Synthesis and Evaluation of Novel α-Oxyalkyl-
α,β-Cyclohexenones as Pro-drugs for the
Intracellular Delivery of Inhibitors of the
Oxidoreductase Enzyme NQO1

A thesis submitted to the University of Manchester for the
degree of Doctor of Philosophy in the Faculty of Science and Engineering

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School of Chemistry
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</tr>
</thead>
<tbody>
<tr>
<td>acac</td>
<td>Acetylacetone</td>
</tr>
<tr>
<td>ADDP</td>
<td>1,1’-(azodicarbonyl)dipiperidine</td>
</tr>
<tr>
<td>ALA</td>
<td>aminolevulinic acid</td>
</tr>
<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>BDA</td>
<td>butane-1,2-diacetal</td>
</tr>
<tr>
<td>Binap</td>
<td>2,2’-bis(diphenylphosphino)-1,1’-binaphthyl</td>
</tr>
<tr>
<td>b.p.</td>
<td>boiling point</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>c or conc.</td>
<td>concentration</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>¹³C</td>
<td>carbon 13</td>
</tr>
<tr>
<td>cat.</td>
<td>catalytic</td>
</tr>
<tr>
<td>Cmpd</td>
<td>compound</td>
</tr>
<tr>
<td>COMC</td>
<td>2-crotonyloxymethyl-cyclohex-2-enone</td>
</tr>
<tr>
<td>COSY</td>
<td>homonuclear correlation spectroscopy</td>
</tr>
<tr>
<td>COTC</td>
<td>2-crotonyloxymethyl-(4R,5R,6R)-4,5,6-trihydroxycyclohex-2-enone</td>
</tr>
<tr>
<td>CSA</td>
<td>camphorsulfonic acid</td>
</tr>
<tr>
<td>DABCO</td>
<td>1,4-diazabicyclo[2.2.2]octane</td>
</tr>
<tr>
<td>DBAD</td>
<td>di-tert-butyl azodicarboxylate</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N'-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DEAD</td>
<td>diethyl azodicarboxylate</td>
</tr>
<tr>
<td>DEPT</td>
<td>distortionless enhancement by polarisation transfer</td>
</tr>
<tr>
<td>DIAD</td>
<td>diiso-propyl azodicarboxylate</td>
</tr>
<tr>
<td>DIBAL-H</td>
<td>diiso-butylaluminium hydride</td>
</tr>
<tr>
<td>dm</td>
<td>decimetre</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
</tr>
<tr>
<td>DME</td>
<td>dimethoxyethane</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N’-dimethylformamide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess-Martin periodinane</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>dppb</td>
<td>1,4-bis(diphenylphosphino)butane</td>
</tr>
<tr>
<td>E. Coli</td>
<td>Escherichia Coli</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>eq.</td>
<td>equivalent</td>
</tr>
<tr>
<td>ETC</td>
<td>electron transport chain</td>
</tr>
<tr>
<td>FAD</td>
<td>flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
</tr>
<tr>
<td>FCS</td>
<td>fetal calf serum</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier Transform infrared</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GDH</td>
<td>glucose dehydrogenase</td>
</tr>
<tr>
<td>GSH</td>
<td>reduced glutathione (γ-L-glutamyl-L-cysteinylglycine)</td>
</tr>
<tr>
<td>GSTP</td>
<td>glutathionyl-S-transferase P</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>$^1$H</td>
<td>proton 1</td>
</tr>
<tr>
<td>His</td>
<td>histidine</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple quantum coherence</td>
</tr>
<tr>
<td>hNQO1</td>
<td>human NQO1</td>
</tr>
<tr>
<td>HSA</td>
<td>human serum albumin</td>
</tr>
<tr>
<td>HSQC</td>
<td>heteronuclear single quantum coherence</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>half maximal Inhibitory Concentration</td>
</tr>
<tr>
<td>IPTG</td>
<td>iso-propyl β-D-1-thiogalactopyranoside</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant</td>
</tr>
<tr>
<td>LB</td>
<td>lysogeny broth</td>
</tr>
<tr>
<td>µM</td>
<td>micromolar</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>m-CPBA</td>
<td>meta-chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>min(s)</td>
<td>minute(s)</td>
</tr>
</tbody>
</table>
Abbreviations

mL  millilitre
mm  millimetre
mmol millimol
m.p. melting point
MTT  (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
NADP+ nicotinamide adenine dinucleotide phosphate
NADPH nicotinamide adenine dinucleotide phosphate hydrogen
NaPi sodium phosphate buffer
Ni-NTA nickle nitrilotracetic acid
nM nanomolar
NMR nuclear magnetic resonance
Np naphthyl
NQO1 NAD(P)H: quinone oxidoreductase 1
PBS phosphate buffer saline
PCC pyridinium chlorochromate
Ph phenyl
Phe phenylalanine
ppm parts per million
pyr pyridine
r.t. room temperature
RPMI Roswell Park Memorial Institute
ROS reactive oxygen species
SDS sodium dodecyl sulfate
SOC super optimal broth with catabolite repression
TBSCl tert-butyldimethylsilyl chloride
TES tert-butyldiethylsilyl
TESOTf tert-butyldiethylsilyl trifluoromethanesulfonate
Tf trifluoromethanesulfonyl
TFA trifluoroacetic acid
THF tetrahydrofuran
TMAD tetramethylazodicarboxylate
p-TsOH p-toluensulfonic acid
Tyr tyrosine
Abstract

The α-oxymethyl-α,β-cyclohexenone moiety is embedded in several bioactive natural products, including 2-crotonyloxymethyl-(4R,5R,6R)-4,5,6-trihydroxycyclohex-2-enone (COTC) and the terpenoid, antheminone A. Both compounds exhibit cytotoxicity towards a variety of cancer cell lines – a finding which has stimulated extensive scientific interest in compounds of this type. A series of mono-hydroxylated analogues of COTC and antheminone A bearing aryl side-chain substituents were synthesised and evaluated in order to study and optimise their structures to achieve better bioactivities.

NAD(P)H: quinone oxidoreductase 1 (NQO1) is a cytosolic homodimeric FAD-dependent flavoprotein that constantly overexpresses in tumour cells. The most commonly used NQO1 inhibitor, dicoumarol, has been found to have unpleasant “off-target” effects and a variety of novel NQO1 inhibitors have hence been designed. Several dicoumarol-based asymmetric NQO1 inhibitors previously designed by the Whitehead group were synthesised and evaluated. In order to overcome poor solubility of the NQO1 inhibitors in terms of drug delivery, they were coupled to different carriers possessing a cyclohexenone moiety as pro-drugs.

A small panel of pro-drugs were synthesised and evaluated. They were designed to undergo a mechanism similar to that of COTC to release the NQO1 inhibitors and also to form alkylating agents to bind crucial biomacromolecules. The synthesised pro-drugs proved to exhibit varying levels of anti-proliferative activity against three tested cancer cell lines.
Declaration

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I. Introduction

The increasing diagnostic prevalence of cancer has made it the focus of widespread research. A distinctive feature of cancer cells is their resistance to apoptosis and unresponsiveness to growth inhibitory signals. Nowadays, cancers are often treated with cancer therapies (e.g. surgery, radiation therapy and chemotherapy) and chemotherapy, since its first use during the period of World War II, remains one of the three mainstream cancer treatments.

Among over 50 currently available chemotherapy compounds, 30% of them are either natural products or their derivatives. These natural compounds exhibit beneficial inhibitory effects on the growth of cancer cells and, therefore, have become synthetic targets of interest. Modifications of the structures of natural products are sometimes made to increase their potencies.

Pro-drugging is a common strategy in medicinal chemistry as well as an important approach in chemotherapy. It secures the delivery of active compounds to the target sites and it is believed to cause fewer side-/adverse-effects.

In this thesis, the syntheses of a small panel of pro-drugs derived from natural product analogues are discussed and their biological properties are examined.

1.1 A Background to COTC and COTC-Related Natural Products

2-(Crotonyloxymethyl-(4R,5R,6R)-4,5,6-trihydroxycyclohex-2-enone (COTC, Figure 1.1) was first isolated in 1975 from a culture broth of *Streptomyces griseosporeus* by Takeuchi and colleagues. Its absolute configuration was subsequently determined by Chimura and co-workers.\(^1\)\(^2\)

COTC became a compound of interest as it displayed certain inhibitory and cancerostatic activities during preliminary studies: it was identified as a glyoxalase I inhibitor and showed inhibition of the growth of HeLa cells as well as of murine Ehrlich ascites carcinoma and L1210 leukemia by daily intraperitoneal injection. It also inhibited the
growth of murine lymphoblastoma L5178Y cells by intervening the biosynthesis of DNA. These results have attracted broad interest in exploring COTC’s potential for biological application.\textsuperscript{1,3}

1.1.1 COTC and the Glyoxalase System

![Figure 1.2: Structure of methylglyoxal (2).]

Methylglyoxal 2 (Figure 1.2) is the product formed by elimination of phosphate from dihydroxyacetonephosphate (DHAP) and glyceraldehyde-3-phosphate (G3P), both of which are intermediates in the glycolysis pathway. It exhibits cytotoxicity which is associated with the inhibition of DNA, RNA and protein synthesis and it can induce cell apoptosis at toxic levels. Methylglyoxal is also the physiological substrate for the glyoxalase system whereby it is inactivated as part of a cell protection scheme (Scheme 1.1).\textsuperscript{4-8}

![Scheme 1.1: Glyoxalase enzymes catalysed methylglyoxal detoxification.]

The glyoxalase system is present in the cytosol of all mammalian cells and is responsible for detoxifying methylglyoxal and its derivatives into the corresponding α-hydroxy acids, in the presence of the co-factor, reduced glutathione (GSH). The system is composed of two enzymes: glyoxalase I (Glo1), which is frequently found over-expressed in malignant tumours, and thioester hydrolase glyoxalase II (Glo2) that works in conjunction with Glo1. The detoxification process begins with a non-enzymatic reaction between methylglyoxal and GSH to give a hemithioacetal adduct 3, which is deprotonated and isomerised into R-D-lactoylglutathione 6 exclusively under the catalysis of Glo1. Glo2 hydrolys...
6 into D-lactate 7 during which GSH is regenerated. From this mechanism it was inferred that by silencing Glo1, or depleting intracellular GSH, methylglyoxal could accumulate, leading to apoptosis. In this regard, inhibitors of Glo1 have previously been reported as novel anti-tumour agents.\textsuperscript{6,8}

Little is known about COTC’s exact mode of action, but it is widely acknowledged that the glyoxalase system is involved to some extent. Based on the initial studies by Takeuchi, Ganem and colleagues proposed that a moderately potent competitive inhibitor of Glo1 (8) was generated by a nucleophilic reaction between COTC and GSH (Scheme 1.2). They also suggested that COTC induced the accumulation of methylglyoxal, not only by reducing the intracellular concentration of GSH, but also by inhibiting the activity of Glo1 via its COTC-GSH adduct.\textsuperscript{9}

\begin{scheme}
\begin{align*}
\text{COTC} & \quad \text{GSH} \\
\text{phosphate buffer (pH = 7.5)} & \\
\text{adduct} & \quad (8)
\end{align*}
\end{scheme}

This proposal was subsequently re-evaluated by the same research group. The COTC-GSH adduct 8 was, in fact, a weak inhibitor when tested against human erythrocyte Glo1 and the anti-tumour activity exhibited by this adduct did not correspond to that of COTC itself (IC\textsubscript{50}). Although it was still plausible that COTC manifested part of its tumour toxicity in this way, Ganem and colleagues advanced an alternative mode of action for COTC on the basis of the results from their kinetic and trapping studies.\textsuperscript{10}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{COMC.png}
\caption{Structure of COMC (9).}
\end{figure}

2-Crotonyloxymethylcyclohex-2-enone (COMC 9, Figure 1.3) was synthesised as a simplified version of COTC for their investigations. It was noticed that under the catalysis of human glutathionyl S-transferase pi (hGSTP1-1), an enzymatic Michael addition between COMC and GSH occurred to afford a transient but highly electrophilic intermediate 11 that further reacted with GSH to give the final product 8 (Scheme 1.3). The
electrophilic intermediate can also react as an alkylating agent with critical cellular molecules, such as nucleic acids and proteins, disrupting the functions of the cells. The tumouricidal activity of COTC was thus explained as a consequence of the interactions between intermediate 11 and important biomacromolecules instead of the inhibition of Glo1 by adduct 8.\textsuperscript{10-13}

Scheme 1.3: Mechanism of the conjugate addition of COMC 9 to GSH catalysed by GST.\textsuperscript{12}

1.1.2 COTC Related Natural Products

1.1.2.1 The Antheminones

The antheminones are a family of terpenoids isolated from the genus *Anthemis*. The ethyl acetate extract from the leaves of *Anthemis maritima* exhibited cytotoxic activity. From this extract three compounds were isolated (Figure 1.4): two cyclohexenones, antheminones A (14) and B (15), and an exocyclic enone, antheminone C (16).\textsuperscript{14}

Figure 1.4: Structures of two cyclohexenone and one exocyclic enone antheminones analogues.\textsuperscript{14}

When tested against a range of cancer cell lines, 14, 15 and 16 all displayed comparable anti-tumour activity with 16 being the most active (Table 1.1).
Table 1.1: Cytotoxicities of antheminone analogues (HCT116 and CaCo-2: human colorectal carcinoma cell lines; MCF-7: human breast carcinoma cell line; HL60: human leukemia cell line; U937: human lymphoma cell line; Jurkat T: human acute T-cell leukemia cell line).14

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (µM)</th>
</tr>
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<tr>
<td></td>
<td>HCT116</td>
</tr>
<tr>
<td>14</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>15</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>16</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

The structural similarity between these compounds and hydrolysed COTC 17 (Figure 1.5) was noted and they are consequently believed to act in a similar manner to COTC with regard to their anti-tumour activity.

![Figure 1.5: Structure of hydrolysed COTC (17).](image)

Compound 16, as an exocyclic enone that resembles the intermediate 11 discussed previously, was suggested to react directly with biomacromolecules and this may also explain its slightly higher activity with respect to the other two compounds.

The antheminone family have been regarded as potential anti-cancer drugs due to their significant anti-proliferative activities.

1.1.2.2 The Phorbasins

Over the course of the last decade, a family of polyene diterpenes, named the phorbasins, have been isolated and characterised by Capon and co-workers. Members of the phorbasin family are metabolites of a marine sponge from the genus *Phorbas* (*Phorbas* sp.). The crude ethanol extract of the sponge inhibited the growth of two strains of Gram positive bacteria. Among the isolated phorbasin analogues, several were reported as exhibiting significant anti-proliferative activities against a variety of cancer cell lines. The biological results are listed in Table 1.2.15-17
Table 1.2: Cytotoxicities (GI_{50}s) of selected phorbasin analogues (NFF: human neonatal foreskin fibroblast healthy cell line; A549: human lung carcinoma cell line; HT29: human colon colorectal carcinoma cell line; MM96L: malignant melanoma cell line).\(^{17}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>GI_{50} (µM)</th>
<th>NFF</th>
<th>A549</th>
<th>HT29</th>
<th>MM96L</th>
</tr>
</thead>
<tbody>
<tr>
<td>18a</td>
<td>6.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>18b</td>
<td>5.3</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>18c</td>
<td>7.4</td>
<td>2.7</td>
<td>4.7</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>18d</td>
<td>6.4</td>
<td>4.5</td>
<td>4.5</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>19a</td>
<td>7.6</td>
<td>2.8</td>
<td>2.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>19b</td>
<td>6.2</td>
<td>2.5</td>
<td>2.5</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>&gt;29.8</td>
<td>11.9</td>
<td>&gt;29.8</td>
<td>6.0</td>
<td></td>
</tr>
</tbody>
</table>

It was concluded by Capon and colleagues that the cyclohex-2-enone functionality was requisite for the anti-tumour activity of the phorbasin analogues. The cytotoxic properties they displayed have made them a group of compounds of interest as potential anti-cancer drugs.\(^{17}\)

1.1.2.3 The Carvotacetones

The carvotacetones are members of a family of compounds that have been isolated from the aerial parts of *Sphaeranthus suaveolens*. Some species of the *Sphaeranthus* genus have been employed as folk medications for treating various diseases, such as skin infections, glandular swellings and bronchitis. The structures of a selection of carvotacetone compounds that have been isolated are depicted in Figure 1.6.\(^{18,19}\)

Figure 1.6: A selection of representative carvotacetone analogues.\(^{18,19}\)
So far little research has been done concerning the bioactivities of carvotacetone analogues, nonetheless, they are compounds of interest due to their structural similarity to the antheminones (14-16) and COTC.

1.1.2.4 The Gabosines

The gabosines are a family of carba-sugars derived from bacteria of the *Streptomyces* strains. Carba-sugars continue to be regarded as biologically-interesting compounds as some have been reported as enzyme inhibitors or isolated as drug precursors. Four different types of gabosines were isolated and investigated by Bach and co-workers, but during initial tests, no significant bioactivities (antifungal, antibacterial, antiviral, *etc.*) were observed. Following later studies, however, weak DNA-binding properties were discovered and more importantly, several of them were found to show inhibition against glutathione S-transferases pi and mu (GSTP1-1 and GSTM1). These compounds (24-27) are depicted in Figure 1.7.20-22

![Figure 1.7: Structures of selected gabosine analogues.](image)

Based on the results of biological testing, a new series of gabosine analogues was designed and prepared by Li and colleagues. 4-*O*-Decyl-gabosine D (27), as a representative of this new family of competitive GST inhibitors, not only showed inhibition against glutathione S-transferase alpha-2 (GSTA2, 37.8 µM), GSTM1 (13.4 µM) and GSTP1-1 (25.3 µM) but also exhibited synergetic effects with cisplatin against the A549 lung cancer cell line through inhibition of GSTM1. The new class of compounds was considered to be useful for developing bioactive compounds that can overcome GSTM1-related drug resistance and cellular stress signalling.22
1.2 Previous Syntheses of COTC

Since its isolation, COTC has been regarded as a compound of research value: several synthetic approaches have been reported for the preparation of this compound. Herein, four total syntheses of COTC are described in the following sections.

1.2.1 Starting from Methyl α-D-mannopyranoside

In 1985, Vasella and colleagues reported the first successful preparation of COTC, starting from methyl α-D-mannopyranoside 28 in an overall yield of 18% (Scheme 1.4). The sequence began with acetonide protection of vicinal cis-diol in 28 followed by benzoate protection of the primary alcohol to give 29. The free hydroxyl group in 29 was oxidised to form ketone 30, which underwent β-elimination to give an intermediate enone. The ketone moiety was then reduced to a secondary alcohol with its stereochemistry inverted to 29 and subsequent oxidative cleavage of the exo-cyclic alkene gave lactone 31. TBS protection of the secondary alcohol in 31 gave 32, which was converted into cyclohexenone 33 by a Fujimoto-Belleau/Horner-Wadsworth-Emmons reaction. 33 underwent a 1,4-addition with dimethylaluminum phenylthiolate to give a thiophenol adduct and in which the enol moiety tautomerised into the corresponding ketone. This thiophenol ketone intermediate then reacted with formaldehyde in an aldol manner to give racemic mixture of 34. Subsequent β-elimination of thiophenol from 34 gave the α-hydroxymethycyclohexenone 35. 35 underwent deprotection and crotonylation to give the final compound COTC, which could also be obtained by reversing the order of the last two steps.23,24
1. Introduction

Scheme 1.4: Synthesis of COTC from α-D-mannopyranoside.23,25
Scheme 1.4: a) i) acetone, DMP, 65 mM sulfuric acid, p-toluenesulfonic acid (cat.), 60 °C, 2 d; ii) benzoyl chloride, pyridine, DCM, <15 °C, 20 min (75%); b) PCC, 3 Å molecular sieves, 2 h; c) i) NEt₃, DCM, r.t., 10 min.; ii) NaBH₄, i-PrOH, 0 °C, 2 h; iii) O₃, DMF, -78 °C; d) (t-Bu)Me₂SiCl, imidazole, DMF, r.t. overnight (72% over b, c and d); e) n-BuLi, dimethyl methylphosphonate, THF, -78 °C, argon, 3.5 h (62%); f) i) trimethylaluminium, thiophenol, Ar, 0 °C, 20 min.; ii) DCM, -78 °C, 1 h; iii) gaseous formaldehyde, -50 °C (66%); g) mCPBA, DCM, 0 °C, 1 h (91%); h) i) 60% aqueous TFA, 0 °C, 4 h (100%); ii) crotonic acid, boron trifluoride diethyl etherate, 4 Å molecular sieves, acetonitrile, 5 °C, 24 h (48%); i) crotonic acid, DMAP (cat.), DCC, DCM, 24 h (85%); j) 50% aqueous TFA, r.t., 2 h (100%).23,25

1.2.2 Adaption of the Asymmetric Diels-Alder Reaction

Koizumi and colleagues reported the first efficient enantioselective total synthesis of COTC via an asymmetric Diels-Alder reaction using the chiral arysulfinylacrylate 38 as a dienophile (Scheme 1.5).26

The optically pure sulfoxide 38 was prepared from 1-methyl propiolate 37 by undergoing an alkyne hydrothiolation followed by oxidation of the sulfide group. The chiral auxiliary was obtained from fractional crystallisation of the resulting sulfoxide intermediate to give compound 38. 38 underwent a diastereoselective and enantioselective Diels-Alder reaction with 2-methoxyfuran to form the endo-cycloadduct 39. Cis-dihydroxylation of the alkene moiety in 39 followed by protection of the resulting diol gave compound 40. The sulfoxide in 40 was then reduced to the sulfide and the menthol ester moiety was reductively cleaved to the primary alcohol in 41. The resulting alcohol was crotonylated and the sulfide was re-
oxidised to a sulfoxide to generate a better leaving group in 42. Subsequent deketalisation, hydrolytic ring-opening and eliminative desulfenylation of 42 was achieved in a single step to give the target compound COTC (1).26,27

Scheme 1.5: Synthesis of COTC using Diels-Alder reaction.26,28

Scheme 1.5: Men = (+)-menthol. a) i) 3-trifluoromethyl-2-mercaptopyridine, NEt₃ (cat.), DMSO-DCM; ii) mCPBA, DCM; iii) fractional crystallisation (20%); b) 2-methoxyfuran, toluene, 0 °C, 6 d; c) i) Me₃NO, OsO₄ (cat.); ii) 2,2-dimethoxypropane, p-TsOH (cat.) (72%); d) i) TiCl₄, EtOH; ii) LiAlH₄, THF (78%); e) i) crotonic anhydride, pyridine; ii) mCPBA, DCM (85%); f) 80% aqueous TFA, -20 °C, 5 h (62%).26,28

1.2.3 Starting from the (−)-Quinic Acid

In 1990, Shing and Tang developed a new approach to the synthesis of COTC starting with (−)-quinic acid, which was based on previous work by Berchtold and Lesuisse (Figure 1.8).29,30

Figure 1.8: Structure of (−)-quinic acid.

The reaction scheme is illustrated in Scheme 1.6: The cis-diol of (−)-quinic acid (43) underwent an acetalisation which was accompanied by a concurrent lactonisation between the carboxylic acid moiety and the C5 hydroxyl group to give lactone 44. Methoxylation of the lactone ring in 44 gave a dihydroxyester intermediate 45 in which the secondary hydroxyl group was oxidised to a ketone and subsequent β-elimination of the remaining tertiary hydroxyl group gave enone 46, which was reduced to give allylic alcohol 47. The resulting hydroxyl group in 47 was TBS protected and subsequent dihydroxylation of the alkene moiety was achieved selectively to afford cis-diol 49. The secondary hydroxyl in 49
was selectively acetylated and the tertiary alcohol was eliminated to give enoate 51. Reduction of both ester groups in 51 gave a diol intermediate in which the resulting primary alcohol was crotonylated to give allylic ester 52. The secondary alcohol in 52 was oxidised and the two protecting groups were removed to give the target compound COTC (I).30

Scheme 1.6: Synthesis of COTC from (−)-quinic acid.30

In 2000, Huntley and co-workers reported an expedient eight-step synthesis of COTC during their investigations into its mode of action (Scheme 1.7).31

(−)-Quinic acid 43 was converted into dihydroxyester 45 in a similar manner described above. The C5 hydroxyl group in 45 underwent tosylation during which C1’s hydroxyl group was spontaneously eliminated to give compound 54. Elimination of the remaining tosyl group in 54 gave diene 55. 55 then underwent an addition of “Br⁺” to give the
bromoformate ester 56 in which aldehyde and ester groups were both reduced to give bromodiol 57. Subsequent ring-closure afforded compound 58, which underwent an oxidative ring-opening mediated by DMSO to give the dihydroxyenone 59. Crotonylation of the primary alcohol in 59 followed by removal of the cyclohexylidene protecting group gave the target compound COTC 1 in an overall yield of 7%. \(^{31}\)

Scheme 1.7: Synthesis of COTC from cyclohexylidene ketal of (−)-methyl quinate. \(^{31,32}\)

Scheme 1.7: a) Tf₂O, pyridine, DCM (65%); b) caesium acetate, DMF; c) N-bromosuccinimide-H₂O, DMF (72% from 45); d) DIBAL-H, benzene-toluene (65%); e) LiN(TMS)₂, THF, -78 °C (87%); f) i) methanesulfonic acid, DMSO, r.t., 1.5 h; ii) NEt₃, r.t., 5 min (71%); g) crotonic anhydride, DCC, DMAP, THF (54%); h) 50% aqueous TFA (73%). \(^{31,32}\)
1.3 NAD(P)H: Quinone Oxidoreductase 1 (NQO1)

1.3.1 Introduction to NQO1

NAD(P)H: quinone oxidoreductase 1 (NQO1), originally referred as DT-diaphorase or menadione reductase 1, is a cytosolic homodimeric FAD-dependent flavoprotein that is widely-distributed in eukaryotes. It was first isolated from rat-liver in 1958 by Ernster and co-workers. NQO1 is regarded as a chemoprotective enzyme that contributes to cellular defence against oxidative stress and redox cycling. It converts endogenous and exogenous quinone species into the more easily excreted, less toxic hydroquinones via a two-electron reduction. This bypasses the one-electron pathway, which results in generation of semiquinone radicals that further give reactive oxygen species (ROS) under aerobic conditions. The increase in ROS level induces NQO1’s expression as a response to the stress signal.

1.3.2 Increased Expression of NQO1

An elevated level of NQO1 has been detected in many malignant tumours, indicating NQO1 is associated with the occurrence or progression of cancers. Ross and co-workers found elevated levels of NQO1 in human non-small cell lung carcinomas (NSCLCs), which were up to 80-fold higher than in normal lung. Schlager and Powis reported the up-regulation of NQO1 in several primary tumours compared to normal tissues of the same origin, such as lung, liver, colon, stomach and breast. Siegel and Ross later reported the over-expression of NQO1 in other tumours including ovary, cornea, adrenal and thyroid gland. An increased level of NQO1 in pancreatic tumours was also observed by Lyn-Cook and colleagues.

It has been suggested that the expression of NQO1 may be transcriptionally controlled and the increase in its expression could be regarded as a biomarker for certain types of neoplasia. These findings supported the development of NQO1-directed anti-tumour agents for therapeutic purposes.

1.3.3 Mechanism of Reduction by NQO1

Reductions catalysed by NQO1 follow a double-displacement sequence, also referred to as a “ping-pong” mechanism, which involves two substrates interacting with the same active site.

The reaction starts with the first substrate, NADH, binding to NQO1 to form a complex,
wherein hydride is transferred to the co-factor FAD (Scheme 1.8). The NQO1-FADH$_2$ intermediate then releases NAD$^+$, vacating the binding site for the second substrate. Quinone then enters the binding site and hydride is transferred from FADH$_2$ to the quinone to convert it into hydroquinone, which is then released as the product.$^{41}$

A detailed mechanism is depicted in Scheme 1.9: the hydrogen of NADH is transferred to N-5 of FAD and the negatively charged O-2 of the NQO1-FADH$_2$ complex then accepts a proton provided by the -OH group of Tyr$^{155}$. The resulting oxyanion of Tyr$^{155}$ can then be stabilised by the protonated His$_{161}$. This step can be summarised as a positive charge moving from His$_{161}$ to NADH.$^{42}$

After the release of NAD$^+$, quinone binds to NQO1 in an orientation that allows the ready acceptance of hydride from FADH$_2$. This reduces the quinone 61 to the hydroquinolate 62 while a reversal of the first step occurs to re-protonate His$_{161}$. The re-protonated His$_{161}$ stabilises the charge on the hydroquinolate, converting it into the hydroquinone 63.$^{42}$
1.3.4 Inhibitors of NQO1

NQO1 has been targeted in the search for anti-cancer treatments due to its close association with tumour development.

There are several compounds that have been used to inhibit NQO1, such as dicoumarol (64), Cibacron blue 3G-A (65) and ES936 (66) (Figure 1.9). Dicoumarol competes with NAD(P)H for binding to NQO1 and has been regarded as the most potent competitive inhibitor of NQO1. Due to its high competence, it has been used for studying the cellular function of NQO1 for decades. Cibacron blue 3G-A is a triazine dye that can bind to the nucleotide-binding site of NQO1 whereas ES936 is a mechanism-based inhibitor that can alkylate Tyr^{126} or Tyr^{128} in NQO1’s active site. It has been found, however, that dicoumarol has some undesired additional effects, which compromise its ability as an NQO1 inhibitor.43,44
1.3.5 “Off-Target” Effects of Dicoumarol

1.3.5.1 Regulation of the Stability of the Tumour Suppressor p53

p53 (Figure 1.10) is known as a tumour suppressor protein. Wild-type p53 is short-lived with a half-life of only a few minutes, resulting in its low expression level under normal circumstances. Activation of p53 occurs when cellular stress signals, such as DNA damage, hypoxia or oxidative stress are detected and as a consequence, an outburst in p53’s expression level is induced and this triggers the activation of transcriptional factors as a consequence. The transcriptional activations include cell cycle arrest during which damaged DNA is repaired, and trigger of cell apoptosis when the damage is deemed to be irreparable. Such p53-dependent activities prevent the damaged or mutated cells from proliferation or clonal expansion.46,47
Mutation of wild-type p53 has commonly been found in various histotypes such as lung, breast and haematopoietic malignancies. These mutants lose the tumour suppressing ability but gain new oncogenic properties, favouring the insurgence, the maintenance, the spreading and the chemo-resistance of malignant tumours. Mutated p53 is also known for its prolonged half-life. It has been reported that NQO1 can stabilise wild-type p53, possibly through a cell-type specific interaction, to suppress its degradation, the pathway of which is currently not completely understood. Shaul and co-workers reported that there is a correlation between inhibition of NQO1 by dicoumarol and the destabilisation of wild-type p53. A significant decrease in endogenous wild-type p53 level of the HCT116 (human colorectal carcinoma) cells occurred after treatment with dicoumarol for 90 minutes and a complete elimination of p53 was observed after 180 minutes. It was therefore concluded that p53 degradation was induced by the inhibition of activity of NQO1.

1.3.5.2 Extensive Protein Binding Ability of Dicoumarol

Dicoumarol is a competitive inhibitor of NQO1 that competes with NAD(P)H for binding to the active site thereby preventing the hydrogen transfer to FAD. On investigating the structures of hNQO1 (human NQO1, recombinant) in complex with different inhibitors, Shaul and colleagues found that dicoumarol bound to the active site of hNQO1 through hydrophobic interactions and hydrogen bonds with residues from two NQO1 monomers and the FAD. Structural changes of the protein caused by dicoumarol binding were reflected in the movement of several residues. The maximum movement was observed between Tyr$^{128}$ and Phe$^{232}$ residues in the catalytic pocket, with the distance approximately 12 Å after binding dicoumarol (7 Å before binding). Cibacron blue 3G-A also interacted with residues from both NQO1 monomers, but the movement between Tyr$^{128}$ and Phe$^{232}$ residues was reduced to 9 Å compared to when dicoumarol was bound. ES936 is a mechanism-based NQO1 inhibitor and its interactions with NQO1 are mainly hydrophobic.
contacts with only one hydrogen bond formed with Tyr$^{126}$ residue: the movement between Tyr$^{128}$ and Phe$^{232}$ residues was reduced to 8 Å compared to when dicoumarol was bound.\textsuperscript{43}

![Figure 1.11: The overall structure of the human NQO1 homodimer bound with dicoumarol determined by Shaul and co-workers. NQO1 is represented as ribbons, FAD is coloured in red and dicoumarol is coloured in blue.\textsuperscript{53}]

Based on the results of Garten and Wosilait’s comparative study, dicoumarol showed the greatest extent of binding to serum albumins from different sources (human, rabbit and bovine) compared to other compounds (i.e. coumarin, 4-hydroxycoumarin, warfarin, Tromexan and Sintrom). It was suggested that electrostatic interaction between the negatively charged hydroxyl groups and the cationic centre on the surface of the protein contributed most to the initial binding. The degree of binding increased when an increased amount of dicoumarol was applied as the inner, hidden hydrophobic binding sites on the serum albumin molecules became available. It is known that human serum albumin (HSA) is the most abundant protein in the blood, and extensive binding interaction with dicoumarol may compromise its role as an NQO1 inhibitor and preclude its delivery into cells.\textsuperscript{52,53}

1.3.5.3 Induction of Production of Intracellular Superoxides and Disruption of MTT Assays

Cullen and co-workers reported that dicoumarol is associated with an increased production of cellular superoxides and hydrogen peroxides. This increase was not only induced by inhibition of NQO1 but also due to the ability of dicoumarol to disturb the mitochondrial electron transport chain (ETC) during oxidative phosphorylation. Villalba and co-workers reported that after treatment with dicoumarol, the inhibition of NQO1 activity as well as reversed electron flow was observed in respiratory Complex II (succinate dehydrogenase).
The disruption of mitochondrial function also causes inaccuracies in mitochondrion-based cell viability assay, the MTT assay.$^{54-56}$

The MTT assay, a commonly used technique for assessing cell proliferation, is dependent on a succinate dehydrogenase-mediated reduction, converting MTT into formazan. Mitochondrial ROS (reactive oxygen species) can also participate in the reduction of MTT. Pritsos and Collier found that the inclusion of dicoumarol in MTT assay led to initial normal cell growth followed by a continuous decrease in cell viability: this corresponded to an initial induction of ROS by dicoumarol and a subsequent manifestation of toxicity of ROS.$^{57}$

In conclusion, the above findings have stimulated a demand for novel NQO1 inhibitors that retain the inhibitory potency of dicoumarol against NQO1 but lack the “off-target” effects.
1.4 Incarviditone

Incarvillea is a genus of plant in the Bignoniaceae family. Several compounds have been isolated from this genus and some of them found to exhibit different types and levels of bioactivities (anti-inflammatory, bacteriostatic and antinociceptive). In 2009, Zhang and co-workers isolated a benzofuranone dimer, incarviditone 67, from the species Incarvillea delavayi and its originally assigned structure is shown in Figure 1.12.58

![Figure 1.12: Structure of incarviditone (67) reported by Zhang and co-workers.](image)

The cytotoxicity of incarviditone against several cell lines was assessed, however it only showed cytotoxicity against HL-60 and 6T-CEM, which are both leukemia cell lines. The monomeric tetrahydrobenzofuran precursor to incarviditone, rengyolone (68), that was also isolated from the same species of Incarvillea, exhibited cytotoxicity against multiple cell lines (Table 1.3).58

Table 1.3: Cytotoxicity of incarviditone (67) and rengyolone (68) against several cell lines (LOVO: human colorectal adenocarcinoma cell line; HL-60 and 6T-CEM are both human leukemia cell lines; HepG2: human hepatocellular carcinoma cell line).58

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>IC50 (μM) incarviditone</th>
<th>IC50 (μM) rengyolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>&gt;97</td>
<td>105.2 ± 5.2</td>
</tr>
<tr>
<td>LOVO</td>
<td>&gt;97</td>
<td>75.3 ± 3.9</td>
</tr>
<tr>
<td>HL-60</td>
<td>48.0 ± 1.0</td>
<td>37.0 ± 1.9</td>
</tr>
<tr>
<td>6T-CEM</td>
<td>72.0 ± 2.9</td>
<td>39.6 ± 2.6</td>
</tr>
<tr>
<td>HepG2</td>
<td>&gt;97</td>
<td>131.1 ± 4.5</td>
</tr>
</tbody>
</table>

In 2012, Tang and colleagues published the biomimetic total synthesis of incarviditone (67) from rengyolone (68) via a stereoselective homodimerisation pathway. They suggested that rengyolone undergoes either an oxa-Michael addition/carba-Michael addition tandem reaction sequence or an oxa-Michael addition/Diels-Alder/retro aldol
reaction sequence to give the homodimer incarviditone (Scheme 1.11).\(^5^9\)

Scheme 1.11: Homodimerisation of rengyolone (68) to form incarviditone (67).\(^5^9\)

Subsequent computational studies by Tang and colleagues on the oxa-Michael addition step found that there were two possible transition states (71 and 72, Scheme 1.12) that lead to different dimerisation products. Compared to 71, 72 was, in fact, more favoured in terms of energy requirement. This finding suggested that the originally proposed structure of incarviditone 67 might require revision if the Michael addition proceeded via 72 instead of 71. Subsequent NMR analysis of their synthetic incarviditone validated this hypothesis.\(^5^9\)

Scheme 1.12: Different transition states lead to different homodimerisation products.\(^5^9\)

The structure of incarviditone was thus revised and the new structure is illustrated in Figure 1.13.\(^5^9\)
1.5 Previous Work by the Whitehead Research Group

1.5.1 COTC and Antheminone Related Research

Over the last decade, the Whitehead research group has been working on the development and evaluation of a number of compounds derived from bioactive natural products possessing the cyclohex-2-enone skeleton, such as the cytotoxic agent COTC and antheminone A. An array of COTC analogues has been prepared with the general structure depicted in Figure 1.14. Three loci, X, Y and Z, provided sites for introducing different substituents in order to probe structure-activity relationships.

![Figure 1.14: General structure of COTC analogues.](image)

The anti-proliferative activities of these COTC analogues were measured against the A549 and H460 cell lines using the MTT assay and the results are listed in Table 1.4.
Table 1.4: MTT assay results of COTC analogues prepared by the Whitehead group (A549 and H460 are both human lung carcinoma cell lines).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (µM)</th>
<th>Compound</th>
<th>IC_{50} (µM)</th>
</tr>
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<tr>
<td></td>
<td>A549</td>
<td>H460</td>
<td>A549</td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>40</td>
<td>31</td>
</tr>
<tr>
<td>74</td>
<td>24</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>75</td>
<td>17</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>76</td>
<td>147</td>
<td>158</td>
<td>18</td>
</tr>
<tr>
<td>77</td>
<td>164</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>78</td>
<td>32</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>74</td>
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</tr>
<tr>
<td>75</td>
<td></td>
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<td>76</td>
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<td>77</td>
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<td></td>
</tr>
<tr>
<td>78</td>
<td></td>
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</tbody>
</table>

The Whitehead group made several conclusions from the results:

1. The extent of hydroxylation influenced the anti-proliferative activity: *i*) non-hydroxylated analogue (COMC, 9) was more active than poly-hydroxylated analogues (76, 77 and 80); *ii*) blockade of the vicinal hydroxyl groups on 76, 77 and 80 (as 78, 79 and 81, respectively) enhanced the activity against both cell lines to the same level as 9; *iii*) mono-hydroxylated analogue 74 and 75 exhibited better activity than the compounds mentioned above, however the stereochemistry of the C4-hydroxyl did not significantly affect the activity;

2. With the same extent of hydroxylation, introduction of an electronegative group (76 and 77) had no beneficial effect on the biological activity;

3. An intact cyclohex-2-enone core appeared to be associated with better biological activity, as compound 82 was 10-fold more active than 83 that lacked such a moiety.
More recently, three more COTC analogues were prepared and evaluated by the Whitehead group. MTT assays were conducted on the A549 cell line and the results are listed below:

Table 1.5: MTT assay results of three new COTC analogues.\textsuperscript{64}

<table>
<thead>
<tr>
<th>Compound</th>
<th>(84)</th>
<th>(85)</th>
<th>(86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC(_{50}) (µM)</td>
<td>1.3 ± 0.2</td>
<td>8 ± 5</td>
<td>14 ± 4</td>
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</tbody>
</table>

From these results, the Whitehead group concluded that: 1. Introduction of an aromatic substituent at C5 was beneficial in terms of activity, given the fact that both \(84\) and \(85\) were more active than the aliphatic-substituted compound \(86\); 2. Reversing the stereochemistry of C5 influences the activity, with \(84\) displaying higher activity than its diastereoisomer \(85\).\textsuperscript{64}
1.5.2 Syntheses of COTC Analogues

The synthetic routes to the COTC analogues discussed above are reported in the following sections and are categorised by the different starting materials that were employed.

1.5.2.1 Starting from the (−)-Quinic Acid

(−)-Quinic acid (43) was employed as the initial starting material for most syntheses of COTC analogues by the Whitehead group, based on the method developed by Shing and Tang (section 1.2.3).

To synthesise the enantiomerically pure mono-hydroxylated COTC analogue 75, the cis-diol group in (−)-quinic acid was initially protected as its cyclohexylidene and a concurrent lactonisation occurred to give 44. The lactone ring in 44 was reductively cleaved and the resulting vicinal diol was oxidised to give hydroxyketone 88. Subsequent β-elimination formed an alkene moiety, which was hydrogenated to give ketone 90. 90 underwent a one-pot reaction during which the cyclohexylidene was removed and the resulting free hydroxyl group was TBS-protected to give enone 91. A Morita-Baylis-Hillman reaction was applied to introduce a hydroxymethylated side chain to the alkene moiety in 91 to afford 92. Subsequent crotonylation followed by deprotection gave the target compound 75 (Scheme 1.13).

Scheme 1.13: Synthesis of mono-hydroxylated COTC analogue 75. 

Scheme 1.13: a) cyclohexanone, DMF/C₆H₆ (1:1), Amberlite® IR 120 (H⁺), reflux (Dean and Stark), 5 h (60%); b) NaBH₄, CH₃OH, 0 °C to r.t., 24 h; c) NaIO₄, H₂O, CH₃OH, 0 °C, 1.5 h (58% from 44); d) triflic anhydride, pyridine, DCM, 0 °C to r.t., 12 h (82%); e) H₂, 10% Pd/C, EtOAc, r.t., 17 h (92%); f) DBU, TBSCl, benzene, reflux, 6 h (80%); g) DMAP (cat.), H₂CO, THF/H₂O (1:1), 40 °C, 24 h (52%); h) i) crotonic anhydride, pyridine, DMAP (cat.), DCM, r.t., 1.5 h (68%); ii) TFA/H₂O (7:1), 0 °C, 1 h (98%).
To synthesise the dihydroxylated COTC analogue 76 and its BDA-protected derivative 78, the trans-diol moiety in (−)-quinic acid 43 was selectively protected and this was accompanied by an esterification of the carboxylic acid group to give 93. The ester group in 93 was reduced and the resulting vicinal diol was oxidised to give hydroxyketone 95, which underwent a β-elimination to give enone 96. Subsequent Morita-Baylis-Hillman reaction furnished 97 with a hydroxymethylene side chain, which was then crotonylated to give compound 78. Final removal of the BDA protecting group gave the target compound 76 (Scheme 1.14).\(^6\)

The enone intermediate 96 was also used in the synthesis of the cyclopropyl compound 82. 96 underwent a Corey-Chaykovsky reaction to convert the alkene moiety into a cyclopropane moiety to give 98. Deprotection of the BDA group afforded the γ-hydroxyenone 99 in which the resulting hydroxyl was TBS-protected to give enone 100. A hydroxymethylated side chain was introduced at the alkene moiety in 100 by a Morita-Baylis-Hillman reaction to afford compound 101. Subsequent crotonylation of the side chain in 101 followed by a deprotection gave the target compound 82 (Scheme 1.15).\(^6\)
Compound 83 could also be prepared from enone 96. 96 underwent a Morita-Baylis-Hillman reaction followed by a TBS protection of the resulting primary alcohol to give compound 103 in which alkene moiety was converted into a cyclopropane to give 104. The TBS protecting group was removed and the primary alcohol was crotonylated. Final removal of the BDA protecting group gave the target compound 83 (Scheme 1.16).62

The synthesis of mono-hydroxylated phenyl-substituted compound 84 also employed 96 as a key intermediate. 96 firstly underwent a conjugate addition reaction to selectively introduce a phenyl group giving 105. The BDA group was removed to afford the γ-hydroxyenone 106 in which the resulting hydroxyl was TES-protected to give 107. A hydroxymethylated side chain was introduced to the alkene moiety in 107 by a Morita-Baylis-Hillman reaction to give 108. Subsequent crotonylation of the resulting side chain in 108 followed by a deprotection gave the target compound 84 (Scheme 1.17).64
1.5.2.2 Starting from the Arene cis-Dihydrodiol

To synthesise the trihydroxylated compound 80 and its BDA-protected derivative 81, *meso* arene *cis*-dihydrodiol (109) was employed as the initial starting material (Scheme 1.18). The vicinal *cis*-diol in 109 was protected followed by a facially selective dihydroxylation of the alkene to give *cis*-diol 110. 111 was obtained as the major product (111:112=9:1) from the mono-protection of the diol group in 110 with a 2-naphthylmethyl group.\(^6^1\)

![Scheme 1.18: Protection of *meso* arene *cis*-dihydrodiol 109.](image)

Scheme 1.18: Protection of *meso* arene *cis*-dihydrodiol 109.\(^6^1\)

The acetonide protecting group of 111 was removed and the vicinal *trans*-diol was protected with a BDA group to give 113 in which secondary alcohol was oxidised to give enone 114. A Morita-Baylis-Hillman reaction introduced a hydroxymethylated side chain to the alkene moiety in 114 to give alcohol 115. Subsequent crotonylation followed by the removal of the 2-naphthylmethyl group gave 81. Removing the BDA protecting group in 81 gave compound 80 (Scheme 1.19).\(^6^1\)
1. Introduction

Scheme 1.19: Synthesis of compound 80 and its BDA-protected derivative 81.\textsuperscript{61}

Scheme 1.19: a) H\textsubscript{2}O/TFA/THF (5:2:1), r.t., 2 h then butan-2,3-dione, (CH\textsubscript{3}O)\textsubscript{3}CH, CSA, CH\textsubscript{3}OH, Δ, 16 h (46%); b) PCC, DCM, r.t., 2 h (76%); c) H\textsubscript{2}CO, imidazole, 1 M NaHCO\textsubscript{3} (aq.), THF, 40 °C, 32 h (68%); d) crotonic anhydride, pyridine, DMAP (cat.), DCM, r.t., 3 h (50%); e) DDQ, DCM/CH\textsubscript{3}OH (4:1), r.t., 6 h (66%); f) TFA/H\textsubscript{2}O (7:1), r.t., 3 h (98%).\textsuperscript{61}

1.5.2.3 Starting from the Cyclohexa-1,3-Diene

The racemic mono-hydroxylated COTC analogue 74 was prepared from cyclohexa-1,3-diene 117. An initial cis-1,4-diacetoxylation transformed 117 into diacetate 118, which was desymmetrised by an enzymatic reaction to give compound 119. The hydroxyl group in 119 was protected and the acetate was removed. The resulting hydroxyl group was oxidised to give racemic enone 120. A hydroxymethylated side chain was introduced to the alkene moiety in 120 and subsequent crotonylation followed by a deprotection gave the target compound 74 (Scheme 1.20).\textsuperscript{63}

Scheme 1.20: Synthesis of mono-hydroxylated COTC analogue 74.\textsuperscript{63}

Scheme 1.20: a) MnO\textsubscript{2}, LiCl, p-benzoquinone, Pd(OAc)\textsubscript{2}, LiOAc-2H\textsubscript{2}O, AcOH, r.t., 3 d (69%); b) electric eel cholinesterase, NaN\textsubscript{3}, phosphate buffer (pH 6.85), 20 °C, 30 h (63%); c) i) TBSOTf, Et\textsubscript{3}N, DMAP, DCM, 0 °C, 1 h (82%); ii) K\textsubscript{2}CO\textsubscript{3}, CH\textsubscript{3}OH, r.t., 3 h (88%); iii) TPAP, NMO, 4 Å mol sieves, DCM, r.t., 7 h (65%); d) i) DMAP (cat.), H\textsubscript{2}CO, THF/H\textsubscript{2}O (1:1), 40 °C, 24 h (52%); ii) crotonic anhydride, pyridine, DMAP (cat.), DCM, r.t., 1.5 h; iii) TFA/H\textsubscript{2}O (7:1), 0 °C, 1 h.\textsuperscript{63}
1.5.3 Studies of Perhydrodibenzofuranones

In 2014, the Whitehead group reported a serendipitous discovery of a reaction for the formation of the perhydrodibenzofuranone 123g during the eliminative deprotection of the conjugate adduct 121g (Scheme 1.21). They found that 123g was structurally similar to the natural product incarviditone (section 1.4) and was thus considered as likely to be bioactive. To further investigate this, a series analogues of 123 were prepared by the Whitehead group with isolated yields ranging between 55 and 86%.65

![Scheme 1.21: Formation of the perhydrodibenzofuranone analogues 123 during the eliminative deprotection of conjugate adducts 121.](image)

The anti-proliferative activities of several 123 analogues were measured using the MTT assay and the results are listed in Table 1.6. Among these compounds, only the 4-tert-butylphenyl substituted compound 123i displayed moderate inhibition against the A549 lung cancer cell line whereas the other candidates were not active. The mode of action of these compounds, however, remains unclear at the present stage and this intrigued the Whitehead group with preparing more members of this family to be tested against other cell lines in order to study their structural features as well as to explore their biological mechanism.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substituent (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>123c</td>
<td>4-methoxyphenyl</td>
</tr>
<tr>
<td>123e</td>
<td>4-iodophenyl</td>
</tr>
<tr>
<td>123g</td>
<td>4-biphenyl</td>
</tr>
<tr>
<td>123h</td>
<td>4-fluorophenyl</td>
</tr>
<tr>
<td>123i</td>
<td>4-tert-butylphenyl</td>
</tr>
</tbody>
</table>

Table 1.6: MTT assay results of analogues 123 against the A549 lung cancer cell line.65
1.5.4 Studies on Novel Inhibitors of NQO1

The Whitehead and the Stratford research groups have been collaboratively working on the development and evaluation of novel coumarin/dicoumarol-based NQO1 inhibitors over the last decade. Two novel series of NQO1 inhibitors were developed and prepared by the team and their inhibitory activities against NQO1 as well as anti-proliferative activities against various cancer cell lines were evaluated.\(^{66}\)

1.5.4.1 Syntheses of Novel Inhibitors of NQO1

A series of symmetric dicoumarol-based NQO1 inhibitors was prepared with a general structure depicted in Figure 1.15. Various substituents, such as electron-donating groups (i.e. methoxy, alkyl), electron-withdrawing groups (e.g. halides, ester) and additional aromatic systems (i.e. phenyl), were introduced in order to study the structure-activity relationships of these compounds.

![General structure of the symmetric NQO1 inhibitors](image)

The synthesis of these compounds was achieved by condensation of the appropriate 4-hydroxy-2H-chromen-2-one with an aldehyde. Some commercially unavailable hydroxychromenone species were synthesised in advance by a base-mediated cyclisation reaction between the appropriate 2-hydroxy acetophenones and diethylcarbonate prior to the condensation reaction (Scheme 1.22).\(^{66}\)

![Synthesis of the symmetric NQO1 inhibitors](image)

Scheme 1.22: Synthesis of the symmetric NQO1 inhibitors.\(^{66}\)
Scheme 1.22: a) diethylcarbonate, NaH, r.t., 0.5 h then 100 °C, 2 h; b) XCHO, EtOH, reflux, 24 h.\(^{66}\)

Commercially unavailable 2-hydroxy acetophenones were synthesised prior to the base-mediated cyclisation reaction, by a phenol acylation followed by an AlCl\(_3\)-mediated Fries rearrangement (Scheme 1.23).
The symmetric dicoumarol-based NQO1 inhibitors prepared by the Whitehead group are listed in Table 1.7.

Table 1.7: The symmetric NQO1 inhibitors prepared by the Whitehead group.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R^5</th>
<th>R^6</th>
<th>R^7</th>
<th>R^8</th>
<th>X</th>
<th>Cmpd</th>
<th>R^5</th>
<th>R^6</th>
<th>R^7</th>
<th>R^8</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>64a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<td>Br</td>
<td>H</td>
</tr>
<tr>
<td>124</td>
<td>H</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>129a</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>125a</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>129b</td>
<td>H</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
</tr>
<tr>
<td>125b</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>130</td>
<td>H</td>
<td>H</td>
<td>7,8-C₆H₄</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>125c</td>
<td>H</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>131</td>
<td>H</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>126a</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>132</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>CO₂Et</td>
</tr>
<tr>
<td>126b</td>
<td>H</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>H</td>
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<td>Cl</td>
<td>H</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*64 = dicoumarol

In order to investigate the possible influence of hydrophobic interactions involving an aromatic moiety on the inhibitory potency, a group of asymmetric NQO1 inhibitors were prepared with one of the 4-hydroxycoumarin systems replaced by an aromatic moiety. The general structure of the asymmetric dicoumarol-based NQO1 inhibitors is illustrated in Figure 1.16.

Figure 1.16: General structure of the asymmetric NQO1 inhibitors.

The synthetic sequence involved an initial tandem aldol condensation/Michael addition reaction sequence between the appropriate hydroxychromenone species and aromatic aldehyde followed by subsequent reductive fragmentation of one unit of hydroxychromenone (Scheme 1.24). The asymmetric NQO1 inhibitors exhibited greater solubility than the symmetric series described above.
Scheme 1.24: Synthesis of asymmetric dicoumarol-based NQO1 inhibitors.\textsuperscript{66}
Scheme 1.24: a) XCHO, EtOH, reflux, 24 h; b) NaBH\textsubscript{3}CN, MeOH, reflux, 24 h.\textsuperscript{66}

The asymmetric NQO1 inhibitors prepared by the Whitehead group are listed below:

Table 1.8: The asymmetric dicoumarol analogues prepared by the Whitehead group.\textsuperscript{66}

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R\textsuperscript{5}</th>
<th>R\textsuperscript{6}</th>
<th>R\textsuperscript{7}</th>
<th>R\textsuperscript{8}</th>
<th>X</th>
<th>Cmpd</th>
<th>R\textsuperscript{5}</th>
<th>R\textsuperscript{6}</th>
<th>R\textsuperscript{7}</th>
<th>R\textsuperscript{8}</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>134a</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>H</td>
<td>1-Np</td>
<td>136b</td>
<td>5,6-C\textsubscript{6}H\textsubscript{4}</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
<td></td>
</tr>
<tr>
<td>134b</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>H</td>
<td>2-Np</td>
<td>136c</td>
<td>5,6-C\textsubscript{6}H\textsubscript{4}</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
<td></td>
</tr>
<tr>
<td>134c</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>H</td>
<td>Ph</td>
<td>137a</td>
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<td>H</td>
<td>H</td>
<td>1-Np</td>
</tr>
<tr>
<td>135a</td>
<td>H</td>
<td>H</td>
<td>7,8-C\textsubscript{6}H\textsubscript{4}</td>
<td>1- Np</td>
<td></td>
<td>137b</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
</tr>
<tr>
<td>135b</td>
<td>H</td>
<td>H</td>
<td>7,8-C\textsubscript{6}H\textsubscript{4}</td>
<td>2- Np</td>
<td></td>
<td>137c</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
</tr>
<tr>
<td>135c</td>
<td>H</td>
<td>H</td>
<td>7,8-C\textsubscript{6}H\textsubscript{4}</td>
<td>Ph</td>
<td></td>
<td>138</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>3,4-Me\textsubscript{2}Ph</td>
</tr>
<tr>
<td>136a</td>
<td>5,6-C\textsubscript{6}H\textsubscript{4}</td>
<td>CH\textsubscript{3}</td>
<td>H</td>
<td>1-Np</td>
<td></td>
<td>139</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>H</td>
<td>3,4-Me\textsubscript{2}Ph</td>
</tr>
</tbody>
</table>

More recently, the Whitehead group employed “borrowing hydrogen” methodology for the synthesis of a new generation of asymmetric NQO1 inhibitors. This methodology allowed the syntheses to be accomplished in one step by coupling the appropriate 4-hydroxy-2H-chromen-2-one with aromatic alcohol (Scheme 1.25).\textsuperscript{41}

Scheme 1.25: Synthesis of the asymmetric NQO1 inhibitors using “borrowing hydrogen” reaction.\textsuperscript{41}
Scheme 1.25: a) (pentamethycyclopentadienyl)iridium (III) chloride dimer, Cs\textsubscript{2}CO\textsubscript{3}, IPA, toluene, XCH\textsubscript{2}OH, reflux, 24 h.\textsuperscript{41}

The asymmetric NQO1 inhibitors prepared using the “borrowing hydrogen” methodology are listed in Table 1.9.
Table 1.9: The NQO1 inhibitors prepared using “borrowing hydrogen” reactions.41

<table>
<thead>
<tr>
<th>Compound</th>
<th>R⁵</th>
<th>R⁷</th>
<th>R⁸</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>140a</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>3-hydroxynaphthalen-2-yl</td>
</tr>
<tr>
<td>140b</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>2-hydroxyphenyl</td>
</tr>
<tr>
<td>140c</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>(1,1'-biphenyl)-4-yl</td>
</tr>
<tr>
<td>140d</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>phenyl</td>
</tr>
<tr>
<td>141a</td>
<td>H</td>
<td>7,8-C₄H₄</td>
<td>3-hydroxynaphthalen-2-yl</td>
<td></td>
</tr>
<tr>
<td>141b</td>
<td>H</td>
<td>7,8-C₄H₄</td>
<td>2-hydroxyphenyl</td>
<td></td>
</tr>
<tr>
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<td>H</td>
<td>7,8-C₄H₄</td>
<td>3,4-dimethylphenyl</td>
<td></td>
</tr>
<tr>
<td>142a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>3-hydroxynaphthalen-2-yl</td>
</tr>
<tr>
<td>142b</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2-hydroxyphenyl</td>
</tr>
<tr>
<td>142c</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>(1,1'-biphenyl)-4-yl</td>
</tr>
<tr>
<td>142d</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>4-hydroxyphenyl</td>
</tr>
</tbody>
</table>

Another family of asymmetric NQO1 inhibitors was prepared by simple condensation of the appropriate 4-hydroxy-2H-chromen-2-ones and hydroxy arylaldehydes (Scheme 1.26). These compounds are structurally similar to the asymmetric NQO1 inhibitors mentioned above but bearing a chromadione core instead of a 4-hydroxycoumarin core.67

Scheme 1.26: Synthesis of chromadione-type NQO1 inhibitors.67

Scheme 1.26: appropriate hydroxy arylaldehyde, EtOH, reflux, 24 h.67

The chromadione-type NQO1 inhibitors prepared by the Whitehead group are listed in Table 1.10.

Table 1.10: Chromadione-type NQO1 inhibitors prepared by the Whitehead group.67

<table>
<thead>
<tr>
<th>Compound</th>
<th>R⁵</th>
<th>R⁷</th>
<th>R⁸</th>
<th>X</th>
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</thead>
<tbody>
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<td>143a</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>3-hydroxynaphthalen-2-yl</td>
</tr>
<tr>
<td>143b</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>2-hydroxyphenyl</td>
</tr>
<tr>
<td>144a</td>
<td>H</td>
<td>7,8-C₄H₄</td>
<td>3-hydroxynaphthalen-2-yl</td>
<td></td>
</tr>
<tr>
<td>144b</td>
<td>H</td>
<td>7,8-C₄H₄</td>
<td>2-hydroxyphenyl</td>
<td></td>
</tr>
<tr>
<td>145a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>3-hydroxynaphthalen-2-yl</td>
</tr>
<tr>
<td>145b</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2-hydroxyphenyl</td>
</tr>
</tbody>
</table>
1.5.4.2 Biological Assessments of Novel NQO1 inhibitors

The inhibitory activity against NQO1 was assessed using the menadione-cytochrome c enzyme activity assay, both in the presence and absence of bovine serum albumin (BSA). The anti-proliferative activity against MIA-PaCa-2 and HCT116 cell lines was determined using the MTT assay.

It is noteworthy that the assay results confirmed dicoumarol (64)'s extensive protein binding ability with an IC\textsubscript{50} value of 2.6 nM in the absence of BSA but 404 nM in the presence of BSA. This was consistent with the previous studies by Shaul and colleagues.\textsuperscript{43}

Several compounds were found to exhibit the same or greater inhibitory potency than dicoumarol (Table 1.11). It was also noted that in the presence of BSA, some compounds showed substantially less protein binding than dicoumarol while still exhibiting significant inhibitory activity against NQO1.\textsuperscript{66}

Table 1.11: NQO1 activity assay results of dicoumarol and selected novel NQO1 inhibitors.\textsuperscript{66}

<table>
<thead>
<tr>
<th>Compound</th>
<th>R\textsuperscript{5}</th>
<th>R\textsuperscript{6}</th>
<th>R\textsuperscript{7}</th>
<th>X</th>
<th>IC\textsubscript{50} (nM) BSA-</th>
<th>IC\textsubscript{50} (nM) BSA+</th>
</tr>
</thead>
<tbody>
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<td>64</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2.6 ± 1.6</td>
<td>404 ± 184</td>
</tr>
<tr>
<td>125a</td>
<td>OCH\textsubscript{3}</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2.8 ± 0.42</td>
<td>38 ± 2.1</td>
</tr>
<tr>
<td>129a</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>H</td>
<td>0.41 ± 0.38</td>
<td>233 ± 68</td>
</tr>
<tr>
<td>129b</td>
<td>H</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>0.42 ± 0.14</td>
<td>96 ± 75</td>
</tr>
<tr>
<td>130</td>
<td>H</td>
<td>H</td>
<td>7,8-C\textsubscript{4}H\textsubscript{4}</td>
<td>H</td>
<td>0.18 ± 0.16</td>
<td>370 ± 198</td>
</tr>
<tr>
<td>134b</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>H</td>
<td>2.7 ± 1.9</td>
<td>167 ± 83</td>
</tr>
<tr>
<td>135b</td>
<td>H</td>
<td>H</td>
<td>7,8-C\textsubscript{4}H\textsubscript{4}</td>
<td>2-Np</td>
<td>2.2 ± 1.6</td>
<td>255 ± 151</td>
</tr>
<tr>
<td>139</td>
<td>H</td>
<td>CH\textsubscript{3}</td>
<td>CH\textsubscript{3}</td>
<td>H</td>
<td>3.4-Me\textsubscript{2}Ph</td>
<td>9.9 ± 4.4</td>
</tr>
</tbody>
</table>

Among the asymmetric compounds, 2-naphthyl substituted compounds 134b and 135b showed stronger inhibitory potency than their 1-napthyl and phenyl counterparts (Table 1.12). This was rationalised by both increased hydrophobic interactions and fewer steric clashes within the active site of the enzyme, according to the results of docking studies.

Compared with the symmetric compounds, however, the asymmetric family lacks the hydrogen bonding capability necessary for holding the molecules steadily in the binding pocket, leading to a slight decrease in the inhibitory potency.\textsuperscript{66}
I. Introduction

Table 1.12: NQO1 activity assay results of selected asymmetric NQO1 inhibitors.66

<table>
<thead>
<tr>
<th>Compound</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
<th>X</th>
<th>IC50 (nM) BSA-</th>
<th>IC50 (nM) BSA+</th>
</tr>
</thead>
<tbody>
<tr>
<td>134a</td>
<td>H</td>
<td>CH3</td>
<td>CH3</td>
<td>H</td>
<td>1-Np</td>
<td>7.7 ± 4.5</td>
<td>1095 ± 290</td>
</tr>
<tr>
<td>134b</td>
<td>H</td>
<td>CH3</td>
<td>CH3</td>
<td>H</td>
<td>2-Np</td>
<td>2.5 ± 1.9</td>
<td>167 ± 83</td>
</tr>
<tr>
<td>134c</td>
<td>H</td>
<td>CH3</td>
<td>CH3</td>
<td>H</td>
<td>Ph</td>
<td>39 ± 12</td>
<td>660 ± 108</td>
</tr>
<tr>
<td>135a</td>
<td>H</td>
<td>H</td>
<td>7,8-C4H5</td>
<td>H</td>
<td>1- Np</td>
<td>6.3 ± 2.7</td>
<td>450 ± 325</td>
</tr>
<tr>
<td>135b</td>
<td>H</td>
<td>H</td>
<td>7,8-C4H5</td>
<td>H</td>
<td>2- Np</td>
<td>2.2 ± 1.6</td>
<td>255 ± 151</td>
</tr>
<tr>
<td>135c</td>
<td>H</td>
<td>H</td>
<td>7,8-C4H5</td>
<td>Ph</td>
<td></td>
<td>35 ± 21</td>
<td>880 ± 364</td>
</tr>
</tbody>
</table>

A cohort of these NQO1 inhibitors was assayed for their anti-proliferative activities against the MIA-PaCa-2 and HCT116 cell lines, but no compounds showed stronger toxicity than dicoumarol after either 24 hours or 96 hours of incubation (Table 1.13).66

Table 1.13: MTT assay results of selected NQO1 inhibitors (MIA-PaCa-2: human pancreas carcinoma cell line; HCT116: human colorectal carcinoma cell line).66

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
<th>X</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>90 ± 42</td>
</tr>
<tr>
<td>125a</td>
<td>OCH3</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>390 ± 14</td>
</tr>
<tr>
<td>129a</td>
<td>H</td>
<td>CH3</td>
<td>CH3</td>
<td>H</td>
<td>H</td>
<td>213 ± 11</td>
</tr>
<tr>
<td>129b</td>
<td>H</td>
<td>CH3</td>
<td>CH3</td>
<td>H</td>
<td>H</td>
<td>359 ± 17</td>
</tr>
<tr>
<td>130</td>
<td>H</td>
<td>H</td>
<td>7,8-C4H5</td>
<td>H</td>
<td></td>
<td>190 ± 15</td>
</tr>
<tr>
<td>134b</td>
<td>H</td>
<td>CH3</td>
<td>CH3</td>
<td>H</td>
<td>2-Np</td>
<td>390 ± 28</td>
</tr>
<tr>
<td>135b</td>
<td>H</td>
<td>H</td>
<td>7,8-C4H5</td>
<td>H</td>
<td>2-Np</td>
<td>215 ± 7.1</td>
</tr>
</tbody>
</table>

The Whitehead and Stratford groups also measured the generation of ROS stimulated by the NQO1 inhibitors, but no systematic structure-activity relationship could be established. This suggested that the toxicity of these compounds is independent of ROS and also explained the lower toxicity exhibited by these compounds in comparison to dicoumarol.66

During subsequent studies, the ability of the NQO1 inhibitors to protect cells against the toxicity of EO9 (146) was investigated. EO9 (Figure 1.17) is an anti-tumour quinone that is exclusively activated by NQO1.
EO9 was shown to kill over 90% of the HT29, A549, and MIA PacCa-2 cells at a concentration as low as 1 µM. The decrease in toxicity of EO9 after the treatment with NQO1 inhibitors indicated the activity of NQO1 was inhibited and therefore less EO9 was activated. In other words, the lower the concentration that is needed to protect cells from the toxicity of EO9, the better the inhibitory potency of the NQO1 inhibitor. The results of a selection of compounds are listed in Table 1.14.

Table 1.14: Results of selected compounds which show a greater NQO1 inhibitory potency in comparison to dicoumarol (HT29: human colon carcinoma cell line).

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R³</th>
<th>R⁶</th>
<th>R⁷</th>
<th>R⁸</th>
<th>X</th>
<th>Conc. of NQO1 inhibitors needed to protect cells from 50% of EO9 toxicity (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HT29</td>
</tr>
<tr>
<td>64</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>211 ± 83</td>
</tr>
<tr>
<td>125a</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>18 ± 9.0</td>
</tr>
<tr>
<td>134b</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
<td>2-Np</td>
<td>50 ± 33</td>
</tr>
<tr>
<td>139</td>
<td>H</td>
<td>CH₃</td>
<td>CH₃</td>
<td>H</td>
<td>3,4-Me₂Ph</td>
<td>97 ± 52</td>
</tr>
</tbody>
</table>

It was also reported that the bioactivity of these compounds is p53-independent. The MTT assays conducted both on the HCT116 cells excreting wild-type p53 and p53-deprived HCT116 showed little difference in terms of anti-proliferative activity, with both cell lines expressing similar levels of NQO1.
1.5.5 Starting Point of Novel Anti-Cancer Pro-drugs

As discussed in the previous section, the Whitehead group has prepared a number of potent NQO1 inhibitors that are of interest with regard to cancer treatment. A disadvantage of these compounds, however, is their extremely poor solubility that may lead to problematic drug delivery. It was suggested to couple them to more soluble carriers to overcome this issue.

It was a preliminary study on the relationship between the pKₐ of the leaving groups of the COTC analogues and bioactivity that laid the foundation for this idea. The pKₐ of the leaving group was believed to directly influence the bioactivity. Three compounds (147-149) were prepared accordingly and their anti-proliferative activities were assessed using the MTT assay against the A549 lung cancer cell line (Table 1.15). The results listed below showed a correlation between the pKₐ of the leaving group and the IC₅₀ values of these compounds. This suggested that modification of the leaving group could alter the anti-proliferative activity.

Table 1.15: MTT assay results of the modified COMC compounds with pKₐ of leaving group provided.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (μM)</th>
<th>pKₐ of leaving group</th>
</tr>
</thead>
<tbody>
<tr>
<td>147</td>
<td>3.5</td>
<td>8.1 ± 4.4</td>
</tr>
<tr>
<td>148</td>
<td>2.2</td>
<td>7.3 ± 6.7</td>
</tr>
<tr>
<td>149</td>
<td>1.6</td>
<td>1.3 ± 0.6</td>
</tr>
</tbody>
</table>

The NQO1 inhibitors were, therefore, to be introduced onto COTC derivatives as the carriers via the hydroxymethylated side chain. It was suggested that once the molecule has been transported into the cells, the carrier would interact with GSH in a fashion similar to that of COTC to generate an alkylating agent while releasing the NQO1 inhibitor to display its enzyme inhibitory function. In order to explore this idea, several compounds (150-152)
were prepared in an initial investigation and they were assayed for their anti-proliferative activities against the A549 lung cancer cell line (Table 1.16).  

Table 1.16: MTT assay results of a series of coupling products (150-152).  

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150a</td>
<td>10.00 ± 1.00</td>
</tr>
<tr>
<td>150b</td>
<td>0.71 ± 0.33</td>
</tr>
<tr>
<td>151a</td>
<td>Not active</td>
</tr>
<tr>
<td>151b</td>
<td>Not active</td>
</tr>
<tr>
<td>152a</td>
<td>4.40 ± 2.5</td>
</tr>
</tbody>
</table>

The trials turned out to be successful. Compounds 150b and 152a showed strong inhibition of the growth of the A549 cells despite the fact that 152a did not embody an NQO1 inhibitor but its precursor, 4-hydroxycoumarin. The inactivity of 151a and 151b indicated that a cyclohexenone core is essential for the exhibition of the anti-proliferative activity by compounds of this type.

A first generation of pro-drugs was systematically synthesised by a former member of the Whitehead group during the same time as this project was being studied. Six of these compounds (153a-f) were assayed for anti-proliferative activities against the A549 cell line. The results are listed in Table 1.17.
Table 1.17: MTT assay results of first generation pro-drugs against the A549 lung cancer cell line.  

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>153a</td>
<td>18.08 ± 4.23</td>
</tr>
<tr>
<td>153b</td>
<td>15.59 ± 2.94</td>
</tr>
<tr>
<td>153c</td>
<td>2.90 ± 0.32</td>
</tr>
<tr>
<td>153d</td>
<td>3.99 ± 0.44</td>
</tr>
<tr>
<td>153e</td>
<td>3.04 ± 0.35</td>
</tr>
<tr>
<td>153f</td>
<td>105.05 ± 5.45</td>
</tr>
<tr>
<td>153g (R = crotonyl)</td>
<td>3.68 ± 0.23</td>
</tr>
<tr>
<td>153h (R = H)</td>
<td>21.28 ± 3.57</td>
</tr>
</tbody>
</table>

The test compounds showed varying levels of activity. Compounds containing a chromadione-type NQO1 inhibitor (153c-e) displayed similar activities to the COTC analogue 153g and compounds containing 4-hydroxycoumarin-type NQO1 inhibitors (153a,b) were as active as the antheminone A analogue 153h. The low potency of 153f might be ascribed to the size as well as comparatively high weight of the molecule (582.18 g/mol) that caused a difficult intracellular delivery. Although the pro-drugs did not show greater anti-proliferative activity than COTC analogues, the results did suggest that they had been delivered into the cells, as NQO1 inhibitors alone did not display strong inhibition of the growth of the A549 cell line (section 1.5.4.2). These studies validated the pro-drug design concept and prompted further interest in carrying out additional investigations in order to establish a structure-activity relationship as well as to achieve improved potency.
II. Results and Discussion

2.1 Project Aims

2.1.1 Preparation and Evaluation of Novel Anti-Cancer Pro-Drugs

The main aim of this project was to synthesise and evaluate novel anti-cancer pro-drugs comprising cyclohex-2-enone carriers for the delivery of NQO1 inhibitors. The idea behind this was to prepare a dual-effect drug molecule that, once it has been delivered into the cells, undergoes a conjugate addition with GSH during which a transient exo-cyclic enone alkylating agent is formed and the NQO1 inhibitor is released. The combination of these two components also solves the problem of the poor solubility of the NQO1 inhibitors, facilitating their transportation.

The general structure of the pro-drug molecules is depicted in Figure 2.1. In order to fulfil the synthetic goal, both cyclohex-2-enone carriers and NQO1 inhibitors required prior preparation.

![Figure 2.1: General structure of the pro-drugs.](image)

According to the previous studies by the Whitehead group into COTC analogues, there is an apparent relationship between hydrophobicity of the drug molecules and their anti-proliferative activities (section 1.5.1). Four different cyclohexenone compounds (154b, 155-157) were therefore selected as carriers with differing hydrophobicities and their structures are illustrated below and they are further discussed in section 2.6.1:

![Figure 2.2: Four cyclohexenone carriers for the synthesis of pro-drugs.](image)

These compounds were to be coupled to the NQO1 inhibitors via the primary hydroxyl
group using the Mitsunobu reaction, hence the secondary alcohol(s) present in the molecule needed prior protection.

Syntheses of the protected pro-drug carriers (159b, 92 and 97) as well as the monool carrier 155 were modelled on those developed previously by the Whitehead group and they are outlined below:

![Scheme 2.1: Synthetic routes to four cyclohexenone carriers in their protected forms.](image)

With respect to the NQO1 inhibitors, several compounds that were previously designed by the Whitehead and Stratford groups were to be re-synthesised, based on their differing NQO1 inhibitory potencies. Although the preparative focus of NQO1 inhibitors was 4-hydroxycoumarin derivatives (164), chromadione derivatives (165), which were investigated more recently by the Whitehead group, were also taken into consideration, as they displayed individual anti-proliferative activity against several cancer cell lines (Figure 2.3).

![Figure 2.3: General structures of the asymmetric NQO1 inhibitors.](image)
The syntheses of these compounds are outlined in Scheme 2.2.

Scheme 2.2: Synthetic routes to the asymmetric NQO1 inhibitors.

2.1.2 Natural Product Studies.

Derivatives of several natural products, including COTC, antheminone A and incarviditone (Figure 2.4), were also to be investigated, as analogues of these natural products exhibited inhibitory properties against the growth of the A549 and/or H460 cell line(s), according to the previous investigations by the Whitehead group. Further studies were therefore planned for discovering new members with enhanced bioactivity.

Figure 2.4: Structures of three natural products of interests.

The mono-hydroxylated COTC analogue bearing a phenyl substituent was the most active compound among all the COTC analogues that the Whitehead group had prepared previously (84, section 1.5.1). The structure of this analogue therefore served as the prototype for the COTC analogues in this project. A general structure of the target COTC analogues is depicted below (Figure 2.5).

Figure 2.5: General structure of the mono-hydroxylated COTC analogues bearing aryl substituents.
The synthetic route leading to aryl substituted COTC analogues 166 is shown in Scheme 2.3. Aryl substituted antheminone A analogues 154 can be acquired via the corresponding intermediate 159.

Scheme 2.3: Synthesis of mono-hydroxylated COTC analogues 166 and antheminone A analogues 154.

The synthetic sequence is considered to be versatile not only because the intermediate 159 can afford antheminone A analogues 154 but intermediates 121 and 122 can also afford diaryl substituted incarviditone analogues via a one-step reaction (Scheme 2.4). Intermediate 89 can afford non-substituted (R = H) incarviditone analogue via its hydrogenated derivative 90.

Scheme 2.4: Synthetic route to incarviditone analogue 123.
2.1.3 Biological Evaluations

It was hoped that biological evaluations of synthesised compounds would facilitate the understanding of structural features and establishment of structure-activity relationships. The compounds that were to be evaluated included the pro-drugs, NQO1 inhibitors, COTC, antheminone A, incarviditone analogues as well as the hydroxyenone intermediates

The MTT assay was used to determine anti-proliferative activity of the compounds mentioned above against four cell lines (A549, MDA-MB-231, FaDu, PNT2). Details regarding the biological assessment (protocols, cell lines information) are provided in section 6.1.1. The results will be discussed in chapter III.

From the next section, the results are divided into five topics and reported as follows:
1. Preparation of COTC and antheminone A analogues as well as the synthesis of four protected pro-drug carriers (section 2.2);
2. Preparation of incarviditone analogues and discussion of their potential mode of action (section 2.3);
3. Potential modifications of the substituents in intermediates derived from the COTC’s synthetic sequence in order to achieve enhanced bioactivity (section 2.4);
4. Preparation of dicoumarol-based asymmetric NQO1 inhibitors (section 2.5);
5. Preparation of novel anti-cancer pro-drugs (section 2.6).

2.2 Preparation of COTC Analogues and Building Blocks of Pro-drugs

This section focuses on the synthetic routes towards mono-hydroxylated COTC, antheminone A analogues and also four important intermediates for the synthesis of pro-drugs. The synthetic sequences are discussed step by step in the following subsections.

2.2.1 Protection of (−)-Quinic Acid

(−)-Quinic acid has been used as the starting material in several published synthetic routes that lead to COTC analogues. Herein, two different protecting groups were employed, affording two alternative products: cyclohexylidene protected (44, section 2.1.1.1) and butane-1,2-diacetal protected (−)-quinic acid (93, section 2.1.1.2).
2.1.1.1 Cyclohexylidene Ketal Protected (−)-Quinic Acid (44)

Géro and colleagues reported a protection of the vicinal cis-diol in (−)-quinic acid (43) with cyclohexanone to give a cyclohexylidene quinide which was accompanied by a concurrent lactonisation (Scheme 2.5).\(^70\)

\[
\text{Scheme 2.5: Cis-diol protection of } (−)-\text{quinic acid 43 using the conditions reported by Géro.} \quad \text{\(^70\)}
\]

The Whitehead group adapted this method and modified the conditions: they replaced benzene with the less toxic toluene and excluded \(N,N\)-dimethylformamide from the reaction system.\(^63\) Under the modified conditions, the yields of this step ranged from 60\% to 80\% (Scheme 2.6). It was noticed that the yields increased when longer reaction times were used but decreased slightly on a larger scale (>10 g).

\[
\text{Scheme 2.6: Formation of cyclohexylidene ketal protected (−)-quinic acid under modified conditions.}
\]

Analysis of NMR and infrared data confirmed the presence of the lactone moiety in compound 44, in accord with selective protection of the vicinal cis-diol.

The mechanism for this step is illustrated in Scheme 2.7. It is not clear whether the lactonisation or the protection occurred at the first place: A higher-energy conformation of (−)-quinic acid is preferred for lactonisation but not for \(cis\)-dial protection, however with cyclohexylidene attached, the conformation thereof would be more difficult to adopt. It was felt, therefore, that the order of the two steps could be influenced by whether the increasing energy for achieving the cyclohexane ring flip after protection could be easily overcome.
II. Results and Discussion

2.1.1.2 Butane-1,2-Diacetal Protected (−)-Quinic Acid

The selective protection of the trans-1,2-diequatorial diol in (−)-quinic acid with butane-2,3-dione was initially reported by Ley and co-workers with the reaction conditions later optimised by Frost and colleagues (Scheme 2.8). 71-74

Scheme 2.8: Protection of the trans-diol in (−)-quinic acid 43 with butane-2,3-dione reported by Frost and co-workers.74

The Whitehead group later modified the conditions reported by these researchers and by adapting the modified conditions, the trans-diol in (−)-quinic acid (43) was protected as its
II. Results and Discussion

butane-1,2-diacetal (BDA), and this was accompanied by esterification of the carboxylic acid moiety to give compound 93 in a yield of 63% (Scheme 2.9).

Scheme 2.9: Conversion of 43 into BDA-protected methyl quinate 93.

The selectivity in the trans-vicinal diol protection can be explained by the formation of a trans-decalin ring system (Figure 2.6):

Figure 2.6: Trans-/cis- protection of diol with BDA.

Although both ring systems are stabilised by anomeric effects, the trans-ring junction is less sterically hindered than the cis-ring, and is more thermodynamically stable.

The mechanism for trans-diol protection is illustrated in Scheme 2.10.

Scheme 2.10: Mechanism for trans-diol protection.
2.2.2 Reduction with Sodium Borohydride

With the compounds 44 and 93 in hand, reduction reactions of the lactone/ester groups were performed (Scheme 2.11). The conversions were accomplished with sodium borohydride in methanol to give the corresponding reduced compounds 87 and 94, respectively.

The reduction of ester species normally requires strong reductants (e.g. lithium aluminium hydride). Sodium borohydride, however, is a comparatively weak reducing agent for esters. The successful reduction of 44 and 93 was made feasible by the presence of the electron-withdrawing α-hydroxyl group to the ester: an intermediate 186 formed between the substrates and sodium borohydride at the α-hydroxyl, which permits an additional intramolecular reaction that, contributes to the ease of the reduction process (Scheme 2.12).

The products 87 and 94 obtained after work-up, normally contained minor amounts of unknown impurities, however these did not interfere with the subsequent step, and thus, no further purification was necessary.

The mechanism for the reduction step is illustrated in Scheme 2.13.
II. Results and Discussion

2.2.3 Formation of Hydroxyketone Compounds

The crude triols 87 and 94 were oxidised using silica-supported sodium meta-periodate, which resulted in cleavage of the vicinal diol moieties to give ketones 88 and 95 (Scheme 2.14).

Historically, due to the poor solubility of sodium meta-periodate in non-polar solvents, the use of sodium meta-periodate as an oxidant was limited. Hodge and colleagues reported a method whereby coating the oxidant onto silica could solve this solubility problem. A mixture of sodium meta-periodate, water and silica was evaporated to dryness in vacuo so that a monolayer of sodium meta-periodate was bound to the surface of silica. Daumas and colleagues later improved Hodge’s method to a less time consuming procedure by adding an aqueous solution of sodium meta-periodate into a stirring suspension of the silica in a non-polar solvent. Column chromatography was generally necessary to purify the crude products from the reactions. Zhong and Shing attempted addition of the aqueous solution of sodium meta-periodate directly to the silica which gave a free-flowing powder and reactions with this modified reagent only required a simple solvent wash for product purification (Scheme 2.15).
Zhong and Shing’s method was adapted to prepare the hydroxyketones 88 and 95 from the crude triols 87 and 94. The reactions proceeded smoothly and were finished in 1.5 hours with acceptable yields in both cases. The mechanism for this step is shown in Scheme 2.16.

2.2.4 Formation of Enone Compounds

The hydroxyketones 88 and 95 were converted into the corresponding enones 89 and 96, in an E1cB fashion, by exposure to methanesulfonyl chloride and triethylamine in dichloromethane (Scheme 2.17).

The transformation is initiated by formation of a sulphene species followed by methylsulfonation of the hydroxyl group. Subsequent β-elimination of the mesylate gives the enones 89 and 96. The mechanism for the conversion is illustrated in Scheme 2.18.
II. Results and Discussion

It was noted that BDA-protected enone 96 is a notably more stable species than compound 89. The instability of 89 can be ascribed to a propensity towards formation of para-benzoquinone (quinone) and hydroquinone in the presence of base (Scheme 2.19). The generation of these by-products therefore required a rapid purification process.

Various attempts to control conditions were investigated, such as lowering the reaction temperature to 0 °C, reducing the reaction time by 1 hour and decreasing the amount of the base by 1 eq.. These changes all failed to minimise the decomposition and the isolated yields were thus compromised.

It was more difficult for the trans-decalin moiety of BDA to undergo the base-mediated decomposition due to the equatorial positions of the protecting group. The preparation of 96 was not compromised by the same difficulties associate with 89.
2.2.5 Rhodium-Mediated Conjugate Addition Reactions

The enone 89 underwent a range of conjugate addition reactions in which boronic acids were used to introduce different aromatic side chains to the alkene moiety (Scheme 2.20).

The reactions were carried out under the catalysis of either hydroxy(cyclooctadiene)rhodium(I) dimer (198) or chloro(cyclooctadiene)rhodium(I) dimer (199) (Figure 2.7). Rhodium(I) complexes such as these are efficient catalysts for conjugate addition reactions and are particularly useful because they allow the reactions to be performed in the presence of water.\textsuperscript{78}

Commonly used group 10 metals in organometallic catalysts, such as nickel, palladium and platinum, shuttle between the oxidation states (0) and (II) within a catalytic cycle. Transmetallation occurs onto the metal(II) species, the overall reaction therefore requires: 1. An electrophile that can oxidise the metal from oxidation state (0) to (II); 2. An organometallic component that can undergo transmetallation with the metal(II) complex and that can also reductively couple with the electrophile. Rhodium complexes, on the other hand, tend to shuttle between oxidation states (I) and (III). This avails two possible catalytic pathways and does not necessarily require an electrophile to oxidise the metal (Scheme 2.21).\textsuperscript{78}
II. Results and Discussion

Scheme 2.21: Two possible catalytic pathways of rhodium catalysts.\textsuperscript{78}

In 1997, Miyaura and colleagues reported a series of rhodium-catalysed conjugate additions between alkenyl-/arylboronic acid and enone: this is now known as the Hayashi-Miyaura reaction. The 1,4-addition between phenyl groups and enone moiety was accomplished in good yields with 3 mol\% of rhodium catalyst loading. The overall transformation is depicted in Scheme 2.22.\textsuperscript{79}

\begin{center}
\[
\begin{array}{c}
\text{Scheme 2.22: 1,4-Addition of organoboronic acids and enones.}\textsuperscript{79}
\end{array}
\end{center}

In 1998, Hayashi and colleagues reported the first rhodium-catalysed asymmetric conjugate addition reaction by modifying their previous method to an enantioselective one, giving the reaction a yield of 93% and an enantiomeric excess (ee) of 97% (Scheme 2.23).\textsuperscript{80}

\begin{center}
\[
\begin{array}{c}
\text{Scheme 2.23: An example of chiral asymmetric 1,4-conjugate addition reaction.}\textsuperscript{80}
\end{array}
\end{center}

Hayashi and co-workers rationalised the mechanism for the rhodium-catalysed conjugate addition using a catalytic cycle (Scheme 2.24): the cycle starts from a transmetallation to form a phenyl-rhodium bond, followed by enone insertion to afford a rhodium enolate, which is then hydrolysed to give the 1,4-adduct.\textsuperscript{81}
II. Results and Discussion

This catalytic process was adapted to accomplish the conversion of enone 89 to the corresponding conjugate adducts 121a-f. Different substituents were introduced at this stage in order to create diversity among compounds (Table 2.1). 4-Bromophenyl (121a) and 4-methoxyphenyl (121c) compounds were synthesised to study the effect of an electron-withdrawing/donating side chain on biological properties, whereas 2-naphthyl (121b) and 5-indolyl (121d) compounds were prepared to study the influence of potential hydrogen bonding on bioactivity. These substituents also slightly differ in hydrophobicity, which may influence bioactivity and are worthy of investigation. Structures of the substituents along with their predicted logarithms of partition coefficient value (milogP) are listed in Table 2.1.

Table 2.1: Introduction of different aromatic substituents to enone 89.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substituent (R)</th>
<th>milogP</th>
<th>Compound</th>
<th>Substituent (R)</th>
<th>milogP</th>
</tr>
</thead>
<tbody>
<tr>
<td>121a</td>
<td>Br</td>
<td>3.19</td>
<td>121d</td>
<td>N</td>
<td>2.58</td>
</tr>
<tr>
<td>121b</td>
<td></td>
<td>3.57</td>
<td>121e</td>
<td>I</td>
<td>3.47</td>
</tr>
<tr>
<td>121c</td>
<td>OMe</td>
<td>2.44</td>
<td>121f</td>
<td>COOH</td>
<td>2.30</td>
</tr>
</tbody>
</table>

Reaction conditions including catalyst loading, reaction time and the molar equivalents of the corresponding boronic acid are listed in Table 2.2.
It is noteworthy that for the reactions to form compound 121a, the quantity of boronic acid used was related to the isolated yields whereas catalyst loading did not have much influence, although the reaction showed limited progression with loading below 1 mol%.

Reactions of compound 121b proceeded readily provided a long reaction time was used. Other factors did not significantly affect the isolated yields.

Catalyst loading showed correlation with isolated yield for the preparation of compound 121c. The yield was reduced by 10% when the amount of catalyst used was halved. A longer reaction time may compensate for this decrease, but this has not been confirmed at present.

Difficulties were encountered when preparation of compounds 121d-f was attempted:

With respect to indolyl compound 121d, the reaction appeared to stall after a period of time, even though additional boronic acid and base were added. Purification by column chromatography failed to provide a pure sample of compound 121d and a large amount of indole was isolated as a by-product from the reaction.

The synthesis of compound 121e turned out to be unreliable with the only successful attempt yielding less than 10%. Subsequent attempts to modify the reaction conditions failed to reproduce this result. These included prolonging the reaction time from 24 h to 45 h and increasing the reaction temperature from r.t. to 40 °C. In order to prepare compound 121e, an aromatic Finkelstein reaction of bromophenyl adduct 121a was thus suggested.
and this requires further studies. All attempts to prepare carboxyphenyl compound 121f failed to give the desired product. It was believed that the low solubility of the carboxyphenylboronic acid was the reason for failure. Alternative solvent systems may improve the situation but this has not been investigated.

2.2.6 Eliminative Deprotection
Eliminative deprotection of the conjugate adducts 121a-d was performed under basic conditions using either NaOH (0.5 M) or DBU (Scheme 2.25). The products, hydroxyenones 122a-d, from this step are important not only as key synthetic intermediates but they are also biologically relevant due to their anti-proliferative activity.

Three products of this reaction, hydroxyenones 122a-c, were obtained in yields ranging between 60% and 80% (Table 2.3). The conversion of 121d into 122d did not proceed at room temperature but progressed once heated to 40-50 °C. Although the reaction was given a much longer time than its counterparts, it did not reach completion. Attempts to acquire pure 5-indolyl compound 122d failed, partly because of the use of the impure starting material 121d.

The details of the reaction conditions used for the elimination reaction are listed in Table 2.3:
Table 2.3: Reaction conditions for eliminative deprotections

<table>
<thead>
<tr>
<th>Product</th>
<th>Substituent (R)</th>
<th>Base</th>
<th>Reaction temperature</th>
<th>Reaction time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>122a</td>
<td>4-bromophenyl</td>
<td>NaOH (0.5 M)</td>
<td>r.t.</td>
<td>2 h</td>
<td>87%</td>
</tr>
<tr>
<td>122a</td>
<td>4-bromophenyl</td>
<td>DBU</td>
<td>r.t.</td>
<td>2.5 h</td>
<td>24%</td>
</tr>
<tr>
<td>122b</td>
<td>2-naphthyl</td>
<td>NaOH (0.5 M)</td>
<td>r.t.</td>
<td>40 min</td>
<td>75%</td>
</tr>
<tr>
<td>122c</td>
<td>4-methoxyphenyl</td>
<td>NaOH (0.5 M)</td>
<td>r.t.</td>
<td>6.5 h</td>
<td>67%</td>
</tr>
<tr>
<td>122c</td>
<td>4-methoxyphenyl</td>
<td>DBU</td>
<td>r.t.</td>
<td>3.5 h</td>
<td>68%</td>
</tr>
<tr>
<td>122d</td>
<td>5-indolyl</td>
<td>NaOH (0.5 M)</td>
<td>45 °C</td>
<td>24 h</td>
<td>Impure</td>
</tr>
</tbody>
</table>

Careful monitoring of the deprotection reactions was ensured, as self-dimerised products 123 (Figure 2.8) are generated after a prolonged period of time in the presence of base. These compounds are of interest as their structures resemble the natural product incarviditone (73). Further discussions regarding these compounds are provided in section 2.2.

The mechanism for the elimination reaction is illustrated in Scheme 2.26.
2.2.7 TES-Protection of C4-Hydroxyl Group

The free C4-hydroxyl group in compounds 122a-c was protected using TESOTf in order to facilitate the subsequent Morita-Baylis-Hillman reaction (Scheme 2.27).

![Scheme 2.27: Protection of hydroxyl group with triethylsilyl trifluoromethanesulfonate.](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substituent (R)</th>
<th>Compound</th>
<th>Substituent (R)</th>
<th>Compound</th>
<th>Substituent (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>158a</td>
<td>Br</td>
<td>158b</td>
<td></td>
<td>158c</td>
<td>OMe</td>
</tr>
</tbody>
</table>

The Whitehead group had previously attempted to prepare TBS-protected compound 210 directly from conjugate adduct 208 using a one-pot reaction. TBSOTf and DBU were used as the reagents and a mixture of hydroxynone 209 and desired product 210 was obtained (Scheme 2.28).^68

![Scheme 2.28: Concerted reaction sequence of eliminative deprotection and re-protection of 208.](image)

A serendipitous observation was made that a higher yield could be achieved when the protection of the hydroxyl group was conducted separately. A trial reaction for protecting hydroxynone 209 was performed using TBSOTf and DBU in DCM, stirring at room temperature for 40 minutes. The reaction resulted in a poor yield (16%) with a significant amount of by-product 211 being isolated instead of the desired product 210 (Figure 2.9). It was suggested that the bulky aromatic substituent (i.e. phenyl) slowed down the reaction rate of silylation of the hydroxyl group. Modified conditions were adapted from method reported by Shibasaki and colleagues, using TESOTf and 2,6-lutidine in DCM at −40 °C. The isolated yield improved but it was still unsatisfactory with both the desired product and the diene by-product being formed in a ratio of 3:1. ^68
The formation of diene 211 was believed to be due to the presence of triflic acid in the reagent (TBSOTf or TESOTf). The Whitehead group decided, therefore, to pre-mix the reagents (TESOTf and 2,6-lutidine) in DCM at −78 °C prior to the addition of substrate in order to neutralise any acid residues. As a consequence, the reaction reached completion within 15 minutes without significant formation of the diene by-product 211, and the TES protected product was obtained in a yield of 81%. These conditions were thus adapted to the conversion from 122a-c to 158a-c, however during the synthesis of compounds 158a-c, an unpredictable relationship between reaction times and isolated yields was noted (Table 2.4):

<table>
<thead>
<tr>
<th>Product</th>
<th>Side chains (R)</th>
<th>Time (mins)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>158a</td>
<td>4-bromophenyl</td>
<td>15</td>
<td>59</td>
</tr>
<tr>
<td>158a</td>
<td>4-bromophenyl</td>
<td>20</td>
<td>58</td>
</tr>
<tr>
<td>158a</td>
<td>4-bromophenyl</td>
<td>30</td>
<td>77</td>
</tr>
<tr>
<td>158b</td>
<td>2-naphthyl</td>
<td>20</td>
<td>81</td>
</tr>
<tr>
<td>158b</td>
<td>2-naphthyl</td>
<td>30</td>
<td>79</td>
</tr>
<tr>
<td>158b</td>
<td>2-naphthyl</td>
<td>40</td>
<td>73</td>
</tr>
<tr>
<td>158c</td>
<td>4-methoxyphenyl</td>
<td>30</td>
<td>78</td>
</tr>
<tr>
<td>158c</td>
<td>4-methoxyphenyl</td>
<td>45</td>
<td>56</td>
</tr>
</tbody>
</table>

In the case of compound 158a, the yields increased with reaction time while the reverse was observed in the case of 158b and 158c. The optimal reaction time was between 20 - 30 minutes and the yields decreased slightly outside of this range.
2.2.8 Hydrogenation of Cyclohexylidene-Protected Enone and the Subsequent One-Pot Deprotection / Re-Protection Reaction

Hydrogenation of the alkene moiety of enone 89 was performed to give the compound 90 (Scheme 2.29). The reaction was conducted in ethyl acetate and 90 was obtained in a good yield (93%). Methanol was initially used as the solvent, but the reaction showed limited progression. Little is known about this solvent preference, but an aprotic solvent appeared to be more suitable for this conversion.

![Scheme 2.29: Hydrogenation of enone 89.](image)

The resulting cyclohexanone 90 was subjected to a one-pot reaction during which the cyclohexylidene protecting group was removed and the free hydroxyl group was protected as its TBS ether to give hydroxyenone 91 (Scheme 2.30). The reaction conditions used were adapted from those developed previously by the Whitehead group.83

![Scheme 2.30: One-pot conversion of 90 into 91.](image)

The reaction was initially attempted with TESOTf and DBU in DCM at room temperature, but this failed to afford the desired TES-protected product with only a small amount of intermediate 212 being recovered (Figure 2.10). Another trial with TBSOTf and DBU in DCM at room temperature also failed. It was only by stirring the compound 90 with TBSCl and DBU in benzene at a reflux temperature could the conversion be accomplished. The reaction was complete in 6 hours, and the product was carried through crude to the next step (section 2.2.10.4).
2.2.10 Morita-Baylis-Hillman Reaction

The Morita-Baylis-Hillman (M-B-H) reaction is a versatile process for providing intermediates for the preparation of COTC and antheminone A analogues as well as building blocks for the pro-drugs. In the following subsections, M-B-H reactions on four different substrates are discussed.

2.2.10.1 M-B-H Reaction on TES-Protected Compounds

The M-B-H reaction on TES-protected compounds 158a-c resulted in the introduction of hydroxymethyl side chains at the C2 position (Scheme 2.31). This step provided important intermediates (159a-c) for the synthesis of both COTC and antheminone A analogues while compound 159b is a building block used subsequently in the synthesis of pro-drugs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substituent (R)</th>
<th>Compound</th>
<th>Substituent (R)</th>
<th>Compound</th>
<th>Substituent (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>159a</td>
<td>Br</td>
<td>159b</td>
<td></td>
<td>159c</td>
<td>OMe</td>
</tr>
</tbody>
</table>

The Whitehead group has previously invested a great deal of effort in searching for optimal reaction conditions: they investigated both the original literature reaction conditions as well as subsequently modified ones, but these all involved long reaction times (10 days minimum). The commonly used catalysts such as DABCO, DBU and imidazole all failed to give satisfactory yields, although these catalysts tend to work efficiently on aliphatic or simpler cyclic substrates.\(^{84-90}\)

Progress was made by adapting Williams’ conditions whereby surfactants were introduced into the reaction medium in which water was used as the solvent (Scheme 2.32).
II. Results and Discussion

The use of surfactants is beneficial for substrates of this type because: 1. Solubility of substrates in the reaction medium is enhanced, as lipophilic substituents of substrates reside in hydrophobic region of surfactant micelles while hydrophilic moieties remain in the aqueous phase; 2. Polar head groups of the surfactants can stabilise the zwitterionic M-B-H intermediates. Following investigations, sodium dodecyl sulfate (SDS) was concluded to be the most suitable candidate in a reaction system with DMAP as nucleophilic catalyst. These surfactant conditions were applied to the conversion of compounds 158a-c into 159a-c. In the initial attempts, isolated yields were below 35% and this was assumed to be due to probable loss of formaldehyde over the course of the reactions. A trial reaction with 158b was then set up with replenishment of formaldehyde after 16 hours. Under these conditions, the reaction was complete after 24 hours and 159b was isolated in a yield of greater than 60% consequently. Yields of M-B-H reactions are reported in Table 2.5.

<table>
<thead>
<tr>
<th>Product</th>
<th>Substituent (R)</th>
<th>Time (h)</th>
<th>Temperature</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>159a</td>
<td>4-bromophenyl</td>
<td>24</td>
<td>r.t.</td>
<td>58</td>
</tr>
<tr>
<td>159b</td>
<td>2-naphthyl</td>
<td>24</td>
<td>r.t.</td>
<td>64</td>
</tr>
<tr>
<td>159c</td>
<td>4-methoxyphenyl</td>
<td>24</td>
<td>r.t.</td>
<td>75</td>
</tr>
</tbody>
</table>

Subsequently, the influence of the quantity of surfactant on isolated yields was studied. The critical micelle concentration (CMC) of SDS in water at 25 ºC is approximately from 6 to 8 nM (as calculated by the manufacturer). Taking the amount of water both as the solvent and present in the formaldehyde (37% aq.) into account, three trial reactions were set up with SDS concentrations: i) at 7 nM; ii) at 10 nM and iii) at 13 nM, respectively. The results showed that the influence of amount of surfactant was almost negligible with all three trial reactions giving similar yields.
The mechanism for the Morita-Baylis-Hillman reaction is illustrated in Scheme 2.33. The process starts with a Michael addition between DMAP and enone 158 to form an enolate 216, which reacts with formaldehyde in an aldol manner. Subsequent proton transfer and elimination of DMAP give the desired product 159.

Based on the theory proposed by Williams and co-workers, the manner in which the surfactant is believed to be involved in the reaction is depicted in Figure 2.11. The TES protecting group and the C5 substituent (R group) reside in the hydrophobic region of the SDS micelles while the hydrophilic cyclohexenone core is located in the aqueous phase, stabilised by the polar head groups of SDS.
2.2.10.2 M-B-H Reaction on 2-Cyclohexen-1-one

The M-B-H reaction of commercially available 2-cyclohexen-1-one 160 to afford 155 was accomplished in a yield of 53% (Scheme 2.34).

Unlike compounds 158a-c discussed above, compound 160 is not a surfactant-like compound and hence it would not benefit from the use of surfactants. Additionally, the reaction medium was changed from water to THF in which compound 160 dissolved readily at room temperature and, therefore, the surfactants were not necessary for this conversion.

The product 155 from this step is another important building block used in the synthesis of pro-drugs (see section 2.6).

2.2.10.3 M-B-H Reaction on BDA-Protected Enone Compound

For the similar reason mentioned in the previous section, the conversion of 96 into 97 was performed without addition of SDS in THF (Scheme 2.35). A better isolated yield (72%) was achieved with a higher reaction temperature 40 °C than at room temperature (35%). The product 97 from this step is also an important intermediate in the synthesis of pro-drugs (see section 2.6).

2.2.10.4 M-B-H Reaction on TBS-Protected Compound

The crude TBS protected hydroxynone 91 was converted into the M-B-H product 92 under the reaction conditions developed previously by the Whitehead group. The reaction proceeded readily in a mixture of THF and water at an elevated reaction temperature
II. Results and Discussion

The product 92 obtained from this reaction is a building block in the synthesis of pro-drugs (see section 2.6).

![Scheme 2.36: M-B-H reaction of TBS-protected compound 91.](image)

After 24 hours of reaction time, 92 was found to be the major product with only a minor amount of starting material 91 remained in the reaction mixture. The reaction did not progress any further leaving after a longer time as well as adding more regents. Subsequent purification by flash column chromatography gave compound 92 in a yield of 69% (over two steps from compound 90, section 2.2.8).

2.2.11 Towards COTC Analogue

The final steps to obtain the COTC analogues were crotonylation of the hydroxymethyl side chain and removal of the TES protecting group. The reaction conditions used were similar to those published by the Whitehead group. The aryl substituted hydroxymethylated compounds 159a-c were all crotonylated in order to furnish them with the side chain present in COTC (Scheme 2.37).

![Scheme 2.37: Crotonylation of hydroxymethylated side chains.](image)

Upon examination of the spectroscopic data for crotonate esters 167a-c, it was noticed that the isolated compounds were all contaminated with an identical impurity. The structure of this impurity could not be identified, although it was assumed to be a crotonic acid-related compound, however, its presence did not interfere with the subsequent step.
The mechanism for this step is illustrated in Scheme 2.38: DMAP behaves as a nucleophilic catalyst and reacts with crotonic anhydride to form $N$-acyl pyridinium species 220 which is a highly reactive acylating agent that reacts with the free hydroxyl group in 159 in order to finally give the target ester 167. The crotonic acid generated during the process is scavenged by the added pyridine.

![Scheme 2.38: Mechanism for the crotonylation of Morita-Baylis-Hillman products.](image)

Having obtained crotonate esters 167a-c, the TES protecting groups were removed to give the final COTC analogues 166a-c (Scheme 2.39). The reactions were performed using TFA and H$_2$O (7:1). These COTC analogues were expected to be bioactive and their anti-proliferative activities are discussed in chapter III.

![Scheme 2.39: Removal of TES protecting group.](image)

The deprotection reactions were accomplished over a short period of time and subsequent purification by flash column chromatography was straightforward. Isolated yields over two steps (crotonylation and deprotection) are listed in Table 2.6.
II. Results and Discussion

Table 2.6: Isolated yields over two steps.

<table>
<thead>
<tr>
<th>Product</th>
<th>Substituent (R)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>166a</td>
<td>4-bromophenyl</td>
<td>39</td>
</tr>
<tr>
<td>166b</td>
<td>2-naphthyl</td>
<td>48</td>
</tr>
<tr>
<td>166c</td>
<td>4-methoxyphenyl</td>
<td>30</td>
</tr>
</tbody>
</table>

2.2.12 Towards Antheminone A Analogues

Deprotection of the Morita-Baylis-Hillman adducts 159a-c gave the diols 154a-c. These compounds bear a structural resemblance to the natural product antheminone A (Figure 2.12).

Figure 2.12: General structure of 154 and antheminone A (14).

The Whitehead group had previously prepared a series of diols 154 with different aromatic substituents (R) and these compounds were found to show moderate anti-proliferative activity against the A549 lung cancer cell line. Three antheminone A analogues were thus prepared to expand the data for establishing a structure-activity relationship (Scheme 2.40).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substituent (R)</th>
<th>Compound</th>
<th>Substituent (R)</th>
<th>Compound</th>
<th>Substituent (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>154a</td>
<td></td>
<td>154b</td>
<td></td>
<td>154c</td>
<td></td>
</tr>
</tbody>
</table>

Scheme 2.40: Deprotection of M-B-H compounds 159a-c.

Similarly to the deprotection of the crotonylated compounds 167a-c, the conversions of TES protected compounds 159a-c into diols 154a-c were accomplished within one hour. The yields following purification are listed in Table 2.7.
Table 2.7: Isolated yields of deprotection reactions.

<table>
<thead>
<tr>
<th>Product</th>
<th>Substituent (R)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>154a</td>
<td>4-bromophenyl</td>
<td>56</td>
</tr>
<tr>
<td>154b</td>
<td>2-naphthyl</td>
<td>46</td>
</tr>
<tr>
<td>154c</td>
<td>4-methoxyphenyl</td>
<td>66</td>
</tr>
</tbody>
</table>

The anti-proliferative activities of compounds 154a-c were measured using the MTT assay against various cell lines. The results are discussed in chapter III.
2.3 Studies on Incarviditone Analogues

This section comprises two subsections: section 2.2.1 focuses on the preparations of incarviditone analogues whereas section 2.2.2 discusses their potential intracellular mode of action.

2.3.1 Discovery and Preparations of Incarviditone Analogues

The Whitehead group has recently focussed its attention on the preparation of incarviditone analogues 123 following the serendipitous discovery of a self-dimerisation reaction during the eliminative deprotection of conjugate adducts 121 (Scheme 2.41). 65,69

Scheme 2.41: Formation of dimer during the eliminative deprotection of conjugate adduct.

In 2013, a member of the Whitehead group isolated compound 123g whose structure resembles the natural product incarviditone 73 (Figure 2.13). A series of analogues were subsequently prepared and several of them were tested for their anti-proliferative activities. The bioassay results showed that 123i displayed moderate anti-proliferative activity against the A549 cell line with an IC50 of 12 μM while the remainder did not exhibit any inhibition of the growth of the A549 cells. 65,69

Figure 2.13: Analogues of incarviditone prepared previously by the Whitehead group. 65,69

The biological assay results prompted interest in further investigations of analogues of this type in order to study their structure-activity relationships. During this work, several additional incarviditone analogues were prepared by treating the appropriate starting
material (121a-d and 90) with base (i.e. NaOH) (Scheme 2.42).

![Scheme 2.42: Preparation of incarviditone analogues 123a-d, j.](image)

The conversion of compounds 121a-c into 123a-c was accomplished in 24 hours at room temperature, giving acceptable yields after purification. Compound 123d was obtained from its corresponding conjugate adduct 121d after heating the reaction mixture at 45 °C for 24 hours, as the reaction barely showed any progression when left at room temperature. The isolated yield was compromised due to the impurities present in the starting material (as discussed in section 2.1.6).

The “core” dimer, 123j, was obtained from unsubstituted compound 90. The reaction appeared to proceed smoothly but due to the high polarity of the product, some material was lost during the purification process, resulting in a reduced isolated yield.

The isolated yields of compounds 123a-d, j are listed in Table 2.8.

![Table 2.8: Yields of dimerisation reactions.](image)

Hydroxynenones 122a-c were also investigated as the starting materials for the dimerisation reactions in order to compare the isolated yields from one-pot reactions with those involving two discrete steps (Scheme 2.43).
Comparative yields are listed in Table 2.9. The reaction conditions used for the conversions of 121a-c into hydroxyenones 122a-c were discussed in section 2.1.7 and subsequent dimerisation reactions lasted approximately 24 hours in each case.

It can be seen that the isolated yields over two steps (121a-c → 122a-c → 123a-c) were similar to those of one-pot reactions (121a-c → 123a-c). The two-step sequence, however, provided a more straightforward purification procedure.

Table 2.9: Comparison of isolated yield between one-pot and two-step reactions.

<table>
<thead>
<tr>
<th>Product</th>
<th>Yield (121 to 122) (%)</th>
<th>Yield (122 to 123) (%)</th>
<th>Yield (Over 2 steps) (%)</th>
<th>Yield (from 121) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>123a</td>
<td>87</td>
<td>66</td>
<td>57</td>
<td>45</td>
</tr>
<tr>
<td>123b</td>
<td>75</td>
<td>77</td>
<td>58</td>
<td>74</td>
</tr>
<tr>
<td>123c</td>
<td>67</td>
<td>74</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

The mechanism for this step is illustrated in Scheme 2.44.
Among the five compounds tested (123a-d, 123j), only 123b showed activity against the A549 cell line with an IC\textsubscript{50} of 13.4 \(\mu\)M, which is, similar to that of 123i (Table 2.10). The fact that only 123b and 123i displaying anti-proliferative activity promoted a suggestion that the hydrophobicity of the compounds may be related to their biological activities. The predicted logarithms of partition coefficient (milogP)\textsuperscript{82} of some analogues prepared to date are listed in Table 2.10. Compounds 123e,h,i were prepared by previous members of the Whitehead group.\textsuperscript{90}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substituents (R)</th>
<th>milogP</th>
<th>Anti-proliferative activity (A549)</th>
</tr>
</thead>
<tbody>
<tr>
<td>123j</td>
<td>H</td>
<td>-0.36</td>
<td>Not active</td>
</tr>
<tr>
<td>123a</td>
<td>4-bromophenyl</td>
<td>4.86</td>
<td>Not active</td>
</tr>
<tr>
<td>123b</td>
<td>2-naphthyl</td>
<td>5.60</td>
<td>Active, 13.4 (\mu)M</td>
</tr>
<tr>
<td>123c</td>
<td>4-methoxyphenyl</td>
<td>3.35</td>
<td>Not active</td>
</tr>
<tr>
<td>123d</td>
<td>5-indolyl</td>
<td>3.63</td>
<td>Not active</td>
</tr>
<tr>
<td>123e</td>
<td>4-iodophenyl</td>
<td>5.40</td>
<td>Not active</td>
</tr>
<tr>
<td>123h</td>
<td>4-fluorophenyl</td>
<td>3.56</td>
<td>Not active</td>
</tr>
<tr>
<td>123i</td>
<td>4-\textit{tert}-butylphenyl</td>
<td>6.65</td>
<td>Active, 12.0 (\mu)M</td>
</tr>
</tbody>
</table>

It is apparent that the active analogues (123b and 123i) have higher predicted logP values than the inactive ones and this hydrophobicity-associated activity was thought to be a reflection of potential hydrophobic interactions within the active site. More investigation is required to establish a structure-activity relationship.

A full list of MTT assay results of the incarviditone analogues 123a-d, j against various
cell lines is provided in chapter III.

2.3.2 Exploring the Mechanism of Action of Incarviditone Analogues against the A549 Cell Line

Unlike COTC analogues, incarviditone analogues are not Michael acceptors that can react with GSH. Their bioactivities are, therefore, assumed to be mediated via other pathways. In this section, a proposal will be discussed regarding incarviditone analogues’ intracellular mode of action.

On investigating the mechanism of quinone-induced cytotoxicity in lung cancer cells, Chakrabarti and coworkers observed that 1,4-benzoquinone (quinone) inhibited cell cycle progression and caused apoptosis of the A549 cells with an IC$_{50}$ of 7.5 μM. They also noted that quinone caused contraction and shrinkage of the A549 cells and this observation led them to believe that damage to the microtubule network might be a cause of quinone’s cytotoxicity.92

Microtubules are formed by the polymerisation of protein tubulin dimers and they have a dynamic cytoskeletal structure. They are associated with cell shaping and morphology and they also take part in various cellular processes such as cell signalling, organelle transport and cell motility. Damage of microtubules can lead to apoptosis of mammalian cells. So far, both tubulin and microtubules have been reported as potential targets for anti-cancer therapies.92-95

Based on the findings of Chakrabarti and coworkers, it was postulated that the core of the dimers 123 might have undergone intracellular and enzymatic oxidation to the para-benzoquinone compound 227 before manifestation of cytotoxicity.

A chemical approach was proposed for oxidation of compound 123 to 227 via intermediate 226 (Scheme 2.45). The active compound 123b was selected for investigation of the proposal.

Scheme 2.45: A proposed oxidation route of the core of dimers 123.
The first attempt employed Corey-Suggs reagent (PCC) as the oxidant: compound 123b was treated with PCC in DCM with the addition of ground 3 Å molecular sieves. The 1H-NMR spectrum of the partially purified product showed signals (singlets) around 6.4 and 6.8 ppm (framed in red, Figure 2.14) that may belong to the hydroxyl groups and alkene moiety in hydroquinone, respectively.

![Figure 2.14: 1H-NMR spectrum of isolated compound.](image)

This indicated the isolated compound could contain the hydroquinone compound 228b instead of the desired intermediate 226b (Figure 2.15).

![Figure 2.15: The desired product 226b and the isolated compound 228b.](image)

Due to the failure of the last attempt, another trial oxidation with 123b was performed using Swern oxidation conditions. Purification by flash column chromatography gave three different fractions and two of which consisted of decomposed reagents and starting material. The 1H-NMR spectrum of the other fraction showed chemical shifts of three groups of signals (framed in red, Figure 2.16) without the appearance of signals that indicated the presence of hydroquinone as described above. These changes suggest a potential formation of diketone 226b during the reaction, however due to the failure of purifying the fraction, this could not be confirmed.
Due to the lack of success with these oxidation attempts, this aspect of the research was brought to a halt, however future investigation is discussed in chapter IV.
2.4 Side Chain Modifications

Over the last decade, the Whitehead group has prepared a considerable amount of analogues of three natural products (COTC, incarviditone and antheminone A) and hydroxyenones with varying anti-proliferative properties. The focus of this aspect of study has been the investigation of hydrophobic interactions between these molecules and target sites, hence various aromatic and aliphatic side chains have been introduced in order to enhance the interactions as such. The intramolecular hydrogen bonding within the active sites, however, has been paid less attention during the previous studies. For the purpose of structure-activity relationship study, it was decided to incorporate hydrogen bond donors, such as nitrogen or oxygen, to the side chain.

In section 2.2 and 2.3, several compounds possessing anti-proliferative activities were described (Figure 2.17). These compounds are of value in terms of potential modification to improve bioactivities, however, they possess multiple functional groups and are, therefore, relatively susceptible to side-reactions and might not be suitable for direct modification. For this reason, their synthetic precursors were considered as alternative starting points.

![Figure 2.17: General structures of compounds exhibiting anti-proliferative activities.](image)

It was attractive to take advantage of the reactivity of the C-Br bond that had already been incorporated into some of the compounds, hence conjugate adduct **121a** and TES-protected compound **158a** were chosen. A proposed synthetic scheme is outlined below (Scheme 2.46):
II. Results and Discussion

2.4.1 Replacements of Halide Group

In 2000, Buchwald and co-workers reported a simple and efficient methodology for building C-N bonds via palladium-catalysed aminations of aryl halides or triflates. They employed several electron-rich phosphine ligands to support palladium complexes as the catalysts (Figure 2.18). Among these, two easily accessible, air-stable ligands 237 and 238 were found to promote such amination reactions at room temperature.\(^97\)

According to Buchwald and co-workers, these amination reactions should be performed in an air-free system to facilitate the formation of the Pd/ligand complex. An excess of amine was also advocated in order to minimise the generation of diarylated by-products (from aryl halides). The functional group tolerance was relatively poor, due to the use of reagents
with strong basicity (NaOEt-Bu or KOt-Bu). Most of the room temperature reactions were accomplished in good yields (70% - 90%), although higher yields were achieved with increased palladium loading. Buchwald’s conditions were applied to 158a in an attempt to introduce morpholine group onto the aromatic substituent with 1 mol% loading of palladium and 2 mol% of ligand 238 (Scheme 2.47). The consumption of starting material was monitored by TLC.

The progress of reaction was checked after 5, 20 and 27 hours and it was stopped after 29 hours, as there was no sign of further conversion. Three compounds generated during the reaction were isolated, however based on the analysis of 1H NMR and mass spectrometry data, none of them were the desired product 239. The reaction was carried out on two occasions with the same disappointing results. The reasons for the failure were not deduced and an alternative method was sought.

In 2002, Nolan and colleagues reported an aryl amination reaction using an N-heterocyclic carbene-supported palladium catalyst: this allowed the reactions to be performed in the presence of air with short reaction time and in relatively high yields. The complex 242 used in the reactions can be prepared in a single step from two commercially available starting materials, bis(benzonitrile)palladium (240) and N,N'-bis(2,6-diiso-propylphenyl)imidazol-2-ylidene (IPr, 241) (Scheme 2.48).

Although the reaction was carefully performed in order to exclude moisture, the IPr supported palladium complex 242 could not be reproduced. The reason for the reproducibility failure was assumed to be high sensitivity to air and moisture of the
reaction system. An alternative commercially available ligand 243, SIPr (Scheme 2.49) was used as a replacement of IPr 241, but the Pd/SIPr complex failed to catalyse the amination reaction of compound 121a.

Several research groups have reported modified Ullmann amination reactions catalysed by copper iodide. These methods generally require relatively harsh reaction conditions, such as high temperatures and were thus considered inapplicable.\(^99-101\)

2.4.2 Sonogashira reaction

Owing to the failed attempts described above, another method was considered. Inspired by an on-going project in the Whitehead group, it was decided to add an alkyne moiety to the side chain in compound 121a in order to ultimately introduce hydrogen bond donors (Scheme 2.50). On the other hand, the incorporation of an alkyne moiety could be of use in terms of tracking the localisations of a drug molecule in the cells via click labelling with a fluorescent azide.

The Sonogashira-Hagihara reaction was firstly reported in 1975 by Sonogashira and colleagues. They developed an efficient Pd-Cu catalytic system for cross-coupling unsaturated halides and terminal acetylenes under mild conditions (Scheme 2.51).\(^102,103\)
Sonogashira reaction of 121a was attempted using modified conditions which had been developed by the Whitehead group (Scheme 2.52).

![Scheme 2.52: Sonogashira reaction of compound 121a.]

Unfortunately, the reaction made little progress and it was assumed that this was due to self-dimerisation of ethynyltrimethylsilane competing with the main reaction (alkynylation) under the catalytic conditions. Ethynyltrimethylsilane was thus added at 0 °C over a course of 30 minutes, however, this gave a similar result. The amount of ethynyltrimethylsilane was increased to 1.5 eq. with the gradual addition over 5.5 hours at 0 °C but the reaction turned out to have progressed even less. The reasons for the failure were unclear, however several modifications reported by other research groups on the reaction conditions were suggested, such as adapting a copper-free system or conducting the reaction in a microwave at elevated temperature. These should be investigated in future studies.104-108

Owing to the failure of the Sonogashira reaction on the 4-bromophenyl compound 121a, the 4-iodophenyl compound 121e was suggested as an alternative substrate, considering the higher reactivity of aryl iodides compared to aryl bromides in cross-coupling reactions (Scheme 2.53). This route, however, was abandoned because the rhodium-mediated cross-coupling reaction failed to provide the 4-iodophenyl compound 121e (as discussed in section 2.1.6).

![Scheme 2.53: An alternative unsuccessful route to give compound 245.]

To summarise, the chemical approaches that have been attempted so far in order to modify side chains all failed to give the desired products and the focus of research transferred to biological aspects, which are discussed in chapter III.
2.5 Preparation of NQO1 Inhibitors

Several of the NQO1 inhibitors previously developed by the Whitehead group were selected for re-synthesis based on their enzyme assay results. Two approaches (reductive fragmentation and “borrowing hydrogen” methodologies) were used for preparing these NQO1 inhibitors. In the following subsections, the two methodologies, along with the syntheses of the precursors to the NQO1 inhibitors, are reported.

2.5.1 Base-mediated Cyclisation

In order to prepare the hydroxychromenone 163a and 163b, precursors to the NQO1 inhibitors, two commercially available acetophenone analogues (162a and 162b) were subjected to base-mediated cyclisation with diethyl carbonate (Scheme 2.54).

![Scheme 2.54: Synthesis of hydroxychromenones from acetophenones.]

A proposed mechanism for this reaction is depicted in Scheme 2.55: the enolate in 248 attacks diethyl carbonate in order to form the intermediate 250, in which the phenoxide attacks the ester carbonyl to force the ethoxy group to leave to give the diketone 252, which finally tautomerises into hydroxychromenone 163 (Scheme 2.55).

![Scheme 2.55: A possible mechanism for the base-mediated ring closure.]

It was noticed that after quenching the reaction for conversion of 162b into 163b with water, a semi-solid component which was generated during the reaction remained in the organic layer, causing difficulties in the subsequent aqueous extraction and resulting in a
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decrease in isolated yield. Attempts to improve the situation, such as prior removal of the mineral oil present with the hydride or adding co-solvent (toluene) to improve the solubility of the semi-solid all turned out to be unsuccessful. The structure of the by-product was not identified, however, considering its insolubility in both aqueous and organic layers, this semi-solid might contain polymer-like components but no further conclusions could be drawn.

2.5.2 Reductive Fragmentation Method

In 1991, Appendino and co-workers reported a two-step synthetic sequence for preparing 3-substituted coumarin derivatives. This method was previously adapted by the Whitehead group for the synthesis of a series of novel NQO1 inhibitors. Herein, two NQO1 inhibitors were re-synthesised using similar conditions (Scheme 2.56).

Scheme 2.56: Preparation of the asymmetric NQO1 inhibitors using reductive fragmentation method.

The first step of this sequence involves an aldol condensation between the hydroxychromenone 163 and 2-naphthaldehyde, followed by a Michael reaction resulting in addition of another molecule of hydroxychromenone. The second step is a reductive cleavage of one of the central C-C bonds of intermediate 253 resulting in loss of one hydroxychromenone unit and generation of the desired NQO1 inhibitor. The yields for this two-step sequence are listed in Table 2.11. Although the first step went smoothly, a less efficient second step compromised the overall yields. It was felt that the reason for this was the poor solubility of the products in organic solvents resulting in inefficient extraction and unpredictable yields.
Table 2.11: Yields of reductive fragmentation sequence.

<table>
<thead>
<tr>
<th>Product</th>
<th>Yield of first step (%)</th>
<th>Yield of second step (%)</th>
<th>Yield over two steps (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>164a</td>
<td>86</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>164c</td>
<td>96</td>
<td>30</td>
<td>29</td>
</tr>
</tbody>
</table>

The mechanism for this reaction sequence was examined by Appendino and colleagues who carried out a deuterium labelling study (deuterated reductant) and other spectroscopic analysis.\(^{109}\) The first step of the sequence starts from the nucleophilic addition between the hydroxychromenone 163 and 2-naphthaldehyde to give aldol adduct 254, which then undergoes elimination of water to give an chromadione species 256. Compound 256 reacts with another molecule of hydroxychromenone (nucleophile) in a Michael addition to generate the bis-adduct 253 (Scheme 2.57).

Scheme 2.57: Mechanism for the first step of reductive fragmentation methodology.\(^{41,109}\)

The second step of the sequence is a retro-Michael addition during which one molecule of hydroxychromenone is released (Scheme 2.58). The mono-deprotonated intermediate 257 is converted into chromadione 256, which is reduced to give the NQO1 inhibitor.
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Appendino and co-workers noticed that the second step proceeded with better yield in the presence of a mild reductant (i.e. sodium cyanoborohydride). The reactions either produced poor yields or made no progress when stronger reducing agents were applied (e.g. LiAlH₄, LiBH₄). Although the exact reasons for this particular superiority of sodium cyanoborohydride were not confirmed, a presumption was put forward by the team: either monodeprotonated intermediate 257 or monoprotonated intermediate 258 (Figure 2.19) can readily undergo the retro-Michael addition to give the desired product whereas strongly basic reductants will convert 253 into a dienolate species, impeding the subsequent retro-Michael reaction and associated loss of one molecule of 163.¹⁰⁹

This method represents a relatively simple operation, however there are disadvantages to the sequence in addition to the poor yields it produced. The first of these is in terms of atom economy: the initial addition and subsequent loss of a molecule of hydroxychromenone results in wastage of this moiety during the reaction. Second is the use of an extremely toxic reagent, sodium cyanoborohydride. The procedure cannot be considered environmentally friendly and offers no sustainability and consequently an alternative method was suggested.
2.5.3 “Borrowing Hydrogen” Methodology

Since the reductive fragmentation methodology has a variety of associated problems described above, an alternative method for preparing NQO1 inhibitors has been developed. Traditionally, C-C bond formation using primary alcohols as electrophilic components has been difficult to achieve due to their limited reactivity. “Borrowing hydrogen” methodology has therefore been developed as an alternative approach to C-C bond formation. This methodology can be viewed as a three-step sequence proceeding in a catalytic cycle: alcohol oxidation, alkene formation and alkene reduction, mediated by either iridium or ruthenium catalytic systems. \[110\]

In 2001, Cho, Shim and co-workers employed ruthenium complexes for their C-C bond forming reaction involving primary alcohols (Scheme 2.59). The process starts from an oxidation of alcohol 259 to aldehyde 260. Subsequent cross aldol reaction between the resulting aldehyde 260 and substrate 261 gives an α, β-unsaturated ketone 262, which is hydrogenated to give the product 263. Ruthenium catalysts shift between low- and high-valent states during the sequence to transfer hydrogen at different stages. \[111\]

![Scheme 2.59: Ru-catalysed C-C bond forming reaction involving primary alcohol.](image)

In 2002, a similar process involving iridium catalysts was reported by Williams and co-workers. It involved an initial alcohol oxidation to give aldehyde 260 and a subsequent in situ Wittig olefination to afford alkene 265, which was then reduced to the product 266 (Scheme 2.60). Iridium catalysts borrow and return hydrogen during the oxidation and reduction steps, respectively. \[112\]
II. Results and Discussion

In 2008, Williams and Pridmore applied the “borrowing hydrogen” methodology to a C-C bond forming reaction between alcohols and malonate half esters, catalysed by Ir or Ru complexes (Scheme 2.61). They noticed that the Ru system generated a large amount of alkene by-product 270 as a result of hydrogen loss during the reaction. This problem was solved by addition of iso-propanol as a hydrogen donor to compensate for the loss. Among all the catalytic systems they investigated, Ru(PPh₃)₃Cl₂/KOH and [Cp*IrCl₂]₂/Cs₂CO₃ turned out to be the most effective.

Eisenstein and co-workers reported a computational study on Ir-mediated alcohol oxidation pathways in the presence of Cs₂CO₃. They stated that the alcohol oxidation stage involves the transfer of the hydroxyl proton to the carbonate ligand which is accompanied by the transfer of a hydride to the metal by β-elimination.

The result of their computational studies is depicted in Scheme 2.62: Ir is activated by Cs₂CO₃ to form a complex 271 which coordinates with the alcohol (i.e. methanol), giving the intermediate 272. After entering into the transition state 273, the proton is transferred from the alcohol to the carbonate to form the intermediate 274. Ir then coordinates a hydrogen from the alkyl group in 274 to reach a new transition state 275. The Ir-H and C-H bonds both become shorter, leading to an associated Ir-O-C bond bending to give the transient intermediate 276. The following transition state 277 undergoes a β-elimination,
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forming the hydride formaldehyde complex 278. Final release of the aldehyde (i.e. formaldehyde) finishes the alcohol oxidation step.\textsuperscript{114}

![Scheme 2.62: Alcohol oxidation pathway.\textsuperscript{114}]

It was noted that the transition state 277 lies at the highest energy point along the pathway, which, implies the β-elimination step is the rate-determining step of the alcohol oxidation.\textsuperscript{113} Elevated temperature is thus needed to overcome the high energy barrier.

The Whitehead group has prepared a series of NQO1 inhibitors by using \([\text{Cp}^*\text{IrCl}_2]_2\) (280)/\(\text{Cs}_2\text{CO}_3\) or \([\text{Ru(PPh}_3)_3\text{Cl}_2]\) (281)/\(\text{Cs}_2\text{CO}_3\) catalytic systems. Although the former of these produced better yields in general, the cost of the Ir complex has made the ruthenium catalyst a preferred substitute (Figure 2.20).\textsuperscript{41,67,69}

![Figure 2.20: Ir and Ru catalysts used in the synthesis of NQO1 inhibitors.]

For the purpose of this project, five NQO1 inhibitors were prepared using “borrowing hydrogen” methodology (Table 2.12). These five compounds were selected for re-synthesis based on their differing NQO1 enzyme assay results, in order to investigate the relationship between inhibitory potency of NQO1 inhibitors and the \textit{in vitro} anti-proliferative activities
of pro-drugs. The NQO1 inhibition assays were conducted by a previous member of the Whitehead group and the results are listed in Table 2.12.

Table 2.12: NQO1 enzyme assay results of five re-synthesised asymmetric NQO1 inhibitors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R^5$</th>
<th>$R^7$</th>
<th>$R^8$</th>
<th>$Ar$</th>
<th>IC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>164a</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>164b</td>
<td>H</td>
<td>7,8-C$_5$H$_4$</td>
<td>Ph</td>
<td>79 ± 10</td>
<td></td>
</tr>
<tr>
<td>164c</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
<td>14 ± 5.8</td>
</tr>
<tr>
<td>164d</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
<td>816 ± 133</td>
</tr>
<tr>
<td>164e</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
<td>144 ± 79</td>
</tr>
</tbody>
</table>

Preparation of the NQO1 inhibitors was carried out using iridium or ruthenium catalysts (Scheme 2.63). The Ir catalytic system generally required lower reaction temperature (110 °C) and shorter reaction time (24 hours) than the Ru system (140 °C, 30 hours). The Ru catalytic system was nevertheless employed in most cases out of economic considerations.

Scheme 2.63: “Borrowing hydrogen” methodology in practice for the preparation of the NQO1 inhibitors.

It was noticed that the concentration of the hydroxychromenone 163 in the reaction mixture directly affected the progress of the reactions. When the concentration was below 1.4 M, the reaction showed little progression, however when between 1.4 M and 1.9 M, the reaction proceed smoothly, provided sufficient time was allowed. It was felt that a higher concentration would also be beneficial, but due to the limited solubility of the reactants and reagents, the highest concentration that could be achieved was 1.9 M.
For compounds 164a and 164c, a comparison of isolated yields arising from the “borrowing hydrogen” methodology (Ru-mediated) and the reductive fragmentation methodology are listed in Table 2.13. The “borrowing hydrogen” methodology gave slightly better yields than the reductive fragmentation methodology and, in addition, the latter took twice the reaction time in total. Preparation of compounds 164b and 164e was achieved in moderate yields, however synthesis of 164d was disappointing with the maximum yield of 23% after several attempts.

Table 2.13: Isolated yields of preparation of asymmetric NQO1 inhibitors.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R⁵</th>
<th>R⁷</th>
<th>R⁸</th>
<th>Ar</th>
<th>“borrowing hydrogen”</th>
<th>reductive fragmentation (over two steps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>164a</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
<td>36</td>
<td>24</td>
</tr>
<tr>
<td>164b</td>
<td>H</td>
<td>7,8-C₆H₆</td>
<td>Ph</td>
<td>66</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>164c</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
<td>35</td>
<td>29</td>
</tr>
<tr>
<td>164d</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
<td>23</td>
<td>N/A</td>
</tr>
<tr>
<td>164e</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
<td>44</td>
<td>N/A</td>
</tr>
</tbody>
</table>

To sum up, although employing “borrowing hydrogen” methodology did not accomplish the conversions in outstanding yields, the method provides a simpler, quicker and more environmentally friendly procedure compared to the reductive fragmentation methodology. A proposed reaction mechanism for the transformation is illustrated in Scheme 2.64.

Scheme 2.64: Proposed mechanism for the “borrowing hydrogen” reaction.
2.5.4 Chromadione-Type NQO1 Inhibitors Syntheses

During the Whitehead group’s previous investigations of the NQO1 inhibitors, compounds that bear a chromadione moiety were also found to show good inhibitory activities against the enzyme. Two of these (165a and 165b) were thus re-synthesised as it was of interest to compare their anti-proliferative activities with the five NQO1 inhibitors described in the last section (2.5.3), following their incorporation into the pro-drugs. The enzyme assay results of these two compounds are reported in Table 2.14.

Table 2.14: NQO1 enzyme assay results of two chromadione-type NQO1 inhibitors.\(^{67}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC(_{50}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>165a</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>165b</td>
<td>20 ± 6</td>
</tr>
</tbody>
</table>

Chromadione-type NQO1 inhibitors have a similar structure to the intermediate (256) formed during the second step of the reductive fragmentation methodology (Scheme 2.58), and thus they can be obtained under similar reaction conditions. Two reactants (hydroxychromenone and 2-hydroxy-1-naphthaldehyde) were used in equimolar amounts to guarantee an efficient process (Scheme 2.65).

Scheme 2.65: Synthesis of chromadione NQO1 inhibitors 165a and 165b.

The reaction times and the isolated yields of this step are listed in Table 2.15.

Table 2.15: Isolated yields of preparation of the chromadione-type NQO1 inhibitors.

<table>
<thead>
<tr>
<th>Product</th>
<th>Reaction time (h)</th>
<th>Conversion yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>165a</td>
<td>3</td>
<td>73</td>
</tr>
<tr>
<td>165b</td>
<td>5</td>
<td>70</td>
</tr>
</tbody>
</table>
Owing to their highly conjugated and planar structures, the products precipitated from the reaction mixtures as distinct yellow solids – this provided a useful means for monitoring the progress of reaction. Purification of these compounds proved to be facile and a simple solvent wash (i.e. methanol) afforded the pure products 165a and 165b. The mechanism for this step is illustrated in Scheme 2.66.

Scheme 2.66: Mechanism for the formation of chromadione-type NQO1 inhibitors.
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2.6 Pro-drugs Syntheses

The preparation of novel anti-cancer pro-drugs involved the coupling of the NQO1 inhibitor to the appropriate carriers that bear a quasi-COTC core. The idea of this combination was to provide dual-effect pro-drugs that, once having entered the cells, could then act in two distinctive ways: the whole drug molecules could behave as COTC analogues that interact with the GSH system during which process they also release the NQO1 inhibitors as the leaving group to inhibit the cancer-associated enzyme NQO1.

These pro-drugs were prepared using the following step(s):
1. Coupling the M-B-H alcohols (section 2.2.10) and the NQO1 inhibitors using the Mitsunobu reaction;
2. Removal of the protecting groups where necessary.

These two steps are discussed in the following subsections.

2.6.1 Protecting Pro-drug Carriers

Following the discussion in sections 1.5.5 and 2.1.1, in order to study the influence of hydrophobicity of the carriers on anti-proliferative activity of pro-drugs, four compounds (154b, 155-157) with varying extent of hydroxylation were selected. Their predicted logarithms of partition coefficient (milogP)\(^{82}\) are listed in Table 2.16.

Table 2.16: Predicted logPs of four pro-drug carriers.\(^{82}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>milogP</th>
<th>Compound</th>
<th>milogP</th>
</tr>
</thead>
<tbody>
<tr>
<td>154b</td>
<td>2.04</td>
<td>156</td>
<td>-0.94</td>
</tr>
<tr>
<td>155</td>
<td>0.45</td>
<td>157</td>
<td>-1.85</td>
</tr>
</tbody>
</table>

The primary alcohols (circled) in these carriers were to be coupled to the appropriate NQO1 inhibitors in order to form pro-drugs. The secondary alcohol groups were hence protected in advance to avoid side reactions, with the exception of the monool 155. The compounds for coupling are depicted in Figure 2.21 (syntheses of these compounds were described in section 2.2.10).
2.6.2 Mitsunobu Reaction

In 1967, Mitsunobu and colleagues found that trivalent phosphorus compounds, phosphines or trialkylphosphites, could be oxidised to the corresponding phosphine oxides or trialkyl phosphates in the presence of diethyl azodicarboxylate (DEAD) and alcohols. A concomitant conversion of the alcohols into their corresponding ethers was also observed (Scheme 2.67). This conversion was then used for inverting the configuration of secondary alcohols and it developed into a useful method as it is mild, stereoselective and compatible with a wide range of functional groups. It was also later adapted for the formation of C-C, C-O, C-N and C-S bonds.

A widely accepted mechanism for the Mitsunobu reaction is illustrated in Scheme 2.68. It commences with a reaction between phosphine and azodicarboxylate to form the betaine species, which deprotonates the acidic nucleophile to give intermediate. The alcohol and react to give hydrazinediacyl oxide (reduced azodicarboxylate) and compound, which is attacked by the deprotonated nucleophile to afford the product along with phosphine oxide.
The formation of the two by-products (phosphine oxide and hydrazinedicarboxylate species) is unavoidable, considering the main coupling is accompanied by a concurrent redox reaction between phosphine and azodicarboxylate. They are difficult to separate from the desired product, which results in low isolated yields. Modifications have been reported aiming at improving this method to a more purification-friendly one (Figure 2.22). Diphenyl-2-pyridylphosphane (294) and di-tert-butyl azodicarboxylate (DBAD) have been used as an alternative reagent system which after acidic work-up would allow ease removal of by-products. Masked carboxylic acid tags have also been attached to the phosphine reagent (e.g. 295) so that on removal of the tags after the reaction, by-products could be removed using ion-exchange resins. Phosphine 296 and diiso-propyl azodicarboxylate (DIAD) system was also reported to provide a simpler separation process.

In 1998, Georg and co-workers reported the use of polymer-supported reagents in etherifications between phenols and alcohols. Polystyrene-bound triphenylphosphine can be easily removed by filtration after reaction workup, and was thus considered as a traceless reagent (Scheme 2.69). Charette and co-workers later used this method in a $S_N2$ reaction between phenols and alcohols, resulting in good yields.
II. Results and Discussion

Alternative azodicarboxylates (301-304, Figure 2.23) such as DIAD and DBAD mentioned before, 1,1’-(azodicarbonyl) dipereridine (ADDP) and N,N,N’,N’-tetramethylazodicarboxamide (TMAD) were also reported to produce satisfactory results.  

In this project, the Mitsunobu reaction was applied to connect the protected pro-drug carriers with the asymmetric NQO1 inhibitors. Previous studies in the Whitehead group on relevant reactions had resulted in a standard reaction procedure: two reagents (DIAD and triphenylphosphine) were added directly into a mixture of alcohol and NQO1 inhibitor in anhydrous THF at 0 °C; the reaction was left at 0 °C for 30 minutes and then allowed to warm to room temperature to stir for a further 20 hours. These reaction conditions were employed during this project for a number of coupling reactions in the initial attempts. All the reactions failed, with the exception of the one between compounds 155 and 165b (Table 2.17).
Table 2.17: Initial attempts at the Mitsunobu reaction using standard reaction conditions.

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Nucleophile</th>
<th>Reagent system</th>
<th>Reaction temperature</th>
<th>Reaction time</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>163c</td>
<td>DIAD/Ph3P</td>
<td>0 °C - r.t.</td>
<td>1 d</td>
<td>failed</td>
</tr>
<tr>
<td>155</td>
<td>164c</td>
<td>DIAD/Ph3P</td>
<td>0 °C - r.t.</td>
<td>2 d</td>
<td>failed</td>
</tr>
<tr>
<td>155</td>
<td>165b</td>
<td>DIAD/Ph3P</td>
<td>0 °C - r.t.</td>
<td>20 h</td>
<td>Successful, 55%</td>
</tr>
<tr>
<td>159b</td>
<td>163c</td>
<td>DIAD/Ph3P</td>
<td>0 °C - r.t.</td>
<td>2 d</td>
<td>failed</td>
</tr>
<tr>
<td>159b</td>
<td>164c</td>
<td>DIAD/Ph3P</td>
<td>0 °C - r.t.</td>
<td>2 d</td>
<td>failed</td>
</tr>
</tbody>
</table>

Two starting materials were recovered from the purification process and no formation of the desired coupling product was observed. Instead, generation of the by-product 308 (Scheme 2.70) was confirmed following analysis of $^1$H-NMR and mass spectrometry data. Formation of the undesired compound 308 was thought to result from the reaction between alcohol and the betaine species.

A similar side reaction was reported by Humphries and colleagues when triphenylphosphine and DEAD were used as reagents. They ascribed the occurrence of the side reaction to the lack of acidity of the nucleophilic reactant. It was pointed out by them that in the Mitsunobu reaction, the pKₐ value of the nucleophilic reactant should ideally be
II. Results and Discussion

lower than 11: when the $pK_a$ is above this value, the desired reaction proceeds slowly or does not occur.\textsuperscript{124} The $pK_a$ values of three nucleophiles (163c, 164c and 165b) from the initial attempts were thus predicted\textsuperscript{125} and are listed in Table 2.18.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Predicted $pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>163c</td>
<td>5.3</td>
</tr>
<tr>
<td>164c</td>
<td>5.7</td>
</tr>
<tr>
<td>165b</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Table 2.18: Predicted $pK_a$s of 163c, 164c and 165b.\textsuperscript{125}

It is clear that the $pK_a$ values of these nucleophiles are all within the required range, implying the failure of the reactions was not due to insufficient acidity. Humphries’ modified conditions were nonetheless employed: a reagent system with ADDP and polymer-supported triphenylphosphine was used to replace the DIAD and triphenylphosphine system.\textsuperscript{124} Three reactions were repeated but, again, none of them afforded the desired products (Table 2.19).

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Nucleophile</th>
<th>Reagent system</th>
<th>Reaction temperature</th>
<th>Reaction time</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>163c</td>
<td>ADDP/PS-Ph$_3$P</td>
<td>0 °C - r.t.</td>
<td>2 d</td>
<td>failed</td>
</tr>
<tr>
<td>159b</td>
<td>163c</td>
<td>ADDP/PS-Ph$_3$P</td>
<td>0 °C - r.t.</td>
<td>5 d</td>
<td>failed</td>
</tr>
<tr>
<td>159b</td>
<td>165b</td>
<td>ADDP/PS-Ph$_3$P</td>
<td>0 °C - r.t.</td>
<td>3 d</td>
<td>failed</td>
</tr>
</tbody>
</table>

Table 2.19: Mitsunobu reactions using Humphries’ conditions.

The failure of these reactions prompted subsequent attempts to modify the experimental procedure. Starting from there, it was decided to change the order of addition of reagents
and reactants in accord with the proposed reaction mechanism. According to this premise, two reagents (triphenylphosphine and DIAD) should firstly be mixed to afford the betaine species; the nucleophiles should then be added to allow deprotonation. Finally the substrate alcohol should be added to form the desired product. Using this procedure, four trial reactions were carried out, three of which proved to be successful. The reaction involving ADDP and polystyrene-supported triphenylphosphine failed once again (Table 2.20).

Table 2.20: Successful Mitsunobu reactions using a modified order of addition of reactants (due to failure to purify the desired coupling product from each reaction mixture, yields are not provided).

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Nucleophile</th>
<th>Reagent system</th>
<th>Reaction temperature</th>
<th>Reaction time</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>159b</td>
<td>163c</td>
<td>DIAD/Ph$_3$P</td>
<td>0 °C - r.t.</td>
<td>20 h</td>
<td>successful</td>
</tr>
<tr>
<td>159b</td>
<td>164c</td>
<td>DIAD/Ph$_3$P</td>
<td>0 °C - r.t.</td>
<td>1 d</td>
<td>successful</td>
</tr>
<tr>
<td>159b</td>
<td>164e</td>
<td>DIAD/Ph$_3$P</td>
<td>0 °C - r.t.</td>
<td>20 h</td>
<td>successful</td>
</tr>
<tr>
<td>159b</td>
<td>164c</td>
<td>ADDP/PS-Ph$_3$P</td>
<td>0 °C - r.t.</td>
<td>1 d</td>
<td>failed</td>
</tr>
</tbody>
</table>

Formation of the desired product appeared within one hour following addition of the alcohol, which was fully consumed by the end of the reaction. The by-product 308, however, was also formed concurrently during the reaction. The similar polarities of the desired product and 308 led to a poor isolated yield in each successful trial.

It was by a serendipitous finding that this result was improved. The amount of DIAD added into the reaction was reduced and a reaction with 25% less DIAD gave less by-product 308. Slow, drop-wise addition of DIAD during the pre-mixing stage further diminished the amount of 308 formed in the subsequent trials. Successful reactions using these conditions are listed in Table 2.21. Due to the difficulties with complete removal of triphenylphosphine oxide and di$\text{iso}$-propyl hydrazine-1,2-dicarboxylate from the desired products, most of the isolated yields are not given.
Table 2.21: Successful Mitsunobu reactions using reduced amount of DIAD.

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Nucleophile</th>
<th>Reagent system</th>
<th>Reaction temperature</th>
<th>Reaction time</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>165a</td>
<td>DIAD/Ph, P</td>
<td>0 °C - r.t.</td>
<td>20 h</td>
<td>successful (44%)</td>
</tr>
<tr>
<td>159b</td>
<td>163a</td>
<td>DIAD/Ph, P</td>
<td>0 °C - r.t.</td>
<td>20 h</td>
<td>successful</td>
</tr>
<tr>
<td>159b</td>
<td>163b</td>
<td>DIAD/Ph, P</td>
<td>0 °C - r.t.</td>
<td>20 h</td>
<td>successful</td>
</tr>
<tr>
<td>159b</td>
<td>163b</td>
<td>ADDP/Ph, P</td>
<td>0 °C - r.t.</td>
<td>20 h</td>
<td>failed</td>
</tr>
<tr>
<td>159b</td>
<td>163c</td>
<td>DIAD/Ph, P</td>
<td>0 °C - r.t.</td>
<td>24 h</td>
<td>successful</td>
</tr>
<tr>
<td>159b</td>
<td>164c</td>
<td>DIAD/Ph, P</td>
<td>0 °C - r.t.</td>
<td>20 h</td>
<td>successful</td>
</tr>
<tr>
<td>159b</td>
<td>165a</td>
<td>DIAD/Ph, P</td>
<td>0 °C - r.t.</td>
<td>24 h</td>
<td>successful</td>
</tr>
</tbody>
</table>

It is noteworthy that under the same conditions, the reaction between 159b and 163b using ADDP instead of DIAD failed to give any coupling product, despite adequate reaction time. It was decided, therefore, to use DIAD and triphenylphosphine as the standard reagent system for subsequent Mitsunobu reactions.

During the following studies, it was found that by leaving the reaction at 0 °C for a longer time (6 hours rather than 1 hour), the overall reaction time could be shortened. The results of reactions under these conditions are listed in Table 2.22.
Table 2.22: Successful Mitsunobu reactions using shortened reaction time (due to failure to purify the desired coupling product from each reaction mixture, yields are not provided).

A mechanism for the Mitsunobu reaction is illustrated in Scheme 2.71.

Scheme 2.1: Proposed mechanism for the Mitsunobu reactions.
2.6.3 Preparation of the Pro-drugs

As discussed in section 2.6.1, carriers with more than one hydroxyl groups were protected prior to the Mitsunobu reaction in order to avoid side reactions, thus the final step towards the pro-drugs is removal of the protecting groups (TES, TBS and BDA), with the exception of the pro-drugs with the monool carrier, 155 (Scheme 2.72).

The deprotection reactions were accomplished over a short reaction time using a mixture of TFA and H₂O in a ratio of 7:1; DCM was added as co-solvent at 0 °C when sensitive substrates (i.e. BDA protected compounds) were deprotected. Considering the reaction mixture was difficult to concentrate directly as both TFA and H₂O have relatively high boiling points, NaHCO₃ solution was added on one occasion to quench the reaction in order to allow extraction with a low boiling point organic solvent. The yield, however, remained the same.

The yields of the pro-drugs synthesised are listed in Table 2.23.
II. Results and Discussion

Table 2.23: Yields of preparation of pro-drugs (for 315, 317 and 318 series, the yields are quoted over two steps).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield (%)</th>
<th>Compound</th>
<th>Yield (%)</th>
<th>Compound</th>
<th>Yield (%)</th>
<th>Compound</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>315a</td>
<td>70</td>
<td>315f</td>
<td>57</td>
<td>316f</td>
<td>27</td>
<td>317f</td>
<td>30</td>
</tr>
<tr>
<td>315b</td>
<td>12</td>
<td>315g</td>
<td>34</td>
<td>316h</td>
<td>31</td>
<td>317h</td>
<td>62</td>
</tr>
<tr>
<td>315c</td>
<td>61</td>
<td>315h</td>
<td>49</td>
<td>316i</td>
<td>59</td>
<td>317j</td>
<td>35</td>
</tr>
<tr>
<td>315d</td>
<td>25</td>
<td>315j</td>
<td>46</td>
<td>316j</td>
<td>44</td>
<td>318j</td>
<td>37</td>
</tr>
<tr>
<td>315e</td>
<td>52</td>
<td>316d</td>
<td>11</td>
<td>317e</td>
<td>57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The reaction proceeded smoothly, but various purification methods that followed (flash column chromatography, solvent wash, crystallisation) caused a loss of some material; the isolated yields were correspondingly compromised.

Attempts to prepare pro-drugs 316e, 316g and 318e were unsuccessful due to it being impossible to separate the desired products from triphenylphosphine oxide and/or diisopropyl hydrazine-1,2-dicarboxylate. Although these two impurities were removed as much as possible following the Mitsunobu reaction, they remained in the final product despite various attempts at purification.

The anti-proliferative activities of the pro-drugs were measured using the MTT assay, the results of which are presented and discussed in the next chapter.
III. MTT Cell Viability Assay and Biotransformation Results

3.1 MTT Cell Viability Assay

In 1983, a quantitative colourimetric approach to measure cytotoxicity, proliferation and activation of mammalian cells was developed by Tim Mosmann. The assay used a yellow tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 319), that could only interact with viable cells in mitochondria to give a purple crystalline product, formazan (320, Scheme 3.1). MTT was pre-dissolved in phosphate-buffered saline (PBS) at a specific concentration. Mitochondrial succinate dehydrogenase present in viable cells then converted yellow MTT into purple formazan, the quantity of which was hence proportional to the number of living cells. Formazan was dissolved in iso-propanol so that the absorbance of the solution could be measured and processed in order to determine cell growth.

![Scheme 3.1: MTT is reduced to formazan by mitochondrial succinate dehydrogenase.](image)

In 1987, Carmichael and co-workers optimised Mosmann’s experimental conditions. Iso-propanol was replaced with DMSO for a better solubilisation of formazan and optical density (OD) was measured at a wavelength of 540 nm instead of 570 nm to achieve the maximum absorbance. Additionally, a 4-day incubation time (96 hours) was recommended in order to implement drug-induced cell death as well as to avoid refeeding of cultures. Since then, the MTT assay has been adopted as a rapid and convenient method for assessing cell viability with reproducible results.

3.2 Procedure for MTT Viability Assay

The procedure used for MTT assays during this project is outlined below and a more detailed procedure can be found in chapter VI.

The cells were seeded in seven columns sextuplicately across the 96-well flat-bottomed microtitre plate at a density of 1000 cells/mL, 180 µL in each well. The plates were
incubated at 37 °C under an atmosphere with 5% CO₂ for 24 hours prior to the addition of test compounds.

Compounds to be tested were added to the pre-incubated cell suspension in which the concentrations of the compounds were at 100 µM, 10 µM, 1 µM, 100 nM, 10 nM and 1 nM. A solvent control using DMSO in which the compounds were dissolved was also included. The compound-treated cells were incubated for 96 hours after which, MTT solution (2.5 g/L) was added to each well. The microtitre plates were incubated for 4 hours following the addition of MTT to allow for formazan crystal formation. The formazan crystals were solubilised in 200 µL of DMSO and mixed evenly. The absorbances of the formazan solutions were measured at a wavelength of 540 nm using multi-well scanning spectrophotometer. The readings were processed to give IC₅₀ values for each compound.
III. MTT Cell Viability Assay and Biotransformation Results

3.3 MTT Cell Viability Assay Results

Four cell lines were subjected to treatment with test compounds during the MTT cell viability assays. Information about the cell lines is provided in Table 3.1 and further details can be found in section 6.1.1. Among them, the A549 cell line has an elevated GSH expression level whereas MDA-MB-231 expresses a distinctively lower NQO1 enzyme level than the other three cell lines. FaDu was chosen for adding diversity to test cell lines and PNT2 is a healthy prostate cell line for comparison with cancer cell lines. For each compound on each cell line, the assay was repeated at least three times and the results reported in this chapter are the average values of three individual experiments; standard deviations were calculated from the results of each individual experiment.

Table 3.1: Four cell lines used in cross-cell line MTT assay.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue of origin and disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>Lung carcinoma</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>FaDu</td>
<td>Pharynx carcinoma</td>
</tr>
<tr>
<td>PNT2</td>
<td>Healthy prostate</td>
</tr>
</tbody>
</table>

3.3.1 MTT Assay Results of Natural Products Analogues

In the following sub-sections, MTT assay results of synthesised natural product analogues are described. Among these compounds, COTC and incarviditone analogues were tested against the four cell lines whereas the remainder (analogues of antheminone A and hydroxyenone intermediates) were tested against the A549 cell line. The standard assay time was 96 hours unless otherwise stated.

3.3.1.1 MTT Assay Results of the Hydroxyenone Intermediates

The hydroxyenone intermediates (122a-e) obtained during the synthesis of COTC analogues are the precursors to incarviditone analogues and can be viewed as analogues of rengyolone (section 1.4). These compounds do not possess a side-chain leaving group and were hence assayed for their anti-proliferative activities in order to compare with analogues of COTC and antheminone A. Such comparison is useful for investigating structure-activity relationship of the natural product analogues synthesised during this project. The results as well as the predicted partition coefficient values (milogP)$^{82}$ are listed in Table 3.2.
III. MTT Cell Viability Assay and Biotransformation Results

Table 3.2: MTT assays results of the hydroxyenone intermediates 122a-c.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>mi$\text{log}$P</th>
</tr>
</thead>
<tbody>
<tr>
<td>122a</td>
<td>5.46 ± 0.49</td>
<td>2.37</td>
</tr>
<tr>
<td>122b</td>
<td>1.93 ± 0.39</td>
<td>2.74</td>
</tr>
<tr>
<td>122c</td>
<td>6.95 ± 0.33</td>
<td>1.61</td>
</tr>
</tbody>
</table>

The growth of the A549 cell line was strongly inhibited by this group of compounds amongst which, the 2-naphthyl substituted compound 122b showed the highest activity. The dataset, however, does not confirm an absolute correlation between hydrophobicity and anti-proliferative activity.

Unlike COTC that undergoes conjugate displacement of its crotonate moiety by intracellular GSH to generate an alkylating agent, these hydroxyenone compounds, lacking a leaving group, could not undergo such a reaction. It was thus assumed that the hydroxyenone compounds may have an alternative mode of action, such as direct alkylation of crucial cellular molecules.

3.3.1.2 MTT Assay Results of the Incarviditone Analogues

The MTT assay results of five incarviditone analogues (123a-d and 123j) against the four cell lines as well as the predicted partition coefficient values (mi$\text{log}$P) are listed in Table 3.3.

Table 3.3: MTT assays results of the incarviditone analogues 123a-d and 123j.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>mi$\text{log}$P</th>
</tr>
</thead>
<tbody>
<tr>
<td>123a</td>
<td>13.44 ± 1.40</td>
<td>5.60</td>
</tr>
<tr>
<td>123b</td>
<td>11.27 ± 0.53</td>
<td>4.86</td>
</tr>
<tr>
<td>123c</td>
<td>21.10 ± 2.49</td>
<td>3.35</td>
</tr>
<tr>
<td>123d</td>
<td>54.93 ± 8.38</td>
<td>3.63</td>
</tr>
<tr>
<td>123j</td>
<td>NA</td>
<td>−0.36</td>
</tr>
</tbody>
</table>

NA = not active (>100 µM)
From this dataset, several observations can be made:

1) In this group of compounds, two out of five exhibited moderate anti-proliferative activity against at least one cell line while the remainder were inactive;

2) Compound 123b was exclusively active against the A549 cell line. Conversely, 123a was active against all but the A549 cell line. The inhibition against the growth of the MDA-MB-231 cell line by 123a was 2-fold and 5-fold higher than that against the FaDu and PNT2, respectively;

3) Noticeably, compounds (123c,d,j) with predicted logP values below 4 were completely inactive, whereas compounds with values above 4 (123a,b) inhibited the growth of some cell lines, indicating that hydrophobicity might be associated with their bioactivities.

Incarviditone analogues do not possess a cyclohexenone skeleton and they are considered to act via a different intracellular pathway, compared to analogues of COTC and antheminone A. Research is still at the stage of deducing the structural features that are prerequisite for manifestation of their anti-proliferative activities. Although hydrophobicity appeared to be associated with their biological properties, this relationship has not been unambiguously verified due to insufficient population of incarviditone analogues.

### 3.3.1.3 MTT Assay Results of the Antheminone A Analogues

The MTT assay results of three antheminone A analogues (154a-c) against the A549 cell line as well as the predicted partition coefficient values (milogPs) are listed in Table 3.4.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>milogP</th>
</tr>
</thead>
<tbody>
<tr>
<td>154a</td>
<td>16.53 ± 1.57</td>
<td>1.67</td>
</tr>
<tr>
<td>154b</td>
<td>15.26 ± 0.22</td>
<td>2.04</td>
</tr>
<tr>
<td>154c</td>
<td>40.76 ± 5.43</td>
<td>0.92</td>
</tr>
</tbody>
</table>

All three compounds exhibited moderate inhibition of the growth of the A549 cell line. Compounds 154a and 154b displayed similar anti-proliferative activities whereas 154c showed comparatively lower activity. A striking difference between 154c and the other two compounds was their hydrophobicities (milogP) and it appeared that a decrease in anti-proliferative activity might occur outside of a certain range of hydrophobicity.
### 3.3.1.4 MTT Assay Results of COTC analogues

The anti-proliferative activities of three analogues of COTC were evaluated. 166a was tested against the A549 cell line whereas 166b and 166c were tested against the four cell lines. The MTT assay results along with the corresponding predicted logP values are listed in Table 3.5.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (μM)</th>
<th>milogP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>MDA-MB-231</td>
<td>FaDu</td>
</tr>
<tr>
<td>166a</td>
<td>2.97 ± 0.16</td>
<td>/</td>
</tr>
<tr>
<td>166b</td>
<td>2.47 ± 0.28</td>
<td>1.22 ± 0.06</td>
</tr>
<tr>
<td>166c</td>
<td>3.17 ± 0.22</td>
<td>2.70 ± 0.14</td>
</tr>
</tbody>
</table>

Several observations were made from this dataset:

*i* This group of compounds exhibited relatively strong inhibition against the growth of the four cell lines with the 2-naphthyl substituted compound 166b displaying the highest general anti-proliferative activity;

*ii* Neither 166b nor 166c showed significant variations of results on different cell lines;

*iii* All three compounds had similar predicted logP values and it was difficult, therefore, to deduce if hydrophobicity was correlated with anti-proliferative activity.

Comparing results of analogues of COTC with those of hydroxyenones and analogues of antheminone A, it could be concluded that:

*i* COTC analogues showed the highest anti-proliferative activity against the four cell lines. Their activity in a descending order is: COTC analogues > hydroxyenones > antheminone A analogues (it would be inappropriate to compare the activity of incarviditone analogues with the cyclohex-2-enone family as they have different structural features). The superior anti-proliferative activity of COTC analogues over antheminone A analogues indicates the leaving ability of the side-chain substituent may influence bioactivity.

*ii* Further biological test data are required to substantiate the relationship between hydrophobicity and anti-proliferative activity, however, an approximation was inferred: a logP range between 2.3 and 2.8 led to good anti-proliferative activity which decreases outside of this range.
iii) It was noteworthy that the 2-naphthyl substituted compounds were the most active in each group. This implied that increased intermolecular π-π interactions may enhance anti-proliferative activity.

3.3.1.5 Timed MTT Assay Results of COTC analogues

To investigate the anti-proliferative activity of compounds 166b and 166c as a function of time, MTT assays were conducted with cells harvested after different periods of time.

166b was tested against the A549 and PNT2 cell lines. Assays were initiated at the same time but terminated after 1, 12, 24, 48, 72 and 96 hours of incubation. The results are listed in Table 3.6.

![Graph of IC₅₀ (µM) vs. Incubation Time (h) for compounds 166b in A549 and PNT2 cell lines.]

Table 3.6: Timed MTT assay results of compound 166b.

<table>
<thead>
<tr>
<th>Incubation Time (h)</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A549</td>
</tr>
<tr>
<td>1</td>
<td>110.35 ± 6.21</td>
</tr>
<tr>
<td>12</td>
<td>52.15 ± 3.43</td>
</tr>
<tr>
<td>24</td>
<td>20.94 ± 3.56</td>
</tr>
<tr>
<td>48</td>
<td>4.37 ± 0.52</td>
</tr>
<tr>
<td>72</td>
<td>3.27 ± 0.22</td>
</tr>
<tr>
<td>96</td>
<td>2.47 ± 0.28</td>
</tr>
</tbody>
</table>

During the first 48 hours of incubation with the A549 cell line, the anti-proliferative activity progressively increased but plateaued out from the 48th hour and after which it slightly increased to the maximum. A similar trend was observed for the PNT2 cell line, in spite of the initial activity being much higher and the plateau arriving after only 12 hours of incubation.

It can thus be concluded that compound 166b acted more efficiently against the PNT2 than the A549 cell line with the inhibition of the growth of the PNT2 cell line being greater than that of the A549 when the same amount of incubation time was provided. It is unclear, however, whether the inhibition of the growth of the A549 by 166b would reach the same level as that of the PNT2 if a longer incubation time was given.
Assays of 166c were conducted on the A549 cell line with cells harvested after 24, 48, 72 and 96 hours of incubation. The results are listed in Table 3.7.

Table 3.7: Timed MTT assay results of compound 166c.

<table>
<thead>
<tr>
<th>Incubation Time (h)</th>
<th>IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>25.73 ± 2.20</td>
</tr>
<tr>
<td>48</td>
<td>13.12 ± 1.98</td>
</tr>
<tr>
<td>72</td>
<td>3.33 ± 0.33</td>
</tr>
<tr>
<td>96</td>
<td>3.17 ± 0.22</td>
</tr>
</tbody>
</table>

During the first 48 hours, the anti-proliferative activity increased steadily until it reached a plateau after 72 hours of incubation. The trend was similar to that of compound 166b, but it took a longer time for 166c to reach the plateau period. The activity of 166c escalated to the maximum level after 72 hours with further negligible increase till the end point.

From these two experiments, it can be summarised that both compounds 166b and 166c require at least three days for the complete expression of their anti-proliferative activities against the A549 cell line. In addition, the fact that 166b exhibited higher activity against a healthy cell line (PNT2) than a cancer cell line (A549) is clearly unsatisfactory. Although the reasons for this are unclear at the current stage, it might be a consequence of cell-type-based preference of compounds. Expanding the assay cell lines would be useful to investigate this issue.
3.3.2 MTT Assay Results of NQO1 Inhibitors

In this section, the MTT assay results of both 4-hydroxycoumarin-type and chromadione-type NQO1 inhibitors are reported and discussed. The results of 4-hydroxycoumarin-type NQO1 inhibitors are listed in Table 3.8. According to the previous studies by the Whitehead group, compounds of this type exhibited very limited cytotoxicity. The test results for the re-synthesised NQO1 inhibitors were consistent with the previous findings: none of them inhibited the growth of tested cell lines at applied concentrations.

### Table 3.8: MTT assay results of 4-hydroxycoumarin-type NQO1 inhibitors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₅</th>
<th>R⁷</th>
<th>R⁸</th>
<th>Ar</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>164a</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
<td>NA</td>
</tr>
<tr>
<td>164b</td>
<td>H</td>
<td>7,8-C₆H₄</td>
<td>Ph</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>164c</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
<td>NA</td>
</tr>
<tr>
<td>164d</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
<td>NA</td>
</tr>
<tr>
<td>164e</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not active

Chromadiones were investigated more recently by the Whitehead group as the second generation of asymmetric NQO1 inhibitors. The MTT assay results of 165a and 165b (Table 3.9) showed their moderate inhibition of the growth of different cell lines. The bioactivity might be a consequence of their Michael accepting capability.

### Table 3.9: MTT assay results of chromadione-type NQO1 inhibitors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₅</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>165a</td>
<td>OMe</td>
<td>13.25 ± 1.15</td>
</tr>
<tr>
<td>165b</td>
<td>H</td>
<td>9.22 ± 0.27</td>
</tr>
</tbody>
</table>

Compound 165a exhibited varying anti-proliferative activity against different cell lines. The growth of the FaDu cell line was inhibited the most by 165a whereas the PNT2 cell line was inhibited the least with an approximate quintuple difference in their IC₅₀ values.
The results of the MTT assays of the NQO1 inhibitors were important prior to the tests of the corresponding pro-drugs. They could help to determine whether the incorporation of NQO1 inhibitors contributed to the anti-proliferative activity.
3.3.3 MTT Assay Results of Pro-drugs

Eighteen pro-drugs were assayed for their anti-proliferative activities against the four cell lines (Table 3.1). The results are categorised by different carriers being incorporated in the pro-drugs and are described in the following sub-sections.

3.3.3.1 Results of 315 Pro-drug Series

In Table 3.10, the MTT assay results of eight pro-drugs with the same cyclohexenone carrier are described. This group of pro-drugs is referred to as the 315 series in this chapter. The predicted partition coefficient values (mlogP)\(^{82}\), enzyme activity assay results (as IC\(_{50}\) values) of the corresponding NQO1 inhibitors and molecular weights are also shown.

Table 3.10: MTT assay results of 315 pro-drug series.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>IC(_{50}) (µM)</th>
<th>mlogP</th>
<th>NQO1 IC(_{50}) (nM) (^{51,67})</th>
<th>M(_{W}) (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>315a</td>
<td>10.24 ± 0.34</td>
<td>4.36</td>
<td>N/A</td>
<td>412.13</td>
</tr>
<tr>
<td>315b</td>
<td>3.66 ± 0.20</td>
<td>4.37</td>
<td>N/A</td>
<td>442.14</td>
</tr>
<tr>
<td>315c</td>
<td>8.68 ± 0.77</td>
<td>5.52</td>
<td>N/A</td>
<td>462.15</td>
</tr>
<tr>
<td>315e</td>
<td>23.43 ± 2.82</td>
<td>7.44</td>
<td>14 ± 5.8</td>
<td>552.19</td>
</tr>
<tr>
<td>315f</td>
<td>21.16 ± 0.68</td>
<td>6.25</td>
<td>25 ± 4</td>
<td>582.20</td>
</tr>
<tr>
<td>315g</td>
<td>19.71 ± 1.24</td>
<td>7.44</td>
<td>144 ± 79</td>
<td>502.18</td>
</tr>
<tr>
<td>315h</td>
<td>8.17 ± 0.42</td>
<td>6.26</td>
<td>816 ± 133</td>
<td>532.19</td>
</tr>
<tr>
<td>315j</td>
<td>3.99 ± 0.52</td>
<td>6.33</td>
<td>25 ± 10</td>
<td>596.18</td>
</tr>
</tbody>
</table>
Several conclusions can be drawn from the above dataset:

1. Within this group of compounds, 315b and 315j exhibited higher general anti-proliferative activities against all tested cell lines than the remainder.

2. The anti-proliferative activity of pro-drugs incorporating the 4-hydroxycoumarin-type NQO1 inhibitors, 315e, f, g, h, did not correlate with the corresponding enzyme inhibitory activity of the NQO1 inhibitors. This could be due to the ionisation of the inhibitors at the physiological pH that altered their activity.

3. Among the pro-drugs carrying the 4-hydroxycoumarin-type NQO1 inhibitors, those with lower molecular weights exhibited greater anti-proliferative activities.

4. All compounds showed higher anti-proliferative activities against the MDA-MB-231 cell line in terms of growth inhibition, with compound 315f showing the most apparent preference.

5. Pro-drugs with lower general anti-proliferative activities showed relatively more varying results on different cell lines and vice versa.

### 3.3.3.2 Results of 316 Pro-drug Series

Below is a group of pro-drugs incorporating the same non-hydroxylated cyclohexenone carrier. They are referred to as the 316 series in this chapter. Compared to the 315 pro-drug series they are less hydrophobic and have lower molecular weights. The MTT assay results of five 316 series pro-drugs are listed in Table 3.11, along with the predicted partition coefficient values (milogP)$^{82}$, enzyme activity assay results (as IC$_{50}$ values) of the corresponding NQO1 inhibitors and molecular weights.
Table 3.1: MTT assay results of 316 pro-drug series.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>M&lt;sub&gt;W&lt;/sub&gt; (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>316d</td>
<td>12.67 ± 0.70</td>
<td>410.15</td>
</tr>
<tr>
<td>316f</td>
<td>Not active</td>
<td>440.16</td>
</tr>
<tr>
<td>316h</td>
<td>8.00 ± 0.51</td>
<td>390.15</td>
</tr>
<tr>
<td>316i</td>
<td>12.81 ± 2.58</td>
<td>424.45</td>
</tr>
<tr>
<td>316j</td>
<td>16.99 ± 1.62</td>
<td>454.14</td>
</tr>
</tbody>
</table>

Several conclusions can be drawn from this dataset:

1. Within this group of compounds, 316h exhibited the highest general anti-proliferative activity against all tested cell lines.
2. A similar propensity to that shown in 315 pro-drug series appeared among compounds 316d, f, h, in that the incorporation of potent NQO1 inhibitors had a "backfiring" effect on anti-proliferative activity of pro-drugs.
3. Compounds 316d, f, h, j displayed a preference for inhibiting the growth of MDA-MB-231 cell line and compound 316f exhibited the most variable results among them.

3.3.3.3 Results of 317 and 318 Pro-drug Series

The pro-drugs incorporating mono- and di-hydroxylated carriers referred to as the 317 and 318 series, respectively, were also assessed for their anti-proliferative activity against the
four cell lines. The results are listed in Table 3.12, along with the predicted partition coefficient values (milogP)\(^8\), enzyme activity assay results (as IC\(_{50}\) values) of the corresponding NQO1 inhibitors and molecular weights.

### Table 3.12: MTT assay results of 317 and 318 pro-drug series.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>IC(_{50}) (µM)</th>
<th>mi logP</th>
<th>IC(_{50}) (nM) (^{41,67})</th>
<th>M(_W) (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>317e</td>
<td>A549 12.67 ± 0.70</td>
<td>3.21 ± 0.22</td>
<td>11.49 ± 1.06</td>
<td>7.99 ± 0.18</td>
</tr>
<tr>
<td>317f</td>
<td>A549 5.74 ± 0.37</td>
<td>3.92 ± 0.16</td>
<td>6.00 ± 0.83</td>
<td>3.96 ± 0.20</td>
</tr>
<tr>
<td>317h</td>
<td>A549 9.07 ± 0.92</td>
<td>3.37 ± 0.33</td>
<td>4.44 ± 0.36</td>
<td>2.79 ± 0.22</td>
</tr>
<tr>
<td>317j</td>
<td>A549 8.43 ± 0.54</td>
<td>2.76 ± 0.24</td>
<td>2.80 ± 0.55</td>
<td>3.27 ± 0.99</td>
</tr>
<tr>
<td>318j</td>
<td>A549 27.35 ± 1.85</td>
<td>18.45 ± 1.23</td>
<td>19.15 ± 3.52</td>
<td>27.41 ± 3.24</td>
</tr>
</tbody>
</table>

Several observations can be made from this dataset:

1. Within this group of compounds, 318j displayed the lowest general anti-proliferative activity.

2. Unlike the previous two groups of compounds, the correlation between inhibitory activity of NQO1 inhibitors and anti-proliferative activity of pro-drugs (317e,f,h,j) was vague.

3. With an exception of compound 317e, which showed greater inhibition of the growth of the MDA-MB-231 cell line than the other three, compounds 317f,h,j and 318j all showed similar levels of activities against the four tested cell lines.
3.3.3.4 Summary

Several conclusions that can be made from the three datasets presented above:

1. It is noteworthy that the incorporation of potent NQO1 inhibitors did not provide the pro-drugs with outstanding anti-proliferative activity, especially among the pro-drug carrying 4-hydroxycoumarin-type NQO1 inhibitors, possibly due to the deprotonation of the 4-hydroxyl in the inhibitors under the physiological environment.

2. Molecular weight showed some correlation with the anti-proliferative activity of the pro-drugs possessing 4-hydroxycoumarin-type NQO1 inhibitors. It appears that pro-drugs with lower molecular weights displayed better activities than their larger, heavier counterparts. This might be due to the difference in permeability of the pro-drug molecules through cell membranes.

3. Unlike pro-drugs that possess 4-hydroxycoumarin type NQO1 inhibitors, pro-drugs that possess chromadione-type NQO1 inhibitors appeared to exhibit better general activities regardless of their higher molecular weights. The increase in anti-proliferative activity might be a consequence of the Michael accepting capability of the incorporated chromadione-type NQO1 inhibitors.

4. Relationship between hydrophobicity and anti-proliferative activity of the pro-drugs was not apparent from the datasets presented above.

5. The tested pro-drugs showed an inclination that compounds with higher general anti-proliferative activities showed similar levels of activity against the four tested cell lines whereas those with more varying results were generally less active.

6. Among the four tested cell lines, the MDA-MB-231 expresses the lowest level of NQO1 enzyme (MDA-MB-231 < PNT2 < FaDu, A549), according to enzyme activity assays conducted by the Stratford group. Interestingly, all the tested pro-drugs showed greater activities against the MDA-MB-231 cell line, which suggests that expression level of NQO1 might have affected anti-proliferative activity of the pro-drugs.

7. It was therefore suspected that the pro-drugs were deactivated by NQO1, in a way that is not known. An MTT assay using compound 317h (Figure 3.1), which was pre-incubated with NQO1 and NADH prior to the drug treatment, however, gave similar results to the one without the pre-incubation. This hence excludes the possibility of NQO1 converting the pro-drugs into less active components.
3.3.3.5 Cross-Series Comparisons

In previous sections, the MTT assay results of 18 pro-drugs were reported. In this section, comparison of different series of pro-drugs is presented (Table 3.13 and 3.14). The two datasets are divided into separate compartments, representing the different pro-drugs series, assorted by the type of carriers. The predicted logarithms of partition coefficient values ($\text{miP}^{32}$), enzyme activity assay results (as IC$_{50}$ values) of the corresponding NQO1 inhibitors and molecular weights are also provided.

A few conclusions can be drawn from the dataset above:

1. **317** pro-drug series showed higher general anti-proliferative activity than **315** and...
316 series.

2. Compounds 315f, 316f and 317e showed greater inhibition of the growth of the MDA-MB-231 cell line than the other three cell lines, however due to these compounds being from different series, it is difficult to determine any superiority of one series over the other.

The results of all pro-drugs bearing a chromadione-type NQO1 inhibitor are listed in Table 3.14.

Table 3.14: MTT assay results of 315-318 pro-drug series.

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC50 (µM)</th>
<th>MDA-MB-231</th>
<th>FaDu</th>
<th>PNT2</th>
<th>logP</th>
<th>NQO1 IC50 (nM)</th>
<th>M_w (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>315j</td>
<td>3.99 ± 0.52</td>
<td>2.25 ± 0.26</td>
<td>5.59 ± 0.44</td>
<td>3.45 ± 0.40</td>
<td>6.33</td>
<td>25 ± 10</td>
<td>596.18</td>
</tr>
<tr>
<td>316j</td>
<td>16.99 ± 1.62</td>
<td>3.70 ± 0.61</td>
<td>4.66 ± 0.37</td>
<td>9.33 ± 0.75</td>
<td>7.44</td>
<td>25 ± 10</td>
<td>454.14</td>
</tr>
<tr>
<td>317j</td>
<td>8.43 ± 0.54</td>
<td>2.76 ± 0.24</td>
<td>2.80 ± 0.55</td>
<td>3.27 ± 0.99</td>
<td>3.35</td>
<td>25 ± 10</td>
<td>470.14</td>
</tr>
<tr>
<td>318j</td>
<td>27.35 ± 1.85</td>
<td>18.45 ± 1.23</td>
<td>19.15 ± 3.52</td>
<td>27.41 ± 3.24</td>
<td>2.44</td>
<td>25 ± 10</td>
<td>486.13</td>
</tr>
</tbody>
</table>

Several observations can be made:

1. The anti-proliferative activities of compounds 315j, 316j and 317j were on a similar level but a significant drop occurred with compound 318j.

2. With an exception of compound 316j, the remainder of this group exhibited very limited variations of anti-proliferative activity against different cell lines.

3.3.3.6 Summary

Based on the cross-series comparisons, several apparent tendencies shown are summarised
as follows:

1. For pro-drugs that possess 4-hydroxycoumarin-type NQO1 inhibitors, introduction of an aromatic moiety at C5 of the cyclohexanone core failed to improve anti-proliferative activity. The extent of hydroxylation of the core did not correlate with the anti-proliferative activity of the pro-drugs.

2. For pro-drugs that possess chromadione-type NQO1 inhibitors, the extent of hydroxylation on the cyclohexenone core appeared to be vaguely related to the anti-proliferative activity. Introducing an aromatic moiety at C5 of the cyclohexanone core had little influence on the anti-proliferative activity of the pro-drugs.

It can be thus concluded that the pro-drug studies confirmed the ability of the synthesised compounds to inhibit the growth of different cancer cell lines, indicating the NQO1 inhibitors were potentially transported into the cells by the carriers, which could be seen from the elevated anti-proliferative activity of the pro-drugs compared to that of the NQO1 inhibitors alone. The pro-drugs, on the other hand, were not as active as COTC analogues, despite the fact that potent NQO1 inhibitors were being incorporated, whereby an enhanced anti-proliferative activity should be observed. The reasons behind this are not fully understood at this time. Considering cells are complicated systems, the pro-drugs might be affected by other interactions which occur inside the cells. Further studies are required to gain a better insight (chapter IV).
3.4 Biotransformation using Cytochrome P450 Oxidoreductase

Following the discussion in section 2.4, modification of the aromatic side chain (Figure 3.2) in bioactive compounds increases diversity and is useful for gaining better understanding of the structure-activity relationship.

In the following sections, a biocatalytical approach involving cytochrome P450 enzymes for demethylating the methoxy group in two compounds of interest is discussed. The desired product will possess a phenolic moiety which is useful in terms of metabolism as well as hydrogen bonding studies of compounds of this type.

3.4.1 Brief Introduction to Cytochrome P450 Enzymes

Cytochrome P450 monooxygenase (also known as P450s) are a family of haemoproteins that can be found in many forms of organisms. The haem cofactor possessed by P450s (Figure 3.3) is involved in their catalytic mechanism (shown in Scheme 3.3). In the human body, they are located mainly in the liver cells and play important roles in drug metabolism. Most P450-catalysed reactions are oxidations such as carbon hydroxylations, \(N\)-demethylations, \(O\)-demethylations and epoxidations.\(^{129,130}\)

Compounds 123 and 166 were selected as the substrates for enzymatic \(O\)-demethylations by the P450 enzymes (Scheme 3.2). Chemical approaches to obtain the demethylated compounds are normally performed under harsh reaction conditions such as using strong acid at high temperature. As a consequence, stable precursors are required in order to avoid
decompositions and side reactions. A mild enzymatic transformation involving cytochrome P450s was hence considered as an alternative, allowing access to the desired products in just one step.

Scheme 3.2: Two potential P450 substrates (123c and 166c) and the corresponding products (321 and 322).

P450-catalysed oxidations essentially involve breakage of the O-O bond of O2 followed by insertion of an oxygen atom into the substrate, aided by NAD(P)H and cytochrome b5 reductase (Scheme 3.3). The catalytic cycle starts with the substrate (ROCH3) binding to the ferric centre of the enzyme which is followed by an NAD(P)H-P450 reductase-mediated one electron reduction. Reduced P450 then binds to oxygen to form an unstable intermediate (Fe2+-O2/ROCH3) which receives the second electron from cytochrome b5. Subsequent protonation of Fe2+-O2 gives an intermediate (FeII-OOH/ROCH3) in which the O-O bond cleaves to form a high-valent FeO3+ complex. This complex then abstracts a proton from the substrate to generate a FeOH3+/radical species which collapses to give the oxidation product (ROCH2OH). The alkoxy oxygen is protonated, followed by the cleavage from the hemiacetal to give the demethylation product (ROH).
3.4.2 Biotransformation Results

During this study, two wild-type P450 enzymes, BM3 and RhF, were used for the desired biotransformation. BM3 and RhF are from different classes of the P450 family and differ in domain compositions as well as redox centres. Pro8 and Pro14 are two commercially available (Prozomix) P450 mutants that were used for initial screening; β-ionone (Figure 3.4) was used as substrate for positive control and pET28a was an empty vector with the absence of P450 gene and was thus used as a negative control during the screening.

Thirty trial reactions were carried out for screening on an analytical scale (Table 3.15). Each enzyme was added as a lysate to a mixture of D-glucose, glucose dehydrogenase (GDH), NADPH/NADP⁺, NaPi buffer (pH = 7.8) and the corresponding substrates. Reactions with 166c and 123c were performed at two concentrations, 250 µM and 2 mM, while reactions with the positive control, β-ionone, were conducted at 2 nM. Further details regarding the experimental conditions are given in section 6.3.5. A control group (without any substrate) was also prepared for each enzyme lysate and the empty pET28a vector.

![Scheme 3.3: Cytochrome P450 catalysed oxidation and demethylation process.](image-url)

**Figure 3.4: Structure of β-ionone (323).**
III. MTT Cell Viability Assay and Biotransformation Results

Reaction numbering and results are explained in Table 3.15.

Table 3.15: Reagents, conditions and results of each biotransformation.

<table>
<thead>
<tr>
<th>Substrate Concentration</th>
<th>2 mM</th>
<th>250 μM</th>
<th>2 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM3</td>
<td>NR</td>
<td>PC</td>
<td>Inconv.</td>
</tr>
<tr>
<td>RhF</td>
<td>NR</td>
<td>PC</td>
<td>Inconv.</td>
</tr>
<tr>
<td>Pro8</td>
<td>NR</td>
<td>PC</td>
<td>Inconv.</td>
</tr>
<tr>
<td>Pro14</td>
<td>NR</td>
<td>PC</td>
<td>Inconv.</td>
</tr>
<tr>
<td>pET28a</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

*NR = no reaction, PC = positive control, Inconv. = inconclusive results, NC = negative control

After 48 hours, the reactions were analysed by GC-MS. As expected, no reactions occurred for blank and negative control groups. Compound 123c was not a substrate for any enzymes at both concentrations. As for 166c (mass = 316 m/z), reactions at a substrate concentration of 250 μM gave inconclusive results, however product signals were detected in the samples from reactions at 2mM with BM3, Pro8 and Pro14. Signals that appeared at 310 and 355 m/z, unfortunately, did not correspond to the desired demethylated product (302 m/z). It was thought that other enzymes present in the lysate might have affected the reactions and BM3 and RhF were later purified by histidine-tagged column. Upon analysing the samples collected from crude lysate solution and eluents during purification, it was found that RhF lost the desired protein expression before purification when the mass was compared with a known protein ladder. Purification of BM3 was successful, giving a relatively pure P450 enzyme (~120 kDa) after dialysis.

Four reactions (Table 3.16) were set up with the purified enzyme under the same conditions at 2 mM.
III. MTT Cell Viability Assay and Biotransformation Results

Table 3.1: Four reactions with purified BM3.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>166c</th>
<th>β-ionone</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM3 (purified)</td>
<td>310 and 355 m/z</td>
<td>PC</td>
<td></td>
</tr>
<tr>
<td>blank</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
</tbody>
</table>

*NC = negative control, PC = positive control

GC-MS and LC-MS analysis of the reaction samples showed identical signals to those using lysates, however the starting material was reacted completely (no signals appeared at 316 m/z). The two products formed during the reaction (mass = 310 and 355 m/z) remained unidentified. Considering P450s are capable of catalysing a variety of reactions, it was possible the biotransformation occurred on other functional groups such as alkenes and methyls in the substrate rather than the methoxy group.

In conclusion, compound 166c was a substrate for the P450 enzyme and other variants of P450 may convert it into the desired product.
IV. Conclusion and Future Work

4.1 Conclusions

4.1.1 Natural Products Studies

By the end of this project, a panel of COTC, antheminone A and incarviditone analogues (166, 154 and 123) were prepared and their anti-proliferative activities were evaluated. All compounds were prepared from the same starting material, (−)-quinic acid (43), and the overall synthetic scheme is outlined below:

![Scheme 4.1: Synthetic scheme of three natural product based analogues.](image)

On assessing anti-proliferative activities of these natural products using the MTT assay, COTC analogues showed the highest activity, whereas incarviditone analogues were the least active. The precursors to the incarviditone analogues, hydroxyenone intermediates (122), were also studied and they exhibited anti-proliferative activity that was higher than antheminone A analogues. It was assumed that their modes of action might differ from COTC and antheminone A analogues in that they do not possess leaving groups for better interactions with GSH.

The relationship between hydrophobicity and activity was studied. Although no systematic correlation could be established, it was concluded that the optimal range of logarithm of partition coefficient (logP) for COTC, antheminone analogues as well as hydroxyenone
compounds was between 2.3 and 2.8. The logP threshold for good activity of incarviditone analogues is above 4.5.

4.1.2 NQO1 Inhibitors Studies
Seven dicoumarol-based asymmetric NQO1 inhibitors (both 4-hydroxycoumarin- and chromadione-type, Table 4.1) were synthesised, following methods previously employed by the Whitehead group. Synthesis of the 4-hydroxycoumarin-type NQO1 inhibitors was mainly achieved using a “borrowing hydrogen” reaction between hydroxychromenone species and aryl alcohols whereas synthesis of the chromadione-type NQO1 inhibitors involved condensation between hydroxychromenones and 2-hydroxy-1-naphthaldehyde.

Table 4.1: Asymmetric NQO1 inhibitors that were synthesized during this project.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R⁵</th>
<th>R⁷</th>
<th>R⁸</th>
<th>Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>164a</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
</tr>
<tr>
<td>164b</td>
<td>H</td>
<td>7,8, C₃H₄</td>
<td>Ph</td>
<td></td>
</tr>
<tr>
<td>164c</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2-Np</td>
</tr>
<tr>
<td>164d</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
</tr>
<tr>
<td>164e</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Ph</td>
</tr>
<tr>
<td>165a</td>
<td>OMe</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>165b</td>
<td>H</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

These compounds were assayed for their anti-proliferative activities against four cell lines and no activity was observed for the 4-hydroxycoumarin-type NQO1 inhibitors. The chromadione-type NQO1 inhibitors showed anti-proliferative activities against the tested cell lines, which may have been a consequence of their Michael accepting capabilities.

4.1.3 Novel Anti-Cancer Pro-drugs Studies
To date, 19 pro-drugs have been prepared and their general structures, and those of the corresponding carriers as well as the NQO1 inhibitors, are depicted in Figure 4.1.
Eighteen pro-drugs were assayed for their anti-proliferative activities. They all displayed some activities against the tested cell lines, but they were not as potent as analogues of COTC. Pro-drugs with carriers 155 and 156 exhibited better activity than the ones with 154b and 157. Some pro-drugs showed a greater extent of variation of activities against tested cell lines but all of them displayed higher activities against the breast carcinoma cell line, MDA-MB-231, used in the assay. Systematic relationship between hydrophobicity and activity could not be established due to insufficient members of the pro-drug series as well as biological test data.

Comparing these results with those of the NQO1 inhibitors alone showed that the pro-drugs displayed higher anti-proliferative activities against the same tested cell lines. This implied that delivery of NQO1 inhibitors via cyclohexenone carriers was potentially successful, which validated the concept of pro-drug design.

**4.2 Future Work**

**4.2.1 Unsolved Problems**

There were several research topics requiring further investigation by the end of this project. The first one is the modification of side chain of the intermediates 121a and 158c. The original idea was to introduce hydrogen bond donor to the side chain in order to investigate its influence on anti-proliferative activity. It is also important to find more suitable reaction
conditions with higher functional group tolerance and lower moisture and air sensitivity. A biocatalytical approach involving enzymes could also be employed to yield the desired products in an efficient and enantioselective manner. Successful conversions of 121a and 158a would offer novel natural product analogues in all three series (COTC, anthemimone A and incarviditone), therefore in this aspect, the modifications are useful in terms of structure-activity relationship study.

Scheme 4.2: An overview of the side chain modifications.

Another aspect requiring further work is the exploration of incarviditone’s mode of action. The research was brought to a halt due to the failure of oxidising the hydroxycyclohexanone core of the incarviditone analogue 123b.

Scheme 4.3: Oxidation of the hydroxycyclohexanone core of incarviditone analogue 123b.

The suggestion that the hydroxycyclohexanone core may be enzymatically oxidised to the quinone moiety might not stand. A full understanding of the mechanism of action of incarviditone analogues requires identification of intracellular target(s) with the assistance of computational analysis and biological aspect of study.
The third research topic worthy of further study is the biotransformation of compounds 123c and 166c. The transformations aimed to provide the corresponding phenol substituted analogues 321 and 322 as these compounds are relatively difficult to obtain using chemical methods. It was confirmed by GC-MS analysis that compound 166c was converted by wild-type P450 BM3 into unidentified products while 123c remained unreacted. Screening different variants of cytochrome P450 enzymes may help to successfully accomplish the transformations.

Scheme 4.4: Proposed biotransformation with cytochrome P450 enzymes.

4.2.2 Continuing Pro-Drugs Studies

The novel anti-cancer pro-drugs prepared during this project did not exhibit high anti-proliferative activity, however this may not be true for all cell lines. Testing these pro-drugs on cancer cell lines originating from other tissues may bring new perspectives.

Further modifications of carriers 155 and 156 could be carried out, as pro-drugs with these two carriers were more active. Enantiomers of 156 could provide different results in terms of anti-proliferative activity. Introduction of small and simple aliphatic groups (R) to these two carriers might be beneficial in terms of cell membrane permeability.
With regard to the NQO1 inhibitors aspect of the research, it appeared that pro-drugs incorporating smaller and lighter NQO1 inhibitors (164d and 164e) tended to give better results. It is thus suggested to further reduce the size and weight of NQO1 inhibitors by replacing the phenyl group with a 5-membered aromatic heterocyclic ring system (e.g. imidazole, oxazole, furan), as they may be useful for achieving better binding interactions by hydrogen bonding with amino acid residues in the binding region. Different substituents (R) that have not been attempted during the previous studies, such as amines, amides, alkenes and quaternary ammonium salts could also be introduced to the 4-hydroxycoumarin system. Varying their positions or shifting the substituents on the 4-hydroxycoumarin ring may result in different binding orientations, which could lead to better activity.

Figure 4.4: Possible modifications of NQO1 inhibitors.

Pro-drug studies discussed in this project are still at a preliminary stage of research. Indeed, attempting the use of different functional groups in order to investigate their effects
on biological activities using *in vitro* tests are essential for structure-activity relationship (SAR) study. With the aid of computational studies such as molecular mechanics calculation, docking and quantitative structure-activity relationship (QSAR), however, the structures may be optimised even before *in vitro* testing. Biological aspects of research regarding pharmacokinetics such as absorption, distribution and metabolism of drugs are also very important for optimising drug structures. Drug design and optimisation are notoriously difficult, and therefore endeavour is required in order to achieve better bioactivities.
V. Experimental

5.1 Experimental Techniques

Chemical Structures
All chemical structures and chemical names in the figures and schemes were generated using ChemDraw Professional v 15.1.

Flash Column Chromatography
Column chromatography was performed using silica-gel (Sigma-Aldrich) 40-63 μm 60 Å, and the solvent system has been stated in each procedure.

Thin Layer Chromatography
TLC was performed using standard silica 60 pre-coated polyester sheets (Macherey-Nagel) with fluorescent indicator 254 nm. Visualisation was by dipping the plates into staining solution (phosphomolybdic acid in ethanol), followed by heating with a heat gun.

Nuclear Magnetic Resonance
$^1$H spectra were recorded using a B400 Bruker Avance III 400 with prodigy probe or B500 Bruker Avance II+ 500. $^1$H assignments were supported by 2D $^1$H-$^1$H COSY, $^{13}$C-$^1$H HSQC and $^{13}$C-$^1$H HMBC. Chemical shifts ($\delta_{\text{H}}$) are quoted in parts per million (ppm) to the nearest 0.01 ppm and referenced to the residual non-deuterated solvent peaks. Coupling constants ($J$) are quoted to the nearest 0.1 Hz. Spectral data are reported as follows: chemical shifts, integration, multiplicity [s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; or as a combination of these, e.g. dd, dt etc.], coupling constant(s) and assignment.

$^{13}$C spectra were recorded using a Bruker Avance III 400 with prodigy probe or Bruker Avance II+ 500. $^{13}$C assignments were supported by 2D $^{13}$C-$^1$H HSQC, $^{13}$C-$^1$H HMBC, $^{13}$C DEPT-135 and $^{13}$C DEPT-90. Chemical shifts ($\delta_C$) are quoted in parts per million (ppm) to the nearest 0.1 ppm and referenced to the deuterated solvent peak.
Infrared Spectroscopy
IR spectra were recorded on a Perkin Elmer FT-IR instrument, absorption ($v_{\text{max}}$) is given in wavenumbers (cm$^{-1}$) and peak intensities are described as strong ($s$.), medium ($m$.), weak ($w$.) or broad ($br$).

Mass Spectrometry
Mass spectrometry was performed by the staff in the Mass Spectrometry Laboratory, School of Chemistry, at the University of Manchester. Only molecular ions, fractions from molecular ions and major peaks are reported as mass/charge (m/z) ratios.

Melting Points
Recorded on a Sanyo Gallenkamp MPD350 heater.

Optical Rotations ([α]D$^T$)
Recorded on an automatic polarimeter AA-100 with sodium D light ($\lambda$=589 nm) at the University of Manchester. [α]D values are given in 10$^{-1}$ deg g$^{-1}$ cm$^2$, and were calculated using the following equation.

$$[\alpha]_D^T = \frac{100\alpha}{1c}$$

$\alpha$ – observed angle of rotation
l – cell length in dm (0.25 dm)
c – weight of sample (g) in 100 mL of solution to the nearest 0.01 g
T – temperature

Solvents
DCM (anhydrous) was either distilled over calcium hydride under an atmosphere of nitrogen or purchased from Sigma-Aldrich; Tetrahydrofuran (anhydrous) was purchased from Sigma-Aldrich.
Solvents were evaporated on an IKA RV-10-B-S99 rotary evaporator equipped with an IKA HB-10 water bath or a Buchi R114 rotary evaporator equipped with a Buchi B-481 water bath.

All chemicals and solvents were purchased from Alfa-Aesar, Fisher, Fluka and Sigma-Aldrich, and were handled in accordance with safety instructions. All reactions were
carried out under an atmosphere of nitrogen and with exclusion of water (unless water was used as the reaction solvents or as part of the reagents). All glasswares used in reactions were pre-dried. Reaction temperatures of 0 °C were obtained using an ice bath and −78 °C obtained using a bath of a mixture of dry ice and acetone. Room temperature (r.t.) refers to 20-25 °C. Brine refers to a saturated sodium chloride solution.
5.2 Experimental procedures

5.2.1 (1S,3R,4R,5R)-3-O,4-O-Cyclohexylidene-7-oxo-6-oxabicyclo[3.2.1]octan-1,3,4-triol (44)

C_{13}H_{18}O_5; M_W = 254.27 g/mol

A solution of (−)-quinic acid (6.50 g, 33.82 mmol) and cyclohexanone (22 mL, 174.40 mmol) in toluene (75 mL) was stirred at the reflux temperature under an atmosphere of nitrogen in a flask fitted with a Dean and Stark trap for 30 min. After cooling to r.t., Amberlite® resin IR 120 H+ (6.5 g, pre-washed with methanol (30 mL) and diethyl ether (2 × 30 mL)) were added to the reaction mixture. The suspension was heated at reflux for 6 h under an atmosphere of nitrogen and then allowed to cool to r.t.. The resin was removed by filtration and the filtrate was washed with NaHCO_3 (sat., aq., 2 × 30 mL), H_2O (2 × 30 mL) and brine (2 × 30 mL). The toluene solution was shaken vigorously with petroleum ether (40-60, 80 mL) and the precipitate was washed with petroleum ether (40-60, 3 × 20 mL) and evaporated to dryness to give the title compound (44) as a white solid (6.9 g, 80%).

M.p. 143-145 °C [Lit.70 139-141 °C]; [α]_D^{29.5} −32.4 (c 1.00 in CH_2Cl_2) [Lit.70 [α]_D^{22} −33.0 (c 1.05 in CHCl_3)]; ν max (film)/cm⁻¹ 3411 br (O-H), 2950w (C-H), 2926w (C-H), 1767s (C=O); δ_H (400 MHz; CDCl_3) 1.41-1.42 (2H, m, CH_2 of cyclohexane), 1.53-1.56 (3H, m, CH_2 of cyclohexane), 1.61-1.73 (5H, m, CH_2 of cyclohexane), 1.61-1.73 (2H, m, CH_2 of cyclohexane), 2.19 (1H, dd, J 14.6, 3.0, C(2)H), 2.27-2.32 (1H, m, C(6)H), 2.32 (1H, dd, J 14.6, 7.6, 2.6, C(2)H), 2.66-2.69 (1H, m, C(6)H), 4.30 (1H, ddd, J 6.4, 2.6, 1.8, C(4)H), 4.48 (1H, ddd, J 7.6, 6.4, 2.6, C(3)H), 4.74 (1H, dd, J 6.4, 2.6, C(5)H); δ_C (100 MHz; CDCl_3) 23.7, 24.1, 25.2, 33.8 (CH_2 of cyclohexane), 34.5 (C(6)H), 37.0 (CH_2 of cyclohexane), 38.6 (C(2)H), 71.1 (C(3)H), 71.2 (C(1)), 71.9 (C(4)H), 76.4 (C(5)H), 110.8 (C), 179.3 (C=O); m/z (+ES) 277 [M+Na]^+, 100%.
5.2.2 \((3aS,4R,6R,7aR)-6-(hydroxymethyl)\text{hexahydrospiro[benzo[d][1,3]dioxole-2, 1'-cyclohexane]}-4,6\text{-diol}\ (87)\)

5.2.3 3-O, 4-O-Cyclohexylidene-(3R,4S,5R)-\text{trihydroxycyclohexanone} (88)

87: \(\text{C}_{13}\text{H}_{22}\text{O}_5; \text{M}_W=258.15\ \text{g/mol}\)  
88: \(\text{C}_{12}\text{H}_{18}\text{O}_4; \text{M}_W=226.27\ \text{g/mol}\)

To a solution of 44 (3.20 g, 12.59 mmol) in methanol (170 mL) was added sodium borohydride (4.76 g, 125.85 mmol) portionwise at 0 °C. Once effervescence ceased, the reaction mixture was stirred at r.t. for 22 h under an atmosphere of nitrogen. The reaction was quenched by the addition of \(\text{NH}_4\text{Cl}\) (sat., aq., 23 mL) and the resulting mixture was concentrated \textit{in vacuo} to give a white solid. The white solid was washed with ethyl acetate (\(5 \times 25\) mL) and the combined washes were dried over \(\text{MgSO}_4\) and concentrated in \textit{vacuo} to give a crude product 87 as an off-white foam.

To a solution of sodium periodate (6.52 g, 30.48 mmol) in hot water (8 mL) was added silica (15 g). The resulting slurry was mixed until it was a free-flowing powder. To the resulting powder were added DCM (40 mL) and a solution of crude 87 in DCM (30 mL). The reaction mixture was stirred at r.t. for 1.5 h under an atmosphere of nitrogen. The powder was collected by filtration, washed with DCM (\(5 \times 20\) mL) and the combined washes were dried over \(\text{MgSO}_4\) and concentrated \textit{in vacuo} to give the title compound (88) as a white solid (1.72 g, 60% over two steps). M.p. 98-99 °C [Lit.\(^{132}\) 97-98 °C]; \([\alpha]_D^{29} +101.1\) (c 0.50 in CH\(_3\)OH) [Lit.\(^{132}\) \([\alpha]_D +100.3\) (c 0.44 in CHCl\(_3\))]; \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3459br (O-H), 2934s (C-H), 2858m (C-H), 1709s (C=O, ketone); \(\delta_{\text{H}}\) (400 MHz; CDCl\(_3\)) 1.37-1.41 (2H, m, \(\text{CH}_2\) of cyclohexane), 1.52-1.62 (8H, m, \(\text{CH}_2\) of cyclohexane), 2.02 (1H, d, \(J 3.4, \text{OH}\)), 2.45 (1H, dt, \(J 17.8, 2.4, \text{C}(2)\text{H}\)), 2.67-2.72 (2H, m, \(\text{C}(2)\text{H}\) and \(\text{C}(6)\text{H}\)), 2.80 (1H, dd, \(J 17.5, 3.4, \text{C}(6)\text{H}\)), 4.24 (1H, ~t, \(J 3.4, \text{C}(4)\text{H}\)), 4.29-4.32 (1H, m, \(\text{C}(3)\text{H}\)), 4.63 (1H, dd, \(J 7.0, 3.4, \text{C}(5)\text{H}\)); \(\delta_{\text{C}}\) (100 MHz; CDCl\(_3\)) 23.7, 24.0, 25.3, 33.4, 36.4 (\(\text{CH}_2\) of cyclohexane), 40.4 (\(\text{C}(2)\text{H}\)), 41.8 (\(\text{C}(6)\text{H}\)), 68.6 (\(\text{C}(3)\text{H}\)), 71.9 (\(\text{C}(5)\text{H}\)), 74.8 (\(\text{C}(4)\text{H}\)), 109.7 (\(\text{C}\) of cyclohexane), 208.1 (\(\text{C}=\text{O}\)); \(m/z\) (+ES) 249 [M+Na]+, 100%.
5.2.4 (3aR,7aS)-3a,4-dihydrospiro[benzo[d][1,3]dioxole-2,1'-cyclohexan]-5(7aH) -one (89)

C₁₂H₁₆O₃; M_W=208.25 g/mol

To a solution of hydroxyketone (88) (1.4 g, 6.72 mmol) in DCM (26 mL) were added methanesulfonyl chloride (0.6 mL, 7.75 mmol) and triethylamine (2.5 mL, 17.92 mmol) at 0 °C under an atmosphere of nitrogen. The reaction was stirred at r.t. for 4 h and quenched with H₂O (8.5 mL). The product was extracted with DCM (2 × 9 mL). The combined organic extracts were washed with H₂O (2 × 9 mL), brine (2 × 9 mL), dried over MgSO₄ and concentrated in vacuo to give the crude product as an orange oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (8:1) gave the title compound (89) as a white solid (985 mg, 70%). M.p. 63-65 °C [Lit. 132 55-58 °C]; [α]D²⁹ +98.4 (c 0.50 in CH₃OH) [Lit. 132 [α]D +100.3 (c 0.44 in CH₃OH)]; ν_max (film)/cm⁻¹ 2946s (C=H), 2861m (C=H), 1674s (C=O); δ_H (400 MHz; CDCl₃) 1.36-1.38 (2H, m, C_H₂ of cyclohexane), 1.56-1.60 (8H, m, C_H₂ of cyclohexane), 2.61 (1H, dd, J 17.7, 3.9, C(6)H), 2.86 (1H, ddd, J 17.7, 2.7, 1.4, C(6)H), 4.67 (1H, tdd, J 4.9, 2.7, 1.4, C(5)H), 4.71 (1H, ddd, J 4.9, 2.7, 1.4, C(4)H), 6.01 (1H, dt, J 10.4, 1.4, C(2)H), 6.64 (1H, ddd, J 10.4, 2.7, 1.4, C(3)H); δ_C (100 MHz; CDCl₃) 23.9, 24.0, 25.0, 36.1, 37.6 (CH₂ of cyclohexane), 39.0 (C(6)H₂), 70.8 (C(5)H), 73.2 (C(4)H), 110.7 (C of cyclohexane), 128.9 (C(2)H), 146.2 (C(3)H), 195.7 (C=O); m/z (+ES) 231 ([M+Na]⁺, 100%); (Found 231.1032, C₁₂H₁₆NaO₃ ([M+Na]⁺) requires 231.0997).

5.2.5 (3aR,7aS)-tetrahydrospiro[benzo[d][1,3]dioxole-2,1'-cyclohexan]-5(4H)-one (90)

C₁₂H₁₈O₃; M_W=210.13 g/mol

Compound 89 (1.35 g, 6.48 mmol) and activated palladium on carbon (5%, 250 mg) were stirred in ethyl acetate (45 mL) at r.t. for 17 h under an atmosphere of hydrogen at atmospheric pressure. The reaction mixture was filtered through a short pad of Celite® and
washed with ethyl acetate (5 × 20 mL). The filtrate was concentrated in vacuo to give the crude product as a yellow solid. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (90) as a white solid (1.26 g, 93%). M.p. 90-92 °C [Lit.\textsuperscript{133} 98 °C]; [α]_D\textsuperscript{20} +82.2 (c 0.5 in CH\textsubscript{2}Cl\textsubscript{2}) [Lit.\textsuperscript{133} [α]_D\textsuperscript{20} +88.8 (c 1.89 in CH\textsubscript{2}Cl\textsubscript{2})]; ν\textsubscript{max} (film)/cm\textsuperscript{-1} 2937m (C-H), 2857w (C-H), 1699s (C=O); δ\textsubscript{H} (400 MHz; CDCl\textsubscript{3}) 1.37-1.43 (2H, m, CH\textsubscript{2} of cyclohexane), 1.50-1.62 (8H, m, CH\textsubscript{2} of cyclohexane), 1.86 (1H, dddd, J\textsubscript{1,6} 1.6, C(2), 2.23 (1H, dddd, J\textsubscript{1,6} 1.74, 8H, m, C(3)), 4.49 (1H, ddd, J\textsubscript{1,6} 1.86, 1H, dddd, J\textsubscript{1,6} 1.98, 4.58 (1H, t, J\textsubscript{1,6} 2.23), 2.44 (1H, dd, J\textsubscript{1,6} 16.8, 4.1, C(2)H), 2.49 (1H, dd, J\textsubscript{1,6} 18.4, 13.6, 4.8, C(6)H), 2.68 (1H, dd, J\textsubscript{1,6} 16.8, 2.7, C(2)H), 4.49-4.58 (1H, m, C(3)H or C(4)H), 4.60-4.67 (1H, m, C(3)H or C(4)H); δ\textsubscript{C} (100 MHz; CDCl\textsubscript{3}) 23.7, 24.1, 25.3 (CH\textsubscript{2} of cyclohexane), 25.8 (C(5)H\textsubscript{2}), 33.4 (C(6)H\textsubscript{2}), 33.6, 35.9 (CH\textsubscript{2} of cyclohexane), 42.1 (C(2)H\textsubscript{2}), 71.2 (C(3)H), 72.5 (C(4)H), 108.4 (ketal C), 210.1 (C(1)=O); m/z (+ES) 211 ([M+H]\textsuperscript{+}, 100%); (Found 233.1143, C\textsubscript{12}H\textsubscript{18}O\textsubscript{3}Na ([M+Na]\textsuperscript{+}) requires 233.1148).

5.2.6 3-\textit{O,4-\textit{O}-cyclohexylidene-5-(4-bromophenyl)-(3R,4S,5S)-dihydroxycyclohexanone (121a)

![Structural formula of 3-O,4-O-cyclohexylidene-5-(4-bromophenyl)-(3R,4S,5S)-dihydroxycyclohexanone](image)

C\textsubscript{18}H\textsubscript{21}O\textsubscript{3}Br; M\textsubscript{W}=365.27 g/mol

To a solution of enone 89 (250 mg, 1.2 mmol) in dioxane and H\textsubscript{2}O (10:1, 2.5 mL) were added 4-bromophenylboronic acid (843 mg, 4.2 mmol), triethylamine (170 µL, 1.2 mmol) and [RhCl(cod)]\textsubscript{2} (30 mg, 5 mol%). The reaction mixture was stirred at r.t. for 20 h under an atmosphere of nitrogen. The solvent was evaporated in vacuo to give the crude product as a deep orange oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (12:1) gave the title compound (121a) as a thick yellow oil (368 mg, 84%). [α]_D\textsuperscript{20} −77.0 (c 1.00 in CH\textsubscript{2}Cl\textsubscript{2}); ν\textsubscript{max} (film)/cm\textsuperscript{-1} 2929s (C-H), 2901w (C-H), 2856m (C-H), 1715s (C=O); δ\textsubscript{H} (400 MHz; CDCl\textsubscript{3}) 1.33-1.44 (2H, m, CH\textsubscript{2} of cyclohexane), 1.50-1.74 (8H, m, CH\textsubscript{2} of cyclohexane), 2.54 (1H, dd, J\textsubscript{1,6} 17.4, 9.6, C(6)H), 2.64 (1H, dd, J\textsubscript{1,6} 17.0, 5.5, C(2)H\textsubscript{2}), 2.72 (1H, dd, J\textsubscript{1,6} 17.4, 4.5, C(6)H), 2.75 (1H, dd, J\textsubscript{1,6} 17.0, 5.1, C(2)H\textsubscript{2}), 3.34 (1H, ddd, J\textsubscript{1,6} 9.6, 6.7, 4.5, C(5)H), 4.49 (1H, t, J\textsubscript{1,6} 6.7, C(4)H), 4.57 (1H, ~dt, J\textsubscript{1,6} 6.7, 5.1, C(3)H\textsubscript{2}), 7.12 (2H, d, J\textsubscript{1,6} 8.4, Ar-CH\textsubscript{2}), 7.47 (2H, d, J\textsubscript{1,6} 8.4, Ar-CH\textsubscript{2}); δ\textsubscript{C} (100
MHz; CDCl₃) some signals coincident 23.7, 24.1, 25.2, 34.0, 37.2 (5 × CH₂ of cyclohexane), 41.1 (C(2)H₂), 42.6 (C(5)H and C(6)H₂), 72.2 (C(3)H), 77.5 (C(4)H), 109.8 (ketal C), 121.1 (C-Br), 129.3, 132.0 (Ar-CH), 139.4 (Ar-C), 208.3 (C=O); m/z (+ES) 389 ([M⁺Br]+Na⁺, 50%), 387 ([M⁺99Br]+Na⁺, 50%), 367 ([M⁺81Br]+H⁺, 50%), 365 ([M⁺99Br]+H⁺, 50%), 160 (100%); (Found 387.0566, C₁₈H₂₁⁹⁹BrNaO₃ ([M+Na⁺]) requires 387.0572).

5.2.7 3-O, 4-O-cyclohexylidene-5-naphthyl-(3R,4S,5S)-dihydroxy-21b-cyclohexanone (121b)

C₂₂H₂₄O₃; Mₚ=336.17 g/mol

To a solution of 89 (273 mg, 1.31 mmol) in dioxane and H₂O (10:1, 2.8 mL) were added 2-naphthylboronic acid (670 mg, 3.93 mmol), triethylamine (130 µL, 1.31 mmol) and [RhCl(cod)]₂ (33 mg, 5 mol%). The reaction mixture was stirred at r.t. for 18 h under an atmosphere of nitrogen. The solvent was evaporated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (13:1) gave the title compound (121b) as a thick yellow oil (383 mg, 87%). [α]D²⁸ = -76 (c 0.50 in CH₃OH); νmax (film)/cm⁻¹ 3051w (ArC-H), 3014w (ArC-H), 2941m (C-H), 2925m (C-H), 2901w (C-H), 2859w (C-H), 1712s (C=O); δH (400 MHz; CDCl₃) 1.34-1.46 (2H, m, CH₂ of cyclohexane), 1.52-1.79 (8H, m, CH₂ of cyclohexane), 2.67 (1H, dd, J 17.2, 5.0, C(2)H), 2.75 (1H, dd, J 17.6, 8.0, C(6)H), 2.79 (1H, dd, J 17.2, 4.3, C(2)H), 2.85 (1H, dd, J 17.6, 5.0, C(6)H), 3.59 (1H, dt, J 8.0, 5.0, C(5)H), 4.62 (1H, ~dt, J 6.6, 5.0, C(3)H), 4.73 (1H, ~t, J 6.6, C(4)H), 7.40 (1H, dd, J 8.5, 1.8, Ar-CH), 7.49-7.49 (2H, m, Ar-CH), 7.61 (1H, d, J 1.8, Ar-CH), 7.78-7.81 (2H, m, Ar-CH), 7.84 (1H, d, J 8.5, Ar-CH); δC (100 MHz; CDCl₃) 23.7, 24.1, 25.3, 33.9, 37.1 (CH₂ of cyclohexane), 40.9 (C(2)H₂), 42.5 (C(5)H), 43.0 (C(6)H₂), 72.4 (C(3)H), 77.3 (C(4)H), 109.5 (ketal C), 125.9, 126.0, 126.1, 126.5, 127.7, 127.9, 128.7 (Ar-CH), 132.5, 133.5, 137.8 (Ar-C), 209.0 (C=O); m/z (+ES) 359 ([M+Na⁺], 100%); (Found 359.1623, C₂₂H₂₄NaO₃ ([M+Na⁺]) requires 359.1628).
5.2.8 (3aS,4S,7aR)-4-(4-methoxyphenyl)tetrahydrospiro[benzo[d][1,3]dioxole-2,1'-cyclo-hexan]-6(3aH)-one (121c)

C_{19}H_{24}O_4; \text{MW}=316.40 \text{ g/mol}

To a solution of 89 (410 mg, 1.97 mmol) in dioxane and H_2O (10:1, 4.1 mL) were added 4-methoxyphenylboronic acid (897 mg, 5.91 mmol), triethylamine (260 µL, 1.97 mmol), and [RhCl(cod)]_2 (49 mg, 5 mol%). The reaction mixture was stirred at r.t. for 6 h under an atmosphere of nitrogen. The solvents were evaporated in vacuo and the residue was dissolved in DCM (10 mL). The resulting solution was washed with H_2O (10 mL) and brine (10 mL), dried over MgSO_4 and concentrated in vacuo to give the crude product as an orange oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (13:1) gave the title compound (121c) as a pale yellow solid (579.6 mg, 93%). M.p. 113-115 °C [Lit. 109.8-111.1 °C]; [α]_D^{22} –68.2 (c 0.50 in CH_2Cl_2) [Lit. 109.8-111.1 °C]; ν_{max} (film)/cm^{-1} 2935m (C-H), 2917m (C-H), 2895w (C-H), 2859w (C-H), 1712s (C=O), 1098s (C-O-C); δ_H (400 MHz; CDCl_3) 1.31-1.47 (2H, m, C_H_2 of cyclohexane), 1.50-1.75 (8H, m, C_H_2 of cyclohexane), 2.58 (1H, dd, J 17.7, 8.4, C(6)H), 2.61 (1H, dd, J 16.9, 4.7, C(2)H), 2.71 (1H, dd, J 16.9, 4.1, C(2)H), 2.73 (1H, dd, J 17.7, 4.8, C(6)H), 3.39 (1H, td, J 8.4, 4.8, C(5)H), 3.79 (3H, s, OCH_3), 4.53-4.59 (2H, m, C(3)H and C(4)H), 6.87 (2H, d, J 8.8, Ar-CH), 7.15 (2H, d, J 8.8, Ar-CH); δ_C (100 MHz; CDCl_3) some signals coincident 23.7, 24.1, 25.3, 33.9, 37.1 (CH_2 of cyclohexane), 41.0 (C(2)H), 42.0 (C(5)H), 42.5 (C(6)H), 55.4 (OCH_3), 72.3 (C(3)H), 77.6 (C(4)H), 109.4 (ketal C), 114.3, 128.5, 128.9 (Ar-CH), 132.2, 158.6 (Ar-C), 209.2 (C=O); m/z (+ES) 339 ([M+Na]^+), 50 %), 102 (100%); (Found 339.1572, C_{19}H_{24}O_4Na ([M+Na]^+) requires 339.1584).
5.2.9 (4R, 5S)-4-hydroxy-5-bromophenylcyclohex-2-enone (122a)

\[\text{C}_{12}\text{H}_{11}\text{O}_2\text{Br}; \text{M}_W=267.12 \text{ g/mol}\]

To a solution of compound 121a (129.8 mg, 0.37 mmol) in THF (1.5 mL) was added NaOH (0.5 M, 5 drops). The reaction was stirred at r.t. for 40 min under an atmosphere of nitrogen and quenched with NH₄Cl (sat., aq., 2 mL). The product was extracted with diethyl ether (3 × 3 mL). The combined organic extracts were washed with brine (3 mL), dried over MgSO₄ and concentrated in vacuo to give the crude product as a thick brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (2:1) gave the title compound (122a) as an off-white wax (86 mg, 87%). [\(\alpha\)]D \(^29\) −105.1 (c 0.50 in CH₃OH); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3388br (O-H), 2956w (C-H), 2904w (C-H), 2880w (C-H), 2846w (C-H), 1665s (C=O); \(\delta\)H (400 MHz; CDCl₃) 2.00 (1H, d, J 4.3, O-H), 2.65-2.67 (2H, m, C(6)H₃), 3.22 (1H, q, J 9.7, C(5)H), 4.64 (1H, ddt, J 9.7, 4.3, 2.0, C(4)H), 6.06 (1H, dd, J 10.2, 2.0, C(2)H), 6.98 (1H, dd, J 10.2, 2.0, C(3)H), 7.19 (2H, d, J 8.4, Ar-CH), 7.52 (2H, d, J 8.4, Ar-CH); \(\delta\)C (100 MHz; CDCl₃) some signals coincident 43.0 (C(6)H₂), 50.2 (C(5)H), 71.8 (C(4)H), 121.9 (C-Br), 129.2 (C(2)H), 129.6, 132.4 (Ar-CH), 138.7 (Ar-C), 152.1 (C(3)H), 197.7 (C=O); m/z (+ES) 291 ([M\(^{+81}\text{Br}+\text{Na}]^+, 100\%\)), 289 ([M\(^{+79}\text{Br}+\text{Na}]^+, 100\%\)); (Found 288.9840, C\(_{12}\)H\(_{11}\)\(^{79}\text{Br}\)NaO\(_2\) ([M+Na]⁺) requires 288.9843).

5.2.10 (4R, 5S)-4-hydroxy-5-naphthylcyclohex-2-enone (122b)

\[\text{C}_{16}\text{H}_{14}\text{O}_2; \text{M}_W=238.10 \text{ g/mol}\]

To a solution of compound 121b (253 mg, 0.73 mmol) in THF (3 mL) was added NaOH (0.5 M, 3 drops). The reaction was stirred at r.t. for 40 min under an atmosphere of nitrogen and quenched with NH₄Cl (sat., aq., 3 mL). H₂O (1 mL) was added to dilute the aqueous layer. Two layers were separated and the product was extracted with diethyl ether.
(3 × 6 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (4:1) gave the title compound (122b) as an off-white solid (131 mg, 75%). Mp 103-105 °C; [α]D²⁹ -150.9 (c 0.50 in CH₃OH); νₚₚₚ (film)/cm⁻¹ 3393br (O-H), 3051w (Ar-C-H), 2962w (C-H), 2931w (C-H), 2901w (C-H), 2874w (C-H), 1667s (C=O); δ(CDCl₃) 2.75 (1H, ddd, J 16.7, 4.8, 1.1, C(6)H₃α), 2.82 (1H, dd, J 16.7, 13.2, C(6)H₃α), 3.42 (1H, ddd, J 13.2, 9.8, 4.8, C(5)H), 4.79 (1H, dt, J 9.8, 2.2, C(4)H), 6.10 (1H, dt, J 10.3, 2.2, 1.1, C(2)H), 7.03 (1H, dd, J 10.3, 2.2, C(3)H), 7.43 (1H, dd, J 8.5, 1.7, Ar-CH), 7.49-7.52 (2H, m, Ar-CH), 7.76 (1H, d, J 1.7, Ar-CH), 7.82-7.86 (2H, m, Ar-CH), 7.89 (1H, d, J 8.5, Ar-CH); δ(CDCl₃) some signals coincident 43.2 (C(6)H₂), 51.1 (C(5)H), 71.9 (C(4)H), 125.2, 126.4, 126.8, 127.2, 127.9 (Ar-CH), 129.2 (C(2)H), 129.3 (Ar-CH), 133.1, 133.6, 136.9 (Ar-C), 152.2 (C(3)H), 198.0 (C=O); m/z (+ES) 261 ([M+Na]⁺, 100%); (Found 261.0891, C₁₆H₁₄NaO₂ ([M+Na]⁺) requires 261.0885).

5.2.11 (1S,6R)-6-hydroxy-4'-methoxy-1,6-dihydro-[1,1'-biphenyl]-3(2H)-one (122c)

C₁₃H₁₄O₃; Mₚ=218.09 g/mol

To a solution of compound 121c (185.5 mg, 0.6 mmol) in THF (2 mL) was added NaOH (0.5 M, 10 drops). The reaction was stirred at r.t. for 6.5 h under an atmosphere of nitrogen and quenched with NH₄Cl (sat., aq., 3 mL). The two layers were separated and the product was extracted with diethyl ether (3 × 3 mL) and DCM (3 × 3 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (2:1) gave the title compound (122c) as a pale yellow wax (87.6 mg, 67%). [α]D²⁷ -128.1 (c 0.5 in CH₂Cl₂) [Lit.⁶⁹ [α]D²⁷ -134 (c 0.5 in CH₂Cl₂)]; νₚₚₚ (film)/cm⁻¹ 3405br (O-H), 2956w (C-H), 2934w (C-H), 2901 (C-H), 2831w (C-H), 1669s (C=O); δ(CDCl₃) 1.97 (1H, s, br, OH), 2.65-2.67 (2H, m, C(6)H₃), 3.20 (1H, td, J 10.0, 7.1, C(5)H), 3.82 (3H, s, OCH₃), 4.61 (1H, dt, J 10.0, 2.2, C(4)H), 6.07 (1H, ddd, J 10.2, 2.2, 0.7, C(2)H), 6.93 (2H, d, J 8.6, Ar-CH), 7.00 (1H, dd, J 10.2, 2.2, C(3)H), 7.22 (2H, d, J 8.6, Ar-CH); δ(CDCl₃) some signals coincident 43.5 (C(6)H₂),
V. Experimental

50.1 (C(5)H), 55.5 (OCH₃), 72.3 (C(4)H), 114.7, 128.9 (Ar-CH), 129.1 (C(2)H), 131.5 (Ar-C), 152.2 (C(3)H), 159.4 (Ar-C), 198.3 (C=O); m/z (+ES) 219 ([M+H]+, 100 %), (Found 219.1005, C₁₃H₁₅O₃ ([M+H]+) requires 219.0995).

5.2.12 (4R, 5S)-4-((triethylsilyl)oxy)-5-[4-bromophenyl]-cyclohex-2-enone (158a)

C₁₈H₂₅BrO₂Si; Mᵢₙ=381.39 g/mol

2,6-Lutidine (250 µL, 2.13 mmol) and TESOTf (0.5 mL, 1.78 mmol) in DCM (2 mL) were mixed at −78 °C for 5 min under an atmosphere of nitrogen. The premixed reagents were added dropwise at −78 °C under an atmosphere of nitrogen to a solution of 122a (189.2 mg, 0.71 mmol) in DCM (4 mL). The reaction was stirred for 30 min and quenched with NH₄Cl (sat., aq., 4 mL). H₂O (4 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with DCM (3 × 8 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (10:1) gave the title compound (158a) as a light yellow oil (208.5 mg, 77%). [α]D₂⁹ −91.7 (c 0.50 in CH₃OH); νmax (film)/cm⁻¹ 3040w (ArC-H), 2953m (C-H), 2910w (C-H), 2873m (C-H), 1685s (C=O); δH (500 MHz; CDCl₃) 0.27-0.44 (6H, m, Si(CH₂CH₃)₃), 0.78 (9H, t, J 8.0, Si(CH₂C₂H₅)₃), 2.63 (1H, ddd, J 16.4, 4.8, 1.1, C(6)H_eq), 2.69 (1H, dd, J 16.4, 13.3, C(6)H_ax), 3.22 (1H, ddd, J 13.3, 9.3, 4.8, C(5)H), 4.51 (1H, dt, J 9.3, 2.0, C(4)H), 6.02 (1H, ddd, J 10.2, 2.0, 1.1, C(2)H), 6.82 (1H, dd, J 10.2, 2.0, C(3)H), 7.14 (2H, d, J 8.4, Ar-CH); 7.47 (2H, d, J 8.4, Ar-CH); δC (100 MHz; CDCl₃) some signals coincident 45.5 (Si(CH₂CH₃)₃), 6.7 (Si(CH₂C₂H₅)₃), 42.7 (C(6)H₂), 50.2 (C(5)H), 72.7 (C(4)H), 121.1 (C-Br), 128.6 (C(2)H), 129.9, 131.7 (Ar-CH), 139.9 (Ar-C), 153.7 (C(3)H), 198.1 (C=O); m/z (+ES) 405 ([M⁺(⁷⁹Br)+Na]⁺, 100%), 403 ([M⁺(⁷⁷Br)+Na]⁺, 100%); (Found, 403.0705, C₁₈H₂₅BrNaO₂Si ([M+Na]⁺) requires 403.0706).
5.2.13 (4R,5S)-5-(naphthalen-2-yl)-4-(triethylsilyloxy)cyclohex-2-en-1-one (158b)

C_{22}H_{28}O_{2}Si; M_{w}=352.55 g/mol
2,6-Lutidine (160 μL, 1.49 mmol) and TESOTf (290 μL, 1.09 mmol) in DCM (4 mL) were mixed at −78 °C for 5 min under an atmosphere of nitrogen. The premixed reagents were added dropwise at −78 °C under an atmosphere of nitrogen to a solution of 122b (118 mg, 0.50 mmol) in DCM (4 mL). The reaction was stirred for 20 min and quenched with NH_{4}Cl (sat., aq., 8 mL). H_{2}O (4 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with DCM (3 × 8 mL). The combined organic extracts were dried over MgSO_{4} and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica chromatography, eluting with petroleum ether (40-60) and ethyl acetate (12:1) gave the title compound (158b) as a light yellow oil (132 mg, 81%). [α]_{D}^{29} = −82.4 (c 0.50 in CH_{3}OH); v_{max} (film)/cm\(^{-1}\) 3056w (ArC-H), 2957m (C-H), 2911w (C-H), 2876m (C-H), 1684s (C=O); δ_{H} (400 MHz; CDCl_{3}) 0.27-0.36 (6H, m, Si(CH_{2}CH_{3})_{3}), 0.69 (9H, t, J 8.0, Si(CH_{2}CH_{3})_{3}), 2.74 (1H, ddd, J 16.5, 4.2, 1.3, C(6)H_{eq}), 2.86 (1H, dd, J 16.5, 13.5, C(6)H_{ax}), 3.44 (1H, ddd, J 13.5, 9.3, 4.2, C(5)H), 4.70 (1H, dt, J 9.3, 2.0, C(4)H), 6.06 (1H, ddd, J 10.3, 2.0, 1.3, C(2)H), 6.88 (1H, dd, J 10.3, 2.0, C(3)H), 7.40 (1H, dd, J 8.4, 1.7, Ar-CH), 7.46-7.49 (2H, m, Ar-CH), 7.70 (1H, d, J 1.7, Ar-CH), 7.78-7.81 (2H, m, Ar-CH), 7.83 (1H, d, J 8.4, Ar-CH); δ_{C} (100 MHz; CDCl_{3}) some signals coincident 4.6 (Si(CH_{2}CH_{3})_{3}), 6.7 (Si(CH_{2}CH_{3})_{3}), 43.0 (C(6)H_{2}), 50.9 (C(5)H), 72.8 (C(4)H), 125.9, 126.3, 127.2, 127.76, 127.79, 128.3 (Ar-CH), 128.6 (C(2)H), 132.9, 133.5, 138.2 (Ar-C), 153.9 (C(3)H), 198.5 (C=O); m/z (+ES) 375 ([M+Na]^+), 100%; (Found 375.1756, C_{22}H_{28}NaO_{2}Si ([M+Na]^+) requires 375.1754).
5.2.14 (1S,6R)-4’-methoxy-6-((triethylsilyl)oxy)-1,6-dihydro-[1,1’-biphenyl]-3(2H)-one (158c)

C_{19}H_{28}O_3Si; M_W=332.18 g/mol

2,6-Lutidine (270 µL, 2.48 mmol) and TESOTf (480 µL, 1.82 mmol) in DCM (3 mL) were mixed at −78 °C for 5 min under an atmosphere of nitrogen. The premixed reagents were added dropwise at −78 °C under an atmosphere of nitrogen to a solution of 122c (179.7 mg, 0.83 mmol) in DCM (3 mL). The reaction was stirred for 30 min and quenched with NH₄Cl (sat., aq., 6 mL). H₂O (4 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with DCM (3 × 10 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo to give the crude product as a yellow oil.

Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (11:1) gave the title compound (158c) as a light yellow oil (215.1 mg, 78%). [α]_D^{26} –132.8 (c 0.50 in CH₂Cl₂) [Lit. [69] [α]_D^{27} –149.6 (c 0.5 in CH₂Cl₂)]. ν_max (film)/cm⁻¹ 3035w (ArC-H), 2953m (C-H), 2901w (C-H), 2877m (C-H), 2834w (C-H), 1683s (C=O); δ_H (400 MHz; CDCl₃) 0.24-0.43 (6H, m, Si(CH₂CH₃)₃), 0.78 (9H, t, J 7.9, Si(CH₂CH₃)₃), 2.63 (1H, ddd, J 16.5, 4.8, 1.2, C(6)H_eq), 2.70 (1H, dd, J 16.5, 13.0, C(6)H_ax), 3.20 (1H, ddd, J 13.0, 9.3, 4.8, C(5)H), 3.80 (3H, s, OCH₃), 4.49 (1H, dt, J 9.3, 2.0, C(4)H), 6.02 (1H, ddd, J 10.2, 2.0, 1.2, C(2)H), 6.83 (1H, dd, J 10.2, 2.0, C(3)H), 6.86 (2H, d, J 8.7, Ar-CH), 7.16 (2H, d, J 8.7, Ar-CH); δ_C (100 MHz; CDCl₃) some signals coincident 4.6 (Si(CH₂CH₃)₃), 6.8 (Si(CH₂CH₃)₃), 43.2 (C(6)H₂), 50.0 (C(5)H), 55.5 (OCH₃), 73.1 (C(4)H), 114.0, 128.6 (Ar-CH), 129.1 (C(2)H), 133.1 (Ar-C), 154.0 (C(3)H), 159.0 (Ar-C), 198.8 (C=O); m/z (+ES) 333 ([M+H]^+), 108 (100%); (Found 333.1884, C_{19}H_{20}O_3Si ([M+H]^+) requires 333.1883).
5.2.15 (4R,5S)-4-((triethylsilyl)oxy)-2-(hydroxymethyl)-5-[4-bromophenyl]-cyclohex-2-enone (159a)

C_{19}H_{27}BrO_3Si; M_W=411.41 g/mol

To a suspension of 158a (195.4 mg, 0.51 mmol) in H_2O (2 mL) were added DMAP (62.3 mg, 0.51 mmol) and SDS (4.6 mg, 0.16 mmol). The solution was stirred until it became cloudy. Formaldehyde (aqueous, 37%, 220 µL, 7.18 mmol) was added and the resulting suspension was stirred at r.t. for 24 h. The reaction was quenched with brine (4 mL). The product was extracted with ethyl acetate (2 × 6 mL) and DCM (2 × 6 mL). The combined organic extracts were washed with brine, dried over MgSO_4, and concentrated in vacuo to give the crude product as a light orange oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (4:1) gave the title compound (159a) as a colorless oil (122.5 mg, 58%). [α]_D^{30} = −74.67 (c 0.50 in CH_3OH);

ν_{max} \text{ (film)/cm}^{-1}: 3341 \text{br (O-H)}, 2959 \text{m (C-H)}, 2907 \text{m (C-H)}, 2871 \text{m (C-H)}, 1675 \text{s (C=O)}, 1096 \text{s (Si-O)};

δ_{H} \text{ (400 MHz; CDCl}_3\text{)}: 0.29-0.44 (6H, m, Si(CH_2CH_3)_3), 0.78 (9H, t, J 8.0, Si(CH_2CH_3)_3), 2.68 (1H, dd, J 16.8, 5.0, C(6)H_3), 2.76 (1H, dd, J 16.8, 12.9, C(6)H_3), 3.23 (1H, ddd, J 12.9, 9.3, 5.0, C(5)H), 4.26 (1H, dd, J 13.7, 6.3, CH_3OH), 4.37 (1H, dd, J 13.7, 6.3, CH_3OH), 4.54 (1H, dd, J 9.3, 1.7, C(4)H), 6.77 (1H, d, J 1.7, C(3)H), 7.13 (2H, d, J 8.4, Ar-CH), 7.47 (2H, d, J 8.4, Ar-CH); δ_{C} \text{ (100 MHz; CDCl}_3\text{)}: some signals coincident 4.6 (Si(CH_2CH_3)_3), 6.8 (Si(CH_2CH_3)_3), 42.9 (C(6)H_2), 50.4 (C(5)H), 61.4 (C(7)H_2), 72.7 (C(4)H), 121.1 (C-Br), 129.9, 131.7 (Ar-CH), 137.2 (C(2)), 139.6 (Ar-C), 149.4 (C(3)H), 198.7 (C=O); m/z (+ES) 435 ([M^{81}Br]+Na^+), 100%, 433 ([M^{79}Br]+Na^+), 100%; (Found 433.0825, C_{19}H_{27}^{79}BrNaO_3Si ([M+Na]^+) requires 433.0811).
5.2.16 (4R,5S)-2-(hydroxymethyl)-5-(naphthalen-2-yl)-4-(triethylsilyloxy)cyclohex-2-en-1-one (159b)

C_{23}\text{H}_{30}\text{O}_3\text{Si}; M_W=382.57 \text{ g/mol}

To a suspension of 158b (234.3 mg, 0.66 mmol) in H_2O (2 mL) were added DMAP (80.6 mg, 0.66 mmol) and SDS (5.8 mg, 0.20 mmol). The solution was stirred until it became cloudy. Formaldehyde (aqueous, 37%, 280 µL, 9.11 mmol) was added and the resulting suspension was stirred at r.t. for 24 h. The reaction was quenched with brine (4 mL). The product was extracted with ethyl acetate (2 × 6 mL) and DCM (2 × 6 mL). The combined organic extracts were washed with brine, dried over MgSO_4 and concentrated in vacuo to give the crude product as a light orange oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (159b) as a colourless oil (162.8 mg, 64%). [α]_D^{28} = -88.8 (c 0.50 in CH_3OH);

ν_{max} (film)/cm^{-1} 3416br (O-H), 2949m (C-H), 2914w (C-H), 2876m (C-H), 1653s (C=O);

δ_H (400 MHz; CDCl_3) 0.25-0.37 (6H, m, Si(CH_2CH_3)_3), 0.69 (9H, t, J 7.9, Si(CH_2CH_3)_3), 2.77 (1H, dd, J 16.6, 4.2, C(6)H_{eq}), 2.90 (1H, dd, J 16.6, 13.6, C(6)H_{ax}), 3.42 (1H, ddd, J 13.6, 9.4, 4.2, C(5)H), 4.30 (1H, dt, J 13.4, 1.4, C(7)H_{ax}), 4.42 (1H, d, J 13.4, 1.4, C(7)H_{eq}), 4.73 (1H, dd, J 9.4, 1.6, C(4)H), 6.81 (1H, d, J 1.6, C(3)H), 7.39 (1H, dd, J 8.5, 1.7, Ar-CH), 7.45-7.50 (2H, m, Ar-CH), 7.69 (1H, d, J 1.7, Ar-CH), 7.78-7.82 (2H, m, Ar-CH), 7.83 (1H, d, J 8.5, Ar-CH); δ_C (100 MHz; CDCl_3) 4.6 (Si(CH_2CH_3)_3), 6.7 (Si(CH_2CH_3)_3), 43.2 (C(6)H_2), 51.0 (C(5)H), 61.5 (C(7)H_2), 72.7 (C(4)H), 125.8, 125.9, 126.4, 127.3, 127.77, 127.79, 128.3 (Ar-CH), 132.9, 133.5 (Ar-C), 137.0 (C(2)), 137.9 (Ar-C), 149.9 (C(3)H), 199.4 (C=O); m/z (+ES) 405 ([M+Na]^+, 100%); (Found 405.1850, C_{23}\text{H}_{30}\text{NaO}_3\text{Si} ([M+Na]^+) requires 405.1852).
5.2.17 (1S,6R)-4-(hydroxymethyl)-4'-methoxy-6-((triethylsilyl)oxy)-1,6-dihydro- [1,1'-biphenyl]-3(2H)-one (159c)

C₂₀H₃₀O₄Si; M₆=362.19 g/mol

To a suspension of 158c (100.2 mg, 0.30 mmol) in H₂O (1 mL) were added DMAP (36.7 mg, 0.30 mmol) and SDS (2.6 mg, 0.09 mmol). The solution was stirred until it became cloudy. Formaldehyde (aqueous, 37%, 130 µL, 4.15 mmol) was added and the resulting suspension was stirred at r.t. for 24 h. The reaction was quenched with brine (2 mL). The product was extracted with ethyl acetate (2 × 3 mL) and DCM (2 × 3 mL). The combined organic extracts were washed with brine, dried over MgSO₄, and concentrated in vacuo to give the crude product as a light orange oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (4:1) gave the title compound (159c) as a colourless oil (81.9 mg, 75%). [α]²⁹D –108.7 (c 0.50 in CH₂Cl₂) [Lit.⁶⁹ [α]²⁶D –91.2 (c 0.5 in CH₂Cl₂)]; νmax (film)/cm⁻¹ 3444br (O-H), 2956m (C-H), 2910w (C-H), 2877m (C-H), 1673s (C=O), 1084s (Si-O); δH (400 MHz; CDCl₃) 0.29-0.42 (6H, m, Si(CH₂CH₃)₃), 0.77 (9H, t, J 7.9, Si(CH₂CH₃)₃), 2.66 (1H, dd, J 16.6, 4.7, C(6)H₆eq), 2.74 (1H, dd, J 16.6, 13.4, C(6)H₆ax), 3.19 (1H, ddd, J 13.4, 9.3, 4.7, C(5)H), 3.81 (3H, s, OC₃H₃), 4.24 (1H, dt, J 13.6, 1.4, C(7)H₃H₆), 4.37 (1H, dt, J 13.6, 1.4, C(7)H₃H₆), 4.53 (1H, dd, J 9.3, 1.7, C(4)H), 6.75 (1H, q, J 1.7, C(3)H), 6.87 (2H, d, J 8.7, Ar-CH), 7.16 (2H, d, J 8.7, Ar-CH); δc (100 MHz; CDCl₃) some signals coincident 4.6 (Si(CH₂CH₃)₃), 6.8 (Si(CH₂CH₃)₃), 43.4 (C(6)H₂), 50.1 (C(5)H), 55.5 (OCH₃), 61.5 (C(7)H₂), 73.0 (C(4)H), 114.0, 129.1 (Ar-CH), 132.7 (Ar-C), 137.0 (C(2)), 149.9 (C(3)H), 159.0 (Ar-C), 199.6 (C=O); m/z (+ES) 363 ([M+H]⁺, 100 %); (Found 363.1989, C₂₀H₃₁O₄Si ([M+H]⁺) requires 363.1993).
5.2.18 (4R,5S)-4-((triethylsilyl)oxy)-2-((E)-crotonyloxymethyl)-5-[4-bromophenyl] -cyclohex-2-enone (167a)

5.2.19 (4R,5S)-4-hydroxy-2-((E)-crotonyloxymethyl)-5-[4-bromophenyl]-cyclohex-2-en-one (166a)

167a: C_{23}H_{31}BrO_{3}Si; M_W = 479.49 g/mol
166a: C_{17}H_{17}BrO_{4}; M_W = 365.22 g/mol

To a solution of 159a (135.4 mg, 0.33 mmol) in DCM (2.5 mL) were added crotonic anhydride (116 µL, 0.72 mmol), DMAP (0.4 mg, 33 µmol) and pyridine (240 µL, 2.90 mmol). The reaction was stirred at r.t. for 1.5 h under an atmosphere of nitrogen and quenched with NaHCO₃ (sat., aq., 5 mL). H₂O (3 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with DCM (3 × 10 mL). The combined organic extracts were washed with NaHCO₃ (sat., aq., 10 mL), dried over MgSO₄ and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40:60) and ethyl acetate (18:1) gave the compound 167a with minor impurity.

A solution of partially purified compound 167a (89.7 mg, 0.19 mmol) in TFA and H₂O (7:1, 1.4 mL) was stirred at r.t. for 1 h. The solvents were evaporated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40:60) and ethyl acetate (7:2) gave the title compound (166a) as an off-white wax (47 mg, 39% over two steps). [α]D^{20} -44.0 (c 0.525 in CH₃OH); ν_{max} (film)/cm⁻¹ 3432br (O-H), 2975w (C-H), 2932w (C-H), 2917w (C-H), 2877w (C-H), 1717s (C=O, ester), 1677s (C=O, enone); δH (400 MHz; CDCl₃) 1.90 (3H, dd J 6.9, 1.7, C(11)H₃), 2.69-2.71 (2H, m, C(6)H₂), 3.24 (1H, td, J 9.9, 7.7, C(5)H), 4.67 (1H, dd, J 9.9, 1.8, C(4)H), 4.85 (1H, dt, J 14.4, 1.7, C(7)H₂H₃b), 4.90 (1H, dt, J 14.4, 1.7, C(7)H₃H₃b), 5.89 (1H, dq, J 15.5, 1.7, C(9)H), 6.92 (1H, d, J 1.8, C(3)H), 7.05 (1H, dt, J 15.5, 6.9, C(10)H), 7.18 (2H, d, J 8.4, Ar-CH), 7.52 (2H, d, J 8.4, Ar-CH); δC (100 MHz; CDCl₃) some signals coincident 18.3 (C(11)H₃), 43.1 (C(6)H₂), 50.1 (C(5)H), 60.2 (C(7)H₂), 71.9 (C(4)H), 122.0 (C-Br), 122.2 (C(9)H), 129.5, 132.4 (Ar-CH), 134.4, 138.4 (C(2) and Ar-C), 146.0 (C(10)H), 148.1 (C(3)H), 166.0 (C(8)=O), 196.0 (C(1)=O); m/z (+ES) 389
([M\(^{81}\text{Br} \text{+ Na}^+]\), 100%), 387 ([M\(^{79}\text{Br} \text{+ Na}^+]\), 100%); (Found 387.0208, C\(_{17}\)H\(_{17}\)BrNaO\(_4\) ([M+Na\(^+\)]) requires 387.0206).

5.2.20 (4\(R\), 5\(S\))- 4-((triethylsilyl)oxy)-2-((E)-crotonyloxymethyl)-5-[4-naphthyl]-cyclohex-2-enone (167b)

5.2.21 (4\(R\), 5\(S\))-4-hydroxy-2-((E)-crotonyloxymethyl)-5-[4-naphthyl]-cyclohex-2-enone (166b)

167b: C\(_{27}\)H\(_{34}\)O\(_4\)Si; M\(_\text{W}\)=450.65 g/mol
166b: C\(_{21}\)H\(_{20}\)O\(_4\); M\(_\text{W}\)=336.39 g/mol

To a solution of 159b (88.1 mg, 0.23 mmol) in DCM (1.8 mL) were added crotonic anhydride (80 \(\mu\)L, 0.51 mmol), DMAP (0.3 mg, 23 \(\mu\)mol) and pyridine (160 \(\mu\)L, 2.02 mmol). The reaction was stirred at r.t. for 3 h under an atmosphere of nitrogen and quenched with NaHCO\(_3\) (sat., aq., 4 mL). H\(_2\)O (2 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with DCM (3 \(\times\) 6 mL). The combined organic extracts were washed with NaHCO\(_3\) (sat., aq., 6 mL), dried over MgSO\(_4\) and concentrated \textit{in vacuo} to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (19:1) gave the compound 167b with minor impurity.

A solution of partially purified compound 167b (59.7 mg, 0.13 mmol) in TFA and H\(_2\)O (7:1, 1.2 mL) was stirred at r.t. for 1 h. The solvents were evaporated \textit{in vacuo} to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (7:2) gave the title compound (166b) as an off-white wax (37.1 mg, 48% over two steps). [\(\alpha\)]\(_D\)\(^{29}\) −90.7 (c 0.5 in CH\(_3\)OH); \(\nu\)\(_\text{max}\) (film)/cm\(^{-1}\) 3426br (O-H), 3054w (C-H), 3020w (C-H), 2956w (C-H), 2917w (C-H), 1718s (C=O, ester), 1674s (C=O, enone); \(\delta\)\(_H\) (400 MHz; CDCl\(_3\)) 1.91 (3H, dd, J 6.9, 1.7, C(11)H\(_3\)), 2.81 (1H, dd, J 16.6, 4.9, C(6)H\(_{eq}\)), 2.86 (1H, dd, J 16.6, 13.2, C(6)H\(_{ax}\)), 3.43 (1H, ddd, J 13.2, 9.9, 4.9, C(5)H), 4.84 (1H, dd, J 9.9, 1.8, C(4)H), 4.88 (1H, dt, J 14.3, 1.5, C(7)H\(_a\)H\(_b\)), 4.94 (1H, dt, J 14.3, 1.5, C(7)H\(_a\)H\(_b\)), 5.91 (1H, dq, J 15.5, 1.7, C(9)H), 6.99 (1H, q, J 1.8, C(3)H), 7.06 (1H, dt, J 15.5, 6.9, C(10)H), 7.42 (1H, dd, J 8.5, 1.7, Ar-
CH), 7.50-7.53 (2H, m, Ar-CH), 7.75 (1H, d, J 1.7, Ar-CH), 7.85-7.89 (2H, m, Ar-CH), 7.90 (1H, d, J 8.5, Ar-CH); δC (100 MHz; CDCl3) some signals coincident 18.2 (C(11)H3), 43.4 (C(6)H2), 51.0 (C(5)H), 60.3 (C(7)H2), 72.0 (C(4)H), 122.3 (C(9)H), 125.1, 126.5, 126.8, 127.2, 127.9, 129.3 (Ar-CH), 133.1, 133.6, 134.4, 136.6 (C), 145.8 (C(10)H), 148.2 (C(3)H), 166.1 (C(8)=O), 196.3 (C(1)=O); m/z (+ES) 359 ([M+Na]+, 100%); (Found 359.1259, C21H20NaO4 ([M+Na]+) requires 359.1259).

5.2.22 ((3R,4S)-4-(naphtalen-2-yl)-6-oxo-3-((triethylsilyl)oxy)cyclohex-1-en-1-yl) meth-yl (E)-but-2-enoate (167c)

5.2.23 ((3R,4S)-3-hydroxy-4-(naphtalen-2-yl)-6-oxocyclohex-1-en-1-yl)methyl (E) -but-2-enoate (166c)

167c: C24H34O5Si; M_W=430.22 g/mol
166c: C18H20O5; M_W=316.13 g/mol

To a solution of 159c (141.9 mg, 0.39 mmol) in DCM (2.9 mL) were added crotonic anhydride (130 µL, 0.88 mmol), DMAP (0.5 mg, 39 µmol) and pyridine (280 µL, 3.46 mmol). The reaction was stirred at r.t. for 3.5 h under an atmosphere of nitrogen and quenched with NaHCO3 (sat., aq., 6 mL). H2O (2 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with DCM (3 × 8 mL). The combined organic extracts were washed with NaHCO3 (sat., aq., 8 mL), dried over MgSO4 and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (19:1) gave the compound 167c with minor impurity.

A solution of partially purified compound 167c (97.0 mg, 0.22 mmol) in TFA and H2O (7:1, 1.9 mL) was stirred at r.t. for 1 h. The solvents were evaporated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (3:1) gave the title compound (166c) as pale yellow solid (36.9 mg, 30% over two steps). M.p. 99-101 °C; [α]_D^{27} −83.5 (c 0.33, CH2Cl2) [Lit.69 [α]_D^{27} −72.5 (c 0.5 in CH2Cl2)]; νmax (film)/cm⁻¹ 3320br (O-H), 2999w (C-H), 2916w (C-H), 2950w (C-H), 2856w (C-H), 1723s (C=O, ester), 1669s (C=O, enone); δH (400
V. Experimental

\[ \text{C}_{13} \text{H}_{13} \text{BrO}_3; M_W=297.15 \text{ g/mol} \]

A solution of 159a (96.4 mg, 0.23 mmol) in TFA and H\(_2\)O (7:1, 2.2 mL) was stirred at r.t. for 1 h. The solvents were evaporated \textit{in vacuo} to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (2:1 to 1:1) gave the title compound (154a) as a pale yellow oil (38 mg, 56%). [\( \alpha \)]\(_D\)\(^{28} \) $-86.4$ (c 0.50 in CH\(_3\)OH); \( \nu_{\text{max}} \) (film)/cm\(^{-1} \) 3368br (O=), 2953w (C-H), 2923w (C-H), 2901w (C-H), 2865w (C-H), 1624s (C=O); \( \delta_{\text{H}} \) (400 MHz; CDCl\(_3\)) 2.62-2.71 (2H, m, C(6)H\(_2\)), 3.20 (1H, ddd, \( J \) 12.9, 9.4, 4.9, C(5)H\(_2\)), 4.25 (1H, dd, \( J \) 13.8, 5.3, C(7)H\(_3\)H\(_b\)), 4.36 (1H, dd, \( J \) 13.8, 5.3, C(7)H\(_3\)H\(_b\)), 4.63 (1H, dd, \( J \) 9.4, 1.7, C(4)H\(_2\)), 6.91 (1H, s, C(3)H\(_2\)), 7.16 (2H, d, \( J \) 8.2, Ar-CH\(_2\)), 7.50 (2H, d, \( J \) 8.2, Ar-CH\(_2\)); \( \delta_{\text{C}} \) (100 MHz; CDCl\(_3\)) some signals coincident 43.3 (C(6)H\(_2\)), 50.1 (C(5)H\(_2\)), 60.6 (C(7)H\(_2\)), 71.7 (C(4)H\(_2\)), 121.8 (C-Br), 129.5, 132.3 (Ar-CH\(_3\)), 137.8 (C(2)), 138.6 (Ar-C), 148.1 (C(3)H\(_2\)), 198.3 (C=O); \( m/z \) (+ES) 321 ([M\(^{\text{61}}\)Br]+Na\(^+\), 100%), 319 ([M\(^{\text{79}}\)Br]+Na\(^+\), 100%); (Found 318.9946, C\(_{13}\)H\(_{13}\)\(^{79}\)BrNaO\(_3\) ([M+Na\(^+\)]) requires 318.9954).
5.2.25 (4R, 5S)-4-hydroxy-2-(hydroxymethyl)-5-(4-naphthyl)-cyclohex-2-enone (154b)

![Chemical Structure](image)

C\textsubscript{17}H\textsubscript{16}O\textsubscript{3}; M\textsubscript{W}=268.31 g/mol

A solution of 159b (87.9 mg, 0.23 mmol) in TFA and H\textsubscript{2}O (7:1, 2.2 mL) was stirred at r.t. for 1 h. The solvents were evaporated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (2:1) gave the title compound (154b) as a pale yellow oil (28.6 mg, 46%). 

\[ \alpha \]\textsubscript{D}\textsuperscript{28} −86.0 (c 0.20 in CH\textsubscript{3}OH); \nu\textsubscript{max} (film)/cm\textsuperscript{-1} 3368br (O-H), 3047w (C-H), 3014w (C-H), 2953w (C-H), 2919w (C-H), 2898w (C-H), 2864w (C-H), 1663s (C=O);

\[ \delta \]H (400 MHz; CDCl\textsubscript{3}) 2.79 (1H, dd, J 16.7, 4.7, C(6)H\textsubscript{eq}), 2.87 (1H, dd, J 16.7, 13.6, C(6)H\textsubscript{ax}), 3.42 (1H, ddd, J 13.6, 10.0, 4.7, C(5)H), 4.33 (1H, dt, J 13.7, 1.5, C(7)H\textsubscript{a}H\textsubscript{b}), 4.40 (1H, dt, J 13.7, 1.5, C(7)H\textsubscript{a}H\textsubscript{b}), 4.83 (1H, dd, J 10.0, 1.8, C(4)H), 6.99 (1H, t, J 1.8, C(3)H), 7.43 (1H, dd, J 8.5, 1.7, Ar-CH), 7.50-7.53 (2H, m, Ar-CH), 7.77 (1H, d, J 1.7, Ar-CH), 7.83-7.87 (2H, m, Ar-CH), 7.90 (1H, d, J 8.5, Ar-CH); \delta\textsubscript{C} (100 MHz; CDCl\textsubscript{3}) 43.4 (C(6)H\textsubscript{2}), 51.1 (C(5)H), 61.4 (C(7)H\textsubscript{2}), 71.9 (C(4)H), 125.1, 126.5, 126.8, 127.2, 127.87, 127.89, 129.3 (Ar-CH), 133.1, 133.6 (Ar-C), 136.5, 137.8 (C(2) and Ar-C), 147.9 (C(3)H), 198.7 (C=O); m/z (+ES) 291 ([M+Na]\textsuperscript{+}, 100%); (Found 291.0997, C\textsubscript{17}H\textsubscript{16}NaO\textsubscript{3} ([M+Na]\textsuperscript{+}) requires 291.1013).

5.2.26 (1S,6R)-6-hydroxy-4-(hydroxymethyl)-4'-methoxy-1,6-dihydro-[1,1'-biphenyl] -3 (2H)-one (154c)

![Chemical Structure](image)

C\textsubscript{14}H\textsubscript{16}O\textsubscript{4}; M\textsubscript{W}=248.10 g/mol

A solution of 159c (146.2 mg, 0.40 mmol) in TFA and H\textsubscript{2}O (7:1, 2.8 mL) was stirred at r.t. for 20 min. The reaction mixture was dried in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (3:1) gave the title compound (154c) as a pale yellow oil (65.4 mg, 66%). [\alpha]\textsubscript{D}\textsuperscript{28} −96.4 (c 0.50 in CH\textsubscript{2}Cl\textsubscript{2}); \nu\textsubscript{max} (film)/cm\textsuperscript{-1} 3405br (O-H), 2959w (C-H),
2917w (C-H), 2947w (C-H), 1668s (C=O); δH (400 MHz; CDCl3) 2.68-2.70 (2H, m, C(6)H2), 3.18 (1H, ddd, J 13.5, 9.3, 4.7, C(5)H), 3.81 (3H, s, OCH3), 4.29 (1H, d, J 13.7, C(7)H2H6), 4.36 (1H, d, J 13.7, C(7)H2H6), 4.64 (1H, dd, J 9.3, 1.6, C(4)H), 6.92 (2H, d, J 8.8, Ar-CH), 6.94 (1H, t, J 1.6, C(3)H), 7.21 (2H, d, J 8.8, Ar-CH); δC (100 MHz; CDCl3) some signals coincident 43.7 (C(6)H2), 50.1 (C(5)H), 55.5 (OCH3), 61.3 (C(7)H2), 72.2 (C(4)H), 114.7, 128.8 (Ar-CH), 131.3, 137.8 (C(2) and Ar-C), 148.0 (C(3)H), 159.4 (Ar-C), 199.0 (C=O); m/z (+ES) 271 ([M+Na]+, 100%); (Found 271.0936, C14H16NaO4 ([M+Na]+) requires 271.0941).

5.2.27 (3S,4S,6S)-3,6-bis(4-bromophenyl)-4-hydroxyoctahydrodibenzo[b,d]furan-1,8 (2H,5aH)-dione (123a)

C24H22Br2O4; Mw=534.24 g/mol

To a solution of 121a (200.5 mg, 0.75 mmol) in THF (2 mL) was added NaOH (0.5 M, 10 drops). The reaction was stirred at r.t. for 24 h under an atmosphere of nitrogen and quenched with NH4Cl (sat., aq., 2 mL). H2O (2 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with diethyl ether (3 × 4 mL) and DCM (3 × 4 mL). The combined organic extracts were washed with brine, dried over MgSO4 and concentrated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (2:1) gave the title compound (123a) as a white solid (90.5 mg, 45%). M.p. 257-258 °C; [α]D28 −104.3 (c 0.50 in CH2Cl2); νmax (film)/cm−1 3368br (O-H); 2962w (C-H); 2928w (C-H); 2898w (C-H), 2852w (C-H), 1710s (C=O); δH (400 MHz; CDCl3) 2.00 (1H, d, J 8.9, O-H), 2.50-2.54 (2H, m, C(12)H2), 2.56-2.58 (2H, m, C(8)H2), 2.62-2.64 (2H, m, C(12)H2), 2.80 (1H, dd, J 4.8, 1.7, C(6)H), 3.14 (1H, ddd, J 12.7, 9.4, 4.8, C(11)H), 3.23 (1H, td, J 10.3, 6.2, C(3)H), 3.56 (1H, dt, J 11.7, 7.3, 1.7, C(9)H), 4.26 (1H, td, J 10.3, 8.9, 3.2, C(4)H), 4.37 (1H, dd, J 9.4, 7.3, C(10)H), 4.70 (1H, dd, J 4.8, 3.2, C(5)H), 7.10 (4H, dd, J 8.4, 1.7, Ar-CH), 7.48 (4H, d, J 8.4, Ar-CH); δC (100 MHz; CDCl3) some signals coincident 36.6 (C(9)H), 40.2 (C(8)H2), 42.6 (C(12)H2), 42.8 (C(11)H), 44.3 (C(3)H), 45.3 (C(2)H2), 56.9 (C(6)H), 72.8 (C(4)H), 78.6 (C(5)H), 81.7 (C(10)H), 121.4, 121.5 (C-Br), 167
129.3, 132.1 (Ar-\(\text{CH}\)), 139.0, 140.2 (Ar-\(\mathcal{C}\)), 205.8 (\(\mathcal{C} = \text{O}\)), 209.0 (\(\mathcal{C} = \text{O}\)); \(m/z\) (+ES) 533 ([M(\(^{79}\text{Br}, \; ^{79}\text{Br}\)]+\(\text{H}\)]\(^+\), 50%), 535 ([M(\(^{79}\text{Br}, \; ^{81}\text{Br}\)]+\(\text{H}\)]\(^+\), 100%), 537 ([M(\(^{81}\text{Br}, \; ^{81}\text{Br}\)]+\(\text{H}\)]\(^+\), 50%); (Found 532.9951, \(\text{C}_{24}\text{H}_{23}\)^{79}\text{Br}_4\). \(\text{[M+H]}\) requires 532.9963).

5.2.28 \((3S,4S,6S)-3,6\text{-bis(4-naphthyl)}-4\text{-hydroxyoctahydrodibenzol[\(\text{b,d}\)]furan-1,8(2\text{H}, \; 5a\text{H})\text{-dione (123b)}}\)

\[
\begin{align*}
\text{C}_{32}\text{H}_{28}\text{O}_4; \; \text{M}_{\text{w}} & = 476.57 \text{ g/mol} \\
\text{To a solution of 122b (136.3 mg, 0.40 mmol) in THF (2 mL) was added NaOH (0.5 M, 10 drops. The reaction was stirred at r.t. for 24 h under an atmosphere of nitrogen and quenched with NH}_4\text{Cl (sat., aq., 2 mL). H}_2\text{O (2 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with diethyl ether (3 \times 4 mL), and DCM (3 \times 4 mL). The combined organic extracts were washed with brine, dried over MgSO}_4 \text{ and concentrated \textit{in vacuo} to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (2:1) gave the title compound (123b) as a white solid (70.5 mg, 74%). M.p. 214-216 °C; }[
\]
\[
\alpha]_{\text{D}}^{28} \; -148.5 \text{ (c 0.50 in CH}_3\text{OH)}; \nu_{\text{max}} \text{(film)}/\text{cm}^{-1} \text{ 3423 br (O-H)}, 3054 \text{ w (C-H)}, 3020 \text{ w (C-H)}, 2953 \text{ w (C-H)}, 2904 \text{ w (C-H)}, 1711 \text{ s (C=O)}; \delta_{\text{H}} (400 \text{ MHz; CDCl}_3) \text{ 2.14 (1H, d, } J \; 8.4, \; \text{O-H}), 2.62-2.66 \text{ (2H, m, C(12)H}_2), 2.66-2.68 \text{ (2H, m, C(8)H}_2), 2.70-2.74 \text{ (1H, m, C(2)H), 2.78-2.82 (1H, m, C(2)H), 2.86 (1H, dd, } J \; 5.1, \; 2.1, \; \text{C(6)H}), 3.35 (1H, ddd, } J \; 11.3, \; 9.3, \; 5.9, \; \text{C(11)H}), 3.45 (1H, ddd, } J \; 11.9, \; 10.0, \; 4.3, \; \text{C(3)H}), 3.62 (1H, ddd, } J \; 12.2, \; 7.4, \; 2.1, \; \text{C(9)H}), 4.41 (1H, td, } J \; 10.0, \; 8.4, \; 3.3, \; \text{C(4)H}), 4.60 (1H, dd, } J \; 9.3, \; 7.4, \; \text{C(10)H}), 4.78 (1H, dd, } J \; 5.1, \; 3.3, \; \text{C(5)H}), 7.36 (2H, ~\text{~td, } J \; 8.2, \; 1.9, \; \text{Ar-CH}), 7.45-7.49 (4H, m, Ar-CH), 7.64 (1H, s, Ar-CH), 7.68 (1H, s, Ar-CH), 7.76-7.82 (4H, m, Ar-CH), 7.85 (2H, d, } J \; 8.2, \; \text{Ar-CH}; \delta_{\text{C}} \text{(100 MHz; CDCl}_3) \text{ some signals coincident 37.1 (C(9)H), 40.5 (C(12)H), 43.1 (C(8)H), 43.5 (C(11)H), 44.7 (C(3)H), 45.3 (C(2)H), 56.9 (C(6)H), 72.9 (C(4)H), 78.7 (C(5)H), 81.8 (C(10)H), 125.2, 125.6, 126.1, 126.2, 126.3, 126.5, 126.7, 127.80, 127.84, 127.9, 128.8, 128.9 (Ar-CH), 132.7, 132.8, 133.5, 137.3, 138.7 (Ar-\(\mathcal{C}\)), 206.5, 209.7 (\(\mathcal{C} = \text{O}\)); }m/z\; (+\text{ES})
\]
V. Experimental

477 ([M+H]+, 80%), 214 (100%); (Found 477.2047, C_{32}H_{29}O_{4} ([M+H]+) requires 477.2066).

5.2.29 (3S,4S,6S)-4-hydroxy-3,6-bis(4-methoxyphenyl)octahydrodibenzo[b,d]furan-1,8 (2H,5aH)-dione (123c)

C_{26}H_{28}O_{6}; M_{W}=436.19 g/mol

To a solution of 122c (139.8 mg, 0.44 mmol) in THF (1.5 mL) was added NaOH (0.5 M, 20 drops). The reaction was stirred at r.t. for 28 h under an atmosphere of nitrogen and quenched with NH_{4}Cl (sat., aq., 3 mL). H_{2}O (2 mL) was added to dilute the aqueous layer. The two layers were separated and the product was extracted with diethyl ether (3 × 5 mL) and DCM (3 × 5 mL). The combined organic extracts were washed with brine, dried over MgSO_{4} and concentrated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (1:1 to 1:3) gave the title compound (123c) as a white solid (48.2 mg, 50%). M.p. 183-185 °C [Lit.\(^{69}\) 179.1-181.9 °C]; [α]_{D}^{26} –185.8 (c 0.50 in CH_{2}Cl_{2}) [Lit.\(^{69}\) [α]_{D}^{28} –192.7(c 0.55 in CH_{2}Cl_{2})]; ν_{max} (film)/cm\(^{-1}\) 3392br (O-H); 2956w (C-H), 2911w (C-H), 2868w (C-H), 2843w (C-H), 1708s (C=O); δ_{H} (400 MHz; CDCl_{3}) 2.11 (1H, d, J 8.3, O-H), 2.51-2.54 (2H, m, C(12)H), 2.55-2.59 (2H, m, C(8)H), 2.63-2.65 (2H, m, C(2)H), 2.78 (1H, dd, J 5.1, 2.1, C(6)H), 3.14 (1H, ddd, J 11.7, 9.3, 5.6, C(11)H), 3.25 (1H, ~q, J 9.7, C(3)H), 3.50 (1H, dtd, J 11.1, 7.4, 2.1, C(9)H), 3.79 (6H, m, OCH_{3}), 4.23 (1H, ddd, J 9.7, 8.3, 3.3, C(4)H), 4.39 (1H, dd, J 9.3, 7.4, C(10)H), 4.70 (1H, dd, J 5.1, 3.3, C(5)H), 6.88 (4H, d, J 8.7, Ar-CH), 7.14 (4H, d, J 8.7, Ar-CH); δ_{C} (100 MHz; CDCl_{3}) some signals coincident 36.9 (C(9)H), 40.5 (C(8)H), 42.4 (C(11)H), 43.1 (C(12)H), 43.8 (C(3)H), 45.5 (C(2)H), 55.4 (OCH_{3}), 56.9 (C(6)H), 73.1 (C(4)H), 78.5 (C(5)H), 82.0 (C(10)H), 114.2, 114.3, 114.5, 114.7, 128.5, 128.7 (Ar-CH), 132.0, 132.4, 158.8, 158.9 (C), 206.7, 209.7 (C=O); m/z (+ES) 454 ([M+NH_{4}]^{+}, 100%); (Found 459.1784, C_{26}H_{28}NaO_{6} ([M+Na]^{+}) requires 459.1776).
5.2.30 (3aS,4S,7aR)-4-(1H-indol-5-yl)tetrahydrospiro[benzo[d][1,3]dioxole-2,1'-cyclohexan]-6(3aH)-one (121d)

5.2.31 (3S,4S,6S)-3,6-bis(4-naphthyl)-4-hydroxyoctahydrodibenzo[b,d]furan-1,8 (2H, 5aH)-dione (123d)

121d: C_{20}H_{23}NO_3; M_w=325.17 g/mol
123d: C_{28}H_{26}N_2O_4; M_w=454.19 g/mol

A solution of 89 (501.2 mg, 2.41 mmol), 5-indolyboronic acid (969.8 mg, 6.03 mmol), triethylamine (310 µL, 2.41 mmol), [RhCl(cod)]_2 (29.9 mg, 2.5 mol%) in dioxane and H_2O (10:1, 5 mL) was stirred at r.t. for 48 h under an atmosphere of nitrogen. The reaction mixture was concentrated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (10:1) gave the compound 121d with minor impurity.

To a solution of partially purified 121d in THF (8 mL) was added NaOH (0.5 M, 40 drops). The reaction was stirred at 45 °C for 24 h and quenched with NH_4Cl (sat., aq., 16 mL). The two layers were separated and the product was washed with brine, dried over MgSO_4 and concentrated in vacuo to give the crude product as a brown oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (1:2) gave the title compound (123d) as an off-white solid (144.8 mg, 26 %). M.p. 189 °C (decomp.); [α]_D^{27} −177.2 (c 0.50 in (CH_3)_2CO); ν_{max} (film)/cm^{-1} 3399m (N-H), 3337br (O-H), 2960w (C-H), 2913w (C-H), 2872w (C-H), 1704s (C=O); δ_H (400 MHz; acetone-d_6) 2.35 (1H, dd, J 17.4, 3.6, C(12)H), 2.42 (1H, dd, J 15.9, 5.8, C(8)H), 2.46 (1H, dd, J 14.5, 4.4, C(2)H), 2.69 (1H, dd, J 17.4, 13.5, C(12)H), 2.77 (1H, dd, J 15.9, 12.0, C(8)H), 2.89 (1H, dd, J 14.5, 11.6, C(2)H), 2.98 (1H, dd, J 5.1, 2.4, C(6)H), 3.28 (1H, ddd, J 11.6, 9.6, 4.4, C(3)H), 3.33 (1H, ddd, J 13.5, 9.7, 3.6, C(11)H), 3.43 (1H, dddd, J 12.0, 7.7, 5.8, 2.4, C(9)H), 4.36 (1H, ddd, J 9.6, 7.2, 3.2, C(4)H), 4.48 (1H, dd, J 9.7, 7.7, C(10)H), 4.73 (1H, dd, J 5.1, 3.2, C(5)H), 6.36 (1H, ddd, J 3.2, 2.0, 1.0, indole CH), 6.38 (1H, ddd, J 3.2, 2.0, 1.0, indole CH), 7.03 (1H, dd, J 8.4, 1.7, Ar-CH),
7.05 (1H, dd, J 8.4, 1.7, Ar-CH), 7.24-7.27 (2H, m, Ar-CH), 7.30 (1H, d, J 8.4, Ar-CH), 7.33 (1H, d, J 8.4, Ar-CH), 7.45 (2H, dt, J 8.4, 1.0, Ar-CH), 10.14 (2H, s, NH); δC (100 MHz; acetone-d6) some signals coincident 36.8 (C(9)H), 40.4 (C(8)H2), 43.2 (C(11)H), 44.0 (C(12)H2), 45.1 (C(3)H), 45.8 (C(2)H2), 57.0 (C(6)H), 73.0 (C(4)H), 79.2 (C(5)H), 82.3 (C(10)H), 101.51, 101.52, 119.2, 121.47, 121.52, 125.1 (Ar-CH), 128.4, 132.4, 133.5, 135.6 (Ar-C), 194.7, 198.6 (C=O); m/z (+ES) 455 ([M+H]+, 80%), 486 (100%); (Found 477.1778, C28H26N2O4Na ([M+Na]+) requires 477.1785).

5.2.32 (4S)-4-hydroxyoctahydrodibenzo[b,d]furan-1,8(2H,5aH)-dione (123j)

C12H16O4; Mw=224.10 g/mol

To a solution of 90 (120.2 mg, 0.54 mmol) in THF (2 mL) was added NaOH (0.5 M, 20 drops). The reaction was stirred at r.t. for 24 h and quenched with NH4Cl (sat., aq., 4 mL). The product was extracted with DCM (3 × 6 mL), ethyl acetate (3 × 6 mL) and diethyl ether (3 × 6 mL). The combined organic extracts were dried over MgSO4 and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (1:2) gave the title compound (123j) as a white solid (19.2 mg, 32%). M.p. 139-141 °C; [α]D 28 +68.0 (c 0.50 in (CH3)2OH); νmax(film)/cm⁻¹ 3419m (O-H), 2960w (C-H), 2921w (C-H), 2851w (C-H), 1698s (C=O); δH (400 MHz; CDCl3) 1.96 (1H, dddd, J 13.1, 9.7, 7.4, 2.3, C(3)H), 2.07-2.14 (3H, m, C(3)H and C(11)H2), 2.23 (1H, dd, J 16.6, 6.0, C(12)H), 2.27-2.30 (1H, m, C(2)H), 2.34 (1H, dd, J 15.2, 7.6, C(8)H), 2.46 (1H, ddd, J 16.6, 8.7, 6.0, C(12)H), 2.52 (1H, dd, J 15.2, 7.6, C(8)H), 2.54-2.57 (1H, m, C(2)H), 2.59 (1H, dd, J 7.4, 4.0, C(6)H), 3.29 (1H,qd, J 7.6, 4.0, C(9)H), 4.20 (1H, ddd, J 7.4, 4.0, 2.3, C(4)H), 4.34 (1H, dd, J 6.0, 4.0, C(10)H), 4.55 (1H, dd, J 7.4, 4.0, C(5)H); δC (100 MHz; CDCl3) 25.6 (C(3)H2), 27.2 (C(11)H2), 34.5 (C(2)H2), 35.2 (C(12)H2), 39.5 (C(9)H), 41.5 (C(8)H2), 56.4 (C(6)H), 67.1 (C(4)H), 76.5 (C(10)H), 80.3 (C(5)H), 208.2, 211.0 (C=O); m/z (+ES) 247 ([M+Na]+, 100%); (Found 247.0932, C12H16O4Na ([M+Na]+) requires 247.0941).
5.2.3 (S)-4-((isopropylidemethylsilyloxy)cyclohex-2-en-1-one (91)

5.2.34 (S)-2-(hydroxymethyl)-4-((isopropylidemethylsilyloxy)cyclohex-2-en-1-one (92)

91: C_{12}H_{22}SiO_2; M_w=226.14 g/mol
92: C_{13}H_{24}SiO_3; M_w=256.15 g/mol

A solution of compound 90 (185.6 mg, 0.89 mmol), TBSCl (159.8 mg, 1.06 mmol) and DBU (148 µL, 0.99 mmol) was stirred in benzene (5 mL) at the reflux temperature for 4 h. A second portion of DBU (37 µL, 0.25 mmol) was added and the reaction was left for another 3 h and then cooled to r.t.. The solvent was evaporated in vacuo and the residue was dissolved in Et_2O (12 mL). The resulting solution was washed with H_2O (10 mL), HCl (1 M, 10 mL), NaHCO_3 (sat. aq., 10 mL) and brine (10 mL). The resulting organic extracts were dried over MgSO_4 and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (12:1) gave the product 91 with minor impurity.

To a solution of partially purified compound 91 (144.2 mg, 0.64 mmol) and DMAP (77.8 mg, 0.64 mmol) in THF and H_2O (1:1, 8 mL) was added formaldehyde (aqueous, 37%, 120 µL, 1.59 mmol). The reaction was stirred at 40 °C for 24 h and quenched with HCl (1 M, 0.8 mL). The product was extracted with DCM (3 × 8 mL). The combined organic extracts were washed with brine (2 × 8 mL), NaHCO_3 (sat. aq., 2 × 8 mL), dried over MgSO_4 and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (6:1) gave the title compound (92) as a colourless oil (157.3 mg, 69% over two steps). [α]_D^{27} –94.2 (c 0.50 in CH_2Cl_2) [Lit. [α]_D^{20} –50.5 (c 1.14 in CH_2Cl_2)]; ν_{max} (film)/cm^{-1} 3443br (O-H), 2953w (C-H), 2929w (C-H), 2886w (C-H), 2856w (C-H), 1673s (C=O); δ_H (400 MHz; CDCl_3) 0.12-0.13 (6H, m, 2 × Si(CH_3)), 0.91 (9H, s, C(CH_3)_3), 2.00 (1H, tdd, J 13.0, 9.0, 4.6, C(5)H), 2.21 (1H, ddd, J 13.0, 4.6, 1.5, C(5)H), 2.37 (1H, ddd, J 16.9, 13.0, 4.6, C(6)H_{2ax}), 2.60 (1H, dt, J 16.9, 4.6, C(6)H_{eq}), 4.19 (1H, dt, J 13.5, 1.4, C(7)H_H_6), 4.31 (1H, dt, J 13.5, 1.4, C(7)H_H_6), 4.57 (1H, ddd, J 9.0, 4.6, 2.4, C(4)H), 6.78 (1H, dt, J 2.4, 1.5, C(3)H); δ_C (100 MHz; CDCl_3) –4.6, –4.5 (Si(CH_3)_2), 18.3 (C(CH_3)_3), 25.9 (C(CH_3)_3), 33.2 (C(5)H_2), 36.0 (C(6)H_2), 61.7 (C(7)H_2), 67.2 (C(4)H), 137.2 (C(2)), 149.9 (C(3)H),
199.9 (C=O); m/z (+ES) 257 ([M+H]^+, 100%); (Found 257.1563, C\textsubscript{13}H\textsubscript{25}SiO\textsubscript{3} ([M+H]^+) requires 257.1567).

5.2.35 2-(Hydroxymethyl)cyclohex-2-enone (155)

\[
\text{C}_7\text{H}_{10}\text{O}_2; \text{M}_W=126.15 \text{ g/mol}
\]

To a solution of 2-cyclohexen-1-one (2.0 mL, 20.60 mmol) in THF (10 mL) were added DMAP (0.24 g, 2.66 mmol) and formaldehyde (aqueous, 37%, 3.8 mL, 51.60 mmol). The reaction was stirred at r.t. for 21 h under an atmosphere of nitrogen and quenched with HCl (1 M, 1.0 mL). The two layers were separated and the product was extracted with DCM (2 \times 20 mL). The combined organic extracts were washed with brine (10 mL), dried over MgSO\textsubscript{4} and concentrated in vacuo to give the crude product as a dark orange oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (155) as a colourless oil (1.38 g, 53%). \(\nu\text{max} (\text{film})/\text{cm}^{-1} 3411 (\text{O-H}), 2935 (\text{C-H}), 2868 (\text{C-H}), 2831 (\text{C-H}), 1662s (\text{C=O}); \delta_H (400 \text{ MHz; CDCl}_3) 2.02 (2H, quint, J 6.1, C(5)H\textsubscript{2}), 2.39-2.47 (4H, m, C(4)H\textsubscript{2} and C(6)H\textsubscript{2}), 4.24 (2H, q, J 1.6, C(7)H\textsubscript{2}), 6.94 (1H, t, J 4.2, C(3)H); \delta_C (100 \text{ MHz; CDCl}_3) 22.8 (C(5)H\textsubscript{2}), 25.8 (C(4)H\textsubscript{2}), 38.3 (C(6)H\textsubscript{2}), 62.4 (C(7)H\textsubscript{2}), 138.3 (C(2)), 147.2 (C(3)H), 201.0 (C=O); m/z (+ES) 149 ([M+Na]^+, 100%); (Found 149.0578, C\textsubscript{7}H\textsubscript{10}O\textsubscript{2}Na ([M+Na]^+) requires 149.0574).

5.2.36 (6-oxocyclohex-1-en-1-yl)methyl (E)-but-2-enoate (COMC, 9)

\[
\text{C}_{11}\text{H}_{14}\text{O}_3; \text{M}_W=194.09 \text{ g/mol}
\]

To a solution of compound 155 (100.2 mg, 0.79 mmol) and DMAP (9.7 mg, 79 \text{ \mu mol}) in DCM (2 mL) were added pyridine (540 \text{ \mu L}, 6.72 mmol) and crotonic anhydride (203 \text{ \mu L}, 1.58 mmol). The reaction was stirred at r.t. for 2.5 h under an atmosphere of nitrogen and quenched with NaHCO\textsubscript{3} (sat., aq., 3 mL). Two layers were separated and the product was extracted with DCM (3 \times 3 mL). The combined organic extracts were washed with
NaHCO₃ (6 mL) and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (8:1) gave the title compound (9) as a colourless oil (45 mg, 29%). ν_max (film)/cm⁻¹ 2940w (C-H), 2867w (C-H), 1719s (C=O), 1671s (C=O); δ_H (400 MHz; CDCl₃) 1.88 (3H, dd, J 6.9, 1.8, C(11)H₃), 2.01 (2H, quint, J 6.2, C(5)H₂), 2.39-2.48 (4H, m, C(4)H₂ and C(6)H₂), 4.79 (2H, q, J 1.5, C(7)H₂), 5.87 (1H, dq, J 15.6, 1.8, C(9)H), 6.99 (1H, t, J 4.2, C(3)H), 6.99 (signals coincident) (1H, dd, J 15.6, 6.9, C(10)H); δ_C (100 MHz; CDCl₃) 18.1 (C(11)H₃), 22.8 (C(5)H₂), 25.9 (C(4)H₂), 38.2 (C(6)H₂), 61.1 (C(7)H₂), 122.5 (C(9)H), 134.7 (C(2)), 145.3 (C(10)H), 148.3 (C(3)H), 166.3 (C(8)=O), 198.1 (C(1)=O); m/z (+ES) 217 ([M+Na]+, 100%); (Found 195.1016, C₁₁H₁₅O₃ ([M+H]+) requires 195.1008).

5.2.37 Methyl (2S,3S,4aR,6S,8R,8aR)-6,8-dihydroxy-2,3-dimethoxy-2,3-dimethyl-octa-hydrobenzo[b][1,4]dioxine-6-carboxylate (93)

C₁₄H₂₄O₈; MW=320.15 g/mol
A solution of (−)-quinic acid (5.00 g, 26.02 mmol), camphorsulfonic acid (0.68 g, 2.93 mmol), butane-2,3-dione (5.4 mL, 60.77 mmol) and trimethylorthoformate (22.3 mL, 0.20 mmol) in methanol (120.0 mL) was stirred at the reflux temperature for 22 h under an atmosphere of nitrogen. The reaction was cooled to r.t. and quenched with triethylamine (10 mL). The reaction mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate (50 mL). The resulting solution was stirred with activated charcoal and filtered through a short pad of Celite®. The filtrate was concentrated in vacuo to give the crude product as a yellow oil. Purification by re-crystalisation gave the title compound (93) as an off-white solid (5.25 g, 63%). M.p. 135-137 °C [Lit.¹³⁴ 138-140 °C]; [α]D²⁸ +114.1 (c 1.00 in CH₂Cl₂) [Lit.¹³⁴ +116.3 (c 1.06 in CH₂Cl₂)]; ν_max (film)/cm⁻¹ 3435br (O-H), 3329br (O-H), 2993w (C-H), 2950w (C-H), 2920w (C-H), 2843w (C-H), 1725s (C=O); δ_H (400 MHz; CDCl₃) 1.29 (3H, s, CH₃), 1.33 (3H, s, CH₃), 1.93 (1H, ~t, J 12.5, C(6)H), 2.04 (1H, dd, J 14.9, 3.1, C(2)H), 2.10 (1H, ddd, J 12.5, 4.7, 3.1, C(6)H), 2.17 (1H, dt, J 14.9, 3.1,
C(2)H), 3.08 (1H, d, J 3.1, C(3)OH), 3.25 (3H, s, OCH₃), 3.26 (3H, s, OCH₃), 3.59 (1H, dd, J 10.2, 3.1, C(4)H), 3.78 (3H, s, COOCH₃), 4.19 (1H, d, J 3.1, C(3)H), 4.21 (1H, s, OH), 4.31 (1H, ddd, J 12.5, 10.2, 4.7, C(5)H); δC (100 MHz; CDCl₃) 17.9 (C(1)H₃), 18.0 (C(2)H₃), 37.6 (C(2)H₂), 38.8 (C(6)H₂), 48.09, 48.10 (OCH₃), 53.1 (COOCH₃), 62.6 (C(5)H); m/z (+ES) 343 ([M+Na]+, 100%); (Found 343.1361, C₁₄H₂₄NaO₈ ([M+Na]+) requires 343.1363).

5.2.38 (2S,3S,4aR,5R,7S,8aR)-7-(hydroxymethyl)-2,3-dimethoxy-2,3-dimethyloctahydrobenzo[b][1,4]dioxine-5,7-diol (94)

5.2.39 (2S,3S,4aR,8R,8aR)-8-hydroxy-2,3-dimethoxy-2,3-dimethylhexahydrobenzo[b][1,4]dioxin-6(SH)-one (95)

94: C₁₃H₂₄O₇; M_w=292.15 g/mol
95: C₁₂H₂₀O₆; M_w=260.13 g/mol

To a solution of 93 (3.50 g, 10.93 mmol) in methanol (84 mL) was added sodium borohydride (4.13 g, 0.11 mol) portionwise at 0 °C. After the effervescence ceased, the reaction was stirred at r.t. for 18 h under an atmosphere of nitrogen and quenched with NH₄Cl (sat., aq., 16 mL). The resulting solution was concentrated in vacuo to give a white solid, which was washed with ethyl acetate (5 × 20 mL). The solid was removed by filtration and the filtrate was dried over MgSO₄ and concentrated to give the crude product (94) as a white solid.

To a solution of sodium periodate (5.80 g, 27.32 mmol) in hot water (7 mL) was added silica (22 g). The slurry was mixed until it was a free-flowing powder. DCM (32 mL) was added to the resulting powder, followed by the addition of 94 in DCM (42 mL). The reaction was stirred at r.t. for 1.5 h under an atmosphere of nitrogen. The powder was collected by filtration and washed with DCM (5 × 20 mL). The combined washes were dried over MgSO₄ and concentrated in vacuo to give the title compound (95) as an off-white solid (1.88 g, 66%). M.p. 174-176 °C [Lit. 135 163-165 °C]; [α]_D²⁹ +125.6 (c 0.50 in
CH$_2$Cl$_2$) [Lit.$^{135} +159.8$ (c 0.59 in CH$_2$Cl$_2$)]; $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3477br (O-H), 2992w (C-H), 2970w (C-H), 2945w (C-H), 2923w (C-H), 1724s (C=O); $\delta_H$(400 MHz; CDCl$_3$) 1.31 (3H, s, CH$_3$), 1.35 (3H, s, CH$_3$), 2.37 (1H, s, OH), 2.46-2.53 (2H, m, C(2)$H_2$ or C(6)$H_2$), 2.63-2.69 (2H, m, C(2)$H_2$ or C(6)$H_2$), 3.24 (3H, s, OCH$_3$), 3.31 (3H, s, OCH$_3$), 3.88 (1H, ddd, $J$ 10.0, 2.9, C(4)$H$), 4.25 (1H, d, $J$ 2.9, C(3)$H$), 4.27 (1H, ddd, $J$ 12.5, 10.0, 5.0, C(5)$H$); $\delta_C$ (100 MHz; CDCl$_3$) 17.8 (CH$_3$), 17.9 (CH$_3$), 44.9, 46.3 (C(2)$H_2$ and C(6)$H_2$), 48.2, 48.3 (OCH$_3$), 63.4, 67.9 (C(3)$H$ and C(5)$H$), 72.4 (C(4)$H$), 99.4, 100.4 (acetal C), 205.6 (C=O); m/z (+ES) 283 ([M+Na]$^+$, 100%); (Found 283.1147, C$_{12}$H$_{20}$NaO$_6$ ([M+Na]$^+$) requires 283.1152).

5.2.40 (2S,3S,4aR,8aR)-2,3-dimethoxy-2,3-dimethyl-2,3,4a,8a-tetrahydrobenzo [b][1,4] dioxin-6(5H)-one (96)

C$_{12}$H$_{18}$O$_5$; M$_W$=242.12 g/mol

To a solution of 95 (1.50 g, 5.76 mmol) in DCM (24 mL) were added methanesulfonyl chloride (640.0 µL, 6.92 mmol) and triethylamine (2.0 mL, 16.71 mmol) at 0 °C under an atmosphere of nitrogen. The reaction was stirred at r.t. for 3 h and quenched with H$_2$O (8 mL). The two layers were separated and the product was extracted with DCM (3 × 8 mL). The combined organic extracts were washed with Na$_2$CO$_3$ (sat., aq., 3 × 8 mL), dried over MgSO$_4$ and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (6:1) gave the title compound (96) as a white solid (1.34 g, 96%). M.p. 189-191 °C [Lit.$^{135}$ 178.6-181.3 °C]; $[\alpha]_D^{29} +40$ (c 0.50 in CH$_2$Cl$_2$) [Lit.$^{135}$ +65.2 (c 1.00 in CH$_2$Cl$_2$)]; $\nu_{\text{max}}$ (film)/cm$^{-1}$ 2996w (C-H), 2958w (C-H), 2949w (C-H), 2928w (C-H), 2858w (C-H), 2833w (C-H), 1675s (C=O); $\delta_H$ (400 MHz; CDCl$_3$) 1.33 (3H, s, CH$_3$), 1.37 (3H, s, CH$_3$), 2.49 (1H, dd, $J$ 16.5, 13.5, C(6)$H_{\text{ax}}$), 2.74 (1H, ddd, $J$ 16.5, 4.8, 1.2, C(6)$H_{\text{eq}}$), 3.26 (3H, s, OCH$_3$), 3.32 (3H, s, OCH$_3$), 4.05 (1H, ddd, $J$ 13.5, 9.2, 4.8, C(5)$H$), 4.51 (1H, ddd, $J$ 9.2, 2.7, 1.7, C(4)$H$), 6.00 (1H, ddd, $J$ 10.1, 2.7, 1.2, C(2)$H$), 6.87 (1H, dd, $J$ 10.1, 1.7, C(3)$H$); $\delta_C$ (100 MHz; CDCl$_3$) 17.8, 17.9 (CH$_3$), 42.2 (C(6)$H_2$), 48.3, 48.4 (OCH$_3$), 68.2 (C(5)$H$), 69.4 (C(4)$H$), 99.9, 100.9 (acetal C), 130.2 (C(2)$H$), 148.7 (C(3)$H$), 197.0 (C=O); m/z
(+ES) 265 ([M+Na]^+, 100%); (Found 265.1049, C_{12}H_{18}NaO_5 ([M+Na]^+) requires 265.1046).

5.2.41 (2S,3S,4aR,8aR)-7-(hydroxymethyl)-2,3-dimethoxy-2,3-dimethyl-2,3,4a,8a-tetra-hydrobenzo[b][1,4]dioxin-6(5H)-one (97)

C_{13}H_{20}O_6; M_W=272.13 g/mol

To a solution of 96 (201.3 mg, 0.83 mmol) in THF and H_2O (1:1, 8 mL) were added DMAP (10.1 mg, 83 µmol) and formaldehyde (37%, 130 µL, 1.65 mmol). The reaction was stirred at 40 °C for 20 h under an atmosphere of nitrogen and quenched with HCl (1 M, 0.4 mL). The product was extracted with DCM (3 × 8 mL). The combined organic extracts were washed with NaHCO_3 (sat., aq., 8 mL), brine (8 mL), dried over MgSO_4 and concentrated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (97) as a white solid (162.6 mg, 72%). M.p. 190-192 °C [Lit.^{63} 179.8-181.2 °C]; [α]_D^{29} +48.3 (c 0.50 in CH_2Cl_2) [Lit.^{63} +34.5 (c 1.2 in CH_2Cl_2)]; ν_max (film)/cm^{-1} 3531br (O-H), 2956w (C-H), 2941w (C-H), 2920w (C-H), 2892w (C-H), 1670s (C=O); δ_H (400 MHz; CDCl_3) 1.33 (3H, s, C_H_3), 1.36 (3H, s, C_H_3), 2.52 (1H, dd, J 16.5, 13.5, C(6)H_{ax}), 2.77 (1H, ddd, J 16.5, 4.8, C(6)H_{eq}), 3.26 (3H, s, CH_3), 3.32 (3H, s, OCH_3), 4.03 (1H, ddd, J 13.5, 9.2, 4.8, C(5)H), 4.21 (1H, d, J 14.0, C(7)H_2H_5), 4.33 (1H, d, J 14.0, C(7)H_6H_6), 4.52 (1H, dd, J 9.2, 1.8, C(4)H), 6.84 (1H, q, J 1.8, C(3)H); δ_C (100 MHz; CDCl_3) 17.8, 17.9 (CH_3), 42.3 (C(6)H_2), 48.3, 48.4 (OCH_3), 61.0 (C(7)H_2), 68.2 (C(5)H), 69.2 (C(4)H), 99.9, 100.9 (acetal C), 139.2 (C(2)), 144.6 (C(3)H), 197.4 (C=O); m/z (+ES) 295 ([M+Na]^+, 100%); (Found 273.1331, C_{13}H_{21}O_6 ([M+H]^+) requires 273.1333).
5.2.42 4-hydroxy-5-methoxy-2H-chromen-2-one (163a)

\[
\begin{array}{c}
\text{O} \\
\text{OH} \\
\text{2} \\
\text{7} \\
\end{array}
\]

C_{10}H_{8}O_4; M_W=192.04 g/mol
To a suspension of NaH (60% dispersion in mineral oil, 2.80 g, 0.12 mol) in diethyl carbonate (10 mL) was added a solution of 2-hydroxy-6-methoxyacetophenone (2.50 g, 15.00 mmol) in diethyl carbonate (25 mL) dropwise at 0 °C. After effervescence ceased, the reaction was stirred at the reflux temperature for 4 h under an atmosphere of nitrogen. The reaction was cooled to 0 °C and quenched with H_2O (10 mL). The two layers were separated and the aqueous layer was washed with diethyl ether (3 × 10 mL) and acidified with HCl (1 M) to pH ≤ 1 to yield a yellow solid. The resulting solid was collected by filtration, washed with H_2O (3 × 10 mL) and petroleum ether (3 × 10 mL) to give the crude product as a light yellow solid. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (3:1) gave the tile compound (163a) as an off-white solid (1.96 g, 68%). M.p. 148-150 °C [Lit.\textsuperscript{136} 155 °C]; \nu_{\text{max}} (film)/cm\textsuperscript{-1} 3265br (O-H), 2947w (C-H), 2911w (C-H), 2880w (C-H), 2846w (C-H), 1705s (C=O), 1644s (C=C); \delta_{\text{H}} (400 MHz; CDCl\textsubscript{3}) 4.08 (3H, s, OCH\textsubscript{3}), 5.69 (1H, s, C(2)H), 6.80 (1H, dd, J 8.4, 0.9, C(5)H or C(7)H), 7.04 (1H, dd, J 8.4, 0.9, C(5)H or C(7)H), 7.49 (1H, t, J 8.4, C(6)H), 9.53 (1H, s, C(3)OH); \delta_{\text{C}} (100 MHz; CDCl\textsubscript{3}) 57.2 (OCH\textsubscript{3}), 93.2 (C(2)H), 105.1 (Ar-C\textsubscript{1}), 105.7, 111.6 (C(5)H and C(7)H), 132.6 (C(6)H), 155.3, 156.3 (Ar-C\textsubscript{3}), 162.9, 166.1 (C(1) and C(3)); m/z (+ES) 215 ([M+Na]\textsuperscript{+}, 100%); (Found 215.0305, C_{10}H_{8}NaO_4 ([M+Na]\textsuperscript{+}) requires 215.0315).

5.2.43 4-hydroxy-2H-benzo[h]chromen-2-one (163b)

\[
\begin{array}{c}
\text{O} \\
\text{OH} \\
\text{2} \\
\text{5} \\
\end{array}
\]

C_{13}H_{8}O_3; M_W=212.05 g/mol
To a suspension of NaH (60% dispersion in mineral oil, 1.30 g, 0.54 mol) in diethyl carbonate (10 mL) was added a solution of 2-hydroxy-6-methoxyacetophenone (2.00 g,
10.74 mmol) in diethyl carbonate (25 mL) dropwise at 0 °C. After effervescence ceased, the reaction was stirred at the reflux temperature for 5 h under an atmosphere of nitrogen. The reaction was cooled to 0 °C and quenched with H2O (10 mL). The two layers were separated and the aqueous layer was washed with diethyl ether (3 × 10 mL) and acidified with HCl (1 M) to pH ≤ 1 to yield a yellow solid. The resulting solid was collected by filtration, washed with H2O (3 × 10 mL) and petroleum ether (3 × 10 mL) to give the crude product as a light yellow solid. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (3:1) gave the title compound (163b) as an off-white solid (550 mg, 24%). M.p. 215 °C (decomp.) [Lit.66 272-273 °C]; νmax (film)/cm⁻¹ 3374br (O-H), 2883w (C-H), 2727w (C-H), 2571w (C-H), 1643s (C=O), 1599s (C=C); δH (400 MHz; DMSO-d6) 5.75 (1H, s, C(2)H), 7.71-7.74 (2H, m, Ar-CH), 7.84 (2H, s, Ar-CH), 8.05-8.09 (1H, m, Ar-CH), 8.34-8.38 (1H, m, Ar-CH), 12.64 (1H, s, C(3)O-H); δC (100 MHz; DMSO-d6) 90.7 (C(2)H), 111.1 (Ar-C), 118.9, 121.7 (Ar-CH), 122.2 (Ar-C), 123.5, 127.2, 128.0, 128.7 (Ar-CH), 134.8, 150.7 (Ar-C), 161.8, 166.6 (C); m/z (+ES) 213 ([M+H]+, 100%); (Found 211.0397, C13H13O3 ([M–H]+) requires 211.0401).

Method A:
General method for the syntheses of dicoumarol-based asymmetric NQO1 inhibitors (164a-e):

The appropriate hydroxycoumarin species (163a-c, 1.4 M) was reacted with the corresponding aryl alcohol in the presence of 5 mol% of RuCl2(Ph3P)3, 10 mol% of Cs2CO3 and 20 mol% of iso-propanol in toluene at 150 °C for 30 h under an atmosphere of nitrogen. The reaction mixture was cooled to r.t. and concentrated in vacuo to give a crude product. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (in an appropriate ratio) gave the corresponding NQO1 inhibitor.

5.2.44 4-hydroxy-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (164c)

C20H14O3; M_W=302.09 g/mol

Using method A, 4-hydroxycoumarin (163c) (500 mg, 3.10 mmol) was coupled to 2-naphthalenemethanol (455 mg, 3.10 mmol) to give the crude product as a yellow solid.
Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (164c) as a white solid (328.5 mg, 35%). M.p. 207-209 °C (Lit.\(^66\) 202-204 °C); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3120br (O-H), 2945w (C-H), 2748w (C-H), 1625s (C=O); \(\delta_H\) (400 MHz; acetone-d\(_6\)) 4.17 (2H, d, J 1.0, C(8)H\(_2\)), 7.35 (1H, td, J 8.3, 1.0, Ar-CH), 7.40 (1H, dd, J 8.3, 1.7, Ar-CH), 7.64 (1H, ddd, J 8.3, 7.6, 1.7, Ar-CH), 7.76-7.77 (1H, m, Ar-CH), 7.78-7.84 (3H, m, Ar-CH), 8.00 (1H, dd, J 8.3, 1.7, Ar-CH), 10.17 (1H, s, OH); \(\delta_C\) (100 MHz; acetone-d\(_6\)) 30.3 (C(8)H\(_2\)), 105.5 (C(2)), 117.1 (Ar-C), 117.2, 123.9, 124.7, 126.1, 126.8, 127.0, 128.2, 128.31, 128.33, 128.6, 132.7 (Ar-CH), 133.1, 134.5, 138.0 (Ar-C), 153.6 (Ar-C), 160.8, 163.6 (C(1) and C(3)); m/z (+ES) 303 ([M+H]\(^+\), 100%); (Found 303.1016, C\(_{20}\)H\(_{15}\)O\(_3\) ([M+H]\(^+\)) requires 303.1014).

5.2.45 4-hydroxy-5-methoxy-3-(naphthalen-2-ylmethyl)-2\(H\)-chromen-2-one (164a)

C\(_{21}\)H\(_{16}\)O\(_4\); M\(_W\)=332.10 g/mol

Using method A, 5-methoxy-4-hydroxycoumarin (163a) (500 mg, 2.60 mmol) was coupled to 2-naphthenemethanol (411 mg, 2.60 mmol) to give the crude product as a yellow solid. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (164a) as an off-white solid (310.8 mg, 36%). M.p. 166-168 °C (Lit.\(^{41}\) 158-160 °C); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3378br (O-H), 2923w (C-H), 2842w (C-H), 1593s (C=O); \(\delta_H\) (400 MHz; acetone-d\(_6\)) 4.00 (2H, d, J 1.1, C(8)H\(_2\)), 4.18 (3H, s, OCH\(_3\)), 6.98 (1H, dd, J 8.3, 1.1, Ar-CH), 7.05 (1H, dd, J 8.3, 1.1, Ar-CH), 7.38-7.46 (2H, m, Ar-CH), 7.55 (1H, dd, J 8.3, 1.6, Ar-CH), 7.57 (1H, t, J 8.3 Ar-CH), 7.77-7.81 (4H, m, Ar-CH), 10.10 (1H, s, OH); \(\delta_C\) (100 MHz; acetone-d\(_6\)) 29.8 (C(8)H\(_2\)), 57.7 (OCH\(_3\)), 105.7 (C(2)), 107.0, 111.0, 126.0, 126.6, 127.4, 128.29, 128.33, 128.4, 128.5, 133.0 (Ar-CH), 133.1, 134.6, 138.9, 139.0 (Ar-C), 154.6, 157.3 (Ar-C), 162.1, 163.2 (C(1) and C(3)); m/z (+ES) 355 ([M+Na]\(^+\), 100%); (Found 355.0938, C\(_{21}\)H\(_{16}\)NaO\(_4\) ([M+Na]\(^+\)) requires 355.0941).
5.2.46 3-benzyl-4-hydroxy-2H-chromen-2-one (164e)

\[
\begin{array}{c}
\text{C}_{16}H_{12}O_3; \quad M_w=252.08 \text{ g/mol} \\
\end{array}
\]

Using method A, 4-hydroxycoumarin (163c) (500 mg, 3.10 mmol) was coupled to benzyl alcohol (640 µL, 6.20 mmol) to give the crude product as a yellow solid. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (164e) as a white solid (343.8 mg, 44%). M.p. 210-212 °C (Lit.\(^6^6\) 205-207 °C); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3056br (O-H), 2923w (C-H), 1650s (C=O); \(\delta_H\) (400 MHz; acetone-\(d_6\)) 3.99 (2H, s, C(8)\(H_2\)), 7.13-7.17 (1H, m, Ar-CH), 7.22-7.26 (2H, m, Ar-CH), 7.29-7.32 (2H, m, Ar-CH), 7.33 (1H, dd, J 8.3, 1.1, Ar-CH), 7.38 (1H, ddd, J 8.3, 7.5, 1.1, Ar-CH), 7.63 (1H, ddd, J 8.3, 7.5, 1.6, Ar-CH), 7.98 (1H, dd, J 8.3, 1.6, Ar-CH), 10.10 (1H, s, OH); \(\delta_C\) (100 MHz; acetone-\(d_6\)) some signals coincident 30.1 (C(8)\(H_2\)), 105.7 (C(2)), 117.1 (Ar-C), 117.2, 123.8, 124.7, 126.8, 129.07, 129.10, 132.6 (Ar-CH), 140.4, 153.5 (Ar-C), 160.6, 163.5 (C(1) and C(3)); \(m/z\) (+ES) 253 ([M+H]\(^+\), 100%); (Found 275.0675, C\(_{16}\)H\(_{12}\)NaO\(_3\) ([M+Na]\(^+\)) requires 275.0679).

5.2.47 3-benzyl-4-hydroxy-5-methoxy-2H-chromen-2-one (164d)

\[
\begin{array}{c}
\text{C}_{17}H_{14}O_4; \quad M_w=282.09 \text{ g/mol} \\
\end{array}
\]

Using method A, 5-methoxy-4-hydroxycoumarin (163a) (500 mg, 2.60 mmol) was coupled to benzyl alcohol (540 µL, 5.20 mmol) to give the crude product as a yellow solid. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (164d) as a white solid (170.8 mg, 23%). M.p. 168-170 °C (Lit.\(^4^1\) 174-177 °C); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3322 (O-H), 2919w (C-H), 2846w (C-H), 1643s (C=O); \(\delta_H\) (400 MHz; acetone-\(d_6\)) 3.82 (2H, s, C(8)\(H_2\)), 4.18 (3H, s, OCH\(_3\)), 6.97 (1H, dd, J 8.3, 1.0, Ar-CH), 7.05 (1H, dd, J 8.3, 1.0, Ar-CH), 7.11-7.16 (1H, m, Ar-CH), 7.20-7.25 (2H, m, Ar-CH), 7.34-7.37 (2H, m, Ar-CH), 7.56 (1H, t, J 8.3, Ar-CH), 10.06 (1H, s, OH); \(\delta_C\) (100 MHz; acetone-\(d_6\)) some signals coincident 29.8 (C(8)\(H_2\)), 57.7 (OCH\(_3\)), 105.3 (C(2)), 107.0, 111.0, 126.7, 128.9, 129.4 (Ar-CH), 131.8 (Ar-C), 132.9 (Ar-...
C\textsubscript{14}H\textsubscript{13}N\textsubscript{4}O\textsubscript{4} ([M+Na]\textsuperscript{+}) requires 305.0784).

5.2.49 \((E)-3-((2\text{-hydroxynaphthalen}-1\text{-yl})\text{methylene})\text{chromane-2,4-dione (165b)}

\[
\begin{align*}
\text{C}_{20}\text{H}_{12}\text{O}_{4}; \text{M}_\text{W}=316.07 \text{ g/mol} \\
\text{A mixture of 4-hydroxycoumarin (489.8 mg, 3.02 mmol) and 2-hydroxy-1-napthaldehyde (516.5 mg, 3.02 mmol) in ethanol (30 mL) was stirred at the reflux temperature for 40 min and cooled to r.t. The resulting precipitate was collected by filtration, washed with methanol (3 × 30 mL) and dried under vacuum to give the title compound (165b) as a bright yellow solid (667.8 mg, 70%). M.p. 257-259 °C; } v_{\text{max}} \text{(film)/cm}^{-1} 3057 \text{br (O-H), } 1615, 1628 \text{ (C(1) and C(3)); m/z (+ES) 303 ([M+H]\textsuperscript{+}, 100%); (Found 325.0846, C}_{20}\text{H}_{14}\text{NaO}_{3} ([M+Na]\textsuperscript{+}) requires 325.0835).}
\end{align*}
\]
1704s (C=O), 1567s (C=O); δH (400 MHz; DMSO-d6) 6.94 (1H, d, J 8.3, Ar-CH), 6.96 (1H, td, J 7.4, 1.0, Ar-CH), 7.50 (1H, ddd, J 8.3, 7.4, 1.7, Ar-CH), 7.65 (1H, d, J 9.0, Ar-CH), 7.64-7.68 (1H, m, Ar-CH), 7.74-7.78 (2H, m, Ar-CH), 8.11 (1H, dd, J 8.3, 1.0, Ar-CH), 8.31 (1H, d, J 9.0, Ar-CH), 8.63 (1H, d, J 9.0, Ar-CH), 9.12 (1H, s, C(8)H), 10.78 (1H, s, OH); δC (100 MHz; DMSO-d6) 112.7 (Ar-C), 116.6, 117.1, 119.4, 122.6 (Ar-CH), 123.3 (Ar-C), 126.4 (Ar-CH), 128.0 (Ar-C), 128.8, 129.0 (Ar-CH), 129.2, 130.0 (Ar-C), 131.2, 134.7, 135.2 (Ar-CH), 138.9 (C(8)H), 154.1, 158.2, 159.0 (C), 192.5 (C=O); m/z (+ES) 317 ([M+H]+, 100%); (Found 339.0633, C20H12NaO4 ([M+Na]+) requires 339.0644).

5.2.50 (E)-3-((1-hydroxynaphthalen-2-yl)methylene)-5-methoxychromane-2,4-dione (165a)

![Image of (E)-3-((1-hydroxynaphthalen-2-yl)methylene)-5-methoxychromane-2,4-dione](image)

C21H14O5; Mw=346.08 g/mol
A mixture of compound 163a (487.2 mg, 2.54 mmol) and 2-hydroxy-1-naphthaldehyde (436.5 mg, 2.54 mmol) in ethanol (20 mL) was stirred at the reflux temperature for 2 h and cooled to r.t. The resulting precipitate was collected by filtration, washed with methanol (3 × 30 mL) and dried under vacuum to give the title compound (165a) as a bright yellow solid (641.7 mg, 73%). M.p. 218-220 °C; νmax (film)/cm⁻¹ 3283br (O-H), 3060w (CH), 2929w (CH), 2834w (CH), 1725s (C=O), 1557s (C=O); δH (400 MHz; DMSO-d6) 3.65 (3H, s, OC2H3), 6.56 (2H, ddd, J 8.3, 7.1, 1.2, Ar-CH), 7.32 (1H, t, J 8.3, Ar-CH), 7.65 (1H, d, J 9.0, Ar-CH), 7.68 (1H, ddd, J 8.3, 7.1, 1.2, Ar-CH), 7.78 (1H, ddd, J 8.3, 7.1, 1.2, Ar-CH), 8.11 (1H, dd, J 8.3, 1.2, Ar-CH), 8.33 (1H, d, J 9.0, Ar-CH), 8.59 (1H, d, J 8.3, Ar-CH), 9.15 (1H, s, C(7)H), 10.47 (1H, s, OH); δC (100 MHz; DMSO-d6) 55.9 (OCH3), 102.5, 109.2 (Ar-CH), 112.5, 115.1 (Ar-C), 116.6, 122.3 (Ar-CH), 126.3 (Ar-C), 126.5, 129.0, 129.1 (Ar-CH), 129.3, 130.0 (Ar-C), 133.4, 135.6 (Ar-CH), 140.3 (C(7)H), 154.7, 157.4, 158.0, 159.0 (C), 191.0 (C=O); m/z (+ES) 347 ([M+H]+, 100%); (Found 369.0737, C21H14NaO5 ([M+Na]+) requires 369.0733).
V. Experimental

5.2.51 4-hydroxy-3-((1-hydroxy-3-oxo-3,4-dihydronaphthalen-2-yl)(naphthalen-2-yl)me-thyl)-2H-chromen-2-one (253c)

\[
\text{C}_{29}\text{H}_{18}\text{O}_6; \quad M_W=462.11 \text{ g/mol}
\]

A mixture of 4-hydroxycoumarin (2.00 g, 12.34 mmol) and 2-naphthaldehyde (0.96 g, 6.15 mmol) was stirred in ethanol (50 mL) at the reflux temperature for 16 h and cooled to r.t.. The resulting precipitate was collected by filtration, washed with ethanol (5 × 20 mL) and dried under vacuum to give the title compound (253c) as a white solid (2.74 g, 96%). M.p. 284-285 °C [Lit. 286-287 °C]; ν\text{max} (film)/cm\(^{-1}\) 3054br (O-H), 2724w (C-H), 2596w (C-H), 1656s (C=O); δ\text{H} (400 MHz; DMSO-d\(_6\)) 6.44 (1H, s, C(8)H), 7.29 (2H, td, J 7.6, 1.1, Ar-CH), 7.30-7.31 (1H, m, Ar-CH), 7.34 (2H, dd, J 8.6, 1.1, Ar-CH), 7.38-7.41 (2H, m, Ar-CH), 7.57 (2H, ddd, J 8.6, 7.6, 1.7, Ar-CH), 7.59-7.60 (1H, m, Ar-CH), 7.73 (1H, d, J 8.6, Ar-CH), 7.74-7.76 (1H, m, Ar-CH), 7.79-7.82 (1H, m, Ar-CH), 7.86 (2H, dd, J 7.6, 1.7, Ar-CH); δ\text{C} (100 MHz; DMSO-d\(_6\)) some signals coincident 36.5 (C(8)H), 103.6 (C(2) and C(10)), 115.7 (Ar-CH), 119.1 (Ar-C), 123.3, 124.1, 124.2, 125.0, 125.7, 126.2, 127.2, 127.3, 127.5, 131.4 (Ar-CH), 131.5, 133.0, 139.2 (Ar-C), 152.5, 164.6, 166.8 (C); m/z (+ES) 485 ([M+Na]\(^+\), 50%, 507, 100%); (Found 485.0995, C\(_{29}\)H\(_{18}\)NaO\(_6\) ([M+Na]\(^+\)) requires 485.0996).

5.2.52 3,3′-(naphthalen-2-ylmethylene)bis(4-hydroxy-5-methoxy-2H-chromen-2-one) (253a)

\[
\text{C}_{31}\text{H}_{22}\text{O}_8; \quad M_W=522.13 \text{ g/mol}
\]

A mixture of compound 163a (385.0 mg, 2.00 mmol) and 2-naphthaldehyde (156.6 mg, 1.00 mmol) in ethanol (10 mL) was stirred at the reflux temperature for 16 h and cooled to r.t.. The resulting precipitate was collected by filtration, washed with ethanol (5 × 20 mL)
and dried under vacuum to give the title compound (253a) as a white solid (449.0 mg, 86%). M.p. 251 °C (decomp.); \( \nu_{\text{max}} \) (film)/cm\(^{-1}\) 3314br (O-H), 3018w (C-H), 3048w (C-H), 3005w (C-H); \( \delta_{\text{H}} \) (400 MHz; DMSO-d\(_6\)) 3.95 (6H, s, OC\(_3\)H), 6.08 (1H, s, C(8)H), 6.99 - 7.03 (4H, m, Ar-C\(_{CH}\)), 7.38 - 7.43 (3H, m, Ar-C\(_H\)), 7.58 (2H, t, J 8.5, Ar-CH), 7.61 (1H, s, Ar-CH), 7.79 - 7.85 (2H, m, Ar-CH); \( \delta_{\text{C}} \) (100 MHz; DMSO-d\(_6\)) some signals coincident 37.5 (C(8)H), 56.8 (OCH\(_3\)), 104.0 (C(2) and C(10)), 106.4, 109.4 (Ar-CH), 115.4 (Ar-C), 124.7, 125.1, 125.6, 125.9, 126.9, 127.2, 127.4, 132.29, 132.34 (Ar-CH), 133.0, 135.75, 135.77, 137.0 (Ar-C), 152.7, 153.3, 154.1, 156.5 (C), 161.9, 162.5 (C); m/z (+ES) 545 ([M+Na]\(^{+}\), 100%); (Found 523.1384, C\(_{31}\)H\(_{23}\)O\(_8\) ([M+H]\(^{+}\)) requires 523.1387).

**Method B:**

General method for the synthesis of pro-drugs (315, 317 and 318 series):

To a solution of triphenylphosphine (2.0 eq.) in THF was added diiso-propyl azodicarboxylate (1.5 eq.) dropwise at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred for 15 min or until it turned cloudy. To this, an NQO1 inhibitor (1.0 - 2.0 eq.) in THF was added and the resulting mixture was stirred for 20 min. A solution of a corresponding substrate (92, 97, 159b, 1.0 eq.) in THF was added subsequently. The reaction was slowly warmed up to r.t. and left until reaction was completed (monitored by TLC analysis). The reaction mixture was concentrated \( \text{in vacuo} \) to give a crude product. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate partially removed the impurities.

A mixture of the intermediate obtained from above in TFA and water (7:1) was stirred (at 0 °C for substrates 92, 97 and r.t. for 159b) for 40 min. The reaction mixture was concentrated \( \text{in vacuo} \) to give a crude product. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate gave the target pro-drug.

**Method C:**

General method for the synthesis of pro-drugs (316 series):

To a solution of triphenylphosphine (2.0 eq.) in THF was added diiso-propyl azodicarboxylate (1.5 eq.) dropwise at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred for 15 min or until it turned cloudy. To this, an NQO1 inhibitor (1.0 -
2.0 eq.) in THF was added and the resulting mixture was stirred for 20 min. A solution of compound 155 (1.0 eq.) in THF was added subsequently. The reaction was slowly warmed up to r.t. and left for 16 h. The reaction mixture was concentrated in vacuo to give a crude product. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate gave the target pro-drug.

5.2.53 4-(((3R,4S)-4-(naphthalen-2-yl)-6-oxo-3-((triethylsilyl)oxy)cyclohex-1-en-1-yl)oxy)-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (333)

5.2.54 4-(((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl)methoxy)-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (315e)

333: C₄₉H₄₂O₅Si; Mₐₐ₉=666.28 g/mol
315e: C₃₇H₂₃O₅; Mₐₐ₉=552.19 g/mol

Using method B, compound 159b (81.1 mg, 0.21 mmol) was coupled to the NQO1 inhibitor 164c (127.9 mg, 0.43 mmol) to give compound 333, which was deprotected to afford the crude product as a yellow oil. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1 to 2:1) gave the title compound (315e) as a white solid (60.7 mg, 52%). M.p. 114-115 °C; [α]D²⁸ −72.8 (c 0.40 in CH₂Cl₂); νmax (film)/cm⁻¹ 3420br (O-H), 3053w (C-H), 2925w (C-H), 2917w (C-H), 2872w (C-H), 1678s (C=O), 1618s (C=O); δH (400 MHz; CDCl₃) 2.73-2.75 (2H, m, C(6)H₃), 3.35 (1H, td, J 10.8, 6.8, C(5)H), 4.19 (2H, s, C(11)H₂), 4.68 (1H, d, J 12.3, C(7)H₃), 4.70 (1H, dd, J 10.8, 17, C(4)H), 4.79 (1H, d, J 12.3, C(7)H₃), 7.13 (1H, d, J 1.7, C(3)H), 7.32-7.36 (2H, m, Ar-CH), 7.39 (1H, dd, J 8.6, 1.0, Ar-CH), 7.42-7.45 (2H, m, Ar-CH), 7.47 (1H, dd, J 8.6, 1.8, Ar-CH), 7.51-7.57 (3H, m, Ar-CH), 7.69 (1H, d, J 1.8, Ar-CH), 7.74 (1H, s, Ar-CH), 7.77-7.88 (7H, m, Ar-CH); δC (100 MHz; CDCl₃) some signals coincident 31.0 (C(11)H₂), 43.2 (C(6)H₃), 50.8 (C(5)H), 70.4 (C(7)H₂), 71.8 (C(4)H), 117.1 (Ar-CH), 117.2 (C(9)), 123.6, 124.4, 125.0, 125.6, 126.2, 126.5, 126.8, 127.1, 127.3, 127.5, 127.8, 127.86, 127.89, 128.4, 129.3, 131.9 (Ar-CH), 132.4, 133.1, 133.6, 133.7, 134.1 (Ar-CH), 136.4, 136.6 (C), 150.3 (C(3)H), 154.0 (C(9)H), 167.8 (C=O).
153.0, 163.3, 163.8 (C), 196.2 (C=O); m/z (+ES) 553 ([M+H]⁺, 100%); (Found 575.1829, C₃₇H₂₈NaO₅ ([M+Na]⁺) requires 575.1839).

5.2.55 3-benzyl-4-(((3R,4S)-4-(naphthalen-2-yl)-6-oxo-3-((triethylsilyl)oxy)cyclohex-1-en-1-yl)methoxy)-2H-chromen-2-one (334)

5.2.56 3-benzyl-4-(((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl)methoxy)-2H-chromen-2-one (315g)

334: C₃₉H₄₀O₅Si; M₇=616.26 g/mol
315g: C₃₃H₂₆O₅; M₇=502.18 g/mol

Using method B, compound 159b (81.1 mg, 0.21 mmol) was coupled to the NQO1 inhibitor 164e (108.5 mg, 0.43 mmol) to give compound 334, which was deprotected to afford the crude product as a yellow oil. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1 to 1:1) gave the title compound (315g) as a white solid (35.9 mg, 34%). M.p. 153-154 °C; [α]D²⁸ –84.3 (c 0.50 in CH₂Cl₂); νmax (film)/cm⁻¹ 3401br (O-H), 3057w (C-H), 3020w (C-H), 2956w (C-H), 2929w (C-H), 1677s (C=O), 1610s (C=O); δH (400 MHz; CDCl₃) 2.75 (1H, dd, J 16.6, 5.7, C(6)H), 2.79 (1H, dd, J 16.6, 12.1, C(6)H), 3.37 (1H, ddd, J 12.1, 9.8, 5.7, C(5)H), 3.93 (2H, s, C(11)H₂), 4.60 (1H, dt, J 12.3, 1.5, C(7)H₂H₉), 4.68 (1H, dt, J 12.3, 1.5, C(7)H₂H₉), 4.78 (1H, dd, J 9.8, 1.8, C(4)H), 7.12-7.14 (1H, m, Ar-CH), 7.15 (1H, d, J 1.8, C(3)H), 7.19-7.23 (3H, m, Ar-CH), 7.25-7.30 (4H, m, Ar-CH), 7.35 (1H, dd, J 8.5, 1.7, Ar-CH), 7.40-7.48 (2H, m, Ar-CH), 7.69 (1H, d, J 1.7, Ar-CH), 7.72 (1H, dd, J 8.5, 1.7, Ar-CH), 7.74-7.83 (3H, m, Ar-CH); δC (100 MHz; CDCl₃) some signals coincident 30.7 (C(11)H₂), 43.3 (C(6)H₂), 50.9 (C(5)H), 70.4 (C(7)H₂), 71.9 (C(4)H), 117.1 (Ar-CH), 117.6 (C(9)), 123.5, 124.4, 125.0, 126.5, 126.6, 126.8, 127.2, 127.86, 127.89, 128.67, 128.69, 129.3, 131.9 (Ar-CH), 133.1, 133.6 (Ar-O), 134.2, 136.4, 139.0 (C), 150.3 (C(3)H), 152.9, 163.0, 163.8 (C), 196.3 (C=O); m/z (+ES) 503 ([M+H]⁺, 100%); (Found 525.1672, C₃₃H₂₆NaO₅ ([M+Na]⁺) requires 525.1673).
5.2.57 4-(((3R,4S)-4-(naphthalen-2-yl)-6-oxo-3-((triethylsilyl)oxy)cyclohex-1-en-1-yl) methoxy)-2H-chromen-2-one (335)

5.2.58 4-(((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl)methoxy)-2H-chromen-2-one (315a)

335: C_{32}H_{34}O_{5}Si; M_w=526.22 g/mol
315a: C_{28}H_{20}O_{5}; M_w=412.13 g/mol

Using method B, compound 159b (480.0 mg, 1.25 mmol) was coupled to 4-hydroxycoumarin (405.4 mg, 2.50 mmol) to give compound 335, which was deprotected to afford the crude product as a yellow oil. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (3:1 to 1:1) gave the title compound (315a) as a white solid (358.4 mg, 70%). M.p. 232-234 °C; νmax (film)/cm⁻¹ 3365br (O-H), 3056w (C-H), 3018w (C-H), 2889w (C-H), 2868w (C-H), 1673s (C=O), 1622m (C=O); δ_H (400 MHz; DMSO-d₆) 2.58 (1H, dd, J 16.2, 3.9, C(6)H_eq), 3.09 (1H, dd, J 16.2, 13.8, C(6)H_ax), 3.42 (1H, ddd, J 13.8, 9.8, 3.9, C(5)H), 4.85 (1H, dd, J 9.8, 1.8, C(4)H), 4.96 (2H, s, C(7)H₂), 6.03 (1H, s, C(9)H), 7.27 (1H, d, J 1.8, C(3)H), 7.39 (1H, t, J 7.4, Ar-CH), 7.43 (1H, d, J 8.3, Ar-CH), 7.47-7.50 (2H, m, Ar-CH), 7.61 (1H, dd, J 8.3, 1.7, Ar-CH), 7.68 (1H, t, J 7.4, Ar-CH), 7.81 (1H, dd, J 8.3, 1.7, Ar-CH), 7.85-7.90 (4H, m, Ar-CH); δ_C (100 MHz; DMSO-d₆) 43.4 (C(6)H₂), 49.9 (C(5)H), 65.7 (C(7)H₂), 69.9 (C(4)H), 91.2 (C(9)H), 115.1 (C), 116.5, 122.9, 124.3, 125.6, 126.0, 126.4, 126.6, 127.45, 127.53, 127.7 (Ar-CH), 132.2, 132.9 (C), 133.0 (Ar-CH), 134.6, 139.2, 152.8 (C), 154.4 (C(3)H), 161.6, 164.6 (C), 196.5 (C=O); m/z (+ES) 413 ([M+H]^+), 100%; (Found 435.1210, C_{28}H_{20}NaO_{5} ([M+Na]^+) requires 435.1203).
5.2.59 4-(((3R,4S)-4-(naphthalen-2-yl)-6-oxo-3-((triethylsilyl)oxy)cyclohex-1-en-1-yl)methoxy)-2H-benzo[h]chromen-2-one (336)

5.2.60 4-(((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl)methoxy)-2H-benzo[h]chromen-2-one (315b)

336: C_{36}H_{36}O_{5}Si; M_w=560.20 g/mol
315b: C_{30}H_{22}O_{5}; M_w=462.15 g/mol

Using method B, compound 159b (81.1 mg, 0.21 mmol) was coupled to the chromenone species 163b (89.9 mg, 0.42 mmol) to give compound 336, which was deprotected to afford the crude product as a yellow oil. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (4:1 to 2:1) gave the title compound (315b) as a white solid (59.6 mg, 61%). M.p. 207-209 °C; ν_{max} (film)/cm^{-1} 3390br (O-H), 3054w (C-H), 2962w (C-H), 2922w (C-H), 2849w (C-H), 1674s (C=O), 1604m (C=O); δ_{H} (400 MHz; DMSO-d_6) 2.60 (1H, dd, J 16.7, 5.0, C(6)H_{eq}), 3.12 (1H, dd, J 16.7, 13.2, C(6)H_{ax}), 3.44 (1H, ddd, J 13.2, 9.8, 5.0, C(5)H), 4.86 (1H, dd, J 9.8, 1.8, C(4)H), 5.00 (2H, s, C(7)H_2), 6.15 (1H, s, C(9)H), 7.33 (1H, d, J 1.8, C(3)H), 7.47-7.53 (2H, m, Ar-CH), 7.62 (1H, dd, J 8.6, 1.6, Ar-CH), 7.73-7.77 (2H, m, Ar-CH), 7.82 (1H, d, J 8.6, Ar-CH), 7.88-7.91 (5H, m, Ar-CH), 8.07-8.09 (1H, m, Ar-CH), 8.38-8.41 (1H, m, Ar-CH); δ_{C} (100 MHz; DMSO-d_6) 43.4 (C(6)H_2), 49.8 (C(5)H), 65.6 (C(7)H_2), 69.9 (C(4)H), 90.7 (C(9)H), 110.5 (C), 118.5, 121.7 (Ar-CH), 122.1 (Ar-C), 123.9, 126.0, 126.4, 126.5, 127.5, 127.7, 128.1, 128.59, 128.63, 129.0, 131.5 (Ar-CH), 131.7, 132.1, 133.0, 134.8, 139.2, 149.9 (C), 154.6 (C(3)H), 161.5, 166.9 (C), 196.4 (C=O); m/z (+ES) 463 ([M+H]^+), 100%; (Found 463.1538, C_{30}H_{23}O_{5} ([M+H]^+) requires 463.1540).
5.2.61 5-methoxy-4-{((3R,4S)-4-(naphthalen-2-yl)-6-oxo-3-((triethylsilyl)oxy)cyclo hex -1-en-1-yl)methoxy}-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (337)

5.2.62 4-{((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl)methoxy)-5 -methoxy-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (315f)

337: C_{44}H_{44}O_{8}Si; M_w=696.29 g/mol
315f: C_{38}H_{39}O_{6}; M_w=582.20 g/mol

Using method B, compound 159b (70.2 mg, 0.18 mmol) was coupled to the NQO1 inhibitor 164a (121.6 mg, 0.37 mmol) to give compound 337, which was deprotected to afford the crude product as a yellow oil. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1 to 2:1) gave the title compound (315f) as a white solid (59.9 mg, 57%). M.p. 201-203 °C; [α]_D^{26} –46.5 (c 0.50 in CH_2Cl_2);
ν_max (film/cm⁻¹) 3414br (O-H), 3051w (C-H), 2953w (C-H), 2922w (C-H), 2852w (C-H),
1670s (C=O), 1597s (C=O); δ_H (400 MHz; CDCl_3) 2.70-2.73 (2H, m, C(6)H_2), 3.34 (1H,
ddd, J 11.5, 9.8, 6.5, C(5)H), 3.91 (3H, s, OCH_3), 4.14 (2H, d, J 5.6, C(11)H_2), 4.62 (1H,
dt, J 13.2, 1.7, C(7)H_2H_9), 4.68 (1H, dt, J 13.2, 1.7, C(7)H_2H_9), 4.72 (1H, dd, J 9.8, 1.8,
C(4)H), 6.77 (1H, dd, J 8.4, 1.0, Ar-CH), 7.00 (1H, dd, J 8.4, 1.0, Ar-CH), 7.19 (1H, d, J
1.8, C(3)H), 7.32 (1H, dd, J 8.4, 1.8, Ar-CH), 7.40-7.43 (2H, m, Ar-CH), 7.44-7.46 (1H, m,
Ar-CH), 7.48-7.54 (3H, m, Ar-CH), 7.67-7.68 (1H, m, Ar-CH), 7.74 (1H, s, Ar-CH), 7.75-
7.88 (6H, m, Ar-CH); δ_C (100 MHz; CDCl_3) 30.5 (C(11)H_2), 43.3 (C(6)H_2), 50.9 (C(5)H),
56.6 (OCH_3), 69.9 (C(7)H_2), 71.9 (C(4)H), 106.6 (Ar-CH), 107.6 (C), 110.1 (Ar-CH),
117.3 (C(9)), 125.0, 125.5, 126.1, 126.4, 126.8, 126.9, 127.1, 127.5, 127.7, 127.74, 127.77,
127.85, 127.88, 128.2, 129.2, 131.9 (Ar-CH), 132.3, 133.1, 133.6, 133.7, 134.6, 136.6,
137.1 (C), 149.0 (C(3)H), 154.8, 156.2, 163.4, 163.7 (C), 196.4 (C=O); m/z (+ES) 583
([M+H]^+, 100%); (Found 583.2115, C_{38}H_{31}O_{6} ([M+H]^+) requires 583.2115).
5.2.63 3-benzyl-5-methoxy-4-(((3R,4S)-4-(naphthalen-2-yl)-6-oxo-3-((triethylsilyl) oxy) cyclohex-1-en-1-yl)methoxy)-2H-chromen-2-one (338)

5.2.64 3-benzyl-4-(((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl) methoxy)-5-methoxy-2H-chromen-2-one (315h)

![Diagram of molecules 338 and 315h]

338: C₄₀H₄₂O₆Si; Mₓ=646.28 g/mol

315h: C₃₄H₂₈O₆; Mₓ=532.19 g/mol

Using method B, compound 159b (83.8 mg, 0.22 mmol) was coupled to the NQO1 inhibitor 164d (123.7 mg, 0.44 mmol) to give compound 338, which was deprotected to afford the crude product as a yellow oil. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (4:1 to 2:1) gave the title compound (315h) as a white solid (57.4 mg, 49%). M.p. 102-103 °C; [α]D⁰ –46.5 (c 0.50 in CH₂Cl₂); νmax (film)/cm⁻¹ 3417br (O-H), 3051w (C-H), 3030w (C-H), 2993w (C-H), 2965w (C-H), 2922w (C-H), 2837w (C-H), 1674s (C=O), 1600s (C=O); δH (400 MHz; CDCl₃) 2.80 (1H, dd, J 16.6, 5.8, C(6)Heq), 2.85 (1H, dd, J 16.6, 12.1, C(6)Hax), 3.42 (1H, ddd, J 12.1, 9.9, 5.8, C(5)H), 3.91 (3H, s, OC₃H₃), 3.96 (2H, d, J 3.8, C(11)H₂), 4.60 (1H, dt, J 12.9, 1.9, C(7)H₄H₅), 4.64 (1H, dt, J 12.9, 1.9, C(7)H₄H₅), 4.85 (1H, dd, J 9.9, 1.8, C(4)H), 6.76 (1H, d, J 8.4, Ar-CH), 6.97 (1H, d, J 8.4, Ar-CH), 7.15-7.19 (1H, m, Ar-CH), 7.24-7.28 (2H, m, C(3)H and Ar-CH), 7.34-7.36 (2H, m, Ar-CH), 7.42 (1H, dd, J 8.4, 1.6, Ar-CH), 7.43 (1H, t, J 8.4, Ar-CH), 7.48-7.53 (2H, m, Ar-CH), 7.76 (1H, d, J 1.6, Ar-CH), 7.82-7.89 (4H, m, Ar-CH); δC (100 MHz; CDCl₃) some signals coincident 30.3 (C(11)H₂), 43.3 (C(6)H₂), 51.0 (C(5)H), 56.6 (OCH₃), 69.8 (C(7)H₂), 71.9 (C(4)H), 106.6 (Ar-CH), 107.5 (C), 110.1 (Ar-CH), 117.7 (C(9)), 125.1, 126.4, 126.8, 127.1, 127.8, 128.6, 128.8, 129.2, 131.9 (Ar-CH), 133.1, 133.6, 134.7, 136.6, 139.5 (C), 149.1 (C(3)H), 154.7, 156.2, 163.6 (C), 196.6 (C=O); m/z (+ES) 533 ([M+H]+, 100%); (Found 533.1959, C₃₄H₂₉O₆ ([M+H]+) requires 533.1959).
5.2.65 5-methoxy-4-(((3R,4S)-4-(naphthalen-2-yl)-6-oxo-3-((triethylsilyl)oxy)cyclohex-1-en-1-yl)methoxy)-2H-chromen-2-one (339)

5.2.66 4-(((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl)methoxy)-5-methoxy-2H-chromen-2-one (315c)

![Chemical Structure](image-url)

**339**: C\textsubscript{33}H\textsubscript{36}O\textsubscript{6}Si; M\textsubscript{W}=556.23 g/mol

**315c**: C\textsubscript{27}H\textsubscript{23}O\textsubscript{6}; M\textsubscript{W}=442.14 g/mol

Using method B, compound **159b** (480.4 mg, 1.25 mmol) was coupled to the chromenone species **163a** (481.9 mg, 2.50 mmol) to give compound **339**, which was deprotected to afford the crude product as a yellow oil. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (3:1 to 1:1) gave the title compound (315c) as a white solid (63.9 mg, 12%). M.p. 216 °C, decomp.; \( \nu_{\text{max}} \) (film)/\( \text{cm}^{-1} \) \( 3493\text{br} \) (O-H), \( 2954\text{w} \) (C-H), \( 2918\text{w} \) (C-H), \( 2848\text{w} \) (C-H), \( 1704\text{s} \) (C=O), \( 1660\text{s} \) (C=O);

\( \delta \)\( H \) (400 MHz; DMSO-\( d_6 \)) 2.56 (1H, dd, \( J \) 16.2, 3.8, C(6)\( H_{eq} \)), 3.06 (1H, dd, \( J \) 16.2, 14.0, C(6)\( H_{ax} \)), 3.40 (1H, ddd, \( J \) 14.0, 9.6, 3.8, C(5)\( H \)), 3.93 (3H, s, OCH\textsubscript{3}), 4.85 (1H, ~dd, \( J \) 9.6, 1.7, C(4)\( H \)), 4.88 (2H, s, C(7)\( H_2 \)), 5.82 (1H, s, C(9)\( H \)), 6.98 (1H, s, Ar-CH), 7.00 (1H, s, Ar-CH), 7.36 (1H, d, \( J \) 1.7, C(3)\( H \)), 7.47-7.51 (2H, m, Ar-CH), 7.59 (1H, t, \( J \) 8.4, Ar-CH), 7.62 (1H, dd, \( J \) 8.4, 1.7, Ar-CH), 7.86-7.90 (4H, m, Ar-CH); \( \delta \)\( C \) (100 MHz; DMSO-\( d_6 \)) 43.6 (C(6)\( H_2 \)), 50.0 (C(5)\( H \)), 56.4 (OCH\textsubscript{3}), 65.7 (C(7)\( H_2 \)), 69.9 (C(4)\( H \)), 90.1 (C(9)\( H \)), 105.1 (C), 107.4, 109.1, 125.6, 126.0, 126.4, 126.5, 127.47, 127.54, 127.6 (Ar-CH), 131.6, 132.2, 133.0 (C), 133.1 (Ar-CH), 139.3 (Ar-\( C \)), 151.9 (C(3)\( H \)), 154.7, 157.4, 161.1, 166.7 (C), 196.4 (C=O);

m/z (+ES) 443 ([M+H]\textsuperscript{+}, 100%); (Found 443.1483, C\textsubscript{27}H\textsubscript{23}O\textsubscript{6} ([M+H]\textsuperscript{+}) requires 443.1489).
5.2.67  \((E)\)-5-methoxy-3-(((3R,4S)-4-(naphthalen-2-yl)-6-oxo-3-((triethylsilyl)oxy)cyclohex-1-en-1-yl)methoxy)naphthalen-1-yl)methylene)chromane-2,4-dione (340)

5.2.68  \((E)\)-3-(((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl)methoxy)naphthalen-1-yl)methylene)-5-methoxycromane-2,4-dione (315j)

\[
\text{340: } C_{44}H_{42}O_7Si; M_W=710.27 \text{ g/mol}
\]
\[
\text{315j: } C_{38}H_{28}O_{7}; M_W=596.18 \text{ g/mol}
\]

Using method B, compound 159b (83.8 mg, 0.22 mmol) was coupled to the NQO1 inhibitor 165a (138.4 mg, 0.44 mmol) to give compound 340, which was deprotected to afford the crude product as a yellow oil. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (5:1) gave the title compound (315j) as a yellow solid (60.4 mg, 46%). M.p. 155 °C (decomp.); \([\alpha]_D^{29} -25.0 (c 0.50 \text{ in } \text{CH}_2\text{Cl}_2);

\nu_{\text{max}} (\text{film})/\text{cm}^{-1} 3425\text{br (O-H), }3052\text{w (C-H), }2958\text{w (C-H), }2919\text{w (C-H), }2842\text{w (C-H), }1736\text{s (C=O), }1668\text{s (C=O), }1595\text{m (C=O);} \delta_{\text{H}} (400 \text{ MHz; CDCl}_3) 2.62-2.65 \text{ (2H, m, C(6)H)}_2, 3.21 \text{ (1H, td, } J 10.2, 7.0, C(5)H), 3.80 \text{ (3H, s, OCH}_3), 4.54 \text{ (1H, dd, } J 10.2, 1.9, C(4)H), 4.79 \text{ (2H, q, J 1.7, C(7)H)}_2, 6.67 \text{ (1H, d, } J 1.2, \text{ Ar-CH), 6.69 \text{ (1H, d, } J 1.2, \text{ Ar-CH), 6.95 \text{ (1H, q, } J 1.9, C(3)H), 7.23 \text{ (1H, dd, } J 8.4, 1.8, \text{ Ar-CH), 7.39 \text{ (1H, t, } J 8.4, \text{ Ar-CH), 7.45-7.50 \text{ (3H, m, Ar-CH), 7.56-7.57 \text{ (1H, m, Ar-CH), 7.60 \text{ (1H, ddd, } J 8.4, 7.3, 1.2, \text{ Ar-CH), 7.74 \text{ (1H, ddd, } J 8.4, 7.3, 1.2, \text{ Ar-CH), 7.76-7.82 \text{ (3H, m, Ar-CH), 7.91 \text{ (1H, dd, } J 8.4, 1.2, \text{ Ar-CH), 8.08 \text{ (1H, d, } J 9.0, \text{ Ar-CH), 8.37 \text{ (1H, d, } J 8.4, \text{ Ar-CH), 9.27 \text{ (1H, s, C(10)H); } \delta_{\text{C}} (100 \text{ MHz; CDCl}_3) 43.4 \text{ (C(6)H), 50.6 \text{ (C(5)H), 56.2 \text{ (OCH}_3), 64.7 \text{ (C(7)H)}_2, 71.6 \text{ (C(4)H), 104.9, 105.6 \text{ (Ar-CH), 113.2 \text{ (C), 116.9 \text{ (Ar-CH), 119.1 \text{ (C), 122.0 \text{ (Ar-CH), 124.8 \text{ (C), 125.2, 126.2, 126.6, 126.7, 126.9, 127.8, 127.9, 128.8, 129.1, 129.2 \text{ (Ar-CH), 130.0, 130.7 \text{ (C), 132.0 \text{ (Ar-CH), 132.7, 133.5, 134.1 \text{ (Ar-C), 135.9 \text{ (Ar-CH), 137.0 \text{ (C), 142.7 \text{ (C(10)H), 148.8 \text{ (C(3)H), 156.1, 156.4, 156.7, 158.3 \text{ (C), 190.2, 196.6 \text{ (C=O); } m/z (\text{ES}) 597 ([M+H]^+, 100\%); (Found 619.1727, C_{38}H_{28}O_{7}Na ([M+Na]^+) requires 619.1727).} \]
5.2.69 3-Benzyl-4-((6-oxyocyclohex-1-en-1-yl)methoxy)-2H-benzo[h]chromen-2-one (316d)

\[
\text{C}_{27}\text{H}_{22}\text{O}_4; \text{M}_W=410.15 \text{ g/mol}
\]

Using method C, compound 155 (65.9 mg, 0.52 mmol) was coupled to the NQO1 inhibitor 162b (316.1 mg, 1.05 mmol) to afford the crude product. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (6:1 to 5:1) gave the title compound (316d) as a white solid (24.2 mg, 11%). M.p. 177-179 °C; \( \nu_{\text{max}} \) (film)/cm\(^{-1}\) 3325br (O-H), 3064w (C-H), 3027w (C-H), 2948w (C-H), 2886w (C-H), 2861w (C-H), 1678s (C=O), 1607s (C=O); \( \delta_\text{H} \) (400 MHz; CDCl\(_3\)) 2.08 (2H, quint, J 6.0, C(5)H\(_2\)), 2.46-2.54 (4H, m, C(4)H\(_2\) and C(6)H\(_2\)), 4.03 (2H, s, C(11)H\(_2\)), 4.70 (2H, q, J 1.3, C(7)H\(_2\)), 7.16 (1H, t, J 4.2, C(3)H), 7.17-7.20 (1H, m, Ar-CH), 7.25-7.29 (2H, m, Ar-CH), 7.35-7.37 (2H, m, Ar-CH), 7.61-7.64 (2H, m, Ar-CH), 7.70 (1H, d, J 8.7, Ar-CH), 7.79 (1H, d, J 8.7, Ar-CH), 7.85-7.88 (1H, m, Ar-CH), 8.53-8.56 (1H, m, Ar-CH); \( \delta_\text{C} \) (100 MHz; CDCl\(_3\)) some signals coincident 22.8 (C(5)H\(_2\)), 26.1 (C(4)H\(_2\)), 30.7 (C(11)H\(_2\)), 38.3 (C(6)H\(_2\)), 71.4 (C(7)H), 112.9, 116.9 (C), 119.5, 122.6 (Ar-CH), 123.2 (Ar-C), 124.3, 126.5, 127.2, 127.9, 128.60, 128.63, 128.69, 128.71 (Ar-CH), 134.86, 134.90, 139.3 (C), 149.7 (C(3)H), 150.3, 163.9, 164.2 (C), 198.1 (C=O); m/z (+ES) 411 ([M+H]\(^+\), 100%); (Found 411.1583, C\(_{27}\)H\(_{22}\)O\(_4\) ([M+H]\(^+\)) requires 411.1591).

5.2.70 5-methoxy-3-(naphthalen-2-ylmethyl)-4-((6-oxyocyclohex-1-en-1-yl)methoxy)-2H-chromen-2-one (316f)

\[
\text{C}_{28}\text{H}_{24}\text{O}_5; \text{M}_W=440.16 \text{ g/mol}
\]

Using method C, compound 155 (44.8 mg, 0.35 mmol) was coupled to a chromenone species 164a (116.2 mg, 0.35 mmol) to afford the crude product. Purification by flash
column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (7:1 to 6:1) gave the title compound (316f) as a white wax (41.8 mg, 27%). ν\text{max} (film)/cm⁻¹ 2960w (C-H), 2929w (C-H), 2873w (C-H), 1677s (C=O), 1602s (C=O); δ\text{H} (400 MHz; CDCl₃) 2.04 (2H, quint, J 6.4, C(5)H₂), 2.42-2.48 (4H, m, C(4)H₂ and C(6)H₂), 3.87 (3H, s, OCH₃), 4.09 (2H, s, C(11)H₂), 4.61 (2H, q, J 1.7, C(7)H₂), 6.75 (1H, dd, J 8.4, 1.0, Ar-CH), 6.98 (1H, dd, J 8.4, 1.0, Ar-CH), 7.16 (1H, t, J 4.2, C(3)H), 7.38-7.41 (2H, m, Ar-CH), 7.43 (1H, t, J 8.4, Ar-CH), 7.48 (1H, dd, J 8.4, 1.8, Ar-CH), 7.71-7.77 (4H, m, Ar-CH); δ\text{C} (100 MHz; CDCl₃) 22.9 (C(5)H₂), 26.0 (C(4)H₂), 30.5 (C(11)H₂), 38.3 (C(6)H₂), 56.5 (OCH₃), 70.7 (C(7)H₂), 106.5 (Ar-CH), 107.7 (C), 110.1 (Ar-CH), 117.5 (C(9)), 125.3, 125.9, 127.0, 127.5, 127.66, 127.71, 128.0, 131.8 (Ar-CH), 132.3, 133.7, 135.2, 137.1 (C), 147.8 (C(3)H), 154.8, 156.2, 163.4, 163.7 (C), 198.1 (C=O); m/z (+ES) 441 ([M+H]+, 50%, 131, 100%); (Found 441.1689, C_{28}H_{25}O_{5} ([M+H]+) requires 441.1697).

5.2.71 3-benzyl-5-methoxy-4-((6-oxocyclohex-1-en-1-yl)methoxy)-2H-chromen-2-one (316h)

C_{28}H_{25}O_{5}; M_w=390.15 g/mol

Using method C, compound 155(44.8 mg, 0.35 mmol) was coupled to a chromenone species 164d (98.7 mg, 0.35 mmol) to afford the crude product. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (6:1 to 4:1) gave the title compound (316h) as a white wax (41.8 mg, 31%). ν\text{max} (film)/cm⁻¹ 3025w (C-H), 2936w (C-H), 2880w (C-H), 2866w (C-H), 2836w (C-H), 1670s (C=O), 1601s (C=O); δ\text{H} (400 MHz; CDCl₃) 2.07 (2H, quint, J 6.1, C(5)H₂), 2.46-2.51 (4H, m, C(4)H₂ and C(6)H₂), 3.87 (3H, s, OCH₃), 3.91 (2H, s, C(11)H₂), 4.59 (2H, q, J 1.7, C(7)H₂), 6.74 (1H, dd, J 8.4, 1.0, Ar-CH), 6.97 (1H, dd, J 8.4, 1.0, Ar-CH), 7.15-7.19 (2H, m, C(3)H and Ar-CH), 7.22-7.26 (2H, m, Ar-CH), 7.32-7.34 (2H, m, Ar-CH), 7.41 (1H, t, J 8.4, Ar-CH); δ\text{C} (100 MHz; CDCl₃) 22.9 (C(5)H₂), 26.0 (C(4)H₂), 30.3 (C(11)H₂), 38.3 (C(6)H₂), 56.5 (OCH₃), 70.7 (C(7)H₂), 106.5 (Ar-CH), 107.7 (C), 110.1 (Ar-CH), 117.8 (C(9)), 126.2, 128.4, 128.5, 128.9, 129.0, 131.7 (Ar-CH), 135.3, 139.6 (C), 147.7 (C(3)H), 154.8, 156.2,
161.2, 163.4 (C), 198.1 (C=O); m/z (+ES) 391 ([M+H]^+, 50%, 129, 100%); (Found 413.1357, C_{24}H_{22}O_{5}Na ([M+Na]^+) requires 413.1359).

5.2.72 (E)-3-((2-((6-oxocyclohex-1-en-1-yl)methoxy)naphthalen-1-yl)methylene) chromane-2,4-dione (316i)

\[
\text{C}_{27}\text{H}_{20}\text{O}_{5}, M_W=424.45 \text{ g/mol}
\]

To a solution of alcohol 155 (49.8 mg, 0.40 mmol) in THF (1.5 mL) were added triphenylphosphine (104.9 mg, 0.40 mmol) and 165b (252.9 mg, 0.80 mmol) at r.t. Diisopropyl azodicarboxylate (85 µL, 0.42 mmol) was added to the resulting solution dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, then at r.t. for 22 h. The solvent was evaporated in vacuo to give the crude product as a yellow oil. Purification by flash silica column chromatography, eluting first with petroleum ether (40-60) and ethyl acetate (6:1 to 4:1), then with DCM and 1% methanol gave the title compound (316i) as a yellow solid (100.5 mg, 59%). M.p. 176-178 °C; ν max (film)/cm⁻¹ 3069 (C-H), 2947 (C-H), 2871 (C-H), 2822 (C-H), 1735 (C=O, ester), 1735 (C=O), 1653 (C=O), 1735 (C=O), 1653 (C=O), 1568s (C=O); δH (400 MHz; CDCl₃) 1.56 (2H, quint, J 6.4, C(5)H₂), 1.67-1.71 (2H, m, C(4)H₂), 2.07-2.10 (2H, m, C(6)H₂), 4.65 (2H, q, J 1.9, C(7)H₂), 6.65 (1H, t, J 4.1, C(3)H), 6.98 (1H, dd, J 8.3, 1.1, Ar-CH), 7.11 (1H, td, J 7.3, 1.1, Ar-CH), 7.47 (1H, d, J 9.0, Ar-CH), 7.52 (1H, ddd, J 8.3, 7.3, 1.8, Ar-CH), 7.62 (1H, ddd, J 8.3, 7.3, 1.1, Ar-CH), 7.75 (1H, ddd, J 8.3, 7.3, 1.1, Ar-CH), 7.78 (1H, dd, J 7.3, 1.8, Ar-CH), 7.94 (1H, dd, J 8.3, 1.1, Ar-CH), 8.08 (1H, d, J 9.0, Ar-CH), 8.35 (1H, d, J 8.3, Ar-CH), 8.95 (1H, s, C(10)H); δC (100 MHz; CDCl₃) 22.4 (C(5)H₂), 25.4 (C(4)H₂), 37.9 (C(6)H₂), 65.6 (C(7)H₂), 112.6 (Ar-CH), 113.1 (C), 116.6, 121.5, 121.9, 126.7 (Ar-CH), 128.2, 128.4 (Ar-C), 129.2, 129.3 (Ar-CH), 129.7, 130.3 (Ar-C), 130.9 (Ar-CH), 134.4 (Ar-C), 134.5, 135.0 (Ar-CH), 139.8 (C(10)H), 147.2 (C(3)H), 155.0, 157.9, 158.7 (C), 191.3, 197.7 (C=O); m/z (+ES) 425 ([M+H]^+, 100%); (Found 463.0948, C_{27}H_{20}O_{5}K ([M+K]^+) requires 463.0954).
5.2.73  \((E)-5\)-methoxy-3-((2-((6-oxocyclohex-1-en-1-yl)methoxy)naphthalen-1-yl)methylene)chromane-2,4-dione (316j)

\[
\text{C}_{28}\text{H}_{22}\text{O}_6; \text{M}_W=454.14 \text{ g/mol}
\]

Using method B, compound 155 (50.0 mg, 0.39 mmol) was coupled to the NQO1 inhibitor 165a (250.6 mg, 0.79 mmol) to afford the crude product. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (6:1 to 4:1) gave the title compound (316j) as a yellow solid (77.5 mg, 44%). M.p. 164 °C (decomp.); $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3060w (C-H), 3011w (C-H), 2929w (C-H), 2886w (C-H), 2837w (C-H), 1737s (C=O), 1666s (C=O), 1594m (C=O); $\delta_H$ (400 MHz; CDCl$_3$) 1.82 (2H, quint, $J_{6.3}$, C(5)H$_2$), 2.09-2.14 (2H, m, C(4)H$_2$), 2.29-2.32 (2H, m, C(6)H$_2$), 3.78 (3H, s, OC$_3$H$_3$), 4.71 (2H, q, $J_{2.0}$, C(7)H$_2$), 6.6 (1H, s, Ar-C), 6.65 (1H, s, Ar-CH), 6.90 (1H, t, J 4.3, C(3)H), 7.36 (1H, t, J 8.3, Ar-CH), 7.47 (1H, d, J 9.0, Ar-CH), 7.51 (1H, d, J 8.3, 7.0, 1.3, Ar-CH), 7.75 (1H, ddd, J 8.3, 7.0, 1.3, Ar-CH), 7.94 (1H, d, J 8.3, 1.3, Ar-CH), 8.09 (1H, d, J 9.0, Ar-CH), 8.34 (1H, d, J 8.3, Ar-CH), 9.24 (1H, s, C(10)H); $\delta_C$ (100 MHz; CDCl$_3$) 22.6 (C(5)H$_2$), 25.6 (C(4)H$_2$), 38.1 (C(6)H$_2$), 56.2 (OCH$_3$), 65.3 (C(7)H$_2$), 104.6, 105.4 (Ar-CH), 113.0 (C), 116.8 (Ar-CH), 118.6 (C), 121.8 (Ar-CH), 124.8 (Ar-C), 126.6, 129.2, 129.3 (Ar-CH), 129.9, 130.3 (C), 132.0 (Ar-CH), 134.4 (C(2)), 135.8 (Ar-CH), 142.5 (C(10)H), 146.8 (C(3)H), 155.9, 157.0, 158.1, 158.2 (C), 190.2, 198.4 (C=O); m/z (+ES) 455 ([M+H]$^+$, 100%); (Found 477.1309, C$_{28}$H$_{22}$O$_6$Na ([M+Na]$^+$) requires 477.1311).
5.2.74 (S)-4-(((3-((tert-butyldimethylsilyl)oxy)-6-oxocyclohex-1-en-1-yl)methoxy))-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (341)

5.2.75 (S)-4-(((3-hydroxy-6-oxocyclohex-1-en-1-yl)methoxy)-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (317e)

341: \( \text{C}_{33}\text{H}_{36}\text{O}_5\text{Si}; M_W=540.23 \text{ g/mol} \)

317e: \( \text{C}_{27}\text{H}_{22}\text{O}_5; M_W=426.15 \text{ g/mol} \)

Using method B, compound 92 (106.6 mg, 0.42 mmol) was coupled to compound 164c (251.6 mg, 0.83 mmol) to give compound 341, which was deprotected to afford the crude product. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (4:1 to 1:1) gave the title compound (317e) as white solid (102.2 mg, 57%). M.p. 138-140 °C; \([\alpha]_D^{29} = -21.3 (c 0.50 \text{ in CH}_2\text{Cl}_2); \nu_{\text{max}} \text{ (film)/cm}^{-1} 3417\text{br (O-H)}, 3054\text{w (C-H)}, 2951\text{w (C-H)}, 2924\text{w (C-H)}, 1679\text{s (C=O)}, 1611\text{s (C=O)}; \delta_H (400 \text{ MHz; CDCl}_3) 2.01 (1H, t\dd, J 12.8, 9.0, 4.6, C(5)H), 2.23 (1H, d\dd, J 12.8, 4.6, 1.6, C(5)H), 2.36 (1H, d, J 16.9, 12.8, 4.6, C(6)H), 2.62 (1H, dt, J 16.9, 4.6, C(6)H), 4.14 (2H, s, C(11)H), 4.57 (1H, d, J 9.0, 4.6, 2.5, C(4)H), 4.61 (1H, dt, J 12.2, 1.4, C(7)H, 4.70 (1H, dt, J 12.2, 1.4, C(7)H), 6.98 (1H, dd, J 2.5, 1.6, C(3)H), 7.30 (1H, d, J 8.5, 7.3, 1.1, Ar-CH), 7.37 (1H, dd, J 8.5, 1.1, Ar-CH), 7.40-7.43 (2H, m, Ar-CH), 7.45 (1H, dd, J 8.5, 1.7, Ar-CH), 7.53 (1H, d, J 8.5, 7.3, 1.7, Ar-CH), 7.71 (1H, d, J 1.7, Ar-CH), 7.74-7.78 (4H, m, Ar-CH), \delta_C (100 \text{ MHz; CDCl}_3)\text{ some signals coincident} 31.0 (\text{C}(11)H), 32.4 (\text{C}(5)H), 35.6 (\text{C}(6)H), 66.5 (\text{C}(4)H), 70.6 (\text{C}(7)H), 117.1 (\text{Ar-CH}), 117.2 (\text{C}(9)), 123.6, 124.4, 125.6, 126.2, 126.8, 127.2, 127.7, 127.8, 128.3, 131.9 (\text{Ar-CH}), 132.4, 133.6, 134.2, 136.6 (\text{C}), 150.3 (\text{C}(3)H), 153.0, 163.3, 163.8 (\text{C}), 196.9 (\text{C}=\text{O}); m/z (+ES) 427 ([M+H]^+, 100%); (Found 427.1539, \text{C}_{27}\text{H}_{23}\text{O}_5 ([M+H]^+)\text{ requires} 427.1540).
5.2.76 (S)-3-benzyl-4-((3-((tert-butyldimethylsilyl)oxy)-6-oxocyclohex-1-en-1-yl) methoxy)-5-methoxy-2H-chromen-2-one (342)

5.2.77 (S)-3-benzyl-4-((3-hydroxy-6-oxocyclohex-1-en-1-yl)methoxy)-5-methoxy-2H-chromen-2-one (317h)

342: C_{30}H_{36}O_{6}Si; M_w=520.23 g/mol
317h: C_{24}H_{22}O_{6}; M_w=406.14 g/mol

Using method B, compound 92 (64.4 mg, 0.25 mmol) was coupled to the NQO1 inhibitor 164d (71.0 mg, 0.25 mmol) to give compound 342, which was deprotected to afford the crude product. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (4:1 to 1:1) gave the title compound (317h) as a white solid (62.7 mg, 62%). M.p. 166-168 °C; [α]_D^{27} −43.4 (c 0.50 in (CH_3)CO); ν_{max} (film)/cm\(^{-1}\) 3381br (O-H), 2957w (C-H), 2922w (C-H), 2854w (C-H), 2833w (C-H), 1699s (C=O), 1651s (C=O); δ_H (400 MHz; CDCl_3) 2.02 (1H, tdd, J 13.3, 9.3, 4.6, C(5)H), 2.38 (1H, dtd, J 13.3, 4.6, 1.6, C(5)H), 2.44 (1H, ~dt, J 16.8, 4.6, C(6)H), 2.66 (1H, dt, J 16.8, 4.6, C(6)H), 3.86 (3H, s, OCH_3), 3.92 (2H, s, C(11)H), 4.54 (1H, dt, J 13.2, 1.8, C(7)H, 4.58 (1H, dt, J 13.2, 1.8, C(7)H), 4.67 (1H, ddt, J 9.3, 4.6, 2.5, C(4)H), 6.74 (1H, dd, J 8.4, 1.0, Ar-CH), 6.97 (1H, dd, J 8.4, 1.0, Ar-CH), 7.13 (1H, dd, J 2.5, 1.6, C(3)H), 7.41-7.18 (1H, m, Ar-CH), 7.22-7.26 (2H, m, Ar-CH), 7.30-7.33 (2H, m, Ar-CH), 7.42 (1H, t, J 8.4, Ar-CH); δ_C (100 MHz; CDCl_3) some signals coincident 30.3 (C(11)H), 32.6 (C(5)H), 35.6 (C(6)H), 56.5 (OCH_3), 66.6 (C(4)H), 69.9 (C(7)H), 106.5 (Ar-CH), 107.5 (C), 110.1 (Ar-CH), 117.7 (C(9)), 126.4, 128.5, 128.8, 131.8 (Ar-CH), 134.9, 139.5 (C), 148.5 (C(3)H), 154.8, 156.2, 163.2, 163.4 (C), 197.1 (C(1)=O); m/z (+ES) 407 ([M+H]^+) 50%, (116, 100%); (Found 407.1483, C_{24}H_{22}O_{6} ([M+H]^+) requires 407.1489).
5.2.78 (S)-4-((3-((tert-butyldimethylsilyl)oxy)-6-oxocyclohex-1-en-1-yl)methoxy)-5-methoxy-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (343)

5.2.79 (S)-4-((3-hydroxy-6-oxocyclohex-1-en-1-yl)methoxy)-5-methoxy-3-(naphthalen-2-ylmethyl)-2H-chromen-2-one (317f)

343: C_{34}H_{38}O_6Si; M_W=570.24 g/mol
317f: C_{28}H_{24}O_6; M_W=456.16 g/mol

Using method B, compound 92 (84.6 mg, 0.33 mmol) was coupled to the NQO1 inhibitor 164a (109.7 mg, 0.33 mmol) to give compound 343, which was deprotected to afford the crude product. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (3:1 to 1:1) gave the title compound (317f) as a white solid (45.2 mg, 30%). M.p. 172-173 °C; [α]D^27 −53.5 (c 0.50 in (CH}_3)_2CO); ν_{max}(film)/cm^{−1} 3443 br (O-H), 2945 w (C-H), 2930 w (C-H), 2869 w (C-H), 1690 s (C=O), 1667 s (C=O); δ_H (400 MHz; CDCl_3) 1.96 (1H, tdd, J 12.5, 9.3, 4.6, C(5)H), 2.33 (1H, ddd, J 9.3, 4.6, 2.4, C(4)H), 4.56 (1H, ddd, J 13.1, 1.8, C(7)H), 7.72-7.78 (4H, m, Ar-C-H); δ_C (100 MHz; CDCl_3) 30.6 (C(11)H_2), 32.5 (C(5)H_2), 35.6 (C(6)H_2), 56.5 (OCH_3), 66.6 (C(4)H), 69.9 (C(7)H_2), 106.6 (Ar-C-H), 107.6 (C(9)), 110.1 (Ar-C-H), 117.5 (C), 125.5, 126.0, 126.9, 127.4, 127.69, 127.71, 128.1, 131.9 (Ar-C-H), 132.3, 133.7, 134.8, 137.1 (C), 148.6 (C(3)H), 154.8, 156.2, 163.3, 163.6 (C), 197.1 (C(1)=O); m/z (+ES) 457 ([M+H]^+, 100%); (Found 479.1455, C_{28}H_{24}O_6Na ([M+Na]^+) requires 479.1465).
5.2.80 (S,E)-3-((2-((3-((tert-butyldimethylsilyl)oxy)-6-oxocyclohex-1-en-1-yl)methoxy)naphthalen-1-yl)methylene)-5-methoxychromane-2,4-dione (344)

5.2.81 (S,E)-3-((2-((3-hydroxy-6-oxocyclohex-1-en-1-yl)methoxy)naphthalen-1-yl)methylene)-5-methoxychromane-2,4-dione (317j)

344: C$_{34}$H$_{36}$O$_7$Si; M$_W$=584.22 g/mol
317j: C$_{28}$H$_{22}$O$_7$; M$_W$=470.14 g/mol

Using method B, compound 92 (67.7 mg, 0.26 mmol) was coupled to the NQO1 inhibitor 165a (91.4 mg, 0.26 mmol) to give compound 344, which was deprotected to afford the crude product. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (4:1 to 1:1) gave the title compound (317j) as a yellow solid (43.1 mg, 35%). M.p. 150-153 °C; $[\alpha]_D^{27}$ -26.1 (c 0.25 in (CH)$_3$CO); $\nu_{\text{max}}$(film)/cm$^{-1}$ 3393br (O-H), 2960w (C-H), 2925w (C-H), 2866w (C-H), 2845w (C-H), 1731m (C=O), 1669s (C=O), 1596m (C=O); $\delta$H (400 MHz; CDCl$_3$) 1.83 (1H, tdd, J 12.6, 9.2, 4.6, C(5)H), 2.21 (1H, ddd, J 12.6, 4.6, 1.6, C(5)H), 2.29 (1H, ddd, J 16.7, 12.6, 4.6, C(6)H), 2.52 (1H, dt, J 16.7, 4.6, C(6)H), 3.78 (3H, s, OCH$_3$), 4.40 (1H, ddt, J 9.2, 4.6, 2.5, C(4)H), 4.70 (1H, dt, J 14.5, 2.0, C(7)H$_2$H$_b$), 4.75 (1H, dt, J 14.5, 2.0, C(7)H$_2$H$_b$), 6.63 (1H, d, J 8.3, Ar-CH), 6.65 (1H, d, J 8.3, Ar-CH), 6.89 (1H, dd, J 2.5, 1.6, C(3)H), 7.36 (1H, t, J 8.3, Ar-CH), 7.47 (1H, d, J 9.0, Ar-CH), 7.61 (1H, ddd, J 8.3, 1.2, Ar-CH), 7.74 (1H, ddd, J 8.3, 7.0, 1.2, Ar-CH), 7.94 (1H, dd, J 8.3, 1.2, Ar-CH), 8.10 (1H, d, J 9.0, Ar-CH), 8.32 (1H, d, J 8.3, Ar-CH), 9.22 (1H, s, C(10)H); $\delta$C (100 MHz; CDCl$_3$) some signals coincident 32.1 (C(5)H$_2$), 35.6 (C(6)H$_2$), 56.2 (OCH$_3$), 64.6 (C(7)H$_2$), 66.3 (C(4)H), 104.7, 105.6 (Ar-CH), 113.2 (C), 116.8 (Ar-CH), 118.9 (C), 121.9 (Ar-CH), 124.6 (C), 126.7, 129.3, 129.4 (Ar-CH), 129.9, 130.4 (C), 132.1 (Ar-CH), 134.2 (C), 136.1 (Ar-CH), 143.0 (C(10)H), 148.6 (C(3)H), 156.0, 156.6, 158.2 (C), 190.4, 197.6 (C=O); m/z (+ES) 493 ([M+Na]$^+$, 100%); (Found 493.1258, C$_{28}$H$_{22}$O$_7$Na ([M+Na]$^+$) requires 493.1251).
5.2.82  \( (E)-3-((2-((2S,3S,4aR,8aR)-2,3-dimethoxy-2,3-dimethyl-7-oxo-2,3,4a,7,8, 8a-hexahydrobenzo[b][1,4]dioxin-6-yl)methoxy)naphthalen-1-yl)methylene)-5-methoxychromane-2,4-dione \) (345)

5.2.83  \( (E)-3-((2-(((3R,4R)-3,4-dihydroxy-6-oxocyclohex-1-en-1-yl)methoxy)naphthalen-1-yl)methylene)-5-methoxychromane-2,4-dione \) (318j)

\[ \text{345: C}_{34}H_{32}O_{10}; M_W=600.20 \text{ g/mol} \]

\[ \text{318j: C}_{28}H_{22}O_{8}; M_W=486.13 \text{ g/mol} \]

Using method B, compound 97 (80.0 mg, 0.29 mmol) was coupled to the NQO1 inhibitor 165a (185.7 mg, 0.79 mmol) to give compound 345 which was deprotected to afford the crude product. Purification by flash column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (3:1 to 1:2) gave the title compound (318j) as a yellow solid (52.4 mg, 37%). M.p. 148 °C (decomp.); \([\alpha]_D^{29} – 25.9 \) (c 0.50 in CHCl₃); \( \nu_{\text{max}} \) (film)/cm⁻¹ 3369br (O-H), 2978w (C-H), 2956w (C-H), 2925w (C-H), 2849w (C-H), 1737s (C=O), 1668s (C=O); \( \delta_H \) (400 MHz; CDCl₃) 2.38 (1H, dd, J 16.5, 12.3, C(6)H₆ax), 2.77 (1H, dd, J 16.5, 4.7, C(6)H₆eq), 3.75 (3H, s, OC₃H₃), 3.88 (1H, ddd, J 12.3, 8.4, 4.7, C(5)H), 4.30 (1H, d, J 8.4, C(4)H), 4.69 (2H, s, C(7)H₂), 6.61 (1H, d, J 8.4, Ar-CH), 6.64 (1H, d, J 8.4, Ar-CH), 6.84 (1H, s, C(3)H), 7.36 (1H, t, J 8.4, Ar-CH), 7.44 (1H, t, J 9.0, Ar-CH) 7.59 (1H, t, J 7.6, Ar-CH), 7.71 (1H, t, J 7.6, Ar-CH), 7.91 (1H, d, J 8.4, Ar-CH), 8.08 (1H, d, J 9.0, Ar-CH), 8.24 (1H, d, J 8.4, Ar-CH), 9.16 (1H, s, C(10)H); \( \delta_C \) (100 MHz; CDCl₃) some signals coincident 44.3 (C(6)H₆), 56.2 (OCH₃), 64.5 (C(7)H), 72.5 (C(5)H), 73.1 (C(4)H), 104.8, 105.6 (Ar-CH), 113.1 (C), 116.8 (Ar-CH), 118.5 (C), 121.8 (Ar-CH), 123.9 (C), 126.8, 129.3, 129.4 (Ar-CH), 129.8, 130.4 (C), 132.2 (Ar-CH), 134.8 (C), 136.5 (Ar-CH), 143.8 (C(10)H), 146.8 (C(3)H), 156.0, 156.5, 158.1 (C), 190.8, 196.1 (C=O); \( m/z \) (+ES) 509 ([M+Na⁺], 100%); (Found 487.1387, C₂₈H₂₃O₈ ([M+H⁺]⁺) requires 487.1386).
5.2.84 N-(2-(((3R,4S)-3-hydroxy-4-(naphthalen-2-yl)-6-oxocyclohex-1-en-1-yl)methyl) t-hio)ethyl)acetamide (346)

\[ \text{C}_{21}\text{H}_{23}\text{NO}_3\text{S; } M_W=369.14 \text{ g/mol} \]

To a solution of N-acetyl cysteamine (47 µL, 0.44 mmol) in DCM (1.3 mL) were added Et₃N (0.1 mL, 0.40 mmol) and a solution of 315a (165 mg, 0.40 mmol) in DCM (2 mL). The reaction was stirred at r.t. for 6 h and quenched with H₂O (3 mL). The product was extracted with DCM (3 × 3 mL) and the combined extract was washed with NaHCO₃ (sat. aq., 6 mL), HCl (0.1 M, 6 mL) and brine (6 mL). The extract was dried over MgSO₄ and concentrated in vacuo to yield the crude product as a yellow oil. Purification by flash silica column chromatography, eluting with petroleum ether (40-60) and ethyl acetate (1:2) partially removed the impurity. \( \left[ \alpha \right]_{D}^{27} = -61.4 \text{ (c 0.50 in (CH}_2\text{Cl}_2); } \nu_{\text{max}}(\text{film})/\text{cm}^{-1} = 3296\text{br} (\text{O}-\text{H}), 3075\text{w} (\text{C}-\text{H}), 3051\text{w} (\text{C}-\text{H}), 2963\text{w} (\text{C}-\text{H}), 2929\text{w} (\text{C}-\text{H}), 2868\text{w} (\text{C}-\text{H}), 1652\text{s} (\text{C}=\text{O}). 1632\text{s} (\text{C}=\text{O}); \delta_{\text{H}} (400 \text{ MHz; CDCl}_3) 2.02 \text{ (3H, s, } \text{C}(10)\text{H}_3), 2.61 \text{ (1H, dt, } J 13.4, 6.9, \text{C}(8)\text{H}), 2.69 \text{ (1H, dt, } J 13.4, 6.9, \text{C}(8)\text{H}), 2.80-2.83 \text{ (2H, m, } \text{C}(6)\text{H}_2), 3.30 \text{ (1H, d, } J 13.8, \text{C}(7)\text{H}), 3.41-3.47 \text{ (4H, m, } \text{C}(7)\text{H}, \text{C}(9)\text{H}_2 \text{ and } \text{C}(5)\text{H}), 4.79 \text{ (1H, d, } J 9.3, \text{C}(4)\text{H}), 6.21 \text{ (1H, s, NH), 7.00 \text{ (1H, d, } J 1.7, \text{C}(3)\text{H}), 7.44 \text{ (1H, dd, } J 8.5, 1.8, \text{Ar-CH}), 7.47-7.52 \text{ (2H, m, Ar-CH), 7.75 \text{ (1H, d, } J 1.8, \text{Ar-CH), 7.81-7.87 \text{ (3H, m, Ar-CH); } \delta_{\text{C}} (100 \text{ MHz; CDCl}_3) 23.4 \text{ (C}(10)\text{H}_3), 29.8 \text{ (C}(7)\text{H}_2), 31.1 \text{ (C}(8)\text{H}_2), 39.3 \text{ (C}(9)\text{H}_2), 43.5 \text{ (C}(6)\text{H}_2), 50.8 \text{ (C}(5)\text{H}), 71.8 \text{ (C}(4)\text{H}), 125.4, 126.2, 126.6, 126.9, 127.8, 127.9, 129.0 \text{ (Ar-CH), 133.0, 133.6, 135.1, 137.2 (Ar-\text{C}, 149.9 \text{ (C}(3)\text{H), 170.8 \text{ (C}=\text{O), 196.9 \text{ (C}(1)=\text{O); } m/z (+ES) 392 ([M+Na]^+), 50\%), 386 (100\%); (Found 370.1467, C}_{21}\text{H}_{24}\text{O}_3\text{NS ([M+H]^+ requires 370.1471).} \right)
VI. Biological Assays and Biotransformation Methods and Materials

6.1 Cell Culture

All chemicals used for cell culturing were supplied by Sigma-Aldrich (Missouri, U.S.A.). All handlings of cells were performed under sterile conditions in a Class II laminar flow microbiological safety cabinet.

6.1.1 The Cell Lines

Information regarding the cell lines used in the MTT cell viability assays is listed in Table 6.1.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell type</th>
<th>Type</th>
<th>Tissue of origin</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>Epithelial</td>
<td>Human</td>
<td>Lung</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Epithelial</td>
<td>Human</td>
<td>Breast</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>FaDu</td>
<td>Epithelial</td>
<td>Human</td>
<td>Pharynx</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>PNT2</td>
<td>Epithelial</td>
<td>Human</td>
<td>Prostate</td>
<td>Healthy prostate</td>
</tr>
</tbody>
</table>

A549, FaDu and PNT2 cell lines were maintained in RPMI-1640 medium with 10% fetal calf serum (FCS) or fetal bovine serum (FBS) and 2 mM L-glutamine (1%); MDA-MB-231 cell line was maintained in DMEM medium with 10% FCS or FBS and 2mM L-glutamine (1%). All media used were free from mycoplasma contamination.

6.1.2 Sub-Culturing the Cell Line

The cell line was cultured in T-75 tissue culture flasks (Falcon, Becton Dickinson, New Jersey, U.S.A.), and kept in a humidified incubator, presetting at 37 °C within a 5% CO₂ atmosphere.

Once the cells monolayer in the culture flask reached 70% confluence, the cell line was sub-cultured. The growth medium in the flask was aspirated and the monolayer of cell was washed with sterile phosphate buffered saline (PBS, pH = 7.4). The cells were trypsinised and incubated at 37 °C for 5 to 10 minutes. The Trypsin/EDTA was then neutralised by the addition of 8 mL of fresh growth medium. The cells were cultured into a new T-75 flask with fresh growth media. The overall dilution ratio varied from 1:2 to 1:4.
6.1.3 Cryopreservation of the Cell Line
The cell lines are preserved at \(-80 \, ^\circ C\) for further use. The freezing was performed when the cells were 70% confluent. The cells were trypsinised, re-suspended in fresh growth medium and centrifuged at 1300 rpm (Megafuge 1.0, Heraeus, Hanau, Germany) for 5 minutes. The medium was aspirated and the cell pellet was re-suspended in freezing medium (see Table 6.2 for components of freezing media). 1 mL of the resulting cell suspension was transferred into a 1.6 mL cryovial (Greiner bioone, Frickenhausen, Germany). The cells were frozen firstly in a \(-20 \, ^\circ C\) then in a \(-80 \, ^\circ C\) freezer.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Components of freezing medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>40% growth medium, 50% FCS/FBS, 10% DMSO</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>70% growth medium, 20% FCS/FBS, 10% DMSO</td>
</tr>
<tr>
<td>FaDu</td>
<td>95% growth medium, 5% DMSO</td>
</tr>
<tr>
<td>PNT2</td>
<td>90% growth medium, 10% DMSO</td>
</tr>
</tbody>
</table>

6.1.4 Thawing Cells
Frozen cells were rapidly thawed at 37 °C. The resulting cell suspension was transferred into 4 mL of pre-warmed growth medium (37 °C) in universal tube (Medline Scientific, Chalgrove, U.K.) and centrifuged for 5 minutes at 1300 rpm. The media was aspirated and the cell pellet was re-suspended in 5 mL of fresh growth medium. The resulting cell suspension was transferred into fresh growth media in T-25 tissue culture flask (Falcon, Becton Dickinson, New Jersey, U.S.A.). The dilution ratio was 1:1. The cells were incubated at 37 °C within an atmosphere of 5% CO\(_2\), and were used for studies after two sub-culture passages.

6.2 MTT Cell Viability Assay
All chemicals used for MTT cell viability assay were supplied by Sigma-Aldrich. All handlings of cells were performed under sterile conditions in a Class II laminar flow microbiological safety cabinet.

6.2.1 MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
MTT powder (50 mg) was dissolved in 20 mL of PBS (pH = 7.4). The resulting solution was kept in 50 mL conical-bottom tubes, wrapped with aluminium foil and stored at 4 °C.
6.2.2 Counting and Seeding Cells
Cells were seeded in seven columns sextuplicately across the 96-well microtitre plates in 180 μL culture media at a seeding density of 1000 cells/mL, incubated at 37 °C for 24 hours prior to drug treatments.

The concentration of the cell suspension is determined using a haemocytometer (Neubauer, Germany). A cover-slip was placed over the counting chamber of the haemocytometer, and 10 μL of cell suspension was transferred onto the chamber. The chamber was placed under a light microscope and the cells in the four corner squares were counted in an order from 1 to 4 (Figure 6.5).

![Figure 6.5: The haemocytometer grid.](image)

The concentration per millilitre of cells is determined using the following equation:

\[
\text{Cells/mL} = \text{Mean cell number of corner squares} \times 10^4
\]

6.2.3 Treating Cells
The compound to be tested was dissolved in DMSO at a concentration of 25 mM as the stock solution. Five serial dilutions with concentrations at 2.5 mM, 0.25 mM, 250 μM, 25 μM and 2.5 μM were prepared from the stock solution. 40 μL of stock solution and each serial dilution were added into 960 μL of growth media in 6-well microtitre plate and 20 μL of each resulting new dilution were added to 180 μL of cell suspension in an ascending order of concentration. The final drug concentrations are, therefore, at 1 nM, 10 nM, 100 nM, 1 μM, 10 μM and 100 μM. The same amount of DMSO was used in solvent control group in a separate column. The plates were incubated at 37 °C for 96 hours.
6.2.4 Ending the MTT Viability Assay

50 μL of MTT solution (2.5 mg/mL) was added to the plate after 96 hours of incubation to terminate the experiment. The plate was incubated at 37 °C for 4 hours, after which the solution in each well was aspirated and 200 μL of DMSO was added to dissolve the resulting formazan crystals. The plate was gently shaken for 5 minutes prior to the reading. Absorbance for each well was read at a wavelength of 540 nm on a multi-well scanning spectrophotometer (μQuant Microplate Spectrophotometer, BioTek, Potton, U.K.) using the Gen5 software package (BioTek, Vermont, U.S.A.). The optical density (OD) values, which are proportional to the number of viable cells, were calculated using average absorption values.

6.2.5 Calculating IC$_{50}$ Concentrations

The OD values were used to determine the half maximal inhibitory concentration (IC$_{50}$) of the test compounds. With the OD values, a dose-response curve (percentage of cell viability against the logarithm of concentration of compound (Log C)) could be generated using GraphPad Prism®. The IC$_{50}$ could be hence obtained from the curve.
6.3 Biotransformation using P450 enzyme

All chemicals used for P450 biotransformation were supplied by Sigma-Aldrich unless otherwise stated. Cell culturing was performed under sterile conditions.

6.3.1 Transfection
Frozen *E. Coli* BL21(DE3) cells were thawed gradually on ice for 10 minutes. 1 μL of pET28a-P450 plasmid (BM3 and RhF) was added into 25 μL of thawed cell suspension. The mixture was left on ice for 30 minutes and heat shocked at 42 °C for 10 seconds. 800 μL of SOC outgrowth medium (New England BioLabs, Massachusetts, U.S.A.) was added and the resulting mixture was incubated (Multitron, Infors HT, Basel, Switzerland) at 250 rpm, 37 °C for 1 hour.

The mixture was centrifuged at 4000 rpm (Centrifuge 5810R, Eppendorf, Hamburg, Germany) for 1 minute and the supernatant was removed. The remaining solution was mixed from which 250 μL was transferred onto an LB agar plate supplemented with kanamycin (50 μg/mL). The plate was incubated at 37 °C overnight.

6.3.2 Inoculation
Multi-colonies were scraped off, preserved in 10 mL of lysogeny broth kanamycin (LB Kan) supplemented with 0.4% glycerol and incubated at 250 rpm, 37 °C overnight.

6.3.3 Large-Scale Protein Expression
8 mL of starter culture was added to 800 mL of sterile LB Broth (Miller) medium (2.5 g/100 mL in deionised water) with 800 μL of kanamycin (50 μg/mL in deionised water). The resulting large culture was incubated at 250 rpm, 37 °C until an OD₆₀₀ between 0.5-0.8 was reached (spectrophotometer Ultrospec 3000, Amersham Pharmacia Biotech, Uppsala, Sweden).

Expression was induced by adding 320 μL of *iso*-propyl β-D-1-thiogalactpyranoside (IPTG) (or 240 μL of δ-aminolevulinic acid (ALA) for RhF) to the culture and the resulting mixture was incubated at 250 rpm, 20 °C overnight.

6.3.4 Enzyme Purification
The cell culture expressing the desired protein was centrifuged at 4500 rpm for 10 minutes and the residue precipitated was re-suspended in binding buffer on ice.
The re-suspended culture was sonicated intermittently (20 s × 20 s × 8) and then centrifuged (Avanti J-E Centrifuge, Beckman Coulter, CA, U.S.A.) at 18000 rpm, 4 °C for 20 minutes, after which the resulting lysate was filtered through a syringe filter with a 0.22 µm pore size.

Ni-NTA agarose in ethanol (Thermo Fischer Scientific, New Hampshire, U.S.A.) was centrifuged at 4000 rpm, 6 °C for 4 minutes and the ethanol layer was removed. Filtered deionised water (5 × volume of agarose) was added to the agarose and the mixture was centrifuged at 4000 rpm for 2 minutes. The aqueous layer was removed and binding buffer (5 × volume of agarose) was added and the mixture was centrifuged at 4000 rpm for 2 minutes, after which the buffer layer was removed.

Washed agarose was added into the crude enzyme solution and the resulting mixture was shaken at 4 °C for 1 hour for binding.

The enzyme-agarose mixture was transferred into a 25 mL gravity column (GE Healthcare, Illinois, U.S.A.), eluting with binding buffer, wash buffer and elution buffer successively. Fractions collected were kept on ice. The fraction containing the desired protein isolated with elution buffer was dialysed against 1 L of NaPi buffer (pH = 7.8) twice.

### 6.3.5 Biotransformation

A mixture of components listed in Table 6.4 was incubated at 400 rpm, 20 °C for 48 hours.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Stock</th>
<th>Final concentration</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>100 mg/mL</td>
<td>10 mg/mL</td>
<td>50</td>
</tr>
<tr>
<td>GDH</td>
<td>20 mg/mL</td>
<td>1 mg/mL</td>
<td>25</td>
</tr>
<tr>
<td>NADPH/NADP⁺</td>
<td>20 mg/mL</td>
<td>1 mg/mL</td>
<td>25</td>
</tr>
<tr>
<td>NaPi buffer</td>
<td>50 mM</td>
<td>50 mM</td>
<td>100</td>
</tr>
<tr>
<td>Enzymes or vector⁺</td>
<td>20 mg/mL (30 mg/mL for RhF)</td>
<td>10 mg/mL (15 mg/mL for RhF)</td>
<td>250</td>
</tr>
<tr>
<td>Substrate⁺</td>
<td>2.5 mM or 20 mM</td>
<td>250 µM or 2 mM</td>
<td>50</td>
</tr>
</tbody>
</table>

* Enzymes used were BM3, RhF, Pro8, Pro14; empty vector used was pET28a.

β-ionone was used as positive control; DMSO was used as blank control.

Acetonitrile was used for extracting the same amount of reaction mixture. The organic extracts were dried (MgSO₄), filtered and submitted for GC-MS (Angilent 6850, CA, U.S.A.) analysis. Methyl t-butyl ether (MTBE) was used for extracting the same volume of
reaction mixture containing β-ionone. The organic extracts were dried (MgSO₄), filtered and submitted for GC-MS analysis.
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