Design, Synthesis and Biological Evaluation of Narciclasine Analogues

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This research has been carried out under the supervision of

Dr Lorenzo Caggiano

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Signed......................................................

Date..........................................................
For Mike.

Thank you.
Abstract

 Whilst the cultivation of the *Amaryllidaceae* genus has primarily been for their ornamental properties, their use in traditional medicine is also well documented. Although over 100 alkaloids from this genus have been isolated, the phenanthridinone analogues narciclasine and pancratistatin isolated from the daffodil bulb, are particularly interesting due to their cytostatic and cytotoxic properties. They have potent and selective anticancer activity which is seemingly unique when compared to currently available chemotherapeutic agents. However, they have yet to be fully exploited as therapeutic agents due to their complex total synthesis and scarce availability from natural sources. The work described herein demonstrates short synthetic sequences which have been developed to gain simple analogues of these natural products. These have been assessed by MTS cell proliferation assays in order to gain more understanding of the SARs of narciclasine and pancratistatin, which in turn will be used to design additional biologically active compounds.

These natural products share a dihydroisoquinolinone core and a series of AB-ring analogues were synthesised using a one-pot procedure previously developed and published by the group. Analogues were tested for anticancer activity by MTS cell proliferation assays against HT29 colon cancer cell line. Although these analogues were not biologically active their isoquinolinone counterparts showed a marked improvement with IC_{50} values below 300 μM. Functionalisation to simulate the C-ring of the natural products had limited success. Allylation gave analogues with IC_{50} values below 250 μM, however attempts at subsequent dihydroxylation proved to be capricious. Acetylation at the benzylic position showed some potential with the most promising compound having an IC_{50} value of 33 μM. Upon N-methylation of the acetylated lactam some activity was lost with the IC_{50} value increasing to 110 μM.

Optimisation of preliminary investigations was undertaken in order to synthesise late stage ABC-ring analogues using Robinson annulation, Curtius rearrangement and Friedel-Craft acylation as key reaction steps. A novel methylenedioxy analogue was synthesised and further functionalisation was briefly explored with very limited success. Preliminary in-house MTS cell proliferation assays against the HT29 cells showed promising biological activity with IC_{50} values ranging from 9 to 50 μM. These selected analogues were sent to the NCI for further analysis.

In order to improve the water solubility of the natural product a quinazolinone AB-ring core was incorporated to generate late stage tricyclic analogues with a basic nitrogen. This utilised a one-step synthesis from anthranilamide and glutaraldehyde starting materials. Unfortunately these compounds were found to be unstable and further functionalisation was problematic due to decomposition of the tricyclic compounds. C-ring analogues were also examined which were derived from sugars such as L- and D-lyxose, furnishing hydroxylated derivatives with known absolute and relative stereochemistry.
Acknowledgments

I would like to start by expressing my sincerest thank you to my supervisor Dr Lorenzo Caggiano who has helped and encouraged me throughout my PhD. He is a passionate researcher who inspires hard work and dedication and I am privileged that he offered me a position within his group. The transition from Pharmacist to Medicinal Chemist has been difficult but he has taken the time to mentor me in the fundamentals of chemistry and laboratory techniques. In addition, he has encouraged me to expand my development into other areas of research and also teaching undergraduate pharmacy students. For this I am grateful.

Learning MTS cell proliferation assay techniques was under the supervision of Dr Pauline Wood and Dr Alex Cuipa (a former member of the group) and they deserve a special mention, particularly Alex. Thank you for the many hours you have sat with me teaching me what I needed to know. I would also like to take this opportunity to thank Dr Tim Woodman for his help with NMR spectroscopic analysis and Dr Anneke Lubben for mass spectrometry.

Life at the University would not have been the same without the past and present members of laboratory 3.11 and 3.5/3.7. You are all an inspiring group of people who have gone on to do amazing things. Thank you for the laughs Gemma, Alex, Ben, Amit, Jo, Helen, Kate and Liz. In particular, Dr Jo Swarbrick who has always been there to help and encourage me and I hope we will not lose touch. Thank you for running great chemistry workshops. Kim Luetchford has also become a good friend, thanks for all of the computer short cuts. This would have been a nightmare without you.

Thank you to all of the members of the Caggiano group, including our many project students, they especially injected some joy and enthusiasm for research when things were tough. Dr Katie Judd deserves a mention as she started this interesting project and Dr Gemma Tunbridge who made me laugh every day. It is a joy to say that they have both become friends I look forward to future parties.

To my family, thank you for supporting me and keeping me grounded. Mum and Dad, I aspire to be like you, working hard but also knowing how to enjoy life. To my brother Chris, you are an inspiration and the brainbox of the family. Thanks for helping me laugh at myself.

Lastly I have to thank my husband Michael. He has been so supportive and encouraging over this PhD period never once complaining about our diminished social life or income. He told me every day that what I was doing was amazing and that gave me support I needed to keep going, especially during the hard times when chemistry was not behaving. I love you and I thank you with all my heart.
Table of contents

Abstract ............................................................................................................................. i

Acknowledgments .......................................................................................................... ii

Table of contents ........................................................................................................... iii

List of Figures .................................................................................................................... vi

List of Schemes ................................................................................................................ viii

List of Tables ................................................................................................................... xii

Compound numbering ................................................................................................... xiii

List of Abbreviations ...................................................................................................... xiv

1. Introduction .................................................................................................................. - 1 -

1.1. Background ............................................................................................................. - 1 -

1.1.1. Galanthamine .................................................................................................... - 1 -

1.1.2. Narciclasine and related derivatives ................................................................. - 2 -

1.1.3. The phenanthridinones, cancer and current cancer therapy .............................. - 3 -

1.2. Biological Mode of Action of the phenanthridinones ............................................ - 4 -

1.2.1. The cytostatic effect of narciclasine ................................................................. - 5 -

1.2.1.1. Inhibition of protein synthesis .................................................................. - 5 -

1.2.1.2. Prevention of cell migration ....................................................................... - 6 -

1.2.2. The cytotoxic effect of the phenanthridinone derivatives ................................. - 6 -

1.2.2.1. Cell death and the induction of apoptosis .................................................. - 6 -

1.2.2.1.1. Extrinsic pathway ............................................................................... - 6 -

1.2.2.1.2. Intrinsic pathway .................................................................................. - 7 -

1.3. Phenanthridinone derivatives and drug development ........................................... - 8 -

1.3.1. Total synthesis of narciclasine ........................................................................ - 9 -

1.3.2. Total synthesis of pancratistatin ................................................................. - 11 -

1.3.3. Structure-activity relationship of the phenanthridinone group ....................... - 13 -

1.3.3.1. A-ring ................................................................................................. - 14 -

1.3.3.2. B-ring .................................................................................................. - 15 -

1.3.3.3. C-ring .................................................................................................. - 16 -

1.3.4. Pro-Drug synthesis and activity ................................................................. - 18 -

1.4. Project aims and objectives .................................................................................... - 20 -

2. Synthesis and evaluation of AB-ring analogues ....................................................... - 22 -

2.1. Introduction to the isoquinolinone motif ............................................................. - 22 -

2.2. Synthesis of dihydroisoquinolinone ................................................................. - 22 -

2.3. Oxidation reaction - background ........................................................................ - 24 -

2.3.1. Oxidation of the lactams using DDQ ............................................................. - 25 -
2.3.2. Oxidation of the lactams using Pd/C ........................................... - 28 -

2.4. Cyclisation of cinnamic acid ................................................................................. - 28 -

2.5. Methylation ........................................................................................................ - 31 -

2.6. Functionalisation of the olefin ............................................................................ - 34 -

2.6.1. Functionalisation at C(4) ............................................................................ - 35 -

2.6.2. Oxygenation at C(3)/C(4) ........................................................................ - 39 -

2.6.2.1. Benzyllic oxidation of the dihydroisoquinolinoine derivatives ............... - 40 -

2.6.2.2. Oxygenation of the isoquinolinoine derivatives ...................................... - 41 -

2.6.3. Functionalisation at C(3) ............................................................................ - 43 -

2.6.3.1. Sharpless dihydroxylation .................................................................... - 46 -

2.7. Biological evaluation .......................................................................................... - 48 -

2.8. Conclusions and future work ............................................................................ - 50 -

3. Synthesis and evaluation of ABC-ring analogues .............................................. - 52 -

3.1. Introduction and preliminary work by the Caggiano group .......................... - 52 -

3.2. Synthesis of 3,4,4a,5-tetrahydro[1,3]dioxolo[4,5-j]phenanthridin-6(2H)-one 148 ... - 52 -

3.2.1. Claisen condensation in the synthesis of β-ketoester 135 .............................. - 52 -

3.2.2. The Robinson annulation as a one-pot procedure ..................................... - 53 -

3.2.3. The Robinson annulation as a two-step procedure ................................. - 54 -

3.2.3.1. Michael Addition ............................................................................ - 54 -

3.2.3.2. Intramolecular aldol cyclisation ......................................................... - 55 -

3.2.4. Reduction of the ketone ............................................................................ - 56 -

3.2.5. Saponification of the ester ........................................................................ - 57 -

3.2.6. Modified Curtius rearrangement and Friedel-Craft acylation .................. - 57 -

3.3. Synthesis of 7-hydroxy-8,9-dimethoxy-3,4,4a,5-tetrahydrophenanthridin-6(2H)-one 155 - 58 -

3.4. Synthesis of 8,9-dimethoxy-3,4,4a,5-tetrahydrophenanthridin-6(2H)-one 162 ...... - 60 -

3.5. Functionalisation of the narcislasine derivatives ............................................. - 61 -

3.5.1. Hydrogenation with palladium .................................................................. - 62 -

3.6. Epoxidation of the Hagemann’s ester .............................................................. - 66 -

3.7. Saponification of epoxide 171 ......................................................................... - 69 -

3.8. Narciprimine 183 ............................................................................................. - 70 -

3.9. Biological evaluation ....................................................................................... - 74 -

3.10. NCI 60 cell line screening ............................................................................. - 76 -

3.11. Conclusions and Future work ....................................................................... - 77 -

4. Synthesis and evaluation of N ABC-ring analogues ........................................... - 79 -
4.1. Introduction to quinazolinones ................................................................. - 79 -
4.2. Synthesis of quinazolinones ..................................................................... - 79 -
4.3. Optimisation of the reaction conditions .................................................. - 82 -
4.4. Ethanol adduct and pro-drug type analogues .......................................... - 83 -
4.5. Attempted synthesis of vinyl dihydroquinazolinone analogues ............... - 87 -
4.6. Attempted functionalisation of the Enamine ........................................... - 88 -
4.7. Synthesis of A-ring analogues..................................................................... - 94 -
  4.7.1. Attempted synthesis of 2-amino-4,5-dimethoxybenzamide from 2-amino-4,5-
            dimethoxybenzoate........................................................................... - 94 -
  4.7.2. Synthesis of 2-amino-4,5-dimethoxybenzamide from 2-amino-4,5-dimethoxybenzoic acid - 97 -
  4.7.3. Proposed synthesis of 6-amino-2,3,4-trimethoxybenzamide ..................... - 98 -
4.8. Tricyclic substituted A-ring analogues.................................................... - 102 -
4.9. Reduction of the enamine by Pd/C ............................................................. - 102 -
4.10. Reduction by NaBH₃CN ........................................................................... - 105 -
4.11. Functionalisation of the enamine by ruthenium ...................................... - 107 -
4.12. Functionalisation of the C-ring using sugars ......................................... - 108 -
4.13. Cyclisation of the sugar side-chain ....................................................... - 111 -
  4.13.1. Oxidation of the primary alcohol..................................................... - 112 -
  4.13.2. Activation of the primary alcohol.................................................... - 113 -
4.14. Conclusions and future work ................................................................. - 114 -
5. Conclusions and future work ...................................................................... - 116 -
6. Experimental .............................................................................................. - 118 -
  6.1. General Experimental ............................................................................. - 118 -
  6.2. MTS cell proliferation assay protocol ..................................................... - 119 -
  6.3. Synthesis of AB-ring analogues .............................................................. - 121 -
  6.4. Synthesis of ABC-ring analogues ........................................................... - 152 -
  6.5. Synthesis of N ABC-ring analogues ...................................................... - 178 -
7. Appendices - NCI 60 cell line screen One-Dose data .................................. - 207 -
8. Bibliography ............................................................................................... - 212 -
List of Figures

Figure 1.01: Examples of compounds generated from norbelladine 1
Figure 1.02: Chemical structure of galanthamine 5 and morphine 6
Figure 1.03: Structure of narciclasine 7 and pancratistatin 8 with 7-deoxy counterparts 9 and 10
Figure 1.04: Elongating peptide chain from eukaryotic ribosome
Figure 1.05: Diverging pathways of narciclasine-mediated induction of apoptosis
Figure 1.06: Numbering of the phenanthridinone narciclasine core
Figure 1.07: Biological activity of narciclasine 7 and pancratistatin 8
Figure 1.08: Spatial similarities of pancratistatin 8 compared to bioisostere 27
Figure 1.09: Activity of aza-analogues against HeLa and MCF7 cancer cell lines
Figure 1.10: Manipulation of the B-ring and observed biological activity
Figure 1.11: C(1) derivatives of the phenanthridinone natural products
Figure 1.12: Structure of C(4) derivative 43 and C(2)-C(3) diol 44
Figure 1.13: Structure of C(2)-C(4) 45 and C(3)-C(4) diol 46
Figure 1.14: Summary of SAR
Figure 1.15: Disodium 7-O-phosphate 47, sodium 3,4-O-cyclic phosphate 48 and sodium 4-O-phosphate 49 prodrugs of pancratistatin
Figure 1.16: C(1) benzoate ester analogues of pancratistatin
Figure 2.01: Dihydroisoquinolinone 52 and highlighted core of narciclasine 7
Figure 2.02: Structure of lactam 60 and lactam 61
Figure 2.03: BF₂ adduct 62
Figure 2.04: Preliminary biological assessment undertaken by the Caggiano group
Figure 2.05: NOESY correlation between proton(s) on C(3) and N-methyl
Figure 2.06: Acetylated isoquinolinone compounds
Figure 2.07: Topoisomerase I inhibitors
Figure 2.08: Novel NSC 314622 analogues 104 and 105
Figure 2.09: BF₂-complex 62
Figure 2.10: Biological evaluation of dihydroisoquinolinone derivatives by MTS cell proliferation assay using HT29 colon cancer cell line
Figure 2.11: Biological activity of oxidised analogues 64 and 65 by MTS cell proliferation assay using HT29 colon cancer cell line
Figure 2.12: Biological activity of C(4) acetylated analogues by MTS cell proliferation assay using HT29 colon cancer cell line
Figure 2.13: Biological activity of C(3) allylated analogues 126, 128 and 129 by MTS cell proliferation assay using HT29 colon cancer cell line

Figure 3.01: Previous preliminary biological assessment undertaken by the Caggiano group by MTS cell proliferation assay using HT29

Figure 3.02: Comparison of pancratistatin 8 with the 103trans and 103cis isomers obtained

Figure 3.03: Structure of narciprimine 183 and proposed analogues 184 and 185

Figure 3.04: IC_{50} values following MTS cell proliferation assay using HT29 colon cancer cells

Figure 4.01: Similarity in structure of dihydroquinazolinone highlighted in the natural products

Figure 4.02: C-ring analogues for the dihydroquinazolinone compounds

Figure 4.03: Suggested compounds extracted from the oxidation reaction with RuO_{4}

Figure 4.04: Percentage ratio ascertained by crude ¹H NMR spectroscopic analysis

Figure 4.05: Benzamide analogues obtained using CDI followed by aqueous washes

Figure 4.06: Structure of compound 253

Figure 4.07: Optimised yields of analogues 275 and 276

Figure 4.08: Biological activity of sugar analogues
# List of Schemes

| Scheme 1.01: Enzymatic dihydroxylation reported by Hudlicky et al. | 10 |
| Scheme 1.02: Key steps in the synthesis reported by Elango et al. | 10 |
| Scheme 1.03: Key steps in the synthesis reported by Keck et al. | 11 |
| Scheme 1.04: Photocyclisation of analogue | 12 |
| Scheme 1.05: Synthesis of late stage pancratistatin intermediates from coupling fragments | 13 |
| Scheme 2.01: General synthesis of lactam analogues by Judd et al. | 22 |
| Scheme 2.02: Isocyanate formation and subsequent cyclisation to afford lactam | 23 |
| Scheme 2.03: Oxidation of dihydroisoquinolinones by DDQ | 26 |
| Scheme 2.04: Dehydrogenation of compound | 26 |
| Scheme 2.05: Expected outcome following the procedure developed by Estevez et al. | 27 |
| Scheme 2.06: Solvent free dehydrogenation of lactam | 28 |
| Scheme 2.07: Isocyanate formation and thermal cyclisation of the 3-methoxy cinnamic acid | 29 |
| Scheme 2.08: Successful cyclisation of cinnamic acid analogues and | 30 |
| Scheme 2.09: Methylation and ester hydrolysis to gain N-methylindole-3-propionic acid | 31 |
| Scheme 2.10: Structure of analogues and | 31 |
| Scheme 2.11: Routes to the observed products | 33 |
| Scheme 2.12: Potential functionalisation at C(4) | 35 |
| Scheme 2.13: Acetylation of isoquinolinone | 35 |
| Scheme 2.14: Reported Friedel-Craft benzoylation | 37 |
| Scheme 2.15: Intramolecular cyclisation to afford indanone analogues | 37 |
| Scheme 2.16: Intramolecular cyclisation of 4-{(indol-3-yl)butanoic acid | 38 |
| Scheme 2.17: Two-step synthesis of phenanthridinone | 38 |
| Scheme 2.18: Possible C(4) and/or C(3) oxidation products | 40 |
| Scheme 2.19: Benzylic oxidation using DDQ in dioxane and water | 40 |
| Scheme 2.20: Benzylic oxidation using SeO$_2$ | 41 |
| Scheme 2.21: Previously reported syntheses of trione compounds | 42 |
| Scheme 2.22: Formation of trione | 43 |
| Scheme 2.23: Proposed route and similarity of the C-ring analogues and narciclasine | 43 |
| Scheme 2.24: Retrosynthetic approach to the bicyclic analogue with side-chain | 44 |
| Scheme 2.25: Allylation of methylenedioxy dihydrocinnamic acid | 44 |
Scheme 3.01: Synthetic route to narciclasine analogues 52
Scheme 3.02: Claisen condensation reaction to furnish β-ketoester 135 53
Scheme 3.03: Robinson annulation 53
Scheme 3.04: Attempts at one-pot Robinson annulation 54
Scheme 3.05: Solvent and solvent-free Robinson annulation, with intact ester 54
Scheme 3.06: Michael addition using MVK 55
Scheme 3.07: Cyclisation of the Michael adduct 142 55
Scheme 3.08: Summary of methods employed to remove the conjugated system of ketone 143 56
Scheme 3.09: Complete reduction of the carbonyl in analogue 139 57
Scheme 3.10: Saponification of the ester 144 57
Scheme 3.11: Isocyanate formation and intramolecular acylation to analogue 148 58
Scheme 3.12: Synthesis of the Michael adduct 151 58
Scheme 3.13: Cyclisation using Soxhlet apparatus 59
Scheme 3.14: Reduction, then saponification of ester 152 59
Scheme 3.15: Cyclisation to afford the narciclasine analogue 155 59
Scheme 3.16: Synthesis of the Michael adduct 158 60
Scheme 3.17: Cyclisation, reduction and then saponification of enone 158 60
Scheme 3.18: Modified Curtius rearrangement and cyclisation 61
Scheme 3.19: Reduction of narciclasine analogue 155 using Pd/C and atmospheric hydrogen 62
Scheme 3.20: Synthesis for the late stage intermediate via a two-step pathway 63
Scheme 3.21: Reduction of narciclasine analogue 162 using Pd/C and atmospheric hydrogen 64
Scheme 3.22: Reduction of narciclasine analogue 148 using Pd/C and atmospheric hydrogen 64
Scheme 3.23: Coupling constants of the B/C-ring junction and idealised dihedral angle 65
Scheme 3.24: Proposed epoxidation and diastereomeric outcomes of analogue 139 66
Scheme 3.25: Epoxidation of analogue 139 and their proposed stereochemistry 67
Scheme 3.26: Epoxidation of enone 174 using TBHP 68
Scheme 3.27: Epoxidation of enone 177 using anhydrous conditions 68
Scheme 3.28: Epoxidation of an enone using mCPBA 69
Scheme 3.29: Saponification of an ester in the presence of an epoxide 69
Scheme 3.30: Retrosynthetic approach to the narciprimine analogues

Scheme 3.31: Synthesis of biphenyl analogue 190 from bromobenzoic acid

Scheme 3.32: Example of ortho-arylation of free benzoic acids by aryl iodides

Scheme 3.33: Attempted coupling of 1-bromo-3,4,5-trimethoxybenzene 192 and salicylic acid 188

Scheme 3.34: Successful synthesis of the coupled benzoic acid 194 and proposed cyclised product

Scheme 4.01: One-pot click synthesis of 2-phenyl-2,3-dihydroquinazolin-4(1H)-one 197

Scheme 4.02: Proposed disconnections to achieve a fused cyclic B/C-ring

Scheme 4.03: Reversibility of the condensation reaction

Scheme 4.04: Isolated products from a combination of reactions

Scheme 4.05: Synthesis of enamine 198 and proposed structure of compound 203

Scheme 4.06: One-pot procedure the synthesis of enamine 198

Scheme 4.07: Proposed reaction mechanism for the tricyclic ring

Scheme 4.08: Suggested structure of analogue 202

Scheme 4.09: Obtaining the methoxy analogue 205

Scheme 4.10: Attempted cyclisation using acrolein

Scheme 4.11: Epoxidation of the nucleophilic alkene of carvone analogue 208

Scheme 4.12: Reported functionalisation of an enamine 210 using oxone

Scheme 4.13: Acetylation of lactam 83, described in Section 2.6.1

Scheme 4.14: Cis-dihydroxylation using catalytic RuO₄

Scheme 4.15: Initial assignment of product 215 based on ¹H NMR spectroscopic analysis

Scheme 4.16: Oxidation, decarboxylation and cyclisation towards product 216

Scheme 4.17: Reduction of C(3a) using NaBH₄

Scheme 4.18: Proposed disconnection to achieving hydroxylated A-ring analogues

Scheme 4.19: Proposed conversion of the dimethoxy ester 222 to an amide 219

Scheme 4.20: Proposed synthetic route to benzamide 219

Scheme 4.21: Reported reaction with hydroxylamine

Scheme 4.22: Reaction with hydrazine reported by Kuemmerle et al.

Scheme 4.23: Protecting the amine with acetyl chloride

Scheme 4.24: Equilibrium of amide formation

Scheme 4.25: Summary mechanism of the CDI reaction

Scheme 4.26: Proposed disconnection in the synthesis of trimethoxybenzamide 236
Scheme 4.27: Synthesis of 4,5-dimethoxyanthranilic acid 241

Scheme 4.28: Suggested structure of compound 242

Scheme 4.29: Model reactions facilitated by AlCl₃

Scheme 4.30: Procedure demonstrated by Kaila et al.

Scheme 4.31: Formation of the trimethoxyistatin analogue 252

Scheme 4.32: Hydrogenation using Pd/C and atmospheric H₂ gas

Scheme 4.33: Potential reaction mechanism

Scheme 4.34: Product 257 isolated following the hydrogenation of the corresponding enamine

Scheme 4.35: Product 258 isolated following the hydrogenation of the corresponding enamine

Scheme 4.36: Two-step reaction for the formation of 261 (not isolated)

Scheme 4.37: Cyclisation and subsequent iminium reduction by NaBH₃CN

Scheme 4.38: Using NaBH₃CN for the synthesis of tricyclic analogue 254

Scheme 4.39: Reduction of the iminium cation by NaBH₃CN

Scheme 4.40: Conditions reported by Nacro et al.

Scheme 4.41: Reduction of the tricyclic analogue by NaBH₃CN

Scheme 4.42: Synthesis of bislactams 268 and 269

Scheme 4.43: Retrosynthetic analysis of C-ring analogues using sugars

Scheme 4.44: Stereochemistry of D- and L-lyxose and possible analogues

Scheme 4.45: Reaction of anthranilamide with D-lyxose

Scheme 4.46: Formation of the sugar derivative 274

Scheme 4.47: Investigation that sugar 274 in an intermediate of analogue 273

Scheme 4.48: Proposed synthetic routes to the cyclised C-ring

Scheme 4.49: Oxidation of a primary alcohol in preference to a secondary alcohol

Scheme 4.50: Synthesis of benzaldehyde 282

Scheme 4.51: Selective tosylation of the primary alcohol
List of Tables

Table 1.01: Summary of the total synthesis of narciclasine 9
Table 1.02: A selection of the total syntheses of pancratistatin 11
Table 2.01: Dehydrogenation of lactams using DDQ 27
Table 2.02: $^1$H and $^{13}$C NMR assignment for analogue 84 32
Table 2.03: Synthesis of N-methylated compounds 34
Table 2.04: Yields of unsubstituted and C(3)-allylated lactam analogues 45
Table 2.05: Yields for Sharpless AD reactions 47
Table 3.01: Optimisation of the reaction conditions to form biaryl analogues 73
Table 3.02: NCI data for the mean growth inhibition using single-dose data 77
Table 4.01: $^1$H NMR assignment for ethanol adduct 202 84
Table 4.02: $^1$H NMR ratio after the enamine 198 is stirred in protic solvent 86
Table 4.03: $^1$H and $^{13}$C NMR assignment for methoxy analogue 205 87
Table 4.04: $^1$H NMR assignment for [6, 9] fused bicyclic ring 217 93
Compound numbering

The numbering of the tricyclic phenanthridinone analogues will be according to Pettit et al. for consistency and are shown below. The IUPAC compound names will be given.\textsuperscript{1}
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>aa-tRNA</td>
<td>Aminoacyl transfer ribonucleic acid</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>Ach</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
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<td>Aluminium chloride</td>
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<tr>
<td>aq.</td>
<td>Aqueous</td>
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<tr>
<td>BF₃·OEt₂</td>
<td>Boron trifluoride diethyl etherate</td>
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<td>CH₂Cl₂</td>
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</tr>
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</tr>
<tr>
<td>DISC</td>
<td>Death inducing signalling complex</td>
</tr>
<tr>
<td>DPPA</td>
<td>Diphenylphosphoryl azide</td>
</tr>
<tr>
<td>DR4</td>
<td>Death receptor 4</td>
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<tr>
<td>ED₅₀</td>
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<td>Ethyl</td>
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1. Introduction
   1.1. Background

Members of the Amaryllidaceae family of bulbous plants include Galanthus (snowdrops), Amaryllis (Lily) and Narcissus (daffodils) and interest in this family has primarily been for their cut flowers and decorative properties. However, of particular interest is their use in traditional medicine which has been well documented around the globe. Indeed, records dating back to ancient Greece show that Hippocrates of Kos (ca. B.C. 460 – 370) recommended the use of a pessary of narcissus oil to treat uterine tumours. To date over 100 alkaloids have been isolated from the Amaryllidaceae family and of particular significance are a structurally diverse group such as lycorine 2, crinine 3 and montanine 4, shown in Figure 1.01, which share a common biosynthetic pathway from the precursors norbelladine 1.3 These alkaloids have so far proved to be a bountiful source of biologically active compounds exhibiting a range of different activities. The exciting promise of these natural products is demonstrated by galanthamine 5, approved by the FDA for the symptomatic relief of Alzheimer’s disease, although it unfortunately remains an incurable disease.

![Figure 1.01: Examples of compounds generated from norbelladine 1](image)

1.1.1. Galanthamine

The anecdotal use of snowdrops to treat poliomyelitis in the villages of Eastern Europe in addition to its similarity to morphine 6, Figure 1.02, led to its development as a drug.4,5 Although galanthamine was found to have some analgesic effects, a more exciting property was its ability to cross the blood brain barrier and selectively inhibit the enzyme acetylcholinesterase. This enzyme is responsible for the removal of acetylcholine (ACh) from the synaptic cleft following neuronal firing and it is well established that a decline in ACh neurons is responsible for the diminishing
cognitive function of Alzheimer’s patients. Galanthamine 5 slows the enzymatic breakdown of ACh, and so prolongs cholinergic neurotransmission leading to the successful treatment of the symptoms of these patients. Galanthamine was licenced in the mid 1990’s as Reminyl in the UK. Although galanthamine can be made by organic synthesis it is interesting to note that Reminyl is manufactured using galanthamine extracted from commercially grown daffodil bulbs.

Figure 1.02: Chemical structure of galanthamine 5 and morphine 6

1.1.2. Narciclasine and related derivatives

Keen gardeners have long known the irritant nature of daffodil bulbs and how cut Amaryllidaceae flowers can hasten the wilting of other flowers within a vase. This physiological effect has been attributed to a series of molecules which are structurally related to lycorine 2 shown in Figure 1.01. Narciclasine 7 was the first to be isolated by Cerrioti et al. in 1967 and pancratistatin 8 isolated in 1984 by the Pettit group, both of which are shown in Figure 1.03 with their 7-deoxy counterparts 9 and 10. It should be noted that the numbering of the tricyclic building block is according to Pettit et al. and will be further defined in Section 1.3.3 and is used throughout this work.

Figure 1.03: Structure of narciclasine 7 and pancratistatin 8 with 7-deoxy counterparts 9 and 10

The naming of this series of analogues is much debated. As they are lacking a basic nitrogen they are not true alkaloids. They have been widely termed the isocarbostyril group containing the
indicative isoquinolinone core, however this is misleading as it fails to capture the C-ring of the natural products. In addition, they have also been referred to as phenanthridinone derivatives, however it should be noted that they lack aromaticity of the C-ring. Within this work they will be referred to as phenanthridinone derivatives in order to fully appreciate the tricyclic nature of the natural products.

Since their discovery narciclasine 7 and pancratistatin 8 have subsequently shown moderate antiviral activity, albeit at near toxic concentrations. However, it is their antineoplastic nature, particularly against some treatment-resistant cancer cell lines, which have shown them to be excellent lead candidates in our fight against cancer as reviewed by Kornienko and Evidente.  

1.1.3. The phenanthridinones, cancer and current cancer therapy

Cancer is well known as one of the leading causes of morbidity and mortality in the western world and although surgery and radiotherapy are valuable treatment options chemical and biological molecules are still the mainstay of treatment. As a consequence much research has been initiated into finding agents used for combating the disease. Cancerous cells are known to have lost normal cellular control of replication and current chemotherapies exploit this increase in cell division by the catastrophic targeting of DNA and/or interfering with the cell division process. Broadly speaking, chemotherapy can be expressed in terms of a drug’s biological mode of action, a brief summary of which is highlighted herein.

Alkylating agents are a prominent feature in current cancer treatment. Interference in cell replication and division is caused by the intercalation between nucleotide bases, modifying DNA (in addition to some other cellular proteins) by alkylation and/or forming crosslinks between DNA strands. This damage to the DNA can stimulate cell death providing a valuable treatment option for some cancers. The nitrogen mustards were the first to be investigated in the 1940’s when chemotherapeutic agents such as chlorambucil and cyclophosphamide were discovered. Platinum agents are often described as having alkylating-like properties and include drugs such as cisplatin and carboplatin. The platinum binds to purine nucleotides forming adducts on the DNA strands, similar to alkylation, which can then go on to form intrastrand crosslinks, thereby preventing normal DNA translation and replication.

A group of compounds which mimic cellular molecules are termed the antimetabolites. This includes pyrimidine analogues 5-fluorouracil, capecitabine and gemcitabine and purine analogues such as 6-mercaptopurine. These drug molecules are incorporated into replicating DNA and RNA in place of pyrimidine-bases cytosine, uracil and thymine or purine-bases adenine and guanine. This
causes replication to fail by preventing chain elongation and subsequently induces apoptosis.\textsuperscript{13} Another subset of antimetabolites are the folate antagonists such as methotrexate and pemetrexed.\textsuperscript{14} These inhibit the dihydrofolate reductase enzyme a key component in the metabolism of nucleotide bases, again preventing cell division and inducing apoptosis.

Microtubules are an important component of a cell’s cytoskeleton, in particular they play a key role in the cell division process by forming the mitotic spindle.\textsuperscript{15} Each microtubule is made from a polymer of tubulin which is a target for a group of chemotherapeutic compounds termed spindle poisons. Tubulin stabilisers, such as paclitaxel, bind to tubulin already in formation preventing the disassembly of microtubules thereby causing prolonged mitotic arrest and cell death. Other agents work by binding tubulin monomers thereby preventing elongation to microtubules and include the vinca alkaloids such as vinblastine.

During DNA translation and replication topoisomerase enzymes release the build-up of DNA torsional strain by instigating controlled single and double strand breaks of the double helix.\textsuperscript{13} Topoisomerase poisons form a complex of this DNA-enzyme intermediate preventing religation of the strand breaks thereby initiating cell death. Such agents include irinotecan, camptothecin and doxorubicin.

Although the intention of these treatments is to target the cancer specifically it is an unfortunate consequence that normal, healthy tissue is also affected. This leads to unwanted side effects sometimes so severe that treatment is terminated. In addition, cancer cells have adapted to evade cell death becoming resistant to a number of the first line treatments currently available. There is therefore an obvious need to continue the development of novel anticancer compounds. They require better side effect profiles in addition to the ability to circumvent resistance developed in some cancer cells. Narciclasine 7 and pancratistatin 8 have both been shown to have unique anticancer activity and will be discussed below in more detail.

\subsection*{1.2. Biological Mode of Action of the phenanthridinones}

The biological mode of action of the phenanthridinone derivatives has yet to be definitively determined although their selectivity for a wide range of cancer cell lines has been proven. Indeed, screening of narciclasine 7 and pancratistatin 8 against 60 cancer cell lines by the National Cancer Institute (NCI) has shown them to exhibit a mean GI\textsubscript{50} of 0.016 μM and 0.091 μM respectively, where GI\textsubscript{50} is defined as the concentration required to inhibit 50 % of cell growth.\textsuperscript{1} Preliminary investigations suggest that narciclasine and related derivatives have a distinctive and selective anticancer activity, which will now be described.
1.2.1. The cytostatic effect of narciclasine

Observations in plant physiology suggest that at low doses the phenanthridinone analogues are cytostatic rather than cytotoxic.\textsuperscript{16} For example, narciclasine is a well-established plant growth modulator which has been found to induce dose dependent mitotic cell cycle arrest in lettuce seedlings.\textsuperscript{17} The cytostatic effect has also been observed \textit{in vitro}, in antiproliferative assays, and also \textit{in vivo} using xenograft animal models of human tumours and is explained below.

1.2.1.1. Inhibition of protein synthesis

Ribosomes are complex molecular machines found within cells which are responsible for translating genetic information into the corresponding protein. In 1975 Carrasco \textit{et al.} reported the inhibition of protein synthesis by narciclasine which specifically targets the peptidyl-transferase centre of the 60S subunit in eukaryotic ribosomes.\textsuperscript{18} Further studies by Jimenez \textit{et al.} in wild-type and mutated yeast confirmed that elongation of the amino acid chain is prevented and suggest that this is due to the inability of the amino acyl-tRNA (aa-tRNA) to move from the A-site to the P-site thereby preventing peptide bond formation, shown in Figure 1.04.\textsuperscript{19,20} A recent study probing the structure-activity relationship (SAR) of eukaryotic ribosome inhibitors used X-ray crystallography to show narciclasine specifically binding to the A-site of the peptidyl-transferase centre thereby preventing normal entry and progression of the aa-tRNA.\textsuperscript{21}

![Figure 1.04: Elongating peptide chain from eukaryotic ribosome](image)

In addition, narciclasine has been found to be a natural ligand for eEF1A, a protein highly involved in the delivery of aa-tRNA to the A-site of the ribosome.\textsuperscript{22} The eEF1A protein is also an important component of cytokinesis by mediating actin organisation and any inhibition would therefore be cytostatic. The role of eEF1A in cell migration is of particular interest as it could assist in tackling the prognosis of terminal metastatic cancer patients, the significance of which will be highlighted further below.
1.2.1.2. Prevention of cell migration.

The disruption of cell migration by narciclasine-mediated inhibition of eEF1A\(^{22}\) is complimented by further work by the Lefranc group into xenograft glioblastoma cells.\(^{23}\) Glioblastoma cancer is a particularly aggressive and invasive brain cancer which can actively migrate over large distances through extracellular spaces in the brain. The coflin pathway is directly responsible for regulating the actin cytoskeleton by controlling the stability of actin filaments and therefore plays a key role in cell migration. Narciclasine moderates Rho GTPase activity which in turn prevents the cascade of events leading to coflin activation. Preclinical studies have demonstrated cell cycle arrest and the inhibition of cell migration of glioblastoma cells which, in turn increases the survival of xenograft-bearing mice. It is worth noting here that narciclasine and pancratistatin show particular promise against brain cancers and this may also be due, in part, to their lipophilic nature which enables them to pass the blood brain barrier.

1.2.2. The cytotoxic effect of the phenanthridinone derivatives

1.2.2.1. Cell death and the induction of apoptosis

Apoptosis is a non-traumatic, highly conserved process of cell death which has been widely characterised in the literature. It presents with defined biochemical hallmarks all leading to phagocytosis.\(^{12}\) This tight control ensures the protection of surrounding tissue from intracellular enzymes and cytokines and may also allow for the recycling of proteins. Conversely, necrosis is an uncontrolled form of cell death often resulting from an extreme insult to the cell. It is characterised by rupturing of the cell wall leading to secretion of cytoplasmic constituents. Apoptosis is preferred and its initiation is the target of many antineoplastic compounds.

It is generally well established that apoptosis is initiated by two main pathways, the intrinsic (mitochondrial) pathway or the extrinsic (death-receptor) pathway.\(^{12,24}\) After stimulation of the extrinsic pathway there are thought to be two divergent responses which are cell type specific. Narciclasine and pancratistatin have been shown to stimulate both pathways and are described in more detail below.

1.2.2.1.1. Extrinsic pathway

Dumont et al. showed that narciclasine-mediated apoptosis can occur via the extrinsic pathway.\(^{25}\) Stimulation of the cell surface TNF death receptors, FAS and DR4 leads to the formation of the Death Inducing Signalling Complex (DISC) and the subsequent recruitment and activation of pro-caspase 8 to caspase 8. A divergence of the downstream signalling cascade then occurs which is highlighted in Figure 1.05.
Figure 1.05: Diverging pathways of narciclasine-mediated induction of apoptosis\textsuperscript{25}

Type I cells, such as PC-3 prostate cancer cells used in the study, show a high concentration of caspase 8 activation.\textsuperscript{25} This leads to the direct recruitment and activation of the caspase cascade triggering apoptosis. Type II cells, such as MCF7 breast cancer described by Dumont \textit{et al.}, show a concentration of caspase 8 activation insufficient to initiate the caspase cascade. Amplification of the signal is therefore required and achieved by the permeabilisation of the mitochondrial outer membrane. This results in a release of the pro-apoptotic protein cytochrome \textit{c} and the subsequent formation of the cytochrome-\textit{c}-Apaf-1-procaspase-9 complex, termed the apoptosome, which commits the cell to die.

Complementary work undertaken by Kekre \textit{et al} shows pancratistatin-promoted stimulation of the Fas receptor, albeit with slightly different downstream consequences.\textsuperscript{26} Apoptosis of Jurkat (human T lymphocyte) cancer cells, preferentially to their non-cancerous counterparts, was achieved with low concentrations of pancratistatin. At a concentration of 500 nM a third of the Jurkat cells were shown to undergo apoptosis, this figure increased to over 70 \% at concentrations of 1 \mu M. Interestingly, it was the activation of caspase 3, rather than the previously described caspase 8, and the flipping of the membrane bound phosphatidylserine from the cytosol to the outside of the cell which induced apoptosis in this study.

1.2.2.1.2. **Intrinsic pathway**

The mitochondrion are extremely important organelles with a dual role of providing energy to the cell in the form of ATP as well as playing a crucial role in the management of apoptosis. Pro- and anti-apoptotic proteins elicit a response from the mitochondria by either preventing or instigating
cell death. The bio-energetic differences between cancerous and non-cancerous cell mitochondria and contradictory function of maintaining life and controlling death have led the mitochondria to becoming an exciting new target for anticancer therapies, either directly or by the initiation of pro-apoptotic proteins described above.

A tumour suppressor protein, p53, is an important regulator in the activation of pro-apoptotic members of the Bcl-2 family of proteins. These proteins govern mitochondrial outer membrane potential and therefore ultimately control the release if cytochrome c, apoptosome formation and the activation of caspase 3. Unfortunately, one of the most common genetic defects in human cancer causes a mutation of p53.

Griffin et al. has shown pancratistatin to induce intrinsic apoptosis in both p53 negative HT29 and p53 wild-type HCT116 colon cancer cell lines with IC_{50} values of 100 nM. This is without relative toxicity to normal colon cells, CCD-18Co, which have an IC_{50} > 10 μM. This implies that pancratistatin elicits a response in a p53 independent manner which was shown to be the case. A collapse in mitochondrial membrane potential was observed with a corresponding loss of membrane integrity and the associated release of cytochrome c and subsequent apoptosome formation described previously.

The unique physiological activity of narciclasine and pancratistatin clearly mark them out as good clinical candidates. Although narciclasine was first observed as being an antimitotic substance by Ceriotti et al. in 1967 it does not share the spindle poison characteristics as shown by paclitaxel or vinblastine. Rather, it is thought to elicit a response by the inhibition of protein synthesis previously described. Narciclasine 7 and pancratistatin 8 induce apoptosis either by the initiation of the extrinsic or intrinsic apoptotic pathway, and not by causing catastrophic interface with DNA or associated metabolites as with alkylating agents, platinum agents, antimetabolite or topoisomerase inhibitors. This again highlights the unique mode of action exhibited by these natural products. Unfortunately, their low natural abundance and complex total synthesis have so far prevented their clinical progression.

1.3. Phenanthridinone derivatives and drug development

The extraction of narciclasine and pancratistatin from a variety of different Amaryllidaceae bulbs is a laborious and costly process, typically producing 100-150 mg of pure compound per kg of bulb. This, in addition to their poor aqueous solubility, has had a prohibitive impact on the development of these compounds as clinical candidates. This is at odds to galanthamine which is easily extracted from natural sources due to the presence of a basic nitrogen. However, their seemingly
unique in addition to specific anticancer activity has stimulated research into the total synthesis of the natural products and truncated and unnatural analogues in order to gain a thorough understanding of the anticancer pharmacophore. Furthermore, research into improving the drug-like characteristics of the natural products is also in development and a variety of potential prodrug analogues will be discussed further in Section 1.3.4.

1.3.1. Total synthesis of narciclasine

The first total synthesis of narciclasine was reported by Rigby et al.\textsuperscript{30} in 1997 (Table 1.01, Entry 1), however the total number of steps and disappointingly low overall yield warranted further investigation by the research community and is summarised in Table 1.01. It should be noted that more recent synthetic work is towards unnatural and truncated analogues of both natural products and will be discussed in more detail in Section 1.3.3.

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<th>Overall yield (%)</th>
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Table 1.01: Summary of the total synthesis of narciclasine

Hudlicky and co-workers approach to the synthesis of narciclasine started from a bio oxidation reaction of a benzene derivative (Table 1.01, Entry 2).\textsuperscript{31} The whole-cell fermentation of 1,3-dibromobenzene 11, using recombinant \textit{Escherichia coli} JM109(pDTG601A), gave the cyclohexadiene diol 12. This was used to construct part of the of the polyhydroxylated C-ring found in narciclasine 7 (Scheme 1.01).\textsuperscript{31} This was followed by multiple reactions, including the key steps of a Suzuki coupling of a boronic acid derivative of 0-vanillin with a vinyl-bromide to introduce the A-ring moiety, and a modified Bischler-Napieralski reaction to form the B-ring lactam.
In 2002, Elango and Yan also applied microbial arene oxidation in their total synthesis of narciclasine \( \text{7} \) gaining a cis-dihydrodiol by whole cell fermentation of benzene with *Pseudomonas putida* 39/D, shown in Scheme 1.02.\(^{33}\) Their overall yield was reported as an impressive 19\% over 9 steps (Table 1.01, Entry 4), although this was later questioned in a review by Rinner and Hudlicky,\(^{34}\) as an accurate step count could not be made from the published data. The key synthetic steps are shown in Scheme 1.02 and were reported as being stereocontrolled epoxide formation followed by intramolecular SnCl\(_4\)-catalysed arene epoxide coupling to gain late stage analogue \( \text{16} \).\(^{33}\)

Keck *et al.* presented the total synthesis of enantiomerically pure narciclasine in 14 steps from D-gulonolactone (Table 1.01, Entry 3).\(^{32}\) After the formation of alkyne \( \text{17} \), intramolecular radical cyclisation was carried out using thiophenol under irradiation and gave the key intermediate \( \text{18} \) in 88\% yield, as shown in Scheme 1.03. Following further steps, B-ring formation occurred by aluminium-catalysed cyclisation and subsequent deprotection furnished (+)-narciclasine \( \text{7} \) in 16\% yield.
1.3.2. Total synthesis of pancratistatin

The first reported synthesis of racemic pancratistatin 8 was by Danishefsky et al. in 1989 over 26 steps with a 0.13 %.35 The first synthesis of enantiomerically pure (+)-pancratistatin was by Tian et al.36 in 1995 (Table 1.02, Entry 1) shortly followed by Trost et al.37 later that year (Table 1.02, Entry 2). There have been subsequent efforts into the stereoselective synthesis of the enantiomerically pure pancratistatin, a selection of which are shown in Table 1.02 and some of which will be discussed herein.

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Table 1.02: A selection of the total syntheses of pancratistatin (a From narciclasine)

An interesting approach to the synthesis of pancratistatin was employed by Pettit et al. using the more abundantly available narciclasine as a precursor (Table 1.02, Entry 5).40 This involved 10 synthetic steps from narciclasine 7 and pancratistatin 8 was isolated in a 3.6 % yield.

Scheme 1.03: Key steps in the synthesis reported by Keck et al.32
Rigby et al. described a 22 step synthesis of pancratistatin (Table 1.02, Entry 4) with a hydrogen bond-controlled aryl enamide photocyclisation as a key step, shown in Scheme 1.04. This furnished the advanced analogue 20 in 30 % yield, a synthetic approach which was initially used to synthesise narciclasine 7 with analogue 20 being the last common intermediate.

![Scheme 1.04: Photocyclisation of analogue 19](image)

Li et al. utilised two coupling fragments, bromide 21 and pinitol analogue 22, in order to gain the A/C-ring 23. They report the total synthesis of pancratistatin from pinitol in 12 steps, 2.7 % yield (Table 1.02, Entry 8). Coupling fragments are also employed by Cagide-Fagin et al. (Table 1.02, Entry 9) who report a [3+3] enantioselective annulation of ketone 24 and methylenedioxy derivative 25 to get the A/C-ring analogue 26, shown below in Scheme 1.05.
A thorough understanding of the structure-activity relationship (SAR) of the phenanthridinone group will enable investigations into more efficacious clinical candidates and will also provide biological probes in order to fully elucidate the true mode of action of this group. The tricyclic phenanthridinone building block, which is numbered according to Pettit et al., and shown in Figure 1.06, is present in a variety of Amaryllidaceae natural products, with various substituents on the aromatic A-ring and a polyhydroxylated C-ring. There are a number of structural analogues of narciclasine and pancratistatin and only selected examples are discussed here to highlight key features in the ring system.
1.3.3.1. A-ring

It has been well established that narciclasine 7 and pancratistatin 8 have a tenfold increase in activity compared to that reported for their 7-deoxy counterparts 9 and 10, shown previously in Figure 1.03, which is attributed to the free phenolic hydroxy at C(7). The anticancer activity was also shown to diminish with subsequent removal of oxygen from the A-ring, with a C(9)-methoxy analogue of pancratistatin showing very poor activity, with a mean GI₅₀ value of 12.5 μM across a minipanel of human cancers.

In order to address the poor bioavailability of the parent compounds, Hudlicky et al. further investigated the tolerance for A-ring manipulation by its replacement with an indole moiety to give pancratistatin analogue 27, shown in Figure 1.08. The H-bond donor-acceptor pairing of the β-ketoamide moiety was thought to contribute to the anticancer pharmacophore and was emulated by the β-carboline-1-one analogue 27 as described in Figure 1.08.
Pancratistatin 8 and analogue 27 were tested against a small panel of human cancer cell lines with analogue 27 being found to have a diminished activity of more than 100 fold. Although molecular modelling showed spatial similarities the difference in electronic density of the analogue to the parent compound was apparent following molecular mapping. The findings complement previous work showing the requirement for electronegative oxygenation of the A-ring for activity. This application of the indole group as a bioisostere for a related analogue was reported by our group, which showed only a slight decrease in activity.

In order to mimic the donor-acceptor functionality of the C(7) phenol the N(7)-oxide 28 was synthesised in addition to 7-aza-nornarciclasine 29 and its HCl salt 30, all shown in Figure 1.09. Each analogue was tested against HeLa cervical cancer and MCF7 breast cancer cell lines. No activity was observed for analogue 29 with moderate activity (IC₅₀ > 100 μM) being observed for analogues 28 and 30. Although the analogues are also missing the methylenedioxy bridge these results suggest that the C(7) phenol is crucial for preserving cancer cell inhibition.

**Figure 1.08:** Spatial similarities of pancratistatin 8 compared to bioisostere 27

**Figure 1.09:** Activity of aza-analogues against HeLa and MCF7 cancer cell lines

1.3.3.2. B-ring

Current understanding suggests that manipulation of the lactam B-ring is poorly tolerated, although this has yet to be fully explored. An investigation into the total synthesis of narciclasine by Chretien...
et al. generated the lactone derivatives 31 and 32, as well as their epi-analogues, all of which were found to be inactive against L1210 murine lymphocytic leukaemia cells (Figure 1.10). In addition, reduction of the lactam to the corresponding basic amine 33 showed biological activity against a minipanel of human cancers lines with mean \(G_I_{50} > 35 \mu M\) compared with \(G_I_{50} < 0.3 \mu M\) of the C(7)-deoxy natural product 9. Interestingly, the hydrochloride salt 34 performed slightly better with an improved activity of \(G_I_{50} > 16 \mu M\).

![Chemical structures](image)

**Figure 1.10:** Manipulation of the B-ring and observed biological activity

The requirement for a cyclic system was established by the synthesis of seco-analogues of narciclasine and lycoricidine. Compounds 35 and 36, lacking the C(10a)/C(10b) bond, showed no activity against L1210 cells.

1.3.3.3. C-ring

The polyoxygenated amino-inositol C-ring of the narciclasine analogues has attracted much synthetic attention in order to ascertain the minimum structural requirements for the anticancer pharmacophore. The natural product 7-deoxy-\textit{trans}-dihydonarciclasine 37, often isolated with narciclasine 7, retains activity in the same order of magnitude as lycoricidine 9, with a corresponding increase in activity for the C(7) phenol 38, as shown in Figure 1.11.
A variety of C(1) derivatives of 7-deoxypancratistatin were developed to further probe the possibility for manipulation at this position. The acid 39 and methyl ester 40 were found to be inactive however, compound 41 and acetate analogue 42 both had moderate activity, with IC₅₀ values of 0.09 and 0.06 μM respectively, against prostate cancer HTB-81 (Figure 1.11). These compounds were also comparable to 7-deoxypancratistatin 10 against P388 cancer cells. In addition, the acetate analogue 42 was found to induce apoptosis in Jurkat cells and not in non-cancerous human fibroblast again showing cancer cell specificity.

Related work by the McNulty group further elaborated key biological features of the C-ring and showed that the C(4) derivative 43 had poor activity against P388 cells (ED₅₀ of 154 μM ) with some improvement being observed with the C(2)-C(3) diol 44, ED₅₀ of 1.62 μM, both of which are shown in Figure 1.12. Continuing their research in this area the McNulty group also showed no induction of apoptosis or cell growth inhibition by the C(2)-C(4) diol 45, proving it to be inactive (Figure 1.13).

Investigations of C-ring substitutions by Rinner et al. generated the C(3)-C(4) diol 46, an analogue of 7-
deoxypancratistatin 10, which had disappointing biological activity showing a mean GI\textsubscript{50} > 36 µM against a minipanel of human cancers.\textsuperscript{46}

![Structure of C(2)-C(4) 45 and C(3)-C(4) diol 46]

**Figure 1.13:** Structure of C(2)-C(4) 45 and C(3)-C(4) diol 46

In summary, a number of key characteristics have been identified as being essential or increasing the anticancer activity of the natural products. Poly-oxygenation of the A-ring is essential for maintaining activity, with a ten-fold increase in anticancer activity being observed when the phenol at C(7) is present, as highlighted in Figure 1.14. An intact, six-membered B-ring is required with an amide moiety. Replacing the lactam with a basic nitrogen or lactone renders the molecule inactive. The C(10b) position should be sp\textsuperscript{2} hybridised, or if saturated, should have a trans B/C-ring junction as seen in pancratistatin. Hydroxylation of the C-ring is required to maintain activity although the absolute and relative stereochemistry of the C-ring has yet to be determined. It appears that the hydroxyl at C(1) is not essential for activity but can be manipulated such as in the formation of prodrugs which will now be described.

![Narciclasine]

**Figure 1.14:** Summary of SAR

1.3.4. Pro-Drug synthesis and activity

Salt derivatives have widely been shown to improve water solubility and bioavailability of drug molecules, for example galanthamine 5 (Reminyl) is orally delivered as the hydrobromide salt. However narciclasine 7 and pancratistatin 8 possess a lactam and are therefore not basic, so simple
salt formation of the natural product is not possible. Alternatively, phosphate prodrugs are encouraging as they increase water solubility and exploit cytosolic nonspecific phosphatases in order to release an active compound and elicit a response. The poor aqueous solubility (53 μg/mL) of pancratistatin and related compounds is thought to be responsible for the poor delivery of these molecules and has, in part, led to their exclusion from clinical testing. This encouraged a series of published works by the Pettit research group into phosphate prodrugs of the phenanthridinone natural products. This has proved to be a very successful approach and has subsequently been patented and will be discussed in more detail below.

Initial investigation focused on the phosphorylation of the C(7) hydroxyl of natural product pancratistatin 8, and the construction of a series of different counterion analogues was examined. Most of the derivatives had comparable \textit{in vitro} activity against the standard murine P388 leukaemia cell line and minipanel of human cancer cells, however it was the sodium 7-O-phosphate prodrug 47, shown in Figure 1.15, which was most soluble at 20 mg/mL in water. The 3,4-O-cyclic phosphate derivatives of pancratistatin were also synthesised in parallel due to the very poor yield of their 7-O-phosphate counterparts however decomposition of the cyclic product led to an investigation of the 4-O-phosphate series. Again it was the sodium phosphate analogues 48 and 49 which showed the best solubility. This procedure has also been extended to the formation of a 3,4-O-cyclic phosphate narciclasine series, with the sodium salt again being the most soluble at 60 mg/mL. It is proposed that the reduced \textit{in vitro} anticancer activity of this series is due to the lack of endogenous nonspecific phosphatases which will be more efficient in \textit{in vivo} models.

![Figure 1.15](image-url)

\textbf{Figure 1.15:} Disodium 7-O-phosphate 47, sodium 3,4-O-cyclic phosphate 48 and sodium 4-O-phosphate 49 prodrugs of pancratistatin

The 3-log improvement in solubility of the 3,4-O-cyclic phosphate prodrug 48 of pancratistatin 8 allowed for subsequent \textit{in vivo} investigations using clinically relevant IV administration. A statistically significant delay in growth of the tumour was observed using a xenograft of DLD-1 colon adenocarcinoma cell line in mice, with negligible harm to the host. The effect was found to be a reduction in operational vasculature in the tumour 24 hours after exposure with the disruption of
mitochondrial function appearing to be the cause, a common mechanism with other vascular targeting agents.

As previously discussed in Section 1.3.3.3 and shown in Figure 1.11, manipulation at C(1) was well tolerated with hydroxymethyl 41 and acetate counterpart 42 showing nM activity. The pro-drug C(1) benzoate ester 50 (shown in Figure 1.16) which would potentially deliver the natural product pancratistatin 8 upon non-specific ester hydrolysis, showed potent biological activity with a GI50 of 1.33 nM across a minipanel of human cancer cell lines, compared to a GI50 of 66 nM across the same panel for pancratistatin 8.40 Manipulation at C(1) therefore shows continued and even improved biological activity with the addition of a lipophilic group whereas an increase in polarity is not tolerated. It has been postulated that an increase in lipophilicity at C(1) assists in cell penetration although it is unclear as to whether intracellular hydrolysis occurs to release the natural product.53 Further development of C(1) benzoate ester 50 led to the synthesis of the phosphate salt 51. This showed good but slightly diminished in vitro activity, GI50 of 244 nM, against the same minipanel of human cancer cell lines as analogue 50.59

![Chemical structures of 50 and 51](attachment:image.png)

**Figure 1.16:** C(1) benzoate ester analogues of pancratistatin40,59

Narciclasine and pancratistatin have both shown interesting and exciting biological activities, in particular against some resistant cancer cell lines and also brain cancers, both of which are notoriously difficult to treat. Their low natural abundance and complex total synthesis has led to much interest around finding short and efficient syntheses of these natural products as well as related and truncated versions in order to probe their SAR.

### 1.4. Project aims and objectives

The aim of this project is to synthesise simplified narciclasine 7 and pancratistatin 8 analogues using short synthetic sequences and to explore their biological activity.
AB-ring analogues will initially be synthesised building on the dihydroisoquinolinone framework present throughout the phenanthridinone natural products. This will be based on published synthesis within the group employing conditions for Curtius rearrangement and intramolecular Friedel-craft acylation. Functionalisation of the AB-ring system will allow for the investigation into the need for a cyclised C-ring.

Late stage narciclasine dimethoxy and dimethoxyhydroxy analogues have been synthesised by the group. Optimisation of this preliminary work will be done in addition to the synthesis of the novel methylenedioxy analogue in order to assess these compounds for biological activity with a view to sending them to the NCI for further testing.

Novel quinazolinone analogues, which would possess a basic nitrogen instead of C(10b) on the late stage ABC-ring intermediates, will be investigated which will be of great interest. In addition, tricyclic analogues will also be examined which are derived from sugars such as L- and D-lyxose. This will give hydroxylated C-ring analogues with known absolute and relative stereochemistry and will be of interest in this field of research.
2. Synthesis and evaluation of AB-ring analogues

2.1. Introduction to the isoquinolinone motif

A common structural motif found throughout the natural world and medicinal chemistry is dihydroisoquinolinone 52, which is also present in narciclasine 7 and related derivatives (Figure 2.01). The Caggiano research group has been interested in developing efficient methods to synthesise dihydroisoquinolinones related to the narciclasine structure and examine their biological activities. Preliminary work in this area was undertaken by the group, which has been expanded and described herein.

![Figure 2.01: Dihydroisoquinolinone 52 and highlighted core of narciclasine 7](image)

2.2. Synthesis of dihydroisoquinolinone

The Caggiano group developed and reported an efficient one-pot procedure for the synthesis of various lactams from the corresponding carboxylic acid precursors (Scheme 2.01).\textsuperscript{63} This method was successfully used to furnish analogues of tetrahydro \( \beta \)-carbolinone 54 and dihydroisoquinolinone 55 as shown below in Scheme 2.01.

![Scheme 2.01: General synthesis of lactam analogues by Judd et al.\textsuperscript{63}](image)

The synthesis of three dihydroisoquinolinone analogues were reported by Judd et al.,\textsuperscript{64} with this reported methodology being successfully employed in this work and is now described.
Commercially available 3,4-dimethoxy dihydrocinnamic acid 56 was treated with triethylamine (Et₃N) and diphenylphosphoryl azide (DPPA) to afford the corresponding acyl-azide 57 shown in Scheme 2.02. Heating the acyl-azide intermediate 57 from room temperature to 90 °C initiated a Curtius rearrangement to give the isocyanate 58 with the evolution of N₂ gas. Upon the removal of toluene, intramolecular Friedel-Crafts acylation was achieved with the addition of neat boron trifluoride diethyl etherate (BF₃·OEt₂), activating the isocyanate which is captured by the electron rich aromatic ring giving the dihydroisoquinolinone 59 in 75 % yield (Scheme 2.02).

\[ \text{56} \xrightarrow{\text{Et₃N, DPPA, Toluene}} \text{57} \]

\[ \text{58} \xrightarrow{\text{Curtius rearrangement}} \text{59} \]

**Scheme 2.02: Isocyanate formation and subsequent cyclisation to afford lactam 59**

Commercially available 3,4-methylenedioxyxycinnamic acid was first reduced using standard conditions to the dihydrocinnamic acid in 93 % yield, which, when subjected to the previously described reaction conditions, gave lactam 60 in 73 % yield (Figure 2.02). When 3,4,5-trimethoxy dihydrocinnamic acid was subjected to the same conditions, again the corresponding lactam product 61 was obtained, in 54 % yield. It should be noted that selective demethylation was observed giving C(8) phenol, shown in Figure 2.02. This is of particular interest as the narciclasine and related derivatives also possess a free hydroxyl group at C(8), as described in Section 1.3.3.1, which when absent results in diminished biological activity. Toke et al. also show regioselective demethylation for a similar phenanthridinone compounds in concentrated BF₃·OEt₂.

\[ \text{60} \xrightarrow{73 \%} \text{61} \xrightarrow{54 \%} \]

**Figure 2.02: Structure of lactam 60 and lactam 61**
Selective demethylation is presumably the result of the strong chelation of the lactam to the BF$_3$·OEt$_2$ Lewis acid, making the methyl group susceptible to nucleophilic attack, similar to BCl$_3$ deprotection of aryl ethers$^{68}$ and is supported by Nicolaou et al.$^{69}$ who report similar chelated structures. Judd et al. obtained an X-ray crystal structure of the isolated of the BF$_2$-adduct 62 and determined C(8) demethylation, as shown in Figure 2.03.$^{63}$

![Image of BF$_2$ adduct 62](image.png)

**Figure 2.03: BF$_2$ adduct 62**

### 2.3. Oxidation reaction - background

Oxidation of the dihydroisoquinolinone to the isoquinolinone derivatives would provide access to an interesting motif seen in a wide variety of biologically active molecules and would mimic the sp$^2$ hybridisation of C(10b) found in narciclasine 7 (Figure 1.03). In addition, the isoquinolinone would provide a precursor to further functionalisation.

The dihydroisoquinolines 61, 59 and 60 were oxidised to their corresponding isoquinolines 63, 64 and 65 using Pd/C following similar reported transformations in the literature,$^{70,71}$ although the yields were poor and often irreproducible. Preliminary anticancer activities of these compounds were investigated by the group using an in-house MTS cell proliferation assay with HT29 colon cancer cell lines, the results of which are shown in Figure 2.04. The dihydroisoquinolinone derivatives 61, 59 and 60 showed relatively poor activity whereas their oxidised counterparts 63, 64 and 65 showed a marked improvement. As expected, and consistent with data previously described in Section 1.3.3.1, the hydroxyl at C(8) improved activity for both series of compounds. With this encouraging preliminary data in hand, further investigations were then conducted in order to improve the yields and validate the results and are now discussed in more detail.
Due to the irreproducible and low yields obtained with Pd/C in the preliminary investigations, and for the potential for palladium contamination effecting the results of the antiproliferative assay, alternative strategies for the oxidation of dihydroisoquinolinones were examined. A commonly used agent for performing dehydrogenation at the benzylic position is 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and this was explored as a possible reagent to achieve oxidation of the lactams.

The typical reaction conditions reported for the oxidation of similar compounds used DDQ in dioxane heated at reflux, as shown in Scheme 2.03.\textsuperscript{72,73} When these procedures were applied to dihydroisoquinolinones 59, 60 and 61 the desired oxidised isoquinolinone analogues were only obtained in small amounts as observed by crude \textsuperscript{1}H NMR spectroscopic analysis. Unfortunately isolation was not possible due to co-eluting of the starting material with the product. No improvement was made upon increasing the amount of DDQ or lengthening the reaction time.
Dough et al.\textsuperscript{72}

Tsai et al.\textsuperscript{73}

Scheme 2.03: Oxidation of dihydroisoquinolines by DDQ

An interesting alternative was described by Estevez et al., in which acetic acid was added dropwise to a gently stirring mixture of starting material 70 and DDQ, shown in Scheme 2.04.\textsuperscript{74} After forming and isolating intermediate 71 the reaction was heated to 250 °C for 30 minutes, giving the oxidised product 72.

Scheme 2.04: Dehydrogenation of compound 70 using DDQ\textsuperscript{74}

The method described by Estevez et al. introduces an acetate intermediate which would also provide an interesting structure for biological testing, which is shown in Scheme 2.05. When these conditions were employed with our substrates we recovered mostly unreacted starting material with only trace amounts of the isoquinolinone identified by \textsuperscript{1}H NMR spectroscopic analysis of the crude. Upon resubmitting the dimethoxy material 59 and leaving for 16 hours at 80 °C, the oxidised product 64 was observed by crude \textsuperscript{1}H NMR spectroscopic analysis in a 3 to 1 ratio of starting material to product, without the need for heating to 250 °C. Trace amounts of a third product were also observed, presumably the acetate 73, but this could not be isolated.
Scheme 2.05: Expected outcome following the procedure developed by Estevez et al.\textsuperscript{74}

This procedure provided some limited success in isolating the oxidised lactams, as shown in Table 2.01, however these results were not consistent. A significant problem was that reactions did not go to completion and therefore required purification. However, the separation of the oxidised product from the dihydroisoquinolinone starting material was problematic, as they possess very similar retention factors in a variety of solvent systems, resulting in poor yields. Manipulation of temperature, reaction time and/or quantity of DDQ failed to improve conversion or yield. In addition, the products of the reaction were often contaminated with residual DDQ preventing accurate biological analysis. An alternative strategy was therefore sought.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Product</th>
<th>DDQ (eq.)</th>
<th>Yield (%)</th>
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<td>55</td>
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<tr>
<td>3</td>
<td><img src="74.png" alt="74" /></td>
<td><img src="75.png" alt="75" /></td>
<td>3</td>
<td>47\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Table 2.01: Dehydrogenation of lactams using DDQ (\textsuperscript{a} Conducted at room temperature for 18 hours. \textsuperscript{b} Conducted 120 °C for 22 hours)
2.3.2. Oxidation of the lactams using Pd/C

Despite the potential for contamination and due to our previous limited success, we investigated the oxidation of dihydroisoquinolinones with Pd/C further. Typically used as a hydrogen transfer agent, dehydrogenation has been successfully achieved using Pd/C in hydrogen-free conditions at elevated temperatures.

Awuah et al. reported the oxidation of dimethoxy lactam 59 in solvent free conditions.\textsuperscript{75} The lactam 59 was ground to a fine powder with Pd/C (10 % wt.) in a mortar prior to being transferred to a round bottom flask. This was heated to 150 °C for 30 minutes, giving the oxidised lactam 64 in 98 % yield, Scheme 2.06. A repeat of these reaction conditions carried out within the group failed to provide lactam 64, returning only starting material. However, increasing the reaction temperature and time, as reported by McNulty and Still, \textsuperscript{76} provided the oxidised product 64 in 81 % yield.\textsuperscript{77} Despite this success, the reaction proved to be inconsistent and afforded an inseparable mix of starting material and oxidised product in various ratios. A similar outcome was also observed for the methylenedioxy analogue 65.

\begin{center}
\includegraphics[width=0.5\textwidth]{Scheme_2.06.png}
\end{center}

\textbf{Scheme 2.06:} Solvent free dehydrogenation of lactam 64\textsuperscript{75}

Limited success was achieved in obtaining the dimethoxyhydroxy lactam 63 using the reported conditions.\textsuperscript{76} Although crude \textsuperscript{1}H NMR spectroscopic analysis suggested a ratio of starting material to product of 18 to 81 only a 6 % yield was achieved, again due to the co-eluting of compounds. Optimisation of the reaction conditions by extending the reaction time, altering the amount of Pd/C and using either open or closed systems failed to improve the yield of all three analogues.

2.4. Cyclisation of cinnamic acid

Due to the capricious nature of the oxidation reactions, an alternative route to isoquinolinones 63, 64 and 65 was pursued. Some success had been described in the literature for the cyclisation of \textit{trans}-cinnamic acids to their corresponding isoquinolinone counterparts.\textsuperscript{78-82} Typical reaction conditions describe the formation of the acyl-azide from the carboxylic acid using ethyl chloroformate followed by sodium azide. The acyl-azide was then heated in diphenyl ether (Ph\textsubscript{2}O) or diphenyl methane (Ph\textsubscript{2}Me) at temperatures above 200 °C to facilitate the Curtius rearrangement,
isomerisation and subsequent cyclisation to the isoquinol. Ray et al. reported that the same transformation could be achieved using DPPA with the similar 3-methoxycinnamic acid 76 to afford lactam 78 (Scheme 2.07).^{81}

![Scheme 2.07: Isocyanate formation and thermal cyclisation of the 3-methoxycinnamic acid](image)

Using DPPA as described by Ray et al. and building on expertise within our group, DPPA was added dropwise to a stirring solution of methylenedioxycinnamic acid 80 and Et₃N in Ph₂O at 25 °C.\(^{63,81}\) The reaction was then heated to 200 °C for one hour. It was interesting to note that as the temperature reached approximately 90 °C a violent effervescence was observed, which is presumably N₂ gas being evolved from the Curtius rearrangement. Analysis by TLC suggested the formation of lactam 65, however this was accompanied by a large amount of other unidentified products. Optimisation of the reaction was conducted, with the initial formation of the acyl-azide in toluene prior to thermal rearrangement,\(^{63,81}\) however the reaction still resulted in complex mixtures of unidentifiable compounds by crude \(^{1}\)H NMR spectroscopic analysis.

The highly reactive nature of the isocyanate group and high temperatures required clearly result in alternative products in addition to the desired capture of the isocyanate by the aromatic ring. There is potential for carbamic acids and amines resulting from hydrolysis of the isocyanate, which can further react forming urea analogues as previously observed within the group. Furthermore, use of DPPA and Et₃N results in phosphate salts which could also result in side reactions. New et al. described the formation of a trimer, but this appears to be an isolated case.\(^{83}\) Some reports isolate the acyl-azide prior to isocyanate formation, either by extraction with toluene\(^{82}\) or filtering through a plug of silica,\(^{84}\) which we investigated in our system.

Using modified conditions, incorporating the procedure reported by Ray et al.\(^{81}\) with the successful azide formation by Judd et al.\(^{63}\) and isolating the acyl-azide, DPPA was added dropwise to a stirring solution of methylenedioxycinnamic acid 80 in toluene and Et₃N. After stirring at room temperature for 120 minutes the reaction was filtered through a plug of silica and washed with toluene, leaving only one spot visible by TLC, \(R_f\) [EtOAc] 0.80. After the toluene was removed under reduced pressure, Ph₂O was added and the reaction heated at 250 °C for 3 hours with the violent evolution of N₂ gas at approximately 90 °C as the isocyanate is formed. After cooling slowly over 18 hours a precipitate was observed and filtered, washed with diethyl ether to give the desired lactam 65 in
good yield without the need for further purification (Scheme 2.08). The filtration process was found to be crucial for the success of the reaction. This is an important result as the oxidised product could be formed in a single step from the cinnamic acid, rather than the previously described method that required 1) reduction of the cinnamic acid; 2) DPPA cyclisation and 3) oxidation, especially as the third step often resulted in low yields and possible palladium or DDQ contamination. Furthermore, the reactions was successfully performed on 25 mmol scale and still afford the product in reasonable yield without the need for further purification. It should also be noted that many processes occur in this transformation, including i) formation of the acyl-azide; ii) Curtius rearrangement; iii) thermal isomerisation to the Z-isomer of the isocyanate and finally iv) thermally-induced intramolecular Friedel-Crafts acylation, therefore the overall yields obtained are good.

![Scheme 2.08: Successful cyclisation of cinnamic acid analogues 79 and 80](image)

As can be seen in Scheme 2.08 the reaction was equally efficient with both the dimethoxy and methylenedioxy cinnamic acids 79 and 80. In both cases $^1$H NMR spectroscopic analysis of the products show the loss of three aromatic protons seen in the starting material, two doublets and a double doublet each integrating for 1H. Instead, there are two aromatic protons, both of which are singlets integrating for 1H, suggesting the cyclisation of the B-ring. In addition, the coupling constants of the olefin present in cinnamic acids 79 and 80 are both 16 Hz, consistent with a trans double bond. In products 64 and 65 the coupling constants are 7 Hz which correlates to a cis double bond, the correct isomer seen in the AB-ring analogues 64 and 65. Regrettably, using these optimised conditions 3,4,5-trimethoxy cinnamic acid failed to provide the corresponding lactam 63. Instead a complex mixture of unidentifiable products was obtained. Presumably, the additional activation caused by the third methoxy group led to other products, possibly due to competing inter- as well as intra-molecular attack of the isocyanate intermediate.
2.5. Methylation

We wished to investigate the effect N-methyl derivatives would have on antiproliferative activity compared to their N-H precursors as a thorough investigation into the requirements for the intact lactam (as previously discussed in Section 1.3.3.2) is yet to be completed. An undergraduate summer student under my guidance followed the procedure reported by Maertens et al. for a similar compound, shown in Scheme 2.09, which was also successfully used previously within the group on similar compounds.

![Scheme 2.09: Methylation and ester hydrolysis to gain N-methylindole-3-propionic acid](image)

Using this procedure with our isoquinolinones, N-methylation was achieved with the dimethoxy analogue 64 to get 83 in 65 % yield (Scheme 2.10 using iodomethane (MeI) in the presence of potassium hydroxide (KOH), with the addition of THF being required to improve solubility. In addition, however, an interesting second compound was also isolated in 23 % yield, identified as compound 84 shown in Scheme 2.10. The \(^1\)H and \(^{13}\)C NMR spectroscopic data matched that previously reported by Seki et al for the same compound 84, albeit isolated via a different synthetic process and is shown in Table 2.02.

![Scheme 2.10: Structure of analogues 83 and 84](image)
<table>
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<tr>
<th>Entry</th>
<th>Carbon</th>
<th>$^{13}$C Chemical Shift (ppm)</th>
<th>$^1$H Chemical Shift (ppm)</th>
<th>Integration</th>
<th>$J$ (Hz)</th>
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**Table 2.02: $^1$H and $^{13}$C NMR assignment for analogue 84**

This remarkable transformation is difficult to understand as several processes have occurred. The $^1$H NMR spectrum clearly shows a tri-substituted aromatic (Table 2.02, Entry 2, 5 and 6) therefore the lactam must be cleaved, followed by a proto-decarboxylation. The urea also suggests that the product has been obtained from two molecules of the isoquinolinone 64. The methoxy groups remain intact (Table 2.02, Entry 13 and 14) furthermore there are an additional two methyl groups (Table 2.02, Entry 15 and 16) which were identified as $N$-methyl moieties. This structure was also found to be consistent with HRMS analysis. Presumably, the reaction proceeds via a deprotonated enamide which can act as a nucleophile through the C(4) carbon, which is part of the product structure (Scheme 2.11).
This methodology was abandoned in order to avoid the side reaction and improve the yield. Using sodium hydride (NaH) as the base a series of N-methylated dihydroisoquinolinone and isoquinolinone derivatives were synthesised in good yield, as shown in Table 2.03 below.

It is worthy of note that selective N-methylation of the phenol analogues 85 and 86 (Table 2.03, Entry 1 and 4) was achieved using these conditions. Using 6 equivalents of NaH ensured the formation of both the phenoxide and amide anions. The ensuing phenoxide anion is stabilised by the aromatic ring allowing for regioselective methylation to occur with the more reactive amide anion, using 1 equivalent of MeI at 0 °C. HRMS confirmed the addition of a single methyl group with $^1$H NMR spectroscopic analysis showing a sharp phenol OH peak consistently found at ~ 13 ppm for similar compounds. The regioselectivity was confirmed by NOESY NMR, which showed a strong correlation between proton(s) on C(3) and the methyl on the nitrogen atom (Figure 2.05).

![Figure 2.05: NOESY correlation between proton(s) on C(3) and N-methyl](image-url)
### Table 2.03: Synthesis of N-methylated compounds

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Product</th>
<th>NaH (eq.)</th>
<th>Mel (eq.)</th>
<th>Yield (%)</th>
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</thead>
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<td>91</td>
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<tr>
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<td><img src="image12" alt="image" /></td>
<td>3</td>
<td>3</td>
<td>95</td>
</tr>
</tbody>
</table>

### 2.6. Functionalisation of the olefin

With the oxidised isoquinolinones in hand, we wished to investigate further functionalisation of the double bond at C(4) and C(3) in order to simulate parts of the oxygenated C-ring of the natural product 8. Initial investigations examined acetylation of the nucleophilic C(4) position, which could afford hydroxylated products which correspond to positions on the C-ring in pancratistatin 8, as shown in Scheme 2.12.
2.6.1. Functionalisation at C(4)

Chao et al. described the acetylation of an N-alkyl isoquinolinone 88 using acetic anhydride in the presence of sulfuric acid, as shown in Scheme 2.13.

Using these reported conditions acetylation of the N-methylated compounds 90 and 91 was achieved, although yields were low (Figure 2.06). Acetylation of the dimethoxy N-H isoquinolinone 92 was also effective even after 16 hours, without possible N-acetylated material being observed. Unfortunately, however, no product was observed with the methylenedioxy N-H analogue 65 despite using the same reaction conditions. Likewise, the dimethoxyhydroxy analogues 63 and 85 gave complicated mixtures of unidentified products, which could not be isolated.

Figure 2.06: Acetylated isoquinolinone compounds
Due to problems with the dimethoxyhydroxy analogue, which would be expected to afford the most active narciclasine analogues, and the low yields obtained with the other substrates, we did not pursue this reaction with 2-propenoyl chloride. In addition, we observed cleaner transformations with the N-methyl derivatives, which again would not be suitable for narciclasine derivatives as N-H substrates were expected to be more potent. Therefore we explored other biologically active compounds which would build on these key observations.

NSC 314622 94, shown in Figure 2.07, was identified as a lead compound following COMPARE analysis of the antiproliferative data of 60 human cancer cell lines by the National Cancer Institute (NCI). It was found to have a cytotoxic profile similar to camptothecin 93, a known topoisomerase I inhibitor.

![Chemical structures of Camptothecin (93) and NSC 314622 (94)](image)

**Figure 2.07:** Topoisomerase I inhibitors

As can be seen, NSC 314622 94 possesses a fused cyclopentanone core. Following our successful acetylation of the C(4) position of the N-methyl isoquinolinones 90 and 91, we wished to investigate the corresponding benzoylation reaction, which would afford novel analogues of NSC 314622 94 such as 95, which does not have a cyclic ketone (Figure 2.07). The proposed analogues would be more flexible and could be accessed by a Friedel-Crafts benzoylation reaction, as previously described.

Initial investigations were conducted using standard conditions for the acylation of aromatic rings, with an acid chloride and either AlCl₃ or BF₃·OEt₂. Unfortunately, none of the expected product was observed and crude ¹H NMR and TLC analysis showed only starting materials were present. Zhang et al. reported that the acylation of 2-chloroethoxy-benzene 97 by piperonylic acid 96 in the presence of trifluoroacetic anhydride (TFAA) could be achieved at room temperature over 96 hours (Scheme 2.14). However, when the N-methyl dimethoxy isoquinolinone 83 was treated with piperonylic acid 96 under the same conditions, no product was obtained.
Dallemagne et al. described the intramolecular cyclisation of dihydrocinnamic acids to the corresponding indanones as a one-pot procedure using a 1 to 1 ratio of trifluoroacetic acid (TFA) and TFAA, shown in Scheme 2.15. Similar work was published by Kolokythas using a 2 to 1 ratio of TFA to TFAA.

Published work from the Caggiano group reported the optimisation of the intramolecular cyclisation of 4-(indol-3-yl)butanoic acid 99 to the corresponding indanone analogue 100 was achieved using just TFAA at 100 °C for 2 hours, Scheme 2.16.
Scheme 2.16: Intramolecular cyclisation of 4-(indol-3-yl)butanoic acid 100

Using these optimised conditions, the group expanded on this methodology with the investigation of the intermolecular reactions between piperonylic acid and cyclohexene.\textsuperscript{77} The resulting indanones 102 were then subjected to Schmidt reaction conditions to obtain the pancratistatin analogue 103, as shown in Scheme 2.17.

Applying these optimised conditions to the synthesis of novel analogues of NSC 314622, N-methyl isoquinolinone 83 and piperonylic acid 96 were treated with TFAA at 100 °C in a sealed thick-walled pressure tube for 18 hours. The $^1$H NMR spectrum of analogue 104 clearly shows the addition of a tri-substituted aromatic compound with a methylenedioxy substituent. The addition of acid 96 at C(4) was confirmed due to the disappearance of the peak at 6.84 ppm. The peak consistent with C(3) remains, however it has moved from 6.98 ppm to 7.47 ppm and was confirmed using correlation data from NOESY and HMBC spectrum. The dimethoxy and aromatic peaks of the AB-ring starting material remain intact. Pleasingly, this procedure generated the novel analogues 104 and 105, both in 46 % yield (Figure 2.08) with $^1$H and $^{13}$C NMR spectroscopic analysis being similar for compound 105 to that previously discussed for analogue 104. HRMS for both compounds was consistent with the proposed structures. As previously observed in the attempted acetylation reaction, benzylation of the dimethoxyhydroxy isoquinolinone 85 was problematic and afforded an inseparable mixture of compounds.
The mechanism is presumably the capture of the electrophile by the nucleophilic C(4) position, followed by elimination of the acidic proton (path a). It is interesting to note that possible cyclisation, resulting from capture of the iminium cation intermediate did not occur (route b), unlike that previously observed in the research group and shown in Scheme 2.17 above. The novel analogues 104 and 105 are of great interest and will be examined for antiproliferative activity by the NCI and the results compared to those obtained for the more rigid NSC 314622 compound.

![Figure 2.08: Novel NSC 314622 analogues 104 and 105](image)

2.6.2. Oxygenation at C(3)/C(4)

Having explored the nucleophilic C(4) position, we next examined oxidation of the C(4) and/or C(3) positions. Oxidation of the olefin would produce biologically interesting small molecules, and could also emulate the oxygen rich nature of the C-ring present in the natural products. In addition these types of small molecules have also been identified as having wide ranging biological activity.

Section 1.2.2 described the process of apoptosis which is highly regulated by the caspase cascade, a series of proteolytic proteins. The importance of these proteins in the cell death pathway has led to their exploitation as drug targets and include isoquinolinone 106, shown in Scheme 2.18, an effective Caspase 3 inhibitor.\(^9\) This framework has also been identified as crucial in a number of other biologically significant compounds.\(^9\) 4H-Isoquinoline-1,3-dione 107 (R=H) is a potent inhibitor of NF-κB inducing kinase (NIK) which, amongst other roles, activates the gene transcription factor NF-κB, a protein important in the immune response.\(^9\)
2.6.2.1. **Benzylic oxidation of the dihydroisoquinolinone derivatives**

Benzylic oxidation has been reported for a similar compound, shown in Scheme 2.19, and was achieved using 2 equivalents of DDQ in a mixture of dioxane and water (95:5). Attempts at this reaction with dihydroisoquinolinone derivatives 59, 60 and 61 unfortunately did not yield any product, and the majority of the mass recovery was unreacted starting material.

Scheme 2.18: Possible C(4) and/or C(3) oxidation products

A more traditional approach is the use of selenium dioxide (SeO₂), as shown in Scheme 2.20 for a similar compound. Unfortunately, submission of our dihydroisoquinolinones 59, 60 and 61 to these reaction conditions again only resulted in the recovery of starting material.
Scheme 2.20: Benzylic oxidation using SeO₂\textsuperscript{94}

2.6.2.2. Oxygenation of the isoquinolinone derivatives

The olefin present in the isoquinolinones is weakly nucleophilic. We attempted various dihydroxylation reactions using the osmium tetroxide (OsO\textsubscript{4}) Sharpless protocol and the preformulated commercial AD mix. This failed to furnish any hydroxylated product and only unreacted starting material was recovered. Ruthenium(VIII) tetroxide (RuO\textsubscript{4}) is a more powerful oxidising agent and is also less toxic than its osmium counterpart.\textsuperscript{98} Although it is typically used for the scission of olefins to carboxylic acids,\textsuperscript{98,99} controlled reaction conditions have been reported which prevent over oxidation and afford cis-diols or diketone products.\textsuperscript{98,99}

Bettoni and co-workers described the unusual transformation of N-benzyl-3,4-dihydroisoquinolin-1(2H)-one 112 to N-benzoyl-homophthalimide 113 using ruthenium (Scheme 2.21).\textsuperscript{100} This was extended by subsequent work published by Yoshifuji et al. (Scheme 2.21) in yields surpassing 88%.\textsuperscript{101} In both cases ruthenium(IV) oxide hydrate (RuO\textsubscript{2}·\textsubscript{3}H\textsubscript{2}O) was oxidised to RuO\textsubscript{4} using a 10% aqueous solution of sodium periodate (NaIO\textsubscript{4}), after which the lactam was added and the reaction followed by TLC. The oxidation of RuO\textsubscript{2} to RuO\textsubscript{4} is identified by a change of colour from a black to a yellow solution.
Bettoni et al.\textsuperscript{100}

![Chemical Reaction](image)

Yoshifuji et al.\textsuperscript{101}

![Chemical Reaction](image)

**Scheme 2.21**: Previously reported syntheses of trione compounds

A common precursor for the generation of RuO\(_2\), and subsequently RuO\(_4\), is ruthenium(III) chloride hydrate (RuCl\(_3\)-xH\(_2\)O) with an oxidising agent such as oxone or NaIO\(_4\). Using conditions reported by Yang et al., dihydroisolquinolone 59 was stirred with RuCl\(_3\)-xH\(_2\)O and oxone in EtOAc:MeCN:H\(_2\)O.\textsuperscript{102} The reaction was followed by TLC analysis, however only starting material persisted which was further confirmed by \(^1\)H NMR spectroscopic analysis of the crude reaction mixture. Altering the solvent system with or without the addition of NaHCO\(_3\) buffer, as reported by Yang et al.\textsuperscript{103} gave the same result. The examples in the literature reported the oxidation of the \(N\)-alkylated lactams,\textsuperscript{100,101} as shown in Scheme 2.21, however, use of the \(N\)-methylated dihydroisolquinolone 87 still only produced unreacted starting material.

When the reaction was attempted with the oxidised dimethoxy isoquinolinone derivative 64, as shown in Scheme 2.22, the colour changed from black to yellow, suggesting the formation of the reactive RuO\(_4\) species. The reaction was followed by TLC analysis, and once the starting material had been consumed the RuO\(_4\) was decomposed by the addition of iPrOH. Column chromatography of the crude reaction mixture gave the trione 117 in 14 % yield. Unfortunately, repeating these conditions with the methylenedioxy analogue 65 only gave an inseparable mixture of compounds, although there was the suggestion of the trione in the \(^1\)H NMR spectroscopic analysis of the crude reaction mixture. Aromatic protons were present without their corresponding C(3) or C(4) peaks,
similar to that seen for analogue 117. Despite this, the isolated trione 117 is of great interest as the structurally similar trione 106 has been reported to inhibit Caspase 3, as previously discussed.\(^9^4\)

![Scheme 2.22: Formation of trione 117](image)

2.6.3. Functionalisation at C(3)

We wished to investigate C(3) allyl dihydroisoquinolines as dihydroxylation would afford analogues which possess part of the highly oxygenated 6-membered C-ring present in the natural products narciclasine 7 and pancratistatin 8. The similarity between the oxygenated analogue 119 and narciclasine 7 is highlighted in blue in Scheme 2.23 and would provide an interesting comparison of biological activity and provide useful information of the rigid C-ring versus the flexible and freely rotating hydroxylated C(3)-sidechain. Sharpless asymmetric dihydroxylation (AD) provides an established route to hydroxylated analogues, utilising AD-mix \(\alpha\) and AD-mix \(\beta\) to introduce the hydroxyl groups with control over stereoselectivity.\(^{10^4}\)

![Scheme 2.23: Proposed route and similarity of the C-ring analogues 118 and narciclasine 7](image)

Scheme 2.24 shows the proposed disconnection to achieve analogue 119, starting with a simple \(\alpha\) allylation of the dihydrocinnamic acid 121 to furnish the allylated carboxylic acid 120.\(^{10^5,10^6}\) Utilisation of the modified Curtius rearrangement and ring closure, developed by our research group and described previously,\(^{63}\) would deliver C(3)-allyl analogues 118. The following investigations into allylation at C(3), and subsequent functionalisation, were carried out in collaboration with undergraduate Erasmus students in the group.
Scheme 2.24: Retrosynthetic approach to the bicyclic analogue with side-chain

Following a literature procedure a solution of methylenedioxy acid 122 in dry THF was added dropwise to an excess of lithium diisopropylamide (LDA) at 0 °C, generating a dianion. After an optimised stirring time of 40 minutes, allyl-bromide was added and the reaction allowed to warm to room temperature. Stirring was continued for 18 hours and following standard aqueous work-up the α-allylated product 123 (Scheme 2.25) was identified by crude ¹H NMR spectroscopic analysis, in addition to the starting material. Unfortunately purification was not possible using standard procedures.

Scheme 2.25: Allylation of methylenedioxy dihydrocinnamic acid 122

Following this successful procedure with either the dimethoxy or trimethoxy dihydrocinnamic acids resulted in precipitation of the dianion and no reaction. Fortunately, addition of the allyl-bromide and heating the dimethoxy-dianion suspension to 65 °C for 18 hours solvated the dianion and resulted in reaction. Optimisation of the trimethoxy analogue 125 proved to be more challenging, however the addition of LDA dropwise to the cooled solution of the trimethoxy acid 124 in THF avoided precipitation of the dianion and the reaction with allyl-bromide proceeded as predicted.

Following standard aqueous work-up of all three analogues ¹H NMR spectroscopic analysis of the crude reaction mixtures showed a combination of starting material and allylated product. Due to the difficulties in separating the starting material from the product, as they are both carboxylic acids, we decided to submit the mixture to the modified Curtius rearrangement and cyclisation and then separate the lactams. Consequently the crude mixtures were subjected to the optimised cyclisation reaction conditions described previously. Both the simplified AB-ring and C(3)-allylated AB-ring analogues were isolated by column chromatography, with selective demethylation of the
trimethoxy derivative 126 being observed as previously noted for similar substrates. The yields over two-steps of the racemic compounds are shown in Table 2.04 below.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Intermediates</th>
<th>Ratio by crude (^1)H NMR analysis</th>
<th>Products</th>
<th>Isolated yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>31</td>
<td>61</td>
<td>17</td>
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<tr>
<td></td>
<td>123</td>
<td>72</td>
<td>129</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 2.04: Yields of unsubstituted and C(3)-allylated lactam analogues
Isoquinolinones were found to be more biologically active than their dihydroisoquinolinone counterparts and, as previously discussed in Section 2.3.2, dehydrogenation of dihydroisoquinolinone using Pd/C in solvent free conditions had been achieved with some limited success. The allylated analogues 126, 128 and 129 were therefore subjected to these conditions in order to gain the corresponding oxidised derivative for further testing. Unfortunately however this failed to furnish the expected products giving instead a mixture of unidentifiable compounds.

2.6.3.1. Sharpless dihydroxylation

The C(3)-allylated products were all obtained as racemic mixtures. Following unsuccessful attempts at separating the allylated enantiomers by chiral HPLC, the racemic mixtures were submitted to the stereoselective asymmetric dihydroxylation reaction as described by Sharpless. The possible outcomes of this reaction include i) chiral resolution – only one starting material enantiomer reacts (matched/mismatched); ii) both enantiomers react, affording diastereomeric mixtures. In both cases we would hope to separate the products by conventional methods, such as column chromatography.

Following the established Sharpless protocol, the AD-mix was stirred at room temperature in tBuOH and water until the aqueous phase was yellow in colour. The solution was then cooled to 0 °C and the C(3)-allylated lactams added and stirring was vigorously continued at 0 °C overnight. Following standard aqueous work-up the dimethoxy 130 and 131 and methylenedioxy 132 and 133 dihydroxylated products were isolated in varying yields shown in Table 2.05. However, although the differences of the diastereoisomers could be seen by 1H NMR, no separation could be observed by TLC analysis with a wide variety of solvent systems, nor could separation be achieved by normal or chiral HPLC. Therefore the compounds were obtained as diastereomeric mixtures and as such, they were not subject to antiproliferative screening.
Entry | AD-mix α | Yield (%) | AD-mix β | Yield (%) |
--- | --- | --- | --- | --- |
1 | ![Image of structure 130](image1) | 25 | ![Image of structure 131](image2) | 17 |
2 | ![Image of structure 132](image3) | 75 | ![Image of structure 133](image4) | 38 |

**Table 2.05:** Yields for Sharpless AD reactions

It is worthy of note that there was no observable reaction when the AD reactions were performed with the dimethoxyhydroxy C(3)-allylated lactam 126. As previously described, Judd et al. reported the crystal structure for the thermally stable BF$_2$-complex 62, shown in Figure 2.09, resulting from treatment with the BF$_3$·OEt$_2$ Lewis acid. This clearly shows that the OH at C(8) and the carbonyl form a potent bidentate ligand, which is capable of co-ordinating to metal ions. It is therefore likely that unproductive coordination of the catalytic osmium in the AD reaction prevents the dihydroxylation reaction, resulting in recovered starting material as observed.

![Image of structure 62](image5)

**Figure 2.09:** BF$_2$-complex 62

Owing to the issues of diastereomeric mixtures, their separation and the inactivity of the most interesting dimethoxyhydroxy lactam, this avenue was not pursued further. Instead we focused our efforts on the synthesis of tricyclic analogues, which is discussed in Chapter 3.
2.7. Biological evaluation

A selected series of AB-ring analogues was tested by MTS cell proliferation assay against the HT29 colon cancer cell lines and this preliminary work is described herein. Data shown is an average of three individual experiments (N=3) unless otherwise stated.

Initial testing on the AB-ring analogues, shown in Figure 2.10, reaffirmed preliminary work by the group and showed that N-methylated compounds were still inactive. Pleasingly, the pattern of increased activity for the C(8) phenol was observed with analogues 61 and 86 showing a slightly higher IC₅₀ of 386 μM and 497 μM respectively, although the N-methyl appears to render the compound less active. It was therefore decided not to biologically evaluate the N-methyl oxidised derivatives. In addition, published SAR studies suggest the lactam must remain unchanged as described in Section 1.3.3.2.

![Figure 2.10: Biological evaluation of dihydroisoquinolinone derivatives by MTS cell proliferation assay using HT29 colon cancer cell line](image)

Biological evaluation of the oxidised analogues 64 and 65 was also undertaken by MTS cell proliferation assay using HT29 colon cancer cells (Figure 2.11) and their IC₅₀ values were 220 μM and 289 μM respectively. Although they still show improved activity compared to their dihydroisoquinolinone counterparts the activity is less than previously thought (shown in Figure 2.11). We suggest that this is due to the potential for palladium contamination affecting the results of the antiproliferative assay. Although purity was previously established by HPLC analysis, palladium contamination would not be observed using this method as it would not absorb UV light and would therefore not be detected. An alternative strategy to determine purity was Elemental (CHN) analysis and was used in this case with the accuracy of each compound being found to be
within 0.4% of the theoretical value. Unfortunately, C(8) phenol analogue 63 could not be tested due to a lack of material.

![Chemical structures](image)

**Figure 2.11:** Biological activity of oxidised analogues 64 and 65 by MTS cell proliferation assay using HT29 colon cancer cell line (*a* This work, *b* Previous work by Judd).

Evaluation of the C(4) acetylated analogues 90, 91 and 92 (shown in Figure 2.12) shows an improved activity with IC$_{50}$ of 176 μM, 110 μM and 33 μM respectively. The pattern already established of the dimethoxy analogues being more active than the methylenedioxy analogues is replicated here. In addition, the N-H analogue has approximately three times the activity observed for the N-methyl counterpart. Data for analogues 91 and 92 should be used with caution however as purity data was not acquired due to limited access to these compounds. Human breast cancer cells (MDA) were also used to assess activity of analogue 90 which was found to have a slightly improved IC$_{50}$ of 117 μM against this cell line.

![Chemical structures](image)

**Figure 2.12:** Biological activity of C(4) acetylated analogues by MTS cell proliferation assay using HT29 colon cancer cell line (*a* N=1, *b* N=2)

Allylation at C(3) also gave some interesting analogues which were also tested and the results are shown in Figure 2.13. These compounds were found to be active with free phenol analogue 126 being the most active, in concordance with the previously reported pattern of activity.
2.8. Conclusions and future work

The one-pot synthesis of AB-ring analogues, published by the group, has been used here to develop methods for further functionalisation and biological evaluation of these analogues. The inactivity of the dihydroisoquinolinone analogues, in addition to their N-methylated counterparts, has been confirmed by MTS cell proliferation assay.

An efficient procedure to furnish oxidised dimethoxy and methylenedioxy analogues and has also been optimised using cinnamic acid starting materials. Formation of the acyl-azide, followed by Curtius rearrangement and thermal isomerisation to the Z-isomer of the isocyanate and subsequent thermally-induced intramolecular Friedel-Crafts acylation gave the isoquinolines in good yields on both a 10 and 25 mmol scale. In addition, the biological results obtained for these compounds show reduced antiproliferative activity compared to previous studies, and suggest that previous samples may have been contaminated with palladium. Therefore, not only is this route more efficient and higher yielding but it also provides cleaner compound, as established by CHN analysis. Analogues with the free phenol have persistently been found to be the most biologically active. It is therefore unfortunate that this analogue has not been synthesised in sufficient quantities for analysis and further functionalisation. The above reaction should be examined further as access to the phenol analogue would be of great interest due to its superior activity.

Functionalisation at C(3) or C(4) has been realised with limited success. With a robust procedure to the oxidised analogues from the corresponding cinnamic acids in place further functionalisation of these analogues could be achieved. For example, further optimisation of the oxidation by ruthenium can be investigated in order to gain access to caspase 3 inhibitors in just two-steps. In addition, preliminary biological evaluation of the NSC-type analogues found them to be insoluble in our in-house MTS assay. However, alternative strategies such as using ethanol in place of DMSO are available and should be investigated.
Synthesis of allylated analogues was successful, however the dihydroxylation of the analogues is worth investigating further. Although separation could not be achieved by traditional methods alternatives could be tried. Separation of the ensuing diastereomers may be achieved by formation of a cyclic carbonate from the diol, which could then be separated by column chromatography or HPLC. Biological evaluation would then provide insight into the need for a tricyclic system.
3. Synthesis and evaluation of ABC-ring analogues

3.1. Introduction and preliminary work by the Caggiano group

The total synthesis of narciclasine 7 and pancratistatin 8, previously discussed in Section 1.3, is a laborious process requiring many synthetic steps and often resulting in low overall yields. Reported total syntheses often preclude the biological investigations of late stage analogues, something which is of interest to this group in order to identify biologically active simplified molecules. An efficient synthesis of late stage analogues would allow for functionalisation and further examination of the structure-activity relationship (SAR) shown by these molecules.

Previous work within the group described our preliminary investigations of synthesising advanced narciclasine analogues utilising our novel methodology, as outlined in Chapter 2. The synthesis of the dimethoxy and trimethoxy analogues were achieved by Judd and employed the Robinson annulation, Curtius rearrangement and intramolecular Friedel-Crafts acylation as the key reactions (Scheme 3.01). This preliminary work was optimised and successfully repeated here, and also extended to novel methylenedioxy narciclasine analogues, which are now discussed together with the results of the optimisation studies.

Scheme 3.01: Synthetic route to narciclasine analogues

3.2. Synthesis of 3,4,4a,5-tetrahydro[1,3]dioxolo[4,5-j]phenanthridin-6(2H)-one 148

3.2.1. Claisen condensation in the synthesis of β-ketoester 135

The initial step towards the simplified tricyclic core is the synthesis of the β-ketoester 135, Scheme 3.02. Following the procedure reported by Jung et al., starting with the commercially available methylenedioxyacetophenone 134, condensation with diethyl carbonate at 80 °C using NaH as base furnished the expected β-ketoester 135 as a yellow oil. Although several attempts were made to purify the resulting product 135, minor residual starting material 134 persisted after column chromatography. The product was therefore used as a mixture of acetophenone 134 to β-ketoester 135 of 17:83 in the subsequent reactions.
3.2.2. The Robinson annulation as a one-pot procedure

During his research into sterol derivatives, Robert Robinson found that the sodium enolate of cyclohexenone 136 and methyl styryl ketone 137 gave the bicyclic analogue 138, shown in Scheme 3.03.\textsuperscript{109} Since then it has become ubiquitous in synthetic chemistry, playing a key part in a number of synthetic processes. The preliminary step is a Michael addition between an enolate and a Michael acceptor, such as methyl styryl ketone or methyl vinyl ketone (MVK). This is followed by an intramolecular aldol reaction to give bicyclic analogues in a one-pot transformation.

Preliminary work on the Robinson annulation within the Caggiano group was achieved by a two-step process. Given that the Robinson annulation can be achieved in a one-pot transformation, this step was investigated further. Previous initial studies also identified that using hydroxide as a base caused decarboxylation of any ensuing enone product, see Section 3.2.4,\textsuperscript{64} and its use was therefore avoided and alternative conditions sought. Reaction conditions were reported for a similar compound using sodium ethoxide in ethanol at 70 °C for 16 hours, as shown in Scheme 3.04, however a mixture of unidentifiable products was recovered.\textsuperscript{110} An alternative base was reported by Chong et al. using potassium tert-butoxide in tert-butanol, but again, a mixture of unidentifiable products was recovered in our system.\textsuperscript{111}
Scheme 3.04: Attempts at one-pot Robinson annulation

Unusual reaction conditions were reported by Miyamoto et al., in which ester 140 was ground in a pestle and mortar with MVK and sodium methoxide (NaOMe), with or without solvent, and left for 3 hours (Scheme 3.05). This was of interest as these conditions gave the cyclohexenone 141 with the ester intact. Using either of these reported conditions with ester 135 unfortunately did not furnish the desired product, giving an unidentifiable mixture of compounds.

Scheme 3.05: Solvent and solvent-free Robinson annulation, with intact ester

Regrettably, these one-pot methods did not produce the expected Hagemann’s ester 139 and so the original two-step process was pursued.

3.2.3. The Robinson annulation as a two-step procedure

3.2.3.1. Michael Addition

Using the conditions reported by Judd for the dimethoxy analogue, the potassium enolate of β-keto ester 135 was made in anhydrous acetonitrile using potassium carbonate (K₂CO₃) at 0 °C, Scheme 3.06. After 10 minutes MVK was added and the reaction warmed to room temperature for 90 minutes to furnish analogue 142 in 79 % yield over two-steps, following column chromatography. Previously, the bis-product had also been reported for the dimethoxy analogue 158, however this was not observed in this reaction.
3.2.3.2. Intramolecular aldol cyclisation

The intramolecular Aldol reaction was previously achieved with catalytic $p$-toluene sulfonic acid ($p$-TsOH) in toluene at reflux under Dean-Stark conditions.$^{64,113,114}$ This method was employed for the synthesis of Hagemann’s ester 139, shown in Scheme 3.07, with varying degrees of success. Unfortunately, low yields between 6 and 16% were achieved together with the recovery of a black insoluble mass. Furthermore, this reaction required 100% conversion of the Michael adduct as this starting material co-eluted with the cyclohexenone 139 product.

The capricious nature of this reaction forced alternative strategies to be sought. Attempts were made to circumvent the need for Dean-Stark conditions. A screen of solvents was tested, both at room temperature and reflux, in the presence of anhydrous magnesium sulfate to promote dehydration. Unfortunately, no observable reaction was detected.

Keeping with the original conditions but changing the apparatus to Soxhlet equipment with molecular sieves proved to be much more successful and reproducible, and was used for all subsequent reactions (Scheme 3.07). In order to improve the yield the catalytic amount of $p$-TsOH was also screened, however no improvements of the 53% yield were observed over 40 mol % of the catalyst.

Scheme 3.06: Michael addition using MVK

Scheme 3.07: Cyclisation of the Michael adduct 142
3.2.4. Reduction of the ketone

With an optimised and reproducible method to the modified Hagemann’s ester 139 in hand, procedures for saponification were considered in order to obtain the carboxylic acid precursor required for the Curtius rearrangement. Previous research by the group had identified decarboxylated by-products, due to the extended conjugate system of ester 143. Methods for the reduction of the carbonyl (Route a) or olefin (Route b) were therefore previously investigated as they would prevent the decarboxylation by this route (Scheme 3.08). However, control over the newly formed stereogenic centres proved difficult, as did separating the ensuing diastereoisomers, therefore these routes were abandoned. Although complete reduction of the carbonyl could be considered counterproductive as the natural product has a polyhydroxylated C-ring, it was found to provide access to the narciclasine core (Route c). The current work would build on the previous results and complete the series of analogues and examine ways of functionalising the ABC-ring products further.

**Scheme 3.08:** Summary of methods employed to remove the conjugated system of ketone 143

Using reaction conditions reported by Freixa et al., sodium borohydride (NaBH₄) was added step-wise to a stirring solution of trifluoroacetic acid (TFA), acetic acid (AcOH) and acetonitrile (MeCN) at 0 °C under inert conditions (Scheme 3.09).¹¹⁵ Cyclohexenone 139 in CH₂Cl₂ was added dropwise and the reaction was allowed to warm to room temperature and followed by TLC analysis. Following aqueous work-up and column chromatography, we were delighted to obtain the desired alkene 144 in 99 % yield.
3.2.5. Saponification of the ester

Standard conditions for hydrolysis were then employed using NaOH\(_{\text{aq}}\) and ethanol at reflux and after 6 hours acid 145 was isolated in quantitative yield following recrystallization, shown in Scheme 3.10. Due to the absence of the enone, decarboxylation was not observed.

3.2.6. Modified Curtius rearrangement and Friedel-Craft acylation

Using our established novel methodology for the formation of the isoquinolinone, as described in Section 2.2 and published by Judd \textit{et al.} for the AB-ring systems,\textsuperscript{63} we successfully obtained the novel methylenedioxy tricyclic analogue 148. The acid 145 in toluene was treated with DPPA and Et\(_3\)N and heated from room temperature to 90 °C for 90 minutes to initially afford the intermediate acyl-azide 146 and subsequent isocyanate 147, as shown in Scheme 3.11. Optimisation of the reaction conditions found that removing the toluene to near dryness, prior to the addition of BF\(_3\)·OEt\(_2\), improved the yield by keeping the reaction in solution. No advantage was observed in performing this step at 0 °C and it was therefore performed at room temperature. The tricyclic analogue 148 was isolated in 57 % yield following column chromatography and no other products were isolated. This represents a 24 % overall yield over 6 steps of the narcilasine analogue 148.
3.3. Synthesis of 7-hydroxy-8,9-dimethoxy-3,4,4a,5-tetrahydrophenanthridin-6(2H)-one 155

Judd reported the synthesis of dimethoxyhydroxy analogue 155 over 6-steps in 18 % overall yield.\textsuperscript{64} Repeated synthesis of this analogue was undertaken in order to obtain compound for further functionalisation studies and evaluation of its biological activity, which is now described.

Diethyl carbonate was treated with NaH base and then 3,4,5-trimethoxyacetophenone 149 to afford the $\beta$-ketoester 150 in 92 % yield (Scheme 3.12). This time the product and starting materials did not co-elute and the product could be purified by column chromatography. As previously outlined the $\beta$-ketoester 150 was then treated with K$_2$CO$_3$ base and MVK to afford the 1,4-Michael adduct 151 in 93 % yield.

Due to the inconsistency observed using the Dean-Stark apparatus for the cyclisation reaction to the Hagemann’s ester 152, which was previously used, the more consistent Soxhlet apparatus was instead employed, shown in Scheme 3.13. This gave a slightly poorer yield of 40 % (Judd reported 53 %)\textsuperscript{64} however the reaction proceeded in a more reliable manner with the yield being consistently around 40 %.
As previously reported for the methylenedioxy analogue 144, described in Section 3.2.4, complete reduction of the carbonyl was achieved in 80 % yield (Scheme 3.14). Subsequent hydrolysis of the ester 153 to afford the carboxylic acid 154 also proceeded in high yield of 97 %.

Pleasingly, cyclisation and concurrent demethylation proceeded smoothly using the previously reported conditions to give the narciclasine analogue 155 in 40 % yield as shown in Scheme 3.15. This is comparable to the 46 % yield previously reported by Judd.64 Although the overall 6-step yield reported here is less than Judd, 11 % compared to 18 % respectively, the reliability of the cyclisation to the Hagemann’s ester is improved.
3.4. Synthesis of 8,9-dimethoxy-3,4,4a,5-tetrahydrophenanthridin-6(2H)-one 162

The previous synthesis of dimethoxy analogue 162 described within the group was achieved in a 15 % overall yield again over 6-steps, with analogue 163 also being isolated in 10 % yield (discussed in Section 3.5 and 3.9). Again, the complete synthesis was repeated to gain more compounds for further analysis, which is now described.

The condensation of 3,4-dimethoxyacetophenone 156 with diethyl carbonate furnished the β-keto ester 157 in 95 % yield after purification by column chromatography (Scheme 3.16). This was followed, as previously described, by a 1,4-Michael addition with methyl vinyl ketone (MVK) to afford the Michael adduct 158 in 94 % yield as a yellow oil following column chromatography (Scheme 3.16).

\[ \text{Diethyl carbonate} \quad \text{NaH (1.5 eq.)} \quad 0 \, ^\circ \text{C to 80} \, ^\circ \text{C} \quad \text{N}_2, \text{90 min} \]

\[ \text{K}_2\text{CO}_3 (1.1 \text{ eq.}) \quad \text{MeCN, 0} \, ^\circ \text{C to r.t.} \quad \text{Ar, 90 min} \]

Scheme 3.16: Synthesis of the Michael adduct 158

Cyclisation of the Michael adduct 158 using the Soxhlet apparatus gave the enone ester 159 in an improved 68 % yield (Scheme 3.17), which is similar to the 70 % yield reported by Judd using the Dean-Stark apparatus. Reduction of the carbonyl 159 and saponification of the ethyl ester 160 proceeded smoothly in 85 % and 96 % yield respectively.

\[ \rho\text{TsOH.H}_2\text{O (40 mol %)} \quad \text{Toluene} \quad \text{N}_2, \text{143} \, ^\circ \text{C oil bath, 24 h} \]

\[ \text{TFA} \quad \text{NaBH}_4 \quad \text{AcOH} \quad (6.5 \text{ eq.}) \]

Scheme 3.17: Cyclisation, reduction and then saponification of enone 158
With the carboxylic acid 161 in hand, treatment with DPPA and Et$_3$N, as previously described, gave the desired dimethoxy isoquinolinone analogue 162 in 36 % yield as the only isolated product and represents an overall yield of 18 % yield over 6-steps. This is comparable to the yield previously obtained by Judd, who obtained a 34 % yield of this product in the final step.$^{64}$ However, in addition to the expected product 162, Judd also isolated the product resulting from double bond migration 163 in 23 % yield and shown in Scheme 3.18. This rearranged product was however not observed in the repeat reactions, and could be the result of slight variations in the reaction or work-up which results in its formation. In an attempt to examine this result, the expected product 162 was treated with neat BF$_3$·OEt$_2$ and the mixture examined by $^1$H NMR spectroscopic analysis which showed no change.

Scheme 3.18: Modified Curtius rearrangement and cyclisation (↑ Reported by Judd.  ♦ This work.)

3.5. Functionalisation of the narciclasine derivatives

The previous syntheses of the ABC-ring analogues gave the following narciclasine analogues 155, 162 and 163 (Figure 3.01). Biological evaluation by MTS cell proliferation assay using a HT29 cell line demonstrated that these compounds have antiproliferative activities in the micromolar range, the values of which are shown in Figure 3.01. These narciclasine analogues lack hydroxyl groups which are present in the natural product C-ring, which obviously improve potency. Strategies to incorporate oxygenation in the C-ring are discussed shortly, but we wished to investigate the corresponding pancratistatin analogues, which are devoid of the olefin. Subjecting analogues 148, 155 and 162 to conditions for hydrogenation would provide an interesting contrast to the potential anticancer activity already observed for C(10b) sp$^2$-hybridisation. The hybridisation of C(10b) of pancratistatin is sp$^3$- in contrast to sp$^2$-hybridisation of narciclasine and our late stage intermediates.
3.5.1. Hydrogenation with palladium

Using standard conditions, the narciclasine analogues 148, 155 and 162 were stirred in ethanol with Pd/C under an atmosphere of hydrogen. The addition of the hydrogen can occur from the top and/or bottom face of the double bond, which would afford cis and/or trans products respectively. Furthermore, given that the starting material is a racemic mixture these diastereoisomers will also be racemic.

Treatment of the dimethoxyhydroxy narciclasine analogue 155 with hydrogen and Pd/C under standard hydrogenation conditions gave a mixture of products. $^1$H NMR spectroscopic analysis of the crude reaction mixture revealed the presence of three compounds, which were identified as a 59:41 mixture of cis:trans 164 in addition to the product of double bond migration 165, as shown in Scheme 3.19. Attempts to separate the products by column chromatography were only partially successful, as the cis/trans mixture proved to be inseparable in a variety of solvents systems. However, following purification the fully reduced pancratistatin analogues were obtained in 54 % yield and the migration product in 33 % yield.

Scheme 3.19: Reduction of narciclasine analogue 155 using Pd/C and atmospheric hydrogen

It is very interesting to note the appearance of the unsaturated B/C-ring junction analogue 165, as it demonstrates isomerisation to afford the more thermodynamically stable tetra-substituted olefin.
is possible, and that presumably the migration product is more resistant to hydrogenation than the trisubstituted olefin starting material. The observed cis/trans mixture could be the result of a poorly diastereoselective reaction, or that the hydrogenation reaction is highly selective for the trans-isomer, but hydrogenation of the migration by-product also results in the formation of the cis-isomer. Given the isolation of the migration product, it seems likely the mixture is a result of low diastereoselectivity.

Analogue 165 has recently been described by our group in a different but related project, and was produced via an alternative synthetic pathway to the late stage pancratistatin analogues described herein (Scheme 3.20).\textsuperscript{77} 3,4,5-Trimethoxy-1-bromobenzene 167 and α,β-cyclohexene carboxylic acid 166 were joined using a Heck cross-coupling reaction. This was followed by the modified Curtius rearrangement to furnish tricyclic 165. Preliminary biological testing showed analogue 165 to have an IC\textsubscript{50} of 9 μM against the HT29 cells following MTS cell proliferation assay. This enhanced anticancer activity complements observations by Judd et al. following bond migration of the dimethoxy 163 shown previously in Figure 3.01. This is in addition to the increase in activity caused by the free phenol and is consistent with the pattern observed for the anticancer activity of the oxidised AB-ring analogues described in Chapter 2.

\begin{center}
\begin{tikzpicture}
\node[draw,rectangle,align=center] (A) at (0,0) {166 \hspace{1cm} \begin{align*}
(PPh_3)_2\text{PdCl}_2 \hspace{1cm} K_2\text{CO}_3(\text{aq}) \hspace{1cm} 100°C, 24 \text{ h}
\end{align*} \hspace{1cm} 167 \hspace{1cm} 168 \hspace{1cm} \text{43 %}};
\node[draw,rectangle,align=center] (B) at (3,0) {168 \hspace{1cm} 43 \%};
\node[draw,rectangle,align=center] (C) at (6,0) {166 \hspace{1cm} \begin{align*}
1) \text{DPPA, Et}_3\text{N} \hspace{1cm} 2) \text{BF}_3\text{OEt}_2
\end{align*} \hspace{1cm} \text{165} \hspace{1cm} 65 \%};
\end{tikzpicture}
\end{center}

\textbf{Scheme 3.20:} Synthesis for the late stage intermediate 165 via a two-step pathway\textsuperscript{77}

The dimethoxy and methylenedioxy narciclasine analogues 162 and 148 were also subject to hydrogenation as previously described, except that the solvent was changed for methanol to improve the solubility of the starting materials. As previously observed, a cis/trans mixture of the fully reduced dimethoxy analogue 169 was observed by \textsuperscript{1}H NMR in 58:42 ratio respectively, in 64 % yield which again could not be separated by column chromatography (Scheme 3.21). Similarly, the product of migration to the tetra-substituted olefin 163 was also identified and could be separated and isolated in 23 % yield.
When the methylenedioxy narciclasine analogue 148 was treated with hydrogen and Pd/C only two products were identified by $^1$H NMR, which were the cis/trans isomers 103 in a 54:46 ratio (Scheme 3.22). As previously noted for the related analogues, these isomers were inseparable by column chromatography and the mixture was obtained in 47 % yield. In this case, the cis-isomer 103 was obtained within our research group, via an alternative method, and an X-ray crystal structure obtained confirming the cis-assignment. The $^1$H NMR spectroscopic data for the major isomer present in our mixture matched perfected data obtained for the isolated and fully characterised cis-methylenedioxy analogue described by Tunbridge. A second compound was isolated by column chromatography which was presumably analogue 170, however it proved to be insoluble in a number of different solvents and was consequently not fully characterised.

The stereochemistry of the reduced compounds is worth noting and is highlighted using analogue 103 in Scheme 3.23.

In all cases the resulting diastereomers were clearly visible by $^1$H NMR spectroscopic analysis and were assigned by extrapolating the data reported by Tunbridge of the cis-analogue 103cis, as there is a high similarity between the $^1$H and $^{13}$C NMR spectra. We consistently observed mixtures which slightly favoured the cis-isomer.
Unfortunately NOESY data did not show correlation between the C(4a)H and the C(10b)H in either isomer. However, using the Karplus equation it is highly suggestive that isomer 103cis, J C(4a)H and C(10b)H is 3 Hz, corresponds to an equatorial/axial interaction. Isomer 103trans, shown in Scheme 3.23, corresponds to an axial/axial interaction as this coupling is approximately 12 Hz. This correlation is consistent throughout the series.

\[ \text{Scheme 3.23: Coupling constants of the B/C-ring junction and idealised dihedral angle} \]

Comparing to the natural product, the trans-analogue would be of particular interest due to its similarity with pancratistatin 8, shown in Figure 3.02. Preliminary antiproliferative investigations of analogue 103cis and 165 were undertaken by Tunbridge,77 and 163 by Judd,64 both of which will be discussed further in Section 3.9. Unfortunately, due to the difficulties in separating the isomers, these mixtures were not investigated for antiproliferative activity.
3.6. Epoxidation of the Hagemann’s ester

The natural products narciclasine 7 and pancratistatin 8 are single enantiomers, however our current synthesis produces analogues which are racemic and this will have an impact on their antiproliferative activity. A possible solution would be to perform enantioselective epoxidation, which could generate diastereoisomers which could then be separated to afford analogues which are not only enantiomerically pure, but contain oxygenation in the C-ring.

Our initial efforts focused on straightforward epoxidation strategies to examine the viability of the reaction and ease of separation. The hope was that the diastereomers would be separable which could then lead to individual testing and is shown in Scheme 3.34 using the methylenedioxy analogue 139 as an example. In addition, the extended conjugated system which previously caused problems with decarboxylation should no longer be a problem as the enone has been removed without having to fully reduce the carbonyl as previously described and outlined in Scheme 3.08. Furthermore, asymmetric reduction of the ketone would install a hydroxyl group with controlled stereochemistry, analogue 172 (Scheme 3.24). Due to their availability, methylenedioxy analogues were initially investigated.

Scheme 3.24: Proposed epoxidation and diastereomeric outcomes of analogue 139

Hydrogen peroxide (H₂O₂) is a well-established source of nucleophilic oxygen for the epoxidation of enones. Using conditions reported by Cabeza et al.,¹¹⁶ enone 139 was stirred in methanol and
NaOH\(_{\text{aq}}\), \(H_2O_2\) was added at room temperature, and stirring continued for 18 hours. A mixture of diastereomers was observed by crude \(^1\)H NMR spectroscopic analysis, shown in Scheme 3.25, with a single diastereomer being isolated in 46 % yield following column chromatography. Unfortunately, it was not possible to determine which isomer was isolated by spectroscopic methods, but one would expect the product to be that from the least hindered face, as observed in literature examples,\(^{117}\) favouring the desired epoxide \(171\).

![Scheme 3.25: Epoxidation products of analogue 139 and their proposed stereochemistry](image)

Although initially these reactions were successful, they were found to be unreliable with starting material often being recovered. Alterations in reaction time and/or the concentration of \(H_2O_2\) or NaOH failed to improve the reliability of the reaction. Replacing the solvent with other water miscible solvents such as THF or MeCN had no beneficial effect.

Keeping the reaction at 0 °C for 48 hours as described by Ringold et al.\(^{118}\) failed to furnish the epoxide, instead only unreacted enone \(139\) was recovered. Conversely, heating the reaction to 50 °C with NaOH (2 eq.) and an excess of \(H_2O_2\) in ethanol,\(^{119}\) gave a single diastereomer on a 0.16 mmol scale. The reaction was repeated and performed on a larger, more viable scale, but regrettably only a mixture of unidentifiable materials was recovered. This was presumably caused by the degradation of the enone \(139\) and/or decarboxylation due to saponification previously discussed in Section 3.2.4. Changing the base to potassium carbonate as described by Goldenstein et al.,\(^{120}\) also failed to furnish the epoxide.

Similar conditions were reported by Das Sarma et al. using sodium carbonate as the base in a biphasic system of methanol and \(CH_2Cl_2\).\(^{121}\) Unfortunately, only starting material was recovered in quantitative yield. The addition of tetrabutylammonium chloride as a phase transfer agent proved to be ineffective and changing the base\(^{122}\) also failed to furnish the epoxide.

An alternative peroxide is \(t\)-butyl hydroperoxide (TBHP). Sabol et al. reported the epoxidation of the enone ester \(174\) using TBHP and 1,8-diazabicyclo[4.5.0]undecane (DBU), Scheme 3.26.\(^{117}\) They
reported a mixture of diastereoisomers, 175 and 176, which could be separated by column chromatography. The major product in this reaction was that obtained from nucleophilic attack from the least hindered face, as desired in our system. Similar transformations and conditions were reported by Schlessinger et al.\textsuperscript{123} in THF and Goldenstein et al.\textsuperscript{120} in CH\textsubscript{2}Cl\textsubscript{2}. Tatsuta et al. also reported using potassium t-butoxide (tBuOK) as the base in THF.\textsuperscript{124} Using enone 139 and following these reported reaction conditions, analysis by TLC showed the presence of starting material, even after 24 hours stirring at room temperature.

Scheme 3.26: Epoxidation of enone 174 using TBHP\textsuperscript{117}

It is possible that ester hydrolysis to the corresponding water-soluble carboxylate, caused by residual water in the H\textsubscript{2}O\textsubscript{2} or TBHP, was responsible for the issues of reproducibility, so alternative conditions were investigated. Yadav and Kapoor reported the capricious nature of epoxidising lactones and cyclic enones using a screen of oxidising agents, bases and solvent systems, all with literature precedent.\textsuperscript{125} They investigated a more reliable non-aqueous epoxidation of electron deficient alkenes using TBHP and DBU. Using conditions reported by Sharpless and Verhoeven for the azeotropic drying of TBHP in 1,2-dichloroethane (DCE) a 2.35mmol/mL solution was prepared.\textsuperscript{126} Yadav et al.\textsuperscript{125} reported that treatment of enone 177 under anhydrous conditions with DBU and TBHP at 0 °C gave epoxide 178, as shown in Scheme 3.27. Unfortunately, using these reported conditions with our enone 139 always gave an unidentifiable mixture of compounds.

Scheme 3.27: Epoxidation of enone 177 using anhydrous conditions\textsuperscript{125}

Although an enone is electrophilic there has been some literature precedent for their epoxidation by m-chloroper oxybenzoic acid (mCPBA). Pirkle and Hoover show the preparation of a single isomer 180 in high yield using mCPBA (1.3 eq.) in CH\textsubscript{2}Cl\textsubscript{2} at reflux, shown in Scheme 3.28.\textsuperscript{127} This is further
corroborated by Tamura\textsuperscript{128} and Cheng\textsuperscript{129} using mCPBA (1 eq.) in CH\textsubscript{2}Cl\textsubscript{2} at room temperature over a longer reaction time. This is an unusual reaction so perhaps unsurprisingly, only starting material was recovered when enone 139 was subjected to these reaction conditions.

\[ \text{179} \xrightarrow{mCPBA (1.3 eq.) \text{, CH}_2\text{Cl}_2, \text{reflux, 8 h}} \text{180} \]

\( 98\% \) (+/−)

\textbf{Scheme 3.28: Epoxidation of an enone using mCPBA\textsuperscript{127}}

\textbf{3.7. Saponification of epoxide 171}

Preliminary investigations into the saponification of the ester in the presence of the epoxide was also investigated. Zhu \textit{et al.} reported the basic hydrolysis of epoxide 181 using NaOH and methanol could be conducted at room temperature to give the acid 182 quantitatively, as shown in Scheme 3.29.\textsuperscript{122} Sakagami \textit{et al.} reported similar conditions gave their product in 92 \% yield.\textsuperscript{130} Despite following this literature precedent, an unidentifiable mixture of products was observed by crude \textsuperscript{1}H NMR spectroscopic analysis when the epoxy ester 171 was treated with NaOH, which could not be separated.

\[ \text{181} \xrightarrow{\text{NaOH:MeOH \text{, r.t.}, 5 h}} \text{182} \]

\textbf{Scheme 3.29: Saponification of an ester in the presence of an epoxide\textsuperscript{122}}

Due to the issues experienced in synthesising the epoxy ester, and in the subsequent hydrolysis of the ester, this synthetic route was not pursued further. The previously described route towards deoxy-narciclasine analogues was successful, and the three desired analogues were achieved. However, owing to the requirement to remove the enone, this current synthetic route is not appropriate for the synthesis of C-ring hydroxylated narciclasine analogues. At present, the exact C-ring substitution pattern required for potent biological activity is not yet known, so a much more straightforward synthesis of hydroxylated analogues was sought to fully explore C-ring substitution, the preliminary results of which are now described.
3.8. Narciprimine 183

A rare compound isolated by Piozzi et al. in 1968 from Narcissus bulbs was identified as a tricyclic narciclasine analogue, narciprimine 183 shown in Figure 3.03. It is related to narciclasine 7 and pancratistatin 8 as it possesses the same substitution patterns on the A-ring, a 6-membered lactam B-ring, however the C-ring is aromatic and only mono-hydroxylated. Due to its scarcity, only minimal investigations have so far been completed into its biological activity. Despite this lack of data, narciprimine 183 is of interest as Sarikaya et al. demonstrated that it interferes with topoisomerase I and has an anti-proliferative effect on HeLa cervical cancer, MCF7 breast cancer and A431 skin cancer cell lines. This is further supported by work by Nair et al. identifying micro-molar activity across a minipanel of human cancer cell lines. This natural product 183 is not as potent as narciclasine 7 or pancratistatin 8 however an investigation into the SAR in this analogue would establish if this was due to the planar nature of narciprimine 183 or its lack of hydroxyl groups on the C-ring.

\[ \text{Figure 3.03: Structure of narciprimine 183 and proposed analogues 184 and 185} \]

The tricyclic structure of analogue 184 could be accessed in a two-step process, the retrosynthetic analysis of which is shown in Scheme 3.30. Initial cross-coupling of two aryl groups, one of which is a benzoic acid derivative, would give A/C-biaryl 186. This could then undergo the modified Curtius rearrangement and cyclisation previously described in Chapter 2. Using aryl-halides 187 with various benzoic acid derivatives such as salicylic acid 188, with different patterns of substitution, would provide a series of analogues which could be used to probe the SAR of narciprimine 183.
The traditional approach for forming the aryl-aryl bond involves the coupling of an aryl-halide and an organometallic reactant, usually in the presence of a transition metal.\textsuperscript{133} This is often an inefficient and costly approach requiring pre-functionalisation of the aromatic groups. Direct arylation via C-H bond activation has received an increasing amount of attention due to its obvious advantages over the traditional approach some of which are reviewed by McGlacken and Bateman.\textsuperscript{133}

Mousseau \textit{et al.} coupled substituted bromobenzene analogues, including benzoic acid 189 shown in Scheme 3.31, with unactivated arenes to get analogue 190.\textsuperscript{134} These reactions are palladium catalysed in the presence of silver carbonate or acetate. Although good yields were reported, problems of regioselectivity occurred when methoxy substituted arenes were used, as would be the case in synthesising A-ring narciprimine analogues. In addition, decarboxylation was often a factor in the synthesis of acid derivatives.

An alternative strategy was reported by Chiong \textit{et al.} in which palladium catalysed coupling was achieved by the direct ortho-arylation of free benzoic acids with aryl iodides and silver acetate, an example of which is shown in Scheme 3.32.\textsuperscript{135} Both electron withdrawing and donating groups on either aryl were tolerated. In addition, aryl bromide derivatives were also reported to be adequate coupling partners.
Using the reported conditions, the inexpensive and commercially available 1-bromo-3,4,5-trimethoxybenzene 192 was chosen to be coupled with salicylic acid 188 in order to more closely replicate the substitution pattern of narciprimine 183. Although specialist equipment was not reported by Chiong et al. 135 preliminary reactions were carried out using a thick walled pressure tube as implied by Mousseau et al. 134 in their work on aryl bromide derivatives. Unfortunately quantitative recovery of starting material was observed, and none of the expected product, as shown in Scheme 3.33 and Entry 1, Table 3.01, was observed.

To understand the scope and limitations of this reaction, benzoic acid was used to examine the effect of the free phenol. In addition, substituted and unsubstituted aryl bromides and aryl iodides were also examined and the results are shown in Table 3.01. The reactions were analysed by ¹H NMR of the crude reaction mixture and comparison with literature spectroscopic data for known compounds enabled positive identification of coupled products.
As can be seen in Table 3.01, there was no observable reaction between 1-bromo-3,4,5-trimethoxybenzene and salicylic acid (Entry 1) or benzoic acid (Entry 2) under these conditions. The reaction was repeated with bromobenzene, which again resulted in no observable reaction by $^1$H NMR spectroscopic analysis of the crude reaction mixture (Entry 3). These results demonstrate the difficulties in performing the cross-coupling reaction with aryl bromides, therefore our attention focused on using the more reactive aryl iodides.

Using iodobenzene under the same reaction conditions gave the desired product in 32 % yield by $^1$H NMR spectroscopic analysis, with the remaining mass being unreacted starting materials (Table 3.01, Entry 4). Reducing the reaction time led to a decrease in the conversion (Entries 4-6) and although increasing the palladium loading from 5 mol % to 10 mol % did lead to an increase from 32 % to 40 % (Entries 4 and 7), it was small so was not adopted. Likewise, an increase in the amount of aryl iodide was also investigated, but the conversion did not improve and even worsened (Entry 8).
With the success of the iodobenzene couplings, we investigated the reaction with 1-iodo-3,4,5-trimethoxybenzene using our optimised conditions (shown in Entry 4). We were delighted to observe good levels of conversion to the corresponding trimethoxyaryl coupled product, which could be repeated (Entries 9 and 10). Unfortunately, despite good conversion the product 194 was only isolated in 12 % yield following column chromatography (Scheme 3.34). Due to the small quantities of the benzoic acid 194 obtained, the cyclisation step could not be fully investigated, although based on our experience in this cyclisation reaction, we are very confident this step will furnish the desired lactam. These results demonstrate the coupling reaction works with aryl iodides and can be further optimised to provide a range of narciprimine analogues readily, via a two-step synthesis.

Scheme 3.34: Successful synthesis of the coupled benzoic acid 194 and proposed cyclised product

3.9. Biological evaluation

Preliminary in-house studies of the tricyclic compounds were undertaken by the group using HT29 colon cancer cells in an MTS cell proliferation assay the results of which are shown in Figure 3.04. Initial IC\textsubscript{50} values for late stage analogues 155 and 162 were 20 \(\mu\)M and 37 \(\mu\)M respectively and these results were subsequently validated following the described synthesis herein and a repeat of the MTS assay. The novel methylenedioxy narciclasine analogue 148, lacking the free phenol group, has a slightly poorer IC\textsubscript{50} of 50 \(\mu\)M, which is consistent with observations previously reported for the AB-ring structures, Chapter 2. All three tricyclic compounds show superior activity compared to their bicyclic counterparts, suggesting that even without the hydroxylation present on the C-ring of the natural products, the tricyclic analogues show improved activity.
The saturated analogues were not examined for antiproliferative activity, as they were mixtures of both *cis* and *trans* isomers which could not be separated by column chromatography using several solvent systems. The *cis*-methylenedioxy analogue 103*\textit{cis}* however was previously isolated within the group and examined and found to be inactive (IC$_{50} >$ 500 $\mu$M). It is interesting to note that this mirrors previous results discussed in Chapter 2 that the saturated AB-ring analogues all displayed little or no activity, whilst the oxidised AB-ring analogues showed some activity. Furthermore, it is also worthy to note that although *cis*-methylenedioxy analogue 103*\textit{cis}* lacks the free phenol and C-ring hydroxyl groups present in the natural product, the unsaturated parent compound 148, also lacking the same substituents, did show some activity (IC$_{50} 50 $ $\mu$M). This clearly demonstrates the need for the double bond, however, it may be that this analogue is inactive because it is the *cis*-isomer, and that the *trans*-isomer would be a closer analogue of pancratistatin and therefore more active. Future investigations should examine separating these diastereomers by HPLC, which would allow for the effect of the *cis*/*trans* configuration to be assessed.

An increase in activity was observed with the oxidised AB-bicyclic analogues, previously discussed in Section 2.7. Data shown in Figure 3.04 suggests that an unsaturated B/C-ring junction also
increases activity with dimethoxy analogue 163 having an IC$_{50}$ value of 17 μM. Predictably, having a free phenol at C(7), analogue 165, shows the best activity with an IC$_{50}$ of 9 μM and is consistent with the pattern previously observed, Section 2.7. Unfortunately, the corresponding product of double bond migration was not isolated using the methylenedioxy analogue 148, so the biological activity of this analogue could not be assessed.

3.10. NCI 60 cell line screening

The National Cancer Institute (NCI) offers a service screening biologically interesting compounds against 60 human cancer cells lines, initially at a single dose of 15 μg/mL. If a fixed threshold of anti-proliferative activity is observed in a minimum number of cell lines then these compounds are carried forward to a five-dose screening programme. The five-dose screen will determine the GI$_{50}$, total growth inhibition (TGI) and LC$_{50}$ of the compounds selected and will suggest whether in vivo testing would be beneficial.

The five active analogues shown in Figure 3.04 were submitted for evaluation and were accepted by the NCI for initial one-dose testing. The growth of the cells is measured relative to the control, in which no cells were treated, and the number of cells at time zero. This is then displayed as a mean graph of the percentage growth relative to the control. The values for growth inhibition lie between 0 and 100, where 100 shows no growth inhibition and therefore a value of 30 means 70% growth inhibition. Lethality is displayed as a value of less than 0 with -100 meaning all the cells had been killed.

Unfortunately the compounds typically showed growth inhibition of less than 10%, as shown in the Appendices, and were not selected by the NCI for further five-dose testing. The best compound was analogue 165 with growth inhibition of 17.30% (Table 3.02, Entry 2). This observation fits with the SAR gathered so far as analogue 165 has a C(7) free phenol and also has the olefin at the B/C-ring junction.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Mean growth inhibition value</th>
<th>Growth inhibition (%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Compound 155" /></td>
<td>91.27</td>
<td>8.73</td>
<td>47.77</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Compound 165" /></td>
<td>82.70</td>
<td>17.30</td>
<td>63.46</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Compound 162" /></td>
<td>97.49</td>
<td>2.51</td>
<td>59.32</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Compound 163" /></td>
<td>97.82</td>
<td>2.18</td>
<td>64.96</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5" alt="Compound 148" /></td>
<td>97.15</td>
<td>2.85</td>
<td>55.68</td>
</tr>
</tbody>
</table>

Table 3.02: NCI data for the mean growth inhibition using single-dose data

### 3.11. Conclusions and Future work

The 6-step synthesis of the late stage ABC-ring narciclasine derivatives developed by the Group has successfully been utilised to furnish the dimethoxy and dimethoxyhydroxy analogues 162 and 155 and has been extended to the novel methylenedioxy analogue 148. Unfortunately, the product of
double bond migration 163 was not isolated following these conditions, as previously reported, however access to the dimethoxy and dimethoxyhydroxy analogues, 163 and 165, was successful using conditions for hydrogenation. Although the methylenedioxy analogue was not isolated it can be presumed that this would be the least active compound as shown by the previously discussed pattern of antiproliferative activity.

The late stage analogues were then subjected to an in-house MTS cell proliferation assay and five were subsequently sent to the NCI for one-dose testing. The results obtained from these investigations highlight the importance for a free phenol group at C(7) in the A-ring and also the presence of hydroxyl groups in the C-ring. In addition, it is worth noting that the fused B/C-ring junction analogues 163 and 165 are achiral and are our most active analogues in this series. Further investigations into the functionalisation of these analogues, providing hydroxylation of the C-ring would be beneficial.

Although our efforts to functionalise the synthesised narciclasine analogues proved challenging the successful coupling strategy outlined here in Section 3.8 could gain narciprimine analogues in an efficient two-step process. Further investigations using substituted C-ring hydroxylated acids, such as salicylic acid or gallic acid, could improve the potency and selectivity of narciprimine as the analogues will more closely resemble the more active natural products narciclasine and pancratistatin.
4. Synthesis and evaluation of N ABC-ring analogues

4.1. Introduction to quinazolinones

Narciclasine and pancratistatin are well established as biologically active lead compounds; however their poor aqueous solubility has hampered their progression into clinical use. Further investigations aiming to improve water solubility, whilst keeping their unique anticancer properties intact, are thereby warranted. The incorporation of a basic nitrogen to the skeleton would improve solubility, with the tertiary amine providing a hydrogen bond acceptor. There would also be an opportunity to make the salt form of any compounds made. The reduction of the lactam to afford the corresponding cyclic amine results in loss of antiproliferative activity,\(^{136}\) therefore another solution needs to be found.

Quinazolinones are important structural motifs present in a large number of pharmacologically diverse active compounds.\(^ {137}\) Of particular interest is 4(3\(H\))-dihydroquinazolinone 195, which has obvious structural similarity to the dihydroisoquinolinone motif of the natural products 7 and 8, as shown in Figure 4.01. Furthermore, the aniline \(N\) would be an effective bioisostere for the \(sp^2\)-hybridised C(10b) present in narciclasine, whilst a protonated analogue would generate a \(sp^3\)-hybridised tetrahedral arrangement seen in pancratistatin. The inclusion of a quinazolinone core would be of great interest, therefore N(10b) analogues were investigated.

![Figure 4.01: Similarity in structure of dihydroquinazolinone highlighted in the natural products](image)

4.2. Synthesis of quinazolinones

Due to the pharmacological importance of the quinazolinone building block, there are multiple procedures for their construction. Traditionally metal-catalysed condensation reactions between substituted anthranilamides and an aldehyde or ketone generated the desired bicyclic dihydroquinazolinone derivative.\(^ {138-140}\)

Recent developments in the field have shown that more facile reaction conditions can be used, often resulting in a higher yield, such as that shown in Scheme 4.01 reported by Shaabani \textit{et al.}\(^ {141}\)
The reaction conditions are mild, using catalytic NH₄Cl in EtOH at room temperature to facilitate the condensation reaction between anthranilamide 196 and a variety of alkyl, aryl and alicyclic aldehydes and ketones, which after intramolecular capture of the intermediate imine by the amide nitrogen, affords the dihydroquinazolinone derivative in high yield.

Scheme 4.01: One-pot click synthesis of 2-phenyl-2,3-dihydroquinazolin-4(1H)-one 197

We wished to employ a similar strategy to afford a C-ring, generating a fused B/C-ring system in the process. Despite no reports of this process in the literature, we postulated that a dialdehyde would initially undergo the transformation shown in Scheme 4.01 (condensation reaction, followed by intramolecular cyclisation of the amide). Then the remaining aldehyde could undergo another condensation reaction with the aniline nitrogen, affording the desired C-ring. A retrosynthetic approach is shown in Scheme 4.02 below, which represents a one-step synthesis of a tricyclic analogue 198.

Scheme 4.02: Proposed disconnections to achieve a fused cyclic B/C-ring

Using the reaction conditions specified by Shaabani et al., treatment of anthranilamide 196 with one equivalent of the symmetrical dialdehyde, glutaraldehyde 200, gave a mixture of compounds observed by crude ¹H NMR spectroscopic analysis with very little separation seen by TLC. Adjustment of the reaction time and temperature failed to improve the outcome, with a mixture of unidentifiable compounds being obtained. We proposed that these mixtures represented the various intermediates possible during the reaction mechanism. The proposed reaction between anthranilamide and glutaraldehyde to obtain the enamine ABC-ring analogue 198 is a reversible process, highlighted in Scheme 4.03. Hydrolysis is continually pushing the reaction towards the starting materials. In addition to the formation of hemi-aminals (R=H) from the reaction of water...
with iminium cations, it is also likely that reaction with the ethanol solvent would afford the corresponding hemi-aminal ethers (R=Et), which would further complicate the process. Furthermore, diastereomeric mixtures are also possible for these hemi-aminal intermediates. This complex equilibrium accounts for the multiple, unidentified products observed in the crude $^1$H NMR spectrum.

Furthermore, diastereomeric mixtures are also possible for these hemi-aminal intermediates. This complex equilibrium accounts for the multiple, unidentified products observed in the crude $^1$H NMR spectrum.

Scheme 4.03: Reversibility of the condensation reaction

Different optimisation conditions were made varying temperature and time which only afforded mixtures of compounds. These were combined and purified by column chromatography from which the expected enamine was isolated, suggesting that reaction fundamentally works and that further modifications should be investigated (Scheme 4.04). Trace amounts of a second compound was also isolated, with an indicative $^1$H NMR peak at approximately 6 ppm. This was proposed, and later identified, as being the hemi-aminal ether 202 which will be discussed further in Section 4.4.

Scheme 4.04: Isolated products from a combination of reactions
4.3. Optimisation of the reaction conditions

Optimisation of the reaction was required to avoid the numerous products and obtain the enamine 198 in good yield for further development. The ability of protic solvents to interact with the iminium cation could be avoided by using tetrahydrofuran (THF), a polar aprotic solvent. Magnesium sulfate (MgSO₄) was also added in order to remove the water generated in the condensation reaction, thereby driving the reaction towards the enamine 198. A standard aqueous work-up was maintained in order to remove any unreacted starting material (Scheme 4.05).

\[
\begin{align*}
\text{NH}_2 & \quad \text{Glutaraldehyde (1 eq.)} \\
\text{NH}_2 & \quad \text{NH}_4\text{Cl (50 mol %)} \\
\text{O} & \quad \text{MgSO}_4 (2.5 \text{ eq.}) \\
196 & \quad \text{THF, r.t., N}_2, 24 \text{ h} \\
& \quad \rightarrow \\
\text{NH} & \quad 198 \\
& \quad + \\
\text{HO} & \quad 203
\end{align*}
\]

Scheme 4.05: Synthesis of enamine 198 and proposed structure of compound 203

Analysis of the crude reaction mixture showed the major product to be enamine 198. Hemi-aminal 203 was also thought to be observed, presumably formed during the aqueous workup. Although the hemi-aminal 203 was never isolated, ¹H NMR spectroscopic analysis of the crude reaction mixture suggested C(1)H at approximately 6 ppm. This is consistent with that reported for similar hemi-aminals reported in the literature.¹⁴²,¹⁴³

Since we still obtained the hemi-aminal, we performed the reaction without the aqueous work-up. Instead, once the reaction was deemed complete by TLC, the MgSO₄ was simply removed by filtration and after removing the solvent the only product observed was the enamine product 198 in quantitative yield without the need for further purification (Scheme 4.06). This is an important result as it represents a one-step synthesis of the tricyclic core of the interesting N(10b) analogues in quantitative yield and furthermore provides a functionalised C-ring for further manipulation.

\[
\begin{align*}
\text{NH}_2 & \quad \text{Glutaraldehyde (1 eq.)} \\
\text{NH}_2 & \quad \text{NH}_4\text{Cl (50 mol %)} \\
\text{O} & \quad \text{MgSO}_4 (2.5 \text{ eq.}) \\
196 & \quad \text{THF, r.t., N}_2, 24 \text{ h} \\
& \quad \rightarrow \\
\text{NH} & \quad 198 \\
& \quad \text{Quantitative}
\end{align*}
\]

Scheme 4.06: One-pot procedure the synthesis of enamine 198
The proposed reaction mechanism is outlined in Scheme 4.07. Initial condensation of the aniline 196 with the carbonyl furnishes an iminium cation with the loss of water. The ensuing iminium cation 204 is captured by the amide group generating the B-ring. Further condensation between the remaining aldehyde and the basic nitrogen affords the C-ring and the initial iminium cation 201 rearranges to afford the observed enamine 198.

![Scheme 4.07: Proposed reaction mechanism for the tricyclic ring](image)

4.4. Ethanol adduct and pro-drug type analogues

Formation of the hemi-aminal ether 202 (Scheme 4.04), mentioned briefly in Section 4.2, is an interesting and possibly advantageous observation and could be used to create pro-drugs. Prodrugs, which have been described in Section 1.3.4, are a well-established method of delivering a drug like molecule into the target cell. The addition of an alcohol would provide the lipophilicity required to pass over the cell membrane followed by subsequent hydrolysis of the group leaving the active molecule to accumulate in the cytosol.

A method for improving the pharmacokinetic and pharmacodynamic profiles of therapeutic proteins is PEGylation and is reviewed by Harris and Chess. PEGylation is the process of adding polyethylene glycol to a candidate molecule reducing renal clearance, improving stability and
masking the potential toxicity of the parent compound, preventing the body from mounting an immune response. Although typically used in the delivery of peptides, PEGylated organic small molecules have also been developed. An opioid antagonist, Naloxegol, reduces the side effects of opioid treatment without interfering with pain relief. A PEGylated version of irinotecan, a topoisomerase I inhibitor anticancer agent is also in development. There is the potential for further improving the drug-likeness of the tricyclic series described here by PEGylation.

Identification of the hemi-aminal ether 202 was possible by $^1$H NMR spectroscopic analysis with a peak at approximately 6 ppm consistent with C(1)H joined to both O and N (Table 4.01, Entry 1). This in accordance with literature data for similar compounds. A large multiplet, integrating for 2H, at 3.56 ppm (Table 4.01, Entry 11) and a triplet, integrating for 3H, at 1.18 ppm (Table 4.01, Entry 12) suggest O(CH$_2$CH$_3$)$_2$ respectively. This is similar to that seen for ethanol.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Carbon</th>
<th>$^1$H Chemical Shift (ppm)</th>
<th>Integration</th>
<th>J (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>6.01</td>
<td>1H</td>
<td>t, J 3.0</td>
</tr>
<tr>
<td>2</td>
<td>2 and 3</td>
<td>2.02-1.88</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td>3</td>
<td>3 and 2</td>
<td>1.69-1.61</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1.87-1.76</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td>5</td>
<td>4a</td>
<td>5.10</td>
<td>1H</td>
<td>ddd, J 9.5, 4.0 and 1.0</td>
</tr>
<tr>
<td>6</td>
<td>5(N)</td>
<td>4.09</td>
<td>1H</td>
<td>brs</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>7.87</td>
<td>1H</td>
<td>dd, J 7.5 and 1.5</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>6.77</td>
<td>1H</td>
<td>ddd, J 8.0, 7.5 and 1.0</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>7.24</td>
<td>1H</td>
<td>ddd, J 8.0, 7.0 and 1.5</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>6.53</td>
<td>1H</td>
<td>dd, J 8.0 and 1.0</td>
</tr>
<tr>
<td>11</td>
<td>1’</td>
<td>3.56</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td>12</td>
<td>2’</td>
<td>1.18</td>
<td>3H</td>
<td>t, J 7.0</td>
</tr>
</tbody>
</table>

Table 4.01: $^1$H NMR assignment for ethanol adduct 202

It is of interest that the hemi-aminal ether 202 was isolated (in 6 % yield, see Section 4.9) as a single diastereoisomer and fully characterised. Owing to the planar nature of the iminium cation 201, ethanol could add from the $Re$ or $Si$ face (Scheme 4.08).
Scheme 4.08: Suggested structure of analogue 202

Attack from the Si face would afford a substituted cyclohexane derivative which would not expect to show a NOESY correlation of the $H^a$ and $H^b$ protons in either conformation (Scheme 4.08). Attack from the Re face would generate a 1,3-diaxial or 1,3-diequatorial cyclohexane derivative, which would mostly exist as the diequatorial species as it is lower in energy. This species would expect to give a strong NOESY correlation between the $H^a$ and $H^b$ protons. However, no correlation was observed by NOESY NMR spectroscopic analysis between C(1)H$^a$ and C(4a)H$^b$, suggesting that the ethanol adds to the least hindered Si face, affording the hemi-aminal ether 202 with high diastereoselectivity as the other epimer was never observed.

We proposed that using the conditions reported by Shaabani et al. (Scheme 4.01) and altering the protic solvent we could furnish the corresponding adduct analogue. Although the indicative peak at 6 ppm was observed using ethanol or methanol isolation of the products was not achieved and attempts at optimising the reaction conditions to favour adduct formation was found to be unpredictable. In addition, owing to the reversibility of the reaction, identified previously in Scheme 4.03, attempts to promote the formation of the iminium ion from the enamine with the addition of an acid resulted in decomposition. However, it was found that simply heating the enamine 198 at 60 °C in the corresponding alcohol overnight gave the best results, which are shown in Table 4.02.
as the ratio of starting material to hemi-aminal ether as determined by crude \(^1\)H NMR spectroscopic analysis. Unfortunately these compounds were unstable and decomposed upon purification and could not be isolated for further analysis.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Solvent pKa</th>
<th>Enamine 198</th>
<th>Hemi-aminal ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH</td>
<td>(\approx 15)</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>EtOH</td>
<td>(\approx 16)</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>(^1)PrOH</td>
<td>(\approx 16.5)</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4.02: \(^1\)H NMR ratio after the enamine 198 is stirred in protic solvent

Despite the decomposition observed during purification, the hemi-aminal methyl ether 205 was isolated (Scheme 4.09), albeit in trace amounts, which allowed for \(^13\)C and \(^1\)H NMR characterisation. The compound again had an indicative \(^1\)H NMR peak at approximately 6 ppm, with a methyl group also being identified at 3.36 ppm, shown in Table 4.03, Entry 1 and 14. The instability of this analogue is perhaps unsurprising as the pKa of methanol, Table 4.02, Entry 1, suggests that this adduct would be the least stable as it would be a better leaving group than either the ethanol or propanol (pKa 16 and 16.5 respectively, Table 4.02, Entry 2 and 3). However, it is the most abundant analogue by \(^1\)H NMR spectroscopic analysis. \(^1\)H NMR NOESY experiments again did not show a correlation between the \(H^a\) and \(H^b\) protons, suggesting the same diastereoisomer was obtained as that previously described.

![Scheme 4.09: Obtaining the methoxy analogue 205](image-url)
<table>
<thead>
<tr>
<th>Entry</th>
<th>Carbon</th>
<th>$^{13}$C Chemical Shift (ppm)</th>
<th>$^1$H Chemical Shift (ppm)</th>
<th>Integration</th>
<th>$J$ (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>80.2</td>
<td>5.91</td>
<td>1H</td>
<td>t, $J$ 3.0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>16.9</td>
<td>1.64-1.68</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>28.7</td>
<td>1.91-1.96</td>
<td>2H</td>
<td>m</td>
</tr>
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<td>4</td>
<td>33.4</td>
<td>1.79-1.87</td>
<td>2H</td>
<td>m</td>
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<td>4a</td>
<td>63.9</td>
<td>5.08</td>
<td>1H</td>
<td>ddd, $J$ 9.5, 4.0 and 1.0</td>
</tr>
<tr>
<td>6</td>
<td>5(N)</td>
<td>na</td>
<td>4.02</td>
<td>1H</td>
<td>brs</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>163.8</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>8</td>
<td>6a</td>
<td>114.9</td>
<td>na</td>
<td>na</td>
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<td>7</td>
<td>129.0</td>
<td>7.89</td>
<td>1H</td>
<td>dd, $J$ 8.0 and 1.5</td>
</tr>
<tr>
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<td>8</td>
<td>118.7</td>
<td>6.79</td>
<td>1H</td>
<td>td, $J$ 8.0 and 1.0</td>
</tr>
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<td>133.8</td>
<td>7.26</td>
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<td>td, $J$ 8.0 and 1.5</td>
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<td>10</td>
<td>113.6</td>
<td>6.54</td>
<td>1H</td>
<td>dt, $J$ 8.0 and 0.5</td>
</tr>
<tr>
<td>13</td>
<td>10a</td>
<td>145.8</td>
<td>na</td>
<td>na</td>
<td>na</td>
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<tr>
<td>14</td>
<td>1’</td>
<td>55.8</td>
<td>3.36</td>
<td>3H</td>
<td>s</td>
</tr>
</tbody>
</table>

Table 4.03: $^1$H and $^{13}$C NMR assignment for methoxy analogue 205

The instability of the hemi-aminal ethers prevented further investigations in this area and other avenues were pursued.

4.5. Attempted synthesis of vinyl dihydroquinazolinone analogues

We previously described the synthesis and biological activity of the allylated AB-ring analogues 126, 128 and 129. Following the success of the glutaraldehyde reaction, we believed that the corresponding allylated dihydroquinazolinone analogue 206 could be accessed using the reaction conditions previously described. This would provide an interesting comparison with the previously reported analogues, and would offer a substrate for further functionalisation (i.e. dihydroxylation) as previously described in Section 2.6.3 and shown below in Figure 4.02.
Initial investigations were undertaken using the commercially available acrolein in order to obtain a vinyl analogue. Using the original conditions as reported by Shaabani et al. for benzaldehyde,\textsuperscript{141} analogue 196 was treated with acrolein in ethanol in the presence of NH\textsubscript{4}Cl. However, the reaction failed to give the expected product 207 and only generated an unidentified precipitate which was insoluble in a range of different solvents. Even using the optimised conditions, which gave the enamine in quantitative yield, failed to furnish the desired product and gave a mixture of unidentified compounds, including the insoluble precipitate (Scheme 4.10).

Presumably the enone present in acrolein is too reactive, leading to the formation of many by-products and polymers. In addition, there are competing reaction pathways to the desired condensation reaction, such as 1,4-conjugate addition. Due to these issues, and the success in obtaining the enamine 198 from glutaraldehyde, we investigated the scope and limitations of this reaction. We examined i) functionalisation of the enamine 198; ii) other anthranilamide derivatives and iii) other aldehyde substrates, the results of which are now described.

4.6. Attempted functionalisation of the Enamine

With ready access to the enamine 198 in a single step, we investigated the further functionalisation of the C-ring which could provide novel narciclasine analogues and allow us to explore the effects of C-ring substitutions on biological activity. A number of procedures are described below.
The formation of an epoxide is a well-established method of adding an oxygen atom to an olefin. The peracid meta-chloroperoxybenzoic acid (mCPBA) is a commonly used reagent, providing an electrophilic source of oxygen to react with the nucleophilic olefin. Using standard procedures the enamine was stirred in CH₂Cl₂ at 0 °C under argon, after which mCPBA was added step-wise and the reaction warmed to room temperature. The reaction was followed by TLC until the enamine 198 was consumed. Crude ¹H NMR spectroscopic analysis showed only a complicated mixture of compounds which could not subsequently be separated and identified. Possible degradation of the compounds was thought to be occurring due to the benzoic acid generated in situ. A buffer was therefore added to the system, following the procedure reported for the carvone analogue 208 in Scheme 4.11.¹⁴⁸ In a model system using carvone the reaction was successful. Unfortunately using enamine 198 resulted in unidentified materials, so alternative methods for the addition of oxygen were sought.

Scheme 4.11: Epoxidation of the nucleophilic alkene of carvone analogue 208¹⁴⁸

A procedure described by Botman et al., shown in Scheme 4.12, shows the oxidation of enamine 210 using oxone, introducing α-methoxy and β-hydroxy substituents in one-step.¹⁴⁹ The reaction proceeds via an unstable epoxide intermediate which is opened immediately by the methanol to give analogue 211.¹⁵⁰ Subsequent removal of the α-methoxy moiety by acid hydrolysis gave compound 212 in high yields. Following the reported procedure with our substrate 198, crude ¹H NMR spectroscopic analysis suggests the possibility of the methanol adduct, with a peak at approximately 6 ppm described previously (Section 4.4). Unfortunately, purification of the products caused degradation, leaving only a mixture of unidentified compounds.

Scheme 4.12: Reported functionalisation of an enamine 210 using oxone¹⁴⁹
Acetylation of oxidised isoquinolinones was achieved using acetic anhydride and sulfuric acid,\textsuperscript{89} as described in Section 2.6.1 and shown in Scheme 4.13. Likewise, the enamine \textbf{198} provides a nucleophilic olefin which could react in a similar fashion, providing an acetylated analogue. Although initial investigations were positive, attempts at optimisation of this reaction and subsequent isolation of compounds were not achieved, giving only a mixture of unidentifiable compounds.

\[
\begin{align*}
\text{Scheme 4.13: Acetylation of lactam 83, described in Section 2.6.1} \\
\text{ Acetates of oxidised isoquinolines was achieved using acetic anhydride and sulfuric acid, as described in Section 2.6.1 and shown in Scheme 4.13. Likewise, the enamine 198 provides a nucleophilic olefin which could react in a similar fashion, providing an acetylated analogue. Although initial investigations were positive, attempts at optimisation of this reaction and subsequent isolation of compounds were not achieved, giving only a mixture of unidentifiable compounds.}
\end{align*}
\]

The use of ruthenium chloride has previously been described in Section 2.6.2.2 for oxidation of a double bond. Following on from this success, we wished to examine the dihydroxylation of the enamine using this methodology. Indeed, this method had been used to achieve \textit{cis}-dihydroxylation in the total synthesis of narciclasine \textbf{7} reported by Hudlicky \textit{et al.} in 2002 and shown in Scheme 4.14.\textsuperscript{151}

\[
\begin{align*}
\text{Scheme 4.14: Cis-dihydroxylation using catalytic RuO}_4\textsuperscript{151} \\
\text{Owing to the previous success in isolation of the dione 117 (Section 2.6.2.2), the enamine 198 was treated with an aqueous solution of RuCl}_3\cdot\text{H}_2\text{O (0.4 mol %) and NaI}_4\text{aq} (5 eq.) in EtOAc at room temperature. The reaction was followed by TLC until the starting material had been consumed. As previously reported, 1PrOH was added and the reaction stirred for a further 3 hours in order to neutralise the active RuO}_4\text{ generated \textit{in situ}. Crude }^1\text{H NMR spectroscopic analysis suggested one major product, although mass recovery was very poor, and was initially assigned as trione 215, Scheme 4.15. This initial assignment could not be confirmed by }^{13}\text{C NMR spectroscopic analysis due to low yield so further investigations were undertaken.}
\end{align*}
\]
Scheme 4.15: Initial assignment of product 215 based on $^1$H NMR spectroscopic analysis

Oxidation reactions involving RuO$_4$ are known to be difficult to control and it was hoped that limiting the amount of oxidant available would prevent over-oxidation. Repeating the reaction using 1 eq. NaIO$_4$(aq) rather than 5 eq., allowed for new compounds to be observed. Very little separation was observed by TLC and isolation was not achieved, however mass spectrometry of the crude products suggested the presence of the compounds shown in Figure 4.03 amongst others.

![Figure 4.03: Suggested compounds extracted from the oxidation reaction with RuO$_4$](image)

The mixture of products was stirred with the mild oxidising agent manganese dioxide (MnO$_2$) in order to further understand what compounds had been synthesised. Following purification by column chromatography, the bislactam 216 shown below in Scheme 4.16 was the sole product isolated in 54 % yield over the two-steps. The bislactam 216 was fully assigned and was the actual compound obtained in Scheme 4.15 as the $^{13}$C NMR clearly shows 11 carbons, highlighting the loss of one carbon from the enamine 198. This then suggests the loss of CO$_2$ during the reaction mechanism and a plausible pathway is shown in Scheme 4.16 below. Oxidative cleavage of the double bond reveals a carbamate, which can undergo decarboxylation leaving an aniline which undergoes lactamisation. This is supported by research undertaken by Lu et al. for a similar compound. Omitting MnO$_2$ led to a mixture of compounds from which the bislactam was isolated in a reduced 26 % yield.
Sodium borohydride (NaBH₄) is a ubiquitous reducing agent typically used for the reduction of aldehydes and ketones to their corresponding alcohols. A more aggressive agent is lithium aluminium hydride which will also reduce amides and lactams. Preliminary investigations were carried out in order to reduce the tertiary amide present in bislactam 216 over the secondary amide using NaBH₄, as reported in the literature. Based on these reported conditions, the tricyclic analogue 216 was treated with NaBH₄ in methanol under nitrogen (Scheme 4.17, the numbering for the product has been kept consistent with analogue 216). The reaction was followed by TLC analysis until the starting material had been consumed.

Crude ¹H NMR spectroscopic analysis showed a single compound which was isolated in quantitative yield. Characteristic carbon peaks are outlined in Table 4.04. It is suggested that the compound is the fused [6, 9] bicyclic ring 217, shown in Scheme 4.17 as it is missing the C(3a) peak at 6.31 ppm.
Instead, there are two triplets, both integrating for 2H, at 2.70 and 3.53 ppm suggesting C(2) (CH$_2$C=O) and C(4) (NCH$_2$) respectively due to their respective chemical shifts and the strong COSY between C(4) and the N(5)H, (Table 4.04, Entry 2 and 4). The 2H multiplet at 1.90-1.97 ppm equates to C(3) (Entry 3) and has a strong COSY with both C(2) and C(4). Unfortunately a lack of material meant that $^{13}$C NMR could not be obtained, however the structure is consistent with HRMS analysis. The reaction was performed on larger scale, but regrettably starting material persisted. Isolation was therefore not possible due to co-eluting of the starting material with the product.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Carbon</th>
<th>$^1$H Chemical Shift (ppm)</th>
<th>Integration</th>
<th>J (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2.70</td>
<td>2H</td>
<td>t, J 7.5</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1.90-1.97</td>
<td>2H</td>
<td>m</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3.53</td>
<td>2H</td>
<td>t, J 6.5</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>7</td>
<td>6a</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>8.13</td>
<td>1H</td>
<td>ddd, J 8.0, 1.5 and 0.5</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>7.51</td>
<td>1H</td>
<td>ddd, J 8.0, 7.0 and 1.0</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>7.82</td>
<td>1H</td>
<td>ddd, J 8.0, 7.0 and 1.5</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>7.64</td>
<td>1H</td>
<td>d, J 7.5</td>
</tr>
</tbody>
</table>

Table 4.04: $^1$H NMR assignment for [6, 9] fused bicyclic ring 217

This result can be explained by appreciating that the C(3a) carbon (highlighted in Scheme 4.17) is at the +2 oxidation level (c.f. aldehydes and ketones) and is therefore susceptible to reduction by NaBH$_4$. This removes B/C-ring junction leaving an expanded 9-membered ring.

In order to ascertain what types of reagent might be used to successfully functionalise the enamine 198 without causing decomposition, stability tests were performed. Enamine 198 was stirred in a 1:1 mixture of THF and either water, NaOH$_{aq}$ or HCl$_{aq}$ at room temperature. TLC analysis was done over a 24 hour period and in each case decomposition of the enamine occurred which was confirmed by crude $^1$H NMR spectroscopic analysis. This study then explains some of the problems encountered in the reactions described above, since the enamine is not compatible with aqueous systems. Future efforts to functionalise the enamine will therefore use non-aqueous reaction conditions.

- 93 -
4.7. Synthesis of A-ring analogues

As described in Section 1.3.3.1, the presence of a free hydroxyl group at C(7) on the A-ring is paramount in ensuring the anticancer activity of the narciclasine analogues. Thus far a series of unsubstituted A-ring compounds have been examined, containing the additional nitrogen at the B/C-ring junction. Using substituted anthranilamides as starting materials will furnish the corresponding A-ring analogues.

We previously investigated dimethoxy and trimethoxy analogues and we wished to examine the corresponding substituted quinazolinones 218 and 220, shown in Scheme 4.18. The benzamide starting materials 219 and 221 for each target compound is not commercially available, however, the corresponding carboxylic acid and ester of the dimethoxy derivatives are and due to the ubiquitous nature of the amide group in medicinal products, as well as biological processes, there are a range of conditions and strategies towards making this important bond.

![Scheme 4.18: Proposed disconnection to achieving hydroxylated A-ring analogues](image)

### 4.7.1. Attempted synthesis of 2-amino-4,5-dimethoxybenzamide from 2-amino-4,5-dimethoxybenzoate

Published procedures describe the direct conversion of the readily available and inexpensive 2-amino-4,5-dimethoxybenzoate 222 to the 2-amino-4,5-dimethoxybenzamide 219, shown in Scheme 4.19. Typical reported conditions describe the ester reacting with ammonia water in a protic solvent over a number of days or at elevated temperatures under pressure. Unfortunately attempts at reproducing the results obtained were unsuccessful, with starting material being recovered in quantitative yield.

![Scheme 4.19: Proposed conversion of the dimethoxy ester 222 to an amide 219](image)
Other literature reports describe a two-step process initially using hydroxylamine or hydrazine to access the corresponding benzamide \( \text{219} \),\(^{156-158}\) as these are more nucleophilic owing to the \( \alpha \)-effect, shown in Scheme 4.20. A second step is then required to cleave the N-N or N-O bond to reveal the desired amide.

![Scheme 4.20: Proposed synthetic route to benzamide 219](image)

From the literature,\(^ {159} \) aminobenzoate \( \text{223} \) was treated with \( \text{NaOH}_\text{aq} \) and \( \text{NH}_2\text{OH-HCl} \), MeOH was added dropwise until all of the constituents were in solution and the reaction was left to stir for 5 days to afford the hydroxylamine product \( \text{224} \) in 80 % yield (Scheme 4.21). However, when this method was applied to the dimethoxy derivative \( \text{222} \), only starting material was recovered. A similar method and transformation was published using \( \text{KOH} \),\(^ {160} \) however when the base was changed to \( \text{KOH} \) and the reaction even heated to reflux, only recovered starting material was obtained.

![Scheme 4.21: Reported reaction with hydroxylamine\(^ {159} \)](image)

A procedure was reported for a similar transformation of benzoate \( \text{225} \) using hydrazine in ethanol at reflux by Kuemmerle et al., shown in Scheme 4.22\(^ {161} \) Again, when dimethoxy ester \( \text{222} \) was subjected to these conditions only unreacted starting material was obtained with good mass recovery.
It was then concluded that the ortho-amino group might be causing interference, preventing the reaction from occurring. Protection of the aniline was therefore examined to observe if this had any effect. Using a well-established procedure, the aniline was acetylated with acetyl chloride and Na₂CO₃ in chloroform (Scheme 4.23). The acetylated product 227 was isolated in 99 % yield and spectroscopic data was consistent with that previously reported for this compound.

The protected aniline 227 was subjected to the reaction conditions described previously with moderate success. Crude ¹H NMR spectroscopic analysis of the reaction with NH₂OH showed a mixture of compounds, including unreacted starting material, the acid and the product (Figure 4.04). These results demonstrated that the unprotected ortho-amino group did prevent the desired reaction, so instead of examining a protecting group strategy and optimising these reaction conditions, we investigated an alternative synthetic route of the desired amides from the corresponding carboxylic acids.

**Scheme 4.22**: Reaction with hydrazine reported by Kuemmerle *et al.*

**Scheme 4.23**: Protecting the amine with acetyl chloride

**Figure 4.04**: Percentage ratio ascertained by crude ¹H NMR spectroscopic analysis
4.7.2. Synthesis of 2-amino-4,5-dimethoxybenzamide from 2-amino-4,5-dimethoxybenzoic acid

Carboxylic acids, rather than esters, are primarily used in the standard approaches to amides, many of which are reviewed by Montalbetti et al. Since conversion of the ester to the corresponding amide was problematic, we investigated the transformation of the 2-amino-4,5-dimethoxybenzoic acid instead, which is also commercially available.

Upon mixing an acid with an amine the stable salt is first to form meaning the coupling of these functional groups has to overcome adverse thermodynamics, the equilibrium of which is depicted in Scheme 4.24. The barriers to this reaction can be overcome at elevated temperature; however this risks denaturing of the rest of the molecule involved.

\[
\text{RCOOH} + \text{R'NH}_2 \rightleftharpoons \text{RCOO}^- + \text{R'NH}_3^+ \xrightarrow{\text{x}} \text{RCONHR'} + \text{H}_2\text{O}
\]

Scheme 4.24: Equilibrium of amide formation

Activation of the acid can be achieved by a variety of methods. 1,1'-Carbonyldiimidazole (CDI) was chosen as the reaction is a well-documented one-pot procedure, giving only imidazole and carbon dioxide as by-products. The initial reaction between the carboxylic acid and CDI forms the activated ester which is susceptible to nucleophilic attack by the ammonia generating the amide with the release of imidazole (Scheme 4.25).

Scheme 4.25: Summary mechanism of the CDI reaction

Substituted 2-amino benzoic acids were treated with CDI and NH₃(aq) and gave the desired benzamide products with isolated yields shown in Figure 4.05. It is worthy of note that the crude reaction mixtures were simply washed with base to remove any unreacted starting material and then washed with iron(II)sulfate (FeSO₄) solution, which complexes the imidazole and removes it.
The products were therefore obtained analytically pure after aqueous washes without the need for further purification.

Figure 4.05: Benzamide analogues obtained using CDI followed by aqueous washes

4.7.3. Proposed synthesis of 6-amino-2,3,4-trimethoxybenzamide

As previously described, narciclasine 7 and pancratistatin 8 analogues with a C(7)OH group have consistently been shown to have superior anticancer activity. We envisioned obtaining the corresponding free phenol 236 from the trimethoxy derivative 220, Scheme 4.26. Based on our previous observations, BF₃·OEt₃ mediated intramolecular Friedel-Crafts acylation with trimethoxy derivatives led to concurrent selective demethylation at the C(7) position. We therefore expected that treatment of the trimethoxy-quinazolinone derivative 220 with BF₃·OEt₃ would result in selective demethylation and afford the desired analogue 236. The construction of 6-amino-2,3,4-trimethoxybenzamide 221 from commercially available 3,4,5-trimethoxyaniline 237 is suggested in Scheme 4.26.

Scheme 4.26: Proposed disconnection in the synthesis of trimethoxybenzamide 236

One potential synthetic route would involve installing a bromine group at the 2 position, followed by treatment with butyl lithium and dry ice to afford the benzoic acid. Finally the amide could be synthesised as previously described. However, this is a multi-step procedure using harsh reaction conditions.

There is growing interest in accessing benzoic acids via an istatin intermediate. A successful strategy reported by Zhang et al. for a similar compound describes ortho carboxylation of the 3,5-dimethoxyaniline salt 238, shown in Scheme 4.27. This transformation has also been reported by
Newman et al. with the suggestion that the reaction proceeds via the oxamyl chloride intermediate \(239\).\(^{166}\) The subsequent intramolecular acylation occurs \textit{ortho} to the amino group without the need for a Lewis acid. This is thought to be due to the \textit{ortho} and \textit{para} activating properties of the methoxy groups, directing the electrophilic attack. Ring opening then occurs with hydrogen peroxide and concentrated sodium hydroxide to get analogue \(241\).

\[
\begin{align*}
\text{O} \quad \text{NH}_2 \cdot \text{HCl} & \quad \underbrace{\text{(COCl)}_2}_{\text{Ar}, 165 \, ^\circ\text{C}, 30 \, \text{min}} & \quad \text{O} \quad \text{NaOH}_{(aq)} \cdot \text{H}_2\text{O}_2 \\
\text{238} & \quad \text{240} & \quad \text{241}
\end{align*}
\]

\textbf{Scheme 4.27: Synthesis of 3,5-dimethoxyanthranilic acid 241}\(^{165,166}\)

Although the procedure required heating compound \(238\) in oxalyl chloride to 165 °C initial attempts were carried out at reflux, 65 °C, as no specialist equipment was mentioned in the experimental procedure.\(^{165}\) In addition, no advantage was apparent for using the salt form of the aniline therefore the free base \(237\) was used.

Following the literature, oxalyl chloride (4 eq.) was added to trimethoxyaniline \(237\) at room temperature under argon forming an insoluble mass of black material which persisted upon heating. Excess oxalyl chloride was therefore required in order to achieve solvation. Upon quenching with methanol, product \(242\) was observed by crude \(^1\text{H}\) NMR spectroscopic analysis, suggesting that cyclisation had not occurred (Scheme 4.28). This was further confirmed by mass spectrometry, with no istatin being observed. Isolation was not attempted due to co-eluting with other compounds in the crude reaction mixture.
Although the published experimental for 3,5-dimethoxyaniline 238 suggested the reaction might proceed without the need for a Lewis acid, other reports suggest a Lewis acid is required. Conditions reported by Browne et al. showed the successful formation of istatin 244 using aluminium chloride (AlCl_3), shown in Scheme 4.29. They describe the aniline 243 treated with oxalyl chloride in CH_2Cl_2 at reflux. After 1 hour, the removal of residual oxalyl chloride and treatment with AlCl_3 facilitated the intramolecular cyclisation and gave the product 244 in 70 % yield. Similar conditions were reported by Shindikar who also described the isolation of the ensuing oxamyl analogue 246 prior to Lewis acid mediated acylation. Unfortunately, only a black, insoluble mass was recovered using these procedures with trimethoxyaniline 237, which could not be separated.

A procedure reported for a similar compound, shown in Scheme 4.30, reported by Kaila et al. described the dropwise addition of oxalyl chloride to a solution of 3-methoxyaniline 248 in benzene. Following heating to reflux, the reaction was then cooled to 0 °C, AlCl_3 was added and the reaction
warmed to room temperature for a further 3 hours. However, when these reaction conditions were applied to trimethoxyaniline they were again unsuccessful, with a black mass of unidentifiable materials being recovered.

Scheme 4.30: Procedure demonstrated by Kaila et al.

The initial methodology was revisited, given the problems with using the Lewis acid mediated reactions. A procedure reported by Kallander et al. for the same compound describes the successful conversion of trimethoxyaniline 237, shown in scheme 4.31, to the benzoic acid. The HCl salt 251 was obtained by treating a solution of trimethoxyaniline 237 in diethyl ether with HCl$_{\text{aq}}$. The product 251 was confirmed by an increased melting point, from 109-110 °C for the starting material 237 to 256-260 °C for the HCl salt, which was isolated as a grey solid without further purification. Using a 0.26 mmol scale, the aniline salt was then stirred in oxalyl chloride at 170 °C in a sealed thick-walled pressure tube for 50 minutes. After cooling to room temperature the reaction was quenched with methanol and refluxed for 10 minutes. Following column chromatography the desired product 252 was finally isolated in 62 % yield.

Scheme 4.31: Formation of the trimethoxyistatin analogue 252

This small amount was subjected to the reported conditions for ring opening, shown in Scheme 4.27. Although this appeared a robust procedure, only a mixture of unidentifiable compounds were unfortunately recovered. In an effort to synthesise a viable amount of the trimethoxyistatin compound 252 for further investigations, the reaction was repeated on a 10 mmol scale. Although the formation of the hydrochloride salt was more successful (90 % yield without further purification) reaction with oxalyl chloride on a 5 mmol scale did not give the expected istatin 252 as previously obtained. Upon purification, $^1$H NMR and HRMS data was consistent with structure 253 shown in Figure 4.06. Attempts at optimising the reaction proved to
be unsuccessful and we summarised that this reaction was capricious. We therefore investigated the reaction of substituted 2-amino benzamides already obtained with glutaraldehyde.

![Structure of compound 253](image)

**Figure 4.06: Structure of compound 253**

### 4.8. Tricyclic substituted A-ring analogues

With the methyl substituted anthranilamide analogues in hand and using the optimised conditions previously described in Section 4.3, cyclisation was investigated with glutaraldehyde. Unfortunately, the reaction to form the enamine with these substituted derivatives did not proceed as efficiently as previously described. Crude $^1$H NMR spectroscopic analysis showed predominantly the expected products, however there were also unidentified contaminants within the reaction mixture. These contaminants could not be removed by column chromatography, as the enamine simply decomposed. Efforts to optimise these reaction conditions failed to eliminate the by-products, so the enamine intermediates were therefore used as crude for subsequent reactions.

It is worthy of note that when dimethoxy anthranilamide 219 was subjected to the reaction shown in Scheme 4.06, only a complex mixture of products was obtained, with no enamine identified. Given the comparative success of the methyl derivatives, this is presumably due to the electron donating properties of the dimethoxy groups affecting the condensation-cyclisation cascade reaction described in Scheme 4.07.

### 4.9. Reduction of the enamine by Pd/C

Due to the instability of the enamine analogues, reduction of the double bond was considered in order to obtain the stable cyclic amine analogues and evaluate the potential benefit of the additional nitrogen in comparison to similar late stage intermediates already synthesised.

Using standard procedures for hydrogenation, mentioned in Section 3.5.1, the intermediate enamine 198 and Pd/C (10 % wt.) were stirred vigorously in EtOH and exposed to an atmosphere
of H₂ for 16 hours. Upon filtering and purification compound 254 was isolated in 39 % yield (Scheme 4.32).

Scheme 4.32: Hydrogenation using Pd/C and atmospheric H₂ gas

Interestingly, the hemi-aminal ethyl ether 202 was also isolated in 6 % yield suggesting that the solvent reacted with the enamine, via the iminium ion, as previously described in Section 4.4 (Scheme 4.08).

An interesting observation was made of the crude ¹H NMR spectrum. In addition to the main cyclic amine product 254 and hemi-aminal ethyl ether 202, another by-product was observed, approximately 4 % by crude ¹H NMR spectroscopic analysis. This revealed triplets at 3 and 4 ppm and it was postulated that this was the [6-10] fused bicyclic analogue 255, shown in Scheme 4.33, formed by the reduction of the C(4a) carbon as previously observed in Scheme 4.17. Data reported for a similar [6-10] fused bicyclic compound are consistent, with reported triplets at 4.44 ppm and 2.86 ppm.¹⁷³ Unfortunately this by-product could not be isolated due to the small mass and attempts at optimising the reaction to favour its formation failed.

Scheme 4.33: Potential reaction mechanism

Performing the condensation-cyclisation reaction of the 3, 4 and 5-methyl 2-amino-benzamides with glutaraldehyde, and subsequent hydrogenation of the enamine intermediate, was largely
successful. With 4-methyl 2-amino-benzamide, in addition to the desired product 257 in 61 % yield, the ethanol adduct 256 was also isolated in 24 % yield, (Scheme 4.34), with $^1$H NMR spectroscopic data being consistent with that reported previously for compound 202 discussed in Section 4.4. Unfortunately decomposition of this unstable hemi-aminal ethyl ether occurred before full characterisation could be achieved.

Scheme 4.34: Product 257 isolated following the hydrogenation of the corresponding enamine

With 5-methyl 2-amino-benzamide, only the corresponding saturated tricyclic analogue 258 was obtained in 31 % yield over the two-steps without the ethanol adduct being observed (Scheme 4.35).

Scheme 4.35: Product 258 isolated following the hydrogenation of the corresponding enamine

Interestingly, when the reaction was performed with 3-methyl 2-amino-benzamide, multiple products were observed, shown in Scheme 4.36. Purification by column chromatography and NMR spectroscopic analysis of the isolated products allowed us to identify that the major component was in fact the imine-amide 260, which was isolated in 40 % yield.

Scheme 4.36: Two-step reaction for the formation of 261 (not isolated)
Palladium is a well-known hydrogen transfer agent and, as previously described in Section 2.3.2, the dehydrogenation of isoquinolinones using Pd/C has been reported, usually at elevated temperatures under an inert atmosphere.\textsuperscript{75} The attempted reduction occurs at room temperature, however the presence of imine-amide \textsuperscript{260} suggests that dehydrogenation at C(4a)/N (to generate the imine) occurs prior to hydrogenation at C(1)/C(2) (the enamine). If the enamine reduced first, we would have expected to obtain some of the expected analogue \textsuperscript{261}, which we do not observe. This is perhaps due to steric interference from the methyl group causing hydrogenation of the enamine to occur slowly over time, with competing dehydrogenation occurring preferentially. Leaving the reaction longer, for 24 hours, did not afford any of the expected lactam product \textsuperscript{261} and instead led to an increased yield of the imine-amide \textsuperscript{260} to 62\%. This is an interesting observation, and together with previous results, demonstrates the sensitivity of the reaction to both steric and electronic factors.

The formation of an enamine, which is then reduced, can be achieved in a single step using sodium cyanoborohydride (NaBH\textsubscript{3}CN), which was also investigated and is now described.

4.10. Reduction by NaBH\textsubscript{3}CN

Sodium cyanoborohydride is a mild reducing agent used in the reduction of imines. The electron withdrawing properties of the cyano group lessen the nucleophilicity of the remaining hydride. This reagent will reduce an iminium cation, preventing the formation of the enamine. Grigg et al. demonstrates the cyclisation of \textsuperscript{262} and subsequent reduction of the ensuing iminium cation \textsuperscript{263} to form bicyclic product \textsuperscript{264} in 89\% yield using NaBH\textsubscript{3}CN, Scheme 4.37.\textsuperscript{174}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {262};
\node (b) at (2,0) {NaBH\textsubscript{3}CN};
\node (c) at (4,0) {HCl, THF, r.t., 24 h};
\node (d) at (6,0) {263};
\node (e) at (8,0) {264};
\node (f) at (10,0) {89\%};
\draw[->] (a) -- (b);
\draw[->] (b) -- (c);
\draw[->] (c) -- (d);
\end{tikzpicture}
\end{center}

\textbf{Scheme 4.37:} Cyclisation and subsequent iminium reduction by NaBH\textsubscript{3}CN\textsuperscript{174}

Using modifications of these reported conditions, optimisation of the reaction conditions was undertaken on anthranilamide \textsuperscript{196}. Anthranilamide \textsuperscript{196} and glutaraldehyde \textsuperscript{200} were stirred in methanol with NH\textsubscript{4}Cl. NaBH\textsubscript{3}CN was added step-wise at room temperature and the reaction stirred for 6 hours. Trace amounts of the reduced product \textsuperscript{254} were recovered, however analogue \textsuperscript{265} was
isolated in 5 % yield, shown in Scheme 4.38, with the rest of the mass being a mixture of unidentifiable products.

Scheme 4.38: Using NaBH₃CN for the synthesis of tricyclic analogue 254

The isolation of the piperidine 265 suggested that the reaction initially proceeded as previously discussed (Section 4.3) however following the initial condensation reaction, the ensuing imine is reduced by the NaBH₃CN before intramolecular cyclisation can occur with the amide (Scheme 4.39). A second condensation and subsequent reduction then affords the observed analogue 265. Allowing for enamine formation prior to the addition of NaBH₃CN should allow for the synthesis of tricyclic analogue 254.

Scheme 4.39: Reduction of the iminium cation by NaBH₃CN

This is supported by conditions reported by Nacro et al. which show a similar step-wise transformation, outlined in Scheme 4.40. Condensation of acetaldehyde with aniline occurs first, followed by the reduction of the ensuing imine by NaBH₃CN to afford compound 267.
The general procedure for the synthesis of enamine 198 was followed, omitting the addition of MgSO₄, and the reaction was monitored by TLC analysis until the starting material was consumed. Methanol was added to the reaction mixture and the temperature reduced to 0 °C. NaBH₃CN was added step-wise, followed by acetic acid until the pH was less than 7 (Scheme 4.41). After stirring at room temperature for 4 hours the reaction was quenched with hydrogen peroxide and ferrous sulfate. Pleasingly, crude ¹H NMR spectroscopic analysis showed mostly the desired product 254 which was isolated in 55 % yield following column chromatography.

Using these optimised one-step conditions, 3-methyl 2-amino-benzamide 233 was investigated. As previously noted, this substrate failed to furnish the desired compound with Pd/C presumably due to steric clashes, which was replicated here as no product was obtained.

4.11. Functionalisation of the enamine by ruthenium

As previously described, treatment of the unsubstituted enamine with RuCl₃·xH₂O and NaIO₄ followed by MnO₂ led to oxidative cleavage, decarboxylation and lactamisation, and gave the corresponding bislactam 216 in 54% yield (Scheme 4.16). Conducting the reaction without MnO₂ led to a reduced yield of 26 %. It is pleasing to note that using either 3, or 4-methyl 2-amino benzamide, 233 and 234, as starting materials, even without MnO₂, gave the corresponding bislactams 268 and 269 in 33 % and 26 % respectively (Scheme 4.42).
Using the novel synthetic approach to quinazolinone analogues of narciclasine and pancratistatin described for the first time here, retrosynthetic analysis of the natural products shows that sugars are a prime candidate to provide hydroxyl groups for the C-ring. This is a major breakthrough, as not only do sugars provide the alcohol groups, they are provided as single enantiomers which are known. Furthermore, a large number of sugars are available to enable a comprehensive structure-activity relationship to be built. This would open up a new avenue for investigation and biological evaluation and has the potential to provide narciclasine 7 and pancratistatin 8 analogues complete with hydroxylated C-ring as single enantiomers in one- or two-steps, Scheme 4.43.

Initially condensation of the anthranilamide analogue 272 with the aldehyde of the sugar will give intermediate 271, subsequent oxidation or activation of the primary alcohol will allow for a second cyclisation and C-ring formation, analogue 270. D- and L-lyxose were initially chosen as they exhibit the same and reversed stereochemistry of the three hydroxyl groups present in the C-ring of narciclasine 7, shown in Scheme 4.44. This would provide an interesting comparison with the anticancer activity of the natural product. Preliminary investigations were undertaken with the economical D-lyxose, as L-lyxose is more expensive.
Using the initial reaction conditions reported by Shaabani et al. (described in Section 4.2),\textsuperscript{141} anthranilamide and D-lyxose were stirred in ethanol at room temperature open to the atmosphere. NH$_4$Cl (50 mol %) was added and the reaction initially stirred at room temperature. Upon monitoring by TLC analysis for a number of hours no reaction was observed therefore the reaction was heated to 60 °C overnight. A precipitate ensued, which upon filtering was found to be pure quinazolinone 273 in 33 % yield, which did not require further purification (Scheme 4.45). $^1$H NMR spectroscopic analysis of the crude filtrate showed a mixture of compounds but was not analysed further as no product 273 was observed.

Scheme 4.44: Stereochemistry of D- and L-lyxose and possible analogues

An interesting intermediate was isolated during attempts at optimising the reaction conditions, as shown in Scheme 4.46. Following the same method, but without the addition of NH$_4$Cl, gave a precipitate which once filtered and analysed was found to be analogue 274 in 26 % yield. Heating the reaction at reflux increased the yield to 89 %. Adding less than 100 mol % of NH$_4$Cl gave solely product 274.

Scheme 4.45: Reaction of anthranilamide with D-lyxose

- 109 -
In addition, using the conditions specified in Scheme 4.45 without the addition of an air condenser again gave exclusively product 274. It was postulated that cyclised analogue 274 was an intermediate to straight chained analogue 273 and that analogue 274 was slightly less soluble and therefore precipitated first, preventing formation of the fused AB-ring. Decreasing the concentration of the reaction by increasing the proportion of solvent failed to furnish consistent results and was found to be highly dependent on the evaporation of ethanol. Conducting the reaction at lower temperatures in order to prevent evaporation again failed to give analogue 273 as did increasing the amount of NH₄Cl to 150 mol % or increasing the temperature.

As previously mentioned it was suggested that analogue 274 was an intermediate to the fused bicyclic analogue 273. Indeed, stirring cyclised 274 in ethanol with NH₄Cl at 60 °C for a further 20 hours (Scheme 4.47) produced a mixture of analogue 273 and analogue 274 of 37 to 63 respectively by ^1H NMR spectroscopic analysis. Solubility was an issue so methanol was added until solvation was achieved.

The reaction was therefore found to be highly dependent on solvent. Better solubility was achieved using methanol (rather than ethanol) and analogue 273 was successfully, and consistently, isolated in 40 % yield.
This improved methodology was repeated with L-lyxose and gave similar results using methanol to gain the AB-ring analogue 275 in 39 % yield, shown in Figure 4.07. Conducting the same reaction using ethanol as the solvent without NH₄Cl instead gave the intermediate analogue 276 in 40 % yield, shown in Figure 4.07. These results were not optimised due to the limited availability of the sugar.

![Figure 4.07: Optimised yields of analogues 275 and 276](image)

The stereochemistry of the newly formed stereogenic center of these sugar derivatives was not assigned. Our next step was to investigate formation of the C-ring, which would afford a rigid structure from which it was hoped the stereochemistry could be assigned based on the coupling with the known adjacent stereocenter.

Other sugars were subjected to these optimised conditions, with different patterns of stereochemistry, such as galactose, glucose and ribose. Regrettably these sugars made a resinous substance which was difficult to manipulate and a crude ¹H NMR spectroscopic analysis was not possible on all but the ribose analogue. This suggested that the ribose straight chain analogue had been synthesised, although it was contaminated with other compounds. Efforts to gain pure compound were not attempted due to time constraints on the project.

4.13. **Cyclisation of the sugar side-chain**

Preliminary investigations were undertaken into the cyclisation of the sugar side-chain with two proposed routes, shown in Scheme 4.48. Route a outlines the oxidation of the primary alcohol to the aldehyde, allowing for cyclisation to occur as previously described, to gain pancratistatin analogue 277. Route b shows the activation of the primary alcohol using tosyl chloride and subsequent cyclisation that would give narciclasine analogue 278. Preliminary investigations into these proposed routes will be described further here.
4.13.1. Oxidation of the primary alcohol

A strategy to cyclise the side-chain is to oxidise the primary alcohol to the aldehyde which would then allow for a condensation reaction to occur as previously mentioned. With a robust procedure in place for the synthesis of analogue 273 and 275 efforts were made to oxidise the primary alcohol, in preference to the secondary alcohols.

A reported procedure by Einhorn et al. demonstrated the oxidation of an aliphatic and benzylic primary alcohols using TEMPO and \(N\)-chlorosuccinimamide (NCS) (Scheme 4.49).\(^{176}\) This was in preference to secondary alcohols with intermolecular (primary and secondary alcohols on different molecules) and intramolecular (primary and secondary alcohols on the same molecule) competition demonstrating high chemoselectivity towards the primary alcohol, consequently forming the aldehyde.

Scheme 4.49: Oxidation of a primary alcohol in preference to a secondary alcohol\(^{176}\)
Following the reported procedure and using the model substrate benzyl alcohol, for which Einhorn et al. report a 99 \% yield, test reactions were carried out. Benzyl alcohol 281 was stirred with TEMPO and tertbutylammonium chloride (TBACl) in dichloromethane at room temperature (Scheme 4.50). NaHCO$_3$(aq) (0.5 M) and K$_2$CO$_3$(aq) (0.05 M) was added and the reaction vigorously stirred after which NCS was added and the reaction followed by TLC. After 3 hours crude $^1$H NMR spectroscopic analysis showed a ratio of starting material to product of 84:16. As reported, improvements in yield were achieved by using recrystallised NCS and also increasing the amount of TBACl in the reaction mixture. However, an optimised ratio of alcohol 281 to aldehyde 282 of 50:50 by crude $^1$H NMR spectroscopic analysis was the best result.

These optimised conditions were implemented on the straight chain analogue 273. Solubility was initially an issue, however the starting material dissolved into the reaction mixture over time. The reaction was followed by TLC and crude $^1$H NMR spectroscopic analysis was done after the starting material had been consumed. This showed one product, however hydroxyl groups were not observed suggesting that over oxidation had occurred. Time constraints prevented further investigations however this methodology may be promising and so more examination of these conditions is warranted.

4.13.2. Activation of the primary alcohol

An alternative strategy is to activate the primary alcohol to a good leaving group, such as p-toluenesulfonyl chloride (pTsCl), in order to promote intramolecular cyclisation. This is a strategy employed by multiple researchers in order to tosylate the primary alcohol in a sugar, in preference to secondary ones.\textsuperscript{177-180} Methodology is consistent throughout, an example of which is work reported by Ulgar et al. shown in Scheme 4.51.\textsuperscript{178} A solution of sugar analogue 283 and anhydrous pyridine at -15 °C was added dropwise to a stirring solution of pTsCl in anhydrous pyridine under inert conditions. The reaction was stirred at 0 °C for 2 hours to furnish tosylated analogue 284 in 77 \% yield.

![Scheme 4.50: Synthesis of benzaldehyde 282](image-url)
Scheme 4.51: Selective tosylation of the primary alcohol\textsuperscript{178}

The standard method was employed using our substrate \textsuperscript{273} and two new spots were observed by TLC analysis, although the starting material persisted. Unfortunately, crude \textsuperscript{1}H NMR spectroscopic analysis showed mostly the pyridine salts. Altering the work-up in order to remove the salt with an aqueous wash and EtOAc extraction only resulted in the recovery of starting materials.

Crude \textsuperscript{1}H NMR spectroscopic analysis suggested that the tosylate was formed due to a new methyl peak at 2.33 ppm, integrating proportionally for 3 hydrogens when compared to the aromatic protons of the AB-ring sugar analogue. Branchaud \textit{et al}. reported using the tosyalted product as crude,\textsuperscript{177} and this was repeated in our system, subjecting the compound to heat in order to promote cyclisation. Unfortunately a mixture of unidentifiable compounds was recovered. Although the tosylated or cyclised product was not isolated analysis of the crude data suggests that this might be a successful avenue for investigation and should be examined further.

4.14. Conclusions and future work

The work in this chapter describes the one-pot procedure to novel quinazolinone analogues of narciclasine and pancratistatin. Although further functionalisation of the enamine was attempted it was found to be unstable in aqueous conditions and reduction of the olefin was required in order to furnish compounds which could be biologically evaluated. It is hoped that these compounds, along with their bislactam counterparts, will be accepted for evaluation by the NCI.

Further analysis of the A-ring methoxy derivatives is warranted. Although initial investigations of the dimethoxy derivative \textsuperscript{219} failed to furnish the desired tricyclic enamine access to the reduced analogue might be achieved in a two-step process. Unfortunately, availability of the benzamide was restrictive, however a robust procedure for aminolysis from the corresponding benzoate has been established here and could be used to gain good yields of starting material for future investigations. In addition, there are alternative strategies to gaining the trimethoxybenzamide \textsuperscript{221} such as installing a bromine group at the C(2) position. This may be followed by treatment with butyl lithium and dry ice to afford the benzoic acid which could then be functionalised to the benzamide with the optimised conditions described in this chapter.
Novel sugar analogues have also been developed using D- and L-lyxose to install hydroxyls with defined stereochemistry mimicking the C-ring of the natural products. Preliminary biological testing, shown in Figure 4.08, suggest that these analogues are inactive following MTS cell proliferation assay against the HT29 colon cancer cells lines. This is presumably due to hydrolysis in the biological media, however, cyclisation has yet to be achieved and should be investigated further as the narciclasine/pancreatistatin analogues outlined in Scheme 4.48 would be of great interest. These preliminary investigations into the synthesis of novel quinazolinone analogues of narciclasine and pancratistatin show great promise. The results so far demonstrate the reaction of substituted anthranilamides and sugars are possible to afford exciting analogues in an efficient and stereoselective route, which requires further investigations.

Figure 4.08: Biological activity of sugar analogues
5. Conclusions and future work

The principal objective of this project was to use short synthetic sequences in order to synthesise simplified narcilasine 7 and pancratistatin 8 analogues. This was carried out with a view to exploring their biological activity, thereby providing new insight into the SAR of these natural products.

Conditions for Curtius rearrangement and intramolecular Friedel-craft acylation previously published by the group for the synthesis of the dihydroisoquinolinone framework was successfully employed in gaining AB-ring systems in comparable yield. Previous studies by Judd et al. had suggested that oxidation of the dihydroisoquinolinone framework to their isoquinolinone counterparts improved their biological activity as observed by MTS cell proliferation assay against the HT29 colon cancer cells. Methods employed by Judd for oxidation to the olefin proved to be unsuccessful when repeated and a variety of other methodologies were investigated all of which were capricious and failed to furnish sufficient clean isoquinolinones for further investigations. 6,7-dimethoxyisoquinolin-1(2H)-one 64 and 1,3-methylenedioxyisoquinolin-1(2H)-one 65 were successfully and reproducibly synthesised from their cinnamic acid counterparts in 46 and 47 % yields respectively. Regrettably, the 3,4,5-trimethoxycinnamic acid failed to furnish the desired AB-ring system presumably due to the additional activation caused by the third methoxy group. Functionalisation of the olefin at both C(3) and C(4) provided some interesting molecules for biological evaluation. In particular the NSC analogues which will be sent for consideration by the NCI.

Judd had completed the 6-step synthesis of the dimethoxy- and dimethoxyhydroxy late stage narcilasine analogues 163 and 155 respectively. This synthetic route was successfully optimised and repeated in addition to being employed in the synthesis of the novel methylenedioxy analogue 148. Interestingly, bond migration was observed for the dimethoxy and dimethoxyhydroxy analogues 163 and 165 using conditions for hydrogenation. These had been isolated previously, albeit via alternative synthetic procedures, and were found to have good biological activity with GI₅₀ of 17 μM and 9 μM respectively. Further biological evaluation of these compounds would be interesting, especially using non-cancerous cells to see if they still elicit an antiproliferative response.

The ABC-ring analogues underwent initial screening by the NCI, but were not selected for further analysis owing to a lack of potency. This is due to the absence of C-ring hydroxyl groups, which as described in the Introduction are required for potency. Functionalisation of these late stage analogues proved to be challenging however further investigations may be beneficial, such as
hydroxylation of the C-ring. Some success was realised in the synthesis of narciprimine analogues using a proposed two-step process. Synthesis of the A/C-ring system was eventually successful, with intact hydroxyls on the C-ring. Although cyclisation of the B-ring was not achieved it deserves more work as we have confidence and expertise in performing the Curtius rearrangement/Friedel-Crafts acylation reactions with similar substrates.

Novel tricyclic quinazolinone analogues of narciclasine and pancratistatin were successfully synthesised in a one-pot procedure in order to incorporate a basic nitrogen into the ABC-ring and evaluate if this improved biological activity. Biological investigations and further functionalisation of the enamine proved to be unsuccessful due to the instability of the compounds in aqueous conditions, however, the more stable hydrogenated and bislactam derivatives will hopefully be accepted by the NCI for further evaluation. In addition, novel sugar analogues were also achieved using D- and L-lyxose which installed hydroxyls with defined stereochemistry mimicking the C-ring. Although preliminary biological testing found these analogues to be inactive this is not surprising as cyclisation of the C-ring is yet to be achieved.

Novel analogues have been synthesised and have been found to have anticancer activity so further investigations into some areas of the project are warranted and have been highlighted above. Current chemotherapeutic agents work by eliciting catastrophic damage to the DNA replicating process, such as alkylating agents or topoisomerase inhibitors, or by preventing mitosis as with the mitotic spindle poisons. However, these methods of targeting cancer will also target any highly replicating cell and therefore cause undesirable side effects. Growing resistance to a number of currents agents is also becoming a problem in treating patients.

The unique anticancer mode of action of the natural products narciclasine and pancratistatin, and potentially our novel compounds, justifies more research in this area. In particular, their effect on the intrinsic apoptotic pathway is intriguing. Recently, the mitochondrion has been identified as an interesting anticancer target which often bypasses the resistance to apoptosis caused by genetic defects common in many human cancers, such as a defective tumour suppressor protein p53.
6. Experimental

6.1. General Experimental

Chemicals, solvents and reagents used were commercially available and were used without further purification. Glassware for anhydrous or air sensitive reactions were dried in an oven and allowed to cool to r.t. under a stream of N₂ or Ar gas. Petroleum ether (PE) refers to the fraction boiling in the range 40 - 60 °C.

TLC was performed on Merck Aluminium-backed TLC plates Silica Gel 60 F254. They were observed using UV light of wavelength 254 nm and then stained with iodine, potassium permanganate (KMnO₄) or 2,4-dinitrophenylhydrazine (DNP).

Merck Silica Gel (0.040-0.063 mm) was used for column chromatography. Compounds were loaded as an oil, CHCl₃ or CH₂Cl₂ solution or dry loaded by adsorption onto silica gel.

Analytical RP-HPLC was performed on a Gilson HPLC system equipped with an Agilent Eclipse XDB 5 μm C18 (150 x 4.6 mm) column with a flow rate of 1.0 mL/min. with detection at 274 nm and 254 nm. The gradient was T = 0-3 min., 20 % MeCN 80 % H₂O, T = 3-13 min., 80 % MeCN 20% H₂O, T = 13-22 min., 80 % MeCN 20% H₂O, 22-25 min., 20% MeCN 80% H₂O.

Melting points (Mp) were obtained using a Reichert-Jung heated-stage microscope and are uncorrected. Solvent for recrystallization is defined in brackets.

Infrared spectra (IR) were recorded on a Perkin-Elmer Spectrum RX I FT-IR system using KBr disk or thin film and all values are recorded in cm⁻¹.

NMR spectra were obtained on a Bruker Avance III (400 MHz) spectrometer or Bruker Avance III (500 MHz) spectrometers. The chemical shifts are recorded in parts per million (ppm) with reference to tetramethylsilane and recorded at 298 K. The coupling constants J are quoted to the nearest 0.5 Hz and are not corrected. The multiplicities are assigned as a broad singlet (brs), singlet (s), doublet (d), triplet (t), doublet of doublets (dd), quartet (q) and multiplet (m). The symbols + and – after the carbon NMR chemical shifts indicate odd (CH and CH₃) and even (C and CH₂) numbers of attached protons. HMBC and HMQC NMR spectrum was used to assign C₆ where data was available.

Mass spectra and high resolution mass spectra were obtained on a microOTOF™ from Bruker Daltonics (Bremen, Germany) coupled with an electrospray source (ESI-TOF) using an autosampler in an Agilent 1100 LC system. Data was processed using external calibration with the Bruker Daltonics software, DataAnalysis™ as part of the overall hardware control software, Compass 1.1™.
6.2. MTS cell proliferation assay protocol

The assay uses a 96-well plate format, with 100 μL per well final volume, to determine cell viability using a single reagent (MTS) and is based on the Promega Cell Titer 96 Aqueous One Solution Cell Proliferation Assay. As this assay is dependent upon the development of a coloured metabolite from viable cells it does not distinguish between cytostatic and cytotoxic compounds. However, this assay provides an interesting and convenient approach to initial anticancer screening.

Human cancer cell lines HT29 colon cancer cell lines were supplied by Cancer Research UK. They were maintained in DMEM with high glucose (4.5 g/L) and L-glutamine, supplemented with penicillin 100 U/mL, streptomycin 100 μg/mL and foetal bovine serum at 10%. Cells were maintained in 75 cm² tissue culture flasks (Nunc) with a weekly 1 in 10 split.

General Procedure:

Seed densities of 500 cells per well in 50 μL culture medium were used for the HT29 cell line, the seed densities having previously been determined to give an acceptable optical density value after a three day incubation. Plates were incubated at 37 °C, in humidified 5% CO₂ in air for between 2 and 4 hours. Test compounds were added to the appropriate wells, to give a final volume of 100 μL. These were prepared at 100 times the final concentration in DMSO (Sigma), diluted 1 in 50 in culture medium to give final drug concentrations of 500 µM, 200 µM, 100 µM, 50 µM, 20 µM, 10 µM, 5 µM, 2 µM, 1 µM and 500 nM in 1% DMSO. Quadruplicate samples were run as follows:

1. Culture medium only (background)
2. Cells only
3. Cells + 1% DMSO
4. Cells + test compound in 1% DMSO

The cells plus test compounds were then incubated at 37 °C, 5 % CO₂ in humidified air for 3 days. This exposure time appears to be adequate to demonstrate anti-proliferative activity, and is routinely used. The MTS reagent was added, 20 μL per well and the plates were returned to the incubator at 37 °C, in humidified 5% CO₂ in air, for colour development.

Optical density readings at 490 nm were taken at 1 to 4 hours on a BMG Labtech FLUOstar Omega plate reader. As the culture medium gives a high OD₄₉₀nm this value was subtracted from all other OD₄₉₀nm readings. Therefore, means and standard deviations were calculated from background corrected OD₄₉₀nm values. IC₅₀ curves and values were generated using the pharmacology function
in SigmaPlot 8 software. Each assay was repeated on three separate occasions and an average value calculated unless otherwise stated.
6.3. Synthesis of AB-ring analogues

General Procedure 1:

Diphenylphosphoryl azide (DPPA) (2.24 mL, 10 mmol) was added dropwise to a rapidly stirred solution of dihydrocinnamic acid (10 mmol) and anhydrous triethylamine (Et$_3$N) (1.39 mL, 10 mmol) in anhydrous toluene (30 mL) at room temperature (r.t.) under Ar. The reaction was heated at 90 °C for 90 min then allowed to cool to r.t., concentrated under reduced pressure and the crude oil was placed back under Ar and boron trifluoride diethyl etherate (BF$_3$:OEt$_2$) (5 mL) was added at r.t.. After 18 h stirring at r.t. the reaction was quenched with 2M NaOH$_{aq}$ to pH 10, diluted with EtOAc (30 mL) and left stirring for a further 90 min at 50 °C. After cooling to r.t. the organic layer was separated and the aqueous fraction washed with EtOAc (3 x 20 mL). The combined organic fractions were washed with sat. brine (40 mL), dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. After column chromatography [silica, PE: EtOAc: MeOH gradient column] the purified product was isolated.
6,7-Dimethoxy-3,4-dihydroisoquinolin-1(2H)-one (59)

Following the general procedure 1, 3-(3,4-dimethoxyphenyl)propanoic acid 56 (5.25 g, 25 mmol), DPPA (5.38 mL, 25 mmol) and Et$_3$N (3.47 mL, 25 mmol) in toluene (75 mL) followed by BF$_3$−OEt$_2$ (12.5 mL) at 50 °C, gave lactam 59 (3.89 g, 75 %) as a white solid.

$^1$H NMR δ$_H$(400 MHz, CDCl$_3$) 7.56 (1H, s, C(8)H), 6.66 (1H, s, C(5)H), 6.33 (1H, brs, NH), 3.91 (3H, s, OCH$_3$), 3.91 (3H, s, OCH$_3$), 3.54 (2H, td, J 6.5 and 3.0 Hz, C(3)H$_2$) and 2.91 (2H, t, J 6.5 Hz, C(4)H$_2$);

$^{13}$C NMR δ$_C$(100 MHz, CDCl$_3$) 166.4− (C(1)q), 152.1− (C(7)q), 148.0− (C(6)q), 134.5− (C(8a)q), 122.8− (C(4a)q), 110.1+ (C(8)H), 109.5+ (C(5)H), 56.0+ (OCH$_3$), 56.0+ (OCH$_3$), 40.4− (C(3)H$_2$) and 28.0− (C(4)H$_2$);

Elemental analysis Found C 63.65 %, H 6.39 % and N 6.86 % requires C 63.76 %, H 6.32 % and N 6.76 %.

Spectroscopic data is consistent with that reported by Judd et al.$^{63}$
3-(3,4-Methylenedioxyphenyl)propionic acid (122)

Following the procedure previously reported,65 a flask containing a vigorously stirred suspension of 3,4-methylenedioxyxycinnamic acid 80 (5 g, 26 mmol) and Pd/C (10 % wt.) (250 mg) in EtOH (125 mL) at r.t. was purged with N2. The flask was degassed and H2 introduced at atmospheric pressure and stirred vigorously for 180 min. The reaction was filtered through filter paper and a plug of cotton wool. After passing through a plug of silica the product 122 was isolated (4.69 g, 92 %) as a white crystalline solid.

\[ \text{H NMR } \delta_{\text{H}}(400 \text{ MHz, CDCl}_3) 10.87 \, (1\text{H, brs, O}H) , \, 6.73 \, (1\text{H, d, } J 8.0 \text{ Hz, C}(5)\text{H}), \, 6.69 \, (1\text{H, d, } J 1.0 \text{ Hz, C}(2)\text{H}), \, 6.66 \, (1\text{H, dd, } J \text{8.0 and 1.0 Hz, C}(6)\text{H}), \, 5.92 \, (2\text{H, s, OCH}_2\text{O}), \, 2.87 \, (2\text{H, t, } J 8.0 \text{ Hz, C}(2')\text{H}_2\text{COOH}) \] and 2.63 (2H, t, J 8.0 Hz, C(3')H$_2$)

\[ \text{C NMR } \delta_{\text{C}}(100 \text{ MHz, CDCl}_3) 177.8−(C(1')_q), \, 147.6−(C(3 or 4')_q), \, 146.0−(C(4 or 3')_q), \, 133.9−(C(1)_q), \, 121.1+ (C(6)H), \, 108.7+ (C(2 or 5)H), \, 108.3+ (C(5 or 2)H), \, 100.8−(OCH$_2$O), \, 35.7−(C(2')H$_2$COOH) \] and 30.3−(C(3')H$_2$).

Spectroscopic data is consistent with that reported by Haga et al.\textsuperscript{66}
7,8-Dihydro[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one (60)

Following the general procedure 1, 3-(3,4-methylenedioxyphenyl)propionic acid 122 (1.94 g, 10 mmol), DPPA (2.24 mL, 10 mmol) and Et₃N (1.39 mL, 10 mmol) in toluene (30 mL) followed by BF₃·OEt₂ (5 mL), gave lactam 60 (1.4 g, 73 %) as a white solid.

$^1$H NMR $\delta$ (400 MHz, CDCl₃) 7.51 (1H, s, C(8)H), 6.64 (1H, s, C(5)H), 5.99 (2H, s, OCH₂O), 5.84 (1H, brs, NH), 3.51 (2H, td, $J$ 6.5 and 2.5 Hz, C(3)H₂) and 2.90 (2H, t, $J$ 6.5 Hz, C(4)H₂);

$^{13}$C NMR $\delta$ (100 MHz, CDCl₃) 166.2– (C(1)a), 150.7– (C(7)a), 146.8– (C(6)a), 134.5– (C(8a)a), 122.8– (C(4a)a), 107.8+ (C(8)H), 107.2+ (C(5)H), 101.4– (OCH₂O), 40.1– (C(3)H₂) and 28.4– (C(4)H₂).

Spectroscopic data is consistent with that reported by Judd et al. 63
Following the general procedure 1, 3-(3,4,5-trimethoxyphenyl)propanoic acid 124 (2.4 g, 10 mmol), DPPA (2.24 mL, 10 mmol) and Et₃N (1.39 mL, 10 mmol) in toluene (30 mL) followed by BF₃·OEt₂ (5 mL) gave lactam 61 (1.2 g, 54 %) as a white solid.

**¹H NMR** δ_H (400 MHz, CDCl₃) 12.35 (1H, s, OH), 6.25 (1H, s, C(5)H), 5.90 (1H, brs, NH), 3.89 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 3.54 (2H, td, J 7.0 and 2.5 Hz, C(3)H₂) and 2.92 (2H, t, J 7.0 Hz, C(4)H₂);

**¹³C NMR** δ_C (100 MHz, CDCl₃) 170.4– (C(1)q), 156.9– (C(6 or 7)q), 155.9– (C(8)q), 135.9– (C(7 or 6)q), 135.2– (C(8a)q), 105.8– (C(4a)q), 101.8+ (C(5)H), 60.7+ (OCH₃), 56.0+ (OCH₃), 40.3– (C(3)H₂) and 28.1– (C(4)H₂);

**Elemental analysis** Found C 59.26 %, H 5.80 % and N 6.33 % requires C 59.19 %, H 5.87 % and N 6.27 %.

Spectroscopic data is consistent with that reported by Judd et al.⁶³
**General Procedure 2:**

**Method A:** Following the previously reported procedure for a similar compound,\textsuperscript{76} lactam (1.0 mmol) and Pd/C (10 % wt.) (100 mg) were ground together to a fine powder using a pestle and mortar. The dry solid was transferred to a flask, flushed with N\textsubscript{2} and heated to 200 °C for 60 min in solvent free conditions. After cooling to r.t. the solid reaction mixture was flushed through a pad of celite using hot methanol until the crude mass was constant. The solvent was removed under reduced pressure and the product was purified by column chromatography [silica, PE: EtOAc: MeOH gradient column].

**Method B:** Following the previously reported procedure for a similar compound,\textsuperscript{74} acetic acid (5 mL) was added dropwise to a stirred mixture of lactam (1.0 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (454 mg, 2.0 mmol) at r.t. under Ar. After 16 h stirring at 80 °C the hot reaction was poured onto water (20 mL) and extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 20 mL). The combined organic layers were washed with 10 % NaOH\textsubscript{(aq)} (10 mL), water (10 mL), sat. brine (10 mL), dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and the solvent removed under reduced pressure. The purified product was isolated by column chromatography [silica, PE: EtOAc: MeOH gradient column].
8-Hydroxy-6,7-dimethoxyisoquinolin-1(2H)-one (63)

Following general procedure 2A, 8-hydroxy-6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 61 (114 mg, 0.51 mmol), using Pd/C (10 % wt.) (115 mg), gave the product 63 (7 mg, 6 %) as a white crystalline solid.

Following general procedure 2B, 8-hydroxy-6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 61 (223 mg, 1.0 mmol), using DDQ (250 mg, 1.1 mmol) and acetic acid (5 mL) at 0 °C after which the reaction was allowed to warm to r.t. and stirred for 18 h, gave the product 63 (64 mg, 29 %) as a white crystalline solid.

Rf [EtOAc] 0.2;
Mp [MeOH] > 230 °C;
IR νmax (KBr disc) 3329 (OH and NH), 1648 (C=O), 1618 (C=C) and 1235 (C-O);

^1H NMR δH (400 MHz, DMSO) 13.26 (1H, s, OH), 11.53 (1H, s, NH), 7.11 (1H, d, J 7.0 Hz, C(3)H), 6.73 (1H, s, C(5)H), 6.56 (1H, d, J 7.5 Hz, C(4)H), 3.87 (3H, s, OCH3) and 3.72 (3H, s, OCH3);

^13C NMR δC (100 MHz, DMSO) 165.7– (C(1)q), 157.8– (C(7)q), 153.7– (C(8)q), 135.1– (C(8a)q), 132.8– (C(6)q), 127.8+ (C(3)H), 106.9– (C(4a)q), 106.7+ (C(4)H), 97.9+ (C(5)H), 59.8+ (OCH3) and 55.8+ (OCH3);

MS (+ESI) m/z 222 (100 %, [M+H]^+) and 244 (56 %, [M+Na]^+);
HRMS (+ESI) m/z Found [M+Na]^+ 244.0619, C_{11}H_{11}NNaO_{4}, requires [M+Na]^+ 244.0586;

Elemental analysis Found C 59.83 %, H 5.07 % and N 6.43 % requires C 59.73 %, H 5.01 % and N 6.33 %.
6,7-Dimethoxyisoquinolin-1(2H)-one (64)

Following general procedure 2B, 6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 59 (201 mg, 0.97 mmol), using DDQ (495 mg, 2.18 mmol) and acetic acid (5 mL), gave the product 64 (108 mg, 55 %) as a white crystalline solid.

Rf [EtOAc] 0.2;

 Mp [MeOH] > 230 °C [Lit.,183 244-245 °C];

IR νmax (KBr disc) 1639 (C=O), 1607 (C=C) and 1273 (C-O);

1H NMR δH (400 MHz, CDCl3) 10.84 (1H, brs, NH), 7.78 (1H, s, C(8)H), 7.08 (1H, d, J 7.0 Hz, C(3)H), 6.91 (1H, s, C(5)H), 6.48 (1H, d, J 7.0 Hz, C(4)H), 4.02 (3H, s, OCH3) and 3.99 (3H, s, OCH3);

13C NMR δC (100 MHz, CDCl3) 163.4 – (C(1)q), 153.8 – (C(7)q), 149.4 – (C(6)q), 133.8 – (C(8a)q), 126.3+ (C(3)H), 120.1 – (C(4a)q), 107.2+ (C(8)H), 106.3+ (C(5)H), 106.2+ (C(4)H), 56.2+ (OCH3) and 56.1+ (OCH3);

MS (+ESI) m/z 433 (100 %, [2M+Na]+) and 228 (45 %, [M+Na]+);


Elemental analysis Found C 64.49 %, H 5.46 % and N 6.91 % requires C 64.38 %, H 5.40 % and N 6.83 %.

Spectroscopic data is consistent with that reported by Awuah et al.75
6-Methyl[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one (75)

Following general procedure 2B 6-methyl-7,8-dihydro[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one 74 (236 mg, 1.15 mmol), using DDQ (784 mg, 3.45 mmol) and acetic acid (5.75 mL) and heating at 120 °C for 22 h, gave the methylated lactam 75 (109 mg, 47 %) as a white solid.

Rf [MeOH:CH2Cl2, 4:96] 0.41;

Mp [MeOH] 167-172 °C;

IR νmax (Thin film) 3007 (CH), 1646 (C=O) and 1611 (C=C);

1H NMR δH(400 MHz, CDCl3) 7.78 (1H, s, C(8)H), 6.98 (1H, d, J 7.0 Hz, C(3)H), 6.84 (1H, s, C(5)H), 6.36 (1H, d, J 7.0 Hz, C(4)H), 6.06 (2H, s, OCH2O) and 3.58 (3H, s, NCH3);

13C NMR δC(100 MHz, CDCl3) 161.8– (C(1)q), 151.8– (C(6)q), 147.8– (C(7)q), 134.4– (C(4a)q), 131.2+ (C(3)H), 121.8– (C(8a)q), 105.8+ (C(4)H), 105.5+ (C(8)H), 103.6+ (C(5)H), 101.6– (OCH2O) and 37.0+ (NCH3);

MS (+ESI) m/z 204 (100 %, [M+H]+), 226 (93 %, [M+Na]+) and 429 (45 %, [2M+Na]+);


Spectroscopic data is consistent with that reported by Jangir et al.184
6,7-Dimethoxyisoquinolin-1(2H)-one (64)

DPPA (5.38 mL, 25 mmol) was added dropwise to a vigorously stirred suspension of 3,4-dimethoxycinnamic acid 79 (5.21 g, 25 mmol) and Et$_3$N (6.96 mL, 50 mmol) in toluene (25 mL) at r.t. open to the atmosphere. After 120 min stirring, the reaction was filtered through a plug of silica and washed with toluene leaving only one spot by TLC, $R_f$ [EtOAc] 0.83. The solvent was removed under reduced pressure and the crude solid was dissolved in diphenyl ether (42 mL). The reaction was heated to 250 °C under a flow of N$_2$ during which time violent effervescence was observed at 90 °C which then subsided. Heating was continued at 250 °C for 180 min after which the reaction was cooled slowly to r.t. and left to stir for 18 h forming a precipitate. The reaction was then filtered and the precipitate washed with diethyl ether to leave the product 64 as a yellow solid (2.36 g, 46 %) without the need for further purification.

$^1$H NMR $\delta$ (400 MHz, CDCl$_3$) 10.84 (1H, brs, NH$_2$), 7.78 (1H, s, C(8)H), 7.08 (1H, d, $J$ 7.0 Hz, C(3)H), 6.91 (1H, s, C(5)H), 6.48 (1H, d, $J$ 7.0 Hz, C(4)H), 4.02 (3H, s, OCH$_3$) and 3.99 (3H, s, OCH$_3$);

$^{13}$C NMR $\delta$ (100 MHz, CDCl$_3$) 163.4–(C(1)q), 153.8–(C(7)q), 149.4–(C(6)q), 133.8–(C(8a)q), 126.3+ (C(3)H), 120.1–(C(4a)q), 107.2+ (C(8)H), 106.3+ (C(5)H), 106.2+ (C(4)H), 56.2+ (OCH$_3$) and 56.1+ (OCH$_3$);

Elemental analysis Found C 64.27 %, H 5.48 % and N 6.91 % requires C 64.38 %, H 5.40 % and N 6.83 %.

Spectroscopic data is consistent with that reported by Awuah et al.$^{75}$
[1,3]Dioxolo[4,5-g]isoquinolin-5(6H)-one (65)

DPPA (2.2 mL, 10 mmol) was added dropwise to a vigorously stirred suspension of 3,4-(methyleneedioxy)cinnamic acid 80 (1.92 g, 10 mmol) and Et₃N (2.78 mL, 20 mmol) in toluene (10 mL) at r.t. open to the atmosphere. After 120 min stirring, the reaction was filtered through a plug of silica and washed with toluene leaving only one spot by TLC, Rₚ [EtOAc] 0.81. The solvent was removed under reduced pressure and the crude solid was dissolved in diphenyl ether (17 mL). The reaction was heated to 250 °C under a flow of N₂ during which time violent effervescence was observed at 90 °C which then subsided. Heating was continued at 250 °C for 180 min after which the reaction was cooled slowly to r.t. and left to stir for 18 h forming a precipitate. The reaction was then filtered and the precipitate washed with diethyl ether to leave the product 65 as a yellow solid (887 mg, 47 %) without the need for further purification.

Rₚ [EtOAc] 0.22;

Mp [EtOAc] > 230 °C;

IR νmax (KBr disc) 2907 (NH) and 1654 (C=O);

¹H NMR δH (400 MHz, DMSO) 11.14 (1H, brs, NH), 7.49 (1H, s, C(8)H), 7.14 (1H, s, C(5)H), 7.05 (1H, dd, J 7.0 and 5.5 Hz, C(3)H), 6.44 (1H, d, J 7.0 Hz, C(4)H) and 6.15 (2H, s, OC₂H₂);

¹³C NMR δC (100 MHz, DMSO) 161.1− (C(1)q), 151.4− (C(7)q), 147.1− (C(6)q), 135.1− (C(4a)q), 127.5+ (C(3)H), 121.3− (C(8a)q), 104.7+ (C(4)H), 104.0+ (C(8)H), 103.9+ (C(5)H) and 101.8− (CH₂);

MS (+ESI) m/z 401 (100 %, [2M+Na]⁺) and 212 (85 %, [M+Na]⁺);

HRMS (+ESI) m/z Found [M+Na]+ 212.0316, C_{10}H_{11}NNaO₃, requires [M+Na]+ 212.0324;

Elemental analysis Found C 63.53 %, H 3.81 % and N 7.32 % requires C 63.49 %, H 3.73 % and N 7.40 %.

Spectroscopic data is consistent with that reported by Xie et al.¹⁸⁵
**General Procedure 3:**

**Method A:** Sodium hydride (NaH) (60% dispersion in mineral oil) (120 mg, 3 mmol) was added portion-wise to a stirred solution of lactam (1 mmol) in anhydrous THF (6 mL) at 0 °C under Ar. After 30 min stirring at 0 °C iodomethane (MeI) (0.2 mL, 3 mmol) was added dropwise and the reaction warmed to r.t. for 4 hours. The reaction was quenched with the addition of NH₄Cl (aq) (15 mL) and extracted with EtOAc (3 x 30 mL). The combined organic fractions were washed with sat. brine (50 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. After column chromatography [silica, PE: EtOAc: gradient column] the product was isolated.

**Method B:** Using modified conditions, potassium hydroxide (KOH) (500 mg, 8.9 mmol) was added to a rapidly stirred solution of lactam (3 mmol) in acetone (30 mL) at 0 °C. MeI (448 µL, 7.2 mmol) and THF (10 mL) was added and the reaction warmed to r.t. and stirred 18 h. MeI was quenched with MeOH (5 mL) and after 5 min stirring the solvent was removed under reduced pressure. EtOAc (20 mL) and HCl (aq) (1 M, 10 mL) were added to the reaction and the organic layer separated. After further extraction with EtOAc (3 x 20 mL) the combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. The purified product was isolated by column chromatography [silica, PE: EtOAc: gradient column].
6,7-Dimethoxy-2-methylisoquinolin-1(2H)-one (83) and 5-(3,4-dimethoxyphenyl)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (84)

Following general procedure 3A, 6,7-dimethoxyisoquinolin-1(2H)-one 64 (126 mg, 0.6 mmol), using NaH (72 mg, 1.8 mmol) in THF (3 mL) and leaving the reaction for 16 h after the addition of MeI (0.11 mL, 1.8 mmol), gave the product 83 (125 mg, 95 %) as a white solid.

Following general procedure 3B, 6,7-dimethoxyisoquinolin-1(2H)-one 64 (615 mg, 3 mmol), using KOH (500 mg, 8.9 mmol) in acetone (30 mL) followed by MeI (0.45 mL, 7.2 mmol) and THF (10 mL), gave product 83 (430 mg, 65 %) and product 84 (96 mg, 23 %) as white solids.

**Lactam (83)**

$R_f [\text{EtOAc}] 0.56$;

$\text{Mp} [\text{EtOAc:Cyclohexane}] 125-127 \, ^\circ\text{C}$;

$\text{IR } \nu_{\text{max}}$ (Thin film) 2908 (CH), 1655 (C=O), 1608 (C=C) and 1145 (C-O);

$^1\text{H NMR} \delta_H(400 \, \text{MHz, CDCl}_3) 7.79$ (1H, s, C(8)H), 6.98 (1H, d, $J$ 7.0 Hz, C(3)H), 6.84 (1H, s, C(5)H), 6.38 (1H, d, $J$ 7.0 Hz, C(4)H), 3.99 (3H, s, OCH$_3$), 3.96 (3H, s, OCH$_3$) and 3.58 (3H, s, NCH$_3$);

$^{13}\text{C NMR} \delta_C(125 \, \text{MHz, CDCl}_3) 161.8$–(C(1)$_\alpha$), 153.1–(C(6)$_\alpha$), 149.1–(C(7)$_\alpha$), 132.5–(C(4a)$_\alpha$), 131.0+(C(3)H), 120.0–(C(8a)$_\alpha$), 107.4+(C(8)H), 105.8+(C(5)H), 105.4+(C(4)H), 56.1+(OCH$_3$), 55.9+(OCH$_3$) and 37.0+(NCH$_3$).

Spectroscopic data is consistent with that reported by Lapa et al.$^{186}$

**Pyrimidine (84)**

$R_f [\text{EtOAc}] 0.47$;

$\text{Mp} [\text{CH}_2\text{Cl}_2] 150-155 \, ^\circ\text{C}$;
IR $\nu_{\text{max}}$ (Thin film) 3007 (CH), 1653 (C=O) and 1028 (C-O); 

$^1$H NMR $\delta$ (500 MHz, CDCl$_3$) 7.27 (1H, s, C(6$'$)H), 7.13 (1H, d, $J$ 2.0 Hz, C(2)H), 7.00 (1H, dd, $J$ 8.0 and 2.0 Hz, C(6)H), 6.88 (1H, d, $J$ 8.5 Hz, C(5)H), 3.91 (3H, s, C(3)OCH$_3$), 3.90 (3H, s, C(4)OCH$_3$), 3.48 (3H, s, N(5$'$)CH$_3$) and 3.43 (3H, s, N(3$'$)CH$_3$); 

$^{13}$C NMR $\delta$ (125 MHz, CDCl$_3$) 162.5– (C(2$'$)$_3$), 151.4– (C(4$'$)$_3$), 148.8– (C(3)$_3$), 148.7– (C(4)$_3$), 139.8+ (C(6$'$)H), 125.6– (C(1$'$)$_3$), 120.4+ (C(6)H), 114.2– (C(1)$_3$), 111.8+ (C(2)H), 111.0+ (C(5)H), 55.9+ (OCH$_3$), 55.9+ (OCH$_3$), 37.1+ (N(5$'$)CH$_3$) and 28.3+ (N(3$'$)CH$_3$); 

MS (+ESI) m/z 575 (100 %, [2M+Na]$^+$), 299 (56 %, [M+Na]$^+$) and 277 (46 %, [M+H]$^+$); 

HRMS (+ESI) m/z Found [M+H]$^+$ 277.1198, C$_{14}$H$_{17}$N$_2$O$_4$ requires 277.1188 [M+H]$^+$. Found [M+Na]$^+$ 299.1028, C$_{14}$H$_{16}$N$_2$NaO$_4$, requires 299.1008 [M+Na]$^+$. 

Spectroscopic data is consistent with that reported by Seki et al.

- 134 -
8-Hydroxy-6,7-dimethoxy-2-methylisoquinolin-1(2H)-one (85)

Following general procedure 3A, 8-hydroxy-6,7-dimethoxyisoquinolin-1(2H)-one 63 (53 mg, 0.24 mmol), using NaH (57 mg, 1.4 mmol) and THF (2 mL) followed by MeI (15 µL, 0.24 mmol), gave the methylated product 85 (56 mg, 99 %).

Rf [EtOAc] 0.58;

Mp [EtOAc] 133-137 °C;

IR ν_max (Thin film) 2939 (OH), 1657 (C=O) and 1597 (C=C);

$^1$H NMR δ_H (400 MHz, CDCl$_3$) 12.98 (1H, s, C(8)OH), 6.91 (1H, d, J 7.5 Hz, C(3)H), 6.42 (1H, s, C(5)H), 6.41 (1H, d, J 7.5 Hz C(4)H), 3.93 (3H, s, OCH$_3$), 3.92 (3H, s, OCH$_3$) and 3.54 (3H, s, N(2)CH$_3$);

$^{13}$C NMR δ_C (100 MHz, CDCl$_3$) 165.2− (C(1)q), 157.9− (C$_3$), 154.2− (C$_4$), 134.6− (C$_5$), 134.3− (C$_6$), 131.1+ (C(3)H), 107.7− (C$_7$), 107.4+ (C(4)H), 97.2+ (C(5)H), 60.6+ (OCH$_3$), 55.9+ (OCH$_3$) and 36.0+ (N(CH$_3$));

MS (+ESI) m/z 258 (100 %, [M+Na]$^+$), 493 (57 %, [2M+Na]$^+$) and 236 (47 %, [M+H]$^+$);

HRMS (+ESI) m/z Found [M+H]$^+$ 236.0926, C$_{12}$H$_{13}$NO$_4$ requires [M+H]$^+$ 236.0923. Found [M+Na]$^+$ 258.0747, C$_{12}$H$_{13}$NaNO$_4$ requires [M+Na]$^+$ 258.0742.
6-Methyl[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one (75)

Following general procedure 3A [1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one 65 (47 mg, 0.25 mmol), using NaH (30 mg, 0.75 mmol) and THF (2 mL) followed by MeI (50 µL, 0.75 mmol), gave the methylated lactam 75 (48 mg, 94 %).

$^1$H NMR $\delta$ (400 MHz, CDCl$_3$) 7.77 (1H, s, C(8)H), 6.98 (1H, d, J 7.0 Hz, C(3)H), 6.84 (1H, s, C(5)H), 6.36 (1H, d, J 7.0 Hz, C(4)H), 6.06 (2H, s, CH$_2$) and 3.58 (3H, s, NCH$_3$);

$^{13}$C NMR $\delta$ (100 MHz, CDCl$_3$) 161.8− (C(1),$\delta$), 151.8− (C(6),$\delta$), 147.8− (C(7),$\delta$), 134.4− (C(4a),$\delta$), 131.2+ (C(3)H), 121.8− (C(8a),$\delta$), 105.8+ (C(4)H), 105.5+ (C(8)H), 103.6+ (C(5)H), 101.6− (CH$_2$) and 37.0+ (NCH$_3$).

Spectroscopic data is consistent with that reported by Jangir et al.$^{184}$
8-Hydroxy-6,7-dimethoxy-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (86)

Following general procedure 3A, 8-hydroxy-6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 61 (223 mg, 1 mmol), using NaH (240 mg, 6 mmol) and THF (6 mL) followed by Mel (60 µL, 1 mmol), gave the methylated lactam 86 (170 mg, 72 %) as a white solid.

R_f [EtOAc] 0.56;

M_p [EtOAc:Cyclohexane] 142 °C;

IR \( \nu_{\text{max}} \) (Thin film) 3034 (CH), 2943 (OH), 1638 (C=O) and 1615 (C=C);

\( ^1H \) NMR \( \delta \) (400 MHz, CDCl\(_3\)) 12.80 (1H, s, C(8)OH), 6.19 (1H, s, C(5)H), 3.87 (3H, s, OCH\(_3\)), 3.86 (3H, s, OCH\(_3\)), 3.51 (2H, t, J 7.0 Hz, C(3)H\(_2\)), 3.09 (3H, s, N(2)CH\(_3\)) and 3.01 (2H, dt, J 7.0 and 0.5 Hz, C(4)H\(_2\));

\( ^13C \) NMR \( \delta \) (100 MHz, CDCl\(_3\)) 168.7 (C(1)q), 156.4 – (C\(_3\)), 155.7 – (C\(_3\)), 135.3 – (C\(_3\)), 134.0 – (C\(_3\)), 106.3 – (C\(_3\)), 101.2+ (C(5)H), 60.6+ (OCH\(_3\)), 55.9+ (OCH\(_3\)), 48.1– (C(3)H\(_2\)), 34.3+ (N(2)CH\(_3\)) and 27.7– (C(4)H\(_2\));

MS (+ESI) \( m/z \) 238 (100 %, [M+H]+), 497 (92 %, [2M+Na]+) and 260 (80 %, [M+Na]+);

HRMS (+ESI) \( m/z \) Found [M+H]+ 238.1183, \( C_{12}H_{16}NO_4 \) requires [M+H]+ 238.1079. Found [M+Na]+ 260.0994, \( C_{12}H_{15}NNaO_4 \) requires [M+Na]+ 260.0899;

Elemental analysis Found C 60.68 %, H 6.42 % and N 6.00 % requires C 60.75 %, H 6.37 % and N 5.90 %.
6,7-Dimethoxy-2-methyl-3,4-dihydroisoquinolin-1(2H)-one (87)

Following general procedure 3A, 6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 59 (207 mg, 1 mmol), using NaH (120 mg, 3 mmol) and THF (6 mL) followed by Mel (200 µL, 3 mmol), gave the methylated lactam 87 (202 mg, 91 %) as a white solid.

Rf [EtOAc] 0.41;

Mp [EtOAc:Cyclohexane] Sublimes 112 °C, melts 133 °C [Lit.,187 125-126 °C];

Mp [MeOH] Sublimes 175-180 °C, melts > 230 °C

IR νmax (KBr disc) 3004 (CH), 1639 (C=O) and 1603 (C=C);

1H NMR δH (400 MHz, CDCl3) 7.60 (1H, s, C(8)H), 6.62 (1H, s, C(5)H), 3.91 (3H, s, C(6)OCH3), 3.90 (3H, s, C(7)OCH3), 3.53 (2H, t, J 7.0 Hz, C(3)H2), 3.12 (3H, s, NCH3) and 2.92 (2H, t, J 7.0 Hz, C(4)H2);

13C NMR δC (100 MHz, CDCl3) 164.8– (C1)q, 151.6– (C6)q, 147.9– (C7)q, 131.5– (C4a)q, 122.0– (C8a)q, 110.5+ (C8)H, 109.2+ (C5)H, 56.0+ (OCH3), 55.9+ (OCH3), 48.3– (C3)H2, 35.1+ (NCH3) and 27.5– (C4)H2);

MS (+ESI) m/z 465 (100 %, [2M+Na]+) and 244 (49 %, [M+Na]+);

HRMS (+ESI) m/z Found [M+Na]+ 244.1063, C12H15NNaO3, requires [M+Na]+ 244.0950;

Elemental analysis Found C 65.23 %, H 6.80 % and N 6.41 % requires C 65.14 %, H 6.83 % and N 6.33 %.

Spectroscopic data is consistent with that reported by Ying et al.188
6-Methyl-7,8-dihydro[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one (74)

Following general procedure 3A, 7,8-dihydro[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one 60 (382 mg, 2 mmol), using NaH (240 mg, 6 mmol) and THF (12 mL) followed by Mel (400 µL, 6 mmol), gave the methylated lactam 74 (389 mg, 95 %) as a white solid.

R$_f$ [EtOAc] 0.51;

M$_p$ [EtOAc:Cyclohexane] 97 °C [Lit.,$^{184}$ 95-97 °C];

IR $\nu_{\text{max}}$ (Thin film) 3007 (CH), 1643 (C=O) and 1610 (C=C);

$^1$H NMR $\delta$(400 MHz, CDCl$_3$) 7.48 (1H, s, C(8)H), 6.55 (1H, s, C(5)H), 5.93 (2H, s, OCH$_2$O), 3.46 (2H, dd, J 6.5 and 7.0 Hz, C(3)H$_2$), 3.07 (3H, s, NCH$_3$) and 2.85 (2H, dd, J 6.5 and 7.0 Hz, C(4)H$_2$);

$^{13}$C NMR $\delta$(100 MHz, CDCl$_3$) 164.4− (C(1)$_2$)$_2$, 150.1− (C$_6$), 146.7− (C$_4$), 133.3− (C$_3$), 123.4− (C$_8$), 108.0+ (C(8)H), 106.7+ (C(5)H), 101.3− (OCH$_2$O), 48.1− (C(3)H$_2$), 35.0+ (NCH$_3$) and 27.9− (C(4)H$_2$);

MS (+ESI) $m/z$ 206 (100 %, [M+H]$^+$), 228 (44 %, [M+Na]$^+$) and 433 (49 %, [2M+Na]$^+$);

HRMS (+ESI) $m/z$ Found [M+H]$^+$ 206.0941, C$_{11}$H$_{12}$NO$_3$ requires [M+H]$^+$ 206.0817. Found [M+Na]$^+$ 228.0718, C$_{11}$H$_{13}$NNaO$_3$, requires [M+Na]$^+$ 228.0637;

Elemental analysis Found C 64.33 %, H 5.35 % and N 6.92 %, requires C 64.38 %, H 5.40 % and N 6.83 %.

Spectroscopic data is consistent with that reported by Jangir et al.$^{184}$
8-Acetyl-6-methyl-[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one (90)

Following a procedure previously reported, concentrated sulfuric acid (4 drops) was added to a stirred solution of 6-methyl[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one 75 (49 mg, 0.24 mmol) in acetic anhydride (3.4 mL) and raised to 119 °C for 1 h. Ice-water was poured onto the reaction slowly and it was stirred for a further 30 min without heat. Upon extraction with CH₂Cl₂ the combined organic fractions were washed with sat. brine (50 mL), dried over Na₂SO₄, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded the product 90 as a white solid (12 mg, 21 %).

Rᶠ[MeOH:CH₂Cl₂, 6:94] 0.39;

Mp [from EtOAc:Cyclohexane] 195-203 °C;

IR νmax (Thin film) 3030 (CH), 1655 (C=O) and 1474 (C=C);

¹H NMR δH(500 MHz, CDCl₃) 8.46 (1H, s, C(5)H), 7.88 (1H, s, C(3)H), 7.78 (1H, s, C(8)H), 6.10 (2H, s, OCH₂O), 3.68 (3H, s, NCH₃) and 2.55 (3H, s, CH₃);

¹³C NMR δC(125 MHz, CDCl₃) 195.6–(C(=O)₃), 161.6–(C(1)₃), 152.8–(C(6 or 7)₃), 148.0–(C(7 or 6)₃), 140.1+(C(3)H), 131.4–(C(4a)₃), 121.2–(C(8a)₃), 115.0–(C(4)₃), 105.5+(C(8)H), 104.6+(C(5)H), 101.9–(OCH₂O), 37.9+(NCH₃) and 27.7+(CH₃);

MS (+ESI) m/z 513 (100 %, [2M+Na]⁺) and 268 (48 %, [M+Na]⁺);

HRMS (+ESI) m/z Found [M+Na]⁺ 268.0598, C₁₃H₁₁NNaO₄, requires [M+Na]⁺ 268.0586;

Elemental analysis Found C 63.62 %, H 4.58 % and N 5.70 %, required C 63.67 %, H 4.52 % and N 5.71 %.
4-Acetyl-6,7-dimethoxy-2-methylisoquinolin-1(2H)-one (91)

Following a procedure previously reported,89 concentrated sulfuric acid (8 drops) was added to a stirred solution of 6,7-dimethoxy-2-methylisoquinolin-1(2H)-one 83 (111 mg, 0.51 mmol) in acetic anhydride (8.67 mL) and raised to reflux for 2 h. Ice-water was poured onto the reaction slowly and it was stirred for a further 30 min without heat. Upon extraction with CH₂Cl₂ the combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc then EtOAc: MeOH: MeOH gradient column] afforded the product 91 as a white solid (70 mg, 53 %).

RF [MeOH:CH₂Cl₂, 6:94] 0.45;

Mp [MeOH] 203-205 °C;

IR νmax (Thin film) 1634 (C=O), 1652 (C=O) and 1508 (C=C);

¹H NMR δH (400 MHz, CDCl₃) 8.54 (1H, s, C(5)H), 7.89 (1H, s, C(3)H), 7.76 (1H, s, C(8)H), 4.01 (3H, s, C(7)OC₃H), 3.98 (3H, s, C(6)OC₃H), 3.67 (3H, s, NCH₃) and 2.54 (3H, s, CH₃);

¹³C NMR δC (100 MHz, CDCl₃) 195.8– (C(=O)q), 161.7– (C(1)q), 153.8– (C(6)q), 149.3– (C(7)q), 140.3+ (C(3)H), 129.6– (C(4a)q), 119.5– (C(8a)q), 114.4– (C(4)q), 107.3+ (C(8)H), 106.7+ (C(5)H), 56.1+ (OCH₃), 56.0+ (OCH₃), 37.9+ (NCH₃) and 27.6+ (CH₃);

MS (+ESI) m/z 262 (100 %, [M+H]+), 545 (89 %, [2M+Na]⁺) and 284 (53 %, [M+Na]⁺);

Following a procedure previously reported, concentrated sulfuric acid (6 drops) was added to a stirred solution of 6,7-dimethoxyisoquinolin-1(2H)-one 64 (68 mg, 0.33 mmol) in acetic anhydride (5.6 mL) at r.t. under Ar and heated to reflux for 18 h. Ice-water was poured onto the reaction slowly and it was stirred for a further 30 min without heat. Upon extraction with CH$_2$Cl$_2$ the combined organic fractions were washed with sat. brine (50 mL), dried over Na$_2$SO$_4$, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc then EtOAc: MeOH gradient column] afforded the product 92 as a yellow solid (31 mg, 38%).

**R$_f$** [MeOH:CH$_2$Cl$_2$, 6:94] 0.32;

**Mp** [MeOH] > 230 °C;

**IR** $\nu_{max}$ (Thin film) 3675 (NH), 1652 (C=O), 1634 (C=O) and 1506 (C=C);

**$^1$H NMR** $\delta$(400 MHz, CDCl$_3$) 9.74 (1H, brs, NH), 8.64 (1H, s, C(5)H), 7.93 (1H, d, J 6.0 Hz, C(3)H), 7.79 (1H, s, C(8)H), 4.05 (3H, s, OCH$_3$), 4.02 (3H, s, OCH$_3$) and 2.57 (3H, s, CH$_3$);

**$^{13}$C NMR** $\delta$(125 MHz, CDCl$_3$) 196.1– (C(=O)$_{q}$), 162.4– (C(1)$_{q}$), 154.1– (C(6)$_{q}$), 149.6– (C(7)$_{q}$), 135.2+ (C(3)$_{q}$), 130.5– (C(5a)$_{q}$), 119.6– (C(8a)$_{q}$), 115.3– (C(4)$_{q}$), 107.2+ (C(5 or 8)$_{q}$), 107.1+ (C(8 or 5)$_{q}$), 56.2+ (OCH$_3$), 56.1+ (OCH$_3$) and 27.7+ (CH$_3$);

**MS** (+ESI) $m/z$ 517 (100 %, [2M+Na]$^+$), 248 (99 %, [M+H]$^+$) and 270 (65 %, [M+Na]$^+$);

**HRMS** (+ESI) $m/z$ Found [M+H]$^+$ 248.0905, C$_{13}$H$_{14}$NO$_4$, requires [M+H]$^+$ 248.0923. Found [M+Na]$^+$ 270.0719, C$_{13}$H$_{13}$NNaO$_4$, requires [M+Na]$^+$ 270.0742.
4-(1,3-Benzodioxol-5-ylcarbonyl)-6,7-dimethoxy-2-methylisoquinolin-1(2H)-one (104)

6,7-Dimethoxy-2-methylisoquinolin-1(2H)-one 83 (36 mg, 0.16 mmol) and piperonylic acid (25 mg, 0.15 mmol) was stirred in TFAA (70 µL, 0.53 mmol) at 100 °C in a thick-walled pressure tube for 18 h. Upon cooling to r.t. the solution was transferred to a round bottom flask with CH$_2$Cl$_2$ and the solvent removed under pressure. The reaction was then stirred for a further 24 h in EtOAc: H$_2$O (1:1, 10 mL) then the organic layer was isolated and the water layer extracted with EtOAc (3 x 5 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na$_2$SO$_4$, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded the product 104 as a white crystalline solid (27 mg, 46%).

R$_f$ [EtOAc] 0.59;

M$_p$ [MeOH] > 230 °C;

IR $\nu_{max}$ (Thin film) 3014 (CH), 1639 (C=O), 1598 (C=C) and 1045 (C-O);

$^1$H NMR $\delta$(500 MHz, CDCl$_3$) 7.84 (1H, s, C(5 or 8)H), 7.81 (1H, s, C(8 or 5)H), 7.47 (1H, s C(3)H), 7.36 (1H, dd, J 8.0 and 1.5 Hz, C(6')H), 7.34 (1H, d, J 1.5 Hz, C(2')H), 6.89 (1H, d, J 8.0 Hz, C(5')H), 6.09 (2H, s, OC$_2$H$_2$O), 4.03 (3H, s, OC$_3$H$_3$), 3.96 (3H, s, OC$_3$H$_3$) and 3.63 (3H, s, NC$_3$H$_3$);

$^{13}$C NMR $\delta$(125 MHz, CDCl$_3$) 192.6– (C(=O)O), 161.6– (C(1)O), 153.7– (C$_3$), 151.6– (C$_5$), 149.6– (C$_6$), 148.2– (C$_7$), 139.3+ (C(3)H), 133.4– (C$_8$), 130.2– (C$_9$), 126.1+ (C(6')H), 119.8– (C$_a$), 115.2– (C$_b$), 109.6+ (C(2')H), 107.9+ (C(5')H), 107.6+ (CH), 105.8+ (CH), 102.0– (OCH$_2$O), 56.2+ (OCH$_3$), 56.1+ (OCH$_3$), 37.7+ (NCH$_3$);

MS (+ESI) m/z 368 (100 %, [M+H]$^+$) and 390 (69 %, [M+Na]$^+$);

HRMS (+ESI) m/z Found [M+H]$^+$ 368.1117 C$_{20}$H$_{18}$NO$_6$ requires [M+H]$^+$ 368.1134. Found [M+Na]$^+$ 390.0954 C$_{20}$H$_{17}$NaO$_6$ requires [M+Na]$^+$ 390.0954;

Elemental analysis Found C 65.29 %, H 4.64 % and N 3.87 % requires C 65.39 %, H 4.66 % and N 3.81 %.

- 143 -
6-Methyl[1,3]dioxolo[4,5-g]isoquinolin-5(6H)-one 75 (93 mg, 0.46 mmol) and piperonylic acid (70 mg, 0.42 mmol) was stirred in TFAA (204 µL, 1.47 mmol) at 100 °C in a thick-walled pressure tube for 18 h. Upon cooling to r.t. the solution was transferred to a round bottom flask with CH₂Cl₂ and the solvent removed under pressure. The reaction was then stirred for a further 24 h in EtOAc: H₂O (1: 1, 25 mL) then the organic layer was isolated and the water layer extracted with EtOAc (3 x 20 mL). The combined organic fractions were washed with sat. brine (40 mL), dried over Na₂SO₄, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded the product 105 as a white crystalline solid (73 mg, 46 %).

**Rf [EtOAc] 0.84;**

**Mₚ [EtOAc] > 230 °C;**

**IR vₜₚ [Thin film] 2927 (CH), 1660 (C=O), 1623 (C=C) and 1040 (C-O);**

**¹H NMR δ (400 MHz, CDCl₃) 8.40 (1H, s, C(5)H), 8.07 (1H, s, C(3)H), 7.79 (1H, s, C(8)H), 7.75 (1H, dd, J 8.5 and 1.5 Hz, C(6')H), 7.53 (1H, d, J 1.5 Hz, C(2')H), 6.89 (1H, d, J 8.0 Hz, C(5')H), 6.13 (2H, s, C(6)OC₆H₅), 6.09 (2H, s, C(3')OC₆H₅O) and 3.72 (3H, s, C₃H₃);**

**¹³C NMR δ (100 MHz, CDCl₃) 177.1– (C(=O)ₖ), 161.0– (C(1)ₖ), 153.4– (C(6)ₖ), 153.0– (C(4')ₖ), 148.2– (C(7)ₖ), 148.1– (C(3')ₖ), 144+ (C(3)ₖ), 130.8– (C(4a)ₖ), 127.0+ (C(6')H), 122.7– (C(1')ₖ), 120.0– (C(8a)ₖ), 110.0+ (C(2')H), 108.3+ (C(5')H), 107.6– (C(2)ₖ), 105.9+ (C(8)H), 103.9+ (C(5)H), 102.2– (2 × CH₃) and 38.5+ (CH₃);**

**MS (+ESI) m/z 204 (100 %, [M+H]^+–ArCO), 226 (75 %, [M+Na]^+–ArCO) and 352 (7 %, [M+H]^+);**

6,7-Dimethoxy-isoquinoline-1,3,4-trione (117)

Sodium periodate\textsubscript{(aq)} (NaIO\textsubscript{4}) (2.2 mL, 10 %) was added to Ruthenium(III) chloride hydrate (RuCl\textsubscript{3}·xH\textsubscript{2}O) (2.2 mg, 5 mol %) and this solution was added dropwise to a stirred solution of 6,7-dimethoxyisoquinolin-1(2\textit{H})-one 64 (45 mg, 0.22 mmol) in EtOAc (4.4 mL) at r.t.. After 40 min stirring, the layers were separated and the aqueous layer washed with EtOAc (3 x 5 mL). The combined organic fractions were stirred with \textup{\textsuperscript{3}PrOH} (10 mL) until a black precipitate was formed after which the reaction was filtered and the solvent removed under reduced pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded the product 117 as a yellow solid (18 mg, 14 %).

R\textsubscript{f} [EtOAc] 0.63;

\textbf{Mp} [MeOH] Sublimes 175-180 °C, melts > 230 °C [Lit.,\textsuperscript{189} sublimes 165-175 °C, melts 275 °C];

\textbf{IR} \textup{\textnu}_{\max} (Thin film) 3354 (NH), 1747 (C=O), 1701 (C=O) and 1516 (C=C);

\textbf{\textsuperscript{1}H NMR} \delta (400 MHz, DMSO) 11.94 (1H, s, NH), 7.58 (1H, s, C(8)H), 7.51 (1H, s, C(5)H), 4.02 (3H, s, CH\textsubscript{3}) and 3.99 (3H, s, CH\textsubscript{3});

\textbf{\textsuperscript{13}C NMR} \delta (100 MHz, DMSO) 174.4– (C(4)\textsubscript{q}), 162.8– (C(1)\textsubscript{q}), 157.6– (C(3)\textsubscript{q}), 154.3– (C(7)\textsubscript{q}), 153.0– (C(6)\textsubscript{q}), 126.2– (C(4a)\textsubscript{q}), 124.3– (C(8a)\textsubscript{q}), 109.4+ (C(8)H), 108.0+ (C(5)H), 56.3+ (CH\textsubscript{3}) and 56.2+ (CH\textsubscript{3});

\textbf{MS} (+ESI) m/z 258 (100 %, [M+Na]\textsuperscript{+}), 493 (35 %, [2M+Na]\textsuperscript{+}) and 236 (15 %, [M+H]\textsuperscript{+});

\textbf{HRMS} (+ESI) m/z Found [M+H]\textsuperscript{+} 236.0789, C\textsubscript{11}H\textsubscript{10}NO\textsubscript{5} requires [M+H]\textsuperscript{+} 236.0559. Found [M+Na]\textsuperscript{+} 258.0368, C\textsubscript{11}H\textsubscript{9}NNaO\textsubscript{5} requires 258.0378 [M+Na]\textsuperscript{+}. 

- 145 -
3-Allyl-8-hydroxy-6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one (126)

LDA (6.11 mL, 11 mmol) was added dropwise to a stirred solution of 3-(3,4,5-trimethoxyphenyl)propanoic acid 124 (1.20 g, 5 mmol) in THF (5 mL) at 0 °C under N₂. After stirring for 40 min at 0 °C the reaction was allowed to warm to room temperature, allyl-bromide (490 µL, 5.5 mmol) was added and the reaction was left stirring for a further 18 h. The reaction was quenched with 6M HCl(aq) to pH 2 and extracted with EtOAc (3 x 10 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford the crude.

DPPA (1.05 mL, 5 mmol) was added dropwise to a stirred solution of crude (2-(3,4,5-trimethoxybenzyl)pent-4-enoic acid and anhydrous Et₃N (700 µL, 5 mmol) in anhydrous toluene (15 mL) under Ar. The reaction was heated at 90 °C for 90 min after which the reaction was concentrated under reduced pressure. Upon being put back under Ar, BF₃·OEt₂ (4 mL, 30 mmol) was added and the reaction left to stir for 16 h at room temperature. The reaction was quenched with 2M NaOH to pH 10, diluted with EtOAc (15 mL) and left stirring for 3 h. After cooling to room temperature the organic layer was removed and the aqueous fraction washed with EtOAc (3 x 15 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded the product 126 (460 mg, 35 %) as a white solid.

Rᶠ[EtOAc] 0.68;

Mp [EtOAc] 104-107 °C;

IR νₘₐₓ (KBr disc) 3685 (OH), 3583 (NH) and 1646 (C=O);

¹H NMR δH(500 MHz, CDCl₃) 12.33 (1H, s, OH), 6.23 (1H, s, C(5)H), 5.81-5.71 (2H, m, NH and C(2’)H), 5.24-5.18 (2H, m, C(3’)H₂), 3.88 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 3.73-3.68 (1H, m, C(3)H), 2.89-2.73 (2H, m, C(4)H₂), 2.46-2.40 (1H, m, C(1’)H) and 2.37-2.24 (1H, m, C(1’)H₂);
\(^{13}\text{C NMR}\) \(\delta_{C}\) (125 MHz, CDCl\(_3\)) 170.2– (C(1)_2), 157.0– (C\(_d\)), 155.8– (C\(_d\)), 135.2– (C\(_d\)), 134.0– (C\(_d\)), 132.8+ (C(2')H), 119.7– (C(3')H\(_2\)), 105.5– (C\(_d\)), 101.9+ (C(5)H), 60.6+ (OCH\(_3\)), 56.0+ (OCH\(_3\)), 50.3+ (C(3)H), 39.6– (C(1')H\(_2\)) and 34.1– (C(4)H\(_2\));

**MS** (+ESI) \(m/z\) 549 (100 %, [2M+Na]\(^+\)), 286 (89 %, [M+Na]\(^+\)) and 264 (38 %, [M+H]\(^+\));

**HRMS** (+ESI) \(m/z\) Found [M+H]\(^+\) 264.1319, \(\text{C}_{14}\text{H}_{18}\text{NO}_4\), requires [M+H]\(^+\) 264.1236. Found [M+Na]\(^+\) 286.1166, \(\text{C}_{14}\text{H}_{17}\text{NNaO}_4\), requires [M+Na]\(^+\) 286.1055;

**Elemental Analysis** Found C 63.78 %, H 6.50 % and N 5.37 %, requires C 63.87 %, H 6.51 % and N 5.32 %.
3-Allyl-6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one (128)

3-(3,4-Dimethoxyphenyl)propanoic acid 56 (1.05 g, 5 mmol) in THF (5 mL) was added dropwise to a stirred solution of LDA (6.11 mL, 11 mmol) at 0 °C under N₂. After stirring for 40 min at 0 °C the reaction was allowed to warm to room temperature, allyl-bromide (490 µL, 5.5 mmol) was added and the reaction was left stirring for a further 18 h at 65 °C. The reaction was quenched with 6M HCl(aq) to pH 2 and extracted with EtOAc (3 x 10 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford the product as a crude.

DPPA (1.05 mL, 5 mmol) was added dropwise to a stirred solution of crude (2-(3,4-dimethoxybenzyl)pent-4-enolic acid and anhydrous Et₃N (700 µL, 5 mmol) in anhydrous toluene (15 mL) under Ar. The reaction was heated at 90 °C for 90 min after which the reaction was concentrated under reduced pressure. Upon being put back under Ar, BF₃·OEt₂ (4 mL, 30 mmol) was added and the reaction left to stir for 16 h at 50 °C. The reaction was quenched with 2M NaOH to pH 10, diluted with EtOAc (15 mL) and left stirring for 1 h. After cooling to room temperature the organic layer was removed and the aqueous fraction washed with EtOAc (3 x 15 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded the product 128 (450 mg, 36 %) as a yellow solid.

Rf [EtOAc] 0.50;

Mp [EtOAc] 194-196 °C;

IR νmax (KBr disc) 3583 (NH) and 1658 (C=O);

¹H NMR δH(500 MHz, CDCl₃) 7.56 (1H, s, C(8)H), 6.65 (1H, s, C(5)H), 5.82-5.71 (1H, m, C(2’)H), 5.67 (1H, brs, NH), 5.23 (1H, s, C(3’)H), 5.20-5.19 (1H, m, C(3’)H), 3.92 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 3.77-3.70 (1H, m, C(3)H), 2.89-2.76 (2H, m, C(4)H₂), 2.46-2.40 (1H, m, C(1’)H₃) and 2.33-2.25 (1H, m, C(1’)H₃);
\textbf{\textit{\textsuperscript{13}C} NMR} \( \delta_c(125 \text{ MHz, CDCl}_3) \) 166.0~(C(1)\text{q}), 152.3~(C(7)\text{q}), 148.1~(C(6)\text{q}), 133.1~(C(2')\text{H}), 131.6~(C(8a)\text{q}), 121.1~(C(4a)\text{q}), 119.4~(C(3')\text{H}₂), 110.2~(C(8)\text{H}), 109.7~(C(5)\text{H}), 56.1~(\text{OCH}_3), 50.6+ (C(3)\text{H}), 39.9~(C(1')\text{H}₂) and 34.0~(C(4)\text{H}₂);

\textbf{MS (ESI)} \textit{m/z} 517 (100 \%, [2M+Na]⁺), 270 (38 \%, [M+Na]⁺) and 248 (27 \%, [M+H]⁺);

\textbf{HRMS (ESI)} \textit{m/z} \textit{Found} [M+H]⁺ 248.1300, \textit{C_{14}H_{18}NO₃}, \textit{requires} [M+H]⁺ 248.1287. \textit{Found} [M+Na]⁺ 270.1145, \textit{C_{14}H_{17}NNaO₃}, \textit{requires} [M+Na]⁺ 270.1106;

\textbf{Elemental Analysis} \textit{Found} C 67.93 \%, H 6.84 \% and N 5.64 \%, \textit{requires} C 68.00 \%, H 6.93 \% and N 5.66 \%.
3-(Benzo[d][1,3]dioxol-5-yl)propanoic acid 122 (0.97 g, 5 mmol) in THF (5 mL) was added dropwise to a stirred solution of LDA (6.11 mL, 11 mmol) at 0 °C under N₂. After stirring for 40 min at 0 °C the reaction was allowed to warm to room temperature, allyl-bromide (490 µL, 5.5 mmol) was added and the reaction was left stirring for a further 18 h. The reaction was quenched with 6M HCl\(_{\text{aq}}\) to pH 2 and extracted with EtOAc (3 x 10 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford the crude.

DPPA (1.05 mL, 5 mmol) was added dropwise to a stirred solution of crude (2-(benzo[d][1,3]dioxol-5-ylmethyl)pent-4-enoic acid and anhydrous Et₃N (700 µL, 5 mmol) in anhydrous toluene (15 mL) under Ar. The reaction was heated at 90 °C for 90 min after which the reaction was concentrated under reduced pressure. Upon being put back under Ar, BF₃∙OEt₂ (4 mL, 30 mmol) was added and the reaction left to stir for 16 h at room temperature. The reaction was quenched with 2M NaOH to pH 10, diluted with EtOAc (15 mL) and left stirring for 1 h. After cooling to room temperature the organic layer was removed and the aqueous fraction washed with EtOAc (3 x 15 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded the product 129 (590 mg, 51 %) as a white solid.

\[ R_f \text{[EtOAc]} 0.69; \]

\[ M_p \text{[EtOAc]} 118-121 ^\circ C; \]

\[ \text{IR } \nu_{\text{max}} \text{ (KBr disc)} 3583 \text{ (NH) and } 1659 \text{ (C=O);} \]

\[ ^1H \text{ NMR } \delta_n(500 \text{ MHz, CDCl}_3) 7.49 \text{ (1H, s, C(8)H), 6.61 (1H, s, C(5)H), 5.99 (2H, dd, } J \text{ 1.0 and 3.0, OCH}_2\text{O), 5.81-5.70 (2H, m, NH and C(2')H), 5.17 (1H, s, C(3')H), 5.14-5.13 (1H, m, C(3')H), 3.74-3.66 (1H, m, C(3)H), 2.86-2.73 (2H, m, C(4)H₂), 2.45-2.37 (1H, m, C(1')H₂) and 2.31-2.23 (1H, m, C(1')H₂); \]
\(^{13}\text{C NMR}\) \(\delta_{(125 \text{ MHz, CDCl}_3)} 165.8– (\text{C(1)}_3), 150.8– (\text{C}_4), 145.8– (\text{C}_3), 133.5– (\text{C}_4), 133.2+ (\text{C(2')}\text{H}), 122.6– (\text{C}_4), 119.1– (\text{C(3')}\text{H}_2), 107.7+ (\text{C(8)}\text{H}), 107.3+ (\text{C(5)}\text{H}), 101.4– (\text{OCH}_2\text{O}), 50.4+ (\text{C(3)}\text{H}), 39.6– (\text{C(1')}\text{H}_2)\) and 34.1– (\text{C(4)}\text{H}_2);

\text{MS (}^{+}\text{ESI)} m/z 485 (100 \%, [2\text{M+Na}]^+), 254 (29 \%, [\text{M+Na}]^+) and 232 (21 \%, [\text{M+H}]^+);

\text{HRMS (}^{+}\text{ESI)} m/z \text{ Found [M+H]}^+ 232.0992, \text{ C}_{13}\text{H}_{14}\text{NO}_3, \text{ requires [M+H]}^+ 232.0974. \text{ Found [M+Na]}^+ 254.0839, \text{ C}_{13}\text{H}_{13}\text{NNaO}_3, \text{ requires [M+Na]}^+ 254.0793;

\text{Elemental Analysis Found C 67.65 \%, H 5.62 \% and N 6.09 \%, requires C 67.52 \%, H 5.67 \% and N 6.06 \%.}
6.4. Synthesis of ABC-ring analogues

Ethyl 2-(1,3-benzodioxol-5-ylcarbonyl)-5-oxohexanoate (142)

NaH (60 % dispersed in mineral oil) (1.20 g, 30 mmol) was added portion wise to a rapidly stirred solution of 3',4'-[methylenedioxy]acetophenone 134 (3.28 g, 20 mmol) in diethyl carbonate (40 mL) at 0 °C under N₂. The reaction was heated to 80 °C for 90 min. Upon cooling to r.t. the reaction was quenched with sat. NH₄Cl (aq) (25 mL), diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with sat. brine (50 mL), dried over Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the crude product 135 was isolated as a yellow oil without further purification.

K₂CO₃ (3.06 g, 22.2 mmol) was added to a rapidly stirred solution of ethyl 3-(1,3-benzodioxol-5-yl)-3-oxopropanoate 135 (4.72 g, 20 mmol) in anhydrous MeCN (90 mL) at 0 °C under Ar. After 10 min vigorous stirring MVK (1.8 mL, 22.2 mmol) was added drop wise and the reaction mixture was allowed to warm to r.t.. After 90 min the reaction was quenched with saturated sat. NH₄Cl (aq) (15 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with sat. brine (50 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. After column chromatography [silica, PE: EtOAc gradient column] the product 142 (4.8 g, 79 %) was isolated as a yellow oil.

Rᶠ [PE:EtOAc, 50:50] 0.53;

IR ν max (thin film) 2983 (CH), 1736 (C=O), 1715 (C=O), 1676 (C=O) and 1605 (C=C);

¹H NMR δ (400 MHz, CDCl₃) 7.64 (1H, dd, J 8.0 and 2.0 Hz, C(6’))H, 7.48 (1H, d, J 2.0 Hz, C(2’))H, 6.85 (1H, d, J 8.0 Hz, C(5’))H, 6.04 (2H, s, OCH₂O), 4.33 (1H, d, J 6.0 Hz, C(2))H, 4.14 (2H, ddd, J 3.0, 7.0...
and 14.0 Hz, OCH₂CH₃), 2.63-2.48 (2H, m, C(4)H₂), 2.27-2.17 (2H, m, C(3)H₂), 2.11 (3H, s, C(6)H₃) and 1.18 (3H, t, J 7.0 Hz, OCH₂CH₃);

¹³C NMR δC (100 MHz, CDCl₃) 207.8- (C(=O)q), 193.1- (C(=O)q), 169.8- (C(=O)q), 152.2- (C₉), 148.3- (C₉), 130.8- (C(1')q), 125.2+ (C(6')H), 108.3+ (C(5')H), 107.9+ (C(2')H), 101.9- (OCH₂O), 61.3- (OCH₂CH₃), 52.4+ (C(2)H), 40.5- (C(4)H₂), 29.9+ (C(6)H₃), 22.9- (C(3)H₂) and 13.9+ (OCH₂CH₃);

MS (+ESI) m/z 329 (100 %, [M+Na]⁺), 635 (55 %, [2M+Na]⁺) and 307 (28 %, [M+H]⁺);

Ethyl 2-{1′,3′-benzodioxol-5-yl)-4-oxocyclohex-2-ene-1-carboxylate (139)

\[
\begin{align*}
\text{Et}_2\text{C}=\text{C} & \quad \text{pTsOH.H}_2\text{O (40 mol %)} \\
\text{Toluene} & \quad 143 ^\circ\text{C oil bath, N}_2, 24 \text{ h} \\
\end{align*}
\]

\[\text{142} \quad \text{139} \quad 53 \%
\]

\(\text{pTsOH.H}_2\text{O (494 mg, 2.6 mmol)}\) in toluene (75 mL) was stirred under Ar for 90 min with the oil bath at 143 \(^\circ\text{C}\) and the azeotropic removal of water using Soxhlet equipment. Ethyl 2-{1′,3′-benzodioxol-5-ylcarbonyl)-5-oxohexanoate 142 (1.99 g, 6.5 mmol) in toluene (10 mL) was added and the reaction heated for a further 24 h. After cooling the toluene was removed under reduced pressure to near dryness and loaded onto a column. After column chromatography [silica, PE: EtOAc gradient column] the purified product 139 (993 mg, 53 \%) was isolated as a light yellow amorphous solid.

\[R_f \ [\text{PE:EtOAc, 50:50}] 0.35;\]

\[\text{MP} \ [\text{EtOAc}] 107-110 ^\circ\text{C};\]

\[\text{\textsuperscript{1}H NMR} \ \delta_{\text{H}}(400 \text{ MHz, CDCl}_3) 7.02 (1\text{H}, \text{dd, } J 8.0 \text{ and } 2.0 \text{ Hz, C(6')H}), 6.99 (1\text{H, d, } J 2.0 \text{ Hz, C(2')H}), 6.82 (1\text{H, d, } J 8.0 \text{ Hz, C(S')H}), 6.38 (1\text{H, s, C(3)H}), 6.00 (2\text{H, s, OCH}_2\text{O}), 4.12 (2\text{H, q, } J 7.0 \text{ Hz, OCH}_2\text{CH}_3), 3.88 (1\text{H, m, C(1)H}), 2.66-2.56 (1\text{H, m, C(4)H}_2), 2.50-2.41 (2\text{H, m, C(6)H}_2 \text{ and C(5)H}_2), 2.40-2.31 (1\text{H, m, C(6)H}_2) \text{ and } 1.18 (3\text{H, t, } J 7.0 \text{ Hz, OCH}_2\text{CH}_3);\]

\[\text{\textsuperscript{13}C NMR} \ \delta_{\text{C}}(100 \text{ MHz, CDCl}_3) 198.4+ (\text{C}(=\text{O})_\text{q}), 171.5+ (\text{C}(=\text{O})_\text{q}), 154.7+ (\text{C}_q), 149.4+ (\text{C}_q), 148.3+ (\text{C}_q), 131.8+ (\text{C}_q), 126.0– (\text{C(3)H}), 120.9– (\text{C(6')H}), 108.4– (\text{C(S')H}), 106.5– (\text{C(2')H}), 101.6+ (\text{OCH}_2\text{O}), 61.4+ (\text{OCH}_2\text{CH}_3), 43.6– (\text{C(1)H}), 33.9+ (\text{C(5)H}_2), 26.5+ (\text{C(6)H}_2) \text{ and } 14.0– (\text{OCH}_2\text{CH}_3);\]

\[\text{MS (+ESI) } m/z \ 289 (100 \%, \ [\text{M+H}]^+), 599 (72 \%, [2\text{M+Na}]^+) \text{ and } 311 (43 \%, [\text{M+Na}]^+);\]

\[\text{HRMS (+ESI) } m/z \text{ Found } [\text{M+H}]^+ 289.1180, \text{ C}_{16}\text{H}_{17}\text{O}_5, \text{ requires } [\text{M+H}]^+ 289.1076. \text{ Found } [\text{M+Na}]^+ 311.0993, \text{ C}_{16}\text{H}_{16}\text{NaO}_5, \text{ requires } [\text{M+Na}]^+ 311.0895.\]
NaBH₄ (378 mg, 10 mmol) was added portion wise over 3 min to a rapidly stirred solution of TFA (2.4 mL), AcOH (2.4 mL) and anhydrous MeCN (2.4 mL) at 0 °C under Ar and effervescent was observed. Ethyl 2-{1',3'-benzodioxol-5-yl}-4-oxocyclohex-2-ene-1-carboxylate 139 (576 mg, 2.0 mmol) in CH₂Cl₂ (12 mL) was added at 0 °C and the reaction was allowed to warm to r.t. over 4 h. The reaction was quenched with 1M NaOH(aq) to pH 6 and extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. After column chromatography [silica, PE: EtOAc gradient column] the product 144 (541 mg, 99 %) was isolated as a pale yellow oil.

Rf[PE:EtOAc, 60:40] 0.57;

IR νmax (thin film) 2936 (OH), 1731 (C=O) and 1505 (C=C);

¹H NMR δH(400 MHz, CDCl₃), 6.83 (1H, d, J 2.0 Hz, C(2')H), 6.76 (1H, dd, J 8.0 and 2.0 Hz, C(6')H), 6.72 (1H, d, J 8.0 Hz, C(5')H), 6.08 (1H, dt, J 4.0 and 1.0 Hz, C(3)H), 5.91 (2H, s, OCH₂O), 4.05-3.99 (2H, m, OCH₂CH₃), 3.61-3.58 (1H, m, C(1)H), 2.31-2.13 (2H, m, C(4)H₂), 2.07-1.93 (2H, m, C(6)H₂), 1.81-1.71 (1H, m, C(5)H₂), 1.69-1.60 (1H, m, C(5)H₂) and 1.10 (3H, t, J 7.0 Hz, OCH₂CH₃);

¹³C NMR δC(100 MHz, CDCl₃) 174.6+ (C(=O)q), 147.5+ (Cq), 146.4+ (Ca), 136.2+ (Ca), 127.4+ (C(3)H), 118.8+ (C(2')H), 107.9+ (C(5')H), 106.4+ (C(6')H), 100.8+ (OCH₂O), 60.3+ (OCH₂CH₃), 43.7– (C(1)H), 27.0+ (C(6)H₂), 25.5+ (C(4)H₂), 19.3+ (C(5)H₂) and 14.0– (OCH₂CH₃);

MS (+ESI) m/z 297 (100 %, [M+Na]+), 571 (38 %, [2M+Na]+) and 275 (30 %, [M+H]+);

2-(1',3'-Benzodioxol-5-yl)cyclohex-2-ene-1-carboxylic acid (145)

A rapidly stirred solution of ethyl 2-(1',3'-benzodioxol-5-yl)cyclohex-2-ene-1-carboxylate 144 (263 mg, 0.96 mmol) in 1M NaOH\(_{aq}\): EtOH (1:1, 6 mL) was heated at reflux for 6 h. The solution was then cooled to 0 °C, acidified to pH 2 using 6M HCl\(_{aq}\) and extracted with EtOAc (3 × 15 mL). The combined organic fractions were washed with sat. brine (10 mL), dried over Na\(_2\)SO\(_4\), filtered and the solvent removed under reduced pressure to give the product 145 (237 mg, 100 %) as a white solid without further purification.

**MP** [EtOAc] 122-126 °C;

**IR** \(\nu_{\text{max}}\) (KBr disc) 2935 (OH), 1703 (C=O) and 1516 (C=C);

**\(^1H\) NMR** \(\delta_H\) (400 MHz, CDCl\(_3\)) 6.83 (1H, d, \(J=2.0\) Hz, C(2')H), 6.78 (1H, dd, \(J=8.0\) and 2.0 Hz, C(6')H), 6.73 (1H, d, \(J=8.0\) Hz, C(5')H), 6.12 (1H, t, \(J=4.0\) Hz C(3)H), 5.93 (2H, s, OCH\(_2\)O), 3.64 (1H, s, C(1)H), 2.25-2.20 (2H, m, C(4)H\(_2\)), 2.18-2.09 (1H, m, C(5)H\(_2\)), 2.02-1.94 (1H, m, C(5)H\(_2\)) and 1.83-1.64 (2H, m, C(6)H\(_2\));

**\(^{13}C\) NMR** \(\delta_C\) (100 MHz, CDCl\(_3\)) 179.0+ (C(=O)\(_3\)), 147.7+ (C\(_3\)), 146.5+ (C\(_4\)), 135.8+ (C\(_5\)), 133.5+ (C\(_6\)), 127.9– (C(3)H), 118.7– (C(5')H), 108.0– (C(6')H), 106.3– (C(2')H), 100.9+ (OCH\(_2\)O), 42.9– (C(1)H), 26.9+ (C(5)H\(_2\)), 25.4+ (C(4)H\(_2\)) and 18.9+ (C(6)H\(_2\));

**MS** (+ESI) \(m/z\) 269 (100 %, [M+Na\(^+\)]), 247 (53 %, [M+H\(^+\)]), and 515 (30 %, [2M+Na\(^+\)]);

**HRMS** (+ESI) \(m/z\) Found [M+H\(^+\)] 247.0973, C\(_{14}\)H\(_{12}\)O\(_4\), requires [M+H\(^+\)] 247.0970. Found [M+Na\(^+\)] 269.0818, C\(_{14}\)H\(_{14}\)NaO\(_4\), requires [M+Na\(^+\)] 269.0790.
**3,4,4a,5-Tetrahydro[1,3]dioxolo[4,5-j]phenanthridin-6(2H)-one (148)**

DPPA (192 μL, 0.89 mmol) was added dropwise to a rapidly stirred solution of 2-\{1',3'-benzodioxol-5-yl\}cyclohex-2-ene-1-carboxylic acid 145 (199 mg, 0.81 mmol) and anhydrous Et$_3$N (124 μL, 0.89 mmol) in anhydrous toluene (3 mL) at r.t. under Ar. The reaction was heated at 90 °C for 90 min after which the reaction was allowed to cool and was concentrated under reduced pressure. The oil was placed back under Ar and BF$_3$∙OEt$_2$ (1 mL) was added. After stirring at r.t. for 16 h the reaction was quenched with 2M NaOH$_{(aq)}$ to pH 10, diluted with EtOAc (10 mL) and left stirring at 50 °C for 60 min. After cooling to r.t. the organic layer was separated and the aqueous fraction washed with EtOAc (3 × 5 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na$_2$SO$_4$, filtered and the solvent removed under pressure. After column chromatography [silica, PE: EtOAc: MeOH gradient column] the product 148 (113 g, 57 %) was isolated as a white crystalline solid.

**Rf [EtOAc] 0.30;**

**MP [EtOAc] 126-129 °C;**

**IR v$_{\text{max}}$ (KBr disc) 3176 (NH), 3055 (CH), 1674 (C=O) and 1615 (C=C);**

**$^1$H NMR δ$_{\text{H}}$(400 MHz, CDCl$_3$) 7.51 (1H, s, C(7)H), 6.89 (1H, s, C(10)H), 6.11 (1H, brt, J 2.0 Hz, C(1)H), 6.00 (2H, dd, J 7.5 and 1.5 Hz, OC$_2$H$_2$O), 5.58 (1H, brs, NH), 4.33 (1H, brs, C(4a)H), 2.39-2.22 (2H, m, C(2)H$_2$), 2.11-2.06 (1H, m, C(3)H$_2$), 1.91-1.87 (1H, m, C(4)H$_2$) and 1.73-1.64 (2H, m, C(3)H$_2$ and C(4)H$_2$);**

**$^{13}$C NMR δ$_{\text{C}}$(100 MHz, CDCl$_3$) 164.6+ (C(6)$_3$), 151.3+ (C$_3$), 147.6+ (C$_3$), 133.3+ (C$_3$), 130.9+ (C$_3$), 124.7– (C(1)H), 121.5+ (C$_3$), 107.4– (C(7 or 10)H), 102.7– (C(10 or 7)H), 101.5+ (OCH$_2$O), 50.7– (C(4a)H), 29.9+ (C(3)H$_2$), 25.7+ (C(2)H$_2$) and 20.1+ (C(4)H$_2$);**

**MS (+ESI) m/z 509 (100 %, [2M+Na]$^+$) and 244 (24 %, [M+H]$^+$);**

**HRMS (+ESI) m/z Found [M+H]$^+$ 244.1010, C$_{14}$H$_{12}$NO$_3$, requires [M+H]$^+$ 244.0974. Found [M+Na]$^+$ 266.0789, C$_{14}$H$_{13}$NNaO$_3$, requires [M+Na]$^+$ 266.0793.**
Ethyl 3-oxo-3-(3',4',5'-trimethoxyphenyl)propanoate (150)

NaH (60 % dispersed in mineral oil) (1.5 g, 37.5 mmol) was added portion wise to a rapidly stirred solution of 3,4,5-trimethoxyacetophenone 149 (5.25 g, 25 mmol) in diethyl carbonate (50 mL) at 0 °C under N₂. The reaction was heated to 80 °C for 90 min. Upon cooling to r.t. the reaction was quenched with sat. NH₄Cl(aq) (25 mL), diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with sat. brine (50 mL), dried over Na₂SO₄ and filtered. The solvent was removed under reduced pressure which was purified by column chromatography [silica, PE: EtOAc gradient column] giving the β-ketoester 150 (6.46 g, 92 %) as a pale yellow solid.

Rᵣ [PE:EtOAc, 50:50] 0.42;

Mp [EtOAc] 84-88 °C;

¹H NMR δ(H) (400 MHz, CDCl₃) 7.20 (2H, s, C(2')H and C(6')H), 4.20 (2H, q, J 7.0 Hz, OCH₂CH₃), 3.95 (2H, s, C(2)H₂), 3.91 (3H, s, OCH₃), 3.90 (6H, s, OCH₃) and 1.25 (3H, t, J 7.0 Hz, OCH₂CCH₃);

¹³C NMR δ(C) (100 MHz, CDCl₃) 191.2− (C(3)ₐ), 167.5− (C(1)ₐ), 153.1− (Cₐ), 143.1− (Cₐ), 131.1− (C(1')ₐ) 106.1+ (C(2')H and C(6')H) 61.5− (C(2)H₂), 61.0+ (OCH₃), 56.3+ (OCH₃), 46.1− (OCH₂CH₃) and 14.1+ (OCH₂CCH₃).

Spectroscopic data is consistent with that reported by Lawrence et al.¹⁹⁰
K₂CO₃ (1.16 g, 8.4 mmol) was added to a rapidly stirred solution of ethyl 3-oxo-3-(3',4',5'-trimethoxyphenyl)propanoate 150 (2.16 g, 7.66 mmol) in anhydrous MeCN (40 mL) at 0 °C under Ar. After 10 min vigorous rapidly stirred methyl vinyl ketone (MVK) (680 μL, 8.4 mmol) was added dropwise and the reaction mixture was allowed to warm to r.t.. After 90 min the reaction was quenched with saturated sat. NH₄Cl (aq) (15 mL) and extracted with EtOAc (3 × 30 mL). The combined organic fractions were washed with sat. brine (50 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. After column chromatography [silica, PE: EtOAc gradient column] the purified product 151 (2.50 g, 93 %) was isolated as a yellow oil.

Rᵥ[PE:EtoAc, 50:50] 0.52;

IR νₓmax (Thin film) 2979 (CH), 1731 (C=O), 1722 (C=O), 1681 (C=O), 1234 (C-O);

¹H NMR δₓH (400 MHz, CDCl₃) 7.36 (2H, s, C(2')H and C(6')H), 4.42 (1H, dd, J 8.5 and 6.0 Hz, C(2)H), 4.19-4.12 (2H, m, OCH₂CH₃), 3.93 (6H, s, OCH₃), 3.91 (3H, s, OCH₃), 2.68-2.52 (2H, m, C(4)H₂), 2.29-2.15 (2H, m, C(3)H₂), 2.13 (3H, s, C(6)H₃) and 1.20 (3H, t, J 7.5 Hz, OCH₂CH₃);

¹³C NMR δₓC (100 MHz, CDCl₃) 208.0– (C₉), 194.2– (C₆), 170.0– (C₅), 153.1– (C₄), 143.0– (C₃), 130.8– (C₄'), 106.2+ (C(2')H and C(6')H), 61.4– (OCH₂CH₃), 60.9+ (OCH₃), 56.3+ (OCH₃), 52.6+ (C(2)H), 40.5– (C(4)H₂), 30.0+ (C(6)H₃), 23.0– (C(3)H₂) and 14.0+ (OCH₂CH₃);

MS (+ESI) m/z 353 (100 %, [M+H]+) and 375 (69 %, [M+Na]+);

HRMS (+ESI) m/z Found [M+H]+ 353.1601, C₁₈H₂₅O₇, requires [M+H]+ 353.1600.

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Ethyl 5-oxo-2-(3',4',5'-trimethoxybenzoyl)hexanoate (151)
Ethyl 4-oxo-2-(3',4',5'-trimethoxyphenyl)cyclohex-2-ene-1-carboxylate (152)

\[ \text{TsOH.H}_2\text{O (40 mol %)} \]\n\[ \text{Toluene} \quad \text{N}_2, \quad 143 \, ^\circ\text{C oil bath, 24 h} \]

\[ \text{151} \quad \text{152} \]

$p$TsOH.H$_2$O (380 mg, 2.0 mmol) in toluene (70 mL) was stirred under Ar for 90 min with the oil bath at 143 °C with the azeotropic removal of water using Soxhlet equipment. Ethyl 5-oxo-2-(3',4',5'-trimethoxybenzoyl)hexanoate 151 (1.76 g, 5.0 mmol) in toluene (10 mL) was added and the reaction heated for a further 24 h. After cooling the toluene was removed under reduced pressure to near dryness and loaded onto a column. After column chromatography [silica, PE: EtOAc gradient column] the purified product 152 (668 mg, 40 %) was isolated as a light yellow amorphous solid.

$R_f$ [PE:EtOAc, 50:50] 0.53;

IR $\nu_{\text{max}}$ (Thin film) 2938 (CH), 1728 (C=O), 1661 (C=O), 1518 (C=C) and 1252 (C-O);

$^1$H NMR $\delta$ (400 MHz, CDCl$_3$) 6.73 (2H, s, C(2')H and C(6')H), 6.44 (1H, s, C(3)H), 4.15-4.08 (2H, m, OCH$_2$CH$_3$), 3.94-3.92 (1H, m, C(1)H), 3.87 (9H, s, 3 × OCH$_3$), 2.68-2.61 (1H, m, C(5)H$_2$), 2.51-2.38 (3H, m, C(5)H$_2$ and C(6)H$_2$) and 1.14 (3H, t, $J$ 7.5 Hz, OCH$_2$CH$_3$);

$^{13}$C NMR $\delta$ (100 MHz, CDCl$_3$) 198.5–(C=O)$_3$, 171.7–(C=O)$_3$, 155.3–(C(1')$_3$), 153.3–(C(3')$_3$), 139.9–(C(4')$_3$), 133.2–(C(1)q), 126.7–(C(3)H), 103.8+ (C(2')H) 61.5–(OCH$_2$CH$_3$) 60.9+ (OCH$_3$) 56.2+ (OCH$_3$) 43.7+ (OCH$_3$) 34.0–(C(5)H$_2$), 26.6–(C(6)H$_2$), 14.1+ (OCH$_2$CH$_3$);

MS (+ESI) $m/z$ 335 (100 %, [M+H]$^+$);

HRMS (+ESI) $m/z$ Found [M+H]$^+$ 335.1495, C$_{18}$H$_{23}$O$_6$ requires [M+H]$^+$ 335.1495.

- 160 -
Ethyl 2-(3',4',5'-trimethoxyphenyl)cyclohex-2-ene-1-carboxylate (153)

NaBH₄ (378 mg, 10 mmol) was added portion wise over 3 min to a rapidly stirred solution of TFA (2.4 mL), AcOH (2.4 mL) and anhydrous MeCN (2.4 mL) at 0 °C under Ar and effervescent was observed. The ethyl 4-oxo-2-(3',4',5'-trimethoxyphenyl)cyclohex-2-ene-1-carboxylate (152) (668 mg, 2 mmol) in CH₂Cl₂ (15 mL) was added at 0 °C and the reaction was allowed to warm to r.t. and was stirred for 4 h. The reaction was quenched with 1M NaOH (aq) to pH 6 and extracted with EtOAc (3 × 15 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. After column chromatography [silica, PE: EtOAc gradient column] the product 153 (514 mg, 80 %) was isolated as a pale yellow solid.

Rᶠ [PE:EtOAc, 50:50] 0.50;

Mp [EtOAc] 75-80 °C;

IR νmax (Thin film) 2937 (CH), 1731 (C=O) and 1243 (C–O);

¹H NMR δH (400 MHz, CDCl₃) 6.53 (2H, s, C(2')H and C(6')H), 6.16 (1H, t, J 4.0 Hz, C(3)H), 4.09-3.95 (2H, m, OCH₂CH₃), 3.84 (6H, s, 2 × OCH₃), 3.81 (3H, s, OCH₃), 3.65-3.63 (1H, m, C(1)H), 2.32-2.18 (2H, m, C(4)H₂), 2.06-1.97 (2H, m, C(6)H₂), 1.84-1.74 (1H, m, C(5)H₂), 1.70-1.61 (1H, m, C(5)H₂) and 1.08 (3H, td, J 7.0 and 0.5 Hz, CH₂C₃H₇);

¹³C NMR δc (100 MHz, CDCl₃) 174.6– (C(=O)₀), 152.9– (C(3')ₐ and C(5')ₐ), 137.5– (C(1' or 4')ₐ), 137.0– (C(4' or 1')ₐ), 134.8– (C(2)ₐ), 128.0+ (C(3)H), 102.8+ (C(2')H and C(6')H), 60.8+ (OCH₃), 60.4– (OCH₂CH₃), 65.6+ (2 × OCH₃), 43.8+ (C(1)H), 27.0– (C(6)H₂), 25.4– (C(4)H₂), 19.4– (C(5)H₂) and 14.0+ (OCH₂CH₃);

MS (+ESI) m/z 321 (100 %, [M+H]⁺);

A rapidly stirred solution of ethyl 2-(3',4',5'-trimethoxyphenyl)cyclohex-2-ene-1-carboxylate 153 (512 mg, 1.6 mmol) in 1M NaOH(aq) : EtOH (1: 1, 10 mL) was heated at reflux for 6 h. The solution was then cooled to 0 °C, acidified to pH 2 using 6M HCl(aq) and extracted with EtOAc (3 × 15 mL). The combined organic fractions were washed with sat. brine (10 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure to give the product 154 (454 mg, 97 %) as a white solid without further purification.

Mp [CHCl₃] 143-147 °C;

IR νmax (Thin film) 3161 (OH), 2937 (CH) and 1073 (C=O);

¹H NMR δH(400 MHz, CDCl₃) 6.54 (2H, s, C(2')H and C(6')H), 6.20 (1H, td, J 4.0 and 1.0 Hz, C(3)H), 3.83 (6H, s, C(3')OCH₃ and C(5')OCH₃), 3.82 (3H, s, C(4')OCH₃), 3.68 (1H, brt, J 4.5 Hz, C(1)H), 2.32-2.20 (2H, m, C(4)H₂), 2.13-2.08 (1H, m, C(6)H₂), 2.05-1.97 (1H, m, C(6)H₂) and 1.82-1.64 (2H, m, C(5)H₂);

¹³C NMR δC(100 MHz, CDCl₃) 180.1– (C(=O)ₗ), 153.0– (C(3')q and C(5')q), 137.2– (C(1')q), 137.1– (C(4')q), 133.9– (C(2')q), 128.6+ (C(3)H), 102.7+ (C(2')H and C(6')H), 60.8+ (C(4')OCH₃), 56.0+ (C(3')OCH₃ and C(5')OCH₃), 42.9+ (C(1)H), 26.9– (C(6)H₂), 25.4– (C(4)H₂) and 19.0– (C(5)H₂);

MS (+ESI) m/z 293 (100 %, [M+H]+) and 315 (20 %, [M+Na]+);

Diphenylphosphoryl azide (DPPA) (106 μL, 0.49 mmol) was added dropwise to a rapidly stirred solution of 2-(3',4',5'-trimethoxyphenyl)cyclohex-2-ene-1-carboxylic acid 154 (131 mg, 0.45 mmol) and anhydrous Et₃N (68 μL, 0.49 mmol) in anhydrous toluene (1.5 mL) at r.t. under Ar. The reaction was heated at 90 °C for 90 min after which the reaction was allowed to cool and was concentrated under reduced pressure. The oil was placed under Ar and BF₃·OEt₂ (1.5 mL) was added and the reaction left stirring for 16 h at 50 °C. After cooling to r.t. the reaction was quenched with 2M NaOH (aq) to pH 10, diluted with EtOAc (5 mL) and left stirring at 50 °C for 4 h. After cooling to r.t. the organic layer was separated and the aqueous fraction washed with EtOAc (3 × 5 mL). The combined organic fractions were washed with sat. brine (10 mL), dried over Na₂SO₄, filtered and the solvent removed under pressure. After column chromatography [silica, PE: EtOAc: MeOH gradient column] the product 155 (50 mg, 40 %) was isolated as a white solid.

Rᵣ [PE:EtOAc, 50:50] 0.37;

Mp [EtOAc] 202-205 °C;

IR νₘₐₓ (Thin film) 3417 (OH and NH), 2935 (CH) and 1651 (C=O);

¹H NMR δₓ (400 MHz, CDCl₃) 12.67 (1H, brs, OH), 6.51 (1H, s, C(10)H), 6.23 (1H, t, J 2.5 Hz, C(1)H), 5.93 (1H, brs, NH), 4.33-4.31 (1H, m, C(4a)H), 3.90 (3H, m, C9OC₃H₃), 3.88 (3H, m, C8OC₃H₃), 2.23-2.41 (2H, m, C(2)H₂), 2.10-2.14 (1H, m, C(4)H₂), 1.88-1.92 (1H, m, C(3)H₂) and 1.57-1.73 (2H, m, C(3)H₂ and C(4)H₂);

¹³C NMR δₖ (100 MHz, CDCl₃) 169.7– (C(6)q), 157.2– (C(9)q), 155.5– (C(7)q), 136.3– (C(8)q), 133.7– (C(10b)q), 130.4– (C(10a)q), 125.9+ (C(1)H), 104.3– (C(6a)q), 97.4+ (C(10)H), 60.7+ (C(8)OCH₃), 50.7+ (C(4a)H), 29.6– (C(4)H₂), 25.9– (C(2)H₂) and 20.0– (C(3)H₂);

MS (+ESI) m/z 276 (100 %, [M+H]+) and 298 (13 %, [M+Na]+);

Ethyl 3-(3’,4’-dimethoxyphenyl)-3-oxopropanoate (157)

NaH (60 % dispersed in mineral oil) (1.20 g, 30 mmol) was added portion wise to a rapidly stirred solution of 3,4-dimethoxyacetophenone 156 (3.6 g, 20 mmol) in diethyl carbonate (40 mL) at 0 °C under N₂. The reaction was heated to 80 °C for 90 min. Upon cooling to r.t. the reaction was quenched with sat. NH₄Cl (aq) (20 mL), diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with sat. brine (50 mL), dried over Na₂SO₄ and filtered. The solvent was removed under reduced pressure which was purified by column chromatography [silica, PE: EtOAc gradient column] giving the β-ketoester 157 (4.79 g, 95 %) as a yellow oil.

³¹H NMR δ (400 MHz, CDCl₃) 7.51 (1H, dd, J 8.0 and 2.0 Hz, C(6’)H), 7.49 (1H, d, J 2.0 Hz, C(2’)H), 6.85 (1H, d, J 8.0 Hz, C(5’)H), 4.17 (2H, q, J 7.0 Hz, OCH₂CH₃), 3.91 (5H, s, OCH₃ and C(2)H), 3.89 (3H, s, OCH₃) and 1.22 (3H, t, J 7.0 Hz, OCH₂CH₃);

¹³C NMR δ (100 MHz, CDCl₃) 190.9− (C(1)q), 167.6− (C(3)q), 153.7− (C(3’ or 4’)q), 149.0− (C(4’ or 3’)q), 129.2− (C(1’)q), 123.4+ (C(6’)H), 110.2+ (C(2’ or 5’)H), 110.0+ (C(5’ or 2’)H), 61.3− (OCH₂CH₃), 56.0+ (OCH₃), 55.9+ (OCH₃), 45.6− (C(2)H₂) and 14.0+ (OCH₂CH₃).

Spectroscopic data is consistent with that reported by Korsager et al.¹⁹¹
Ethyl 2-(3',4'-dimethoxybenzoyl)-5-oxohexanoate (158)

K₂CO₃ (800 mg, 5.8 mmol) was added to a rapidly stirred solution of ethyl 3-(3',4'-dimethoxyphenyl)-3-oxopropanoate 157 (1.34 g, 5.3 mmol) in anhydrous MeCN (20 mL) at 0 °C under Ar. After 10 min vigorous stirring MVK (470 μL, 5.8 mmol) was added drop wise and the reaction mixture was allowed to warm to r.t.. After 90 min the reaction was quenched with saturated sat. NH₄Cl(aq) (20 mL) and extracted with EtOAc (3 × 30 mL). The combined organic fractions were washed with sat. brine (50 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. After column chromatography [silica, PE: EtOAc gradient column] the product 158 (1.6 g, 94 %) was isolated as a yellow oil.

Rₛ [PE:EtOAc, 50:50] 0.52;

IR νₘₐₓ (Thin film) 1731 (C=O), 1721 (C=O), 1673 (C=O) and 1594 (C=C);

¹H NMR δH (400 MHz, CDCl₃) 7.71 (1H, dd, J 8.5 and 2.0 Hz, C(6’)H), 7.60 (1H, d, J 2.0 Hz, C(2’)H), 6.91 (1H, d, J 8.5 Hz, C(5’)H), 4.42 (1H, dd, J 8.0 and 6.0 Hz, C(2)H), 4.14 (2H, qd, J 7.0 and 2.0 Hz, OCH₂CH₃), 3.95 (6H, s, OCH₃), 2.59 (2H, qt, J 18.0 and 7.0 Hz, C(4)H₂), 2.29-2.13 (2H, m, C(3)H₂), 2.13 (3H, s, C(6)H₃) and 1.18 (3H, t, J 7.0 Hz, OCH₂CH₃);

¹³C NMR δC (100 MHz, CDCl₃) 208.0–(C(5)ₐ), 193.8–(C(1)ₐ), 170.0–(C(=O)ₐ), 153.7–(C(4’)ₐ), 149.1–(C(3’)ₐ), 128.9–(C(1’)ₐ), 123.6+ (C(6’)H), 110.6+ (C(2’)H), 110.0+ (C(5’)H), 61.3–(OCH₃CH₃), 56.1+ (OCH₃), 56.0+ (OCH₃), 52.2+ (C(2)H), 40.5– (C(4)H₂), 30.0+ (C(6)H₃), 22.9– (C(3)H₂) and 14.0+ (OCH₂CH₃);

MS (+ESI) m/z 345 (100 %, [M+Na]*) and 323 (29 %, [M+H]*);

Ethyl 2-(3',4'-dimethoxyphenyl)-4-oxocyclohex-2-ene-1-carboxylate (159)

\[
\begin{align*}
\text{158} & \quad \xrightarrow{\text{pTsOH.H}_2\text{O (40 mol %)}} \quad \text{159} \\
\text{Toluene} & \quad \text{N}_2, \quad 143 \, ^\circ\text{C oil bath, 24 h} & \text{159} \quad 68 \%
\end{align*}
\]

\(\text{pTsOH.H}_2\text{O (304 mg, 1.6 mmol)}\) in toluene (40 mL) was stirred under Ar for 90 min with the oil bath at 143 °C with the azeotropic removal of water using Soxhlet equipment. Ethyl 2-(3',4'-dimethoxybenzoyl)-5-oxohexanoate 158 (1.26 g, 3.9 mmol) in toluene (15 mL) was added and the reaction heated for a further 24 h. After cooling the toluene was removed under reduced pressure to near dryness and loaded onto a column. After column chromatography [silica, PE: EtOAc gradient column] the purified product 159 (811 mg, 68 %) was isolated as a light yellow amorphous solid.

\(R_f\) [PE:EtOAc, 50:50] 0.30;

\text{IR } \nu_{\text{max}} \text{ (thin film)} 1728 (C=O), 1661 (C=O) and 1518 (C=C);

\text{\textsuperscript{1}H NMR } \delta_H (400 MHz, CDCl\textsubscript{3}) 7.10 (1H, dd, \textit{J} 8.5 and 2.0 Hz, C(6')\textsubscript{H}), 7.04 (1H, d, \textit{J} 2.0 Hz, C(2')\textsubscript{H}), 6.86 (1H, d, \textit{J} 8.5 Hz, C(5')\textsubscript{H}), 6.45 (1H, s, C(3)\textsubscript{H}), 4.11 (2H, q, \textit{J} 7.0 Hz, OCH\textsubscript{2}CH\textsubscript{3}), 3.96-3.94 (1H, m, C(1)\textsubscript{H}), 3.89 (3H, s, C(4')OC\textsubscript{H}\textsubscript{3}), 3.88 (3H, s, C(3')OC\textsubscript{H}\textsubscript{3}), 2.67-2.58 (1H, m, C(5)H\textsubscript{2} and C(5)H\textsubscript{2}), 1.14 (3H, t, \textit{J} 7.0 Hz, OCH\textsubscript{2}CH\textsubscript{3});

\text{\textsuperscript{13}C NMR } \delta_C (100 MHz, CDCl\textsubscript{3}) 198.6– (C(4)\textsubscript{C}), 171.7– (C(=O)\textsubscript{C}), 154.8– (C(2)\textsubscript{C}), 150.9– (C(4')\textsubscript{C}), 149.1– (C(3')\textsubscript{C}), 130.1– (C(1')\textsubscript{C}), 125.4+ (C(3)\textsubscript{C}), 119.6+ (C(6')\textsubscript{C}), 110.9+ (C(5')\textsubscript{C}), 109.1+ (C(2')\textsubscript{C}), 61.4– (OCH\textsubscript{2}CH\textsubscript{3}), 55.9+ (C(3')OCH\textsubscript{3} and C(4')OCH\textsubscript{3}), 43.3+ (C(1)\textsubscript{C}), 33.9– (C(5)H\textsubscript{2}), 26.5– (C(6)H\textsubscript{2}) and 14.0+ (OCH\textsubscript{2}CH\textsubscript{3});

\text{MS (+ESI)} m/z 305 (100 %, [M+H]\textsuperscript{+}) and 327 (9 %, [M+Na]\textsuperscript{+});

\text{HRMS (+ESI)} Found [M+Na]\textsuperscript{+} 324.1311, C\textsubscript{17}H\textsubscript{20}NaO\textsubscript{5}, requires 324.1208 [M+Na]\textsuperscript{+}. 

- 166 -
Ethyl 2-(3',4'-dimethoxyphenyl)cyclohex-2-ene-1-carboxylate (160)

NaBH₄ (491 mg, 13 mmol) was added portion wise over 3 min to a rapidly stirred solution of TFA (2.4 mL), AcOH (2.4 mL) and anhydrous MeCN (2.4 mL) at 0 °C under Ar and effervescent was observed. The ethyl 2-(3',4'-dimethoxyphenyl)-4-oxocyclohex-2-ene-1-carboxylate 159 (608 mg, 2.0 mmol) in CH₂Cl₂ (15 mL) was added at 0 °C and the reaction was allowed to warm to r.t. and was stirred for 4 h. The reaction was quenched with 1M NaOH (aq) to pH 6 and extracted with EtOAc (3 × 15 mL). The combined organic fractions were washed with sat. brine (20 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. After column chromatography [silica, PE: EtOAc gradient column] the product 160 (494 mg, 85 %) was isolated as a pale yellow amorphous solid.

Rᶠ [PE:EtOAc, 60:40] 0.57;

IR νmax (Thin film) 2935 (C–H), 1731 (C=O) and 1603 (C=C);

¹H NMR δ(H, 400 MHz, CDCl₃) 6.87 (1H, d, J 2.0 Hz, C(2')H), 6.84 (1H, dd, J 8.5 and 2.0 Hz, C(6')H), 6.77 (1H, d, J 8.5 Hz, C(5')H), 6.12 (1H, td, J 4.0 and 1.5 Hz, C(3)H), 4.06-3.94 (2H, m, OCH₂CH₃), 3.86 (3H, s, OC₃H₃), 3.84 (3H, s, OC₃H₃), 3.66-3.63 (1H, m, C(1)H), 2.32-2.14 (2H, m, C(4)H₂), 2.06-1.94 (2H, m, C(6)H₂), 1.82-1.72 (1H, m, C(5)H₂), 1.69-1.60 (1H, m, C(5)H₂) and 1.06 (3H, t, J 7.0 Hz, OCH₂CH₃);

¹³C NMR δ(C, 100 MHz, CDCl₃) 174.7–(C(=O)ₗ), 148.6–(C(3' or 4')ₗ), 148.0–(C(4' or 3')ₗ), 134.6–(C(1' or 2)ₗ), 134.3–(C(2 or 1')ₗ), 127.0+ (C(3)H), 117.6+ (C(6')H), 110.8+ (C(5')H), 109.0+ (C(2')H), 60.4–(OCH₂CH₃), 55.8+ (OCH₃), 55.7+ (OCH₃), 43.6+ (C(1)H), 27.0–(C(6)H₂), 25.5–(C(4)H₂), 19.3–(C(5)H₂) and 14.0+ (OCH₂CH₃);

MS (+ESI) m/z 291 (93 %, [M+H]+);

2-(3',4'-Dimethoxyphenyl)cyclohex-2-ene-1-carboxylic acid (161)

A rapidly stirred solution of ethyl 2-(3,4-dimethoxyphenyl)cyclohex-2-ene-1-carboxylate 160 (406 mg, 1.4 mmol) in 1M NaOH(aq) : EtOH (1: 1, 16 mL) was heated at reflux for 6 h. The solution was then cooled to 0 °C, acidified to pH 2 using 6M HCl(aq) and extracted with EtOAc (3 x 15 mL). The combined organic fractions were washed with sat. brine (10 mL), dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure to give the product 161 (352 mg, 96 %) as a white solid without further purification.

Mp [CHCl$_3$] 102-106 °C;

IR $\nu_{\text{max}}$ (Thin film) 2935 (OH) and 1703 (C=O);

$^1$H NMR $\delta_H$(400 MHz, CDCl$_3$) 6.89 (1H, d, $J$ 2.0 Hz, C(2')H), 6.86 (1H, dd, $J$ 8.5 and 2.0 Hz, C(6')H), 6.79 (1H, d, $J$ 8.5 Hz, C(5')H), 6.17 (1H, td, $J$ 4.5 and 1.0 Hz, C(3)H), 3.86 (6H, s, OCH$_3$), 3.69-3.67 (1H, m, C(1)H), 2.33-2.22 (2H, m, C(4)H$_2$), 2.15-2.01 (1H, m, C(6)H$_2$), 2.04-1.95 (1H, m, C(6)H$_2$) and 1.83-1.62 (2H, m, C(5)H$_2$);

$^{13}$C NMR $\delta_C$(100 MHz, CDCl$_3$) 179.3– (C(=O)$_2$), 148.9– (C(4')$_2$), 148.3– (C(3')$_2$), 134.3– (C(2)$_2$), 133.6– (C(1')$_2$), 127.6+ (C(3)H), 117.6+ (C(6')H), 111.1+ (C(5')H), 109.2+ (C(2')H), 55.9+ (OCH$_3$), 55.8+ (OCH$_3$), 42.8+ (C(1)H), 27.0– (C(6)H$_2$), 25.5– (C(4)H$_2$) and 19.4– (C(5)H$_2$);

MS (+ESI) $m/z$ 285 (100 %, [M+Na]$^+$) and 263 (44 %, [M+H]$^+$);

HRMS (+ESI) $m/z$ Found [M+H]$^+$ 263.1284, C$_{15}$H$_{19}$O$_4$, requires [M+H]$^+$ 263.1283.
8,9-Dimethoxy-3,4,4a,5-tetrahydrophenanthridin-6(2H)-one (162)

DPPA (366 μL, 1.7 mmol) was added dropwise to a rapidly stirred solution of 2-(3’4’-dimethoxyphenyl)cyclohex-2-ene-1-carboxylic acid 161 (445 mg, 1.7 mmol) and anhydrous Et₃N (237 μL, 1.7 mmol) in anhydrous toluene (10 mL) at r.t. under Ar. The reaction was heated at 90 °C for 90 min after which the reaction was allowed to cool and was concentrated under reduced pressure. The oil was placed under Ar and BF₃·OEt₂ (1.5 mL) was added and the reaction left stirring for 16 h at 50 °C. After cooling to r.t. the reaction was quenched with 2M NaOH (aq) to pH 10, diluted with EtOAc (5 mL) and left stirring at 50 °C for 4 h. After cooling to r.t. the organic layer was separated and the aqueous fraction washed with EtOAc (3 × 5 mL). The combined organic fractions were washed with sat. brine (10 mL), dried over Na₂SO₄, filtered and the solvent removed under pressure. After column chromatography [silica, PE: EtOAc: MeOH gradient column] the product 162 (158 g, 36 %) was isolated as a white crystalline solid.

R<sub>f</sub> [EtOAc] 0.38;

M<sub>p</sub> [EtOAc] > 230 °C;

IR ν<sub>max</sub> (Thin film) 3175 (NH), 2905 (CH) and 1659 (C=O);

¹H NMR δ<sub>H</sub> (400 MHz, CDCl₃) 7.56 (1H, s, C(7)H), 6.91 (1H, s, C(10)H), 6.16 (1H, s, C(1)H), 5.60 (1H, brs, NH), 4.36-4.38 (1H, m, C(4a)H), 3.95 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 2.35-2.41 (1H, m, C(2)H₂), 2.24-2.29 (1H, m, C(2)H₂), 2.08-2.11 (1H, m, C(4)H₂), 1.90-1.93 (1H, m, C(3)H₂) and 1.63-1.75 (2H, m, C(3)H₂ and C(4)H₂);

¹³C NMR δ<sub>C</sub> (100 MHz, DMSO) 163.7– (C(6)c), 152.0– (C(9)c), 148.5– (C(8)c), 130.7– (C(10a or 10b)c), 130.6– (C(10b or 10a)c), 123.8+ (C(1)H), 119.9– (C(6a)a), 109.1+ (C(7)H), 105.5+ (C(10)H), 55.7+ (C(9)OCH₃), 55.4+ (C(8)OCH₃), 50.3+ (C(4a)H), 29.2– (C(4)H₂), 25.2– (C(2)H₂) and 19.8– (C(3)H₂);

MS (+ESI) m/z 260 (100 %, [M+H]<sup>+</sup>) and 282 (33 %, [M+Na]<sup>+</sup>);

HRMS (+ESI) m/z Found [M+H]<sup>+</sup> 260.1305, C₁₅H₁₈NO₃, requires [M+H]<sup>+</sup> 260.1281.
7-Hydroxy-8,9-dimethoxy-1,3,4,4a,5,10b-hexahydrophenanthridin-6(2H)-one (164) and 7-hydroxy-8,9-dimethoxy-1,3,4,5-tetrahydrophenanthridin-6(2H)-one (165)

Pd/C (10 % wt.) (24 mg) was added to a vigorously stirred solution of 7-hydroxy-8,9-dimethoxy-3,4,4a,5-tetrahydrophenanthridin-6(2H)-one 155 (119 mg, 0.43 mmol) in EtOH (5 mL) under N₂. The reaction was degassed and then placed under an atmosphere of H₂ at r.t. for 3 h. Upon filtering the solvent was removed under reduced pressure and column chromatography [silica, CH₂Cl₂: Acetone gradient column] afforded 164 (64 mg, 54 %) and 165 (38 mg, 33 %) both as white crystalline solids.

**Lactam (164)**

Rᵣ [EtOAc] 0.64;

Mp > 230 °C;

IR ν max (Thin film) 3430 (OH and NH) and 1649 (C=O);

³¹H NMR δₑ(500 MHz, CDCl₃, cis:trans, 59:41) 12.56 (1H, s, OH, trans), 12.47 (1H, s, OH, cis), 6.53 (1H, brs, N(5)H, trans), 6.35 (1H, brs, N(5)H, cis), 6.32 (1H, s, C(10)H, trans), 6.24 (1H, s, C(10)H, cis), 3.90 (3H, s, OCH₃, trans), 3.88 (3H, s, OCH₃, cis), 3.87 (6H, s, 2 × OCH₃, cis), 3.87 (1H, m, C(4a)H, cis), 3.24 (1H, td, J 12.0 and 4.0 Hz, C(4a)H, trans), 2.68 (1H, m, C(10b)H, cis), 2.57 (1H, td, J 12.0 and 4.0 Hz, C(10b)H, trans), 2.38 (1H, dd, J 12.5 and 2.5 Hz, C(1)H₂, trans), 1.99-1.92 (2H, m, C(3)H₂ and C(4)H₂, trans), 1.88-1.83 (2H, m, C(3)H₂ and C(4)H₂, cis), 1.74-1.71 (1H, m, C(2)H₂, trans), 1.69-1.65 (1H, m, C(4)H₂, cis), 1.62-1.55 (4H, m, C(1)H₂ and C(2)H₂, cis) and 1.54-1.51 (1H, m, C(4)H₂, trans), 1.44-1.35 (1H, m, C(3)H₂, cis), 1.44-1.35 (2H, m, C(2)H₂ and C(3)H₂, trans) and 1.33-1.27 (1H, m, C(1)H₂, trans);

¹³C NMR δₑ(125 MHz, CDCl₃, cis:trans, 59:41) 170.9– (C(6)₀, cis), 170.2– (C(6)₀, trans), 157.0– (C(8 or 9)₀, trans or cis), 156.9– (C(8 or 9)₀, cis or trans), 155.9– (C(10a)₀, trans or cis), 155.8– (C(10a)₀, cis or trans), 140.7– (C(7)₀, cis), 138.5– (C(7)₀, trans), 135.1– (C(9 or 8)₀, trans or cis), 134.9– (C(9 or 8)₀,
trans or cis), 105.9− (C(6a)\text{trans}), 104.5− (C(6a)\text{cis}), 101.0+ (C(10)H, cis), 98.3+ (C(10)H, trans), 60.6+ (2 × CH₃, cis), 55.9+ (2 × CH₃, trans), 55.6+ (C(4a)H, trans), 50.2+ (C(4a)H, cis), 41.5+ (C(10b)H, trans), 40.2+ (C(10b)H, cis), 32.0− (C(3 or 4)H₂, trans), 29.8− (C(3 or 4)H₂, cis), 29.4− (C(2)H₂, cis), 26.7− (C(1)H₂, trans), 25.3− (C(1)H₂, cis); MS (+ESI) m/z 577 (100 %, [2M+Na]⁺), 278 (78 %, [M+H]⁺) and 300 (34 %, [M+Na]⁺);


Lactam (165)

R_f [EtOAc] 0.5;

Mp [EtOAc] >230 °C;

IR v_max (liquid film) 3400 (OH and NH), 2990 (CH) and 1649 (C=O);

$^{1}H$ NMR δ_H (500 MHz, CDCl₃) 13.00 (1H, s, O\text{H}), 10.85 (1H, brs, N(5)\text{H}), 6.43 (1H, s, C(10)\text{H}), 3.97 (3H, s, C(9)OCCH₃), 3.94 (3H, s, C(8)OCCH₃), 2.64 (2H, brt, J 5.5 Hz, C(4)\text{H}), 2.59 (2H, brt, J 5.5 Hz, C(1)\text{H}) and 1.87 (4H, m, C(2)H₂ and C(3)H₂);

$^{13}C$ NMR δ_C (125 MHz, CDCl₃) 165.7− (C(6)d), 158.3− (C(9)d), 154.5− (C(7)d), 136.0− (C(10a)d), 134.5− (C(4a)d), 133.8− (C(8)d), 111.3− (C(10b)d), 106.0− (C(6a)d), 93.7+ (C(10)H), 60.7+ (C(8)OCH₃), 55.8+ (C(9)OCH₃), 27.0− (C(4)H₂), 23.4− (C(1)H₂), 22.4− (C(2 or 3)H₂) and 21.8− (C(3 or 2)H₂);

MS (+ESI) m/z 573 (100 %, [2M+Na]⁺), 276 (85 %, [M+H]⁺) and 298 (66 %, [M+Na]⁺);

8,9-Dimethoxy-1,3,4,4a,5,10b-hexahydrophenanthridin-6(2H)-one (169) and 8,9-dimethoxy-1,3,4,5-tetrahydrophenanthridin-6(2H)-one (163)

Pd/C (10 % wt.) (27 mg) was added to a vigorously stirred solution of 8,9-dimethoxy-3,4,4a,5-tetrahydrophenanthridin-6(2H)-one 162 (135 mg, 0.52 mmol) in MeOH (10 mL) under \( \text{N}_2 \). The reaction was degassed and then placed under an atmosphere of \( \text{H}_2 \) at r.t. for 4 h. Upon filtering the solvent was removed under reduced pressure and column chromatography [silica, \( \text{CH}_2\text{Cl}_2 \): Acetone gradient column] afforded lactam 169 (87 mg, 64 %) and lactam 163 (31 mg, 23 %) both as white crystalline solids.

**Lactam (169)**

\( R_f \) [EtOAc] 0.45;

\( \text{Mp} \) [MeOH] > 230 °C;

\(^1\text{H NMR} \quad \delta_\text{H} (400 \text{ MHz, CDCl}_3, \text{cis:trans, 58:42}) 7.59 (1\text{H, s, CH, trans}), 7.56 (1\text{H, s, CH, cis}), 6.75 (1\text{H, s, CH, trans}), 6.65 (1\text{H, s, CH, cis}), 5.74 (1\text{H, brs, NH, trans}), 5.49 (1\text{H, brs, NH, cis}), 3.92 (6\text{H, s, OCH}_3, \text{trans}), 3.92 (6\text{H, s, OCH}_3, \text{cis}), 3.26 (1\text{H, td, } J = 12.0 \text{ and } 4.0 \text{ Hz, CH, trans}), 2.69 (1\text{H, brs, CH, cis}), 2.66 (1\text{H, td, } J = 12.0 \text{ and } 4.0 \text{ Hz, CH, trans}), 2.30 (1\text{H, dd, } J = 12.0 \text{ and } 2.5 \text{ Hz, CH}_2, \text{trans}) \text{ and } 1.95-1.29 (15\text{H, m, CH}_2 \text{ trans and cis});

\(^{13}\text{C NMR} \delta_\text{C} (100 \text{ MHz, CDCl}_3, \text{cis:trans, 58:42}) 152.3 (\text{C}_3), 152.2 (\text{C}_3), 147.9 (\text{C}_3), 147.7 (\text{C}_3), 136.0 (\text{C}_3), 110.4 (\text{CH, trans}), 110.2 (\text{CH, cis}), 108.9 (\text{CH, cis}), 106.0 (\text{CH, trans}), 56.1 (2 \times \text{OCH}_3, \text{trans or B}, 56.0 (2 \times \text{OCH}_3, \text{cis or A}), 55.9 (\text{CH, trans}), 50.2 (\text{CH, cis}), 41.7 (\text{CH, trans}), 40.0 (\text{CH, cis}), 32.1 (\text{CH}_2, \text{trans}), 30.3 (2 \times \text{CH}_2, \text{cis}), 29.2 (2 \times \text{CH}_2, \text{cis}), 27.0 (\text{CH}_2, \text{trans}), 25.4 (\text{CH}_2, \text{trans}) \text{ and } 24.1 (\text{CH}_2, \text{trans});

\textbf{MS (+ESI) } m/z 545 (100 \%, [2\text{M+Na}]^+), 284 (50 \%, [\text{M+Na}]^+) \text{ and } 262 (44 \%, [\text{M+H}]^+);

\textbf{HRMS (+ESI) } m/z \text{ Found [M+H]^+ 262.1425, } C_{15}H_{20}\text{NO}_3 \text{ requires [M+H]^+ 262.1443. Found [M+Na]^+ 284.1245, } C_{15}H_{19}\text{NNaO}_3 \text{ requires [M+Na]^+ 284.1263.}
Lactam (163)

R_f [EtOAc] 0.25;

Mp [EtOAc] > 230 °C;

IR v_{max} (Thin film) 3735 (NH), 2918 (CH), 1637 (C=O) and 1609 (C=C);

^1H NMR δ_H (500 MHz, CDCl₃) 10.07 (1H, brs, N(5)H), 7.79 (1H, s, CH), 6.92 (1H, s, CH), 4.01 (3H, s, OCH₃), 4.00 (3H, s, OCH₃), 2.66 (4H, brs, CH₂) and 1.88 (4H, brs, CH₂);

^13C NMR δ_C (125 MHz, CDCl₃) 162.6 – (C(6))q, 153.5 – (C(9))q, 148.2 – (C(8))q, 134.1 – (C(4a))q, 133.8 – (C(10a))q, 118.6 – (C(6a))q, 109.3 – (C(10b))q, 107.5+ (C(10)H), 102.3+ (C(7)H), 56.1+ (C(9)OCH₃), 55.9+ (C(8)OCH₃), 27.3+ (C(1)H₂), 23.3+ (C(4)H₂), 22.6+ (C(2 or 3)H₂) and 22.0+ (C(3 or 2)H₂);

MS (+ESI) m/z 260 (100 %, [M+H]^+) and 282 (9 %, [M+Na]^+);

Pd/C (10 % wt.) (17 mg) was added to a vigorously rapidly stirred solution of 3,4,4a,5-tetrahydro[1,3]dioxolo[4,5-j]phenanthridin-6(2H)-one 148 (73 mg, 0.30 mmol) in MeOH (6 mL) under N₂. The reaction was degassed and then placed under an atmosphere of H₂ at r.t. for 4 h. Upon filtering the solvent was removed under reduced pressure and column chromatography [silica, CH₂Cl₂: Acetone gradient column] afforded lactam 103 (34 mg, 47 %).

**Lactam (103)**

Rᵣ[EtOAc] 0.54;

Mp [EtOAc] > 230 °C;

IR νₖₑₑₑₑ (Thin film) 3402 (NH), 2987 (CH) and 1664 (C=O);

³¹H NMR δₜₛ(400 MHz, CDCl₃, cis:trans, 54:56) 7.52 (1H, s, C(7)H, trans), 7.51 (1H, s, C(7)H, cis), 6.75 (1H, s, C(10)H, trans), 6.62 (1H, s, C(10)H, cis), 5.99-5.98 (3H, m, OCH₂O and NH, trans), 5.99-5.98 (2H, m, OCH₂O, cis), 5.72 (1H, brs, NH, cis), 3.87 (1H, brd, J 3.0 Hz, C(4a)H, cis), 3.22 (1H, td, J 11.5 and 3.5 Hz, C(4a)H, trans), 2.67 (1H, brs, C(10b)H, cis), 2.60 (1H, td, J 11.5 and 3.5 Hz, C(10b)H, trans), 2.32 (1H, brdd, J 7.5 and 3.5 Hz, C(1)H₂, trans), 1.95-1.91 (3H, m, C(3)H₂ and C(4)H₂ trans), 1.87-1.81 (1H, m, CH₂, trans), 1.78-1.64 (4H, m, 2 x CH₂, cis), 1.62-1.53 (4H, m, 2 x CH₂, cis) and 1.48-1.27 (3H, m, C(1)H₂ and C(2)H₂, trans);

³¹C NMR δₜₛ(100 MHz, CDCl₃, cis:trans, 54:46) 166.2+ (C(6)₀, cis), 165.6+ (C(6)₀, trans), 151.1+ (C(9)₀, trans), 150.9+ (C(9)₀, cis), 146.7+ (C(8)₀, cis), 146.4+ (C(8)₀, trans), 140.0+ (C(6a)₀, cis), 138.1+ (C(6a)₀, trans), 123.3+ (C(10a)₀, trans), 121.7+ (C(10a)₀, cis), 108.1– (C(7)H, trans), 107.9– (C(7)H, cis), 106.6– (C(10)H, cis), 103.9– (C(10)H, trans), 101.5+ (OCH₂O, trans or cis), 101.4+ (OCH₂O, cis or trans), 55.7– (C(4a)H, trans), 50.0– (C(4a)H, cis), 41.9– (C(10b)H, trans), 40.3– (C(10b)H, cis), 32.0+ (C(4 or 3)H₂,
trans), 30.1+ (CH$_2$, cis), 29.9+ (CH$_2$, cis), 29.1+ (CH$_2$, cis), 27.0+ (C(1)H$_2$, trans), 25.3+ (C(3 or 4)H$_2$, trans), 24.0+ (C(2)H$_2$, trans) and 19.8+ (CH$_2$, cis);

**MS (±ESI) m/z 246 (39 %, [M+H]$^+$) and 268 (8.4 %, [2M+Na]$^+$);**

**HRMS (±ESI) m/z** Found [M+H]$^+$ 246.1128, C$_{14}$H$_{16}$NO$_3$, requires [M+H]$^+$ 246.1130. Found [M+Na]$^+$ 268.0939, C$_{14}$H$_{15}$NNaO$_3$, requires [M+Na]$^+$ 268.0949.
Ethyl 1-{benzo[d][1,3]dioxol-5-yl}-5-oxo-7-oxabicyclo[4.1.0]heptane-2-carboxylate (171)

![Chemical Structure](image)

NaOH\(_{\text{aq}}\) (240 μL, 10 %) and H\(_2\text{O}_2\)\(_{\text{aq}}\) (480 μL, 36 %) was added to a rapidly stirred solution of cyclic ketone in EtOH at r.t.. After 20 h stirring the EtOH was removed under reduced pressure and the reaction mixture diluted with water (10 mL) and extracted with EtOAc (3 x 15 mL). The combined organic fractions were washed with sat. brine (10 mL), dried over Na\(_2\text{SO}_4\) and filtered. After column chromatography [silica, PE: EtOAc gradient column] the epoxide product 171 (56 g, 21 %) was isolated as a yellow oil.

\( \text{R}_f \) [EtOAc] 0.67;

\( \text{IR} \) \( \nu_{\text{max}} \) (Thin film) 1718 (C=O), 1173 (C-O) and 1109 (C-O);

\( ^1\text{H NMR} \) \( \delta \) (500 MHz, CDCl\(_3\)) 6.83 (1H, dd, \( J = 1.5 \) and 8.0 Hz, C(6')H), 6.82 (1H, d, \( J = 1.5 \) Hz, C(2')H), 6.76 (1H, d, \( J = 8.0 \) Hz, C(5')H), 5.96 (2H, s, OCH\(_2\)O), 4.17-4.12 (2H, m, OCH\(_2\)CH\(_3\)), 3.59 (1H, s, C(6)H), 3.49-3.47 (1H, m, C(2)H), 2.50-2.44 (2H, m, C(3)H\(_a\)H\(_b\) and C(4)H\(_a\)H\(_b\)), 2.37-2.32 (1H, m, C(4) H\(_a\)H\(_b\)), 1.95-1.90 (1H, m, C(3) H\(_a\)H\(_b\)) and 1.20 (3H, t, \( J = 7.0 \) Hz, OCH\(_2\)CH\(_3\));

\( ^{13}\text{C NMR} \) (125 MHz, CDCl\(_3\)) 203.8- (C\(_q\)), 171.9- (C\(_a\)), 148.0- (C\(_b\)), 147.9- (C\(_a\)), 130.5- (C\(_b\)), 120.7+ (C(6')H), 108.3+ (C(5')H), 107.2+ (C(2')H), 101.3- (OCH\(_2\)O), 62.9+ (C(6)H), 61.4- (OCH\(_2\)), 44.7+ (C(2)H), 32.2- (C(4)H\(_2\)), 20.4- (C(3)H\(_2\)) and 14.0+ (CH\(_3\));

\( \text{MS} (+\text{ESI}) \) \( m/z \) 631 (100 %, [2M+Na]\(^+\)), 327 (57 %, [M+Na]\(^+\)) and 305 (16 %, [M+H]\(^+\));

\( \text{HRMS} (+\text{ESI}) \) \( m/z \) Found [M+H]\(^+\) 305.1010, C\(_{16}\)H\(_{23}\)O\(_6\) requires [M+H]\(^+\) 305.1025. Found [M+Na]\(^+\) 327.0874, C\(_{16}\)H\(_{16}\)NaO\(_6\) requires [M+Na]\(^+\) 327.0845.
3',4',5'-Trimethoxy-[1,1'-biphenyl]-2-carboxylic acid (194)

Acetic acid (0.3 mL) was added dropwise to a rapidly stirred mixture of 5-iodo-1,2,3-trimethoxybenzene (882 mg, 3 mmol), benzoic acid (122 mg, 1 mmol), Pd(OAc)$_2$ (38 mg, 5 mol %) and AgOAc (228 mg, 1.3 mmol) in a 2 dram vial. The sealed vial was then stirred at 130 °C for 24 h. Upon cooling to room temperature the solution was transferred to a round bottom flask with CH$_2$Cl$_2$ and the solvent removed under reduced pressure. The reaction was then re-suspended in KOH$_{aq}$ (15 mL, 5 %) and extracted with CH$_2$Cl$_2$ (3 x 20 mL). The aqueous layer was then acidified to pH 2 with 6M HCl$_{aq}$ and extracted with EtOAc (3 x 20 mL). The combined EtOAc was dried over Na$_2$SO$_4$, filtered and the solvent removed under pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded the product 194 as an off-white solid (35 mg, 12 %).

**Mp** [EtOAc] 138-140 °C [Lit.,$^{192}$ 136-137 °C];

$^1$H NMR $\delta$(500 MHz, CDCl$_3$) 7.91 (1H, dd, J 8.0 and 1.0 Hz, C(6)H), 7.56 (1H, dt, J 7.5 and 1.5 Hz, C(4)H), 7.43 (1H, dt, J 7.5 and 1.5 Hz, C(5)H), 7.39 (1H, dd, J 8.0 and 1.0 Hz, C(3)H), 6.56 (2H, s, C(2')H) and C(6')H), 3.89 (3H, s, OC$_3$H$_3$) and 3.85 (6H, s, OC$_3$H$_6$);

$^{13}$C NMR $\delta$(125 MHz, CDCl$_3$) 172.5– (Cq), 152.9– (2 x Cq), 142.9– (Cq), 137.5– (Cq), 136.5– (Cq), 131.9+ (C(4 or 5)H), 130.9+ (C(3 or 6)H), 130.4+ (C(6 or 3)H), 129.5– (Cq), 127.3+ (C(5 or 4)H), 105.8+ (C(2')H) and C(6')H), 60.9+ (OCH$_3$) and 56.1+ (2 x OCH$_3$);

**MS** (+ESI) m/z 599 (100 %, [2M+Na]$^+$), 311 (26 %, [M+Na]$^+$) and 289 (20 %, [M+H]$^+$);

**HRMS** (+ESI) m/z Found [M+H]$^+$ 289.1070, C$_{16}$H$_{17}$O$_5$ requires [M+H]$^+$ 289.1076. Found [M+Na]$^+$ 311.0902, C$_{16}$H$_{16}$NaO$_5$, requires [M+Na]$^+$ 311.0895.
6.5. Synthesis of N ABC-ring analogues

3,4,4a,5-Tetrahydro-6H-pyrido[1,2-α]quinazolin-6-one (198)

Glutaraldehyde (50 % wt. in water) (1.9 mL, 10 mmol) was added dropwise to a stirred solution of anthranilamide 196 (1.36 g, 10 mmol) and MgSO₄ (3.00 g, 25 mmol) in THF (30 mL) at r.t. under N₂. NH₄Cl (266 mg, 50 mol %) was added and the reaction stirred at r.t. for 24 h. EtOAc (150 mL) was added and the THF removed under reduced pressure. After filtering the solvent was removed under reduced pressure to give the enamine 198 (2.00 g, 100 %) as a light yellow solid in quantitative yield without further purification.

Rᶠ [EtOAc] 0.44;

Mp [EtOAc] Sublimes 120 °C, melts 150 °C;

IR ν_max (KBr disc) 3415 (NH), 1680 (C=O) and 1650 (C=C);

¹H NMR δ_H (500 MHz, CDCl₃) 7.98 (1H, dd, J 8.0 and 1.5 Hz, C(7)H), 7.40 (1H, dt, J 1.5 and 7.0 Hz, C(9)H), 6.93 (1H, t, J 8.0 Hz, C(8)H), 6.91 (1H, d, J 8.0 Hz, C(10)H), 6.60 (1H, dd, J 8.0 and 2.5 Hz, C(1)H), 6.13 (1H, brs, NH), 5.04 (1H, dd, J 10.0 and 4.0 Hz, C(4a)H), 4.94 (1H, brt, J 6.0 and 2.5 Hz, C(2)H), 2.31-2.28 (1H, m, C(3)H₂) and 2.20-2.10 (3H, m, C(3)H₂ and C(4)H₂);

¹³C NMR δ_C (125 MHz, CDCl₃) 164.4− (C(6)α), 144.2− (C(10α))ₐ, 134.0+ (C(9)H), 129.2+ (C(7)H), 124.6+ (C(1)H), 119.8+ (C(8)H), 116.9− (C(6a)ₐ), 111.9+ (C(10)H), 103.1+ (C(2)H), 64.7+ (C(4a)H), 28.8− (C(4)H₂) and 19.4− (C(3)H₂);

MS (+ESI) m/z 201 (100 %, [M+H]+), 423 (97 %, [2M+Na]+), 455 (88 %, [2M+Na]+)+MeOH) and 223 (74 %, [M+Na]+);

A solution of sodium periodate(aq) (NaIO₄) (10%, 10 mL) was mixed with RuCl₃×H₂O (10 mg, 0.04 mmol) and then added dropwise to a stirred solution of 4a,5-dihydro-3H-pyrido[1,2-a]quinazolin-6(4H)-one 196 (200 mg, 1 mmol) in EtOAc (20 mL) at r.t.. After 150 min stirring the layers were separated and the aqueous layer extracted with EtOAc (3 × 20 mL). iPrOH (8 mL) was added to the combined organic fractions and stirred for 4 h. After filtering the black precipitate the reaction was washed with water (10 mL), sat. brine (10 mL), dried over Na₂SO₄ and filtered. Column chromatography [silica, CH₂Cl₂: Acetone gradient column] afforded the product 216 (53 mg, 26%) as a white crystalline solid.

Rf [EtOAc] 0.19;

Mp [EtOAc] 148-154 °C;

IR νmax (Thin film) 3580 (NH), 2963 (CH), 1670 (C=O) and 1632 (C=O);

1H NMR δH (500 MHz, CDCl₃) 8.25 (1H, dd, J 8.0 and 1.5 Hz, C(6)H), 7.75 (1H, ddd, J 8.5 and 1.5 Hz, C(8)H), 7.66 (1H, d, J 8.0 Hz, C(9)H), 7.45 (1H, ddd, J 8.5 and 1.0 Hz, C(7)H), 6.31 (1H, dd, J 6.5 and 2.5 Hz, C(3a)H), 5.00 (1H, brs, N(4)H), 3.36 (1H, ddd, J 17.5, 9.5 and 8.0 Hz, C(2)H₂), 3.05 (1H, ddd, J 18.0, 9.5 and 4.5 Hz, C(2)H₂), 2.51 (1H, C(3)H), and 2.23 (1H, dddd, J 12.5, 9.5, 4.5 and 3.0 Hz, C(3)H₂);

13C NMR δC (125 MHz, CDCl₃) 161.7– (C(5)ₐ), 158.4– (C(1)ₐ), 149.6– (C(9a)ₐ), 134.7+ (C(8)H), 127.0+ (C(9)H), 126.4+ (C(7)H), 126.4+ (C(6)H), 120.6– (C(5a)ₐ), 83.1+ (C(3a)H), 29.8– (C(2)H₂) and 27.6– (C(3)H₂);

MS (+ESI) m/z 225 (100%, [M+Na]+), 203 (15%, [M+H]+) and 427 (10%, [2M+Na]+);

General Procedure 4:

1,1’-Carbonyldiimidazole (CDI) (324 mg, 2 mmol) was added to a stirred solution of benzoic acid (2 mmol) in MeCN (10 mL) at r.t. under N₂. The reaction was heated to 80 °C for 120 min then cooled to r.t. and NH₃(aq) (35 %, 1 mL) was added. The reaction was stirred at r.t. for 18 h after which MeCN was removed under reduced pressure and 1 M NaOH(aq) (10 mL) added. The reaction was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with water (10 mL) followed by 0.5 M FeSO₄(aq) (2 × 10 mL) then sat. brine (10 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford the product without the need for further purification.

2-Amino-4,5-dimethoxybenzamide (219)

Following the general procedure 4 using CDI (324 mg, 2 mmol) and 2-amino-4,5-dimethoxybenzoic acid (394 mg, 2 mmol) in MeCN (10 mL) followed by NH₃(aq) (35 %, 1 mL) to give the benzamide 219 (168 mg, 43 %) without further purification.

Rᵢ[EtOAc] 0.23;

Mp [EtOAc] 139-142 °C [Lit.,[193] 138-140 °C];

¹H NMR δ_H(400 MHz, DMSO) 7.56 (1H, brs, CONH₂), 7.11 (1H, s, C(6)H), 6.83 (1H, s, CONH₂), 6.43 (2H, brs, NH₂), 6.28 (1H, s, C(3)H), 3.70 (3H, s, C(4)OCH₃) and 3.66 (3H, s, C(5)OCH₃);

¹³C NMR δ_C(100 MHz, DMSO) 170.9– (C=O), 153.0– (C(4)ₐ), 146.8– (C(2)ₐ), 138.9– (C(5)ₐ), 113.0+ (C(6)H), 104.3– (C(1)ₐ), 99.5+ (C(3)H), 56.6+ (C(5)OCH₃) and 55.1+ (C(4)OCH₃).

Spectroscopic data is consistent with that reported by Stroganova et al. and Kanişkan et al.[194,195]
2-Amino-3-methylbenzamide (233)

Following the general procedure 4 using CDI (1.62 g, 10 mmol) and 2-amino-3-methylbenzoic acid (1.51 g, 10 mmol) in MeCN (50 mL) followed by NH₃(aq) (35 %, 5 mL) to give the benzamide 233 (1.07 mg, 71 %) without further purification.

Rᵥ [EtOAc] 0.41;

Mp [EtOAc] 148-150 °C;

¹H NMR δH (400 MHz, DMSO) 7.72 (1H, brs, CONH₂), 7.41 (1H, dd, J 8.0 and 1.0 Hz, C(4)H), 7.06 (2H, d, J 7.0 Hz, C(6)H and CONH₂), 6.44 (1H, t, J 7.5 Hz, C(5)H), 6.39 (2H, brs, C(2)NH₂) and 2.06 (3H, s, CH₃);

¹³C NMR δC (100 MHz, DMSO) 172.5 – (C(=O)q), 149.0 – (C(2)q), 133.4+ (C(4)H), 127.4+ (C(6)H), 123.8– (C(3)q), 114.9+ (C(5)H), 114.3– (C(1)q) and 18.4+ (CH₃);

MS (+ESI) m/z 173 (100 %, [M+Na]+) and 306 (16 %, [2M+Na]+ –NH₃);


Spectroscopic data is consistent with that reported by Nathubhai et al.¹⁹⁶
Following the general procedure 4 using CDI (1.59 g, 9.8 mmol) and 2-amino-4-methylbenzoic acid (1.48 g, 9.8 mmol) in MeCN (50 mL) followed by NH$_3$ (aq) (35 %, 10 mL) to give the benzamide 234 (993 mg, 67 %) without further purification.

$R_f$ [PE:EtoAc, 20:80] 0.41;

Mp [EtOAc] 149-150 °C [Lit.,$^{197}$ 148 °C];

$^1$H NMR $\delta_{\text{H}}$ (400 MHz, DMSO) 7.41 (1H, brs, CON$_2$H), 7.39 (1H, d, $J$ 8.0 Hz, C(6)H), 6.92 (1H, brs, CON$_2$H), 6.49 (2H, brs, NH$_2$), 6.43 (1H, d, $J$ 0.5 Hz, C(3)H), 6.26 (1H, ddd, $J$ 0.5, 1.5 and 0.5 Hz, C(5)H) and 2.12 (3H, s, CH$_3$);

$^{13}$C NMR $\delta_{\text{C}}$ (100 MHz, DMSO) 171.8– (C(=O)$_2$), 150.9– (C$_3$), 142.1– (C$_3$), 129.3+ (C(5)H), 117.0+ (C(6)H), 116.2+ (C(3)H), 111.6– (C$_3$) and 21.6+ (CH$_3$);

MS (+ESI) $m/z$ 173 (100 %, [M+Na]$^+$);


Spectroscopic data is consistent with that reported by Zhao et al.$^{197}$
2-Amino-5-methylbenzamide (235)

Following the general procedure 4 using CDI (324 mg, 2.0 mmol) and 2-amino-5-methylbenzoic acid (303 mg, 2.0 mmol) in MeCN (10 mL) followed by NH$_3$(aq) (35 %, 1 mL) to give the benzamide 235 (184 mg, 61 %) without further purification.

R$_f$[PE:EtOAc, 50:50] 0.40; 

M$_p$ [EtOAc] 179-180 °C [Lit.,$^{198}$ 175-177 °C];

$^1$H NMR $\delta$(400 MHz, DMSO) 7.72 (1H, brs, CONH$_2$), 7.40 (1H, d, $J$ 1.0 Hz, C(6)H), 7.05 (1H, brs, CONH$_2$), 7.01 (1H, ddd, $J$ 8.5, 2.0 and 0.5 Hz, C(4)NH$_2$), 6.44 (1H, d, $J$ 8.5 Hz, C(3)H), 6.38 (2H, brs, NH$_2$) and 2.20 (3H, s, CH$_3$);

$^{13}$C NMR $\delta$(100 MHz, DMSO) 171.3− (C(=O)$_2$), 147.9− (C$_9$), 132.7+ (C(4)H), 128.7+ C(6)H), 122.7− (C$_9$), 116.5+ (C(3)H), 113.7− (C$_9$) and 20.0+ (CH$_3$);

MS (+ESI) m/z 173 (100 %, [M+Na]$^+$);

HRMS (+ESI) m/z Found [M+Na]$^+$ 173.0712, C$_8$H$_{10}$N$_2$NaO, requires 173.0691 [M+Na]$^+$. 
4,5,6-Trimethoxy-1H-indole-2,3-dione (252)

HCl_{(aq)} (6 M, 0.5 mL) was added dropwise to a vigorously stirred solution of trimethoxyaniline 237 (183 mg, 1 mmol) in diethyl ether (3 mL) r.t. until a precipitate was observed. The reaction was filtered and the solid washed with diethyl ether (2 x 5 mL) to give the HCl salt 251 (130 mg, 59%) as a grey crystalline solid which was used without further purification.

3,4,5-Trimethoxyaniline hydrochloride (58 mg, 0.26 mmol) was stirred in oxalyl chloride (150 μL) at 160 °C in a sealed thick-walled pressure tube for 50 min. Upon cooling to r.t. MeOH was added and the reaction heated at reflux for 10 min. The solvent was removed under reduced pressure and column chromatography [silica, PE: EtOAc gradient column] afforded the product 252 (38 mg, 62%) as a red-brown crystalline solid.

Salt (251)

Mp [Et₂O] 258 °C [Lit., 172 256 °C]

Istatin (252)

^1H NMR δ_H(400 MHz, CDCl₃) 7.72 (1H, brs, NH), 6.14 (1H, s, CH), 4.22 (3H, s, CH₃), 3.96 (3H, s, CH₃) and 3.77 (3H, s, CH₃);

^13C NMR δ_C(100 MHz, DMSO) 178.6–(C(=O)ₓ), 162.4–(C(=O)ₓ), 160.4–(Cₓ), 152.8–(Cₓ), 148.9–(Cₓ), 135.3–(Cₓ), 102.6+ (CH), 91.7–(Cₓ), 61.5+ (CH₃), 60.9+ (CH₃) and 56.7+ (CH₃).

Spectroscopic data is consistent with that reported by Mason et al.199
Glutaraldehyde (50 % wt. in water) (190 μL, 1 mmol) was added dropwise to a stirred solution of 2-amino-3-methylbenzamide 233 (150 mg, 1 mmol) and MgSO₄ (300 mg, 2.5 mmol) in THF (3 mL) at r.t. under N₂. NH₄Cl (27 mg, 50 mol %) was added and the reaction stirred at r.t. for 24 h. EtOAc (10 mL) was added and the THF removed under reduced pressure. After filtering the solvent was removed under reduced pressure to give the crude enamine 285 which was used without further purification.

$^1$H NMR δ_H (400 MHz, CDCl₃) 7.83 (1H, d, J 7.5 Hz, C(7)H), 7.30 (1H, dd, J 8.5 and 2.5 Hz, C(1)H), 7.18 (1H, dd, J 7.5 and 1.0 Hz, C(9)H), 6.80 (1H, t, J 7.5 Hz, C(8)H), 5.16-5.12 (1H, m, C(2)H), 5.03-5.00 (1H, m, C(4a)H), 2.23-2.19 (2H, m, C(3)H₂), 2.17 (3H, s, CH₃) and 2.16-2.10 (2H, m, C(4)H₂);

$^{13}$C NMR δ_C (100 MHz, CDCl₃) 160.5– (C(6)q), 144.7– (C₆), 134.4+ (C(9)H), 126.9+ (C(7)H), 122.4+ (C(1)H), 121.7– (C₆), 119.2+ (C(8)H), 116.1– (C₆), 107.6+ (C(2)H), 65.5+ (C(4a)H), 29.0– (CH₂), 20.0– (CH₂) and 16.3+ (CH₃).
9-Methyl-3,4,4a,5-tetrahydro-6H-pyrido[1,2-α]quinazolin-6-one (286)

Glutaraldehyde (50 % wt. in water) (190 μL, 1.0 mmol) was added dropwise to a stirred solution of 2-amino-4-methylbenzamide 234 (150 mg, 1.0 mmol) and MgSO₄ (360 mg, 3.0 mmol) in THF (5 mL) at r.t. under N₂. NH₄Cl (27 mg, 50 mol %) was added and the reaction stirred at r.t. for 24 h. EtOAc (15 mL) was added and the THF removed under reduced pressure. After filtering the solvent was removed under reduced pressure to give the crude enamine 286 which was used without further purification.

¹H NMR δ (400 MHz, CDCl₃) 7.85 (1H, d, J 8.0 Hz, C(7)H), 6.73 (1H, d, J 8.0 Hz, C(8)H), 6.71 (1H, s, C(10)H), 6.59 (1H, dd, J 8.0 and 2.5 Hz, C(1)H), 6.47 (1H, brs, NH), 5.00 (1H, dd, J 10.0 and 4.0 Hz, C(4a)H), 4.89-4.94 (1H, m, C(2)H), 2.34 (3H, s, CH₃) and 2.06-2.17 (4H, m, C(3)H₂ and C(4)H₂);

¹³C NMR δ (100 MHz, CDCl₃) 165.8+ (C(6)c), 144.8+ (C(6a or 10a)c), 144.2+ (C(10a or 6a)c), 129.0– (C(7)H), 124.5– (C(1)H), 120.9– (C(8)H), 114.4+ (C(9)c), 112.2– (C(10)H), 102.9– (C(2)H), 64.7– (C(4a)H), 28.7+ (C(4)H₂), 22.1– (CH₃) and 19.3+ (C(3)H₂);

8-Methyl-3,4,4a,5-tetrahydro-6H-pyrido[1,2-α]quinazolin-6-one (284)

Glutaraldehyde (50 % wt. in water) (190 μL, 1 mmol) was added dropwise to a stirred solution of 2-amino-5-methylbenzamide 235 (150 mg, 1 mmol) and MgSO₄ (300 mg, 2.5 mmol) in THF (3 mL) at r.t. under N₂. NH₄Cl (27 mg, 50 mol %) was added and the reaction stirred at r.t. for 24 h. EtOAc (10 mL) was added and the THF removed under reduced pressure. After filtering the solvent was removed under reduced pressure to give the crude enamine 287 which was used without further purification.

¹H NMR δ(H, 400 MHz, CDCl₃) 7.76 (1H, dd, J 0.5 and 1.5 Hz, C(7)H), 7.20 (1H, ddd J 0.5, 2.5 and 8.5 Hz, CH), 6.80 (1H, d, J 8.5 Hz, CH), 6.55 (1H, dd, J 2.5 and 8.0 Hz, C(1)H), 4.99 (1H, dd, J 3.5 and 10.0 Hz, CH), 4.90-4.86 (1H, m, CH) and 2.28-2.13 (7H, s, CH₃ and m, 2 x CH₂).
1,2,3,4,4a,5-Hexahydro-6H-pyrido[1,2-a]quinazolin-6-one (254)

A flask containing a vigorously stirring suspension of 4a,5-dihydro-3H-pyrido[1,2-a]quinazolin-6(4H)-one 198 (200 mg, 1 mmol) and Pd/C (10 % wt.) (40 mg) in EtOH (10 mL) at r.t. was purged with N₂. The flask was degassed and H₂ introduced at atmospheric pressure. Vigorous stirring was continued at r.t. for 16 h. Upon filtering through a pad of celite the solvent was removed under reduced pressure and column chromatography [silica, PE: EtOAc gradient column] afforded the product 254 (137 mg, 68 %) as a white solid.

Rf [EtOAc] 0.67;

Mp [MeOH] 170-172 °C [Lit.,²⁰⁰ 184-186 °C];

IR νmax (Thin film) 3413 (NH), 1672 (C=O) and 1607 (C=C);

¹H NMR δH (500 MHz, CDCl₃) 7.95 (1H, dd, J 7.5 and 1.5 Hz, C(7)H), 7.39 (2H, dt, J 7.0 and 1.5 Hz, C(9)H), 7.09 (1H, brs, NH), 6.90 (1H, t, J 7.5 Hz, C(8)H), 6.82 (1H, d, J 8.5 Hz, C(10)H), 4.46 (1H, dd, J 10.5 and 3.0 Hz, C(4a)H), 3.74 (1H, brd, J 12.0 Hz, C(1)H₂), 2.62 (1H, dt, J 12.5 and 3.0 Hz, C(1)H₂), 2.00-1.98 (1H, m, C(4)H₂), 1.89-1.78 (3H, m, C(2)H₂, C(3)H₂ and C(4)H₂), 1.69 (1H, qt, J 13.5 and 4.0 Hz, C(2)H₂) and 1.46 (1H, qt, J 13.0 and 3.5 Hz, C(3)H₂);

¹³C NMR δC (125 MHz, CDCl₃) 164.7– (C(6)₃), 149.7– (C(10a)₃), 133.9+ (C(9)H), 128.7+ (C(7)H), 119.2+ (C(8)H), 117.7– (C(6a)₃), 112.3+ (C(10)H), 68.2+ (C(4a)H), 45.5– (C(1)H), 31.8– (C(4)H), 24.2– (C(2)H₁) and 21.9– (C(3)H₁);

MS (+ESI) m/z 427 (100 %, [2M+Na]+), 225 (58 %, [M+Na]+) and 203 (32 %, [M+H]+);

1,2,3,4,4a,5-Hexahydro-6H-pyrido[1,2-α]quinazolin-6-one (254) and 1-ethoxy-1,2,3,4,4a,5-hexahydro-6H-pyrido[1,2-α]quinazolin-6-one (202)

A flask containing a vigorously stirring suspension of 4a,5-dihydro-3H-pyrido[1,2-α]quinazolin-6(4H)-one 196 (1.00 g, 5 mmol) and Pd/C (10 % wt.) (200 mg) in EtOH (25 mL) at r.t. was purged with N₂. The flask was degassed and H₂ introduced at atmospheric pressure. Vigorous stirring was continued at r.t. for 16 h. Upon the removal of H₂ the reaction was heated to reflux for 2 h after which it was filtered through celite and further washed with hot EtOH until the crude mass was constant. The solvent was removed under reduced pressure and column chromatography [silica, PE: EtOAc gradient column] afforded the ethanol adduct 202 (77 mg, 6 %) as a yellow amorphous solid and product 254 (392 mg, 39 %) white crystalline solid.

**Ethanol Adduct (202)**

R₇ [EtOAc] 0.76;

IR ν(max) (KBr disc) 3420 (NH);

¹H NMR δ(H) (400 MHz, CDCl₃) 7.87 (1H, dd, J 7.5 and 1.5 Hz, C(7)H), 7.24 (1H, ddd, J 8.0, 7.0 and 1.5 Hz, C(9)H), 6.77 (1H, ddd, J 8.0, 7.5 and 1.0 Hz, C(8)H), 6.53 (1H, dd, J 8.0 and 1.0 Hz, C(10)H), 6.01 (1H, t, J 3.0 Hz, C(1)H), 5.11-5.09 (1H, m, C(4a)H), 4.09 (1H, brs, NH), 3.56-5.52 (2H, m, OC₂H₂CH₃), 2.02-1.88 (2H, m, C(2)H₂ and C(3)H₂), 1.87-1.76 (2H, m, C(4)H₂), 1.69-1.61 (2H, m, C(2)H₂ and C(3)H₂) and 1.18 (3H, t, J 7.0 Hz, OCH₂CH₃);

¹³C NMR δ(C) (100 MHz, CDCl₃) 163.5–(C(6)H), 145.8–(C(10a)H), 133.7+ (C(9)H), 128.9+ (C(7)H), 118.5+ (C(8)H), 114.8–(C(6a)H), 113.6+ (C(10)H), 78.6+ (C(1)H), 64.0+ (C(4a)H), 63.4–(OCH₂CH₃), 33.5–(C(4)H₂), 29.2–(C(2 or 3)H₂), 17.1–(C(3 or 2)H₂) and 15.2+ (OCH₂CH₃);

MS (+ESI) m/z 515 (100 %, [2M+Na]⁺), 269 (52 %, [M+Na]⁺) and 201 (39 %, [M+H]⁺–EtOH);


**Analogue (254)**

Spectroscopic data are consistent with those previously reported for analogue 254.
1-Ethoxy-9-methyl-1,2,3,4,4a,5-hexahydro-6H-pyrido[1,2-α]quinazolin-6-one (256) and 9-methyl-1,2,3,4,4a,5-hexahydro-6H-pyrido[1,2-α]quinazolin-6-one (257)

Glutaraldehyde (50 % wt. in water) (190 µL, 1 mmol) was added dropwise to a stirred solution of 2-amino-4-methylbenzamide 234 (150 mg, 1 mmol) and MgSO₄ (480 mg, 4 mmol) in THF (5 mL) at r.t. under N₂. NH₄Cl (27 mg, 50 mol %) was added and the reaction stirred at r.t. for 24 h. EtOAc (15 mL) was added and the THF removed under reduced pressure. After filtering the solvent was removed under reduced pressure to afford the crude enamine 286 without further purification.

A vigorously stirred suspension of crude 9-methyl-3,4,4a,5-tetrahydro-6H-pyrido[1,2-α]quinazolin-6-one 286 (214 mg, 1 mmol) in EtOH (20 mL) at r.t. was purged with N₂ and Pd/C (10 % wt.) (100 mg) was added. The flask was degassed and H₂ introduced at atmospheric pressure. Vigorous stirring was continued at r.t. for 16 h. Upon filtering through a pad of celite the solvent was removed under reduced pressure and column chromatography [silica, PE: EtOAc gradient column] afforded the ethanol adduct 256 (62 mg, 24 %) and reduced product 257 (131 mg, 61 %) as a white solid.

**Reduced product (257)**

Rₙ[EtOAc] 0.32;

Mp [CH₂Cl₂] Sublimes 195 °C, melts 215 °C;

IR ν_max (Thin film) 3420 (NH) and 1670 (C=O);

³H NMR δ_H(400 MHz, CDCl₃) 7.83 (1H, d, J 8.0 Hz, C(7)H), 6.94 (1H, brs, N(5)H), 6.71 (1H, dd, J 8.0 and 0.5 Hz, C(8)H), 6.62 (1H, s, C(10)H), 4.42 (1H, ddd, J 10.0, 3.0 and 0.5 Hz, C(4a)H), 3.73 (1H, d, J 12.0 and 2.0 Hz, C(1)H₂), 2.61 (1H, dd, J 12.0 and 3.0 Hz, C(1)H₂), 2.33 (3H, s, CH₃), 1.99-1.93 (1H, m, C(4)H₂), 1.89-1.74 (3H, m, C(2)H₂, C(3)H₂ and C(4)H₂), 1.67 (1H, qt, J 13.0 and 3.0 Hz, C(2)H₃) and 1.44 (1H, qt, J 13.0 and 3.5 Hz, C(3)H₂);
$^{13}$C NMR $\delta$ (100 MHz, CDCl$_3$) 164.8+ (C(6)q), 149.7+ (C(10a)q), 144.5+ (C(9)q), 128.6− (C(7)H), 120.3− (C(8)H), 115.2+ (C(6a)q), 112.8− (C(10)H), 68.2− (C(4a)H), 45.4+ (C(1)H$_2$), 31.7+ (C(4)H$_2$), 24.1+ (C(2)H$_2$), 22.2− (CH$_3$) and 21.8+ (C(3)H$_2$);

**MS** (+ESI) $m/z$ 217 (100 %, [M+H]$^+$) and 239 (69 %, [M+Na]$^+$);

**HRMS** (+ESI) $m/z$ Found [M+H]$^+$ 217.1327, C$_{13}$H$_{17}$N$_2$O, requires [M+H]$^+$ 217.1341. Found [M+Na]$^+$ 239.1146, C$_{13}$H$_{16}$N$_2$NaO, requires [M+Na]$^+$ 239.1160.
**8-Methyl-1,2,3,4,4a,5-hexahydro-6H-pyrido[1,2-\(\alpha\)]quinazolin-6-one (258)**

Glutaraldehyde (50 % wt. in water) (100 \(\mu\)L, 0.5 mmol) was added dropwise to a stirred solution of 2-amino-5-methylbenzamide 235 (75 mg, 0.5 mmol) and Na\(\text{SO}_4\) (213 mg, 1.5 mmol) in THF (3 mL) at r.t. under \(\text{N}_2\). NH\(_4\)Cl (27 mg, 100 mol %) was added and the reaction stirred at r.t. for 9 h, monitoring by TLC. EtOAc (10 mL) was added and the THF removed under reduced pressure. After filtering the solvent was removed under reduced pressure to afford the crude enamine 287 without further purification.

A vigorously stirred suspension of 8-methyl-3,4,4a,5-tetrahydro-6H-pyrido[1,2-\(\alpha\)]quinazolin-6-one 287 (107 mg, 0.5 mmol) in EtOH (5 mL) at r.t. was purged with \(\text{N}_2\) and Pd/C (10 % wt.) (50 mg) was added. The flask was degassed and \(\text{H}_2\) introduced at atmospheric pressure. Vigorous stirring was continued at r.t. for 16 h. Upon filtering through a pad of celite the solvent was removed under reduced pressure and column chromatography [silica, PE: EtOAc gradient column] afforded the reduced product 258 (34 mg, 31 %) as a white solid.

\(\text{R}_f\) [EtOAc] 0.5;

\(\text{Mp}\) [\(\text{CH}_2\text{Cl}_2\)] 119 °C;

\(\text{IR} \ \nu_{\text{max}}\) (Thin film) 3430 (NH) and 1650 (C=O);

\(\text{\(^1\)H NMR}\) \(\delta\) (400 MHz, CDCl\(_3\)) 7.75 (1H, d, \(J\ 2.5\ Hz, C(7)H\)), 7.20 (1H, ddd, \(J\ 1.0, 2.5\ and\ 8.5\ Hz, C(9)H\)), 7.08 (1H, brs, N(5)H), 6.73 (1H, d, \(J\ 8.5\ Hz, C(10)H\)), 4.38 (1H, ddd, \(J\ 1.0, 3.0\ and\ 10.5\ Hz, C(4a)H\)), 3.68 (1H, brd, \(J\ 12.0\ Hz, C(1)H_2\)), 2.58 (1H, td, \(J\ 3.0\ and\ 12.5\ Hz, C(1)H_2\)), 2.27 (3H, s, CH\(_3\)), 2.00-1.94 (1H, m, C(4)H\(_2\)), 1.89-1.78 (3H, m, C(2)H\(_2\), C(3)H\(_2\) and C(4)H\(_2\)), 1.76-1.63 (1H, m, C(2)H\(_2\)) and 1.44 (1H, qt, \(J\ 3.5\ and\ 12.5\ Hz, C(3)H\(_2\));

\(\text{\(^{13}\)C NMR}\) \(\delta\) (100 MHz, CDCl\(_3\)) 165.0+ (C(6)\(_\alpha\)), 147.7+ (C(10a)\(_\alpha\)), 134.6– (C(9)H), 128.7– (C(7)H and C(8)\(_\alpha\)), 117.6+ (C(6a)\(_\alpha\)), 112.4– (C(10)H), 68.2– (C(4a)H), 45.6+ (C(1)H\(_2\)), 31.5+ (C(4)H\(_2\)), 24.2+ (C(2)H\(_2\)), 21.8+ (C(3)H\(_2\)), 20.2– (CH\(_3\)).
**MS** (+ESI) m/z 215 (100 %, [M+H]^+–H$_2$), 237 (69 %, [M+Na]^+–H$_2$), 217 (48 %, [M+H]^+) and 239 (34 %, [M+Na]^+);

**HRMS** (+ESI) m/z Found [M+H]^+ 217.1327, C$_{13}$H$_{17}$N$_2$O, requires [M+H]^+ 219.1341. Found [M+Na]^+ 239.1146, C$_{13}$H$_{16}$N$_2$NaO, requires [M+Na]^+ 239.1160.
Glutaraldehyde (50 % wt. in water) (67 μL, 0.35 mmol) was added dropwise to a stirred solution of 2-amino-3-methylbenzamide 233 (53 mg, 0.35 mmol) and MgSO₄ (105 mg, 0.88 mmol) in THF (2 mL) at r.t. under N₂. NH₄Cl (9.0 mg, 50 mol %) was added and the reaction stirred at r.t. for 24 h. EtOAc (10 mL) was added and after filtering the solvent was removed under reduced pressure to give the crude enamine 285 which was used without further purification.

A flask containing a vigorously stirring suspension of crude 10-methyl-3,4,4a,5-tetrahydro-6H-pyrido[1,2-α]quinazolin-6-one 285 (75 mg, 0.35 mmol) and Pd/C (10 % wt.) (35 mg) in EtOH (2.5 mL) at r.t. was purged with N₂. The flask was degassed and H₂ introduced at atmospheric pressure. Vigorous stirring was continued at r.t. for 18 h. Upon the removal of H₂ the reaction was filtered through celite and further washed with warm EtOH until the crude mass was constant. The solvent was removed under reduced pressure and column chromatography [silica, PE: EtOAc gradient column] afforded analogue 260 (30 mg, 40 %) as a light yellow crystalline solid.

**Analogue (260)**

Rᶠ [PE:EtOAc, 30:70] 0.43;

Mp [CHCl₃] 95-98 °C;

IR νₘₐₓ (Thin film) 1663 (C=O), 1518 (C=N) and 1239 (CN);

¹H NMR δ(H (400 MHz, CDCl₃) 8.10 (1H, ddd, J 8.0, 1.5 and 1.0 Hz, C(7)H), 7.54 (1H, ddd, J 7.0, 1.5 and 1.0 Hz, C(9)H), 7.29 (1H, t, J 7.5 Hz, C(8)H), 4.07 (1H, t, J 6.5 Hz, C(1)H₂), 3.00 (1H, t, J 7.0 Hz, C(4)H₂), 2.58 (3H, s, CH₃) and 2.03-1.90 (4H, m, C(2)H₂ and C(3)H₂);

¹³C NMR δ(C (100 MHz, CDCl₃) 162.5– (C(6)C), 153.4– (C₆), 146.0– (C₄), 134.8– (C₉), 134.5+ (C(9)H), 124.2+ (C(8)H), 123.9+ (C(7)H), 120.3– (C₆), 42.1– (CH₂), 32.1– (CH₂), 22.1+– (C(2 or 3)H₂), 19.4– (C(3 or 2)H₂) and 17.2– (CH₃);

MS (+ESI) m/z 215 (100 %, [M+H]⁺), 237 (67 %, [M+Na]⁺) and 451 (35 %, [2M+Na]⁺);
HRMS (+ESI) m/z Found [M+H]\(^+\) 215.1181, C\(_{13}\)H\(_{15}\)N\(_2\)O, requires [M+H]\(^+\) 215.1184. Found [M+Na]\(^+\) 237.0990, C\(_{13}\)H\(_{14}\)N\(_2\)NaO, requires [M+Na]\(^+\) 237.1004;
2-(Piperidin-1-yl)benzamide (265) and 1,2,3,4,4a,5-hexahydro-6H-pyrido[1,2-α]quinazolin-6-one (254) and 1,2,3,4,4a,5-hexahydro-6H-pyrido[1,2-α]quinazolin-6-one (254)

Glutaraldehyde (50 % wt. in water) (190 μL, 1 mmol) was added dropwise to a stirred solution of anthranilamide 196 (136 mg, 1 mmol) and MgSO₄ (300 mg, 2.5 mmol) in MeOH (3 mL) at r.t. under Ar. NH₄Cl (27 mg, 50 mol %) was added and the reaction stirred at r.t. for 60 mins. NaBH₃CN (251 mg, 4 mmol) was added and the reaction stirred at r.t. for 24 h. The reaction was quenched with NaHSO₄ and extracted with EtOAc (3 x 10 mL). The combined organic fractions were washed with sat. brine (10 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure.

Column chromatography [silica, PE: EtOAc gradient column] afforded product 265 (10 mg, 5 %) and product 254 (trace) as a white crystalline solids.

**Compound (265)**

$^1$H NMR δ$_H$(400 MHz, CDCl$_3$) 9.80 (1H, brs, NH$_2$), 8.16 (1H, ddd, J 7.5, 2.0 and 1.0 Hz, C(6)H), 7.44 (1H, ddd, J 8.0, 7.0 and 2.0 Hz, C(3)H), 7.22-7.18 (2H, m, C(4)H and C(5)H), 5.82 (1H, brs, NH$_2$), 2.93 (4H, t, J 5.0 Hz, C(2')H$_2$ and C(6')H$_2$), 1.74 (4H, p, J 5.5 Hz, C(3')H$_2$ and C(5')H$_2$) and 1.62-1.58 (2H, m, C(4')H$_2$);

$^{13}$C NMR δ$_C$(100 MHz, CDCl$_3$) 168.7 (C(=O)$_a$), 152.9 (C(2))$_a$, 132.4+ (C(3)H), 131.6+ (C(6)H), 127.3 (C(1))$_a$, 124.3+ (C(4 or 5)H), 120.4+ (C(5 or 4)H), 54.9 (C(2')H$_2$ and C(6')H$_2$), 26.7+ (C(3')H$_2$ and C(5')H$_2$) and 23.6 (C(4')H$_2$).

Spectroscopic data is consistent with that reported by Sun et al.$^{201}$

**Analogue (254)**

Spectroscopic data are consistent with those previously reported for analogue 254.
Glutaraldehyde (50 % wt. in water) (190 μL, 1 mmol) was added dropwise to a stirred solution of anthranilamide 196 (136 mg, 1 mmol) in THF (3 mL) at r.t. under Ar. NH₄Cl (27 mg, 50 mol %) was added and the reaction stirred at r.t. until the starting material had been consumed as determined by TLC spectroscopic analysis. Methanol was added (3 mL) and the reaction cooled to 0 °C. NaBH₃CN (188 mg, 3 mmol) was added portion wise followed by AcOH until the pH was less than 7. The reaction was warmed to r.t. and stirred for 4 h. The reaction was quenched with H₂O₂(aq) (2 mL) and 1M NaOH(aq) (5 mL) and stirred until two separate layers were visible. 0.5 M FeSO₄(aq) (10 mL) was added and the reaction stirred overnight, extracted with EtOAc (3 x 10 mL) and the combined organic fractions were washed with sat. brine (10 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Column chromatography [silica, PE: EtOAc gradient column] afforded product 254 (112 mg, 55 %) as a white crystalline solids.

¹H NMR δH (500 MHz, CDCl₃) 7.95 (1H, dd, J 7.5 and 1.5 Hz, C(7)H), 7.39 (2H, dt, J 7.0 and 1.5 Hz, C(9)H), 7.09 (1H, brs, NH), 6.90 (1H, t, J 7.5 Hz, C(8)H), 6.82 (1H, d, J 8.5 Hz, C(10)H), 4.46 (1H, dd, J 10.5 and 3.0 Hz, C(4a)H), 3.74 (1H, brd, J 12.0 Hz, C(1)H₂), 2.62 (1H, dt, J 12.5 and 3.0 Hz, C(1)H₂), 2.00-1.98 (1H, m, C(4)H₂), 1.89-1.78 (3H, m, C(2)H₂, C(3)H₂ and C(4)H₂), 1.69 (1H, qt, J 12.5 and 4.0 Hz, C(2)H₂) and 1.46 (1H, qt, J 13.0 and 3.5 Hz, C(3)H₂);

¹³C NMR δC (125 MHz, CDCl₃) 164.7–(C(6)ₙ), 149.7–(C(10a)ₙ), 133.9+ (C(9)H), 128.7+ (C(7)H), 119.2+ (C(8)H), 117.7– (C(6a)ₙ), 112.3+ (C(10)H), 68.2+ (C(4a)H), 45.5– (C(1)H₂), 31.8– (C(4)H₂), 24.2– (C(2)H₂) and 21.9– (C(3)H₂).

Spectroscopic data are consistent with those previously reported for analogue 254.
Glutaraldehyde (50 % wt. in water) (100 µL, 0.5 mmol) was added dropwise to a stirred solution of 2-amino-3-methylbenzamide \textit{233} (75 mg, 0.5 mmol) and MgSO$_4$ (180 mg, 1.5 mmol) in THF (3 mL) at r.t. under N$_2$. NH$_4$Cl (27 mg, 100 mol %) was added and the reaction stirred at r.t. for 24 h. EtOAc (10 mL) was added and the THF removed under reduced pressure. After filtering the solvent was removed under reduced pressure to afford the crude enamine without further purification.

A solution of NaIO$_4$\textit{(aq)} (10 %, 5 mL) was mixed with RuCl$_3$·$\times$H$_2$O (5 mg, 0.02 mmol) and then added dropwise to a stirred solution of crude 10-methyl-3,4a,5-tetrahydro-6H-pyrido[1,2-\textit{a}]quinoxalin-6-one \textit{285} (107 mg, 0.5 mmol) in EtOAc (10 mL) at r.t.. After 105 min stirring the layers were separated and the aqueous layer extracted with EtOAc (3 × 10 mL). iPrOH (8.0 mL) was added to the combined organic fractions and stirred for 18 h. After filtering the black precipitate the reaction was washed with water (10 mL), sat. brine (10 mL) and dried over Na$_2$SO$_4$ and filtered. Column chromatography [silica, CH$_2$Cl$_2$; Acetone gradient column] afforded the product \textit{268} (36 mg, 33 %) as a white crystalline solid.

**Dione (268)**

\textit{R}$_f$ [CH$_2$Cl$_2$; Acetone, 50:50] 0.61;

\textit{Mp} [CH$_2$Cl$_2$] 135-139 °C;

\textit{IR} $\nu_{max}$ (Thin film) 3585 (NH) 1665 (C=O) and 1632 (C=O);

\textit{H NMR} $\delta$ (400 MHz, CDCl$_3$) 8.12 (1H, dt, $J$ 7.5 and 1.0 Hz, C(6)H), 7.60 (1H, dq, $J$ 7.0 and 1.0 Hz, C(8)H), 7.34 (1H, t, $J$ 7.5 Hz, C(7)H), 6.30 (1H, dd, $J$ 7.0 and 3.0 Hz, C(3a)H), 4.49 (1H, brs, N(4)H), 3.36 (1H, C(2)H$_2$), 3.06 (1H, C(2)H$_2$), 2.60 (3H, s, CH$_3$), 2.50 (1H, C(3)H$_2$) and 2.21 (1H, C(3)H$_2$);

\textit{C NMR} $\delta$ (100 MHz, CDCl$_3$) 162.2– (C(5)\textit{d}), 156.8– (C(1)\textit{d}), 148.3– (C(9)\textit{d}), 135.5– (C(5a)\textit{d}), 135.3+ (C(8)H), 125.8+ (C(7)H), 123.9+ (C(6)H), 120.4– (C(9)\textit{d}), 83.1+ (C(3a)H), 29.8– (C(2)H$_2$), 27.3– (C(3)H$_2$) and 17.7+ (CH$_3$);
**MS (±ESI) m/z** 239 (100 %, [M+Na]⁺) and 217 (39 %, [M+H]⁺);

8-Methyl-2,3,3a,4-tetrahydropyrrolo[1,2-α]quinazoline-1,5-dione (269)

Glutaraldehyde (50 % wt. in water) (190 mL µL, 1 mmol) was added dropwise to a stirred solution of 2-amino-4-methylbenzamide 234 (150 mg, 1 mmol) and MgSO₄ (300 mg, 2.5 mmol) in THF (5 mL) at r.t. under N₂. NH₄Cl (27 mg, 50 mol %) was added and the reaction stirred at r.t. for 24 h. EtOAc (10 mL) was added and the THF removed under reduced pressure. After filtering the solvent was removed under reduced pressure to afford the crude enamine without further purification.

A solution of NaIO₄(aq) (10 %, 10 mL) was mixed with RuCl₃·xH₂O (10 mg, 0.04 mmol) and then added dropwise to a stirred solution of crude 9-methyl-3,4,4a,5-tetrahydro-6H-pyrido[1,2-α]quinazolin-6-one 286 (214 mg, 1 mmol) in EtOAc (20 mL) at r.t.. After 105 min stirring the layers were separated and the aqueous layer extracted with EtOAc (3 × 10 mL). iPrOH (20 mL) was added to the combined organic fractions and stirred for 18 h. After filtering the black precipitate the reaction was washed with water (10 mL), sat. brine (10 mL) and dried over Na₂SO₄ and filtered. Column chromatography [silica, CH₂Cl₂: Acetone gradient column] afforded the product 269 (56 mg, 26 %) as a white crystalline solid.

**Dione (269)**

Rf [EtOAc] 0.47;

Mp [MeOH] 135-145 °C;

**IR** νmax (Thin film) 3575 (NH), 1670 (C=O) and 1627 (C=O);

**¹H NMR** δH (400 MHz, CDCl₃) 8.12 (1H, d, J 8.5 Hz, C(6)H), 7.44 (1H, s, C(9)H), 7.26 (1H, d, J 8.5 Hz, C(7)H), 6.30 (1H, dd, J 3.0 and 7.0 Hz, C(3a)H), 4.89 (1H, brs, N(4)H), 3.39-3.31 (1H, m, C(2)H₂), 3.07-2.99 (1H, m, C(3)H₂) and 2.49 (3H, s, CH₃), 2.44-2.53 (1H, m, C(3)H₂) and 2.17-2.25 (1H, m, C(3)H₂);

**¹³C NMR** δC (100 MHz, CDCl₃) 161.7+ (C(5)₃), 158.3+ (C(1)₃), 149.7+ (C(9a)₃), 145.8+ (C(8)₃), 128.0–(C(7)H), 126.7– (C(9)H), 126.1– (C(6)H), 118.1+ (C(5a)₃), 83.0– (C(3a)H), 29.7+ (C(2)H₂), 27.4+ (C(3)H₂) and 21.9– (CH₃);
**MS** (+ESI) m/z 239 (100 %, [M+Na]^+), 455 (21 %, [2M+Na]^+) and 217 (19 %, [M+H]^+);

**HRMS** (+ESI) m/z Found [M+H]^+ 217.0972, C_{12}H_{13}N_{2}O_{2}, requires [M+H]^+ 217.0977. Found [M+Na]^+ 239.0794, C_{12}H_{12}N_{2}NaO_{2}, requires [M+H]^+ 239.0796;
2-\{(1R,2R,3R)-1,2,3,4-tetrahydroxybutyl\}-2,3-dihydroquinazolin-4(1H)-one (273)

Anthranilamide 196 (272 mg, 2 mmol) and D-lyxose (300 mg, 2 mmol) were stirred in MeOH (12 mL). NH₄Cl (54 mg, 100 mol %) was added and the reaction stirred at 60 °C for 18 h. The white precipitate was filtered and rinsed with MeOH (2 x 10 mL) affording the product 273 as a white solid (177 mg, 33 %) without further purification.

Mp [MeOH] 215-216 °C;

IR νmax (KBr disc) 3387, 3295, 3235, 1654, 1610, 1103;

$^1$H NMR δH (400 MHz, DMSO) 7.59 (1H, brs, NH), 7.58 (1H, dd, J 8.0 and 1.5 Hz, C(8)H), 7.23 (1H, ddd, J 8.0, 7.0 and 1.5 Hz, C(6)H), 6.84 (1H, dd, J 8.0 and 0.5 Hz, C(5)H), 6.64 (1H, td, J 7.5 and 1.0 Hz, C(7)H), 6.32 (1H, brs, N), 5.06 (1H, d, J 6.5 Hz, C(1')OH), 4.89 (1H, d, J 6.5 Hz, C(3')H), 4.57 (2H, m, C(2')OH and C(4')OH), 4.44 (1H, d, J 6.5 Hz, C(3')OH), 3.76 (1H, m, C(3')H), 3.64-3.57 (2H, m, C(2')H and C(1')H) and 3.50-3.46 (2H, m, C(4')H$_2$);

$^{13}$C NMR δC (100 MHz, DMSO) 163.7 (C(=O)$_2$), 148.5 (C(4a)$_2$), 132.8+ (C(6)H), 126.9+ (C(8)H), 116.2+ (C(7)H), 114.7 (C(8a)$_2$), 114.2+ (C(5)H), 72.2+ (C(1')H), 70.4+ (C(2')H), 70.1+ (C(3')H), 66.3+ (C(3)H) and 62.7– (C(4')H$_2$);

MS (+ESI) m/z 157 (100 %, [Anthranilamide+Na]$^+$–H$_2$), 291 (24 %, [M+Na]$^+$) and 559 (25 %, [2M+Na]$^+$);

HRMS (+ESI) m/z Found [M+Na]$^+$ 291.0942, C$_{12}$H$_{16}$N$_2$NaO$_5$, requires [M+Na]$^+$ 291.0957.
2-((E)-((2R,3R,4R)-2,3,4,5-Tetrahydroxypentylidene)amino) benzamide (274)

Anthranilamide 196 (272 mg, 2 mmol) and D-lyxose (300 mg, 2 mmol) were stirred in EtOH (12 mL) at 60 °C for 18 h. The white precipitate was filtered and rinsed with EtOH (2 x 10 mL) affording the product 274 as a white solid (477 mg, 89 %) without further purification.

Mp [MeOH] 178-185 °C;

IR  ν max (KBr disc) 3433, 3288, 3186, 1646, 1514, 1039;

1H NMR δ (400 MHz, DMSO) 8.78 (1H, d, J 9.0 Hz, NH), 7.83 (1H, brs, NH₂), 7.61 (1H, dd, J 1.5, and 8.0 Hz, C(3)H), 7.31 (1H, dt, J 1.5 and 8.5 Hz, C(5)H), 7.16 (1H, brs, NH₂), 6.87 (1H, d, J 8.0 Hz, C(6)H), 6.67 (1H, dt, J 1.0 and 8.0 Hz, C(4)H), 4.93 (1H, d, J 6.0 Hz, C(2')O), 4.88 (1H, d, J 5.5 Hz, C(3')OH), 4.84 (1H, d, J 5.0 Hz, C(4')OH), 4.83 (1H, brd, J 10.0 Hz, C(1')H), 3.76 (1H, brdd, J 1.5, 3.5 and 5.5 Hz, C(2')H), 3.71-3.61 (2H, m, C(4')H and C(5')H₂), 3.44-3.40 (1H, m, C(3')H) and 3.15 (1H, dd, J 9.0 and 10.5 Hz, C(5')H₂);

13C NMR δ (125 MHz, DMSO) 171.1 (C(=O)q), 147.3 (C(1)q), 132.0+ (C(5)H), 128.7+ (C(3)H), 116.0 (C(2)q), 115.5+ (C(4)H), 113.1+ (C(6)H), 81.3+ (C(1')H), 74.1+ (C(3')H), 70.3+ (C(2')H), 66.5+ (C(4')H) and 66.0– (C(5')H₂);

1H NMR δ (400 MHz, DMSO-D₂O shake) 7.57 (1H, dd, J 1.5 and 8.0 Hz, C(3)H), 7.32 (1H, ddd, J 1.5, 7.0 and 8.5 Hz, C(5)H), 6.87 (1H, d, J 8.0 Hz, C(6)H), 6.69 (1H, dt, J 1.0 and 8.0 Hz, C(4)H), 4.82 (1H, d, J 1.5 Hz, C(1')H), 3.77 (1H, brdd, J 1.5 and 3.5 Hz, C(2')H), 3.71-3.66 (1H, m, C(5')H), 3.64-3.60 (1H, m, C(4')H), 3.43 (1H, dd, J 3.5 and 8.5 Hz, C(3')H) and 3.15 (1H, dd, J 9.0 and 10.5 Hz, C(5')H);

13C NMR δ (125 MHz, DMSO-D₂O shake) 171.1 (C(=O)q), 147.0 (C(1)q), 132.3+ (C(5)H), 128.7+ (C(3)H), 116.0+ (C(2)H), 115.6 (C(4)q), 113.2+ (C(6)H), 81.1+ (C(1')H), 73.7+ (C(3')H), 70.0+ (C(2')H), 66.4+ (C(4')H) and 65.6 (C(5')H₂);

MS (+ESI) m/z 291 (100 %, [M+Na]⁺) and 559 (58 %, [2M+Na]⁺);
HRMS (ESI) \( m/z \) Found \([M+Na]^+ \) 291.0942, \( \text{C}_{12}\text{H}_{16}\text{N}_{2}\text{NaO}_{5} \), requires \([M+Na]^+ \) 291.0957;
2-({15,25,35}-1,2,3,4-tetrahydroxybutyl)-2,3-dihydroquinazolin-4(1H)-one (275)

Anthranilamide 196 (272 mg, 2 mmol) and L-lyxose (300 mg, 2 mmol) were stirred in MeOH (12 mL). NH₄Cl (54 mg, 100 mol %) was added and the reaction stirred at 60 °C for 18 h. The white precipitate was filtered and rinsed with MeOH (2 x 10 mL) affording the product 275 as a white solid (209 mg, 39 %) without further purification.

**Mp [MeOH]** 213-215 °C;

**IR** νₓ max (KBr disc) 3375, 3237, 1654, 1504, 1103;

**¹H NMR** δₓ (400 MHz, DMSO) 7.62 (1H, brs, NH), 7.57 (1H, dd, J 8.0 and 1.5 Hz, C(8)H), 7.30 (1H, brs, NH), 7.22 (1H, ddd, J 8.0, 7.0 and 1.5 Hz, C(6)H), 6.84 (1H, dd, J 8.0 and 0.5 Hz, C(5)H), 6.63 (1H, td, J 8.0 and 1.0 Hz, C(7)H), 6.38 (1H, brs, NH), 5.06 (1H, d, J 6.5 Hz, C(1')OH), 4.89 (1H, d, J 6.5 Hz, C(3')H), 4.64-4.57 (2H, m, C(2')OH and C(4')OH), 4.44 (1H, d, J 6.5 Hz, C(3')OH), 3.76 (1H, q, J 6.0 Hz, C(3')H), 3.64-3.57 (2H, m, C(2')H and C(1')H) and 3.50-3.46 (2H, m, C(4')H₂);

**¹³C NMR** δₓ (100 MHz, DMSO) 163.7 (C(1)q), 148.5 (C(4a)q), 132.8+ (C(6)H), 126.9+ (C(8)H), 116.2+ (C(7)H), 114.7 (C(8a)q), 114.2+ (C(5)H), 72.2+ (C(1')H), 70.4+ (C(2')H), 70.1+ (C(3')H), 66.3+ (C(3)H) and 62.7– (C(4')H₂);

**MS (+ESI)** m/z 157 (100 %, [Anthranilamide+Na]⁺ – H₂), 559 (25 %, [2M+Na]⁺) and 291 (24 %, [M+Na]⁺);

2-((E)-((2S,3S,4S,5S)-2,3,4,5-Tetrahydroxypentylidene)amino) benzamide (276)

Anthranilamide 196 (272 mg, 2 mmol) and L-lyxose (300 mg, 2 mmol) were stirred in EtOH (12 mL) at 60 °C for 18 h. The white precipitate was filtered and rinsed with EtOH (2 x 10 mL) affording the product 276 as a white solid (214 mg, 40 %) without further purification.

Mp [MeOH] 173-175 °C;

IR \( \nu_{\text{max}} \) (KBr disc) 3433, 3288, 3186, 1646, 1514, 1039;

\(^1\)H NMR \( \delta (400 \text{ MHz, DMSO}) \)

8.87 (1H, d, \( J \) 9.0 Hz, \( \text{NH} \)), 7.85 (1H, brs, \( \text{NH}_2 \)), 7.61 (1H, dd, \( J \) 8.0 and 1.5 Hz, \( C(3)H \)), 7.31 (1H, dt, \( J \) 8.5 and 1.5 Hz, \( C(5)H \)), 7.21 (1H, brs, \( \text{NH}_2 \)), 6.87 (1H, d, \( J \) 8.5 Hz, \( C(6)H \)), 6.67 (1H, d, \( J \) 7.5 Hz, \( C(4)H \)), 4.97 (1H, d, \( J \) 6.0 Hz, \( C(2')\text{OH} \)), 4.93 (1H, d, \( J \) 5.0 Hz, \( C(3')\text{OH} \)), 4.88 (1H, d, \( J \) 5.0 Hz, \( C(4')\text{OH} \)), 4.82 (1H, brd, \( J \) 10.0 Hz, \( C(1')H \)), 3.77 (1H, brt, \( J \) 3.5 Hz, \( C(2')H \)), 3.71-3.61 (2H, m, \( C(4')H \) and \( C(5')H \)), 3.44-3.40 (1H, m, \( C(3')H \)) and 3.15 (1H, dd, \( J \) 9.0 and 10.5 Hz, \( C(5')H \));

\(^{13}\)C NMR \( \delta (125 \text{ MHz, DMSO}) \)

171.0 (\( C(=\text{O})_2 \)), 147.3 (\( C(1)_2 \)), 132.0+ (\( C(5)H \)), 128.7+ (\( C(3)H \)), 115.9 (\( C(2)_2 \)), 115.6+ (\( C(4)H \)), 113.1+ (\( C(6)H \)), 81.3+ (\( C(1')H \)), 74.1+ (\( C(3')H \)), 70.3+ (\( C(2')H \)), 66.5+ (\( C(4')H \)) and 66.0– (\( C(5')H_2 \));

MS (+ESI) \( m/z \) 559 (100 %, [2M+Na]⁺) and 291 (70 %, [M+Na]⁺);

HRMS (+ESI) \( m/z \) Found [M+Na]⁺ 291.0942, \( C_{12}H_{16}N_2NaOs \), requires [M+Na]⁺ 291.0957;
7. Appendices - NCI 60 cell line screen One-Dose data

7-Hydroxy-8,9-dimethoxy-1,3,4,5-tetrahydrophenanthridin-6(2H)-one (165)

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- 207 -
### Developmental Therapeutics Program

#### One Dose Mean Graph

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| Mean           | 67.92                               |
| Delta          | 42.90                               |
| Range          | 64.95                               |

**Developmental Therapeutics Program**

**One Dose Mean Graph**

**Experiment ID:** 14082633

**Test Date:** Aug 11, 2014

**Report Date:** Aug 27, 2014

[Graph showing data distribution]
### 8,9-Dimethoxy-3,4,4a,5-tetrahydrophenanthridin-6(2H)-one (162)

**Developmental Therapeutics Program**

**One Dose Mean Graph**

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#### Developmental Therapeutics Program

**One Dose Mean Graph**

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- 211 -
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