SYNTHESIS OF 2-AMINO-3-CYANO-4H-CHROMENES
A THESIS
SUBMITTED TO THE GRADUATE SCHOOL
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE
MASTERS OF SCIENCE

BY
RYAN W. MCCLURG
ADVISOR: ROBERT E. SAMMELSON

BALL STATE UNIVERSITY
MUNCIE, INDIANA
MAY 2010
Acknowledgments

I would like to sincerely express my gratitude toward the people of this department. I have learned an unquantifiable amount of knowledge from the various faculty, staff, and students I have interacted with. This time and these experiences have allowed me to mature and grow in both my personal and professional life. I genuinely appreciate the opportunity I have been granted and strongly feel that the knowledge and experience I have gained here has afforded great future opportunity and will continue to benefit me as I continue on to PhD studies in this field.

Particularly, I would like to acknowledge those on the graduate committee whom allowed me to attend and those faculty who have taught me in class, I have continually admired their professional competence and very much appreciated their patience.

I would absolutely like to recognize the members of my thesis committee and recommendation writers for taking time out of their lives to help me become successful, and of course my research advisor Dr. Sammelson for persistently challenging me to work hard...even though admittedly at times I may have been reluctant to do so.

Thank you very much.
Abstract

Thesis: Synthesis of Substituted 2-Amino-3-Cyano-4H-Chromenes

Student: Ryan McClurg

Degree: Master of Science

College: Science and Humanities

Date: April 2010

Pages: 157

The Knoevenagel reaction is defined by the condensation of an aldehyde or ketone with a carbon nucleophile produced by the deprotonation of a methylene species whose acidity is dramatically increased by bonds to strongly electron withdrawing groups. Previously, our group developed an effective one-pot method for the preparation of 4H-chromenes using sodium borohydride reduction of the cyclized intermediates formed by the Knoevenagel condensation of malononitrile with salicylaldehydes in aqueous ethanol. In this study we outline the extension of these strategies to include 2′-hydroxyphenylketones as the starting material. Many of these compounds are also unique and were prepared by Friedel-Crafts acylation of phenols with acyl chlorides and/or Fries Rearrangement of the corresponding phenyl ester.
The objective of this project has been to expand the application of the methods optimized in our lab for the simple and efficient formation of carbon-carbon bonds via the selective reduction of the alkylidene portion of the Knoevenagel reaction products. These methods have allowed for the production of several important classes of natural product-like compounds. Specifically, in this investigation, we have adapted these methods to the production of various 4-alkyl and 4-aryl substituted 3-amino-2-cyano-4H-chromenes. These types of molecules exhibit diverse pharmacological activity and have been shown to be potentially useful for the treatment of various diseases. A subset of the synthesized compounds will be submitted to Eli Lilly through their PD² program.

Further variation of substrates included the reaction of salicylaldehydes with ethyl cyanoacetate or cyanoacetamide which provided products unreported in the literature. Reactions with cyanoacetates gave the expected 3-carboethoxy(ester) functionalized 4H-chromene compounds. Products from cyanoacetamide were found to occur in open rather than cyclized forms.
# Table of Contents

<table>
<thead>
<tr>
<th>List of Figures</th>
<th>iii.</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Schemes</td>
<td>iv.</td>
</tr>
<tr>
<td>List of Tables</td>
<td>v.</td>
</tr>
</tbody>
</table>

**Chapter 1  Introduction and Background Literature**

1.1 Introduction
1.2 Production of Monosubstituted Malononitriles
1.3 Knoevenagel Condensation with Aryl Substrates
1.4 Knoevenagel Condensation with Salicylaldehydes
1.5 One-pot Synthesis of Substituted 4H-Chromenes

**Chapter 2  Synthesis of 2'-Hydroxyphenylketones**

2.1 Introduction
2.2 Other Applications of Benzophenones
2.3 Literature Methods for the Preparation of 2'-Hydroxyphenylketones
2.4 Results and Discussion
2.5 General Experimental
2.6 Data

**Chapter 3  Synthesis of 2-Amino-3-Cyano-4H-Chromenes**

3.1 Introduction
3.2 Background for HTS Identification of Medicinally Relevant Compounds
3.3 Applications of Chromene Compounds
3.4 Literature Methods
3.5 Results and Conclusions
3.6 General Experimental
3.7 Data
# Table of Contents

<table>
<thead>
<tr>
<th>Chapter 4</th>
<th>One pot method applied to Salicylaldehydes with Ethylcyanoacetate or Cyanoacetamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>4.2</td>
<td>Applications of other 4H-Chromenes</td>
</tr>
<tr>
<td>4.3</td>
<td>Literature Methods for Preparation of Other 4H-Chromenes</td>
</tr>
<tr>
<td>4.4</td>
<td>Discussion and Conclusions</td>
</tr>
<tr>
<td>4.5</td>
<td>General Experimental</td>
</tr>
<tr>
<td>4.6</td>
<td>Data</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Appendix</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix</td>
<td>70</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>Introduction and Background Literature</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>Synthesis of 2′-Hydroxyphenylketones</td>
</tr>
<tr>
<td>Figure</td>
<td>2.1 Anti-Inflammatory Benzophenones</td>
</tr>
<tr>
<td>Page Number</td>
<td>14</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>Synthesis of 2-Amino-3-Cyano-4H-Chromenes</td>
</tr>
<tr>
<td>Figure</td>
<td>3.1 2-amino-4H-chromene Medicinal Scaffold</td>
</tr>
<tr>
<td></td>
<td>3.2 Relevant Chromene Compounds</td>
</tr>
<tr>
<td></td>
<td>3.3 Amlexanox</td>
</tr>
<tr>
<td></td>
<td>3.4 Intermediates for One-Pot Method</td>
</tr>
<tr>
<td>Page Number</td>
<td>30 32 33 37</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>One-Pot Method Applied to Salicylaldehydes with Ethyl Cyanoacetate or Cyanoacetamide</td>
</tr>
<tr>
<td>Figure</td>
<td>4.1 HA 14-1 / sHA 14-1</td>
</tr>
<tr>
<td></td>
<td>4.1 HA 14-1 <em>In vitro</em> Decompostion Products</td>
</tr>
<tr>
<td>Page Number</td>
<td>53 54</td>
</tr>
</tbody>
</table>
# List of Schemes

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>Introduction and Background Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schemes</td>
<td></td>
</tr>
<tr>
<td>1.1</td>
<td>General Scheme for Knoevenagel Condensation</td>
</tr>
<tr>
<td>1.2</td>
<td>Proposed Mechanism for One-Step/One-Pot Condensation/Reduction Method</td>
</tr>
<tr>
<td>1.3</td>
<td>Uncatalyzed Condensation</td>
</tr>
<tr>
<td>1.4</td>
<td>Condensation of Malononitrile with Salicylaldehyde</td>
</tr>
<tr>
<td>1.5</td>
<td>Further Methods for Catalyzed Condensation of Malononitrile with Salicylaldehyde</td>
</tr>
<tr>
<td>1.6</td>
<td>Intermediates in the Reaction of Malononitrile with Salicylaldehyde</td>
</tr>
<tr>
<td>1.7</td>
<td>Substituted Benzopyranopyridenes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 2</th>
<th>Synthesis of 2′-Hydroxyphenylketones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schemes</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>Fries Rearrangement of p-Nitrophenyl Acetate</td>
</tr>
<tr>
<td>2.2</td>
<td>Metal Promoted Fries Rearrangement</td>
</tr>
<tr>
<td>2.3</td>
<td>Palladium Catalyzed Coupling of Salicylaldehydes with Aryl Iodides</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 3</th>
<th>Synthesis of 2-Amino-3-Cyano-4H-Chromenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schemes</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>Three Component Production of 4-Substituted 2-Amino-3-Cyano-4H-Chromene</td>
</tr>
<tr>
<td>3.2</td>
<td>Malononitrile Condensation with 2′-Hydroxyphenylketones</td>
</tr>
<tr>
<td>3.3</td>
<td>Oxidation/Reduction of 2H/4H-Chromenes</td>
</tr>
</tbody>
</table>
## List of Schemes

<table>
<thead>
<tr>
<th>Chapter 4</th>
<th>One-Pot Methods Applied to Salicylaldehydes with Ethyl Cyanoacetate or Cyanoacetamide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Schemes</strong></td>
<td><strong>Page Number</strong></td>
</tr>
<tr>
<td>4.1 Preparation of Ester Functionalized 4H-Chromenes</td>
<td>56</td>
</tr>
<tr>
<td>4.2 Electrocatalytic Condensation of Salicylaldehyde with Activated Methylene Compounds</td>
<td>56</td>
</tr>
<tr>
<td>4.3 Multicomponent Preparation of Ester Functionalized 4H-Chromenes</td>
<td>57</td>
</tr>
<tr>
<td>4.4 Further Multicomponent Preparation of Ester Functionalized 4H-Chromenes</td>
<td>57</td>
</tr>
<tr>
<td>4.5 Reaction Sequence For Preparation of Ester Functionalized 4H-Chromenes</td>
<td>58</td>
</tr>
</tbody>
</table>
# List of Tables

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Section</th>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 1</td>
<td>Introduction and Background Literature</td>
<td>1.1</td>
<td>Reductive Alkylation of Malononitrile with Aromatic Aldehydes</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2</td>
<td>Results of One-Pot Production of 4H-Chromenes</td>
<td>10</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>Synthesis of 2’-Hydroxyphenylketones</td>
<td>2.1</td>
<td>2’-Hydroxyphenylketones</td>
<td>18</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>Synthesis of 2-Amino-3-Cyano-4H-Chromenes</td>
<td>3.1</td>
<td>Substituted 4-H Chromene Products</td>
<td>39</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>One Pot Methods Applied to Salicylaldehydes with Ethyl Cyanoacetate or Cyanoacetamide</td>
<td>4.1</td>
<td>Ethyl Cyanoacetate/ Cyanoacetamide Products</td>
<td>60</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction and Background Literature

1.1 Introduction
1.2 Production of Monosubstituted Malononitriles
1.3 Knoevenagel Condensation with Aryl Substrates
1.4 Knoevenagel Condensation with Salicylaldehydes
1.5 One-Pot Synthesis of Substituted 4H-Chromenes
1.1 Introduction

As part of the ongoing project in our laboratory investigating the carbon-carbon bond forming Knoevenagel reaction, it was shown that the technique could be used to produce a variety of interesting and potentially useful compounds. This includes efficient access to several fundamental heterocyclic ring systems that are of particular biological interest. This reaction is the condensation of an aldehyde or ketone carbonyl with a carbon nucleophile produced by proton abstraction of compounds that have form Z-CH₂-Z’ or Z-CHR-Z’ where Z and Z’ may be CHO, COR, COOH, COOR, CN, NO₂, SOR, SO₂R, SO₂R.¹

Scheme 1.1 General Scheme for Knoevenagel Condensation

The methylene carbon atom of these molecules is activated by the adjoining electron withdrawing groups which reduce the electron density of the carbon sufficiently to make the C-H bond somewhat acidic and help to stabilize the resulting negative charge.

1.2 Production of Monosubstituted Malononitriles

The process was shown applicable by Sammelson and Allen for the production of monosubstituted malononitrile compounds by alumina catalyzed Knoevenagel reaction of malononitrile with n-alkylketones; and sodium borohydride reduction of the
alkylidene intermediate in a two step one-pot process. This was an improvement over methods such as direct alkylation of malononitrile$^{3a}$, or other condensation/reduction methods carried out with reducing agents like Indium metal in aqueous ethanolic ammonium chloride$^{3b}$, indium (III) chloride with sodium borohydride in acetonitrile$^{3c}$, or Hantzsch 1,4-dihydropyridine ester.$^{3d}$ In the case of direct alkylation the reaction has been known to over alkylate the substrate producing only the symmetrically disubstituted malononitrile. While some condensation reduction methods use more expensive reagents or require involved preparation and in the case of the Hantzsch 1,4-dihydropyridine ester suffer from lack of carbon efficiency. Further improvement was achieved by Dunham et. al. when they realized that sodium borohydride could be used as the Knoevenagel catalyst and as the reducing agent for the intermediate electron deficient olefin in a single step one pot process using aldehydes or ketones.$^{4}$ The general mechanism proposed for this reaction is presented in Scheme 1.1.
In this mechanism, sodium borohydride acts to produce an alkoxide ion from the starting ketone or aldehyde which in turn deprotonates malononitrile (pKa = 11.2) (or possibly the hydride itself deprotonates malononitrile). The carbanion then condenses with the carbonyl group and proton transfer and/or protonation precedes the elimination of water. The formed alkylidene malononitrile intermediate is an electron deficient olefin that reacts with hydride to give the substituted malononitrile anion (III). This is able to accept a proton from another malononitrile molecule, yielding the final product and another equivalent of the carbon nucleophile (II) to continue the process. The utility of this reaction was shown on a variety of alkyl ketones and aldehydes eventually leading to the production of benzyl substituted malononitriles from
benzaldehydes. Unfortunately, when these conditions were extended to aryl substrates the competing reduction of the aldehyde took precedence and lower yields of the desired product were obtained. Additionally, these alcohol side-products were no longer volatile and/or water soluble.

### 1.3 Knoevenagel Condensation with Aryl Substrates

The Knoevenagel condensation of these aryl aldehydes with malononitrile were shown to proceed in water\(^5\) or ethanol\(^6\) without other catalyst, and the reaction being driven toward completion by the precipitation of the product. These examples are represented in Scheme 1.2.

![Scheme 1.3 Uncatalyzed Condensation](image)

This work provided the foundation for Tayyari et. al. to develop the one-pot method for the reductive alkylation of malononitrile with aromatic aldehydes, which was a vast improvement on other procedures giving increased efficiency and yields.\(^7\) Also the one-pot procedure avoided the handling of hazardous intermediates as seen in entry 6 of Table 1.1, whose intermediate alkylidene species is known to be a lachrymator and used as a riot control agent. It was seen that when water was used as the solvent the reaction
mixture had a tendency to oil out and in absolute ethanol the reaction rate was very slow. The conditions were optimized by the use of a 95% ethanol/water solution at a concentration of 1.0M for the arylaldehyde and malononitrile. The procedure gave the desired products in good yields with appropriate reaction times on a variety of aryl substrates. These results are shown in Table 1.1.

![Reaction Scheme](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar</th>
<th>Product</th>
<th>Time(h)(^a)</th>
<th>Yield (%)(^b)</th>
<th>Yield (%)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>1</td>
<td>1</td>
<td>98</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>4-MeOPh</td>
<td>2</td>
<td>1</td>
<td>94</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>4-MePh</td>
<td>3</td>
<td>3.5</td>
<td>92</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>4-BrPh</td>
<td>4</td>
<td>2</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>4-ClPh</td>
<td>5</td>
<td>2</td>
<td>96</td>
<td>91</td>
</tr>
<tr>
<td>6</td>
<td>2-ClPh</td>
<td>6</td>
<td>24</td>
<td>94</td>
<td>X(^d)</td>
</tr>
<tr>
<td>7</td>
<td>4-NO(_2)Ph</td>
<td>7</td>
<td>2</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>8</td>
<td>3-NO(_2)Ph</td>
<td>8</td>
<td>1.5</td>
<td>97</td>
<td>88</td>
</tr>
<tr>
<td>9</td>
<td>2-NO(_2)Ph</td>
<td>9</td>
<td>0.5</td>
<td>97</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>4-HOPh</td>
<td>10</td>
<td>18</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>11</td>
<td>3-HOPh</td>
<td>11</td>
<td>18</td>
<td>76</td>
<td>X(^d)</td>
</tr>
<tr>
<td>12</td>
<td>4-HO-3-MeOPh</td>
<td>12</td>
<td>24</td>
<td>84</td>
<td>74</td>
</tr>
<tr>
<td>13</td>
<td>2-(4-ClBnO)Ph</td>
<td>13</td>
<td>3</td>
<td>75</td>
<td>X(^d)</td>
</tr>
<tr>
<td>14</td>
<td>4-Me(_2)NPh</td>
<td>14</td>
<td>2.5</td>
<td>98</td>
<td>90</td>
</tr>
<tr>
<td>15</td>
<td>4-(2-pyridyl)Ph</td>
<td>15</td>
<td>24</td>
<td>98</td>
<td>-(^e)</td>
</tr>
<tr>
<td>16</td>
<td>2,4,6-Cl(_3)Ph</td>
<td>16</td>
<td>24</td>
<td>97</td>
<td>-(^e)</td>
</tr>
<tr>
<td>17</td>
<td>2-fluorene</td>
<td>17</td>
<td>3</td>
<td>62</td>
<td>-(^e)</td>
</tr>
<tr>
<td>18</td>
<td>2,4-(MeO)(_2)Ph</td>
<td>18</td>
<td>24</td>
<td>-(^f)</td>
<td>98</td>
</tr>
<tr>
<td>19</td>
<td>2-(BnO)Ph</td>
<td>19</td>
<td>24</td>
<td>-(^f)</td>
<td>87</td>
</tr>
</tbody>
</table>

\(^a\) Time refers to the condensation step.

\(^b\) Isolated yield of purified compound using extraction method work-up.

\(^c\) Isolated yield of purified compounds using filtration method work-up.

\(^d\) No precipitate formed and extraction method work-up must be used.

\(^e\) Filtration method work-up was not attempted.

\(^f\) Extraction method work-up was not attempted.

Table 1.1 Reductive Alkylation of Malononitrile with Aromatic Aldehydes
When the two step one pot condensation/reduction procedure was performed on 2-hydroxybenzaldehydes (salicylaldehydes) the resulting product was not the expected 2′-hydroxybenzylmalononitrile. Instead it was found that an intramolecular cyclization occurs by the nucleophilic attack of the o-hydroxyl oxygen on one of the cyano carbons after the condensation, and upon reduction gave 2-amino-3-cyano-4H-chromenes in good to high yields. These methods allow access to this very important class of compounds and efficient production of the chromene moiety is a highly desirable and sought after process.

1.4 Knoevenagel Condensation with Salicylaldehydes

The reaction of malononitrile with salicylaldehyde has been reported to give several different products. In their paper Pande et al. described the condensation of several aromatic aldehydes with malononitrile including a 1:1 molar ratio of salicylaldehyde:malononitrile in water at 35 °C with 1-methylimidazole as a catalyst and they show an uncyclized product as the result (Scheme 1.3).

Deb et al. performed a similar reaction with a 1:1 molar ratio o-hydroxybenzaldehyde:malononitrile in water without catalyst and describe the...
product as a 2-imino-2H-1-benzopyran-3-carbonitrile.\textsuperscript{5} Zahouily \textit{et al.} report the use of potassium fluoride-doped natural phosphate as a catalyst in methanol solvent at room temperature and describe a 2-amino-3-cyano-4-malononitrile-4H-chromene as the result.\textsuperscript{9} Elinson \textit{et al.} generated malononitrile substituted 4H-chromene upon reaction of salicylaldehyde and malononitrile in alcohols in the presence of sodium bromide as an electrolyte in an undivided electrocatalytic cell.\textsuperscript{10} In ethanol with a piperidine catalyst Volmajer \textit{et al.} reported the product to be the 3-cyano-2-imino-2H-chromene.\textsuperscript{11}

\begin{equation}
\text{Scheme 1.5 Catalyzed Condensation of Malononitrile with Salicylaldehyde}
\end{equation}

Finally, Costa \textit{et al.} showed that reaction conditions dictated which product would be formed and careful control of these conditions allowed them to synthesize multiple compounds from the reaction of malononitrile and salicylaldehyde.\textsuperscript{12} Among the procedures was the production of the 2-imino-2H-chromene-carbonitrile using 1.7-2.1 equivalents of malononitrile to 1 equivalent salicylaldehyde in 0.05M aqueous sodium bicarbonate or 0.05M aqueous sodium carbonate at room temperature for 2-24 hours (Scheme 1.4) or alternatively the production of (2-amino-3-cyano-4H-chromen-4-yl)malononitrile with 1.7-2.1 molar equivalents of malononitrile to 1 equivalent salicylaldehyde in dichloromethane or methanol with triethylamine catalyst or 1.7-2:1 equivalents malononitrile to 1 equivalent salicylaldehyde in 0.05M aqueous sodium.
bicarbonate at room temperature for 5-40 minutes (Scheme 1.4). Costa also performed a $^1$H NMR study that provided a description of the intermediate products formed over the course of the condensation/cyclization. A depiction of their results is shown in Scheme 1.6.

Most notable among the results was the initial benzylidene malononitrile condensate product (A), an electron deficient olefin and good Michael acceptor, reacts a second equivalent of malononitrile to give the addition product (B). This yields the cyclized imino chromane species (C) which then tautomerizes to the enamine giving the 4-substituted 4H-aminochromene (D) that equilibrates with the 2H-iminochromene (E) by the simultaneous addition/elimination of the second malononitrile equivalent.
1.5 One-Pot Synthesis of Substituted 4H-Chromenes

Tayyari et al. were able to apply the one-pot procedure to the production of a variety of substituted 4H-chromene compounds. The reaction was carried out in 95% ethanol and it was found that 2 equivalents of malononitrile were required for good yields and the intermediate formed was the (2-amino-3-cyano-4H-chromen-4-yl)malononitrile. Table 1.2 lists the results of these experiments.

![Reaction Diagram]

<table>
<thead>
<tr>
<th>R_1</th>
<th>R_2</th>
<th>R_3</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>Br</td>
<td>91</td>
</tr>
<tr>
<td>CH_3CH_2O</td>
<td>H</td>
<td>H</td>
<td>93</td>
</tr>
<tr>
<td>Cl</td>
<td>H</td>
<td>Cl</td>
<td>87</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>NO_2</td>
<td>89</td>
</tr>
<tr>
<td>CH_3O</td>
<td>H</td>
<td>H</td>
<td>66</td>
</tr>
<tr>
<td>CH_3O</td>
<td>H</td>
<td>Br</td>
<td>61</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>89</td>
</tr>
<tr>
<td>CH=CH-CH=CH</td>
<td>H</td>
<td></td>
<td>73</td>
</tr>
</tbody>
</table>

Table 1.2 Results of One-Pot Production of 4H-Chromenes

In two of the experiments when the salicylaldehyde was substituted with a strong electron donating group the reaction proceeded by an additional pathway. In these cases the second equivalent of malononitrile added in a different arrangement. The malononitrile activated methylene carbon reacted with the chromene cyano carbon and in turn the chromene 2-amino group reacted with a malononitrile nitrile carbon through another cyclization process giving the corresponding substituted
benzopyranopyridine. These types of compounds are also of great interest and methods for their preparation are of importance. This reaction sequence can be seen in Scheme 1.7.³

![Scheme 1.7 Substituted Benzopyranopyridenes](image_url)
Chapter 2

Synthesis of 2’-Hydroxyphenylketones

2.1 Introduction
2.2 Other Applications of Benzophenones
2.3 Literature Methods for the Preparation of 2’-Hydroxyketones
2.4 Results and Conclusions
2.5 General Experimental
2.6 Data
2.1 Introduction

For the production of our 4H-chromene target molecules it was found that many of the 2’-hydroxyphenylketone precursor compounds were not readily available through commercial sources. These starting materials were prepared by the application of literature methods for Friedel-Crafts acylation and Fries rearrangement of the corresponding phenol and acyl substrates or by palladium catalyzed coupling of salicylaldehydes with iodoarenes.

2.2 Other Applications of Benzophenones

It is worth noting that benzophenone analogues have been well documented as chemotherapeutic agents especially for anti-inflammatory applications.\textsuperscript{35} Recently molecules containing the benzophenone core were reported to be a novel class of p38 MAP kinase inhibitors with high anti-inflammatory activity and are shown in Figure 2.1.\textsuperscript{21} Several classes of hydroxyl substituted benzophenones have been pursued and have shown promise as potential medicinal products.
2.3 Literature Methods for the Preparation of 2'-Hydroxyphenylketones

Among the various methods typically used to prepare aryl ketones, Friedel-Crafts acylation reactions are the most important. This reaction employs a Lewis acid catalyst (usually aluminum chloride) and often an acyl halide, although other reagents have been applied such as carboxylic acids, anhydrides, and ketenes. A common variation used particularly for the ortho acylation of phenols is referred to as the Fries rearrangement and involves the esterification of the phenol hydroxyl group before treatment with aluminum chloride. It is often reported that carrying out the Fries Rearrangement at high temperature produces an increased ratio of the ortho substituted product. (Scheme 2.1).
Numerous examples of the Fries Rearrangement can be found in the literature with many different variations using several different reagents. It has been reported frequently that as the acyl group becomes larger and more sterically hindered the para product becomes increasingly prevalent. To overcome this problem Miller utilized a lithiation procedure to direct acyl migration primarily to the ortho position. This metal promoted variation is known as the anionic Fries rearrangement (Scheme 2.2).
Satoh et al. were able to efficiently produce 2-hydroxybenzphenones by the direct coupling of salicylaldehydes and iodobenzene, using palladium(II) chloride as a catalyst in the presence of lithium chloride. This reaction can be seen in Scheme 2.3.

2.4 Results and Discussion

It was often observed that while following the general procedure for the Fries rearrangement consistently the product obtained was approximately a 50:50 mixture of the ortho / para isomers. The optimized procedure used most often simply involved the addition of the acid chloride directly to the phenol without prior efforts to ensure esterification. The isomers were often separated by exploiting their respective solubility in hexane (the ortho isomer proved much more nonpolar due to intramolecular
hydrogen bonding). Ultimately the most frequently used procedure was the Friedel-Crafts / Fries reaction for its ability to produce both alkyl and aryl ketones and general availability of starting materials. The coupling of salicylaldehydes with aryl iodides was quite efficient and produced the desired benzophenone derivatives without any unwanted isomers. This reaction was apparently limited to the formation of benzophenones and access to novel salicylaldehydes and aryl iodides was somewhat limited. The materials produced are listed in Table 2.1.
<table>
<thead>
<tr>
<th>( \text{R}_1 )</th>
<th>( \text{R}_2 )</th>
<th>( \text{R}_3 )</th>
<th>( \text{R}_4 )</th>
<th>\text{Product}</th>
<th>\text{Time (h)}</th>
<th>\text{Yield}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>Propyl</td>
<td>Methyl</td>
<td>H</td>
<td>H</td>
<td>2</td>
<td>2</td>
<td>79</td>
</tr>
<tr>
<td>i-propyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>3</td>
<td>2</td>
<td>42</td>
</tr>
<tr>
<td>i-butyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>4</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>Neopentyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>6</td>
<td>1.75</td>
<td>47</td>
</tr>
<tr>
<td>1-ethylpropyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>7</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>Phenyl</td>
<td>H</td>
<td>Methyl</td>
<td>H</td>
<td>8</td>
<td>2</td>
<td>38</td>
</tr>
<tr>
<td>Phenyl</td>
<td>Bromo</td>
<td>H</td>
<td>H</td>
<td>9</td>
<td>2</td>
<td>91</td>
</tr>
<tr>
<td>4-fluorophenyl</td>
<td>Methyl</td>
<td>H</td>
<td>H</td>
<td>10</td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>2-furanyl</td>
<td>Methyl</td>
<td>H</td>
<td>H</td>
<td>11</td>
<td>2</td>
<td>73</td>
</tr>
<tr>
<td>Phenyl</td>
<td>H</td>
<td>H</td>
<td>Ethoxy</td>
<td>12</td>
<td>8</td>
<td>82(^a)</td>
</tr>
<tr>
<td>Phenyl</td>
<td>Chloro</td>
<td>H</td>
<td>H</td>
<td>13</td>
<td>6</td>
<td>56(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Product produced by palladium coupling procedure method

\textbf{Table 2.1 2'-Hydroxyphenylketones}
2.5 General Experimental

Analytical thin-layer chromatography (TLC) was performed using Baker-Flex silica gel IB-F plates and visualized using UV lamp and basic KMnO$_4$ (2.3 g KMnO$_4$, 15 g K$_2$CO$_3$, 1.9 mL 2.5M NaOH in 300 mL DI H$_2$O). Column chromatography was performed using silica gel (35-70mm, 6nm pore) from Acros. Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer using an ATR accessory with a diamond element. Proton nuclear magnetic resonance ($^1$H NMR) spectra and carbon-13 nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on a JEOL Eclipse spectrometer at 400 MHz or 300 MHz and 100 or 75 MHz, respectively. Chemical shifts are reported downfield from referenced values for CDCl$_3$ ($^1$H NMR = 7.26ppm, $^{13}$C = 77.16 ± 0.06ppm). Purchased chemicals were used without further purification; prepared materials were purified by column chromatography or recrystallization prior to use.

**Representative procedure for Friedel-Crafts Acylation / Fries Rearrangement**

15 mmol of phenol was added to a round bottom flask followed by 15 mmol of acid chloride and then 22.5 mmol aluminum chloride. The mixture was heated at 140-160 °C for 2 hours and cooled to room temperature. 15 mL of 6M HCl was added to the glassy solid and the resultant solution was heated to boiling to complete hydrolysis. The mixture was extracted with hexanes (3x20 mL) the extracts were combined, dried over magnesium sulfate and stripped of solvent under vacuum. If necessary, the product was purified by recrystallization from ethanol or by column chromatography.
General Procedure for Preparation of Acyl Chlorides

When only the carboxylic acid was available the corresponding acid chloride was produced immediately prior to use by adding 1.2 molar equiv. of thionyl chloride and heating to 70°C for 45min.²⁶

Representative Procedure for the Direct Coupling of Salicylaldehydes with Iodobenzene²⁵

To a solution of 3-ethoxysalicylaldehyde (1 mmol) and iodobenzene (2 mmol) was added PdCl₂ (0.05 mmol) followed by LiCl (0.2 mmol) then Na₂CO₃ (2 mmol) and finally anhydrous DMF (5 mL). The mixture was heated at 100 °C for 8 hours or until complete as indicated by TLC. The solids were filtered out and 25 mL DI H₂O was added. This solution was extracted 3 times with CH₂Cl₂ and the extracts were combined and thoroughly washed with DI H₂O to remove traces of DMF, dried over magnesium sulfate, and concentrated under vacuum. This crude product was purified by column chromatography.
2.6 Data

2-butanoxyphenol (1)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using phenol (1.44g, 15.3 mmol), butyryl chloride (1.64g, 15.0 mmol), and aluminum chloride (2.91g, 22.5mmol; yielded a yellow oil (0.88g, 36%). $^1$H NMR (300MHz, CHLOROFORM-d) δ 12.41 (s, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.46 (t, J = 7.7Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 6.89 (t, J = 7.5Hz, 1H), 2.97 (t, J = 7.4 Hz, 2H), 1.79 (sex, J = 7.4 Hz, 2H), 1.03 (t, J = 7.4 Hz, 3H). $^{13}$C (75MHz, CHLOROFORM-d) δ 206.7, 162.4, 136.1, 129.9, 119.3, 118.8, 118.4, 17.8, 13.7.

2-butanoxy-4-methylphenol (2)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using p-cresol (0.54 g, 5.0 mmol), butyryl chloride (0.59 g, 5.5 mmol), and aluminum chloride (1.00 g, 7.5 mmol); yielded an off white solid (0.70 g, 79%). $^1$H NMR (300MHz, CHLOROFORM-d) δ 12.23 (s, 1H), 7.53 (s, 1H), 7.28 (d, J = 8.2 Hz, 1H), 6.88 (d, J = 8.5 Hz,
1H), 2.96 (t, J = 7.4 Hz, 2H), 2.31 (s, 3H), 1.78 (sex, J = 7.4 Hz, 2H), 1.02 (t, J = 7.4 Hz, 3H). $^{13}$C (75MHz, CHLOROFORM-d) δ 206.6, 160.4, 137.1, 129.7, 127.8, 119.0, 118.2, 40.1, 20.5, 17.8, 13.8.

2-isobutanoylphenol (3)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using phenol (2.02 g, 11.5 mmol), isobutyryl chloride (1.10 g, 10.3 mmol), and aluminum chloride (1.6 g, 12.0 mmol); yielded pale yellow oil (1.46 g, 42%). $^1$H NMR (300MHz, CHLOROFORM-d) δ 12.53 (s, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.46 (t, J = 8.5 Hz, 1H), 6.99 (d, J = 7.4 Hz, 1H), 6.90 (t, J = 8.0 Hz, 1H), 3.62 (sep, J = 6.9 Hz, 1H), 1.26 (d, J = 6.9 Hz, 6H). $^{13}$C (75MHz, CHLOROFORM-d) δ 210.8, 162.9, 136.1, 129.8, 118.8, 118.5, 118.0, 115.2, 34.8, 19.2.

2-isovaleroylphenol (4)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using phenol (1.76 g, 18.7 mmol), isovaleryl chloride (2.10 g, 17.6 mmol), and aluminum
chloride (2.90 g, 22.5 mmol); yielded a pale yellow oil (1.49 g, 47%). $^1$H NMR (300MHz, CHLOROFORM-d) δ 12.48 (s, 1H), 7.76 (d, J = 9.63 Hz, 1H), 7.46 (t, J = 8.52 Hz, 1H), 6.99 (d, J = 9.6 Hz, 1H), 6.89 (t, J = 8.3 Hz, 1H), 2.85 (d, J = 6.9 Hz, 2H), 2.30 (sep, J = 6.9 Hz, 1H), 1.02 (d, J = 6.6 Hz, 6H). $^{13}$C (75MHz, CHLOROFORM-d) δ 206.6, 162.6, 136.2, 130.1, 119.6, 118.7, 118.4, 47.0, 25.5, 22.7.

2-(3,3-dimethyl)butanoylphenol (5)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using phenol (1.42g, 15.0 mmol), t-butylacetyl chloride (2.01g, 15.0 mmol), and aluminum chloride (3.00g, 22.5mmol); yielded a pale oil (1.36 g, 47%). $^1$H NMR (300MHz, CHLOROFORM-d) δ 12.48 (s, 1H), 7.76 (d, J = 9.63 Hz, 1H), 7.46 (t, J = 8.49 Hz, 1H), 6.98 (d, J = 9.60, 1H), 6.89 (t, J = 8.25 Hz, 1H), 2.85 (d, J = 6.87, 2H), 2.30 (sep, J = 6.60 Hz, 1H), 1.02 (d, J = 6.60 Hz). $^{13}$C (75MHz, CHLOROFORM-d) δ 206.7, 162.6, 136.2, 130.1, 119.6, 118.7, 118.5, 47.0, 25.5, 22.7.
2-(2-ethyl)butanoylphenol (6)

![Chemical Structure](image)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using phenol (1.75 g, 15.1 mmol), 2-ethylbutyryl chloride (2.01 g, 15.0 mmol), and aluminum chloride (2.40 g, 18 mmol); yielded a tan oil (1.27 g, 44%). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ 12.71 (s, 1H), 7.81 (d, $J = 9.6$, 1H), 7.47 (t, $J = 8.4$ Hz, 1H), 6.99 (d, $J = 9.4$ Hz, 1H), 6.90 (t, $J = 8.1$ Hz, 1H), 3.38-3.20 (m, 1H), 1.88-1.74 (m, 2H), 1.67-1.53 (m, 2H), 0.89 (t, $J = 7.1$ Hz, 6H). $^{13}$C (75MHz, CHLOROFORM-d) $\delta$ 210.9, 163.0, 136.2, 129.9, 119.8, 118.7, 118.7, 48.5, 25.1, 11.9.

2-benzoyl-5-methylphenol (7)

![Chemical Structure](image)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using m-cresol (1.08 g, 10.0 mmol), benzoyl chloride (1.40 g, 10.0 mmol), aluminum chloride (2.08 g, 15.0 mmol); yielded a pale yellow solid (0.80 g, 38%). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ 12.13 (s, 1H), 7.67-7.46 (m, 6H), 6.89 (s, 1H), 6.68 (d, $J = 9.0$ Hz, 1H), 2.38 (s, 3H). $^{13}$C (75MHz, CHLOROFORM-d) $\delta$ 201.1, 163.5, 148.1, 138.1, 133.5, 131.7, 129.0, 128.3, 120.0, 118.5, 116.9, 22.0.
2-benzoyl-4-bromophenol (8)

![Structure of 2-benzoyl-4-bromophenol](image)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using p-bromophenol (0.37 g, 2.14 mmol), benzoyl chloride (0.30 g, 2.13 mmol), and aluminum chloride (0.57 g, 4.27 mmol); yielded a yellow solid (0.54 g, 91%). $^1$H NMR (300MHz, CHLOROFORM-d) δ 11.93 (s, 1H), 7.70-7.51 (m, 7H), 6.98 (d, J = 8.79 Hz, 1H).

$^{13}$C (100MHz, CHLOROFORM-d) δ 200.5, 162.1, 138.9, 137.2, 135.4, 132.4, 129.1, 128.6, 120.5, 120.4, 110.2.

2-(4'-fluorobenzoyl)-4-methylphenol (9)

![Structure of 2-(4'-fluorobenzoyl)-4-methylphenol](image)

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using p-cresol (0.58 g, 5.4 mmol), 4-fluorobenzoyl chloride (0.84 g, 5.3 mmol), and aluminum chloride (0.98 g, 7.5 mmol); yielded a yellow solid (0.92 g, 75%). $^1$H NMR (300MHz, CHLOROFORM-d) δ 11.70 (s, 1H), 7.74-7.69 (m, 2H), 7.34 (d, J = 7.4 Hz, 2H), 7.20 (t, J = 8.8 Hz, 2H), 6.98 (d, J = 7.4 Hz, 1H), 2.27 (s, 3H). $^{13}$C (75MHz, CHLOROFORM-d) δ 200.0, 164.9 (d, J = 253.3 Hz), 161.1, 137.4, 134.2 (d, J = 3.5 Hz), 132.8, 131.7 (d, J = 9.2 Hz), 127.9, 118.7, 118.3, 115.5 (d, J = 21.9 Hz), 20.5.
2-(2-furanoyl)-4-methylphenol (10)

![Chemical Structure]

The general procedure for the Friedel-Crafts/Fries Rearrangement was followed to scale using 2-furanoyl chloride (1.08 g, 10.00 mmol), p-cresol (1.31 g, 10.04 mmol), and aluminum chloride (2.00 g, 15.02 mmol); yielded a yellow solid (1.07 g, 53%). $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ 11.88 (s, 1H), 8.04-8.03 (m, 1H), 7.75 (m, 1H), 7.38 (d, J = 4.0 Hz, 1H), 7.33 (dd, J = 8.5 Hz, 1H), 6.95 (d, J = 8.5 Hz, 1H), 2.35 (s, 1H). $^{13}$C (100MHz, CHLOROFORM-d) $\delta$ 184.9, 161.3, 152.0, 147.1, 137.2, 131.1, 128.1, 120.8, 118.4, 118.2, 112.4, 20.6.

2-benzoyl-6-ethoxyphenol (11)

![Chemical Structure]

The general procedure for the direct coupling of salicylaldehydes with iodobenzene was followed to scale using 3-ethoxysalicylaldehyde (1.02 g, 6.13 mmol), iodobenzene (2.45 g, 12.0 mmol), palladium chloride (0.08 g, 0.30 mmol), lithium chloride (0.05 g, 1.2 mmol), sodium carbonate (1.23 g, 12.04 mmol); yielded a dark yellow oil (1.53 g, 82%). IR (ATR): 3058, 2980, 2929, 1623, 1599, 1575, 1445, 1249, 703 cm$^{-1}$. $^1$H NMR (300MHz, CHLOROFORM-d) $\delta$ 12.18 (s, 1H), 7.69 (d, J = 6.3 Hz, 2H), 7.59 (t, 5.49 Hz, 1H), 7.50 (t, J =
5.5, 2H), 7.18 (d, J = 6.0, 1H), 7.10 (d, J = 5.2, 1H), 6.80 (t, J = 6.0, 1H), 4.16 (q, J = 5.2 Hz, 2H), 1.51 (t, J = 5.2 Hz, 3H). $^{13}$C (75MHz, CHLOROFORM-d) δ 201.7, 153.6, 148.2, 138.0, 131.9, 129.2, 128.2, 124.9, 119.4, 118.5, 117.9, 64.8, 14.8

2-benzoyl-4-chlorophenol (12)

The general procedure for the direct coupling of salicylaldehydes with iodobenzene was followed to scale using 5-chlorosalicylaldehyde (1.01 g, 6.45 mmol), iodobenzene (2.65 g, 13.0 mmol), palladium chloride (0.055 g, 0.31 mmol), lithium chloride (0.051g, 1.3 mmol), sodium carbonate (1.38 g, 12.98 mmol); yielded a yellow solid (0.84 g, 56%). $^1$H NMR (300MHz, CHLOROFORM-d) δ 11.91 (s, 1H), 7.69-7.43 (m, 7H), 7.05-7.02 (m, 1H). $^{13}$C (75MHz, CHLOROFORM-d) δ 200.6, 161.7, 137.2, 136.2, 132.4, 129.1, 128.6, 123.4, 120.1, 119.7.
Chapter 3

Synthesis of
2-Amino-3-Cyano-4H-Chromenes

3.1 Introduction
3.2 Background for HTS Identification of Medicinally Relevant Compounds
3.3 Applications of Chromene Compounds
3.4 Literature Methods
3.5 Results and Conclusions
3.6 General Experimental
3.7 Data
3.1 Introduction

Numerous natural products have been identified as potential therapeutic agents and play important roles in modern drug discovery.\textsuperscript{13} These compounds are often classified as primary or secondary metabolites. Primary metabolites serve as vital components in structure and function often exerting effect within the cell or organism and secondary metabolites have a variety of supporting roles. Some roles are not well understood and many others seem to be present to elicit response in other organisms, regulating interactions between these organisms. A substantial portion of modern medicines have originated from natural product compounds.\textsuperscript{14} The idea of producing molecules based on common structural themes found in natural products that bind with high affinity to a variety of unrelated protein receptors has become a fundamental principal guiding the drug discovery process. These structural themes have been termed “privileged medicinal scaffolds” and a number of these scaffolds are present in many commercially available drugs. Often these scaffolds consist of a heterocyclic ring system possessing functionalities with specific orientation to allow appropriate recognition at target receptors. Simple and efficient methodologies that produce the various functionalized versions of these scaffolds are highly desirable for preparation of large natural product-like libraries that can be assessed for potential therapeutic activity. Elinson \textit{et al.} have identified the 2-amino-4H-chromene core as one such medicinally privileged scaffold. This structure is shown in Figure 3.1.\textsuperscript{10a}
3.2 Background for HTS Identification of Medicinally Relevant Compounds

Common chemotherapeutic agents used in modern cancer treatments are frequently recognized by cell growth inhibition assay. Species found to be active in this type of assay are classified as cytostatic or cytotoxic. Substances considered cytotoxic are further classified by their mode of action. A necrotic mode of action is a general term for an unspecific type of cell death resulting from traumatic acute injury, while induced apoptosis occurs by the careful regulation of events that result in the termination of the cell. The natural apoptotic regulation of cell proliferation is found to be altered in many types of cancer cells. Modern cytotoxic agents considered clinically relevant act primarily through the induction of apoptosis.\textsuperscript{17f} Prevailing proapoptotic cancer treatments target tubulin which is a small family of proteins that make up microtubules a major component of the cytoskeletal framework responsible for cell structure, movement, and mitotic division. Examples of agents that target tubulin are the taxanes (Taxol and Taxotere) and the Vinca alkaloids (Vincristine, Vinblastine, and Vinorelbine). Effector caspases that cleave a variety of proteins have been determined to play essential roles in apoptotic cell death.\textsuperscript{17e} Cai et al. have developed caspase
substrates that fluoresce when cleaved. This has allowed them to develop a high
throughput screening assay to identify potential proapoptotic compounds.\textsuperscript{17g}

3.3 Applications of Chromene Compounds

The Chromene moiety is found in a large number of natural products that are
present in many different kinds of plants with varying biological activities and have long
been known to prevent or treat disease. Among the many classes of compounds that
contain this subunit are alkaloids, flavonoids, tocopherols, and anthocyanins. Many
synthetic analogs containing the chromene moiety are now produced as
pharmaceuticals for their antifungal and antimicrobial properties.\textsuperscript{12} Compounds of
particular interest are shown in Figure 3.2. These molecules have been recognized for
their potential treatment of diseases such as rheumatoid or psoriatic arthritis and
cancer.\textsuperscript{10b} Each of the compounds shown in Figure 3.2, represent excellent starting
points for the design of synthetic libraries to be scrutinized for biological activity, and
are the types of frameworks accessible through the methods optimized by our group.
Through the high throughput screening fluorescent caspase substrates mentioned above Kemnitzer et al. have identified aryl substituted 4H-chromene compounds like compound A (Figure 3.2) as potent apoptosis inducers and have since prepared libraries of compounds based on SAR studies.\textsuperscript{17b} Compound B (Figure 3.2) has also been identified by fluorescent caspase high throughput screening methods as an apoptosis inducing agent. It has also been reported to be a good antagonist for the Bcl-2/Bak family of proteins. It has been shown that overexpression of the Bcl-2 protein leads to the protection of cancer cells from apoptotic regulatory events.\textsuperscript{28,29}
Benzopyranopyridine compounds similar to compound C (Figure 3.2) have been shown to modify production of the proinflammatory TNFα (tumor necrosis factor alpha) protein. The TNFα protein is a type of cytokine able to induce apoptotic cell death which produces inflammation and inhibits tumorgenesis. Figure 3.3 shows the structure of Amlexanox, which is currently a commonly prescribed drug for its antiallergenic and topical antiulcer effects.

![Amlexanox](image)

**3.4 Literature Methods**

Kemnitzer *et al.* extensively report the synthesis of 4-aryl substituted 2-amino-3-cyano-4H-chromenes (Method A, Scheme 3.1). Many of these compounds were synthesized in a three component reaction consisting of a slight excess of the corresponding phenol derivative with respect to the aryl aldehyde, 1 equivalent malononitrile with respect to the phenol, and 2 molar equivalents of piperidine all in solution with ethanol. Sometimes these reactions were carried out at reflux or at room temperature with reaction times usually varying from 12-24 hours. Similarly, these types of compounds are produced essentially following the same procedure in a stepwise process allowing the aryl aldehyde to condense with malononitrile creating the benzylidene product and then reacting this species with the phenol. These reactions
appear to be limited by the necessity for the presence of strong activating groups on the phenol compound. Similarly, the three component method is described in high yields by Kidwai et al. using equimolar amounts of aryl aldehyde, resorcinol, and malononitrile in aqueous potassium carbonate under microwave irradiation (Method B, Scheme 3.1).\textsuperscript{18}

\textbf{Scheme 3.1 Three Component Production of 4-substituted 2-amino-3-cyano-4H-chromenes}

Cai et al. have described the preparation of 3-Cyano-2-imino-4-phenyl-2H-chromene by reaction of malononitrile with 2-hydroxybenzophenone at a 1:1 mol ratio in ethanol catalyzed by a half molar equivalent of piperidine (Method C, Scheme 3.2).\textsuperscript{20}

A similar reaction was reported by Junek using a 1:1 malononitrile: 2′-hydroxyacetophenone with 10% aqueous sodium hydroxide or sodium ethoxide solution in ethanol (Method D, Scheme 3.2).\textsuperscript{16}
For the preparation of 4H-chromene compounds with deactivating substituents, Kemnitzer et al. chose diazotization of the corresponding 7-amino (Ia) compound and nucleophillic substitution to produce a halogenated product (IIa).\textsuperscript{17a} To avoid interference with the diazotization process the 2-amino group was oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to the imino-2H-chromene (IIa) and after halogenation reduced back to the 4H-chromene (I) with sodium borohydride (Scheme 2.3). This is the only instance we have seen sodium borohydride used in the preparation of 4H-chromenes. The lengthy and low yielding (10-14%) conversion of NH\textsubscript{2} to Cl or Br further justifies the use of synthetic methods developed by our group.
3.5 Results and Conclusions

The one-pot methods developed by Tayyari et al. were expanded and applied with modification to prepare 4H-chromenes substituted at the 4 position. The procedure was modified by the addition of aqueous sodium hydroxide solution during the condensation step and a series of 4H-chromene compounds were produced. This
study has shown that the methods developed by our group could be expanded to produce a new series of potentially useful compounds. Some of the compounds we produced suffered from relatively low yields. A particular example being the isopropyl derivative in which we were able to isolate 23% of the expected product (Table 3.1). In this case were able to identify a second major product through mass spectral and $^1$HNMR to be a substituted benzopyranopyridine. These types of compounds were observed to be the major product in earlier experiments using strongly activated salicylaldehydes and two equivalents of malononitrile. At that time two equivalents of malononitrile were necessary to facilitate the formation of the intermediate (IIIa, Figure 3.4).

![Figure 3.4 Intermediates for One-Pot Method](image)

The ratio of reactants for the production of the 4-alkyl or 4-aryl derivatives was deemed most suitable to be one molar equivalent of malononitrile to one molar equivalent 2’-hydroxyketone. The intermediates for these reactions were continually shown to be of the 2-imino-2H-chromene type (IIIb, Figure 3.4). One of the goals of the project was to incorporate gradually more sterically hindered groups to probe the tolerance for such groups during the initial condensation. Our assessment is that the sterically hindered
ketone carbonyl is less available for the reaction with malononitrile and lower reactivity results. This lag potentially allows for more opportunity for the competing reaction to become more prevalent as evidenced by the isolation of the major side product. Future inquiries should involve the adjustment of reaction conditions such that the desired products could be prepared preferentially. Our hopeful expectation is that through collaborative effort, some of the molecules we have prepared will show promising biological activity. This will guide the future of the project allowing us to focus on functionalities that improve activity and justify further optimization of the current methods. Table 3.1 describes each of the products and reaction times. The compounds prepared demonstrate the importance of this methodology in its ability to produce functionalities of interest for the possible treatment and prevention of disease.
<table>
<thead>
<tr>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>$R_4$</th>
<th>Time (h)</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>*</td>
<td>13</td>
<td>71</td>
</tr>
<tr>
<td>Methyl</td>
<td>Bromo</td>
<td>H</td>
<td>H</td>
<td>*</td>
<td>14</td>
<td>52</td>
</tr>
<tr>
<td>Methyl</td>
<td>Nitro</td>
<td>H</td>
<td>H</td>
<td>*</td>
<td>15</td>
<td>Low</td>
</tr>
<tr>
<td>Ethyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>*</td>
<td>16</td>
<td>76</td>
</tr>
<tr>
<td>Propyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>3</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Propyl</td>
<td>Methyl</td>
<td>H</td>
<td>H</td>
<td>0.75</td>
<td>18</td>
<td>68</td>
</tr>
<tr>
<td>Isopropyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>2.5</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>Isobutyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>20</td>
<td>57</td>
</tr>
<tr>
<td>Neopentyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>21</td>
<td>53</td>
</tr>
<tr>
<td>Phenyl</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>3.5</td>
<td>22</td>
<td>58</td>
</tr>
<tr>
<td>Phenyl</td>
<td>H</td>
<td>H</td>
<td>Ethoxy</td>
<td>0.5</td>
<td>23</td>
<td>75</td>
</tr>
<tr>
<td>Phenyl</td>
<td>H</td>
<td>Methyl</td>
<td>H</td>
<td>1</td>
<td>24</td>
<td>57</td>
</tr>
<tr>
<td>Phenyl</td>
<td>Bromo</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>25</td>
<td>55</td>
</tr>
<tr>
<td>4-Fluorophenyl</td>
<td>Methyl</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>Phenyl</td>
<td>Chloro</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>27</td>
<td>64</td>
</tr>
<tr>
<td>2-Furanyl</td>
<td>Methyl</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>28</td>
<td>51</td>
</tr>
</tbody>
</table>

*Prepared by modified condensation procedure method. Precipitate usually formed immediately upon cooling.

Table 3.1 Substituted 4-H Chromene products
3.6 General Experimental

Analytical thin-layer chromatography (TLC) was performed using Baker-Flex silica gel IB-F plates and visualized using UV lamp or basic KMnO₄ (2.3 g KMnO₄, 15 g K₂CO₃, 1.9 mL 2.5M NaOH in 300 mL DI H₂O). Column chromatography was performed using silica gel (35-70mm, 6nm pore) from Acros. Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer using an ATR accessory with a diamond element. Proton (^1H NMR) and carbon-13 nuclear magnetic resonance (^13C NMR) spectra were recorded on JEOL Eclipse multinuclear FT-NMR spectrometer (400 MHz or 300 MHz). Chemical shifts are reported from referenced values for CDCl₃ (^1H NMR = 7.26ppm, ^13C = 77.16 ± 0.06ppm), ACETONE-d₆ (^1H NMR = 2.05ppm, ^13C = 29.84 ± 0.01ppm), and DMSO-d₆ (^1H NMR = 2.50ppm, ^13C = 39.52 ± 0.01ppm). Purchased chemicals were used without further purification, prepared materials were purified by column chromatography or recrystallization prior to use.

Representative Procedure

2'-Hydroxyphenylketone (1 mmol) was dissolved (by heating if a solid) in 95% ethanol (1-2 mL) then malononitrile (1 mmol) and 10% aqueous sodium hydroxide (5-6 drops). The solution was stirred at room temperature until precipitation was complete. If the precipitate did not form at 1 hour, DI H₂O (<1mL) was added drop-wise (to facilitate precipitation) and stirring was allowed to continue until precipitation was complete. Times for the condensation step varied and are recorded in Table 3.1.
Additional 95% ethanol (5 mL) was then added and the mixture was cooled on an ice bath to 0 °C. The cooled mixture continued to stir while sodium borohydride (1 mmol) was added. The reduction step was usually complete at 4 hours but was often left overnight out of convenience and to ensure completion of the reaction. DI H₂O was added (10 mL) to quench the reaction and precipitate the product, and the mixture cooled on an ice bath to complete precipitation. The solid was isolated by vacuum filtration, washed with cold DI H₂O, and dried.

-A slightly modified procedure was used in early experiments which incorporated heating the solution of 2-hydroxyphenylketone, malononitrile, and 10% aqueous sodium hydroxide in 95% ethanol to 70 °C for 1 minute then cooling to room temperature. The precipitate formed immediately upon cooling and the remainder of the process was as described in the representative procedure. This variation did not prove advantageous over the course of the study and was discontinued. This procedure will be referred to as the modified procedure.
3.7 Experimental Data

2-Amino-3-cyano-4-methyl-4H-chromene (13)

![Chemical structure of 2-Amino-3-cyano-4-methyl-4H-chromene (13)]

The modified procedure was followed using 2'-hydroxyacetophenone (1.365 g, 10.0 mmol), malononitrile (0.639 g, 9.7 mmol), and sodium borohydride (0.364 g, 9.6 mmol); yielded an off white solid (1.778 g, 71%); mp 124-125 °C. IR (ATR): 3436, 3330, 3244, 3212, 3063, 2968, 2928, 2864, 2181, 1652, 1607, 1577, 1405, 969, 754 cm⁻¹. ¹H NMR (300MHz, ACETONE-d₆) δ 7.30 (d, J = 7.41, 1H), 7.25-7.20 (m, 1H), 7.16-7.11 (m, 1H), 6.96 (d, J = 7.95 Hz, 1H), 6.00 (s, 2H), 3.63 (q, J = 6.57 Hz, 1H), 1.38 (d, J = 6.87, 3H). ¹³C NMR (75 MHz, ACETONE-d₆) δ 160.2, 148.9, 128.3, 127.9, 125.2, 124.7, 119.5, 116.1, 58.5, 24.4.

2-Amino-6-bromo-3-cyano-4-methyl-4H-chromene (14)

![Chemical structure of 2-Amino-6-bromo-3-cyano-4-methyl-4H-chromene (14)]

The modified procedure was followed using 5-bromo-2-hydroxyacetophenone (0.461g, 2.14 mmol), malononitrile (0.141 g, 2.13 mmol), and sodium borohydride (0.081 g, 2.14 mmol); yielded a white solid (0.291 g, 52%); mp 137-138°C. IR (ATR) 3443, 3320, 3286, 3236, 3198, 2977, 2962, 2927, 2866, 2192, 1664, 1604, 1571, 1398, 817 cm⁻¹. ¹H NMR (400MHz, CHLOROFORM-d) δ 7.30-7.27 (m, 2H), 6.82 (d, J = 9.16 Hz, 1H), 4.57 (s, 2H),
3.63 (q, J = 6.60 Hz, 1H), 1.43 (d, J = 6.60, 3H). $^{13}$C NMR (100 MHz, CHLOROFORM-$d$) δ 159.3, 147.8, 131.0, 127.0, 119.8, 118.2, 117.5, 60.7, 29.3, 24.8.

**2-Amino-3-cyano-4-methyl-6-nitro-4H-chromene (15)**

![Chemical structure of 2-Amino-3-cyano-4-methyl-6-nitro-4H-chromene](image)

The modified procedure was followed using 5-nitro-2-hydroxyacetophenone (0.090 g, 0.54 mmol), malononitrile (0.053 g, 0.80 mmol), and sodium borohydride (0.081 g, 0.57 mmol); yielded solid (low yield). $^1$H NMR (400MHz, DMSO-$d_6$) δ 8.26-8.25 (m, 1H), 8.13-8.09 (m, 1H), 7.23-7.20 (m, 1H), 7.05 (s, 2H), 3.75 (q, J = 8.8 Hz, 1H), 1.35 (d, J = 8.8 Hz, 3H).

**2-Amino-3-cyano-4-ethyl-4H-chromene (16)**

![Chemical structure of 2-Amino-3-cyano-4-ethyl-4H-chromene](image)

The modified procedure was followed using 2'-hydroxypropiophenone (0.755 g, 5.0 mmol), malononitrile (0.300 g, 4.5 mmol), and sodium borohydride (0.187 g, 4.9 mmol); yielded a solid (0.678 g, 76%); mp 77-78 °C. IR (ATR): 3456, 3330, 3279, 3215, 3049, 2976, 2925, 2879, 2183, 1649, 1606, 1577, 1409, 1188, 755 cm$^{-1}$. $^1$H NMR (300MHz, ACETONE-$d_6$) δ 7.26-7.21 (m, 2H), 7.16-7.12 (m, 1H), 6.96 (d, J = 8.1Hz, 1H), 6.05 (s, 1H), 3.62 (t, J = 4.8 Hz, 1H), 1.77-1.70 (m, 2H), 0.75 (t, J = 5.5 Hz, 3H). $^{13}$C NMR (100 MHz, ACETONE-$d_6$) δ 162.2, 150.9, 129.1, 128.6, 125.4, 124.4, 120.5, 116.6, 56.7, 36.6, 31.2, 8.9.
2-Amino-3-cyano-4-propyl-4H-chromene (17)

Representative procedure followed to scale with 2'-hydroxybutaphenone (0.082 g, 0.52 mmol), malononitrile (0.038 g, 0.58 mmol), and sodium borohydride (0.020 g, 0.53 mmol); yielded a solid (0.026 g, 25%); mp 67-68 °C. IR (ATR) 3429, 3333, 3200, 3044, 2955, 2927, 2870, 2187, 2179, 1647, 1603, 1578, 1412, 1221, 747 cm⁻¹. ¹H NMR (300 MHz, ACETONE-d₆) δ 7.28-7.20 (m, 2H), 7.14 (t, J = 7.4 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 6.04 (s, 2H), 3.60 (t, J = 4.9 Hz, 1H), 1.71-1.60 (m, 2H), 1.42-1.27 (m, 1H), 1.22-1.05 (m, 1H), 0.88-0.84 (d, J = 7.4 Hz, 3H). ¹³C NMR (100 MHz, ACETONE-d₆) δ 162.1, 150.8, 129.1, 128.6, 125.4, 125.2, 120.61, 116.7, 57.4, 41.5, 35.8, 18.7, 14.3.

2-Amino-3-cyano-6-methyl-4-propyl-4H-chromene (18)

Representative procedure followed to scale with 2-hydroxy-5-methylbutaphenone (0.091 g, 0.51 mmol), malononitrile (0.035 g, 0.53 mmol), and sodium borohydride (0.029 g, 0.44 mmol); yielded a white solid (0.077 g, 68%); mp 139-149 °C. IR (ATR): 3403, 3326, 3243, 3211, 2955, 2928, 2872, 2184, 1648, 1612, 1588, 1405, 1221, 808 cm⁻¹. ¹H NMR (300 MHz, ACETONE-d₆) δ 7.07-7.02 (m, 2H), 6.85 (d, J = 8.0 Hz, 1H), 6.00 (s, 1H), 3.56 (t, J = 5.2 Hz, 1H), 2.29 (s, 3H), 1.72-1.60 (m, 2H), 1.44-1.27 (m, 1H), 1.22-1.05 (m,
1H), 0.86 (t, J = 7.41 Hz, 3H). $^{13}$C NMR (75 MHz, ACETONE-$d_6$) δ 162.3, 148.7, 134.9, 129.3, 129.1, 124.8, 120.7, 116.5, 57.7, 41.5, 35.8, 20.7, 18.7, 14.3.

2-Amino-3-cyano-4-isopropyl-4H-chromene (19)

Representative procedure followed to scale with 2-t-butanoylphenol (0.081g, 0.49 mmol), malononitrile (0.038 g, 0.58 mmol), and sodium borohydride (0.028 g, 0.74 mmol); yielded a white solid (0.024 g, 23%); mp 142-143 °C. IR (ATR): 3410, 3325, 3208, 2965, 2934, 2901, 2867, 2181, 1638, 1608, 1578, 1411, 745 cm$^{-1}$. $^1$H NMR (300MHz, CHLOROFORM-d) δ 7.24-7.17 (m, 1H), 7.14-7.11 (m, 2H), 6.96 (d, J = 8.0 Hz, 1H), 4.58 (s, 2H), 3.45 (d, J = 3.6 Hz, 1H), 2.01-1.91 (m, 1H), 0.95 (d, J = 6.9 Hz, 3H), 0.81 (d, J = 6.6 Hz, 3H). $^{13}$C NMR (75 MHz, CHLOROFORM-d) δ 161.7, 150.4, 128.8, 127.9, 124.8, 123.5, 121.1, 116.1, 57.1, 41.8, 37.0, 19.2, 18.0.

2-Amino-3-cyano-4-isobutyl-4H-chromene (20)

Representative procedure followed to scale with 2-isovaleroylphenol (0.086 g, 0.48 mmol), malononitrile (0.034 g, 0.51mmol), and sodium borohydride (0.026 g, 0.68 mmol); yielded a off white solid (0.064 g, 57%); mp 108-108.5 °C. IR (ATR): 3425, 3327, 3236, 3206, 2955, 2924, 2868, 2180, 1640, 1607, 1577, 1409, 1224, 759 cm$^{-1}$. $^1$H NMR
(300MHz, ACETONE-\textit{d}_6) \delta 7.24 (t, J = 7.7 Hz, 2H), 7.14 (t, J = 7.4 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 6.07 (s, 2H), 3.57 (t, J = 6.30 Hz, 1H), 1.90-1.77 (m, 1H), 1.60-1.40 (m, 2H), 0.97 (d, J = 6.3 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H). $^{13}$C NMR (75 MHz, ACETONE-\textit{d}_6) \delta 162.3, 150.7, 129.2, 128.5, 126.5, 125.4, 120.9, 116.9, 58.0, 50.2, 34.4, 25.4, 23.5, 22.9.

2-Amino-3-cyano-4-neopentyl-4H-chromene (21)

![Diagram of 2-Amino-3-cyano-4-neopentyl-4H-chromene](image)

Representative procedure followed to scale using 2-(3,3-dimethyl)butanoylphenol (0.115 g, 0.60 mmol), malononitrile (0.041 g, 0.62 mmol), and sodium borohydride (0.020 g, 0.53 mmol); yielded a yellow-white solid (0.076 g, 53%); mp 100-101 °C. IR (ATR): 3458, 3319, 3225, 3196, 2953, 2902, 2865, 2187, 1636, 1611, 1599, 1576, 1417, 754 cm$^{-1}$. $^1$H NMR (300MHz, ACETONE-\textit{d}_6) \delta 7.30-7.20 (m, 2H), 7.14 (t, J = 7.4 Hz, 1H), 6.99 (d, J = 7.9 Hz, 1H), 6.09 (s, 2H), 3.59 (t, J = 5.8 Hz, 1H), 1.64-1.55 (m, 2H), 0.98 (s, 9H). $^{13}$C NMR (75 MHz, ACETONE-\textit{d}_6) \delta 162.9, 151.0, 128.9, 128.7, 128.3, 125.6, 121.2, 116.9, 59.8, 54.4.

2-Amino-3-cyano-4-phenyl-4H-chromene (22)

![Diagram of 2-Amino-3-cyano-4-phenyl-4H-chromene](image)

Representative procedure followed to scale using 2-hydroxybenzophenone (0.224 g, 1.10 mmol), malononitrile (0.073 g, 1.11 mmol), and sodium borohydride (0.042 g, 1.11
mmol); yielded a white solid (0.156 g, 58%); mp 206.0-206.5 °C. IR (ATR): 3444, 3326, 3241, 3204, 3027, 2883, 2189, 1647, 1609, 1579, 1405, 744 cm⁻¹. ¹H NMR (400MHz, ACETONE-d₆) δ 7.34-7.31 (m, 2H), 7.28-7.20 (m, 4H), 7.09-7.04 (m, 3H), 6.18 (s, 2H), 4.79 (s, 1H)

2-Amino-3-cyano-8-ethoxy-4-phenyl-4H-chromene (23)

Representative procedure followed to scale using 2-benzoyl-6-ethoxyphenol (0.256 g, 1.06 mmol), malononitrile (0.067 g, 1.01 mmol), and sodium borohydride (0.038 g, 1.00 mmol); yielded a slightly pink-white solid (0.232 g, 75%); mp 199.5-200.5 °C. IR (ATR): 3474, 3288, 3324, 3242, 3177, ¹H NMR (400MHz, ACETONE-d₆) δ 7.34-7.20 (m, 5H), 6.98-6.89 (m, 2H), 6.62 (d, J = 7.68, 1H), 6.19 (s, 2H), 4.75 (s, 1H), 4.12 (q, J = 6.96 Hz, 2H), 1.41 (t, J = 6.96 Hz, 3H). ¹³C (100MHz, CHLOROFORM-d) δ 159.4, 147.0, 144.8, 138.7, 128.9, 128.0, 127.4, 124.8, 124.1, 121.0, 120.0, 111.6, 64.8, 60.9, 41.3, 14.9

2-Amino-3-cyano-7-methyl-4-phenyl-4H-chromene (24)

Representative procedure followed to scale using 2-benzoyl-5-methylphenol (0.102 g, 0.48 mmol), malononitrile (0.033 g, 0.50 mmol), and sodium borohydride (0.020 g, 0.53
mmol); yielded a white solid (0.071 g, 57%); mp 207-208 °C. IR (ATR): 3449, 3324, 3230, 3202, 3026, 2924, 2187, 1646, 1626, 1607, 1572, 1404, 1404, 698 cm⁻¹. ¹H NMR (300MHz, CHLOROFORM-d) δ 7.33-7.17 (m, 5H), 6.85-6.82 (m, 3H), 4.70 (s, 1H), 4.55 (s, 2H), 2.31 (s, 3H). ¹³C NMR (75 MHz, ACETONE-d₆) δ 161.1, 149.5, 147.0, 139.2, 130.1, 129.5, 128.6, 127.7, 126.5, 121.5, 120.3, 117.2, 59.3, 41.9, 20.9

2-Amino-6-bromo-3-cyano-4-phenyl-4H-chromene (25)

![structure](image)

Representative procedure followed to scale using 2-benzoyl-4-bromophenol (0.135 g, 0.49 mmol), malononitrile (0.034 g, 0.51 mmol), and sodium borohydride (0.019 g, 0.50 mmol); yielded a solid (0.085 g, 55%); mp 215.0-216.0 °C. IR (ATR) 3456, 3312, 3275, 3227, 3192, 3065, 3023, 2199, 1643, 1603, 1570, 1218, 825, 700 cm⁻¹. ¹H NMR (400MHz, ACETONE-d₆) δ 7.41-7.23 (m, 7H), 7.03 (d, J = 8.52, 1H), 6.28 (s, 2H), 4.82 (s, 1H). ¹³C NMR (75 MHz, ACETONE-d₆) δ 160.9, 149.0, 146.2, 132.9, 132.0, 129.7, 128.6, 128.1, 127.1, 119.8, 119.3, 117.3, 59.1, 41.9.
2-Amino-3-cyano-4-(4-fluorophenyl)-6-methyl-4H-chromene (26)

![Image of chemical structure]

Representative procedure followed to scale using 2-(4'-fluorobenzoyl)-4-methylphenol (0.105 g, 0.49 mmol), malononitrile (0.034 g, 0.51 mmol), and sodium borohydride (0.019 g, 0.50 mmol); yielded a pale pink solid (0.089 g, 65%); mp 172-173 °C. IR (ATR): 3436, 3324, 3211, 3044, 2919, 2199, 1647, 1614, 1605, 1496, 1401, 1219, 809 cm⁻¹. H NMR (300MHz, CHLOROFORM-d) δ 7.19-7.14 (m, 2H), 7.04-6.89 (m, 4H), 6.73 (s, 1H), 4.69 (s, 1H), 4.60 (s, 2H), 2.21 (s, 3H). C NMR (75 MHz, ACETONE-d₆) δ 162.1 (d, J = 242.1 Hz), 159.4, 146.6, 140.7, 134.9, 129.8, 129.6 (d, J = 8.1 Hz), 129.2, 122.3, 119.9, 116.1 (d, J = 21.9 Hz), 115.7, 60.8, 40.4, 20.8.

2-Amino-6-chloro-3-cyano-4-phenyl-4H-chromene (27)

![Image of chemical structure]

Representative procedure followed to scale using 2-benzoyl-4-chlorophenol (0.116 g, 0.50 mmol), malononitrile (0.036 g, 0.54 mmol), and sodium borohydride (0.020 g, 0.53 mmol); yielded a solid (0.090 g, 64%); mp 206-207 °C. IR (ATR): 3458, 3311, 3275, 3228, 3193, 3066, 3024, 2198, 1642, 1604, 1574, 1411, 826 cm⁻¹. H NMR (300MHz, ACETONE-d₆) δ 7.37-7.23 (m, 6H), 7.12-7.07 (m, 2H), 6.28 (s, 2H), 4.82 (s, 1H). C NMR (100 MHz,
ACETONE-$d_6$ $\delta$ 160.1, 147.7, 145.4, 129.1, 128.9, 128.2, 127.8, 127.3, 125.8, 119.0, 118.2, 58.2, 41.1.

2-Amino-3-cyano-4-furanyl-6-methyl-4H-chromene (28)

Representative procedure followed to scale using 2-furanoyl-4-methylphenol (0.140 g, 0.70 mmol), malononitrile (0.048 g, 0.73 mmol), and sodium borohydride (0.029 g, 0.77 mmol); yielded a pale blue-green solid (0.089 g, 51%); mp 191.0-191.5 °C. IR (ATR): 3440, 3327, 3241, 3208, 2929, 2186, 1647, 1609, 1587, 1407, 736 cm$^{-1}$. $^1$H NMR (300MHz, CHLOROFORM-$d$) $\delta$ 7.33 (m, 1H), 6.99-6.90 (m, 2H), 6.88 (d, $J=8.22$, 1H), 6.30-6.29 (m, 1H), 6.16 (d, $J=4.04$, 1H), 4.83 (s, 1H), 4.65 (s, 2H), 2.28 (s, 3H). $^{13}$C (75MHz, CHLOROFORM-$d$) $\delta$ 160.3, 155.7, 146.6, 142.4, 134.7, 129.3, 129.2, 119.8, 119.7, 116.2, 110.3, 106.4, 57.4, 34.6, 20.7.
Chapter 4

One Pot Method Applied to Salicylaldehydes with Ethylcyanoacetate or Cyanoacetamide

4.1 Introduction
4.2 Applications of Other 4H-Chromene Compounds
4.3 Literature Methods
4.4 Results and Conclusions
4.5 General Experimental
4.6 Data
4.1 Introduction

As noted above, the benzopyran subunit is well known as a biologically relevant functionality and the many variant classes of this structure have shown broad utility in agriculture and medicine. Similarly, the 4H-chromene moiety in particular is of interest for use as antifungal, antimicrobial, anti-inflammatory, and antitumor agents.\textsuperscript{12} Specifically, ester functionalized 4-H chromenes have been hypothesized on the basis of computational protein binding data, to be capable of affecting apoptotic events.\textsuperscript{28} Since then various analogues have been produced and their activity documented. To further investigate the application of our one-pot production of 4H-chromenes, the substrates were expanded to include the reaction of salicylaldehydes with ethyl cyanoacetate or cyanoacetamide. Our products, generated through by this methodology, are anticipated to be biologically active, as the share many characteristics of known 4H-chromenes. These compounds have not been reported in the literature.
4.2 Applications of other 4H-Chromenes

![Figure 4.1 HA 14-1/ sHA 14-1](image)

The structure shown above in Figure 4.1 is often referred to as HA 14-1 (IVa) and has been reported to be an antagonist for the Bcl-2 protein by competing for the binding site of the Bak protein. It has also been shown that overexpression of the Bcl-2 proteins leads to the protection of cancer cells from apoptotic regulatory events.\textsuperscript{28,29} This activity was first recognized by computationally screening nearly 200,000 compounds for binding affinity to Bcl-2. High throughput screening confirmed inhibition, by demonstrating that at 50µM 90% of the HL-60 cells underwent apoptosis. This a somewhat high concentration in comparison with more recently discovered compounds but indicates proof of concept.\textsuperscript{32} Doshi et al. were able to show that HA 14-1 was not stable \textit{in vitro} and was decomposed into ethylcyanoacetate (IVc), 5- bromosalicylaldehyde (IVd), and both the ester (IVf) and carboxylic acid (IVe) substituted coumarin derivatives. The decomposition products are shown in Figure 4.2.
They have also shown that none of these compounds alone or together were able to induce apoptosis to the extent of HA 14-1. The mechanism of action seemed to be primarily through the generation of ROS (reactive oxygen species). This was illustrated by the profound reduction in cytotoxicity after the introduction of a ROS scavenger into the assay. This mode of action is the apparent result of the decomposition process and does not allow for Bcl-2 antagonist activity. The conclusion from this study was that a more stable version of the compound should be pursued such that it would resist degradation and allow for greater efficacy of the active parent compound. The result was the preparation of sHA 14-1 (IVb) shown in Figure 4.1 and justifies the continued exploration of related analogues. The general sequence for the preparation of this compound is shown in Scheme 4.5 of the next section.
Through SAR studies the sHA 14-1 structure was determined to be a more stable and potent analogue than HA 14-1. Das et al. were able to further optimize these results and produce analogues that have shown to have much higher toxicity in a variety of cancer cell lines, without generating ROS and maintaining the lack of drug resistance and synergistic activity with other therapeutic agents that was characteristic of the predecessor compound.33

4.3 Literature Methods for Preparation of Other 4H-Chromenes

Various methods exist for the preparation of ester functionalized 4H-Chromenes. Gree et al. reported that the reaction of 1 equivalent of 5-bromosalicylaldehyde with 2 equivalents of ethyl cyanoacetate in ethanol in the presence of molecular sieves generated the product HA 14-1 in good yields (Scheme 4.1, Method A).29 In order to vary the substituents on the group attached to the chromene 4-position, they opted or were required to protect the salicylaldehyde phenol group to prevent cyclization prior to the Michael addition step (Scheme 4.1, Method B).28
Elinson et al. describe a similar reaction to produce the HA 14-1 compound using electrocatalysis with a 1:2 mixture of salicylaldehydes and ethyl cyanoacetate respectively in ethanol with sodium bromide. This sequence is shown in Scheme 4.2.
Jin et al. report the preparation of ester functionalized naphthopyran chromene derivatives in good to high yields by a multicomponent reaction mixture of benzaldehydes, ethyl cyanoacetate (or malononitrile), and 1- or 2-naphthol with HTMAB (Hexadecyltrimethylammonium bromide) in refluxing water.\textsuperscript{30} A representative example is depicted in Scheme 4.3.

Likewise Kumar et al. report good to high yields for preparation of ester functionalized aryl substituted 4H-chromenes using a similar multicomponent reaction of phenols, ethyl cyanoacetate (or malononitrile), and benzaldehydes catalyzed with TiCl\textsubscript{4} under solvent-free conditions at room temperature.\textsuperscript{31} This is depicted in scheme 4.4.
Scheme 4.5 Reaction sequence for preparation of ester sHA 14-1 analogues

Scheme 4.5 depicts the preparation of sHA 14-1 analogues, the synthesis begins by formylation of 4-phenylphenol or Suzuki coupling of 5-bromosalicylaldehyde to phenylboronic acid. The resulting 5-phenylsalicylaldehyde is reacted with N,N-dimethylacetamide and phosphorous oxychloride which upon treatment with bicarbonate gives coumarin. Coumarin is a good Michael acceptor and is able to react with ethyl cyanoacetate or malononitrile under varied conditions to give the sHA 14-1 analogue.33
4.4 Discussion and Conclusions

The reaction of salicylaldehyde with ethyl cyanoacetate and subsequent reduction with sodium borohydride produced the expected ethyl substituted 4H-chromene. The reaction was quite consistent with the analogous reaction of malononitrile and salicylaldehyde although additional catalyst was necessary to enable the condensation. When these methods were applied to salicylaldehyde with cyanoacetamide the product upon reduction was shown not to be the amide substituted 4H-chromene derivative. The intermediate formed was isolated on several occasions and was consistently observed to be the 2-imino-2H-chromene-3-carboxamide, after the sodium borohydride reduction the pyran ring would open to give the unexpected 2-cyano-3-(2-hydroxyphenyl)propanamide product. The reaction was carried out with the more activated 3-ethoxysalicylaldehyde and the open form still resulted. Similarly, deactivated salicylaldehydes were reacted with ethyl cyanoacetate to explore the possibility that the products may be of the open form though the 4H-chromene was produced for each of the substrates. Products are described in Table 4.1. Our assertion is that the methods outlined in this investigation allow for the preparation of multiple variations of the medicinally relevant 4H-chromene core. The ability to functionalize this core particularly at the 4-position with alkyl substituents easily allows for speculation that other variations are possible. To produce compounds less susceptible to degradation in vivo and yield greater efficacy. Also the simple production of the biologically active 4-aryl substituted chromenes with better reaction tolerance for
activating or deactivating substituents is also of great importance. It is our hope that collaborations with Eli Lilly & Co. will yield further insight into this investigation and guide future efforts.

Cyclized Form

Open Form

<table>
<thead>
<tr>
<th>Form</th>
<th>R</th>
<th>R’</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclized (ester)</td>
<td>H</td>
<td>H</td>
<td>29</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>Cl</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>Cl</td>
<td>31</td>
<td>56</td>
</tr>
<tr>
<td>Open (amide)</td>
<td>H</td>
<td>H</td>
<td>32</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>Cl</td>
<td>33</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>Cl</td>
<td>34</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 4.1 Ethylcyanoacetate/Cyanoacetamide One-Pot Products

4.5 General Experimental

Analytical thin-layer chromatography (TLC) was performed using Baker-Flex silica gel IB-F plates and visualized using UV lamp or basic KMnO₄ stain (2.3g KMnO₄, 15g K₂CO₃, 1.9mL 2.5M NaOH in 300mL DI H₂O). Column chromatography was performed using silica gel (35-70mm, 6nm pore) from Acros. Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer using an ATR accessory with a diamond
element. Proton nuclear magnetic resonance \((^1\text{H NMR})\) spectra and carbon-13 nuclear magnetic resonance \((^{13}\text{C NMR})\) spectra were recorded on a JEOL Eclipse spectrometer at 400 MHz or 300 MHz and 100 or 75 MHz, respectively. Chemical shifts are reported downfield from referenced values for CDCl\(_3\) \((^1\text{H NMR} = 7.26\text{ppm}, ^{13}\text{C} = 77.16 \pm 0.06\text{ppm})\), ACETONE-d\(_6\) \((^1\text{H NMR} = 2.05\text{ppm}, ^{13}\text{C} = 29.84 \pm 0.01\text{ppm})\), and DMSO-d\(_6\) \((^1\text{H NMR} = 2.50\text{ppm}, ^{13}\text{C} = 39.52 \pm 0.01\text{ppm})\).\(^{19}\) Purchased chemicals were used without further purification, prepared materials were purified by column chromatography or recrystallization prior to use.

**Representative Procedure for the Preparation of Ethyl 2-Amino-4H-Chromene-3-Carboxylates**

Salicylaldehyde (1 mmol) was added to the reaction vessel followed by Ethylcyanoacetate (1 mmol) then 95% ethanol (1mL) and a catalytic amount of piperidine (approximately 2-3 drops) or alumina (Al\(_2\)O\(_3\))\(^*\). The solution was stirred at room temperature until precipitation was complete. Additional 95% ethanol was added (3-4 mL) and the mixture was cooled on an ice bath to 0 °C. To the cooled mixture sodium borohydride was added (1 mmol) and the reaction was typically finished by 4 hours but often allowed to stir overnight for convenience and to ensure completion. DI H\(_2\)O was added (10 mL) to quench the reaction and precipitate the product. This mixture was cooled on ice bath to complete precipitation and solid was separated by vacuum filtration and washed with DI H\(_2\)O.
*When Al₂O₃ is used as the catalyst the reaction mixture is hot filtered prior to the reduction step, the solid alumina collected in the filter is then washed with boiling ethanol. The ethanol mother liquor is cooled and subjected to the reduction step (if the intermediate imino 2H-chromene was desired DI H₂O was added to facilitate precipitation).

**Procedure for the Preparation of 2-Cyano-3-(2-Hydroxyphenyl)Propanamides**

Cyanoacetamide (1 mmol) was dissolved (with heating) in 95% ethanol (4-5 mL) and to this solution was added salicylaldehyde (1 mmol) and a catalytic amount of piperidine (approximately 2-3 drops). The solution was stirred at room temperature until precipitation was complete. Additional 95% ethanol was added (3-4 mL) and the mixture was cooled on an ice bath to 0 °C. To the cooled mixture sodium borohydride was added (1 mmol) and the reaction was typically complete at 4 hours. DI H₂O was added (10 mL) to quench the reaction and precipitate the product. This mixture was cooled on ice bath to complete precipitation and solid was separated by vacuum filtration and washed with DI H₂O.
4.6 Data

**ethyl 2-amino-4H-chromene-3-carboxylate (29)**

![Structure of ethyl 2-amino-4H-chromene-3-carboxylate (29)](structure.png)

Representative procedure followed to scale with salicylaldehyde (0.623 g, 5.10 mmol), ethylcyanoacetate (0.585 g, 5.17 mmol), and sodium borohydride (0.181 g, 4.78 mmol); yielded a solid (0.682 g, 63%); mp 76-77 °C. IR (ATR): 3410, 3298, 2987, 2901, 2838, 1737, 1670, 1607, 1521, 1488, 1457, 1226, 752 cm⁻¹. ¹H NMR (400 MHz, DMSO-δ₆) δ 7.50 (s, 2H), 7.25-7.18 (m, 2H), 7.08 (t, J = 7.4 Hz, 1H), 6.96 (d, J = 7.9 Hz, 1H), 4.10 (q, J = 7.2 Hz, 2H), 3.49 (s, 2H), 1.22 (t, J = 7.2 Hz, 3H). ¹³C NMR (100 MHz, ACETONE-δ₆) δ 170.0, 161.7, 150.7, 130.0, 128.3, 125.0, 122.8, 116.5, 71.8, 59.6, 23.9, 15.0.

**ethyl 2-amino-6-chloro-4H-chromene-3-carboxylate (30)**

![Structure of ethyl 2-amino-6-chloro-4H-chromene-3-carboxylate (30)](structure.png)

Representative procedure followed to scale with 5-chlorosalicylaldehyde (0.497 g, 3.17 mmol), ethylcyanoacetate (0.369 g, 3.26 mmol), and sodium borohydride (0.111 g, 2.93 mmol); yielded a solid (0.482 g, 60%); mp 82-83°C. IR (ATR): 3471, 3330, 2980, 2907, 2850, 1684, 1624, 1610, 1523, 1476, 1184, 1083, 809, 766 cm⁻¹. ¹H NMR (400 MHz, DMSO-δ₆) δ 7.53 (s, 2H), 7.35 (s, 1H), 7.26-7.23 (m, 1H), 6.99 (d, J = 8.4 Hz, 1H), 4.10 (q, J
ethyl 2-amino-6,8-dichloro-4H-chromene-3-carboxylate (31)

Representative procedure followed to scale with 5-chlorosalicylaldehyde (0.190 g, 1.00 mmol), ethylcyanoacetate (0.113 g, 1.00 mmol), and sodium borohydride (0.040 g, 1.05 mmol); yielded a solid (0.162 g, 56%); mp 122.5-123°C. IR (ATR): 3396, 3286, 2983, 2911, 1685, 1628, 1520, 1459, 1408, 1281, 1198, 1184, 1031 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 7.60 (s, 2H), 7.52 (d, J = 2.2 Hz, 1H) 7.37 (d, J = 2.2 Hz, 1H), 4.10 (q, J = 7.1Hz, 2H), 3.53 (s, 2H), 1.22 (t, J = 7.1Hz, 3H). ^13C NMR (100 MHz, ACETONE-d_6) δ 168.3, 159.7, 142.7, 127.6, 127.4, 125.6, 120.8, 69.6, 58.9, 23.1, 14.6.

2-cyano-3-(2-hydroxyphenyl)propanamide (32)

Representative procedure followed to scale with salicylaldehyde (0.600 g, 4.9 mmol), cyanoacetamide (0.430 g, 5.11 mmol), and sodium borohydride (0.200 g, 5.28 mmol); yielded a solid (0.701 g, 73%); mp 154-155°C. IR(ATR): 3373, 3315, 3152, 2930, 2862, 2811, 2252, 1691, 1605, 1423, 1227, 687 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 9.63 (s,
1H), 7.81 (s, 1H), 7.46 (s, 1H), 7.11-7.06 (m, 2H), 6.82 (d, J = 7.32 Hz, 1H), 6.74 (t, J = 7.28, 1H), 3.92 (t, J = 7.68, 1H), 3.04-2.97 (m, 2H). $^{13}$C NMR (100 MHz, ACETONE-$d_6$) δ 167.7, 156.1, 132.0, 129.4, 124.0, 120.5, 118.8, 116.1, 38.3, 31.8.

2-cyano-3-(3-ethoxy-2-hydroxyphenyl)propanamide (33)

Representative procedure followed to scale with 3-ethoxysalicylaldehyde (0.843 g, 5.07 mmol), cyanoacetamide (0.444 g, 5.28 mmol), and sodium borohydride (0.201 g, 5.31 mmol); yielded a solid (0.901 g, 77%); mp 149.5-150°C. IR (ATR): 3443, 3323, 3032, 2976, 2928, 2874, 2248, 1697, 1608, 1495, 1470, 1270, 1077, 729 cm$^{-1}$. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.61 (s, 1H), 7.82 (s, 1H), 7.47 (s, 1H), 6.86 (d, J = 7.32, 1H), 6.73-6.68 (m, 2H), 4.04 (q, J = 6.96, 2H), 3.89 (t, J = 6.96 1H), 3.08-2.97 (m, 2H), 1.35 (t, J = 6.96, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 166.4, 146.4, 144.6, 123.3, 122.3, 118.7, 118.6, 112.0, 64.0, 37.2, 30.1, 14.7.

2-cyano-3-(5-chloro-2-hydroxyphenyl)propanamide (34)

Representative procedure followed to scale with 5-chlorosalicylaldehyde (0.156 g, 1.00 mmol), cyanoacetamide (0.088 g, 1.33 mmol), and sodium borohydride (0.040 g, 1.05
mmol); yielded a solid (0.090 g, 40%); mp 140.5-141.5 °C. IR (ATR): 3381, 3178, 2249, 1688, 1632, 1606, 1498, 1419, 1274, 1115, 807 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ 9.97 (s, 1H), 7.81 (s, 1H), 7.50 (s, 1H), 7.17-7.11 (m, 6H), 6.84-6.81 (m, 2H), 3.97-3.92 (m, 1H), 3.02-2.99 (m, 2H). ¹³C NMR (100 MHz, DMSO-d₆) δ 166.7, 155.0, 130.6, 128.5, 125.6, 122.7, 118.9, 117.1, 37.4, 30.3.
Bibliography

3.) Tayyari, F., *M.S. Thesis*, Ball State University, Muncie, IN, **2008**.
14.) [http://www.rsc.org/pdf/tct/natprods1.pdf](http://www.rsc.org/pdf/tct/natprods1.pdf)


Appendix
2-isovalerylphenol(4)
2-(3-tert-butyl-2-methyl)phenoxy(1,3)
2-(2-furanylidene)-4-methylyphenol (10)
ethyl 2-amino-6,8-dichloro-4H-chromene-3-carboxylate (21)
ethyl 2-amin-6,8-dichloro-4H-chromene-3-carboxylate (31)
2-cyano-3-(2-hydroxyphenyl)propanamide (32)
2-Amino-3-cyano-4-ethyl-4H-chromene (16)
ethyl 2-amino-4H-chromene-3-carboxylate (2')
1.1 Introduction

1.2 Production of Malononitrile

1.3 Knoevenagel Condensation with Aryl Substrates

1.4 Knoevenagel Condensation with Salicylaldehydes

1.5 One-pot Synthesis of Substituted 4H-Chromenes

2-benzoyl-6-ethoxyphenol (11)