Development of Small Molecule Inhibitors of the Bromodomain-Histone Interaction



Timothy P. C. Rooney

St Catherine's College, University of Oxford

DPhil Dissertation

Organic Chemistry

(Systems Approaches to Biomedical Science CDT Programme)

October 2014

Supervisors: Prof Stuart Conway and Prof Paul Brennan

Development of Small Molecule Inhibitors of the Bromodomain-Histone Interaction

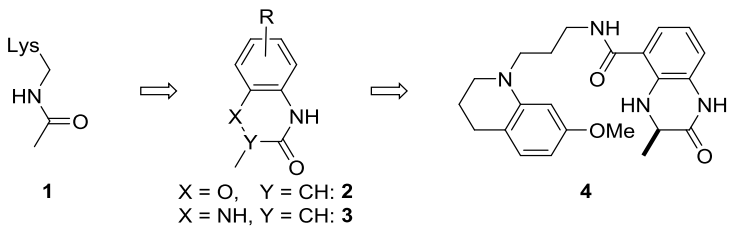
Timothy P. C. Rooney *St Catherine's College* *University of Oxford*

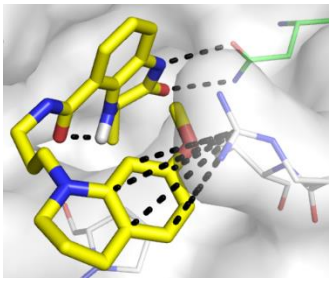
Bromodomains bind to acetylated lysine residues **1** to mediate a wide range of biological processes, including the assembly of transcriptional machinery at modified histones. This thesis describes the design of small molecule inhibitors of bromodomains, with particular focus on the bromodomain of CREBBP.

A fragment based approach was employed to investigate bicyclic amides as acetyl lysine mimics. Initially the benzoxazinone scaffold (BNZ) **2** was shown to be a novel, ligand efficient bromodomain inhibitor. Structure based elaboration of the BNZ scaffold was employed to direct substitutions towards the region of CREBBP with greatest variability compared to other bromodomains. Ultimately, the compounds in this series were limited to micromolar affinity for CREBBP, but provided useful structure-activity relationships.

Subsequently the dihydroquinoxalinone scaffold (DHQN) **3** was also shown to be a novel acetyl lysine mimic. Attachment of the optimum side group identified in the BNZ series led to the discovery of the first sub-micromolar inhibitor of CREBBP. A co-crystal structure with CREBBP revealed that the side group of this compound bound in a newly identified induced-fit pocket, mediated by a cation-π interaction. A combination of structural, functional and computational studies confirmed that the cation-π interaction contributed significantly towards the binding affinity of these ligands. Further work to elaborate the DHQN core, or develop an alternative acetyl lysine mimic into a CREBBP inhibitor, did not lead to an improvement. However, the optimum compound **4** was shown to displace CREBBP from chromatin in a cell-based assay.

Overall, cyclic amide based fragments were developed as CREBBP inhibitors, providing some of the first bromodomain ligands with nanomolar affinity outside of the BET family. In the process, key structural information about binding of ligands to CREBBP was revealed. Compound **4** provides a tool with which to study the biological implications of aberrant CREBBP activity and to investigate the therapeutic potential of bromodomain inhibition.





SCHOOL OF CHEMISTRY

DECLARATION OF AUTHORSHIP

Name: TIMOTHY P C ROONEY **Candidate number:** 221711

College: ST CATHERINE'S **Supervisor:** PROF STUART CONWAY

Title of thesis: Development of small molecule inhibitors of the bromodomain-histone interaction

Word count: 69758

Please tick to confirm the following:

I have read and understood the University's disciplinary regulations concerning conduct in examinations and, in particular, the regulations on plagiarism.	☑
I have read and understood the Education Committee's information and guidance on academic good practice and plagiarism at www.admin.ox.ac.uk/edc/goodpractice.	☑
The dissertation I am submitting is entirely my own work except where otherwise indicated.	☑
It has not been submitted, either partially or in full, for another Honour School or qualification of this University (except where the Special Regulations for the subject permit this), or for a qualification at any other institution.	☑
I have clearly indicated the presence of all material I have quoted from other sources, including any diagrams, charts, tables or graphs.	☑
I have clearly indicated the presence of all paraphrased material with appropriate references.	☑
I have acknowledged appropriately any assistance I have received in addition to that provided by my supervisor.	☑
I have not copied from the work of any other candidate.	☑
I have not used the services of any agency providing specimen, model or ghostwritten work in the preparation of this dissertation.	☑
I agree to retain an electronic copy of this work until the publication of my final examination result, except where submission in hand-written format is permitted.	☑
I agree to make any such electronic copy available to the examiners should it be necessary to confirm my word count or to check for plagiarism.	☑

Candidate's signature: Timothy Rooney Date: 01/09/15

Contents

Acknowledgements xii

Abbreviations xiv

1 Introduction

1.1 Lysine acetylation 2

1.1.1 Post translational modification 2
1.1.2 Histone modification 2
1.1.3 Role of histone modifications 3
1.1.4 Modulators of histone modifications 4
1.1.5 Non-histone lysine acetylation 5
1.1.6 Targeting histone modifications as a therapeutic strategy 6

1.2 Bromodomains 7

1.2.1 Occurrence of bromodomains 7
1.2.2 Structure of bromodomains 8
1.2.3 Bromodomains as drug targets 9

1.3 Structure and function of CREBBP 10

1.3.1 Role of CREBBP 10
1.3.2 CREBBP dysfunction 12
1.3.2.1 Implications of CREBBP in cancer 12
1.3.2.2 Implications of CREBBP in inflammation 13
1.3.2.3 Implications of CREBBP in neurodegeneration 14
1.3.3 Prospect of CREBBP inhibitors 14

1.4 Bromodomain inhibitors 16

1.4.1 Peptides 16
1.4.2 *N*-Acylated fragments 18
1.4.3 Azobenzenes 20
1.4.4 Cyclic amide fragments 21
1.4.5 Dimethylisoxazoles 22
1.4.6 Triazoles 24
1.4.7 Benzoxazepines 25
1.4.8 Summary 26

1.5 Project Aims 28

2 Investigation of benzoxazinones as bromodomain inhibitors

2.1 Dihydroquinazolinones as bromodomain inhibitors 30

2.1.1 Identification of cyclic amide fragments as acetyl lysine mimics 30
2.1.2 Limitations of the dihydroquinazolinone core 31
2.2 Benzoxazinones as acetyl lysine mimics 33

2.2.1 Rationale behind the change to a benzoxazinone core 33
2.2.2 Biologically active benzoxazinones 34
2.2.3 Synthesis of benzoxazinone fragments 35
2.2.4 Biological evaluation of benzoxazinone fragments 36
2.3 Derivatisation of the benzoxazinone core 37

2.3.1 Structural analysis of the CREBBP bromodomain 37
2.3.2 Computational screening of proposed benzoxazinone analogues 39
2.3.3 Synthesis of benzoxazinone analogues 41
2.3.4 Biological analysis of benzoxazinone analogues 44
2.3.5 Analysis of techniques for identifying benzoxazinone analogues 45
2.4 Investigation of chiral non-racemic benzoxazinones 48

2.4.1 Synthesis of chiral non-racemic benzoxazinone analogues 48
2.4.2 Analysis of chiral benzoxazinone analogues 49
2.5 Summary 50

3 Development of dihydroquinoxalinones as potent bromodomain inhibitors

3.1 Dihydroquinoxalinones as acetyl-lysine mimics 52

3.1.1 Rationale behind changing the core 52
3.1.2 Biologically active dihydroquinoxalinones 53
3.2 Synthesis of dihydroquinoxalinones 54

3.2.1 Retrosynthetic analysis of dihydroquinoxalinones 54
3.2.2. Synthesis of dihydroquinoxalinones – methyl ester route 55
3.2.3 Synthesis of dihydroquinoxalinones - benzyl ester route 57
3.2.3.1 Installation of benzyl protecting group 58
3.2.3.2 *N*-Arylation using Buchwald-Hartwig conditions 59

3.2.3.3 *N*-Arylation using nucleophilic aromatic substitution conditions 59
3.2.3.4 Cyclisation to form dihydroquinoxalinone core 61
3.2.4 Assessment of enantiopurity 62
3.2.5 Extension from dihydroquinoxalinone core 62
3.3 Biological testing of dihydroquinoxalinones 64
3.3.1 Inhibition of CREBBP bromodomain 64
3.3.2 Activity against other bromodomains 64
3.4 Crystal structure of dihydroquinoxalinone bound to CREBBP 66
3.5 Tetrahydroquinoline analogues to investigate the cation-π interaction 69
3.5.1 Background to the cation-π interaction 69
3.5.2 Route to afford tetrahydroquinoline analogues 70
3.5.3 Selection of starting materials 71
3.5.4 Addition of amino-propyl linker to tetrahydroquinolines 72
3.5.5 Biological analysis of tetrahydroquinoline analogues 74
3.5.6 Crystal structure of a tetrahydroquinoline analogue 76
3.6 Further analysis of CREBBP ligands 78
3.6.1 Rationale 78
3.6.2 NMR analysis of internal hydrogen bond. 78
3.6.3 Computational analysis of internal hydrogen bond 79
3.6.4 Computational analysis of cation-π interaction 82
3.7 Biological analysis of a dihydroquinoxalinone ligand as a CREBBP inhibitor 83
3.8 Summary 84
4 Further bicyclic amides as bromodomain inhibitors
4.1 Aims 86
4.2 Further analogues to probe cation-π interaction 86
4.2.1 Rationale 86
4.2.2 Selection and preparation of starting materials 87
4.2.2.1 Preparation of 6-nitro-1,2,3,4-tetrahydroquinoline 87
4.2.2.2 Preparation of 7-(benzyloxy)-1,2,3,4-tetrahydroquinoline 88
4.2.2.3 Preparation of dialkoxy-substituted tetrahydroquinolines 88
4.2.3 Synthesis of tetrahydroquinoline analogues 90
4.2.4 Attachment to dihydroquinoxalinone core 93

4.2.5 Computational analysis of tetrahydroquinoline analogues 94
4.2.6 Summary 96
4.3 Rigidification of linker 97
4.3.1 Rationale 97
4.3.2 Retrosynthetic analysis of cyclopropyl tetrahydroquinolines 100
4.3.3 Route A – Addition of methylene 101
4.3.4 Route B – Addition of functionalised carbenoid 102
4.3.5 Route C – Addition of amido-carbenoid 103
4.3.6 Summary 104
4.4 Fluorination of dihydroquinoxalinone core 105
4.4.1 Rationale 105
4.4.2 Retrosynthetic analysis of fluorinated dihydroquinoxalinones 107
4.4.3 Synthesis of 7-fluoro analogue 108
4.4.4 Synthesis of 6-fluoro analogue 109
4.4.5 Attachment of tetrahydroquinoline side group 110
4.4.6 Biological analysis of fluorinated DHQNs 112
4.4.7 Summary 112
4.5 Variation at C(3) of dihydroquinoxalinone 113
4.5.1 Rationale 113
4.5.2 Route and starting material preparation 114
4.5.3 Synthesis of cyclopropyl DHQN 114
4.5.4 Synthesis of gem-dimethyl DHQN 116
4.5.5 Stability of spirocyclopropyl compounds 118
4.5.6 Summary 119
4.6 Methylquinolinones as acetyl-lysine mimics 120
4.6.1 Rationale 120
4.6.2 Synthesis of fragments 121
4.6.3 Attachment of tetrahydroquinoline side group 122
4.6.4 Biological analysis of methylquinolinones 123
4.6.5 Summary 124
5 Summary
5.1 Summary 126

6 Experimental

6.1 General Experimental 130

6.1.1 Computational Methods 130
6.1.2 Physical Methods 130
6.1.3 Biochemical Methods 131
6.1.4 Synthetic Methods 132

6.2 General Procedures 136

6.3 Chapter 2 Procedures 139

6.4 Chapter 3 Procedures 156

6.4 Chapter 4 Procedures 187

7 References

Appendix

A1 Chiral HPLC of benzoxazinone analogues 278

A2 Chiral HPLC of dihydroquinoxalinone carboxylic acids 280

A3 NMR analysis of internal H-bond 281

A4 NMR assignments using NOE-spectroscopy 283

A5 Publications arising from this thesis 287

Acknowledgements

I would like to first thank Prof Stuart Conway for his supervision throughout my DPhil. Your enthusiasm for the project has made it all the more enjoyable. Thanks also for your continued support and encouragement in career planning. Thanks also to Dr Paul Brennan for your supervision over the last couple of years and for providing great MedChem advice. Many thanks to Dr Tom Heightman for 'selling' the project to me in the first instance and for helping get things off to a great start. I'd also like to acknowledge Pfizer for their support of my project, in particular Dr Andrew Cook and Dr David Pryde for providing very insightful discussions whenever we've had the chance to meet. Finally, thanks also to Prof Chris Schofield and Prof Stefan Knapp for helping to oversee the project.

I have been lucky to have had the opportunity to collaborate with some great groups that have helped me immensely. Particular thanks to Martin, Oleg and Panagis from the SGC for help with screening compounds. Also, thanks to Wilian and Prof Rob Paton for doing the computational work with me that really improved the project. Many thanks to all the support staff at the CRL that make the department such a great place to work. In particular, thanks to Barbra and Tina for always finding time to run NMR samples for me, but also to all other members of the NMR, Mass Spec, Facilities and Stores teams. Also, thanks Sonia, Angie and most of all Diane for such superb proof reading of my thesis.

It's difficult to appropriately acknowledge members of the Conway group, past and present, all of whom have made being in the lab an enjoyable experience. All the Part IIs and project students that have gone through the group have left me with fond memories, like Boat Trips and Crazy Tuesdays. A particular thank you to Natalie for being a great person to supervise and for leaving us with enough acronyms to throw into any situation. Good luck to all the DPhils who are still working away. Thanks to Angie and Sam for being great desk buddies and tolerating my mess, and thanks Laura for being an excellent fumehood neighbour. Thanks

Michael for tolerating me in your fumehood. Well done Brian for always picking out great songs, even on a manic Monday, and thanks to Liam for encouraging the TGIF feeling at the end of the week. Thanks to Jess, Megan, Dan, Aine and Yulin for helping me settle in and for making my DPhil fun from the start. Thanks also to all the Post-Docs who have joined the group since, for your advice and experience. David, it's been great working with you throughout our time in the CRL, I hope that we get the chance to do it again someday. Finally, thanks Diane for all your help and support; I couldn't have got through it without you.

I feel very thankful to have met so many great people whilst being part of the DTC. You guys have all been fantastic colleagues to discuss projects with, and even more importantly great friends to hang out with. I also owe thanks to the O'Hare group for providing such great housemates during my time in Oxford. Particular thanks to Saul for all those years of being housemates, I'll miss them.

Thanks to the old St Catz group for being great friends over the years. It's been great to always have some event with you guys in the near future to look forward to – long may it continue! I'm sorry I've held up the party for so long.

Finally thanks to my Mum, Dad and Sister for all your continuous love and support.

Abbreviations

Ac: Acetyl

ACC: 1-Aminocyclopropane-1-carboxylic acid

Ach: Acetylcholine

B2AR: β_2 Adrenergic receptor

BAZ1B: Bromodomain adjacent to zinc finger domain protein 1B

BET: Bromodomain and extra C-terminal domain

BINAP: (1,1′-Binaphthalene-2,2′-diyl)bis(diphenylphosphine)

Bn: Benzyl

BNZ: 1,4-Benzoxazin-3(4*H*)-one

BRCA2: Breast cancer 2, early onset

BRCT(s): BRCA1 C Terminus

BRD4: Bromodomain-containing protein 4

cAMP: Cyclic adenosine monophosphate

CECR2: Cat eye syndrome chromosome region, candidate 2

COX-2: Cyclooxygenase-2

CREB: cAMP response element binding protein

CREBBP: CREB binding protein

DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene

DFT: Density functional theory

DHQ: Dihydroquinazolinone

DHQN: 3,4-dihydroquinoxalin-2(1*H*)-one

DIAD: Di*iso*propyl azodicarboxylate

DIBAL: Di*iso*butylaluminium hydride

DIMBOA: 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one

DIPEA: *N*,*N*-Di*iso*propylethylamine

DMF: *N*,*N*-Dimethylformamide

DMI: Dimethylisoxazole

DMSO: Dimethyl sulfoxide

DNA: Deoxyribose nucleic acid

dppf: 1,1'-Bis(diphenylphosphino)ferrocene

DSF: Differential scanning fluorimetry

e.*e*: Enantiomeric excess

ELSD: Evaporative light scattering detector

EP300: E1A binding protein p300

eq: equivalents

ES: Electrospray

Et: Ethyl

FG: Functional group

Fmoc: 9-Fluorenylmethoxycarbonyl

FRAP: Fluorescence Recovery After Photobleaching

GCN5L2:

GFP: Green fluorescent protein

GPCR: G protein-coupled receptor

GSK: GlaxoSmithKline

$H3_{46\text{-}66}K_{56}Ac$: H2N-ALREIRRYQK(Ac)STELLIRKLK(Biotin)-COOH

HAT(s): Histone acetyltransferase(s)

HBTU: *N*,*N*,*N*′,*N*′-Tetramethyl-O-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate

HDAC(s): Histone deacetylase(s)

HIV: Human immunodeficiency virus

HMT(s): Histone methyltransferase

HOESY: Heteronuclear NOE spectroscopy

HPLC: High-performance liquid chromatography

HRMS: High resolution mass spectrometry

HSQC: Heteronuclear Single Quantum Correlation

HyAT: Hydrogen atom transfer

IC_{50}: Half maximal inhibitory concentration

IPA: *iso*-Propyl alcohol

ITC: Isothermal titration calorimetry

KAc: Acetyl lysine

KAT: Lysine acetyltransferase

LA: Lewis acid

LC-MS: Liquid chromatography-mass spectrometry

LE: Ligand efficiency

LRMS: Low resolution mass spectrometry

MBT(s): Malignant Brain Tumour

MD: Molecular dynamics

Me: Methyl

MeQ: 1-Methylquinolin-2(1*H*)-one

MLL: Methyltransferase

MORF: MOZ-related factor

MOZ: Monocytic leukaemia zinc-finger protein

Ms: Mesyl

Mw: Molecular weight

MW: Microwave

NBS: *N*-bromosuccinimide

NF-κB: nuclear factor of κ light polypeptide gene enhancer in B-cells

NMP: 1-Methyl-2-pyrrolidinone

NMR: Nuclear Magnetic Resonance

NOE: Nuclear Overhauser Effect

NUT: Nuclear protein in testis

PCAF: EP300/CREBP-associated factor

PDB: Protein Data Bank

Ph: Phenyl

PHD(s): Plant Homeo Domain(s)

Piv: Pivaloyl/Trimethylacetyl

PPI(s): Protein-protein interaction(s)

PPTS: Pyridinium *p*-toluenesulfonate

PTMs: Post-translational modification(s)

RAS: Renin-angiotension system

R_f: Retention factor

rt: Room temperature

SAHA: Suberoylanilide hydroxamic acid

SAR: Structure-activity relationship

SD: Standard deviation

SET: Single electron transfer

SGC: Structural Genomics Consortium

siRNA: Small interfering ribonucleic acid

S_NAr: Nucleophilic aromatic substitution

SPR: Surface plasmon resonance

STD: Saturation transfer difference

Tf: Triflic/triflate

TFA: Trifluoroacetic acid

THF: Tetrahydrofuran

THQ: 1,2,3,4-Tetrahydroquinoline

TMSOI: Trimethylsulfoxonium iodide

Chapter 1

Introduction

This chapter will introduce lysine acetylation as a post-translational modification with particular regards to its role in controlling gene transcription through histone modifications. Then the structure and function of bromodomains, the protein motifs which bind acetylated lysine residues, will be discussed. The biology of one particular bromodomain containing protein, CREBBP, will be profiled within the context of human disease. Progress to date on the development of bromodomain inhibitors will be outlined, with a focus on those that have been shown to target CREBBP. Finally, the aims of this project will be outlined.

1.1 Lysine acetylation

1.1.1 Post translational modification

Post-translational modifications (PTMs) are a crucial mechanism for the modulation of protein function in eukaryotes. From the addition of chemical groups as small as a methyl group, to kilo-dalton sized proteins such as ubiquitin, PTMs have been implicated in almost every cellular process.[1] Addition of an acetyl group to the terminal amine of lysine, forming *N*-ε-acetyl-L-lysine (acetyl lysine, KAc), is one such ubiquitous PTM. A recent study identified 3600 lysine acetylation sites on 1750 proteins with a diverse array of cellular functions such as cell cycle regulation, transcription and replication, nuclear transport and chromatin remodelling.[2] Furthermore, one public database has identified over 27 000 lysine acetylation sites within human cells.[3] The high abundance of this modification has led to speculation that this PTM could play a central role in signal transduction, in a similar fashion to phosphorylation.[4]

1.1.2 Histone modification

PTM of histone proteins was first identified by Allfrey *et al.*, who proposed that acetylation and methylation could play a role in the control of gene expression.[5] Histones are small globular proteins, around which DNA is wound to form a nucleosome, and are the principle unit of DNA packing in eukaryotes (Figure 1.1A). The histone core consists of two copies of four conserved histone proteins, namely H2A, H2B, H3 and H4, to form an octomer around which a section of DNA (~146 base pairs) is wound (Figure 1.1B). The disordered *N* and *C*-terminal tails of each of these proteins protrude away from the central body and are subject to many different PTMs, such as acetylation, but also including methylation, phosphorylation, crotonylation and ubiquitination. The transcriptional programme of a cell is closely related to the physical packaging of its DNA. Tightly packed heterochromatin is associated with transcriptionally

silent genes whereas the more loosely packed euchromatin is found in regions of active gene transcription. Consequently, structural changes to chromatin can be expected to have a strong influence on biological processes.[6]

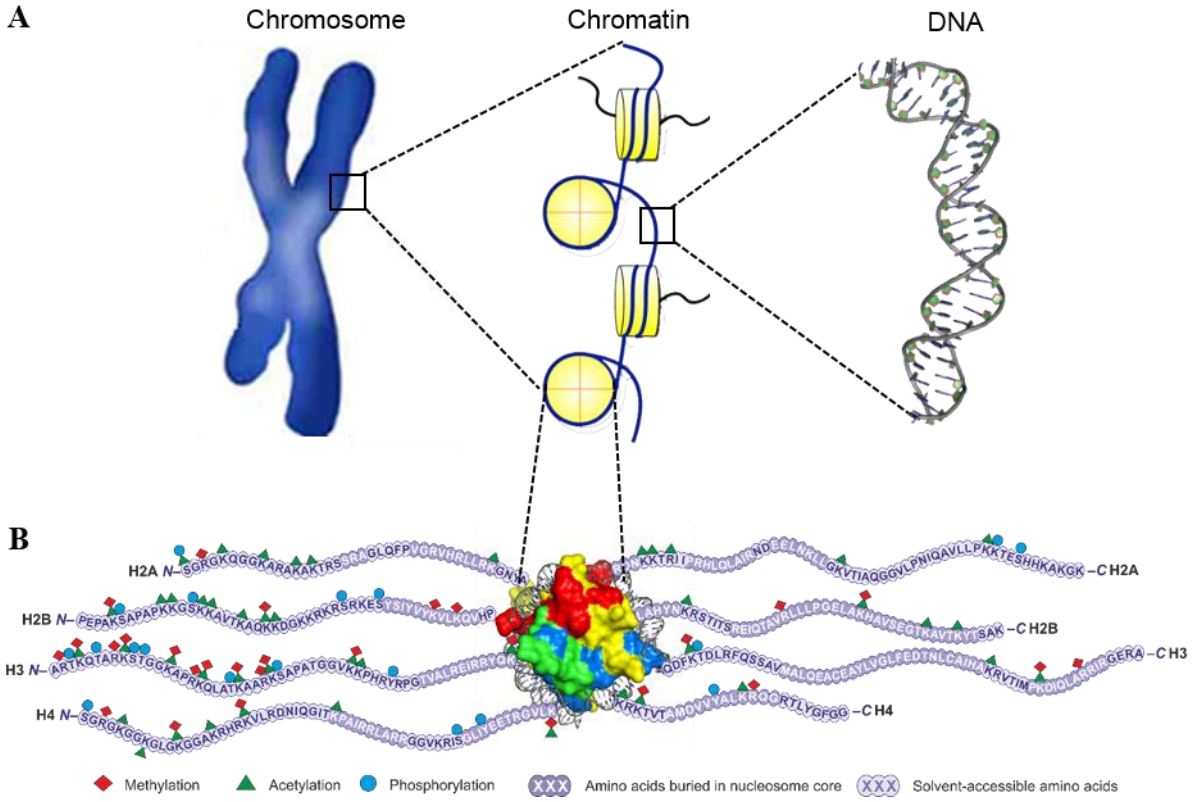


Figure 1.1: **A**: In eukaryotes DNA is packaged into chromatin by wrapping around histone proteins to form a nucleosome. **B**: A nucleosome consists of two copies of four histone proteins, which are subject to numerous PTMs on both the *N* and *C*-terminal tails. Image shows an X-ray crystal structure of a nucleosome (PDB ID: 1AOI) with detail of positioning of modifications to histone tails adapted from Jennings *et al*.[7]

1.1.3 Role of histone modifications

The biological functions of covalent modifications to histones are complex and varied, and are only just beginning to be understood. Initially only changes to the physical nature of the residue were considered, such as *N*-ε-acetylation of a lysine residue, neutralising the positive charge on the terminal amino group. Accordingly, this modification has been broadly associated with transcriptional activation, due to the weakened interaction between the histone and the negatively charged backbone of DNA, leading to a "more open" chromatin state.[8] Subsequent research has revealed how these modifications also recruit proteins and assemble complexes

involved in the remodelling of chromatin and the control of transcription.[9] It is through these mechanisms that covalent modifications, or marks, on histones have been proposed to constitute a "code" that exerts control over gene expression.[10]

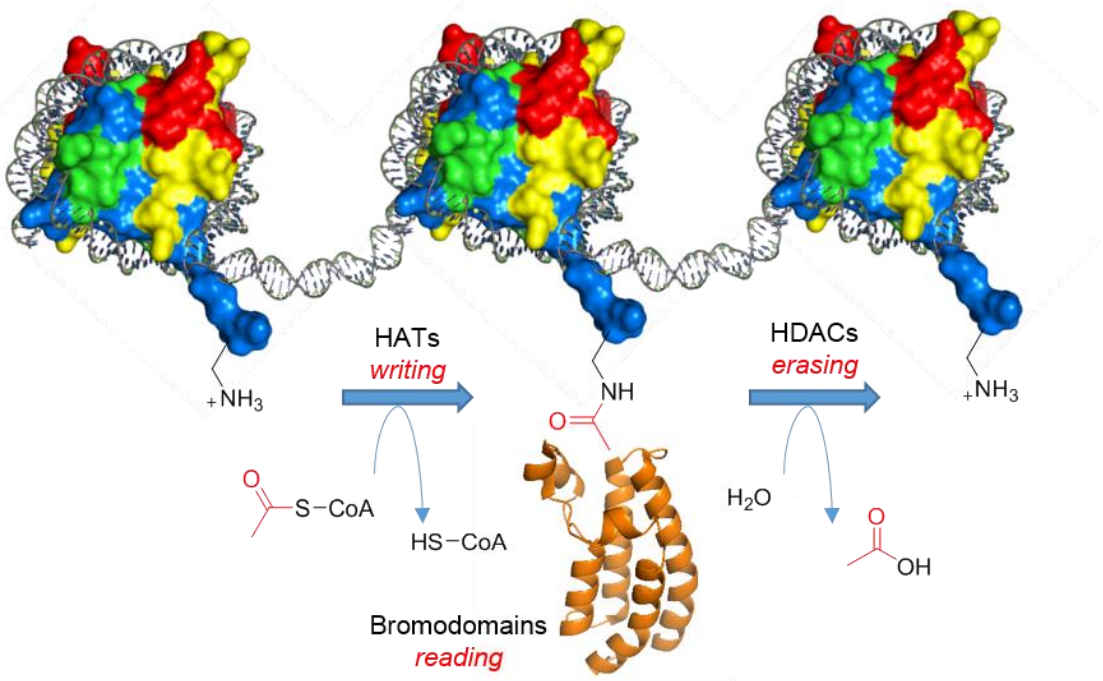
It is common for a particular mark to be correlated with positive or negative transcriptional states.[11] For example, methylation at lysine 4 on histone 3 ($H3K_4$) is associated with transcriptionally active genes, whereas methylation at $H3K_9$ is associated with repression.[12] However, there is extensive cross-talk between marks that adds another layer of complexity to the process.[13] Therefore, the biological outcome of a specific histone modification should also be considered a consequence of the proteins that recognise it and the marks that surround it, i.e. context dependent, rather than being directed by the modification alone.[14]

Since these dynamic markers exert a control on biological processes and can be subtly altered by the environment, they have been proposed to play an important role in the epigenetic memory of cells.[15] However, many questions still remain unanswered, such as how the information is passed across cell division and between generations, and therefore closer study of the proteins involved is required.

1.1.4 Modulators of histone modifications

The concept of a histone code has been fortified by characterisation of the proteins that are involved in the control of PTMs. Many of the enzymes that add (writers) or remove (erasers) PTMs have been identified, which is consistent with the process being dynamic.[9] In the case of lysine acetylation, acetyl groups are added to the terminal amine by histone acetyl transferases (HATs) and removed by histone deacetylases (HDACs), while similar catalytic partners are found for other PTMs (Figure 1.2). Recently, the proteins (readers) that bind to the post-translationally modified residues have also been identified. Bromodomains bind to

acetylated lysine residues and, through the formation of this protein-protein interaction (PPI), serve to assemble multi-protein complexes.



Modification	Writer	Reader	Eraser
Acetylation	HAT	Bromodomain	HDAC
Methylation	HMT	PHD, tudor, MBT	DMT
Phosphorylation	Kinase	14-3-3	Phosphatase

Figure 1.2: The post-translational modification of histone proteins is mediated by distinct families of enzymes that write and erase the histone code. Reader domains, which bind to these marks, play a role in the interpretation of the code by recruiting multidomain complexes to the nucleosome.

1.1.5 Non-histone lysine acetylation

It is important to note that, in addition to histones, there are many other proteins that are subject to PTMs and can therefore act as substrates to HATs, HDACs and bromodomains. These include transcription factors, nuclear transporters and non-nuclear proteins such as tubulin.[4] One recent study identified lysine acetylation as a contributing factor in the activity of most

enzymes involved in metabolism.[16] As the number of cellular processes involving lysine acetylation increases, so does the importance of understanding the proteins involved.

1.1.6 Targeting histone modifications as a therapeutic strategy

The writers, readers and erasers involved in histone modification are attractive drug targets since modulation of their activity could offer control over the expression, and corresponding activity, of particular proteins.[17] HDAC inhibitors were among the first to emerge with a number now used in the treatment of cancer.[18] However, the broader use of these inhibitors in areas other than oncology has been hampered by a lack of selectivity between the HDAC enzymes. In addition, questions still remain over whether a compound with selectivity for one epigenetic target will have enough of an effect on the complex network of modifications to observe a phenotypic response.[19] One way in which these issues can be addressed is through the development of small molecule inhibitors as chemical probes for effectors of the histone code.[20,21] It is important for these compounds to be well characterised, with known selectivity for a subset of targets, in order to properly correlate the phenotypic response to target inhibition.[22]

1.2 Bromodomains

1.2.1 Occurrence of bromodomains

The bromodomain motif was first reported in the *Drosophila* gene *brahma*, from where the name derives.[23,24] To date, 61 bromodomains have been identified in the human genome and these have been classified into eight families according to their sequence homology (Figure 1.3).[25] Of particular note is family II, the bromodomain and extra C-terminal domain (BET) family, which have been particularly well studied to date. Also included is family III, containing the bromodomains CREBBP and EP300, which will be discussed in more detail in the following sections. Bromodomains form part of larger multidomain scaffolds, and the 46 distinct proteins that contain bromodomains have various nuclear and cytoplasmic roles. Many histone-modifying enzymes (such as the methyltransferase MLL, and the acetyltransferase CREBBP) contain a bromodomain, highlighting the importance of cooperativity between different histone modifications. Bromodomains are also found in transcription factors (many of the BET family) and chromatin remodelling factors (BAZ1B) demonstrating the central role that acetylation plays in the control of transcriptional programs.

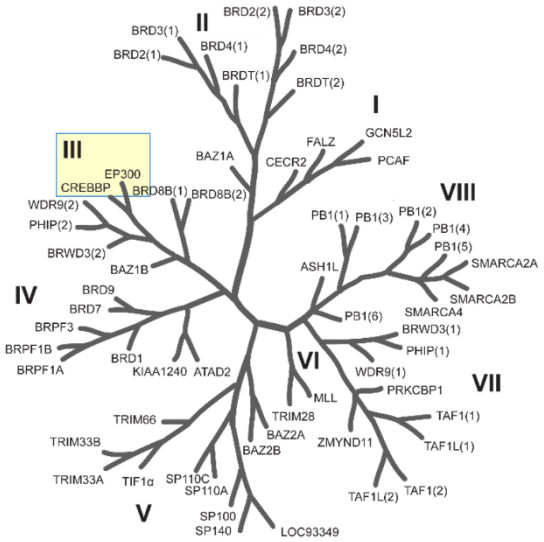


Figure 1.3: Phylogenetic tree of the human bromodomain family, adapted from Hewings *et. al*.[26] Bromodomains are divided into 8 families according to their sequence homology and, where more than one bromodomain exists in a protein, are numbered according to their position from the *N*-terminus.[25] Highlighted are CREBBP and EP300 which are discussed in this work.

1.2.2 Structure of bromodomains

The structural fold of the bromodomain is largely conserved despite low sequence homology between the different families.[25] It consists of around 110 amino acids that adopt a left-handed bundle of four α-helices and encompass a deep hydrophobic pocket, which binds the acetylated lysine residue (Figure 1.4). Within this binding pocket lies a conserved asparagine residue that forms a hydrogen bond with the *N*-acetyl group (N1168 in CREBBP). In the few bromodomains where this asparagine is not present, a different hydrogen bond-donor (threonine or tyrosine) occupies this position. A water-mediated hydrogen bond also forms between the carbonyl of KAc and a conserved tyrosine in the BC loop. This water molecule is part of a network of four water molecules bound at the bottom of the pocket, which form a series of concerted hydrogen bonds, and are not easily displaced. Much of the sequence variation between bromodomains exists in the loop regions (ZA and BC) which vary in length and charge.

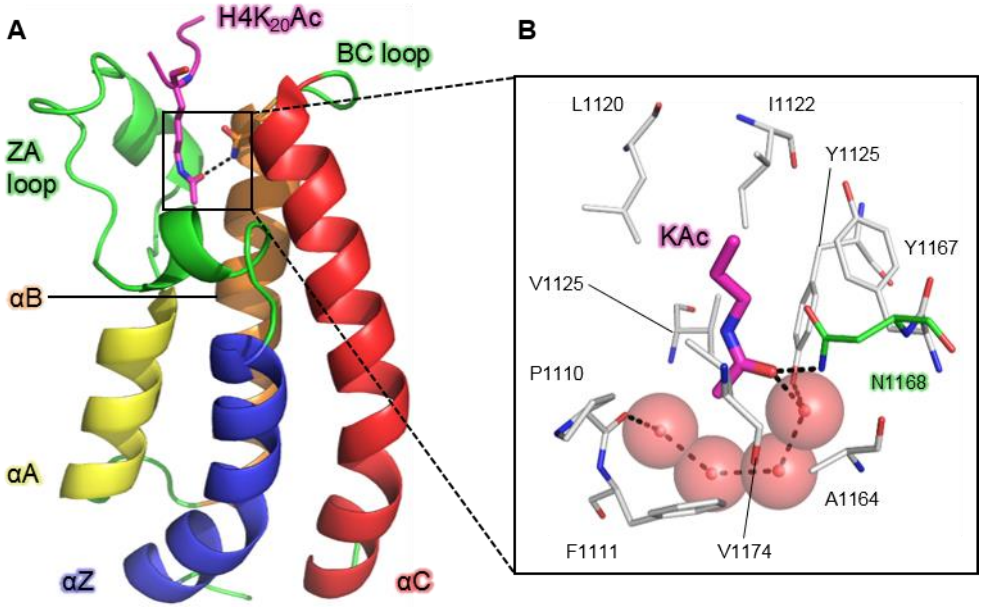


Figure 1.4: Structure of the CREBBP bromodomain. **A**: X-ray crystal structure of $H4_{13\text{-}27}K_{20}Ac$ bound to CREBBP (PDB ID: 4N3W). The bromodomain fold consists of four α-helices, labelled from the *N*-terminus αZ, αA, αB and αC. The ZA-loop and BC-loop (coloured green) define the KAc-binding pocket. **B**: X-ray crystal structure of KAc bound to CREBBP (PDB ID: 3P1C). KAc forms a hydrogen-bond to a conserved asparagine residue (N1168 in CREBBP) and a water mediated hydrogen-bond to a tyrosine residue (Y1125 in CREBBP)

Through these differences in the residues at the opening of the binding site, each bromodomain could be expected to interact with acetylated lysine in a sequence dependent manner, allowing selectivity for a specific KAc at a certain position on a histone protein.

1.2.3 Bromodomains as drug targets

Given the central role that they play in chromatin biology, it is not surprising that bromodomains have emerged as attractive therapeutic targets.[27–29] A number of disease areas have been identified in which the activity or expression of a disease causing protein is dependent on a bromodomain;[30] some of those resulting from CREBBP dysfunction are described in the following section (Section 1.3.2).

From a medicinal chemistry point of view there a number of factors that support bromodomains as being 'ligand-able' – i.e. possible to design ligands for. Traditionally, targeting PPIs has been challenging due to larger contact surfaces that generally lack pockets for binding small molecules.[31] However, the bromodomain has a well-defined lypophilic binding site that represents a good target for drug-like compounds. Indeed, a recent druggability analysis of the bromodomains family using SiteMap (which identifies and scores a binding site based on its enclosure, volume and hydrophobicity) found that all bromodomains were more druggable than a typical protein involved in a PPI.[32] The discovery of bromodomain inhibitors, with particular regards to inhibitors of CREBBP, is described in a later section (Section 1.4).

1.3 Structure and function of CREBBP

1.3.1 Role of CREBBP

CREBBP [cyclic adenosine monophosphate (cAMP) response element binding (CREB) binding protein, also known as CBP and KAT3A] is a multi kilo-dalton protein that is present in all higher order eukaryotes and is ubiquitously expressed during development.[33] It was first identified as a cofactor of the transcription factor CREB,[34] and has since been shown to possess acetyl-transferase activity.[35,36] The domain architecture of CREBBP consists of regions of relatively low disorder that function as binding sites for a plethora of biological partners (Figure 1.5). Of particular interest is the catalytic core of CREBBP which consists of two binding domains, the bromodomain and the PHD finger, and an acetyl transferase domain. Structural characterisation of these domains has been aided by X-ray crystal structures (Figure 1.5B). It seems likely that the two binding domains (readers) and the HAT domain (writer) corroborate each other's function and play important roles in consolidating the histone code.[37]

Another region that has been structurally characterised is the KIX domain, which utilises two allosterically connected binding sites to engage a variety of transcription factors. Despite its high degree of conformational malleability, a crystal structure of this domain has been obtained by tethering to a small molecule to improve stability (Figure 1.5D).[38] No crystal structures of the remaining domains are available, although the use of NMR to solve solution structures has been reported (Figure 1.5C,E-F). These include the cysteine-histidine rich regions CH-1 and CH-3,[39] and the nuclear receptor co-activator binding domain (NCBD), which have all been proposed to interact with many transcription factors.

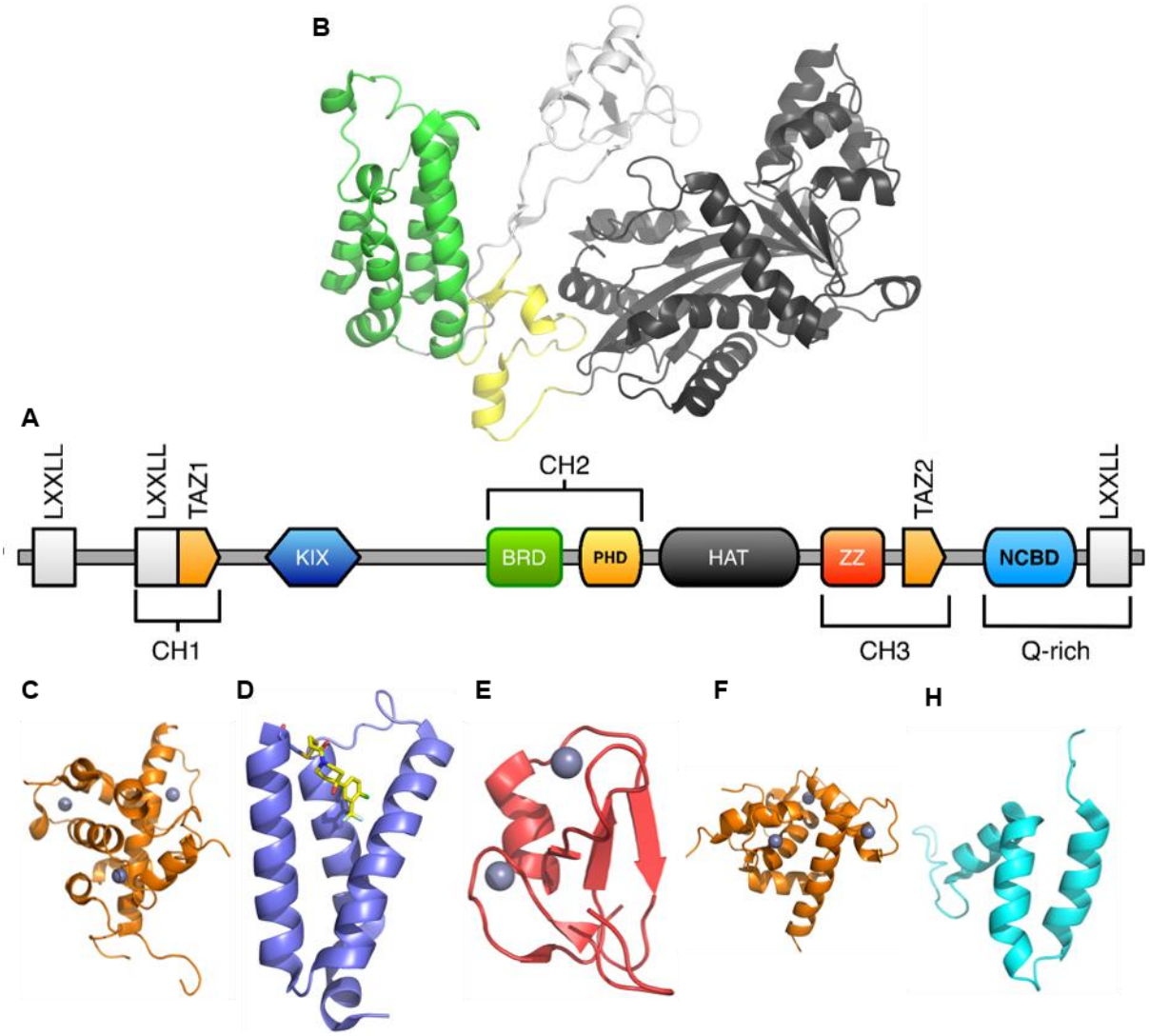


Figure 1.5: **A**: Schematic representation of the domain structure of CREBBP. **B**: X-Ray crystal structure of the catalytic core of EP300 consisting of a bromodomain (yellow), a PHD finger (green) and HAT domain (blue) (PDB ID: 4BHW).[40] **C**: NMR structure of TAZ1 domain of CREBBP (PDB ID: 2KA4).[41] **D**: X-ray crystal structure of KIX domain of CREBBP tethered to a small molecule inhibitor (PDB ID: 4I9O).[38] **E**: NMR structure of ZZ domain of CREBBP (PDB ID: 1TOT).[42] **F**: NMR structure of TAZ2 domain of CREBBP (PDB ID: 2KA6).[41] **G**: NMR structure of NCBD domain of CREBBP (PDB ID: 2KKJ).[43]

CREBBP has a closely related homologue EP300 (also known as p300 and KAT3B) and the two proteins share the same key domains, with an overall homology of 63%.[44] The bromodomains have 97% homology, with the main differences located in the helix regions rather than the peptide-binding loop regions. Despite their similarity, the bromodomains of CREBBP and EP300 have been shown to have different affinities for histone marks, both in binding and acetylation preference.[25,45] For example, bromodomain binding to the histone

$H4_{1-11}K_5Ac$ and $H4_{1-11}K_8Ac$ has been detected for EP300 but not for CREBBP.[25] In addition, while many cell-based studies have found CREBBP and EP300 to be interchangeable, others have functional differences.[46]

Through the domains described above, CREBBP and EP300 interact with over 400 protein partners and appear as hubs in the protein-protein interactome.[47] By binding at the promoter or enhancer regions of target genes and recruiting the transcriptional machinery, these proteins are proposed to play a key role in stimulating transcription.[48,49] Both the catalytic and binding activities of CREBBP are important for this role and aberrant activity of either can have severe consequences on cellular function.

1.3.2 CREBBP dysfunction

Homozygous knockout of either CREBBP or EP300 results in embryonic lethality, as does heterozygous knockout of both, demonstrating the importance of these genes for normal development.[50] In addition, using small interfering RNA (siRNA) to deplete CREBBP causes cell death by mitotic catastrophe as a result of abnormal gene expression, suggesting a continued role in cellular function.[51] Conditional knockout models have also been developed and used to demonstrate that CREBBP plays a significant role in maintaining histone acetylation levels.[52] Given the central role that CREBBP plays in regulation of cell growth and genomic stability, as well as in DNA replication and repair, it is not surprising that it has been implicated in numerous disorders.[53] Some of these diseases are discussed below.

1.3.2.1 Implications of CREBBP in cancer

Since CREBBP operates as a cofactor to both tumour suppressors (such as p53 and BRCA2)[54,55] and oncoproteins (such as c-Myb),[56] whether it operates as an anti- or pro-oncogenic factor is clearly context dependent. Nevertheless, there are a number of examples of tumourigenesis clearly driven by dysfunction of CREBBP.[48]

Inactivating mutations of CREBBP have been frequently observed in B-cell non-Hodgkin's lymphoma.[57] Of the 30 missense mutations identified, 27 were clustered in the HAT domain, implicating compromised acetylation in the pathogenic mechanism. Additionally, since CREBBP serves as a coactivator of the tumour suppressor p53, loss of CREBBP activity is likely to prevent p53-mediated cell-growth arrest or apoptosis.[54]

Overexpression of CREBBP and EP300 has been reported in prostate cancer and implicated in the regulation of oestrogen receptor beta-mediated transcription.[58] EP300 in particular was found to play a role in cancer migration, with expression levels linked to patient survival.

Chromosomal translocations that result in oncogenic fusion proteins are a common occurrence in cancer. Translocation of CREBBP to two proteins that possess histone modifying domains, MORF (an acetyltransferase) and MLL (a methyltransferase), has been observed in acute myeloid leukaemia.[59,60] In the case of the MLL fusion, the presence of both the bromodomain and HAT of CREBBP is required for the leukaemic phenotype.[61] Another chromosomal translocation, which occurs in NUT midline carcinoma, between the NUT and BRD4 genes, has been extensively studied in the context of BET bromodomain.[62] The proposed mechanism of sequestration of CREBBP and EP300 by the oncogenic NUT-BRD4 fusion protein, leading to inactivation of p53, demonstrates the intriguing relationship between bromodomain-containing proteins from different families in a disease context.[63]

1.3.2.2 Implications of CREBBP in inflammation

Activation of proinflammatory genes by the transcription factor NF-κB is enhanced by binding to CREBBP.[64] Whether CREBBP acts as a cofactor to p53 or NF-κB appears to be mediated by phosphorylation of the acetyltransferase domain, potentially a critical factor in the promotion of tumour growth.[65] In addition, EP300 (but not CREBBP) has been shown to be essential for

cyclooxygenase 2 (COX-2) mediated transcriptional activation, and expression of target genes was be abolished through inhibition of HAT activity.[66]

1.3.2.3 Implications of CREBBP in neurodegeneration

Aberrant CREB signalling has been identified in many neurological diseases and consequently its cofactors CREBBP and EP300 have also been implicated.[67,68] Mutations in CREBBP are a cause of the human congenital disorder Rubinstein-Taybi syndrome, which is characterised by physical abnormalities and mental retardation.[69] Loss of CREBBP function has also been shown to lead to neuronal cell death in other neurodegenerative diseases including Alzheimer's disease and Huntington's disease.[70,71] Conditional knockout of CREBBP within the hippocampus of adult mice has been shown to have a deleterious effect on long-term potentiation and long-term memory.[72] A similar study knocking out CREBBP from the nuclear accumbens has demonstrated a role of CREBBP in mediating cocaine-induced gene transcription.[73]

1.3.3 Prospect of CREBBP inhibitors

Many of the studies described above relate disease phenotype to levels of expression or repression of the CREBBP or EP300 proteins as a whole. In order to investigate the effect of a particular domain, missense mutations were employed, but could not be so severe as to cause embryonic lethality. Consequently, these approaches are limited in their tuneability and the timescale over which observations can be made. In contract, the use of small molecule inhibitors can allow protein activity to be perturbed in a rapid, tuneable, reversible and highly selective manner.[74] In addition, a bromodomain chemical probe could be used to investigate the crosstalk between different domains, which is of particular use for a protein such as CREBBP which forms so many interactions.

As well as being useful as a tool to investigate the biology of their respective proteins, inhibitors of CREBBP and EP300 may also have a therapeutic potential. As seen by the severe effects of

homozygous knockout of CREBBP and EP300, inhibitors are likely to be cytotoxic, but may also offer an opportunity to target cancer in other ways. Inhibition of EP300 has been shown to activate senescence,[75] and impair T regulatory cell function,[76] thereby presenting opportunities to limit tumour growth. Also, by modulating the activity of transcription factors such as NF-κB, CREBBP inhibitors may have a use as anti-inflammatory treatments. For example, the huge promise of COX-2 inhibitors for the treatment of pain and inflammation and subsequent demise due to major side effects has been well documented.[77] Identification of EP300 as an upstream mediator of COX-2 could offer new strategies for controlling this target in the clinic.

Clearly there is a need for selective inhibitors of the CREBBP bromodomain that can be used to investigate the underlying biology of this multifaceted protein. The current state of progress for CREBBP inhibitors is described in the following section.

1.4 Bromodomain inhibitors

The first bromodomain inhibitors reported in the literature were fragment-like molecules that inhibited the binding between the bromodomain of PCAF and an acetylated lysine residue in HIV-1 Tad, albeit with relatively low affinity.[78] In 2009, a patent from Mitsubishi pharmaceuticals reported potent BET inhibition by triazolothienodiazepine compounds.[79] Subsequently, workers at the SGC and GSK independently reported the discovery of (+)-JQ1 and I-BET762, both analogues of the triazole scaffold **1**, as potent and selective inhibitors of the BET bromodomains (Figure 1.6).[80,81] Encouraged by the potential promised during the biological assessment of these early bromodomain inhibitors, many academic and industrial groups have sought compounds to further probe the acetyl-lysine reading process. These efforts have been reviewed in great detail in the literature.[7,26,82–84] This section will chronical the development of inhibitors of the CREBBP bromodomain, with additional references to cases where cross-reactivity of a chemical scaffold has been used to develop inhibitors for other bromodomain families.

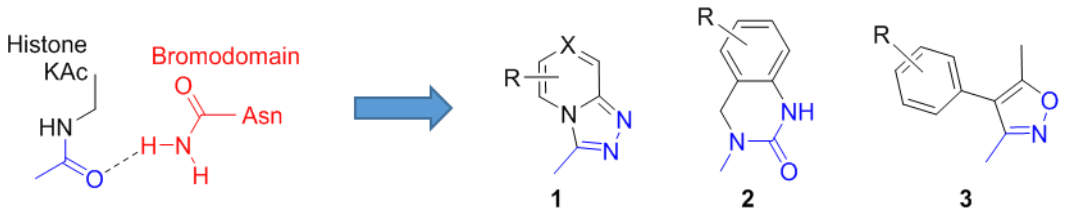


Figure 1.6: Development of inhibitors of the bromodomain-KAc interaction. Triazoles **1**, cyclic amides **2** and isoxazoles **3** have all been employed as KAc mimics in the development of bromodomain ligands. The region of the molecule that binds at the KAc site is shown in blue.

1.4.1 Peptides

Some of the earliest structural information on ligand recognition by the CREBBP bromodomain was obtained by the Zhou group while investigating the interaction with p53.[85] An NMR structure of CREBBP bound to $p53_{367\text{-}386}K_{382}Ac$ confirmed that a similar fold was adopted by the bromodomain compared to the previously reported structures of PCAF and GCN5L2

(Figure 1.7A). By making a series of point mutations to residues around the KAc binding site of CREBBP, residues that made important contributions to the CREBBP/p53 association were identified. From this study, the selectivity of different bromodomains for various peptide sequences could be attributed to variations within the loop regions of the bromodomain.

Investigations into the binding of the CREBBP bromodomain to histone substrates was conducted more recently, again using NMR techniques (Figure 1.7B).[86] An NMR structure of CREBBP bound to $H4_{13\text{-}27}K_{20}Ac$ was obtained and compared to structures of other bromodomains bound to other histone peptides to determine the basis of sequence specific recognition of lysine acetylation. A preference for peptides with hydrophobic residues at (KAc + 1) and (KAc + 2), a basic residue at (KAc – 1) and an aromatic at (KAc – 2) were reported.

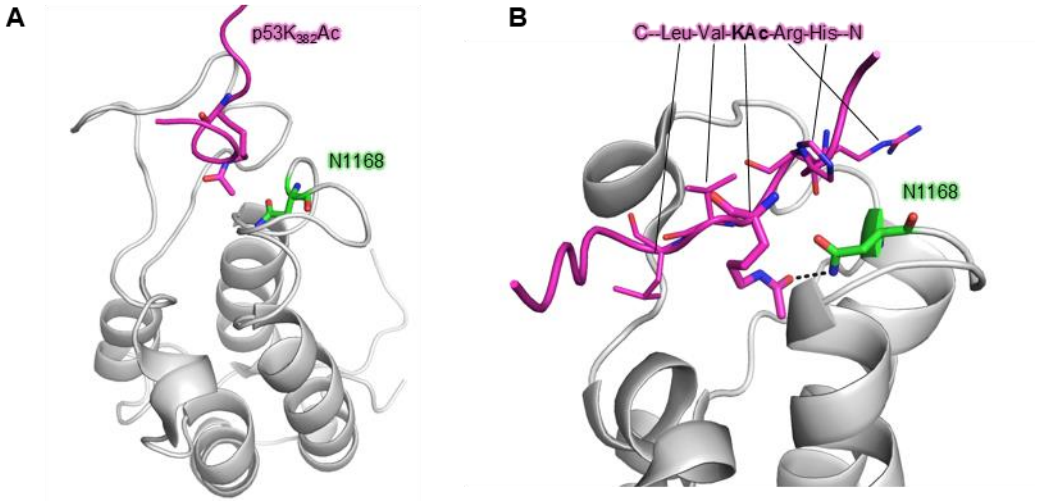
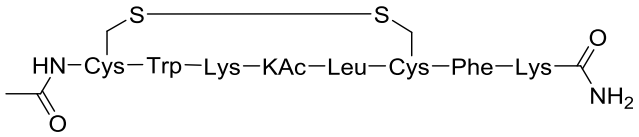


Figure 1.7: Peptide bound CREBBP bromodomain structures. **A**: NMR structure of an acetylated p53 peptide bound to CREBBP (PDB ID 1JSP). **B**: NMR structure of an acetylated histone peptide bound to CREBBP (PDB ID 2RNY). The preferred amino acid sequence either side of the KAc residue is shown.

Aided by much of this structural information a cyclic peptide inhibitor of CREBBP based on the p53 sequence was developed.[87] MD simulations were employed to identify which residues could be converted to cysteines and chemically linked while still maintaining the β-turn adopted by the bound peptide. Optimisation of the amino acid sequence and linker strategy resulted in a cyclic peptide **4** that bound CREBBP with a K_D value of 8 μM compared to a K_D value of

190 μM for the native p53 peptide (Figure 1.8). Treatment of colorectal carcinoma and embryonic kidney cells with **4** showed a reduction in p53-induced p21 expression, implicating the importance of CREBBP bromodomain activity in p53 activity. Fluorescent derivatives of **4** were also used as tools in the development of small molecule inhibitors of CREBBP.



4

CREBBP K_D = 8.0 μM

Figure 1.8: Cyclic peptides have been developed into ligands of CREBBP bromodomain.

1.4.2 *N*-Acylated fragments

The first attempt to screen for small molecule inhibitors of the CREBBP bromodomain was reported by the Zhou group in 2007.[88] Having recently solved the NMR structure of CREBBP bound to p53, a structure based screening program was initiated. A focused library of 200 compounds containing an anticipated acetyl lysine mimic was screened by NMR spectroscopy, looking for perturbations in the 2D ^{1}H-^{15}N HSQC spectra. From this screen, 14 bromodomain-binding compounds were identified, of which two, **5** (MS2129) and **6** (MS7179), were shown to block the interaction between p53 and CREBBP in a concentration dependent manner (Figure 1.9A). In addition, in cells treated with doxorubicin to stimulate DNA damage, both **5** and **6** were shown to supress the levels of p53 and the expression of p21 (a target of p53). Further characterisation using a fluorescence assay determined **6** to bind with a K_D of around 20 μM. An NMR structure of **6** bound to CREBBP was solved which showed that while the compound partly resided in the KAc binding site, no specific interactions were formed with the conserved asparagine (Figure 1.9B).

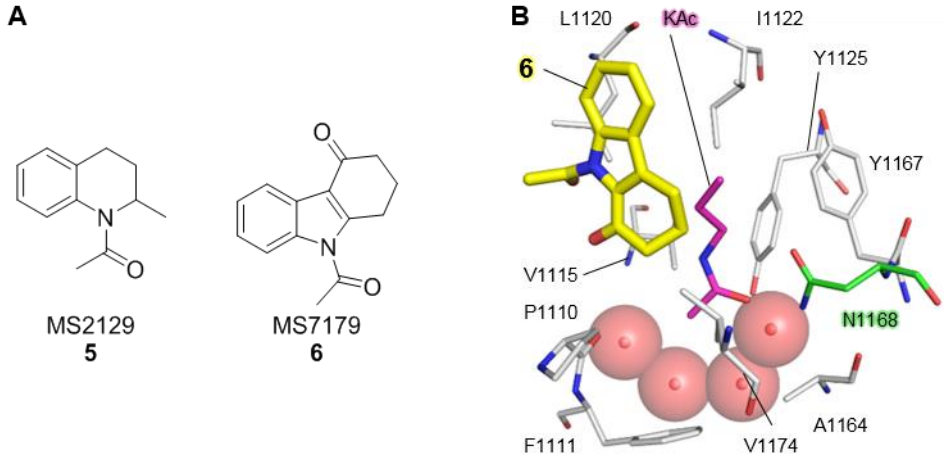


Figure 1.9: **A**: *N*-acyl based fragment inhibitors of the CREBBP bromodomain. **B**: Overlay of X-ray crystal structure of KAc (carbon = purple) bound to CREBBP (PDB ID 3P1C) with NMR structure of **6** bound to CREBBP (PDB ID 2D82). Rather than bind to N1168 the *N*-acyl group was directed towards the ZA loop region.

Interestingly, although no further optimisation of these fragments against CREBBP has been reported, both **5** and **6** have been investigated as BET bromodomain inhibitors. Olinone **7** was developed by addition of a longer acetamidoalkyl substituent to the indole of **6** allowed a hydrogen bond to form to the asparagine within the KAc pocket, resulting in low micromolar inhibition of BRD4(1) (Figure 1.10A).[89] The *N*-acyl-tetrahydroquinoline **5** was later identified in a fragment screen against BRD4 performed at GSK,[90] and features in the BET-selective inhibitor I-BET726 **8** (Figure 1.10B).[91] With a K_D value of 4.4 nM against BRD4 and a K_D value of 6.3 μM against CREBBP,[92] this is a clear example of how a fragment that binds to multiple bromodomains can be optimised for one family alone by forming interactions with residues surrounding the KAc pocket.

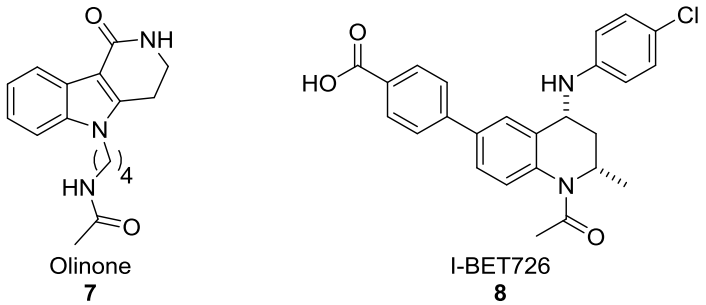


Figure 1.10: Further development of *N*-acyl based bromodomains inhibitors into compounds with BET selectivity.

1.4.3 Azobenzenes

In a subsequent NMR screen on a larger set of compounds, the Zhou group identified a number of azobenzene-based ligands of CREBBP (Figure 1.11A).[93] Several analogues of this framework were synthesised and screened in a cell-based assay measuring p53-dependent expression of p21 in response to doxorubicin. The optimised compound **9** had an IC_{50} value of 5 µM in the cellular assay and a K_D of 19 µM in a fluorescence assay counter-screen, and was up to 5-fold selective over PCAF, BRD4(1) and BAZ2B. An NMR structure revealed that **9** bound to CREBBP at the entrance to the KAc pocket, packing between the ZA and BC loops (Figure 1.11B). An hydrogen bond formed between a phenyl group on **9** and the conserved asparagine, although in a disparate manner to KAc itself. Two substituents on the azobenzene scaffold, an aniline amine and a sulfonyl group, formed interactions with R1173 from the BC loop, potentially contributing to the observed selectivity. Treatment of cardiomyocytes cells with **9** was shown to prevent p53-induced apoptosis upon doxorubicin treatment (a model for ischemic stress), giving **9** the name ischemin.

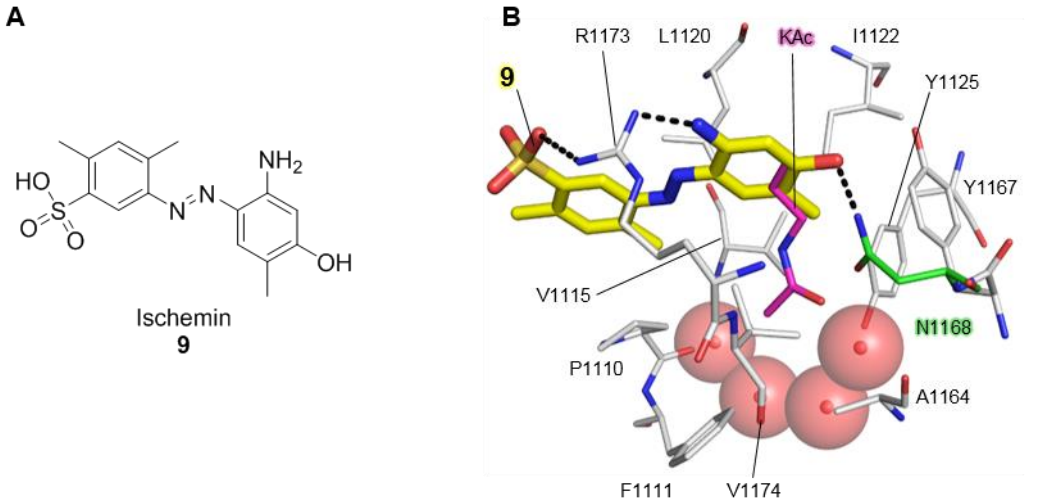
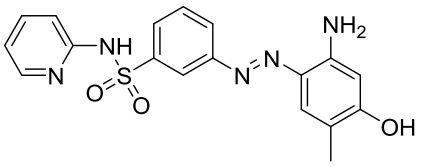


Figure 1.11: **A**: Ischemin, an azobenzene based inhibitor of the CREBBP bromodomain. **B**: Overlay of X-ray crystal structure of **9** bound to CREBBP (PDB ID 2L84) with X-ray crystal structure of KAc bound to CREBBP (PDB ID 3P1C). The compound blocked the entrance to the KAc pocket by forming a hydrogen bond to N1168 and forms two hydrogen bonds to R1173.

The diazobenzene scaffold has also been adapted into an inhibitor of the BET bromodomains (Figure 1.12).[94] The most potent analogue, MS436 **10**, had similar low micromolar inhibition of CREBBP but vastly improved activity against BRD4(1) with an estimated K_i of 30-50 nM. A crystal structure confirmed that the improved azobenzenes bound further into the KAc pocket than the NMR structure of **9** bound to CREBBP implied (not shown).



MS436
10

Figure 1.12: Azobenzenes have also been investigated as inhibitors of the BET bromodomains.

1.4.4 Cyclic amide fragments

The propensity of solvent molecules to displace histone peptides from bromodomains was explored by workers at the SGC during the development of screening assays.[95] Of the solvents investigated, the cyclic amide *N*-methyl-pyrrolidinone **11** (NMP) displaced $H3_{27\text{-}45}K_{36}Ac$ from CREBBP with an IC_{50} of 1.9 mM, and binding was further confirmed by saturation transfer difference (STD) NMR and X-ray crystallography. A subsequent screen of NMP analogues identified many bicyclic amides, such as compound **12**, to be potent and ligand efficient inhibitors of bromodomains (Figure 1.13). The optimisation of bicyclic amides as CREBBP bromodomain inhibitors is described in the following chapters and will therefore not be discussed further at this point.

The DHQ scaffold has also been employed in the development of BET selective bromodomain inhibitors. It was identified in a fragment screen performed by GSK along with a more potent sulfonamide analogue that bound with an IC_{50} value of 30-50 μM against BRD2/3/4.[90] The DHQ fragment was also optimised into the BET-selective inhibitor PFI-1 **13** in a collaboration between the SGC and Pfizer (Figure 1.13A).[96] With an IC_{50} value of 220 nM for BRD4(1) and

a K_D of 49 µM for CREBBP, this compound again demonstrates how selectivity can be gained by favourably substituting an unselective scaffold.

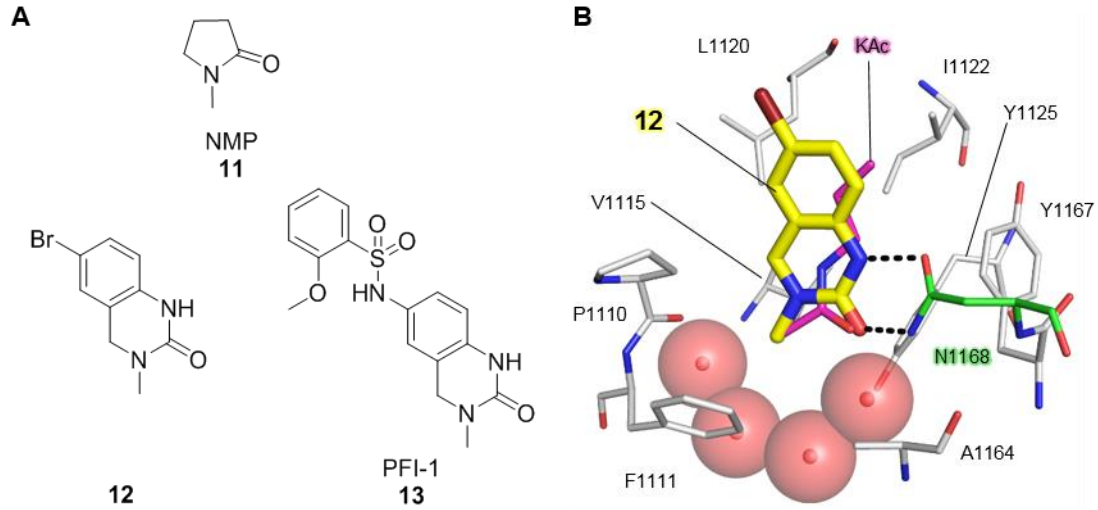


Figure 1.13: **A**: Cyclic amide bromodomain inhibitors. **B**: Overlay of X-ray crystal structure of **12** bound to CREBBP (PDB ID 4NYV) with X-ray crystal structure of KAc bound to CREBBP (PDB ID 3P1C). The compound bound deep into the KAc pocket and formed two hydrogen bonds to N1168.

1.4.5 Dimethylisoxazoles

The 3,5-dimethylisoxazole (DMI) scaffold was independently identified by Hewings *et al.* and workers at GSK as a ligand efficient acetyl lysine mimetic.[81,97,98] Having observed by crystallography that the DMI bound in the KAc pocket of BRD4(1), forming hydrogen bonds to the conserved asparagine and a water molecule, Hewings *et al.* undertook a structure-guided design approach to develop new BET inhibitors.[97] In the process, a number of analogues that bind to CREBBP in addition to the BET bromodomains were identified. The most potent CREBBP analogue, **14**, had an IC_{50} of 32 µM and similar potencies for BET bromodomains (Figure 1.14A). A crystal structure of **14** bound to CREBBP confirmed the DMI moiety bound to N1168 in accordance with the BRD4(1) structure. A carboxylate substitution directed towards the BC loop, which contributed to the affinity of **14** for CREBBP, potentially formed a long range interaction with R1173 (Figure 1.14B).

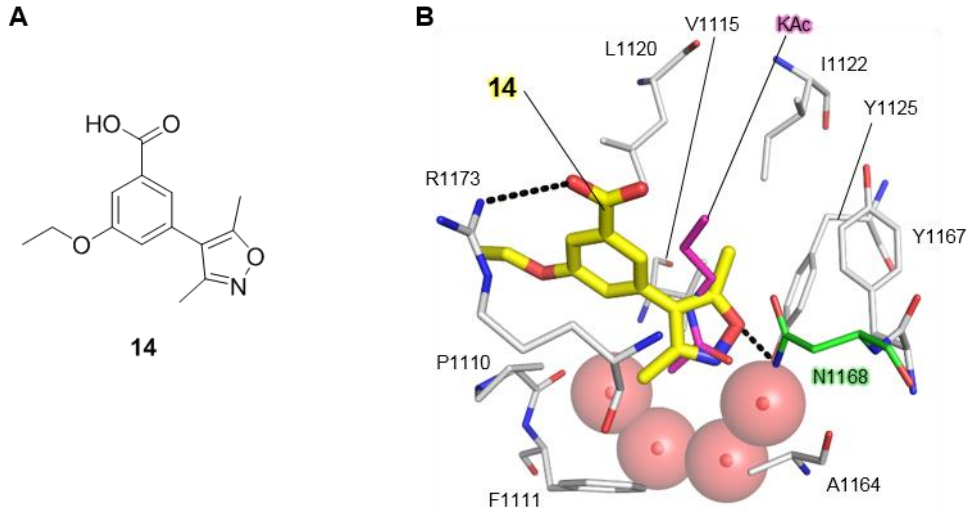


Figure 1.14: **A**: A DMI based bromodomain inhibitor. **B**: Overlay of X-ray crystal structure of **14** bound to CREBBP (PDB ID 3SVH) with X-ray crystal structure of KAc bound to CREBBP (PDB ID 3P1C). The DMI core bound in the KAc pocket and formed a hydrogen bond to N1168 while the carboxylic acid formed a long range interaction (5.3 Å) with R1173.

Benzimidazole substituted DMIs, such as **15**, have also been identified as promiscuous BET and CREBBP bromodomain inhibitors (Figure 1.15A).[99] An extensive optimisation of this fragment has recently resulted in the development of SGC-CBP30 **16**, which has a K_D of 21 nM for CREBBP and 40-fold selectivity over BRD4(1).[100] Throughout the development of **16**, selectivity over BRD4(1) remained an issue and it was only through numerous substitutions at the N(1) and C(2) positions that the final compound was derived. A morpholine group at N(1) was deemed optimum, while a stereogenic methyl group in the linker was integral to the eventual selectivity. Aryl substituents at C(2) were screened and electron rich analogues found to be favoured. A co-crystal structure of the final compound **16** with CREBBP revealed that the DMI bound as expected in the KAc pocket (Figure 1.15B). The morpholine group was directed into the ZA channel, while the aryl substituent formed a cation-π interaction with R1173, consistent with findings that were first reported in this project.[101] Using a fluorescence recovery after photobleaching (FRAP) assay, **16** was shown to displace CREBBP from chromatin, and in a luciferase reporter assay was shown to inhibit the CREBBP co-activation of p53 target genes.

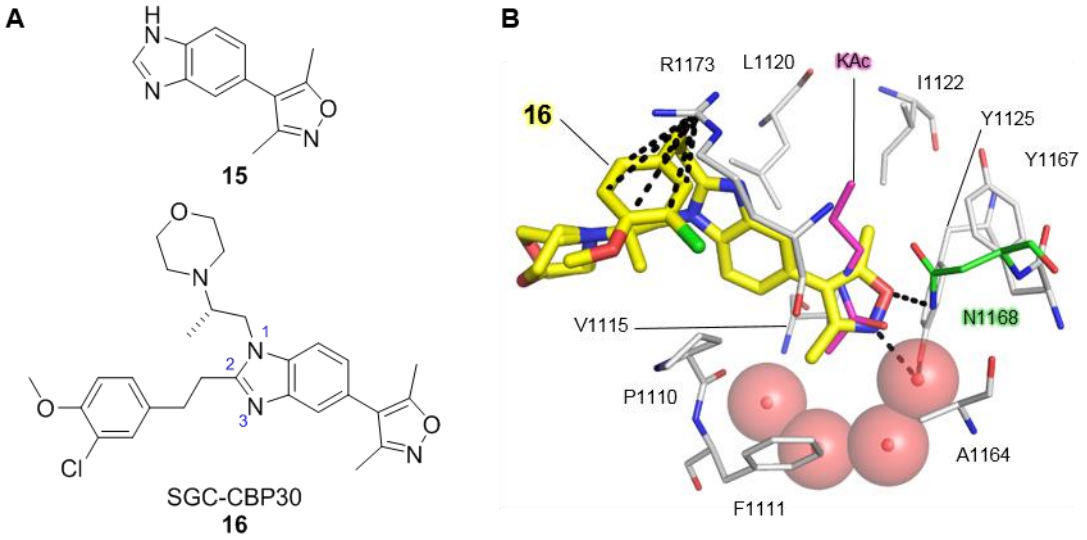


Figure 1.15: **A**: Development of DMIs as CREBBP bromodomain inhibitors. **B**: Overlay of X-ray crystal structure of **16** bound to CREBBP (PDB ID 4NR7) with X-ray crystal structure of KAc bound to CREBBP (PDB ID 3P1C). The DMI core bound in the KAc pocket while an aryl-group at C(2) formed a cation-π interaction with R1173.

1.4.6 Triazoles

Methyltriazolodiazepines were the first potent inhibitors of the BET bromodomains to be reported, catalysing the intense interest in bromodomain inhibitors seen in recent years.[80,81] While they have generally shown high selectivity for the BET family, the triazole moiety has also been modified to bind other bromodomains as part of a triazolopyridazine or triazolophthalazine scaffold. The triazolopyridazine scaffold, also reported in a patent by Constellation and as a BRD9 inhibitor,[102,103] was developed by the SGC into a pan-bromodomain inhibitor bromosporine **17** (Figure 1.16A).[104] Comparable bromodomain binding affinities of **17** are yet to be reported, however, inhibition of CREBBP at 1 μM has been demonstrated in a cell based FRAP assay. In a separate study, derivatives of the triazolophthalazine scaffold were designed to improve CREBBP selectively, for example, by targeting an interaction with R1173, but were met with limited success.[105] Ultimately, a number of analogues that bind to CREBBP, BRD4(1) and BRD9 in the nanomolar range were identified, of which **18** had the highest affinity for CREBBP (IC_{50} of 200 nM), although a co-crystal structure in CREBBP was not reported (Figure 1.16).

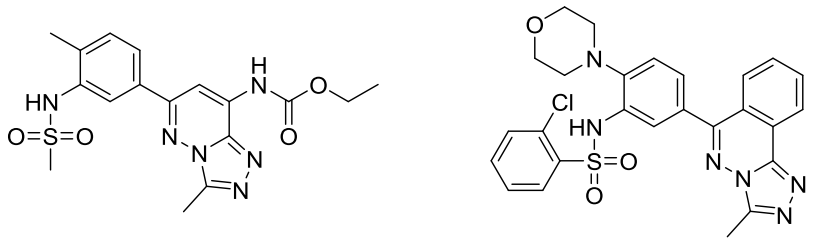


Figure 1.16: Triazole based inhibitors of the CREBBP bromodomain. Bromosporine **17** is a broad spectrum bromodomain inhibitor that was active in a cell based assay against CREBBP. Compound **18** also bound to numerous bromodomains, including CREBBP, with sub-micromolar affinity.

1.4.7 Benzoxazepines

Recently, the SGC and GSK developed a benzoxazepine based CREBBP inhibitor, I-CBP112 **19**, which binds to the bromodomains of CREBBP and EP300 with K_D values of 151 nM and 625 nM respectively (Figure 1.17A).[106] Selectivity over other bromodomains appeared satisfactory and the compound was active in a FRAP assay demonstrating an on target effect in cells. Although the design of **19** has yet to be published, a structure is available in the PDB which highlighted a number of interesting features (Figure 1.17B). The benzoxazepine core bound across the entrance to the KAc pocket, while the acyl group attached to N(4) acted as the acetyl lysine mimic forming the typical direct hydrogen bond to N1168 and water mediated hydrogen bond to Y1125. The aryl group at C(7) formed a cation-π interaction to R1173 and the two methoxy substituents suggest that this interaction was optimised during the design process. A unique feature of **19** is the presence of a propionate rather than acetate group in the KAc mimic. The longer ethyl group extended towards, but did not displace, the conserved water molecules at the base of the KAc pocket. While this deeper occupancy was not seen in any other bromodomain ligand to date, most notably propionylated lysine,[107] it is possible that the cation-π interaction that **19** forms to the BC loop makes this deeper occupancy of the KAc pocket possible.

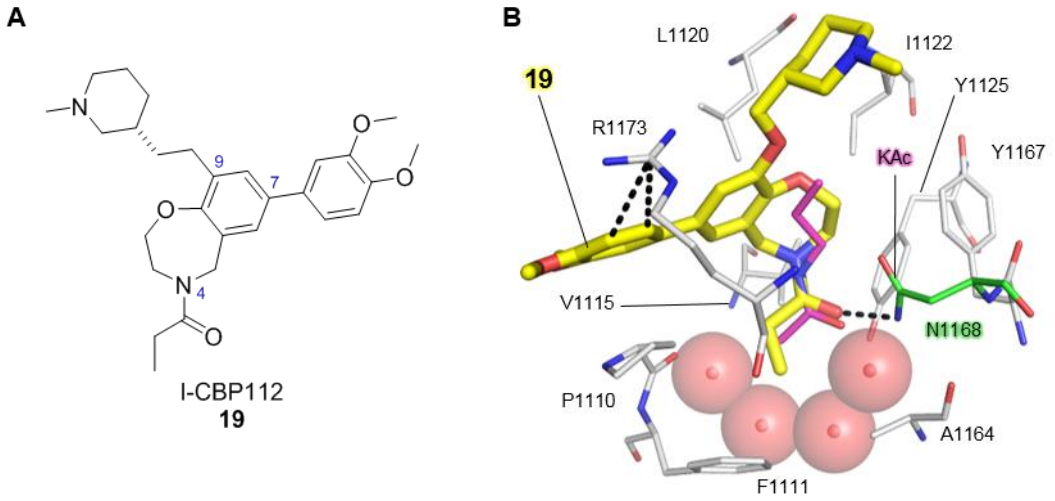
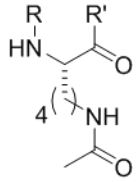
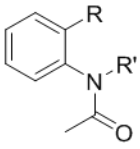
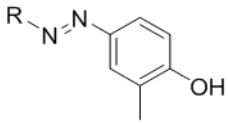
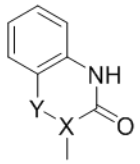
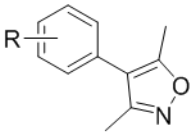
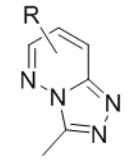
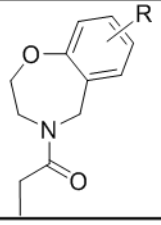


Figure 1.17: **A**: I-CBP112 **19**, a benzoxazepine based CREBBP bromodomain inhibitor. **B**: Overlay of the X-ray crystal structure of **19** bound to CREBBP (PDB ID 4NR6) with X-ray crystal structure of KAc bound to CREBBP (PDB ID 3P1C). The acetyl group at C(4) formed a hydrogen bond to N1168 while an aryl-group at C(2) formed a T-shaped cation-π interaction with R1173.

1.4.8 Summary

The number of reported inhibitors of CREBBP has expanded rapidly in recent years, from fragments with micromolar affinity in 2007 to a number of distinct chemical scaffolds with nanomolar affinity and proven on-target effect in cells within the last year (Table 1.1). Since the majority of these compounds have only recently been reported, relatively few biological studies have been conducted. During its development the CREBBP inhibitor ischemin **9** was shown to prevent apoptosis in ischemic cardiomyocytes, offering a potential therapeutic application.[93] Very recently, I-CBP112 **19** was used to demonstrate a clear relationship between the HAT and bromodomains of CREBBP, since inhibition of the bromodomain caused a drop in $H3K_{56}$ (but not $H3K_{27}$) acetylation.[108] The precedent set by (+)-JQ1 suggests that further biological studies will not be far off.

Table 1.1: Summary of CREBBP bromodomain inhibitors.

Structure	Example compound	CREBBP activity	CREBBP structure	Reference
	**4**	IC_{50} = 8.0 μM (*Fluorescence polarisation*)	NA	Gerona-Navarro *et al.*[87]
	**5** (MS2129)	NA	NA	Sachchidanand *et al.*[88]
	6 (MS7179)	K_D = 20 μM (*Fluorescence polarisation*)	2D82 (*NMR*)	Sachchidanand *et al.*[88]
	**9** (ischemin)	K_D = 19 μM (*Tryptophan fluorescence*)	2L84 (*X-ray*)	Borah *et al.*[93]
	**11** (NMP)	IC_{50} = 1.9 mM (*AlphaScreen*)	3P1D (*X-ray*)	Philpott *et al.*[95]
	(*R*)-**156**	K_D = 391 nM (*ITC*)	4NYX (*X-ray*)	Rooney *et al.*[101]
	**14**	IC_{50} = 32 μM (*AlphaScreen*)	3SVH (*X-ray*)	Hewings *et al.*[97]
	15	IC_{50} = 4.0 μM (*AlphaScreen*)	NA	Hay *et al.*[99]
	16 (SGC-CBP30)	K_D = 21 nM (*ITC*)	4NR7 (*X-ray*)	Hay *et al.*[100]
	**17** (bromosporine)	NA	NA	SGC[104]
	18	IC_{50} = 200 nM (*AlphaScreen*)	NA	Fedorov *et al.*[105]
	**19** (I-CBP112)	K_D = 151 nM (ITC)	4NR6 (*X-ray*)	SGC[106]

1.5 Project Aims

At the start of this project the published record of bromodomain inhibitors was limited to fragment hits against PCAF and CREBBP described by the group of Ming-Ming Zhou.[78,88] The BET bromodomain inhibitor (+)-JQ1 had just been characterised by co-workers at the SGC, demonstrating that potent and selective ligands could be developed to inhibit the bromodomain-histone interaction *in vivo*. However, to further validate BRDs as therapeutic targets and to expand to tools available for probing histone modifications, potent inhibitors of other bromodomains were required.

CREBBP was chosen as the primary target of this project, due in part to the availability of structural information but also with the aim of providing a tool to further investigate some of the diverse biology that had been previously described (Section 1.3.2). The desired compound would have sub-micromolar affinity *in vitro* and show activity against the desired target in a cellular setting. Good selectivity over other bromodomain families was also desired. Due to their structural similarity around the binding site, design of a bromodomain inhibitor with selectivity for CREBBP over EP300 was not considered possible. However, with a tool in hand it may well be possible to investigate the differential functions of the two proteins by using a combination of domain inhibitors or by careful choice of cellular system. Finally, any information about key binding interactions could be fed into ligand discovery efforts of other chemical series and to aid in the development of probes for other bromodomain targets.

Chapter 2

Investigation of benzoxazinones as bromodomain inhibitors

This chapter describes the identification and investigation of cyclic amide-containing structures as bromodomain inhibitors. The result of an initial fragment screen will be considered, along with the structural data generated in relation these compounds. The development of a novel acetyl-lysine mimic will be outlined, followed by a computational screen and parallel chemistry experiments designed to elaborate the fragment. Throughout, analysis of the biological activity of the resulting compounds will be provided.

2.1 Dihydroquinazolinones as bromodomain inhibitors

2.1.1 Identification of cyclic amide fragments as acetyl lysine mimics

Work by Philpott *et al.* on the development of assays to measure the binding affinities of acetylated histone peptides for bromodomain proteins found that the solvents dimethylsulfoxide (DMSO) and 1-methyl-2-pyrrolidinone (NMP) **11** bind to some bromodomains in a concentration dependent manner (Table 2.1, Figure 2.1A).[95] Co-crystal structures were obtained confirming that these solvents bound to the KAc-binding pocket of bromodomains (Figure 2.1). This result prompted the screen of a series of NMP analogues, which identified that bicyclic amide compounds based on the dihydroquinazolinone (DHQ) core, such as 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one **20** and its brominated analogue **12**, are ligand efficient inhibitors of a number of bromodomains (Table 2.1).

Table 2.1: A selection of solvent and early fragment inhibitors of the CREBBP and BRD4 bromodomains. Shown is the compound IC_{50} (AlphaScreen), ligand efficiency (LE), and PDB code of a crystal structure where available.

Name	Number	CREBBP			BRD4		
		IC_{50} (mM)	LE	PDB	IC_{50} (mM)	LE	PDB
KAc	-	1.2[a]	0.41	3P1C	7.2[a]	0.30	NA
DMSO	-	39.5[a]	0.39	3P1E	281.0[a]	0.15	4IOR[c]
NMP	**11**	1.9[a]	0.54	3P1D	6.0[a]	0.43	4IOQ[c]
DHQ	**20**	0.029	0.53	3P1F	0.039[b]	0.51	4DON[c]
6-Br-DHQ	**21**	0.019	0.55	4NYV	0.023[b]	0.54	4HBV

[a] Data from Philpott[95] *et al.* [b] Data from Fish[96] *et al*. [c] Not available at the start of this project.

An X-ray crystal structure of **20** bound to CREBBP confirmed that this fragment was binding, as expected, in the KAc pocket of the bromodomain (Figure 2.1C). Synergistic hydrogen bonds, between the amide of DHQ and the conserved asparagine residue, anchored the fragment in a position such that the *N*-methyl group occupied a similar position to the terminal methyl group

in KAc, and the aromatic group projected into the opening of the pocket. This result provided an ideal opportunity to investigate whether more productive substitutions could be made to the aromatic ring of **20** in order to develop this fragment into a potent bromodomain inhibitor.

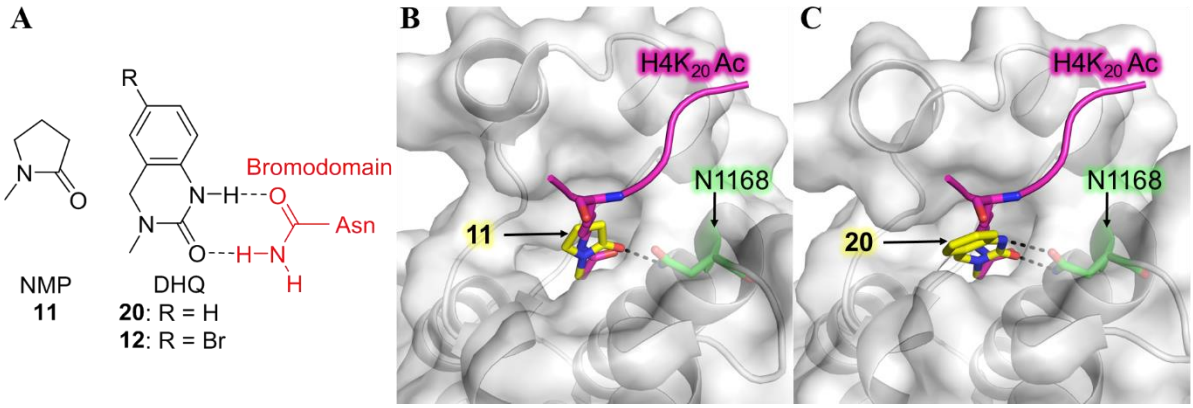


Figure 2.1: **A**: Cyclic amide bromodomain inhibitors. **B**: Crystal structure of NMP **11** (yellow) bound to CREBBP (PDB ID: 3P1D) overlaid with crystal structure of $H4_{13\text{-}27}K_{20}Ac$ bound to CREBBP (4N3W). The hydrogen bond to N1168 is highlighted. **C**: Crystal structure of DHQ **20** bound to CREBBP (PDB ID: 4NYV) overlaid with crystal structure of $H4_{13\text{-}27}K_{20}Ac$ bound to CREBBP (4N3W). The double hydrogen bond to N1168 is highlighted.

2.1.2 Limitations of the dihydroquinazolinone core

Despite these promising results, the synthesis of DHQ analogues met with limited success.[109] Metal-catalysed coupling reactions with the aryl-bromide **12** were unsuccessful due to oxidation at the benzylic position. Confirmation of this behaviour was obtained when the DHQ analogue **21**, which also contained a 1,3-dimethylisoxazole group (DMI), was crystallised with BRD4. As well as demonstrating for the first time that the DMI group acts as an KAc mimic, the structure showed an unexpected area of electron density at the benzylic position of the DHQ ring (Figure 2.2A).[110] The ligand was resolved as compound **23**, indicating that substitution of one hydrogen atom by an ethylene glycol group had occurred (Figure 2.2B). This reaction was determined to have proceeded *via* oxidation of **21** to the iminium ion **22** during storage in DMSO, followed by addition of ethylene glycol, which was present in the crystallisation buffer (Figure 2.2B).

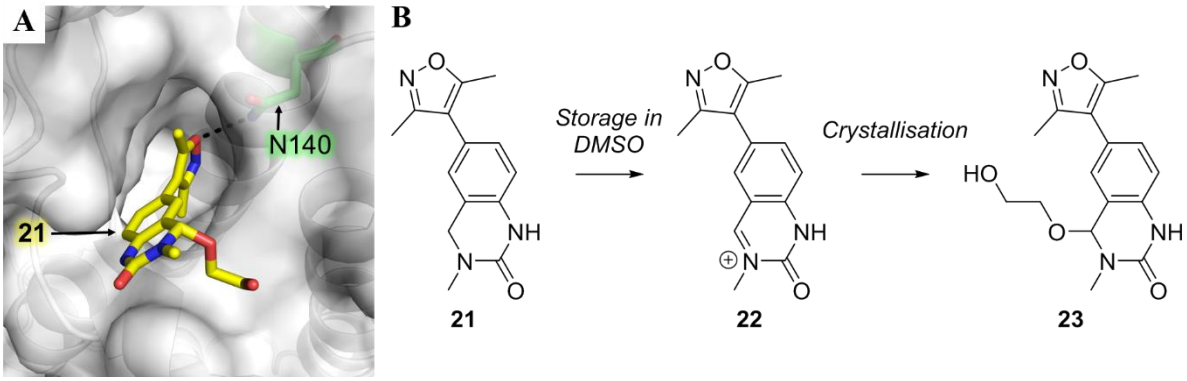


Figure 2.2: **A**: Structure of DHQ containing DMI **21** bound to BRD4 (PDB ID: 3SVF).[110] **B**: Proposed mechanism for oxidation of DHQ core.

The DHQ core was subsequently developed by Fish *et al.* into PFI-1 **13** (Figure 1.13), a potent and selective BET-family bromodomain inhibitor, although the susceptibility to oxidation was also noted in this study.[96] Due to this observed instability, further work on the DHQ core was not pursued in the course of this project.

2.2 Benzoxazinones as acetyl lysine mimics

2.2.1 Rationale behind the change to a benzoxazinone core

Whilst the ligand efficiency and structural information available for the DHQ **20** made this fragment an ideal starting point for ligand development, the susceptibility to oxidation necessitated a means to prevent this instability. Substitution of the benzylic C(4) with oxygen and the N(3) with carbon results in the 1,4-benzoxazin-3(4*H*)-one (BNZ) **24** core structure (Figure 2.3A), which is less susceptible to oxidation.

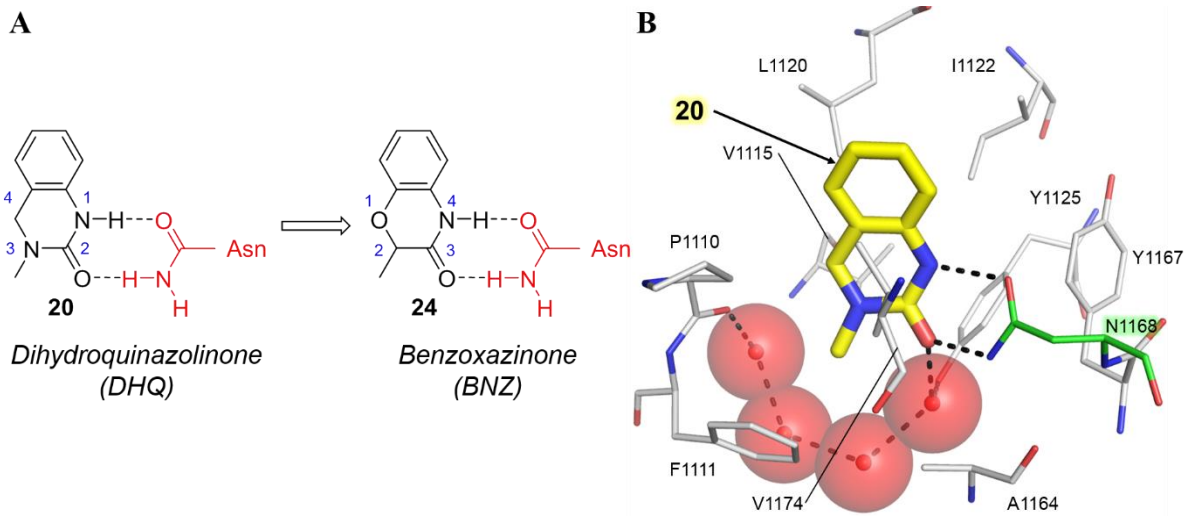


Figure 2.3: **A**: Proposed change to a BNZ core. **B**: Crystal structure of DHQ **20** bound to CREBBP (PDB ID: 3PIC). The double hydrogen bond to N1168 and water mediated hydrogen bond to Y1125 are highlighted.

Analysis of the crystal structure of the DHQ **20** bound to the CREBBP bromodomain suggested that a BNZ ligand would bind in much the same way, maintaining the synergistic hydrogen bonds to the asparagine residue and projecting aromatic substitutions out of the pocket (Figure 2.3B). In addition, installation of a methyl group at the C(2) position (equivalent to N(3) in DHQ) would introduce a stereogenic centre to a position that is bound deep within the KAc pocket. All other KAc mimics are planar, so incorporation of a greater degree of 3D shape would be interesting to explore and may be useful for gaining selectivity over other bromodomains. It may also be of benefit since, when inversion of a stereogenic centre results

in a significant drop in affinity, the inactive enantiomer can be used as a negative control in biological studies, as exemplified in the case of (−)- and (+)-JQ1.[80]

2.2.2 Biologically active benzoxazinones

BNZs have frequently been studied as, and developed into, biologically active compounds; primarily as phytotoxic, antimicrobial and antifungal agents but also as pharmacologically active compounds (Figure 2.4).[111] For example, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one **25** (DIMBOA) is produced by maize and wheat and is thought to play an allelopathic role by inhibiting growth of the plant pathogen *Agrobacterium tumifaciens*.[112,113] In the area of human pharmacology, compounds based on a 6-(2,4-diaminopyrimidinyl)-1,4-benzoxazin-3-one template **26** have been targeted as renin inhibitors to modulate the renin-angiotension system (RAS) control of blood pressure.[114] This series was optimised to have a favourable pharmacokinetic profile but not carried forward to *in vivo* studies. Analogues of 5-hydroxy-1,4-benzoxazin-3-one were developed into β_2 adrenergic receptor (B2AR) agonists as long-lasting bronchodilating agents, but failed to show improved therapeutic potential in an animal model.[115] Interestingly, compound **27** was later crystallised in human B2AR to provide the first structure of a ligand bound G-protein coupled receptor (GPCR) in its

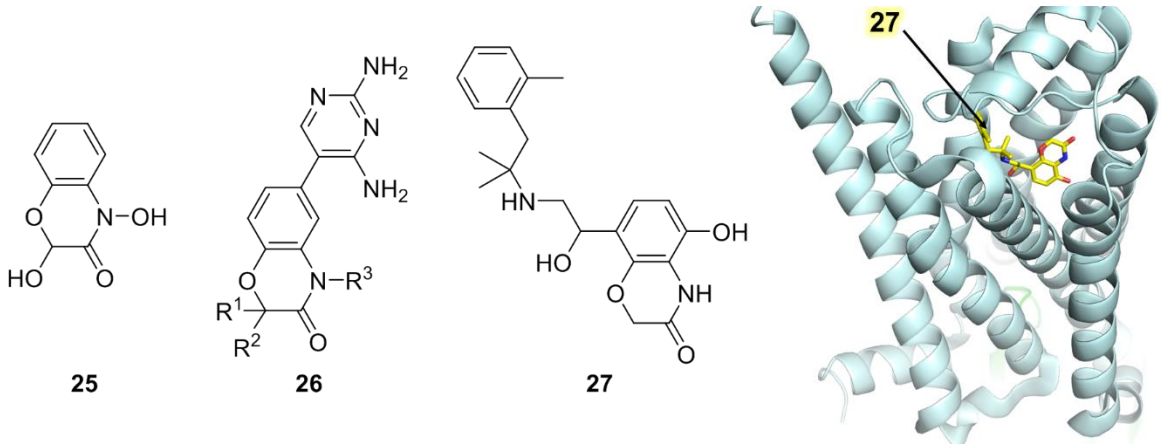


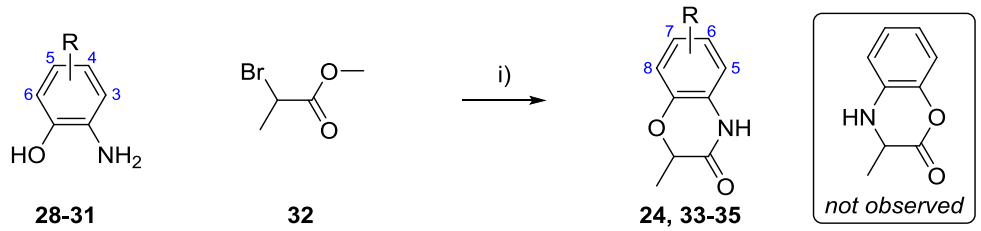
Figure 2.4: Biologically active BNZ-based compounds. **25** is an allelochemical produced by maize and wheat.[111] Analogues of **26** were developed as renin inhibitors.[114] **27** was developed as a B2AR agonist, and is shown crystallised with the GPCR in its active form (PDB ID: 3P0G).[116]

active form, which has helped provide invaluable information on this protein class as a drug target.[116,117] Overall, the presence of a BNZ scaffold in compounds that have been optimised against various biological targets supports the use of this moiety as a chemical probe.

2.2.3 Synthesis of benzoxazinone fragments

The synthesis of BNZs has been reported in the literature in a convenient one-step process from amino-phenols and α-bromoesters.[118] Under basic conditions, alkylation of the phenol group is favoured, followed by concomitant acylation of the amino group to afford the cyclised 1,4-benzoxazin-3(4*H*)-one **24** as the major regioisomer (as confirmed by NMR shift of C(3)*H*). Microwave irradiation was preferable, although thermal heating was also possible with longer reaction times. Treatment of 2-aminophenol **28** with methyl bromopropionate **32** and DBU afforded the desired BNZ **24** in 79% yield (Scheme 2.1). The same conditions allowed synthesis of a selection of BNZ **33-35** with substitution at either the 7- or 8-position. Reasonable yields were obtained in all cases (Table 2.2) and the other regioisomer was not observed.

Scheme 2.1, Table 2.2: Synthesis of BNZ fragments. *Reagents and conditions*: i) DBU, DMF, 110 °C, 33-79%.



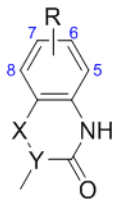
28-31 **32** **24, 33-35**

Amino phenol	BNZ	R	Conditions	Time (h)	Yield
28	**24**	H	MW	1.5	79%
28	**24**	H	Thermal	18	33%
29	**33**	7-Br	MW	1	51%
30	**34**	8-Br	MW	1	78%
30	**34**	8-Br	Thermal	24	77%
31	**35**	8-CO_2Me	Thermal	16	67%

2.2.4 Biological evaluation of benzoxazinone fragments

With a selection of BNZs in hand, their propensity to act as KAc mimics and inhibit CREBBP was investigated. An AlphaScreen assay was employed to evaluate the competitive displacement of a histone peptide ($H3_{46\text{-}66}K_{56}Ac$) from the CREBBP bromodomain by compounds **24**, **33-35** (Table 2.3). The results indicate that compared to the original fragment hit DHQ **20**, the change to a BNZ core resulted in a 3.6-fold drop in activity. Part of the reduction in activity might be attributed to the introduction of a stereogenic centre and only one enantiomer being active. Substitution at the 8-position, **34** and **35**, was tolerated, however, a bromide at the 7-position **33** caused a large drop in activity. This result was surprising considering the tolerance of the same substitution on a DHQ core (**12**, Table 2.1), but could be due to a combination of two inductively electron-withdrawing groups (O and Br) altering the electronics of the amide. Nevertheless compound **24**, as a new micromolar inhibitor of CREBBP with a LE of 0.46, indeed proved that the BNZ template was a suitable fragment to build upon to develop bromodomain inhibitors.

Table 2.3: IC_{50} values obtained by AlphaScreen for BNZ fragments against CREBBP. 95% confidence intervals are shown in parentheses.



Compound	X	Y	R	IC_{50} (μM)
20	CH_2	N	H	29.4 (25.5-33.8)
24	O	CH	H	107.1 (93.1-123.3)
35	O	CH	8-CO_2Me	222.2 (158.7-311.1)
34	O	CH	8-Br	396.2 (198.4-791.0)
33	O	CH	7-Br	7452.1 (very wide)

2.3 Derivatisation of the benzoxazinone core

Having established the BNZ scaffold as a ligand efficient KAc mimic, attention turned to the identification of substitutions that would improve potency and selectivity for the CREBBP bromodomain. In order to achieve this, a virtual screening and parallel chemistry approach was employed.

2.3.1 Structural analysis of the CREBBP bromodomain

Although no crystal structure of a BNZ bound to any bromodomain was available, the binding mode could be envisaged to be similar to that of a DHQ (Figure 2.3B). Analysis of the binding of the brominated DHQ **12** was performed since structures of this compound bound to both CREBBP and BRD4(1) were available at this time in the project (Figure 2.5).

Despite the fact that the bromodomains of CREBBP and the BET family have low sequence homology (30% for CREBBP vs BRD4(1)), the ZA and BC loop regions that define the receptor pocket have a relatively similar overall structure (Figure 2.5A).[32] This similarity presents a challenge when designing ligands that are selective between the two and is reflected in the activity of many BET inhibitors against CREBBP. For example, in both a series of DMI and a series of triazolophthalazine ligands,[105,110] the CREBBP and BRD4(1) activities were difficult to separate. An analysis of structures of both CREBBP and BRD4(1) bound to brominated DHQ **12** demonstrated why this is the case (Figure 2.5B).

Of the 14 residues that were identified to be in close proximity to the ligand, six (43%) were identical and only three (21%) showed a difference more substantial than addition of a CH_3 group (Figure 2.5B). Of particular interest was a leucine residue (L1109) of CREBBP in place of a much larger tryptophan residue (W40) in BRD4(1). A common feature of BET-family ligands is the formation of close contacts around the tryptophan residue (within the WPF shelf region),[119] whereas in CREBBP there is a more open surface. By directing substitutions towards

this region it was envisaged that some would bind tightly in CREBBP but clash with the bulky tryptophan residue in BRD4, thereby contributing to the selectivity for CREBBP.

An additional residue of interest was an arginine residue (R1173) in CREBBP in place of a glutamic acid (D140) residue in BRD4(1) at the edge of the KAc binding-pocket. At physiological pH, the guanidine in CREBBP is positively charged whereas the carboxylic acid in BRD4 has a complete negative charge. Furthermore, analysis of the equivalent residues identified by Vidler *et al.*,[32] showed that only 5 of the 61 bromodomains analysed contain a positively-charged residue in this position (Figure 2.5C). Forming interactions with this arginine could again be expected to confer some selectivity for CREBBP.

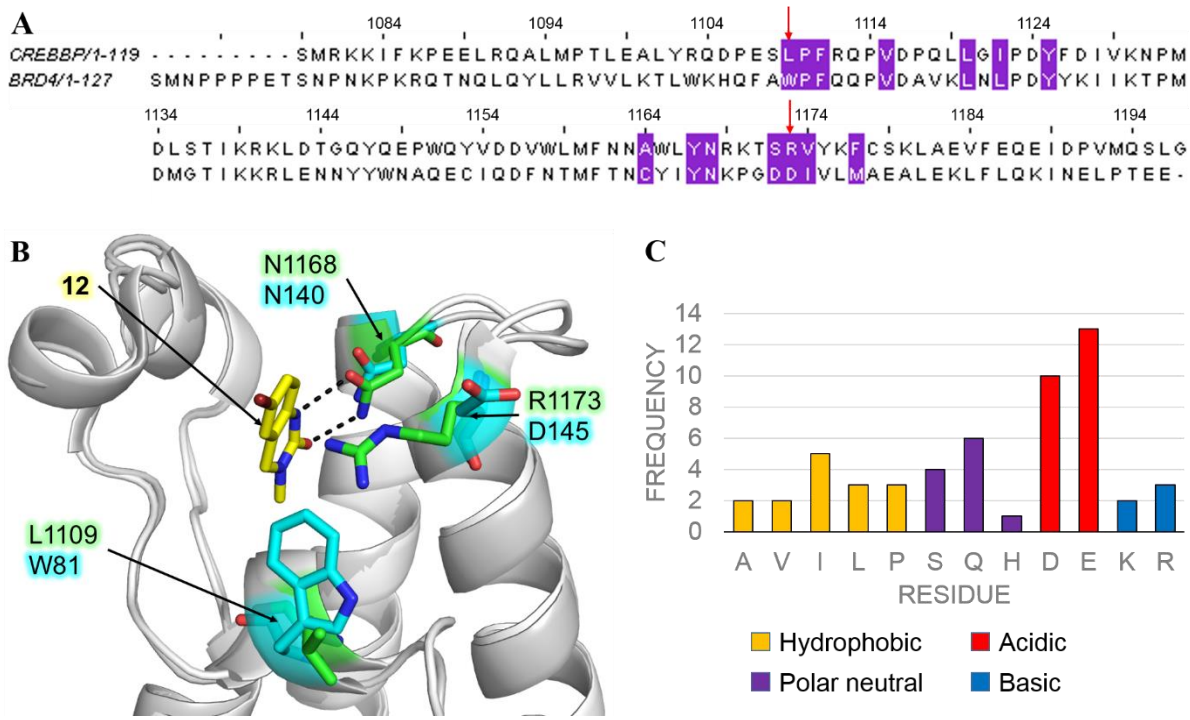


Figure 2.5: **A**: Sequence alignment of CREBBP and BRD4. Highlighted are the 14 identified binding site residues. **B**: Overlaid X-ray structures of DHQ **12** bound to CREBBP (green, PDB ID: 4NYV) and BRD4(1) (cyan, PDB ID 4HBV). Highlighted are the asparagine in the KAc pocket, and two residues that show the greatest difference in size or charge **C**: Comparison of residues across the bromodomain family in an equivalent position to R1173 of CREBBP.

2.3.2 Computational screening of proposed benzoxazinone analogues

Analysis of the crystal structure of DHQ **12** bound to CREBBP predicted that substitutions at the 7- and 8-position of a BNZ would be directed into the ZA channel and towards the aforementioned residues of interest (Figure 2.6A). In addition, it was noted that amide substitutions at the 8-position could form an internal hydrogen bond between the O of the BNZ and the NH of the exocyclic amide (Figure 2.6B). By directing the amide substituent along a vector that interacts with the surface of the protein, this internal hydrogen bond could improve ligand potency by reducing the entropic cost of binding.

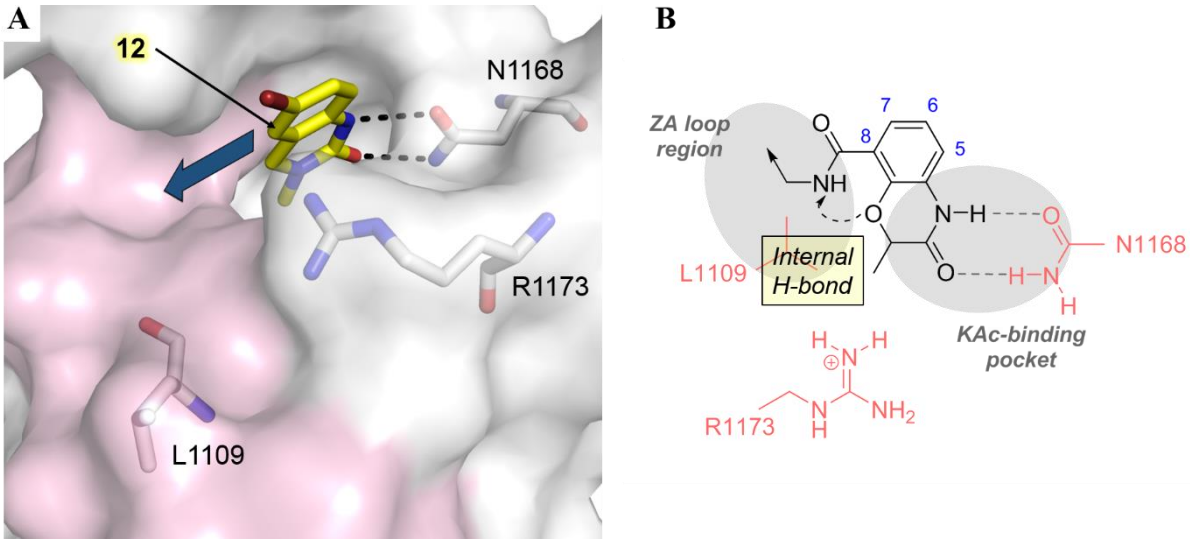


Figure 2.6: **A**: Crystal structure of DHQ **12** bound to CREBBP (PDB ID: 3P1F). The ZA loop region, towards which 7- and 8-position substituents are directed, is highlighted pink. **B**: Amide substituents at the 8-position form an internal hydrogen bond to the BNZ core and are directed towards the ZA loop.

Anticipating that a focused library of amides could be synthesised *via* coupling of the BNZ core to a diverse set of amines, a docking study was conducted to rank the compounds that could be formed in this way according to their estimated binding affinity. Commercially available amines from three suppliers (taken from the SGC vendor collection) were coupled to carboxylic acid **36** *in silico*. The resulting set was expanded to include all possible stereoisomers and tautomers giving a library of 12 358 compounds. This library was docked into the crystal structure of CREBBP bound to **12** using the crystallised ligand as a template to anchor the BNZ

core in the appropriate pocket. A binding score was then calculated for each entry using the inbuilt scoring function in ICM-Pro,[120] which takes into account, among other factors, energies associated with formed hydrogen bonds, hydrophobic interactions and desolvation of the ligand. Although the binding score calculated by docking programs cannot be reliably be used to predict the actual binding affinity of each compound, it does serve as a good measure to rank a library of compounds.[121] This *in silico* process is summarised in Figure 2.7.

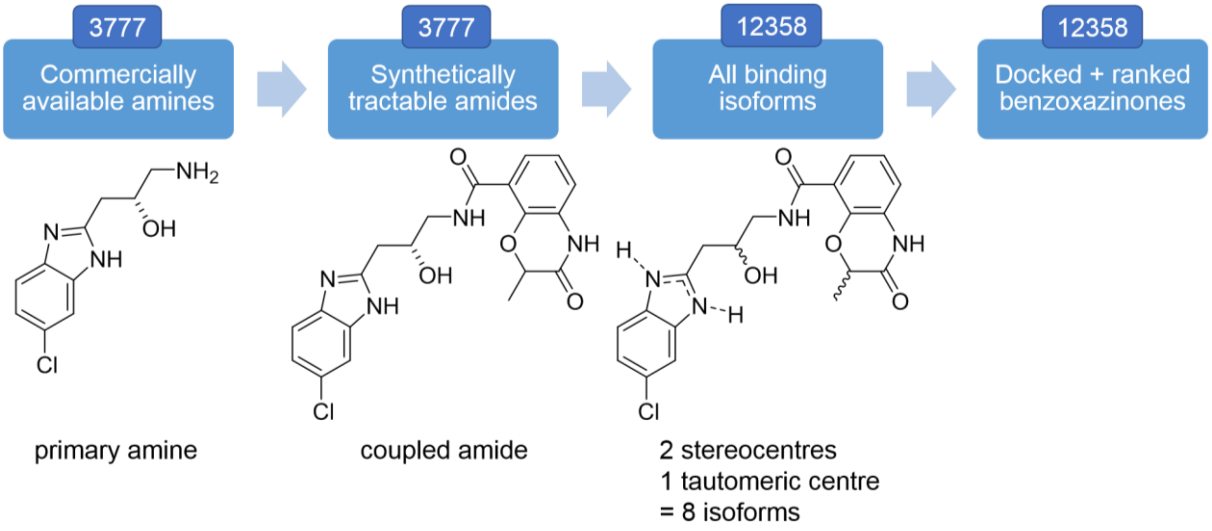


Figure 2.7: Workflow for the generation and analysis of a library of BNZ analogues. A collection of commercially available amines was assembled, attached to the BNZ core, expanded to include all stereoisomers and tautomers and then docked into the structure of CREBBP.

As predicted in the design of the compounds, the majority of amide side groups of the docked structures bound in the ZA channel (Figure 2.8A). This result was encouraging considering the differences between residues in this region compared to the BET-family bromodomains. It was also interesting to observe that binding of the (*R*)-enantiomer of the BNZ was predicted to be favoured (Figure 2.8B). Of the 3756 compounds with an achiral side-group, the (*R*)-BNZ bound with an average score of −29.6 kcal/mol whereas the (*S*)-BNZ had an average score of −22.0 kcal/mol.

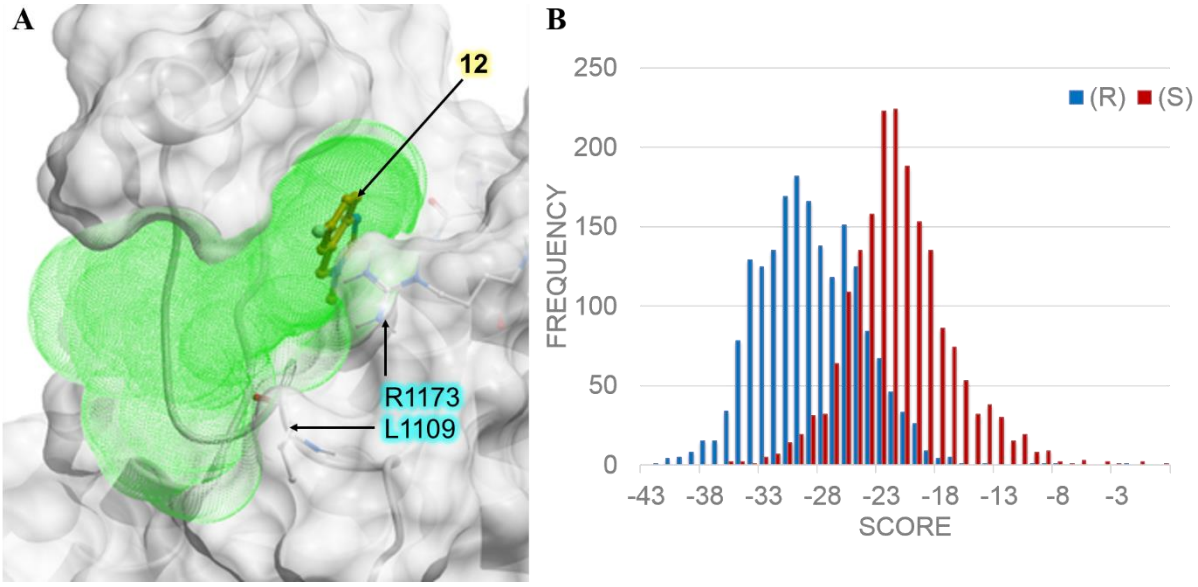
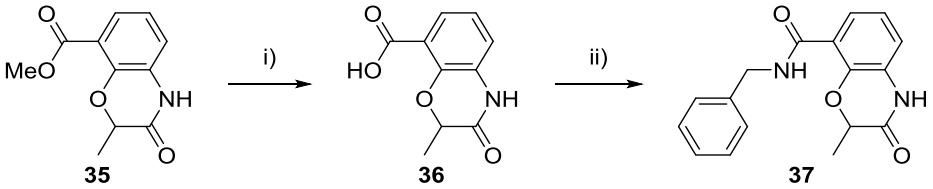


Figure 2.8: **A**: Predicted occupancy of the ZA loop of CREBBP by docked BNZs substituted at the 8-position (green dot representation). **B**: Histogram of docking scores for each enantiomer of 1878 BNZ.

2.3.3 Synthesis of benzoxazinone analogues

With docking studies confirming the suitability of a number of BNZ targets, attention turned to their synthesis. The key carboxylic acid **36** was synthesised *via* base-mediated hydrolysis of the previously prepared methyl ester **35** (Scheme 2.1 and Scheme 2.2). Notably, in its neutral form, **36** was insoluble in most solvents immiscible with water, so the work-up consisted of a straightforward filtration, followed by a wash with ethyl acetate. Next, coupling conditions were sought that would provide the desired compounds in good yields over a single step, with by-products that could be removed *via* an aqueous work-up. 1-*H*-Benzotriazole-based coupling reagents had the potential to satisfy these criteria since excess coupling reagent and by-products could be removed during work-up. Therefore, the reaction of **36** with benzylamine was investigated using HBTU as the coupling reagent, which afforded the desired product **37** in 95% yield and adequate purity after only an aqueous work-up (Scheme 2.2).

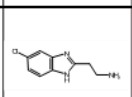
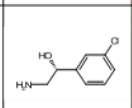
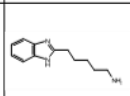
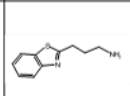
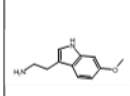
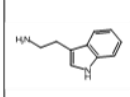
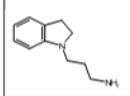
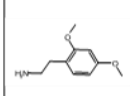
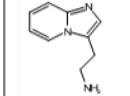
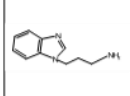
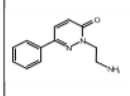
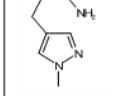
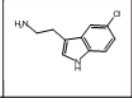


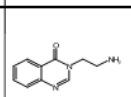
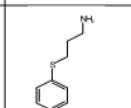
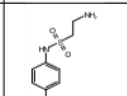
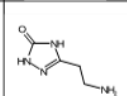
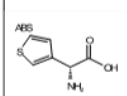
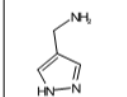
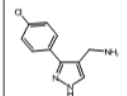
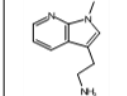
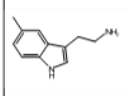
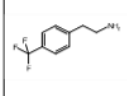
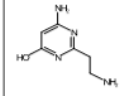
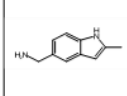
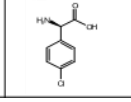
Scheme 2.2: *Reagents and conditions*: i) LiOH (aq.), MeOH, THF, rt, 91%; ii) Benzylamine, HBTU, DIPEA, DMF, rt, 95%.

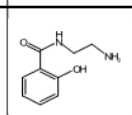
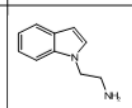
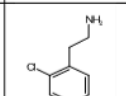
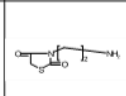
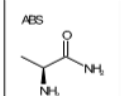
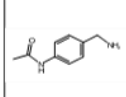
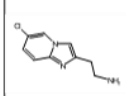
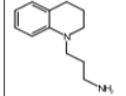
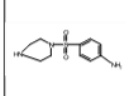
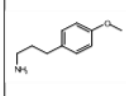
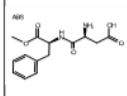
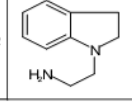
Having established suitable conditions for the coupling reaction, the synthesis of a focused library of analogues was pursued. With the aid of Andrew Cook and Abid Masood at Pfizer, the top binding compounds from the *in silico* study were screened against the Pfizer in-house collection of compounds to identify 50 available amines on which to perform parallel chemistry. These amines were coupled to carboxylic acid **36** in a plate format using the HBTU-mediated conditions established previously (Table 2.4).

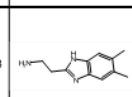
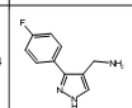
Overall, 58% of reactions were completed successfully with the principle reason for failure of the other reactions being the insolubility of the product during work-up. Analysis of the crude reaction mixtures by LC-MS indicated that 16 of the 29 reactions had afforded a product with the desired mass in a purity of greater than 90% by ELSD, which was deemed pure enough for testing *in vitro*. An overall success rate of 32% for obtaining compounds *via* parallel chemistry is lower than could be expected. On reflection, an analysis of the predicted solubilities of the products prior to performing this chemistry, or using a more adaptable work-up procedure, could have improved the success rate of the reaction. Deliberately targeting compounds with an internal hydrogen bond and therefore fewer rotatable bonds may have been a confounding factor. In addition, had more time been available whilst at Pfizer it would have been possible to carry out HPLC purification of all of the products. Nevertheless, 16 compounds were carried forward for analysis of CREBBP inhibition.

Table 2.4: Synthesis of a library of compounds *via* an amide coupling reaction performed in parallel. Shown are the amines to which coupling to **36** was performed, according to the protocol in Scheme 2.2. Also indicated is whether the work-up (WU) was successful and the purity (P) of the amide product as determined by LC-MS with ELSD detection. Highlighted compounds were carried forward for analysis (Section 2.3.4).

Prod	Well	Amine	WU	P
38	A1		N	69
39	A2		Y	100
40	A3		N	100
41	A4		Y	91
42	A5		Y	92
43	A6		Y	85
44	B1		Y	91
45	B2		Y	100
46	B3		Y	20
47	B4		Y	77
48	B5		Y	87
49	B6		Y	66
50	C1		N	100

Prod	Well	Amine	WU	P
51	C2		N	67
52	C3		Y	87
53	C4		N	100
54	C5		Y	ND
55	C6		Y	56
56	D1		Y	31
57	D2		N	100
58	D3		Y	90
59	D4		Y	100
60	D5		Y	90
61	D6		N	ND
62	E1		N	ND
63	E2		N	52

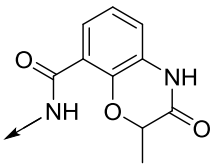
Prod	Well	Amine	WU	P
64	E3		Y	100
65	E4		Y	100
66	E5		Y	100
67	E6		N	100
68	F1		N	ND
69	F2		N	17
70	F3		N	80
71	F4		Y	94
72	F5		Y	89
73	F6		Y	91
74	G1		N	ND
75	G2		Y	100

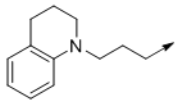
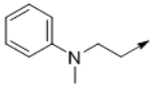
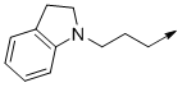
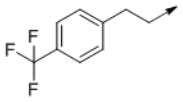
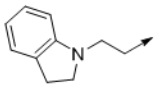
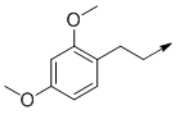
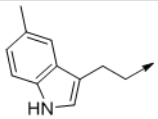
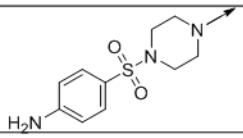
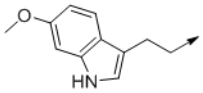
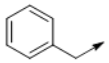
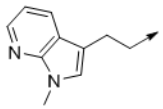
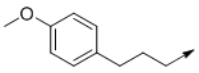
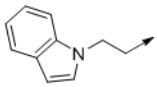
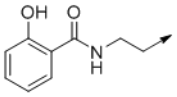
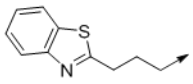
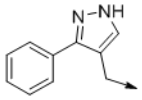
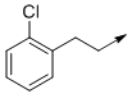
Prod	Well	Amine	WU	P
76	G3		N	100
77	G4		N	100
78	G5		Y	88
79	G6		Y	100
80	H1		Y	88
81	H2		N	91
82	H3		N	70
83	H4		Y	ND
84	H5		N	87
85	H6		Y	100
86	I1		N	ND
87	I2		N	ND

2.3.4 Biological analysis of benzoxazinone analogues

An AlphaScreen assay was again used to test the prepared BNZs for CREBBP bromodomain inhibition (Table 2.5). Due to variability in the performance of the assay, the IC_{50} values of reference compounds appeared approximately two fold lower than in Table 2.3. So although a direct comparison between the two would be misleading, the relative order of the compounds could be analysed. The attachment of some groups to the parent carboxylic acid **36**, such as a short linker followed by a phenyl group with either electron withdrawing (**60**) or donating (**73**) groups attached, caused a drop in ligand potency. On the other hand indolines (**44** and **75**) and quinolones (**71**) connected *via* a short linker enhanced ligand binding. It was also interesting to note that semi-saturated bicyclic compounds were more active than their oxidised analogues (**75** vs **58**). The most potent analogue, compound **71**, contained a tetrahydroquinoline (THQ) side group and a 3-carbon chain. However, attachment of a large lipophilic side group only achieved a modest improvement in affinity, and therefore resulted in a drop in ligand efficiency (now 0.21). These results suggested that further optimisation was required to enable the ligands to form quality interactions with the protein and to achieve the nanomolar *in vitro* potency required to investigate cell activity.

Table 2.5: IC_{50} values obtained by AlphaScreen for BNZ analogues against CREBBP. 95% confidence intervals are shown in parentheses.



	Structure	IC_{50} (μM)		Structure	IC_{50} (μM)
71		51.4 (46.7-56.4)	**79**		241.5 (227.5-256.3)
44		68.6 (62.6-75.2)	**60**		262.1 (very wide)
75		76.9 (70.6-83.8)	**45**		328.8 (294.4-367.3)
57		81.2 (74.9-88.1)	**72**		370.4 (304.1-451.2)
42		97 (89.0-105.6)	**37**		498.7 (336.3-739.6)
47		167.4 (151.1-185.6)	**73**		1280 (682.1-2,402)
58		173.1 (161.0-186.1)	**64**		1532 (wide)
36		198.8 (173.9-227.4)	**41**		15050 (very wide)
85		213.5 (201.3-226.4)	**66**		2938000 (very wide)

2.3.5 Analysis of techniques for identifying benzoxazinone analogues

The challenges of using large compound libraries obtained from combinatorial chemistry to screen for drug discovery candidates have been widely discussed.[122,123] It has become apparent that rather than the number of compounds, it is the diversity and ‘drug-likeness’ that determines

the quality of a library.[124] In order to avoid synthesising a large number of random attachments to the BNZ core, potential analogues were docked and ranked according to their binding affinity, with the hope of prioritising the synthesis of strongly binding compounds. This approach relied on the docking being reasonably accurate, and on the subsequent filtering steps maintaining a good quality set of compounds. An analysis of whether this was the case is discussed below.

Table 2.5 demonstrates that a range of both active and inactive compounds were synthesised. Since these were all compounds that had high docking scores, it indicates that the initial docking study had not been entirely dependable. This is, to some extent, to be expected, but some further methods could have been adopted to improve the reliability of the virtual screen. It is possible, although more computationally demanding, to consider the protein to be flexible during docking study.[125] Also, subsequent results (Section 3.4) have revealed that an analysis of the displaceability of water molecules in the protein might have improved the correlation between predicted and experimentally observed activity.[126]

In order to assess the quality of the compound set used throughout the processes outlined above, and to determine whether a specific type of compound was being biased for, an analysis of the properties of the compound set was performed (Figure 2.9). The set of compounds docked into CREBBP sampled a good selection of chemical space represented by molecular weight and cLogP, while still falling within the desirable range defined by Lipinski (Mw < 500, cLogP < 5) (Figure 2.9C).[127] Upon selecting the top 1000 hits from docking there was an increase in the mean Mw, as would be expected when filtering out low potency binding compounds, but the variation was still high (Figure 2.9D). Selecting compounds that were available for synthesis from the Pfizer set caused a slight drop in mean Mw but little change in cLogP, suggesting the compound library was well suited to this approach (Figure 2.9E). However, there was a substantial change apparent after the synthesis had been performed, with the mean cLogP

increasing from 1.95 to 2.48 (Figure 2.9F). This result would suggest that the chemistry was biased towards more lipophilic compounds. In order to improve the number of compounds synthesised successfully, the parallel chemistry and purification techniques could be improved as described in Section 2.3.3, or the library could be targeted towards successful chemistry initially. In order to place less reliance on the docking scores and improve the area of chemical space sampled, future work may benefit from grouping the compounds according to their similarity, prior to any filtering steps.

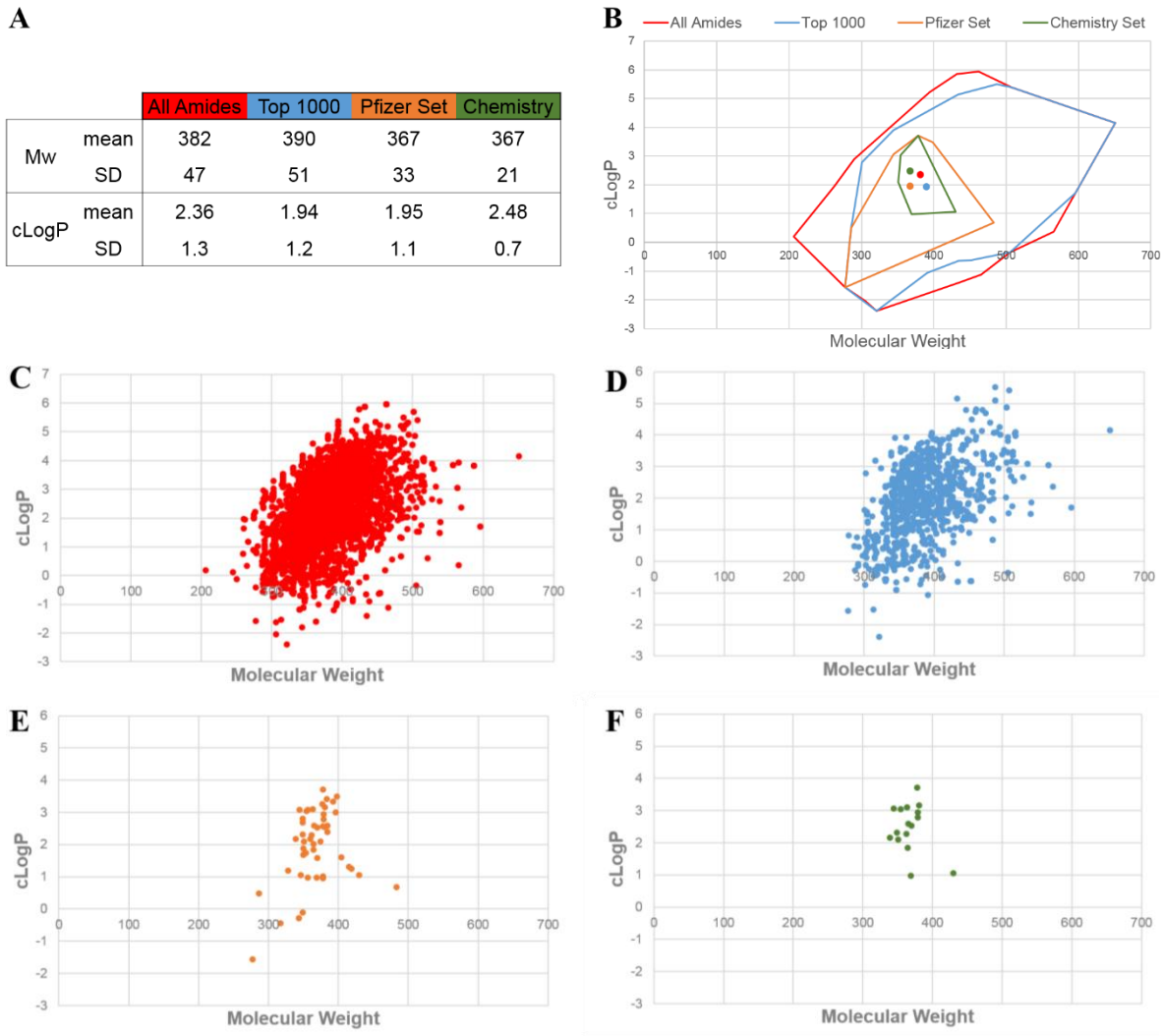


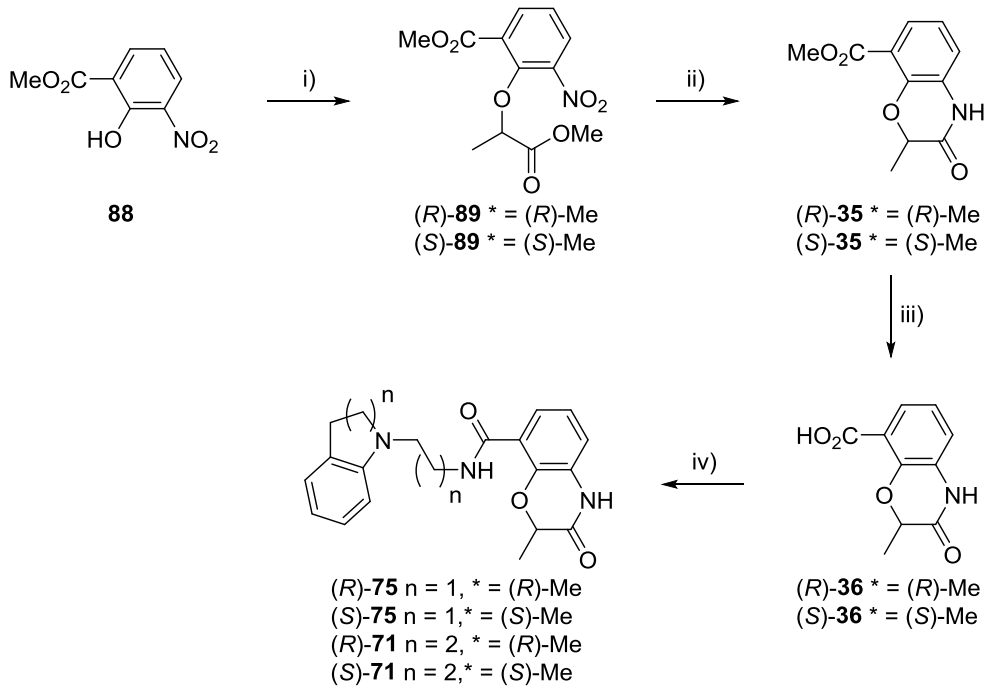
Figure 2.9: **A**: Mean and standard deviation of the molecular weight and cLogP of compounds at each stage. **B**: Convex hull scatter plot for compound properties at each stage with centre of mass (mean) also indicated. **C**-**F**: Scatter plots of molecular weight *vs* cLogP for; **C**: Compounds docked into CREBBP, **D**: Top 1000 ranked compounds, **E**: Compounds available from Pfizer library, **F**: 16 compounds successfully synthesised.

2.4 Investigation of chiral non-racemic benzoxazinones

2.4.1 Synthesis of chiral non-racemic benzoxazinone analogues

In order to explore the influence of chirality within the KAc-binding pocket of bromodomains, the synthesis of each enantiomer of the BNZs **71** and **75** was pursued. A new route was required since methyl bromopropionate is not commercially available as a single enantiomer and would potentially racemise under the basic reaction conditions. Instead, the stereogenic centre could be installed *via* a Mitsunobu coupling between an α-hydroxy carboxylic ester (a derivative of lactic acid widely available as single enantiomers) and the requisite nitro-phenol.[114,128] Treatment of methyl 3-nitro-2-hydroxybenzoate **88** with (*S*)-methyl 2-hydroxypropanoate, DIAD and PPh_3 yielded (*R*)-**89** in 89% yield (Scheme 2.3). Reduction of the nitro group under hydrogenation conditions and concomitant cyclisation then afforded the desired (*R*)-**35** in 91% yield. Ester hydrolysis proceeded in 76% yield to afford (*R*)-**36**. The same route was followed to provide a sample of (*S*)-**36**, which was prepared in 61% yield over the 3 steps.

The subsequent coupling reaction was performed on two amines to afford enantiomerically pure samples of **71** and **75**. These amines were chosen because **71** was the most potent of the previously tested BNZs (Table 2.5), and **75** could be isolated in solid form making it a suitable candidate for future crystallisation trials. Chiral HPLC was performed on both the intermediate methyl ester **35** and on the final product **75** to establish enantiopurity (Appendix A1). The enantiopurity was found to be >98% after Mitsunobu coupling and reduction, however, a drop to 91% for (*R*)-**75** indicated some racemisation had occurred, presumably during the basic ester hydrolysis.



Scheme 2.3: *Reagents and conditions*: i) (*S*)- or (*R*)-Methyl 2-hydroxypropanoate, DIAD, PPh_3, CH_2Cl_2, rt, 89-98%. ii) H_2, Pd/C, EtOAc, rt, 81-91%. iii) LiOH, MeOH, THF, rt, 63-76%. iv) R-NH_2, HBTU, DIPEA, DMF, 69-88%.

2.4.2 Analysis of chiral benzoxazinone analogues

It was hoped that with samples of (*R*)-**71** and (*S*)-**71** in hand, the effect of chirality within the KAc-binding pocket could be investigated. However, the binding of these compounds proved to be too weak for visualisation by thermal shift analysis. Evidence for preferential binding of one enantiomer over the other was eventually obtained in a later, more strongly binding series of compounds, therefore, this problem was not investigated further for the BNZ series.

It was possible, however, to crystallise compound (*R*)-**75**, such that a crystal structure was obtained by X-ray diffraction (Figure 2.10). This structure revealed that an internal hydrogen bond was indeed forming between the O of the BNZ core and the NH of the 8-position amide (NH···O = 2.696(2) Å), thereby fixing the vector of the amide bond. This structure also allowed conformation of the absolute stereochemistry at the 2-position (Flack parameter = 0.03) and it was therefore possible to infer that the Mitsunobu coupling had proceeded with inversion of configuration as expected.

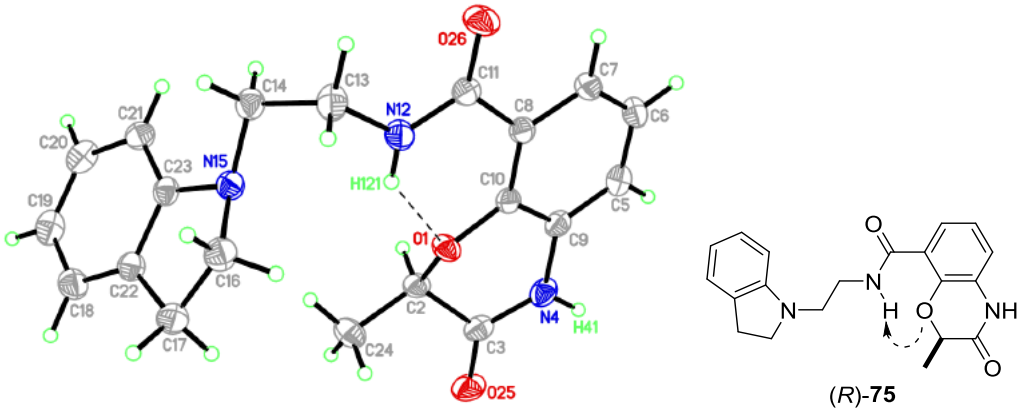


Figure 2.10: X-Ray crystal structure of (*R*)-**75**. The close proximity of the cyclic O and exocyclic NH, measured at 2.696(2) Å, indicates the presence of an internal hydrogen bond.

2.5 Summary

This chapter has described how cyclic amide structures were identified and investigated as bromodomain inhibitors. The bicyclic amide dihydroquinazolinone was identified from a screen of analogues of the solvent NMP, however, limitations in stability and synthetic tractability of this fragment prompted a change to a BNZ core. This core was shown to be a suitable scaffold from which to develop more potent bromodomain inhibitors. By screening a series of amide analogues at the 8-position (directed by a prior *in silico* screen), compound **71**, with a propyl-linker to a tetrahydroquinoline side group, was identified as a low micromolar inhibitor of CREBBP. In addition, an X-ray crystal structure confirmed the presence of an internal hydrogen bond. The data obtained during the study of these BNZ formed an invaluable basis on which to develop a more potent series of compounds, which will be described in the subsequent chapters.

Chapter 3

Development of dihydroquinoxalinones as potent bromodomain inhibitors

This chapter describes the development of dihydroquinoxalinones as selective inhibitors of the CREBBP bromodomain. A move away from the benzoxazinone core that featured in Chapter 2 will be rationalised and a novel acetyl-lysine mimic introduced. Optimisation of the synthetic route for the desired dihydroquinoxalinone heterocycle will be outlined, allowing the SAR generated from the benzoxazinone series to be applied to this new core. The biological evaluation of the compounds will be discussed, with reference to the affinity and selectivity of these inhibitors for the CREBBP bromodomain. Structural information on the binding of the optimum ligands will be discussed and further analysed in computational studies. Finally, the efficacy of the optimum compound as a CREBBP inhibitor, assessed in a cellular assay, is reported.

3.1 Dihydroquinoxalinones as acetyl-lysine mimics

3.1.1 Rationale behind changing the core

In the previous chapter the use of the BNZ core as an KAc mimic was discussed. The ease with which this core could be synthesised allowed the rapid screening of a series of analogues that extended towards the ZA loop, in an effort to gain potency and selectivity for CREBBP. However, the most potent analogues only reached the low micromolar range, still some way from the desired nanomolar potency required to test inhibition in a cellular setting. Therefore, a change to the core of the KAc mimic was considered. Re-examination of the crystal structure of DHQ **20** bound to CREBBP revealed that the benzylic C(4) was in close proximity to the backbone carbonyl of P1110, therefore substituting an oxygen into this position, as in the BNZ core, may have introduced a degree of lone-pair repulsion (Figure 3.1A). This unfavourable interaction could be ameliorated by substitution of O(1) with N, resulting in a 3,4-dihydroquinoxalin-2(1*H*)-one (DHQN) core (Figure 3.1B). This modification also represented a change from a hydrogen bond acceptor to donor, thereby altering the nature of the intramolecular hydrogen bond to the exocyclic amide (at the 8-position in BNZ or 5-position

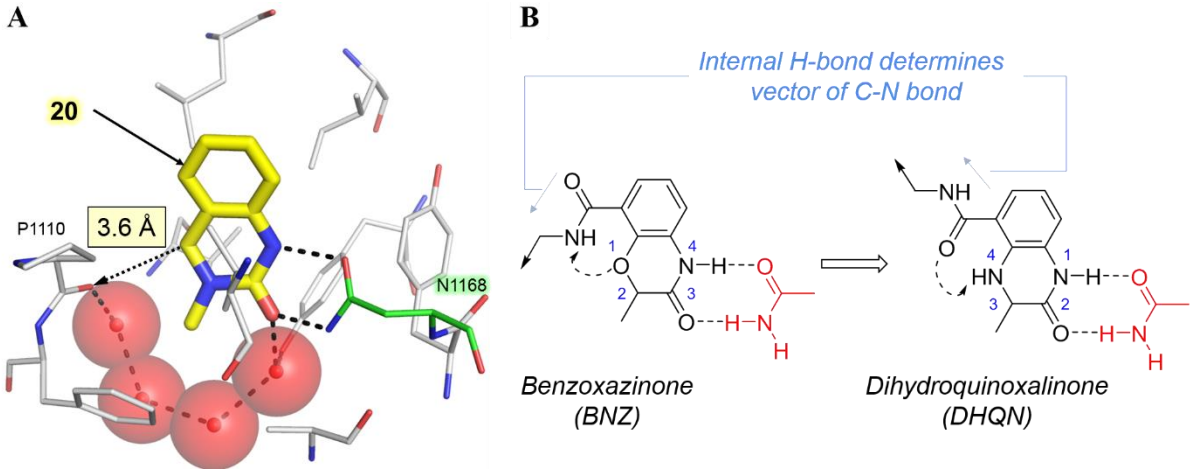
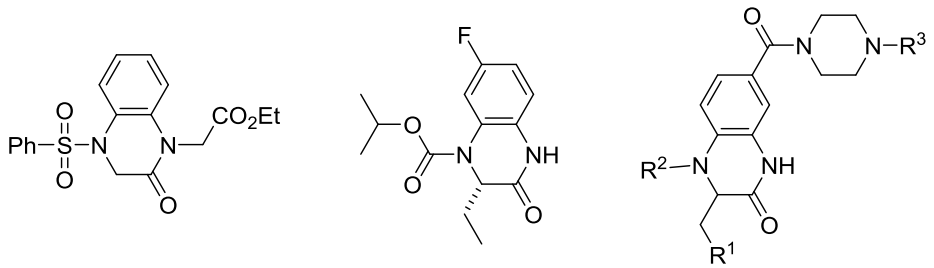


Figure 3.1: **A**: Crystal structure of DHQ **20** bound to CREBBP (PDB ID: 3P1F) with the distance between the benzylic carbon of the ligand and a backbone carbonyl of P1110 highlighted. **B**: The change from a BNZ to DHQN core reverses the sense of the internal hydrogen bond and alters the vector of the amide side group.

in DHQN), and consequently the vector along which substituents are directed. To investigate the consequence of these variations, synthesis of the DHQN core was sought.

3.1.2 Biologically active dihydroquinoxalinones

An early example of a biologically active DHQN was compound **90**, shown to be an inhibitor of aldose reductase.[129] DHQNs have also emerged as promising candidates in the treatment of type-1 variant human immunodeficiency virus (HIV-1). A compound developed by GSK, GW420867X **91** (Figure 3.2), demonstrated effective antiviral activity when used in combination with established nucleoside based reverse-transcriptase inhibitors.[130] It entered clinical trials under the name opaviraline, and was found to have a favourable pharmacokinetic and safety profile,[131] but appears to have been discontinued at Phase II. Further work has continued on this series, both on the development of further analogues,[132] and on investigating the structural basis of drug resistance.[133] A similar series of compounds, with the general structure **92**, have been developed as probes of the C-type lectin receptor DC-SIGN, in order to study the mechanisms by which pathogens (including HIV) mitigate a host's immune response.[134] With these examples outlined above, and others, DHQNs have proved themselves as amenable candidates for biological studies, and therefore represent a good core to pursue.



90 **91** GW420867X Opaviraline **92**

Figure 3.2: Biologically active DHQN derivatives. **90** is an inhibitor of aldose reductase.[129] **91** entered clinical trials for the treatment of HIV-1.[131] **92** is a chemical probe for DC-SIGN.[134]

3.2 Synthesis of dihydroquinoxalinones

3.2.1 Retrosynthetic analysis of dihydroquinoxalinones

In order to make amide substitutions to the 5-position of a DHQN core, the key carboxylic acid **94** intermediate was desired. The synthesis of DHQN analogues in a single step from diaminobenzenes (in a manner similar to the BNZs in Scheme 2.1) was not considered since, with two amino groups, regioselectivity was expected to be an issue. A two-step synthesis, where one of the amines is masked as a nitro group, was therefore considered (Figure 3.3).[135] Unfortunately, the attempted alkylation of methyl 2-amino-3-nitrobenzoate **96** (R = Me) with methyl bromopropionate **32** was unsuccessful. This was perhaps a result of both *ortho*-positions being occupied by electron-withdrawing groups, which strongly deactivate the aniline for nucleophilic attack. Performing the acylation on C(3)*N* first, with C(2)*N* masked as a nitro-group, was not possible due to unavailability of the starting material.

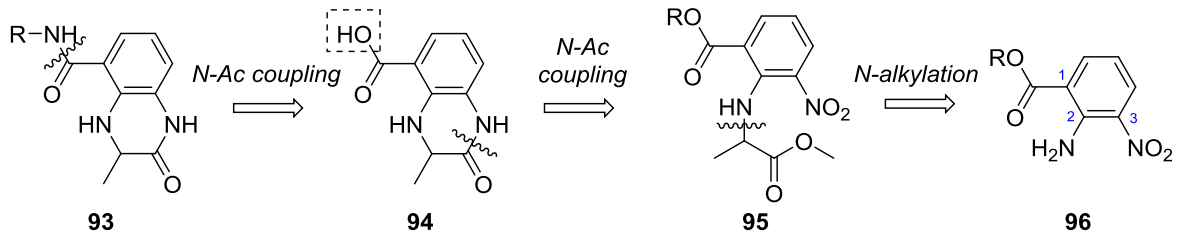


Figure 3.3: Retrosynthetic route to DHQN involving an *N*-alkylation step.

An alternative to attempting the formation of an *N*-alkyl bond on a poorly nucleophilic nitrogen was to instead form the *N*-aryl bond. There are a number of versatile synthetic methods for the formation of C-N bonds to aromatic compounds, including transition metal-catalysed Ullmann-Goldberg, Buchwald-Hartwig and Chan-Lam methodologies.[136,137] In order to complete the synthesis of the DHQN core, an *N*-acyl bond must be formed. The order in which these two steps are performed determines whether the arylation (route A) or acylation (route B) is intramolecular (Figure 3.4). An advantage of these approaches is that the coupling partner is

an appropriately protected amino acid, either **99** or **102**, and therefore readily available as a single enantiomer.

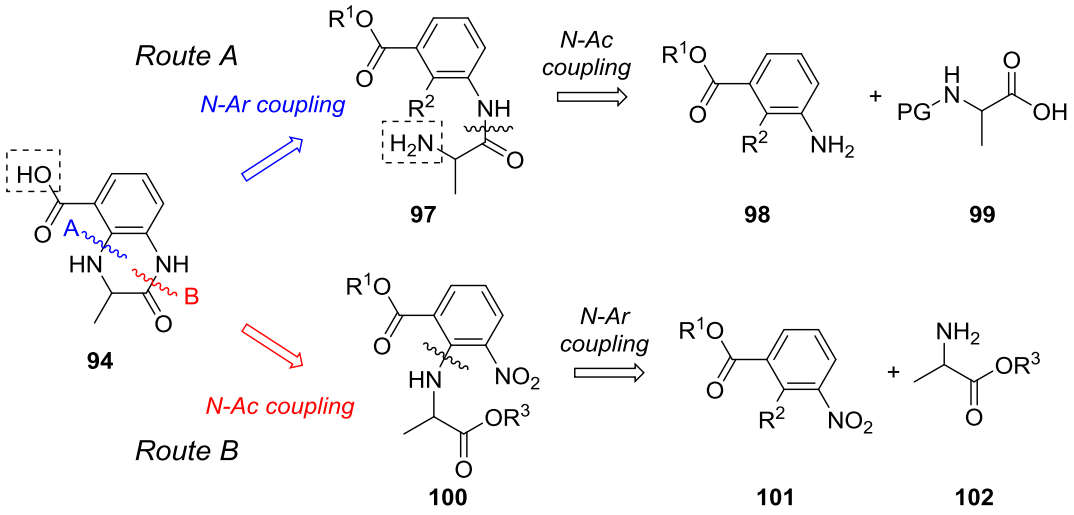


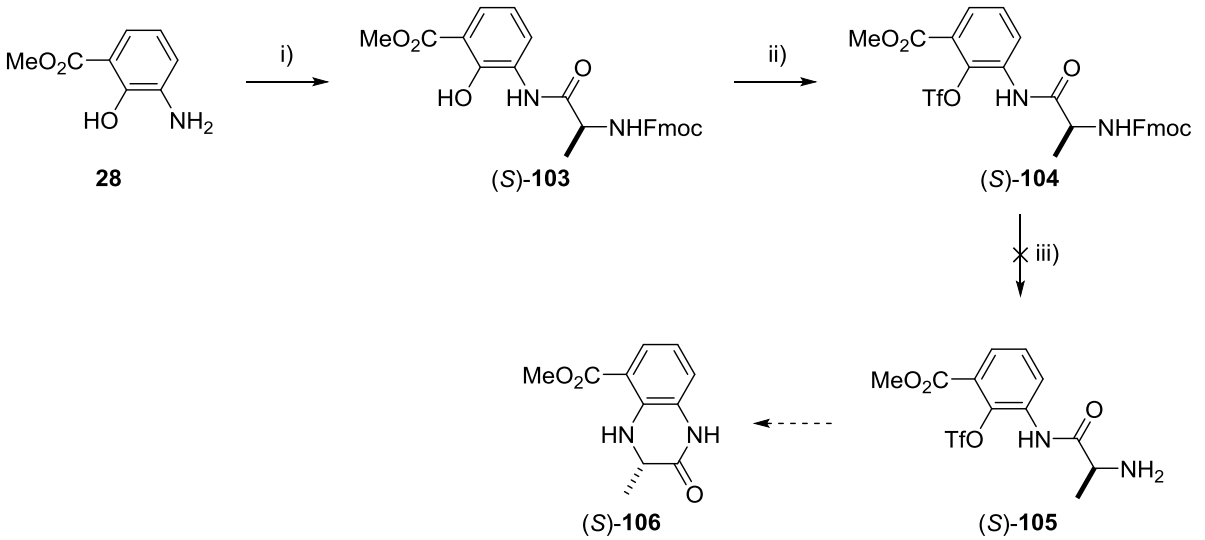
Figure 3.4: Retrosynthetic route to DHQN involving an *N*-arylation step that is either intramolecular (route A) or intermolecular (route B).

3.2.2. Synthesis of dihydroquinoxalinones – methyl ester route

A Buchwald-Hartwig coupling was investigated as a method for the formation of the *N*-aryl bond.[138,139] Whilst most commonly performed on aryl-bromides, the reaction has also been performed on aryl-triflates, which can be easily accessed from the corresponding phenol. This method would allow a synthesis that started with a readily available 2-hydroxy-3-aminobenzoate derivative (complementary to the BNZ synthesis), with the carboxylic acid protected, in the first instance, as the methyl ester (**98** or **101** with R^1 = OMe, R^2 = OTf, Figure 3.4). Two routes were considered in which the *N*-aryl bond was formed in either an intramolecular (route A) or intermolecular (route B) reaction.

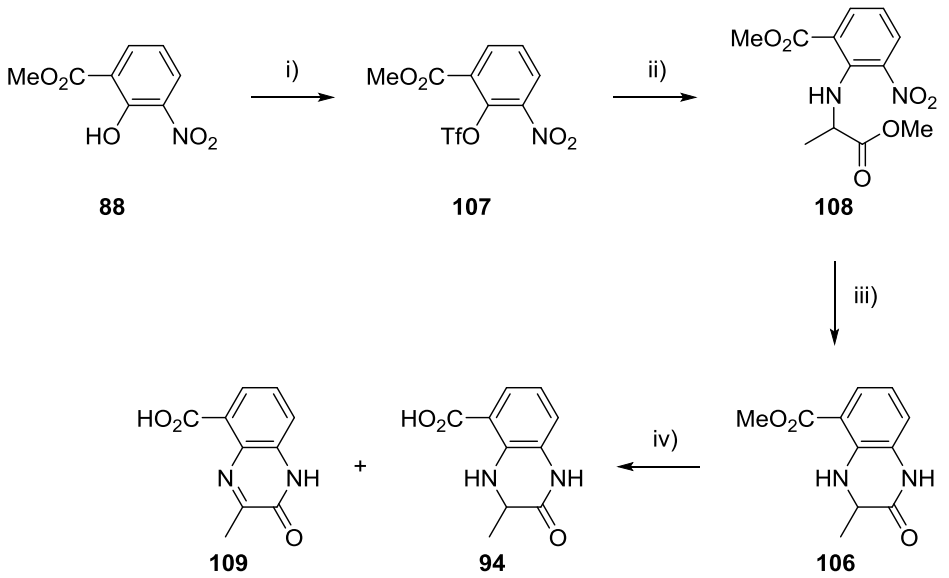
The first step of Route A involved coupling amino-phenol **28** to Fmoc-protected L-alanine (Scheme 3.1). Standard peptide coupling conditions allowed isolation of (*S*)-**103** in a satisfactory 52% yield. Subsequent conversion of the phenol to triflate (*S*)-**104** was achieved with triflic anhydride in 49% yield. However Fmoc deprotection using 10% piperidine was

unsuccessful due to difficulties experienced in purification of the resulting primary amine (*S*)-**105**. This route was therefore aborted in favour of route B, which is one step shorter and more atom efficient.



Scheme 3.1: *Reagents and conditions*: i) Fmoc-L-Ala-OH, HBTU, DIPEA, DMF, rt, 52%. ii) Tf_2O, Et_3N, CH_2Cl_2, 0 °C → rt, 49%. iii) Piperidine, DMF, rt, 0%.

The first step of Route B involved conversion of nitro-phenol **88** to the triflate **107** in 86% yield (Scheme 3.2). Next, the Buchwald-Hartwig coupling to D/L-alanine methyl ester was attempted. It has been reported in the literature that electron deficient aryl triflates are susceptible to triflate cleavage.[140,141] Consequently, reagent **107** was added slowly to the reaction mixture in order to minimise the undesired side reaction. Overall, the desired coupled product **108** was obtained in an encouraging 76% yield. Reduction of the nitro-group in **108** using standard hydrogenation conditions with concomitant cyclisation afforded the DHQN **106** in 83% yield. Finally, base promoted ester hydrolysis afforded the key carboxylic acid **94** in 65% yield.



Scheme 3.2: *Reagents and conditions*: i) Tf_2O, Et_3N, CH_2Cl_2, rt, 90%. ii) D/L-Ala-OMe·HCl, Cs_2CO_3, $Pd(OAc)_2$, (*S*)-BINAP, toluene, 85 °C, 76%. iii) H_2, Pd/C, EtOAc, rt, 83%. iv) LiOH, MeOH, THF, rt, 65%.

It was noticed during the synthesis that once the DHQN core had been formed the compounds became susceptible to oxidation when left for an extended period of time under acidic conditions (such as dissolution in unbuffered $CDCl_3$). Such behaviour of DHQNs has been reported previously, for example during the cleavage of compounds from a solid support with TFA.[142] During the workup of the methyl ester deprotection, treatment with aqueous 1 M HCl was necessary to extract the carboxylic acid into the organic layer. This resulted in some of the oxidised product **109**, which could only be removed after coupling of the carboxylic acid to an amine. In addition, the basic ester hydrolysis conditions could potentially cause racemisation at the C(3) position of the DHQN core when performed on a non-racemic sample of **106** (the enantiopurity of each route is considered in Section 3.2.4). An alternative protecting group strategy for the carboxylic acid was therefore investigated.

3.2.3 Synthesis of dihydroquinoxalinones - benzyl ester route

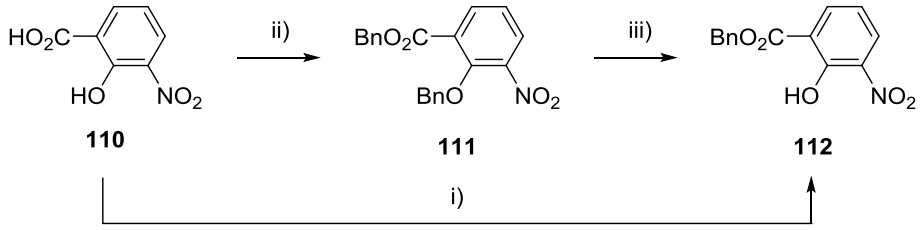
Protection of the carboxyl group as the benzyl ester was considered to be a promising strategy since it could be removed under neutral hydrogenolysis conditions. Indeed, this approach would

shorten the reaction sequence since the carboxylate deprotection could be incorporated into the nitro reduction step to encompass a one-step hydrogenation-cyclisation-hydrogenolysis conversion of **100** (R = Bn) to **94** (Figure 3.4). Installation of the benzyl ester proved more challenging than expected as outlined in the following sections.

3.2.3.1 Installation of benzyl protecting group

In order to selectively protect the carboxyl of 2-hydroxy-3-nitrobenzoic acid **110**, a number of approaches were considered (Scheme 3.3). Subjecting **110** to standard esterification conditions, namely heating with benzyl alcohol and PPTS in toluene, did not afford any of the desired benzyl ester **112**.[143] It was hoped instead that the difference in pKa between the carboxylic acid and the phenol could be exploited by using $KHCO_3$ as a base and alkylating with benzyl bromide, as has been reported for unsubstituted phenolic acids.[144] However, the reaction proved sluggish and could not be forced to completion (Scheme 3.3). During the work-up a $NaHCO_3$ wash, intended to remove unreacted starting material, caused the monobenzylated product **112** to precipitate as its sodium salt. This approach provided a useful way of isolating the desired product, albeit in 29% yield due to the incomplete reaction.

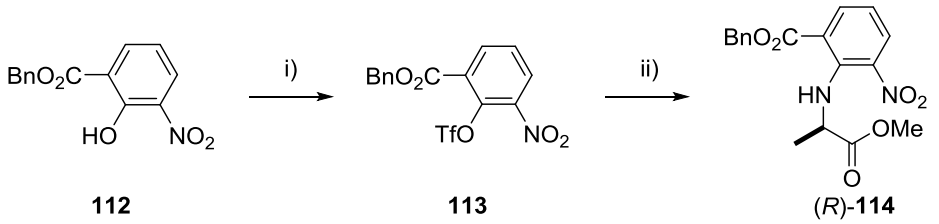
Given that the electron withdrawing effect of the nitro group appeared to lower the pKa of the phenol sufficiently to allow deprotonation by $NaHCO_3$, a selective deprotection of the dibenzylated product **111** was considered. Debenzylation of phenols can be achieved with TFA, and the presence of π–electron-withdrawing groups (such as ester or nitro groups) has been shown to rapidly increase the reaction rate.[145] With this in mind, benzylation of both the carboxyl and phenol groups of **110** was achieved using K_2CO_3 as a base in an acceptable 67% yield (Scheme 3.3). Subsequent treatment of **111** with a 1:1 mixture of TFA:toluene accomplished the desired mono-deprotection to afford **112** in 99% yield.



Scheme 3.3: *Reagents and conditions*: i) BnBr, $KHCO_3$, DMF, 40 °C, 29%. ii) BnBr, K_2CO_3, DMF, 70 °C, 67%. iii) TFA, toluene, rt, 99%.

3.2.3.2 *N*-Arylation using Buchwald-Hartwig conditions

With the suitably protected phenol **112** in hand, conversion to the triflate **113** was achieved under the previously described conditions and in equally good yield (Scheme 3.4). Next, the coupling to D-alanine was attempted using Buchwald-Hartwig conditions, which again proceeded well to afford (*R*)-**114** in 91% yield.



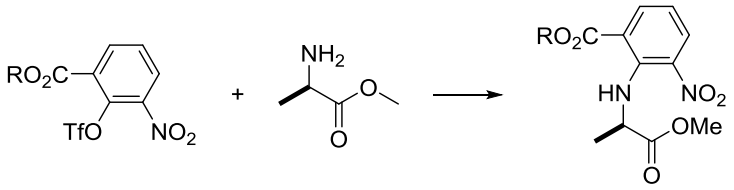
Scheme 3.4: *Reagents and conditions*: i) Tf_2O, Et_3N, CH_2Cl_2, rt, 92%. ii) D-Ala-OMe·HCl, Cs_2CO_3, $Pd(OAc)_2$, (*S*)-BINAP, toluene, 85 °C, 91%.

3.2.3.3 *N*-Arylation using nucleophilic aromatic substitution conditions

It was noted in Section 3.2.2 that the Buchwald-Hartwig coupling of electron-poor aryl triflates is known to suffer from triflate cleavage. However, the presence of π-electron withdrawing groups in each *ortho*-position also make compound **113** a possible candidate for nucleophilic aromatic substitution (S_NAr). In order to investigate the propensity of this reaction to occur, the coupling of triflate **113** to alanine was performed without addition of palladium or a ligand (Table 3.1). Whilst the presence of trace palladium in the reaction mixture could not be ruled out,[146] the product was isolated successfully in 79% yield. The reaction required a longer reaction time, which is consistent with S_NAr reactions requiring harsher conditions. Whether

the reaction mechanism is metal-catalysed or not, these modified conditions are preferred since they constitute a more cost effective and operationally simple procedure.

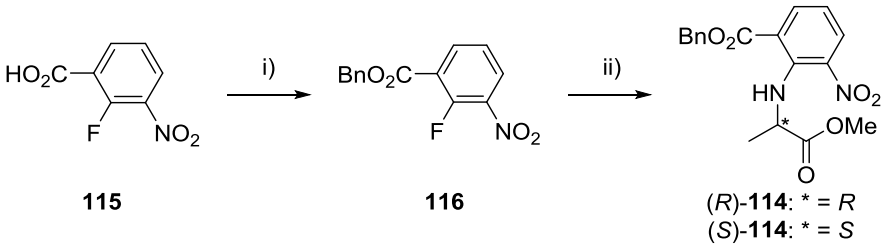
Table 3.1: Cross coupling of aryl triflate to alanine. *Reagents and conditions*: i) D-Ala-OMe·HCl, Cs_2CO_3, $Pd(OAc)_2$, (*S*)-BINAP, toluene, 85 °C. ii) D-Ala-OMe·HCl, Cs_2CO_3, Toluene, 85 °C.



Aryl-triflate	Product	R	Conditions	Time (h)	Average Yield
107	(*R*)-**108**	Me	i)	2.5	92% (n = 3)
113	(*R*)-**114**	Bn	i)	2	84% (n = 4)
113	(*R*)-**114**	Bn	ii)	19	79% (n = 2)

n = number of repeats

Aryl fluorides are commonly used as substrates for nucleophilic aromatic substitution reactions since the polarised C-F bond aids the rate-determining nucleophilic attack. To investigate whether the DHQN core could be synthesised from commercially available 2-fluoro-3-nitrobenzoic acid **115**, potentially circumventing the need to selectively benzylate the carboxyl of **110** described in Section 3.2.3.1, an alternative route was proposed (Scheme 3.5). Conversion of **115** to the benzyl ester was performed under standard reaction conditions to yield aryl-fluoride **116** in 97% yield. Subsequent reaction with D-alanine was achieved under the previously identified ('Pd-free') conditions, again with a longer reaction time of 24 h, to afford the desired aniline (*R*)-**114** in 90% yield. To access the opposite enantiomer of the DHQN core, coupling between **116** and L-alanine was also performed. On this occasion the reaction was worked up after 5 h and (*S*)-**114** could only be isolated in a lower yield of 52% with a significant amount of starting material remaining, corroborating that longer reaction times are necessary for S_NAr of **116** compared to Buchwald-Hartwig coupling of **113**.

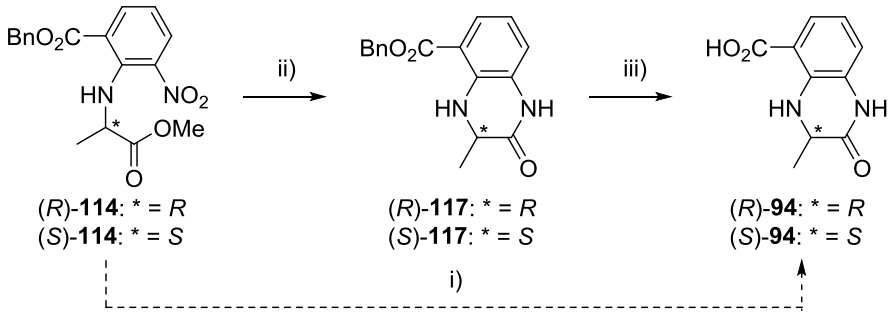


Scheme 3.5: *Reagents and conditions*: i) BnBr, K_2CO_3, DMF, 60 °C, 97%. ii) D- or L-Ala-OMe·HCl, Cs_2CO_3, Toluene, 85 °C, 52-90%.

3.2.3.4 Cyclisation to form dihydroquinoxalinone core

With compound (*R*)-**114** in hand, simultaneous debenzylation and nitro reduction to prepare the carboxylic acid (*R*)-**94** in a single step was attempted by treatment with Pd/C under a H_2 atmosphere (Scheme 3.6). Although some of the desired product was formed, the crude reaction mixture was impure and (*R*)-**94** was difficult to isolate. Therefore, a two-step process, involving reduction of the nitro-group whilst maintaining the benzyl ester, was investigated. Reagents that would not interfere with the cyclisation were desired and strongly acidic conditions (which could cause oxidation) had to be avoided; consequently two transition metal reductants were investigated.[147,148]

Tin(II) chloride in EtOAc or DMF achieved the desired reduction and cyclisation to afford (*R*)-**117**, however, the yields were inconsistent and, at best only, 35%. The use of zinc and NH_4Cl in DMF proved preferable and reproducibly furnished (*R*)-**117** in excellent yields (Scheme 3.6). Deprotection of the benzyl ester was performed under hydrogenolysis conditions, with a slightly modified work-up to account for the poor solubility of the product, to afford carboxylic acid (*R*)-**94** in 98% yield without the need for further purification. The cyclisation and deprotection of (*S*)-**114** proceeded in a similar fashion, furnishing the other enantiomer of the DHQN core (*S*)-**94**.



Scheme 3.6: *Reagents and conditions*: i) H_2, Pd/C, EtOAc, rt. ii) Zn, NH_4Cl, DMF, rt, 88-96%. iii) H_2, Pd/C, EtOAc, rt, 98-99%.

In summary, the key DHQN carboxylic acid (*R*)-**94** was successfully synthesised from 2-hydroxy-3-nitrobenzoic acid (£10 per gram) in 6 steps and 52% yield, or from 2-fluoro-3-nitrobenzoic acid (£60 per gram) in 4 steps and 82% yield. The opposite enantiomer (*S*)-**94** was also prepared.

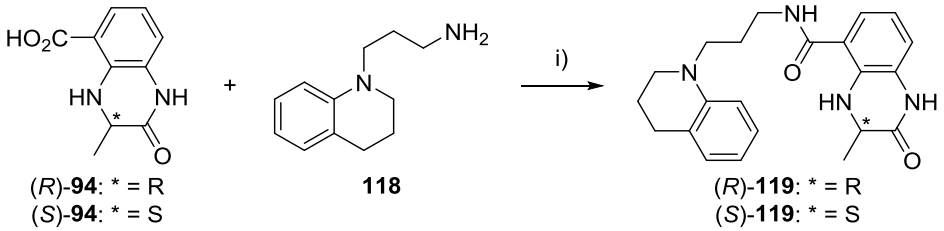
3.2.4 Assessment of enantiopurity

In order to compare the susceptibility of the DHQN to racemisation during the two syntheses with the carboxyl group protected as either a methyl ester (Section 3.2.2) or a benzyl ester (Section 3.2.3), a chiral HPLC analysis was obtained on the carboxylic acid **94** (Appendix A2). The methyl ester protecting group route afforded (*R*)-**94** in 91% *e.e.* indicating some racemisation had occurred, potentially during the basic ester hydrolysis, as also observed in the BNZ series. On the other hand, the benzyl ester protecting group route had proceeded without any detectable racemisation to afford (*R*)-**94** in >98% *e.e.*

3.2.5 Extension from dihydroquinoxalinone core

Derivatisation of carboxylic acids (*R*)-**94** and (*S*)-**94** followed, in pursuit of analogues that would show good CREBBP activity. The most potent analogue from the BNZ series of compounds was **71**, containing a THQ side group with a 3 carbon linker. Coupling of amine **118** to both enantiomers of carboxylic acid **94** was accomplished under standard conditions to furnish (*R*)-**119** and (*S*)-**119** in 32% and 48% yield respectively (Scheme 3.7). These

compounds were still unstable under acidic conditions but were stable in neutral solvents such as DMSO and acetone.



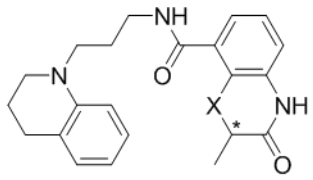
Scheme 3.7: *Reagents and conditions*: i) HBTU, DIPEA, DMF, rt, 32-48%.

3.3 Biological testing of dihydroquinoxalinones

3.3.1 Inhibition of CREBBP bromodomain

The two DHQN analogues were tested for CREBBP inhibition using the AlphaScreen assay (Table 3.2). Pleasingly, it was found that replacement of the O(1) in the BNZ core with N(4) in the DHQN core resulted in a large jump in IC_{50} as determined by AlphaScreen; from 51 µM for **71** to 760 nM for (*R*)-**119**. The magnitude of this improvement, which must be due to more than just resolution of the racemic mixture, highlights the importance of having the correct heteroatom at this position in the ring. It was also interesting to note that the (*R*)-enantiomer was nine times more active than the (*S*)-enantiomer, supporting the prediction from the initial docking study of the BNZ core that binding of the (*R*)-enantiomer would be favoured (see Figure 2.8B).

Table 3.2: AlphaScreen data for initial DHQN compounds with a comparison to the most potent BNZ from Section 2.3.4. 95% confidence intervals are shown in parentheses.



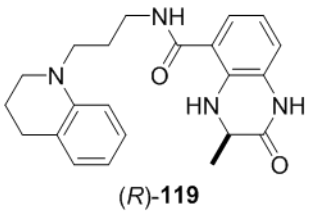
Compound	X	*	IC50 (nM)
(*R*)-**119**	NH	*R*	758 (637-853)
(*S*)-**119**	NH	*S*	6870 (5964-7905)
71	O	*rac*	51400 (46700-56400)

3.3.2 Activity against other bromodomains

This improved potency made it possible to obtain selectivity data for (*R*)-**119** against a panel of bromodomains sampling 5 of the 8 bromodomain families (Table 3.3). A differential scanning fluorimetry (DSF) assay was used to assess the binding of (*R*)-**119** by measuring the change in melting temperature (ΔTm) of each protein. It is important to note that the thermal shift caused by binding of a compound to different proteins cannot be quantitatively compared because each

result is dependent on the protein's initial stability, which is variable. Whilst it has been shown that there is often reasonable correlation between the ΔTm measured by DSF and the IC_{50} measured by AlphaScreen for bromodomains,[105] this is protein and compound dependent. The results showed that whilst (*R*)-**119** induced a significant shift in the melting temperature of CREBBP (ΔTm = 6.5 °C), the temperature shift of the other proteins did not rise above the noise of the assay (ΔTm = ~1 °C). Of particular interest was the low ΔTm for (*R*)-**119** against the BET family, a promising indication that some degree of selectivity for CREBBP over these closely related bromodomains had been achieved.

Table 3.3: DSF data for binding of (*R*)-**119** to a panel of bromodomains. The results from the table are also plotted onto the bromodomain family tree, with circle size and colour representative of the ΔTm of each protein tested.

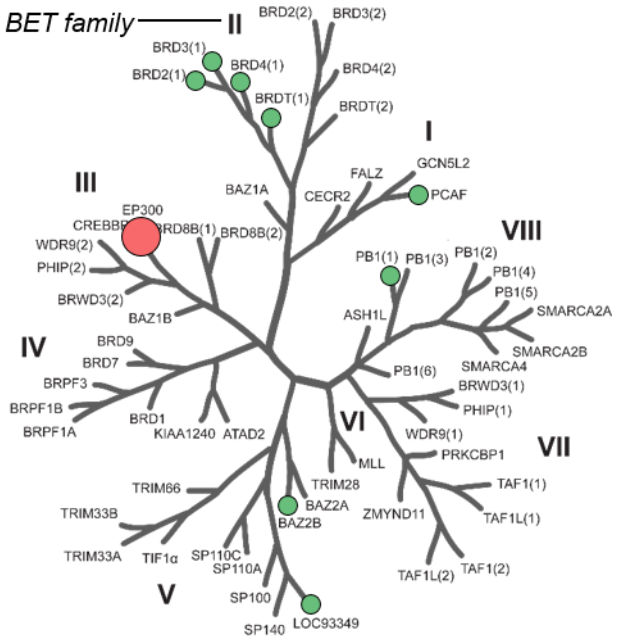


(*R*)-**119**

BRD2(1)	BRD3(1)	BRD4(1)
0.7	1.0	1.0

BRDT	PCAF	CREBBP
0.6	0.1	6.5

LOC093349A	BAZ2B	PB1(1)
0.0	0.5	0.3



3.4 Crystal structure of dihydroquinoxalinone bound to CREBBP

With a nanomolar inhibitor of CREBBP in hand, it was hoped that a crystal structure could be obtained in order to identify the important binding interactions and evaluate the success of the docking predictions. A sample of (*R*)-**119** was submitted to the SGC to screen for crystallisation conditions, allowing diffraction quality crystals to be grown within a few days. Diffraction data was collected for the co-crystal at Diamond (beamline I04.1), allowing a structure of (*R*)-**119** bound to CREBBP to be solved at a resolution of 1.41 Å (Figure 3.5). This structure showed that the compound bound to the CREBBP bromodomain, as predicted, in the KAc-binding pocket. The DHQN core formed synergistic hydrogen bonds to the amide of N1168 and the methyl group at the stereogenic C(3) occupied a similar position to the methyl of acetyl lysine above the network of four conserved water molecules (Figure 3.5A). This structure was in accordance with the observed binding mode of the initial DHQ fragment and the low deviation between the two was a reflection of the key binding interactions being maintained. The conformation of the amide at the C(5) position was, as expected, influenced by the formation of an internal hydrogen bond between the exocyclic carbonyl and N(4)*H*.

A consequence of the vector adopted by the amide was that the exocyclic carbonyl formed a hydrogen bond to a water molecule (numbered 5) situated at the boundary between the KAc pocket and ZA loop (Figure 3.5B). Furthermore, the conformation adopted by the THQ side chain was not as predicted in the initial docking studies. Rather than being directed into the ZA channel and forming interactions with residues in this region, the aromatic group bound in an induced pocket beneath R1173. Two water molecules occupying the ZA channel (5 and 6) remained in much the same position as in the fragment crystal structure. These water molecules had been removed in the initial docking study that was performed, but evidence from this crystal structure, and others, suggest these water molecules should be considered structurally important.[26]

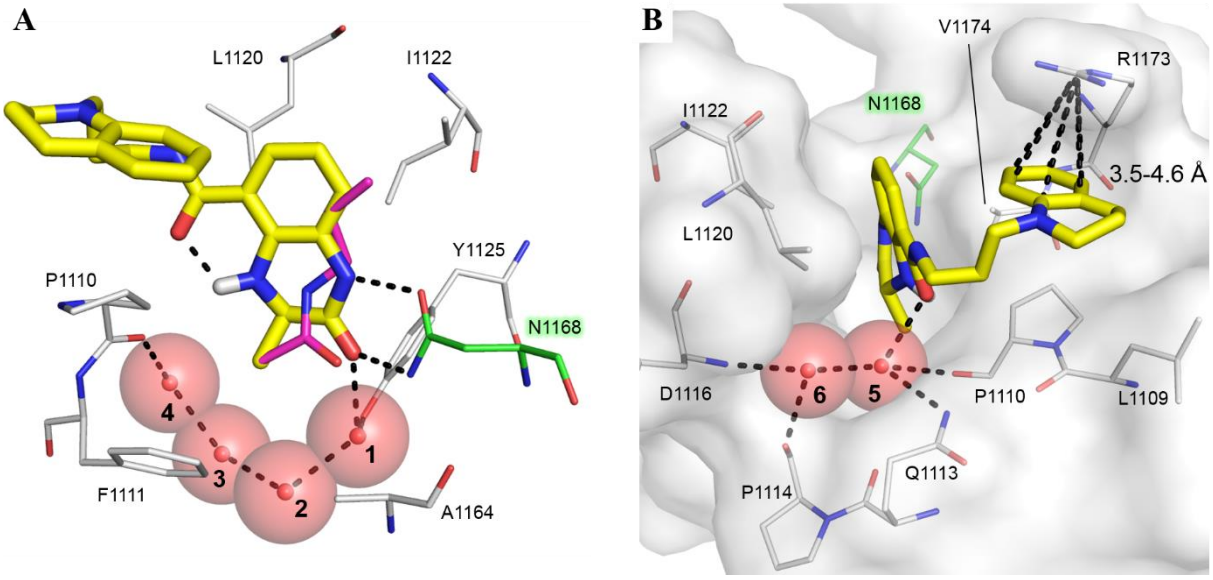


Figure 3.5: Co-crystal structure of (*R*)-**119** bound to CREBBP (PDB ID: 4NYW). **A**: An overlay with the structure of KAc bound to CREBBP (PDB ID: 3P1C) reveals that the DHQN head-group occupied the same pocket as the native substrate and formed two hydrogen bonds to the key asparagine residue. An internal hydrogen bond is present between the amide carbonyl and cyclic-NH. **B**: The ZA channel remains occupied by water molecules, one of which forms a hydrogen-bond to the amide carbonyl. The THQ side group is directed into an inducted fit pocket beneath R1173 with an average distance between the cation and the aryl group measured as 4.0 Å.

Compared to the crystal structure of DHQ **20** that was used for docking analogues into CREBBP, the conformation of R1173 had changed significantly (Figure 3.6A). A distance of 3.5-4.6 Å between the electron rich aryl group of the THQ and the positively charged guanidine of the arginine residue suggests that a cation-π interaction is being formed.[149] This interaction was of particular interest since, as mentioned in the design of these compounds, only three (of 61) bromodomains other than CREBBP and EP300 contain a cationic residue in this position.

The THQ side group is also binding in close proximity to L1109, another residue that was targeted in the initial design of the compounds. An overlay with the structure of BRD4 demonstrates how occupancy in this region of the protein may result in a steric clash between the THQ group and W81, potentially enhancing selectivity for CREBBP over the BET bromodomains (Figure 3.6B).

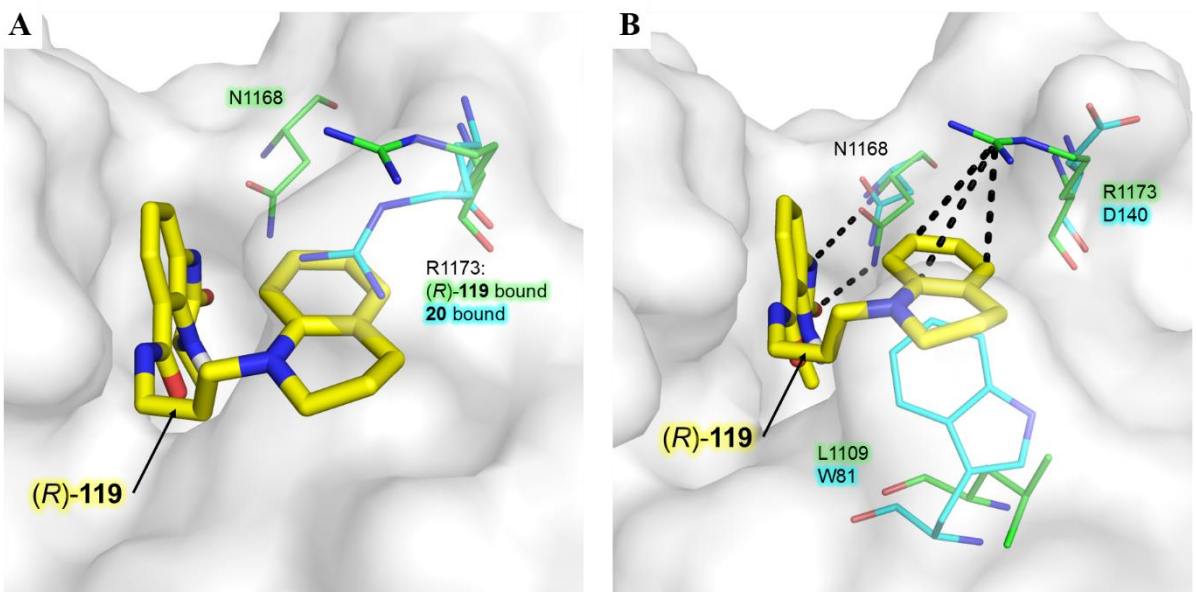


Figure 3.6: Co-crystal structure of (*R*)-**119** bound to CREBBP (PDB ID: 4NYW). **A**: The conformation of R1173 is altered significantly from the **20** bound structure (PDB ID = 4NYV) in order to adopt the cation-π interaction. **B**: An overlay with the structure of BRD4(1) bound to DHQ **20** (PDB ID: 4HBV) indicates that the THQ group exploits the more open pocket of CREBBP, whereas a clash would occur with W81 in BRD4.

In summary, a co-crystal structure of (*R*)-**119** and CREBBP revealed that the THQ side group bound in a novel inducted fit pocket beneath R1173 and is stabilised by a cation-π interaction. The following sections describe a number of approaches undertaken to investigate the significance of this binding interaction.

3.5 Tetrahydroquinoline analogues to investigate the cation-π interaction

3.5.1 Background to the cation-π interaction

A series of studies by Docherty and others in the 1990s established the cation-π interaction as an important force in controlling protein structure and function alongside hydrophobic interactions, hydrogen bonding and ion pairs.[150] Since then the cation-π interaction has been studied extensively, either computationally, in model systems, or in increasingly complex biological systems.[151–153] Electrostatic attraction between the cation and the electron rich aryl system is thought to be the main contributing force to the interaction, although charge-transfer and polarisation effects should also be taken into consideration.[154] The cation-π interaction is thought to play an important role in stabilising the secondary structure of proteins through the interaction of positively charged side chains of lysine and arginine with the aromatic side chains of phenylalanine, tyrosine or tryptophan. In a study of almost 600 protein crystal structures by Gallivan, one energetically significant cation-π interaction was found per 77 residues.[155] A strikingly large 26% of tryptophan residues and 18% of arginine residues were found to be involved in a cation-π interaction, demonstrating the prevalence of this interaction.

In addition to stabilising secondary structure, many examples of a cation-π interaction between a small molecule and a protein that contribute significantly to the ligand binding energy have been reported.[156] One example of the occurrence of this interaction in biological systems is the signalling of cationic ligands such as acetylcholine (ACh) **120** and nicotine **121**.[157] The proteins that bind these ligands are complex integral membrane proteins, for which it can be difficult to obtain structural information by X-ray crystallography. One of the ways in which the importance of the cation-π interaction has been elucidated has been through the fluorination of a tryptophan residue within the binding site (Figure 3.7A).[158,159] A subsequent reduction in the binding affinities of the natural ligands ACh **120** and nicotine **121** provided an indication that

binding *via* a cation-π interaction was being compromised. The cation-π interaction has also been exploited in the development of a series of glycoside-based inhibitors **122** of gelatin-3 (Figure 3.7B).[160,161] When a crystal structure revealed that benzamide substitutions at the 3'-position **123** stacked against an arginine residue, analogues were designed with methoxy-substitutions **124** that were found to improve the affinity through enthalpic contributions.

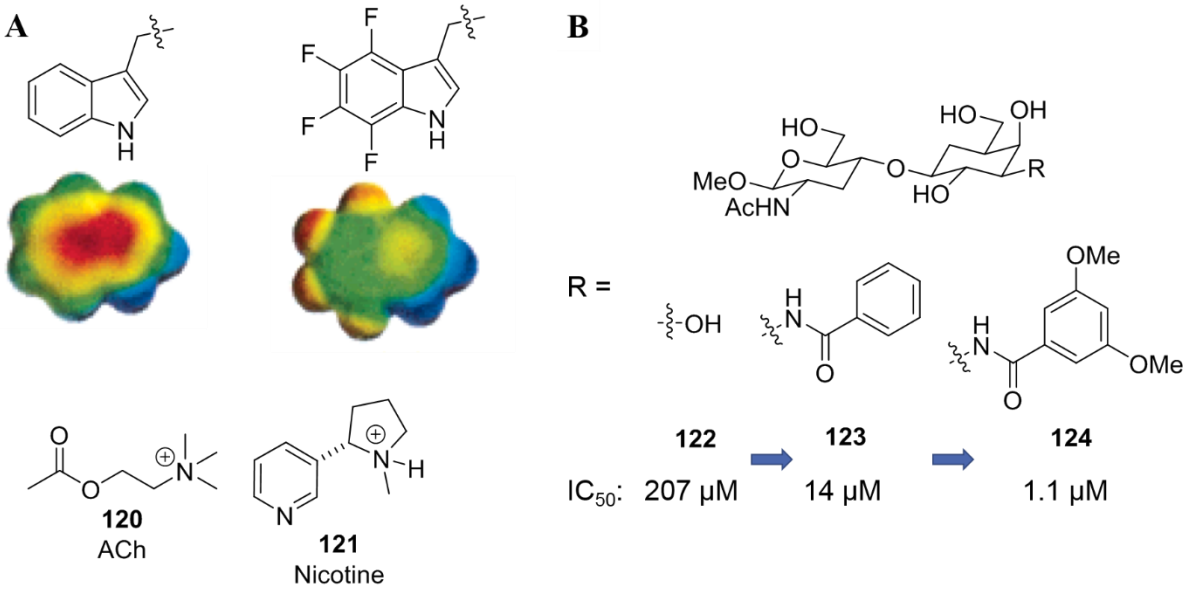


Figure 3.7: Examples of mediating a cation-π interaction. **A**: The binding of nicotinic receptors to neurotransmitters **120** and **121**, thought to be dependent on a cation-π interaction, was probed by introducing fluorinated analogues of tryptophan.[158] **B**: The potency of gelatin-3 inhibitors were improved by exploiting a cation-π interaction.[160]

3.5.2 Route to afford tetrahydroquinoline analogues

In order to synthesise a set of THQ amines **125**, which could be coupled to the DHQN core, a robust route for the attachment of the propyl-amine linker to a series of THQs **127** was required (Figure 3.8). Masking of the primary amine in **125** was deemed necessary in order to prevent any cross reactivity during the initial alkylation step. Due to the poor nucleophilicity of the aniline **127**, it was anticipated that forceful conditions would be required to achieve the desired alkylation.

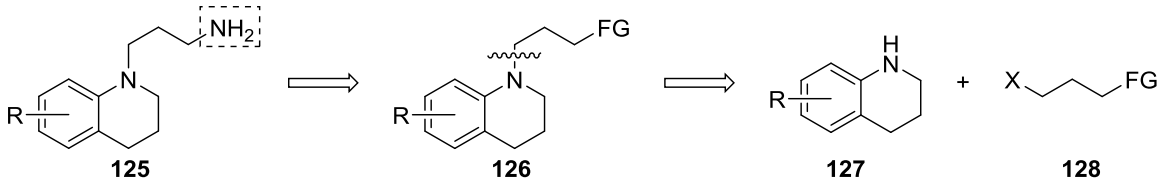


Figure 3.8: Retrosynthetic analysis for attachment of a propyl-amine linker to a THQ.

Alkylation of THQs can be achieved using alkyl halides, electrophilic alkenes or by reductive amination.[162–164] Considering the requirement of a masked amine and 3-carbon linker in the alkylating reagent, conjugate addition to an α,β-unsaturated ester was considered. Alkylation of THQ derivatives **127** with methyl acrylate has been reported to proceed in good yield and would allow the preparation of **126** with FG = CO_2Me.[165] Subsequently, the transformation of the ester to a primary amide could be achieved *via* reduction to a primary alcohol followed by displacement with a suitable nitrogen nucleophile.

3.5.3 Selection of starting materials

A small selection of THQs containing electron-donating groups was sought to test the hypothesis that the cation-π interaction plays a significant role in the affinity of (*R*)-**119** for the CREBBP bromodomain (Figure 3.9).

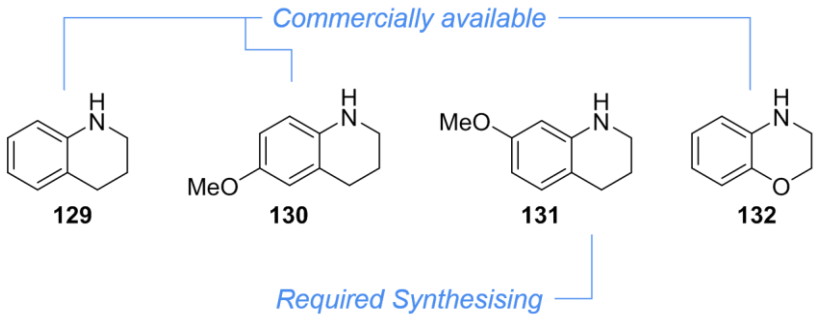
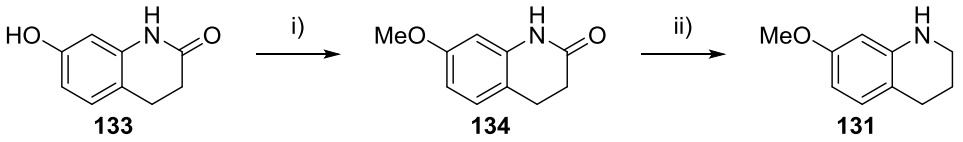


Figure 3.9: Availability of THQ analogues with electron donating groups attached.

The 6-methoxy and benzoxazole analogues of THQ, **130** and **132**, were available commercially, whilst the 7-methoxy analogue **131** was prepared *via* a short reaction sequence (Scheme 3.8). Methylation of dihydroquinolinone **133**, followed by reduction with $LiAlH_4$, afforded the desired 7-methoxy analogue **131**.

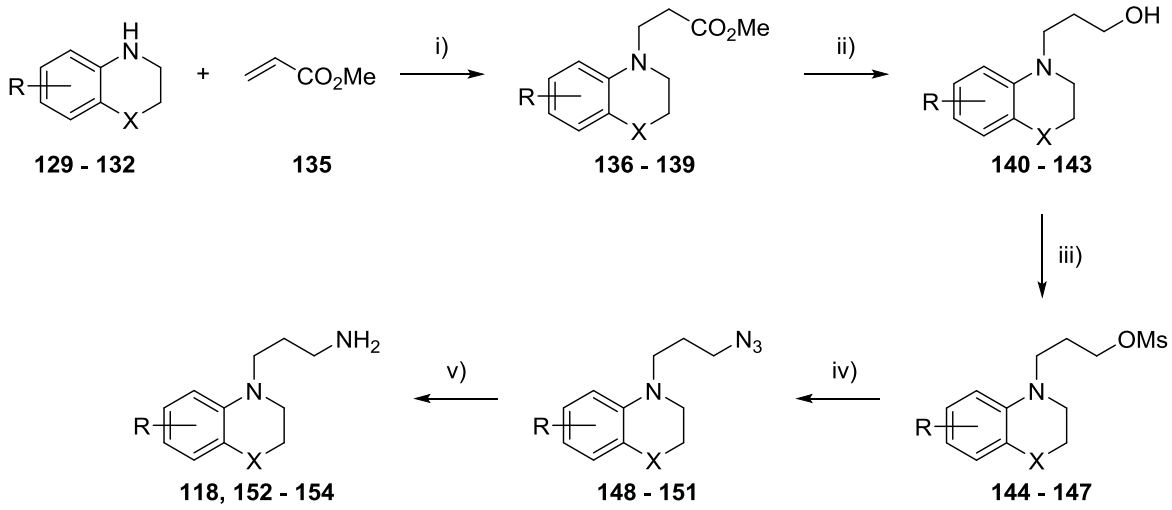


133 134 131

Scheme 3.8: *Reagents and conditions*: i) MeI, K_2CO_3, DMF, 60 °C, 57%. ii) $LiAlH_4$, THF, 0 °C → rt, 99%.

3.5.4 Addition of amino-propyl linker to tetrahydroquinolines

The first step for the derivatisation of THQs **129**-**132** was conjugate addition with methyl acrylate **135** (Scheme 3.9, Table 3.4). Heating a solution of the two reactants at 120 °C in AcOH was sufficient to allow full consumption of the THQs. In all cases the crude reaction mixture contained mostly the desired esters **136**-**139**, which were carried forward to the next reaction without further purification. Reduction with $LiAlH_4$ achieved the desired reduction to the primary alcohols **140**-**143** and, after purification *via* silica gel chromatography, the combined yield for the two steps was between 50-60% in all cases. No improvement in yield was observed when a later batch of ester **138** was purified, supporting the combined approach. In order to perform the desired substitution, the primary alcohols were first functionalised as the mesylates **144**-**147** in yields >80% for all analogues. Consequently, reaction with sodium azide proceeded well to allow isolation of azides **148**-**151**. Finally, reduction of the azides to the desired amines **118**, **152**-**154** was achieved under hydrogenation conditions in near 100% yields.



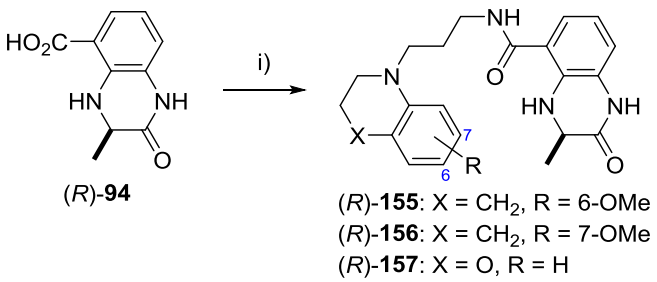
Scheme 3.9: *Reagents and conditions*: i) AcOH, 120 °C. ii) $LiAlH_4$, THF, 0 °C → rt, 51-59% (2-step yield). iii) MsCl, Et_3N, CH_2Cl_2, rt, 83-94%. iv) NaN_3, DMF, 60° C, 56- >99%. v) H_2, Pd/C, EtOAc, 86-99%.

Table 3.4: Preparation of THQ amine analogues.

Step	X = CH_2, R = H		X = CH_2, R = 6-OMe		X = CH_2, R = 7-OMe		X = O, R = H	
	Product	Yield	Product	Yield	Product	Yield	Product	Yield
i)	**136**	-	**137**	-	**138**	-	**139**	-
ii)[a]	**140**	59%	**141**	55%	**142**	57%	**143**	51%
iii)	**144**	83%	**145**	88%	**146**	84%	**147**	94%
iv)	**148**	56%	**149**	>99%	**150**	77%	**151**	98%
v)	**118**	99%	**152**	86%	**153**	99%	**154**	96%
Σ[b]		27%		42%		36%		45%

[a] Yield quoted over 2-steps. [b] Overall 5-step yield.

Overall, the 4 primary amines **118**, **152-154** were synthesised from their corresponding starting materials in 27-45% yield over 5 steps. With these amines in hand, the final step was to perform a peptide coupling reaction to carboxylic acid (*R*)-**94**, which was achieved using HBTU-mediated peptide coupling conditions with yields of 48-76% (Scheme 3.10). The purification of these compounds proved slightly challenging since ethyl acetate was difficult to remove, requiring azeotropic distillation with acetone.

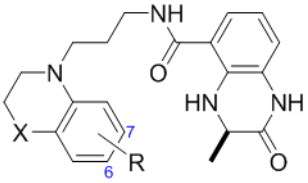


Scheme 3.10: *Reagents and conditions*: i) **152-154**, HBTU, DIPEA, DMF, rt, 48-76%.

3.5.5 Biological analysis of tetrahydroquinoline analogues

The THQ-derivatised DHQN compounds were first submitted for testing by AlphaScreen to analyse their inhibition of the CREBBP bromodomain (Table 3.5). Satisfyingly, the addition of an electron-donating methoxy group to the 7-position of the THQ ring, (*R*)-**156**, resulted in a more potent CREBBP inhibitor in comparison to the unsubstituted analogue (*R*)-**119**. The benzoxazole analogue (*R*)-**157** also showed slightly improved binding, while addition of a methoxy group to the 6-position of the THQ ring (*R*)-**155** actually led to a drop in the binding affinity. These data largely support the initial hypothesis that addition of an electron-donating group to the THQ ring would improve binding through fortification of the cation-π interaction. However, the location of the electron-donating group is important and the reduction in potency for the 6-methoxy analogue could be due to a steric clash with the protein or subtle differences in the electronics of the ring.

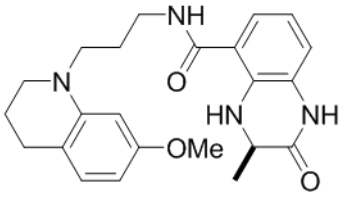
Table 3.5: AlphaScreen data for THQ analogues. 95% confidence intervals are shown in parentheses.



Compound	X	R	IC_{50} (nM)
(*R*)-**156**	CH_2	(7)-OMe	323 (298-351)
(*R*)-**157**	O	H	549 (511-590)
(*R*)-**119**	CH_2	H	758 (637-853)
(*R*)-**155**	CH_2	(6)-OMe	1410 (1220-1630)

Following the identification of (*R*)-**156** as a more potent inhibitor of the CREBBP bromodomain, selectivity analysis against a selection of bromodomains was performed using a DSF assay (Table 3.6). The results obtained were similar to the data for compound (*R*)-**119** (Section 3.2.2), with the observed shift in melting temperature around 1 °C or less for all bromodomains other than CREBBP. It was interesting to note that the value for CREBBP had dropped from 6.5 to 5.4 °C. This might simply be a result of the experiment being run at a different time and therefore on a different batch of protein, or could be a reflection of the fact that the compounds are causing a conformational change upon binding and may therefore have a more complex relationship to the protein melting temperature.

Table 3.6: DSF data for binding of (*R*)-**156** to a panel of bromodomains. The results from the table are also plotted onto the bromodomain family tree, with circle size and colour representative of the ΔTm of each protein tested.



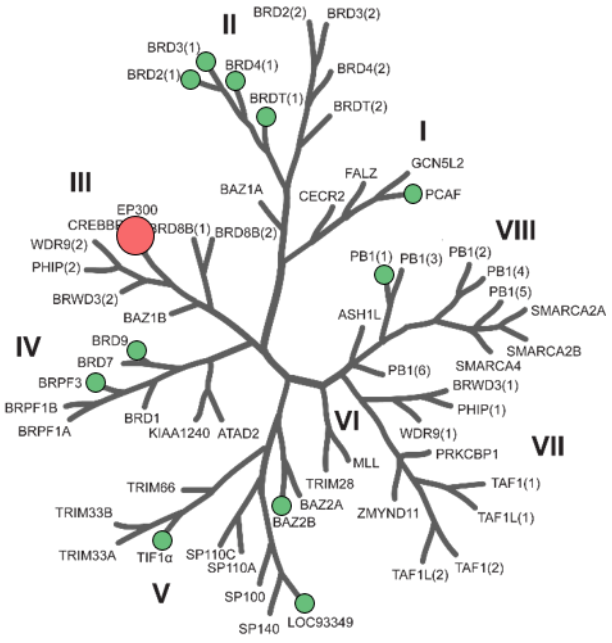
(*R*)-**156**

BRD2(1)	BRD3(1)	BRD4(1)
0.6	0.6	0.7

BRDT(1)	PCAF	CREBBP
0.7	0.0	5.4

BRD9	BRPF3	TIF1α
-1.1	0.5	0.7

LOC093349A	BAZ2B	PB1(1)
0.3	-0.5	-0.1



Finally, the binding of (*R*)-**156** was assessed by isothermal titration calorimetry (ITC) by co-workers at the SGC. ITC provides a direct measure of the thermodynamic components when a ligand binds to a protein, independent of a competing ligand or secondary readout. Pleasingly, the K_D for (*R*)-**156** binding to CREBBP, measured as 391 nM, correlated well with the IC_{50} of 323 nM from the AlphaScreen assay (Figure 3.10A). The binding of (*R*)-**156** to BRD4(1) was

also assessed by ITC and shown to have a K_D of 1.38 µM (Figure 3.10B). A selectivity of 3.5-fold for CREBBP over BRD4(1) was surprisingly low given the key binding interaction, the cation-π interaction, cannot exist in BRD4(1). An analysis of the thermodynamic contributions to the Gibbs free energy of binding (ΔG) revealed that (*R*)-**156** bound to both bromodomains with a major exothermic enthalpic component (ΔH) and a minor favourable entropic component (−TΔS) (Figure 3.10C). The selectivity for CREBBP over BRD(4) is principally due to a difference in their entropic components, which were −2.40 kcal/mol and −1.36 kcal/mol respectively. Since the DSF assay was run at variable temperatures depending on the melting temperature of the target protein, this offers some explanation for why the DSF assay underestimated the selectivity for this compound.

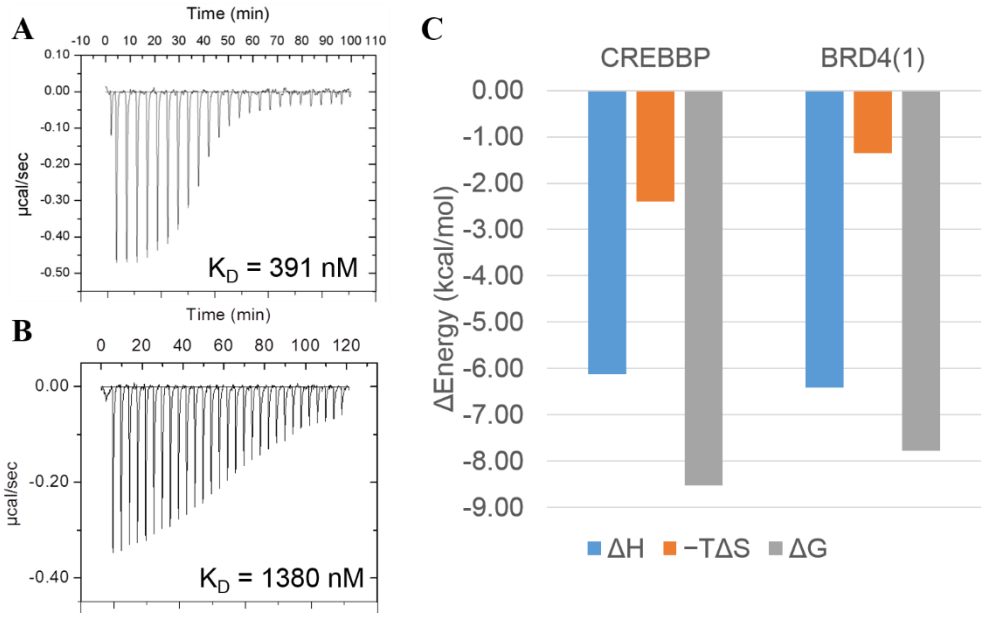


Figure 3.10: **A**: ITC measurement of the binding of (*R*)-**156** to CREBBP. **B**: ITC measurement of the binding of (*R*)-**156** to BRD4(1). **C**: Comparison of relative contributions of enthalpy (ΔH) and entropy (−TΔS) to the Gibbs free energy of binding (ΔG).

3.5.6 Crystal structure of a tetrahydroquinoline analogue

Having identified the more potent DHQN-based compound (*R*)-**156** it was hoped that a crystal structure could be obtained for comparison to the original (*R*)-**119**/CREBBP structure. Again a sample of (*R*)-**156** was submitted to the SGC, where a co-crystal with CREBBP was grown.

The diffraction data were collected from Diamond (beamline I04) allowing the structure to be solved at a resolution of 1.10 Å. This revealed that the DHQN moiety of (*R*)-**156** bound in much the same way as (*R*)-**119** with hydrogen bonds to N1168 and the internal hydrogen bond maintained (Figure 3.11A). The orientation of the side chain was conserved with the THQ group binding in the same pocket beneath R1173 (Figure 3.11B). The perpendicular distance between the aryl ring of (*R*)-**156** and the cation had not changed compared to (*R*)-**119**, although addition of the methoxy group had caused the aryl ring to shift slightly. This could be a consequence of the steric demands of the pocket, or due to a maximisation of the interaction between the electron rich part of the ring and the guanidinium side chain. Given the higher than expected affinity of the ligand to BRD4, crystallisation of (*R*)-**156** with this protein was also attempted but without success.

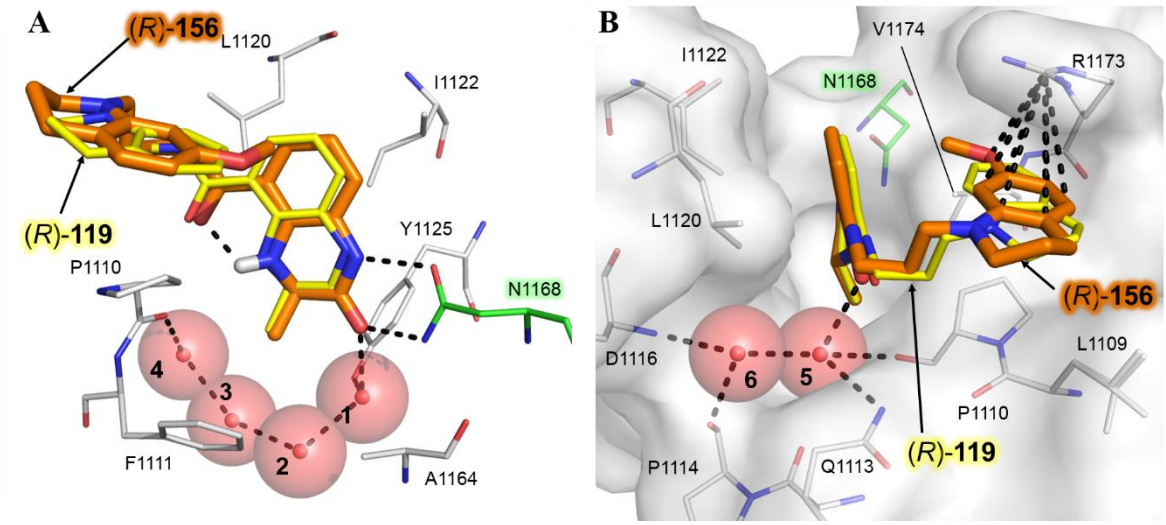


Figure 3.11: Crystal structure of (*R*)-**156** (orange) bound to CREBBP (PDB ID: 4NYX) overlaid with (*R*)-**119** (yellow) bound to CREBBP (PDB ID: 4NYW). **A**: View of the KAc-binding pocket with hydrogen-bonds to N1168 and water-mediated hydrogen-bond to Y1125 illustrated, along with the internal hydrogen-bond. **B**: View of the THQ-binding pocket illustrating the shift in the aromatic ring of (*R*)-**156** compared to (*R*)-**119**.

3.6 Further analysis of CREBBP ligands

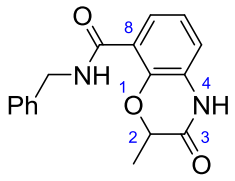
3.6.1 Rationale

It was proposed in the initial design of the BNZ compounds (Section 2.3.2) that an internal hydrogen bond would form to an amide in the 8-position, and that upon changing to a DHQN core this hydrogen bond would reverse. Structural evidence for the existence of the hydrogen bond in the BNZ series was obtained from a small molecule crystal structure of (*R*)-**75** (Figure 2.10), while evidence for the hydrogen bond in the DHQN series can be seen in the protein bound crystal structures of (*R*)-**119** and (*R*)-**156** (Figures 3.5 and 3.11). However, since crystal structures only offer a snapshot of the conformation of molecules, other methods for measuring the proclivity of the hydrogen bond to form in solution were investigated.

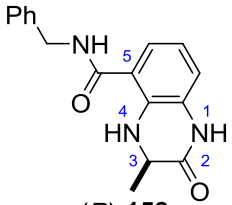
3.6.2 NMR analysis of internal hydrogen bond.

It is commonly found that hydrogen bonding results in a downfield shift (higher δ) in the NMR signal of the corresponding hydrogen.[166] It has also been shown that the signal of intramolecularly hydrogen bonded protons are much less dependent on external factors such as solvent and concentration.[167] In order to investigate whether a similar effect could be observed in the BNZ and DHQN analogues, a comparison of the NMR spectra of **37** and (*R*)-**158** in DMSO and $CDCl_3$ was performed (Table 3.7). For the BNZ **37**, both amide protons overlap at around 7.9 ppm in $CDCl_3$, but in d_6-DMSO the cyclic N(4)*H* is shifted further downfield than the exocyclic N*H*. This observation supports the hypothesis that the exocyclic N*H* is less affected by a change to a solvent that forms hydrogen bonds since one already exists to O(1). The same effect is seen in the DHQN (*R*)-**158** for which altering the solvent causes a large change in chemical shift of both N(1)*H* and the exocyclic N*H*, but a minimal change in the shift of N(4)*H* which forms the intramolecular hydrogen bond.

Table 3.7: NMR shifts for amine and amide protons in **37** and (*R*)-**158**. All experiments were performed at 2 mg/mL on an AV500 spectrometer. Chemical shifts are calibrated to the solvent peak and reported in ppm relative to the 1H signal of TMS.



37



(*R*)-**158**

	N(4)*H*	**C(8)CO_2N*H***	**N(4)*H***	**N(1)*H***	**C(5)CO_2N*H***
$CDCl_3$	~7.9	~7.9	7.79	7.74	6.38
d_6-DMSO	10.79	8.59	7.87	10.37	8.96
Difference	~2.9	~0.7	0.08	2.63	2.58

The NMR for (*R*)-**158** was also run at different concentrations, and whilst the solubility limited the range over which measurements could be taken (max 2 mg/mL), it was apparent that N(1)*H* showed the greatest change in chemical shift (Appendix A3). This is, perhaps, a reflection of the tendency of this amide to dimerise at higher concentrations in solution.

The possible observation of *cis* and *trans* amide conformers on an NMR timescale has been exploited to calculate the thermodynamic parameters of exchange by using variable temperature NMR.[168,169] In addition, a recent publication reports an NMR methodology to determine the different conformers in solution of streptavidin and rank them according to their occupancy to help lead medicinal chemistry design.[170] Unfortunately, none of the BNZ or DHQN compounds synthesised had rotamers that could be distinguished by NMR, prohibiting the use of these techniques. Therefore, in order to obtain more detail on the strength of the hydrogen bonds an alternative technique had to be found.

3.6.3 Computational analysis of internal hydrogen bond

In collaboration with the Paton group (University of Oxford), two computational techniques were employed to investigate the significance of the intramolecular hydrogen bond. Density

functional theory (DFT) calculations were performed to calculate the energetics for rotation at 30° increments about the $C_{carbonyl}$-C_{aryl} bond in a BNZ and DHNQ analogue containing a simple amide substitution off the core. These results showed that the BNZ (*R*)-**37** adopts a single low energy conformation in solution where the carbonyl oxygen and quaternary aryl carbon are on opposite faces; that is to say, a dihedral angle (θ) of around 180° (Figure 3.12A). The DHQN (*R*)-**158** has two low energy conformations in solution, with dihedral angles of 30° and 330° (Figure 3.12B). It is possible that the completely planar conformation (θ = 0) was predicted to be slightly disfavoured due to a steric clash between the amide N*H* and the aryl C*H*, but the low barrier between the two would suggest an overall energy minimum around 0°. Satisfyingly, the lowest energy dihedral angles that were calculated by DFT and those observed *via* X-ray crystallography show good agreement (Figure 3.12C). It can therefore be concluded from these results that a hydrogen bond exists between *O*(1) and the amide N*H* in the BNZ series, and between the amide C=*O* and N(4)*H* in the DHQN series.

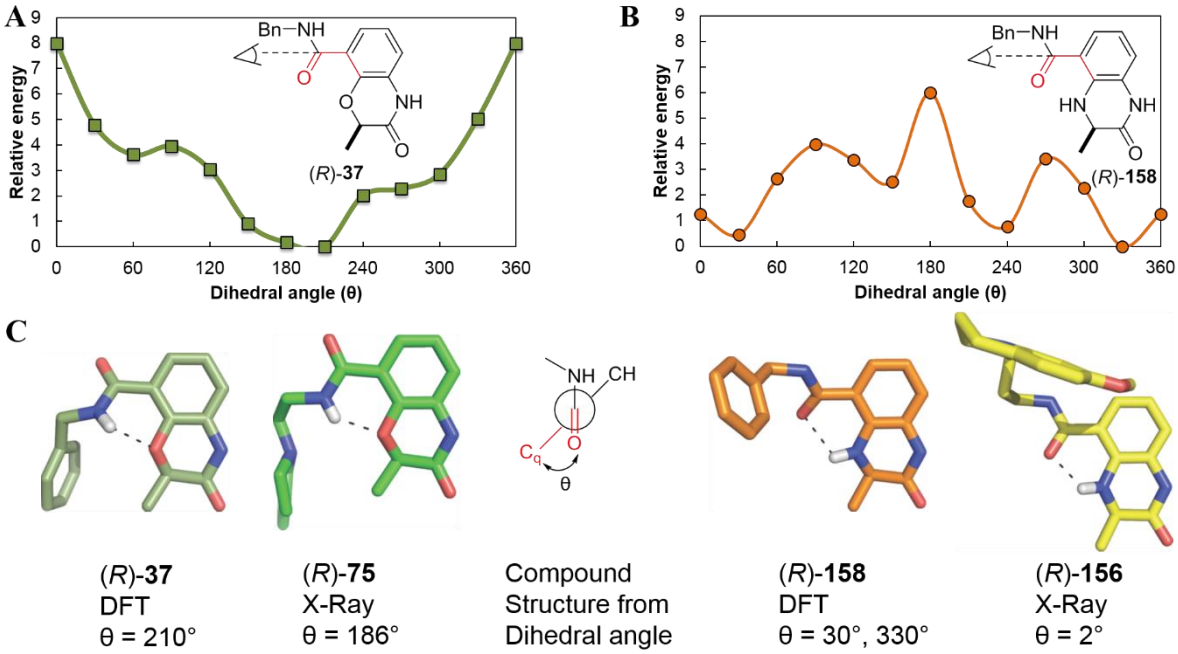


Figure 3.12: **A**: DFT calculations for rotation about the $C_{carbonyl}$-C_{aryl} bond for the model BNZ (*R*)-**37**. **B**: DFT calculations for rotation about the $C_{carbonyl}$-C_{aryl} bond for the model DHQN (*R*)-**158**. **C**: Comparison of experimentally and computationally obtained dihedral angles for BNZ and DHQN compounds. The angle θ is measured as the angle between the four atoms highlighted in red.

Molecular dynamics (MD) simulations were also performed on (*R*)-**156** in explicit water to validate the results of the DFT calculations (Figure 3.13). During the timescale of the simulation the dihedral angle of the $C_{carbonyl}$-C_{aryl} bond remained within 330° to 30°, consistent with the energy minimum predicted by DFT (Figure 3.12B). This result supports the hypothesis that the hydrogen bond in (*R*)-**156** exists both when bound to the protein and free in solution.

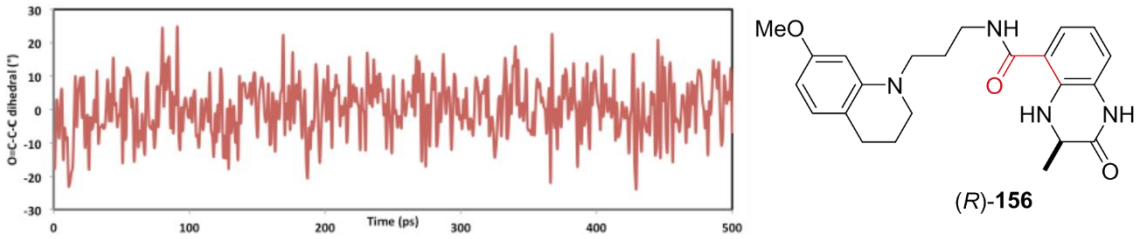


Figure 3.13: MD simulation to measure the impact of solvent on the conformation bias of DHQN (*R*)-**156**. The dihedral angle for the $C_{carbonyl}$-C_{aryl} bond is maintained within the range of 330° to 30°.

These calculations demonstrated that the lowest energy conformations of both BNZ-amides and DHQN-amides in solution possess the internal hydrogen bond. This helps to explain, to some extent, the difference in activity between the two series (Figure 3.14). In the case of the BNZ series the hydrogen bond directs the amide substituent too close to the ZA channel, and therefore must deviate from the predicted low energy conformation. In the case of the DHQN series the hydrogen bond stabilises the bound like conformation and projects the amide substituent towards the induced fit pocket.

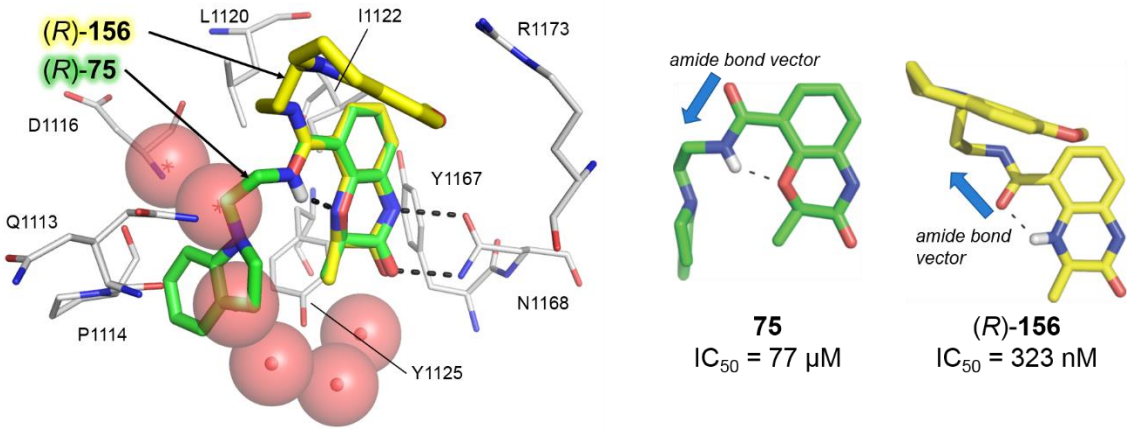


Figure 3.14: An overlay of the small molecule X-ray crystal structure of (*R*)-**75** with the X-ray crystal structure of (*R*)-**156** bound to CREBBP allows a comparison of the influence of the internal hydrogen bond on the positioning of amide group attached to either a BNZ or DHQN core.

3.6.4 Computational analysis of cation-π interaction

In a further collaboration with the Paton group, to explore the significance of the cation-π interaction in the solution phase, MD simulations were performed on the crystal structure of CREBBP bound to (*R*)-**156**. Monitoring the distance between the centre of masses of the arginine residue and the THQ aryl ring gave an indication of how often the cation-π interaction formed (Figure 3.15A). After the initial minimisation of the protein structure, a cation-π interaction was not observed since the distance between the two centres of mass was 12 Å (Figure 3.15B). However, the interaction did form early in the simulation, and by applying a 6.0 Å distance cut off,[155] was found to be present for more than 40% of the experiment time. It was also interesting to monitor the conformation of the cation-π interaction (by measuring the angle between the aryl ring and the guanidine) which showed that as well as the parallel conformation (present in the crystal structure) the T-shaped conformation was also observed (Figure 3.14C and D). The parallel conformation formed more frequently (70%), consistent with this geometry being favoured in solution.[155]

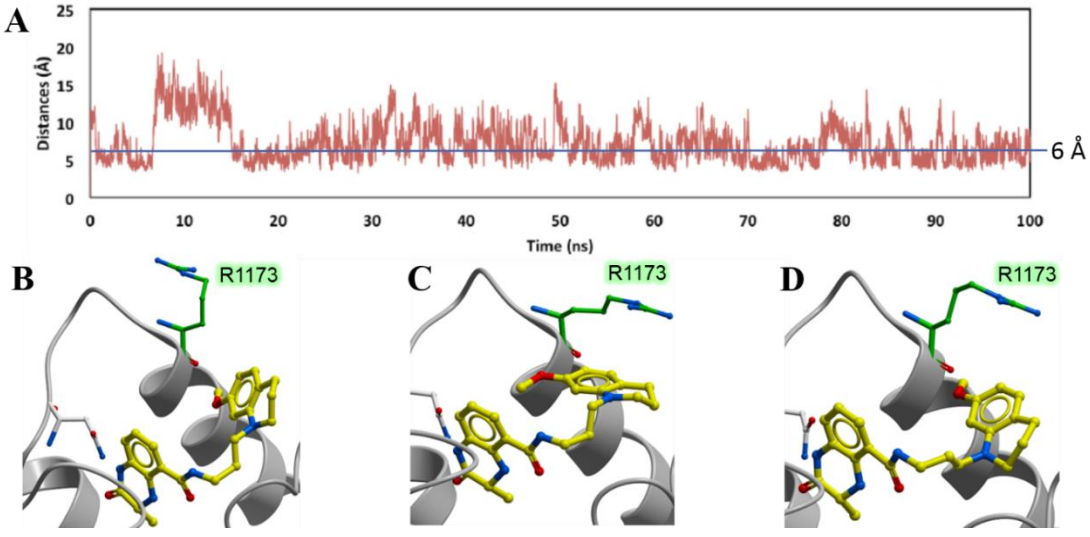


Figure 3.15: MD simulation of (*R*)-**156** bound to CREBBP. A: Plot showing the distance between the centre of masses of the cation and the aromatic ring over the course of the simulation. A distance of <6.0Å between the two was indicative of the presence of a cation-π interaction, which occurred for 40% of the time. **B**: The conformation at the start of the simulation had R1173 and the THQ 12 Å apart. **C**: An angle between the two planes of 0-45° and 135-180° was considered in the parallel conformation. **D**: In the T-shaped conformation the two planes are perpendicular with an angle between 45° and 135°.

The MD study also corroborated the importance of the internal hydrogen bond, which was not broken during the course of the simulation, and hydrogen bonds to N1168, which anchored the DHQN core in the KAc-binding pocket. Overall, it helped to validate the key binding interactions that were observed in the crystal structures of (*R*)-**119** and (*R*)-**156**.

3.7 Biological analysis of a dihydroquinoxalinone ligand as a CREBBP inhibitor

In order to evaluate whether (*R*)-**156** could displace the bromodomain of CREBBP from chromatin in a cellular setting, a fluorescence recovery after photobleaching (FRAP) assay was performed by co-workers at the SGC. FRAP provides an indication of the mobility of fluorescently-labelled intracellular molecules. Bleaching the fluorescence with laser irradiation and then recording the time taken for the fluorescence signal to recover gives an indication of how free the molecule is to diffuse, since less mobile molecules have longer recovery times.[171] In this instance, the mobility of a GFP labelled CREBBP chimera, with three tandem repeats of the bromodomain, in U2OS cells was measured (Figure 3.16). Treatment with the histone deacetylase inhibitor SAHA was required to increase lysine acetylation levels and improve the assay window. Subsequently, the fluorescence recovery time in the presence of (*R*)-**156** is dose-dependently reduced compared to the wild type (WT). At 5 μM and 10 μM of compound the recovery time is similar to the positive control in which N1168 has been mutated to a phenylalanine, indicating that (*R*)-**156** interacts with the KAc binding site of CREBBP in cells and competitively displaces it from chromatin.

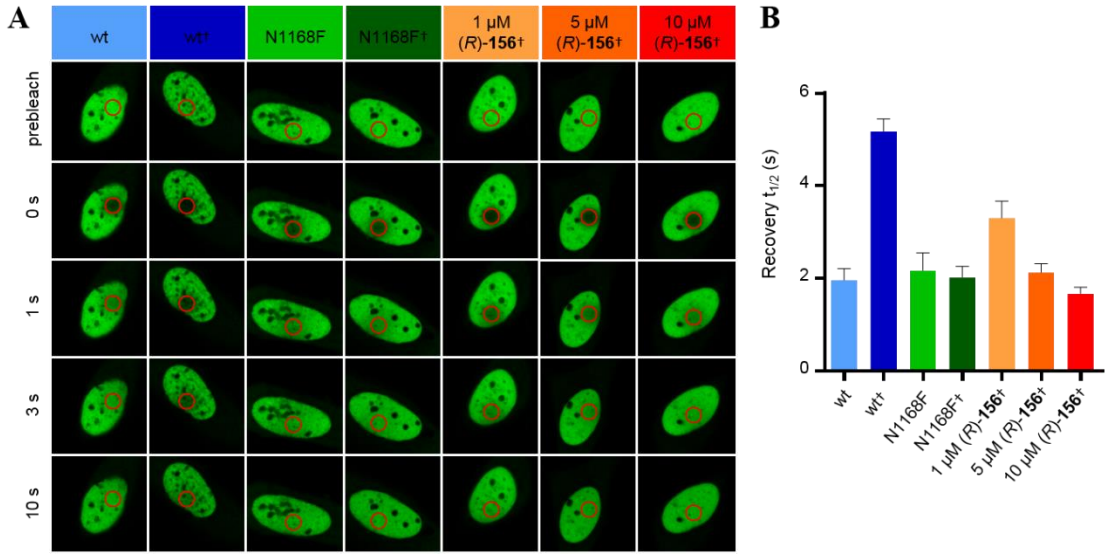


Figure 3.16: **A**: Nuclei of U2OS cells at time points after photobleaching with the bleached area indicated by a red circle. **B**: Half times of fluorescence recovery ($t_{1/2}$) of U2OS cells. Bars represent the mean $t_{1/2}$ calculated from individual recovery curves of 15 cells per group. † indicates treatment with 2.5 mM SAHA. * significantly different from WT, $p<0.0001$. ** significantly different from wt†, $p<0.0001$.

3.8 Summary

This chapter describes how a series of dihydroquinoxalinones have been developed at potent inhibitors of the CREBBP bromodomain. A number of synthetic routes to the core DHQN were investigated, with the optimum providing the key carboxylic acid in an 82% yield over 4 steps. Coupling to the previously established aminopropyl THQ side chain from the BNZ series of compounds furnished (*R*)-**119**, a sub-micromolar inhibitor of CREBBP. An X-ray crystal structure of (*R*)-**119** bound to CREBBP identified that the THQ group bound in a previously unknown induced fit pocket, stabilised by a cation-π interaction to R1173. Further analogues of the THQ side group established that the cation-π interaction could be optimised *via* addition of electron-donating groups. The optimum inhibitor, (*R*)-**156**, had a K_D of 391 nM and was found to be partially selective for CREBBP over BRD(4). Computational studies on the binding of (*R*)-**156** to CREBBP validated many of the key aspects of binding that had previously been proposed. Finally, (*R*)-**156** was shown to competitively displace CREBBP from chromatin in a cellular setting.

Chapter 4

Further bicyclic amides as bromodomain inhibitors

This chapter describes work towards the optimisation of dihydroquinoxalinone based inhibitors of CREBBP by aiming to improve the potency, selectivity and stability of the compounds described in Chapter 3. Alterations to the tetrahydroquinoline side group, propyl linker and dihydroquinoxalinone core will be described. In addition, progress towards developing an isofunctional CREBBP inhibitor with a different molecular scaffold will be described.

4.1 Aims

The crystal structure of (*R*)-**119** bound to CREBBP identified a number of opportunities to improve potency (Figure 3.11). The binding of the THQ side group to R1173 in a cation-π interaction was unprecedented and therefore not acknowledged during the initial design phase of the compounds. However, subsequent computational studies indicated that the cation-π interaction contributed significantly to the ligand's binding affinity (Section 3.6.4), and increasing the electron density in the aryl ring was found to improve potency (Table 3.5). Therefore, to further explore the influence that substituents on the THQ-group have on the cation-π interaction, a more diverse set of analogues was investigated. A further point of interest was the flexible three carbon linker (attaching the THQ side group to the DHQN core) of (*R*)-**119**. Restriction of this linker in the unbound ligand, such that it more closely resembled the protein bound conformation was therefore considered, with a view to reducing the entropic cost of binding. In order to assess whether any additional interactions within the lipophilic KAc-binding pocket could also be formed, fluorination of the DHQN core was considered. Finally, an improvement in the stability of the KAc-mimic was targeted, *via* two discrete approaches. Firstly, the preparation of DHQN analogues with substitutions that prevent oxidation were sought. In addition, an alternative core was investigated as a new KAc mimic, to which the SAR from previous compound series was applied with the aim of maintaining CREBBP inhibitory activity.

4.2 Further analogues to probe cation-π interaction

4.2.1 Rationale

It was described in Section 3.5 how a small set of analogues of the THQ side group were developed to probe the cation-π interaction. It was found that the addition of an electron-donating methoxy group did improve ligand affinity, but that this was dependent on

the position of substitution. In order to further probe the relationship between substitution pattern and ligand affinity a larger set of compounds was designed and synthesised.

4.2.2 Selection and preparation of starting materials

A range of THQs substituted with both electron-donating and electron-withdrawing groups were sought. Compounds **159-161** were available from commercial suppliers, while compounds **162-164** were available from the Pfizer compound collection (Figure 4.1). Further analogues that could be synthesised following a known literature procedure were considered, such as the benzyl ether **165**, which could be deprotected at a late stage to furnish the free phenol. In addition, the di-substituted THQs **166** and **167** were considered. Finally analogues **168** and **170**, which could be prepared from an alkylated THQ intermediate, were also considered.

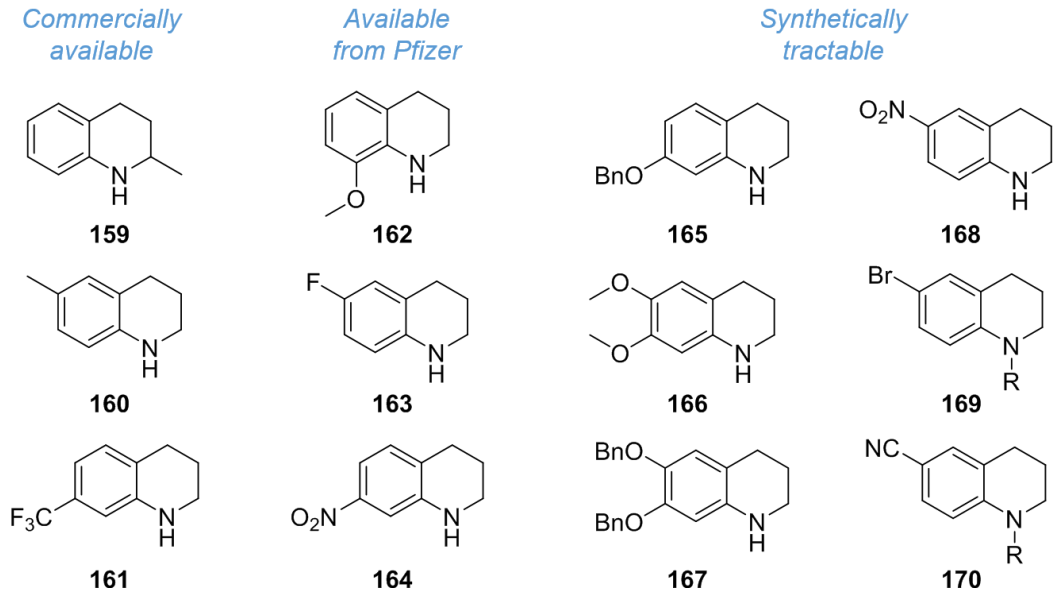
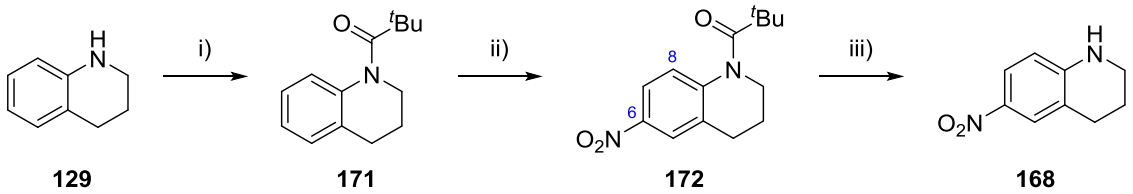


Figure 4.1: THQ analogues that were available commercially, in house, or *via* a short synthetic route.

4.2.2.1 Preparation of 6-nitro-1,2,3,4-tetrahydroquinoline

In order to prepare THQ **168**, a regioselective nitration of THQ **129** recently studied by Cordeiro *et al*, was persued.[172] Bulky amino protecting groups at the N(1)-position were found to improve selectivity for nitration at the 6-position over the 8-position. The highest selectivity for nitration

at the 6-position was observed with the Fmoc protecting group (>99%), whilst the pivaloyl group also achieved good selectivity (80%). In this work, the Fmoc protecting group was initially employed, but due to difficulties with the solubility of Fmoc-protected THQ, the pivaloyl group was also investigated. Nitration of **171** using conditions reported in the literature gave a 76:24 mixture of nitration at the 6-position and 8-position (Scheme 4.1). Fortunately the two could be easily separated by silica chromatography to afford the desired **172** in 63% yield. Subsequent deprotection with HCl furnished **168** with an overall yield from the three steps of 42%.

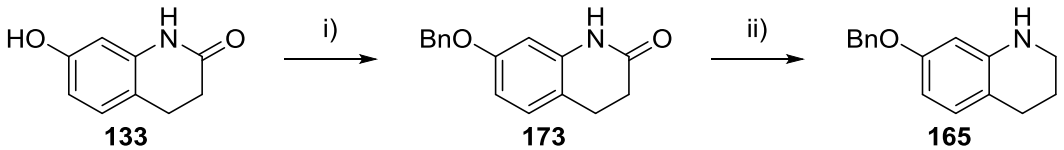


129 **171** **172** **168**

Scheme 4.1: *Reagents and conditions*: i) PivCl, pyridine, 0 °C → rt, 94%. ii) HNO_3, Ac_2O, −10 °C → rt, 63%. iii) HCl (aq.), EtOH, 100 °C, 71%.

4.2.2.2 Preparation of 7-(benzyloxy)-1,2,3,4-tetrahydroquinoline

Having previously synthesised the 7-methoxy derivative **131** (Scheme 3.8), the preparation of the 7-benzyloxy analogue **165** was achieved in a similar fashion. Alkylation of dihydroquinoxalinone **133** with benzyl bromide followed by reduction with $LiAlH_4$ furnished **165**, in an overall yield of 66% over two steps (Scheme 4.2).



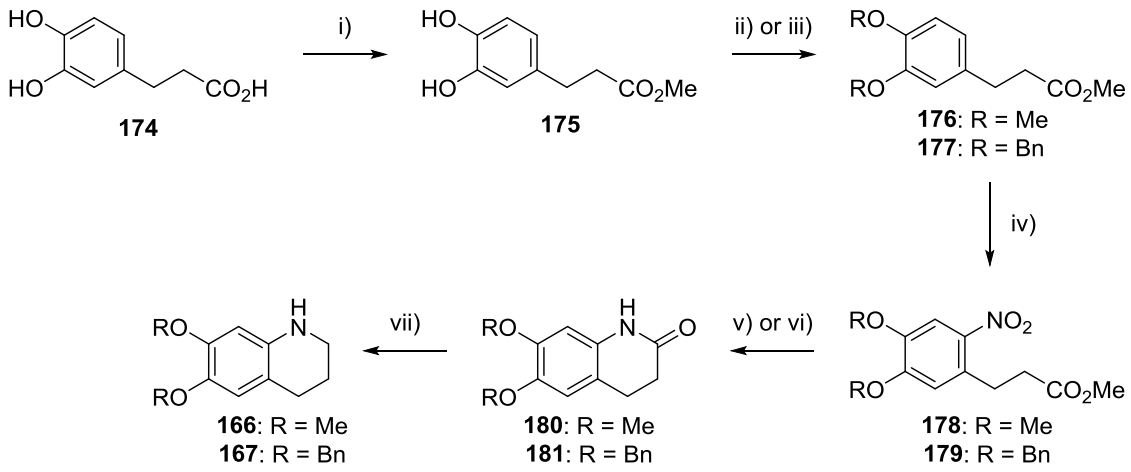
133 **173** **165**

Scheme 4.2: *Reagents and conditions*: i) BnBr, K_2CO_3, DMF, 60 °C, 70%. ii) $LiAlH_4$, THF, 0 °C → rt, 94%.

4.2.2.3 Preparation of dialkoxy-substituted tetrahydroquinolines

Dialkoxy-THQ derivatives have been synthesised previously to study the biosynthetic pathways involving catecholamines, such as dopamine, and have also been developed into

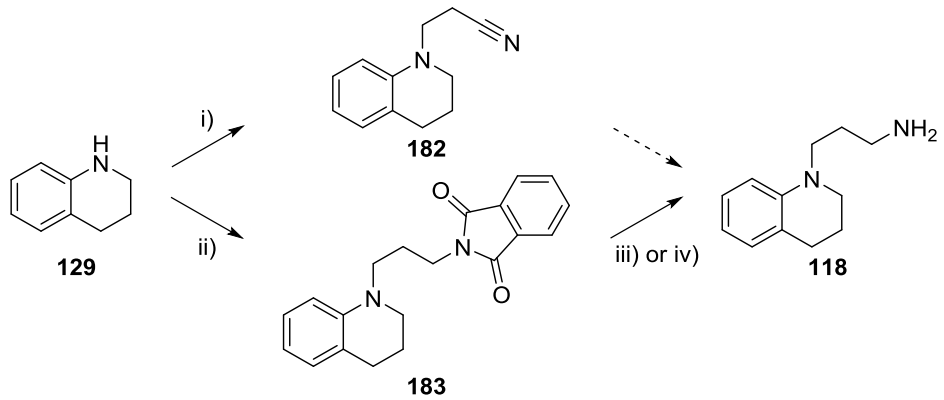
potent inhibitors of the sigma-2 receptor.[173,174] A synthetic route similar to that reported in those studies was employed, starting from 3,4-dihydroxycinnamic acid **174** (Scheme 4.3). Conversion of the carboxylic acid **174** to a methyl ester **175** was achieved using thionyl chloride in methanol in 95% yield. In order to maximise the number of steps before alkylation of the phenol with different groups, nitration of the free phenol **175** was attempted, but without success. Consequently, dialkylation with either methyl iodide or benzyl bromide was performed, with the former proceeding smoothly to afford **176** in 99% yield. However, dibenzylation to afford **177** was slow and the intermediate monobenzylated phenol, resulting from incomplete reaction, was isolated if the reaction was not left for long enough. Nitration of both **176** and **177** proceeded smoothly to afford **178** and **179** without any of the possible regioisomers being observed. Reduction of the nitro group with concomitant cyclisation to form the THQ core was achieved under hydrogenation conditions for **178** to afford **180**, or using zinc as the reductant of **179** to afford **181**. Finally, reduction of the amide to the THQs **166** and **167** was achieved using $LiAlH_4$ in 84% and 90% yields respectively. Overall the THQs **166** and **167** were prepared from 3,4-dihydroxycinnamic acid **174** over 5 steps in 64% and 35% yield respectively.



Scheme 4.3: *Reagents and conditions*: i) $SOCl_2$, MeOH, 0 °C → rt, 95%. ii) MeI, K_2CO_3, DMF, rt, >99%. iii) BnBr, K_2CO_3, NaI, DMF, 85 °C, 69%. iv) HNO_3, AcOH, 0 °C → rt, 78-84%. v) H_2, Pd/C, EtOAc, rt, 96%. vi) Zn, NH_4Cl, DMF, rt, 77%. vii) $LiAlH_4$, THF, 0 °C → rt, 84-90%.

4.2.3 Synthesis of tetrahydroquinoline analogues

In Section 3.5.4, a 5-step synthetic route was developed for the attachment of a propylamine linker to the THQ group *via* a conjugate addition reaction. Whilst this was robust, it was relatively time consuming and therefore, for the derivatisation of the 13 THQs in Figure 4.1, a shorter route was sought. Conditions to achieve the desired alkylation with an alkyl halide containing a protected amine that could then be unmasked in one step were investigated. Treatment of THQ **129** with 3-bromopropionitrile did not afford any conversion to **182**, while using 3-(bromopropyl)-phthalimide afforded **183** in a moderate 54%, reflecting the poor nucleophilicity of the aniline (not shown). It was subsequently found that reaction concentration was a key factor. By increasing the concentration 10-fold to 1.6 M, the nitrile derivative **182** could be isolated, albeit in only 18% yield, while the phthalimide derivative **183** was obtained in an excellent 90% yield (Scheme 4.4). Deprotection of the phthalimide group was investigated using either $NaBH_4$ or NH_2NH_2,[175,176] with the latter proving favourable since the phthalazine by-product could be removed *via* filtration to afford a cleaner crude sample of the product **118**.

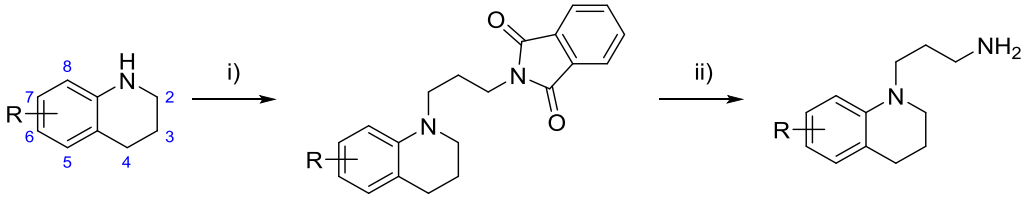


Scheme 4.4: i) 3-bromopropionitrile, K_2CO_3, DMF, 85 °C, 18%. ii) 3-(bromopropyl)-phthalimide, K_2CO_3, DMF, 85 °C, 90%. iii) $NaBH_4$, H_2O, CH_2Cl_2, IPA, rt. iv) $NH_2NH_2 \cdot H_2O$, MeOH, CH_2Cl_2, 60 °C, 41%.

With a suitable route for installing the propylamine linker established, derivatisation of the THQ analogues described in Section 4.2.1 was performed (Table 4.1). It was noted during the purification of the final primary amines that an acid-base extraction using NH_4Cl as the proton

source allowed separation of the deprotected amine **118** from any unreacted THQ **129**. Therefore, a two-step process without purification of the intermediate was investigated. Whilst this was successful in the preparation of the 6-methoxy analogue **152**, which was achieved in an overall 71% yield, the yield of the 7-benzyloxy analogue **201** was much lower due to limited solubility of this more lipophilic species. Consequently, purification of the intermediate phthalimide was performed for the remaining analogues.

Table 4.1: *Reagents and conditions*: i) 3-(Bromopropyl)-phthalimide, K_2CO_3, DMF, 28-92%. ii) $NH_2NH_2{\cdot}H_2O$, MeOH, CH_2Cl_2, 70 °C, 64-96%.



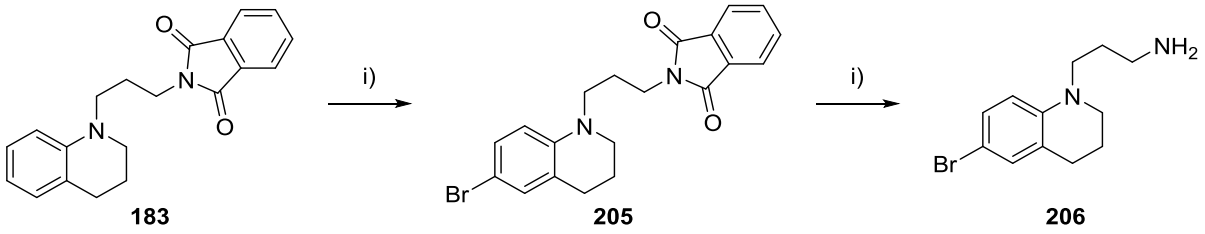
130-132, 159-168 **184-194** **152-154, 195-204**

Starting Material		Step i)		Step ii)	
Compound	R	Product	Yield	Product	Yield
130	6-OCH_3	NA	-	**152**	73%[b]
131	7-OCH_3	**184**	69%	**153**	90%
132	C(4) = O	**185**	44%	**154**	89%
159	2-CH_3	**186**	54%	**195**	64%
160	6-CH_3	**187**	92%	**196**	91%
161	7-CF_3	**188**	60%	**197**	90%
162	8-OCH_3	**189**	81%	**198**	96%
163	6-F	**190**	85%	**199**	93%
164	7-NO_2	**191**	28%	**200**	95%
165	7-OBn	NA	-	**201**	41%[b]
166	6,7-OCH_3	**192**	85%	**202**	89%
167	6,7-OBn	**193**	85%	**203**	85%
168	6-NO_2	**194**	32%[a]	**204**	87%

[a] Modified conditions; 3-(iodopropyl)-phthalimide, NaH, DMF, 0 °C → rt. [b] Yield over 2 steps.

The alkylation generally proceeded well with an average yield of 65%. Lower yields were obtained for analogues with electron-withdrawing groups attached, as demonstrated by 7- and 6-nitro substitutions **191** and **194**. A modified procedure was employed that succeeded in improving the alkylation of **168** and, although the yield was still only 35%, this allowed enough material to be prepared. The phthalimide deprotections with NH_2NH_2 gave high yields in most cases to afford the desired primary amines **152**-**154** and **195**-**204**.

Further THQ substitutions that could be installed on the phthalimide protected intermediate **183** were sought. Literature reports show that *N*-bromosuccinimide (NBS) has been used to brominate both anilines and *N*-alkyl-tetrahydroquinolines in good yields.[177,178] Subjecting **183** to the same conditions allowed conversion to the 6-bromo analogue **205** in 44% yield and subsequent deprotection with NH_2NH_2 afforded the primary amine **206** (Scheme 4.5).



Scheme 4.5: *Reagents and conditions*: i) NBS, MeCN, rt, 44%. ii) $NH_2NH_2 \cdot H_2O$, MeOH, CH_2Cl_2, 70 °C, 35%.

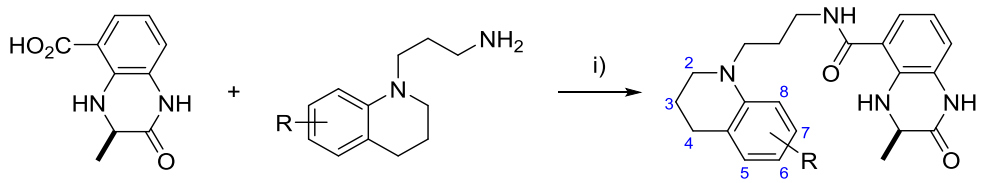
With the brominated analogue in hand, further derivatisation was possible using the multitude of metal-catalysed coupling conditions available to functionalise aryl halides. It was proposed that a nitrile analogue was an interesting target; being a strongly electron-withdrawing group it may be expected to weaken the cation-π interaction, however, it has been reported to form hydrogen bonds to arginine,[179] which could also occur when the compound binds to CREBBP. A nitrile group can be installed through the Rosenmund-von Braun reaction of aryl halides and copper cyanide, and has been reported on 6-bromo-tetrahydroquinoline.[180] However, reaction of **205** under similar conditions returned only starting material. This transformation may be

possible by performing the coupling at higher temperature. Alternatively, a 2-step procedure proceeding *via* the 6-position aldehyde, has been reported on THQ **129** but would require additional steps.[181] No further analogues were sought at this time, although **205** represents a valuable starting point should the introduction of groups to the 6-position of THQs be required in the future.

4.2.4 Attachment to dihydroquinoxalinone core

With the 11 novel amines **195**-**204**, **206** in hand, HBTU-mediated coupling to the DHQN carboxylic acid (*R*)-**94** was performed (Table 4.2). The reaction proceeded well for almost all the analogues, with an average yield of 57%. The 6-bromo analogue **217** had poor solubility, accounting for the lower yield, whilst the 6-fluoro analogue **211** was unstable and could not be isolated.

Table 4.2: *Reagents and conditions*: i) HBTU, DIPEA, DMF, rt, 25-80%.



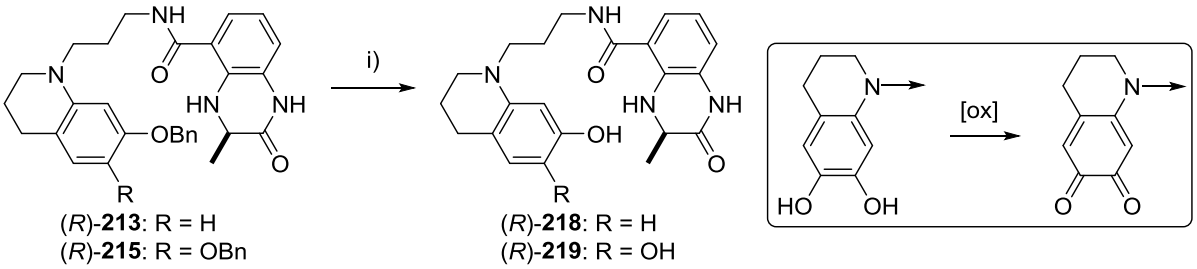
(*R*)-**94** | **195-204**, **206** | (*R*)-**207** - (*R*)-**217**

Product	R	Yield
(*R*)-**207**	2-CH_3	39%
(*R*)-**208**	6-CH_3	74%
(*R*)-**209**	7-CF_3	54%
(*R*)-**210**	8-OCH_3	49%
(*R*)-**211**	6-F	0%
(*R*)-**212**	7-NO_2	73%

Product	R	Yield
(*R*)-**213**	7-OBn	55%
(*R*)-**214**	6,7-OCH_3	56%
(*R*)-**215**	6,7-OBn	67%
(*R*)-**216**	6-NO_2	80%
(*R*)-**217**	6-Br	25%

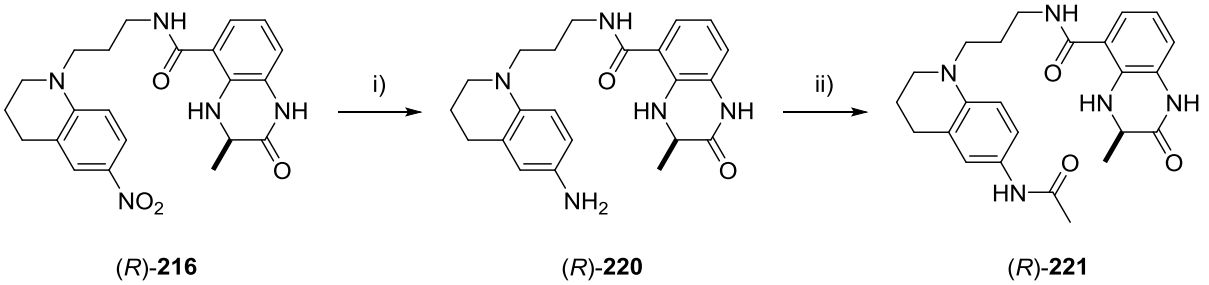
Some analogues had deliberately been chosen as they offered the opportunity for further derivatisation. The benzyloxy THQs (*R*)-**213** and (*R*)-**215** were subject to hydrogenolysis conditions to allow the preparation of the free phenols (*R*)-**218** and (*R*)-**219** (Scheme 4.6).

Whilst this was successful for (*R*)-**218**, which was isolated in 69% yield, the diphenol (*R*)-**219** oxidised as soon as the hydrogen atmosphere was removed. The propensity of similar catechols to oxidise has been well studied given their occurrence in melanin and their biological role as a protecting agent against oxidative damage.[182]



Scheme 4.6: *Reagents and conditions*: i) H_2, Pd/C, EtOAc, 0%-69%. *Inset*: Oxidation of catechols.

The nitro THQ was also subjected to hydrogenation conditions in order to prepare the amine (*R*)-**220** (Scheme 4.7). This compound could be isolated and purified by silica chromatography but was found to be unstable. To confer stability, the amine was acetylated using acetic anhydride furnishing a stable acetate (*R*)-**221** in 72% yield.



Scheme 4.7: *Reagents and conditions*: i) H_2, Pd/C, EtOAc, 42%. ii) Ac_2O, CH_2Cl_2, rt, 72%.

4.2.5 Computational analysis of tetrahydroquinoline analogues

Unfortunately, it was not possible to obtain *in vitro* data for the inhibition of CREBBP by compounds (*R*)-**207**-(*R*)-**221** at this time and future work will continue to pursue this aim. It was possible, however, in collaboration with the Paton group, to obtain computational data relating to the predicted strength of the cation-π interaction these compounds could form. Whilst there is much debate about the effects that substituents have on the strength of a cation-π

interaction, it has been reported that considering the electrostatic potential on the surface of an aryl ring is sufficient to predict the trend in affinity.[154] Analysis was therefore performed on simplified versions of the synthesised THQ analogues **222-225** and revealed how the addition of electron-donating groups, such as the 6-methoxy substituent in **223**, increased the negative potential in the ring (Figure 4.2A vs B). In contrast, attachment of electron-withdrawing groups caused a shift in the negative potential from the centre to the outside of the ring, which could be expected to lead to a weakening of the cation-π interaction (Figure 4.2D). It also highlighted the importance of the position of the substitution, as evidenced by comparison of the 6- and 7-methoxy THQs **223** and **224**, with the former showing a greater degree of negative charge in the centre of the ring (Figure 4.2B vs C).

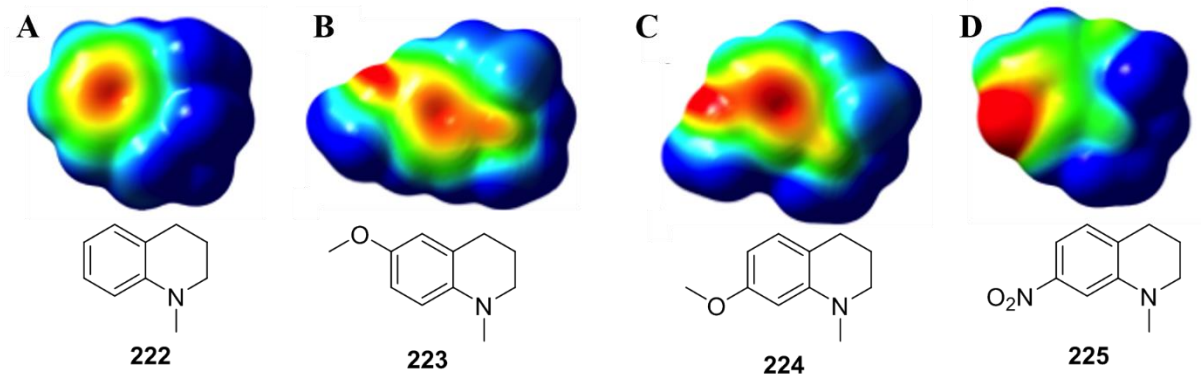


Figure 4.2: Electrostatic potential of some THQ analogues.

Whilst these calculations are valuable, they do not take into account the steric demands associated with the protein bound interactions. In the future, it would be interesting to compare the theoretical strength of the cation-π interaction with the *in vitro* activity of the compounds, which would reveal whether this computational assessment could be used as a rapid method for selecting suitable side chains. Additionally, if the thermodynamic parameters of binding could be determined experimentally (using ITC or SPR), the enthalpic contribution would be of particular interest for validating the computational method.

4.2.6 Summary

In summary a number of THQ analogues, in addition to those encountered in Section 3.5, were prepared containing both electron-donating and withdrawing groups at various positions. Attachment of a propyl amine linker to N(1) of the THQs allowed these analogues to be coupled to the DHQN core. *In silico* calculations were performed on this set of compounds to predict the propensity of the analogues to form a cation-π interaction to an arginine residue. Whilst it was not possible to assess the *in vitro* inhibition of CREBBP within the timeframe of this project, future work on the analysis of these compounds will allow evaluation of the computational model as a technique for predicting the formation of cation-π interactions.

4.3 Rigidification of linker

4.3.1 Rationale

A common approach in the optimisation of protein-ligand interactions is to rigidify the conformation of the ligand such that it more closely resembles the protein-bound conformation. Performing this preorganisation is expected to improve the binding affinity of a compound by lowering the entropic cost of binding. This principle has been widely employed in the design peptidomimetics, where cyclisation of a short peptide segment can induce a desired secondary structure to the backbone. For example, the β-turn adopted by CREBBP-binding region of p53 was stabilised by cyclising the linear peptide **226** to give **4**, leading to a 6.6-fold improvement in affinity (Figure 4.3A).[87] Further control over the spatial orientation of side chains in a peptidomimetic has been achieved by incorporating cyclopropane rings into the peptide backbone.[183] Preorganisation has also been employed to improve both the selectivity and bioavailability of compounds by reducing the chance of binding to undesired proteins in alternative conformations. These effects were exemplified in the development of the cathepsin K inhibitor **228**, where cyclisation of **227** led to a 4000 fold increase in potency and higher oral bioavailability (%F) (Figure 4.3B).[184]

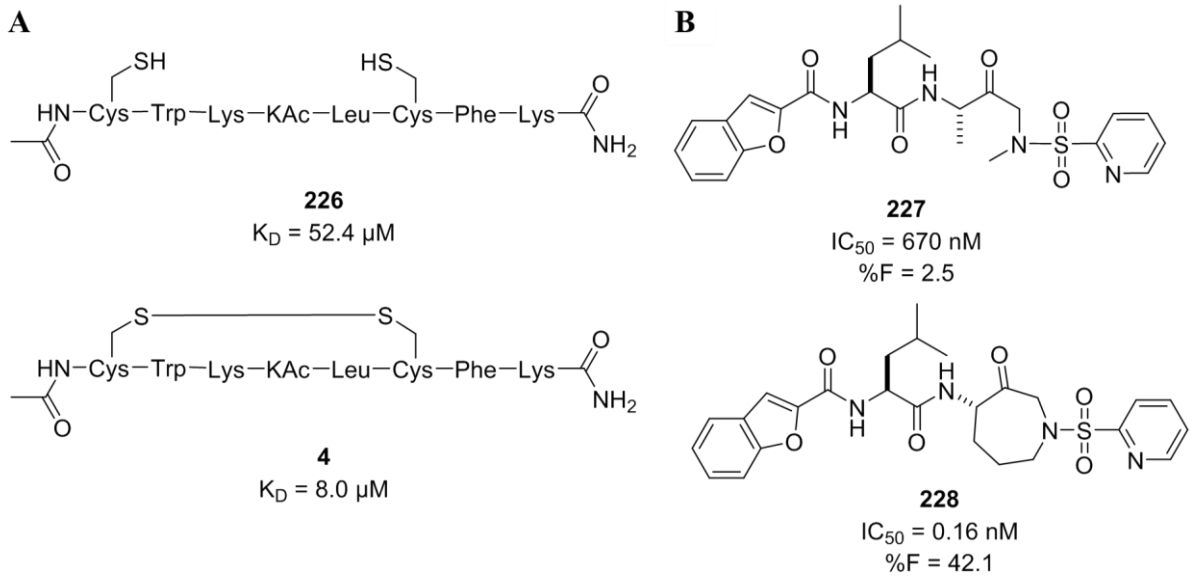


Figure 4.3: **A**: Rigidification of the CREBBP-binding peptide **226** by cyclisation to **4** stabilised the β-turn structure and improved binding affinity.[87] **B**: The cathepsin K ligand **228** was more potent and had improved bioavailability in the cyclised form.[184]

An examination of the crystal structures of (*R*)-**119** and (*R*)-**156** bound to CREBBP revealed that the propyl linker adopts a non-linear conformation in order for the THQ to access the induced fit pocket beneath R1173. It was tempting, therefore, to pursue a method for rigidifying the conformation of the chain to make the CREBBP bound conformation more accessible, thereby improving potency by reducing the entropic cost of binding. This approach might also improve selectivity over BRD4 by making the bound conformation of (*R*)-**156** with this protein inaccessible. Attachment of a *trans*-cyclopropyl group between C(2') and C(3') was therefore considered (Figure 4.4A). This modification would reduce the number of rotatable bonds in the linker to two and would achieve the desired *trans* arrangement of the C(1')-C(2') and the C(3')-N(THQ) bonds. Indeed, the predicted lowest energy conformation of the side chain, represented by **230**, shows good agreement with the protein bound structure of (*R*)-**119** (Figure 4.4B and C).

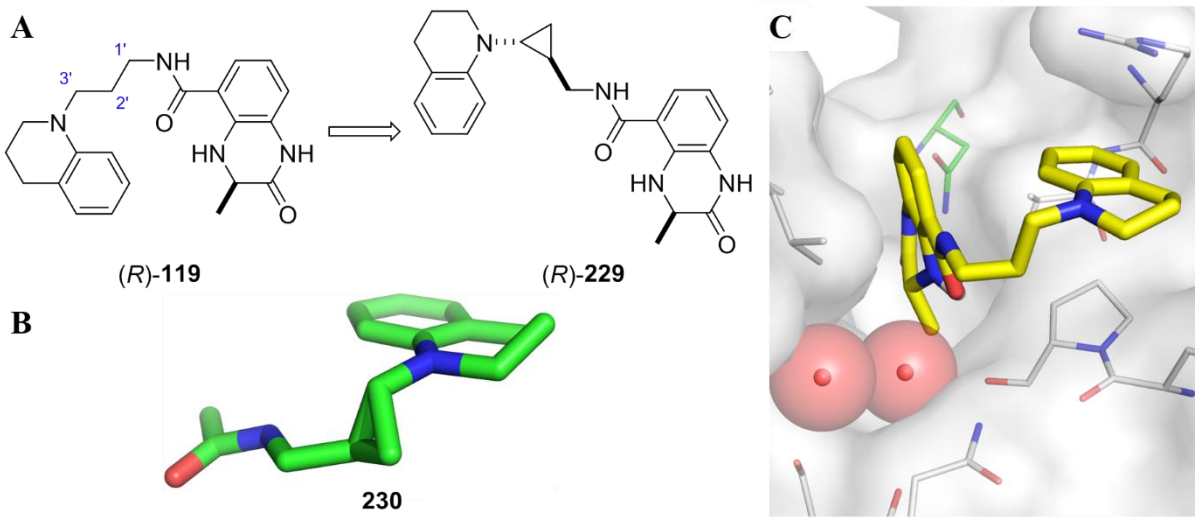


Figure 4.4: **A** Proposed addition of a cyclopropyl group between C(2') and C(3') of the propyl-linker. **B**: ChemBio3D model of a *trans*-cyclopropyl group. The conformation was obtained by performing an MM2 energy minimisation. **C**: Crystal structure of (*R*)-**119** bound to CREBBP (PDB ID: 4NYW).

It is important to note that ligand preorganisation is not always accompanied by an entropic advantage.[185] The factors that contribute to the binding affinity of a compound are complex and in many cases attempting to match the conformation of a ligand to the bound state has failed to improve potency.[183] One factor that is often overlooked is the entropic cost to the protein in forming and maintaining a favourable enthalpic interaction with a ligand (the relevance of this so called enthalpy-entropy compensation has received much debate). Since in solution, a protein-ligand complex adopts many different poses, it is often the case that flexible domains require dynamic ligands in order to form and maintain favourable interactions.[149] Therefore, restricting a ligand's conformation to completely match that observed in a crystal structure, which is a static snapshot of the protein conformation, may be detrimental. This is exemplified in the MD studies performed on the binding of CREBBP to (*R*)-**156** (Section 3.6.4), during which the mobility of the arginine residue often requires the propyl chain to change conformation in order to maintain the cation-π interaction. Nevertheless, it would be interesting to investigate whether installing some rigidity had an effect on either the potency or selectivity of the compound.

4.3.2 Retrosynthetic analysis of cyclopropyl tetrahydroquinolines

A retrosynthetic analysis of the desired compound identified a number of possible ways to install the cyclopropyl group. Cyclopropyl groups are commonly formed *via* the reaction of a carbene with an alkene and, for a di-functionalised cyclopropyl group such as **232**, there are 3 possible carbene-alkene combinations (Figure 4.5). Potentially the most simple is the addition of methylene **234** to an alkene **233**, which contains a functional group that could be converted to the desired primary amine (Route A). Alternatively, addition of a carbene **236** containing a suitable functional group to alkene **235** could be performed to access the desired intermediate (Route B). Finally, a methyl carbene could be generated on the THQ containing species **237** and coupled to an appropriate alkene **238** (Route C). Each of these approaches is discussed below.

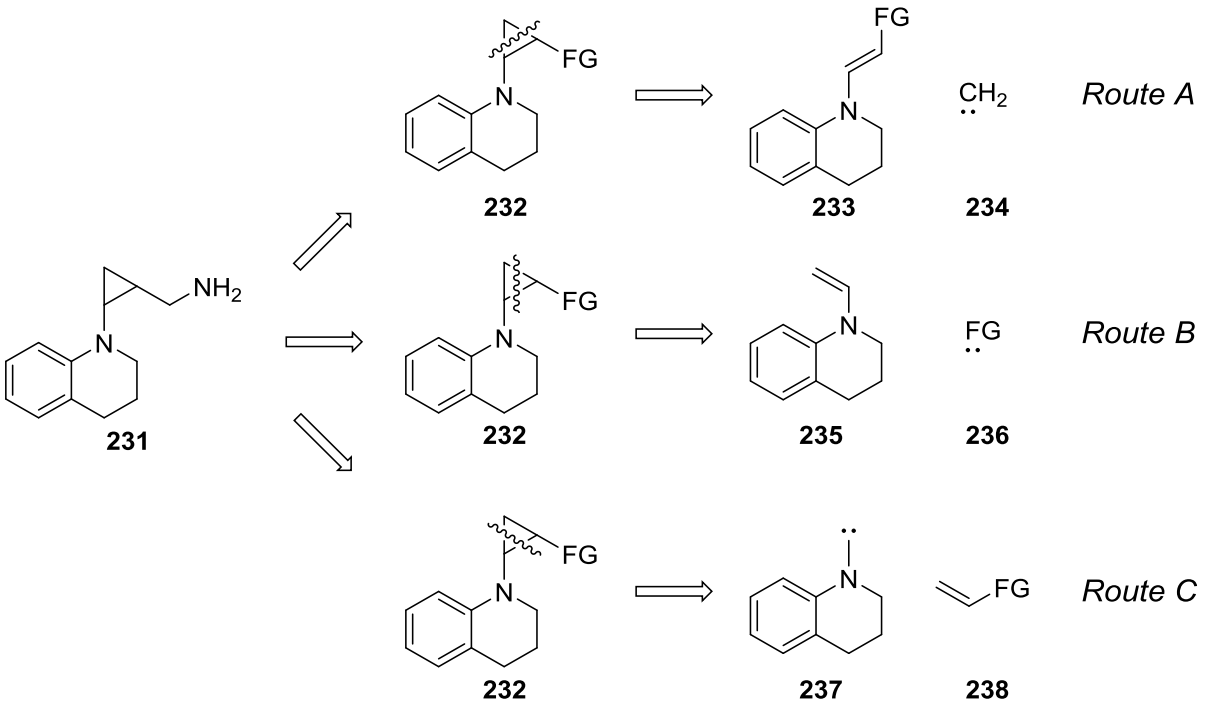
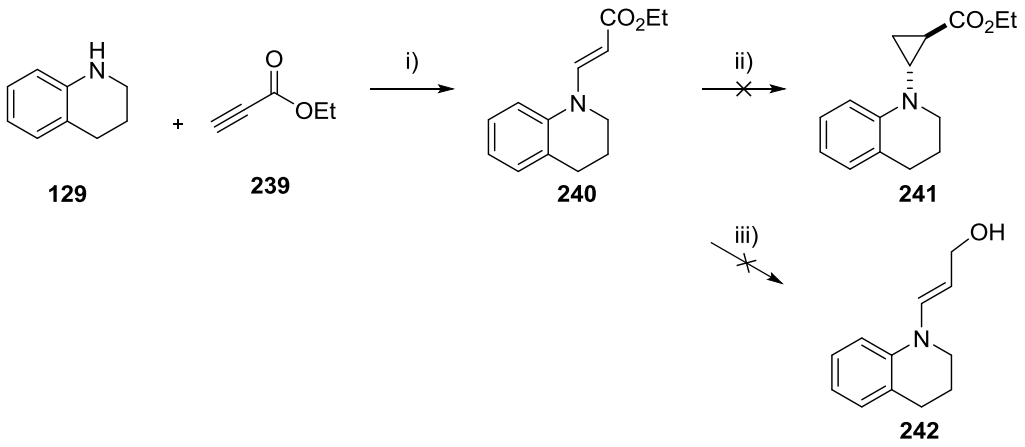


Figure 4.5: Retrosynthetic analysis of the di-functionalised cyclopropyl amine target **231**.

4.3.3 Route A – Addition of methylene

In this route, a methylene unit would be added to a suitably functionalised alkene in the cyclopropanation step. In order to synthesise the desired alkene, a route similar to the THQ derivatisation discussed previously (Section 3.5.4) was employed. Conjugate addition between an amine and an alkyne has been reported as a facile method for the synthesis of enaminones.[186,187] Reaction of THQ **129** and ethyl propiolate **239** in THF at room temperature according to one set of literature conditions resulted in no consumption of starting material.[186] However, upon addition of a protic solvent and heating under reflux the reaction proceeded well, affording the conjugated enamine **240** in 68% yield (Scheme 4.9). An analysis of **240** by NOE-spectroscopy showed that the desired (*E*)-double bond had been formed, which was important for establishing the *trans*-cyclopropyl geometry in the subsequent steps (Appendix A4.1).

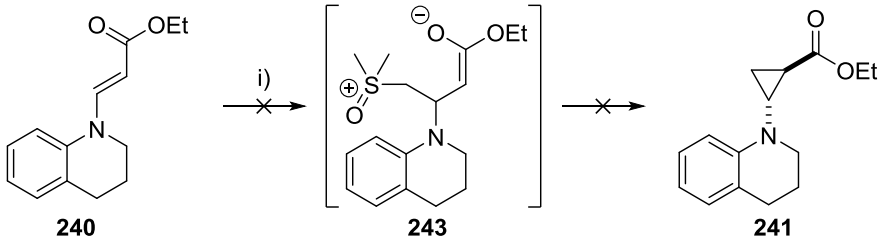


Scheme 4.9: *Reagents and conditions*: i) Ethyl propiolate, THF, EtOH, 80 °C, 68%. ii) CH_2I_2, Et_2Zn, CH_2Cl_2, rt, 0%. iii) DIBAL (1 M in hexanes), CH_2Cl_2, –40 °C, 0%.

With alkene **240** in hand, conditions to achieve the desired cyclopropanation were investigated. One of the most common methods for transfer of a methylene group is a [2+1] cycloaddition using a zinc carbenoid in the Simmons-Smith reaction. The broad substrate scope of this reaction has resulted in its extensive use on both electron poor and electron rich alkenes, including enamines.[188] A multitude of reagents exist for generation of the halomethylmetal

reagent. The use of Et_2Zn and CH_2I_2 was chosen in this instance due to commercial availability (Scheme 4.9). However, despite observing the formation of a colourless precipitate, indicating the formation of the Wittig-Furukawa reagent ($EtZnCH_2I$),[189] upon addition of olefin **240**, no evidence of cyclopropanation was observed. Hydroxyl groups have been employed to direct a zinc carbenoid to a certain face of an alkene, so in an effort to improve the reactivity of the olefin, reduction of the ester to primary alcohol **242** was proposed. However, upon treatment of **240** with DIBAL, only THQ **129** was isolated, indicating that hydrolysis of the enamine had occurred and suggesting the ester group was a requirement for its stability (Scheme 4.9).

An alternative approach to concurrent addition of the methylene unit is a sequential formation of the cyclopropyl bonds. This can be achieved *via* conjugate addition to an electrophilic olefin, resulting in the formation of an enolate **243** that can then undergo an intramolecular ring closing reaction (Scheme 4.10). The already prepared α,β-unsaturated ester **240** was considered to be a suitable substrate for this reaction, with a possible methylene transfer reagent being a sulfonium ylid.[190] The ylid was generated by treating a solution of trimethylsulfonium iodide in DMSO with NaH before the addition of a solution of the alkene **240**. However, none of the desired cyclopropyl product **241** could be isolated.



Scheme 4.10: *Reagents and conditions*: i) NaH, TMSOI, DMSO, rt, 0%.

4.3.4 Route B – Addition of functionalised carbenoid

In this route a suitably functionalised carbenoid would be added to the THQ-alkene **235**. There is one reported synthesis of **235** in the literature, a palladium-catalysed amination of allyl alcohol with $Pd(PPh_3)_4$ and $SnCl_2$.[191] However, given that compound **240** had been found to

hydrolyse back to **129** upon reduction of the ester, it was envisaged that the stability of **235** would limit its use. Therefore no further studies were undertaken for this route.

4.3.5 Route C – Addition of amido-carbenoid

A method for the direct addition of amido-organozinc carbenoid to alkenes has recently been reported by the Sheppard group.[192,193] By starting from a diethoxymethylamide **244**, treatment with a Lewis acid (LA) and zinc led to the generation of the organozinc carbenoid **246**, which then underwent cyclopropanation with a range of alkenes **247** (Figure 4.6). This method was reported to have been successful with various amides including lactams and oxazolidinones and was therefore applied to the cyclopropanation of 3,4-dihydro-2(1*H*)-quinolinone **249**.

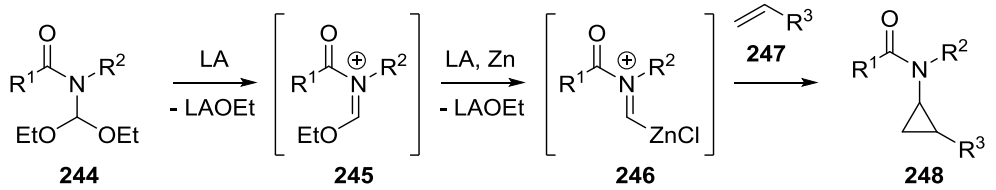
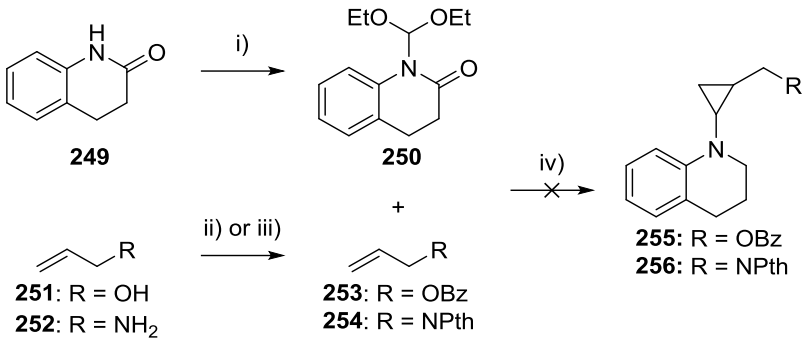


Figure 4.6: Cyclopropanation *via* generation of an amido-organozinc carbenoid from an orthoester.[192]

Addition of a diethoxymethyl group to **249** has been reported on one occasion and proposed as a protecting group for amide nitrogens.[194] Treatment of **249** with triethylorthoformate and $AlCl_3$ resulted in slow conversion to **250** and, after 70 h of heating at 155 °C, the product could only be isolated in 33% yield (Scheme 4.11). The low yield, similar to that reported in the literature,[194] is probably due to the poor nucleophilicity of the aniline nitrogen and may have been improved by using the more active diethylphenyl orthoformate.



Scheme 4.11: i) Triethylorthoformate, $AlCl_3$, 155 °C, 33%. ii) Benzoyl chloride, Et_3N, Et_2O, rt, 81%. iii) Phthalic anhydride, AcOH, 150 °C, 78%. iv) Zn, CuCl, $ZnCl_2$, Me_3SiCl, Et_2O, 40 °C, 0%.

Two alkenes were sought as suitably functionalised coupling partners for use in the cyclopropanation. Benzoyl protected alcohol **253** was prepared by treatment of allyl alcohol **251** with benzoyl chloride,[195] and phthalimide protected amine **254** was prepared by treatment of allyl amine **252** with phthalic anhydride (Scheme 4.11).[196] Subsequently, the cyclopropanation was attempted using Zn and catalytic CuCl as the metal source and freshly distilled Me_3SiCl as the Lewis acid. Unfortunately, neither cyclopropyl **255** nor **256** could be isolated from reaction of **250** with either olefin. It was found that the diethoxymethylated THQ **250** was unstable to storage at rt and perhaps had decomposed during the reaction. It may be possible to form the orthoester *in situ* during the cyclopropanation reaction, although it is probable that the poor nucleophilicity of the amine may again hinder this approach.

4.3.6 Summary

In summary, a number of routes to obtain cyclopropylamine **231** were proposed and attempted, although none were successful. Work to access **231** is being continued by other members of the Conway group and alternative methods for achieving the desired rigidification of the linker are also under investigation.

4.4 Fluorination of dihydroquinoxalinone core

4.4.1 Rationale

Addition of a fluorine atom is a popular approach in the optimisation of biologically active compounds.[197,198] This is evident in the fact that at least one fluorine atom is contained in 30% of the top 20 small molecule drugs by 2013 sales and around 20% of all pharmaceuticals administered in the clinic.[199] There are several ways in which addition of fluorine can improve the efficacy of a compound, enhancing both physiochemical properties and the strength of protein-ligand interactions. Addition of fluorine is commonly performed to block a position that is susceptible to metabolic oxidation by cytochrome P450 enzymes. The strong electron-withdrawing effect of an added fluorine has also been employed to modulate the pKa of nearby groups,[200] most commonly to reduce the basicity of a neighbouring basic centre in order to improve bioavailability. Both of these motives were exemplified in the optimisation of the 5-HT_{2A} receptor antagonist **257** (Figure 4.7A). A fluorine was introduced to the piperidine ring in order to reduce the pKa of the basic nitrogen and improve bioavailability, whilst a second fluorine was introduced to the indole to improve metabolic stability.[201] Another reason for performing aryl-fluorination is the resultant increase in lipophilicity, due to excellent overlap between the fluorine and carbon 2s or 2p orbitals making the C-F bond highly non-polarisable.[197] This effect is often exploited to aid in the formation of non-specific lipophilic interactions with a protein, or to reduce the cLogP of a compound to improve cell permeability. Whether fluorine can form true hydrogen bonds is a controversial topic due to the low polarisability of the C-F bond,[202] however, close C-F···H-N contacts that can be rationalised as favourable dipolar interactions are becoming increasingly abundant in the PDB.[203] The influence of a favourable intramolecular C-F···H-N interaction appears to be a contributing factor in the activity of two isomers of fluoronorepinephrine; **259** being an α-adrenergic agonist and **258** a β-adrenergic agonist (Figure 4.7B).[204] In summary, addition of fluorine to a ligand

can be used to tune both physical properties (metabolic stability, pKa) and interactions with the protein (lipophilic or dipolar interactions).

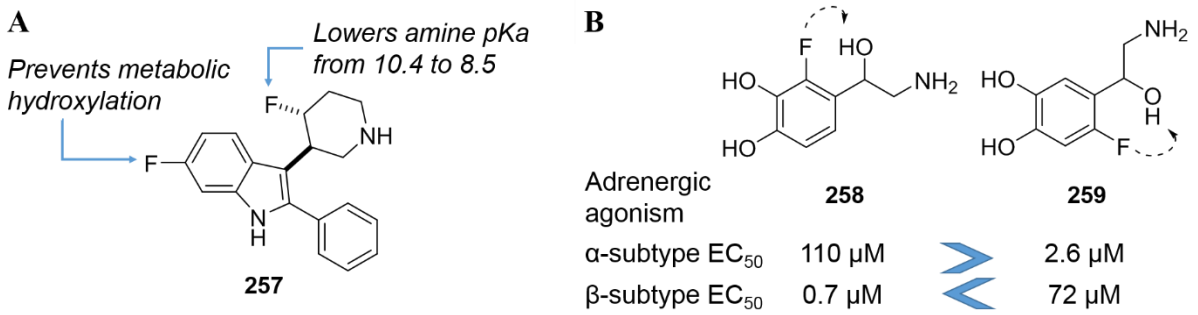


Figure 4.7: **A**: The physiochemical properties of **257** were optimised through the addition of fluorine. **B**: An intramolecular F···H-O interaction has been proposed to explain the differences in adrenergic agonistic properties of fluoronorepinephrine isomers.

There were a number of reasons why introduction of a fluorine atom to the DHQN-core would be interesting to investigate (Figure 4.8): Since the core binds in a largely hydrophobic pocket, the introduction of a fluorine could lead to an increased binding affinity through non-specific lipophilic interactions. Altering the electronics of the ring could affect the nature of the hydrogen bonds, both intramolecularly and to the protein, although it is not clear from the outset whether this would be beneficial or detrimental. A fluorine in the 6-position of **260** would be of additional interest since it would be in close contact with the amide N-H, and an interaction between the two could help further direct the vector of the amine bond [in addition to the existing H-bond to N(4)].

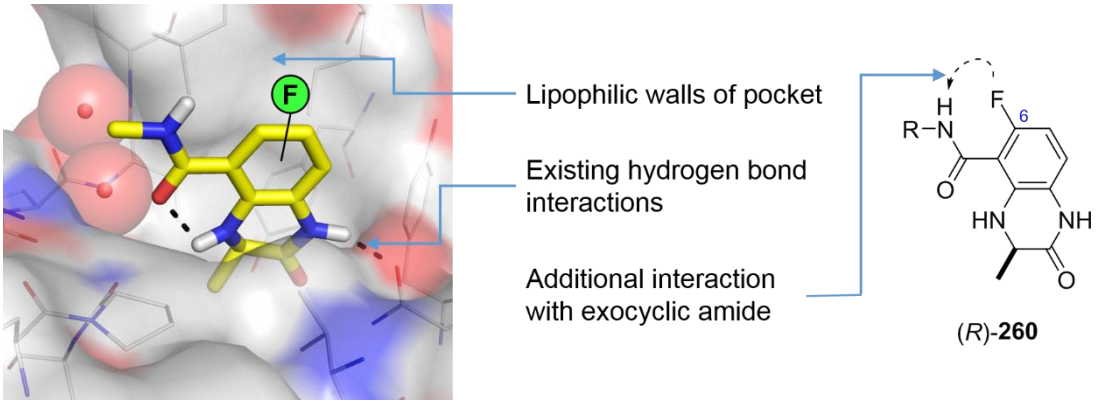


Figure 4.8: Potential influence of fluorine substitution on DHQNs as bromodomain inhibitors.

4.4.2 Retrosynthetic analysis of fluorinated dihydroquinoxalinones

In order to obtain the desired fluorinated DHQN analogues (*R*)-**261**, a route similar to that used for the unfluorinated analogues was considered (Figure 4.9). However, due to the limited commercial availability of the desired tetrasubstituted phenyls, it was expected that at least one of the groups would have to be installed. By starting from the readily available fluorinated-salicylic acid derivatives **264**, nitration could be expected to be directed to the desired 3-position by the 2-positioned phenol. The resulting phenols could then be converted to the triflate **263** (R^2 = OTf) allowing installation of the chiral centre *via* an S_NAr coupling reaction with D-alanine to yield **262**. Importantly, the selectivity of such a reaction for the desired substitution of R^2 at the 2-position, rather than the newly introduced fluorine group, would have to be monitored. Reduction of the nitro-group to form the cyclised core, followed by ester deprotection, would afford the desired carboxylic acid analogues (*R*)-**261**.

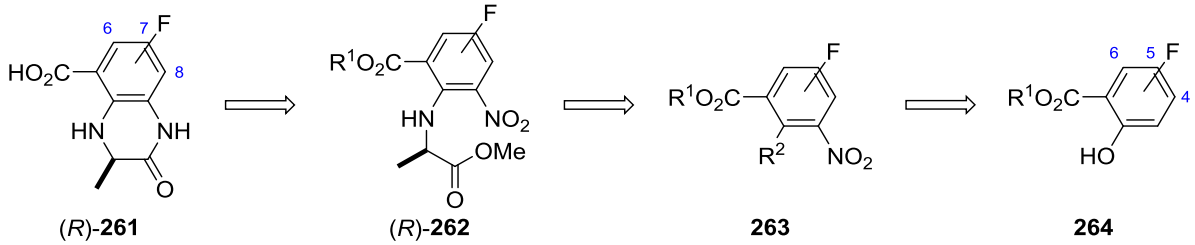
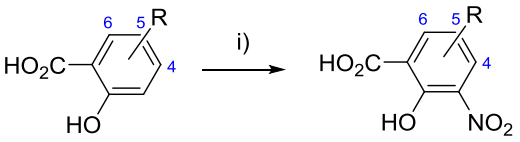


Figure 4.9: Retrosynthetic analysis of carboxylic acid (*R*)-**261** with a fluorine group at the 6-, 7- or 8-position.

Following the conditions for nitration of 5-fluoro-2-hydroxybenzoic reported in a patent,[205] the desired nitrophenol **269**, fluorinated at the 5-position, was successfully synthesised (Scheme 4.12). The splitting of the single fluorine peak in the ^{19}F NMR (an apparent triplet, J = 8.3 Hz, from coupling to protons at the 4- and 6-position) supported the expected addition of the nitro-group to the 3-position. However, application of the same conditions in pursuit of the 4- and 6-fluoro compounds **268** and **270**, afforded a mixture of products, potentially a consequence of the unsubstitued 5-position also being susceptible to nitration.



265: R = 4-F
266: R = 5-F
267: R = 6-F

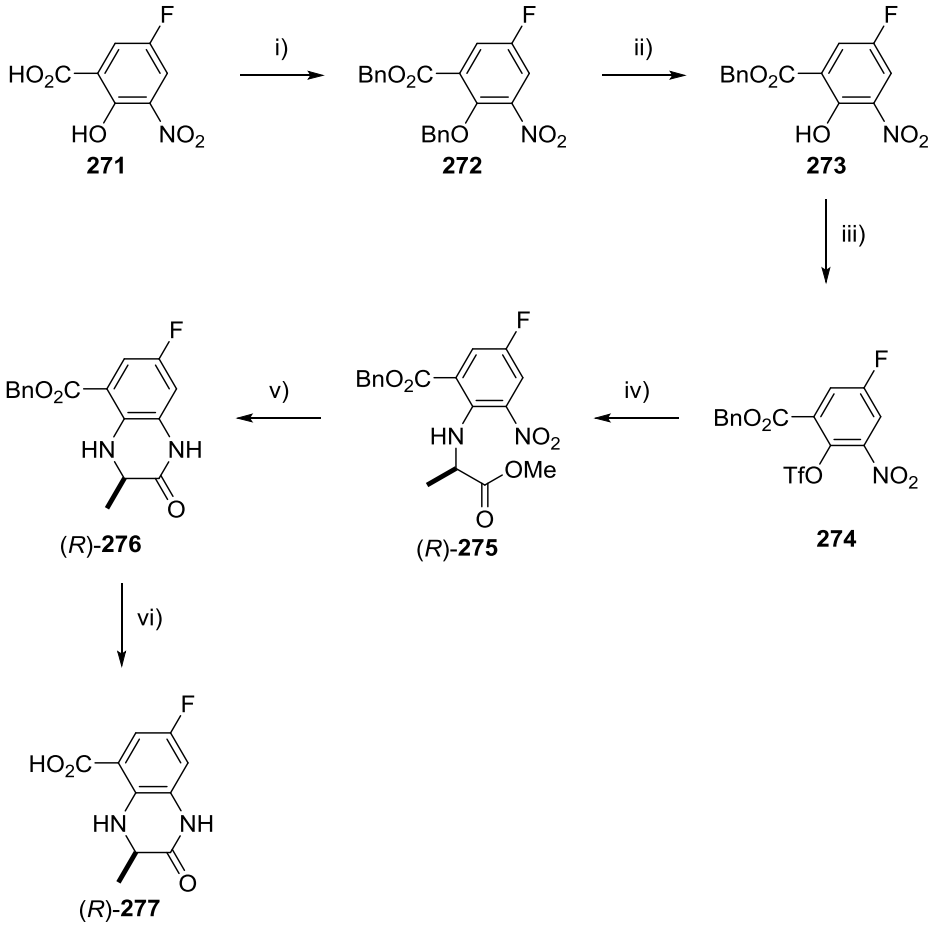
268: R = 4-F
269: R = 5-F
270: R = 6-F

Scheme 4.12: *Reagents and conditions*: i) HNO_3, H_2SO_4, 0 °C, 0–86%.

With carboxylic acid **269** in hand, the synthesis of the 7-fluoro-DHQN analogue of **261** could be pursued (Section 4.4.3). In order to access the 6-fluoro or 8-fluoro-DHQNs, an alternative approach was required. Fortunately 2,6-difluoro-3-nitrobenzoic acid was commercially available, so to access the 6-fluoro-DHQN analogue of **261**, the retrosynthetic route outlined in Figure 4.9 could still be applied with **263** R^2 = F (Section 4.4.4). The 8-fluoro-DHQN was not investigated further.

4.4.3 Synthesis of 7-fluoro analogue

In a similar fashion to the unfluorinated analogue (Section 3.2.3), selective formation of the benzyl ester of **271** was best achieved using a two-step process. Dibenzylation of **271** was achieved using BnBr and K_2CO_3 in 46% yield, then deprotection of the benzyl ether of **272** could be accomplished with TFA to afford **273** in 59% yield (Scheme 4.13). The drop in yield compared to the unfluorinated analogue was in part due the greater acidity of the phenol making purification of **273** by silica gel chromatography difficult. The presence of the fluoride did not cause any issues during the triflation reaction, which proceeded well to afford the aryl-triflate **274** in 83% yield. Equally, the S_NAr reaction with D-alanine yielding (*R*)-**275**, followed by reduction with zinc to prepare the cyclised (*R*)-**276** proceeded with similar yields to those achieved previously. Deprotection of the benzyl ester afforded the carboxylic acid (*R*)-**277** in a slightly lower (65%) yield due to the poor solubility of the free acid causing some material to be lost during filtration. Overall, the DHQN **277**, containing a fluorine substitution at the 7-position, was synthesised from **266** in seven steps with an overall yield of 8%.

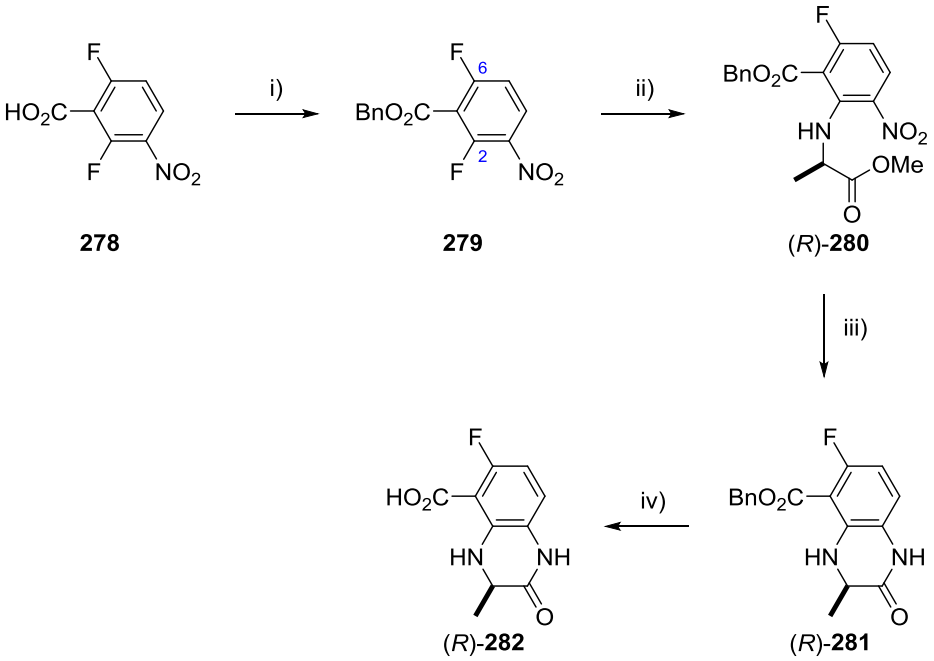


Scheme 4.13: *Reagents and Conditions*: i) BnBr, K_2CO_3, DMF, 70 °C, 46%. ii) TFA, toluene, rt, 59%. iii) Tf_2O, Et_3N, CH_2Cl_2, 0 °C to rt, 83%. iv) D-Ala-OMe·HCl, Cs_2CO_3, toluene, 85 °C, 81%. v) Zn, NH_4Cl, DMF, 74%. vi) H_2, Pd/C, EtOAc, 65%.

4.4.4 Synthesis of 6-fluoro analogue

Protection of the carboxylic acid **278** was less complicated than for the analogue described above because of the absence of cross reactivity with a hydroxyl group, so preparation of the benzyl ester **279** was achieved in 59% yield (Scheme 4.14). The S_NAr reaction of **279** with D-alanine could have been complicated since two positions are activated for substitution; C(2)F has two *ortho* electron-withdrawing groups, whereas C(6)F has an *ortho* and a *para* electron-withdrawing group. Consequently, substitution at C(2) was expected to be favoured electronically due to the larger partial positive charge, yet disfavoured sterically since both *ortho* positions are occupied. In practice, when one equivalent of D-alanine was used to minimise disubstitution, a single product was obtained in 86% yield. This was confirmed to be

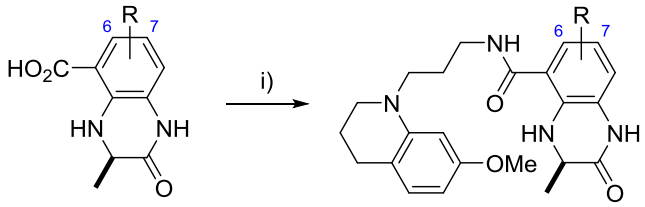
the desired regioisomer of (*R*)-**280** by heteronuclear NOE spectroscopy (HOESY), which showed correlation of C(6)*F* with C(5)*H* (Appendix A4.2). The reduction with zinc and NH_4Cl afforded the cyclised (*R*)-**281** in a reasonable 63% yield, then ester deprotection afforded the free carboxylic acid (*R*)-**282** in 95% yield. Overall, the DHQN (*R*)-**282**, containing a fluorine substitution at the 6-position, was synthesised from 2,6-difluoro-3-nitrobenzoic acid **278** in four steps and an overall yield of 30%.



Scheme 4.14: *Reagents and conditions*: i) BnBr, K_2CO_3, DMF, 70 °C, 59%. ii) D-Ala-OMe·HCl, Cs_2CO_3, toluene, 85 °C, 86%. iii) Zn, NH_4Cl, DMF, 63%. iv) H_2, Pd/C, EtOAc, 95%.

4.4.5 Attachment of tetrahydroquinoline side group

With carboxylic acids (*R*)-**277** and (*R*)-**282** in hand the HBTU-mediated coupling to amine **153** was performed (Scheme 4.15). Both compounds had to be purified *via* silica gel chromatography numerous times before suitable purity could be achieved, resulting in rather disappointing yields of 29% and 27% for (*R*)-**283** and (*R*)-**284**, respectively.



Scheme 4.15: *Reagents and conditions*: i) **153**, HBTU, DIPEA, DMF, rt, 27-29%.

Close inspection of the ^{19}F-NMR spectrum of the 6-fluorinated analogue was of particular interest. While the splitting for (*R*)-**281** was the expected double doublet, with coupling constants of 11.3 Hz and 5.1 Hz to the *ortho* and *meta* hydrogens (Figure 4.10A), the ^{19}F peak for (*R*)-**284** was split further (Figure 4.10B). By calculating the $^{3}J_{\text{F-H}}$ and $^{4}J_{\text{F-H}}$ from the ^{1}H NMR as 11.8 and 5.3 respectively, the remaining coupling of 9.5 Hz is too large for a simple through bond effect. This extra coupling is also observed in a similar series of benzamides containing a fluorine, such as **285**,[206] and can be attributed to a through space coupling constant for the N*H*···F groups. This indicates that the N*H* and F are in close contact much of the time, supporting the proposal that a weak interaction exists between the two.

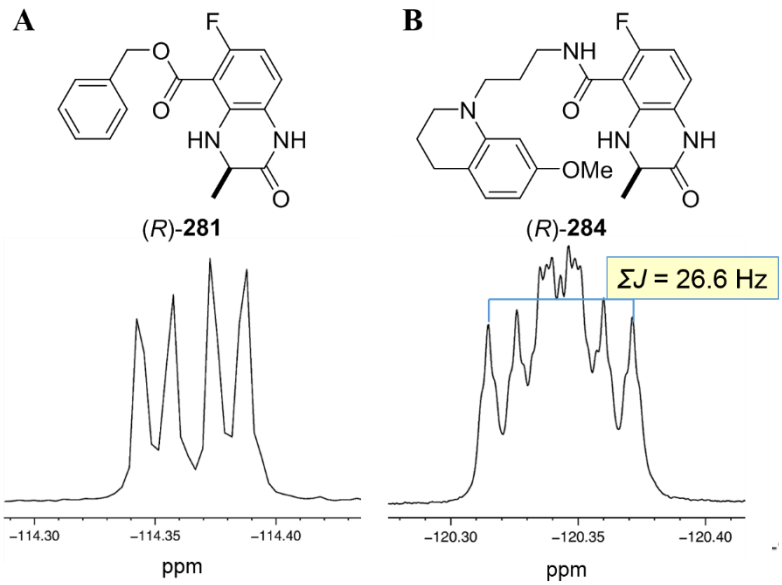
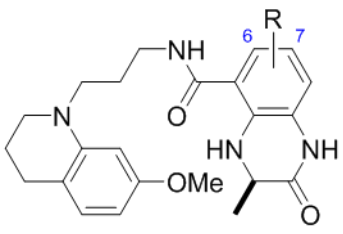


Figure 4.10: **A**: ^{19}F NMR of (*R*)-**281**. **B**: ^{19}F NMR of (*R*)-**284**. **C**: Redacted

4.4.6 Biological analysis of fluorinated DHQNs

Inhibition of CREBBP by the fluorinated DHQN analogues prepared above was assessed using the AlphaScreen assay. Unfortunately, the addition of a fluorine to either the 6- or the 7-position of the DHQN core led to a marked reduction in potency (Table 4.3). These results suggest that no favourable interactions could form between fluorine and the hydrophobic pocket, and that attachment of an electron-withdrawing group to the ring was disfavoured. It was interesting that of the two, (*R*)-**284** was less potent despite the NMR studies above suggesting some interaction between the fluorine and amide NH, which would direct the amide into a conformation similar to that observed in the crystal structure. Therefore, either the extra conformational rigidity is disfavoured, or the interaction is too weak to compensate for any detrimental effects associated with fluorination, such as the potential weakening of hydrogen bonds to the protein.

Table 4.3: AlphaScreen data for fluorinated-DHQN analogues. 95% confidence intervals are shown in parentheses.



Compound	R	IC_{50} (nM)
(*R*)-**156**	H	323 (298-351)
(*R*)-**283**	(7)-F	1530 (1340-1740)
(*R*)-**284**	(6)-F	2550 (2170-3010)

4.4.7 Summary

The fluorinated DHQN analogues (*R*)-**283** and (*R*)-**284** were synthesised from fluorinated starting materials using similar methodology to that developed previously. Evidence of an interaction between the fluorine and the amide NH of (*R*)-**284** was observed by NMR. It would have been interesting to determine the dependence of this NH···F interaction on the existing internal hydrogen bond between N(4)H and the exocyclic carbonyl. However, limited material prevented further investigation at this time, and both (*R*)-**283** and (*R*)-**284** were less potent inhibitors of CREBBP than the unfluorinated analogue (*R*)-**156**.

4.5 Variation at C(3) of dihydroquinoxalinone

4.5.1 Rationale

The structures obtained previously revealed that the methyl group occupying the stereogenic carbon atom at the 3-position of the DHQN core is bound deep within the KAc-binding pocket of CREBBP, in a similar position to the methyl group of KAc (Figure 3.5). It was also found that the (*R*)-enantiomer was favoured in this position, but that the core was susceptible to oxidation under acidic conditions. In an effort to prevent this instability, attachment of additional groups to the 3-position of the DHQN core was investigated. The observation that the (*S*)-enantiomer was a less potent inhibitor limited what groups could be used to replace the hydrogen but still retain potency. A spirocyclopropyl-containing DHQN **286** was considered an interesting prospect (Figure 4.11A). As a consequence of the inherent ring strain of the cyclopropyl group, the two methyl groups are forced closer together (1.5 Å) than in the un-cyclised analogue **287** (2.5 Å). Subsequently, whilst it would be expected that the introduction of a gem-dimethyl group to the 3-position would result in a steric clash with the walls of the KAc pocket, the more compact spiro-cyclopropyl group might avoid this. Indeed, an overlay of **286** with (*R*)-**119** bound to CREBBP suggested there may be enough space between the flanking V1115 and V1174 residues to accommodate this change (Figure 4.11B).

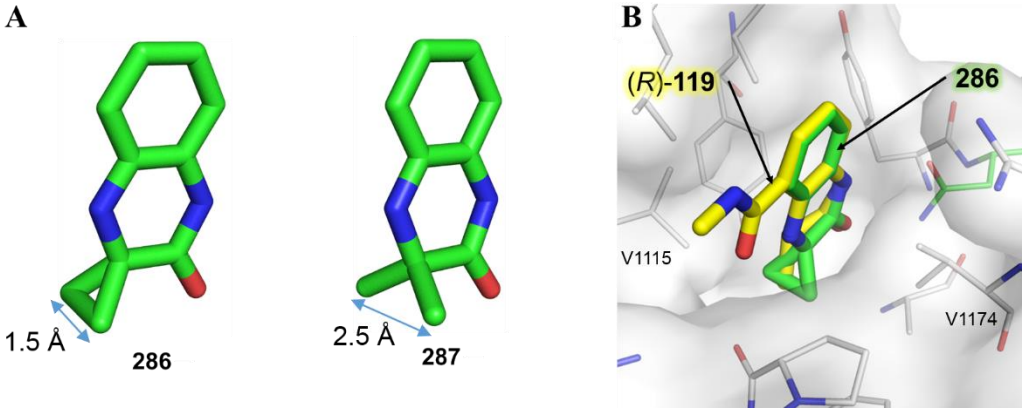
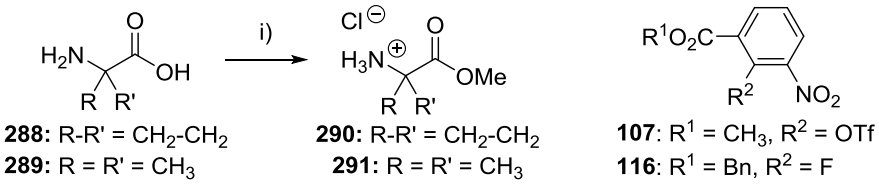


Figure 4.11: **A**: DHQN analogues substituted at the 3-position by spirocyclopropyl **286** and gem-dimethyl **287** groups. **B**: Overlay of **286** (green) with the crystal structure of (*R*)-**119** (gold) bound to CREBBP (PDB ID: 4NYW) (side chain not shown).

4.5.2 Route and starting material preparation

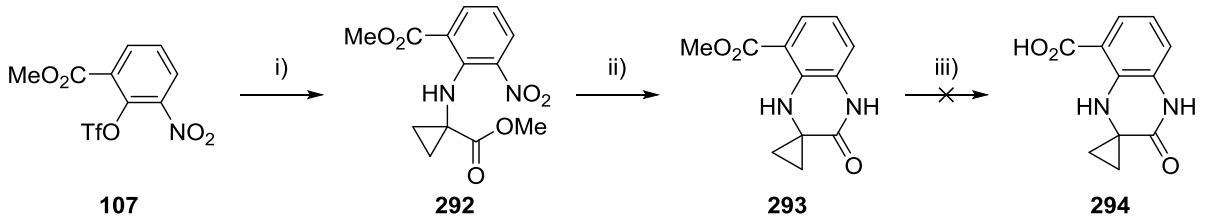
The synthesis of the two analogues was expected to be possible using a similar approach to that adopted in Section 3.2, where installation of the cyclopropyl or gem-dimethyl moiety could be achieved *via* coupling of an appropriate amino-ester to the previously prepared aryl-triflate **107** or aryl fluoride **116**. The required methyl esters **290** and **291** were prepared from the commercially available carboxylic acids **288** and **289** by reaction with thionyl chloride in methanol (Scheme 4.16). The subsequent coupling reactions are discussed in the following sections.



Scheme 4.16: *Reagents and conditions*: i) $SOCl_2$, MeOH, 0° C → rt, 66-88%. **107** and **116** were prepared according to Scheme 3.2 and 3.5, respectively.

4.5.3 Synthesis of cyclopropyl DHQN

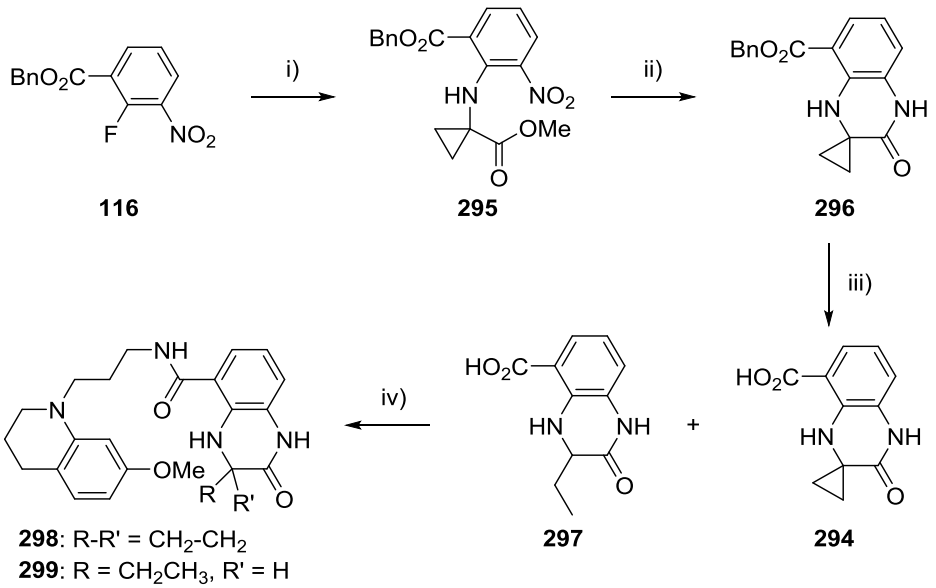
In order to obtain the cyclopropyl analogue, the route using the Buchwald-Hartwig coupling (in the manner of Section 3.2.2) was considered. Reaction of aryltriflate **107** with amine **290** was performed to afford aniline **292** in 59% yield (Scheme 4.17). A longer reaction time of 20 h was required, compared to 2.5 h for coupling to alanine (Table 3.1), potentially a reflection of the cyclopropylamine **290** being a worse nucleophile. Indeed, it has been shown that cyclopropylamine is less basic than cyclohexylamine (ΔpKa = 1.5-1.8), possibly due to the cyclopropyl carbon having more sp^2 character and therefore being more electronegative.[207] With methyl ester **292** in hand, reduction of the nitro group with cyclisation to form the DHQN **293** was attempted under hydrogenation conditions. Unfortunately, too little material was isolated for characterisation and when this was subjected to a test of the methyl ester deprotection conditions, it decomposed.



107 **292** **293** **294**

Scheme 4.17: *Reagents and conditions*: i) **290**, Cs_2CO_3, $Pd(OAc)_2$, (*S*)-BINAP, toluene, 85 °C, 59%. ii) H_2, Pd/C, EtOAc, rt, 0%. iii) LiOH, MeOH, THF, rt, 0%.

Considering the difficulties associated with the reduction and cyclisation in the presence of Pd, as well as with the methyl ester deprotection, an alternative route, using a benzyl-ester (in the manner of Section 3.2.3), was considered. S_NAr between aryl fluoride **116** and amine **290** proceeded slowly and, after a reaction time of 72 h, the desired aniline **295** was only isolated in 37% yield (Scheme 4.18). Subsequently, reduction of the nitro group with zinc and NH_4Cl was performed to afford the cyclised product **296** in 72% yield. Finally, the ester deprotection was performed using hydrogenolysis conditions, which afforded the desired carboxylic acid **294**. Unfortunately, there was also an impurity present corresponding to compound **297**, which has an ethyl group in place of the cyclopropyl group (see Section 4.5.5). It was hoped that performing the coupling reaction between the mixture of carboxylic acids **294** and **297** with amine **153** would allow separation at a later stage, however products **298** and **299** remained inseparable by silica chromatography, HPLC and crystallisation.



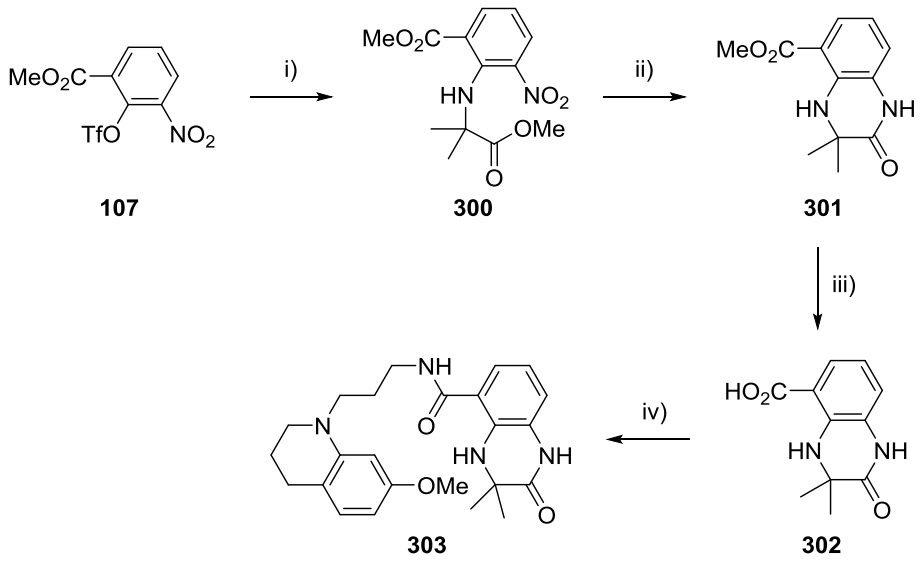
116 **295** **296** **297** **294**

Scheme 4.18: *Reagents and conditions*: i) **290**, Cs_2CO_3, toluene, 85 °C, 37%. ii) Zn, NH_4Cl, DMF, rt, 72%. iii) H_2, Pd/C, EtOAc, rt, 69%. iv) **153**, HBTU, DIPEA, DMF, rt.

In summary, the preparation of the cyclopropyl DHQN **298** could not be achieved due to Pd-mediated opening of the cyclopropyl ring. It is possible that the synthesis could be completed by avoiding the use of Pd, either by using some other conditions for the benzyl ester deprotection, by using a different carboxylate protecting group, or by changing the order of the steps to obviate the need for a protecting group altogether.

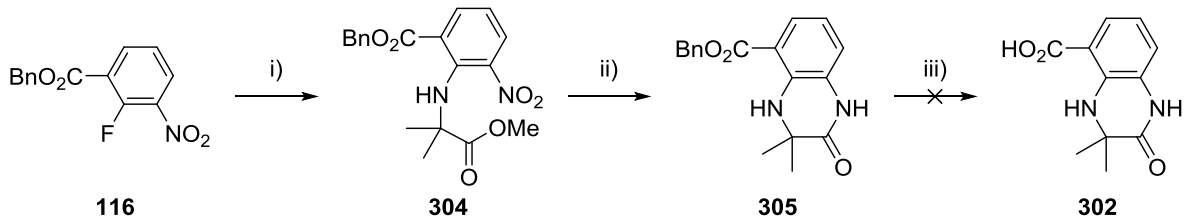
4.5.4 Synthesis of gem-dimethyl DHQN

In order to synthesise the gem-dimethyl analogue of the DHQN core, aryl triflate **107** was reacted with amine **291** under Buchwald-Hartwig coupling conditions (Scheme 4.19). Again, a longer reaction time than with alanine was necessary (19 h c.f. 2.5 h), and the aniline **300** could only be isolated in 29% yield. Nevertheless, reduction of the nitro-group, followed by base-mediated deprotection of the methyl ester, both proceeded smoothly to yield the carboxylic acid **302**. The coupling with amine **153** was straightforward, furnishing the desired final compound **303** in 72% yield.



Scheme 4.19: *Reagents and conditions*: i) **291**, Cs_2CO_3, $Pd(OAc)_2$, (*S*)-BINAP, toluene, 85 °C, 29%. ii) H_2, Pd/C, EtOAc, rt, 90%. iii) LiOH, MeOH, THF, rt, 85%; iv) **153**, HBTU, DIPEA, DMF, rt, 72%.

The benzyl ester protecting group strategy was also attempted to access the gem-dimethyl analogue (Scheme 4.20). The S_NAr gave a lower yield of **304** than the Buchwald-Hartwig conditions described above, as appears to be the trend in this series of less nucleophilic amines. The reduction to **305** proceeded well, but the Pd-catalysed deprotection of **305** did not proceed smoothly. Despite the addition of more Pd (up to 0.25 eq.), the application of high pressure (5 atm. H_2) or the addition of AcOH (to prevent chelation of the product to the catalyst) the reaction could not be forced to completion. Consequently, the methyl ester protecting group strategy was determined to be the best approach, allowing the preparation of **303** from methyl 2-hydroxy-3-nitrobenzyl ester **88** in five steps and overall 19% yield.



Scheme 4.20: *Reagents and conditions*: i) **291**, Cs_2CO_3, toluene, 85 °C, 18%. ii) Zn, NH_4Cl, DMF, rt, 60%. iii) H_2, Pd/C, EtOAc, rt, 0%.

4.5.5 Stability of spirocyclopropyl compounds

As noted above, compounds **293** and **296** were found to be unstable to the reaction conditions involving palladium-catalysed nitro group reduction or benzyl ester deprotection. The opening of cyclopropylamines has been used as a tool to study and to irreversibly inhibit oxidative enzymes such as cytochrome p450 enzymes,[208] so this instability is not entirely unexpected. The enzyme-mediated mechanism by which opening occurs is a matter of debate, with different studies providing data in favour of either the single electron transfer (SET) or hydrogen atom transfer (HyAT) mechanisms (Figure 4.12B).[209] However, the mechanism for the formation of **297** from **296** will likely differ, both due to the absence of an oxidising enzyme and because of the germinal carbonyl group. Such geminally donor-acceptor substituted cyclopropyl compounds are rare, but 1-aminocyclopropane-1-carboxylic acid (ACC) **288** is a naturally occurring amino acid, thought to play a role in the biosynthesis of ethylene (Figure 4.12A).[210] The influence of each group on the stability of the cyclopropyl group is unclear,[211] and further investigation on the behaviour of the spirocyclopropyl compounds synthesised above may contribute to this understanding.

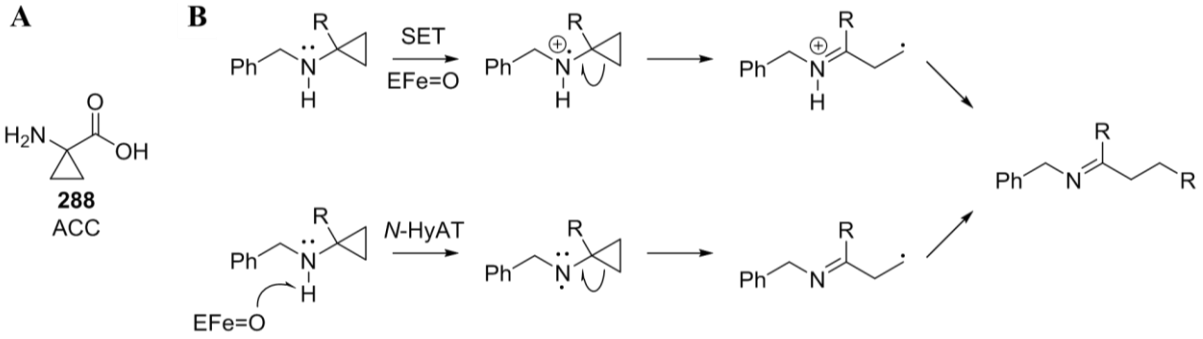


Figure 4.12: A: Structure of the plant hormone AAC. B: Proposed mechanism for oxidation of cyclopropylamines by cytochrome p450 enzymes by either single electron transfer (SET) or by hydrogen atom transfer (HyAT). EFe=O represents the enzymatic oxidant.[209]

4.5.6 Summary

In an effort to explore substitutions to the 3-position of the DHNQ core a gem-dimethyl analogue **303** was successfully synthesised but a cyclopropyl analogue **298** could not be isolated. Without a clean sample of both it was not deemed worthwhile testing *in vitro*, but should the synthesis of **298** be completed at a later date it would be interesting to compare the propensity of the DHQN core to tolerate substitution at this position. However, the observed lability of the cyclopropyl analogues would require further investigation before installing this group as a means of improving the stability of the DHQN core. A better option to improve the stability of the DHQN core may be to replace the hydrogen on the 3-position with fluorine, or to expand the ring size to reduce the propensity for oxidation. Efforts towards this effect are currently ongoing in the group.

4.6 Methylquinolinones as acetyl-lysine mimics

4.6.1 Rationale

The data obtained in the previous two chapters concerning BNZ- and DHQN-based ligands illustrated the importance of the internal hydrogen bond for inhibition of CREBBP. A fragment incorporating this interaction as a permanent bond could also provide a good starting point for the development of bromodomain inhibitors. Transformation of the 6-membered hydrogen bonding ring into a phenyl group and discarding the top portion of the DHQN aryl group results in a 1-methylquinolin-2(1*H*)-one (MeQ) core (Figure 4.13A). This fragment could be expected to form a hydrogen bond from the carbonyl at C(2) to the bromodomain asparagine residue, whilst the methyl attached to N(1) would occupy the same position as the *N*-methyl of KAc (Figure 4.13B). In addition, provided the interactions within the KAc-binding pocked are maintained, amine substitutions at the 6-position would be directed along the same vector as the amide side groups of DHQN analogues (Figure 4.13B). This would offer the opportunity to transfer the SAR observed in previous series onto the new MeQ scaffold, to develop an isofunctional CREBBP selective inhibitor. To investigate these hypotheses, the synthesis of MeQ analogues was sought.

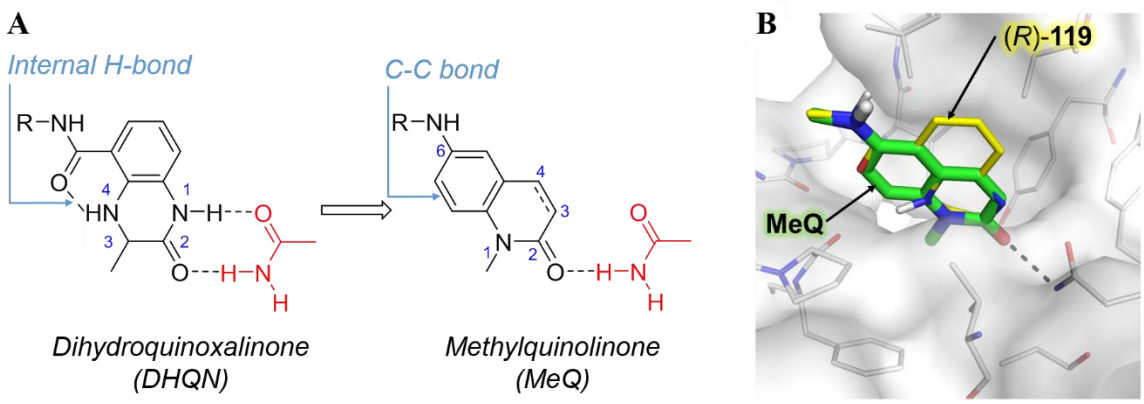
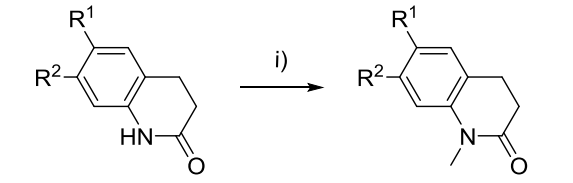


Figure 4.13: **A**: Proposed change to a methylquinolinone (MeQ) core. **B**: Overlay of MeQ with the crystal structure of (*R*)-**119** bound to CREBBP (PDB ID: 4NYW) (side chain not shown).

4.6.2 Synthesis of fragments

During the previous work a number of dihydroquinolinones were synthesised as precursors to access THQ analogues (Section 4.2.2). These could be alkylated using methyl iodide to afford analogues **306**-**308** (Scheme 4.21). For **307**, the chemoselectivity for *N*-alkylation (as well as the assignment of each methoxy group) was confirmed by NOE-spectroscopy (Appendix A4.3).

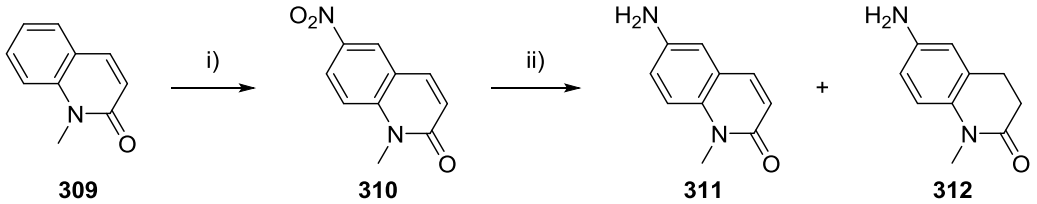


134: R^1 = H, R^2 = OMe
180: R^1 = R^2 = OMe
181: R^1 = R^2 = OBn

306: R^1 = H, R^2 = OMe
307: R^1 = R^2 = OMe
308: R^1 = R^2 = OBn

Scheme 4.21: i) MeI, K_2CO_3, DMF, rt, 65-91%.

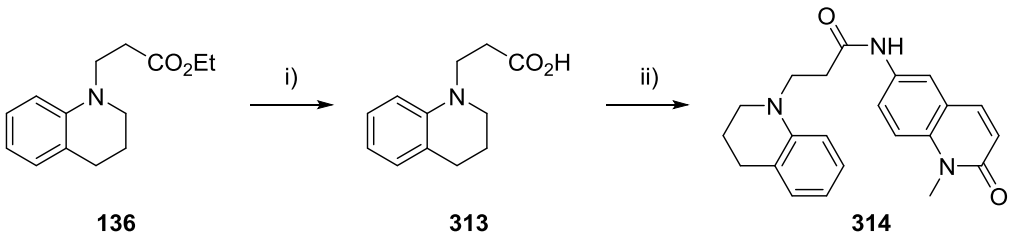
In addition, the fully unsaturated 1-methyl-2(1*H*)-quinolone **309** was commercially available and offered an attractive starting point for the synthesis of further analogues. Conditions to achieve multiple nitrations on **309** have been reported, along with the observation that nitration at the (6)-position is introduced first.[212] Heating a solution of **309** in HNO_3 and H_2SO_4, according to the literature conditions, afforded a mixture of mono- and di-nitrated products (Scheme 4.22). However, performing the reaction at room temperature allowed selective conversion to the (6)-nitro analogue **310**, although a low yield of 31% resulted from loss of material in a crystallisation step. Subsequent reduction of the nitro group under hydrogenation conditions was performed to afford aniline **311** in 62% yield. These conditions also resulted in the formation of the dihydroquinolinone **312**, which was of benefit on this occasion as it provided another analogue to test, but could be avoided in the future by using different reduction conditions such as Fe/AcOH.



Scheme 4.22: *Reagents and conditions*: i) HNO_3, H_2SO_4, 0 °C → rt, 31%. ii) H_2, Pd/C, EtOAc, rt, 62% **311** and 26% **312**.

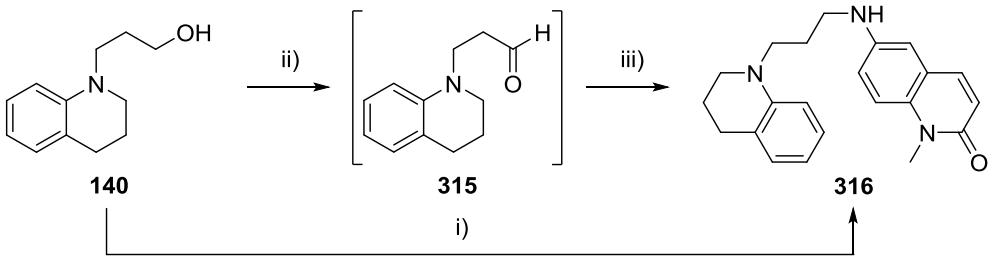
4.6.3 Attachment of tetrahydroquinoline side group

With the amine **311** in hand, further derivatisation was considered in order to target inhibition of the CREBBP bromodomain. The attachment of the THQ side group, which had been shown to improve the potency of both BNZ and DHQN fragments, either *via* an acylation or alkylation was considered. To achieve the acylation, the carboxylic acid THQ **313**, which was obtained from ester hydrolysis of the previously prepared methyl ester **136** (Scheme 3.9), was coupled to the quinolinone **311** to afford the amide **314** in 67% yield (Scheme 4.23).



Scheme 4.23: i) LiOH, THF, MeOH, rt, 22%. ii) **311**, HBTU, DIPEA, DMF, rt, 67%

Reductive amination was chosen as a strategy to achieve a single alkylation of **311** (Scheme 4.24). The alcohol **140** could be attached onto the amine either by pre-forming aldehyde **315** or *via* a one-step 'hydrogen borrowing' strategy, as had been reported for various aniline compounds.[213] The hydrogen borrowing approach was unsuccessful, with no evidence of the desired product obtained. However, a Swern oxidation of alcohol **140** afforded aldehyde **315**, which was used immediately for the reductive amination of **311**, allowing the preparation of the secondary aniline **316** albeit in a low 12% yield.

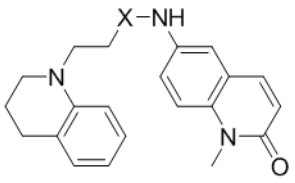


Scheme 4.24: i) **311**, Ru(p-cymene)Cl_2, dppf, toluene, 100 °C, 0%. ii) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C. iii) **311**, $NaBH_3CN$, MeCN, AcOH, rt, 12% (two-step yield).

4.6.4 Biological analysis of methylquinolinones

The THQ-containing MeQs **314** and **316** synthesised above were tested by AlphaScreen for inhibition of a set of bromodomains (Table 4.4). Unfortunately, neither exhibited greater than 50% inhibition of CREBBP at a concentration of 20 µM. This result suggests that the MeQ core does not bind favourably in the pocket of CREBBP, which could be due to the loss of the synergistic hydrogen-bond to N1168 or due to a steric clash resulting from the shift in the positioning of the bicycle. However, it was interesting to observe that both compounds were more active against the bromodomains of CECR2 and BRD9. In particular, **316** has an IC_{50} of 580 nM against BRD9, suggesting that the MeQ core may provide a valuable starting point from which to develop inhibitors for these bromodomains in the future.

Table 4.4: AlphaScreen data for methyl-quinolinone compounds.



Compound	R	SS[a]	IC_{50} (µM)		
		CREBBP	CECR2	BRD4	BRD9
314	X = CO	36	2.48	54.9	3.12
316	X = CH_2	47	9.39	19.7	0.58

[a]: single shot % inhibition at 20 µM

4.6.5 Summary

In summary, the methylquinolinone structure was investigated as an alternative core for inhibitors of the CREBBP bromodomain. The core was synthesised from either a cinnamic acid derivative, or a handle for further derivatisation was attached to an unsubstituted analogue. Analysis of these compounds indicated that whilst they do not bind favourably to CREBBP, they have the potential to be developed into inhibitors for other bromodomains in the future.

Chapter 5

Summary

5.1 Summary

Chapter 2 described how the benzoxazinone core was shown to be a ligand efficient acetyl lysine mimic from which more selective bromodomain inhibitors could be developed. Amide substituents at the 8-position were proposed to bind to the region of CREBBP with greatest variability compared to other bromodomains, and docking studies were performed to prioritise targets from an *in silico* library. The most potent BNZ analogue, with an aminopropyl-linked THQ side group, was a low micromolar inhibitor of CREBBP.

Chapter 3 described how the structure-activity relationships determined in the BNZ series was applied to a new acetyl lysine mimic based on the dihydroquinoxalinone scaffold (DHQN). Attachment of the aminopropyl-linked THQ side group resulted in a CREBBP inhibitor with nanomolar affinity. A crystal structure of this compound bound to CREBBP confirmed that the DHQN core bound, as predicted, within the acetyl lysine pocket. In addition, the THQ side group bound in a newly identified induced-fit pocket, forming a cation-π interaction to an arginine residue. Computational studies indicated that, despite the intrinsic flexibility of arginine residues, the cation-π interaction is not transient and contributes significantly towards the binding affinity of these ligands for CREBBP. Optimisation of the cation-π interaction was achieved by attachment of electron-donating substituents, to provide a compound with 323 nM affinity for CREBBP. This compound was shown to competitively displace CREBBP from chromatin in a cell-based assay.

Chapter 4 described further work to elaborate the DHQN core, either through fluorination or variation at the stereogenic centre, which unfortunately did not lead to an improvement. Also, attempts to improve the selectivity of inhibitors for CREBBP by rigidification of the side chain could not be realised. The application of the SAR generated from the BNZ and DHQN series to a new acetyl lysine mimic based on the methylquinolinone scaffold did not generate a

CREBBP ligand, but instead identified this core as an attractive target for the development of inhibitors for other bromodomains in the future.

In conclusion, bicyclic amides were developed from novel acetyl lysine mimics into potent inhibitors of the CREBBP bromodomain. The optimum compound showed on target effects for CREBBP inhibition in a cellular setting and provides a tool with which to investigate the therapeutic potential of CREBBP bromodomain inhibition.

Chapter 6

Experimental Procedures

6.1 General Experimental

6.1.1 Computational Methods

Docking Studies on 8-amido substituted benzoxazinones was carried out using ICM-Pro 3.6-1 (Molsoft LLC). The X-ray crystal structure of DHQ **12** bound to CREBBP (PDB ID: 4NYV) was used to define the receptor and the bicyclic core used as a template for placement of ligands. The four structurally important water molecules at the base of the ligand pocket were retained in the model of the receptor. A database of primary amines compiled from commercially available sources was used to generate an *in silico* library of benzoxazinones. The ligands were then put through a flexible-ligand docking procedure using the in-build procedure in ICM-Pro. Briefly, the compound set was expanded to include all possible stereoisomers and tautomers, and protonation state was assigned using an in-built prediction of pKa/pKb. A receptor pocket was defined around the DHQ **12** and expanded to encompass the ZA channel. The binding free energy between ligand and receptor was then determined for sampled conformations of the flexible ligand, with the highest score chosen in the ranking of the benzoxazinone library.

Calculated lipophilicity cLogP was determined using I-Lab (ACD) or ICM-Pro (Molsoft).

Ligand efficiency was calculated according to the equation:[214]

$$LE = \frac{pIC50 \times\ 1.4}{Heavy_atom_count}$$

Molecular Dynamics and Density Functional Theory calculations: were performed by Wilian A. Cortopassi and Prof. Robert S. Paton (Department of Chemistry, University of Oxford) as described previously.[101]

6.1.2 Physical Methods

X-Ray Diffraction: data was collected by Amber L. Thompson (Department of Chemistry, University of Oxford) as described previously.[101]

6.1.3 Biochemical Methods

Peptide Synthesis, Protein Expression and Purification, Crystallization, Data Collection and Structure Solution: was carried out by Sarah Picaud and Panagis Filippakopoulos (Structural Genomics Consortium, University of Oxford) as described previously.[25] CREBBP bromodomain protein was expressed as a His-tagged fusion and purified *via* Ni-affinity chromatography. Buffer conditions for crystallography, data collection and refinement statistics are as reported by Rooney *et al*.[101]

AlphaScreen® Peptide Displacement Assay, Differential Scanning Fluorimetry and Isothermal Titration Calorimetry: was carried out by Sarah Martin, Anthony Tumber, Martin Philpott, Oleg Fedorov, Duncan Hay and Stefan Knapp (Structural Genomics Consortium, University of Oxford) as described previously.[101] Briefly, AlphaScreen was performed using HIS-tagged protein, biotinylated peptide, streptavidin-coated donor beads and nickel chelate acceptor beads. Experiments were carried out in duplicate and, within a table, on the same plate. DSF was performed using an Mx3005p real-time PCR machine with final concentrations of 2 μM protein and 10 μM compound. ITC was carried out on a VP-ITC microcalorimeter and a single binding site model was employed.

Fluorescence recovery after photobleaching (FRAP) assay: was carried out by Catherine M. Rogers, Martin Philpott and Susanne Müller (Structural Genomics Consortium, University of Oxford) as described previously.[101] In brief, U2OS cells expressing a GFP chimera with three tandem repeats of the CREBBP bromodomain were treated with SAHA and compound (*R*)-**156**. A region of the GFP-positive nucleus was photobleached and then a time-lapse series of images taken to record GFP recovery.

6.1.4 Synthetic Methods

Reagents and solvents used, unless otherwise stated, were of commercially available reagent grade quality and were used without further purification. Where appropriate and if not stated otherwise, all non-aqueous reactions were carried out in a flame dried flask under an inert atmosphere of nitrogen or argon. Anhydrous solvent was purchased from SigmaAldrich UK in SureSeal™ bottles, or dried according to the procedure outlined by Pangborn *et al.*,[215] and used without purification unless otherwise indicated. Solvents for use in organometallic reactions were degassed by three freeze-thaw cycles under vacuum and were stored under an argon atmosphere over 3 Å molecular sieves. Triethylamine for use in organometallic reactions was dried with KOH and distilled onto KOH pellets. *In vacuo* refers to solvent removal under reduced pressure using a Buchi™ rotary evaporator. Brine refers to a saturated aqueous solution of sodium chloride. Petroleum ether refers to the fraction of light petroleum ether boiling in the range 40-60 °C.

Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} aluminium-supported thin layer chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 or 365 nm), or thermal development after dipping in one of: **a** ethanolic solution of phosphomolybdic acid; **b** aqueous solution of potassium permanganate, potassium carbonate and sodium hydroxide; **c** ethanolic solution of ninhydrin.

Flash Column chromatography was carried out either on Merck silica gel 60 (240-400 mesh), eluting with solvents as supplied under a positive pressure of compressed air, or on a Biotage® SP1 system using KP-Sil™ cartridges.

Melting points were determined using a Kofler hot stage microscope and are uncorrected.

Specific optical rotations were measured using a Perkin-Elmer 341 polarimeter with a water-jacketed 1 dm path-length cell maintained at 20 °C. The light source was maintained at 589 nm.

The concentration (*c*) is expressed in g/100 mL and specific rotations are denoted $[\alpha]_D$ with implied units of 10^{-1} deg cm^2 g^{-1}.

Infrared spectra were obtained as a thin film on sodium chloride discs or from neat samples using a diamond ATR module. The spectra were recorded on a Bruker Tensor 27 spectrometer and a representative number of absorption maxima are reported in wavenumbers (cm^{-1}) and assigned as s (strong), m (medium), w (weak) or br (broad).

^{1}H NMR spectra were recorded on Bruker DPX400, AVII400, AVIII400, Bruker DRX500, AVII500 with cryoprobe and AVII700 spectrometers. The specified deuterated solvent was used as a reference for internal deuterium lock. The chemical shift data for each signal are given as δ_H in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_H (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets); dt (doublet of triplets); dq (doublet of quartets); td (triplet of doublets); tt (triplet of triplets); qd (quartet of doublets); ddd (doublet of doublet of doublets); ddt (doublet of doublet of triplets); dddd (doublet of doublet of doublet of doublets); or m (multiplet). Where appropriate, a broadened signal is denoted by the prefix br (broad). The number of protons (n) for a given resonance signal is indicated by nH. Coupling constants (*J*) are expressed in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined using Bruker TopSpin software.

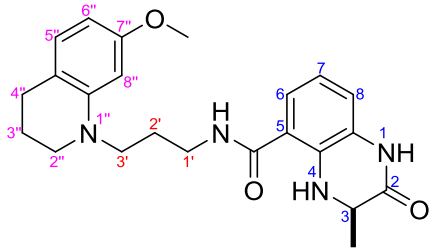
^{13}C NMR spectra were recorded on Bruker AVII400 or AVIII400 (101 MHz) and Bruker DRX500 or AVII500 with cryoprobe (126 MHz) spectrometers using broadband proton decoupling and an internal deuterium lock. The chemical shift data for each signal are given as δ_C in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_C (TMS) = 0.0 ppm. Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest

0.1 Hz. ^{1}H and ^{13}C spectra were assigned using 2D NMR experiments including COSY, HSQC and HMBC.

^{19}F NMR spectra were recorded on Bruker AVIII400 (377 MHz) and Bruker AVII500 (470 MHz) spectrometers using deuterochloroform (unless indicated otherwise) as a reference for internal deuterium lock. The chemical shift data for each signal are given as δ_F in units of parts per million (ppm) relative to trichlorofluoromethane where $\delta F = 0.00$ ppm. ^{19}F spectra with multiple ^{19}F signals were assigned using a HOESY 2D NMR experiment.

Compound numbering in the assignment of NMR signals is done according to the IUPAC name with an additional dash applied for each subspecies contained within a bracket. For example;

(3*R*)-*N*-[3-(7-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide



Mass spectra were acquired on either a Micromass LCT Premier spectrometer, Agilent 6120 Quadrapole spectrometer or Bruker MicroTOF spectrometer using electrospray ionization, operating in positive or negative mode, from solutions of methanol. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses.

Elemental analyses were obtained by the microanalysis service of the London Metropolitan University, UK.

High-Performance Liquid Chromatography was carried out using a PerkinElmer Flexar system with a Binary LC pump and UV/VIS LC detector. For determination of compound purity a Dionex Acclaim 120 column (C18, 5 µm, 120 Å, 4.6 × 150 mm) was used with the

method described below (Table A). Samples were injected in $(CH_3)_2SO$ or $(CH_3)_2CHOH$. For determination of enantiopurity a Daicel ChiralPak® (AD-H, 5 µm 4.6 × 250 mm) column was used with the method described below (Table B). Samples were injected in $(CH_3)_2CHOH$ or $(CH_3)_2SO$. Chromera software was used to determine purity and enantiomeric excess from relative peak areas of UV/VIS absorbance at 254 nm.

Table A: Solvents; A = 95%H_2O/5%MeCN + 0.1% TFA. B = 95%MeCN/5%H_2O + 0.1% TFA.

Step length (min)	Elapsed time (min)	%A	%B
1	1	100	0
10	11	0	100
3	14	0	100
1	15	100	0
5	20	100	0

Table B: Solvents; A = *n*-hexane. B = $(CH_3)_2CHOH$.

Step length (min)	Elapsed time (min)	%A	%B
1	1	90	10
12	13	50	50
10	23	50	50
1	24	90	10
6	30	90	10

6.2 General Procedures

General Procedure 1 for coupling of carboxylic acids to amines

To a solution of the requisite carboxylic acid (40 mg, 0.19 mmol, 1.0 eq) and HBTU (81 mg, 0.21 mmol, 1.1 eq) in DMF (1 mL) was added *N*,*N*-di*iso*propylethylamine (68 μL, 50 mg, 0.39 mmol, 2.0 eq). The reaction mixture was stirred for 10 min, then a solution of the requisite amine (0.21 mmol, 1.1 eq) in DMF (1 mL) was added and stirring continued for 19 h. The reaction mixture was diluted with EtOAc (50 mL), washed with H_2O (3 × 50 mL), brine (50 mL), dried (Mg_2SO_4), filtered and concentrated *in vacuo*.

General procedure 2 for coupling of benzoxazinone-carboxylic acid 36 to a library of amines

Stock solutions of carboxylic acid **36** (312 mM in DMF) and HBTU (250 mM in DMF) were prepared. Pre-weighed amines (125 μM, 1.3 eq) in 7.5 mL vials were arranged into the desired plate format. DMF (600 μL) and *N*,*N*-diisopropylethylamine (74 mg, 100 μL, 574 μM, 4.6 eq) were added to each. If required, vials were sonicated and gently heated to aid dissolution. Aliquots of **36** (400 μL, 1.0 eq) and HBTU (400 μL, 1.2 eq) were added sequentially to each amine vial using a multi-dispenser pipette. The reaction mixtures were placed on a rocking platform for 20 h before being placed in a centrifugal evaporator to remove DMF and volatile reagents. To the resulting residue was added CH_2Cl_2 (1 mL) and H_2O (1 mL) and dissolution encouraged through gentle shaking and sonication. Mixtures that remained insoluble were put aside for a separate work-up. The organic components were transferred into a 96-well plate *via* a phase separator and concentrated using a centrifugal evaporator. Conversion of the reaction to the desired product was determined by LC-MS with an ELSD trace. Further purification was not carried out and consequently only samples with a purity > 90% (ELSD) were considered for *in vitro* evaluation.

General procedure 3 for preparation of alcohols 140-143

A solution of the requisite quinoline/benzoxazine (190 mg, 1.43 mmol, 1.0 eq) and methyl prop-2-enoate (258 µL, 247 mg, 2.87 mmol, 2.0 eq) in acetic acid (1 mL) was heated at 120 °C for 16 h. The reaction mixture was concentrated *in vacuo* and azeotroped with methanol (15 mL) to yield the methyl ester as a brown oil that was used without further purification. To a solution of the ester (287 mg, 1.31 mmol, 1.0 eq) in THF (14 mL) at 0 °C was added a solution of $LiAlH_4$ in THF (1 M, 1.57 mL, 1.57 mmol, 1.2 eq) dropwise over 5 min. The reaction mixture was allowed to warm to rt and stirred for 23 h before being cooled to 0 °C and quenched by sequential addition of EtOAc (2 mL) and H_2O (1 mL). The resulting mixture was dried ($MgSO_4$), filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*.

General Procedure 4 for preparation of azides 148-151

To a solution of the requisite alcohol (82 mg, 0.43 mmol, 1.0 eq) in CH_2Cl_2 (3 mL) at 0 °C was added Et_3N (87 mg, 120 µL, 0.86 mmol, 2.0 eq) and stirred for 5 min. Mesyl chloride (74 mg, 50 µL, 0.65 mmol, 1.5 eq) was added and the reaction mixture was stirred at rt for 40 min before being concentrated *in vacuo*. The resulting residue was passed through a plug of silica gel (eluent EtOAc) and concentrated *in vacuo* to give 3-(3,4-dihydroquinolin-1(2*H*)-yl)propyl methanesulfonate (96 mg, 0.36 mmol) as a brown oil. A solution of the sulfonate (96 mg, 0.36 mmol, 1.0 eq) and NaN_3 (93 mg, 1.43 mmol, 4.0 eq) in DMF (3 mL) was heated at 60 °C for 1 h. The reaction mixture was cooled to rt, then diluted with EtOAc (25 mL), washed with H_2O (2 × 25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*.

General Procedure 5 for preparation of amines 152-154.

A mixture of the requisite azide (43 mg, 0.20 mmol, 1.0 eq) and Pd/C (10% w/w, 11 mg, 0.010 mmol, 0.05 eq) in EtOAc (2 mL) was stirred under a H_2 atmosphere for 30 h. The reaction mixture was filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*.

General Procedure 6 for preparation of phthalimide protected amines.

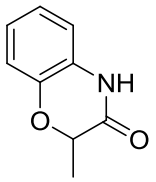
A mixture of the requisite quinoline/benzoxazine (200 mg, 1.23 mmol, 1.0 eq), *N*-(3-bromopropyl)phthalimide (394 mg, 1.47 mmol, 1.2 eq), NaI (220 mg, 1.47 mmol, 1.2 eq) and K_2CO_3 (203 mg, 1.47 mmol, 1.2 eq) in DMF (1.0 mL) was heated at 80 °C for 48 h. Upon cooling to rt the reaction mixture was diluted with EtOAc (50 mL), washed with aq. K_2CO_3 (2 M, 3 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*.

General Procedure 7 for phthalimide deprotection of amines.

To a solution of the requisite phthalimide (260 mg, 0.742 mmol, 1.0 eq) in CH_2Cl_2 (2 mL) and MeOH (2 mL) was added hydrazine monohydrate (65%, 144 µL, 149 mg, 3.02 mmol, 4.1 eq) and the reaction mixture heated at 70 °C for 3 h. Upon cooling to rt the reaction mixture was diluted with CH_2Cl_2 (20 mL), filtered through Celite® (eluent CH_2Cl_2) and concentrated *in vacuo*. Purification *via* acid base extraction was performed as follows: The crude reaction mixture was dissolved in EtOAc (25 mL) and extracted with 20% NH_4Cl (3 × 25 mL). The combined aqueous layers were basified with NaOH aq. (2 M, 75 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extractions were washed with brine (50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*.

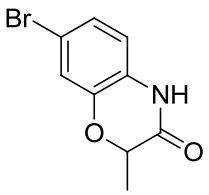
6.3 Chapter 2 Procedures

2-Methyl-2*H*-1,4-benzoxazin-3(4H)-one (24)[118]



A dry 2-5 mL microwave flask was charged with 2-aminophenol (100 mg, 0.916 mmol, 1.1 eq) and sealed under N_2. DMF (2.5 mL) was added, followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (152 mg, 149 µL, 1.07 mmol, 1.2 eq) and methyl bromopropionate (139 mg, 93 µL, 0.83 mmol, 1.0 eq). The reaction mixture was heated at 110 °C under microwave irradiation for 90 min, then diluted with EtOAc (20 mL), washed with H_2O (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) gave **24** (108 mg, 0.662 mmol, 79%) as a colourless solid. R_f 0.33 (1:2 EtOAc:petroleum ether); mp 141-142 °C (from EtOAc, lit.[118] 143-144 °C); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.78 (1H, s, N*H*), 7.01-6.93 (3H, m, C(Ar)*H* & C(Ar)*H* & C(Ar)*H*), 6.81-6.77 (1H, m, C(Ar)*H*), 4.67 (1H, q, *J* 6.8 Hz, C(2)*H*), 1.60 (3H, d, *J* 6.8 Hz, C(2)CH_3); LRMS *m/z* (ES+) 186 ([M+Na]$^+$, 100%). These data are in accordance with the literature.[118]

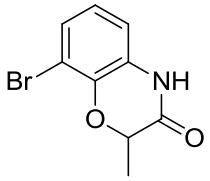
7-Bromo-2-methyl-2*H*-1,4-benzoxazin-3(4H)-one (33)



A dry 2-5 mL microwave flask was charged with 2-amino-5-bromophenol (350 mg, 1.86 mmol, 1.1 eq) and sealed under N_2. DMF (2.5 mL) was added, followed by and 1,8-diazabicyclo[5.4.0]undec-7-ene (308 mg, 304 µL, 2.03 mmol, 1.2 eq) and methyl bromopropionate (283 mg, 189 µL, 1.69 mmol, 1.0 eq The reaction mixture was heated at 110 °C under microwave irradiation for 60 min, then diluted with EtOAc (20 mL), washed with

H_2O (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo.* Purification *via* silica gel chromatography (gradient elution 6 to 48% EtOAc in petroleum ether) gave **33** (230 mg, 0.954 mmol, 51%) as a brown solid. R_f 0.25 (1:2 EtOAc:petroleum ether); mp 127-128 °C (from $(CH_3)_2CO$); ν_{max}/cm^{-1} 3048 (w), 1682 (s), 1499 (m), 1396 (m); ^1H NMR (500 MHz; CD_3OD): δ 7.14 (1H, d, *J* 2.1 Hz, C(8)*H*), 7.12 (1H, dd, *J* 8.3 2.1 Hz, C(6)*H*), 6.82 (1H, d, *J* 8.3 Hz, C(5)*H*), 4.67 (1H, q, *J* 6.8 Hz, C(2)*H*), 1.52 (3H, d, *J* 6.8 Hz, C(2)CH_3); ^{13}C NMR (126 MHz; CD_3OD): δ 169.3 (*C*(3)=O), 145.5 (*C*(10)), 128.0 (*C*(9)), 126.5 (*C*(6)H), 121.0 (*C*(8)H), 118.0 (*C*(5)H), 116.3 (*C*(7)Br), 74.7 (*C*(2)H), 16.6 (C(2)*C*H_3); LRMS *m/z* (ES+) 264 ([M(^{79}Br)+Na]$^+$, 100%), 266 ([M(^{81}Br)+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (^{79}M+Na)$^+$ 263.9625. $C_9H_8{}^{79}BrNNaO_2^+$ requires 263.9631], [Found: (^{81}M+Na)$^+$ 265.9604. $C_9H_8{}^{81}BrNNaO_2^+$ requires 265.9610]; Anal. calcd for $C_9H_8BrNO_2$: C 44.66%, H 3.33%, N 5.79%; Found: C 44.79%, H 3.30%, N 5.79%.

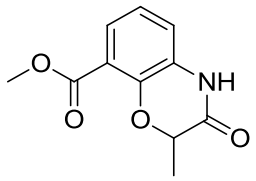
8-Bromo-2-methyl-2*H*-1,4-benzoxazin-3(4H)-one (34)



A dry 2-5 mL microwave flask was charged with 2-amino-6-bromophenol (185 mg, 0.986 mmol, 1.1 eq) and sealed under N_2. DMF (2.5 mL) was added, followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (164 mg, 161 µL, 1.07 mmol, 1.2 eq) and methyl bromopropionate (150 mg, 100 µL, 0.896 mmol, 1.0 eq). The reaction mixture was heated at 110 °C under microwave irradiation for 60 min, then diluted with EtOAc (20 mL), washed with H_2O (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) gave a brown solid. The solid was redissolved in EtOAc, stirred with charcoal for 2 h, filtered through Celite® (eluent EtOAc) and concentrated *in vacuo* to give **34** (168 mg, 0.697 mmol, 78%) as a colourless solid.

R_f 0.26 (1:2 EtOAc:petroleum ether); mp 134-136 °C (from $(CH_3)_2CO$); ν_{max}/cm^{-1} 3055 (w), 1682 (s), 1597(m), 1478 (m); ^1H NMR (400 MHz; $CDCl_3$): δ 9.97 (1H, br s, N*H*), 7.21 (1H, dd, *J* 7.8 1.7 Hz, C(Ar)*H*), 6.85 (1H, dd, *J* 7.9 7.8 Hz, C(6)*H*), 6.81 (1H, dd, *J* 7.9 1.7 Hz, C(Ar)*H*), 4.79 (1H, q, *J* 6.9 Hz, C(2)*H*), 1.63 (3H, d, *J* 6.9 Hz, C(2)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.9 (*C*(3)=O), 140.4 (*C*(Ar)), 127.8 (*C*(Ar)H), 127.4 (*C*(Ar)), 123.3 (*C*(Ar)H), 115.0 (*C*(Ar)H), 111.0 (*C*(Ar)), 73.9 (*C*(2)H), 16.4 (C(2)*C*H_3); LRMS *m/z* (ES+) 264 ([M(^{79}Br)+Na]$^+$, 98%), 266 ([M(^{81}Br)+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (^{79}M+Na)$^+$ 263.9632. $C_9H_8{}^{79}BrNNaO_2^+$ requires 263.9631], [Found: (^{79}M+Na)$^+$ 265.9604. $C_9H_8{}^{81}BrNNaO_2^+$ requires 265.9610]; Anal. calcd for $C_9H_8BrNO_2$: C 44.66%, H 3.33%, N 5.79%; Found: C 44.63%, H 3.21%, N 5.71%.

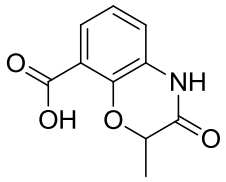
Methyl 2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylate (35)



A dry 10-20 mL microwave flask was charged with methyl 3-amino-2-hydroxybenzoate (2.01 g, 12.0 mmol, 1.0 eq) and sealed under N_2. DMF (10 mL) was added, followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (2.02 g, 1.98 mL, 13.3 mmol, 1.1 eq) and methyl bromopropionate (2.22 g, 1.48 mL, 13.3 mmol, 1.1 eq). The reaction mixture was heated at 110 °C in an oil bath for 16 h, then diluted with EtOAc (100 mL), washed with H_2O (3 × 100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:4 EtOAc:petroleum ether) gave **35** (1.77 g, 8.00 mmol, 67%) as a colourless solid. R_f 0.13 (1:2 EtOAc:petroleum ether); mp 144-146 °C (from $(CH_3)_2CO$); ν_{max}/cm^{-1} 3076 (w), 1730 (s), 1703 (s), 1477 (m), 1286 (m); ^1H NMR (500 MHz; $CDCl_3$): δ 9.44 (1H, br s, N*H*), 7.53 (1H, dd, *J* 7.4 2.4 Hz, C(7)*H*), 7.03 (1H, dd, *J* 7.7 2.4 Hz, C(5)*H*), 7.00 (1H, dd, *J* 7.7 7.4 Hz, C(6)*H*), 4.79 (1H, q, *J* 6.9 Hz, C(2)*H*), 3.92 (3H, s, OCH_3), 1.62 (3H, d, *J* 6.9 Hz,

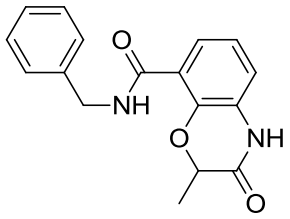
C(2)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.5 (*C*(3)=O), 165.6 (C(8)*C*=O), 143.3 (*C*(10)), 127.6 (*C*(9)), 126.1 (*C*(7)H), 122.0 (*C*(6)H), 120.5 (*C*(8)), 119.5 (*C*(5)H), 73.6 (*C*(2)H), 52.2 (O*C*H_3), 16.3 (C(2)*C*H_3); LRMS *m/z* (ES+) 465 ([2M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 244.0578. $C_{11}H_{11}NNaO_4^+$ requires 244.0580]; Anal. calcd for $C_{11}H_{11}NO_4$: C 59.73%, H 5.01%, N 6.33%; Found: C 59.63%, H 4.94%, N 6.24%.

2-Methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid (36)



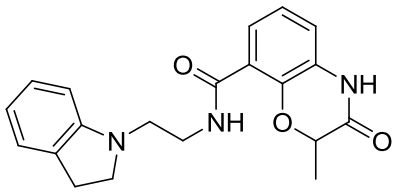
To a solution of **35** [methyl 2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylate] (1.94 g, 8.77 mmol, 1.0 eq) in MeOH (70 mL) and THF (70 mL) was added a solution of LiOH aq. (1 M, 44 mL, 5.0 eq). The reaction mixture was stirred at rt for 32 h, then concentrated *in vacuo*, diluted with H_2O (50 mL) and acidified with HCl aq. (1 M). The resulting precipitate was filtered and washed with 1 M HCl (10 mL) and EtOAc (20 mL) to give **36** (1.65 g, 91%) as a colourless solid which was used without further purification. R_f 0.53 (1:99 AcOH:EtOAc); mp 211 °C (dec., from 1 M HCl); ν_{max}/cm^{-1} 3001 (w), 1739 (m), 1671 (s), 1481 (m), 1220 (m); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 12.81 (1H, br s, CO_2H), 10.80 (1H, s, N*H*), 7.31 (1H, dd, *J* 7.7 1.8 Hz, C(7)*H*), 7.05 (1H, dd, *J* 7.7 1.8 Hz, C(5)*H*), 7.01 (1H, dd, *J* 7.7 7.7 Hz, C(6)*H*), 4.73 (1H, q, *J* 6.8 Hz, C(2)*H*), 1.41 (3H, d, *J* 6.8 Hz, C(2)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 166.9 (*C*=O), 166.4 (*C*=O), 142.4 (*C*(10)), 128.7 (*C*(Ar)), 124.3 (*C*(7)H), 121.8 (*C*(6)H), 121.2 (*C*(Ar)), 118.7 (*C*(5)H), 72.7 (*C*(2)H), 16.0 (C(2)*C*H_3); LRMS *m/z* (ES−) 206 ([M−H]$^-$, 100%); HRMS *m/z* (ES−) [Found: (M−H)$^-$ 206.0458. $C_{10}H_8NO_4^-$ requires 206.0459]; Anal. calcd for $C_{10}H_9NO_4$: C 57.97%, H 4.38%, N 6.76%; Found: C 57.88%, H 4.47%, N 6.68%.

***N*-Benzyl-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (37)**



Compound **36** [2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid] (50 mg, 0.24 mmol, 1.0 eq) and benzylamine (28 mg, 29 µL, 0.27 mmol, 1.1 eq) were reacted according to general procedure 1 for 18 h to give **37** (68 mg, 0.23 mmol, 95%) as a colourless solid. R_f 0.32 (2:1 EtOAc:petroleum ether); mp 137-139 °C (EtOAc); ν_{max}/cm^{-1} 3323 (m), 2926 (m), 2360 (w), 1685 (s), 1627 (s), 1589 (m), 1311 (m); ^{1}H NMR (400 MHz; $(CD_3)_2SO$): δ 10.81 (1H, s, N(4)*H*), 8.61 (1H, dd, *J* 5.9 5.8 Hz, C(8)CON*H*), 7.42-7.20 (6H, m, C(7)*H* & 5 × C(Ar')*H*), 7.08-6.99 (2H, m, C(5)*H* & C(6)*H*), 4.80 (1H, q, *J* 6.8 Hz, C(2)*H*), 4.54 (1H, dd, *J* 15.3 5.9 Hz, CH_{AB}Ph), 4.47 (1H, dd, *J* 15.3 5.8 Hz, CH_{AB}Ph), 1.44 (3H, d, *J* 6.8 Hz, C(2)CH_3); ^{13}C NMR (101 MHz; $(CD_3)_2SO$) δ 166.7 (*C*(3)=O), 164.3 (C(8)*C*=O), 140.8, 139.4, 128.3, 128.2, 126.9, 126.7, 123.9, 123.4, 122.2, 117.6, 73.1 (*C*H_2Ph), 42.5 (*C*(2)H), 15.8 (C(2)*C*H_3); LRMS *m/z* (ES−) 295 ([M−H]$^-$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 319.1052. $C_{17}H_{16}N_2NaO_3^+$ requires 319.1053]; Anal. calcd for $C_{17}H_{16}N_2O_3$: C 68.91%, H 5.44%, N 9.45%; Found: C 69.03%, H 5.38%, N 9.38%.

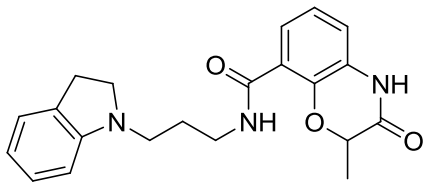
***N*-[2-(2,3-Dihydro-1*H*-indol-1-yl)ethyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (75)**



Compound **36** [2-methyl-3-oxo-3,4-dihydro-2*H*-1,4benzoxazine-8-carboxylic acid] (75 mg, 0.36 mmol, 1.0 eq) and 2-(2,3-dihydro-1*H*-indol-1-yl)ethanamine (65 mg, 0.40 mmol, 1.1 eq)

were reacted according to general procedure 1 for 44 h. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) gave **75** (112 mg, 0.319 mmol, 88%) as a colourless solid. R_f 0.4 (2:1 EtOAc:petroleum ether); mp 142-144 °C (from MeOH); ν_{max}/cm^{-1} 3391 (w), 3231 (w), 2821 (w), 1693 (s), 1650 (s), 1534 (m), 1305 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.05 (1H, s, N(4)*H*), 8.12 (1H, s, C(1')N*H*), 7.90 (1H, dd, *J* 8.0 1.6 Hz, C(7)*H*), 7.14-7.02 (3H, m, C(6)*H* & 2 × C(Ar'')*H*), 6.96 (1H, dd, *J* 7.7 1.6 Hz, C(5)*H*), 6.69 (1H, dd, *J* 7.6 7.6 Hz, C(Ar'')*H*), 6.54 (1H, d, *J* 7.9 Hz, C(Ar'')*H*), 4.49 (1H, q, *J* 6.9 Hz, C(2)*H*), 3.83-3.68 (2H, m, C(1')H_2), 3.51-3.26 (4H, m, C(2')H_2 & C(2'')H_2), 3.01 (2H, t, *J* 8.3 Hz, C(3'')H_2), 1.34 (3H, t, *J* 6.9 Hz, C(2)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.3 (*C*(3)), 164.2 (*C*(1')), 152.5 (*C*(9'')), 141.5 (*C*(10)), 129.6 (*C*(8'')), 127.4 (*C*(Ar'')H), 127.0 (*C*(9)), 126.4 (*C*(7)H), 124.7 (*C*(Ar'')H), 122.8 (*C*(6)H), 122.1 (*C*(8)), 118.9 (*C*(5)H), 118.3 (*C*(Ar'')H), 107.0 (*C*(Ar'')H), 74.1 (*C*(2)H), 53.3 (*C*(2'')H_2), 49.0 (*C*(2')H_2), 38.0 (*C*(1'')H_2), 28.7 (*C*(3'')H_2), 15.7 (C(2)*C*H_3); LRMS *m/z* (ES+) 352 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 374.1476. $C_{20}H_{21}N_3NaO_3^+$ requires 374.1475]; Anal. calcd for $C_{20}H_{21}N_3O_3$: C 68.36%, H 6.02%, N 11.96%; Found: C 68.36%, H 5.93%, N 11.86%.

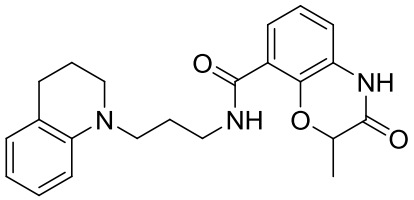
_N_-[3-(2,3-Dihydro-1*H*-indol-1-yl)propyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (44)



Compound **36** [2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid] (75 mg, 0.362 mmol, 1.0 eq) and 3-(2,3-dihydro-1*H*-indol-1-yl)propan-1-amine (70 mg, 0.398 mmol, 1.1 eq) were reacted according to general procedure 1 for 44 h. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) gave **44** (99 mg, 0.271 mmol, 75%) as a colourless oil. Crystallisation from MeOH afforded a pale grey solid.

R_f 0.4 (2:1 EtOAc:petroleum ether); mp 128-130 °C (MeOH); ν_{max}/cm^{-1} 3292 (w), 3062 (w), 2925 (w), 1680 (s), 1633 (s), 1470 (m), 1314 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.07 (1H, s, N(4)*H*), 7.89 (1H, dd, *J* 7.9 1.6 Hz, C(7)*H*), 7.87-7.81 (1H, m, N(2')*H*), 7.13-7.03 (3H, m, C(6)*H* & 2 × C(Ar'')*H*), 7.00 (1H, dd, *J* 7.9 1.6 Hz, C(5)*H*), 6.68 (1H, dd, *J* 7.3 7.3 Hz, C(Ar'')*H*), 6.49 (1H, d, *J* 7.7 Hz, C(Ar'')*H*), 4.63 (1H, q, *J* 6.9 Hz, C(2)*H*), 3.71-3.57 (2H, m, C(1')H_2), 3.41-3.31 (2H, m, C(2'')H_2), 3.18 (2H, t, *J* 6.9 Hz, C(3')H_2), 2.95 (2H, t, *J* 8.3 Hz, C(3'')H_2), 2.02-1.92 (2H, m, C(2')H_2), 1.53 (3H, d, *J* 6.9 Hz, C(2)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.3 (*C*(3)=O), 164.2 (C(8)*C*=O), 152.5 (*C*(9'')), 141.3 (*C*(10)), 130.0 (*C*(8'')), 127.3 (*C*(Ar'')H), 126.9 (*C*(9)), 126.5 (*C*(7)H), 124.5 (*C*(Ar'')H), 122.9 (*C*(6)H), 122.4 (*C*(8)), 118.9 (*C*(5)H), 117.9 (*C*(Ar'')H), 106.9 (*C*(Ar'')H), 74.1 (*C*(2)H), 53.4 (*C*(2'')H_2), 47.5 (*C*(3')H_2), 38.2 (*C*(1')H_2), 28.6 (*C*(3'')H_2), 27.3 (C(2')H_2), 16.2 (C(2)*C*H_3); LRMS *m/z* (ES+) 366 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 388.1630. $C_{21}H_{23}N_3NaO_3^+$ requires 388.1632]; Anal. calcd for $C_{21}H_{23}N_3O_3$: C 69.02%, H 6.34%, N 11.50%; Found: C 69.02%, H 6.41%, N 11.58%.

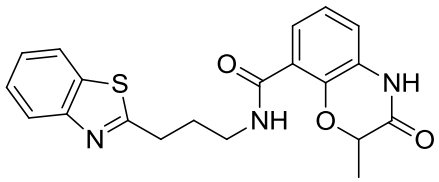
_N_-[2-(3,4-Dihydroquinolin-1(2*H*)-yl)propyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (71)



Compounds **36** [2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid] (50 mg, 0.241 mmol, 1.0 eq) and **118** [2-(3,4-dihydroquinolin-1(2*H*)-yl)ethanamine] (51 mg, 0.268 mmol, 1.1 eq) were reacted according to general procedure 1 for 44 h. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) gave **71** (79 mg, 0.208 mmol, 86%) as a brown oil. R_f 0.3 (2:1 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3400 (w), 2928 (m), 1700 (s), 1643 (m), 1591 (m), 1301 (m); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.47

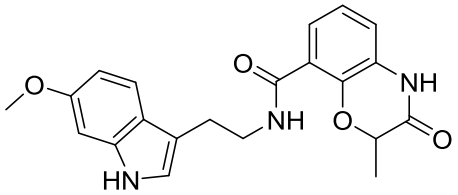
(1H, br s, N(4)*H*), 7.88 (1H, dd, *J* 7.9 1.6 Hz, C(7)*H*), 7.68 (1H, t, *J* 5.8 Hz, C(1')H_2N*H*), 7.09 (1H, dd, *J* 7.9 7.9 Hz, C(6)*H*), 7.06-7.00 (1H, m, C(7'')*H*), 6.98-6.93 (2H, m, C(5)*H* & C(5'')*H*), 6.60-6.55 (2H, m, C(6'')*H* & C(8'')*H*), 4.71 (1H, q, *J* 6.8 Hz, C(2)*H*), 3.57 (2H, td, *J* 6.5 5.8 Hz, C(1')*H*), 3.37 (2H, td, *J* 7.1 2.5 Hz, C(3')H_2), 3.28 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.74 (2H, t, *J* 6.3 Hz, C(4'')H_2), 2.00-1.90 (4H, m, C(2')H_2 & C(3'')H_2), 1.59 (3H, d, *J* 6.8 Hz, C(2)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$) δ 167.0 (*C*(3)=O), 164.3 (C(8)*C*=O), 145.3 (*C*(10'')), 141.3 (*C*(10)), 129.5 (*C*(5'')H), 127.2 (*C*(7'')H), 127.0 (*C*(9)), 126.7 (*C*(7)H), 123.2 (*C*(6)H), 122.7-122.6 (*C*(8) & *C*(9'')), 118.9 (*C*(5)H), 116.0 (*C*(Ar'')H), 110.5 (*C*(Ar'')H), 74.3 (*C*(2)H), 49.9 (*C*(2'')H_2), 49.5 (*C*(3'')H_2), 38.2 (*C*(1')H_2), 28.2 (*C*(4'')H_2), 26.8 (*C*(2')H_2), 22.3 (*C*(3'')H_2), 16.5 (C(2)*C*H_3); LRMS *m/z* (ES+) 781 ([2M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 380.1963. $C_{22}H_{26}N_3O_3^+$ requires 380.1969].

_N_-[3-(1,3-Benzothiazol-2-yl)propyl]-2-methyl-3-oxo-3,4-dihydro-2_H_-1,4-benzoxazine-8-carboxamide (41)



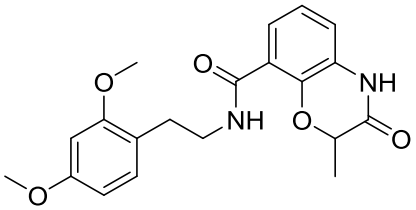
3-(1,3-Benzothiazol-2-yl)propan-1-amine and **36** were reacted according to general procedure 2 to afford **41**. LC-MS retention time = 2.89 min (91%), *m/z* (ES+) 382 ([M+H]$^+$, 100%).

***N*-[2-(6-Methoxy-1*H*-indol-3-yl)ethyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (42)**



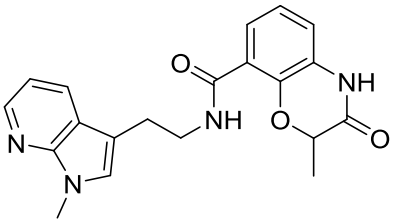
2-(6-Methoxy-1*H*-indol-3-yl)ethan-1-amine and **36** were reacted according to general procedure 2 to afford **42**. LC-MS retention time = 2.87 min (92%), *m/z* (ES+) 380 ($[M+H]^+$, 100%).

***N*-[2-(2,4-Dimethoxyphenyl)ethyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (45)**



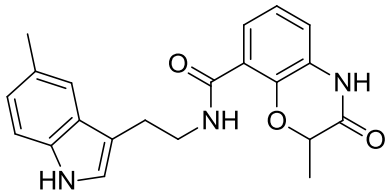
2-(2,4-Dimethoxyphenyl)ethan-1-amine and **36** were reacted according to general procedure 2 to afford **45**. LC-MS retention time = 3.08 min (100%), *m/z* (ES+) 371 ($[M+H]^+$, 100%).

2-Methyl-*N*-[2-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)ethyl]-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (58)



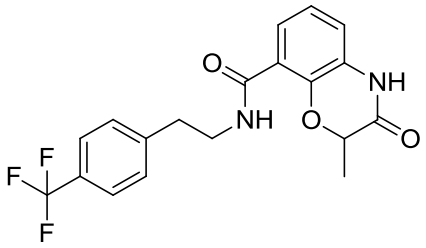
2-(1-Methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)ethan-1-amine and **36** were reacted according to general procedure 2 to afford **58**. LC-MS retention time = 2.61 min (90%), *m/z* (ES+) 365 ($[M+H]^+$, 100%).

2-Methyl-*N*-[2-(5-methyl-1*H*-indol-3-yl)ethyl]-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (59)



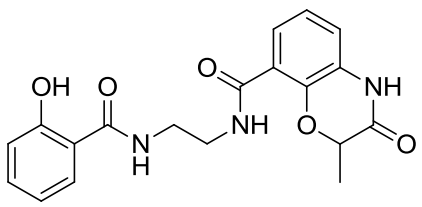
2-(5-Methyl-1*H*-indol-3-yl)ethan-1-amine and **36** were reacted according to general procedure 2 to afford **59**. LC-MS retention time = 3.03 min (100%), *m/z* (ES+) 364 ($[M+H]^+$, 100%).

2-Methyl-3-oxo-*N*-{2-[4-(trifluoromethyl)phenyl]ethyl}-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (60)



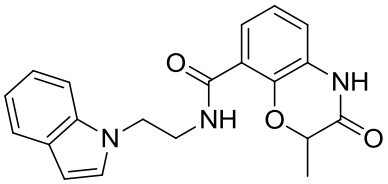
2-[4-(Trifluoromethyl)phenyl]ethan-1-amine and **36** were reacted according to general procedure 2 to afford **60**. LC-MS retention time = 3.20 min (90%), *m/z* (ES+) 379 ($[M+H]^+$, 100%).

***N*-{2-[(2-Hydroxybenzoyl)amino]ethyl}-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (64)**



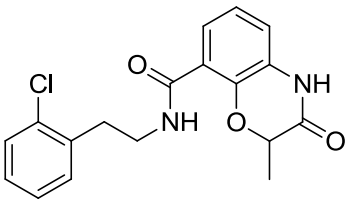
2-[(2-Hydroxybenzoyl)amino]ethan-1-amine and **36** were reacted according to general procedure 2 to afford **64**. LC-MS retention time = 2.71 min (100%), *m/z* (ES+) 370 ($[M+H]^+$, 100%).

***N*-[2-(1*H*-Indol-1-yl)ethyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (65)**



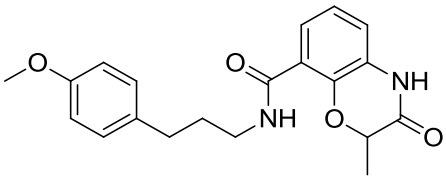
2-(1*H*-Indol-1-yl)ethan-1-amine and **36** were reacted according to general procedure 2 to afford **65**. LC-MS retention time = 3.02 min (100%), *m/z* (ES+) 350 ($[M+H]^+$, 100%).

***N*-[2-(2-Chlorophenyl)ethyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (66)**



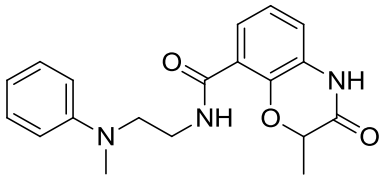
2-(2-Chlorophenyl)ethan-1-amine and **36** were reacted according to general procedure 2 to afford **66**. LC-MS retention time = 3.07 min (100%), *m/z* (ES+) 345 ($[M+H]^+$, 100%).

***N*-[3-(4-Methoxyphenyl)propyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (73)**



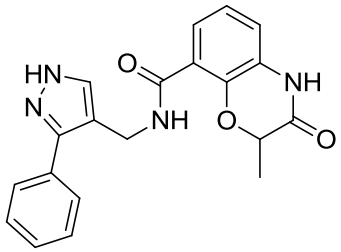
3-(4-Methoxyphenyl)propan-1-amine and **36** were reacted according to general procedure 2 to afford **73**. LC-MS retention time = 3.12 min (91%), *m/z* (ES+) 355 ($[M+H]^+$, 100%).

2-Methyl-*N*-{2-[methyl(phenyl)amino]ethyl}-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (79)



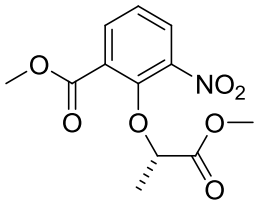
2-[Methyl(phenyl)amino]ethan-1-amine and **36** were reacted according to general procedure 2 to afford **79**. LC-MS retention time = 2.99 min (100%), *m/z* (ES+) 340 ([M+H]$^+$, 100%).

2-Methyl-3-oxo-*N*-[(3-phenyl-1*H*-pyrazol-4-yl)methyl]-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (85)



1-(3-Phenyl-1*H*-pyrazol-4-yl)methanamine and **36** were reacted according to general procedure 2 to afford **85**. LC-MS retention time = 2.66 min (100%), *m/z* (ES+) 363 ([M+H]$^+$, 100%).

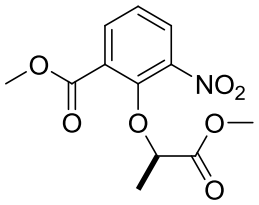
Methyl 2-{[(2*S*)-1-methoxy-1-oxopropan-2-yl]oxy}-3-nitrobenzoate [(*S*)-89]



To a solution of methyl 3-nitro-2-hydroxybenzoate (1.00 g, 5.07 mmol, 1.0 eq) in CH_2Cl_2 (15 mL) were added (*R*)-methyl 2-hydroxypropanoate (792 mg, 727 µL, 7.61 mmol, 1.5 eq) and PPh_3 (2.20 g, 8.37 mmol, 1.7 eq). The mixture was stirred at rt for 10 min before being cooled to 0 °C and di*iso*propyl azodicarboxylate (1.29 g, 1.26 mL, 6.40 mmol, 1.3 eq) was added dropwise *via* syringe. The reaction mixture was stirred at rt for 41 h, then concentrated

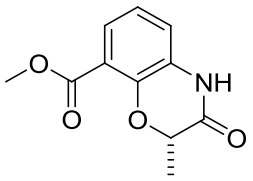
in vacuo and purified *via* silica gel chromatography (1:5 EtOAc:petroleum ether) to give (*S*)-**89** (1.47 mg, 4.95 mmol, 98%) as a yellow oil. R_f 0.45 (1:2 EtOAc:petroleum ether); $[\alpha]_D$ +53.6 (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 2955 (w), 1726 (w), 1533 (s), 1446 (m), 1360 (m), 1267 (m); ^1H NMR (400 MHz; $CDCl_3$): δ 8.04 (1H, dd, *J* 7.9 1.8 Hz, C(6)*H*), 7.93 (1H, dd, *J* 8.0 1.8 Hz, C(4)*H*), 7.27 (1H, dd, *J* 8.0 7.9 Hz, C(5)*H*), 4.70 (1H, q, *J* 6.8 Hz, C(2')*H*), 3.93 (3H, s, C(1)$CO_2C\mathit{H}_3$), 3.67 (3H, s, C(1')$O_2C\mathit{H}_3$), 1.63 (3H, d, *J* 6.8 Hz, (C(2')$C\mathit{H}_3$); ^{13}C NMR (101 MHz; $CDCl_3$): δ 170.9 (*C*(1')=O), 164.8 (C(1)*C*=O), 150.6 (*C*(2)), 145.8 (*C*(3)), 135.7 (*C*(6)H), 128.7 (*C*(4)H), 126.8 (*C*(1)), 123.9 (*C*(5)H), 80.4 (*C*(2')H), 52.8 (C(1)$CO_2C\mathit{H}_3$), 52.4 (C(1')$O_2C\mathit{H}_3$), 18.2 (C(2')$C\mathit{H}_3$); LRMS *m/z* (ES+) 589 ($[2M+Na]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 306.0573. $C_{12}H_{13}NNaO_7$ requires 306.0584]; Anal. calcd for $C_{12}H_{13}NO_7$: C 50.89%, H 4.63%, N 4.95%; Found: C 51.00%, H 4.62%, N 4.94%.

Methyl 2-{[(2*R*)-1-methoxy-1-oxopropan-2-yl]oxy}-3-nitrobenzoate [(*R*)-89]



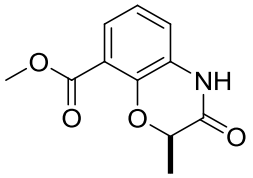
Methyl 3-nitro-2-hydroxybenzoate (1.00 g, 5.07 mmol, 1.0 eq) and (*S*)-methyl 2-hydroxypropanoate (792 mg, 727 µL, 7.61 mmol, 1.5 eq) were reacted as described above to prepare (*R*)-**89** (1.34 mg, 4.51 mmol, 89%) as a yellow oil. R_f 0.45 (1:2 EtOAc:petroleum ether); $[\alpha]_D$ −43.3 (*c* 1.0 in $CHCl_3$); ^1H NMR (400 MHz; $CDCl_3$): δ 8.05 (1H, dd, *J* 7.8 1.8 Hz, C(6)*H*), 7.95 (1H, dd, *J* 8.0 1.8 Hz, C(4)*H*), 7.29 (1H, t, *J* 8.0 7.8 Hz, C(5)*H*), 4.71 (1H, q, *J* 6.8 Hz, C(2')*H*), 3.95 (3H, s, C(1)$CO_2C\mathit{H}_3$), 3.69 (3H, s, C(1')$O_2C\mathit{H}_3$), 1.66 (3H, d, *J* 6.8 Hz, (C(2')$C\mathit{H}_3$); LRMS *m/z* (ES+) 289 ($[2M+Na]^+$, 100%). These data are in accordance with the enantiomer (*S*)-**89**.

Methyl (2*S*)-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylate [(*S*)-35]



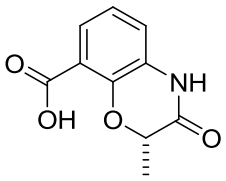
A mixture of (*S*)-**89** (191 mg, 0.643 mmol, 1.0 eq) and Pd/C (10% w/w, 34 mg, 0.032 mmol, 0.05 eq) in EtOAc (8 mL) was placed under a H_2 atmosphere. The reaction mixture was stirred at rt for 17 h, then filtered through Celite® (eluent EtOAc), concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 8 to 64% EtOAc in petroleum ether) yielded (*S*)-**35** (115 mg, 0.520 mmol, 81%) as a colourless solid. R_f 0.13 (1:2 EtOAc:petroleum ether); mp 136-138 °C (from EtOAc); $[\alpha]_D$ +38.3 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 9.19 (1H, br s, N*H*) 7.56-7.51 (1H, m, C(7)*H*), 7.03-6.98 (2H, m, C(5)*H* & C(6)*H*), 4.79 (1H, q, *J* 6.8 Hz, C(2)*H*), 3.92 (3H, s, OCH_3), 1.63 (3H, d, *J* 6.8 Hz, C(2)CH_3); LRMS *m/z* (ES+) 465 ([2M+Na]$^+$, 100%); Anal. calcd for $C_{11}H_{11}NO_4$: C 59.73%, H 5.01%, N 6.33%; Found: C 59.67%, H 4.91%, N 6.18%. These data are in accordance with the racemate **35**.

Methyl (2*R*)-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylate [(*R*)-35]



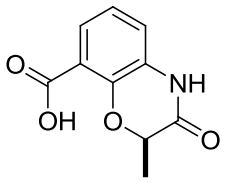
Compound (*R*)-**89** (1.34 mg, 4.51 mmol, 1.0 eq) was reacted as described above to prepare (*R*)-**35** (909 mg, 4.11 mmol, 91%) as a colourless solid. R_f 0.13 (1:2 EtOAc:petroleum ether); mp 134-135 °C (from EtOAc); $[\alpha]_D$ −32.0 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.43 (1H, br s, N*H*) 7.54 (1H, dd, *J* 7.7 1.9 Hz, C(7)*H*), 7.04-6.95 (2H, m, C(5)*H* & C(6)*H*), 4.78 (1H, q, *J* 6.8 Hz, C(2)*H*), 3.92 (3H, s, OCH_3), 1.63 (3H, d, *J* 6.8 Hz, C(2)CH_3); LRMS *m/z* (ES+) 465 ([2M+Na]$^+$, 100%); Anal. calcd for $C_{11}H_{11}NO_4$: C 59.73%, H 5.01%, N 6.33%; Found: C 59.54%, H 4.90%, N 6.25%. These data are in accordance with the racemate **35**.

(*S*)-2-Methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid [(*S*)-36]



To a solution of (*S*)-**35** [Methyl (2*S*)-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylate] (1.09 g, 4.94 mmol, 1.0 eq) in MeOH (40 mL) and THF (40 mL) was added a solution of LiOH aq. (1 M, 25 mL, 5.0 eq). The reaction mixture was stirred at rt for 44 h, then concentrated *in vacuo*, diluted with H_2O (50 mL) and acidified with HCl aq. (1 M). The resulting precipitate was filtered and washed with HCl aq. (1 M, 10 mL) and EtOAc (20 mL) to give (*S*)-**36** (640 mg, 3.09 mmol, 63%) as a colourless solid. R_f 0.46 (1:99 AcOH:EtOAc); mp 224 °C (dec., from 1 M HCl); $[\alpha]_D$ +34.8 (*c* 0.5 in $(CH_3)_2SO$); ^{1}H NMR (400 MHz; $(CD_3)_2SO$): δ 12.83 (1H, br s, CO_2H), 10.80 (1H, br s, N*H*), 7.31 (1H, dd, *J* 7.6 1.9 Hz, C(7)*H*), 7.08-6.98 (2H, m, C(5)*H* & C(6)*H*), 4.73 (1H, q, *J* 6.8 Hz, C(2)*H*), 1.41 (3H, d, *J* 6.8 Hz, C(2)CH_3); LRMS *m/z* (ES−) 206 ($[M-H]^-$, 100%). These data are in accordance with the racemate **36**.

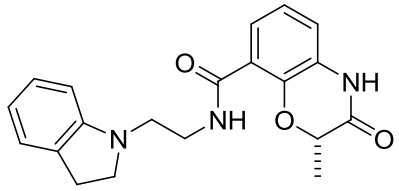
(*R*)-2-Methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid [(*R*)-36]



Compound (*R*)-**35** [methyl (2*R*)-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylate] (909 mg, 4.11 mmol, 1.0 eq) was reacted as described above to prepare (*R*)-**36** (643 mg, 3.10 mmol, 76%) as a colourless solid. R_f 0.49 (1:99 AcOH:EtOAc); mp 212 °C (dec.); $[\alpha]_D$ −36.8 (*c* 0.5 in $(CH_3)_2SO$); ^{1}H NMR (400 MHz; $(CD_3)_2SO$): δ 12.83 (1H, br s, CO_2H), 10.79 (1H, br s, N*H*), 7.31 (1H, dd, *J* 7.5 1.9 Hz, C(7)*H*), 7.08-6.98 (2H, m, C(5)*H* &

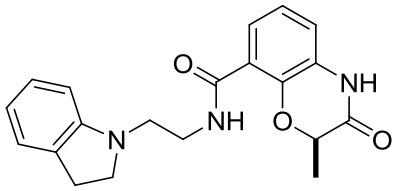
C(6)*H*), 4.73 (1H, q, *J* 6.8 Hz, C(2)*H*), 1.41 (3H, d, *J* 6.8 Hz, C(2)CH_3); LRMS *m/z* (ES−) 206 ([M−H]$^-$, 100%). These data are in accordance with the racemate **36**.

(2*S*)-*N*-[2-(2,3-Dihydro-1*H*-indol-1-yl)ethyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide [(*S*)-75]



Compound (*S*)-**36** [(*S*)-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid] (75 mg, 0.27 mmol, 1.0 eq) and 2-(2,3-dihydro-1*H*-indol-1-yl)ethanamine (48 mg, 0.30 mmol, 1.1 eq) were reacted according to general procedure 1 for 68 h. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) gave (*S*)-**75** (66 mg, 0.19 mmol, 71%) as a colourless solid. R_f 0.4 (2:1 EtOAc:petroleum ether); mp 161-163 °C (from MeOH); $[\alpha]_D$ +27.4 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.12 (1H, br s, N*H*), 7.93 (1H, br s, N*H*), 7.91 (1H, dd, *J* 7.8 1.6 Hz, C(7)*H*), 7.14-7.01 (3H, m, C(6)*H* & 2× C(Ar')*H*), 6.90 (1H, dd, *J* 7.8 1.6 Hz, C(5)*H*), 6.69 (1H, dd, *J* 7.0 7.0 Hz, C(Ar')*H*), 6.54 (1H, d, *J* 7.8 Hz, C(Ar')*H*), 4.48 (1H, q, *J* 6.8 Hz, C(2)*H*), 3.84-3.67 (2H, m, C(1')H_2), 3.51-3.25 (4H, m, C(2')H_2 & C(2'')H_2), 3.01 (2H, t, *J* 8.3 Hz, C(3'')H_2), 1.34 (3H, d, *J* 6.8 Hz, C(2)CH_3); LRMS *m/z* (ES+) 374 ([M+Na]$^+$, 100%); Anal. calcd for $C_{20}H_{21}N_3O_3$: C 68.36%, H 6.02%, N 11.96%; Found: C 68.38%, H 5.96%, N 11.83%. These data are in accordance with the racemate **75**.

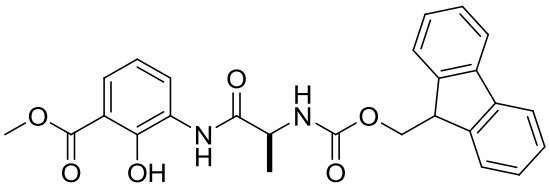
(2*R*)-*N*-[2-(2,3-Dihydro-1*H*-indol-1-yl)ethyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide [(*R*)-75]



Compound (*R*)-**36** [(*R*)-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid] (57 mg, 0.275 mmol, 1.0 eq) and 2-(2,3-dihydro-1*H*-indol-1-yl)ethanamine (49 mg, 0.30 mmol, 1.1 eq) were reacted according to general procedure 1 for 69 h. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) gave (*R*)-**75** (85 mg, 0.242 mmol, 88%) as a colourless solid. Crystals for X-ray crystallography were prepared *via* slow evaporation from EtOH. R_f 0.4 (2:1 EtOAc:petroleum ether); mp 160-162°C (from EtOH); $[\alpha]_D$ −28.9 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.12 (1H, br s, N*H*), 7.97 (1H, br s, N*H*), 7.91 (1H, dd, *J* 7.9 1.6 Hz, C(7)*H*), 7.14-7.02 (3H, m, C(6)*H* & 2× C(Ar')*H*), 6.91 (1H, dd, *J* 7.9 1.6 Hz, C(5)*H*), 6.69 (1H, dd, *J* 7.4 7.4 Hz, C(Ar')*H*), 6.54 (1H, d, *J* 7.8 Hz, C(Ar')*H*), 4.48 (1H, q, *J* 6.8 Hz, C(2)*H*), 3.84-3.67 (2H, m, C(1')H_2), 3.52-3.25 (4H, m, C(2')H_2 & C(2'')H_2), 3.01 (2H, t, *J* 8.3 Hz, C(3'')H_2), 1.34 (3H, d, *J* 6.8 Hz, C(2)CH_3); LRMS *m/z* (ES+) 374 ($[M+Na]^+$, 100%); Anal. calcd for $C_{20}H_{21}N_3O_3$: C 68.36%, H 6.02%, N 11.96%; Found: C 68.43%, H 5.94%, N 11.83%. These data are in accordance with the racemate **75**.

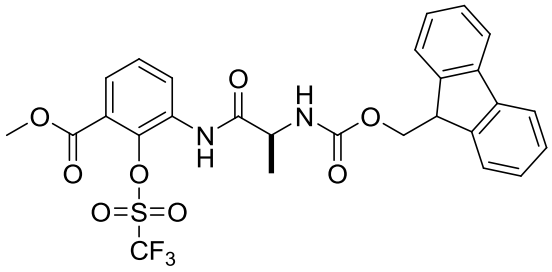
6.4 Chapter 3 Procedures

Methyl 3-[(2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}propanoyl)amino]-2-hydroxybenzoate (103)



To a solution of Fmoc-L-alanine (784 mg, 2.52 mmol, 1.0 eq) and HBTU (1.43 g, 3.78 mmol, 1.5 eq) in DMF (18 mL) were added sequentially methyl 3-amino-2-hydroxybenzoate (463 mg, 2.77 mmol, 1.1 eq) and *N*,*N*-diisopropylethylamine (877 µL, 651 mg, 5.04 mmol, 2.0 eq). The reaction mixture was stirred at rt for 96 h. The reaction mixture was diluted with EtOAc (100 mL), washed with H_2O (3 × 100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 5 to 62% EtOAc in petroleum ether) gave **103** (326 mg, 0.708 mmol, 28%) as a colourless solid. R_f 0.43 (1:1 EtOAc:petroleum ether); mp 188-190 °C (from $CHCl_3$); ν_{max}/cm^{-1} 3394 (br m), 1671 (s), 1526 (s), 1439 (m), 1248 (s); ^{1}H NMR (400 MHz; $(CD_3)_2SO$): δ 10.99 (s, 1H, O*H*), 9.26 (s, 1H, N*H*), 8.24 (d, 1H, *J* 7.9 Hz, C(6)*H*), 7.89 (d, 2H, *J* 7.4 Hz, C(4'')*H* & C(5'')*H*), 7.83 (d, 1H, *J* 7.0 Hz, N*H*), 7.77-7.69 (m, 2H, C(1'')*H* & C(8'')*H*), 7.55 (d, 1H, *J* 8.0 Hz, C(4)*H*), 7.41 (dd, 2H, *J* 7.4 7.3 Hz, C(3'')*H* & C(6'')*H*), 7.37-7.27 (m, 2H, C(2'')*H* & C(7'')*H*), 6.95 (dd, 1H, *J* 8.0 7.9 Hz, C(5)*H*), 4.42-4.17 (m, 4H, C(2')*H* & C(9'')*H* & C(9'')CH_2), 3.92 (s, 3H, OCH_3), 1.33 (d, 3H, *J* 7.08 Hz, C(3')H_3); LRMS *m/z* (ES+) 943 ($[2M+Na]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 483.1509. $C_{26}H_{24}N_2NaO_6^+$ requires 483.1527].

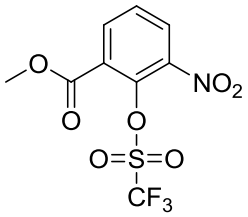
Methyl 3-[(2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}propanoyl)amino]-2-[(trifluoromethyl)sulfonyl]benzoate (104)



To a solution of **103** [methyl 3-[(2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}propanoyl)amino]-2-hydroxybenzoate] (64 mg, 0.139 mmol, 1.0 eq) and triethylamine (29 µL, 0.208 mmol, 1.5 eq) in CH_2Cl_2 (2 mL) at 0 °C was added triflic anhydride (47 µL, 79 mg, 0.278 mmol, 2.0 eq) dropwise over 5 min. The reaction mixture was allowed to warm to rt and stirred for a further 4 h. The reaction mixture was concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 6 to 48% EtOAc in petroleum ether) to give **104** (40 mg, 0.0675 mmol, 49%) as a pale yellow oily solid. R_f 0.14 (1:3 EtOAc:petroleum ether); mp 104-108 °C (from EtOAc); ν_{max}/cm^{-1} 3297 (w), 1720 (w), 1672 (s), 1526 (m), 1426 (s); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 10.23 (s, 1H, N*H*), 7.92-7.83 (m, 3H, C(6)*H* & C(4'')*H* & C(5'')*H*), 7.79 (dd, 1H, *J* 7.8 1.1 Hz, C(4)*H*), 7.76-7.68 (m, 3H, C(1'')*H* & C(8'')*H* & N*H*), 7.58 (dd, 1H, *J* 8.0 7.8 Hz, C(5)*H*), 7.41 (dd, 2H, *J* 7.5 7.4 Hz, C(3'')*H* & C(6'')*H*), 7.33 (dd, 2H, *J* 7.4 7.3 Hz, C(2'')*H* & C(7'')*H*), 4.35-4.19 (m, 4H, C(2')*H* & C(9'')*H* & C(9'')CH_2), 3.87 (s, 3H, OCH_3), 1.35 (d, 3H, *J* 7.1 Hz, C(3')H_3);^{13}C NMR (126 MHz; $(CD_3)_2SO$): δ 172.0 (*C*(1')=O), 164.1 (C(1)*C*=O), 155.8 (C(2')NH*C*=O), 143.9 (*C*(12'')), 143.8 (*C*(13'')), 140.7 (*C*(10'') & *C*(11'')), 140.4 (*C*(2)), 131.5 (*C*(3)), 131.3 (*C*(6)H), 128.9 (*C*(5)H), 128.2 (*C*(4)H), 127.6 (*C*(3'')H & *C*(6'')H), 127.0 (*C*(2'')H & *C*(7'')H), 125.3 (*C*(1'')H & *C*(8'')H), 124.9 (*C*(1)), 120.1 (*C*(4'')H & *C*(5'')H), 117.9 (q, *J* 320.5 Hz, O*C*F_3), 65.7 (C(9'')*C*H_2), 52.8 (O*C*H_3), 50.4 (*C*(2')H), 46.6 (*C*(9'')H), 17.6 (*C*(3')H_3); ^{19}F NMR (377 MHz, $CDCl_3$): δ −73.14 (s, 3F, OCF_3); LRMS *m/z* (ES+) 615

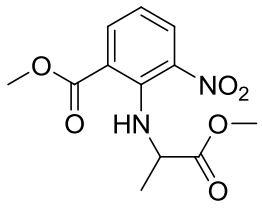
([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 615.1002. $C_{26}H_{24}N_2NaO_6^+$ requires 615.1019].

Methyl 3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate (107)



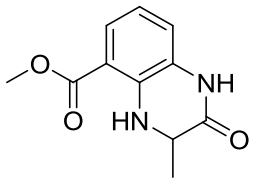
To a solution of methyl 3-nitro-2-hydroxybenzoate (556 mg, 2.82 mmol, 1.0 eq) and Et_3N (428 mg, 590 µL, 4.23 mmol, 2.0 eq) in CH_2Cl_2 (12 mL) at 0 °C was added triflic anhydride (839 mg, 500 µL, 2.97 mmol, 1.1 eq) dropwise over 5 min. The reaction mixture was allowed to warm to rt, stirred for a further 2 h, then concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 6 to 42% EtOAc in petroleum ether) to give **107** (836 mg, 2.54 mmol, 90%) as a yellow oil. R_f 0.29 (1:4 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2973 (w), 1735 (s), 1543 (s), 1431 (s), 1353 (m), 1208 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.32 (1H, dd, *J* 8.0 1.8 Hz, C(6)*H*), 8.23 (1H, dd, *J* 8.1 1.8 Hz, C(4)*H*), 8.02 (1H, dd, *J* 8.1 8.0 Hz, C(5)*H*), 4.01 (3H, s, CO_2CH_3); ^{13}C NMR (100 MHz; $CDCl_3$): δ 163.2 (*C*O_2CH_3), 143.2 (*C*(3)), 140.2 (*C*(2)), 136.9 (*C*(6)H), 129.9 (*C*(4)H), 128.6 (*C*(5)H), 127.5 (*C*(1)), 118.4 (d, *J* 322.2 Hz, *C*F_3), 53.3 (CO_2CH_3); ^{19}F NMR (377 MHz; $CDCl_3$): δ −73.6 (3F, s, OC*F*$_3$); LRMS *m/z* (ES+) 352 ([M+Na]$^+$, 30%), 413 (100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 351.9702. $C_9H_6F_3NNaO_7S^+$ requires 351.9709]; Anal. calcd for $C_9H_6F_3NO_7S$: C 32.84%, H 1.84%, N 4.25%; Found: C 32.98%, H 1.84%, N 4.19%.

Methyl 2-[(1-methoxy-1-oxopropan-2-yl)amino]-3-nitrobenzoate (108)



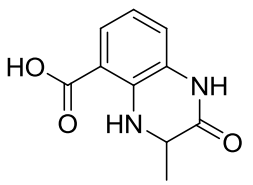
A microwave flask was charged with DL-alanine methyl ester hydrochloride (237 mg, 1.70 mmol, 1.4 eq), $Pd(OAc)_2$ (13 mg, 0.058 mmol, 0.05 eq), BINAP (70 mg, 0.112 mmol, 0.1 eq), and Cs_2CO_3 (1.11 g, 3.41 mmol, 2.9 eq) and sealed under Ar. Toluene (20 mL) was added and the resulting solution was heated to 80 °C for 5 min. A solution of **107** [methyl 3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate] (390 mg, 1.18 mmol, 1 eq) in toluene (5 mL) was added dropwise over 30 min and the reaction mixture stirred at 80 °C for 2.5 h. After cooling to rt the slurry was washed with H_2O (3 × 25 mL) and the organic components concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) gave **108** (253 mg, 0.896 mmol, 76%) as a yellow oil. R_f 0.60 (1:2 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2955 (w), 1745 (s), 1698 (s), 1532 (s), 1346 (m), 1260 (s); ^1H NMR (500 MHz; $CDCl_3$): δ 8.76 (1H, d, *J* 8.0 Hz, N*H*), 8.12 (1H, dd, *J* 7.9 1.7 Hz, C(6)*H*), 7.97 (1H, dd, *J* 8.1 1.7 Hz, C(4)*H*), 6.79 (1H, dd, *J* 8.1 7.9 Hz, C(5)*H*), 4.09-4.01 (1H, m, C(1')*H*), 3.94 (3H, s, $ArCO_2CH_3$), 3.72 (3H, s, $C(2')O_2CH_3$), 1.46 (3H, d, *J* 7.0 Hz, $C(1')CH_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 173.4 ($C(2')O_2CH_3$), 167.5 ($C(1)CO_2CH_3$), 144.0 (*C*(1)), 138.7 (*C*(3)), 137.0 (*C*(6)H), 131.2 (*C*(4)H), 118.4 (*C*(2)), 116.3 (*C*(5)H), 53.9 (*C*(1')H), 52.5 ($ArCO_2CH_3$), 52.5 ($C(2')O_2CH_3$), 19.1 ($C(1')CH_3$); LRMS *m/z* (ES+) 587 ($[2M+Na]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 305.0744. $C_{12}H_{14}N_2NaO_6$ requires 305.0748]; Anal. calcd for $C_{12}H_{14}N_2O_6$: C 51.06%, H 5.00%, N 9.93%; Found: C 51.13%, H 4.96%, N 9.88%.

Methyl 3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate (106)



A mixture of **108** (238 mg, 0.843 mmol, 1.0 eq) and Pd/C (10% w/w, 45 mg, 0.42 mmol, 0.05 eq) in EtOAc (10 mL) was placed under a H_2 atmosphere. The reaction mixture was stirred for 18 h, then filtered through Celite® (eluent EtOAc) and concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 8 to 64% EtOAc in petroleum ether) to give **106** (154 mg, 0.699 mmol, 83%) as a colourless solid. R_f 0.3 (1:2 EtOAc:petroleum ether); mp 152-154 °C (from EtOAc); ν_{max}/cm^{-1} 3348 (w), 2953 (w), 1671 (s), 1436 (m), 1238 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.03 (1H, br s, N*H*), 7.62-7.55 (2H, m, C(6)*H* & N*H*), 6.88 (1H, d, *J* 7.7 Hz, C(8)*H*), 6.66 (1H, dd, *J* 7.9 7.7 Hz, C(7)*H*), 4.19 (1H, qd, *J* 6.6 1.6 Hz, C(3)*H*), 3.89 (3H, s, OCH_3), 1.52 (3H, d, *J* 6.6 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.8 (*C*(2)=O), 168.4 (C(5)*C*O_2CH_3), 137.6 (*C*(Ar)), 126.0 (*C*(Ar)), 125.7 (*C*(6)H), 119.1 (*C*(8)H), 116.6 (*C*(7)H), 111.3 (*C*(Ar)), 51.8 (O*C*H_3), 51.1 (*C*(3)H), 18.9 (C(3)*C*H_3); LRMS *m/z* (ES+) 463 ([2M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 243.0735. $C_{11}H_{12}N_2NaO_3^+$ requires 243.0740]; Anal. calcd for $C_{11}H_{12}N_2O_3$: C 59.99%, H 5.49%, N 12.72%; Found: C 59.89%, H 5.42%, N 12.69%.

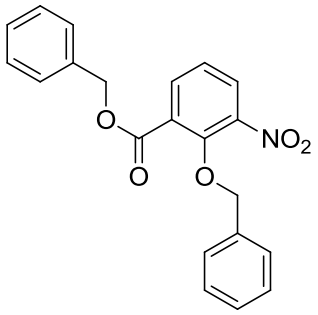
3-Methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid (94)



To a solution of **106** [methyl (2*S*)-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylate] (54 mg, 0.245 mmol, 1.0 eq) in MeOH (2 mL) and THF (2 mL) was added a solution of LiOH aq. (2 M, 1.25 mL, 10 eq). The reaction mixture was stirred at rt for 24 h, then

concentrated *in vacuo*, diluted with H_2O (50 mL) and acidified with HCl aq. (1 M). The resulting precipitate was extracted into EtOAc (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **94** (33 mg, 0.160 mmol, 65%) as a yellow solid that was used without further purification. R_f 0.4 (EtOAc); mp 230 °C (dec., from 1 M HCl); ^{1}H NMR (400 MHz; $(CD_3)_2SO$): δ 12.91 (1H, br s, CO_2H), 10.46 (1H, s, N*H*), 7.60 (1H, s, N*H*), 7.42 (1H, dd, *J* 7.9 1.4 Hz, C(Ar)*H*), 6.92 (1H, dd, *J* 7.7 1.4 Hz, C(Ar)*H*), 6.63 (1H, dd, *J* 7.9 7.7 Hz, C(7)*H*), 4.09-4.00 (1H, m, C(3)*H*), 1.29 (3H, d, *J* 6.7 Hz, C(3)CH_3); LRMS *m/z* (ES−) 205 ($[M-H]^-$, 100%). These data are in accordance with the (*R*)-enantiomer **94**.

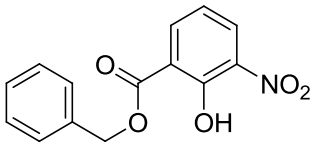
Benzyl 2-benzyloxy-3-nitrobenzoate (111)



A 5 mL microwave vial was charged with 2-hydroxy-3-nitrobenzoic acid (200 mg, 1.09 mmol, 1.0 eq), K_2CO_3 (755 mg, 5.46 mmol, 5.0 eq) and DMF (3 mL). The vial was sealed and the mixture stirred at rt for 5 min before BnBr (650 µL, 933 mg, 5.46 mmol, 5.0 eq) was added *via* syringe. The reaction mixture was stirred at 70 ° C for 39 h. After cooling to rt the mixture was diluted with EtOAc (50 mL), washed with 2 M K_2CO_3 (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 2 to 20% EtOAc in petroleum ether) gave **111** (267 mg, 0.735 mmol, 67%) as a pale orange oil. R_f 0.52 (1:4 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3034 (w), 1728 (s), 1531 (s), 1361 (m), 1260 (m); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.09 (1H, dd, *J* 7.9 1.8 Hz, C(6)*H*), 7.93 (1H, dd, *J* 8.0 1.8 Hz, C(4)*H*), 7.47-7.34 (10H, m, 10 × C(Ar')*H*), 7.29 (1H, dd, *J* 8.0 7.9 Hz, C(5)*H*), 5.38 (2H, s, C(1)CO_2CH_2Ph), 5.17 (2H, s, C(2)OCH_2Ph); ^{13}C NMR (101 MHz;

$CDCl_3$): δ 164.3 (C(1)*C*=O), 151.6 (*C*(Ar')), 145.9 (*C*(Ar')), 135.70 (*C*(Ar')), 135.68 (*C*(6)H), 135.2 (*C*(Ar')), 128.73 (*C*(Ar')H), 128.69 (*C*(Ar')H), 128.62 (*C*(Ar')H), 128.58 (*C*(Ar')H), 128.5 (*C*(Ar)H), 128.2 (*C*(Ar')), 124.1 (*C*(5)H), 78.6 (C(2)O*C*H_2Ph), 67.7 (C(1)CO_2*C*H_2Ph); LRMS *m/z* (ES+) 386 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 386.0985. $C_{21}H_{17}NNaO_5^+$ requires 386.0999]; Anal. calcd for $C_{21}H_{17}NO_5$: C 69.41%, 4.72%, 3.85%; Found: C 69.32%, H 4.68%, 3.93%.

Benzyl 3-nitro-2-hydroxybenzoate (112)



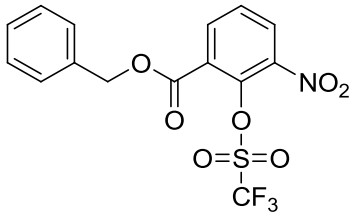
Method 1: 2-hydroxy-3-nitrobenzoic acid (800 mg, 4.37 mmol, 1.0 eq) and $KHCO_3$ (525 mg, 5.24 mmol, 1.2 eq) were dissolved in DMF (12 mL) and stirred at rt for 10 min. BnBr (897 mg, 624 µL, 5.24 mmol, 1.2 eq) was added and the reaction mixture was stirred at 40 °C for 23 h. The reaction mixture was diluted with EtOAc (50 mL) and washed with H_2O (2 × 50 mL). Addition of 5% $NaHCO_3$ (50 mL) caused the precipitation of an orange solid that was isolated *via* filtration (eluent EtOAc). The solid was dissolved in 1 M HCl (50 mL) and extracted with EtOAc (50 mL). The organic components were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **112** (350 mg, 1.28 mmol, 29%) as a yellow solid.

Method 2: To a solution of **111** [benzyl 2-benzyloxy-3-nitrobenzoate] (961 mg, 2.65 mmol, 1.0 eq) in trifluoroacetic acid (13 mL) and toluene (13 mL) was stirred at rt for 1.5 h. The reaction mixture was concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 2 to 80% EtOAc in petroleum ether) to give **112** (721 mg, 2.64 mmol, 99%) as a yellow solid.

R_f 0.38 (1:6 EtOAc:petroleum ether); mp 41-43 °C (from Et_2O); ν_{max}/cm^{-1} 2909 (w), 1672 (s), 1523 (s), 1350 (m), 1266 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.20-8.14 (2H, m, C(4)*H* &

C(6)*H*), 7.49-7.37 (5H, m, 5 × C(Ar')*H*), 6.99 (1H, t, *J* 8.0 Hz, C(5)*H*), 5.44 (2H, s, OC*H*$_2$Ph); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.5 (C(1)*C*=O), 155.8 (*C*(Ar)), 138.0 (*C*(Ar)), 135.8 (*C*(Ar)H), 134.4 (*C*(Ar')), 131.4 (*C*(Ar)H), 128.9 (*C*(Ar')H), 128.8 (*C*(Ar')H), 128.6 (*C*(Ar')H), 118.4 (*C*(5)H), 115.9 (*C*(Ar)), 68.0 (O*C*H$_2$Ph); LRMS *m/z* (ES+) 296 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 296.0527. $C_{14}H_{11}NNaO_5^+$ requires 296.0529]; Anal. calcd for $C_{14}H_{11}NO_5$: C 61.54%, 4.06%, 5.13%; Found: C 61.60%, H 3.92%, 5.13%.

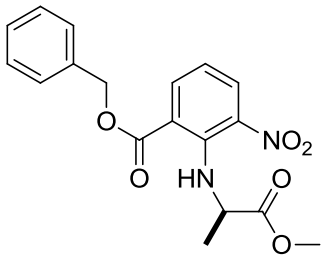
Benzyl 3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate (113)



To a solution of **112** [benzyl 3-nitro-2-hydroxybenzoate] (1.65 g, 6.04 mmol, 1.0 eq) and Et_3N (915 mg, 1.26 mL, 9.06 mmol, 1.5 eq) in CH_2Cl_2 (50 mL) at 0 °C was added triflic anhydride (2.05 g, 1.22 mL, 7.25 mmol, 1.2 eq) dropwise over 5 min. The reaction mixture was allowed to warm to rt, stirred for a further 3 h, then concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) to give **113** (2.25 g, 5.55 mmol, 92%) as a yellow oil. R_f 0.22 (1:10 EtOAc:petroleum ether to 1:5 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3265 (w), 1732 (s), 1542 (s), 1431 (s), 1352 (m), 1267 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.30 (1H, dd, *J* 8.0 1.7 Hz, C(6)H), 8.21 (1H, dd, *J* 8.0 1.7 Hz, C(4)*H*), 7.63 (1H, dd, *J* 8.0 8.0 Hz, C(5)*H*), 7.51-7.45 (2H, m, 2 × C(Ar')*H*), 7.45-7.36 (3H, m, C(4')*H* & 2 × C(Ar')*H*), 5.45 (2H, s, OC*H*$_2$Ph); ^{13}C NMR (101 MHz; $CDCl_3$): δ 162.4 (C(1)C=O), 143.1 (C(Ar)), 140.1 (C(Ar)), 136.7 (C(6)H), 134.4 (C(1')), 129.8 (C(4)H), 128.9 (2 × C(Ar')H), 128.9 (C(4')H), 128.7 (2 × C(Ar')H), 128.6 (C(5)H), 127.8 (C(Ar)), 118.4 (q, *J* 320.9 Hz, O*C*F$_3$), 68.5 (O*C*H$_2$Ph); ^{19}F NMR (377 MHz; $CDCl_3$): δ −73.43 (3F, s, OC*F*$_3$); LRMS *m/z* (ES+) 833 ([2M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 428.0015.

$C_{15}H_{10}F_3NNaO_7S^+$ requires 428.0022]; Anal. calcd for $C_{15}H_{10}F_3NO_7S$: C 44.45%, H 2.49%, N 3.46%; Found: C 44.49%, H 2.40%, N 3.42%.

Benzyl 2-{[(2'*R*)-1'-methoxy-1'-oxopropan-2'-yl]amino}-3-nitrobenzoate [(*R*)-114]



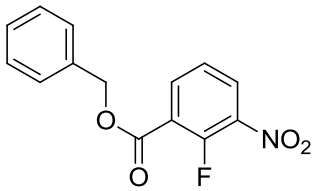
Method 1: A mixture of D-alanine methyl ester hydrochloride (437 mg, 2.08 mmol, 1.5 eq), $Pd(OAc)_2$ (23 mg, 0.10 mmol, 0.05 eq), BINAP (130 mg, 0.209 mmol, 0.1 eq), and Cs_2CO_3 (2.04 g, 6.26 mmol, 2.9 eq) in toluene (30 mL) was placed under an argon atmosphere. The mixture was heated to 80 °C for 30 min causing a deep red colour to form. A solution of **113** [benzyl 3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate] (845 mg, 2.08 mmol, 1.0 eq) in toluene (10 mL) was added dropwise over 30 min and the reaction mixture stirred at 80 °C for 2 h. After cooling to rt the slurry was washed with H_2O (3 × 50 mL) and the organic components concentrated *in vacuo*. Purification *via* silica gel chromatography (1:10 EtOAc:petroleum ether) gave (*R*)-**114** (680 mg, 1.90 mmol, 91%).

Method 2: To a solution of **113** [benzyl 3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate] (3.37 g, 8.31 mmol, 1.0 eq) in toluene (150 mL) was added sequentially Cs_2CO_3 (8.13 g, 24.9 mmol, 3.0 eq) and D-alanine methyl ester hydrochloride (1.74 g, 12.5 mmol, 1.5 eq). The resulting yellow solution was heated to 85 ºC causing a deep orange colour to form and stirring was continued at 85 ºC for 19 h. Upon cooling to rt the reaction mixture was filtered through Celite® (eluent toluene) and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 3 to 28% EtOAc in petroleum ether) yielded (*R*)-**114** (2.36 g, 6.59 mmol, 79%) as a yellow oil.

Method 3: Reported later.

R_f 0.56 (1:3 EtOAc:petroleum ether); $[\alpha]_D$ −10.9 (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3266 (w), 1745 (m), 1690 (s), 1530 (s), 1344 (m), 1251 (s); ^1H NMR (400 MHz; $CDCl_3$): δ 8.78 (1H, br d, *J* 8.4 Hz, N*H*), 8.14 (1H, dd, *J* 7.9 1.7 Hz, C(6)*H*), 7.96 (1H, dd, *J* 8.1 1.7 Hz, C(4)*H*), 7.48-7.33 (5H, m, 5 × C(Ar')*H*), 6.77 (1H, dd, *J* 8.1 7.9 Hz, C(5)*H*), 5.36 (2H, s, OCH_2Ph), 4.05 (1H, dq, *J* 8.4 7.0 Hz, C(2')*H*), 3.69 (3H, s, C(1')O_2CH_3), 1.43 (3H, d, *J* 7.0 Hz, C(2')CH_3); ^{13}C NMR (101 MHz; $CDCl_3$): δ 173.5 (*C*(1')O_2CH_3), 166.8 (C(1)*C*=O), 144.1 (*C*(2)), 138.8 (*C*(3)), 137.1 (*C*(6)H), 135.3 (*C*(Ar')), 131.3 (*C*(4)H), 128.7 (*C*(Ar')H), 128.6 (*C*(Ar')H), 128.5 (*C*(Ar')H), 118.4 (*C*(1)), 116.3 (*C*(5)H), 67.3 (O*C*H_2Ph), 54.0 (*C*(2')H), 52.6 (C(1')O_2*C*H_3), 19.1 (C(2')*C*H_3); LRMS *m/z* (ES+) 359 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 381.1071. $C_{18}H_{18}N_2NaO_6^+$ requires 381.1057]; Anal. calcd for $C_{18}H_{18}N_2O_6$: C 60.33%, H 5.06%, N 7.82%; Found: C 60.37%, H 4.97%, N 7.74%.

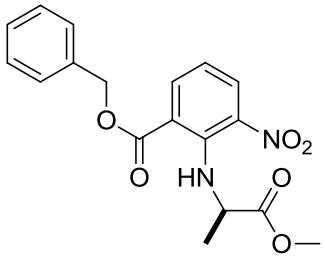
Benzyl 2-fluoro-3-nitrobenzoate (116)



A suspension of 2-fluoro-3-nitrobenzoic acid (4.15 g, 22.4 mmol, 1.0 eq) and K_2CO_3 (4.65 g, 33.1 mmol, 1.5 eq) in DMF (50 mL) was stirred at rt for 5 min, then BnBr (4.01 mL, 5.75 g, 33.1 mmol, 1.5 eq) was added and the reaction mixture stirred at 60 ºC for 3 h. After cooling to rt the suspension was diluted with EtOAc (250 mL), washed with 2 M K_2CO_3 (3 × 250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:10 EtOAc:petroleum ether) yielded **116** (5.95 g, 21.6 mmol, 97%) as a pale yellow oil. R_f 0.35 (1:6 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3092 (w), 2361 (w), 1730 (s), 1535 (s), 1350 (m), 1263 (m); ^1H NMR (500 MHz; $CDCl_3$): δ 8.24 (1H, ddd, *J* 7.8 6.2 1.6 Hz,

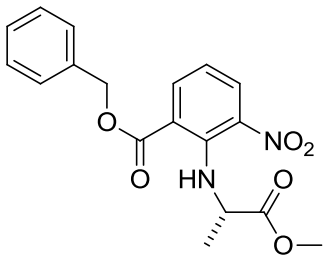
C(6)*H*), 8.19 (1H, ddd, *J* 8.2 6.2 1.6 Hz, C(4)*H*), 7.49-7.45 (2H, m, 2 × C(Ar')*H*), 7.44-7.34 (4H, m, C(5)*H* & 3 × C(Ar')*H*), 5.43 (2H, s, C(1)$CO_2C\mathit{H}_2$Ph); ^{13}C NMR (126 MHz; $CDCl_3$): δ 162.5 (d, *J* 3.6 Hz, C(1)*C*=O), 154.9 (d, *J* 278.3 Hz, *C*(2)F), 138.7 (d, *J* 8.8 Hz, *C*(3)), 137.3 (*C*(6)H), 135.0 (*C*(Ar')), 129.9 (d, *J* 1.4 Hz, *C*(4/6)H), 128.7 (*C*(Ar')H), 128.6 (*C*(Ar')H), 128.3 (*C*(Ar')H), 124.0 (d, *J* 5.6 Hz, *C*(5)H), 121.6 (d, *J* 9.7 Hz, *C*(1)), 67.9 (O*C*H_2Ph); ^{19}F NMR (377 MHz; $CDCl_3$): δ −116.13 (1F, dd, *J* 6.2 6.2 Hz, C(2)*F*); LRMS *m/z* (ES+) 298 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 298.0476. $C_{14}H_{10}FNNaO_4^+$ requires 298.0486]; Anal. calcd for $C_{14}H_{10}FNO_4$. C 61.09%, H 3.66%, N 5.09%; Found: C 61.21%, H 3.57%, N 5.23%.

Benzyl 2-{[(2'*R*)-1'-methoxy-1'-oxopropan-2'-yl]amino}-3-nitrobenzoate [(*R*)-114]



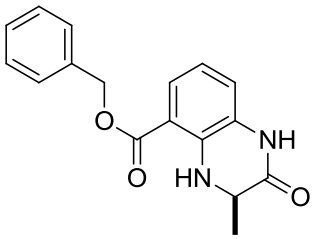
Method 3: To a solution of **116** [benzyl 2-fluoro-3-nitrobenzoate] (2.50 g, 9.08 mmol, 1.0 eq) in toluene (150 mL) was added Cs_2CO_3 (6.51 g, 20.0 mmol, 2.2 eq) and D-alanine methyl ester hydrochloride (1.39 g, 9.99 mmol, 1.1 eq). The resulting yellow solution was stirred at 85 ºC for 24 h. Upon cooling to rt the reaction mixture was diluted with EtOAc (300 mL), washed with 2 M K_2CO_3 (3 × 250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:10 EtOAc:petroleum ether) yielded (*R*)-**114** (2.93 g, 8.18 mmol, 90%) as a yellow oil. Data reported above.

Benzyl 2-{[(2'*S*)-1'-methoxy-1'-oxopropan-2'-yl]amino}-3-nitrobenzoate [(*S*)-114]



Compound **116** [benzyl 2-fluoro-3-nitrobenzoate] (305 mg, 1.11 mmol, 1.0 eq) and L-alanine methyl ester hydrochloride (186 mg, 1.33 mmol, 1.2 eq) were reacted as described above for 4.5 h. Purification *via* silica gel chromatography (gradient elution 12 to 100% CH_2Cl_2 in petroleum ether) yielded (*S*)-**114** (212 mg, 0.592 mmol, 53%) as a yellow oil. R_f 0.38 (1:1 CH_2Cl_2:petroleum ether); $[\alpha]_D$ +4.4 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.77 (1H, br d, *J* 8.4 Hz, N*H*), 8.14 (1H, dd, *J* 7.9 1.7 Hz, C(6)*H*), 7.97 (1H, dd, *J* 8.1 1.7 Hz, C(4)*H*), 7.48-7.33 (5H, m, 5 × C(Ar')*H*), 6.77 (1H, dd, *J* 8.1 7.9 Hz, C(5)*H*), 5.37 (2H, s, OCH_2Ph), 4.04 (1H, dq, J 8.4 6.9, C(2')*H*), 3.70 (3H, s, C(1')O_2CH_3), 1.43 (3H, d, *J* 6.9 Hz, C(2')CH_3); LRMS *m/z* (ES+) 359 ([M+H]$^+$, 100%). These data are in accordance with the enantiomer (*R*)-**114**.

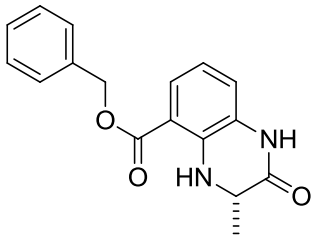
Benzyl (3*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate [(*R*)-117]



To a solution of (*R*)-**114** [benzyl 2-{[(2*R*)-1-methoxy-1-oxopropan-2-yl]amino}-3-nitrobenzoate] (106 mg, 0.296 mmol, 1.0 eq) in DMF (6 mL) was added zinc dust (484 mg, 7.40 mmol, 25 eq) and NH_4Cl (396 mg, 7.40 mmol, 25 eq). The reaction mixture was stirred at rt for 24 h, then diluted with EtOAc (50 mL) and filtered through Celite® (eluent EtOAc). The filtrate was washed with brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*.

Purification *via* silica gel chromatography (1:4 EtOAc:petroleum ether) yielded (*R*)-**117** (84 mg, 0.28 mmol, 96%) as a colourless solid. R_f 0.40 (1:1 EtOAc:petroleum ether); mp 199-200 °C (from EtOAc); $[\alpha]_D$ −48.4 (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3354 (w), 3065 (w), 1687 (s), 1498 (m), 1239 (s); ^1H NMR (500 MHz; $CDCl_3$): δ 8.80 (1H, br s, N*H*), 7.65 (1H, d, *J* 7.8 Hz, C(6)*H*), 7.60 (1H, br s, N*H*), 7.51-7.33 (5H, m, 5 × C(Ar')*H*), 6.87 (1H, d, *J* 7.5 Hz, C(8)*H*), 6.65 (1H, dd, *J* 7.8 7.5 Hz, C(7)*H*), 5.34 (2H, s, OCH_2Ph), 4.24-4.14 (1H, m, C(3)*H*), 1.52 (3H, d, *J* 6.5 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.6 (*C*(2)=O), 167.7 (C(5)*C*=O), 137.8 (*C*(9)), 135.9 (*C*(Ar')), 128.6 (*C*(Ar')H), 128.3 (*C*(Ar')H), 128.0 (*C*(Ar')H), 126.0 (*C*(10)), 125.8 (*C*(6)H), 119.1 (*C*(8)H), 116.6 (*C*(7)H), 111.2 (*C*(5)), 66.4 (O*C*H_2Ph), 51.1 (*C*(3)H), 18.9 (C(3)*C*H_3); LRMS *m/z* (ES+) 615 ($[2M+Na]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 319.1051. $C_{17}H_{16}N_2NaO_3^+$ requires 319.1053]; Anal. calcd for $C_{17}H_{16}N_2O_3$: C 68.91%, H 5.44%, N 9.45%; Found: C 69.01%, H 5.39%, N 9.39%.

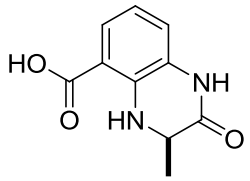
Benzyl (3*S*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate [(*S*)-117]



Compound (*S*)-**114** [benzyl 2-{[(2*S*)-1-methoxy-1-oxopropan-2-yl]amino}-3-nitrobenzoate] (186 mg, 0.512 mmol, 1.0 eq) was reacted as described above for 24 h. Purification *via* silica gel chromatography (gradient elution 5 to 80% EtOAc in petroleum ether) yielded (*S*)-**117** (134 mg, 0.452 mmol, 88%) as a colourless solid. R_f 0.29 (1:2 EtOAc:petroleum ether); mp 204-206 °C (from EtOAc); $[\alpha]_D$ +42.0 (*c* 1.0 in $CHCl_3$); ^1H NMR (400 MHz; $CDCl_3$): δ 7.83 (1H, br s, N*H*), 7.65 (1H, d, *J* 8.0 1.1 Hz, C(6)*H*), 7.59 (1H, br s, N*H*), 7.46-7.32 (5H, m, 5 × C(Ar')*H*), 6.80 (1H, d, *J* 7.7 1.1 Hz, C(8)*H*), 6.65 (1H, dd, *J* 8.0 7.7 Hz, C(7)*H*), 5.34 (2H, d, *J*

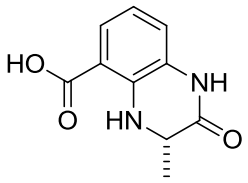
1.0 Hz, OC*H*$_2$Ph), 4.16 (1H, qd, *J* 6.8 1.3 Hz, C(3)*H*), 1.51 (3H, d, *J* 6.8 Hz, C(3)C*H*$_3$); LRMS *m/z* (ES+) 297 ([M+H]$^+$, 100%). These data are in accordance with the enantiomer (*R*)-**117**.

(*R*)-3-Methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid [(*R*)-94]



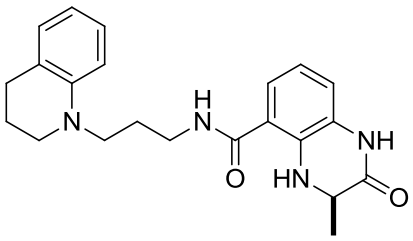
A mixture of (*R*)-**117** [benzyl (3*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate] (1.01 g, 3.41 mmol, 1.0 eq) and Pd/C (10% w/w, 72 mg, 0.068 mmol, 0.02 eq) in EtOAc (250 mL) was placed under a H_2 atmosphere. The reaction mixture was stirred for 16 h then diluted with EtOAc (250 mL), filtered through Celite® (eluent EtOAc) and the filtrate retained. The filter cake was resuspended in EtOAc (500 mL), stirred for 3 h, then filtered through Celite®, combined with the previous filtrate and concentrated *in vacuo* to give (*R*)-**94** (686 mg, 3.33 mmol, 98%) as a colourless solid that was used without further purification. R_f 0.43 (1:99 AcOH:EtOAc); mp 230 $^\circ$ C (dec.); $[\alpha]_D$ −124.6 (*c* 0.5 in MeOH); ν_{max}/cm^{-1} 2972 (br), 1666 (s), 1420 (m), 1231 (s); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 12.89 (1H, br s, CO_2H), 10.44 (1H, s, N*H*), 7.61 (1H, s, N*H*), 7.43 (1H, dd, *J* 8.0 1.3 Hz, C(6)*H*), 6.93 (1H, dd, *J* 7.7 1.3 Hz, C(8)*H*), 6.63 (1H, dd, *J* 8.0 7.7 Hz, C(7)*H*), 4.05 (1H, q, *J* 6.7 Hz, C(3)*H*), 1.30 (3H, d, *J* 6.7 Hz, C(3)C*H*$_3$); ^{13}C NMR (500 MHz; $(CD_3)_2SO$): δ 169.7 (C(5)*C*O$_2$H), 167.8 (*C*(2)), 137.6 (*C*(9)), 127.4 (*C*(10)), 125.2 (*C*(6)H), 118.8 (*C*(8)H), 116.6 (*C*(7)H), 111.8 (*C*(5)), 50.6 (*C*(3)H), 19.0 (C(3)*C*H$_3$); LRMS *m/z* (ES+) 207 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 229.0592. $C_{10}H_{10}N_2NaO_3^+$ requires 229.0584].

(*S*)-3-Methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid [(*S*)-94]



Compound (*S*)-**117** [benzyl (3*S*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate] (121 mg, 0.408 mmol, 1.0 eq) was reacted as described above to afford (*S*)-**94** (84 mg, 0.407 mmol, 99%) as a colourless solid that was used without further purification. R_f 0.57 (1:99 AcOH:EtOAc); mp 227 ° C (dec.); $[\alpha]_D$ +127.8 (*c* 0.5 in MeOH); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 12.93 (1H, br s, CO_2H), 10.44 (1H, s, N*H*), 7.63 (1H, s, N*H*), 7.41 (1H, dd, *J* 7.9 1.4 Hz, C(6)*H*), 6.91 (1H, dd, *J* 7.7 1.4 Hz, C(8)*H*), 6.62 (1H, dd, *J* 7.9 7.7 Hz, C(7)*H*), 4.03 (1H, q, *J* 6.7 Hz, C(3)*H*), 1.29 (3H, d, *J* 6.7 Hz, C(3)CH_3); LRMS *m/z* (ES−) 205 ($[M-H]^-$, 100%). These data are in accordance with the enantiomer (*R*)-**94**.

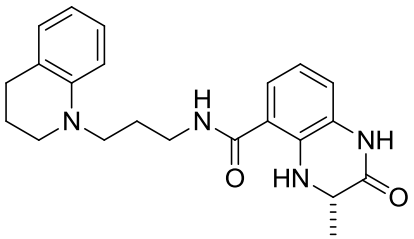
(3*R*)-*N*-[3-(3,4-Dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-119]



Compound (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **118** [3-(3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (47 mg, 0.21 mmol, 1.1 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (gradient elution 8 to 100% EtOAc in petroleum ether) gave (*R*)-**119** (23 mg, 0.061 mmol, 32%) as a brown oil. R_f 0.50 (2:1 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3328 (br), 2925 (s), 2854 (m), 1682 (s), 1601 (m), 1505 (m), 1288 (s); $[\alpha]_D$ −23.8 (*c* 0.5 in $(CH_3)_2CO$); ^{1}H NMR (400 MHz; $(CD_3)_2CO$): δ 9.34 (1H, br s, N*H*), 8.03 (1H, br s, N*H*), 7.80

(1H, br s, N*H*), 7.30 (1H, dd, *J* 8.0 1.3 Hz, C(6)*H*), 7.00-6.90 (2H, m, C(8)*H* & C(7'')*H*), 6.88-6.84 (1H, m, C(5'')*H*), 6.65 (1H, dd, *J* 8.0 7.9 Hz, C(7)*H*), 6.64-6.60 (1H, m, C(8'')*H*), 6.46 (1H, ddd, *J* 7.3 7.3 1.0 Hz, C(6'')*H*), 4.02 (1H, qd, *J* 6.7 1.6 Hz, C(3)*H*), 3.51-3.43 (2H, m, C(1')*H*), 3.39 (2H, t, *J* 7.4 Hz, C(3')H_2), 3.31 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.71 (2H, t, *J* 6.4 Hz, C(4'')H_2), 1.97-1.87 (4H, m, C(2')H_2 & C(3'')H_2), 1.39 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.4 (C(5)*C*=O), 168.7 (*C*(2)=O), 146.2 (*C*(10'')), 137.4 (*C*(9)), 129.8 (*C*(5'')H), 128.8 (*C*(10)), 127.7 (*C*(7'')H), 123.1 (*C*(9'')), 122.2 (*C*(6)H), 117.8 (*C*(8)H), 117.5 (*C*(7)H), 116.9 (*C*(5)), 116.3 (*C*(6'')H), 111.5 (*C*(8'')H), 51.6 (*C*(3)H), 50.1 (*C*(2'')H_2), 49.7 (*C*(3')H_2), 38.2 (*C*(1')H_2), 28.8 (*C*(4'')H_2), 27.2 (*C*(2')H_2), 23.1 (*C*(3'')H_2), 18.7 (C(3)*C*H_3); LRMS *m/z* (ES+) 379 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 401.1934. $C_{22}H_{26}N_4NaO_2^+$ requires 401.1948]; HPLC (C-18) retention time = 9.48 min, 96.8%; (AD-H) retention time = 13.31 min, 98.6%.

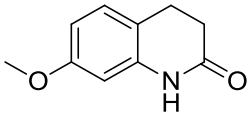
(3*S*)-*N*-[3-(3,4-Dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*S*)-119]



Compound (*S*)-**94** [(*S*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (35 mg, 0.17 mmol, 1.0 eq) and **118** [3-(3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (36 mg, 0.19 mmol, 1.1 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (gradient elution 8 to 100% EtOAc in petroleum ether) gave (*S*)-**119** (31 mg, 0.082 mmol, 48%) as a brown oil. R_f 0.37 (1:1 EtOAc:petroleum ether); $[\alpha]_D$ +20.8 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz; $(CD_3)_2CO$): δ 9.33 (1H, br s, N*H*), 8.03 (1H, br s, N*H*), 7.80 (1H, br s, N*H*), 7.30 (1H, dd, *J* 8.0 1.3 Hz, C(6)*H*), 6.99-6.91 (2H, m, C(8)*H* & C(7'')*H*),

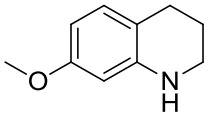
6.89-6.84 (1H, m, C(5'')*H*), 6.65 (1H, dd, *J* 8.0 7.9 Hz, C(7)*H*), 6.64-6.59 (1H, m, C(8'')*H*), 6.46 (1H, ddd, *J* 7.3 7.3 1.0 Hz, C(6'')*H*), 4.02 (1H, qd, *J* 6.6 1.5 Hz, C(3)*H*), 3.51-3.43 (2H, m, C(1')*H*), 3.39 (2H, t, *J* 7.4 Hz, C(3')H_2), 3.32 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.71 (2H, t, *J* 6.4 Hz, C(4'')H_2), 1.96-1.87 (4H, m, C(2')H_2 & C(3'')H_2), 1.39 (3H, d, *J* 6.6 Hz, C(3)CH_3); LRMS *m/z* (ES+) 379 ($[M+H]^+$, 100%); HPLC (C-18) retention time = 9.42 min, 95.6%; (AD-H) retention time = 14.82 min, 97.1%. These data are in agreement with the enantiomer (*R*)-**119**.

7-methoxy-3,4-dihydroquinolin-2(1*H*)-one (134)



To a solution of 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (900 mg, 5.52 mmol, 1.0 eq) and K_2CO_3 (1.52 g, 11.0 mmol, 2.0 eq) in DMF (9 mL) was added MeI (378 µL, 861 mg, 6.07 mmol, 1.1 eq). The reaction mixture was heated to 60 °C for 16 h before being diluted with EtOAc (200 mL), washed with sat. aq. K_2CO_3 (3 × 200 mL) and brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* crystallisation from EtOH yielded **134** (554 mg, 3.13 mmol, 57%) as colourless needles. R_f 0.22 (1:2 EtOAc:petroleum ether); mp 142-143 °C (from EtOH, lit.[216] 147 °C); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.51 (1H, br s, N*H*), 7.07 (1H, d, *J* 8.3 Hz, C(5)*H*), 6.54 (1H, dd, *J* 8.3 2.4 Hz, C(6)*H*), 6.29 (1H, d, J 2.4 Hz, C(8)H), 3.79 (3H, s, C(7)OCH_3), 2.91 (2H, t, *J* 2.91 Hz, C(3)H_2), 2.63 (2H, t, *J* 7.5 Hz, C(4)H_2); LRMS *m/z* (ES+) 200 ($[M+Na]^+$, 100%). These data are in accordance with the literature.[216]

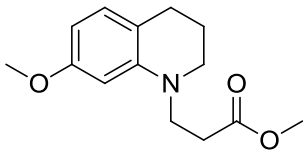
7-methoxy-1,2,3,4-tetrahydroquinoline (131)



To a solution of **134** [7-methoxy-3,4-dihydroquinolin-2(1*H*)-one] (251 mg, 1.42 mmol, 1.0 eq) in THF (17 mL) at 0 °C was added a solution of $LiAlH_4$ in THF (1M, 1.70 mL, 1.70 mmol,

1.2 eq) dropwise over 5 min. The reaction mixture was allowed to warm to rt and stirred for 13 h before being cooled to 0 °C and quenched by sequential addition of EtOAc (2 mL) and H_2O (1 mL). The resulting mixture was dried ($MgSO_4$), filtered through Celite® (eluent EtOAc) and concentrated *in vacuo* to give **131** (230 mg, 1.41 mmol, 99%) as a yellow oil which was used without further purification. R_f 0.51 (1:2 EtOAc:petroleum ether); ^{1}H NMR (400 MHz; $CDCl_3$): δ 6.85 (1H, d, *J* 8.3 Hz, C(5)*H*), 6.21 (1H, dd, *J* 8.3 2.5 Hz, C(6)*H*), 6.05 (1H, d, *J* 2.5 Hz, C(8)*H*), 3.92 (1H, br s, N*H*), 3.74 (3H, s, C(7)OCH_3), 3.29 (2H, t, *J* 5.5 Hz, C(2)H_2), 2.70 (2H, t, *J* 6.4 Hz, C(4)H_2), 1.96-1.89 (2H, m, C(3)H_2); LRMS *m/z* (ES+) 164 ([M+H]$^+$, 100%). These data are in accordance with the literature.[217]

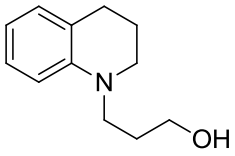
Methyl 3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propanoate (138)



A solution of **131** [7-methoxy-1,2,3,4-tetrahydroquinoline] (507 mg, 3.11 mmol, 1.0 eq) and methyl prop-2-enoate (560 µL, 535 mg, 6.21 mmol, 2.0 eq) in acetic acid (2.5 mL) was heated at 120 °C for 39 h. The reaction mixture was concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 3 to 28% EtOAc in petroleum ether) to yield **138** (359 mg, 1.44 mmol, 46%) as a brown oil. R_f 0.17 (1:4 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2943 (m), 1732 (s), 1611 (s), 1508 (s), 1161 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 6.87 (1H, d, J 8.64 Hz, C(5)*H*), 6.22-6.16 (2H, m, 2 × C(Ar)*H*), 3.78 (3H, s, C(7)OCH_3), 3.70 (3H, s, C(1')O_2CH_3), 3.60 (2H, t, *J* 7.2 Hz, C(3')H_2), 3.28 (2H, t, *J* 5.6 Hz, C(2)H_2), 2.69 (2H, t, *J* 6.4 Hz, C(4)H_2), 2.62 (2H, t, *J* 7.2 Hz, C(2')H_2), 1.97-1.88 (2H, m, C(3)H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 172.8 (*C*(1')O_2CH_3), 159.2 (*C*(7)), 145.3 (*C*(10)), 129.7 (*C*(5)H), 115.5 (*C*(9)), 110.3 (*C*(Ar)H), 97.4 (*C*(Ar)H), 55.2 (C(7)O*C*H_3), 51.7 (C(1')O_2*C*H_3), 49.4 (*C*(2)H_2), 47.2 (*C*(3')H_2), 31.0 (*C*(2')H_2), 27.3 (*C*(4)H_2), 22.4 (*C*(3)H_2); LRMS *m/z* (ES+) 250 ([M+H]$^+$, 100%); HRMS *m/z*

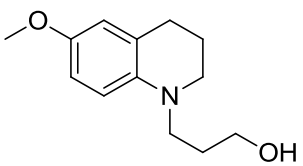
(ES+) [Found: $(M+Na)^+$ 272.1266. $C_{14}H_{19}NNaO_3$ requires 272.1257]; Anal. calcd for $C_{14}H_{19}NO_3$: C 67.45%, H 7.68%, N 5.62%; Found: C 67.38%, H 7.79%, N 5.72%.

3-(3,4-Dihydroquinolin-1(2*H*)-yl)propan-1-ol (140)



1,2,3,4-tetrahydroquinoline (190 mg, 1.43 mmol, 1.0 eq) was reacted according to general procedure 3. Purification *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) yielded **140** (161 mg, 0.843 mmol, 59%) as a brown oil. R_f 0.14 (1:4 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3352 (br) 2930 (m), 1601 (s), 1505 (s); ^1H NMR (500 MHz; $CDCl_3$): δ 7.06 (1H, dd, *J* 8.0 7.5, C(7)*H*), 6.96 (1H, d, *J* 7.0, C(5)*H*), 6.67 (1H, d, *J* 8.0, C(8)*H*), 6.59 (1H, ddd, *J* 7.5 7.0 0.9 Hz, C(6)*H*), 3.77 (2H, t, *J* 6.0 Hz, C(1')H_2), 3.41 (2H, t, *J* 7.0 Hz, C(3')H_2), 3.30 (2H, t, *J* 5.7 Hz, C(2)H_2), 2.77 (2H, t, *J* 6.4 Hz, C(4)H_2), 2.00-1.94 (2H, m, C(3)H_2), 1.91-1.84 (2H, m, C(2')H_2), 1.83 (1H, br s, O*H*); ^{13}C NMR (126 MHz; $CDCl_3$): δ 145.4 (*C*(10)), 129.2 (*C*(7)H), 127.1 (*C*(5)H), 122.7 (*C*(9)), 115.8 (*C*(6)H), 111.0 (*C*(8)H), 61.1 (*C*(1')H_2), 49.6 (*C*(2)H_2), 48.7 (*C*(3')H_2), 29.3 (*C*(2')H_2), 28.1 (*C*(4)H_2), 22.2 (*C*(3)H_2); LRMS *m/z* (ES+) 214 ($[M+Na]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 214.1204. $C_{12}H_{17}NNaO^+$ requires 214.1202]; Anal. calcd for $C_{12}H_{17}NO$: C 75.35%, H 8.96%, N 7.32%; Found: C 75.22%, H 9.14%, N 7.19%.

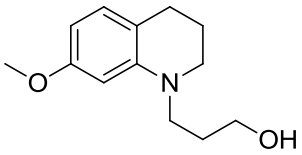
3-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-ol (141)



6-methoxy-1,2,3,4-tetrahydroquinoline (206 mg, 1.26 mmol, 1.0 eq) was reacted according to general procedure 3. Purification *via* silica gel chromatography (gradient elution 8 to 66%

EtOAc in petroleum ether) yielded **141** (152 mg, 0.687 mmol, 55%) as a brown oil. R_f 0.28 (1:2 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3372 (br), 2933 (m), 1504 (s), 1240 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 6.73-6.67 (2H, m, C(7)*H* & C(8)*H*), 6.63-6.60 (1H, m, C(5)*H*), 3.79 (2H, t, *J* 6.2 Hz, C(1')H_2), 3.77 (3H, s, C(6)OCH_3), 3.36 (2H, t, *J* 6.6 Hz, C(3')H_2), 3.22 (2H, t, *J* 5.6 Hz, C(2)H_2), 2.78 (2H, t, *J* 6.5 Hz, C(4)H_2), 2.54 (1H, br s, O*H*) 2.01-1.92 (2H, m, C(3)H_2), 1.86 (2H, tt, *J* 6.6 6.2 Hz, C(2')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 151.2 (*C*(6)), 140.2 (*C*(10)), 124.7 (*C*(9)), 115.2 (*C*(5)H), 113.2 (*C*(Ar)H), 112.5 (*C*(Ar)H), 61.6 (*C*(1')H_2), 55.8 (C(6)O*C*H_3), 50.2 (*C*(3')H_2), 49.5 (*C*(2)H_2), 29.2 (*C*(2')H_2), 28.3 (*C*(4)H_2), 22.3 (*C*(3)H_2); LRMS *m/z* (ES+) 222 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 222.1483. $C_{13}H_{20}NO_2^+$ requires 222.1489].

3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-ol (142)

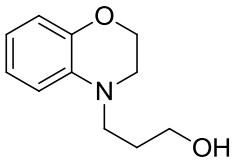


Method 1: Compound **131** [7-methoxy-1,2,3,4-tetrahydroquinoline] (200 mg, 1.23 mmol, 1.0 eq) was reacted according to general procedure 3. Purification *via* silica gel chromatography (gradient elution 8 to 66% EtOAc in petroleum ether) yielded **142** (130 mg, 0.587 mmol, 57%) as a brown oil.

Method 2: To a solution of **138** [methyl 3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propanoate] (338 mg, 1.54 mmol, 1.0 eq) in THF (10 mL) at 0 °C was added a solution of $LiAlH_4$ in THF (1M, 1.85 mL, 1.85 mmol, 1.2 eq) dropwise over 5 min. The reaction mixture was allowed to warm to rt and stirred for 23 h before being cooled to 0 °C and quenched by sequential addition of EtOAc (2 mL) and H_2O (1 mL). The resulting mixture was dried ($MgSO_4$), filtered through Celite® (eluent EtOAc) and concentrated *in vacuo* to give **142** (275 mg, 1.24 mmol, 81%) as a brown oil that was used without further purification.

R_f 0.36 (2:1 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3370 (br) 2934 (m), 1611 (s), 1508 (s), 1166 (s); ^{1}H NMR (300 MHz; $CDCl_3$): δ 6.86 (1H, d, *J* 8.1 Hz, C(5)*H*), 6.24 (1H, d, *J* 2.4 Hz, C(8)*H*), 6.17 (1H, dd, *J* 8.1 2.4 Hz, C(6)*H*), 3.78 (3H, s, OCH_3), 3.75 (2H, t, *J* 6.0 Hz, C(1')H_2), 3.38 (2H, t, *J* 7.0 Hz, C(3')H_2), 3.28 (2H, t, *J* 5.6 Hz, C(2)H_2), 2.71 (2H, t, *J* 6.4 Hz, C(4)H_2), 2.02 (1H, br s, O*H*), 1.99-1.81 (4H, m, C(3)H_2 & C(2')H_2); ^{13}C NMR (75.5 MHz; CDCl3): δ 159.2 (*C*(7)), 146.3 (*C*(10)), 129.5 (*C*(5)H), 115.5 (*C*(9)), 100.0 (*C*(6)H), 97.7 (*C*(8)H), 60.9 (*C*(1')H_2), 55.5 (O*C*H_3), 49.5 (*C*(2)H_2), 48.5 (*C*(3')H_2), 29.4 (*C*(2')H_2), 27.4 (*C*(4)H_2), 22.4 (*C*(3)H_2); LRMS *m/z* (ES+) 221 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 244.1315. $C_{13}H_{19}NNaO_2$ requires 244.1308]; Anal. calcd for $C_{13}H_{19}NO_2$: C 70.56%, H 8.65%, N 6.33%; Found: C 70.50%, H 8.63%, N 6.39%.

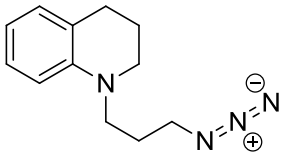
3-(2,3-Dihydro-4*H*-1,4-benzoxazin-4-yl)propan-1-ol (143)



A solution of 3,4-dihydro-2*H*-1,4-benzoxazine (179 mg, 1.32 mmol, 1.0 eq) was reacted according to general procedure 3. Purification *via* silica gel chromatography (gradient elution 8 to 66% EtOAc in petroleum ether) yielded **143** (131 mg, 0.483 mmol, 51%) as a brown oil. R_f 0.20 (1:2 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3359 (br), 2935 (m), 2877 (m), 1503 (s), 1056 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 6.87-6.81 (1H, m, C(6)*H*), 6.79 (1H, dd, *J* 7.7 1.5 Hz, C(8)*H*), 6.74 (1H, dd, *J* 8.1 1.3 Hz, C(5)*H*), 6.63 (1H, ddd, *J* 7.7 7.5 1.3 Hz, C(7)*H*), 4.24 (2H, t, *J* 4.4 Hz, C(2)H_2), 3.76 (2H, t, *J* 6.2 Hz, C(1')H_2), 3.39 (2H, t, *J* 6.7 Hz, C(3')H_2), 3.34 (2H, t, *J* 4.4 Hz, C(3)H_2), 1.86 (2H, tt, *J* 6.7 6.2 Hz, C(2')H_2), 1.82 (1H, br s, O*H*); ^{13}C NMR (101 MHz; $CDCl_3$): δ 144.2 (*C*(10)), 135.3 (*C*(9)), 121.6 (*C*(6)H), 117.7 (*C*(7)H), 116.4 (*C*(8)H), 112.6 (*C*(5)H), 64.4 (*C*(2)H_2), 60.9 (*C*(1')H_2), 48.4 (*C*(3')H_2), 47.2 (*C*(3)H_2), 29.1

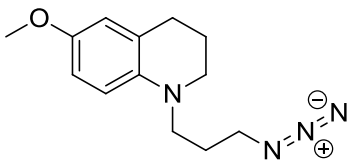
(*C*(2')H_2); LRMS *m/z* (ES+) 194 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 194.11780. $C_{11}H_{16}NO_2^+$ requires 194.1176].

1-(3-Azidopropyl)-1,2,3,4-tetrahydroquinoline (148)



Compound **140** [3-(3,4-dihydroquinolin-1(2*H*)-yl)propan-1-ol] (82 mg, 0.43 mmol, 1.0 eq) was reacted according to general procedure 4. The crude material was passed through a pad of silica (eluent EtOAc) to give **148** (43 mg, 0.95 mmol, 46%) as a brown oil. R_f 0.88 (1:2 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2929 (m), 2090 (s), 1601 (s), 1503 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.10 (1H, dd, *J* 7.8 7.8 Hz, C(7)*H*), 6.99 (1H, d, *J* 7.5 Hz, C(5)*H*), 6.66-6.58 (2H, m, C(6)*H* & C(8)*H*), 3.46-3.35 (4H, m, C(1')H_2 & C(3')H_2), 3.31 (2H, t, *J* 5.8 Hz, C(2)H_2), 2.80 (2H, t, *J* 6.2 Hz, C(4)H_2), 1.99 (2H, tt, *J* 6.2 5.8 Hz, C(3)H_2), 1.91 (2H, tt, *J* 6.8 6.8 Hz, C(2')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 145.1 (*C*(10)), 129.3 (*C*(5)H), 127.2 (*C*(7)H), 122.5 (*C*(9)), 115.8 (*C*(6/8)H), 110.5 (*C*(6/8)H), 49.8 (*C*(2)H_2), 49.3 (*C*(1'/3')H_2), 48.5 (*C*(1'/3')H_2), 28.1 (*C*(4)H_2), 25.9 (*C*(2')H_2), 22.2 (*C*(3)H_2); LRMS *m/z* (ES+) 217 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 217.1444. $C_{12}H_{17}N_4^+$ requires 217.1448].

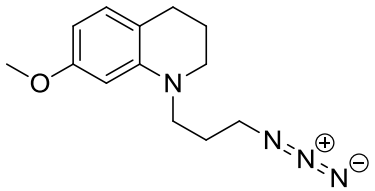
1-(3-Azidopropyl)-6-methoxy-1,2,3,4-tetrahydroquinoline (149)



Compound **141** [3-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-ol] (107 mg, 0.484 mmol, 1.0 eq) was reacted according to general procedure 4. The crude material was passed through a pad of silica (eluent EtOAc) to give **149** (104 mg, 0.422 mmol, 87%) as a red oil. R_f 0.80 (1:2 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2951 (m), 2092 (s), 1504 (s), 1240 (s); ^{1}H

NMR (400 MHz; $CDCl_3$): δ 6.66 (1H, dd, *J* 8.8 3.0 Hz, C(7)*H*), 6.59 (1H, d, *J* 3.0 Hz, C(5)*H*), 6.53 (1H, d, *J* 8.8 Hz, C(8)*H*), 3.73 (3H, s, C(6)OCH_3), 3.38 (2H, t, *J* 6.7 Hz, C(3')H_2), 3.30 (2H, t, *J* 6.9 Hz, C(1')H_2), 3.20 (2H, t, *J* 5.6 Hz, C(2)H_2), 2.74 (2H, t, *J* 6.4 Hz, C(4)H_2), 1.99-1.90 (2H, m, C(3)H_2), 1.85 (2H, tt, *J* 6.9 6.7 Hz, C(2')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 150.9 (*C*(6)), 139.8 (*C*(10)), 124.1 (*C*(9)), 115.4 (*C*(5)H), 112.5 (*C*(7)H), 112.0 (*C*(8)H), 55.8 (C(6)O*C*H_3), 49.7 (*C*(2)H_2), 49.4 (*C*(3')H_2), 49.2 (*C*(1')H_2), 28.3 (*C*(4)H_2), 25.9 (*C*(2')H_2), 22.4 (*C*(3)H_2); LRMS *m/z* (ES+) 247 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 247.1560. $C_{13}H_{19}N_4O^+$ requires 247.1553].

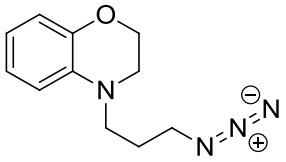
1-(3-azidopropyl)-7-methoxy-1,2,3,4-tetrahydroquinoline (150)



To a solution of **142** [3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-ol] (265 mg, 1.20 mmol, 1.0 eq) in CH_2Cl_2 (7 mL) at 0 °C was added Et_3N (242 mg, 334 µL, 2.39 mmol, 2.0 eq) and stirred for 5 min. MsCl (164 mg, 111 µL, 1.44 mmol, 1.2 eq) was added and the reaction mixture was stirred at rt for 1 h before being concentrated *in vacuo*. The resulting residue was passed through a plug of silica gel (eluent EtOAc) and concentrated *in vacuo* to give 3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl methanesulfonate (348 mg, 1.16 mmol, 97%) as a brown oil. A solution of the sulfonate (348 mg, 1.16 mmol, 1.0 eq) and NaN_3 (302 mg, 4.65 mmol, 4.0 eq) in DMF (10 mL) was heated at 60 °C for 75 min. The reaction mixture was cooled to rt, then diluted with EtOAc (40 mL), washed with H_2O (2 × 40 mL) and brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified *via* silica gel chromatography (gradient elution 1 to 12% EtOAc in petroleum ether) to give **150** (213 mg, 0.946 mmol, 82%) as a brown oil. R_f 0.77 (1:2 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2933 (m), 2091 (s), 1609 (s), 1507 (s), 1263 (s); ^{1}H NMR

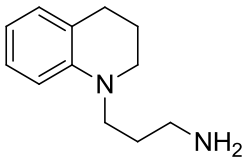
(400 MHz; $CDCl_3$): δ 6.91-6.87 (1H, m, C(5)*H*), 6.21-6.17 (2H, m, C(6)*H* & C(8)*H*), 3.80 (3H, s, OCH_3), 3.41 (2H, t, *J* 6.5 Hz, C(3')H_2), 3.36 (2H, t, *J* 7.1 Hz, C(1')H_2), 3.28 (2H, t, *J* 5.6 Hz, C(2)H_2), 2.72 (2H, t, *J* 6.3 Hz, C(4)H_2), 1.99-1.87 (4H, m, C(3)H_2 & C(2')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 159.3 (*C*(7)), 145.9 (*C*(10)), 129.6 (*C*(5)H), 115.3 (*C*(9)), 100.0 (*C*(Ar)H), 97.4 (*C*(Ar)H), 55.2 (O*C*H_3), 49.7 (*C*(2)H_2), 49.3 (*C*(3')H_2), 48.6 (*C*(1')H_2), 27.4 (*C*(4)H_2), 25.8 (*C*(2')H_2), 22.4 (*C*(3)H_2); LRMS *m/z* (ES+) 247 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 247.1556. $C_{13}H_{19}N_4O^+$ requires 247.1553].

4-(3-Azidopropyl)-3,4-dihydro-2*H*-1,4-benzoxazine (151)



Compound **143** [3-(2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)propan-1-ol] (105 mg, 0.543 mmol, 1.0 eq) was reacted according to general procedure 4. The crude material was passed through a pad of silica (eluent EtOAc) to give **151** (109 mg, 0.499 mmol, 92%) as a brown oil. R_f 0.77 (1:2 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2933 (m), 2878 (m), 2092 (s), 1502 (s), 1057 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.87-6.81 (1H, m, C(6)*H*), 6.79 (1H, dd, J 7.7 1.5 Hz, C(8)*H*), 6.67 (1H, dd, J 8.1 1.3 , C(5)*H*), 6.63 (1H, ddd, J 7.7 7.6 1.3 Hz, C(7)*H*), 4.24 (2H, t, *J* 4.4 Hz, C(2)H_2), 3.41 (2H, t, *J* 6. 6 Hz, C(3')H_2), 3.38-3.30 (4H, m, C(3)H_2 & C(1')H_2), 1.88 (2H, tt, *J* 6.8 6.6 Hz, C(2')H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 144.2 (*C*(10)), 135.1 (*C*(9)), 121.8 (*C*(6)H), 117.8 (*C*(7)H), 116.6 (*C*(8)H), 112.2 (*C*(5)H), 64.6 (*C*(2)H_2), 49.3 (*C*(3')H_2), 48.3 (*C*(1')H_2), 47.5 (*C*(3)H_2), 26.0 (*C*(2')H_2); LRMS *m/z* (ES+) 193 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 193.1329. $C_{11}H_{17}N_2O^+$ requires 193.1335].

3-(3,4-Dihydroquinolin-1(2*H*)-yl)propan-1-amine (118)

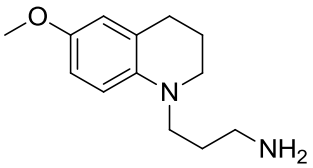


Method 1: Compound **148** [1-(3-azidopropyl)-7-methoxy-1,2,3,4-tetrahydroquinoline] (43 mg, 0.20 mmol, 1.0 eq) was reacted according to general procedure 5 to give **118** (37 mg, 0.20 mmol, 99%) as a grey oil without need for further purification.

Method 2: Reported later.

R_f 0.16 (1:9 MeOH:$CHCl_3$); ν_{max}/cm^{-1} 3344 (br), 2928 (m), 1600 (s), 1500 (s); ^{1}H NMR (500 MHz; CD_3OD): δ 6.96 (1H, dd, *J* 7.9 7.4 Hz, C(7)*H*), 6.85 (1H, d, *J* 7.3 Hz, C(5)*H*), 6.59 (1H, d, *J* 7.9 Hz, C(8)*H*), 6.48 (1H, dd, *J* 7.4 7.3 Hz, C(6)*H*), 3.30 (2H, t, *J* 7.4 Hz, C(3')H_2), 3.25 (2H, t, *J* 5.6 Hz, C(2)H_2), 2.77-2.67 (4H, m, C(1')H_2 & C(4)H_2), 1.95-1.86 (2H, m, C(3)H_2), 1.77 (2H, tt, *J* 7.4 7.3 Hz, C(2')H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 146.9 (*C*(10)), 130.5 (*C*(5)H), 128.4 (*C*(7)H), 124.2 (*C*(9)), 117.3 (*C*(6)H), 112.5 (*C*(8)H), 51.0 (*C*(2)H_2), 50.4 (*C*(3')H_2), 40.8 (*C*(1')H_2), 30.1 (*C*(2')H_2), 29.7 (*C*(4)H_2), 23.9 (*C*(3)H_2); LRMS *m/z* (ES+) 191 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 191.1547. $C_{12}H_{19}N_2^+$ requires 191.1543].

3-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (152)

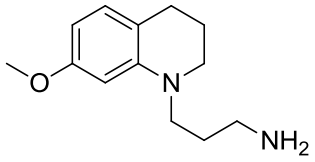


Method 1: Compound **149** [1-(3-azidopropyl)-6-methoxy-1,2,3,4-tetrahydroquinoline] (90 mg, 0.37 mmol, 1.0 eq) was reacted according to general procedure 5 to give **152** (69 mg, 0.31 mmol, 86%) as a yellow oil without need for further purification.

Method 2: Reported later.

R_f 0.11 (1:9 MeOH:$CHCl_3$); ν_{max}/cm^{-1} 3289 (br), 2931 (m), 1503 (s), 1238 (s); ^1H NMR (500 MHz; CD_3OD): δ 6.62 (1H, dd, *J* 8.9 2.8 Hz, C(7)*H*), 6.59 (1H, d, *J* 8.9 Hz, C(8)*H*), 6.55 (1H, d, *J* 2.8 Hz, C(5)*H*), 3.67 (3H, s, C(6)OCH_3), 3.24 (2H, t, *J* 7.3 Hz, C(3')H_2), 3.18 (2H, t, *J* 5.6 Hz, C(2)H_2), 2.73-2.66 (4H, m, C(4)H_2 & C(1')H_2), 1.95-1.88 (2H, m, C(3)H_2), 1.72 (2H, tt, *J* 7.3 7.3 Hz, C(2')H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 152.8 (*C*(6)), 141.7 (*C*(10)), 126.0 (*C*(9)), 116.6 (*C*(5)), 114.3 (*C*(Ar)), 113.9 (*C*(Ar)), 56.6 (C(6)O*C*H_3), 51.3 (*C*(3')), 50.9 (*C*(2)), 41.2 (*C*(1')), 30.8 (*C*(2')), 29.8 (*C*(4)), 24.0 (*C*(3)); LRMS *m/z* (ES+) 221 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 221.1644. $C_{13}H_{21}N_2O^+$ requires 221.1648].

3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (153)



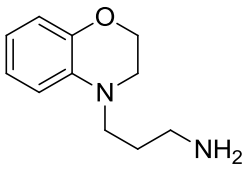
Method 1: A mixture of **150** [1-(3-azidopropyl)-7-methoxy-1,2,3,4-tetrahydroquinoline] (213 mg, 0.865 mmol, 1.0 eq) and Pd/C (10% w/w, 46 mg, 0.043 mmol, 0.05 eq) in EtOAc (10 mL) was stirred under a H_2 atmosphere for 25 h. The reaction mixture was filtered through Celite® (eluent EtOAc) and concentrated *in vacuo* to give **153** (190 mg, 0.862 mmol, 99%) as a grey oil without need for further purification.

Method 2: Reported later.

R_f 0.18 (1:9 MeOH:$CHCl_3$); ν_{max}/cm^{-1} 3353 (br), 2931 (m), 1611 (s), 1508 (s), 1166 (s); ^1H NMR (500 MHz; CD_3OD): δ 6.76 (1H, d, *J* 8.1 Hz, C(5)*H*), 6.14 (1H, d, *J* 2.3 Hz, C(8)*H*), 6.09 (1H, dd, *J* 8.1 2.3 Hz, C(6)*H*), 3.71 (3H, s, C(7)OCH_3), 3.31-3.26 (2H, m, C(3')H_2), 3.25 (2H, t, *J* 5.6 Hz, C(2)H_2), 2.71 (2H, t, *J* 7.3 Hz, C(1')H_2), 2.64 (2H, t, *J* 6.3 Hz, C(4)H_2), 1.92-1.86 (2H, m, C(3)H_2), 1.80-1.72 (2H, m, C(2')H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 161.0 (*C*(7)), 147.7 (*C*(10)), 130.9 (*C*(5)H), 116.9 (*C*(9)), 101.8 (*C*(6)H), 99.0 (*C*(8)H), 55.9 (C(7)O*C*H_3),

50.9 (*C*(2)H_2), 50.5 (*C*(3')H_2), 41.0 (*C*(1')H_2), 30.7 (*C*(2')H_2), 28.9 (*C*(4)H_2), 24.1 (*C*(3)H_2); LRMS *m/z* (ES+) 221 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 221.1644. $C_{13}H_{21}N_2O^+$ requires 221.1648].

3-(2,3-Dihydro-4*H*-1,4-benzoxazin-4-yl)propan-1-amine (154)

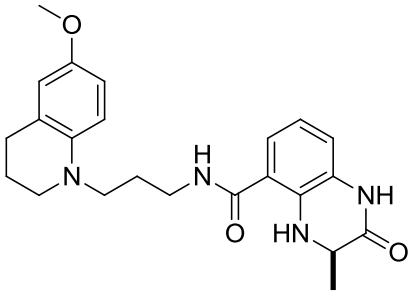


Method 1: Compound **151** [4-(3-azidopropyl)-3,4-dihydro-2*H*-1,4-benzoxazine] (90 mg, 0.41 mmol, 1.0 eq) was reacted according to general procedure 5 to give **154** (76 mg, 0.40 mmol, 96%) as a colourless oil without need for further purification.

Method 2: Reported later.

R_f 0.08 (1:9 MeOH:$CHCl_3$); ν_{max}/cm^{-1} 3302 (br), 2933 (m), 2874 (m), 1502 (s), 1056 (s); ^{1}H NMR (500 MHz; CD_3OD): δ 6.79-6.69 (2H, m, C(6)*H*) & C(5)*H*), 6.67 (1H, dd, *J* 7.9 1.3 Hz, C(8)*H*), 6.55 (1H, m, C(7)*H*), 4.19 (2H, t, *J* 4.4 Hz, C(2)H_2), 3.37-3.30 (4H, m, C(3)H_2 & C(3')H_2), 2.83 (2H, t, *J* 7.4 Hz, C(1')H_2), 1.84 (2H, tt, *J* 7.4 7.4 Hz, C(2')H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 145.6 (*C*(10)), 136.6 (*C*(9)), 122.5 (*C*(6)H), 118.3 (*C*(7)H), 117.1 (*C*(8)H), 113.5 (*C*(5)H), 65.6 (*C*(2)H_2), 49.6 (*C*H_2), 48.1 (*C*H_2), 40.5 (*C*(1')H_2), 30.2 (*C*(2')H_2); LRMS *m/z* (ES+) 193 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 193.1329. $C_{11}H_{17}N_2O^+$ requires 193.1335].

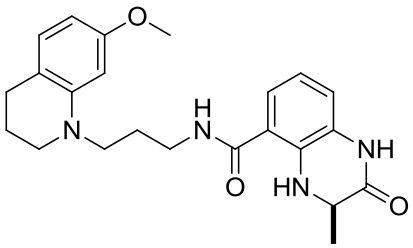
(3*R*)-*N*-[3-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-155]



Compound (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **152** [3-(6-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (47 mg, 0.21 mmol, 1.1 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) gave (*R*)-**155** (37 mg, 0.091 mmol, 48%) as a brown oil. R_f 0.25 (1:2 $(CH_3)_2CO$:petroleum ether); $[\alpha]_D$ −41.0 (*c* 0.1 in MeOH); ν_{max}/cm^{-1} 3305 (br), 2927 (s), 2829 (w), 1681 (s), 1505 (m), 1288 (s); ^{1}H NMR (400 MHz; $(CD_3)_2CO$): δ 9.33 (1H, br s, N*H*), 8.03 (1H, br s, N*H*), 7.80 (1H, br s, N*H*), 7.29 (1H, dd, *J* 8.0 1.0 Hz, C(6)*H*), 6.96 (1H, dd, *J* 7.7 1.0 Hz, C(8)*H*), 6.65 (1H, dd, *J* 8.0 7.7 Hz, C(7)*H*), 6.60-6.57 (2H, m, C(7'')*H* & C(8'')*H*), 6.55-6.53 (1H, m, C(5'')*H*), 4.01 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.66 (3H, s, C(6'')OCH_3), 3.51-3.42 (2H, m, C(1')H_2), 3.33 (2H, t, *J* 7.3 Hz, C(3')H_2), 3.23 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.71 (2H, t, *J* 6.4 Hz, C(4'')H_2), 1.95-1.85 (4H, m, C(2')H_2 & C(3'')H_2), 1.39 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.8 (C(5)*C*=O), 169.1 (*C*(2)=O), 152.2 (*C*(6'')), 141.2 (*C*(10'')), 137.8 (*C*(9)), 129.2 (*C*(10)), 125.1 (*C*(9'')), 122.6 (*C*(6)H), 118.3 (*C*(8)H), 117.9 (*C*(7)H), 117.3 (*C*(5)), 116.3 (*C*(5'')H), 113.5 (*C*(Ar'')H), 113.4 (*C*(Ar'')H), 56.1 (C(6'')O*C*H_3), 52.0 (*C*(3)H), 50.8 (*C*(3')H_2), 50.5 (*C*(2'')H_2), 38.8 (*C*(1')H_2), 29.4 (*C*(4'')H_2), 27.5 (*C*(2')H_2), 23.7 (*C*(3'')H_2), 19.1 (C(3)*C*H_3); LRMS *m/z* (ES+) 409 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found:

$(M+Na)^+$ 431.2054. $C_{23}H_{28}N_4NaO_3^+$ requires 431.2054]; HPLC (C-18) retention time = 9.12 min, 98.5%; (AD-H) retention time = 16.57 min, 95.1%.

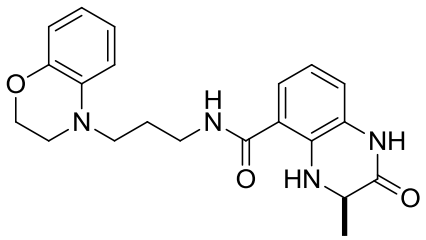
(3*R*)-*N*-[3-(7-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-156]



Compound (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (83 mg, 0.40 mmol, 1.0 eq) and **153** [3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (98 mg, 0.44 mmol, 1.1 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (gradient elution 40 to 100% Et_2O in petroleum ether) gave (*R*)-**156** (107 mg, 262 mmol, 65%) as a colourless solid. R_f 0.30 (1:1 EtOAc:petroleum ether); mp 101-103 °C (from $(CH_3)_2CO$); $[\alpha]_D$ −23.2 (*c* 0.5 in $(CH_3)_2CO$); ν_{max}/cm^{-1} 3328 (br), 2933 (m), 1682 (s), 1609 (m), 1509 (s), 1288 (s); ^1H NMR (500 MHz; $(CD_3)_2CO$): 9.40 (1H, br s, N*H*), 8.04 (1H, br s, N*H*), 7.83 (1H, br s, N*H*), 7.30 (1H, dd, *J* 7.9 1.1 Hz, C(6)*H*), 6.95 (1H, d, *J* 7.6 Hz, C(8)*H*), 6.75 (1H, d, *J* 8.1 Hz, C(5'')*H*), 6.64 (1H, dd, *J* 7.9 7.6 Hz, C(7)*H*), 6.16 (1H, d, *J* 2.4 Hz, C(8'')*H*), 6.07 (1H, dd, *J* 8.1 2.4 Hz, C(6'')*H*), 4.01 (1H, qd, *J* 6.7 1.4 Hz, C(3)*H*), 3.65 (3H, s, C(7'')OCH_3), 3.50-3.44 (2H, m, C(1')H_2), 3.36 (2H, t, *J* 7.4 Hz, C(3')H_2), 3.27 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.62 (2H, t, *J* 6.3 Hz, C(4'')H_2), 1.96-1.82 (4H, m, C(2')H_2 & C(3'')H_2), 1.38 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.5 (C(5)*C*=O), 168.8 (*C*(2)=O), 160.3 (*C*(7'')), 146.9 (*C*(10'')), 137.4 (*C*(9)), 130.2 (*C*(5'')H), 128.8 (*C*(10)), 122.3 (*C*(6)H), 117.9 (*C*(8)H), 117.5 (*C*(7)H), 116.9 (*C*(5)), 115.7 (*C*(9'')), 101.1 (*C*(6'')H), 97.9 (*C*(8'')H), 55.1 (C(12')O*C*H_3), 51.7 (*C*(3)H), 50.0 (*C*(2'')H_2), 49.8 (*C*(3')H_2), 38.2 (*C*(1')H_2), 28.1 (*C*(4'')H_2), 27.1 (*C*(2')H_2), 23.3 (*C*(3'')H_2), 18.7 (C(3)*C*H_3);

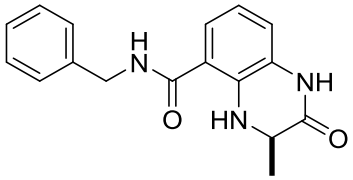
LRMS *m/z* (ES+) 431 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 409.2236. $C_{23}H_{29}N_4O_3^+$ requires 409.2234]; HPLC (C-18) retention time = 10.19 min, 98.7%; (AD-H) retention time = 14.96 min, 98.2%.

(3*R*)-*N*-[3-(2,3-Dihydro-4*H*-1,4-benzoxazin-4-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide ((*R*)-157)



Compound (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (60 mg, 0.29 mmol, 1.0 eq) and **154** [3-(2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)propan-1-amine] (62 mg, 0.32 mmol, 1.1 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) gave (*R*)-**157** (84 mg, 0.22 mmol, 76%) as a colourless oil. R_f 0.17 (1:1 EtOAc:petroleum ether); $[\alpha]_D$ −41.6 (*c* 0.5 in $(CH_3)_2CO$); ν_{max}/cm^{-1} 3320 (br), 2932 (m), 2875 (m), 1695 (s), 1502 (m), 1286 (s); ^{1}H NMR (400 MHz; $(CD_3)_2CO$): δ 9.32 (1H, br s, N*H*), 8.02 (1H, br s, N*H*), 7.82 (1H, br s, N*H*), 7.30 (1H, dd, *J* 7.8 1.2 Hz, C(6)*H*), 6.96 (1H, d, *J* 7.8 Hz, C(8)*H*), 6.76-6.71 (2H, m, C(Ar'')*H* & C(Ar'')*H*), 6.69-6.61 (2H, m, C(Ar'')*H* & C(7)*H*), 6.55-6.47 (1H, m, C(Ar'')*H*), 4.22-4.18 (2H, m, C(2'')H_2), 4.01 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.51-3.44 (2H, m, C(1')H_2), 3.42-3.35 (4H, m, C(3'')H_2 & C(3')H_2), 1.93 (2H, tt, *J* 7.4 7.4 Hz, C(2')H_2), 1.37 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 168.6, 167.8, 144.3, 136.5, 135.4 , 127.9, 121.4, 121.3, 116.99, 116.97, 116.92, 116.6, 116.0, 112.2, 64.3, 50.8, 48.3, 46.8, 37.2, 26.0, 17.8; LRMS *m/z* (ES+) 381 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 403.1734. $C_{21}H_{24}N_4NaO_3^+$ requires 403.1741]; HPLC (C-18) retention time = 10.74 min, 98.3%; (AD-H) retention time = 15.00 min, 99.5%.

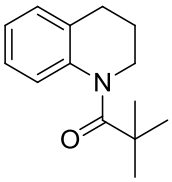
(3*R*)-*N*-[3-(2,3-Dihydro-4*H*-1,4-benzoxazin-4-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-158]



Compound (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (68 mg, 0.33 mmol, 1.0 eq) and benzylamine (39 mg, 0.36 mmol, 1.1 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (gradient elution 8 to 100% EtOAc in petroleum ether) gave (*R*)-**158** (63 mg, 0.21 mmol, 65%) as a white solid. R_f 0.36 (1:1 EtOAc:petroleum ether); $[\alpha]_D$ −55.4 (*c* 0.5 in $(CH_3)_2CO$); ν_{max}/cm^{-1} 3311 (m), 2972 (w), 1680 (s), 1523 (m), 1380 (w); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 10.36 (1H, s, N(1)*H*), 8.96 (1H, t, *J* 6.1 Hz, C(5)CO_2N*H*), 7.86 (1H, s, N(4)*H*), 7.35 (1H, dd, *J* 8.0 1.0 Hz, C(6)*H*), 7.34-7.27 (4H, m, 4 × C(Ar')*H*), 7.25-7.19 (1H, m, C(Ar')*H*), 6.85 (1H, dd, *J* 7.7 1.0 Hz, C(8)*H*), 6.63 (1H, dd, *J* 8.0 7.7 Hz, C(7)*H*), 4.43 (2H, d, *J* 6.1 Hz, NCH_2), 3.93 (1H, dq, *J* 6.7 1.45 Hz, C(3)*H*), 1.25 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): 168.2 (C(5)*C*=O), 167.8 (*C*(2)=O), 139.7 (*C*(Ar)), 136.0 (*C*(9)), 128.3 (2 × *C*(Ar')H), 127.5 (*C*(10)), 127.2 (2 × *C*(Ar')H), 126.8 (*C*(Ar')H), 121.8 (*C*(6)H), 117.1 (*C*(5)), 116.4 (*C*(8)H), 115.2 (*C*(7)H), 50.2 (*C*(3)H), 42.3 (N*C*H_2), 18.2 (C(3)*C*H_3); LRMS *m/z* (ES+) 296 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 296.1386. $C_{17}H_{18}N_3O_2^+$ requires 296.1394].

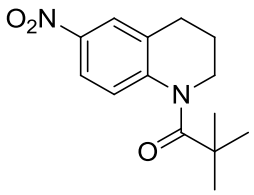
6.4 Chapter 4 Procedures

1-(3,4-Dihydroquinolin-1(2*H*)-yl)-2,2-dimethylpropan-1-one (171)



To a solution of 1,2,3,4-tetrahydroquinoline (5.31 g, 5.00 mL, 39.8 mmol, 1.0 eq) in pyridine (50 mL) at 0 °C was added trimethylacetyl chloride (9.60 g, 9.81 mL, 79.7 mmol, 2.0 eq) dropwise over 5 min. The reaction mixture was warmed to rt and stirred for 2 h, then concentrated *in vacuo*. The residue was redissolved in EtOAc (250 mL), washed with 1M HCl (3 × 250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **171** (8.17 mg, 37.6 mmol, 94%) as a pale brown crystalline solid. R_f 0.51 (1:4 EtOAc:Petrol); mp 96-98 ° C (from EtOAc, lit.[172] 96-98 ° C); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.38 (1H, d, *J* 8.0 Hz, C(Ar')*H*), 7.19-7.12 (2H, m, 2 × C(Ar)*H*), 7.12-7.05 (1H, m, C(Ar)*H*), 3.79 (2H, t, *J* 6.5 Hz, C(2')H_2), 2.77 (2H, t, *J* 6.9 Hz, C(4')H_2), 2.00 (2H, tt, *J* 6.9 6.5 Hz, C(3')*H*), 1.30 (9H, s, C(1)(CH_3)$_3$); LRMS *m/z* (ES+) 218 ([M+Na]$^+$, 100%). These data are in accordance with the literature.[172]

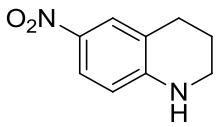
2,2-dimethyl-1-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-one (172)



In a 3-necked flask equipped with a dropping funnel and thermometer, a solution of **171** [1-(3,4-dihydroquinolin-1(2*H*)-yl)-2,2-dimethylpropan-1-one] (4.96 g, 21.9 mmol, 1.0 eq) in Ac_2O (180 mL) was cooled to −10 °C. A solution of HNO_3 (70%, 2.95 mL, 32.8 mmol, 1.5 eq) in Ac_2O (40 mL) was added dropwise over 20 min whilst maintaining a temperature between −10 °C and −5 °C. Upon complete addition the reaction was warmed to rt and stirred for 20 min,

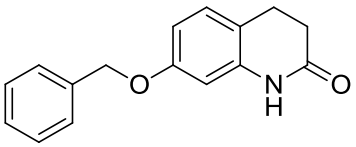
then quenched by pouring onto crushed ice (200 mL). The resulting precipitate was extracted with EtOAc (3 × 150 mL), washed with 10% $NaHCO_3$ (3 × 250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:4 EtOAc:petroleum ether) gave **172** (3.61 g, 13.8 mmol, 63%) as a pale yellow solid. R_f 0.35 (1:4 EtOAc:Petrol); mp 83-85 ° C (from EtOAc, lit.[172] 81-83 ° C); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.03 (1H, d, *J* 2.6 Hz, C(5')*H*), 7.98 (1H, dd, *J* 9.1 2.6 Hz, C(7')*H*), 7.61 (1H, d, *J* 9.1 Hz, C(5')*H*), 3.89-3.83 (2H, m, C(2')H_2), 2.94 (2H, t, *J* 7.0 Hz, C(4')H_2), 2.11-2.01 (2H, m, C(3')*H*), 1.39 (9H, s, C(1)(CH_3)$_3$); LRMS *m/z* (ES+) 263 ([M+H]$^+$, 100%). These data are in accordance with the literature.[172]

6-nitro-1,2,3,4-tetrahydroquinoline (168)



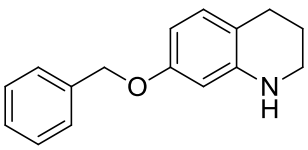
To a solution of **172** [2,2-dimethyl-1-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-one] (3.18 g, 12.1 mmol, 1.0 eq) in EtOH (90 mL) was added HCl aq. (4.8 M, 16 mL, 77 mmol, 6.3 eq) and the reaction mixture was heated at 100 °C for 48 h. The reaction mixture was concentrated in vacuo, then diluted with H_2O (50 mL), basified with sat. aq. K_2CO_3 (50 mL), extracted with EtOAc (3 × 100 mL), washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification *via* crystallisation from EtOH gave **168** (1.53 g, 8.61 mmol, 71%) as a yellow crystalline solid. Rf 0.15 (1:6 EtOAc:Petrol); mp 158-160 ° C (from EtOH, lit.[172] 160-162 ° C); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.93-7.86 (2H, m, 2 × C(Ar)*H*), 6.40-6.34 (1H, m, C(Ar)*H*), 4.70 (1H, br s, N(1)*H*), 3.42 (2H, t, *J* 5.8 Hz, C(2')H_2), 2.81 (2H, t, *J* 6.1 Hz, C(4')H_2), 1.96 (2H, tt, *J* 6.1 5.8 Hz, C(3')*H*); LRMS *m/z* (ES+) 201 ([M+Na]$^+$, 100%). These data are in accordance with the literature.[172]

7-(benzyloxy)-3,4-dihydroquinolin-2(1*H*)-one (173)



To a solution of 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (1.50 g, 9.19 mmol, 1.0 eq) and K_2CO_3 (2.54 g, 18.4 mmol, 2.0 eq) in DMF (15 mL) was added BnBr (1.21 mL, 1.74 g, 10.1 mmol, 1.1 eq). The reaction mixture was heated to 80 °C for 17 h before being diluted with EtOAc (250 mL), washed with sat. aq. K_2CO_3 (3 × 250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* crystallisation from EtOH yielded **173** (1.62 g, 3.13 mmol, 70%) as a colourless crystalline solid. R_f 0.26 (1:1 EtOAc:Petrol); mp 152-154 °C (from EtOH); ν_{max}/cm^{-1} 3197 (w), 2896 (w), 1674 (s), 1594 (m), 1410 (m), 1168 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.54 (1H, br s, N*H*), 7.44 (2H, d, *J* 7.4 Hz, C(2')*H* & C(6')*H*), 7.40 (2H, dd, *J* 7.4 7.4, C(3')*H* & C(5')*H*), 7.37-7.31 (1H, m, C(4')*H*), 7.05 (1H, d, *J* 8.3 Hz, C(5)*H*), 6.62 (1H, dd, *J* 8.3 2.4 Hz, C(6)*H*), 6.56 (1H, d, *J* 2.4 Hz, C(8)*H*), 5.04 (2H, s, OCH_2Ph), 2.90 (2H, t, *J* 7.6 Hz, C(4)H_2), 2.64 (2H, t, *J* 7.6 Hz, C(3)H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 172.7 (*C*(2)=O), 158.4 (*C*(7)), 138.4 (*C*(10)), 136.9 (*C*(1')), 128.6 (*C*(8)H & *C*(3')H & *C*(5')), 128.0 (*C*(4')H), 127.6 (*C*(2')H & *C*(6')H), 116.0 (*C*(9)), 109.1 (*C*(6)H), 102.8 (*C*(8)H), 70.2 (O*C*H_2Ph), 31.1 (*C*(3)H_2), 24.6 (*C*(4)H_2); LRMS *m/z* (ES+) 254 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 276.1001. $C_{16}H_{15}NNaO_2^+$ requires 276.0995]; Anal. calcd for $C_{16}H_{15}NO_2$: C 75.87%, H 5.97%, N 5.53%; Found: C 75.69%, H 6.01%, N 5.61%.

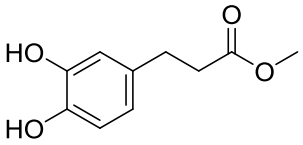
7-(benzyloxy)-1,2,3,4-tetrahydroquinoline (165)



To a solution of **173** [7-(benzyloxy)-3,4-dihydroquinolin-2(1*H*)-one] (800 mg, 3.16 mmol, 1.0 eq) in THF (50 mL) at 0 °C was added a solution of $LiAlH_4$ in THF (1M, 5.70 mL,

5.70 mmol, 1.8 eq) dropwise over 5 min. The reaction mixture was allowed to warm to rt and stirred for 18 h before being cooled to 0 °C and quenched by sequential addition of EtOAc (5 mL) and H_2O (2 mL). The resulting mixture was dried (Na_2SO_4), filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:8 EtOAc:petroleum ether) gave **165** (714 mg, 2.98 mmol, 94%) as an orange waxy solid. R_f 0.69 (1:1 EtOAc:Petrol); mp 52-54 °C (from EtOAc); ν_{max}/cm^{-1} 3400 (m), 2946 (w), 1618 (m), 1499 (m), 1080 (s); ^1H NMR (500 MHz; $CDCl_3$): δ 7.46 (2H, d, *J* 7.5 Hz, C(2')*H* & C(6')*H*), 7.41 (2H, dd, *J* 7.5 7.5 Hz, C(3')*H* & C(5')*H*), 7.37-7.32 (1H, m, C(4')*H*), 6.89 (1H, d, *J* 8.2 Hz, C(5)*H*), 6.32 (1H, dd, *J* 8.2 2.5 Hz, C(6)*H*), 6.15 (1H, d, *J* 2.5 Hz, C(8)*H*), 5.03 (2H, s, OCH_2Ph), 3.68 (1H, br s, N(1)*H*), 3.29 (2H, t, *J* 5.5 Hz, C(4)H_2), 2.73 (2H, t, *J* 6.4 Hz, C(2)H_2), 1.99-1.92 (2H, m, C(3)H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 158.2 (*C*(7)), 145.6 (*C*(10)), 137.6 (*C*(1')), 130.2 (*C*(5)H), 128.6 (*C*(3')H & *C*(5')H), 127.8 (*C*(4')H), 127.5 (*C*(2')H & *C*(6')H), 114.4 (*C*(9)), 103.7 (*C*(6)H), 100.5 (*C*(8)H), 70.0 (O*C*H_2Ph), 42.0 (*C*(2)H_2), 26.4 (*C*(4)H_2), 22.5 (*C*(3)H_2); LRMS *m/z* (ES+) 240 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 240.1374. $C_{16}H_{18}NO^+$ requires 240.1383]; Anal. calcd for $C_{16}H_{17}NO$: C 80.30%, H 7.16%, N 5.85%; Found: C 80.40%, H 7.11%, N 6.00%.

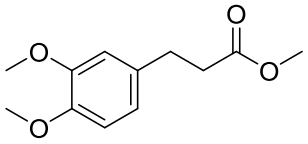
Methyl 3-(3,4-dihydroxyphenyl)propanoate (175)



3-(3,4-Dihydroxyphenyl)propionic acid (2.00 g, 11.0 mmol, 1.0 eq) was added in one portion to a solution of thionyl chloride (1.84 g, 1.12 mL, 15.4 mmol, 1.4 eq) in MeOH (40 mL) at 0 °C. The reaction mixture was warmed to rt and stirred for 4 h then concentrated *in vacuo*. The resulting residue was resuspended in EtOAc (250 mL), washed with sat. aq. $NaHCO_3$ (3 × 250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give

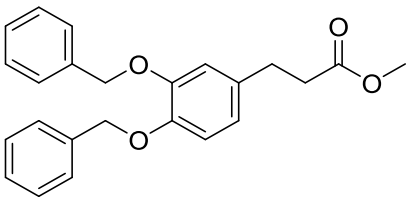
175 (2.06 g, 10.5 mmol, 95%) as a waxy yellow solid that was used without further purification. R_f 0.76 (1:9:0.01 MeOH:EtOAc:AcOH); mp 72-73 ° C (from EtOAc, lit.[218] 72-74 ° C); ^{1}H NMR (400 MHz; $CDCl_3$): δ 6.78 (1H, d, *J* 8.1 Hz, C(5')*H*), 6.71 (1H, d, *J* 1.9 Hz, C(2')*H*), 6.62 (1H, dd, *J* 8.1 1.9 Hz, C(6')*H*), 5.69 (1H, br s, O*H*), 5.53 (1H, br s, O*H*), 3.68 (3H, s, OCH_3), 2.84 (2H, t, *J* 7.7 Hz, C(3)H_2), 2.61 (2H, t, *J* 7.7 Hz, C(2)H_2); LRMS *m/z* (ES+) 219 ([M+Na]$^+$, 100%). This data is in agreement with the literature.[219]

Methyl 3-(3,4-dimethoxyphenyl)propanoate (176)



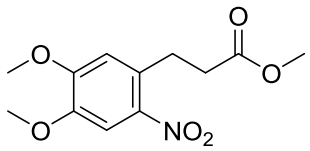
To a solution of **175** [methyl 3-(3,4-dihydroxyphenyl)propanoate] (1.00 g, 5.10 mmol, 1.0 eq) in DMF (10 mL) was added K_2CO_3 (2.82 g, 20.4 mmol, 4.0 eq) and iodomethane (1.59 g, 698 µL, 11.2 mmol, 2.2 eq) and the reaction mixture stirred at rt for 16 h. The reaction mixture was diluted with EtOAc (200 mL), washed with sat. aq. K_2CO_3 (3 × 200 mL) and brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **176** as a white solid (1.14 g, 5.08 mmol, 100%) that was used without further purification. R_f 0.41 (1:4 EtOAc:Petrol); mp 34-35 ° C (from EtOAc, lit.[220] 37 ° C); ^{1}H NMR (400 MHz; $CDCl_3$): δ 6.80 (1H, d, *J* 6.8 Hz, C(5')*H*), 6.77-6.72 (2H, m, C(2')*H* & C(6')*H*), 3.88 (3H, s, C(Ar)OCH_3), 3.87 (3H, s, C(Ar)OCH_3), 3.68 (3H, s, C(1)O_2CH_3), 2.92 (2H, t, *J* 7.8 Hz, C(3)H_2), 2.62 (2H, t, *J* 7.8 Hz, C(2)H_2); LRMS *m/z* (ES+) 247 ([M+Na]$^+$, 100%). This data is in agreement with the literature.[221]

Methyl 3-(3,4-bis(benzyloxy)phenyl)propanoate (177)



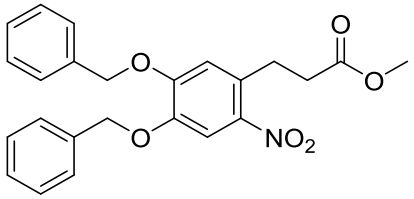
To a solution of **175** [methyl 3-(3,4-dihydroxyphenyl)propanoate] (705 mg, 3.59 mmol, 1.0 eq), K_2CO_3 (1.24 g, 8.98 mmol, 2.5 eq) and NaI (539 mg, 3.60 mmol, 1.0 eq) in DMF (8 mL) was added BnBr (1.35 g, 943 µL, 7.91 mmol, 2.2 eq). The reaction mixture stirred at 85 °C for 16 h then diluted with EtOAc (200 mL), washed with sat. aq. K_2CO_3 (3 × 200 mL) and brine (200 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:10 EtOAc:petroleum ether) gave **177** (418 mg, 1.11 mmol). Monobenzylated-**175** [methyl 3-(3,4-dihydroxyphenyl)propanoate] (658 mg, 2.30 mmol, 0.64 eq) was also isolated so was resubjected to the reaction conditions for 24 h. Purification *via* silica gel chromatography (1:10 EtOAc:Petrol) yielded a further **177** (517 mg, 1.37 mmol). The two batches were combined to give **177** (935 mg, 2.48 mmol, 69%) as a colourless oil. R_f 0.44 (1:4 EtOAc:Petrol); ^{1}H NMR (400 MHz; $CDCl_3$): δ7.49-7.43 (4H, m, 2 × C(Ar'')*H* & 2 × C(Ar''')*H*), 7.40-7.35 (4H, m, 2 × C(Ar'')*H* & 2 × C(Ar''')*H*), 7.35-7.29 (2H, m, C(4'')*H* & C(4''')*H*), 6.88 (1H, d, *J* 8.2 Hz, C(5')*H*), 6.82 (1H, d, *J* 2.0 Hz, C(2')*H*), 6.73 (1H, dd, *J* 8.2 2.0 Hz, C(6')*H*), 5.16 (2H, s, OCH_2Ph), 5.14 (2H, s, OCH_2Ph), 3.67 (3H, s, OCH_3), 2.87 (2H, t, *J* 7.8 Hz, C(3)H_2), 2.58 (2H, t, *J* 7.8 Hz, C(2)H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 173.5 (*C*(1)=O), 149.1 (*C*(3'/4')), 147.6 (*C*(3'/4')), 137.6 (*C*(1'')), 137.4 (*C*(1''')), 134.1 (*C*(1')), 128.6 (4 × *C*(Ar)H), 127.9 (*C*(4'')H), 127.8 (*C*(4''')H), 127.5 (2 × *C*(Ar)H), 127.4 (2 × *C*(Ar)H), 121.2 (*C*(6')H), 115.6 (*C*(2')H), 115.5 (*C*(5')H), 71.6 (O*C*H_2Ph), 71.5 (O*C*H_2Ph), 51.7 (O*C*H_3), 36.0 (*C*(2)H_2), 30.6 (*C*(3)H_2); LRMS *m/z* (ES+) 399 ([M+Na]$^+$, 100%). This data is in agreement with the literature.[219]

Methyl 3-(4,5-dimethoxy-2-nitrophenyl)propanoate (178)



To a solution of **176** [methyl 3-(3,4-dimethoxyphenyl)propanoate] (885 mg, 3.95 mmol, 1.0 eq), in AcOH (9 mL) was added a solution of HNO_3 (70%, 336 µL, 1.5 eq) in AcOH (2 mL). The reaction mixture was stirred at rt for 40 min before being quenched by pouring onto ice. The resulting precipitate was extracted into EtOAc (3 × 100 mL) washed with sat. aq. $NaHCO_3$ (3 × 200 mL) and brine (200 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give **178** as a yellow crystalline solid (892 mg, 3.31 mmol, 84%) that was used without further purification. R_f 0.30 (1:4 EtOAc:Petrol); mp 67-70 ° C (from EtOAc); ν_{max}/cm^{-1} 2952 (w), 1732 (m), 1521 (s), 1438 (w), 1330 (m), 1268 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.60 (1H, s, C(5')*H*), 6.79 (1H, s, C(2')*H*), 3.94 (3H, s, C(Ar)OC*H*$_3$), 3.91 (3H, s, C(Ar)OC*H*$_3$), 3.65 (3H, s, C(1)CO_2C*H*$_3$), 3.22 (2H, t, *J* 7.4 Hz, C(3)H_2), 2.71 (2H, t, *J* 7.4 Hz, C(2)H_2); ^{13}C NMR (126 MHz; CDCl3): δ 173.1 (*C*(1)O_2CH_3), 153.1 (*C*(Ar')), 147.5 (*C*(Ar')), 141.1 (*C*(Ar')), 131.0 (*C*(Ar')), 113.7 (*C*(2')H), 108.3 (*C*(5')H), 56.4 (*C*(Ar')OCH_3), 56.3 (*C*(Ar')OCH_3), 51.7 (C(1)O_2*C*H$_3$), 34.6 (*C*(2)H_2), 29.2 (*C*(3)H_2); LRMS *m/z* (ES+) 292 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 292.0787. $C_{12}H_{15}NNaO_6^+$ requires 292.0792]; Anal. calcd for $C_{12}H_{15}NO_6$: C 53.53%, H 5.62%, N 5.20%; Found: C 53.57%, H 5.73%, N 5.31%.

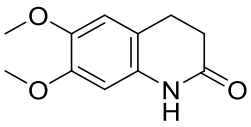
Methyl 3-(4,5-bis(benzyloxy)-2-nitrophenyl)propanoate (179)



Compound **177** [methyl 3-(3,4-bis(benzyloxy)phenyl)propanoate] (822 mg, 2.18 mmol, 1.0 eq), was reacted as above to give **179** as a yellow solid (712 mg, 1.69 mmol, 78%) that was used

without further purification. R_f 0.78 (1:1 EtOAc:Petrol); mp 112-113 °C (from EtOAc); ν_{max}/cm^{-1} 3062 (w), 2949 (w), 1736 (m), 1530 (m), 1268 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.71 (1H, s, C(3')*H*), 7.48-7.43 (4H, m, 2 × C(Ar'')*H* & 2 × C(Ar''')*H*), 7.42-7.37 (4H, m, 2 × C(Ar'')*H* & 2 × C(Ar''')*H*), 7.37-7.32 (2H, m, C(4'')*H* & C(4''')*H*), 6.88 (1H, s, C(3')*H*), 5.24 (2H, s, OCH_2Ph), 5.19 (2H, s, OCH_2Ph), 3.65 (3H, s, OCH_3), 3.19 (2H, t, *J* 7.4 Hz, C(3)H_2), 2.68 (2H, t, *J* 7.4 Hz, C(2)H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 173.2 (*C*(1)=O), 153.0 (*C*(4'/5')), 147.1 (*C*(4'/5')), 141.3 (*C*(2')), 136.1 (*C*(1'')), 135.9 (*C*(1''')), 131.3 (*C*(1')), 128.82 (2 × *C*(Ar'')H), 128.77 (2 × *C*(Ar''')H), 128.39 (*C*(4'')H), 128.34 (*C*(4''')H), 127.5 (2 × *C*(Ar'')H), 127.3 (2 × *C*(Ar''')H), 116.3 (*C*(6')H), 111.4 (*C*(3')H), 71.4 (O*C*H_2Ph), 71.2 (O*C*H_2Ph), 51.8 (O*C*H_3), 34.6 (*C*(2)H_2), 29.2 (*C*(3)H_2); LRMS *m/z* (ES+) 444 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 444.1421. $C_{24}H_{23}NNaO_6^+$ requires 444.1418]; Anal. calcd for $C_{24}H_{23}NO_6$: C 68.4%, H 5.50%, N 3.32%; Found: C 68.52%, H 5.57%, N 3.40%.

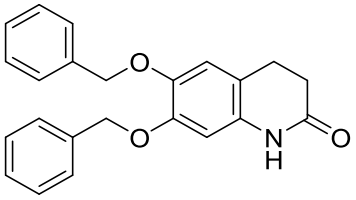
6,7-Dimethoxy-3,4-dihydroquinolin-2(1*H*)-one (180)



A solution of **178** [methyl 3-(4,5-dimethoxy-2-nitrophenyl)propanoate] (1.04 g, 3.86 mmol, 1.0 eq) and Pd/C (10% w/w, 205 mg, 0.193 mmol 0.05 eq) in EtOAc (40 mL) was placed under a H_2 atmosphere. The reaction mixture was stirred for 20 h, then filtered through Celite® (eluent EtOAc) and concentrated *in vacuo* to give **180** (767 mg, 3.70 mmol, 96%) as a colourless solid. R_f 0.18 (4:1 EtOAc:Petrol); mp 128-130 °C (from EtOAc, lit.[222] 128-130 °C); ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.29 (1H, br s, N*H*), 6.67 (1H, s, C(5)*H*), 6.42 (1H, s, C(8)*H*), 3.85 (3H, s, C(7)OCH_3), 3.84 (3H, s, C(6)OCH_3), 2.89 (2H, t, *J* 7.7 Hz, C(4)H_2), 2.61 (2H, t, *J* 7.7 Hz, C(3)H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 172.1 (*C*(1)=O), 148.4 (*C*(7)), 144.7 (*C*(6)), 130.7

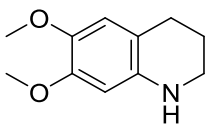
(*C*(10)), 114.8 (*C*(9)), 111.6 (*C*(5)H), 100.4 (*C*(8)H), 56.3 (C(7)O*C*H$_3$), 56.2 (C(6)O*C*H$_3$), 30.9 (*C*(3)H$_2$), 25.0 (*C*(4)H$_2$); LRMS *m/z* (ES+) 437 ([2M+Na]$^+$, 100%), 208 ([M+H]$^+$, 47%). This data is in agreement with the literature.

6,7-Bis(benzyloxy)-3,4-dihydroquinolin-2(1*H*)-one (181)



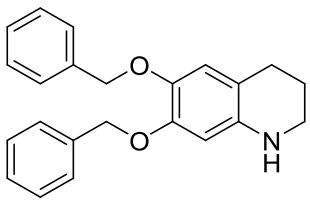
To a solution of **179** [methyl 3-(4,5-bis(benzyloxy)-2-nitrophenyl)propanoate] (625 mg, 1.48 mmol, 1.0 eq) in DMF (14 mL) was added zinc dust (2.42 g, 38.2 mmol, 26 eq) and NH_4Cl (1.98 mg, 37.0 mmol, 25 eq). The reaction mixture was stirred at rt for 3 h, then diluted with EtOAc (250 mL) and filtered through Celite® (eluent EtOAc). The filtrate was washed with brine (3 × 250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* crystallisation from propan-2-ol yielded **181** (412 mg, 1.15 mmol, 77%) as a pale brown solid. R_f 0.20 (1:1 EtOAc:Petrol); mp 139-142 °C (from propan-2-ol); ν_{max}/cm^{-1} 3207 (w), 2898 (w), 1673 (s), 1518 (m), 1228 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.51 (1H, br s, N(1)*H*), 7.47-7.42 (4H, m, 2 × C(Ar')*H* & 2 × C(Ar'')*H*), 7.40-7.35 (4H, m, 2 × C(Ar')*H* & 2 × C(Ar'')*H*), 7.35-7.30 (2H, m, C(Ar')*H* & C(Ar'')*H*), 6.77 (1H, s, C(5)*H*), 6.46 (1H, s, C(8)*H*), 5.12 (2H, s, OC*H*$_2$Ph), 5.10 (2H, s, OC*H*$_2$Ph), 2.85 (2H, t, *J* 7.5 Hz, C(4)*H*$_2$), 2.59 (2H, t, *J* 7.5 Hz, C(3)*H*$_2$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 171.8 (*C*(2)=O), 148.7 (*C*(6/7)), 144.8 (*C*(6/7)), 137.4 (*C*(1')), 137.1 (*C*(1'')), 131.7 (*C*(10)), 128.65 (2 × *C*(Ar)H), 128.60 (2 × *C*(Ar)H), 128.1 (*C*(4')H), 128.0 (*C*(4'')H), 127.59 (2 × *C*(Ar)H), 127.57 (2 × *C*(Ar)H), 116.4 (*C*(5)H), 116.1 (*C*(9)), 103.8 (*C*(8)H), 72.5 (O*C*H$_2$Ph), 71.8 (O*C*H$_2$Ph), 31.0 (*C*(3)H$_2$), 25.1 (*C*(4)H$_2$); LRMS *m/z* (ES+) 382 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 382.1401. $C_{23}H_{21}NNaO_3^+$ requires 382.1414].

6,7-Dimethoxy-1,2,3,4-tetrahydroquinoline (166)



To a solution of **180** [6,7-dimethoxy-3,4-dihydroquinolin-2(1*H*)-one] (746 mg, 3.60 mmol, 1.0 eq) in THF (50 mL) at 0 °C was added $LiAlH_4$ (1M in THF, 7.34 mL, 7.34 mmol, 2.0 eq). The reaction mixture was stirred at rt for 20 h, then recooled to 0 °C and quenched by sequential addition of EtOAc (5 mL) and H_2O (2 mL). The resulting mixture was concentrated *in vacuo*, resuspended in EtOAc (250 mL), washed with brine (3 × 250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:4 EtOAc:petroleum ether) gave **166** (584 mg, 3.02 mmol, 84%) as a brown oil. R_f 0.40 (2:1 EtOAc:Petrol); ν_{max}/cm^{-1} 3386 (m), 2930 (m), 1517 (s), 1229 (s), 1136 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.52 (1H, s, C(5)*H*), 6.10 (1H, s, C(8)*H*), 3.78 (6H, s, 2 × OCH_3), 3.40 (1H, br s, N(1)*H*), 3.24 (2H, t, *J* 5.4 Hz, C(2)H_2), 2.69 (2H, t, *J* 6.4 Hz, C(4)H_2), 1.95-1.88 (2H, m, C(3)H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 148.2 (*C*(6/7)), 141.4 (*C*(6/7)), 138.7 (*C*(10)), 114.0 (*C*(5)H), 112.9 (*C*(9)), 99.8 (*C*(8)H), 56.7 (O*C*H_3), 55.9 (O*C*H_3), 42.3 (*C*(2)H_2), 26.4 (*C*(4)H_2), 22.7 (*C*(3)H_2); LRMS *m/z* (ES+) 194 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 194.1171. $C_{11}H_{16}NO_2^+$ requires 194.1176].

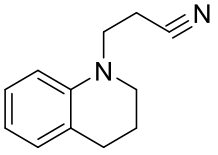
6,7-Bis(benzyloxy)-1,2,3,4-tetrahydroquinoline (167)



To a solution of **181** [6,7-bis(benzyloxy)-3,4-dihydroquinolin-2(1*H*)-one] (353 mg, 0.982 mmol, 1.0 eq) in THF (14mL) at 0 °C was added $LiAlH_4$ (1M in THF, 1.2 mL, 1.20 mmol, 1.2 eq). The reaction mixture was stirred at rt for 4 h, then recooled to 0 °C and quenched by

sequential addition of EtOAc (2 mL) and H_2O (1 mL). The resulting mixture was concentrated *in vacuo*, resuspended in EtOAc (100 mL), washed with brine (3 × 100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:10 then 1:1 EtOAc:petroleum ether) gave **167** (304 mg, 0.880 mmol, 90%) as a pale yellow waxy solid. R_f 0.77 (1:1 EtOAc:Petrol); mp 84-86 °C (from EtOAc); ν_{max}/cm^{-1} 3395 (w), 2926 (w), 1514 (s), 1226 (m), 1191 (s); ^1H NMR (500 MHz; $CDCl_3$): δ7.48-7.42 (4H, m, 2 × C(Ar')*H* & 2 × C(Ar'')*H*), 7.39-7.33 (4H, m, 2 × C(Ar')*H* & 2 × C(Ar'')*H*), 7.33-7.28 (2H, m, C(4')*H* & C(4'')*H*), 6.64 (1H, s, C(5)*H*), 6.17 (1H, s, C(8)*H*), 5.08 (2H, s, OCH_2Ph), 5.04 (2H, s, OCH_2Ph), 3.55 (1H, br s, N(1)*H*), 3.23 (2H, t, *J* 5.5 Hz, C(2)H_2), 2.66 (2H, t, *J* 6.4 Hz, C(4)H_2), 1.94-1.87 (2H, m, C(3)H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 148.8 (*C*(6/7)), 141.1 (*C*(6/7)), 139.9 (*C*(10)), 138.2 (*C*(1')), 137.8 (*C*(1'')), 128.6 (2 × *C*(Ar')H), 128.4 (2 × *C*(Ar'')H), 127.80-127.76 (2 × *C*(Ar')H & *C*(4')H), 127.7 (*C*(4'')H), 127.4 (2 × *C*(Ar'')H), 119.1 (*C*(5)H), 114.2 (*C*(9)), 102.7 (*C*(8)H), 73.4 (O*C*H_2Ph), 71.5 (O*C*H_2Ph), 42.2 (*C*(2)H_2), 26.4 (*C*(4)H_2), 22.6 (*C*(3)H_2); LRMS *m/z* (ES+) 346 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 346.1802. $C_{23}H_{24}NO_2^+$ requires 346.1802].

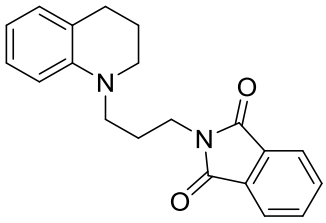
3-(3,4-dihydroquinolin-1(2*H*)-yl)propanenitrile (182)



To a microwave flask containing a suspension of 1,2,3,4-tetrahydroquinoline (400 µL, 424 mg, 3.19 mmol, 1.0 eq), K_2CO_3 (484 mg, 3.51 mmol, 1.1 eq) and NaI (525 mg, 3.51 mmol, 1.1 eq) in DMF (2 mL) was added 3-bromopropionitrile (291 µL, 470 mg, 3.51 mmol, 1.1 eq). The flask was sealed and the reaction mixture was heated at 80 °C for 71 h. Upon cooling to rt, the mixture was diluted with EtOAc (50 mL), washed with K_2CO_3 (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography

(1:10 EtOAc:petroleum ether) gave **182** (106 mg, 0.569 mmol, 18%) as a brown oil. R_f 0.57 (1:4 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2931 (w), 2246 (w), 1601 (m), 1498 (s), 1346 (m); ^1H NMR (400 MHz; $CDCl_3$): δ 7.10 (1H, dd, *J* 8.0 7.5 Hz, C(7')*H*), 7.01 (1H, d, *J* 7.3 Hz, C(5')*H*), 6.67 (1H, ddd, *J* 7.5 7.3 0.6 Hz, C(6')*H*), 6.53 (1H, d, *J* 8.0 Hz, C(8')*H*), 3.67 (2H, t, *J* 6.9 Hz, C(3)H_2), 3.40 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.79 (2H, t, *J* 6.3 Hz, C(4')H_2), 2.61 (2H, t, *J* 6.9 Hz, C(2)H_2), 2.04-1.97 (2H, m, C(3')H_2); ^{13}C NMR (101 MHz; CD_3Cl): δ 143.5 (*C*(10')), 129.7 (*C*(5')H), 127.3 (*C*(7')H), 123.1 (*C*(9')), 118.7 (*C*(1)), 117.0 (*C*(6')H), 110.2 (*C*(8')H), 49.9 (*C*(2'')H_2), 47.6 (*C*(3)H_2), 27.9 (*C*(4')H_2), 22.1 (*C*(3')H_2), 14.8 (*C*(2)H_2); LRMS *m/z* (ES+) 187 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 187.1223. $C_{12}H_{15}N_2^+$ requires 187.1223].

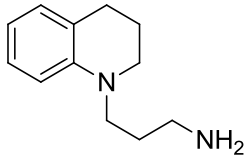
2-[3-(3,4-Dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (183)



1,2,3,4-Tetrahydroquinoline (400 µL, 424 mg, 3.19 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (1:10 then 1:8 EtOAc:petroleum ether) yielded **183** (915 mg, 2.86 mmol, 90%) as a pale yellow solid. R_f 0.35 (1:6 EtOAc:Petrol); mp 67-68 °C (from EtOAc); ν_{max}/cm^{-1} 2932 (w), 1705 (s), 1601 (m), 1501 (m), 1395 (m); ^1H NMR (400 MHz; $CDCl_3$): δ 7.86 (2H, dd, *J* 5.4 3.1 Hz, C(4)*H* & C(7)*H*), 7.73 (2H, dd, *J* 5.4 3.1 Hz, C(5)*H* & C(6)*H*), 7.04 (1H, dd, *J* 7.7 7.7 Hz, C(7'')*H*), 6.94 (1H, d, *J* 7.6 Hz, C(5'')*H*), 6.61-6.54 (2H, m, C(6'')*H* & C(8'')*H*), 3.77 (2H, t, *J* 7.3 Hz, C(1')H_2), 3.34 (2H, t, *J* 7.4 Hz, C(3')H_2), 3.29 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.75 (2H, t, *J* 6.3 Hz, C(4'')H_2), 2.08-1.91 (4H, m, C(2')H_2 & C(3'')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O & *C*(3)=O), 145.2 (*C*(10'')), 134.1 (*C*(5)H & *C*(6)H), 132.2 (*C*(8) & *C*(9)), 129.3 (*C*(5'')H), 127.2 (*C*(7'')H), 123.3 (*C*(4)H & *C*(7)H), 122.7 (*C*(9'')), 115.8 (*C*(6'')H), 110.6

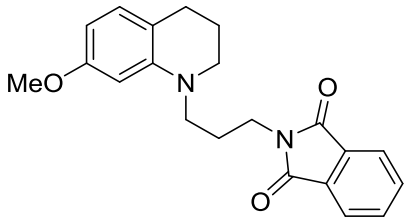
(*C*(8’’)H), 49.4 (*C*(2’’)H_2), 49.0 (*C*(3’)H_2), 36.2 (*C*(1’)H_2), 28.2 (*C*(4’’)H_2), 25.6 (*C*(2’)H_2), 22.3 (*C*(3’’)H_2); LRMS *m/z* (ES+) 321 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 321.1602. $C_{20}H_{21}N_2O_2^+$ requires 321.1598].

3-(3,4-Dihydroquinolin-1(2*H*)-yl)propan-1-amine (118)



Method 2: Compound **183** [2-[3-(3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (390 mg, 1.22 mmol, 1.0 eq) was reacted according to general procedure 7. Purification *via* acid-base extraction yielded **118** (93 mg, 0.49 mmol, 41%) as a grey oil. Data reported previously.

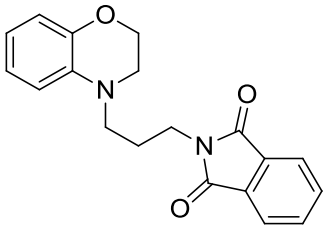
2-[3-(7-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (184)



Compound **131** [7-methoxy-1,2,3,4-tetrahydroquinoline] (200 mg, 1.23 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 3 to 24% EtOAc in petroleum ether) yielded **184** (299 mg, 0.853 mmol, 69%) as a yellow oil. R_f 0.16 (1:7 EtOAc:Petrol); ν_{max}/cm^{-1} 2936 (w), 1709 (s), 1612 (w), 1509 (m), 1396 (m); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.85 (2H, dd, *J* 5.5 3.1 Hz, C(4)*H* & C(7)*H*), 7.72 (2H, dd, *J* 5.5 3.1 Hz, C(5)*H* & C(6)*H*), 6.83 (1H, d, *J* 8.6, C(5’’)*H*), 6.15-6.11 (2H, m, C(6’’)*H* & C(8’’)*H*), 3.76 (2H, t, *J* 7.2 Hz, C(1’)H_2), 3.73 (3H, s, C(7’’)OCH_3), 3.32 (2H, t, *J* 7.3 Hz, C(3’)H_2), 3.26 (2H, t, *J* 5.6 Hz, C(2’’)H_2), 2.68 (2H, t, *J* 6.3 Hz, C(4’’)H_2), 2.07-1.97 (2H, m, C(2’)H_2), 1.97-1.88 (2H, m, C(3’’)H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O &

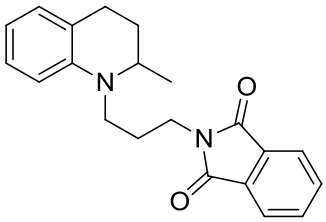
C(3)=O), 159.3 (*C*(7’’)), 146.0 (*C*(10’’)), 134.1 (*C*(5)H & *C*(6)H), 132.2 (*C*(8) & *C*(9)), 129.7 (*C*(5’’)H), 123.3 (*C*(4)H & *C*(7)H), 115.5 (*C*(9’’)), 100.5 (*C*(Ar’’)H), 97.4 (*C*(Ar’’)H), 55.2 (O*C*H_3), 49.4 (*C*(2’’)H_2), 49.1 (*C*(3’)H_2), 36.2 (*C*(1’)H_2), 27.5 (*C*(4’’)H_2), 25.7 (*C*(2’)H_2), 22.6 (*C*(3’’)H_2); LRMS *m/z* (ES+) 351 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 373.1528. $C_{21}H_{22}N_2NaO_3^+$ requires 373.1523].

2-[3-(2,3-Dihydro-4*H*-1,4-benzoxazin-4-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (185)



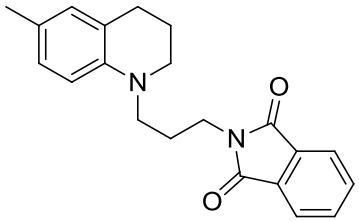
3,4-Dihydro-2*H*-1,4-benzoxazine (561 mg, 4.15 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) yielded **185** (583 mg, 1.80 mmol, 44%) as a yellow solid. R_f 0.18 (1:4 EtOAc:Petrol); mp 93-98 °C; ν_{max}/cm^{-1} 2938 (w), 1707 (s), 1503 (m), 1396 (m), 1235 (w); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.86 (2H, dd, *J* 5.4 3.1 Hz, C(4)*H* & C(7)*H*), 7.74 (2H, dd, *J* 5.4 3.1 Hz, C(5)*H* & C(6)*H*), 6.82 (1H, ddd, *J* 7.9 7.6 1.5 Hz, C(6’’)*H*), 6.77 (1H, dd, *J* 7.7 1.5 Hz, C(8’’)*H*), 6.66 (1H, dd, *J* 7.9 1.3 Hz, C(5’’)*H*), 6.62 (1H, ddd, *J* 7.7 7.6 1.3 Hz, C(7’’)*H*), 4.26 (2H, t, *J* 4.4 Hz, C(4’’)H_2), 3.78 (2H, t, *J* 7.1 Hz, C(1’)H_2), 3.35 (2H, t, *J* 4.4 Hz, C(3’’)H_2), 3.32 (2H, t, *J* 7.1 Hz, C(3’)H_2), 2.02 (2H, tt, *J* 7.1 7.1 Hz, C(2’)H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.5 (*C*(1)=O & *C*(3)=O), 144.3 (*C*(10’’)), 135.1 (*C*(9’’)), 134.2 (*C*(5)H & *C*(6)H), 132.2 (*C*(8) & *C*(9)), 123.4 (*C*(4)H & *C*(7)H), 121.7 (*C*(6’’)H), 117.8 (*C*(7’’)H), 116.5 (*C*(8’’)H), 112.3 (*C*(5’’)H), 64.7 (*C*(4’’)H_2), 48.6 (*C*(3’)H_2), 47.0 (*C*(3’’)H_2), 36.0 (*C*(1’)H_2), 25.5 (*C*(2’)H_2); LRMS *m/z* (ES+) 323 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 345.1205. $C_{19}H_{18}N_2NaO_3^+$ requires 345.1210].

2-[3-(2-Methyl-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (186)



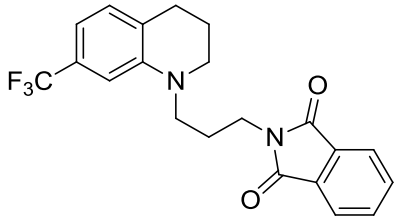
2-Methyl-1,2,3,4-tetrahydroquinoline (224 mg, 1.52 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 5 to 35% EtOAc in petroleum ether) yielded **186** (274 mg, 0.819 mmol, 54%) as a yellow solid. R_f 0.35 (1:4 EtOAc:Petrol); mp 114-116 °C (from EtOAc); ν_{max}/cm^{-1} 2932 (w), 1706 (s), 1601 (w), 1497 (m), 1394 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.86 (2H, dd, *J* 5.5 3.0 Hz, C(4)*H* & C(7)*H*), 7.73 (2H, dd, *J* 5.5 3.0 Hz, C(5)*H* & C(6)*H*), 7.04 (1H, dd, *J* 8.0 7.5 Hz, C(7'')*H*), 6.97 (1H, d, *J* 7.7 Hz, C(5'')*H*), 6.56 (1H, dd, *J* 8.0 7.7 Hz, C(6'')*H*), 6.53 (1H, d, *J* 7.5 Hz, C(8'')*H*), 3.78 (1H, dt, *J* 13.7 7.3 Hz, C(1')H_{AB}), 3.75 (1H, dt, *J* 13.7 7.2 Hz, C(1')H_{AB}), 3.56-3.49 (1H, m, C(2'')*H*), 3.44 (1H, ddd, *J* 15.1 8.0 6.5 Hz, C(3')H_{AB}), 3.23 (1H, ddd, *J* 15.1 8.1 7.0 Hz, C(3')H_{AB}), 2.84 (1H, ddd, *J* 16.1 12.4 5.3 Hz, C(4'')H_{ax}), 2.67 (1H, ddd, *J* 16.1 4.6 4.1 Hz, C(4'')H_{eq}), 2.02 (2H, tt, *J* 7.4 7.3 Hz, C(2')H_2), 1.92 (1H, dddd, *J* 12.7 12.4 4.8 4.6 Hz, C(3'')H_{ax}), 1.76 (1H, dddd, *J* 12.7 5.3 4.1 3.7 Hz, C(3'')H_{eq}), 1.13 (3H, d, *J* 6. 5 Hz, C(2'')CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.5 (*C*(1)=O & *C*(3)=O), 144.1 (*C*(10'')), 134.1 (*C*(5)H & *C*(6)H), 132.2 (*C*(8) & *C*(9)), 129.2 (*C*(5'')H), 127.2 (*C*(7'')H), 123.4 (*C*(4)H & *C*(7)H), 122.0 (*C*(9'')), 115.3 (*C*(6'')H), 110.7 (*C*(8'')H), 52.5 (*C*(2'')H), 47.2 (*C*(3')H_2), 36.2 (*C*(1')H_2), 28.0 (*C*(3'')H_2), 26.7 (*C*(2')H_2), 23.9 (*C*(4'')H_2), 18.8 (C(2'')*C*H_3); LRMS *m/z* (ES+) 335 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 357.1565. $C_{21}H_{22}N_2NaO_2^+$ requires 357.1573].

2-[3-(6-Methyl-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (187)



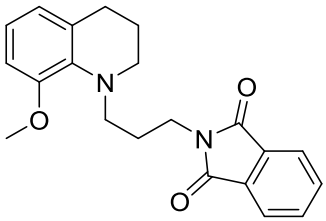
6-Methyl-1,2,3,4-tetrahydroquinoline (519 mg, 3.53 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) yielded **187** (1.09 g, 3.26 mmol, 92%) as a brown oil. R_f 0.28 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2931 (w), 1706 (s), 1509 (m), 1394 (m), 1341 (w); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.86 (2H, dd, *J* 5.4 3.0 Hz, C(4)*H* & C(7)*H*), 7.73 (2H, dd, *J* 5.4 3.0 Hz, C(5)*H* & C(6)*H*), 6.85 (1H, dd, *J* 8.3 1.4 Hz, C(7'')*H*), 6.77 (1H, d, *J* 1.4 Hz, C(5'')*H*), 6.50 (1H, d, *J* 8.3 Hz, C(8'')*H*), 3.76 (2H, t, *J* 7.3 Hz, C(1')H_2), 3.32 (2H, t, *J* 7.4 Hz, C(3')H_2), 3.25 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.72 (2H, t, *J* 6.4 Hz, C(4'')H_2), 2.20 (3H, s, C(6'')CH_3), 2.06-1.91 (2H, m, C(2')H_2 & C(3'')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O & *C*(3)=O), 143.0 (*C*(10'')), 134.0 (*C*(5)H & *C*(6)H), 132.2 (*C*(8) & *C*(9)), 130.0 (*C*(5'')H), 127.5 (*C*(7'')H), 125.0 (*C*(6'')), 123.3 (*C*(4)H & *C*(7)H), 122.8 (*C*(9'')), 111.0 (*C*(8'')H), 49.4 (*C*(2'')H_2), 49.1 (*C*(3')H_2), 36.2 (*C*(1')H_2), 28.1 (*C*(4'')H_2), 25.5 (*C*(2')H_2), 22.5 (*C*(3'')H_2), 20.4 (C(6'')*C*H_3); LRMS *m/z* (ES+) 335 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 357.1571. $C_{21}H_{22}N_2NaO_2^+$ requires 357.1573].

2-{3-[7-(Trifluoromethyl)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-1*H*-isoindole-1,3(2*H*)-dione (188)



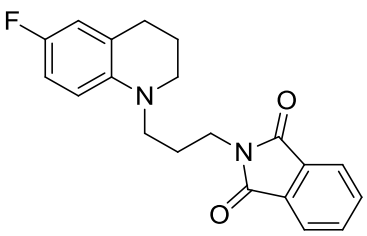
7-(trifluoromethyl)-1,2,3,4-tetrahydroquinoline (209 mg, 1.04 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 4 to 40% EtOAc in petroleum ether) yielded **188** (243 mg, 0.626 mmol, 60%) as a colourless oil. R_f 0.18 (1:8 EtOAc:Petrol); ν_{max}/cm^{-1} 2935 (w), 1707 (s), 1439 (m), 1395 (m), 1318 (m), 1110 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.85 (2H, dd, *J* 5.4 3.1 Hz, C(4)*H* & C(7)*H*), 7.72 (2H, dd, *J* 5.4 3.1 Hz, C(5)*H* & C(6)*H*), 6.98 (1H, d, *J* 7.7 Hz, C(5'')*H*), 6.76 (1H, d, *J* 7.7 Hz, C(6'')*H*), 6.67 (1H, s, C(8'')*H*), 3.77 (2H, t, *J* 7.2 Hz, C(1')H_2), 3.36 (2H, t, *J* 7.3 Hz, C(3')H_2), 3.31 (2H, t, *J* 5.8 Hz, C(2'')H_2), 2.76 (2H, t, *J* 6.1 Hz, C(4'')H_2), 2.01 (2H, tt, *J* 7.3 7.2 Hz, C(2')H_2), 1.95 (2H, tt, *J* 6.1 5.8 Hz, C(3'')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O & *C*(3)=O), 145.2 (*C*(10'')), 134.1 (*C*(5)H & *C*(6)H), 132.1 (*C*(8) & *C*(9)), 129.4 (q, *J* 31.5 Hz, *C*(7'')), 129.3 (*C*(5'')H), 126.2 (*C*(9'')), 124.6 (q, *J* 272.1 Hz, C(7'')*C*F_3), 123.4 (*C*(4)H & *C*(7)H), 112.1 (q, *J* 3.9 Hz, *C*(6'')H), 106.5 (q, *J* 3.8 Hz, *C*(8'')H), 49.2 (*C*(2'')H_2), 48.7 (*C*(3')H_2), 35.9 (*C*(1')H_2), 28.1 (*C*(4'')H_2), 25.4 (*C*(2')H_2), 21.8 (*C*(3'')H_2); ^{19}F NMR (377 MHz; $CDCl_3$): δ −62.5 (3F, s, C(7'')C*F*$_3$); LRMS *m/z* (ES+) 389 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 411.1276. $C_{21}H_{19}F_3N_2NaO_2^+$ requires 411.1291].

2-[3-(8-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (189)



8-Methoxy-1,2,3,4-tetrahydroquinoline (223 mg, 1.37 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) yielded **189** (388 mg, 1.11 mmol, 81%) as an orange oil. R_f 0.22 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2934 (w), 1706 (s), 1395 (m), 1250 (w), 1084 (w); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.87-7.81 (2H, m, C(4)*H* & C(7)*H*), 7.74-7.68 (2H, m, C(5)*H* & C(6)*H*), 6.77 (1H, dd, *J* 7.8 7.8 Hz, C(6'')*H*), 6.66-6.59 (2H, m, C(5'')*H* & C(7'')*H*), 3.80 (2H, t, *J* 7.4 Hz, C(1')H_2), 3.75 (3H, s, OCH_3), 3.15-3.10 (2H, m, C(2'')H_2), 3.08-3.01 (2H, m, C(3')H_2), 2.74 (2H, t, *J* 6.5 Hz, C(4'')H_2), 2.12-2.00 (2H, m, C(2')H_2), 1.83-1.74 (2H, m, C(3'')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 168.5 (*C*(1)=O & *C*(3)=O), 144.3 (*C*(10'')), 135.1 (*C*(9'')), 134.2 (*C*(5)H & *C*(6)H), 132.2 (*C*(8) & *C*(9)), 123.4 (*C*(4)H & *C*(7)H), 121.7 (*C*(6'')H), 117.8 (*C*(7'')H), 116.5 (*C*(8'')H), 112.3 (*C*(5'')H), 64.7 (*C*(4'')H_2), 48.6 (*C*(3')H_2), 47.0 (*C*(3'')H_2), 36.0 (*C*(1')H_2), 25.5 (*C*(2')H_2); LRMS *m/z* (ES+) 351 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 351.1701. $C_{21}H_{23}N_2O_3^+$ requires 351.1703].

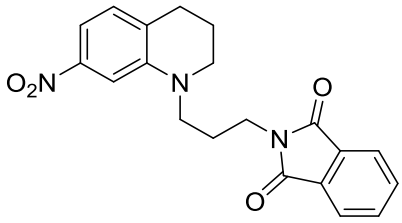
2-[3-(4-Fluoro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (190)



4-Fluoro-1,2,3,4-tetrahydroquinoline (112 mg, 0.741 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 3 to 28% EtOAc in petroleum ether) yielded **190** (214 mg, 0.632 mmol, 85%) as a yellow waxy solid. R_f

0.46 (1:3 EtOAc:Petrol); ν_{max}/cm^{-1} 2936 (w), 1708 (s), 1504 (m), 1395 (m), 1170 (w); ^{1}H NMR (500 MHz; $(CD_3)_2CO$): δ 7.87-7.78 (4H, m, C(4)*H* & C(5)*H* & C(6)*H* & C(7)*H*), 6.71-6.62 (2H, m, C(5'')*H* & C(7'')*H*), 6.55 (1H, dd, *J* 8.9 4.7 Hz, C(8'')*H*), 3.72 (2H, t, *J* 7.2 Hz, C(1')*H*), 3.33 (2H, t, *J* 7.3 Hz, C(3')*H*), 3.24 (2H, t, *J* 5.8 Hz, C(2'')*H*), 2.69 (2H, t, *J* 6.2 Hz, C(4'')*H*), 1.96 (2H, tt, *J* 7.3 7.2 Hz, C(2')*H*), 1.89 (2H, tt, *J* 6.2 5.8 Hz, C(3'')*H*); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 168.9 (*C*(1)=O & *C*(3)=O), 155.4 (d, *J* 232.5 Hz, *C*(6'')F), 142.9 (*C*(10'')), 135.0 (*C*(5)H & *C*(7)H), 133.3 (*C*(8) & *C*(9)), 125.1 (d, *J* 6.5 Hz, *C*(9'')), 123.8 (*C*(4)H & *C*(6)H), 116.2 (d, *J* 21.5 Hz, *C*(5'')H), 113.7 (d, *J* 21.9 Hz, *C*(7'')H), 112.5 (d, *J* 7.4 Hz, *C*(8'')H), 50.0 (*C*(3')H_2), 49.8 (*C*(2'')H_2), 36.7 (*C*(1')H_2), 28.9 (*C*(4'')H_2), 26.1 (*C*(2')H_2), 23.0 (*C*(3'')H_2); ^{19}F NMR (377 MHz; $(CD_3)_2CO$): δ –131.9 (ddd, *J* 8.8 8.8 4.7 Hz, C(6'')*F*); LRMS *m/z* (ES+) 339 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 361.1322. $C_{20}H_{19}FN_2NaO_2^+$ requires 361.1323].

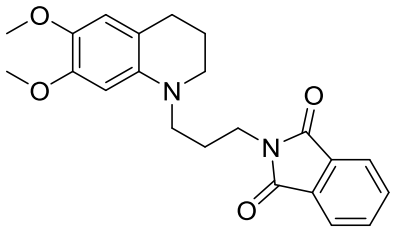
2-[3-(7-Nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (191)



7-nitro-1,2,3,4-tetrahydroquinoline (211 mg, 1.18 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) yielded **191** (122 mg, 0.334 mmol, 28%) as an orange oil. R_f 0.41 (1:2 EtOAc:Petrol); ν_{max}/cm^{-1} 2944 (w), 1710 (s), 1508 (s), 1393 (m), 1328 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.84 (2H, dd, *J* 5.4 3.1 Hz, C(4)*H* & C(7)*H*), 7.72 (2H, dd, *J* 5.4 3.1 Hz, C(5)*H* & C(6)*H*), 7.34 (1H, dd, *J* 8.1 2.2 Hz, C(6'')*H*), 7.26 (1H, d, *J* 2.2 Hz, C(8'')*H*), 6.98 (1H, d, *J* 8.1 Hz, C(5'')*H*), 3.78 (2H, t, *J* 7.1 Hz, C(1')H_2), 3.38 (2H, t, *J* 7.3 Hz, C(3')H_2), 3.32 (2H, t, *J* 5.9 Hz, C(2'')H_2), 2.77 (2H, t, *J* 6.2 Hz, C(4'')H_2), 2.03 (2H, tt, *J* 7.3 7.1 Hz, C(2')H_2),

1.95 (2H, tt, *J* 6.2 5.9 Hz, C(3'')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O & *C*(3)=O), 147.7 (*C*(7'')), 145.4 (*C*(10'')), 134.1 (*C*(5)H & *C*(6)), 132.0 (*C*(8) & *C*(9)), 129.8 (*C*(9'')), 129.3 (*C*(5'')H), 123.4 (*C*(4)H & *C*(7)H), 110.5 (*C*(6'')H), 104.2 (*C*(8'')H), 49.0 (*C*(2'')H_2), 48.7 (*C*(3')H_2), 35.8 (*C*(1')H_2), 28.3 (*C*(4'')H_2), 25.3 (*C*(2')H_2), 21.4 (*C*(3'')H_2); LRMS *m/z* (ES+) 366 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 388.1265. $C_{20}H_{19}N_3NaO_4^+$ requires 388.1268].

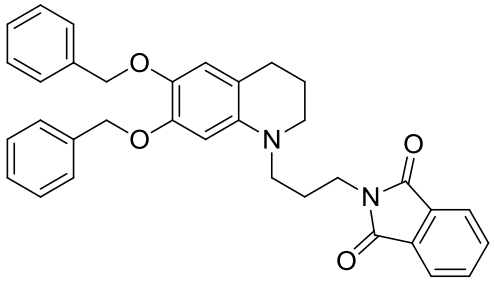
2-[3-(6,7-Dimethoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (192)



Compound **166** [6,7-dimethoxy-1,2,3,4-tetrahydroquinoline] (450 mg, 2.33 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 6 to 50% EtOAc in petroleum ether) yielded **192** (751 mg, 1.97 mmol, 85%) as a yellow solid. R_f 0.11 (1:4 EtOAc:Petrol); mp 102-104 °C (from EtOAc); ν_{max}/cm^{-1} 2934 (m), 1707 (s), 1517 (m), 1395 (m), 1235 (m); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.83 (2H, dd, *J* 5.4 3.1 Hz, C(4)*H* & C(7)*H*), 7.71 (2H, dd, *J* 5.4 3.1 Hz, C(5)*H* & C(6)*H*), 6.51 (1H, s, C(5'')*H*), 6.20 (1H, s, C(8'')*H*), 3.79 (3H, s, C(Ar'')OCH_3), 3.77 (3H, s, C(Ar'')OCH_3), 3.76 (2H, t, *J* 7.2 Hz, C(1')H_2), 3.29 (2H, t, *J* 7.5 Hz, C(3')H_2), 3.19 (2H, t, *J* 5.5 Hz, C(2'')H_2), 2.66 (2H, t, *J* 6.4 Hz, C(4'')H_2), 2.05-1.96 (2H, m, C(2')H_2), 1.96-1.88 (2H, m, C(3'')H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O & *C*(3)=O), 148.2 (*C*(10'')), 140.3 (*C*(Ar'')OCH_3), 139.8 (*C*(Ar'')OCH_3), 134.1 (*C*(5)H & *C*(6)H), 132.1 (*C*(8) & *C*(9)), 123.3 (*C*(4)H & *C*(7)H), 114.5 (*C*(9'')), 114.4 (*C*(5'')H), 97.6 (*C*(8'')H), 56.9 (C(Ar'')O*C*H_3), 56.0 (C(Ar'')O*C*H_3), 49.8 (*C*(3')H_2), 49.2 (*C*(2'')H_2), 36.1 (*C*(1')H_2), 27.5 (*C*(4'')H_2), 25.5 (*C*(2')H_2), 22.5 (*C*(3'')H_2);

LRMS *m/z* (ES+) 381 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 403.1628. $C_{22}H_{24}N_2NaO_4^+$ requires 403.1628].

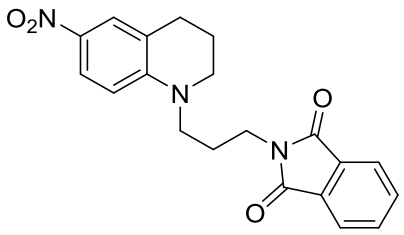
2-{3-[6,7-Bis(benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-1*H*-isoindole-1,3(2*H*)-dione (193)



Compound **167** [6,7-bis(benzyloxy)-1,2,3,4-tetrahydroquinoline] (282 mg, 0.816 mmol, 1.0 eq) was reacted according to general procedure 6. Purification *via* silica gel chromatography (gradient elution 3 to 28% EtOAc in petroleum ether) yielded **193** (751 mg, 1.97 mmol, 85%) as an orange oil. R_f 0.17 (1:6 EtOAc:Petrol); ν_{max}/cm^{-1} 2933 (w), 1707 (s), 1514 (m), 1395 (m), 1195 (m); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.85 (2H, dd, *J* 5.4 3.0 Hz, C(4)*H* & C(7)*H*), 7.70 (2H, dd, *J* 5.4 3.0 Hz, C(5)*H* & C(6)*H*), 7.50-7.43 (4H, m, C(2’’’)*H* & C(6’’’)*H* & C(2’’’’)*H* & C(6’’’’)*H*), 7.39-7.33 (4H, m, C(3’’’)*H* & C(5’’’)*H* & C(3’’’’)*H* & C(5’’’’)*H*), 7.33-7.26 (2H, m, C(4’’’)*H* & C(4’’’’)*H*), 6.64 (1H, s, C(5’’)*H*), 6.24 (1H, s, C(8’’)*H*), 5.13 (2H, s, OCH_2Ph), 5.04 (2H, s, OCH_2Ph), 3.70 (2H, t, *J* 7.2 Hz, C(1’)H_2), 3.24-3.15 (4H, m, C(3’)H_2 & C(2’’)H_2), 2.64 (2H, *J* 6.4 Hz, C(4’’)H_2), 1.96-1.86 (4H, m, C(2’)H_2 & C(3’’)H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O & *C*(3)=O), 148.6 (*C*(10’’)), 140.7 (*C*(Ar’’)OCH$_2$Ph), 140.0 (*C*(Ar’’)OCH$_2$Ph), 138.2 (*C*(1’’’)), 138.0 (*C*(1’’’’)), 134.1 (*C*(5)H & *C*(6)H), 132.1 (*C*(8) & *C*(9)), 128.5 (*C*(3’’’)H & *C*(5’’’)H), 128.4 (*C*(3’’’’)H & *C*(5’’’’)H), 127.72 (*C*(2’’’)H & *C*(6’’’)H), 127.67 (*C*(4’’’)), 127.6 (*C*(4’’’’)), 127.5 (*C*(2’’’’)H & *C*(6’’’’)H), 123.3 (*C*(4)H & *C*(7)H), 119.0 (*C*(5’’)H), 115.6 (*C*(9’’)), 100.7 (*C*(8’’)H), 73.3 (O*C*H$_2$Ph), 71.8 (O*C*H$_2$Ph), 49.6 (*C*(3’)H$_2$), 49.2 (*C*(2’’)H$_2$), 36.1 (*C*(1’)H$_2$), 27.5 (*C*(4’’)H$_2$),

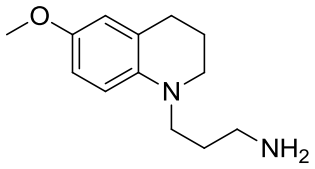
25.5 (*C*(2')H_2), 22.5 (*C*(3'')H_2); LRMS *m/z* (ES+) 533 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 555.2242. $C_{34}H_{32}N_2NaO_4^+$ requires 555.2254].

2-[3-(6-Nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (194)



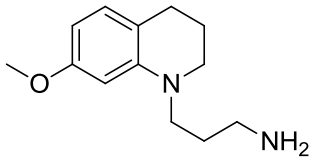
To a solution of **167** [6-nitro-1,2,3,4-tetrahydroquinoline] (200 mg, 1.12 mmol, 1.0 eq) in DMF (1 mL) at 0 °C was added NaH (57 mg, 2.38 mmol, 2.1 eq) in one portion causing a deep red colour to develop. *N*-(3-Iodopropyl)phthalimide (707 mg, 2.24 mmol, 2.0 eq) was added and the reaction mixture was stirred at rt for 110 h. The reaction was quenched *via* addition of H_2O (1 mL), then diluted with EtOAc (50 mL), washed with brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 6 to 50% EtOAc in petroleum ether) yielded **194** (130 mg, 0.356 mmol, 32%) as a yellow solid. R_f 0.28 (1:2 EtOAc:Petrol); mp 154-156 °C (from $CHCl_3$); ν_{max}/cm^{-1} 2942 (w), 1708 (s), 1601 (m), 1518 (w), 1491 (w), 1397 (m), 1291 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.93 (1H, dd, *J* 9.2 2.7 Hz, C(7'')*H*), 7.86 (2H, dd, *J* 5.5 3.0 Hz, C(4)*H* & C(7)*H*), 7.83-7.81 (1H, m, C(5'')*H*), 7.74 (2H, dd, *J* 5.5 3.0 Hz, C(5)*H* & C(6)*H*), 6.46 (1H, d, *J* 9.2 Hz, C(8'')*H*), 3.77 (2H, t, *J* 7.3 Hz, C(1')H_2), 3.49-3.39 (4H, m, C(3')H_2 & C(2'')H_2), 2.77 (2H, t, *J* 6.1 Hz, C(4'')H_2), 2.05 (2H, tt, *J* 7.4 7.3 Hz, C(2')H_2), 1.96 (2H, tt, *J* 6.1 6.0 Hz, C(3'')H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O & *C*(3)=O), 150.2 (*C*(10'')), 136.5 (*C*(6'')), 134.3 (*C*(5)H & *C*(6)H), 132.0 (*C*(8) & *C*(9)), 125.3 (*C*(5'')H), 124.8 (*C*(7'')H), 123.5 (*C*(4)H & *C*(7)H), 121.7 (*C*(9'')), 108.8 (*C*(8'')H), 49.8 (*C*(2'')H_2), 49.2 (*C*(3')H_2), 35.8 (*C*(1')H_2), 28.0 (*C*(4'')H_2), 25.6 (*C*(2')H_2), 21.4 (*C*(3'')H_2); LRMS *m/z* (ES+) 326 ($[M+H]^+$, 95%), 107.1 (100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 388.1274. $C_{20}H_{19}N_3NaO_4^+$ requires 388.1268].

3-(6-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (152)



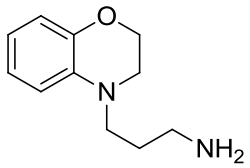
Method 2: 6-Methoxy-1,2,3,4-tetrahydroquinoline (500 mg, 3.06 mmol, 1.0 eq) was reacted according to general procedure 6 to give 2-[3-(6-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (1.17 g) which was used without further purification. The phthalimide (1.12 mg) was reacted according to general procedure 7. Purification *via* acid base extraction yielded **152** (474 mg, 2.15 mmol, 73%) as a yellow oil. Data reported previously.

3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (153)



Method 2: Compound **184** [2-[3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (260 mg, 0.742 mmol, 1.0 eq) was reacted according to general procedure 7. Purification *via* acid-base extraction yielded **153** (147 mg, 0.667 mmol, 90%) as a grey oil. Data reported previously.

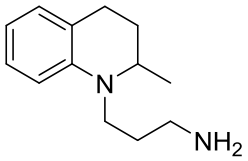
3-(2,3-Dihydro-4*H*-1,4-benzoxazin-4-yl)propan-1-amine (154)



Method 2: Compound **185** [2-[3-(2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (356 mg, 1.10 mmol, 1.0 eq) was reacted according to general procedure 7 to

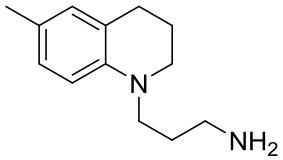
afford **154** (189 mg, 0.983 mmol, 89%) as a colourless oil which was used without further purification. Data reported previously.

3-(2-Methyl-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (195)



Compound **186** [2-[3-(2-methyl-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (189 mg, 0.565 mmol, 1.0 eq) was reacted according to general procedure 7. Purification *via* acid-base extraction yielded **195** (74 mg, 0.36 mmol, 64%) as a yellow oil. R_f 0.00 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2930 (m), 2361 (w), 1601 (m), 1498 (s), 1337 (w); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.10-7.03 (1H, m, C(7)*H*), 7.01-6.95 (1H, m, C(5)*H*), 6.62-6.50 (2H, m, C(6)*H* & C(8)*H*), 3.54-3.47 (1H, m, C(2')*H*), 3.47-3.37 (1H, m, C(3)H_AH_B), 3.31-3.16 (1H, m, C(3)H_AH_B), 2.90-2.61 (4H, m, C(1)H_2 & C(4')H_2), 2.00-1.68 (6H, m, C(2)H_2 & C(3')H_2 & NH_2), 1.15 (3H, d, *J* 6.5 Hz, C(2')CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 144.2 (*C*(10')), 129.1 (*C*(5')H), 127.1 (*C*(7')H), 121.7 (*C*(9')), 114.9 (*C*(Ar')H), 110.5 (*C*(Ar')H), 52.7 (*C*(2')H), 47.2 (*C*(3)H_2), 40.2 (*C*(1)H_2), 31.4 (*C*(2)H_2), 27.9 (*C*(3')H_2), 23.9 (*C*(4')H_2), 18.9 (C(2')*C*H_3); LRMS *m/z* (ES+) 205 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 205.1697. $C_{13}H_{21}N_2^+$ requires 205.1699].

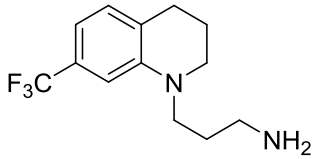
3-(6-Methyl-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (196)



Compound **187** [2-[3-(6-methyl-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (562 mg, 1.68 mmol, 1.0 eq) was reacted according to general procedure 7. The crude material was redissolved in EtOAc and filtered through Celite® to yield **196** (314 mg,

1.54 mmol, 91%) as a yellow oil. R_f 0.00 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2956 (m), 2857 (w), 1509 (s), 1297 (m), 1201 (m); ^{1}H NMR (500 MHz; CD_3OD): δ 6.77 (1H, d, *J* 8.3 Hz, C(7')*H*), 6.68 (1H, s, C(5')*H*), 6.51 (1H, d, *J* 8.3 Hz, C(8')*H*), 3.24 (2H, t, *J* 7.3 Hz, C(3)H_2), 3.20 (2H, t, *J* 5.6 Hz, C(2')H_2), 2.72-2.60 (4H, m, C(1)H_2 & C(4')H_2), 2.14 (3H, s, C(6)CH_3), 1.93-1.85 (2H, m, C(3')H_2), 1.71 (2H, tt, *J* 7.3 7.3 Hz, C(2)H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 144.4 (*C*(10')), 130.8 (*C*(5')H), 128.4 (*C*(7')H), 125.9 (*C*(6')), 123.9 (*C*(9')), 112.4 (*C*(8')H), 50.5 (*C*(2')H_2), 50.3 (*C*(3)H_2), 40.6 (*C*(1)H_2), 30.2 (*C*(2)H_2), 29.2 (*C*(4')H_2), 23.6 (*C*(3')H_2), 20.4 (C(6)*C*H_3); LRMS *m/z* (ES+) 205 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 205.1697. $C_{13}H_{21}N_2^+$ requires 205.1699].

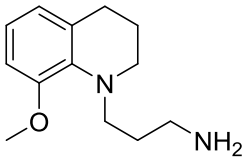
3-[7-(Trifluoromethyl)-3,4-dihydroquinolin-1(2*H*)-yl]propan-1-amine (197)



Compound **188** [2-{3-[7-(trifluoromethyl)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-1*H*-isoindole-1,3(2*H*)-dione] (188 mg, 0.484 mmol, 1.0 eq) was reacted according to general procedure 7 to give **197** (113 mg, 0.438 mmol, 90%) as a colourless oil that was used without further purification. R_f 0.00 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2932 (w), 2863 (w), 1579 (w), 1440 (m), 1317 (s), 1111 (s); ^{1}H NMR (500 MHz; CD_3OD): δ 7.00 (1H, d, *J* 7.7 Hz, C(5')*H*), 6.75 (1H, s, C(8')*H*), 6.71 (1H, d, *J* 7.7 Hz, C(6')*H*), 3.38-3.28 (4H, m, C(3)H_2 & C(2')H_2), 2.76 (2H, t, *J* 6.2 Hz, C(4')H_2), 2.72 (2H, t, *J* 7.3 Hz, C(1)H_2), 1.93 (2H, tt, *J* 6.2 6.0 Hz, C(3')H_2), 1.77 (2H, tt, *J* 7.3 7.3 Hz, C(2)H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 146.7 (*C*(10')), 130.4 (*C*(5')H), 130.3 (q, *J* 31.2 Hz, *C*(7')), 127.6 (*C*(9')), 126.2 (q, *J* 271.1 Hz, C(7)*C*F_3), 112.6 (q, *J* 4.0 Hz, *C*(6')H), 107.4 (q, *J* 4.0 Hz, *C*(8')H), 50.4 (*C*(2')H_2), 49.8 (*C*(3)H_2), 40.4 (*C*(1)H_2), 30.2 (*C*(2)H_2), 29.1 (*C*(4')H_2), 22.9 (*C*(3')H_2); ^{19}F NMR (377 MHz; $CDCl_3$): δ –62.48 (3F, s,

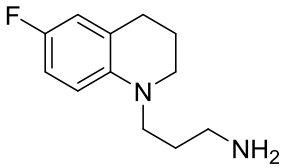
C(7)*C*F_3); LRMS *m/z* (ES+) 259 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 259.1420. $C_{13}H_{18}NF_3N_2^+$ requires 259.1417].

3-(8-Methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (198)



Compound **189** [2-[3-(8-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (332 mg, 0.947 mmol, 1.0 eq) was reacted according to general procedure 7 to afford **198** (201 mg, 0.912 mmol, 96%) as a yellow oil which was used without further purification. R_f 0.00 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2933 (m), 2836 (w), 1576 (s), 1474 (m), 1083 (w); ^{1}H NMR (500 MHz; CD_3OD): δ 6.83 (1H, dd, *J* 7.9 7.7 Hz, C(6')*H*), 6.73 (1H, d, *J* 7.9 Hz, C(7')*H*), 6.63 (1H, d, *J* 7.7 Hz, C(5')*H*), 3.83 (3H, s, C(8)OCH_3), 3.09-3.04 (2H, m, C(2')H_2), 3.02 (2H, t, *J* 6.9 Hz, C(3)H_2), 2.84 (2H, t, *J* 6.7 Hz, C(1)H_2), 2.74 (2H, t, *J* 6.5 Hz, C(4')H_2), 1.84-1.75 (4H, m, C(2)H_2 & C(3')H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 153.5 (*C*(8')), 138.6 (*C*(10')), 130.8 (*C*(9')), 123.1 (*C*(5')H), 122.9 (*C*(6')H), 109.9 (*C*(7')H), 55.7 (C(8)O*C*H_3), 53.5 (*C*(3)H_2), 48.3 (*C*(2')H_2), 40.9 (*C*(1)H_2), 30.9 (*C*(2)H_2), 28.7 (*C*(4')H_2), 18.5 (*C*(3')H_2); LRMS *m/z* (ES+) 221 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 221.1642. $C_{13}H_{21}N_2O^+$ requires 221.1648].

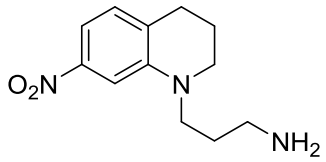
3-(7-Fluoro-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (199)



Compound **190** [2-[3-(7-fluoro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (68 mg, 0.20 mmol, 1.0 eq) was reacted according to general procedure 7. Purification *via* acid-base extraction yielded **199** (39 mg, 0.19 mmol, 93%) as a yellow oil. R_f 0.00 (1:4

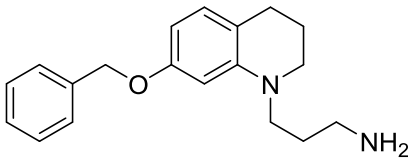
EtOAc:Petrol); ν_{max}/cm^{-1} 2931 (w), 2860 (w), 1502 (s), 1262 (m), 1233 (m), 1200 (m); ^{1}H NMR (500 MHz; CD_3OD): δ 6.69 (1H, ddd, *J* 8.8 8.7 3.0 Hz, C(7')*H*), 6.63 (1H, dd, *J* 9.1 3.0 Hz, C(5')*H*), 6.55 (1H, dd, *J* 8.8 4.7 Hz, C(8')*H*), 3.26 (2H, t, *J* 7.3 Hz, C(3)H_2), 3.21 (2H, t, *J* 5.6 Hz, C(3')H_2), 2.73-2.64 (4H, m, C(4')H_2 & C(1)H_2), 1.94-1.84 (2H, m, C(2')H_2), 1.72 (2H, tt, *J* 7.3 7.3 Hz, C(2)H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 156.0 (d, *J* 232.7 Hz, *C*(6)F), 143.2 (*C*(10')), 125.4 (d, *J* 6.5 Hz, *C*(9')), 116.2 (d, *J* 22.0 Hz, *C*(5')), 113.8 (d, *J* 21.9 Hz, *C*(7')), 112.9 (d, *J* 7.4 Hz, *C*(8')), 50.6 (*C*(3)H_2), 50.4 (*C*(2')H_2), 40.6 (*C*(1)H_2), 30.2 (*C*(2)H_2), 29.2 (*C*(4')H_2), 23.3 (*C*(3')H_2); ^{19}F NMR (470 MHz; CD_3OD): δ –132.20 - –132.33 (1F, m, C(6)*F*); LRMS *m/z* (ES+) 209 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 209.1440. $C_{12}H_{18}FN_2^+$ requires 209.1449].

3-(7-Nitro-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (200)



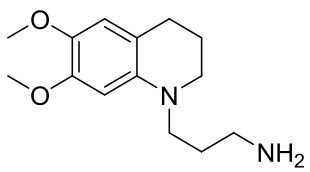
Compound **191** [2-[3-(7-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (114 mg, 0.312 mmol, 1.0 eq) was reacted according to general procedure 7 to give **200** (70 mg, 0.30 mmol, 95%) as a red oil that was used without further purification. R_f 0.00 (1:2 EtOAc:Petrol); ν_{max}/cm^{-1} 2930 (w), 2862 (w), 1513 (s), 1431 (w), 1341 (s); ^{1}H NMR (500 MHz; CD_3OD): δ 7.35 (1H, d, *J* 2.2 Hz, C(8')*H*), 7.31 (1H, dd, *J* 8.1 2.2 Hz, C(7')*H*), 7.05 (1H, d, *J* 8.1 Hz, C(5')*H*), 3.40 (2H, t, *J* 7.4 Hz, C(3)H_2), 3.36 (2H, t, *J* 5.8 Hz, C(2')H_2), 2.83-2.74 (4H, m, C(1)H_2 & C(4')H_2), 1.94 (2H, tt, *J* 6.0 5.8 Hz, C(3')H_2), 1.82 (2H, tt, *J* 7.4 7.4 Hz, C(2)H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 149.1 (*C*(7')), 147.0 (*C*(10')), 131.2 (*C*(9')), 130.4 (*C*(5')H), 111.0 (*C*(7')H), 105.0 (*C*(8')H), 50.1 (*C*(2')H_2), 49.8 (*C*(3)H_2), 40.2 (*C*(1)H_2), 29.6 (*C*(2)H_2), 29.2 (*C*(4')H_2), 22.5 (*C*(3')H_2); LRMS *m/z* (ES+) 236 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 236.1387. $C_{12}H_{18}N_3O_2^+$ requires 236.1394].

3-[7-(Benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propan-1-amine (201)



Compound **165** [7-(benzyloxy)-1,2,3,4-tetrahydroquinoline] (453 mg, 1.89 mmol, 1.0 eq) was reacted according to general procedure 6 to give 2-{3-[7-(benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-1*H*-isoindole-1,3(2*H*)-dione (709 mg) which was used without further purification. The phthalimide (679 mg) was reacted according to general procedure 7. Purification *via* acid base extraction yielded **201** (231 mg, 0.779 mmol, 41%) as a yellow oil. R_f 0.00 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2927 (m), 2856 (w), 1611 (m), 1507 (s), 1307 (m), 1170 (s); ^{1}H NMR (400 MHz; CD_3OD): δ 7.41-7.36 (2H, m, 2 × C(Ar)*H*), 7.36-7.30 (2H, m, 2 × C(Ar)*H*), 7.29-7.24 (1H, m, C(4'')*H*), 6.74 (1H, d, *J* 8.1 Hz, C(5')*H*), 6.18 (1H, d, *J* 2.4 Hz, C(8')*H*), 6.15 (1H, dd, *J* 8.1 2.4 Hz, C(6')*H*), 4.97 (2H, s, OCH_2Ph), 3.24-3.17 (4H, m, C(3)H_2 & C(2')H_2), 2.64-2.57 (4H, m, C(1)H_2 & C(4')H_2), 1.89-1.82 (2H, m, C(3')H_2), 1.65 (2H, tt, *J* 7.3 7.3 Hz, C(2)H_2); ^{13}C NMR (101 MHz; CD_3OD): δ 159.7 (*C*(7')), 147.3 (*C*(10')), 139.3 (*C*(1'')), 130.5 (*C*(5')H), 129.4 (2 × *C*(Ar'')H), 128.6 (*C*(4'')H), 128.4 (2 × *C*(Ar'')H), 116.7 (*C*(9')), 102.6 (*C*(6')H), 99.4 (*C*(8')H), 70.8 (O*C*H_2Ph), 50.4 (*C*(2')H_2), 50.1 (*C*(3)H_2), 40.6 (*C*(1)H_2), 30.4 (*C*(2)H_2), 28.5 (*C*(4')H_2), 23.6 (*C*(3')H_2); LRMS *m/z* (ES+) 297 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 297.1952. $C_{19}H_{25}N_2O^+$ requires 297.1961].

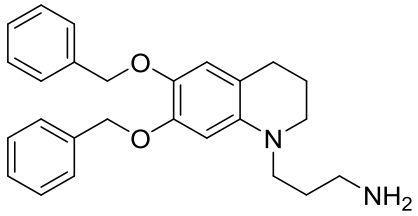
3-(6,7-Dimethoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (202)



Compound **192** [2-[3-(6,7-dimethoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (433 mg, 1.14 mmol, 1.0 eq) was reacted according to general procedure 7 to

afford **202** (254 mg, 1.02 mmol, 89%) as a brown oil which was used without further purification. R_f 0.00 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2933 (m), 2837 (w), 1517 (s), 1268 (m), 1239 (m), 1223 (m), 1201 (m); ^{1}H NMR (500 MHz; CD_3OD): δ 6.56 (1H, s, C(5')*H*), 6.30 (1H, s, C(8')*H*), 3.78 (3H, s, C(Ar')OCH_3), 3.71 (3H, s, C(Ar')OCH_3), 3.29 (2H, t, *J* 7.4 Hz, C(3)H_2), 3.18 (2H, t, *J* 5.6 Hz, C(2')H_2), 2.81 (2H, t, *J* 7.4 Hz, C(1)H_2), 2.65 (2H, t, *J* 6.4 Hz, C(4')H_2), 1.93-1.87 (2H, m, C(3')H_2), 1.81 (2H, tt, *J* 7.4 7.4 Hz, C(2)H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 149.8 (*C*(Ar')OCH_3), 141.8 (*C*(Ar')OCH_3), 141.6 (*C*(10')), 116.6 (*C*(5')H), 116.5 (*C*(9')), 99.7 (*C*(8')H), 57.8 (C(Ar')O*C*H_3), 56.8 (C(Ar')O*C*H_3), 50.7 (*C*(3)H_2), 50.4 (*C*(2')H_2), 40.1 (*C*(1)H_2), 28.9 (*C*(2)H_2), 28.5 (*C*(4')H_2), 23.6 (*C*(3')H_2); LRMS *m/z* (ES+) 251 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 251.1754. $C_{14}H_{23}N_2O_2^+$ requires 251.1754].

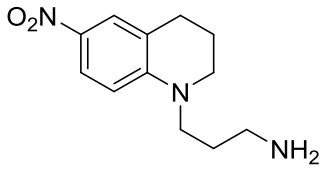
3-[6,7-Bis(benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propan-1-amine (203)



Compound **193** [2-{3-[6,7-bis(benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-1*H*-isoindole-1,3(2*H*)-dione] (290 mg, 0.544 mmol, 1.0 eq) was reacted according to general procedure 7 to give **203** (186 mg, 0.462 mmol, 85%) as a brown oil that was used without further purification. R_f 0.00 (1:4 EtOAc:Petrol); ν_{max}/cm^{-1} 2930 (m), 2860 (m), 1513 (s), 1271 (m), 1198 (m); ^{1}H NMR (500 MHz; CD_3OD): δ 7.45-7.38 (4H, m, 4 × C(Ar'')*H*), 7.37-7.25 (6H, m, 6 × C(Ar'')*H*), 6.63 (1H, s, C(5')*H*), 6.28 (1H, s, C(8')*H*), 5.08 (2H, s, OCH_2Ph), 4.95 (2H, s, OCH_2Ph), 3.19 (2H, t, *J* 7.3 Hz, C(3)H_2), 3.15 (2H, t, *J* 5.7 Hz, C(2')H_2), 2.71 (2H, t, *J* 7.4 Hz, C(1)H_2), 2.60 (2H, t, *J* 6.2 Hz, C(4')H_2), 1.87 (2H, tt, *J* 6.2 5.7 Hz, C(3')H_2), 1.69 (2H, tt, *J* 7.4 7.3 Hz, C(2)H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 149.7 (*C*(Ar')OCH_2Ph), 142.2 (*C*(10')), 141.0 (*C*(Ar')OCH_2Ph), 139.3 (2 × *C*(Ar'')), 129 5 (2 × *C*(Ar'')H), 129.3 (2 ×

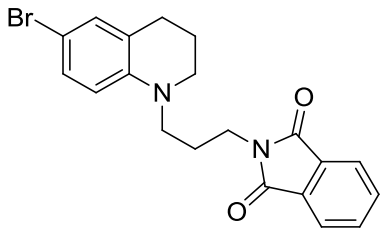
C(Ar'')H), 129.0 (2 × *C*(Ar'')H), 128,8 (2 × *C*(Ar'')H), 128.6 (2 × *C*(Ar'')H), 120.2 (*C*(5')H), 117.2 (*C*(9')), 102.2 (*C*(8')H), 74.4 (O*C*H_2Ph), 72.8 (O*C*H_2Ph), 50.4 (*C*(3)H_2), 50.3 (*C*(2')H_2), 39.9 (*C*(1)H_2), 28.5-28.4 (*C*(2)H_2 & *C*(4')H_2), 23.6 (*C*(3')H_2); LRMS *m/z* (ES+) 403 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 403.2367. $C_{26}H_{31}N_2O_3^+$ requires 403.2380].

3-(6-Nitro-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (204)



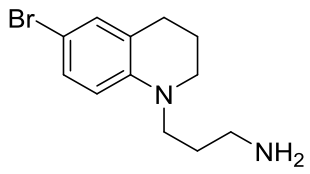
Compound **194** [2-[3-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (270 mg, 0.739 mmol, 1.0 eq) was reacted according to general procedure 7 to give **204** (151 mg, 0.642 mmol, 87%) as a yellow oil that was used without further purification. R_f 0.00 (1:3 EtOAc:Petrol); ν_{max}/cm^{-1} 2934 (w), 2864 (w), 1601 (m), 1519 (w), 1284 (s), 1176 (m); ^{1}H NMR (500 MHz; CD_3OD): δ 7.90 (1H, d, *J* 9.3 Hz, C(7')*H*), 7.78 (1H, s, C(5')*H*), 6.63 (1H, d, *J* 9.3 Hz, C(8')*H*), 3.47-3.41 (4H, m, C(3)H_2 & C(2')H_2), 2.76 (2H, t, *J* 6.1 Hz, C(4')H_2), 2.72 (2H, t, *J* 7.3 Hz, C(1)H_2), 1.94 (2H, tt, *J* 6.1 6.0 Hz, C(3')H_2), 1.79 (2H, tt, *J* 7.4 7.3 Hz, C(2)H_2); ^{13}C NMR (126 MHz; CD_3OD): δ 152.0 (*C*(10')), 136.9 (*C*(6')), 125.9 (*C*(5')H), 125.6 (*C*(7')H), 123.0 (*C*(9')), 110.1 (*C*(8')H), 50.8 (*C*(2')H_2), 50.1 (*C*(3)H_2), 40.2 (*C*(1)H_2), 30.4 (*C*(2)H_2), 29.0 (*C*(4')H_2), 22.4 (*C*(3')H_2); LRMS *m/z* (ES+) 236 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 236.1395. $C_{12}H_{18}N_3O_2^+$ requires 236.1394].

2-[3-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione (205)



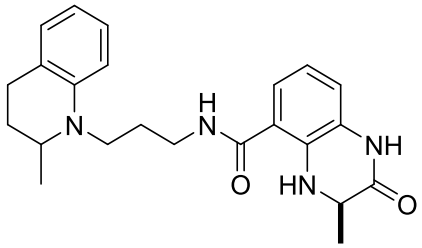
To a solution of **183** [2-[3-(3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (1.02 g, 3.17 mmol, 1.0 eq) in MeCN (16 mL) was added *N*-bromosuccinimide (677 mg, 3.80 mmol, 1.2 eq) causing a deep brown colour to immediately develop. The reaction mixture was stirred at rt for 3 h, then diluted with EtOAc (250 mL), washed with 10% $Na_2S_2O_3$ (250 mL), 2 M K_2CO_3 (3 × 250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 3 to 24% EtOAc in petroleum ether) followed by crystallisation from EtOH yielded **205** (554 mg, 1.39 mmol, 44%) as a yellow solid. R_f 0.23 (1:1 CH_2Cl_2:Petrol); mp 109-112 °C (from $CHCl_3$); ν_{max}/cm^{-1} 2936 (w), 1706 (s), 1497 (m), 1395 (m), 1340 (w); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.85 (2H, dd, *J* 5.5 3.1 Hz, C(4)*H* & C(7)*H*), 7.72 (2H, dd, *J* 5.5 3.1 Hz, C(5)*H* & C(6)*H*), 7.07 (1H, dd, *J* 8.8 2.4 Hz, C(7'')*H*), 7.00 (1H, d, *J* 2.4 Hz, C(5'')*H*), 6.40 (1H, d, *J* 8.8 Hz, C(8'')*H*), 3.74 (2H, t, *J* 7.3 Hz, C(1')H_2), 3.30 (2H, t, *J* 7.4 Hz, C(3')H_2), 3.25 (2H, t, *J* 5.8 Hz, C(2'')H_2), 2.69 (2H, t, *J* 6.2 Hz, C(4'')H_2), 1.98 (2H, tt, *J* 7.4 7.3 Hz, C(2')H_2), 1.92 (2H, tt, *J* 6.2 5.8 Hz, C(3'')H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.4 (*C*(1)=O & *C*(3)=O), 144.1 (*C*(10'')), 134.1 (*C*(5)H & *C*(6)H), 132.1 (*C*(8) & *C*(9)), 131.6 (*C*(5'')H), 129.7 (*C*(7'')H), 124.8 (*C*(9'')), 123.4 (*C*(4)H & *C*(7)), 112.2 (*C*(8'')H), 107.4 (*C*(6'')), 49.2 (*C*(2'')H_2), 48.9 (*C*(3')H_2), 36.1 (*C*(1')H_2), 28.0 (*C*(4'')H_2), 25.4 (*C*(2')H_2), 22.0 (*C*(3'')H_2); LRMS *m/z* (ES+) 399 ([M(^{79}Br)+H]$^+$, 100%), 401 ([M(^{81}Br)+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (^{79}M+Na)$^+$ 421.0516. $C_{20}H_{19}{}^{79}BrN_2NaO_2^+$ requires 421.0522], [Found: (^{81}M+Na)$^+$ 423.0498. $C_{20}H_{19}{}^{81}BrN_2NaO_2^+$ requires 423.0503].

3-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (205)



Compound **205** [2-[3-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1*H*-isoindole-1,3(2*H*)-dione] (237 mg, 0.604 mmol, 1.0 eq) was reacted according to general procedure 7. Purification *via* acid-base extraction yielded **206** (57 mg, 0.21 mmol, 35%) as a pale yellow oil which was used without further characterisation.

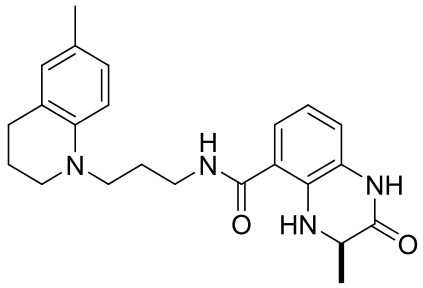
(3*R*)-*N*-[3-(2-Methyl-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-207]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **195** [3-(2-methyl-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (44 mg, 0.21 mmol, 1.1 eq) were reacted according to general procedure 1 for 15 h. Purification *via* silica gel chromatography (gradient elution 8 to 100% EtOAc in petroleum ether) gave (*R*)-**207** (30 mg, 0.076 mmol, 39%) as a yellow oil. R_f 0.33 (1:2 $(CH_3)_2CO$:Petrol); $[\alpha]_D$ −37.1 (*c* 1.0 in $(CH_3)_2CO$); ^{1}H NMR (400 MHz; $(CD_3)_2CO$): δ 9.37 (1H, br s, N*H*), 8.00 (1H, br s, N*H*), 7.78 (1H, br s, C(1')N*H*), 7.29 (1H, d, *J* 7.9 Hz, C(6)*H*), 6.98-6.91 (2H, m, C(8)*H* & C(7'')*H*), 6.89 (1H, d, *J* 7.3 Hz, C(5'')*H*), 6.64 (1H, dd, *J* 7.9 7.8 Hz, C(7)*H*), 6.59 (1H, d, *J* 8.3 Hz, C(8'')*H*), 6.46 (1H, dd, *J* 7.3 7.3 Hz, C(6'')*H*), 4.01 (1H, q, *J* 6.7 Hz, C(3)*H*), 3.60-3.52 (1H, m, C(2'')*H*), 3.52-3.39 (3H, m, C(1')H_2 & C(3')H_{AB}), 3.30 (1H, ddd, *J* 15.3 8.1 6.9 Hz, C(3')H_{AB}), 2.79 (1H, ddd, *J* 16.1 12.1 5.4 Hz, C(4'')H_{AB}), 2.66-2.57 (1H, m, C(4'')H_{AB}),

2.00-1.87 (2H, m, C(2')H_2), 1.87-1.79 (1H, m, C(3'')H_{AB}), 1.78-1.70 (1H, m, C(3'')H_{AB}), 1.38 (3H, d, *J* 6.7 Hz, C(3)CH_3), 1.11 (3H, d, *J* 6.4 Hz, C(2'')CH_3); ^{13}C NMR (101 MHz; $(CD_3)_2CO$): δ 169.5 (C(5)*C*=O), 168.8 (*C*(2)=O), 145.1 (*C*(10'')), 137.3 (*C*(9)), 129.7 (*C*(5'')H), 128.7 (*C*(10)), 127.8 (*C*(7'')H), 122.5 (*C*(9'')), 122.3 (*C*(6)H), 117.9 (*C*(8)H), 117.5 (*C*(7)H), 116.9 (*C*(5)), 115.8 (*C*(6'')H), 111.5 (*C*(8'')H), 53.2 (*C*(2'')H), 51.7 (*C*(3)H), 47.9-47.8 (*C*(3')H_2), 38.2 (*C*(1')H_2), 28.7 (*C*(3'')H_2), 28.3 (*C*(2')H_2), 24.4 (*C*(4'')H_2), 19.0-18.9 (C(2'')*C*H_3), 18.7 (C(3)*C*H_3); LRMS *m/z* (ES+) 393 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 415.2103. $C_{23}H_{28}N_4NaO_2^+$ requires 415.2104]; HPLC (C-18) retention time = 9.65 min, 94.3%.

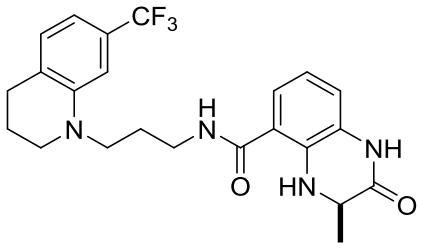
(3*R*)-*N*-[3-(6-methyl-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-208]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **196** [3-(6-methyl-3,4-dihydroquinolin-1(2H)-yl)propan-1-amine] (44 mg, 0.21 mmol, 1.1 eq) were reacted according to general procedure 1 for 15 h. Purification *via* silica gel chromatography (gradient elution 6 to 50% EtOAc in petroleum ether) gave (*R*)-**208** (56 mg, 0.14 mmol, 74%) as a brown solid. R_f 0.53 (EtOAc); mp 206-209 °C (from EtOAc); ν_{max}/cm^{-1} 3372 (br), 2932 (w), 1682 (s), 1510 (s), 1376 (m), 1287 (m); $[\alpha]_D$ −35.4 (*c* 0.3 in $(CH_3)_2CO$); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 10.36 (1H, br s, N*H*), 8.41 (1H, br t, *J* 5.5 Hz, C(1')N*H*), 7.79 (1H, br s, N*H*), 7.28 (1H, dd, *J* 7.9 0.7 Hz, C(6)*H*), 6.85 (1H, dd, *J* 7.7 0.7 Hz, C(8)*H*), 6.72 (1H, d, *J* 8.3 Hz, C(7'')*H*), 6.67 (1H, s, C(5'')*H*), 6.64 (1H, dd, *J* 7.9 7.7 Hz, C(7)*H*), 6.46 (1H, d, *J* 8.3 Hz, C(8'')*H*), 3.93 (1H, qd, *J* 6.7 1.3 Hz, C(3)*H*), 3.30-3.21

(4H, m, C(1')H_2 & C(3')H_2), 3.18 (2H, t, *J* 5.8 Hz, C(2'')H_2), 2.62 (2H, t, *J* 6.1 Hz, C(4'')H_2), 2.10 (3H, s, C(6'')CH_3), 1.82 (2H, tt, *J* 6.1 5.8 Hz, C(3'')H_2), 1.75 (2H, tt, *J* 7.2 7.2 Hz, C(2')H_2), 1.26 (3H, d, *J* 6.7 Hz, C(3)H_3); ^{13}C NMR (126 MHz; $(CD_3)_2SO$): δ 168.1 (C(5)*C*=O), 167.8 (*C*(2)=O), 142.8 (*C*(10'')), 135.7 (*C*(9)), 129.5 (*C*(5'')H), 127.4 (*C*(10)), 127.2 (*C*(7'')H), 123.4 (*C*(6'')), 121.8 (*C*(9'')), 121.7 (*C*(6)H), 116.9 (*C*(8)H), 116.4 (*C*(7)H), 115.7 (*C*(5)), 110.6 (*C*(8'')H), 50.1 (*C*(3)H), 48.7 (*C*(2'')H_2), 48.5 (*C*(3')H_2), 37.0 (*C*(1')H_2), 27.5 (*C*(4'')H_2), 25.6 (*C*(2')H_2), 21.9 (*C*(3'')H_2), 19.9 (C(6'')*C*H_3), 18.2 (C(3)*C*H_3); LRMS *m/z* (ES+) 393 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 415.2093. $C_{23}H_{28}N_4NaO_2^+$ requires 415.2104]; HPLC (C-18) retention time = 9.46 min, 96.8%; (AD-H) retention time = 12.59 min, >98%.

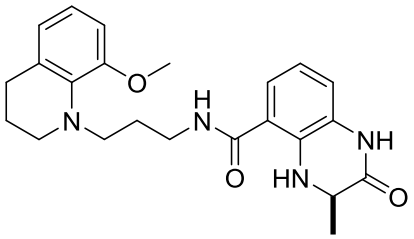
(3*R*)-*N*-{3-[7-(Trifluoromethyl)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-209]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **197** [3-[7-(trifluoromethyl)-3,4-dihydroquinolin-1(2*H*)-yl]propan-1-amine] (55 mg, 0.21 mmol, 1.1 eq) were reacted according to general procedure 1 for 15 h. Purification *via* silica gel chromatography (gradient elution 6 to 50% $(CH_3)_2CO$ in petroleum ether) gave (*R*)-**209** (47 mg, 0.11 mmol, 54%) as a colourless solid. R_f 0.21 (1:3 $(CH_3)_2CO$:Petrol); mp 165-167 °C (from $(CH_3)_2CO$); ν_{max}/cm^{-1} 3331 (br), 2936 (w), 1684 (s), 1520 (m), 1442 (m), 1318 (s); $[\alpha]_D$ −40.2 (*c* 0.5 in $(CH_3)_2CO$); ^{1}H NMR (500 MHz; $(CD_3)_2CO$): δ 9.35 (1H, br s, N*H*), 8.02 (1H, br s, N*H*), 7.83 (1H, br s, C(1')N*H*), 7.31 (1H, dd, *J* 7.9 1.0 Hz, C(6)*H*), 7.04 (1H, d, *J* 7.6 Hz, C(5'')*H*), 6.96 (1H, d, *J* 7.5 Hz, C(8)*H*), 6.82 (1H,

s, C(8'')*H*), 6.74 (1H, d, *J* 7.6 Hz, C(6'')*H*), 6.64 (1H, dd, *J* 7.9, 7.7, C(7)*H*), 4.01 (1H, qd, *J* 6.7 1.4 Hz, C(3)*H*), 3.52-3.43 (4H, m, C(1')H_2 & C(3')H_2), 3.38 (2H, t, *J* 5.7 Hz, C(2'')H_2), 2.77 (2H, t, *J* 6.2 Hz, C(4'')H_2), 1.98-1.90 (4H, m, C(2')H_2 & C(3'')H_2), 1.38 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.5 (C(5)*C*=O), 168.7 (*C*(2)=O), 146.4 (*C*(10'')), 137.4 (*C*(9)), 130.2 (*C*(5'')H), 129.7 (q, *J* 31.1 Hz, *C*(7'')), 128.3 (*C*(9'')), 126.9 (*C*(10)), 125.9 (q, *J* 271.(7'')*C*F_3), 122.3 (*C*(6)H), 117.9 (*C*(8)H), 117.4 (*C*(7)H), 116.8 (*C*(5)), 112.1 (q, *J* 4.0 Hz, *C*(6'')H), 107.0 (q, *J* 3.9 Hz, *C*(8'')H), 51.6 (*C*(3)H), 49.9 (*C*(2'')H_2), 49.5 (*C*(3')H_2), 38.1 (*C*(1')H_2), 28.7 (*C*(4'')H_2), 27.1 (*C*(2')H_2), 22.4 (*C*(3'')H_2), 18.7 (C(3)*C*H_3); ^{19}F NMR (470 MHz; $(CD_3)_2CO$): δ –62.85 (3F, s, C(7'')*C*F_3); LRMS *m/z* (ES+) 447 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 469.1819. $C_{23}H_{25}F_3N_4NaO_2^+$ requires 469.1822]; HPLC (C-18) retention time = 13.19 min, 98.7%; (AD-H) retention time = 11.65 min, >98%.

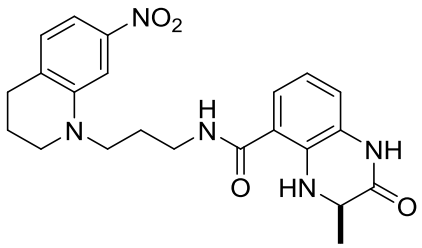
(3*R*)-*N*-[3-(8-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-210]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **198** [3-(8-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (47 mg, 0.21 mmol, 1.1 eq) were reacted according to general procedure 1 for 17 h. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) then azeotroped with $(CH_3)_2CO$ to give (*R*)-**210** (39 mg, 0.095 mmol, 49%) as a colourless solid. R_f 0.56 (1:1 EtOAc:Petrol); mp 124-126 °C (from $(CH_3)_2CO$); ν_{max}/cm^{-1} 3296 (br), 2933 (w), 1682 (s), 1476 (m), 1287 (m) 1252 (m); $[\alpha]_D$ −39.4 (*c* 1.0 in $(CH_3)_2CO$); ^{1}H NMR (500 MHz; $(CD_3)_2CO$): δ 9.36 (1H, br s, N*H*), 8.10 (1H, br s, N*H*), 8.04 (1H, br s,

C(1')N*H*), 7.19 (1H, dd, *J* 8.1 1.0 Hz, C(6)*H*), 6.93 (1H, d, *J* 7.6 Hz, C(8)*H*), 6.75 (1H, dd, *J* 7.7 7.7 Hz, C(6'')*H*), 6.73-6.67 (1H, m, C(7'')*H*), 6.62-6.55 (2H, m, C(7)*H* & C(5'')*H*), 4.00 (1H, qd, *J* 6.6 1.4 Hz, C(3)*H*), 3.75 (3H, s, C(8'')OCH_3), 3.55 (2H, td, *J* 6.5 6.3 Hz, C(1')H_2), 3.12 (2H, t, *J* 6.7 Hz, C(3')H_2), 3.11-3.07 (2H, m, C(2'')H_2), 2.71 (2H, t, *J* 6.5 Hz, C(4'')H_2), 1.90 (2H, tt, *J* 6.7 6.5 Hz, C(2')H_2), 1.80-1.73 (2H, m, C(3'')H_2), 1.38 (3H, d, *J* 6.6 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.0 (C(5)*C*=O), 168.8 (*C*(2)=O), 152.9 (*C*(8'')), 138.9 (*C*(10'')), 137.4 (*C*(9)), 129.6 (*C*(9'')), 128.8 (*C*(10)), 122.7 (*C*(5'')H), 122.0 (*C*(6)H), 121.4 (*C*(6'')H), 117.7 (*C*(8)H), 117.5 (*C*(7)H), 117.1 (*C*(5)), 110.1 (*C*(7'')H), 55.6 (C(8'')O*C*H_3), 53.7 (*C*(3')H_2), 51.7 (*C*(3)H), 48.4 (*C*(2'')H_2), 39.1 (*C*(1')H_2), 28.9 (*C*(2')H_2), 28.6 (*C*(4'')H_2), 19.0 (*C*(3'')H_2), 18.7 (C(3)*C*H_3); LRMS *m/z* (ES+) 409 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 409.2215. $C_{23}H_{29}N_4O_3^+$ requires 409.2234]; HPLC (C-18) unstable; (AD-H) retention time = 13.59 min, >98%.

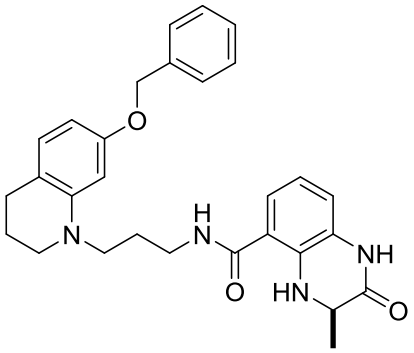
(3*R*)-*N*-[3-(7-Nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-212]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **200** [3-(7-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (53 mg, 0.23 mmol, 1.1 eq) were reacted according to general procedure 1 for 15 h. Purification *via* silica gel chromatography (gradient elution 8 to 66% $(CH_3)_2CO$ in petroleum ether) gave (*R*)-**212** (60 mg, 0.14 mmol, 73%) as an orange oil. R_f 0.23 (1:2 $(CH_3)_2CO$:Petrol); ν_{max}/cm^{-1} 3321 (br), 2935 (w), 1682 (s), 1515 (s), 1342 (s), 1288 (m); $[\alpha]_D$ −39.5 (*c* 1.0 in $(CH_3)_2CO$); ^{1}H NMR (500 MHz; $(CD_3)_2CO$): δ 9.37 (1H, br s, N*H*), 8.00 (1H, br s, N*H*), 7.84 (1H, br s,

C(1')N*H*), 7.37 (1H, d, *J* 2.2 Hz, C(8'')*H*), 7.35-7.29 (2H, m, C(6)*H* & C(6'')*H*), 7.09 (1H, d, *J* 8.2 Hz, C(5'')*H*), 6.96 (1H, dd, *J* 7.7 0.4 Hz, C(8)*H*), 6.64 (1H, dd, *J* 7.8 7.7 Hz, C(7)*H*), 4.01 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.54-3.46 (4H, m, C(1')H_2 & C(3')H_2), 3.41 (2H, t, *J* 5.7 Hz, C(2'')H_2), 2.81 (2H, t, *J* 6.3 Hz, C(4'')H_2), 1.98 (2H, tt, *J* 7.2 7.2 Hz, C(2')H_2), 1.96-1.91 (2H, m, C(3'')H_2), 1.38 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.6 (C(5)*C*=O), 168.7 (*C*(2)=O), 148.7 (*C*(7'')), 146.7 (*C*(10'')), 137.4 (*C*(9)), 130.7 (*C*(9'')), 130.1 (*C*(5'')H), 128.7 (*C*(10)), 122.3 (*C*(6)H), 117.9 (*C*(8)H), 117.5 (*C*(7)H), 116.8 (*C*(5)), 110.4 (*C*(6'')H), 104.6 (*C*(8'')H), 51.6 (*C*(3)H), 49.6 (*C*(2'')H_2), 49.5 (*C*(3')H_2), 38.0 (*C*(1')H_2), 28.8 (*C*(4'')H_2), 26.8 (*C*(2')H_2), 22.0 (*C*(3'')H_2), 18.6 (C(3)*C*H_3); LRMS *m/z* (ES+) 424 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 446.1797. $C_{22}H_{25}N_5NaO_4^+$ requires 446.1799]; HPLC (C-18) retention time = 12.34 min, 95.5.

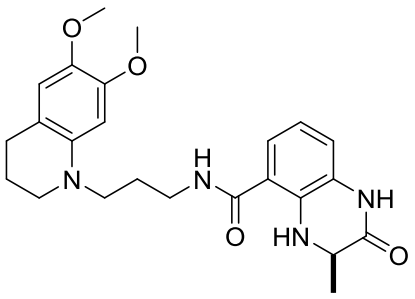
(3*R*)-*N*-{3-[7-(Benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-213]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (80 mg, 0.39 mmol, 1.0 eq) and **201** [3-[7-(benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propan-1-amine] (126 mg, 0.426 mmol, 1.1 eq) were reacted according to general procedure 1 for 16 h. Purification *via* silica gel chromatography (gradient elution 8 to 100% $(CH_3)_2CO$ in petroleum ether) gave (*R*)-**213** (102 mg, 0.212 mmol, 55%) as a colourless oil. R_f 0.10 (1:3 $(CH_3)_2CO$:Petrol); ν_{max}/cm^{-1} 3330 (w), 2932 (w), 1683 (s), 1609 (m), 1508 (m), 1288 (m);

$[\alpha]_D$ −33.4 (*c* 1.0 in $(CH_3)_2CO$); ^{1}H NMR (400 MHz; $(CD_3)_2CO$): δ 9.30 (1H, br s, N*H*), 8.03 (1H, br s, N*H*), 7.80 (1H, br s, C(1')N*H*), 7.42-7.38 (2H, m, C(Ar''')*H*), 7.38-7.32 (2H, m, C(Ar''')*H*), 7.32-7.25 (2H, m, C(6)*H* & C(4''')*H*), 6.93 (1H, dd, *J* 7.8 0.7 Hz, C(8)*H*), 6.76 (1H, d, *J* 8.1 Hz, C(5'')*H*), 6.60 (1H, dd, *J* 7.8 7.8 Hz, C(7)*H*), 6.25 (1H, d, *J* 2.4 Hz, C(8'')*H*), 6.16 (1H, dd, *J* 8.1 2.4 Hz, C(6'')*H*), 4.99 (2H, s, OCH_2Ph), 3.97 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.49-3.42 (2H, m, C(1')H_2), 3.36 (2H, t, *J* 7.5 Hz, C(3')H_2), 3.29 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.63 (2H, t, *J* 6.3 Hz, C(4'')H_2), 1.94-1.84 (4H, m, C(2')H_2 & C(3'')H_2), 1.35 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.5 (C(5)*C*=O), 168.7 (*C*(2)=O), 159.4 (*C*(7'')), 146.9 (*C*(10'')), 139.0 (*C*(1''')), 137.3 (*C*(9)), 130.2 (*C*(5'')), 129.1 (*C*(Ar''')H & *C*(Ar''')H), 128.7 (*C*(10)), 128.33 (*C*(4''')H), 128.29 (*C*(Ar''')H & *C*(Ar''')H), 122.3 (*C*(6)), 117.9 (*C*(8)), 117.4 (*C*(7)), 116.8 (*C*(5)), 115.9 (*C*(9'')), 102.3 (*C*(6'')), 98.7 (*C*(8'')), 70.1 (O*C*H_2Ph), 51.6 (*C*(3)), 49.9 (*C*(2'')), 49.8 (*C*(3')), 38.2 (*C*(1')), 28.1 (*C*(4'')), 27.0 (*C*(2')), 23.2 (*C*(3'')), 18.7 (*C*(3)CH_3); LRMS *m/z* (ES+) 485 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 507.2371. $C_{29}H_{32}N_4NaO_3^+$ requires 507.2367]; HPLC (C-18) retention time = 11.95 min, 97.7%; (AD-H) retention time = 17.29 min, >98%.

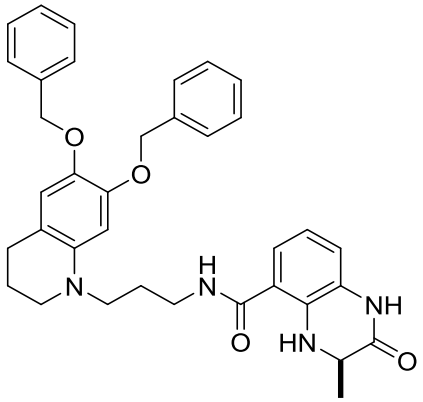
(3*R*)-*N*-[3-(6,7-dimethoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-214]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **202** [3-(6,7-dimethoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (53 mg, 0.21 mmol, 1.1 eq) were reacted according to general procedure 1 for 15 h.

Purification *via* silica gel chromatography (gradient elution 8 to 66% $(CH_3)_2CO$ in petroleum ether) gave (*R*)-**214** (48 mg, 0.11 mmol, 56%) as a yellow oil. R_f 0.55 (1:1 $(CH_3)_2CO$:Petrol); ν_{max}/cm^{-1} 3295 (br), 2933 (w), 1683 (s), 1518 (s), 1285 (m), 1268 (m); $[\alpha]_D$ −33.5 (*c* 1.0 in $(CH_3)_2CO$); ^1H NMR (500 MHz; $(CD_3)_2SO$): δ 10.36 (1H, br s, N*H*), 8.45 (1H, br t, *J* 5.9 Hz, C(1')N*H*), 7.84 (1H, br s, N*H*), 7.30 (1H, d, *J* 7.9 Hz, C(6)*H*), 6.85 (1H, d, *J* 7.7 Hz, C(8)*H*), 6.63 (1H, dd, *J* 7.9 7.7 Hz, C(7)*H*), 6.52 (1H, s, C(5'')*H*), 6.19 (1H, s, C(8'')*H*), 3.93 (1H, q, *J* 6.6 Hz, C(3)*H*), 3.60 (3H, s, C(Ar'')OCH_3), 3.59 (3H, s, C(Ar'')OCH_3), 3.29 (2H, td, *J* 6.7 7 5.9 Hz, C(1')H_2), 3.24 (2H, t, *J* 7.2 Hz, C(3')H_2), 3.14 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.58 (2H, t, *J* 6.0 Hz, C(4'')H_2), 1.82 (2H, tt, *J* 6.0 5.6 Hz, C(3'')H_2), 1.76 (2H, tt, *J* 7.2 6.7 Hz, C(2')H_2), 1.26 (2H, d, *J* 6.6 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2SO$): δ 168.2 (C(5)*C*=O), 167.8 (*C*(2)=O), 148.1 (*C*(Ar'')OCH_3), 139.8 (*C*(Ar'')OCH_3), 139.6 (*C*(10'')), 135.7 (*C*(9)), 127.4 (*C*(10)), 121.7 (*C*(6)H), 116.9 (*C*(8)H), 116.3 (*C*(7)H), 115.5 (*C*(5)), 115.2 (*C*(5'')H), 113.6 (*C*(9'')), 97.7 (*C*(8'')H), 56.6 (C(Ar'')O*C*H_3), 55.5 (C(Ar'')O*C*H_3), 50.1 (*C*(3)H), 49.0 (*C*(3')H_2), 48.6 (*C*(2')H_2), 37.0 (*C*(1')H_2), 27.0 (*C*(4'')H_2), 25.7 (*C*(2')H_2), 22.1 (*C*(3'')H_2), 18.2 (C(3)*C*H_3); LRMS *m/z* (ES+) 439 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 439.2319. $C_{24}H_{31}N_4O_4^+$ requires 439.2340]; HPLC (C-18) unstable; (AD-H) retention time = 17.67 min, >98%.

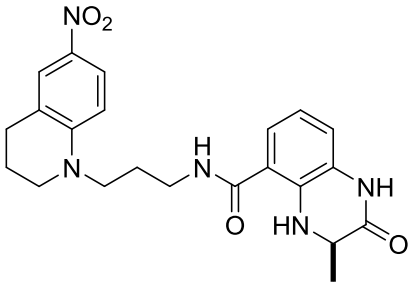
(3*R*)-*N*-{3-[6,7-Bis(benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-215]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (60 mg, 0.29 mmol, 1.0 eq) and **203** [3-[6,7-bis(benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propan-1-amine] (115 mg, 0.32 mmol, 1.1 eq) were reacted according to general procedure 1 for 17 h. Purification *via* silica gel chromatography (gradient elution 6 to 50% $(CH_3)_2CO$ in petroleum ether) gave (*R*)-**215** (115 mg, 195 mmol, 67%) as a colourless oil. R_f 0.16 (1:3 $(CH_3)_2CO$:Petrol); ν_{max}/cm^{-1} 3311 (br), 2931 (w), 1683 (s), 1516 (s), 1285 (m), 1269 (m); $[\alpha]_D$ −34.1 (*c* 1.0 in $(CH_3)_2CO$); ^1H NMR (400 MHz; $(CD_3)_2CO$): δ 9.37 (1H, br s, N*H*), 8.05 (1H, br s, N*H*), 7.81 (1H, br s, C(1')N*H*), 7.49-7.43 (2H, m, 2 × C(Ar''')*H*), 7.43-7.38 (2H, m, 2 × C(Ar''')*H*), 7.37-7.23 (7H, m, C(6)*H* & 6 × C(Ar''')*H*), 6.93 (1H, dd, *J* 7.7 0.6 Hz, C(8)*H*), 6.66 (1H, s, C(5'')*H*), 6.61 (1H, dd, *J* 7.8 7.7 Hz, C(7)*H*), 6.38 (1H, s, C(8'')*H*), 5.04 (2H, s, OCH_2Ph), 4.98 (2H, s, OCH_2Ph), 3.95 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.47-3.40 (2H, m, C(1')H_2), 3.35-3.28 (2H, m, C(3')H_2), 3.20 (2H, t, *J* 5.5 Hz, C(2'')H_2), 2.61 (2H, t, *J* 6.4 Hz, C(4'')H_2), 1.90-1.80 (4H, m, C(2')H_2 & C(3'')H_2), 1.34 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.5 (C(5)*C*=O), 168.6 (*C*(2)=O), 149.6 (*C*(Ar'')OCH$_2$Ph), 141.8 (*C*(10'')), 140.6 (*C*(Ar'')OCH$_2$Ph), 139.5 (*C*(Ar''')), 139.0 (*C*(Ar''')), 137.4 (*C*(9)), 129.1 (2 × *C*(Ar''')H), 129.0 (2 × *C*(Ar''')H), 128.8 (*C*(10)), 128.5 (2 × *C*(Ar''')H), 128.4 (3 × *C*(Ar''')H), 128.3 (*C*(Ar''')H), 122.2 (*C*(6)H), 119.8 (*C*(5'')H), 117.9 (*C*(8)H), 117.5 (*C*(7)H),

116.7 (*C*(5)), 115.8 (*C*(9'')), 101.1 (*C*(8'')H), 73.5 (O*C*H$_2$Ph), 71.8 (O*C*H$_2$Ph), 51.6 (*C*(3)H), 50.4 (*C*(3')H$_2$), 49.9 (*C*(2'')H$_2$), 38.3 (*C*(1')H$_2$), 28.1 (*C*(4'')H$_2$), 26.9 (*C*(2')H$_2$), 23.3 (*C*(3'')H$_2$), 18.7 (C(3)*C*H$_3$); LRMS *m/z* (ES+) 591 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 613.2773. $C_{36}H_{38}N_4NaO_4^+$ requires 613.2785]; HPLC (C-18) retention time = 11.54 min, 97.2%

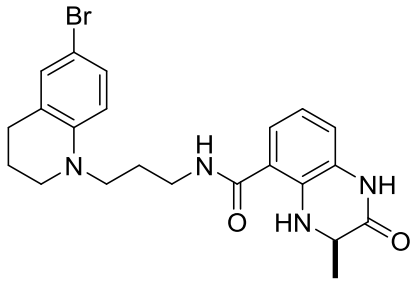
(3*R*)-*N*-[3-(6-Nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-216]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (80 mg, 0.39 mmol, 1.1 eq) and **204** [3-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (100 mg, 0.425 mmol, 1.1 eq) were reacted according to general procedure 1 for 15 h. Purification *via* silica gel chromatography (gradient elution 8 to 66% $(CH_3)_2CO$ in petroleum ether) gave (*R*)-**216** (131 mg, 0.309 mmol, 80%) as a yellow solid. R_f 0.67 (EtOAc); mp 207-209 °C (from $(CH_3)_2CO$); ν_{max}/cm^{-1} 2932 (w), 1695 (m), 1602 (m), 1522 (m), 1378 (w), 1279 (s); $[\alpha]_D$ −29.0 (*c* 0.5 in $(CH_3)_2CO$); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 10.4 (1H, s, N*H*), 8.44 (1H, br t, *J* 6.0 Hz, C(1')N*H*), 7.85 (1H, dd, *J* 9.3 2.8 Hz, C(7'')*H*), 7.80-7.75 (2H, m, N*H* & C(5'')*H*), 7.29 (1H, dd, *J* 8.1 1.1 Hz, C(6)*H*), 6.86 (1H, d, *J* 7.1 Hz, C(8)*H*), 6.70-6.62 (2H, m, C(7)*H* & C(8'')*H*), 3.93 (1H, qd, *J* 6.7 1.4 Hz, C(3)*H*), 3.45 (2H, t, *J* 7.4 Hz, C(3')*H*$_2$), 3.42 (2H, t, *J* 5.6 Hz, C(2'')*H*$_2$), 3.30 (2H, td, *J* 6.5 6.0 Hz, C(1')*H*$_2$), 2.74 (2H, t, *J* 6.1 Hz, C(4'')*H*$_2$), 1.89-1.73 (4H, m, C(2')*H*$_2$ & C(3'')*H*$_2$), 1.26 (3H, d, *J* 6.7 Hz, C(3)C*H*$_3$); ^{13}C NMR (126 MHz; $(CD_3)_2SO$): δ 168.2 (C(5)*C*=O), 167.8 (*C*(2)=O), 150.4 (*C*(10'')), 135.7 (*C*(9)), 134.7 (*C*(6'')),

127.4 (*C*(10)), 124.5 (*C*(5’’)H), 124.3 (*C*(7’’)H), 121.7 (*C*(9’’)), 121.5 (*C*(6)H), 117.0 (*C*(8)H), 116.4 (*C*(7)H), 115.5 (*C*(5)), 109.0 (*C*(8’’)H), 50.1 (*C*(3)H), 49.2 (*C*(2’’)H_2), 48.6 (*C*(3’)H_2), 36.7 (*C*(1’)H_2), 27.2 (*C*(4’’)H_2), 25.9 (*C*(2’)H_2), 20.6 (*C*(3’’)H_2), 18.2 (*C*(3)H_3); LRMS *m/z* (ES+) 446 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 446.1785. $C_{22}H_{25}N_5NaO_4^+$ requires 446.1799]; HPLC (C-18) retention time = 11.60 min, 95.8%.

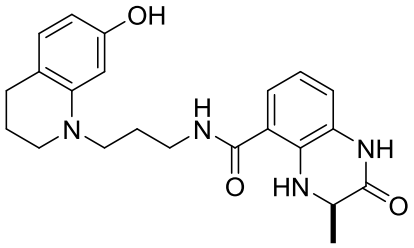
(3*R*)-*N*-[3-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-217]



Compounds (*R*)-**94** [(*R*)-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (40 mg, 0.19 mmol, 1.0 eq) and **206** [3-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (57 mg, 0.21 mmol, 1.1 eq) were reacted according to general procedure 1 for 15 h. Purification *via* silica gel chromatography (gradient elution 2 to 18% $(CH_3)_2CO$ in CH_2Cl_2) gave (*R*)-**217** (22 mg, 0.048 mmol, 25%) as a colourless solid. R_f 0.26 (1:10 $(CH_3)_2CO$:CH_2Cl_2); mp 230-232 °C (from $(CH_3)_2CO$); ν_{max}/cm^{-1} 3324 (br), 2931 (w), 1682 (s), 1535 (m), 1498 (s), 1289 (m); $[\alpha]_D$ −45.6 (*c* 0.2 in $(CH_3)_2CO$); ^{1}H NMR (500 MHz; $(CD_3)_2CO$): δ 9.32 (1H, br s, N*H*), 8.00 (1H, br s, N*H*), 7.79 (1H, br s, C(1’)N*H*), 7.29 (1H, dd, *J* 7.9 1.1 Hz, C(6)*H*), 7.04 (1H, dd, *J* 8.8 2.4 Hz, C(7’’)*H*), 6.99 (1H, d, *J* 2.4 Hz, C(5’’)*H*), 6.96 (1H, d, *J* 7.6 Hz, C(8)*H*), 6.64 (1H, dd, *J* 7.9 7.6 Hz, C(7)*H*)6.56 (1H, d, *J* 8.8 Hz, C(8’’)*H*), 4.01 (1H, qd, *J* 6.7 1.4, C(3)*H*), 3.45 (2H, dt, *J* 6.5 6.5 Hz, C(1’)H_2), 3.38 (2H, t, *J* 7.4 Hz, C(3’)H_2), 3.32 (2H, t, *J* 5.7 Hz, C(2’’)H_2), 2.71 (2H, t, *J* 6.4 Hz, C(4’’)H_2), 1.95-1.86 (4H, m, C(2’)H_2 & C(1’)H_2), 1.38 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.5 (C(5)*C*=O), 168.6

(*C*(2)=O), 145.4 (*C*(10'')), 137.4 (*C*(9)), 132.0 (*C*(5'')H), 130.2 (*C*(7'')H), 128.8 (*C*(10)), 125.6 (*C*(9'')), 122.2 (*C*(6)H), 117.9 (*C*(8)H), 117.5 (*C*(7)H), 116.9 (*C*(5)), 113.2 (*C*(8'')H), 107.1 (*C*(6'')), 51.6 (*C*(3)H), 49.9 (*C*(2'')H_2), 49.6 (*C*(3')H_2), 38.1 (*C*(1')H_2), 28.6 (*C*(4'')H_2), 26.9 (*C*(2')H_2), 22.6 (*C*(3'')H_2), 18.7 (C(3)*C*H_3); LRMS *m/z* (ES+) 457 ([M(^{79}Br)+H]$^+$, 100%), 459 ([M(^{81}Br)+H]$^+$, 88%); HRMS *m/z* (ES+) [Found: (^{79}M+Na)$^+$ 479.1053. $C_{22}H_{25}{}^{79}BrN_2NaO_2^+$ requires 479.1053], [Found: (^{81}M+Na)$^+$ 481.1039. $C_{22}H_{25}{}^{79}BrN_4NaO_2^+$ requires 481.1034]; HPLC (C-18) unstable.

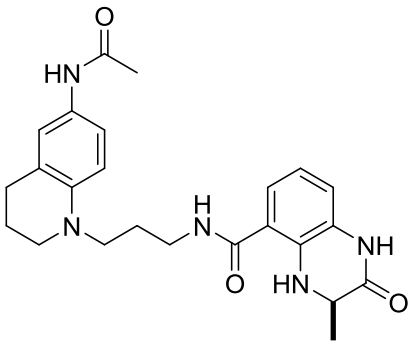
(3*R*)-*N*-[3-(7-hydroxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-218]



Compound (*R*)-**213** [(3*R*)-*N*-{3-[7-(benzyloxy)-3,4-dihydroquinolin-1(2*H*)-yl]propyl}-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide] (100 mg, 0.206 mmol, 1.0 eq) and Pd/C (10% w/w, 11 mg, 0.010 mmol, 0.05 eq) in EtOAc (2 mL) was stirred under a H_2 atmosphere for 48 h. After this time starting material still remained so additional Pd/C (0.05 eq) was added and the reaction mixture was stirred for a further 14 h before being filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 12 to 100% $(CH_3)_2CO$ in petroleum ether) gave **218** (56 mg, 0.14 mmol, 69%) as a colourless oil. R_f 0.62 (EtOAc); ν_{max}/cm^{-1} 3318 (br), 2934 (w), 1679 (s), 1533 (s), 1364 (m), 1261 (m); $[\alpha]_D$ −43.3 (*c* 0.3 in $(CH_3)_2CO$); ^{1}H NMR (500 MHz; $(CD_3)_2CO$): δ 9.38 (1H, br s, N*H*), 8.00 (1H, br s, N*H*), 7.78 (1H, br s, C(1')N*H*), 7.71 (1H, s, C(7'')O*H*), 7.29 (1H, dd, *J* 8.0 0.8 Hz, C(6)*H*), 6.95 (1H, dd, *J* 7.7 0.8 Hz, C(8)*H*), 6.66 (1H, d, *J* 8.0 Hz, C(5'')*H*), 6.63 (1H, dd, *J* 8.0 7.7 Hz, C(7)*H*), 6.14 (1H, d, *J* 2.3 Hz, C(8'')*H*), 6.01 (1H, dd, *J* 8.0 2.3 Hz,

C(6’’)*H*), 4.01 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.46 (2H, td, *J* 6.9 6.6 Hz, C(1’)H_2), 3.32 (2H, t, *J* 7.3 Hz, C(3’)H_2), 3.25 (2H, t, *J* 5.6 Hz, C(2’’)H_2), 2.60 (2H, t, *J* 6.3 Hz, C(4’’)H_2), 1.90 (2H, tt, *J* 7.3 6.9 Hz, C(2’)H_2), 1.89-1.82 (2H, m, C(3’’)H_2), 1.38 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.4 (C(5)*C*=O), 168.8 (*C*(2)=O), 157.5 (*C*(7’’)), 147.0 (*C*(10’’)), 137.3 (*C*(9)), 130.3 (*C*(5’’)H), 128.7 (*C*(10)), 122.3 (*C*(6)H), 117.9 (*C*(8)H), 117.5 (*C*(7)H), 116.9 (*C*(5)), 114.3 (*C*(9’’)), 103.4 (*C*(6’’)H), 98.9 (*C*(8’’)H), 51.6 (*C*(3)H). 50.1 (*C*(2’’)H_2). 49.8 (*C*(3’)H_2). 38.3 (*C*(1’)H_2). 28.1 (*C*(4’’)H_2). 27.2 (*C*(2’)H_2). 23.4 (*C*(3’’)H_2). 18.6 (C(3)*C*H_3); LRMS *m/z* (ES+) 395 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 417.1907. $C_{22}H_{26}N_4NaO_3^+$ requires 417.1897]; HPLC (C-18) unstable; (AD-H) retention time = 18.82 min, 96.3%.

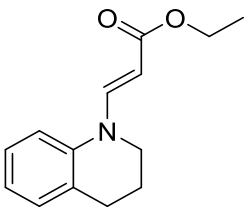
(3*R*)-*N*-[3-(6-acetamido-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-221]



A mixture of (*R*)-**216** [(3*R*)-*N*-[3-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide] (90 mg, 0.21 mmol, 1.0 eq) and Pd/C (10% w/w, 11 mg, 0.010 mmol, 0.05 eq) in EtOAc (15 mL) and MeOH (15 mL) was placed under a H_2 atmosphere. The reaction mixture was stirred for 11 h after which time starting material still remained so additional Pd/C (0.05 eq) was added. The reaction was continued for a further 5 h, then diluted with EtOAc (50 mL), filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 8 to 100% $(CH_3)_2CO$ in petroleum ether) gave (3*R*)-*N*-[3-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-3-methyl-2-

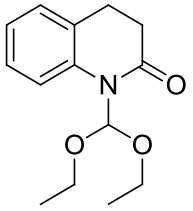
oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide (35 mg, 0.089 mmol, 42%) as an unstable brown oil. To a solution of the amine (20 mg, 0.051 mmol, 1.0 eq) in CH_2Cl_2 (1 mL) was added acetic anhydride (10 drops, ~50 mg, 0.49 mmol, 10 eq). The reaction mixture was stirred for 1 h, then concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 12 to 100% $(CH_3)_2CO$ in petroleum ether) to give (*R*)-**221** (16 mg, 0.037 mmol, 72%) as a brown oil. R_f 0.16 (1:1 $(CH_3)_2CO$:Petrol); ν_{max}/cm^{-1} 3304 (br), 2932 (w), 1681 (s), 1510 (s), 1372 (w), 1287 (m); $[\alpha]_D$ −37.4 (*c* 0.5 in $(CH_3)_2CO$); ^1H NMR (500 MHz; $(CD_3)_2CO$): δ 9.38 (1H, br s, N*H*), 8.74 (1H, br s, N*H*), 8.00 (1H, br s, N*H*), 7.81 (1H, br s, C(1')N*H*), 7.29 (1H, dd, *J* 8.0 0.8 Hz, C(6)*H*), 7.21-7.15 (2H, m, C(5'')*H* & C(7'')*H*), 6.95 (1H, dd, *J* 7.7 0.8 Hz, C(8)*H*), 6.63 (1H, dd, *J* 8.0 7.7 Hz, C(7)*H*), 6.54 (1H, d, *J* 8.7 Hz, C(8'')*H*), 4.01 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.45 (2H, dt, *J* 6.6 6.6 Hz, C(1')H_2), 3.34 (2H, t, *J* 7.3 Hz, C(3')H_2), 3.25 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.66 (2H, t, *J* 6.4 Hz, C(4'')H_2), 2.00 (3H, s, C(6'')NHCOCH_3), 1.94-1.84 (4H, m, C(2')H_2 & C(3'')H_2), 1.37 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.4 (C(5)*C*=O), 168.7 (*C*(2)=O), 168.0 (C(6'')NH*C*=O), 142.6 (*C*(10'')), 137.3 (*C*(9)), 129.5 (*C*(7'')), 128.7 (*C*(10)), 123.2 (*C*(9'')), 122.3 (*C*(6)H), 121.9 (*C*(5'')H), 119.7 (*C*(7'')H), 117.8 (*C*(8)H), 117.4 (*C*(7)H), 116.9 (*C*(5)), 111.6 (*C*(8'')H), 51.6 (*C*(3)H), 50.0 (*C*(2'')H_2), 49.9 (*C*(3')H_2), 38.3 (*C*(1')H_2), 28.9 (*C*(4'')H_2), 27.0 (*C*(2')H_2), 24.0 (C(6'')NHCO*C*H_3), 23.2 (*C*(3'')H_2), 18.7 (C(3)*C*H_3); LRMS *m/z* (ES+) 436 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 458.2159. $C_{24}H_{29}N_5NaO_3^+$ requires 458.2163]; HPLC (C-18) unstable.

Ethyl (2*E*)-3-(3,4-dihydroquinolin-1(2*H*)-yl)prop-2-enoate (240)



To a solution of 1,2,3,4-tetrahydroquinoline (1.06 g, 1.00 mL, 7.97 mmol, 1.2 eq) in THF (30 mL) and EtOH (30 mL) was added ethyl prop-2-ynoate (651 mg, 673 µL, 6.64 mmol, 1.0 eq). The reaction mixture heated at 80 °C for 71 h, then concentrated *in vacuo* and purified via silica gel chromatography (gradient elution 1 to 14% EtOAc in petroleum ether) yielded **240** (1.04 g, 4.50 mmol, 68%) as a yellow oil. R_f 0.27 (1:8 EtOAc:petroleum ether); ν_{max}/cm^{-1} 2938 (w), 1689 (m), 1613 (s), 1597 (s), 1575 (s), 1495 (m), 1240 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.19 (1H, d, *J* 13.2 Hz, C(3)*H*), 7.21 (1H, dd, *J* 7.9 7.6 Hz, C(7')*H*), 7.15 (1H, d, *J* 7.9 Hz, C(8')*H*), 7.08 (1H, d, *J* 7.3 Hz, C(5')*H*), 6.96 (1H, dd, *J* 7.6 7.3 Hz, C(6')*H*), 5.04 (1H, d, *J* 13.2 Hz, C(2)*H*), 4.21 (2H, q, *J* 7.1 Hz, OCH_2), 3.47 (2H, t, *J* 6.4 Hz, C(2')H_2), 2.72 (2H, t, *J* 6.1 Hz, C(4')H_2), 2.06-1.99 (2H, m, C(3')H_2), 1.30 (3H, t, *J* 7.1 Hz, OCH_2CH_3);^{13}C NMR (126 MHz; $CDCl_3$): δ 169.6 (*C*(1)=O), 146.1 (*C*(3)H), 140.7 (*C*(10')), 129.2 (*C*(8')H), 128.6 (*C*(9')), 128.1 (*C*(7')H), 122.8 (*C*(6')H), 116.3 (*C*(5')H), 91.1 (*C*(2)H), 59.8 (O*C*H_2), 46.3 (*C*(2')H_2), 27.9 (*C*(4')H_2), 22.8 (*C*(3')H_2), 15.0 (OCH_2CH_3); LRMS *m/z* (ES+) 254 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 232.1338. $C_{14}H_{18}NO_2^+$ requires 232.1332].

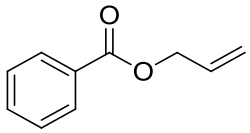
1-(Diethoxymethyl)-3,4-dihydroquinolin-2(1*H*)-one (250)



A solution of 3,4-dihydroquinolin-2-one (1.00 g, 6.79 mmol, 1.0 eq) and $AlCl_3$ (272 mg, 2.04 mmol, 0.3 eq) in triethylorthoformate (22 mL, 20 eq) was heated at 155 ºC for 69 h. The

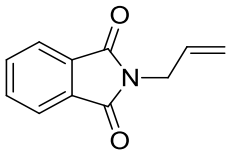
reaction mixture was allowed to cool to rt, quenched with $NaHCO_3$ (sat. aq., 300 mL) and extracted with Et_2O (3 × 150 mL). The combined organics were washed with brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 3 to 28% EtOAc in petroleum ether) yielded **250** (566 mg, 2.27 mmol, 33%) as a yellow oil. R_f 0.34 (1:4 EtOAc:petroleum ether); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.81 (1H, dd, J 8.2 0.8 Hz, C(8)*H*), 7.21-7.10 (2H, m, C(5)*H* & C(7)*H*), 7.02 (1H, ddd, J 7.4 7.4 0.8 Hz, C(6)*H*), 6.80 (1H, s, NC*H*), 3.75-3.67 (2H, m, OCH_2), 3.55-3.48 (2H, m, OCH_2), 2.87 (2H, t, J 7.2 Hz, C(4)H_2), 2.65 (2H, t, J 7.2 Hz, C(3)H_2), 1.19 (6H, t, J 7.1 Hz, 2 × CH_3); LRMS *m/z* (ES+) 272 ($[M+Na]^+$, 100%). These data are in accordance with the literature.[194]

Prop-2-en-1-yl benzoate (253)



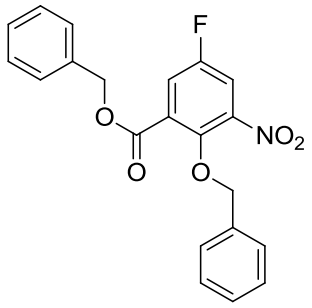
To a solution of benzoyl chloride (3.88 g, 3.20 mL, 27.6 mmol, 1.1 eq) in Et_2O (25 mL) at 0 °C was added allyl alcohol (1.45 g, 1.70 mL, 25.0 mmol, 1.0 eq) and triethylamine (2.76 g, 3.80 mL, 27.3 mmol, 1.1 eq). The reaction mixture was stirred at rt for 3.5 h, then diluted with EtOAc (200 mL), washed with HCl (aq. 2M, 3 × 50 mL), $NaHCO_3$ (sat. aq., 3 × 50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:20 EtOAc:petroleum ether) yielded **253** (3.27 g, 20.2 mmol, 81%) as a colourless oil. R_f 0.69 (1:4 EtOAc:petroleum ether); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.08 (2H, d, *J* 7.4 Hz, C(2')*H* & C(6')*H*), 7.58 (1H, t, *J* 7.5 Hz, C(4')*H*), 7.46 (2H, dd, *J* 7.5 7.4 Hz, C(3')*H* & C(5')*H*), 6.04 (1H, ddt, *J* 17.2 10.4 5.6 Hz, C(2)H), 5.43 (1H, dd, *J* 17.2 1.4 Hz, C(3)H_YH_Z), 5.30 (1H, dd, *J* 10.4 1.3 Hz, C(3)H_YH_Z), 4.84 (2H, ddd, *J* 5.6 1.4 1.3 Hz, C(1)H_2); HRMS *m/z* (FI) [Found: $(M)^+$ 162.0678. $C_{10}H_{10}O_2^+$ requires 162.0681]. These data are in accordance with the literature.

2-(prop-2-en-1-yl)-1*H*-isoindole-1,3(2*H*)-dione (254)



To a solution of phthalic anhydride (1.00 g, 6.75 mmol, 1.0 eq) in acetic acid (2.7 mL) was added allyl amine (424 mg, 557 µL, 7.43 mmol, 1.1 eq). The reaction mixture was heated at 150 ºC for 80 m, then cooled to rt and stirred for a further 15.5 h. After this time the reaction was diluted with EtOAc (50 mL), washed with $NaHCO_3$ (50 mL), H_2O (50 mL) and brine (50 mL), dried and concentrated *in vacuo* to give **254** (981 mg, 5.24 mmol, 78%) as a colourless solid which was used without further purification. R_f 0.39 (1:4 EtOAc:petroleum ether); mp 69-71 ° C (from EtOAc, lit. 65-69 ° C); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.90-7.83 (2H, m, C(4)*H* & C(7)*H*), 7.76-7.70 (2H, m, C(5)*H* & C(6)*H*), 5.96-5.83 (1H, m, C(2')H), 5.29-5.23 (1H, m, C(3')H_YH_Z), 5.23-5.18 (1H, m, C(3')H_YH_Z), 4.30 (2H, ddd, *J* 5.62 1.39 1.39 Hz, C(1')H_2); LRMS *m/z* (ES+) 210 ($[M+Na]^+$, 100%). These data are in accordance with the literature.

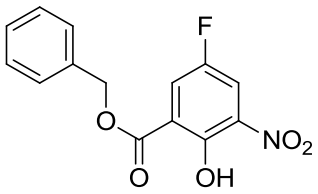
Benzyl 2-(benzyloxy)-5-fluoro-3-nitrobenzoate (272)



To a solution of 5-fluoro-2-hydroxybenzoic acid (1.39 g, 8.90 mmol, 1.0 eq) at 0 ° C in H_2SO_4 (98%, 15 mL) was added a solution of HNO_3 (70% w/w, 882 µL, 1.25 g, 13.8 mmol, 1.6 eq) in H_2SO_4 (3 mL) over 5 min. The reaction mixture was stirred at 0 ° C for 2 h and then quenched by pouring onto crushed ice (200 mL). The resulting yellow precipitate was extracted with EtOAc (3 × 100 mL), the combined organics were washed with brine (100 mL), dried ($MgSO_4$),

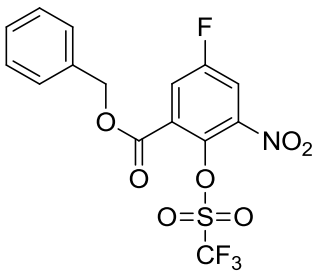
filtered and concentrated *in vacuo* to give 5-fluoro-2-hydroxy-3-nitrobenzoic acid as a yellow solid (1.54 g, 7.66 mmol) that was used without further purification. A 5 mL microwave vial was charged with the carboxylic acid (200 mg, 0.994 mmol, 1.0 eq), K_2CO_3 (412 mg, 2.98 mmol, 3.0 eq) and DMF (3 mL). The vial was sealed and the mixture stirred at rt for 5 min before BnBr (355 µL, 510 mg, 2.98 mmol, 3.0 eq) was added *via* syringe. The reaction mixture was stirred at 110 °C for 1 h before being cooled to rt and stirred for an additional 48 h. The mixture was diluted with EtOAc (50 mL), washed with 2 M K_2CO_3 (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 1 to 14% EtOAc in petroleum ether) gave **272** (173 mg, 0.454 mmol, 40%) as a yellow oily solid. R_f 0.16 (1:12 EtOAc:petroleum ether); mp 72-73 °C (from Et_2O); ν_{max}/cm^{-1} 3089 (w), 1734 (s), 1539 (s), 1378 (m), 1247 (m); ^1H NMR (500 MHz; $CDCl_3$): δ 7.80 (1H, dd, *J* 7.9 3.3 Hz, C(6)*H*), 7.69 (1H, dd, *J* 7.0 3.3 Hz, C(4)*H*), 7.43-7.33 (10H, m, 10 × C(Ar')*H*), 5.36 (2H, s, C(1)$CO_2C\mathit{H}_2$Ph), 5.12 (2H, s, C(2)O$C\mathit{H}_2$Ph); ^{13}C NMR (126 MHz; $CDCl_3$): δ 163.1 (d, *J* 1.9 Hz, C(1)*C*=O), 156.8 (d, *J* 250.0 Hz, *C*(5)F), 148.0 (d, *J* 3.7 Hz, *C*(2)), 146.0 (d, *J* 8.5 Hz, *C*(3)), 135.4 (C(2)O$CH_2C(C_5H_5)$), 134.8 (C(1)$CO_2CH_2C(C_5H_5)$), 129.6 (d, *J* 7.4 Hz, *C*(1)), 128.8 – 128.7 (3 × *C*(Ar')H), 128.65 (*C*(Ar')H), 128.61 (*C*(Ar')H), 128.5 (*C*(Ar')H), 122.6 (d, *J* 24.5 Hz, *C*(6)H), 116.0 (d, *J* 27.0 Hz, *C*(4)H), 78.9 (C(2)O$C\mathit{H}_2$Ph), 68.0 (C(1)$CO_2C\mathit{H}_2$Ph); ^{19}F NMR (377 MHz; $CDCl_3$): δ −113.48 (1F, dd, *J* 7.9 7.0 Hz, C(5)*F*); LRMS *m/z* (ES+) 404 ($[M+Na]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 404.0904. $C_{21}H_{16}FNNaO_5^+$ requires 404.0905]; Anal. calcd for $C_{21}H_{16}FNO_5$: C 66.14%, H 4.23%, N 3.67%; Found: C 66.04%, H 4.29%, N 3.72%.

Benzyl 5-fluoro-2-hydroxy-3-nitrobenzoate (273)



A solution of **272** [benzyl 2-(benzyloxy)-5-fluoro-3-nitrobenzoate] (996 mg, 2.61 mmol, 1.0 eq) in trifluoroacetic acid (13 mL) and toluene (13 mL) was stirred at rt for 23 h. The reaction mixture was concentrated *in vacuo* and azeotroped with toluene (2 × 50 mL). Purification *via* crystallisation from Et_2O yielded **273** (449 mg, 1.54 mmol, 59%) as yellow needles. R_f 0.48 (1:4 EtOAc:petroleum ether); mp 58 °C (from Et_2O); ν_{max}/cm^{-1} 3092 (w), 1687 (m), 1534 (s), 1396 (m), 1208 (s); ^1H NMR (400 MHz; $CDCl_3$): δ 11.7 (1H, s, O*H*), 7.93 (1H, dd, *J* 7.7 3.3 Hz, C(Ar)*H*), 7.90 (1H, dd, *J* 7.8 3.3 Hz, C(Ar)*H*), 7.49-7.38 (5H, m, 5 × C(Ar')*H*), 5.45 (2H, s, OCH_2Ph); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.7 (d, *J* 2.5 Hz, C(1)*C*=O), 153.1 (d, *J* 244.0 Hz, *C*(5)F), 152.4 (d, *J* 2.2 Hz, *C*(2)), 137.8 (d, *J* 7.6 Hz, *C*(3)), 134.2 (*C*(Ar')), 129.3 (*C*(Ar')H), 129.0 (2 × *C*(Ar')H), 128.9 (2 × *C*(Ar')H), 122.7 (d, *J* 23.8 Hz, *C*(6)H), 119.0 (d, *J* 26.7 Hz, *C*(4)H), 116.6 (d, *J* 6.7 Hz, *C*(1)), 68.7 (O*C*H_2Ph); ^{19}F NMR (377 MHz; $CDCl_3$): δ −121.0 (1F, dd, *J* 7.8 7.7 Hz, C(5)*F*); LRMS *m/z* (ES+) 314 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 314.0431. $C_{14}H_{10}FNNaO_5^+$ requires 314.0435].

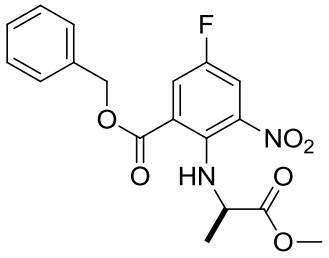
Benzyl 5-fluoro-3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate (274)



To a solution of **273** [benzyl 5-fluoro-2-hydroxy-3-nitrobenzoate] (230 mg, 0.790 mmol, 1.0 eq) and Et_3N (120 mg, 165 µL, 1.18 mmol, 1.5 eq) in CH_2Cl_2 (8 mL) at 0 °C was added

triflic anhydride (267 mg, 159 µL, 0.947 mmol, 1.2 eq) dropwise over 5 min. The reaction mixture was allowed to warm to rt, stirred for a further 1.5 h, then concentrated *in vacuo* and purified by passing through a short path of silica gel (eluent 1:1 EtOAc:petroleum ether) to give **274** (279 mg, 0.659 mmol, 83%) as a colourless oil. R_f 0.51 (1:4 EtOAc:petroleum ether); ν_{max}/cm^{-1} 3094 (w), 1737 (s), 1552 (s), 1433 (m), 1306 (m), 1222 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.99 (1H, dd, *J* 7.1 3.3 Hz, C(6)*H*), 7.94 (1H, dd, *J* 6.8 3.3 Hz, C(4)*H*), 7.48-7.43 (2H, m, 2 × C(Ar')*H*), 7.43-7.36 (3H, m, 3 × C(Ar')*H*), 5.43 (2H, s, OCH_2Ph); ^{13}C NMR (126 MHz; $CDCl_3$): δ 161.6 (C(1)*C*=O), 159.7 (d, *J* 256.8 Hz, *C*(5)F), 143.9 (d, *J* 8.1 Hz, *C*(Ar)), 136.5 (d, *J* 4.5 Hz, *C*(Ar)), 134.2 (*C*(1')), 129.6 (d, *J* 7.5 Hz, *C*(Ar)), 129.2 (2 × *C*(Ar')H), 128.9 (*C*(4')H & 2 × *C*(Ar')H), 123.9 (d, *J* 24.8 Hz, *C*(6)H), 118.5 (q, *J* 321.1 Hz, O*C*F_3), 117.6 (d, *J* 27.8 Hz, *C*(4)H), 69.1 (O*C*H_2Ph); ^{19}F NMR (377 MHz; $CDCl_3$): δ −73.3 (3F, s, OC*F*$_3$), −105.9 (1F, dd, J 7.1 6.8 Hz, C(5)*F*); LRMS *m/z* (ES+) 446 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 445.9927. $C_{15}H_9F_4NNaO_7S^+$ requires 445.9928].

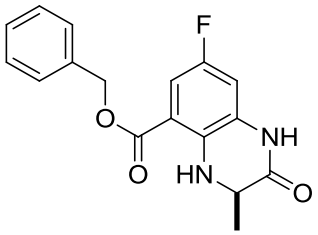
Benzyl 5-fluoro-2-{[(2*R*)-1-methoxy-1-oxopropan-2-yl]amino}-3-nitrobenzoate [(*R*)-275]



A solution of **274** [benzyl 5-fluoro-3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate] (397 mg, 0.938 mmol, 1.0 eq), D-alanine methyl ester hydrochloride (144 mg, 1.03 mmol, 1.1 eq) and Cs_2CO_3 (672 mg, 2.06 mmol, 2.2 eq) was heated to 85 °C, causing a deep orange colour to develop, and stirring was continued for 13 h. The reaction mixture was cooled to rt, diluted with EtOAc (200 mL), washed with 2 M K_2CO_3 (3 × 100 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:10

EtOAc:petroleum ether) yielded (*R*)-**275** (285 mg, 0.757 mmol, 81%) as a yellow oil. R_f 0.30 (1:10 EtOAc:petroleum ether); $[\alpha]_D$ +25.0 (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3281 (w), 2360 (w), 1744 (s), 1703 (m), 1539 (s), 1342 (m), 1201 (s); ^1H NMR (500 MHz; $CDCl_3$): δ 8.37 (1H, br s, N*H*), 7.90 (1H, dd, *J* 8.2 3.2 Hz, C(6)*H*), 7.77 (1H, dd, *J* 7.6 3.2 Hz, C(4)*H*), 7.48-7.36 (5H, m, $CH_2C_6H_5$), 5.38 (2H, s, OCH_2Ph), 3.99 (1H, q, *J* 6.9 Hz, C(2')*H*), 3.68 (3H, s, C(1')O_2CH_3), 1.44 (3H, d, *J* 6.9 Hz, C(2')CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 173.4 (s, *C*(1')=O), 165.6 (d, *J* 2.6 Hz, C(1)*C*=O), 152.4 (d, *J* 241.7 Hz, *C*(5)F), 141.2 (d, *J* 1.6 Hz, *C*(2)), 138.7 (d, *J* 7.6 Hz, *C*(3)), 134.9 (C(1')), 128.8-128.7 (*C*(4')H & 2 × *C*(Ar')H), 128.6 (2 × *C*(Ar')H), 124.2 (d, *J* 24.1 Hz, *C*(6)H), 120.0 (d, *J* 6.1 Hz, *C*(1)), 118.0 (d, *J* 26.6 Hz, *C*(4)H), 67.7 (OCH_2Ph), 54.4 (*C*(2')), 52.5 (OCH_3), 19.0 (C(2')CH_3); ^{19}F NMR (377 MHz; $CDCl_3$): δ −124.29 (1F, dd, *J* 8.2 7.6 Hz); LRMS *m/z* (ES+) 377 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 399.0962. $C_{18}H_{17}FN_2NaO_6^+$ requires 399.0963]; Anal. calcd for $C_{18}H_{17}FN_2O_6$: C 57.45%, H 4.55%, N 7.44%; Found: C 57.56%, H 4.59%, N 7.32%.

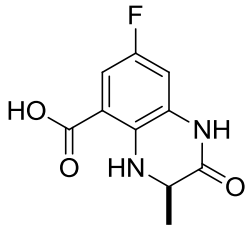
Benzyl (3*R*)-7-fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate [(*R*)-276]



To solution of (*R*)-**275** [benzyl 5-fluoro-2-{[(2*R*)-1-methoxy-1-oxopropan-2-yl]amino}-3-nitrobenzoate] (261 mg, 0.694 mmol, 1.0 eq) in DMF (14 mL) was added zinc dust (1.13 g, 17.3 mmol, 25 eq) and NH_4Cl (1.85 g, 34.5 mmol, 50 eq). The reaction mixture was stirred at rt for 19 h, then diluted with EtOAc (100 mL) and filtered through Celite® (eluent EtOAc). The filtrate was washed with brine (3 × 100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:6 EtOAc:petroleum ether) yielded (*R*)-**276**

(161 mg, 0.512 mmol, 74%) as a pale yellow solid. R_f 0.60 (1:1 EtOAc:petroleum ether); mp 191-192 °C (from $CHCl_3$); $[\alpha]_D$ −47.9 (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3341 (w), 2969 (w), 1678 (s), 1319 (m), 1217 (s); ^{1}H NMR (500 MHz; $(CD_3)_2CO$): δ 9.57 (1H, br s, N*H*), 7.54-7.49 (2H, m, 2 × C(Ar')*H*), 7.48 (1H, br s, N*H*), 7.44-7.40 (2H, m, 2 × C(Ar')*H*), 7.39-7.34 (1H, m, C(4')*H*), 7.25 (1H, dd, *J* 9.6 2.9 Hz, C(6)*H*), 6.90 (1H, dd, *J* 9.2 2.9 Hz, C(8)*H*), 5.37 (2H, s, OCH_2Ph), 4.12 (1H, qd, *J* 6.7 1.6 Hz, C(3)*H*), 1.41 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 168.4 (*C*(2)=O), 167.5 (d, *J* 2.9 Hz, C(5)*C*=O), 154.8 (d, *J* 233.3 Hz, *C*(7)F), 137.2 (*C*(1')), 135.8 (*C*(9)), 130.0 (d, *J* 10.4 Hz, *C*(10)), 129.4 (2 × *C*(Ar')H), 129.10 (*C*(4')H), 129.05 (2 × *C*(Ar')H), 111.6 (d, *J* 8.4 Hz, *C*(5)), 109.5 (d, *J* 23.6 Hz, *C*(6)H), 107.7 (d, *J* 27.9 Hz, *C*(8)H), 67.2 (O*C*H_2Ph), 51.7 (*C*(3)H), 18.8 (C(3)*C*H_3); ^{19}F NMR (470 MHz; $(CD_3)_2CO$): δ −127.9 (1F, dd, *J* 9.6 9.2 Hz, C(7)*F*); LRMS *m/z* (ES+) 315 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 337.0965. $C_{17}H_{15}FN_2NaO_3^+$ requires 337.0959].

(3*R*)-7-Fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid [(*R*)-277]

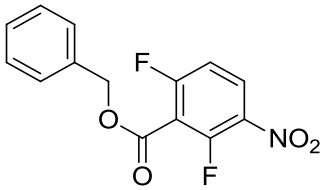


A mixture of (*R*)-**276** [benzyl (3*R*)-7-fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate] (136 mg, 0.433 mmol, 1.0 eq) and Pd/C (10% w/w, 23 mg, 0.022 mmol, 0.05 eq) in EtOAc (30 mL) was placed under a H_2 atmosphere. The reaction mixture was stirred for 18 h, then diluted with EtOAc (60 mL), filtered through Celite® (eluent EtOAc) and concentrated *in vacuo* to give (*R*)-**277** (63 mg, 0.28 mmol, 65%) as a yellow solid.

R_f 0.58 (1:99 AcOH:EtOAc); mp 240 °C (dec.); $[\alpha]_D$ −78.8 (*c* 0.4 in MeOH); ν_{max}/cm^{-1} 3343 (w), 2923 (br), 1688 (s), 1668 (s), 1315 (m), 1231 (s); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 13.3 (1H, br s, O*H*), 10.56 (1H, s, N*H*), 7.51 (1H, br s, N*H*), 7.13 (1H, dd, *J* 9.5 3.0 Hz, C(6)*H*), 6.77

(1H, dd, *J* 9.3 3.0 Hz, , C(8)*H*), 4.03 (1H, q, *J* 6.7 Hz, , C(3)*H*), 1.29 (3H, d, *J* 6.7 Hz, , C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2SO$): δ 168.4 (d, *J* 2.7 Hz, C(5)*C*=O), 167.9 (*C*(1)), 153.1 (d, *J* 232.0 Hz, *C*(7)F), 134.2 (*C*(9)), 128.6 (d, *J* 10.7 Hz, *C*(10)), 111.8 (d, *J* 7.7 Hz, *C*(5)), 108.9 (d, *J* 23.0 Hz, *C*(6)H), 106.24 (d, *J* 27.6 Hz, *C*(8)H), 50.2 (*C*(3)), 18.3 (C(3)*C*H_3); ^{19}F NMR (377 MHz; $(CD_3)_2SO$): δ −126.6 (1F, dd, *J* 9.5 9.3 Hz, C(7)*F*); LRMS *m/z* (ES−) 223 ($[M-H]^-$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 247.0492. $C_{10}H_9FN_2NaO_3^+$ requires 247.0489].

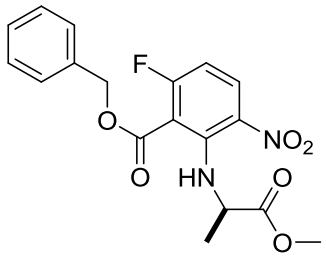
Benzyl 2,6-difluoro-3-nitrobenzoate (279)



A 2 mL microwave vial was charged with 2,6-difluoro-3-nitrobenzoic acid (100 mg, 0.492 mmol, 1.0 eq), K_2CO_3 (206 mg, 1.49 mmol, 3.0 eq) and DMF (1.5 mL). The vial was sealed and the mixture stirred at rt for 5 min before BnBr (178 µL, 255 mg, 1.49 mmol, 3.0 eq) was added *via* syringe. The reaction mixture was stirred at 110 °C for 30 min before being cooled to rt and stirred for an additional 16 h. The mixture was diluted with EtOAc (25 mL), washed with 2 M K_2CO_3 (3 × 25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 2 to 20% EtOAc in petroleum ether) gave **279** (86 mg, 0.29 mmol, 59%) as a waxy yellow solid. R_f 0.32 (1:10 EtOAc:petroleum ether); mp 74 °C; ν_{max}/cm^{-1} 3095 (w), 1736 (s), 1537 (s), 1351 (s), 1292 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.24 (1H, ddd, *J* 9.4 8.2 5.5 Hz, C(4)*H*), 7.49-7.35 (5H, m, 5 × *C*(Ar')H), 7.12 (1H, ddd, *J* 9.4 7.9 1.7 Hz, C(5)*H*), 5.44 (2H, s, OCH_2Ph); ^{13}C NMR (126 MHz; $CDCl_3$): δ 163.2 (dd, *J* 267.0 5.6 Hz, *C*(2)F), 159.5 (C(1)*C*=O), 154.7 (dd, *J* 274.8 7.4 Hz, *C*(6)F), 134.6 (*C*(Ar')), 134.5 (br, *C*(3)), 129.5 (d, *J* 11.6 Hz, *C*(4)H), 128.93 (*C*(Ar')H), 128.88 (*C*(Ar')H), 128.6 (*C*(Ar')H), 113.8 (dd, *J* 19.9 18.4 Hz, *C*(1)), 112.8 (dd, *J* 23.7 4.5 Hz,

C(5)H), 68.7 (O*C*H$_2$Ph); ^{19}F NMR (377 MHz; CDCl$_3$): δ −97.9 (1F, ddd, *J* 9.5 7.9 5.5 Hz, C(6)*F*), −112.5 (1F, ddd, *J* 9.5 8.2 1.7 Hz, C(2)*F*); LRMS *m/z* (ES+) 316 ([M+Na]$^+$, 50%), 404 (100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 316.0394. $C_{14}H_9F_2NNaO_4^+$ requires 316.0392]; Anal. calcd for $C_{14}H_9F_2NO_4$: C 57.35%, H 3.09%, N 4.78%; Found: C 57.49%, H 2.98%, N 4.80%.

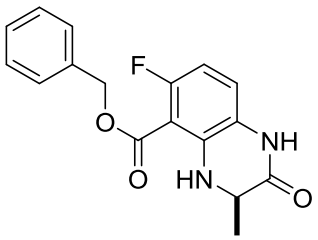
Benzyl 6-fluoro-2-{[(2*R*)-1-methoxy-1-oxopropan-2-yl]amino}-3-nitrobenzoate [(*R*)-280]



A solution of **279** [benzyl 2,6-difluoro-3-nitrobenzoate] (261 mg, 0.890 mmol, 1.0 eq), D-alanine methyl ester hydrochloride (124 mg, 0.890 mmol, 1.0 eq) were reacted as described above. Purification *via* silica gel chromatography (gradient elution 1 to 15% EtOAc in petroleum ether) yielded (*R*)-**280** (288 mg, 0.765 mmol, 86%) as a yellow oil. R$_f$ 0.45 (1:4 EtOAc:petroleum ether); [α]$_D$ −96.1 (*c* 1.0 in CHCl$_3$); ν_{max}/cm^{-1} ν_{max}/cm^{-1} 3328 (w), 2956 (w), 1739 (s), 1614 (s), 1500 (s), 1261 (s); ^{1}H NMR (400 MHz; CDCl$_3$): δ 8.53 (1H, br d, *J* 8.6 Hz, N*H*), 8.26 (1H, dd, J 9.4 6.5 Hz, C(4)*H*), 7.50-7.43 (2H, m, 2 × C(Ar')*H*), 7.43-7.33 (3H, m, 3 × C(Ar')*H*), 6.52 (1H, dd J 9.4 7.6, C(5)*H*), 5.43 (2H, d, *J* 12.1 Hz, OC*H*$_A$H$_B$Ph), 5.33 (2H, d, *J* 12.1 Hz, OCH$_A$*H*$_B$Ph), 4.07 (1H, dq, *J* 8.6 6.9 Hz, C(2')*H*), 3.65 (3H, s, OC*H*$_3$), 1.38 (3H, d, *J* 6.9 Hz, C(2')C*H*$_3$); ^{13}C NMR (101 MHz; CDCl$_3$): δ 172.7 (*C*(1')=O), 164.5 (d, *J* 260.5 Hz, *C*(6)F), 164.3 (C(1)*C*=O), 144.1 (d, *J* 7.9 Hz, *C*(2)), 134.4 (*C*(Ar')), 132.8 (*C*(3)), 131.1 (d, *J* 12.8 Hz, *C*(4)H), 128.84 (2 × *C*(Ar')H), 128.82 (*C*(Ar')H), 128.76 (2 × *C*(Ar')H), 110.6 (d, *J* 21.2 Hz, *C*(1)), 105.4 (d, *J* 25.1 Hz, *C*(5)H), 68.5 (O*C*H$_2$Ph), 53.5 (*C*(2')H), 52.7 (O*C*H$_3$), 19.5 (C(2')*C*H$_3$); ^{19}F NMR (470 MHz; CDCl$_3$): δ −99.9 (1F, dd, *J* 7.6 6.5 Hz, C(6)*F*);

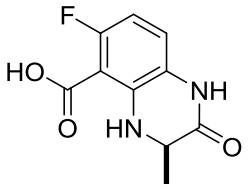
LRMS *m/z* (ES+) 377 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 399.0962. $C_{18}H_{17}FN_2NaO_6^+$ requires 399.0963]; Anal. calcd for $C_{18}H_{17}FN_2O_6$: C 57.45%, H 4.55%, N 7.44%; Found: C 57.36%, H 4.48%, N 7.52%.

Benzyl (3*R*)-6-fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate [(*R*)-281]



Compound (*R*)-**280** [benzyl 6-fluoro-2-{[(2*R*)-1-methoxy-1-oxopropan-2-yl]amino}-3-nitrobenzoate] (265 mg, 0.704 mmol, 1.0 eq) was reacted as described above. Purification *via* silica gel chromatography (1:6 EtOAc:petroleum ether) yielded (*R*)-**281** (140 mg, 0.445 mmol, 63%) as a colourless solid. R_f 0.10 (1:4 EtOAc:petroleum ether); mp 162-163 °C (from $CHCl_3$); $[\alpha]_D$ −30.3 (*c* 0.9 in $CHCl_3$); ν_{max}/cm^{-1} 3320 (w), 2361 (w), 1683 (s), 1312 (m), 1232 (s); ^{1}H NMR (500 MHz; $(CD_3)_2CO$): δ 9.43 (1H, br s, N*H*), 7.55 (1H, br s, N*H*), 7.50 (2H, d, *J* 7.4 Hz, C(2')*H* & C(6')*H*), 7.40 (2H, dd, *J* 7.4 7.4 Hz, C(3')*H* & C(5')*H*), 7.37-7.31 (1H, m, C(4')*H*), 7.00 (1H, dd, *J* 8.6 5.1 Hz, C(8)*H*), 6.45 (1H, dd, *J* 11.3 8.6 Hz, C(7)*H*), 5.40 (2H, s, OCH_2Ph), 4.11 (1H, qd, *J* 6.7 1.6 Hz, C(3)*H*), 1.40 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 167.6 (*C*(2)=O), 166.9 (d, J 4.0 Hz, C(5)*C*=O), 159.3 (d, J 251.4 Hz, *C*(6)F), 139.1 (d, J 4.5 Hz, *C*(9)), 137.1 (*C*(1')), 129.3 (*C*(3')H & *C*(5')H), 128.9 (*C*(4')H), 128.7 (*C*(2')H & *C*(6')H), 124.4 (d, J 2.8 Hz, *C*(10)), 119.3 (d, J 11.0 Hz, *C*(8)H), 104.4 (d, J 25.7 Hz, *C*(7)H), 102.4 (d, J 15.9 Hz, *C*(5)), 67.2 (O*C*H_2Ph), 51.5 (*C*(3)H), 18.9 (C(3)*C*H_3); ^{19}F NMR (377 MHz; $(CD_3)_2CO$): δ −114.4 (1F, dd, *J* 11.3 5.1 Hz, C(6)*F*); LRMS *m/z* (ES−) 313 ([M−H]$^-$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 337.0968. $C_{17}H_{15}FN_2NaO_3^+$ requires 337.0959].

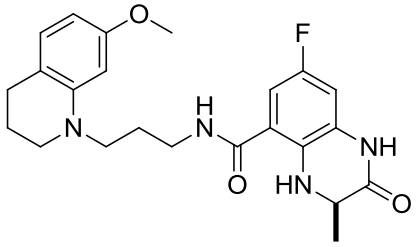
(3*R*)-6-Fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid [(*R*)-282]



Compound (*R*)-**281** [benzyl (3*R*)-7-fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate] (126 mg, 0.401 mmol, 1.0 eq) was reacted as described above to afford (*R*)-**282** (85 mg, 0.38 mmol, 95%) as a colourless solid, which was used without further purification.

R_f 0.55 (1:99 AcOH:EtOAc); mp 222-226 °C (from EtOAc); $[\alpha]_D$ −92.2 (*c* 0.5 in MeOH); ν_{max}/cm^{-1} 3310 (w), 2973 (br), 1684 (s), 1488 (m), 1205 (m); ^{1}H NMR (500 MHz; $(CD_3)_2SO$): δ 13.30 (1H, br s, O*H*), 10.40 (1H, s, N*H*), 7.49 (1H, s, N*H*), 6.84 (1H, dd, *J* 8.6 4.8 Hz, C(8)*H*), 6.44 (1H, dd, *J* 11.1 8.6 Hz, C(7)*H*), 4.00 (1H, q, *J* 6.7 Hz, C(3)*H*), 1.27 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2SO$): δ 167.6 (d, *J* 2.7 Hz, *C*=O), 169.4 (*C*=O), 158.1 (d, *J* 249.0 Hz, *C*(6)F), 137.7 (d, *J* 3.9 Hz, *C*(Ar)), 123.4 (d, *J* 2.3 Hz, *C*(Ar)), 118.24 (d, *J* 11.1 Hz, *C*(8)H), 103.7 (d, *J* 103.7 Hz, *C*(7)H), 102.6 (d, *J* 15.8 Hz, *C*(Ar)), 50.4 (*C*(3)), 18.8 (C(3)*C*H_3);; ^{19}F NMR (377 MHz; $(CD_3)_2SO$): δ −114.5 (1F, dd, *J* 11.1 4.8 Hz, C(6)*F*); LRMS *m/z* (ES−) 223 ([M−H]$^-$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 247.0501. $C_{10}H_9FN_2NaO_3^+$ requires 247.0489].

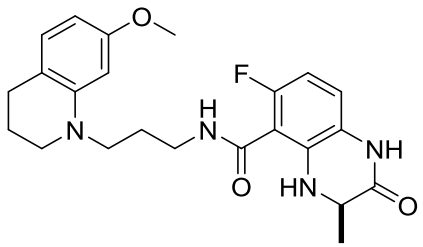
(3*R*)-*N*-[3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-7-fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-283]



Compounds (*R*)-**277** [(3*R*)-7-fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (38 mg, 0.17 mmol, 1.0 eq) and **153** [3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (42 mg, 0.19 mmol, 1.1 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (2:1 then 1:0 Et_2O:petroleum ether) yielded (*R*)-**283** (21 mg, 0.049 mmol, 29%) as a pale yellow oil. R_f 0.45 (Et_2O); $[\alpha]_D$ −21.2 (*c* 0.5 in $(CH_3)_2CO$); ν_{max}/cm^{-1} 3322 (br), 2935 (s), 1686 (s), 1611 (s), 1509 (s), 1308 (s); ^1H NMR (500 MHz; $(CD_3)_2CO$): δ 9.46 (1H, br s, N*H*), 7.85 (2H, br s, 2 × N*H*), 7.11 (1H, dd, *J* 9.8 2.7 Hz, C(6)*H*), 6.81 (1H, dd, *J* 9.3 2.7 Hz, C(8)*H*), 6.75 (1H, d, *J* 8.1 Hz, C(5'')*H*), 6.16 (1H, d, *J* 2.3 Hz, C(8'')*H*), 6.07 (1H, dd, *J* 8.1 2.3, C(6'')*H*), 3.98 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.66 (3H, s, C(7'')OCH_3), 3.47 (2H, q, *J* 6.6 Hz, C(1')H_2), 3.37 (2H, t, *J* 7.4 Hz, C(3')H_2), 3.28 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.63 (2H, t, *J* 6.3 Hz, C(4'')H_2), 1.96-1.84 (4H, m, C(2')H_2 & C(3'')H_2), 1.37 (3H, d, *J* 6.7, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 169.0 (*C*(2)=O), 168.5 (d, *J* 2.7 Hz, (C(5)*C*=O), 160.3 (*C*(7')), 155.3 (d, *J* 232.8 Hz, *C*(7)F), 146.9 (*C*(10'')), 134.1 (*C*(9)), 130.2 (*C*(5'')H), 130.1 (*C*(10)), 116.8 (d, *J* 6.8 Hz, (*C*(5)), 115.7 (*C*(9'')), 107.3 (d, *J* 23.5 Hz, (*C*(6)H)), 105.5 (d, *J* 27.4 Hz, (*C*(8)H)), 101.1 (*C*(6'')H), 98.0 (*C*(8'')H), 55.1 (O*C*H_3), 51.6 (*C*(3)H), 50.0 (*C*(2'')H_2), 49.7 (*C*(3')H_2), 38.3 (*C*(1')H_2), 28.1 (*C*(4'')H_2), 27.0 (*C*(2')H_2), 23.3 (*C*(3'')H_2), 18.4 (C(3)*C*H_3); ^{19}F NMR (470 MHz; $(CD_3)_2CO$): δ −125.9 (1F, dd, J 9.8 9.3 Hz, C(7)*F*); LRMS *m/z* (ES+) 427 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found:

$(M+H)^+$ 427.2137. $C_{23}H_{28}FN_4O_3^+$ requires 427.2140]; HPLC (C-18) retention time = 10.51 min, 98.1%; (AD-H) retention time = 15.76 min, 96.1%.

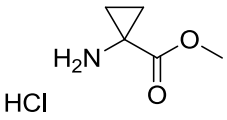
(3*R*)-*N*-[3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-6-fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxamide [(*R*)-284]



Compounds (*R*)-**282** [(3*R*)-6-fluoro-3-methyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (39 mg, 0.17 mmol, 1.0 eq) and **153** [3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (42 mg, 0.19 mmol, 1.1 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (3:1 then 1:0 Et_2O:petroleum ether) yielded (*R*)-**284** (20 mg, 0.047 mmol, 27%) as a pale yellow oil. R_f 0.70 (2:1 EtOAc:petroleum ether); $[\alpha]_D$ −6.1 (*c* 0.5 in $(CH_3)_2CO$); ν_{max}/cm^{-1} 3270 (br), 2937 (s), 1685 (s), 1508 (s), 1307 (s); ^1H NMR (500 MHz; $(CD_3)_2CO$): δ 9.34 (1H, br s, N*H*), 7.84 (1H, br s, N*H*), 7.64 (1H, br s, N*H*), 6.92 (1H, dd, *J* 8.6 5.3 Hz, C(8)*H*), 6.77 (1H, d, *J* 8.1 Hz, C(5'')*H*), 6.46 (1H, dd, *J* 11.8 8.6 Hz, C(7)*H*), 6.19 (1H, d, *J* 2.4 Hz, C(8'')*H*), 6.09 (1H, dd, *J* 8.1 2.4 Hz, C(6'')*H*), 4.03 (1H, qd, *J* 6.7 1.5 Hz, C(3)*H*), 3.69 (3H, s, C(7'')OCH_3), 3.53 (2H, dt, *J* 6.7 6.2 Hz, C(1')H_2), 3.39 (2H, t, *J* 7.3 Hz, C(3')H_2), 3.30 (2H, t, *J* 5.6 Hz, C(2'')H_2), 2.65 (2H, t, *J* 6.3 Hz, C(4'')H_2), 1.95 (2H, tt, *J* 7.3 6.7 Hz, C(2')H_2), 1.93-1.87 (2H, m, C(3'')H_2), 1.39 (3H, d, *J* 6.7 Hz, C(3)CH_3); ^{13}C NMR (126 MHz; $(CD_3)_2CO$): δ 168.0 (*C*(2)=O), 165.8 (d, *J* 2.0 Hz, C(5)*C*=O), 160.3 (*C*(7'')), 157.9 (d, *J* 239.4 Hz, *C*(6)F), 146.9 (*C*(10'')), 138.1 (d, *J* 5.7 Hz, *C*(9)), 130.2 (*C*(5'')H), 124.8 (d, *J* 1.8, *C*(10)), 117.4 (d, *J* 11.6 Hz, *C*(8)H), 115.7 (*C*(5)), 106.4 (d, *J* 17.9 Hz, *C*(5)), 103.9 (d, *J* 27.2 Hz, *C*(7)H), 101.2 (*C*(6'')H), 98.0 (*C*(8'')H), 55.1 (C(7'')O*C*H_3), 51.4 (*C*(3)H), 50.0 (*C*(2'')H_2), 49.7 (*C*(3')H_2), 38.3 (*C*(1')H_2), 28.1 (*C*(4'')H_2), 27.0 (*C*(2')H_2), 23.3

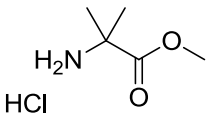
(*C*(3'')H_2), 18.7 (C(3)*C*H_3); ^{19}F NMR (470 MHz; $(CD_3)_2CO$): δ −120.3 - −120.4 (1F, m, C(6)*F*); LRMS *m/z* (ES+) 427 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 427.2137. $C_{23}H_{28}FN_4O_3^+$ requires 427.2140]; HPLC (C-18) retention time = 10.72 min, 97.5%; (AD-H) retention time = 13.31 min, 98.4%.

Methyl 1-aminocyclopropanecarboxylate hydrochloride (290)



To a solution of 1-aminocyclopropane carboxylic acid (150 mg, 1.48 mmol, 1.0 eq) in MeOH (3.5 mL) at 0 °C was added thionyl chloride (433 µL, 5.93 mmol, 4.0 eq). The reaction mixture was stirred at rt for 24 h before being concentrated *in vacuo*. The resulting residue was stirred with Et_2O for 16 h and filtered to give **290** (197 mg, 1.30 mmol, 88%) as a white solid. R_f 0.51 (1:9 MeOH:CH_2Cl_2); mp 120 °C (sublimes); ν_{max}/cm^{-1} 2881 (br s), 2699 (m), 1737 (m), 1537 (m), 1211 (s); ^{1}H NMR (500 MHz; CD_3OD): δ 3.81 (3H, s, OC*H*$_3$), 1.57 (2H, dd, J_{trans} 8.4 Hz J_{gem} 5.6 Hz, C(2)*H*$_Y$H$_Z$ & C(3)*H*$_Y$H$_Z$), 1.41 (2H, dd, J_{trans} 8.4 Hz J_{gem} 5.6 Hz, C(2)H$_Y$*H*$_Z$ & C(3)H$_Y$*H*$_Z$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 171.0 (*C*O_2CH$_3$), 53.8 (CO_2*C*H$_3$), 35.3 (*C*(1)), 14.3 (*C*(2)H_2 & *C*(3)H_2); LRMS *m/z* (ES+) 116 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 116.0704. $C_5H_{10}NO_2^+$ requires 116.0706]; Anal. Calcd for $C_5H_{10}NO_2$: C 39.62%, H 6.65%, N 9.24%; Found: C 39.58%, H 6.73%, N 9.19%.

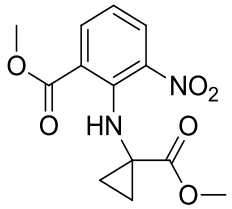
Methyl 2-amino-2-methylpropanoate hydrochloride (291)



To a solution of 2-aminoisobutyric acid (200 mg, 1.94 mmol, 1.0 eq) in MeOH (5.0 mL) at 0 °C was added thionyl chloride (566 µL, 7.76 mmol, 4.0 eq). The reaction mixture was stirred at rt for 37 h before being concentrated *in vacuo*. The resulting residue was stirred with Et_2O for

16 h and filtered to give **291** (198 mg, 1.29 mmol, 66%) as a white solid. R_f 0.44 (1:9 MeOH:CH_2Cl_2); mp ~140 (sublimes, lit.140 °C); ^{1}H NMR (400 MHz; CD_3OD): δ 3.87 (3H, s, CO_2CH_3), 1.59 (6H, s, C(2)(CH_3)$_2$); LRMS *m/z* (ES+) 118 ([M+H]$^+$, 100%). These data are in accordance with the literature.

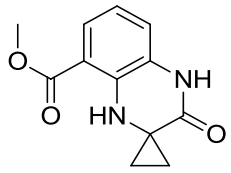
Methyl 2-{[1-(methoxycarbonyl)cyclopropyl]amino}-3-nitrobenzoate (292)



A dry microwave flask was charged with **290** [methyl 2-amino-2-methylpropanoate hydrochloride] (180 mg, 1.19 mmol, 1.3 eq), $Pd(OAc)_2$ (10 mg, 0.046 mmol, 0.05 eq), (S)-BINAP (57 mg, 0.092 mmol, 0.10 mmol) and Cs_2CO_3 (775 mg, 2.38 mmol, 2.6 eq) and sealed under Ar. Toluene (12 mL) was added and the resulting solution heated to 80 °C for 15 min. A solution of **107** [methyl-3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate] (316 mg, 0.915 mg, 1.0 eq) in toluene (5 mL) was added portionwise over 1 h and the reaction mixture stirred at 80 °C for 20 h. After cooling to rt the slurry was filtered (eluent toluene) and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 4 to 22% EtOAc in petroleum ether) followed by recrystallisation from Et_2O gave **292** (151 mg, 513 mmol, 56%) as a yellow crystalline solid. R_f 0.40 (1:4 EtOAc:petroleum ether); mp 92-95 °C (from CH_3Cl); ν_{max}/cm^{-1} 3312 (w), 1730 (m), 1692 (m), 1531 (m), 1258 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.06 (1H, br s, N*H*), 8.03 (1H, dd, *J* 7.9 1.7 Hz, C(6)*H*), 7.93 (1H, dd, *J* 8.0 1.7 Hz, C(4)*H*), 6.74 (1H, dd, *J* 8.0 7.9 Hz, C(5)*H*), 3.89 (3H, s, C(1')CO_2CH_3), 3.82 (3H, s, C(1)CO_2CH_3), 1.52 (2H, dd, J_{trans} 7.8 Hz J_{gem} 4.7 Hz, C(2')H_YH_Z & C(3')H_YH_Z), 1.09 (2H, dd, J_{trans} 7.8 Hz J_{gem} 4.7 Hz, C(2')H_YH_Z & C(3')H_YH_Z); ^{13}C NMR (126 MHz; $CDCl_3$): δ 173.0 (C(1')CO_2CH_3), 167.8 (C(1)CO_2CH_3), 143.2 (*C*(2)), 137.8 (*C*(3)), 136.5 (*C*(6)H), 131.0

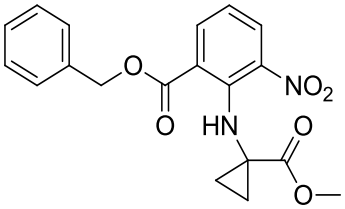
(*C*(4)H), 117.2 (*C*(1)), 115.6 (*C*(5)H), 52.7 (C(1)CO_2CH_3), 52.5 (C(1')CO_2CH_3), 39.1 (*C*(1')), 19.2 (*C*(2')H_2 & *C*(3')H_2); LRMS *m/z* (ES+) 611 ($[2M+Na]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 317.0750. $C_{13}H_{14}N_2NaO_6^+$ requires 317.0744]; Anal. Calcd for $C_{13}H_{14}N_2O_6$: C 53.06%, H 4.80%, N 9.52%; Found: C 52.99%, H 4.72%, N 9.64%.

Methyl 3'-oxo-3',4'-dihydro-1'*H*-spiro[cyclopropane-1,2'-quinoxaline]-8'-carboxylate (293)



A solution of **292** [Methyl 2-{[1-(methoxycarbonyl)cyclopropyl]amino}-3-nitrobenzoate] (136 mg, 0.462 mmol, 1.0 eq) and Pd/C (10% w/w, 25 mg, 0.023 mmol, 0.05 eq) in EtOAc (6 mL) was stirred under a H_2 atmosphere for 25 h. The reaction mixture was filtered through Celite® (eluent EtOAc), concentrated *in vacuo* and purified attempted *via* silica gel chromatography (1:5 EtOAc:petroleum ether). However, the product could not be isolated in a sufficient quantity to characterise.

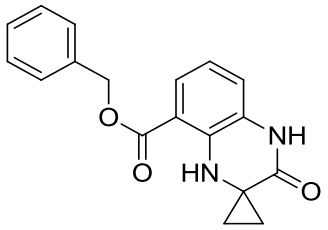
Benzyl 2-{[1-(methoxycarbonyl)cyclopropyl]amino}-3-nitrobenzoate (295)



To a solution of **116** [benzyl 2-fluoro-3-nitrobenzoate] (306 mg, 1.11 mmol, 1.0 eq) in toluene (25 mL) was added Cs_2CO_3 (797 mg, 1.22 mmol, 2.2 eq) and **290** [methyl 2-amino-2-methylpropanoate hydrochloride] (185 mg, 1.22 mmol, 1.1 eq). The resulting yellow solution was heated to 85 ºC for 72 h. Upon cooling to rt the reaction mixture was diluted with EtOAc (50 mL), washed with 2 M K_2CO_3 (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 14 to 100%

CH_2Cl_2 in petroleum ether) yielded **295** (144 mg, 0.408 mmol, 37%) as a yellow oil. R_f 0.11 (1:1 CH_2Cl_2:Petrol); ν_{max}/cm^{-1} 3309 (w), 1731 (m), 1690 (m), 1532 (m), 1253 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 9.09 (1H, br s, N*H*), 8.08 (1H, dd, *J* 7.9 1.6 Hz, C(6)*H*), 7.91 (1H, dd. *J* 8.1 1.6 Hz, C(4)*H*), 7.47-7.34 (5H, m, 5 × C(Ar')*H*), 6.72 (1H, dd, *J* 8.1 7.9 Hz, C(5)*H*), 5.32 (2H, s, OCH_2Ph), 3.81 (3H, s, OCH_3), 1.51 (2H, dd, J_{trans} 7.7 Hz J_{gem} 4.7 Hz, C(2')H_YH_Z & C(3')H_YH_Z), 1.09 (2H, dd, J_{trans} 7.8 Hz J_{gem} 4.7 Hz, C(2')H_YH_Z & C(3')H_YH_Z); ^{13}C NMR (101 MHz; $CDCl_3$): δ 173.0 (C(1')*C*=O), 167.1 (C(1)*C*=O), 143.4 (*C*(2)), 137.9 (*C*(3)), 136.5 (*C*(6)H), 135.2 (*C*(Ar')), 131.2 (*C*(4)H), 128.7 (2 × *C*(Ar')H), 128.6 (*C*(Ar')H), 128.4 (2 × *C*(Ar')H), 116.8 (*C*(1)), 115.5 (*C*(5)H), 67.2 (O*C*H_2Ph), 52.8 (O*C*H_3), 39.1 (*C*(1')), 19.2 (*C*(2')H_2 & *C*(3')H_2); LRMS *m/z* (ES+) 393 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 393.1042. $C_{19}H_{18}N_2NaO_6^+$ requires 393.1057].

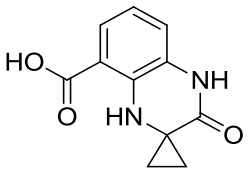
Benzyl 3'-oxo-3',4'-dihydro-1'*H*-spiro[cyclopropane-1,2'-quinoxaline]-8'-carboxylate (296)



To a solution of **295** [benzyl 2-{[1-(methoxycarbonyl)cyclopropyl]amino}-3-nitrobenzoate] (108 mg, 0.292 mmol, 1.0 eq) in DMF (5 mL) was added zinc dust (477 mg, 7.29 mmol, 25 eq) and NH_4Cl (390 mg, 7.29 mmol, 25 eq). The reaction mixture was stirred at rt for 16 h, then diluted with EtOAc (50 mL) and filtered through Celite® (eluent EtOAc). The filtrate was washed with brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 14 to 100% EtOAc in petroleum ether) yielded **296** (65 mg, 0.21 mmol, 72%) as a yellow solid. R_f 0.18 (1:4 EtOAc:Petrol); mp 216-220 °C (from $(CH_3)_2O$); ν_{max}/cm^{-1} 3346 (w), 2362 (w), 1679 (m), 1425 (m), 1244 (s); ^{1}H NMR

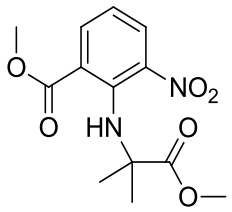
(700 MHz; $CDCl_3$): δ 8.02 (1H, br s, N*H*), 7.59 (1H, d, *J* 8.0 Hz, C(7')*H*), 7.44-7.37 (4H, m, 4 × C(Ar'')*H*), 7.37-7.33 (1H, m, C(Ar'')*H*), 7.23 (1H, br s, N*H*), 6.74 (1H, d, *J* 7.6 Hz, C(5')*H*), 6.60 (1H, dd, *J* 8.0 7.6 Hz, C(6')*H*), 5.29 (2H, s, OCH_2Ph), 1.57 (2H, dd, J_{trans} 7.6 Hz J_{gem} 5.1 Hz, C(2)H_YH_Z & C(3)H_YH_Z), 0.92 (2H, dd, J_{trans} 7.6 Hz J_{gem} 5.1 Hz, C(2)H_YH_Z & C(3)H_YH_Z); ^{13}C NMR (176 MHz; $CDCl_3$): δ 170.0 (*C*(4')=O), 167.9 (C(8')*C*=O), 138.7 (*C*(9')), 136.0 (*C*(Ar'')), 128.8 (2 × *C*(Ar'')H), 128.5 (*C*(Ar'')H), 128.1 (2 × *C*(Ar'')H), 126.2 (*C*(10')), 125.8 (*C*(7')H), 118.6 (*C*(5')H), 116.6 (*C*(6')H), 110.5 (*C*(8')), 66.5 (O*C*H_2Ph), 38.8 (*C*(1)), 17.0 (*C*(2)H_2 & *C*(3)H_2); LRMS *m/z* (ES+) 309 ($[M+H]^+$, 90%), 119 (100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 331.1049. $C_{18}H_{16}N_2NaO_3^+$ requires 331.1053].

3'-Oxo-3',4'-dihydro-1'*H*-spiro[cyclopropane-1,2'-quinoxaline]-8'-carboxylic acid (294)



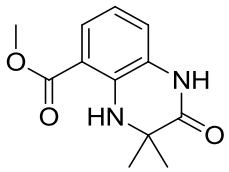
A mixture of **296** [benzyl 3'-oxo-3',4'-dihydro-1'*H*-spiro[cyclopropane-1,2'-quinoxaline]-8'-carboxylate] (53 mg, 0.17 mmol, 1.0 eq) and Pd/C (10% w/w, 4 mg, 0.004 mmol, 0.02 eq) in EtOAc (30 mL) was placed under a H_2 atmosphere. The reaction mixture was stirred for 13 h, but since starting material remained additional Pd/C was added (10 mg, 0.009 mmol, 0.06 eq). After a further 6 h the reaction mixture was diluted with EtOAc (80 mL), filtered through Celite® (eluent EtOAc) and concentrated *in vacuo* to give a mixture of **294** and **297** [3-ethyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (26 mg, 0.119 mmol, 69%) as a yellow solid that was used without further purification.

Methyl 2-[(1-methoxy-2-methyl-1-oxopropan-2-yl)amino]-3-nitrobenzoate (300)



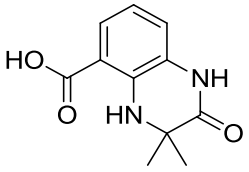
A dry microwave flask was charged with **291** [methyl 2-amino-2-methylpropanoate hydrochloride] (180 mg, 1.19 mmol, 1.5 eq), $Pd(OAc)_2$ (9 mg, 0.04 mmol, 0.05 eq), (*S*)-BINAP (49 mg, 0.076 mmol, 0.10 mmol) and Cs_2CO_3 (765 mg, 2.35 mmol, 3.0 eq) and sealed under Ar. Toluene (10 mL) was added and the resulting solution heated to 80 °C for 20 min. A solution of **107** [methyl-3-nitro-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate] (316 mg, 0.915 mg, 1.0 eq) in toluene (5 mL) was added dropwise over 25 min and the reaction mixture stirred at 80 °C for 19 h. After cooling to rt the slurry was filtered (eluent toluene) and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 3 to 26% EtOAc in petroleum ether) followed by recrystallisation from Et_2O gave **300** (68 mg, 0.23 mmol, 29%) as a yellow crystalline solid. R_f 0.48 (1:2 EtOAc:petroleum ether); mp 113-115 °C (from CH_3Cl); ν_{max}/cm^{-1} 3316 (w), 1687 (m), 1601 (m), 1538 (m), 1262 (s) (C=O); ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.20 (1H, br s, N*H*), 8.11 (1H, dd, *J* 7.9 1.7 Hz, C(4)*H*), 8.03 (1H, dd, *J* 8.1 1.7 Hz, C(6)*H*), 6.90 (1H, dd, *J* 8.1 7.9 Hz, C(5)*H*), 3.92 (3H, s, C(3)CO_2CH_3), 3.79 (3H, s, C(1')CO_2CH_3), 1.45 (6H, s, C(1')(CH_3)$_2$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 175.1 (C(1')CO_2CH_3), 167.5 (C(3)CO_2CH_3), 142.7 (*C*(Ar)), 141.4(*C*(Ar)), 136.6 (*C*(4)H), 130.8 (*C*(6)H), 121.9 (*C*(Ar)), 118.2 (*C*(5)H), 60.7 (*C*(1')), 52.6 (C(3)CO_2CH_3) & C(1')CO_2CH_3), 26.8 (C(1')(CH_3)$_2$); LRMS *m/z* (ES+) 615 ([2M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 319.0905. $C_{13}H_{16}N_2NaO_6^+$ requires 319.0901]; Anal. Calcd for $C_{13}H_{16}N_2O_6$: C 52.70%, H 5.44%, N 9.46%; Found: C 52.58%, H 5.51%, N 9.33%.

Methyl 3,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate (301)



A solution of **300** [Methyl 2-[(1-methoxy-2-methyl-1-oxopropan-2-yl)amino]-3-nitrobenzoate] (56 mg, 0.19 mmol, 1.0 eq) and Pd/C (10% w/w, 10 mg, 0.094 mmol, 0.05 eq) in EtOAc (2 mL) was stirred under a H_2 atmosphere for 25 h. The reaction mixture was filtered through Celite® (eluent EtOAc), concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 5 to 40% EtOAc in petroleum ether) to give **301** (40 mg, 0.17 mmol, 90%) as a white solid. R_f 0.33 (2:1 EtOAc:Petrol); mp 158-161 °C (from EtOAc); ν_{max}/cm^{-1} 3354 (w), 2963 (w), 2360 (w), 1672 (s), 1223 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.59 (1H, br s, N*H*), 7.59 (1H, dd, *J* 8.0 1.2 Hz, C(6)*H*), 7.50 (1H, br s, N*H*), 6.93 (1H, dd, *J* 7.7 1.2 Hz, C(8)*H*), 6.67 (1H, dd, *J* 8.0 7.7 Hz, C(7)*H*), 3.89 (3H, s, OCH_3), 1.48 (6H, s, 2 × C(3)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 171.8 (*C*(2)=O), 170.0 (C(5)*C*=O), 137.7 (*C*(9)), 126.7 (*C*(10)), 126.0 (*C*(6)H), 119.5 (*C*(8)H), 117.1 (*C*(7)H), 111.8 (*C*(5)), 55.5 (*C*(3)), 52.2 (O*C*H_3), 26.8 (2 × C(3)*C*H_3); LRMS *m/z* (ES+) 491 ([2M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 257.0901. $C_{12}H_{14}N_2NaO_3^+$ requires 257.0897]; Anal. Calcd for $C_{12}H_{14}N_2O_3$: C 61.53%, H 6.02%, N 11.96%; Found: C 61.39%, H 6.05%, N 11.82%.

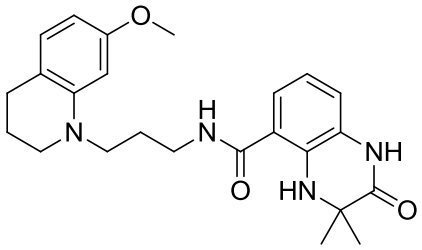
3,3-Dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid (302)



To a solution of **301** [3,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (25 mg, 0.11 mmol, 1.0 eq) in MeOH (1 mL) and THF (1 mL) was added a solution of LiOH aq. (2 M, 550 µL, 10 eq). The reaction mixture was stirred at rt for 43 h, then concentrated *in vacuo*,

diluted with H_2O (25 mL) and acidified with HCl aq. (1 M). The resulting precipitate was extracted with EtOAc (3 × 25 mL), washed with brine (25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **302** (20 mg, 0.091 mmol, 85%) as a colourless solid which was used without further purification. R_f 0.60 (1:99 AcOH:EtOAc); mp 235 °C (dec.); ν_{max}/cm^{-1} 3710 (w), 2981 (m), 1656 (w), 1309 (w), 1033 (s); ^1H NMR (400 MHz; $(CD_3)_2SO$): δ 12.89 (1H, br s, O*H*), 10.42 (1H, br s, N*H*), 7.52 (1H, br s, N*H*), 7.50 (1H, dd, *J* 7.9 1.3 Hz, C(6)*H*), 7.00 (1H, dd, *J* 7.7 1.3 Hz, C(8)*H*), 6.71 (1H, dd, *J* 7.9 7.7 Hz, C(7)*H*), 1.30 (1H, s, 2 × C(3)CH_3); ^{13}C NMR (101 MHz; $(CD_3)_2SO$): δ 169.4 (*C*=O), 169.3 (*C*=O), 136.7 (*C*(Ar)), 127.1 (*C*(Ar)), 124.8 (*C*(Ar)), 118.3 (*C*(Ar)), 116.6 (*C*(Ar)), 111.7 (*C*(Ar)), 54.0 (*C*(3)), 25.7 (2 × C(3)*C*H_3); LRMS *m/z* (ES+) 221 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 243.0744. $C_{11}H_{12}N_2NaO_3^+$ requires 243.0740].

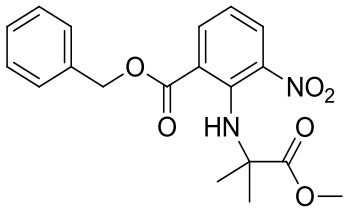
3,3-Dimethyl-2-oxo-*N*-[3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propyl]-1,2,3,4-tetrahydroquinoxaline-5-carboxamide (303)



Compound **302** [3,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylic acid] (18 mg, 0.082 mmol, 1.0 eq) and **153** [3-(7-methoxy-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine] (22 mg, 0.10 mmol, 1.2 eq) were reacted according to general procedure 1. Purification *via* silica gel chromatography (gradient elution 12 to 100% EtOAc in petroleum ether) gave **303** (25 mg, 0.079 mmol, 72%) as a brown oil. R_f 0.23 (1:1 EtOAc:Petrol); mp 174-175 °C (from $(CH_3)_2O$); ν_{max}/cm^{-1} 3326 (m), 2932 (m), 1680 (s), 1610 (m), 1509 (s), 1291 (s); ^1H NMR (400 MHz; $(CD_3)_2O$): δ 9.29 (1H, br s, N*H*), 8.00 (1H, br s, N*H*), 7.78 (1H, br s, N*H*), 7.31 (1H, dd, *J* 8.0 0.8 Hz, C(6)*H*), 6.97 (1H, dd, *J* 7.8 0.8 Hz, C(8)*H*), 6.75 (1H, d, *J* 8.1 Hz, C(5'')*H*),

6.65 (1H, dd, *J* 8.0 7.8 Hz, C(7)*H*), 6.17 (1H, d, *J* 2.4 Hz, C(8'')*H*), 6.07 (1H, dd, *J* 8.1 2.4 Hz, C(6'')*H*), 3.66 (3H, s, OCH_3), 3.47 (2H, dt, *J* 6.5 6.5 Hz, C(1')*H*), 3.37 (2H, t, *J* 7.4 Hz, C(3')*H*), 3.28 (2H, t, *J* 5.6 Hz, C(2'')*H*), 2.63 (2H, t, *J* 6.3 Hz, C(4'')*H*), 1.98-1.83 (4H, m, C(2')H_2 & C(3'')H_2), 1.35 (6H, s, 2 × C(3)CH_3); ^{13}C NMR (101 MHz; $(CD_3)_2O$): δ 170.7 (*C*(2)=O), 169.5 (C(5)*C*=O), 160.3 (*C*(7'')), 147.0 (*C*(10'')), 136.8 (*C*(9)), 130.2 (*C*(5'')H), 128.8 (*C*(10)), 122.3 (*C*(6)H), 117.6 (*C*(8)H), 117.5 (*C*(7)H), 117.1 (*C*(5)), 115.7 (*C*(9'')), 101.2 (*C*(6'')H), 98.0 (*C*(8'')H)), 55.2 (*C*(3)), 55.1 (O*C*H_3), 50.0 (*C*(2'')H_2), 49.8 (*C*(3')H_2), 38.2 (*C*(1')H_2), 28.1 (*C*(4'')H_2), 27.2 (*C*(2')H_2), 26.2 (2 × C(3)*C*H_3), 23.3 (*C*(3'')H_2); LRMS *m/z* (ES+) 423 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 423.2394. $C_{24}H_{31}N_4O_3^+$ requires 423.2391].

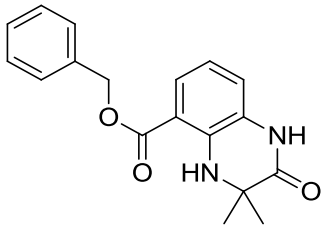
Benzyl 2-[(1-methoxy-2-methyl-1-oxopropan-2-yl)amino]-3-nitrobenzoate (304)



To a solution of **116** [benzyl 2-fluoro-3-nitrobenzoate] (603 mg, 2.19 mmol, 1.0 eq) in toluene (50 mL) was added Cs_2CO_3 (1.57 g, 4.82 mmol, 2.2 eq) and 291 [methyl 2-amino-2-methylpropanoate hydrochloride] (370 mg, 2.41 mmol, 1.1 eq). The resulting yellow solution was heated to 85 ºC for 21 h. Upon cooling to rt the reaction mixture was diluted with EtOAc (100 mL), washed with 2 M K_2CO_3 (3 × 100 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 14 to 100% CH_2Cl_2 in petroleum ether) yielded **304** (144 mg, 0.387 mmol, 18%) as a yellow oil. R_f 0.15 (1:1 CH_2Cl_2:petroleum ether); ν_{max}/cm^{-1} 3323 (w), 1736 (m), 1692 (m), 1534 (m), 1253 (s); ^{1}H NMR (700 MHz; $CDCl_3$): δ 8.20 (1H, br s, N*H*), 8.14 (1H, dd, *J* 7.9 1.6 Hz, C(6)*H*), 8.02 (1H, dd, *J* 8.1 1.6 Hz, C(4)*H*), 7.44 (2H, d, *J* 7.3 Hz, C(2'')*H* & C(6'')*H*), 7.41 (2H, dd, *J*

7.3 7.2 Hz, C(3’’)*H* & C(5’’)*H*), 7.38 (1H, d, *J* 7.2 Hz, C(4’’)*H*), 6.88 (1H, dd, *J* 8.1 7.9 Hz, C(5)*H*), 5.34 (2H, s, OCH_2Ph), 3.78 (3H, s, C(1’)O_2CH_3), 1.44 (6H, s, 2 × C(2’)CH_3); ^{13}C NMR (176 MHz; $CDCl_3$): δ 175.1 (*C*(1’)=O), 166.8 (C(1)*C*=O), 142.9 (*C*(2)), 141.5 (*C*(3)), 136.7 (*C*(6)H), 135.2 (*C*(1’’)), 130.9 (*C*(4)H), 128.7 (*C*(3’’)H & *C*(5’’)H), 128.6 (*C*(4’’)H), 128.5 (*C*(2’’)H & *C*(6’’)H), 121.8 (*C*(1)), 118.2 (*C*(5)H), 67.4 (C(1’)O_2*C*H_3), 60.7 (*C*(2’)CH_3), 52.6 (C(1’)O_2*C*H_3), 26.8 (2 × C(2’)*C*H_3); LRMS *m/z* (ES+) 373 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+H)$^+$ 373.1404. $C_{19}H_{21}N_2O_6^+$ requires 373.1394].

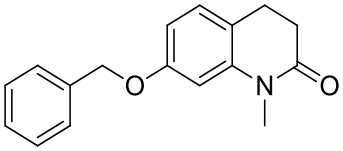
Benzyl 3,3-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoxaline-5-carboxylate (305)



To a solution of **304** [benzyl 2-[(1-methoxy-2-methyl-1-oxopropan-2-yl)amino]-3-nitrobenzoate] (123 mg, 0.330 mmol, 1.0 eq) in DMF (6 mL) was added zinc dust (540 mg, 8.26 mmol, 25 eq) and NH_4Cl (442 mg, 8.26 mmol, 25 eq). The reaction mixture was stirred at rt for 14 h, then diluted with EtOAc (50 mL) and filtered through Celite® (eluent EtOAc). The filtrate was washed with brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 14 to 100% EtOAc in petroleum ether) yielded **305** (61 mg, 0.20 mmol, 60%) as a colourless solid. R_f 0.23 (1:4 EtOAc:Petrol); mp 204-206 ° C (from $(CH_3)_2O$); ν_{max}/cm^{-1} 3330 (w), 2928 (w), 2359 (w), 1682 (s), 1244 (m), 1152 (m); ^{1}H NMR (400 MHz; $(CD_3)_2O$): δ 9.45 (1H, br s, N*H*), 7.59 (1H, dd, *J* 8.0 1.3 Hz, C(6)*H*), 7.54-7.48 (3H, m, 2 × C(Ar’)*H* & N*H*), 7.46-7.39 (2H, m, 2 × C(Ar’)*H*), 7.39-7.34 (1H, m, C(Ar’)*H*), 7.07 (1H, d, *J* 7.7 Hz, C(8)*H*), 6.71 (1H, dd, *J* 8.0 7.7 Hz, C(7)*H*), 5.36 (2H, s, OCH_2Ph), 1.39 (6H, s, 2 × C(3)CH_3); ^{13}C NMR (176 MHz; $(CD_3)_2O$): δ 169.2 (*C*(2)=O), 167.3 (C(5)*C*=O), 137.2 (*C*(9)), 136.4 (*C*(Ar’)), 128.4 (2 × *C*(Ar’)H), 128.03 (*C*(Ar’)H), 127.99

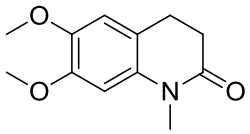
(2 × *C*(Ar')H), 124.6 (*C*(6)H), 118.5 (*C*(8)H), 118.4 (*C*(10)), 116.7 (*C*(7)H), 111.2 (*C*(5)), 65.9 (O*C*H_2Ph), 54.6 (*C*(3)), 25.4 (2 × C(3)*C*H_3); LRMS *m/z* (ES+) 311 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 333.1209. $C_{18}H_{18}N_2NaO_3^+$ requires 333.1210].

7-(Benzyloxy)-1-methyl-3,4-dihydroquinolin-2(1H)-one (306)



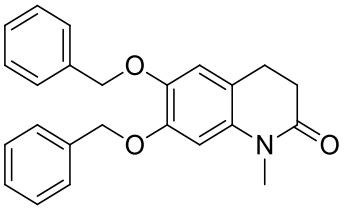
To a solution of **173** [7-(benzyloxy)-3,4-dihydroquinolin-2(1H)-one] (158 mg, 0.624 mmol, 1.0 eq) in THF (6 mL) was added NaH (60% dispersion in oil, 37 mg, 0.94 mmol, 1.5 eq) and the mixture stirred for 5 min. MeI (58 µL, 132 mg, 0.936 mmol, 1.5 eq) was added and the reaction mixture stirred for 20 h. The reaction mixture was diluted with EtOAc (50 mL), washed with H_2O (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (gradient elution 6 to 50 % EtOAc in petroleum ether) yielded **306** (151 mg, 0.565 mmol, 91%) as a colourless solid. R_f 0.23 (1:3 EtOAc:Petrol); mp 72-73 °C (from EtOAc); ν_{max}/cm^{-1} 3062 (w), 2973 (w), 1666 (s), 1385 (s), 1128 (s); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.49-7.38 (4H, m, 4 × C(Ar)*H*), 7.38-7.31 (1H, m, C(Ar)*H*), 7.06 (1H, d, *J* 8.1 Hz, C(5)*H*), 6.65 (1H, d, *J* 2.3 Hz, C(8)*H*), 6.62 (1H, dd, *J* 8.1 2.3 Hz, C(6)*H*), 5.08 (2H, s, OCH_2Ph), 3.32 (3H, s, N(1)CH_3), 2.84 (2H, t, *J* 7.3 Hz, C(4)H_2), 2.64 (2H, t, *J* 7.3 Hz, C(3)H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 170.7 (*C*(2)=O), 158.5 (*C*(7)), 141.8 (*C*(10)), 136.9 (*C*(Ar)), 128.8 (2 × *C*(Ar)H), 128.3 (*C*(5)H), 128.2 (*C*(Ar)H), 127.6 (2 × *C*(Ar)H), 118.8 (*C*(9)), 107.6 (*C*(6)H), 103.5 (*C*(8)H), 70.4 (O*C*H_2Ph), 32.2 (*C*(3)H_2), 29.7 (N(1)*C*H_3), 24.8 (*C*(4)H_2); LRMS *m/z* (ES+) 268 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 290.1153. $C_{17}H_{17}NNaO_2^+$ requires 290.1151]; HPLC (C-18) retention time = 12.78 min, 100%.

6,7-Dimethoxy-1-methyl-3,4-dihydroquinolin-2(1H)-one (307)



To a solution of **180** [6,7-dimethoxy-3,4-dihydroquinolin-2(1H)-one] (20 mg, 0.097 mmol, 1.0 eq) in THF (0.5 mL) was added NaH (60% dispersion in oil, 8 mg, 0.2 mmol, 2.1 eq) and the mixture stirred for 5 min. MeI (15 mg, 0.11 mmol, 1.1 eq) in THF (0.5 mL) was added and the reaction mixture stirred for 20 h. The reaction mixture was diluted with EtOAc (50 mL), washed with H_2O (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:2 then 1:1 EtOAc:petroleum ether) yielded **307** (14 mg, 0.063 mmol, 65%) as a waxy yellow solid. R_f 0.24 (1:1 EtOAc:Petrol); mp 130-132 °C (from EtOAc); ν_{max}/cm^{-1} 2936 (w), 1644 (s), 1524 (m), 1351 (m), 1210 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.69 (1H, s, C(5)*H*), 6.56 (1H, s, C(8)*H*), 3.89 (3H, s, C(7)OC*H*$_3$), 3.85 (3H, s, C(6)OC*H*$_3$), 3.34 (3H, s, N(1)C*H*$_3$), 2.82 (2H, t, *J* 7.4 Hz, C(4)*H*$_2$), 2.61 (2H, t, *J* 7.4 Hz, C(3)*H*$_2$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 170.2 (*C*(2)=O), 148.0 (*C*(7)), 144.4 (*C*(6)), 134.1 (*C*(10)), 117.9 (*C*(9)), 111.6 (*C*(5)H), 100.5 (*C*(8)H), 56.3 (C(6)O*C*H$_3$ & C(7)O*C*H$_3$), 32.0 (*C*(3)H$_2$), 29.7 (N(1)*C*H$_3$), 25.0 (*C*(4)H$_2$); LRMS *m/z* (ES+) 222 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 244.0950. $C_{12}H_{15}NNaO_3^+$ requires 244.0944]; HPLC (C-18) retention time = 9.53 min, 98.2%.

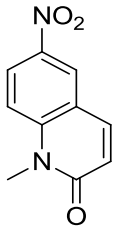
6,7-Bis(benzyloxy)-1-methyl-3,4-dihydroquinolin-2(1H)-one (308)



Compound **181** [6,7-bis(benzyloxy)-3,4-dihydroquinolin-2(1*H*)-one] (76 mg, 0.21 mmol, 1.0 eq) in THF (1 mL) was added NaH (60% dispersion in oil, 9 mg, 0.2 mmol, 1.1 eq) and the

mixture stirred for 5 min. MeI (33 mg, 0.23 mmol, 1.1 eq) in THF (1 mL) was added and the reaction mixture stirred for 40 h. The reaction mixture was diluted with EtOAc (50 mL), washed with H_2O (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica gel chromatography (1:4 then 1:2 EtOAc:petroleum ether) yielded **308** (57 mg, 0.15 mmol, 73%) as a waxy yellow solid. R_f 0.24 (1:2 EtOAc:Petrol); mp 65-68 °C (from CH_3CN); ν_{max}/cm^{-1} 2888 (w), 1665 (s), 1517 (m), 1348 (m), 1248 (s); ^1H NMR (400 MHz; $CDCl_3$): δ 7.45 (4H, d, *J* 7.2 Hz, C(2')*H*, C(6')*H*, C(2'')*H* & C(6'')*H*), 7.38 (4H, dd, *J* 7.2 7.2 Hz, C(3')*H*, C(5')*H*, C(3'')*H* & C(5'')*H*), 7.35-7.30 (2H, m, C(4')*H* & C(4'')*H*), 6.79 (1H, s, C(5)*H*), 6.62 (1H, s, C(8)*H*), 5.17 (2H, s, OCH_2Ph), 5.13 (2H, s, OCH_2Ph), 3.25 (3H, s, N(1)CH_3), 2.78 (2H, t, *J* 7.3 Hz, C(4)H_2), 2.60 (2H, t, *J* 7.3 Hz, C(3)H_2); ^{13}C NMR (101 MHz; $CDCl_3$): δ 170.3 (*C*(2)=O), 148.2 (*C*(6/7)), 144.7 (*C*(7/6)), 137.4 (*C*(1')), 137.3 (*C*(1'')), 135.0 (*C*(10)), 128.7 (*C*(3')H & *C*(5')H), 128.6 (*C*(3'')H & *C*(5'')H), 128.1 (*C*(4')H), 128.0 (*C*(4'')H), 127.7 (*C*(2')H & *C*(6')H), 127.6 (*C*(2'')H & *C*(6'')H), 119.4 (*C*(9)), 115.9 (*C*(5)H), 104.9 (*C*(8)H), 72.5 (O*C*H_2Ph), 72.3 (O*C*H_2Ph), 32.0 (*C*(3)H_2), 29.7 (N(1)*C*H_3), 25.1 (*C*(4)H_2); LRMS *m/z* (ES+) 396 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 396.1577. $C_{24}H_{23}NNaO_3^+$ requires 396.1570]; HPLC (C-18) retention time = 13.76 min, 99.6%.

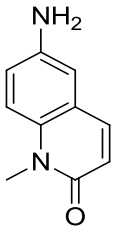
1-Methyl-6-nitroquinolin-2(1*H*)-one (310)



To a solution of 1-methyl-2(1*H*)-one (1.00 g, 6.28 mmol, 1.0 eq) in fuming sulfuric acid (98%, 7.0 mL) at 0 °C was added nitric acid (70%, 7 mL) dropwise over 5 min. The reaction mixture was allowed to warm to rt and stirred for 90 min. The reaction was quenched by pouring onto crushed ice and the resulting yellow precipitate was extracted into EtOAc (3 × 100 mL). The

combined organics were washed with brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Recrystallisation from EtOAc (25 mL) afforded **310** (400 mg, 1.96 mmol, 31%) as a yellow solid. R_f 0.28 (1:1 EtOAc:Petrol); mp 212-214 °C (from EtOAc, with sublimation, lit. 225-226 °C); ν_{max}/cm^{-1} 3068 (w), 1662 (s), 1603 (s), 1522 (m), 1339 (s), 1296 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.48 (1H, d, *J* 2.6 Hz, C(5)*H*), 8.40 (1H, dd, *J* 9.3 2.6 Hz, C(7)*H*), 7.77 (1H, d, *J* 9.5 Hz, C(4)*H*), 7.46 (1H, d, *J* 9.3 Hz, C(8)*H*), 6.83 (1H, d, *J* 9.5 Hz, C(3)*H*), 3.77 (3H, s, N(1)CH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 162.1 (*C*(2)=O), 144.1 (*C*(10)), 142.3 (*C*(6)), 138.7 (*C*(4)H), 125.4 (*C*(7)H), 124.7 (*C*(5)H), 124.1 (*C*(3)H), 120.2 (*C*(9)), 115.0 (*C*(8)H), 30.2 (N(1)*C*H_3); LRMS *m/z* (ES+) 227 ([M+Na]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 227.0434. $C_{10}H_8N_2NaO_3^+$ requires 227.0427].

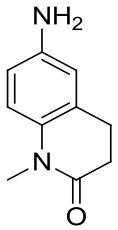
6-Amino-1-methylquinolin-2(1*H*)-one (311)



A solution of **310** [1-methyl-6-nitroquinolin-2(1*H*)-one] (300 mg, 1.47 mmol, 1.0 eq) and Pd/C (10% w/w, 78 mg, 0.73 mmol, 0.05 eq) in EtOAc (10 mL) was stirred under a H_2 atmosphere for 65 h. The reaction mixture was filtered through Celite® (eluent EtOAc), concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 50 to 100 % EtOAc in petroleum ether) to give **311** (160 mg, 0.918 mmol, 62%) as a yellow solid. R_f 0.11 (100% EtOAc); mp 166-167 °C (from EtOAc, lit. 168 °C); ν_{max}/cm^{-1} 3326 (m), 3222 (m), 1641 (m), 1564 (s), 1449 (m); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.48 (1H, d, *J* 9.4 Hz, C(4)*H*), 7.16 (1H, d, *J* 8.9 Hz, C(8)*H*), 6.95 (1H, dd, *J* 8.9 2.6 Hz, C(7)*H*), 6.80 (1H, d, *J* 2.6 Hz, C(5)*H*), 6.64 (1H, d, *J* 9.4 Hz, C(3)*H*), 3.70 (2H, br s, NH_2), 3.65 (3H, s, N(1)CH_3); ^{13}C NMR (101 MHz; $CDCl_3$): δ 161.8 (*C*(2)=O), 141.4 (*C*(6)), 138.2 (*C*(4)H), 133.3 (*C*(Ar)), 122.0 (*C*(3)H), 121.6 (*C*(Ar)),

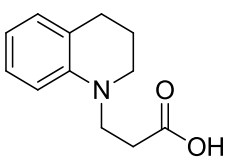
119.5 (*C*(7)H), 115.1 (*C*(8)H), 112.7 (*C*(5)H), 29.4 (N(1)*C*H$_3$); LRMS *m/z* (ES+) 175 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 197.0687. $C_{10}H_{10}N_2NaO^+$ requires 197.0685]; Anal. calcd for $C_{10}H_{10}N_2O$: C 68.95%, H 5.79%, N 16.08%; Found: C 69.08%, H 5.87%, N 15.99%.

6-Amino-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (312)



From purification of the above reaction could be isolated **312** (69 mg, 0.39 mmol, 26%) as a pale yellow solid. R_f 0.23 (100% EtOAc); mp 99-101 °C (from EtOAc); ν_{max}/cm^{-1} 3203 (m), 2911 (w), 1641 (s), 1471 (m), 1372 (s), 1126 (s); ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.78 (1H, d, *J* 8.5 Hz, C(8)*H*), 6.53 (1H, dd, *J* 8.5 2.6 Hz, C(7)*H*), 6.53 (1H, d, *J* 2.6 Hz, C(5)*H*), 3.44 (2H, br s, C(6)N*H*$_2$), 3.30 (3H, s, N(1)C*H*$_3$), 2.80 (2H, t, *J* 7.3 Hz, C(4)*H*), 2.59 (2H, t, *J* 7.3 Hz, C(3)*H*); ^{13}C NMR (126 MHz; $CDCl_3$): δ 170.1 (*C*(2)=O), 141.9 (*C*(6)), 132.9 (*C*(10)), 127.7 (*C*(9)), 115.8 (*C*(8)H), 115.1 (*C*(5)H), 113.7 (*C*(7)H), 31.9 (*C*(3)H$_2$), 29.7 (N(1)*C*H$_3$), 25.7 (*C*(4)H$_2$); LRMS *m/z* (ES+) 177 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 119.0844. $C_{10}H_{12}N_2NaO^+$ requires 199.0842]; Anal. calcd for $C_{10}H_{12}N_2O$: C 68.16%, H 6.86%, N 15.90%; Found: C 68.27%, H 6.97%, N 15.81%.

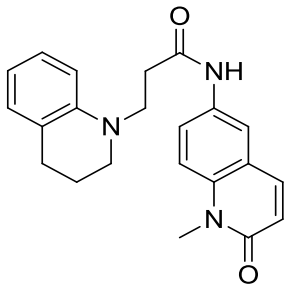
3-(3,4-dihydroquinolin-1(2*H*)-yl)propanoic acid (313)



To a solution of **136** [methyl 3-(3,4-dihydroquinolin-1(2*H*)-yl)propanoate] (1.68 g, 7.66 mmol, 1.0 eq) in MeOH (40 mL) was added a solution of LiOH. (917 mg, 38.3 mmol, 5 eq) in H_2O

(10 mL). The reaction mixture was stirred at rt for 19 h, then concentrated *in vacuo*. The resulting residue was resuspended in H_2O (250 mL), acidified with HCl aq. (1 M), extracted into EtOAc (250 mL), washed with brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **313** (341 mg, 1.66 mmol, 22%) as a brown oil that was used without further purification. R_f 0.67 (1:99 AcOH:EtOAc); ν_{max}/cm^{-1} 3020 (s), 1704 (s), 1601 (m), 1499 (m); ^1H NMR (500 MHz; $CDCl_3$): δ 10.4 (1H, br s, O*H*), 7.10 (1H, dd, *J* 8.4 8.4 Hz, C(Ar)*H*), 6.00 (1H, dd, *J* 7.5 0.5 Hz, C(Ar)*H*), 6.68-6.62 (2H, m, C(Ar)*H* & C(Ar)*H*), 3.66 (2H, t, *J* 7.3 Hz, C(3)H_2), 3.32 (2H, t, *J* 5.6 Hz, C(2')H_2), 2.78 (2H, t, *J* 6.4 Hz, C(4')H_2), 2.68 (2H, t, *J* 7.2 Hz, C(2)H_2), 2.02-1.95 (2H, m, C(3')H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 178.8 (*C*(1)=O), 144.5 (*C*(10')), 129.5 (*C*(Ar)H), 127.3 (*C*(Ar)H), 123.1 (*C*(9')), 116.6 (*C*(Ar)H), 110.9 (*C*(Ar)H), 49.5 (*C*(2')H_2), 47.2 (*C*(3)H_2), 31.2 (*C*(2)H_2), 28.1 (*C*(4')H_2), 22.2 (*C*(3')H_2); LRMS *m/z* (ES+) 206 ($[M+H]^+$, 100%); HRMS *m/z* (ES+) [Found: $(M+H)^+$ 206.1179. $C_{12}H_{16}NO_2^+$ requires 206.1176].

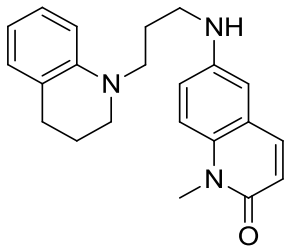
6-{[3-(3,4-dihydroquinolin-1(2*H*)-yl)propanoyl]amino}-1-methylquinolin-2(1*H*)-one (314)



Compounds **311** [6-amino-1-methylquinolin-2(1*H*)-one] (33 mg, 0.19 mmol, 1.0 eq) and **313** [3-(3,4-dihydroquinolin-1(2*H*)-yl)propanoic acid] (49 mg, 0.24 mmol, 1.25 eq) were reacted according to general procedure 1 for 47 h. Purification *via* silica gel chromatography (gradient elution 20 to 100 % EtOAc in petroleum ether) yielded **314** (46 mg, 0.13 mmol, 67%) as a pale yellow solid. R_f 0.13 (2:1 EtOAc:Petrol); mp 195-197 °C (from EtOAc); ν_{max}/cm^{-1} 3274 (w),

2936 (w), 2243 (w), 1643 (s), 1500 (m), 1439 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.97 (1H, br s, N*H*), 7.89 (1H, d, *J* 2.4 Hz, C(5)*H*), 7.61 (1H, d, *J* 9.5 Hz, C(4)*H*), 7.50 (1H, dd, *J* 9.1 2.4 Hz, C(7)*H*), 7.28 (1H, d, *J* 9.1 Hz, C(8)*H*), 7.09 (1H, dd, *J* 7.7 7.7 Hz, C(7'')*H*), 7.02 (1H, d, *J* 7.2 Hz, C(5'')*H*), 6.76-6.66 (3H, m, C(6'')*H* & C(8'')*H* & C(3)*H*), 3.73 (2H, t, *J* 6.4 Hz, C(3')H_2), 3.69 (3H, s, N(1)CH_3), 3.34 (2H, t, *J* 5.8 Hz, C(2'')H_2), 2.77 (2H, t, *J* 6.2 Hz, C(4'')H_2), 2.71 (2H, t, *J* 6.4 Hz, C(2')H_2), 1.96 (2H, tt, *J* 6.2 5.8 Hz, C(3'')H_2); ^{13}C NMR (126 MHz; $CDCl_3$): δ 170.2 (*C*(1')=O), 162.1 (*C*(2)=O), 144.3 (*C*(10'')), 138.8 (*C*(4)H), 136.8 (*C*(10)), 132.54 (*C*(6)), 129.6 (*C*(5'')H), 127.3 (*C*(7'')H), 123.8 (*C*(9'')), 122.9 (*C*(7)H), 122.4 (*C*(3)H), 120.9 (*C*(9)), 119.2 (*C*(5)H), 117.6 (*C*(6'')H), 114.6 (*C*(8)H), 111.8 (*C*(8'')H), 49.8 (*C*(2'')H_2), 48.6 (*C*(3')H_2), 34.6 (*C*(2')H_2), 29.5 (N(1)*C*H_3), 27.8 (*C*(4'')H_2), 21.9 (*C*(3'')H_2); LRMS *m/z* (ES+) 362 ([M+H]$^+$, 100%); HRMS *m/z* (ES+) [Found: (M+Na)$^+$ 384.1683. $C_{22}H_{23}N_3NaO_2^+$ requires 384.1682]; Anal. calcd for $C_{22}H_{23}N_3O_2$: C 73.11%, H 6.41%, N 11.63%; Found: C 73.03%, H 6.33%, N 11.67%.

6-{[3-(3,4-dihydroquinolin-1(2*H*)-yl)propyl]amino}-1-methylquinolin-2(1*H*)-one (316)



A solution of dimethyl sulfoxide (51 µL, 56 mg, 0.71 mmol, 2.2 eq) in CH_2Cl_2 (0.5 mL) was added dropwise over 20 min to a solution of oxalyl chloride (30 µL, 45 mg, 0.36 mmol, 1.1 eq) in CH_2Cl_2 (0.5 mL) at −78 °C. The mixture was stirred at −78 °C for 20 min before a solution of **140** [3-(3,4-dihydroquinolin-1(2*H*)-yl)propan-1-ol] (62 mg, 0.32 mmol, 1.0 eq) in CH_2Cl_2 (0.5 mL) was added. The resulting pale red solution was stirred for a further 3, quenched *via* addition of Et_3N and warmed to rt. The solution was diluted with CH_2Cl_2 (25 mL), washed with H_2O (3 × 25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give

315 [3-(3,4-dihydroquinolin-1(2*H*)-yl)propanal] (60 mg, 0.32 mmol). The crude material was used without further purification. To a solution of the aldehyde **312** (50 mg, 0.29 mmol, 1.0 eq) in MeCN (2 mL) was added sequentially a solution of **311** [3-(3,4-dihydroquinolin-1(2*H*)-yl)propanal] (60 mg, 0.32 mmol, 1.1 eq) in MeCN (2 mL), $NaBH_3CN$ (20 mg, 0.32 mmol, 1.1 eq) and acetic acid (2 drops, ~ 0.2 eq). The reaction mixture was stirred at rt for 16 h then concentrated *in vacuo* and purified *via* silica gel chromatography (gradient elution 12 to 100 % EtOAc in petroleum ether) to give **316** (12 mg, 0.035 mmol, 12%) as a yellow oil. R_f 0.23 (2:1 EtOAc:Petrol); ν_{max}/cm^{-1} 3326 (w), 2925 (w), 1728 (s), 1647 (s), 1571 (s), 1503 (m); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.52 (1H, d, J 9.5 Hz, C(4)*H*), 7.20 (1H, d, J 9.0 Hz, C(8)*H*), 7.02 (1H, dd, J 7.0 7.0 Hz, C(7'')*H*), 6.96 (1H, d, J 7.0 Hz, C(5'')*H*), 6.91 (1H, dd, J 9.0 2.7 Hz, C(7)*H*), 6.70 (1H, d, J 2.7 Hz, C(5)*H*), 6.67 (1H, d, J 9.5 Hz, C(3)*H*), 6.62-6.56 (2H, m, C(6'')*H* & C(8'')*H*), 3.69 (3H, s, N(1)CH_3), 3.41 (2H, t, J 7.0 Hz, C(3')H_2), 3.29 (2H, t, J 5.7 Hz, C(2'')H_2), 3.25 (2H, t, J 6.8 Hz, C(1')H_2), 2.76 (2H, t, J 6.4 Hz, C(4'')H_2), 2.00-1.92 (4H, m, C(2')H_2 & C(3'')H_2); ^{13}C NMR (500 MHz; $CDCl_3$): δ 161.9 (*C*(2)=O), 145.3 (*C*(10'')), 143.5 (*C*(Ar)), 138.6 (*C*(4)H), 133.0 (*C*(Ar)), 129.5 (*C*(5'')H), 127.3 (*C*(7'')H), 122.8 (*C*(9'')), 122.2 (*C*(3)H), 121.9 (*C*(Ar)), 118.5 (*C*(7)H), 116.1 (*C*(6'')H), 115.3 (*C*(8)H), 110.8 (*C*(8'')H), 109.7 (*C*(5)H), 49.9 (*C*(2'')H_2), 49.6 (*C*(3')H_2), 42.7 (*C*(1')H_2), 29.5 (N(1)*C*H_3), 28.2 (*C*(4'')H_2), 26.6 (*C*(2')H_2), 22.4 (*C*(3'')H_2); LRMS *m/z* (ES–) 346 ([M–H]$^-$, 100%); HRMS *m/z* (ES+) [Found: $(M+Na)^+$ 370.1883. $C_{22}H_{25}N_3NaO^+$ requires 370.1890]; HPLC (C-18) retention time = 9.71 min, 93.7%.

Chapter 7

References

(1) Deribe, Y.; Pawson, T.; Dikic, I. *Nat. Struct. Mol. Biol.* **2010**, *17*, 666–672.

(2) Choudhary, C.; Kumar, C.; Gnad, F.; Nielsen, M.; Rehman, M.; Walther, T.; Olsen, J.; Mann, M. *Science* **2009**, *325*, 834–840.

(3) Hornbeck, P.; Kornhauser, J.; Tkachev, S.; Zhang, B.; Skrzypek, E.; Murray, B.; Latham, V.; Sullivan, M. *Nucleic Acids Res.* **2012**, *40*, D261–D270.

(4) Kouzarides, T. *EMBO J.* **2000**, *19*, 1176–1179.

(5) Allfrey, V.; Faulkner, R.; Mirsky, A. *Proc. Natl. Acad. Sci.* **1964**, *51*, 786–794.

(6) Wang, G. G.; Allis, C. D.; Chi, P. *Trends Mol. Med.* **2007**, *13*, 363-372.

(7) Jennings, L.; Measures, A.; Wilson, B.; Conway, S. *Future Med. Chem.* **2014**, *6*, 179–204.

(8) Marushige, K. *Proc. Natl. Acad. Sci.* **1976**, *73*, 3937–3941.

(9) Kouzarides, T. *Cell* **2007**, *128*, 693–705.

(10) Strahl, B. D.; Allis, C. D. *Nature* **2000**, *403*, 41–45.

(11) Jenuwein, T.; Allis, D. *Science* **2001**, *293*, 1074–1080.

(12) Martin, C.; Zhang, Y. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 838–849.

(13) Bannister, A.; Kouzarides, T. *Cell Res.* **2011**, *21*, 381–395.

(14) Lee, J.-S.; Smith, E.; Shilatifard, A. *Cell* **2010**, *142*, 682–685.

(15) Turner, B. *Nat. Cell Biol.* **2007**, *9*, 2–6.

(16) Zhao, S.; Xu, W.; Jiang, W.; Yu, W.; Lin, Y.; Zhang, T.; Yao, J.; Zhou, L.; Zeng, Y.; Li, H.; Li, Y.; Shi, J.; An, W.; Hancock, S.; He, F.; Qin, L.; Chin, J.; Yang, P.; Chen, X.; Lei, Q.; Xiong, Y.; Guan, K.-L. *Science* **2010**, *327*, 1000–1004.

(17) Arrowsmith, C.; Bountra, C.; Fish, P.; Lee, K.; Schapira, M. *Nat. Rev. Drug Discov.* **2012**, *11*, 384–400.

(18) Bolden, J.; Peart, M.; Johnstone, R. *Nat. Rev. Drug Discov.* **2006**, *5*, 769–784.

(19) Cherblanc, F.; Davidson, R.; Di Fruscia, P.; Srimongkolpithak, N.; Fuchter, M. *Nat. Prod. Rep.* **2013**, *30*, 605–624.

(20) Cole, P. *Nat. Chem. Biol.* **2008**, *4*, 590–597.

(21) Edwards, A.; Bountra, C.; Kerr, D.; Willson, T. *Nat. Chem. Biol.* **2009**, *5*, 436–440.

(22) Bunnage, M.; Chekler, E.; Jones, L. *Nat. Chem. Biol.* **2013**, *9*, 195–199.

(23) Tamkun, J.; Deuring, R.; Scott, M.; Kissinger, M.; Pattatucci, A.; Kaufman, T.; Kennison, J. *Cell* **1992**, *68*, 561–572.

(24) Haynes, S.; Dollard, C.; Winston, F.; Beck, S.; Trowsdale, J.; Dawid, I. *Nucleic Acids Res.* **1992**, *20*, 2603–2603.

(25) Filippakopoulos, P.; Picaud, S.; Mangos, M.; Keates, T.; Lambert, J.-P.; Barsyte-Lovejoy, D.; Felletar, I.; Volkmer, R.; Müller, S.; Pawson, T.; Gingras, A.-C.; Arrowsmith, C. H.; Knapp, S. *Cell* **2012**, *149*, 214–231.

(26) Hewings, D.; Rooney, T.; Jennings, L.; Hay, D.; Schofield, C.; Brennan, P.; Knapp, S.; Conway, S. *J. Med. Chem.* **2012**, *55*, 9393–9413.

(27) Prinjha, R. K.; Witherington, J.; Lee, K. *Trends Pharmacol. Sci.* **2012**, *33*, 146–153.

(28) Zhang, G.; Sanchez, R.; Zhou, M.-M. *J. Med. Chem.* **2012**, *55*, 7342–7345.

(29) Conway, S. *ACS Med. Chem. Lett.* **2012**, *3*, 691–694.

(30) Muller, S.; Filippakopoulos, P.; Knapp, S. *Expert Rev. Mol. Med.* **2011**, *13*.

(31) Wells, J.; McClendon, C. *Nature* **2007**, *450*, 1001–1009.

(32) Vidler, L. R.; Brown, N.; Knapp, S.; Hoelder, S. *J. Med. Chem.* **2012**, *55*, 7346–7359.

(33) Partanen, A.; Motoyama, J.; Hui, C. C. *Int. J. Dev. Biol.* **1999**, *43*, 487–494.

(34) Chrivia, J.; Kwok, R.; Lamb, N.; Hagiwara, M.; Montminy, M.; Goodman, R. *Nature* **1993**, *365*, 855–859.

(35) Ogryzko, V. *Cell* **1996**, *87*, 953–959.

(36) Bannister, A. J.; Kouzarides, T. *Nature* **1996**, *384*, 641–643.

(37) Plotnikov, A.; Yang, S.; Zhou, T.; Rusinova, E.; Frasca, A.; Zhou, M.-M. *Structure* **2014**, *22*, 353–360.

(38) Wang, N.; Majmudar, C.; Pomerantz, W.; Gagnon, J.; Sadowsky, J.; Meagher, J.; Johnson, T.; Stuckey, J.; Brooks, C.; Wells, J.; Mapp, A. *J. Am. Chem. Soc.* **2013**, *135*, 3363–3366.

(39) Newton, A.; Sharpe, B.; Kwan, A.; Mackay, J.; Crossley, M. *J. Biol. Chem.* **2000**, *275*, 15128–15134.

(40) Delvecchio, M.; Gaucher, J.; Aguilar-Gurrieri, C.; Ortega, E.; Panne, D. *Nat. Struct. & Mol. Biol.* **2013**, *20*, 1040–1046.

(41) Wojciak, J.; Martinez-Yamout, M.; Dyson, J.; Wright, P. *EMBO J.* **2009**, *28*, 948–958.

(42) Legge, G.; Martinez-Yamout, M.; Hambly, D.; Trinh, T.; Lee, B.; Dyson, J.; Wright, P. *J. Mol. Biol.* **2004**, *343*, 1081–1093.

(43) Kjaergaard, M.; Teilum, K.; Poulsen, F. *Proc. Natl. Acad. Sci.* **2010**, *107*, 12535–12540.

(44) Giles, R.; Peters, D.; Breuning, M. *Trends Genet.* **1998**, *14*, 178–183.

(45) Henry, R.; Kuo, Y.-M.; Andrews, A. *Biochemistry* **2013**, *52*, 5746–5759.

(46) Kalkhoven, E. *Biochem. Pharmacol.* **2004**, *68*, 1145–1155.

(47) Bedford, D.; Kasper, L.; Fukuyama, T.; Brindle, P. *Epigenetics* **2010**, *5*, 9–15.

(48) Wang, F.; Marshall, C.; Ikura, M. *Cell & Mol. Life Sci.* **2013**, *70*, 3989–4008.

(49) Ong, C.-T.; Corces, V. *Nat Rev Genet.* **2011**, *12*, 283–293.

(50) Goodman, R. H.; Smolik, S. *Genes & Dev.* **2000**, *14*, 1553–1577.

(51) Stauffer, D.; Chang, B.; Huang, J.; Dunn, A.; Thayer, M. *J. Biol. Chem.* **2007**, *282*, 9678–9687.

(52) Kasper, L.; Lerach, S.; Wang, J.; Wu, S.; Jeevan, T.; Brindle, P. *EMBO J.* **2010**, *29*, 3660–3672.

(53) Muller, S.; Filippakopoulos, P.; Knapp, S. *Expert Rev. Mol. Med.* **2011**, *13*, e29.

(54) Gu, W.; Roeder, R. *Cell* **1997**, *90*, 595–606.

(55) Pao, G. M.; Janknecht, R.; Ruffner, H.; Hunter, T.; Verma, I. M. *Proc. Natl. Acad. Sci.*

2000, *97*, 1020–1025.

(56) Dai, P.; Akimaru, H.; Tanaka, Y.; Hou, D. X.; Yasukawa, T.; Kanei-Ishii, C.; Takahashi, T.; Ishii, S. *Genes & Dev.* **1996**, *10*, 528–540.

(57) Pasqualucci, L.; Dominguez-Sola, D.; Chiarenza, A.; Fabbri, G.; Grunn, A.; Trifonov, V.; Kasper, L.; Lerach, S.; Tang, H.; Ma, J.; Rossi, D.; Chadburn, A.; Murty, V.; Mullighan, C.; Gaidano, G.; Rabadan, R.; Brindle, P.; Dalla-Favera, R. *Nature* **2011**, *471*, 189–195.

(58) Bouchal, J.; Santer, F.; Höschele, P.; Tomastikova, E.; Neuwirt, H.; Culig, Z. *Prostate* **2011**, *71*, 431–437.

(59) Panagopoulos, I.; Fioretos, T.; Isaksson, M.; Samuelsson, U.; Billström, R.; Strömbeck, B.; Mitelman, F.; Johansson, B. *Hum. Mol. Genet.* **2001**, *10*, 395–404.

(60) Sobulo, O. M.; Borrow, J.; Tomek, R.; Reshmi, S.; Harden, A.; Schlegelberger, B.; Housman, D.; Doggett, N. A.; Rowley, J. D.; Zeleznik-Le, N. J. *Proc. Natl. Acad. Sci.* **1997**, *94*, 8732–8737.

(61) Lavau, C.; Du, C.; Thirman, M.; Zeleznik-Le, N. *EMBO J.* **2000**, *19*, 4655–4664.

(62) French, C. *Annu. Rev. Pathol. Mech. Dis.* **2012**, *7*, 247–265.

(63) Reynoird, N.; Schwartz, B.; Delvecchio, M.; Sadoul, K.; Meyers, D.; Mukherjee, C.; Caron, C.; Kimura, H.; Rousseaux, S.; Cole, P.; Panne, D.; French, C.; Khochbin, S. *EMBO J.* **2010**, *29*, 2943–2952.

(64) Zhong, H.; Voll, R.; Ghosh, S. *Mol. Cell* **1998**, *1*, 661–671.

(65) Huang, W.-C.; Ju, T.-K.; Hung, M.-C.; Chen, C.-C. *Mol. Cell* **2007**, *26*, 75–87.

(66) Deng, W. G.; Zhu, Y.; Wu, K. K. *Blood* **2004**, *103*, 2135–2142.

(67) Rouaux, C.; Loeffler, J.-P.; Boutillier, A.-L. *Biochem. Pharmacol.* **2004**, *68*, 1157–1164.

(68) Saura, C.; Valero, J. *Rev. Neurosci.* **2011**, *22*, 153–169.

(69) Murata, T.; Kurokawa, R.; Krones, A.; Tatsumi, K.; Ishii, M.; Taki, T.; Masuno, M.; Ohashi, H.; Yanagisawa, M.; Rosenfeld, M. G.; Glass, C. K.; Hayashi, Y. *Hum. Mol. Genet.* **2001**, *10*, 1071–1076.

(70) Rouaux, C.; Jokic, N.; Mbebi, C.; Boutillier, S.; Loeffler, J.-P.; Boutillier, A.-L. *EMBO J.* **2003**, *22*, 6537–6549.

(71) Steffan, J. S.; Kazantsev, A.; Spasic-Boskovic, O.; Greenwald, M.; Zhu, Y. Z.; Gohler, H.; Wanker, E. E.; Bates, G. P.; Housman, D. E.; Thompson, L. M. *Proc. Natl. Acad. Sci.* **2000**, *97*, 6763–6768.

(72) Barrett, R.; Malvaez, M.; Kramar, E.; Matheos, D.; Arrizon, A.; Cabrera, S.; Lynch, G.; Greene, R.; Wood, M. *Neuropsychopharmacol.* **2011**, *36*, 1545–1556.

(73) Malvaez, M.; Mhillaj, E.; Matheos, D.; Palmery, M.; Wood, M. *J. Neurosci. : Off. J. Soc. Neurosci.* **2011**, *31*, 16941–16948.

(74) Frye, S. *Nat. Chem. Biol.* **2010**, *6*, 159–161.

(75) Bandyopadhyay, D.; Okan, N.; Bales, E.; Nascimento, L.; Cole, P.; Medrano, E. *Cancer Res.* **2002**, *62*, 6231–6239.

(76) Liu, Y.; Wang, L.; Predina, J.; Han, R.; Beier, U.; Wang, L.-C.; Kapoor, V.; Bhatti, T.; Akimova, T.; Singhal, S.; Brindle, P.; Cole, P.; Albelda, S.; Hancock, W. *Nat. Med.* **2013**, *19*,

1173–1177.

(77) Melnikova, I. *Nat. Rev. Drug Discov.* **2005**, *4*, 453–454.

(78) Zeng, L.; Li, J.; Muller, M.; Yan, S.; Mujtaba, S.; Pan, C.; Wang, Z.; Zhou, M.-M. *J. Am. Chem. Soc.* **2005**, *127*, 2376–2377.

(79) Miyoshi, S.; Ooike, S.; Iwata, K.; Hikawa, H.; Sugahara, K. Antitumor agent. *WO2009084693*, 2009.

(80) Filippakopoulos, P.; Qi, J.; Picaud, S.; Shen, Y.; Smith, W. B.; Fedorov, O.; Morse, E. M.; Keates, T.; Hickman, T. T.; Felletar, I.; Philpott, M.; Munro, S.; McKeown, M. R.; Wang, Y.; Christie, A. L.; West, N.; Cameron, M. J.; Schwartz, B.; Heightman, T. D.; La Thangue, N.; French, C. A.; Wiest, O.; Kung, A. L.; Knapp, S.; Bradner, J. E. *Nature* **2010**, *468*, 1067–1073.

(81) Dawson, M.; Prinjha, R.; Dittmann, A.; Giotopoulos, G.; Bantscheff, M.; Chan, W.-I.; Robson, S.; Chung, C.; Hopf, C.; Savitski, M.; Huthmacher, C.; Gudgin, E.; Lugo, D.; Beinke, S.; Chapman, T.; Roberts, E.; Soden, P.; Auger, K.; Mirguet, O.; Doehner, K.; Delwel, R.; Burnett, A.; Jeffrey, P.; Drewes, G.; Lee, K.; Huntly, B.; Kouzarides, T. *Nature* **2011**, *478*, 529–533.

(82) Furdas, S.; Carlino, L.; Sippl, W.; Jung, M. *Med. Chem. Commun.* **2012**, *3*, 123–134.

(83) Gallenkamp, D.; Gelato, K.; Haendler, B.; Weinmann, H. *ChemMedChem* **2014**, *9*, 438–464.

(84) Filippakopoulos, P.; Knapp, S. *Nat. Rev. Drug Discov.* **2014**, *13*, 337–356.

(85) Mujtaba, S.; He, Y.; Zeng, L.; Yan, S.; Plotnikova, O.; Sachchidanand; Sanchez, R.; Zeleznik-Le, N.; Ronai, Z.; Zhou, M.-M. *Mol. Cell* **2004**, *13*, 251–263.

(86) Zeng, L.; Zhang, Q.; Gerona-Navarro, G.; Moshkina, N.; Zhou, M.-M. *Structure* **2008**, *16*, 643–652.

(87) Gerona-Navarro, G.; Yoel-Rodríguez; Mujtaba, S.; Frasca, A.; Patel, J.; Zeng, L.; Plotnikov, A.; Osman, R.; Zhou, M.-M. *J. Am. Chem. Soc.* **2011**, *133*, 2040–2043.

(88) Sachchidanand; Resnick-Silverman, L.; Yan, S.; Mutjaba, S.; Liu, W.-J.; Zeng, L.; Manfredi, J. J.; Zhou, M.-M. *Chem. & Biol.* **2006**, *13*, 81–90.

(89) Gacias, M.; Gerona-Navarro, G.; Plotnikov, A.; Zhang, G.; Zeng, L.; Kaur, J.; Moy, G.; Rusinova, E.; Rodriguez, Y.; Matikainen, B.; Vincek, A.; Joshua, J.; Casaccia, P.; Zhou, M.-M. *Chem. & Biol.* **2014**, *21*, 841–854.

(90) Chung, C.; Dean, A.; Woolven, J.; Bamborough, P. *J. Med. Chem.* **2011**, *55*, 576–586.

(91) Gosmini, R.; Nguyen, V.; Toum, J.; Simon, C.; Brusq, J.-M.; Krysa, G.; Mirguet, O.; Riou-Eymard, A.; Boursier, E.; Trottet, L.; Bamborough, P.; Clark, H.; Chung, C.; Cutler, L.; Demont, E.; Kaur, R.; Lewis, A.; Schilling, M.; Soden, P.; Taylor, S.; Walker, A.; Walker, M.; Prinjha, R.; Nicodème, E. *J. Med. Chem.* **2014**.

(92) Wyce, A.; Ganji, G.; Smitheman, K.; Chung, C.; Korenchuk, S.; Bai, Y.; Barbash, O.; Le, B.; Craggs, P.; McCabe, M.; Kennedy-Wilson, K.; Sanchez, L.; Gosmini, R.; Parr, N.; McHugh, C.; Dhanak, D.; Prinjha, R.; Auger, K.; Tummino, P. *PLoS ONE* **2013**, *8*.

(93) Borah, J.; Mujtaba, S.; Karakikes, I.; Zeng, L.; Muller, M.; Patel, J.; Moshkina, N.; Morohashi, K.; Zhang, W.; Gerona-Navarro, G.; Hajjar, R.; Zhou, M.-M. *Chem. & Biol.* **2011**, *18*, 531–541.

(94) Zhang, G.; Plotnikov, A.; Rusinova, E.; Shen, T.; Morohashi, K.; Joshua, J.; Zeng, L.; Mujtaba, S.; Ohlmeyer, M.; Zhou, M.-M. *J. Med. Chem.* **2013**, *56*, 9251–9264.

(95) Philpott, M.; Yang, J.; Tumber, T.; Fedorov, O.; Uttarkar, S.; Filippakopoulos, P.; Picaud, S.; Keates, T.; Felletar, I.; Ciulli, A.; Knapp, S.; Heightman, T. D. *Mol. Biosyst.* **2011**, *7*, 2899–2908.

(96) Fish, P.; Filippakopoulos, P.; Bish, G.; Brennan, P.; Bunnage, M.; Cook, A.; Federov, O.; Gerstenberger, B.; Jones, H.; Knapp, S.; Marsden, B.; Nocka, K.; Owen, D.; Philpott, M.; Picaud, S.; Primiano, M.; Ralph, M.; Sciammetta, N.; Trzupek, J. *J. Med. Chem.* **2012**, *55*, 9831–9837.

(97) Hewings, D.; Wang, M.; Philpott, M.; Fedorov, O.; Uttarkar, S.; Filippakopoulos, P.; Picaud, S.; Vuppusetty, C.; Marsden, B.; Knapp, S.; Conway, S.; Heightman, T. *J. Med. Chem.* **2011**, *54*, 6761–6770.

(98) Bamborough, P.; Diallo, H.; Goodacre, J.; Gordon, L.; Lewis, A.; Seal, J.; Wilson, D.; Woodrow, M.; Chung, C. *J. Med. Chem.* **2011**.

(99) Hay, D.; Fedorov, O.; Filippakopoulos, P.; Martin, S.; Philpott, M.; Picaud, S.; Hewings, D.; Uttakar, S.; Heightman, T.; Conway, S.; Knapp, S.; Brennan, P. *Med. Chem. Commun.* **2013**, *4*, 140–144.

(100) Hay, D.; Fedorov, O.; Martin, S.; Singleton, D.; Tallant, C.; Wells, C.; Picaud, S.; Philpott, M.; Monteiro, O.; Rogers, C.; Conway, S.; Rooney, T.; Tumber, A.; Yapp, C.; Filippakopoulos, P.; Bunnage, M.; Müller, S.; Knapp, S.; Schofield, C.; Brennan, P. *J. Am. Chem. Soc.* **2014**, *136*, 9308–9319.

(101) Rooney, T.; Filippakopoulos, P.; Fedorov, O.; Picaud, S.; Cortopassi, W.; Hay, D.; Martin, S.; Tumber, A.; Rogers, C.; Philpott, M.; Wang, M.; Thompson, A.; Heightman, T.; Pryde, D.; Cook, A.; Paton, R.; Müller, S.; Knapp, S.; Brennan, P.; Conway, S. *Angew. Chem. Int. Ed.* **2014**, *53*, 6126–6130.

(102) Albrecht, B.; Harmange, J.-C.; Côté, A.; Taylor, A. Bromodomain inhibitors and uses thereof. *WO2012174487*, 2012.

(103) Guetzoyan, L.; Ingham, R.; Nikbin, N.; Rossignol, J.; Wolling, M.; Baumert, M.; Burgess-Brown, N.; Strain-Damerell, C.; Shrestha, L.; Brennan, P.; Fedorov, O.; Knapp, S.; Ley, S. *Med. Chem. Commun.* **2014**, *5*, 540–546.

(104) Bromosporine. *http://www.thesgc.org/chemical-probes/bromosporine*, 2014.

(105) Fedorov, O.; Lingard, H.; Wells, C.; Monteiro, O.; Picaud, S.; Keates, T.; Yapp, C.; Philpott, M.; Martin, S.; Felletar, I.; Marsden, B.; Filippakopoulos, P.; Müller, S.; Knapp, S.; Brennan, P. *J. Med. Chem.* **2013**, *57*, 462–476.

(106) *http://www.thesgc.org/chemical-probes/ICBP112* **2014**.

(107) Vollmuth, F.; Geyer, M. *Angew. Chem.* **2010**, *49*, 6768–6772.

(108) Das, C.; Roy, S.; Namjoshi, S.; Malarkey, C.; Jones, D.; Kutateladze, T.; Churchill, M.; Tyler, J. *Proc. Natl. Acad. Sci.* **2014**, *111*, E1072–E1081.

(109) Rooney, T. P. C. *SABS-IDC short project report, University of Oxford* **2010**.

(110) Hewings, D.; Wang, M.; Philpott, M.; Fedorov, O.; Uttarkar, S.; Filippakopoulos, P.; Picaud, S.; Vuppusetty, C.; Marsden, B.; Knapp, S.; Conway, S.; Heightman, T. *J. Med. Chem.* **2011**, *54*, 6761–6770.

(111) Macias, F.; Marin, D.; Oliveros-Bastidas, A.; Molinillo, J. *Nat. Prod. Rep.* **2009**, *26*, 478–489.

(112) Copaja, S.; Nicol, D.; Wratten, S. *Phytochemistry* **1999**, *50*, 17–24.

(113) Sahi, S. V.; Chilton, M. D.; Chilton, W. S. *Proc. Natl. Acad. Sci.* **1990**, *87*, 3879–3883.

(114) Powell, N. A.; Ciske, F. L.; Cai, C.; Holsworth, D. D.; Mennen, K.; Van Huis, C. A.; Jalaie, M.; Day, J.; Mastronardi, M.; McConnell, P.; Mochalkin, I.; Zhang, E.; Ryan, M. J.; Bryant, J.; Collard, W.; Ferreira, S.; Gu, C.; Collins, R.; Edmunds, J. J. *Bioorganic & Med. Chem.* **2007**, *15*, 5912–5949.

(115) Hoenke, C.; Bouyssou, T.; Tautermann, C. S.; Rudolf, K.; Schnapp, A.; Konetzki, I. *Bioorganic & Med. Chem. Lett.* **2009**, *19*, 6640–6644.

(116) Rasmussen, S. G. F.; Choi, H.-J.; Fung, J. J.; Pardon, E.; Casarosa, P.; Chae, P. S.; Devree, B. T.; Rosenbaum, D. M.; Thian, F. S.; Kobilka, T. S.; Schnapp, A.; Konetzki, I.; Sunahara, R. K.; Gellman, S. H.; Pautsch, A.; Steyaert, J.; Weis, W. I.; Kobilka, B. K. *Nature* **2011**, *469*, 175–180.

(117) Tautermann, C. S.; Pautsch, A. *ACS Med. Chem. Lett.* **2011**, *2*, 414–418.

(118) Dai, W.-M.; Wang, X.; Ma, C. *Tetrahedron* **2005**, *61*, 6879–6885.

(119) Chung, C.-W.; Witherington, J. *J. Biomol. Screen.* **2011**, *16*, 1170–1185.

(120) Abagyan, R.; Totrov, M.; Kuznetsov, D. *J. Comput. Chem.* **1994**, *15*, 488–506.

(121) Perola, E.; Walters, W. P.; Charifson, P. S. *Proteins: Struct. Funct. Bioinforma.* **2004**, *56*, 235–249.

(122) Golebiowski, A.; Klopfenstein, S. R.; Portlock, D. E. *Curr. Opin. Chem. Biol.* **2001**, *5*, 273–284.

(123) Geysen, H. M.; Schoenen, F.; Wagner, D.; Wagner, R. *Nat. Rev. Drug Discov.* **2003**, *2*, 222–230.

(124) Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem. Int. Ed.* **2002**, *41*, 2879–2890.

(125) Morris, G.; Huey, R.; Lindstrom, W.; Sanner, M.; Belew, R.; Goodsell, D.; Olson, A. *J. Comput. Chem.* **2009**, *30*, 2785–2791.

(126) Ross, G. A.; Morris, G. M.; Biggin, P. C. *PLoS ONE* **2012**, *7*.

(127) Lipinski, C.; Hopkins, A. *Nature* **2004**, *432*, 855–861.

(128) Akazome, M.; Sukegawa, J.; Goto, Y.; Matsumoto, S. *Tetrahedron Lett.* **2009**, *50*, 5382–5385.

(129) Sarges, R.; Lyga, J. *J. Heterocycl. Chem.* **1988**, *25*, 1475–1479.

(130) Balzarini, J.; De Clercq, E.; Carbonez, A.; Burt, V.; Kleim, J. P. *AIDS Res. Hum. Retroviruses* **2000**, *16*, 517–528.

(131) Moore, K.; Cass, L.; Dallow, N.; Hardman, T.; Jones, A.; Boyce, M.; Prince, W. *J. Clin. Pharmacol.* **2001**, *41*, 1098–1105.

(132) Zhou, J.; Ba, M.; Wang, B.; Zhou, H.; Bie, J.; Fu, D.; Cao, Y.; Xu, B.; Guo, Y. *Med. Chem. Comm.* **2014**, *5*, 441–444.

(133) Ren, J.; Nichols, C.; Chamberlain, P.; Weaver, K.; Short, S.; Chan, J.; Kleim, J.-P.;

Stammers, D. *J. Med. Chem.* **2007**, *50*, 2301–2309.

(134) Mangold, S.; Prost, L.; Kiessling, L. *Chem. Sci.* **2012**, *3*, 772–777.

(135) Jacobsen, J.; TenBrink, R.; Stelzer, L.; Belonga, K.; Carter, D.; Im, H.; Im, W.; Sethy, V.; Tang, A.; VonVoigtlander, P.; Petke, J. *J. Med. Chem.* **1996**, *39*, 158–175.

(136) Fischer, C.; Koenig, B. *Beilstein J. Org. Chem.* **2011**, *7*, 59–74.

(137) Bariwal, J.; Van der Eycken, E. *Chem. Soc. Rev.* **2013**, *42*.

(138) Hartwig, J. *Angew. Chem. Int. Ed.* **1998**, *37*, 2046–2067.

(139) Wolfe, J.; Wagaw, S.; Marcoux, J.-F.; Buchwald, S. *Accounts Chem. Res.* **1998**, *31*, 805–818.

(140) Wolfe, J.; Buchwald, S. *J. Org. Chem.* **1997**, *62*, 1264–1267.

(141) Louie, J.; Driver, M.; Hamann, B.; Hartwig, J. *J. Org. Chem.* **1997**, *62*, 1268–1273.

(142) Lee, J.; Murray, W.; Rivero, R. *J. Org. Chem.* **1997**, *62*, 3874–3879.

(143) Hu, Z.; Jiang, X.; Han, W. *Tetrahedron Lett.* **2008**, *49*, 5192–5195.

(144) Guo, W.; Li, J.; Fan, N.; Wu, W.; Zhou, P.; Xia, C. *Synth. Commun.* **2005**, *35*, 145–152.

(145) Fletcher, S.; Gunning, P. *Tetrahedron Lett.* **2008**, *49*, 4817–4819.

(146) Leadbeater, N. *Nat. Chem.* **2010**, *2*, 1007–1009.

(147) Bellamy, F. D.; Ou, K. *Tetrahedron Lett.* **1984**, *25*, 839–842.

(148) Cazares-Körner, C.; Pires, I.; Swallow, D.; Grayer, S.; O'Connor, L.; Olcina, M.; Christlieb, M.; Conway, S.; Hammond, E. *ACS Chem. Biol.* **2013**, *8*, 1451–1459.

(149) Bissantz, C.; Kuhn, B.; Stahl, M. *J. Med. Chem.* **2010**, *53*, 5061–5084.

(150) Dougherty, D. *Science* **1996**, *271*, 163–168.

(151) Dougherty, D. *Acc. Chem. Res.* **2012**, *46*, 885–893.

(152) Mahadevi, S.; Sastry, N. *Chem. Rev.* **2012**, *113*, 2100–2138.

(153) Salonen, L.; Ellermann, M.; Diederich, F. *Angew. Chem. Int. Ed.* **2011**, *50*, 4808–4842.

(154) Ma, J.; Dougherty, D. *Chem. Rev.* **1997**, *97*, 1303–1324.

(155) Gallivan, J. P.; Dougherty, D. A. *Proc. Natl. Acad. Sci.* **1999**, *96*, 9459–9464.

(156) Zacharias, N.; Dougherty, D. A. *Trends Pharmacol. Sci.* **2002**, *23*, 281–287.

(157) Dougherty, D. A.; Stauffer, D. A. *Science* **1990**, *250*, 1558–1560.

(158) Beene, D.; Brandt, G.; Zhong, W.; Zacharias, N.; Lester, H.; Dougherty, D. *Biochemistry* **2002**, *41*, 10262–10269.

(159) Xiu, X.; Puskar, N.; Shanata, J.; Lester, H.; Dougherty, D. *Nature* **2009**, *458*, 534–537.

(160) Sörme, P.; Arnoux, P.; Kahl-Knutsson, B.; Leffler, H.; Rini, J.; Nilsson, U. *J. Am. Chem. Soc.* **2005**, *127*, 1737–1743.

(161) Sörme, P.; Qian, Y.; Nyholm, P.-G.; Leffler, H.; Nilsson, U. *ChemBioChem* **2002**, *3*, 183–189.

(162) Wang, H.; Lu, Z.; Lord, S.; Willets, K.; Bertke, J.; Bunge, S.; Moerner, W. E.; Twieg, R. *Tetrahedron* **2007**, *63*, 103–114.

(163) Alcaide, B.; Almendros, P.; Alonso, J. *Chem. Eur. J.* **2003**, *9*, 5793–5799.

(164) Konishi, H.; Itoh, T.; Manabe, K. *Chem. & Pharm. Bull.* **2010**, *58*, 1255–1258.

(165) Agbo, S.; Hallas, G.; Towns, A. *Dyes Pigment.* **2000**, *47*, 33–43.

(166) Perrin, C.; Nielson, J. *Annu. Rev. Phys. Chem.* **1997**, *48*, 511–544.

(167) Gorobets, N.; Yermolayev, S.; Gurley, T.; Gurinov, A.; Tolstoy, P.; Shenderovich, I.; Leadbeater, N. *J. Phys. Org. Chem.* **2012**, *25*, 287–295.

(168) Aguirre, G.; Somanathan, R.; Hellberg, L.; Dwyer, T.; North, R. *Magn. Reson. Chem.* **2003**, *41*, 131–134.

(169) Li, F.; Zhang, H.; Jiang, L.; Zhang, W.; Nie, J.; Feng, Y.; Yang, M.; Liu, M. *Magn. Reson. Chem.* **2007**, *45*, 929–936.

(170) Blundell, C.; Packer, M.; Almond, A. *Bioorg. Med. Chem.* **2013**, *21*, 4976–4987.

(171) Dey, A.; Ellenberg, J.; Farina, A.; Coleman, A.; Maruyama, T.; Sciortino, S.; Lippincott-Schwartz, J.; Ozato, K. *Mol. Cell. Biol.* **2000**, *20*, 6537–6549.

(172) Cordeiro, A.; Shaw, J.; O'Brien, J.; Blanco, F.; Rozas, I. *Eur. J. Org. Chem.* **2011**, *2011*, 1504–1513.

(173) Bethuel, Y.; Gademann, K. *J. Org. Chem.* **2005**, *70*, 6258–6264.

(174) Fan, K.-H.; Lever, J. R.; Lever, S. Z. *Bioorg. Med. Chem.* **2011**, *19*, 1852–1859.

(175) Osby, J.; Martin, M.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 2093–2096.

(176) Khan, N. *J. Org. Chem.* **1995**, *60*, 4536–4541.

(177) Das, B.; Venkateswarlu, K.; Majhi, A.; Siddaiah, V.; Reddy, K. *J. Mol. Catal. A: Chem. Chem.* **2007**, *267*, 30–33.

(178) Kudo, Y.; Furumoto, S.; Okamura, N. Preparation of fluorine-containing quinoline derivatives as Tau imaging probes for positron-emission tomography (PET). *WO2012057312*, 2012.

(179) Fleming, F.; Yao, L.; Ravikumar, P. C.; Funk, L.; Shook, B. *J. Med. Chem.* **2010**, *53*, 7902–7917.

(180) Ryo, S.; Ayako, M.; Hiroaki, T.; Hiroshi, F. Preparation of N-(adamantan-4-yl)-3,4-dihydroquinoline-1(2H)-carboxamide and -3,4-dihydroquinoxaline-1(2H)-carboxamide derivatives and related compounds as having 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitory activity. *WO2009001817*.

(181) Zachariasse, K.; Druzhinin, S.; Bosch, W.; Machinek, R. *J. Am. Chem. Soc.* **2004**, *126*, 1705–1715.

(182) Land, E.; Ramsden, C.; Riley, P. *Acc. Chem. Res.* **2003**, *36*, 300–308.

(183) Reichelt, A.; Martin, S. *Acc. Chem. Res.* **2006**, *39*, 433–442.

(184) Marquis, R.; Ru, Y.; LoCastro, S.; Zeng, J.; Yamashita, D.; Oh, H.-J.; Erhard, K.; Davis, L.; Tomaszek, T.; Tew, D.; Salyers, K.; Proksch, J.; Ward, K.; Smith, B.; Levy, M.; Cummings, M.; Haltiwanger, C.; Trescher, G.; Wang, B.; Hemling, M.; Quinn, C.; Cheng, H. Y.; Lin, F.; Smith, W.; Janson, C.; Zhao, B.; McQueney, M.; D'Alessio, K.; Lee, C.-P.; Marzulli, A.; Dodds, R.; Blake, S.; James, I.; Gress, C.; Bradley, B.; Lark, M.; Gowen, M.; Veber, D. *J. Med. Chem.* **2001**, *44*, 1380–1395.

(185) DeLorbe, J.; Clements, J.; Teresk, M.; Benfield, A.; Plake, H.; Millspaugh, L.; Martin, S. *J. Am. Chem. Soc.* **2009**, *131*, 16758–16770.

(186) Jiang, Y.; Khong, V.; Lourdusamy, E.; Park, C.-M. *Chem. Commun.* **2012**, *48*, 3133–3135.

(187) Müller, T.; Karpov, A. *Synthesis* **2003**, 2815–2826.

(188) Loeppky, R.; Elomari, S. *J. Org. Chem.* **1999**, *65*, 96–103.

(189) Csatayová, K.; Davies, S.; Lee, J.; Ling, K.; Roberts, P.; Russell, A.; Thomson, J. *Org. Lett.* **2010**, *12*, 3152–3155.

(190) De, P.; Baltas, M.; Lamoral-Theys, D.; Bruyère, C.; Kiss, R.; Bedos-Belval, F.; Saffon, N. *Bioorganic & Med. Chem.* **2010**, *18*, 2537–2548.

(191) Masuyama, Y.; Kagawa, M.; Kurusu, Y. *Chem. Lett.* **1995**, 1121–1122.

(192) Motherwell, W.; Bégis, G.; Cladingboel, D.; Jerome, L.; Sheppard, T. *Tetrahedron* **2007**, *63*, 6462–6476.

(193) Bégis, G.; Cladingboel, D.; Jerome, L.; Motherwell, W.; Sheppard, T. *Eur. J. Org. Chem.* **2009**, *2009*, 1532–1548.

(194) Gmeiner, P.; Bollinger, B. *Synthesis* **1995**, *1995*, 168–170.

(195) Yasui, K.; Fugami, K.; Tanaka, S.; Tamaru, Y. *J. Org. Chem.* **1995**, *60*, 1365–1380.

(196) Prediger, P.; Barbosa, L.; Génisson, Y.; Correia, C. *J. Org. Chem.* **2011**, *76*, 7737–7749.

(197) Purser, S.; Moore, P.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320–330.

(198) Böhm, H.-J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M. *ChemBioChem* **2004**, *5*, 637–643.

(199) O'Hagan, D. *J. Fluor. Chem.* **2010**, *131*, 1071–1081.

(200) Morgenthaler, M.; Schweizer, E.; Hoffmann-Röder, A.; Benini, F.; Martin, R. E.; Jaeschke, G.; Wagner, B.; Fischer, H.; Bendels, S.; Zimmerli, D.; Schneider, J.; Diederich, F.; Kansy, M.; Müller, K. *ChemMedChem* **2007**, *2*, 1100–1115.

(201) Rowley, M.; Hallett, D.; Goodacre, S.; Moyes, C.; Crawforth, J.; Sparey, T.; Patel, S.; Marwood, R.; Patel, S.; Thomas, S.; Hitzel, L.; O'Connor, D.; Szeto, N.; Castro, J.; Hutson, P.; MacLeod, A. *J. Med. Chem.* **2001**, *44*, 1603–1614.

(202) Dunitz, J.; Taylor, R. *Chem. Eur. J.* **1997**, *3*, 89–98.

(203) Müller, K.; Faeh, C.; Diederich, F. *Science* **2007**, *317*, 1881–1886.

(204) Cantacuzene, D.; Kirk, K. L.; McCulloh, D. H.; Creveling, C. R. *Science* **1979**, *204*, 1217–1219.

(205) Chu, D.; Wang, B. **2009**, *WO2009099736 A2*.

(206) Hennig, L.; Ayala-Leon, K.; Angulo-Cornejo, J.; Richter, R.; Beyer, L. *J. Fluor. Chem.* **2009**, *130*, 453–460.

(207) Perrin, C.; Fabian, M.; Rivero, I. *Tetrahedron* **1999**, *55*, 5773–5780.

(208) Hanzlik, R.; Kishore, V.; Tullman, R. *J. Med. Chem.* **1979**, *22*, 759–761.

(209) Cerny, M.; Hanzlik, R. *J. Am. Chem. Soc.* **2006**, *128*, 3346–3354.

(210) Adams, D. O.; Yang, S. F. *Proc. Natl. Acad. Sci.* **1979**, *76*, 170–174.

(211) Reissig, H.-U.; Zimmer, R. *Chem. Rev.* **2003**, *103*, 1151–1196.

(212) Nishiwaki, N.; Sakashita, M.; Azuma, M.; Tanaka, C.; Tamura, M.; Asaka, N.; Hori, K.; Tohda, Y.; Ariga, M. *Tetrahedron* **2002**, *58*, 473–478.

(213) Hamid, M. H. S. A.; Allen, L.; Lamb, G.; Maxwell, A.; Maytum, H.; Watson, A.; Williams, J. M. J. *J. Am. Chem. Soc.* **2009**, *131*, 1766–1774.

(214) Hopkins, A.; Groom, C.; Alex, A. *Drug Discov. Today* **2004**, *9*, 430–431.

(215) Pangborn, A.; Giardello, M.; Grubbs, R.; Rosen, R.; Timmers, F. *Organometallics* **1996**, *15*, 1518–1520.

(216) Tsuritani, T.; Yamamoto, Y.; Kawasaki, M.; Mase, T. *Org. Lett.* **2009**, *11*, 1043–1045.

(217) Pauff, S.; Miller, S. *Org. Lett.* **2011**, *13*, 6196–6199.

(218) Bourne, G.; Golding, S.; McGeary, R.; Meutermans, W.; Jones, A.; Marshall, G.; Alewood, P.; Smythe, M. *J. Org. Chem.* **2001**, *66*, 7706–7713.

(219) Dorrestein, P.; Poole, K.; Begley, T. *Org. Lett.* **2003**, *5*, 2215–2217.

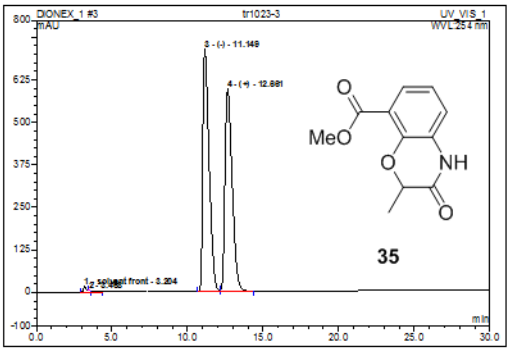
(220) Lewis, H. J.; Ramage, G. R.; Robinson, R. *J. Chem. Soc.* **1935**.

(221) Moreira, D. D.; Guimarães, E. F.; Kaplan, M. A. *Phytochemistry* **2000**, *55*, 783–786.

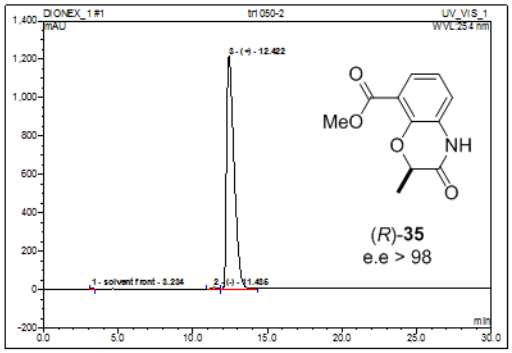
(222) Mali, R. S.; Yadav, V. J. *Synthesis* **1984**, *1984*, 862–865.

Appendix

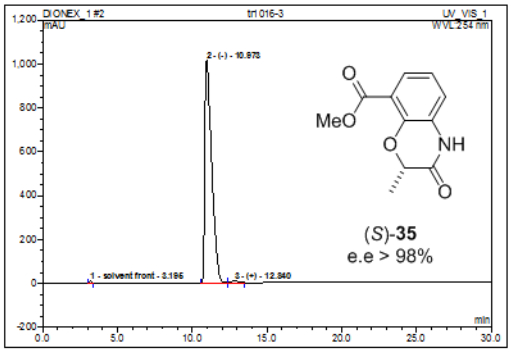
A1 Chiral HPLC of benzoxazinone analogues



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.20	solvent front	18.487	5.130	0.76	n.a.	BMB
2	3.47	n.a.	1.307	0.136	0.02	n.a.	Rd
3	11.15	(-)	713.556	345.189	50.91	n.a.	BM
4	12.66	(+)	595.292	327.617	48.32	n.a.	MB
Total:			1328.642	678.072	100.00	0.000	

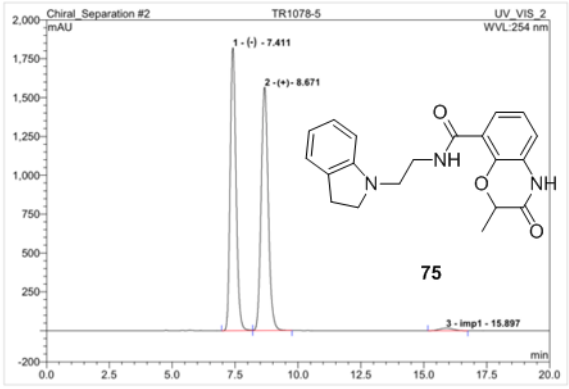


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.23	solvent front	9.311	1.418	0.20	n.a.	BMB
2	11.44	(-)	8.036	3.004	0.43	n.a.	BM
3	12.42	(+)	1212.477	697.502	99.37	n.a.	MB
Total:			1229.824	701.924	100.00	0.000	

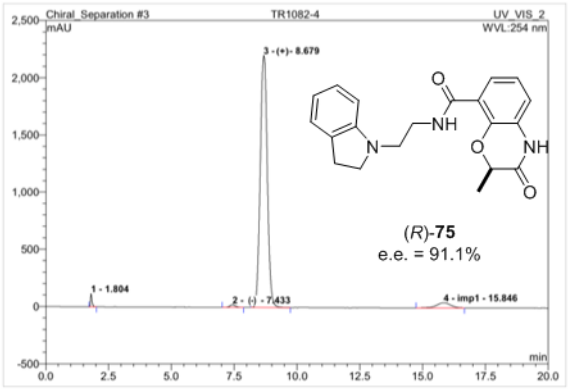


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.19	solvent front	10.739	1.614	0.30	n.a.	BMB
2	10.97	(-)	1016.701	540.011	98.88	n.a.	BM
3	12.84	(+)	8.624	4.520	0.83	n.a.	MB
Total:			1036.064	546.145	100.00	0.000	

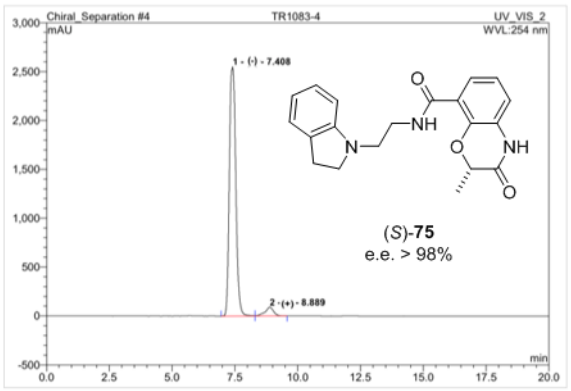
Figure A1.1: Chiral HPLC separation of methyl 2-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylate (**35**). Enantiomeric excess (ee) is calculated using the relative areas of each peak.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	7.41	(-)	1820.757	495.142	48.68	n.a.	BM
2	8.67	(+)	1566.308	511.288	50.27	n.a.	MB
3	15.90	imp1	16.889	10.655	1.05	n.a.	BMB
Total:			3403.955	1017.085	100.00	0.000	



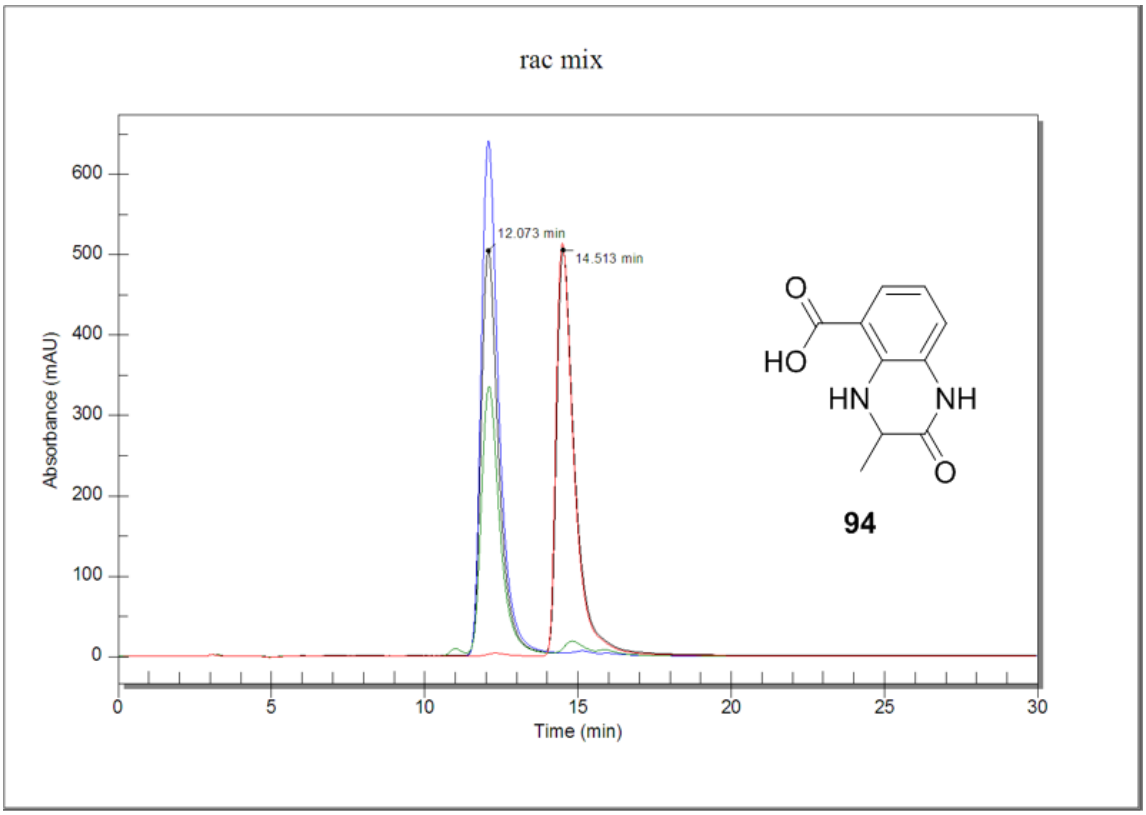
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.80	n.a.	115.648	7.803	1.02	n.a.	BMB
2	7.43	(-)	23.305	6.193	0.81	n.a.	BMB
3	8.68	(+)	2204.896	718.212	94.30	n.a.	BMB
4	15.85	imp1	44.978	29.407	3.86	n.a.	BMB
Total:			2388.828	761.615	100.00	0.000	



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	7.41	(-)	2552.714	754.774	95.56	n.a.	BM
2	8.89	(+)	88.164	35.097	4.44	n.a.	MB
Total:			2640.878	789.872	100.00	0.000	

Figure A1.2: Chiral HPLC separation of *N*-[2-(2,3-Dihydro-1*H*-indol-1-yl)ethyl]-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (**75**). Enantiomeric excess (ee) is calculated using the relative areas of each peak.

A2 Chiral HPLC of dihydroquinoxalinone carboxylic acids



Racemic sample (black)

Time	Area	Area %
2.984	5689.9	0.01
3.084	7896.5	0.02
12.073	19891567	49.76
14.513	20069239	50.21
Total	39974392.1	100.00

e.e. = NA

Methyl ester route (green)

Time	Area	Area %
2.977	6618.2	0.05
3.082	7869.7	0.05
11.000	214387	1.47
12.095	13866121	95.05
14.810	492976	3.38
Total	14587971.1	100.00

e.e. = 93%

Benzyl ester route (blue)

Time	Area	Area %
2.979	6723.4	0.03
3.083	9441.9	0.04
12.075	25529517	99.94
Total	25545682.2	100.00

e.e. > 98%

Figure A2.1: HPLC traces of compound **94** synthesised either *via* a methyl ester protecting group (Section 3.2.2) or *via* a benzyl ester protecting group (Section 3.2.3). The latter provided (*R*)-**94** in higher e.e.

A3 NMR analysis of internal H-bond

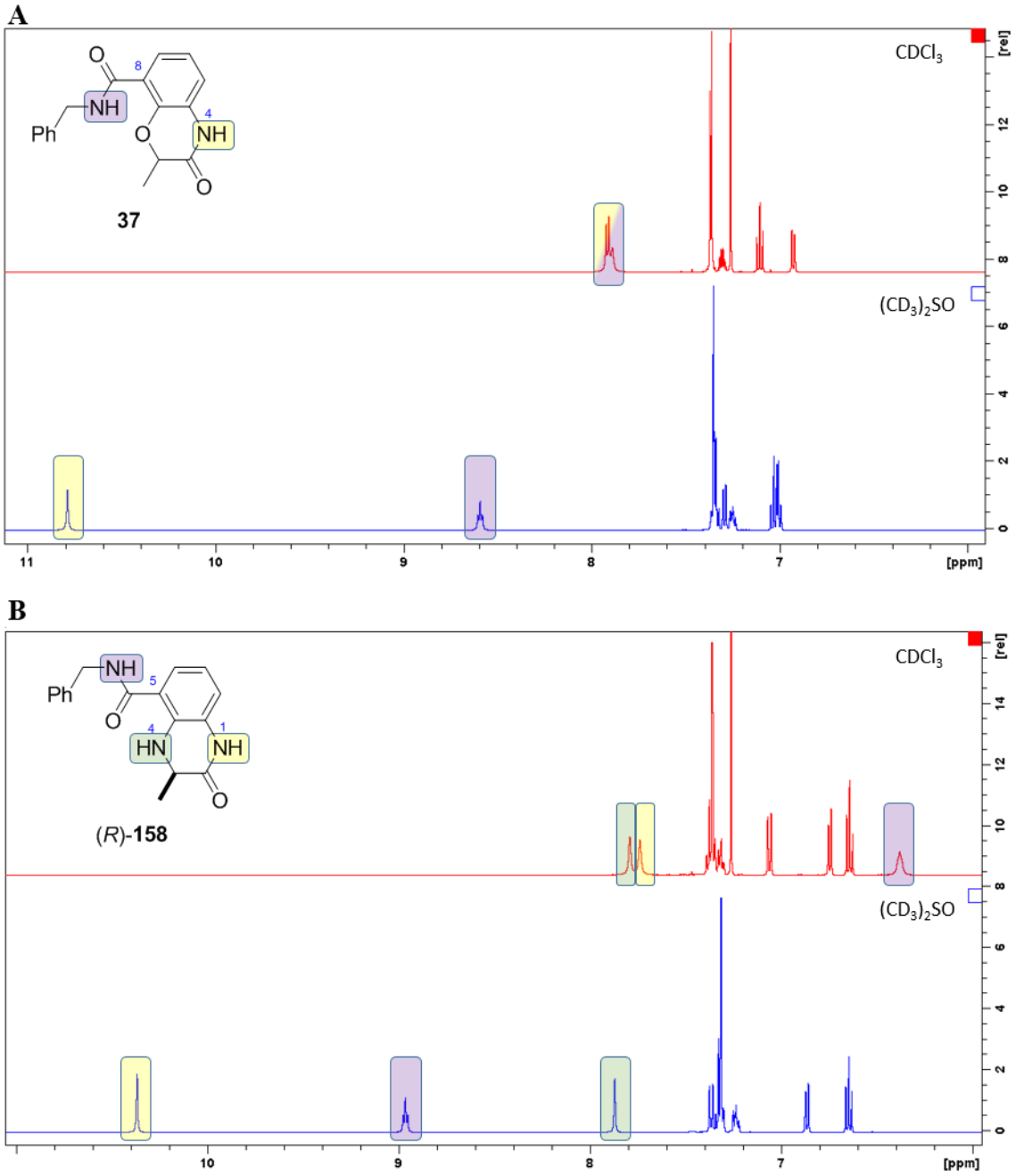


Figure A3.1: NMR traces of **37** (**A**) and (*R*)-**158** (**B**) in $CDCl_3$ and $(CD_3)_2SO$ (all at 2 mg/mL). Assigned N*H* signals are highlighted and the difference in chemical shift resulting from the change in solvent are reported in Table 3.7.

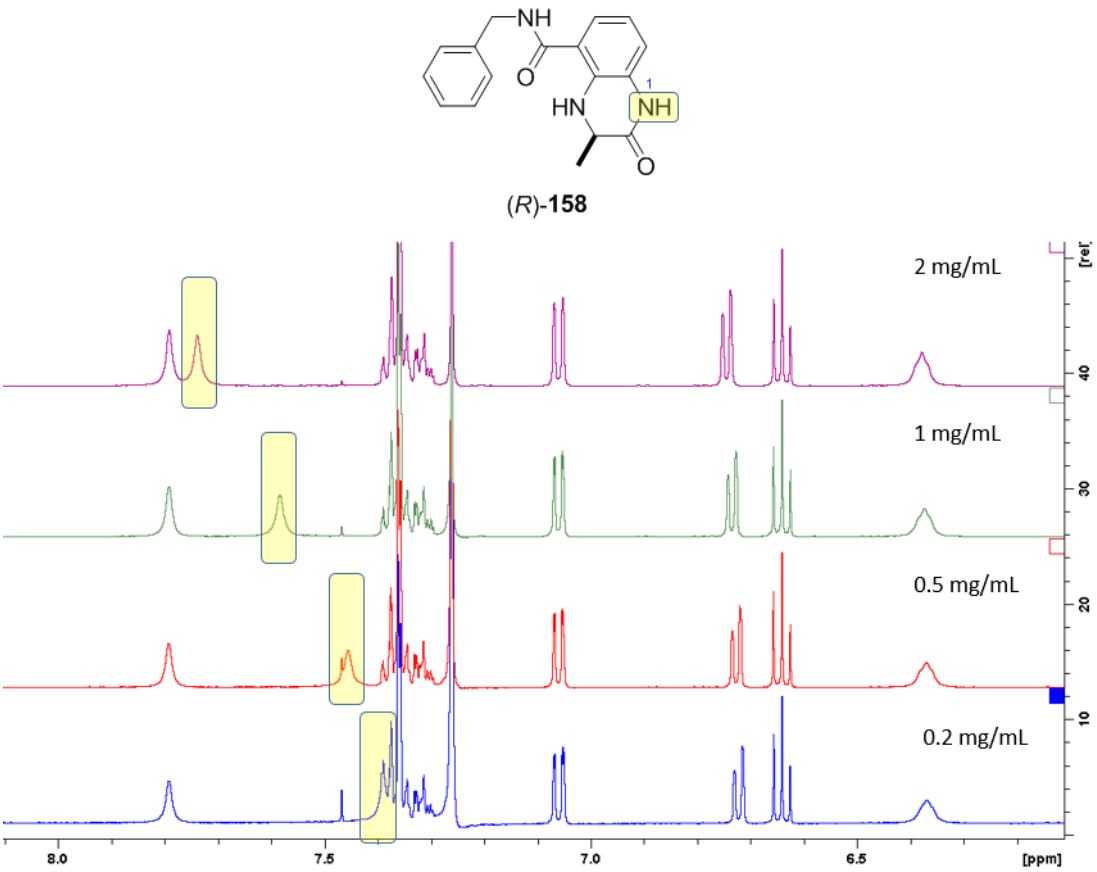


Figure A3.2: NMR traces of (*R*)-**158** run at different concentrations in $CDCl_3$. The proton assigned to N(1)*H* showed the greatest degree of concentration dependence.

A4 NMR assignments using NOE-spectroscopy

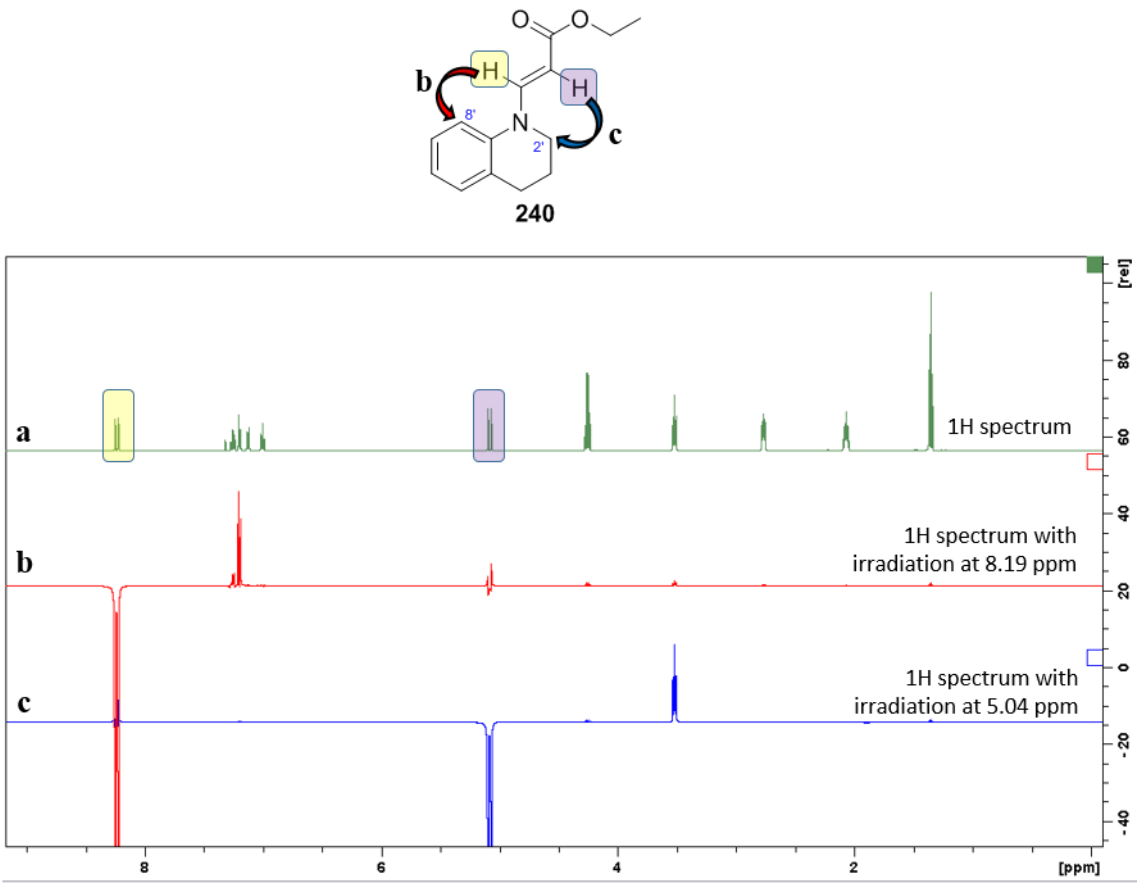


Figure A4.1: NOE analysis of **240**. Irradiation at the two alkene protons let to enhancement of either C(8')*H* or C(2')H_2, confirming the (*E*)-double bond geometry.

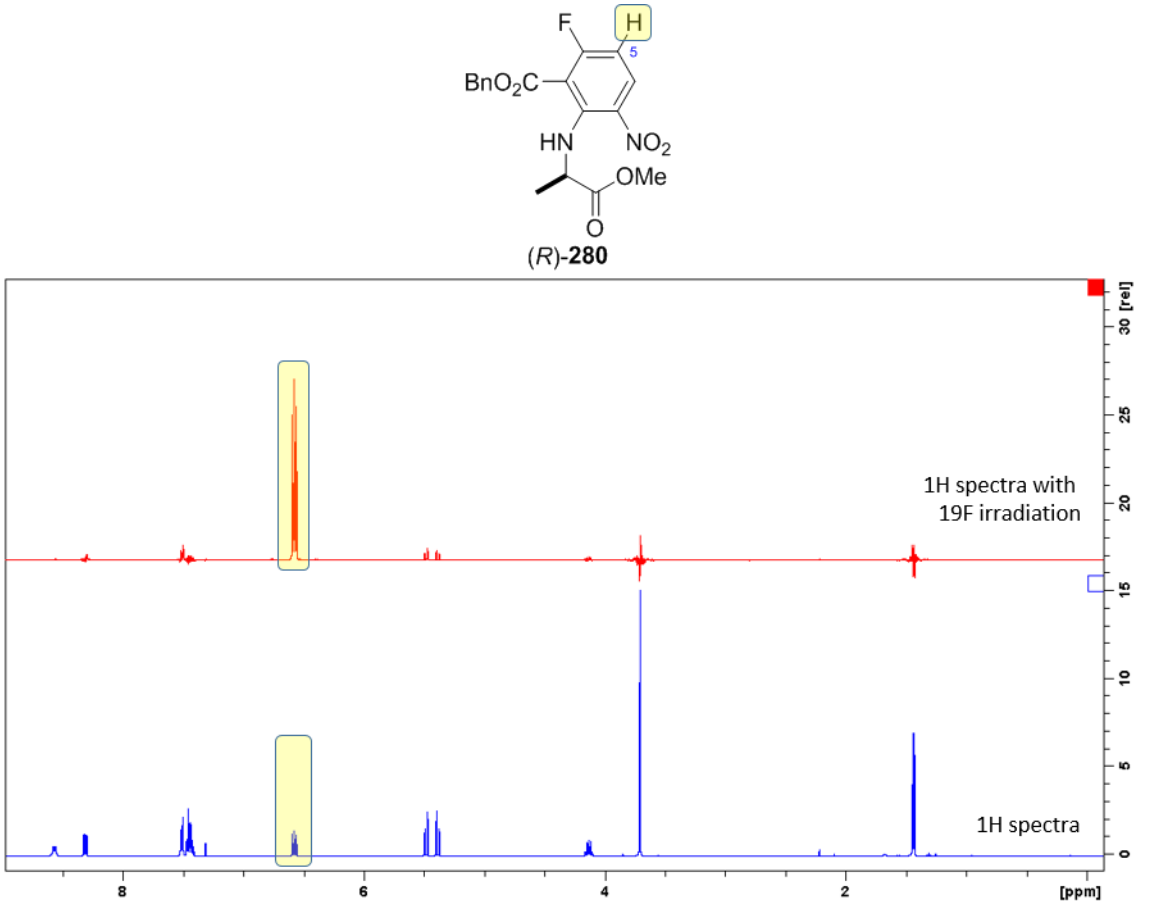


Figure A4.2: HOSEY analysis of (*R*)-**280**. Irradiation of the 19F signal led to enhancement of the C(5)*H* signal, supporting the regiochemistry reported in Scheme 4.14.

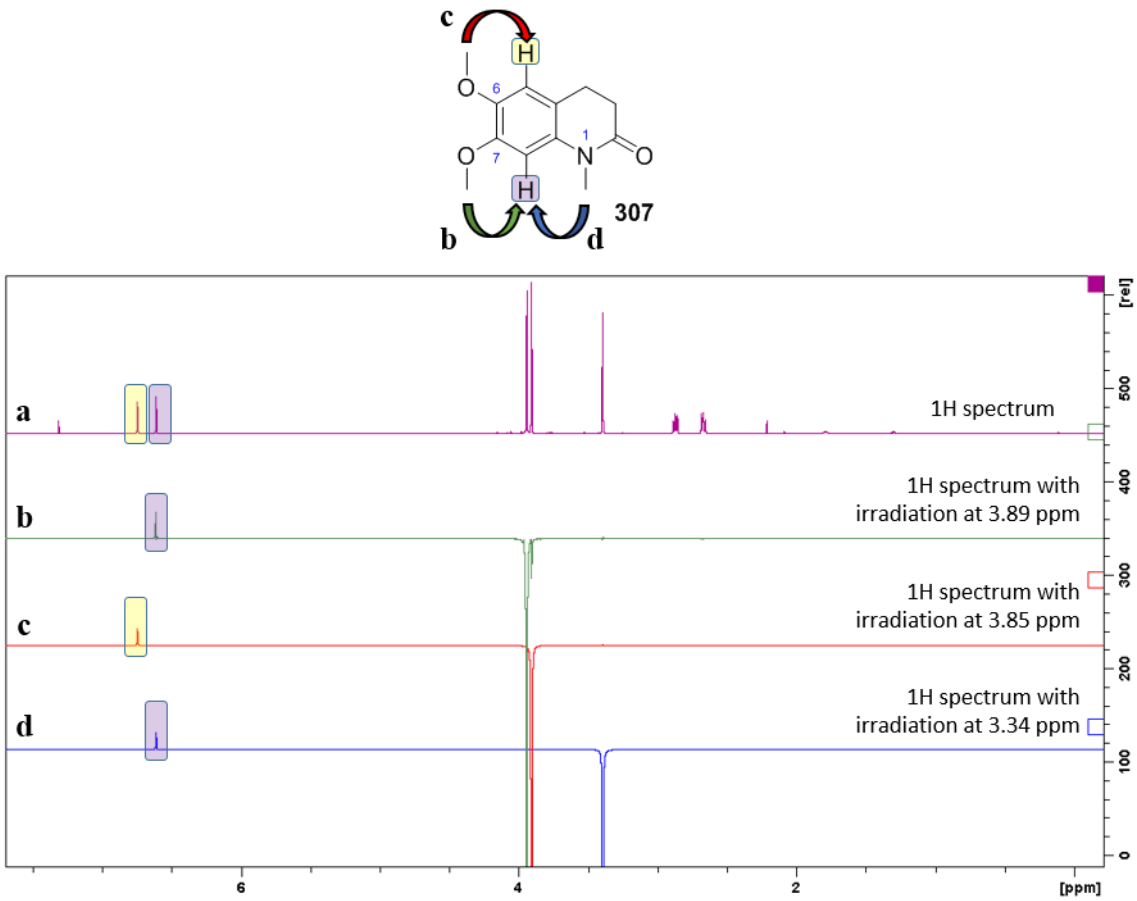


Figure A4.3: NOE analysis of **307**. Irradiation at the three CH_3 signals showed enhancement of either C(5)*H* or C(8)*H*, confirming the chemoselectivity for N-alkylation (Scheme 4.21) and the assignment of each methoxy signal.

A5 Publications arising from this thesis

Rooney, T.; Filippakopoulos, P.; Fedorov, O.; Picaud, S.; Cortopassi, W.; Hay, D.; Martin, S.; Tumber, A.; Rogers, C.; Philpott, M.; Wang, M.; Thompson, A.; Heightman, T.; Pryde, D.; Cook, A.; Paton, R.; Müller, S.; Knapp, S.; Brennan, P.; Conway, S. *Angew. Chem. Int. Ed.* **2014**, *53*, 6126–6130.