Novel synthetic route towards the synthesis of mono-, di- and tri-substituted quinoxalines

by

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DISSERTATION

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Declaration

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Chemistry has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Ndlovu, NT (Mr) **Date**

Abstract

2-Benzenesulfonyloxyquinoxaline was prepared following literature procedure followed by palladium-catalysed Negishi coupling reactions to yield the corresponding, 2-mono-substituted quinoxaline derivatives, 2-phenylquinoxaline and 2-butylquinoxaline. These Negishi cross-coupled derivatives were treated with various nucleophiles, in tetrahydrofuran at room temperature, to yield a series of di-substituted quinoxaline derivatives containing; aryl-, heteroaryl-, arylalkynyl- and alkyl-substituents. Tri-substitution was successful with reaction of 6-chloro-2-benzenesulfonyloxyquinoxaline with excess phenyl-magnesium bromide to yield 2,3,6-triphenylquinoxaline.

Sonogashira cross-coupled compounds were successfully synthesised by reacting 2-benzenesulfonyloxyquinoxaline, 6-nitro-2-benzenesulfonyloxyquinoxaline and 6-chloro-2-benzenesulfonyloxyquinoxaline using phenylacetylene, respectively. Nucleophilic substitution was only successful on 2-(2-phenylethynyl)quinoxaline to yield 3-butyl-2-(2-phenylethynyl)quinoxaline.

The formation of carbon-nitrogen bonds was accomplished *via* palladium-catalysed Buchwald-Hartwig amination of 2-benzenesulfonyloxyquinoxaline with arylamines to afford *N*-phenylquinoxalin-2-amine and *N*-benzylquinoxalin-2-amine in good to high yields. *N*-phenylquinoxalin-2-amine was subsequently treated with iodomethane to synthesise *N*-methyl-*N*-phenylquinoxalin-2-amine. Nucleophilic substitution on Buchwald-Hartwig coupled compounds was only successful when using alkyl nucleophiles.

The reaction of all these quinoxaline derivatives with various nucleophiles does not stop at the stage of α-adduct formation, but continues with the oxidation of these compounds to aromatic substitution products. All synthesised compounds were characterised by NMR, and mass spectral data as well as melting points where applicable.

N-Methyl-*N*-phenylquinoxalin-2-amine and 2,3,6-triphenylquinoxaline showed percentage parasite viability of 42.64% and 58.12%, respectively, against the *Plasmodium falciparum* strain 3D7. *N*-Methyl-*N*-phenylquinoxalin-2-amine showed MIC_{90} of 16.4 and MIC_{99} of 19 μM, while 6-chloro-2-(2-phenylethynyl)quinoxaline

showed MIC_{90} of 8.15 and MIC_{99} of 9.26 µM values against *Mycobacterium tuberculosis* (*Mtb*)-$H_{37}Rv$ strains.

Dedication

This dissertation is dedicated to my family; my father Dumisani Douglas Ndlovu and my late mother Lettie Alphosinah Ndlovu, my sisters Zinhle Ndlovu and Nombulelo Mahlobo, my brother Thembelani Mazibuko, my son Dumisa and Ms. U Msengana for all the support.

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List of abbreviations

KI	Potassium iodide
HIV	Human Immunodeficiency Virus
TB	Tuberculosis
ZOI	Zone of inhibition
E. Coli	Escherichia coli
Mtb	Mycobacterium tuberculosis
DNA	Deoxyribonucleic acid
AcOH	Acetic acid
HFIP	Hexafluoroisopropanol
$POCl_3$	Phosphorous oxychloride
LDA	Lithium diisopropylamide
n-BuLi	n-Butyllithium
TMP	2,2,6,6-Tetramethylpiperamidyl
$ZnCl_2$	Zinc chloride
LiCl	Lithium chloride
MgCl	Magnesium chloride
$K_2S_2O_8$	Potassium persulfate
DMF	*N, N*-Dimethylformamide
NMR	Nuclear Magnetic Resonance
DMAP	4-Dimethylaminopyridine

Et_3N	Triethylamine
DCM	Dichloromethane
Sat	Saturated
EtOAc	Ethyl acetate
$NaHCO_3$	Sodium hydrogen carbonate
$MgSO_4$	Magnesium sulfate
MeI	Methyl iodide
PhMgBr	Phenyl magnesium bromide
$PdCl_2(PPh_3)_2$	Dichlorobis-triphenylphosphine palladium chloride
TLC	Thin layer chromatography
I_2	Iodine
MS	Mass Spectroscopy
*i*PrMgCl	Isopropyl-magnesium chloride
THF	Teterahydrofuran
NH_4Cl	Ammonium chloride
MIC	Minimum inhibition concentration

CHAPTER

1

Chapter 1: Introduction

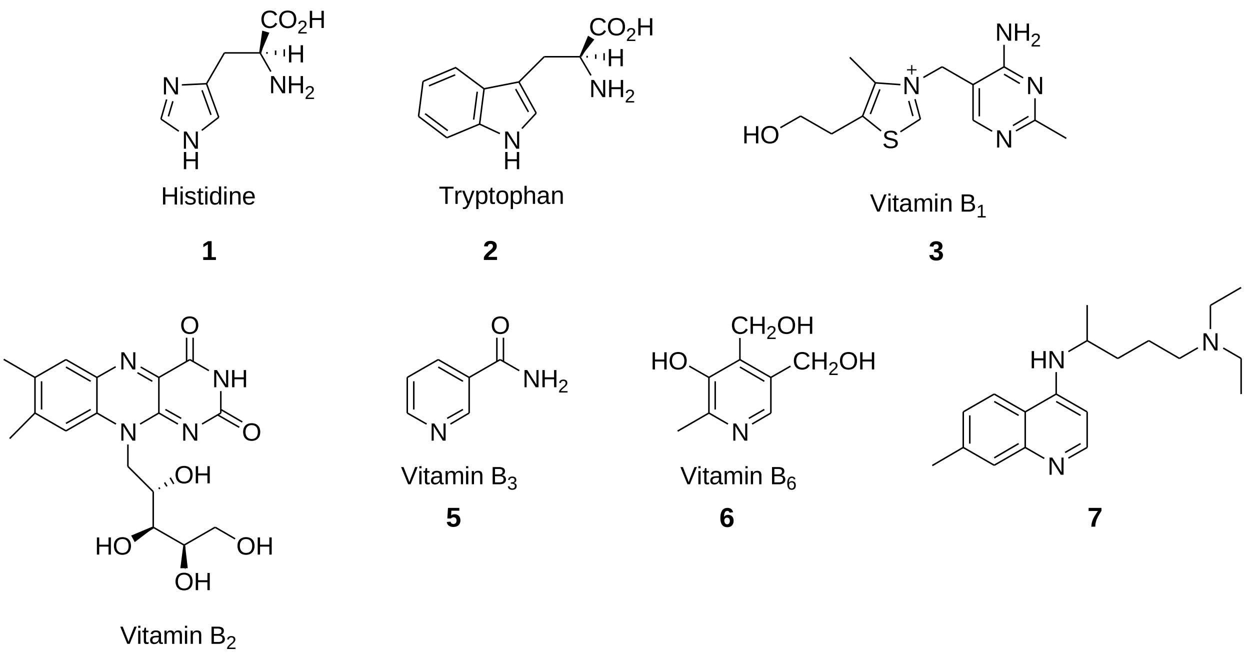
1.1 Heterocyclic Chemistry

There are many organic compounds which contain heteroatoms; these are atoms other than carbon (C) or hydrogen (H). Heterocyclic chemistry deals with heterocyclic organic compounds containing C, H and one or more heteroatoms such as nitrogen (N), sulfur (S), oxygen (O) or phosphorus (P), as part of the ring.[1,2] These ring structures that have the abovementioned atoms within their framework can show both aromatic and aliphatic properties.[2,3]

The importance of heterocyclic compounds comes from their diverse physical, chemical and biological properties.[2] Heterocyclic compounds are found as key components in biological processes, which stems from their wide distribution in nature. Derivatives of the pyrimidine and purine ring systems can be identified in nucleic acid bases, of which are crucial in DNA replication.[4] Heterocyclic compounds are predominantly used as pharmaceuticals, agrochemicals and as vertinary products. Their applications have been extended to brightening agents, antioxidants, corrosion inhibitors, additives, chemical sensors, polymer lasers and transistors.[4-6]

Derivatives of the porphyrin ring system, which are found in chlorophyll and heme, are the components required for photosynthesis and for oxygen transport in plants and animals, respectively.[4] There are two ‘essential amino acids’ which cannot be biosynthesised by human beings, which contain a hetero-aromatic side chain – histidine **1**, with an imidazole, and tryptophan **2**, with an indole (Fig. 1.1).[7]

Thiamin (vitamin B_1) **3**, riboflavin (vitamin B_2) **4**, pyridoxol (vitamin B_6) **5**, nicotinamide (vitamin B_3) **6** and ascorbic acid (vitamin C) **6** (Fig. 1.1) are essential dietary ingredients which contain natural heterocyclic compounds.[4,7]



Histidine **1**

Tryptophan **2**

Vitamin B_1 **3**

Vitamin B_2 **4**

Vitamin B_3 **5**

Vitamin B_6 **6**

7

Figure 1.1: Examples of heterocyclic compounds

Quinoline is a six-membered heterocyclic fused ring compound containing benzene and a pyridine ring. It is a high boiling liquid, which is derived from naphthalene by replacement of one of its α-CH groups by nitrogen.[8] It has a sweetish odour and was first isolated from coal tar in 1834.[7] The quinoline moiety, which is found in clinical drugs such as chloroquine **7**, has been used as the basis for the synthesis of anti-malarial compounds. The traditional anti-malarial drug (alkaloid quinine), also used as a tonic, is another example of a compound containing a quinoline skeleton that exhibits pharmacological properties.[4]

Amongst the various classes of heterocyclic compounds, quinoxalines are an important component of pharmacologically active compounds.[9] Although quinoxaline derivatives contain two nitrogen atoms fused to a benzo ring and quinolines contain one nitrogen fused to a benzo ring, they have similarities as bioisosteres – which exhibit similar biological functions but modulate physical properties.[10].

1.2 Quinoxaline moiety and its derivatives

Quinoxaline **8** is a low melting solid which is miscible with water. It is considerably a weaker base (pKa 0.56) than its counterparts, the isomeric diazonapthalenes namely; cinnoline (pKa 2.42), pthalizine (pKa 3.47) and quinozaline (pKa 1.95).[11] Quinoxaline is an aromatic fused ring heterocycle, consisting of benzene and a pyrazine ring. It is commonly referred to as 1,4-benzodiazine, benzoparadiazine and phenpiazine and its numbering is shown as below (Fig. 1.2). The 2- and 3- positions are equivalent and are designated as α-positions.[12]

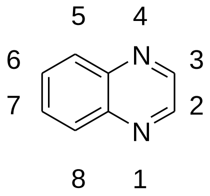


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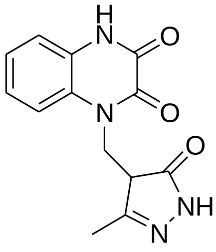
Figure 1.2: Numbering of quinoxaline

Quinoxaline derivatives have been reported in other applications outside medicinal chemistry, in organic optoelectronic applications and dye-sensitised solar cells.[13,14] The useful biological properties and numerous applications of quinoxaline derivatives, has led to considerable interest amongst both academia and industries. This dissertation will detail the applications of quinoxaline derivatives in a medicinal chemistry context.

1.3 Biological activity of quinoxalines

Quinoxaline derivatives possess extensive applications in medicinal chemistry due to their broad spectrum of biological activity.[15,16] A large number of synthetic quinoxalines have been reported to exhibit anti-diabetic, anti-HIV, anti-fungal, anti-parasitic, anti-TB and anti-cancer activities.[17] Changes in the substituent(s) attached to the quinoxaline moiety remarkably affect biological, chemical and physical properties of the compound.[18]

Quinoxaline derivatives like, quinoxaline-2-ones and quinoxaline-2,3-diones have been reported to exhibit anti-microbial, anti-pain and anti-inflammatory activities while pyrazoloquinoxalines are used as anti-fungal agents.[19,20] Ramalingam *et al*.,[21] synthesized some 1-substituted quinoxaline-2,3(1*H*,4*H*)-diones. All the compounds were evaluated for anti-microbial activity against the gram-positive and gram-negative bacteria, fungi and the *Mycobacterium tuberculosis* $H_{37}Rv$ species. The results against $H_{37}Rv$ showed that compounds **9**, **10** and **11** (Fig. 1.3) were the most effective, with minimum inhibitory concentrations (MIC_{99}) of 8.01, 8.56, and 8.93 µg/mL, respectively.



MIC_{99}-8.01

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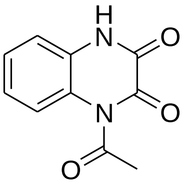

MIC_{99}-8.56

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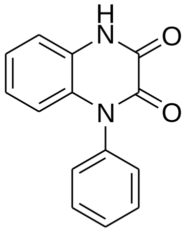

MIC_{99}-8.93

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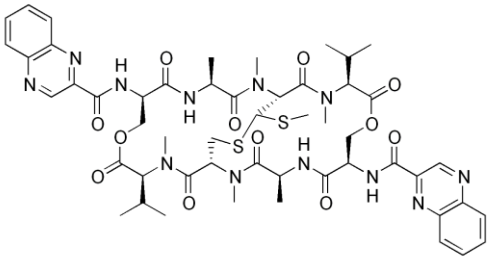
Figure 1.3: Examples of quinoxalinediones

Quinoxaline derivatives like Echinomycin **12** and Triostin A **13** (Fig. 1.4), which possess one or more quinoxalinyl residues, are used as antibiotics.[14] The chromophore moiety of quinoxaline-2-carboxylic acid plays an important biogenetic role in the synthesis of antibiotic Triostin A.[22] These compounds exhibit anti-microbial activity due to their DNA cleaving property.[19,23]



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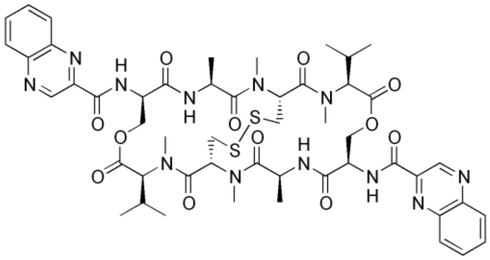

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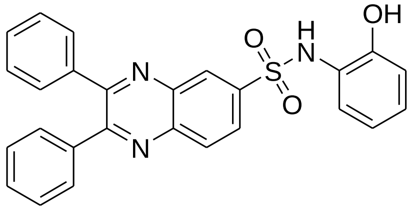
Figure 1.4: Antimicrobial compounds

Potey *et al.*,[24] prepared sulfonamide-substituted quinoxaline derivatives which were subjected to anti-microbial susceptibility testing against-gram positive (*Enterobacteria, S. aureus*) and gram-negative bacteria (*E. coli, P. vulgaries, Vibrio cholorie*). It was found that sulfonamide-quinoxaline **14** (Fig. 1.5) has pronounced effect against all gram-positive and gram-negative bacteria compared to 2,3-diphenylquinoxaline. The zone of inhibition (ZOI) for *S. aureus* and *E. coli*, were reported to be 19 mm and 22 mm, respectively. The combination of Piperacillin **15** and Tazobactum **16** (Fig. 1.5), on the other hand, show similar ZOI activity against the two test organisms; *E. coli* (10.0 mm – 16.0 mm) and *S. aureus* (21.0 mm – 31.0 mm).[25]



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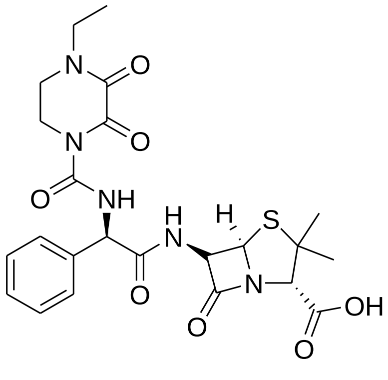

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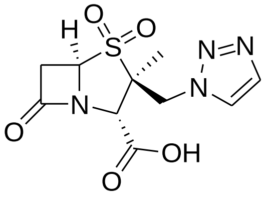

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Figure 1.5: Structure of compounds with similar ZOI

A study by Suter *et al.*,[26] showed the effect of quindoxin **17** on the synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein of different strains in *Escherichia coli* was examined under aerobic and anaerobic conditions (Fig. 1.6). The study revealed that all the strains were more resistant in the presence of oxygen with values of IC_{50} = 1.2-100 µg/mL. However, in the absence of oxygen the DNA synthesis was partially inhibited with values of IC_{50} = 0.05-1.2 µg/mL, without affecting the RNA and protein synthesis.

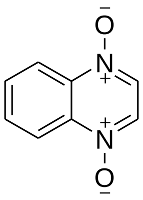

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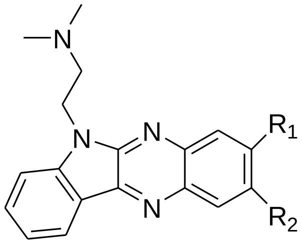
Figure 1.6: Structure of quindoxin

A study reported by Patel *et al.*,[27] in the early 1990s revealed that Elipticine analogues, 2,3-dimethyl-6-(2-dimethylaminoethyl)-6*H*-indolo-[2,3-*b*]quinoxaline **18a** and 6-(2-dimethylaminoethyl)-6*H*-indolo-[2,3-*b*]quinoxaline **18b** (Fig. 1.7), showed highest activity against the herpes virus in comparison to the Ellipticine **19** (Fig. 1.7). These quinoxaline derivatives showed improved DNA binding properties due to the 6-(2-dimethlaminoethyl) side chain.

A computational study prepared by Balasubramanian *et* al.,[28] has revealed the type of interaction that different quinoxaline analogues **20a**, **20b** and **20c** (Fig. 1.7) have on the Human Immunodeficiency Virus 1 (HIV-1). The type of interactions that these compounds exhibit, suggest that they are good inhibitors of HIV-1 integrase. Inhibition was based on glide score, glide energy and interaction with residues in the active site of the HIV integrase.



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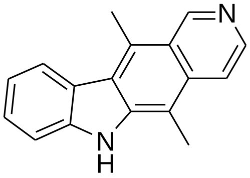

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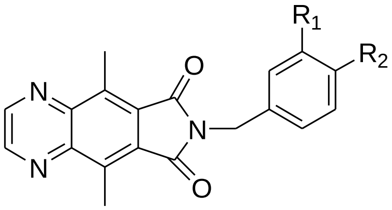

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18: R_1, R_2 = CH_3 **(a)**; R_1, R_2 = H **(b)**

20: R_1, R_2 = Cl **(a)**; R_1 = Cl, R_2 = H **(b)**; R_1 = OF_3, R_2 = H **(c)**

Figure 1.7: Compounds with anti-viral activity

Brimonidine (Alphagan) **21** (Fig. 1.8) is a quinoxaline based drug used for the treatment of glaucoma and it acts by reducing intraocular pressure, thus mitigate the symptoms of glaucoma.[29] This further qualifies quinoxaline containing compounds as important and versatile in its applications in medicinal chemistry.

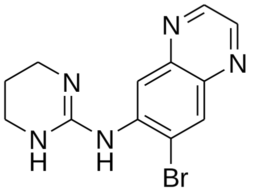

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Figure 1.8: Structure of Brimonidine

Quinoxaline acts as a core unit in a number of biologically active compounds and is also used as a pharmaceutically important moiety in the treatment of a wide variety of diseases. Due to the useful biological properties and numerous applications of quinoxaline derivatives, new and improved synthetic methods would be beneficial.

1.4 Synthetic quinoxaline derivatives

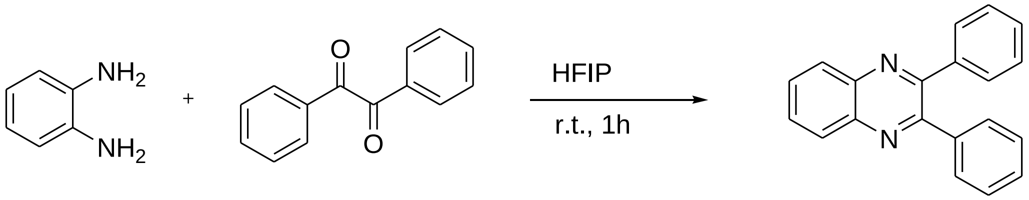
Various routes have been developed for the synthesis of quinoxalines and their derivatives, but the most common method for the preparation of these compounds relies on the condensation of an aryl 1,2-diamine with a 1,2-dicarbonyl compound.[18,30,31] Condensation, intramolecular cyclisation and modified C-H activation methods generally fall under the classical approach for the synthesis of quinoxaline derivatives. The non-conventional methods for the synthesis of quinoxaline derivatives rely on the use of transition metal cross-coupling reactions, the use of organo-lithium type bases and nucleophilic substitution.

1.4.1 Classical methods for the synthesis of quinoxaline derivatives

The classical method used for the synthesis of quinoxalines is applicable for the synthesis of mono-, di- and tri-substituted quinoxalines. The variations in these methods stem from the use of different catalysts, catalyst loadings and solvents, which remarkably affect the yields. Quinoxaline derivatives have been prepared by some of the following methods:

a) Condensation of an aryl 1,2-diamine with a 1,2-dicarbonyl compound

Khaksar *et al.*,[32] reported a method that involved the condensation of *o*-phenylenediamine **22** with benzil **23** in a solution of hexafluoroisopropanol (HFIP) at room temperature to afford 2,3-diphenylquinoxaline **24** in 95% yield (Scheme 1). HFIP is envisaged to activate the carbonyl group of **23** due to its polarity, high ionization power and strong hydrogen bond donation ability.[32] The advantage of this method is the short reaction time to yield **24** and it can be extended to the synthesis of the di- or tri-substituted quinoxalines. However, the limitation is that this method requires the use of different substrates with pre-defined groups to yield a variety of compounds.

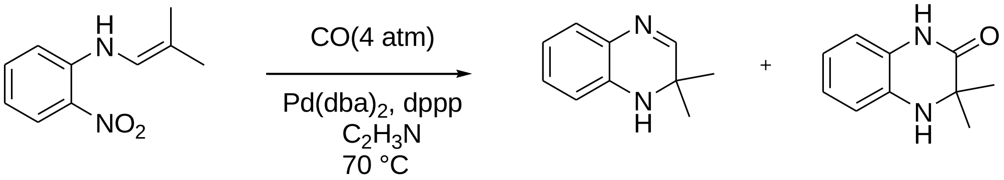


22 **23** **24**

Scheme 1: Synthesis of 2,3-diphenylquinoxaline in HFIP

b) Intramolecular cyclisation of *N*-substituted aromatic *o*-diamines:

Soderberg *et al*.,[33] reported the reaction of enamine **25** with carbon monoxide (4 atm) in the presence of bis(dibenzylideneacetone)palladium(0) (Pd(dba)$_2$) and 1,3-bis(diphenylphosphino)propane (dppp) catalyst complex in acetonitrile to afford 1,2-dihydroquinoxaline **26** and 3,4-dihydroquinoxalinone **27** in 71% and 11% yield, respectively (Scheme 2). The enamine was used as a substrate for reductive annulation for the synthesis of these quinoxaline derivatives. Treatment of **27** with phosphorous oxychloride (POCl$_3$), followed by reactions with amine nucleophiles yielded the 2-aminoquinoxalines.[34] The chemoselectivity in this case depends on the solvent that is used, which can affect the yield of the desired compound.[33]

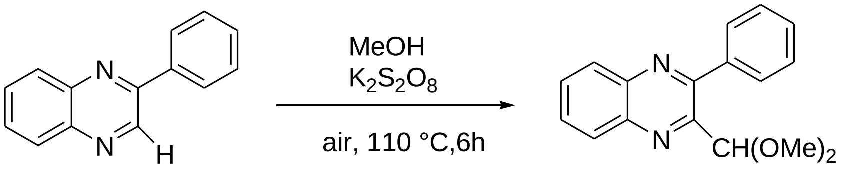


25 **26** **27**

Scheme 2: *N*-heteroannulation of enamines

c) Multibond cleavage and formation:

Liu *et al*.,[35] reported an efficient potassium persulfate($K_2S_2O_8$)-mediated C-C bond forming procedure involving 2-phenylquinoxaline **28** in anhydrous methanol under reflux to afford 2-(dimethoxymethyl)-3-phenylquinoxaline **29** in 72% yield (Scheme 3). The group was able to establish that all the three carbon atoms in the dimethoxymethyl group of the target acetals originated from methanol. The main drawback is that the reaction is done at high temperature.



28 **29**

Scheme 3: $K_2S_2O_8$-mediated synthesis of 2-quinaoxalinyl carbaldehyde dimethyl acetals

An alternative to the use of the more classical approach of synthesising mono- and di-substituted quinoxalines with pre-defined starting material is the use of C-C bond forming reactions. Non-conventional methods for the synthesis of substituted quinoxalines, which make use of transition metals to effect C-C or C-heteroatom bond formation, have also been described in literature. These methods are briefly described below.

1.4.2 Metal mediated methods for the synthesis of substituted quinoxalines

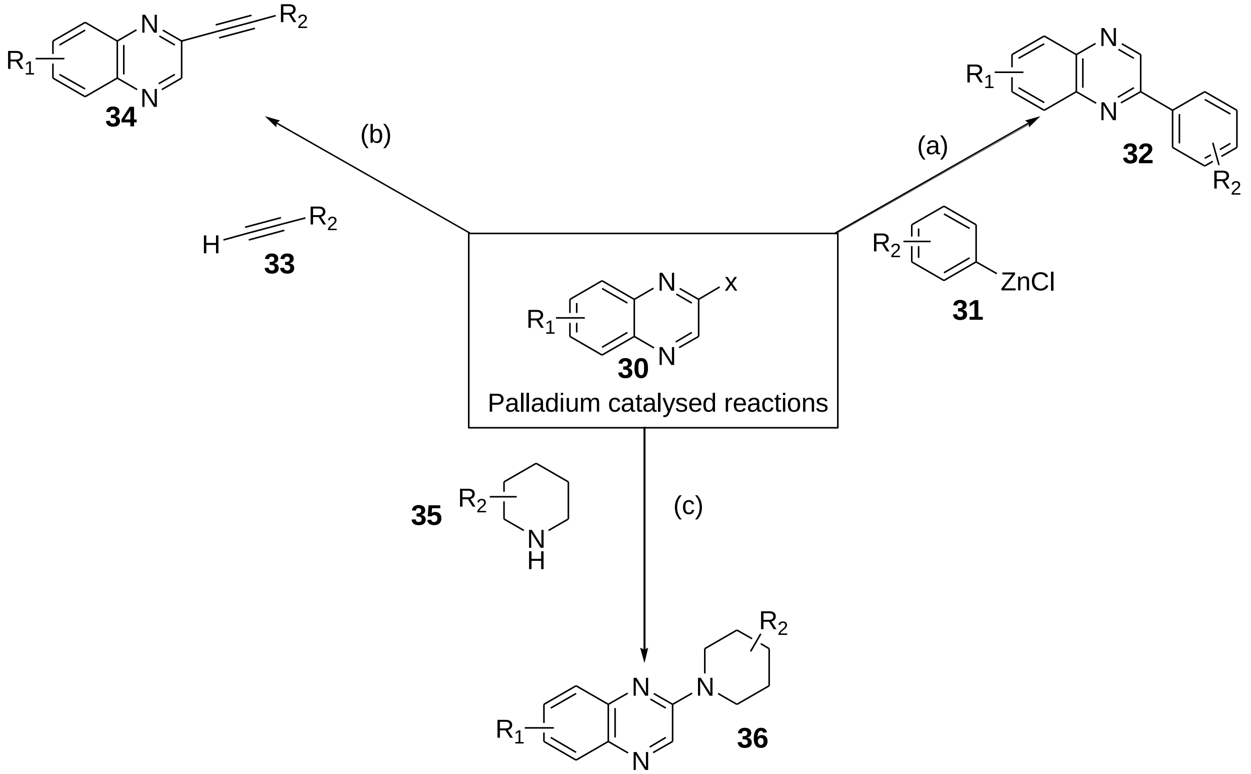
Examples of Pd-catalysed cross-coupling reactions include Kumada, Negishi, Sonogashira, Suzuki, Stille reactions to form Csp^2-Csp^2, Csp^2-Csp and Csp^2-Csp^3 bonds, while the Buchwald-Hartwig reaction effects Csp^2-nitrogen bonds.[36-38] Cross-coupling reactions have grown into powerful strategies for forming C–C, and C–heteroatom bonds.

The most common leaving groups used in palladium catalysed cross-coupling reactions are halogens (I>Br>Cl) or triflates. The relative reactivity of the most common leaving groups has been estimated to substituted benzoates< halides < sulfonates <

perfluoroalkane sulfonates.[39] Ideal leaving groups are the chlorides due to their availability and cost effectiveness, but they are less reactive than the bromides and iodides due to different bond dissociation energies. Iodides and bromides are highly reactive, but they are not economical in terms of scale and triflates are very expensive.[40] A cheaper, yet efficient alternative is the use of aryl-*o*-sulfonates or alkyl-*o*-sulfonates. Some of the advantages of using aryl-*o*-sulfonates are:[41-43]

i. They generate high to excellent yields
ii. A wide spectrum of substrates can be used for the Pd-catalysed cross-coupling reactions.

In this work, attention is focused on Negishi, Sonogashira and Buchwald-Hartwig amination, designated as (a), (b) and (c), respectively (Scheme 4).



$R_1 = R_2 =$ Aryl, alkyl, akenyl, heteroycle

X = Cl, Br, I, OTf, OSO_2Ph

Scheme 4: Palladium-catalysed cross-coupling reactions on quinoxaline

(a) Negishi cross-coupling reaction

Negishi cross-coupling reaction was first pioneered by Negishi *et al*., in 1977.[44] It is a palladium- or nickel-catalysed cross-coupling reaction involving the use of an organozinc reagent as the nucleophilic component, to form carbon-carbon bonds in the process.[45,46] Negishi coupling is often associated with air and moisture sensitivity but Knochel *et al*. reported a method to synthesise solid and stable organozinc reagents, thus overcoming this issue.[47]

(b) Sonogashira cross-coupling reaction

Kenkichi Sonogashira discovered the Sonogashira coupling reaction in 1975.[48] It is a cross-coupling reaction used to form carbon-carbon bonds. It uses a palladium catalyst and normally requires a co-catalyst, copper iodide (CuI), which plays a significant role in the transmetallation step.[49]

The traditional reactants in the Sonogashira coupling are vinyl or aryl halides and terminal alkynes.[50] The Sonogashira reaction is used in the synthesis of various organic compounds and in the production of pharmaceuticals, agricultural chemicals, and natural products.[51] Many factors such as; leaving group, solvent, base and catalyst used influence the outcomes of Sonogashira cross-coupling reaction.[52]

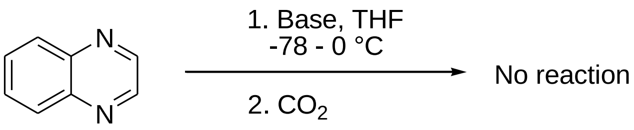
(c) Buchwald-Hartwig coupling reaction

In 1995 Buchwald and Hartwig independently reported the palladium catalysed coupling of aryl halides with amine nucleophiles in the presence of stoichiometric amounts of base.[53, 54] The Buchwald-Hartwig amination is a cross-coupling reaction of an aryl halide with an amine using palladium as a catalyst and a strong base to make carbon-nitrogen bonds (C-N).[55] Buchwald-Hartwig hetero cross-coupling reactions have been successful in the use of Pd(0)-catalysts by using suitable ligand based palladium complexes, preferably with bis-phosphine ligands.[56, 57]

The use of Palladium-catalysed cross-coupling reactions for the functionalisation of quinoxaline on the second position allows the opportunity for further exploration of other methods for functionalisation of the 3-position. The synthesis of quinoxaline derivatives, by deprotonation of acidic protons followed by the reaction with electrophiles, is described in detail below.

1.4.3 Substitution on quinoxaline framework by electrophiles

In a study reported by Harms, for the synthesis of 2-quinoxalinecarboxylic acid, various methods were investigated.[58] One method in particular was the attempted deprotonation of quinoxaline **37** with a variety of bases such as lithium diisopropylamide (LDA), potassium (KHMDS) and lithium bis-(trimethylsilyl)amide (LHMDS), which failed to yield desired results (Scheme 5).



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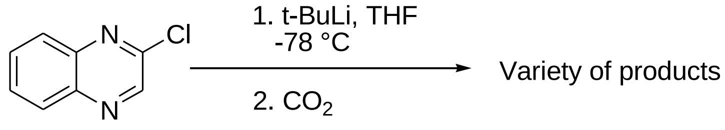
Base = LDA, KHMDS, LHMDS

Scheme 5: Deprotonation of quinoxaline

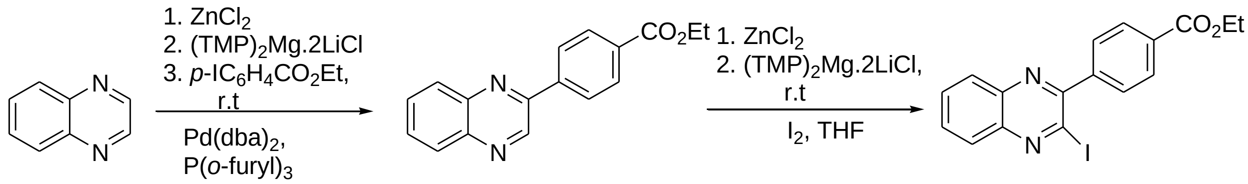
In a similar approach, 2-chloroquinoxaline **38** was subjected to conditions of lithium-halogen exchange. Under these conditions, the starting material was consumed with no detection of the desired quinoxalinecarboxylic acid, instead a wide variety of products were isolated (Scheme 6).[58] The main drawback of using organolithium or Grignard reagents is due to their high nucleophilicity and basicity, which cannot tolerate various functional groups in the substrates.



38

Scheme 6: Deprotonation of 2-chloroquinoxaline

Dong *et al*.,[59] reported a direct method for the deprotonation and functionalisation of sensitive aromatic and heteroaromatic substrates by using $(TMP)_2Mg.2LiCl$ (TMP = 2,2,6,6-tetramethylpiperamidyl) in the presence of zinc chloride ($ZnCl_2$) at room temperature. Direct metalation of quinoxaline **37** with $(TMP)_2Zn.2MgCl_2.2LiCl$, followed by reaction with ethyl 4-iodobenzoate using $[Pd(dba)_2]$ and $P(o\text{-furyl})_3$ as a catalyat complex, afforded the functionalised quinoxaline **39** in 82% yield. A subsequent zincation of **39** followed by the reaction with iodine afforded iodide **40** in 73% overall yield (Scheme 7). This transformation resulted in double functionalisation of quinoxaline **37**.[59] The drawback of using this type of magnesium base is the use of stoichiometric amounts of LiCl, MgCl and $ZnCl_2$ needed to synthesise them. This makes the use of these bases impractical when considering economies of scale.



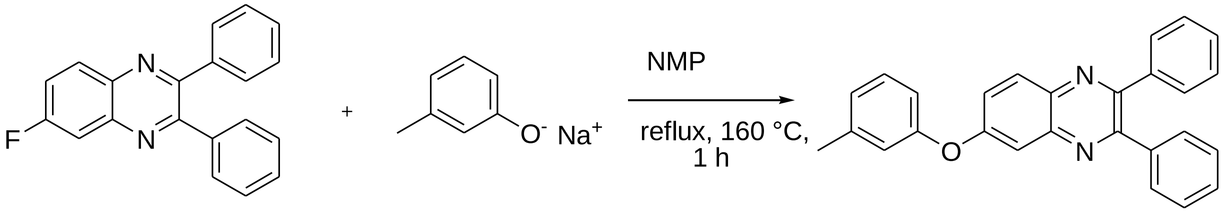
37 **39** **40**

Scheme 7: Double functionalisation of quinoxaline

Due to the difficulties in deprotonation of the α-positions on the quinoxaline moiety, an alternative method for further functionalisation of quinoxaline derivatives without the use of pre-defined starting material is described in literature. Alternatively, the reaction of C-nucleophiles with quinoxaline and its derivatives is described in detail below.

1.4.4 Nucleophilic substitution on quinoxaline framework

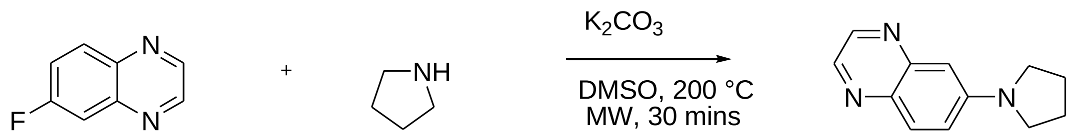
Hedrick *et al*.,[60] reported a general method for the preparation of aryl ether-phenylquinoxalines. The reaction between 2,3-diphenyl-6-fluoroquinoxaline **41** and sodium *m*-methylphenolate **42** was carried out in *N*-methyl-2-pyrrolidone (NMP), under reflux, at 160 °C for 1 hour (Scheme 8). The reaction afforded 6-(3-methylphenoxy)-2,3-diphenylquinoxaline **43** in 97% yield. These researchers found that the electron-deficient pyrazine of the quinoxaline ring system activated the 6-fluoro substituent toward nucleophilic aromatic substitution. The main advantage of this method is the short reaction time but these type of reactions are done at high temperatures.[61]



41 **42** **43**

Scheme 8: Nucleophilic substitution on flouro-substituted-quinoxaline

A similar method to that of Hedrick *et al.* was reported by Zhang *et al*.[62] With the aid of microwave irradiation (MW), 6-fluoroquinoxaline **44** was reacted with pyrrolidine **45** in dimethylsulfoxide (DMSO) in the presence of potassium carbonate(K_2CO_3) at 200 °C to afford 6-(1-pyrrolidinyl)-quinoxaline **46** in 93% yield (Scheme 9). The use of MW shortens the reaction time and can potentially improve yields. Although this method is efficient, it involves high temperatures.

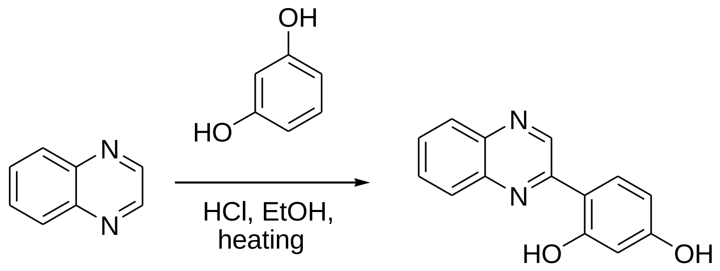


44 **45** **46**

Scheme 9: Nucleophilic substitution on 6-fluoroquinoxaline

The method reported by Azev *et al*.,[63] which involves heating of quinoxaline **37** with resorcinol **47** in ethanol (EtOH) in the presence of acid, afforded a resorcinol derivative, 2-(2,4-dihydroxyphenyl)quinoxaline **48** in 25% (Scheme 10). The reaction of quinoxaline with this nucleophile continues to oxidation of the compound to an aromatic substitution product without the need of having an oxidizing agent. The drawback is the use of a concentrated acid.

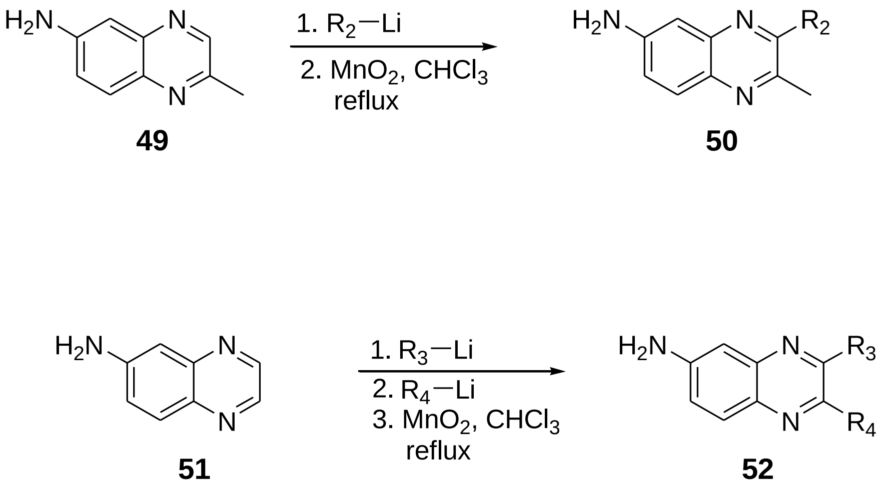
47



37 **48**

Scheme 10: Synthesis of 2-(2,4-dihydroxyphenyl)quinoxaline

Hui *et al*.,[64] reported the synthesis of quinoxaline derivatives from alkyl lithium bases and phenyl lithium by nucleophilic substitution (Scheme 11). 2-Methylquinoxalin-6-amine **49** and quinoxalin-6-amine **50** were treated with various nucleophiles in chloroform ($CHCl_3$) under reflux and then oxidized with manganese dioxide (MnO_2) to afford substituted-quinoxalines **51** and **52** in low to high yields. Organolithium reagents are highly basic and nucleophilic and can deprotonate $–NH_2$ or $–CH_3$, but do not in these examples. It would be interesting to try and expand the scope of nucleophilic substitution on quinoxaline derivatives by introducing alkynyl, amine and heterocyclic groups.



50	
R_2	% Yield
- Ph	75
-$(CH_2)_5$-Me	65
- Bu	75
- sBu	65
- tBu	25

52		
R_3	R_4	% Yield
-$(CH_2)_5$-Me	- Bu	35
- Bu	-$(CH_2)_5$-Me	30
- Bu	- Ph	30

Scheme 11: Nucleophilic substitution using organo-lithiated compounds

These methods deal with the reactivity of quinoxalines to afford substituted derivatives. Although efficient some of these methods are not compatible with some functional groups, and this led to the need for the development or modification of efficient methods for the synthesis of these compounds.

1.5 Purpose of current study

We are mainly interested in the synthesis of di- and tri-substituted quinoxalines. This interest was prompted by a literature search (May 2014) of which there was an overwhelming report of mono-substituted quinoxaline derivatives than di- and tri-substituted quinoxaline derivatives. In this dissertation metal-catalysed cross-coupling reactions are used to synthesise 2-mono-substituted quinoxaline and 2,6-disubstituted quinoxalines, in order to further selectively functionalise the 3-position. This is achieved by avoiding the use of pre-defined starting materials, since appropriately substituted *o*-phenylenediamines and 1,2-dicarbonyl compounds are not always readily available. Negishi coupling is used to introduce non-reactive aryl- and alkyl-groups while Sonogashira coupling will be used to introduce a non-reactive arylalkynyl-group. Buchwald-Hartwig amination is useful for the introduction of Csp^2-nitrogen bonds (arylamine-groups).

Thus, this study proposes convenient and cost-effective synthetic designs of incorporating substituents on the 3- and 6-position on the quinoxaline moiety.

1.5.1 Aim

The aim of this study is to apply/modify various synthetic procedures for preparing mono-, di- and tri-substituted quinoxalines and evaluate their biological activity.

1.5.2 Objectives

The objectives of the study will be to:

i. modify methods for the synthesis of mono-, di- and tri-substituted quinoxalines, which may include the use of Negishi coupling, Buchwald-Hartwig coupling and Sonogashira coupling reactions,

ii. test the synthesised compounds for biological activity against Tuberculosis and Malaria and do cytotoxicity studies.

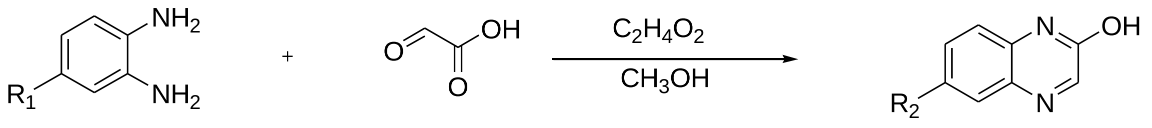
CHAPTER

2

Chapter 2: Results and Discussion

2.1 Synthesis of quinoxalinone derivatives

We started by first preparing quinoxalin-2-ol **55a**, through a condensation reaction of *o*-phenylenediamine substituted derivative **53** and glyoxylic acid **54** in acetic acid and methanol, at room temperature (Scheme 12). Following recrystallisation of the crude product in *N,N*-dimethylformamide (DMF), the desired product was obtained in 60% yield. Spectroscopic data of the prepared compound compare favorably with the literature data.[43]



53 **54** **55**

53: R_1 = H **(a)**; R_1 = NO_2 **(b)**; R_1 = Cl **(c)**

Compound	R_2	% Yield	m.p °C (Lit.^{ref})
55a	6-H	60	265-267 (266-267[42])
55b	6-NO_2	74	301-302 (298-302[83])
55c	6-Cl	58	-

Scheme 12: Synthesis of quinoxalin-2-ol derivatives

Compound **55a** was recovered as a tan solid which was characterised by ^1H and ^{13}C NMR spectroscopy in DMSO-d_6. The ^1H NMR spectrum showed a total of 6 protons with a characteristic singlet at δ 8.16 ppm, which corresponds to a –N=C-H group. The 4 remaining protons were attributed to the benzene rings with peaks in the region δ 7.77–7.25 ppm. The ^{13}C NMR spectrum showed a total number of 8 carbons.

We then synthesised 6-nitroquinoxalin-2-ol **55b** and 6-chloroquinoxalin-2-ol **55c** using a similar method as with **55a** (Scheme 12). The target compound **55b** was obtained as a light brown solid in 74% yield, following recrystallisation of the crude product in DMF. The ^{1}H NMR spectrum showed a total of 4 protons with a characteristic singlet at δ 8.32 ppm, which corresponds to a –N=C-H group. The absence of one proton indicated that there is a $–NO_2$ substituent.

Compound **55c** was obtained as a purple solid in 58% yield, and characterised by ^{1}H and ^{13}C NMR spectroscopy in $CDCl_3$. The ^{1}H NMR spectrum showed a total of 5 protons with an –OH peak at δ 11.48 ppm and a characteristic singlet at δ 8.25 ppm for –N=C-H. There was also an appearance of another singlet at δ 8.30 with a ratio of 1 : 0.5 to the peak at δ 8.25 ppm. The spectrum showed an apparent doublet in the region δ 7.75 – 7.74 ppm integrating for 1 proton, but there was also another doublet in the region δ 7.84 – 7.83 ppm with a ratio of 1 : 0.4 to the peak at 8.25 ppm. A multiplet in the region δ 7.28 – 7.26 ppm integrating for 2 protons was observed with another adjacent multiplet in the region δ 7.48 – 7.45 ppm with a ratio of 1 : 0.5 to the peak at δ 8.25 ppm. The appearance of additional minor peaks suggests that there is a presence of the isomer 7-chloroquinoxalin-2-ol. The ^{13}C NMR spectrum showed a total of 8 carbons. The reaction was repeated and readily prepared in gram quantities.

2.2 Preparation of 2-benzenesulfonyloxyquinoxaline derivatives

The title compounds were prepared following literature method, which involves the treatment of **55a**, **55b** and **55c** with benzenesulfonylchloride, in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine (Et_3N) in dichloromethane (DCM).[43] The reaction mixture was cooled to 0 °C and left to stir for 1 hour while being monitored by TLC. Upon completion of the reaction, it was quenched with saturated sodium hydrogen carbonate ($NaHCO_3$) and the crude product purified by column chromatography on silica gel. The synthesis of 2-benzenesulfonyloxyquinoxaline derivatives was achieved by the method shown in Scheme 13.

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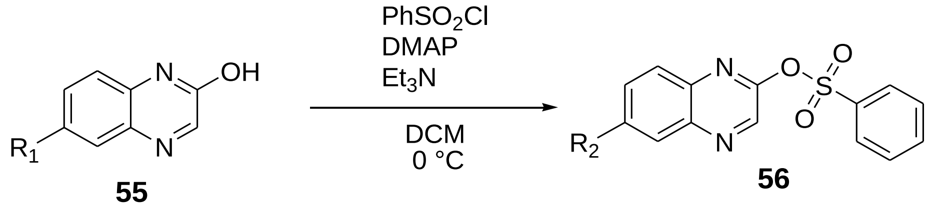


55: R_1 = H **(a)**; R_1 = NO_2 **(b)**; R_1 = Cl **(c)**

Compound	R_2	% Yield	m.p °C (Lit.^{ref})
56a	6-H	85	89-93 (91[43])
56b	6-NO_2	66	-
56c	6-Cl	74	-

Scheme 13: Synthesis of 2-benzenesulfonyloxyquinoxaline derivatives

The target compound 2-benzenesulfonyloxyquinoxaline **56a** was obtained as a brown solid in 85% yield with the melting point of 89-93 °C. Compound **56a** was analysed by ^{1}H NMR and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum showed a total of 10 protons, with a characteristic singlet at δ 8.67 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 was assigned to the benzene ring. Two multiplets in the region δ 7.69 – 7.77 ppm and δ 7.58 – 7.63 ppm, integrating for 2 and 3 protons respectively, were observed. The ^{13}C NMR spectrum showed the presence of 12 carbons instead of 14 carbons, which indicates that there are equivalent carbons in the aromatic ring. All the peaks in the aromatic region were accounted for and there was an indication that there was an addition of a phenyl ring to the quinoxaline. All the peaks obtained were consistent with those reported in literature.[43]

We then synthesised 6-nitro-2-benzenesulfonyloxyquinoxaline **56b** in 66% yield (Scheme 13). Compound **56b** was difficult to purify by column chromatography even when the mobile phase was changed. The compound was recrystallised from petroleum ether and n-hexane but still the compound was not clean enough.

The ^{1}H NMR spectrum showed two singlets at δ 8.80 ppm and δ 8.78 ppm, both integrating for 1 proton. There was a doublet of doublets in the region δ 8.53 – 8.52 ppm integrating for 1 proton. A doublet was observed in the region δ 8.28 – 8.26 ppm integrating for 1 proton. Two multiplets were observed in the region δ 8.04 – 8.03 ppm and δ 7.73 – 7.77 ppm, integrating for 2 and 3 protons, respectively. There was also evidence of an excess of benzenesulfonyl protons which were observed as an apparent triplet in the region δ 8.22 – 8.21 ppm and as a doublet in the region δ 8.19 – 8.17 ppm. A multiplet in the region δ 7.65 – 7.74 ppm was also observed. This suggests that the substrate was not completely consumed. Despite the difficulty of purifying compound **56b**, the compound was subjected to subsequent reactions that will be outlined in this section.

The compound 6-chloro-2-benzenesulfonyloxyquinoxaline **56c** was synthesised in 74% yield (Scheme 13). Compound **56c** was also difficult to purify due to the presence of a small quantity of the isomeric 7-chloro-2-benzenesulfonyloxyquinoxaline.

The ^{1}H NMR spectrum showed a singlet at δ 8.67 ppm integrating for 1 proton, which was attributed to a –N=C-H group but there was also an adjacent singlet at δ 8.64 ppm with a ratio of 1 : 0.5 to the peak at δ 8.67 ppm. There was a doublet in the region δ 8.13 – 8.14 ppm integrating for 1 proton but there was also a multiplet in the region δ 8.18 – 8.17 ppm with a ratio of 1:0.5 to the peak at δ 8.67 ppm. A doublet in the region δ 8.05 – 8.03 ppm was observed with a ratio of 1:0.6 to the peak at δ 8.67 ppm. There was also evidence of a doublet of doublets in the region δ 7.84 – 7.81 ppm integrating for 1 proton but also an apparent doublet in the region δ 7.87 – 7.86 ppm was observed with a ratio of 1:0.5 to the peak at δ 8.67 ppm. A multiplet in the region δ 7.73 – 7.71 ppm and δ 7.65 – 7.59 ppm, integrating for 2 and 3 protons respectively, was observed. There was a multiplet in the region δ 7.76 – 7.74 ppm with a ratio of 1:0.5 to the peak at δ 8.67 ppm.

56c **57**

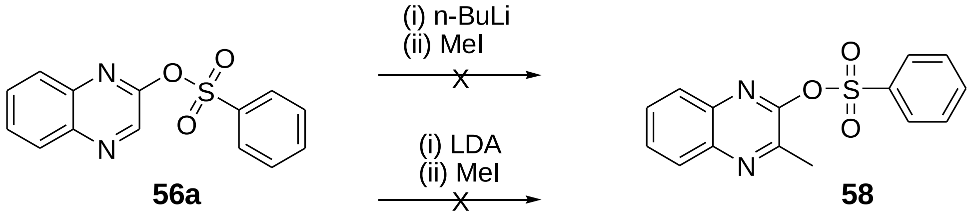
Figure 2.1: Structure of chloro-benzenesulfonyloxyquinoxaline positional isomers

The appearance of these minor peaks further suggested the presence of an isomer. In order to verify the structure of the major product, ^{15}N – ^{1}H Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy studies were done. There was a spot at δ 8.67 ppm indicating ^{2}J coupling between 4a and 3a. There was also a spot at 8.10 ppm indicating ^{3}J coupling between 1a and 8a. This peak appeared as a doublet with J = 2.4 Hz. ^{3}J coupling between 4a and 5a was not observed, due to the magnetic properties of the chloro-substituent which has influence on 5a for compound **56c**. There was neither any observation of ^{3}J coupling between 4b and 5b nor ^{2}J coupling between 4b and 3b for compound **57**. From the information gathered from ^{15}N – ^{1}H HMBC spectroscopy, we were confident that the major product came from the 6-chloro-2-benzenesulfonyloxyquinoxaline **56c.**

2.3 Synthesis of mono- and di-substituted quinoxaline derivatives

We wanted to determine the influence that the benzene-*O*-sulfonate group has on the acidic proton on the –N=C-H group. 2-Benzenesulfonyloxyquinoxaline **56a** required deprotonation by a strong base and our first choice was the lithium bases. Previous work done by Harms employed lithiumdiisopropylamide (LDA), n-butyllithium (n-BuLi), potassium bis-(trimethylsilyl)amide and lithium bis-(trimethylsilyl)amide.[58] Our attention was drawn by the attempt to use these bases on unsubstituted quinoxaline **37** and 2-chloroquinoxaline **38**. But, the use of lithium bases failed to yield any positive results.

Treatment of 2-benzenesulfonyloxyquinoxaline **56a** with n-BuLi followed by quenching with methyl iodide (MeI) did not yield the desired product (Scheme 14). Based on the crude ^{1}H-NMR spectrum, a mixture of compounds was detected but could not be separated. We then attempted to synthesise the desired product by treating **56a** with LDA and quenching with MeI, but we recovered the starting material unchanged (Scheme 14).

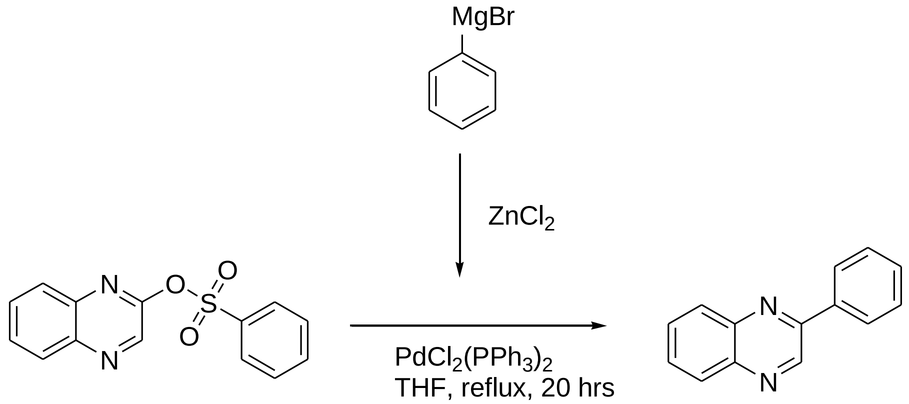


Scheme 14: Attempt to synthesise 2-methylquinoxalin-3-yl benzenesulfonate

The 2-benzenesulfonyloxyquinoxaline **56a** was then investigated as a potential coupling partner to undergo Negishi coupling reaction as described in the next section.

2.3.1 Negishi coupling of quinoxaline-*o*-sulfonate

We synthesised 2-phenylquinoxaline **59** *via* the preparation of organozinc reagent using direct magnesium insertion. Phenylmagnesium bromide (PhMgBr) was prepared using a similar method reported in literature.[65] Phenylmagnesium bromide was added to a solution of zinc chloride (ZnCl) and left to stir for 1 hour. The Zn-substrate and benzenesulfonyloxyquinoxaline **56a** were subjected to Negishi coupling conditions in THF, using 5% $PdCl_2(PPh_3)_2$ as a catalyst source (Scheme 15). After 20 hours the reaction was quenched with sat. $NaHCO_3$ workup and purified by flash column chromatography on silica gel to afford compound **59** as an orange solid with a melting point of 73-74 °C in 75% yield. The compound **59** was analysed with NMR and mass spectroscopy (MS) (Fig. 2.2).

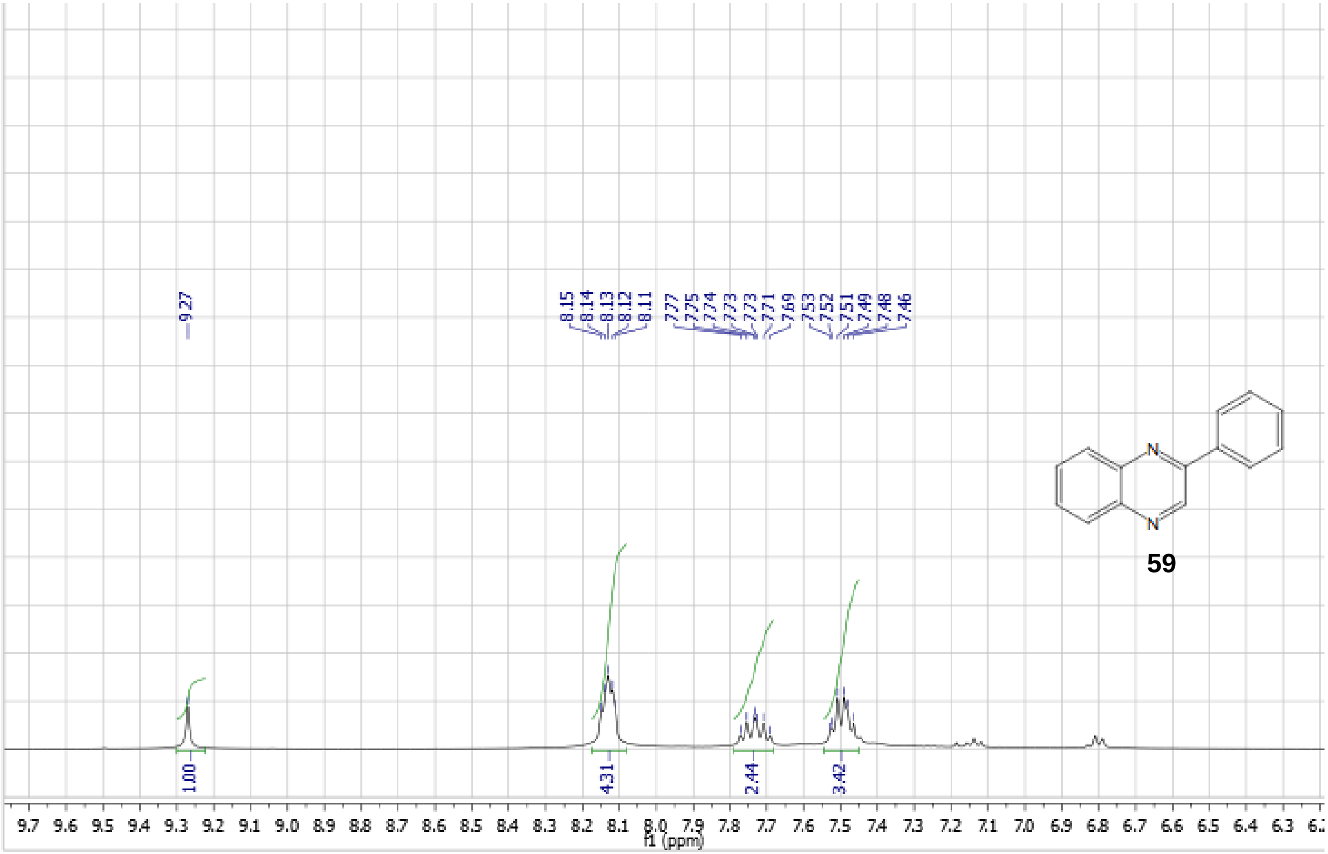


56a

59

Scheme 15: Synthesis of 2-phenylquinoxaline

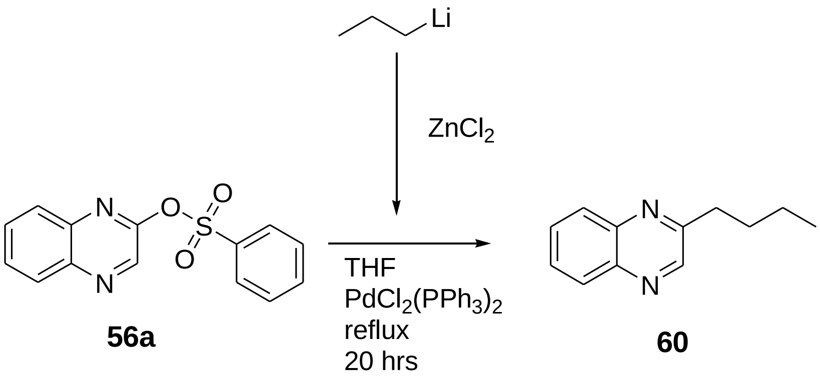
The ^{1}H NMR spectrum of **59** showed a total of 10 protons with a characteristic singlet at δ 9.27 ppm, integrating for 1 proton, which corresponds to a –N=C-H group. Multiplets were observed in the region δ 8.12 – 8.15 ppm and δ 7.69 – 7.77 ppm, integrating for 4 and 2 protons, respectively. Another multiplet integrating for 3 protons was observed in the region δ 7.46 – 7.52 ppm. The low resolution mass spectrum revealed the presence of a molecular ion peak at *m/z* 207.10 (M+1), consistent with the calculated value of *m/z* $C_{14}H_{10}N_2^+$ (207.10). All the peaks obtained were consistent with those reported in literature.[66]



59

Figure 2.2: ^{1}H NMR spectrum of 2-phenylquinoxaline

We synthesised 2-butylquinoxaline **60** by Negishi coupling on the 2-benzenesulfonyloxyquinoxaline **56a** using n-BuLi (2.5 M solution in hexane). Zincyl-butyl was prepared from n-BuLi by transmetallation reaction with $ZnCl_2$ at -78 °C. The Zn-substrate and **56a** were subjected to Negishi coupling conditions in THF, using 5% $PdCl_2(PPh_3)_2$ as a catalyst source. The solution was refluxed, under nitrogen, for 20 hours. The reaction was quenched with sat. $NaHCO_3$ workup and purified by column chromatography on silica gel to afford compound **60** as orange oil in 61% yield. One fraction was isolated and further analysed (Scheme 16).



56a **60**

Scheme 16: Synthesis of 2-butylquinoxaline

The ^{1}H NMR spectrum showed a singlet more downfield at δ 8.75 ppm integrating for 1 proton. There was an absence of 5 protons, in the aromatic region. There was an appearance of a triplet at δ 3.02 ppm integrating for 2 protons, and an appearance of multiplets in the region δ 1.79 – 1.86 ppm and δ 1.44 – 1.49 ppm both integrating for 2 protons. In addition, there was an appearance of a triplet at δ 1.00 ppm integrating for 3 protons. This gave a total number of 14 protons. The ^{13}C NMR spectrum showed a total number of 11 carbons signifying overlapping of carbons at the same chemical shift.

The low resolution mass spectrum showed a molecular ion peak at *m/z* 188.12 (M+1) which was consistent with the calculated value of *m/z* $C_{12}H_{14}N_2^+$ (188.12). The NMR and MS data was in agreement with previously reported data in literature.[67] Nucleophilic substitution on compounds **59** and **60** is described in detail in the next section.

2.3.2 Nucleophilic substitution on Negishi cross-coupled quinoxaline

2-Phenylquinoxaline **59** was treated with n-BuLi at -78 °C and quenched with iodine (I) which gave two fractions after purification by column chromatography and they were characterised by ^{1}H NMR spectroscopy. The first fraction that was collected was found to be the starting material **59**. The second fraction appeared to be 3-butyl-2-phenylquinoxaline **59a** due to the absence of the singlet at 9.27 ppm and the appearance of additional peaks at the upfield region. Using this method, **59a** was isolated in 12% yield with no evidence to suggest the formation of the expected compound, 2-iodo-3-phenylquinoxaline. We came to the conclusion that n-BuLi acted as a nucleophile instead of acting as a base. With that in mind, we decided to try to improve the percentage yield of **59a**.

The number of equivalents (eq) of n-BuLi was increased from 0.5 eq to 1.5 eq. Thus, 2-phenylquinoxaline **59a** was treated with n-BuLi (1.5 eq.) and left to stir for 18 hours at room temperature (Scheme 17). After work-up with sat. $NaHCO_3$ and purification on column chromatography, 3-butyl-2-phenylquinoxaline **59a** was isolated as tan oil in 66% yield. The compound was characterised by NMR and MS (Fig. 2.3).

The ^{1}H NMR spectrum showed the absence of the singlet at δ 9.27 ppm and the presence of additional peaks in the aliphatic region. The 9 protons on the aromatic ring in the region δ 7.44 – 8.06 ppm were accounted for and there was an appearance of a triplet in the region δ 2.96 – 2.99 ppm, integrating for 2 protons. Other multiplets were observed in the region δ 1.62 – 1.65 ppm and δ 1.18 – 1.27 ppm both integrating for 2 protons. There was also an appearance of a triplet in the region δ 0.75 – 0.79 ppm integrating for 3 protons.

The ^{13}C NMR spectrum showed a total of 16 carbons which suggests that there is an overlap of equivalent carbon peaks. The low resolution mass spectrum showed a molecular ion peak at *m/z* 263.15 (M+1), consistent with the calculated value of *m/z* $C_{17}H_{16}N_2^+$ (263.15) and the data reported in literature.[68]

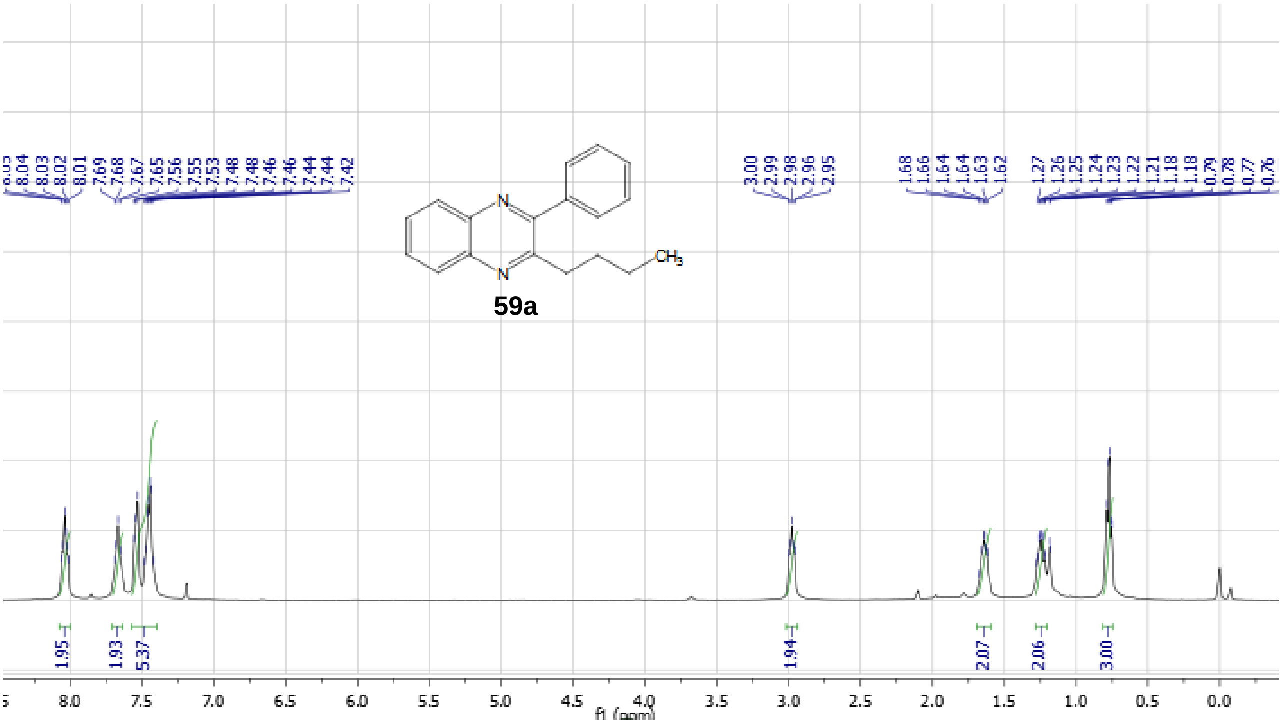
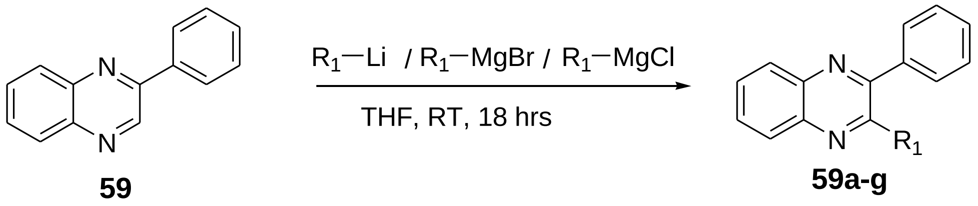


Figure 2.3: ^{1}H NMR spectrum of 3-butyl-2-phenylquinoxaline



Compound	R$_1$	% Yield	m.p °C (Lit.^{ref})
59a	-Butyl	66	-
59b	-Isopropyl	54	96-98 (98-99[69])
59c	-Phenyl	22	126-128 (127-129[70])
59d	-2-Phenylethynyl	47	110-112 (109-112[71])
59e	-Oct-1-ynyl	14	-
59f	-Thiophen-2-yl	22	126-128 (128[72])
59g	-furan-2-yl	46	102-103 (103-104[73])

Scheme 17: General procedure for di-substitution on 2-phenylquinoxaline

We tried deprotonation on 2-phenylquinoxaline **59** using a weaker base than n-BuLi. We used LDA and isopropyl-magnesium chloride (*i*PrMgCl) on 2-phenylquinoxaline **59**, following a similar approach as in Scheme 17. We used LDA because dialkyl amides act solely as a base. The use of *i*PrMgCl was motivated by its dual nature of acting as a base and a nucleophile. The reaction between **59** and LDA was unsuccessful, only the starting material **59** was isolated. But, when **59** was treated with *i*PrMgCl and quenched with iodine, two spots appeared on the TLC. After purification on column chromatography, the two fractions were characterised using ^{1}H NMR spectroscopy. The first fraction was the starting material **59** and the second fraction was attributed to 3-isopropyl-2-phenylquinoxaline **59b** which was isolated in 7% yield. These results suggest that the Grignard reagent is reactive toward electrophiles but not basic enough for deprotonation.

We then tried to improve the percentage yield of **59b** by increasing the number of equivalents of the Grignard reagent from 0.5 eq to 1.5 eq. The reaction was left to stir for 18 hours at room temperature (Scheme 17). After aqueous work-up and purification on column chromatography, 3-isopropyl-2-phenylquinoxaline **59b** was isolated as yellowish solid in 54% yield and melting point of 96-98 °C.

The ^{1}H NMR spectrum showed a total of 16 protons, in which the characteristic singlet at δ 9.27 ppm was no longer evident. There was a presence of new peaks in the upfield region where there was a multiplet in the region δ 3.39 – 3.46 ppm integrating for 1 proton and a doublet in the region δ 1.17 – 1.25 ppm integrating for 6 protons.

The ^{13}C NMR spectrum showed a total of 14 carbons which suggests that there is an overlap of equivalent carbon peaks. The low resolution mass spectrum gave a molecular ion peak at *m/z* 249.13 (M+1), consistent with the calculated value of *m/z* $C_{17}H_{16}N_2^+$ (249.13) and the data reported in literature.[69]

The successful synthesis of compounds 3-butyl-2-phenylquinoxaline **59a** and 3-isopropyl-2-phenylquinoxaline **59b** prompted us to further explore other types of nucleophiles that can be added on the third position on 2-phenylquinoxaline **59**. All the synthesised compounds **59a-g** were characterised by ^{1}H NMR, ^{13}C NMR and MS.

2-Phenylquinoxaline **59** was treated with 1.5 eq phenyl-MgBr (1.14M solution in THF) in THF following Scheme 17. After work-up and purification on column chromatography, 2,3-diphenylquinoxaline **59c** was isolated as orange solid in 22% yield and a melting point of 126-128 °C. Attempts to try and improve the percentage yield of 2,3-diphenylquinoxaline **59c** proved to be unsuccessful. When the reaction was run at higher temperatures the percentage yield decreased significantly. Compound **59c** was characterised by NMR spectroscopy and low resolution MS.

The absence of the characteristic singlet at δ 9.27 ppm confirmed that nucleophilic substitution on the third position was successful. There were two multiplets in the region δ 8.05 – 8.13 ppm and δ 7.62 – 7.73 ppm both integrating for 2 protons. There was also an apparent doublet in the region δ 7.44 – 7.52 ppm integrating for 4 protons. A multiplet was observed in the region δ 7.27 – 7.29 ppm integrating for 6 protons. The emergence of new peaks in the aromatic region further supports the proposed compound **59c**.

The ^{13}C NMR spectrum showed a total of 8 carbons instead of 20 carbons, which can be attributed to the symmetry on the phenyl ring. The low resolution mass spectrum of compound **59c** gave a molecular ion peak at *m/z* 283.12 (M+1) which is consistent with the calculated value of *m/z* $C_{20}H_{14}N_2^+$ (283.12) and there is consistency with the data reported in literature.[66,70]

Following the reaction on Scheme 17, the compound 2-phenyl-3-(2-phenylethynyl)quinoxaline **59d** was isolated as a light brown solid in 47% yield with a melting point of 110-112 °C.

The ^{1}H NMR spectrum showed a total number of 14 protons and the absence of the characteristic singlet at δ 9.27 ppm (Fig. 2.4). The presence of new peaks in the aromatic region coming from the phenylethynyl supports the structure of compound **59d**.

The total number of carbon atoms on 2-phenyl-3-(2-phenylethynyl)quinoxaline **59d** is 22, but the ^{13}C NMR spectrum showed a total number of 18 carbons. This can be attributed to the symmetry on the phenyl ring. The low resolution mass spectrum gave a molecular ion peak at *m/z* 307.12 (M+1), which is consistent with the calculated value of *m/z* $C_{22}H_{14}N_2^+$ (307.12). The data strongly agreed with data reported in literature.[71]

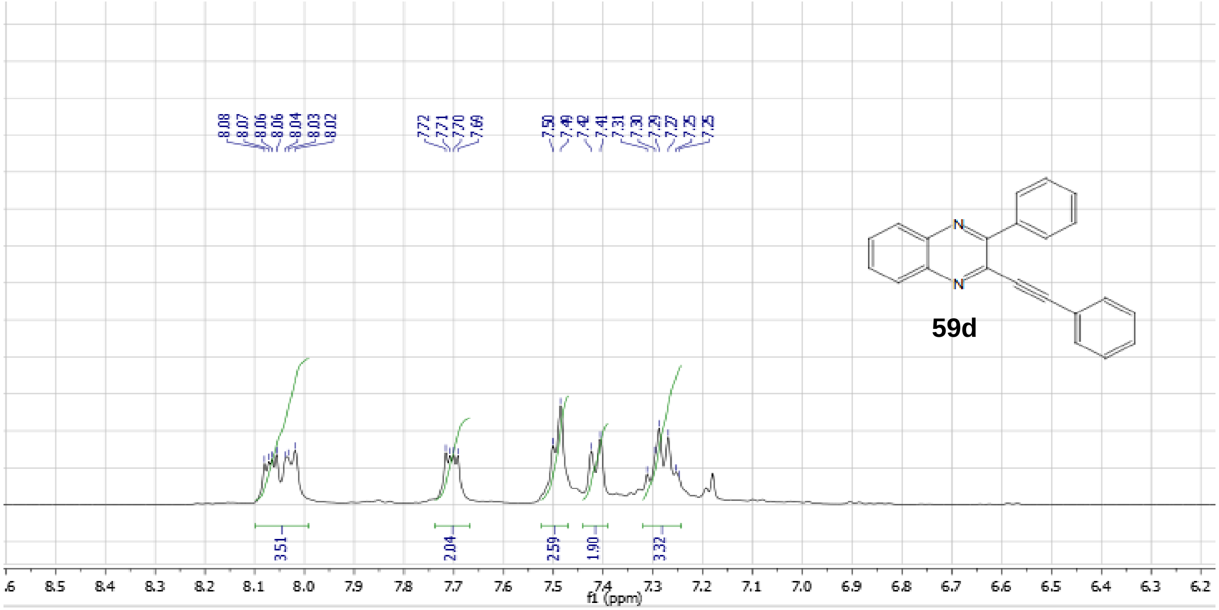


Figure 2.4: ^{1}H NMR spectrum of 2-phenyl-3-(2-phenylethynyl)quinoxaline

A similar approach was used as in **59d** to synthesise compound 3-(oct-1-ynyl)-2-phenylquinoxaline **59e**. The compound **59e** was isolated as orange oil and characterised by NMR spectroscopy and high resolution MS. To our knowledge compound **59e** has not been reported in literature.

The ^{1}H NMR spectrum showed the absence of the characteristic singlet with the emergence of new peaks in the upfield region. A -CH_2– peak was observed in the region δ 2.42 – 2.46 ppm as a triplet integrating for 2 protons. The two -CH_2- peaks in the region δ 1.52 – 1.60 ppm appeared as a multiplet, integrating for 4 protons indicating that these protons are equivalent. The two -CH_2- peaks in the region δ 1.25 – 1.32 ppm also appeared as a multiplet integrating for 4 protons. There was a triplet at δ

0.86 – 0.89 ppm that was observed which could be attributed to the -CH_3- group. A total of 22 protons were accounted for.

The ^{13}C NMR spectrum showed a total of 20 carbons but the structure has a total of 22 carbons. The high resolution mass spectrum gave a molecular ion peak at *m/z* MH^+ calculated for $[C_{22}H_{22}N_2]^+$: 315.1783, found: 315.1861. Given the NMR and MS data collected we were confident that compound **59e** is indeed 3-(oct-1-ynyl)-2-phenylquinoxaline.

We investigated the potential for heteroaryl groups to be incorporated within the 2-phenylquinoxaline **59** moiety. 2-Phenyl-3-(thiophen-2-yl)quinoxaline **59f** was isolated as a lime solid in 22% yield with a melting point of 126-128°C.

The characteristic singlet at δ 9.27 ppm was absent and new peaks in the aromatic region were present. The appearance of a doublet, due to ^3J coupling, in the region δ 7.40 – 7.41 ppm integrating for 1 proton can be attributed to the proton adjacent to the heteroatom. A multiplet in the region δ 6.86 – 6.88 ppm was observed also integrating for 1 proton. The appearance of a doublet, integrating for 1 proton, in the region δ 6.75 – 6.76 ppm further confirms the structure of compound **59f**. The remaining 9 protons were assigned to the benzene rings. A total of 12 protons were accounted for.

The low resolution mass spectrum gave a molecular ion peak at *m/z* at 289.07 (M+1) which is consistent with the calculated value of *m/z* $C_{18}H_{12}N_2S^+$ (289.07). The data strongly agreed with the data reported in literature.[72]

A similar approach was used as in **59f** to synthesise the compound 2-(furan-2-yl)-3-phenylquinoxaline **59g** as a brown solid in 47% yield with a melting point of 102-103 °C.

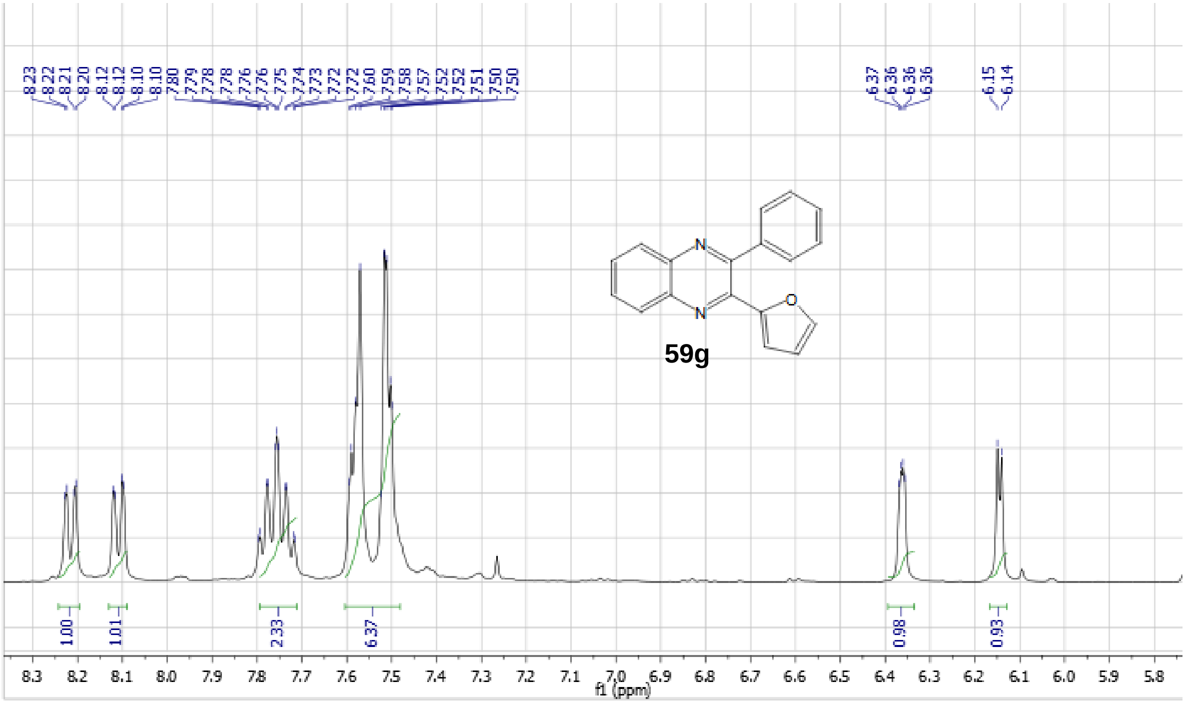


Figure 2.5: ^{1}H NMR of 2-(furan-2-yl)-3-phenylquinoxaline

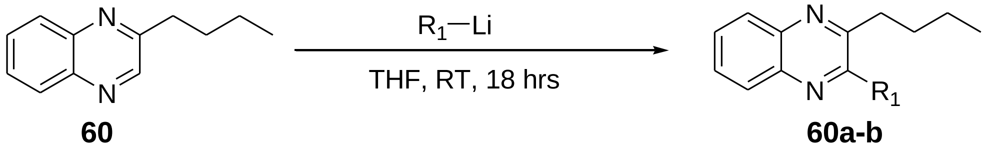
The ^{1}H NMR spectrum showed a total number of 12 protons and there was an absence of the singlet at δ 9.27 ppm. There was the presence of a peak at δ 7.49 ppm, integrating for 1 proton, overlapping with peaks from the phenyl ring. There was also the presence of a multiplet in the region δ 6.35 – 6.36 ppm integrating for 1 proton. A doublet was observed in the region δ 6.14 – 6.15 ppm integrating for 1 proton. The remaining 8 protons were assigned to the two benzene rings in the aromatic region (Fig. 2.5).

The ^{13}C NMR spectrum showed a total number of 16 carbons instead of the expected 18 carbons due to symmetry. The low resolution mass spectrum gave a molecular ion peak at *m/z* 273.09 (M+1) which was consistent with the calculated value of *m/z* $C_{18}H_{12}N_2O^+$ (273.09). Physical and spectroscopic data agree with those reported literature.[73]

With the success of nucleophilic substitution on **59**, we decided to check how the yield would be affected if we start with a less bulky group on the second position of the quinoxaline moiety. Having previously made 2-butylquinoxaline **60**, we then subjected it to nucleophilic substitution using the same conditions mentioned for **59a-g**.

2,3-Dibutylquinoxaline **60a** was isolated as brown oil in 76% yield. This result indicates that it is easier for nucleophilic substitution to occur when starting with a less bulky quinoxaline derivative **60**.

We then tried to increase the percentage yield of **60a**, by increasing the number of equivalents of n-BuLi to 3.0 eq. There was a significant increase in the percentage yield. There was only one spot evident on the TLC plate which suggests that the reaction ran to completion without any traces of the starting material **60**. The compound **60a** was isolated in 97% yield. The reaction is shown in Scheme 18.



Compound	R_1	% Yield	m.p °C (Lit.^{ref})
60a	-Butyl	97	-
60b	-furan-2-yl	91	-

Scheme 18: General procedure for di-substitution on 2-butylquinoxaline

The ^{1}H NMR spectrum showed a total number of 22 protons. There were two multiplets in the region δ 7.98 – 8.00 ppm and δ 7.63 – 7.66 ppm both integrating for 2 protons. A triplet was observed in the region δ 2.99 – 3.03 ppm integrating for 4 protons. There was an appearance of a multiplet in the region δ 1.49 – 1.53 ppm integrating for 4 protons. A triplet in the region δ 0.89 – 1.11 ppm was observed integrating for 6 protons. The spectrum shows an overlap of all the $–CH_2–$ and $–CH_3–$ peaks.

The ^{13}C NMR spectrum showed a total number of 8 carbons instead of 16 carbons. This is due to the symmetry on the molecule. The data strongly agreed with the data reported in literature.[74]

Following a similar method as with **60a**, 2-butyl-3-(furan-2-yl)quinoxaline **60b** was isolated as a brown oil in 91% yield. To our knowledge compound **60b** has not been reported in literature.

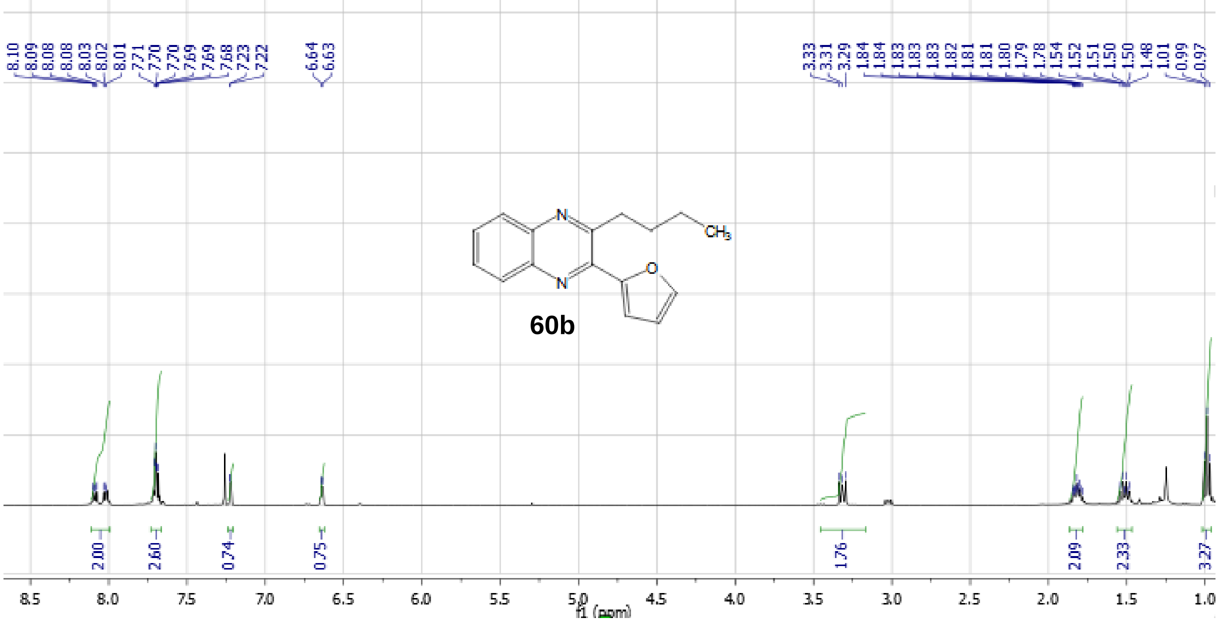


Figure 2.6: ^{1}H NMR of 2-butyl-3-(furan-2-yl)quinoxaline

The absence of the characteristic peak at δ 8.75 ppm confirmed that nucleophilic substitution on the third position was successful. There was the presence of a peak at δ 7.15 ppm, integrating for 1 proton, overlapping with peaks from the phenyl ring. There was also the presence of a doublet in the region δ 7.22 – 7.23 ppm integrating for 1 proton. A multiplet was observed in the region δ 6.63 – 6.64 ppm integrating for 1 proton. The remaining 4 protons were assigned to the benzene ring and the other 9 protons were assigned to the butyl chain in the upfield region (Fig. 2.6).

The ^{13}C NMR spectrum showed a total number of 16 carbons which are consistent with the proposed structure. The high resolution mass spectrum gave a molecular ion peak at MH^+ calculated for $[C_{16}H_{16}N_2O]^+$: 253.1263, found: 253.1339. Given the NMR data collected we were confident that compound **60b** is indeed 2-butyl-3-(furan-2-yl)quinoxaline.

The use of Negishi cross-coupling gave moderate to high yields. Different methods were used to synthesise compounds **59** and **60**. One method involved the lithiation of the substrate with n-BuLi followed by transmetallation with $ZnCl_2$, while the other method involved the preparation of the organozinc reagent by direct magnesium insertion.

Most of the di-substituted compounds that we were able to synthesise have been reported previously, but the methods used for the synthesis of these compounds were different to the ones reported in this dissertation. Compounds **59a**, **59b**, **59f** and **59g** were reported to be synthesised by condensation of an aryl 1, 2-diamine with a 1, 2-dicarbonyl compound. The synthesis of 3-butyl-2-phenylquinoxaline **59a** involved the condensation of 2-hydroxy-1-phenylhexan-1-one with *o*-phenylenediamine (copper-catalysed) in 59% yield.[68]

The compound 2-phenyl-3-(2-phenylethynyl)quinoxaline **59d** was reported to be synthesised by the cyclisation of *o*-phenylenediamine with phenylacetylene in the presence of Cu(II) as a catalyst.[71] The synthesis of 2, 3-diphenylquinoxaline **59c** was reported to be achieved by Stille cross-coupling reaction between 2,3-bis(tributylstannyl)quinoxaline and iodobenzene to give the product in 39% yield.[66] These methods required the use of different substrates with pre-defined groups while the method reported herein only required 2-phenylquinoxaline **59**, as a starting material, to synthesise compounds **59a**-**59g**.

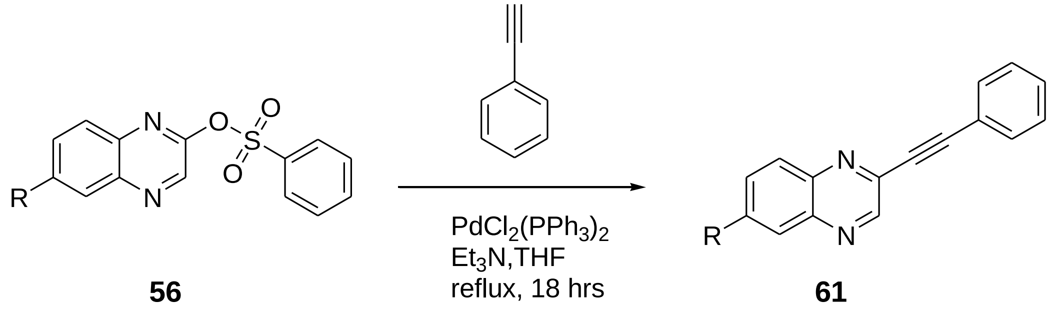
We wanted to have a non-reactive group at the second position of the quinoxaline moiety, hence the use of 2-phenylquinoxaline **59** for nucleophilic substitution. The phenyl-group is a large group, thus the difficulty of nucleophilic substitution, due to steric hindrance, which led to the observation of low to moderate yields with compounds **59a**-

59g. The use of 2-butylquinoxaline **60** for the synthesis of **60a** and **60b** further supports this assumption due to the yields observed. The butyl-substituent is a less bulky substituent, in comparison to the phenyl group, which made nucleophilic substitution easier. The use of a less bulky substituent, in this case, makes nucleophilic substitution occur readily and the reactions run to completion due to the complete consumption of starting material **60**.

The successful synthesis of compounds **59a-59g**, **60a** and **60b**, encouraged us to further investigate the possibilities of nucleophilic substitution on compounds with a different functional group on the second position of the quinoxaline moiety. We wanted to investigate the possibilities for nucleophilic substitution to incorporate an alkynyl group at the second position of the quinoxaline moiety. We thus employed Sonogashira cross-coupling, which represents a convenient method for Csp^2-Csp bond formation, described in detail below.

2.3.3 Sonogashira coupling on quinoxaline-*o*-sulfonate

Phenylacetylene was coupled with 2-benzensulfonyloxyquinoxaline derivatives **56** under Sonogashira coupling conditions in THF, using 5% $PdCl_2(PPh_3)_2$ as a catalyst with triethylamine (Et_3N) used as a base. The solution was refluxed at approximately 55 °C for atleast 18 hours. The reaction was monitored on TLC and after 18 hours the reaction was stopped and quenched with sat. $NaHCO_3$ and purified by column chromatography on silica gel to afford 2-(2-phenylethynyl)quinoxaline derivatives **61**. The synthesised compounds were analysed by NMR spectroscopy and MS, where applicable (Scheme 19).



56 **61**

56: R = H **(a)**; R = NO_2 **(b)**; R = Cl **(c)**

Compound	R	% Yield	m.p °C (Lit.[ref])
61a	6-H	61	66-63 (91[75])
61b	6-NO_2	77	76-79
61c	6-Cl	70	116-118(118-120[76])

Scheme 19: General procedure for Sonogashira-coupling reaction

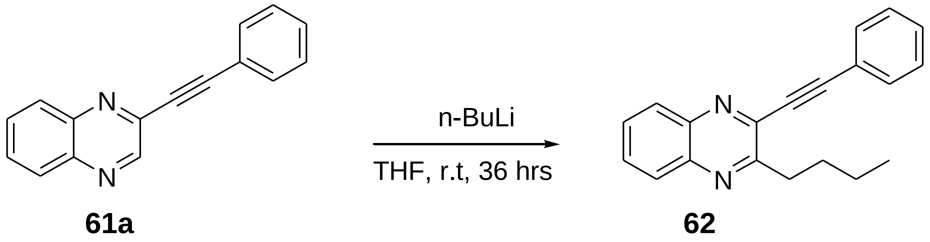
The ^{1}H NMR spectrum showed a characteristic singlet at δ 8.99, 9.10 and 8.96 ppm for compound **61a-c**, respectively, integrating for 1 proton. The chemical shift of the proton signals in the region δ 7.42 – 9.00 ppm confirms the incorporation of the phenylethynyl group. A multiplet in the region δ 7.78 – 7.82 integrating for 2 protons, was observed. This peak is attributed to compound **61a**. The presence of a doublet in the region δ 8.99 – 9.00 ppm (compound **61b**) and in the region δ 8.09 – 8.08 ppm (compound **61c**), due to ^4J coupling, confirms the presence of Csp^2-NO_2 and Csp^2-Cl bond formation, respectively. The ^{13}C NMR spectrum showed a total number of 14 carbons instead of 16 carbons, which means that there are equivalent carbons due to the symmetry on the phenyl ring. The low resolution mass spectrum of compound **61a** gave a molecular ion peak at *m/z* 231.08 (M+1) which is consistent with calculated value of *m/z* $C_{16}H_{10}N_2^+$ (231.08). The results obtained were consistent with those reported in literature.[75,76]

Nucleophilic substitution on compound **61a** is described in detail in the next section.

2.3.4 Nucleophilic substitution on Sonogashira cross-coupled quinoxaline

Under the same conditions previously mentioned in section 2.3.2, 2-(2-phenylethynyl)quinoxaline **61a** was reacted with n-BuLi (3.0 eq). The reaction was left to stir for 18 hours at room temperature. After aqueous work-up and purification on column chromatography the compound 3-butyl-2-(2-phenylethynyl)quinoxaline **62** was isolated as an orange solid in 20% yield and melting point of 76-78 °C. The starting material **56a** was also recovered.

We tried to increase the percentage yield by increasing the reaction time. We monitored the reaction on TLC plate and only stopped the reaction after consumption of the starting material. The reaction was stopped after 36 hours when only one spot was clearly visible on the TLC. The percentage yield improved to 56%. To our knowledge compound **62** has never been reported in literature (Scheme 20).



Scheme 20: Synthesis of 3-butyl-2-(2-phenylethynyl)quinoxaline

The absence of the characteristic peak, –N=C-H group, at 8.99 ppm confirmed that nucleophilic substitution on the third position was successful. There was a presence of new peaks in the upfield region where there was a triplet in the region δ 3.19 – 3.23 ppm followed by two multiplets in the region δ 1.82 – 1.89 ppm and δ 1.44 – 1.50 ppm all integrating for 2 protons coming from –CH_2–. There was the presence of a triplet in the region δ at 0.93 – 0.96 ppm integrating for 3 protons from –CH_3–. The 9 remaining protons can be attributed to the benzene rings. There were a total number of 18 protons.

The ^{13}C NMR spectrum showed a total number of 18 carbons instead of 20 carbons, which means that there are equivalent carbons due to the symmetry on the phenyl ring. The high resolution mass spectrum gave a molecular ion peak at MH^+ calculated for $[C_{20}H_{18}N_2]^+$: 287.1470, found: 287.1545. Given the NMR data collected we were confident that compound **62** is indeed 3-butyl-2-(2-phenylethynyl)quinoxaline

We then used all the nucleophiles, previously reported in section 2.3.2, on 2-(2-phenylethynyl)quinoxaline **61a** but this attempt proved to be futile since only the starting material was recovered. We also attempted to use these nucleophiles on 6-nitro-2-(2-phenylethynyl)quinoxaline **61b** and 6-chloro-2-(2-phenylethynyl)quinoxaline **61c**, but only the starting material was recovered. Surprisingly, not even n-BuLi was able to undergo nucleophilic substitution with **61b** and **61c**. We varied the parameters as shown below (Table 2.1).

Table 2.1: Variation of parameters on compounds **61a**-**61c**

Deprotonation/Lithiation (hrs)	No. of equivalents (eq.)	Reaction time (hrs)	Yield
1	3.0	36	No reaction
2	3.0	36	No reaction
1	5.0*	36*	No reaction*
2	5.0	36	No reaction
2	5.0*	48*	No reaction*

Variation when only using n-BuLi*

The use of Sonogashira cross-coupling also gave moderate to high yields, which further demonstrates the versatility of aryl-*o*-sulfonate, as a good leaving group. Only one compound 3-butyl-2-(2-phenylethynyl)quinoxaline **62** was synthesised by nucleophilic substitution. Nucleophilic substitution on 2-(2-phenylethynyl)quinoxaline **61a** was difficult due to the electron donating nature of the aryl alkynyl attached at the second position of the quinoxaline moiety. This is due to the high electron density caused by highly delocalized pi-electron clouds from the phenyl ring and the acetylenic moiety.[77] The donation of electrons to the quinoxaline moiety, makes the –N=C-H site less electrophilic. This suggests that only very strong nucleophiles, which are stronger than n-BuLi, can work.

The -NO_2 group is a strong deactivating group, thus when attached to the quinoxaline at the sixth position it acts as a directing metalation group. It is also possible that there is interaction between the -NO_2 and n-BuLi by coordination of the lithium on the heteroatom-containing nitro group, which results in n-BuLi being pulled away from the –N=C-H site.[78] The chloro- group is weakly deactivating and can undergo lithium-halogen exchange with n-BuLi, making nucleophilic substitution on the –N=C-H site extremely difficult.

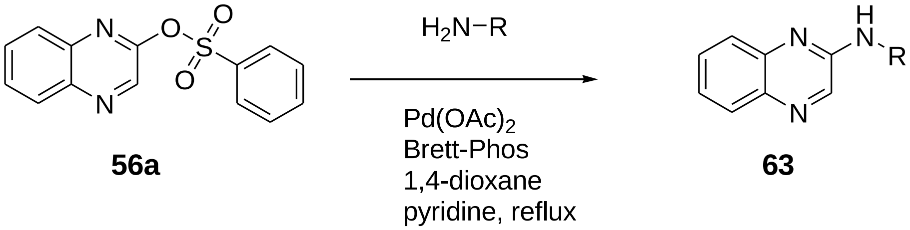
We realized that the type of bonding atom on the second position of the quinoxaline ring (C-C or C-C-alkynyl bond) plays a significant role in the ability to direct nucleophilic attack towards the –N=C-H site. Thus, we opted to investigate the possibility of nucleophilic attack if the bonding atom on the second position of the quinoxaline was C-N.

2.3.5 Buchwald-Hartwig coupling on quinoxaline-*o*-sulfonate

The *N*-arylquinoxalin-2-amine derivatives **63** were prepared by reacting 2-benzenesulfonyloxyquinoxaline **56a** with arylamine substrates under Buchwald-Hartwig conditions in 1,4-dioxane, 5% palladium acetate ($Pd(OAc)_2$) as a catalyst, pyridine as a base and the ligand Brett-Phos was used (Scheme 21). The solution was heated under reflux for 18 hours and quenched with sat. $NaHCO_3$, purified on column chromatography and characterised. The ^1H NMR spectrum showed a characteristic singlet at δ 8.47 and 8.21 ppm for compound **63a** and **63b**, respectively, integrating for 1 proton. Resonances in the region δ 7.14 – 7.94 ppm correspond to aromatic protons while an NH proton signal resonates as a singlet at δ 7.08 ppm (compound **63a**) and δ 5.15 ppm (compound **63b**).

The low resolution mass spectrum for compound **63a** gave a molecular ion peak at *m/z* 222.10 (M+1) which is consistent with calculated value of *m/z* $C_{14}H_{11}N_3^+$ (222.10). The results obtained were consistent with those reported in literature.[79,80]

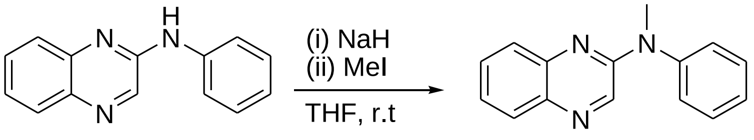
The high resolution mass spectrum for compound **63b** gave a molecular ion peak MH^+ calculated for $[C_{15}H_{13}N_3]^+$: 236.1109, found: 236.1189. To our knowledge **63b** has not been reported in literature.



Compound	R	δ(Aromatic H)	δ(N-H)	δ(CH_2)	% Yield	m.p °C (Lit.[ref])
63a	Phenyl	7.14 – 7.97	7.08	-	52	135-137(137[79])
63b	Benzyl	7.28 – 7.87	5.15	4.74 – 4.76	94	-

Scheme 21: General approach of Buchwald-Hartwig reaction

With the free N-H bond on **63a**, we then decided to use a methyl group as a protecting group. *N*-phenylquinoxalin-2-amine **63a** was dissolved in THF and treated with sodium hydride (NaH). After 1 hour the resulting solution was reacted with iodomethane, quenched with sat. NH_4Cl and purified by column chromatography (Scheme 22). *N*-methyl-*N*-phenylquinoxalin-2-amine **63c** was isolated as orange oil in 78 % yield. To our knowledge compound **63c** has not been reported literature.



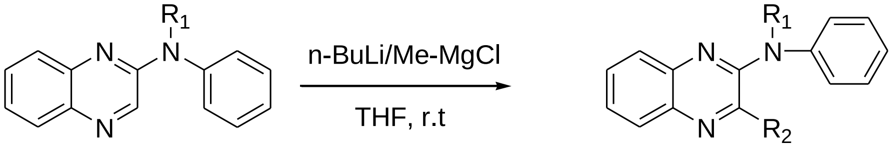
63a **63c**

Scheme 22: Synthesis of *N*-methyl-*N*-phenylquinoxalin-2-amine

The ^1H NMR spectrum showed a total of 13 protons, in which a characteristic singlet peak at δ 8.47 ppm corresponding to an –N=C-H group, was observed, integrating for 1 proton. There was a presence of a singlet at 3.60 ppm assigned for 3 protons. There was also an appearance of a carbon peak at 36.68 ppm on the ^{13}C NMR spectrum. The high resolution mass spectrum for compound **63c** gave a molecular ion peak MH^+ calculated for $[C_{15}H_{13}N_3]^+$: 236.1109, found: 236.1191.

2.3.6 Nucleophilic substitution on Buchwald-Hartwig cross-coupled quinoxaline

When *N*-phenylquinoxalin-2-amine **63a** was reacted with n-BuLi (3.0 eq) and left to stir for 18 hours, at room temperature, the target compound 3-butyl-*N*-phenylquinoxalin-2-amine **65a** was isolated in very low yields and there was also recovery of the starting material *N*-phenylquinoxalin-2-amine **63a**. When the reaction time was increased to 36 hours, compound 3-butyl-*N*-phenylquinoxalin-2-amine **64a** was isolated as an orange solid in yield of 32% and a melting point of 99-102 °C (Scheme 23). There was still recovery of the starting material. The low yield could be due to the competition between the free N-H and the –N=C-H site. To our knowledge **64a** has never been reported in literature.



63 **64**

63: R_1 = H **(a)**; R_1 = CH_3 **(c)**

Compound	R_1	R_2	% Yield	m.p °C (Lit.^{ref})
64a	H	-Butyl	32	99-102
64b	H	-Methyl	42	-
64c	CH_3	-Butyl	62	-

Scheme 23: General approach for nucleophilic substitution on Buchwald coupled derivatives

The absence of the characteristic peak, –N=C-H group, at δ 8.47 ppm confirmed that nucleophilic substitution on the third position was successful. There was a presence of new peaks in the upfield region. There was a presence of a $–CH_2–$ peak in the region δ 2.87 – 2.91 ppm, appearing as a triplet and integrating for 2 prortons. There were two multiplets appearing in the region δ 1.81 – 1.89 ppm and δ 1.46 – 1.51 ppm both integrating for 2 protons followed by the appearance of a triplet at 0.95 – 0.98 ppm integrating for 3 protons. The ^{1}H NMR showed a total of 19 protons.

The ^{13}C NMR spectrum showed the appearance of four new carbon sites at 14.03, 22.78, 28.91and 33.94 ppm. The high resolution mass spectrum for compound **64a** gave a molecular ion peak MH^+ calculated for $[C_{18}H_{19}N_3]^+$: 278.1579, found: 278.1659.

Compound **64b** showed a presence of a singlet at 2.43 ppm integrating for 3 protons. The ^{13}C NMR spectrum showed an emergence of a new carbon peak at 15.32 ppm. Physical and spectroscopic data agree with those reported in literature.[81]

We synthesised 3-butyl-*N*-methyl-*N*-phenylquinoxalin-2-amine **64c** by reacting *N*-methyl-*N*-phenylquinoxalin-2-amine **63c** with n-BuLi (Scheme 23). With the protecting group (methyl-) on the nitrogen, the n-BuLi was able to fully react with the starting material *N*-methyl-*N*-phenylquinoxalin-2-amine **63c** since none of the compound **63c** was recovered. To our knowledge **64c** has never been reported in literature.

The absence of the characteristic peak, –N=C-H group, at δ 8.21 ppm confirmed that nucleophilic substitution on the third position was successful. There was a presence of a triplet in the region δ 2.36 – 2.40 ppm integrating for 2 protons followed by two multiplets in the region δ 1.52 – 1.58 ppm and δ 1.09 – 1.19 ppm both integrating for 2 protons from –CH_2–. The –CH_3– peak appeared in the region δ 0.75 – 0.79 ppm as a triplet (Fig. 2.7).

The ^{13}C NMR showed the appearance of four new carbon sites at 13.84, 22.56, 29.78, 35.44 ppm. The high resolution mass spectrum for compound **64c** gave a molecular ion peak MH^+ calculated for $[C_{19}H_{21}N_3]^+$: 292.1735, found: 292.1817.

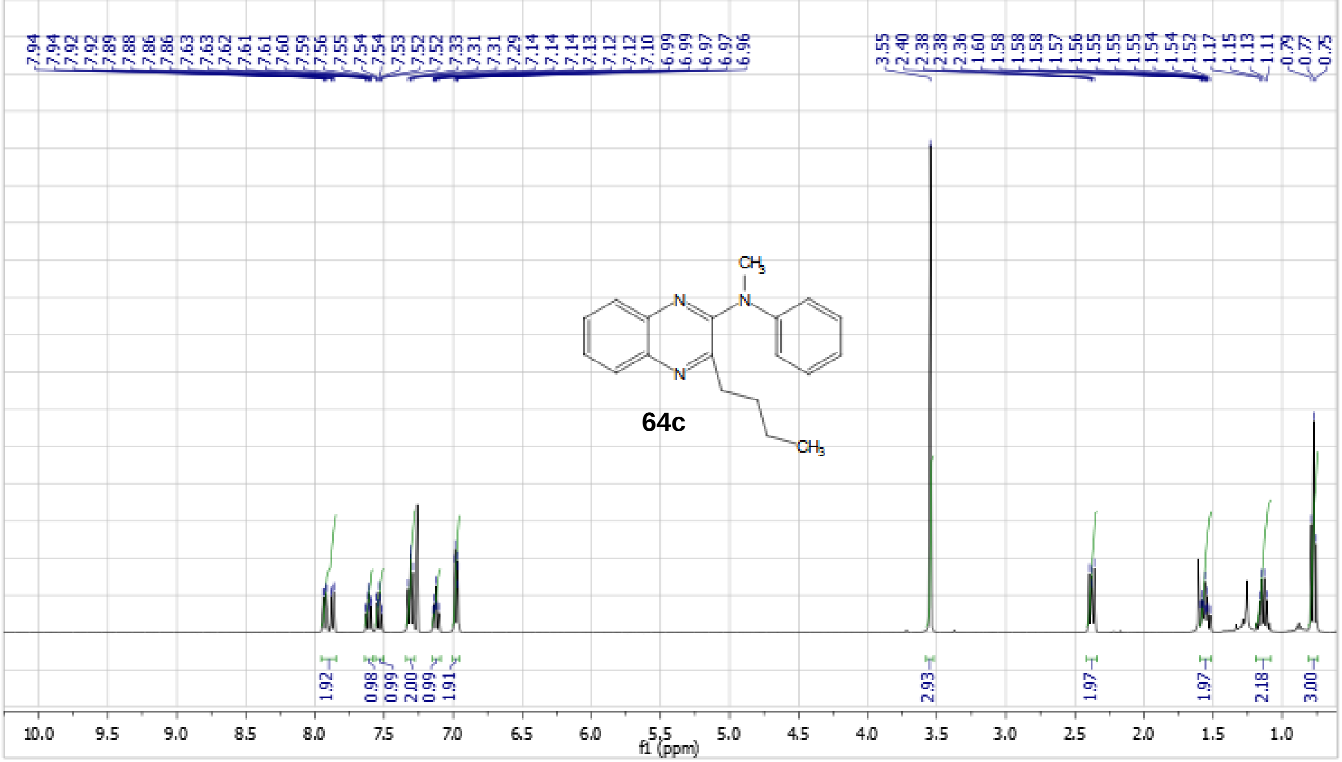


Figure 2.7: ^{1}H NMR of 3-butyl-*N*-methyl-*N*-phenylquinoxalin-2-amine

The attempt to use nucleophiles from furan and phenylacetylene on *N*-phenylquinoxalin-2-amine **63a** and *N*-methyl-*N*-phenylquinoxalin-2-amine **63c** were unsuccessful. The variations of parameters are shown below (Table 2.2).

Table 2.2: Variations of parameters on compounds **63a** and **63b**

Deprotonation/Lithiation (hours)	No. of equivalents (eq.)	Reaction time (hours)	Yield
1	5.0	36	No reaction
2	5.0	36	No reaction
2	5.0	48	No reaction

Variation using Furan and Phenylacetylene nucleophiles

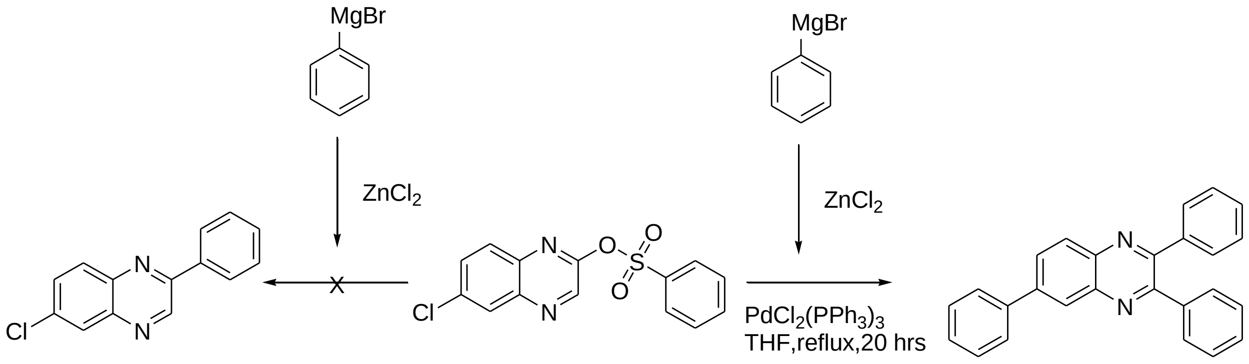
Buchwald-Hartwig cross-coupling reaction on 2-benzenesulfonyloxyquinoxaline **56a** generated a number of novel compounds and known compounds with moderate to high yields. During nucleophilic substitution though, the C-N group on the second position of the quinoxaline moiety acts in a similar manner as with the terminal alkyne. This could be due to steric hindrance accompanied by the presence of the bulky N-phenyl substituent making the –N=C-H site inaccessible by other larger nucleophiles. This suggests that only alkyl nucleophiles can readily access the third position of the quinoxaline moiety, in this case.

Badr et al. reported the synthesis of 3-methyl-N-phenylquinoxalin-2-amine **64b** by the reaction of 2-chloro-3-methylquinoxaline with aniline as a nucleophilic reagent in dry benzene in the presence of anhydrous potassium carbonate and potassium iodide, which shows that there are minor similarities to the synthesis of compound **64b**, to the method reported herein.[81]

2.4 Synthesis of tri-substituted quinoxaline derivatives

We initially used Negishi coupling on 6-chloro-benzenesulfonyloxyquinoxaline **56c** in order to incorporate a phenyl ring on the second position on **56c** (Scheme 24). We followed a similar reaction as previously reported for the synthesis of **59**. Phenyl magnesium bromide was added to a solution of zinc chloride ($ZnCl_2$) and left to stir for 1 hour. The Zn-substrate and **56c** were subjected to Negishi coupling conditions in THF, using 5% $PdCl_2(PPh_3)_3$ as a catalyst. After 20 hours the reaction was stopped and quenched with sat. $NaHCO_3$ and purified by flash column chromatography on silica gel. The expected compound 6-chloro-2-phenylquinoxaline **66** was not isolated, instead 2,3,6-triphenylquinoxaline **65** and an inconclusive variety of compounds were synthesised. The compound **65** was isolated as brown oil in 36% yield.

The compound 6-chloro-benzenesulfonyloxyquinoxaline **56c** had undergone coupling at the sixth and second position, as well as nucleophilic substitution at the second position. This could be due to an excess amount of phenyl-magnesium bromide.



Scheme 24: Synthesis of 2,3,6-triphenylquinoxaline

The absence of the characteristic peak, –N=C-H group, at 8.67 ppm confirmed that nucleophilic substitution on the third position was successful. There was an apparent doublet of doublets in the region δ 7.75 – 7.77 ppm integrating for 2 protons.

Resonances in the region δ 6.89 – 7.61 can be attributed to the remaining protons in the aromatic region. A total of 18 protons were observed on the ^{1}H NMR spectrum (Fig. 2.8).

The ^{13}C NMR spectrum showed a total of 20 carbons instead of 26 carbons. This is due to the symmetry on the phenyl ring. Physical and spectroscopic data agree with those reported elsewhere.[82]

Since the chloro-substituent on compound 6-chloro-2-benzenesulfonyloxyquinoxaline **56c** can undergo coupling, we then attempted to use 6-nitro-2-benzenesulfonyloxyquinoxaline **56b** for Negishi coupling and nucleophilic substitution. This attempt proved to be futile since only an inconclusive compound was isolated of which it was difficult to purify even when different solvent systems were used.

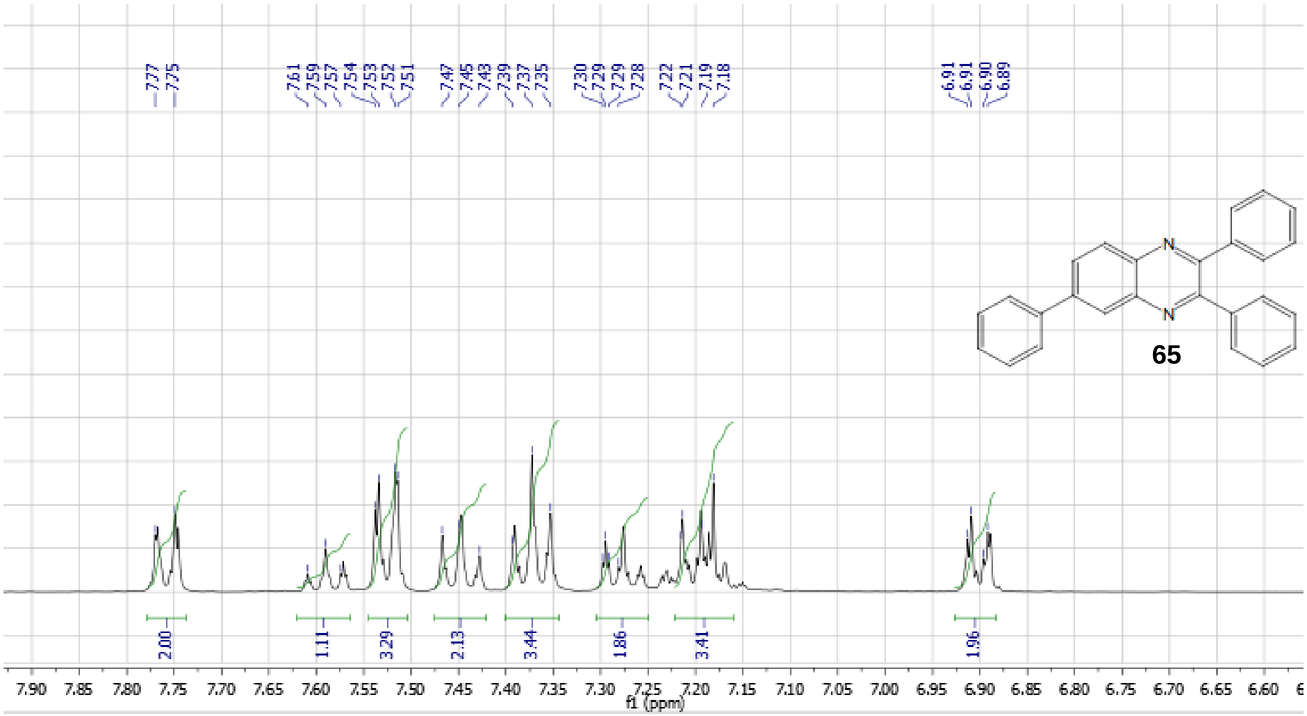


Figure 2.8: ^{1}H NMR spectrum of 2,3,6-triphenylquinoxaline

The synthesis of tri-substituted quinoxalines proved to be very difficult due to having the chloro- group on the sixth position, which is very reactive. The synthesis of quinoxaline moiety with a NO_2- group on the sixth position also made it difficult for tri-substitution due to the deactivating property of the NO_2- group.

CHAPTER

3

Chapter 3: Biological activity of the synthesised compounds

3.1 Cytotoxicity studies

For the cytotoxicity assay, results are expressed as % cell viability, based on fluorescence reading in treated wells vs. untreated control well (Table 3.2). Emetine (which induces cell apoptosis) is used as a positive control drug standard (Table 3.1).

Table 3.1: Emetine

		Emetine
Conc (μM)	**Log(Conc)**	**Percentage Viability**
50	1.69897	2.635418
5	0.69897	1.731299
0.5	-0.30103	3.334907
0.05	-1.30103	8.739463
0.005	-2.30103	63.80681
0.0005	-3.30103	78.32607
0.00005	-4.30103	98.34043
0.000005	-5.30103	100.7783

Table 3.2: Cell toxicity assay

	Percentage viability		
Compound ID	**100 µM**	**25µ M**	**6.5 µM**
59b	108.04	133.52	128.71
59c	102.99	99.69	105.47
59a	107.10	103.66	102.70
59d	15.08	14.77	81.05
59f	96.12	84.94	104.63
59g	88.02	108.55	133.62
59e	17.58	119.02	110.06
62	7.73	74.08	99.20
63a	42.22	138.70	114.87
64a	114.57	100.29	111.67
64b	92.64	98.17	132.26
63c	115.77	122.11	133.75
64c	110.46	114.36	101.09
63b	84.72	94.53	90.54
65	87.08	107.98	98.60
61c	3.38	7.34	42.85

The compounds **59d**, **59e**, **61c**, **62** and **63a** significantly decreased the cell viability after 24 hours. Compounds **59d** and **61c** showed cytotoxicity at concentrations exceeding 6.5 µM while compounds **59e**, **62** and **63a** showed cytotoxicity at concentrations exceeding 25 µM. The other compounds on Table 3.2, showed no significant effect on HeLa cells at high concentrations (100 µM).

3.2 Tuberculosis (TB) screening

TB activity studies were done to determine the minimum inhibition concentration (MIC) required in inhibiting 90% (MIC_{90}) and 99% (MIC_{99}) growth of *Mycobacterium tuberculosis (Mtb)*-$H_{37}Rv$ strains. The relevant critical concentrations of the synthesised compounds were compared with the MIC of a clinically and laboratory-determined susceptible drug (Rifampacin).

Table 3.3: MIC$_{90}$ and MIC$_{99}$ (*Mtb*-H$_{37}$Rv strain activity) data on synthesised compounds

Compound ID	**MIC$_{90}$ (µg/ml)**	**MIC$_{99}$ (µg/ml)**
Rifimpacin	0.00541	0.00648
59	>20	>20
59a	>20	>20
59b	>20	>20
59c	>20	>20
59d	>20	>20
59e	>20	>20
59f	>20	>20
59g	>20	>20
61a	>20	>20
61b	>20	>20
61c	8.15	9.26
62	>20	>20
64a	>20	>20
64c	>20	>20
64b	>20	>20
63c	16.4	19
65	>20	>20

Only two compounds showed MIC$_{90}$ and MIC$_{99}$< 20 against *Mtb*-H$_{37}$Rv strains (Table 3.3). The compounds **61c** and **63c** showed MIC$_{90}$ of 8.15 and 16.4; MIC$_{99}$ 9.26 and 19, respectively. The results have shown that most of the compounds were exhibiting very high MIC$_{90}$ and MIC$_{99}$ values. Although the compounds **61c** and **63c** are not considered to be active in comparison to the standard, they show some potential for further development.

3.3 Malaria screening

For each compound concentration, % parasite viability– the PLDH activity in compound-treated wells relative to untreated controls–was calculated (Table 3.5). For comparative purposes, Chloroquine (an anti-malarial drug) was used as a drug standard and yields IC_{50} values in the range 0.01-0.05 µM.

Table 3.4: Chloroquine

Chloroquine standard (uM)	**Percentage parasite viability**
100	5.039732
10	6.603931
1	6.407361
0.1	9.038059
0.01	80.326230
0.001	109.556700
0.0001	106.545400
0.00001	116.457600

Table 3.5: *Plasmodium falciparum* strain 3D7 data on synthesised compounds

Compound ID	**Percentage parasite viability at 20 µM**
59b	92.50
59c	86.97
59a	82.03
59d	85.60
59f	85.72
59g	87.20
59e	89.96
62	98.14
63a	93.49
64a	80.07
64b	80.85
63c	42.64
64c	89.49
63b	89.67
65	58.12
61c	91.07

The compounds **63c** and **65** showed a percentage parasite viability of 42.64% and 58.12%, respectively. Although not active, these compounds have a potential to be developed further, by changing or including other functional groups. The results have shown that most of the compounds were exhibiting higher percentage parasite viability values.

3.4 Conclusion

A series of mono-substituted quinoxalines were successfully synthesised by Negishi, Sonogashira and Buchwald-Hartwig coupling reactions. Di-substituted quinoxalines were synthesised by nucleophilic substitution. The proton on –N=C-H is not acidic enough for the use of bases such as n-BuLi, LDA and *i*PrMgCl, for deprotonation. Only one tri-substituted quinoxaline compound was synthesised. The synthesised compounds were isolated in low to high yields.

The ^{1}H NMR, ^{13}C NMR and MS data analysis were 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 showed that deprotonation is not possible when using traditional bases on quinoxaline and its derivatives. There is also evidence that that the type of bonding atom on the second position of the quinoxaline ring plays a significant role in the ability to direct nucleophilic attack towards the –N=C-H site. The most promising type of bonding was the C-C bonding but nucleophilic attack on the –N=C-H site becomes easier when there is a less bulky group. The bonding types of C-C-alkynyl and C-N, on the second position, make nucleophilic attack more difficult on the –N=C-H site.

Although all the synthesised compounds showed no biological activity, further studies will be done on compounds **61c**, **63c** and **65** to try and improve their activity against *M.tb* $H_{37}Rv$ and *Plasmodium falciparum* 3D7 strains and to improve their cytotoxicity levels on HeLa cells. It will be interesting to see what kind of effects the combination of the substituents of compounds **61c**, **63c**, and **65** will have on *M.tb* $H_{37}Rv$ and *Plasmodium falciparum* 3D7 strains.

3.5 Future work

We will investigate alternative methods for the synthesis of tri-substituted quinoxalines and also investigate the synthesis of C-O bonds on the second position of the quinoxaline ring and attempt nucleophilic substitution on them. We will employ CH activation, using different metal catalysts and ligands, as an alternative for the synthesis of novel mono-, di- and tri-substituted quinoxaline compounds. We will furthermore, try to optimise metal-amine bases for deprotonation of less acidic protons and make them more selective.

CHAPTER

4

Chapter 4: Experimental section

4.1 General

a) Purification of reagents and solvents

- THF was distilled from sodium and benzophenone

b) Spectroscopic and physical data

- Melting points were obtained using Lasec/ SA-melting point apparatus from Lasec Company, SA.
- ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on Bruker Avance-400 spectrometer. Spectra were recorded in deuterated chloroform ($CDCl_3$) unless otherwise specified. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard at zero ppm. The ^{1}H NMR chemical shifts are reported: value (number of hydrogens, description of signal, assignment) and the ^{13}C NMR chemical shifts are reported: value (assignment). Abbreviations used: s = singlet, d = doublet, dd = doublet of doublets, t = triplet and m = multiplet.

c) Nomenclature and numbering of compounds

The compounds prepared for this project are named in the following experimental section 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.

4.2 Synthetic procedures of Grignard reagents

Phenylmagnesium bromide (Phenyl-MgBr)[43]

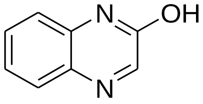
To an oven dried 3-neck 100 mL round bottom flask containing magnesium turnings (1.01 g, 41.55 mmol), pre-dried under vacuum for 15 minutes, was fitted with a rubber stopper, equipped with a stirrer bar, reflux condenser and a positive pressure of dry nitrogen. THF (10 mL) was transferred to the flask followed by a solution of bromobenzene (4.82 ml, 45.70 mmol, 1.1 eq), with 2 pellets of iodine. The solution was stirred at room temperature until reaction starts (3-5 minutes). Gentle reflux was maintained by cooling in a room temperature water bath. More THF (12.5 mL) was added after the reaction had begun to subside (20 minutes) and stirred for 2 hours. More THF (12.5 mL) was added, with the fine precipitates allowed to settle and the supernatants transferred to an oven dried Schlenk tube. The concentration of the phenyl-MgBr was assumed to be 1.14 M.

Isopropylmagnesium chloride (*i*PrMgCl)[43]

Magnesium turnings (2.02 g, 83.1 mmol) were dried under vacuum for 15 minutes. 2-chloropropane (~ 6 mL) was added drop-wise, with THF (20 mL) and 2 pellets of iodine, to the oven dried 3-neck 250 mL round bottom flask equipped with a stirrer bar, reflux condenser and a positive pressure of dry nitrogen. Reflux was maintained with an ice-water bath once the reaction started. The remaining 2-chloropropane was added drop-wise to maintain gentle reflux. THF (25 mL) was added after the reaction had begun to subside and stirred for 2 hours. Further THF (25 mL) was added and the solution was left to stir until all the magnesium had reacted. The resulting solution was transferred to an oven dried Schlenk tube. The concentration of the *i*PrMgCl was assumed to be 0.9 M.

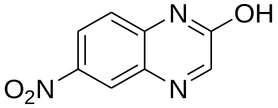
4.3 Synthetic procedures of quinoxaline derivatives

Synthesis of quinoxalin-2-ol (55a)[42]



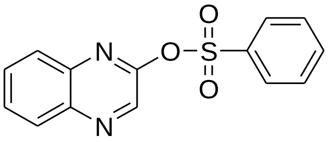
O-Phenylenediamine (30.30 g, 0.276 mol) was dissolved in acetic acid (30 mL) and methanol (30 mL), and the solution cooled to -15 °C with stirring. Glyoxylic acid (25.50 g, 0.276 mol) in water (60 mL) was added drop-wise over 30 minutes to the solution, while maintaining the temperature at -15 °C. The final solution was allowed to warm up to 0 °C over 90 minutes, filtered, then washed with water (45 mL) then methanol (15 mL), and air dried to give a dark grey solid. Recrystallisation from DMF gave the 2-quinoxalinone as a tan solid (24.30 g, 60%); m.p 265-267 °C (Lit.[42] 266-267 °C); δ_H (400 MHz, DMSO-d_6) 2.99 (1H, s) 7.25 – 7.34 (2 H, m), 7.54 (1 H, m), 7.77 (1 H, m) and 8.16 (1 H, s); δ_C (100 MHz, DMSO-d_6) 116.17, 123.75, 129.22, 131.23, 132.24, 132.47, 152.03 and 155.38. Spectroscopic data agree with those reported in literature.

Synthesis of 6-nitroquinoxalin-2-ol (55b)[83]



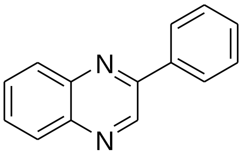
4-Nitro-O-phenylenediamine (10.0 g, 65 mmol) was dissolved in a mixture of acetic acid (5 mL) and methanol (5 mL). The reaction was left to stir for 10 minutes at room temperature. A solution of glyoxylic acid (4.25 g, 46 mmol) in water (10 mL) was added to the solution, drop-wise for 30 minutes, maintaining the temperature at -15 °C while stirring. The final solution was allowed to warm up to 0 °C over 90 minutes, filtered, then washed with water (15 mL) then methanol (5 mL), and air dried to give an orange solid. Recrystallisation from DMF gave the 6-nitroquinoxalin-2-ol as a light brown solid (10.72 g, 74%); m.p 301-302 °C (Lit.[83] 298-302 °C); δ_H (400 MHz, DMSO-d_6) 7.44 – 7.46 (1H, d, *J* 8.7 Hz), 8.32 (1H, s), 8.37 – 8.40 (1H, dd, *J* 8.7 and 2.3 Hz) and 8.55 – 8.56 (1H, d, *J* 8.6 Hz); δ_C (100 MHz, DMSO-d_6) 111.40, 117.87, 130.66, 132.88, 135.76, 148.04, 155.03 and 155.96. Spectroscopic data agree with those reported in literature.

Synthesis of 2-benzensulfonyloxyquinoxaline (56a)[43]



In a round bottom flask, quinoxalinone **55a** (5 g, 34 mmol), DMAP (0.416 g, 3.4 mmol) and benzenesulfonyl chloride (8.72 mL, 68 mmol) were dissolved in DCM (100 mL), cooled to 0 °C and stirred for 5 minutes. Et_3N (12 mL, 88 mmol) was added drop-wise over 5 minutes, the solution allowed to stir at room temperature for 1 hour, and the reaction quenched with aqueous $NaHCO_3$ (80 mL). The two layers were separated and the aqueous layer washed with DCM (2 × 60 mL). The combined organic layers were dried over $MgSO_4$ filtered, concentrated and dissolved in EtOAc (15 mL). The filtrate was re-filtered and concentrated to give 2-benzenesulfonyloxyquinoxaline as a brown solid (8.23 g, 85%); m.p 89-93 °C (Lit[43]. 91 °C); δ_H (400 MHz, $CDCl_3$) 7.58 – 7.63 (2H, m), 7.69 – 7.77 (3H, m), 7.87 – 7.90 (1H, m), 8.09 – 8.17 (3H, m) and 8.67 ppm (1H, s). Spectroscopic data agree with those reported in literature.

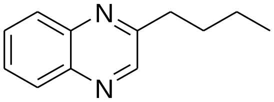
Synthesis of 2-phenylquinoxaline (59)[66]



$ZnCl_2$ (0.95 g, 6.99 mmol, 2.0 eq, taken directly from oven) was added to an oven dried 2-neck flask with a stirrer bar, which was further dried under vacuum for 15 minutes. Dry THF (10 mL) was added to the flask under dry nitrogen. The solution was left to stir until all dried $ZnCl_2$ was dissolved. Phenyl-MgBr (6.3 mL, 6.29 mmol, 1 M) was added to the solution and left to stir for 1 hour.

To a solution of Phenyl-ZnCl (6.29 mmol), 2-benzenesulfonyloxyquinoxaline **56a** (1 g, 3.49 mmol) and $PdCl_2(PPh_3)_2$ (122.6 mg, 0.18 mmol, 5 mol%) were added and set to reflux while stirring under nitrogen for 20 hours. The solution was allowed to cool to room temperature, diluted with EtOAc (25 mL), quenched with sat. $NaHCO_3$ (4 × 15 mL). The two layers were separated and the aqueous layer diluted with EtOAc (15 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated, and purified on silica gel eluting with 20% EtOAc/Hexane to give 2-phenylquinoxaline as a yellow solid (0.537 g, 75%); m.p 73-74 °C (Lit.[66] 74-75 °C); δ_H (400 MHz, $CDCl_3$) 7.46 – 7.52 (3H, m), 7.69 – 7.77 (2H, m), 8.12 – 8.15 (4H, m) and 9.27 (1H, s); δ_C (100 MHz, $CDCl_3$) 127.63, 128.54, 129.32, 129.51, 129.65, 130.58, 138.87, 132.16, 136.24, 140.41, 142.51 and 152.00; *m/z* (ES-API, +ve) 207.1 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

Synthesis of 2-butylquinoxaline (60)[67]



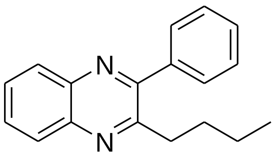
$ZnCl_2$ (475.7 mg, 3.49 mmol, 2.0 eq, taken directly from oven) was added to a pre-oven dried round bottom flask with a stirrer bar. Dry THF (20 mL) was added to the flask and left to stir until all $ZnCl_2$ was dissolved, under nitrogen. n-BuLi (1.4 mL, 3.49 mmol, 2.0 eq, 2.5 M) was added to the flask at -78 °C, left to stir for 1 hour at -78 °C. To the solution, 2-benzenesulfonyloxyquinoxaline **56a** (500 mg, 1.75 mmol) and $PdCl_2(PPh_3)_3$ (61.3 mg, 0.0874 mmol, 5 mol %) were added and set to reflux while stirring under nitrogen for 18 hours. The solution was allowed to cool to room temperature, diluted with EtOAc (12.5 mL) and quenched with sat. $NaHCO_3$ (2 × 15 mL). The two layers separated and the aqueous layer diluted with EtOAc (8 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, and purified on silica gel eluting with

10% EtOAc/Hexane to give 2-butylquinoxaline as an orange oil (200 mg, 61%); δ_H (400 MHz, $CDCl_3$) 1.00 (3H, t, *J* 7.4), 1.44 – 1.49 (2H, m), 1.79 – 1.86 (2H, m), 3.02 (2H, t, *J* 7.8 Hz), 7.70 – 7.74 (2H, m), 8.03 – 8.08 (2H, m) and 8.75 (1H, s); δ_C (100 MHz, $CDCl_3$) 14.13, 22.62, 31.61, 36.37, 128.94, 129.16, 129.29, 139.99, 141.26, 142.20, 145.84 and 157.73; *m/z* (ES-API, +ve) 188 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

General procedure for nucleophilic substitution on 2-Phenylquinoxaline (method 1)

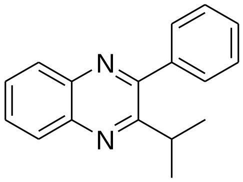
A solution of either an alkynyl or hetero-aryl substrate (0.97 mmol) in THF (5 ml) was lithiated with n-BuLi (0.4 mL, 0.97 mmol, 2 eq, 2.5 M) and left to stir for 15 minutes under a nitrogen atmosphere. 2-Phenylquinoxaline **59** (100 mg, 0.485 mmol) was added to the flask, and the solution allowed to stir at room temperature for 18 hours. The final solution was diluted with EtOAc (10 mL), quenched with sat. $NaHCO_3$ (2 × 10 mL). The organic layers were combined and dried over Na_2SO_4. Filtered, concentrated and purified over flash column chromatography on silica gel.

Synthesis of 3-butyl-2-phenylquinoxaline (59a)[68]



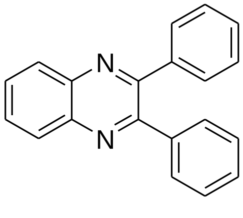
2-Phenylquinoxaline **59** (100 mg, 0.485 mmol) was treated with n-BuLi (0.3 mL, 0.728 mmol, 1.5 eq, 2.5 M) at -78 °C under nitrogen atmosphere. The solution was left to stir for 18 hours at room temperature, followed by aqueous work up and purification by flash column chromatography on silica gel, eluting with 5% EtOAc/hexane to give 3-butyl-2-phenylquinoxaline as tan oil (84 mg, 66%); δ_H (400 MHz, $CDCl_3$) 0.75 – 0.79 (3H, t, *J* 7.4 Hz); 1.18 – 1.27 (2H, m), 1.62 – 1.65 (2H, m), 2.96 – 2.99 (2H, t, *J* 7.8 Hz), 7.44 – 7.54 (5H, m), 7.65 – 7.68 (2H, m) and 8.01 – 8.06 (2H, m); δ_C (100 MHz, $CDCl_3$) 13.83, 22.62, 29.72, 31.25, 35.76, 128.45, 128.57, 128.86, 129.22, 129.27, 129.73, 139.11, 140.72, 141.32, 146.38, 155.12 and 156.35; *m/z* (ES-API,+ve) 263.1 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

Synthesis of 3-isopropyl-2-phenylquinoxaline (59b)[69]



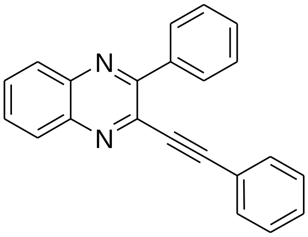
2-Phenylquinoxaline **59** (100 mg, 0.485 mmol) was treated with *i*PrMgCl (0.8 mL, 0.728 mmol, 1.5 eq, 0.9 M) at 0 °C under nitrogen atmosphere. The solution was left to stir for 18 hours, followed by aqueous work up and purification by flash column chromatography on silica gel, eluting with 5% EtOAc/hexane to give 2-isopropyl-3-phenylquinoxaline as yellowish solid (65.28 mg, 54%); m.p 96-98 °C (Lit.[69] 98-99 °C); δ_H (400 MHz, $CDCl_3$) 1.17 – 1.25 (6H, d, *J* 6.8 Hz), 3.39 – 3.46 (1H, m), 7.41 – 7.51 (5H, m), 7.61 – 7.68 (2H, m) and 8.03 – 8.05 (2H, m); δ_C (100 MHz, $CDCl_3$) 21.80, 30.99, 127.50, 127.66, 127.71, 127.79, 128.07, 128.18, 128.51, 138.12, 139.42, 140.66, 153.53 and 159.74; *m/z* (ES-API,+ve) 249.1 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

Synthesis of 2, 3-diphenylquinoxaline (59c)[70]



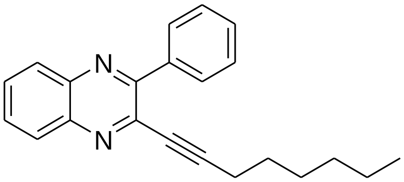
2-Phenylquinoxaline **59** (100 mg, 0.485 mmol) was treated with Phenyl-MgBr (0.64 mL, 0.728 mmol, 1.5 eq, 1.14 M) at 0 °C under nitrogen atmosphere. The solution was left to stir for 18 hours, followed by aqueous work up and purification by flash column chromatography on silica gel, eluting with 5% EtOAc/hexane to give 2,3-diphenylquinoxaline as orange solid (29.8 mg, 22%); m.p 126-128 °C (Lit.[70] 127-129 °C); δ_H (400 MHz, $CDCl_3$) 7.27 – 7.29 (6H, m), 7.44 – 7.52 (4H, d, *J* 7.4 Hz), 7.62 – 7.73 (2H, dd, *J* 8.7 and 2.3 Hz) and 8.05 – 8.13 (2H, dd, *J* 8.7 and 2.3 Hz); δ_C (100 MHz, $CDCl_3$) 126.88, 127.47, 127.91, 128.05, 129.12, 137.73, 140.01 and 152.38; *m/z* (ES-API,+ve) 283.1 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

Synthesis of 2-phenyl-3-(2-phenylethynyl)quinoxaline (59d)[71]



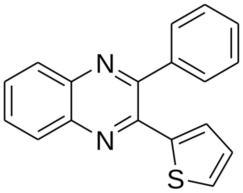
According to method 1, the resulting solution from phenylacetylene (0.1 mL, 0.97 mmol, 2.0 eq) was treated with **59**. Eluting with 10% EtOAc/hexane to give 2-phenyl-3-(2-phenylethynyl)quinoxaline as light brown solid (69.2 mg, 47%); m.p 110-112 °C (Lit.[71] 109-11 °C); δ_H (400 MHz, $CDCl_3$) 7.33 – 7.39 (3H, m), 7.48 – 7.50 (2H, d, *J* 6.8 Hz), 7.56 – 7.57 (3H, d, *J* 6.8 Hz), 7.77 – 7.79 (2H, m) and 8.09 – 8.15 (4H, m); δ_C (100 MHz, $CDCl_3$) 87.31, 94.06, 120.66, 127.15, 127.44, 127.74, 128.29, 128.60, 128.67, 128.71, 129.31, 129.69, 131.11, 136.60, 137.08, 139.70, 139.99 and 154.10; *m/z* (ES-API,+ve) 307.1 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

Synthesis of 3-(oct-1-ynyl)-2-phenylquinoxaline (59e)



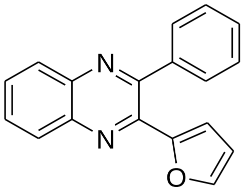
According to method 1, the resulting solution from 1-octyne (0.14 mL, 0.97 mmol, 2.0 eq) was treated with **59**. Eluting with 10% EtOAc/hexane to give *2-(oct-1-ynyl)-3-phenylquinoxaline* as orange oil (20.5 mg, 14%); δ_H (400 MHz, $CDCl_3$) 0.86 – 0.89 (3H, t, *J* 6.8 Hz), 1.25 – 1.32 (4H, m), 1.52 – 1.60 (4H, m), 2.42 – 2.46 (2H, t, *J* 7.1 Hz), 7.50 – 7.52 (3H, m), 7.74 – 7.76 (2H, m) and 8.02 – 8.03 (4H, m) ; δ_C (100 MHz, $CDCl_3$) 14.10, 19.76, 22.53, 27.81, 28.64, 29.72, 31.35, 97.92, 128.08, 128.64, 129.23, 129.53, 129.58, 130.13, 130.36, 137.75, 138.50, 140.60,140.93 and 154.92; HRMS (ES): MH^+ calcd for $[C_{22}H_{22}N_2]^+$: 315.1783, found: 315.1861. To our knowledge compound **59e** has not been reported in literature.

Synthesis of 2-phenyl-3-(thiophen-2-yl)quinoxaline (59f)[72]



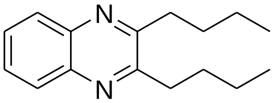
According to method 1, the resulting solution from thiophene (0.08 mL, 0.97 mmol, 2.0 eq) was treated with **59**. Eluting with 10% EtOAc/hexane to give 2-phenyl-3-(thiophen-2-yl)quinoxaline as a lime solid (30.5 mg, 22%); m.p 126-128 °C (Lit.[72] 128 °C) ; δ_H (400 MHz, $CDCl_3$) 6.75 – 6.76 (1H, d, *J* 3.6 Hz), 6.86 – 6.88 (1H, m), 7.40 – 7.41 (1H, d, *J* 4.8 Hz), 7.48 – 7.54 (3H, m), 7.59 – 7.61 (2H, m), 7.70 – 7.79 (2H, m) and 8.08 – 8.12 (2H, m); δ_C (100 MHz, $CDCl_3$) 127.78, 128.76, 128.85, 129.07, 129.14, 129.25, 129.34, 129.77, 129.95, 130.23, 139.42, 140.45, 141.14, 142.74, 146.91 and 152.58; *m/z* (ES-API,+ve) 289.1 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

Synthesis of 2-(furan-2-yl)-3-phenylquinoxaline (59g)[73]



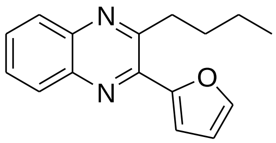
According to method 1, the resulting solution from furan (0.08 mL, 0.97 mmol, 2.0 eq) was treated with **59**. Eluting with 10% EtOAc/hexane to give 2-(*furan*-2-*yl*)-3-*phenylquinoxaline* as brown solid (60.6 mg, 46%); m.p 102-103 °C (Lit.[73] 103-104 °C); δ_H (400 MHz, $CDCl_3$) 6.14 – 6.15 (1H, d, *J* 3.5 Hz), 6.35 – 6.36 (1H, m), 7.50 – 7.59 (6H, m), 7.71 – 7.79 (2H, m), 8.10 – 8.12 (1H, dd, *J* 8.0 and 1.1 Hz) and 8.20 – 8.28 (1H, dd, *J* 8.0 and 1.1 Hz); δ_C (100 MHz, $CDCl_3$) 111.79, 114.88, 128.70, 128.82, 129.07, 129.13, 129.25, 129.96, 130.35, 139.28, 140.35, 141.01, 143.09, 144.74, 150.88 and 152.57; *m/z* (ES-API,+ve) 273.1 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

Synthesis of 2, 3-dibutylquinoxaline (60a)[74]



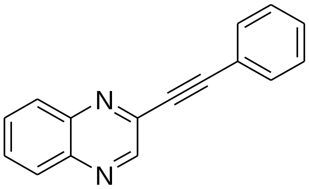
2-butylquinoxaline **60** (50 mg, 0.269 mmol) was treated with n-BuLi (0.32 mL, 0.807 mmol, 3.0 eq, 2.5 M) at -78 °C under nitrogen atmosphere. The solution was left to stir for 18 hours at room temperature, followed by aqueous work up and purification by flash column chromatography on silica gel, eluting with 10% EtOAc/hexane to give 2,3-dibutylquinoxaline as brown oil (63 mg, 97%); δ_H (400 MHz, $CDCl_3$) 0.89 – 1.11 (6H, t, *J* 7.4 Hz), 1.49 – 1.53 (4H, m), 2.99 – 3.03 (4H, t, *J* 7.4 Hz), 7.63 – 7.66 (2H, m) and 7.98 – 8.00 (2H, m); δ_C (100 MHz, $CDCl_3$) 14.01, 22.91, 31.05, 35.21, 128.47, 128.69, 140.99 and 156.72. Physical and spectroscopic data agree with those reported in literature.

Synthesis of 2-butyl-3-(furan-2-yl)quinoxaline (60b)



Furan (0.06 mL, 0.807 mmol, 3.0 eq) was treated with n-BuLi (0.32 mL, 0.807 mmol, 3.0 eq, 2.5 M) at -78 °C under nitrogen atmosphere. The resulting solution from furan was treated with **60** and the solution was left to stir for 18 hours at room temperature, followed by aqueous work up and purification by flash column chromatography on silica gel, eluting with 10% EtOAc/hexane to give *2-butyl-3-(furan-2-yl)quinoxaline* as brown oil (62 mg, 91%); δ_H (400 MHz, $CDCl_3$) 0.97 – 1.01 (3H, t, *J* 7.4 Hz), 1.48 – 1.54 (2H, m), 1.78 – 1.84 (2H, m), 3.29 – 3.33 (2H, t, *J* 7.8 Hz), 6.63 – 6.64 (1H, m), 7.22 – 723 (1H, d, *J* 3.5 Hz), 7.68 – 7.71 (3H, m) and 8.01 – 8.10 (2H, m) ; δ_C (100 MHz, $CDCl_3$) 14.01, 22.87, 30.81, 36.69, 112.12, 113.96, 128.41, 128.93, 129.41, 129.62 and 144.49. HRMS (ES): MH^+ calcd for $[C_{16}H_{16}N_2O]^+$: 253.1263, found: 253.1339. To our knowledge compound **60b** has not been reported in literature.

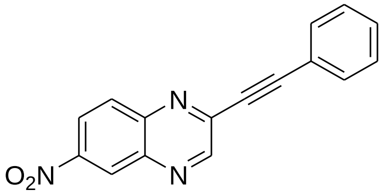
Synthesis of 2-(2-phenylethynyl)quinoxaline (61a)[75]



Under a nitrogen atmosphere, in a round bottom flask equipped with a stirrer bar, 2-benzenesulfonyloxyquinoxaline **56a** (0.301 g, 1.05 mmol), $PdCl_2(PPh_3)_2$ (38.0 mg, 0.052 mmol, 5 mol%), Et_3N (0.2 mL, 2.1 mmol, 2 eq.) and phenylacetylene (154 µL, 1.26 mmol, 1.2 eq) were dissolved in THF (10 mL). The solution was refluxed for 18 hours, under nitrogen followed by aqueous work up and purification by flash column chromatography on silica gel, eluting with 1:9 EtOAc/n-hexane to give 2-(2-phenylethynyl)quinoxaline as a yellowish solid (0.178 g, 61%); m.p 63-66°C (Lit.[75] 65-66

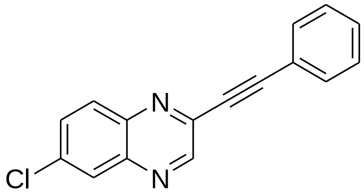
°C); δ_H (400 MHz, $CDCl_3$) 7.42 – 7.45 (3H, m), 7.69 – 7.71 (2H, m), 7.78 – 7.82 (2H, m), 8.11 – 8.12 (2H, m) and 8.99 (1H, s); δ_C (100 MHz, $CDCl_3$) 121.41, 128.58, 129.05, 129.19, 129.23, 129.27, 129.80, 130.45, 130.73, 132.40, 139.14, 139.62, 140.92, 142.22 and 147.36; *m/z* 231 (M+1). Physical and spectroscopic data agree with those reported in literature.

Synthesis of 6-nitro-2-(2-phenylethynyl)quinoxaline (61b)



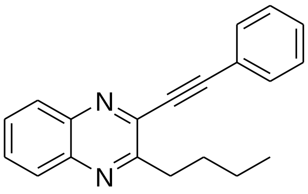
Under a nitrogen atmosphere, in a round bottom flask equipped with a stirrer bar, 6-nitro-2-benzenesulfonyloxyquinoxaline **56b** (0.301 g, 1.05 mmol), $PdCl_2(PPh_3)_2$ (38.0 mg, 0.052 mmol, 5 mol%), Et_3N (0.2 mL, 2.1 mmol, 2 eq.) and phenylacetylene (154 µL, 1.26 mmol, 1.2 eq) were dissolved in THF (10 mL). The solution was refluxed for 18 hours under nitrogen, followed by aqueous work up and purification by flash column chromatography on silica gel, eluting with 1:9 EtOAc/n-hexane to *give 6-nitro-2-(2-phenylethynyl)quinoxaline* as a yellowish solid (0.191 g, 77%); m.p 76-79°C (Lit.[76] 65-66 °C); δ_H (400 MHz, $CDCl_3$) 7.43 – 7.50 (3H, m), 7.70 – 7.72 (2H, m), 8.23 – 8.25 (1H, d, *J* 3.4), 8.55 – 8.58 (1H, dd, *J* 9.0 and 2.4 Hz), 8.99 – 9.00 (1H, d, *J* 8.6 Hz) and 9.10 (1H, s); δ_C (100 MHz, $CDCl_3$) 86.63, 91.20, 110.89, 111.61, 120.24, 121.44, 124.64, 127.17, 129.06, 129.76, 130.87, 141.16 and 147.72. To our knowledge compound **61b** has not been reported in literature.

Synthesis of 6-chloro-2-(2-phenylethynyl)quinoxaline (61c)[76]



Under a nitrogen atmosphere, in a round bottom flask equipped with a stirrer bar, 6-chloro-2-benzenesulfonyloxyquinoxaline **56c** (500 mg, 1.55 mmol), $PdCl_2(PPh_3)_2$ (56.6 mg, 0.0775 mmol, 5 mol%), Et_3N (0.27 mL, 1.86 mmol, 1.2 eq.) and phenylacetylene (0.23 mL, 1.86 mmol, 1.2 eq) were dissolved in THF (10 mL). The solution was refluxed for 18 hours, under nitrogen, followed by aqueous work up and purification by flash column chromatography on silica gel eluting with 1:9 EtOAc/n-hexane to give 6-chloro-2-(2-phenylethynyl)quinoxaline as a grey solid (285.5 mg, 70%); m.p 116-118 °C (Lit.[76] 118-120 °C); δ_H (400 MHz, $CDCl_3$) 7.43 – 7.42 (2H, m), 7.72 – 7.67 (3H, m), 7.75 – 7.74 (1H, dd, *J* 9.0 and 2.4 Hz), 8.04 – 8.02 (1H, d, *J* 2.3 Hz), 8.09 – 8.08 (1H, d, *J* 8.6 Hz) and 8.96 (1H, s); δ_C (100 MHz, $CDCl_3$) 90.12, 94.80, 123.92, 128.09, 128.63, 129.44, 132.14,132.55, 134.36, 135.66, 137.30, 139.29, 145.23 and 149.40. Physical and spectroscopic data agree with those reported in literature.

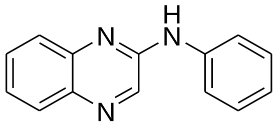
Synthesis of 3-butyl-2-(2-phenylethynyl)quinoxaline (62)



2-(2-phenylethynyl)quinoxaline **61a** (100 mg, 0.434 mmol) was treated with n-BuLi (0.5 mL, 0.302 mmol, 3 eq, 2.5 M) at -78 °C under nitrogen atmosphere. The solution was left to stir for 36 hours at room temperature, followed by aqueous work up and purification by flash column chromatography on silica gel eluting with 10%

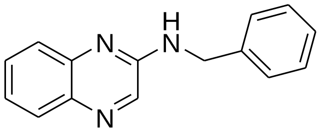
EtOAc/hexane to give *3-butyl-2-(2-phenylethynyl)quinoxaline* as orange solid (69.1 mg, 56%); m.p 76-78 °C ; δ_H (400 MHz, $CDCl_3$) 0.93 – 0.96 (3H, t, *J* 7.4 Hz), 1.44 – 1.50 (2H, m), 1.82 – 1.89 (2H, m), 3.19 – 3.23 (2H, t, *J* 7.8 Hz), 7.35 – 7.37 (3H, m), 7.60 – 7.66 (4H, m) and 7.95 – 8.97 (2H, m); δ_C (100 MHz, $CDCl_3$) 14.02, 22.90, 29.72, 30.93, 36.34, 86.87, 94.90, 121.74, 128.62, 128.84, 129.51, 129.70, 130.34, 132.24, 139.52, 140.73,140.85 and 158.86. HRMS (ES): MH^+ calcd for $[C_{20}H_{18}N_2]^+$: 287.1470, found: 287.1545. To our knowledge compound **62** has not been reported in literature.

Synthesis of N-phenylquinoxalin-2-amine (63a)[79]



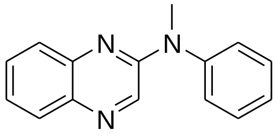
In a 50 mL 2-neck round bottom flask equipped with a stirrer bar under nitrogen, 2-benzenesulfonyloxyquinoxaline **56a** (100 mg, 0.349 mmol), $Pd(OAc)_2$ (4 mg, 0.0175 mmol, 5 mol %), Brett-Phos (9.4 mg, 0.0175 mmol, 5 mol %) and aniline (0.1 mL, 1.047 mmol, 3.0 eq) were dissolved in 1,4-dioxane (5 mL). Pyridine (0.08 mL, 1.047 mmol, 3.0 eq) was added to the solution, and refluxed for 18 hours. The solution was diluted with EtOAc (10 mL), and quenched with sat. $NaHCO_3$ (2 × 10 mL). The organic layers were combined and dried over Na_2SO_4, filtered then concentrated and purified by flash column chromatography on silica gel eluting with 40% EtOAc/hexane and on prep TLC with 5% MeOH/DCM to give N-phenylquinoxalin-2-amine as yellowish solid (40 mg, 52%); m.p 135-137 °C (Lit.[79] 137°C); δ_H (400 MHz, $CDCl_3$) 7.08 (1H, s), 7.14 – 7.17 (1H, m), 7.40 – 7.45 (2H, m), 7.48 – 7.52 (1H, m), 7.64 – 7.68 (1H, m), 7.76 – 7.97 (3H, m) and 8.47 (1H, s); δ_C (100 MHz, $CDCl_3$) 119.85, 123.59, 125.65, 126.95, 128.83, 129.32, 130.37, 137.84, 138.48, 139.21, 141.19 and 149.32. *m/z* (ES-API,+ve) 222.1 (M+1,100). Physical and spectroscopic data agree with those reported in literature.

Synthesis of N-benzylquinoxalin-2-amine (63b)



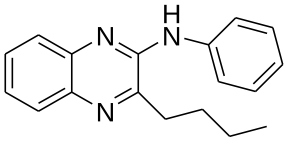
In a 50 ml 2-neck round bottom flask equipped with a stirrer bar under nitrogen, 2-benzenesulfonyloxyquinoxaline **56a** (200 mg, 0.698 mmol), $Pd(OAc)_2$ (7.83 mg, 0.0349 mmol, 5 mol %), Brett-Phos (18.73 mg, 0.0349 mmol, 5 mol %) and benzylamine (0.3 mL, 2.094 mmol, 3.0 eq) were dissolved in 1,4-dioxane (5 mL). Potassium tert-butoxide (9.5 mL, 2.094 mmol, 3.0 eq, 0.22 M) was added to the solution, and refluxed for 18 hours. The solution was diluted with EtOAc (10 mL), and quenched with sat. $NaHCO_3$ (2×10 mL). The organic layers were combined and dried over Na_2SO_4, filtered then concentrated and purified by flash column chromatography on silica gel eluting with 40% EtOAc/hexane to give *N-benzylquinoxalin-2-amine* as an orange oil (155 mg, 94%); δ_H (400 MHz, $CDCl_3$) 4.74 – 4.76 (2H, d, *J* 3.4), 5.15 (1H, s), 7.28 – 7.43 (6H, m), 7.52 – 7.58 (1H, m), 7.71 – 7.87 (2H, m) and 8.21 (1H, s); δ_C (100 MHz, $CDCl_3$) 45.39, 124.54, 126.32, 127.72, 127.88, 127.89, 128.06, 128.07, 128.89, 129.19, 135.18, 137.31, 141.9 and 155.9. *m/z* (ES-API, +ve) 236.1 (M+1, 100). HRMS (ES): MH^+ calcd for $[C_{15}H_{13}N_3]^+$: 236.1109, found: 236.1189. To our knowledge **63b** has not been reported in literature.

Synthesis of N-methyl-N-phenylquinoxalin-2-amine (63c)



2-Quinoxalineamine **63a** (200 mg, 0.904 mmol) was dissolved in THF (20 mL) and was treated with NaH (43 mg, 1.81 mmol, 2.0 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 sat. NH_4Cl 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, $CDCl_3$) 3.60 (3H, s), 7.32 – 7.34 (3H, m), 7.38 – 7.43 (1H, m), 7.45 – 7.49 (2H, m), 7.58 – 7.62 (1H, m), 7.76 – 7.88 (2H, m) and 8.27 (1H, s); δ_C (100 MHz, $CDCl_3$) 36.68, 124.83, 126.42, 126.57, 126.82, 128.76, 128.76, 130.02, 130.23, 136.92, 141.67, 144.96 and 152.20. HRMS (ES): MH^+ calcd for $[C_{15}H_{13}N_3]^+$: 236.1109, found: 236.1191. To our knowledge compound **63c** has not been reported literature.

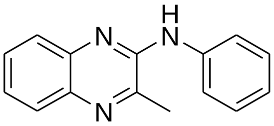
Synthesis of 3-butyl-N-phenylquinoxalin-2-amine (64a)



2-Quinoxalineamine **63a** (50 mg, 0.226 mmol) was treated with n-BuLi (0.3 mL, 0.678 mmol, 3 eq, 2.5 M) at -78 °C under nitrogen atmosphere. The solution was left to stir for 36 hours at room temperature, followed by aqueous work up and purification by flash column chromatography on silica gel eluting with 10% EtOAc/hexane to give *3-butyl-N-phenylquinoxalin-2-amine* as orange solid (22 mg, 32%); m.p 99-102 °C ; δ_H (400 MHz, $CDCl_3$) 0.95 – 0.98 (3H, t, *J* 7.4 Hz), 1.46 – 1.51 (2H, m), 1.81 – 1.89 (2H, m), 2.87 – 2.91 (2H, t, *J* 7.8 Hz), 6.68 (1H, s), 7.03 – 7.06 (1H, m), 7.32 – 7.41 (3H, m), 7.48 – 7.52

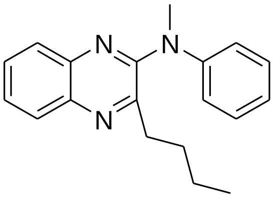
(1H, m) and 7.72 – 7.83 (4H, m); δ_C (100 MHz, $CDCl_3$) 14.03, 22.78, 28.91, 33.94, 119.85, 123.17, 125.45, 126.68, 128.17, 129.07, 137.51, 138.25, 139.44, 140.25, 147.58 and 148.01. HRMS (ES): MH^+ calcd for $[C_{18}H_{19}N_3]^+$: 278.1579, found: 278.1659. To our knowledge **64a** has never been reported in literature.

Synthesis of 3-methyl-N-phenylquinoxalin-2-amine (64b)[81]



2-Quinoxalineamine **63a** (50 mg, 0.226 mmol) was treated with Me-MgCl (0.3 mL, 3 eq, 3.0 M) at 0 °C under nitrogen atmosphere. The solution was left to stir for 36 hours at room temperature, followed by aqueous work up and purification by flash column chromatography on silica gel eluting with 10% EtOAc/hexane to give 3-methyl-N-phenylquinoxalin-2-amine as orange oil (22 mg, 42%); δ_H (400 MHz, $CDCl_3$) 2.43 (3H, s), 6.61 (1H, s), 7.39 – 7.41 (2H, m), 7.45 – 7.48 (2H, m), 7.53 – 7.57 (3H, m), 7.94 – 7.97 (1H, m) and 8.09 – 8.12 (1H, m); δ_C (100 MHz, $CDCl_3$) 15.32, 116.36, 119.22, 124.82, 125.44, 128.34, 128.82, 129.63, 136.44, 140.28, 143.62, 145.99 and 160.32. Physical and spectroscopic data agree with those reported in literature.

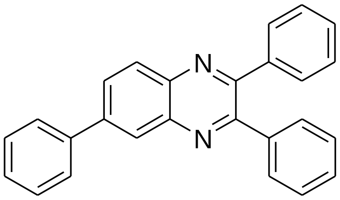
Synthesis of 3-butyl-N-methyl-N-phenylquinoxalin-2-amine (64c)



N-Methyl-N-phenylquinoxalin-2-amine **63c** (50 mg, 0.213 mmol) was treated with n-BuLi (0.26 mL, 0.639 mmol, 3 eq, 2.5 M) at -78 °C under nitrogen atmosphere. The solution was left to stir for 36 hours at room temperature, followed by aqueous work up and purification by flash column chromatography on silica gel eluting with 10%

EtOAc/hexane to give *3-butyl-N-methyl-N-phenylquinoxalin-2-amine* as yellow oil (52.2 mg, 62%); δ_H (400 MHz, $CDCl_3$) 0.75 – 0.79 (3H, t, *J* 7.4 Hz), 1.09 – 1.19 (2H, m), 1.52 – 1.58 (2H, m), 2.36 – 2.40 (2H, t, *J* 7.8 Hz), 3.55 (1H, s), 6.97 – 6.99 (2H, m), 7.10 – 7.14 (1H, m), 7.28 – 7.34 (2H, m), 7.51 – 7.56 (1H, m), 7.60 – 7.63 (1H, m), 7.86 – 7.89 (1H, d) and 7.92 – 7.94 (1H, d); δ_C (100 MHz, $CDCl_3$) 13.84, 22.56, 29.78, 35.44, 41.73, 122.96, 124.33, 126.66, 127.05, 127.97, 128.81, 129.62, 138.83, 140.29, 148.92, 153.34 and 154.28. HRMS (ES): MH^+ calcd for $[C_{19}H_{21}N_3]^+$: 292.1735, found: 292.1817. To our knowledge **64c** has never been reported in literature.

Synthesis of 2, 3, 6-triphenylquinoxaline (65)[82]



$ZnCl_2$ (845 mg, 6.2 mmol, 2.0 eq, taken directly from oven) was added to an oven dried 2-neck flask with a stirrer bar, which was further dried under vacuum for 15 minutes. Dry THF (10 mL) was added to the flask under dry nitrogen. The solution was left to stir until all dried $ZnCl_2$ was dissolved. Phenyl-MgBr (7 mL, 5.598 mmol, 0.8 M) was added to the solution and left to stir for 1 hour.

To a solution of Phenyl-ZnCl (6.2 mmol), 6-chloro-benzenesulfonyloxyquinoxaline **56c** (1 g, 3.11 mmol) and $PdCl_2(PPh_3)_2$ (113.63 mg, 0.155 mmol, 5 mol%) were added and set to reflux while stirring under nitrogen for 20 hours. The solution was allowed to cool to room temperature, diluted with EtOAc (25 mL), and quenched with sat. $NaHCO_3$ (4 × 15 mL). The two layers were separated and the aqueous layer diluted with EtOAc (15 mL). The combined organic layers were dried over Na_2SO_4, filtered then concentrated, and purified by flash column chromatography on silica gel eluting with 20% EtOAc/Hexane to give 2,3,6-triphenylquinoxaline as brown oil (401.9 mg, 36%); δ_H (400 MHz, $CDCl_3$) 6.89 – 6.91 (2H, m), 7.18 – 7.22 (3H, m), 7.28 – 7.30 (2H, m), 7.35 – 7.39 (3H, m), 7.43 – 7.47 (2H, m), 7.51 – 7.54 (3H, m), 7.57 – 7.61 (1H, m) and 7.75 – 7.77

(2H, dd, *J* 8.3 and 1.1 Hz); δ_C (100 MHz, $CDCl_3$) 126.42, 128.54, 131.36, 136.51, 139.72, 142.22, 144.86 and 153.17. Physical and spectroscopic data agree with those reported elsewhere.[83]

4.4 Biological studies

4.4.1 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.

4.4.2 Tuberculosis (TB) assay

Mycobacterium tuberculosis (Mtb.) $H_{37}Rv$ strains were isolated into tissue culture plates in liquid medium with 0.02% Tween 80 and 0.5% albumin using a visual readout after 14 days of growth. The plates were treated with different concentrations (0.244-125 µM), to determine the minimum inhibition concentration required to inhibit 90% (MIC_{90}) and 99% (MIC_{99}) growth of *Mtb*-$H_{37}Rv$ strains.

4.4.3 Malaria (PLDH) assay

Malaria parasites (*Plasmodium falciparum* strain 3D7) were maintained in RPMI 1640 medium containing 2 mM L-glutamine and 25 mM Hepes (Lonza). The medium was further supplemented with 5% Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamycin and 2-4% hematocrit human red blood cells. The parasites were cultured at 37 ^{o}C under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2 in sealed T25 or T75 culture flasks.

For screening compounds against malaria parasites, compounds at 20 µM or 25 µg/mL (natural extracts) were added to parasite cultures in 96-well plates and incubated for 48 hours in a 37 ^{o}C CO_2 incubator. After 48h the plates were removed from the incubator. Twenty µL of culture was 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 is formed when PLDH is present, and this product can be quantified in a 96-well plate reader by absorbance at 620 nm (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.

CHAPTER

5

Chapter 5: References

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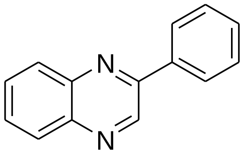
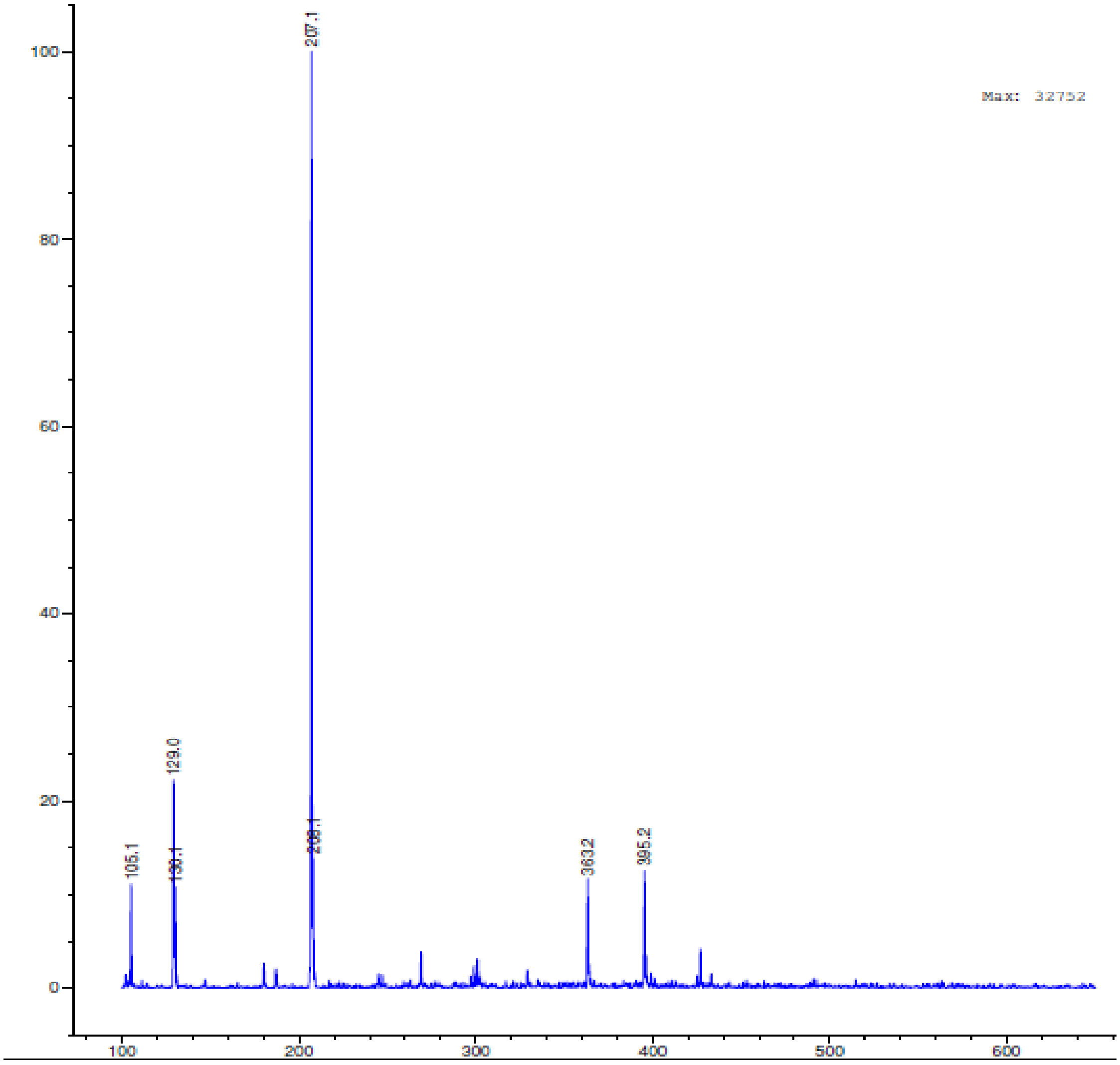
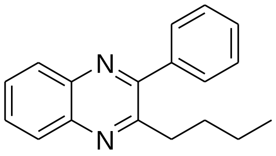
APPENDIX

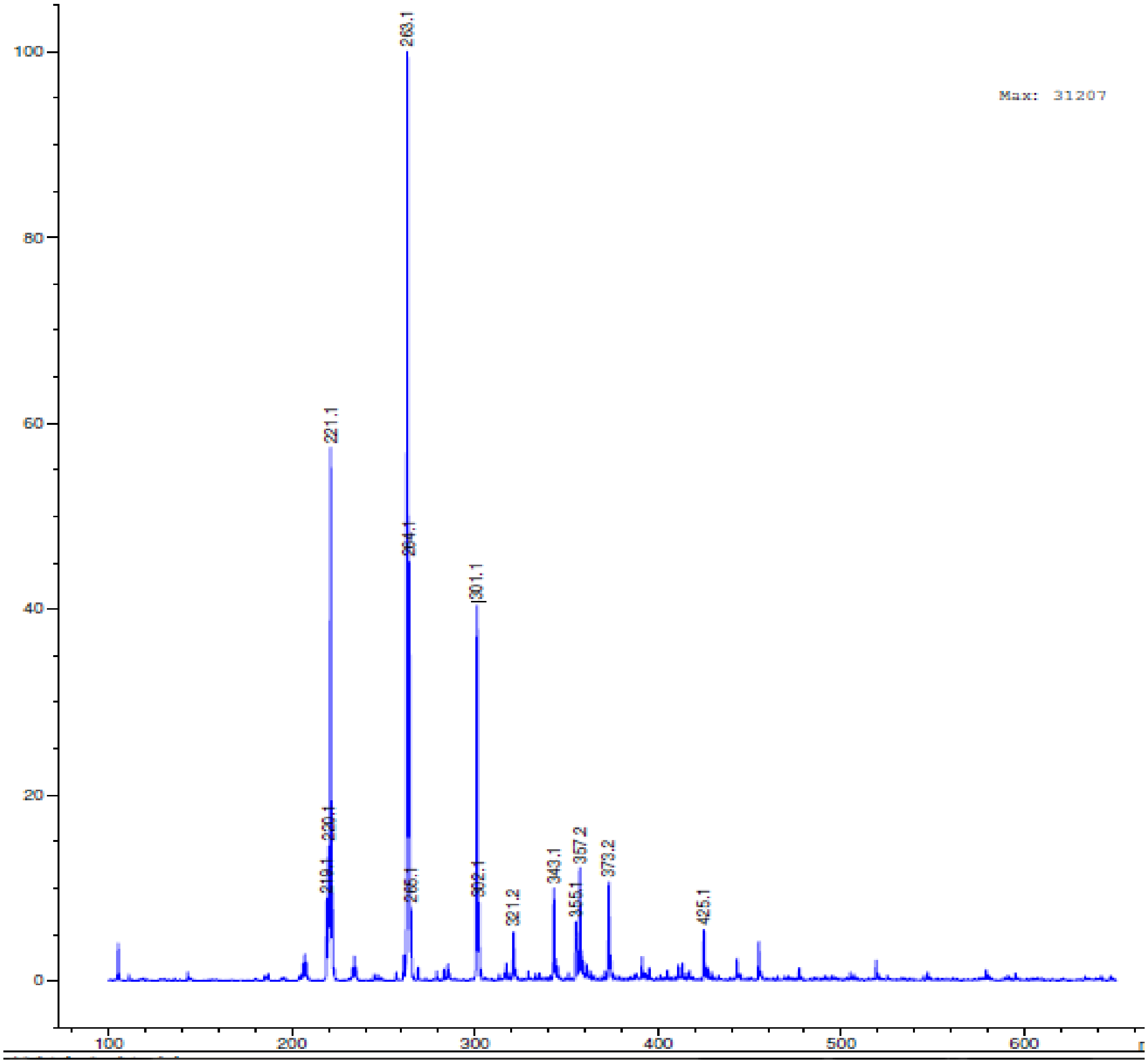
Mass spectra of **59**, **59a**, **59e**, **59f**, **59g**, **63b**, **63c** and **64c**



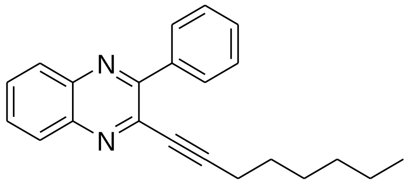


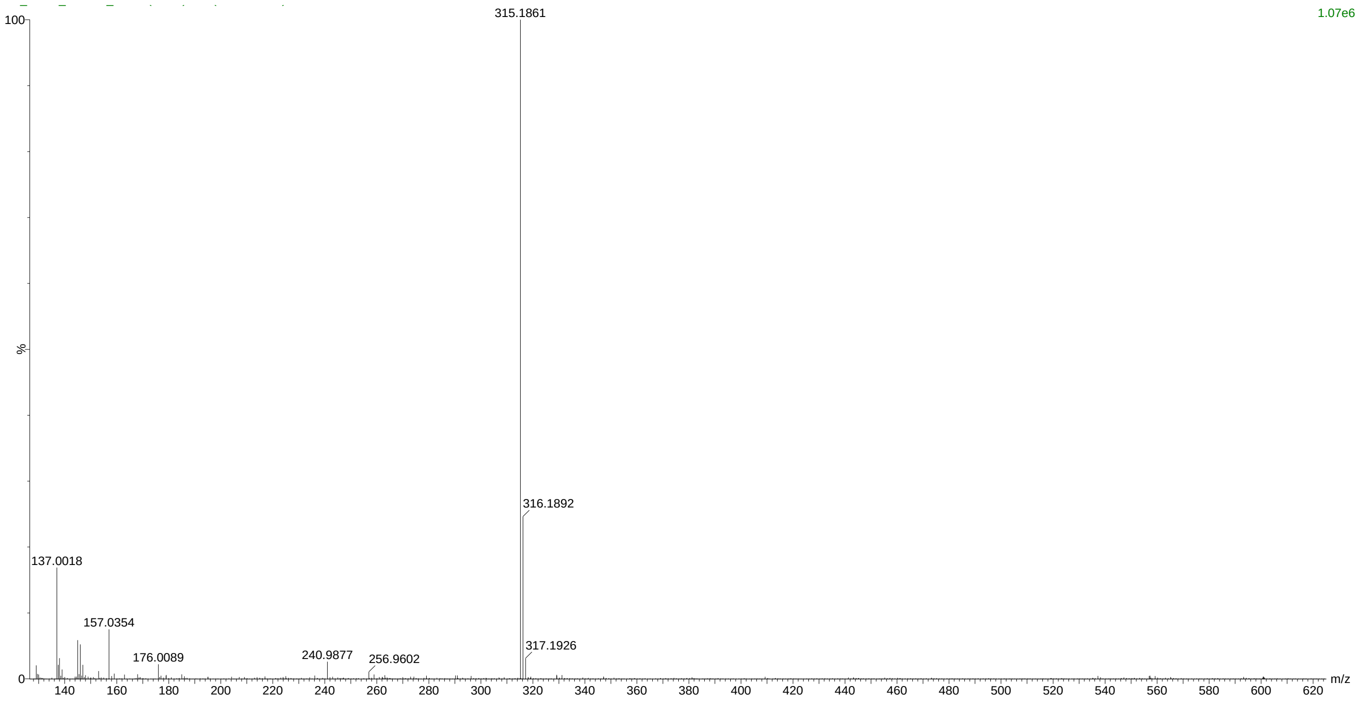
2-phenylquinoxaline **59**



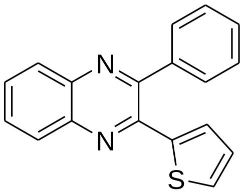


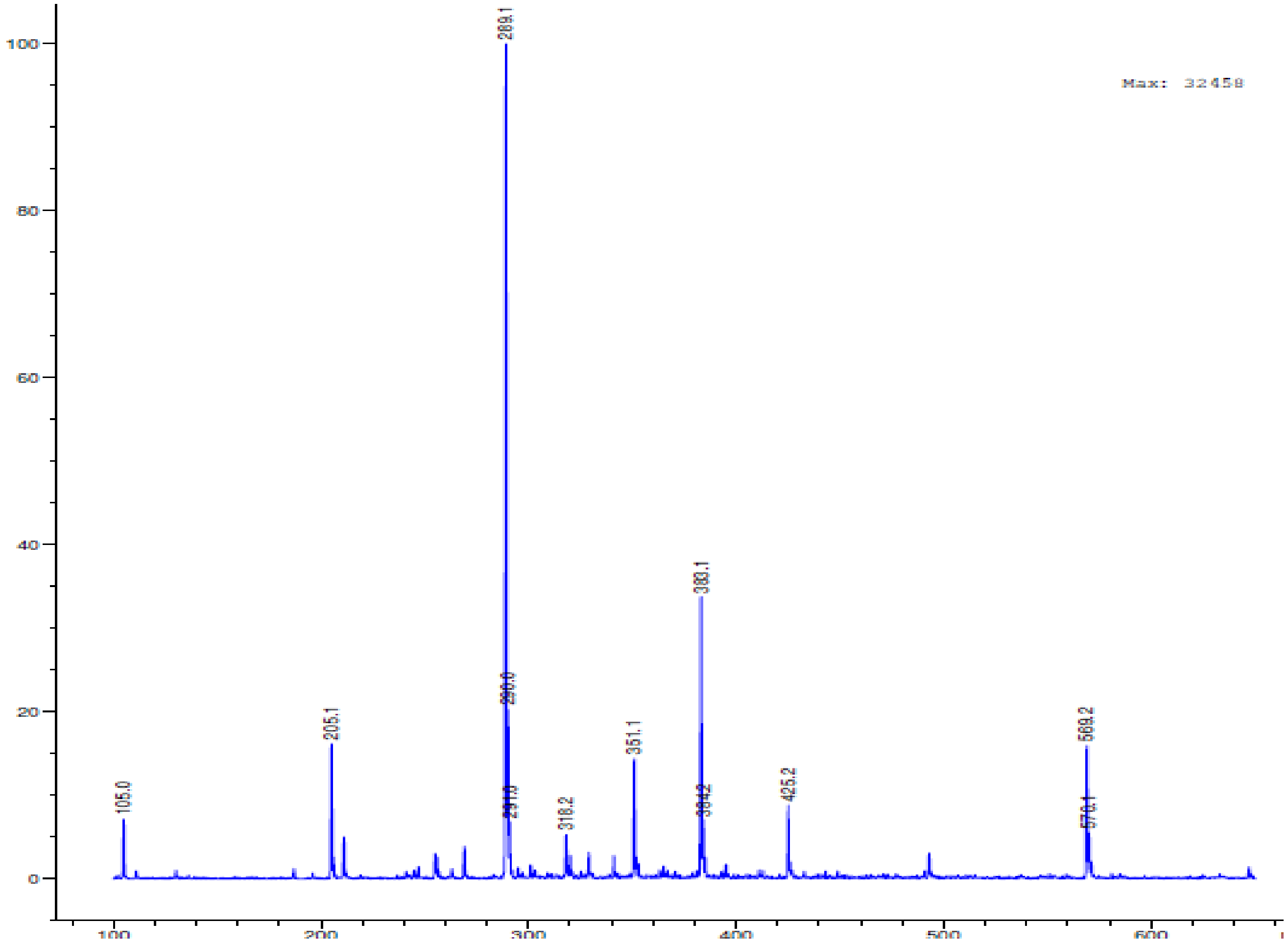
3-butyl-2-phenylquinoxaline **59a**



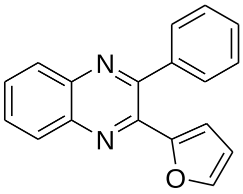


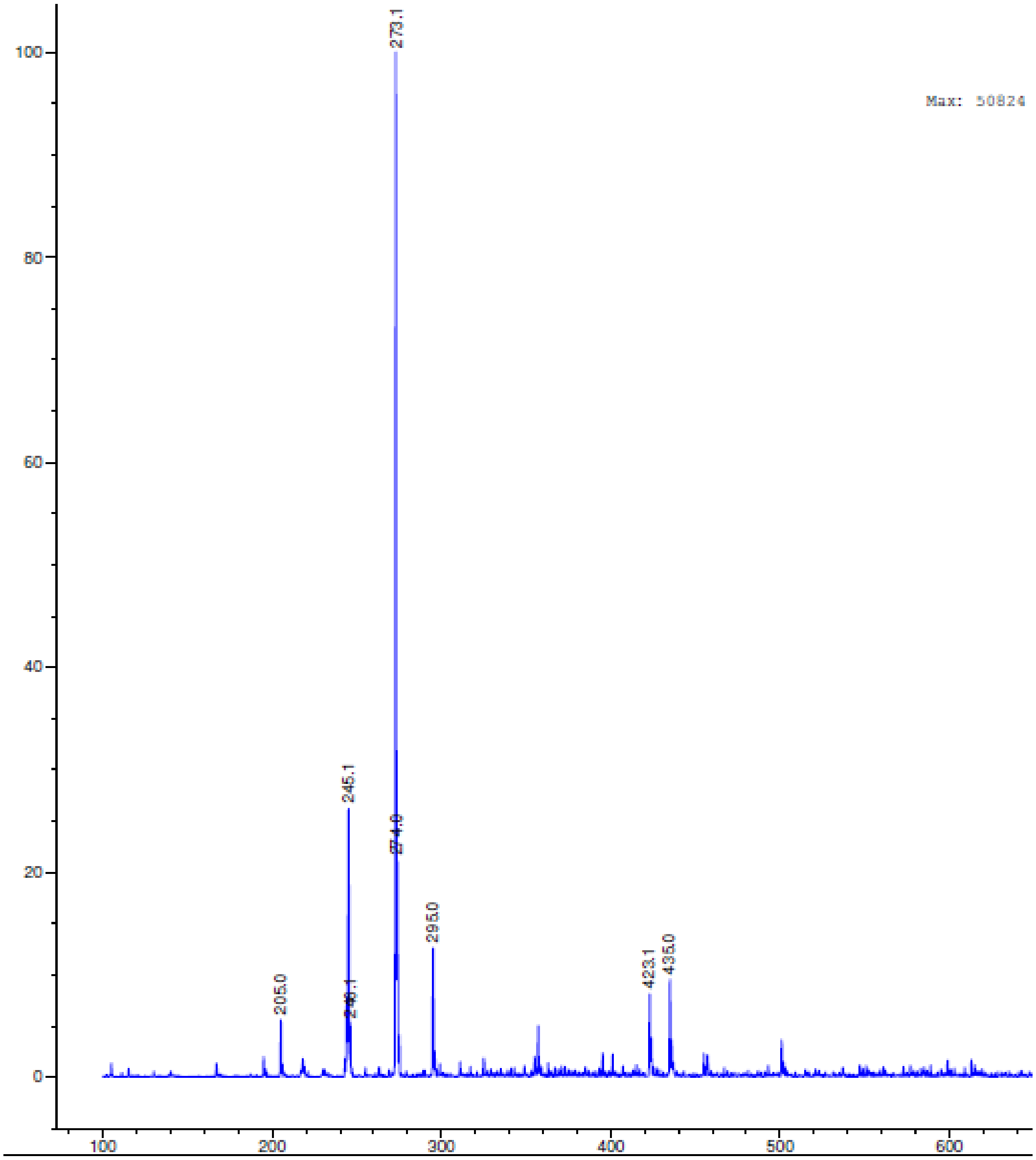
3-(oct-1-ynyl)-2-phenylquinoxaline **59e**



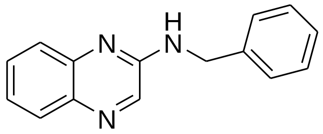


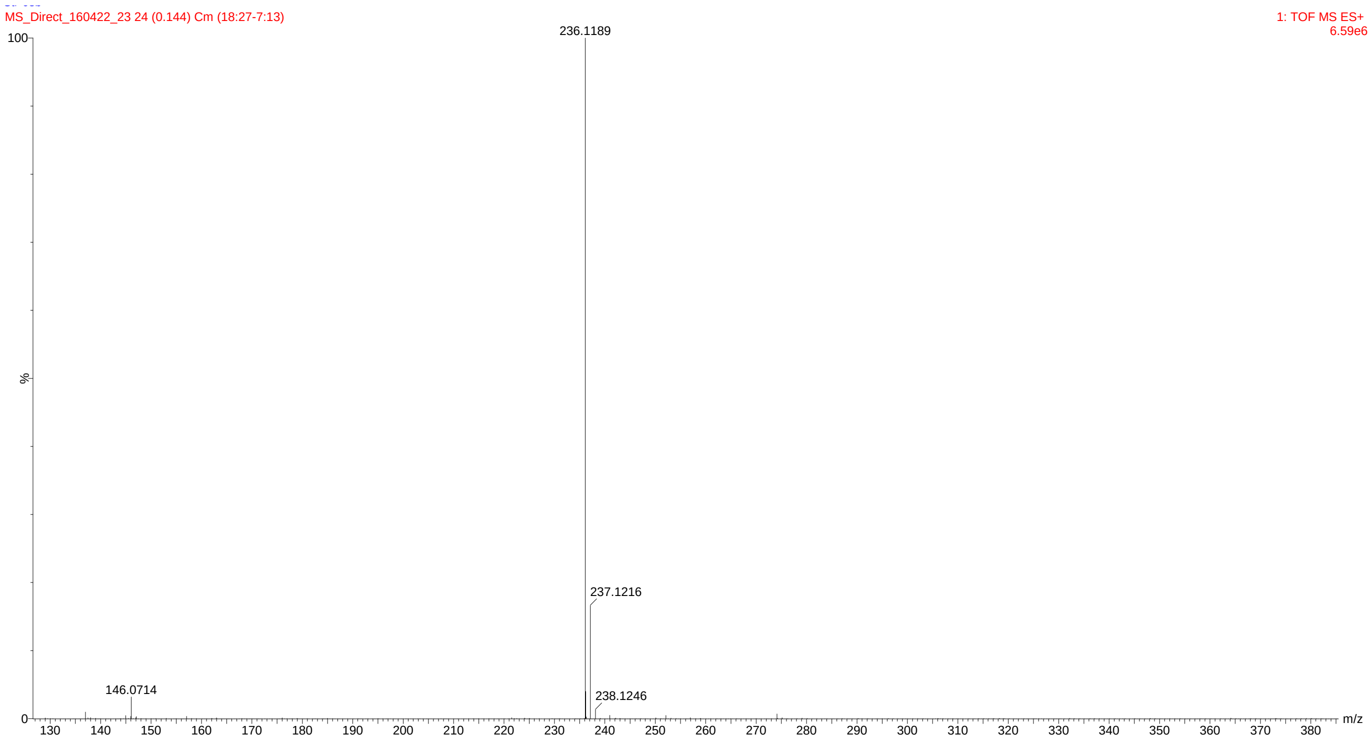
2-phenyl-3-(thiophen-2-yl)quinoxaline **59f**



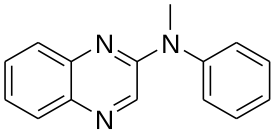


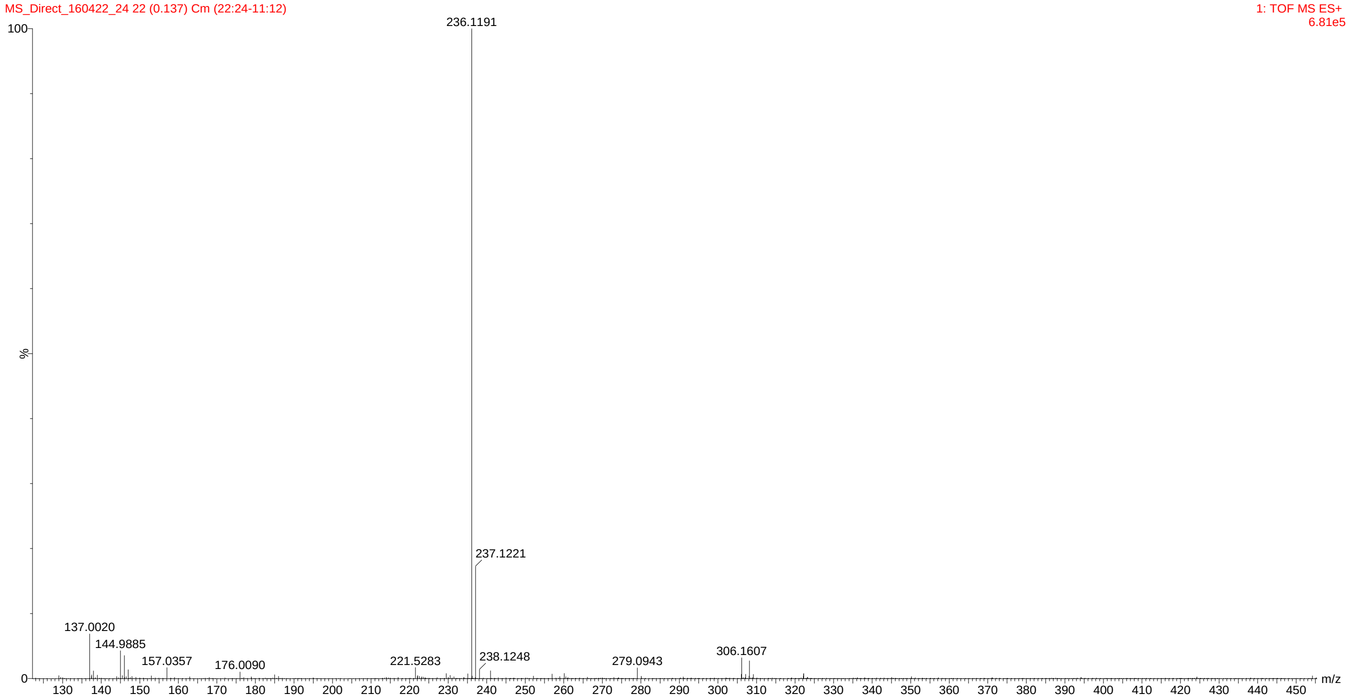
2-(furan-2-yl)-3-phenylquinoxaline **59g**



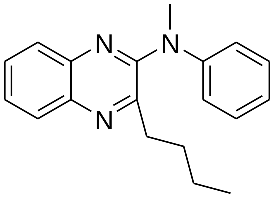


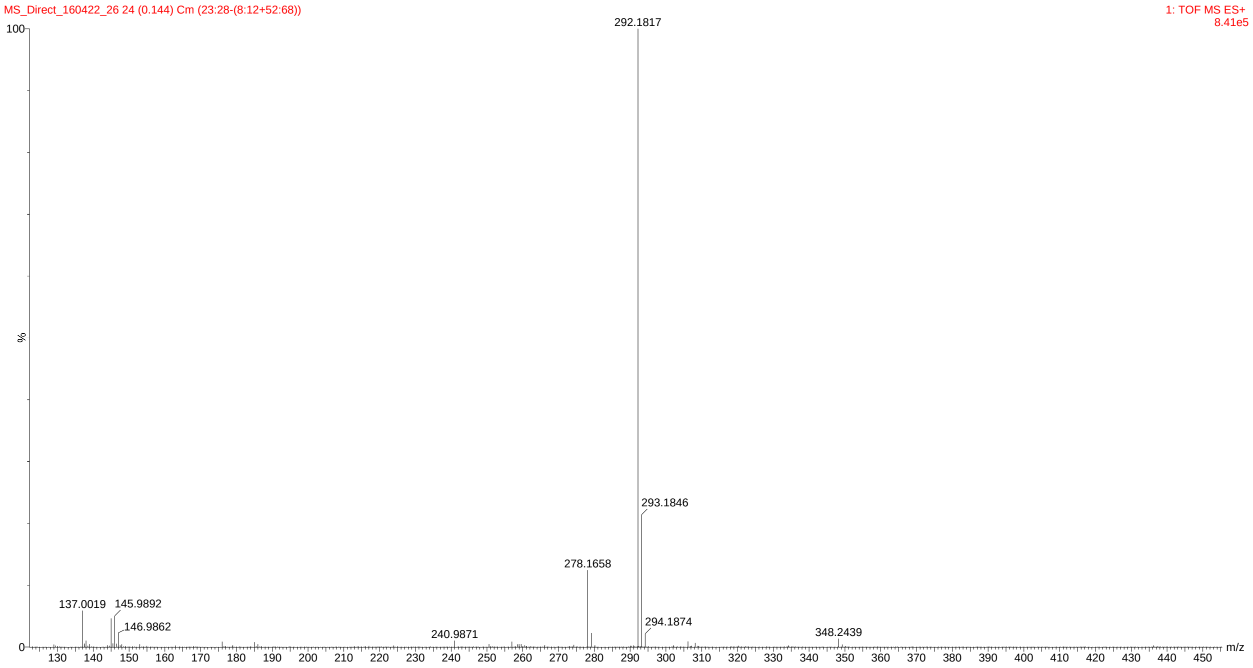
N-benzylquinoxalin-2-amine **63b**





N-methyl-*N*-phenylquinoxalin-2-amine **63c**





3-butyl-*N*-methyl-*N*-phenylquinoxalin-2-amine **64c**