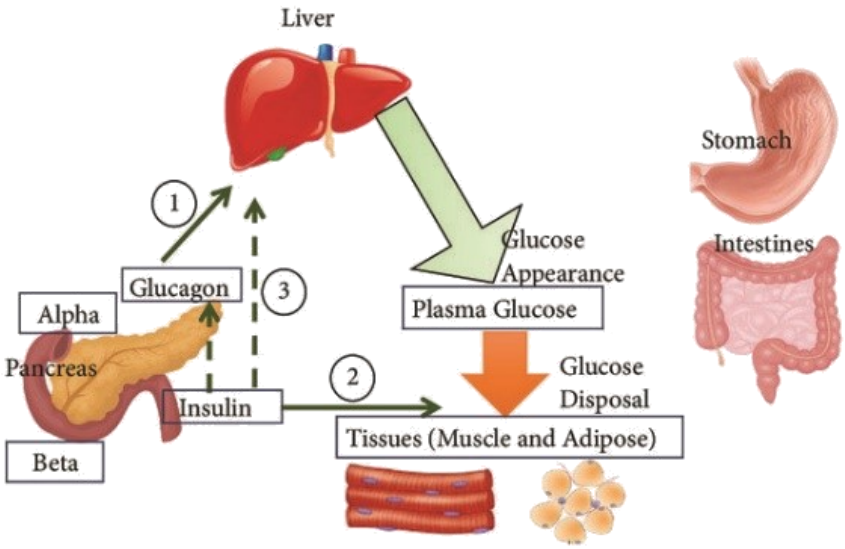
Glucose is a predominant metabolic substrate of all mammalian cells, as most body tissues and organs regularly consume glucose as a vital energy source as well as to fulfil many other anabolic requirements (Szablewski, 2011). The balance of glucose concentration in the blood, also known as glucose homeostasis, is critical for health and vitality (Wahren *et al*., 1978). The normal fasting plasma glucose concentration is required to be maintained within a narrow range, between 3.9 and 5.6 mmol/L (Shi *et al*., 2021). Long lasting deprivation of glucose concentration in the blood, also known as hypoglycemia, may lead to seizures, unconsciousness and fatality (Gerich *et al*., 1991). Hypoglycemia is often characterized by a plasma glucose concentration below 3.9 mmol/L (Cryer *et al*., 2003). However, a prolonged elevation of glucose concentrations in blood, also known as hyperglycemia, may cause wide-range side effects such as blindness, renal failure, vascular disease, and neuropathy *(Kumar et al*.,

2014). Hyperglycemia is defined when plasma glucose levels above 7.0 mmol/L in the fasting state and exceeding 10.0 mmol/L 2 hours postprandial (Gerich, 2003).

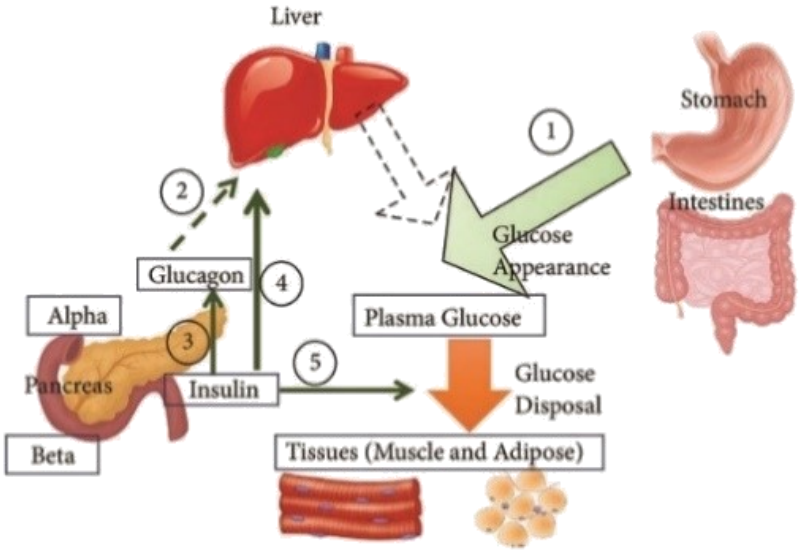
To maintain glucose homeostasis, the human body has a highly sophisticated network of hormones and neuropeptides that are released by various body organs and tissues. Within this network, the pancreas is the essential organ to accomplish a tight plasma glucose control with its abilities in secreting of glucose-lowering hormone insulin and its counterpart hormone glucagon (Röder *et al*., 2016). Insulin is an endocrine polypeptide hormone that consists of 51 amino acids and is primarily secreted by β cells in the islets of Langerhans of the pancreas (Rahman *et al*., 2021). The main function of insulin is to promote glucose uptake in liver, fat and skeletal muscle cells when plasma glucose concentrations are elevated *(Chao et al*., 2019). On the other hand, glucagon is another endocrine polypeptide hormone composed of 51 amino acids that primarily secreted by the α-cell glucagon in the islets of Langerhans of the pancreas (Gilon, 2020). When plasma glucose is deficient, glucagon is secreted to trigger glycogenolysis, in which stored glycogen is converted into glucose before releasing into the blood (Magnusson *et al*., 1995).

The daily body mechanism of glucose control can be visualised as depicted in Figure 1.1. In the fasting state, plasma glucose will be drained out for metabolic needs, resulting in a drop of glucose concentration. In this state, the serum glucose primarily originates from glycogenolysis regulated by glucagon (1). At basal insulin levels, glucose disposal is controlled (2), however due to the low glucose concentration, insulin plays a minimal role in suppressing glucose production in the serum *via* glycogenolysis and gluconeogenesis (3). Conversely, In the fed state (b), plasma glucose is mainly derived from dietary intake, specifically from the stomach and intestine (1). The secretion and action of glucagon are inhibited by insulin secretion

(2,3), with intra-islet communication in the pancreas contributing to this suppression (4). Consequently, gluconeogenesis and glycogenolysis in the liver are inhibited, while glucose disposal in peripheral tissues is enhanced (5).



(a)



(b)

Figure 1.1 Illustration of glucose homeostasis and roles of both insulin and glucagon in fasting state (a) and in fed state (b). (Shanak *et al*., 2019).

1.2 Research background

Diabetes mellitus is a ubiquitous metabolic disease associated with chronic hyperglycemia; caused by impairment of the bodies in insulin secretion. Based on available data sources, diabetes mellitus is termed as a global threat as its epidemic nature is observed at an alarming rate worldwide, with middle- and low-income regions encompassing for 80% of reported cases (Saeedi *et al*., 2019). The global prevalence of impaired glucose tolerance was 9.1% (464 million), and according to the latest statistical studies, the increasing trend continues and will reach approximately 10.0% of the global population (638 million) in 2045 (Rooney *et al*., 2023).

Figure 1.2 Number of people with diabetes worldwide per IDF region in 2021-2045. Published by IDF Diabetes Atlas 2021 (Sun *et al*., 2022).

As depicted in Figure 1.2, according to the latest International Diabetes Federation (IDF) report, the number of adults living with diabetes is approximately 537 million in 2021, with a projected leap of 46% to 783 million; in 2045 (Sun *et al*.,

2022). Remarkably, the Southeast Asia region is anticipated a 68% increase to a total of 152 million diabetes patients over the next 25 years. The reported statistic for 2021 shows that 90% of patients are diagnosed with Type 2 Diabetes Mellitus (T2DM).

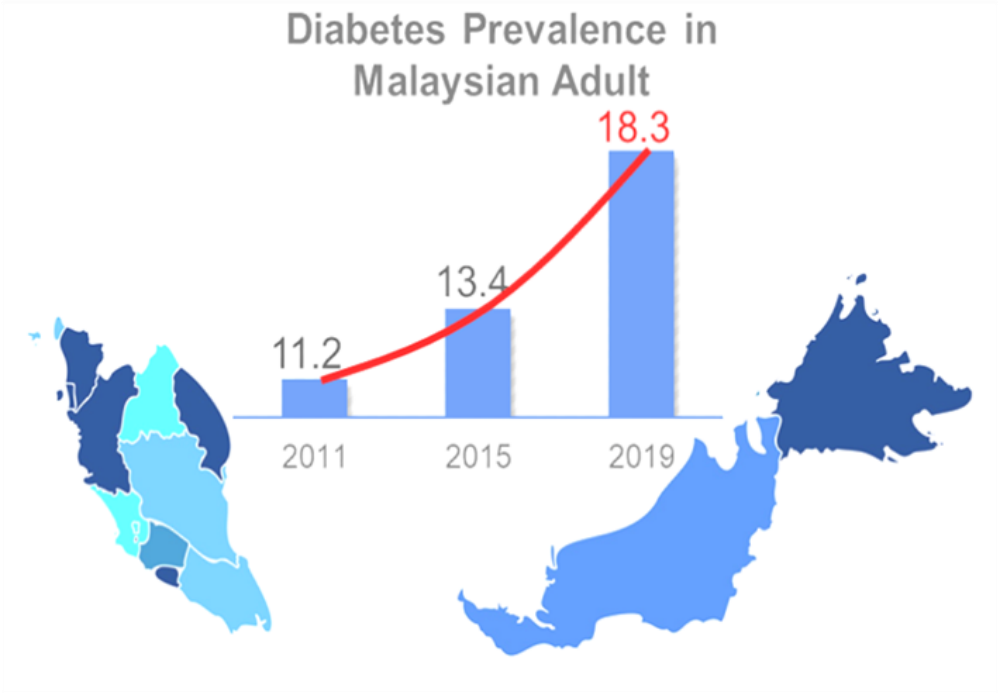


Figure 1.3 Percentage of Diabetes Prevalence in Malaysian Adults (National Health and Morbidity Survey (NHMS) (IPH, 2020).

According to the National Health and Morbidity Surveys (NHMS) performed by Institute for Public Health, Ministry of Health, Malaysia, the number of Malaysian patients with diabetes has increased dramatically from 11.2% in 2011 to 13.4% in 2015 and 18.3% in 2019 as shown in Figure 1.3 (IPH, 2020). Breaking these statistics down to simpler numbers, it implies that one in five Malaysians, or cumulatively about 3.9 million individuals aged 18 years and above suffers to have diabetes in 2019. The reported figure is aligned with IDF studies on the Southeast Asia region in 2021; and is a possible indication of a higher number of diabetes patients in the next two decades (Sun *et al*., 2022).

The widespread of diabetes are deeply concerning due to the associated complications. The World Health Organization (WHO) has highlighted that diabetes is the direct cause of 1.5 million deaths and 460,000 deaths from kidney disease in 2019, with 48% of all deaths occurring before the age of 70 (Anta *et al.,* 2023). In addition to diabetes, elevated blood glucose levels are also directly contributed to approximately 20% of cardiovascular deaths provided by Institute for Health Metrics and Evaluation (IHME, 2022). The severity is marked by a recorded 3% increase in age-standardized mortality rates between 2000 and 2019. Consistent with the recent events of COVID-19 pandemic outbreaks, the systematic analysis report found that the prevalence of diabetes in 2009 influenza A (Badawi & Ryoo, 2016), Middle East respiratory syndrome coronavirus (MERS) (Badawi & Ryoo, 2016), and COVID-19 (Abdi *et al*., 2020) to be 14.6%, 54.5%, and 14.5%, respectively, with significantly more acute complications and higher mortality rates reported (Huang *et al*., 2020; Lu *et al*., 2020).

To address this global threat, the classification of diabetes mellitus is critical for study and treatment. Diabetes mellitus is commonly classified into Type 1 diabetes (T1DM), which also known as insulin-dependent diabetes mellitus. Type 1 diabetes is caused by the loss of insulin-producing β-cells in pancreas (DiMeglio *et al*., 2018) and is thought to develop when environmental triggers stimulate an autoimmune reaction against pancreatic β-cells in a genetically susceptible individual (T1DM). Meanwhile, Type 2 diabetes (T2DM) is the body impairment in regulating insulin secretion by pancreatic β-cells, resulting in excessive glucose absorption (Baron & Practice, 1998; Galicia-Garcia *et al*., 2020). In view of the severity of T2DM, which accounts for over 90% of adult diabetes cases in 2019, one of the trending approaches has been taken to prolong glucose absorption in the gastrointestinal tract and inhibit hyperglycemia, by

impeding the action of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase (Rabasa-Lhoret & Chiasson, 2003).

The enzymes α-amylase and α-glucosidase enzymes plays a crucial role in breaking down carbohydrates into small molecules such as glucose, prior they absorbed by the body to support vital bodily functions. The *α*-amylase enzyme begins the digestion of carbohydrate (for instances starch, glycogen); by randomly cleaving the *α*-1-4 glycosidic linkages of amylose to yield low molecular weight products such as dextrin, maltose, maltotriose; or disaccharides in general *via* hydrolysis. While, *α*-glucosidase enzyme further catalysed the disaccharides to monosaccharides known as glucose (Hara *et al*., 1990; Matsui *et al*., 2007). Through the tremendous efforts in research on carbohydrate digestion and absorption, it has been found that inhibition of these enzymes leads to a significant reduction in postprandial blood glucose levels following a mixed carbohydrate diet. Therefore, such an approach is emphasized as an important strategy in the treatment of T2DM (Adefegha *et al*., 2010; Kwon *et al*., 2006). The discovery of effective novel bioactive pharmacophores that selectively inhibit these carbohydrate-hydrolysing enzymes is an important milestone for the field of pharmaceutics, biology, and organic chemical synthesis in combating T2DM.

At present, research on synthesis medicinal compounds for the treatment of diabetes has attracted much attention of scientists. Many of the health-promoting characteristics of nitrogen bearing heterocyclic are partly associated with the presence of an array of important therapeutic agents in recent drug discovery (Vitaku *et al*., 2014). Upon reviewing the literature for lead compounds with anti-hyperglycemic activity, the structure featuring a benzoparadiazine backbone known as quinoxaline (**1**) was attracted significant interest due to it is a nitrogen bearing heterocyclic and it is the core scaffold of many potential drug candidates as shown in Figure 1.4.

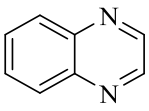


Figure 1.4 Quinoxaline structure.

Quinoxaline represent a six-membered ring compound of the benzoparadiazine family that exhibits a broad spectrum of pharmaceutical properties (Suthar *et al.,* 2022), including anti-bacterial *(*El-Gendy *et al.,* 1995*;* Badran *et al.,* 2003*;* Ajani *et al.,* 2009; Parh*i et al*., 2013), anti-malarial (Rangisetty *et al*., 2001; Guillon *et a*l., 2011; Shekhar *et al*., 2014), anti-HIV (De Clercq, 1995), anticancer (Marcu & Olver, 2006; Undevia *et al*., 2008; Lee *et al.*, 2013), and anti-diabetic (Ibrahim *et al*., 2017; Mohamed, K *et al*., 2017). Additionally, quinoxaline and its derivatives offer multiple sites for structural modification where the introduction of various pharmacodynamic groups can significantly influence their biological activity (Jia *et al*., 2023).

Furthermore, the isoxazole heterocyclic compound that is beneficial in the field of organic synthesis is isoxazole (**2**), or also known as 1,2-oxazole. This scaffold is one of the five-membered rings of heterocyclic compounds contain both nitrogen and oxygen atoms, as shown in Figure 1.5. Isoxazole also presents various medicinal applications such as anti-bacterial (Ma *et al*., 2007; Temiz *et al*., 2008), anti-HIV (Srivastava *et al*., 1999; Kumar & Jayaroopa, 2013), anticancer (Li *et al*., 2003; Yong *et al*., 2014), anti-depressant (Andrés *et al.*, 2005; Youn *et al*., 2009), and anti-diabetic agents (Nie *et al*., 2020).

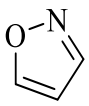


Figure 1.5 Isoxazole structure.

Hence, in this thesis, in-depth studies and literature reviews were conducted on the synthesis protocols and motifs of the nitrogen-bearing heterocyclic compound candidatures, namely quinoxaline and isoxazole. The quinoxaline moiety involved in carbohydrate metabolism, such as α-amylase and α-glucosidase, which are critical targets in diabetes management (Khan *et al*., 2017; Missioui *et al*., 2022 & Mortada *et al*., 2023), while the isoxazole component is often linked to their ability to modulate glucose metabolism which is commonly associated with diabetes (Algethami *et al*., 2021 & Saidi *et al*., 2022). By incorporating potential synergistic activity, molecular hybrid was playing important role in combining of different bioactive pharmacophores. Further efforts were made to facilitate the first reported novel series of quinoxaline scaffolds hybridised with isoxazole moieties. This inventive approach could lead to the discovery and development of more effective, efficacious anti-diabetic agents as shown in Figure 1.6.

With thorough characterisation, *in vitro* bioactivity studies were performed using commercial acarbose as a control, as the first gate to investigate both mono- and dual-inhibitory potency against the α-amylase and/or α-glucosidase enzymes. Cheminformatics is a modern discipline that integrates the use of physical chemistry theory with computational approaches to support the analysis, visualisation, modelling and rationalisation experimental results of both chemical and biological activities (Martinez-Mayorga *et al*., 2020). Information science and computational techniques, also known as "*in silico*" techniques, are trending as simulation and computational capabilities are frequently incorporated into recent research publications, especially in exploring and describing the structural relationship between the structures of chemicals (Begam & Kumar, 2012).

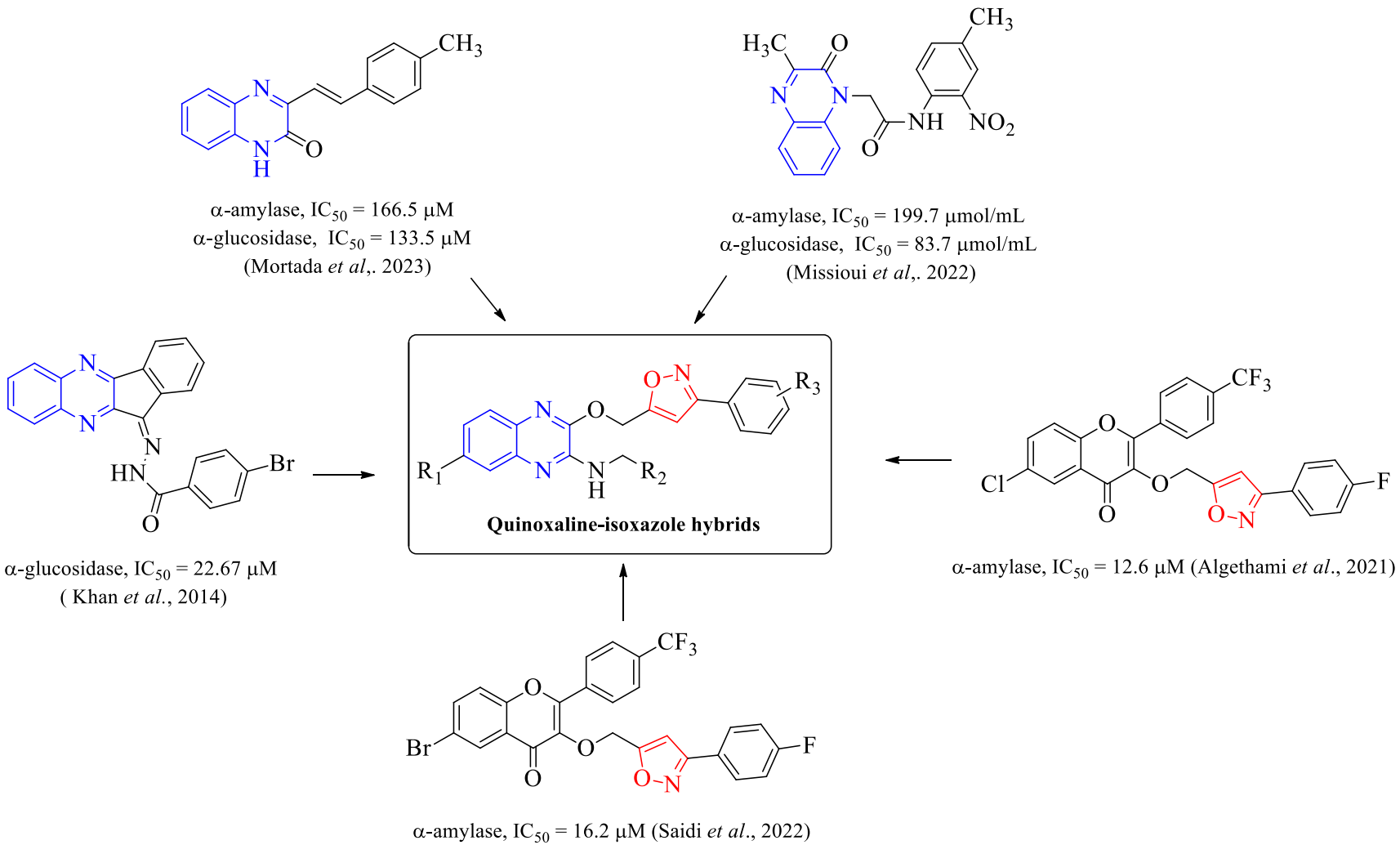


Figure 1.6 Design scaffold of quinoxaline-isoxazole hybrids.

Molecular docking is one of the *in silico* techniques that allow prediction of the binding mode and corresponding binding energy of bioactive pharmacophores with targeted ligands, and serve as the core supplementary information to validate *in vitro* bioactivity results.

To expand the scope and extend the insights of the synthesised quinoxaline-isoxazole of the study, computational analytical approaches such as molecular docking simulations and molecular dynamics studies of the synthesised quinoxaline-isoxazole ligands with high inhibitory activity and the active site of the protein structures of human maltase-glucoamylase (ctMGAM) (PDB ID: 3TOP) and human pancreatic α-amylase complexed (PDB ID: 2QV4) (Huang *et al*., 2014) were adapted in this research to validate the experimental bioactivity.

1.3 Problem statement

Diabetes is a chronic disease that occur whether the pancreas does not produce insulin, or the body is resistant to insulin secretion. It represents a major challenge in healthcare worldwide. Diabetic disease is non-communicable diseases (NCDs), which is also one of the top 10 leading causes of death worldwide and a global health problem. The tremendous burden of this growing threat is illustrated by the four most common NCDs: cardiovascular diseases (such as heart attacks and strokes), cancers, chronic respiratory diseases (such as chronic obstructive pulmonary disease and asthma), and diabetes. Every year, 41 million people die from NCDs, accounting for more than 7 in 10 deaths worldwide (World Health Organization, 2018).

The treatment of T2DM has been researched since the 1950s. At that time, Metformin was the first drug approved for T2DM and treated the diabetes patients (Bell & Hadden, 1997), and drug discovery for diabetes has been intensively developed for decades. Although, the oral medications such as acarbose, miglitol,

voglibose are widely used for the treatment of T2DM, these commercial drugs still show some minor side effects including bloating, nausea, and diarrhea (Nathan, 2015). To the best of our knowledge, there is no previous study on quinoxaline-isoxazole derivatives as α-amylase and α-glucosidase inhibitors. In addition, some of the reports of quinoxaline derivatives still showed weak inhibition of the α-amylase enzyme. Inspired by the above-mention, this research focuses on the synthesis of novel quinoxaline-isoxazole hybrids and their biological activity as *in vitro* α-amylase and α-glucosidase inhibitors. *In silico* drug-likeness and molecular docking studies have also been validated which expected to give us the promising results for T2DM drug development.

1.4 Research objective

The objectives of the study are as follows:

a) To synthesise and characterise a new series of quinoxaline-isoxazole derivatives with various substituent groups.

b) To evaluate the α-amylase and/or α-glucosidase inhibitor properties of selected quinoxaline-isoxazole compounds *via in vitro* bioactivity assessment.

c) To predict the mechanism interaction of the synthesised compounds with the targeted protein *via in silico* molecular docking and drug-likeness assessment.

1.5 Significance of the study

Thorough this study, a new series of quinoxaline skeletons bearing isoxazole moieties, a new library of hybrids of two classes of nitrogen-bearing heterocyclic is synthesised and evaluated for the application of anti-hyperglycemic. While both

individual structures are widely reported as medicinally important compounds, there is limited information on this hybrid compound. Therefore, the deployment of modified 5-steps cyclisation synthetic protocol could be a remarkable milestone in achieving the targeted bioactive pharmacophores onto a single scaffold. Subsequent *in vitro* bioactivity assessment against α-glucosidase and α-amylase, with standard acarbose as a positive control, will demonstrate the efficacy of inhibition, including mono-inhibition against either of the enzymes and dual-inhibitions. Meanwhile, incorporation of *in silico* techniques and drug-likeness studies will be equivalently essential to justify the experimental outcome as well as enhancing the understanding of these quinoxaline-isoxazole hybrids.

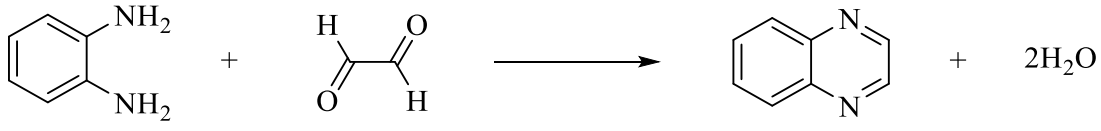
CHAPTER 2

LITERATURE REVIEW

2.1 Quinoxaline

Nitrogen-bearing heterocyclic scaffolds have been widely reported in association with medicinal importance and therapeutic potential (Irfan *et al*., 2021). Out of these, 1,4-benzodiazine better known as quinoxaline, is a member of benzodiazine family characterized by fused bicycle heterocycle compounds with 1,4-nitrogen as heteroatoms. Quinoxaline is a versatile class of nitrogen-bearing heterocycles as the structure constitute prominent intermediates in both organic synthesis and natural products. It can be afforded by a relatively simple condensation reaction between *o*-phenylenediamine **3** with substituted 1,2-diketone **4.** The synthesis of the quinoxaline pathway has been studied extensively for over a century, with the very first report published by Hinsberg in 1884 (Hinsberg, 1884; Suthar *et al*., 2022) (Scheme 2.1). Since then, remarkable unified efforts have been exerted to establish various synthesis protocols for more complex quinoxaline-based compounds, as discussed thoroughly.

Quinoxaline moieties are found present in very broad spectrum of potent biological applications, including antibacterial (Tang *et al*., 2022), anti-hepatitis C virus (HCV) (Liu *et al*., 2011), anti-cancer , human immunodeficiency virus (HIV) (Fabian *et al*., 2020) as shown in Figure 2.1.



3 **4** **1**

Scheme 2.1 First report on quinoxaline reaction (Hinsberg, 1884).

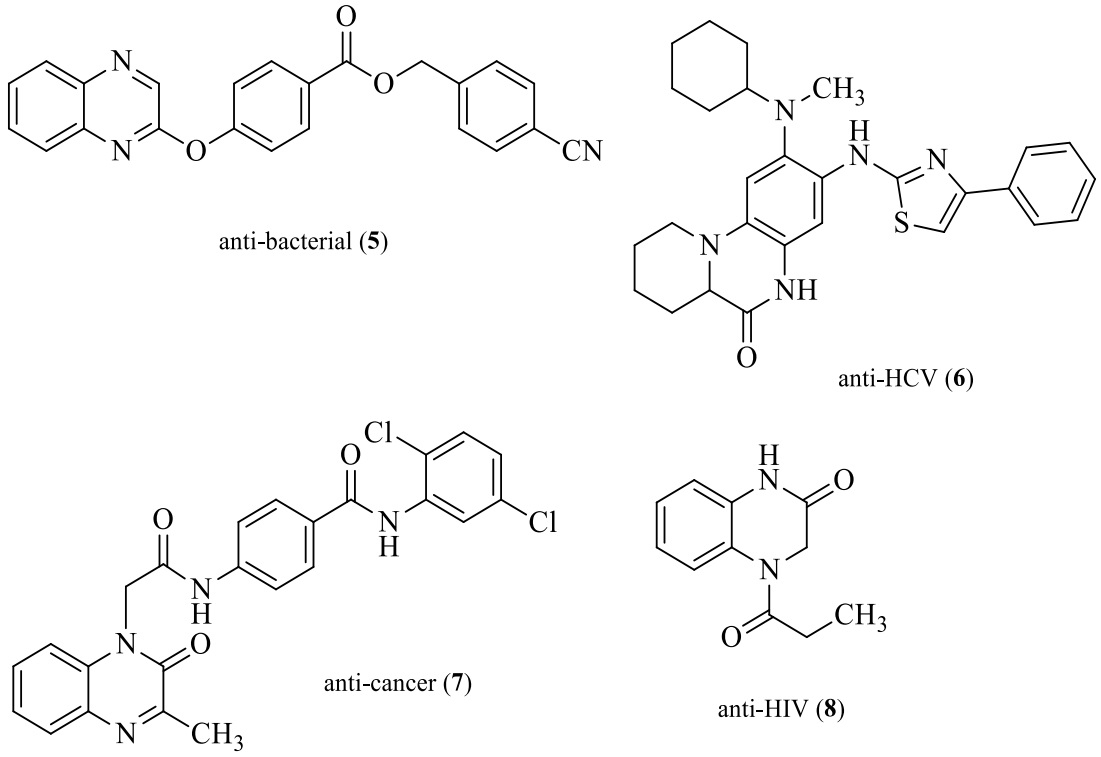
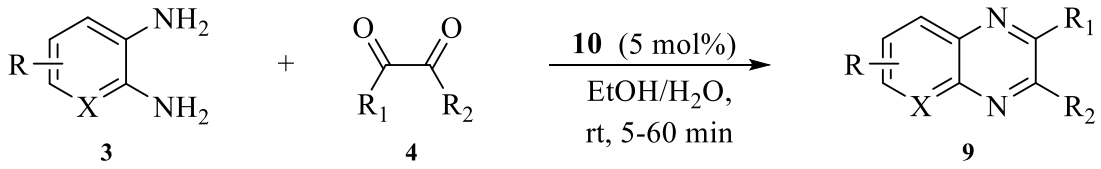


Figure 2.1 Drug agents with quinoxaline moiety (Liu *et al*., 2011; Fabian *et al*., 2020; Tang *et al*., 2022).

In view of the significance and research value of quinoxaline scaffold, various synthetical routes have been extensively studied and introduced. The synthetic protocol ranges from simple condensation to complex rearrangement reactions. The preparation methods are discussed in detail in the following sections.

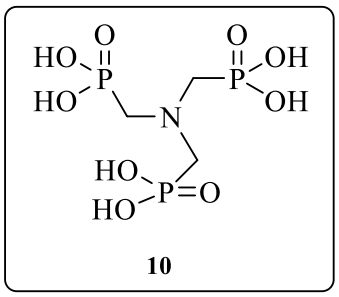
2.1.1 Reaction of 1,2-diamines and 1,2-dicarbonyl

As reported, aside from the simple condensation of 1,2-diamines **3** with 1,2-dicarbonyl compounds **4**, Fathi and team introduced an improvised preparatory protocol involving an organocatalyst for the synthesis of quinoxaline **9** from the condensation of 1,2-diamines **3** and 1,2-dicarbonyl **4** (Fathi *et al*., 2015). This reaction was carried out with numerous substituents of 1,2-diamines **3** in EtOH/H_2O catalysed by 5% nitrilotris(methylenephosphonic acid) **10**. All target compounds afforded excellent yields (80-97%), demonstrating effectiveness of the strategy in Scheme 2.2.



80-97% yield

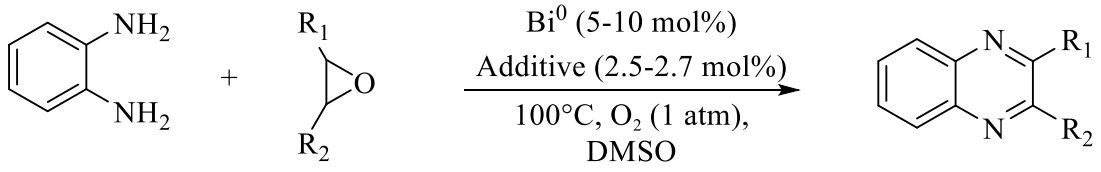
R = H, 4-Cl, 4-CH_3, 4-COC_6H_5, 4-NO_2, 5-CH_3
X = C, N
R_1 = Ph, 4-OCH_3-Ph, 4-CH_3-C_6H_5, CH_3
R_2 = CH_3, C_6H_5, 4CH_3-C_6H_5, 4-OCH_3-C_6H_5



Scheme 2.2 Reaction of 1,2-diamines with 1,2-dicarbonyl.

2.1.2 Reaction of epoxides and *o*-phenylenediamines

The new strategy of the synthesising quinoxaline derivatives **12** from simple epoxides has been reported by Antoniotti *et al*. This protocol was carried out using the *o*-phenylenediamines (**3**) and the internal epoxides (**11**) in an oxidative coupling catalysed by bismuth powder. The reaction also used $Cu(Otf)_2$ or TfOH as an additive and DMSO as a solvent. The results were desired compounds in moderate yield (53-71%), as in Scheme 2.3 (Antoniotti & Duñach, 2002).



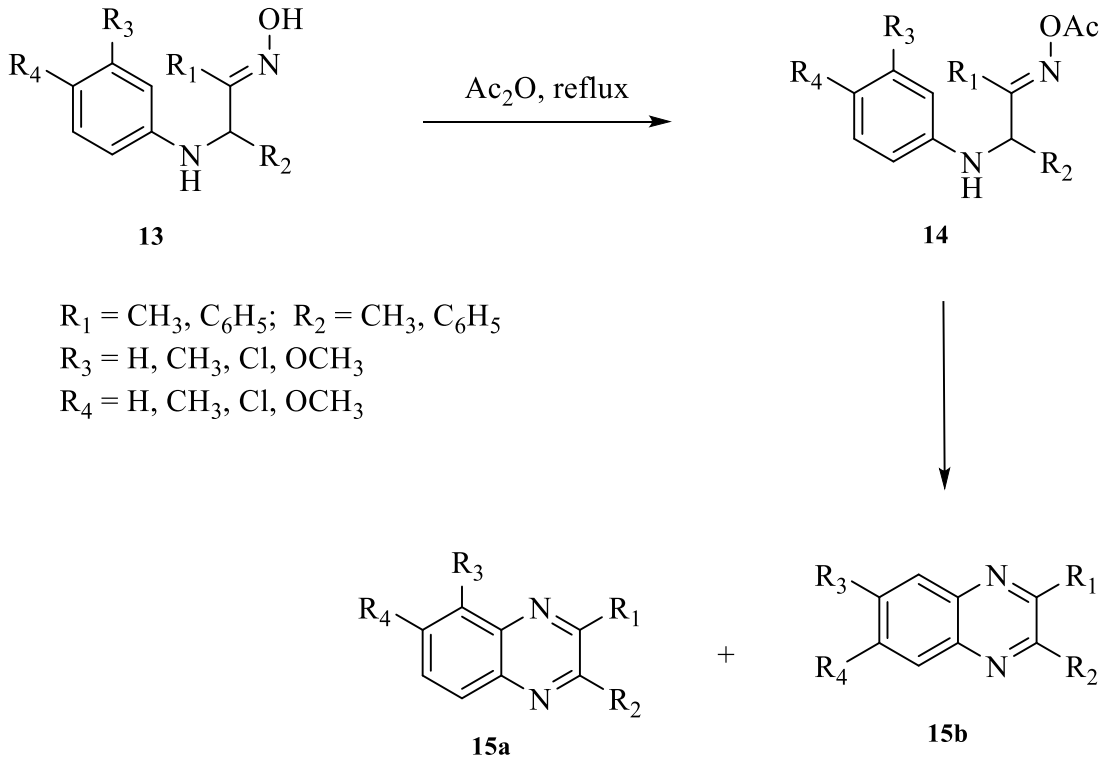
53-71%

Additive = $Cu(OTf)_2$, TfOH

Scheme 2.3 Reaction of epoxide with *o*-phenylenediamines.

2.1.3 Reaction of *α*-arylimino oxime and dicarbonyl compounds

Xekoukoulotakis and team demonstrated a one-pot straightforward synthesis of quinoxaline derivatives *via* oxidative cyclisation of *α*-arylimino oxime **13** (Xekoukoulotakis *et al*., 2000). The reaction involved thermal conversion in the presence of acetic anhydride to form oxime acetate **14**, which subsequently forms quinoxaline **15a** in moderate yield (Scheme 2.4). The authors believe that the cyclisation of this analogue occurred after acetylation through iminyl radical and spirodienylone.

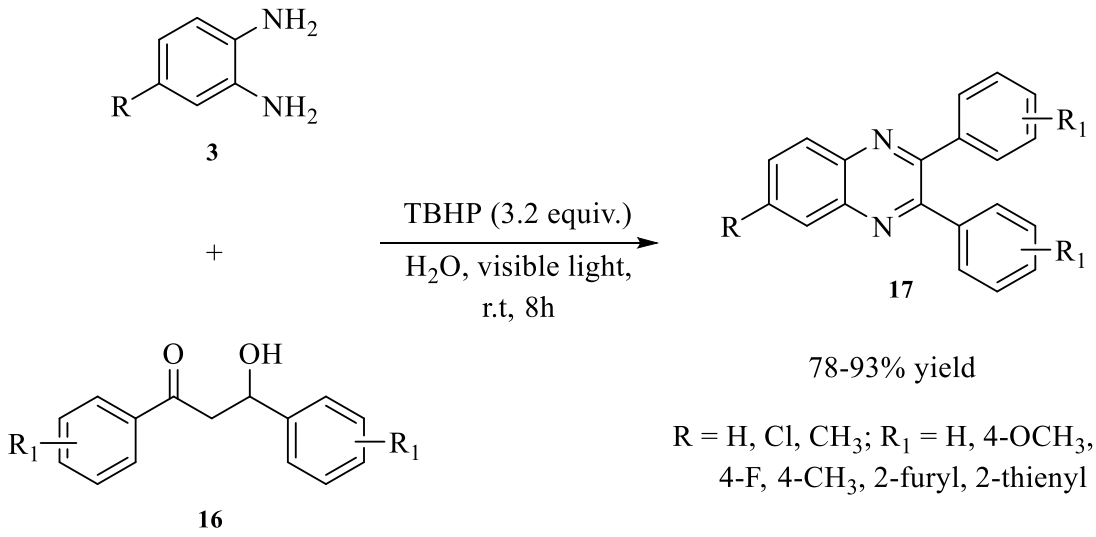


Scheme 2.4 Reaction of dicarbonyl and *α*-arylimino oxime.

2.1.4 Reaction of *α*-hydroxy ketones and *o*-phenylenediamine

Sarma and co-workers have disclosed a visible light-assisted catalyst-free green protocol toward the synthesis of a series of quinoxaline derivatives by employing easily accessible *o*-phenylenediamine **3** and *α*-hydroxy ketone **16** as starting materials

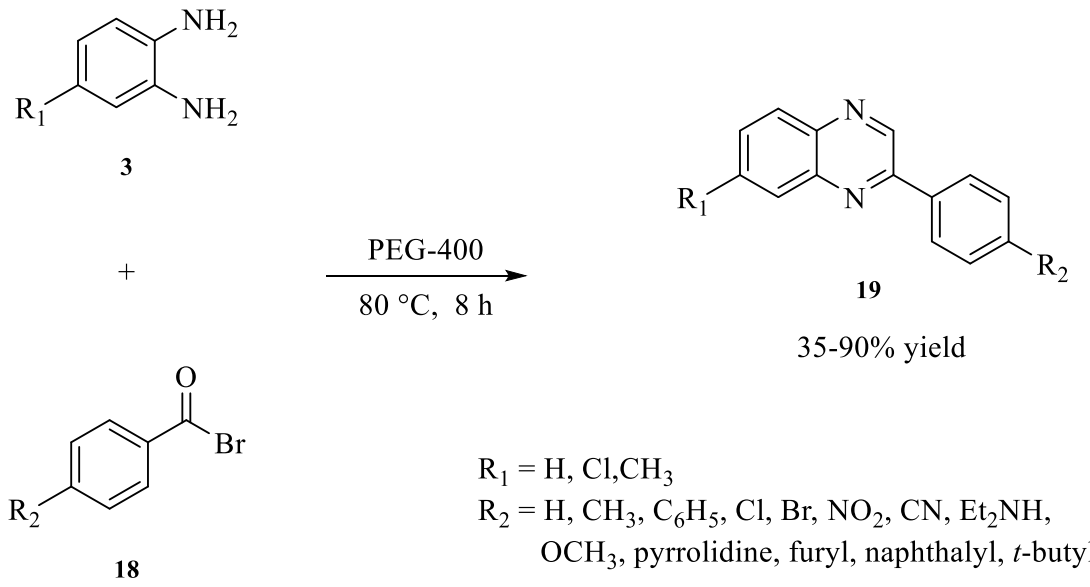
(Scheme 2.5). Using *tert*-butyl hydroperoxide (TBHP) as an oxidizing agent, the corresponding quinoxaline products **17** were obtained in 78–93% yield. The decomposition of TBHP into its radical was predominately promoted by metal- or photo-catalysts, or other reagents. However, in this reaction, the decomposition of TBHP for the radical formation and its acceleration for the completion of the reaction was simply achieved by visible light irradiation, thus no additional metal-based catalyst or photocatalyst is required. The successful utilisation of the photoinduced catalyst-free protocol in an aqueous medium makes this approach very efficient for green and sustainable practices (Sarma *et al*., 2021).



Scheme 2.5 Reaction of *α*-hydroxy ketones and *o*-phenylenediamine.

2.1.5 Reaction of phenacyl bromides *o*-phenylenediamine

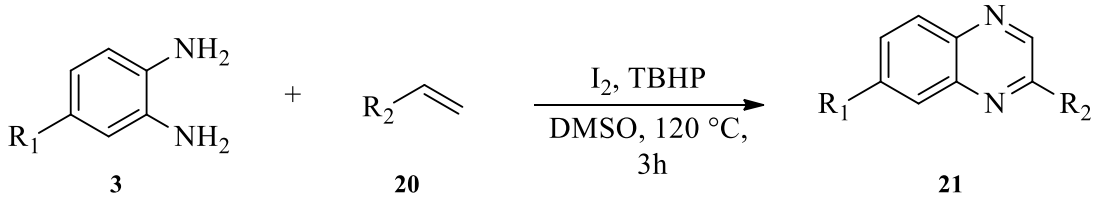
Another synthesis pathway of quinoxaline **19** was introduced by Nagarapu and coworkers. The team performed the reaction of *o*-phenylenediamine **3** with phenacyl bromide **18** in PEG-400 and reacted at 80 ºC. This protocol proceeded smoothly in the absence of a catalyst with the promising results up to 90% yield (Nagarapu *et al*., 2010) (Scheme 2.6).



Scheme 2.6 Reaction of phenyl bromide and *o*-phenylenediamine.

2.1.6 Reaction of alkene and *o*-phenylenediamine

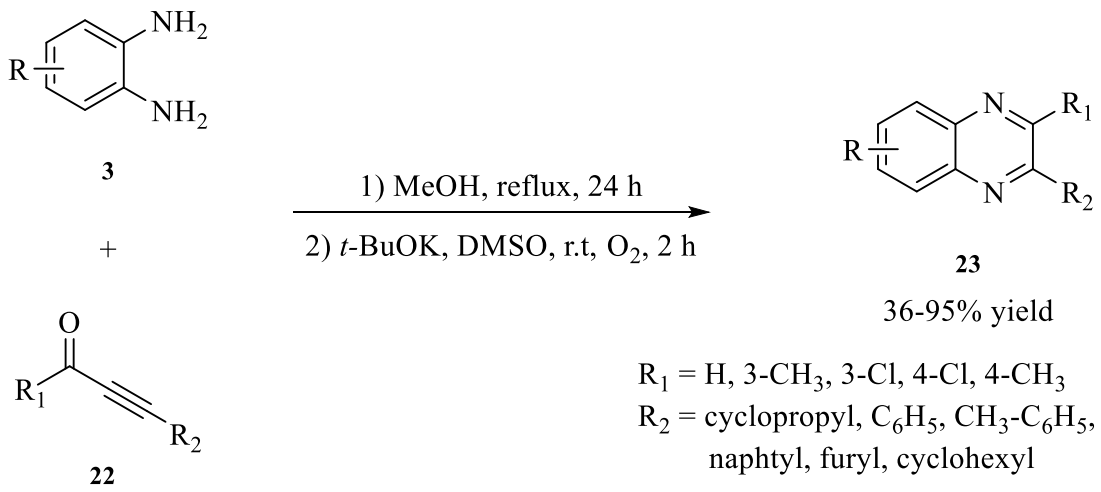
In the presence of alkene to afford quinoxaline **21**, Chaskar et al. conducted studies on the condensation reaction between various *o*-phenyldiamine **3** with alkene **20**, using iodine (I_2) as catalyst, TBHP as an oxidant agent and dimethyl sulfoxide (DMSO) as solvent, as shown in Scheme 2.7. With the optimized condition, the reaction successfully obtained corresponding compounds with the yield of 31-93%. It is noteworthy that the reported protocol was also applicable to heteroaromatic alkenes (Vadagaonkar *et al*., 2015).



Scheme 2.7 Reaction of alkene and *o*-phenylenediamine.

2.1.7 Reaction of alkyne and *o*-phenylenediamine

One-pot approach for the regiospecific synthesis of quinoxaline derivatives **23** from various aryl, heteroaryl, and alkyl-substituted ynones **22** with *o*-phenyldiamine **3** has been reported by Cui *et al*, as in Scheme 2.8. In the suppression of metal catalysts, the reported synthetic protocol, which occurred *via* an intramolecular Micheal addition, followed by dehydration condensation and subsequent base-promoted C–α-CH_2- extrusion process has successfully obtained quinoxaline derivatives **23** with the yield 36-95% (Shen *et al*., 2016).

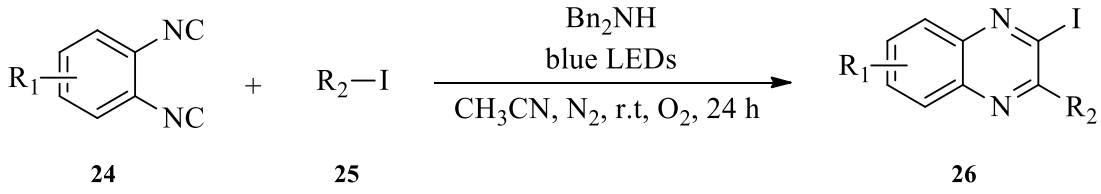


Scheme 2.8 Reaction of alkyne and *o*-phenylenediamin.

2.1.8 Reaction of isonitrile and perfluoroalkyl iodides

Sun and the team reported the development and deployment of non-covalent bond-mediated radical reactions, as an alternative metal- and additive-free synthesis protocol incorporating visible-light-induced single electron transfer for the synthesis of aromatic scaffoldings including quinoxaline derivatives (Cheng *et al., 2015;* Sun *et al*., 2016). With presence of visible light from blue light emitting diodes (LED), *o*-diisocyanoarene **24** traps the radical perfluoroalkyl iodides **25** to form quinoxaline ring

26 with halogen bonds. The reaction proceeded smoothly at room temperature for 24 h given the yield of 50-82%, as in Scheme 2.9.



24 **25** **26**

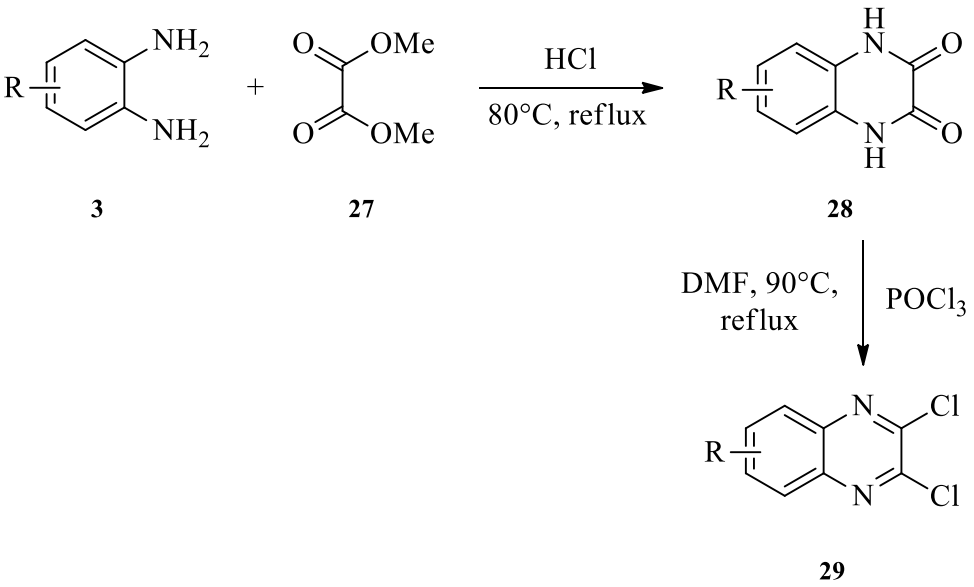
50-82% yield

R_1 = H, 4-CH_3, 5-CH_3, 4-OCH_3, 5-OCH_3, 6-OCH_3
R_2 = CF_3, C_3F_7, C_4F_9, C_6F_{13}, C_7F_{15}, C_8F_{17}

Scheme 2.9 Reaction of isonitrile and perfluoroalkyl iodides.

2.2 Synthesis route of 2,3-Dichloroquinoxaline

Lin and co-workers have successfully synthesised quinoxaline derivatives with dichloro-substituents *via* a two-step's chlorination reaction (Lin *et al*., 2020). The reaction was initially carried out in the presence of *o*-phenyldiamines **3** with dimethyl oxalate **27** to form quinoxaline-2,3-dione **28** in hydrochloric acid (HCl). Subsequently, 2,3-dichloroquinoxaline **29** can be readily produced from the use of quinoxaline-2,3-dione **28** as precursor and phosphorus oxychloride ($POCl_3$) as shown (Scheme 2.10). The reported synthesis protocol is in close agreement with a procedure pioneered by Hinsberg (Hinsberg, 1884).



Scheme 2.10 Synthesis route of 2,3-dichloroquinoxaline.

2.3 Role of quinoxaline moiety as antidiabetic

In line with the alarming global prevalence of diabetes in recent decades, researchers have carried out numerous studies on nitrogen-bearing heterocycle and their scaffolds as potential candidatures for anti-hyperglycemia with promising inhibitory effects on certain enzymes.

Bahekar and coworkers reported two series of 3,6,7-trisubstituted-2-(1H-imidazol-2-ylsulfanyl)-quinoxalines and 2-(quinoxalin-2-yl)-isothioureas, that were screened *in vitro* in a RIN5F (rat insulinoma) cell assay for glucose-dependent insulinotropic activity (Bahekar *et al*., 2007). The series of (imidazole-2-ylsulfonyl)-quinoxaline derivatives, especially 2-((6,7-dichloro-3-phenylquinoxalin-2-yl)thio)-1,3,4-thiadia-zole (**30**) had high insulin secretion with the IC_{50} of 16.2 pg/hr (Figure 2.2). In contrast, none of quinoxaline-2yl-isothiourea is favourable for the insulinotropic effect.

Kulkami and co-workers studied extensively on the new transition metal complexes of quinoxaline-thiosemicarbazine ligands, which obtained by using

quinoxaline-2,3-diones as precursor and incorporated with thiosemicarbazide, before treating the ligands with metal (II) chloride ($CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$ and $ZnCl_2$) (Kulkarni *et al*., 2012). The complexes of ligands were assessed for their antidiabetic activity in the diabetes-induced Wister rats by doing the blood glucose level test and oral glucose tolerance test (OGTT). The complex of ligand (*E*)-2-(3-oxo-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-*N*-phenylhydrazine-1-carbothioamide (**31)** as shown in (Figure 2.2) exhibited the best activity among the synthesised metal complexes of ligands and with comparable efficiency as the standard drug antidiabetic glibenclamide in both blood glucose levels reduction and OGTT test with notably low toxicity.

Yang and the coworkers presented a novel and facile synthesis of new class of quinoxalinone derivatives as a potent aldose reductase inhibitor (Yang *et al*., 2012; Wu *et al*., 2013). The protocol was claimed to be superior in the preparation of a wide variety of bioactive quinoxaline-based compounds, targeting for anti-diabetes. The synthesised compounds were mainly with an N1-acetic acid head group and a substituted C3-phenoxy side chain. Among these target compounds, the most dominant compound that showed good selectivity and inhibitory strength against aldose reductase were 2-(3-(4-bromophenoxy)-7-fluoro-2-oxoquinoxalin-1(2*H*)-yl)acetic acid (**32**), as depicted in Figure 2.2, with the IC_{50} value of 11.40 nM.

The same research team led by Wu has reported another series of 2-(3-benzyl-2-oxoquinoxalin-1(2*H*)-yl)acetic acid derivatives as potent aldose reductase inhibitors (Wu *et al.*, 2013). Quinoxaline acts as the core scaffold with targeted substitution at the C3-benzyl side chain with phenoxy or anilino group. As demonstrated in this study, there were 9 synthesised compounds with good inhibitory strength against aldose reductase. Among these compounds, 2-(3-(4-bromo-2-fluorobenzyl)-7-fluoro-2-

oxoquinoxalin-1(2*H*)-yl)acetic acid (**33**) (Figure 2.2) with an IC_{50} value of 6.44 nM, representing a remarkable 13 folds improvements over the commercial aldose reductase inhibitor drug epalrestat (IC_{50} value of 85.68 nM). Based on structure-activity relationship (SAR) studies, the author concluded that in terms of biological activity, the substituent of C3-benzyl side chain was more impactful than C3-phenoxy side chain due to conformational shape of benzyl side chain was favoured for binding in the active site.

Hussain *et al*., introduced a novel series of quinoxalinone with nitro-benzyl and piperazine substitutions as an effective aldose reductase inhibitor (Hao *et al*., 2019; Hussain *et al*., 2014). The results revealed that 2-(2-oxo-3-phenethylquinoxalin-1(2*H*)-yl)acetic acid **(34)** (Figure 2.2) bearing a C3-phenethyl side chain was the most potent inhibitor with an IC_{50} value of 0.143 μM. The research team extended their investigations into SAR of the synthesised structures and suggested that the substituent of both the phenyl and NO_2 groups plays an important role in enhancing the activity and selectivity of the quinoxalinone-based inhibitors. Nevertheless, this research group led by Hao intensified research efforts into exploration of potent aldose reductase inhibitors by expanding new quinoxaline series with hydroxypyrazole group replacing carboxylic acid (Hao et al., 2019). Among the synthesised compounds of this series, 7-fluoro-1-(1-hydroxy-1*H*-pyrazol-4-yl)-3-(4-hydroxybenzyl)quinoxalin-2(1*H*)-one (**35**) (Figure 2.2) exhibited the lowest IC_{50} value of 0.107 μM, which was comparative to that of epalrestat as the positive control. The study highlighted that the significant enhancing bioactivity of synthesised compounds were contributed by excellent bioisostere of the 1-hydroxypyrazole head group and its synergetic effects with phenolic hydroxyl and vinyl spacer as C3 side chain at quinoxaline scaffolding.