Development of an approach to novel hybrid analogues of COTC and Antheminone A as potential anticancer agents

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School of Chemistry
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Abstract

The glyoxalase pathway is vital for the survival of many organisms and is an important focus of research in the area of cancer. A number of antiproliferative agents have been isolated from natural sources, including COTC, antheminone A, the phorbasins and the gabosines, which share a common α-oxyalkylcyclohex-2-enone moiety. These compounds are believed to interfere with the glyoxalase pathway, however their precise mechanism of action still remains unknown. As a continuation of previous research this study was concerned with the synthesis of novel hybrid analogues of COTC and antheminone A, which were selected in order to gain important information regarding the ideal C5 substituent for optimum bioactivity. Compounds which contained a bromophenyl substituent were therefore synthesised which subsequently will be assayed for their antiproliferative activity towards non-small-cell lung cancer cell lines.
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Declaration

No portion of the work referred to in the dissertation has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Finally, I want to express my great appreciation to my mother, father and my sister for their help and motivation throughout my postgraduate degree. This dissertation is dedicated to my mother, Yeter Kaskun.
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Abbreviations

°C  degree celcius
δ  chemical shift
[α]_D  optical rotation
+ES  positive ion electroscopy
Ac  Acetyl
acac  acetylacetonyl
aq.  aqueous
ax  axial
BDA  butane-1,2-diacetal
n-Bu  n-butyl
cat.  catalytic
cm⁻¹  wavenumber (s)
cod  1,5-cyclooctadiene
COMC  2-crotonyloxymethylcyclohex-2-enone
COSY  correlation spectroscopy
COTC  2-crotonyloxymethyl-(4R,5R,6R)-4,5,6-trihydroxy cyclohex-2-enone
DBSA  dodecylbenzenesulfonic acid
DBU  1,8-diazabicyclo[5.4.0]undec-7-ene
DCM  dichloromethane
DIBAL-H  diisobutylaluminium hydride
DMAP  4-dimethylaminopyridine
DME  1,2-dimethoxyethane
DMF  N,N-dimethylformamide
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>eq</td>
<td>equatorial</td>
</tr>
<tr>
<td>eq.</td>
<td>equivalent(s)</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GI&lt;sub&gt;50&lt;/sub&gt;</td>
<td>half maximal growth inhibitory concentration</td>
</tr>
<tr>
<td>Glx I</td>
<td>glyoxalase I</td>
</tr>
<tr>
<td>Glx II</td>
<td>glyoxalase II</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>GST</td>
<td>glutathione S-transferase</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple bond correlation</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>KHMDS</td>
<td>potassium hexamethyldisilazide</td>
</tr>
<tr>
<td>IR</td>
<td>infrared spectroscopy</td>
</tr>
<tr>
<td>L</td>
<td>litre(s)</td>
</tr>
<tr>
<td>LiHMDS</td>
<td>lithium bis(trimethyl)amide</td>
</tr>
<tr>
<td>m</td>
<td>metre(s)</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>m.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>m/z</td>
<td>mass/charge ratio</td>
</tr>
</tbody>
</table>
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M-B-H Morita-Baylis-Hillman
MDR multidrug resistant
min minute(s)
mol mole(s)
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR nuclear magnetic resonance
NBS N-bromosuccinimide
Nu nuclophile
PEPPSI-IPr dichloro-[1,3-bis(di-isopropylphenyl)imidazolidene]
RNA ribonucleic acid
ROESY rotating frame Overhauser effect spectroscopy
r.t. room temperature
SDS sodium dodecyl sulfate
t tert
TBS tert-butylidimethylsilyl
TES triethylsilyl
Tf trifluoromethanesulfonyl
TFA trifluoroacetic acid
THF tetrahydrofuran
TGI total growth inhibition
TIPS triisopropylsilyl
TLC thin layer chromatography
TMS trimethylsilyl
Rf Retention factor
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1. Introduction

1.1. Importance of COTC and the Glyoxalase System

COTC is a natural compound which was isolated from cultures of *Streptomyces griseosporeus* in 1975 by Takeuchi and co-workers and was shown to exhibit cytotoxic properties toward human lung cancer cells (Figure 1).1

![COTC structure](image1)

**Figure 1**: COTC, 2-crotonyloxymethyl-(4R, 5R, 6R)-4,5,6-trihydroxycyclohex-2-enone

Takeuchi and collaborators proposed that the antitumor properties of COTC were a consequence of its reaction with the thiol group of glutathione. After conjugation with glutathione, the glyoxalase pathway was believed to be inhibited, thus leading to cell apoptosis (Scheme 1).1

![Reaction scheme](image2)

**Scheme 1**: Reaction of COTC with glutathione

Glyoxalase I is an enzyme that is responsible for the degradation of methylglyoxal, a side-product arising from cellular metabolism: methylglyoxal, also known as pyruvaldehyde, is an antiproliferative agent because of its toxicity.1 Many detoxification mechanisms for methylglyoxal have been evolved in order to protect cells, and perhaps the best documented of these is the glyoxalase pathway.2

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The glyoxalase pathway involves two enzyme catalysed reactions: a hemithioacetal is initially formed by conjugation of methylglyoxal with glutathione (GSH), then glyoxalase I catalyses the conversion of this adduct to S-D-lactoyl-glutathione (Scheme 2). Subsequently, glyoxalase II catalyses the conversion of S-D-lactoyl-glutathione to D-lactate. Many researchers have targeted inhibition of glyoxalase I in the context of cancer treatment, due to the resulting increase in methylglyoxal concentration.²

\[ \text{Scheme 2: Detoxification of methylglyoxal catalysed by glyoxalase I} \]

Tumor cells show elevated levels of methylglyoxal and consequently, glyoxalase I inhibitors are one of interest as potential anticancer agents.

1.2. Mechanism of antitumor activity of COTC and COMC

Aghil and co-workers demonstrated that 2-crotonyloxymethyl-2-cyclohexenone-(COMC), the synthetic non-hydroxlated analogue of COTC, possessed greater potency than COTC in all cases toward a panel of cancer cells (Figure 2).³

\[ \text{Figure 2: COMC, (2-crotonyloxymethylcyclohex-2-enone)} \]

As described above, COTC and its analogues have been shown to exhibit antitumor properties, however their mechanism of action which, in principle, may involve a simple
alkylation via $S_N2$ substitution, still remains unproven.\textsuperscript{4} Takeuchi and co-workers proposed initially that inhibition of glyoxalase I by COTC, proceeded by the reaction of the thiol group of GSH displacing its crotonate moiety at pH 7.4.\textsuperscript{1} This reaction was proposed to be a vital feature of the mechanism of glyoxalase inhibition.

Alternative findings reported by Joseph and collaborators indicated that Glx I only binds weakly to the putative inhibitor which is derived from COTC. This investigation therefore, suggested a new mechanism whereby the exocyclic enone reacts with DNA/RNA or intracellular proteins leading to apoptosis. This mechanism could explain why cancer cells exhibit enhanced sensitivity to the cyclohexenone analogues as they upregulate GSH/GST production compared with normal cells in order to protect themselves.\textsuperscript{5}

Research conducted by Ganem and co-workers has illustrated that rather than a direct $S_N2$ substitution, there is an additional step whereby COMC behaves as a Michael acceptor towards GSH and a highly reactive exocyclic enone is generated.\textsuperscript{6,7} Thus, COTC, and its nonhydroxlated analogue COMC, react with the thiol group of reduced glutathione to form a glutathionylated exocyclic enone 9 which may then alkylate proteins or DNA/RNA leading to apoptosis. Alternatively, reaction with a second molecule of GSH may lead to the product 10, formerly derived from direct $S_N2$ reaction at C-2\textsuperscript{1} (Scheme 3).\textsuperscript{17}
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Scheme 3: Proposed mechanism of action of COTC and COMC

1.3. Other natural products related to COTC

1.3.1. Antheminone A

Antheminone A is another example of a cyclohexenone-containing natural product which exhibits anticancer properties. It was isolated from Anthemis maritima and is believed to react with glutathione in a similar manner to COMC. Collu and collaborators isolated antheminone A from the ethyl acetate extract of Anthemis Maritima and the structure of antheminone A was established using $^1$H NMR and $^{13}$C NMR spectroscopy. HMBC experiments helped identify the structure of antheminone A and $^1$H NMR data were used to assign its relative stereochemistry, although that at C5$^1$ is still unknown (Figure 3).

Figure 3: Antheminone A, 4-hydroxy-5-((1-hydroxy-1,5-dimethyl-4-hexenyl)-2-(hydroxymethyl)-2-cyclohexen-1-one

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Antheminone A exhibits weaker antitumor activity than its synthetic analogue COMC, but its mechanism of reaction with GSH is believed to be similar. Table 1 illustrates the IC₅₀ values for antheminone A towards a range of cancer cell lines after 72 hours of exposure. It is clear that antheminone A has a powerful effect towards those tested cancer cell lines except the MCF-7 cell line. Antheminone A is, therefore, a reasonable lead compound for subsequent drug discovery.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>HCT-116 (Colon)</th>
<th>CaCo-2 (Colon)</th>
<th>MCF-7 (Breast)</th>
<th>HL-60 (Leukemia)</th>
<th>U-937 (Leukemia)</th>
<th>Jurkat-T (Leukemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀(µM)</td>
<td>15 ± 2</td>
<td>11 ± 1</td>
<td>21 ± 2</td>
<td>7.6 ± 0.6</td>
<td>6.2 ± 3</td>
<td>9.0 ± 0.4</td>
</tr>
</tbody>
</table>

Table 1: IC₅₀ values for antheminone A towards a range of cancer cell lines after 72 hours exposure to the drug

1.3.2. The Gabosines

The gabosines are a family of poly-hydroxylated carbocyclic natural products which can also be viewed as carbosugars. Although gabosines B, F and O are saturated cyclohexanones, the other gabosines, isolated from a *Streptomyces* strain, are unsaturated cyclohexenones. Gabosines C and E are a non-crotonylated analogue of COTC and their structures are depicted in Figure 4. Many members of the family of natural products have been synthesised including gabosines A, C, D, E, F and O.

![Figure 4](image-url)

Figure 4: Structures of gabosine C and E and a generic structure for all gabosines
For the most part, the gabosines have not been found to possess powerful biological properties such as anticancer, antibiotic or antifungal activities: they do, however, bind weakly with DNA. Gabosine E has also been shown to be a weak inhibitor of cholesterol biosynthesis.\textsuperscript{11}

1.3.3. The Phorbasins

The phorbasins are a family of diterpenes which were extracted from a \textit{Phorbas Sp.}, in the Great Australian Bight.\textsuperscript{14} The structures of Phorbasins B, C, J and K are depicted in \textbf{Figure 5}.\textsuperscript{15}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{The structures of Phorbasins B, C, J and K.}\textsuperscript{15}
\end{figure}

Phorbasins B, C, J and K were tested against cancer cell lines NFF, A549, HT29 and MM96L and were found to show significant activities. \textbf{Table 2} shows the value of GI\textsubscript{50}, TGI and LC\textsubscript{50} (the concentration needed to inhibit cell growth by 50\%, the concentration needed to inhibit cell growth by 100\% and the concentration needed to kill 50\% of the cells respectively) towards NFF, A549, HT29 and MM96L cancer cells.\textsuperscript{15}
Table 2: The GI\text{50}, TGI and LC\text{50} values of Phorbasins B, C, J and K towards NFF, A549, HT29 and MM96L cell lines\textsuperscript{15}

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>NFF</th>
<th>A549</th>
<th>HT29</th>
<th>MM96L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phorbasin B</td>
<td>GI\text{50}</td>
<td>6.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>TGI</td>
<td>13.5</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>LC\text{50}</td>
<td>26.9</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Phorbasin C</td>
<td>GI\text{50}</td>
<td>5.3</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>TGI</td>
<td>12.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>LC\text{50}</td>
<td>23.9</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Phorbasin J</td>
<td>GI\text{50}</td>
<td>&gt;29.8</td>
<td>11.9</td>
<td>&gt;29.8</td>
</tr>
<tr>
<td></td>
<td>TGI</td>
<td>&gt;29.8</td>
<td>&gt;29.8</td>
<td>&gt;29.8</td>
</tr>
<tr>
<td></td>
<td>LC\text{50}</td>
<td>&gt;29.8</td>
<td>&gt;29.8</td>
<td>&gt;29.8</td>
</tr>
<tr>
<td>Phorbasin K</td>
<td>GI\text{50}</td>
<td>&gt;70</td>
<td>&gt;70</td>
<td>&gt;70</td>
</tr>
<tr>
<td></td>
<td>TGI</td>
<td>&gt;70</td>
<td>&gt;70</td>
<td>&gt;70</td>
</tr>
<tr>
<td></td>
<td>LC\text{50}</td>
<td>&gt;70</td>
<td>&gt;70</td>
<td>&gt;70</td>
</tr>
</tbody>
</table>

On the other hand, Phorbasins J and K, which do not possess a cyclohexenone moiety, showed the weakest cytotoxicity of all the family of natural products. This study indicates that the cyclohex-2-enone moiety is an important structural feature for inhibition of cancer cell growth\textsuperscript{15}.

1.4. Synthesis of the natural products

1.4.1. COTC synthesis

COTC and antheminone A are naturally occurring compounds, but because of the small quantities that can be isolated, they are important targets for organic synthesis. Due to the powerful antitumor activity of COTC in particular, a number of research groups have attempted to synthesise COTC and its analogues. A notable synthesis of COTC, which
employed cyclohexylidene protected (-)-quinic acid 20 as a starting material, is shown below in Scheme 4.\textsuperscript{16}

\textbf{Scheme 4.} Synthesis of COTC from (-)-quinic acid\textsuperscript{16}
Reagents and conditions: i) Tf\textsubscript{2}O (2.2 eq.), pyridine, CH\textsubscript{2}Cl\textsubscript{2}, 65\%; ii) CsOAc, DMF; iii) NBS-H\textsubscript{2}O, DMF, 72\%; iv) DIBAL-H, benzene-toluene, 65\%; v) LiN(TMS), -78 °C, 87\%; vi) CH\textsubscript{3}SO\textsubscript{3}H, DMSO, r.t., 1.5 h; then Et\textsubscript{3}N, r.t., 5 min, 71\%; vii) crotonic anhydride, DCC, DMAP, THF, 54\%; viii) 1:1 TFA/H\textsubscript{2}O, 73\%.

Compound 20 was treated with an excess of 2 equivalents of Tf\textsubscript{2}O then with cesium acetate to give diene 22 via a triflate intermediate 21. Subsequent electrophilic addition to the more reactive alkene with N-bromosuccinimide, and DMF gave bromoformate 23. Reduction of the formate ester 23 was achieved using DIBAL-H to give bromo alcohol 24.

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20
which was treated with lithium hexamethyldisilazide to give epoxide 25. The course of this reaction sequence involves epoxide ring opening followed by Kornblum oxidation of 25 using DMSO and Et$_3$N, furnishing hydroxyketone 26 which was selectively esterified to give crotonate 27. Acid mediated removal of the cyclohexylidene protecting group from 27 gave 2-crotonyloxymethyl-(4R, 5R, 6R)-4,5,6-trihydroxycyclohex-2- enone (COTC, 1).

1.4.2. COMC synthesis

A number of research groups have also investigated the synthesis of the simplified non-hydroxylated analogue, COMC. In 1992, Aghil and co-workers employed 1-bromo-2-cyclohexenone 28 as a starting material; the route, they employed is illustrated in Scheme 5.\(^3\)

Scheme 5: Synthesis of COMC from 1-bromo-2-cyclohexenone\(^3\)
Reagents and conditions: i) ethylene glycol, H\(^+\), benzene, 22%; ii) n-BuLi, THF, DMF, -78 °C, 74%; iii) CeCl$_3$, NaBH$_4$, CH$_3$OH, -78 °C; iv) oxalic acid, H$_2$O, CH$_2$Cl$_2$, 90%; v) crotonic anhydride then pyridine, DMAP, CH$_2$Cl$_2$, 92%.

Subsequently in 2002 Ganem and co-workers reported a more efficient route for the synthesis of COMC. This route involved only 2 steps which are illustrated in Scheme 6.\(^17\)

In this approach 2-cyclohexenone 33 was used as the substrate in a Morita-Baylis-Hillman

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reaction to give 2-methanolcyclohexenone 34 which was esterified using crotonic anhydride and DMAP to give COMC 7.\textsuperscript{17}

\[
\begin{align*}
33 & \rightarrow \text{HOCH}_2\text{C} & 34 & \rightarrow & 7 \\
\text{H}_2\text{O} & & & & \\
\text{i)} & & & & ii)
\end{align*}
\]

**Scheme 6**: Synthesis of COMC from 2-cyclohexenone 33\textsuperscript{17}
Reagents and conditions: i) DMAP (cat.) H\textsubscript{2}CO, THF/H\textsubscript{2}O (1:1), 24 h, 40 °C, 65%; ii) crotonic anhydride, pyridine, DMAP (cat.), CH\textsubscript{2}Cl\textsubscript{2}, rt, 1.5 h, 38%.

1.5. Previous work carried out by the Whitehead research group

A number of analogues of COTC, with the generic structure 35, have been prepared by the Whitehead group. These structures contain 3 loci for diversification (C4, C5 and C6) were chosen in order to identify the structural features necessary for potent antitumor activity (Figure 6).\textsuperscript{18-21}

**Figure 6**: Generic structure of COTC analogues prepared by the Whitehead group\textsuperscript{18}
1.5.1. **Analogues synthesised previously by the Whitehead group**

![Chemical structures](image1)

**Figure 7:** The initial series of COTC analogues synthesised by the Whitehead group reported in 2007\(^\text{18}\)

![Chemical structures](image2)

**Figure 8:** The second series of COTC analogues reported by the Whitehead group in 2008\(^\text{19}\)

![Chemical structures](image3)

**Figure 9:** The most recent series of COTC analogues reported by the Whitehead group in 2013\(^\text{21}\)
Development of an approach to novel hybrid analogues of COTC and Antheminone A as potential anticancer agents

Members of the Whitehead group have synthesised number of analogues of COTC and antheminone A which are shown in Figures 7, 8 and 9. The synthesis of compounds 36, 37 and 41 was accomplished in order to investigate the effect of the degree of hydroxylation of the cyclohexenone skeleton on antitumour activity. Moreover, these compounds were selected in order to discover the importance of absolute stereochemistry on the anticancer activity of the analogues. Compound 38 was selected in order to identify the influence of a highly electronegative flourine atom at C5 and compound 39 was chosen to discover whether a conjugated ketone moiety was necessary for antiproliferative activity.\(^\text{18-19}\)

Compound 42, which contains an \(\alpha,\beta\)-cyclopropyl and \(\alpha,\beta\)-enone moiety, was selected in order to elucidate whether a second electrophilic site on the cyclohexenone core had any influence on anti-cancer activity. Enantiomerically pure 40 was selected in order to identify the importance of absolute stereochemistry of a C4 bearing residue.\(^\text{19}\)

Compounds 43 and 44, which can be viewed as hybrid analogues of COTC and antheminone A, were synthesised to identify the influence of stereochemistry at C5, bearing a phenyl substituent, on bioactivity. Compound 45 was chosen to discover whether an alkyl or aryl substituent at C5 had a different effect on potency. Finally, compound 46 was prepared to elucidate whether an ester moiety on the oxyalkyl side-chain was vital for anticancer activity.\(^\text{21}\)

These three series of compounds were synthesised, therefore, in order to identify the effect of stereochemistry, degree of hydroxylation, and the nature of C5 substituent on toxicity towards the cancer cell lines.
Development of an approach to novel hybrid analogues of COTC and Antheminone A as potential anticancer agents

Table 3: Toxicity results for COMC and its analogues against A549 and H460 cancer cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>7</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
<th>40</th>
<th>41</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ (µM)-A549</td>
<td>55</td>
<td>24</td>
<td>147</td>
<td>164</td>
<td>&gt;200</td>
<td>17</td>
<td>170</td>
</tr>
<tr>
<td>IC$_{50}$ (µM)-H460</td>
<td>40</td>
<td>10</td>
<td>158</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>11</td>
<td>158</td>
</tr>
</tbody>
</table>

Table 4: Toxicity results for COMC analogues against A549 and H460 cancer cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>42</th>
<th>63</th>
<th>54</th>
<th>43</th>
<th>44</th>
<th>45</th>
<th>46</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ (µM)-A549</td>
<td>18</td>
<td>32</td>
<td>31</td>
<td>1.3±0.2</td>
<td>8±5</td>
<td>14±4</td>
<td>159±28</td>
</tr>
<tr>
<td>IC$_{50}$ (µM)-H460</td>
<td>20</td>
<td>60</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The results of the MTT cell-viability assays of the analogues towards A549 and H460 cancer cell lines indicate that some of the analogues may be candidates for rational drug design. The two cell lines both show elevated levels of GSH and the bioassay results are summarized in Table 3 and Table 4.$^{18-21}$

The value of inhibitory concentration (IC$_{50}$) represents the power of a particular analogue to prevent proliferation of the cancer cell lines. According to the data in Table 3 and Table 4, it is apparent that the diastereoisomeric compounds 43 and 44 are the most potent towards the A549 non-small-cell lung cancer cell line. Compound 43, which possesses an anti-relationship between the phenyl substituent at C5 and hydroxyl group at C4, was substantially more potent than its syn-related diastereoisomer 44.

Monohydroxylated compounds 40 and 42 also showed considerable toxicity towards representative cancer cell lines. These compounds have opposite stereochemistry at the hydroxylated C4 position, which may indicate that absolute stereochemistry at this position is not important, for biological potency.
The IC\textsubscript{50} values for compounds 43 and 45 indicate that an aryl moiety at C5 is important for high potency as 45 was not as effective an antiproliferative agent as 43.

Hydroxylated analogues 38 and 39 and 41 of COTC 1 all displayed weak anti-proliferative activity, which suggests a requirement for a degree of hydrophobicity. Compound 46, which lacks a crotonyl side chain, was also only weakly active, thus indicating a need for a reasonable leaving group at that position.

Overall these results indicate that a hydroxyl group at C4, an anti-related aromatic moiety at C5, and a reasonable leaving group attached to the hydroxymethyl C2-substituent are all important requirements for good anti-cancer activity.

### 1.5.2. Synthetic routes for the most potent analogues of COTC

(-)-Quinic acid 47 was used as a starting material to synthesise most of the analogues apart from compounds 36 and 41. The highly potent antiproliferative agents were compounds 40 and 42, and the routes used for their synthesis are depicted in Schemes 7 and 8 respectively.\textsuperscript{19} For the synthesis of 40, the cis-diol moiety of (-)-quinic acid 47 was selectively protected with cyclohexanone to give lactone quinide 48. The lactone ring was then reduced using NaBH\textsubscript{4} to give a crude triol which was treated with silica supported NaIO\textsubscript{4} to give ketone 49. Cyclohexylidene protected enone 50 was obtained by dehydration of 49 \textit{via} an intermediate mesylate. After hydrogenation of the alkene moiety in 50, the reaction with DBU resulted in eliminative deprotection to give an allylic alcohol which was protected \textit{in situ} as its TBS ether to give 52. Morita-Baylis-Hillman functionalization of 52 afforded the hydroxymethyl compound 53, which on esterification (crotonic anhydride) and final deprotection with TFA gave the mono-hydroxylated analogue 40.\textsuperscript{19}
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Scheme 7: Synthetic route to Analogue 40

Reagents and Conditions: i) cyclohexanone, DMF/C₆H₆ (1:1), Amberlite® IR 120(H), reflux (Dean and Stark), 5 h, 60%; ii) NaBH₄, CH₃OH, 0 °C to r.t., 24 h; iii) NaO₂, H₂O, CH₃OH, 0 °C, 1.5 h, 58%; iv) (CF₃SO₂)₂O, pyridine, CH₂Cl₂, 0 °C to r.t., 12 h, 82%; v) H₂, 10% Pd on C, EtOAc, r.t., 17 h, 92%; vi) DBU, TBSCI, C₆H₆, reflux, 6 h, 80%; vii) DMAP (cat.), H₂CO (37% in H₂O), THF/H₂O (1:1), 40 °C, 24 h, 52%; viii) crotonic anhydride, pyr., DMAP (cat.), CH₂Cl₂, r.t., 1.5 h, 68%; ix) TFA/H₂O (7:1), 0 °C, 1 h, 98%.

The synthetic route employed for preparation of 42 was slightly different in that (-)-quinic acid 47 was protected as its butane diacetal rather than its cyclohexyldiene ketal. Furthermore, intermediate allylic alcohol 56 was generated under acidic conditions (TFA) rather than the basic conditions (DBU) used during the synthesis of 40. The remaining transformation employed for the synthesis of 42 was similar to those described previously.¹⁹
Scheme 8: Synthetic route for cyclopropane containing analogue 42\textsuperscript{19}

Reagents and conditions: i) trimethylsulfoxonium iodide, NaH, DMSO, r.t., 1.5 h, 61%; ii) TFA/H\textsubscript{2}O (7:1), r.t., 12 h, then K\textsubscript{2}CO\textsubscript{3}, H\textsubscript{2}O, CH\textsubscript{3}OH, r.t., 30 min, 99%; iii) TBSCI, DMAP (cat.), Et\textsubscript{3}N, CH\textsubscript{2}Cl\textsubscript{2}, r.t., 6 d, 78%; iv) imidazole (cat.), H\textsubscript{2}CO (37\% in H\textsubscript{2}O), THF, Na\textsubscript{2}CO\textsubscript{3}(aq), 40 °C, 7 d, 23%; v) crotonic anhydride, pyridine, DMAP (cat.), CH\textsubscript{2}Cl\textsubscript{2}, r.t., 24 h, 48%; vi) TFA/H\textsubscript{2}O (7:1), 0°C, 1 h, 94%.

Similar approaches to those described above were used for the synthesis of analogues 37 and 38, as illustrated in Schemes 9\textsuperscript{18} and 10\textsuperscript{20} respectively.

Scheme 9: Synthetic route for the preparation of dihydroxylated analogue 37\textsuperscript{18}

i) DMAP(cat.), H\textsubscript{2}CO, THF/H\textsubscript{2}O (1:1), 40 °C, 24 h, 80%; ii) crotonic anhydride, pyridine, DMAP (cat.), CH\textsubscript{2}Cl\textsubscript{2}, r.t., 1.5 h, 67%; iii) TFA/H\textsubscript{2}O (7:1), 0 °C, 30 min, then purification by HPLC, 75%.
In the case of analogue 38, the key fluorination step (54→61) was accomplished using the electrophilic fluorinating reagent SelectFluor® and proceeded with excellent diastereoselectivity (>100:1) (Scheme 10).20

Scheme 10: Synthesis of fluorinated analogue 38
Reagents and conditions: i) KHMDS (0.75 M in toluene), TMSCl, THF, -78 °C to 0 °C, 1 h then SelectFluor®, CH₃CN, 0 °C, 30 min, 57%; ii) DMAP (cat.), H₂CO (37% in H₂O), THF/H₂O (1:1), 40 °C, 24 h, 94%; (iii) crotonic anhydride, pyridine, DMAP (cat.), CH₂Cl₂, r.t., 1.5 h, 39%; iv) TFA/H₂O (7:1), r.t., 3 h, then purification by HPLC, 95%.

The most potent antiproliferative agent synthesised so far, compound 43, was prepared from the butane-diacetal protected enone 54 (Scheme 11).21
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Scheme 11: Synthetic route to the most potent crotonylated analogue 43\textsuperscript{21}

Reagents and conditions: i) (C\textsubscript{6}H\textsubscript{5})\textsubscript{2}CuMgBr, (CH\textsubscript{3})\textsubscript{3}SiCl (5 eq), r.t., THF, 0 °C, 37%; ii) dodecylbenzene-sulfonic acid (5 mol %), H\textsubscript{2}O, 100 °C, 1.5 h, 65%; iii) TESOTf (2.2 eq.); 2,6-lutidine, CH\textsubscript{2}Cl\textsubscript{2}, -78 °C, 10 min, 74%; iv) DMAP, H\textsubscript{2}CO (37% in H\textsubscript{2}O), sodiumdodecyl sulphate (10 mol %), r.t., 18 h, 58%; v) crotonic anhydride, DMAP, pyridine, CH\textsubscript{2}Cl\textsubscript{2}, r.t., 2 h, 86%; (vi) TFA/H\textsubscript{2}O (7:1), r.t., 2.5 h, 70%.

The key step of the sequence was introduction of the aromatic substituent at C3 of 54 which was accomplished in low yield due to the poor reaction diastereoselectivity. Acid mediated eliminative removal of the diacetal protecting group of 64 was then accomplished using dodecylbenzene-sulphonic acid in water, at elevated temperature to give hydroxy enone 65. Subsequent protection of the allylic alcohol in 65 using TESOTf gave silyl ether 66 which underwent a M-B-H reaction with formaldehyde to give 67. The M-B-H adduct 67 was esterified using crotonic anhydride and final deprotection with TFA gave the phenyl substituted analogue 43.\textsuperscript{21} A Similar approach to that described above was used for the synthesis of analogue 44.
1.6. Project aims

The aim of this project is to prepare novel hybrid analogues, 68, of COTC and antheminone A, using an approach related to that previously developed by the Whitehead group.

**Figure 10**: General structure of the novel target hybrid analogues of antheminone A and COTC

General target structure 68 contains two loci for diversification, R\(^1\) and R\(^2\). The project focuses on the variation of substituent R\(^1\) in order to probe its influence on bioactivity (Figure 10).

**Figure 11**: 3-O, 4-O-Cyclohexylidene-5-(4-bromophenyl)-(3R,4S,5S)-dihydroxycyclohexanone

Based upon previous work in the group, (-)-quinic acid 47 was used as a chiral building block for the preparation of a new series of α-β unsaturated cyclohexenones (Figure 10). The approach will be modelled on that used previously by the Whitehead group and a key
intermediate will be a 4-bromophenyl substituted cyclohexanone 70 which will be prepared using a 5-step process (Scheme 12).

Scheme 12: Synthetic route to synthesise an intermediate 70

The first step of the sequence would be to selectively protect the hydroxyl groups of (-)-quinic acid 47. Thus, the vicinal cis-diol of (-)-quinic acid would be protected as a cyclohexylidene ketal using cyclohexanone in the presence of a sulphonic acid and Amberlite®IR 120 H, as an acid catalyst. During this reaction lactonization of the carboxylic acid and C5 hydroxyl will also occur to give lactone 48.

The second step of the sequence will be reductive ring-opening of the lactone ring in 48 using a solution of NaBH₄ in CH₃OH to give ‘crude’ triol 69. Oxidative cleavage of the vicinal diol moiety of 69 will then be carried out using silica supported NaIO₄ to give β-hydroxy ketone 49. Dehydration of hydroxy ketone 49 to give enone 50 will then be accomplished via formation of an intermediate mesylate, using methanesulfonyl chloride and triethylamine in dichloromethane. The key step of the sequence was to be the introduction of a para-bromophenyl moiety at C5 using a conjugate addition reaction. We
envisaged that this could be accomplished via conjugate addition of para-bromophenylboronic acid with 50 in the presence of a rhodium catalyst, where we anticipated that 70 would be the major product.

**Scheme 13**: Synthetic route for the preparation of novel analogues of COTC and antheminone A

The synthetic route for the preparation of novel analogues of COTC and antheminone A is depicted in Scheme 13. As previously observed it was anticipated that adduct 70 would suffer eliminative removal of the protecting group to give allylic alcohol 71 on reaction with 1,8-diazabicycloundec-7-ene (DBU) in dichloromethane. The resulting free hydroxyl group in compound 71 was then to be protected as its triethylsilyl ether using TES-OTf under carefully controlled condition to give 72. Introduction of the pivotal α-hydroxymethyl moiety in 73 could then be accomplished using a Morita-Baylis-Hillman reaction, using formaldehyde as the electrophile under surfactant conditions. The first
target compound of the project, diol 74 will be obtained by deprotection of 73 using aqueous trifluoroacetic acid. After esterification of 73, using crotonic anhydride, followed by acid mediated deprotection, the second target 76 will be obtained.

Scheme 14: Synthetic route to synthesise the novel analogues of COMC

It is suggested that intermediate 70 could represent a useful divergent building block for the preparation of a range of compounds bearing different aromatic substituents. In order to probe the synthetic potential of 70 its utility in Suzuki cross-coupling reaction, (to give biphenyl derivative 77), and also a Sonogashira reaction (to give aryl acetylene 78) was to be investigated (Scheme 14).

2. Results and discussion

2.1. Vicinal cis-diol protection of quinic acid and concomitant lactonization

(-)-Quinic acid is a readily available chiral natural product that is often employed as an economical starting material for the synthesis of complex molecules. The trans-diequatorial diol of (-)-quinic acid 47 can be protected with butane-2,3-dione to give conformationally rigid and very stable butane diacetal 79 (Scheme 15). Previous work by the Whitehead group\(^{19}\) has involved the use of this protecting group which was slightly different to that described by Brückner and Gebaur.\(^{22}\)
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Scheme 15: Protection of (-)-quinic acid as its butane diacetal\textsuperscript{19}

Hydroxyl groups can also be protected in a number of ways, such as an alkyl ether, a tert-butyldimethylsilyl ether, a tetrahydropyranyl (THP) ether, a methoxymethyl ether, or a benzyl ether.\textsuperscript{23}

In our synthetic sequence, cyclohexanone was employed to protect the cis vicinal diol of (-)-quinic acid 47 using Amberlite\textsuperscript{®} 120 (H) resin as an acid catalyst to give a stable cyclohexylidene ketal 48 in 65\% yield (Scheme 16).

Scheme 16: Synthesis of cyclohexylidene ketal 48

2.1.2. Mechanism of lactonization

During the reaction, lactonization also occurred via intramolecular condensation of the syn-related carboxylic acid at C1 and hydroxyl group at C5. The mechanism for the reaction is shown in Scheme 17.\textsuperscript{25}
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Scheme 17: The mechanism for the formation of cyclohexylidene ketal 48 from (-)-quinic acid 47

Water is generated as a by-product from the reaction and was removed during the course of the reaction by the use of the Dean and Stark trap. This process helps to drive the reaction to completion according to Le Chatelier’s principle.  

2.2. Lactone reduction

2.2.1. Synthesis of crude triol

A procedure described by Schulz and co-workers employed NaBH₄ to reductively open the lactone ring of 48 to give the crude triol 69.  

Although LiAlH₄ is the more nucleophilic reducing agent generally used for lactone reduction, NaBH₄ was used in this case, as the inductive effect of the hydroxyl group enhanced the reaction of the lactone carbonyl group of 48. The relatively mild reagent NaBH₄ consequently reduced lactone 48 to the crude triol 69 (Scheme 18).  

Songül Kaskun
Scheme 18: Reduction of protected lactone quinide to crude triol\textsuperscript{26}

2.3. Oxidative cleavage

Oxidative cleavage of the vicinal diol moiety of 69 was accomplished using NaIO\textsubscript{4}. There are many reasons to use NaIO\textsubscript{4} as an oxidative reagent; it is an inexpensive compound and does not require very strict reaction conditions. That is why NaIO\textsubscript{4} is a popular reagent for the oxidative cleavage of vicinal diols into carbonyl compound.\textsuperscript{27} Unfortunately though, NaIO\textsubscript{4} is not soluble in apolar solvents and this characteristic originally limited its potential application. Gupta and co-workers solved this solubility problem by introducing a silica gel supported NaIO\textsubscript{4} procedure in 1981.\textsuperscript{28} Thanks to this method, solvents such as dichloromethane and diethylether can be used for the oxidation of hydroquinones and primary alcohols despite their apolar properties. However, this procedure has a few problematic features including the need for a long reaction time and often low yields following removal of the solvent.\textsuperscript{28}

In the light of this previous work, Daumas and co-workers improved upon the solution with a method using wet silica gel supported NaIO\textsubscript{4} which overcame some of the difficulties. Latterly Dauma et al.\textsuperscript{29} have described a modification of this procedure, which is utilised NaIO\textsubscript{4}

These workers mixed silica gel in DCM and NaIO\textsubscript{4} in H\textsubscript{2}O with a vicinal diol in DCM. Unfortunately, when stirring NaIO\textsubscript{4} and silica gel normally it was observed that silica gel generated colloidal forms. In order to overcome these problems Zhong and co-workers
developed a silica gel supported NaIO₄ reagent, in the form of powder, which they reported in 1996. The free flowing powder was obtained by mixing aqueous NaIO₄ with silica gel which was active for one month. An additional benefit of this method is that, further purification is rarely required.⁵⁰

Previous work by the Whitehead group²⁰ has involved the use of silica supported NaIO₄ which was slightly different as described by Schulz and co-workers.²⁶ The free-flowing powder form of silica gel supported sodium peridodate was consequently used for the oxidation of triol 69 to hydroxy ketone 49 in 93% yield as illustrated in Scheme 19.

![Scheme 19: Oxidative cleavage mechanism during the synthesis of hydroxy ketone²⁰](image)

2.4. **Dehydration of β-hydroxy ketone**

Methane sulfonyl chloride is a reagent commonly employed in organic chemistry for elimination reactions. The dehydration of hydroxyketone 49 was consequently accomplished by reaction with methanesulfonyl chloride and triethylamine to give enone 50 in 79% yield (Scheme 20). The procedure employed by the Whitehead group¹⁹ was modified from that described by Audia and co-workers.³¹
Scheme 20: Dehydration of hydroxy ketone 49 to enone 50\textsuperscript{19}

2.4.1. Mechanism of dehydration of β-hydroxy ketone

The dehydration of hydroxyketone 49 to give enone 50 involves initial conversion of the hydroxyl group to a better leaving group (mesylate), which is accomplished using triethylamine and methanesulfonyl chloride. Triethylamine acts to deprotonate methanesulfonyl chloride and the resulting carbanion eliminates chloride to give a sulphene intermediate. Reaction of a hydroxyl group with the sulphene then gives mesylated ketone 81. Triethylamine then acts again as the base for an E1cB elimination of methanesulphonic acid to give enone 50 (Scheme 21).
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Scheme 21: The mechanism for eliminative removal of the cyclohexylidene protecting group of 49 to give enone 50

2.5. Nucleophilic conjugate addition

Nucleophilic conjugate addition reactions are commonly used for the generation of C-C bonds via the use of organometallic reagents which possess a partially ionic carbon-metal bond. Organometallic conjugate addition has a variety of different catalysts including copper, rhodium and palladium species. The formation of C-C bonds without the use of organometallic reagents as the ‘nucleophilic’ partner can sometimes present difficulties.³²

2.5.1. Copper catalysed conjugate addition

Organometallic reactions can involve many reagents including Grignard reagents and organocuprate reagents. The latter can be used in a stoichiometric manner or under
catalytic conditions and are generally prepared from the reaction of a Group I or Group II organometallic reagent with a copper (I) salt.\textsuperscript{32-33}

In 1941 Kharasch and co-workers described the conjugate addition of Grignard reagents to $\alpha,\beta$-unsaturated carbonyl groups in the presence of a copper (I) salt. In their procedure, CuCl was employed as catalyst for the conjugate addition of CH$_3$MgBr to enone 82 to give compound 83 in 83% yield and diene 85 in 7% yield. In the absence of CuCl the products of this reaction were 84 (43%) and 85 (48%), both derived from a 1,2-addition pathway (Scheme 22).\textsuperscript{34}

\[ \text{Scheme 22: Kharasch reaction of } \alpha,\beta-\text{unsaturated enone 83 with or without CuCl catalysis.} \textsuperscript{34} \]

Further work in this area was conducted by Henry Gilman who found that methyl lithium underwent reaction with copper (II) chloride to give ethane and copper (I) chloride which on further treatment with methyl lithium gave methylcopper (CH$_3$Cu). Further reaction of the latter species with methyl lithium gave a ‘stable’ lithium diorganocopper reagent (CH$_3$)$_2$CuLi, which is now regarded as a typical Gilman reagent (Scheme 23).\textsuperscript{35}

\[ \text{2CH}_3\text{Li} + 2\text{CuCl}_2 \rightarrow \text{C}_2\text{H}_6 + \text{Cu}_2\text{Cl}_2 + 2\text{LiCl} \]
\[ \text{2CH}_3\text{Li} + \text{Cu}_2\text{Cl}_2 \rightarrow 2\text{[CH}_3\text{Cu]} + 2\text{LiCl} \]
\[ \text{CH}_3\text{Cu} + \text{CH}_3\text{Li} \rightarrow (\text{CH}_3)_2\text{CuLi} \]

\[ \text{Scheme 23: Preparation of a Gilman reagent} \textsuperscript{35} \]
In 1965, Costa and co-workers reported that a stable phenyl copper species, \((\text{C}_6\text{H}_5\text{Cu})_4\text{C}_6\text{H}_3\text{Li}_{3.5}(\text{C}_2\text{H}_3)_2\text{O}\) could be obtained from reaction of phenyl lithium and copper (I) bromide in diethylether and was identified by IR spectroscopy. Initially the reaction with phenyl lithium was successful but when phenyl magnesium bromide was used the corresponding copper species was not obtained.\(^{35}\) Exchanging \(\text{Et}_2\text{O}\) by \(\text{THF}\) resulted in the formation of two copper species \((\text{CuBr})_{2.5}(\text{C}_6\text{H}_5)_2\text{Mg.nTHF}\) and \((\text{C}_6\text{H}_5)_2\text{Cu-}(\text{C}_6\text{H}_5)_2\text{Mg.nTHF}\).\(^{36}\)

Following these initial investigations, House and collaborators described the addition of an organocopper reagent \((\text{Me}_2\text{CuLi})\) to an \(\alpha,\beta\)-unsaturated ketone in 1966. These experiments showed that the presence of a copper salt enormously affected the rate of conjugate-addition as well as the overall yield of reaction.\(^{37}\) A general outline of the reaction of an organocopper species with an electrophile is illustrated in Scheme 24.\(^{38}\)

Scheme 24: A general mechanistic scheme for organocuprate reaction\(^{38}\)

2.5.1.1. Stereochemical outcome of organocuprate conjugate addition

In 1985, Corey and Boaz reported that the conjugate addition of cuprate reagent reaction to the \(\gamma\)-oxygenated \(\alpha,\beta\)-unsaturated ketone 86 gave two products - the syn- and anti-
diastereoisomers. This study highlighted the importance of TMSCl in both the regiochemical and stereochemical outcome of this reaction type. In the absence of TMSCl the principal product 88 had a syn-relationship between the newly added substituent and the γ-oxygen: in the presence of TMSCl the major product 87 had an anti-relationship between these substituents (Scheme 25).  

Scheme 25: Conjugate addition of a Gilman cuprate to α-β unsaturated ketones.  

Furthermore, Audia and co-workers found γ-oxygenated cyclohexenone 89 undergoes conjugate addition with Me₂CuLi in the presence of TMSCl to give the anti-product 90 (Scheme 26).  

Scheme 26: Conjugate addition of Me₂CuLi to a γ-oxygenated cyclohexenone
In 1991, Bhatt and co-workers described the conjugate addition reaction to an enone of an organostannane, which on treatment with CuCN (8 mmol%) in THF, gave the desired adduct 92 in 50% yield. The general transformation is illustrated in Scheme 27 and a specific example of relevance to this project is shown in Scheme 28.40

**Scheme 27**: Conjugate addition of an organostannane to an α,β-unsaturated ketone catalysed by CuCN40

The conjugate addition reactions to an α,β-unsaturated ketone derived from (-)-quinic acid have been carried out by the Whitehead group using a range of Gilman cuprates in order to understand the influence of the C4 oxygen substituent on the stereochemical outcome (Scheme 29).21 Despite the many experiments that have been carried out so far, prediction of the stereochemical outcome of these reactions remains unreliable (Scheme 29).21 From these studies alone, it is clear that prediction of the stereochemical outcome of such reactions is extremely difficult.
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Scheme 29: Conjugate addition reactions of Gilman cuprates to an α,β-unsaturated ketone derived from (-)-quinic acid

2.5.2. Rhodium catalysed conjugate addition reactions

Rhodium-catalysed conjugate addition reactions are attracting an increasing amount of attention because of the advantages they have over copper-mediated reactions. The advantages are that reactions can be carried out with high enantioselectivity and under quite mild reaction conditions, including aqueous solvent systems and readily accessible reaction temperatures (20 °C – 90 °C).

In 1997, Sakai and co-workers described a new procedure which employed a rhodium catalyst and 1,4-bis(diphenylphosphino)butane (dppb) for conjugate addition reactions to α,β-unsaturated ketones. Sakai has proposed that this reaction proceed via transmetallation from boron to rhodium prior to C-C bond forming, which is in contrast to other conjugate addition reactions.

According to the Sakai procedure, a methanol/water solvent system was optimal successful conjugate additions, a general example of which is illustrated in Scheme 30.
Hayashi and collaborators investigated an asymmetric variant of the rhodium-catalysed conjugate addition reaction using ([Rh(acac)((S)-binap)]) and proposed the presence of three intermediates. In 2002, NMR investigations supported the presence of phenylrhodium 99, oxa-δ-allyl rhodium 100 and hydroxyrhodium 102 which are shown in Scheme 31. During the catalytic cycle the arylboronic acid 98 undergoes transmetallation with a hydroxyrhodium complex 102 to generate Rh-Ph complex 99 which subsequently inserts into the cyclohexenone to give intermediate 100. Finally enolate 100 undergoes hydrolysis to generate 4-aryl cyclohexanone 101.

**Scheme 31:** The reaction schemes for the three intermediates 99, 100, 102

Boiteau and co-workers reported a similar reaction which proceeded in 89% ee as illustrated in Scheme 32. The reaction was carried out at 100 °C and used 7.5 mol % of the
chiral ligand H8-monophos: when other bisphenol based ligands were employed the ee was just 15% demonstrating the importance of the nature of the ligand.\textsuperscript{43}

\begin{equation}
\text{Scheme 32: Asymmetric synthesis of 3-phenylcyclohexanone}^{43}
\end{equation}

Martina and co-workers modified the Boiteau procedure using lower quantities of reagents ([Rh(OH)(cod)]\textsubscript{2}, [RhCl(cod)]\textsubscript{2}/KOH), together with a monodentate phosphoramidite ligand (BINAP, amidomonophosphines, chiral bicyclodienes) and also carried the reaction out at room temperature. The product was obtained in 99% ee however a longer reaction time (5 hours) was required (\textbf{Scheme 33}).\textsuperscript{44}

\begin{equation}
\text{Scheme 33: Asymmetric rhodium-catalysed conjugate addition to cyclohexenone as described by Sebastian and collaborators.}\textsuperscript{44}
\end{equation}

\textbf{2.5.2.1. Conjugate addition of 4-bromophenylboronic acid}

During the course of this project, the (-)-quinic acid derived enone 50 was found to undergo conjugate addition reaction with 4-bromophenylboronic acid using [RhOH(cod)]\textsubscript{2} as catalyst in the presence of Et\textsubscript{3}N. The reaction proceeded smoothly and with complete
diastereoselectivity to give the 4-bromophenyl adduct 70, wherein the newly introduced substituent was anti to the C4 oxygen substituent (Scheme 34).

Scheme 34: Synthesis of bromophenyl adduct 70

The reaction was attempted several times with 4-bromophenylboronic acid in order to discover the optimum conditions. A summary of the outcome of these reactions is given in Table 5. Reaction time was the parameter that was varied and an optimum yield of 81% was obtained after 6 hours. However, the isolated yield was very low and starting material still remained when the reaction was left overnight.

<table>
<thead>
<tr>
<th>Entry</th>
<th>[RhOH(cod)]₂ (mol %)</th>
<th>Conversion (%)</th>
<th>Yield</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>100</td>
<td>71</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>100</td>
<td>37</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>100</td>
<td>81</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>100</td>
<td>72</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>100</td>
<td>58</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 5: Summary of the outcome of conjugate addition reactions of 4-bromophenylboronic acid and enone 50

2.5.2.2. The catalytic cycle for the Rh catalysed conjugate addition

A general mechanism for the reaction of enone 50 with 4-bromophenylboronic acid is depicted in Scheme 35. During the reaction, the aryl boronic acid undergoes transmetallation with [RhOH(cod)]₂ to give an intermediate [RhOH(cod)]₂-Ph-Br complex.
which further interacts with the $\alpha,\beta$-unsaturated cyclohexenone to give intermediate 106. Ultimately, enolate 106 undergoes hydrolysis to give the target compound 70.

![Scheme 35: Mechanism for the conjugate addition of 4-bromophenyl boronic acid with enone 50 using a Rh(I) catalyst.](image)

2.6. **Elimination of cyclohexylidene protecting group**

In 1989, Audia and co-workers reported the synthesis of enantiomerically pure cyclohexenol 108 from cyclohexanone 107 which is depicted in Scheme 36. Unfortunately, however, alcohol 108 proved to be quite unstable and was therefore converted into the corresponding TBS-protective derivative 52.
The eliminative removal of a protecting group from cyclohexanone substrates can be quite problematic. Gebauer and Brückner reported a representative example of such a difficult reaction in 1996. Despite their efforts, in order to eliminate the bis-acetal moiety of 109 using CH$_3$CO$_2$H at reflux to give enantiomerically pure alcohol 111, the desired product was only obtained in a disappointing yield of 48% (Scheme 37).²²

The difficulties associated with the eliminative removal of the bis-acetal moiety may be a consequence of the pseudo-equatorial oxygen substituent at C3; as efficient elimination of a pseudo-axial leaving group, orthogonal to the π-system of the intermediate enol, is required.² Thus, Et$_3$N or DBU were required for eliminative removal of the cis-fused acetonide moiety in 107 whereas acidic condition were required for eliminative removal of the trans-fused bis acetal moiety in 109.
2.6.1. Synthesis of γ-bromophenylated alcohol

During the research programme described herein, elimination of the cyclohexylidene was initially carried out using 0.05 mL of 0.5 M NaOH to give allylic alcohol 71 in a maximum yield of 34% (Scheme 38).

Scheme 38: Synthesis of 4-hydroxy-5-(4-bromophenyl)cyclohex-2-enone 6 yield with the dimeric perhydro-dibenzo-furanone by-product 112.

Unfortunately, dimeric perhydro-dibenzo-furanone 112, a second product, was formed under these reaction conditions. Several attempts were made to improve the yield and optimise reaction conditions, but the best yield obtained was 34%. This prompted investigations into finding alternative conditions for the reaction.

Hence, in an attempt to improve the yield, it was decided to use diazobicycloundec-7-ene (DBU) which is a weaker base than NaOH; this base elimination method is the same as previously described by Audia and co-workers.\textsuperscript{31} Synthesis of 4-hydroxy-5-(4-bromophenyl)cyclohex-2-enone 71 was achieved using this method in 85% maximum yield as depicted in Scheme 39.
Scheme 39: Synthesis of 4-hydroxy-5-(4-bromophenyl)cyclohex-2-enone 71

Table 6 summarizes the results of all the elimination reactions of 70 which were carried out during this investigation. From this, it is clear that the NaOH method was not a good choice for the elimination.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Extraction solvent</th>
<th>Time (h)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5M NaOH</td>
<td>0.02mL</td>
<td>Et₂O</td>
<td>2</td>
<td>20%</td>
</tr>
<tr>
<td>0.5M NaOH</td>
<td>0.05mL</td>
<td>Et₂O</td>
<td>5</td>
<td>28%</td>
</tr>
<tr>
<td>0.5M NaOH</td>
<td>008ml</td>
<td>Et₂O</td>
<td>8</td>
<td>28%</td>
</tr>
<tr>
<td>0.5M NaOH</td>
<td>008ml</td>
<td>EtOAc</td>
<td>8</td>
<td>34%</td>
</tr>
<tr>
<td>DBU</td>
<td>1.1 eq.</td>
<td>DCM</td>
<td>1.25h</td>
<td>85%</td>
</tr>
<tr>
<td>DBU</td>
<td>1.1 eq.</td>
<td>DCM</td>
<td>1.3h</td>
<td>72%</td>
</tr>
<tr>
<td>DBU</td>
<td>1.1 eq.</td>
<td>DCM</td>
<td>1.5</td>
<td>65%</td>
</tr>
<tr>
<td>DBU</td>
<td>1.1 eq.</td>
<td>DCM</td>
<td>2.5</td>
<td>67%</td>
</tr>
</tbody>
</table>

Table 6: Summary of the condition used for synthesis of 4-Hydroxy-5-(4-bromophenyl)cyclohex-2-enone.

In the case of the use of DBU for conjugate adduct 70, the optimum reaction condition found involved 1.25 hours at room temperature which gave the allylic alcohol 71 in a maximum 85% yield. The reaction was monitored by TLC in order to discover when it had reached completion.
2.6.2. The mechanism for eliminative deprotection of cyclohexyldene (70)

The mechanism for eliminative removal of the cyclohexyldene protecting group from the conjugate adduct 70 involves initial deprotonation at C2 by DBU to give enolate 113. Subsequent E1cb-type cleavage of the C3-Oxygen bond gives deprotonated hemiketal 114, which collapses with expulsion of cyclohexanone to give alkoxide 115. Proton-transfer between 115 and the incipient conjugate acid of DBU gives hydroxy enone 71 (Scheme 40).

Scheme 40: The mechanism for eliminative removal of the cyclohexyldene protecting group of 70 to give hydroxy enone 71.

2.7. TES Protection of the secondary alcohol group at C4

A number of silyl ethers including tert-butyldimethylsilyl (TBDMS), triisopropylsilyl (TIPS) and triethylsilyl (TES) are widely used in organic synthesis as protecting groups. The nature of the hydroxyl group and the reaction conditions to be used indicate which
silyl ether is more appropriate for the protection. For instance, the triethylsilyl group is commonly used because of its ease of the introduction. In this project TESOTf was selected as the silylating reagent because the resulting TES ether can be readily removed, whilst also being stable in basic conditions such as those involved in the subsequent Morita-Baylis-Hillman reaction.

The protection of secondary alcohol 71, therefore, was carried out using triethylsilyl trifluoromethanesulfonate (TESOTf) and 2,6 lutidine in DCM, using the same procedure as had previously been described by the Whitehead group (Scheme 41).

Scheme 41: Synthesis of TES protected analogue 72

2.7.1. Mechanism of TES protection of the secondary alcohol

Nucleophilic attack of the γ-hydroxyl on TES-OTf, via an associative/dissociative process, gives charged intermediate 116. Deprotonation by the hindered base, 2,6-lutidine, then gives the TES protected compound 72.

Scheme 42: Mechanism of TES-protection of secondary alcohol 71
2.8. Morita-Baylis-Hillman reaction

The Morita-Baylis-Hillman (M-B-H) reaction is a constructive catalytic reaction for C-C bond formation between ketones, aldehydes and imines and electron deficient alkene which engage nucleophilic catalysts such as amines or phosphines to produce the derived adduct. M-B-H addition has been frequently used as a synthetic precursor for the preparation of complex natural product as well as other biologically imperative compound (Scheme 43).

\[
\begin{align*}
\text{R} = & \text{ alkyl, aryl, heteroaryl} \\
\text{R}^1 = & \text{ H, COOR, alkyl} \\
\text{X} = & \text{ O, NCOOR, NTs, NSO}_2\text{Ph} \\
\text{EWG} = & \text{ electron withdrawing group (COR, CHO, CN, COOR, PO(OEt)}_2, \text{ SOPh, SO}_2\text{Ph, SO}_3\text{Ph)}
\end{align*}
\]

Scheme 43: Generic representation of the Morita-Baylis-Hillman reaction

There are a number of benefits related to the Morita-Baylis-Hillman reaction for C-C bond formation including basic reaction conditions and the reaction can generally be obtained in good yield.

In 1998 Rezgui and El Gaied described an efficient M-B-H reaction of 2-cyclohexenone using aqueous formaldehyde in the presence of 10 mol% DMAP (dimethylamino pyridine) to give hydroxymethyl cyclohexenone 117 in 82 % yield (Scheme 44).

\[
\begin{align*}
\text{33} & \xrightarrow{\text{H}_2\text{C}=\text{O (37% in } \text{H}_2\text{O)}} \text{ THF, DMAP, r.t., 75-82%} \\
& \text{117}
\end{align*}
\]

Scheme 44: Hydroxymethylation of cyclohex-2-enone using ‘aqueous’ M-B-H condition
The Whitehead group employed this method in 2010, an example of which is illustrated in Scheme 45, but the isolated product yield of 52% was felt to be unsatisfactory. The reason for the low yield of this reaction remains unknown but may be a consequence of the hydrophobic nature of the substrate.

Scheme 45: TBS protective M-B-H adduct to TBS protective cyclohex-2-enone

The M-B-H reaction attempted to employ water as one of the solvents, but found this could lead to difficulties due to the insolubility of more organic compounds. Porzelle and co-workers have overcome this by employing surfactants such as sodium dodecyl sulfate (SDS) or cetyl trimethylammonium bromide (CTAB) in aqueous solvents. Accordingly, the Whitehead group employed, SDS successfully in a M-B-H reaction and using formaldehyde as an electrophile, managed to introduce a hydroxymethyl group in good yield (Scheme 46).

Scheme 46: M-B-H reaction of phenyl substituted cyclohexenone 66 under surfactant condition
2.8.1. **M-B-H reaction of bromophenyl substituted cyclohexenone (72)**

The bromophenyl compound, with its TES protected oxygen 72, is very hydrophobic and the M-B-H reaction on this compound was therefore carried out in the presence of the anionic surfactant, SDS (Scheme 47).

![Scheme 47: M-B-H reaction of bromophenyl substituted cyclohexenone 72 under surfactant condition](image)

2.8.2. **Mechanism of the M-B-H reaction of bromophenyl substituted cyclohexenone (72)**

DMAP was employed as the nucleophilic catalyst which adds in conjugate fashion to TES protected enone 72 to give enolate 119. Subsequent ‘aldol’ reaction with formaldehyde gives alkoxide intermediate 120 which, following proton transfer undergoes elimination of the DMAP catalyst to give M-B-H adduct 73 (Scheme 48).
Scheme 48: Mechanism of the M-B-H reaction catalysed by DMAP

2.9. Deprotection of TES ether (73)

The efficient deprotection of TES ether 73 was accomplished using a method that the Whitehead group had previously developed.\textsuperscript{21} Thus diol 74 was obtained from exposure of 73 to aqueous TFA (TFA:H\textsubscript{2}O (7:1)). The reaction was monitored by TLC and was found to have reached completion after 40 minutes. The transformation is depicted in Scheme 49.

Scheme 49: Transformation of TES ether 73 to diol 74 under aqueous acidic conditions
The mechanism of deprotection of TES protected MBH adduct 73 is shown in Scheme 50 and involves initial protonation of the ethereal oxygen at C4 followed by loss of the silyl moiety to give the diol 73. The incipient triethylsilyl cation 122 may be quenched via attack by $\text{CF}_3\text{CO}_2^-$ 123 as depicted or alternatively via attack by water.

Scheme 50: Mechanism of silyl ether deprotection using TFA:H$_2$O, (7:1)

2.10. Crotonylation of TES ether (73)

The crotonylation of M-B-H adduct has been carried out previously by the Whitehead group, and the same method was again applied to esterify the allylic alcohol 73 using crotonic anhydride, DMAP and pyridine in DCM for 2 hours under an atmosphere of N$_2$ (Scheme 51). An attempt to purify the crotonylated product 75 by flash column chromatography, was only partially purified as indicated by spectroscopic analysis.
Scheme 51: Synthesis of the first crotonylated compound during this project from the allylic alcohol 73

It was not possible to purify crotonate 75 any further, but $^1$H NMR spectroscopic analysis did confirm its structural identity. The partially purified material 75 was therefore exposed to the same deprotection condition used for preparation of diol 74, TFA: H$_2$O, (7:1). The reaction progress was monitored by TLC and found to have reached completion after 30 minutes. The target crotonate compound 76 was finally obtained after purification using two successive flash silica columns ethyl acetate: petroleum ether (1:5) in 16% yield over the two steps from M-B-H adduct 73.

2.11. An unexpected dimerisation reaction leading to dibenzofuranone (112)

During the eliminative removal of cyclohexylidiene protecting group a dimeric perhydro-dibenzofuranone 112 was also formed together with the desired allylic alcohol 71 (Scheme 52). In order to optimise the formation of the dimeric compound 112, a modified procedure was carried out whereby the ketone 70 was exposed to 0.5 M NaOH for a prolonged reaction time.
Scheme 52: The synthesis of dimeric perhydro-dibenzofuranone 112

Interestingly the dimeric perhydro-dibenzofuranone 112 is structurally related to incarviditone 124 a natural product derived from rengyolone 125, both of which were isolated from Incarvillea delewayi in 2009 (Figure 12). Incarviditone 124 and rengyolone 125 were assayed against A549, LOVO, HL-60, 6T-CEM and HepG2 cancer cell lines and the IC50 [µg/ml] values of these compound make them of interest as possible anticancer agents (Table 7).50

Figure 12: Structure of incarviditone 124 and rengyolone 125

<table>
<thead>
<tr>
<th>IC50 [µg/ml]</th>
<th>A549</th>
<th>LOVO</th>
<th>HL-60</th>
<th>6T-CEM</th>
<th>HepG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>124</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>14.8±0.3</td>
<td>22.2±0.9</td>
<td>&gt;30</td>
</tr>
<tr>
<td>125</td>
<td>16.2±0.8</td>
<td>11.6±0.6</td>
<td>5.7±0.3</td>
<td>6.1±0.4</td>
<td>20.2±0.7</td>
</tr>
</tbody>
</table>

Table 7: The IC50 [µg/ml] values of incarviditone 3 and rengyolone 4 against to the cancer cell lines

In light of these findings, the unexpected dimeric product 112 will be tested for its toxicity towards non-small-cell lung cancer cell lines. A plausible mechanism for the formation of the dimer 112 is depicted in Scheme 53. The Whitehead group suggest that eliminative
removal of the cyclohexylidene protecting group of 70, to give allylic alcohol 71, followed by a tandem Michael-Michael reaction of 126 with its conjugate acid 71, affords 112. Michael reaction to give enolate 128. Finally, protonation of 128 give the perhydro-dibenzofuranone 112.

Scheme 53: A proposed mechanism for formation of dimer 112 from bromophenyl adduct 70

2.12. Suzuki-Miyaura reaction

In 1979, Suzuki, Miyaura and Yamada described an innovative and versatile cross-coupling reaction between 1-alkenyl boranes and alkenyl halides which employed tetrakis(triphenylphosphine) palladium as catalyst (Scheme 54). Pivotal to this discovery was the importance of an added base as, in the absence of such, the product could not be obtained in more than 2% yield: when the reaction was carried out using sodium hydroxide or sodium ethoxide in THF, the yield increased to 59% and 81% respectively.51
Development of an approach to novel hybrid analogues of COTC and Antheminone A as potential anticancer agents

Scheme 54: The first example of a tetrakis(tri-phenylphosphine)palladium-catalysed Suzuki-Miyaura reaction.

2.12.1. Suzuki-Miyaura reaction mechanism

Since 1979, the Suzuki-Miyaura reaction has developed into one of the most common reactions for the formation of C-C bonds between sp²-sp² centers, employing a palladium catalyst and an appropriate base. The general cycle of these cross-coupling reactions is depicted in Scheme 55. During the catalytic cycle, the organic halide (Ar₁-X) undergoes oxidative addition to Pd(0) to give Ar₁-Pd(II)-X. Transmetalation of this Pd(II) species with the organoboron component affords a diaryl Pd(II) species, Ar₁-Pd(II)-Ar₂-Ln. Reductive elimination at Pd(0) leads to the formation of a new C-C bond, undergoes reductive elimination resulting in the generation of a new C-C bond.

Scheme 55: General mechanism for the Pd catalysed Suzuki-Miyaura reaction.
2.12.2. Attempted Suzuki-Miyaura coupling of bromophenyl adduct (70)

During the course of this project, the use of Suzuki-Miyaura reactions were investigated for the coupling of phenylboronic acid with bromide 77, Scheme 56.

Scheme 56: Attempted Suzuki-Miyaura cross-coupling of bromophenyl adduct 70

Table 8, summarises the data obtained in the initial screening of the Suzuki-Miyaura reaction carried out during this project. Unfortunately the coupled product 77 was not obtained using the conditions employed. In allylic alcohol 71 was formed using the conditions, in entry 2 and 3 due to the presence of a weak base.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst (mol %)</th>
<th>Reagent (g.)</th>
<th>Bases</th>
<th>Solvent</th>
<th>Ligand</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>palladium acetate 3 %</td>
<td>phenylboronic acid (37 mg)</td>
<td>CsF</td>
<td>dimethyl ether (DME)</td>
<td>PPh3</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>palladium acetate 5%</td>
<td>phenylboronic acid (35 mg)</td>
<td>Na2CO3</td>
<td>2-propanol</td>
<td>PPh3</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>PEPPSI 2%</td>
<td>phenylboronic acid (40 mg)</td>
<td>K2CO3</td>
<td>THF</td>
<td>-</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 8: The data for the Suzuki-Miyaura reactions carried out in this project.
2.13. Sonogashira coupling

The Sonogashira cross-coupling reaction is generally carried out between aryl or vinyl halides (sp^2) and alkynes (sp) using an appropriate base in the presence of a palladium catalyst and copper co-catalyst to give aryl acetylenes or conjugated enynes (Scheme 57).^{54}

\[
\begin{align*}
\text{H} & \quad \text{R}^1 \quad + \quad \text{R}^2 - \text{X} \\
\text{Pd cat., Cu cocat., base} & \rightarrow \\
\text{R}^1 & \quad \text{R}^2
\end{align*}
\]

- R^1 = alkyl, alkenyl, hetaryl, aryl, SiR_3
- R^2 = aryl, hetaryl, vinyl
- X = br, Cl, I, OTf

Scheme 57: A general Sonogashira cross-coupling reaction^{55}

This extremely useful carbon-carbon bond forming reaction has attracted a great deal of attention from chemists due to its several advantages including: i) the ready availability of appropriate starting materials and catalysts; ii) the lack of necessity for strict reaction conditions; iii) the usual high yields are obtained.^{55}

2.13.1. Attempted Sonogashira coupling of bromophenyl adduct 70

Attempted the coupling of bromide 70 was treated with ethynyltrimethylsilane in the presence of triethylamine Pd(PPh_3)_2Cl_2 (2 mol %) and CuI (4 mol %) (Scheme 58). Again we were not able to obtained any product from this coupling reaction. The reaction was carried out under an atmosphere of N_2 at room temperature and its progress was monitored by TLC for 2 days. Unfortunately, there was no evidence of formation of the aryl acetylene 78 despite the extended reaction time.
Scheme 58: Attempted Sonogashira reaction between bromophenyl adduct 1 and ethynytrimethylsilane

The reason for the failure of this reaction remains unknown although it may be a consequence of the relative strength of the sp\(^2\) C-Br bond compared to the more commonly employed sp\(^2\) C-I bond.

3. Conclusion

The outcome of this project has been the preparation of novel hybrid analogues of COTC and antheminone A using (-)-quinic acid 47 as a chiral starting material. The synthetic sequence that was followed was based on an approach that the Whitehead group had previously investigated.

Figure 13: A divergent building block for this project

The bromide 70 is a potentially versatile intermediate for subsequent C-C bond formations, although its functionalisation remains problematic (Figure 13). Optimum conditions for the preparation of this novel intermediate were developed. During the synthetic sequence to the analogues, every transformation proceeded smoothly apart from eliminative removal of the cyclohexyldiene moiety to give alcohol 71. Interestingly, when NaOH was used for this step a dimeric product 112 was also obtained resulting in a lower yield of alcohol than

Songül Kaskun
desired. This problem during the elimination step has been overcome by using 1,8-diazabicycloundec-7-ene (DBU) as the base for the reaction. The product from the elimination step was then successfully converted into the target compound 74 and 76 using 3 and 4 step sequences respectively.

Figure 14: New hybrid analogues of COTC and Antheminone A which were synthesised during the project together with a novel dimer

Compounds 74 and 76 were successfully synthesised in order to probe the importance of the crotonate ester moiety on bioactivity and these compounds, together with the unusual dimer 112, will be assayed against non-small-cell lung cancer cell lines. The serendipitous synthesis of 112 provides a novel scaffold for future investigations.

Figure 15: Future target structure of this project

A future direction for the project would be to discover appropriate conditions for the Sonogashira reaction to generate aryl acetylene 47. Despite an attempt to obtain this product, there has been no evidence of its formation during the project. Furthermore, optimisation of the conditions required for the Suzuki coupling to generate 46 and other
biaryl-substituted compounds is necessary in order to increase yield and shorten the reaction time (Figure 15).

In the future, the analogues shown in Figure 14, which have been successfully synthesised, as well as the analogues 46 and 47 (Figure 15), will be assessed for their anti-proliferative effect towards cancer cell lines in vitro.
4. Experimental section

4.1. Experimental techniques

Chromatography

Column chromatography was performed using silica gel (Sigma Aldrich) 40-63 µm 60 Å treated with solvent system as described in individual procedures. The eluents are specified in individual procedures.

Infra-Red Spectroscopy

Recorded as KBr discs using a Perkin Elmer FT-IR system. Absorption maxima \((\nu_{\text{max}})\) are recorded in wavenumbers \((\text{cm}^{-1})\) and the abbreviations used to describe the intensity and appearance are: w, weak; m, medium; s, strong; br, broad.

Mass Spectroscopy

Mass spectroscopy was carried out by the staff in the Mass Spectrometry Laboratory, School of Chemistry, at the University of Manchester. Only molecular ions, fragments from molecular ions and other major peaks are reported as mass/charge \((m/z)\) ratios. Reported mass values are within \(\pm 5\) ppm mass units for electrospray and \(\pm 10\) ppm for HRMS.

Melting Points

Recorded on a Sanyo Gallenkamp MPD350 heater. Readings are uncorrected.

Nuclear Magnetic Resonance

\(^1\text{H}\) were recorded using a BrukerAvance 400 and a BrukerAvance 300. \(^1\text{H}\) assignments were supported by 2D \(^1\text{H} - ^1\text{H}\) COSY. Chemical shifts \((\delta_{\text{H}})\) are quoted in parts per million \((\text{ppm})\) to the nearest 0.01 ppm and referenced to the residual non-deuterated solvent peak.
Coupling constants ($J$) are quoted to the nearest 0.1 Hz. Spectral data is reported as follows: chemical shift, integration, multiplicity [s, singlet; d, doublet; t, triplet; m, multiplet; or as a combination of these e.g. dd, dt etc], coupling constant(s) and assignment. $^{13}$C were recorded using a BrukerAvance 400. $^{13}$C assignments were supported by 2D one-bond, $^{13}$C-$^1$H HMQC. Chemical shifts ($\delta_C$) are reported in ppm to the nearest 0.1 ppm and referenced to the residual non-deuterated solvent peak.

**Solvents**

Dichloromethane was dried over calcium hydride; THF was distilled from sodium and benzophenone under nitrogen atmosphere; Petroleum ether (b.p. 40-60) was distilled before use.

All chemicals were handled in accordance with safety instructions.

Solvents were evaporated on a Buchi RE111 rotary evaporator equipped with a Buchi 461 water bath or a Buchi RE111 rotary evaporator equipped with a Buchi 481 water bath.

Unless otherwise stated, all reactions were carried out with exclusion of water in an inert nitrogen atmosphere using a nitrogen balloon. All glassware was pre-dried in an oven at 110 °C and cooled in a nitrogen atmosphere prior to use. Reaction temperatures of ~0 °C were obtained using an ice water bath. Room temperature refers to 20-25 °C.

**Optical Rotation**

Taken with an AA-Polarimeter, Optical Activity Ltd. The sensitivity is one millidegree and the path length is 0.25 dm.

**Thin Layer Chromatography**

The plates used were plastic sheets, precoated with Macharey - Nagel, 0.2 mm silica gel with fluorescent indicator UV$_{254}$. Visualization was achieved by the quenching of UV fluorescence ($\lambda_{max} = 254$ nm) or by staining with potassium permanganate solution, followed by heating. Retention factors ($R_f$) are quoted to 0.01.
4.2. Experimental procedure

4.2.1. (1S,3R,4R,5R)-3-O,4-O-Cyclohexylidene-7-oxo-6-oxabicyclo[3.2.1]-octan-1,3,4-triol (48)

A solution of (-)-quinic acid 47 (5.0 g, 26.0 mmol), cyclohexanone (16 mL, 158 mmol) and toluene was heated in a flask which was fitted with a Dean and Stark trap and condensor, under reflux for 30 minutes. After, it was allowed to cool to room temperature, Amberlite® resin IR 120 (H) (5.0 g) [cleaned by washing with MeOH (20 mL) then with Et₂O (20 mL) and then drying under vacuum] was added to the solution. The resulting suspension was heated at reflux for 5 hours, and after cooling the flask to room temperature, the Amberlite® resin was removed by filtration. The filtrate was washed with a saturated aqueous solution of NaHCO₃ (2 x 20 mL) then brine (2 x 20 mL) and dried over MgSO₄. The solvent was removed in vacuo affording a yellow oil to which petroleum ether was added resulting in precipitation of a white solid which was collected by filtration and dried (4.34 g, 65%). Rf 0.28 [(petroleum ether (40:60): ethyl acetate (2:1)]; m.p.: 141-143 °C [Lit.²⁴ m.p 139-141°C]; [α]D₂⁸ -33.6 (c 1.0 in CH₂Cl₂) [Lit.²⁴ [α]D²⁵ -33 (c 1.05 in CHCl₃)]; νmax/cm⁻¹ 3426br (O-H), 2929m (C-H), 2851m (C-H), 1765s (C=O, lactone); δH (400 MHz, CDCl₃) 1.41-1.72 (10H, m, 5 x CH₂ of cyclohexane), 2.22 (1H, dd, J 14.6, 3.0, C(2)Hax), 2.28-2.40 (2H, m, C(2)Hox and C(6)Heq), 2.66 (1H, d, J 11.6, C(6)Hax), 4.31 (1H, ddd, J 6.5, 2.5, 1.3, C(4)H), 4.49 (1H, td, J 6.5, 3.0, C(3)H), 4.75 (1H, dd, J 6.3, 2.5, C(5)H); δC (100 MHz, CDCl₃) 23.5, 23.9, 25.0, 33.6 (4 x CH₂, of cyclohexane), 34.3 (C(6)H₂), 36.8 (CH₂...
of cyclohexane), 38.4 (C(2)H), 71.0 (C(3)H), 71.5 (C(1)), 71.7 (C(4)H), 76.0 (C(5)H), 110.6 (acetal C), 178.8 (C=O); m/z (+ES) 277.2 ([M+Na]^+, 100%).

4.2.2. 3-O,4-O-Cyclohexyldiene-(3R,4S,5R)-trihydroxycyclohexanone (49)

To a mixture of the lactone 48 (1.87 g, 7.35 mmol) in methanol (100 mL) at 0 °C, NaBH₄ (2.78 g, 73.5 mmol) was added portionwise. After effervescence had ceased, the solution was stirred overnight at room temperature under an atmosphere of N₂. A saturated aqueous solution of ammonium chloride (30 mL) was added to quench the reaction mixture. A white solid was then obtained after the resulting mixture was concentrated in vacuo. EtOAc (100mL) was added and the resulting suspension was filtered. EtOAc (3 x 30mL) was then added to extract additional product from the residue and the combined washings were concentrated in vacuo to give the crude triol 69 as an off-white oil (1.88g, 99%).

Warm water (4.5 mL) was added to dissolve sodium periodate (3.89 g, 18.2 mmol) in a flask. To the resulting solution, silica gel (8.6 g) was added portionwise to prepare silica-supported sodium periodate as a free flowing powder. DCM (21 mL) was added to this solution, followed by a solution of crude triol 69 (1.88 g, 7.28 mmol) in DCM (17 mL). The solution was stirred at room temperature under an atmosphere of N₂ for 1.5 hours, and then the resulting mixture was filtered to remove the silica. The silica was washed with a further three portions of DCM (3 x 20 mL). The combined filtrate was dried over MgSO₄ and concentrated in vacuo to give the title compound 49 as a pale yellow solid (1.54g, 93%). Rₜ 0.25 [(petroleum ether (40:60): ethyl acetate (3:1)]; m.p.: 96-98 °C [Lit. 56 m.p
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98 °C; [α]$_D$$_{28}$ +94.6 (c 0.5 in CH$_2$Cl$_2$), [Lit.$^{56}$ [$α$]$_D$$_{25}$ +103 (c 1.36 in CHCl$_3$)]; $v$$_{max}$/cm$^{-1}$ 3456br (O-H), 2936s (C-H), 2861w (C-H), 1709s (C=O); δ$_H$ (400 MHz, CDCl$_3$) 1.42-1.64 (10H, m, 5 x CH$_2$ of cyclohexane), 1.76 (1H, br, s, OH), 2.46 (1H, ddd, J 17.7, 2.9, 1.8, C(6)Heq), 2.69-2.84 (2H, m, C(2)Heq and C(6)Hax), 2.81 (1H, dd, J 17.7, 3.3, C(2)Hax), 4.25-4.29 (1H, m, C(5)H), 4.32 (1H, dt, J 6.8, 2.9, C(4)H), 4.71 (1H, d~t, J 6.8, 3.5, C(3)H); δ$_C$ (100 MHz, CDCl$_3$) 23.5, 23.9, 25.1, 33.2, 36.2 (5 x CH$_2$, of cyclohexane), 40.2 (C(2)H$_2$), 41.6 (C(6)H$_2$), 68.4 (C(5)H), 71.7 (C(3)H), 74.7 C(4)H, 109.5 (acetal C), 204.9 (C=O); m/z (+ES) 249 ([M+Na]$^+$, 100%).

4.2.3. 4-O,5-O-Cyclohexylidene-(4S,5R)-dihydroxy-cyclohex-2-en-1-one (50)

To a solution of hydroxyketone 49 (2.80 g, 12.37 mmol) in distilled DCM (55 mL) at 0 °C, under an atmosphere of N$_2$, were added methanesulfonyl chloride (1.5 mL, 18.6 mmol) and triethylamine (5.1 mL, 36.9 mmol). The mixture was stirred at room temperature for 4 hours when it was quenched by the addition of water (18 mL) followed by extraction with DCM (2 x 18 mL). 0.1 M HCl (2 x 18 mL) was added to wash the combined organic extracts which were then dried over MgSO$_4$ and concentrated in vacuo to give an orange oil. The resulting residue was purified by flash silica chromatography, eluting with petroleum ether 40-60: ethyl acetate (3:1), to yield enone 50 as an off-white solid (2.03 g, 79%). R$_f$ 0.23 [(petroleum ether (40:60): ethyl acetate (3:1)]; m.p.: 52-54 °C [Lit.$^{57}$ m.p 56-58 °C]; [α]$_D$$_{24}$+156.5 (c 0.8 in CH$_2$Cl$_2$) [Lit.$^{57}$ [$α$]$_D$$_{26}$ +135 (c 1.00 in CH$_2$Cl$_2$)]; $v$$_{max}$/cm$^{-1}$ 2941m (C-H), 2862w (C-H), 1647s (C=O); δ$_H$ (400 MHz, CDCl$_3$) 1.38-1.60 (10H, m, 5 x CH$_2$ of cyclohexane), 1.76 (1H, br, s, OH), 2.46 (1H, ddd, J 17.7, 2.9, 1.8, C(6)Heq), 2.69-2.84 (2H, m, C(2)Heq and C(6)Hax), 2.81 (1H, dd, J 17.7, 3.3, C(2)Hax), 4.25-4.29 (1H, m, C(5)H), 4.32 (1H, dt, J 6.8, 2.9, C(4)H), 4.71 (1H, d~t, J 6.8, 3.5, C(3)H); δ$_C$ (100 MHz, CDCl$_3$) 23.5, 23.9, 25.1, 33.2, 36.2 (5 x CH$_2$, of cyclohexane), 40.2 (C(2)H$_2$), 41.6 (C(6)H$_2$), 68.4 (C(5)H), 71.7 (C(3)H), 74.7 C(4)H, 109.5 (acetal C), 204.9 (C=O); m/z (+ES) 249 ([M+Na]$^+$, 100%).
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CH\textsubscript{2} of cyclohexane), 2.68 (1H, dd, \(J 17.7, 3.8, \text{C(6)Hax}\)), 2.97 (1H, ddd, \(J 17.7, 2.5, 1.0, \text{C(6)Heq}\)), 4.66-4.69 (1H, m, \text{C(4)H}), 4.71-4.73 (1H, m, \text{C(5)H}), 6.01 (1H, dt, \(J 10.4, 1.0, \text{C(2)H}\)), 6.65 (1H, ddd, \(J 10.4, 2.8, 2.0, \text{C(3)H}\)); \(\delta_C\) (100 MHz, CDCl\textsubscript{3}) 24.1, 24.2, 25.2, 36.2, 37.7 (5 x CH\textsubscript{2} of cyclohexane), 39.1 (C(6)H\textsubscript{2}), 70.9 (C(4)H), 73.3 (C(5)H), 110.8 (acetal C), 129.0 (C(2)H), 146.4 (C(3)H), 195.9 (C=O); \text{m/z} (+ES) 231.0 ([M+Na]\textsuperscript{+}, 100%).

4.2.4. 3-O, 4-O-Cyclohexylidene-5-(4-bromophenyl)-(3R,4S,5S)-dihydroxycyclohexanone (70)

To a solution of enone 50 (100 mg, 0.48 mmol), in dioxane: water (10:1, 0.7 mL), was added 4-bromophenylboronic acid (240 mg, 1.2 mmol) and [RhOH(cod)]\textsubscript{2} (10.7 mg, 0.023 mmol) followed by Et\textsubscript{3}N (0.062 ml, 0.48 mmol). The solution was stirred at r.t. for 6 hours when it was concentrated in vacuo to give a brown oil. This residue was purified by flash silica chromatography, eluting with (petroleum ether 40-60: ethyl acetate (5:1), to give the conjugate adduct 70 as a light yellow oil (0.14g, 81%). \(R_f\) 0.55 [(petroleum ether 40-60: ethyl acetate (5:1)); m.p.: 71-73.8; \([\alpha]_D^{24}\) -105.5 (c 0.77 in CH\textsubscript{2}Cl\textsubscript{2}); \(\nu_{\text{max}}/\text{cm}^{-1}\) 2931 m (C-H), 2859 w (C-H), 1712 s (C=O); \(\delta_H\) (300 MHz, CDCl\textsubscript{3}) 1.48-1.75 (10H, m, 5 x CH\textsubscript{2} of cyclohexane), 2.55 (1H, dd, 17.5, 10.2 C(6)Heq), 2.61-2.80 (3H, m, C(2)H\textsubscript{2} and C(6)Hax), 3.35 (1H, ddd, \(J 10.2, 6.5, 4.7, \text{C(5)H}\)), 4.50 (1H, t, \(J 6.5, \text{C(4)H}\)), 4.56-4.61 (1H, m, C(3)H); 7.48 (2H, d, \(J 8.4, \text{Ar-H}\)), 7.12 (2H, d, \(J 8.4, \text{Ar-H}\)); \(\delta_C\) (100 MHz, CDCl\textsubscript{3}) 23.6, 24.0, 25.1, 33.8, 37.1 (5 x CH\textsubscript{2} of cyclohexane), 41.0 (C(6)H\textsubscript{2}), 42.5 (C(2)H\textsubscript{2} and C(5)H

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(overlapping)), 72.1 (C(4)H), 76.6 (C(3)H), 109.6 (acetal C), 121.0 (Ar-CH), 129.1 (Ar-CH), 131.9 (Ar-C), 139.3 (Ar-C), 208.1 (C=O); m/z (+ES) 389 ([M(81Br)+Na]+, 100%), 387 ([M(79Br)+Na]+, 95%).

4.2.5 (4R,5S)-4-Hydroxy-5-(4-bromophenyl)-cyclohex-2-enone (71)

![Chemical structure of 71](image)

**Method 1:**

To a solution of the adduct 70 (0.125 g, 0.34 mmol) in THF (2.2 ml) was added 0.5M NaOH (1 drop [~0.01 mL]) every hour at 0 °C and the reaction was monitored by TLC. After 2 hours, the reaction was quenched by the addition of a saturated aqueous solution of NH₄Cl (10 mL) and extracted with Et₂O (2 x 10 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo. The residue was purified by flash silica chromatography, eluting with petroleum ether 40-60: ethyl acetate (2:1), to give the title compound 71 as a colourless oil (33 mg, 34%). Rf 0.17 [petroleum ether 40-60: ethyl acetate, (2:1)]; [α]D²⁸ -76.8 (c 0.25 in CH₂Cl₂); νmax/cm⁻¹ 3381br (O-H), 2924w (C-H), 2850w (C-H), 1666 s (C=O); δH (400 MHz, CDCl₃) 2.01 (1H, d, J 4.5, O-H), 2.63-2.69 (2H, m, C(6)H₂), 3.23 (1H, ~q, J 9.6, C(5)H), 4.65 (1H, dt, J 9.6, 2.0, C(4)H), 6.07 (1H, dd, J 10.3, 2.0, C(2)H), 6.99 (1H, dd, J 10.3, 2.0, C(3)H), 7.19 (2H, d, J 8.6, ArCH), 7.53 (2H, d, J 8.6, ArCH); δC (100 MHz, CDCl₃) 42.5 (C(6)H₂), 58.2 (C(5)H), 72.6 (C(4)H), 127.2 (Ar-CH), 129.0 (Ar-CH), 129.4 (C(2)H), 139.2 (Ar-C), 140.0 (Ar-C), 152.9 (C(3)H), 197.7 (C=O); m/z (+ES) 291 ([M(81Br)+Na]+, 95%), 289 ([M(79Br)+Na]+, 100%).

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Method 2:

To a solution of conjugate adduct 70 (0.27 g, 0.73 mmol) in DCM (10 mL) was added DBU (0.120 mL). The solution was stirred at r.t. and the reaction was monitored by TLC. After 1.5 hours, the reaction was quenched with a 0.1 M aqueous solution of HCl (10 mL) and extracted with DCM (2 x 10 mL). The combined organic extracts were washed with brine then dried over MgSO₄ and concentrated in vacuo. The residue was purified by flash silica chromatography, eluting with petroleum ether 40-60: ethyl acetate (5:1), to give the title compound 71 as a colourless oil (165 mg, 65%).

4.2.6. (4R,5S)-4-((Triethylsilyl)oxy)-5-(4-bromophenyl)-cyclohex-2-enone (72)

To a solution of 2,6 lutidine (0.09 ml, 1.4 mmol) and TESOTf (0.30 mL, 1.1 mmol) in DCM (4 mL) at -78 °C was added, dropwise, a solution of alcohol 71 (128 mg, 0.48 mmol) in DCM (4 mL). The reaction mixture was stirred at -78 °C under an atmosphere of N₂ for 15 min. After this time, the reaction was quenched by the addition of a saturated aqueous solution of ammonium chloride (5 mL) and extracted with DCM (3 x 5 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo. Purification of the residue by flash column chromatography, eluting with petroleum ether 40-60: ethyl acetate (5:1), gave the title compound 72 as a colourless oil (123 mg, 67%). Rₐ 0.75 [petroleum ether 40-60: ethyl acetate (5:1)]; [α]D²⁸⁻¹⁰⁰.₄ (c 1.00 in CH₂Cl₂); νmax/cm⁻¹ 2955w (C-H), 2876w (C-H), 1678s (C=O enone); δH (400 MHz, CDCl₃) 0.26-0.45 (6H, m,
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Si(CH$_2$CH$_3$)$_3$, 0.76-0.81 (9H, m, Si(CH$_2$CH$_3$)$_3$), 2.65 (1H, ddd, J 16.8, 4.8, 1.1, C(6)H$_{eq}$), 2.7 (1H, dd, J 16.8, 13.6, C(6)H$_{ax}$), 3.23 (1H, ddd, J 13.6, 9.5, 4.8, C(5)H), 4.52 (1H, dt, J 9.5, 2.1, C(4)H), 6.03 (1H, ddd, J 10.3, 2.1, 1.3, C(2)H), 6.83 (1H, dd, J 10.3, 2.1, C(3)H), 7.14 (2H, d, J 8.3, ArCH), 7.47 (2H, d, J 8.3, ArCH); $\delta$C (100 MHz, CDCl$_3$) 4.4 (Si(CH$_2$CH$_3$)$_3$), 6.6 (Si(CH$_2$CH$_3$)$_3$), 42.5 (C(6)H$_2$), 50.1 (C(5)H), 72.5 (C(4)H), 128.4 (Ar-CH), 129.7 (Ar-CH), 131.5 (C(2)H), 138.8 (Ar-CH), 139.7 (Ar-CH), 153.6 (C(3)H), 197.9 (C=O); $m/z$ (+ES) 405.2 ([M($^{81}$Br)+Na]$^+$, 95%), 403.2 ([M($^{79}$Br)+Na]$^+$, 100%).

4.2.7. **(4R,5S)-4-((Triethylsilyl)oxy)-2-(hydroxymethyl)-5-(4-bromophenyl)-cyclohex-2-enone (73)**

![Chemical Structure](image)

To a mixture of enone 72 (133 mg, 0.35 mmol) in water (1.2 mL) was added SDS (34 mg, 0.12 mmol) and DMAP (0.35 mmol, 43 mg). The reaction mixture was stirred until a cloudy suspension was observed, then aqueous formaldehyde (37%) (4.9 mmol, 0.36 mL) was added and the reaction mixture was stirred at r.t. for 18 hours under an atmosphere of N$_2$. Brine (1.5 mL) was added to quench the reaction and the resulting mixture was extracted with EtOAc (3 x 8 mL). The combined organic extracts were washed with brine, dried over MgSO$_4$ and concentrated in vacuo to give a light yellow oil. Purification by flash column chromatography, eluting with petroleum ether 40-60: ethyl acetate (4:1), gave the title compound 73 as a viscous white oil (78 mg, 54%). R$_f$ 0.26 [petroleum ether (40:60): ethyl acetate (4:1)]; $[\alpha]_D^{28}$-68.8 (c 0.5 in CH$_2$Cl$_2$); $\nu_{max}$/cm$^{-1}$ 3338br (O-H), 2953w (C-H), 2875w (C-H), 1674s (C=O); $\delta$H (400MHz, CDCl$_3$) 0.28-0.45 (6H, m, Si(CH$_2$CH$_3$)$_3$), 0.79 (9H, t, J 8.6, Si(CH$_2$CH$_3$)$_3$), 2.68 (1H, dd, J 16.5, 4.9, C(6)H$_{eq}$), 2.75 (1H, dd, J 16.5, 4.9, C(6)H$_{eq}$), 7.14 (2H, d, J 8.3, ArCH), 7.47 (2H, d, J 8.3, ArCH); $\delta$C (100 MHz, CDCl$_3$) 4.4 (Si(CH$_2$CH$_3$)$_3$), 6.6 (Si(CH$_2$CH$_3$)$_3$), 42.5 (C(6)H$_2$), 50.1 (C(5)H), 72.5 (C(4)H), 128.4 (Ar-CH), 129.7 (Ar-CH), 131.5 (C(2)H), 138.8 (Ar-CH), 139.7 (Ar-CH), 153.6 (C(3)H), 197.9 (C=O); $m/z$ (+ES) 405.2 ([M($^{81}$Br)+Na]$^+$, 95%), 403.2 ([M($^{79}$Br)+Na]$^+$, 100%).

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13.4, C(6)Hax), 3.24 (1H, ddd, J 13.4, 9.3, 4.9, C(5)H), 4.26 (1H, dd, J 13.9, 1.2, CH,OH), 4.38 (1H, dd, J 13.9, 1.2, CH,OH), 4.55 (1H, ~q, J 9.3, 1.3, C(4)H), 6.77 (1H, ~s, C(3)H), 7.14 (2H, d, J 8.3, Ar-CH), 7.48 (2H, d, J 8.3, Ar-CH); δC (100 MHz, CDCl3), 4.5 (Si(C6H2CH3)3), 6.6 (Si(CH2C6H3)3), 42.7 (C(6)H2), 50.2 (C(5)H), 72.5 (C(4)H), 129.7 (Ar-CH), 131.6 (Ar-CH), 134.7 (C(2)), 139.5 (Ar-Ç), 142.0 (Ar-Ç), 151.1 (C(3)H), 198.7 (C=O); m/z 414.9 ([M(81Br) +H]+, 100%), 412.9 ([M(79Br) +H]+, 100%).

4.2.8. (4R,5S)-4-(Hydroxy- 2-(hydroxymethyl)-5-(4-bromophenyl)-cyclohexa-2-enone (74)

A solution of hydroxymethyl compound 73 (53 mg, 0.13 mmol) in TFA:H2O (7:1) (1.2 mL: 0.17) was stirred at r.t. for 40 minutes. The resulting mixture was concentrated in vacuo to give a brown oil which was purified by flash silica chromatography, eluting with petroleum ether 40-60: ethyl acetate (1:1), to give diol 74 as a viscous film (34 mg, 88%). Rf 0.2 [(petroleum ether (40:60): ethyl acetate (1:1)]; [α]D30 -46.2 (c 0.39 in CH2Cl2); νmax/cm⁻¹ 3369br (O-H), 2900w (C-H), 2869w (C-H), 1663s (C=O, enone); δH (400MHz, CDCl3) 2.33 (1H, br, s, C(4)H), 2.64-2.73 (2H, m, C(6)H2), 3.21 (1H, ~q, J 9.3, C(5)H), 4.37 (1H, d, J 14.2, CHaHbOH), 4.42 (1H, d, J 14.2, CHaHbOH), 4.65 (1H, dd, J 9.3, 1.3, C(4)H), 6.93 (1H, d, J 1.3, C(3)H), 7.18 (2H, d, J 7.9, Ar-CH), 7.53 (2H, d, J 7.9, Ar-CH); δC (100 MHz, CDCl3) 43.1 (C(6)H2), 50.0 (C(5)H), 60.8 (CH2OH) 71.5 (C(4)H), 121.7 (Ar-CH), 129.4 (Ar-CH), 132.2(C(2)), 137.7 (Ar-Ç), 138.4 (Ar-Ç), 147.8 (C(3)H), 198.1 (C=O); m/z (+ES) 299.1 ([M(81Br) +H]+, 90%), 297.1 ([M(79Br) +H]+, 90%).

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4.2.9. (4R,5S)-4-(Hydroxy-2-((E)(crotonyloxymethyl))-5-(4-bromophenyl)-cyclohexa-2-enone (76)

![Chemical Structure](image)

To a solution of hydroxymethyl compound 73 (78 mg, 0.19 mmol) in DCM (1mL) were added, crotonic anhydride (0.06 mL, 0.42 mmol), DMAP (2.3 mg, 0.019 mmol) and pyridine (0.13 mL, 1.67 mmol). The reaction was stirred r.t. under an atmosphere of N\textsubscript{2} for 2 hours. The reaction was then quenched by the addition of saturated aqueous NaHCO\textsubscript{3} (2 mL) and diluted with H\textsubscript{2}O (3 mL) and DCM (5 mL). The two layers were separated and the aqueous phase was extracted with DCM (3 x 5 mL). The combined organic extracts were washed with brine (5 mL), dried over MgSO\textsubscript{4} and evaporated in vacuo to give a dark yellow oil. The residue was purified by silica column chromatography, eluting with petroleum ether 40-60: ethyl acetate (18:1) to give partially purified crotonate ester 75 as a dark yellow oil.

A solution of partially purified crotonate ester 75 in TFA:H\textsubscript{2}O (7:1) (1.6 mL) was stirred at r.t. for 30 minutes. The solvents were evaporated in vacuo to give a brown oil. The residue was purified by flash silica chromatography, eluting with petroleum ether 40-60: ethyl acetate (5:1), to give the title compound 76 a pale yellow film (11 mg, 16%). R\textsubscript{f} 0.12 [petroleum ether 40-60: ethyl acetate (5:1)]; [α]\textsubscript{D}\textsuperscript{28} -45.8 (c 1.0 in CH\textsubscript{2}Cl\textsubscript{2}); ν\textsubscript{max}/cm\textsuperscript{-1} 3431br (O-H), 2917w (C-H), 2853w (C-H), 1717s (C=O, α,β-unsaturated ester), 1674s (C=O, enone); δ\textsubscript{H} (CDCl\textsubscript{3}, 400MHz) 1.91 (3H, dd, J 6.8, 1.5, CHCH\textsubscript{3}), 2.70- 2.73 (2H, m, C(6)H\textsubscript{2}), 3.21-3.28 (1H, m, C(5)H), 4.68 (1H, br, d, J 9.8, C(4)H), 4.86 (1H, d-t, J 14.0, 1.4, CH\textsubscript{3}H\textsubscript{5}O), 4.91 (1H, ddd, J 14.0, 2.5, 1.4, CH\textsubscript{3}CH\textsubscript{3}O), 5.91 (1H, dq, J 15.6, 1.5, 1.4, CH\textsubscript{3}H\textsubscript{5}O).

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CHCHCH₃), 6.94 (1H, ~q, J 1.5, C(3)H), 7.04 (1H, dq, 15.6, 7.1, CHCHCH₃) 7.19 (2H, d, J 7.7, Ar-CH), 7.53 (2H, d, J 7.7, Ar-CH); δC (100 MHz, CDCl₃) 18.1 (CHCHCH₃), 43.0 (C(6)H₂), 50.0 (C(5)H), 60.1 (CH₂O), 71.7 (C(4)H), 122.1 (CHCHCH₃), 129.4 (Ar-CH), 132.3 (Ar-CH), 134.3 (C(2)), 140.6 (Ar-C), 141.1 (Ar-C), 145.8 (CHCHCH₃), 148.0 (C(3)H), 165.9 (C=O, ester), 198.1 (C=O, enone); m/z (+ES) 367.2 ([M⁺⁻²⁸Br]⁺, 100%), 365.2 ([M⁺⁻²⁹Br]+H⁺, 95%).

4.2.10. 4-hydroxy-3,11-di-(4-bromophenyl)-perhydro-dibenzofuranone (112)

To a solution of adduct 70 (50 mg, 0.14 mmol) in THF (10 mL) was added 5 drops of 0.5 M NaOH (~0.25 mL). The reaction mixture was stirred at room temperature overnight when it was quenched with aqueous NH₄Cl (10 mL) and extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo to give a white solid which was purified by flash silica chromatography eluting with petroleum ether 40-60: ethyl acetate (2:1) to give the title compound 112 as a white solid (31 mg, 85%). Rf 0.6 [petroleum ether 40-60: ethyl acetate (2:1)]; [α]D¹⁷⁺⁻¹⁴⁰.3 (c 1.3 CH₂Cl₂), νmax/cm⁻¹ 3366br (O-H), 2899w (C-H), 2856w (C-H), 1710s (C=O, ketone), 1697s (C=O, ketone); δH (400MHz, CDCl₃) 2.05 (1H, d, J 4.2, OH), 2.46-2.69 (6H, m, C(2)H₂, C(8)H₂ and C(12)H₂), 2.81 (1H, dd, J 4.5, 1.7, C(6)H), 3.15 (1H, ddd, J 12.0, 9.4, 5.2, C(11)H), 3.24 (1H, td, J 10.1, 6.3, C(3)H), 3.55 (1H, ddd, J 11.1, 7.8, 1.7, C(9)H), 4.24 (1H, dd, J 10.1, 4.2, C(4)H), 4.38 (1H, dd, J 9.4, 7.8, C(10)H), 4.71 (1H, dd, J 4.5, 3.0, C(5)H), 7.11 (4H, d, J 8.5, Ar-CH), 7.48 (4H, d, J 8.5, Ar-CH); δC (100 MHz, CDCl₃),
36.6 (C(9)H), 40.1 (C(2)H₂ or C(8)H₂ or (C(12)H₂), 42.5 (C(2)H₂ or C(8)H₂ or (C(12)H₂), 42.6 (C(11)H), 44.1 (C(3)H), 45.1 (C(2)H₂ or C(8)H₂ or (C(12)H₂), 56.8 (C(6)H), 72.7 (C(4)H), 78.6 (C(10)H), 81.6 (C(5)H), 121.2 (Ar-C), 121.3 (Ar-C), 129.2 (Ar-CH, Ar-CH (overlapping)), 131.9 (Ar-CH), 132.0 (Ar-CH), 138.9 (Ar-C), 140.11 (Ar-C), 205.6 (C=O), 208.8 (C=O); m/z (+ES) 581.22 ([M(81Br +Na81Br) +Na]+, 30%), 579.33 ([M(81Br +Na79Br) +Na]+, 80%), 577.27 ([M(79Br +Na79Br) +Na]+, 40%).

4.2.11. Attempted synthesis of 3-O, 4-O-Cyclohexylidene-5-(4-biphenyl)-(3R,4S,5S)-dihydroxyxyclohexanone (77)

![Chemical Structure](image)

**Method 1**

To a solution of conjugate adduct 70 (0.1 g, 0.27 mmol) in DME (5 mL) were added Pd(OAc)₂ (1.85 mg, 3% mol), phenylboronic acid (36.7 mg, 0.3 mmol) and triphenyl phosphine (6.47 mg, 9% mol) followed by CsF (91.6 mg, 0.602 mmol). The reaction mixture was heated at reflux and it was monitored by TLC. The resulting solution was cooled to r.t and quenched by the addition of water (5 mL) and extracted with EtOAc (2 x 5 mL). The combined organic extracts were washed with brine (10 mL) and the aqueous extracts were washed with EtOAc (10 mL). The combined organic washes were dried over MgSO₄ and evaporated in vacuo to give a brown oil. This residue was purified by flash silica chromatography, eluting with petroleum ether 40-60: ethyl acetate (1:1), to yield starting material 70 back as brown oil. The title compound biphenyl derivative 77 was not observed.
Method 2
To a solution of conjugate adduct 70 (0.1 g, 0.27 mmol) in 2-propanol (4 mL) was added phenylboronic acid (35 mg, 0.29 mmol) under an atmosphere of N₂. Pd(OAc)₂ (3.07 mg, 5 mol %), triphenylphosphine (10.76 mg, 15 mol %) and 2M Na₂CO₃ (0.16 mL) were added to the resulting solution. The reaction mixture was heated at reflux an atmosphere of N₂ for 2 days and its completion was monitored by TLC. The resulting solution was cooled to r.t. and then extracted with water (2 x 5 mL) and EtOAc (2 x 5 mL). Then the combined organic extracts were washed with saturated aqueous NaHCO₃ (2 x 5 mL) solution then were dried over MgSO₄ and evaporated in vacuo to give an orange film. This residue was purified by flash silica chromatography, eluting with petroleum ether 40-60: ethyl acetate (1:1), to yield alcohol 71 as a colourless oil (data as reported for compound 71). The title compound biphenyl derivative 77 was not obtained.

Method 3
To a mixture (5 mL) of freshly distilled THF and 2 M K₂CO₃ (0.4 mL), stirred under an atmosphere of N₂, conjugate adduct 70 (0.1 g, 0.27 mmol), PEPPSI (3.73 mg, 2 mol %) and phenylboronic acid (40 mg, 0.33 mmol) were added. The resulting mixture was heated at reflux for overnight. After the reaction mixture had cooled to r.t., it was diluted with water (5 mL) and extracted with Et₂O (2 x 5 mL). The combined extracts were washed with water, then dried over MgSO₄ and evaporated in vacuo to give a brown oil. This residue was purified by flash silica chromatography, eluting with petroleum ether 40-60: ethyl acetate (1:1), to yield alcohol 71 as a colourless oil (data as reported for compound 71). The title biphenyl derivative 77 was not obtained.
4.2.12. Attempted synthesis of 3-O,4-O-Cyclohexylidene-5-(4-(trimethylsilyl)phenylacetylene)-(3R,4S,5S)-dihydroxycyclohexanone (78)

To a solution of conjugate adduct 70 (0.1 g, 0.27 mmol) in freshly distilled THF (1.5 mL) and freshly distilled triethylamine (0.09 mL, 0.65 mmol), ethynyltrimethylsilane (0.06 mL, 0.41 mmol) was added under an atmosphere of N₂. The reaction solution was stirred for overnight and its completion was monitored by TLC. The resulting mixture concentrated in vacuo to give a black oil which was quenched by the addition of water (5 mL) and organic material extracted into EtOAc (2 x 5 mL). The combined organic extracts were dried over MgSO₄ and evaporated in vacuo to give a black oil. The title compound was not obtained.
Development of an approach to novel hybrid analogues of COTC and Antheminone A as potential anticancer agents

References:


Songül Kaskun


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