# Synthesis and Evaluation of Novel Quinolines and Quinazolinediones as Potential Anti-Cancer Agents

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# Table of Chapters

# *Acknowledgements*

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Most of all I would like to thank my immediate and extended family for their constant love and support throughout this project. Mum and Dad I constantly fail to find the words to express my love for you both, thank you so very much for everything!

# *Declaration*

I hereby confirm that the body of work described within this thesis for the degree of Doctor of Philosophy, is my own research work, and has not been submitted for any other degree, either at University College Cork or elsewhere.

_____ Date: _____

*For Mum and Dad*

# *Abstract*

This thesis outlines the design and effectuation of novel chemical routes towards a nascent class of functionalised quinoline-5,8-diones and the expansion of a series of contemporary quinazolinediones towards an innovative family of pyridinoquinazolinetetrone derivatives. This fragment based approach is envisaged to lead to advancements in the three scaffolds, expanding the SAR pool of both quinolines and quinazolinediones with subsequent evaluation of chemotherapeutic potential as well as furnishing a new class of tricycle for biological investigation.

Development of novel quinoline-5,8-diones is provided for by expanding on existing methodology. By using a variety of selected nucleophiles on a critical intermediate, a broad range of novel compounds was afforded which serve as molecular probes into the chemotherapeutic potency of this class of compounds, while also serving as integral intermediates for accomplishing novel pyridinoquinazolinetetrone congeners using contemporary cyclisation methodology.

In order to incorporate functionality into our quinazolinedione template, an efficient synthetic strategy was constructed which provided a robust route to effectuate a highly derivatised pyrimidinedione ring from simple starting materials. As derivatisation of this template is unreported our chief priority was to synthesise a range of diverse quinazolinediones. The application of annulation methodology using functionalised precursors provided a library of *N*-3 derivatised quinazolinedione analogues. These, along with their *N*-1 functionalised derivatives provide a wide scope from which to construct a series of pyridinoquinazolinetetrone derivatives while also serving as a unique class of molecules whose biological potential is uncharted.

Although the actualisation of the pyridinoquinazolinetetrone was ultimately unsuccessful, our work has led to the development of novel quinoline-5,8-diones which were found to possess excellent anti-cancer activity when assessed by the NCI screen. Preliminary results indicated appreciable cytotoxicity across several tumour types.

Of the quinazolinediones synthesised eight compounds were accepted for screening by the NCI. Results from the single-dose tests however indicated that these compounds possessed little cytotoxic activity at 10 µM. The development of this novel template in conjunction with the highly active quinolinediones serves as an excellent rostrum for future synthetic endeavours.

# *Abbreviations*

| | |
|---|---|
| Anhyd. | Anhydrous |
| ATP | Adenosine triphosphate |
| s | Singlet |
| bs | Broad singlet |
| CAK | CdK-activating kinase |
| Cdc | Cell-division cycle |
| $CDCl_3$ | Deuterated chloroform |
| CdK | Cyclin-dependant kinase |
| d | Doublet |
| DCM | Dichloromethane |
| DBU | 1,8-Diazabicyclo[5.4.0]undec-7-ene |
| dd | Doublet of doublets |
| ddt | Doublet of doublet of triplets |
| dt | Doublet of triplets |
| DEPT | Distortionless enhancement of polarisation transfer |
| DMF | *N,N*-Dimethylformamide |
| DMF-$d_7$ | Deuterated *N,N*-Dimethylformamide |
| DMSO-$d_6$ | Deuterated dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| DPP-4 | Dipeptidyl peptidase-4 |
| DSP | Dual specifity phosphatase |

| | |
|---|---|
| EDC | 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide |
| EGFR | Epidermal growth factor receptor |
| Et | Ethyl |
| EtOH | Ethanol |
| g | Gram |
| GGPP | Geranylgeranyldiphosphate |
| $GI_{50}$ | Concentration at which growth is inhibited to 50% |
| Hz | Hertz |
| HCl | Hydrochloric acid |
| HIV | Human immunodeficiency virus |
| $IC_{50}$ | 50% Inhibition concentration |
| IR | Infrared |
| *J* | Coupling constant |
| $LC_{50}$ | Concentration required for 50% cell death |
| lit. | Literature |
| LNCaP | Androgen-sensitive human prostate adenocarcinoma cell-line |
| Me | Methyl |
| MeOH | Methanol |
| MHz | Megahertz |
| MiaPaCa2 | Human pancreatic carcinoma cell-line |
| $MIC_{90}$ | Minimum concentration which inhibits 90% of organisms |
| m.p. | Melting point |
| µg | Microgram |

| | |
|---|---|
| µM | Micromolar |
| mg | Milligram |
| mL | Millilitre |
| mmol | Millimolar |
| m | Multiplet |
| mRNA | Messenger ribonucleic acid |
| nM | Nanomolar |
| NMP | *N*-Methyl-2-pyrrolidone |
| NMR | Nuclear magnetic resonance |
| PDE3 | Phosphodiesterase type 3 |
| PDE4 | Phosphodiesterase type 4 |
| PGGTase | Protein geranylgeranyl transferase |
| *p*-TsOH | *para*-Toluenesulfonic acid |
| Ph | Phenyl |
| $POCl_3$ | Phosphorous oxychloride |
| q | Quartet |
| qt | Quintet |
| r.t. | Room temperature |
| SAR | Structure-activity relationship |
| sep | Septet |
| st | Sextet |
| 1,1,3,3-TMP | 1,1,3,3-Tetramethoxypropane |
| TFA | Trifluoroacetic acid |
| TGI | Total growth inhibition |

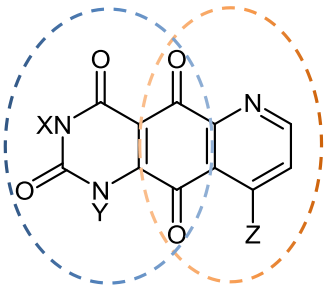
| | |
|---|---|
| THF | Tetrahydrofuran |
| Thr | Threonine |
| t | Triplet |
| td | Triplet of doublets |
| TLC | Thin-layer chromatography |
| TMS | Trimethylsilane |
| Tyr | Tyrosine |
| UV | Ultraviolet |

# *1.0 Biological Introduction*

## Contents

## 1.0 Biological introduction

The following chapter is divided into two sections based on the parent bicyclic fragment which pyridinoquinazolinetetrone **1** is derived from. Each section opens by briefly outlining the applications each pharmacophore has in medicinal chemistry followed by a detailed description of the biological profile of the scaffolds most relevant to this work.




**1**

***Fig 1.1.1*** *Illustrates both bicycles from which* ***1*** *is inferred. Highlighted in orange is the quinoline pharmacophore (***Section 1.1***) and the quinazoline fragment is highlighted in blue (***Section 1.2***).*

## 1.1 Quinolines

The quinoline **2** scaffold which is comprised of a benzene ring fused with pyridine at two adjacent carbon atoms forms the foundation of a vast array of diverse compounds with extensive pharmacological properties. The quinoline structure is perhaps most notably associated with anti-malarial drugs arising from the isolation of the natural alkaloid quinine. Until the 1940's it was the drug of choice for treatment of malaria until it was superseded by chloroquine, another quinoline, which possessed a more favourable pharmacological profile.[1]

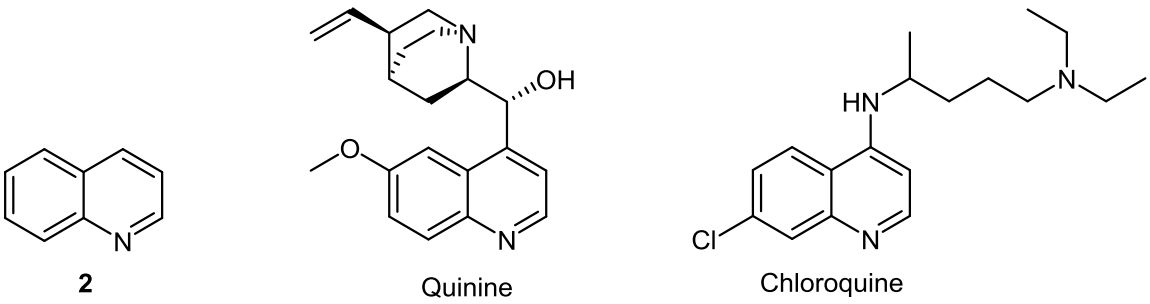



***Fig 1.1.2***

Due to much work being carried out on effective syntheses of quinoline compounds as well as diversification, a range of quinoline type compounds now possess among others, anti-tubercular,[2] anti-hypertensive[3] and anti-Alzheimer[4] activity. Quinolines have also been shown to exhibit anti-cancer activity, highlighted in a recent review by Solomon *et al.*.[5] One subclass in the vast pedigree of quinolines which have received attention as antineoplastics in the last ten to fifteen years are quinoline-5,8-diones **3** due to their ability to inhibit Cdc25 phosphatase, a key regulator of the eukaryotic cell cycle, with some derivatives exhibiting nanomolar activity in *in vitro* assays.[6]

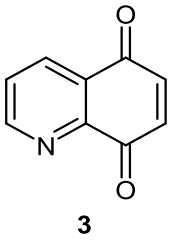



***Fig 1.1.3***

The following section outlines the role that quinoline-5,8-diones have in generating effective chemotherapeutic agents against Cdc25 phosphatase which has been shown to be overexpressed in a multitude of cancers, ***Table 1.1.1*** (page 18). This focus stems from the direct relationship of **3** with the quinoline fragment of **1**, ***Fig 1.1.1*** (page 14).

### 1.1.1 Introduction to regulation of the cell cycle

Common to all cancers is a disordered cell cycle and irregularities such as overexpression, deletion or mutations in the molecules which govern the cell cycle.[7] A family of proteins known collectively as cell division cycle 25 (Cdc25) proteins are highly conserved dual specifity (acting on tyrosine or serine/threonine residues) phosphatases (DSP) which activate cyclin-dependant kinase (CdK) complexes, by dephosphorylating the Thr 14 and Tyr 15 residues of CdK. A consequent phosphorylation of Thr 161 by CdK-activating kinase (CAK) results in complete activation leading to cell-cycle progression ***Fig 1.1.5*** (page 19).[7,8] Cdc25 phosphatases also play a role in checkpoint pathways, (e.g. $G_1$/S or $G_2$/M) which are activated as a result of DNA damage. When DNA damage occurs the cell responds by activating a relevant checkpoint mechanism, resulting in cell-cycle arrest which either leads to repair of the damaged DNA or apoptosis.[9] Overexpression of Cdc25 is thought to lead to a loss of cell cycle checkpoint control, uncontrolled cell proliferation and a loss of genome integrity. From this it is easy to see that Cdc25 phosphatases make ideal targets for cancer therapy.

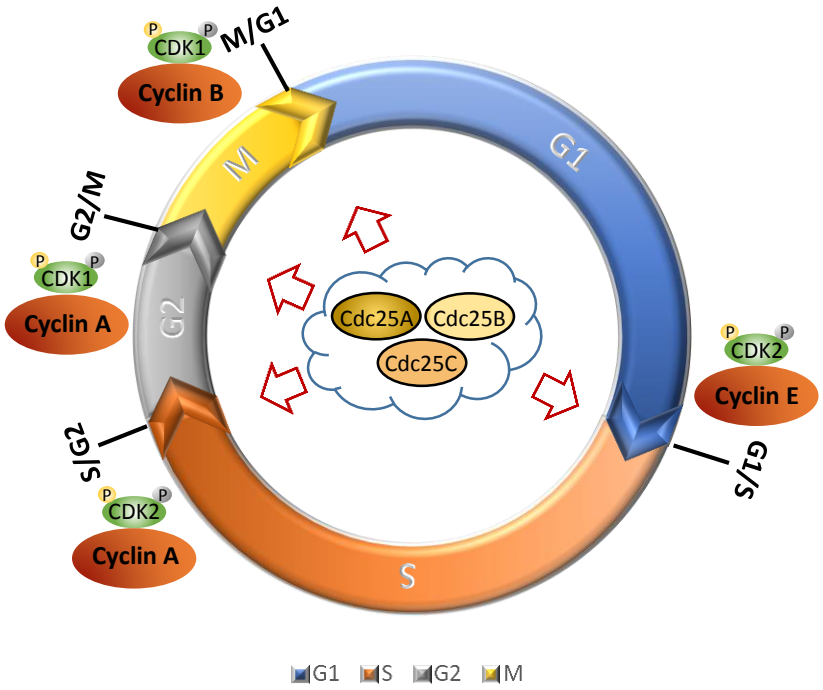



***Fig. 1.1.4*** *Illustrates the four stages of the cell cycle. Thr 14 is represented as the yellow P and Tyr 15 is represented by the grey P. During the gap 1 (G1) phase (blue) cells increase in mass and synthesise mRNA and proteins for DNA synthesis. Synthesis (S) phase (brown) is where DNA synthesis occurs. Following completion of DNA replication the cell enters the gap 2 (G2) phase (grey) where cells continue to grow and synthesise proteins necessary for mitosis. The mitosis (M) phase, (yellow) involves cells duplicating into two identical cells.*

### 1.1.2 Cdc25 in cell-cycle control

In the human genome three Cdc25 genes have been identified. These three isoforms are Cdc25A, Cdc25B and Cdc25C.[10,11] Each gene can produce alternative splicing variants which generate two Cdc25A variants[12] and five variants each for Cdc25B[13,14] and Cdc25C.[12,15] Of the three isoforms it is the overexpression of Cdc25A and Cdc25B which are linked to a variety of human malignancies in the majority of cases. ***Table 1.1.1*** illustrates the percentage of cancers which show the overexpression of Cdc25 proteins.[9]

| Cancer type | Cdc25 A % | Cdc25 B % | Cdc25 C % |
|---|---|---|---|
| Thyroid | 17-69 | 36-64 | ND |
| Breast | 70 | 57 | ND |
| Hepatocellular | 56 | 20 | ND |
| Ovarian | 30 | 30 | ND |
| Colorectal | 47-53 | 43-67 | 27 |
| Non-Hodgkin lymphoma | 50 | 65 | 15 |
| Gliomas | ND | 47 | ND |
| Laryngeal | 41 | 57 | ND |
| Oesophageal | 46-66 | 48-79 | ND |
| Gastric | ND | 78 | ND |
| Endometrial | ND | 73 | 13 |
| Prostate | ND | 30 | ND |

***Table 1.1.1*** *Percentage of tumours which exhibit overexpression of Cdc25A, Cdc25B or Cdc25C proteins, ND-not determined* [9].

Cdc25 proteins are responsible for activating CdKs which are a family of highly conserved serine/threonine protein kinases associated with regulatory cyclin subunits. CdKs are held in an inactive state by WEE1 and MYT1 kinases which phosphorylate the Thr14 and Tyr15 residues of CdK1, located within the ATP binding loop of CdK.[16] When CdK activity is necessary for the progression of the cell-cycle to the next phase the dual specifity phosphatases (Cdc25s) dephosphorylate both residues thereby activating the CdK-cyclin complex, ***Fig 1.1.5***. CAK then phosphorylates Thr 161 yielding the fully active complex.[8]

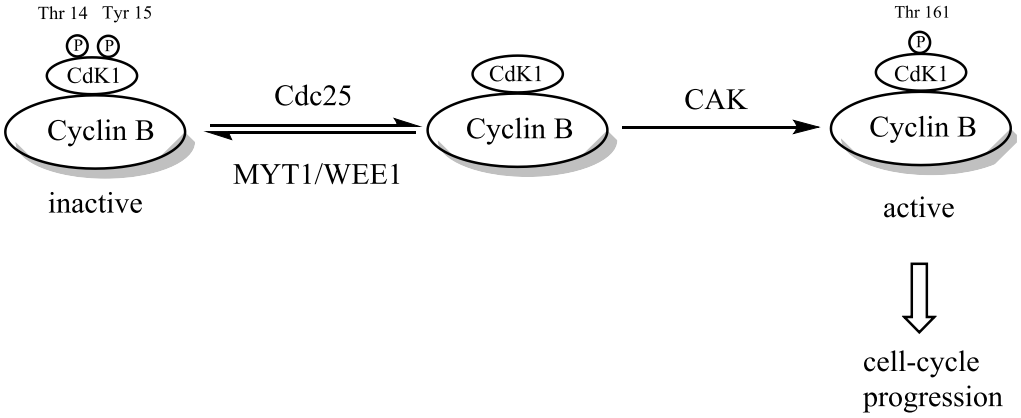



***Fig 1.1.5*** *Catalytic cycle of Cdc25*

In mammalian cells the three isoforms of Cdc25 are implicated in cell cycle regulation as a result of their ability to dephosphorylate CdK1 and CdK2. Although initially it was thought that there was a specific role for each Cdc25 phosphatase at defined stages of the cell cycle in reality it is emerging that all the Cdc25 isoforms are involved in phosphorylating CdK-cyclin complexes ***Fig 1.1.4*** (page 17).[9]

### 1.1.3 Structure and catalytic mechanism of Cdc25 phosphatase

Cdc25 phosphatases are between 470 and 566 residues long and consist of two primary domains, an N-terminus and a C-terminus. The N-terminus contains a regulatory domain which modulates the activity of the enzyme. Contained within the C-terminus is the catalytic domain which is highly conserved among the three Cdc25 proteins.[17] Within the catalytic domain is a phosphate binding loop (P-loop) also known as a $HCX_5R$ motif where H is a highly conserved histidine residue, C is the catalytic cysteine, $X_5$ are the five residues which create the loop in which all amide nitrogens bond to the phosphate of the substrate and R is a highly conserved arginine which hydrogen bonds to the phosphorylated amino acid of the substrate,[18] ***Fig 1.1.6***.

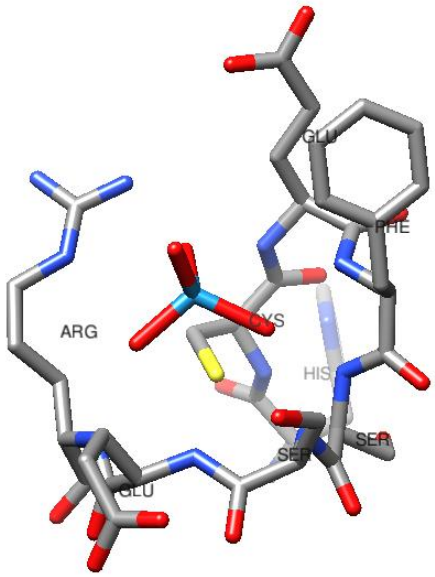



***Fig 1.1.6*** *Catalytic site P-loop bound to a tungstate anion*[19]

This motif is common to all tyrosine phosphatases. The structures of Cdc25A and Cdc25B have been solved by X-ray crystallography and showed similar catalytic domains.[19,20] A key difference between these two isoforms is the relatively shallow active site of Cdc25A compared to Cdc25B whose active site is similar to other DSPs. The Cdc25A catalytic domain also contains no flexible peptide loops proximal to the active site that might facilitate substrate binding.

Cdc25B contains a flat active site within a shallow pocket. Adjacent to the active site is a cavity known as the "swimming pool" due to the abundance of well-ordered water molecules contained within the pocket.[21] The mechanism of catalysis occurs in two distinct steps.[22] Firstly the catalytic cysteine acts as a nucleophile to the phosphate ester substrate, generating a thiophosphorylated intermediate. A proton transfer to the leaving group also occurs in this step however the origin of this proton is debatable. In other DSP proteins an Asp residue located in a mobile loop distal to the active site acts as the proton source (Cdc25s lack this catalytic site residue). The final step involves hydrolysis of the thiophosphorylated intermediate to regenerate the free enzyme as a free CdK-cyclin

complex which promotes cell cycle progression, ***Fig. 1.1.7***. From this it is obvious to see that regulating this transfer would lead to control of the cell cycle.

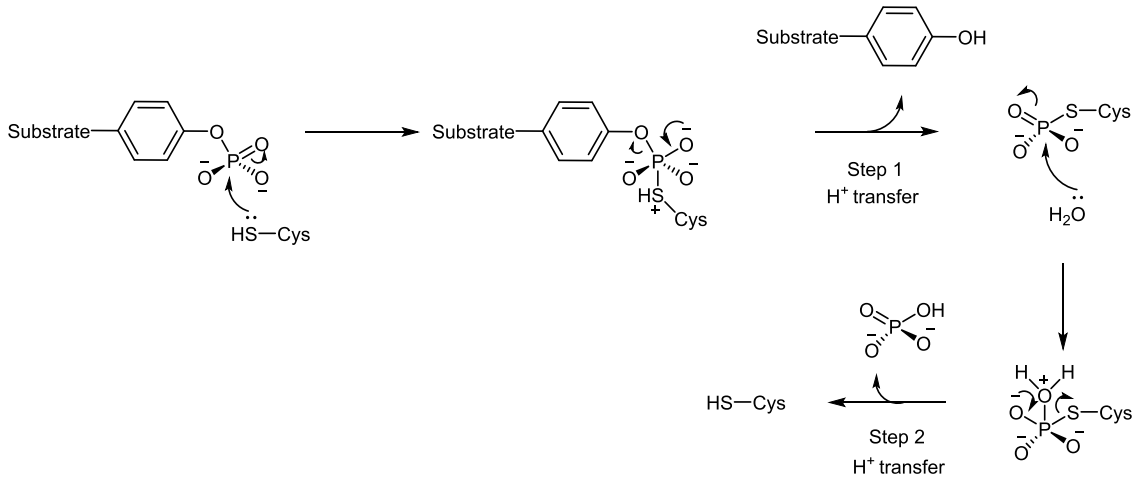
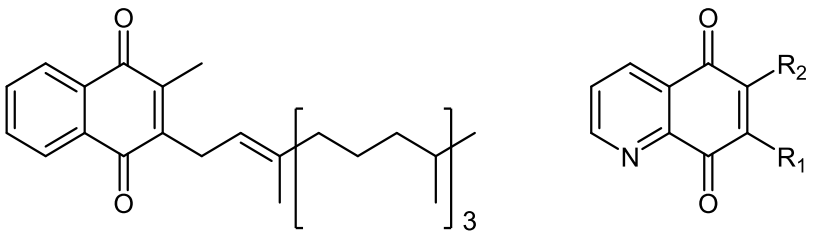



***Fig. 1.1.7*** *Catalytic cycle of Cdc25B*

### 1.1.4 Inhibition of Cdc25 in anti-cancer therapy

The main families of compounds which have been identified to be potent Cdc25 inhibitors include quinoline-5,8-diones, phosphomimetics and electrophilic entities.[23] Quinoline-5,8-dione compounds which are congeners of vitamin K are some of the most numerous and active compounds ***Fig. 1.1.8***.




Phylloquinone (vitamin $K_1$) Quinoline-5,8-dione

***Fig. 1.1.8*** *Illustrates the similarity between the vitamin $K_1$ and quinoline-5,8-dione pharmacophores.*

To date there has been no crystal structure of quinoline-5,8-diones docked in the Cdc25 active site which hampers the establishment of a definite mechanism of action, however it is thought to involve either covalent adduct formation with a serine residue adjacent to the catalytic site,[24] or irreversible oxidation of the cysteine residue in the catalytic domain to a sulphonic acid (Cys-$SO_3^-$).[24] The two most potent compounds in this family are JUN1111 and its 6-chloro derivative NSC663284 (DA3003-1) which were synthesised by Lazo and co-workers.[25,26] Adociaquinone B which is a derivative of a marine sponge extract also showed an excellent inhibition profile. Collaborative work between IPSEN pharmaceuticals and the research group of Boutros resulted in the discovery of two potent Cdc25 inhibitors, BN82685 and IRC083864, the latter displaying the most efficacy to date.[9] ***Table 1.1.2*** illustrates the nanomolar activity of the five most potent quinone derivatives against Cdc25 in an enzyme assay. Both BN82685 and IRC083864 were found to possess excellent inhibition properties against MiaPaCa2 (0.1 μM) and LNCaP (0.02 μM) cell lines respectively *in vitro*. Furthermore *in vivo* testing also showed encouraging results with both compounds showing activity against their respective cell lines in xenografted tumours in nude mice.

Given the infancy of this research the selectivity of these compounds is an issue which requires future SAR development. It is envisaged that Cdc25 phosphatase inhibitors are not selective for tumour cell lines and would inhibit the cell-cycle progression of any cell type but the upregulation of Cdc25s in various cancers means increased sensitivity may exist. For example, both colon adenocarcinoma (HCT116) and pancreatic ductal adenocarcinoma show increased expression of Cdc25B and also show increased sensitivity to chemical inhibition of Cdc25 phosphatase activity.[9,27]

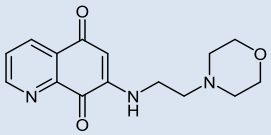
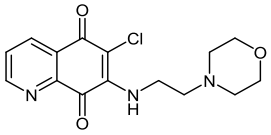
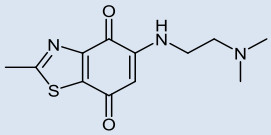
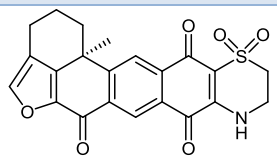
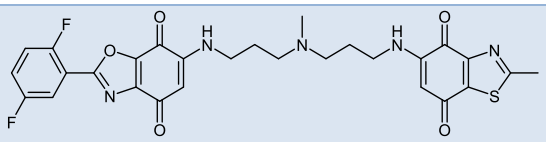
| Compound | Structure | $IC_{50}$ *in vitro* µM |
|---|---|---|
| **JUN1111** |  | 0.38-1.8 |
| **NSC663284 (DA3003-1)** |  | 0.2-0.9 |
| **BN82685** |  | 0.17-0.25 |
| **Adociaquinone B** |  | 0.07 |
| **IRC083864** |  | 0.02 |

***Table 1.1.2*** In vitro *$IC_{50}$ values for five of the most potent compounds identified so far against the three Cdc25 isoforms.*

DA3003-1 was first reported by Lazo *et al.* in 2001 and showed sub micromolar inhibition of Cdc25, ***Fig. 1.1.9***. Owing to the encouraging results from this study further elaboration of the pharmacophore was investigated by Wipf *et al.* in 2008.[28] This study was based on inverting the six and seven positions of DA3003-1 type structures leading to the synthesis and biological evaluation of analogous quinoline-5,8-diones.

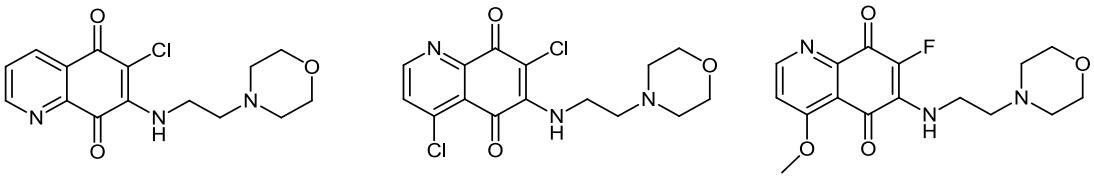



***Fig. 1.1.9*** *Inverse quinoline-5,8-diones WDP1079 and WDP1149 synthesised by Wipf* et al..[28]

The $IC_{50}$ values of WDP1079 and WDP1149 against the Cdc25B catalytic domain as well as subsequent cytotoxicity assays against the Cdc25B expressing lung cancer cell line A549 are detailed below in ***Table 1.1.3***. As a comparison the $IC_{50}$ of DA3003-1 against A549 is also shown.[24] Of the two quinolines synthesised by Wipf *et al.* it is apparent that halogenation of both the four and seven position results in greater activity against Cdc25B and A549. These encouraging preliminary findings provide the rationale to diversify this inchoate branch of quinolines and form the basis of our synthetic venture into quinoline-5,8-diones as Cdc25 phosphatase inhibitors ***Section 6.2.1***.

| Compound | Cdc25B $IC_{50}$ ± SEM/µM | A549 $IC_{50}$ ± SEM/µM |
|---|---|---|
| DA3003-1 | 0.91 ± 0.36 | 1.48 ± 0.04 |
| WDP1079 | 1.10 ± 0.1 | 2.69 ± 0.08 |
| WDP1149 | 5.30 ± 0.6 | 9.52 ± 0.33 |

***Table 1.1.3*** *Comparison of* in vitro *efficacy of inverted quinoline-5,8-diones versus DA3003-1 against Cdc25B and human lung carcinoma cell line (A549).*

### 1.1.5 Conclusion

The pivotal role CdKs play in cell cycle regulation makes them an attractive target for the ontogenesis of antineoplastic agents. As CdK activators, Cdc25 phosphatases are discernible targets for the development of novel approaches to indirectly inhibit CdKs and their ramifications on cell cycle regulation. The quinoline-5,8-dione pharmacophore represents a privileged template which serves as a molecular probe to investigate the consequence of Cdc25 phosphatase inhibition.

To date, the exact role and mechanism of Cdc25 phosphatases remains vague largely due to the paucity of suitable exploratory templates, a niche where quinoline-5,8-diones apply. Given the importance of Cdc25 inhibition and its subsequent effects in cell cycle

control, the elaboration of the quinoline-5,8-dione pharmacophore is of eminent importance in order to expatiate the biological knowledge of this key process.

## 1.2 Quinazolines

The quinazoline scaffold, consisting of a core bicyclic structure **4**, represents a family of molecules containing diverse pharmacophores which possess a broad spectrum of activity. Up until the late 1960's, only two quinazolines were used medically, methaqualone **5**, a soporific and anti-convulsant, and the diuretic quinethazone **6**.[29]

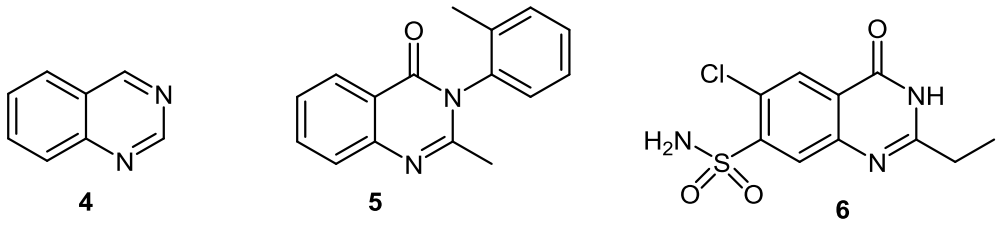
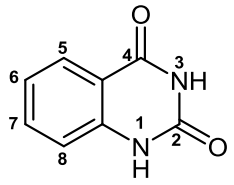



***Fig 1.2.1***

However, in recent years there have been significant advances in this field leading to the generation of quinazoline derivatives possessing a range of activities including analgesic and anti-inflammatory, anti-malarial, anti-fungal, anti-diabetic, diuretic, anti-hypertensive, sedative/soporific, anti-cancer as well as the treatment of benign prostatic hyperplasia.[29] Between 2007 and 2010 alone eighty eight world patents were filed for 4-anilinoquinazolines, a family of tyrosine kinase inhibitors which are at the forefront of chemotherapy.[30] Our specific interest lies in the exploration of the quinazoline2,4-(1*H*,3*H*)-dione scaffold **7** as they represent an underdeveloped domain of quinazolines.

### 1.2.1 Quinazoline-2,4-(1*H*,3*H*)-dione scaffold

Quinazoline-2,4-(1*H*,3*H*)-diones **7** represent a branch of quinazoline derivatives which have also been found to possess a vast array of pharmacological properties ranging from serotonin receptor antagonists,[31,32] glutamate receptor antagonists,[33] α-adrenoceptor antagonists,[34] acetylcholine receptor antagonists,[35] anti-bacterial and anti-cancer agents.[36-40] Owing to the intrinsic nature of quinazoline-2,4-(1*H*,3*H*)-dione fragment **7**, ***Fig 1.2.2***, in pyridinoquinazolinetetrone **1**, ***Fig 1.1.1*** (page 14) the following section outlines the most prevalent biological applications of this pharmacophore.




**7**

***Fig 1.2.2*** *Structure and numbering sequence of quinazoline-2,4-(1*H*,3*H*)-dione.*

### 1.2.2 Anti-cancer activity

A recently published paper by Zhou *et al.* documents the synthesis of a range of quinazoline-2,4-(1*H*,3*H*)-dione derivatives which were found to possess significant anticancer activity when tested against the NCI 60-cell line screen.[40] Following SAR studies a total of forty-two relevant compounds were synthesised of which seventeen exhibited anti-proliferative activity. Four of these compounds were found to possess sub-micromolar activity, ***Table 1.2.1*** (page 27). The final two compounds (NSC D-752221/1 and NSC D-751371/1) appear to have remarkable cytotoxic activity given their similarity to other compounds assayed. Due to the lack of selectivity of these compounds for a specific cancer sub-type, no plausible mechanism of action was identified, however these compounds may be useful as leads for future SAR studies.

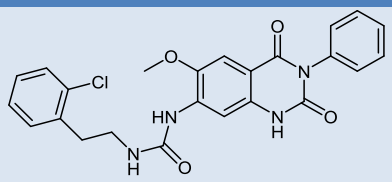
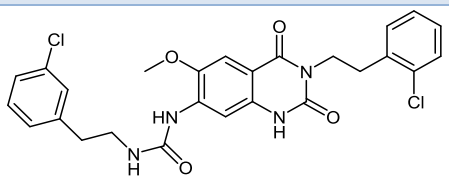
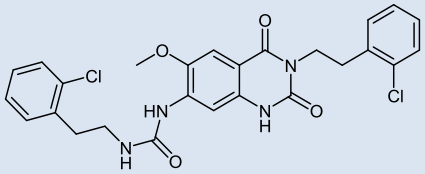
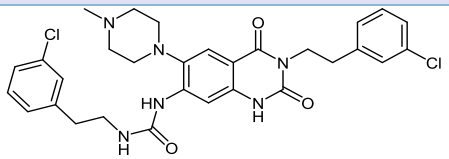
| Compound | % mean growth at 10 µM | $GI_{50}$ (µM) average value over 56 cell lines |
|---|---|---|
|  NSC D-750471/1 | 20.39 | 0.794 |
|  NSC D-752951/1 | 14.59 | 0.741 |
|  NSC D-752221/1 | -3.62 | 0.363 |
|  NSC D-751371/1 | -81.75 | 0.407 |

***Table 1.2.1*** *The four most potent compounds synthesised by Zhou* et al. *when tested against the NCI 60-cell line screen.*

### 1.2.3 Geranylgeranyltransferase-I inhibitors

Carrico *et al.* outlines the employment of quinazoline-2,4-dione congeners as perspective protein geranylgeranyltransferase-I (PGGTase-I) inhibitors as a potential

chemotherapeutic target.[39] Geranylgeranyltransferase-I belongs to the prenyltransferase family which catalyse the lipidation of proteins. Specifically PGGTase-I catalyses the transfer of a geranylgeranyl moiety to the cysteine residue of specific proteins. PGGTase-I is part of the CAAX prenyltransferase category where C is a cysteine residue, A represents any aliphatic amino acid moiety and X is leucine, isoleucine or phenylalanine. The precedence for this study is based on the fact that many PGGTase-I substrates were found to play a critical role in the development of tumours and subsequent metastasis.

The PGGTase-I enzyme is a heterodimer with both α and β subunits containing primarily alpha helices, ***Fig 1.2.3***.[41] The α-subunit forms a crescent shape around the β-subunit. The β-subunit forms a compact, α–α barrel domain which contains a central cavity. At the α–β interface is the substrate binding site which extends into the funnel-shaped cavity of the β-subunit. Contained along this funnel are hydrophobic residues with a catalytic zinc ion at the top of the funnel which binds to the cysteine of the CAAX system.

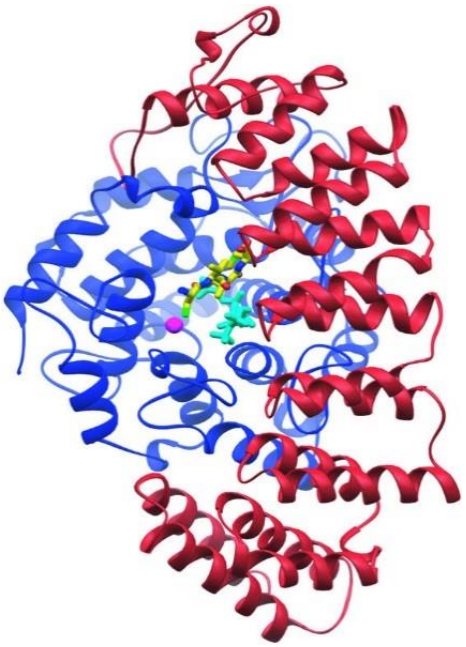


***Fig 1.2.3*** *PGGTase-I enzyme with the α- and β-subunits highlighted in red and blue respectively. The catalytic zinc is highlighted as the magenta sphere. Highlighted in cyan is 3'azaGGPP, a non-reactive analogue of geranylgeranyldiphosphate. Also shown in yellow is the CAAX (CVIL) residue of the peptide substrate.*[41]

Design of peptidomimetics was based around the adaption of the CAAX residues.[39] The central AA units were replaced with a rigid linker which lead to the synthesis of **GGTI-2154** a potent PGGTase-I inhibitor, $IC_{50}$=21nM, ***Fig 1.2.4***. The cysteine residue has also been replaced with an imidazole bioisostere which was previously shown to increase metabolic stability and selectivity for PGGTase-I.

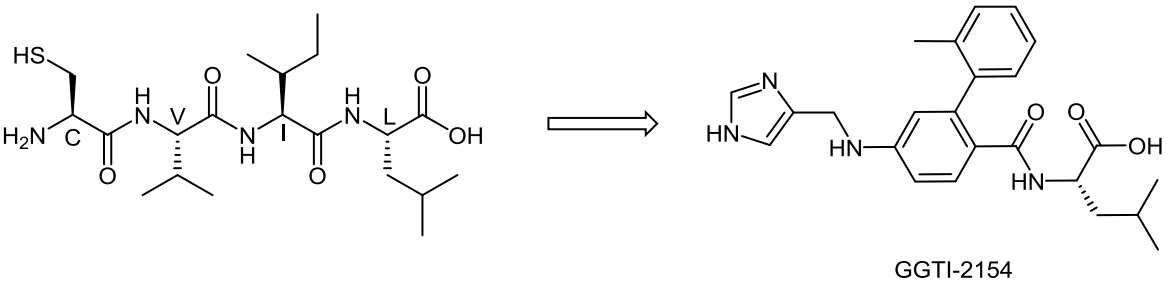



***Fig 1.2.4***

To further this work, a series of molecular modelling studies were carried out in order to explore the development of novel PGGTase-I inhibitors, which revealed that the quinazoline-2,4-dione scaffold would be an attractive alternative to the biphenyl linker present in **GGTI-2154**. As a result a series of quinazoline-2,4-dione congeners were furnished in order to investigate their efficacy against PGGTase-I. Of the sixteen compounds two produced $IC_{50}$ values in the nano-molar region, ***Fig 1.2.5***.

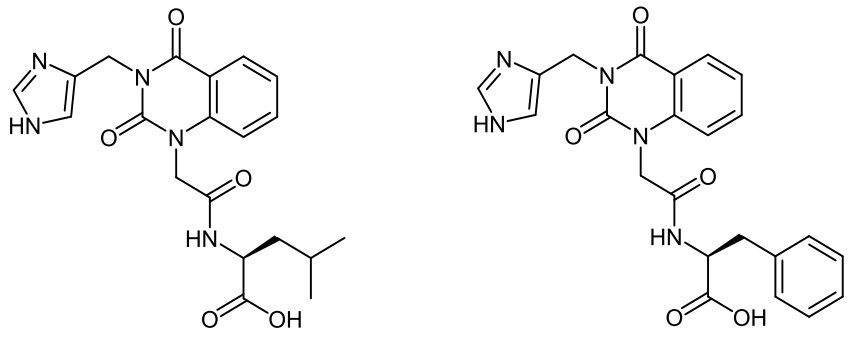



***Fig 1.2.5***

Comparison of derivatives **8** and **9** shows that the phenylalanine derivative **9** exhibits superior activity when compared to the leucine derivative **8**. Further studies determined that an unsubstituted imidazole was necessary for effective binding to zinc. Conversion of the amino acid residues to the D-series lead to a complete drop off in activity most likely due to size restrictions in the X pocket. Docking studies of the leucine derivative **8** revealed that the compounds bind in the CVIL site suggesting the activity of these compounds may be due to competitive inhibition of PGGTase-I substrates.

### 1.2.4 Anti-bacterial agents

Quinazoline-2,4-(1*H*,3*H*)-diones have also been the subject of much interest as new avenues of therapy for resistant gram-positive infections.[36,37] ***Fig. 1.2.6*** (page 31) shows two of the existing antibiotics used in the treatment of gram-positive infections. The need to develop new therapies arises from the emergence of resistant strains of bacteria namely methicillin resistant *Staphlococcus aureus* (MRSA) and vancomycin resistant *enterococci* (VRE) which are of particular concern. There has also been an emergence of vancomycin resistant *Staphylococcus aureus* infection (VRSA), a drug which is usually associated as a last line therapy. Therefore it is easy to see the urgent need to develop original therapies to combat this serious clinical problem.

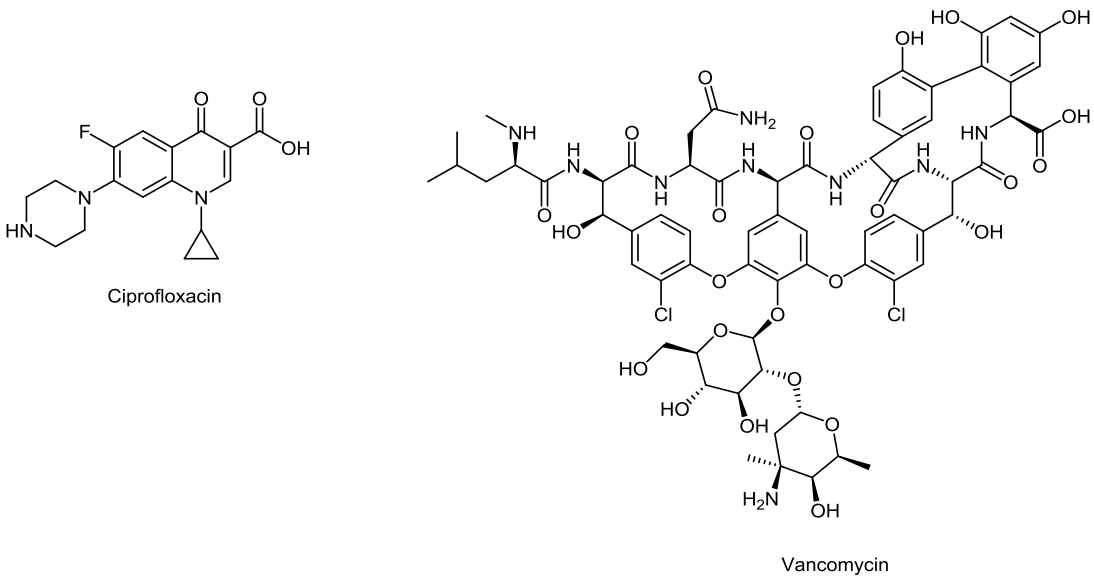



***Fig. 1.2.6*** *Structures of ciprofloxacin and vancomycin.*

A study carried out by Huband *et al.* details the antibiotic activity of two novel quinazoline-2,4-(1*H*,3*H*)-diones, PD 0305970 and the 3-desamino analogue PD 0326448 ***Fig. 1.2.7***, which serve as next generation therapies for resistant and susceptible strains of bacteria.[36] *In vitro* testing of the compounds was carried out against 1,036 clinically significant strains of bacteria. Both analogues were found to possess exceptional antibiotic activity versus gram-positive, including resistant strains.

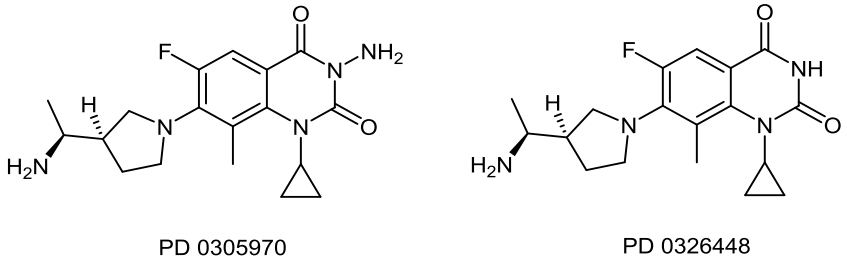



***Fig 1.2.7***

The $MIC_{90}$ values of PD 0305970 ranged from 0.008-0.5 μg/ml against staphylococci, streptococci, *Corynebacterium* spp., while PD 0326448 exhibited values which were two- to fourfold higher in an identical study. When compared against existing treatments for gram-positive resistant strains, *Streptococcus pneumonia, Enterococcus faecalis,*

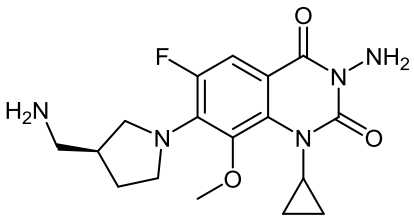
*Enterococcus faecium* and staphylococci, PD 0305970 is bestowed with an exemplary 8- to 512-fold $MIC_{90}$ advantage over existing treatments.

The quinazoline-2,4-(1*H*,3*H*)-diones excel in the treatment of quinolone resistant mutants with similar or superior anti-bacterial properties as current quinolones to susceptible strains of gram-positive bacteria. A study using PD 0305970 showed that this activity is most likely due to the targeting of the gyrB and parE subunits in contrast to the quinolones which targets the gyrA and parC.

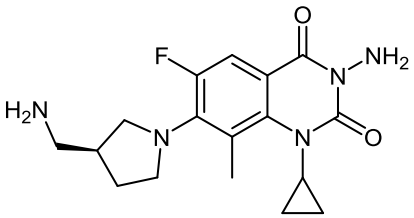
In 2010 Oppegard *et al.* published work detailing the biological evaluation of novel quinazoline-2,4-(1*H*,3*H*)-diones as potential alternatives to quinolone type antibiotics.[37] In order to be viable these compounds had to fulfil two criteria; (i) possess activity against known quinolone-resistant mutants and (ii) to display similar activity as quinolone antibiotics towards DNA gyrase and Topoisomerase IV. It is thought that dual targeting agents assist in slowing the emergence of drug resistant mutants.

In a previous study the same group demonstrated that *gyr*A and *gyr*B mutations of *E. coli* which are resistant to quinolone therapies displayed sensitivity to 8-methoxy-quinazoline-2,4-diones. Both 8-methoxy and 8-methyl quinazoline-2,4-diones were then tested against three mutant gyrases in order to assess the efficacy of these compounds against high, moderate and low resistance strains, ***Table 1.2.2***.

As can be seen below both of the compounds show increased activity towards mutant strains relative to wild-type gyrase than ciprofloxacin, ***Table 1.2.2***. Another study which examined these two compounds against the catalytic activity of *S. aureus* gyrase and *S. aureus* Topo IV demonstrated comparable efficacy which suggests that quinazoline-2,4-diones may function as dual-target antibiotics. From these studies it can be seen that quinazoline-2,4-diones represent a class of drugs with promising potential in the fight against antibiotic resistance.




8-methoxy




8-methyl

| Compound | Wild-type gyrase $IC_{50}$ (μM) | GyrA S83W gyrase $IC_{50}$ (μM) High | GyrA G81C gyrase $IC_{50}$ (μM) Moderate | GyrA A67S gyrase $IC_{50}$ (μM) Low |
|---|---|---|---|---|
| Ciprofloxacin | 0.45 ± 0.004 | 101 ± 1.9 | 28 ± 7.0 | 1.0 ± 0.15 |
| 8-Methoxy | 2.8 ± 0.1 | 5.9 ± 0.9 | 4.3 ± 0.4 | 2.4 ± 0.1 |
| 8-Methyl | 0.95 ± 0.15 | 3.8 ± 0.6 | 1.7 ± 0.2 | 1.2 ± 0.3 |

***Table 1.2.2*** *Inhibition of catalytic activities of* E. coli *gyrases*

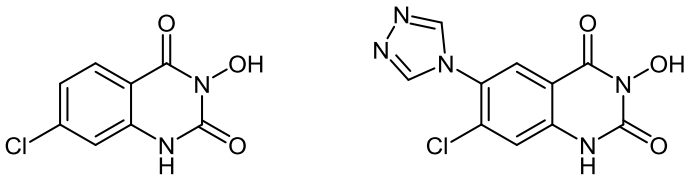
### 1.2.5 Glutamate receptor antagonists

Quinazoline-2,4-diones have also garnered attention as potential glutamate receptor antagonists. Glutamate (Glu) is the primary excitatory neurotransmitter in the central and peripheral nervous system and is involved in a range of physiological processes such as learning and memory. Excess glutamate transmission has also been implicated in a range of neurological disorders such as Alzheimer's,[42,43] Parkinson's,[44] epilepsy,[45] multiple sclerosis[46,47] as well as the transmission of pain.[48-50]

Glutamate expends its effects by acting on two sets of receptors, metabotropic (mGluRs) and ionotropic (iGluRs) receptors. The ionotropic receptors are classified into three

subsets, *N*-methyl-D-aspartate (NMDA), α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (KA) receptors each of which contain six, four and five subunits respectively. While many insights have been provided by research into the role of NMDA and AMPA receptors, knowledge of the role of KA receptors is sparse owing to the lack of selective antagonists.

Work carried out by Colotta *et al.* in 2004 detailed the synthesis of 3-hydroxy-1*H*-quinazoline-2,4-diones and subsequent bioassays showed that these compounds were antagonistic towards NMDA and AMPA receptors.[51] By manipulating the substituents on the quinazoline scaffold it was found that the affinity for a specific receptor type could be effected, ***Fig 1.2.8***.



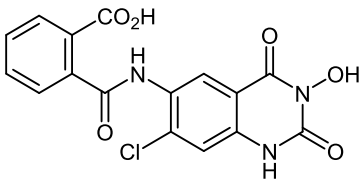

**10** **11**

***Fig. 1.2.8*** *NMDA antagonist 7-chloro-3-hydroxy-1*H*-quinazoline-2,4-dione* ***10*** *and AMPA antagonist 7-chloro-3-hydroxy-6-(4H-1,2,4-triazol-4-yl)quinazoline-2,4(1H,3H)-dione* ***11***.

With this in mind the same group published a later paper exploring the SAR of this pharmacophore with the aim of developing KA specific antagonists to use as probes for the characterisation of the KA receptor.[33] Diversification was investigated at the three and six positions, using the compounds in ***Fig 1.2.8*** as lead compounds. Exploration of the three position was carried out on 7-chloro-3-hydroxy-1*H*-quinazoline-2,4-dione **10** with a variety of ethers being synthesised however these compounds lacked any affinity for AMPA, Gly/NMDA or KA receptors illustrating the necessity of the 3-hydroxyl group.

Derivatisation of the six position led to the discovery of 6-(2-carboxybenzoylamino)-3-hydroxy-1*H*-quinazoline-2,4-dione, **12**, ***Fig 1.2.9***. This compound exhibits a good affinity

for both high and low-affinity KA receptors with $IC_{50}$ values of 0.62 and 1.6 μM respectively. The compound also shows good selectivity versus Gly/NMDA and AMPA receptors. Since few selective KA receptor antagonists are known, quinazoline-2,4-diones represent a family of compounds which are pivotal in the emergence of research in this field.




**12**

***Fig 1.2.9***

### 1.2.6 Conclusion

The quinazoline-2,4-(1*H*,3*H*)-dione heterocycle represents a privileged pharmacophore with which to develop novel chemotherapeutic agents due to their widespread and distinct biopharmaceutical properties. The limited exploration of this moiety as antineoplastic agents has shown promising preliminary results, ***section 1.2.2***, but much work is necessary in order to expound the mode of action of these drugs, congenerous to Carrico's work on PGGTase-I inhibitors, ***section 1.2.3***, so as to implement more judicious investigation.[39]

Quinazolinedione derivatives have also been bequeathed with excellent anti-bacterial properties mediated by the inhibition of bacterial gyrase and topoisomerase IV and represent a new avenue in the treatment of multidrug and fluoroquinolone resistant strains of bacteria.

Given the wide range of disorders implicated with excess glutamate transmission, antagonism of its receptors represents an attractive target for developing effective therapies. The quinazoline-2,4-(1*H*,3*H*)-dione scaffold represents an exemplary research tool in this field. Targeted elaboration of the quinazolinedione backbone conferred

remarkable selectivity profiles, leading to the generation of receptor specific antagonists. Owing to the sparsity of detailed knowledge of these receptors quinazoline-2,4-(1*H*,3*H*)-diones serve as principle templates to expand the biological understanding of glutamate receptor antagonists.

## 1.3 Perspectives

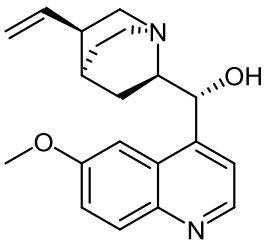
In ***Sections 1.1*** and ***1.2*** critical insight into the prevalence of both pharmacophores in medicinal chemistry was highlighted. The validated bioactivity of both classes of compounds proffers the paradigm of synthesising a tricyclic hybrid in the quest for novel chemotherapeutic agents. It is foreseen that the fusion of these structures to generate the unheralded pyridinoquinazolinetetrone **1** will lead to a cogent new template with which to pioneer new avenues of drug discovery.

# *2.0 Quinoline Chemical Introduction*

## Contents

## 2.1 Introduction

The quinoline moiety is of great interest to chemistry due to its prominence in biologically active natural products, in particular alkaloids, most notably as antimalarial drugs.[52-54] Perhaps the most known quinoline derivative is the alkaloid quinine, **13** which occurs naturally in the bark of the cinchona tree and was the antimalarial drug of choice from the 17th century until the 1940's.




**13**

A wide range of quinoline derivatives display a broad range of pharmacological properties ranging from anti-cancer,[55] anti-HIV,[56] anti-hypertensive,[57] anti-tuberculosis[58] to anti-Alzheimer activities.[59] Due to their importance, continuing research is focused on the development of more efficient methods of synthesis as well as derivatisation.

A key subset in the synthesis of quinolines are quinolones (hydroxyquinolines), in this chapter synthetic routes will be classified based on the substitution pattern of quinolones followed by the substitution pattern of quinolines;

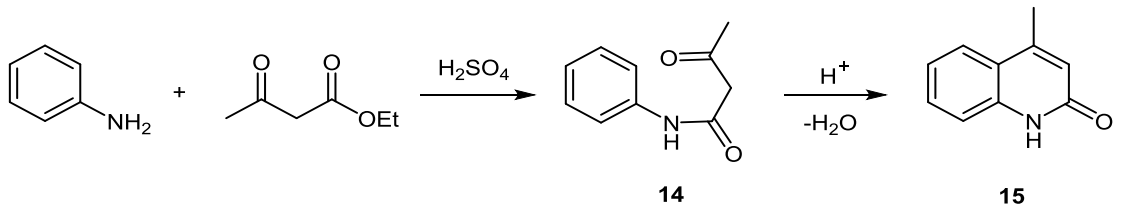
- 2-Quinolones
- 4-Quinolones
- Substituted quinolines

In each case syntheses will be classified based on the named reaction. The final section addresses the synthesis of quinoline-5,8-diones, a family of quinolines central to this work.

## 2.2 Synthesis of 2-quinolones

### 2.2.1 Knorr Synthesis

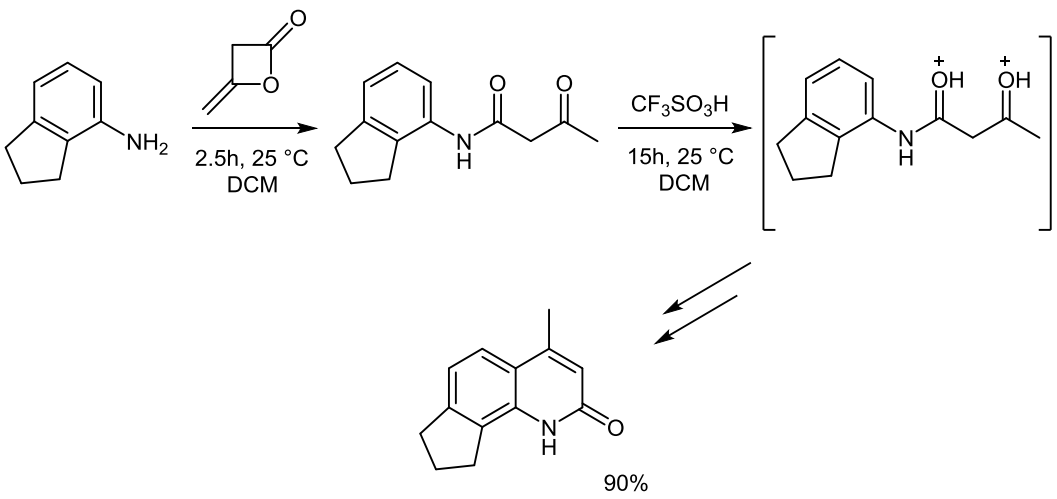
The Knorr quinoline synthesis, first described in 1886 by Ludwig Knorr, is an intramolecular cyclisation reaction which converts a β-ketoanilide **14** to a 2-quinolone **15** under strongly acidic conditions.[60] The β-ketoanilide **14** is generated from the acid-catalysed condensation of primary arylamines and β-ketoesters, ***Scheme 2.2.1.1.***




**14** **15**

***Scheme 2.2.1.1***

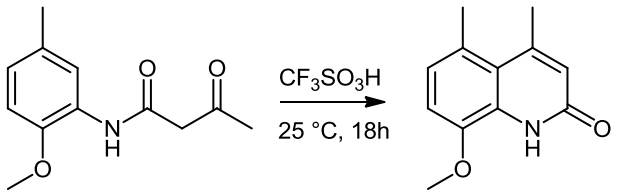
Recently, Klumpp *et al.* published an investigation into the mechanism of the cyclisation.[61] His results, supported by low-temperature $^{1}H$, $^{13}C$ and $^{15}N$ NMR indicated that the β-ketoanilide **14** undergoes diprotonation at the two carbonyl oxygen atoms to form a distonic superelectrophile. Computational studies also showed that this conformation was the most stable, being at least 8 kcal $mol^{-1}$ more stable than other dications. Klumpp synthesised a range of substituted 2-quinolones from acetoacetanilides (generated from the respective anilines and diketene) in the presence of trifluoromethanesulfonic (triflic) acid at ambient temperature in high to excellent yields for activated aryl groups e.g., ***Scheme 2.2.1.2***.




***Scheme 2.2.1.2***

This chemistry was unsuccessful for deactivated aryl groups with the exception of a *para*-fluoro acetanilide which gave a moderate yield of 44 % (this was increased to 69 % with the addition of 10 mol % $SbF_5$).

Studies on acid equivalents were also performed, ***Scheme 2.2.1.3***, where it was found that the yield decreased with decreasing quantities of triflic acid, in accordance with an earlier paper published by Staskun who observed that the Knorr cyclisation required heating with excess strong acid which is consistent with the formation of supercationic species.[62]



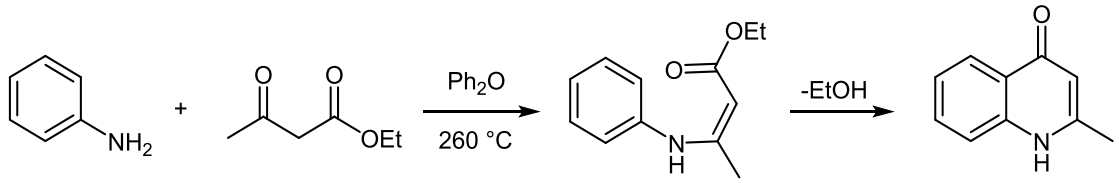

| Acid eq. | Yield% |
|---|---|
| **25** | 97 |
| **2.5** | 86 |
| **1.0** | 0 |

***Scheme 2.2.1.3***

## 2.3 Synthesis of 4-quinolones

### 2.3.1 Conrad-Limpach synthesis

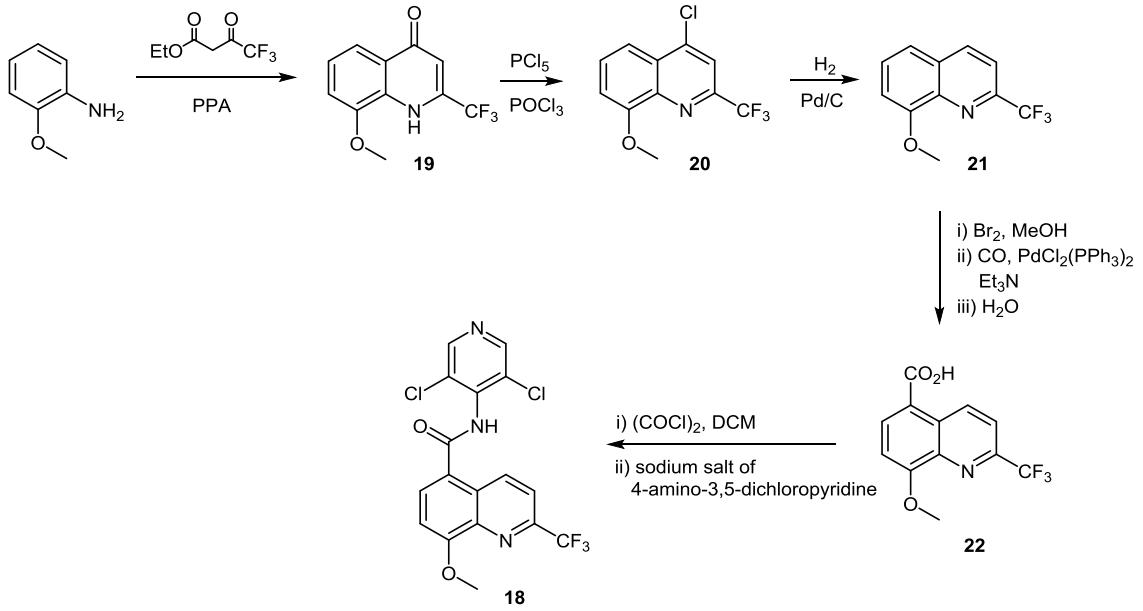
The Conrad-Limpach synthesis, first described in 1891, involves the thermal or acid-catalysed cyclisation of primary arylamines with β-ketoesters to generate the imine **16**, which cyclises to 4-quinolone **17**.[63]




**16** **17**

***Scheme 2.3.1.1***

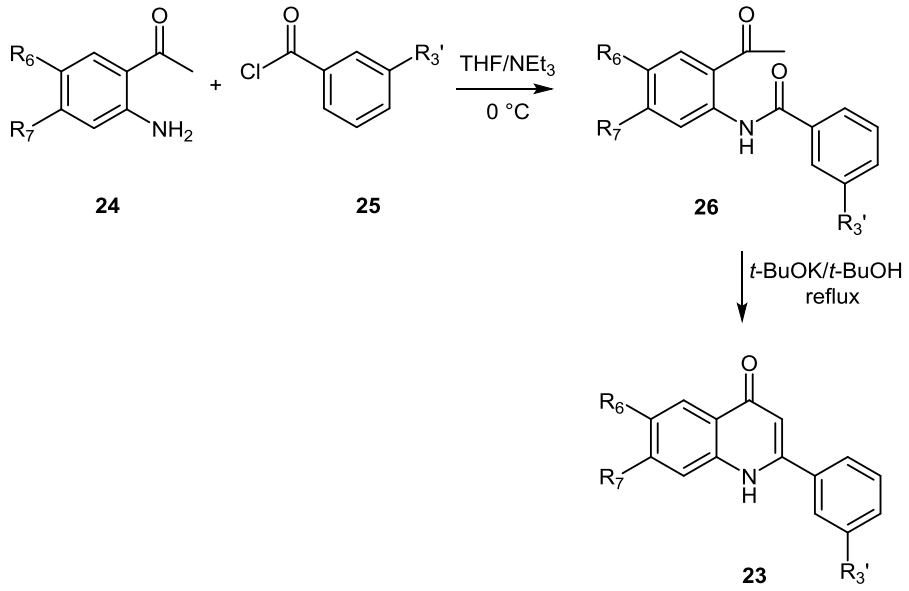
In a search for novel PDE4 inhibitors, Billah *et al.* employed this synthesis to yield the 8-methoxyquinoline **18**.[64] *o*-Anisidine was condensed with ethyl 4,4,4-trifluoroacetoacetate in the presence of polyphosphoric acid to give the quinolone **19**. Chlorination at the 4-position generated **20** and subsequent catalytic dehydrogenation furnished the 8-substituted quinoline **21**. Bromination at the 5-position followed by carbonylation afforded 8-methoxyquinoline-5-carboxylic acid **22**. Activation of the acid residue followed by reaction with the sodium salt of 4-amino-3,5-dichloropyridine gave 8-methoxyquinoline-5-carboxamide **18**, ***Scheme 2.3.1.2***. Derivatisation of **18** in a subsequent publication resulted in the synthesis of the dichloropyridine-*N*-oxide derivative.[65] Both of these compounds showed very promising results as selective inhibitors of PDE4.




***Scheme 2.3.1.2***

### 2.3.2 Camps Synthesis

Li *et al.* used the Camps synthesis to synthesise a range of 6,7-substituted-2-phenyl-4-quinolones **23** in a search for anticancer drug candidates due to the known anti-microtubular activity of 2-phenyl-4-quinolones, which interact with tubulin at the colchicine (***Figure 2.3.2.1)*** site.[66] Access to these compounds was achieved *via* two synthetic routes. The first, ***Scheme 2.3.2.1***, involves reacting *o*-amino acetophenones **24**, and benzoyl chlorides **25** to form diarylamides **26**. Potassium *tert*-butoxide mediated cyclisation of **26** resulted in the formation of **23**.




**24** **25** **26** **23**

***Scheme 2.3.2.1***

Li found that substitution at the 3'-position of 6,7-methylenedioxy derivatives is well tolerated with no significant change in activity for a range of electron-donating and electron-withdrawing substituents, the same was also true for steric bulk with OBz and H at the 3'-position showing similar results. 6-Amino derivatives (morpholine and pyrrolidine) showed high activity with $IC_{50}$ values in the nano-molar range against tubulin polymerisation. Yields for the reactions ranged from 27-80% for a diverse range of substituents. All the compounds synthesised exhibited cytotoxic effects against a variety of human tumour cell lines including solid tumours. The most potent compound synthesised **27**, ***Fig. 2.3.2.1***, possessed $GI_{50}$ values in the nano and subnanomolar range across the majority of cell lines tested in the NCI programme and is also a potent inhibitor of radiolabelled colchicine binding to tubulin with activity comparable to the anti-mitotic products colchicine, podophyllotoxin and combretastatin A-4.

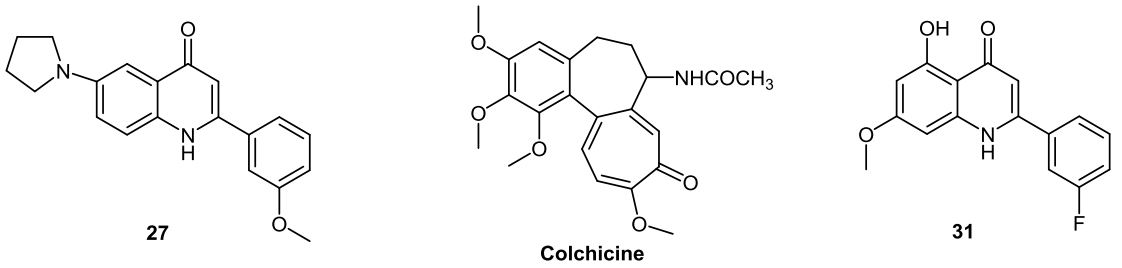
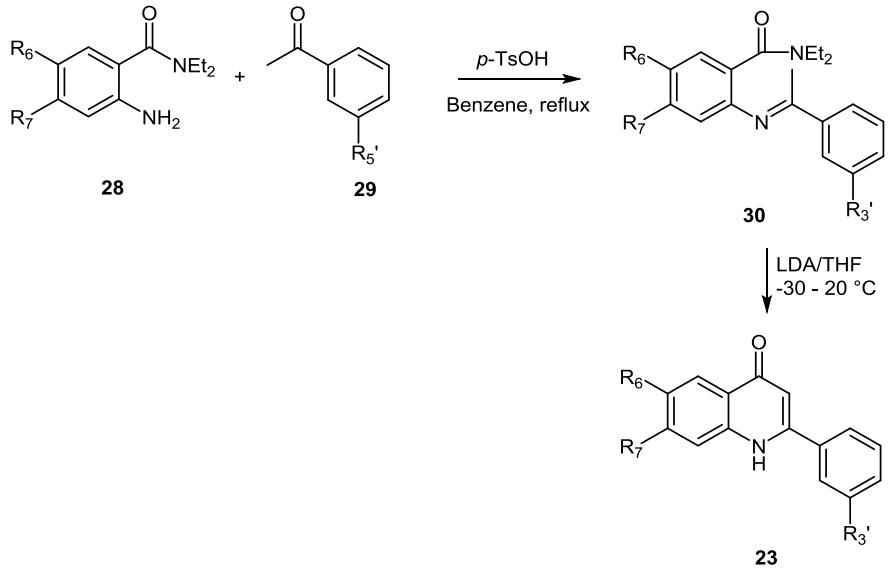



***Fig. 2.3.2.1***

The second method involves the acid catalysed condensation of substituted anthranilamides **28**, with substituted acetophenones **29** to generate the corresponding imines **30**. Lithium diisopropylamide mediated cyclisation of **30** gave the respective 2-phenyl-4-quinolones **23** in good yields, ***Scheme 2.3.2.2***.

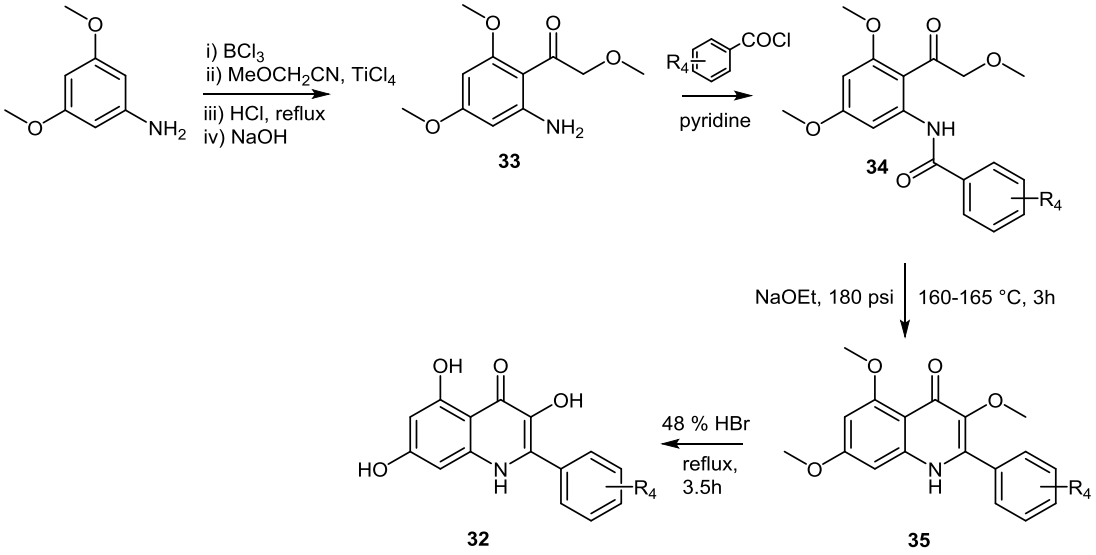



***Scheme 2.3.2.2***

More recently Hadjeri *et al.* reported the synthesis and anti-mitotic activity of 5-hydroxy-7-methoxy-2-phenyl-4-quinolones.[67] The compounds were synthesised in a similar fashion to ***Scheme 2.3.2.2***. In terms of structural requirements they found that a 7-methoxy, a 5-hydroxyl group and a free N-1 were all necessary for anti-mitotic activity. The presence of a fluorine at the 3'- or 2'- position and a methoxy or chloro group at the

7-position resulted in high activity for cell cycle arrest and antiproliferation, with **31** being the most potent, ***Fig. 2.3.2.1***.

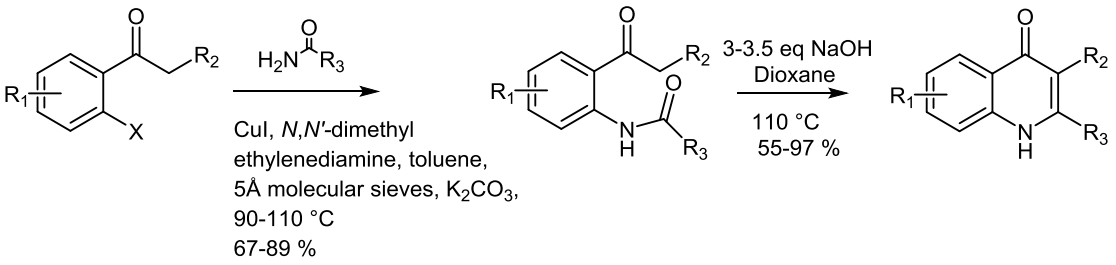
Sui *et al.* used the Camps synthesis to synthesise a series of novel quinolones **32** as potential topoisomerase II inhibitors.[68] In all twenty-six compounds were synthesised and tested against topoisomerase II using ellipticine, as a reference. The most potent of the compounds synthesised was over 400 times more potent than ellipticine.




***Scheme 2.3.2.3***

Ketones **33** were synthesised by regioselective electrophilic aromatic substitution at the *ortho* position by the corresponding nitriles using titanium tetrachloride as catalyst. *N*-Acylation of **33** using benzoyl chlorides under standard conditions gave the amides **34**. Cyclisation of **34** under pressure in the presence of sodium ethoxide gave quinolones **35**, which were demethylated using hydrogen bromide to yield quinolones **32**, ***Scheme 2.3.2.3***.

Buchwald *et al.* [69] developed a novel two-step synthesis of 2-aryl quinolones *via* a copper catalysed amidation of *o*-halophenones followed by a base catalysed Camps cyclisation of the resultant *N*-(2-ketophenyl)amides, ***Scheme 2.3.2.4***.



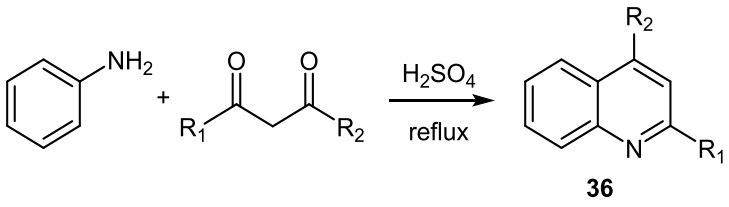

***Scheme 2.3.2.4***

From these findings a series of *N*-(2-ketophenyl)amides were synthesised in good yield. Cyclisation in the presence of 3-3.5 equivalents of base resulted in the generation of a library of 2-aryl (phenyl, chlorophenyl, pyridyl, thiophenyl and styryl) quinolones.

## 2.4 Synthesis of substituted quinolines

### 2.4.1 Combes synthesis

The Combes synthesis of quinolines was first described in 1888 by the French chemist Alphonse-Edmund Combes.[70] The reaction involves the acid catalysed condensation of *ortho*-unsubstituted anilines with β-diketones to generate 2,4-disubstituted quinolines, **36**, or β-keto aldehydes to give 4-substituted quinolines *via* an imine intermediate, ***Scheme 2.4.1.1***.



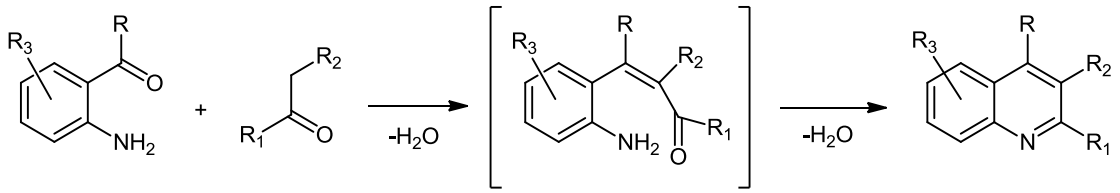

**36**

***Scheme 2.4.1.1***

Though one of the less utilised cyclisation methods the Combes synthesis has found applications in the synthesis of benzoquinolines[71,72] and pyrido[3,2'-*b*]carbazoles.[73]

### 2.4.2 Friedländer synthesis

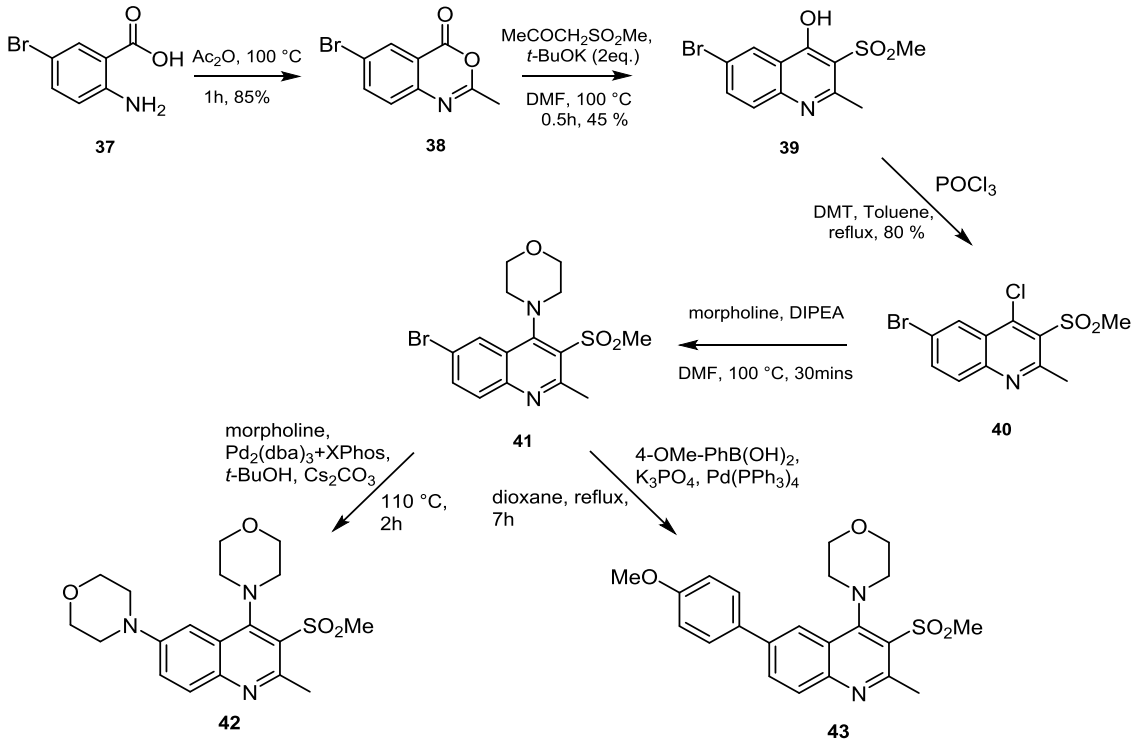
First described in 1882 by German chemist Paul Friedländer, the Friedländer reaction involves the condensation of *o*-aminoaryl aldehydes or ketones with an aldehyde or ketone possessing an α-$CH_2$ group under basic or acidic conditions, ***Scheme 2.4.2.1***.




***Scheme 2.4.2.1***

The Friedländer synthesis has the advantage of being one of the simplest and most straightforward methods for synthesising polysubstituted quinolines. The reaction is catalysed by both acid and base. Brønsted acids like sulfimic acid, hydrochloric acid, sulphuric acid, *p*-toluene sulfonic acid and phosphoric acid are widely reported as catalysts, though reaction conditions are usually harsh and lead to reduced efficiency and hence lower yields. Fehnel *et al.* reported that under thermal or base catalysed conditions simple ketones fail to react with *o*-aminobenzophenone.[74]

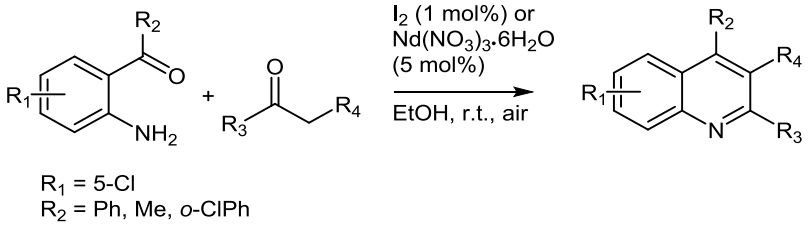
A novel synthesis of 3-(methanesulfonyl)quinolines was developed by Atechian *et al.* as previous literature reported low to mediocre yields and long reaction times. Anthranilic acid **37** was firstly cyclised to benzoxazinone **38** followed by conversion to 3-(methanesulfonyl)quinoline **39** in a 39% overall yield. The synthesis of **39** allowed access to derivatives at the 4-position. Chlorination of **39** using $POCl_3$ and *N*,*N*-dimethyl-*p*-toluidine in refluxing toluene for 7 hours gave **40**, as a crystalline solid**.** Further derivatisation of **40** with secondary amines, like morpholine, gave **41** in an 80% yield. Elaboration of the 6-position of **41** was achieved using both Buchwald and Suzuki-Miyaura protocols to generate **42** and **43** in 78% and 56% yields respectively, ***Scheme 2.4.2.2***.[75]




***Scheme 2.4.2.2***

### 2.4.2.1 Lewis acid catalysed Friedländer synthesis

Wu *et al.* published work detailing the use of molecular iodine as an efficient, mild and environmentally friendly catalyst in the Friedländer reaction.[76] After screening several reaction conditions it was found that 1 mol% of iodine at room temperature for 16 hours produced a variety of 2,3,4-trisubstituted quinolines in good to excellent yields, ***Scheme 2.4.2.1.1***. The reaction was also shown to tolerate a wide range of ketones both cyclic and acyclic.

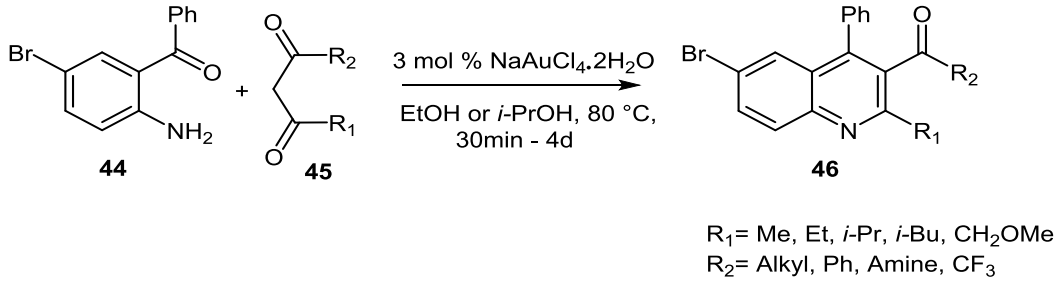



$R_1$ = 5-Cl
$R_2$ = Ph, Me, *o*-ClPh

***Scheme 2.4.2.1.1***

A similar study was carried out by Adapa and co-workers[77] where neodymium(III) nitrate hexahydrate (5 mol%) was used as the Lewis acid catalyst for the reaction, ***Scheme 2.4.2.1.1***. This reaction, like Wu's was applicable to a broad range of substrates, both cyclic and acyclic.

In 2007, Atechian and co-workers[75] published work detailing the synthesis of poly-substituted quinolines *via* a gold(III)catalysed Friedländer synthesis first described by Arcadi *et al.*[78] In this reaction the gold acts as a Lewis acid much like neodymium in Adapa's synthesis, ***Scheme 2.4.2.1.1.*** The general reaction is outlined in ***Scheme 2.4.2.1.2*****.**



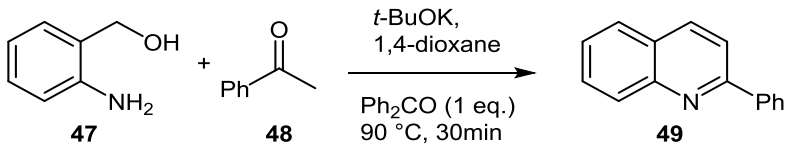

***Scheme 2.4.2.1.2***

The condensation of 2-aminobenzophenones **44** with **45** led to the formation of **46** however, if the carbonyl groups of **45** had similar reactivity a regioisomeric mixture was formed as was the case where $R_1$= *i*-Bu/$CH_2OMe$ and $R_2$= Me. The reaction proceeds smoothly giving moderate to good yields.

### 2.4.2.2 Synthesis of quinolines from alcohols

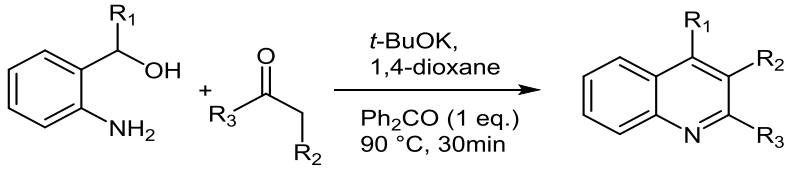
Ricardo and Yus developed an indirect Friedländer synthesis by reacting 2-aminobenzylic alcohols with a series of ketones.[79] They proposed a metal free Meerwein-Ponndorf-Verley reaction between 2-aminobenzylic alcohols and benzophenone using potassium *tert*-butoxide, followed by a Friedländer annulation to generate the quinoline. The mechanism was supported with deuterium labelling experiments and the fact that the reaction does not proceed in the absence of benzophenone. After optimisation using the

alcohol **47** and ketone **48** the desired quinoline **49** was isolated in a 99% yield, ***Scheme 2.4.2.2.1***.




***Scheme 2.4.2.2.1***

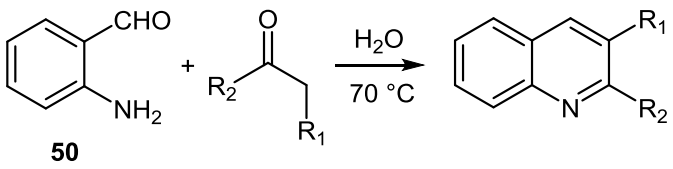
Expanding on this, a range of 2,3 and 4-tri-substituted quinolines were regioselectively synthesised in excellent yields, ***Scheme 2.4.2.2.2*.**




***Scheme 2.4.2.2.2***

**2.4.2.3 Catalyst-free Friedländer annulation in water**

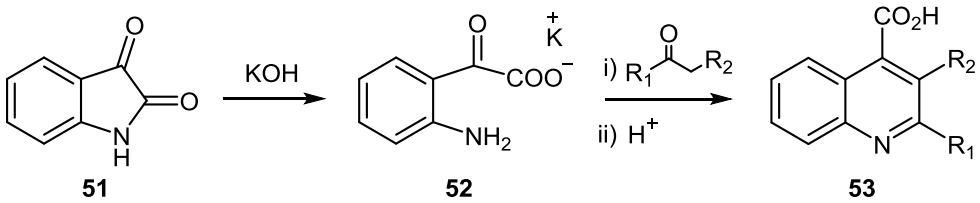
Wang *et al.* reported the synthesis of various quinolines from 2-aminobenzaldehyde **50** in aqueous conditions without the use of catalysts.[80] A series of 2,3-disubstituted and polycyclic quinolines were synthesised in excellent yields, ***Scheme 2.4.2.3.1***. This method is tolerant of a wide variety of substrates and is a useful, green addition to existing chemistry.




***Scheme 2.4.2.3.1***

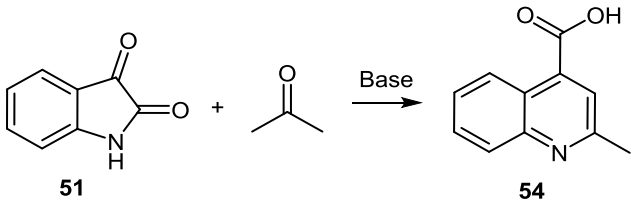
## 2.5 Pfitzinger synthesis of quinolines

The Pfitzinger reaction involves the reaction of isatin **51** or its derivatives with methylene ketones in an alkaline medium to generate quinoline-4-carboxylic acid derivatives. The reaction was first described in 1886 by W. Pfitzinger and is the most important variant of the Friedlander synthesis.[81] Isatin **51**, in the presence of base is converted into an isatinic acid salt **52** which cyclocondenses *via* the α-keto function with methylene ketones. Treatment with acid results in the formation of quinoline-4-carboxylic acid derivatives **53**, ***Scheme 2.5.1***.



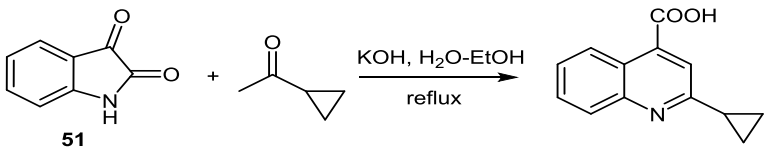

***Scheme 2.5.1***

Pfitzinger studied the reaction of isatin **51** with acetone in the presence of an aqueous base to generate 2-methyl-4-quinolinecarboxylic acid **54**, ***Scheme 2.5.2***. In further publications Pfitzinger reported optimised reaction conditions for the synthesis of **54** using 33% NaOH at 100 °C for 8 hours giving up to an 80% yield while various other groups reported a drop in yield using more dilute sodium hydroxide solutions.[82,83]



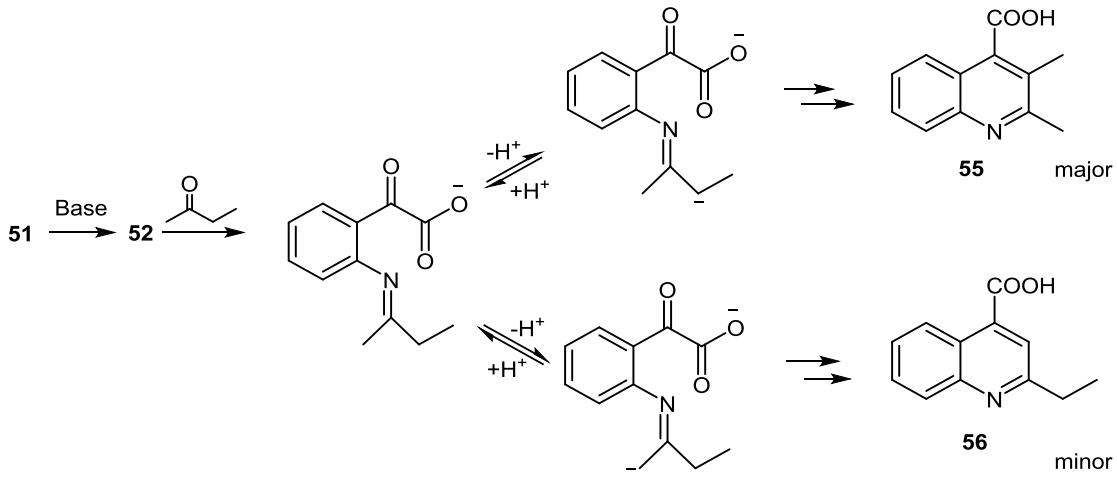

***Scheme 2.5.2***

As is shown in ***Scheme 2.5.2*** the reaction of **51** with symmetrical ketones results in the formation of only one product, similarly only one product is formed in the case of unsymmetrical ketones containing only one methyl/methylene group, ***Scheme 2.5.3***.




***Scheme 2.5.3***

Borsche *et al.*[84] and Braun *et al.*[85] carried out studies of unsymmetrical ketones (e.g. butanone) with **51** and found that 2,3-dimethyl-4-quinolinecarboxylic acid **55** was the major product with 2-ethyl-4-quinolinecarboxylic acid **56** being the minor. Palmer and McIntyre[86] published a mechanism explaining these observations, ***Scheme 2.5.4***.

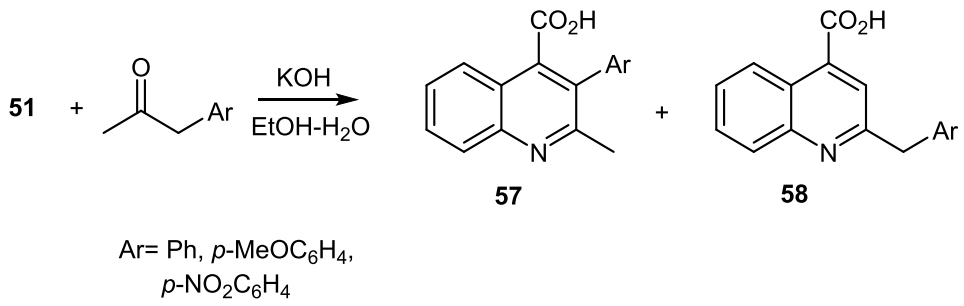



***Scheme 2.5.4***

Buu-Hoi *et al.* demonstrated that 7-halogen-substituted isatins, when reacted with methyl ethyl ketone only give 2,3-dimethyl-8-haloquinoline acids.[87,88] Generally the reactivity of carbanions for these reactions is 2°>1°>3° with the lack of 3° carbanion reactivity being due to steric restrictions. This rule is consistent with the findings of Palmer and McIntyre, ***Scheme 2.5.4***.[86]

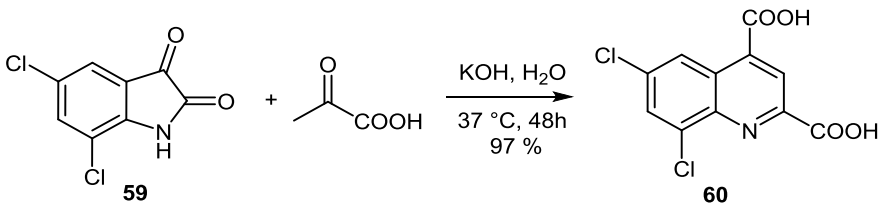
Unsymmetrical ketones which contain aryl substituents were investigated by Palmer and McIntyre[86], who found that the nature of the aryl substituent had an influence on the reaction products. Electron-withdrawing substituents at the *para*-position of the aryl group exclusively gave 3-aryl quinolinecarboxylic acids, **57**. Unsubstituted or electron-

donating groups at the *para*-position gave a mixture of compounds, **57** and **58** with the major product being 3-aryl quinolinecarboxylic acids, **57**, ***Scheme 2.5.5***.



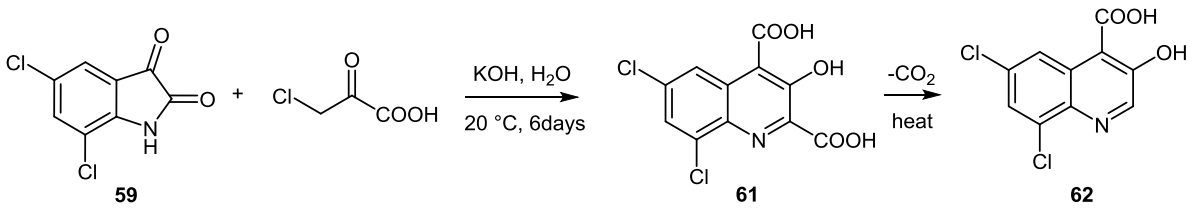

***Scheme 2.5.5***

Synthesis of quinolinedicarboxylic acids from isatin **59** and its derivatives and α-keto acids is well documented in the literature, chiefly due to the search for anti-malarial drugs. Many groups studied the reaction of **59** and its derivatives with pyruvic acid to generate substituted quinoline dicarboxylic acids. Buchman and co-workers[89] successfully synthesised 6,8-dichloroquinoline-2,4-dicarboxylic acid **60** in excellent yield using this protocol, ***Scheme 2.5.6***.




***Scheme 2.5.6***

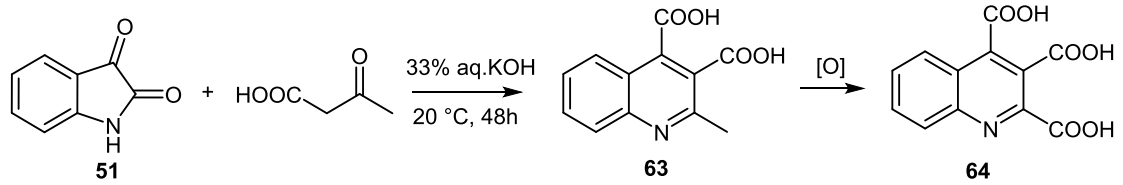
As can be seen in ***Scheme 2.5.6*** the reaction conditions are quite mild. Cragoe demonstrated that when halo-substituted acids or their esters are used the reaction takes place even at room temperature, an example of which is shown in ***Scheme 2.5.7***.[90,91]




***Scheme 2.5.7***

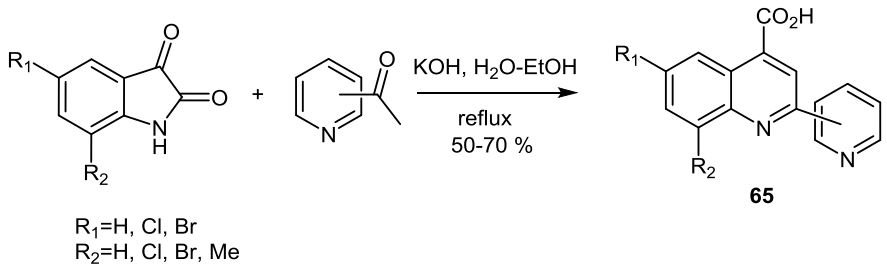
The reaction of isatin **59** with chloropyruvic acid generated the dicarboxylic acid **61** which was decarboxylated *in situ* to give **62** in an 85% yield.[92]

The reaction of isatin **51** with acetoacetic acid (β-keto acid) was investigated by Pfitzinger and structure **63** was proposed.[83] Enhelhard later proved the structure of **63** by oxidation to **64**, ***Scheme 2.5.8***.[93]



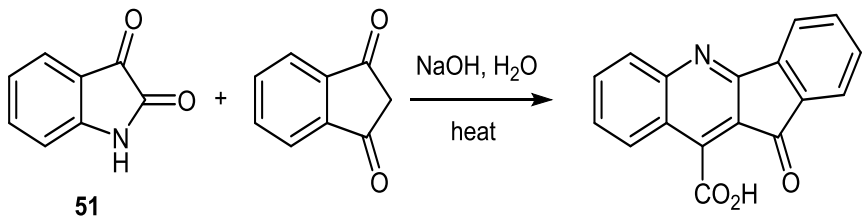

***Scheme 2.5.8***

Alkyl aryl ketones are common reactants used in the Pfitzinger reaction and generate 2-aryl quinoline-4-carboxylic acids exclusively.[83] Similarly alkyl hetaryl ketones yield 2-hetaryl quinolinecarboxylic acids. Due to the large number of publications only one example is shown, ***Scheme 2.5.9*****.** Gilman[94] and Atwell[95] synthesised compounds of type **65** in a search for anti-malarial drugs.




***Scheme 2.5.9***

The Pfitzinger reaction provides a convenient method for the synthesis of polycyclic systems when cyclic ketones/diketones are used resulting in a wide variety of fused quinoline derivatives e.g., ***Scheme 2.5.10***.[96]

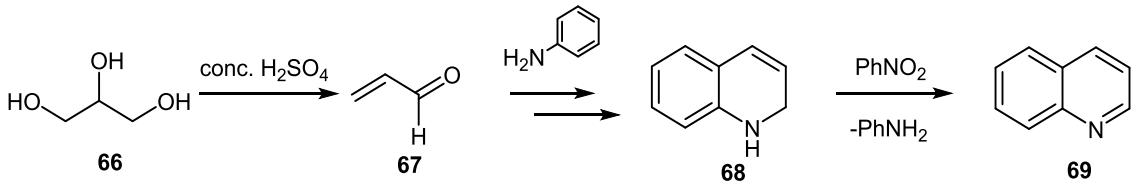



51

***Scheme 2.5.10***

## 2.6 Skraup type synthesis

The Skraup and Doebner-von Miller synthesis of quinolines involves the reaction of an aromatic amine containing at least one unsubstituted *ortho*-position with an electrophilic three carbon fragment. The archetypal Skraup synthesis involves the reaction of aniline with glycerol **66**, sulphuric acid and nitrobenzene as an oxidising agent, although more recent non-organic oxidising agents such as arsenic pentoxide, boric acid, iron(III) salts and iodine have replaced nitrobenzene due to the reduction of resin formation, leading to purer isolates.[97] The sulphuric acid acts to generate acrolein **67** *in situ* by catalysing the dehydration of **66** which then undergoes conjugate addition with aniline resulting in the formation of **68**. Nitrobenzene then oxidises **68** to quinoline **69**, ***Scheme 2.6.1***.

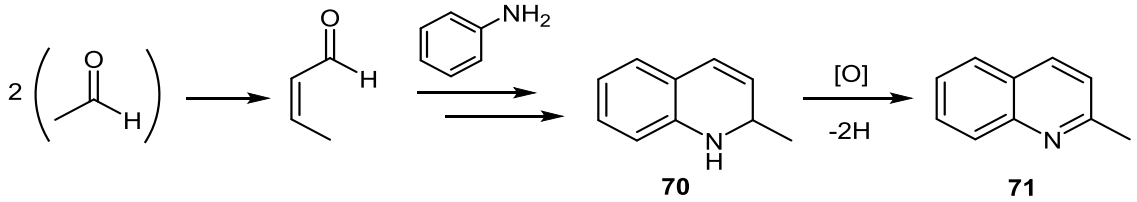



66 67 68 69

***Scheme 2.6.1***

The Doebner-von Miller reaction is classically described as the reaction of aniline with the crotonic condensation product of an aldehyde or ketone, in this case acetaldehyde,

generating dihydroquinoline **70** and oxidation results in 2-methylquinoline **71**, ***Scheme 2.6.2***.

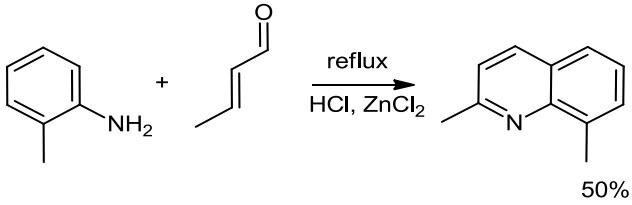



***Scheme 2.6.2***

Due to the low-yielding nature of the Skraup and Doebner-von Miller syntheses, recent investigations have been devoted to finding optimal reaction conditions. Li *et al.* used a system of 12 M HCl, toluene and tetrabutylammonium chloride when synthesising 2-alkyl-8-quinolinecarboxylic acid which gave a 57% yield.[98] Matsugi *et al.* also reported improved yields by using a mixture of 6M HCl and toluene in their syntheses.[99]

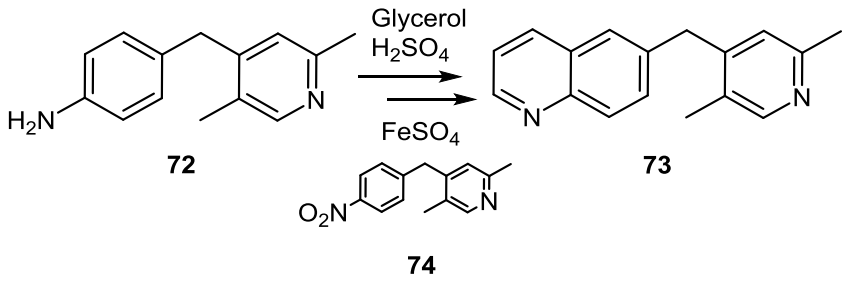
### 2.6.1 Substituent effects on cyclisation

Aromatic amines substituted at the *ortho*-position lead to the formation of 8-substituted quinolines, ***Scheme 2.6.1.1***.[100]




***Scheme 2.6.1.1***

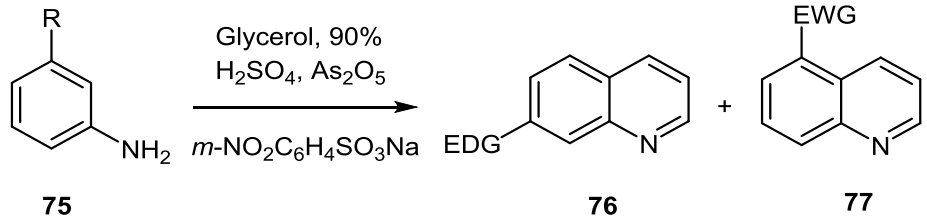
*para*-Substituted aminobenzenes cyclise at any symmetrical *ortho*-position to give 6-substituted quinolines. 2,5-Dimethyl-4-(*p*-nitrobenzyl)pyridine **72** was reacted under Skraup conditions to generate 6-[(2,5-dimethyl-4-pyridyl)methyl]quinoline **73**. 2,5-Dimethyl-4-(*p*-nitrobenzyl)pyridine **74** from which **72** was synthesised also acted as the oxidising agent, ***Scheme 2.6.1.2***.[101]




***Scheme 2.6.1.2***

*meta*-Substituted anilines, **75** cyclise to give a mixture of 5 and 7 substituted quinolines with the outcome dependant on the substituent at the *meta* position.

Strong electron-donating substituents preferentially give 7-substituted quinolines, **76** (78:22). Weaker electron-donating substituents also give 7-substituted quinolines with only a slight preference however (56:44). Strong electron-withdrawing groups promote cyclisation at the 2-position of **75** giving 5-substituted quinolines, **77** as the main product (78:22), ***Scheme 2.6.1.3***.[102-108]

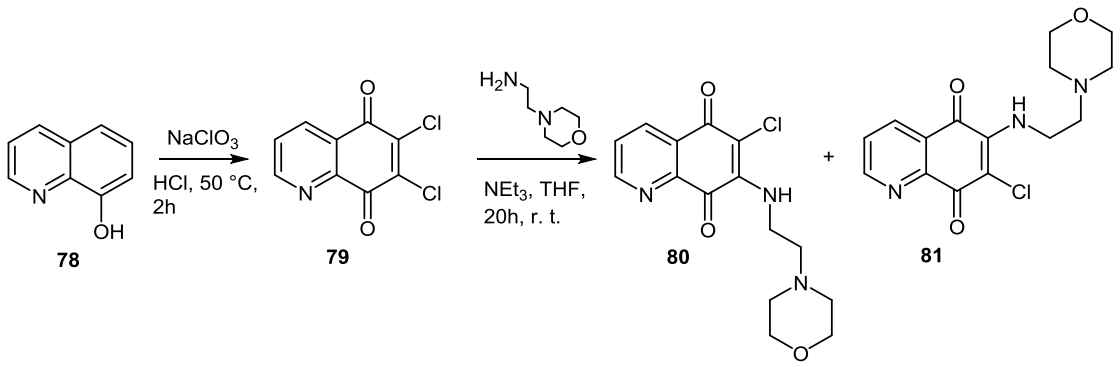



***Scheme 2.6.1.3***

## 2.7 Quinoline-5,8-diones

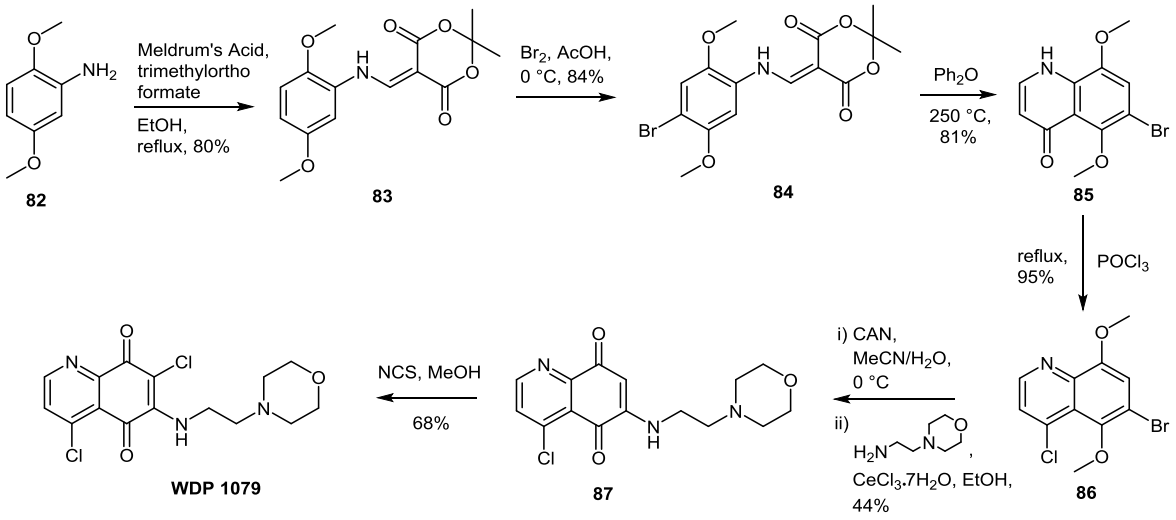
Central to this project is the synthesis of novel quinoline-5,8-diones. Interest in this family of compounds as antineoplastics arose from a study carried out in 2001 by Lazo and co-workers.[6] The search for novel inhibitors of Cdc25 in the NCI repository lead to the identification of 30 quinolinediones, of which 8 had micromolar activity. Outlined in ***Scheme 2.7.1*** is the synthetic route used by Lazo to access quinoline-5,8-diones using

syntheses previously described.[109-111] Oxidation and chlorination of quinoline-8-ol **78** was achieved in one step to generate **79** in a 30% yield. Amination of **79** was carried out at ambient temperature using functionalised ethyl amines in the presence of triethylamine, leading to the synthesis of a mixture of regioisomers **80** (DA3003-1) and **81** (DA3003-2) in a 2:1 mixture (measured by NMR) which were separated using column chromatography.



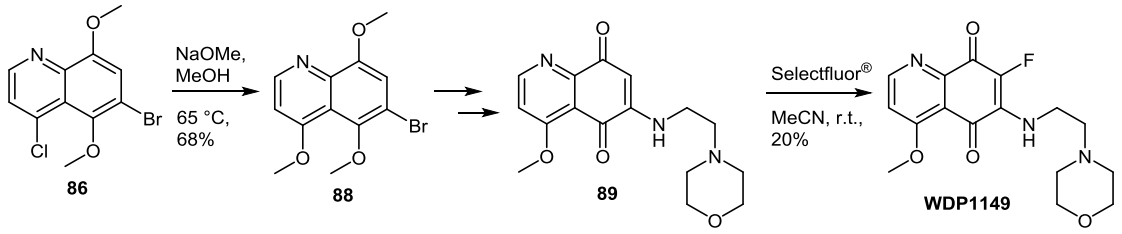

***Scheme 2.7.1***

More recently Wipf *et al.* published work detailing the synthesis of compounds of type **81** in an effort to address the problems associated with quinoline-5,8-dione redox cycling leading to undesired off-target mechanisms. Synthesis starts from 2,5-dimethoxyaniline **82** which is refluxed with Meldrum's acid and trimethyl orthoformate resulting in the formation of **83** in an 80% yield. Bromination of **83** at the four-position was achieved using a mixture of bromine and acetic acid to afford **84** in an 84% yield. Pyridone **85** is realised in an 81% yield by refluxing **84** in diphenyl ether at 250 °C. Treatment of **85** with $POCl_3$ results in the formation of the 4-chloroquinoline **86**. Synthesis of the desired quinoline-5,8-diones was carried out in a two-step process. Firstly **86** is oxidatively demethylated using ceric ammonium nitrate. Following completion the isolated crude product is aminated at the 6-position using 4-(2-aminoethyl)morpholine to afford the functionalised quinone **87**. Finally chlorination of the 7-position was achieved by treating a methanolic solution of **87** with *N*-chlorosuccinimide to generate WDP1079 in a 65% yield, ***Scheme 2.7.2***.[28]




***Scheme 2.7.2***

Wipf also used this synthesis to generate the 4-methoxy-7-fluoro derivative of WDP1079, WDP1149. Generation of the methyl ether was afforded by heating **86** with sodium methoxide in methanol to generate **88**. Synthesis of the 6-amino quinoline was achieved using analogous conditions to ***Scheme 2.7.2***. Fluorination of the 7-position was achieved using Selectfluor® to afford WDP1149 albeit in low yield, ***Scheme 2.7.3***.[28]




***Scheme 2.7.3***

## 2.8 Conclusion

Due to their widespread applications in medicinal chemistry considerable progress has been made in the development of the efficient synthesis of the quinoline pharmacophore. While traditional methods remain firmly rooted in many syntheses, pullulating biological interest has led to the genesis of numerous novel methodologies which offer highly derivatised quinolines from simple precursors.

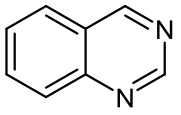
Given the promising preliminary biological results attributed to quinoline-5,8-diones this family of compounds make an attractive synthetic target. The sum total of literature in this area is outlined in ***Section 2.7*** making it obvious that the scope for development of this area is tremendous. With the importance of their application highlighted in ***Section 1.1.4*** development of this pharmacophore is imperative in order to elucidate their mode of action and ameliorate this area of medicinal chemistry.

# *3.0 Quinazoline Chemical Introduction*

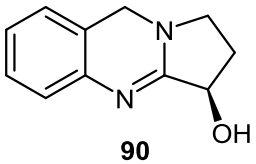
## Contents

## 3.1 Quinazoline introduction

Quinazolines **89**, ***Scheme 3.1.1*** and their related derivatives can be found embedded in a wide range of biologically active compounds, for example, anti-cancer, anti-fungal, diuretic, anti-inflammatory, anti-convulsant and anti-hypertensive drugs.[112-115] This class of compound forms a substantial part of heterocyclic chemistry and is of considerable interest to the pharmaceutical industry.




**89**




**90**

***Scheme 3.1.1***

The first quinazoline-based alkaloid natural product, vasicine (peganine) **90**, ***Scheme 3.1.1*** was isolated from the plant material of *Adhatoda vasica* in 1888.[116] Research remained dormant until the 1950's when the effectiveness of quinazoline derivatives as anti-bacterial, anti-viral and anti-parasitic agents were discovered.[117] It has only been the last fifteen years that research has seen significant advances, which is driven by their diverse applications.

Many literature syntheses of quinazolines require long reaction times and are often low yielding.[118-120] As a result much attention has been focussed to develop more efficient methods for the construction of quinazolines. Although in the majority of cases no new chemistry was employed, the use of microwave-enhanced processes and new catalysts offer clear advantages both in yield and reaction time.

In this chapter synthetic routes will be classified based on the increasing substitution patterns of the pyrimidine ring system converging on syntheses most relevant to this work;

- 2-Substituted quinazolines
- 4-Substituted quinazolines

- 2,4-Disubstituted quinazolines
- Quinazoline-2,4-diones
- *N*-3 Substituted quinazoline-2,4-diones
- *N*-1, *N*-3 Substituted quinazoline-2,4-diones

## 3.2 Synthesis of 2-substituted quinazolines

2-Substituted quinazolines represent a medicinally important branch of quinazolines, finding uses in the treatment of essential thrombocytosis, chronic myeloid leukaemia, hypertension and heart disease. Anagrelide is used in the treatment of essential thrombocytosis (overproduction of blood platelets) and has also been used for treating chronic myeloid leukaemia.[121] Its congener, quazinone is a PDE3 inhibitor used for the treatment of heart disease. Quinethazone is a thiazide-type diuretic used in the treatment of hypertension,[122] ***Fig. 3.2.1***.

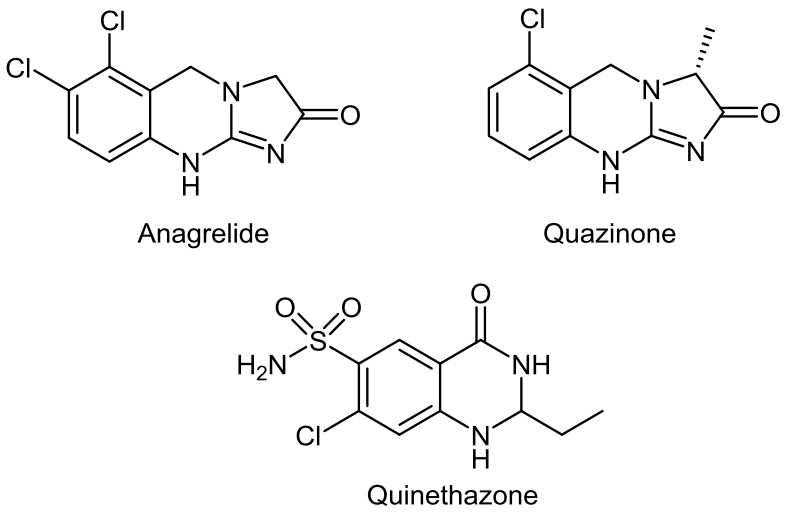
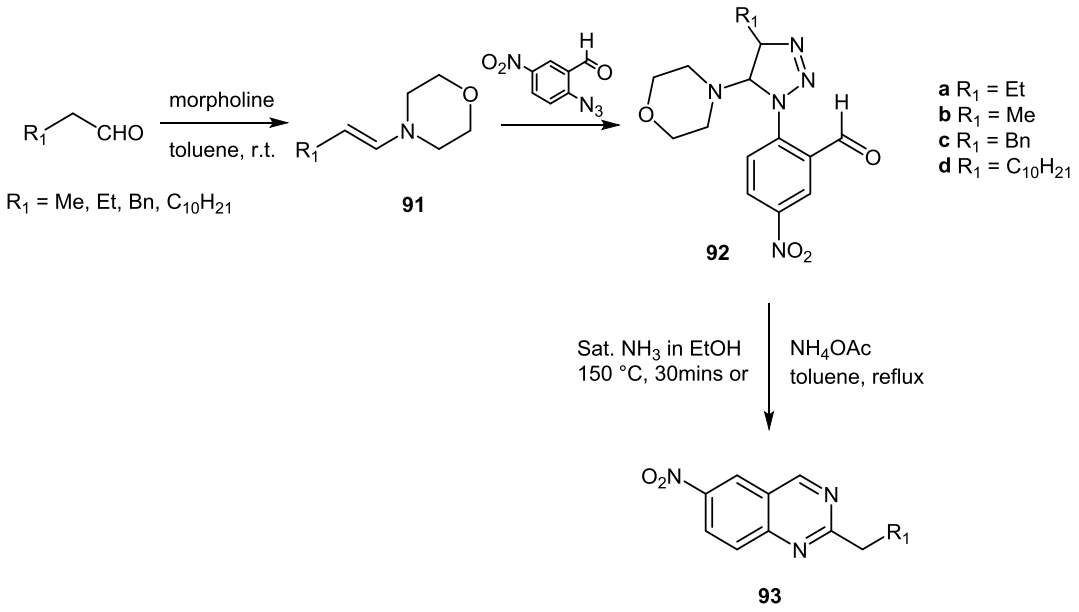



***Fig. 3.2.1***

### 3.2.1 Synthesis from triazoline intermediates

In 1999 Erba and co-workers published a method for the synthesis of 2-alkylquinazolines from triazolines, ***Scheme 3.2.1.1***.[123] Synthesis of the triazoline intermediates began by reacting a functionalised aldehyde with morpholine in toluene at room temperature to generate the enamine **91** and subsequent reaction with an aryl azide to generate triazoline **92**.

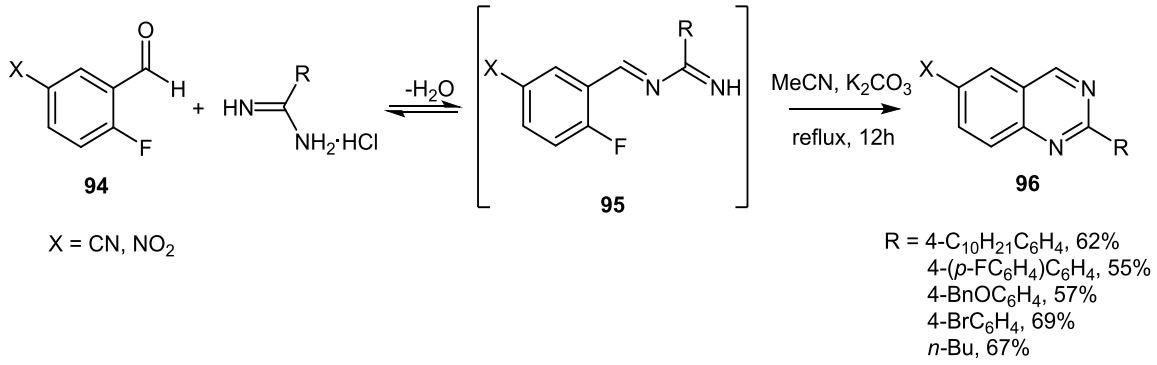
Cyclisation to 2-alkylquinazolines **93** was afforded by treatment of **92** with either a saturated ethanolic solution of ammonia at 150 °C, or in ammonium acetate in refluxing toluene. Yields for this reaction varied from excellent **93a** (92%) and **93b** (95%) to moderate for **93c** (38%) and **93d** (37%). Overall this synthesis proved to be a robust and reliable method for the synthesis of 2-substituted quinazolines that possess electron-withdrawing groups at the 6-position.




***Scheme 3.2.1.1***

### 3.2.2 Reaction of amidines with 2-fluorobenzaldehydes

In 1999 Kotsuki *et al.* published their work on the reaction of cyano/nitro activated *o*-fluorobenzaldehydes with a variety of arylamidines to generate 2-aryl quinazolines in moderate yields after chromatography, ***Scheme 3.2.2.1***.[124] The reaction involves the condensation of *o*-fluorobenzaldehydes **94** with aryl amidines resulting in the formation of imines **95** followed by a nucleophilic aromatic substitution at the *ortho*-position in the presence of potassium carbonate in acetonitrile at reflux to generate quinazolines **96**.




***Scheme 3.2.2.1***

## 3.3 Synthesis of 4-substituted quinazolines

Some of the most promising 4-substituted quinazolines synthesised to date are shown in ***Fig. 3.3.1***. In 2003 Iressa™ was the first epidermal growth factor receptor inhibitor, for the treatment of lung cancer.[113] Similar compounds such as afatinib and dacomitinib are currently undergoing phase III clinical trials, ***Fig 3.3.1***. The prevalence of this family of compounds in chemotherapy was previously highlighted in ***Section 1.2***.

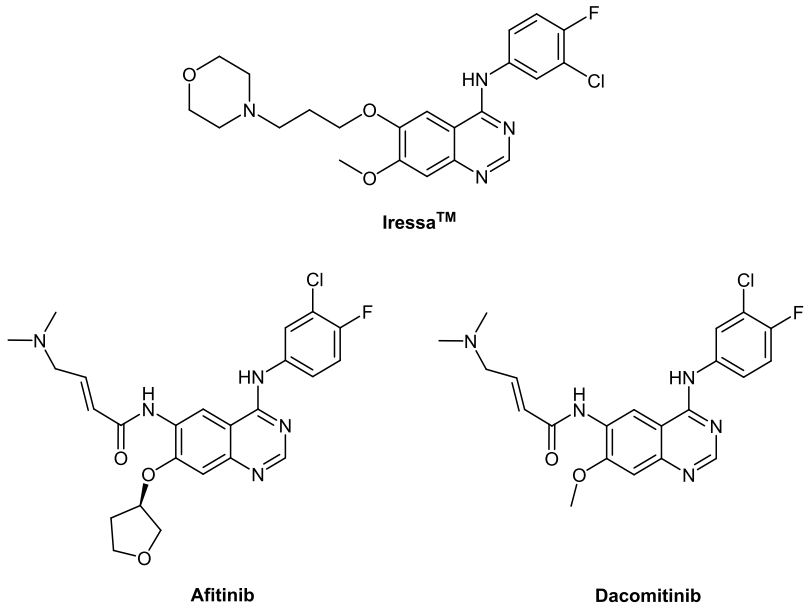
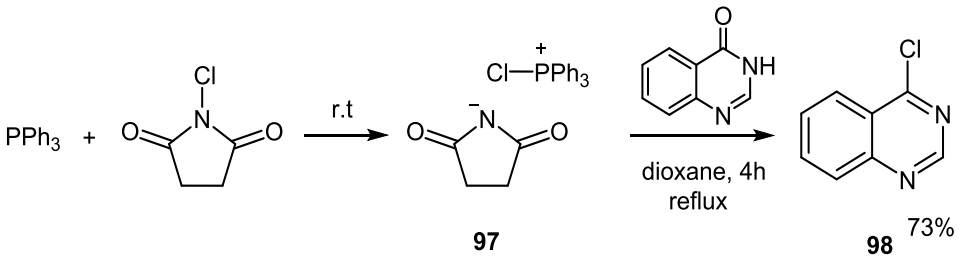



***Fig. 3.3.1***

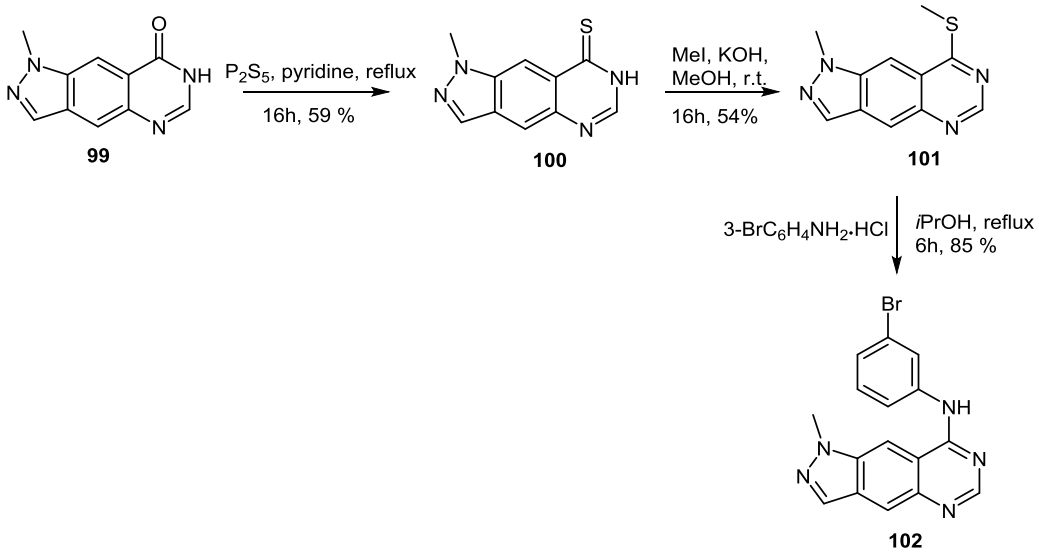
### 3.3.1 Derivatisation of 4(3*H*)-quinazolinones

Conversion of 4(3*H*)-quinazolinones to 4-chloroquinazolines is well documented in the literature and is most commonly achieved by treating a quinazolin-4-one with $POCl_3$ or thionyl chloride.[125-127] An alternative synthesis was published by Sugimoto *et al.* which involves the use of a phosphonium salt of *N*-chlorosuccinimide, **97** in refluxing dioxane to give good yields of 4-chloroquinazolines, **98**, ***Scheme 3.3.1.1***.[128] 4-Chloroquinazolines are very versatile intermediates as they can be derivatised further through nucleophilic attack at the C-4 position.



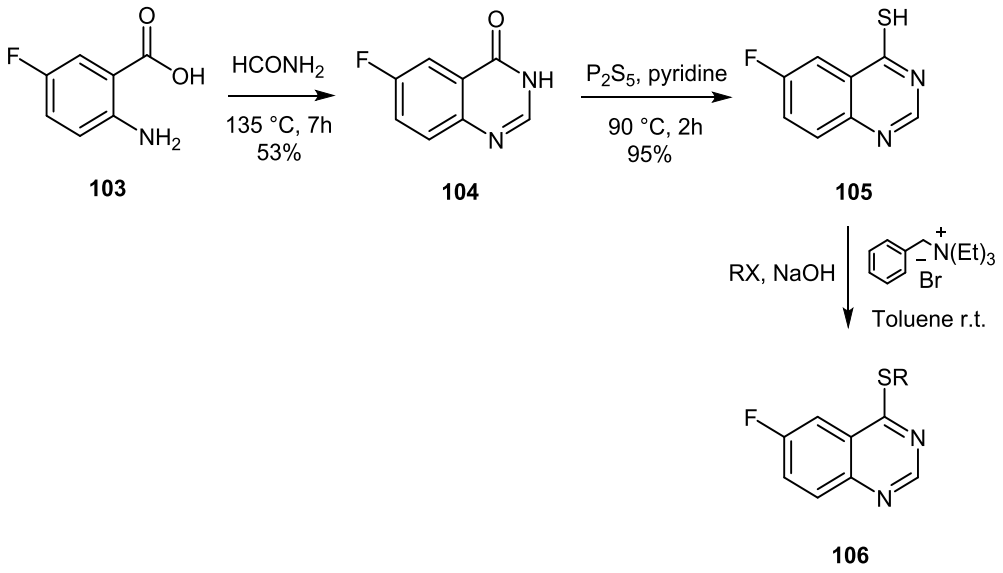

***Scheme 3.3.1.1***

The 4-position can also be activated using a thiomethyl substituent as reported by Rewcastle *et al.*[126] Treatment of quinazolinone **99** with Lawesson's reagent affords quinazolinethione, **100** which is converted to the thiomethyl ether **101** by treatment with potassium hydroxide and iodomethane. Displacement of the thioether with a nucleophile affords the 4-substituted quinazoline **102**, in good yield, ***Scheme 3.3.1.2***. 4-Arylaminoquinazolines are of particular interest due to their potential as antitumour agents, ***Scheme 3.3.2.2***.[113]




***Scheme 3.3.1.2***

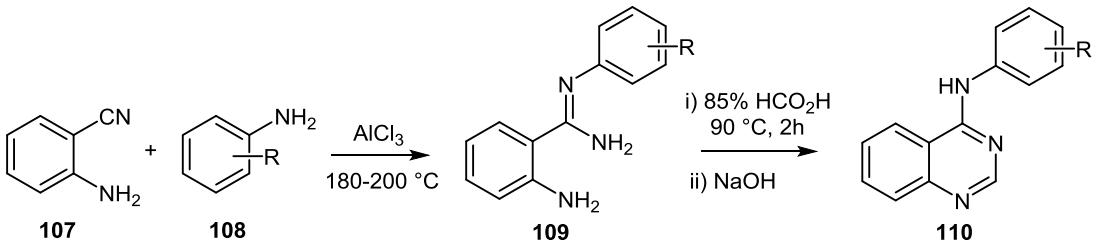
As well as useful intermediates 4-thioquinazolines also garnered some attention as potential antifungal agents. Xu and co-workers synthesised a range of 6-fluoro-4-alkylthioquinazolines with derivatives containing 4-thioallyl, 4-thio-*n*-propyl and 4-thioethyl showing good antifungal activity.[129] Synthesis began by reacting 2-amino-5-fluorobenzoic acid **103** with formamide to generate the quinazolinone **104**. Thiol **105** was afforded from treatment of **104** with Lawesson's reagent. Alkylthioquinazolines **106** were synthesised in good to excellent yield by treating **105** with a number of alkyl halides under phase-transfer conditions, ***Scheme 3.3.1.3***.




***Scheme 3.3.1.3***

### 3.3.2 Reaction of anilines with 2-aminobenzonitrile

4-Anilinoquinazolines can also be synthesised from the reaction of 2-aminobenzonitrile **107** and anilines **108** as detailed by Szczepankiewicz *et al.*[130,131] These reactions proceed *via* amidines **109** which are heated with 85% formic acid to give 4-arylaminoquinazolines **110** in good yields, ***Scheme 3.3.2.1***.




***Scheme 3.3.2.1***

Compounds of similar structure were reported by Foote *et al.* which exhibited anti-tumour activity, **111**, ***Fig. 3.3.2.2***.[132] Vasdev and co-workers published work on $^{18}F$ labelled 4-anilinoquinazolines as potential EGFR imaging probes, **112**, ***Fig. 3.3.2.2***.[133]

4-Anilinoquinazolines have also been studied as potential tyrosine kinase inhibitors by pharmaceutical companies, AstraZeneca[134] and Qilu, **113**, ***Fig. 3.3.2.2***.[135,136]

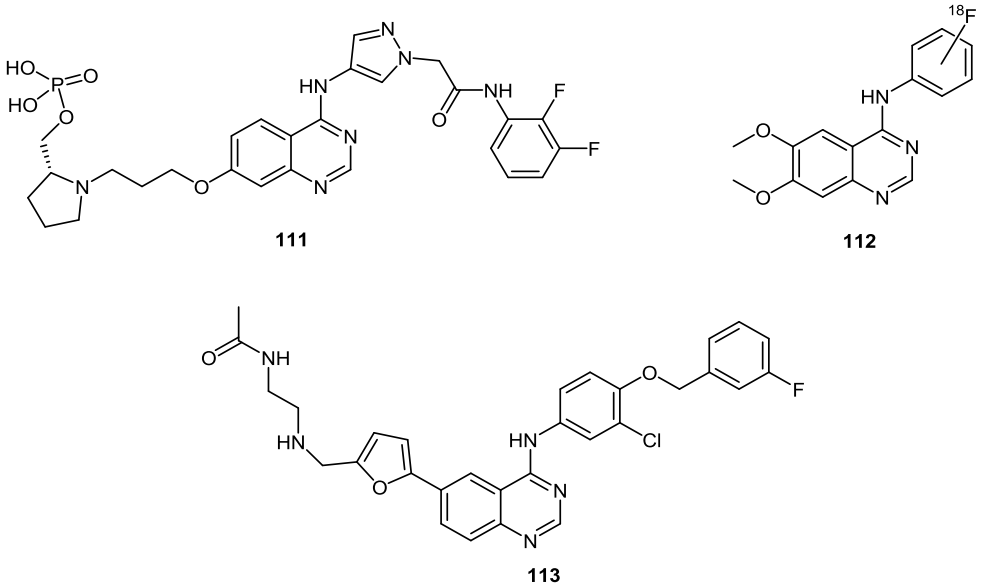
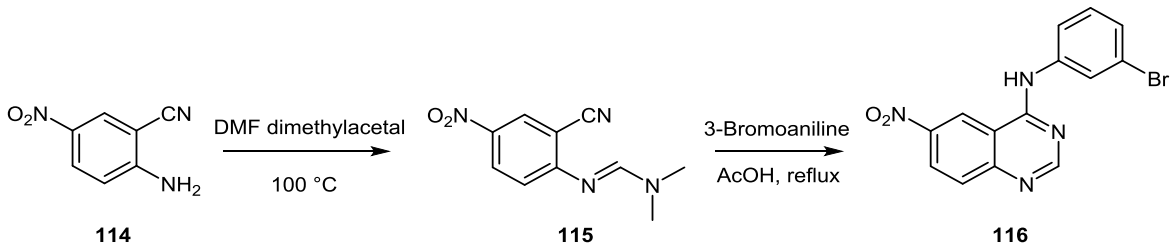



***Fig. 3.3.2.2***

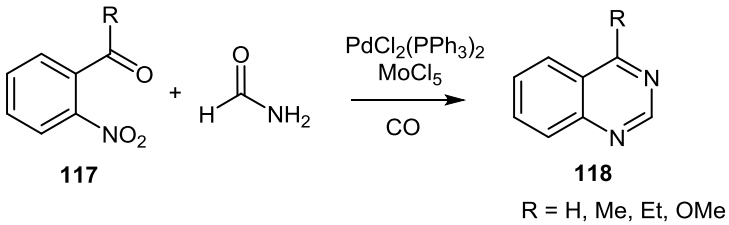
Tsou *et al.* reported an efficient method for the synthesis of 4-anilinoquinazolines where incorporation of the 4-anilino group and ring closure were achieved in one step.[137] The first step of the reaction involved the condensation of 2-amino-5-nitrobenzonitrile **114** with DMF dimethylacetal. Refluxing the resultant imine **115** in acetic acid with 3-bromoaniline gave the desired compound **116** in an 89% yield, ***Scheme 3.3.2.3***. A similar method was reported by Yoon *et al.* using microwave conditions to generate substituted 4-aminoquinazolines in excellent yield.[138]




***Scheme 3.3.2.3***

### 3.3.3 Palladium mediated quinazoline synthesis

A novel method for synthesising 4-substituted quinazolines involving a palladium catalysed reaction was developed by Akazome *et al*.[139] The reaction involves an intermolecular reductive *N*-heterocyclisation between 2-nitrophenyl ketones **117** and formamide to give a variety of 4-substituted quinazolines **118**, ***Scheme 3.3.3.1***. It was speculated by the authors that the reaction proceeds *via* an active nitrene intermediate which was generated by selective deoxygenation of the nitro group by carbon monoxide.




***Scheme 3.3.3.1***

## 3.4 Synthesis of 2,4-disubstituted quinazolines

There are many examples of 2,4-disubstituted quinazolines in medicine, three of which are highlighted in ***Fig. 3.4.1***. Bunazosin was initially developed to treat benign prostatic hyperplasia.[140] Its congener prazosin is an alpha-adrenergic antagonist used for the treatment of high blood pressure, anxiety and panic disorder.[141] Linagliptin is a 2,4-disubstituted quinazoline which was approved in 2011 for the treatment of type-II diabetes, which acts by inhibiting DPP-4.[142]

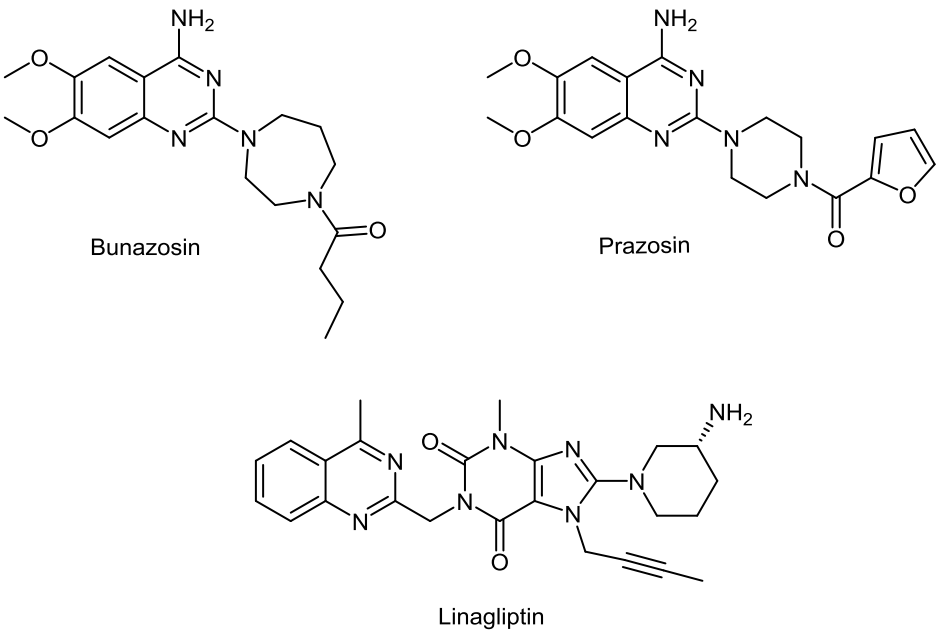
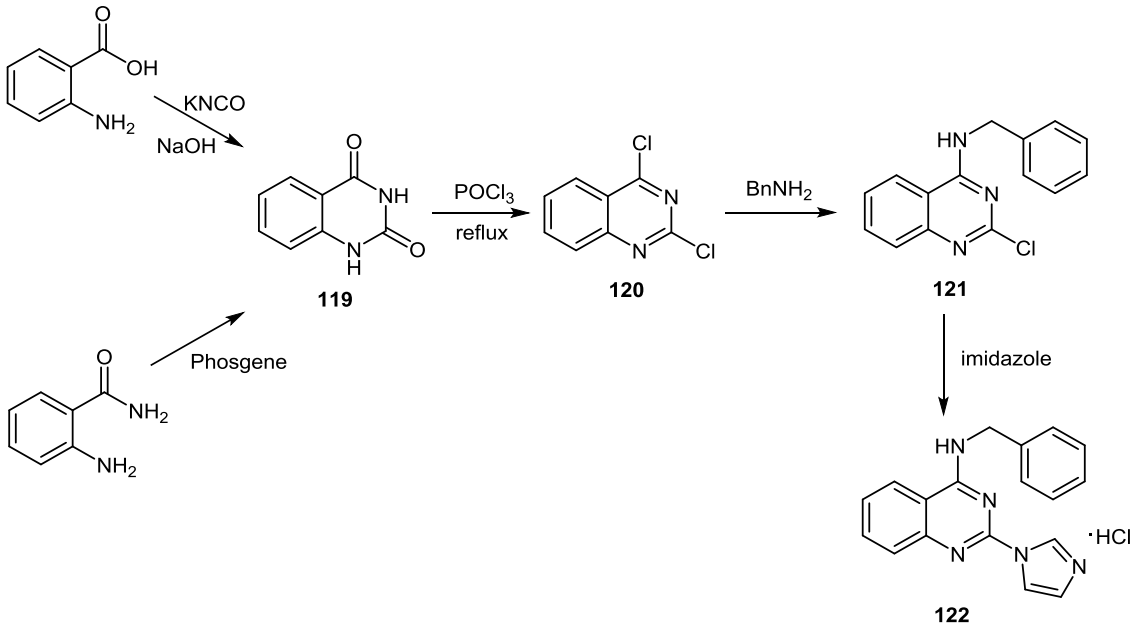



***Fig. 3.4.1***

### 3.4.1 Reactivity of 2,4-dichloroquinazolines

As was mentioned in ***Section 3.3.1***, chloroquinazolines serve as useful intermediates when derivatising quinazolines. 2,4-Dichloroquinazolines can be accessed in good yields from chlorinating the corresponding quinazoline-2,4-dione using phosphorous oxychloride or thionyl chloride. Due to the increased electrophilicity of the 4-position, regiospecific substitution can be achieved by nucleophilic substitution. Lee *et al.* exploited this regioselectivity in the synthesis of potential phosphodiesterase inhibitors, ***Scheme 3.4.1.1***.[143]




***Scheme 3.4.1.1***

Quinazoline-2,4-dione **119** was prepared from anthranilamide using phosgene or anthranilic acid using potassium isocyanate followed by cyclisation. 2,4-Dichloroquinazoline **120** was furnished by refluxing **119** in $POCl_3$. The 4-position was then selectively aminated using benzylamine. An imidazole moiety was subsequently introduced at the 2-position by heating **121** with excess imidazole to give **122** in a 63% yield. The inherent reactivity of both the 2 and 4 positions allows expansive diversification of the quinazoline pharmacophore in a regioselective manner.

Undheim *et al.* investigated the use of trialkylalanes in palladium catalysed coupling reactions, ***Fig. 3.4.1.2***.[144] Synthesis of 2-chloro-4-methylquinazoline, **123** is achieved in a 76% yield. The mechanism involves the oxidative addition at the more electrophilic 4-position of **120** to the palladium (0) complex. The methyl group is transferred to the palladium (II) complex from the aluminium and subsequent reductive elimination generates **123** in good yield. Repeating the process using tri-isobutylalane affords 2-isobutyl-4-methylquinoline, **124** in an 80% yield. This method provides a regiospecific route to bioactive 2,4-disubstituted quinazolines.

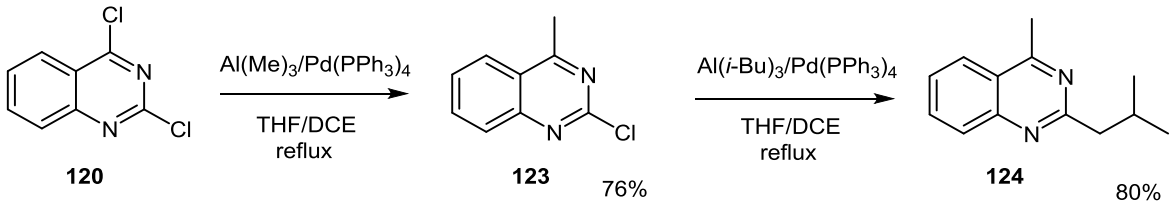
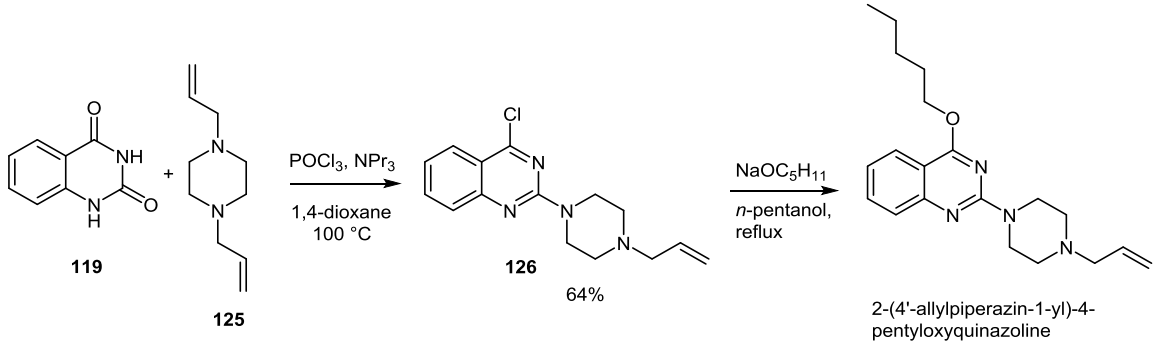



***Fig. 3.4.1.2***

### 3.4.2 Chlorination of quinazoline-2,4-dione in the presence of cyclic amines

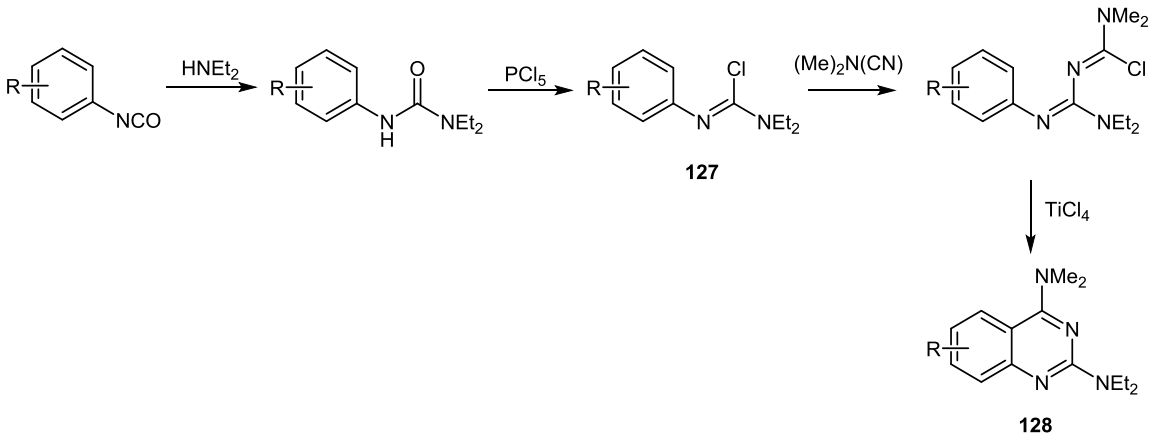
As was alluded to in ***Section 3.4.1*** the 4-position of **120** is more reactive towards nucleophiles than the 2-position. Based on work initially carried out by Miki, Yoshida *et al.* published work which demonstrated that the 2-position could be selectively substituted using a tertiary cyclic amine in the presence of $POCl_3$ in good yield.[145,146] This work was conducted while developing a more frugal route to the potential anti-dementia drug, 2-(4'-allylpiperazin-1-yl)-4-pentyloxyquinazoline. Reaction of quinazoline-2,4-dione **119** and 1,4-diallylpiperazine **125** afforded the key intermediate, 2-(4'-allylpiperazin-1-yl)-4-chloroquinazoline **126**, ***Scheme 3.4.2.1*.** If a primary or secondary amine is used in place of a tertiary the reaction proceeds typically with substitution occurring at the more favoured 4-position.[118]




***Scheme 3.4.2.1***

### 3.4.3 Synthesis of 2,4-diaminoquinazolines

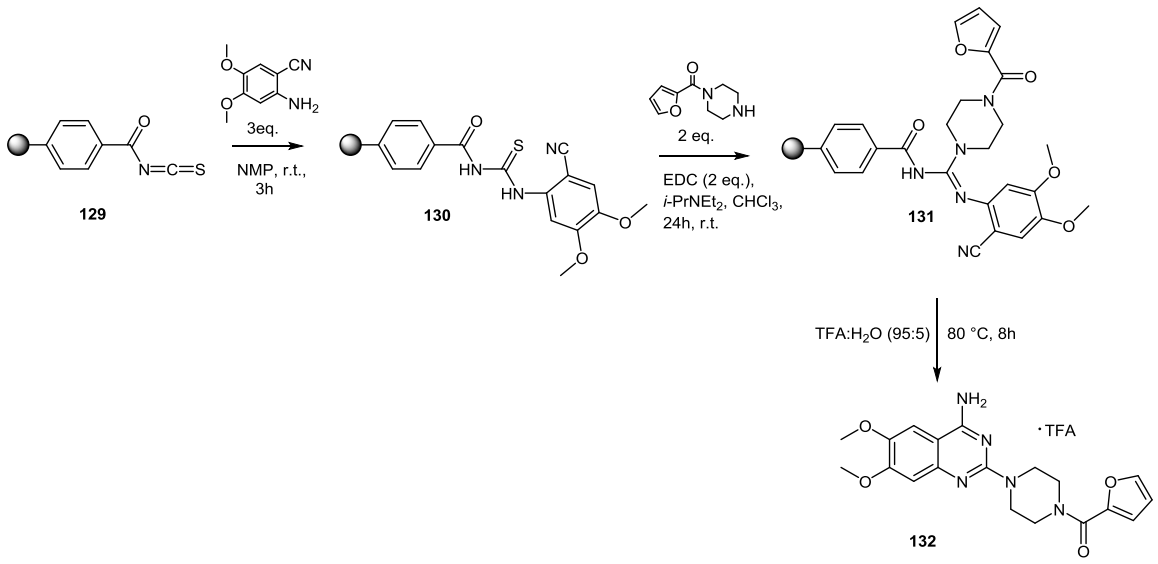
Zielenski *et al.* reported the synthesis of 2,4-diaminoquinazolines from chloroamidines and dialkylcyanamides.[147] The chloroamidines were synthesised in two steps from substituted phenyl isocyanates *via* reaction with *N*,*N*-diethylamine to afford substituted ureas. Treatment with phosphorous pentachloride generated the chloroamidines **127**. Reaction with *N,N*-dimethylcyanamide followed by cyclisation furnished 2,4-diaminoquinazolines **128**, ***Scheme 3.4.3.1***. Although a wide variety of substituted phenylisocyanates were tolerated, purification of the quinazolines proved difficult in some cases leading to lower yields.




***Scheme 3.4.3.1***

Wilson *et al.* reported the synthesis of 2,4-disubstituted quinazolines using a resin bound isothiocyanate **129** generated from the parent carboxystyrene resin. Their investigation began with the synthesis of the antihypertensive drug prazosin, which was achieved in three steps from **129**. 2-Amino-4,5-dimethoxybenzonitrile was dissolved in NMP, added to the resin and stirred for 3 hours to generate **130**. Treatment of the intermediate with 1-(2-furoyl)-piperazine and EDC under basic conditions to furnish the resin bound guanidine **131**. Cleavage and cyclisation was achieved using a mixture of trifluoroacetic acid and water yielding prosazin **132** as the TFA salt in a 24% overall yield, ***Scheme***

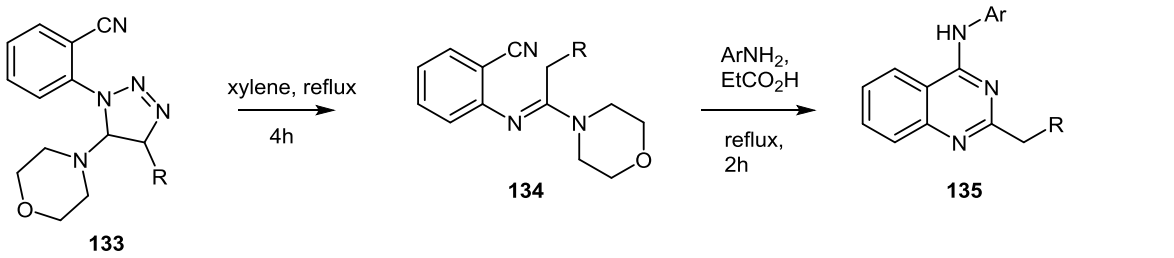
***3.4.3.2***.[148] This method offers an alternative to sequential chlorine displacement of 2,4-dichloroquinazolines employed by *Lee et al.*.[143]




***Scheme 3.4.3.2***

### 3.4.4 Rearrangement of triazolines to 2-alkyl-4-arylaminoquinazolines

Previous to his work on 2-substituted quinazolines (***Scheme 3.2.1.1***) Erba *et al.* reported the synthesis of 2-alkyl-4-anilinoquinazolines *via* the cyclisation of arylamines with amidines. Refluxing triazoline **133** in xylene induces thermal elimination of nitrogen followed by rearrangement to the tertiary amidine **134**. Reaction of **134** with anilines afforded a range of 2-alkyl-4-anilinoquinazolines **135** in moderate to low yields, ***Scheme 3.4.4.1***.[149] The low yields of **135d** and **135e**, which could not be improved upon with longer reaction times, is due to the lower nucleophilicity of the substituted arylamines used for the cyclisation. Conversely **135c** shows a higher yield due to the increased nucleophilicity of the aryl substituent. It also seems that the steric bulk of the R group influences yields, which is illustrated when comparing **135a** and **135b**.

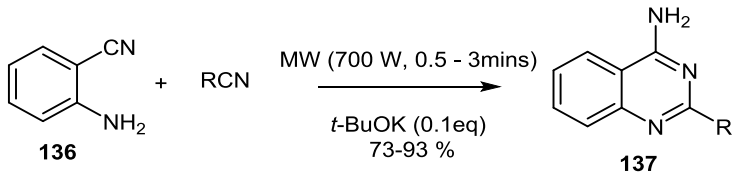



**a** R = Me, Ar = Ph, 45%
**b** R = Bz, Ar = Ph, 30%
**c** R = Et, Ar = 4-$OMeC_6H_4$, 65%
**d** R = Et, Ar = 4-$ClC_6H_4$, 28 %
**e** R = Et, Ar = 3-$CF_3C_6H_4$, 15%

***Scheme 3.4.4.1***

### 3.4.5 Microwave synthesis of 2-substituted-4-aminoquinazolines

The synthesis of 2-substituted-4-aminoquinazolines was reported by Seijas and co-workers in 2000.[150] 2-Aminobenzonitrile **136** was reacted with a variety of nitriles in the presence of potassium *tert*-butoxide under microwave conditions to give quinazolines **137** in excellent yield, ***Scheme 3.4.5.1*.** These reactions represent a significant improvement in methodology not only because of their short reaction time but improved yields, catalytic amount of base and absence of solvent.

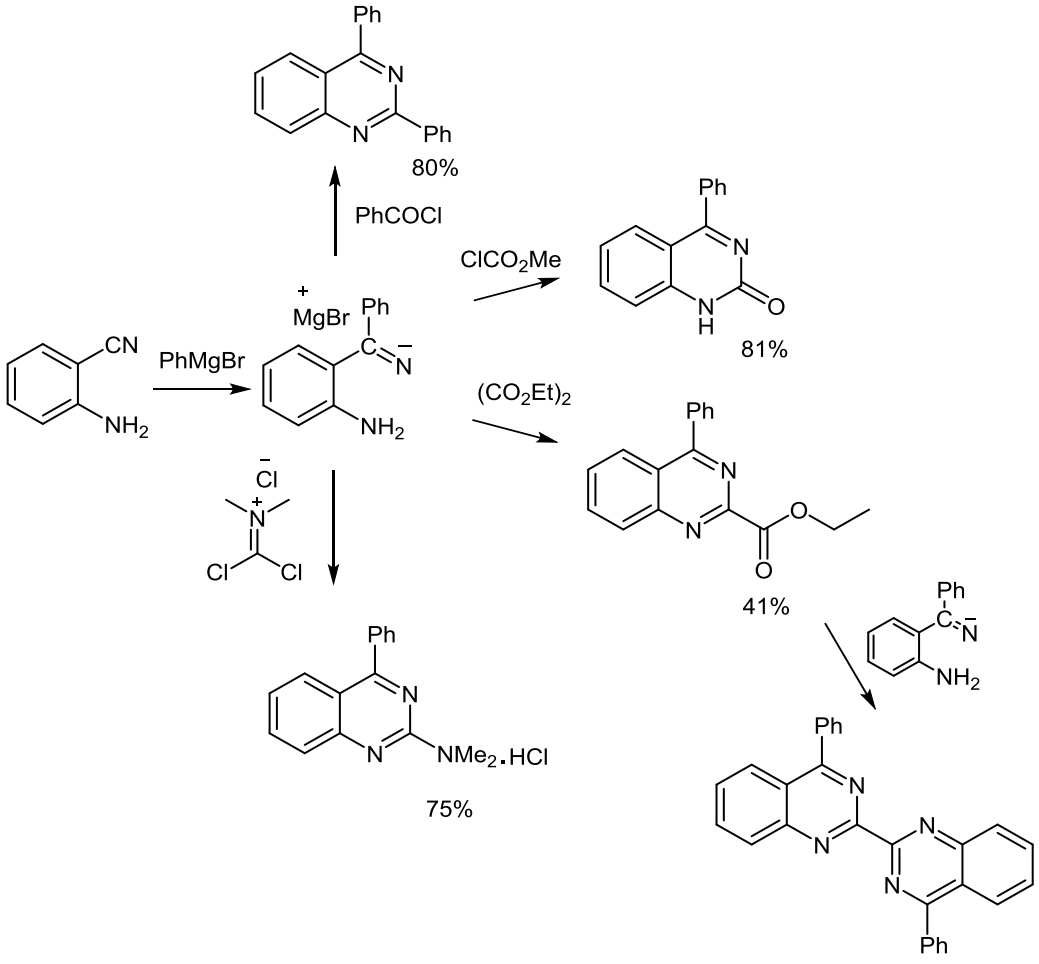



R = Ph
R = 3-$CNC_6H_4$
R = 4-$OMeC_6H_4$
R = 2-$NH_2C_6H_4$
R = 2-Thiophenyl
R = Bn
R = 2-Furanyl
R = 2/3/4-Pyridyl

***Scheme 3.4.5.1***

### 3.4.6 Use of Grignard reagents

Bergman *et al.* demonstrated that when 2-aminobenzonitrile is reacted with Grignard reagents the resulting intermediate was useful in accessing a variety of quinazoline derivatives in good to excellent yields, when quenched with suitable electrophiles (acid chlorides, formates, oxalates and Viehe's salt).[151,152] It was found that when quenched with diethyl oxalate the quinazoline product generated was susceptible to reaction with a second mole of the intermediate leading to the formation of 2,2'-coupled bis-quinazoline, accounting for the lower yield of the desired product. This general approach for synthesis of 2,4-disubstituted quinazolines is a useful addition to existing procedures, given the range of available Grignard reagents, ***Scheme 3.4.6.1***.



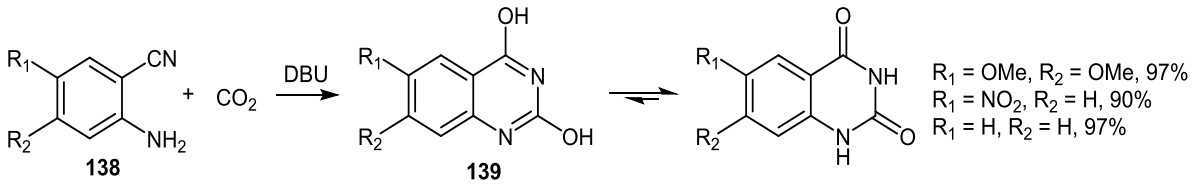

***Scheme 3.4.6.1***

## 3.5 Synthesis of Quinazoline-2,4-diones

There are many examples of this reaction type in the literature.[40] Some of the more recent publications are discussed below which detail more bespoke preparations that display clear advantages over conventional methods.

### 3.5.1 Quinazoline-2,4-diones from aminobenzonitrile precursors

Mizuno *et al.* first described the synthesis of quinazoline-2,4-diones in 2000 using 2-aminobenzonitrile **138** and carbon dioxide in the presence of DBU, ***Scheme 3.5.1.1***.[153] More recently the same group used supercritical carbon dioxide in place of organic solvents to synthesise a range of quinazoline-2,4-diones **138** in good to excellent yields.[154] Gao *et al.* published similar work detailing the use of guanidines as catalysts for this reaction with very encouraging results.[155] Skibo and Sung published work which included more conventional methods where an anthranilamide is treated with phosgene to generate compounds of type **139**. The preceding anthranilic acid can also be cyclised directly upon treatment with potassium isocyanate, ***Scheme 3.4.1.1***.[156,157]



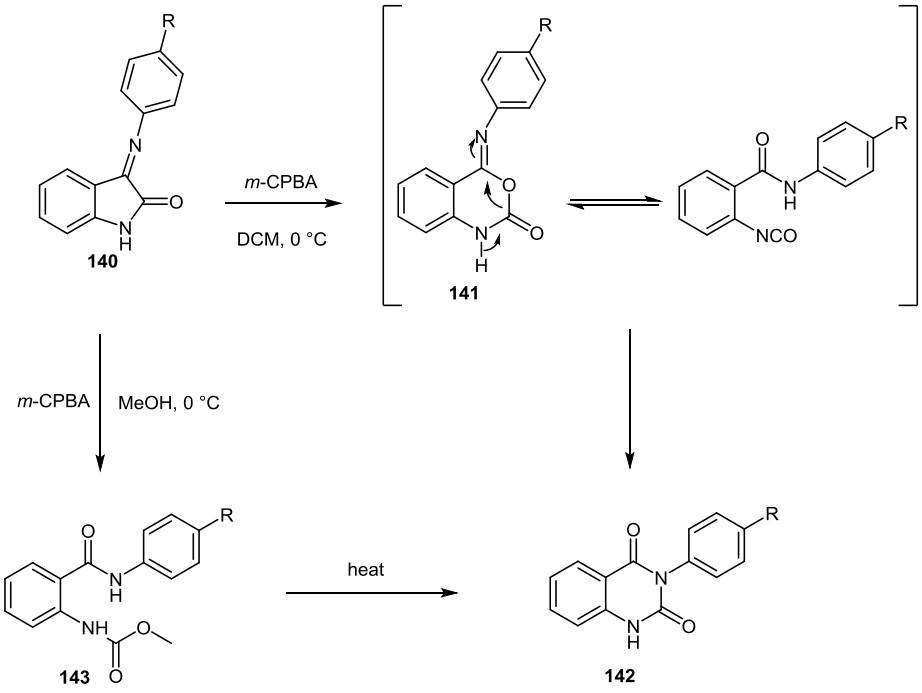

***Scheme 3.5.1.1***

## 3.6 N-3 substituted quinazolinediones

The application of *N*-3 substituted quinazolinediones has been previously discussed in ***Section 1.2.2***, which highlights the promising anti-cancer activity of some recently described *N*-3 substituted quinazolinediones.

### 3.6.1 Baeyer-Villiger oxidation to 3-arylquinazoline-2,4-diones

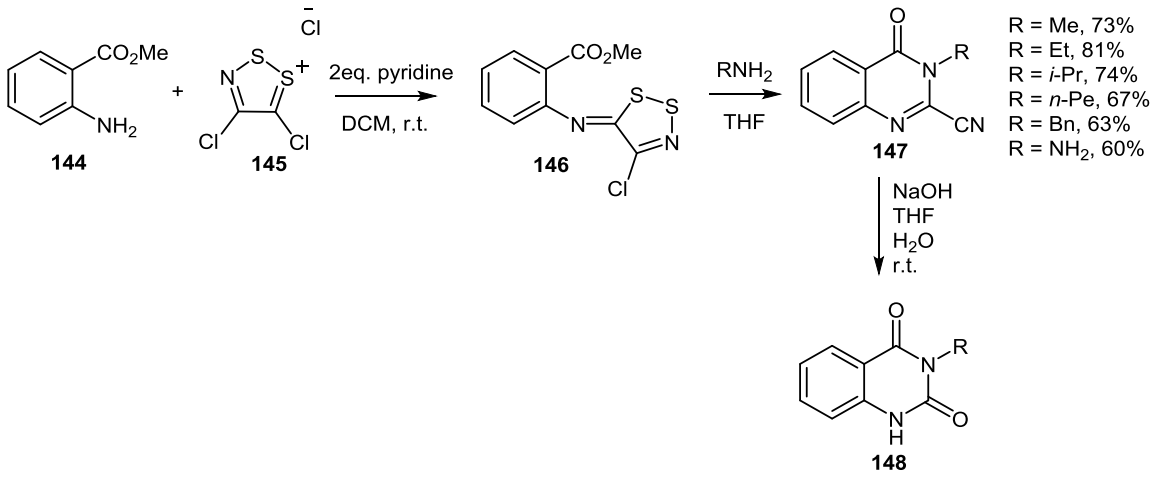
Azizian *et al.* described the synthesis of 3-arylquinazoline-2,4-diones *via* the rearrangement of benzoxazinones.[158] The procedure involves the oxidation of 3-arylimino-2-indolinones **140** with *m*-chloroperbenzoic acid at 0 °C to generate **141**. The expected quinazolinedione **142** was formed in excellent yield after separation from impurities by flash chromatography, ***Scheme 3.6.1.1***. A range of quinazolinediones were synthesised demonstrating the versatility of this procedure. When the reaction was carried out in methanol it was found that the carbamate, **143** was returned in high yield. Cyclisation of **143** to the corresponding quinazolinedione **142** was afforded by heating **143** to its melting point.




***Scheme 3.6.1.1***

### 3.6.2 Preparation using Appel's salt

Kim *et al.* developed a facile synthesis of a range of 3-substituted-2-cyano-4(3*H*)-quinazolinones. Reaction of methyl anthranilate **144** with Appel's salt **145** in the presence of pyridine returned dithiazolium **146** in a 50% yield.[159] 3-Substituted-2-cyano-4(3*H*)-quinazolinones **147** are afforded by reacting primary alkylamines with dithiazolium **146**. The nitrile group can readily be displaced by a variety of nucleophiles to generate a range of corresponding 2-substituted analogues. Hydrolysis of the nitrile offers a convenient method for synthesising *N*-3 substituted quinazolinediones **148** in moderate to good yields (R=Me, 56% overall), ***Scheme 3.6.2.1***.

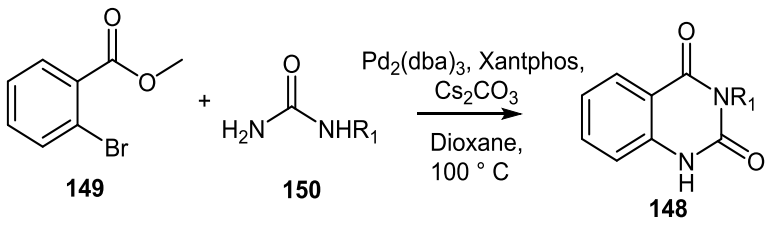



***Scheme 3.6.2.1***

### 3.6.3 Palladium-catalysed synthesis of *N*-3 substituted quinazoline-2,4-diones

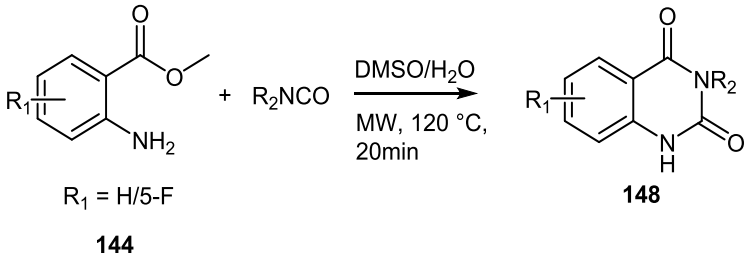
Willis *et al.* developed an efficient synthesis of *N*-3 substituted quinazolinediones **148** by reacting methyl *o*-bromobenzoate **149** with mono-*N*-substituted ureas **150** in the presence of a palladium catalyst, ***Scheme 3.6.3.1***.[160] This method is tolerant of a wide variety of substituted ureas as well as both electron-donating and electron-withdrawing substituents on the benzene ring of **149**. Regioselectivity was determined by comparison

with existing literature, 2D NMR studies and conversion to known compounds. Willis speculated that selectivity is due to an initial arylation reaction followed by a ring-closing amidation reaction, with the arylation occurring at the least hindered nitrogen of the urea.




***Scheme 3.6.3.1***

Li *et al.* described the synthesis of analogous compounds from methyl anthranilate **144** using microwave conditions, ***Scheme 3.6.3.2***.[161] Initial use of THF as the solvent in these reactions led to dimerization of the urea, so DMF and DMSO were screened, however this lead to the isolation of uncyclised product. It was thought that a nucleophilic solvent may be necessary in order to facilitate the elimination of methanol so a 1:1 DMSO:$H_2O$ solvent system was employed which lead to the formation of **148** with minimal dimerization. A range of compounds were synthesised probing substitution patterns, electron distribution and steric hindrance. A variety of *N*-3 aryl derivatives were all synthesised in good to excellent yields, however, *N*-3 alkyl analogues returned low yields. Steric hindrance did prove problematic as was evident in the use of 2,6-diisopropylphenylisocyanate where no product was formed. Overall this method was proved to be a rapid and green alternative to Willis' synthesis.



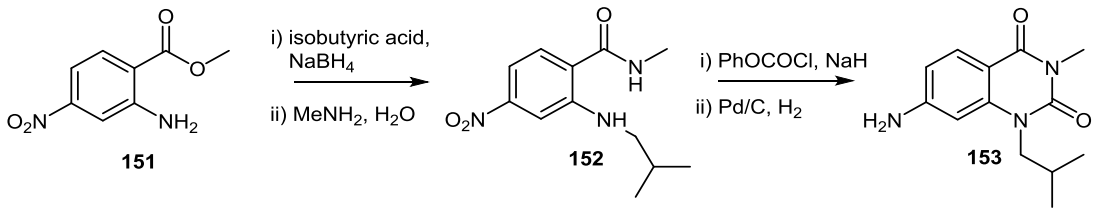

***Scheme 3.6.3.2***

## 3.7 *N*-1, *N*-3-Disubstituted quinazolinediones

***Section 1.2.3*** and ***Section 1.2.4*** highlight the relevance of *N*-1, *N*-3-disubstituted quinazolinediones in the development of prospective chemotherapeutic agents, specifically in the design of peptidomimetics for use as anti-cancer agents and also as new therapies in the fight against antibiotic resistance.

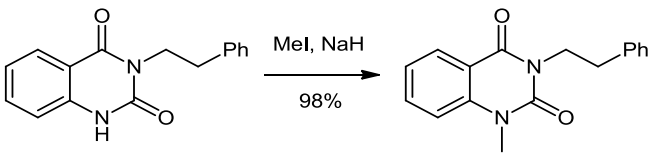
### 3.7.1 Synthesis from anthranilate precursors

While searching for potential immunosuppressive and anti-inflammatory agents Michne *et al.* described a method for synthesising *N*-1, *N*-3-disubstituted quinazolinediones, ***Scheme 3.7.1.1***.[162] Alkylation of **151** followed by the formation of the amide using methylamine gave **152**. Reaction of **152** with phenyl chloroformate in the presence of sodium hydride followed by reduction gave **153** in a 51% overall yield.




***Scheme 3.7.1.1***

Although not the most conventional method it does provide a viable route to *N*-1, *N*-3-disubstituted quinazolinediones. More commonly *N*-3-substituted quinazolinediones are prepared and the *N*-1 position is substituted using an alkylating/arylating agent in the presence of a base. These reactions usually give high to excellent yields and a range of nucleophiles can be used. Michne *et al.* also described this method for compounds of type **153** as did Willis, ***Scheme 3.7.1.2***.




***Scheme 3.7.1.2***

## 3.8 Conclusion

Quinazolines represent a family of compounds which possess extensive and diverse biological profiles and as a result have gained significant interest in the field of medicinal chemistry. As a result of this interest much research has been carried out into the development of efficient routes of synthesis. This interest has lead to the development of many prospective novel chemotherapeutic agents, perhaps the most pronounced in this area is the development of 4-anilinoquinazolines which are currently at the cutting edge of cancer chemotherapeutics.

In contrast elaboration of the quinazoline-2,4-dione pharmacophore remains an underdeveloped demesne of the quinazoline family. Given the beseeching biological modes of action attributed to a range of quinazoline-2,4-diones, the development of novel synthetic routes as well as elaboration of the pharmacophore is imperative in order to advance this area of chemotherapeutics.

# *9.0 References*

## 9.0 References


1. A. A. Yakoub, L. L. Gustafsson O. Ericsson, U. Hellgren, *Handbook of Drugs for Tropical Diseases*, **1995**, 2.

2. K. D. Thomas, A. V. Adhikari, S. Telkar, I. H. Chowdhury, R. Mahmood, N. K. Pal, G. Row, E. Sumesh, *Eur. J. Med. Chem.*, **2011**, 46(11), 5283-5293.

3. N. Muruganantham, R. Sivakumar, *Biol. Pharm. Bull.*, **2004**, 27(10), 1683-1687.

4. P. Camps, X. Formosa, C. Galdeano, D. Muñoz-Torrero, L. Ramírez, E. Gómez, N. Isambert, R. Lavilla, A. Badia, M. V. Clos, M. Bartolini, F. Mancini, V. Andrisano, M. P. Arce, M. I. Rodríguez-Franco, Ó. Huertas, T. Dafni, F. J. Luque, *J. Med. Chem.*, **2009**, 52(17), 5365-5379.

5. V. R. Solomon, H. Lee, *Curr. Med. Chem.*, **2011**, 18(10), 1488-1508.

6. J. S. Lazo, D. C. Aslan, E. C. Southwick, K. A. Cooley, A. P. Ducruet, B. Joo, A. Vogt, P. Wipf, *J. Med. Chem.*, **2001**, 44(24), 4042-4049.

7. K. Kristjánsdóttir, J. Rudolph, *Chem. Biol.*, **2004**, 11(8), 1043-1051.

8. P. Kaldis, *Cell. Mol. Life Sci.*, **1999**, 55(2), 284-296.

9. R. Boutros, V. Lobjois, B. Ducommun, *Nat. Rev. Cancer*, **2007**, 7(7), 495-507.

10. K. Galaktionov, A. K. Lee, J. Eckstein, D. Beach, *Science*, **1991**, 269, 1575-1577.

11. K. Sadhu, S. I. Reed, H. Richardson, P. Russell, *Proc. Natl. Acad. Sci.*, **1990**, 87, 5139-5143.

12. S. Wegener, W. Hampe, D. Herrmann, H. C. Schaller, *Eur. J. Cell Biol.*, **2000**, 79(11), 810-815.

13. V. Baldin, C. Cans, G. Superti-Furga, B. Ducommun, *Oncogene*, **1997**, 14, 2485-2495.

14. A. R. R. Forrest, A. K. McCormack, C. P. C. DeSouza, J. M. Sinnamon, I. D. Tonks, N. K. Hayward, K. A. O. Ellem, B. G. Gabrielli, *Biochem. Biophys.l Res. Commun.*, **1999**, 260(5), 510-515.

15. N. Rief, R. Drescher, A. Jungbluth, M. Montenarh, P. Wagner, M. Bureik, *Int. J. Oncol.*, **2000**, 17, 1251-1258.

16. M. Malumbras, M. Barbacid, *Trends Biochem. Sci.*, **2005**, 30(11), 630-641.

17. J. Rudolph, D. M. Epstein, L. Parker, J. Eckstein, *Anal. Biochem.*, **2001**, 289(1), 43-51.

18. S. M. Keyse, M. Ginsburg, *Trends Biochem. Sci.*, **1993**, 18(10), 377-378.

19. R. A. Reynolds, A. W. Yem, C. L. Wolfe, M. R. Deibel Jr., C. G. Chidester, K. D. Watenpaugh, *J. Mol. Biol.*, **1999**, 293(3), 559-568.

20. E. B. Fauman, J. P. Cogswell, B. Lovejoy, W. J. Rocque, W. Holmes, V. G. Montana, H. Piwnica-Worms, M. J. Rink, M. A. Saper, *Cell*, **1998**, 93(4), 617-625.

21. J. Rudolph, *Mol. Pharm.*, **2004**, 66(4), 780-782.

22. A. Lavecchia, C. Di Giovanni, E. Novellino, *Expert Opin. Ther. Patents*, **2010**, 20(3), 405-425.

23. M. O. Contour-Galcera, A. Sidhu, G. Prévost, D. Bigg, B. Ducommun, *Pharmacol. Ther.*, **2007**, 115, 1-12.

24. M. Brisson, T. Nguyen, P. Wipf, B. Joo, B. W. Daly, J. S. Skoko, E. M. Schreiber, C. Foster, P. Bansal, J. S. Lazo, *Mol. Pharmacol.*, **2005**, 68(6), 1810-1820.

25. M. Brisson, T. Nguyen, A. Vogt, J. Yalowich, A. Giorgianni, D. Tobi, I. Bahar, C. R. J. Stephenson, P. Wipf, J. S. Lazo, *Mol. Pharmacol.*, **2004**, 66(4), 824-833.

26. M. A. Lyon, A. P. Ducruet, P. Wipf, J. S. Lazo, *Nat. Rev. Drug Discovery*, **2002**, 1(12), 961-976.

27. J. Guo, J. Kleeff, J. Li, J. Ding, J. Hammer, Y. Zhou, T. Giese, M. Korc, M. W. Buchler, H. Friess, *Nature*, **2004**, 23(1), 71-81.

28. S. Keinan, W. D. Paquette, J. J. Skoko, D. N. Beratan, W. Yang, S. Shinde, P. A. Johnston, J. S. Lazo, P. Wipf, *Org. and Biomol. Chem.*, **2008**, 6(18), 3256-3263.

29. T. P. Selvam, P. V. Kumar, *Res. Pharm.*, **2011**, 1(1), 1-21.

30. G. Marzaro, A. Guiotto, A. Chilin, *Expert Opin. Ther. Patents*, **2012**, 22(3), 223-251.

31. T. W. Butler, A. F. J. Fliri, R. J. Gallaschun, **2002**, EP 1178048.

32. C. Seong, N. Park, J. Choi, C. M. Park, W. Park, J. Kong, 2008, WO 2008004716, **2008-01-10**.

33. V. Colotta, D. Catarzi, F. Varano, O. Lenzi, G. Filacchioni, C. Costagli, A. Galli, C. Ghelardini, N. Galeotti, P. Gratteri, J. Sgrignani, F. Deflorian, S. Moro, *J. Med. Chem.*, **2006**, 49, 6015-6026.

34. R. K. Russell, J. B. Press, R. A. Rampulla, J. J. McNally, R. Falotico, J. A. Keiser, D. A. Bright, A. Tobia, *J. Med. Chem.*, **1988**, 31, 1786-1793.

35. P. C. Astles, S. R. Baker, C. Bonnefous, J. M Vernier, M Keenan, A. J. Sanderson, 2002, WO 03062224, **2003-07-31**.

36. M. D. Huband, M. A. Cohen, M. Zurack, D. L. Hanna, L. A. Skerlos, M. C. Sulavik, G. W. Gibson, J. W. Gage, E. Ellsworth, M. A. Stier, S. J. Gracheck, *Antimicrob. Agents Chemother.*, **2007**, 51(4), 1191-1201.

37. L. M. Oppegard, K. R. Streck, J. D. Rosen, H. A. Schwanz, K. Drlica, R. J. Kerns, H. Hiasa, *Antimicrob. Agents Chemother.*, **2010**, 54(7), 3011-3014.

38. J. Mortier, B. Masereel, C. Remouchamps, C. Ganeff, J. Piette, R. Frederick, *Bioorg. Med. Chem. Lett.*, **2010**, 20, 4515-4520.

39. D. Carrico, M. A. Blaskovich, C. J. Bucher, S. M. Sebti, A. D. Hamilton, *Bioorg. Med. Chem.*, **2005**, 13, 677-688.

40. X. Zhou, X. Xie, G. Liu, *Mol. Divers.*, **2013**, 17(2), 197-219.

41. J. S. Taylor, T. S. Reid, K. L. Terry, P. J. Casey, L. S. Beese, *Embo J.*, **2003**, 22(22), 5963-5974.

42. F. Sellal, A. Nieoullon, G. Michel, B. F. Michel, L. Lacomlez, H. Geerts, A. Delini-Stula, R. Bordet, D. Bentuè-Ferrer, H. Allain, *Dementia Geriatr. Cognit. Disord.*, **2005**, 19, 229-245.

43. M. R. Hynd, H. L. Scott, P. R. Dodd, *Nuerochem Int.*, **2004**, 45, 583-595.

44. P. J. Hallett, D. G. Standaert, *Pharmacol. Ther.*, **2004**, 102, 155-174.

45. H. Bräuner-Osborne, J. Egebjerg, E. Ø. Nielsen, U. Madsen, P. Krogsgaard-Larsen *J. Med. Chem.*, **2000**, 43, 2609-2654.

46. G. Lombardi, G. Miglio, P. L. Canonico, P. Naldi, C. Comi, F. Monaco, *Neurosci. Lett.*, **2003**, 340, 5-8.

47. D. Pitt, P. Werner, C. S. Raine, *Nature*, **2000**, 6, 67-70.

48. A. B. Petrenko, T. Yamakura, H. Baba, K. Shimoji, *Anesth. Analg.*, **2003**, 97, 1108-1116.

49. J. I. Székely, K. Török, G. Mate, *Curr. Pharm. Des.*, **2002**, 8, 125-133.

50. R. Ruscheweyh, J. Sandkühler, *Brain Res. Rev.*, **2002**, 40, 215-222.

51. V. Colotta, D. Catarzi, F. Varano, F. R. Calabri, G. Filacchioni, C. Costagli, A. Galli, *Bioorg. Med. Chem. Lett.*, **2004**, 14, 2345-2349.

52. J. P. Michael, *Nat. Prod. Rep.*, **2005**, *22*(5), 627-646.

53. J. P. Michael, *Nat. Prod. Rep.*, **2007**, *24*(1), 223-246.

54. P. G. Bray, S. A. Ward, P. M. O' Nell, *Curr. Top. Microbiol. Immunol.*, **2005**, 3.

55. M. Brisson, T. Nguyen, P. Wipf, *Mol. Pharmacol.*, **2005**, 1810-1820.

56. M. A. Fakhfakh, A. Fournet, E. Prina, J. F. Mouscadet, X. Franck, R. Hocquemiller, B. Figadere, *Bioorg. Med. Chem.*, **2003**, *11*(23), 5013-5023.

57. N. Muruganantham, R. Sivakumar, N. Anbalagan, V. Gunasekaran, J. T. Leonard, *Biol. Pharm. Bull.*, **2004**, *27*(10), 1683-1687.

58. S. Vangapandu, M. Jain, R. Jain, S. Kaur, P. Pal Singh, *Bioorg. Med. Chem.*, **2004**, *12*(10), 2501-2508.

59. P. Camps, E. Gomez, D. Munoz-Torrero, A. Badia, N. M. A. Vivas, X. Barril, M. Orozco, F. J. Luque, *J. Med. Chem.*, **2001**, *44*(26), 4733-4736.

60. L. Knorr, *Ann*., **1886**, 236, 290.

61. K. K. S. Sai, T. M. Gilbert, D. A. Klumpp, *J. Org. Chem.,* **2007**, *72*(25), 9761-9764.

62. B. Staskun, *J. Org. Chem.*, **1964**, *29*(5), 1153-1157.

63. M. Conrad, L. Limpach, *Ber*, **1891**, 944.

64. M. Billah, G. M. Buckley, N. Cooper, H. J. Dyke, R. Egan, A. Ganguly, L. Gowers, A. F. Haughan, H. J. Kendall, C. Lowe, M. Minnicozzi, J. G. Montana, J. Oxford, J. C. Peake, C. L. Picken, J. J. Piwinski, R. Naylor, V. Sabin, N. Y. Shih, J. B. H. Warneck, *Bioorg. Med. Chem. Lett.*, **2002**, *12*(12), 1617-1619.

65. M. Billah, N. Cooper, F. Cuss, R. J. Davenport, H. J. Dyke, R. Egan, A. Ganguly, L. Gowers, D. R. Hannah, A. F. Haughan, H. J. Kendall, C. Lowe, M. Minnicozzi, J. G. Montana, R. Naylor, J. Oxford, J. C. Peake, J. J. Piwinski, K. A. Runcie, V. Sabin, A. Sharpe, N. Y. Shih, J. B. H. Warneck, *Bioorg. Med. Chem. Lett.*, **2002**, *12*(12), 1621-1623.

66. L. Li, H. K. Wang, S. C. Kuo, T. S. Wu, A. Mauger, C. M. Lin, E. Hamel, K. H. Lee, *J. Med. Chem.*, **1994**, *37*(20), 3400-3407.

67. M. Hadjeri, E. L. Peiller, C. Beney, N. Deka, M. A. Lawson, C. Dumontet, A. N. Boumendjel, *J. Med. Chem.*, **2004**, *47*(20), 4964-4970.

68. Z. Sui, V. N. Nguyen, J. Altom, J. Fernandez, J. J. Hilliard, J. I. Bernstein, J. F. Barrett, A. K. Ohemeng, *Eur. J. Med. Chem.*, **1999**, *34*(5), 381-387.

69. C. P.Jones, K. W. Anderson, S. L. Buchwald, *J. Org. Chem.*, **2007**, *72*(21), 7968-7973.

70. A. Combes, *Bull. Soc. Chim*., **1888**, 89.

71. W. S. Johnson, F. J. Matthews, *J. Am. Chem. Soc.*, **1944**, 66(2), 210-215.

72. J. L. Born, *J. Org. Chem.*, **1972**, 37(24), 3752-3753.

73. J. C. Perche, G. Saint-Ruf, N. P. Buu-Hoi, *J. Chem. Soc. Perk. Trans. I*, **1972**, 260-262.

74. E. A. Fehnel, P. Friedlander, *J. Org. Chem.*, **1966**, *31*(9), 2899-2902.

75. S. Atechian, N. Nock, R. D. Norcross, H. Ratni, A. W. Thomas, J. Verron, R. Masciadri, *Tetrahedron*, **2007**, *63*(13), 2811-2823.

76. J. Wu, H. G. Xia, K. Gao, *Org. Biomol. Chem.*, **2006**, *4*(1), 126-129.

77. R. Varala, R. Enugala, S. R. Adapa, *Synthesis*, **2006**, 3825-3830.

78. A. Arcadi, M. Chiarini, S. Di Giuseppe, F. Marinelli, *Synlett*, **2003**, 203-206.

79. R. Martinez, D. J. Ramon, M. Yus, *J. Org. Chem.*, **2008**, *73*(24), 9778-9780.

80. Q. Shen, L. Wang, J. Yu, M. Liu, J. Qiu, L. Fang, F. Guo, J. Tang, *Synthesis*, **2012**, *44*, 389-392.

81. W. Pfitzinger, *J. Prakt. Chem*., **1886**, 100.

82. W. Pfitzinger, *J. Prakt. Chem*, **1888**, 582.

83. W. Pfitzinger, *J. Prakt. Chem*, **1897**, 283.

84. W. Borsche, O. Vorbach, *Justus Liebigs Ann. Chem*., **1938**, 22.

85. J. Braun, W. Gmelin, A. Schuelthesiss, *Berichte*, **1923**, 1344.

86. M. N. Palmer, P. S. McIntyre, *J. Chem. Soc.,* **1969**, 539.

87. N. Buu-Hoi, R. Royer, *J. Chem. Soc.*, **1948**, 106-110.

88. N. Buu-Hoi, R. Royer, N. Xuong, P. Jacquignon, *J. Org. Chem.*, **1953**, *18*(9), 1209-1224.

89. E. R. Buchman, H. Sargent, T. C. Myers, J. A. Seneker, *J. Am. Chem. Soc.*, **1946**, *68*(12), 2692-2695.

90. E. J. Cragoe, C. M. Robb, M. D. Bealor, *J. Org. Chem.*, **1953**, *18*(5), 552-560.

91. E. J. Cragoe, C. M. Robb, *Syn. Org. Prod.*, **1964**, 123.

92. R. R. Renshaw, H. L. Friedman, *J. Am. Chem. Soc.*, **1939**, 61(12), 3320-3322

93. C. Enhelhard, *J. Prakt. Chem*, **1898**, 467.

94. H. Gilman, L. Tolman, S. P. Massie, *J. Am. Chem. Soc.*, **1946**, *68*(11), 2399-2400.

95. G. J. Atwell, B. C. Baguley, W.A. Denny, *J. Med. Chem.*, **1989**, *32*(2), 396-401.

96. Noelting, Herzbaum, *Ber*, **1911**, 44, 2585.

97. S. A. Yamashkin, E. A. Oreskina, *Chem. Het. Compounds*, **2006**, 42(6), 701-718.

98. X. G. Li, X. Cheng, Q. Zhou, *Syn. Comm.*, **2002**, 32(16), 2477-2481.

99. M. Matsugi, F. Tabusa, J. I. Minamikawa, *Tet. Lett.*, **2000**, 41(44), 8523-8525.

100. A. Köppl, H. G. Alt, *J. Mol. Cat. A:Chemical*, **2000**, 154, 45-53.

101. N. S. Prostakov, A. P. Krapivko, A. T. Soldatenkov, N. D. Sergeeva, *Chem. Het. Compounds*, **1980**, 16(5), 529-532.

102. H. Walter, H. Sauter, T. Winkler, *Helv. Chim. Acta*, **1992**, *75*(4), 1274-1280.

103. H. Walter, H. Sauter, J. S. Schneider, *Helv. Chim. Acta*, **1993,** *76*(4), 1469-1475.

104. H. Walter, *Helv. Chim. Acta*, **1994,** *77*(3), 608-614.

105. H. Walter, *Heterocycles,* **1995**, 1251-1269.

106. H. Walter, *Heterocycles*, **1995**, 2427-2435.

107. H. Walter, *Heterocycles*, **1998**, 1581-1591.

108. H. Walter, *J. Prakt. Chem.*, **1998**, *340*(4), 309-314.

109. D. W. Cameron, K. R. Deutscher, G. I. Feutrill, *Aust. J. Chem.*, **1982**, 35, 1439.

110. A. Vogt, T. Adachi, A. P. Ducruet, J. Chesebrough, K. Nemato, B. I. Carr, J. S. Lazo, *J. Biol. Chem.*, **2001**, 276(23), 20544-20550.

111. E. Y. Yoon, H. Y. Choi, K. J. Shin, K. H. Yoo, Y. Dae, D. J. Kim, *Tetrahedron Lett.*, **2000**, 41(39), 7475-7480.

112. H. J. Chan, D. P. Baccanari, R. L. Tansik, C. M. Boytos, S. K. Rudolph, A. D. Brown, S. J. Hong, L. F. Kuyper, M. L. Jones, *J. Het. Chem.*, **1997**, *34*(1), 145-151.

113. A. J. Barker, K. H. Gibson, W. Grundy, A. A. Godfrey, J. J. Barlow, M. P. Healy, J. R. Woodburn, S. E. Ashton, B. J. Curry, L. Scarlett, L. Henthorn, L. Richards, *Bioorg. & Med. Chem. Lett.*, **2001**, *11*(14), 1911-1914.

114. M. A. H. Ismail, S. Barker, D. A. Abou El Ella, K. A. M. Abouzid, A. R. Toubar, M. H. Todd, *J. Med. Chem.*, **2006**, *49*(5), 1526-1535.

115. G. M. Buckley, N. Davies, H. J. Dyke, P. J. Gilbert, D. R. Hannah, A. F. Haughan, C. A. Hunt, W. R. Pitt, R. H. Profit, N. C. Ray, M. D. Richard, A. Sharpe, A. J. Taylor, M. J. Whitworth, S. C. Williams, *Bioorg. & Med. Chem. Lett.*, **2005**, *15*(3), 751-754.

116. D. Hooper, *Pharm. J.*, **1888**, 18, 841.

117. A. Vijaychand, *Int. J. Pharma. Bio. Sci.*, 2011, 2(1), 780-809.

118. W. L. F. Armarego, *Fused pyrimidines, Part 1: Quinazolines*, Interscience, New York, **1967**.

119. T. L. Gilchrist, *J. Chem. Soc , Perkin Trans. 1*, **2001**, 2491-2515.

120. T. L. Gilchrist, *J. Chem. Soc , Perkin Trans. 1*, **1999**, 2849-2866.

121. http://www.drugs.com/cdi/anagrelide.html (visited on 7/12/2014).

122. http://www.drugbank.ca/drugs/DB01325 (visited on 07/12/2014).

123. E. Erba, D. Pocar, M. Valle, *J. Chem. Soc., Perkin Trans. 1* **1999**, 421-426.

124. H. Kotsuki, H. Sakai, H. Morimoto, H. Suenaga, *Synlett,* **1999**, 12, 1993.

125. F. R. Alexandre, A. Berecibar, R. Wrigglesworth, T. Besson, *Tetrahedron,* **2003**, *59*(9), 1413-1419.

126. G. W. Rewcastle, B. D. Palmer, A. J. Bridges, H. D. H. Showalter, L. Sun, J. Nelson, A. McMichael, A. J. Kraker, D. W. Fry, W. A. Denny, *J. Med. Chem.*, **1996**, *39*(4), 918-928.

127. M. Tobe, Y. Isobe, H. Tomizawa, T. Nagasaki, H. Takahashi, T. Fukazawa, H. Hayashi, *Bioorg. & Med. Chem.*, **2003**, *11*(3), 383-391.

128. O. Sugimoto, M. Mori, K. Moriya, K. I. Tanji, *Helv. Chim. Acta,* **2001**, *84*(5), 1112-1118.

129. G. F. Xu, B. A. Song, P. S. Bhadury, S. Yang, P. Q. Zhang, L. H. Jin, W. Xue, D. Y. Hu, P. Lu, *Bioorg. & Med. Chem.*, **2007**, *15*(11), 3768-3774.

130. W. Szczepankiewicz, J. Suwinski, R. Bujok, *Tetrahedron*, **2000**, *56*(47), 9343-9349.

131. W. Szczepankiewicz, J. Suwinski, *Tetrahedron Lett.*, **1998**, *39*(13), 1785-1786.

132. K. M. Foote, A. A. Mortlock, N. M. Heron, F. D. R. H. Jung, G. B. Hill, G. Pasquet, M. C. Brady, S. Green, S. P. Heaton, S. Kearney, N. J. Keen, R. Odedra, S. R. Wedge, R. W. Wilkinson, *Bioorg. & Med. Chem. Lett.*, **2008**, *18*(6), 1904-1909.

133. N. Vasdev, P. N. Dorff,J. P. O' Neil, F. T. Chin, S. Hanrahan, H. F. VanBrocklin, *Bioorg. & Med. Chem.*, **2011**, *19*(9), 2959-2965.

134. B. C. Barlaam, (AstraZeneca AB, AstraZeneca UK Ltd.), WO2007/63291, **2007**.

135. J. Wang, C. Fan, L. Zhang, Z. Guo, Y. Li, S. Yang, S. Yan, J. Zhu, Q. Yang, M. Minghui , (Qilu Pharmaceutical Co. Ltd.), WO2011/35540, **2011**.

136. J. Wang, C. Fan, L. Zhang, Z. Guo, Y. Li, S. Yang, S. Yan, J. Zhu, Q. Yang, M. Minghui , (Qilu Pharmaceutical Co. Ltd.), EP2484678, **2012**.

137. H. R. Tsou, N. Mamuya, B. D. Johnson, M. F. Reich, B. C. Gruber, F. Ye, R. Nilakantan, R. Shen, C. Discafani, R. DeBlanc, R. Davis, F. E. Koehn, L. M. Greenberger, Y. F. Wang, A. Wissner, *J. Med. Chem.,* **2001**, *44*(17), 2719-2734.

138. D. S. Yoon, Y. Han, T. M. Stark, J. C. Haber, B. T. Gregg, S. B. Stankovich, *Org. Lett.*, **2004**, *6*(25), 4775-4778.

139. M. Akazome, J. Yamamoto, T. Kondo, Y. Watanabe, *J. Organomet. Chem.*, **1995**, *494*, 229-233.

140. H. Hara, M. Ichikawa, H. Oku, M. Shimazawa, M. Araie, *Cardiovasc. Drug Rev.*, **2005**, *23*(1), 43-56.

141. http://www.drugs.com/cdi/prazosin.html (visited on 7/12/2014).

142. http://www.drugs.com/cdi/linagliptin.html (visited on 7/12/2014).

143. S. J. Lee, Y. Konishi, D. T. Yu, T. A. Miskowski, C. M. Riviello, O. T. Macina, M. R. Frierson, K. Kondo, M. Sugitani, *J. Med. Chem.*, **1995**, *38*(18), 3547-3557.

144. I. Mangalagiu, T. Benneche, K. Undheim, *Tetrahedron Lett.*, **1996**, *37*(8), 1309-1312.

145. H. I. D. E. Miki, *Chem. Pharm Bull.,* **1982**, *30*(6), 1947-1951.

146. K. Yoshida, T. Tanaka, H. Ohtaka, *J. Chem. Soc., Perkin Trans. 1*, **1991**, 1279-1282.

147. W. Zielinski, A. Kudelko, E. M. Holt, *Heterocycles*, **1998**, 319.

148. L. J. Wilson, *Org. Lett.,* **2001**, *3*(4), 585-588.

149. E. Erba, D. Sporchia, *J. Chem. Soc. , Perkin Trans. 1,* **1997**, 3021-3024.

150. J. A. Seijas, T. Vazquez, M. Montserrat Martinez, *Tetrahedron Lett.*, **2000**, *41*(13), 2215-2217.

151. P. Wiklund, J. Bergman, *Org. Biomol. Chem.*, **2003**, *1*(2), 367-372.

152. J. Bergman, A. Brynolf, B. Elman, E. Vuorinen, *Tetrahedron*, **1986**, *42*(13), 3697-3706.

153. T. Mizuno, N. Okamoto, T. Ito, T. Miyata, *Tetrahedron Lett.*, **2000**, *41*(7), 1051-1053.

154. T. Mizuno, T. Iwai, Y. Ishino, *Tetrahedron Lett.*, **2004**, *45*(38), 7073-7075.

155. J. Gao, L. N. He, C. X. Miao, S. Chanfreau, *Tetrahedron*, **2010**, *66*(23), 4063-4067.

156. E. B. Skibo, *J. Org. Chem.*, **1985**, *50*(24), 4861-4865.

157. S. J. Lee, Y. Konishi, D. T. Yu, T. A. Miskowski, C. M. Riviello, O. T. Macina, M. R. Frierson, K. Kondo, M. Sugitani, *J. Med. Chem.*, **1995**, *38*(18), 3547-3557.

158. J. Azizian, M. Mehrdad , K. Jadidi, Y. Sarrafi, *Tetrahedron Lett.*, **2000**, *41*(27), 5265-5268.

159. H. S. Lee, Y. G. Chang, K. Kim, *J. Het. Chem.*, **1998**, *35*(3), 659-668.

160. M. C. Willis, R. H. Snell, A. J. Fletcher, R. L. Woodward, *Org. Lett.*, **2006**, *8*(22), 5089-5091.

161. Z. Li, H. Huang, H. Sun, H. Jiang, H. Liu, *J. Comb. Chem.*, **2008**, *10*(3), 484-486.

162. W. F. Michne, J. D. Schroeder, J. W. Guiles, A. M. Treasurywala, C. A. Weigelt, M. F. Stansberry, E. McAvoy, C. R. Shah, E. Bump, *J. Med. Chem.* **1995,** *38* (14), 2557-2569.

163. R. Pettengell, B. Coiffier, G. Narayanan, F. H. de Mendoza, R. Digumarti, H. Gomez, P. L. Zinzani, G. Schiller, D. Rizzieri, G. Boland, P. Cernohous, L. Wang, C. Kuepfer, I. Gorbatchevsky, J. W. Singer, *The Lancet Oncology*, **2012**, 13, 7, 696-706.

164. M. M. Cahill, *PhD Thesis*, NUI Cork, **2013**.

165. D. L. J. Clive, M. Sannigrahi, S. Hisaindee, *J. Org. Chem.*, **2001**, 66(3), 954-961.

166. E. B. Skibo, *J. Org. Chem.*, **1985**, *50*(24), 4861-4865.

167. Menke Nitration, *Comprehensive Organic Name Reactions and Reagents*, **2010**, 425, 1893-1896.

168. A. McCarroll, Chemspider Synthetic Pages, 201, 261, (visited on 6/5/2012).

169. E. Gomez-Bengoa, A. M. Echavarren, *J. Org. Chem.*, **1991**, 56(11), 3497-3501.

170. C. M. Miller, *PhD Thesis*, NUI Cork, **2012**.

171. J. K. Stille, *Angew. Chem. Int. Ed. Engl.*, **1986**, 25(6), 508-524.

172. C. E. Masse, M. Yang, J. Solomon, J. S. Panek, *J. Am. Chem. Soc.*, **1998**, 120(17), 4123-4134.

173. S. F. Martin, J. M. Humphrey, A. Ali, M. C. Hillier, *J. Am. Chem. Soc.*, **1999**, 121(4), 866-867.

174. A. D. Lebsack, J. T. Link, L. E. Overman, B. A. Stearns, *J. Am. Chem. Soc.*,**2002**, 124(31), 9008-9009.

175. N. J. Kirwan, *PhD Thesis*, NUI Cork, **2000**.

176. F. Popowycz, P. Bernard, P. Raboisson, B. T. Joseph, *Synthesis*, **2007**, 3, 367-374.

177. G. Calvin, G. E. Coates, *J. Chem. Soc.*, **1960**, (0), 2008-2016.

178. C. M. Lindsay, D. A. Widdowson, *J. Chem. Soc. Perkin Trans. 1*, **1988**, (3), 569-573.

179. J. Feng, Z. Zhang, M. B. Wallace, J. A. Stafford, S. W. Kaldor, D. B. Kassel, M. Navre, L. Shi, R. J. Skene, T. Asakawa, K. Takeuchi, R. Xu, D. R. Webb, S. L. Gwaltney, *J. Med. Chem.*, **2007**, 50(10), 2297-2300.

180. H. K. Hall Jr., *J. Am. Chem. Soc.*, **1957**, 79, 5441.

181. H. C. Brown, *Determination of Organic Structures by Physical Methods*, Academic Press, New York, **1955.**

182. N. P. Peet, S. Sunder, *J. Org. Chem.*, **1974**, 39(13), 1931-1935.

183. J. L. Jiménez Blanco, C. Saitz Barría, J. M. Benito, C. Ortiz Mellet, J. Fuentes, F. Santoyo-González, J. M. García Fernández, *Synthesis*, **1999**, 11, 1907-1914.

184. W. Q. Tian, Y. A. Wang, *J. Org. Chem.*, **2004**, 69, 4299.

185. M. J. S. Dewar, P. M. Maitlis, *J. Chem.l Soc.*, **1957**, (0), 2518-2521.

186. M. T. Bogert, G. Scatchard, *J. Am. Chem. Soc.*, **1919**, 41(12), 2052-2068.

187. R. H. Shoemaker, *Nat. Rev. Cancer*, **2006**, 6, 813-823.

188. K. Paull, R. H. Shoemaker, L. Hodes, A. Monks, D. A. Scudiero, L. Rubinstein, J. Plowman, M. R. Boyd, *J. Natl. Cancer Inst.*, **1989**, 81, 1088-1092.

189. M. C. Alley, D. A. Scudiero, P.A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.*, **1988**, 48, 589-601.

190. R. Eagan, R. Dinapoli, T. Cascino, B. Scheithauer, B. O'Neill, J, O'Fallon, *J. Neuro-Oncol*, **1987**, 5, 4, 309-314.

191. J. Rubin, G. A. van Hazel, A. J. Schutt, C. G. Moertel, M. J. O'Connell, *Am. J. Clin. Oncol.*, **1982**, 5, 539-540.

192. http://www.cancer.ie/cancerinformation/treatments/chemotherapy/drugs/

Daunorubicin, (visited on 04/04/2014).

193. M. A. Horstmann, W. A. Hassenpflug, U. zur Stadt, G. Escherich, G. Janka, H. Kabisch, *Haematologica*, **2005**, 90, 12, 1701-1703.

194. P. L. Gutierrez, S. Siva, *Chem. Res. Toxicol.*, **1995**, 8, 455-464.

195. B. M. Hoey, J. Butler, A. J. Swallow, *Biochemistry*, **1988**, 27, 7, 2608-2614.

196. M. Lehmann, K. d. S. P. Vilar, A. Franco, M. L. z. Reguly, H. s. H. R. de Andrade, *Environ. Mol. Mutagen*, **2004**, 43, 4, 250-257.

197. A. C. Ketron, W. A. Denny, D. E. Graves, N. Osheroff, *Biochemistry,* **2012**, 51, 8, 1730-1739.

198. M. M. Mossoba, M. Alizadeh, P. L. Gutierrez, *J. Pharm. Sci.,* **1985**, 74, 12, 1249-1254.

199. G. C. Pais, T. R. Burke, *Drugs Future*, **2002**, 27, 11, 1101-1111.

200. C. L. Dwyer, C. W. Holzapfel, *Tetrahedron,* **1998**, 54, 27, 7843-7848.

201. G. T. Wang, X. Zhao, Z. T. Li, *Tetrahedron,* **2011**, 67, 1, 48-57.

202. L. Malatesia, M. Angoletta, *J. Chem. Soc.*, **1957**, 0, 1186-1188.

203. D. Zhahrevitz, *J. Mol. Grap. Model.,* **2002**, 297-303.