BUCHWALD COUPLING OF QUINOXALINE- O-SULFONATES LEADING TO THE HETEROCYCLIC COMPOUNDS WITH POTENTIAL MEDICINAL PROPERTIES AGAINST TB

by

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DISSERTATION

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Declaration

I Tselane Geneva Ramakadi, hereby declare and certify that the information in this booklet is true and original, the result of my own investigations under the supervision of Prof W Nxumalo. I submit this dissertation for the master's degree in the chemistry department at the University of Limpopo. This work has not previously been submitted; all sources that were used or quoted have been duly acknowledged.

Ramakadi TG (Ms) Date

…… **13/06/2018**

Dedication

I am dedicating this project to the following people:

- My son Mahube and my two nephews, Mmasehwana and Temosho for being my pillar of strength
- My mother Lina and my late father Moraswi for their never ending love, care and support.
- My sisters Makgomo, Mokgadi, Mabagwe and Nape for their unconditional love and encouragement.
- My brothers Selaelo, Rephang and Mmasehwana for being my Inspiration

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Completing this work would have been difficult without the support and friendship provided by the past and present members in our Organic Group especially Tshepiso Tema whom I worked closely in this project. I am also extremely thankful to Herman, Charlotte, Ndumiso, Welly, Reagan and Lerato for their valuable discussions and inputs. I felt humbled for spending my laboratory days around people who are ambitious, motivated and enthusiastic.

My deepest gratitude also goes to my family, for the endless love and support throughout my career. To my mother Lina and my late father Moraswi, thank you for all the sacrifices, I wouldn't be where I am without you. To my younger sister Abrina Kgoetja Ramakadi, thank you for being a constant and solid support system during the ups and downs of my life. Lastly and most importantly to my son Mahube, and my two nephews Mmasehwana and Temosho for making me smile, you will always be my best cheerleaders.

Table of Contents

List of Abbreviations

A

A549	Cells adenocarcinomic human alveolar basal epithelial cells
AIDS	Acquired immune deficiency syndrome

B

BrettPhos	dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxybiphenyl- 2-yl)phosphine

C

C-C	Carbon-carbon bond
δ	Chemical shift

D

^{o}C	Degree Celsius
DNA	Deoxyribonucleic acid
DCM	Dichloromethane
DCC	*N,N'*-Dicyclohexylcarbodiimide
DMAP	4-Dimethylamino pyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
DOTS	Directly observed treatment short-course
d	doublet
dd	doublet of doublets
H3-D	Drug Discovery and Development Centre -UCT

E

EtOH	Ethanol
EtOAc	Ethyl acetate
eq	Equivalents
XDR	Extensive drug resistance

G

g	Gram
GFP	Green fluorescent protein

H

HRMS	High-resolution mass spectrometry
HIV	Human Immunodeficiency Virus
J	
J	Coupling constants
I	
IR	Infra-red
IC	Inhibitory concentration
INH	Isonicotinic Hydrazide or isoniazid
*i*Pr	iso-*p*ropyl
L	
Lit	Literature
M	
m	multiplet
MHz	Mega Hertz
m/z	Mass-to-charge ratio
m.p	Melting point
MeOH	Methanol
µL	Microlitre
µM	Micromolar
min	Minutes
mg/ml	Milligram per millilitre
mmol	Millimole
MIC90	Minimum inhibitory concentration of compound needed to inhibit cells growth by 90%
MIC99	Minimum inhibitory concentration of compound needed to inhibit cells growth by 99%
MDR	Multi drug resistance
N	
NMR	Nuclear Magnetic Resonance
O	

OTf	triflate (trifluoromethanesulfonate)
OTs	tosylate (*p*-toluenesulfonate)
P	
ppm	Parts per million
%	Percentage
% Yield	Percentage Yield
R	
RNA	Ribonucleic acid
RIF	Rifampicin
Rf	Retention Factor
RuPhos	dicyclohexyl(2',6'-diisopropoxybiphenyl-2-yl)phosphine
S	
SI	Selective index
SA	South Africa
T	
THF	Tetrahydrofuran
TB	Tuberculosis
TLC	Thin layer chromatography
t	triplet
tBu-XPhos	di-tert-butyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine
TMS	trimethylsilyl
X	
XPhos	dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl) phosphine
W	
WHO	World Health Organization

Abstract

The dissertation describes the use of 2-benzenesulfonyloxyquinoxaline as a good coupling partner for different amine substrates. The palladium-mediated cross-coupling of aryl electrophiles and amines has become a widely used method of constructing arylamine frameworks. The formation of carbon-nitrogen bonds was accomplished via palladium-catalysed Buchwald-Hartwig amination employing different amine substrates to yield substituted quinoxaline-2-amines compounds in good to moderate yields. Buchwald ligands (Xphos, tButylxphos and BrettPhos), were varied with different amine substrates in an attempt of improving the yields. Compounds **81a** *N*-phenylquinoxalin-2-amine and **82b,** *N*-benzylquinoxalin-2-amine were obtained with the yield over 70 % employing Xphos as the ligand.

Significant attention has also been given to the application of cross coupling reaction protocols in substrates bearing electron withdrawing substituents. The presence of deactivating groups on the arylamine such as fluoro, nitro and iodo proved to be a challenge as only few compounds were synthesised in moderate yields. Compound **81b,** *N*-(4-fluorophenyl)quinoxalin-2-amine which has electronegative atom attached**,** showed significant improvement when employing tButyl-Xphos ligand rather than XPhos since the yield improved from 10 % to 71 %. Furthermore, nucleophilic substitution on Buchwald-Hartwig coupled compounds by treating them with alkyl iodides was successful when using methyl and ethyl electrophiles on the N-H group of **81a** 2-quinoxalineamine.

The synthesised quinoxaline derivatives comprised 7 novel compounds. The *in vitro* analysis on anti-tubercular screening against H37RvMA strains of *Mycobacterium tuberculosis* was conducted on 9 compounds. The results revealed none of the compounds to have promising inhibition percentages against *Mycobacterium tuberculosis* when compared with rifampicin which was used as a positive control. Screening against malaria with chloroquine as the control also did not yield any active compounds.

Chapter 1

CHAPTER 1

1. Introduction

Heterocyclic compounds are a class of chemical compounds that contains other atoms in addition to carbon and hydrogen[1]. They constitute the largest and most varied family of organic compounds by comprising at least half of all organic chemistry research worldwide[2]. They are also known as the largest classical synthetic compounds where the majority are present in most therapeutic agents currently marketed by the pharmaceutical industry[3].

For more than a century, heterocyclic compounds have dominated the largest areas of research in organic chemistry. They have contributed to the development of society from a biological and industrial point of view as true cornerstones of medicinal chemistry due to their biologically interesting properties[4]. They are widely found in nature particularly in nucleic acids, plant alkaloids, anthocyanin and flavones, as well as some vitamins and proteins[5]. These type of compounds are fundamental building blocks of most biologically active compounds which possess antitumor, antibiotic, anti-inflammatory, anti-depressant, anti-malarial, anti-HIV, anti-microbial, anti-bacterial, anti-fungal, anti-viral, anti-diabetic, herbicidal, and insecticidal properties[6,7]. In fact, heterocyclic moieties are present in the structures of all top 10 brand name small molecule drugs[8].

One striking structural feature inherent to heterocycles, which continues to be exploited to great advantage by the drug industry, lies in their ability to manifest substituents around a core scaffold in defined three dimensional representations[9]. The type and size of ring structures, together with the substituent groups on the core structure impact strongly on the physicochemical properties[10]. After all, every carbocyclic compound, regardless of structure and functionality, may in principle be converted into a collection of heterocyclic derivative by replacing one or more of the ring carbon atoms with a different atom[11]. The ring bonds of heterocycles are polarised, due to the electronegativity differences between the carbons and the heteroatoms which implies that they may participate in polar interactions, such as hydrogen bridge bonds. Heterocycles with three atoms in the ring are more reactive because of ring strain while those containing one heteroatom are in general, stable and those consisting of two heteroatoms are more likely to occur as reactive intermediates[11].

1.1 Nitrogen containing heterocyclic compounds

Nitrogen heterocycles exhibit diverse biological and pharmacological activities because of their similarity with many natural and synthetic molecules with known biological activities[4]. Compounds with *N*-aryl moieties often exhibit improved solubility and can facilitate salt formation properties both of which are known to be important for oral absorption and bioavailability[12]. Examples of some common *N*-heterocycles include some of the compounds shown in **Figure 1** below.

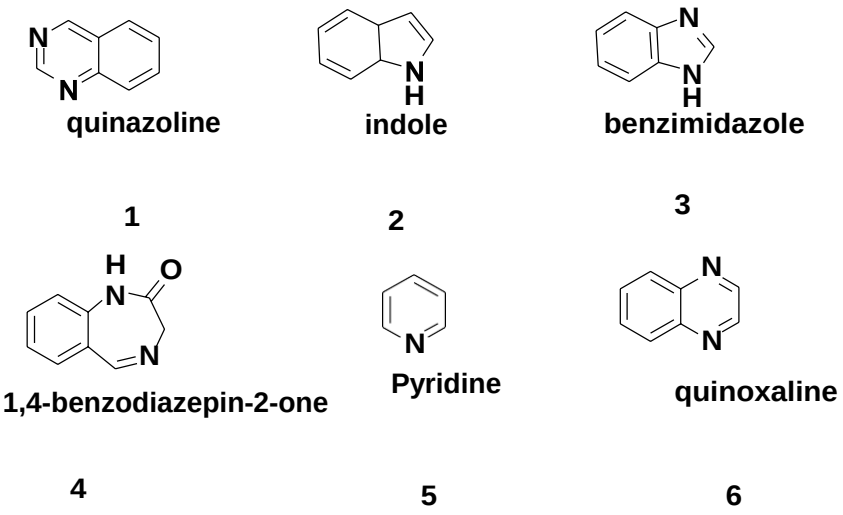


Figure 1: Examples of nitrogen heterocyclic compounds

1.1.1 Quinoxalines

Quinoxaline and its derivatives which form the basis of this study are important nitrogen containing heterocyclic compounds with multiple potential applications[13].The scaffold of this compound **6**, which is made up of a benzene ring and a pyrazine ring is numbered according to IUPAC rules of numbering as shown in **Figure 2**, where the carbons at position two and three are chemically equivalent. Quinoxalines are soluble in water, and produce monoquaternary salts when treated with quaternising agents, like dimethyl sulfate and methyl *p*-toluene sulphonate[13].

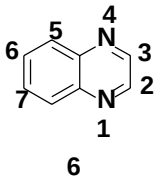
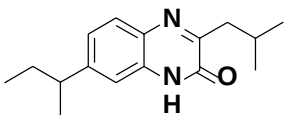
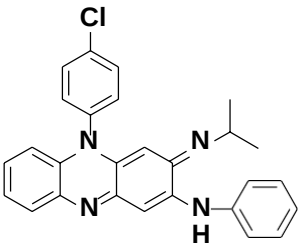


Figure 2: Quinoxaline ring and its IUPAC numbering.

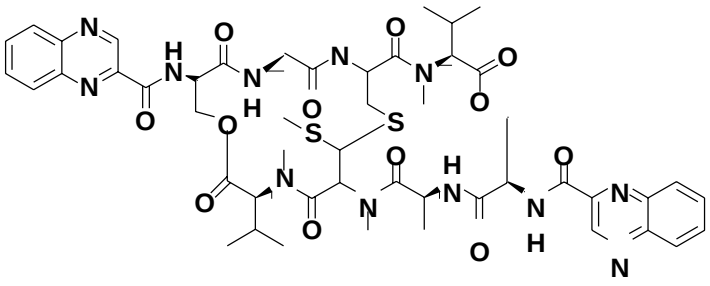
There are two classes of quinoxaline compounds **(Figure 3)** namely synthetic and natural quinoxaline compounds. Natural quinoxaline compounds include aspergillic acid **7** and clofazimine **8**. The broad spectrum of natural quinoxalines compounds also features echinomycin **9**, and quinoxalines are contained in several drugs and antibiotics, which have been reported to possess anticancer, antiviral and antibacterial activities. Like many other natural quinoxaline compounds, Echinomycin cannot be easily synthesised in the lab due to its high stereochemistry which cannot be easily obtained through synthesis but can only be obtained by isolation from bacteria such as *Streptomyces lasalienis*[14]*.*



Aspergillic acid
7



Clofazimine
8



Echinomycin
9

Figure 3: Naturally occurring quinoxaline compounds

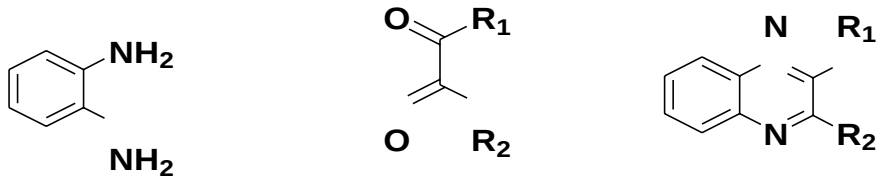
Quinoxaline derivatives have emerged as lead compounds in drug discovery research because of their diverse pharmacological and biological properties like antibacterial, antifungal, anti-tubercular, anti-inflammatory, anti-hyperglycemic, and anti-tumor activities[6,7,8]. They are an important class of nitrogen containing heterocyclic compounds which act as important intermediates in organic synthesis where a number of synthetic strategies have been developed for its derivatives[15].

1.1.2 Synthesis of quinoxalines derivatives

Substituted quinoxalines are an important class of benzo-heterocycles that have received considerable attention during the last two decades as they provide a variety of biological activities including wide range of therapeutic properties[14]. Several methods of preparation of quinoxalines have been published in the literature. These include condensation of 1,2-diamines with α-diketones[16],1,4-addition of 1,2-diamines to diazenylbutenes[17], cyclisation–oxidation of phenacyl bromides and oxidative coupling of epoxides with ene-1,2-diamines[18]. Recently, researchers have published reports concerning the synthesis of different quinoxaline derivatives involving several green methodologies, including recyclable catalysts, microwave-assisted synthesis and reactions in aqueous medium[19].

1.1.2.1 Preparation of quinoxalines from *o*-diamines

Among reported methods, the classical synthesis[16,17,18] of quinoxalines involves the condensation of an aromatic *o*-diamine and α-dicarbonyl compounds **(Scheme 1)**. The reaction is very facile and is most widely used for the synthesis of quinoxaline itself and its alkyl substituted derivatives. The condensation of glyoxal with *o*-phenylenediamine **10** yields quinoxaline **11** in almost quantitative yield[16].



10 **11**

R = H, Alkyl, Aryl

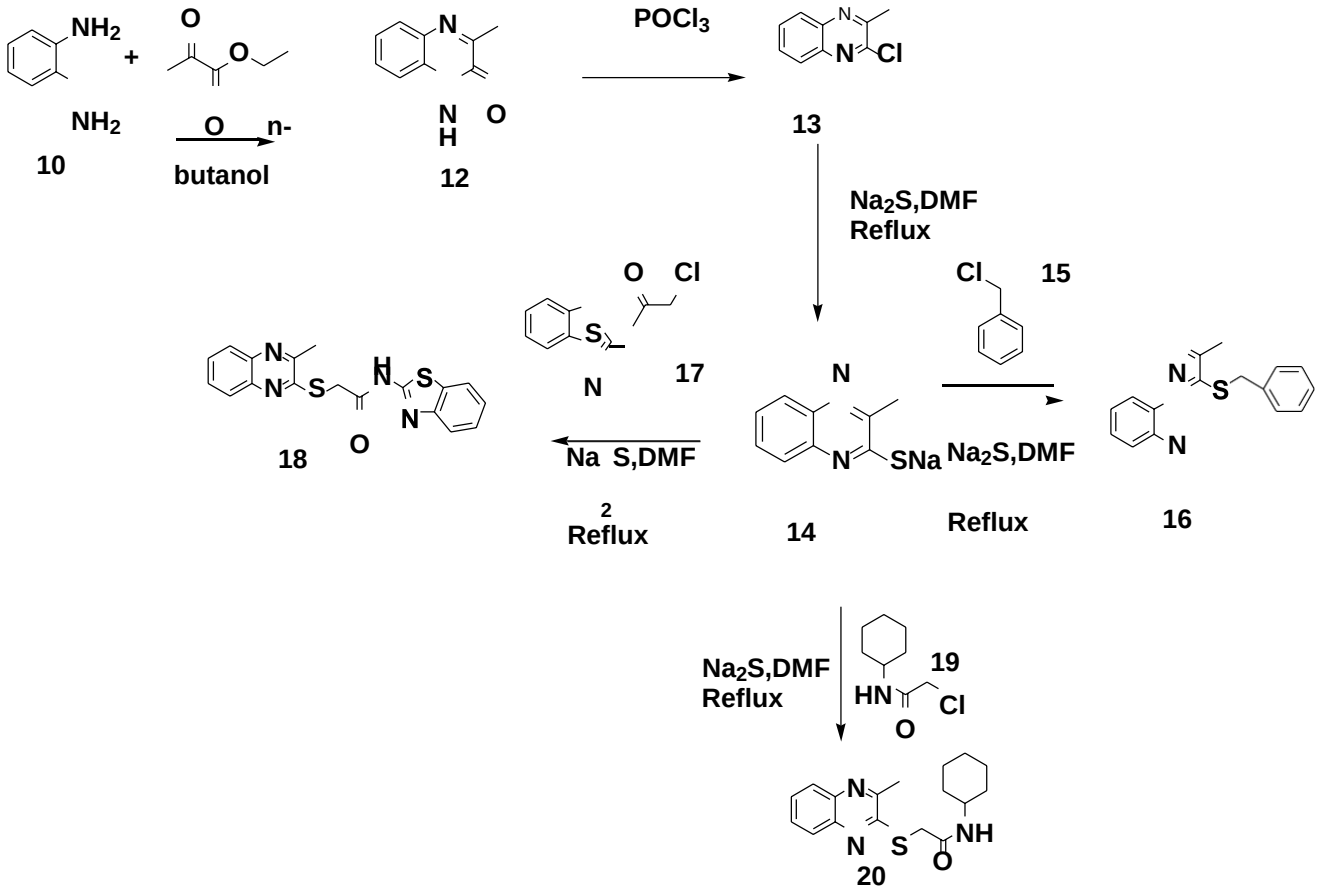
Scheme 1: Classical synthesis of Quinoxalines

Numerous data of biological applications of quinoxaline-based scaffolds offering excellent pathways to new biomolecular targets, qualifying them to be excellent precursors in drug design and future candidates in therapeutic research has been unveiled. A considerable amount of research activity is directed towards potent, more specific and less toxic antibiotics[20]. The approach which is more convenient in the synthesis of quinoxaline derivatives is the substitution of hydrogen at the 2-or 3-position of quinoxaline by other nucleophillic compounds[20,21].

The synthesis of thioester derivatives **(Scheme 2)** was initiated with the reaction of *o*-

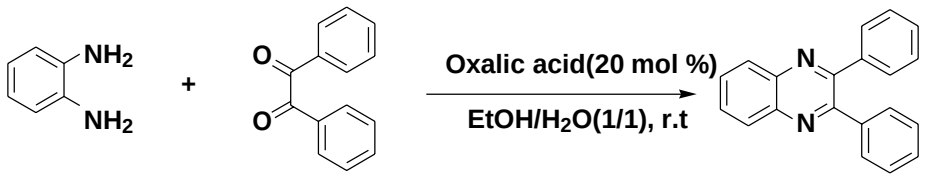
phenylenediamine **10** with ethyl pyruvate in n-butanol to yield 2-hydroxy-3-methyl

quinoxaline **12** which on treatment with $POCl_3$, yielded 2-chloro-3-methylquinoxaline **13.** A mixture of the compound **13** and sodium sulphide in DMF was refluxed to yield 3-methylquinoxalin-2-thiosodium **14** which on treatment with different benzyl chloride **15**, and chloroacetamides **17 & 19** afforded the one pot synthesis of different quinoxaline derivatives **16**,**18**, and **20** in good yields[22].



Scheme 2: Synthesis of 2-thio-3-methylquinoxaline derivatives.

In another example, Hasaninejad *et. al*[23] developed a new route for the synthesis of quinoxaline derivatives via the condensation of 1,2-diamines with α-diketones which is efficient for organic transformations using economic and eco-friendly materials as catalysts and reagents. The condensation of *o*-phenylenediamine with bibenzoyl **21** was chosen as a model to provide compound **22 (Scheme 3)**, and its behaviour was studied in the presence of various catalysts in $EtOH/H_2O$ at room temperature. This new strategy had several advantages, such as excellent yield, short reaction time, low cost, simple experimental as well as isolation procedures, and finally, it is in agreement with the green chemistry protocols[24].



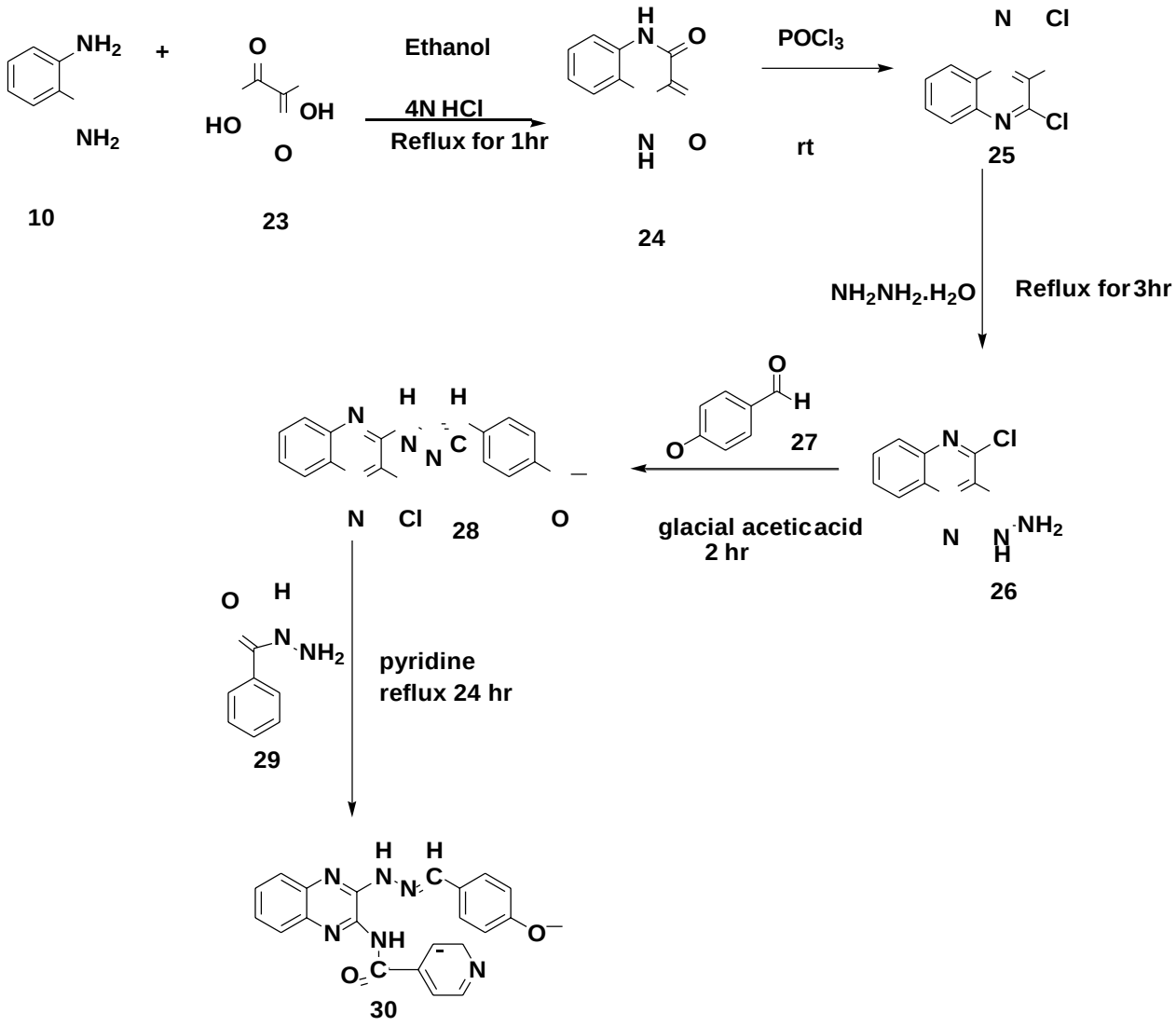
10 21 22

Scheme 3: Condensation of benzene-1,2 diamine with bibenzoyl

1.1.2.2 Synthesis of quinoxaline derivatives with aryl amines

The importance of amination reaction originates from the prevalence of aromatic amines in biologically active molecule where significant classes include kinase inhibitors, antibiotics and CNS active agents[25]. Compounds with a quinoxaline scaffold have a broad spectrum of biological activity as it was previously mentioned that they possess extensive applications in medicinal chemistry[26]. A continuous study into chemistry of quinoxaline is inevitable for its pharmacological influence.

Substituted novel quinoxaline derivatives were synthesised by incorporating the isoniazide moiety into the quinoxaline scaffold to evaluate them for biological activities such as anti-bacterial potency and anti-inflammatory activity[27]. Isoniazid, is a first line drug used for the treatment of tuberculosis along with rifampicin and ethambutol[28]
The synthetic route (**Scheme 4)** started with the preparation of the starting material, quinoxaline-1,2-diol **24** prepared from *o*-phenylenediamine **10** and diethyloxalate **23** under reflux for 1 hour in single step. The quinoxaline-1,2-diol was refluxed with phosphorous oxychloride, ($POCl_3$), to afford 2,3-dichloroquinoxaline **25**. The progress of the reaction was followed by preparation of 3-chloro-2-hydrazinoquinoxaline **26** by reacting of 2,3-dichloroquinoxaline and hydrazine hydrate. Different Schiff's bases of 3-chloro-2 -hydrazinoquinoxaline were also obtained by refluxing the appropriate substituted benzaldehyde **27** and 3-chloro-2-hydrazinoquinoxaline in acetic acid medium. The obtained quinoxaline Schiff base **28** was further converted into the quinoxaline derivatives **30** by reacting the Schiff's base with Isoniazide **29** in pyridine [28].

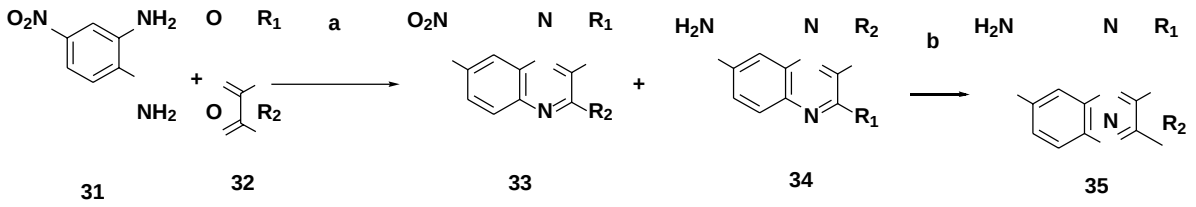


Scheme 4: Synthesis of quinoxaline derivatives containing Isoniazid[27]

The results established the fact that quinoxaline can be a rich source for exploitation in the drug discovery process. Some of the synthesised compounds were found to possess good antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. Quinoxaline and isoniazid though responsible for antibacterial activity, when fused with other moieties showed a broad spectrum of antibacterial activity. Therefore, in search of new generations of antibiotics it may be worthwhile to explore the possibility in this area by fusing different moieties and increase the potency[28].

A second generation of quinoxaline-derived molecules based on structure-activity relationship was designed and synthesised (**Scheme 5**) to discover new active neuroprotective compounds for dopamine neurons[29]. Parkinson's disease is a neurodegenerative disorder of aging characterised by motor symptoms that result from the loss of midbrain dopamine neurons and the disruption of dopamine-mediated neurotransmission[30]*. N*-alkyl-6-aminoquinoxaline **35** derivatives were synthesised in

order to obtain better therapeutic agents where compounds with no aliphatic side chain showed potential of interesting results[29].



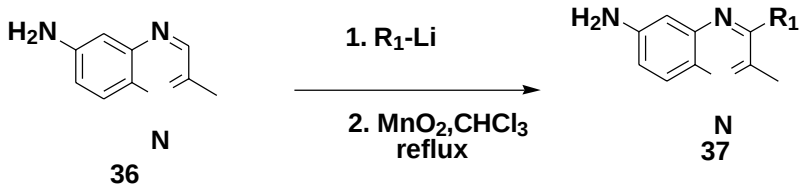
31 32 33 34 35

Reagents and conditions : (a) H_2o, Reflux, (b) H_2, Pd/C in EtOH, 60 °C, then purificaion by flash chromatography

Compound	R_1	R_2	Yield
35a	H	H	90 %
35b	H	CH_3	90%
35c	CH_3	CH_3	74 %
35d	H	Ph	98 %

Scheme 5: Synthesis of 6-Amino and 2-Substituted-6-aminoquinoxalines by Hinsberg Condensation[29].

Furthermore, various 2,3-disubstituted quinoxalines were obtained from 2-substituted 6-aminoquinoxalines obtained through Hinsberg condensation[29] by addition of an organolithium reagent at -78 °C followed by oxidation with manganese oxide **(Scheme 6)**.



36 37

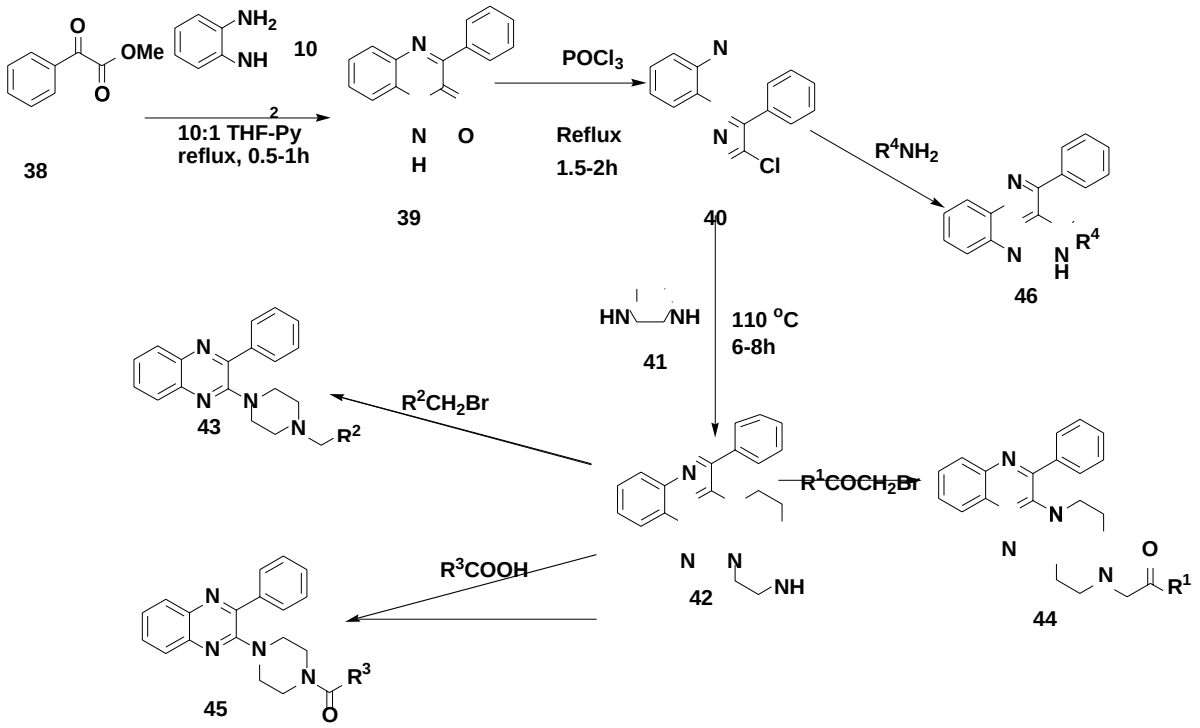
Compound	R_1	Yield
37a	Ph	75 %
37b	-Bu	75%
37c	-sBu	65 %
37d	-tBu	25%

Scheme 6: Synthesis of 2,3-disubstituted quinoxalines using organolitiated compounds.

A series of novel quinoxaline derivatives with biological properties containing amino

substitution at C-2 were also synthesised by *Rao et.al*[31]. The key intermediate required

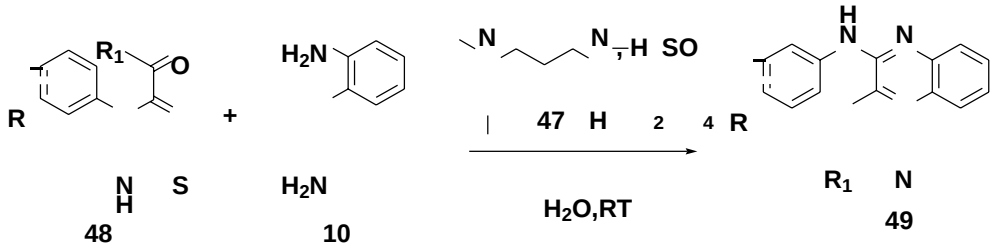
for the synthesis of these derivatives has been established as per conditions in **(Scheme 7)**.



Scheme 7: Synthesis of biologically active novel quinoxaline derivatives

The synthetic methodology resulted in four different substituted 2-amino quinoxalines in excellent yields **(Scheme 7)**. Quinoxaline derivatives substituted with a piperizine ring at C-2 have shown tremendous anti-deppression activity for phosphodiesterase type 4 inhibitor (PDE4B) enzymes with reference to rolipram, a drug used to treat depression[31].

Furthermore, 2,3-disubstituted quinoxaline derivatives were synthesised succesfully with recyclable task-specific ionic liquid *N,N,N*-trimethyl–*N*-propane–sulfonic acid ammonium sulfates [TMPSA] **47** employing H_2SO_4 as a catalyst. This was achieved by treatment of *N*-substituted aniline **48** with *o*-phenylenediamine **10** in water in the presence of TMPSA, affording the 2,3-disubstituted quinoxaline derivatives **49**. The reaction can be performed in water as well as organic solvent, and the satisfactory results were obtained under the mild conditions as shown in **Scheme 8**[32].



R = H, Alkyl, Phenyl

Scheme 8: Synthesis of 2,3-quinoxalines by oxidation[32].

Many improved synthetic routes were also reported in addition to the latter but most of them suffer from one or more limitation such as harsh conditions, low yields, long reaction times, tedious product isolation procedures and co-occurrence of several side products.

Catalysis has attracted attention in the last few years as a result of efficiency and selectivity of many catalytic reactions. Applications of palladium catalysis in heterocyclic chemistry have grown lately particularly Buchwald–Hartwig amination reactions for the formation of C-N bonds[33]. Transition metal-catalysed reactions play a vital role in the production of many industrially important chemicals where homogeneous catalysis is rapidly growing. The emergence of cross-coupling as a popular method in synthesis arises from both the diversity of organometallic reagents utilized in these reactions and the broad range of functional groups which can be incorporated into these reagents[34].

1.2 Buchwald-Hartwig carbon-nitrogen bond formation

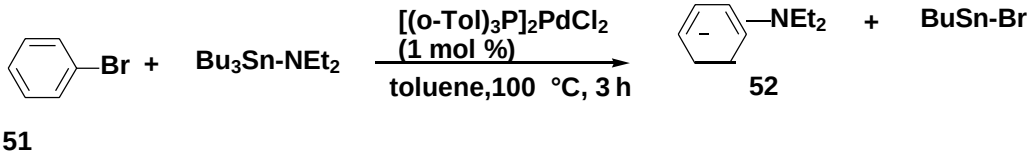
There can be no question that the coupling of aryl electrophiles and nucleophiles to form new carbon-carbon bonds is of great utility. However, although carbon-carbon bond formation processes dominated the beginnings of cross-coupling chemistry, in recent years the scope of metal-mediated cross-coupling has expanded immensely, with carbon-nitrogen cross-coupling emerging to the forefront as a versatile and useful method of preparing aryl amines[35,36]. Nitrogen heterocycles as previously mentioned are structural constituents of a wide variety of biologically active natural products, medicinally important compounds and organic materials, and their construction by cross-coupling processes is wide spread[4,6,7,15]. Aryl amines are also important because they are common precursors to or substructures within active pharmaceutical

ingredients and herbicides produced on ton scales, as well as conducting polymers and layers of organic light-emitting diodes produced on a small scale[37] .

Due to the diversity of nitrogen nucleophiles, Pd-catalyzed C–N bond formation is more versatile than analogous C–C bond forming processes[38]. Effective C–N coupling conditions have been discovered for a large number of different classes of nitrogen-containing substrates, including 1° aliphatic amines, cyclic and acyclic 2° aliphatic amines, imines, 1° and 2° anilines, amides, carbamates, sulfonamides, sulfoximes, ureas and hydrazones[38]. Over a decade, many conditions have been developed, allowing the coupling of a broad range of amines and amides with haloaromatic compounds and other suitable partners such as triflates, tosylates and sulphonates[39]. In addition to extending the scope of nitrogen nucleophiles, research efforts have focused on improving the practical aspects of the reaction, such as reducing catalyst loadings, development of room-temperature coupling reactions, and use of aryl chlorides as coupling partners. The discovery of improved procedures has gone hand-in-hand with the development of novel ligands[40].

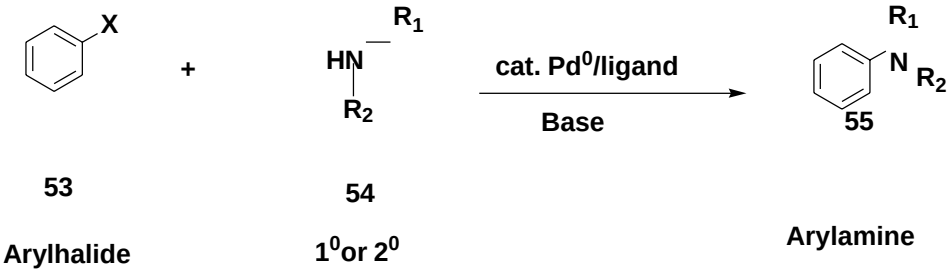
Buchwald-Hartwig coupling has become particularly important for developing compounds containing carbon–nitrogen bonds for applications in industry as well as α-arylation of carbonyl compounds such as ketones, esters, amides, aldehydes and nitriles[41]. Such nitrogen-containing fragments are present in biologically-relevant molecules, pharmaceuticals, herbicides, as well as their organic precursors, making their efficient preparation of great interest[4-7]. Indeed, synthetic methods forming the carbon-nitrogen bonds in aromatic amines have to be considered fundamental in introductory sections of undergraduate Organic chemistry courses.

Historically, aryl amines were prepared from classical methods, such as nitration, reduction and reductive alkylation, at high temperatures, addition to benzene intermediates, or direct nucleophilic substitution on particularly electron-poor aromatic or heteroaromatic halides[42]. The first palladium-catalysed carbon-nitrogen bond forming process **(Scheme 9)** was reported by Migita and Kosugi in 1983[43]. The treatment of bromobenzene with the amino-tin compound in the presence of a palladium catalyst provided *N,N*-diethyl aniline. However, this method requires the use of a stoichiometric quantity of a toxic and sensitive tin reagent.



Scheme 9: Palladium-catalysed aromatic amination reaction by Migita and Kosugi[43].

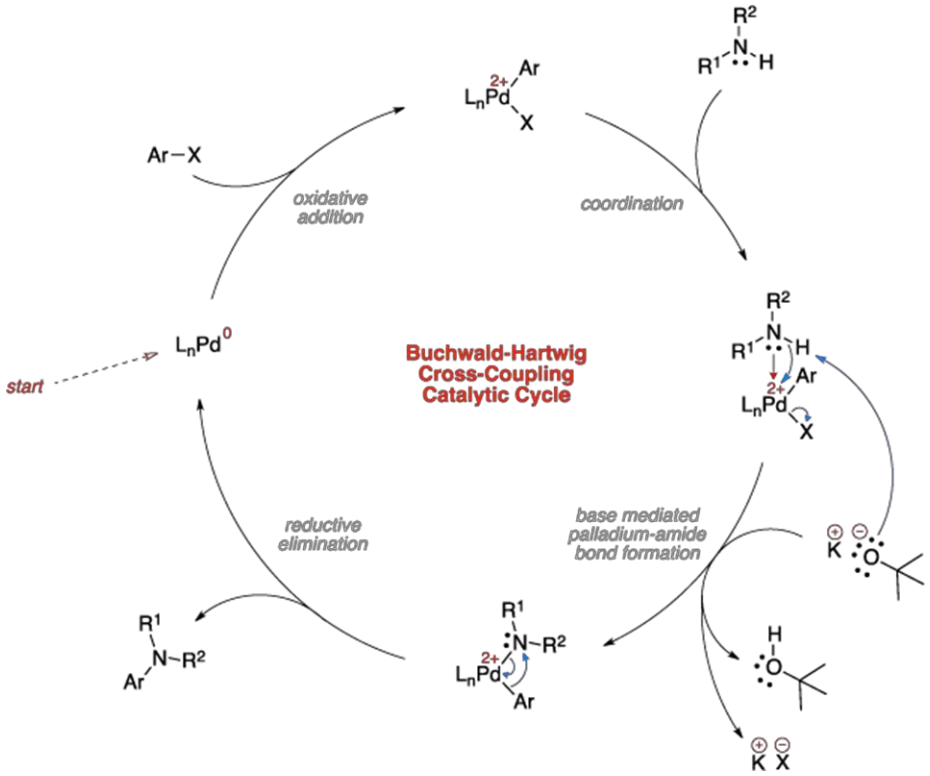
Therefore, it was of great interest when the Buchwald-Hartwig amination was reported in 1995 **(Scheme 10)**. This coupling reaction has matured from a synthetic laboratory procedure to a technique that is widely used in natural product synthesis as well as in other fields of academic interest. The reaction involves the coupling of the aryl halides **53** with amines **54** by employing suitable palladium catalyst to form aryl amines **55** which acts as important intermediates in organic synthesis of biologically active compounds[44].



X = Cl, Br, I, OTf, OSulphonates
R_1 = Phenyl, Alkyl
R_2 = Phenyl, Alkyl

Scheme 10: Synthetic route of Buchwald coupling

The reaction requires a catalytic system **(Scheme 11)** containing four components to efficiently generate the desired C-N bond. Palladium precursor is typically stabilised in solution by an adequate ligand that also raises the electron density at the metal to facilitate oxidative addition and provides sufficient bulkiness to accelerate reductive elimination. A base is required to deprotonate the amine substrate and reductive elimination of the resulting amido species yields the arylamine product while regenerating the active catalyst [45].



Scheme 11: Catalytic cycle for Pd-catalysed amination reaction

The steric and electronic characteristics of the substrates also influence reaction rates while sterically unhindered complexes promote oxidative addition more favourably. The nature of the halide itself is also of great importance both in terms of halide-carbon bond strength, and due to the fact that the mechanism of oxidative addition can differ based on the type of halide in question. The rate of reductive elimination of the aryl amine product is primarily a function of the metal/ligand characteristics. Furthermore, in complexes containing bidentate phosphine ligands, it has been proved that reductive elimination to form a new carbon-nitrogen bond proceeds more quickly when a more electron-rich amido reacting ligand is involved[46].

1.2.1 Buchwald ligands

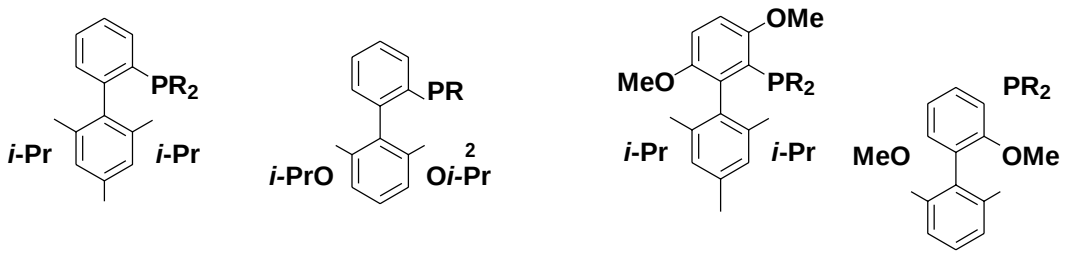
The development of phosphine ligands for transition metal-catalysed reactions has had a huge impact on catalytic processes in chemistry. Bulky and electron-rich phosphine ligands[47], which can dramatically improve the efficiency and selectivity of such cross-coupling reactions, have been introduced in C-N bond-forming reactions. Phosphine ligands which have the general formula PR_3 where R = alkyl, aryl, H, halide

etc. are extremely reactive in transition metal chemistry. The use of these ligands enhanced the rate of both the oxidative addition and reductive elimination processes in the Buchwald amination reactions[48].

Ligands play a key role in stabilizing and activating the central metal atom and fine-tuning the selectivity of the transformation. The steric and electronic properties of ligands led to the creation of organometallic complexes as highly effective catalysts. The reactions utilising these ligand systems have progressed rapidly and now applied in numerous synthetic reactions[47,48].

1.2.2 Notable Buchwald phosphine ligands

Buchwald ligands are highly active reagents which are extensively applied in the synthesis of pharmaceuticals, natural products, polymers, and new materials[49]. The use of dialkylbiaryl phosphine ligands often allows reactions to proceed with short reaction times, low catalyst loadings and under mild reaction conditions. Several studies have been directed towards finding the origin of these effects to further optimize catalyst design. Many of these ligands have been commercialised and various important ones have been reported in leading publications. The structural differences between Ruphos, XPhos, SPhos and BrettPhos **(Figure 4)** are important to acknowledge and illustrate the fact that minor changes in ligand structure can ultimately have a large impact on reactivity[49,50].



i-Pr

XPhos

L1

R = Cy

RuPhos

L2

i-Pr

BrettPhos

L3

SPhos

L4

Figure 4: Some notable types of Buchwald ligands

XPhos **L1** discovery came with a major breakthrough by supplying improved reactivity

in a diversity of amination reactions. This ligand facilitates the amination of aryl chlorides under mild conditions and proved to be efficient for chemoselective

amination of amines with multiple reaction sites such as amides, aliphatic amines or indoles[45]. RuPhos L**2** has found use as an excellent ligand for the arylation of secondary amines using low catalyst loadings, which can be challenging for other ligands, even structurally similar biaryl monodentate phosphines, due to their steric bulk[46]. More recently, a new biaryldialkylphosphine ligand BrettPhos **L3** was disclosed as the most active ligand that shows excellent reactivity and stability in C-N cross coupling which can overcome many restrictions that some catalysts systems have possessed[47]. This improved ligand enables highly selective monoarylation of primary amines using low catalyst loadings of a monophosphine-based catalyst [48].

Furthermore, BrettPhos demonstrated its ability to promote the amination of aryl mesylates, an area where XPhos, despite successfully promoting couplings of aryl sulfonates, was found lacking[49]. The corresponding characteristics of BrettPhos and RuPhos as ligands for the arylation of primary and secondary amines respectively, led to the

discovery of a multi-ligand catalyst system based upon both ligands which not only comprises of the substrate scope of both, but also offers additional reactivity that neither can manifest on their own, demonstrating that even these well-established ligands can offer novel reactivity in some instances[50].

1.2.3 Other key variables reaction parameters

The reaction parameters in Buchwald coupling reaction can vary for different substrate combinations due to wide variation in the electronic and steric properties of the nitrogen-based nucleophiles when compared to other cross-coupling reactions. It is also important to mention that the selection is typically determined by both the structure of the amine and the electrophile[51].

1.2.3.1 Bases

The most challenging key factor in C-N coupling reactions is choosing a suitable base which influences rate and functional group tolerance. $NaOBu^t$ was the first base utilised and it has been extensively used with dialkylbiaryl phosphine ligand systems by Buchwald and co-workers, but because it is a relatively strong base it can participate in undesirable side reactions with various electrophilic functional groups[50]. KO^tBu exhibits the same efficiency in some of these reactions, but both of these bases

have some limitations because the functional group tolerance for substrates is limited[51].

Weak inorganic bases such as Cs_2CO_3, K_3PO_4 or K_2CO_3 can play significant role in the functional group tolerance of Pd-catalysed amination reactions. These bases provide favourable conditions for substrates containing electrophilic functional groups such as ketones, esters and nitro aromatics and facilitate the exploitation of aryl sulfonates as electrophiles in cross-coupling reaction[52].

1.2.3.2 Electrophile

Aryl chlorides have attracted increased attention from aryl bromides recently because of their lower cost and environmental friendliness[53]. The new ligands mentioned above, overcame the oxidative addition challenge of the C-Cl bond strength which is higher than C-Br[53]. Aryl tosylates and benzenesulfonates are more demanding substrates due to their low propensity to undergo oxidative addition. They are however attractive from economic point of view due to their lower cost than aryl triflates. The presence of electron-donating or withdrawing substituents on the aromatic ring also affects the rate of all steps in the catalytic cycle. If these heteroatoms are capable of coordination for example pyridines, displacement of the phosphine ligand can occur resulting in catalyst deactivation[54].

1.2.3.3 Nucleophile

Aromatic primary amines, such as aniline derivatives, are some of the easiest substrates because they lack β-hydrogen atoms, although double arylation can be a competing pathway[55]. It is worth noting that, by employing the right ligand, it is possible to achieve the desired selectivity. Buchwald[56] reported that in the presence of primary amines, using XPhos allows the selective arylation of anilines. Arylation of aromatic secondary amines such as piperidine, pyrrolidine, piperazine, *N*-methylpiperazine and morpholine are among the first reported palladium-catalysed amination reactions and most ligands are efficient. This can be due to the fact that cyclic palladium(II) amide intermediates are less prone to β-hydride elimination compared to acyclic amide intermediates[56].

1.2.3.4 Leaving groups

The reactivity of electrophilic substrate in Pd-catalysed reactions depends highly on its leaving group[57]. Good leaving group species are determined by their electronegativity, size, and resonance which tend to affect the basicity.

1.2.3.4.1 Halides

Atoms or groups that are good leaving groups are usually conjugate bases of strong acids which have a lower pKa value (**Table 1.1**). The table below shows that HI has the most stable conjugate base with pKa of -11 making it the excellent leaving group. Water on the contrary has a pKa Value of 15.7 therefore its conjugate base ($OH^{-)}$ will be a bad leaving group[58].

Table 1.1 Good Leaving Groups

No	Acid	pKa	Conjugate Base
1	HI	-11	I^-
2	HBr	-9	Br^-
3	HCl	-7	Cl^-
4	H_2O	7	OH^-

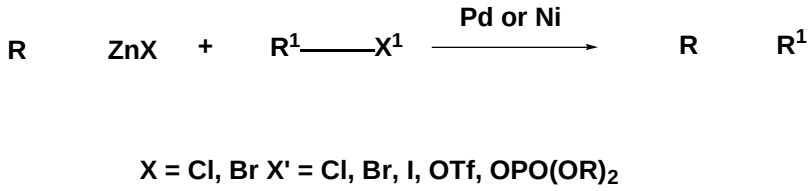
Initial studies on Pd-catalysed amination reactions were carried out predominantly with aryl bromides as electrophiles. Buchwald coupling reactions of aryl bromides and aryl chlorides with amines has been reported in the past years, even though iodine is a better leaving group than the other halogens. The problem with the latter is that aryl iodides are often more expensive and less readily available than aryl bromides[59]. Aryl chlorides on the contrary, are less reactive than aryl bromides in Pd-catalyzed aminations even though they are cheaper as compared to other leaving groups. Furthermore, hetero aryl halides have proved to be challenging in reactions where substrates contain heteroatoms capable of coordination like pyridines. In this case it leads to displacement of the phosphine ligand resulting in catalyst deactivation[60].

1.2.3.4.2 Sulfonates

Besylates (benzenesulfonate esters) fall under sulfonate ester leaving groups, which are said to be good leaving groups because of their stability and poor reactivity. Benzenesulfonate ester is derived from benzoic acid and its anion comes in a form of cheap purchasable benzenesulfonyl chloride. Besylates are prepared by incorporating alcohol with benzenesulfonyl chloride. Aryl benzene sulfonates are attractive from an economic point of view due to their affordability[61]. Benzenesulfonyloxy leaving group has been recently reported to be an efficient leaving group employing Negishi and Sonogashira coupling reactions on quinoxalines and pteridines[62].

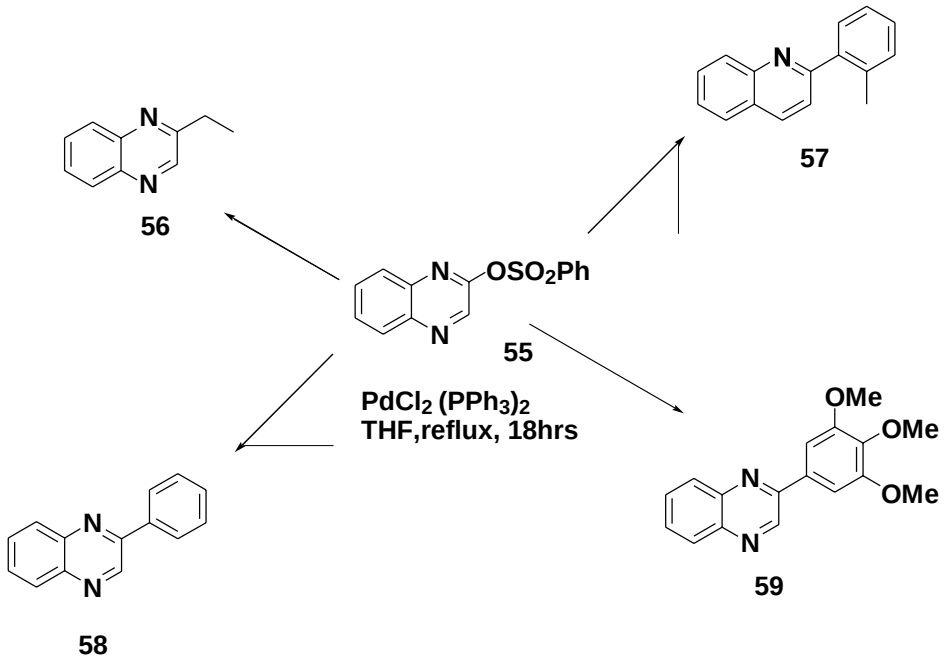
1.3 Negishi coupling

Negishi cross-coupling reaction was first pioneered by Negishi et al., in 1977 and allows coupling between a wide range groups, including aryl, heteroaryl, alkenyl and alkyl substituents. The chemistry has received much attention over the past three decades improving on many variations to suit synthesis of different groups of compounds. The variations include the replacement of palladium with a nickel catalyst, the replacement of the halogen leaving group with oxygen containing groups such as triflates and phosphate-ester groups, and also replacing the triphenylphosphine ligands with bulkier electron-rich ligands[63].



Scheme 12: Negishi Cross Coupling Reaction

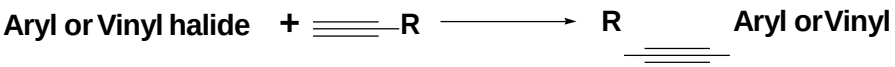
Nxumalo et. al investigated the possibility of quinoxaline and pteridine-*o*-sulfonate substrates to be cross coupled using Negishi reaction conditions. Subjecting phenyl-ZnCl and benzenesulfonyl quinoxaline with different substrates gave good to excellent yields. The results obtained demonstrated the ability of attaching different substituents i.e. Aryl, heterocycles, and alkyl, at the 2-position for quinoxaline, and 6-position for pteridines[62]



Scheme 13: Negishi coupling reaction of quinoxaline *o*- sulfonates

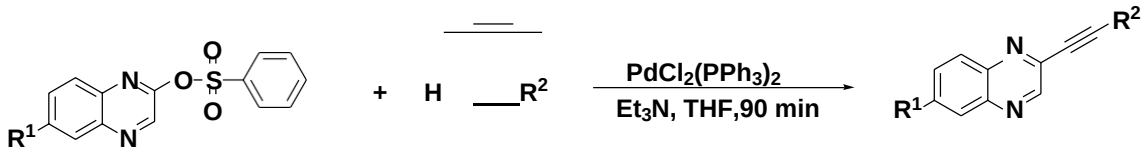
1.4 Sonogashira coupling with quinoxaline *o*-sulfonate

The Sonogashira reaction is used in the synthesis of various organic compounds and in the production of pharmaceuticals, agricultural chemicals, and natural products[64]. It has recently become the third most popular organic reaction for C-C bond formation between a terminal alkyne and an aryl or vinyl halide employing palladium catalyst[65].



Scheme 14: Sonogashira Coupling reaction

Encouraged by the success on the previous results, Nxumalo et.al investigated the possibility of benzene sulfonate group on Sonogashira coupling of quinaxolines. A series of alkyne-quinoxaline derivatives were successfully synthesised by Sonogashira coupling reaction using various alkyne substrates and were obtained in moderate to good yields[66].



55 60 61

R^1 = H, NO_2
R^2 = Aryl, alkyl

Scheme 15: Sonogashira Coupling with quinoxaline *o*-sulfonates

The results showed that benzene sulphonate is a good coupling partner for the formation of C-N bonds. Buchwald-Hartwig hetero-cross-coupling reactions have been successful with the use of suitable ligand based palladium complexes, preferably with bis-phosphine ligands. The investigation on the palladium-catalysed cross-coupling reactions for the functionalisation on the second position will help in compiling a library of new arylamines bearing a quinoxaline scaffold.

1.5. Purpose of the study

Significant research effort was directed towards evaluating how modifying various reaction parameters, including the choice of solvent, base, palladium precursor, and most notably dialkylbiaryl phosphine ligand, influences the outcome of the cross-coupling reaction. Consequently, several highly effective classes of catalysts for Buchwald-Hartwig amination that offer broad substrate scope and excellent functional group tolerance at relatively low catalyst loadings have been reported, including the cross-coupling of less expensive and more abundant (but less reactive), (hetero)aryl chloride substrates[67]. Despite such progress, a number of significant challenges remain in Buchwald-Hartwig amination chemistry, including the establishment of catalysts with predictably high yield and chemoselectivity.

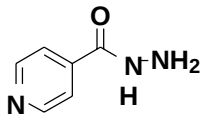
In the light of these facts we decided to synthesise some new quinoxaline derivatives employing alternative reaction conditions for the synthesis of active biological compounds through Buchwald-Hartwig reaction. QuinoxalineS bearing a benzenesulfonyl substituent which has been reported to be a good leaving group in of coupling with primary anilines were used to acquire more libraries of novel compounds which can be used as biological agents for TB.

1.6 TB

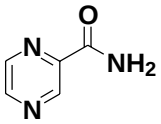
Tuberculosis remains a major global health problem which causes ill-health among millions of people each year and is also ranked as the second leading cause of death from an infectious disease worldwide, after human immune-deficiency virus[68]. Quinoxaline analogues exhibit anti-tuberculosis activity since they possess similarity with some anti-tuberculosis drugs. Quinoxalines are also bioisosters of quinolones which are also used as 2nd line drugs in the treatment of TB. These compounds have received a great deal of attention in research because of their diverse biological potential and easy synthetic routes[4-7].

1.6.1 First-line TB drugs

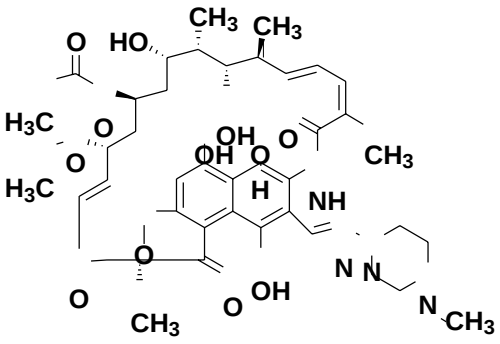
Of the approved drugs, the first-line anti-TB agents that form the core of treatment regimens include: isoniazid (INH), rifampin (RIF), ethambutol (EMB), streptomycin (SM) and pyrazinamide (PZA) which are shown in Figure 6. The standard treatment regimen involves taking isoniazid, rifampicin, pyrazinamide and ethambutol for two months in a combination called Rifafour, and then Isoniazid and rifampicin for the next four months[69].



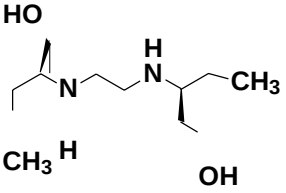
Isoniazid



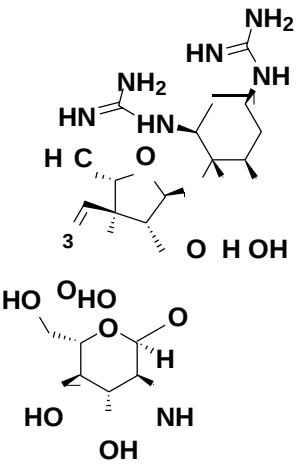
Pyrazinamide



Rifampin



Ethambutol

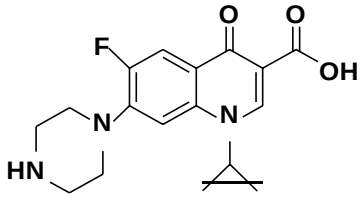


Streptomycin

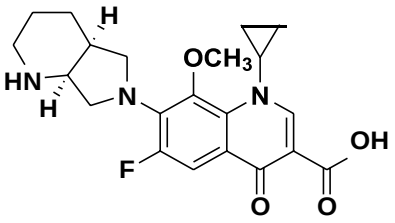
Figure 5: Structures of first-line anti-tuberculosis drugs[69].

1.6.2 Second-line drugs

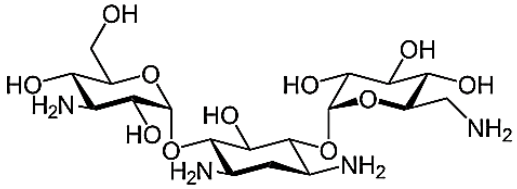
Compared to the first-line drugs, the second-line therapeutic drugs used for the treatment of *Mycobacterium tuberculosis* infection are less effective, more expensive and have higher toxicities associated with them[70]. They are however, essential for the treatment of drug resistant forms of the bacteria (MDR-TB)[71]. Several members of the fluoroquinolones class of drugs are currently used as second line TB drugs for the treatment of multi-drug resistant TB[72]. They include ciprofloxacin, moxifloxacin, kanamycin and cysloserine **(Figure 6).**



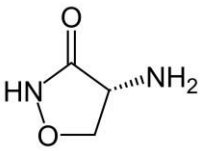
Ciprofloxacin



Moxifloxacin



Kanamycin



Cycloserine

Figure 6: Structures of Second Line TB Drugs[70].

1.6.3 Current TB drugs on trial

Bedaquiline **(Figure 7),** the first TB drug with a novel method of action to be approved after 40 years, has generated considerable interest and some countries have already begun using it to treat patients with MDR-TB[73]. The WHO and other groups involved in TB control are urging caution in the use of the drug, both to prevent the development of resistance to this new agent and to protect patients from potential adverse events

associated with a new drug that has not yet completed the final phase of clinical testing[74].

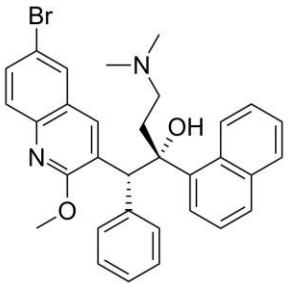


Figure 7: Structure of bedaquiline[73]

Sutezolid **(Figure 8)** is a Phase 2 oxazolidinone antibiotic that is in development to battle tuberculosis[75].It demonstrated encouraging activity in a Phase 2a Early Bactericidal Activity (EBA) study in TB patients in South Africa. It also demonstrated potent antibacterial activity against *Mycobacterium tuberculosis* in the laboratory and in animal models of TB. The potential indications for sutezolid include multidrug and extensively drug resistant tuberculosis (M/XDR-TB), HIV-associated tuberculosis (HIV-TB), drug-sensitive tuberculosis (DS-TB), and suspected M/XDR latent *Mycobacterium tuberculosis* infection (M/XDR-LTBI)[76].

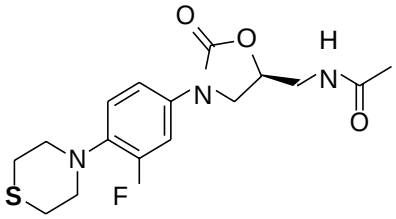


Figure 8: Structure of Sutezolid[75].

1.6.4 Current TB drugs with quinoxaline moiety

There is little information about quinoxaline derivatives used as agents against *M tuberculosis*, but there are some compounds that have been shown to be active against TB. Several new molecules which are currently in clinical development encourage the scientific community to find new drug targets and new drug leads. Some quinoxaline 1,4-*di*-*N*-oxides derivatives **(Figure 9)** with very different substituents in 2, 3, 6 and 7 positions have been synthesized in order to obtain new

selective agents. Some of these products have been tested as anti-tuberculosis agents and very interesting results have been obtained from the first screening[77].

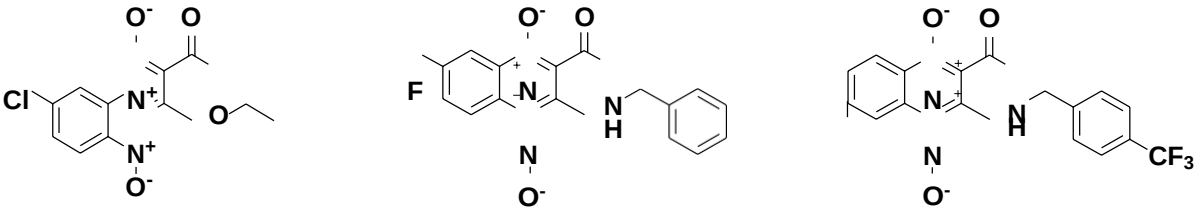


Figure 9: Potential TB Drugs with quinoxaline scaffold

The study focused mainly on the synthesis and coupling of quinoxaline derivatives which would possibly lead to potential anti-TB drugs because it is an important skeleton, which is playing a role in the preparation of substances with different biological activities.

1.7 Aim and Objectives

The aim of the study was to synthesise, characterise and evaluate the biological activity of quinoxaline compounds against tuberculosis

The objective of the study was to:

i. synthesise quinoxaline derivatives using Buchwald-coupling reactions.
ii. investigate the ability of benzenesulphonyl as a good coupling partner in Buchwald–coupling reactions
iii. evaluate biological activity of the synthesised compounds.

CHAPTER 2

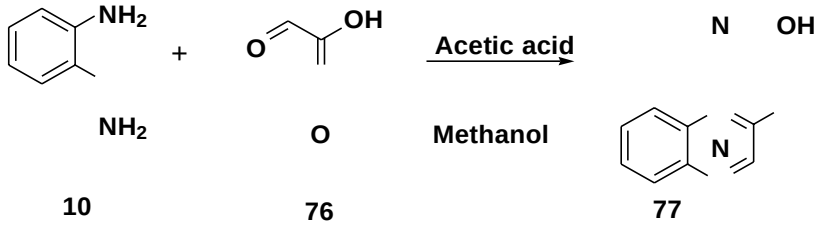
CHAPTER 2 RESULTS AND DISCUSSION

2. Introduction

Numerous quinoxaline derivatives were prepared with the aim of obtaining new anti-tuberculosis agents which can improve the current chemotherapeutic anti-tuberculosis treatments. The versatility of the quinoxaline scaffold in addition to its relative chemical simplicity and accessibility makes these compounds amongst the most promising sources of bioactive compounds.

2.1 Synthesis of 2-benzenesulfonyloxyquinoxaline

In an effort to establish new derivatives utilised as suitable skeletons for the design of biologically active compounds, our investigation started with the preparation of quinoxali-2-ol **(Scheme 16)** [78].



Scheme 16: Synthesis of quinoxalin-2-ol

The condensation reaction between *o*-phenylenediamine **10** and glyoxylic acid **76** in acetic acid gave 80% yield of the desired product 2-quinoxalinone **77**, after recrystallization of the crude product using DMF. The cream white solid material obtained as the product was characterised by NMR spectroscopy **(Figure 10).** The proton NMR spectrum obtained showed peaks integrating for 5 protons with singlet peak characterising quinoxaline ring appearing at 8.30 ppm which corresponds to a –N=C-H group. The peak resonating at 7.30 ppm as a triplet is due to two chemically equivalent protons attached to carbon number 8 and nine of the benzene ring attached to pyrazine ring. Two sets of doublets integrating for one proton each are due to hydrogen on C7 & C9 of the benzene ring. While a broad singlet at 12.5ppm is due can be assigned to the hydrogen of the alcohol group. The ^{13}C NMR spectrum showed a total number of 8 carbons around the aromatic region. The results were consistent with those reported in the literature[78].

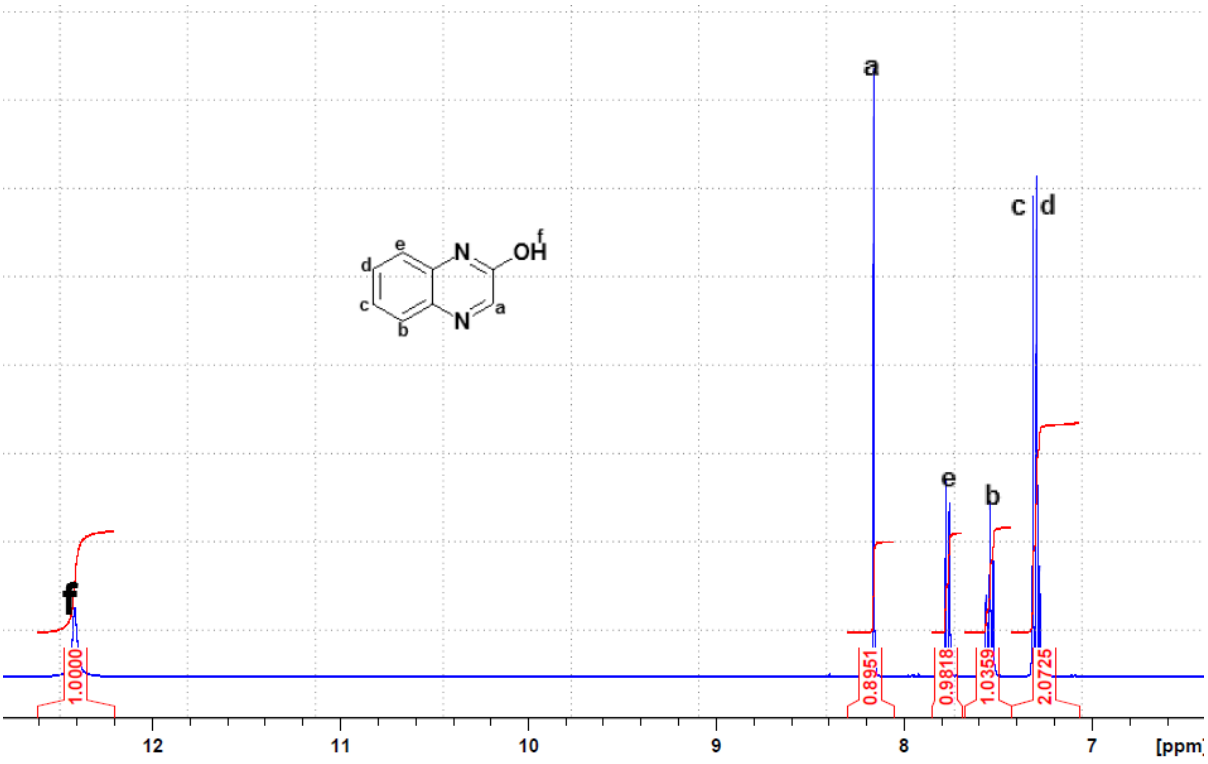
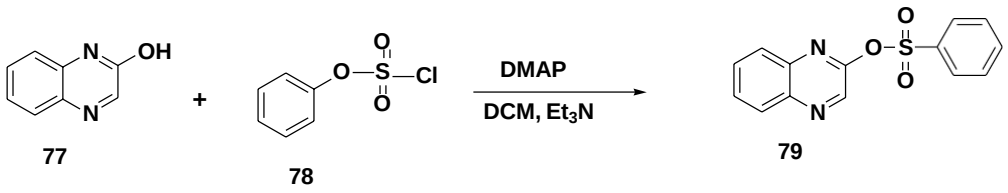


Figure 10: ^{1}H- NMR of quinoxalin-2-ol

Alcohols are poor substrates for substitution reactions and they must firstly be converted into good leaving groups for easily displacement by a variety of nucleophiles. Sulfonation of the hydroxyl group by treatment with a sulfonyl chloride provides a valuable method towards nucleophilic substitution **(Scheme 17)**. Aryl sulfonates have distinct properties with respect to ease of oxidative addition[79].



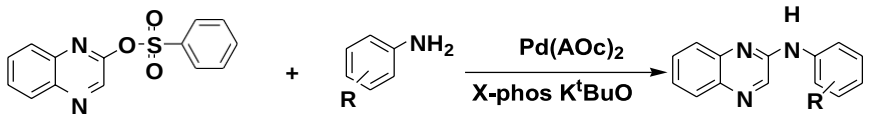
Scheme 17: Synthesis of 2-benzenesulfonyloxyquinoxaline

2-Quinoxalinone **77** was reacted with benzenesulfonylchloride **78**, DMAP and Et_3N in DCM while stirring for 2 hours in an effort to introduce a good leaving group replacing the proton on the –OH group in quinoxalin-2-ol **77**. The reaction was quenched after 2 hours with Na_2SO_4 followed by purification of the crude product on flash silica yielding the desired 2-benzenesulfonyloxyquinoxaline **79** in 95% yield.

Nuclear magnetic resonance was employed to characterise product obtained in $CDCl_3$. The ^1H NMR spectrum showed a total of 10 protons, with a characteristic singlet at δ 8.66 ppm, which corresponds to a –N=C-H group. A multiplet in the region δ 8.09 – 8.17 ppm integrating for 3 protons and another multiplet in the region δ 7.87 – 7.90 ppm integrating for 1 proton were assigned to protons of the benzene ring of the quinoxaline. The ^{13}C NMR gave a total of 12 carbons instead of 14 fourteen around the aromatic region due to element of symmetry to account for the two missing carbons. All peaks appeared in the aromatic region, and this indicated addition of a phenyl ring to the system. The results depicted by the NMR were consistent with those reported in the literature[78] verifying that compound, 2-benzenesulfonyloxyquinoxaline **79**, had been synthesised.

2.2 Buchwald coupling reactions

After obtaining the correct starting material, the aim was to further investigate the ability of 2-benzenesulfonyloxyquinoxaline as a good coupling partner of different aryl amines through Buchwald Coupling reactions. The focus was mainly on evaluating various reaction parameters, including the choice of solvent, base and most notably dialkybiaryl phosphine ligand on how it affects the reaction results **(Scheme 18)**.



79 **80** **81**

R= H

Coumpound 81	Amine substituent
81 a	**H**
81 b	**F (para)**
81c	**F(ortho)**
81 d	**-OCH_3**

Scheme 18: Buchwald coupling of 2-benzenesulfonyloxy with aniline substrates.

We began by employing Buchwald coupling conditions that were recently used in literature with aniline as substrate[34,38]. The cross-coupling was performed using the 5 mol % of Palladium acetate as a catalyst, X-phos as ligand, 3 eq K^{t}BuO in 1.4 dioxane solvent medium to obtain our target compound, *N*-phenylquinoxalin-2-amine **81a** in 80% yield.

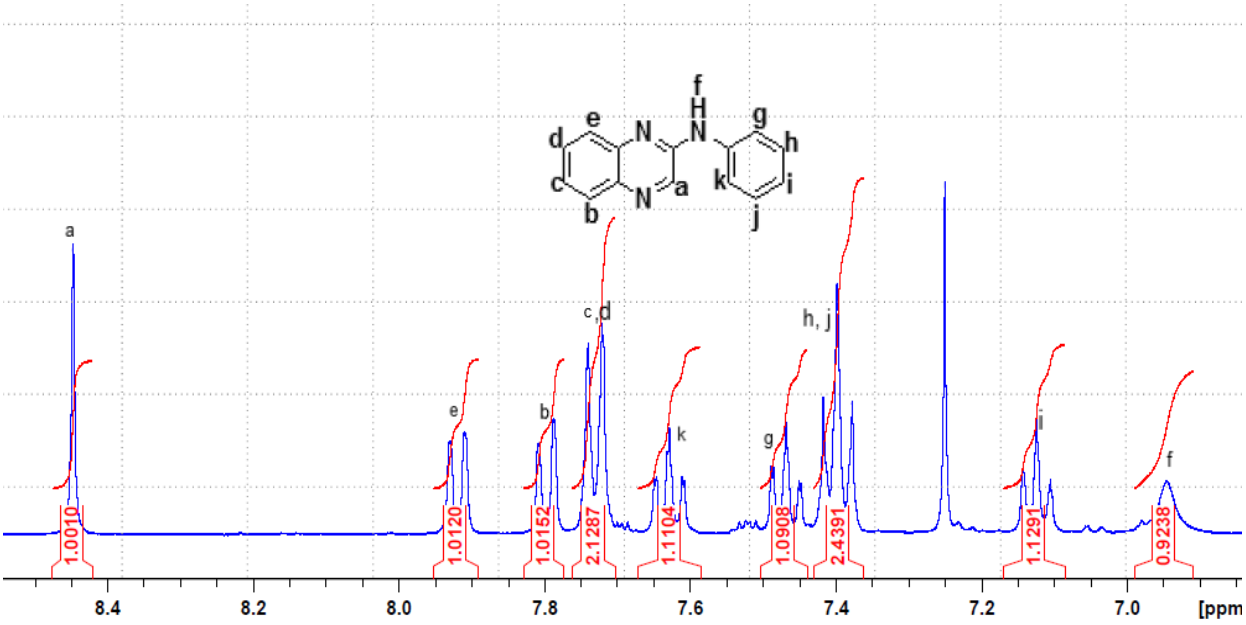


Figure 11: ^{1}H NMR of *N*-phenylquinoxalin-2-amine

The ^{1}H NMR obtained for this compound showed a total of 11 protons with the presence of the quinoxaline ring moiety characteristic singlet peak at 8.44 ppm assigned to a –N=C-H group. The broad singlet resonating at 6.94 ppm was assigned to the N-H group. The two multiplets resonating at 7.46 and 7.72 ppm integrating for one proton each can be assigned to the aromatic protons. The other peaks appearing on the spectrum can be accounted by remaining hydrogens in the structure. Further characterisation was done by using LC-ES-API MS and it gave a peak of 222.1 [M+H] which is in good agreement to the calculated value for $C_{14}H_{11}N_3$ of 221.1 amu including the data reported in literature[80].

Encouraged by these results, we evaluated the efficacy of other functionalised anilines to synthesise corresponding aryl amines in excellent yields. The same reaction conditions were employed beginning with 4-fluoro aniline as the potential cross-coupling partner. The ^{1}H NMR analysis of the crude mixture showed the presence of

the desired product with traces of the two starting materials meaning the reaction didn't reach completion. After purification using prep TLC eluting with 10% Methanol/DCM solvent fraction, the product **81b** was obtained in 10% yield.

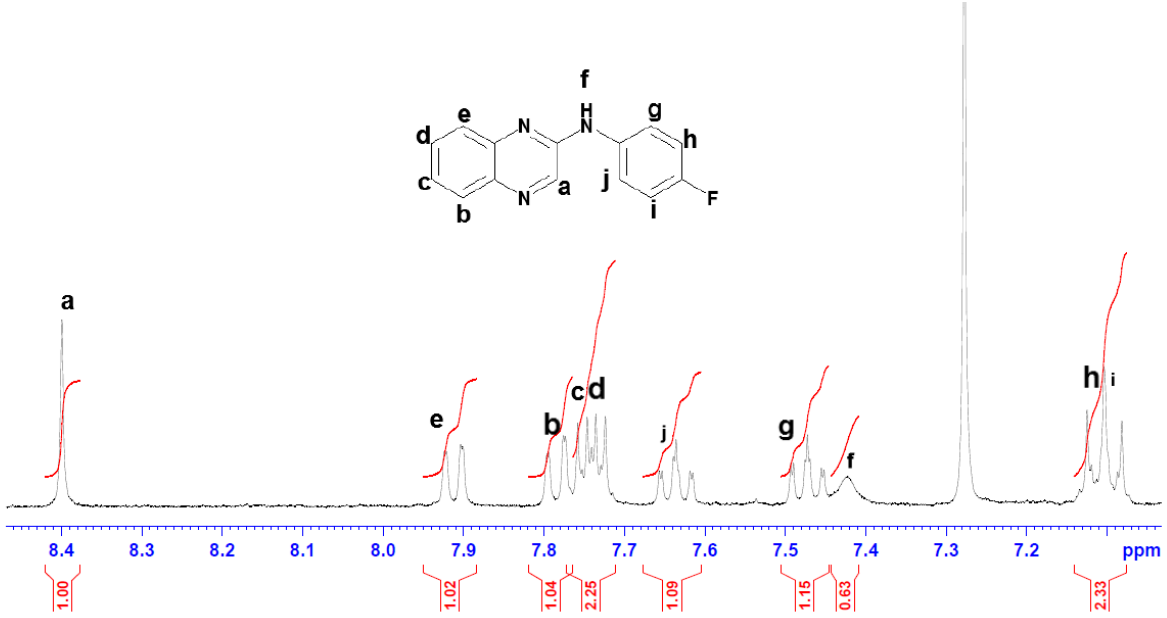


Figure 12: Proton NMR of *N*-(4-fluorophenyl)quinoxalin-2-amine.

The ^{1}HNMR study **(Figure 12**) on the product indicated different signals with a total of 10 protons corresponding with number of the hydrogens on the product. The presence of a quinoxaline ring moiety characteristic singlet peak at resonating 8.40 ppm for the –N=C-H group. The broad singlet peak assigned to a proton bonded to nitrogen atom appears at 7.43 ppm. The target compound consists of a para- substituted fluorine atom which cannot be decoupled. The triplet resonating at 7.12 ppm is assigned to two hydrogen atoms attached to the carbons ortho to the amine group Coupling between fluorine and hydrogen is very strong making the latter to appear as a triplet due to $^{4}J_{HF}$ coupling of 4Hz. The other signal with 2 protons attached to the carbons of the benzene ring at the meta position to the amine group appear at 7.75 ppm with $^{3}J_{HF}$ coupling value of 7.6Hz. The 4 remaining hydrogens appearing at 7.91 ppm, 7.80 ppm,7.65 ppm, 7.50 ppm on the spectra belongs to the benzene ring. The ^{13}C NMR was also used to characterize the synthesized compound since it has never been synthesized (**this was checked by Scifinder on the 10/06/2017)** The number of carbons peaks obtained in the NMR corresponds to the total number of 14 carbons on the structure of the product with the quaternary carbon attached to the fluorine atom appearing at 163.3ppm with a $^{1}J_{CF}$ value of 243.2 Hz. We also observed two signals

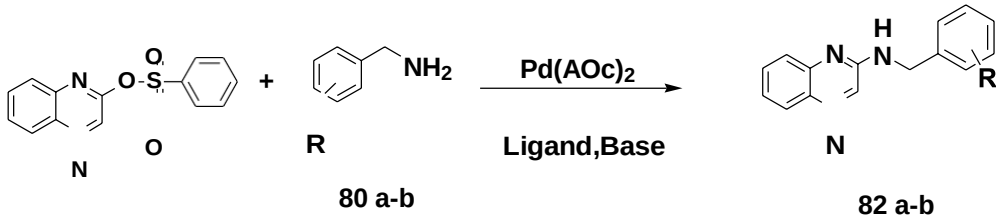
appearing as doublets at 106.8-106.6 with a coupling with $^{2}J_{CF}$ of 26.3Hz and 109.1-109.7 ppm with $^{2}J_{CF}$ of 21.6 Hz representing the two adjacent carbons to the fluorine atom. Resonance at 114.7-114.6 can be accounted to two the carbon atoms at the ortho position of the amine ring where the peak has been splitted through C-F coupling with $^{3}J_{CF}$ of 2.9 Hz. The other peaks appearing on the spectrum represent the remaining carbons in the structure Characterisation by LC- ES-API MS, gave a peak of 240.1 [M+H] which is in good agreement to the calculated value for $C_{14}H_{10}N_3F$ of 239.1 amu. confirming that the desired product, **81b** *N*-(4-fluorophenyl)quinoxalin-2-amine.

Further attempts were done this time employing 2-fluoro aniline as a potential coupling partner in the reaction maintaining similar reaction conditions. The product obtained was characterised as above with NMR and Mass spectroscopy. The NMR gave a total of 10 protons which agreed with the number hydrogen in the target structure. The –N=C-H group resonated at 8.60 ppm and two sets of doublets appearing at 7.91 ppm and 7.77 ppm assigned to four hydrogens of the benzene ring attached to the pyrazine. The hydrogens attached to amine benzene ring did not show any element of symmetry due to the ortho-substituted fluorine atom as compared to the previously para-substituted product making each proton unique. The multiplet signal resonating at 6.82 ppm is due to the hydrogen attached to the carbon adjacent to the fluorine substituent. The remaining hydrogens appearing as doublets are due to the hydrogens on the amine ring. Characterisation with ^{13}C NMR gave a total of 14 carbons which correlates with number of carbons in the compound. Mass spectroscopy gave a peak of 240.1 [M+H] which is in good agreement to the calculated value for $C_{14}H_{10}N_3F$ of 239.1 amu and **81c** proved that indeed this is the desired compound which is also novel obtained in 5% yield.

We then employed an electron donating substrate to further probe the possibility of attaining Buchwald coupling while increasing the library of C-N quinoxaline derivatives. *p*-Anisidine was employed with the aim of increasing substrate scope. The proton NMR results showed a total of 15 hydrogens. The long intense signal in the aliphatic region appearing at 3.80 ppm is due to the methoxy group. The –N=C-H group appears as a singlet at 8.44 ppm, and the NH peak has overlapped with a multiplet intergrating for three hydrogens at 7.40 ppm. The 4 hydrogens of the *p*-anisisdine are chemically equivalent and their signals are resonated at 6.8 and 7.6 ppm as multiplets.

The remaining peaks can be attributed to the remaining hydrogens in the structure. It was then deduced that a pure compound *N*-(4-methoxyphenyl) quinoxalin-2-amine **81d** was obtained in 5% yield[81]. The chelating X-phos ligand in combination with $Pd(AOc)_2$ seems to form poor catalyst in coupling substrates containing electron withdrawing and donating substituents.

We were then more interested in exploring the scope of X-phos with monoarylation of benzyl amines using the previously mentioned conditions **(Scheme 19).**



Compound No	R
82a	H
82b	-OCH_3

Scheme 19: Synthesis of 2-amine quinoxalines with benzyl amines

Firstly, treatment of 2-benzosulfonylsulfonyloxyquinoxaline with benzyl amine under Xphos catalyst system with K^{t}BuO conditions mentioned above gave the desired product *N*-benzylquinoxalin-2-amine **82a** as tan oil in 78% yield[66].

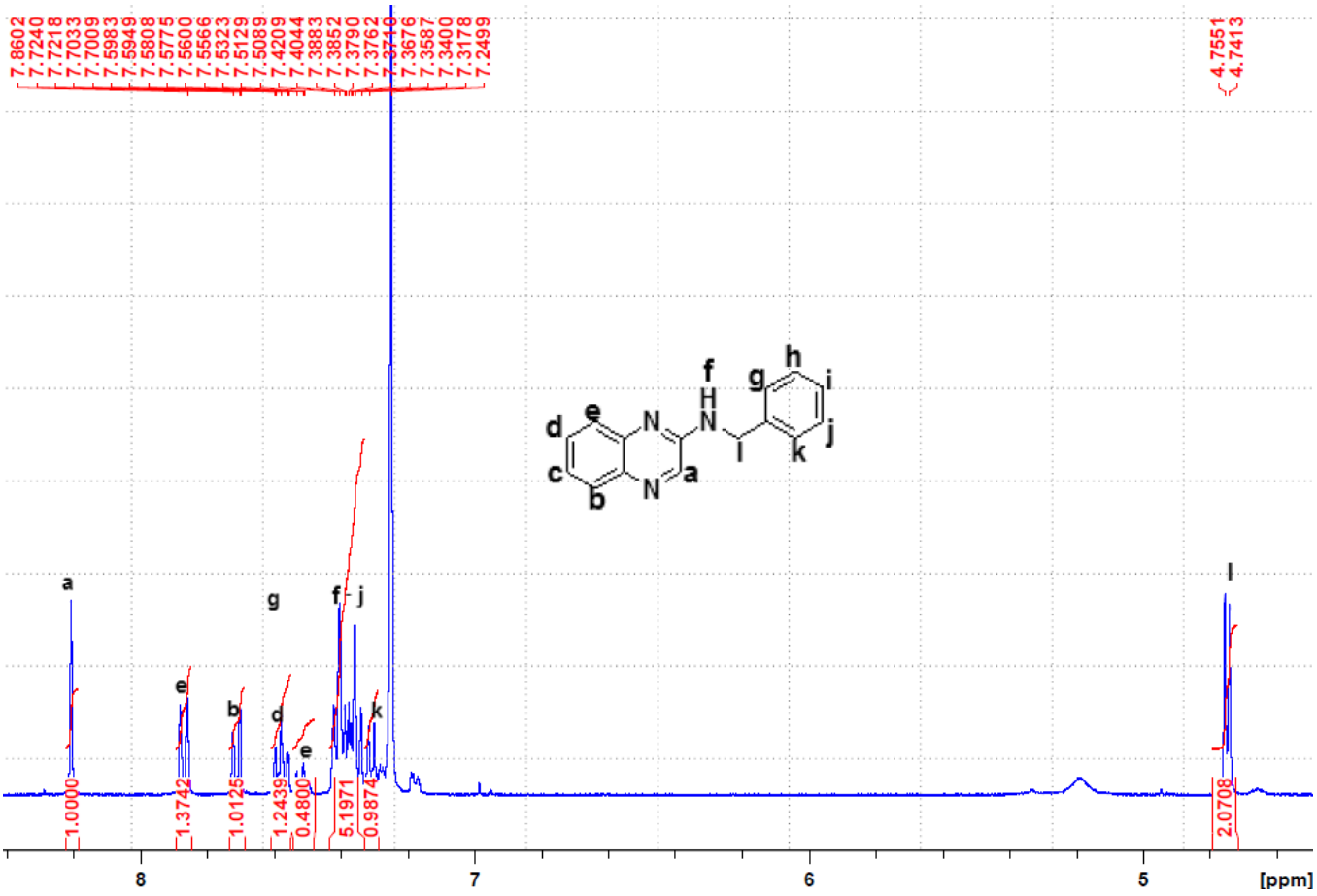


Figure 13: Proton NMR spectrum of *N*-benzylquinoxalin-2-amine

The ^{1}H NMR spectrum showed a singlet at 8.30 ppm integrating for 1 proton, which was attributed to a –N=C-H group. A doublet intergrating for two protons at 4.80 ppm assigned to the deshielded –CH_2 group and a broad singlet resonating at 5.00 ppm for the amino group.The carbon NMR gave a total of 15 signals which is in agreement with the total number of carbons. Mass spectroscopy gave a peak of 236.1 [M+H] which is in good agreement to the calculated value for $C_{15}H_{13}N_3$ of 235.1 amu. The NMR and mass spectras correspond with those reported previously in the literature[66].

Compound **82b** was obtained by employing para mrthoxy substituted benzyl amine. The Pd/Xphos catalytic complex was employed and the product obtained was analysed as in **82a**. The proton NMR showed an appearance of the methoxy group resonating at 3.87 ppm in the aliphatic region in addition to the already existing CH_2 at 4.80 ppm yielding the desired *N*-(4-methoxybenzyl)quinoxalin-2-amine in 10%. To our knowledge **82b** has never been reported in literature.

2.2 Optimisation of Buchwald-coupling reaction conditions

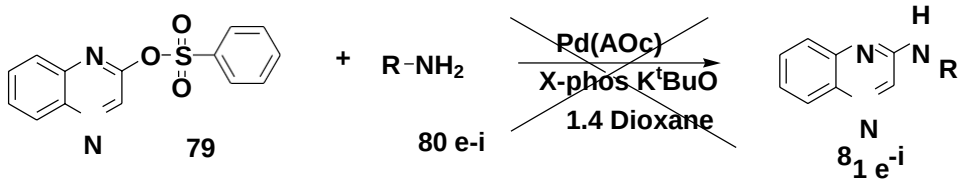
In an effort to improve the yield of the product and to optimise reaction conditions, t-Butylxphos was used on Scheme 5 replacing Xphos for the Buchwald coupling reaction of 2-benzenesulfonyloxyquinoxaline with 4-flouroaniline. The yield of desired product N-(4-fluorophenyl)quinoxalin-2-amine **81b** improved significantly as it was obtained in 71%yield. The recently employed catalytic complex proved to be better in coupling amines substituted with electron withdrawing flouro groups. t-Butylxphos proved to be a suitable ligand to be utilised in amination reactions of fluoro-anilines as the yield was successively increased from 10% to 70%.

In an effort to further probe reactivity of various amine substrates with different steric properties using t-Butylxphos ligand, the same reaction conditions were used on coupling *p*-anisidine with the aim of increasing the yield of compound **81d.** The results showed less conversion of the starting material at the end of the reaction with only 31 % yield.

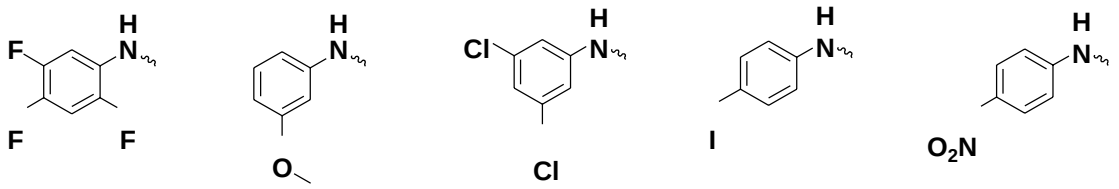
The improvement in yield of *N*-(4-methoxybenzyl)quinoxalin-2-amine **82b** was also observed when employing t-butylxphos catalyst system in the cross coupling reaction. Treatment of 2-benzosulfonyloxyquinoxaline with 4-methoxybenzylamine under Buchwald coupling reaction conditions mentioned above gave the desired 2-(4-methoxybenzylamino) quinoxaline in a yield of 57%.

In an effort to further investigate sterically hindered amines **80 e-i** with 2-benzenesulphonoloxy quinoxaline employing t-ButylXphos catalyst complex. The reaction was performed in 1, 4 dioxane medium and after 24 hours we failed to get the desired coupling products **(Scheme 19**).

The possibility of increasing the library of compounds employing X-phos/Pd(Aoc) catalyst with other various aniline substrates did not yield results.



Where R =

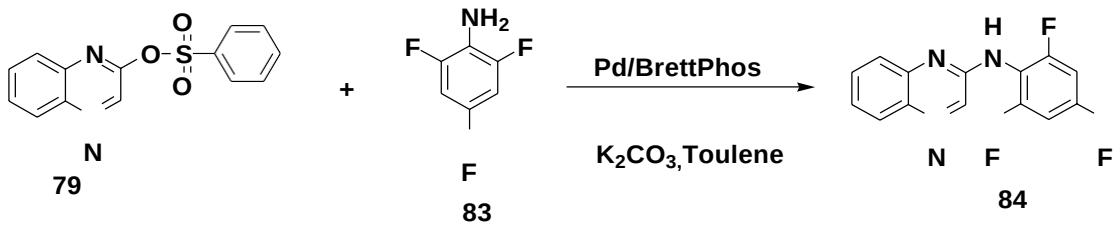


Scheme 19: Attempt of Buchwald coupling with amines containing electron withdrawing atoms

Poor nucleophilic aromatic amines failed to yield results in the cross coupling reactions. All attempts on the amines constituting electron withdrawing substituents were not tolerated under the above reaction conditions (Scheme 15). Our postulation based on the facts that halide substituted anilines will couple effectively with aryl sulphonyl under the conditions reported for the cross coupling of fluoro aniline did not yield expected results as only starting material was recovered at the end of the reaction. Attempts on the reaction between 3,5-dichloro aniline employing Xphos catalytic complex again did not yield expected results. Further trials with nitro-aniline did not generate any product mainly because nitro group is deactivating substituent as it tends to withdraw electrons from the amino group making it impossible for the C-N bond formation step on the catalytic cycle. The electron withdrawing substituents on the amine substrate tend to cause weaker coordination on the formation of the amido complex on the catalytic cycle[46].

We then decided to employ a different ligand BrettPhos which has shown excellent reactivity in literature and overcomes many restrictions which the previous catalyst possess. The ligand constitutes electron-donating methoxy group which tends to increase electron density around the metal centre. The use of electron rich phosphines has turned out to be effective in accelerating oxidative addition as it increases electron density on the metal centre[47].

We started our investigation by coupling 2,4,6-trifluoro aniline **83** with a weaker base K_2CO_3 and toluene as the solvent medium **(Scheme 20)**.



Scheme 20: Buchwald coupling of 2,4,6-tri fluoro aniline

The choice of base has a large bearing on the functional groups that may be present in amination substrates and thus has been the subject of considerable interest, including detailed mechanistic studies with some ligand systems[44].

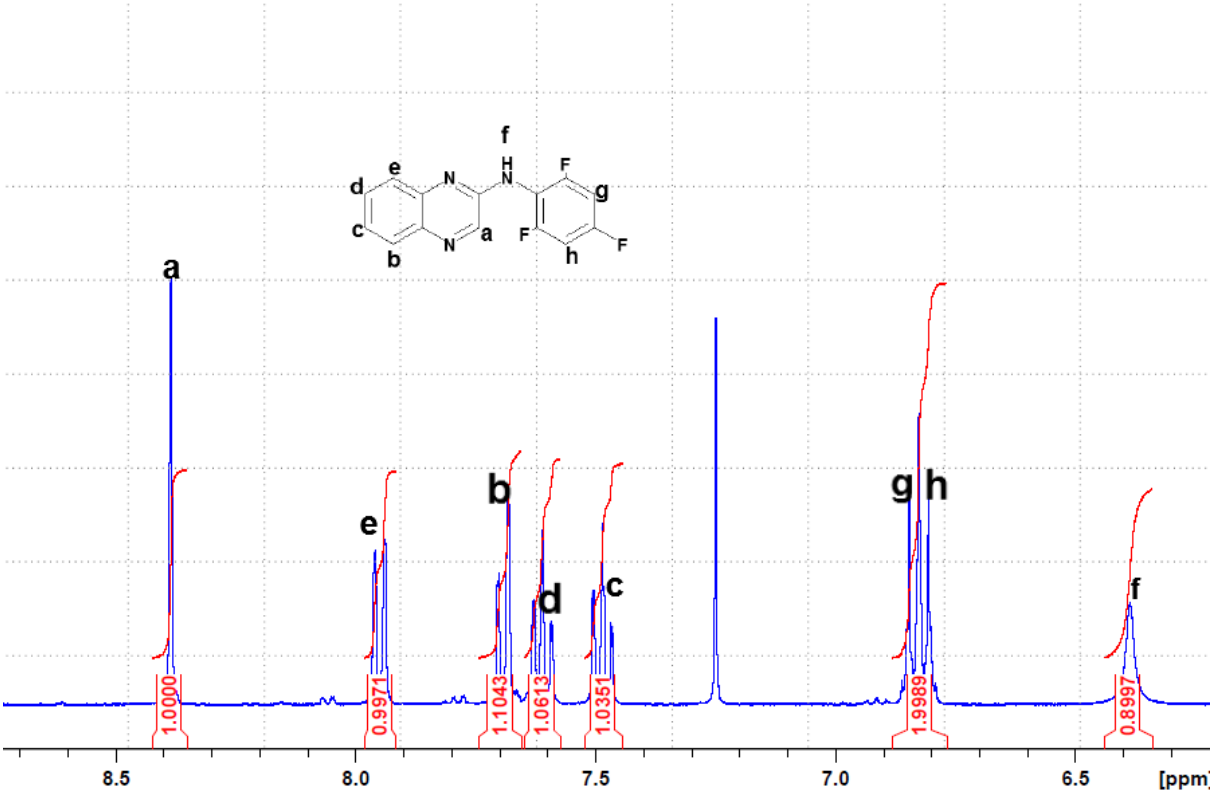
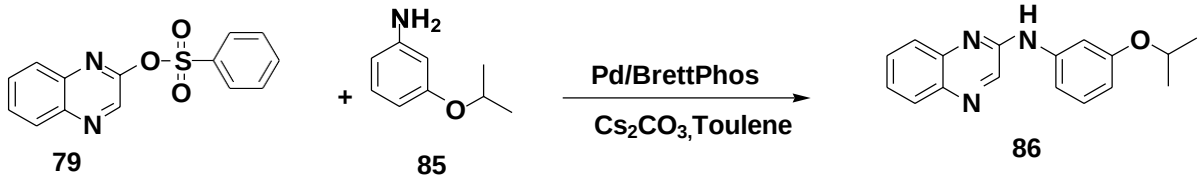


Figure 14: ^{1}HNMR of *N*-(2,4,6-trifluorophenyl)quinoxalin-2-amine

The NMR data obtained **(Figure 14)** above showed the presence of 8 the peaks in agreement with the 8 hydrogen atoms in the structure. Resonance at 6.58 ppm is due to the quartert from two protons adjancent to the two carbons with fluorine substituents by $^{3}J_{CF}$ coupling. The singlet appearing at 8.35 is due to proton attached to –N=C-H group. The remaining peaks integrating for four protons belong to the benzene ring of quinoxaline. Further structure elucidation was validated with carbon NMR since the compound *N*-(2,4,6-trifluorophenyl)quinoxalin-2-amine **84** proved to be novel after being checked on **Scifinder on 10 June 2017**. The carbon NMR showed total of 13 carbons due to elmet of symmetry to account to the missing carbon and mass spectroscopy gave a peak of 276.0 [M+H] which agree with the calculated value for $C_{14}H_8N_3$ of 275.1 amu. The reaction upon completion yielded 25% of the product as yellow oil.

Functional group compatibility was demonstrated again with BrettPhos via the successful incorporation of sterically hindered 3-isopropoxyaniline **85** substrate.

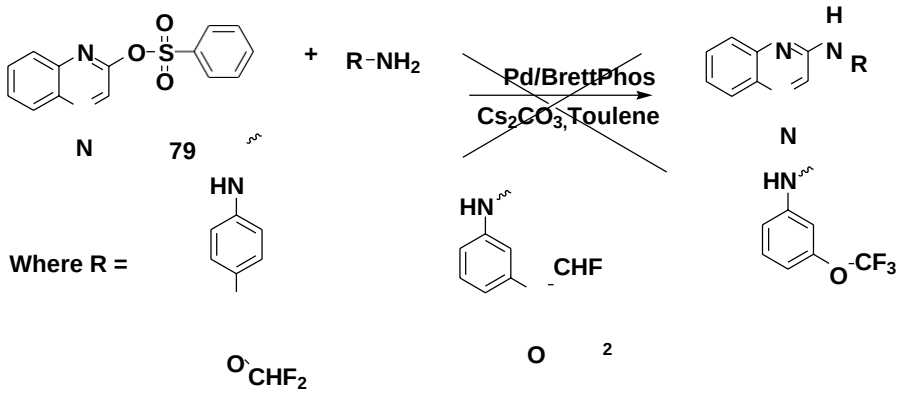


79 85 86

Scheme 21: Buchwald coupling of 3-Isopropoxy aniline

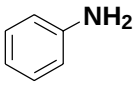
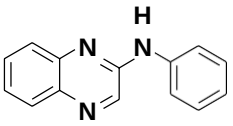
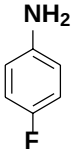
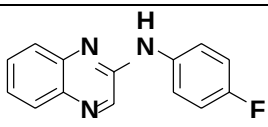
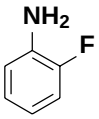
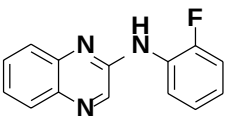
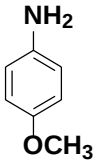
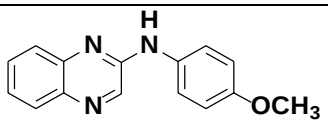
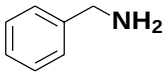
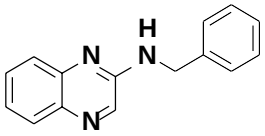
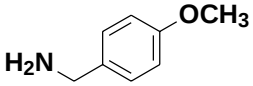
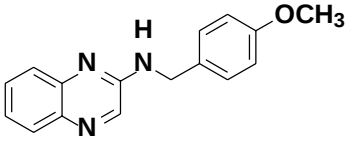
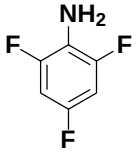
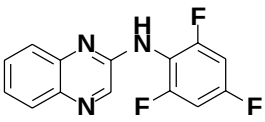
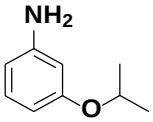
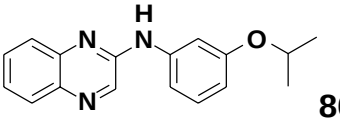
The ^{1}H NMR spectrum showed a total of 16 protons, in which a characteristic singlet peak at δ 8.44 ppm corresponding to an –N=C-H group, was observed, integrating for 1 proton. There was a presence of a septed at 4.60 ppm assigned for C-H proton of the isopropoxy group which is deshielded due to the adjacent oxygen atom. There was also a doublet peak at 1.40 ppm assigned to 6 methyl protons of the isopropyl group with a coupling constant of 8Hz. The ^{13}C NMR spectrum showed the presence of 16 carbons with two peaks appearing in the aliphatic region due to the Isopropyl group carbons. All the peaks in the aromatic region were accounted for the remaining carbons in the molecule and we deduced that compound *N*-(4- isopropoxyphenyl)quinoxalin-2-amine **86** was obtained in 5% yield. Employing a weaker cesium carbonate base and more catalyst loadings afforded the reaction producing our desired product, **86** in moderate yields even though the full substrate conversion was not experienced. We postulated that weak inorganic bases can bring significant benefits in the functional group tolerance of Pd-catalyzed amination reactions for substrates containing electron donating functional groups. To our knowledge compound **86** has never been reported in literature.

Attempts on other amines containing electron withdrawing groups (**Scheme 22**) did not yield expected results.



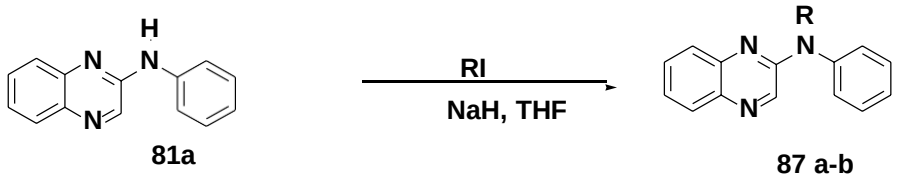
Scheme 22: Attempt of Buchwald coupling with fluoro-methoxy amines.

Table 2.1: Summary of compounds synthesised through Buchwald Coupling

Entry	Amine	Ligand	Base	Product	Yield
1		X-phos	K^{t}BuO	 **81a**	**71%**
2		t-butylXphos	K^{t}BuO	 **81b**	**70%**
3		t-butylXphos	K^{t}BuO	 **81c**	**50%**
4		t-butylXphos	K^{t}BuO	 **81d**	**31%**
5		X-phos	K^{t}BuO	 **82a**	**78%**
6		t-butylXphos	K^{t}BuO	 **82b**	**57%**
7		BrettPhos	K_2CO_3	 **84**	**12%**
8		BrettPhos	$CsCO_3$	 **86**	**5%**

2.3 Alkylation of *N*-phenylquinoxalin-2-amine

With the free N-H bond present on the different products synthesized it was decided that there is a possibility of an alkylation reaction that can occur on the N-H group of the products, whereby different alkyl and acid groups are incorporated to the nitrogen by removing the hydrogen bonded to it with a strong base (**Scheme 23)**. From the products synthesised, *N*-phenylquinoxalin-2-amine **81a** was chosen for the alkylation reaction.



R = CH_3, CH_2CH_3

Scheme 23: Alkylation reaction of *N*-phenylquinoxalin-2-amine

The N-methyl product was obtained in 63% yield as an orange oil. The ^{1}H NMR spectrum showed a total of 13 protons, in which a characteristic singlet peak at 8.48 ppm corresponding to an –N=C-H group integrating for 1 proton. There was a presence of a singlet at 3.60 ppm assigned for 3 protons of deshieldeded methyl group. The ^{13}C NMR spectrum showed a total of 15 carbon signals. There was also an appearance of a carbon peak at 29.3 ppm in the aliphatic region assigned to the carbon of the newly attached methyl group. The results confirmed that pure product *N*-methyl-*N*-phenylquinoxalin-2-amine **87a**, phenyl was obtained in 63% yield. Physical and spectroscopic data agree with those reported in literature[66].

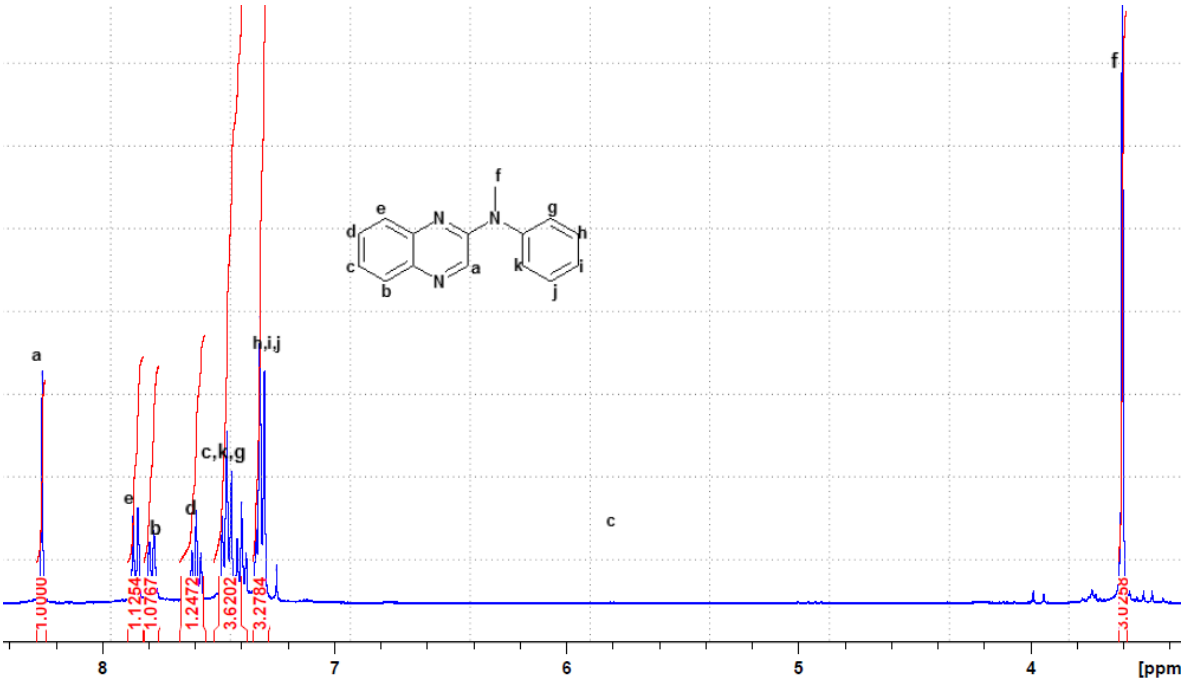
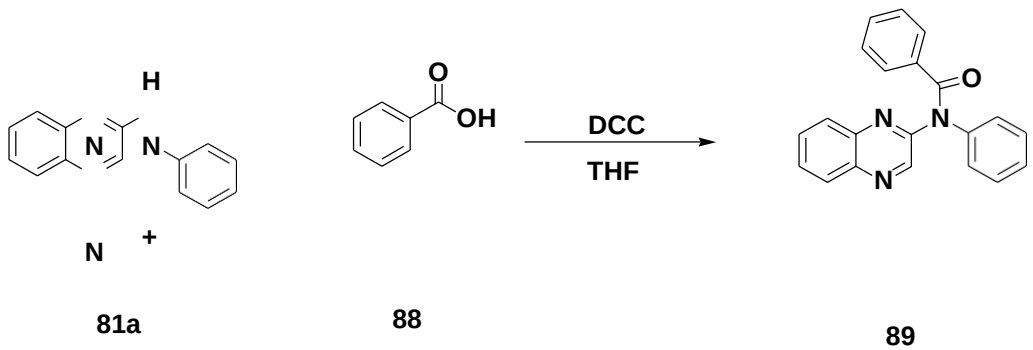


Figure 15: Proton NMR of *N*-methyl-*N*-phenylquinoxalin-2-amine.

Treatment of the *N*-phenylquinoxalin-2-amine **81a** under the same reaction conditions were followed as mentioned but employing iodoethane to alkylate the free -NH. The proton NMR spectrum showed the presence of a –N=C-H group at 8.47 ppm and the disappearance of the –NH broad singlet. There was an appearance of new peaks belonging to the ethyl group resonating at 4.20 (quartet) and 1.30 ppm (triplet). The proton NMR has confirmed that the product obtained is indeed the desired product, N-ethyl-*N*-phenylquinoxalin-2-amine **87b** in 75% yield. To our knowledge compound **87b** has never been reported in literature.

N-phenyl-*N*-(quinoxalin-3-yl)benzamide **89** was formed by treating *N*-phenylquinoxalin-2-amine **81a** with benzoic acid **88** to form an amide bond on the free N-H bond. The ^{1}H NMR spectra confirmed the product was pure N-phenyl-N-(quinoxalin-3-yl)benzamide **88** in 73% yield.



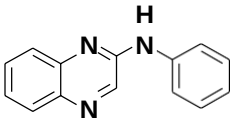
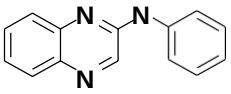
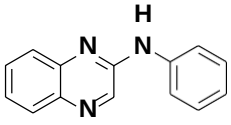
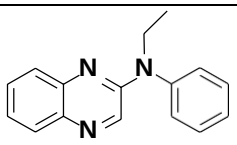
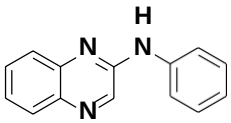
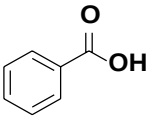
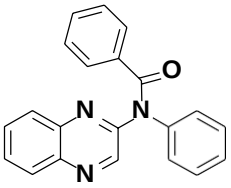
81a **88** **89**

Scheme 24: Synthesis of *N*-phenyl-*N*-(quinoxalin-3-yl)benzamide.

The ^{1}H NMR obtained showed the quinoxaline ring moiety characteristic singlet peak at 8.54 ppm and integration of all the other peaks present relative to this peak gave a total of 15 protons which are consistent with the total number of protons in the desired product.^{13}C NMR spectra was also used to check the presence of the carbonyl carbon peak belonging to the amide bond formed in the reaction which appeared at the downfield of the spectrum having a chemical shift of 171.19 ppm on the ^{13}C NMR spectrum. To our knowledge compound **89** has never been reported in literature.

The alkylation of *N*-phenylquinoxalin-2-amine was successful and good product yields were obtained.

Table 2.2: Summary of compounds from successful alkylation reactions performed

Entry		Reagents	Product	Yield
1		CH_3I	 **87a**	63 %
2		CH_3CH_2I	 **87b**	75 %
3			 **89**	73 %

CHAPTER

3

CHAPTER 3. BIOLOGICAL ACTIVITY TESTING

3.1 TB

In recent years, medicinal chemistry has undergone a revolutionary change. Rapid advances in the biological sciences have resulted in a much better understanding of how the body functions at the cellular and the molecular level. As a result, most research projects in the pharmaceutical industry or university sector now begin by identifying a suitable target in the body and designing a drug to interact with that target. An understanding of the structure and function of the target, as well as the mechanism by which it interacts with potential drugs is crucial to this approach.

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*, which most commonly affects the lungs. It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory disease. In healthy people, infections with *Mycobacterium tuberculosis* often cause no symptoms since the person's immune system acts to "wall off" the bacteria. The symptoms of active TB of the lung are coughing, sometimes sputum with blood, chest pains, weakness, weight loss, fever, and night sweats. TB is a worldwide pandemic and still remains one of the foremost among infectious diseases in the world causing the maximum number of deaths due to the spread of a single microorganism[82].

The quinoxaline derivatives show very interesting biological properties (anti-bacterial, anti-viral, anti-cancer, anti-fungal, anti-helminthic, and insecticidal)[6]. In an effort of obtaining new and more potent anti-tubercular compounds that can improve the current chemotherapeutic treatments, we managed to synthesise quinoxaline derivatives and evaluate their antimicrobial activity against *Mycobacterium tuberculosis*. The biological activity of the synthesised compounds against TB was done at the University of Cape Town, drug discovery and development centre (H3-D).

Biological investigations of *in vitro* anti-TB activity of the amine-quinoxaline compounds were done for the anti-mycobacterial activity against *Mycobacterium tuberculosis* H37RvMA strain. None of the compounds showed promising inhibition percentages against *Mycobacterium tuberculosis* when compared with rifampicin which was used as a positive control. The antibacterial activities of the compounds were evaluated by means of the minimum inhibitory concentrations (MIC) which are

MIC_{90} and MIC_{99} against *M tuberculosis* (Table 3). MIC_{90} and MIC_{99} are defined as the minimum concentration of the compound that inhibits growth of bacteria by 90% and 99% respectively.

Table 3.1: Results of the *in vitro* evaluation against *Mycobacterium tuberculosis*

Entry	Compound ID	MIC_{90} (µg/ml)	MIC_{99} (µg/ml)
1	Rifampicin	0.0188	0.0037
2	81s	>20	>20
3	81 b	> 20.0	> 20.0
4	81 c	> 20.0	> 20.0
5	81 d	> 20.0	> 20.0
6	82a	> 20.0	> 20.0
7	82b	> 20.0	> 20.0
8	83 a	> 20.0	> 20.0
9	83b	> 20.0	> 20.0
10	84	> 20.0	> 20.0
11	86	> 20.0	> 20.0
12	87a	> 20.0	> 20.0
13	87b	> 20.0	> 20.0

The results revealed that none of the compounds have promising inhibition percentages against *M tuberculosis* when compared with rifampicin which was used as a positive control. They all exhibited MIC_{90} and MIC_{99} greater than 20 µg/ml.

3.2 Malaria

Malaria is one of the world's deadliest diseases which causes approximately 881,000 deaths every year, with nine out of ten deaths occurring in sub-Saharan Africa, and 85% of malaria-related deaths in children under five years of age. This is the equivalent of a child dying of malaria in Africa every 30 seconds[83]. The most serious forms of the disease are caused by the parasite *Plasmodium falciparum*; malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* results in milder disease in humans that is not generally fatal. In 2008, 109 countries were reported to be endemic with malaria, with 45 of the countries within the African region. There were an estimated 247 million episodes of malaria in 2006, with 86% of cases reported in African countries[84].

The control and eradication of malaria demands many sided approach. Currently a range of good tools are employed such as insecticide spraying and long-lasting insecticide-treated bed nets to prevent the transmission of the infection via the mosquito vector. This preventative strategy is not 100% effective which makes it a serious challenge. The current WHO-recommended first-line treatment for the majority of malaria cases is artemisinin-based combination therapy (ACT). These medicines, in addition to diagnostics, are available to treat and in some cases prevent malaria[85].

Continued and sustainable improvements in antimalarial medicines through focused research and development are essential for the world's future ability to treat and control malaria. Unfortunately, malaria is a disease of poverty, and despite a wealth of scientific knowledge there is insufficient market incentive to generate the competitive, business-driven industrial antimalarial drug research and development that is normally needed to deliver new products. Mechanisms of partnering with industry have been established to overcome this obstacle and to open up and build on scientific opportunities for improved chemotherapy in the future[86].

After failing to get activity on our synthesized compounds for TB, we then decided to further investigate some of our compounds against malaria. Chloroquine (an anti-malarial drug) was used as a drug standard and showed IC50 values in the range 0.01-0.05 µM.

Table 3.2: Results Malaria screening with Chloroquine used as a control

Compound	Viability %
81a	93.49
81b	118.94
81c	117.45
82a	89.67
84	99.39
86	93.83
87a	42.64

Compound **87a** showed a percentage parasite viability of 42.64%. Although not active, this compound has a potential to be developed further, by modifying other functional groups. The results have shown that most of the compounds exhibited higher percentage parasite viability values as majority of compounds exceeded 90%.

3.3 Cytotoxicity Studies

For the cytotoxicity assay, results are expressed as % cell viability, based on fluorescence reading in treated wells vs. untreated control well (Table 3.3.2). Emetine (which induces cell apoptosis) is used as a positive control drug standard (Table 3.3.1).

Table 3.3.1: Emetine

		Emetine
Conc (µM)	Log(Conc)	Percentage Viability
1	0	12.395200
0.333333	-0.47712	12.824640
0.111111	-0.95424	14.059700
0.037037	-1.43136	40.566510
0.012346	-1.90849	101.749000
0.004115	-2.38561	102.784600
0.001372	-2.86273	112.576800
0.000457	-3.33985	110.937700

Table 3.3.2: Cell toxicity assay

Compound	Viability %
81a	138.70
81b	47.13
81c	19.97
82a	84.72
84	91.48
86	87.29
87a	115.77

The compounds **81b and 81c** significantly decreased the cell viability after 24 hours at concentrations exceeding 25 µM. The other compounds on Table 3.3.2, showed no significant effect on HeLa cells.

3.4 Conclusion

Buchwald coupling of 2-benzesulfonyloxyquinoxaline with a series of amine substrates was investigated. The purity of the isolated cross coupling products was found to agree with the expected compounds which were confirmed by NMR and HPLC-MS spectroscopy. The ^{1}H NMR, ^{13}C NMR found to be in good agreement with the proposed structures of the compounds that were synthesised. Where applicable, the synthesised compounds were in agreement with those reported in literature.

The results presented showed higher yields obtained when coupling unsubstituted aryl amines **81a** and **82a** employing Pd/Xphos catalytic complex in 1.4 dioxane solvent. Despite significant attempts with amines bearing electron withdrawing substituents we, observed limitation in the substrate scope as mostly the yields were below 50%. Catalytic complex constituting of tButyl-Xphos proved to be success when coupling fluoro-aniline and *p*-anisidine after the yield increased tremendously as compared to conditions with Xphos. Employing a weaker inorganic base Cs_2CO_3 brought significant benefits in the functional group tolerance when coupling 2,4,6-trifluoro aniline **83** substrate as compared to using K^{t}Buo because we managed to obtain the yield of our desired product **84** in 25%

Despite the successes with other subsitituted amines, the application of this strategy towards the synthesis of substrates bearing bulky electron withdrawing groups remains a challenge. The coupling can be difficult to accomplish as a result of their difficulty in undergoing reductive elimination due to multiple electron withdrawing groups attached.

The quinoxaline derivatives obtained were evaluated for their biological activity against TB but none of them proved to be active as they gave MIC_{99} greater than 20%. Compound **87a** although not active, showed a percentage parasite viability of 42 % against *Plasmodium falciparum* 3D7 strains and has a potential for further investigation. Cytotoxicity studies showed that compounds **81b** and **81c** significantly decreased the cell viability after 24 hours at concentrations exceeding 25 µM,while other compounds had no significant effect on HeLa cells.

3.5 Future work

Carbon-nitrogen cross-coupling reaction has revolutionised the synthesis of organic molecules on both bench-top and industrial scales as a versatile and useful method of preparing aryl amines. However, the very limited substrate scope and the observations that in most instances the products isolated during our investigation were obtained in less than 50 % yield highlights the significant limitations of the synthetic protocols of this coupling reaction. The anticipated results on the coupling of these substrates employing different reaction parameters were not successful. This work highlights challenges of designing experimental parameters needed to influence amination of substrates containing multiple reaction sites. We need to overcome difficulty of some experimental parameters that influence chemoselectivity in the Buchwald-Hartwig amination of substrates containing multiple reactive amine sites, including the complex dependence of substrate structure on reactivity and the potential reactivity benefits of employing various bidentate ligands.

Developing a method which will employ different catalyst systems that favour electronic properties of both coupling partners will increase the library of our amination reaction products. The catalyst must tolerate a high degree of steric congestion on both 2-benzesulfonylquinoxaline and amine substrate. Currently, the most extensive investigations of chemoselective amine arylation have been published by the Buchwald group, using five variants of their biarylphosphine ligands (XPhos, tBu-XPhos, SPhos, BrettPhos, and RuPhos)[49,50]. The design and implementation of novel ancillary ligand frameworks has played a particularly important role in advancing the outcomes of the reaction[88]. New catalyst systems can allow more challenging substrate transformations to be addressed with greater selectivity and increasingly mild conditions.

Although all the synthesised compounds showed no biological activity, further studies will be done on compound **87a** to try and improve its activity against *Plasmodium falciparum* 3D7 strains and to improve all compounds cytotoxicity levels on HeLa cells. Further investigations will now be based in optimising parameters of the reaction in order to improve the yields. Application of Buchwald coupling on the secondary amines to increase the library of the quinoxaline derivatives and test their biologically activity against TB and other opportunistic diseases is also a subject available for investigation.

CHAPTER 4

CHAPTER 4: EXPERIMENTAL

4.1 Reagents and solvents

All reactions involving moisture-sensitive reactants were performed under nitrogen (N_2) atmosphere using oven-dried glassware. Tetrahydrofuran (THF) was freshly distilled over sodium/benzophenone under N_2 atmosphere for 4–6 hours before use. Commercially available reagents and solvents were purchased from Sigma Aldrich, Merck, Fluka analytical and Rochelle chemicals. All chemicals were used directly as received, unless otherwise stated. All measurements were performed at room temperature unless otherwise mentioned. Anhydrous solvents were achieved by using standard desiccation/drying methods and stored over activated molecular sieves[87]. Column chromatography was used as a method to purify isolated products.

4.2 Physical and spectroscopic properties compounds

The structural properties of the compounds were recorded and confirmed by nuclear magnetic resonance (NMR) (Bruker Ascend 400 MHz Topspin 3.2), HPLC-MS (LCMS-2020-Shimadzu Scientific Instruments), ^{1}H NMR and ^{13}C NMR spectra were referenced internally using solvent signals, ^{1}H NMR: 7.26 ppm for $CDCl_3$, 2.50 ppm for DMSO-d6; ^{13}C NMR: 77.0 ppm for $CDCl_3$, 39.4 ppm for DMSO-d_6, respectively which were used as the solvents at room temperature. Chemical shifts are expressed in δ-values parts per million (ppm) and the coupling constants (*J*) in Hertz (Hz). Multiplicity of the signals is given as follows: br. = broad, s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet.

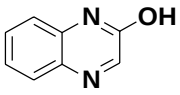
4.3 Nomenclature and numbering of compounds

The compounds generated in this work are named in the following experimental sections according to systematic nomenclature. However, the numbering system used to illustrate the diagrams of these compounds is one adopted for convenience and is not meant to reflect systematic numbering of these compounds.

4.4 Synthesis

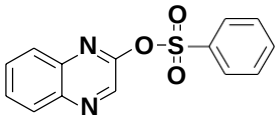
4.4.1 Preparation of starting material

Preparation of quinoxalin-2-ol (77)[79]



O-Phenylenediamine (10.0 g, 0.0920 mol) was dissolved in acetic acid (10 mL) and methanol (10 mL), and then the solution cooled to -15°C with stirring. Glyoxylic acid (8.50 g, 0.0920 mol) in water (20 mL) was added drop-wise for over 30 minutes to the solution, maintaining the temperature at -15°C. The final solution was allowed to warm up to 0˚C over 3 hours and then filtered. The filtrate was washed with water (15 mL) then methanol (15 mL), and air dried to give a dark grey solid. Recrystallization from DMF gave quinoxalin-2-ol **77** as a tan solid (9.40 g, 70%); M.p 265-267°C (Lit. 266-267 °C); δ_H (400 MHz, DMSO-d_6) 2.99 (s,1H) 7.25-7.34 (m, 2H) 7.54 (m, 1H), 7.77 (m, 1H) and 8.16 (s,1H); δ_C (100 MHz, DMSO-d_6) 116.17, 123.75, 129.22, 131.23, 132.24, 132.47, 152.03 and 155.38 ppm. Spectroscopic data agreed with those reported in literature.

Synthesis of 2-benzosulfonyloxyquinoxaline (78)[79]

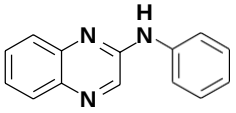


In a 50 mL round bottom flask, quinoxalin-2-ol **77** (1.25 g, 8.5 mmol), DMAP (0.104 g, 0.85 mmol) and benzenesulfonyl chloride (1.32 mL, 17 mmol) were dissolved in dry DCM (20 mL), cooled to 0 ˚C and stirred for 5 minutes. Et_3N (3 mL, 22 mmol) was added drop-wise over 5 minutes, the solution allowed to stir at room temperature for 3 hours, and the reaction quenched with $NaHCO_3$ (20 mL). The two layers were separated and the aqueous layer was washed with DCM (2 x 15 mL). The combined organic layers were dried over Na_2SO_4, concentrated and then purified on flash silica eluting with DCM to give 2-benzenesulfonyloxyquinoxaline **79** as a brown solid (2.3 g, 95 %). M.p 89–92 °C (Lit. 91 °C). δ_H (400 MHz, $CDCl_3$), 7.72 (m, 2H), 7.80–7.72 (m, 3H) 7.92 (d ,1H, *J* 9.1 Hz), 8.19 (3H, m), 8.70 (1H, s). δ_C (100 MHz, $CDCl_3$), 128.5,

128.6, 129.0, 129.2, 129.8, 131.2, 134.6, 136.5, 139.2, 139.7, 141.31 and 150.9 ppm. Spectroscopic data agree with those reported in literature.

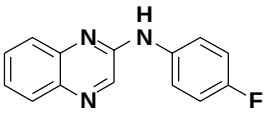
4.4.2 Buchwald Coupling experimental reactions

Synthesis of *N*-phenylquinoxalin-2-amine (81a)[80]



In a 50 ml 2 neck round bottom flask, equipped with a stirrer bar and under nitrogen atmosphere, 2-benzenesulfonyloxyquinoxaline **79** (200 mg, 0.698 mmol), $Pd(OAc)_2$ (7.80 mg, 0.350 mmol, 5 mol%), X-phos (16.6 mg, 0.0390 mmol) and aniline (260.8 mg, 1.4 mmol, 3eq, were dissolved in 1,4-dioxane. K^tBuO (0.48 mL, 2.09 mmol, 3 eq) was added and solution was refluxed for 18 hours, quenched using anhydrous $NaHCO_3$ solution, the layers separated and aqueous layer washed with EtOAc (15 mL). The organic layer was dried over Na_2SO_4, concentrated and then purified on flash silica and prep TLC with 5% methanol/DCM to give *N*-phenylquinoxalin-2-amine **81a** as yellowish solid (108.2 mg, 70.2%); M.p 135-137 °C (Lit. 137 °C); δ_H (400 MHz, $CDCl_3$), 8.45 (s, 1H), 7.93 (m, 1H), 7.81 (m, 1H), 7.73 (d, 2H, *J* = 8.8 Hz), 7.66-7.61 (m, 1H), 7.49-7.45 (m, 1H), 7.4 (m, 2H), 7.15-7.12 (m, 1H) and 7.04 ppm (m, 1H). δ_C (100 MHz, $CDCl_3$), 119.8, 121.8, 123.6, 125.6, 126.9, 128.8, 129.3, 130.3, 131.9, 137.8, 138.4, 139.1, 141.1 and 149.2 ppm, *m/z* (ES-API, +ve) 222.1 [$M+H^+$, 100]. Spectroscopic data agree with those reported in literature.

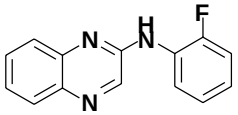
Synthesis of *N*-(4-fluorophenyl)quinoxalin-2-amine (81b)



In a 50 mL round bottom flask, equipped with a stirrer bar and under nitrogen atmosphere, 2-benzenesulfonyloxyquinoxaline **79** (200 mg, 0.698 mmol), $Pd(OAc)_2$ (7.8 mg, 0.35 mmol, 5 mol%), t-Butylxphos (16.6 mg, 0.039 mmol) and 4-flouroanaline (0.233 mg, 3 eq.) were dissolved in 1,4-dioxane (5 mL). K^tBuO (0.48 mL, 2.099 mmol, 3 eq) was added and solution was refluxed for 18 hours, quenched using anhydrous $NaHCO_3$, the layers separated and aqueous layer washed with EtOAc (15 mL). The

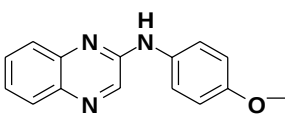
organic layer was dried over Na_2SO_4, concentrated and purification on flash silica with 40% ethyl-acetate/hexane followed by prep TLC with 5% methanol/DCM gave *N*-(4-fluorophenyl)quinoxalin-2-amine as yellow solid (140 mg, 70%). M.p (150.1-152.4 °C), δH (400 MHz, $CDCl_3$): 8.40 (s, 1H), 7.91 (d,1H *J* = 9.6 Hz) ,7.77 (m,1H), 7.75 (t, 2H, $^3J_{HF}$ = 7.6 Hz), 7.63 (m, 1H), 7.47(m, 1H), 7.42 (m, 1H) and 7.12 ppm (t, 2H, $^4J_{HF}$ = 4 Hz), δ_C (100 MHz, $CDCl_3$),106.8-106.6 (d, 1x C,$^2J_{CF}$ =26.3 Hz) 109.1-109.7 (d,1x C, $^2J_{CF}$ =21.6 Hz) 114.7-114.6 (d, 1x C $^3J_{CF}$ = 2.9 Hz), 126.1, 127.1, 128.8, 130.1, 130.5, 137.9, 138.5, 140.8, 141.0, 148.6, 163.1-164.5 ppm (d, 1x C,$^1J_{CF}$ = 243.2 Hz) m/*z* (ES-API, +ve) 240.1 [$M+H^+$, 100].9

Synthesis of *N*-(2-fluorophenyl)quinoxalin-2-amine 81c



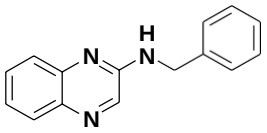
In a 50 mL round bottom flask, equipped with a stirrer bar and under nitrogen, 2-benzenesulfonyloxyquinoxaline **79** (200 mg, 0.698 mmol), $Pd(OAc)_2$ (7.8 mg, 0.35 mmol, 5 mol%), t-Butylxphos (16.6 mg, 0.039 mmol) and 2-flouroanaline (0.233 mg, 3 eq.) were dissolved in 1,4-dioxane (5 mL). K^tBuO (0.48 mL, 2.099 mmol, 3 eq) was added and solution was refluxed for 18 hours, quenched using anhydrous $NaHCO_3$, the layers separated and aqueous layer washed with EtOAc (15 mL). Purification on flash silica eluting with 40% ethyl-acetate/hexane followed by 5% methanol/DCM gave *N*-(2-fluorophenyl)quinoxalin-2-amine as orange solid (85 mg, 50%). M.p. (151.0-156.7 °C) δ_H (400 MHz, DMSO), 8.40 (s, 1H), 7.91 (m, 2H), 7.77 (m, 2H), 6.96 (d, 1H *J* = 7.97 Hz), 6.82 (d, 1H, *J* = 8.37 Hz), 6.56 (t, 1H, $^3J_{HF}$ = 7.14 Hz), 6.32 (m, 2H). δ_C (100 MHz, DMSO) :114.6, 114.5, 114.8, 114.9, 115.7, 120.4, 124.6, 125.8, 127.8, 129.3, 136.8, 137.2,139.9 ,141.5 (d, 1x C, J_{CF} = 160 Hz) and 148.1 ppm; *m/z* (ES-API, +ve) 240.1 [$M+H^+$, 100].

Synthesis of *N*-(4-methoxyphenyl)quinoxalin-2-amine (81d)[81]



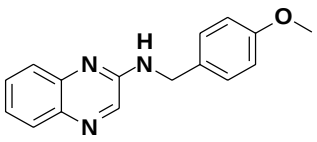
In a 50 mL round bottom flask, equipped with a stirrer bar and under nitrogen atmosphere, 2-benzenesulfonyloxyquinoxaline **79** (200 mg, 0.698 mmol), $Pd(OAc)_2$ (7.8 mg, 0.35 mmol, 5 mol%), t-Butylxphos (16.6mg, 0.039 mmol) and 4-anisidine (0.127 g, 0.116 mL, 1.031 mmol, 3 eq.) were dissolved in 1,4-dioxane (5 mL). K^{t}BuO (0.48 mL, 2.099 mmol, 3 eq) was added and solution was refluxed for 18 hours, quenched using anhydrous $NaHCO_3$, the layers separated and aqueous layer washed with EtOAc (15 mL). Purification on flash silica and prep TLC eluting with 40% ethyl-acetate/hexane gave *N*-(4-methoxyphenyl)quinoxalin-2-amine **81d** as yellow oil (29mg, 21%). δ_H (400 MHz, $CDCl_3$),8.4 (s, 1H), 7.9 (d,1H, *J* = 7.6 Hz),7.75(d, 1H, *J* = 8Hz), 7.64-7.58 (m, 3H), 7.45 (m, 1H), 6.9 (m, 2H), 6.73 (m, 1H), 3.80 (s, 3H). δ_C (100 MHz, $CDCl_3$) 55.9, 115.1, 117.3, 125.0, 125.9, 127.8, 128.9,135.3, 135.4, 135.9, 142.2 and 161.5 ppm. Physical and spectroscopic data agree with those reported in literature.

Synthesis of *N*-benzylquinoxalin-2-amine (82a)[66]



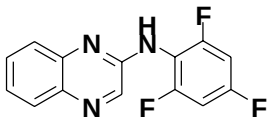
In a 50 mL 2 neck round bottom flask, equipped with a stirrer bar and under nitrogen atmosphere, 2-benzenesulfonyloxyquinoxaline **79** (200mg, 0.698 mmol), Pd $(OAc)_2$ (7.8 mg, 0.35 mmol, 5 mol%), X-phos (16.6mg, 0.039 mmol) and benzyl amine (0.23 mL, 3eq). were dissolved in 1,4-dioxane (5 mL). K^{t}BuO (0.48 mL, 2.099 mmol, 3 eq) was added and solution was refluxed for 18 hours, quenched using anhydrous $NaHCO_3$, the layers separated and aqueous layer washed with EtOAc (15 mL). Purification on flash silica and on prep TLC eluting with 40% ethyl-acetate /hexane gave *N*-benzylquinoxalin-2-amine as tan oil (129 mg, 78%), δ_H (400 MHz, $CDCl_3$), 8.21(s, 1H), 7.87 (d, 1H *J* 4 Hz), 7.73 (d, 1H, *J* = 8Hz), 7.58 (m, 1H),7.43-7.28 (m, 6H), 5.15 (s, 1H) and 4.76-4.74 ppm (d, 2H, *J* = 3.4 Hz). δ_C (100 MHz, $CDCl_3$): 45.3, 124.5, 126.3, 127.1, 127.7, 127.8, 127.8, 128.0, 128.8, 128.8, 129.1, 130.1, 137.3, 138.3 and 138.43 ppm. *m/z* (ES-API, +ve) 236.1 [M+H$^+$, 100]. Physical and spectroscopic data agree with those reported in literature

Synthesis of *N*-(4-methoxybenzyl)quinoxalin-2-amine (82b)



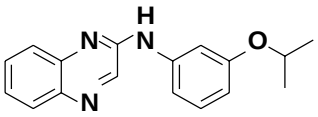
In a 50 mL round bottom flask, equipped with a stirrer bar and under nitrogen atmosphere, 2-benzenesulfonyloxyquinoxaline **79** (200 mg, 0.698 mmol), $Pd(OAc)_2$ (7.8 mg, 0.35 mmol, 5 mol%), t-Butylxphos (16.6 mg, 0.039 mmol) was treated with 4-methoxybenzyl-amine (0.224 g, 2.093 mmol, 3eq.) in 1,4-dioxane (5 mL) K^tBuO (0.48 mL, 2.099 mmol, 3 eq) was added and solution was refluxed for 18 hours, quenched using anhydrous $NaHCO_3$, the layers separated and aqueous layer washed with EtOAc (15 mL). Purification on flash silica prep TLC eluting with 40% ethyl-acetate/hexane gave *N*-(4-methoxybenzyl)quinoxalin-2-amine as tan oil (0.098 g, 57 %). δ_H (400 MHz, $CDCl_3$): 8.21 (s, 1H), 7.87 (d, 1H, *J* = 8.75 Hz), 7.731 (d, 1H, *J* = 9.26 Hz), 7.58 (m, 1H), 7.43-7.28 (m, 5H), 5.15 (s, 1H), 4.76-4.74 (d, 2H, *J* = 5.2 Hz) and 3.84 ppm (s, 3H). δ_C (100 MHz, $CDCl_3$): 46.2, 55.9, 114.1,125.0, 125.9,127.8, 128.0, 128.9, 134.0, 135.3, 142.2, 142.2 and 158.7 ppm.

Synthesis of *N*-(2,4,6-trifluorophenyl)quinoxalin-2-amine (84)



In a 50 mL round bottom flask, equipped with a stirrer bar and under nitrogen atmosphere, 2-benzenesulfonyloxyquinoxaline **79** (200 mg, 0.698 mmol), $Pd(OAc)_2$ (7.8 mg, 0.35 mmol, 5 mol%), Brettphos (16.6 mg,0.039 mmol) and 2,4,6-trifluoroaniline (0.23 mg,3eq), were dissolved in 1.4 dioxane (5 mL). K^tBuO (0.48 mL, 2.099 mmol, 3 eq) was added and solution was refluxed for 18 hours, quenched using anhydrous $NaHCO_3$, the layers separated and aqueous layer washed with EtOAc (15 mL). Purification on flash silica with 40% ethyl-acetate/hexane and on prep TLC with 5% methanol/DCM gave *N*-(2,4,6-trifluorophenyl)quinoxalin-2-amine as yellow oil (25 mg, 13%). δ_H (400 MHz, $CDCl_3$): 8.40 (s, 1H), 7.91-7.90 (d,1H, 8.12 Hz), 7.71-7.70 (d,1H, 8.4 Hz), 7.64-7.60 (m, 1H), 7.50-7.47 (m, 1H), 6.86-6.81 (t, 2H, $^2J_{HF}$ = 8.03 Hz). δ_C (100 MHz, $CDCl_3$):100.9, 125.9, 126.9, 128.8, 130.4, 136.6, 138.4, 141.09,141.1, 161.4, 159.5 and 158, 9. *m/z* (ES-API, +ve) 276.1 [$M+H^+$, 100]

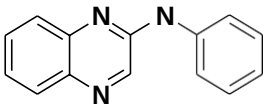
Synthesis of *N*-(4-isopropoxyphenyl)quinoxalin-2-amine (86)



In a 50 mL round bottom flask, equipped with a stirrer bar and under nitrogen atmosphere, 2-benzenesulfonyloxyquinoxaline **79** (200mg, 0.698 mmol), $Pd(OAc)_2$ (7.8 mg, 0.35 mmol,5 mol%), Brettphos (16.6mg, 0.039 mmol) was treated with 3-isopropoxyaniline (0.127g, 0.116 mL, 1.031 mmol, 3 eq.) in 1.4 dioxane (5 mL). K^{t}BuO (0.48 mL, 2.099 mmol, 3 eq) was added and solution was refluxed for 18 hours, quenched using anhydrous $NaHCO_3$, the layers separated and aqueous layer washed with EtOAc (15 mL). Purification on flash silica and on prep TLC eluting with 40% ethyl-acetate/hexane gave *N*-(4-isopropoxyphenyl)quinoxalin-2-amine as yellow oil (29 mg, 21%). δ_H (400 MHz, $CDCl_3$): 8.45 (s, 1H) ,7.93-7.9 (d,1H, *J* = 8Hz), 7.80-7.77 (d, 1H, *J* = 12Hz), 7.65-763 (m, 1H), 7.52 (m, 1H), 7.49-7.47 (m, 1H), 7.12-7.10 (d, 1H, *J* = 8Hz) 6.85 (m ,1H), 6.68-6.65 (m, 1H), 3.80 (m, 1H), 1.43-1.41 (d, 6H, *J* = 8Hz). δ_C (100 MHz, $CDCl_3$): 22.0, 70.0, 107.4, 111.6, 111.8, 125.6, 126.1 128.8, 129.9, 130.2,137.8, 138.4,140.3,141.1,149.2, and 158.7 ppm

4.4.3 Alkylation Reactions

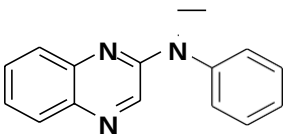
Synthesis of *N*-methyl-N-phenylquinoxalin-2-amine (87a)[66]



In a 50mL round bottom flask, *N*-phenylquinoxalin-2-amine **81a** (200 mg, 0.904 mmol) was dissolved in THF (20 mL) and was treated with NaH (43 mg, 1.81 mmol, 2 eq) at 0 °C and left to stir for 1 hour at room temperature. Iodomethane (0.2 mL, 0.712 mmol, 3.0 eq) was added to the solution under nitrogen. The solution was left to stir for 5 hours at room temperature. The solution was quenched with aqueous NH_4Cl solution and purified by flash column chromatography on silica gel eluting with 5% MeOH/DCM to give *N*-methyl-*N*-phenylquinoxalin-2-amine as orange oil (165 mg, 78%); δ_H (400 MHz, $CDCl_3$) and 8.27 (1H, s); 7.76 – 7.88 (2H, m), 7.58 – 7.62 (1H, m), 7.45 – 7.49 (2H, m), 7.38 – 7.43 (1H, m), 7.32 – 7.34 (3H, m), 3.60 (3H, s), δ_C (100 MHz, $CDCl_3$): 29.73, 119.89, 121.34, 123.61, 125.65, 126.94, 128.94, 139.33, 130.37, 131.37,

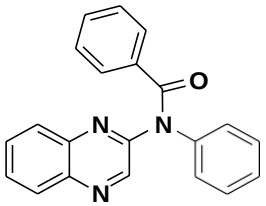
137.85, 138.45, 139.45, 141.17 and 149.23 ppm. *m/z* (ES-API, +ve) found: 236.1191, [M+H$^+$, 100]. Physical and spectroscopic data agree with those reported in literature.

Synthesis of *N*-ethyl-*N*-phenylquinoxalin-2-amine (87b)



In a 50mL round bottom flask, *N*-phenylquinoxalin-2-amine **81a** (60 mg, 0.271mmol) was dissolved in THF (15 mL) and treated with NaH (13mg) and iodoethane (76 mg,0.813 mmol, 3eq). at 0 °C and left to stir for 1 hour at room temperature. Iodoethane (76 mg, 0.813mmol, 3.0 eq) was added to the solution under nitrogen atmosphere. The solution was left to stir for 5 hours at room temperature. The solution was quenched with sat. NH_4Cl and purified by flash column chromatography on silica gel eluting with 5% MeOH/DCM to give *N*-ethyl-*N*-phenylquinoxalin-2-amine as a yellow oil (50 mg, 75%). δ_H (400 MHz, $CDCl_3$): 8.1 (s, 1H), 7.8 (m,1H), 7.55 (m, 1H), 7.45 (m, 2H), 7.35(m, 2H), 7.25 (m, 3H), 4.17 (q, 2H), 1.26 (s, 3H). δ_C (100 MHz, $CDCl_3$): 12.6, 42.4, 113.5, 113.5, 117.2, 125.0, 128.9, 127.8, 128.9, 129.6, 135.3, 135.9, 142.2 and 161.5 ppm.

Synthesis of *N*-phenyl-*N*-(quinoxalin-3-yl)benzamide (88)



In a 50mL round bottom flask N-phenylquinoxalin-2-amine,**81a** *N*-phenyl (100 mg, 0.45 mmol) was dissolved in THF (15 mL) and was treated with DCC (186 mg, 0.90 mmol) and Benzoic acid (110 mg, 2.7 mmol, 3.0 eq). The solution was left to stir for 5 hours at room temperature. Purification on flash silica prep TLC eluting with 5% methanol/DCM then with 40% ethyl-acetate/hexane gave *N*-phenyl-*N*-(quinoxalin-3-yl)benzamide as yellow crystals (83 mg, 73%). δ_H (400 MHz, $CDCl_3$): 8.53 (s, 1H), 8.05 (m, 1H), 7.90 (1H, dd, *J* = 4 Hz), 7.76 (m, 1H), 7.64 (m, 2H), 7.53 (2H, dd, *J* = 12 Hz), 7.47 (m, 2H), 7.38 (m, 4H) and 7.20 ppm (m, 1H). δ_C (100 MHz, $CDCl_3$): 123.2,

125.9, 126.5, 126.6, 128.3, 128.5, 128.9, 129.3, 129.4, 129.6, 129.8, 130.0, 130.7, 131.9, 133.2, 136.76, 137.0, 138.3, 147.8 and 171.1 ppm.

4.5 Biological Studies

4.5.1 Antimicrobial activity against TB

The broth inoculation method was used to test antimicrobial activity against *M. tubercolosis a*t University of Capetown, H3-D laboratories. The broth microdilution method allowed a range of antibiotic concentrations to be tested on a single 96-well microtitre plate in order to determine the minimum inhibitory concentration (MIC). Briefly, a 10 mL culture of a mutant *Mycobacterium tuberculosis* (H37RvMA) strain constitutively expressing recombinant green fluorescent protein (GFP) off a plasmid integrated at the attB locus was grown to an OD600 of 0.6–0.7. The H37 RvMA:gfp culture was then diluted 1:100 in GAST/Fe medium. In a 96-well microtitre plate, 50 µL of GAST/Fe medium was added to all wells from Rows 2–12. The compounds to be tested are added to Row 1 in duplicate, at a final concentration of 640 µM (stocks were made up to a concentration of 12.8 mM in DMSO, and diluted to 640 µM in GAST/Fe medium). A two-fold serial dilution was prepared, by transferring 50 µL of the liquid in Row 1 to Row 2 and aspirating to mix. 50 µL of the liquid in Row 2 then transferred to Row 3 and aspirated, and so on. This procedure was repeated until Row 12 is reached, from which 50 µL of the liquid was discarded so as to bring the final volume in all wells to 50 µl. Finally, 50 µL of the 1:100 diluted *M. tuberculosis* cultures were added to all wells in Rows 2-12. Cells were not added to Row 1, as this served as a contamination control. Controls include media only, 5% DMSO, rifampicin and kanamycin. The microtitre plate was stored in a secondary container and incubated at 37 °C with humidifier to prevent evaporation of the liquid. The lowest concentration of drug that inhibits growth of more than 90% of the bacterial population was considered to be the MIC_{90} and MIC_{99}. MIC_{90} and MIC_{99} values were scored by quantitative fluorescence using a Fluostar Optima microplate reader (BMG Labtech) at 7-days and 14-days post inoculation, and digital images captured and stored.

4.5.2 Antimicrobial activity against Malaria

The screening of compounds against malaria was done at Rhodes University. Firstly, compounds at 20 uM or 25 ug/mL were added to parasite cultures in 96-well plates and incubated for 48 h in a 37 ^{o}C CO_2 incubator. After 48 h the plates were removed

from the incubator.20 µL of culture is removed from each well and mixed with 125 µL of a mixture of Malstat solution and NBT/PES solution in a fresh 96-well plate. These solutions measure the activity of the parasite lactate dehydrogenase (pLDH) enzyme in the cultures. A purple product was formed when pLDH is present, and this product can be quantified in a 96-well plate reader by absorbance at 620nm (Abs_{620}). The Abs_{620} reading in each well is thus an indication of the pLDH activity in that well and also the number of parasites in that well.

For each compound concentration, **% parasite viability** – the pLDH activity in compound-treated wells relative to untreated controls – was calculated. Compounds were tested in duplicate wells, and a standard deviation (SD) was derived. For comparative purposes, Chloroquine (an anti-malarial drug) was used as a drug standard and yielded IC_{50} values in the range 0.01-0.05 µM.

4.5.3 Cytotoxicity assay

To assess the overt cytotoxicity, the compounds were incubated at a fixed concentration of 100 µM (µg/mL), 25 µM and 6.5 µM for pure compounds in 96-well plates containing HeLa (human cervix adenocarcinoma) cells/HEK 293 (human embryonic kidney) cells for 24 hours. The numbers of cells surviving drug exposure were also determined by using the resazurin based reagent and reading resorufin fluorescence in a multiwell plate reader.

CHAPTER 5

CHAPTER 5 REFERENCES

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