SYNTHESIS AND MOLECULAR DOCKING STUDIES OF NEW PHENYLISOXAZOLE QUINOXALINE-2-AMINE HYBRIDS AS POTENTIAL α-AMYLASE AND α-GLUCOSIDASE INHIBITORS

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by

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LIST OF SYMBOLS

α	Alpha
Å	Angstrom
β	Beta
cm^{-1}	Per centimeter
δ	Chemical shift
δ_c	Chemical shift carbon
δ_H	Chemical shift proton
°C	Degree Celsius
g	Grams
$gmol^{-1}$	Grams per mol
Hz	Hertz
J	Coupling constant
µg	Microgram
µL	Microlitre
µM	Micromolar
M	Molarity
mg	Milligram
mm	Millimeter
mM	Millimolar
MHz	Megahertz
mL	Milliliter
mmol	Millimole
nm	Nanometer
o-	Ortho
ppm	Parts per million

%	Percentage
%T	Percentage transmittance
υ	Wavenumber

LIST OF ABBREVIATIONS

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
AMPK	Adenosine monophosphate-activated protein kinase
ATP	Adenosine triphosphate
ATR-FTIR	Attenuated total reflectance−Fourier-transform infrared
aq.	Aqueous
BF_3OEt_2	Boron trifluoride etherate
br. s	Broad singlet
cat.	Catalyst
$CDCl_3$	Deuterated chloroform
CF_3COOEt	Ethyl trifluoroacetate
CH_2Cl_2	Dichloromethane
COX-2	Cyclooxygenase-2
d	Doublet
dd	Doublet of doublet
DCM	Dichloromethane
DM	Diabetes Mellitus
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
DNS	3,5-Dinitrosalicylic acid
DPP-4	Dipeptidyl peptidase-4
DPPH	α,α-diphenyl-β-picrylhydrazyl
EC_{50}	Half-maximal effective concentration
Et	Ethyl
Et_3N	Triethylamine
EtOH	Ethanol
FDA	Food and Drug Administration
FRAP	Ferric reducing antioxidant power
FTIR	Fourier-transform infrared
GLP-1	Glucagon-like peptide-1
GLP-1 RA	Glucagon-like peptide-1 receptor agonist

GPR40	G protein-coupled receptor 40
GIP	Gastric inhibitory polypeptide
HCl	Hydrochloric acid
HMBC	Heteronuclear multiple bond correlation
HRMS	High-resolution mass spectroscopy
hr	Hour(s)
HSQC	Heteronuclear single quantum coherence
IC_{50}	Inhibition concentration at 50%
LC_{50}	Lethal concentration at 50%
m	Multiplet
MIC	Minimal inhibitory concentration
mins	Minutes
MS	Mass spectrometer
N_2	Nitrogen gas
n-BuLi	*n*-Butyllithium
ND	Not determined
NMR	Nuclear magnetic resonance
NaOCl	Sodium hypochlorite
Na_2CO_3	Sodium carbonate
NPOQA	*N*-(4-methyl-2-nitrophenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide
NSAIDs	Non-steroidal anti-inflammatory drugs
OS	Operating system
PDB	Protein data bank
Ph	Phenyl
pNPG	4-Nitrophenyl α-D-glucopyranoside
$POCl_3$	Phosphoryl chloride
PPARs	Peroxisome proliferator-activated receptors
PTP1B	Protein tyrosine phosphatase 1B
RCSB	Research Collaboratory for the Structural Bioinformatics
r.t.	Room temperature
s	Singlet
SAR	Structure-activity relationship
SGLT-2	Sodium-glucose cotransporter-2
$SOCl_2$	Thionyl chloride

STZ	Streptozotocin
SUR	Sulfonylurea receptors
t	Triplet
t	tert
t-BuOK	Potassium *tert*-butoxide
T1DM	Type-1 diabetes mellitus
T2DM	Type-2 diabetes mellitus
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography
THF	Tetrahydrofuran
TMS	Tetramethylsilane
TZD	Thiazolidinediones
UCSF	University of California, San Francisco
UV	Ultraviolet

LIST OF APPENDICES

SINTESIS DAN KAJIAN PENDOKKAN MOLEKUL KEPADA TERBITAN FENILISOKAZOLA KUINOKSALINA-2 AMINA YANG BERPOTENSI SEBAGAI PERENCAT *A*-AMILASE DAN *A*-GLUKOSIDASE

ABSTRAK

Hibrid fenilisoksazola kuinoksalina-2-amina **55a-i** telah berjaya disintesis dengan peratus hasilan antara 53-85%. Sebatian yang telah disintesis telah dipencilkan dan dicirikan dengan menggunakan kaedah spektroskopi 1D- dan 2D-NMR (^{1}H, ^{13}C, HSQC, HMBC dan COSY NMR), FTIR dan HRMS. Setelah itu, sebatian diuji dalam kajian *in vitro* perencat enzim *α*-amilase dan *α*-glukosidase bersama acarbose sebagai kawalan positif. Daripada ujian keaktifan biologi yang telah dijalankan, didapati bahawa sebatian **55i** adalah perencat terkuat *α*-amilase dengan nilai IC_{50} = 16.4 μM manakala sebatian **55a**, **55c-f**, dan **55i** merupakan perencat kuat *α*-glukosidase, dengan sebatian **55e** merupakan perencat terkuat *α*-glukosidase (IC_{50} = 15.2 μM). Tambahan pula, potensi sebatian sebagai perencat *α*-amilase dan *α*-glukosidase telah disokong melalui ujian analisis pendokkan molekul. Bagi kedua-dua enzim, sebatian yang berpotensi sebagai perencat menunjukkan nilai tenaga pengikat yang tinggi. Sebatian **55i** menunjukkan interaksi yang penting dengan tapak aktif *α*-amilase dan merekodkan tenaga pengikat tertinggi dengan nilai -8.9 ± 0.10 kcal/mol, manakala sebatian **55e** menunjukkan tenaga pengikat tertinggi iaitu sebanyak -9.0 ± 0.20 kcal/mol dengan membentuk interaksi penting dengan tapak aktif *α*-glukosidase. Daripada ujian *in vitro* dan *in silico* yang telah dijalankan, didapati bahawa sebatian **55c** dan **55i** menunjukkan potensi sebagai dwiperencat bagi kedua-dua enzim. Oleh itu, dapat disimpulkan bahawa sebatian hibrid fenilisokazola kuinoksalina-2-amina yang telah disintesis

menunjukkan potensi yang tinggi sebagai perencat α-amilase dan α-glukosidase bagi diabetes jenis 2 berbanding kawalan positif, acarbose.

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ABSTRACT

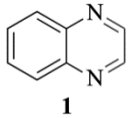
New phenylisoxazole quinoxaline-2-amine hybrids **55a-i** were successfully synthesised with percentage yield ranging from 53% to 85%. These compounds were purified and characterised by 1D- and 2D- NMR (^{1}H, ^{13}C, HSQC, HMBC and COSY), FTIR, and HRMS analyses. Then, the hybrids underwent *in vitro* α-amylase and α-glucosidase inhibitory assays, with acarbose as the positive control. Through the biological study, compound **55i** exhibits the most potent α-amylase inhibitory activity with IC_{50} = 16.4 μM, while compounds **55a**, **55c-f**, and **55i** exhibit good potential as α-glucosidase inhibitors, with **55e** being the most potent inhibitor (IC_{50} = 15.2 μM). Moreover, through the molecular docking studies, the inhibition potential for both α-amylase and α-glucosidase were affirmed, where all selected compounds exhibit good binding energy with both enzymes. Compound **55i** showed important interactions with α-amylase enzyme active site and exhibited the highest binding energy of -8.9 ± 0.10 kcal/mol, while compound **55e** exhibited the highest binding energy of -9.0 ± 0.20 kcal/mol by forming important interactions with the α-glucosidase enzyme active site residues. From the *in vitro* and *in silico* studies conducted, it can be concluded that compounds **55c** and **55i** exhibit the potential as dual inhibitors for both enzymes. Thus, it can be concluded that the quinoxaline-isoxazole hybrids synthesised in this study exhibit promising potential as α-amylase and α-glucosidase inhibitors for type 2 diabetes mellitus.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Organic synthesis involves the science of synthesising both designed and natural substances containing carbon as the primary element (Nicolaou, 2014). The development of organic synthesis is said to be marked by Friedrich Wöhler's synthesis of urea, the first organic compound synthesised from an inorganic substance back in 1828 (Nicolaou, 2014; Shampo & Kyle, 1985). Heterocyclic compounds are a cyclic ring of atoms containing at least one heteroatom; the most common of them being nitrogen, oxygen, and sulphur (Kabir & Uzzaman, 2022). Heterocyclic compounds are regarded as a key component in medicinal chemistry, and they have been utilised as various medical agents such as antifungal, anti-inflammatory, antibacterial, anticancer and antidiabetic agents (Kabir & Uzzaman, 2022; Saini *et al.*, 2013). Nitrogen-containing heterocyclic compounds have demonstrated great importance towards recent drug discovery, with this group being present in a handful of recently FDA-approved therapeutic drugs (Suthar *et al.*, 2022; Vitaku *et al.*, 2014). Quinoxaline, also known as 1,4-diazanapthalene or benzopyrazines, is a nitrogen-containing heterocyclic compound, with its structure composing of a benzene ring and a pyrazine ring condensed together, as shown in Figure 1.1.



1

Figure 1.1 Quinoxaline.

Quinoxalines have been shown to have good biological activities such as antidiabetic, antibacterial, antimalarial, antituberculosis, anticancer, antiviral, and anti-inflammatory activities (Suthar *et al.*, 2022). Quinoxalines are also used in the agriculture industries, as it is a major component in insecticides, herbicides, and fungicides (Meyes, 1985). This class of compound has also been used as dyes for solar cell applications, organic conductors and fluorescent materials (Suthar *et al.*, 2022).

Azoles are a class of nitrogen-containing five-membered heterocyclic compounds, with an addition of at least one non-carbon heteroatom. Azoles are critical for various types of applications in the pharmaceutical field and are used in the organic synthesis of various complex molecules (Hu & Szostak, 2015). Isoxazoles is a class of azoles, with its structure containing a nitrogen and an oxygen atom in a five-membered aromatic ring. The skeletal structure of isoxazole is depicted in Figure 1.2.

2

Figure 1.2 Isoxazole.

Isoxazoles have applications in various industries including the pharmaceutical and agricultural industry (Gutiérrez *et al.*, 2013). The isoxazole derivatives can be found in herbicides, and are used as a foundation in medicines such as COX-2 inhibitors and nitric acid donors (Shrivastava, 2022). Isoxazoles have also been researched to have good biological activities as antidiabetic, antimicrobial, antiviral, anticancer, and anti-inflammatory agents (Sysak & Obmińska-Mrukowicz, 2017).

A hybrid compound can be defined as a compound that composes of artificially assembled two or more classes of compounds synthesised to discover a much more potent and efficient drug to combat a certain disease (Alkhzem *et al.*, 2022; Mller-Schiffmann *et al.*, 2012). These compounds are usually combined and attached with a molecular linker through a covalent bond, which can be made cleavable or non-cleavable. A cleavable linker is expected to bio-transform when the hybrid compounds reach the active site, whereas a hybrid with a non-cleavable linker remains its structure throughout the period where it remains in the body (Alkhzem *et al.*, 2022).

Diabetes Mellitus, commonly known as diabetes, is a metabolic disorder caused by defects in insulin production, insulin secretion, or both (American Diabetes Association, 2005). Chronic cases of diabetes are often associated with long-term complications, dysfunction, or damage of internal organs (American Diabetes Association, 2005). Generally, diabetes cases are often categorized into two types; type 1 and type 2 diabetes (T2DM). Type 1 diabetes (T1DM) is caused by the failure of the pancreas to produce and secrete insulin (American Diabetes Association, 2005). More prevalently, T2DM is heterogenous in that the occurrence is contributed by both genetic and environmental factors, with a majority of the cases caused by the latter factor (Riddle *et al.*, 2021). This disease can cause one or more of these three primary defects; insulin resistance, the failure of β-cells found in the pancreas and the overproduction of hepatic glucose, all of which contribute to hyperglycaemia (Weber & Thornberry, 2010). There have been many types of drugs approved for T2DM treatment; Metformin is considered a first-line drug to be used for diabetic patients, followed by sulfonylureas, DPP-4 inhibitors, GLP-1 receptor agonists, meglitinides and α-glucosidase inhibitors (Lahiri, 2012).

α-Amylase and *α*-glucosidase enzymes are enzymes that are in charge of breaking down carbohydrates into simpler forms such as glucose. The *α*-amylase enzymes can be found in the pancreas and break down dietary carbohydrates such as starch and glycogen into simpler chains in the digestive system, whilst *α*-glucosidase enzymes are situated on the surface border of the small intestine cells and break down oligosaccharides into glucose to be absorbed into the blood. The inhibition of these enzymes can help slow down postprandial hyperglycaemia by decreasing the breaking down of complex sugars into glucose, thus lowering the rate of absorption of glucose into the blood (Kumar *et al.*, 2011). These inhibitors competitively bind to the enzyme *via* the lock-and-key model. In the case of T2DM treatment, *α*-glucosidase inhibitors are much more common compared to *α*-amylase inhibitors. In the inhibitory bioassay studies for these enzymes, the rate of inhibition is measured with IC_{50} values calculated by measuring the concentration of indicators present *via* UV absorbance. The results of the bioassay studies can be further confirmed by conducting *in silico* molecular docking studies of our compounds as the ligands, with the enzymes. The docking process calculates the binding energy of the ligand-enzyme complexes by measuring the different types of interactions formed in the complex. The lowest value of binding energy indicates the ligand-enzyme complex contain the strongest interactions formed within the complex when compared to other ligands.

Hence, based on past research conducted on the potential inhibitory activity exhibited by selected quinoxaline (Hameed *et al.*, 2022; Missioui *et al.*, 2021; Settypalli *et al.*, 2019; Khan *et al.*, 2014) and isoxazole derivatives (Saidi *et al.*, 2021 and Lin *et al.*, 2020) as *α*-glucosidase and *α*-amylase, these compounds are hybridised and selected to undergo bioassay and molecular docking studies with the aim to synthesise more potent *α*-glucosidase and *α*-amylase inhibitors as T2DM treatment.

1.2 Problem statement

Diabetes Mellitus has been a chronic disease that has been affecting a huge portion of the world population for decades. According to the International Diabetes Federation, there are approximately 537 million adults currently living with diabetes in 2021, of which 90% of them have T2DM and the number of patients is expected to rise to 645 million by 2030 (International Diabetes Federation, 2022). Since T2DM is a heterogenous disease, it is a more complicated type of diabetes to treat, compared to T1DM, where most patients are treated with daily insulin treatment.

Treatment for T2DM has been an ongoing mission spanning decades of research. Metformin, a first-line drug for T2DM treatment has been approved and in use in most countries since the 1950s (Bell & Hadden, 1997). The wide usage of this drug compared to other oral agents can be contributed to its ability of it to reduce blood glucose levels by 1-2% (Lahiri, 2012). It also does not induce hypoglycaemia, or weight gain, and is generally cost-effective (Lahiri, 2012). This drug, however, still has its side effects, some of which include lactic acidosis, nausea, and diarrhoea (Nathan, 2015). Some of the other drugs used for T2DM treatment are sulfonylureas, DPP-4 inhibitors, GLP-1 receptor agonists, meglitinides and α-glucosidase inhibitors (Nathan, 2015). These drugs also have major side effects when ingested or administrated to patients, some of which include hypoglycaemia, weight gain, respiratory infections, congestive heart failure and diabetic ketoacidosis (Nathan, 2015).

α-Glucosidase and α-amylase inhibitors are a more recent type of T2DM treatment approved compared to other therapies. Some of the approved α-glucosidase and α-amylase inhibitors clinically used are acarbose, voglibose and miglitol, where

these drugs mostly inhibit α-glucosidase enzymes and only weakly inhibit α-amylase enzymes (Bedekar *et al.*, 2010). The side effects of these drugs can vary by patient. Generally, some of the adverse side effects of these drugs can include abdominal pain, diarrhoea, bloating, fluctuance, nausea and constipation (Nathan, 2015). These inhibitors have a limit of usage for monotherapy among other drugs due to their low efficacy compared to other treatments. Hence, although there is an abundance of other treatments available for T2DM, the major side effects of these treatments are a major setback for efficient use by diabetic patients. Moreover, since the research on α-glucosidase and α-amylase inhibitors are still limited, there is a lack of more effective therapy *via* this class of drug with minimal side effects. Hence, this project focuses on the synthesis of new quinoxaline-isoxazole hybrids and their biological evaluation as potential α-amylase and α-glucosidase inhibitors. The *in silico* molecular docking studies were conducted to further verify the potency of the synthesised hybrids as good α-amylase and α-glucosidase inhibitors for T2DM treatment.

1.3 Objectives

a) To synthesise new quinoxaline-isoxazole derivatives.
b) To characterise newly synthesised quinoxaline-isoxazole derivatives with various spectroscopy methods.
c) To evaluate the potency of the newly synthesised compounds as potential α-amylase and α-glucosidase inhibitors through *in vitro* bioassays.
d) To perform *in silico* molecular docking studies on selected quinoxaline-isoxazole derivatives.

CHAPTER 2

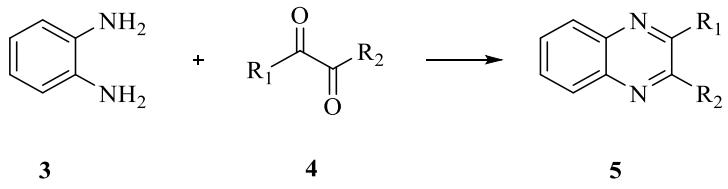
LITERATURE REVIEW

2.1 Overview

This chapter discusses the literature findings for the synthesis and biological activities of quinoxaline and isoxazole compounds, centering around their potential as potent α-glucosidase and α-amylase inhibitors. The mechanisms of multiple organic synthesis reactions are also discussed. Metabolic disorders, more importantly Diabetes Mellitus, and the general concept of molecular docking studies are also reviewed.

2.2 Quinoxaline

These past few decades have seen great progress in quinoxaline synthesis. The synthesis of quinoxaline can be done through several pathways. Scheme 2.1 shows the general synthesis of the quinoxaline skeleton involving the condensation reaction between 1,2-diamine (**3**) and 1,2-dicarbonyl derivatives (**4**).



3 **4** **5**

Scheme 2.1 General scheme of synthesis of quinoxaline.

The reported conditions for this reaction have evolved towards a greener chemistry. As reported by Rahmatpour in 2012, polystyrene-supported $AlCl_3$ has been used as a stable and reusable catalyst for the reaction with ethanol as a solvent. The quinoxaline derivatives were synthesised with percentage yield of up to 96% and short reaction time of 10-60 mins. In 2014, research by Shekhar *et al.* successfully synthesised quinoxaline using aqueous hydrofluoric acid as the catalyst at room

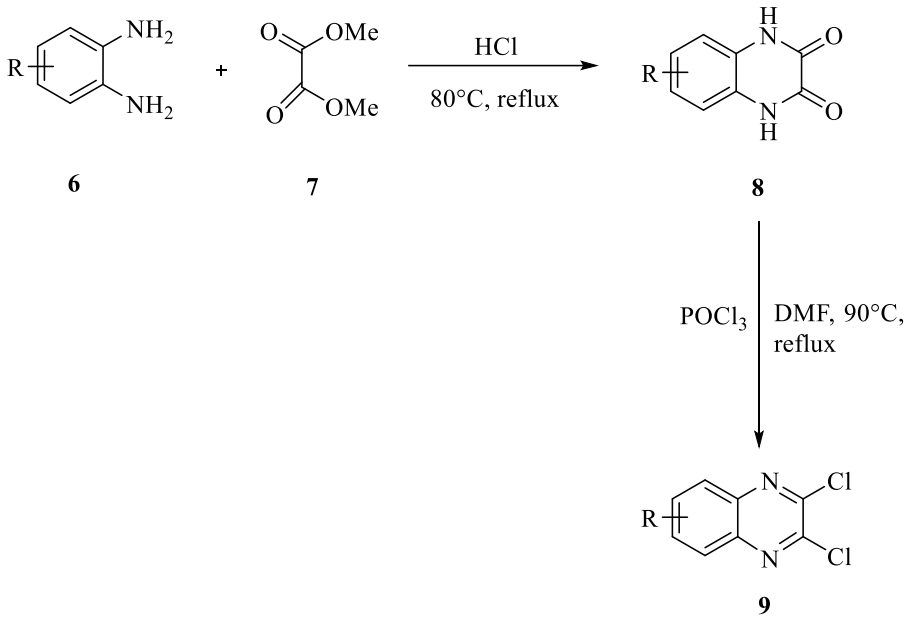
temperature, producing a percentage yield of up to 98% with reaction time of 10-90 mins. This method shows its advantage in the inexpensive catalyst, short reaction time and mild reaction conditions. More recently, Indalkar *et al.* in 2017 have reported a green method to synthesise this compound, with the use of sulfated polyborate as a recyclable catalyst in a solvent-free reaction. This method proved to be efficient as a high percentage yield of up to 99% was achieved in a mere 3 minutes, stirred at 100 °C. The different reaction conditions for the synthesis of quinoxaline have been tabulated in Table 2.1 below.

Table 2.1. Reaction conditions of the synthesis of quinoxaline.

Reaction Conditions	**Catalysts**	**Reaction Time**	**Yield (%)**	**Reference**
Ethanol, reflux	PS/$AlCl_3$	10-60 mins	84-96	Rahmatpour, 2012
Aq. Hydrofluoric acid, r.t.	Aq. Hydrofluoric acid	10-90 mins	95-98	Shekhar *et al.*, 2014
Solvent-free, 100 °C	Sulfated polyborate	3-10 mins	95-99	Indalkar *et al.*, 2017

To achieve disubstituted quinoxaline derivatives, one highlighted method has been reported by multiple research groups. This method involves a two-step reaction, the first step is initiated with the synthesis of the quinoxaline-2,3-dione intermediate followed by the synthesis of the disubstituted quinoxaline with a chlorinating agent. To illustrate this method, a report by Lin *et al.* in 2020 used this exact synthetic route to synthesise 2,3-dichloroquinoxaline derivatives (Scheme 2.2). The first step involves the reflux of substituted *o*-phenyldiamine derivatives **6** with dimethyl oxalate (**7**) to yield quinoxaline-2,3-dione derivatives **8**, and the reaction is continued with the chlorination

of **8** to yield substituted 2,3-dichloroquinoxalines compounds **9**, with reported percentage yield of the range 60% to 81%.



Scheme 2.2 Synthesis route of 2,3-dichloroquinoxaline derivatives.

A variation of this method has been reported by using different chlorinating agents such as thionyl chloride ($SOCl_2$) in CH_2Cl_2, as reported by Yang *et al.* in 2012, which have reported an excellent yield of around 94%.

Quinoxaline derivatives exhibit great potential as T2DM treatment, as DPP-4 inhibitors, GLP-1 receptor agonists, PPARγ and SUR agonists, *α*-amylase inhibitors, and *α*-glucosidase inhibitors. Knudsen *et al.* in 2007 studied the potential of 6,7-dichloro-2-methylsulfonyl-3-*N*-tert-butylaminoquinoxaline (**10**) shown in Figure 2.1 as a GLP-1 receptor agonist *via* functional screening assays. In 2010, a further study of compound **10** by Irwin *et al.* concluded that this compound has the potential to stimulate insulin secretion and lower blood glucose levels. However, it is also found that this compound shows to be less potent than stable peptide GLP-1 receptor mimics.

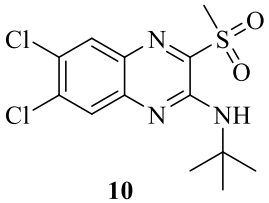


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Figure 2.1 6,7-dichloro-2-methylsulfonyl-3-*N*-tert-butylaminoquinoxaline (**10**) as potential GLP-1 agonist.

In 2017, Ibrahim and co-workers synthesised novel quinoxaline derivatives as potential PPARγ and SUR agonists as anti-hyperglycaemic agents. Figure 2.2 shows *N*-(4-[*N*-(Cyclohexylcarbamoyl)sulfamoyl]phenyl)2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide (**11)** that exhibited the most potent insulin-secreting activity as well as exhibits the ability to bind to PPARγ which is responsible for glucose metabolism when activated, with EC_{50} = 0.92 μM and IC_{50} = 0.482 μM compared to positive control Glimiperide and Rosiglitazone, respectively (Ibrahim *et al.*, 2017).

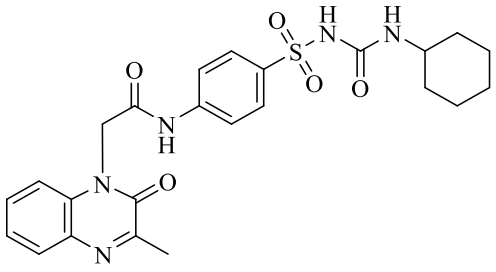

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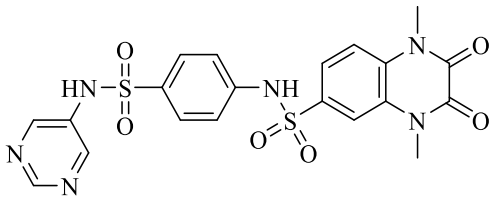
Figure 2.2 *N*-{4-[*N*-(Cyclohexylcarbamoyl)sulfamoyl]phenyl}2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide (**11**) as potential PPARγ agonist.

Some quinoxaline derivatives have also been studied as DPP-4 inhibitor. A study by Syam *et al.* in 2021 reported that 1,4-Dimethyl-2,3-dioxo-*N*-(4-(*N*-pyrimidin-2-yl)sulfamoyl)phenyl-1,2,3,4-tetrahydroquinoxaline-6-sulfonamide (**12**) (Figure 2.3) has shown to have potent DPP-4 inhibition, and also exhibits a promising glucose-

controlling effect (IC_{50} = 0.48 μM) in comparison to linagliptin, an approved DPP-4 inhibitor (Syam *et al.*, 2021).



12

Figure 2.3 1,4-Dimethyl-2,3-dioxo-*N*-(4-(*N*-pyrimidin-2-yl)sulfamoyl)phenyl-1,2,3,4-tetrahydroquinoxaline-6-sulfonamide (**12**) as DPP-4 inhibitor.

There has also been widespread research on the potency of quinoxaline derivatives as a good *α*-amylase and *α*-glucosidase inhibitors these past few years. A study by Khan *et al.* in 2014 researched the potential of new *N*-(11H-Indeno[1,2-b]quinoxalin-11-ylidene)benzohydrazide derivatives as potential *α*-glucosidase inhibitors. Their study found that compound **13** shown in Figure 2.4 exhibits the highest potency as *α*-glucosidase inhibitor with IC_{50} value of 22.7 μM, which is lower than that of acarbose (IC_{50} = 38.3 μM) as the positive control in the inhibitory assay (Khan *et al.*, 2014).

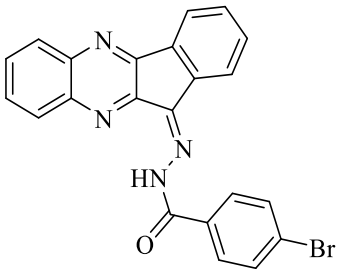


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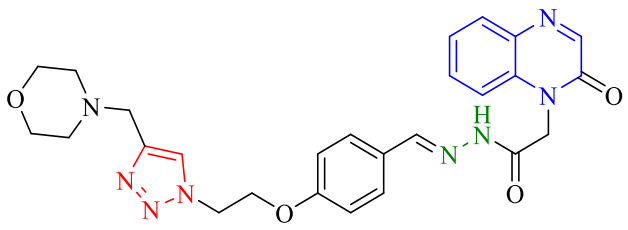
Figure 2.4 *N*-(11H-Indeno[1,2-b]quinoxalin-11-ylidene)benzohydrazide (**13**) as potential *α*-glucosidase inhibitor.

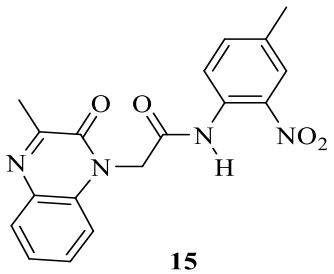
Settypalli and co-workers synthesised new quinoxaline-hydrazide hydrazone-1,2,3-triazole hybrids as potential *α*-glucosidase inhibitors. Their research found that the quinoxaline derivative, (*E*)-*N*'-(4-(2-(4-(morpholinomethyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)benzylidene)-2-(2-oxoquinoxalin-1(2*H*)-yl)acetohydrazide (**14**) in Figure 2.5, displayed the most potent inhibition of *α*-glucosidase with IC_{50} = 21.9 μg/mL, comparable to acarbose (IC_{50} = 22.3 μg/mL). It is reported that this might be due to the presence of the morpholino rings attached to the triazole group, compared to other compounds where the morpholino ring is replaced by piperidino and pyrrolidino rings (Settypalli *et al.*, 2019).



14

Figure 2.5 (*E*)-*N*'-(4-(2-(4-(Morpholinomethyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)benzylidene)-2-(2-oxoquinoxalin-1(2*H*)-yl)acetohydrazide as potential *α*-glucosidase inhibitor.

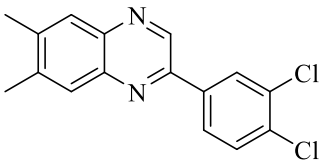
A new quinoxaline derivative, *N*-(4-methyl-2-nitrophenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide (NPOQA) (**15**) as shown in Figure 2.6, was synthesised by Missioui *et al.* in 2021 and tested for its *in vitro* biological activity against *α*-amylase and *α*-glucosidase enzymes. This study reported that this synthesised compound exhibits potent inhibitory activity against *α*-glucosidase (IC_{50}= 83.78 μM) and considerable *α*-amylase inhibition (IC_{50}= 199.70 μM) when compared to the positive control, acarbose (IC_{50}= 72.58 and 115.9 μM respectively) (Missioui *et al.*, 2021).



15

Figure 2.6 NPOQA (**15**) as potential α-amylase and α-glucosidase inhibitor.

More recently, 2-aryl quinoxaline derivatives have been synthesised and evaluated its potential of as α-amylase and α-glucosidase inhibitors by Hameed and co-researchers in 2022. 2-(3,4-Dichlorophenyl)-6,7-dimethylquinoxaline (**16)** shown in Figure 2.7 revealed most potent inhibitory activity towards both α-amylase and α-glucosidase enzymes with IC_{50} values of 294.35 nM and 198.21 nM respectively, compared to other synthesised analogues when put against the positive control acarbose (IC_{50}= 10.0 µM and 22.8 µM respectively) (Hameed *et al.*, 2022).



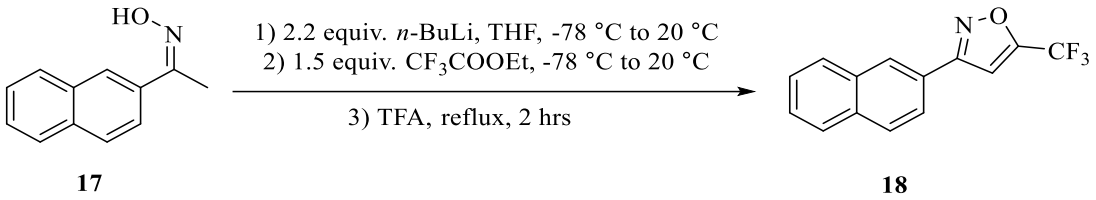
16

Figure 2.7 2-(3,4-Dichlorophenyl)-6,7-dimethylquinoxaline (**16**) as potential α-amylase and α-glucosidase inhibitor.

2.3 Isoxazoles

In recent years, there has been various methods developed for synthesising isoxazoles, some of which includes condensation, cycloisomerization, cycloaddition and functionalisation reactions. To demonstrate an example of a condensation reaction, a study by Ngo *et al.* in 2015 have synthesised 5-trifluoromethylated isoxazoles such as

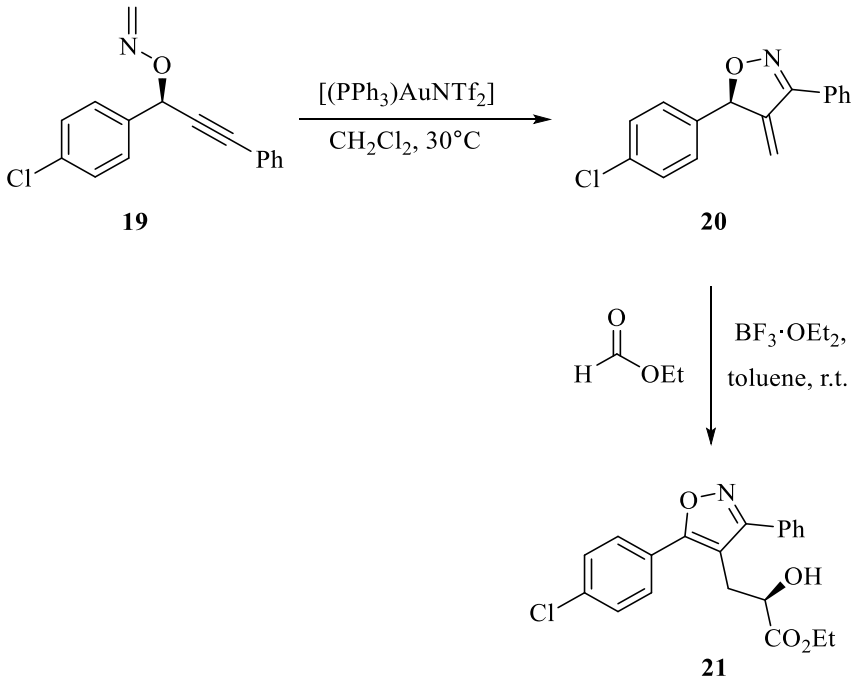
shown in Scheme 2.3 with a 3-step reaction of the synthesis of 3-(naphthalen-2-yl)-5-(trifluoromethyl)isoxazole (**18**) and yielded 62% of the product.



17 **18**

Scheme 2.3 Synthesis of 3-(naphthalen-2-yl)-5-(trifluoromethyl)isoxazole (**18**).

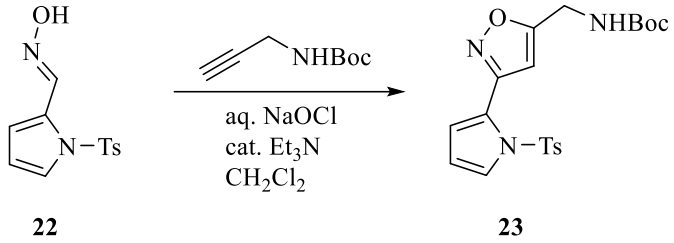
A research report by Gima *et al.* in 2017 approached the cycloisomerization method in synthesising isoxazole compounds. Scheme 2.4 demonstrates the synthesis method to yield a chiral isoxazole, which is initiated with a gold-catalysed reaction of **19** to afford substituted isoxazoline **20**, followed by the carbonyl ene reaction of **20** to afford Ethyl (*R*)-3-(5-(4-chlorophenyl)-3-phenylisoxazol-4-yl)-2-hydroxypropanoate (**21**) (Gima *et al.*, 2017).



19 **20** **21**

Scheme 2.4 Synthesis of ethyl (*R*)-3-(5-(4-chlorophenyl)-3-phenylisoxazol-4-yl)-2-hydroxypropanoate (**21**) *via* cycloisomerization reaction.

This reaction produced a 88% yield for the final product, which demonstrates the efficiency of this method. However, the use of gold catalyst might be a setback, as it is difficult and costly to acquire. In 2013, the cycloaddition reaction have been utilised by Frederich *et al.* to synthesise isoxazole derivatives. This is demonstrated in Scheme 2.5 below, where *tert*-butyl((3-(1-tosyl-1*H*-pyrrol-2-yl)isoxazol-5-yl)methyl)carbamate (**23**) was synthesised *via* the cycloaddition reaction in the presence of NaOCl and triethylamine (Frederich *et al.*, 2013).



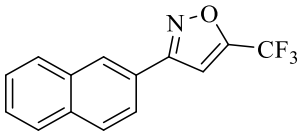
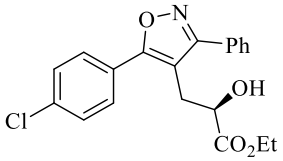
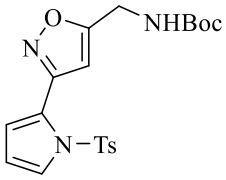
22 **23**

Scheme 2.5 Synthesis of *tert*-butyl ((3-(1-tosyl-1*H*-pyrrol-2-yl)isoxazol-5-yl)methyl)carbamate (**23**) *via* cycloaddition reaction.

This method has been reported to be one of the most established routes, with the above conditions proving to be more efficient compared to other methods. There have also been modifications of the conditions of this method, where some reactions are metal-catalysed (Himo *et al.*, 2005; Zhang *et al.*, 2014), and some involved the 1,3-dipolar cycloaddition of substituted alkynes (Hanamoto *et al.*, 2004; Moore *et al.*, 2005). The different reaction conditions for the synthesis of different isoxazole derivatives have been tabulated in Table 2.2 below.

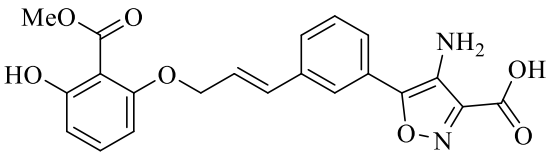
Table 2.2. Reaction conditions for the synthesis of different isoxazole derivatives.

Reaction Conditions	**Catalysts**	**Isoxazole synthesised**	**Time (hrs)**	**Yield (%)**	**Reference**

3-step condensation reaction: 1) *n*-BuLi, THF, -78 to 20 °C 2) CF_3COOEt, -78 to 20 °C 3) TFA, reflux, 2 hrs	-	 **18**	2-8	57-62	Ngo *et al.*, 2015
2-step cycloisomerization reaction: 1) CH_2Cl_2, 30 °C 2) $BF_3.OEt_2$, toluene, r.t.	$[(PPh_3)AuNTf_2]$	 **21**	0.5	80-86	Gima *et al.*, 2017
One-step cycloaddition reaction: Aq. NaOCl, CH_2Cl_2, 0 °C	Et_3N	 **23**	5	95	Fredrich *et al.*, 2013

Isoxazoles have been proven to play an important role, exhibiting good biological activities such as antimicrobial, antibacterial, antiviral, anticancer, anti-inflammatory and antidiabetic agents (Sysak & Obmińska-Mrukowicz, 2017). Isoxazoles have been reported to exhibit good potential as a component for T2DM treatment, some of which includes protein tyrosine phosphatase 1B (PTP1B) inhibitors, GPR40 agonists, *α*-amylase inhibitors, and *α*-glucosidase inhibitors (Sysak & Obmińska-Mrukowicz, 2017). The PTP1B protein is known to be responsible for the negative regulation of insulin secretion. In 2004, Zhao *et al.* synthesised isoxazole carboxylic acid derivatives as potential PTP1B inhibitors, guided by x-ray

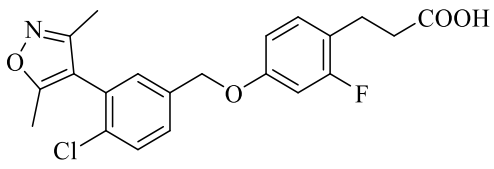
crystallography. Figure 2.8 shows (*E*)-4-amino-5-(3-(3-(3-hydroxy-2-(methoxycarbonyl)phenoxy)prop-1-en-1-yl)phenyl)isoxazole-3-carboxylic acid (**24**) reported to have good cellular activity, which is said to be contributed by its lower molecular weight and polarity compared to other synthesised derivatives (Zhao *et al.*, 2004).



24

Figure 2.8 (*E*)-4-Amino-5-(3-(3-(3-hydroxy-2-(methoxycarbonyl)phenoxy)prop-1-en-1-yl)phenyl)isoxazole-3-carboxylic acid (**24**) as PTP1B inhibitor.

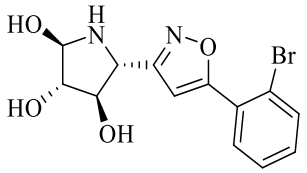
Besides that, isoxazoles have been studied to have the potential as GPR40 agonists, a protein enzyme that when activated, can enhance insulin secretion *via* glucose stimulation (Yang *et al.*, 2016). Yang and co-researchers synthesised a series of analogues containing 3,5-dimethylisoxazole in 2016 and tested them for their *in vitro* and *in vivo* biological activity. It is found that 3-(4-((4-chloro-3-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluorophenyl)propanoic acid (**25**) in Figure 2.9 exhibits the most potent glycaemic activity *in vitro* (EC_{50} = 15.9 nM) and *in vivo* ($T_{1/2}$ = 2.87 hrs) when compared with TAK-875, the positive control (Yang *et al.*, 2016).



25

Figure 2.9 3-(4-((4-chloro-3-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluorophenyl)propanoic acid (**25**) potential GPR40 agonist.

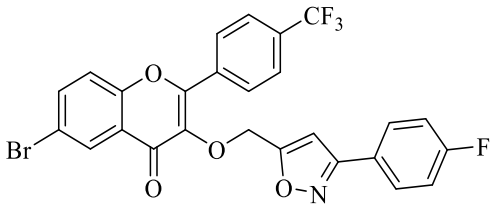
There has also been widespread research conducted on the potential of isoxazoles as potential *α*-amylase and *α*-glucosidase inhibitors. In 2020, Lin *et al.* have synthesised novel polyhydroxylated pyrrolidine-isoxazole hybrids *via* 1,3-dipolar cycloaddition and tested for their inhibitory activity against *α*-glucosidase and β-glucosidase enzymes. Figure 2.10 below shows (2*R*,3*S*,4*R*,5*R*)-5-(5-(2-bromophenyl)isoxazol-3-yl)pyrrolidine-2,3,4-triol (**26**) reported to have the most potent *α*-glucosidase inhibitory activity (IC_{50}= 0.2 μM), when compared to other synthesised analogues, such as the triazole derivatives. It is found that isoxazole hybrids synthesised have much higher inhibitory potency against *α*-glucosidase compared to the other derivatives.



26

Figure 2.10 (2*R*,3*S*,4*R*,5*R*)-5-(5-(2-bromophenyl)isoxazol-3-yl)pyrrolidine-2,3,4-triol (**26**) as potential *α*-glucosidase inhibitor.

In 2022, Saidi *et al.* synthesised new halogenated flavonoid-based isoxazoles and evaluated them for their inhibitory activity against *α*-amylase. 6-bromo-3-((3-(4-fluorophenyl)isoxazol-5-yl)methoxy)-2-(4-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (**27**) in Figure 2.11 shows the isoxazole derivative reported to exhibit the most potent inhibitory activity against *α*-amylase (IC_{50} = 16.20 μM), when compared to other synthesised analogues against the positive control, acarbose (IC_{50} = 15.74 μM).



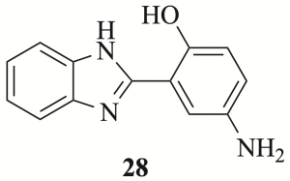
27

Figure 2.11 6-bromo-3-((3-(4-fluorophenyl)isoxazol-5-yl)methoxy)-2-(4-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (**27**) as potential α-amylase inhibitor.

2.4 Hybrid compounds and their biological importance

The hybridisation of two or more potent pharmacophores is a popular technique used in these past few years where molecular hybridised drugs are used to target multiple active sites, making them more efficient and potent than single-targeting treatments for a certain disease (Choudhary *et al.*, 2021). Two of the most common hybrids are hybrids with cleavable and non-cleavable linkers. Hybrids with cleavable linkers contain two pharmacophores that separate and independently act on their target active sites. Hybrids with non-cleavable linkers contain two pharmacophores that act on different targets with the linkage intact (Choudhary *et al.*, 2021).

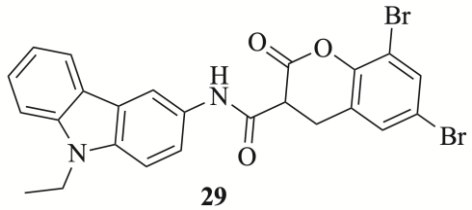
According to a report in 2014, Bansal and Silakari synthesised a series of benzimidazole-non-steroidal anti-inflammatory drugs (NSAIDs) hybrids that were evaluated for their anti-inflammatory, immunomodulatory, antioxidant and ulcerogenic effects. In the hybrids, the benzimidazole moiety provides immune-modulatory effects, while the different NSAIDs provide potential for anti-inflammatory and antioxidant effects for the hybrid drug. Based on the study reported, it is found that compound **28** in Figure 2.12 exhibited the most potential as a multi-functional drug for anti-inflammatory, antioxidant, and immune-modulatory effects with % paw oedema inhibition for anti-inflammatory effect = 1.5 ± 0.11%, carbon clearance index for immunomodulatory activity = 0.053 ± 0.002 K and antioxidant EC_{50} = 0.03 µM/ml.



28

Figure 2.12 Benzimidazole-mesalamine hybrid.

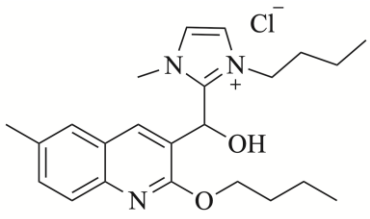
A new series of carbazole-coumarin hybrids were synthesised and evaluated for their anticancer potential, as seen in the study by Bondock and co-researchers. Among the newly-synthesised hybrids, compound **29** shown in Figure 2.13 was reported to be the most potent, which showed special effectiveness against the human HCT-116 and HepG-2 cell lines with IC_{50} =1.50 ± 0.07 μM and 0.90 ± 0.02 μM respectively (Bondock *et al.*, 2019). Thus, it can be said that the hybridisation of carbazole and coumarin can facilitate the increase in the anticancer and antitumor potential compared to the individual moiety.



29

Figure 2.13 Carbazole-coumarin hybrid.

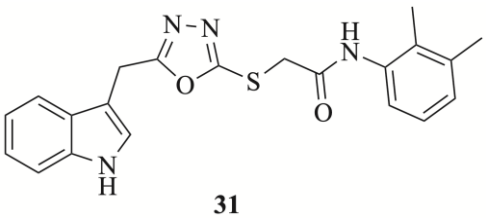
In 2019, Insuasty *et al.* synthesised eight quinoline-based hydroxyimidazolium hybrids and their antimicrobial and antifungal activity were evaluated. The quinoline moiety is present in several antibacterial treatments such as bedaquiline. Synthetic analogues of the imidazolium are also reported to exhibit potent antimicrobial and antitumoral activity. Among the hybrids synthesised, hybrid **30** shown in Figure 2.14 demonstrated high selectivity in inhibiting *S. aureus* and *M. tuberculosis* H37Rv with MIC values of 5 μM and 24 μM respectively, as well as moderate inhibitory activity against *E. Coli*, *K. pneumoniae* and *M. bovis* BCG (Insuasty *et al.*, 2019).



30

Figure 2.14 Quinoline-based hydroxyimidazolium hybrid.

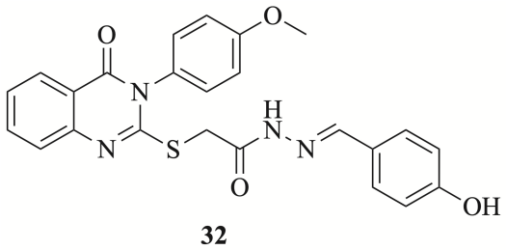
New indole-based hybrid oxadiazole scaffolds were synthesised and their antidiabetic activity was evaluated by Nazir *et al.* in 2018. The indole moiety is present as a core scaffold in many recent drugs for antineoplastic, antihypertensive, and antimitotic treatment. Indole-based oxadiazole hybrid **31** shown in Figure 2.15 exhibited the most potent inhibitory activity against α-glucosidase, with IC_{50} = 9.46 ± 0.03 μM when compared to acarbose (Nazir *et al.*, 2018).



31

Figure 2.15 Indole-based oxadiazole hybrid.

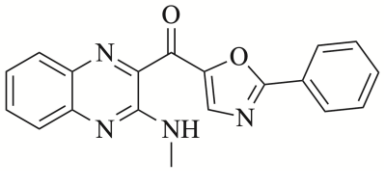
Khalifa *et al.* synthesised novel benzylidene-quinazolinone hybrids and the compounds were evaluated for their *in vitro* α-glucosidase inhibitory activity and further tested for their *in vivo* anti-hyperglycaemic activities against STZ-induced hyperglycaemic rats. When compared with the positive control (i.e acarbose), hybrid **32** (Figure 2.16) exhibited the most potent inhibitory activity against α-glucosidase, with IC_{50} of 561.0 μM (Khalifa *et al.*, 2022).



32

Figure 2.16 Benzylidene-quinazolinone hybrid.

A series of 1,3-oxazole-quinoxaline amine hybrids were synthesised and evaluated for their antibacterial activity, based on a study by Keivanloo *et al.* in 2022. The quinoxaline and oxazole moiety both play crucial roles as intermediates in organic synthesis and as basic scaffolds for the treatments of various types of diseases. Hybrid **33** (Figure 2.17) exhibited one of the most potent antibacterial activities against two bacterial strains, *Micrococcus luteus* and *Pseudomonas aeruginosa* with MIC values of 62.5 and 31.25 mg/mL respectively (Keivanloo *et al.*, 2022).



33

Figure 2.17 1,3-Oxazole quinoxaline amine hybrid.

In 2022, Lingala *et al.* synthesised a new series of isoxazole containing 1,3-oxazole-1,3,4-oxadiazole heterocyclic hybrids and the compounds were evaluated for their cytotoxic activity towards human prostate, lung and breast cancer cell lines. It is known that the 1,3,4-oxadiazole ring is associated with a wide range of biological activities including anti-inflammatory, antibacterial, antidiabetic, antifungal and anticancer properties (Lingala *et al.*, 2022). 1,2-isoxazoles also play a key role in medicinal chemistry as they exhibit various biological activities such as antimicrobial,

antifungal, anti-inflammatory and anticancer activities (Lingala *et al.*, 2022). From the compounds synthesised, hybrid **34** shown in Figure 2.18 exhibited the most potent anticancer activity with IC_{50} = 0.04 ± 0.01, 0.01 ± 0.01 and 0.46 ± 0.06 μM for human prostate, lung, and breast cancer lines respectively, when compared to etoposide (IC_{50} = 2.39 ± 1.56, 3.08 ± 0.13 and 2.11 ± 0.02 μM).

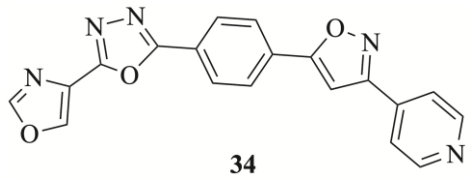


Figure 2.18 Isoxazole contained 1,3-oxazole-1,3,4-oxadiazole hybrid.

Dabhi and co-workers synthesised a series of novel spiro quinoxaline-pyrimidone based heterocyclic compounds and evaluated their anticancer potential. Both moieties, the quinoxaline and pyrimidone, possess good biological activity as antimalarial, antidiabetic, antioxidant, and anticancer agents. Spiro quinoxaline-pyrimidone based heterocyclic hybrid **35** (Figure 2.19) exhibited the most potent anticancer activity among the hybrids synthesised with LC_{50} value of 5.66 μg/mL (Dabhi *et al.*, 2022).

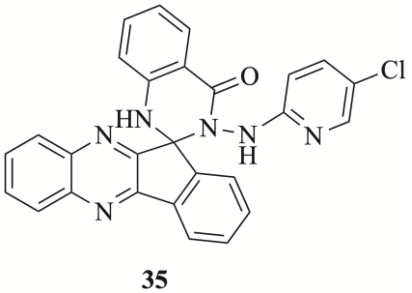


Figure 2.19 Spiro quinoxaline-pyrimidone based heterocyclic hybrid.