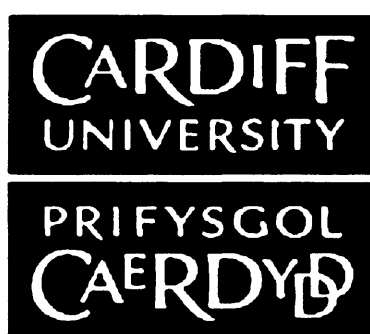


**The Design and Synthesis of Combretastatin A-4
like chalcones and their analogues, and
other Anticancer Agents.**

Edward Simon Marco Armitage



A THESIS SUBMITTED AS PART REQUIREMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY AT CARDIFF UNIVERSITY

DEPARTMENT OF CHEMISTRY

CARDIFF UNIVERSITY

2007

UMI Number: U584965

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



UMI U584965

Published by ProQuest LLC 2013. Copyright in the Dissertation held by the Author.
Microform Edition © ProQuest LLC.

All rights reserved. This work is protected against
unauthorized copying under Title 17, United States Code.

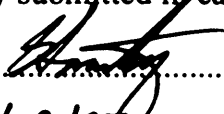


ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Preface

Declaration

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed.....(candidate)

Date 22/02/07

Statement One

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed.....(candidate)

Date 22/02/07

Statement Two

I hereby give consent for my thesis, if accepted, to be available for photocopying and interlibrary loan, and for the title and summary to be made available to outside organisations.

Signed.....(candidate)

Date 22/02/07

Preface
Content

Title Page	i
Declaration	ii
Content	iii
Acknowledgements	v
Abstract	vii
Publications	viii
Numbering System	ix
Abbreviations and Glossary	x
Chapter 1 - Cancer Chemotherapy	1
1.1 Introduction	1
1.2 Social Awareness and Cancerphobia in Modern Society of the US.	1
1.3 Cancer Epidemiology	3
1.4 The History of Cancer.	6
1.5 How Does a Normal Cell Become a Cancer Cell?	8
1.6 Cancer Chemotherapy	9
1.6.1 The Mustard Gases	10
1.6.2 Platinum Complexes	12
1.6.3 Antimetabolites and the Cure for Leukaemia	13
1.6.4 Anticancer Drugs from Nature	17
Chapter 2 - Development of Agents that Target Tubulin	24
2.1 Developing Antimitotic Agents	24
2.2 The Cell Cycle and Mitosis	24
2.3 The Formation of Microtubules from Tubulin	26
2.4 The Role and Dynamic Instability of Microtubules	27
2.5 Classes of Mitotic Spindle Poison	27
2.5.1 The Colchicine Binding Site	28
2.5.2 Vinca Alkaloid Binding Site	33
2.5.3 Rhizoxin/Maytansine (RZX/MAY) Binding Site	35
2.5.4 Taxane Binding Site	36
2.6 Antivascular Effect	37
Chapter 3 – Combretastatin A-4 like Chalcones and Analogues.	
Results and Discussion	39
3.1 History and Development of the Chalcone Project	39
3.2 α -Arylchalcones	48
3.2.1 α -Arylchalcones - Synthesis, Results and Discussion	48
3.2.1.1 Biological Results for α -Substituted Chalcones	56
3.2.2 α -Benzylchalcones - Synthesis, Results and Discussion	57
3.2.2.1 Biological Results for the Reduced Chalcones	59
3.2.3 Another Route to α -Arylchalcones - Synthesis, Results and Discussion	59
3.2.3.1 Biological Results for the α -Arylchalcones	63
3.3 Indanones and Indenones	64
3.3.1 Indanones - Synthesis, Results and Discussion	64
3.3.1.1 Biological Results for the Indanones and Precursor Chalcones	75
3.3.2 Indenones - Synthesis, Results and Discussion	77

Preface

3.3.2.1	Biological Results for the Indenones and the Precursor 2'-Bromochalcones	90
	Chapter 4 – Other Cytotoxic Chalcones.	
	Results and Discussion	92
4.1	The Development of Other Cytotoxic Chalcones	92
4.2	The Total Synthesis of 4-Hydroxyderricin and Analogues.	96
4.2.1	The Synthesis of 4-Hydroxyderricin	97
4.2.2	Biological Results for the 4-Hydroxyderricin and Similar Chalcone Derivatives.	109
4.3	p53-MDM2 Complex Disrupting Chalcones	109
4.3.1	Synthesis of the 3',4'-Dichlorochalcones	114
4.3.2	Biological Activities of 3',4'-Dichlorochalcones	117
	Chapter 5 – Heat Shock Proteins as Targets for Drug Design	119
5.1	Hyperthermia and the Prevention of Thermotolerance via Drug like Compounds	119
5.2	Synthesis of KNK437 and Similar Analogues.	120
5.3	Biological Results of KNK437 and its Analogues	125
	Chapter 6 – Experimental	127
	Chapter 7 – Reference	237
	Chapter 8 – Appendix A – Supplementary X-ray Crystal Data	248

Preface

Acknowledgements

First of all I would like to thank my supervisor Professor Nick Lawrence, your expertise, guidance, and patience though out my PhD has been both inspirational and invaluable.

I would like to thank everyone at the Centre for Molecular Drug Design at the University of Salford who were involved in the cytotoxic testing of my compounds especially Dr. Sylvie Ducki and Darren Cook.

I am obliged to the Engineering and Physical Sciences Research Council for the funds necessary to carry out this research.

Likewise I am obliged to members of the technical staff at Cardiff University, and moreover Dr. Abdul Malik and Dr. Li-ling Ooi for their assistance in the collection of X-ray crystal data and Rob Jenkins for his assistance in the collection of NMR data.

I would like to thank the H. Lee Moffitt Cancer Center & Research Institute for giving me the opportunity to work in the USA and express my gratitude to all off its staff for their support and making me feel welcome. I would especially like to thank Dr. Harshani Lawrence for all the help and advice she gave me in the lab. Acknowledgements also go to Professor Mark McLaughlin and his research group for the support they gave through out my stay in the USA. Again I am obliged to members of the technical staff at the H. Lee Moffitt Cancer Center & Research Institute and the University of South Florida, especially Umut Oguz and Ted Gauthier for their assistance in the collection of NMR and mass spectroscopy data respectively. I would also like to thanks also Prof Jiandong Chen and Dr Hu Baoli for the p53-MDM2 bioassay and Prof Bhalla and Fei Guo for the bioassays of the KNK compounds.

I would like to express my appreciation for the past and present members of the Lawrence group. Especially Dan Pernazza, Argha Dutta, Chris Davies, Richard Patterson, Abdul Hannan, Nick Richards, Pedro Morchón, Ben Greedy, Roberta Pireddu, Huw Davies, Chris Rees and Suzanne Gregson. These are the people who have made my years in the group enjoyable and memorable. I would also like to thank Dr. Michael Coogan and his research group for sharing their lab space with me and to positively contributing to my experience in Cardiff.

Special thanks are expressed to those brave souls who have lived with me in Cardiff especially Neal Potter, Andrew Watts, and Dave King.

At this point I would also like to express my gratitude again to Nick Lawrence, Harshani Lawrence, Ben Greedy, Dan Pernazza, Roberta Pireddu and Nick Richards for being there

Preface

during my American experience and surviving the Florida weather; with out you it wouldn't have been the same.

I would like to express my heart felt appreciation to all my family for all the love and support they have giving me over the last few years. Thanks to David, Susan, Jenny, Susan and Peter and the rest of my extended family, but most of all I want to thank Mother for everything she has given me, I am eternally grateful.

Finally I wish to thank Clare for constantly believing in me, encouraging me, and being there for me on every step of my journey; I couldn't have done it without you.

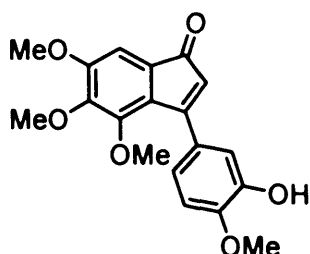
Preface

Abstract

This thesis covers work investigating the design, synthesis and evaluation of Combretastatin A-4 like chalcones and their analogues, and other anticancer agents.

The first two chapters are an introduction to cancer chemotherapy and the development of tubulin as a target for chemotherapy. Chapter one includes information on cancer epidemiology, the history of cancer and the development of important cancer chemotherapy agents. While the second chapter describes the role of tubulin in mitosis, and the development of anticancer agents that target tubulin.

The third chapter describes the background to the chalcone project, as well as the design and synthesis of new Combretastatin A-4 like chalcones and their analogues. To further the investigation of the substitution of the α -hydrogen of the chalcone with various groups, a series of α -arylchalcones was synthesized. The substitution of α -hydrogen with various aryl groups generally shows a significant increase in the anticancer activity of the chalcone. The effect of the conformation of the chalcone on its anticancer activity was also investigated by synthesizing two series of chalcone analogues which mimic the *s-trans* arrangement of chalcone, the indanones and indenones. Several of the indanones and indenones showed high levels of cytotoxicity with the CA-4 like indenone having an $IC_{50}(K562)$ value of $0.019 \mu\text{M}$.



Chapter four looks at other chalcones with anticancer activity including the naturally occurring chalcone 4-hydroxyderricin, which has been isolated from the roots of *Angelica keiskei* and has shown to have anti-tumour promoting properties. As part of my PhD study the natural product 4-hydroxyderricin and a series of analogues was synthesized to investigate its anticancer activity.

In chapter five I synthesized the anticancer agent KNK437 which has been shown to be a dose dependant inhibitor of the acquisition of thermotolerance in several different tumours. The study found that KNK437 sensitizes human leukaemia cells to the 17-allylamino-demethoxy geldanamycin induced apoptosis.

Preface

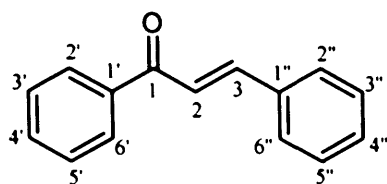
Publications

The synthesis of indanones related to combretastatin A-4 via microwave-assisted Nazarov cyclization of chalcones. N. J. Lawrence, E. S. M. Armitage, B. Greedy, D. Cook, S. Ducki and A. T. McGown, *Tetrahedron Letters*, **2006**, *47*, 1637-1640.

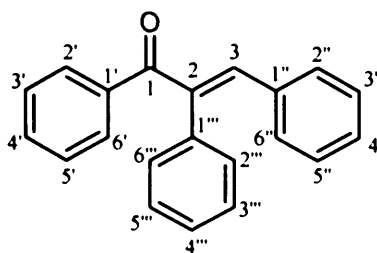
Abrogation of heat shock protein 70 induction as a strategy, to increase antileukaemia activity of heat shock protein 90 inhibitor 17-allylamino-demethoxy geldanamycin. F. Guo, K. Rocha, P. Bali, M. Pranpat, W. Fiskus, S. Boyapalle, S. Kumaraswamy, M. Balasis, B. Greedy, E. S. M. Armitage, N. Lawrence and K. Bhalla, *Cancer Res.*, **2005**, *65*, 10536-10544.

Preface

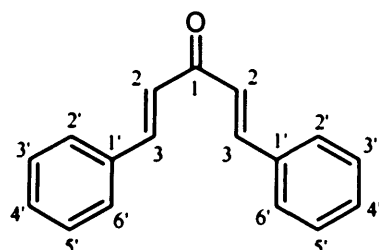
Numbering System



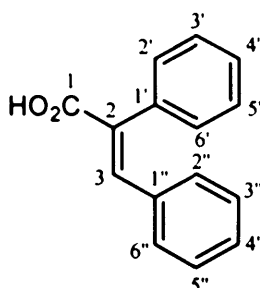
Chalcone



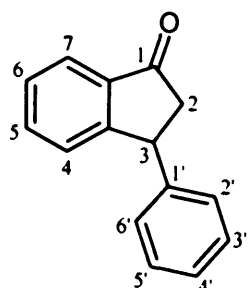
α -Arylchalcone



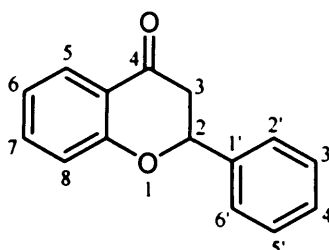
Dibenzylideneacetone



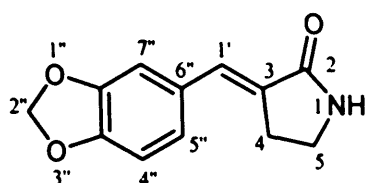
Acrylic acid



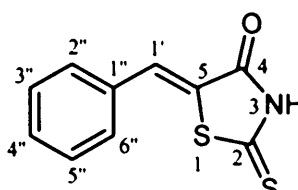
Indanone/Indenone



Flavanone



Benzylidene lactam



Benzylidene rhodanine

Preface

Abbreviations and Glossary

Å	Angstrom
Ac	Acetyl
AcOH	Acetic acid
AD	<i>Anno domini</i> (Latin: "in the year of our Lord")
AKR	Aldo keto reductase
aq	Aqueous
Ar	Aryl
BC	Before Christ
Bn	Benzyl
bp	Boiling point
br.	Broad
<i>ca.</i>	<i>Circa</i> (Latin: "about")
Celite	High-grade diatomaceous earth used as a filter aid
CIP	Cahn Ingold Prelog
CML	Chronic myelogenous leukaemia
cm ⁻¹	Wavenumber
δ (delta)	Chemical shift used in NMR (units are in ppm)
d	Doublet
dba	Dibenzylideneacetone
DBU	1,8-Diazobicyclo[4.3.0]undec-7-ene
DCM	Dichloromethane
dd	Double doublet
ddt	Double doublet of triplets
DHFR	Dihydrofolate reductase
dm	Double multiplet
DMBA	7,12-dimethylbenz[<i>a</i>]anthracene
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dq	Double quartet
dt	Double triplet
<i>e.g.</i>	<i>exempli gratia</i> (Latin: "for example")
ELISA	Enzyme-Linked ImmunoSorbent Assay

Preface

eqv	Equivalent, equivalents
<i>et al.</i>	<i>Et alia</i> (Latin: “and others”)
GTP	Guanosine 5'-triphosphate
HDM2	Human double minute 2
HRMS	High resolution mass spectrometry
HSE	Heat shock element
HSF	Heat shock factor
HSP	Heat shock protein
HUVEC	Human umbilical vein endothelial cell
IC	Inhibition concentration
<i>in situ</i>	<i>in the reaction mixture</i> [Latin: “in its (original place)”]
<i>in vacuo</i>	<i>under vacuum</i> [Latin: “empty space”]
<i>in vitro</i>	[Latin: “in glass”]
<i>in vivo</i>	[Latin: “in a living (thing)”]
ITP	Inhibition of tubulin polymerization
kDa	KiloDaltons
LLC	Lewis lung carcinoma
m	Multiplet
<i>m</i>	<i>Meta</i>
MAP	Microtubule associated proteins
MDM2	Mouse double minute 2
MDR	Multidrug resistance
MOM	Methoxymethyl
m.p.	Melting point
MS	Molecular sieves
MTOC	Microtubules organising centres
MTT	3-(4,5-Dimethoxythiazol-2-yl)-2,5-diphenyl tetrazolium bromide
<i>m/z</i>	Mass to charge ratio measured by mass spectroscopy
n	Nano
NBS	<i>N</i> -bromosuccinimide
NCI	National Cancer Institute
<i>o</i>	<i>Ortho</i>
<i>p</i>	<i>Para</i>
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffer solution

Preface

PBSMT	PBS + 5% nonfat dairy milk + 0.1% Tween 20
PBST	PBS + 0.1% Tween 20
PCC	Pyridinium chlorochromate
<i>per capita</i>	Latin: "for each head"
ppm	Parts per million
q	Quartet
R _f	Retardation factor
s	Singlet
SAR	Structure-activity-relationship
sp	Septuplet
t	Triplet
TBAF	Tetra- <i>n</i> -butylammonium fluoride
TBDMS	<i>Tert</i> -butyldimethylsilyl
td	Triple doublet
<i>tert</i>	Tertiary
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
t.l.c	Thin-layer chromatography
tm	Triple multiplet
TMS	Trimethylsilyl
TPA	12- <i>O</i> -tetradecanoyl phorbol-13-acetate
μwave	microwave

Angiogenesis growth of new blood vessels from existing vasculature.

Apoptosis death of individual cells within a tissue as a result of normal cell turnover within a tissue or toxicity of an environmental agent.

Benign Not cancerous.

Cancer a malignant neoplasm.

Chromosome a structure within a cell nucleus of eukaryotes, consisting of DNA in association with proteins and other molecules.

Cytostatic inhibiting or suppressing cellular growth and multiplication

Cytotoxic cell-killing

Denaturation a structural change in macromolecules caused by extreme conditions.

Preface

Differentiation make or become different in the process of growth or development.

Enzyme an organic catalyst, usually a protein in nature, that catalyses chemical reactions while in most cases remaining chemically unchanged.

Epidemiology the study of the incidence and distribution of diseases and other factors relating to health

Hormone a chemical made by glands in the body which control the actions of certain cells or organs

Hyperthermia a type of treatment in which body tissue is exposed to high temperatures to damage and kill cancer cells or to make cancer cells more sensitive to the effects of radiation and certain anticancer drugs.

Hypoxia a condition in which there is a decrease in the oxygen supply to a tissue.

Leukaemia a cancer that starts in blood-forming tissue such as the bone marrow, and causes large numbers of blood cells to be produced and enter the bloodstream.

Malignant tending to invade normal tissue or to recur after removal

Metastasis the dissemination and/or growth of cells originating from a primary neoplasm at another site.

Microfilament the smallest of fibrous cytoskeletal elements, which have diameters 30-60 Å, lack periodicity, and are chemically related to actins.

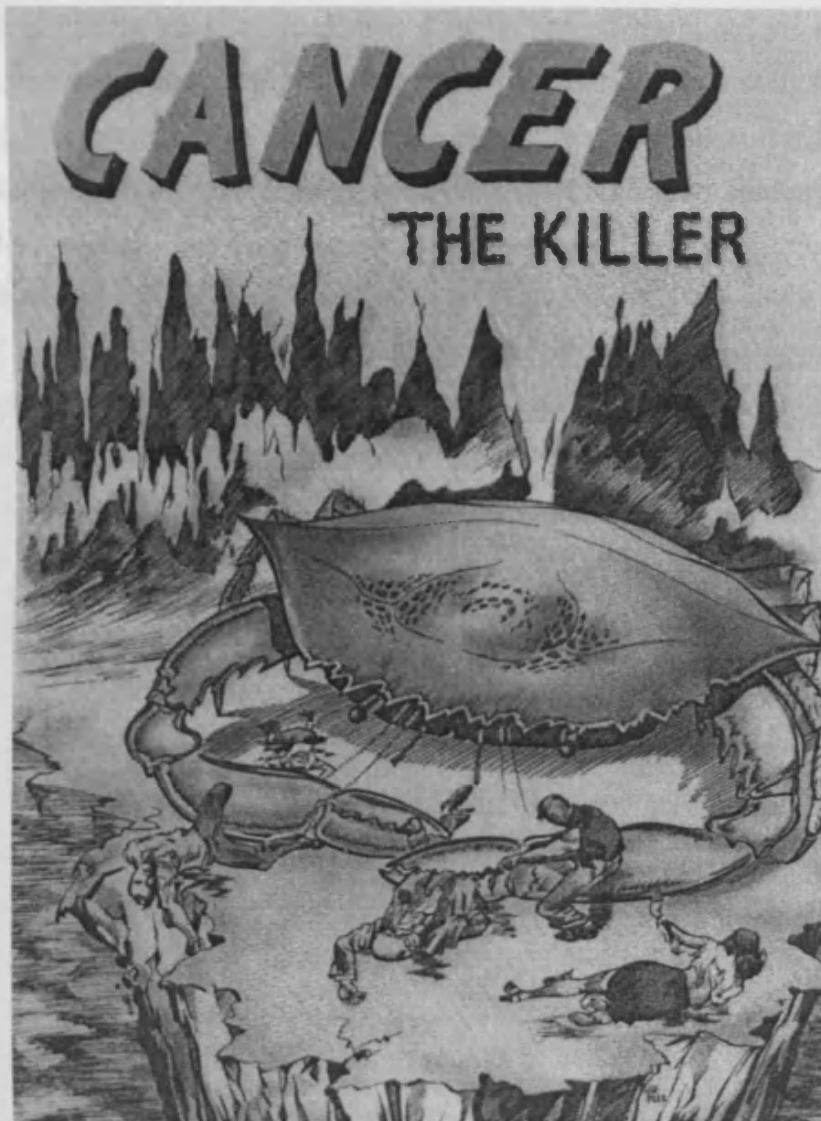
Microtubule a widely occurring cylindrical cytoplasmic element 200 to 270 Å in diameter and of variable length, increasing in number during mitosis.

Mitosis process resulting in the replication of cells which contain the same number of chromosomes as the parent cell.

Mutation a change in the structure and/or expression of a gene that is perpetuated in subsequent progeny of the cells in which it first occurred.

Neoplasm a heritably altered, relatively autonomous growth of tissue.

Tumour an abnormal mass of tissue that results when cells divide more than they should or do not die when they should. Tumours may be benign, or malignant.



Cartoon booklet from the 1950s¹

1.1 Introduction

In the late 19th century tuberculosis, also known as the “great destroyer” and the “white plague”, was the most feared disease of its time. In 1900 it killed 200 Americans per 100,000 of the population. But early on in the 20th century due to advances in medical treatment and understanding of the disease, the number of cases started to drop as the disease was brought under control. When this happened the number of cases of cancer and heart disease related deaths started to increase. This was especially apparent in the elderly population.

1.2 Social awareness and cancerphobia in modern society of the US.¹

At the turn of the 20th century cancer was a disease that was beyond the ability of contemporary medical science to treat or even understand. One theory at the time was that cancer may be caused by germs and that it could be contagious, while other theories also

Chapter 1 - Cancer Chemotherapy

included that it could be heredity, or that it may be caused by luxurious living, depraved sexual acts or even depression and mental stress. It was an indiscriminating killer, striking at random, caring not of race, class or age. One US physician described cancer as a “Loathsome beast, which seized upon the breast, drove its long claws into the surrounding tissues, derived its sustenance by sucking out the juices of its victims, and never even relaxed its hold in death.” It became a social stigma, a disease that people would not talk about or see doctors if they suspected they had it, relatives of people who died of cancer often begged the coroner to record the death to be the cause of something else so the family would not be scandalized by it. Because of this, there was a general lack of hard evidence on the number of incidences and deaths each year. With such sentiments it is understandable why cancer became the new dread disease.

However by the mid 1930s the message of early detection and medical intervention was starting to spread, bringing a larger public interest. This led to several important news articles carried by Time, Life and Fortune and series of dedicated news reels shown in cinemas. In July 1937 a group of scientists and politicians secured \$700,000 to support cancer research and a further \$750,000 from the federal government, to build the National Cancer Institute in Bethesda, Maryland which would be a place to provide for and foster the continuous study of the cause, prevention, diagnosis and treatment of cancer. This action by the federal government strongly committed them to quest for the cure for cancer and further cancer research in the US.

By the late 1960s it was argued that the federal government needed to increase spending on cancer research. The price of scientific equipment was increasing faster than the increase in funds the government made to the NCI. The argument stated that in the 4 year course of the Vietnam War a total of 41,000 Americans had died, however in the last year over 320,000 Americans had died of cancer, and due to the war less money was being spent on scientific research. One writer noted that in one year America spent \$410 *per capita* on defense, \$125 on the war and only 89 cents on cancer research. With growing pressure and the chance to out flank one of his opponents, President Nixon made the following statement in his State of the Union message of January 1971 “The time has come when the same kind of concentrated effort that split the atom and took man to the moon should be turned towards conquering this dread disease. Let us make a total commitment to achieve this goal.” On the 23rd of December 1971 Nixon signed the National Cancer Act of 1971 into law; it stipulated that the NCI director was to be appointed directly by the president and to submit NCI financial requests to the budget office. Nixon also stated at the time that “I hope in years ahead we

Chapter 1 - Cancer Chemotherapy

will be able to look back and see this action has been the most important action taken by this administration.” By 1973 NCI funding had increased to over \$400 million, and by the end of the decade the funding had nearly reached a billion dollars.

1.3 Cancer Epidemiology

In the Western world cancer is the 2nd leading cause of death after heart disease (*Figure 1*). In 2003 cancer accounted for over a quarter of the deaths in the UK, with 154,547 people registered as dying from cancerous tumours.² In the USA it is estimated that there will be 570,280 deaths due to cancer in the year 2005, over 1500 deaths a day.²

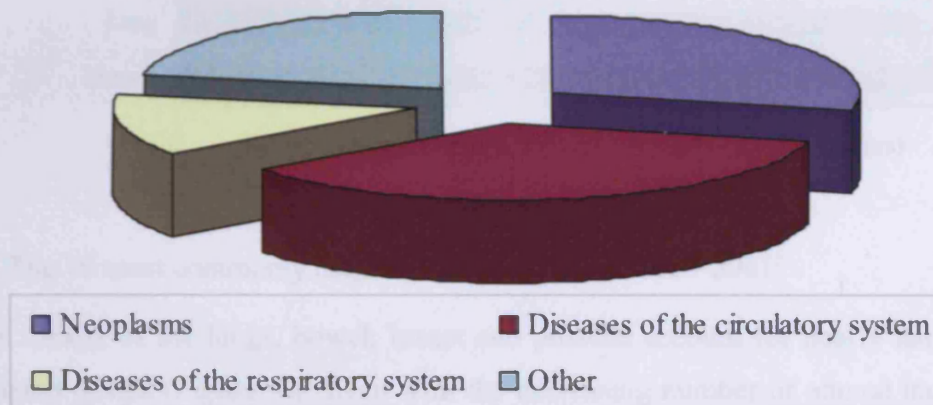


Figure 1: Mortality Statistics in England and Wales – 2003²

It is estimated that 1 in every 3 people in the UK will develop some form of cancer during their life, with over quarter of a million new cases of cancer being diagnosed each year. Even though there are over 200 different types of cancer, only 4 of them account for over half the new cases, these are breast, lung, large bowel and prostate cancer (*Figure 2*). In recent years prostate cancer has overtaken lung cancer as the most commonly diagnosed cancer in UK male, with over 30,000 cases in 2001. The most prolific cancer in women is breast cancer, which is the highest occurring cancer in the British population, with over 40,000 in 2001. Children 14 years and younger count for less than 1% of total number of new cases of cancer each year. Nearly two thirds of all the new cases of cancer are diagnosed in those aged 65 and older. With a growing elderly population due to increased life expectancy, it is predicted that by the year 2025, there will be an extra 100,000 incidences a year.²

Chapter 1 - Cancer Chemotherapy

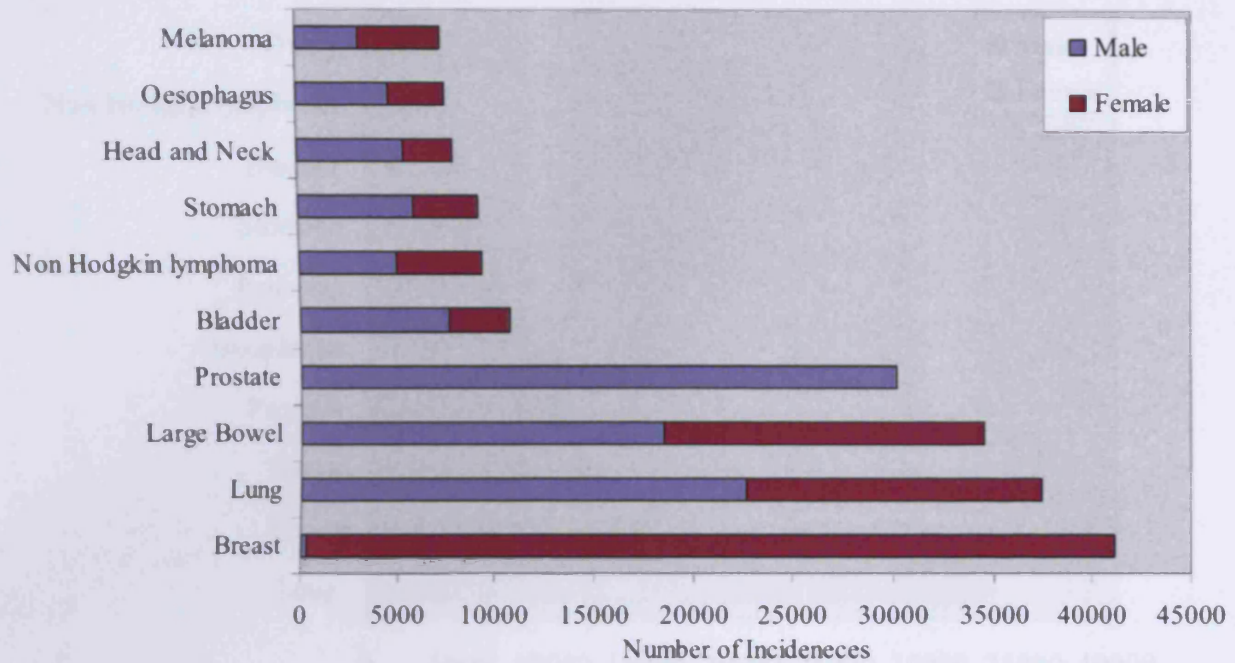


Figure 2: The 10 most commonly diagnosed cancers in the UK – 2001²

In the UK cancer of the lungs, bowel, breast and prostate account for nearly half of all the cancer related deaths (Figure 3). Even with the decreasing number of annual incidences of lung cancer it is still accountable for over a fifth of the annual cancer deaths. More than three quarters of deaths due to cancer occur to persons aged 65 and over. However cancer accounts for over two fifths of the deaths in the population under 65 years old, and outnumbers the deaths due to circulatory systems including stroke and ischaemic heart disease.²

Despite the increasing number of incidents a year, the overall mortality rate from cancer is going down. From 1994 to 2003 the age standardized mortality rate from cancerous tumours has dropped by 13% for men and 10% for women. For instance there have been 1758 fewer deaths due to breast cancer in 2003 than 1994, even though there has been an increase in the number of incidences for breast cancer.²

Chapter 1 - Cancer Chemotherapy

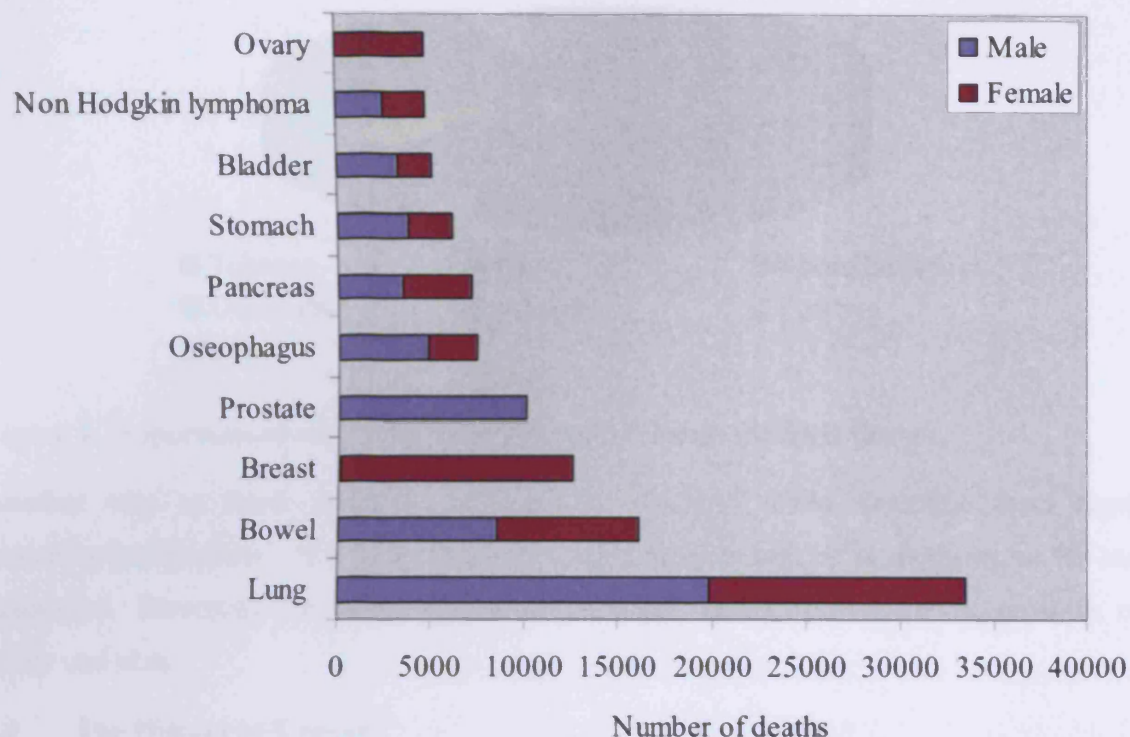


Figure 3: The 10 most common causes of death from cancer in the UK – 2003²

As well as the previously mentioned drop in cancer related mortality rate, the 5-year survival rate for people diagnosed with cancer in the US has also increased from 50% in 1974 – 1976 to 64% in 1995 - 2000. These 5-year survival rates vary greatly depending on the type of cancer, for instance the largest increase has been for prostate cancer, the 5-year survival rate was increased to 99% in 1995 - 2000 from 67% in 1974 – 1976. However there has been a small decrease in the 5-year survival rates for cancers of the larynx and uterine corpus. ²

Tobacco smoking has been identified as the single most important cause of preventable cancer in the UK, and is linked to one third of the annual cancer related deaths.² While in the US over one third of all cancer deaths are linked to nutrition, physical inactivity and weight/obesity, all of which are preventable.² A study done on the factors that contribute to cancer in the US by R. Doll and R. Peto³ in 1981 (*Figure 4*) shows that a large percentage of these risks can be reduced by taking proper precautions. It has been suggested that pollution and occupation exposure to carcinogenic sources are relatively low risk factors when compared to our own lifestyle choices such as smoking, diet, alcohol consumption, sexual behaviour and exposure to sunlight.

Chapter 1 - Cancer Chemotherapy

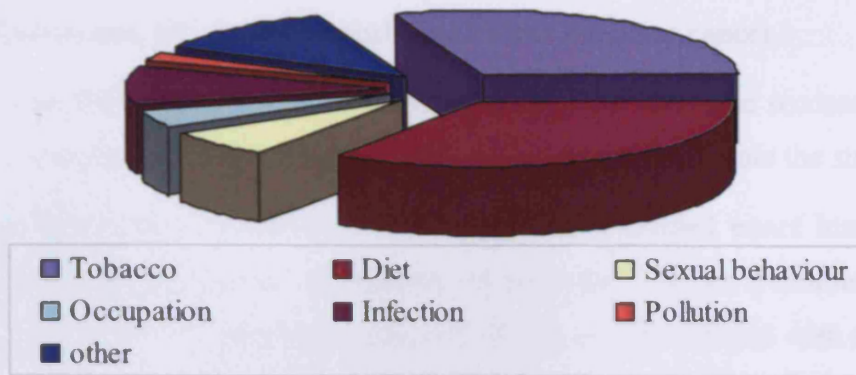


Figure 4: Proportions of cancer deaths attributed to various different factors.³

Another way to lower the risks of terminal cancer is early detection from regular screening/examination. If a cancer can be caught early treatment is likely to be far more successful. Screening can detect cancers of the breast, colon, rectum, cervix, prostate, oral cavity and skin.

1.4 The History of Cancer.⁴

Cancer is often thought of as a modern disease, however traces of tumour have been found in fossil remains of dinosaurs from the Cretaceous period, 144 to 65 million years ago.⁵ The earliest recorded history of the treatment of cancer is in the Ebers Papyrus (Figure 5). The Ebers Papyrus is ancient Egyptian medical scroll dating from around 1500 BC; it is over 20 metres long and details over 700 medical magic's using different animal and plant extracts as well as surgery. The scrolls show evidence of Egyptian surgeons regularly identifying and removing tumours.⁶ There are also a number of tomb paintings showing evidence of tumours.



Figure 4: Frontispiece of the Ebers Papyrus.⁶

Hippocrates (460 BC – 380 BC) noted in his Aphorisms about the dangers of cancer spreading due to incomplete surgery. Also it was Hippocrates who described a tumour as

Chapter 1 - Cancer Chemotherapy

being crab like due to the distended veins radiating from central lump, the Greek word for crabs being Karkinoma, which when translated to Latin becomes cancer.¹

On a similar note the Roman physician Celsus (25 BC – 50 AD) also recommended surgery for early diagnosed tumours, claiming late intervention would aggravate the situation.

The Greek medical writer Galen (131 AD – 201 AD) provided exact instructions in his books, the therapeutic method on the removal of tumours. “When a tumour has risen to a noticeable size, there is no cure without surgery. The goal is to proceed with a round incision so that the entire tumour can be excised.” Galen’s medical views dominated European medicine for over a thousand years, cancer and all ailments were thought to be caused by an imbalance in the four body humours: blood, phlegm, black bile and yellow bile. The cause of cancer was said to be due to a concentration of black bile.

Cancer was a rare disease in Hippocrates and Galen’s time due to the average life span being much shorter then, 30 – 40 years, with modern day cancer happening most frequently to the people aged 65 and over. Physician and surgeons of this time were far more concerned with treatment of injuries from battles, sexually transmitted diseases and food poisoning. The true causes of cancer remained a mystery until the 19th century.

The Italian physician Bernardino Ramazzini, carried out the first study of cancer in the early 18th century. He noted that nuns have a much higher incident rate of breast cancer compared to mothers who had breast fed their children. At the time no one knew of the importance of hormones and the development of breast cancer. Also in the 18th century an English physician John Hill⁷ commented on snuff users getting nasal cancer; this was probably the first link of cancer and tobacco. But it was Percivall Pott⁸ in 1775, who reported that boys who had worked as chimney sweeps, often developed scrotal cancer later on in life. This was first link between environmental carcinogens in this case, soot and cancer. Despite this warning, the practice of small boys cleaning chimney flues continued for a further hundred years before it became illegal, and even then it was due to humanitarian rights rather than health issues.

The discovery that organisms were made of cells which divide to replicate themselves in the 1850s - 1860s by European scientists, especially the German Rudolf Virchow revolutionized medical theory. The idea that all cells come from pre-existing cells eliminated the accepted ideas of Galen’s theories on the bodies 4 humours. Virchow stated that cancer cells sprung from other cells in the body, just like any other cells. But unlike normal cells their growth was anarchic, invasive, metastatic and all too often disastrous. Later researchers disproved some of Virchow’s theories on the origins of some cancers; however no one was able to

Chapter 1 - Cancer Chemotherapy

describe the reason why some cells became cancerous. This remained a mystery. Near the end of the 19th century a leading Philadelphia surgeon, Samuel Gross admitted that the basic questions of what cancers were and how they developed were simply beyond contemporary medical science.¹

Virchow also speculated that cells that were exposed to certain irritants would be more prone to developing cancer cells. This helped to explain why some habits such as smoking tobacco seemed to provoke the disease. This idea also worked well to explain the link between chemical exposure and cancer. In the late 19th century these links became more apparent with increased cases of skin cancers in workers employed in the paraffin, tar and oil industry in the north of England and Scotland. Another case of exposure to hazardous materials occurred to German miners who were contracting lung cancer. The idea at the time was that mine dust was to blame, but the tumours were probably due to the presence of radioactive pitchblende, a mineral which contains radium.¹

The first scientific proof between chemical exposure and cancer came from a study done by Yamagiwa & Ichikawa⁹ in 1915, where they had produced malignant tumours on the ears of rabbits by applying coal tar to them. Similar experiments were carried out on mice by Passey¹⁰ who smeared them with an extract of soot and reported the growth of malignant tumours.

1.5 How does a normal cell become a cancer cell?

The average adult is made up of 30 million million (3×10^{12}) cells which can all undergo mitosis to produce two new cells which can replace dead or damaged cells. But during mitosis there are numerous points where a mistake can occur or where the delicate control mechanism can break down, this can lead to a number of events where a normal cell can be turned into a rogue cell and perhaps a cancer cell.

Two particular types of genes have the main responsibility of controlling the life cycle of a cell. These are the proto-oncogenes such as src protein and ras protein, the other being tumour suppressor genes like p53. The proto-oncogenes control the growth, differentiation (to change the function of a cell) and proliferation (rapid reproduction) of cells. While tumour suppressor genes code for the production of proteins that control DNA transcription, DNA repair, and are key components in biological pathways activated in response to DNA damage which will stop the cell from growing and undergo apoptosis. Damage to genes can occur through the effects of carcinogens, viruses and ionizing radiation and will disrupt their normal function. Proto-oncogenes when mutated will become an oncogene. Oncogenes are

Chapter 1 - Cancer Chemotherapy

formed by a minor DNA sequence change to the proto-oncogenes; one amino acids difference will completely change the activity of the encoded enzyme and can ensure that the cell is constantly activated for growth and proliferation. In about half of tumours, tumour suppressors are found to be mutated. This ensures that the growth of the cell is uncontrolled and does not undergo apoptosis.

One of these mutations will not instantly cause tumour growth, normally half a dozen such mutations must accumulate before the tumour cell line is established. Even then the tumour may not be cancerous and will remain benign and localized; such tumours can be dealt with by surgery or destroyed via irradiation. For a tumour to become malignant it must undergo a process know as metastasis where it will become invasive and spread through out the body. To become invasive the localized tumour must overcome several more control systems and enter the bloodstream or lymphatic system. Once the tumour has become malignant it can only be treated with chemotherapy to stop the spread of the disease through out the body.

So how do cancer cells migrate when normal cells stay in the same place? Normal cells release adhesion molecules which bind them to their neighbours and the underlying structure of the body, the extracellular matrix. However in cancer cells these adhesion molecules are missing or have a higher affinity for the tissues that they came from, also cancer cells can avoid detection and therefore destruction by white blood cells. A better understanding of how these migrations take place will help in fight against cancer.

1.6 Cancer Chemotherapy

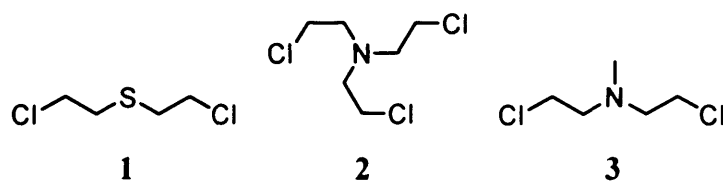
In the past 25 years there have been major advancements in surgery and radiotherapy in the treatment of cancer, but the most progress has been made in the field of chemotherapy. At the time of detection a tumour will probably weigh about a gram and be about one billion cells (1×10^9 cells). The overall strategy to deal with tumours is to eliminate 99.999% of it, leaving around ten thousand cells (1×10^4 cells) where hopefully the bodies immune system will destroy the remaining cells. However if a single cell remains it can reproduce itself again and start another tumour. The time taken for a tumour to double in size varies greatly, for instant people who have Hodgkin's lymphoma will only have a short remission time because the tumour doubles in 3 - 4 days, while the remission time for breast cancer can be 10 – 15 years before a tumour re-emerges, this is due to the long doubling time of 80 – 100 days. Anticancer drugs are most effective against cells that are going though mitosis, and therefore cancers with short remission times are the easiest to cure compared to ones with long remission time, this is because the cells are more frequently in the mitosis stage.

Chapter 1 - Cancer Chemotherapy

1.6.1 The Mustard Gases

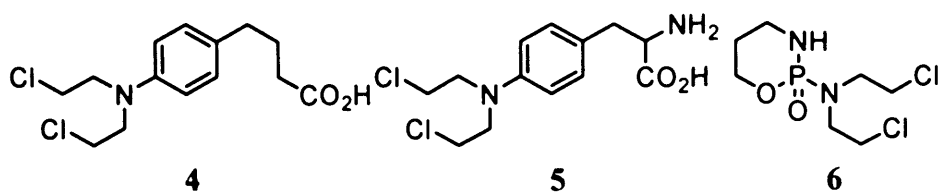
Mustard gas, bis(2-chloroethyl)sulfane (1), was first used by the Germans against the Allies in the 1st world war on the night of 12/13 July 1917. By the end of July it had claimed 15,000 casualties, by the end of the war a further 125,000 casualties would be caused by the use of mustards. People who had been exposed suffered with severe eye irritation which could lead to blindness and would get a rash that turned to blisters. After 4 weeks, the blisters would burst and then if they became infected, it would take a further 5 to 6 weeks to heal. Also many of the exposed also suffered with inflamed throats and subsequent lung problems.⁴

However mustard gases were not made with the intention of killing, only to sow panic and confusion. Between the wars further research was done on the mustards to examine their properties.^{11,12} Various nitrogen analogues of mustard gas was made. They were found to have similar blistering properties and were also found to be toxic to many cells of the body, especially cells going through mitosis.¹³



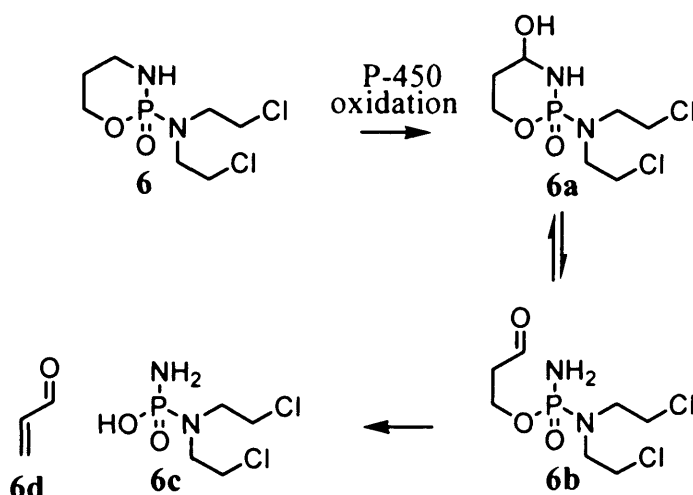
Research done at Yale University showed that mice bearing tumours that were treated with N-mustard analogues showed almost immediate effects and the tumours shrunk and eventually disappeared, only to reappear a month later. Subsequent treatments proved to be less successful; this was thought to be due to drug resistance.¹³

In August 1942 a man with terminal lymphoma whose tumours had stopped responding to radiotherapy was given a 10 day treatment with the nitrogen mustard, tris(2-chloroethyl)amine (2). After 7 days the tumours which had covered his chest, neck and lower face had completely disappeared. However like the mice this was only temporary and he died 3 months later. Further clinical trials with 2 were done and another 5 people showed similar responses. A more widespread clinical trial took place with mechlorethamine (3) with over 150 patients and showed results against various lymphomas and leukaemias.¹³ It proved to be especially effective against Hodgkin's lymphoma with one patient being in remission for 3 years upon being given periodic doses of 3.



Chapter 1 - Cancer Chemotherapy

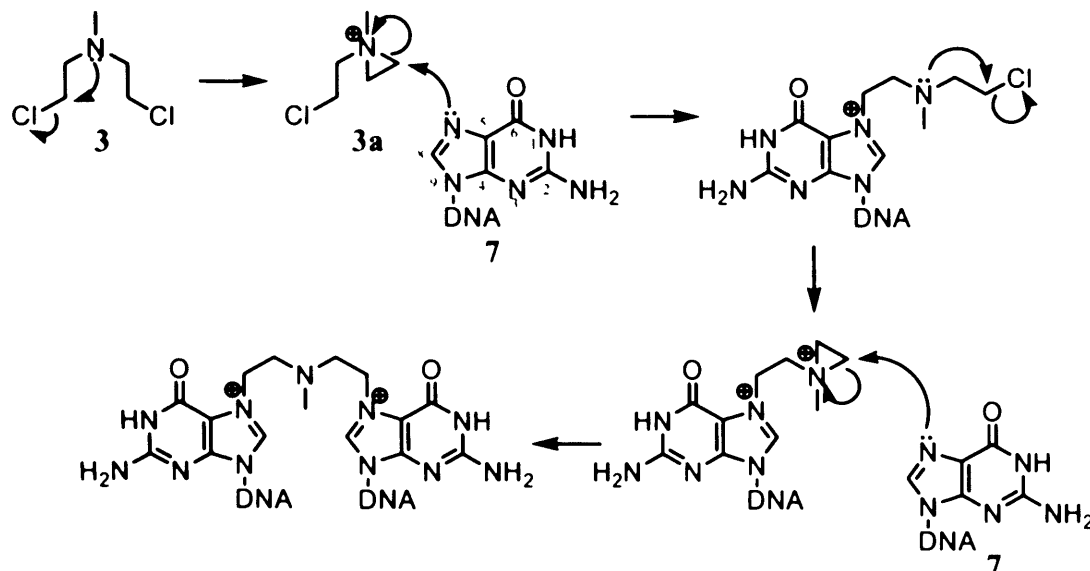
The side effects of the mustards include suppression of the bone marrow and therefore careful dosage due to the toxicity of the compounds was required. But they did not cause vomiting which is common with other anticancer drugs. In 1946 the first paper on this subject was released in *Science*, detailing the therapeutic results of **3**. This was the first the public knew of this because until then all the research had been done in secret due to the war.¹³ The paper stated that only two nitrogen mustards had been tested so far, of literally hundreds of possible analogues. Over the next few years several improvements were made with drugs like Chlorambucil (**4**) and Melphalan (**5**) being water soluble and orally active. The most successful nitrogen mustard is Cyclophosphamide (**6**), which was designed as a prodrug to target prostate cancer. Designed to release **1**, the actual mechanism of activation proved to be more complex. Cytochrome P-450, in the liver, is thought to oxidize C-4 of the prodrug **6** to give 4-hydroxycyclophosphamide (**6a**). This hemiaminal exists in equilibrium with the aldehyde **6b**. The aldehyde then undergoes a retro-Michael reaction to give the true alkylating agent phosphoramidate mustard (**6c**) and acrolein (**6d**) (*Scheme 1*).^{14,15} It was found to be highly active against a whole range of cancers. It can be taken orally and remains active in the body for up to 48 hours and has none of the adverse blistering effects that the early mustards had. Side effects from the mustards include bone marrow toxicity, hair loss and a reduction in fertility.



Scheme 1

The mode of action of these compounds involves nucleic acid alkylation and they are therefore described as alkylating agents. DNA exists in a double helical structure and for a cell to replicate itself, the DNA strands need to be unwound so that they can be copied. The nitrogen mustards and other related alkylating agents work by reacting with various groups on the DNA. When this takes place it changes the structure of the DNA, it also leads to the alkylating agents making inter and intra cross links on the DNA, which will prevent the DNA from being unwound. For example mechlorethamine (**3**) eliminates a chlorine anion by an internal nucleophilic attack to produce the reactive species **3a**. The product can then react with the electron donor groups within DNA (**7**) such as the nitrogen 7 on guanine to form covalent bonds. The alkylated mustard can then form another aziridinium species which can then react with a further guanine to cross link the DNA (*Scheme 2*).

Chapter 1 - Cancer Chemotherapy

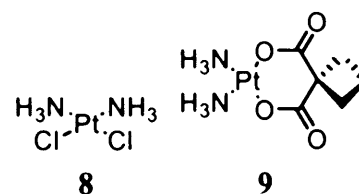


Scheme 2

1.6.2 Platinum Complexes

The next class of alkylating agent was found again by serendipity. In 1965, Prof. Barnett Rosenberg of the Biophysics Department at Michigan State University was investigating the effects of an electric current on cell division

in cultures of *Escherichia coli*. He found that in the presence of platinum electrodes, ammonium chloride and oxygen that the bacteria cells did not undergo mitosis. He proposed that the acidified chloride salts attacked the platinum electrodes during electrolysis causing it to form a soluble platinum salt which could disrupt mitosis. Several experiments were then



carried out to prove this idea and it was reported that mitosis was prevented in the presence of $\text{PtCl}_6(\text{NH}_4)_2$ and several other transition metal salts.¹⁶ Further research showed $\text{PtCl}_6(\text{NH}_4)_2$ was unstable and that it was cis-diaminodichloroplatinate (8) (now known as cisplatin) which prevented mitosis at concentrations as low as 5 ppm.¹⁷ Further research by Rosenberg's coworker Loretta Van Camp showed that cisplatin (8) had anticancer activity on tumours in mice.



Figure 5: X-ray crystal structure of DNA containing a cross linked Cisplatin¹⁸

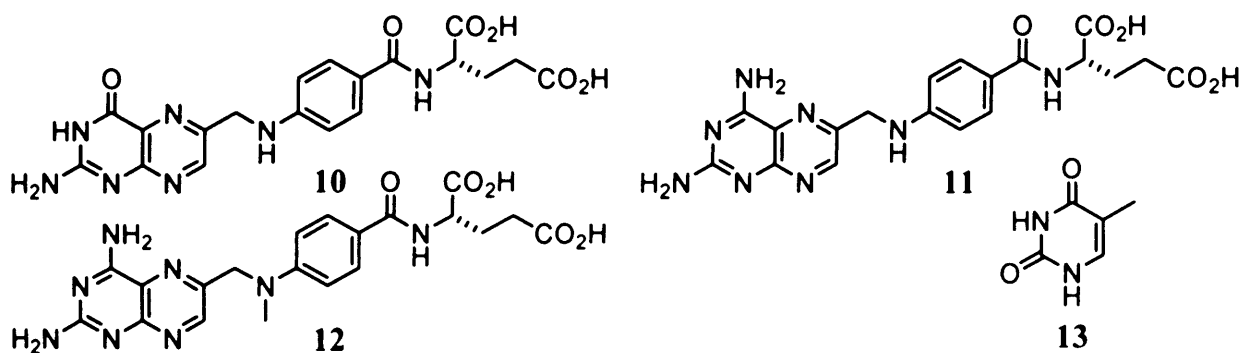
Chapter 1 - Cancer Chemotherapy

By 1973 preliminary results on humans with **8** had shown it to be highly effective at treating testicular cancer, and certain ovarian cancers. The side effects were high toxicity to the kidney and severe vomiting, but it had only a little toxicity to the bone marrow. By 1986 a new less toxic analogue of **8** was licensed for clinical use, known as carboplatin (**9**). The modes of action for these platinum complexes have been shown to be similar to that of the mustards and are also alkylating agents (*Figure 5*) where the nitrogen 7 on guanine (**7**) forms a covalent bond to the platinum complex, displacing a chlorine atom from **8**. The X-ray crystal structure¹⁸ (*Figure 5*) shows two separate stands of DNA one in blue, the other in green which have been cross-linked by the platinum complex (shown in red) from the nitrogen at position 7 on guanines of each strand of DNA.

1.6.3 Antimetabolites and the cure for Leukaemia

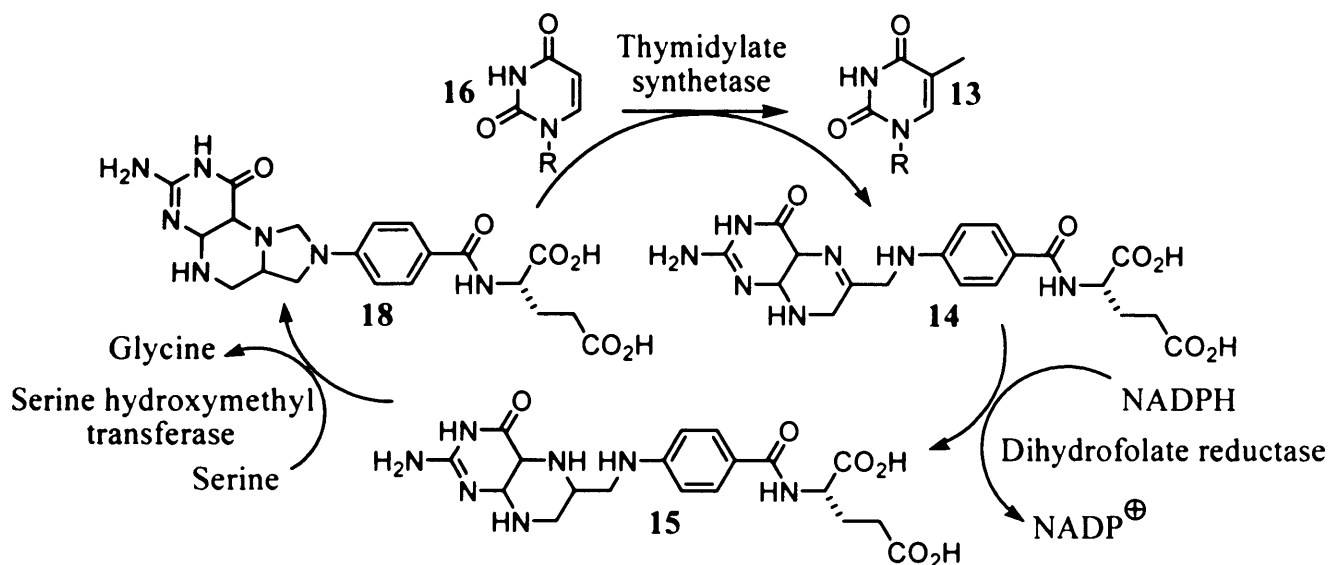
Leukaemia is caused through uncontrolled proliferation of the white blood cells, it is a common childhood cancer. Fifty years ago, the life expectancy for patients with leukemia was 3 to 4 months. With modern treatments, nearly 80% of all children go on to survive for 5 years, and most make a full recovery.

The breakthrough in the treatment of leukaemia started with the study of antimetabolites which disrupt cellular function due to the similarity of these compounds with cellular metabolites. Previous studies on leukaemia patients showed that the disease was accelerated when they were treated with folic acid (**10**) conjugates.¹⁹ This led Farber to believe that using antifolates (folic acid antagonists) might be effective in treating leukaemia cases. In 1947 Farber found that 10 out of 16 children with leukaemia responded favourably to the antifolate, aminopterin (**11**), going into full remission. However this remission was only temporary and repeated treatments with aminopterin had no effect.²⁰ The use of aminopterin (**11**) caused ulceration to the mouth and left the patient at risk of infection due to their immune system being compromised. Aminopterin (**11**) was soon replaced with the safer drug methotrexate (**12**), which had first been synthesized by Seeger.²¹



Chapter 1 - Cancer Chemotherapy

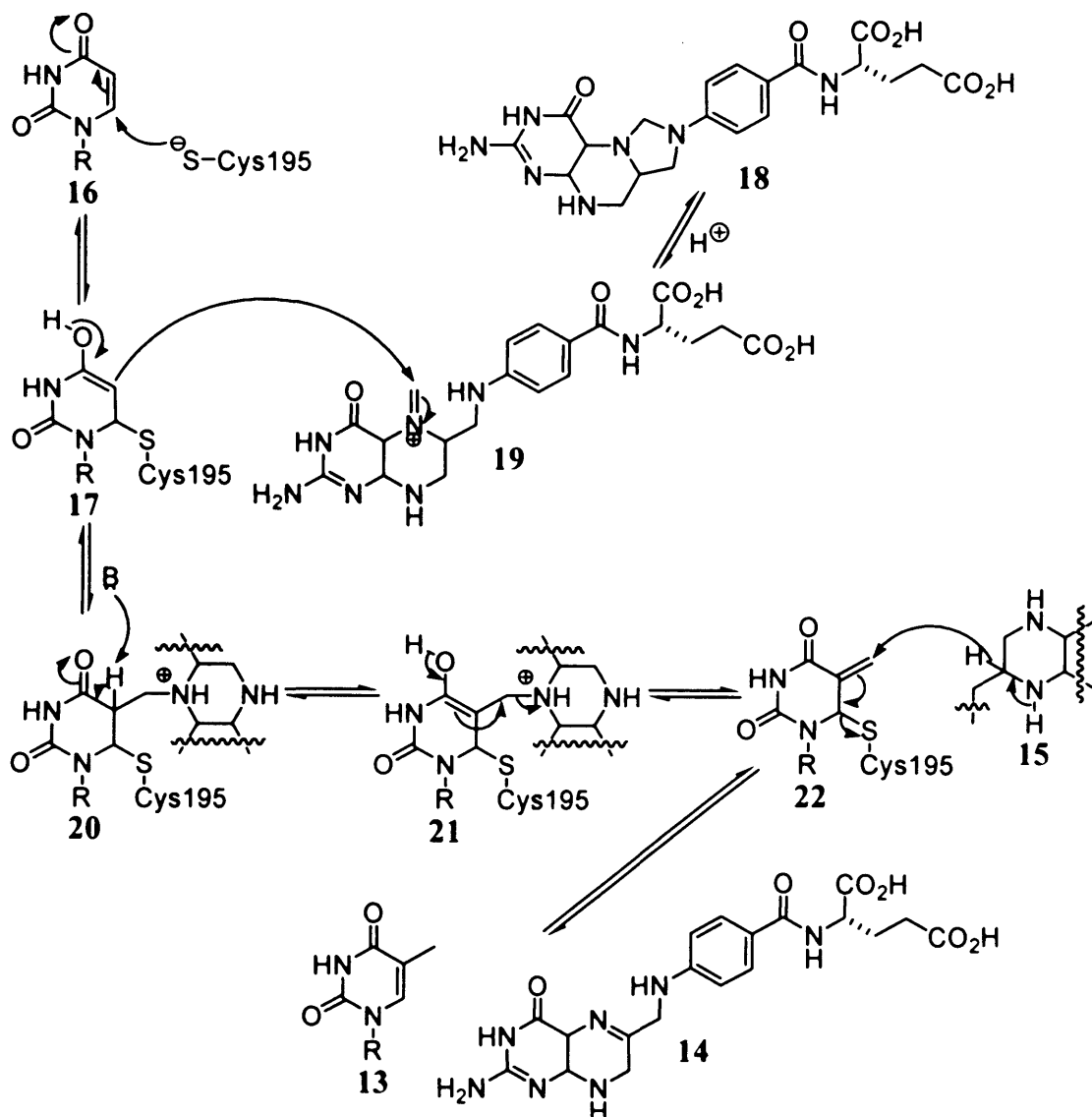
Synthesis of the nucleotide thymidine (**13**) which is needed for the synthesis of DNA, requires the reduction of dihydrofolic acid (**14**) to tetrahydrofolic acid (**15**) by dihydrofolate reductase (DHFR) (*Scheme 3*). The antifolates aminopterin (**11**) and methotrexate (**12**) are dihydrofolate analogues which bind to DHFR and inhibits DHFR reduction of **14**, this halts the synthesis of DNA by disrupting the synthesis of the nucleotide thymidine (**13**) and cause apoptosis.²²



Scheme 3: The Folate Catalytic Cycle

The methylation mechanism of thymidylate synthase (*Scheme 4*) starts with the Michael attack of cysteine residue at the C6 position of the uridine (**16**) to form the enolate **17**. The tetrahydrofolate (**18**) is then activated by iminium ion **19** formation via acid catalysis. The methylene group of the activated tetrahydrofolate iminium ion **19** then forms a bond with the enolate **17** at its C5 position, giving the methylene bridged product **20**. The proton on the C5 position of **20** is then deprotonated and the compound undergoes a E1cB elimination of tetrahydrofolic acid (**15**), via the enol **21**, leaving the methylene group attached to C5 of **22**. The methylene group of **22** is then reduced by the tetrahydrofolic acid (**15**) and releases the thioenzyme, giving the newly formed thymidine (**13**) and dihydrofolic acid (**14**) which is then recycled back tetrahydrofolate (**18**) again using the folate catalytic cycle (*Scheme 3*).²³

Chapter 1 - Cancer Chemotherapy

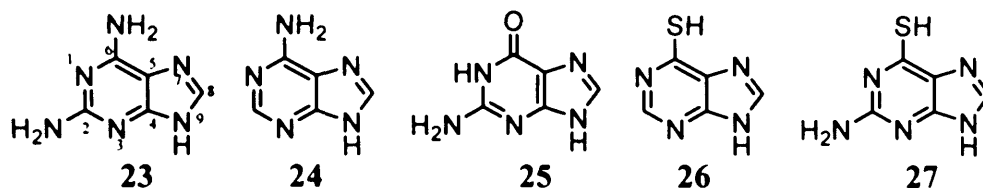


Scheme 4: The mechanism of Thymidylate Synthase

With the success of the antifolates, research was done into the other 2 antimetabolites, antipurines and antypyrimidines. Pioneering work on antipurines carried out by Elion and Hitchings in the late 1940s and early 1950s led to the initial development of antipurines as anticancer agents.²⁴ At this time the basic knowledge of the nucleic acids was rather rudimentary. Watson and Crick's publications on DNA were released in 1953.²⁵ Hitching theorized that it might be possible to stop the growth of rapidly dividing cells with antipurines because they would interfere with nucleic acid synthesis. By 1948, 2,6-diaminopurine (**23**) was found to inhibit the growth of the microorganism *Lactobacillus casei*, the inhibition could be reversed by the addition of adenine (**24**). From this they deduced that adenine and 2,6-diaminopurine were anabolized by the same enzyme and somehow the diaminopurine interfered with purine interconversion. Further testing showed that 2,6-diaminopurine was a strong inhibitor of aldo keto reductase (AKR) mouse leukaemia. Unfortunately 2,6-diaminopurine proved to be too toxic for clinical trials, producing severe nausea and severe

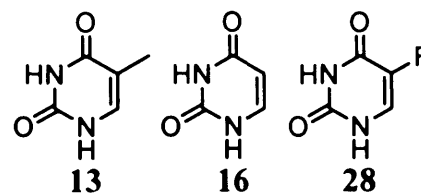
Chapter 1 - Cancer Chemotherapy

bone marrow depression. By 1951 over 100 purine analogues had been tested in the screen against *Lactobacillus casei*, the substitution of the oxygen by sulfur at the 6 position of guanine (**25**) produced 6-mercaptopurine (**26**) and 6-thioguanine (**27**) which proved to be active against a wide range of rodent tumours and leukaemias.



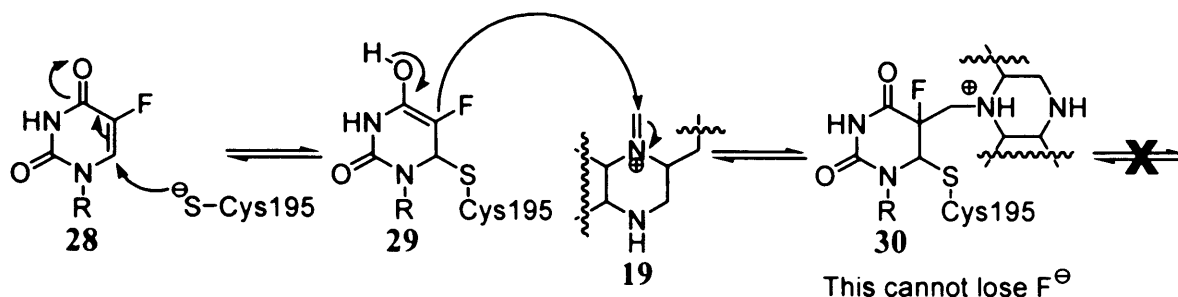
In 1953 the Food and Drug Administration approved 6-mercaptopurine (**26**) for general use, after clinical trials showed that it could induce complete remission in acute leukaemia in children, however most of the cases would eventually relapse. Today 6-mercaptopurine (**26**) is used in combination chemotherapy with other drugs, including methotrexate (**12**) and induces remission in nearly 80 % of all children with acute leukaemia. Hitchings and Elion were awarded the Nobel Prize in 1988 for their advances in finding anticancer drugs.

Other purine and pyrimidine analogues have also shown anticancer activity. Prof Heidelberger at McArdle Memorial Labs at the University of Wisconsin in 1957 reported that the analogue of uracil (**16**), 5-fluorouracil (**28**) showed tumour inhibitory properties.²⁶ 5-Fluorouracil (**28**) was first synthesized at Hoffmann-La Roche, Nutley, New Jersey in 1957.²⁷ Heidelberger chose to make analogues of uracil



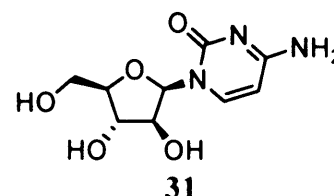
because he believed that uracil was a precursor to thymidine (**13**) which is a key component of DNA. His idea for replacing the hydrogen for fluorine, came from the difference between acetic acid and fluoroacetic acid (which is highly toxic), and that the same may be true of uracil. Clinical trials of 5-fluorouracil (**28**) have shown it to be highly effective at targeting colon and rectal cancers. It was also used in combination chemotherapy along with methotrexate to treat some cases of breast cancer in women. Fluorouracil (**28**) will become incorporated into the thymidylate synthase and will proceed normally like uracil (**16**). With the cysteine residue attacking the C6 position of fluorouracil (**28**), forming the enolate **29**. The enolate **29** will then form a bond with the activated tetrahydrofolate iminium ion **19**, giving the methylene bridged product **30**. However the E1cB elimination of the linked tetrahydrofolic acid (**15**) is not possible, because the fluorine on the C5 position of **30** cannot be eliminated like the hydrogen in the same position of **20** through deprotonation, stopping the synthesis of **13** and thus disrupting DNA synthesis and inducing apoptosis (*Scheme 5*).

Chapter 1 - Cancer Chemotherapy



Scheme 5: Mode of action for 5-fluorouracil (29)

Cytarabine or cytosine arabinoside (31) was originally isolated from a Caribbean sea sponge, *Cryptotethia crypta* in 1951. Since then it has been found to have *in vivo* antitumour activity against leukaemia and a variety of transplanted rodent tumours as well as an effective agent in the treatment of acute leukaemia in adults.²⁸ It is presently used against leukaemia and in combination chemotherapy.



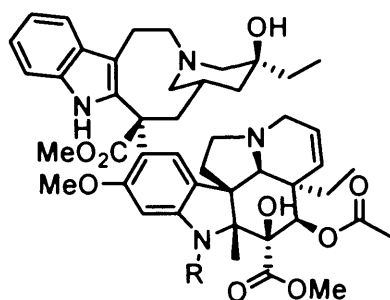
1.6.4 Anticancer drugs from Nature

Man's search for a cure to his ailments isn't a new one. As previously mentioned, the Ebers Papyrus, an ancient Egyptian scroll from around 1500 BC details over 700 remedies using animal and plant extracts.⁶ Another ancient source of remedies using animal and plant extracts is Chinese Shennong herbal text from around 200 BC. Medical knowledge like this has been passed down the generations. In the 19th century scientists started to isolate and characterize compounds from these remedies. However many of the compounds proved to be too complex to be characterised until the 20th century. In the late 1940s scientists started a systematic search for novel natural products with interesting biological properties.⁴ These studies have given us several highly active anticancer drugs, such as vinblastine and vincristine.

In the Philippines the extract of the Madagascar periwinkle was used as a treatment of diabetes. The extract was also used in the same way in South Africa. In England the extract was sold as "Vin-q-lin" and was believed to reduce blood sugar. However experiments done by Ralph Noble²⁹ at the University of Western Ontario, Canada, on rats showed that the extract did not lower their blood sugar level. All the rats tested died due to infections; more tests showed that the extract lowered the white blood cell count. With the help of an English chemist Charles Beer in 1958 they isolated a pure compound that depleted white blood cells and called it vincalukoblastine, later known as vinblastine (32).³⁰ At the same time Eli Lilly made similar discoveries and had found that the plant extract prolongs the life of mice carrying the leukaemia cell line P1534. They isolated a total of four vinca alkaloids that

Chapter 1 - Cancer Chemotherapy

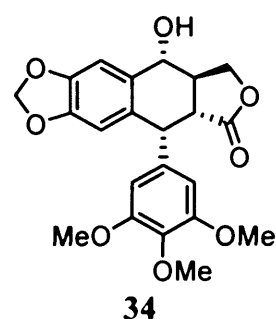
showed antileukaemia activity. Only Vinblastine (32) and Vincristine (33) went into clinical trials.³¹ Vinblastine is now used in the treatment of Hodgkin's lymphoma, testicular teratoma, and bladder cancer. Vincristine is used in



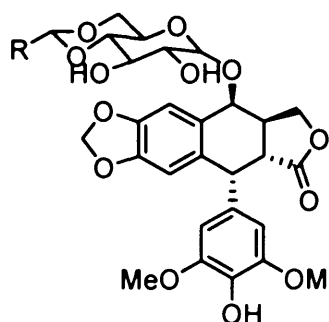
	R
32 Vinblastine	Me
33 Vincristine	CHO

the treatment of Hodgkin's lymphoma and a host of rare childhood cancers including Wilm's tumour, neuroblastoma, rhabdomyosarcoma and Ewing's sarcoma. Both compounds have serious adverse effects, Vinblastine leaves people susceptible to infection and depresses bone marrow cell production, while use of Vincristine can cause loss of sensation in fingers and toes, constipation and severe abdominal pain.

Another natural extract that has had anticancer activity was derived from *Podophyllum peltatum* (Mayapple) (34). The Leech book of Bald, around 950 AD, reports the use of wild chervils in the treatment of tumours, wild chervils are thought to be *Myrrhis Odorata* which is a source of a podophyllotoxin analogue. The NCI screening of natural compounds showed that podophyllotoxin (34) isolated from the American Mayapple had antitumour activity. However it proved to be too toxic in clinical trials.



Further studies done by the Swiss pharmaceutical company Sandoz, making semisynthetic glycosides analogues of podophyllotoxin, finally yielded two successful drugs Etoposide (35) and Teniposide (36). Etoposide is used as a less toxic replacement for Vinblastine (32) in testicular cancer as well as small cell lung cancer. Teniposide (36) is used to treat acute lymphoblastic leukaemia and rare childhood cancers like neuroblastoma. They work by inhibiting the topoisomerase II enzyme, which is involved in the process of unwinding supercoiled DNA. Inhibition of topoisomerase II effects the S phase of the cell cycle and blocks the continuation to the G2/M phase and leads to apoptosis.³²



	R
35 Etoposide	CH ₃
36 Teniposide	

The natural product Camptothecin (37) was isolated from *Camptotheca acuminata*, a tree native to China and Tibet which is used in traditional Chinese medicine, by Monroe Wall in 1958.³³ It was found to have potent antitumour activity against leukaemia L1210. The structure of Camptothecin (37) was determined in 1966.³⁴ More water soluble analogues Topotecan (38) and Irinotecan (39) are used in the clinic for treatment of colon and ovarian

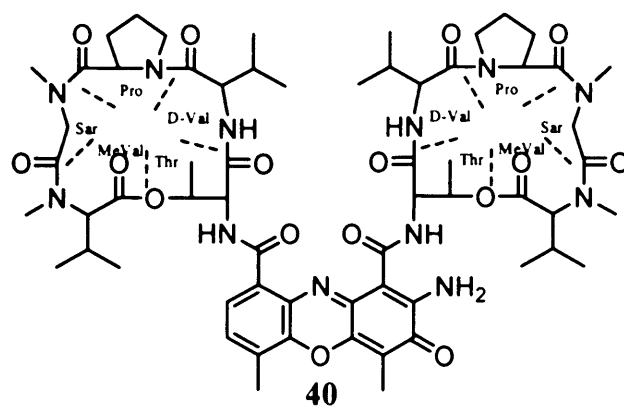
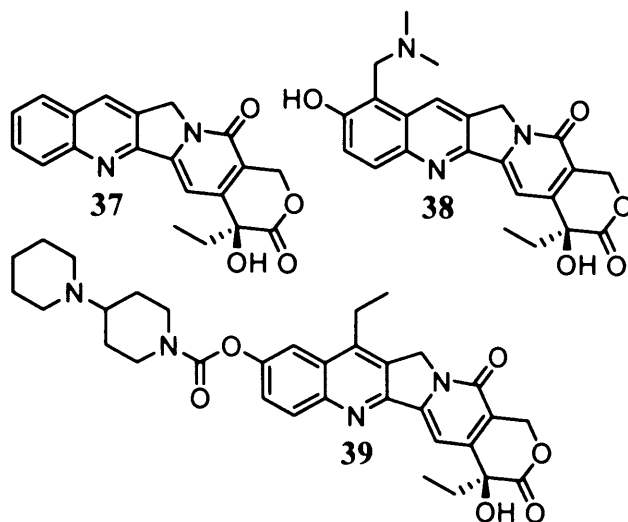
Chapter 1 - Cancer Chemotherapy

cancers, respectively.³³ The mode of action of Camptothecin (**37**) was found in 1985,³⁵ it inhibits the topoisomerase I enzyme which is needed in the S phase of the cell cycle to cause the DNA to unwind.³³

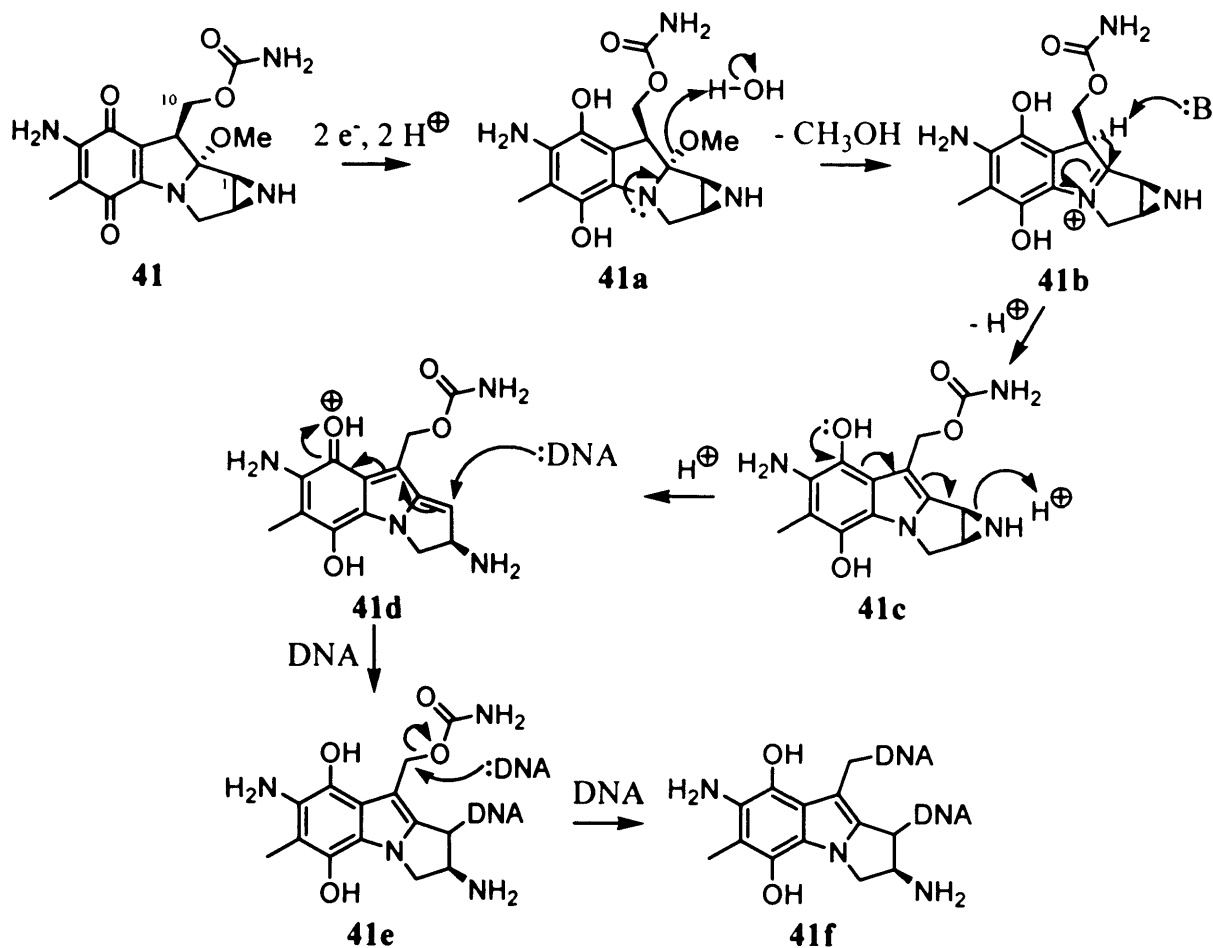
Since the discovery of penicillium in mould by Fleming in 1928,³⁶ scientists have been studying the biological activities of microorganisms, this has led to hundreds of antibiotics and has also led to several antitumour agents.

In 1960 Faber reported about the antitumour properties of actinomycin D (**40**) in treating Wilm's tumour in children.³⁷ Actinomycin D (**40**) had been isolated from *Streptomyces parvullus* by Waksman in his studies of possible new antibiotics in 1954.³⁸ The successes of actinomycin D led the way for a new class of anticancer drug into the clinic.

Mitomycin C (**41**) was first isolated from *Streptomyces caespitosus* 1958 by Wakaki.³⁹ It was also isolated from *Streptomyces verticillatus* and characterized by Lefemine and coworkers.⁴⁰ It proved to be highly toxic against a range of tumour cells as well as normal cells, causing severe side effects including vomiting, bone-marrow depression, alopecia, kidney and liver toxicity and heart damage. However it was widely used in Japan in the 1960s. Mitomycin C is bioreduced *in vivo*, producing reactive intermediates that are capable of forming covalent bonds to DNA, similar to the alkylating agents.⁴¹ The quinone of Mitomycin C (**41**) is reduced to the dihydroquinone **41a**. The bioreduction of Mitomycin C **41**, to the dihydroquinone **41a** then triggers the loss of methanol (since the nitrogen lone pair is no longer delocalized) to give the conjugated electron rich system of leucoaziridinomitosenone (**41c**). The hydroxy group on the C8 position of **41b** then donates its electron pair causing the aziridine ring to open and give **41d** (Scheme 6). The C1 position of **41d** then undergoes nucleophilic attack from DNA to form the first DNA cross linking bond to give the **41e**. Another nucleophilic group within DNA then attacks the C10 position of **41e** and eliminates the carbamate group forming the second DNA covalent bond, and giving the alkylated DNA product **41f**² (Scheme 6)



Chapter 1 - Cancer Chemotherapy



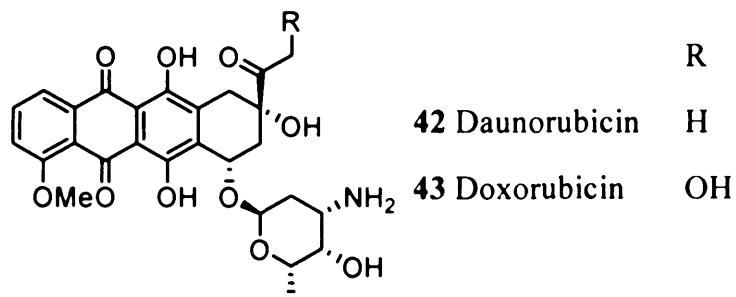
Scheme 6

A more useful class of antitumour antibiotics is the anthracyclines. They were first isolated by the Arcamone group in the early 1960s and were found to strongly to inhibit the growth of a variety of tumours.⁴³ Many hundreds of analogues have been made, including Daunorubicin (42) and Doxorubicin (43). Doxorubicin (42) has a broad spectrum of anticancer activity; it is used today for the treatment of solid tumours and childhood leukaemia. Daunorubicin (43) is one of the drugs of choice used against adult leukaemia.

The flat aromatic unit of these compounds slide between the DNA bases by intercalation so that the DNA can't separate (Figure 6). They also inhibit the topoisomerases II enzyme.

The other mode of action these

compounds have is that they undergo a chemical reaction to form radicals, the radicals react with DNA and damage it. The main side effect of these drugs is damage to the heart muscle and cardiotoxicity.



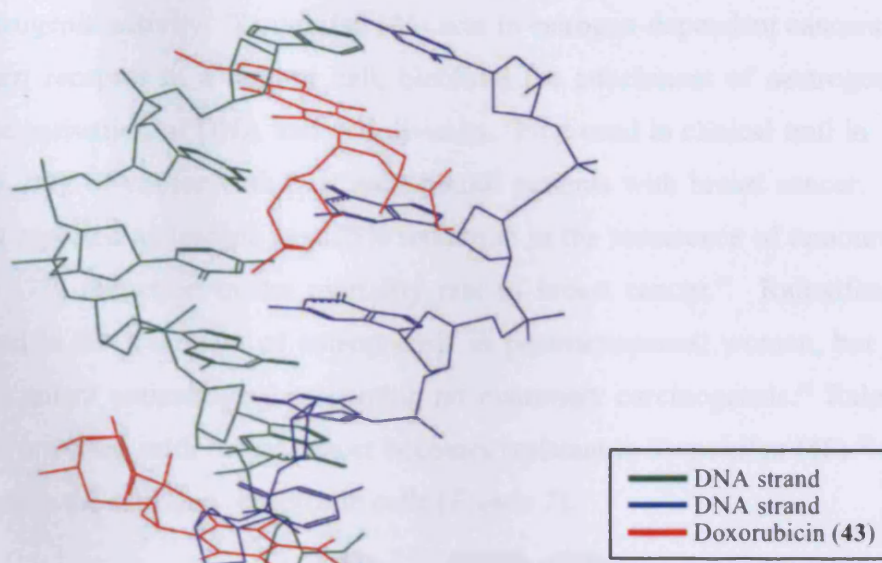
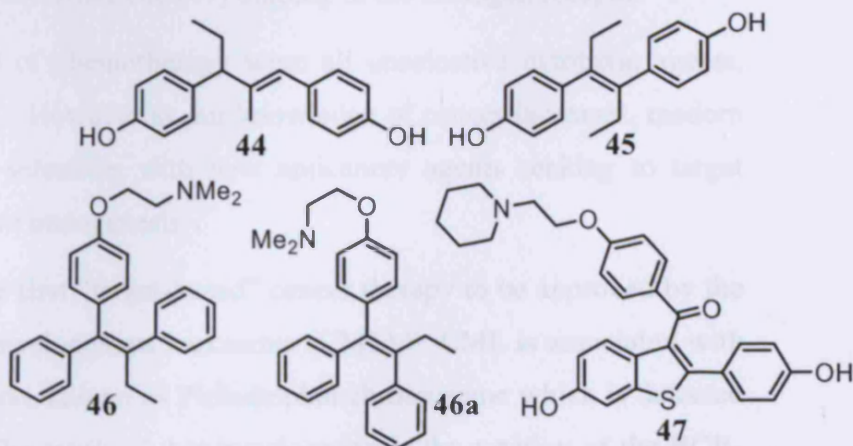


Figure 6: X-ray crystal structure of the intercalation of DNA base by Doxorubicin (43)⁴⁴

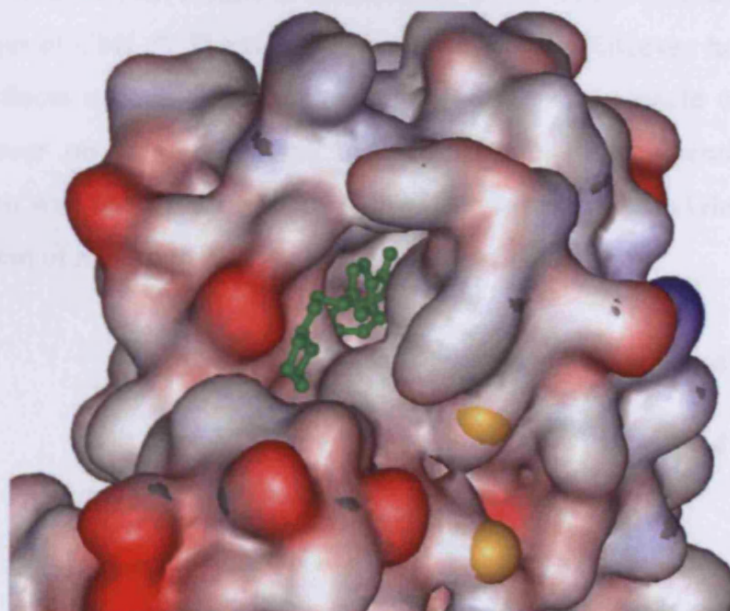
Another area of development in anticancer drugs is the use of hormone mimics to treat cancer. In 1896 a Scottish surgeon George Beatson reported in *Lancet* that tumours of women with breast cancer ceased growing and regressed after the removal of their ovaries and fallopian tubes.⁴⁵ At the time nothing was known about hormones and this was inspired by the idea of the link between the mammary glands and the ovaries, which was shown that when a mother was breast feeding her menstrual cycle stopped. Since then it has been discovered that cell growth and differentiation is controlled by hormone signals, and that by switching off production of hormones or interfering with the receptor-hormone complex provides a means of cancer therapy.

In the 1930s Dodd and coworkers investigated hormone mimics of estrogen. They found that a dimer of *p*-propenyl phenol (44) had potent oestrogenic activity. This led to a range of dimeric structures being synthesized, in 1938 diethylstilbestrol (45) was reported to be 2-3 times more active than estrone.⁴⁶ In 1944 Haddow reported that some breast cancer patients could be helped by treating them with diethylstilbestrol (45). Further modification of diethylstilbestrol led to Tamoxifen (46) first made by ICI. This exists as two



Chapter 1 - Cancer Chemotherapy

isomers, the *Z*-isomer (**46a**) having potent oestrogenic activity, the *E*-isomer (**46**) having potent antiestrogenic activity. Tamoxifen (**46**) acts in estrogen-dependent cancers by binding to the estrogen receptor in a tumour cell, blocking the attachment of oestrogen, thus also preventing the activation of DNA and cell division. First used in clinical trial in 1971, it has proven to be the drug of choice with postmenopausal patients with breast cancer. Tamoxifen (**46**) has been reported as leading to a 25% reduction in the recurrence of tumours after their removal and 17% reduction in the mortality rate in breast cancer.⁴⁷ Raloxifene (**47**) was originally used in the treatment of osteoporosis in postmenopausal women, but it was also found to be a potent antiestrogen, preventing rat mammary carcinogenesis.⁴⁸ Raloxifene (**47**) can be used if a woman with breast cancer becomes resistant to Tamoxifen (**46**).⁴⁹ Raloxifene (**47**) also binds in the estrogen receptor in cells (*Figure 7*).



*Figure 7: X-ray crystal structure of Raloxifene (47) binding in the Estrogen receptor*⁵⁰

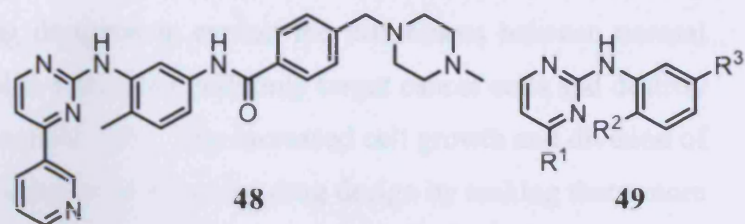
The early discoveries in the field of chemotherapy were all unselective cytotoxic agents, many of which are still used today. However as our knowledge of cancer increases, modern chemotherapy is becoming more selective, with new anticancer agents seeking to target specific biological models to prevent oncogenesis.

In May 2001, Gleevec (**48**) was the first “target-based” cancer therapy to be approved by the FDA for the treatment of chronic myelogenous leukaemia (CML).⁵¹ CML is associated with a specific chromosomal translocation, known as Philadelphia chromosome which is detected in 95% of patients with CML.⁵² The result of this translocation is the creation of the BCR-ABL fusion protein, which is an activated form of tyrosine protein kinase.^{51,53} The oncogenic properties of the BCR-ABL fusion protein were further demonstrated by several studies done

Chapter 1 - Cancer Chemotherapy

in mice, where the introduction of the BCR-ABL fusion protein to healthy mice would induce chronic myelogenous leukaemias.⁵⁴⁻⁵⁶

Therefore the inhibition of BCR-ABL tyrosine kinase activity in CML cells became an attractive drug target for the treatment of the disease.⁵¹



Extensive optimization of phenylamino-pyrimidines (49) by Zimmermann⁵⁷ *et al.* yielded a highly potent ($IC_{50} = 38$ nM) and selective inhibitor of BCR-ABL kinase, Gleevec (48).⁵⁷ X-ray crystallography shows that 48 inhibits the BCR-ABL kinase activity, by binding with high specificity to the inactive form of the ABL kinase (Figure 8).⁵⁸

Treatment with Gleevec (48) results in remission in nearly 100% of newly diagnosed patients in the early stages of CML.⁵⁹ The side effects from the use of Gleevec have mainly been mild with adverse effects such as nausea, vomiting, oedema and muscle cramps being mostly reported, however on rarer occasions liver toxicity or fluid retention have also been reported.⁵¹ Even with these rarer serious side effects, treatment with Gleevec is far less toxic than the treatment of a more traditional chemotherapy.

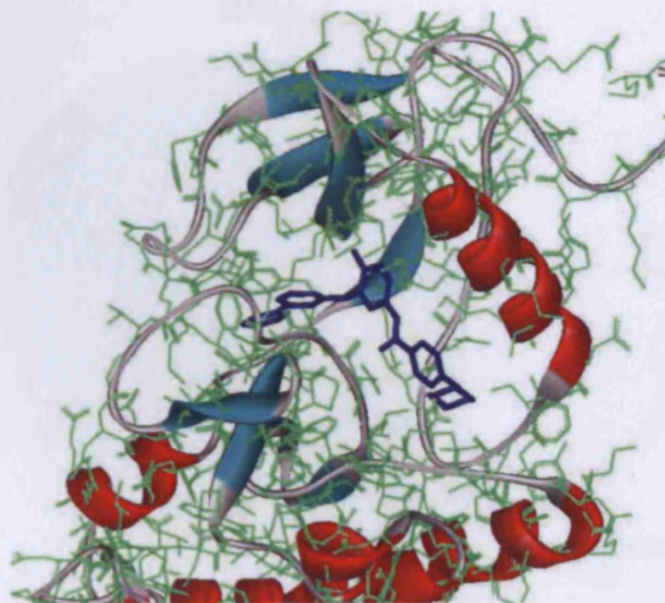


Figure 8: X-ray crystal structure of Gleevec (48) binding to inactive form of the ABL kinase.⁵⁸

Chapter 2 - Development of agents that target Tubulin

2.1 Developing Antimitotic Agents

Cancer chemotherapy agents are being designed to exploit the differences between normal and malignant cells. The ultimate goal is a drug that will only target cancer cells and destroy or render them benign, and not affect normal cells. The increased cell growth and division of cancer cells presents an attractive and achievable target for drug design by making them more selective to rapidly dividing cells. The biochemical target we are interested in is tubulin and the formation of microtubules, which plays a vital role in cell division. The disruption of microtubule formation will impede the replication of the dividing cancer cells and will lead to cell death.

2.2 The Cell Cycle and Mitosis

Cell division is arguably one of the most complex and demanding process undertaken by the body. During this process the DNA of the cell will become unraveled and duplicated. The cell will then reorder the DNA strands into 2 new identical sets of chromosomes. The 2 new duplicate sets of DNA will be moved to opposite sides of the cell to make new nuclei, which will eventually separate into 2 new daughter cells.

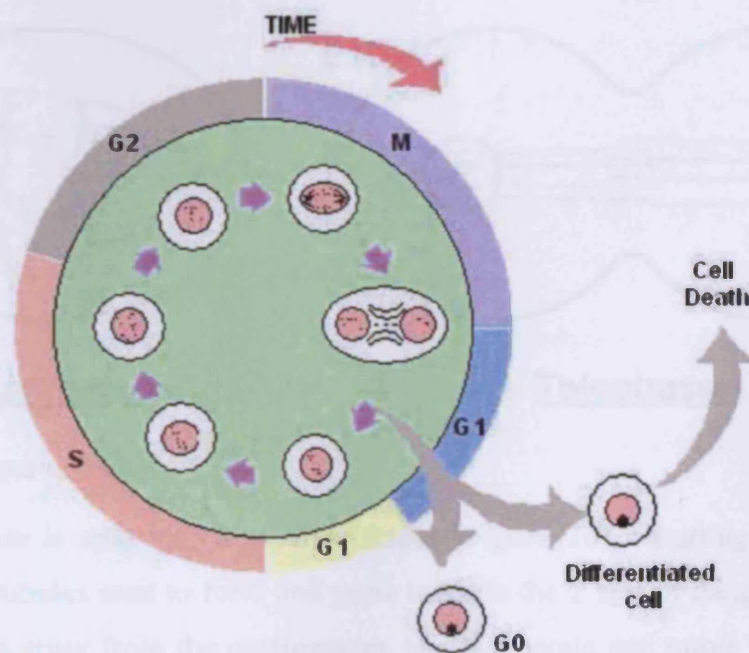


Figure 9: The Cell Cycle

The cell cycle is split into 5 different phases (*Figure 9*). The first is the G₁ phase or intermitotic phase where the cell checks the DNA integrity, cell size, presence of nutrients and growth factors in preparation of cell division. This is followed by the S phase or DNA synthesis phase where replication of the DNA and chromosomes takes place. The cell cycle

Chapter 2 - Development of agents that target Tubulin

then enters the G₂ phase or premitotic phase, where the integrity of new DNA is checked. These 3 stages are known as the interphase and it accounts for 95% of the time taken for a cell to divide. The M phase or mitosis stage is then entered, during which the chromosomes are segregated and the cell divides, this stage takes about an hour to complete. The fifth phase of the cell cycle can take place during the G₁ phase if there is a lack of sufficient stimuli, it can become indefinite and the cells will become quiescent, this is known as the G₀ phase.^{60,61}

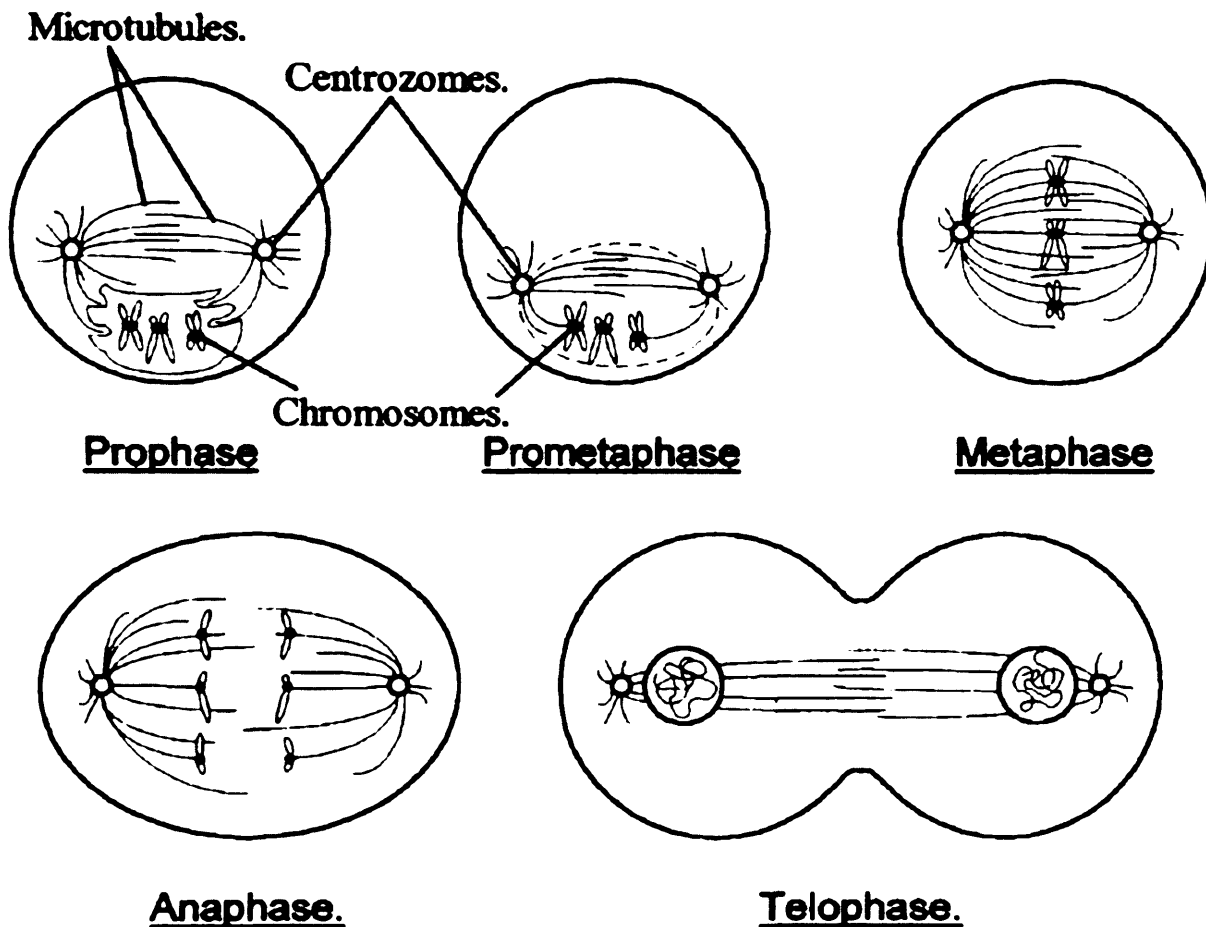


Figure 10: Stages of Mitosis⁶²

The Mitosis phase is split into 5 separate stages (Figure 10). Starting with the prophase where the microtubules start to form and grow towards the 2 sets of daughter chromosomes. The microtubules grow from the centrosomes which separate and move to opposite ends of the cell. The next phase is the prometaphase where the nuclear envelope around the nucleus disintegrates and the microtubules attach themselves to the centre point of the chromosomes known as the Kinetichore. The chromosomes gradually arrange themselves between the 2 centrosomes. This is the metaphase. Once the chromosomes are accurately arranged between the 2 centrosomes, the cell abruptly enters the anaphase. The daughter chromosomes begin to separate and move towards the centrosomes as the microtubules

Chapter 2 - Development of agents that target Tubulin

attached to them begin to decay. Once the chromosomes reach other ends of the molecule a new nuclear envelope is formed around them. This is the end of mitosis with only the surrounding cytoplasm needing to divide in the process known as cytokinesis, giving 2 identical daughter cells.⁶²

2.3 The formation of Microtubules from Tubulin⁶²

As mentioned above microtubules play a vital role in cell division and disruption of the microtubules can have a major effect on the cell. Tubulin exists as 2 spherical proteins, the α and β tubulin subunits, each with a molecular weight ~ 50 kDa. The 2 subunits come together to make a heterodimer. In the presence of GTP and 37°C , the heterodimers combine in a head to tail fashion to form protofilaments comprised of a long line of alternating α and β -tubulin subunits. After several minutes of protofilament formation, 12 to 13 protofilaments will come together and form a protein sheet, that curls up into a C-shape to give the pipe-like structure of the microtubule. The external diameter of the microtubule is ~ 24 nm and the internal diameter is ~ 15 nm across (*Figure 11*). There are several types of proteins that are associated with the microtubules and their formation, which are known as microtubule associated proteins (MAPs). The presence of the MAPs increases the speed of microtubule formation and protect it from depolymerization agents like low temperature and Ca^{2+} ions. There are also the microtubules organising centres (MTOCs) or centrosomes, where the microtubule starts its growth. The 3rd type of protein involved in formation of microtubules is γ -tubulin, which may bind to the centrosome, from there it may act as a site for nucleation for incoming α,β -tubulin heterodimers.

Once formed the microtubule maintains its shape by remaining in a dynamic equilibrium where heterodimers constantly add to one end of the microtubule (+ end) and leave from the other end of the microtubule (- end). This is a finely balanced equilibrium which controls the length and function of the microtubule. Disruption of this equilibrium will affect a number of cell functions including cellular division.

Chapter 2 - Development of agents that target Tubulin

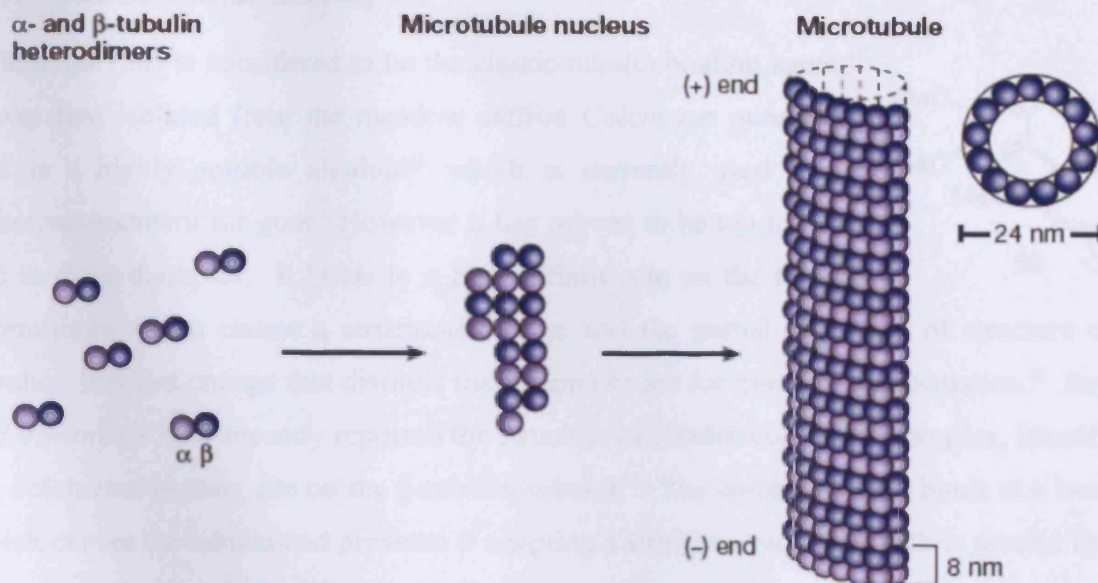


Figure 11: Polymerization of Tubulin to Microtubules⁶³

2.4 The Role and Dynamic Instability of Microtubules

As well as being vital to mitosis, microtubules are also key components of the cytoskeleton and are critical in the development of and maintenance of cell shape. They are also involved with the transport of vesicles, mitochondria and other components throughout the cells, needed for cell signaling and mitosis.⁶³ One of the most remarkable features of microtubules is its dynamic instability, with it constantly growing or shrinking depending on the needs of the cell. The half life of a microtubule can range from several hours in the interphase when the cell is preparing itself for replication of the DNA, to tens of seconds in the metaphase where the new strands of DNA are separated and moved to other ends of the cell so it can divide.⁶¹ If the equilibrium of formation of microtubules from tubulin can be disrupted by using an agent that will inhibit the decay of microtubules or the binding of tubulin to form microtubules, then the separation of chromosomes can be prevented and cell division stopped. This makes microtubules and tubulin an interesting biological target.

2.5 Classes of Mitotic Spindle Poison⁶¹⁻⁶³

Tubulin is the target of several drugs used clinically in the treatment of cancer. These drugs can be classified by which site they bind to on Tubulin. These sites include the Colchicine binding site, the Vinca Alkaloid binding site, the Rhizoxin/Maytansine binding site and the Taxane binding site. However some agents do not bind to any of these sites and instead have an affinity for reactive groups on tubulin like cysteine sulfhydryl groups.

Chapter 2 - Development of agents that target Tubulin

2.5.1 The Colchicine Binding Site

Colchicine (50) is considered to be the classic tubulin binding agent.⁶⁴

It was first isolated from the meadow saffron *Colchicum autumnale* and is a highly soluble alkaloid⁶⁵ which is currently used as an effective treatment for gout. However it has proven to be too toxic to use in other therapies. It binds to a high affinity site on the tubulin

heterodimer which causes a structural change and the partial unfolding of structure of β -tubulin. It is this change that disrupts the region needed for microtubule formation.⁶⁶ Ravelli and coworkers have recently reported the structure of tubulin-colchicine complex, identifying the colchicine binding site on the β -tubulin subunit.⁶⁷ The colchicine (**50**) binds at a location which curves the tubulin and prevents it adopting a straight structure, which is needed for the formation of microtubules (*Figure 12*).⁶⁷

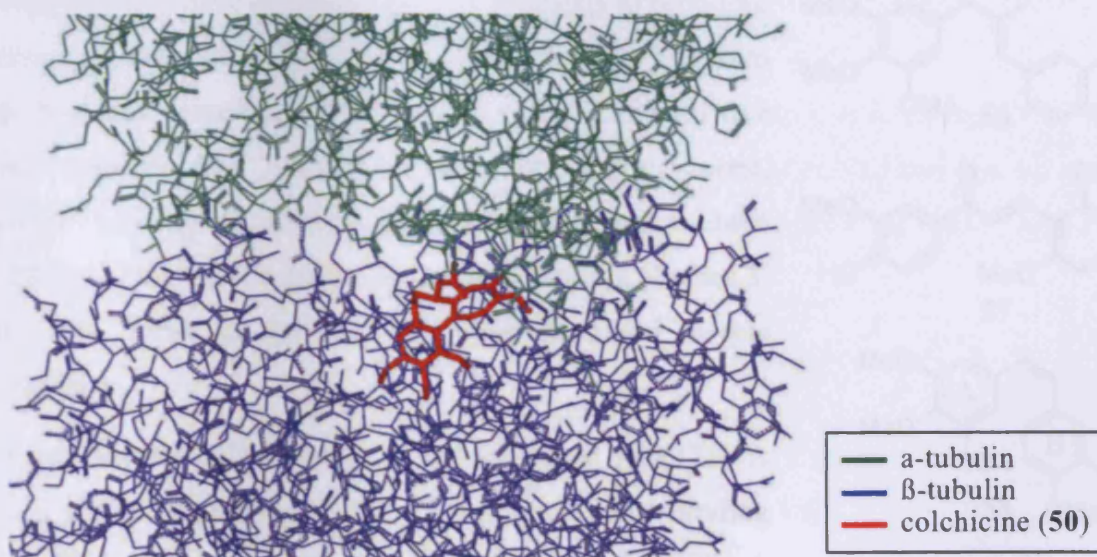
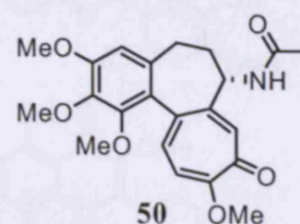
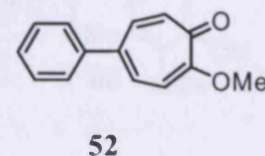
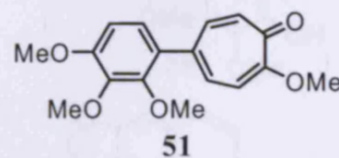


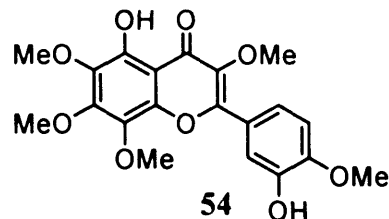
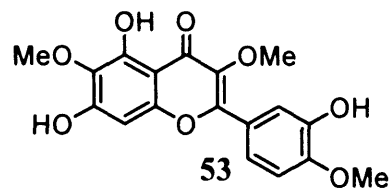
Figure 12: X-ray crystal structure of colchicine binding site to the β -tubulin subunit.⁶⁷

Structural related analogues of Colchicine (**50**) that retain their binding affinity to the colchicine site of tubulin include 2-methoxy-5-(2',3',4'-trimethoxyphenyl)tropone (**51**), which prevented microtubule assembly at concentrations comparable to Colchicine (**50**) itself. However the analogue that has no methoxy groups on the B ring (**52**) is almost inactive.⁶⁸

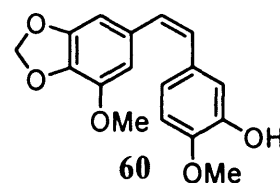
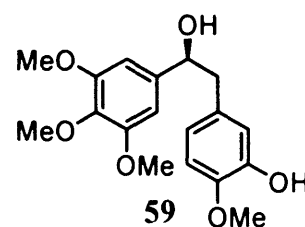
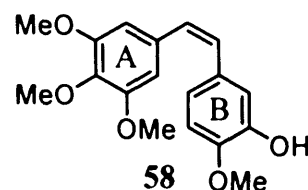
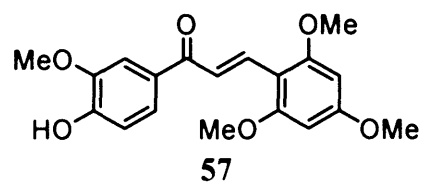
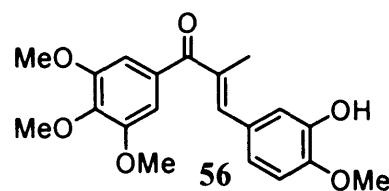
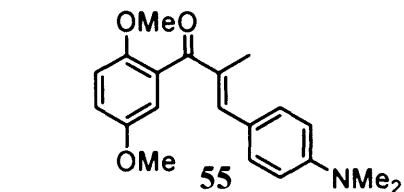


Chapter 2 - Development of agents that target Tubulin

Centauriedin (53) a natural flavone isolated from *Polymnia fruticosa*, prevents the binding of Colchicine (**50**) to tubulin and the formation of microtubules.⁶⁹ Since the discovery that centauriedin (**53**) causes mitotic arrest by destabilizing the mitotic spindle, other flavones have shown tubulin inhibiting properties such as the flavone (**54**) isolated from both *Zieridium pseudobtusifolium* and *Polansia dodecandra*.^{70,71}



Chalcones. A series of chalcones, typified by **55** were synthesized by Edwards and co-workers and were shown to be potent cytotoxic agents.⁷² Lawrence and co-workers revealed **56** as a potent anticancer agent⁷³ with many other chalcones displaying high levels of cytotoxicity. Lawrence and co-workers have also used parallel synthesis to produce a library of over 600 chalcones, yielding 16 positive hits from their bioassay screening of the library, with the most active being chalcone **57**.⁷⁴ Such agents induce mitotic arrest, strongly binding to tubulin and preventing microtubule assembly. This will be discussed in more detail in chapter 3, since this forms the basis of the work described in this dissertation.

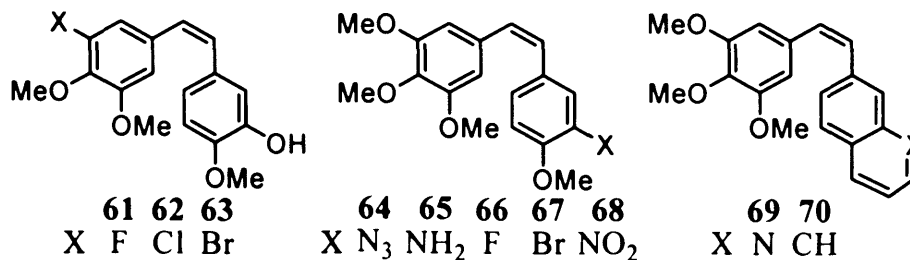


Combretastatins. The stilbene Combretastatin A-4 (**58**) is one of the most potent inhibitors of the colchicine binding site.⁷⁵ It was first isolated from the South African Willow, *Combretum caffrum*, along with the less active Combretastatin **59** and Combretastatin A-2 (**60**).⁷⁶ These compounds are some of the simplest structures that show antimetabolic effects by interaction with the colchicine binding site of tubulin. Combretastatin A-4 (**58**) has also generated lots of interest due to its effects on the inhibition of angiogenesis, the growth and development of blood vessels⁷⁷ as well as its antimetabolic effects.⁷⁸

Due to the biological effects of the Combretastatin A-4 (**58**) there have been many structure-activity relationship (SAR) studies reported in recent years to try and maximize their biological properties. It has been generally accepted that A-ring of **58** must be kept as 3,4,5-

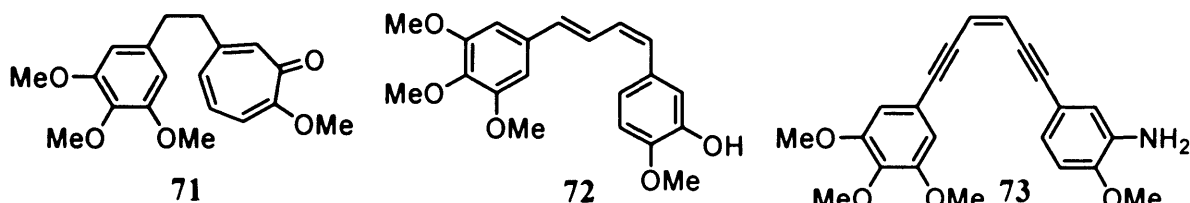
Chapter 2 - Development of agents that target Tubulin

trimethoxyphenyl for compound to maintain its potent antimitotic effects.⁷⁹ However recent work reported

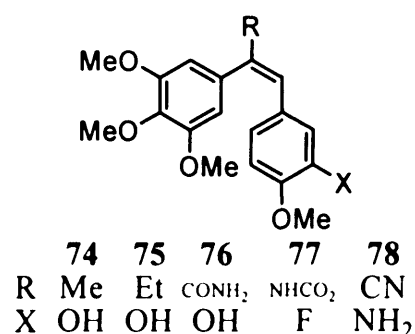


by Pettit⁸⁰ has shown that the methoxy group on the C3 position of the A-ring can be replaced with a fluorine group in **61**, chlorine group in **62** or bromine group in **63** and still retain the antimitotic effects of **58**. The hydroxy group on C3 position of the B-ring of **58** can be replaced by several different groups including azido group in **64**,⁸¹ amine groups in **65**,^{79,81,82} fluorine groups in **66**,⁸³ and still maintain high levels of cytotoxicity comparable to **58**. The hydroxy group on C3 position can also be replaced by bromine groups in **67**,⁸⁴ and nitro groups in **68**⁸⁴ or the whole B ring can be replaced with quinoline groups in **69**⁸⁵ or a naphthalene group in **70**^{86,87} and still retain good high levels of cytotoxicity, but not as high as **58**.

The SAR studies have also involved modification of the group that links the A and B rings. The *cis*-ethene linker has been replaced with an ethane linker in **71**,⁸⁸ and had an additional double bond in **72**⁸⁹ or triple bond in **73**⁹⁰ unit introduced to the linker as well. These changes to the linkers have all resulted in a general loss of cytotoxicity.

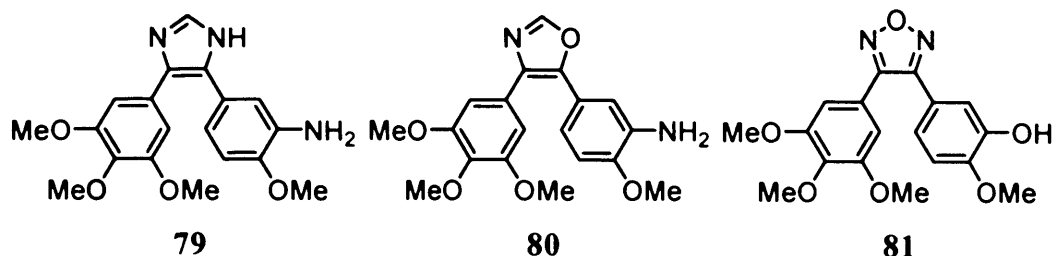


The introduction of various alkyl groups in **74** – **75**,⁹¹ acylamide groups in **76** – **77**⁹² and other bulky groups^{79,91,92} attached to the ethene linker also gives CA4 analogues with reduced cytotoxicity. The nitrile group in **78**⁷⁹ appears to be largest group tolerated on the ethene cross linker with out causing a large loss cytotoxicity.



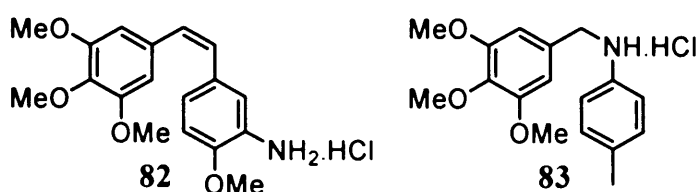
Various *cis*-locked analogues of **58** have also been investigated, replacing the *cis*-ethene linker with either an 1,2,-disubstituted five membered heterocycle such as imidazole **79** or oxazole **80** gave several compounds which inhibited tubulin polymerization more potently than **58** while still being less cytotoxic.⁹³ The replacement of the *cis*-ethene linker with a furazan ring gave a compound **81**⁹⁴ that was 4 times more cytotoxic than Combretastatin A-4 (**58**) and inhibited tubulin polymerization.

Chapter 2 - Development of agents that target Tubulin

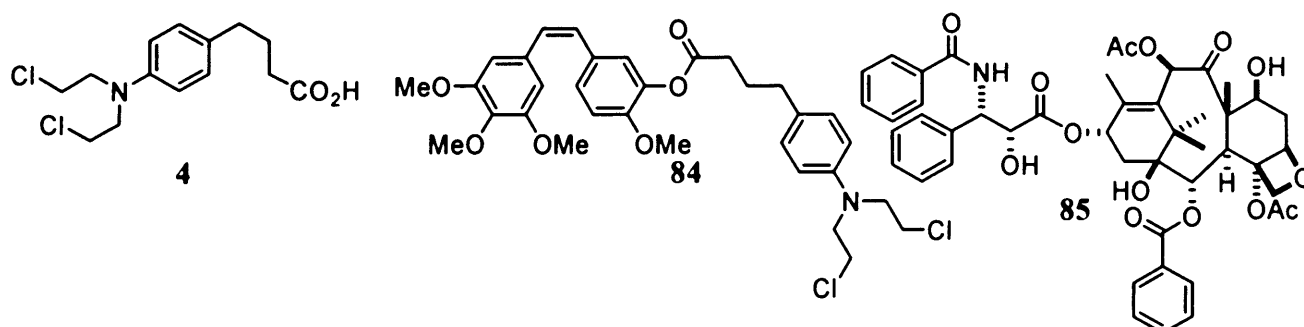


Clinical trials of Combretastatin A-4 (**58**) have been hindered by its low water solubility, prompting research into Combretastatin analogues with enhanced solubility. Ohsumi and co-workers synthesized a more water soluble analogue of Combretastatin A-4 (**58**) in which the phenol group of the B ring was replaced with an amino group, (AC-7739, **82**).⁷⁹ This analogue proved to have a more potent antitubulin activity than its parent molecule.

Cushman and co-workers made a series of benzylamines hydrochlorides typified by **83**, these compounds displayed good water solubility and bind to tubulin in a similar manner to Combretastatin A-4 (**58**).⁷⁵



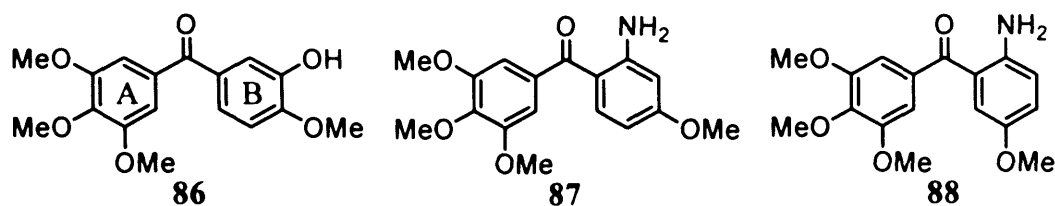
Other recent areas of Combretastatin research is the development of a chimeric compound **84** bearing the Combretastatin and nitrogen mustard core to see if this combination could overcome multidrug resistance (MDR) protein induced by various alkylating agents. The chimeric compound **84** which is a cross between Chlorambucil (**4**) and Combretastatin A-4 (**58**) is reported to inhibit tubulin polymerization concentrations as low as would be expected for **58**. At higher concentrations the nitrogen mustard alkylates tubulin and inhibit tubulin depolymerization much like Paclitaxel (**85**).⁹⁵



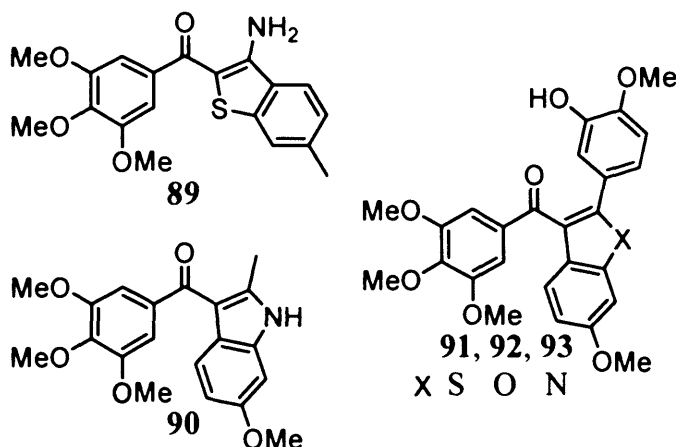
Benzophenones. The benzophenone analogue of Combretastatin A-4 (**58**), Phenstatin (**86**) was isolated as a side product from the Jacobsen oxidation of **58**.⁹⁶ Phenstatin (**86**) proved to be a potent inhibitor of tubulin polymerization, comparable to Combretastatin A-4 (**58**).⁹⁶ It is also highly cytotoxicity, but not as cytotoxic as **58**.⁹⁷ Several SAR studies⁹⁶⁻⁹⁹ have been carried out on the benzophenone scaffold, trying to improve the biological activity of the Combretastatin A-4 like benzophenone. Many of the compounds made have inhibition of

Chapter 2 - Development of agents that target Tubulin

tubulin polymerization activity comparable to **58**, many of them weren't as cytotoxic as **58**. However the 2-aminobenzophenones **87** and **88** proved to be as cytotoxic as Combretastatin A-4 (**58**).⁹⁷



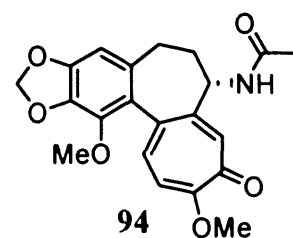
Further investigation showed that the 2-amino-4-methoxyphenyl ring of benzophenone analogue **87**, can be replaced 3-amino-6-methylbenzo[b]thiophene to give **89** which proved to be more active than **58** as an inhibitor of tubulin polymerization, however it wasn't as cytotoxic.¹⁰⁰ The B ring of phenstatin (**86**) has also been replaced by various heterocycles like 3-arylindoles **90**,¹⁰¹ benzo[b]thiophenes **91**,¹⁰²⁻¹⁰⁴ benzo[b]furans **92**¹⁰⁴ or indoles **93**,¹⁰⁴ many of which proved to be more potent inhibitors of tubulin polymerization than Combretastatin A-4 (**58**), however none of



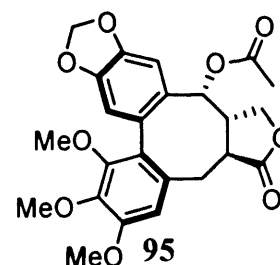
them are as cytotoxic as **58**. The replacement of the carbonyl linker group between the 2 aryl rings with a methylene group led to a complete lose of biological activity.^{99,104} The presence of 3,4,5-trimethoxyphenyl ring has also proved to be vital for biological activity.¹⁰⁰

Cornigerine (94) a natural product isolated from *Colchicum*

cornigerum, resembles a hybrid of Colchicine (**50**), podophyllotoxin (**34**) and steganacin (**95**). As would be expected from the similarity of its structure, **62** displays a similar effect binding to the colchicine site with high affinity.¹⁰⁵



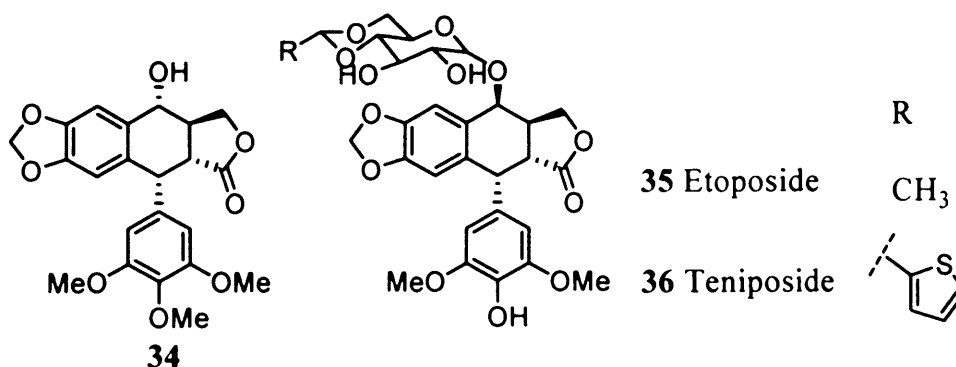
Steganacin⁷⁶ (**95**) was isolated in 1973^{106,107} from the stems and stem bark of the east African tree *steganataenia araliacea* Hochest, and was found to have *in vivo* antitumour activity against P388 leukaemia in mice and *in vitro* against human nasopharynx carcinoma cell line KB.¹⁰⁶ Steganacin (**95**) completely inhibits cleavage in fertilized sea urchin eggs at concentration of 0.3 μ M by preventing the formation of mitotic apparatus.¹⁰⁸ Steganacin (**95**) shares structurally features with both Colchicine (**50**) and



Chapter 2 - Development of agents that target Tubulin

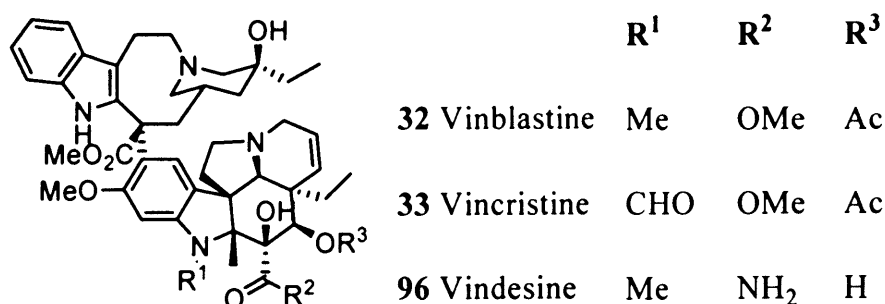
Podophyllotoxin (34), with all 3 compounds sharing a trimethoxybenzene ring. Like Podophyllotoxin¹⁰⁹ (34), Steganacin (95) binds to tubulin with an affinity similar to Colchicine (50), and is, in fact, a competitive inhibitor of colchicine binding to tubulin.^{108,110-113} Steganacin (95) has also been shown to inhibit *in vitro* polymerization of microtubules,^{108,110,111} with an IC₅₀ value of 1.5 – 3.5 μM,^{110,111} which makes it slightly less active than both Colchicine [50, IC₅₀(polymerization of microtubules) 2.5 μM]¹¹¹ and Podophyllotoxin [34, IC₅₀(polymerization of microtubules) 0.6 – 1.7 μM]^{110,111}

Podophyllotoxin (34), Etoposide (35) and Teniposide (36). Podophyllotoxin (34) is isolated from the dried roots of the American mandrake *Podophyllum peltatum*. It has been used as a medical treatment for hundreds of years for conditions ranging from sclerosis of the liver through to constipation, rheumatism and cancer. Podophyllotoxin (34) has been shown to bind reversibly to at least part of the colchicine site of tubulin and binds as a competitive inhibitor.¹⁰⁹ However Podophyllotoxin (34) proved to be too toxic for clinical use in the treatment of cancer, so two less toxic semisynthetic analogues have been developed Etoposide (35) and Teniposide (36).¹¹⁴ Although both compounds bind to tubulin, it is thought now that they function predominantly by inhibition of DNA topoisomerases II, an enzyme involved in the unfolding of DNA in cell replication, rather than a microtubule interaction.¹¹⁵



2.5.2 Vinca Alkaloid Binding Site.

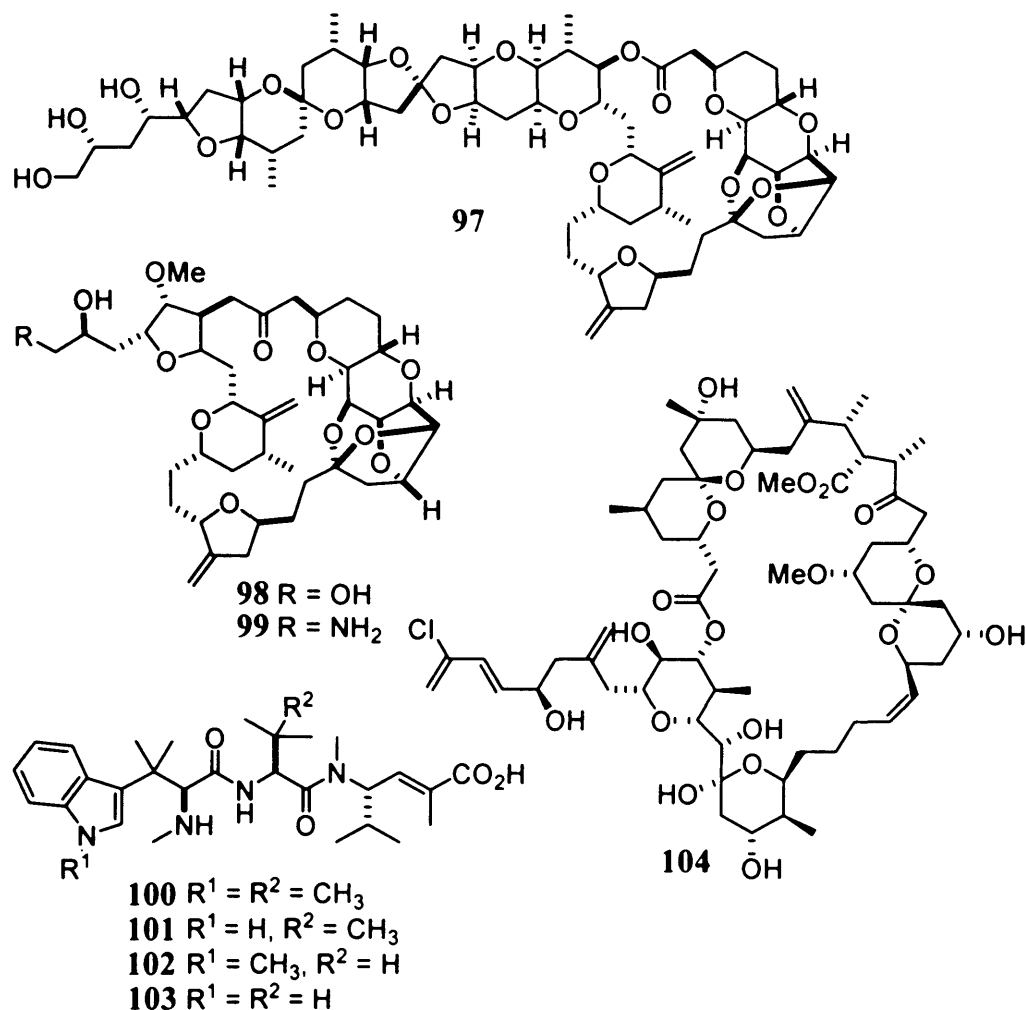
The two vinca alkaloids Vinblastine (32) and Vincristine (33) were isolated from the *Catharanthus roseus* periwinkle. They are both used as clinical anticancer drugs for the treatment of leukaemias, lymphomas and some solid tumours.¹¹⁶ Both these agents bind to a site on the β-tubulin subunit of the heterodimer¹¹⁷



Chapter 2 - Development of agents that target Tubulin

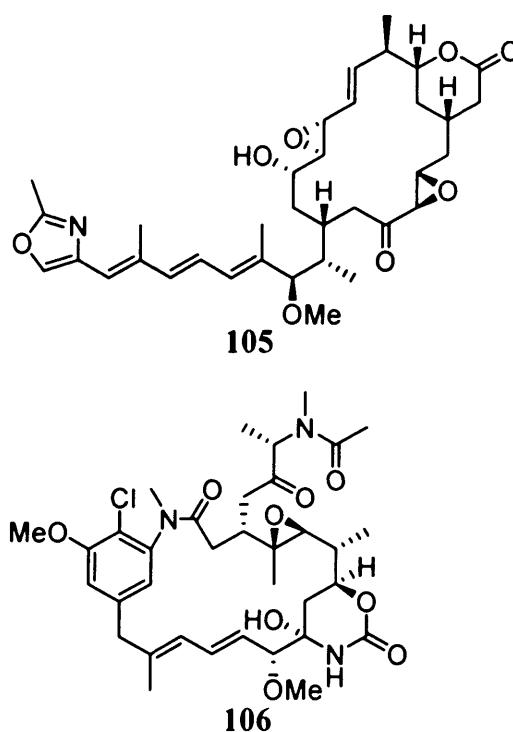
with a high affinity for the protein¹¹⁸ and induce destabilization of polymerized tubulin. The destabilizing effect results from the stoichiometric endwise poisoning of the tubulin heterodimer, presumably preventing polymerization from occurring by blocking the region involved in heterodimer attachment. Due to the dynamic nature of microtubules constantly polymerizing and depolymerizing, a vinca alkaloid poisoned heterodimer can be easily introduced to the microtubule, thus preventing further growth. The incorporation of the vinca alkaloid onto the heterodimer is rapidly reversible and occurs at 2 sites per tubulin dimer. As well as the two natural products **32** and **33**, a third effective alkaloid has also been produced by functional group transformation, Vindesine (**96**) and works by the same mechanism. Other Vinca Alkaloid binding site agents include halichondrin B (**97**), the hemiasterlins¹¹⁹ and the spongistatins,¹²⁰ all isolated from various marine sponges. Halichondrin B (**97**) has been isolated from several sponges *Halichondria okadai*,¹²¹ *Axinella sp.*,¹²² *Phankellia carteri*¹²³ and *Lissodendoryx sp.*,¹²⁴ and have potent *in vitro* and *in vivo* activities against melanomas and leukaemias.¹²⁵ Halichondrin B (**97**) is extremely rare in the marine sponges, with only 12.5 mg of **97** being isolated from 600 kg of *Halichondria okadai*,¹²¹ and would have limited the study of **97** as a viable tubulin targeting agent. Fortunately the complete synthesis of **97** was reported by Aicher and coworkers¹²⁶ and further research into the structure has led to the structurally simpler analogs, ER-076349 (**98**) and ER-086526 (**99**) which still retain the biological activity of **97**.¹²⁵ The hemiasterlins are a family of cytotoxic and antimitotic tripeptides that have all been isolated from various marine sponges, such as *Avletta sp* [hemiasterlin (**100**), hemiasterlin A (**101**) and hemiasterlin C (**102**)]¹²⁷ and *Cymbastela sp* [hemiasterlin A (**101**) and hemiasterlin B (**103**)].¹²⁸ Despite their relatively simple structures the hemiasterlins A and B (**101** and **103**) are more potent in both cytotoxic and antimitotic activity than other microtubule agents such as Vinblastine (**32**) and Paclitaxel (**85**).¹¹⁹ Spongistatins 1 – 9¹²⁹ are a group of macrocyclic lactones compounds¹³⁰ which have been isolated from various marine sponges, Spongistatin 1 (**104**) was isolated from the Republic of Maldives *Spongia sp.*¹³¹ The Spongistatins have all proven to be highly cytotoxic, with Spongistatins 1 (**104**) having an IC₅₀ value of 0.03 nM for the inhibition of L1210 murine leukaemia cells.¹²⁹

Chapter 2 - Development of agents that target Tubulin



2.5.3 Rhizoxin/Maytansine (RZX/MAY) Binding Site.

Rhizoxin (**105**) was isolated from the fungus *Rhizopus chinensis* and is an antifungal agent which also acts as an antimetabolic agent. Like many of the agents that bind to the RZX/MAY binding site, **105** was originally thought to be binding to the Vinca alkaloid binding site. However Rhizoxin (**105**) has been shown to have its own binding site that slightly overlaps with the Vinca alkaloid binding site.¹³² Rhizoxin (**105**) is effective against hundreds of cancer cell lines and some vinca alkaloid resistance tumours, it also competitively inhibits Maytansine (**106**) binding and exhibits similar effects.⁶² Maytansine (**106**) is isolated from various plants in the *Maytenus* family. It is the most potent of a family of highly cytotoxic macrolides.¹³³ Maytansine (**106**) binds to tubulin and in a reversible manner and can competitively inhibit the vinca alkaloids **32** and **33**.¹³⁴ Maytansine

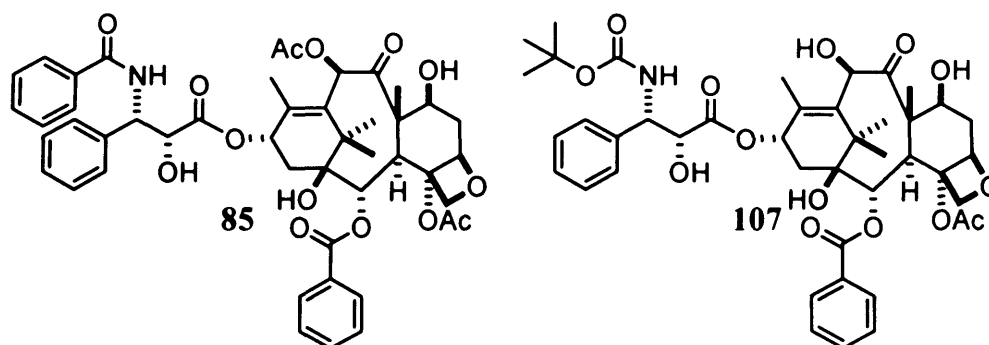


Chapter 2 - Development of agents that target Tubulin

(106) binding to tubulin causes disassembly of the microtubules and prevents tubulin spiralization.¹³⁵ Other RZX/MAY binding site agents include Cryptophycin 1,¹³⁶ the Dolastatins¹³⁷ and Phomopsin A.¹³⁸

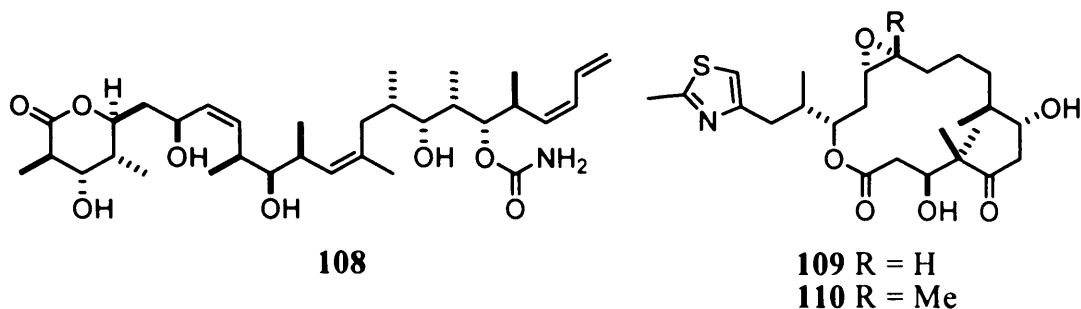
2.5.4 Taxane Binding Site.

Paclitaxel (Taxol™) (85) first isolated by Monroe Wall and Mansukh Wani in 1967 from the bark of a yew tree.¹³⁹ It was largely ignored as antimetabolic agent, due to low availability and poor water solubility, until 1979 when the discovery that it stimulated microtubule polymerization, unlike the Vinca alkaloids which induce destabilization of polymerized tubulin.¹⁴⁰ Four groups have succeeded in the total synthesis¹⁴¹⁻¹⁴⁵ of Paclitaxel (85), and it is now used to treat breast and ovarian cancer as well as non-small-cell lung cancer and Kaposi's sarcoma.⁶³ The binding site of Paclitaxel (85) is on the β -subunit of tubulin and is on the inside of the surface of the microtubule,¹⁴⁶ and this binding stabilizes the microtubule and increases its polymerization. Taxotere (107) is a late stage intermediate in a synthesis of Paclitaxel (85) from a precursor readily obtained from needles from a yew tree. It displays a similar spectrum of activity as 85 but with a four fold increase in potency and improved water solubility.¹⁴⁷ It is currently used in clinical treatments of prostate, brain and lung tumours.⁶³



Discodermolide (108) is a potent immunosuppressive lactone isolated from Caribbean Sea sponge *Discodermia dissolute*. It also inhibits the dynamic nature of microtubules by binding to the microtubule and making it abnormally stable. Studies show that 108 shares the same binding site as Paclitaxel (85), has a greater affinity for this site.^{148,149} Discodermolide (108) is currently in phase I clinical trials.⁶³ The Epothilones (e.g. 109 and 110) a family of 16 membered macrolides isolated from the myxobacterium *Sorangium cellulosum*. Like Discodermolide (108) they are competitive inhibitors of the binding of Paclitaxel (85) to tubulin, exhibiting activity similar to 85 at the same concentrations.¹⁵⁰ It is thought that Paclitaxel (85) and the Epothilones may share a common pharmacophore and adopt similar conformations *in vivo*.¹⁵¹ The Epothilones are currently used clinically in the treatment of Paclitaxel-resistant tumours.⁶³

Chapter 2 - Development of agents that target Tubulin

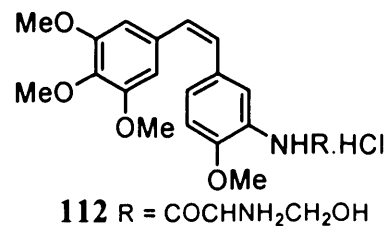
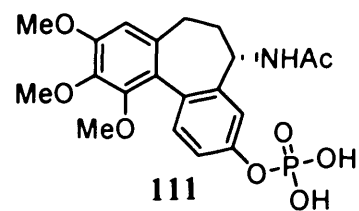
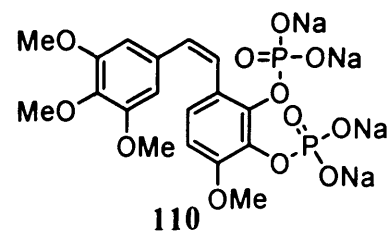
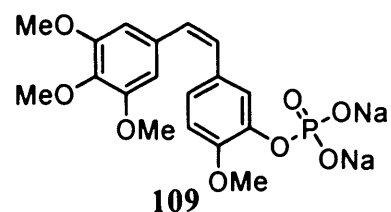


2.6 Antivascular effect^{152,153}

The tumour vasculature network is an important cancer therapy target. By disrupting the tumour vasculatures, the vital supply of oxygen and nutrients in blood that feeds the tumour will be cut off causing most tumours to starve and die. Antivascular agents inhibit tumour vascular function in 2 different ways; some inhibit the formation of new blood vessels, angiogenesis,¹⁵⁴ to tumours, while other compounds, especially tubulin targeting agents, have been shown to rapidly shut down existing tumour vasculatures.¹⁵² Several Colchicine binding site agents are currently in clinical trials, including Combretastatin A-4 3-*O*-phosphate (CA-4-P) (**111**), Combretastatin A-1-phosphate (CA-1-P) (**112**), ZD6126 (**113**) and AVE8062A (**114**).¹⁵² Certain dolastatins like TZT-1027 are also in clinical trials for their antivascular effect, but target the vinca alkaloid binding site instead.^{63,155}

Combretastatin A-4 3-*O*-phosphate (CA-4-P) (**111**) has been shown to cause microtubules in endothelial cells to undergo rapidly depolymerization in a matter of minutes, which causes the cells to become round and the vasculature to become leaky, also known as blebbing. This eventually leads to secondary tumour cell death.¹⁵⁶ *In vivo* testing on P22 tumour in rats with CA-4-P (**111**) showed that the blood flow rate to the interior of the P22 tumour dropped to <5 % of the starting value within a minute after the administration of **111**, and this value fell to almost zero after an hour.¹⁵⁷

Also importantly further *in vivo* studies on P22 tumour in rats with **111** showed that its antivascular effects are selective against tumour vasculature without causing significant harm to normal tissue.¹⁵⁸ The reason for the selective nature is not known, however it has been suggested that it may be due to the difference between mature normal vasculature and immature tumour vasculature. Normal vasculature can sustain more endothelial cell injury without the



Chapter 2 - Development of agents that target Tubulin

vascular collapse than the less developed cytoskeleton of the tumour vasculature.¹⁵⁷ The selectivity could also be due to the difference in blood flow rate of tumours and normal tissue, with tumour blood flow rate being far more slow than that of normal tissue, making the tumour more susceptible to CA-4-P (111).¹⁵⁷ Also differences in tumour and normal tissue endothelial cells, like proliferation rate,⁷⁸ post-translational modifications of tubulin and the interaction of actin cytoskeleton and tubulin might also contribute to the specificity of vascular-targeting agents like CA-4-P (110).¹⁵⁷

Many of the classic mitotic spindle poisons, such as Colchicine (50), Vincristine (33) and Vinblastine (34) induce similar deleterious effects on endothelial cells in culture and damage tumour vasculature in animals at high concentrations. However it has been shown that these new vascular targeting agents are effective at much lower concentrations, for instance 10 % of its maximum tolerated dose of CA-4-P (111) has been shown to shut down tumour blood flow rate after 24 hours.¹⁵² However at even lower concentrations 1 – 3 % of the maximum tolerated dose, similar reductions in tumour blood flow did occur but after 24 hours the blood flow rate had recovered, showing that the antivascular effects of the CA-4-P (111) are reversible at to low a concentration. The desired affects of microtubule targeting agents as either antiproliferative agents or as a vascular targeting agent may depend on its pharmacodynamics and pharmacokinetic characteristics, like the reversibility of the tubulin binding and its retention time in cells. Agents that can rapidly bind to tubulin/microtubules and cause microtubules to depolymerize and are rapidly metabolized by the body may act best as antivascular agents. While those agents are retained in cells and induce long term mitotic block might work best as antiproliferative agents causing apoptosis.⁶³

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

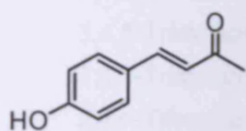
3.1 History and development of the chalcone project.¹⁵⁹

In 1994 our group launched a plant screening program¹⁶⁰⁻¹⁶² to investigate the cell growth inhibitory properties of plant extracts from plants used in traditional Chinese medicine. The dried whole plant of *Scutellaria barbata* D. Don (*Labiatae*) (*Figure 13*) is used in traditional Chinese Medicine as an anti-inflammatory, anti-tumour agent and as a diuretic.¹⁶³ It is used clinically to treat cancers of the digestive system, lung, breast, liver.¹⁶³ Clinical studies have shown that 62% of patients suffering with primary liver cancer hepatoma were totally cured when treated with *S. barbata*.¹⁶³ The book *Anticancer Medicinal Herbs* details the use of *Scutellaria barbata* in several different anti-cancer herbal therapies, for instance the ingredients for the treatment of ovarian cancer consists of *Scutellaria barbata* (50 g), *Solani nigri* (30 g), *Solani lyrati* (30 g), *Oldenlandia diffusa* (30 g) and *Carapax trionycis* (30 g).¹⁶⁴



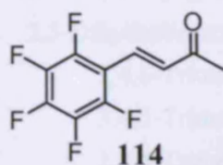
Figure 13: *Scutellaria barbata*

The herb is known to contain several alkaloids and flavones, however the active anti-tumour ingredients of *Scutellaria barbata* were unknown. Bioassay guided fractionation of the methanol extract of *Scutellaria barbata*, using the MTT assay¹⁶⁵ to determine the cytotoxicity of each fraction against the K562 human leukaemia cell line, led to the discovery of a fraction which had an IC_{50} (K562) of 7.5 $\mu\text{g/mL}$.¹⁶¹ Further chromatography of the active fraction showed that the major component of this fraction was the α,β -unsaturated ketone *E*-1-(4'-hydroxyphenyl)-but-1-en-3-one (**113**) which displayed weak cell growth inhibitory properties, IC_{50} (K562) 60 μM .



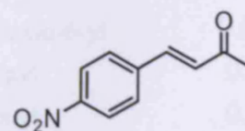
113

IC_{50} (K562) 60 μM



114

IC_{50} (K562) 1.9 μM



115

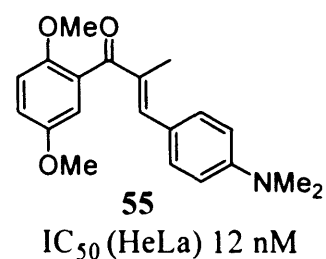
IC_{50} (K562) 2.6 μM

A library of over 30 *trans*-phenylbutenones was then prepared and screened for its cell growth inhibitory properties.¹⁶⁶ It was found that the butenones processing electron withdrawing groups were the most active. For instance the pentafluorophenylbutenone **114**

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

was over 30 times more active than **113** and the nitrophenylbutenone **115** was over 20 times more active than **113**.

Such findings prompted the investigation of replacing the methyl group of the *trans*-phenylbutenone with substituted aryl groups to make chalcones to see what effect this substitution had on its anti-cancer properties. The chalcone scaffold, 1,3-diarylprop-2-en-1-one, is commonly found throughout the plant kingdom. Chalcones have been shown to elicit various biological effects including anti-fungal,^{167,168} anti-inflammatory,^{169,170} anti-bacterial,^{169,171} anti-malarial,^{172,173} and anti-mutagenic^{174,175} properties. It was Edwards *et al.*⁷² who reported the anticancer activity of chalcones, with the chalcone MDL 27048 (**55**) being a very potent antimetabolic agent.



Various substituted chalcones **116** – **127**^{73,176,177} were then prepared to further investigate the prerequisites needed for high cytotoxicity. The chalcones were prepared through the base catalysed Claisen-Schmidt aldol condensation¹⁷⁸ of substituted benzaldehydes and substituted acetophenones. It became apparent that the aryl ring substitution was again an important factor, as the anticancer activity of the chalcone may be improved or diminished depending on the arrangements selected (see *table 1*).

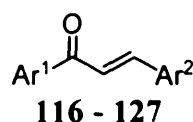


Table 1: Cell growth inhibitory properties of substituted chalcones **116** – **127**

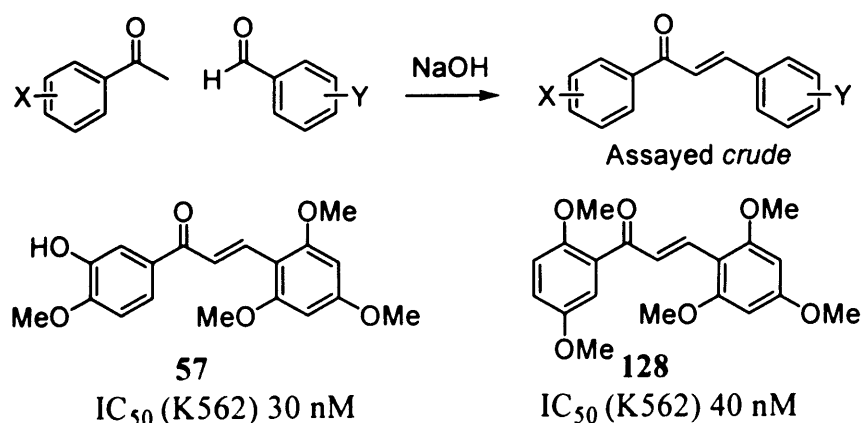
Chalcone	Ar ¹	Ar ²	IC ₅₀ (K562) μM
116	3,4,5-Trimethoxyphenyl	3,4-Dimethoxyphenyl	0.3
117	3,4,5-Trimethoxyphenyl	3-Hydroxy-4-methoxyphenyl	0.0043
118	3,4,5-Trimethoxyphenyl	4-Methoxyphenyl	0.3
119	3,4,5-Trimethoxyphenyl	Benzo[<i>d</i>][1,3]dioxol-5-yl	0.3
120	3,4,5-Trimethoxyphenyl	2,3-Dihydrobenzo[<i>b</i>][1,4]dioxin-6-yl	0.08
121	3,4,5-Trimethoxyphenyl	2,4,6-Trimethoxyphenyl	0.2
122	2,4,6-Trimethoxyphenyl	3,4,5-Trimethoxyphenyl	0.3
123	3,4-Dimethoxyphenyl	2,4,6-Trimethoxyphenyl	0.3
124	3,4-Dimethoxyphenyl	3-Hydroxy-4-methoxyphenyl	0.6
125	3-Hydroxy-4-methoxyphenyl	3,4,5-Trimethoxyphenyl	0.12
126	Thiophen-2-yl	3,4,5-Trimethoxyphenyl	1.2
127	2,5-Dimethylfuran-3-yl	3,4,5-Trimethoxyphenyl	2.8

The Combretastatin A-4 (**58**) chalcone analog **117** was the most potent cell growth inhibitor prepared, with a cytotoxicity in the nanomolar region (**117**, IC₅₀ 4.3 nM). The introduction of

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

the 3-hydroxy substitution on the B-ring greatly increases the cytotoxicity of chalcone **117**, the chalcone **118** without the 3-hydroxy substitution is nearly 70 times less active than **117**. The presence the 3,4,5-trimethoxyphenyl A-ring of chalcone **117** also appears vital to the cytotoxicity, which is shown when compared to the 3,4-dimethoxyphenyl analogue **124** which is nearly a 140 times less active than **117**.

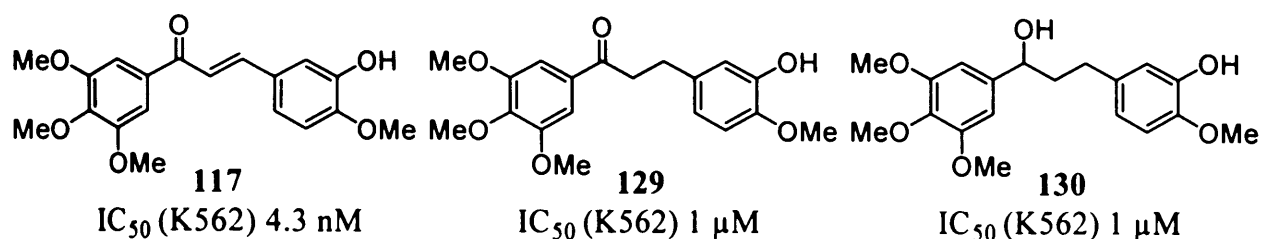
The ease of the synthesis of chalcones provided an opportunity to make a large number of analogues. The Claisen-Schmidt condensation¹⁷⁸ method is attractive because it predominantly generates the *E*-isomer, normally in high yields, from substituted benzaldehydes and acetophenones, a large number of both are commercially available and inexpensive. A 644-membered library of chalcones was prepared by parallel synthesis using the Claisen-Schmidt condensation¹⁷⁸ of 23 substituted acetophenones and 28 substituted benzaldehydes all commercial available and selected randomly in a conventional 96-well tissue culture test-plate.⁷⁴ The cytotoxicity of these chalcones was conveniently determined upon the crude products directly in the 96-well tissue culture test-plate, at a concentration of 5 μ M, by conventional MTT assay¹⁶⁵ Sixteen wells gave a positive bioassay response; a “success” rate 2.5 %. The 16 chalcones were then prepared conventionally on a 10 mmol scale, and the chalcones were indeed all active with an IC_{50} (K562) less than 5 μ M. Seven of these chalcones had an IC_{50} (K562) less than 1 μ M, with chalcones **57** and **128** being the most active. Chalcone **57** was also reported to cause cell cycle arrest at the G_2/M point and binds to the colchicine binding site of tubulin, further testing shows that the chalcone inhibits the polymerization of tubulin with an IC_{50} value of 1.5 μ M which is comparable to Combretastatin A-4 (**58**, reported IC_{50} for the inhibition of tubulin polymerization varies between 0.5¹⁷⁹ – 2¹⁸⁰ μ M).



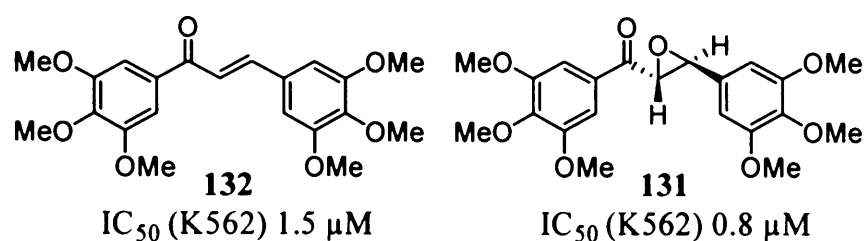
Attention was then moved onto the effects of modifying the “bridge” linking the two aryl groups. The reduction of the carbon – carbon double bond of chalcone **117** to the ketone **129**

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

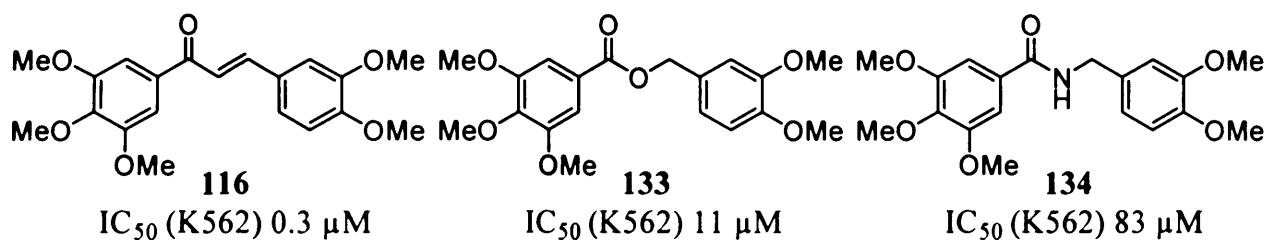
resulted in a loss of activity, as did the subsequent reduction of the carbonyl functionality of **129** to **130**.¹⁸¹



Changing the carbon-carbon double bond into an epoxide has been reported to increase the chalcones mutagenic properties by Rashid and co-workers.¹⁸² This prompted the investigation of the effects of introducing an epoxide to see what effect this would have on cytotoxicity. Interestingly the results obtained showed no significant loss of activity and the chalcone oxide **131** of chalcone **132** displays a slight increase in activity.¹⁷⁷



The importance of the α,β-unsaturated ketone was shown again in a series of ester **133** and amide **134** chalcone derivatives where the α-carbon had been replaced with either an oxygen or a nitrogen respectively. Both linker changes caused significant loss in activity when compared to the equivalent chalcone **116**.¹⁸³



It has been speculated that the importance of the α,β-unsaturated ketone to the activity of the compound is down to two possible reasons. The first is that the enone system could undergo nucleophilic attack via a Michael-type addition. Thus strong binding at the colchicine binding site may be the result of the enone system covalent binding to the tubulin. The second reason is that the role of the carbonyl and alkene group is to simply present the two aryl groups in a way that favours strong non-covalent binding to the tubulin.

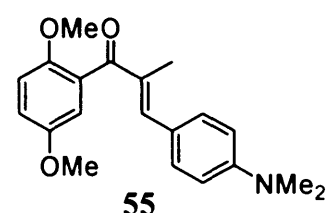
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

Edwards⁷² chalcone study showed that the presence of a methyl group on α -position to the carbonyl increased the cytotoxicity of the chalcone when compared to its α -hydrogen bearing chalcone equivalent. For instance the α -methylchalcone **55** is twice as active as chalcone **135**. Edwards gave no explanation for this.

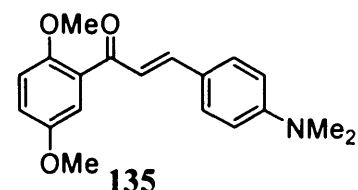
The paper⁷² also reports data on a selection of α -halogen bearing chalcones. It showed that the introduction of bromine group onto the α -position of the chalcone **136** increased its cytotoxicity, compared to the α -bearing chalcone equivalent **135**. While the introduction of the chlorine group decreased the activity of the chalcone **137**.

We then decided to investigate the introduction of various groups on the C-2 position of the chalcone to see the effects upon anticancer activity of the compounds and to try and account for the effects these groups have on the activity of the chalcone. It was suspected that the biological effects may be caused by the conformation that the chalcone adopts.

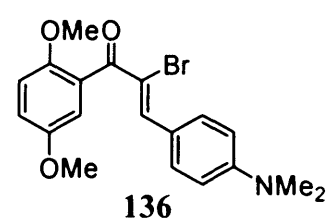
The α -methylchalcone of the CA-4 like chalcone **117** was quickly prepared. The *E*-isomer of the CA-4 like α -methyl chalcone **56** was isolated and had an exceptionally impressive IC_{50} value of 0.21 nM, a 20 fold increase in activity over chalcone **117**. While a (*Z*)-enriched mixture of **138** (*E*:*Z*, 1:5 determined by ¹H NMR) gave an IC_{50} value of 60 nM.⁷³



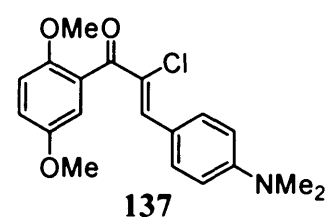
IC_{50} (HeLa) 12 nM



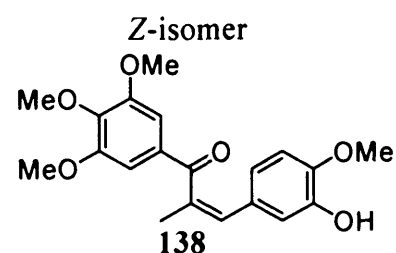
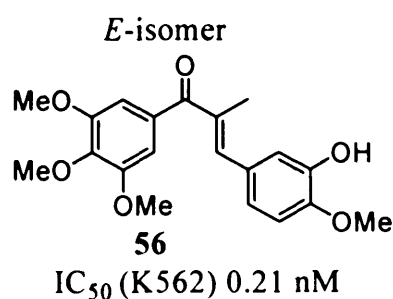
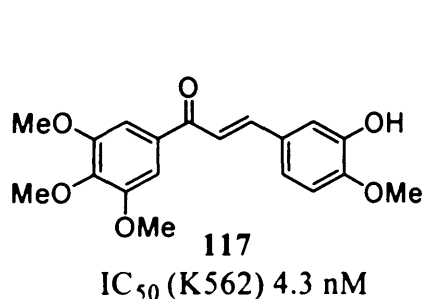
IC_{50} (HeLa) 24 nM



IC_{50} (HeLa) 19 nM



IC_{50} (HeLa) 43 nM



Single crystals of chalcones **117** and **56** were obtained by careful diffusion crystallization and their structures determined by X-ray crystal structure analysis (*Figure 14* and *15*). The crystal structure of **56** confirmed the (*E*)-configuration of the carbon-carbon double bond.

The X-ray crystal structure of **117** and **56** revealed an interesting relationship between the conformation of the chalcone and its cytotoxicity. The structure of chalcone **117** shows that the carbon-oxygen double bond and the carbon-carbon double bond are positioned in a *cis*

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

conformation relative to each other around the C1-C2 single bond (illustrated in the *s-cis* conformer *Figure 14*). While the more active α -methyl chalcone **56** structure shows that its carbon-oxygen double bond and the carbon-carbon double bond are positioned in a *trans* conformation relative to each other around its C1-C2 single bond (illustrated in the *s-trans* conformer *Figure 15*).

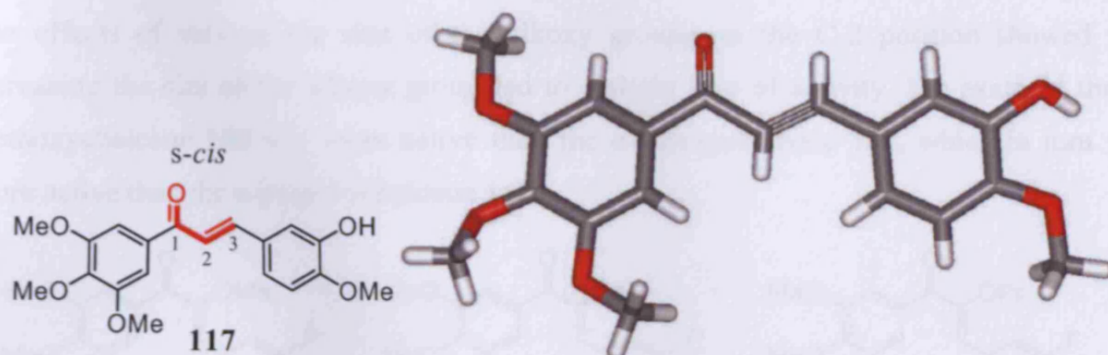


Figure 14: X-ray crystal structure of chalcone 117

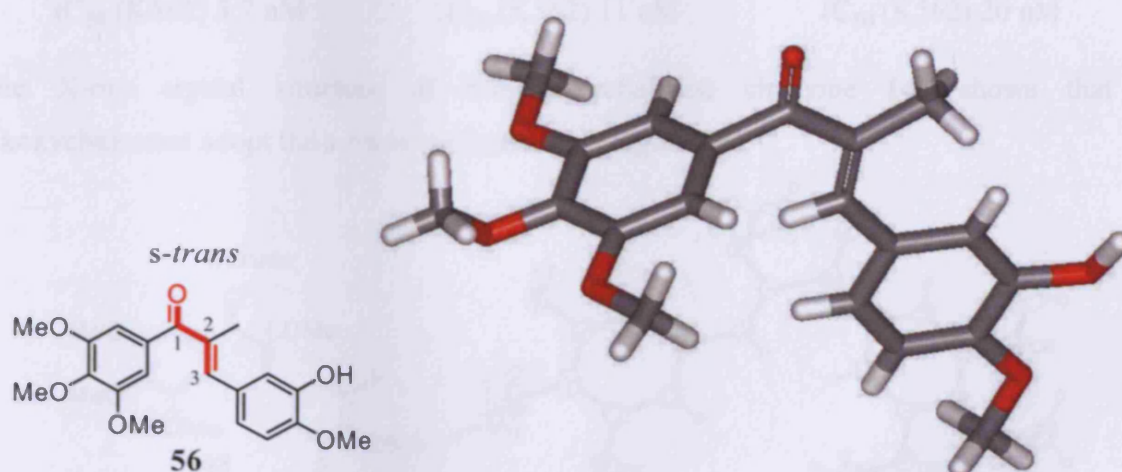
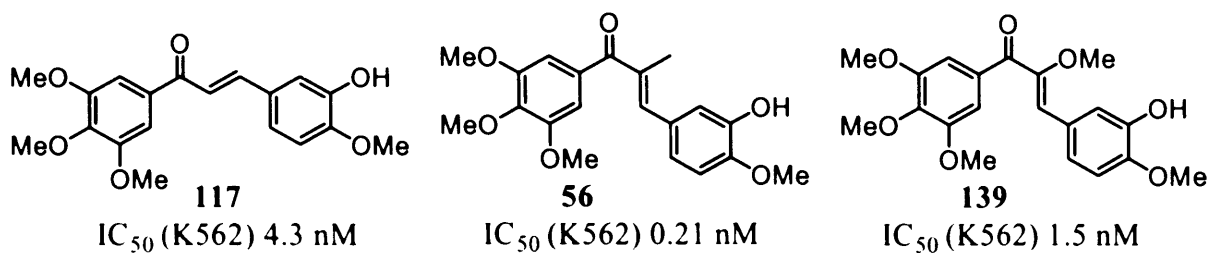


Figure 15: X-ray crystal structure of chalcone 56

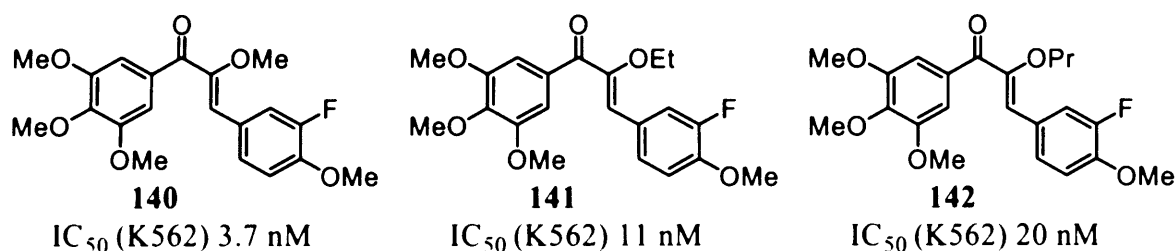
These results were supported by a number of examples in the literature showing that the X-ray crystal structures of chalcones bearing hydrogen on the α -position to the carbonyl adopt the *s-cis* conformation.¹⁸⁴⁻¹⁸⁷ A number of chalcones bearing various bulky groups at the α -position to the carbonyl, including methoxy,¹⁸⁸ azido¹⁸⁹ and pyridinium¹⁹⁰ groups, for which X-ray crystal structures have been determined, adopt the *s-trans* conformation.

The introduction of various alkoxy groups at the C-2 position of the chalcone has been investigated.¹⁹¹ The CA-4 like α -methoxychalcone **139** showed the greatest anti-cancer activity of all of the α -alkoxychalcones, with an increase in cytotoxicity over the unsubstituted chalcone **117**. However it proved to be less toxic than the α -methylchalcone **56**.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



The effects of varying the size of the alkoxy groups on the C-2 position showed that increasing the size of the alkoxy group led to a slight loss of activity. For example the α -methoxychalcone **140** was more active than the α -ethoxychalcone **141**, which in turn was more active than the α -propoxychalcone **142**.



The X-ray crystal structure of α -methoxychalcone chalcone **143** shows that α -alkoxychalcones adopt the *s-trans* conformation (Figure 16).¹⁹¹

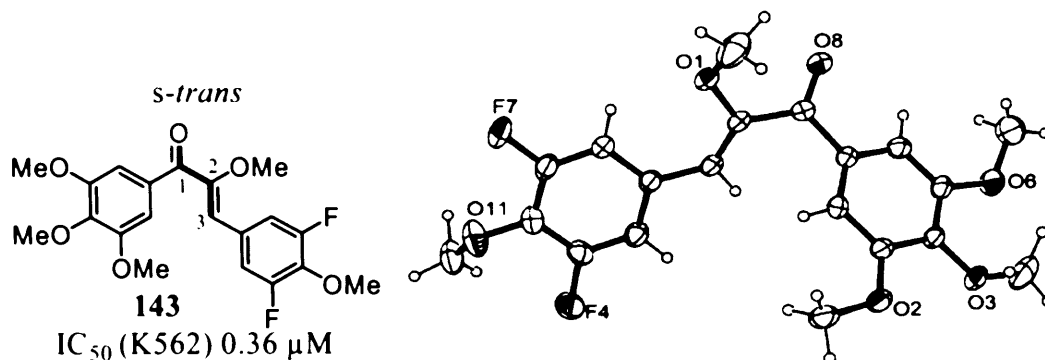


Figure 16: X-ray crystal structure of chalcone 143

A series of aurones **144** (conformationally-restricted analogues of the chalcones) was prepared and assessed for anti-cancer activity.¹⁹² This was done to investigate the effects of the aryl ring orientation about the rotatable bonds a and c and their influence on the cytotoxicity of the molecule (Figure 17).

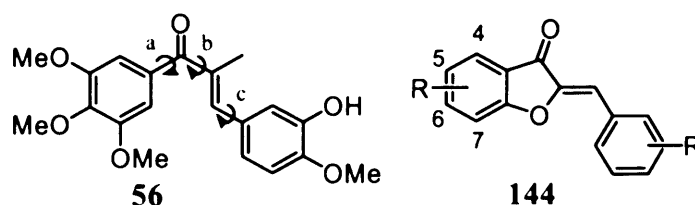
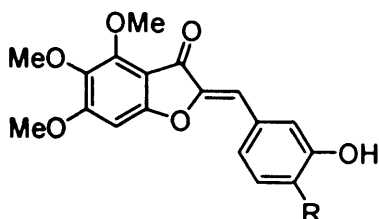


Figure 17: Structure of aurones

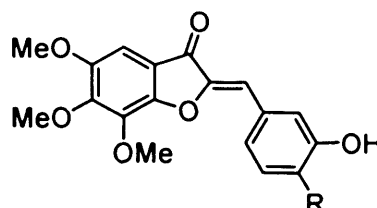
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

This study also provided a route for the total synthesis of the aurone **145** isolated from *Uvaria hamiltonii* by Wani and co-workers.¹⁹³ The 3',4'-dihydroxy B-ring of extracted aurone **145** and aurone **146** made both aurones fairly inactive, however the replacement of the 4'-hydroxy on the B-ring of **146** with a 4'-methoxy to give the CA-4 like aurone **147** causes the cytotoxicity to be significantly increased. The same replacement of the 4'-hydroxy group on **145** with the 4'-methoxy group **148** decreases the cytotoxicity of the aurone. It seems to suggest that the 5,6,7-trimethoxy A-ring of **147** is vital for good cytotoxicity, while the 4,5,6-trimethoxy A-ring of **148** is detrimental to its activity.¹⁹²



145 R = OH, IC₅₀ (K562) 12 μM

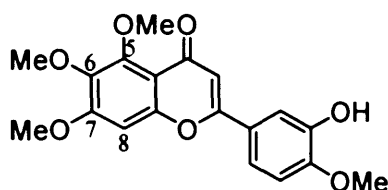
148 R = OMe, IC₅₀ (K562) 18 μM



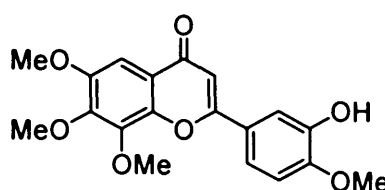
146 R = OH, IC₅₀ (K562) 10 μM

147 R = OMe, IC₅₀ (K562) 50 nM

Using the protocol developed by Wheeler and co-workers¹⁹⁴ the aurones were transformed into their corresponding flavones by simply heating in the presence of KCN. The most active flavone was the CA-4 like flavone **149**. Similar results to those of the aurones were found, with the CA-4 like 6,7,8-trimethoxy A-ring of **149** being vital to the cytotoxicity, and the 5,6,7-trimethoxy A-ring of **150** having a detrimental effect on the activity of the molecule.¹⁹²



150 IC₅₀ (K562) 22 μM

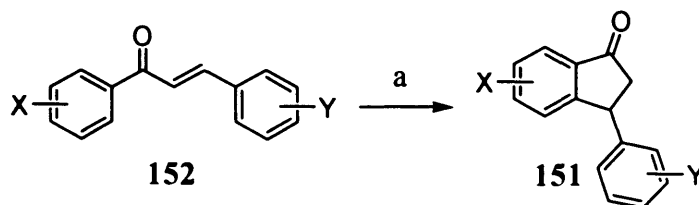


149 IC₅₀ (K562) 40 nM

The activities of the aurones and flavones nevertheless show a significant decrease in activity compared to the chalcones **117** and **56**, indicating the importance of rotational freedom around bond a (*Figure 17*).

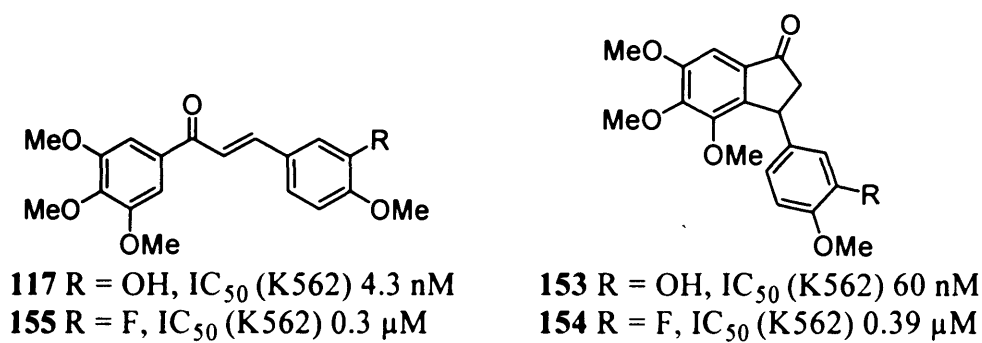
Another group of conformationally-restricted chalcone analogues were prepared, the indanones **151**.¹⁹¹ This was done to further investigate if the importance of the α,β-unsaturated ketone to the activity of the compound. The indanones **151** were prepared via Nazarov cyclization by refluxing the precursor chalcone **152** in trifluoroacetic acid (*Scheme 7*).¹⁹⁵

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

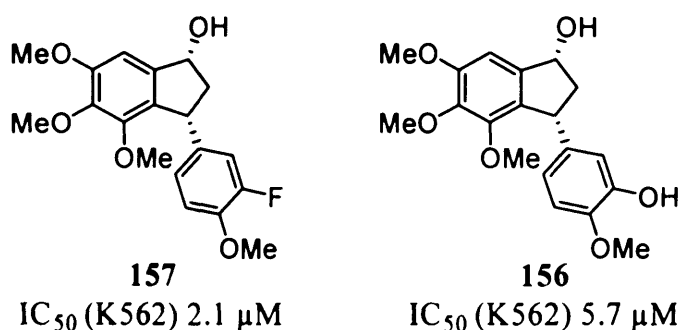


Scheme 7: a. TFA, reflux

The indanones show reasonable cytotoxicity.¹⁹¹ However the CA-4 like indanone **153** is significantly less active than its precursor chalcone **117**.¹⁹¹ This difference in activities isn't really noticed in the fluoro indanone **154** and its precursor chalcone **155**. The indanones **153** and **154** both show modest inhibition of tubulin polymerization (ITP) with IC_{50} (ITP) values of 1.9 μM and 4.0 μM respectively.¹⁹¹ The IC_{50} (ITP) of **153** is comparable to that of Combretastatin A-4 (**58**, reported IC_{50} (ITP) varies between 0.5¹⁷⁹ – 2¹⁸⁰ μM).



The indanones were then reduced to give the indanols. The indanols show major loss in cytotoxicity, with the CA-4 like indanol **156** being 95 times less active than the precursor indanone **153**.¹⁹¹



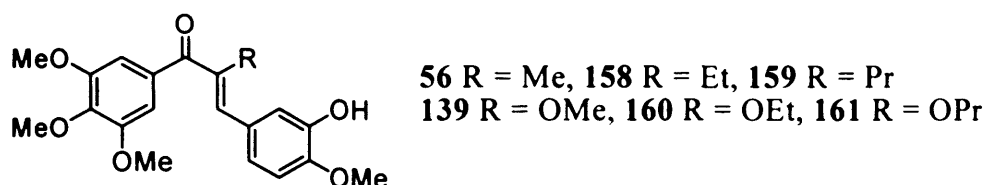
The biological properties of the indanols again show that the carbonyl group is vital to the cytotoxicity of the molecule. However while the indanones are less cytotoxic than their precursor chalcones, they still retain significant anti-cancer activity, supporting the idea that the purpose of the α,β -unsaturated ketone is to maintain the distance of the two aryl groups in a way that favours strong non-covalent binding to the tubulin. It appears that the ability of

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

the enone system to undergo nucleophilic attack via a Michael-type is not an important determinant of biological activity.

3.2 α -Arylchalcones

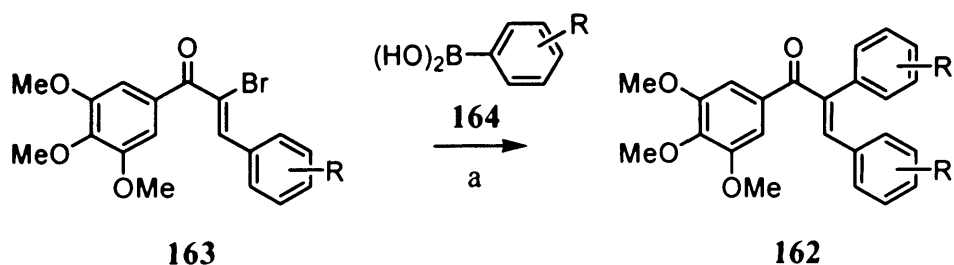
Previous studies in our research group have investigated the substitution of the hydrogen on the C-2 of the chalcone with various other groups. This has given several active compounds including (*E*)-3-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-2-methylprop-2-en-1-one (**56**), IC_{50} (K562) = 0.21 nM.⁷³ Other C-2 substitutions that have also been investigated include alkyl (**158**, **159**) or an alkoxy (**139**, **160**, **161**) groups.^{177,181,191,196}



To further the investigation into the effects of the substitution of the hydrogen on the C-2 of the chalcone, we set out to make a series of α -arylchalcones. The effects of this substitution upon the structure (especially the *s-cis/s-trans* preference) and the biological activity will feature highly in our study.

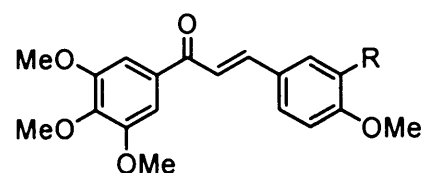
3.2.1 α -Arylchalcones - Synthesis, Results and Discussion

The approach to making the series of α -arylchalcones **162** we chose was the Suzuki coupling of an α -bromo chalcone **163** and an arylboronic acid **164** to give the α -arylchalcone **162**. This route was chosen due to the large number of commercially available arylboronic acids (*Scheme 8*).



Scheme 8: a. Suzuki coupling conditions

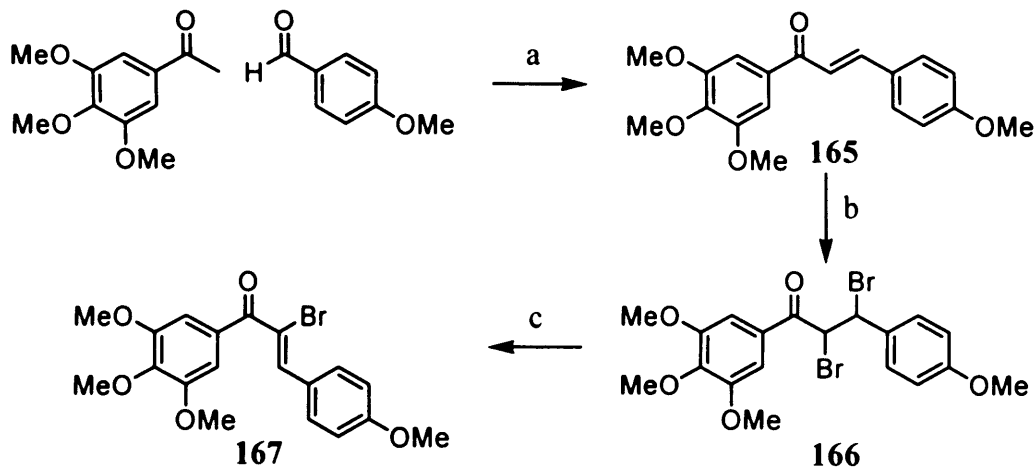
The first step in the synthesis of the α -arylchalcones is the preparation of the chalcone (*E*)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**165**). The chalcone **165**, was chosen over the more biological active chalcone **117**^{73,74} due to the ease of synthesis and no requirement of protecting groups later in the



165 R = H, IC_{50} (K562) = 0.1 μ M
117 R = OH, IC_{50} (K562) = 4.3 nM

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

synthesis. With the chemistry validated we would return to the chalcone **117** later. The chalcone **165** was prepared in an excellent yield using the Claisen-Schmidt condensation¹⁷⁸ of 3,4,5,-trimethoxyacetophenone with *p*-anisaldehyde (*Scheme 9*). Inspection of the ¹H NMR spectrum clearly shows that the product chalcone is geometrically pure with only the *trans* isomer ($J_{H_2-H_3}$ 15.6 Hz) being obtained.



Scheme 9: a. NaOH (2M) (aq), MeOH, r.t., overnight, 90%; b. Br₂ (1M in CHCl₃), CHCl₃, N₂, 0 °C, 1 h, 99%; c. Et₃N, CHCl₃, N₂, 95 °C, 1 h, 60%.

A 1 molar solution of bromine in chloroform was then prepared; this solution was used to brominate the double bond of the chalcone **165**. The bromine solution was added to the chalcone in chloroform at 0 °C (*Scheme 9*), giving a pink solid **166**. The TLC of the product **166** showed 2 spots, however the separation of these 2 products by column chromatography proved to be not trivial. The ¹H NMR spectrum of the crude product showed that it was approximately 90% pure (*Figure 18*) so it was used in the next step without purification.

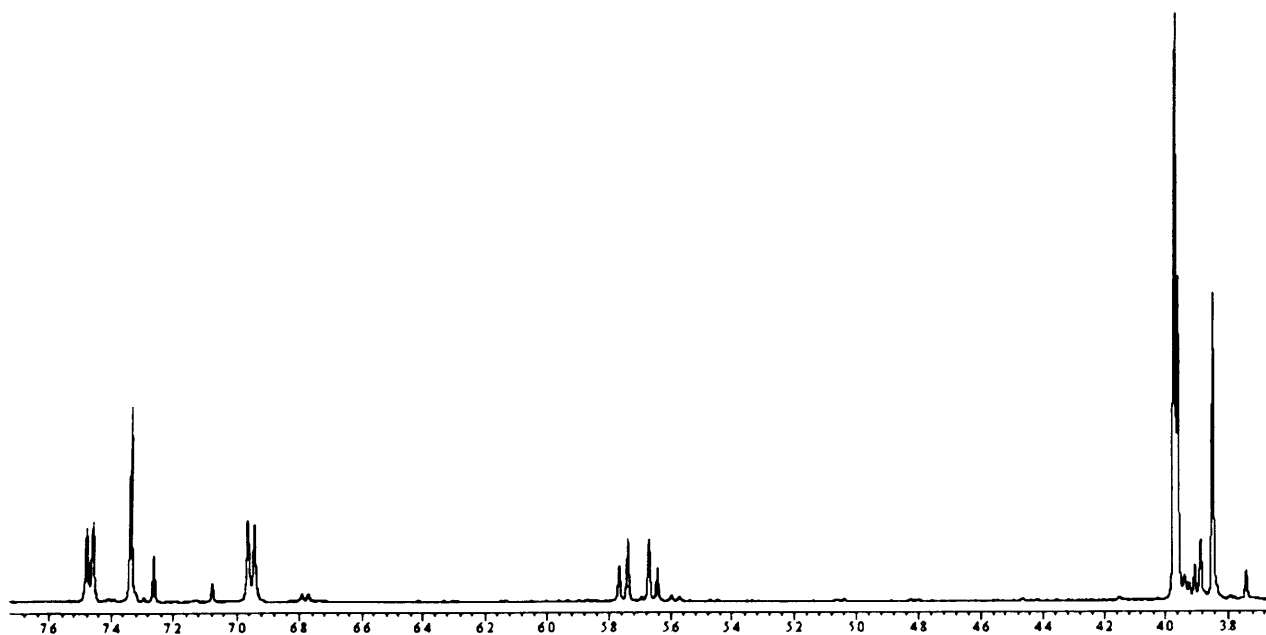


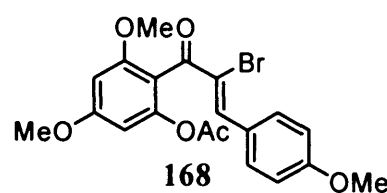
Figure 18: ¹H NMR spectrum of dibrominated chalcone **166**

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

We then needed to carry out a dehydrohalogenation reaction to give the α -bromo chalcone **167**. This was first tried by treating **166** dissolved in chloroform, with DBU at room temperature for 30 minutes. This gave a mixture of *E* and *Z* isomers. We also performed the reaction at $-30\text{ }^{\circ}\text{C}$ for 2 hours and this also gave a mixture of isomers. Other α -bromo chalcones have been made by treating the precursor dibrominated chalcone with triethylamine.^{197,198} So the chalcone in chloroform was refluxed in excess triethylamine for 1 hour. This procedure gave mainly one isomer; however it was noticed that the amount of the secondary isomer started to increase when the NMR sample was left out in the light. So it appeared that the product was light sensitive

The reaction was then repeated but wrapped in aluminium foil, purified by column chromatography and recrystallization from ethyl acetate and hexane, to give fine cream crystals of (*Z*)-2-bromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**167**) in a reasonable yield (*Scheme 9*), that was geometrically

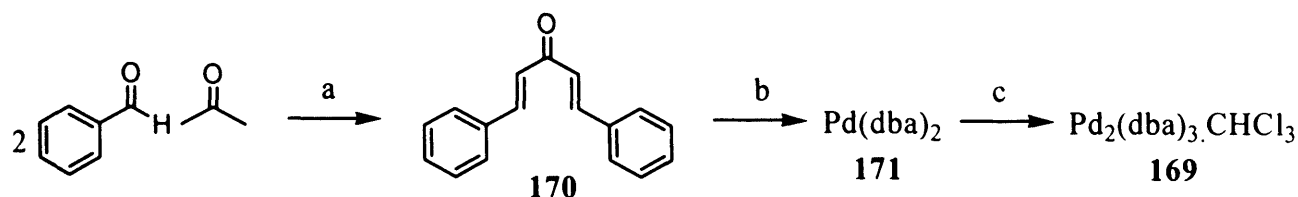
pure. The *Z* configuration of the compound was assigned by ^1H NMR spectral comparison to another (*Z*)- α -bromo chalcone **168** reported by Bose¹⁹⁷, the peak for the hydrogen attached to



C3 of **168** was reported to occur at 7.72 ppm. The peak for the hydrogen attached to C3 of the geometrically pure **167** occurs at 7.69 ppm, looking at the crude mixture of the 2 isomers of **167** the peak for the hydrogen on C3 of the other isomer occurs at 7.31 ppm. Therefore we concluded that the pure isomer isolated was the *Z*-isomer due to the similarity of the 2 spectral values. The structure was also conformed later by the X-ray crystals of several of the α -arylchalcones made from **167** (*Figure 19 and 20*).

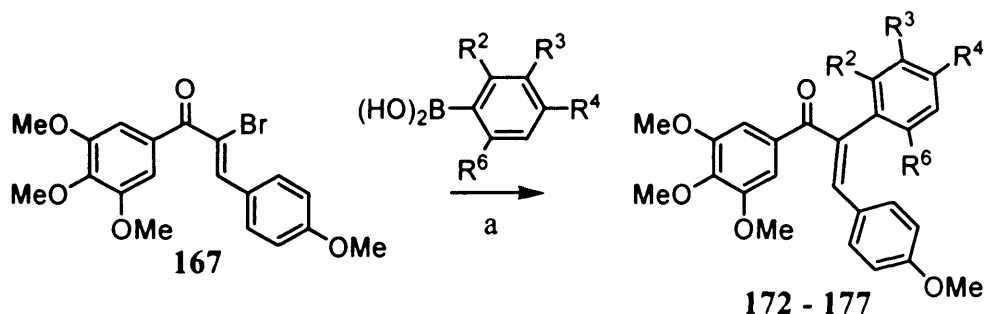
The α -arylchalcone library was then to be synthesized by palladium-catalyzed Suzuki coupling of **167** and a selection of boronic acids.^{199,200} First, the palladium catalyst tris(dibenzylideneacetone)dipalladium(0).chloroform (**169**) had to be made as it is often most active when freshly prepared. The dibenzylideneacetone (**170**) was prepared by stirring acetone and benzaldehyde in the presence of sodium hydroxide in aqueous ethanol to give the Claisen-Schmidt condensation product as bright yellow crystals (*Scheme 10*).²⁰¹ Only the *trans* isomer of dibenzylideneacetone (**170**) was obtained, this was clearly shown in ^1H NMR spectrum with 2 doublets at 7.09 and 7.75 ppm with a coupling constant of 16.2 Hz. Bis(dibenzylideneacetone)palladium(0) (**171**) was then prepared by heating a mixture of **170**, sodium acetate and palladium chloride in methanol at $40\text{ }^{\circ}\text{C}$ for 4 hours, forming a purple precipitate. This purple precipitate, bis(dibenzylideneacetone) palladium(0) (**171**), was then recrystallized from hot chloroform to give deep purple crystals of **169** (*Scheme 10*).²⁰²

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



Scheme 10: a. 5M NaOH, ethanol, r.t. 1 hr, 50%; b. NaOAc, PdCl₂, MeOH, 40 °C, 4 h, 99%; c. CHCl₃, 74%.

The Suzuki coupling reaction of α -bromo chalcone **167** and phenylboronic acid using the conditions described by Mull²⁰⁰ (*Scheme 11*) gave (*E*)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)-2-phenylprop-2-en-1-one (**172**) isolated as cream fine needles after purification by chromatography and crystallization. Since this method proved successful a series of α -arylchalcones **173** – **177** was made from **167** using various substituted commercially available boronic acids.



Scheme 11: a. Pd₂(dba)₃.CHCl₃ (2 mol%), PPh₃ (4 mol%), Et₂NH, Toluene, *n*PrOH, H₂O, 120 °C, 1 hr, yield (see *table 2*).

Table 2: α -Arylchalcones prepared by Suzuki coupling (see *scheme 11*).

	R ²	R ³	R ⁴	R ⁶	Yield (%)
172	H	H	H	H	43
173	H	H	OMe	H	30
174	OMe	H	OMe	H	37
175	H	H	NMe ₂	H	26
176	OMe	OMe	OMe	H	61
177	OMe	H	H	OMe	40

All the α -arylchalcones except **177** were isolated as a single isomer. X-ray crystal structures of the α -arylchalcones **173** (*Figure 19*) and **175** (*Figure 20*) show that these α -arylchalcones have the *s-cis* conformation around the C1 – C2 single bond. The X-ray crystal structures of the α -arylchalcones also show their carbon carbon double bond is of the *E* configuration.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

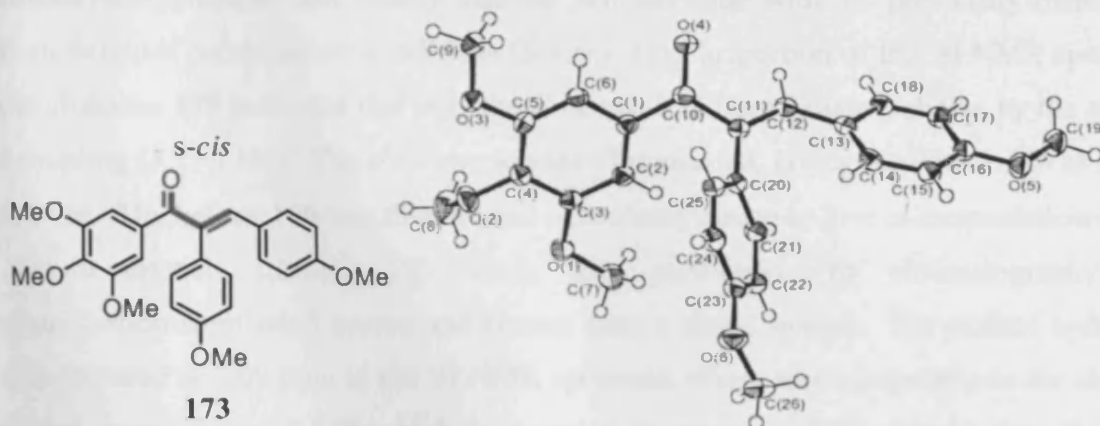


Figure 19: X-ray crystal structure of chalcone 173

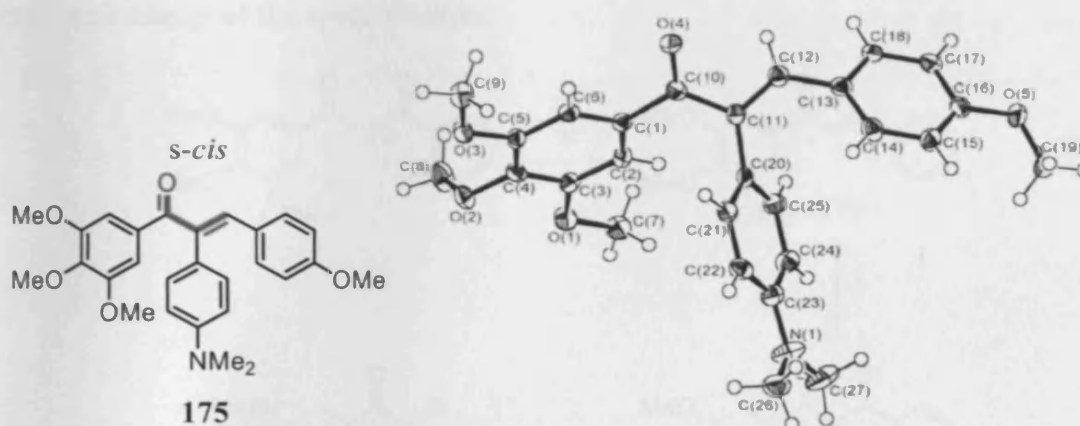


Figure 20: X-ray crystal structure of chalcone 175

The ^1H NMR spectrum of α -arylchalcone 177 shows that it is a mixture of the *E* and *Z* isomers. The 3:2 ratio favours for the *Z*-isomer. The ^1H NMR signal of the hydrogen on C3, appears around 7.10 – 7.25 ppm in the other *E*-conFigured α -arylchalcones. For 177 the olefinic signals occur at 7.13 ppm and 7.40 ppm (Figure 21).

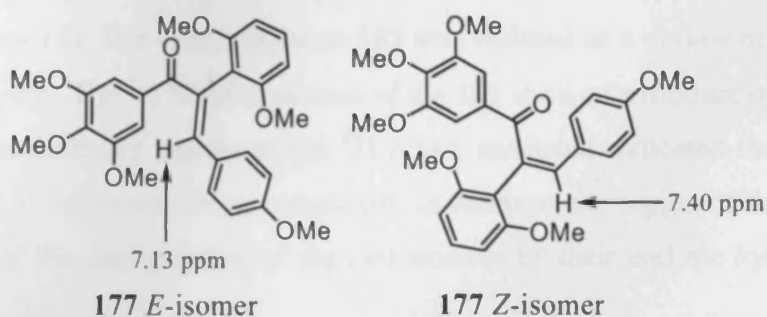
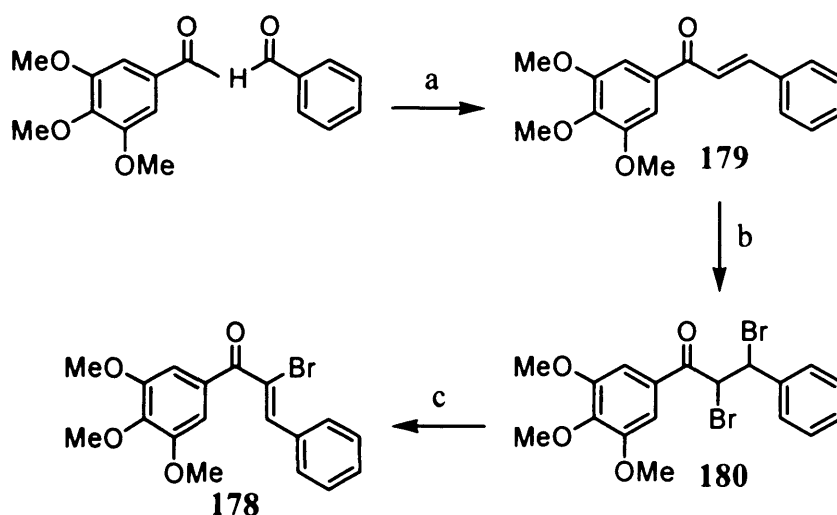


Figure 21: Structure of *E* and *Z* isomers of α -arylchalcone 177

We then decided to make another α -bromo-chalcone 178 bearing the unsubstituted phenyl group. The starting chalcone 179 was isolated as light yellow crystals after treating 3,4,5-

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

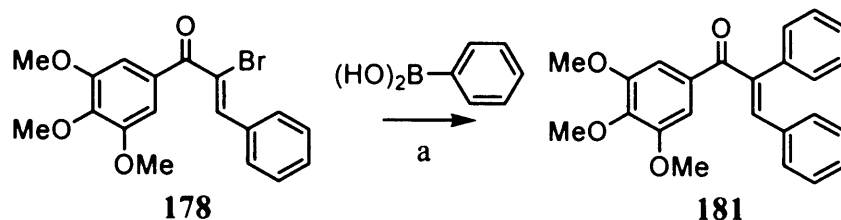
trimethoxyacetophenone and freshly distilled benzaldehyde with the previously mentioned Claisen-Schmidt condensation conditions (*Scheme 12*). Inspection of the ^1H NMR spectrum of the chalcone **179** indicated that only the *E*-isomer had been isolated, shown by the alkene HH coupling (J 15.7 Hz). The chalcone was then brominated, giving the ketone **180** as a pale pink solid. The ketone **180** was then treated with triethylamine to give α -bromo-chalcone **178** as brown crystals (*Scheme 12*) which after purification by chromatography and recrystallization from ethyl acetate and hexane gave a single isomer. The olefinic hydrogen of **178** occurred at 7.69 ppm in the ^1H NMR spectrum, which was comparable to the olefinic hydrogen peak of the *Z*-**168** which is reported to occur at 7.72 ppm in the ^1H NMR spectrum.¹⁹⁷ Therefore we concluded that the pure isomer of **178** isolated was the *Z*-isomer due to the similarity of the spectral values.



CHCl_3 , N_2 , 0 $^\circ\text{C}$, 1 h, 99%; c. Et_3N , CHCl_3 , N_2 , 95 $^\circ\text{C}$, 1 h, 41%; b. Br_2 (1M in CHCl_3), CHCl_3 , N_2 , 0 $^\circ\text{C}$, 1 h, 99%; c. Et_3N , CHCl_3 , N_2 , 95 $^\circ\text{C}$, 1 h, 41%.

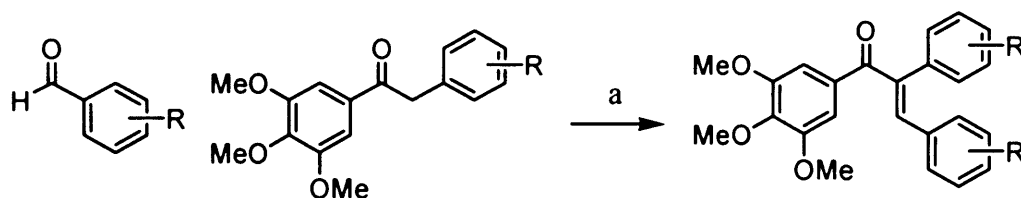
Suzuki coupling was then carried out with the α -bromo-chalcone **178** and phenylboronic acid as before (*Scheme 13*). The α -arylchalcone **181** was isolated as a yellow oil after purification by chromatography. The ^1H NMR spectrum of the **181** showed a mixture of *E* and *Z* isomers. Inspection of the methoxy signals in the ^1H NMR spectrum indicated that the ratio of the isomers was 1:2.3. However due to complexity of the aromatic region of the spectrum of **181** the assignment of the configuration of the two isomers by their olefinic hydrogens could not be made.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



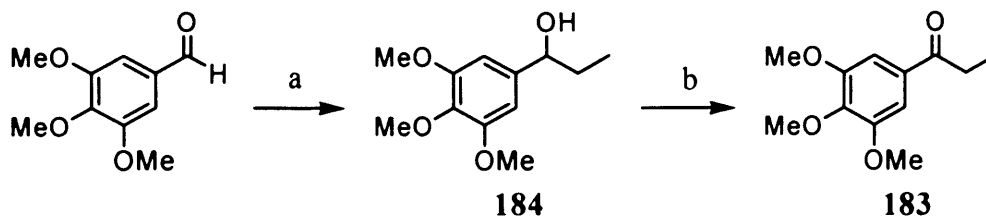
Scheme 13: a. $\text{Pd}_2(\text{dba})_3 \cdot \text{CHCl}_3$ (2 mol%), PPh_3 (4 mol%), Et_2NH , Toluene, *n*PrOH, H_2O , 120 °C, 1 hr, 52 %

Only one α -arylchalcone was made from **178**, the synthesis of other α -arylchalcone was then carried on by others in the group. The other α -arylchalcones were synthesized by the acid catalyzed condensation of 1-(3,4,5-trimethoxyphenyl)-2-phenylethanone derivatives and various benzaldehydes (*Scheme 14*). This route proved to be easier for the synthesis of a larger library of α -arylchalcones. However it gave a mixture of *E* and *Z*-isomers as the product.



Scheme 14: a. Piperidine, AcOH, EtOH, Ar, 110 °C, 4 days, 30%.

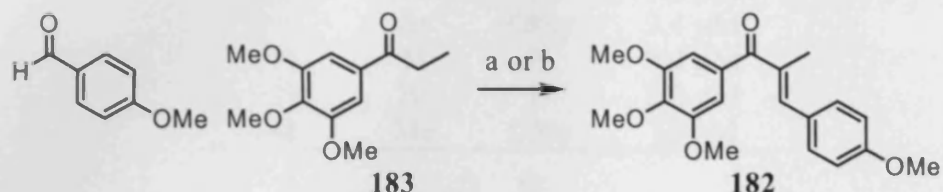
The α -methylchalcone analogue **182** of α -bromochalcone **167** was also synthesized so that its biological properties could be compared with those of the α -arylchalcones **172** – **177** and **181**. To make α -methylchalcone **182**, we first needed to synthesise the propiophenone **183**. One practical route to this compound was *via* a Grignard reaction to make the alcohol **184**, followed by the oxidation with pyridinium chlorochromate (PCC) to give the desired propiophenone **183** in a good yield (*Scheme 15*).



Scheme 15: a. Magnesium turnings, bromoethane, THF, Ar, r.t., overnight, 97%; b. PCC, DCM, Ar, r.t., overnight, 76%.

The α -methylchalcone **182** was prepared using the acid-catalyzed condensation method described by Edwards and co-workers.⁷² The propiophenone **183** and *p*-anisaldehyde, dissolved in ethanol, were refluxed in the presence of glacial acetic acid and piperidine

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion (*Scheme 16*). The water generated by the reaction was removed from the refluxing ethanol as it passed over activated 3-Å molecular sieves packed in the condenser. After purification by column chromatography and recrystallization **182** was isolated in a moderate yield as clear white crystals. The reaction was repeated but using base catalyzed conditions, refluxing the propiophenone **183** and *p*-anisaldehyde in ethanol and the presence of sodium hydroxide for 2 hours. After purification this method gave **182** in only 15% yield.



Scheme 16: a. Piperidine, AcOH, EtOH, Ar, 110 °C, 4 days, 30%; b. NaOH, EtOH, Ar, 110 °C, 2 hours, 15%.

The ¹H NMR spectrum of **182** showed that only a single isomer had been made, and after diffusion crystallisation from ethyl acetate and hexane, the X-ray structure of the crystals showed that the *E*-isomer had been isolated. The structure shown in *Figure 22*, clearly shows that the molecule adopts the *s-trans* configuration as we expected.

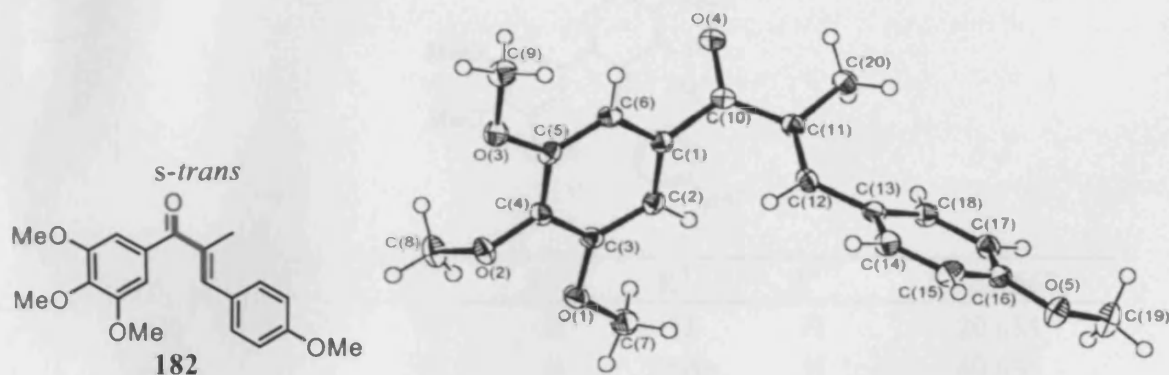
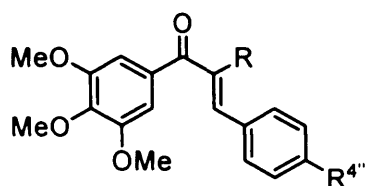
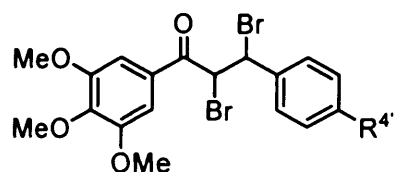


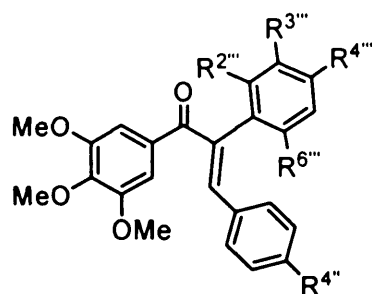
Figure 22: X-ray crystal structure of α -methylchalcone **182**

3.2.1.1 Biological results for α -substituted chalcones

Chalcone	R	R ^{4''}	IC ₅₀ (K562)
165	H	OMe	1.6 μ M
167	Br	OMe	3.4 μ M
179	H	H	9.4 μ M
178	Br	H	2.0 μ M
182	Me	OMe	10 nM



Chalcone	R ^{4''}	IC ₅₀ (K562)
166	OMe	3.3 μ M
180	H	3.5 μ M



Chalcone	R ^{4''}	R ^{2'''}	R ^{3'''}	R ^{4'''}	R ^{6'''}	IC ₅₀ (K562)
172	OMe	H	H	H	H	20 nM
173	OMe	H	H	OMe	H	40 nM
174	OMe	OMe	H	OMe	H	90 nM
175	OMe	H	H	NMe ₂	H	150 nM
176	OMe	OMe	OMe	H	H	2.2 μ M
177	OMe	OMe	H	H	OMe	>10 μ M
181	H	H	H	H	H	7.4 μ M

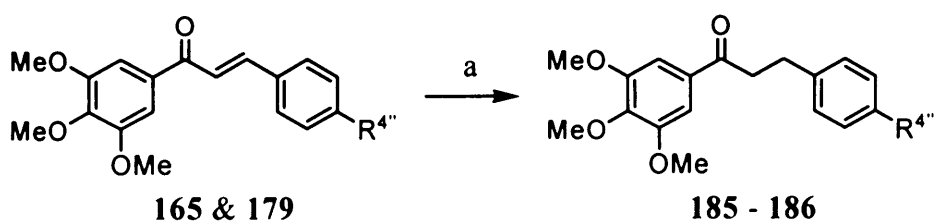
The biological activity of the chalcone **165** had been previously reported as having an IC₅₀(K562) of 0.1 μ M, however the experimental value this time proved to be significantly lower. The biological results show that the α -bromochalcone **167**, and its precursor **166**, are not as active as the original chalcone **165**. The lack of the methoxy in the 4 position on the B ring of chalcone **179** decreases the activity of the chalcone compared to the chalcone **165** which has a methoxy on the 4 position of its B ring. Therefore the presence of a methoxy in the 4 position on B ring increases the activity of the compound. The substitution of α -

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

hydrogen with various substituted phenyl groups **172** – **177** does generally show a significant change in biological activity. The exceptions to this are **176** where there seems to be a slight decrease in activity and **177** which the ^1H NMR shows as a mixture of *E* and *Z* isomers and has had a severe reduction in biological activity. The α -methylchalcone **182**, as expected also showed a significant increase in biological activity, this increase appears to be more than the increase due to the addition of α -phenyl groups. The difference in activity of the 2 compounds may be due to the favoured configuration around the C1 – C2 single bond, with the single isomer α -phenylchalcones favouring the *s-cis* conformation (*Figures 19* and *20*) and the α -methylchalcone **182** favouring the *s-trans* conformation around the C1 – C2 single bond (*Figure 22*).

3.2.2 α -Benzylchalcones - Synthesis, Results and Discussion

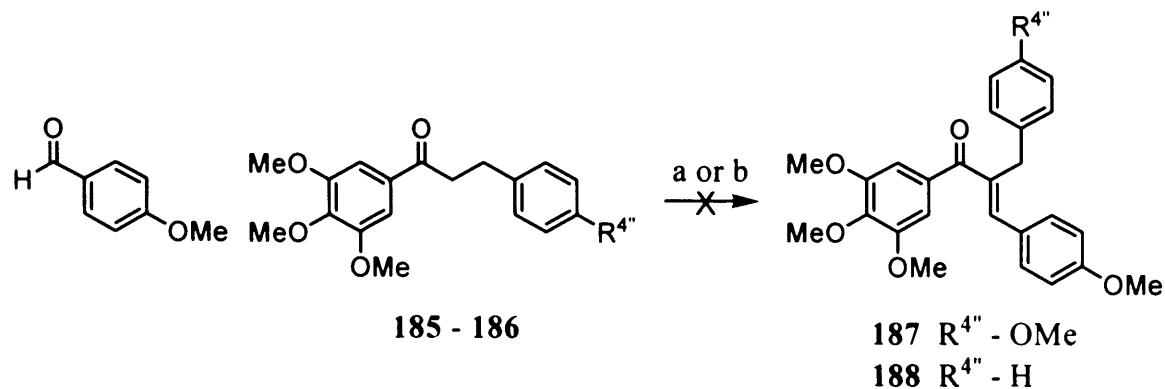
Seeing that the reaction to make the α -methylchalcone **182** from propiophenone **183** had worked, we decided to try the same reaction but with a chalcone that had been reduced at its carbon-carbon double bond, to make an α -benzylchalcone. We took the 2 chalcones that had already been prepared, **165** and **179** and reduced them using a hydrogenation method previously used in the Lawrence group by M. Woo.²⁰³ The chalcone was dissolved in ethyl acetate, a catalytic amount of 10 % palladium on carbon was added and then the mixture was stirred under a hydrogen atmosphere for 3 hours at room temperature (*Scheme 17*). The reduced chalcones were then both purified via column chromatography in excellent yields. The ^1H NMR of the product showed that peaks for the alkene HH coupling were no longer present and that there were now 2 triplets both with an integration of 2 hydrogen's in the 3.00 – 3.30 region of the spectrum for the newly created methylene groups.



Scheme 17: a. H₂, 10% Pd/C, EtOAc, r.t., 3 hrs, yield (**185**, R^{4''} = OMe, 82%; **186** R^{4''} = H, 95%).

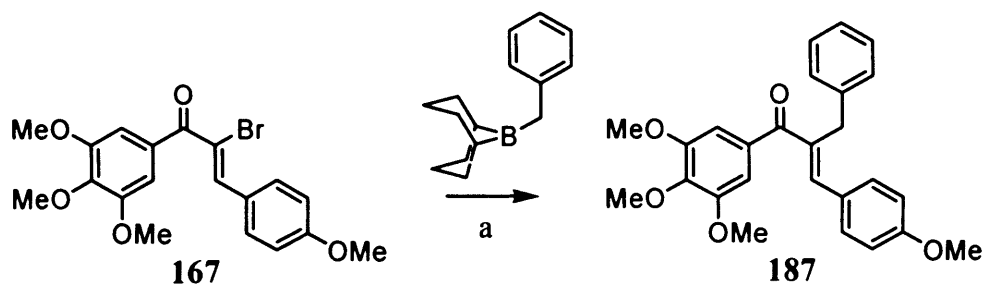
Both the acid catalyzed condensation⁷² and the base catalyzed condensation were attempted with the reduced chalcone and *p*-anisaldehyde (*Scheme 18*). Unfortunately neither of these conditions worked and the starting materials were recovered in both cases.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



Scheme 18: a. Piperidine, AcOH, EtOH, Ar, 110 °C, 4 days, 0 %; b. NaOH, EtOH, Ar, 110 °C, 24 hours, 0 %.

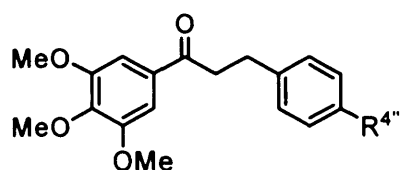
Another route to the α -benzylchalcone **187** was then tried. This time we attempted the Suzuki coupling reaction of the α -bromo-chalcone **167** with 0.5 M *B*-benzyl-9-BBN in THF (*Scheme 19*). This reaction also didn't work, the ¹H NMR spectrum of the crude product showed that it was mainly the α -bromo-chalcone **167**, the spectrum also showed traces of the benzyl group in spectrum, with peaks at 4.69 ppm with an integration of two representing the methylene group of the benzyl compound and a multiplet at 7.33 – 7.39 ppm with an integration of five representing the phenyl group of the possible benzyl compound. The α -bromo-chalcone **167**, was then recovered by column chromatography. The *B*-benzyl-9-BBN had probably been removed in the work up of the reaction, which would explain for the trace amounts of it in the ¹H NMR spectrum of the crude product.



Scheme 19: a. Pd₂(dba)₃.CHCl₃ (2 mol%), PPh₃ (4 mol%), Et₂NH, Toluene, *n*PrOH, H₂O, 120 °C, 1 hr, 0%;

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

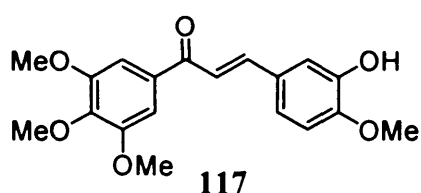
3.2.2.1 Biological results for the reduced chalcones



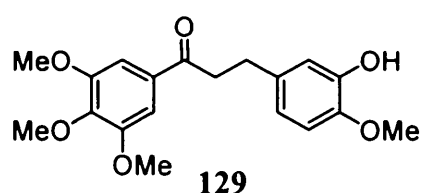
185 - 186

Chalcone	R ^{4''}	IC ₅₀ (K562) (μM)
185	OMe	2.5
186	H	>10

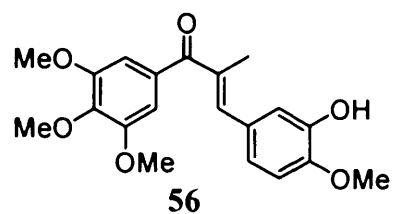
The effects on the biological activity of the chalcone by reducing its double bond to **185** and **186** resulted in a fall in activity. Thus showing the importance of the double bond to the biological activity of the chalcone structure. The decrease in biological activity resulting from the reduction of the double bond has been previously reported in the group, with chalcones **117** and **56**, being more active than their reduced analogues **129** and **189**.^{181,203}



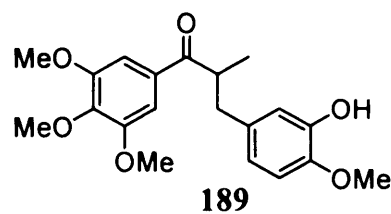
IC₅₀ (K562) 4.3 nM



IC₅₀ (K562) 1 μM



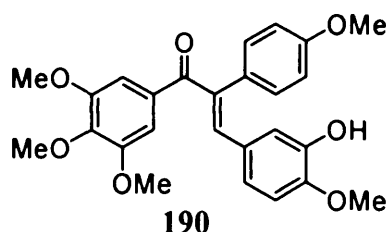
IC₅₀ (K562) 0.21 nM



IC₅₀ (K562) 30 nM

3.2.3 Another route to α-arylchalcones - Synthesis, Results and Discussion

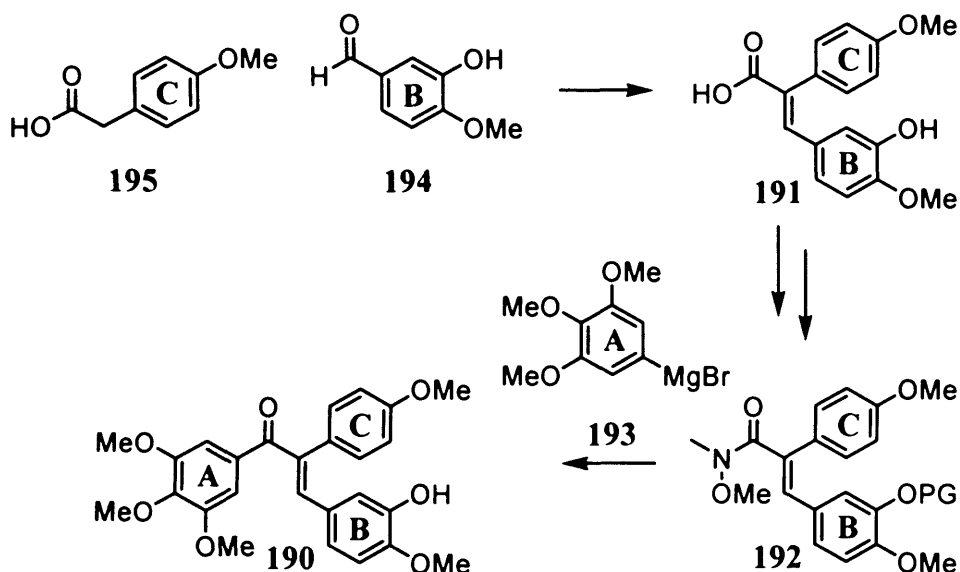
As previously mentioned other members of the group made another series of α-arylchalcones by the acid catalyzed condensation of 1-(3,4,5-trimethoxyphenyl)-2-phenylethanone derivatives and various benzaldehydes (*Scheme 14*). The most active compound of this series was the α-arylchalcone **190**, IC₅₀(K562) 12 nM. However this chalcone was a mixture of *E* and *Z* isomers.



To try and prepare **190** as a single isomer we devised a new approach to the synthesis of the α-arylchalcones. The key steps in this new synthesis would be the synthesis of acrylic acid

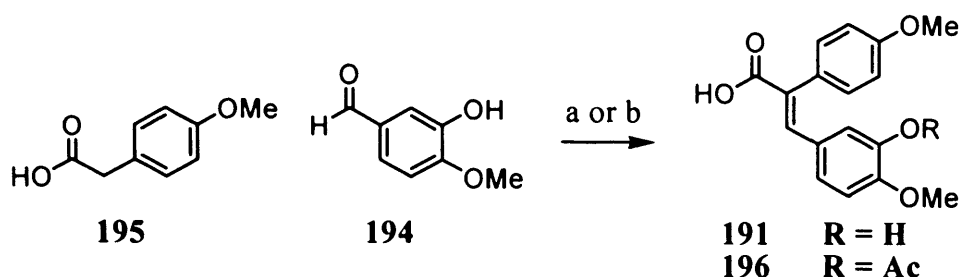
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

191 to give the B and C ring system and the conversion of the acid **191** into the Weinreb amide **192**. Finally we hoped that the reaction of **192** with the Grignard reagent **193** would introduce the A ring (*Scheme 20*).



Scheme 20

The acrylic acid was synthesized by means of a Perkin reaction,²⁰⁴ where isovanillin (**194**) and 4-methoxyphenylacetic acid (**195**) were heated in a mixture of triethylamine and acetic anhydride at 140 °C for 3 hours, the mixture was then left to cool to room temperature and then acidified with concentrated hydrochloric acid and stirred overnight causing the acrylic acid **191** to precipitate as a cream solid which was then purified by crystallization from hot ethanol and isolated in a moderate yield. The reaction was then repeated again, except this time in the microwave at 170 °C for 25 min and without the acid workup to see if the phenoxy acetate **196** could be isolated directly. The acetic anhydride and triethylamine were removed via vacuum distillation (Genevac), the product was then purified by chromatography and crystallization from hot ethanol to give the acetate of the corresponding acrylic acid **196** in a moderate yield (*Scheme 21*).



Scheme 21: a. i $(\text{CH}_3\text{CO})_2\text{O}$, Et_3N , $140\text{ }^\circ\text{C}$, 3 hr. ii. Conc. HCl . To give **191** 37%; b. $(\text{CH}_3\text{CO})_2\text{O}$, Et_3N , $170\text{ }^\circ\text{C}$, 25 min, μwave . To give **196** 39%.

By comparing the values of the ^1H NMR chemical shifts for hydrogen on C3 of the 2 acrylic acid obtained to similar compounds in literature it appears that we have isolated the desired *E* conFigured isomers. The *E* and *Z* isomers of 3-(4'-methoxy-3'-nitrophenyl)-2-(3'',4'',5''-trimethoxyphenyl)acrylic acids (**197**) were reported by Borrel²⁰⁴, and the ^1H NMNR chemical shift value for hydrogen on C3 for the *E* isomer appears at 7.70 ppm while the *Z* isomer appears at 7.10 ppm. The ^1H NMNR chemical shift value for hydrogen on C3 of **191** is 7.56 ppm and **196** 7.62 ppm, and both appear to be the *E* isomer when compared to the values of **197** (Figure 23).

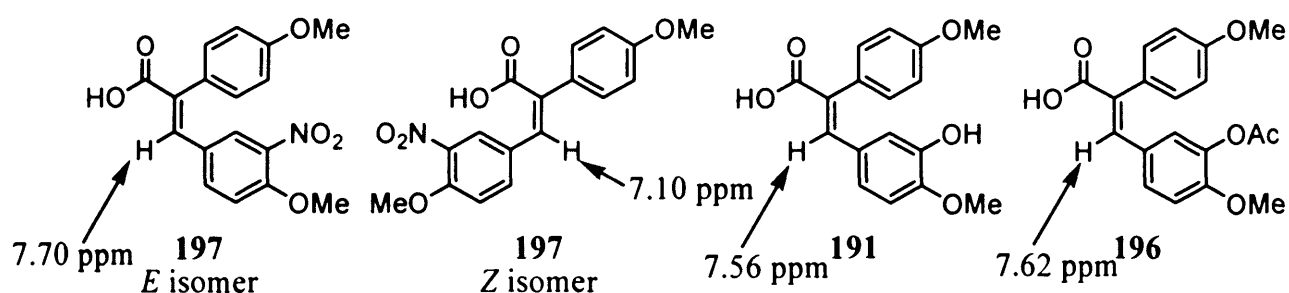
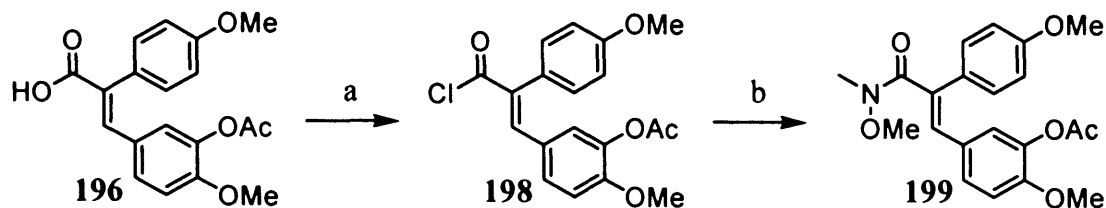


Figure 23: ^1H NMR comparisons of **191** and **196** to **197**

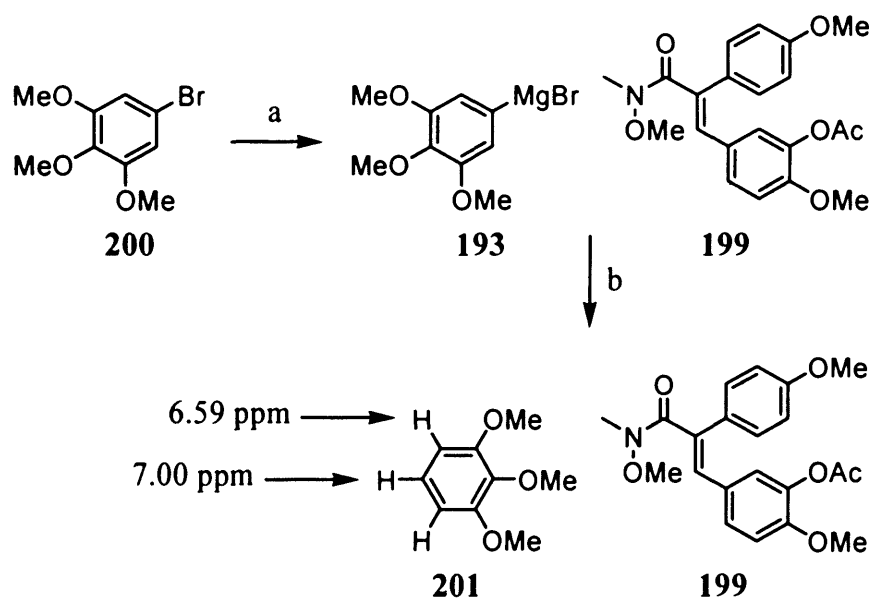
The protected acrylic acid **196** was then dissolved in chloroform and refluxed with thionyl chloride for 4 hours²⁰⁵ to give the acid chloride **198** in an excellent yield (*Scheme 22*). The Weinreb amide **199** was then obtained by treating the acid chloride **198** with *N,O*-dimethylhydroxylamine hydrochloride, in the presence of pyridine at $0\text{ }^\circ\text{C}$.²⁰⁶ The amide **199** was purified by chromatography as a pale yellow oil in good yield (*Scheme 22*).

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



Scheme 22: a. SOCl_2 , CHCl_3 , 75°C , 4 hr, 93%; b. $\text{MeNHOMe}\cdot\text{HCl}$, pyridine, CHCl_3 , 0°C , 1 hr, 70%.

The Grignard reagent **193** was prepared in freshly distilled anhydrous THF, by gently heating magnesium turnings with 5-bromo-1,2,3-trimethoxybenzene (**200**) in the presence of a single iodine crystal, under an argon atmosphere.^{99,207} When the reaction started the iodine discolouring disappeared and the mixture became clear, it was heated at 65°C for an hour, and then stirred at room temperature for an hour (*Scheme 23*). The Weinreb amide **199** was then treated with a slight excess of the Grignard reagent **193** at 0°C , and then left to stir at room temperature overnight (*Scheme 23*). The NMR spectrum of the crude reaction product showed that the reaction had not been successful and Weinreb amide **199** was recovered by chromatography, in a 66% yield. The spectrum also showed the presence of the quenched Grignard reagent **201**, with a triplet with an integration of one proton at 7.00 ppm and doublet with an integration of two protons at 6.59 ppm. The trimethoxy benzene **201** was not isolated.

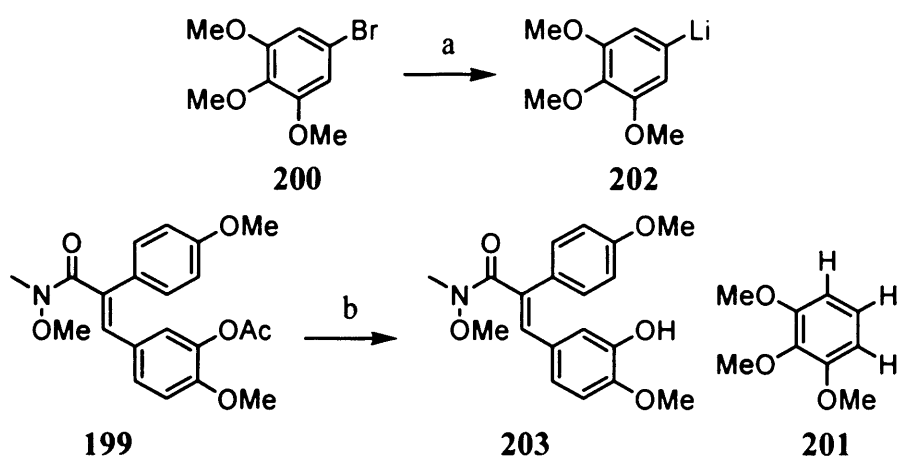


Scheme 23: a. Mg, THF, I_2 , 65°C , 1hr; b. THF, 0°C to r.t., overnight, **199** 66%.

The reaction was then attempted again, however this time the organolithium reagent **202** was used instead. The organolithium reagent **202** was prepared using the procedure reported by Chen²⁰⁸, where 5-bromo-1,2,3-trimethoxybenzene (**200**) dissolved in ether was added to

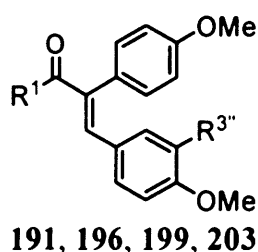
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

solution of *n*-butyllithium in ether at -78 °C, and stirred for an hour at this temperature (*Scheme 24*). The Weinreb amide **199** was then dissolved in THF and chilled to -78 °C under an argon atmosphere, then 2.3 equivalents of the organolithium reagent **202** in ether was added to the mixture, and stirred at -78 °C for a further hour. The mixture was then stirred overnight at room temperature. Unfortunately this reaction did not work, and the ¹H NMR spectrum of the crude reaction mixture showed that the Weinreb amide **199** had become deprotected giving **203**, which was isolated by chromatography (*Scheme 24*). It would have been deprotected by the excess amount of *n*-butyllithium used to make **202**. The ¹H NMR spectrum of the crude product also showed the presence of the quenched organolithium reagent **201** as previously mentioned.



Scheme 24: a. *n*BuLi (2 eqv), ether, -78 °C, 1hr; b. **202** (2.3 eqv), THF, -78 °C to r.t., overnight, 58%.

3.2.3.1 Biological results for the α -arylchalcones



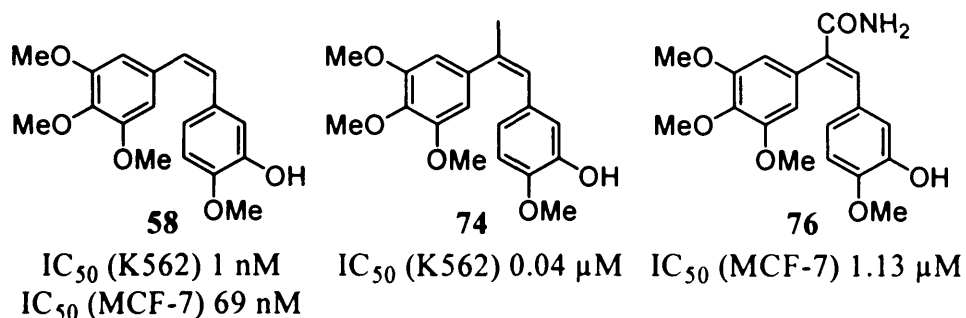
191, 196, 199, 203

Compound	R^1	$R^{3''}$	IC ₅₀ (K562) (μ M)
191	OH	OH	>10
196	OH	OAc	>10
199	N(OMe)Me	OAc	>10
203	N(OMe)Me	OH	8.3

None of the above compounds proved to be particularly biologically active. The compounds are similar to Combretastatin A-4 (**58**), with a 4-methoxyphenyl A-ring instead of a 3,4,5-trimethoxyphenyl A-ring and a substituted group on the ethene linker. It has generally been accepted that A-ring of CA4 (**58**) must be 3,4,5-trimethoxyphenyl for the compound to maintain its cytotoxicity,⁷⁹ and the presence of a 4-methoxyphenyl A-ring may partially be the cause of the low biological activity of the compounds **191**, **196**, **199** and **203**. Recent SAR studies of Combretastatin A-4 (**58**) has shown that introduction of alkyl groups⁹¹ and

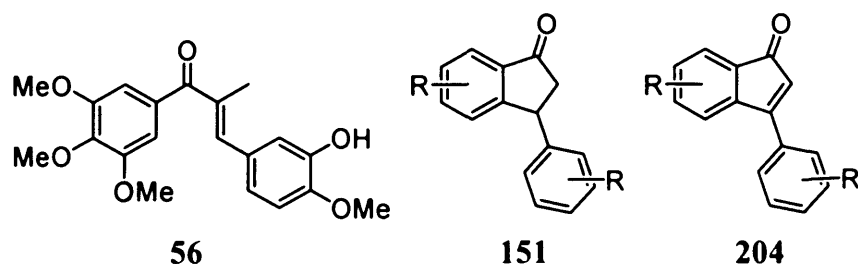
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

acylamide groups⁹² onto the ethene linker decreases the cytotoxicity of the CA4 analogues, this shows that the presence of various groups attached to the ethene linker of CA4 (**58**) will reduce its cytotoxicity. Therefore the low activity of **191**, **196**, **199** and **203** is also be due to the presence of the carboxylic acid group on **191** and **196** or the Weinreb amide on **199** and **203** attached to the ethene linker.



3.3 Indanones and Indenones

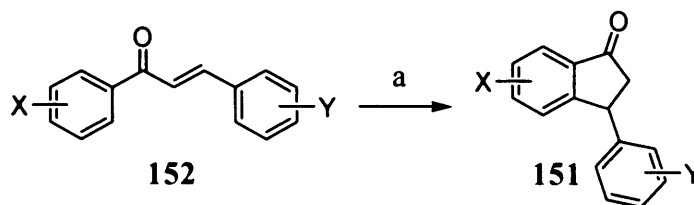
Research previously done by the group has shown that the *s-trans* conformation around the double bond of the chalcone **56** has proven important to its biological activity.⁷³ Therefore we have decided to investigate this effect, by making indanones **151** and indenones **204** as conformationally constrained analogs of the chalcones that mimic the *s-trans* arrangement.



3.3.1 Indanones - Synthesis, Results and Discussion

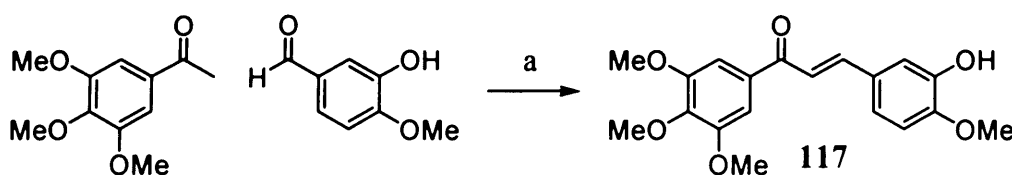
The indanone scaffold can be constructed from chalcones through catalyzed Nazarov cyclization.²⁰⁹⁻²¹¹ Literature shows that the preparation of indanones involves heating a chalcone in the presence of Lewis acids like aluminum chloride²¹² and boron trifluoride²¹³ or strong protic acids such as polyphosphonic acid²¹⁴ or trifluoroacetic acid (TFA).¹⁹⁵ The key step in this synthesis to make the indanones will be TFA catalyzed Nazarov cyclization of chalcones (*Scheme 25*).

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



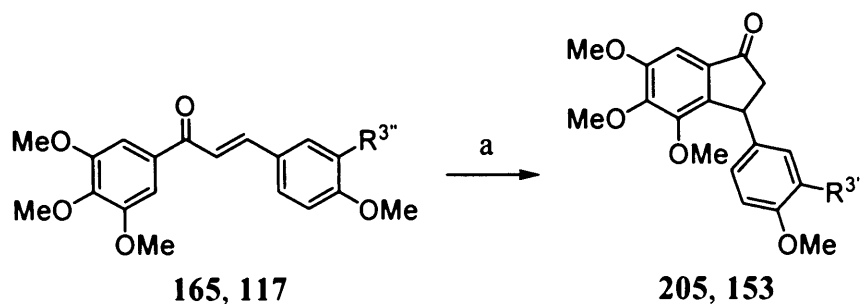
Scheme 25: a. TFA, reflux

The chalcone **117** was prepared in a good yield using the Claisen-Schmidt condensation¹⁷⁸ of 3,4,5-trimethoxyacetophenone with isovanillin (Scheme 26). After an acid work up and recrystallization from aqueous methanol, the geometrically pure chalcone **117** was obtained, with the ¹H NMR spectrum showing that only the *trans* isomer (J_{H2-H3} 15.4 Hz) was present.



Scheme 26: a. NaOH (2M) (aq), MeOH, r.t., overnight, 69%.

Then using the conditions described by Rice and coworkers²¹⁵ the indanones **205** and **153** were prepared respectively from the chalcones **165** and **117** which were dissolved in neat TFA and heated at 120 °C for 4 hours in a sealed tube (Scheme 27).



Scheme 27: a. TFA, sealed tube, 120 °C, 4 hours, yield (**205**, R^{3''} = H, 72%; **153** R^{3''} = OH, 63%).

The indanones were then purified by column chromatography and characterised. The ¹H NMR spectrum of the indanone **153** (Figure 24) shows key diagnostic peaks of a classic ABX system that occur at 2.57 (1H_a, dd, J 2.5, 19.3 Hz, H-2a), 3.15 (1H_b, dd, J 7.9, 19.3 Hz, H-2b) and 4.50 (1H_x, dd, J 2.5, 7.9 Hz, H-3) ppm.

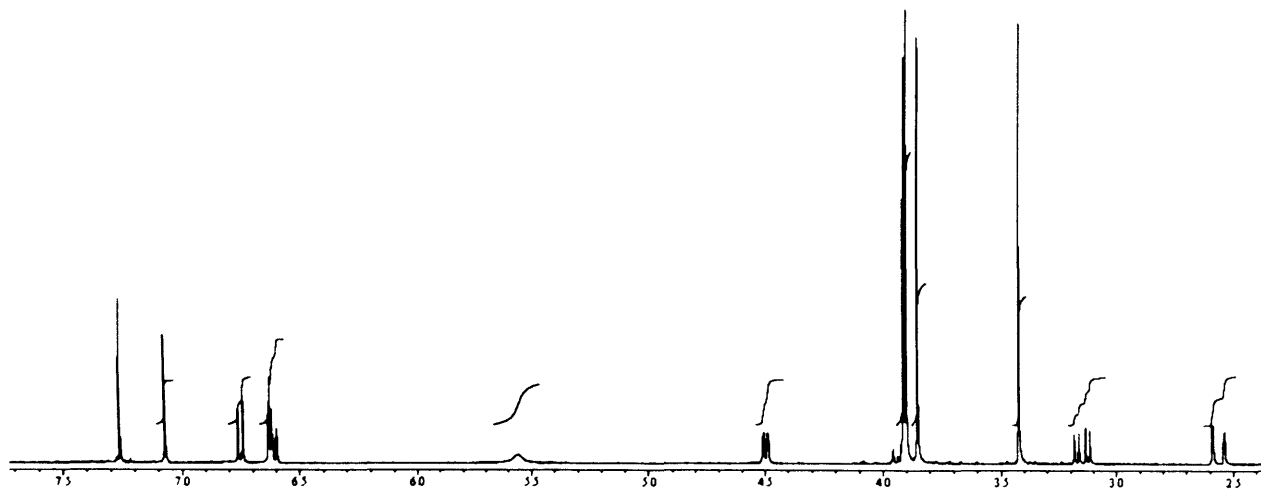
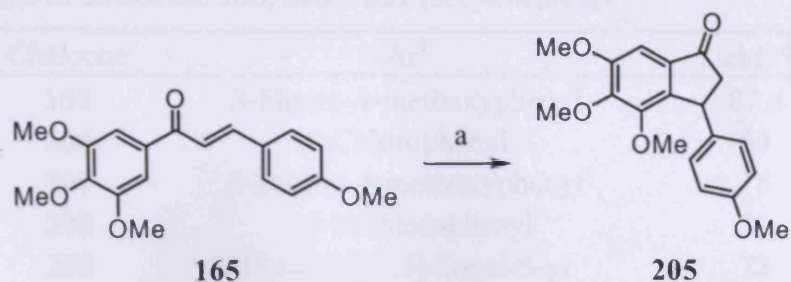


Figure 24: ^1H NMR of indanone **153**

We were then intrigued to see if the use of a microwave to heat the reaction would shorten the time of the reaction and give us a way of quickly producing a library of indanones. The use of microwave irradiation in organic synthesis has now become a widely adopted process as an alternative to traditional heating methods. The process of microwave irradiation often results in higher yields, shorter reaction time and less impurities,²¹⁶⁻²²⁰ and is more energy efficient than traditional heating.²²¹ It is also an operationally simple process. There is no need to set up an oil bath. The reactor also allows the reaction to be performed safely at temperatures well above the usual boiling point of the solvent.

The first attempt at using microwave irradiation instead of traditional heating was performed on chalcone **165** on a 600 mg scale. The chalcone was dissolved in TFA (0.7 mL) in a microwave reaction vessel and heated to 60 °C (power 50 W) for 5 minutes. Analysis of the resulting mixture by TLC showed that the reaction was still mainly starting material. Further heating at 70 °C (power 100 W) for 10 minutes showed that the resulted in an incomplete reaction; the indanone **205** was visible in the TLC plate. The mixture was then heated at 120 °C (power 100 W) for 10 minutes and the TLC of reaction showed that the reaction had gone to completion. The indanone was then isolated by column chromatography in a 50% yield. The reaction was then repeated using the following conditions, 900 mg scale, TFA (1 mL), 120 °C (power 100 W) for 20 minutes, after purification by column chromatography the indanone was obtained in a 60% yield (*Scheme 28*).

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



Scheme 28: a. TFA, μ wave, 120 °C (100 W), 20 mins, 60%.

A series of chalcones **155**, **206** – **220**, were then prepared using the Claisen-Schmidt condensation¹⁷⁸ of 3,4,5-trimethoxyacetophenone and various substituted benzaldehydes (*Scheme 29*). The chalcones, which precipitate from the reaction mixture were filtered off and purified by recrystallization from methanol. The ¹H NMR spectra of the obtained chalcones showed that they had all been isolated as the geometrically pure *E* isomer, shown by the alkene coupling \approx 15 – 16 Hz. Whilst this assignment was not in doubt the X-ray crystal structures of chalcone **209** further showed that the *E* isomer had been obtained (*Figure 24*).

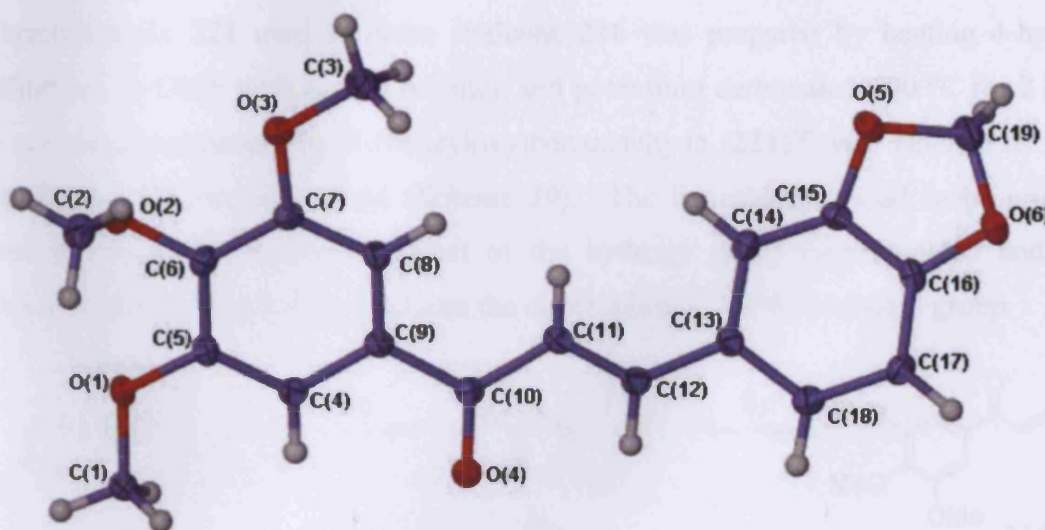
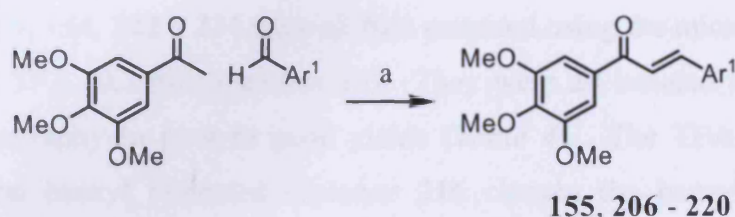


Figure 24: X-ray crystal structure of chalcone **209**



Scheme 29: a. NaOH (2M) (aq), MeOH, r.t., overnight, yields (see *table 3*).

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

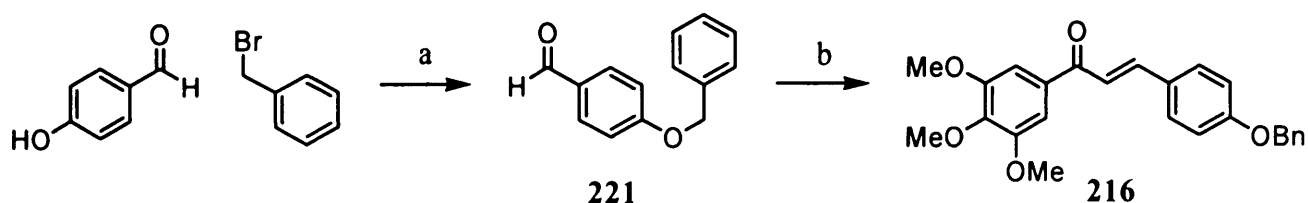
Table 3: Yields of chalcones 155, 206 – 221 (see scheme 29).

Chalcone	Ar ¹	Yield (%)
155	3-Fluoro-4-methoxyphenyl	87 ^o
206	4-Chlorophenyl	55
207	3-Bromo-4-methoxyphenyl	75
208	3,4-Dichlorophenyl	50
209	Benzo[<i>d</i>][1,3]dioxol-5-yl	73
210	4-Bromophenyl	88
211	Phenyl-4-oxyacetic acid	52
212	2,3,4-Trimethoxyphenyl	78
213	2,6-Dichlorophenyl	92
214	2,4-Dichlorophenyl	83
215	3-Nitro-4-methoxyphenyl	68 ^o
216	4-(Benzyloxy)phenyl	42
217	2,3-Dihydrobenzo[<i>b</i>][1,4]dioxin-6-yl	96 [†]
218	3,4,5-Trimethoxyphenyl	83
219	2,4-Dimethoxyphenyl	73
220	3,5-Dimethoxyphenyl	83

^o Prepared by Dr. B. Greedy, Post doctoral research assistant, Cardiff, 2003

[†] Prepared by S. Ducki, PhD Student, UMIST, 1997

The benzaldehyde **221** used to make chalcone **216** was prepared by heating 4-hydroxy benzaldehyde in DMF with benzyl bromide and potassium carbonate at 90 °C for 2 hours. After column chromatography 4-(benzyloxy)benzaldehyde (**221**)²²² was isolated as a pale yellow solid in an excellent yield (Scheme 30). The benzaldehyde had to be protected because of the electron-donating effect of the hydroxy group (deprotonated under the reaction conditions) significantly reduces the electrophilicity of the carbonyl group.

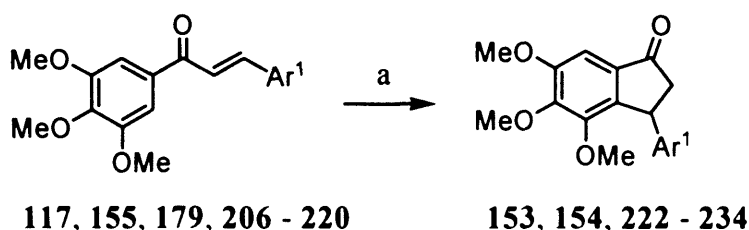


Scheme 30: a. K₂CO₃, DMF, 90 °C, 2 hours, 96%; b. 3,4,5-trimethoxyacetophenone, NaOH (2M) (aq), MeOH, r.t., overnight, 42%.

The indanones **153**, **154**, **222** – **234** were all then prepared using the microwave method, on a 250 mg scale in TFA (0.3 mL) (Scheme 31). They were all isolated after purification by column chromatography in poor to good yields (Table 4). The TFA promoted Nazarov cyclization of the benzyl protected chalcone **216** cleaves the benzyl group giving the unprotected indanone **235**, but only in a poor yield. The chalcones **218** – **220** were also used in the microwave synthesis of the indanones, however these reactions failed and were not

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

further investigated. These examples have electron rich B-rings which generally reduce the efficiency of the Nazarov cyclization (see later).



Scheme 31: a. TFA, μ wave, 120 °C (100 W), 20 mins, yield (see *table 4*).

Table 4: Yields of indanones **153, 154, 222 - 234** via microwave assisted Nazarov reaction (see *scheme 31*).

Indanone	Ar ¹	Yield (%)
153	3-Hydroxy-4-methoxyphenyl	56
154	3-Fluoro-4-methoxyphenyl	37
222	4-Chlorophenyl	53
223	3-Bromo-4-methoxyphenyl	59
224	3,4-Dichlorophenyl	69
225	Benzo[<i>d</i>][1,3]dioxol-5-yl	54
226	4-Bromophenyl	33
227	Phenyl-4-oxyacetic acid	26
228	2,3,4-Trimethoxyphenyl	25
229	2,6-Dichlorophenyl	95
230	2,4-Dichlorophenyl	83
231	Phenyl	23
232	3-Nitro-4-methoxyphenyl	39
233	4-Hydroxyphenyl	15
234	2,3-Dihydrobenzo[<i>b</i>][1,4]dioxin-6-yl	52

Two of the indanones (**228** and **230**) ¹H NMR spectra (*Figure 26* and *Figure 27*) showed line broadening for peaks on the B ring and hydrogens H-2 and H-3 of the indanone due to bulky groups on the B ring causing steric hindrance and inhibiting its free rotation around the C-3 C-1' bond (*Figure 25*). While the 2 bulky chlorine groups on C-2' and C-6' of the B ring of indanone **229** allow for no rotation at all (or at least slow rotation on the NMR timescale) and gives three signals for the hydrogen's on the B ring, since H3'' and H5'' are no longer equivalent (*Figure 28*). All three indanones **228 - 230** have the hydrogen H-3 signal shifted slightly down field due to the bulky effect of the groups on there C-2' and C-6'. The degree of shielding from the B ring is dependent on its rotation around the C-3 – C-1' bond. The shielding effect on the H-3 hydrogen from the B ring attached to C-3 is reduced due to the orientation the B ring is forced to take by its bulky ortho group. The decreased shielding effect causes the signal for the hydrogen H-3 signal to be shifted further downfield.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

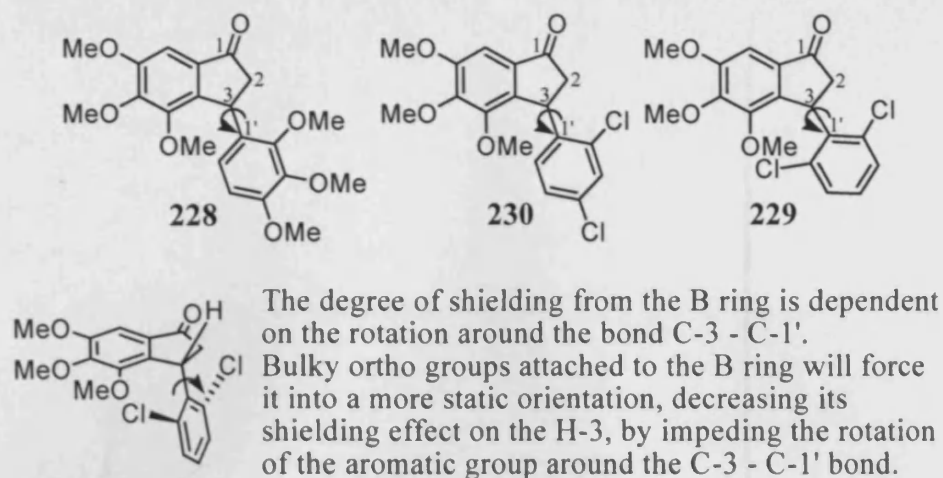


Figure 25: Steric hindrance inhibiting free rotation around C-3 - C-1' bond.

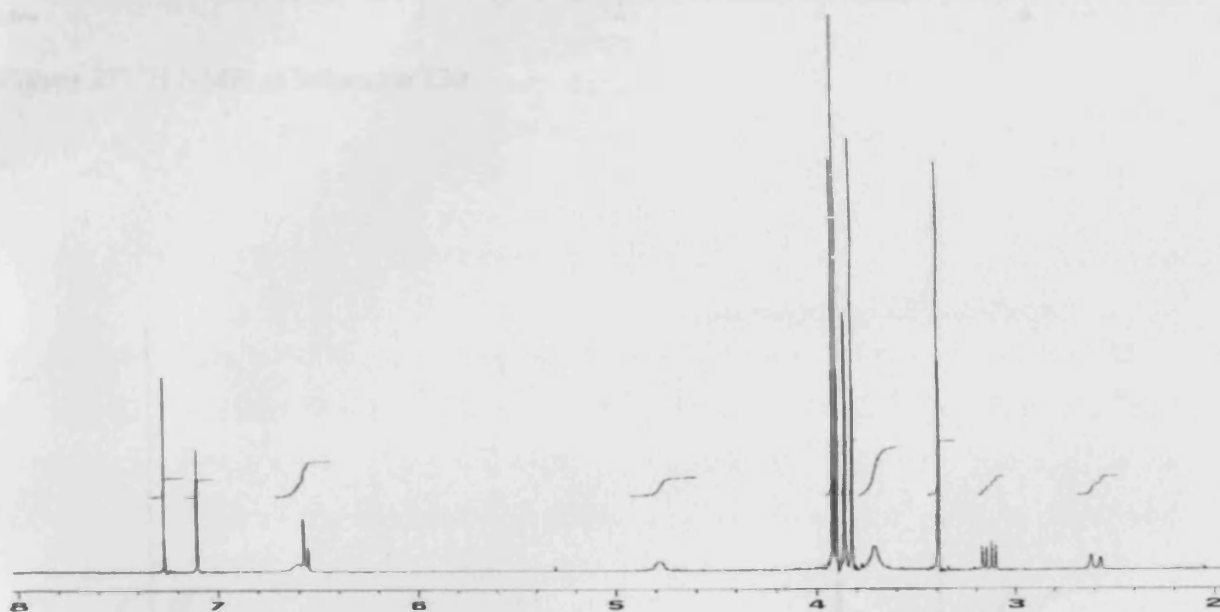


Figure 26: ¹H NMR of indanone 228

A small series of chalcones 203 – 204 were made from 3,4-dimethoxyacetophenone and various benzaldehydes using the Claisen-Schmidt condensation¹⁷ (Scheme 3.2). All of the chalcones were isolated as the E isomers after recrystallization from methanol. The geometry of the chalcones was as usual determined by the alkene coupling value $a = 15 - 16$ Hz in the ¹H NMR spectra of the chalcones.

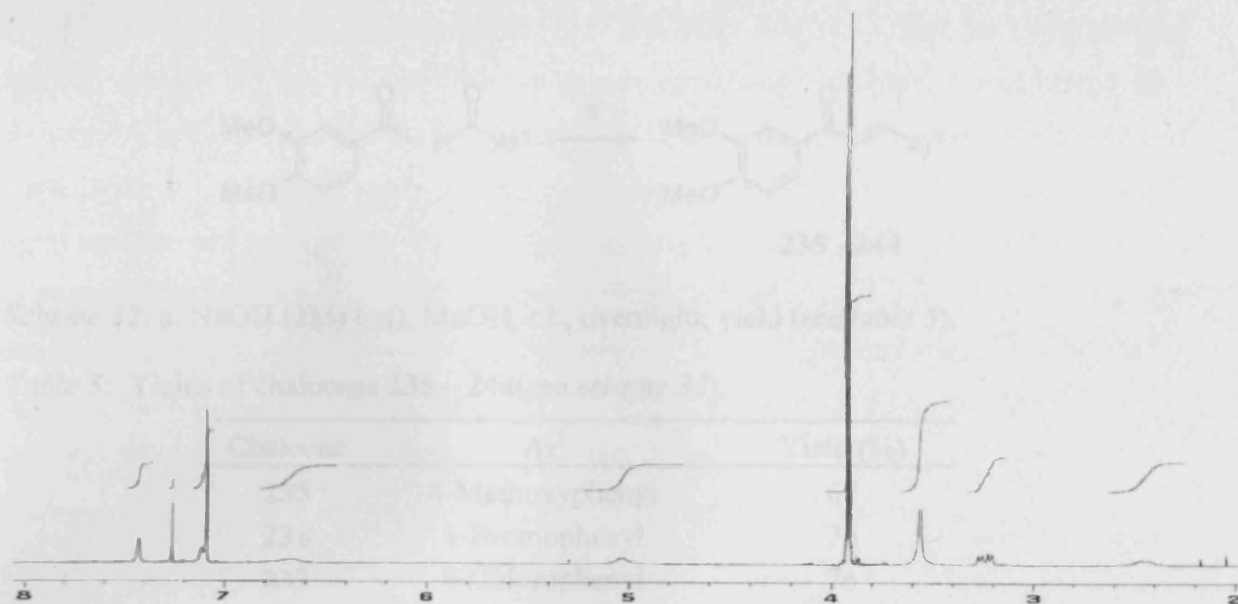


Figure 27: ¹H NMR of indanone 230

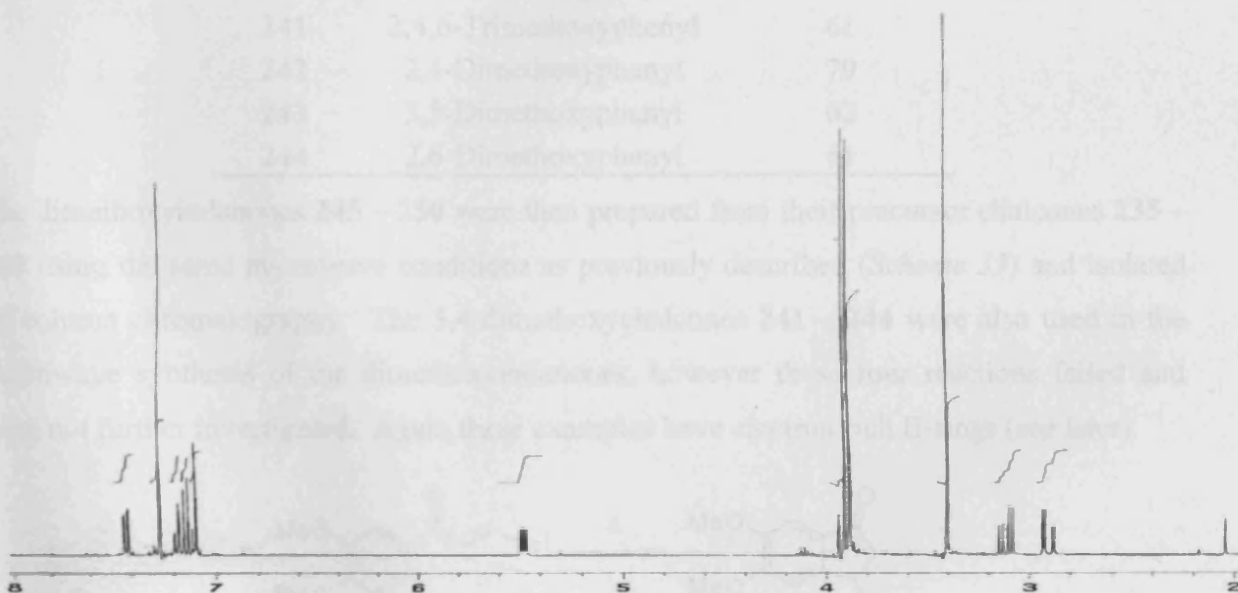
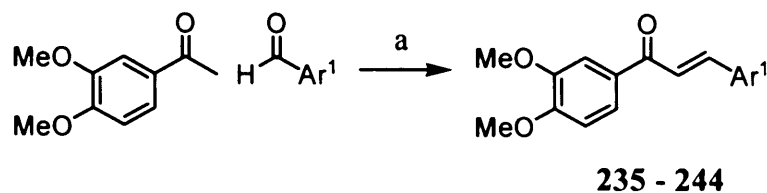


Figure 28: ¹H NMR of indanone 229

A small series of chalcones **235** – **244** were made from 3,4-dimethoxyacetophenone and various benzaldehydes using the Claisen-Schmidt condensation¹⁷⁸ (Scheme 32). All of the chalcones were isolated as the *E* isomers after recrystallization from methanol. The geometry of the chalcones was as usual determined by the alkene coupling value $\approx 15 - 16$ Hz in the ¹H NMR spectra of the chalcones.

235	4-Methoxyphenyl	32
237	4-Chlorophenyl	34
240	1,6-Dichlorophenyl	71
241	3,5-Dimethoxyphenyl	41
244	3,4-Dichlorophenyl	13

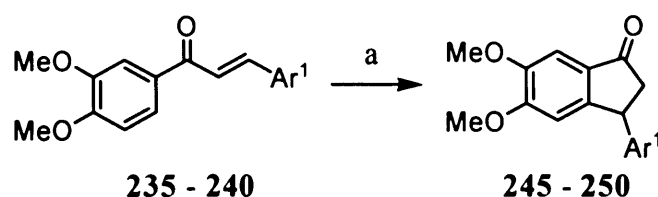


Scheme 32: a. NaOH (2M) (aq), MeOH, r.t., overnight, yield (see *table 5*).

Table 5: Yields of chalcones **235 – 244**(see *scheme 32*).

Chalcone	Ar ¹	Yield (%)
235	4-Methoxyphenyl	67
236	4-Bromophenyl	76
237	4-Chlorophenyl	76
238	2,6-Dichlorophenyl	77
239	3,4,5-Trimethoxyphenyl	90
240	3,4-Dichlorophenyl	57
241	2,4,6-Trimethoxyphenyl	61
242	2,4-Dimethoxyphenyl	79
243	3,5-Dimethoxyphenyl	62
244	2,6-Dimethoxyphenyl	61

The dimethoxyindanones **245 – 250** were then prepared from their precursor chalcones **235 – 240** using the same microwave conditions as previously described (*Scheme 33*) and isolated by column chromatography. The 3,4-dimethoxychalcones **241 – 244** were also used in the microwave synthesis of the dimethoxyindanones, however these four reactions failed and were not further investigated. Again these examples have electron rich B-rings (see later).



Scheme 33: a. TFA, μ wave, 120 °C (100 W), 20 mins, yield (see *table 6*).

Table 6: Yields of indanones **245 – 250** via microwave assisted reaction (see *scheme 33*).

Indanone	Ar ¹	Yield (%)
245	4-Methoxyphenyl	7
246	4-Bromophenyl	52
247	4-Chlorophenyl	34
248	2,6-Dichlorophenyl	71
249	3,4,5-Trimethoxyphenyl	41
250	3,4-Dichlorophenyl	13

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

The ^1H NMR spectra of the indanones **245** – **247** and **249** – **250** all showed the key peaks and coupling patterns for the H-2 and H-3 atoms as previously described for indanone **153**. However the indanone **248** 2,6-dichlorophenyl B ring inhibited the free rotation of the phenyl around the C-3–C-1'' bond, again causing the hydrogens at position H-3' and H-5' on the B ring to become non equivalent. The ^1H NMR spectrum of indanone **248** shows that one of the hydrogens at position H-3' or H-5' occurs at 7.43 (1H, dd, J 1.4, 7.8 Hz) ppm the other signal for the hydrogen at position H-3' or H-5' occurs under the multiplet at 7.16 – 7.23 ppm (Figure 29). Also the hydrogen H-3 has its peak shifted down field from around 4.50 ppm to 5.43 ppm by reduced shielding effect of the bulky 2,6-dichlorophenyl B ring of **248**, similar to same affect seen in the hindered indanones **228** – **230**.

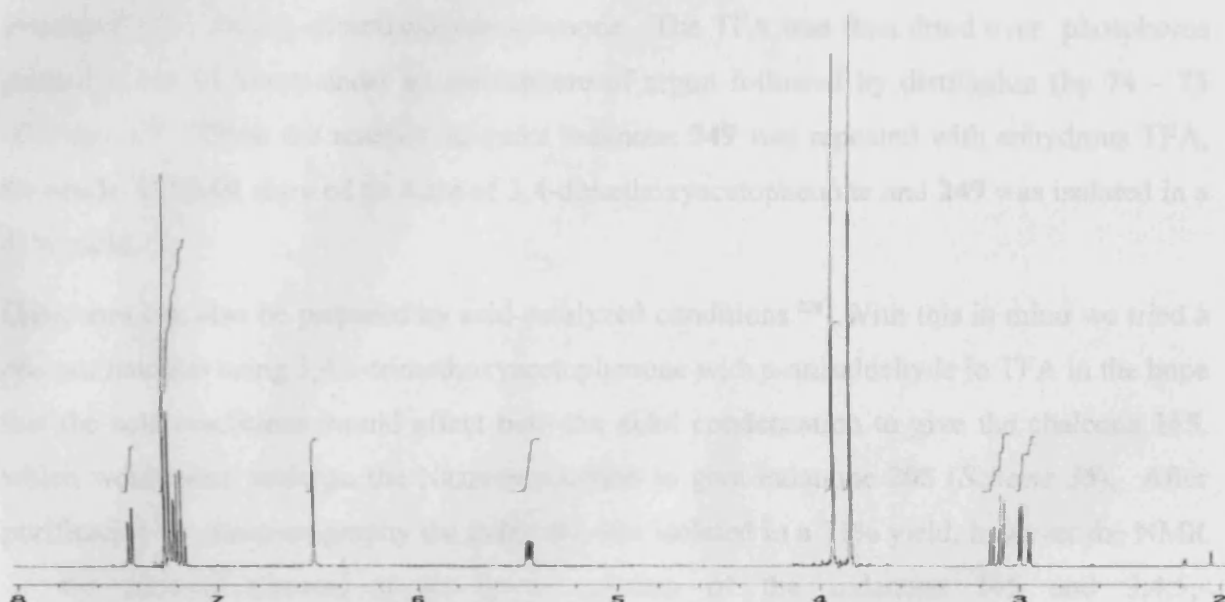
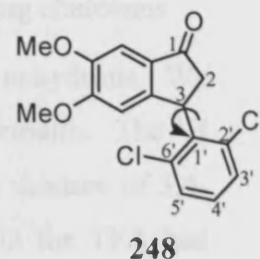
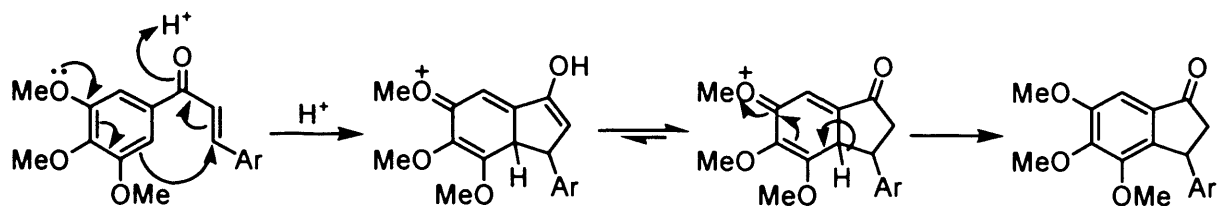


Figure 29: ^1H NMR of indanone **248**

Inspection of the tables 4 and 6 show that the Nazarov cyclization works best for the electron-deficient B-ring systems like **229**, **230** and **248**. The presence of an electron donating *p*-alkoxy group on the B-ring like **227**, **228** and **245** has a detrimental effect on the yield of the indanone. The detrimental effect on the indanone formation of electron donating *p*-alkoxy group on the B-ring was further shown in the failed indanone formations from chalcones **218** – **219** and **241** – **243**. This is expected because the Nazarov cyclization is an electrophilic substitution process which requires an electron-rich A-ring system which can donate electrons to an electron deficient enone reaction partner (Scheme 34). So the methoxy groups on the A ring are not only necessary for good tubulin binding properties but also increase the precursor chalcone reactivity in the cyclization.

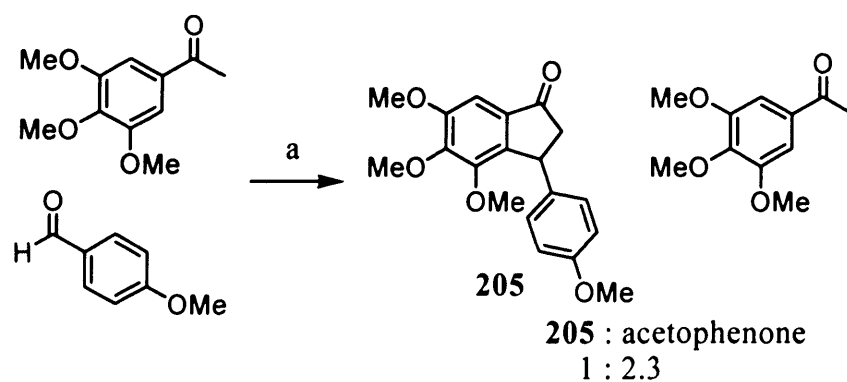
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



Scheme 34: The mechanism of the Nazarov cyclization of electron-rich A-ring chalcones

It was found that the TFA used in the microwave reaction should ideally be anhydrous. We found that the synthesis of indanone **249** using TFA as supplied was problematic. The ^1H NMR spectrum of the crude product of **249** showed the formation of a 2.5:1 mixture of 3,4-dimethoxyacetophenone and **249**. It appeared that the presence of water in the TFA had caused the precursor chalcone **249** to undergo a water promoted retro-Michael retro-aldol process to give the 3,4-dimethoxyacetophenone. The TFA was then dried over phosphorus pentoxide for 12 hours under an atmosphere of argon followed by distillation (bp 74 – 75 $^\circ\text{C}/1$ atm.).²²³ When the reaction to make indanone **249** was repeated with anhydrous TFA, the crude ^1H NMR showed no trace of 3,4-dimethoxyacetophenone and **249** was isolated in a 41% yield.

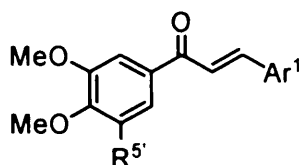
Chalcones can also be prepared by acid-catalyzed conditions.²²⁴ With this in mind we tried a one-pot reaction using 3,4,5-trimethoxyacetophenone with *p*-anisaldehyde in TFA in the hope that the acid conditions would affect both the aldol condensation to give the chalcone **165**, which would then undergo the Nazarov reaction to give indanone **205** (*Scheme 35*). After purification by chromatography the indanone was isolated in a 31% yield, however the NMR of the product showed it to be a mixture of the indanone **205** and 3,4,5-trimethoxyacetophenone in a ratio of 1:2.3 respectively. Further purification of the indanone was not trivial and this method was not investigated any further. For comparison the two step synthesis of indanone **205** give an overall yield of 54%, 90% for the chalcone formation and 60% for the Nazarov cyclization.



Scheme 35: a. TFA, μwave , 120 $^\circ\text{C}$ (100 W), 20 mins, 31%.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

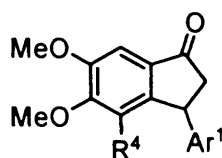
3.3.1.1 Biological results for the indanones and precursor chalcones



Chalcone	R ^{5'}	Ar ¹	IC ₅₀ (K562) (μM)
117	OMe	3-Hydroxy-4-methoxyphenyl	0.26
155	OMe	3-Fluoro-4-methoxyphenyl	0.30 ^o
165	OMe	4-Methoxyphenyl	1.6
179	OMe	Phenyl	9.4
206	OMe	4-Chlorophenyl	> 10
207	OMe	3-Bromo-4-methoxyphenyl	6.57
208	OMe	3,4-Dichlorophenyl	7.0
209	OMe	Benzo[<i>d</i>][1,3]dioxol-5-yl	1.48
210	OMe	4-Bromophenyl	> 10
211	OMe	Phenyl-4-oxyacetic acid	> 10
212	OMe	2,3,4-Trimethoxyphenyl	3.67
213	OMe	2,6-Dichlorophenyl	4.3
214	OMe	2,4-Dichlorophenyl	6.71
215	OMe	3-Nitro-4-methoxyphenyl	4.0 ^o
216	OMe	4-(Benzyloxy)phenyl	> 10
217	OMe	2,3-Dihydrobenzo[<i>b</i>][1,4]dioxin-6-yl	0.08 [†]
218	OMe	3,4,5-Trimethoxyphenyl	> 10
219	OMe	2,4-Dimethoxyphenyl	1.66
220	OMe	3,5-Dimethoxyphenyl	0.64
235	H	4-Methoxyphenyl	> 10
236	H	4-Bromophenyl	> 10
237	H	4-Chlorophenyl	8.9
238	H	2,6-Dichlorophenyl	1.2
239	H	3,4,5-Trimethoxyphenyl	5.0
240	H	3,4-Dichlorophenyl	> 10
241	H	2,4,6-Trimethoxyphenyl	1.94
242	H	2,4-Dimethoxyphenyl	9.76
243	H	3,5-Dimethoxyphenyl	6.31
244	H	2,6-Dimethoxyphenyl	4.64

^o Prepared by Dr. B. Greedy, Post doctoral research assistant, Cardiff, 2003

[†] Prepared by S. Ducki, PhD Student, UMIST, 1997



Indanone	R ⁴	Ar ¹	IC ₅₀ (K562) (μM)
153	OMe	3-Hydroxy-4-methoxyphenyl	0.13
154	OMe	3-Fluoro-4-methoxyphenyl	0.105
205	OMe	4-Methoxyphenyl	0.49
222	OMe	4-Chlorophenyl	30
223	OMe	3-Bromo-4-methoxyphenyl	1.2
224	OMe	3,4-Dichlorophenyl	24
225	OMe	Benzo[<i>d</i>][1,3]dioxol-5-yl	2.41
226	OMe	4-Bromophenyl	1.85
227	OMe	Phenyl-4-oxyacetic acid	> 10
228	OMe	2,3,4-Trimethoxyphenyl	2.78
229	OMe	2,6-Dichlorophenyl	7.37
230	OMe	2,4-Dichlorophenyl	1.97
231	OMe	Phenyl	> 10
232	OMe	3-Nitro-4-methoxyphenyl	0.081
233	OMe	4-Hydroxyphenyl	> 10
234	OMe	2,3-Dihydrobenzo[<i>b</i>][1,4]dioxin-6-yl	0.22
245	H	4-Methoxyphenyl	0.17
246	H	4-Bromophenyl	5.35
247	H	4-Chlorophenyl	> 10
248	H	2,6-Dichlorophenyl	> 10
249	H	3,4,5-Trimethoxyphenyl	34
250	H	3,4-Dichlorophenyl	> 10

Generally the chalcones with the 3,4,5-trimethoxyphenyl A rings tend to show more biological activity against the leukaemia cell line K562 than their 3,4-dimethoxyphenyl A ring analogues. For instance looking at the two chalcones with 4-methoxyphenyl B ring **165** and **235**, the 3,4,5-trimethoxyphenyl A ring chalcone **165** has much higher IC₅₀(K562) value of 1.6 μM, compared to the 3,4-dimethoxyphenyl A ring chalcone **235**, which has an IC₅₀(K562) value of >10 μM. This shows that the presence of the 3,4,5-trimethoxyphenyl A ring is an important feature of the overall cytotoxicity of the chalcone. However the 3,4-dimethoxyphenyl A ring chalcones with either a 4-chlorophenyl B ring **237** [IC₅₀(K562) = 8.9 μM] or a 2,6-dichlorophenyl B ring **238** [IC₅₀(K562) = 1.2 μM] show a higher biological activity than their 3,4,5-trimethoxyphenyl A ring chalcone equivalents with a 4-chlorophenyl B ring **206** [IC₅₀(K562) > 10 μM] and 2,6-dichlorophenyl B ring **213** [IC₅₀(K562) = 4.3 μM].

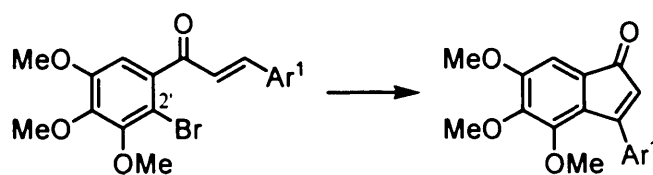
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

In some cases the indanones show increased biological activity over their precursor chalcones. For instance the indanones **232** [$IC_{50}(K562)$ 0.081 μ M] and **245** [$IC_{50}(K562)$ 0.17 μ M] show a significant increase in biological activity compared to their precursor chalcones **215** [$IC_{50}(K562)$ 4.0 μ M] and **235** [$IC_{50}(K562)$ >10 μ M]. However this is not always the case. The indanones **225** [$IC_{50}(K562)$ 2.41 μ M] and **234** [$IC_{50}(K562)$ 0.22 μ M] both show a decrease in the biological activity when compared to their precursor chalcones **209** [$IC_{50}(K562)$ 1.48 μ M] and **217** [$IC_{50}(K562)$ 0.08 μ M].

The biological results of the indanones **153**, **154**, **205**, **232**, **234** and **245**, show that they are all potent inhibitors of K562 cell growth with IC_{50} values less than 0.5 μ M.

3.3.2 Indenones - Synthesis, Results and Discussion

The indenone scaffold is another conformationally constrained analog of the CA-4 like chalcones locked in the *s-trans* arrangement. It retains an α,β -unsaturated enone and lacks the indanone stereogenic centre and is therefore worthy of study. Clark and co-workers used an internal Heck reaction to form the Indenone scaffold from a 2'-bromo chalcone.²²⁵ Using the same idea the key step in making the CA-4 like Indenone scaffold will be the preparation of the precursor chalcone and the internal Heck reaction of a 2'-bromo chalcone (*Scheme 36*).

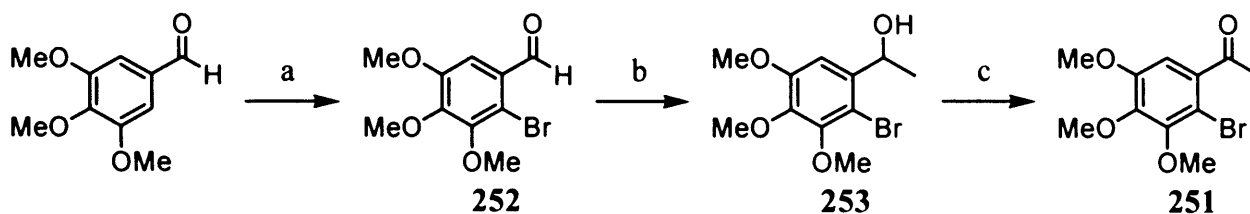


Scheme 36

To make the 2'-bromo chalcone precursors we first had to prepare the 2'-bromoacetophenone **251**. We set about doing this by first refluxing 3,4,5-trimethoxybenzaldehyde with *N*-bromo succinimide (NBS) in chloroform for 4 hours to give the 2-bromo-3,4,5-trimethoxybenzaldehyde (**252**), which was isolated after column chromatography in a good yield (*Scheme 37*).²²⁶ We then used commercially available 3 molar methylmagnesium chloride in THF to carry out the Grignard reaction on **252** to give the secondary alcohol **253** as pale yellow oil in an excellent yield (*Scheme 37*). We originally tried to make the Grignard reagent *insitu* by reacting iodomethane with magnesium turnings in anhydrous THF, however this caused a grey precipitate to form. Since methylchloride is a gas we thought the preparation of methylmagnesium chloride would be troublesome. The alcohol **253** was then oxidized with pyridinium chlorochromate (PCC) to give the desired acetophenone **251** in a good yield (*Scheme 37*). The PCC oxidation was carried out in the presence of silica gel (5 times the mass of PCC) to stop

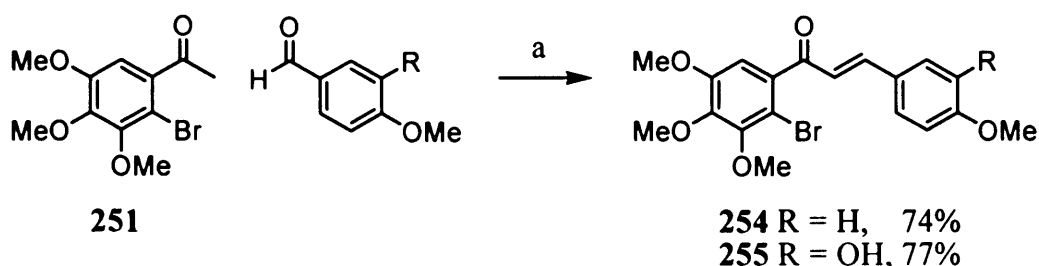
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

the reaction from becoming too viscous, by providing a support upon which reduced chromium species can precipitate. The reaction work-up is also convenient as the solvent can simply be removed to provide the crude reaction product supported on silica for use directly on a chromatography column.



Scheme 37: a. NBS, CHCl₃, 75 °C, 4 hours, 83%; b. MeMgCl (3M in THF), THF, 0 °C to r.t., 12 hours, 99%; c. PCC, DCM, r.t., 12 hours, 83%.

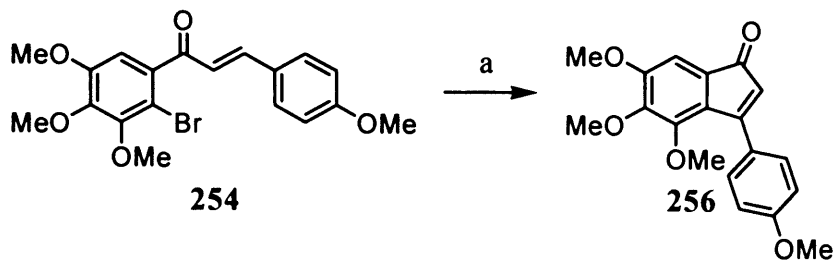
The two chalcones **254** and **255** were prepared using the Claisen-Schmidt condensation¹⁷⁸ of **251** and their corresponding benzaldehyde, and purified by recrystallization from methanol (*Scheme 38*). The chalcones were only isolated as *E* isomers, as shown by the usual alkene coupling value $\approx 15 - 16$ Hz in the ¹H NMR spectra.



Scheme 38: a. NaOH (2M) (aq), MeOH, r.t., overnight.

Then using the conditions described by Clark²²⁵ the internal Heck reaction was carried out on 1 mmol scale of chalcone **254** (*Scheme 39*). After purification by chromatography and recrystallization from ethyl acetate and hexane the indenone **256** was isolated in a high yield as bright red crystals. The key diagnostic peak of the indenone **256** is singlet at 5.73 ppm for the alkene hydrogen H-2 (*Figure 30*). The reaction was repeated using chalcone **255**, however no indenone was isolated and only starting material was recovered. This prompted us to investigate the use of various protecting groups to mask the phenol group, which we believe was the cause of the failure to make the indenone.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion



Scheme 39: a. PdCl₂ (2.5 mol%), PPh₃ (7.5 mol%), K₂CO₃, DMF, 110 °C, 30 mins, 88%.

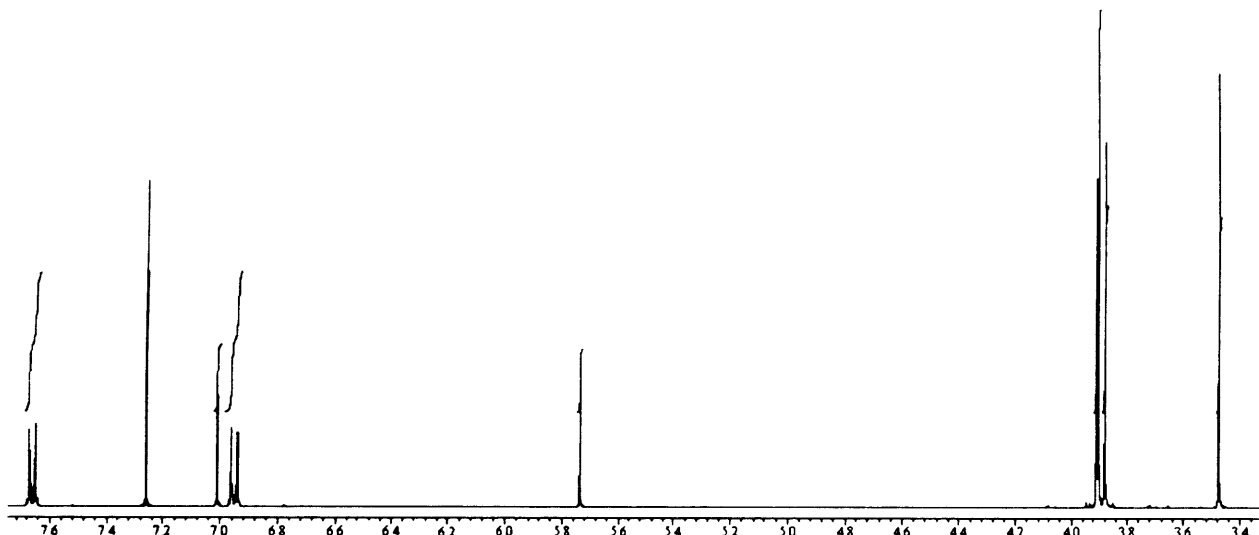
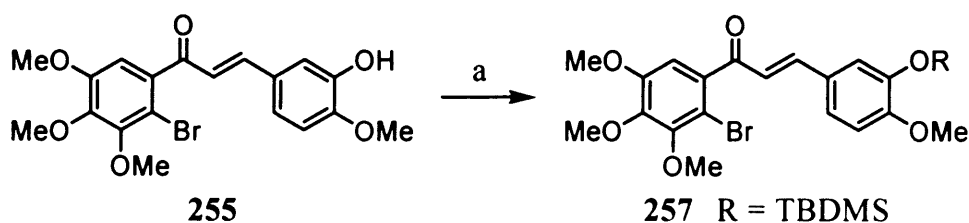


Figure 30: ¹H NMR spectrum of indenone **256**

Several protected chalcones **257** – **260** were prepared. The chalcone **255** was protected with *tert*-butylchlorodimethylsilane chloride (TBDMSCl), by reaction with TBDMSCl for 3 days at room temperature. The protected chalcone **257** was isolated via column chromatography in a good yield as a pale yellow oil that eventually solidified (*Scheme 40*).

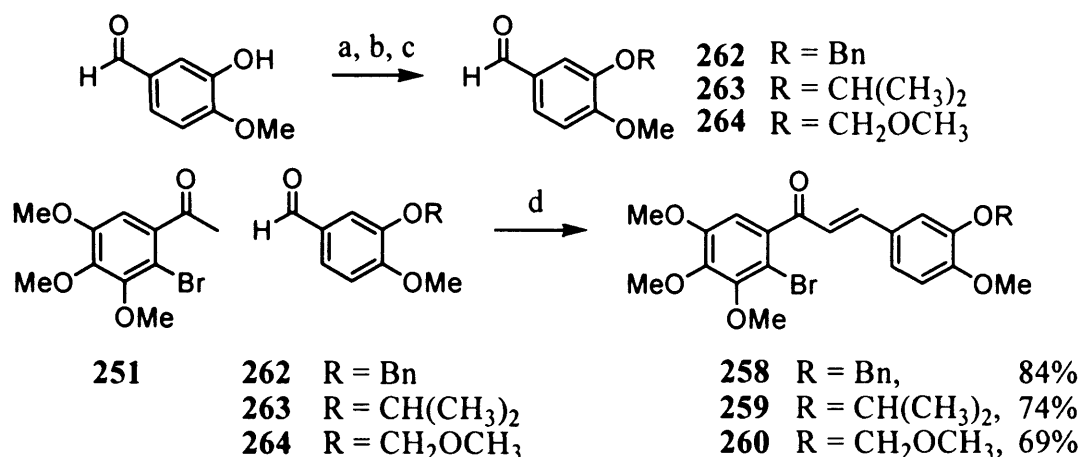


Scheme 40: a. TBDMSCl, imidazole, DMF, r.t., 3 days, 89%;

The intramolecular Heck reaction²²⁵ of the TBDMS protected chalcones **257** was then carried out (*Scheme 42*). Unfortunately the preparation of the protected indenone **261** failed and the unprotected chalcone **255** was recovered. Clearly the TBDMS group is not suitable as a phenol protecting group for the reaction conditions used.

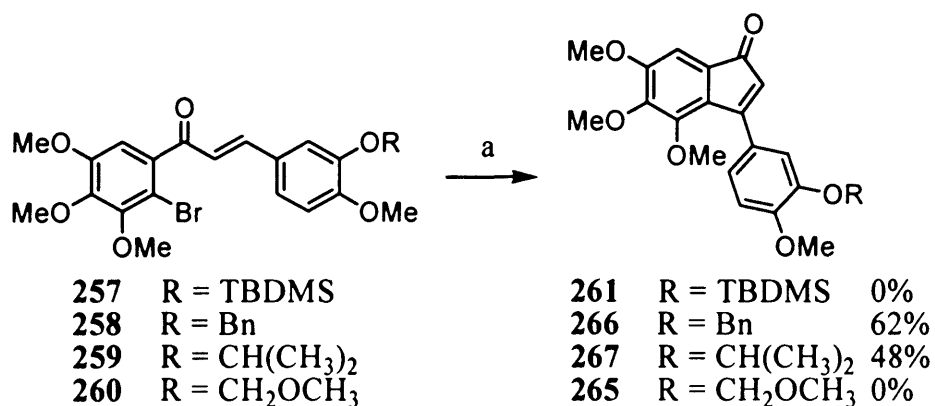
Other protected chalcones were therefore required. Three protected versions of the isovanillin starting material were prepared, benzyl protected²²² **262**, isopropyl protected²²⁷ **263**

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion
 and methoxymethyl (MOM) protected²²⁸ **264** (Scheme 41). These protected benzaldehydes were then reacted with **251** using the Claisen-Schmidt condensation¹⁷⁸ to make the protected chalcones **258** – **260**. Chalcones **258** – **260** were all isolated as pure *E* isomers after recrystallization from methanol.



Scheme 41: a. Benzyl bromide, K₂CO₃, DMF, 90 °C, 2 hours, 99%; b. Isopropyl bromide, K₂CO₃, DMF, r.t. 2 days, 88%; c. MOMCl, NaH, DMF, 0 °C to r.t., 88%; d. NaOH (2M) (aq), MeOH, r.t., overnight.

After heating the MOM protected chalcone **260** in the previous conditions used for the intramolecular Heck reaction²²⁵ for 30 minutes the reaction had not gone to completion (*Scheme 42*). So the mixture was heated at 110 °C for 8 hours, however after this time the reaction still had not gone to completion and the ¹H NMR spectrum of the crude product showed that it was a 1:1 mix of chalcone **260** and indenone **265**. The separation of **260** and **265** by column chromatography proved to be hard because the 2 compounds co-spotted on the TLC plate.

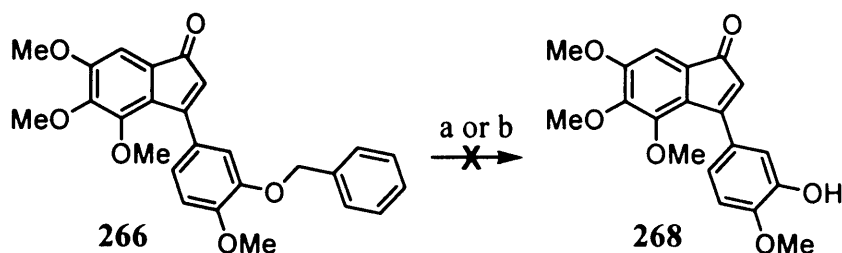


Scheme 42: a. PdCl₂ (2.5 mol%), PPh₃ (7.5 mol%), K₂CO₃, DMF, 110 °C, 30 mins;

The benzyl protected indenone was prepared from **258** using the intramolecular Heck reaction²²⁵ previously described as a red solid which was isolated via chromatography in a

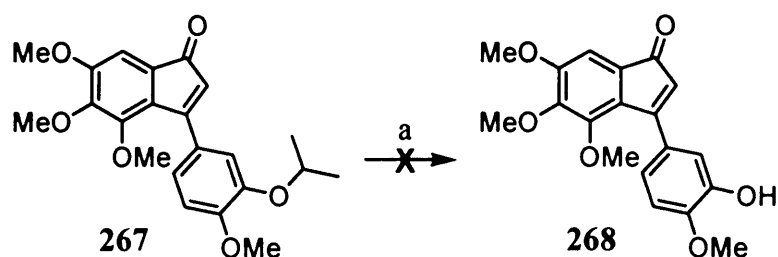
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

good yield (*Scheme 42*). To remove the benzyl group from the indenone **266** hydrogenation with 10% palladium on carbon and hydrogen was attempted (*Scheme 43*). Unfortunately this reaction failed and the starting indenone **266** was recovered. We then tried using trimethylsilyl iodide²²⁹ to cleave the benzyl ether. After ten minutes the reaction was quenched with methanol and was worked up. Unfortunately the ¹H NMR spectrum showed that the compound had decomposed and neither starting material nor product could be recovered from the mixture (*Scheme 43*). Trimethylsilyl iodide cleaves ether groups, so it may have reacted with the methoxy groups on the indenone as well as the benzyl ether.



Scheme 43: a. H₂, 10% Pd/C, EtOAc, r.t., 30 mins; b. Me₃SiI, CHCl₃, r.t., 10 mins.

The intramolecular Heck reaction²²⁵ was carried out on the isopropyl protected chalcone **259** to prepare the isopropyl-protected indenone **267** in a moderate yield as red crystals after recrystallization from ethyl acetate and hexane (*Scheme 42*). We then tried to deprotect the isopropyl-protected indenone **267** by treating it with 1M boron trichloride in heptane (*Scheme 44*).²²⁷ Unfortunately the reaction caused the compound **267** to decompose and no phenol could be recovered from the resulting reaction product.

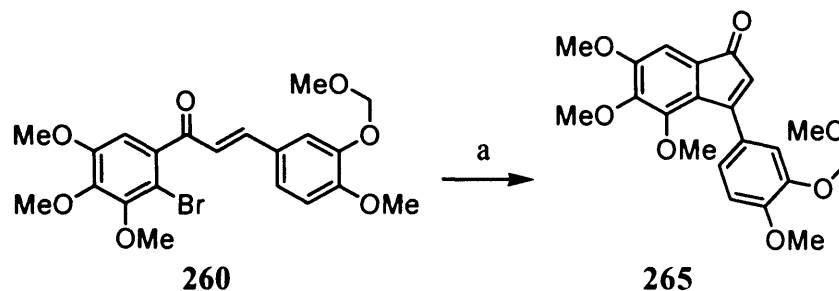


Scheme 44: a. 1M BCl₃ in heptane, EtOAc, 0 °C, 6 hrs.

The preparation of the MOM-protected indenone **265** was then attempted again, this time using microwave irradiation instead of traditional heating, to see if the reaction could be pushed to completion. The reaction was performed in a microwave vessel at 160 °C, 150 W, for 15 minutes (*Scheme 45*). The reaction proved to be capricious, one attempt on a 100 mg scale gave a 91% crude yield of **265** and another time using the same conditions but on a larger scale of 260 mg the reaction did not go to completion. After careful column chromatography using the Flashmaster system only 19% of the indenone **265** was isolated

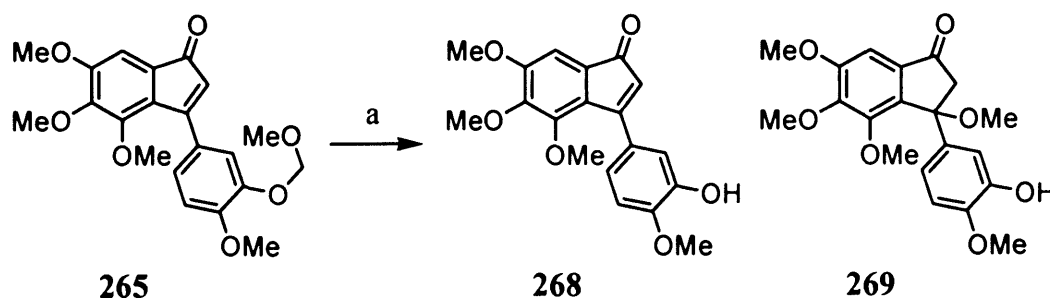
Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

from the chalcone **260**/indenone **265** mix. More indenone could possibly have been isolated from the mixture by repeat chromatography (*Scheme 45*). Maybe if the reaction was repeated for a longer time on a 260 mg scale the reaction would have gone to completion.



Scheme 45: a. PdCl₂ (2.5 mol%), PPh₃ (7.5 mol%), K₂CO₃, DMF, μ wave, 160 °C (150 W), 15 mins, 100 mg scale crude 91%, 260 mg scale 19%.

The deprotection of the MOM-protected indenone **265** was carried out by refluxing the compound in methanol and in the presence of 3 M hydrochloric acid for 45 minutes (*Scheme 46*).²³⁰ This deprotection method gave only a 12% yield of indenone **268**, however it also gave the side product **269** in a 30% yield corresponding to the addition of methanol across the C2-C3 double bond. The ¹H NMR spectrum of **269** showed the presence of an extra methoxy signal, also the key diagnostic peak of the alkene hydrogen H-2 of the indenone **268** which appears at 5.73 ppm was no longer present in the spectrum of **269** indicating that the alkene bond was no longer present in the molecule. The signal at 51.73 ppm in the ¹³C NMR and DEPT spectrum of **269** showed that the molecule possesses a methylene group, this was further confirmed by the key diagnostic peaks of a AB system that occur at δ 2.92 (1H_a, d, *J* 19.2 Hz, H-2a), 3.17 (1H_b, d, *J* 19.2 Hz, H-2b) ppm in the ¹H NMR spectrum. The HRMS of **269** also showed the molecular weight for the [MH]⁺ ion to be 375.14396, HRMS which confirms that the molecule has undergone the addition of methanol, when compared to HRMS **268** found [MH]⁺ 343.11840 for C₁₉H₁₉O₆, where the difference in weight is 36 (CH₃OH).

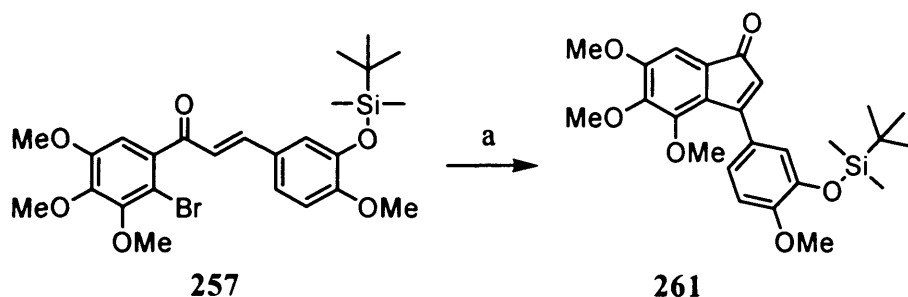


Scheme 46: a. 3M HCl (aq), MeOH, 75 °C, 45 mins, **268** 12%, **269** 30%;

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

Seeing that the microwave assisted Heck reaction of the MOM-protected chalcone **260** was successful we then attempted the same reaction but with the TBDMS protected chalcone **257**. Using these Heck reaction conditions, the chalcone **257** was heated in a microwave reactor at 160 °C (150 W) for 15mins, giving a 81% crude yield of indenone **261** which was then taken without any further purification to be deprotected (*Scheme 47*).

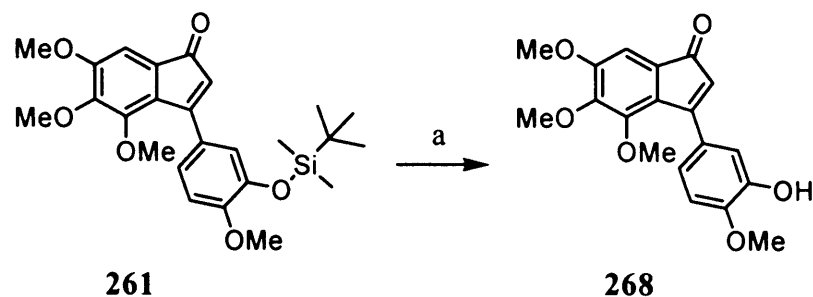
The indenone **261** was fully characterised with API ES+ MS showing a molecular ion $[MH^+]$ at m/z 457, corresponding to a molecular formula of $C_{25}H_{33}O_6Si$. 1H NMR spectroscopy further supported the formation of the indenone **261** with the key indenone diagnostic peak appearing at δ 5.71 (1H, s, H-2) ppm.



Scheme 47: a. $PdCl_2$ (2.5 mol%), PPh_3 (7.5 mol%), K_2CO_3 , DMF, μ wave, 160 °C (150 W), 15 mins, crude 81%.

This transformation of **257** into **261** also proved to be capricious. When it was repeated on a slightly larger scale of 160 mg the results varied dramatically from the original reaction. After purification, in this case by column chromatography only a 24% yield of the TBDMS protected indenone **261** was isolated, however the unprotected indenone **268** was also isolated in a 25% yield.

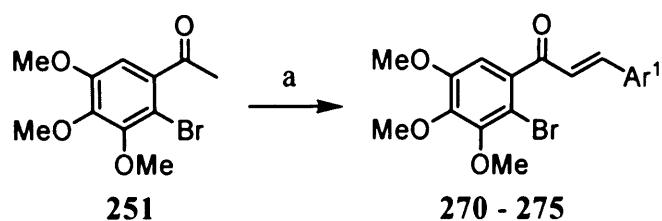
The TBDMS-protected indenone **261** was then treated with tetrabutylammonium fluoride (TBAF) in THF at 0 °C to give the unprotected indenone **268** in a good yield (*Scheme 48*). The indenone **268** was fully characterised with API ES+ MS showing a molecular ion $[MH^+]$ at m/z 343, corresponding to a molecular formula of $C_{19}H_{19}O_6$. 1H NMR spectrum further supported the formation of the indenone **261** with the key indenone diagnostic peak appearing at δ 5.73 (1H, s, H-2) ppm.



Scheme 48: a. 1M TBAF in THF, THF, 0 °C, 30 mins, 66%.

A small series of 2'-bromochalcones **270** – **275** were prepared to make a small library of compounds with the indenone scaffold. They were prepared using the typical Claisen-Schmidt condensation¹⁷⁸ method previous mentioned and isolated in good yields as crystals after recrystallization from methanol (*Scheme 49*). The ¹H NMR spectra of the compounds showed that they were isolated as the pure *E* isomer by their alkene coupling value $\approx 15 - 16$ Hz. The fluoro chalcone **271** ¹H NMR spectrum showed a more complex coupling pattern due to the fluorine hydrogen coupling on its aromatic B ring (*Figure 31*), with with ¹H NMR signals at 6.97 (1H, d, *J* 8.4 Hz, H-5''), 7.29 – 7.33 (1H, dm, *J* 9.0 Hz, H-6'') and 7.36 (1H, dd, *J* 2.0 Hz, *J* 12.0

Hz, H-2'') ppm.



Scheme 49: a. Ar¹CHO, NaOH (2M) (aq), MeOH, r.t., overnight.

Chalcone	Ar ¹	%
270	3-Nitro-4-methoxyphenyl	70
271	3-Fluoro-4-methoxyphenyl	84
272	Phenyl	71
273	3,5-Dimethoxyphenyl	73
274	3,4,5-Trimethoxyphenyl	79
275	3,4-Dichlorophenyl	71

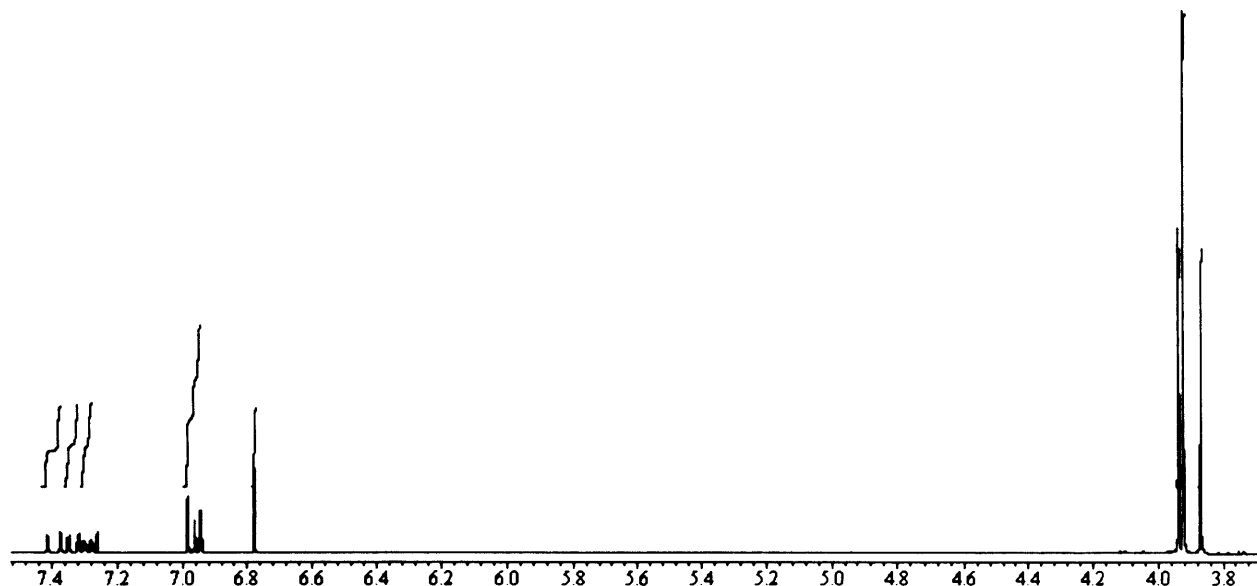
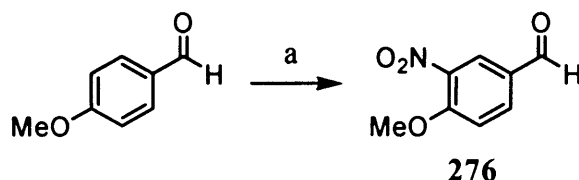


Figure 31: ^1H NMR of chalcone 271

To make chalcone 270 the 4-methoxy-3-nitrobenzaldehyde (276) was prepared from *p*-anisaldehyde by nitration with concentrated nitric acid and concentrated sulfuric acid at $-15\text{ }^\circ\text{C}$ (Scheme 50). The benzaldehyde 276 was isolated after an hour by pouring the reaction into ice cold water. The benzaldehyde 276 that precipitated as a light brown solid was then filtered off, recrystallized from ethanol and then dried *in vacuo*. This benzaldehyde was then used to prepare chalcone 270.

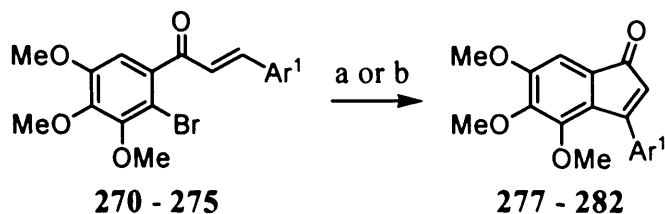


Scheme 50: a. HNO_3 , H_2SO_4 , $-15\text{ }^\circ\text{C}$, 1 hour, 69%.

Each of the 2'-bromochalcones 270 – 275 were then treated with the conditions described by Clark²²⁵ for the intramolecular Heck reaction, on a 1 mmol scale (Scheme 51, route a). This route worked well for all the chalcones, except for nitro chalcone 270 and dichloro chalcone 275. The indenones 277 – 280 were isolated pure after column chromatography and recrystallization from EtOAc and hexane. The formation of the dichloro indenone 281 was problematic because the reaction did not go to completion and the ^1H NMR spectrum of the crude product showed that it was a mixture of the starting chalcone 275 and the indenone 281. Unfortunately both chalcone 275 and indenone 281 were not resolved by TLC, however we did manage to isolate the indenone by recrystallization from ethyl acetate and hexane in a 31% yield of peach coloured fluffy crystals. The reaction to make the nitro indenone 282 via traditional heating techniques did not work and the starting chalcone was recovered. We next tried the microwave conditions used to make the MOM and TBDMS protected indenone 265

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

and **261** on the chalcone **270** (Scheme 51, route b). The indenone **282** was prepared running the experiment on a 100 mg scale of the chalcone **270** five times and then combining the crude mixtures and working them up together. After recrystallization from ethyl acetate and hexane **282** was isolated as red crystals in a 31% yield.

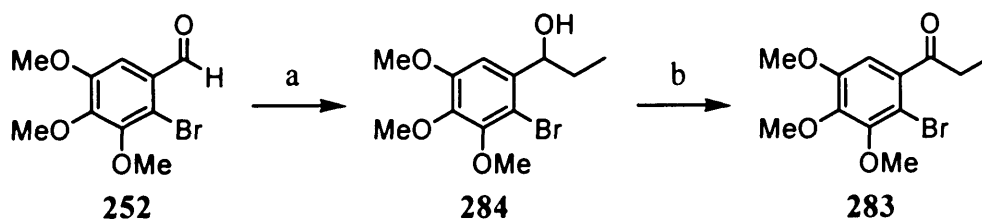


Scheme 51: a. PdCl₂ (2.5 mol%), PPh₃ (7.5 mol%), K₂CO₃, DMF, 110 °C, 30 mins, yield (see table 7); b. PdCl₂ (2.5 mol%), PPh₃ (7.5 mol%), K₂CO₃, DMF, μ wave, 160 °C (150 W), 15 mins, yield (see table 7).

Table 7: Yields of indenones **277** – **282** (see scheme 51).

Indenones	Route	Ar ¹	%
277	a	3-Fluoro-4-methoxyphenyl	70
278	a	Phenyl	65
279	a	3,5-Dimethoxyphenyl	54
280	a	3,4,5-Trimethoxyphenyl	63
281	a	3,4-Dichlorophenyl	32
282	b	3-Nitro-4-methoxyphenyl	31

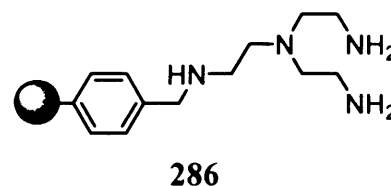
We then wanted to investigate the effects of replacing the α -hydrogen with a methyl on the indenone scaffold, due to the marked improvement of the biological activity of chalcones such as **182**. The 2'-bromopropiophenone **283** was prepared via the Grignard addition of commercially available ethylmagnesium chloride in THF to the 2-bromobenzaldehyde **252** to make the secondary alcohol **284**. This alcohol was oxidized with PCC to give the desired 2'-bromo propiophenone **283** in a good yield (Scheme 52).



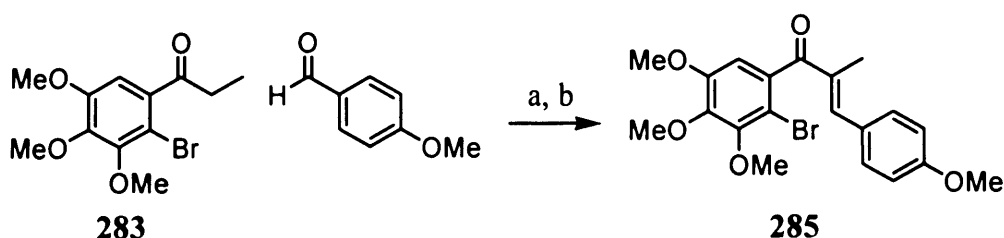
Scheme 52: a. EtMgCl (2M in THF), THF, 0 °C to r.t., 12 hours, 99%; b. PCC, DCM, r.t., 12 hours, 89%.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

The α -methylchalcone **285** was then prepared via the acid-catalyzed condensation method described by Edwards,⁷² by refluxing 2'-bromopropiophenone **283** and *p*-anisaldehyde in ethanol, glacial acetic acid and piperidine (*Scheme 53*). The reaction generates water which was removed from the reaction as the refluxing reaction condenses in the condenser tube packed with activated 3-Å molecular sieves. The reaction was then worked up and purified by chromatography. However the desired chalcone was isolated with *p*-anisaldehyde which co-eluted on the TLC plate. The ¹H NMR spectrum showed the ratio of α -methylchalcone **285** to *p*-anisaldehyde was 2.9:1. To remove the *p*-anisaldehyde from the desired α -methylchalcone **285** the mixture was shaken in deuterated chloroform in the presence of the scavenger agent PS-trisamine (**286**) at room temperature for 2 hours. PS-trisamine (**286**) can scavenge a range of compounds²³¹ including acid chlorides, aldehydes, epoxides and isocyanates. To scavenge benzaldehydes the primary amine groups of PS-trisamine (**286**) react with the carbonyl of the benzaldehyde forming imines. Deuterated chloroform was used as the solvent so that the reaction can be monitored by taking ¹H NMR spectra periodically. After which time the mixture was passed through a filter to remove the PS-trisamine to give the pure α -methylchalcone **285** as a yellow oil in a fair yield (*Scheme 53*).



The α -methylchalcone **285** was fully characterised with AP+ mass spectrometry showing a molecular ion [MH⁺] at *m/z* 421, corresponding to the molecular formula C₂₀H₂₂BrO₅. The ¹H NMR spectrum also supports the formation of the α -methylchalcone **285**, the key diagnostic peak being the β -H of the enone system appearing at δ 7.09 (1H, s, H-3) ppm. Similar α -methylchalcone **56**,^{177,191} **287** and **288**³⁶ prepared previously in our group and the α -methylchalcone **x23** were all isolated as the *E*-isomer with the β -H appearing 7.08 – 7.17 ppm (*Figure 32*). The β -H signal of the α -methylchalcone **285** appears at δ 7.09 ppm suggesting that the *E*-isomer of **285** has also been isolated.



Scheme 53: a. Piperidine, AcOH, EtOH, Ar, 110 °C, 7 days; b. PS-trisamine **286**, CHCl₃, r.t., 2 hours, 61% over 2 steps.

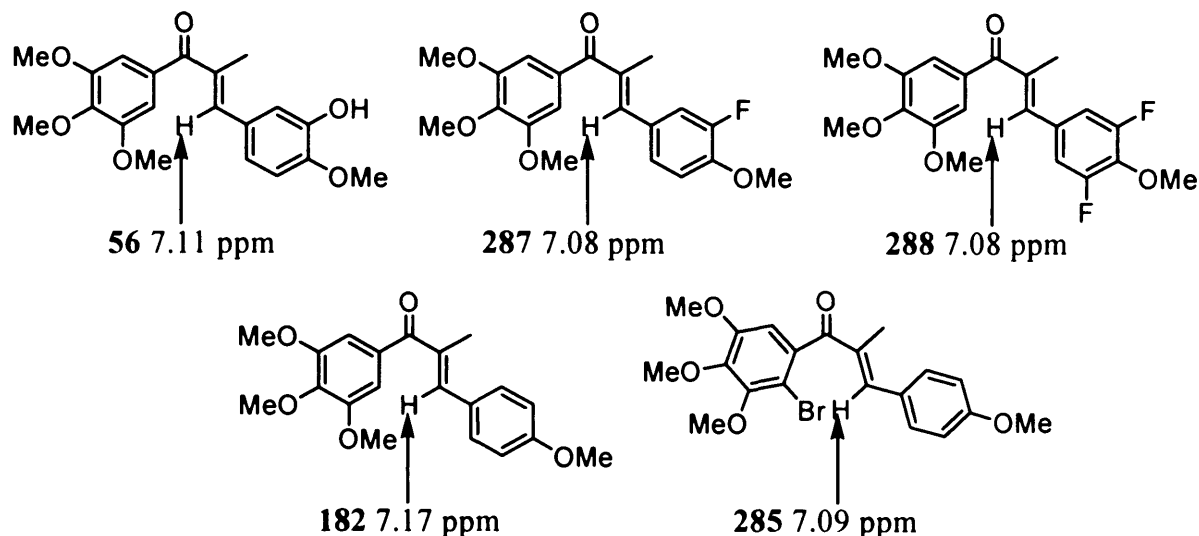
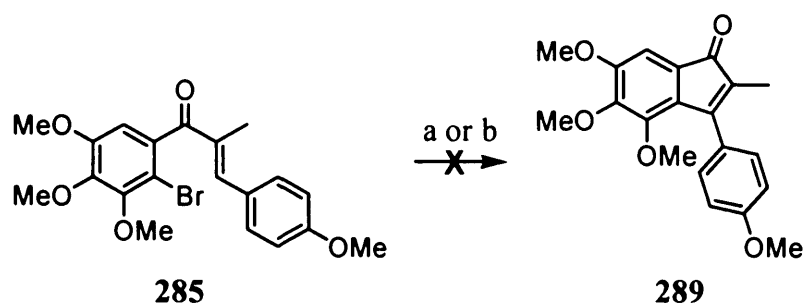


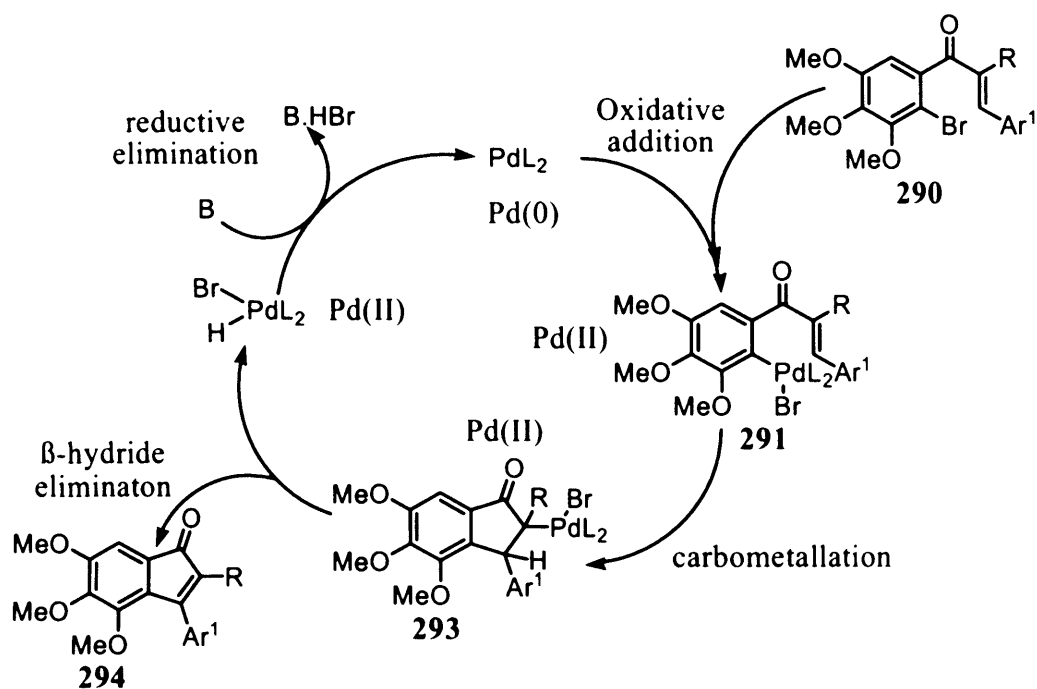
Figure 32: ^1H NMR signals for the $\beta\text{-H}$ of the $\alpha\text{-methylchalcone}$ chalcones

The Clark indenone formation technique,²²⁵ as previously described, was then applied to $\alpha\text{-methylchalcone}$ **285**. Unfortunately this reaction did not work and the $\alpha\text{-methylchalcone}$ **285** was recovered. The microwave indenone technique was then also tried, this reaction also failed and the starting material was recovered (Scheme 54).



Scheme 54: a. PdCl_2 (2.5 mol%), PPh_3 (7.5 mol%), K_2CO_3 , DMF, 110 $^\circ\text{C}$, 1 hour; b. PdCl_2 (2.5 mol%), PPh_3 (7.5 mol%), K_2CO_3 , DMF, μwave , 160 $^\circ\text{C}$ (150 W), 15 mins.

One possible reason why the internal Heck reaction of $\alpha\text{-methylchalcone}$ **285** failed, may be due to the difficulty in forming the carbometallation intermediate **293** (Scheme 55). The carbometallation intermediate of $\alpha\text{-methylchalcone}$ **285**, **295** (Figure 33) has two bulky groups attached to the C-2 position, the methyl group and the palladium (II) species, these will make the formation of this intermediate unfavourable due to steric hindrance of these groups. This is not a problem for the formation for indenones from the 2'-bromo-chalcones which only have a hydrogen on the $\alpha\text{-position}$ instead of a methyl group, because this gives a carbometallation intermediate **296** (Figure 33) has less steric hindrance from the interaction of the hydrogen and the palladium (II) species attached to the C-2 position which makes its formation more favourable than the formation of **295** (Figure 33).



Scheme 55: The internal Heck reaction mechanism for the formation of the indenones.

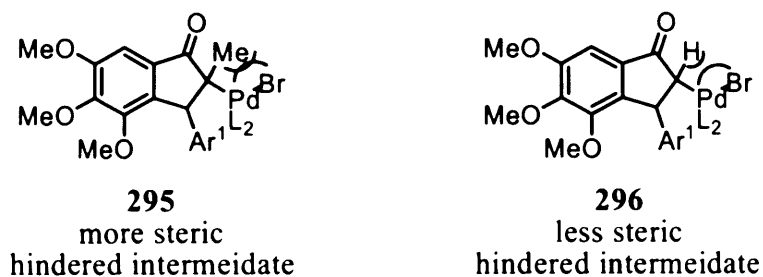
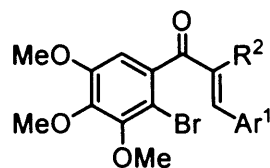


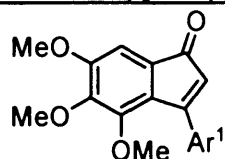
Figure 33: The steric hindrance of the carbometallation intermediate.

Chapter 3 – Combretastatin A-4 like chalcones and analogues. Results and Discussion

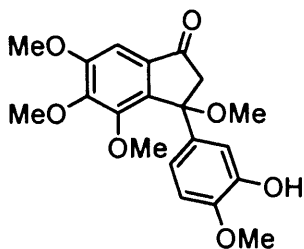
3.3.2.1 Biological results for the indenones and the precursor 2'-bromo-chalcones



Indanone	R ²	Ar ¹	IC ₅₀ (K562) (μM)
254	H	4-Methoxyphenyl	4.25
255	H	3-Hydroxy-4-methoxyphenyl	0.32
257	H	3- <i>tert</i> -Butyldimethylsilyloxy-4-methoxyphenyl	4.1
258	H	3-(Benzyloxy)-4-methoxyphenyl	>10
259	H	3-Isopropoxy-4-methoxyphenyl	3.2
260	H	4-Methoxy-3-(methoxymethoxy)phenyl	6.15
270	H	3-Nitro-4-methoxyphenyl	5.5
271	H	3-Fluoro-4-methoxyphenyl	7.6
272	H	Phenyl	8.1
273	H	3,5-Dimethoxyphenyl	7.4
274	H	3,4,5-Trimethoxyphenyl	>10
275	H	3,4-Dichlorophenyl	>10
285	Me	4-Methoxyphenyl	0.24



Indenone	Ar ¹	IC ₅₀ (K562) (μM)
256	4-Methoxyphenyl	0.090
261	3- <i>tert</i> -Butyldimethylsilyloxy-4-methoxyphenyl	1.86
266	3-(Benzyloxy)-4-methoxyphenyl	2.0
267	3-Isopropoxy-4-methoxyphenyl	3.32
265	4-Methoxy-3-(methoxymethoxy)phenyl	2.21
268	3-Hydroxy-4-methoxyphenyl	0.019
282	3-Nitro-4-methoxyphenyl	0.44
277	3-Fluoro-4-methoxyphenyl	0.037
278	Phenyl	7.6
279	3,5-Dimethoxyphenyl	> 10
280	3,4,5-Trimethoxyphenyl	1.4
281	3,4-Dichlorophenyl	0.6



IC ₅₀ (K562) (μM)	
269	0.024

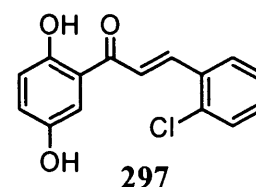
The biological results show that the addition of a bromine on the C2 position of the A ring of the chalcone generally decreases the biological activity against leukaemia cell line K562, for instance the α -methylchalcone **285** [IC₅₀(K562) = 0.24 μM] with the bromine on the C2 position of its A ring is much less effective against K562 than the α -methylchalcone **182** [IC₅₀(K562) = 0.010 μM] without the bromine. The conformationally *s-trans* locked indenones generally show an increased activity against K562 when compared against their 2'-bromochalcone precursors. This activity is also an improvement on their conformationally flexible chalcone analogues, for instance the activity of chalcone **165** [IC₅₀(K562) = 1.6 μM] is considerably increased when compared to its indenone **256** [IC₅₀(K562) = 0.090 μM]. As is expected from previous biological results the indenone **268** with the CA-4 like 3-hydroxy-4-methoxyphenyl B ring is the most active indenone prepared [IC₅₀(K562) = 0.019 μM] and is a potent inhibitor of K562 cells. It is a shame that the α -methyl indenone **289** could not be prepared because the addition of the methyl group alpha to the carbonyl of chalcones has shown a vast improvement on their biological activity,⁷³ and it would be interesting to see if this improvement also takes place on the indenones. The side product **269** from the deprotection of the MOM protected indenone **265** also has a high biological activity against K562 [IC₅₀(K562) = 0.024 μM] and further investigation into this product may lead to other compounds of interest, one area specifically that may give interesting results on this compound is the investigation of the separation of the two enantiomers of **269** to see what effects they have on its biological activity.

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

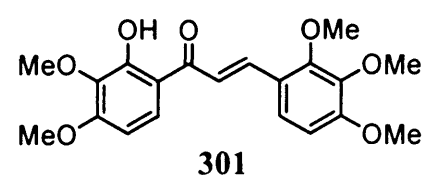
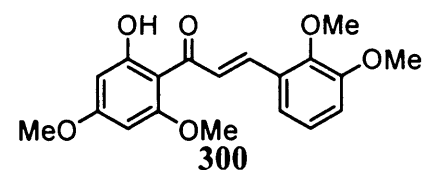
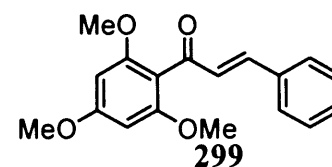
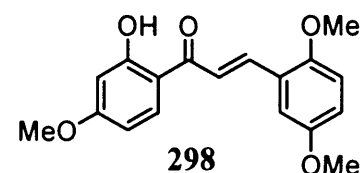
4.1 The Development of other Cytotoxic Chalcones²³²

The biological properties of the chalcone scaffold have been widely investigated and have shown a diverse range of pharmacological activities including cytotoxicity,²³² antitumour,²³² antiinflammatory,^{170,233,234} antiplasmodial,²³⁵ and antioxidant²³⁶ properties. There has been sustained interest in the cytotoxic properties of chalcones and in recent years a number of cytotoxic chalcones have been reported

Nam^{237,238} and co-workers prepared a 50+ library of 2',5'-dihydroxychalcones developed from the active extracts of the Vietnamese medicinal plant *Notopterygium incisum*.^{239,240} These chalcones were then tested against a variety of tumour cell lines including B16 murine melanoma, HCT 116 human colon cancer and A31 human epidermoid carcinoma to investigate their cytotoxicity. The majority of the 2',5'-dihydroxychalcones proved to have potent cytotoxicity against these cell lines, with the 2-chloro substituted B ring chalcone **297** being one of the most cytotoxic chalcone prepared. Several of the chalcones prepared also had strong angiogenesis inhibitory properties, especially **297** [The IC₅₀ for growth inhibition of human umbilical vein endothelial cells (HUVEC) was 0.11 μM].²³⁷



The 2'-oxygenated chalcones have previously shown anticancer activity including the inhibition of the growth of the MCF-7 human breast cancer cells.²⁴¹ Rao and co-workers²⁴² decided to synthesis ten 2'-oxygenated chalcones to further investigate their cytotoxic effects against the Jurkat and U937 human tumour cell lines, as well as against normal peripheral blood mononuclear cells (PBMCs), to explore their potential clinical application. The 2'-oxygenated chalcone **298** proved to be selectively toxic to Jurkat cells, IC₅₀ (Jurkat) 3.2 μM, but showed little cytotoxicity to U937 cancer cells, IC₅₀ (U937) 16.0 μM or the normal cells, IC₅₀ (PBMCs) 39.8 μM. The 2'-oxygenated chalcones **299** – **301** also showed good cytotoxicity to both Jurkat and U937 cells with IC₅₀ < 7 μM, however they were all fairly cytotoxic to the normal cell line PBMC as well with IC₅₀ < 11 μM.



The 2'-amino chalcone analogues of structurally rigid 2-phenyl-4-quinolones²⁴³ were shown to possess potent cytotoxic activity to a panel of human tumour cells, with the 2'-amino chalcone **302** being the most potent.²⁴⁴ The presence of the 2'-amino group was shown to be

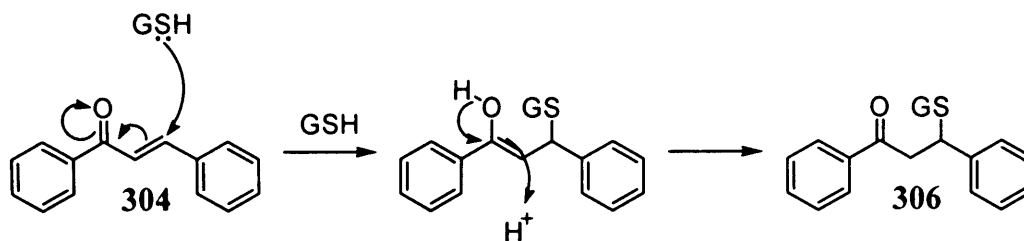
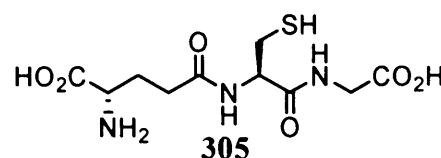
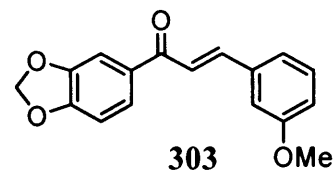
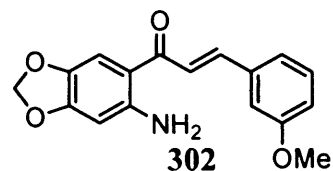
Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

vital to the activity of the chalcone, with **303** being 40 fold less active than the 2'-amino chalcone **302**.

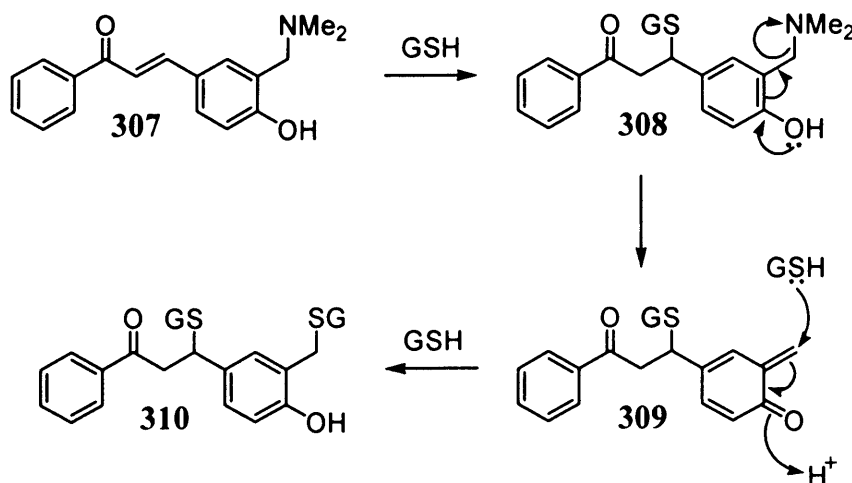
Dimmock *et al.*²²⁴ investigated the cytotoxicity of various Mannich base chalcones, many of which displayed a significant cytotoxicity towards murine P388, L1210 leukaemia cells and a number of human tumour cells as well. The idea that caused these Mannich base chalcones to be investigated for possible cytotoxicity is that a number of α,β -unsaturated ketones have demonstrated reactivity towards soft nucleophiles like thiols^{245,246} instead of hard nucleophiles like hydroxy or amino groups, and therefore avoid the problems of mutagenicity and carcinogenicity associated with a number of alkylating agents used in cancer chemotherapy.²⁴⁷ The Mannich base should also be able to alkylate the thiol as well, causing the compound to be more cytotoxic than the normal chalcone

(Scheme 57). The chalcone **304** may alkylate the common cellular thiol glutathione (GSH, **305**) to give the adduct **306** (Scheme 56). This could also happen to the Mannich base

chalcone **307** to give the thiol adduct **308**, the Mannich base of **308** could then form the intermediate enone **309** which could then undergo further thiol alkylation to give the thiol bisadduct **310** (Scheme 57).



Scheme 56: The mechanism of the thiol alkylation of chalcone **304**



Scheme 57: The mechanism of thiol alkylation of Mannich base chalcone **307**

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

Biological testing of the Mannich base chalcone **311** showed that in a buffered solution it was stable and it did not react with the excess GSH (**305**). However the chalcone **311** did react with GSH (**305**) when it was in the presence of glutathione *S*-transferase. Therefore under these biological conditions the chalcones may undergo thiol alkylation.²²⁴

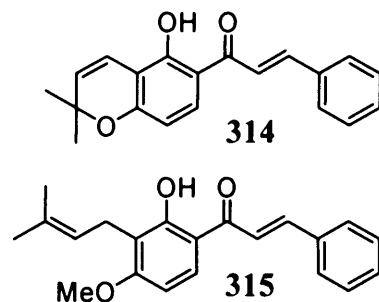
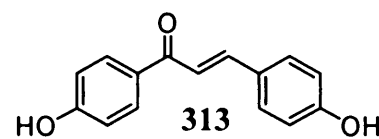
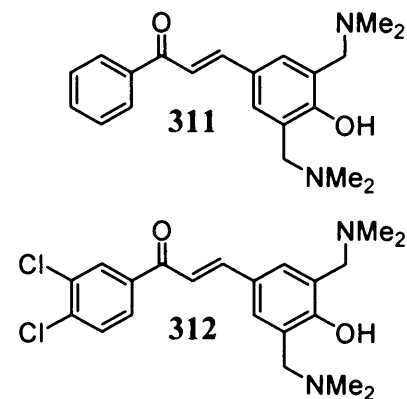
The most promising lead molecule found in Dimmock study was the Mannich base chalcone **312** which displayed potent cytotoxicity to the L1210 [IC₅₀(L1210) 2.5 μM] and human tumour cells [IC₅₀(Molt 4/C8) 7.0 μM, IC₅₀(CEM) 2.5 μM].²²⁴

The 4',4''-hydroxychalcone RVC-588 (**313**) has also been shown recently to be cytotoxic to the human myeloid HL-60 leukaemia cell line (IC₅₀ 2 μM).²⁴⁸ The mode of action of **313** is not known, however a previous study²³⁶ has shown that hydroxy substituted compounds were found to be the most potent antioxidant and cytotoxic in tumour cells, and it may be the hydroxy groups that play a role in the cytotoxicity of RVC-588 (**313**). It was also proposed that the cytotoxicity of RVC-588 (**313**) could be attributed in part to the thiol alkylating ability of the α,β-unsaturated carbonyl system in the chalcone.²²⁴

Hexane extracts of the common northeastern Brazilian plant *Lonchocarpus sericeus*, were found to be cytotoxic against the CEM leukemic cell line, IC₅₀ 17.6 μg mL⁻¹.²⁴⁹ The main components of the extract were Lonchocapin (**314**) [IC₅₀(CEM) 33.9 μM] and Derricin (**315**) [IC₅₀(CEM) 40.3 μM] in roughly equal amounts. Derricin (**315**) proved to be the most potent of the two chalcone isolated causing a maximum inhibition of CEM

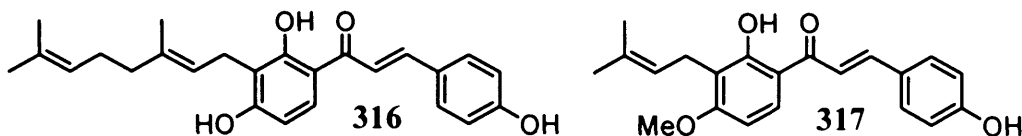
cells of 96%, while Lonchocapin (**314**) maximum inhibition of the CEM cells was 77%. Further testing of the prenylated chalcone Derricin (**315**) showed that it disrupted the development of fertilized eggs of sea urchins by disrupting the cell cycle. Further testing suggested that the cytotoxicity of the two chalcones **314** and **315** was not due to microtubule disruption.²⁴⁹

Two similar chalcones have been recently isolated from the Japanese plant *Angelica keiskei* Koizumi, xanthoangelol (**316**)²⁵⁰ and 4-hydroxyderricin (**317**).²⁵¹ Xanthoangelol (**316**)²⁵⁰ and 4-hydroxyderricin (**317**)²⁵¹ were both shown to inhibit the growth of Lewis lung carcinoma (LLC) tumours and have antimetastatic properties. It was proposed that the mechanism for

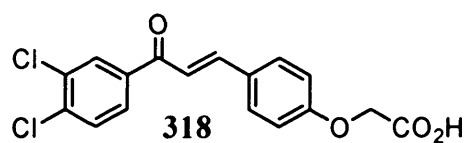


Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

the antitumour and antimetastatic actions of **316** might involve the inhibition of DNA synthesis in LLC cells and inhibition of tumour induced angiogenesis.²⁵⁰ However it was found that **317** did not inhibit DNA synthesis and its mechanism of antitumour and antimetastatic actives of **317** may be associated with the inhibition of the reduction of the immune system and tumour-induced angiogenesis.²⁵¹

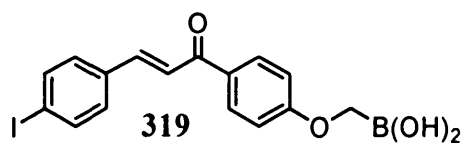


The human homolog of MDM2 (mouse double minute 2) oncogene is over expressed in human breast cancer, and it binds to the tumour suppressor protein p53 at its transactivation site, inhibiting p53's ability to initiate apoptosis and leading to deregulation of the cell cycle. This makes disruption of the p53/HDM2 complex an attractive target for cancer therapy. Stoll²⁵² and co workers found that chalcones with carboxylic acid substituents bound to the tryptophan pocket of the p53 binding site of MDM2 and promote dissociation of the p53/MDM2 complex. The most active carboxylic acid chalcone **318** inhibited MDM2 binding to p53 with an IC₅₀ value of 49 μM. The carboxylic acid chalcone **318** was shown by multidimensional NMR spectroscopy to dock in the tryptophan subsite of human MDM2 p53 binding site and disrupt the MDM2/p53 protein complex. The carboxylic acid is thought to be important to the activity of **318** by disrupting the salt bridge between lysine51 and glutamic acid 54 in the tryptophan subsite of human MDM2 bound P53.



The replacement of the carboxylic acid group with a boronic acid was further investigated by Kumar *et al.*²⁵³ The boronic acids are weaker acids pK_a 9-10 and will be largely non-ionized at physiological pH and are therefore unlikely to form a salt bridge with lysine51. However it was proposed that they may form a covalent bond to the amino of lysine51 instead, resulting in a stronger interaction with p53 binding site of MDM2.

The carboxylic acid chalcone **318** was not selectively toxic to cancer cell lines, with IC₅₀ values ranging from



13 – 18 μM for the human breast cancer cell lines and IC₅₀ values ranging from 12 – 28 μM for the normal human breast cell lines, however the boronic acid chalcones showed a greater selectivity to cancer cells. For example the iodo derivative boronic acid **319** had the highest selective cytotoxicity against human breast cancer cell lines IC₅₀ 9.5 – 18 μM, compared to the normal human breast cell line IC₅₀ 38 – 100 μM.²⁵³

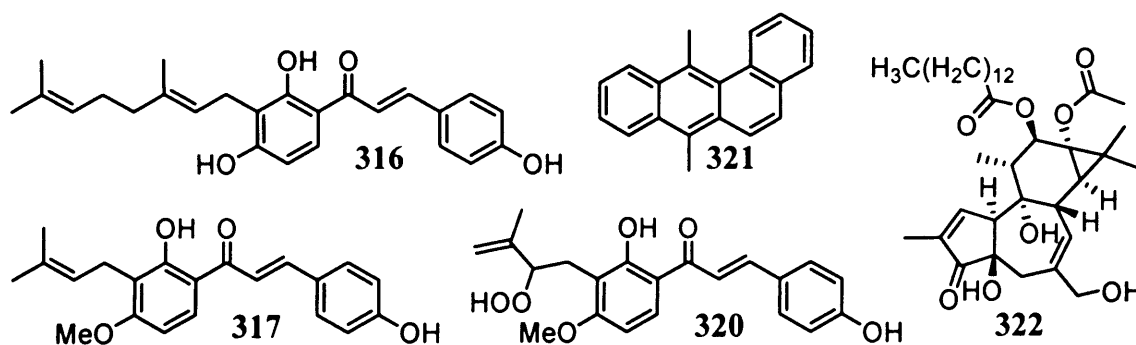


Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

4.2 The total synthesis of 4-hydroxyderricin (317) and analogues.

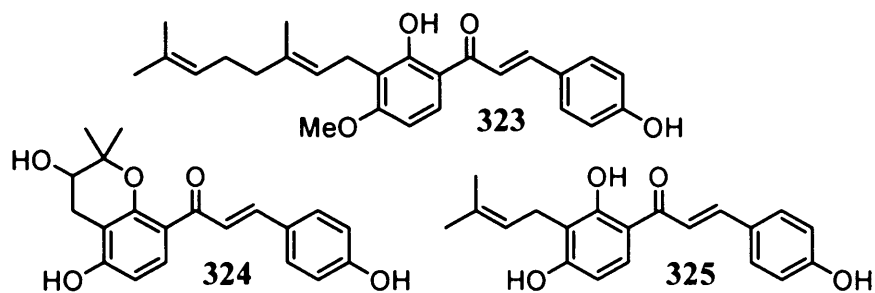
The hardy Japanese plant *Angelica keiskei* Koizumi (Japanese name “Ashitaba”, belonging to the Umbelliferae family) is found mainly on the Pacific coast of Japan. The Umbelliferae are used in traditional Chinese medicines,²⁵⁴ *Angelica keiskei* Koizumi has traditionally been used as a diuretic, laxative, analeptic and galactagogue.²⁵⁵ The plant has also shown beneficial effects such as the suppression of gastric acid secretion,²⁵⁶ antithrombotic effects,²⁵⁷ reduction of blood pressure,²⁵⁸ suppression of histamine release and the promotion of blood circulation.²⁵⁹ The roots and herbs of *A. keiskei* are also thought to have preventive effects against coronary heart disease, hypertension and cancer, however the basis for this is unclear.^{250,251}

In a study of the anti-tumour-promoting activities of the Umbelliferae family, Okuyama²⁵⁴ and co-workers reported the potent anti-tumour activity of the nonpolar extracts of the root of *Angelica keiskei*. Eight compounds were isolated from the nonpolar extract including three chalcones xanthoangelol (316), 4-hydroxyderricin (317) and the novel ashitaba-chalcone (320). The chalcones xanthoangelol (316) and 4-hydroxyderricin (317) proved to have anti-tumour promoting activity in mouse skin carcinogenesis that had been induced by exposure to the carcinogen 7,12-dimethylbenz[α]anthracene (DMBA, 321) plus 12-*O*-tetradecanoyl phorbol-13-acetate (TPA, 322).²⁵⁴



Akihisa²⁶⁰ and co-workers have recently reported the isolation of seventeen different compounds from the ethyl acetate extract of the stems of *Angelica keiskei* including two of the previously reported chalcones 316 and 317, as well as three other chalcones xanthoangelol F (323), xanthoangelol H (324), isobavachalcone (325). All 5 of the chalcones isolated showed potent antitumour inhibition of the induction of Epstein-Barr virus early antigen (EBV-EA) by TPA (322), with the chalcones 317 and 325 being the most potent inhibitors.²⁶⁰

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion



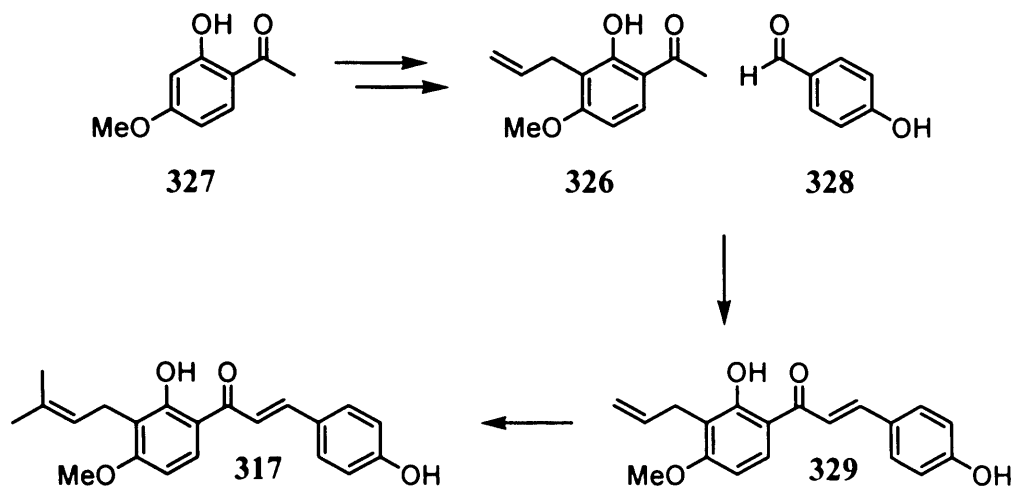
In another recent study by Kimura and co-workers the two chalcones xanthoangelol (**316**)²⁵⁰ and 4-hydroxyderricin (**317**)²⁵¹ were isolated from *Angelica keiskei*. Both of the chalcones **316** and **317** were shown to inhibit the growth of Lewis lung carcinoma (LLC) tumours, and were also reported to have antimetastatic properties. It was proposed that the mechanism for the antitumour and antimetastatic actions of **316** might involve the inhibition of DNA synthesis in LLC cells and inhibition of tumour induced angiogenesis.²⁵⁰ However when 4-hydroxyderricin (**317**) was further investigated it did not inhibit DNA synthesis. The mechanism of antitumour and antimetastatic actives of **317** are now proposed to be associated with the inhibition of the reduction of the immune system and tumour-induced angiogenesis.²⁵¹ The chalcone 4-hydroxyderricin (**317**) has been shown to exert a beneficial effect on the prevention of strokes in mice.²⁶¹

So we have chosen to further investigate the total synthesis of 4-hydroxyderricin (**317**) and then create a small library of similar compounds to assess their biological activity.

4.2.1 The synthesis of 4-hydroxyderricin (**317**).

Our idea for the synthesis of 4-hydroxyderricin (**317**) was the preparation of the 3'-allylacetophenone **326** from 2-hydroxy-4-methoxyacetophenone (**327**), followed by the Claisen-Schmidt condensation¹⁷⁸ with 4'-hydroxybenzaldehyde (**328**) to give the 3'-allylchalcone **329**. Metathesis of the 3'-allylchalcone **329** would then hopefully provide the prenylated chalcone 4-hydroxyderricin (**317**) (*Scheme 58*).

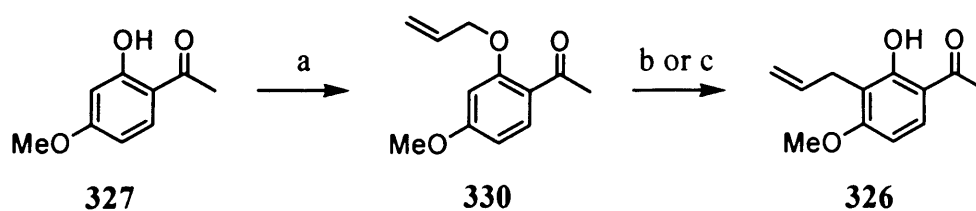
Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion



Scheme 58

Using the method described by Anjaneyulu²⁶² *et al.*, the hydroxy group of 2'-hydroxy-4'-methoxyacetophenone (327) was allylated by refluxing 327 in acetone with allyl bromide and potassium carbonate at 75 °C for 12 hours (Scheme 59). The mixture was then allowed to cool to room temperature, the potassium carbonate was then removed by filtration and then the acetone was removed *in vacuo*. The crude product was then dissolved in ethyl acetate and the base washed with 5% sodium hydroxide solution to remove any remaining 327, the allyl ether acetophenone 330 was then isolated in an excellent yield as a white solid.

The allyl ether acetophenone 330 was then dissolved in *N,N*-dimethylaniline, and heated at 210 °C for 12 hours to cause the allyl group to undergo a [3,3]-sigmatropic Claisen rearrangement to give the 3'-allylacetophenone 326 (Scheme 59)²⁶² as yellow crystals in a moderate yield.



Scheme 59: a. allyl bromide, K₂CO₃, acetone, 75 °C, 12 hours, 99 %; b. *N,N*-dimethylaniline, 210 °C, 6 hours, 326 58%; c. *N,N*-dimethylaniline, μ wave, 260 °C, 200 W, 12 mins, 326 66%.

The key diagnostic peaks of the ¹H NMR spectrum of 3'-allylacetophenone 326 are the peaks for the allyl group which appear at 3.42 (2H, dt, *J* 6.1, 1.7 Hz, H-1''), 4.93 – 4.98 (1H, dm, *J* 10.1 Hz, *cis*-H-3''), 4.97 – 5.04 (1H, dm, *J* 17.0 Hz, *trans*-H-3'') and 5.95 (1H,

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

ddt, J 10.1, 17.0 and 6.1 Hz, H-2'') ppm (Figure 34). The ^1H NMR spectrum of **326** corresponds to the literature values reported by Anjaneyulu²⁶² *et al.*

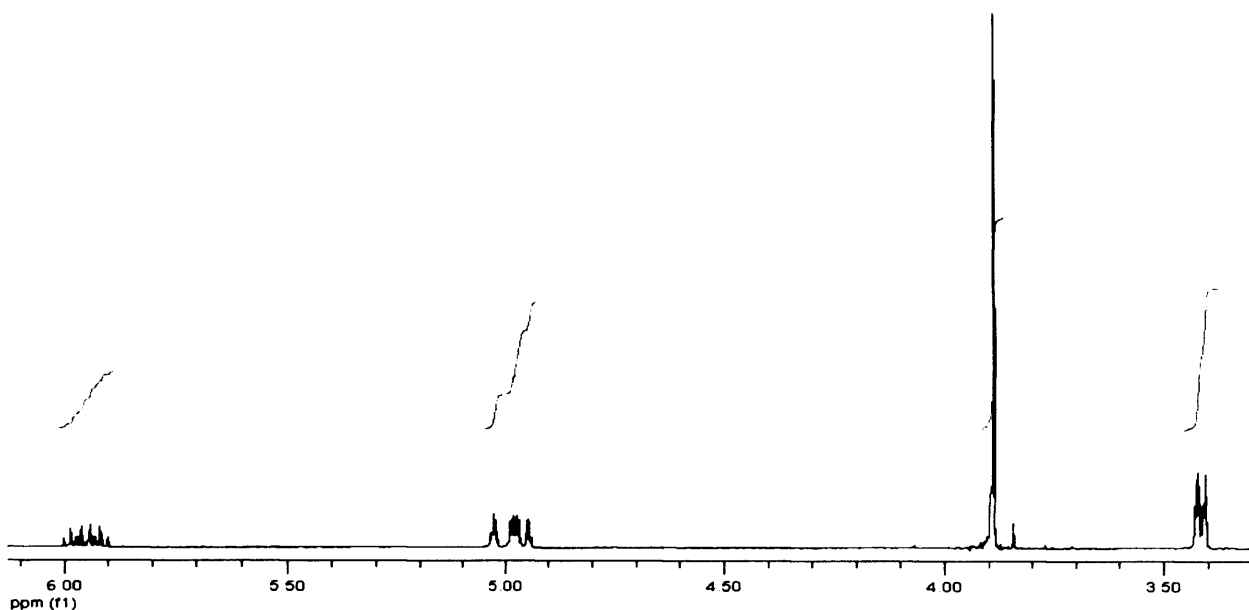


Figure 34: ^1H NMR spectra signals for the allyl group of **326**

The Claisen rearrangement of the allyl ether acetophenone **330** to prepare the 3'-allyl acetophenone **326** (Scheme 59) was tried again. However instead of heating this reaction conventionally we used the microwave reactor to see if we could reduce the reaction time. It is generally expected that each time the temperature of a reaction is increased in the microwave reactor by 10 °C the time taken for the reaction is halved. Therefore the Claisen rearrangement was attempted at 260 °C for 12 minutes, after which time the TLC showed that the reaction had gone to completion (Scheme 59, route c). The 3'-allyl acetophenone **326** was then isolated by column chromatography as a yellow solid in a moderate yield.

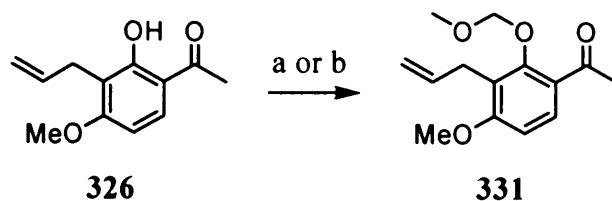
The 2'-hydroxy group of **326** had to be protected before the acetophenone **326** could be used to prepare a chalcone, to prevent the *in situ* formation of the possible flavanone side product from the 2'-hydroxychalcone under the basic conditions needed for the Claisen-Schmidt condensation.²³²

We first attempted the MOM protection of **326** using the conditions that had previous worked for protecting isovanillin in chapter 3.²²⁸ A solution of **326** in DMF was treated with 1.2 equivalents of sodium hydride, followed by 1.2 equivalents of methoxymethyl chloride. This technique for MOM protection did not work well and only gave a low yield of 28% of **331**, and 70% of the starting acetophenone **326** (Scheme 60).

We then tried using a modified version of the MOM protecting strategy described recently by Romano²⁶³ and coworkers for the protection of 2,4-dihydroxybenzaldehyde. In this protocol a

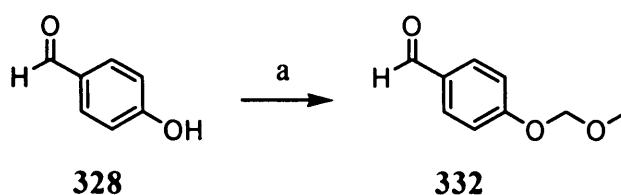
Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

slightly larger excess of sodium hydride and MOMCl is used. But the significant change is the reaction temperature (65 °C for 18 hours). The MOM protected acetophenone **331** was isolated after column chromatography in a moderate yield of 43%. The starting acetophenone **326** was also recovered from the column chromatography in a 42% yield (*Scheme 60*).



Scheme 60: a. MOMCl (1.2 eqv), NaH (1.2 eqv), DMF, 0 °C to r.t., 12 hrs, 28%; b. MOMCl (1.5 eqv), NaH (1.5 eqv), THF, 0 °C to 65 °C, 18 hrs, 43%.

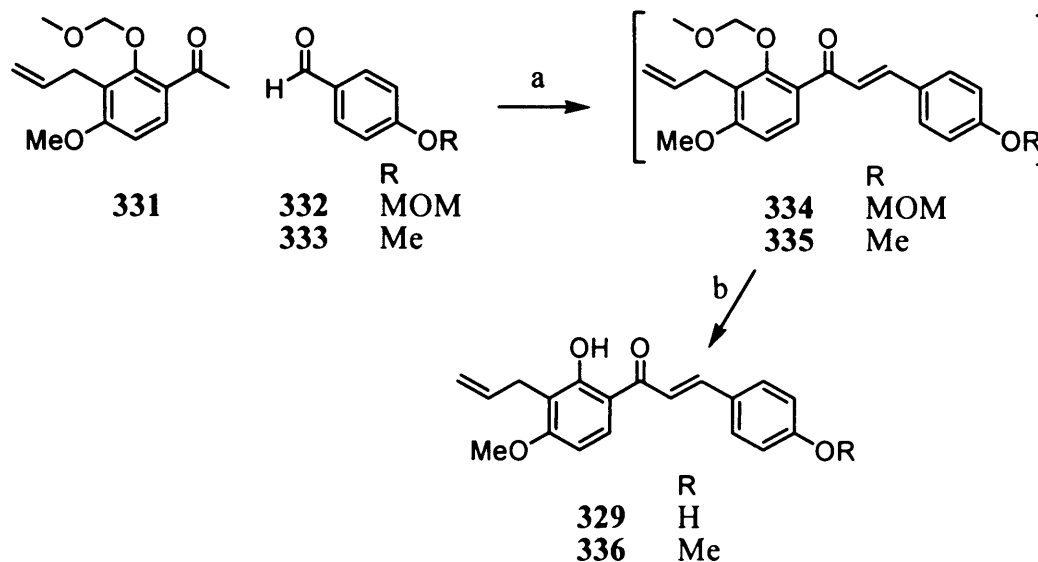
Before the chalcone **329** could be made the 4-hydroxybenzaldehyde **328** had to be protected because of the electron-donating effect of the hydroxy group (deprotonated under the reaction conditions) significantly reduces the electrophilicity of the carbonyl group. Therefore we MOM protected the 4-hydroxybenzaldehyde **328** using the conditions described for the MOM protection of isovanillin in chapter 3 (*Scheme 61*). This gave the MOM protected benzaldehyde **332** in a high yield of 94% as a pale yellow oil.



Scheme 61: a. MOMCl, NaH, DMF, 0 °C to r.t., 12 hrs, 94%.

The MOM protected acetophenone **331** was then treated sodium hydroxide and the benzaldehydes **332** and **333** using the typical Claisen-Schmidt condensation¹⁷⁸ conditions to give the MOM protected chalcones **334** and **335**. The ¹H NMR spectrum of crude products of **334** and **335** showed that they were a mixture of the desired chalcone and their precursor benzaldehyde. Neither of the chalcones were isolated and instead they were deprotected, by refluxing the mixtures in methanol in the presence of 3 M hydrochloric acid for 45 minutes (*Scheme 62*).²³⁰ The two chalcones **329** and **336** were then both isolated after column chromatography in good yields. Both chalcones were isolated as their *E* isomer and were fully characterised.

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion



Scheme 62: a. 2M NaOH (aq), MeOH, r.t., overnight; b. 3M HCl (aq), MeOH, 75 °C, 45 mins, **329** 81%, **336** 72%.

The API ES⁺ mass spectrometry of 3'-allylchalcone **329** showed the expected molecular ion [MH⁺] at *m/z* 311. The key diagnostic peaks in the ¹H NMR spectrum of **329** were the hydrogen signals of the enone system appearing at δ 7.47 (1H, d, *J* 15.0 Hz, H-2) and 7.84 (1H, d, *J* 15.0 Hz, H-3) ppm proving that the chalcone **329** was isolated as its *E*-isomer. The hydrogen signals for the allyl group which appear at 3.45 (2H, dt, *J* 6.2 and 1.8 Hz, H-1'''), 4.95 – 5.00 (1H, dm, *J* 10.0 Hz, *cis*-H-3'''), 5.00 – 5.07 (1H, dm, *J* 17.1 Hz, *trans*-H-3'''), 5.98 (1H, ddt, *J* 10.0 Hz, 17.1, 6.2 Hz, H-2''') ppm (*Figure 35*).

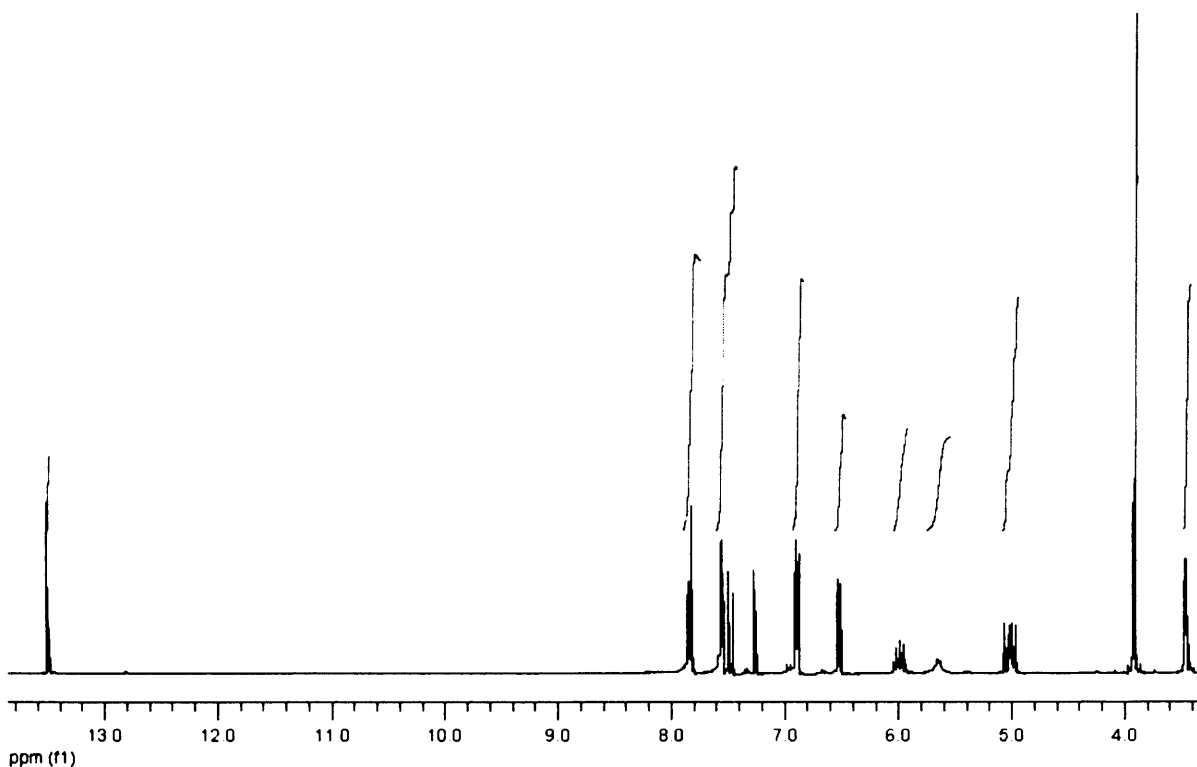
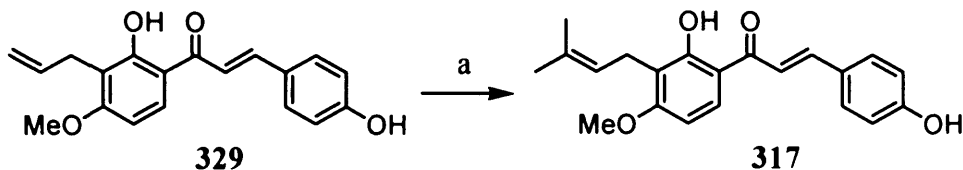


Figure 35: ¹H NMR spectrum of 3'-allylchalcone **329**

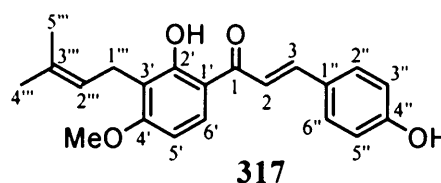
Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

Using Grubbs second generation catalyst (**339**) the cross metathesis of the 3'-allylchalcone **329** and 2-methylbut-2-ene (**340**) was carried out in anhydrous DCM under an argon atmosphere in a Schlenk tube at 50 °C for 12 hours.²⁷⁹ After column chromatography the 3'-prenylchalcone 4-hydroxyderricin (**317**) was isolated in a 66% yield as a yellow solid (*Scheme 65*).



Scheme 65: a. 2-methylbut-2-ene (**340**), **339** (2 mol%), DCM, 50 °C, 12 hrs, 66%.

The 3'-prenylchalcone **317** was characterised and deemed to be identical to the 4-hydroxyderricin (**317**) isolated by Baba *et al*²⁸⁰ and Okuyama *et al*²⁵⁴ from the plant *Angelica keiskei* Koizumi. The melting point of **317** was recorded



at 133 – 134 °C (literature m.p. 134 – 135 °C²⁵⁴) and the ¹H and ¹³C NMR spectra (*Table 8*) of **317** support the formation of 4-hydroxyderricin (**317**). The key diagnostic peaks in the ¹H NMR spectrum of **317** are the hydrogen signals of the enone system appearing at δ 7.47 (1H, d, *J* 15.4 Hz, H-2) and 7.83 (1H, d, *J* 15.4 Hz, H-3) ppm proving that the chalcone **317** was isolated as its *E*-isomer (*Figure 36*). The hydrogen signals for the prenyl group which appear at 1.68 (3H, s, H-5'''), 1.80 (3H, s, H-4'''), 3.36 (2H, broad d, *J* 6.5 Hz, H-1'''), 5.23 (1H, broad t, *J* 6.5 Hz, H-2''') ppm, showing that the 2-methylbut-2-ene (**340**) cross-metathesis reaction gave the desired prenylated product (*Figure 36*).

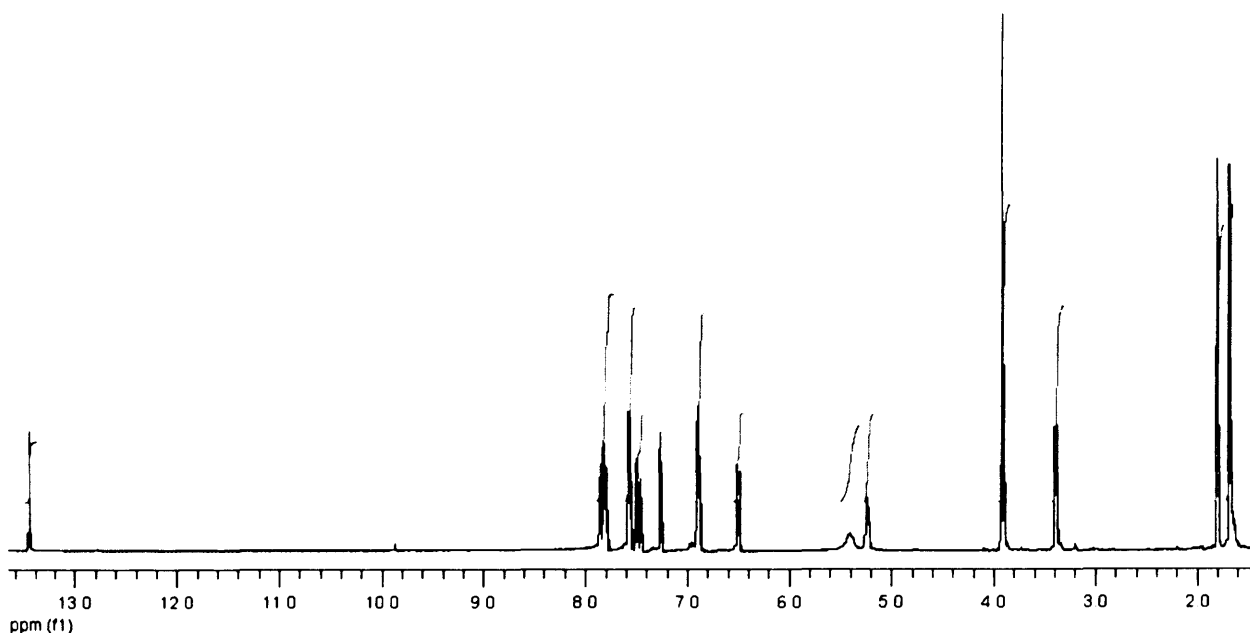


Figure 36: ¹H NMR spectrum of 4-hydroxyderricin (**317**)

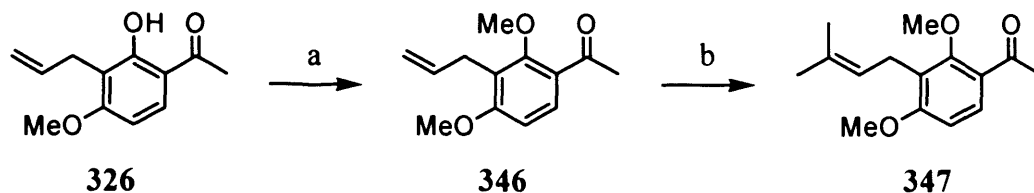
Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

Table 8: ^1H NMR and ^{13}C NMR Spectral data for 4-hydroxyderricin (**317**) and literature comparison

Position	^1H NMR (ppm)		^{13}C NMR (ppm)	
	Synthetic product (CDCl_3)	Natural product ²⁸⁰ (CDCl_3)	Synthetic product (CDCl_3)	Natural product ²⁸⁰ (CDCl_3)
1	-	-	192.56	193.39
2	7.47 (1H, d, J 15.4 Hz)	7.48 (1H, d, J 15.4 Hz)	118.45	118.26
3	7.83 (1H, d, J 15.4 Hz)	7.84 (1H, d, J 15.4 Hz)	144.15	145.04
1'	-	-	114.89	115.07
2'	-	-	163.23	163.51
3'	-	-	117.81	118.02
4'	-	-	163.47	164.04
5'	6.50 (1H, d, J 9.2 Hz)	6.50 (1H, d, J 9.0 Hz)	102.31	102.77
6'	7.79 (1H, d, J 9.2 Hz)	7.80 (1H, d, J 9.0 Hz)	129.35	129.90
1''	-	-	128.07	127.81
2''	7.56 (2H, d, J 8.2 Hz)	7.57 (2H, d, J 8.5 Hz)	130.75	131.16
3''	6.88 (2H, d, J 8.2 Hz)	6.89 (2H, d, J 8.5 Hz)	116.22	116.58
4''	-	-	158.13	159.20
5''	6.88 (2H, d, J 8.2 Hz)	6.89 (2H, d, J 8.5 Hz)	116.22	116.58
6''	7.56 (2H, d, J 8.2 Hz)	7.57 (2H, d, J 8.5 Hz)	130.75	131.16
1'''	3.36 (2H, br.d, J 6.5 Hz)	3.40 (2H, d, J 7.6 Hz)	21.96	21.94
2'''	5.23 (1H, br.t, J 6.5 Hz)	5.23 (1H, t, J 7.6 Hz)	122.25	122.53
3'''	-	-	132.17	132.57
4'''	1.80 (3H, s)	1.80 (3H, s)	26.05	26.03
5'''	1.68 (3H, s)	1.69 (3H, s)	18.05	18.03
2'-OH	13.46 (1H, s)	13.50 (1H, s)	-	-
4'-OMe	3.91 (3H, s)	3.92 (3H, s)	56.01	(not reported)
4''-OH	5.41 (1H, bd.s)	5.78 (1H, s)	-	-

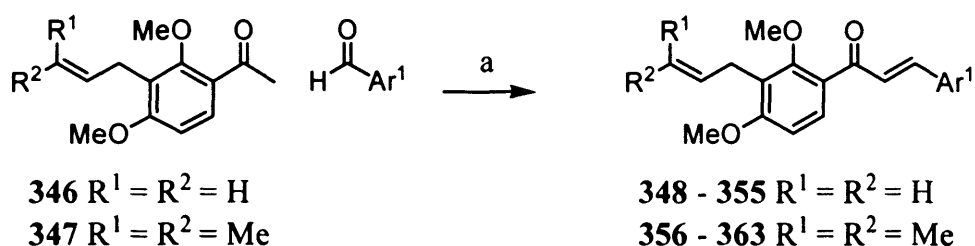
We then decided to make a small library of similar 3'-allylchalcones and 3'-prenylchalcones. We methylated the 2'-hydroxy group on the acetophenone **326** to make the synthesis of the chalcone library easier by eliminating the requirement of protecting and deprotecting this hydroxy group. The 2'-hydroxy group was methylated by treating **326** in THF with sodium hydride at 0 °C under an argon atmosphere and then adding 1M solution of dimethyl sulfate in THF and then refluxing the mixture for 18 hours at 65 °C (*Scheme 66*).²⁶³ This gave the methylated acetophenone **346** which was isolated by column chromatography in an excellent yield as a yellow oil. The ^1H NMR spectrum of **346** showed that the 2-hydroxy group of the acetophenone **326** had been methylated by the presence of a new methoxy signal at 3.75 ppm, and the signal for the 2'-hydroxy group which appears at 12.77 ppm in the ^1H NMR spectrum of **326** was no longer present. The acetophenone **346** was then subjected to the cross-metathesis reaction²⁷⁹ with 2-methylbut-2-ene (**340**) to give the prenylated acetophenone **347** which was isolated in an excellent yield as a light brown oil after column chromatography (*Scheme 66*).

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion



Scheme 66: a. 1M (MeO)₂SO₂ in THF, NaH, THF, 0 °C to 65 °C, 18 hrs, 96%; b. 2-methylbut-2-ene (**340**), **339** (2 mol%), DCM, 50 °C, 12 hrs, 98%.

A small library of chalcones **348** – **363**, were then prepared from the two acetophenones **346** and **347** and various substituted benzaldehydes using the Claisen-Schmidt condensation¹⁷⁸ conditions (*Scheme 67*). The chalcones **350** – **352** and **358** were purified by recrystallization from methanol, while the other chalcones were purified by column chromatography. All of the chalcones were isolated as the *E*-isomer as shown by their ¹H NMR spectra.



Scheme 67: a. 2M NaOH (aq), MeOH, r.t., overnight, yields see *table 9*.

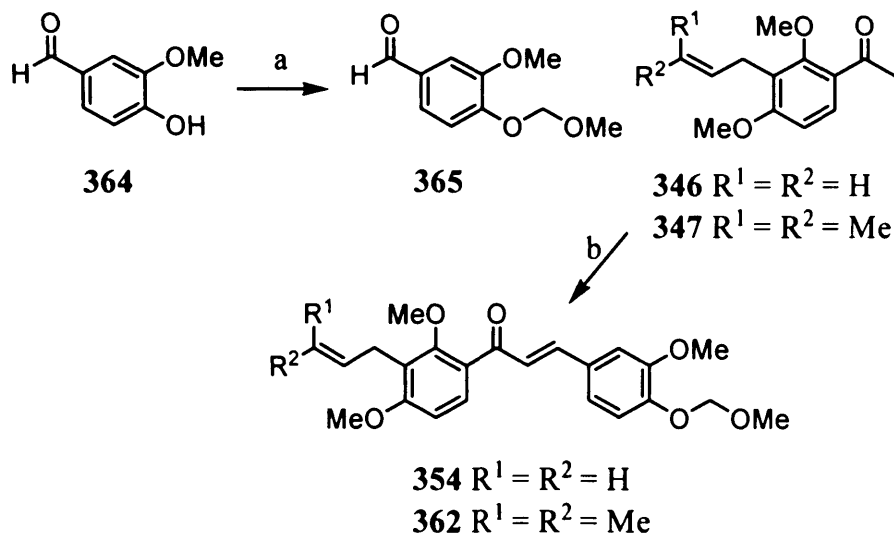
Table 9: Yields of the 3'-allylchalcones **348** – **355** and 3'-prenylchalcones **356** – **363**.

Chalcone	R ¹	R ²	Ar ¹	Yield (%)
348	H	H	4-Methoxyphenyl	62
349	H	H	3-Fluoro-4-methoxyphenyl	84
350	H	H	Phenyl-4-oxyacetic acid	71
351	H	H	3,4-Dichlorophenyl	69
352	H	H	2,4-Dichlorophenyl	82
353	H	H	4-(Methoxymethoxy)phenyl	77
354	H	H	3-Methoxy-4-(methoxymethoxy)phenyl	89
355	H	H	4-Methoxy-3-(methoxymethoxy)phenyl	73
356	Me	Me	4-Methoxyphenyl	54
357	Me	Me	3-Fluoro-4-methoxyphenyl	75
358	Me	Me	Phenyl-4-oxyacetic acid	85
359	Me	Me	3,4-Dichlorophenyl	25
360	Me	Me	2,4-Dichlorophenyl	89
361	Me	Me	4-(Methoxymethoxy)phenyl	86
362	Me	Me	3-Methoxy-4-(methoxymethoxy)phenyl	85
363	Me	Me	4-Methoxy-3-(methoxymethoxy)phenyl	91

Before the chalcones **354** and **362** could be prepared the 4-hydroxy group of vanillin (**364**) had to be protected, otherwise the electrophilicity of the carbonyl group of **364** would have

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

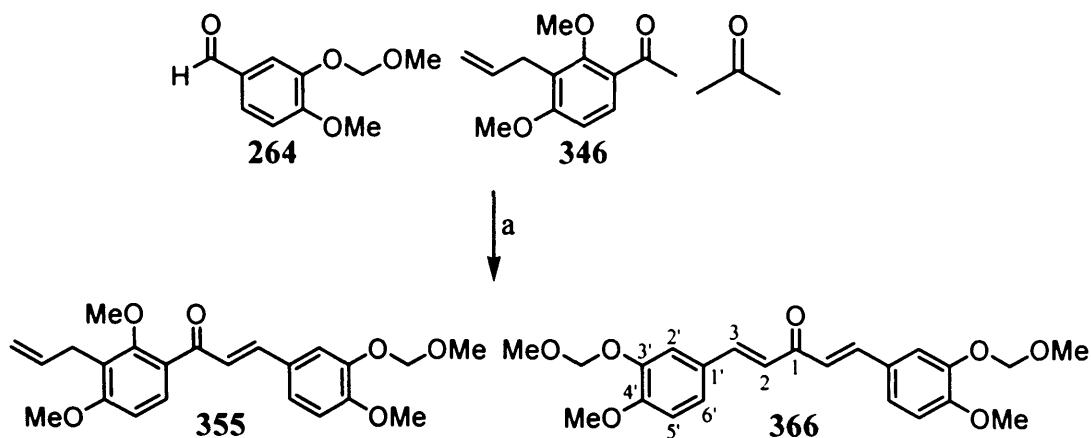
been significantly reduced by the electron-donating effects of the 4-hydroxy group (deprotonated under the reaction conditions). The MOM protected benzaldehyde **365** was prepared from vanillin (**364**) using the same conditions as previously described for **264** and **332** (Scheme 68).²²⁸ This gave the MOM protected benzaldehyde **365** as a pale yellow oil which was isolated in a 80% yield after column chromatography.



Scheme 68: a. MOMCl, NaH, DMF, 0 °C to r.t., 12 hrs, 80%; b. 2M NaOH (aq), MeOH, r.t., overnight, **354** 89%, **362** 85%.

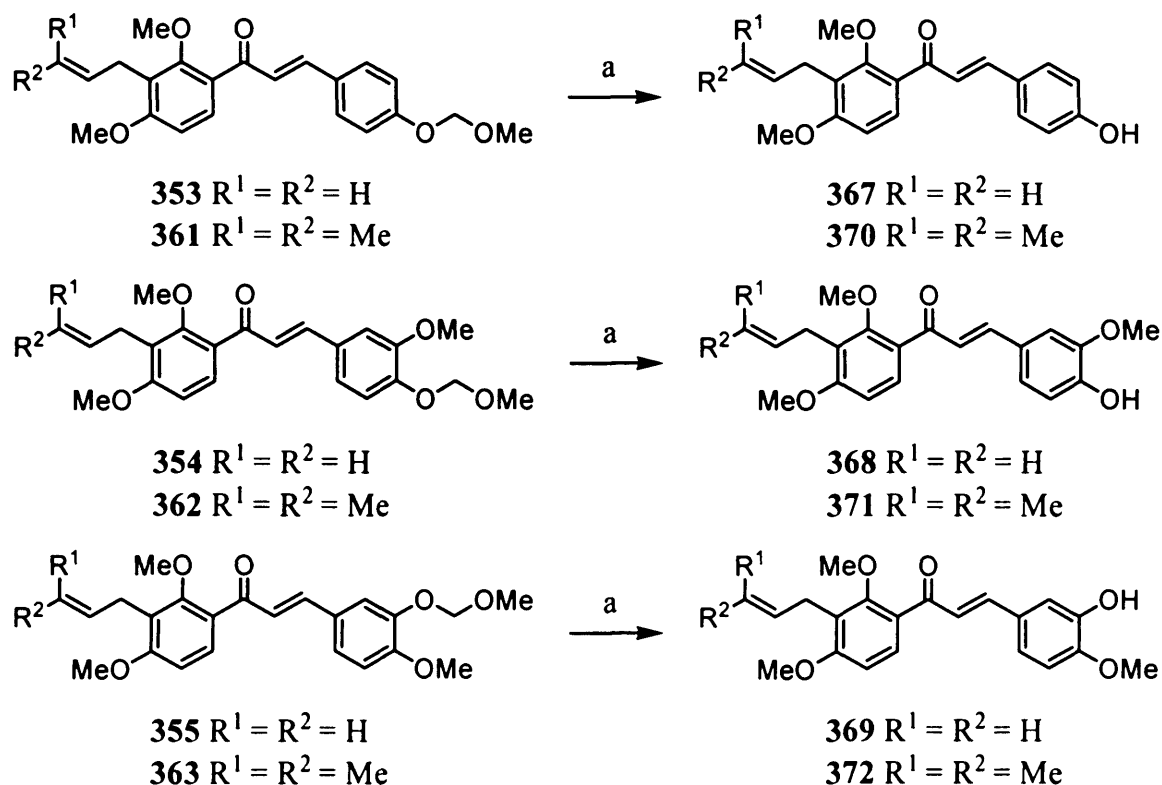
From the column of the isolation of the 3'-allylchalcone **355** a side product was also isolated. The side product was a yellow solid which had a melting point of 105 - 107 °C, the ¹H NMR spectrum showed the presence of the MOM protected B-ring of the chalcone **355** with the signals at 3.56 (3H, s, CH₂OCH₃), 3.94 (3H, s, OCH₃), 5.30 (2H, s, OCH₂O), 6.92 (1H, d, *J* 8.4 Hz, H-5'), 7.27 (1H, dd, *J* 1.9 Hz, *J* 8.4 Hz, H-6'), 7.47 (1H, d, *J* 1.9 Hz, H-2') ppm. The ¹H NMR spectrum also showed the presence of an *E*-isomer enone system with signals at 6.95 (1H, *J* 15.8 Hz, H-2) and 7.67 (1H, *J* 15.8 Hz, H-3) ppm. The ¹³C NMR spectrum of the side product showed the presence of 12 non-equivalent carbons, with the presence of a carbonyl signal at 188.92 (s, C-1) ppm. Finally the API-ES+ mass spectrometry of the side product gave a molecular ion [MH⁺] at *m/z* 415, corresponding to the molecular formula C₂₃H₂₇O₇. We therefore decided that the side product must be **366**, therefore the reaction mixture must have been contaminated with acetone, which had most probably been used to clean the reaction flask before hand. So that the acetone and benzaldehyde undergo Claisen-Schmidt condensation to give **366** (Scheme 69).

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion



Scheme 69: a. 2M NaOH (aq), MeOH, r.t., overnight, **355** 73%, **366** 17%.

The 6 MOM protected chalcones **353** – **355** and **361** – **363** were then deprotected by refluxing the chalcones in methanol and in the presence of 3 M hydrochloric acid for 45 minutes (*Scheme 70*).²³⁰ The chalcones **367** – **370** were isolated by column chromatography in excellent yields, and the chalcones **371** and **372** were crystallized from methanol.

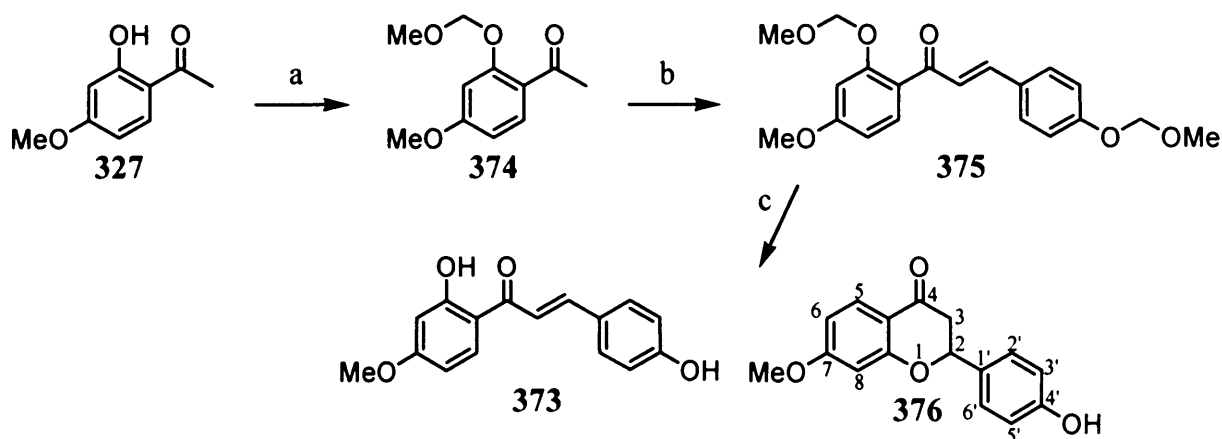


Scheme 70: a. 3M HCl (aq), MeOH, 75 °C, 45 mins, **367** 95%, **368** 99%, **369** 98%, **370** 89%, **371** 72%, **372** 59%.

We also prepared the 2'-hydroxychalcone **373** without the 3'-allyl or 3'-prenyl group to test for biological activity, because several 2'-hydroxychalcones have been reported in the literature as being cytotoxic agents^{241,242} and we wanted to investigate the importance of the allyl/prenyl group. The 2'-hydroxy group of the acetophenone **327** was MOM protected by

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

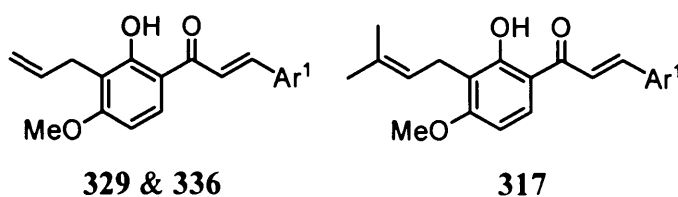
treating it with sodium hydride and MOMCl in THF at 65 °C for 18 hrs. This gave **374** as a pale yellow oil in a moderate yield after chromatography (*Scheme 71*).²⁶³ The MOM protected acetophenone **374** and MOM protected benzaldehyde **332** were then treated with aqueous sodium hydroxide in the typical Claisen-Schmidt condensation¹⁷⁸ conditions, yielding the MOM protected chalcone **375** as a yellow oil which was isolated via column chromatography (*Scheme 71*). The MOM protected chalcone **375** was then deprotected by refluxing it in methanol and 3 molar hydrochloric acid for 45 minutes (*Scheme 71*). This MOM deprotection led to 2 separate products which were isolated by chromatography, the first being the desired 2'-hydroxychalcone **373** in a moderate yield as a yellow solid and the flavanone side product **376** as yellow crystals. The 2'-hydroxychalcone **373** was isolated as the *E*-isomer as which was shown in the ¹H NMR spectrum by its alkene coupling of 15.4 Hz. The ¹H NMR spectrum of the flavanone **376** corresponds to literature values given by Pouget *et al*,²⁸¹ with the key signals in the spectrum being the signals for the classic ABX system of the flavanone **376** which appear at 2.81 (1H_a, dd, *J* 2.9 Hz, *J* 16.9 Hz, H-3a), 3.06 (1H_b, dd, *J* 13.2 Hz, *J* 16.9 Hz, H-3b) and 5.41 (1H_x, dd, *J* 2.9 Hz, *J* 13.2 Hz, H-2) ppm.



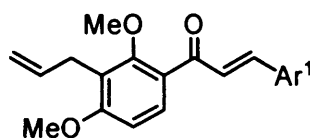
Scheme 71: a. MOMCl, NaH, THF, 0 °C to 65 °C, 18 hrs, 66%; b. **332**, 2M NaOH (aq), MeOH, r.t., overnight 62%; c. 3M HCl (aq), MeOH, 75 °C, 45 mins, **373** 41%, **376** 34%.

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

4.2.2 Biological results for the 4-hydroxyderricin (317) and similar chalcone derivatives.



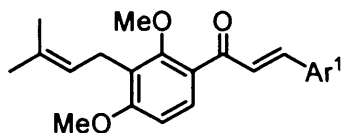
Chalcone	Ar ¹	IC ₅₀ (K562) (μM)
329	4-Hydroxyphenyl	>10
336	4-Methoxyphenyl	>10
317	4-Hydroxyphenyl	>10



348 - 355 & 367 - 369

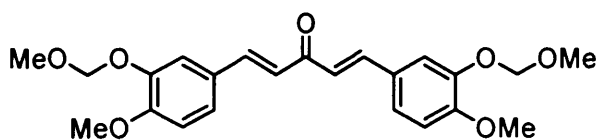
Chalcone	Ar ¹	IC ₅₀ (K562) (μM)
348	4-Methoxyphenyl	7.9
349	3-Fluoro-4-methoxyphenyl	4.2
350	Phenyl-4-oxyacetic acid	>10
351	3,4-Dichlorophenyl	>10
352	2,4-Dichlorophenyl	>10
353	4-(Methoxymethoxy)phenyl	>10
354	3-Methoxy-4-(methoxymethoxy)phenyl	7.5
355	4-Methoxy-3-(methoxymethoxy)phenyl	8.4
367	4-Hydroxyphenyl	>10
368	4-Hydroxy-3-methoxyphenyl	>10
369	3-Hydroxy-4-methoxyphenyl	0.2

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

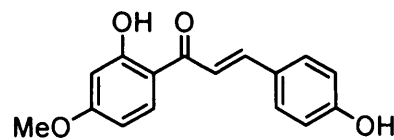


356 - 363 & 370 - 372

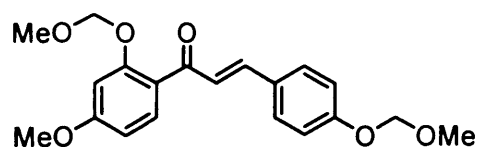
Chalcone	Ar ¹	IC ₅₀ (K562) (μM)
356	4-Methoxyphenyl	>10
357	3-Fluoro-4-methoxyphenyl	>10
358	Phenyl-4-oxyacetic acid	>10
359	3,4-Dichlorophenyl	>10
360	2,4-Dichlorophenyl	>10
361	4-(Methoxymethoxy)phenyl	>10
362	3-Methoxy-4-(methoxymethoxy)phenyl	>10
363	4-Methoxy-3-(methoxymethoxy)phenyl	>10
370	4-Hydroxyphenyl	>10
371	4-Hydroxy-3-methoxyphenyl	>10
372	3-Hydroxy-4-methoxyphenyl	8.4



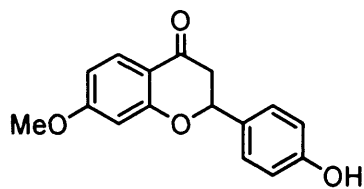
366 IC₅₀(K562) 3.9 μM



373 IC₅₀(K562) >10 μM



375 IC₅₀(K562) >10 μM



376 IC₅₀(K562) >10 μM

The 4-hydroxyderricin (**317**) and chalcone derivatives generally show low cytotoxicity towards the human leukaemia cell line K562. Only 7 of the compounds prepared show cytotoxicity below 10 μM.

The most active of these compounds is the chalcone (*E*)-1-(3-allyl-2,4-dimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one (**369**) which displays an IC₅₀ value of 0.2 μM. Our studies of the CA-4 like chalcones (see chapter 3) have shown that chalcones which bear the 3-hydroxy-4-methoxyphenyl *B*-ring display potent cytotoxicity towards the K562 cell line and the activity of **369** may be due to presence of its 3-hydroxy-4-methoxyphenyl *B*-ring.

The prenylated version of **369**, (*E*)-3-(3-hydroxy-4-methoxyphenyl)-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)prop-2-en-1-one (**372**) has an IC₅₀ value of 8.4 μM, shows that the

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

addition of the two methyl groups on the allyl group to make the prenyl group cause a 40 fold decrease in activity of this chalcone.

The side product (1*E*,4*E*)-1,5-bis(4-methoxy-3-(methoxymethoxy)phenyl)penta-1,4-dien-3-one (**366**) is the second most active compound isolated, with an IC₅₀ value of 3.9 μM. The deprotected version of this compound may have been proven to be more active, and may prove interesting for future studies. Several similar compounds have been reported by Artico and co-workers²⁸² for having anti-HIV activity.

4.3 p53-MDM2 complex disrupting chalcones^{283,284}

The tumour suppressor p53 is a key player in the prevention of cancer. When a cell is exposed to oncogenic stress, p53 in the cell nucleus acts as a transcription factor capable of activating multiple target genes, leading to cell cycle arrest, apoptosis and senescence as well as enhancing DNA repair.²⁸⁵⁻²⁸⁷ Therefore p53 ensures that cells carrying cancer promoting alterations such as damaged genomes are either fixed or permanently deleted. In normal cells the p53 is tightly regulated by the oncoprotein HDM2, which binds to p53 and promotes p53 ubiquitination, causing the degradation of p53 within the nucleus and prevents it causing cell death in normal cells.²⁸⁸ However amplification of the HDM2 protein has been observed in several human tumours,^{283,289-292} inhibiting the ability of p53 to initiate apoptosis of cancer cells. This makes disruption of the p53/HDM2 complex an attractive target for cancer therapy.

However due to the nature of proteins-protein interactions, with their relatively large and flat interacting surfaces they are often viewed as high-risk targets because they are not easily disrupted by small drug like molecules.²⁹³ The crystal structure of a p53 bound to HDM2 revealed that the interaction of the two proteins is due to the projection of three amino acid residues (Phenylalanine19, Tryptophan23 and Leucine26) from p53 into the deep hydrophobic binding pocket on the surface of MDM2 (*Figure 37*).²⁹⁴ This realization has raised the hope that finding a pharmacological inhibitor of this interaction may be possible.

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

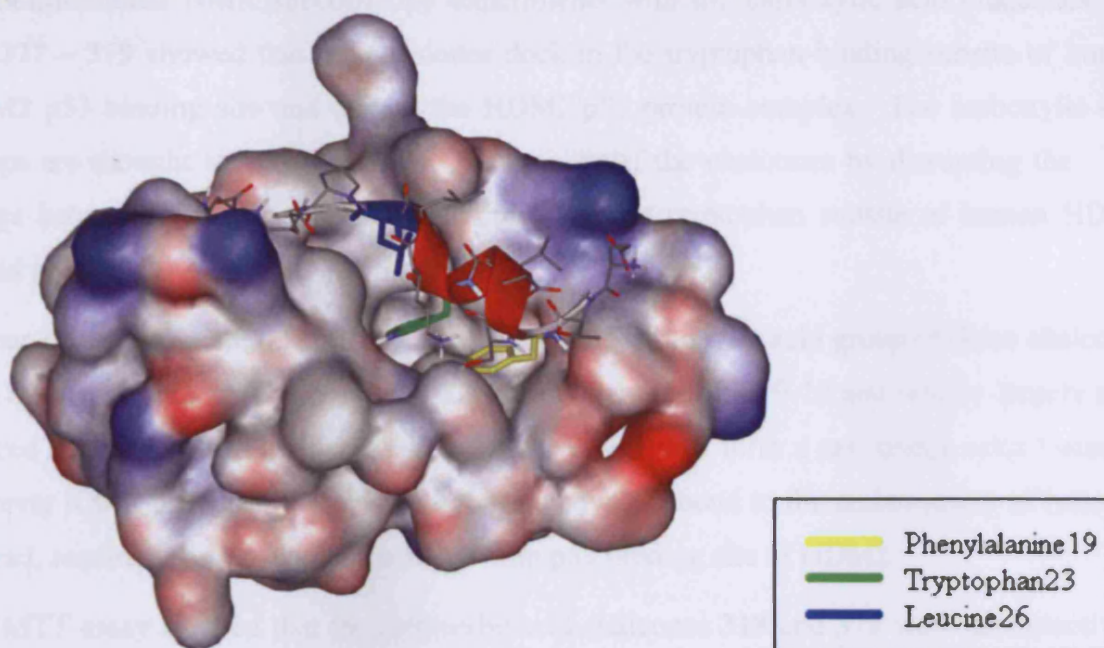


Figure 37: The p53 helix binding to the hydrophobic binding pocket on the surface of MDM2.²⁹⁴

Stoll *et al.*²⁵² reported chalcones are MDM2 antagonists. They found that chalcones with carboxylic acid substituents could disrupt the MDM2 binding of p53 with ELISA (Enzyme-Linked Immunosorbent Assay) data giving IC_{50} values of 49 – 250 μM for the disruption of p53/MDM2 complex (*Figure 38*).

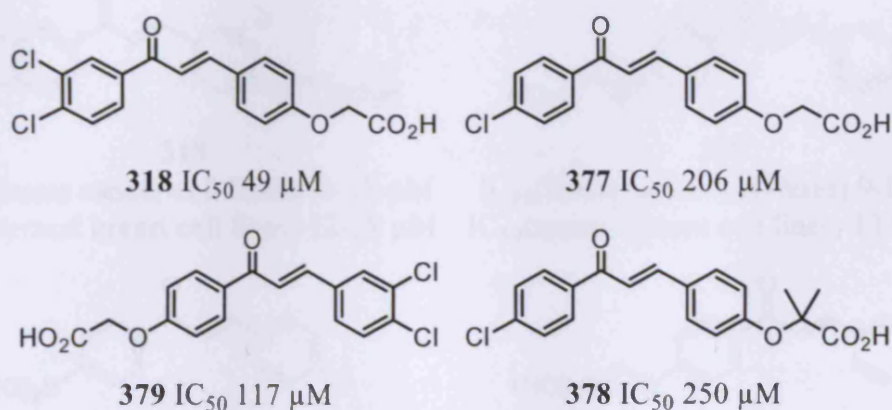


Figure 38: Inhibition of MDM2 binding to p53 measured by ELISA²⁵²

The chalcones were then tested to see if they could dissociate preincubated p53/MDM2 complexes and release p53 active for DNA-binding in an electrophoretic gel mobility shift assay (EMSA). The EMSA showed that the carboxylated chalcones 377 and 378 did not release active p53, while chalcones 318 and 379 removed MDM2 from the complex with p53, with 379 fully removing MDM2.²⁵²

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

Multidimensional NMR spectroscopy experiments with the carboxylic acid chalcones **318** and **377 – 379** showed that the chalcones dock in the tryptophan-binding subsite of human HDM2 p53 binding site and disrupt the HDM2/p53 protein complex. The carboxylic acid groups are thought to be important to the activity of the chalcones by disrupting the salt bridge between lysine51 and glutamic acid 54 in the tryptophan subsite of human HDM2 bound p53.²⁵²

Kumar *et al* investigated the effects of replacing the carboxylic acid group of these chalcones with a boronic acid.²⁵³ The boronic acids are weaker acids pK_a 9-10 and will be largely non-ionized at physiological pH, they are therefore unlikely to form a salt bridge with lysine51, however it was proposed that they may form a covalent bond to the amino group of lysine51 instead, resulting in a stronger interaction with p53 binding site of HDM2.

The MTT assay showed that the carboxylic acid chalcones **318** and **379** were not selectively toxic to cancer cell lines, with IC_{50} values ranging from 9 – 18 μ M for the human breast cancer cell lines and IC_{50} values ranging from 12 – 28 μ M for the normal human breast cell lines, however several of the boronic acid chalcones **319** and **380** showed a greater selectivity to cancer cells with IC_{50} values ranging from 7 – 18 μ M for the human breast cancer cell lines and IC_{50} values ranging from 38 – 100 μ M for the normal human breast cell lines, (*Figure 39*).²⁵³

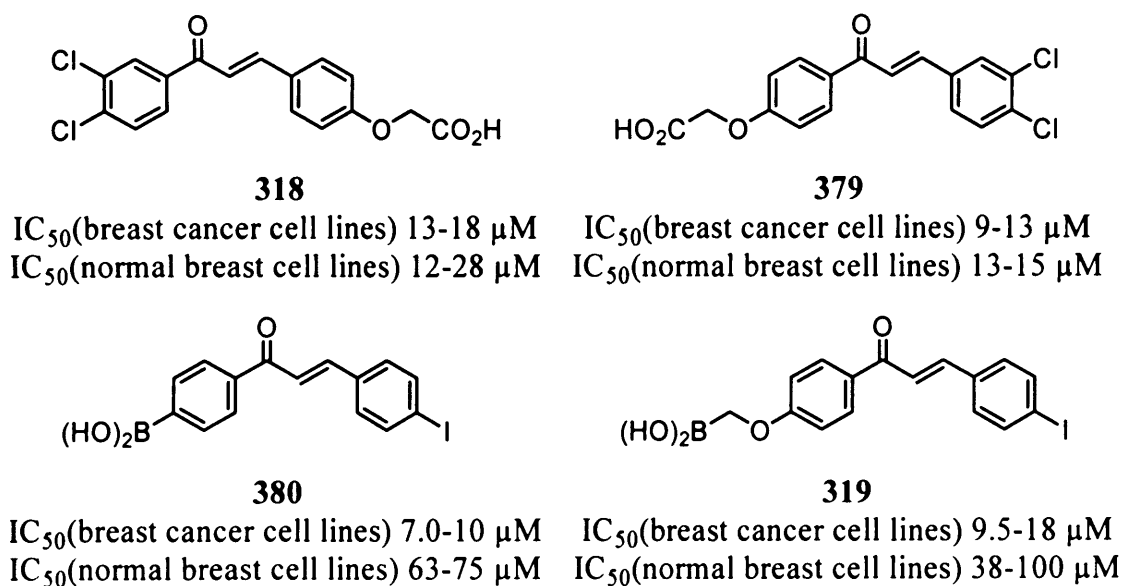
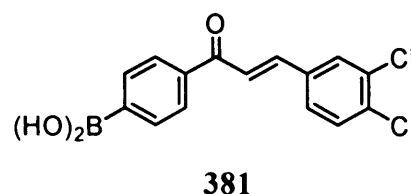


Figure 39: IC_{50} values of carboxylic acid chalcones **318** & **379** compared against boronic acid chalcones **380** & **319** against human breast cancer cell lines (MDA-MB-435, MDA-MB-231 & Wt-MCF7) and normal breast cell lines (MCF-10A & MCF-12A)²⁵³

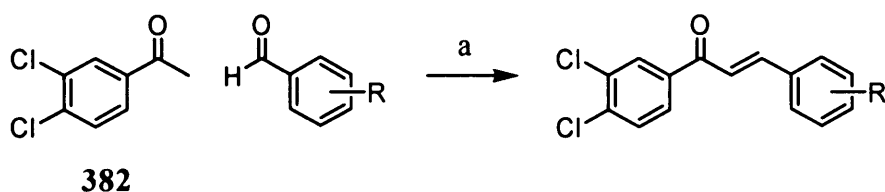
Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

However multidimensional NMR spectroscopy experiments carried out by D'Silva²⁹⁵ and co-workers recently, show that the boronic chalcone **381** does not dissociate the p53-HDM2 complex, but **381** does bind to the tryptophan-binding subsite of human MDM2 p53-binding cleft, and that they may be extremely weak inhibitors of the MDM2/p53 interaction.



4.3.1 Synthesis of the 3',4'-dichlorochalcones

We decided to make a small series of 3',4'-dichlorochalcones without the carboxylic acid group to see if it was a requirement for disrupting the HDM2/p53 interaction. The chalcones were prepared by the Claisen-Schmidt condensation¹⁷⁸ of the 3',4'-dichloroacetophenone (**382**) and various substituted benzaldehydes (*Scheme 72*).



Scheme 72: a. 2M NaOH (aq), MeOH, r.t., overnight

The 3',4'-dichlorochalcones were prepared by treating 3',4'-dichloroacetophenone (**382**) and a substituted benzaldehyde in methanol with 2M sodium hydroxide. The product chalcone was left overnight to precipitate out of the methanol solution. They were then filtered off and recrystallized from methanol (*Scheme 73*).

382 **383 - 390**

Chalcone	Ar ¹	Yield (%)
383	4-Chlorophenyl	66
384	3,4-Dichlorophenyl	83
385	2,6-Dichlorophenyl	66
386	4-Bromophenyl	56
387	2,4-Dimethoxyphenyl	72
388	3,5-Dimethoxyphenyl	70
389	2,3,4-Trimethoxyphenyl	90
390	3,4,5-Trimethoxyphenyl	48

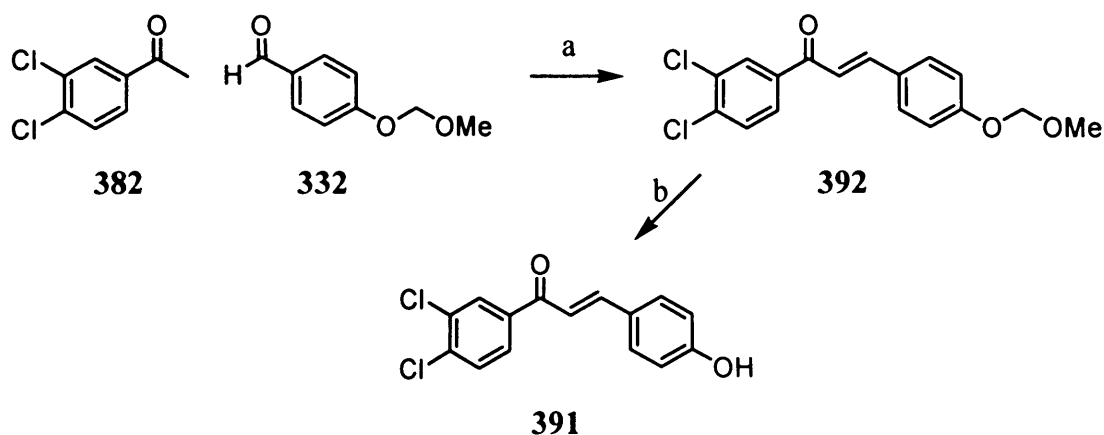
Scheme 73: a. Ar¹CHO, 2M NaOH (aq), MeOH, r.t., overnight.

The 3',4'-dichlorochalcones **383** – **390** were all isolated in moderate to excellent yields as the *E*-isomers as shown by their ¹H NMR spectra.

We then tried to prepare (*E*)-1-(3',4'-dichlorophenyl)-3-(4''-hydroxyphenyl)prop-2-en-1-one (**391**) via the basic Claisen-Schmidt condensation¹⁷⁸ of the 3',4'-dichloroacetophenone (**382**)

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

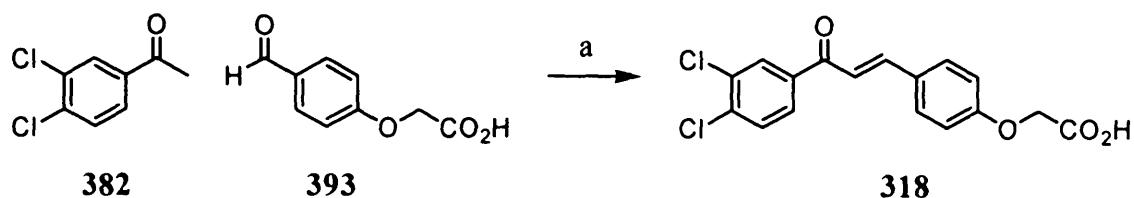
and 4-hydroxybenzaldehyde (**328**) This reaction failed due to the reduced electrophilicity of the carbonyl group of **328** because the electron donating effect of the deprotonated 4-hydroxy group. The starting materials were recovered. We attempted the acidic Claisen-Schmidt condensation of the 3',4'-dichloroacetophenone (**382**) and 4-hydroxybenzaldehyde (**328**) reported by Dimmock²²⁴ *et al*, whereby chalcone was prepared by bubbling hydrogen chloride through a solution of **382** and **328** in ethanol, the mixture turned red and was left stirring at room temperature overnight. However this reaction also failed and the 3',4'-dichloroacetophenone (**382**) was recovered. We then prepared the MOM protected chalcone **392** from the MOM protected benzaldehyde **332** and the acetophenone **382** (*Scheme 74*). The MOM protected chalcone **392** was then deprotected by refluxing the chalcone in methanol and in the presence of 3 M hydrochloric acid for 45 minutes (*Scheme 74*).²³⁰ This gave **391** as yellow solid in an excellent yield, as the *E*-isomer shown by inspection of its ¹H NMR spectrum ($J_{\text{H}\alpha,\beta}$ 15.4 Hz).



Scheme 74: a. 2M NaOH (aq), MeOH, r.t., overnight, 65%; b. 3M HCl (aq), MeOH, 75 °C, 45 mins, 99%.

We also prepared the carboxylic acid chalcone **318** so we could directly compare the biological results of the 3',4'-dichlorochalcones with and without the carboxylic acid group. The chalcone **318** was prepared using the Claisen-Schmidt condensation¹⁷⁸ of the 3',4'-dichloroacetophenone (**382**) and 4-formylphenoxyacetic acid (**393**) in methanol under basic conditions (*Scheme 75*). The chalcone was isolated by ethyl acetate extraction and recrystallization from methanol to give **318** in good yield as yellow crystals.

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion



Scheme 75: a. 2M NaOH (aq), MeOH, r.t., overnight, 66%.

The key diagnostic peak of the ^1H NMR spectrum of carboxylic acid chalcone **318** are the peak for the methylene group which appears at 4.76 (2H, s, H-2'') ppm and the peaks for enone system of **318** at 7.74 (1H, d, J 15.4 Hz, H-2) and 7.83 (1H, d, J 15.4 Hz, H-3) ppm showing that chalcone was isolated as the *E*-isomer (*Figure 40*). The ^1H NMR spectrum of **318** corresponds to the literature values reported by Kumar²⁵³ *et al.*

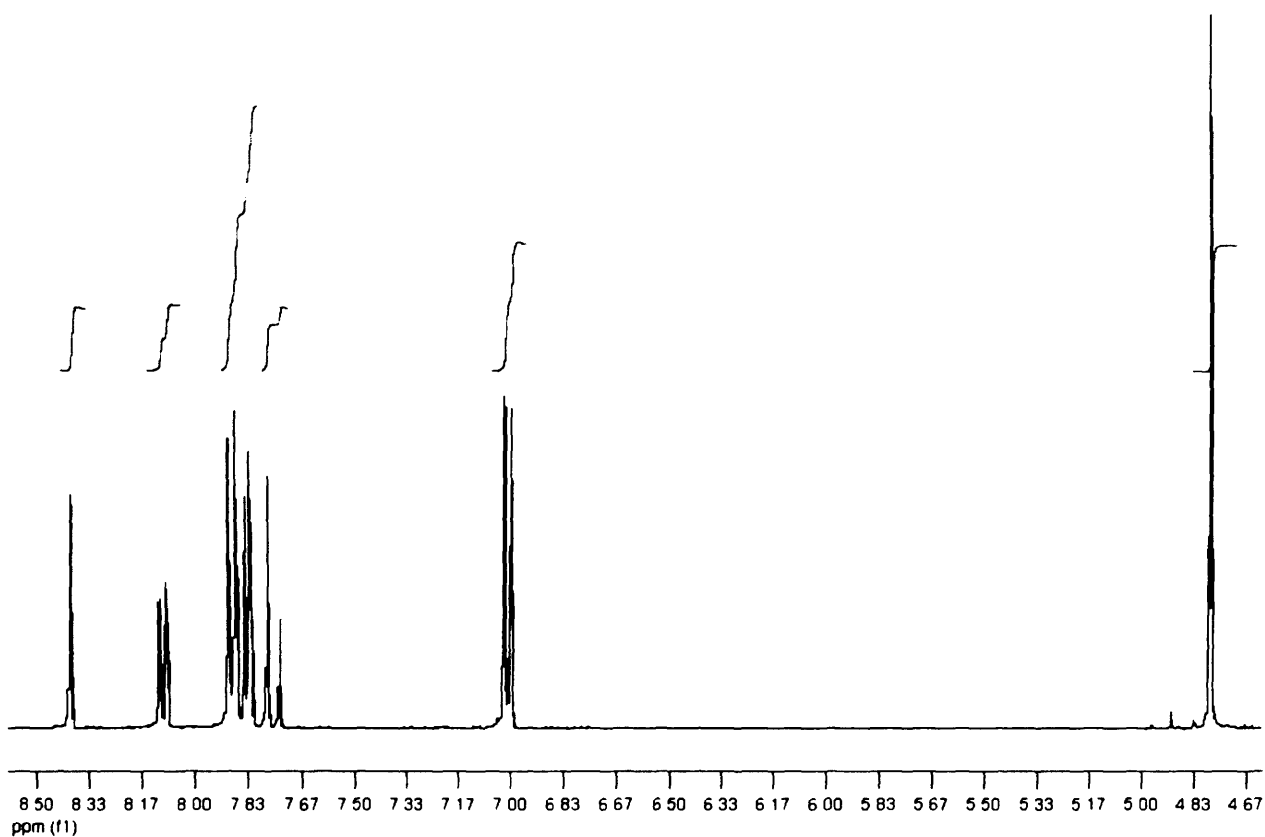
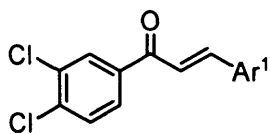


Figure 40: ^1H NMR spectrum of the carboxylic acid chalcone **318**

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

4.3.2 Biological activities of 3',4'-dichlorochalcones



318, 383 - 392

Chalcone	Ar ¹	IC ₅₀ (p53-MDMX) (μM)
318	Phenyl-4-oxyacetic acid	250
383	4-Chlorophenyl	>300
384	3,4-Dichlorophenyl	>300
385	2,6-Dichlorophenyl	>300
386	4-Bromophenyl	>300
387	2,4-Dimethoxyphenyl	>300
388	3,5-Dimethoxyphenyl	>300
389	2,3,4-Trimethoxyphenyl	>300
390	3,4,5-Trimethoxyphenyl	>300
391	4-Hydroxyphenyl	>300
392	4-(Methoxymethoxy)phenyl	>300

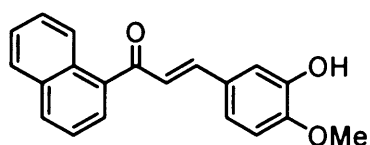
Our chalcones were tested for their ability to disrupt the p53-MDMX complex. The chalcones were screened in an ELISA (Enzyme-Linked ImmunoSorbent Assay) assay²⁹⁶ using p53 and MDMX proteins purified from *E. coli*. This ELISA assay measures the binding of the MDMX protein to the p53 protein which is immobilized on the surface of the ELISA plate, by looking at the absorbance of the plate at a wavelength of 450 nm. The ELISA plates are first treated with p53 and incubated in PBS (phosphate buffered saline) for 16 hours, so the p53 can bind to the ELISA plate. The plate was then washed with PBST (PBS + 0.1% Tween 20), and then blocked with PBSMT (PBS + 5% nonfat dairy milk + 0.1% Tween 20) for 30 minutes. The chalcones were then dissolved in DMSO. The MDMX (5 μg/ml) was then mixed with the DMSO dissolved chalcone (at various concentrations) in PBSMT. The MDMX/chalcone mixture was then added to the wells of the plate. The plate was then incubated for an hour and then washed with PBST. The antibody 5B10 in PBSMT was then added to the plates for 1 hour, followed by washing and incubation with horseradish peroxidase-rabbit anti-mouse immunoglobulin antibody for an hour. The antibody 5B10 is used to detect the MDMX binding to P53. The plate was then developed by incubation with tetramethyl benzidine peroxides substrate and measured by absorbance at 450 nm.²⁹⁶

MDMX is a protein related to MDM2 and shares several regions of homology with MDM2 including the p53 binding site.²⁹⁷ The only chalcone to show any activity below a concentration of 300 μM, was the chalcone **318** with an IC₅₀(p53-MDMX) of 250 μM. This

Chapter 4 – Other Cytotoxic Chalcones. Results and Discussion

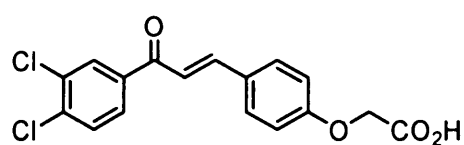
shows that the presence of the carboxylic acid group on 3,4-dichalcone **318** plays an important role in the disruption of the HDM2/p53 interaction.

The chalcone **394** which was prepared by another member of the our group exhibited an IC_{50} value of 80 μM for the inhibition of the p53-MDMX interaction, however further testing showed that **394** had no effect on p53-MDM2 interaction, up to a concentration of 500 μM . While the chalcone **319** shows a weak activity at inhibiting both the p53-MDMX and p53-MDM2 interaction. This makes **394** a good lead structure for the optimization of potency of a selective p53-MDMX inhibitor. Presently the crystal structure of MDMX is unknown, so a model of the p53 binding site, based on the MDM2 model will be constructed by the computer modelers, which can be used cautiously for structure-based design for the optimization of **394**. Traditional QSAR (quantitative structure-activity relationships) studies will also be used in the optimization of **394** as a selective p53-MDMX inhibitor.



394

IC_{50} (p53-MDMX) 80 μM
 IC_{50} (p53-MDM2) >500 μM



318

IC_{50} (p53-MDMX) 250 μM
 IC_{50} (p53-MDM2) 250 μM

Chapter 5 – Heat Shock Proteins as targets for drug design

5.1 Hyperthermia and the prevention of thermotolerance via drug like compounds

Hyperthermia is a type of cancer treatment in which body tissue is exposed to high temperatures, 40 – 44 °C. *In vivo* tests show that hyperthermia treatment selectively kills tumour cells at temperatures between 40 – 44 °C,²⁹⁸ while normal tissues are undamaged at exposure to these temperatures.²⁹⁹ Tumour tissue is more sensitive to hyperthermia because of the chaotic architecture of the tumour vasculature in solid tumours, which result in regions of the tumour tissue which have low pH and suffer from hypoxia.^{298,300} These environmental differences make tumour cells more sensitive to hyperthermia treatment. The main mechanism for cell death is thought to be caused by protein denaturation, which is observed at temperatures >40 °C, this can lead to the alteration of cellular structures like the cytoskeleton and membranes, and changes in enzyme complexes used for DNA synthesis and repair.³⁰¹

The use of hyperthermia in conjunction with radiotherapy³⁰²⁻³⁰⁶ or chemotherapy³⁰⁷ has been shown to increase the anticancer effects of both therapies. However one of the major problems with hyperthermia is the development of thermotolerance, which is a transient resistance to the effects of hyperthermia induced by prior exposure to heat treatment.^{308,309} When cells are exposed to heat they respond by synthesizing a group of proteins called the heat shock proteins (HSPs)³¹⁰ which are involved in the acquisition of thermotolerance in cells.^{311,312} The HSPs bind to denatured proteins caused by cellular stress such as heat shock and renature them or bring them to degradation pathways.³⁰² Several studies³¹²⁻³¹⁴ have shown the relationship between the level of thermotolerance and cellular content of the HSPs, one study showed that the introduction of HSP72 specific antibodies to fibroblast cells made them more sensitive to thermal stress.³¹⁵ These studies suggest that a strong relationship between the induction of stress proteins in the prevention of apoptosis.³⁰²

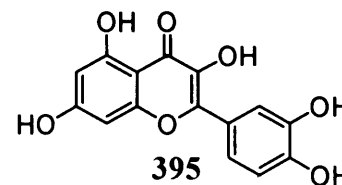
The regulation of the synthesis of the HSP is mediated by the interaction of the heat shock factor (HSF1) with the heat shock elements (HSEs) in the heat shock protein gene promoter region.^{316,317} HSF1 exists in unstressed cells in an inactive form and is rapidly activated after heat shock.³⁰²

It is critical for the further development of hyperthermia as a cancer therapy to find drug like compounds that can specifically inhibit the induction of HSPs in cancer cells after their first heat treatment to help improve the effects of fractionated hyperthermia.^{302,318}

The flavonoid quercetin (**395**) has been shown to inhibit the synthesis of several HSPs (including HSP70) induced by heat shock on 2 human cancer cell lines, HeLa and COLO

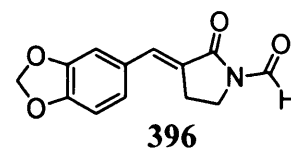
Chapter 5 – Heat Shock Proteins as targets for drug design

320DM.³¹⁹ *In vivo* and *in vitro* tests show that the quercetin (**395**) inhibits the induction of the HSP by suppressing the activation of the HSF1, or the interaction of the HSF1 with the HSEs.³²⁰

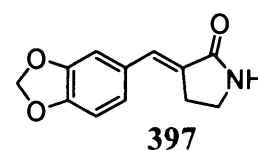


In vitro testing of the novel benzylidene lactam compound KNK437

(**396**) by Yokota³⁰² and co-workers showed that it was a dose dependant inhibitor of the acquisition of thermotolerance in COLO 320DM (human colon carcinoma) cells and in HeLa S3 cells after fractionated heat treatment. Further testing showed that **396** inhibits the synthesis of various HSPs including HSP70, HSP40 and HSP105.³⁰²



Further *in vivo* testing of KNK437 (**396**) by Koishi³¹⁸ and co-workers, showed that KNK437 (**396**) inhibited the induction of thermotolerance at a concentration of 200 mg/kg when administrated 6 hours before initial heating. The time taken for the tumour to triple in volume was on average 2.9 days longer than that for tumours that had been heat shocked without the KNK437.³¹⁸ Showing that KNK437 had contributed to the inhibition of the synthesis of HSP *in vivo*. They also found that the KNK437 (**396**) was metabolized to KNK423 (**397**) which was observed by HPLC at high levels in tumours that had been administered with KNK437 (**396**).³¹⁸



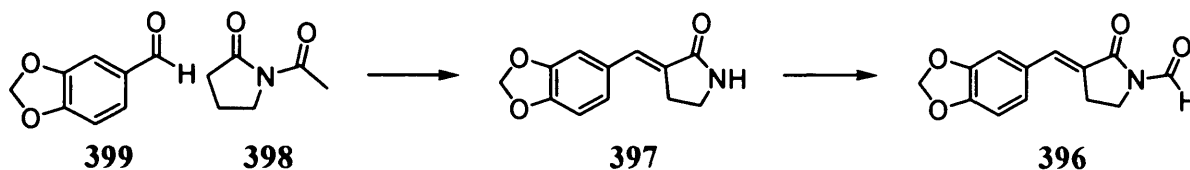
The effects of KNK 437 (**396**) proved to be cytostatic and reversible over time, rather than being cytotoxic.³⁰² Further testing showed that KNK 437 (**396**) was a very weakly toxic drug, with tumour free mice recovering the lost body weight by 2 days after treatment of **396** at concentrations up to 400mg/kg.³¹⁸

It is thought that the inhibitory mechanism of KNK437 may be attributed to the inhibition of the HSF1, or the interaction of the HSF1 with the HSEs, as reported for the bioflavonoid Quercetin.^{302,320}

5.2 Synthesis of KNK437 and similar analogues.

Our route for the synthesis of KNK 437 (**396**) involved the aldol condensation of 1-acetylpyrrolidin-2-one (**398**) and piperonal (**399**) to give KNK423 (**397**)³²¹ (*Scheme 76*). KNK 437 (**396**) would then be obtained via the formylation of the amine on the 2-pyrrolidinone ring of **397** (*Scheme 76*).³²²

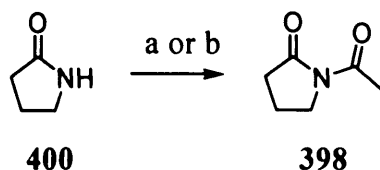
Chapter 5 – Heat Shock Proteins as targets for drug design



Scheme 76

Before we carried out the aldol condensation of piperonal (**399**) and 1-acetylpyrrolidin-2-one (**398**), the 1-acetylpyrrolidin-2-one (**398**) had to be prepared by acetylating the nitrogen of 2-pyrrolidinone (**400**) (Scheme 77). Using the procedure described by Sasaki³²³ and coworkers 1-acetylpyrrolidin-2-one (**398**) was prepared by adding acetyl chloride to a mixture of triethylamine and 2-pyrrolidinone in THF at 0 °C (Scheme 77, route a). The isolation of **398** as described by Sasaki³²³ was done by distillation, however after collecting the distillate at 152 – 155 °C the ¹H NMR spectrum showed that the distillate was an 1:1 mixture of the starting pyrrolidinone (**400**) and desired acetylated product **398**. The acetylated product **398** was then isolated from **400** by column chromatography in a moderate yield.

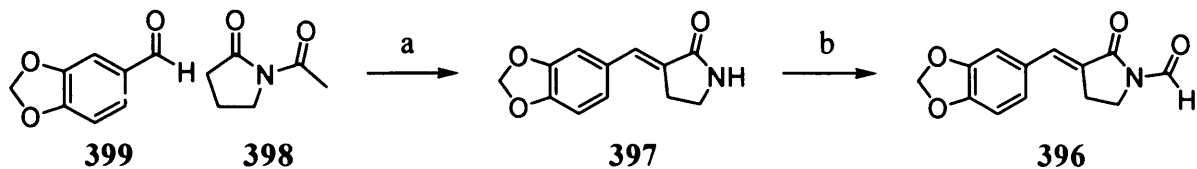
It was also found by other members of the group that the acetylation of **400** can be achieved by treating the 2-pyrrolidinone (**400**) in anhydrous THF with 2.5 M *n*-butyllithium in hexanes at -20 °C and then by adding acetyl chloride to a mixture and then leaving the mixture stirring overnight (Scheme 77, route b). The acetylated product **398** was then isolated by chromatography in a good yield.



Scheme 77: a. acetyl chloride, triethylamine, THF, 0 °C – r.t., 1hr, 52%; b. i. *n*BuLi, THF, -20 °C, ii. acetyl chloride, THF, -20 °C to r.t., overnight, 71%.

Then using the technique described by Zimmer³²¹ *et al.* the benzylidene lactam compound KNK423 (**397**) was prepared by treating 1-acetylpyrrolidin-2-one (**398**) and piperonal (**399**) in anhydrous THF at 0 °C with sodium hydride (Scheme 78). After an hour the reaction was quenched by treating the mixture with methanol, and then tipped into 6M H₂SO₄. The product was then extracted with chloroform, and was recrystallized from *iso*-propyl alcohol as a fine cream powder in a low yield. However, this provides sufficient material for the biological studies.

Chapter 5 – Heat Shock Proteins as targets for drug design



Scheme 78: a. NaH, THF, 0 °C, 1hr, 27%; b. formic acid, 105 °C, 18hrs, 55%.

The KNK 423 (**397**) was fully characterised; the HRMS of **397** gave a molecular ion $[MH^+]$ of m/z 218.0812 which corresponds to the expected formula $C_{12}H_{12}O_3N$. The key diagnostic peaks in the 1H NMR spectrum of **397** are two signals for the 2-pyrrolidinone ring 3.12 (2H, td, J 6.5 Hz, J 2.8 Hz, H-4) and 3.56 (2H, t, J 6.5 Hz, H-5) ppm and the signal for methylene proton at 7.28 (1H, t, J 2.8 Hz, H-1') ppm (*Figure 41*).

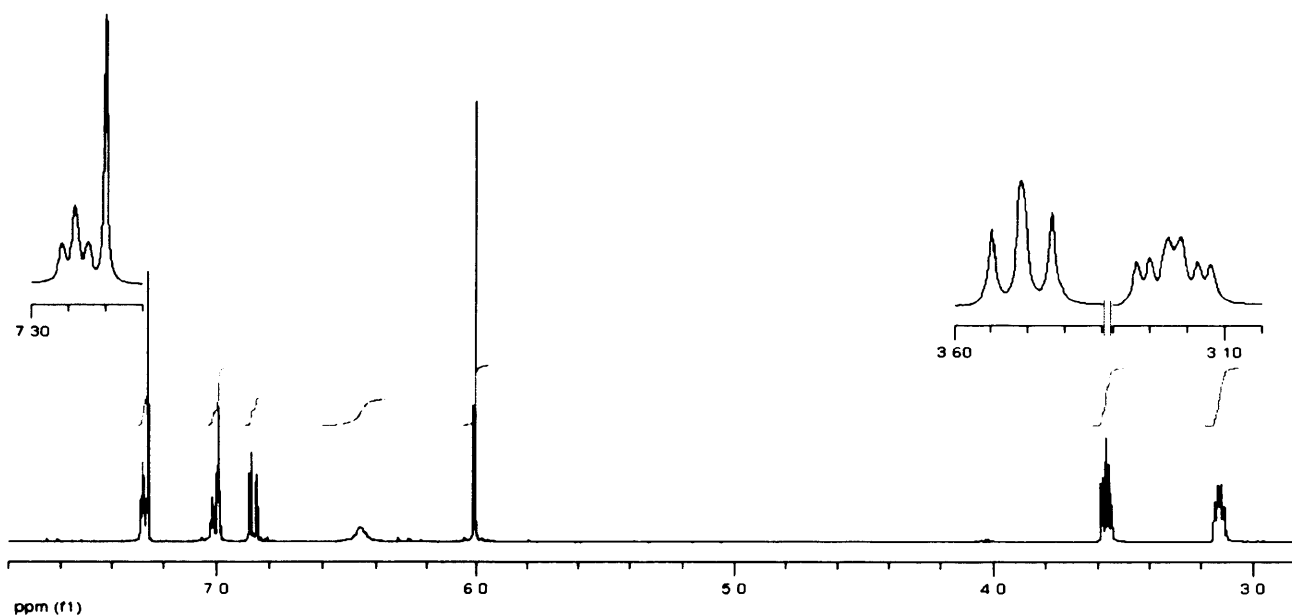
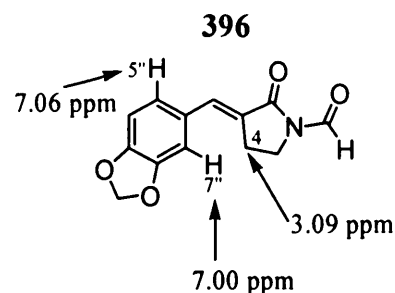


Figure 41: 1H NMR spectrum of KNK423 (**397**)

Using the procedure described by Rigo³²² *et al.* **397** was formylated by heating in neat refluxing formic acid for 18 hrs (*Scheme 78*). After which time the reaction mixture was a 1:1.9 ratio of the starting KNK423 (**397**) to the product KNK437 (**396**). The KNK437 (**396**) was then isolated via column chromatography as a white solid in a moderate yield.

The KNK 437 (**396**) was fully characterised; the HRMS of **396** gave a molecular ion $[MH^+]$ of m/z 246.0758 which corresponds to the required $C_{13}H_{12}O_4N$ formula. The key diagnostic peaks in the 1H NMR spectrum of **396** is the loss of the signal for the **397** amine proton of at 6.45 (1H, br. s, NH-1) ppm (*Figure 41*) and introduction of the signal for the formyl group at 9.24 (1H, s, H-1'') ppm (*Figure 42*).



Chapter 5 – Heat Shock Proteins as targets for drug design

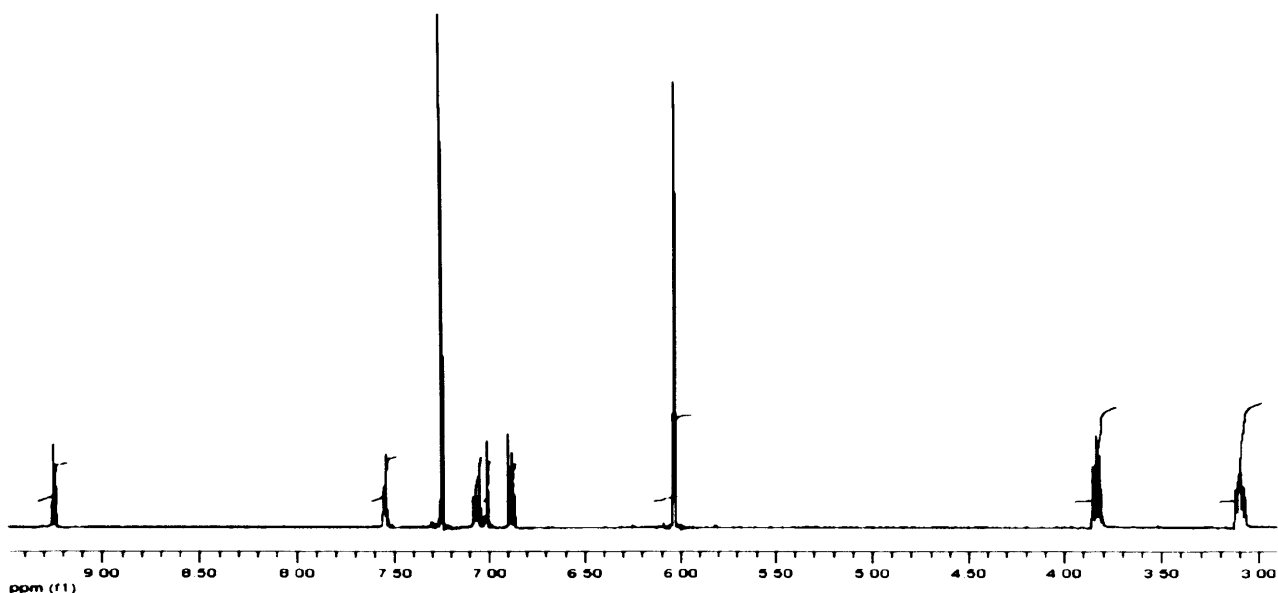


Figure 42: ^1H NMR spectrum of KNK437 (**396**)

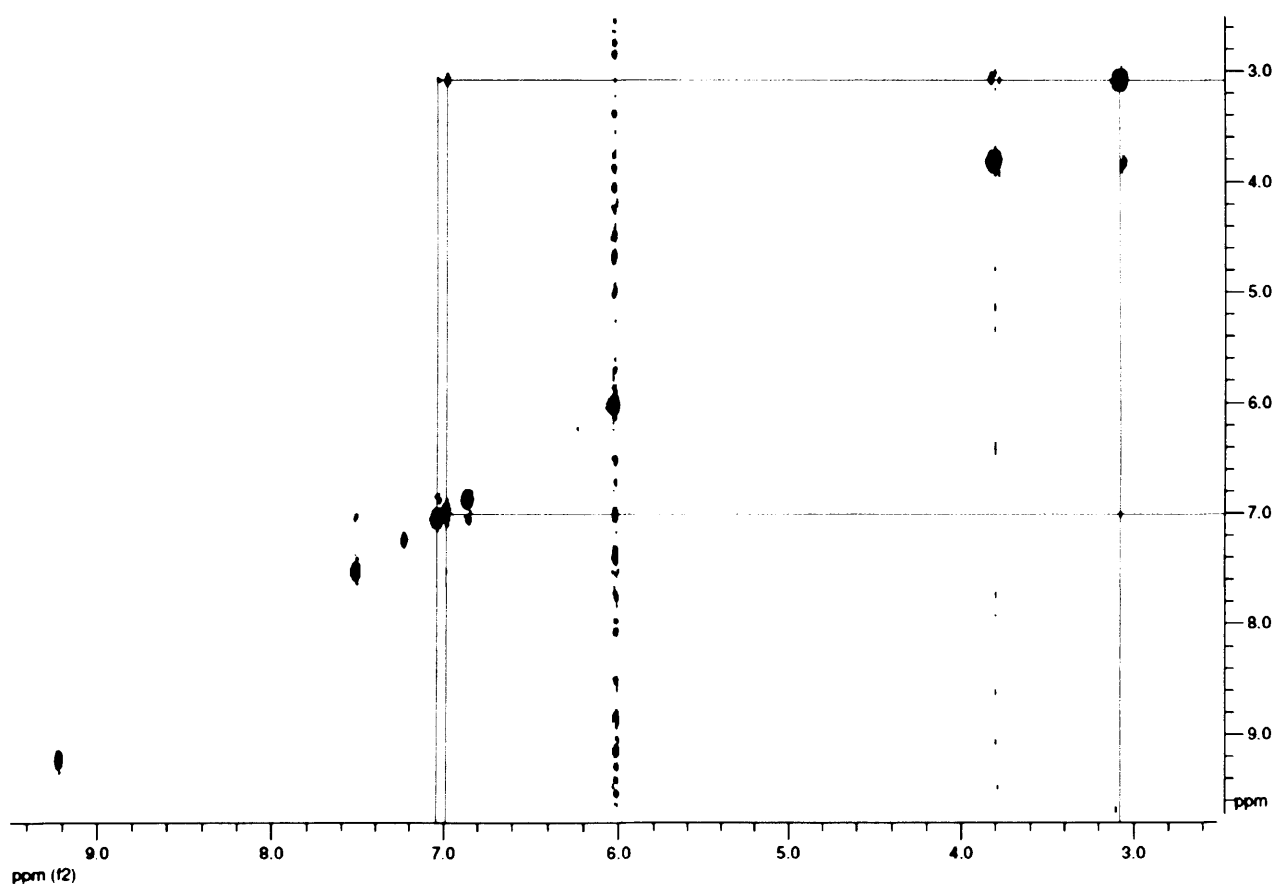


Figure 43: NOESY NMR spectrum of KNK437 (**396**) showing the nOe correlations between the signals of H-4 and H-7'', and H-4 and H-5'' proving the *E*-geometry of **396**.

The geometry of double bond of KNK437 (**396**) was determined by a NOESY NMR experiment (Figure 43). The NOESY experiment of **396**, shows nOe correlations between the signals for the H-4 hydrogens at 3.09 ppm to the H-7'' hydrogen at 7.00 ppm, as well as

Chapter 5 – Heat Shock Proteins as targets for drug design

the H-5'' hydrogen at 7.06 ppm proving that the *E*-isomer of KNK437 (**396**) was isolated from the reaction.

One of the main problems with KNK437 (**396**) and KNK423 (**397**) is their poor solubility in DMSO, KNK437 (**396**) solubility in DMSO ~ 25 mg/ml, while KNK423 (**397**) solubility in DMSO ~20 mg/ml.

We therefore decide to investigate producing a more soluble version of the KNK compounds. To do this we decided to use rhodanine (**401**) instead of 1-acetylpyrrolidin-2-one (**398**) in the synthesis. The introduction of the rhodanine (**401**) into the KNK structure gives the molecule an extra sulfide group and thioamide group, which should improve the solubility of the molecule. The heteroatom should increase the number of hydrogen bonds that the KNK rhodanine analogues can make with a polar solvent, thus enhancing the overall solubility of these compounds in polar solvents.

The rhodanine analogue **402** of KNK423 (**397**) was prepared using the same conditions for the synthesis of KNK423 (**397**)³²¹ (Scheme 79).

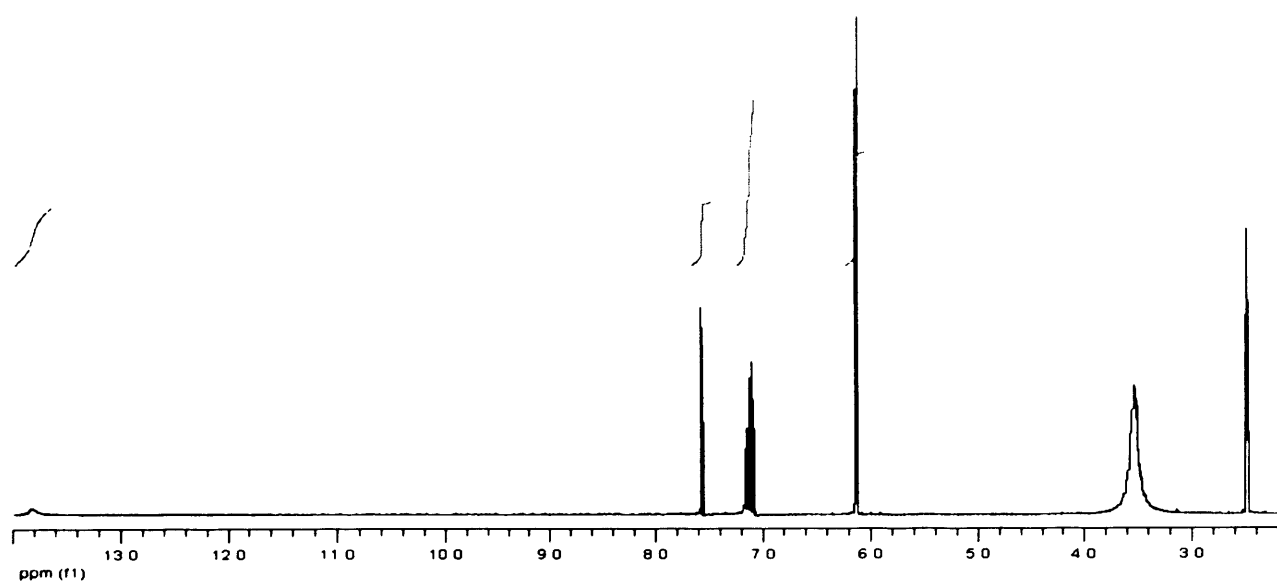
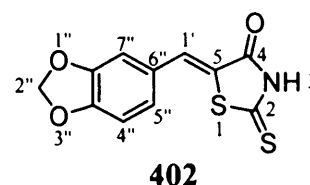


Figure 44: ¹H NMR spectrum of rhodanine analogue **402** of KNK423

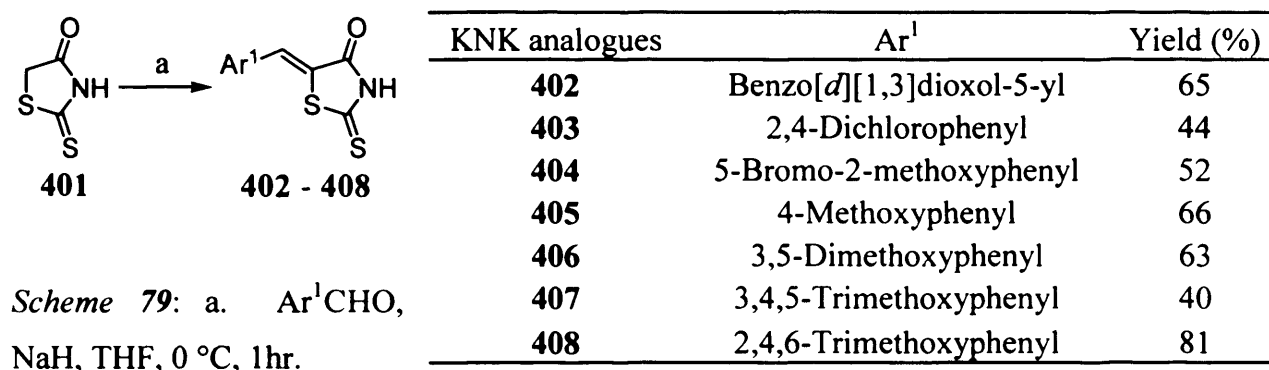
The key diagnostic peak of the ¹H NMR spectrum of rhodanine analogue **402** of KNK423 (**397**) are the signals for the aromatic hydrogens which appear at 7.08 (1H, d, *J* 8.0 Hz, H-4''), 7.11 (1H, d, *J* 1.9 Hz, H-7'') and 7.15 (1H, dd, *J* 1.9 Hz, *J* 8.0 Hz, H-5'') ppm, and the olefinic proton at 7.56 (1H, s, H-1') ppm (Figure 44).



It was found that we could dissolve up to 35 mg of **402** in 1 ml of DMSO. This made it more soluble than KNK423 (**397**) (ca. 20 mg could be dissolved in 1 ml of DMSO).

Chapter 5 – Heat Shock Proteins as targets for drug design

We then made a small series of other KNK like rhodanine analogues using various substituted benzaldehydes to investigate the effects of changing the aromatic group on the biological properties of the molecule. Another six KNK rhodanine analogues **403** – **408** were prepared using the same method as previously described (*Scheme 79*).

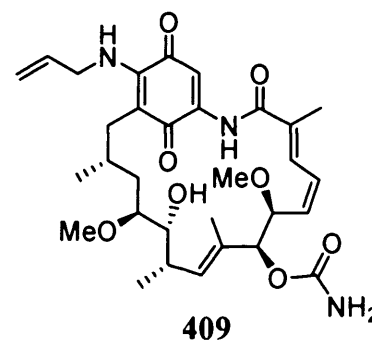


The ¹H NMR spectrum of the KNK rhodanine analogue **405** corresponded to *Z*-isomer of **405** which had been previously prepared by Khodair,³²⁴ therefore the *Z*-isomer of **405** had been prepared. This is the same geometrical sense as for the KNK437 (**396**), but **405** is labeled *Z*, since the CIP rankings have changed.

Others members of our group continued the investigation into developing a more soluble version of the KNK compounds.

5.3 Biological results of KNK437 and its analogues

A number of proteins associated with leukaemia require interaction with HSP90 to maintain a mature and functional conformation.^{325,326} The semi-synthetic derivative 17-allylamino-demethoxygeldanancin (17-AGG, **409**) has been shown to inhibit the function of HSP90,³²⁷ and leads to the degradation of its HSP90 associated proteins.^{325,326} 17-AGG (**409**) is currently being investigated in phase 1 and 2 clinical trials, administered alone or in combination with other anticancer agents.^{328,329}



The HSP90 also binds HSF1.³³⁰ When 17-AGG (**409**) binds to HSP90, it displaces bound HSF1. This increased level of HSF1 led to the up-regulation of the antiapoptotic HSP72.³³⁰⁻³³² Therefore the question arises, whether the elimination of the antiapoptotic HSP72 by co-treating with KNK437 (**396**) will further sensitize the human leukaemia (HL-60) cells to 17-AGG (**409**) treatment?³³³

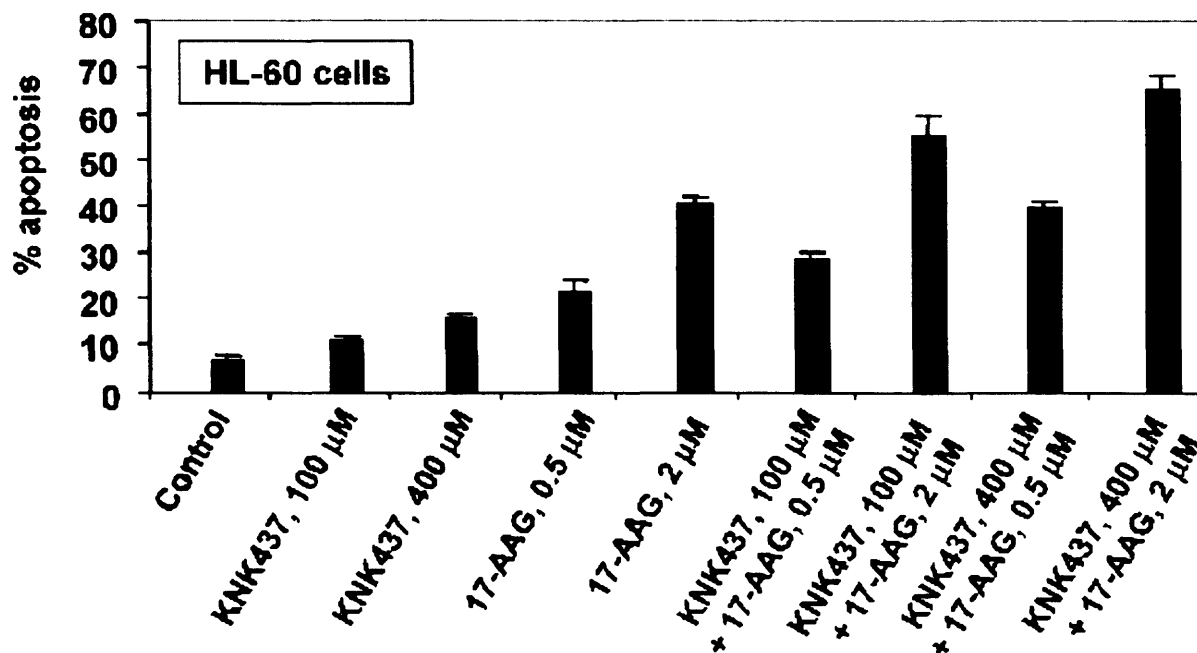


Figure 45: The effect on apoptosis of HL-60 cells after being pretreated with KNK437 (396) for an hour, followed by 17-AAG (409) + KNK437 (396) for 72 hours.³³³

The KNK437 (396) we made was then used in a study by Guo³³³ *et al.* The study found that KNK437 (396) sensitizes human leukaemia (HL-60) cells to 17-AGG (409) induced apoptosis (Figure 45).³³³ For instance treating the HL-60 cells with only 2.0 μM 17-AGG (409) caused apoptosis in about 40% of the HL-60 cells after 72 hours, while treatment with 2.0 μM 17-AGG (409) and 100 μM KNK437 (396) increased apoptosis to about 55%. Treating the HL-60 cells with 2.0 μM 17-AGG (409) and 400 μM KNK437 (396) increased the apoptosis again to 65% of the HL-60 cells (Figure 45).³³³

Chapter 6 – Experimental

General Experimental Information

Nuclear Magnetic Resonance Spectrometry

^1H NMR and ^{13}C NMR were carried out on a Bruker 400 MHz spectrometre and Varian 400 MHz spectrometre; chemical shifts for ^1H NMR are referenced to tetramethylsilane (TMS) as an internal standard, and chemical shifts for ^{13}C NMR are referenced to the solvent used.

Infra-red Spectroscopy

IR spectras were obtained for the compounds using a Perkin Elmer 1600 series FTIR instrument, Perkin-Elmer Spectrum One FTIR Spectrometre and JASCO FT/IR 4100 Spectrometre

Mass Spectrometry

Low resolution mass spectra were determined using a Fisons VG Platform II Quadrupole instrument and Agilent Technologies LC/MSD VL instrument. High resolution mass spectroscopy was carried out by the EPSRC national mass spectrometry service centre at Swansea University, as well as on an Agilent Technologies LC/MSD TOF instrument at the University of South Florida.

Elemental Analysis and Melting Point

Elemental analyses were performed by Warwick Analytical Services Ltd. Melting points were determined using Electrothermal Mel-Temp* manual melting point apparatus and Electrothermal Digital melting point apparatus model 9100, they were not corrected.

Chromatography and Solvents

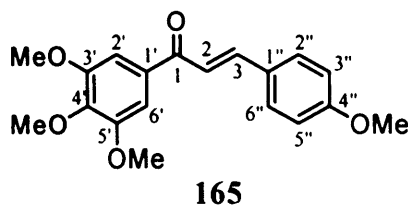
Column chromatography was performed using silica gel 60, 220-440 mesh (Apollo in the UK and Fisher in the US). Automated flash chromatography was conducted using a Flashmaster II system (Argonaut-Biotage), using Biotage silica cartridges. Thin layer chromatography was performed using silica gel 60 F₂₅₄ plates (Apollo in the UK and Fisher in the US), with observation under UV when necessary. Anhydrous solvents were obtained as follows: dichloromethane (anhydrous, 99.8%, contains 50-150 ppm hydrocarbon as stabilizer from Aldrich), dimethyl formamide (anhydrous, 99.8% from Aldrich), tetrahydrofuran (distilled over sodium/benzophenone under nitrogen as required).

Chapter 6 – Experimental

Protocol A

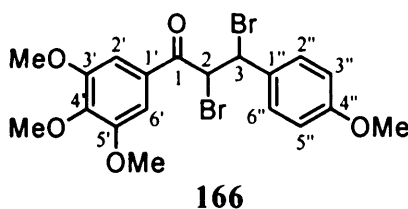
To a stirring solution of substituted acetophenone and substituted benzaldehyde in methanol was added a quantity of an aqueous solution of sodium hydroxide and the mixture stirred at room temperature overnight allowing the chalone to precipitate. The precipitate was then filtered off and washed with cold aqueous methanol (4:1 MeOH:H₂O). Purified by recrystallization from methanol.

(E)-1-(3,4,5-Trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **165**^{334,335}



The chalcone **165** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (8.65 g, 41.1 mmol), *p*-anisaldehyde (5.0 cm³, 41.1 mmol) and aqueous sodium hydroxide solution (6.9 cm³, 12M) in methanol (25 cm³). Recrystallization from methanol afforded **165** as cream crystals (12.1 g, 37.0 mmol, 90%); m.p. 98 – 99 °C; *R_f* 0.73 (SiO₂, hexane:ethyl acetate 1:1); ν_{\max} (cm⁻¹) 3007, 2941, 2843, 1655, 1591, 1567, 1505, 1459, 1447, 1420, 1411, 1345, 1308, 1248, 1153, 1121, 998, 979, 812, 824, 709; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.87 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 3.95 (6H, s, OCH₃), 6.95 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.27 (2H, s, H-2' & H-6'), 7.37 (1H, d, *J* 15.6 Hz, H-2), 7.62 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.80 (1H, d, *J* 15.6 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.46 (s, OCH₃), 56.39 (s, OCH₃), 61.02 (s, OCH₃), 105.90 (s, CH), 114.44 (s, CH), 119.37 (s, CH), 127.59 (s, C), 130.28 (s, CH), 133.85 (s, C), 142.19 (s, C), 144.68 (s, CH), 153.12 (s, C), 161.70 (s, C), 189.32 (s, CO); *m/z*(AP⁺) 329 (MH⁺, 100%); HRMS found [MH]⁺ 329.1385 for C₁₉H₂₁O₅, C₁₉H₂₁O₅ requires [MH]⁺ 329.1384; CHN found C 69.16%, H 6.14%, CHN requires C 69.50%, H 6.14%.

2,3-Dibromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)propan-1-one **166**³³⁴

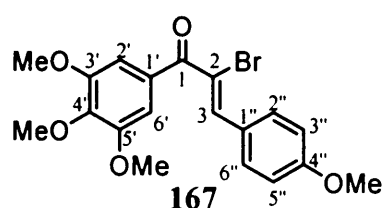


To a stirring solution of *(E)*-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **165** (0.92 g, 2.8 mmol), in chloroform (15cm³) at 0 °C under nitrogen, was added a solution of bromine in chloroform (2.8 cm³, 1M). The mixture was then stirred at 0 °C for

Chapter 6 – Experimental

one hour. The chloroform was then removed *in vacuo* to give the crude product **166** as a light pink solid (1.35 g, 2.8 mmol, 99%); m.p. 129 - 130 °C; R_f 0.85 & 0.66 (SiO₂, ethyl acetate:hexane 1:1); ν_{\max} (cm⁻¹) 3006, 2941, 2841, 1663, 1607, 1580, 1515, 1504, 1455, 1414, 1332, 1244, 1123, 991, 767; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.77 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.90 (6H, s, OCH₃), 5.59 (1H, d, J 11.3 Hz, H-3), 5.69 (1H, d, J 11.3 Hz, H-2), 6.88 (2H, d, J 8.7 Hz, H-3'' & H-5''), 7.27 (2H, s, H-2' & H-6'), 7.40 (2H, d, J 8.7 Hz, H-2'' & H-6''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 47.04 (s, CHBr), 50.42 (s, CHBr), 55.39 (s, OCH₃), 56.52 (s, OCH₃), 61.08 (s, OCH₃), 106.52 (s, CH), 114.24 (s, CH), 129.43 (s, C), 129.71 (s, CH), 130.23 (s, C), 143.69 (s, C), 153.33 (s, C), 160.17 (s, C), 190.17 (s, C=O).

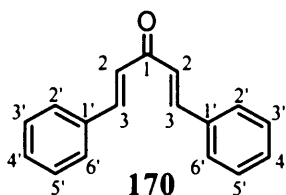
(*Z*)-2-Bromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **167**



Triethylamine (50 cm³) was slowly added to a stirring solution of (2,3-dibromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)propan-1-one **166** (2.44 g, 5.0 mmol), in chloroform (5cm³) at 0 °C under nitrogen. The mixture was then stirred overnight at room temperature, and then heated to 95 °C for 1 hour. The triethylamine was then evaporated *in vacuo*, the crude compound was then dissolved in chloroform (30 cm³) and washed with water (3 × 20 cm³) and saturated sodium bicarbonate solution (2 × 20 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from ethyl acetate and hexane afforded **167** as fine cream crystals (1.22g, 3.0 mmol, 60%) m.p. 111-112 °C; R_f 0.64 (SiO₂, hexane:ethyl acetate 1:1); ν_{\max} (cm⁻¹) 2935, 2837, 1642, 1581, 1504, 1461, 1448, 1411, 1327, 1249, 1121, 1099, 1028, 827, 712; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.87 (3H, s, OCH₃), 3.89 (6H, s, OCH₃), 3.94 (3H, s, OCH₃) 6.97 (2H, d, J 8.9 Hz, H-3'' & H-5''), 7.03 (2H, s, H-2' & H-6'), 7.69 (1H, s, H-3), 7.92 (2H, d, J 8.9 Hz, H-2'' & H-6''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.47 (s, OCH₃), 56.38 (s, OCH₃), 61.05 (s, OCH₃), 107.14 (s, CH), 114.04 (s, CH), 119.77 (s, C), 126.02 (s, CBr), 131.89 (s, C), 132.55 (s, CH), 141.81 (s, C), 142.47 (s, CH), 152.96 (s, C), 161.47 (s, C), 190.89 (s, C=O); m/z (AP+) 407 (MH⁺, 80%), 409 (MH⁺, 100%); HRMS found [MH]⁺ 407.0489 for C₁₉H₂₀O₅Br, C₁₉H₂₀O₅Br requires [MH]⁺ 407.0489; CHN found C 56.05%, H 4.78%, CHN requires C 56.03%, H 4.70%.

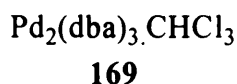
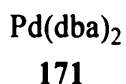
Chapter 6 – Experimental

(1*E*,4*E*)-1,5-Diphenylpenta-1,4-dien-3-one **170**³³⁶



The method adopted was that of Shoppee²⁰¹ *et al.* To a stirring solution of acetone (3.7 cm³, 50.4 mmol) and benzaldehyde (10.3 cm³, 101.3 mmol), in ethanol (80 cm³) was added an aqueous sodium hydroxide solution (100 cm³, 2.5 M). The reaction was then allowed to stand for 1 hour, allowing the bright yellow solid to precipitate. The yellow solid was then filtered off and washed with water (3 × 25 cm³). Recrystallization from ethyl acetate afforded **170** as bright yellow crystals (5.90 g, 25.2 mmol, 50%); m.p. 107 - 108 °C; R_f 0.32 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 3054, 3026, 1649, 1626, 1589, 1574, 1495, 1447, 1342, 1192, 980, 883, 760; δ ¹H NMR (400 MHz; CDCl₃; ppm) 7.09 (2H, d, *J* 16.2 Hz, H-2), 7.39 – 7.44 (6H, m, H-3', H-4' & H-5'), 7.60 – 7.65 (4H, m, H-2' & H-6'), 7.75 (2H, d, *J* 16.2 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 125.66 (s, CH), 128.64 (s, CH), 129.21 (s, CH), 130.74 (s, CH), 135.04 (s, C), 143.55 (s, CH), 189.16 (s, CO).

Bis(dibenzylideneacetone)palladium(0) **171** and *tris*(dibenzylideneacetone)dipalladium(0) chloroform **169**²⁰²



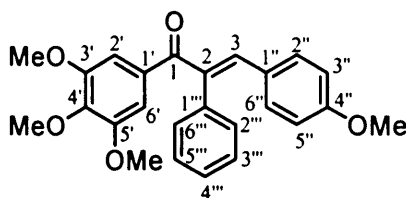
The method adopted was that of Hegedus.²⁰² To a stirring solution of (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one **170** (4.60 g, 19.7 mmol) and sodium acetate (3.90 g, 47.5 mmol) in methanol (150 cm³) under argon at 50 °C, was added palladium chloride (1.00 g, 5.6 mmol). The reaction was then cooled to 40 °C and stirred at this temperature for 4 hours. The mixture was then left to cool to room temperature and allow the product to precipitate. The purple precipitate was then collected by filtration and washed with water (10 cm³) and acetone (10 cm³). The purple precipitate was then dried *in vacuo* and afforded **171** as a purple precipitate (3.21 g, 5.6 mmol, 99%). *Bis*(dibenzylideneacetone)palladium(0) **171** (2.42 g, 4.2 mmol) was then dissolved in hot chloroform (90 cm³) and filtered to give a deep purple solution. Diethyl ether (120 cm³) was then slowly added to the purple solution and the mixture was then left to crystallize over night. The crystals were then filtered and dried *in vacuo* to afford **169** as deep purple crystals (1.61 g, 1.6 mmol, 74%).

Chapter 6 – Experimental

Protocol B

The method adopted was that of Mull²⁰⁰ *et al.* In a Schlenk tube a mixture of the α -bromo chalcone (1.0 mmol), boronic acid (1.2 mmol), tris(dibenzylideneacetone)dipalladium(0) chloroform **169** (0.023g, 0.02 mmol) and triphenylphosphine (0.011 g, 0.04 mmol) was dissolved in toluene (6 cm³) and *n*-propanol (2 cm³), and then degassed by evacuation. After stirring for 10mins, diethylamine (0.13 cm³, 1.3 mmol) and H₂O (1.6 cm³) were added to the reaction. The mixture was degassed a second time, refluxed for a 1 hour at 100 °C, then cooled to room temperature. The reaction was then poured into ethyl acetate (100 cm³) and washed with aqueous sodium hydroxide solution (30 cm³, 0.2 M), aqueous hydrochloric acid (30 cm³, 0.05 M) and water (2 × 30 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. The product chalcone was then purified by means of column chromatography and/or recrystallization.

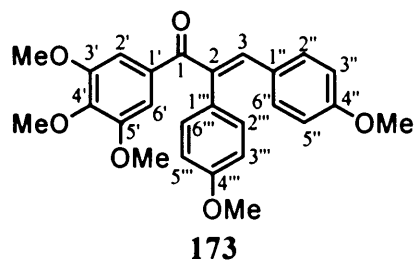
(E)-1-(3,4,5-Trimethoxyphenyl)-3-(4-methoxyphenyl)-2-phenylprop-2-en-1-one **172**



The α -arylchalcone **172** was obtained following Protocol B. Using (*Z*)-2-bromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **167** (0.407 g, 1.0 mmol), phenylboronic acid (0.147 g, 1.2 mmol). Recrystallization from ethyl acetate and hexane afforded **172** as cream fine needles (0.172 g, 0.42 mmol, 43%) m.p. 136-137°C; *R_f* 0.75 (SiO₂, hexane:ethyl acetate 1:1); ν_{max} (cm⁻¹) 2936, 1642, 1580, 1505, 1459, 1411, 1328, 1249, 1170, 1028, 827, 712; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.70 (3H, s, OCH₃), 3.75 (6H, s, OCH₃), 3.83 (3H, s, OCH₃), 6.65 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 6.98 (2H, s, H-2' & H-6'), 6.99 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.19-7.31 (6H, m, H-3 & H-2''' to H-6'''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.28 (s, OCH₃), 56.21 (s, OCH₃), 60.97 (s, OCH₃), 107.31 (s, CH), 113.77 (s, CH), 127.33 (s, C), 127.86 (s, CH), 128.98 (s, CH), 129.74 (s, CH), 132.22 (s, CH), 133.31 (s, C), 137.33 (s, C), 138.58 (s, C), 140.02 (s, CH), 141.37 (s, C), 152.71 (s, C), 160.27 (s, C), 196.17 (s, C=O); *m/z*(AP⁺) 405 (MH⁺, 100%), HRMS found [MH]⁺ 405.1697 for C₂₅H₂₅O₅, C₂₅H₂₅O₅ requires [MH]⁺ 405.1697; CHN found C 73.65%, H 5.97%, CHN requires C 74.24%, H 5.98%.

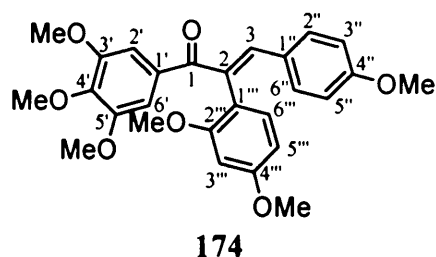
Chapter 6 – Experimental

(E)-1-(3,4,5-Trimethoxyphenyl)-2,3-bis(4-methoxyphenyl)prop-2-en-1-one **173**



The α -arylchalcone **173** was obtained following Protocol B. Using (*Z*)-2-bromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **167** (0.407 g, 1.0 mmol), 4-methoxyphenylboronic acid (0.182 g, 1.2 mmol). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) followed by recrystallization from ethyl acetate and hexane afforded **173** as yellow solid (0.131 g, 0.30 mmol 30%) m.p. 112-114°C; *R_f* 0.19 (SiO₂, hexane:ethyl acetate 3:1); ν_{max} (cm⁻¹) 2937, 2838, 1644, 1602, 1578, 1555, 1504, 1453, 1413, 1329, 1253, 1120, 1029, 1004, 823; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.71 (3H, s, OCH₃), 3.75 (6H, s, OCH₃), 3.76 (3H, s, OCH₃), 3.83 (3H, s, OCH₃), 6.66 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 6.83 (2H, d, *J* 8.7 Hz, H-3''' & H-5'''), 6.98 (2H, s, H-2' & H-6'), 7.03 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.13 (2H, d, *J* 8.7 Hz, H-2''' & H-6'''), 7.16 (H, s, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.27 (s, OCH₃), 56.21 (s, OCH₃), 60.96 (s, OCH₃), 107.29 (s, CH), 113.76 (s, CH), 114.40 (s, CH), 127.56 (s, C), 129.40 (s, C), 130.95 (s, CH), 132.11 (s, CH), 133.40 (s, C), 138.26 (s, C), 139.43 (s, CH), 141.32 (s, C), 152.70 (s, C), 159.19 (s, C), 160.14 (s, C), 196.61 (s, C=O); *m/z*(AP⁺) 435 (MH⁺, 100%); HRMS found [MH]⁺ 435.1799 for C₂₆H₂₇O₆, C₂₆H₂₇O₆ requires [MH]⁺ 435.1802; CHN found C 71.46%, H 6.04%, CHN requires C 71.87%, H 6.03%.

(E)-2-(2,4-Dimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **174**

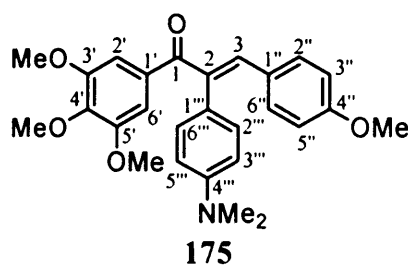


The α -arylchalcone **174** was obtained following Protocol B. Using (*Z*)-2-bromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **167** (0.407 g, 1.0 mmol), 2,4-dimethoxyphenylboronic acid (0.219 g, 1.2 mmol). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) followed by recrystallization from ethyl acetate and hexane afforded **174**

Chapter 6 – Experimental

as cream crystals (0.171 g, 0.37 mmol, 37%); m.p. 124 – 126 °C; R_f 0.12 (SiO₂, hexane:ethyl acetate 3:1); $\nu_{\max}(\text{cm}^{-1})$ 2935, 2835, 1651, 1580, 1503, 1463, 1439, 1412, 1328, 1303, 1208, 1155, 1121, 1008, 830, 766; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.59 (3H, s, OCH₃), 3.78 (3H, s, OCH₃), 3.81 (6H, s, OCH₃), 3.83 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 6.44 – 6.51 (2H, m, H-3''' & H-5'''), 6.73 (2H, d, J 8.6 Hz, H-3'' & H-5''), 7.07 (2H, s, H-2' & H-6'), 7.10 (1H, d, J 8.0 Hz, H-6'''), 7.14 (2H, d, J 8.6 Hz, H-2'' & H-6''), 7.23 (H, s, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.25 (s, OCH₃), 55.39 (s, OCH₃), 55.49 (s, OCH₃), 56.15 (s, OCH₃), 60.93 (s, OCH₃), 99.07 (s, CH), 105.23 (s, CH), 106.96 (s, CH), 113.63 (s, CH), 119.44 (s, C), 128.04 (s, C), 131.69 (s, CH), 131.75 (s, CH), 133.87 (s, C), 135.42 (s, C), 139.68 (s, CH), 141.00 (s, C), 152.52 (s, C), 158.05 (s, C), 159.98 (s, C), 161.08 (s, C), 197.09 (s, CO); $m/z(\text{AP}^+)$ 465 (MH⁺, 100%); HRMS found [MH]⁺ 465.1901 for C₂₇H₂₉O₇, C₂₇H₂₉O₇ requires [MH]⁺ 465.1908; CHN found C 69.45%, H 6.06%, CHN requires C 69.81%, H 6.08%.

(E)-2-(4-(Dimethylamino)phenyl)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **175**

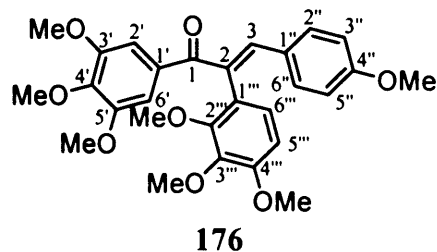


The α -arylchalcone **175** was obtained following Protocol B. Using (*Z*)-2-bromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **167** (0.407 g, 1.0 mmol), 4-(dimethylamino)phenylboronic acid (0.198 g, 1.2 mmol). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) followed by recrystallization from ethyl acetate and hexane afforded **175** as yellow crystals (0.116 g, 0.26 mmol, 26%); m.p. 129 – 131 °C; R_f 0.20 (SiO₂, hexane:ethyl acetate 3:1); $\nu_{\max}(\text{cm}^{-1})$ 2945, 1642, 1604, 1576, 1526, 1507, 1465, 1412, 1326, 1119, 997, 764; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.97 (6H, s, N(CH₃)₂), 3.78 (3H, s, OCH₃), 3.80 (6H, s, OCH₃), 3.89 (3H, s, OCH₃), 6.68 (2H, d, J 8.8 Hz, H-3'' & H-5''), 6.74 (2H, d, J 8.8 Hz, H-3''' & H-5'''), 7.07 (2H, s, H-2' & H-6'), 7.10 – 7.14 (3H, m, H-3 & H-2''' & H-6'''), 7.18 (2H, d, J 8.8 Hz, H-2'' & H-6''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 40.79(s, N(CH₃)₂), 55.62 (s, OCH₃), 56.55 (s, OCH₃), 61.30 (s, OCH₃), 107.76 (s, CH), 112.97 (s, CH), 114.04 (s, CH), 125.07 (s, C), 128.52 (s, C), 130.89 (s, CH), 132.32 (s, CH), 133.82 (s, C), 137.82 (s, CH), 139.41 (s, C), 150.32(s, C), 152.98 (s, C), 153.43 (s, C-N), 160.19 (s, C), 197.36 (s, CO); $m/z(\text{AP}^+)$ 447 (M, 100%) 448 (MH⁺, 30%); HRMS found

Chapter 6 – Experimental

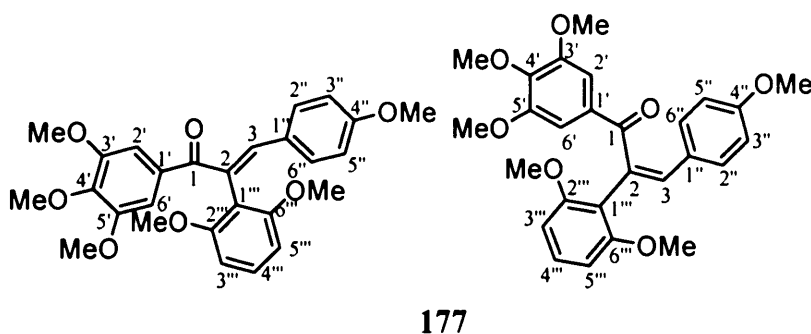
$[MH]^+$ 448.2112 for $C_{27}H_{30}NO_5$, $C_{27}H_{30}NO_5$ requires $[MH]^+$ 448.2118; CHN found C 71.82%, H 6.59%, N 2.94%, CHN requires C 72.46%, H 6.53%, N 3.13%.

(E)-2-(2,3,4-Trimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **176**



The α -arylchalcone **176** was obtained following Protocol B. Using (*Z*)-2-bromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **167** (0.407 g, 1.0 mmol), 2,3,4-trimethoxyphenylboronic acid (0.255 g, 1.2 mmol). Column chromatography (SiO_2 , ethyl acetate:hexane 1:3) afforded **176** as brown oil (0.30 g, 0.61 mmol, 61%); R_f 0.18 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 2938, 1649, 1581, 1509, 1493, 1460, 1409, 1327, 1254, 1122, 1026, 1005, 798; δ 1H NMR (400 MHz; $CDCl_3$; ppm) 3.63 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.78 (6H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 6.59 (H, d, J 8.6 Hz, H-5'''), 6.67 (2H, d, J 8.9 Hz, H-3'' & H-5''), 6.82 (H, d, J 8.6 Hz, H-6'''), 7.04 (2H, d, J 8.9 Hz, H-2'' & H-6''), 7.05 (2H, s, H-2' & H-6'), 7.15 (H, s, H-3); δ ^{13}C NMR (100 MHz; $CDCl_3$; ppm) 55.26 (s, OCH_3), 55.98 (s, OCH_3), 56.21 (s, OCH_3), 60.69 (s, OCH_3), 60.91 (s, OCH_3), 60.93 (s, OCH_3), 107.15 (s, CH), 107.61 (s, CH), 113.61 (s, CH), 124.43 (s, C), 125.45 (s, CH), 127.56 (s, C), 131.86 (s, CH), 133.82 (s, C), 135.38 (s, C), 140.39 (s, CH), 141.20 (s, C), 142.42 (s, C), 151.45 (s, C), 152.64 (s, C), 153.94 (s, C), 160.17 (s, C), 197.07 (s, CO); m/z (AP $^+$) 117 (Mfragment, 100%), 495 (MH^+ , 50%); HRMS found $[MH]^+$ 495.2008 for $C_{19}H_{18}O_6$, $C_{19}H_{18}O_6$ requires $[MH]^+$ 495.2013.

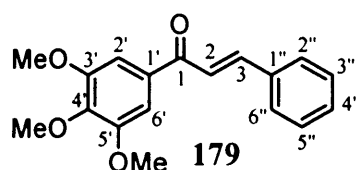
(E & Z)-2-(2,6-Dimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl) prop-2-en-1-one **177**



Chapter 6 – Experimental

The α -arylchalcone **177** was obtained following Protocol B. Using (*Z*)-2-bromo-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **167** (0.407 g, 1.0 mmol), 2,6-dimethoxyphenylboronic acid (0.219 g, 1.2 mmol). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **177** as a mixture of *E* and *Z* isomers as a light brown solid (0.186 g, 0.40 mmol, 40%); m.p. 123 - 124 °C; R_f 0.19 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2938, 1662, 1644, 1582, 1501, 1474, 1414, 1327, 1254, 1157, 1023, 998, 810, 773, 739; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.57 (9H, s, OCH₃), 3.69 (6H, s, OCH₃, *E*), 3.73 (3H, s, OCH₃, *E*), 3.75 (6H, s, OCH₃, *E*), 3.77 (4.5H, s, OCH₃, *Z*), 3.78 (9H, s, OCH₃, *Z*), 3.83 (3H, s, OCH₃, *E*), 3.87 (4.5H, s, OCH₃, *Z*), 6.52 – 6.58 (5H, m, H-3''' & H-5''', *E* & *Z*), 6.68 (2H, d, *J* 9.2 Hz, H-3'' & H-5'', *E*), 6.69 (3H, d, *J* 8.8 Hz, H-2'' & H-6'', *Z*), 7.05 (3H, s, H-2' & H-6', *Z*), 7.12 (3H, d, *J* 8.8 Hz, H-2'' & H-6'', *Z*), 7.13 (1H, s, H-3, *E*), 7.16 (2H, d, *J* 9.2 Hz, H-2'' & H-6'', *E*), 7.21 (1H, t, *J* 8.6 Hz, H-4''', *E*), 7.24 (2H, s, H-2' & H-6', *E*), 7.27 (1.5 H, t, *J* 8.4 Hz, H-4''', *Z*) 7.40 (1.5H, s, H-3, *Z*); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.22 (s, OCH₃), 55.73 (s, OCH₃), 55.84 (s, OCH₃), 56.04 (s, OCH₃), 60.85 (s, OCH₃), 60.90 (s, OCH₃), 104.37 (s, CH), 104.49 (s, CH), 106.52 (s, CH), 106.79 (s, CH), 113.43 (s, CH), 115.82 (s, C), 118.88 (s, C), 128.87 (s, C), 129.02 (s, C), 129.28 (s, CH), 129.96 (s, CH), 130.67 (s, CH), 130.79 (s, C), 131.06 (s, CH), 131.14 (s, C), 132.65 (s, C), 134.09 (s, C), 138.59 (s, CH), 140.73 (s, C), 141.03 (s, CH), 141.38 (s, C), 152.42 (s, C), 157.70 (s, C), 158.08 (s, C), 159.26 (s, C), 160.06 (s, C), 197.12 (s, CO), 197.41 (s, CO); *m/z*(API-ES) 465 (MH⁺, 100%); HRMS found [MH]⁺ 465.19053 for C₂₇H₂₉O₇, C₂₇H₂₉O₇ requires [MH]⁺ 465.19078.

(*E*)-1-(3,4,5-Trimethoxyphenyl)-3-phenylprop-2-en-1-one **179**^{72,337}

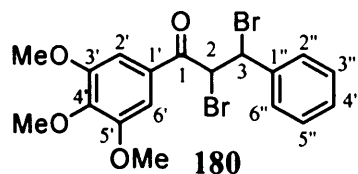


The chalcone **179** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (3.15 g, 15.0 mmol), freshly distilled benzaldehyde (1.6 cm³, 15.7 mmol) and aqueous sodium hydroxide solution (2.5 cm³, 12M) in methanol (9 cm³). Recrystallization from methanol afforded **179** as light yellow crystals (3.71 g, 12.4 mmol, 83%); m.p. 75 – 77 °C; R_f 0.38 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2941, 1656, 1573, 1501, 1451, 1412, 1337, 1156, 991, 763; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.94 (3H, s, OCH₃), 3.96 (6H, s, OCH₃), 7.28 (2H, s, H-2' & H-6'), 7.41 – 7.46 (3H, m, H-3'', H-4'' & H-5''), 7.49 (1H, d, *J* 15.7 Hz, H-2), 7.63 – 7.68 (2H, m, H-2'' & H-6''), 7.82 (1H, d, *J* 15.7 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.41 (s, OCH₃), 61.04 (s, OCH₃), 106.03 (s, CH), 121.77 (s, CH),

Chapter 6 – Experimental

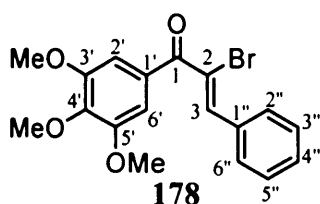
128.48 (s, CH), 129.01 (s, CH), 130.61 (s, CH), 133.52 (s, C), 134.89 (s, C), 142.44 (s, C), 144.82 (s, CH), 153.17 (s, C), 189.30 (s, CO).

2,3-Dibromo-1-(3,4,5-trimethoxyphenyl)-3-phenylpropan-1-one **180**



To a stirring solution of (*E*)-1-(3,4,5-trimethoxyphenyl)-3-phenylprop-2-en-1-one **179** (3.05 g, 10.2 mmol), in chloroform (45cm³) at 0 °C under nitrogen, was added a solution of bromine in chloroform (5.2 cm³, 2M). The mixture was then stirred at 0 °C for one hour. The chloroform was then removed *in vacuo* to give the crude product **180** as a pale pink solid (4.66 g, 10.2 mmol, 99%); m.p. 128 -134 °C; R_f 0.31 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 2937, 1682, 1579, 1504, 1454, 1412, 1335, 997, 696; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.90 (3H, s, OCH₃), 3.91 (6H, s, OCH₃), 5.58 (1H, d, *J* 11.26 Hz, H-3), 5.69 (1H, d, *J* 11.26 Hz, H-2), 7.27 (2H, s, H-2' & H-6'), 7.32 – 7.40 (3H, m, H-3'', H-4'' & H-5''), 7.46 – 7.49 (2H, m, H-2'' & H-6''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 46.71 (s, CH), 50.35 (s, CH), 56.90 (s, OCH₃), 61.47 (s, OCH₃), 106.90 (s, CH), 128.80 (s, CH), 129.28 (s, CH), 129.75 (s, CH), 138.57 (s, C), 144.11 (s, C), 153.72 (s, C), 190.44(s, CO); *m/z*(AP⁺) 299 (MH⁺-Br₂, 100%), 377(M-Br, 30%), 379(M-Br, 40%), 457 (MH⁺, 25%), 459 (MH⁺, 40%), 461(MH⁺, 25%).

(*Z*)-2-Bromo-1-(3,4,5-trimethoxyphenyl)-3-phenylprop-2-en-1-one **178**

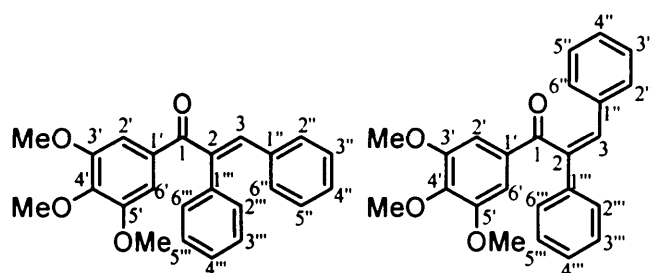


Triethylamine (35 cm³) was slowly added to a stirring solution of 2,3-dibromo-1-(3,4,5-trimethoxyphenyl)-3-phenylpropan-1-one **180** (1.60 g, 3.5 mmol), in chloroform (3cm³) at 0 °C under nitrogen. The mixture was then stirred overnight at room temperature, and then heated to 95 °C for 1 hour. The triethylamine was then evaporated *in vacuo*, the crude compound was then dissolved in chloroform (30 cm³) and washed with water (3 × 20 cm³) and saturated sodium bicarbonate solution (2 × 20 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from ethyl acetate and hexane afforded **178** as brown crystals (0.54 g, 1.4 mmol, 41%); m.p. 108 -

Chapter 6 – Experimental

110 °C; R_f 0.35 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2975, 1651, 1582, 1505, 1488, 1463, 1443, 1411, 1334, 1245, 1100, 1005, 770, 692; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.83 (6H, s, OCH₃), 3.88 (3H, s, OCH₃), 7.02 (2H, s, H-2' & H-6'), 7.36 – 7.41 (3H, m, H-3'', H-4'' & H-5''), 7.62 (1H, s, H-3), 7.78 – 7.82 (2H, m, H-2'' & H-6''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.39 (s, OCH₃), 61.06 (s, OCH₃), 107.34 (s, CH), 121.74 (s, C), 128.60 (s, CH), 130.23 (s, CH), 130.41 (s, CH), 131.21 (s, C), 133.59 (s, C), 141.66 (s, CH), 142.23 (s, C), 153.00 (s, C), 190.69 (s, CO); m/z (AP+) 377 (MH⁺, 100%) 379 (MH⁺, 100%); HRMS found [MH]⁺ 377.0389 for C₁₉H₁₈O₆, C₁₉H₁₈O₆ requires [MH]⁺ 377.0383.

(*E* & *Z*)-1-(3,4,5-Trimethoxyphenyl)-2,3-diphenylprop-2-en-1-one **181**

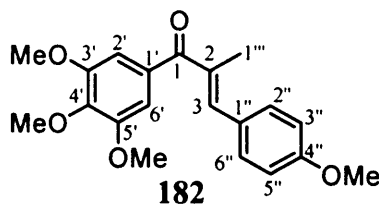


181

The α -arylchalcone **181** was obtained following Protocol B. Using (*Z*)-2-bromo-1-(3,4,5-trimethoxyphenyl)-3-phenylprop-2-en-1-one **178** (0.337 g, 1.0 mmol), phenylboronic acid (0.147 g, 1.2 mmol). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **181** as a mixture of *E* and *Z* isomers as yellow oil (0.194 g, 0.52 mmol, 52%); R_f 0.44 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2936, 1651, 1578, 1499, 1447, 1412, 1323, 1154, 1000, 764, 692; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.77 (14H, s, OCH₃), 3.81 (6H, s, OCH₃), 3.86 (7H, s, OCH₃), 3.90 (3H, s, OCH₃), 7.09 – 7.47 (42 H, m, Ar-H); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.15 (s, OCH₃), 56.22 (s, OCH₃), 60.92 (s, OCH₃), 60.98 (s, OCH₃), 107.02 (s, CH), 107.45 (s, CH), 126.38 (s, CH), 128.02 (s, CH), 128.17 (s, CH), 128.28 (s, CH), 128.31 (s, CH), 128.58 (s, CH), 128.79 (s, CH), 128.92 (s, CH), 128.94 (s, CH), 129.61 (s, CH), 130.27 (s, CH), 130.33 (s, CH), 131.32 (s, C), 132.71 (s, C), 134.87 (s, C), 135.51 (s, C), 136.85 (s, C), 138.40 (s, C), 138.87 (s, CH), 140.70 (s, C), 140.76 (s, C), 142.93 (s, C), 152.77 (s, C), 153.10 (s, C), 196.11 (s, CO), 198.17 (s, CO); m/z (API-ES) 375 (MH⁺, 100%); HRMS found [MH]⁺ 375.15914 for C₂₄H₂₃O₄, C₂₄H₂₃O₄ requires [MH]⁺ 465.19078.

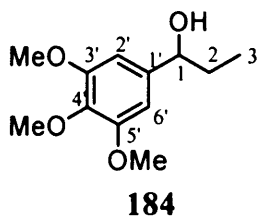
Chapter 6 – Experimental

(E)-1-(3,4,5-Trimethoxyphenyl)-3-(4-methoxyphenyl)-2-methylprop-2-en-1-one **182**



The method adopted was that of Edwards⁷² *et al.* A solution of 1-(3,4,5-trimethoxyphenyl)propan-1-one **183** (2.73 g, 12.2 mmol), *p*-anisaldehyde (1.5 cm³, 12.3 mmol), piperidine (7 cm³) and acetic acid (3.5 cm³) in ethanol (13 cm³) was heated at reflux at 110 °C under argon for 4 days. The mixture was then cooled to room temperature and diluted with dichloromethane (60 cm³) and washed with water (3 × 20 cm³). The aqueous fraction was then extracted with dichloromethane (4 × 50 cm³). The organic fractions were then combined and dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from ethyl acetate and hexane afforded **182** as clear white crystals (1.24 g, 3.7 mmol, 30%); mp 119 – 121 °C; R_f 0.38 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2996, 2936, 2825, 1632, 1604, 1582, 1502, 1458, 1412, 1331, 1175, 1031, 1015, 821, 771; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.28 (3H, d, *J* 1.3 Hz, H-1'''), 3.85 (3H, s, OCH₃), 3.89 (6H, s, OCH₃), 3.92 (3H, s, OCH₃), 6.95 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 6.99 (2H, s, H-2' & H-6'), 7.17 (1H, br. s, H-3), 7.42 (2H, d, *J* 8.8 Hz, H-2'' & H-6''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 14.72 (s, CH₃), 55.38 (s, OCH₃), 56.30 (s, OCH₃), 60.99 (s, OCH₃), 106.94 (s, CH), 114.03 (s, CH), 128.33 (s, C), 131.60 (s, CH), 133.91 (s, C), 134.56 (s, C), 141.06 (s, C), 141.85 (s, CH), 152.80 (s, C), 159.99 (s, C), 198.70 (s, CO); *m/z*(AP+) 343 (MH⁺, 100%); HRMS found [MH]⁺ 343.1544 for C₂₀H₂₃O₅, C₂₀H₂₃O₅ requires [MH]⁺ 343.1540; CHN found C 70.22%, H 6.49% CHN requires C 70.16%, H 6.48%.

1-(3,4,5-Trimethoxyphenyl)propan-1-ol **184**^{338,339}

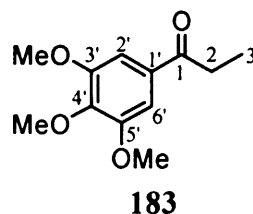


A solution of bromoethane (3.7 cm³, 49.6 mmol) in anhydrous tetrahydrofuran (40 cm³) was slowly added to a stirred mixture of magnesium turnings (1.46 g, 60.1 mmol) in anhydrous tetrahydrofuran (40 cm³) under argon. On consumption of the magnesium a solution of 3,4,5-trimethoxybenzaldehyde (7.85 g, 40.0 mmol) in anhydrous tetrahydrofuran (40 cm³) was added over 30 minutes. The mixture was stirred at room temperature under argon overnight

Chapter 6 – Experimental

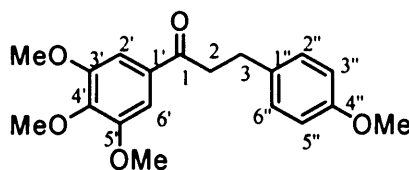
and quenched by pouring carefully onto a mixture of ice and aqueous hydrochloric acid (70 cm³, 1 M). The mixture was extracted with ethyl acetate (5 × 60 cm³) The organic fraction was dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo* to give **184** as a yellow oil (8.77 g, 38.8 mmol, 97%); R_f 0.14 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3448, 2937, 2811, 2823, 1590, 1507, 1456, 1417, 1326, 1121, 1004, 830; δ ¹H NMR (400 MHz; CDCl₃; ppm) 0.91 (3H, t, *J* 7.6 Hz, H-3), 1.61 – 1.86 (2H, m, H-2), 2.09 (1H, s, OH-1), 3.81 (3H, s, OCH₃), 3.84 (6H, s, OCH₃), 4.50 (1H, t, *J* 6.7 Hz, H-1), 6.54 (2H, s, H-2' & H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 10.30 (s, CH₃), 31.96 (s, CH₂), 56.04 (s, OCH₃), 60.83 (s, OCH₃), 76.18 (s, CH(OH)), 102.67 (s, CH), 136.94 (s, C), 140.58 (s, C), 153.13 (s, C); *m/z*(AP+) 209 (M(-H₂O)H⁺, 100%).

1-(3,4,5-Trimethoxyphenyl)propan-1-one **183**³³⁸



Pyridinium chlorochromate (12.93 g, 60 mmol) was added to a stirred solution of 1-(3,4,5-trimethoxyphenyl)propan-1-ol **184** (8.70 g, 38.5 mmol) in dichloromethane (100 cm³). The reaction was then put under argon gas and stirred at room temperature under argon overnight. It was then filtered through Celite[®], dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **183** as a pale cream solid (6.55 g, 29.3 mmol, 76%); mp 64 – 66 °C; R_f 0.32 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3004, 2968, 2939, 2839, 1673, 1586, 1505, 1454, 1411, 1309, 1157, 1123, 857, 799; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.21 (3H, t, *J* 7.2 Hz, H-3), 2.97 (2H, q, *J* 7.2 Hz, H-2), 3.90 (3H, s, OCH₃), 3.91 (6H, s, OCH₃), 7.21 (2H, s, H-2' & H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 8.44 (s, CH₃), 31.59 (s, CH₂), 56.28 (s, OCH₃), 60.96 (s, OCH₃), 105.40 (s, CH), 132.24 (s, C), 142.33 (s, C), 153.04 (s, C), 199.65 (s, CO); *m/z*(AP+) 225 (MH⁺, 100%).

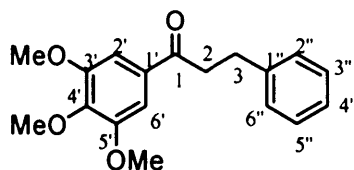
1-(3,4,5-Trimethoxyphenyl)-3-(4-methoxyphenyl)propan-1-one **185**



Chapter 6 – Experimental

(*E*)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **165** (2.09 g, 6.4 mmol) in ethyl acetate (40 cm³) was added to a stirring activated suspension of 10% Pd/C (1 spatula) in ethyl acetate (10 cm³). The mixture was stirred at room temperature under a hydrogen atmosphere for 3 hours, filtered through celite and evaporated *in vacuo*. Purification by column chromatography (SiO₂ hexane:ethyl acetate 3:1) afforded **185** as white crystals (1.72 g, 5.2 mmol, 82%); m.p. 88 – 90 °C; R_f 0.46 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3006, 2964, 2939, 2834, 1673, 1615, 1585, 1514, 1503, 1482, 1451, 1411, 1323, 1247, 1151, 1033, 1007, 816, 762; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.00 (2H, t, *J* 7.6 Hz, H-3), 3.23 (2H, t, *J* 7.6 Hz, H-2), 3.79 (3H, s, OCH₃), 3.89 (6H, s, OCH₃), 3.91 (3H, s, OCH₃), 6.85 (2H, d, *J* 8.5 Hz, H-3'' & H-5''), 7.18 (2H, d, *J* 8.5 Hz, H-2'' & H-6''), 7.20 (2H, s, H-2' & H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 29.50 (s, CH₂), 40.67 (s, CH₂), 55.30 (s, OCH₃), 56.29 (s, OCH₃), 60.98 (s, OCH₃), 105.42 (s, CH), 113.96 (s, CH), 129.43 (s, CH), 132.19 (s, C), 133.33 (s, C), 142.44 (s, C), 153.05 (s, C), 158.03 (s, C), 198.19 (s, CO); *m/z*(AP+) 331 (MH⁺, 100%); HRMS found [MH]⁺ 331.1537 for C₁₉H₂₃O₅, C₁₉H₂₃O₅ requires [MH]⁺ 331.1540.

1-(3,4,5-Trimethoxyphenyl)-3-phenylpropan-1-one **186**

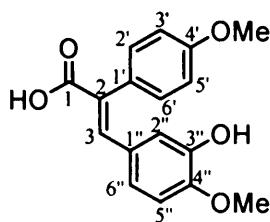


186

(*E*)-1-(3,4,5-trimethoxyphenyl)-3-phenylprop-2-en-1-one **179** (3.00 g, 10.1 mmol) in ethyl acetate (50 cm³) was added to a stirring activated suspension of 10% Pd/C (1 spatula) in ethyl acetate (10 cm³). The mixture was stirred at room temperature under a hydrogen atmosphere for 3 hours, filtered through celite and evaporated *in vacuo*. Purification by column chromatography (SiO₂ hexane:ethyl acetate 3:1) afforded **186** as an off white solid (2.87 g, 9.6 mmol, 95%); m.p. 90 – 91 °C; R_f 0.45 (SiO₂ hexane:ethyl acetate 3:1 v/v); ν_{max}(cm⁻¹) 2998, 2940, 2839, 1674, 1583, 1501, 1465, 1452, 1411, 1324, 1001, 712; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.06 (2H, t, *J* 7.6 Hz, H-3), 3.27 (2H, t, *J* 7.6 Hz, H-2), 3.89 (6H, s, OCH₃), 3.91 (3H, s, OCH₃), 7.20 (2H, s, H-2' & H-6'), 7.21 – 7.34 (5H, m, H-2'' to H-6''); δ ¹³C (100MHz; CDCl₃; ppm), 30.36 (s, CH₂), 40.42 (s, CH₂), 56.30 (s, OCH₃), 60.99 (s, OCH₃), 105.40 (s, CH), 126.23 (s, CH), 128.52 (s, CH), 128.60 (s, CH), 132.14 (s, C), 141.35 (s, C), 142.45 (s, C), 153.06 (s, C), 198.04 (s, CO); *m/z*(AP+) 301 (MH⁺, 100%); HRMS found [MH]⁺ 301.14398 for C₁₈H₂₁O₄, C₁₈H₂₁O₄ requires [MH]⁺ 301.14344.

Chapter 6 – Experimental

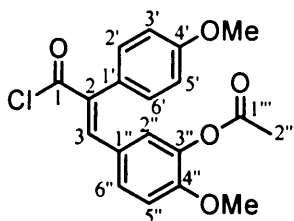
(E)-3-(3-Hydroxy-4-methoxyphenyl)-2-(4-methoxyphenyl)acrylic acid **191**



191

The method adopted was that of Borrel⁹² *et al.* A mixture of 4-methoxyphenylacetic acid (1.66 g, 10.0 mmol), 3-hydroxy-4-methoxybenzaldehyde (0.76 g, 5.0 mmol) and triethylamine (2.3 cm³, 16.5 mmol) in acetic anhydride (4.5 cm³) under argon was heated to 140 °C for 3 hours. The mixture was then cooled to room temperature and was acidified with concentrated hydrochloric acid (6.9 cm³). The mixture was then stirred overnight at room temperature allowing the product to precipitate. The cream precipitate was then filtered off and recrystallization from ethanol afforded **191** as cream crystals (0.56 g, 1.9 mmol, 37%); m.p. 207 – 209 °C; R_f 0.16 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max.}(\text{cm}^{-1})$ 3323, 2939, 2847, 1661, 1598, 1509, 1434, 1268, 1241, 1133, 800; δ ¹H NMR (400 MHz; d₆-DMSO; ppm) 3.71 (3H, s, OCH₃), 3.77 (3H, s, OCH₃), 6.48 (1H, d, *J* 1.7 Hz, H-2''), 6.55 (2H, d, *J* 1.7, 8.7 Hz, H-6''), 6.76 (1H, d, *J* 8.7 Hz, H-5''), 6.92 (2H, d, *J* 9.0 Hz, H-3' & H-5'), 7.05 (2H, d, *J* 9.0 Hz, H-2' & H-6'), 7.56 (1H, s, H-3), 8.91 (1H, s, OH-3''), 12.41 (1H, s, CO₂H-1); δ ¹³C NMR (100 MHz; d₆-DMSO; ppm) 55.71 (s, OCH₃), 56.17 (s, OCH₃), 122.22 (s, CH), 114.63 (s, CH), 117.84 (s, CH), 123.41 (s, CH), 128.01 (s, C), 129.29 (s, C), 130.86 (s, C), 131.41 (s, CH), 139.66 (s, CH), 146.54 (s, C), 149.36 (s, C), 159.20 (s, C), 169.57 (s, CO₂H); *m/z*(API-ES +) 301 ([MH]⁺, 100%); HRMS found [MH]⁺ 301.10765 for C₁₇H₁₇O₅, C₁₇H₁₇O₅ requires [MH]⁺ 301.10705.

5-((*E*)-2-(Chlorocarbonyl)-2-(4-methoxyphenyl)vinyl)-2-methoxyphenyl acetate **198**



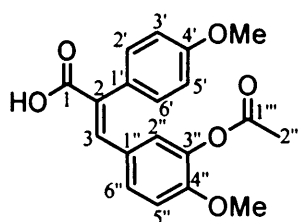
198

The method adopted was that of Hosoda²⁰⁵ *et al.* To a stirred suspension of (*E*)-3-(3-acetoxy-4-methoxyphenyl)-2-(4-methoxyphenyl)acrylic acid **196** (0.38 g, 1.1 mmol) in dry chloroform and anhydrous dimethyl formamide (1 drop) under argon was added thionyl chloride (0.1 cm³, 1.4 mmol). The mixture was then heated to reflux at 75 °C for 4 hours.

Chapter 6 – Experimental

The solvent was then removed at evaporated *in vacuo* at 80 °C to afford **198** as a thick yellow brown oil (0.37g, 1.0 mmol, 93%); R_f 0.17 (SiO₂, ethyl acetate:hexane 1:1); ν_{max} (cm⁻¹) 2937, 2841, 1765, 1735, 1601, 1571, 1508, 1462, 1439, 1274, 1195, 1071, 1020, 813; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.26 (3H, s, H-2'''), 3.81 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 6.79 (1H, d, J 8.4 Hz, H-5''), 6.80 (1H, d, J 2.3 Hz, H-2''), 6.96 (2H, d, J 9.2 Hz, H-3' & H-5'), 6.98 (1H, dd, J 2.3, 8.4 Hz, H-6'), 7.14 (2H, d, J 9.2 Hz, H-2' & H-6'), 7.99 (1H, s, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 20.55 (s, CH₃), 55.33 (s, OCH₃), 55.95 (s, OCH₃), 111.98 (s, CH), 114.73 (s, CH), 126.08 (s, CH), 126.56 (s, C), 126.78 (s, C), 130.82 (s, CH), 131.02 (s, CH), 134.09 (s, C), 139.42 (s, C), 146.57 (s, CH), 153.10 (s, C), 159.88 (s, C), 168.63 (s, CO), 169.40 (s, CO).

(*E*)-3-(3-Acetoxy-4-methoxyphenyl)-2-(4-methoxyphenyl)acrylic acid **196**



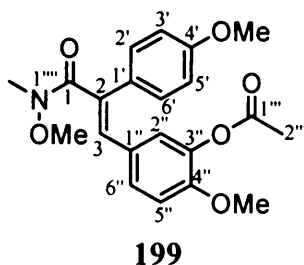
196

A mixture of 4-methoxyphenylacetic acid (1.66 g, 10.0 mmol), 3-hydroxy-4-methoxybenzaldehyde (0.76 g, 5.0 mmol) and triethylamine (2.3 cm³, 16.5 mmol) in acetic anhydride (4.5 cm³) was sealed in a pressure-rated reaction vial (10 cm³). The reaction vial was then irradiated in a self-turning single-mode CEM Discovery™ Focused Synthesizer. The reaction was maintained at 170 °C (power: 200 W) for 25 minutes. The mixture was then rapidly cooled to room temperature. The solvent was evaporated *in vacuo* using a gene vac. The residue was then dissolved in ethyl acetate (50 cm³), washed with water (3 × 20 cm³), dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:1) followed by recrystallization from ethyl acetate and hexane afforded **196** as white crystals (0.67 g, 1.9 mmol, 39%); m.p. 163 – 165 °C; R_f 0.26 (SiO₂, ethyl acetate:hexane 1:1); ν_{max} (cm⁻¹) 2967, 2933, 2837, 2624, 1769, 1734, 1687, 1666, 1606, 1575, 1504, 1468, 1431, 1244, 1201, 1173, 1123, 1031, 815, 772; δ ¹H NMR (400 MHz; d₆-DMSO; ppm) 2.17 (3H, s, H-2'''), 3.70 (3H, s, OCH₃), 3.76 (3H, s, OCH₃), 6.78 (1H, d, J 1.9 Hz, H-2''), 6.91 (1H, dd, J 1.9, 9.1 Hz, H-6''), 6.93 (2H, d, J 8.6 Hz, H-3' & H-5'), 6.96 (1H, d, J 9.1 Hz, H-5''), 7.06 (2H, d, J 8.6 Hz, H-2' & H-6'), 7.62 (1H, s, H-3), 12.52 (1H, br. s, CO₂H-1); δ ¹³C NMR (100 MHz; d₆-DMSO; ppm) 20.94 (s, CH₃), 55.79 (s, OCH₃), 56.58 (s, OCH₃), 113.19 (s, CH), 114.80 (s, CH), 125.43 (s, CH), 128.01 (s, C), 128.96 (s, C), 129.72 (s, CH), 131.35 (s, CH), 132.12 (s, C), 138.32 (s, CH),

Chapter 6 – Experimental

139.49 (s, C), 152.08 (s, C), 159.38 (s, C), 168.94 (s, CO₂H), 169.31 (s, CO); *m/z*(API-ES +) 342 ([MH]⁺, 100%); HRMS found [MH]⁺ 343.11800 for C₁₉H₁₉O₆, C₁₉H₁₉O₆ requires [MH]⁺ 343.11761.

5-((*E*)-2-(*N*-Methoxy-*N*-methylcarbamoyl)-2-(4-methoxyphenyl)vinyl)-2-methoxyphenyl acetate **199**

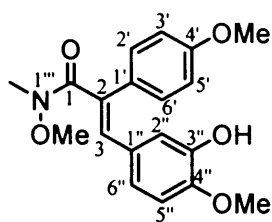


The method adopted was that of Nahm²⁰⁶ *et al.* To a stirred mixture of 5-((*E*)-2-(chlorocarbonyl)-2-(4-methoxyphenyl)vinyl)-2-methoxyphenyl acetate **198** (0.36 g, 1.0 mmol) and *N,O*-dimethylhydroxyamine hydrochloride (0.11 g, 1.2 mmol) in dry chloroform (10 cm³) under argon at 0 °C was added pyridine (0.2 cm³, 2.6 mmol). The mixture was then at room temperature for 1 hour. The solvent was then evaporated *in vacuo*, the residue as then dissolved in dichloromethane (10 cm³) and washed with water (3 × 10 cm³). The aqueous fraction was then extracted with dichloromethane (2 × 10 cm³), the organic fractions were then combined, then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:1) afforded **199** as a pale yellow oil (0.27 g, 0.7 mmol, 70%); *R_f* 0.30 (SiO₂, ethyl acetate:hexane 1:1); *v*_{max}(cm⁻¹) 2936, 2840, 1765, 1643, 1607, 1573, 1508, 1460, 1442, 1268, 1246, 1175, 1124, 1109, 1020, 836; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.25 (3H, s, H-2'''), 3.20 (3H, s, NOCH₃-1'''), 3.30 (3H, s, NCH₃-1'''), 3.78 (3H, s, OCH₃), 3.82 (3H, s, OCH₃), 6.75 (1H, d, *J* 8.5 Hz, H-5''), 6.79 (1H, d, *J* 2.0 Hz, H-2''), 6.81 (1H, s, H-3), 6.86 (2H, d, *J* 8.8 Hz, H-3' & H-5'), 6.95 (1H, dd, *J* 2.0, 8.5 Hz, H-6''), 7.23 (2H, d, *J* 8.8 Hz, H-2' & H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 20.85 (s, CH₃), 33.75(s, NCH₃), 55.47(s, OCH₃), 56.03(s, OCH₃), 60.85(s, OCH₃), 112.00 (s, CH), 114.34 (s, CH), 124.34 (s, CH), 128.45, 128.55, 128.67 (s, CH), 130.50 (s, CH), 130.94 (s, CH), 136.03 (s, C), 139.41 (s, C), 150.98 (s, C), 159.42 (s, C), 169.01 (s, CO), 172.31 (s, CO); *m/z*(API-ES +) 386 ([MH]⁺, 100%);HRMS found [MH]⁺ 386.16039 for C₂₁H₂₄O₆N, C₂₁H₂₄O₆N requires [MH]⁺ 386.15981.

Chapter 6 – Experimental

(E)-3-(3-Hydroxy-4-methoxyphenyl)-N-methoxy-2-(4-methoxyphenyl)-N-methylacrylamide

203

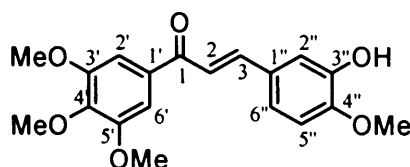


203

A mixture 5-bromo-1,2,3-trimethoxybenzene (0.500 g, 2.02 mmol) in anhydrous ether (4 cm³) was slowly added to n-butyllithium in hexane (2.5 cm³, 1.6 M) in anhydrous ether (30 cm³) under argon at -78 °C. The mixture was then stirred for an hour at -78 °C, to make a milky white solution of 3,4,5-trimethoxyphenyl lithium. A portion of the 3,4,5-trimethoxyphenyl lithium in ether (20 cm³) was then added slowly to a mixture of 5-((E)-2-(N-methoxy-N-methylcarbamoyl)-2-(4-methoxyphenyl)vinyl)-2-methoxyphenyl acetate **199** (0.183 g, 0.47 mmol) in anhydrous tetrahydrofuran (10 cm³) under argon at -78 °C. The reaction was then stirred at -78 °C for 1 hour, before being allowed to warm to room temperature and being stirred overnight. Any excess lithium reagent was then quenched by adding methanol (0.5 cm³) to the mixture. The mixture was then washed with water (3 × 10 cm³) and the aqueous layer was extracted with ethyl acetate (4 × 20 cm³). The organic fractions were then combined, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:19 to 1:1) afforded **203** as a yellow residue (0.094 g, 0.27 mmol, 58%); m.p. 54 – 55 °C; R_f 0.30 (SiO₂, ethyl acetate:hexane 1:1); ν_{\max} (cm⁻¹) 3352, 2961, 2935, 1631, 1606, 1578, 1508, 1459, 1440, 1415, 1275, 1244, 1175, 1022, 836, 797, 762; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.20 (3H, s, NOCH₃-1'''), 3.30 (3H, s, NCH₃-1'''), 3.82 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 5.44 (1H, s, OH-3''), 6.63 (1H, dd, *J* 1.7, 8.5 Hz, H-6''), 6.66 (1H, d, *J* 8.5 Hz, H-5''), 6.71 (1H, d, *J* 1.7 Hz, H-2''), 6.82 (1H, s, H-3), 6.85 (2H, d, *J* 8.6 Hz, H-3' & H-5'), 7.23 (2H, d, *J* 8.6 Hz, H-2' & H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 33.81 (s, NCH₃), 55.41 (s, OCH₃), 56.03 (s, OCH₃), 60.79 (s, OCH₃), 110.42 (s, CH), 114.24 (s, CH), 115.94 (s, CH), 122.55 (s, CH), 128.70 (s, C), 129.00 (s, C), 130.53 (s, CH), 132.06 (s, CH), 135.41 (s, C), 145.28 (s, C), 146.74 (s, C), 159.33 (s, C), 172.55 (s, CO); *m/z*(API-ES +) 344 ([MH]⁺, 100%); HRMS found [MH]⁺ 344.15096 for C₁₉H₂₂O₅N, C₁₉H₂₂O₅N requires [MH]⁺ 344.14925.

Chapter 6 – Experimental

(E)-3-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **117**⁷³



117

To a stirring mixture of 3,4,5-trimethoxyacetophenone (2.51 g, 11.9 mmol) and 3-hydroxy-4-methoxybenzaldehyde (1.81 g, 11.9 mmol) in methanol (10 cm³) was added an aqueous sodium hydroxide solution (4.0 cm³, 12M). The mixture was then stirred overnight at room temperature forming a red solution; the mixture was then acidified with aqueous hydrochloric acid (1 M) till the pH ~ 5 – 6, solution turns yellow. The mixture was then extracted with chloroform (4 × 25 cm³), the organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from methanol afforded **117** as yellow crystals (2.83 g, 8.2 mmol, 69%); mp 150 – 151 °C; R_f 0.32 (SiO₂, ethyl acetate:hexane 1:1); ν_{\max} (cm⁻¹) 3398, 3003, 2935, 2837, 1652, 1571, 1507, 1453, 1414, 1244, 1233, 1127, 990, 813; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.92 (6H, s, OCH₃), 3.94 (6H, s, OCH₃), 5.81 (1H, s, OH-3''), 6.86 (1H, d, *J* 8.2 Hz, H-5''), 7.12 (1H, *J* 2.0, 8.2 Hz, H-6''), 7.26 (2H, s, H-2' & H-6'), 7.30 (1H, d, *J* 2.0 Hz, H-2''), 7.34 (1H, d, *J* 15.4 Hz, H-2), 7.74 (1H, d, *J* 15.4 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.06 (s, OCH₃), 56.38 (s, OCH₃), 61.02 (s, OCH₃), 105.87 (s, CH), 110.57 (s, CH), 112.70 (s, CH), 119.80 (s, CH), 123.10 (s, CH), 128.52 (s, C), 133.76 (s, C), 142.24 (s, C), 144.73 (s, CH), 145.89 (s, C), 148.86 (s, C), 153.13 (s, C), 189.15 (s, CO); CHN found C 66.17%, H 5.88%, CHN requires C 66.27%, H 5.85%.

Anhydrous Trifluoroacetic acid

The method adopted was that of Armarego²²³ *et al.* Trifluoroacetic acid (25 cm³) stirred over phosphorus pentoxide (5.2 g) for 12 hours under an atmosphere of argon, followed by distillation (bp 74 – 75 °C/1 atm.).

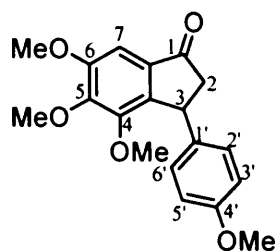
Protocol C

The chalcone was dissolved in anhydrous trifluoroacetic acid and sealed in a pressure-rated reaction vial (10 cm³). The reaction tube was irradiated in a self-turning single-mode CEM Discovery™ Focused Synthesizer. The reaction was maintained at 120 °C (power: 100 W) for 20 minutes. The mixture was then rapidly cooled to room temperature and poured into water, extracted with ethyl acetate. The organic fraction was then washed with saturated

Chapter 6 – Experimental

aqueous sodium bicarbonate solution, dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. The crude indanone was purified by means of column chromatography.

2,3-Dihydro-4,5,6-trimethoxy-3-(4-methoxyphenyl)inden-1-one **205**



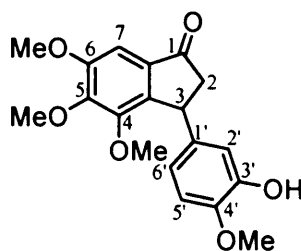
205

The method adopted was that of Rice²¹⁵ *et al.* In a sealed tube (*E*)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **165** (2.25 g, 6.9 mmol) was dissolved in anhydrous trifluoroacetic acid (13 cm³). The sealed tube is then heated to 120 °C for 4 hours. Once the reaction had cooled to room temperature it was tipped into cold water (30 cm³), and then extracted with ethyl acetate (5 × 30 cm³). The organic fraction was then washed with saturated aqueous sodium bicarbonate solution (30 cm³), brine (30 cm³), dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **205** as a cream solid (1.63 g, 5.0 mmol, 72%).

The indanone **205** was obtained following Protocol C. Using (*E*)-1-(3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **165** (0.99 g, 6.9 mmol) in anhydrous trifluoroacetic acid (1 cm³). Tipped into water (10 cm³), extracted with ethyl acetate (2 × 10 cm³) washed with saturated aqueous sodium bicarbonate solution (10 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **205** as a cream solid (0.59 g, 1.8 mmol, 60%); m.p. 66 - 67 °C; R_f 0.35 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2967, 2938, 2836, 1706, 1610, 1594, 1512, 1470, 1456, 1417, 1429, 1417, 1340, 1303, 1248, 1177, 1125, 1091, 1025, 830; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.59 (1H, dd, *J* 2.4, 19.2 Hz, H-2), 3.19 (1H, dd, *J* 7.6, 19.2 Hz, H-2), 3.35 (3H, s, OCH₃), 3.77 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.54 (1H, dd, *J* 2.4, 7.6 Hz, H-3), 6.81 (2H, d, *J* 8.8 Hz, H-3' & H-5'), 7.02 (2H, d, *J* 8.8 Hz, H-2' & H-6'), 7.08 (1H, s, H-7); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 40.89 (s, CH), 47.33 (s, CH₂), 55.26 (s, OCH₃), 56.25 (s, OCH₃), 60.14 (s, OCH₃), 60.92 (s, OCH₃), 100.31 (s, CH), 113.97 (s, CH), 128.23 (s, CH), 132.00 (s, C), 136.27 (s, C), 145.17 (s, C), 148.98 (s, C), 150.35 (s, C), 154.86 (s, C), 158.27 (s, C), 206.19 (s, CO); *m/z*(AP⁺) 329 (MH⁺, 100%); HRMS found [MH]⁺ 329.13853 for C₁₉H₂₁O₅, C₁₉H₂₁O₅ requires [MH]⁺ 329.13835.

Chapter 6 – Experimental

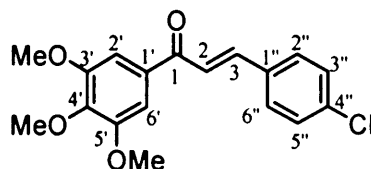
2,3-Dihydro-3-(3-hydroxy-4-methoxyphenyl)-4,5,6-trimethoxyinden-1-one **153**



153

The indanone **153** was obtained following Protocol C. Using (*E*)-3-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **117** (0.92 g, 2.7 mmol) in anhydrous trifluoroacetic acid (1 cm³). Tipped into water (10 cm³), extracted with ethyl acetate (2 × 15 cm³) washed with saturated aqueous sodium bicarbonate solution (10 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **153** as brown solid (0.51 g, 1.5 mmol, 56%); m.p. 116-117 °C; R_f 0.36 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 3253, 2959, 2835, 1693, 1586, 1509, 1463, 1417, 1270, 1215, 1130, 1096, 1024, 806; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.57 (1H, dd, *J* 2.5, 19.3 Hz, H-2), 3.15 (1H, dd, *J* 7.9, 19.3 Hz, H-2), 3.42 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.50 (1H, dd, *J* 2.5, 7.9 Hz, H-3), 5.56 (1H, s, OH-3'), 6.61 (1H, dd, *J* 2.0, 8.3 Hz, H-6'), 6.63 (1H, d, *J* 2.0 Hz, H-2'), 6.75 (1H, d, *J* 8.3 Hz, H-5'), 7.07 (1H, s, H-7) ; δ ¹³C NMR (100 MHz; CDCl₃; ppm) 41.04 (s, CH), 47.32 (s, CH₂), 55.95 (s, OCH₃), 56.27 (s, OCH₃), 60.18 (s, OCH₃), 60.94 (s, OCH₃), 100.26 (s, CH), 110.55 (s, CH), 113.32 (s, CH), 118.72 (s, CH), 132.19 (s, C), 137.69 (s, C), 144.66 (s, C), 145.23 (s, C), 145.66 (s, C), 148.79 (s, C), 150.40 (s, C), 154.85 (s, C), 205.54 (s, C=O); *m/z* (AP⁺) 345 (MH⁺, 100%); HRMS found [MH]⁺ 345.13353 for C₁₉H₂₁O₆, C₁₉H₂₁O₆ requires [MH]⁺ 345.13326.

(*E*)-3-(4-Chlorophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **206**



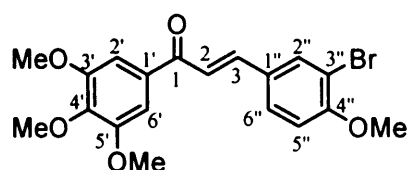
206

The chalcone **206** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (2.10 g, 10.0 mmol), 4-chlorobenzaldehyde (1.41 g, 10.0 mmol) and aqueous sodium hydroxide solution (1.7 cm³, 12M) in methanol (5 cm³). Recrystallization from methanol afforded **51** as light yellow crystals (1.87 g, 5.6 mmol, 55%); m.p. 115-116 °C; R_f 0.50 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2936, 2837, 1655, 1579, 1505, 1490, 1455, 1410, 1323,

Chapter 6 – Experimental

1157, 1090, 1001, 809; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.87 (3H, s, OCH_3), 3.89 (6H, s, OCH_3), 7.20 (2H, s, H-2' & H-6'), 7.33 (2H, d, J 8.5 Hz, H-3'' & H-5''), 7.39 (1H, d, J 15.6 Hz, H-2), 7.52 (2H, d, J 8.5 Hz, H-2'' & H-6''), 7.70 (1H, d, J 15.6 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 56.43 (s, OCH_3), 61.05 (s, OCH_3), 106.04 (s, CH), 122.08 (s, CH), 129.28 (s, CH), 129.64 (s, CH), 133.32 (s, C), 133.36 (s, C), 136.47 (s, C), 142.57 (s, C), 143.30 (s, CH), 153.19 (s, C), 188.94 (s, CO); m/z (AP+) 333 (MH^+ , 100%), 335 (MH^+ , 25%); HRMS found $[\text{MH}]^+$ 333.0887 for $\text{C}_{18}\text{H}_{18}\text{O}_4\text{Cl}$, $\text{C}_{18}\text{H}_{18}\text{O}_4\text{Cl}$ requires $[\text{MH}]^+$ 333.0888; CHN found C 64.88%, H 5.11% CHN requires C 64.97%, H 5.15%.

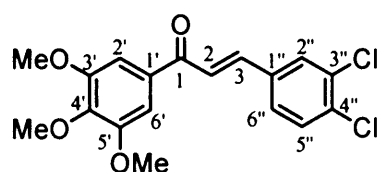
(*E*)-3-(3-Bromo-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **207**



207

The chalcone **207** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (1.07 g, 5.1 mmol), 3-bromo-4-methoxybenzaldehyde (1.09 g, 5.1 mmol) and aqueous sodium hydroxide solution (1.7 cm^3 , 12M) in methanol (20 cm^3). Recrystallization from methanol afforded **207** as light yellow crystals (1.55 g, 3.8 mmol, 75%); mp 108-110 $^\circ\text{C}$; Rf 0.28 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 2939, 1654, 1575, 1494, 1453, 1412, 1341, 1306, 1263, 1157, 1126, 807; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.88 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.90 (6H, s, OCH_3), 6.87 (1H, d, J 8.5 Hz, H-5''), 7.20 (2H, s, H-2' & H-6'), 7.29 (1H, d, J 15.6 Hz, H-2), 7.48 (1H, dd, J 2.2, 8.5 Hz, H-6''), 7.66 (1H, d, J 15.6 Hz, H-3), 7.84 (1H, d, J 2.2 Hz, H-2''); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 56.24 (s, OCH_3), 56.45 (s, OCH_3), 61.04 (s, OCH_3), 105.96 (s, CH), 111.85 (s, CH), 112.41 (s, C), 120.43 (s, CH), 128.94 (s, C), 130.00 (s, CH), 132.57 (s, CH), 133.54 (s, C), 142.43 (s, C), 143.06 (s, CH), 153.16 (s, C), 157.63 (s, C), 188.91 (s, C=O); m/z (CI+) 407 (MH^+ , 70%), 409 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 407.0487 for $\text{C}_{19}\text{H}_{20}\text{BrO}_5$, $\text{C}_{19}\text{H}_{20}\text{BrO}_5$ requires $[\text{MH}]^+$ 407.0489; CHN found 55.10%, H 4.80% CHN requires C 56.03%, H 4.70%.

(*E*)-3-(3,4-Dichlorophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **208**

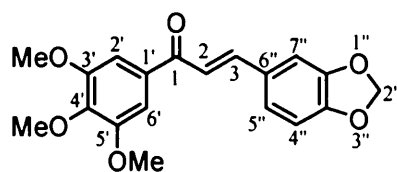


208

Chapter 6 – Experimental

The chalcone **208** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (1.03 g, 4.9 mmol), 3,4-dichlorobenzaldehyde (0.89 g, 5.1 mmol) and aqueous sodium hydroxide solution (1 cm³, 10M) in methanol (2.5 cm³). Recrystallization from methanol afforded **208** as light yellow crystals (1.17 g, 3.2 mmol, 65%); mp 137 – 139 °C; R_f 0.78 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max}(\text{cm}^{-1})$ 2943, 2833, 1664, 1607, 1577, 1505, 1467, 1413, 1337, 1125, 996, 812; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.88 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 7.20 (2H, s, H-2' & H-6'), 7.36-7.42 (2H, m, H-2 & H-6''), 7.44 (1H, d, *J* 8.5 Hz, H-5''), 7.64 (1H, d, *J* 15.6 Hz, H-3), 7.67 (1H, d, *J* 2.0 Hz, H-2''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.46 (s, OCH₃), 61.05 (s, OCH₃), 106.08 (s, CH), 123.10 (s, CH), 127.63 (s, CH), 129.73 (s, CH), 130.98 (s, CH), 133.05 (s, C), 133.31 (s, C), 134.41 (s, C), 134.93 (s, C), 141.88 (s, CH), 142.76 (s, C), 153.22 (s, C=O), 188.52 (s, C=O); *m/z* (AP+) 366 (MH⁺, 100%), (368 MH⁺, 60%); HRMS found [MH]⁺ 367.0499 for C₁₈H₁₇O₄Cl₂, C₁₈H₁₇O₄Cl₂ requires [MH]⁺ 367.0498; CHN found C 57.67%, H 4.29% CHN requires C 58.87%, H 4.39%.

(E)-3-(Benzo[*d*][1,3]dioxol-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **209**³⁴⁰

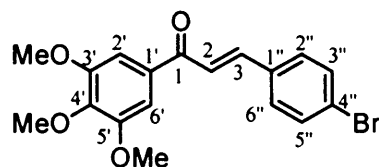


209

The chalcone **209** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (0.64 g, 3.0 mmol), piperonal (0.45 g, 3.0 mmol) and aqueous sodium hydroxide solution (1 cm³, 6M) in methanol (3.0 cm³). Recrystallization from methanol afforded **209** as light yellow needle (0.75 g, 2.2 mmol, 73%); m.p. 133-135 °C; R_f 0.48 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max}(\text{cm}^{-1})$ 2944, 1655, 1576, 1492, 1464, 1444, 1410, 1328, 1240, 1157, 1121, 992, 724; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.95 (3H, s, OCH₃), 3.97 (6H, s, OCH₃), 6.06 (2H, s, H-2''), 6.87 (1H, d, *J* 8.0 Hz, H-4''), 7.15 (1H, dd, *J* 8.0, 1.6 Hz, H-5''), 7.20 (1H, d, *J* 1.6 Hz, H-7''), 7.28 (2H, s, H-2'' & H-6''), 7.34 (1H, d, *J* 15.5 Hz, H-2), 7.76 (1H, d, *J* 15.5 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.39 (s, OCH₃), 61.02 (s, OCH₃), 101.70 (s, CH₂), 105.89 (s, CH), 106.62 (s, CH), 108.73 (s, CH), 119.67 (s, CH), 125.35 (s, CH), 129.33 (s, C), 133.72 (s, C), 142.27 (s, C), 144.66 (s, CH), 148.41 (s, C), 149.95 (s, C), 153.13 (s, C), 189.11 (s, CO); *m/z* (AP+) 343 (MH⁺, 100%); HRMS found [MH]⁺ 343.1175 for C₁₉H₁₈O₆, C₁₉H₁₈O₆ requires [MH]⁺ 343.1176; CHN found C 66.55%, H 5.28% CHN requires C 66.66%, H 5.30%.

Chapter 6 – Experimental

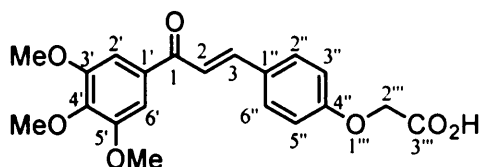
(E)-3-(4-Bromophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **210**⁷²



210

The chalcone **210** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (0.41 g, 1.9 mmol), 4-bromobenzaldehyde (0.37 g, 2.0 mmol) and aqueous sodium hydroxide solution (2.0 cm³, 2M) in methanol (3 cm³). Recrystallization from methanol afforded **210** as fluffy white crystals (0.62 g, 1.6 mmol, 84%); m.p. 124 – 125 °C; R_f 0.64 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2933, 2836, 1668, 1610, 1580, 1506, 1485, 1461, 1451, 1435, 1415, 1336, 1160, 1063, 996, 802, 730; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.94 (3H, s, OCH₃), 3.95 (6H, s, OCH₃), 7.27 (2H, s, H-2' & H-6'), 7.47 (1H, d, *J* 15.6 Hz, H-2), 7.51 (2H, d, *J* 8.6 Hz, H-3'' & H-5''), 7.56 (2H, d, *J* 8.6 Hz, H-2'' & H-6''), 7.75 (1H, d, *J* 15.6 Hz, H-3); δ ¹³C (100MHz; CDCl₃; ppm) 56.65 (s, OCH₃), 61.23 (s, OCH₃), 106.35 (s, CH), 122.44 (s, CH), 125.03 (s, C), 130.02 (s, CH), 132.45 (s, CH), 133.51 (s, C), 134.03 (s, C), 142.89 (s, C), 143.51 (s, C), 153.42 (s, CH), 189.10 (s, CO); *m/z*(API ES⁺) 377 (MH⁺, 100%), 379 (MH⁺, 90%).

(E)-3-(4-Carboxymethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **211**



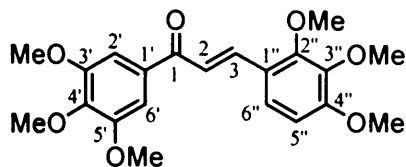
211

To a stirring mixture of 3,4,5-trimethoxyacetophenone (0.50 g, 2.4 mmol) and 4-formylphenoxyacetic acid (0.43 g, 2.4 mmol) in methanol (2.5 cm³) was added an aqueous sodium hydroxide solution (1.2 cm³, 4 M). The mixture was then stirred overnight at room temperature; the mixture was then acidified with aqueous hydrochloric acid (1 M) till the pH ~ 5 – 6, causing the product to precipitate.. Recrystallization from methanol afforded **211** as a pale yellow solid (0.46 g, 1.2 mmol, 52%); m.p. 148 – 151 °C; R_f 0.46 (SiO₂, ethyl acetate:methanol 9:1); ν_{max}(cm⁻¹) 3489, 3001, 2943, 2833, 1718, 1652, 1596, 1575, 1455, 1423, 1412, 1336, 1253, 1159, 992, 826; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.94 (3H, s, OCH₃), 3.94 (6H, s, OCH₃), 4.74 (2H, s, H-2'''), 6.96 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.26 (2H, s, H-2' & H-6'), 7.38 (1H, d, *J* 15.8 Hz, H-2), 7.61 (2H, d, *J* 8.8 Hz, H-2'' & H-6''),

Chapter 6 – Experimental

7.79 (1H, d, J 15.8 Hz, H-3); $\delta^{13}\text{C}$ (100MHz; CDCl_3 ; ppm) 56.64 (s, OCH_3), 61.23 (s, OCH_3), 64.90 (s, CH_2), 106.36 (s, CH), 115.28 (s, CH), 120.39 (s, CH), 129.11 (s, C), 130.55 (s, CH), 133.77 (s, C), 142.73 (s, C), 144.59 (s, CH), 153.36 (s, C), 159.60 (s, C), 172.49 (s, CO_2H), 189.79 (s, CO); m/z (API ES+) 373 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 373.12852 for $\text{C}_{20}\text{H}_{21}\text{O}_7$, $\text{C}_{20}\text{H}_{21}\text{O}_7$ requires $[\text{MH}]^+$ 373.12818.

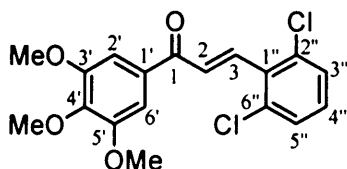
(*E*)-3-(2,3,4-Trimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **212**



212

The chalcone **212** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (0.51 g, 2.4 mmol), 2,3,4-trimethoxybenzaldehyde (0.47 g, 2.4 mmol) and aqueous sodium hydroxide solution (1.2 cm^3 , 4M) in methanol (2.5 cm^3). Recrystallization from methanol afforded **212** as yellow crystals (0.72 g, 1.9 mmol, 78%); m.p. 118 – 120 °C; R_f 0.36 (SiO_2 , ethyl acetate:hexane 1:1); ν_{max} (cm^{-1}) 2935, 2833, 1651, 1568, 1494, 1465, 1416, 1343, 1296, 1162, 1123, 993, 804; $\delta^1\text{H}$ NMR (400 MHz; CDCl_3 ; ppm) 3.88 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.93 (6H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.72 (1H, d, J 8.6 Hz, H-5''), 7.26 (2H, s, H-2' & H-6'), 7.37 (1H, d, J 8.6 Hz, H-6''), 7.50 (1H, d, J 15.8 Hz, H-2), 7.97 (1H, d, J 15.8 Hz, H-3); $\delta^{13}\text{C}$ (100MHz; CDCl_3 ; ppm) 56.32 (s, OCH_3), 56.56 (s, OCH_3), 61.16 (s, OCH_3), 61.19 (s, OCH_3), 61.60 (s, OCH_3), 106.24 (s, CH), 107.83 (s, CH), 121.44 (s, CH), 122.22 (s, C), 124.22 (s, CH), 134.11 (s, C), 140.34 (s, CH), 142.40 (s, C), 142.70 (s, C), 153.31 (s, C), 153.99 (s, C), 156.04 (s, C), 189.97 (s, CO); m/z (API ES+) 389 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 389.16097 for $\text{C}_{21}\text{H}_{25}\text{O}_7$, $\text{C}_{21}\text{H}_{25}\text{O}_7$ requires $[\text{MH}]^+$ 389.15948.

(*E*)-3-(2,6-Dichlorophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **213**



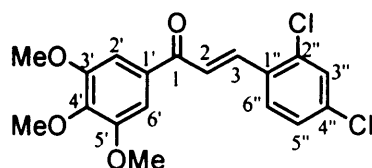
213

The chalcone **213** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (0.50 g, 2.4 mmol), 2,6-dichlorobenzaldehyde (0.42 g, 2.4 mmol) and aqueous sodium hydroxide solution (1.2 cm^3 , 4M) in methanol (2.5 cm^3). Recrystallization from methanol afforded **213** as fluffy white crystals (0.72 g, 1.9 mmol, 82%); m.p. 100 – 101 °C; R_f 0.64

Chapter 6 – Experimental

(SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max}(\text{cm}^{-1})$ 2936, 2839, 1671, 1621, 1582, 1507, 1459, 1429, 1411, 1340, 1160, 991, 963, 771; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.95 (9H, OCH₃), 7.22 (1H, t, *J* 8.0 Hz, H-4''), 7.29 (2H, s, H-2' & H-6'), 7.39 (2H, d, *J* 8.0 Hz, H-3'' & H-5''), 7.59 (1H, d, *J* 16.2 Hz, H-2), 7.81 (1H, d, *J* 16.2 Hz, H-3); δ ¹³C (100MHz; CDCl₃; ppm) 56.55 (s, OCH₃), 61.23 (s, OCH₃), 106.50 (s, CH), 129.09 (s, CH), 130.08 (s, CH), 130.86 (s, CH), 133.00 (s, C), 133.07 (s, C), 135.33 (s, C), 137.79 (s, CH), 142.94 (s, C), 153.41 (s, C), 189.33 (s, CO); *m/z*(API ES+) 367 (MH⁺, 100%), 369 (MH⁺, 60%); HRMS found [MH]⁺ 367.05060 for C₁₈H₁₇O₄Cl₂, C₁₈H₁₇O₄Cl₂ requires [MH]⁺ 367.04984.

(*E*)-3-(2,4-Dichlorophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **214**

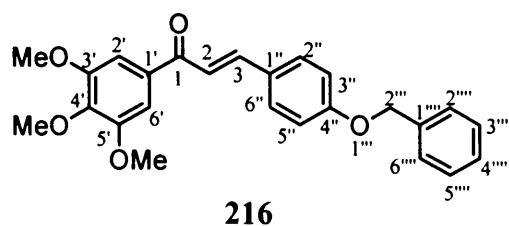


214

The chalcone **214** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (0.63 g, 3.0 mmol), 2,4-dichlorobenzaldehyde (0.52 g, 3.0 mmol) and aqueous sodium hydroxide solution (1.0 cm³, 6M) in methanol (3 cm³). Recrystallization from methanol afforded **214** as yellow crystals (0.91 g, 2.5 mmol, 83%); m.p. 151-153 °C; R_f 0.63 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max}(\text{cm}^{-1})$ 3090, 2939, 1657, 1597, 1572, 1504, 1451, 1413, 1341, 1314, 1159, 986, 815; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.96 (9H, s, OCH₃), 7.27 (2H, s, H-2' & H-6'), 7.32 (1H, dd, *J* 8.4, 2.1 Hz, H-5''), 7.40 (1H, d, *J* 15.8 Hz, H-2), 7.44 (1H, d, *J* 2.1 Hz, H-3''), 7.70 (1H, d, *J* 8.4 Hz, H-6''), 8.09 (1H, d, *J* 15.8 Hz, H-3'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.40 (s, OCH₃), 61.05 (s, OCH₃), 106.19 (s, CH), 125.05 (s, CH), 127.61 (s, CH), 128.55 (s, CH), 130.14 (s, CH), 131.88 (s, C), 132.95 (s, C), 135.99 (s, C), 136.49 (s, C), 139.32 (s, CH), 142.64 (s, C), 153.1 (s, C), 189.18 (s, CO); *m/z* (API-ES+) 367 (MH⁺, 100%), 369 (MH⁺, 65%); HRMS found [MH]⁺ 367.4966 for C₁₈H₁₇Cl₂O₄, C₁₈H₁₇Cl₂O₄ requires [MH]⁺ 367.04984; CHN found C 58.74%, H 4.33% CHN requires C 58.87%, H 4.39%.

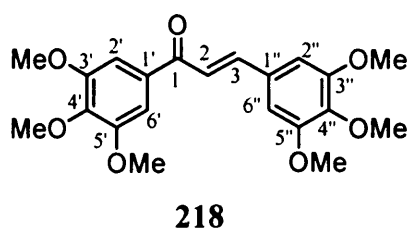
Chapter 6 – Experimental

(*E*)-3-(4-(Benzyloxy)phenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **216**



The chalcone **216** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (0.63 g, 3.0 mmol), 4-(benzyloxy)benzaldehyde **x64** (0.64 g, 3.0 mmol) and aqueous sodium hydroxide solution (1.0 cm³, 6M) in methanol (3 cm³). Recrystallization from methanol afforded **216** as yellow needles (0.50 g, 1.2 mmol, 42%); m.p. 90-93 °C; R_f 0.52 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2937, 2835, 1649, 1571, 1504, 1463, 1424, 1410, 1326, 1250, 1157, 1009, 980, 812; δ¹H NMR (400 MHz; CDCl₃; ppm) 3.94 (3H, s, OCH₃), 3.95 (6H, s, OCH₃), 5.13 (2H, s, H-2'''), 7.02 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.27 (2H, s, H-2' & H-6') 7.33 – 7.46 (6H, m, H-2 & H-2'''' – H-6''''), 7.61 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.80 (1H, d, *J* 15.6 Hz, H-3); δ¹³C NMR (100 MHz; CDCl₃; ppm) 56.39 (s, OCH₃), 61.03 (s, OCH₃), 70.12 (s, CH₂), 105.91 (s, CH), 115.31 (s, CH), 119.48 (s, CH), 127.52 (s, CH), 127.82 (s, C), 128.24 (s, CH), 128.73 (s, CH), 130.30 (s, CH), 133.83 (s, C), 136.36 (s, C), 142.21 (s, C), 144.62 (s, CH), 153.12 (s, C), 160.85 (s, C), 189.30 (s, CO); *m/z* (AP⁺) 405 (MH⁺, 100%), 406 (MH⁺, 30%); HRMS found [MH]⁺ 405.1695 for C₂₅H₂₄O₅, C₂₅H₂₄O₅ requires [MH]⁺ 405.1697; CHN found C 74.10%, H 5.99% CHN requires C 74.24%, H 5.98%.

(*E*)-1,3-Bis(3,4,5-trimethoxyphenyl)prop-2-en-1-one **218**⁴¹

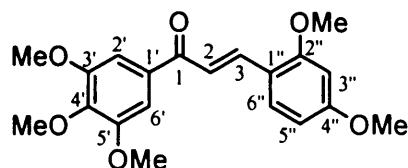


The chalcone **218** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (2.10 g, 10.0 mmol), 3,4,5-trimethoxybenzaldehyde (1.96 g, 10.0 mmol) and aqueous sodium hydroxide solution (2.0 cm³, 10M) in methanol (10 cm³). Recrystallization from methanol afforded **218** as pale yellow crystals (3.61 g, 8.3 mmol, 83%); m.p. 125-128 °C; R_f 0.08 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2936, 2833, 1677, 1662, 1579, 1503, 1465, 1451, 1412, 1326, 1243, 1227, 994, 972, 813; δ¹H NMR (400 MHz; CDCl₃; ppm) 3.90 (3H, s, OCH₃), 3.92 (6H, s, OCH₃), 3.94 (3H, s, OCH₃), 3.95 (6H, s, OCH₃), 6.86 (2H, s, H-2'' &

Chapter 6 – Experimental

H-6''), 7.25 (2H, s, H-2' & H-6'), 7.33 (1H, d, J 15.6 Hz, H-2), 7.72 (1H, d, J 15.6 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 56.29 (s, OCH_3), 56.51 (s, OCH_3), 61.04 (s, OCH_3), 61.06 (s, OCH_3), 105.71 (s, CH), 106.21 (s, CH), 121.33 (s, CH), 130.39 (s, C), 133.64 (s, C), 140.47 (s, C), 142.49 (s, C), 145.03 (s, CH), 153.16 (s, C), 153.51 (s, C), 189.50 (s, CO); $m/z(\text{AP}^+)$ 389 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 389.1596 for $\text{C}_{21}\text{H}_{25}\text{O}_7$, $\text{C}_{21}\text{H}_{25}\text{O}_7$ requires $[\text{MH}]^+$ 389.1595; CHN found C 64.55%, H 6.22% CHN requires C 64.94%, H 6.23%.

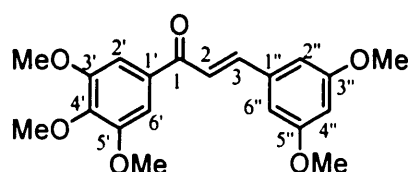
(*E*)-3-(2,4-Dimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **219**



219

The chalcone **219** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (0.51 g, 2.4 mmol), 2,4-dimethoxybenzaldehyde (0.40 g, 2.4 mmol) and aqueous sodium hydroxide solution (1.2 cm^3 , 4M) in methanol (2.5 cm^3). Recrystallization from methanol afforded **219** as yellow crystals (0.63 g, 1.8 mmol, 73%); m.p. 129 – 130 $^\circ\text{C}$; R_f 0.43 (SiO_2 , ethyl acetate:hexane 1:1); $\nu_{\text{max}}(\text{cm}^{-1})$ 2941, 2837, 1647, 1604, 1568, 1502, 1468, 1455, 1439, 1409, 1323, 1299, 1274, 1157, 1104, 1027, 1003, 828; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.85 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.94 (6H, s, OCH_3), 6.47 (1H, d, J 2.4 Hz, H-3''), 6.54 (1H, dd, J 2.4, 8.7 Hz, H-5''), 7.26 (2H, s, H-2' & H-6'), 7.47 (1H, d, J 15.6 Hz, H-2), 7.57 (1H, d, J 8.7 Hz, H-6''), 8.04 (1H, d, J 15.6 Hz, H-6''); δ ^{13}C (100MHz; CDCl_3 ; ppm) 55.74 (s, OCH_3), 55.77 (s, OCH_3), 56.54 (s, OCH_3), 61.18 (s, OCH_3), 98.67 (s, CH), 105.67 (s, CH), 106.24 (s, CH), 117.35 (s, C), 120.52 (s, CH), 131.05 (s, CH), 134.40 (s, C), 140.68 (s, CH), 142.21 (s, C), 153.25 (s, C), 160.57 (s, C), 163.28 (s, C), 190.32 (s, CO); $m/z(\text{API ES}^+)$ 359 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 359.1493 for $\text{C}_{20}\text{H}_{23}\text{O}_6$, $\text{C}_{20}\text{H}_{23}\text{O}_6$ requires $[\text{MH}]^+$ 359.1489.

(*E*)-1-(3,4,5-Trimethoxyphenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one **220**

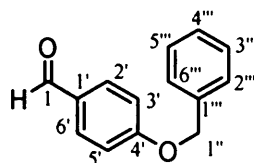


220

Chapter 6 – Experimental

The chalcone **220** was obtained following Protocol A. Using 3,4,5-trimethoxyacetophenone (0.51 g, 2.4 mmol), 3,5-dimethoxybenzaldehyde (0.42 g, 2.4 mmol) and aqueous sodium hydroxide solution (1.2 cm³, 4M) in methanol (2.5 cm³). Recrystallization from methanol afforded **220** as yellow crystals (0.72 g, 2.0 mmol, 83%); m.p. 107 – 109 °C; R_f 0.50 (SiO₂, ethyl acetate:hexane 1:1); ν_{\max} (cm⁻¹) 2947, 2839, 1648, 1589, 1570, 1507, 1461, 1416, 1334, 1160, 827; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.83 (6H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.94 (6H, s, OCH₃), 6.52 (1H, t, *J* 2.3 Hz, H-4), 6.77 (2H, d, *J* 2.3 Hz, H-2'' & H-6''), 7.25 (2H, s, H-2' & H-6'), 7.41 (1H, d, *J* 15.6 Hz, H-2), 7.71 (1H, d, *J* 15.6 Hz, H-3); δ ¹³C (100MHz; CDCl₃; ppm) 55.72 (s, OCH₃), 56.65 (s, OCH₃), 61.22 (s, OCH₃), 102.68 (s, CH), 106.39 (s, CH), 106.69 (s, CH), 122.59 (s, CH), 133.65 (s, C), 137.02 (s, C), 142.77 (s, C), 144.98 (s, CH), 153.38 (s, C), 161.29 (s, C), 189.54 (s, CO); *m/z*(API ES⁺) 359 (MH⁺, 100%); HRMS found [MH]⁺ 359.15056 for C₂₀H₂₃O₆, C₂₀H₂₃O₆ requires [MH]⁺ 359.14891.

4-(Benzyloxy)benzaldehyde **221**³⁴²

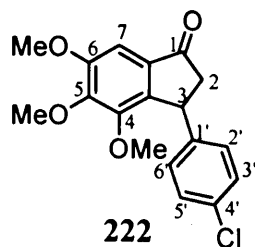


221

To a stirring mixture of 4-hydroxybenzaldehyde (1.00 g, 8.2 mmol) and potassium carbonate (4.53 g, 32.7 mmol) in dimethyl formamide (40 cm³) was added benzyl bromide (1.2 cm³, 10.1 mmol). The mixture was then put under argon and heated to 90 °C for 2 hours. The dimethyl formamide was then evaporated *in vacuo*. The crude product was then dissolved in chloroform (120 cm³) and filtered through celite, and washed with water (3 × 40 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:1) afforded **221** as a pale yellow solid (1.67 g, 7.9 mmol, 96%); m.p. 73 – 74 °C; R_f 0.76 (SiO₂, ethyl acetate:hexane 1:1); ν_{\max} (cm⁻¹) 2829, 2745, 1685, 1598, 1574, 1509, 1462, 1452, 1425, 1259, 1163, 1018, 829; δ ¹H NMR (400 MHz; CDCl₃; ppm) 5.15 (2H, s, H-1''), 7.08 (2H, d, *J* 8.8 Hz, H-3' & H-5'), 7.33 – 7.47 (5H, m, H-2''' – H-6'''), 7.84 (2H, d, *J* 8.8 Hz, H-2' & H-6'), 9.89 (1H, s, H-1); δ ¹³C (100MHz; CDCl₃; ppm) 70.28 (s, OCH₂O), 115.16 (s, CH), 127.54 (s, CH), 128.39 (s, CH), 128.78 (s, CH), 130.09 (s, C), 132.07 (s, CH), 135.94 (s, C), 163.76 (s, C), 190.92 (s, CHO).

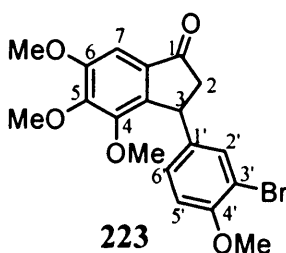
Chapter 6 – Experimental

3-(4-Chlorophenyl)-2,3-dihydro-4,5,6-trimethoxyinden-1-one **222**



The indanone **222** was obtained following Protocol C. Using (*E*)-3-(4-chlorophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **206** (0.24 g, 0.72 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **222** as yellow solid (0.13 g, 0.39 mmol, 53%); m.p. 74-75 °C; R_f 0.50 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2960, 2840, 1703, 1598, 1491, 1469, 1431, 1417, 1341, 1306, 1127, 1090, 1014, 793; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.49 (1H, dd, *J* 2.8, 19.3 Hz, H-2), 3.12 (1H, dd, *J* 8.0, 19.3 Hz, H-2) 3.33 (3H, s, OCH₃), 3.83 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 4.48 (1H, dd, *J* 2.8, 8.0 Hz, H-3), 6.98 (2H, d, *J* 8.5 Hz, H-3' & H-5'), 7.01 (1H, s, H-7), 7.19 (2H, d, *J* 8.5 Hz, H-2' & H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 40.97 (s, CH), 47.01 (s, CH₂), 56.28 (s, OCH₃), 60.11 (s, OCH₃), 60.93 (s, OCH₃), 100.29 (s, CH), 128.63 (s, CH), 128.76 (s, CH), 132.17 (s, C), 132.33 (s, C), 142.93 (s, C), 144.02 (s, C), 148.73 (s, C), 150.27 (s, C), 155.10 (s, C), 204.93 (s, C=O); *m/z* (AP+) 333 (MH⁺, 100%), 335 (MH⁺, 30%); HRMS found [MH]⁺ 333.0890 for C₁₈H₁₈O₄Cl, C₁₈H₁₈O₄Cl requires [MH]⁺ 333.088.

3-(3-Bromo-4-methoxyphenyl)-2,3-dihydro-4,5,6-trimethoxyinden-1-one **223**

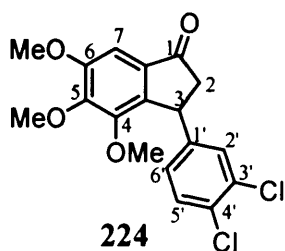


The indanone **223** was obtained following Protocol C. Using (*E*)-3-(3-bromo-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **207** (0.26 g, 0.64 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **223** as brown solid (0.15 g, 0.37 mmol, 59%); m.p. 128-129 °C; R_f 0.39 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2956, 2834, 1699, 1601, 1497, 1462, 1442, 1419, 1342, 1281, 1092, 1021, 814; δ ¹H NMR

Chapter 6 – Experimental

(400 MHz; CDCl₃; ppm) 2.51 (1H, dd, *J* 2.6, 19.3 Hz, H-2), 3.10 (1H, dd, *J* 8.0, 19.3 Hz, H-2) 3.36 (3H, s, OCH₃), 3.80 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 4.44 (1H, dd, *J* 2.6, 8.0 Hz, H-3), 6.74 (1H, d, *J* 8.5 Hz, H-5'), 6.94 (1H, dd, *J* 2.3, 8.5 Hz, H-6'), 7.01 (1H, s, H-7), 7.22 (1H, d, *J* 2.3 Hz, H-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 40.46 (s, CH), 47.06 (s, CH₂), 56.26 (s, OCH₃), 56.28 (s, OCH₃), 60.20 (s, OCH₃), 60.94 (s, OCH₃), 100.31 (s, CH), 111.61 (s, CBr), 111.88 (s, CH), 127.17 (s, CH), 132.06 (s, CH), 132.12 (s, C), 137.93 (s, C), 144.76 (s, C), 148.76 (s, C), 150.29 (s, C), 154.53 (s, C), 155.06 (s, C), 205.01 (s, C=O); *m/z* (AP+) 407 (MH⁺, 100%), 409 (MH⁺, 70%); HRMS found [MH]⁺ 407.0495 for C₁₉H₂₀O₅Br, C₁₉H₂₀O₅Br requires [MH]⁺ 407.0489.

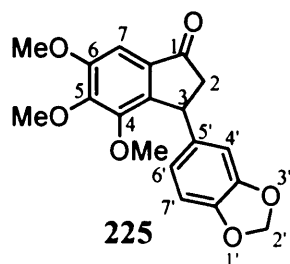
3-(3,4-Dichlorophenyl)-2,3-dihydro-4,5,6-trimethoxyinden-1-one **224**



The indanone **224** was obtained following Protocol C. Using (*E*)-3-(3,4-dichlorophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **208** (0.25 g, 0.68 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **224** as light brown solid (0.17 g, 0.46 mmol, 69%); m.p. 129 - 132°C; R_f 0.32 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2941, 2898, 2834, 1710, 1601, 1557, 1471, 1431, 1417, 1346, 1316, 1121, 1095, 832; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.73 (1H, dd, *J* 2.8, 19.3 Hz, H-2), 3.11 (1H, dd, *J* 8.0, 19.3 Hz, H-2), 3.41 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 4.46 (1H, dd, *J* 2.8, 8.0 Hz, H-3), 6.87 (H, dd, *J* 2.3, 8.3 Hz, H-6'), 7.02 (H, s, H-7), 7.14 (H, d, *J* 2.3 Hz, H-2'), 7.29 (H, d, *J* 8.3 Hz, H-5'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 40.72 (s, CH), 46.72 (s, CH₂), 56.31 (s, OCH₃), 60.20 (s, OCH₃), 60.97 (s, OCH₃), 100.36 (s, CH), 126.62 (s, CH), 129.27 (s, CH), 130.58 (s, CH), 132.17 (s, C), 132.57 (s, C), 143.11 (s, C), 144.68 (s, C), 148.65 (s, C), 150.18 (s, C), 155.31 (s, C), 204.34 (s, C=O); *m/z* (AP+) 367 (MH⁺, 100%), 369 (MH⁺, 60%); HRMS found [MH]⁺ 367.0501 for C₁₈H₁₇O₄Cl₂, C₁₈H₁₇O₄Cl₂ requires [MH]⁺ 367.0498.

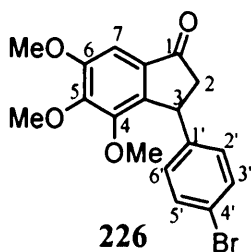
Chapter 6 – Experimental

3-(Benzo[d][1,3]dioxol-5-yl)-2,3-dihydro-4,5,6-trimethoxyinden-1-one **225**



The indanone **225** was obtained following Protocol C. Using (*E*)-3-(benzo[*d*][1,3]dioxol-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **209** (0.26 g, 0.76 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **225** as light brown solid (0.14 g, .41 mmol, 54%); m.p. 105-107 °C; R_f 0.62 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2973, 2938, 2837, 1706, 1593, 1506, 1487, 1478, 1468, 1456, 1444, 1431, 1417, 1340, 1308, 1246, 1127, 1036, 1021, 923, 875, 858; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.48 (1H, dd, *J* 2.5, 19.1 Hz, H-2), 3.09 (1H, dd, *J* 8.0, 19.1 Hz, H-2), 3.39 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 4.45 (1H, dd, *J* 2.5, 8.0 Hz, H-3), 5.85 (2H, dd, *J* 1.5, 4.1 Hz, H-2'), 6.45 (1H, d, *J* 1.8 Hz, H-4'), 6.54 (1H, dd, *J* 1.8, 8.0 Hz, H-6'), 6.66(1H, d, *J* 8.0 Hz, H-7'), 7.01 (1H, s, H-7); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 41.33 (s, CH), 47.30 (s, CH₂), 56.27 (s, OCH₃), 60.20 (s, OCH₃), 60.94 (s, OCH₃), 100.28 (s, CH), 101.00 (s, OCH₂O), 107.49 (s, CH), 108.27 (s, CH), 120.36 (s, CH), 132.18 (s, C), 138.29 (s, C), 144.44 (s, C), 146.19 (s, C), 147.84 (s, C), 148.80 (s, C), 150.37 (s, C), 154.94 (s, C), 205.28 (s, C=O); *m/z* (API-ES⁺) 343 (MH⁺, 100%); HRMS found [MH]⁺ 343.11762 for C₁₉H₁₉O₆, C₁₉H₁₉O₆ requires [MH]⁺ 343.11761.

3-(4-Bromophenyl)-2,3-dihydro-4,5,6-trimethoxyinden-1-one **226**

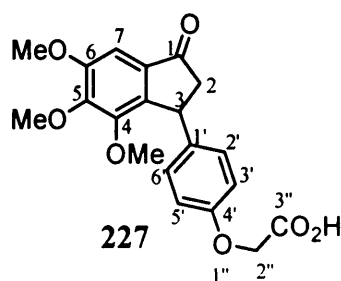


The indanone **226** was obtained following Protocol C. Using (*E*)-3-(4-bromophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **210** (0.22 g, 0.58 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column

Chapter 6 – Experimental

chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **226** as a pale yellow oil (0.07 g, 0.19 mmol, 33%); R_f 0.34 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max.}(\text{cm}^{-1})$ 2938, 2837, 1702, 1599, 1468, 1417, 1340, 1309, 1125, 1009, 825; $\delta^1\text{H NMR}$ (400 MHz; CDCl₃; ppm) 2.55 (1H, dd, J 2.5, 19.3 Hz, CH₂), 3.18 (1H, dd, J 7.8, 19.3 Hz, CH₂), 3.41 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 4.54 (1H, dd, J 2.4, 7.8 Hz, H-3), 6.99 (2H, d, J 8.2 Hz, H-2' & H-6'), 7.08 (1H, s, H-7), 7.41 (2H, d, J 8.2 Hz, H-3' & H-5'); $\delta^{13}\text{C NMR}$ (100MHz; CDCl₃; ppm) 41.25 (s, CH), 47.16 (s, CH₂), 56.48 (s, OCH₃), 60.32 (s, OCH₃), 61.12 (s, OCH₃), 100.52 (s, CH), 120.56 (s, C), 129.21 (s, CH), 131.91 (s, CH), 132.39 (s, C), 143.69 (s, C), 144.06 (s, C), 148.92 (s, C), 150.48 (s, C), 155.34 (s, C), 204.97 (s, CO); m/z (API ES+) 377 (MH⁺, 100%), 379 (MH⁺, 100%); HRMS found [MH]⁺ 377.03851 for C₁₈H₁₈O₄Br, C₁₈H₁₈O₄Br requires [MH]⁺ 377.03830.

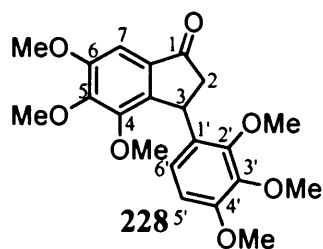
2,3-Dihydro-3-(4-carboxymethoxyphenyl)-4,5,6-trimethoxyinden-1-one 227



The indanone **227** was obtained following Protocol C. Using (*E*)-3-(4-carboxymethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **211** (0.24 g, 0.64 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Aqueous layer then washed acidified aqueous hydrochloric acid (1 M) till the pH ~ 5 – 6, then extracted with ethyl acetate (2 × 10 cm³). Column chromatography (SiO₂, ethyl acetate:methanol 49:1) afforded **227** as a cream solid (0.06 g, 0.16 mmol, 26%); m.p. 169 – 171 °C; R_f 0.63 (SiO₂, ethyl acetate:methanol 49:1); $\nu_{\max.}(\text{cm}^{-1})$ 2930, 2726, 2668, 1740, 1667, 1599, 1512, 1468, 1439, 1416, 1344, 1101, 830; $\delta^1\text{H NMR}$ (400 MHz; CDCl₃; ppm) 2.57 (1H, dd, J 2.7 Hz, J 19.2 Hz, H-2), 3.18 (1H, dd, J 8.1 Hz, J 19.2 Hz, H-2), 3.36 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.55 (1H, dd, J 2.7 Hz, J 8.1 Hz, H-3), 4.65 (2H, s, H-2''), 6.85 (2H, d, J 8.8 Hz, H-3' & H-5'), 7.05 (2H, d, J 8.8 Hz, H-2' & H-6'), 7.08 (1H, s, H-7); $\delta^{13}\text{C NMR}$ (100MHz; D₆-DMSO; ppm) 47.62 (s, CH₂), 56.81 (s, OCH₃), 60.51 (s, OCH₃), 61.22 (s, OCH₃), 65.18 (s, CH₂), 100.94 (s, CH), 115.13 (s, CH), 128.72 (s, CH), 132.41 (s, C), 137.52 (s, C), 144.56 (s, C), 148.73 (s, C), 150.61 (s, C), 155.17 (s, C), 156.95 (s, C), 170.87 (s, CO₂H), 204.81 (s, CO); m/z (API ES+) 373 (MH⁺, 100%); HRMS found [MH]⁺ 373.12851 for C₂₀H₂₁O₇, C₂₀H₂₁O₇Br requires [MH]⁺ 373.12818.

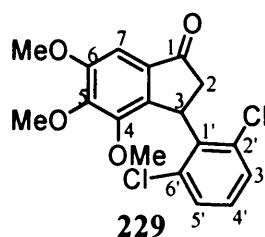
Chapter 6 – Experimental

2,3-Dihydro-4,5,6-trimethoxy-3-(2,3,4-trimethoxyphenyl)inden-1-one **228**



The indanone **228** was obtained following Protocol C. Using (*E*)-3-(2,3,4-trimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **212** (0.23 g, 0.59 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **228** as a pale brown solid (0.06g, 0.15 mmol, 25%); m.p. 115 – 117 °C; R_f 0.31 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2932, 2838, 1699, 1597, 1497, 1469, 1434, 1418, 1337, 850; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.59 (1H, broad d, *J* 20.8 Hz, H-2), 3.13 (1H, dd, *J* 8.2, 19.0 Hz, H-2), 3.39 (3H, s, OCH₃), 3.71 (3H, br. s, OCH₃), 3.82 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 4.78 (1H, d, *J* 5.6 Hz, H-3), 6.56 (1H, d, *J* 8.4 Hz, H-5'), 6.60 (1H, br. s, H-6'), 7.10 (1H, s, H-7); δ ¹³C NMR (100MHz; CDCl₃; ppm) 36.41 (broad s, CH), 46.61 (s, CH₂), 56.15 (s, OCH₃), 56.44 (s, OCH₃), 60.25 (s, OCH₃), 60.75 (broad s, OCH₃), 60.93 (s, OCH₃), 61.11 (s, OCH₃), 100.56 (s, CH), 107.37 (s, CH), 122.75 (broad s, CH), 130.09 (broad s, C), 132.83 (s, C), 142.54 (s, C), 144.67 (s, C), 148.77 (s, C), 150.50 (s, C), 151.80 (s, C), 152.76 (s, C), 154.81 (s, C), 205.99 (s, CO); *m/z*(API ES⁺) 389 (MH⁺, 100%); HRMS found [MH]⁺ 389.16112 for C₂₁H₂₅O₇, C₂₁H₂₅O₇ requires [MH]⁺ 389.15948.

3-(2,6-Dichlorophenyl)-2,3-dihydro-4,5,6-trimethoxyinden-1-one **229**

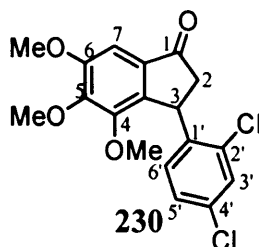


The indanone **229** was obtained following Protocol C. Using (*E*)-3-(2,6-dichlorophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **213** (0.24 g, 0.65 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **229** as a pink solid (0.23 g, 0.63 mmol, 95%); m.p. 98 – 102 °C; R_f 0.46 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2999,

Chapter 6 – Experimental

2974, 2938, 2831, 1706, 1598, 1582, 1561, 1470, 1459, 1435, 1414, 1340, 1308, 1124, 1096, 1044, 1006, 848, 753; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 2.91 (1H, dd, J 4.0, 19.1 Hz, H-2), 3.11 (1H, dd, J 8.1, 19.1 Hz, H-2), 3.41 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 5.48 (1H, dd, J 4.0, 8.1 Hz, H-3), 7.09 (1H, s, H-7), 7.14 (1H, d, J 7.9 Hz, H-4'), 7.18 (1H, dd, J 1.5, 7.9 Hz, H-3' or H-5'), 7.42 (1H, dd, J 1.5, 7.9 Hz, H-3' or H-5'); δ ^{13}C NMR (100MHz; CDCl_3 ; ppm) 37.85 (s, CH), 42.59 (s, CH_2), 56.38 (s, OCH_3), 60.32 (s, OCH_3), 61.05 (s, OCH_3), 100.99 (s, CH), 128.50 (s, CH), 128.55 (s, CH), 130.08 (s, CH), 133.22 (s, C), 134.84 (s, C), 136.49 (s, C), 137.25 (s, C), 142.68 (s, C), 148.41 (s, C), 150.31 (s, C), 154.87 (s, C), 204.31 (s, CO); m/z (API ES+) 367 (MH^+ , 100%), 369 (MH^+ , 60%); HRMS found $[\text{MH}]^+$ 367.05008 for $\text{C}_{18}\text{H}_{17}\text{Cl}_2\text{O}_4$, $\text{C}_{18}\text{H}_{17}\text{Cl}_2\text{O}_4$ requires $[\text{MH}]^+$ 367.04984.

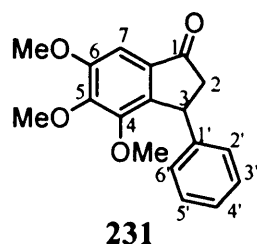
3-(2,4-Dichlorophenyl)-2,3-dihydro-4,5,6-trimethoxyinden-1-one **230**



The indanone **230** was obtained following Protocol C. Using (*E*)-3-(2,4-dichlorophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **214** (0.25 g, 0.68 mmol) in anhydrous trifluoroacetic acid (0.3 cm^3). Tipped into water (5 cm^3), extracted with ethyl acetate (2 \times 5 cm^3) washed with saturated aqueous sodium bicarbonate solution (5 cm^3). Column chromatography (SiO_2 , ethyl acetate:hexane 1:3) afforded **230** as a light brown solid (0.21 g, 0.57 mmol, 83%); m.p. 103 – 105 $^\circ\text{C}$; R_f 0.48 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 2945, 1703, 1603, 1587, 1559, 1469, 1417, 1346, 1317, 1157, 830; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 2.45 (1H, br. d, J 15.2 Hz, H-2), 3.24 (1H, dd, J 8.0, 19.2 Hz, H-2), 3.56 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.03 (1H, br. s, H-3), 6.67 (1H, br s, H-6'), 7.09 (1H, s, H-7), 7.11 (1H, br. s, H-5'), 7.42 (1H, br. s, H-3'); δ ^{13}C NMR (100MHz; CDCl_3 ; ppm) 37.60 (broad s, CH), 45.92 (broad s, CH_2), 56.45 (s, OCH_3), 60.38 (s, OCH_3), 61.14 (s, OCH_3), 100.60 (s, CH), 127.57 (s, CH), 128.67 (broad s, CH), 129.46 (s, C), 132.93 (s, C), 133.00 (s, C), 134.37 (s, C), 140.57 (broad s, CH), 142.49 (s, C), 148.65 (s, C), 150.29 (s, C), 155.44 (s, C), 204.27 (s, CO); m/z (API ES+) 367 (MH^+ , 100%), 369 (MH^+ , 60%); HRMS found $[\text{MH}]^+$ 367.05035 for $\text{C}_{18}\text{H}_{17}\text{O}_4\text{Cl}_2$, $\text{C}_{18}\text{H}_{17}\text{O}_4\text{Cl}_2$ requires $[\text{MH}]^+$ 367.04984.

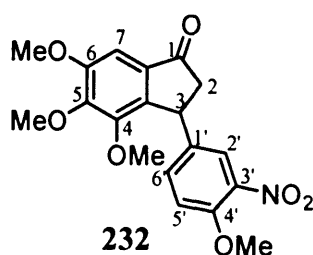
Chapter 6 – Experimental

2,3-Dihydro-4,5,6-trimethoxy-3-phenylinden-1-one **231**



The indanone **231** was obtained following Protocol C. Using (*E*)-1-(3,4,5-trimethoxyphenyl)-3-phenylprop-2-en-1-one **179** (0.23 g, 0.77 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **231** as a yellow oil (0.05 g, 1.7 mmol, 23%); R_f 0.36 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2938, 2837, 1702, 1600, 1493, 1467, 1417, 1339, 1309, 1125, 699; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.62 (1H, dd, *J* 2.6, 19.2 Hz, H-2), 3.19 (1H, dd, *J* 8.0, 19.2 Hz, H-3), 3.31 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 4.57 (1H, dd, *J* 2.6, 8.0 Hz, H-3), 7.09 – 7.13 (3H, m, H-7, H-2' & H-6'), 7.16 – 7.23 (1H, m, H-4'), 7.26 – 7.31 (2H, m, H-3' & H-5'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 41.84 (s, CH), 47.40 (s, CH₂), 56.46 (s, OCH₃), 60.19 (s, OCH₃), 61.08 (s, OCH₃), 100.50 (s, CH), 126.86 (s, CH), 127.51 (s, CH), 128.83 (s, CH), 132.45 (s, C), 144.61 (s, C), 144.92 (s, C), 149.01 (s, C), 150.62 (s, C), 155.14 (s, C), 205.55 (s, CO); *m/z*(API ES⁺) 299 (MH⁺, 100%); HRMS found [MH]⁺ 299.12893 for C₁₈H₁₉O₄, C₁₈H₁₉O₄ requires [MH]⁺ 299.12779.

2,3-Dihydro-4,5,6-trimethoxy-3-(4-methoxy-3-nitrophenyl)inden-1-one **232**

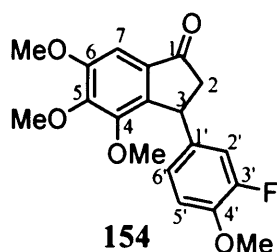


The indanone **232** was obtained following Protocol C. Using (*E*)-3-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **215** (0.25 g, 0.67 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **232** as a yellow solid (0.10 g, 0.27 mmol, 39%); m.p. 136 – 137 °C; R_f 0.14 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2999, 2926, 2837, 1696, 1623, 1596, 1570, 1525, 1461, 1422, 1342, 1275, 1248, 1093, 1004, 824; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.55 (1H, dd, *J* 2.8 Hz, *J* 19.3 Hz, H-

Chapter 6 – Experimental

2), 3.21 (1H, dd, J 8.1 Hz, J 19.3 Hz, H-2), 3.50 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 4.58 (1H, dd, J 2.8 Hz, J 8.1 Hz, H-3), 7.01 (1H, d, J 9.1 Hz, H-5'), 7.09 (1H, s, H-7), 7.26 (1H, dd, J 2.4, 9.1 Hz, H-6'), 7.64 (1H, d, J 2.4 Hz, H-2'); $\delta^{13}\text{C}$ NMR (100MHz; CDCl₃; ppm) 40.53 (s, CH), 46.89 (s, CH₂), 56.49 (s, OCH₃), 56.80 (s, OCH₃), 60.41 (s, OCH₃), 61.16 (s, OCH₃), 100.61 (s, CH), 114.03 (s, CH), 124.61 (s, CH), 132.36 (s, C), 132.93 (s, CH), 136.96 (s, C), 139.67 (s, C), 143.09 (s, C), 148.84 (s, C), 150.38 (s, C), 151.83 (s, C), 155.56 (s, C), 204.34 (s, CO); m/z (API ES+) 374 (MH⁺, 100%), 396 (MNa⁺, 40%); HRMS found [MH]⁺ 374.12469 for C₁₉H₂₀O₇N, C₁₉H₂₀O₇N requires [MH]⁺ 374.12343.

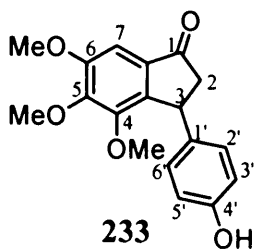
3-(3-Fluoro-4-methoxyphenyl)-2,3-dihydro-4,5,6-trimethoxyindan-1-one **154**



The indanone **154** was obtained following Protocol C. Using (*E*)-3-(3-fluoro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **155** (0.24 g, 0.69 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **154** as a yellow solid (0.09g, 0.26 mmol, 37%); m.p. 111 – 114 °C; R_f 0.31 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 3008, 2927, 2853, 1705, 1625, 1586, 1518, 1455, 1430, 1415, 1290, 1263, 1092, 1025, 1006, 760; $\delta^1\text{H}$ NMR (400 MHz; CDCl₃; ppm) 2.55 (1H, dd, J 2.3, 19.1 Hz, H-2), 3.16 (1H, dd, J 7.8, 19.1 Hz, H-2), 3.44 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 4.52 (1H, dd, J 2.3, 7.8 Hz, H-3), 6.79 – 6.90 (3H, m, H-2', H-5' & H-6'), 7.08 (1H, s, H-7); $\delta^{13}\text{C}$ NMR (100MHz; CDCl₃; ppm) 40.90 (s, CH), 47.29 (s, CH₂), 56.45 (s, OCH₃), 56.49 (s, OCH₃), 60.34 (s, OCH₃), 61.11 (s, OCH₃), 100.51 (s, CH), 100.51 (s, CH), 113.68 (d, J 2.1 Hz, CH), 115.14 (d, J 18.3 Hz, CH), 122.97 (d, J 3.7 Hz, CH), 132.34, (s, C), 137.67 (d, J 5.1 Hz, CH), 144.15 (s, C), 146.44 (d, J 10.2 Hz, C), 148.95 (s, C), 150.53 (s, C), 152.54 (d, J 244.7 Hz, CF), 155.27 (s, C), 205.06 (s, CO); m/z (API ES+) 374 (MH⁺, 100%); HRMS found [MH]⁺ 347.13028 for C₁₉H₂₀O₅F, C₁₉H₂₀O₅F requires [MH]⁺ 347.12893.

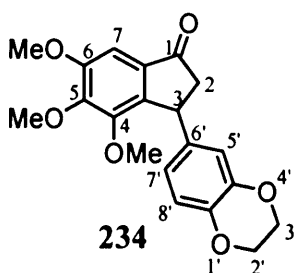
Chapter 6 – Experimental

2,3-Dihydro-3-(4-hydroxyphenyl)-4,5,6-trimethoxyindan-1-one **233**



The indanone **233** was obtained following Protocol C. Using (*E*)-3-(4-(benzyloxy)phenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **216** (0.22 g, 0.56 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **233** as a Brown gum (0.03g, 0.10 mmol, 15%); R_f 0.44 (SiO₂, ethyl acetate:hexane 1:1); ν_{max} (cm⁻¹) 3299 (broad m), 2938 (m), 1682 (s), 1596 (s), 1514 (s), 1468 (s), 1418 (s), 1340 (s), 1311 (s), 1207 (s), 1127 (s), 834 (s); δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.58 (1H, dd, *J* 2.7, 19.3 Hz, H-2), 3.17 (1H, dd, *J* 7.6, 19.3 Hz, H-2), 3.36 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.53 (1H, dd, *J* 2.7, 7.6 Hz, H-3), 4.75 (1H, broad s, OH-4'), 6.75 (2H, d, *J* 8.6 Hz, H-3' & H-5'), 6.98 (2H, d, *J* 8.6 Hz, H-2' & H-6'), 7.08 (1H, s, H-7); δ ¹³C NMR (100MHz; CDCl₃; ppm) 41.10 (s, CH), 47.59 (s, CH₂), 56.45 (s, OCH₃), 60.34 (s, OCH₃), 61.11 (s, OCH₃), 100.52 (s, CH), 115.73 (s, CH), 128.56 (s, CH), 132.26 (s, C), 136.18 (s, C), 141.34 (s, C), 149.13 (s, C), 150.59 (s, C), 155.06 (s, C), 162.98 (s, C), 206.20 (s, CO); *m/z*(API ES⁺) 315 (MH⁺, 100%); HRMS found [MH]⁺ 279.15910 for C₁₈H₁₉O₅, C₁₈H₁₉O₅ requires [MH]⁺ 279.14602.

2,3-Dihydro-3-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-4,5,6-trimethoxyindan-1-one **234**

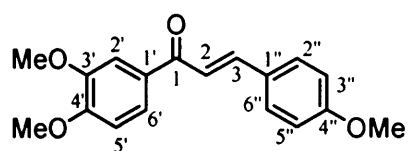


The indanone **234** was obtained following Protocol C. Using (*E*)-3-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **217** (0.27 g, 0.76 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **234** as a brown solid (0.14 g, 0.39 mmol, 52%); m.p. 93 – 96 °C; R_f 0.26 (SiO₂, ethyl acetate:hexane 1:3);

Chapter 6 – Experimental

$\nu_{\max}(\text{cm}^{-1})$ 2931 (m), 1705 (s), 1587 (s), 1505 (s), 1466 (s), 1427 (s), 1414 (s), 1281 (s), 1125 (s), 1100 (s), 841 (s), 810 (s); $\delta^1\text{H NMR}$ (400 MHz; CDCl_3 ; ppm) 2.57 (1H, dd, J 2.7, 19.2 Hz, H-2), 3.15 (1H, dd, J 7.9, 19.2 Hz, H-2), 3.45 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.22 (4H, s, H-2' & H-3'), 4.47 (1H, dd, J 2.7, 7.9 Hz, H-3), 6.57 – 6.61 (2H, m, H-5' & H-7'), 6.77 (1H, d, J 8.4 Hz, H-8'), 7.07 (1H, s, H-7); $\delta^{13}\text{C NMR}$ (100MHz; CDCl_3 ; ppm) 41.12 (s, CH), 47.46 (s, CH_2), 56.42 (s, OCH_3), 60.34 (s, OCH_3), 61.08 (s, OCH_3), 64.48 (s, $\text{OCH}_2\text{CH}_2\text{O}$), 64.55 (s, $\text{OCH}_2\text{CH}_2\text{O}$), 100.46 (s, CH), 116.05 (s, CH), 117.43 (s, CH), 120.34 (s, CH), 132.35 (s, C), 137.89 (s, C), 142.37 (s, C), 143.69 (s, C), 144.70 (s, C), 148.95 (s, C), 150.57 (s, C), 155.06 (s, C), 205.52 (s, CO); $m/z(\text{API ES}^+)$ 357 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 357.13332 for $\text{C}_{20}\text{H}_{21}\text{O}_6$, $\text{C}_{20}\text{H}_{21}\text{O}_6$ requires $[\text{MH}]^+$ 357.13326.

(*E*)-1-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **235**³⁴³

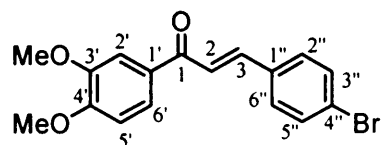


235

The chalcone **235** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.36 g, 2.0 mmol), *p*-anisaldehyde (0.25 cm³, 2.0 mmol) and aqueous sodium hydroxide solution (1.0 cm³, 4M) in methanol (2.0 cm³). Recrystallization from methanol afforded **235** as light yellow crystals (0.40 g, 1.3 mmol, 67%); m.p. 81 – 83 °C; R_f 0.54 (SiO_2 , ethyl acetate:hexane 1:1); $\nu_{\max}(\text{cm}^{-1})$ 3055, 3007, 2931, 2836, 1648, 1592, 1568, 1508, 1452, 1439, 1422, 1240, 1161, 1148, 1025, 995, 822; $\delta^1\text{H NMR}$ (400 MHz; CDCl_3 ; ppm) 3.86 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.93 (1H, d, J 8.5 Hz, H-5'), 6.94 (2H, d, J 8.4 Hz, H-3'' & H-5''), 7.45 (1H, d, J 15.6 Hz, H-2), 7.61 (2H, d, J 8.4 Hz, H-2'' & H-6''), 7.62 (1H, d, J 1.5 Hz, H-2'), 7.68 (1H, dd, J 1.5, 8.5 Hz, H-6'), 7.79 (1H, d, J 15.6 Hz, H-3); $\delta^{13}\text{C NMR}$ (100MHz; CDCl_3 ; ppm) 55.62 (s, OCH_3), 56.26 (s, OCH_3), 56.30 (s, OCH_3), 110.19 (s, CH), 111.01 (s, CH), 114.61 (s, CH), 119.56 (s, CH), 123.05 (s, CH), 128.02 (s, C), 130.34 (s, CH), 131.80 (s, C), 144.03 (s, CH), 149.42 (s, C), 153.31 (s, C), 161.75 (s, C), 188.82 (s, CO); $m/z(\text{API ES}^+)$ 299 (MH^+ , 100%).

Chapter 6 – Experimental

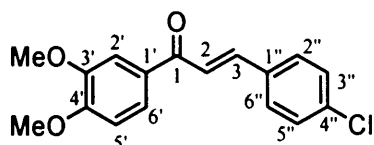
(E)-3-(4-Bromophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **236**



236

The chalcone **236** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.36 g, 2.0 mmol), 4-bromobenzaldehyde (0.37 g, 2.0 mmol) and aqueous sodium hydroxide solution (2.0 cm³, 2M) in methanol (3.0 cm³). Recrystallization from methanol afforded **236** as yellow crystals (0.52 g, 1.5 mmol, 75%); m.p. 130 – 132 °C; R_f 0.63 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3003, 2941, 2844, 1654, 1599, 1577, 1561, 1511, 1485, 1451, 1417, 1256, 1239, 1157, 1144, 1018, 993, 757; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.97 (6H, s, OCH₃), 6.93 (1H, d, *J* 8.5 Hz, H-5'), 7.49 – 7.57 (5H, m, H-2, H-2'', H-3'', H-5'' & H-6''), 7.62 (1H, d, *J* 2.0 Hz, H-2'), 7.68 (1H, dd, *J* 2.0, 8.5 Hz, H-6'), 7.74 (1H, *J* 15.6 Hz, H-3); δ ¹³C NMR (100MHz; CDCl₃; ppm) 56.28 (s, OCH₃), 56.34 (s, OCH₃), 110.19 (s, CH), 110.96 (s, CH), 122.37 (s, CH), 123.30 (s, CH), 124.77 (s, C), 129.95 (s, CH), 131.34 (s, C), 132.38 (s, CH), 134.2 (s, C), 142.71 (s, CH), 149.53 (s, C), 153.64 (s, C), 188.43 (s, CO); *m/z*(API ES⁺) 347 (MH⁺, 100%), 349 (MH⁺, 100%); HRMS found [MH]⁺ 347.02896 for C₁₇H₁₆O₃Br, C₁₇H₁₆O₃Br requires [MH]⁺ 347.02773.

(E)-3-(4-Chlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **237**



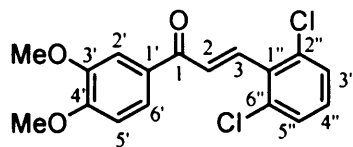
237

The chalcone **237** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.36 g, 2.0 mmol), 4-chlorobenzaldehyde (0.28 g, 2.0 mmol) and aqueous sodium hydroxide solution (1.0 cm³, 4M) in methanol (2.0 cm³). Recrystallization from methanol afforded **237** as white crystal (0.47 g, 1.5 mmol, 76%); m.p. 123 – 125 °C; R_f 0.63 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 3078, 2933, 2837, 1651, 1600, 1575, 1566, 1513, 1490, 1459, 1446, 1417, 1255, 1240, 1148, 1018, 760; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.96 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.92 (1H, d, *J* 8.8 Hz, H-5'), 7.38 (2H, d, *J* 8.6 Hz, H-3'' & H-5''), 7.51 (1H, d, *J* 15.6 Hz, H-2), 7.56 (2H, d, *J* 8.6 Hz, H-2'' & H-6''), 7.61 (1H, d, *J* 2.0 Hz, H-2'), 7.67 (1H, dd, *J* 2.0, 8.8 Hz, H-6'), 7.74 (1H, d, *J* 15.6 Hz, H-3); δ ¹³C NMR (100MHz; CDCl₃; ppm) 56.28 (s, OCH₃), 56.33 (s, OCH₃), 110.22 (s, CH), 111.02 (s, CH),

Chapter 6 – Experimental

122.32 (s, CH), 123.28 (s, CH), 129.42 (s, CH), 129.73 (s, CH), 131.38 (s, C), 133.81 (s, C), 136.42 (s, C), 142.66 (s, CH), 149.55 (s, C), 153.65 (s, C), 188.47 (s, CO); m/z (API ES+) 303 (MH^+ , 100%), 305 (MH^+ , 30%); HRMS found $[MH]^+$ 303.07929 for $C_{17}H_{16}O_3Cl$, $C_{17}H_{16}O_3Cl$ requires $[MH]^+$ 303.07825.

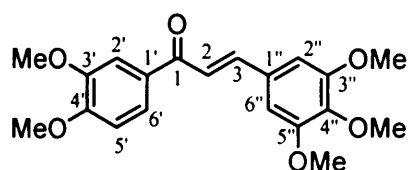
(*E*)-3-(2,6-Dichlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **238**



238

The chalcone **238** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.36 g, 2.0 mmol), 2,6-dichlorobenzaldehyde (0.35 g, 2.0 mmol) and aqueous sodium hydroxide solution (1.0 cm³, 4M) in methanol (2.0 cm³). Recrystallization from methanol afforded **238** as yellow crystals (0.52 g, 1.5 mmol, 77%); m.p. 131 – 133 °C; R_f 0.71 (SiO₂, ethyl acetate:hexane 1:1); ν_{max} (cm⁻¹) 3057, 2937, 1652, 1599, 1575, 1554, 1513, 1451, 1439, 1420, 1304, 1265, 1256, 1242, 1161, 1141, 1022, 760; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.96 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.93 (1H, d, J 8.4 Hz, H-5'), 7.19 (1H, t, J 8.3 Hz, H-4''), 7.37 (2H, d, J 8.3 Hz, H-3'' & H-5''), 7.62 – 7.69 (3H, m, H-2, H-2' & H-6'), 7.83 (1H, d, J 16.0 Hz, H-3); δ ¹³C NMR (100MHz; CDCl₃; ppm) 56.26 (s, OCH₃), 56.34 (s, OCH₃), 110.28 (s, CH), 111.04 (s, CH), 123.71 (s, CH), 129.06 (s, CH), 129.93 (s, CH), 130.53 (s, CH), 131.06 (s, C), 133.09 (s, C), 135.37 (s, C), 137.18 (s, CH), 149.52 (s, C), 153.76 (s, C), 188.57 (s, CO); m/z (API ES+) 337 (MH^+ , 100%), 339 (MH^+ , 60%); HRMS found $[MH]^+$ 337.04008 for $C_{17}H_{15}O_3Cl_2$, $C_{17}H_{15}O_3Cl_2$ requires $[MH]^+$ 337.03928.

(*E*)-3-(3,4,5-Trimethoxyphenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **239**



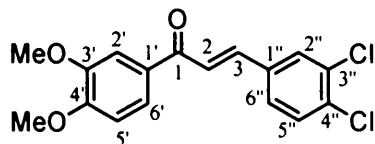
239

The chalcone **239** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.36 g, 2.0 mmol), 3,4,5-trimethoxybenzaldehyde (0.39 g, 2.0 mmol) and aqueous sodium hydroxide solution (2.0 cm³, 2M) in methanol (3.0 cm³). Recrystallization from methanol afforded **239** as light yellow crystals (0.65 g, 1.8 mmol, 91%); m.p. 125 – 126 °C; R_f 0.43 (SiO₂, ethyl acetate:hexane 1:1); ν_{max} (cm⁻¹) 2999, 2949, 2832, 1656, 1605, 1582, 1505, 1470, 1450, 1418, 1262, 1244, 1000, 981, 817; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.89

Chapter 6 – Experimental

(3H, s, OCH₃), 3.92 (6H, s, OCH₃), 3.96 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.86 (2H, s, H-2'' & H-6''), 6.92 (1H, d, *J* 8.4 Hz, H-5'), 7.42 (1H, *J* 15.6 Hz, H-2), 7.61 (1H, d, *J* 2.0 Hz, H-2'), 7.68 (1H, dd, *J* 2.0, 8.4 Hz, H-6'), 7.71 (1H, *J* 15.6 Hz, H-3); $\delta^{13}\text{C}$ NMR (100MHz; CDCl₃; ppm) 56.31 (s, OCH₃), 56.32 (s, OCH₃), 56.46 (s, OCH₃), 61.22 (s, OCH₃), 105.82 (s, CH), 110.14 (s, CH), 111.05 (s, CH), 121.28 (s, CH), 123.22 (s, CH), 130.80 (s, C), 131.59 (s, C), 140.51 (s, C), 144.36 (s, CH), 149.52 (s, C), 153.48 (s, C), 153.69 (s, C), 188.79 (s, CO); *m/z*(API ES+) 359 (MH⁺, 100%).

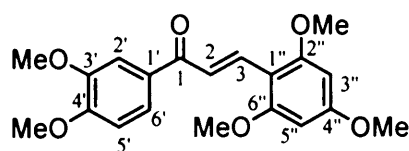
(*E*)-3-(3,4-Dichlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **240**²¹⁵



240

The chalcone **240** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.90 g, 5.0 mmol), 3,4,-dichlorobenzaldehyde (0.88 g, 5.0 mmol) and aqueous potassium hydroxide solution (6.0 cm³, 3M) in ethanol (13.0 cm³). Recrystallization from ethanol afforded **240** as cream crystals (0.96 g, 2.8 mmol, 57%); mp 119 – 121 °C; R_f 0.36 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 2953, 2832, 1661, 1599, 1583, 1514, 1472, 1417, 1261, 1242, 1165, 1150, 1027, 766; $\delta^1\text{H}$ NMR (400 MHz; CDCl₃; ppm) 3.98 (6H, s, OCH₃), 6.94 (1H, d, *J* 8.4 Hz, H-5'), 7.46 (1H, dd, *J* 1.8, 8.4 Hz, H-6'), 7.49 (1H, d, *J* 8.3 Hz, H-5''), 7.53 (1H, d, *J* 15.6 Hz, H-2), 7.62 (1H, d, *J* 2.0 Hz, H-2''), 7.66 – 7.72 (2H, m, H-3 & H-6''), 7.73 (1H, d, *J* 1.8 Hz, H-2'); $\delta^{13}\text{C}$ NMR (100 MHz; CDCl₃; ppm) 56.10 (s, OCH₃), 56.17 (s, OCH₃), 109.94 (s, CH), 110.65 (s, CH), 123.09 (s, CH), 123.21 (s, CH), 127.54 (s, CH), 129.65 (s, CH), 130.91 (s, C), 130.94 (s, CH), 133.26 (s, C), 134.17 (s, C), 135.14 (s, C), 141.09 (s, CH), 149.36 (s, C), 153.56 (s, C), 187.89 (s, CO); *m/z*(AP+) 337 (MH⁺, 100%) 339 (MH⁺, 60%).

(*E*)-3-(2,4,6-Trimethoxyphenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **241**³⁴⁴



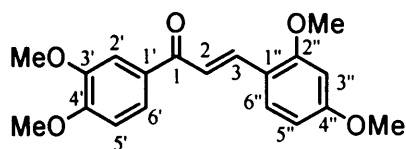
241

The chalcone **241** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.55 g, 3.0 mmol), 2,4,6-trimethoxybenzaldehyde (0.59 g, 3.0 mmol) and aqueous sodium hydroxide solution (1.0 cm³, 6M) in methanol (3.0 cm³). Recrystallization from methanol

Chapter 6 – Experimental

afforded **241** as yellow plates (0.65 g, 1.8 mmol, 61%); m.p. 171-173 °C; R_f 0.30 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max.}(\text{cm}^{-1})$ 2983, 2840, 1637, 1597, 1555, 1515, 1464, 1417, 1319, 1258, 1235, 1154, 1022, 819; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.88 (3H, s, OCH₃), 3.93 (6H, s, OCH₃), 3.97 (3H, s, OCH₃), 3.99 (3H, s, OCH₃), 6.16 (2H, s, H-3'' & H-5''), 6.95 (1H, d, J 8.4 Hz, H-5'), 7.65 (1H, d, J 1.9 Hz, H-2'), 7.68 (1H, dd, J 8.4, 1.9 Hz, H-6'), 7.92 (1H, d, J 15.9 Hz, H-2), 8.25 (1H, d, J 15.9 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.43 (s, OCH₃), 55.83 (s, OCH₃), 55.98 (s, OCH₃), 56.05 (s, OCH₃), 90.50 (s, CH), 106.60 (s, C), 109.88 (s, CH), 110.91 (s, CH), 121.73 (s, CH), 122.70 (s, CH), 132.35 (s, C), 135.27 (s, CH), 148.90 (s, C), 152.52 (s, C), 161.62 (s, C), 162.95 (s, C), 190.48 (s, CO); m/z (AP⁺) 359 (MH⁺, 100%); HRMS found [MH]⁺ 359.1490 for C₂₀H₂₂O₆, C₂₀H₂₂O₆ requires [MH]⁺ 359.1489; CHN found C 66.97%, H 6.19% CHN requires C 67.03%, H 6.19%.

(E)-3-(2,4-Dimethoxyphenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **242**

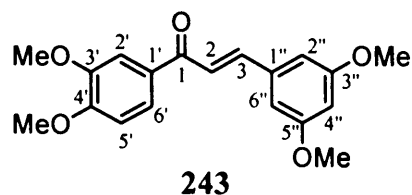


242

The chalcone **242** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.37 g, 3.0 mmol), 2,4,-dimethoxybenzaldehyde (0.33 g, 2.0 mmol) and aqueous sodium hydroxide solution (1.0 cm³, 4M) in methanol (2.0 cm³). Recrystallization from methanol afforded **242** as yellow crystals (0.51 g, 1.6 mmol, 79%); m.p. 111 – 112 °C; R_f 0.42 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max.}(\text{cm}^{-1})$ 2999, 2938, 2836, 1646, 1592, 1578, 1554, 1506, 1468, 1454, 1437, 1417, 1307, 1242, 1210, 1151, 1025, 1009, 838, 786, 745; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.85 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.47 (1H, d, J 1.7 Hz, H-3''), 6.53 (1H, dd, J 1.7, 8.6 Hz, H-5''), 6.92 (1H, d, J 8.4 Hz, H-5'), 7.56 (1H, d, J 15.7 Hz, H-2), 7.57 (1H, d, J 8.6 Hz, H-6''), 7.62 (1H, d, J 1.9 Hz, H-2'), 7.66 (1H, dd, J 1.9, 8.4 Hz, H-6'), 8.04 (1H, d, J 15.7 Hz, H-3); δ ¹³C (100MHz; CDCl₃; ppm) 55.71 (s, OCH₃), 55.77 (s, OCH₃), 56.23 (s, OCH₃), 56.28 (s, OCH₃), 98.68 (s, CH), 105.59 (s, CH), 110.18 (s, CH), 111.13 (s, CH), 117.53 (s, C), 120.36 (s, CH), 122.99 (s, CH), 131.05 (s, CH), 132.15 (s, C), 139.91 (s, CH), 149.30 (s, C), 153.06 (s, C), 160.53 (s, C), 163.09 (s, C), 189.57 (s, CO); m/z (API ES⁺) 329 (MH⁺, 100%).

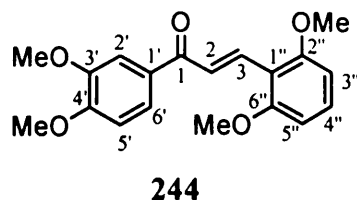
Chapter 6 – Experimental

(*E*)-1-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one **243**



The chalcone **243** was obtained following Protocol A. Using 3,4-dimethoxyacetophenone (0.36 g, 3.0 mmol), 2,4,-dimethoxybenzaldehyde (0.33 g, 2.0 mmol) and aqueous sodium hydroxide solution (2.0 cm³, 2M) in methanol (3.0 cm³). Recrystallization from methanol afforded **243** as white crystals (0.39 g, 1.6 mmol, 62%); m.p. 82 – 84 °C; R_f 0.58 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2968, 2844, 1652, 1594, 1579, 1510, 1459, 1418, 1286, 1260, 1162, 1020, 834; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.83 (6H, s, OCH₃), 3.96 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.51 (1H, t, *J* 2.0 Hz, H-4''), 6.78 (2H, d, *J* 2.0 Hz, H-2'' & H-6''), 6.92 (1H, d, *J* 8.8 Hz, H-5'), 7.50 (1H, d, *J* 15.8 Hz, H-2), 7.61 (1H, d, *J* 2.0 Hz, H-2'), 7.67 (1H, dd, *J* 2.0, 8.8 Hz, H-6'), 7.71 (1H, d, *J* 15.8 Hz, H-3); δ ¹³C (100MHz; CDCl₃; ppm) 55.70 (s, OCH₃), 56.28 (s, OCH₃), 56.32 (s, OCH₃), 102.67 (s, CH), 106.43 (s, CH), 106.53 (s, CH), 110.18 (s, CH), 111.00 (s, CH), 122.45 (s, CH), 123.30 (s, CH), 131.48 (s, C), 137.21 (s, C), 144.18 (s, CH), 149.49 (s, C), 153.53 (s, C), 161.27 (s, C), 188.80 (s, CO); *m/z*(API ES+) 329 (MH⁺, 100%).

(*E*)-3-(2,6-Dimethoxyphenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **244**

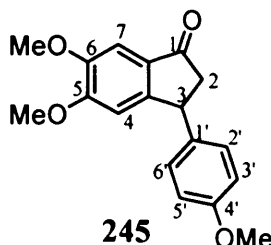


The chalcone **244** was obtained following Protocol A. Using 2,6-dimethoxyacetophenone (0.36 g, 3.0 mmol), 2,4,-dimethoxybenzaldehyde (0.34 g, 2.1 mmol) and aqueous sodium hydroxide solution (1.0 cm³, 1M) in methanol (2.0 cm³). Recrystallization from methanol afforded **244** as pale cream crystals (0.40 g, 1.2 mmol, 61%); m.p. 119 – 120 °C; R_f 0.50 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2932, 2837, 1644, 1593, 1567, 1509, 1478, 1454, 1435, 1417, 1255, 1238, 1144, 1108, 1022, 1003; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.92 (6H, s, OCH₃), 3.96 (3H, s, OCH₃), 3.97 (3H, s, OCH₃), 6.59 (2H, d, *J* 8.5 Hz, H-3'' & H-5''), 6.93 (1H, d, *J* 8.4 Hz, H-5'), 7.29 (1H, t, *J* 8.5 Hz, H-4''), 7.64 (1H, d, *J* 2.0 Hz, H-2'), 7.68(1H, dd, *J* 2.0, 8.4 Hz, H-6'), 8.00 (1H, d, *J* 16.2 Hz, H-2), 8.25(1H, d, *J* 16.2 Hz, H-3); δ ¹³C (100MHz; CDCl₃; ppm) 56.09 (s, OCH₃), 56.21 (s, OCH₃), 56.27 (s, OCH₃), 104.01

Chapter 6 – Experimental

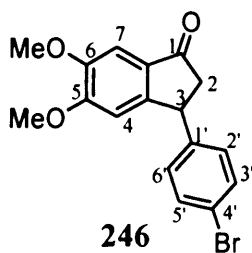
(s, CH), 110.17 (s, CH), 111.21 (s, CH), 113.30 (s, C), 123.13 (s, CH), 125.02 (s, CH), 131.49 (s, CH), 132.30 (s, C), 135.18 (s, CH), 149.23 (s, C), 152.98 (s, C), 160.53 (s, C), 190.66 (s, CO); m/z (API ES+) 329 (MH^+ , 100%); HRMS found $[MH]^+$ 329.13849 for $C_{19}H_{21}O_5$, $C_{19}H_{21}O_5$ requires $[MH]^+$ 329.13835.

2,3-Dihydro-5,6-dimethoxy-3-(4-methoxyphenyl)inden-1-one **245**



The indanone **245** was obtained following Protocol C. Using (*E*)-1-(3,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **235** (0.23 g, 0.77 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **245** as a yellow brown gum (0.016 g, 0.05 mmol, 7%); R_f 0.22 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 2933, 2836, 1695, 1590, 1510, 1496, 1463, 1440, 1418, 1246, 1210, 1044, 1030, 832; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.59 (1H, dd, J 3.3, 19.0 Hz, H-2), 3.19 (1H, dd, J 7.7, 19.0 Hz, H-2), 3.80 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 4.44 (1H, dd, J 3.3, 7.7 Hz, H-3), 6.63 (1H, s, H-4), 6.85 (2H, d, J 8.4 Hz, H-3' & H-5'), 7.04 (2H, d, J 8.4 Hz, H-2' & H-6'), 7.22 (1H, s, H-7); δ ¹³C NMR (100MHz; CDCl₃; ppm) 45.68 (s, CH), 47.62 (s, CH₂), 55.52 (s, OCH₃), 56.38 (s, OCH₃), 56.50 (s, OCH₃), 103.84 (s, CH), 107.63 (s, CH), 114.50 (s, CH), 128.77 (s, CH), 130.02 (s, C), 136.11 (s, C), 150.02 (s, C), 153.68 (s, C), 155.99 (s, C), 158.76 (s, C), 204.98 (s, CO); m/z (API ES+) 299 (MH^+ , 100%); HRMS found $[MH]^+$ 299.12734 for $C_{18}H_{19}O_4$, $C_{18}H_{19}O_4$ requires $[MH]^+$ 299.12779.

3-(4-Bromophenyl)-2,3-dihydro-5,6-dimethoxyinden-1-one **246**

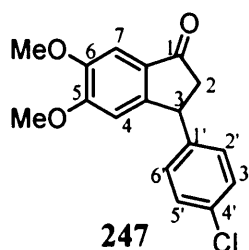


The indanone **246** was obtained following Protocol C. Using (*E*)-3-(4-bromophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **236** (0.23 g, 0.66 mmol) in anhydrous trifluoroacetic acid

Chapter 6 – Experimental

(0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **246** as a yellow solid (0.12 g, 0.35 mmol, 52%); m.p. 106 – 111 °C; R_f 0.21 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3062, 3019, 2926, 2854, 1701, 1689, 1587, 1502, 1488, 1468, 1455, 1434, 1418, 1301, 1283, 1212, 1009, 854, 819; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.57 (1H, dd, *J* 3.5, 18.9 Hz, H-2), 3.20 (1H, dd, *J* 8.0, 18.9 Hz, H-2), 3.86 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 4.45 (1H, dd, *J* 3.5, 8.0 Hz, H-3), 6.60 (1H, s, H-4), 7.00 (2H, d, *J* 8.2 Hz, H-2' & H-6'), 7.22 (1H, s, H-5), 7.44 (2H, d, *J* 8.2 Hz, H-3' & H-5'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 43.87 (s, CH), 47.25 (s, CH₂), 56.40 (s, OCH₃), 56.53 (s, OCH₃), 103.97 (s, CH), 107.49 (s, CH), 121.02 (s, C), 129.53 (s, CH), 130.09 (s, C), 132.26 (s, CH), 143.14 (s, C), 150.23 (s, C), 152.63 (s, C), 156.10 (s, C), 204.25 (s, CO); *m/z*(API ES+) 347 (MH⁺, 100%), 349 (MH⁺, 100%); HRMS found [MH]⁺ 347.02820 for C₁₇H₁₆O₃Br, C₁₇H₁₆O₃Br requires [MH]⁺ 347.02773.

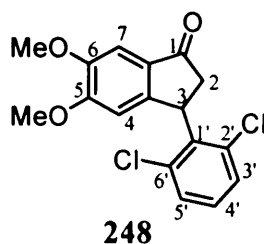
3-(4-Chlorophenyl)-2,3-dihydro-5,6-dimethoxyinden-1-one **247**



The indanone **247** was obtained following Protocol C. Using (*E*)-3-(4-chlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **237** (0.21 g, 0.71 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **247** as a orange solid (0.073 g, 0.24 mmol, 34%); m.p. 112 – 113 °C; R_f 0.26 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2923, 1691, 1592, 1501, 1489, 1469, 1459, 1442, 1402, 1300, 1287, 1046, 818; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.57 (1H, dd, *J* 3.4, 18.9 Hz, H-2), 3.21 (1H, dd, *J* 7.9, 18.9 Hz, H-2), 3.86 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 4.46 (1H, dd, *J* 3.4, 7.9 Hz, H-3), 6.60 (1H, s, H-4), 7.05 (2H, d, *J* 8.6 Hz, H-2' & H-6'), 7.23 (1H, s, H-7), 7.29 (2H, d, *J* 8.6 Hz, H-3' & H-5'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 43.79 (s, CH), 47.29 (s, CH₂), 56.38 (s, OCH₃), 56.50 (s, OCH₃), 103.95 (s, CH), 107.51 (s, CH), 129.15 (s, CH), 129.29 (s, CH), 130.07 (s, C), 132.95 (s, C), 142.62 (s, C), 150.21 (s, C), 152.72 (s, C), 156.08 (s, C), 204.26 (s, CO); *m/z*(API ES+) 303 (MH⁺, 100%), 305 (MH⁺, 30%); HRMS found [MH]⁺ 303.07854 for C₁₇H₁₆O₃Cl, C₁₇H₁₆O₃Cl requires [MH]⁺ 303.07825.

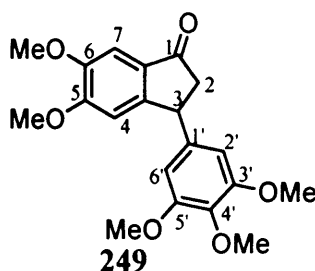
Chapter 6 – Experimental

3-(2,6-Dichlorophenyl)-2,3-dihydro-5,6-dimethoxyinden-1-one **248**



The indanone **248** was obtained following Protocol C. Using (*E*)-3-(2,6-dichlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **238** (0.26 g, 0.76 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **248** as a light brown solid (0.18 g, 0.54 mmol, 71%); m.p. 140 – 143 °C; R_f 0.35 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2924, 2853, 1700, 1592, 1560, 1498, 1465, 1436, 1422, 1292, 1254, 1211, 1115, 773; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.99 (1H, dd, *J* 4.3, 19.0 Hz, H-2), 3.12 (1H, dd, *J* 7.7, 19.0 Hz, H-2), 3.85 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 5.43 (1H, dd, *J* 4.3, 7.7 Hz, H-3), 6.52 (1H, s, H-4), 7.23 – 7.16 (3H, m, H-7, H-4' & H-3' or H-5'), 7.43 (1H, dd, *J* 1.4, 7.8 Hz, H-3' or H-5'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 40.23 (s, CH), 42.70 (s, CH₂), 56.28 (s, OCH₃), 56.50 (s, OCH₃), 104.41 (s, CH), 106.25 (s, CH), 128.61 (s, CH), 1129.04 (s, CH), 130.34 (s, C), 130.53 (s, CH), 135.64 (s, C), 136.59 (s, C), 136.70 (s, C), 149.80 (s, C), 151.61 (s, C), 155.92 (s, C), 203.73 (s, CO); *m/z*(API ES⁺) 337 (MH⁺, 100%), 339 (MH⁺, 60%); HRMS found [MH]⁺ 337.0395 for C₁₇H₁₅O₃Cl₂, C₁₇H₁₅O₃Cl₂ requires [MH]⁺ 337.0392.

2,3-Dihydro-5,6-dimethoxy-3-(3,4,5-trimethoxyphenyl)inden-1-one **249**

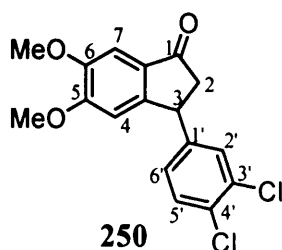


The indanone **249** was obtained following Protocol C. Using (*E*)-3-(3,4,5-trimethoxyphenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **239** (0.25 g, 0.70 mmol) in anhydrous trifluoroacetic acid (0.3 cm³). Tipped into water (5 cm³), extracted with ethyl acetate (2 × 5 cm³) washed with saturated aqueous sodium bicarbonate solution (5 cm³). Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **249** as dark brown solid (0.10 g, 0.28 mmol, 41%); m.p. 173-175 °C; R_f 0.28 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹)

Chapter 6 – Experimental

2957 (s), 2917 (s), 2848, 1690, 1587, 1495, 1462, 1421, 1296, 1259, 1247, 1212, 1110, 1052, 1008, 835; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 2.56(1H, dd, J 3.5, 19.1 Hz, H-2), 3.13 (1H, dd, J 7.8, 19.3 Hz, H-2), 3.73 (6H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.35 (1H, dd, J 3.5, 7.8 Hz, H-3), 6.24 (2H, s, H-2' & H-6'), 6.61 (H, s, H-4), 7.16 (H, s, H-7); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 44.59 (s, CH), 47.23 (s, CH_2), 56.13 (s, OCH_3), 56.17 (s, OCH_3), 56.40 (s, OCH_3), 60.88 (s, OCH_3), 103.61 (s, CH), 104.38 (s, CH), 107.41 (s, CH), 129.88 (s, C), 136.76 (s, C), 139.56 (s, C), 149.88 (s, C), 152.86 (s, C), 153.54 (s, C), 155.77 (s, C), 204.57 (s, C=O); m/z (AP+) 359 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 359.1489 for $\text{C}_{20}\text{H}_{23}\text{O}_6$, $\text{C}_{20}\text{H}_{23}\text{O}_6$ requires $[\text{MH}]^+$ 359.1489.

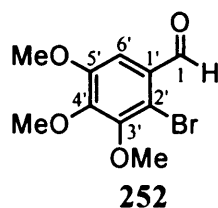
3-(3,4-Dichlorophenyl)-2,3-dihydro-5,6-dimethoxyindan-1-one **250**²¹⁵



The indanone **250** was obtained following Protocol C. Using (*E*)-3-(3,4,5-trimethoxyphenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one **240** (0.24 g, 0.70 mmol) in anhydrous trifluoroacetic acid (0.3 cm^3). Tipped into water (5 cm^3), extracted with ethyl acetate (2 \times 5 cm^3) washed with saturated aqueous sodium bicarbonate solution (5 cm^3). Column chromatography (SiO_2 , ethyl acetate:hexane 1:3) afforded **250** as a light brown solid (0.031 g, 0.09 mmol, 13%); m.p. 144 – 146 $^\circ\text{C}$; R_f 0.30 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 2925, 1694, 1588, 1498, 1463, 1417, 1285, 1254, 1211, 1112, 1044, 826; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 2.56 (1H, dd, J 3.5, 19.1 Hz, H-2), 3.21 (1H, dd, J 7.6, 19.1 Hz, H-2), 3.87 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.44 (1H, dd, J 3.5, 7.6 Hz, H-3), 6.60 (1H, s, H-6), 6.84 (1H, dd, J 2.1, 8.4 Hz, H-6'), 7.22 (1H, d, J 2.1 Hz, H-2'), 7.23 (1H, s, H-7), 7.85 (1H, d, J 8.4 Hz, H-5'); δ ^{13}C NMR (100MHz; CDCl_3 ; ppm) 43.57 (s, CH), 47.10 (s, CH_2), 56.43 (s, OCH_3), 56.59 (s, OCH_3), 104.07 (s, CH), 107.42 (s, CH), 127.14 (s, CH), 129.85 (s, CH), 130.13 (s, C), 131.16 (s, CH), 131.30 (s, C), 133.16 (s, C), 144.44 (s, C), 150.40 (s, C), 151.93 (s, C), 156.21 (s, C), 203.74 (s, CO); m/z (API ES+) 337 (MH^+ , 100%), 339 (MH^+ , 60%).

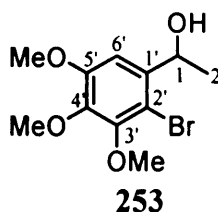
Chapter 6 – Experimental

2-Bromo-3,4,5-trimethoxybenzaldehyde **252**^{226,345}



The method adopted was that of Beugelmans²²⁶ *et al.* To a stirring solution of 3,4,5-trimethoxybenzaldehyde (3.96 g, 20.2 mmol) in chloroform (40 cm³) was added *N*-bromosuccinimide (3.84 g, 21.6 mmol). The reaction was then put under argon and refluxed at 75 °C for 4 hours. The reaction was then left stirring overnight at room temperature. The solvent was then evaporated *in vacuo*, and column chromatography (SiO₂, dichloromethane) afforded **252** as a white solid (4.60 g, 16.7 mmol, 83%); m.p. 69 - 70 °C; R_f 0.40 (SiO₂, DCM); ν_{\max} (cm⁻¹) 2943, 2866, 1684, 1578, 1564, 1471, 1449, 1426, 1404, 1383, 1326, 1197, 1165, 1002, 981, 920, 859, 726; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.91 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 3.98 (3H, s, OCH₃), 7.31 (1H, s, H-6'), 10.30 (1H, s, H-1); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.27 (s, OCH₃), 61.24 (s, OCH₃), 61.31 (s, OCH₃), 107.44 (s, CH), 115.69 (s, C), 128.80 (s, C), 148.72 (s, C), 150.79 (s, C), 153.03 (s, C), 191.15 (s, CHO).

1-(2-Bromo-3,4,5-trimethoxyphenyl)ethanol **253**³⁴⁶

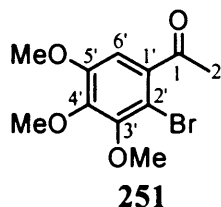


To a stirring solution of methylmagnesium chloride in tetrahydrofuran (8.8 cm³, 26.4 mmol, 3M) in anhydrous tetrahydrofuran (30 cm³) under argon at 0 °C, was slowly added 2-bromo-3,4,5-trimethoxybenzaldehyde **252** (4.53 g, 16.5 mmol) in anhydrous tetrahydrofuran (20 cm³). The reaction was then stirred overnight at room temperature and then poured into mixture of concentrated aqueous ammonium chloride solution (100 cm³) and ice, and then extracted with ethyl acetate (6 × 20 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo* to afford **253** as a yellow oil (4.74 g, 16.3 mmol, 99%); R_f 0.33 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3476, 2972, 2939, 1664, 1570, 1478, 1450, 1426, 1391, 1316, 1196, 1160, 1045, 999, 857, 796; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.44 (3H, d, *J* 6.4 Hz, H-2), 2.16 (1H, d, *J* 1.2 Hz, OH-1), 3.86 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 5.22 (1H, dq, *J* 1.2 Hz, 6.4 Hz,

Chapter 6 – Experimental

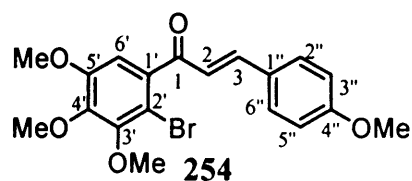
H-1), 6.98 (1H, s, H-6'); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 23.65 (s, CH_3), 56.12 (s, OCH_3), 61.03 (s, OCH_3), 61.11 (s, OCH_3), 69.23 (s, CHOH), 105.08 (s, CH), 107.64 (s, C), 140.48 (s, C), 142.04 (s, C), 150.41 (s, C), 153.10 (s, C); $m/z(\text{AP}^+)$ 273 ($\text{M}-\text{H}_2\text{O}$ 100%), 275 ($\text{M}-\text{H}_2\text{O}$, 90%).

1-(2-Bromo-3,4,5-trimethoxyphenyl)ethanone **251**^{226,346}



Pyridinium chlorochromate (1.75 g, 8.1 mmol) was added to a stirred solution of 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanol **253** (1.58 g, 5.4 mmol) and silica gel (9.0 g) in dichloromethane (20 cm^3). The reaction was then put under argon gas and stirred at room temperature under argon overnight. The solvent was then evaporated *in vacuo*. Column chromatography (SiO_2 , ethyl acetate:hexane 1:9) of the crude product supported on silica afforded **251** as a yellow solid (1.30 g, 4.5 mmol, 83%); m.p. 37-38 $^\circ\text{C}$; R_f 0.46 (SiO_2 , ethyl acetate:hexane 1:3); $\nu_{\text{max}}(\text{cm}^{-1})$ 2937, 2847, 1693, 1580, 1560, 1510, 1480, 1438, 1426, 1381, 1325, 1265, 1208, 1167, 1152, 992, 933, 856; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 2.64 (3H, s, H-2), 3.87 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 6.81 (H, s, H-6'); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 30.70 (s, CH_3), 56.26 (s, OCH_3), 61.11 (s, OCH_3), 61.20 (s, OCH_3), 106.50 (s, CBr), 107.84 (s, CH), 137.29 (s, C), 145.15 (s, C), 151.08 (s, C), 152.83 (s, C), 201.18 (s, CO); $m/z(\text{AP}^+)$ 289 (MH^+ , 100%), 291 (MH^+ , 95%).

(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **254**



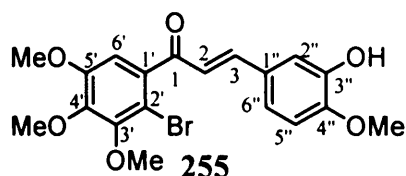
The chalcone **254** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (1.00 g, 3.5 mmol), *p*-anisaldehyde (0.40 cm^3 , 3.5 mmol) and aqueous sodium hydroxide solution (0.7 cm^3 , 10M) in methanol (3.0 cm^3). Recrystallization from methanol afforded **254** as white crystals (1.04 g, 2.6 mmol, 74%); m.p. 98 - 100 $^\circ\text{C}$; R_f 0.27 (SiO_2 , ethyl acetate:hexane 1:3); $\nu_{\text{max}}(\text{cm}^{-1})$ 2936, 2837, 1636, 1616, 1600, 1572, 1510, 1480, 1464, 1455, 1423, 1382, 1344, 1246, 1166, 1021, 1004, 987, 829; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.85 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.95 (3H, s,

Chapter 6 – Experimental

OCH₃), 6.78 (1H, s, H-6'), 6.92 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 6.98 (1H, d, *J* 16.0 Hz, H-2), 7.44 (1H, d, *J* 16.0 Hz, H-3), 7.53 (2H, d, *J* 8.8 Hz, H-2'' & H-6''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.47 (s, OCH₃), 56.29 (s, OCH₃), 61.19 (s, OCH₃), 61.23 (s, OCH₃), 106.46 (s, CBr), 107.95 (s, CH), 114.50 (s, CH), 123.85 (s, CH), 127.16 (s, C), 130.48 (s, CH), 137.09 (s, C), 144.52 (s, C), 146.15 (s, CH), 151.09 (s, C), 152.99 (s, C), 161.99 (s, C), 194.17 (s, CO); *m/z*(AP+) 407 (MH⁺, 100%), 409 (MH⁺, 95%); HRMS found [MH]⁺ 407.04991.

(*E*)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one

255



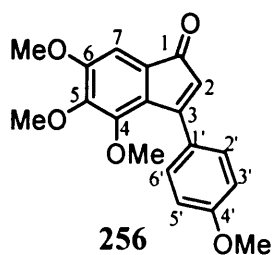
To a stirring mixture of Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (2.23 g, 7.7 mmol) and 3-hydroxy-4-methoxybenzaldehyde (1.18 g, 7.7 mmol) in methanol (13 cm³) was added an aqueous sodium hydroxide solution (3.1 cm³, 10M). The mixture was then stirred overnight at room temperature forming a red solution; the mixture was then acidified with aqueous hydrochloric acid (1 M) till the pH ~ 5 – 6, solution turns yellow. The mixture was then extracted with dichloromethane (3 × 30 cm³), the organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from methanol afforded **255** as cream crystals (2.51 g, 5.9 mmol, 77%); m.p. 157 - 159 °C; R_f 0.18 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 3459, 3004, 2945, 2846, 1640, 1605, 1564, 1508, 1482, 1459, 1441, 1385, 1343, 1259, 1217, 1158, 1012, 1002, 906, 810, 760; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.87 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 5.67 (1H, s, OH-3''), 6.78 (1H, s, H-6'), 6.86 (1H, d, *J* 8.4 Hz, H-5''), 6.96(1H, d, *J* 16.0 Hz, H-2), 7.08(1H, dd, *J* 2.0, 8.4 Hz, H-6''), 7.20(1H, d, *J* 2.0 Hz, H-2''), 7.40(1H, d, *J* 16.0 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.07 (s, OCH₃), 56.29 (s, OCH₃), 61.19 (s, OCH₃), 61.24 (s, OCH₃), 106.52 (s, CBr), 108.00 (s, CH), 110.57 (s, CH), 113.31 (s, CH), 122.85 (s, CH), 124.33 (s, CH), 128.09 (s, C), 137.04 (s, C), 144.58 (s, C), 145.95 (s, C), 146.13 (s, CH), 149.10 (s, C), 151.09 (s, C), 152.98 (s, C), 194.04 (s, CO); *m/z*(AP+) 423 (MH⁺, 100%), 425 (MH⁺, 100%); HRMS found [MH]⁺ 423.04471 for C₁₉H₂₀O₆Br, C₁₉H₂₀O₆Br requires [MH]⁺ 423.04378; CHN found C 53.56%, H 4.49% CHN requires C 53.92%, H 4.52%.

Chapter 6 – Experimental

Protocol D

The method adopted was that of Clark²²⁵ *et al.* To a mixture of the 2'-bromochoalcone (1 mmol) and potassium carbonate (0.350 g, 2.53 mmol) in anhydrous dimethyl formamide (8 cm³) in a Schlenk tube was added palladium(II) chloride (0.005 g, 0.028 mmol) and triphenylphosphine (0.019 g, 0.071 mmol). The Schlenk tube was then degassed by evacuation, and put under argon. The reaction was then heated to 110 °C for 30 minutes. The reaction was then cooled to room temperature and had water (5 cm³) added to it, and stirred overnight. The precipitate was then filtered off, washed with water (2 cm³). The wet solid was then dissolved in ethyl acetate (3 cm³), and was heated to 65 °C with an aqueous potassium thiocyanate solution (1.5 cm³, 2M) under argon for 1 hour. The mixture was then cooled to room temperature and extracted with ethyl acetate (2 × 3 cm³), the combined organic fractions were then washed with water (2 × 3 cm³) and brine (3 cm³) then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*.

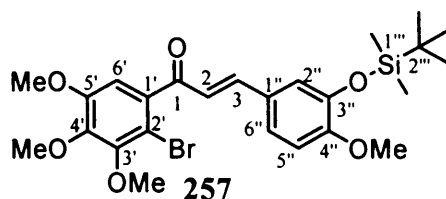
4,5,6-Trimethoxy-3-(4-methoxyphenyl)-1H-inden-1-one **256**



The indenone **256** was obtained following Protocol D. Using (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **254** (0.407 g, 1.00 mmol). Recrystallization from ethyl acetate and hexane afforded **256** as bright red crystals (0.287 g, 0.88 mmol, 88%); m.p. 107 – 109 °C; R_f 0.63 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2940, 1696, 1600, 1505, 1463, 1404, 1360, 1247, 1103, 1025, 970, 830; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.48 (3H, s, OCH₃), 3.79 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 5.73 (1H, s, H-2), 6.95 (2H, d, J 8.9 Hz, H-3' & H-5'), 7.01 (1H, s, H-7), 7.66 (2H, d, J 8.9 Hz, H-2' & H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.38 (s, OCH₃), 56.47 (s, OCH₃), 61.22 (s, OCH₃), 61.64 (s, OCH₃), 104.45 (s, CH), 113.269 (s, CH), 122.29 (s, CH), 126.49 (s, CH), 127.21 (s, C), 129.02 (s, C), 129.63 (s, CH), 146.83 (s, C), 149.17 (s, C), 154.54 (s, C), 161.18 (s, C), 164.55 (s, C), 195.93 (s, CO); m/z (AP⁺) 327 (MH⁺, 100%); HRMS found [MH]⁺ 327.12425 for C₁₉H₁₉O₅, C₁₉H₁₉O₅ requires [MH]⁺ 327.12270.

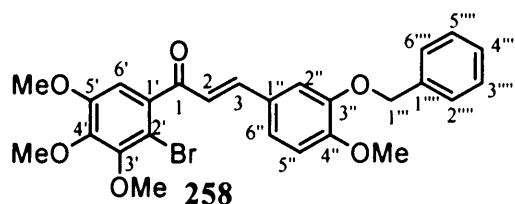
Chapter 6 – Experimental

(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(3-*tert*-butyldimethylsilanoxy-4-methoxyphenyl)prop-2-en-1-one **257**



A mixture of *(E)*-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one **255** (1.99 g, 4.7 mmol), imidazole (0.39 g, 5.7 mmol) and *tert*-butylchlorodimethylsilane (0.85 g, 5.6 mmol) in anhydrous dimethyl formamide (10 cm³) under argon was stirred at room temperature for 3 days. The mixture was then diluted with dichloromethane (15 cm³) and washed with water (2 × 15 cm³), the organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **257** a yellow oil which solidified as a cream solid (2.24 g, 4.2 mmol, 89%); m.p. 53 - 55 °C; R_f 0.58 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2937, 2854, 1640, 1618, 1510, 1481, 1439, 1427, 1382, 1337, 1263, 1106, 1012, 997, 985, 861, 838; δ ¹H NMR (400 MHz; CDCl₃; ppm) 0.16 (6H, s, Si(CH₃)₂), 1.00 (9H, s, SiC(CH₃)₃), 3.85 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 6.77 (1H, s, H-6'), 6.85 (1H, d, *J* 8.4 Hz, H-5'), 6.93 (1H, d, *J* 16.1 Hz, H-2), 7.10 (1H, d, *J* 2.1 Hz, H-2''), 7.14 (1H, dd, *J* 2.1, 8.4 Hz, H-6''), 7.37 (1H, d, *J* 16.1 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) -4.59 (s, Si(CH₃)₂), 18.47 (s, SiC(CH₃)₃), 25.70 (s, SiC(CH₃)₃), 55.48 (s, OCH₃), 56.30 (s, OCH₃), 61.18 (s, OCH₃), 61.23 (s, OCH₃), 106.49 (s, CBr), 107.97 (s, CH), 111.71 (s, CH), 120.10 (s, CH), 123.95 (s, CH), 124.11 (s, CH), 127.47 (s, C), 137.06 (s, C), 144.51 (s, C), 145.36 (s, C), 146.42 (s, CH), 151.08 (s, C), 152.97 (s, C), 153.80 (s, C), 194.20 (s, CO); *m/z*(AP+) 537 (MH⁺, 90%), 539 (MH⁺, 100%); HRMS found [MH]⁺ 537.13138 for C₂₅H₃₃BrO₆Si, C₂₅H₃₃BrO₆Si requires [MH]⁺ 537.13025; CHN found C 55.78%, H 6.16%, CHN requires C 55.86%, H 6.19%.

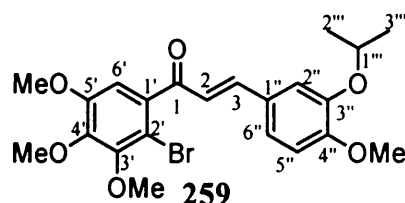
(E)-3-(3-(Benzyloxy)-4-methoxyphenyl)-1-(2-bromo-3,4,5-trimethoxyphenyl)prop-2-en-1-one **258**



Chapter 6 – Experimental

The chalcone **258** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (1.24 g, 4.3 mmol), 3-(benzyloxy)-4-methoxybenzaldehyde **262** (1.06 g, 4.4 mmol) and aqueous sodium hydroxide solution (0.9 cm³, 10M) in methanol (3.0 cm³). Recrystallization from methanol afforded **258** as cream crystals (1.85 g, 3.6 mmol, 84%); m.p. 111 - 112 °C; R_f 0.68 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max}(\text{cm}^{-1})$ 2935, 1629, 1615, 1593, 1510, 1480, 1444, 1426, 1386, 1342, 1256, 1230, 1157, 1132, 1101, 1008, 979, 805, 702; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.87 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 5.18 (2H, s, H-1'''), 6.77 (1H, s, H-6'), 6.90 (1H, d, *J* 15.6 Hz, H-2), 6.90 (1H, d, *J* 8.4 Hz, H-5''), 7.13 (1H, d, *J* 2.0 Hz, H-2''), 7.16 (1H, dd, *J* 2.0, 8.4 Hz, H-6'') 7.29 – 7.48 (6H, m, H-3 & H-2'''' – H-6'''''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.09 (s, OCH₃), 56.30 (s, OCH₃), 61.20 (s, OCH₃), 61.25 (s, OCH₃), 71.04 (s, CH₂), 106.50 (s, CBr), 107.99 (s, CH), 111.48 (s, CH), 112.67 (s, CH), 123.98 (s, CH), 127.28 (s, CH), 127.37 (s, CH), 128.11 (s, CH), 128.70 (s, CH), 136.55 (s, C), 137.02 (s, C), 144.56 (s, C), 146.28 (s, CH), 148.38 (s, C), 151.07 (s, C), 152.40 (s, C), 152.99 (s, C), 194.04 (s, CO) missing CH and C peak; *m/z*(AP⁺) 513 (MH⁺, 95%), 515 (MH⁺, 100%); HRMS found [MH]⁺ 513.0915 for C₂₆H₂₆O₆Br, C₂₆H₂₆O₆Br requires [MH]⁺ 513.0907; CHN found C 59.65%, H 4.78% CHN requires C 60.83%, H 4.91%.

(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(3-isopropoxy-4-methoxyphenyl)prop-2-en-1-one
259

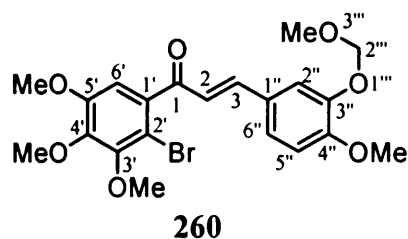


The chalcone **259** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (1.52 g, 5.3 mmol), 3-isopropoxy-4-methoxybenzaldehyde **263** (1.04 g, 5.4 mmol) and aqueous sodium hydroxide solution (0.9 cm³, 12M) in methanol (5.0 cm³). Recrystallization from methanol afforded **259** as fine light yellow needles (1.82 g, 3.9 mmol, 74%); m.p. 138 – 140 °C; R_f 0.83 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max}(\text{cm}^{-1})$ 3087, 2980, 2939, 2836, 1634, 1615, 1591, 1512, 1482, 1470, 1443, 1427, 1388, 1339, 1261, 1162, 1137, 999, 977; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.38 (6H, d, *J* 6.1 Hz, H-2''' & H-3'''), 3.87 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 4.57 (1H, sp, *J* 6.1 Hz, H-1'''), 6.77 (1H, s, H-6'), 6.88 (1H, d, *J* 8.3 Hz, H-5'), 6.94 (1H, d, *J* 16.0 Hz, H-2), 7.12 (1H, d, *J* 1.9 Hz, H-2''), 7.15 (1H, dd, *J* 1.9, 8.3 Hz, H-6''), 7.39 (1H, d, *J* 16.0 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 22.02 (s, CH(CH₃)₂), 56.04 (s, OCH₃),

Chapter 6 – Experimental

56.30 (s, OCH₃), 61.20 (s, OCH₃), 61.24 (s, OCH₃), 71.59 (s, CH(CH₃)₂), 106.46 (s, CBr), 107.95 (s, CH), 111.65 (s, CH), 114.51 (s, CH), 123.63 (s, CH), 123.96 (s, CH), 127.28 (s, C), 137.05 (s, C), 144.52 (s, C), 146.57 (s, CH), 147.52 (s, C), 151.07 (s, C), 153.00 (s, C), 153.14 (s, C), 194.19 (s, CO); *m/z*(API-ES+) 465 (MH⁺, 100%), 467 (MH⁺, 100%); HRMS found [MH]⁺ 465.0975 for C₂₂H₂₅BrO₆, C₂₂H₂₅BrO₆ requires [MH]⁺ 465.09073; CHN found C 56.20%, H 5.31%, CHN requires C 56.78%, H 5.42%.

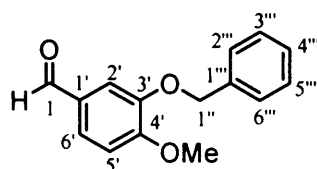
(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(4-methoxy-3-(methoxymethoxy)phenyl)prop-2-en-1-one **260**



The chalcone **260** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (0.44 g, 1.5 mmol), 4-methoxy-3-(methoxymethoxy)benzaldehyde **264** (0.30 g, 1.5 mmol) and aqueous sodium hydroxide solution (1.5 cm³, 2M) in methanol (6.0 cm³). Recrystallization from methanol afforded **260** as white crystals (0.48 g, 1.0 mmol, 69%); m.p. 99 – 100 °C; R_f 0.20 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3004, 2963, 2939, 2837, 1629, 1595, 1510, 1482, 1466, 1451, 1429, 1248, 1138, 1104, 979, 828; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.51 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 5.24 (2H, s, H-2'''), 6.75 (1H, s, H-6'), 6.90 (1H, d, *J* 8.5 Hz, H-5'), 6.95 (1H, d, *J* 16.0 Hz, H-2), 7.22 (1H, dd, *J* 2.0, 8.5 Hz, H-6''), 7.37 (1H, d, *J* 16.0 Hz, H-3), 7.38 (1H, d, *J* 2.0 Hz, H-2''); δ ¹³C NMR (100MHz; CDCl₃; ppm) 56.26 (s, OCH₃), 56.52 (s, OCH₃), 56.57 (s, OCH₃), 61.38 (s, OCH₃), 61.42 (s, OCH₃), 95.77 (s, OCH₂O), 106.64 (s, CBr), 108.15 (s, CH), 111.87 (s, CH), 115.82 (s, CH), 124.61 (s, CH), 124.69 (s, CH), 127.76 (s, C), 137.21 (s, C), 144.75 (s, C), 146.48 (s, CH), 147.06 (s, C), 151.31 (s, C), 152.54 (s, C), 153.22 (s, C), 194.42 (s, CO); *m/z*(API ES+) 467 (MH⁺, 100%), 469 (MH⁺, 100%); HRMS found [MH]⁺ 467.07094 for C₂₁H₂₃BrO₇, C₂₁H₂₃BrO₇ requires [MH]⁺ 467.06999.

Chapter 6 – Experimental

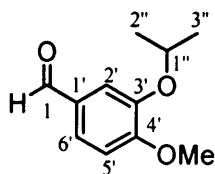
3-(Benzyloxy)-4-methoxybenzaldehyde **262**³⁴⁷



262

Benzyl bromide (2.3 cm³, 19.4 mmol) was added to a mixture of 3-hydroxy-4-methoxybenzaldehyde (2.86 g, 18.8 mmol) and potassium carbonate (10.5 g, 76.0 mmol) in anhydrous dimethyl formamide (100 cm³). The mixture was then put under argon and heated to 90 °C for 2 hours. The dimethyl formamide was then evaporated *in vacuo*. The crude product was then dissolved in chloroform (300 cm³) and filtered through celite, and washed with water (6 × 50 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*, to afford **262** as a pale yellow solid (4.49 g, 18.5 mmol, 99%); m.p. 53 – 55 °C; R_f 0.63 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2966, 2934, 2812, 2749, 2718, 1677, 1596, 1582, 1505, 1456, 1431, 1383, 1235, 1128, 1008, 805, 734, 697; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.88 (3H, s, OCH₃), 5.11 (2H, s, H-1''), 6.91 (1H, d, *J* 8.3 Hz, H-5'), 7.21-7.42 (7H, m, H-2', H-6', H-2''' – H-6'''), 9.74 (1H, s, H-1); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.22 (s, OCH₃), 70.82 (s, CH₂Ph), 110.75 (s, CH), 111.23 (s, CH), 126.98 (s, CH), 127.51 (s, CH), 128.16 (s, CH), 128.68 (s, CH), 129.96 (s, C), 136.27 (s, C), 148.68 (s, C), 155.03 (s, C), 190.92 (s, CHO).

3-Isopropoxy-4-methoxybenzaldehyde **263**²²⁷



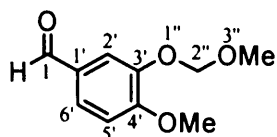
263

The method adopted was that of Ridley²²⁷ et al. A mixture 3-hydroxy-4-methoxybenzaldehyde (1.54 g, 10.1 mmol), isopropyl bromide (1.45 cm³, 15.4 mmol) and potassium carbonate (2.27 g, 16.5 mmol) in anhydrous dimethyl formamide (8 cm³) was stirred at room temperature for 96 hours. The mixture was then poured in water (24 cm³) and extracted with diethyl ether (3 × 20 cm³). The organic fraction was then washed with aqueous sodium hydroxide solution (3 × 30 cm³, 1M), dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:9) afforded **263** as a yellow brown oil (1.73 g, 8.9 mmol, 88%); R_f 0.23 (SiO₂, ethyl acetate:hexane 1:9); ν_{max}(cm⁻¹) 2977, 2935, 2841, 1685, 1582, 1506, 1464, 1432, 1238, 1128, 1109; δ ¹H NMR

Chapter 6 – Experimental

(400 MHz; CDCl₃; ppm) 1.40 (6H, d, *J* 6.1 Hz, H-2'' & H-3''), 3.94 (3H, s, OCH₃), 4.65 (1H, sp, *J* 6.1 Hz, H-1''), 6.98 (1H, d, *J* 8.2 Hz, H-5'), 7.41 (1H, d, *J* 1.8 Hz, H-2'), 7.44 (1H, dd, *J* 1.8, 8.2 Hz, H-6'), 9.84 (1H, s, H-1); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 21.91 (s, CH(CH₃)₂), 56.18 (s, OCH₃), 71.26 (s, CH(CH₃)₂), 110.87 (s, CH), 112.46 (s, CH), 126.55 (s, CH), 130.01 (s, C), 147.84 (s, C), 155.60 (s, C), 191.04 (s, CHO); *m/z*(AP⁺) 125 (C₇H₉O₂, 50%), 153 (C₉H₁₃O₂, 35%), 195 (MH⁺, 100%).

4-Methoxy-3-(methoxymethoxy)benzaldehyde **264**³⁴⁸

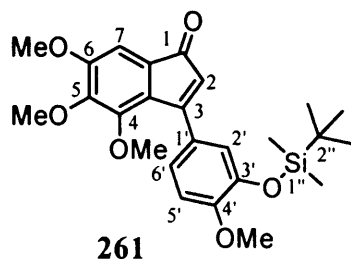


264

The method was adapted from that of Crombie²²⁸ *et al.* To a stirring solution of sodium hydride (0.32 g, 8.0 mmol, 60% dispersion in mineral oil) in anhydrous dimethyl formamide (4 cm³) at 0 °C under argon 3-hydroxy-4-methoxybenzaldehyde (1.00 g, 6.6 mmol) in anhydrous dimethyl formamide (4 cm³) was added slowly drop wise over 20 minutes. The reaction was then allowed to warm to room temperature and was stirred at room temperature for 30 minutes, before being cooled to 0 °C. Chloromethyl methyl ether (0.60 cm³, 7.9 mmol) in anhydrous dimethyl formamide (2 cm³) was then added drop wise over 30 minutes to the mixture at 0 °C. The mixture was then stirred for 30 minutes at 0 °C and then stirred overnight at room temperature. The reaction was then tipped into a mixture of water (10 cm³) and ice (10 g), and extracted with dichloromethane (4 × 15cm³). The organic fraction was washed with an aqueous sodium hydroxide solution (2 × 15 cm³, 1.0M) and water (2 × 15 cm³), it was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **264** as a cream solid (1.14 g, 5.8 mmol, 88%); m.p. 29 – 31 °C; R_f 0.69 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2952, 2839, 2767, 1678, 1595, 1584, 1507, 1464, 1434, 1231, 1151, 1125, 1073, 997, 916, 803, 730; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.53 (3H, s, OCH₃), 3.97 (3H, s, OCH₃), 5.29 (2H, s, H-2''), 7.01 (1H, d, *J* 8.4 Hz, H-5'), 7.55 (1H, dd, *J* 1.9, *J* 8.4 Hz, H-6'), 7.67 (1H, d, *J* 1.9 Hz, H-2'), 9.86 (1H, s, H-1); δ ¹³C NMR (100MHz; CDCl₃; ppm) 56.20 (s, OCH₃), 56.42 (s, OCH₃), 95.41 (s, OCH₂O), 111.13 (s, CH), 115.39 (s, CH), 126.78 (s, CH), 130.17 (s, C), 146.97 (s, C), 155.08 (s, C), 190.77 (s, CHO); *m/z*(API ES⁺) 197 (MH⁺, 100%).

Chapter 6 – Experimental

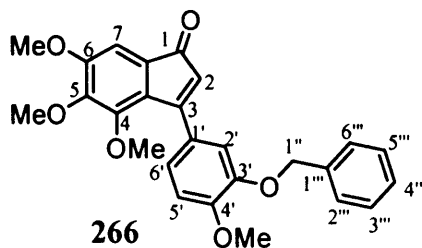
3-(3-*Tert*-butyldimethylsilaneoxy-4-methoxyphenyl)-4,5,6-trimethoxy-1*H*-inden-1-one 261



A mixture of (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3-*tert*-butyldimethylsilaneoxy-4-methoxyphenyl)prop-2-en-1-one **257** (0.104 g, 0.19 mmol), potassium carbonate (0.075 g, 0.54 mmol), palladium(II) chloride (0.001 g, 0.0056 mmol) and triphenylphosphine (0.005 g, 0.019 mmol) was dissolved in anhydrous dimethyl formamide (1.5 cm³) and sealed in a pressure-rated reaction vial (10 cm³). The reaction vial was then irradiated in a self-turning single-mode CEM Discovery™ Focused Synthesizer. The reaction was maintained at 160 °C (power: 150 W) for 15 minutes. The mixture was then rapidly cooled to room temperature, then had water (1.5 cm³) added to it and left at room temperature overnight. The precipitate was then filtered off, washed with water (1 cm³). The wet solid was then dissolved in ethyl acetate (3 cm³), and was heated to 65 °C with an aqueous potassium thiocyanate solution (1 cm³, 2M) under argon for 1 hour. The mixture was then cooled to room temperature and extracted with ethyl acetate (3 cm³), the combined organic fractions were then washed with water (2 × 1 cm³) and brine (2 × 1 cm³) then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to afford **261** as a red gum (0.072 g, 0.16 mmol, 81%); *R*_f 0.63 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\text{max}}(\text{cm}^{-1})$ 2930, 2855, 1703, 1597, 1508, 1465, 1415, 1338, 1262, 1106, 1026, 834; δ ¹H NMR (400 MHz; CDCl₃; ppm) 0.19 (6H, s, Si(CH₃)₂), 1.01 (9H, s, C(CH₃)₃), 3.47 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 5.71 (1H, s, H-2), 6.88 (1H, d, *J* 8.7 Hz, H-5'), 7.00 (1H, s, H-7), 7.21 (1H, d, *J* 2.1 Hz, H-2'), 7.31 (1H, dd, *J* 2.1, 8.7 Hz, H-6'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 0.00 (s, Si(CH₃)₂), 23.10 (s, C(CH₃)₃), 30.34 (s, C(CH₃)₃), 60.11 (s, OCH₃), 61.13 (s, OCH₃), 65.82 (s, OCH₃), 66.25 (s, OCH₃), 109.11 (s, CH), 115.65 (s, CH), 125.31 (s, CH), 126.64 (s, CH), 127.06 (s, CH), 131.50 (s, C), 131.97 (s, C), 133.68 (s, C), 148.95 (s, C), 151.55 (s, C), 153.93 (s, C), 157.41 (s, C), 157.41 (s, C), 159.15 (s, C), 169.15 (s, C), 200.47 (s, CO); *m/z*(API ES⁺) 457 (MH⁺, 100%); HRMS found [MH]⁺ 457.20440 for C₂₅H₃₃O₆Si, C₂₅H₃₃O₆Si requires [MH]⁺ 457.20409.

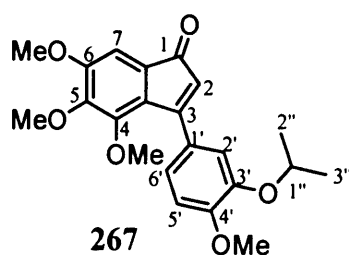
Chapter 6 – Experimental

3-(3-(Benzyloxy)-4-methoxyphenyl)-4,5,6-trimethoxy-1H-inden-1-one 266



The indenone **266** was obtained following Protocol D. Using (*E*)-3-(3-(benzyloxy)-4-methoxyphenyl)-1-(2-bromo-3,4,5-trimethoxyphenyl)prop-2-en-1-one **258** (0.54 g, 1.05 mmol). Recrystallization from ethyl acetate and hexane afforded **266** as a Red solid (0.28 g, 0.65 mmol, 62%); m.p. 107 – 109 °C; R_f 0.71 (SiO₂, ethyl acetate:hexane 1:1); ν_{\max} (cm⁻¹) 2943, 1696, 1595, 1545, 1505, 1465, 1439, 1418, 1404, 1357, 1275, 1255, 1135, 1023, 836, 756, 702; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.40 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 5.20 (2H, s, H-1''), 5.67 (1H, s, H-2), 6.94 (1H, d, J 8.2 Hz, H-5'), 7.00 (1H, s, H-7), 7.29 – 7.48 (7H, m, H-2', H-6' & H-2''' – H-6'''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.03 (s, OCH₃), 56.48 (s, OCH₃), 61.26 (s, OCH₃), 61.75 (s, OCH₃), 71.05 (s, CH₂), 104.48 (s, CH), 110.70 (s, CH), 113.78 (s, CH), 121.74 (s, CH), 122.47 (s, CH), 126.58 (s, C), 127.14 (s, C), 127.34 (s, CH), 127.99 (s, CH), 128.62 (s, CH), 129.00 (s, C), 136.88 (s, C), 146.84 (s, C), 147.33 (s, C), 149.17 (s, C), 151.37 (s, C), 154.51 (s, C), 164.50 (s, C), 195.81 (s, CO); m/z (AP⁺) 433 (MH⁺, 100%); HRMS found [MH]⁺ 433.1649 for C₂₆H₂₅O₆, C₂₆H₂₅O₆ requires [MH]⁺ 433.1646.

3-(3-Isopropoxy-4-methoxyphenyl)-4,5,6-trimethoxy-1H-inden-1-one 267

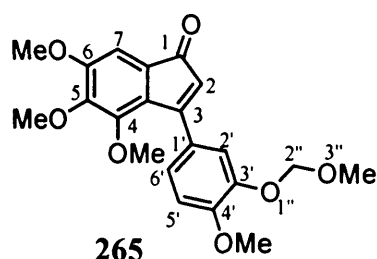


The indenone **267** was obtained following Protocol D. Using (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3-isopropoxy-4-methoxyphenyl)prop-2-en-1-one **259** (0.46 g, 1.00 mmol). Recrystallization from ethyl acetate afforded **267** as a red powder (0.18 g, 0.48 mmol, 48%); m.p. 111 – 112 °C; R_f 0.83 (SiO₂, ethyl acetate:hexane 1:1); ν_{\max} (cm⁻¹) 2992, 2975, 2937, 1692, 1611, 1590, 1538, 1507, 1464, 1438, 1404, 1368, 1271, 1109, 1020, 1001, 776; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.40 (6H, d, J 6.1 Hz, H-2'' & H-3''), 3.45 (3H, s, OCH₃), 3.90 (6H, s, OCH₃), 3.92 (3H, s, OCH₃), 4.57 (1H, sp, J 6.1 Hz, H-1''), 5.73 (1H, s, H-2), 6.91 (1H, d, J 9.0 Hz, H-5'), 7.01 (1H, s, H-7), 7.27 – 7.31 (2H, m, H-2' & H-6'); δ ¹³C

Chapter 6 – Experimental

(100MHz; CDCl₃; ppm) 22.10 (s, CH(CH₃)₂), 55.97 (s, OCH₃), 56.48 (s, OCH₃), 61.24 (s, OCH₃), 61.77 (s, OCH₃), 71.55 (s, CH(CH₃)₂), 104.51 (s, CH), 110.85 (s, CH), 115.34 (s, CH), 121.39 (s, CH), 122.46 (s, CH), 126.66 (s, C), 127.25 (s, C), 129.03 (s, C), 146.50 (s, C), 146.87 (s, C), 149.22 (s, C), 152.04 (s, C), 154.50 (s, C), 164.67 (s, C), 195.81 (s, CO); *m/z*(AP⁺) 385 (MH⁺, 100%); HRMS found [MH]⁺ 385.16672 for C₂₂H₂₅O₆, C₂₂H₂₅O₆ requires [MH]⁺ 385.16456.

4,5,6-Trimethoxy-3-(4-methoxy-3-(methoxymethoxy)phenyl)-1H-inden-1-one **265**



A mixture of (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(4-methoxy-3-(methoxymethoxy)phenyl)prop-2-en-1-one **260** (0.101 g, 0.22 mmol), potassium carbonate (0.081 g, 0.59 mmol), palladium(II) chloride (0.001 g, 0.0056 mmol) and triphenylphosphine (0.005 g, 0.019 mmol) was dissolved in anhydrous dimethyl formamide (1.5 cm³) and sealed in a pressure-rated reaction vial (10 cm³). The reaction vial was then irradiated in a self-turning single-mode CEM Discovery™ Focused Synthesizer. The reaction was maintained at 160 °C (power: 150 W) for 15 minutes. The mixture was then rapidly cooled to room temperature, then had water (1.5 cm³) added to it and left at room temperature overnight. The precipitate was then filtered off, washed with water (1 cm³). The wet solid was then dissolved in ethyl acetate (1 cm³), and was heated to 65 °C with an aqueous potassium thiocyanate solution (0.75 cm³, 2M) under argon for 1 hour. The mixture was then cooled to room temperature and extracted with ethyl acetate (3 cm³), the combined organic fractions were then washed with water (2 × 1 cm³) and brine (2 × 3 cm³) then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to afford **265** as a red solid (0.075 g, 0.19 mmol, 91%).

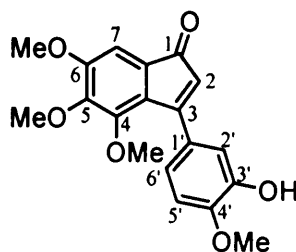
A mixture of (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(4-methoxy-3-(methoxymethoxy)phenyl)prop-2-en-1-one **260** (0.264 g, 0.56 mmol), potassium carbonate (0.211 g, 1.53 mmol), palladium(II) chloride (0.005 g, 0.019 mmol) and triphenylphosphine (0.030 g, 0.11 mmol) was dissolved in anhydrous dimethyl formamide (4.5 cm³) and sealed in a pressure-rated reaction vial (10 cm³). The reaction vial was then irradiated in a self-turning single-mode CEM Discovery™ Focused Synthesizer. The reaction was maintained at 160 °C (power: 150 W) for 15 minutes. The mixture was then rapidly cooled to room temperature, then had water (4.5 cm³) added to it and left at room temperature overnight. The precipitate

Chapter 6 – Experimental

was then filtered off, washed with water (3 cm³). The wet solid was then dissolved in ethyl acetate (3 cm³), and was heated to 65 °C with an aqueous potassium thiocyanate solution (2.5 cm³, 2M) under argon for 1 hour. The mixture was then cooled to room temperature and extracted with ethyl acetate (9 cm³), the combined organic fractions were then washed with water (2 × 3 cm³) and brine (2 × 9 cm³) then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:19 to 1:3) afforded **265** as a red solid (0.041 g, 0.11 mmol, 19%).

m.p. 109 – 111 °C; R_f 0.15 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2993, 2934, 2844, 1691, 1599, 1553, 1506, 1465, 1445, 1432, 1406, 1363, 1262, 1111, 1073, 918, 821; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.49 (3H, s, OCH₃), 3.53 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 5.27 (3H, s, H-2''), 5.75 (1H, s, H-2), 6.94 (1H, d, *J* 8.7 Hz, H-5'), 7.00 (1H, s, H-7), 7.38 (1H, dd, *J* 2.1, 8.7 Hz, H-6'), 7.54 (1H, d, *J* 2.1 Hz, H-2'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 56.18 (s, OCH₃), 56.49 (s, OCH₃), 56.70 (s, OCH₃), 61.38 (s, OCH₃), 61.84 (s, OCH₃), 95.94 (s, OCH₂O), 104.70 (s, CH), 111.04 (s, CH), 116.88 (s, CH), 122.87 (s, CH), 122.99 (s, CH), 127.11 (s, C), 127.41 (s, C), 129.16 (s, C), 145.94 (s, C), 147.11 (s, C), 149.42 (s, C), 151.72 (s, C), 154.76 (s, C), 164.52 (s, C), 196.01 (s, CO); *m/z*(API ES+) 387 (MH⁺, 100%); HRMS found [MH]⁺ 387.14368 for C₂₁H₂₃O₇, C₂₁H₂₃O₇ requires [MH]⁺ 387.14383.

3-(3-Hydroxy-4-methoxyphenyl)-4,5,6-trimethoxy-1H-inden-1-one **268**



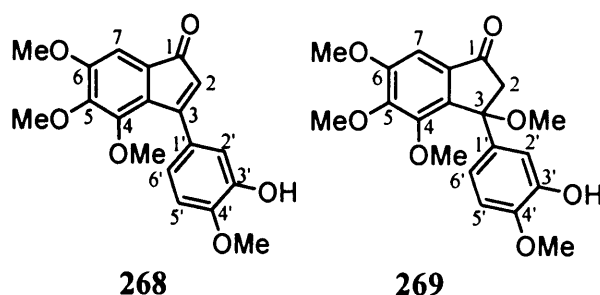
268

To a stirring mixture of 3-(3-*tert*-butyldimethylsilaneoxy-4-methoxyphenyl)-4,5,6-trimethoxy-1H-inden-1-one **261** (0.040 g, 0.088 mmol) in anhydrous tetrahydrofuran (1.4 cm³) at 0 °C under argon was added a solution of tetrabutylammonium fluoride in tetrahydrofuran (0.09 cm³, 0.09 mmol, 1 M). The reaction was stirred at 0 °C for 30 minutes, the solvent was evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:19 to 1:3) afforded **268** as a red solid (0.020 g, 0.058 mmol, 66%); m.p. 131 – 133 °C; R_f 0.30 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3319, 2931, 1683, 1603, 1556, 1501, 1454, 1405, 1363, 1285, 1236, 1023, 847, 800; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.52 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 5.67 (1H, br. s, OH-3'),

Chapter 6 – Experimental

5.73 (1H, s, H-2), 6.83 (1H, d, J 8.0 Hz, H-5'), 7.00 (1H, s, H-7), 7.24 – 7.28 (2H, m, H-2' & H-6'); $\delta^{13}\text{C}$ NMR (100MHz; CDCl_3 ; ppm) 56.22 (s, OCH_3), 56.70 (s, OCH_3), 61.39 (s, OCH_3), 61.82 (s, OCH_3), 104.69 (s, CH), 110.00 (s, CH), 114.51 (s, CH), 120.68 (s, CH), 122.94 (s, CH), 127.41 (s, C), 127.72 (s, C), 129.16 (s, C), 145.17 (s, C), 147.12 (s, C), 148.35 (s, C), 149.44 (s, C), 154.77 (s, C), 164.68 (s, C), 196.08 (s, CO); m/z (API ES+) 343 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 343.11840 for $\text{C}_{19}\text{H}_{19}\text{O}_6$, $\text{C}_{19}\text{H}_{19}\text{O}_6$ requires $[\text{MH}]^+$ 343.11761.

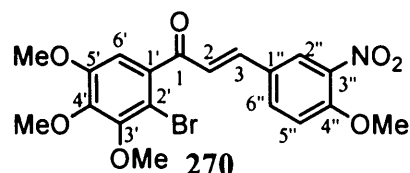
3-(3-Hydroxy-4-methoxyphenyl)-4,5,6-trimethoxy-1H-inden-1-one **268** & *2,3-Dihydro-3-(3-hydroxy-4-methoxyphenyl)-3,4,5,6-tetramethoxyinden-1-one* **269**



The method adopted was that of Wang²³⁰ *et al.* To a stirring solution of 4,5,6-trimethoxy-3-(4-methoxy-3-(methoxymethoxy)phenyl)-1H-inden-1-one **265** (0.040 g, 0.10 mmol) in methanol (3 cm^3) under argon was added aqueous hydrochloric acid (0.6 cm^3 , 3M). The reaction was then heated to reflux at 75 °C for 45 minutes. The reaction was cooled to room temperature and tipped into water (3 cm^3), it was then extracted with dichloromethane (3 \times 10 cm^3). The organic fraction was then washed dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO_2 , ethyl acetate:hexane 1:19 to 2:3) afforded **268** as a red solid (0.004 g, 0.01 mmol, 12%) and then afforded **269** as a yellow gum (0.011 g, 0.03 mmol, 30%); R_f 0.12 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 3412, 2938, 2838, 1708, 1595, 1509, 1467, 1416, 1334, 1019, 762; $\delta^1\text{H}$ NMR (400 MHz; CDCl_3 ; ppm) 2.92 (1H, d, J 19.2 Hz, H-2), 3.17 (1H, d, J 19.2 Hz, H-2), 3.19 (3H, s, OCH_3), 3.48 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 5.55 (1H, s, OH-3'), 6.80 (1H, d, J 8.5 Hz, H-5'), 6.88 (1H, dd, J 2.0, 8.5 Hz, H-6'), 6.90 (1H, d, J 2.0 Hz, H-2'), 7.08 (1H, s, H-7); $\delta^{13}\text{C}$ NMR (100 MHz; CDCl_3 ; ppm) 51.73 (s, CH_2), 51.86 (s, OCH_3), 56.17 (s, OCH_3), 56.51 (s, OCH_3), 60.53 (s, OCH_3), 61.13 (s, OCH_3), 83.01 (s, C), 100.18 (s, CH), 110.36 (s, CH), 112.14 (s, CH), 116.89 (s, CH), 133.31 (s, C), 138.43 (s, C), 141.22 (s, C), 145.52 (s, C), 145.60 (s, C), 149.32 (s, C), 151.03 (s, C), 156.36 (s, C), 202.7 (s, CO) m/z (API-ES -) 373 ($[\text{M}]^-$, 100%); HRMS found $[\text{MH}]^+$ 375.14396 for $\text{C}_{20}\text{H}_{23}\text{O}_7$, $\text{C}_{20}\text{H}_{23}\text{O}_7$ requires $[\text{MH}]^+$ 375.14383.

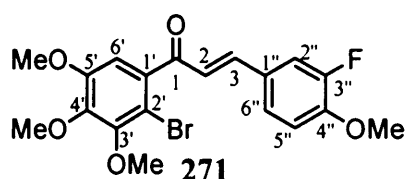
Chapter 6 – Experimental

(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(4-methoxy-3-nitrophenyl)prop-2-en-1-one **270**



The chalcone **270** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (1.04 g, 3.6 mmol), 4-methoxy-3-nitrobenzaldehyde **276** (0.65 g, 3.6 mmol) and aqueous sodium hydroxide solution (0.8 cm³, 10M) in methanol (6.0 cm³). Recrystallization from methanol afforded **270** as yellow fluffy crystals (1.14 g, 2.5 mmol, 70%); m.p. 95 – 110 °C; R_f 0.24 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2942, 2845, 1662, 1618, 1600, 1579, 1562, 1532, 1498, 1483, 1459, 1445, 1427, 1415, 1343, 1276, 1001, 818; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.88 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 4.02 (3H, s, OCH₃), 6.80 (1H, s, H-6'), 7.48 (1H, d, *J* 16.2 Hz, H-2), 7.14 (1H, d, *J* 8.7 Hz, H-5''), 7.44 (1H, d, *J* 16.2 Hz, H-3), 7.76 (1H, dd, *J* 2.1, 8.7 Hz, H-6''), 8.06 (1H, d, *J* 2.1 Hz, H-2''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.33 (s, OCH₃), 56.85 (s, OCH₃), 61.23 (s, OCH₃), 61.25 (s, OCH₃), 106.60 (s, CBr), 108.07 (s, CH), 113.97 (s, CH), 125.56 (s, CH), 126.32 (s, CH), 127.21 (s, C), 133.99 (s, CH), 136.47 (s, C), 139.85 (s, C), 142.39 (s, CH), 144.96 (s, C), 151.13 (s, C), 153.13 (s, C), 154.41 (s, C), 193.29 (s, CO); *m/z*(AP+) 452 (MH⁺, 100%), 454 (MH⁺, 100%); HRMS found [MH]⁺ 452.03438 for C₁₉H₁₉O₇NBr, C₁₉H₁₉O₇NBr requires [MH]⁺ 452.03394; CHN found C 50.28%, H 3.96%, N 2.95% CHN requires C 50.46%, H 4.01%, N 3.10%.

(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(3-fluoro-4-methoxyphenyl)prop-2-en-1-one **271**



The chalcone **271** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (0.93 g, 3.2 mmol), 3-fluoro-4-methoxybenzaldehyde (0.53 g, 3.4 mmol) and aqueous sodium hydroxide solution (0.8 cm³, 10M) in methanol (3.0 cm³). Recrystallization from methanol afforded **271** as white crystals (1.15 g, 2.7 mmol, 84%); m.p. 116 – 117 °C; R_f 0.71 (SiO₂, ethyl acetate:hexane 1:1); ν_{\max} (cm⁻¹) 2934, 1642, 1611, 1559, 1511, 1482, 1457, 1437, 1425, 1383, 1345, 1266, 1129, 1019, 1008, 986, 804; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.89 (3H, s, OCH₃), 3.95 (6H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.80 (1H, s, H-6'), 6.97 (1H, t, *J* 8.5 Hz, H-5''), 6.99 (1H, d, *J* 16.1 Hz, H-2), 7.29-7.33 (1H,

Chapter 6 – Experimental

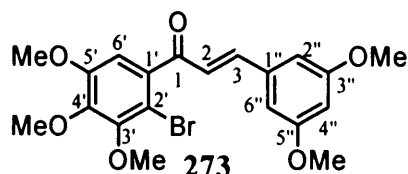
br d, J 9.0 Hz, H-6''), 7.36 (1H, dd, J 2.0 Hz, J 12.0 Hz, H-2''), 7.41 (1H, d, J 15.5 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 56.28 (s, OCH_3), 56.30 (s, OCH_3), 61.20 (s, OCH_3), 61.24 (s, OCH_3), 106.52 (s, CBr), 108.00 (s, CH), 113.136 (d, J 2.0 Hz, CH), 115.09 (d, J 18.1 Hz, CH), 124.94 (s, CH), 126.22 (d, J 3.7 Hz, CH), 127.70 (d, J 6.6 Hz, C), 136.82 (s, C), 144.57 (d, J 2.9 Hz, CH), 144.71 (s, C), 150.03 (d, J 11.0 Hz, C), 151.09 (s, C), 152.39 (d, J 247.2 Hz, CF), 153.62 (s, C), 193.72 (s, CO); $m/z(\text{AP}^+)$ 425 (MH^+ , 100%), 427 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 425.0388 for $\text{C}_{19}\text{H}_{19}\text{O}_5\text{BrF}$, $\text{C}_{19}\text{H}_{19}\text{O}_5\text{BrF}$ requires $[\text{MH}]^+$ 425.0394; CHN found C 53.70%, H 4.21% CHN requires C 53.66%, H 4.27%.

(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-phenylprop-2-en-1-one **272**



The chalcone **272** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (1.15 g, 4.0 mmol), benzaldehyde (0.4 cm³, 3.9 mmol) and aqueous sodium hydroxide solution (0.8 cm³, 10M) in methanol (3.0 cm³). Recrystallization from methanol afforded **272** as white crystals (0.86 g, 2.7 mmol, 71%); m.p. 79 – 80 °C; R_f 0.77 (SiO_2 , ethyl acetate:hexane 1:1); $\nu_{\text{max}}(\text{cm}^{-1})$ 2939, 1643, 1623, 1564, 1479, 1464, 1447, 1426, 1385, 1341, 1099, 1005, 907, 778, 689; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.80 (1H, s, H-6'), 7.12 (1H, d, J 16.0 Hz, H-2), 7.38 – 7.45 (3H, m, H-3'' – H-5''), 7.49 (1H, d, J 16.0 Hz, H-3), 7.56 – 7.60 (2H, m, H-2'' & H-6''); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 56.30 (s, OCH_3), 61.20 (s, OCH_3), 61.25 (s, OCH_3), 106.58 (s, CBr), 108.05 (s CH), 126.03 (s CH), 128.64 (s CH), 129.04 (s CH), 130.93 (s CH), 134.47 (s, C), 136.79 (s, C), 144.74 (s, C), 146.00 (s CH), 151.12 (s, C), 153.04 (s, C), 194.07 (s, CO); $m/z(\text{AP}^+)$ 377 (MH^+ , 100%), 379 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 377.380 for $\text{C}_{18}\text{H}_{18}\text{O}_4\text{Br}$, $\text{C}_{18}\text{H}_{18}\text{O}_4\text{Br}$ requires $[\text{MH}]^+$ 377.0383; CHN found C 57.35%, H 4.49%, CHN requires C 57.31%, H 4.54%.

(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one **273**

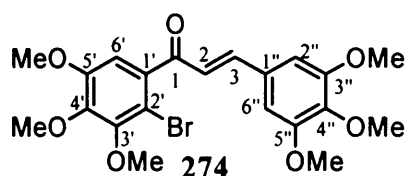


The chalcone **273** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (1.04 g, 3.6 mmol), 3,5-dimethoxybenzaldehyde (0.60 g, 3.6

Chapter 6 – Experimental

mmol) and aqueous sodium hydroxide solution (0.8 cm³, 10M) in methanol (3.0 cm³). Recrystallization from methanol afforded **273** as white crystals (1.15 g, 2.6 mmol, 73%); m.p. 81 – 83 °C; R_f 0.74 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2938, 2833, 1664, 1594, 1562, 1479, 1467, 1448, 1428, 1337, 1279, 1199, 1164, 1055, 1003, 977, 922, 826, 669; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.75 (6H, s, OCH₃), 3.81 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 6.45(1H, t, *J* 2.4 Hz, H-4''), 6.64 (2H, d, *J* 2.4 Hz, H-2'' & H-6''), 6.72 (1H, s, H-6'), 7.00 (1H, d, *J* 16.0 Hz, H-2), 7.33 (1H, d, *J* 16.0 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.50 (s, OCH₃), 56.31 (s, OCH₃), 61.21 (s, OCH₃), 61.25 (s, OCH₃), 103.19 (s, CH), 106.39 (s, CH), 106.59 (s, CBr), 108.06 (s, CH), 126.50 (s, CH), 136.32 (s, C), 136.70 (s, C), 144.75 (s, C), 146.01 (s, CH), 151.09 (s, C), 153.04 (s, C), 161.06 (s, C), 194.05 (s, CO); *m/z*(AP+) 437 (MH⁺, 100%), 439 (MH⁺, 95%); HRMS found [MH]⁺ 437.0598 for C₂₀H₂₂O₆Br, C₂₀H₂₂O₆Br requires [MH]⁺ 437.0594; CHN found C 54.93%, H 4.78% CHN requires C 54.93%, H 4.84%.

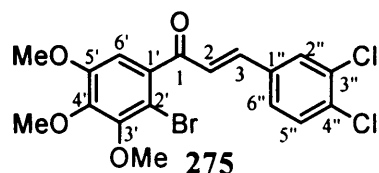
(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **274**



The chalcone **274** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (1.02 g, 3.5 mmol), 3,4,5-trimethoxybenzaldehyde (0.71 g, 3.6 mmol) and aqueous sodium hydroxide solution (0.8 cm³, 10M) in methanol (3.0 cm³). Recrystallization from methanol afforded **274** as white crystals (1.30 g, 2.8 mmol, 79%); m.p. 114 – 115 °C; R_f 0.61 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 3005, 2967, 2943, 1636, 1619, 1578, 1501, 1483, 1466, 1448, 1420, 1388, 1334, 1253, 1122, 1002, 841; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.90 (3H, s, OCH₃), 3.90 (6H, s, OCH₃), 3.91 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 3.97 (3H, s, OCH₃), 6.80 (H, s, H-6'), 6.81 (2H, s, H-2'' & H-6''), 7.01 (2H, d, *J* 15.9 Hz, H-2), 7.38 (2H, d, *J* 15.9 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.20 (s, OCH₃), 56.32 (s, OCH₃), 61.04 (s, OCH₃), 61.22 (s, OCH₃), 61.25 (s, OCH₃), 105.68 (s, CH), 106.47 (s, CBr), 107.97 (s, CH), 125.44 (s, CH), 129.89 (s, C), 136.81 (s, C), 140.62 (s, C), 144.64 (s, C), 146.28 (s, CH), 151.06 (s, C), 153.07 (s, C), 153.49 (s, C), 194.08 (s, CO); *m/z*(AP+) 467 (MH⁺, 100%), 469 (MH⁺, 90%); HRMS found [MH]⁺ 467.0705 for C₂₁H₂₄O₇Br, C₂₁H₂₄O₇Br requires [MH]⁺ 467.0700; CHN found C 53.89%, H 4.89% CHN requires C 53.97%, H 4.96%.

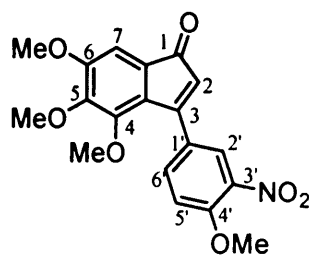
Chapter 6 – Experimental

(E)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one **275**



The chalcone **275** was obtained following Protocol A. Using 1-(2-bromo-3,4,5-trimethoxyphenyl)ethanone **251** (1.08 g, 3.7 mmol), 3,4-dichlorobenzaldehyde (0.66 g, 3.7 mmol) and aqueous sodium hydroxide solution (0.8 cm³, 10M) in methanol (3.0 cm³). Recrystallization from methanol afforded **275** as white crystals (1.14 g, 2.6 mmol, 71%); m.p. 122 – 124 °C; R_f 0.87 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 3055, 2940, 1643, 1622, 1587, 1552, 1477, 1466, 1426, 1386, 1261, 1202, 1160, 1099, 994, 908, 855, 822; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.90 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 3.97 (3H, s, OCH₃), 6.82 (1H, s, H-6'), 7.13 (1H, d, *J* 16.0 Hz, H-2), 7.39 - 7.46 (2H, m, H-3 & H-6''), 7.50 (1H, d, *J* 8.4 Hz, H-5''), 7.67 (1H, d, *J* 2.0 Hz, H-2''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.32 (s, OCH₃), 61.23 (s, OCH₃), 61.26 (s, OCH₃), 106.68 (s, CBr), 108.15 (s, CH), 127.40 (s, CH), 127.46 (s, CH), 130.06 (s, CH), 131.02 (s, CH), 133.39 (s, C), 134.57 (s, C), 134.72 (s, C), 136.39 (s, C), 142.42 (s, CH), 145.04, (s, C), 151.12 (s, C), 153.12 (s, C), 193.27 (s, CO); *m/z*(AP⁺) 445 (MH⁺, 50%), 447 (MH⁺, 100%), 449 (MH⁺, 50%); HRMS found [MH]⁺ 444.9606 for C₁₈H₁₅O₄Cl₂Br, C₁₈H₁₅O₄Cl₂Br requires [MH]⁺ 444.9605; CHN found C 48.42%, H 3.32% CHN requires C 48.46%, H 3.39%.

4,5,6-Trimethoxy-3-(4-methoxy-3-nitrophenyl)-1*H*-inden-1-one **282**

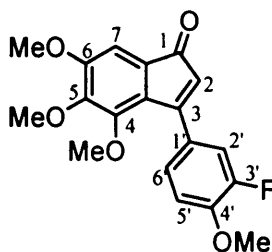


In 5 individual pressure-rated reaction vial (10 cm³), a mixture of *(E)*-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(4-methoxy-3-nitrophenyl)prop-2-en-1-one **270** (0.100 g, 0.22 mmol), potassium carbonate (0.076 g, 0.55 mmol), palladium(II) chloride (0.001 g, 0.0056 mmol) and triphenylphosphine (0.005 g, 0.019 mmol) was dissolved in anhydrous dimethyl formamide (1.7 cm³) and sealed. Each reaction vial was then irradiated in a self-turning single-mode CEM Discovery™ Focused Synthesizer. The reaction was maintained at 160 °C (power: 150 W) for 15 minutes. The vials were then rapidly cooled to room temperature,

Chapter 6 – Experimental

then had water (1.8 cm³) added to each vial and left at room temperature overnight. The precipitate from each vial was then filtered off, washed with water (5 × 3 cm³). The combined wet solid was then dissolved in ethyl acetate (5 cm³), and was heated to 65 °C with an aqueous potassium thiocyanate solution (1.5 cm³, 2M) under argon for 1 hour. The mixture was then cooled to room temperature and extracted with dichloromethane (30 cm³), the combined organic fractions were then washed with water (10 cm³) and brine (2 × 15 cm³) then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Recrystallization from ethyl acetate afforded **282** as dark red crystals (0.127 g, 0.34 mmol, 31%); m.p. 155 – 159 °C; R_f 0.34 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2958 (m), 1694 (s), 1617 (s), 1566 (m), 1526 (s), 1497 (s), 1466 (s), 1411 (s), 1356 (s), 1283 (s), 1113 (s), 1027 (s); δ¹H NMR (400 MHz; CDCl₃; ppm) 3.85 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.04 (3H, s, OCH₃), 5.79 (1H, s, H-2), 7.02 (1H, s, H-7), 7.14 (1H, d, *J* 8.4 Hz, H-5'), 7.85 (1H, dd, *J* 2.3, 8.4 Hz, H-6'), 8.23 (1H, d, *J* 2.3 Hz, H-2''); δ¹³C NMR (100MHz; CDCl₃; ppm) 56.73 (s, OCH₃), 56.94 (s, OCH₃), 61.36 (s, OCH₃), 61.60 (s, OCH₃), 105.02 (s, CH), 113.00 (s, CH), 123.90 (s, CH), 125.76 (s, CH), 126.61 (s, C), 126.79 (s, C), 128.47 (s, C), 133.91 (s, CH), 139.31 (s, C), 147.14 (s, C), 149.19 (s, C), 154.03 (s, C), 155.18 (s, C), 161.74 (s, C), 195.46 (s, CO); *m/z*(API ES⁺) 372 (MH⁺, 100%); HRMS found [MH]⁺ 372.10805 for C₁₉H₁₈NO₇, C₁₉H₁₈NO₇ requires [MH]⁺ 372.10778.

3-(3-Fluoro-4-methoxyphenyl)-4,5,6-trimethoxy-1H-inden-1-one **277**



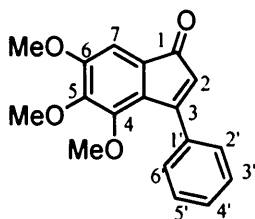
277

The indenone **277** was obtained following Protocol D. Using (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3-fluoro-4-methoxyphenyl)prop-2-en-1-one **271** (0.43 g, 1.01 mmol). Recrystallization from ethyl acetate and hexane afforded **277** as fine orange crystals (0.24 g, 0.71 mmol, 70%); m.p. 148-149 °C; R_f 0.47 (SiO₂, ethyl acetate:hexane 1:1); ν_{max} (cm⁻¹) 2947, 2903, 2850, 1699, 1598, 1514, 1463, 1410, 1376, 1343, 1317; δ¹H (400 MHz; CDCl₃; ppm), 3.45 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 5.68 (1H, s, H-2), 6.91-6.97 (2H, m, H-7 & H-5''), 7.37-7.44 (2H, m, H-2'' & H-6''); δ¹³C (100MHz; CDCl₃; ppm), 56.24 (s, OCH₃), 56.49 (s, OCH₃), 61.21 (s, OCH₃), 61.56 (s, OCH₃), 104.59 (s, CH), 112.29 (d, *J* 2.0 Hz, CH), 116.04 (d, *J* 19.7 Hz, CH), 123.01 (s, CH),

Chapter 6 – Experimental

124.42 (d, J 3.3 Hz, CH), 126.86 (s, C), 126.94 (d, J 6.9 Hz, C), 128.68 (s, C), 146.89 (s, C), 149.09 (s, C), 149.11 (d, J 10.8 Hz, C), 151.48 (d, J 245.7 Hz, CF), 154.68 (s, C), 163.18 (s, C), 195.64 (s, CO); m/z (AP+) 345 (MH^+ , 100); HRMS found $[MH]^+$ 345.1136 for $C_{19}H_{17}O_5F$, $C_{19}H_{17}O_5F$ requires $[MH]^+$ 345.1133.

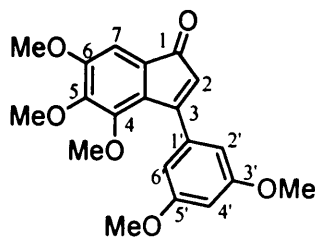
4,5,6-Trimethoxy-3-phenyl-1H-inden-1-one 278



278

The indenone **278** was obtained following Protocol D. Using (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-phenylprop-2-en-1-one **272** (0.31 g, 1.00 mmol). Recrystallization from ethyl acetate and hexane afforded **278** as light red crystals (0.15 g, 0.66 mmol, 65%); m.p. 148 – 149 °C; R_f 0.76 (SiO_2 , ethyl acetate:hexane 1:1); ν_{max} (cm^{-1}) 3073, 2935, 1692, 1603, 1553, 1487, 1460, 1443, 1432, 1402, 1364, 1299, 1099, 1020, 853, 770; δ 1H NMR (400 MHz; $CDCl_3$; ppm) 3.43 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 5.76 (1H, s, H-2), 7.01 (1H, s, H-7), 7.40 – 7.45 (3H, m, H-3' – H-5'), 7.61 – 7.65 (2H, m, H-2' & H-6'); δ ^{13}C NMR (100 MHz; $CDCl_3$; ppm) 56.49 (s, OCH_3), 61.19 (s, OCH_3), 61.51 (s, OCH_3), 104.62 (s, CH), 123.56 (s, CH), 127.43 (s, C), 127.68 (s, CH), 127.88 (s, CH), 128.53 (s, CH), 129.87 (s, C), 134.29 (s, CH), 134.29 (s, C), 146.98 (s, C), 149.20 (s, C), 154.62 (s, C), 164.58 (s, C), 195.98 (s, CO); m/z (AP+) 297 (MH^+ , 100%); HRMS found $[MH]^+$ 297.11257 for $C_{18}H_{17}O_4$, $C_{18}H_{17}O_4$ requires $[MH]^+$ 297.11214.

4,5,6-Trimethoxy-3-(3,5-dimethoxyphenyl)-1H-inden-1-one 279



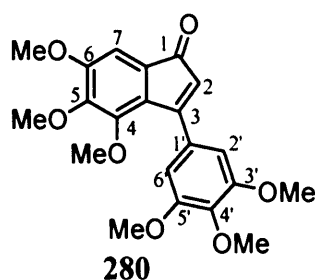
279

The indenone **279** was obtained following Protocol D. Using (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one **273** (0.44 g, 1.01 mmol). Recrystallization from ethyl acetate and hexane afforded **279** as dark red crystals (0.23 g, 0.64 mmol, 63%); m.p. 113 - 114 °C; R_f 0.20 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1})

Chapter 6 – Experimental

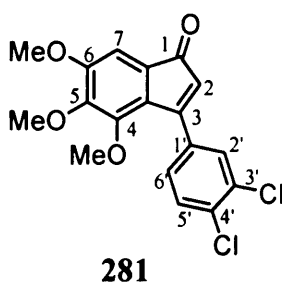
2943, 2842, 1691, 1591, 1551, 1462, 1450, 1423, 1404, 1361, 1307, 1293, 1210, 1156, 1105, 1025, 836, 822; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.50 (3H, s, OCH_3), 3.84 (6H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 5.77 (1H, s, H-2), 6.55 (1H, t, J 3.6 Hz, H-4'), 6.80 (2H, d, J 3.6 Hz, H-2' & H-6'), 7.01 (1H, s, H-7); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 55.52 (s, OCH_3), 56.49 (s, OCH_3), 61.20 (s, OCH_3), 61.77 (s, OCH_3), 102.00 (s, CH), 104.62 (s, CH), 105.64 (s, CH), 123.68 (s, CH), 127.35 (s, C), 128.44 (s, C), 136.15 (s, C), 146.99 (s, C), 149.26 (s, C), 154.59 (s, C), 160.26 (s, C), 164.45 (s, C), 195.95 (s, CO); $m/z(\text{AP}^+)$ 357 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 357.1329 for $\text{C}_{20}\text{H}_{21}\text{O}_6$, $\text{C}_{20}\text{H}_{21}\text{O}_6$ requires $[\text{MH}]^+$ 357.1333.

4,5,6-Trimethoxy-3-(3,4,5-trimethoxyphenyl)-1H-inden-1-one **280**



The indenone **280** was obtained following Protocol D. Using (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **274** (0.49 g, 1.04 mmol). Recrystallization from ethyl acetate and hexane afforded **280** as fine red crystals (0.22 g, 0.56 mmol, 54%); m.p. 117 – 118 °C; R_f 0.45 (SiO_2 , ethyl acetate:hexane 1:1); $\nu_{\text{max}}(\text{cm}^{-1})$ 2936, 2826, 1698, 1603, 1580, 1554, 1500, 1456, 1413, 1366, 1331, 1234, 1102, 1006, 807; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.42 (3H, s, OCH_3), 3.84 (6H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 5.72 (1H, s, CH), 6.89 (2H, s, H-Ar), 6.95 (1H, s, H-Ar); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 56.25 (s, OCH_3), 56.49 (s, OCH_3), 61.06 (s, OCH_3), 61.24 (s, OCH_3), 61.92 (s, OCH_3), 104.66 (s, CH), 105.20 (s, CH), 123.14 (s, CH), 127.11 (s, C), 128.71 (s, C), 129.48 (s, C), 139.53 (s, C), 146.95 (s, C), 149.20 (s, C), 152.66 (s, C), 154.58 (s, C), 164.57 (s, C), 195.73 (s, CO); $m/z(\text{AP}^+)$ 386 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 387.1439 for $\text{C}_{21}\text{H}_{22}\text{O}_7$, $\text{C}_{21}\text{H}_{22}\text{O}_7$ requires $[\text{MH}]^+$ 387.1438.

3-(3,4-Dichlorophenyl)-4,5,6-trimethoxy-1H-inden-1-one **281**



Chapter 6 – Experimental

The indenone **281** was obtained following Protocol D. Using (*E*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one **275** (0.45 g, 1.00 mmol). Recrystallization from ethyl acetate and hexane afforded **281** as peach coloured crystals (0.12 g, 0.32 mmol, 32%); m.p. 147 – 149 °C; R_f 0.61 (SiO₂, ethyl acetate:hexane 1:1); $\nu_{\max.}(\text{cm}^{-1})$ 3074, 2946, 1705, 1606, 1546, 1463, 1409, 1361, 1304, 1028, 973, 817, 664; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.55 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 5.77 (1H, s, H-2), 7.02 (1H, s, H-7), 7.48 (1H, dd, *J* 1.5, 8.4 Hz, H-6'), 7.51 (1H, d, *J* 8.4 Hz, H-5'), 7.75 (1H, d, *J* 1.5 Hz, H-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.90 (s, OCH₃), 61.54 (s, OCH₃), 61.82 (s, OCH₃), 105.22 (s, CH), 124.59 (s, CH), 126.63 (s, C), 127.55 (s, CH), 128.44 (s, C), 130.04 (s, CH), 130.31 (s, CH), 132.56 (s, C), 134.17 (s, C), 134.56 (s, C), 147.35 (s, C), 149.39 (s, C), 155.29 (s, C), 162.07 (s, C), 195.73 (s, CO); *m/z*(AP⁺) 365 (MH⁺, 100%), 367 (MH⁺, 70%); HRMS found [MH]⁺ 365.03436 for C₁₈H₁₅Cl₂O₄, C₁₈H₁₅Cl₂O₄ requires [MH]⁺ 365.03419.

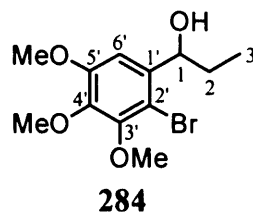
4-Methoxy-3-nitrobenzaldehyde **276**³⁴⁹



To stirred mixture of concentrated nitric acid (5.3 cm³, 83.0 mmol) and concentrated sulfuric acid (150 cm³) at -15 °C, was added p-anisaldehyde (9.8 cm³, 80.7 mmol). The reaction was then stirred at -15 °C for an hour, the solution turns a golden brown colour. The mixture was then tipped into cold water (500 cm³) and chilled to 0 °C for an hour. The cream precipitate was then filtered, washed with water (100 cm³). Recrystallization from ethanol (~ 100 cm³) afforded **276** as cream crystals (10.06 g, 55.6 mmol, 69%); m.p. 77 – 79 °C; R_f 0.32 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max.}(\text{cm}^{-1})$ 3074, 2873, 1704, 1682, 1610, 1569, 1530, 1497, 1468, 1446, 1424, 1352, 1280, 1206, 1153, 1001, 684; δ ¹H NMR (400 MHz; CDCl₃; ppm) 4.07 (3H, s, OCH₃), 7.24 (1H, d, *J* 8.8 Hz, H-5'), 8.09 (1H, dd, *J* 2.0, 8.8 Hz, H-6'), 8.35 (1H, d, *J* 2.0 Hz, H-2'), 9.94 (1H, s, H-1); δ ¹³C NMR (100MHz; CDCl₃; ppm) 57.31 (s, OCH₃), 114.06 (s, CH), 127.65 (s, CH), 129.29 (s, C), 134.94 (s, CH), 157.38 (s, C), 188.96 (s, CO).

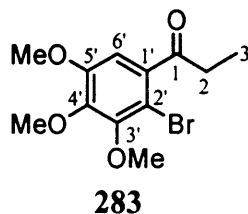
Chapter 6 – Experimental

1-(2-Bromo-3,4,5-trimethoxyphenyl)propan-1-ol **284**



To a stirring solution of ethylmagnesium chloride in tetrahydrofuran (10.5 cm³, 21.0 mmol, 2M) in anhydrous tetrahydrofuran (10 cm³) under argon at 0 °C, was slowly added 2-bromo-3,4,5-trimethoxybenzaldehyde **252** (3.07g, 11.2 mmol) in anhydrous tetrahydrofuran (20 cm³). The reaction was then stirred overnight at room temperature and then poured into mixture of concentrated aqueous ammonium chloride solution (100 cm³) and ice, and then extracted with ethyl acetate (6 × 20 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:9) afforded **284** as a yellow oil (1.99 g, 6.5 mmol, 58%); R_f 0.71 (SiO₂, ethyl acetate:hexane 1:1); δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.02 (3H, t, *J* 7.4 Hz, H-3), 1.62 – 1.70 (1H, m, H-2), 1.76 – 1.84 (1H, m, H-2), 1.97 (1H, d, *J* 3.4 Hz, OH-1), 3.87 (6H, s, OCH₃), 3.89 (3H, OCH₃), 4.98 – 5.03 (1H, m, H-1) 6.94 (1H, s, H-2').

1-(2-Bromo-3,4,5-trimethoxyphenyl)propan-1-one **283**

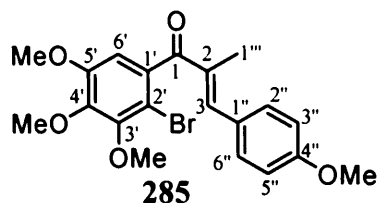


Pyridinium chlorochromate (2.19 g, 10.2 mmol) was added to a stirred solution of 1-(2-bromo-3,4,5-trimethoxyphenyl)propan-1-ol **284** (1.99 g, 6.5mmol) in dichloromethane (20 cm³). The reaction was then put under argon gas and stirred at room temperature under argon overnight. It was then filtered through Celite[®], dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded **283** as cream crystals (1.73 g, 1.9 mmol, 88%); m.p. 62 – 63 °C; R_f 0.64 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2972, 2938, 2895, 1698, 1582, 1563, 1482, 1447, 1430, 1401, 1384, 1351, 1318, 1167, 999, 857, 793; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.15 (3H, t, *J* 7.2 Hz, H-3), 2.85 (2H, q, *J* 7.2 Hz, H-2), 3.80 (3H, s, OCH₃), 3.83 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 6.63 (1H, s, H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 8.29 (s, CH₃), 36.23 (s, CH₂), 56.27 (s, OCH₃), 61.12 (s, OCH₃), 61.18 (s, OCH₃), 105.65 (s, CBr), 107.10(s, CH), 137.81 (s, C), 144.62 (s, C), 151.07 (s, C), 152.96 (s, C), 204.99 (s, CO); *m/z*(AP⁺) 303(MH⁺,

Chapter 6 – Experimental

100%), 305 (MH^+ , 80%); HRMS found $[MH]^+$ 303.02254 for $C_{12}H_{16}BrO_4$, $C_{12}H_{16}BrO_4$ requires $[MH]^+$ 303.02265.

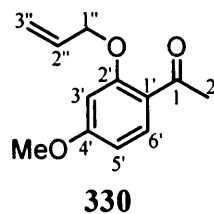
(*E*)-1-(2-Bromo-3,4,5-trimethoxyphenyl)-3-(4-methoxyphenyl)-2-methylprop-2-en-1-one **285**



The method adopted was that of Edwards⁷² *et al.* A solution of 1-(2-bromo-3,4,5-trimethoxyphenyl)propan-1-one **283** (0.92 g, 3.0 mmol), *p*-anisaldehyde (0.35 cm³, 3.1 mmol), piperidine (1.2 cm³) and acetic acid (0.6 cm³) in ethanol (3.5 cm³) was heated at reflux at 110 °C under argon for 7 days. The mixture was then cooled to room temperature and diluted with dichloromethane (30 cm³) and washed with water (3 × 10 cm³). The aqueous fraction was then extracted with dichloromethane (3 × 10 cm³). The organic fractions were then combined and dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Column chromatography (SiO₂, ethyl acetate:hexane 1:3) afforded a mixture of **283** and *p*-anisaldehyde (2.9:1), the mixture was shaken in deuterated chloroform (15 cm³) in the presence of the scavenger agent PS-trisamine (1.18 g, 5.36 mmol) at room temperature for 2 hours, reaction monitored by ¹H NMR. The mixture was , filtered and evaporated *in vacuo* to afforded **285** as yellow oil (0.78 g, 1.8 mmol, 61%); *R*_f 0.32 (SiO₂, ethyl acetate:hexane 1:1); *v*_{max}(cm⁻¹) 2937, 2839, 1648, 1599, 1565, 1509, 1479, 1462, 1426, 1382, 1333, 1251, 1174, 1005, 828; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.25 (3H, s, CH₃), 3.84 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 6.65 (1H, s, H-6'), 6.93 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.09 (1H, s, H-3), 7.40 (2H, d, *J* 8.8 Hz, H-2'' & H-6''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 12.87 (s, CH₃), 55.35 (s, OCH₃), 56.25 (s, OCH₃), 61.18 (s, OCH₃), 106.24 (s, CBr), 107.46 (s, CH), 114.01 (s, CH), 128.111 (s, C), 131.99 (s, CH), 134.44 (s, C), 137.08 (s, C), 143.69 (s, C), 145.41 (s, CH), 150.90 (s, C), 152.96 (s, C), 160.39 (s, C), 198.13 (s, CO); *m/z*(AP⁺) 421 (MH^+ , 100%), 423 (MH^+ , 100%); HRMS found $[MH]^+$ 421.06569 for $C_{20}H_{22}BrO_5$, $C_{20}H_{22}BrO_5$ requires $[MH]^+$ 421.06451; CHN found C 55.78%, H 6.16%, CHN requires C 55.86%, H 6.19%.

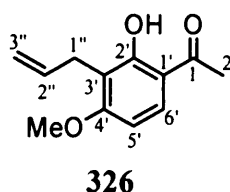
Chapter 6 – Experimental

1-(2-(Allyloxy)-4-methoxyphenyl)ethanone **330**²⁶²



The method adopted was that of Anjaneyulu²⁶² *et al.* To a stirring solution of 2-hydroxy-4-methoxyacetophenone (1.66 g, 10.0 mmol), and potassium carbonate (5.00 g, 36.2 mmol) in acetone (10 cm³) at room temperature, was added allyl bromide (1.72 cm³, 19.9 mmol). The mixture was then put under argon and heated to reflux overnight at 75 °C. The reaction was then cooled to room temperature and filtered to remove the potassium carbonate, and then the acetone was evaporated *in vacuo*. The crude product was then diluted with ethyl acetate (30 cm³) and washed with an aqueous sodium hydroxide solution (3 × 20 cm³, 1.3 M) and water (3 × 20 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo* to afford **330** as a white solid (2.05 g, 10.0 mmol, 99%); m.p. 36 – 37 °C; R_f 0.36 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 3090, 2988, 2928, 2846, 1647, 1597, 1572, 1501, 1480, 1465, 1465, 1439, 1419, 1326, 1201, 1010, 923, 827; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.59 (3H, s, H-2), 3.83 (3H, s, OCH₃), 4.60 (2H, td, *J* 1.5, 5.3 Hz, H-1''), 5.32 (1H, dq, *J* 10.6, 1.5 Hz, cis H-3''), 5.44 (1H, dq, *J* 17.2, 1.5 Hz, trans H-3''), 6.18 (1H, tdd, *J* 5.3, 10.6, 17.2 Hz, H-2''), 6.43 (1H, d, *J* 2.4 Hz, H-3'), 6.51 (1H, dd, *J* 2.4, 8.9 Hz, H-5'), 7.83 (1H, d, *J* 8.9 Hz, H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 32.28 (s, CH₃), 55.74 (s, OCH₃), 69.66 (s, OCH₂), 99.60 (s, CH), 105.57 (s, CH), 118.53 (s, CH₂), 121.65 (s, C), 132.74 (s, CH), 132.90 (s, CH), 160.25 (s, C), 164.59 (s, C), 197.98 (s, CO); *m/z* (API ES+) 207 (MH⁺, 100%).

1-(3-Allyl-2-hydroxy-4-methoxyphenyl)ethanone **326**²⁶²

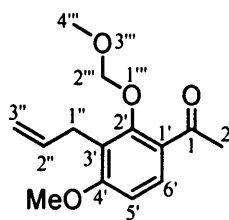


The method was adapted from that of Anjaneyulu²⁶² *et al.* 1-(2-(allyloxy)-4-methoxyphenyl)ethanone **330** (0.50 g, 2.4 mmol) was dissolved in *N,N*-dimethylaniline (5 cm³) and sealed in a pressure-rated reaction vial (10 cm³). The reaction vial was then irradiated in a self-turning single-mode CEM Discovery™ Focused Synthesizer. The reaction was maintained at 260 °C (power: 250 W) for 12 minutes. The mixture was then rapidly cooled to room temperature

Chapter 6 – Experimental

and poured into hydrochloric acid (50 cm³, 2M) and extracted with ethyl acetate (5 × 10 cm³). The organic fraction as then washed with hydrochloric acid (5 × 10 cm³, 2M) and saturated sodium bicarbonate aqueous solution (10 cm³), it was then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9) afforded **326** as a cream solid (0.33g, 1.6 mmol, 66%); m.p. 49 – 51 °C; R_f 0.48 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3002, 2949, 2914, 2842, 1608, 1496, 1466, 1414, 1366, 1268, 1123, 1070, 995, 918, 818; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.57 (3H, s, H-2), 3.42 (2H, td, *J* 1.7, 6.1 Hz, H-1''), 3.89 (3H, s, OCH₃), 4.93 – 4.98 (1H, dm, *J* 10.1 Hz, cis-H-3''), 4.97 – 5.04 (1H, dm, *J* 17.0 Hz, trans-H-3''), 5.95 (1H, ddt, *J* 10.1, 17.0, 6.1 Hz, H-2''), 6.47 (1H, d, *J* 8.8 Hz, H-5'), 7.64 (1H, d, *J* 8.8 Hz, H-6'), 12.77 (1H, s, OH-2'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 26.49 (s, CH₃), 26.77 (s, CH₂), 56.07 (s, OCH₃), 102.31 (s, CH), 144.58 (s, C), 144.70 (s, CH₂), 115.68 (s, C), 130.86 (s, CH), 136.10 (s, CH), 162.09 (s, C), 163.59 (s, C), 203.13 (s, CO).

1-(3-Allyl-4-methoxy-2-(methoxymethoxy)phenyl)ethanone **331**



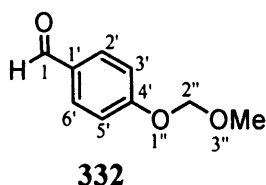
331

To a stirring solution of 1-(3-allyl-2-hydroxy-4-methoxyphenyl)ethanone **326** (1.00g, 4.8 mmol) in anhydrous tetrahydrofuran (10 cm³) at 0 °C under argon, sodium hydride (0.29 g, 7.3 mmol, 60% dispersion in mineral oil) was added. The reaction was then stirred for 5 minutes at 0 °C, before adding Chloromethyl methyl ether (0.56 cm³, 7.4 mmol) to the mixture. The reaction was then stirred for a further 10 minutes at 0 °C. The reaction was then heated to 65 °C for 18 hours. The reaction was then allowed to cool to room temperature; it was then tipped into water (10 cm³) and extracted with ethyl acetate (3 × 15 cm³). The organic fraction was then washed with water (2 × 10 cm³) and dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9) afforded **331** as a yellow oil (0.52 g, 2.1 mmol, 43%); R_f 0.54 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2941, 2840, 1675, 1588, 1482, 1465, 1427, 1264, 972, 921, 806; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.58 (3H, s, H-2), 3.48 (2H, td, *J* 1.6, 5.6 Hz, H-1''), 3.52 (3H, s, H-4'''), 3.89 (3H, s, OCH₃), 4.96 (2H, s, H-2'''), 4.95 – 5.01 (2H, m, H-3''), 5.92 – 6.03 (1H, m, H-2''), 6.71 (1H, d, *J* 8.8 Hz, H-5'), 7.57 (1H, d, *J* 8.8 Hz, H-6'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 28.46 (s, CH₂), 30.25 (s,

Chapter 6 – Experimental

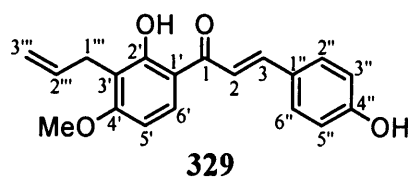
CH), 56.09 (s, OCH₃), 58.07 (s, OCH₃), 101.60 (s, OCH₂O), 115.12 (s, CH₂), 122.86 (s, C), 127.06 (s, C), 129.65 (s, CH), 136.51 (s, CH), 156.00 (s, C), 161.83 (s, C), 199.59 (s, CO); *m/z*(API ES+) 251 (MH⁺, 100%); HRMS found [MH]⁺ 251.12800 for C₁₄H₁₉O₄, C₁₄H₁₉O₄ requires [MH]⁺ 251.12779.

4-(Methoxymethoxy)benzaldehyde **332**³⁵⁰



The method was adapted from that of Crombie²²⁸ *et al.* To a stirring solution of sodium hydride (0.81 g, 20.3 mmol, 60% dispersion in mineral oil) in anhydrous dimethyl formamide (10 cm³) at 0 °C under argon 4-methoxybenzaldehyde (2.00 g, 16.4 mmol) in anhydrous dimethyl formamide (10 cm³) was added slowly drop wise over 20 minutes. The reaction was then allowed to warm to room temperature, before being cooled to 0 °C. Chloromethyl methyl ether (1.5 cm³, 19.7 mmol) in anhydrous dimethyl formamide (4 cm³) was then added drop wise over 20 minutes to the mixture at 0 °C. The mixture was then stirred for 30 minutes at 0 °C and then stirred overnight at room temperature. The reaction was then tipped into a mixture of water (20 cm³) and ice (20 g), and extracted with ethyl acetate (4 × 30cm³). The organic fraction was washed with an aqueous sodium hydroxide solution (2 × 10 cm³, 0.1 M) and water (2 × 10 cm³), it was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:1) afforded **332** as a pale yellow oil (2.30 g, 13.8 mmol, 84%); R_f 0.46 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2903, 2829, 2741, 1684, 1597, 1578, 1508, 1444, 1427, 1237, 1213, 1201, 1079, 974, 921, 831; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.49 (3H, s, OCH₃), 5.25 (2H, s, H-2''), 7.15 (2H, d, *J* 8.6 Hz, H-3' & H-5'), 7.84 (2H, d, *J* 8.6 Hz, H-2' & H-6'), 9.90 (1H, s, H-1); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.58 (s, CH₃), 94.33 (s, OCH₂O), 116.50 (s, CH), 130.97 (s, C), 132.10 (s, CH), 162.43 (s, C), 191.12 (s, CHO).

(*E*)-1-(3-Allyl-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **329**

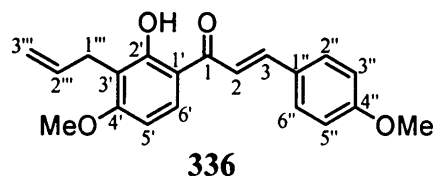


To a stirring solution of 1-(3-allyl-4-methoxy-2-(methoxymethoxy)phenyl)ethanone **331** (0.150 g, 0.60 mmol) and 4-(methoxymethoxy)benzaldehyde **332** (0.100 g, 0.60 mmol) in

Chapter 6 – Experimental

methanol (1.8 cm³) was added an aqueous solution of sodium hydroxide (0.6 cm³, 2M) and the mixture stirred at room temperature overnight. The mixture was then extracted with dichloromethane (3 × 5 cm³), the organic fraction was then washed with water (3 × 5 cm³), dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The crude product was then dissolved in methanol (12 cm³), and had hydrochloric acid (2.4 cm³, 3M) added to it. The reaction under argon was then heated to reflux at 75 °C for 45 minutes. The reaction was then cooled to room temperature and tipped into water (12 cm³), it was then extracted with ethyl acetate (3 × 12 cm³). The organic fraction was then washed with water (2 × 10 cm³) and brine (10 cm³), dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:1) afforded **329** as a yellow solid (0.150 g, 0.48 mmol, 81%); m.p. 140 – 142 °C; R_f 0.39 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3166, 2903, 1666, 1628, 1605, 1582, 1543, 1511, 1490, 1458, 1413, 1280, 1236, 1210, 1165, 1120, 783; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.45 (2H, td, *J* 1.8, 6.2 Hz, H-1'''), 3.91 (3H, s, OCH₃), 4.98 (1H, dq, *J* 10.0, 1.8 Hz, cis-H-3'''), 5.04 (1H, dq, *J* 17.1, 1.8 Hz, trans-H-3'''), 5.65 (1H, br. s, OH-4''), 5.98 (1H, ddt, *J* 10.0, 17.1, 6.2 Hz, H-2'''), 6.52 (1H, d, *J* 9.2 Hz, H-5'), 6.89 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.47 (1H, d, *J* 15.0 Hz, H-2), 7.55 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.83 (1H, d, *J* 9.2 Hz, H-6'), 7.84 (1H, d, *J* 15.0 Hz, H-3), 13.50 (1H, br. s, OH-2'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 26.89 (s, CH₂), 56.09 (s, OCH₃), 102.35 (s, CH), 114.73 (s, CH₂), 114.85 (s, C), 115.95 (s, C), 116.25 (s, CH), 118.29 (s, CH), 127.96 (s, C), 129.82 (s, CH), 130.80 (s, CH), 136.15 (s, CH), 144.40 (s, CH), 158.27 (s, C), 163.33 (s, C), 163.64 (s, C), 192.64 (s, CO); *m/z*(API ES+) 311 (MH⁺, 100%), 333 (MNa⁺, 50%); HRMS found [MH]⁺ 311.12800 for C₁₉H₁₉O₄, C₁₉H₁₉O₄ requires [MH]⁺ 311.12779.

(*E*)-1-(3-Allyl-2-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one **336**



To a stirring solution of 1-(3-allyl-4-methoxy-2-(methoxymethoxy)phenyl)ethanone **331** (0.025 g, 0.10 mmol) and *p*-anisaldehyde in methanol (0.1 cm³, 1M), in methanol (0.1 cm³) was added an aqueous solution of sodium hydroxide (0.1 cm³, 2M) and the mixture stirred at room temperature overnight. The mixture was then extracted with chloroform (3 × 3 cm³), the organic fraction was then evaporated *in vacuo*. The crude product was then dissolved in methanol (3 cm³), and had hydrochloric acid (0.6 cm³, 3M) added to it. The reaction under

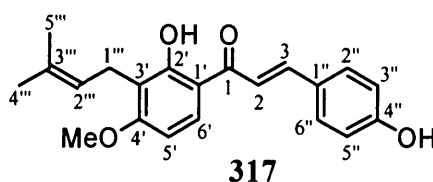
Chapter 6 – Experimental

argon was then heated to reflux at 75 °C for 45 minutes. The reaction was then cooled to room temperature and tipped into water (3 cm³), it was then extracted with chloroform (3 × 10 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:1) afforded **336** as yellow needle crystals (0.023 g, 0.07mmol, 72%); m.p. 94 – 95 °C; R_f 0.48 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3004, 2978, 2908, 2841, 1633, 1603, 1584, 1559, 1510, 1491, 1461, 1442, 1415, 1281, 1235, 1183, 1112, 1076, 856, 825; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.45 (2H, td, *J* 1.7, 6.2 Hz, H-1'''), 3.87 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.97 (1H, dq, *J* 9.9, 1.7 Hz, cis-H-3'''), 5.03 (1H, dq, *J* 17.2, 1.7 Hz, trans-H-3'''), 5.98 (1H, ddt, *J* 9.9, 17.2, 6.2 Hz, H-2''), 6.52 (1H, d, *J* 9.0 Hz, H-5'), 6.95 (2H, d, *J* 8.6 Hz, H-3'' & H-5''), 7.49 (1H, d, *J* 15.4 Hz, H-2), 7.62 (2H, d, *J* 8.6 Hz, H-2'' & H-6''), 7.84 (1H, d, *J* 9.0 Hz, H-6'), 7.87 (1H, d, *J* 15.4 Hz, H-3), 13.52 (1H, s, OH-2'); δ ¹³C NMR (100MHz; CDCl₃; ppm) 26.90 (s, CH₂), 55.66 (s, OCH₃), 56.08 (s, OCH₃), 102.25 (s, CH), 114.69 (s, CH + CH₂), 114.89 (s, C), 115.90 (s, C), 118.26 (s, CH), 127.84 (s, C), 129.73 (s, CH), 130.55 (s, CH), 136.19 (s, CH), 144.34 (s, CH), 161.98 (s, C), 163.36 (s, C), 163.53 (s, C), 192.50 (s, CO); *m/z*(API ES+) 325 (MH⁺, 100%); HRMS found [MH]⁺ 325.14338 for C₂₀H₂₁O₄, C₂₀H₂₁O₄ requires [MH]⁺ 325.14344.

Protocol E

To a stirring mixture of the allyl compound, 2-methylbut-2-ene and anhydrous dichloromethane in a Schlenk tube under an argon atmosphere, was added a quantity of Grubbs catalyst, 2nd generation. The mixture was then degassed, put under an argon atmosphere and heated to reflux at 50 °C for 12 hours. The dichloromethane was then evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:1).

(E)-1-(2-Hydroxy-4-methoxy-3-(3-methylbut-2-enyl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **317**

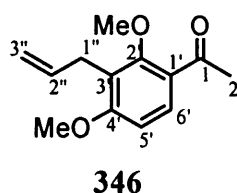


The chalcone **317** was obtained following Protocol E using *(E)*-1-(3-allyl-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **329** (0.050 g, 0.16 mmol), 2-methylbut-2-ene (0.33 cm³, 3.1 mmol) and Grubbs catalyst, 2nd generation (0.003 g, 0.0035 mmol) in anhydrous dichloromethane (2.3 cm³). Purification by column chromatography

Chapter 6 – Experimental

(SiO₂, ethyl acetate:hexane 1:9 to 1:1) afforded **317** as a yellow solid (0.036 g, 0.11 mmol, 66%); m.p. 130 – 131 °C; R_f 0.15 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max.}(\text{cm}^{-1})$ 3282, 2924, 1622, 1603, 1579, 1548, 1513, 1489, 1435, 1408, 1365, 1279, 1244, 1218, 1167, 1119, 833, 794; $\delta^1\text{H NMR}$ (400 MHz; CDCl₃; ppm) 1.68 (3H, s, CH₃), 1.80 (3H, s, CH₃), 3.36 (2H, d, J 6.5 Hz, H-1'''), 3.91 (3H, s, OCH₃), 5.23 (1H, br. d, J 6.5 Hz, H-2''') 5.41 (1H, br. s, OH-4''), 6.50 (1H, d, J 9.2 Hz, H-5'), 6.88 (2H, d, J 8.2 Hz, H-3'' & H-5''), 7.47 (1H, d, J 15.4 Hz, H-2), 7.56 (2H, d, J 8.2 Hz, H-2'' & H-6''), 7.79 (1H, d, J 9.2 Hz, H-6'), 7.83 (1H, d, J 15.4 Hz, H-3), 13.46 (1H, s, OH-2'); $\delta^{13}\text{C NMR}$ (100 MHz; CDCl₃; ppm) 18.05 (s, CH₃), 21.96 (s, CH₂), 26.05 (s, CH₃), 56.01 (s, OCH₃), 102.31 (s, CH), 114.89 (s, C), 116.22 (s, CH), 117.81 (s, C), 118.45 (s, CH), 122.25 (s, CH), 128.07 (s, C), 129.35 (s, CH), 130.75 (s, CH), 132.17 (s, C), 144.15 (s, CH), 158.13 (s, C), 163.23 (s, C), 163.47 (s, C), 192.56 (s, CO).

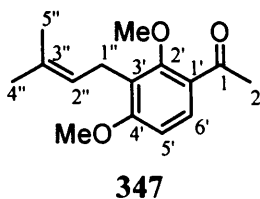
1-(3-Allyl-2,4-dimethoxyphenyl)ethanone **346**³⁵¹



To a stirring solution of *1-(3-allyl-2-hydroxy-4-methoxyphenyl)ethanone* **326** (1.29g, 6.3 mmol) in anhydrous tetrahydrofuran (50 cm³) at 0 °C under argon, sodium hydride (0.39 g, 9.8 mmol, 60% dispersion in mineral oil) was added. The reaction was then stirred for 5 minutes at 0 °C, before adding dimethyl sulfate (0.91 cm³, 9.6 mmol) to the mixture. The reaction was then stirred for a further 10 minutes at 0 °C and then heated to 65 °C for 18 hours. The reaction was then allowed to cool to room temperature; it was then tipped into water (80 cm³) and extracted with ethyl acetate (6 × 40 cm³). The organic fraction was then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9) afforded **346** as a yellow oil (1.32 g, 6.0 mmol, 96%); R_f 0.40 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max.}(\text{cm}^{-1})$ 3079, 2941, 2840, 1672, 1638, 1586, 1482, 1457, 1408, 1232, 1115, 1091, 1071, 808; $\delta^1\text{H NMR}$ (400 MHz; CDCl₃; ppm) 2.61 (3H, s, H-2), 3.44 (2H, td, J 1.6, 6.0 Hz, H-1''), 3.75 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 4.95 – 5.01 (2H, m, H-3''), 5.98 (1H, ddt, J 9.5, 17.8, 6.0 Hz, H-2''), 6.71 (1H, d, J 8.8 Hz, H-5'), 7.63 (1H, d, J 8.8 Hz, H-6'); $\delta^{13}\text{C NMR}$ (100 MHz; CDCl₃; ppm) 28.11 (s, CH₃), 30.23 (s, CH₂), 56.09 (s, OCH₃), 63.33 (s, OCH₃), 106.51 (s, CH), 115.05 (s, CH₂), 122.49 (s, C), 126.15 (s, C), 130.09 (s, CH), 136.75 (s, CH), 159.53 (s, C), 162.15 (s, C), 199.33 (s, CO).

Chapter 6 – Experimental

*1-(2,4-Dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone 347*³⁵²

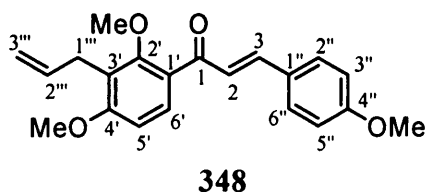


The acetophenone **347** was obtained following Protocol E. Using *1-(3-allyl-2,4-dimethoxyphenyl)ethanone 346* (1.0 g, 4.5 mmol), 2-methylbut-2-ene (9.1 cm³, 85.6 mmol) and Grubbs catalyst, 2nd generation (0.077 g, 0.0905 mmol) in anhydrous dichloromethane (65 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:19 to 1:3) afforded **347** as a light brown oil (1.1 g, 4.5 mmol, 98%); R_f 0.47 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max.}(\text{cm}^{-1})$ 2939, 1671, 1586, 1482, 1455, 1409, 1265, 807; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.68 (3H, d, *J* 1.2 Hz, CH₃), 1.78 (3H, s, CH₃), 2.61 (3H, s, H-2), 3.37 (2H, d, *J* 6.9 Hz, H-1''), 3.74 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 5.13 – 5.20 (1H, tm, *J* 6.9 Hz, H-2''), 6.69 (1H, d, *J* 8.6 Hz, H-5'), 7.60 (1H, d, *J* 8.6 Hz, H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 18.08 (s, CH₃), 23.06 (s, CH₂), 25.97 (s, CH₃), 30.23 (s, CH₃), 56.04 (s, OCH₃), 63.05 (s, OCH₃), 106.47 (s, CH), 122.79 (s, CH), 124.37 (s, C), 126.09 (s, C), 129.65 (s, CH), 131.97 (s, C), 159.39 (s, C), 162.14 (s, C), 199.36 (s, CO).

Protocol F

To a stirring solution of substituted acetophenone and substituted benzaldehyde in methanol was added a quantity of an aqueous solution of sodium hydroxide and the mixture stirred at room temperature overnight. The mixture was then extracted with ethyl acetate or chloroform or dichloromethane, the organic fraction was then washed with water, dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The chalcone was then isolated by column chromatography or recrystallization.

(E)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one 348

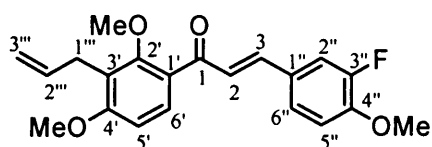


The chalcone **348** was obtained by following Protocol F. Using *1-(3-allyl-2,4-dimethoxyphenyl)ethanone 346* (0.087 g, 0.39 mmol), *p*-anisaldehyde in methanol (0.39 cm³, 1M), and aqueous sodium hydroxide solution (0.39 cm³, 2M) in methanol (1.2 cm³). The mixture was extracted with ethyl acetate (2 × 5 cm³) and washed with water (2 × 5 cm³).

Chapter 6 – Experimental

Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9) afforded **348** as a yellow oil (0.082 g, 0.24 mmol, 62%); R_f 0.30 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max}(\text{cm}^{-1})$ 2937, 1655, 1638, 1587, 1571, 1509, 1482, 1458, 1421, 1408, 1235, 1170, 1076, 804; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.48 (2H, td, *J* 1.5, 6.0 Hz, H-1'''), 3.71 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 4.97 – 5.03 (2H, m, H-3'''), 6.01 (1H, ddt, *J* 9.6, 17.6, 6.0 Hz, H-2'''), 6.74 (1H, d, *J* 8.6 Hz, H-5'), 6.92 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.42 (1H, d, *J* 15.8 Hz, H-2), 7.58 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.63 (1H, d, *J* 8.6 Hz, H-6'), 7.70 (1H, d, *J* 15.8 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 24.04 (s, CH₂), 55.63 (s, CH₃), 56.12 (s, CH₃), 63.53 (s, CH₃), 106.61 (s, CH), 114.58 (s, CH), 144.94 (s, CH₂), 122.23 (s, C), 124.23 (s, CH), 126.60 (s, C), 128.10 (s, C), 130.25 (s, CH), 130.41 (s, CH), 136.91 (s, CH), 143.39 (s, CH), 159.20 (s, C), 161.66 (s, C), 161.67 (s, C), 191.91 (s, CO); *m/z*(API ES+) 399 (MH⁺, 100%); HRMS found [MH]⁺ 339.15954 for C₂₁H₂₃O₄, C₂₁H₂₃O₄ requires [MH]⁺ 339.15909.

(E)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(3-fluoro-4-methoxyphenyl)prop-2-en-1-one **349**



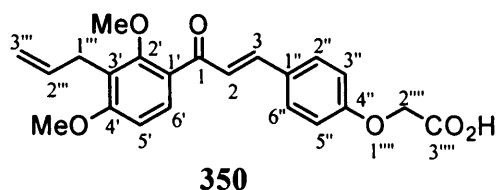
349

The chalcone **349** was obtained by following Protocol F. Using 1-(3-allyl-2,4-dimethoxyphenyl)ethanone **346** (0.110 g, 0.50 mmol), 3-fluoro-4-methoxybenzaldehyde (0.076 g, 0.50 mmol), and aqueous sodium hydroxide solution (0.50 cm³, 2M) in methanol (2.0 cm³). The mixture was extracted with chloroform (2 × 5 cm³) and washed with water (2 × 5 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9) afforded **349** as a yellow solid (0.148 g, 0.42 mmol, 84%); m.p. 77 – 79 °C; R_f 0.25 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max}(\text{cm}^{-1})$ 2939, 1651, 1616, 1584, 1514, 1484, 1456, 1436, 1411, 1276, 1262, 1242, 1209, 1096; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.48 (2H, td, *J* 1.5, 6.0 Hz, H-1'''), 3.70 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 4.97 – 5.03 (2H, m, H-3'''), 6.01 (1H, ddt, *J* 9.6, 17.6, 6.0 Hz, H-2'''), 6.75 (1H, d, *J* 8.7 Hz, H-5'), 6.97 (1H, t, *J* 8.5 Hz, H-5''), 7.33 (1H, br. d, *J* 8.5 Hz, H-6''), 7.39 (1H, dd, *J* 2.0 Hz, *J* 12.1 Hz, H-2''), 7.42 (1H, d, *J* 15.6 Hz, H-2), 7.64 (1H, d, *J* 8.7 Hz, H-6'), 7.65 (1H, br. d, *J* 15.6 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 28.03 (s, CH₂), 56.14 (s, CH₃), 56.49 (s, CH₃), 63.61 (s, CH₃), 106.71 (s, CH), 113.38 (d, *J* 2.2 Hz, CH), 115.01 (s, CH₂), 115.11 (d, *J* 21.3 Hz, CH), 122.32 (s, C), 125.41 (s, CH), 126.09 (d, *J* 2.9 Hz, CH), 126.37 (s, C), 128.75 (d, *J* 7.4 Hz, C) 130.37 (s, CH), 136.84 (s, CH), 142.03 (d, *J* 2.9 Hz, CH), 149.74 (s, C), 152.66 (d, *J* 245.4

Chapter 6 – Experimental

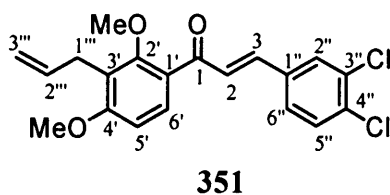
Hz, CF), 159.34 (s, C), 161.89 (s, C), 191.43 (s, CO); m/z (API ES+) 357 (MH^+ , 100%); HRMS found $[MH]^+$ 357.15081 for $C_{21}H_{22}O_4F$, $C_{21}H_{22}O_4F$ requires $[MH]^+$ 357.14966.

(*E*)-3-(4-Carboxymethoxyphenyl)-1-(3-allyl-2,4-dimethoxyphenyl)prop-2-en-1-one **350**



To a stirring solution of 1-(3-allyl-2,4-dimethoxyphenyl)ethanone **346** (0.110 g, 0.50 mmol), and 4-formyl phenoxyacetic acid (0.090 g, 0.50 mmol) in methanol (2.0 cm³), was added an aqueous sodium hydroxide solution (1 cm³, 2M) and the mixture stirred at room temperature overnight. The mixture was then acidified with hydrochloric acid (until pH = 1, 1M), and extracted with ethyl acetate (3 × 5 cm³). The organic fraction was then washed with water (2 × 5 cm³) dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from methanol afforded **350** as cream crystals (0.136 g, 0.36 mmol, 71%); m.p. 173 – 175 °C; R_f 0.26 (SiO₂, methanol:ethyl acetate 1:9); ν_{max} (cm⁻¹) 2938, 2559, 1735, 1703, 1645, 1574, 1561, 1505, 1467, 1453, 1417, 1225, 1173, 1079, 1015, 987, 917, 834; δ ¹H NMR (400 MHz; CD₃OD; ppm) 3.45 (2H, td, J 1.4, 6.1 Hz, H-1'''), 3.67 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 4.72 (2H, s, H-2'''), 4.93 – 5.01 (2H, m, H-3'''), 5.96 (1H, ddt, J 10.2, 17.0, 6.1 Hz, H-2''), 6.87 (1H, d, J 8.7 Hz, H-5'), 6.99 (2H, d, J 8.7 Hz, H-3'' & H-5''), 7.42 (1H, d, J 15.9 Hz, H-2), 7.58 (1H, d, J 8.7 Hz, H-6'), 7.62 (2H, d, J 8.7 Hz, H-2'' & H-6''), 7.63 (1H, d, J 15.9 Hz, H-3); δ ¹³C NMR (100 MHz; CD₃OD; ppm) 27.54 (s, CH₂), 55.26 (s, OCH₃), 62.67 (s, OCH₃), 64.65 (s, OCH₂O), 106.41 (s, CH), 113.97 (s, CH₂), 115.03 (s, CH), 122.27 (s, C), 124.10 (s, CH), 125.95 (s, C), 128.50 (s, C), 129.90 (s, CH), 130.15 (s, CH), 136.64 (s, CH), 143.88 (s, CH), 159.11 (s, C), 160.38 (s, C), 161.99 (s, C), 171.03 (CO₂H), 192.82 (s, CO); m/z (API ES+) 383 (MH^+ , 100%); HRMS found $[MH]^+$ 383.15032 for $C_{22}H_{23}O_6$, $C_{22}H_{23}O_6$ requires $[MH]^+$ 383.14891.

(*E*)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one **351**

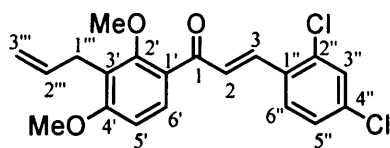


The chalcone **351** was obtained by following Protocol F. Using 1-(3-allyl-2,4-dimethoxyphenyl)ethanone **346** (0.110 g, 0.50 mmol), 3,4-dichlorobenzaldehyde (0.088 g,

Chapter 6 – Experimental

0.50 mmol), and aqueous sodium hydroxide solution (0.50 cm³, 2M) in methanol (2.0 cm³). The mixture was extracted with dichloromethane (3 × 5 cm³) and washed with water (2 × 15 cm³). Recrystallization from methanol afforded **351** as pale yellow crystals (0.130 g, 0.35 mmol, 69%); m.p. 78 – 79 °C; R_f 0.52 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2940, 1655, 1638, 1603, 1583, 1551, 1469, 1454, 1408, 1321, 1268, 1258, 1228, 1215, 1119, 1077, 976, 917, 823; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.48 (2H, td, *J* 1.4, 6.0 Hz, H-1'''), 3.70 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 4.97 – 5.03 (2H, m, H-3'''), 6.00 (1H, ddt, *J* 11.1, 16.1, 6.0 Hz, H-2'''), 6.76 (1H, d, *J* 8.7 Hz, H-5'), 7.44 (1H, dd, *J* 1.8, 8.3 Hz, H-6''), 7.47 (1H, d, *J* 8.3 Hz, H-5''), 7.54 (1H, d, *J* 15.8 Hz, H-2), 7.63 (1H, d, *J* 15.8 Hz, H-3), 7.67 (1H, d, *J* 8.7 Hz, H-6'), 7.69 (1H, d, *J* 1.8 Hz, H-2''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 28.02 (s, CH₂), 56.18 (s, OCH₃), 63.75 (s, OCH₃), 106.88 (s, CH), 115.08 (s, CH₂), 122.42 (s, C), 126.02 (s, C), 127.64 (s, CH), 127.93 (s, CH), 130.03 (s, CH), 130.56 (s, CH), 131.09 (s, CH), 133.43 (s, C), 134.20 (s, C), 135.54 (s, C), 136.73 (s, CH), 140.21 (s, CH), 159.53 (s, C), 162.24 (s, C), 190.91 (s, CO); *m/z*(API ES⁺) 377 (MH⁺, 100%), 379 (MH⁺, 70%); HRMS found [MH]⁺ 377.07085 for C₂₀H₁₉O₃Cl₂, C₂₀H₁₉O₃Cl₂ requires [MH]⁺ 377.07058.

(E)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one **352**



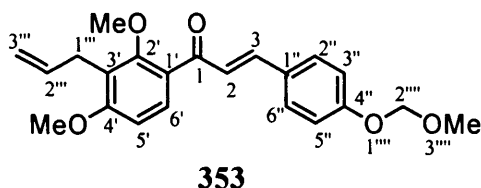
352

The chalcone **352** was obtained following Protocol A using 1-(3-allyl-2,4-dimethoxyphenyl)ethanone **346** (0.110 g, 0.50 mmol), 2,4-dichlorobenzaldehyde (0.088 g, 0.50 mmol) and aqueous sodium hydroxide solution (0.5 cm³, 2M) in methanol (2.0 cm³). Recrystallization from methanol afforded **352** as pale yellow crystals (0.155 g, 0.41 mmol, 82%); m.p. 91 – 92 °C; R_f 0.56 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2941, 1645, 1601, 1590, 1462, 1409, 1327, 1271, 813; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.47 (2H, td, *J* 1.4, 5.9 Hz, H-1'''), 3.70 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 4.97 – 5.03 (2H, m, H-3'''), 6.00 (1H, ddt, *J* 9.4, 17.8, 5.9 Hz, H-2'''), 6.77 (1H, d, *J* 8.7 Hz, H-5'), 7.27 (1H, dd, *J* 2.1, 8.7 Hz, H-5''), 7.45 (1H, d, *J* 2.1 Hz, H-3''), 7.52 (1H, d, *J* 15.8 Hz, H-2), 7.67 (1H, d, *J* 8.7 Hz, H-6'), 7.68 (1H, d, *J* 8.4 Hz, H-6''), 8.06 (1H, d, *J* 15.8 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 28.01 (s, CH₂), 56.17 (s, OCH₃), 63.76 (s, OCH₃), 106.87 (s, CH), 115.06 (s, CH₂), 122.36 (s, C), 126.04 (s, C), 127.72 (s, CH), 128.67 (s, CH), 129.09 (s, CH), 130.23 (s, CH), 130.62 (s, CH), 132.32 (s, C), 136.23 (s, C), 136.32 (s, C), 136.73 (s, CH), 137.49 (s, CH), 159.53 (s, C), 162.20 (s, C), 190.94 (s, CO); *m/z*(API ES⁺) 377 (MH⁺,

Chapter 6 – Experimental

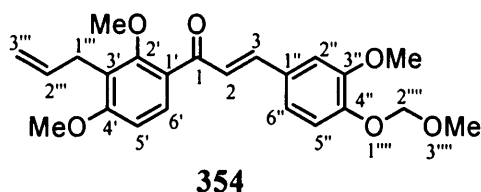
100%), 379 (MH^+ , 70%); HRMS found $[\text{MH}]^+$ 377.07103 for $\text{C}_{20}\text{H}_{19}\text{O}_3\text{Cl}_2$, $\text{C}_{20}\text{H}_{19}\text{O}_3\text{Cl}_2$ requires $[\text{MH}]^+$ 377.07058.

(E)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **353**



The chalcone **353** was obtained by following Protocol F. Using 1-(3-allyl-2,4-dimethoxyphenyl)ethanone **346** (0.110 g, 0.50 mmol), 4-(methoxymethoxy)benzaldehyde **332** (0.084 g, 0.50 mmol), and aqueous sodium hydroxide solution (0.50 cm^3 , 2M) in methanol (2.0 cm^3). The mixture was extracted with dichloromethane ($2 \times 5 \text{ cm}^3$) and washed with water (5 cm^3). Purification by column chromatography (SiO_2 , ethyl acetate:hexane 1:19 to 1:4) afforded **353** as a yellow oil (0.142 g, 0.39 mmol, 77%); R_f 0.29 (SiO_2 , ethyl acetate:hexane 1:3); $\nu_{\text{max}}(\text{cm}^{-1})$ 2938, 1655, 1638, 1589, 1508, 1482, 1456, 1421, 1408, 1272, 1232, 1151, 1075, 807; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.45 – 3.49 (2H, m, H-1'''), 3.49 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.97 – 5.03 (2H, m, H-3'''), 5.21 (2H, s, H-2'''), 6.01 (1H, ddt, J 9.6, 17.6, 6.0 Hz, H-2'''), 6.75 (1H, d, J 8.7 Hz, H-5'), 7.06 (2H, d, J 8.7 Hz, H-3'' & H-5''), 7.44 (1H, d, J 15.8 Hz, H-2), 7.57 (2H, d, J 8.7 Hz, H-2'' & H-6''), 7.63 (1H, d, J 8.7 Hz, H-6'), 7.70 (1H, d, J 15.8 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 28.04 (s, CH_2), 56.12 (s, OCH_3), 56.39 (s, OCH_3), 63.55 (s, OCH_3), 94.42 (s, OCH_2O), 106.63 (s, CH), 114.96 (s, CH_2), 116.69 (s, CH), 122.25 (s, C), 124.68 (s, CH), 126.54 (s, C), 129.15 (s, C), 130.31 (s, CH), 136.89 (s, CH), 143.17 (s, CH), 159.20 (s, C), 159.26 (s, C), 161.72 (s, C), 191.84 (s, CO); m/z (API ES+) 369 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 369.17007 for $\text{C}_{22}\text{H}_{25}\text{O}_5$, $\text{C}_{22}\text{H}_{25}\text{O}_5$ requires $[\text{MH}]^+$ 369.16965.

(E)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(3-methoxy-4-(methoxymethoxy)phenyl)prop-2-en-1-one **354**

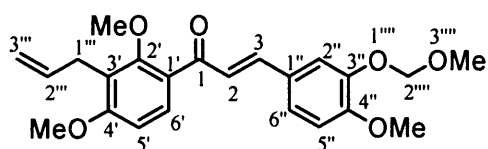


The chalcone **354** was obtained by following Protocol F. Using 1-(3-allyl-2,4-dimethoxyphenyl)ethanone **346** (0.110 g, 0.50 mmol), 3-methoxy-4-(methoxymethoxy)benzaldehyde **365** (0.098 g, 0.50 mmol), and aqueous sodium hydroxide solution (0.50 cm^3 ,

Chapter 6 – Experimental

2M) in methanol (2.0 cm³). The mixture was extracted with dichloromethane (2 × 5 cm³) and washed with water (5 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:5) afforded **354** as a yellow oil (0.177 g, 0.45 mmol, 89%); R_f 0.21 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2937, 1655, 1638, 1588, 1508, 1482, 1463, 1409, 1216, 1155, 1118, 1075, 978, 806; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.48 (2H, td, *J* 1.5, 6.0 Hz, H-1'''), 3.52 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 4.98 – 5.04 (2H, m, H-3'''), 5.28 (2H, s, H-2'''), 6.01 (1H, ddt, *J* 9.6, 17.7, 6.0 Hz, H-2'''), 6.75 (1H, d, *J* 8.7 Hz, H-5'), 7.15 (1H, d, *J* 1.7 Hz, H-2''), 7.16 (1H, d, *J* 8.3 Hz, H-5''), 7.19 (1H, dd, *J* 1.7, 8.3 Hz, H-6''), 7.40 (1H, d, *J* 15.8 Hz, H-2), 7.62 (1H, d, *J* 8.7 Hz, H-6'), 7.67 (1H, d, *J* 15.8 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 28.05 (s, CH₂), 56.13 (s, OCH₃), 56.23 (s, OCH₃), 56.58 (s, OCH₃), 63.55 (s, OCH₃), 95.42 (s, OCH₂O), 106.61, 111.18, 114.97 (s, CH₂), 116.08 (s, CH), 122.27 (s, C), 122.72 (s, CH), 125.00 (s, CH), 126.51 (s, C), 129.75 (s, C), 130.25 (s, CH), 136.89 (s, CH), 143.55 (s, CH), 148.79 (s, C), 150.02 (s, C), 159.19 (s, C), 161.72 (s, C), 191.94 (s, CO); *m/z*(API ES+) 399 (MH⁺, 100%); HRMS found [MH]⁺ 399.18062 for C₂₃H₂₇O₆, C₂₃H₂₇O₆ requires [MH]⁺ 399.18022.

(E)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(4-methoxy-3-(methoxymethoxy)phenyl)prop-2-en-1-one **355**



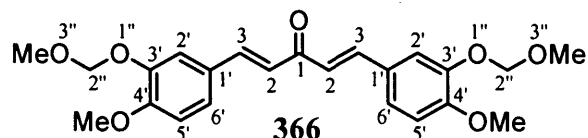
355

The chalcone **355** was obtained by following Protocol F. Using 1-(3-allyl-2,4-dimethoxyphenyl)ethanone **346** (0.110 g, 0.50 mmol), 4-methoxy-3-(methoxymethoxy) benzaldehyde **264** (0.098 g, 0.50 mmol), and aqueous sodium hydroxide solution (0.50 cm³, 2M) in methanol (2.0 cm³). The mixture was extracted with dichloromethane (2 × 10 cm³) and washed with water (2 × 10 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:1) afforded **355** as a yellow oil (0.145 g, 0.37 mmol, 73%); R_f 0.18 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2937, 1654, 1637, 1588, 1508, 1482, 1461, 1442, 1407, 1075, 993, 804; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.48 (2H, td, *J* 1.4, 6.1 Hz, H-1'''), 3.54 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 4.97 – 5.04 (2H, m, H-3'''), 5.27 (2H, s, H-2'''), 6.01 (1H, ddt, *J* 9.5, 17.7, 6.1 Hz, H-2'''), 6.74 (1H, d, *J* 8.7 Hz, H-5'), 6.91 (1H, d, *J* 8.5 Hz, H-5''), 7.29 (1H, dd, *J* 1.9, 8.5 Hz, H-6''), 7.36 (1H, d, *J* 15.8 Hz, H-2), 7.42 (1H, d, *J* 1.9 Hz, H-2''), 7.60 (1H, d, *J* 8.7 Hz, H-6'), 7.64

Chapter 6 – Experimental

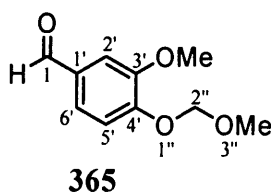
(1H, d, J 15.8 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 28.06 (s, CH_2), 56.12 (s, OCH_3), 56.23 (s, OCH_3), 56.56 (s, OCH_3), 63.48 (s, OCH_3), 95.86 (s, OCH_2O), 106.54 (s, CH), 111.87 (s, CH), 114.96 (s, CH_2), 116.25 (s, CH), 122.28 (s, C), 124.17 (s, CH), 124.81 (s, CH), 126.50 (s, C), 128.49 (s, C), 130.15 (s, CH), 136.92 (s, CH), 143.64 (s, CH), 146.90 (s, C), 152.05 (s, C), 159.14 (s, C), 161.62 (s, C), 192.07 (s, CO); m/z (API ES+) 399 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 399.18047 for $\text{C}_{23}\text{H}_{27}\text{O}_6$, $\text{C}_{23}\text{H}_{27}\text{O}_6$ requires $[\text{MH}]^+$ 399.18022.

(1E,4E)-1,5-Bis(4-methoxy-3-(methoxymethoxy)phenyl)penta-1,4-dien-3-one **366**



(1E,4E)-1,5-bis(4-methoxy-3-(methoxymethoxy)phenyl)penta-1,4-dien-3-one **366** was also isolated from the column chromatography of (E)-1-(3-allyl-2,4-dimethoxyphenyl)-3-(4-methoxy-3-(methoxymethoxy)phenyl)prop-2-en-1-one **355**. Purification by column chromatography (SiO_2 , ethyl acetate:hexane 1:9 to 1:1) afforded **366** as yellow solid (0.18 g, 0.04 mmol, 17%); m.p. 105 - 107 °C; R_f 0.07 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 3002, 2919, 2839, 1646, 1622, 1584, 1507, 1466, 1441, 1429, 1401, 1260, 1155, 1126, 1076, 974, 919, 799; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.56 (6H, s, OCH_3), 3.94 (6H, s, OCH_3), 5.30 (4H, s, H-2''), 6.92 (2H, d, J 8.4 Hz, H-5'), 6.95 (2H, J 15.8 Hz, H-2), 7.27 (2H, dd, J 1.9, 8.4 Hz, H-6'), 7.47 (2H, d, J 1.9 Hz, H-2'), 7.67 (2H, J 15.8 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 56.24 (s, OCH_3), 56.57 (s, OCH_3), 95.79 (s, OCH_2O), 111.85 (s, CH), 115.35 (s, CH), 124.14 (s, CH), 124.66 (s, CH), 128.26 (s, C), 143.03 (s, CH), 147.06 (s, C), 152.13 (s, C), 188.92 (s, CO); m/z (API-ES +) 415 ($[\text{MH}]^+$, 100%); HRMS found $[\text{MH}]^+$ 415.17558 for $\text{C}_{23}\text{H}_{27}\text{O}_7$, $\text{C}_{23}\text{H}_{27}\text{O}_7$ requires $[\text{MH}]^+$ 415.17513.

3-Methoxy-4-(methoxymethoxy)benzaldehyde **365**³⁵³

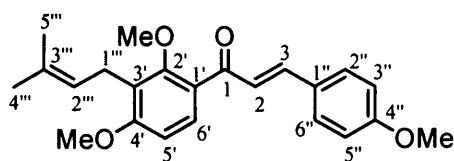


The method was adapted from that of Crombie²²⁸ *et al.* To a stirring solution of sodium hydride (0.16 g, 4.0 mmol, 60% dispersion in mineral oil) in anhydrous dimethyl formamide (2 cm^3) at 0 °C under argon 4-hydroxy-3-methoxybenzaldehyde (0.50 g, 3.3 mmol) in anhydrous dimethyl formamide (2 cm^3) was added slowly drop wise over 20 minutes. The reaction was then allowed to warm to room temperature, before being cooled to 0 °C.

Chapter 6 – Experimental

Chloromethyl methyl ether (0.30 cm³, 3.9 mmol) in anhydrous dimethyl formamide (1 cm³) was then added drop wise over 30 minutes to the mixture at 0 °C. The mixture was then stirred for 30 minutes at 0 °C and then stirred overnight at room temperature. The reaction was then tipped into a mixture of water (5 cm³) and ice (5 g), and extracted with ethyl acetate (4 × 6 cm³). The organic fraction was washed with an aqueous sodium hydroxide solution (2 × 3 cm³, 0.1M) and water (2 × 3 cm³), it was then dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:3) afforded **365** as pale yellow oil (0.51 g, 2.6 mmol, 80%); R_f 0.22 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2956, 2932, 2872, 2829, 1680, 1596, 1586, 1506, 1466, 1451, 1442, 1421, 1259, 1230, 1150, 1126, 1082, 919, 868, 817, 728; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.53 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 5.37 (3H, s, H-2''), 7.28 (1H, d, J 8.8 Hz, H-5'), 7.42 – 7.45 (2H, m, H-2' & H-6'), 9.88 (1H, s, H-1); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.27 (s, OCH₃), 56.76 (s, OCH₃), 95.24 (s, OCH₂O), 109.73 (s, CH), 114.91 (s, CH), 126.67 (s, CH), 131.30 (s, C), 150.30 (s, C), 152.23 (s, C), 191.24 (s, CHO).

(E)-1-(2,4-Dimethoxy-3-(3-methylbut-2-enyl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one
356



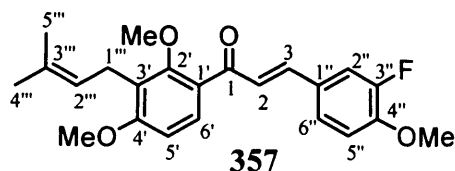
356

The chalcone **356** was obtained by following Protocol F. Using 1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone **347** (0.087 g, 0.35 mmol), *p*-anisaldehyde in methanol (0.35 cm³, 1M), and aqueous sodium hydroxide solution (0.35 cm³, 2M) in methanol (1.4 cm³). The mixture was extracted with dichloromethane (2 × 5 cm³) and washed with water (5 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9) afforded **356** as a pale yellow oil (0.069 g, 0.19 mmol, 54%); R_f 0.35 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2934, 1655, 1588, 1571, 1509, 1481, 1456, 1421, 1409, 1235, 1170, 802; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.69 (3H, d, J 1.2 Hz, CH₃), 1.79 (3H, s, CH₃), 3.40 (2H, d, J 6.9 Hz, H-1'''), 3.69 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 5.15 – 5.22 (1H, tm, J 6.9 Hz, H-2'''), 6.72 (1H, d, J 8.6 Hz, H-5'), 6.92 (2H, d, J 8.8 Hz, H-3'' & H-5''), 7.42 (1H, d, J 15.8 Hz, H-2), 7.58 (2H, d, J 8.8 Hz, H-2'' & H-6''), 7.59 (1H, d, J 8.6 Hz, H-6'), 7.70 (1H, d, J 15.8 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 18.10 (s, CH₃), 23.02 (s, CH₂), 26.02 (s, CH₃), 55.53 (s, OCH₃), 56.07 (s, CH₃), 63.29 (s, CH₃), 106.56 (s, CH), 114.57 (s, CH), 122.92 (s, CH), 124.12 (s, C), 124.34 (s, CH), 126.56 (s, C), 128.16 (s,

Chapter 6 – Experimental

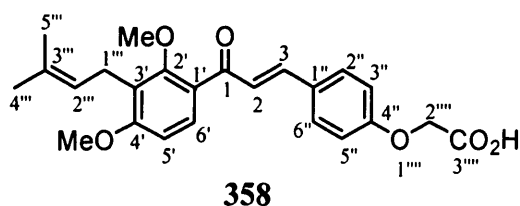
C), 129.80 (s, CH), 130.39 (s, CH), 131.85 (s, C), 143.28 (s, CH), 159.09 (s, C), 161.64 (s, C), 191.95 (s, CO); m/z (API ES+) 367 (MH^+ , 100%); HRMS found $[MH]^+$ 367.19117 for $C_{23}H_{27}O_4$, $C_{23}H_{27}O_4$ requires $[MH]^+$ 367.19039.

(E)-3-(3-Fluoro-4-methoxyphenyl)-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)prop-2-en-1-one **357**



The chalcone **357** was obtained by following Protocol F. Using 1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone **347** (0.087 g, 0.35 mmol), 3-fluoro-4-methoxybenzaldehyde (0.053 g, 0.35 mmol), and aqueous sodium hydroxide solution (0.35 cm^3 , 2M) in methanol (1.4 cm^3). The mixture was extracted with dichloromethane (2 \times 5 cm^3) and washed with water (5 cm^3). Purification by column chromatography (SiO_2 , ethyl acetate:hexane 1:19) afforded **357** as a yellow solid (0.100 g, 0.26 mmol, 75%); m.p. 64 – 66 $^{\circ}C$; R_f 0.29 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 2911, 1647, 1583, 1514, 1468, 1438, 1407, 1274, 1254, 1086, 976, 761; δ^1H NMR (400 MHz; $CDCl_3$; ppm) 1.69 (3H, d, J 0.8 Hz, CH_3), 1.80 (3H, s CH_3), 3.40 (2H, d, J 6.9 Hz, H-1'''), 3.69 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 5.15 – 5.22 (1H, tm, J 6.9 Hz, H-2'''), 6.73 (1H, d, J 8.4 Hz, H-5'), 6.96 (1H, t, J 8.4 Hz, H-5''), 7.33 (1H, br. d, J 8.4 Hz, H-6''), 7.39 (1H, dd, J 2.2 Hz, J 11.8 Hz, H-2''), 7.42 (1H, d, J 16.2 Hz, H-2), 7.60 (1H, d J 8.4 Hz, H-6'), 7.64 (1H, d, J 16.2 Hz, H-3); $\delta^{13}C$ NMR (100 MHz; $CDCl_3$; ppm) 18.11 (s, CH_3), 23.01 (s, CH_2), 26.01 (s, CH_3), 56.08 (s, OCH_3), 56.48 (s, OCH_3), 63.36 (s, OCH_3), 106.67 (s, CH), 113.38 (d, J 2.2 Hz, CH), 115.09 (d, J 18.3 Hz, CH), 122.84 (s, CH), 124.20 (s, C), 125.51 (s, CH) 126.08 (d, J 3.0 Hz, CH), 126.32 (s, C), 128.80 (d, J 6.6 Hz, C), 129.91 (s, CH), 131.93 (s, C), 141.91 (d, J 2.2 Hz, CH), 149.65 (d, J 11.0 Hz, C), 153.66 (d, J 245.4 Hz, CF), 159.23 (s, C), 161.87 (s, C), 191.46 (s, CO); m/z (API ES+) 385 (MH^+ , 100%); HRMS found $[MH]^+$ 385.18150 for $C_{23}H_{26}O_4F$, $C_{23}H_{26}O_4F$ requires $[MH]^+$ 385.18096.

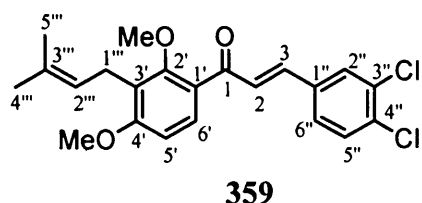
(E)-3-(4-Carboxymethoxyphenyl)-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl) prop-2-en-1-one **358**



Chapter 6 – Experimental

To a stirring solution of 1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone **347** (0.087 g, 0.35 mmol), and 4-formyl phenoxyacetic acid (0.063 g, 0.35 mmol) in methanol (1.4 cm³), was added an aqueous sodium hydroxide solution (0.70 cm³, 2M) and the mixture stirred at room temperature overnight. The mixture was then acidified with hydrochloric acid (until pH = 1, 2M), and extracted with ethyl acetate (4 × 5 cm³). The organic fraction was then washed with water (2 × 5 cm³) dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from methanol afforded **358** as pale brown crystals (0.119 g, 0.29 mmol, 85%); m.p. 148 – 150 °C; R_f 0.14 (SiO₂, methanol:ethyl acetate 1:4); ν_{max}(cm⁻¹) 2909, 1736, 1705, 1645, 1591, 1561, 1506, 1453, 1417, 1227, 1173, 808; δ ¹H NMR (400 MHz; CD₃OD; ppm) 1.67 (3H, d, *J* 0.8 Hz, CH₃), 1.78 (3H, s, CH₃), 3.38 (2H, d, *J* 6.9 Hz, H-1'''), 3.65 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 4.72 (2H, s, H-2'''), 5.11 – 5.19 (1H, tm, *J* 6.9 Hz, H-2'''), 6.85 (1H, d, *J* 8.8 Hz, H-5'), 6.98 (2H, d, *J* 8.6 Hz, H-3'' & H-5''), 7.42 (1H, d, *J* 16.6 Hz, H-2), 7.54 (1H, d, *J* 8.8 Hz, H-6'), 7.61 (2H, d, *J* 8.6 Hz, H-2'' & H-6''), 7.63 (1H, d, *J* 16.6 Hz, H-3); δ ¹³C NMR (100 MHz; CD₃OD; ppm) 16.80 (s, CH₃), 22.43 (s, CH₂), 24.74 (s, CH₃), 55.22 (s, CH₃), 62.29 (s, CH₃), 64.62 (s, CH₂), 106.37 (s, CH), 115.02 (s, CH), 122.72 (s, CH), 124.00 (s, C), 124.12 (s, CH), 125.89 (s, C), 128.51 (s, C), 129.50 (s, CH), 130.15 (s, CH), 131.13 (s, C), 143.80 (s, CH), 158.98 (s, C), 160.35 (s, C), 161.97 (s, C), 171.02 (s, CO₂H), 192.83 (s, CO); *m/z*(API ES⁺) 411 (MH⁺, 100%); HRMS found [MH]⁺ 411.18079 for C₂₄H₂₇O₆, C₂₄H₂₇O₆ requires [MH]⁺ 411.18022.

(E)-3-(3,4-Dichlorophenyl)-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)prop-2-en-1-one
359

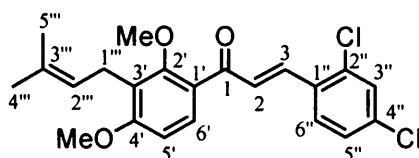


The chalcone **359** was obtained by following Protocol F. Using 1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone **347** (0.087 g, 0.35 mmol), 3,4-dichlorobenzaldehyde (0.062 g, 0.35 mmol), and aqueous sodium hydroxide solution (0.35 cm³, 2M) in methanol (1.4 cm³). The mixture was extracted with dichloromethane (2 × 5 cm³) and washed with water (5 cm³). Purification by column chromatography (SiO₂, dichloromethane:hexane 3:1) afforded **359** as a yellow oil (0.037g, 0.09 mmol, 25%); R_f 0.42 (SiO₂, dichloromethane:hexane 3:1); ν_{max}(cm⁻¹) 2934, 1659, 1586, 1552, 1470, 1410, 1322, 1270, 805; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.70 (3H, d, *J* 1.2 Hz, CH₃), 1.80 (3H, s, CH₃), 3.40 (2H, d, *J* 6.9 Hz, H-1'''), 3.68 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 5.15 – 5.21 (1H, tm, *J*

Chapter 6 – Experimental

6.9 Hz, H-2'''), 6.75 (1H, d, J 8.8 Hz, H-5'), 7.44 (1H, dd, J 1.7, 8.3 Hz, H-6''), 7.47 (1H, d, J 8.3 Hz, H-5''), 7.55 (1H, d, J 16.0 Hz, H-2), 7.62 (1H, d, J 16.0 Hz, H-3), 7.63 (1H, d, J 8.8 Hz, H-6'), 7.69 (1H, d, J 1.7 Hz, H-2''); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 18.12 (s, CH_3), 22.99 (s, CH_2), 26.00 (s, CH_3), 56.12 (s, OCH_3), 63.49 (s, OCH_3), 106.84 (s, CH), 122.74 (s, CH), 124.31 (s, C), 125.97 (s, C), 127.62 (s, CH), 128.03 (s, CH), 130.03 (s, CH), 130.11 (s, CH), 131.08 (s, CH), 132.04 (s, C), 133.41 (s, C), 134.15 (s, C), 135.60 (s, C), 140.10 (s, CH), 159.40 (s, C), 162.23 (s, C), 196.96 (s, CO); m/z (API ES+) 405 (MH^+ , 100%), 407 (MH^+ , 60%); HRMS found $[\text{MH}]^+$ 405.10242 for $\text{C}_{22}\text{H}_{23}\text{Cl}_2\text{O}_3$, $\text{C}_{22}\text{H}_{23}\text{Cl}_2\text{O}_3$ requires $[\text{MH}]^+$ 405.10188.

(E)-3-(2,4-Dichlorophenyl)-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)prop-2-en-1-one
360

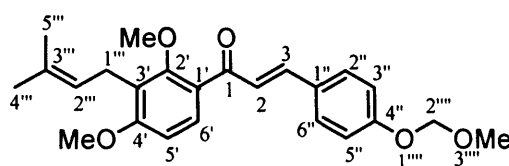


360

The chalcone **360** was obtained by following Protocol F. Using 1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone **347** (0.087 g, 0.35 mmol), 2,4-dichlorobenzaldehyde (0.062 g, 0.35 mmol), and aqueous sodium hydroxide solution (0.35 cm^3 , 2M) in methanol (1.4 cm^3). The mixture was extracted with dichloromethane ($2 \times 5 \text{ cm}^3$) and washed with water (5 cm^3). Purification by column chromatography (SiO_2 , ethyl acetate:hexane 1:9) afforded **360** as a yellow oil (0.126 g, 0.31 mmol, 89%); R_f 0.56 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 2937, 1657, 1583, 1466, 1410, 1272, 1256, 806; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 1.69 (3H, d, J 1.2 Hz, CH_3), 1.79 (3H, s, CH_3), 3.40 (2H, d, J 6.9 Hz, H-1'''), 3.69 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 5.14 – 5.20 (1H, tm, J 6.9 Hz, H-2'''), 6.75 (1H, d, J 8.8 Hz, H-5'), 7.27 (1H, dd, J 1.7, 8.4 Hz, H-5''), 7.45 (1H, d, J 1.7 Hz, H-3''), 7.53 (1H, d, J 15.8 Hz, H-2), 7.64 (1H, d, J 8.8 Hz, H-6'), 7.68 (1H, d, J 8.4 Hz, H-6''), 8.05 (1H, d, J 15.8 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 18.11 (s, CH_3), 22.98 (s, CH_2), 26.00 (s, CH_3), 56.11 (s, OCH_3), 63.51 (s, OCH_3), 106.82 (s, CH), 122.75 (s, CH), 124.26 (s, C), 125.99 (s, C), 127.72 (s, CH), 128.67 (s, CH), 129.19 (s, CH), 130.17 (s, CH), 130.23 (s, CH), 132.00 (s, C), 132.38 (s, C), 136.21 (s, C), 136.27 (s, C), 137.39 (s, CH), 159.41 (s, C), 162.19 (s, C), 191.00 (s, CO); m/z (API ES+) 405 (MH^+ , 100%), 407 (MH^+ , 70%); HRMS found $[\text{MH}]^+$ 405.10239 for $\text{C}_{22}\text{H}_{23}\text{Cl}_2\text{O}_3$, $\text{C}_{22}\text{H}_{23}\text{Cl}_2\text{O}_3$ requires $[\text{MH}]^+$ 405.10188.

Chapter 6 – Experimental

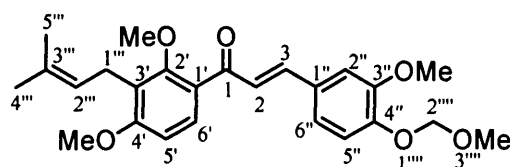
(E)-1-(2,4-Dimethoxy-3-(3-methylbut-2-enyl)phenyl)-3-(4-(methoxymethoxy)phenyl) prop-2-en-1-one **361**



361

The chalcone **361** was obtained by following Protocol F. Using 1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone **347** (0.087 g, 0.35 mmol), 4-(methoxymethoxy)benzaldehyde **332** (0.059 g, 0.35 mmol), and aqueous sodium hydroxide solution (0.35 cm³, 2M) in methanol (1.4 cm³). The mixture was extracted with dichloromethane (2 × 5 cm³) and washed with water (5 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:19) afforded **361** as a yellow oil (0.120, 0.30 mmol, 86%) R_f 0.37 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2933, 1655, 1590, 1508, 1481, 1455, 1421, 1409, 1232, 1151, 982; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.69 (3H, d, *J* 0.8 Hz, CH₃), 1.79 (3H, s, CH₃), 3.40 (2H, d, *J* 6.7 Hz, H-1'''), 3.48 (3H, s, OCH₃), 3.69 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 5.16 – 5.22 (1H, tm, *J* 6.7 Hz, H-2'''), 5.21 (2H, s, H-2'''), 6.73 (1H, d, *J* 8.8 Hz, H-5'), 7.06 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.44 (1H, d, *J* 16.0 Hz, H-2), 7.57 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.60 (1H, d, *J* 8.8 Hz, H-6'), 7.69 (1H, d, *J* 16.0 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 18.10 (s, CH₃), 23.02 (s, CH₂), 26.02 (s, CH₃), 56.07 (s, OCH₃), 56.39 (s, OCH₃), 63.30 (s, OCH₃), 94.43 (s, OCH₂O), 106.58 (s, CH), 116.69 (s, CH), 122.90 (s, CH), 124.13 (s, C), 124.78 (s, CH), 126.50 (s, C), 129.21 (s, C), 129.84 (s, CH), 130.29 (s, CH), 131.87 (s, C), 143.06 (s, CH), 159.13 (s, C), 159.17 (s, C), 161.69 (s, C), 191.90 (s, CO); *m/z* (API ES⁺) 397 (MH⁺, 100%); HRMS found [MH]⁺ 397.20159 for C₂₄H₂₉O₅, C₂₄H₂₉O₅ requires [MH]⁺ 397.20095.

(E)-1-(2,4-Dimethoxy-3-(3-methylbut-2-enyl)phenyl)-3-(3-methoxy-4-(methoxymethoxy)phenyl) prop-2-en-1-one **362**



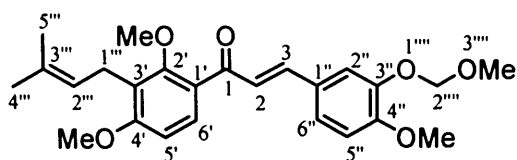
362

The chalcone **362** was obtained by following Protocol F. Using 1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone **347** (0.087 g, 0.35 mmol), 3-methoxy-4-(methoxymethoxy)benzaldehyde **365** (0.069 g, 0.35 mmol), and aqueous sodium hydroxide

Chapter 6 – Experimental

solution (0.35 cm³, 2M) in methanol (1.4 cm³). The mixture was extracted with dichloromethane (2 × 5 cm³) and washed with water (5 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:19) afforded **362** as a yellow oil (0.128 g, 0.30 mmol, 85%); R_f 0.25 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2934, 1655, 1588, 1507, 1482, 1453, 1410, 1217, 1088, 978, 806; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.69 (3H, d, *J* 0.8 Hz, CH₃), 1.80 (3H, s, CH₃), 3.41 (2H, d, *J* 6.9 Hz, H-1'''), 3.52 (3H, s, OCH₃), 3.69 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 5.16 - 5.22 (1H, tm, *J* 6.9 Hz, H-2'''), 5.28 (2H, s, H-2'''''), 6.73 (1H, d, *J* 8.8 Hz, H-5'), 7.15 (1H, d, *J* 1.7 Hz, H-2''), 7.17 (1H, d, *J* 8.3 Hz, H-5''), 7.20 (1H, dd, *J* 1.7, 8.3 Hz, H-6''), 7.41 (1H, d, *J* 15.6 Hz, H-2), 7.59 (1H, d, *J* 8.8 Hz, H-6'), 7.66 (1H, d, *J* 15.6 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 18.10 (s, CH₃), 23.03 (s, CH₂), 26.02 (s, CH₃), 56.07 (s, OCH₃), 56.23 (s, OCH₃), 56.57 (s, OCH₃), 63.30 (s, OCH₃), 95.42 (s, OCH₂O), 106.57 (s, CH), 111.19 (s, CH), 116.09 (s, CH), 122.66 (s, CH), 122.88 (s, CH), 124.15 (s, C), 125.13 (s, CH), 126.48 (s, C), 129.78 (s, CH), 129.81 (s, C), 131.89 (s, C), 143.41 (s, CH), 148.74 (s, C), 150.02 (s, C), 159.06 (s, C), 161.68 (s, C), 192.01 (s, CO); *m/z*(API ES+) 427 (MH⁺, 100%); HRMS found [MH]⁺ 427.21192 for C₂₅H₃₁O₆, C₂₅H₃₁O₆ requires [MH]⁺ 427.21152.

(E)-1-(2,4-Dimethoxy-3-(3-methylbut-2-enyl)phenyl)-3-(4-methoxy-3-(methoxymethoxy)phenyl)prop-2-en-1-one **363**



363

The chalcone **363** was obtained by following Protocol F. Using 1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)ethanone **347** (0.087 g, 0.35 mmol), 4-methoxy-3-(methoxymethoxy)benzaldehyde **264** (0.069 g, 0.35 mmol), and aqueous sodium hydroxide solution (0.35 cm³, 2M) in methanol (1.4 cm³). The mixture was extracted with dichloromethane (2 × 5 cm³) and washed with water (5 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:19) afforded **363** as a pale yellow oil (0.136 g, 0.32 mmol, 91%); R_f 0.20 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 2934, 1655, 1588, 1509, 1482, 1442, 1409, 1218, 1154, 1132, 1088, 996, 804; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.69 (3H, d, *J* 0.8 Hz, CH₃), 1.80 (3H, s, CH₃), 3.41 (2H, d, *J* 6.9 Hz, H-1'''), 3.54 (3H, s, OCH₃), 3.70 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 5.16 - 5.22 (1H, tm, *J* 6.9 Hz, H-2'''), 5.27 (2H, s, H-2'''''), 6.73 (1H, d, *J* 8.8 Hz, H-5'), 6.91 (1H, d, *J* 8.3 Hz, H-5''), 7.28 (1H, dd, *J* 2.0 Hz, *J* 8.3 Hz, H-6''), 7.37(1H, d, *J* 16.0 Hz, H-2), 7.43 (1H, d, *J* 2.0

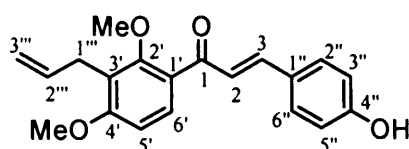
Chapter 6 – Experimental

Hz, H-2''), 7.57 (1H, d, J 8.8 Hz, H-6'), 7.63 (1H, d, J 15.6 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 18.09 (s, CH_3), 23.04 (s, CH_2), 26.02 (s, CH_3), 56.07 (s, OCH_3), 56.23 (s, OCH_3), 56.55 (s, OCH_3), 63.22 (s, OCH_3), 95.87 (s, OCH_2O), 106.50 (s, CH), 111.87 (s, CH), 116.24 (s, CH), 122.91 (s, CH), 124.15 (s, C), 124.17 (s, CH), 124.94 (s, CH), 126.47 (s, C), 128.55 (s, C), 129.69 (s, CH), 131.85 (s, C), 143.51 (s, CH), 146.91 (s, C), 152.01 (s, C), 159.01 (s, C), 161.58 (s, C), 192.14 (s, CO); m/z (API ES+) 427 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 427.21212 for $\text{C}_{25}\text{H}_{31}\text{O}_6$, $\text{C}_{25}\text{H}_{31}\text{O}_6$ requires $[\text{MH}]^+$ 427.21152.

Protocol G

The method adopted was that of Wang²³⁰ *et al.* To a stirring solution of MOM-protected compound in methanol under argon was added hydrochloric acid (3M). The reaction was then heated to reflux at 75 °C for 45 minutes. The reaction was cooled to room temperature and tipped into water (6 cm^3), it was then extracted with ethyl acetate (3 \times 6 cm^3). The organic fraction was then washed with water (2 \times 5 cm^3), dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Purified by column chromatography or recrystallization.

(*E*)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **367**



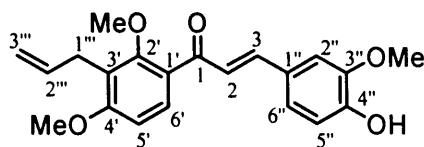
367

The chalcone **367** was obtained by following Protocol G. Using (*E*)-1-(3-allyl-2,4-dimethoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **353** (0.134 g, 0.36 mmol), methanol (7.3 cm^3) and hydrochloric acid (1.3 cm^3 , 3M). Purification by column chromatography (SiO_2 , ethyl acetate:hexane 1:9 to 1:4) afforded **367** as a yellow oil (0.112 g, 0.35 mmol, 95%); R_f 0.13 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 3232, 2938, 1638, 1576, 1510, 1483, 1438, 1408, 1272, 1216, 1095, 808; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.47 (2H, td, J 1.5, 6.0 Hz, H-1'''), 3.71 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.97 – 5.03 (2H, m, H-3'''), 5.93 (1H, s, OH-4''), 6.00 (1H, ddt, J 9.6, 17.6, 6.0 Hz, H-2'''), 6.75 (1H, d, J 8.7 Hz, H-5'), 6.88 (2H, d, J 8.6 Hz, H-3'' & H-5''), 7.41 (1H, d, J 15.8 Hz, H-2), 7.52 (2H, d, J 8.6 Hz, H-2'' & H-6''), 7.63 (1H, d, J 8.7 Hz, H-6'), 7.70 (1H, d, J 15.8 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 26.04 (s, CH_2), 56.13 (s, OCH_3), 65.56 (s, OCH_3), 106.65 (s, CH), 114.97 (s, CH_2), 116.18 (s, CH), 122.29 (s, C), 124.06 (s, CH), 126.44 (s, C), 128.03 (s, C), 130.29 (s, CH), 130.68 (s, CH), 136.85 (s, CH), 143.75 (s, CH), 158.19 (s, C), 159.24 (s,

Chapter 6 – Experimental

C), 161.77 (s, C), 192.30 (s, CO); m/z (API ES⁺) 325 (MH⁺, 100%); HRMS found [MH]⁺ 325.14524 for C₂₀H₂₁O₄, C₂₀H₂₁O₄ requires [MH]⁺ 325.14344.

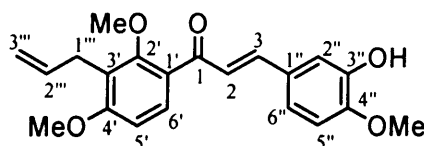
(E)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one **368**



368

The chalcone **368** was obtained by following Protocol G. Using *(E)*-1-(3-allyl-2,4-dimethoxyphenyl)-3-(3-methoxy-4-(methoxymethoxy)phenyl)prop-2-en-1-one **354** (0.152 g, 0.38 mmol), methanol (7.6 cm³) and hydrochloric acid (1.4 cm³, 3M). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:3) afforded **368** as a yellow oil (0.134 g, 0.38 mmol, 99%); R_f 0.13 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3355, 2937, 1638, 1583, 1508, 1482, 1454, 1427, 1408, 1207, 1117, 1076, 807; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.48 (2H, td, J 1.5, 6.0 Hz, H-1'''), 3.71 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 4.97 – 5.03 (2H, m, H-3'''), 5.91 (1H, s, OH-4''), 6.00 (1H, ddt, J 9.4, 17.7, 6.0 Hz, H-2''), 6.74 (1H, d, J 8.7 Hz, H-5'), 6.93 (1H, d, J 8.3 Hz, H-5''), 7.04 (1H, d, J 1.8, H-2''), 7.18 (1H, dd J 1.8, 8.3 Hz, H-6''), 7.36 (1H, d, J 15.8 Hz, H-2), 7.61 (1H, d, J 8.7 Hz, H-6'), 7.66 (1H, d, J 15.8 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 28.05 (s, CH₂), 56.12 (s, OCH₃), 56.25 (s, OCH₃), 63.53 (s, OCH₃), 106.58 (s, CH), 110.17 (s, CH), 114.94 (s, CH₂), 115.00 (s, CH), 122.22 (s, C), 123.57 (s, CH), 124.20 (s, CH), 126.58 (s, C), 127.93 (s, C), 130.18 (s, CH), 136.90 (s, CH), 143.96 (s, CH), 146.97 (s, C), 148.25 (s, C), 159.10 (s, C), 161.62 (s, C), 192.01 (s, CO); m/z (API ES⁺) 355 (MH⁺, 100%); HRMS found [MH]⁺ 355.15441 for C₂₁H₂₃O₅, C₂₁H₂₃O₅ requires [MH]⁺ 355.15400.

(E)-1-(3-Allyl-2,4-dimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one **369**



369

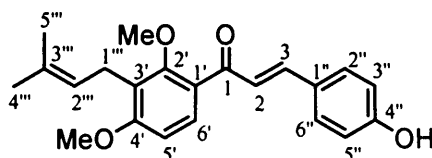
The chalcone **369** was obtained by following Protocol G. Using *(E)*-1-(3-allyl-2,4-dimethoxyphenyl)-3-(4-methoxy-3-(methoxymethoxy)phenyl)prop-2-en-1-one **355** (0.137 g, 0.34 mmol), methanol (6.9 cm³) and hydrochloric acid (1.3 cm³, 3M). Purification by column chromatography (SiO₂, ethyl acetate:hexane 3:17 to 1:3) afforded **369** as a yellow solid (0.120 g, 0.34 mmol, 98%); m.p. 91 – 94 °C; R_f 0.15 (SiO₂, ethyl acetate:hexane 1:3);

Chapter 6 – Experimental

$\nu_{\text{max.}}(\text{cm}^{-1})$ 3377, 2937, 2839, 1638, 1583, 1508, 1483, 1456, 1439, 1211, 1118, 1076; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.47 (2H, td, J 1.4, 6.0 Hz, H-1'''), 3.70 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 4.97 – 5.03 (2H, m, H-3'''), 5.66 (1H, s, OH-3'''), 6.00 (1H, ddt, J 9.5, 17.7, 6.0 Hz, H-2''), 6.74 (1H, d, J 8.7 Hz, H-5'), 6.86 (1H, d, J 8.4 Hz, H-5''), 7.12 (1H, dd, J 2.0, 8.4 Hz, H-6''), 7.26 (1H, d, J 2.0 Hz, H-2''), 7.41 (1H, d, J 15.7 Hz, H-2), 7.63 (1H, d, J 8.7 Hz, H-6'), 7.66 (1H, d, J 15.7 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 28.04 (s, CH_2), 56.11 (s, OCH_3), 56.24 (s, OCH_3), 63.52 (s, OCH_3), 106.60 (s, CH), 110.75 (s, CH), 113.38 (s, CH), 114.96 (s, CH_2), 122.26 (s, C), 122.69 (s, CH), 124.76 (s, CH), 126.53 (s, C), 129.06 (s, C), 130.27 (s, CH), 136.88 (s, C), 143.39 (s, CH), 146.06 (s, C), 148.76 (s, C), 159.28 (s, C), 161.70 (s, C), 191.75 (s, CO); m/z (API ES+) 355 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 355.15431 for $\text{C}_{21}\text{H}_{23}\text{O}_5$, $\text{C}_{21}\text{H}_{23}\text{O}_5$ requires $[\text{MH}]^+$ 355.15400.

(E)-3-(4-Hydroxyphenyl)-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)prop-2-en-1-one

370

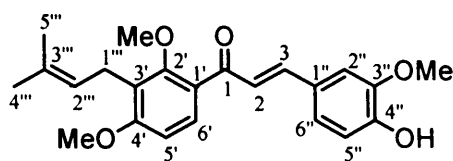


370

The chalcone **370** was obtained by following Protocol G. Using *(E)*-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)-3-(4-(methoxymethoxy)phenyl) prop-2-en-1-one **361** (0.101 g, 0.25 mmol), methanol (5.1 cm^3) and hydrochloric acid (0.9 cm^3 , 3M). Purification by column chromatography (SiO_2 , ethyl acetate:hexane 3:17) afforded **370** as a pale yellow solid (0.080 g, 0.23 mmol, 89%); m.p. 131 – 132 $^\circ\text{C}$; R_f 0.13 (SiO_2 , ethyl acetate:hexane 1:3); $\nu_{\text{max.}}(\text{cm}^{-1})$ 3362, 2935, 1651, 1589, 1557, 1541, 1483, 1436, 1273, 1244, 1068, 811; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 1.69 (3H, d, J 1.0 Hz, CH_3), 1.79 (3H, s, CH_3), 3.40 (2H, d, J 6.7 Hz, H-1'''), 3.70 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.16 – 5.20 (1H, tm, J 6.7 Hz, H-2'''), 6.49 (1H, s, OH-4''), 6.73 (1H, d, J 8.7 Hz, H-5'), 6.89 (2H, d, J 8.5 Hz, H-3'' & H-5''), 7.42 (1H, d, J 15.8 Hz, H-2), 7.51 (2H, d, J 8.5 Hz, H-2'' & H-6''), 7.60 (1H, d, J 8.7 Hz, H-6'), 7.70 (1H, d, J 15.8 Hz, H-3); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 18.10 (s, CH_3), 23.02 (s, CH_2), 26.00 (s, CH_3), 56.08 (s, OCH_3), 63.33 (s, OCH_3), 106.63 (s, CH), 116.23 (s, CH), 122.84 (s, CH), 123.98 (s, CH), 124.22 (s, C), 126.34 (s, C), 127.87 (s, C), 129.86 (s, CH), 130.71 (s, CH), 131.91 (s, C), 144.02 (s, CH), 158.47 (s, C), 159.13 (s, C), 161.81 (s, C), 192.63 (s, CO); m/z (API ES+) 353 (MH^+ , 100%); HRMS found $[\text{MH}]^+$ 353.17624 for $\text{C}_{22}\text{H}_{25}\text{O}_4$, $\text{C}_{22}\text{H}_{25}\text{O}_4$ requires $[\text{MH}]^+$ 353.17474.

Chapter 6 – Experimental

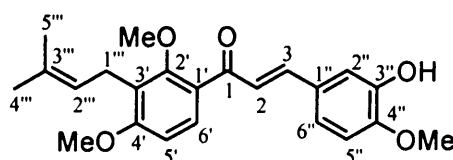
(E)-3-(4-Hydroxy-3-methoxyphenyl)-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)prop-2-en-1-one **371**



371

The chalcone **371** was obtained by following Protocol G. Using *(E)*-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)-3-(3-methoxy-4-(methoxymethoxy)phenyl)prop-2-en-1-one **362** (0.104 g, 0.24 mmol), methanol (4.9 cm³) and hydrochloric acid (0.9 cm³, 3M). Purification by recrystallization from methanol afforded **371** as light yellow crystals (0.067 g, 0.17 mmol, 72%); m.p. 91 – 92 °C; R_f 0.13 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3455, 2936, 1647, 1584, 1509, 1456, 1429, 1411, 1085, 1062, 810; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.69 (3H, d, J 1.1 Hz, CH₃), 1.79 (3H, s, CH₃), 3.41 (2H, d, J 6.9 Hz, H-1'''), 3.70 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 5.16 – 5.21 (1H, tm, J 6.9 Hz, H-2'''), 5.94 (1H, s, OH-4''), 6.73 (1H, d, J 8.7 Hz, H-5''), 6.94 (1H, d, J 8.2 Hz, H-5''), 7.11 (1H, d, J 1.9 Hz, H-2''), 7.18 (1H, dd, J 1.9, 8.2 Hz, H-6''), 7.37 (1H, d, J 15.8 Hz, H-2), 7.58 (1H, d, J 8.7 Hz, H-6'), 7.65 (1H, d, J 15.8 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 18.10 (s, CH₃), 23.02 (s, CH₂), 26.01 (s, CH₃), 56.06 (s, OCH₃), 56.23 (s, OCH₃), 63.28 (s, OCH₃), 106.53 (s, CH), 110.20 (s, CH), 115.00 (s, CH), 122.90 (s, CH), 123.50 (s, CH), 124.10 (s, C), 124.32 (s, CH), 126.55 (s, C), 127.98 (s, C), 129.71 (s, CH), 131.85 (s, C), 143.85 (s, CH), 146.97 (s, C), 148.21 (s, C), 158.96 (s, C), 161.58 (s, C), 192.08 (s, CO); m/z (API ES+) 383 (MH⁺, 100%); HRMS found [MH]⁺ 383.18531 for C₂₃H₂₇O₅, C₂₃H₂₇O₅ requires [MH]⁺ 383.18530.

(E)-3-(3-Hydroxy-4-methoxyphenyl)-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)prop-2-en-1-one **372**



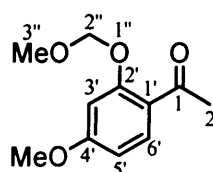
372

The chalcone **372** was obtained by following Protocol G. Using *(E)*-1-(2,4-dimethoxy-3-(3-methylbut-2-enyl)phenyl)-3-(4-methoxy-3-(methoxymethoxy)phenyl)prop-2-en-1-one **363** (0.123 g, 0.29 mmol), methanol (5.8 cm³) and hydrochloric acid (1.1 cm³, 3M). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:4) followed by recrystallization

Chapter 6 – Experimental

from methanol afforded **372** as light yellow needles (0.065g, 0.17 mmol, 59%); m.p. 111 – 113 °C; R_f 0.17 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3483, 2938, 1647, 1584, 1510, 1481, 1451, 1440, 1267, 1089, 1010, 805; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.69 (3H, d, J 1.0 Hz, CH₃), 1.80 (3H, s, CH₃), 3.40 (2H, d, J 6.9 Hz, H-1'''), 3.69 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 5.16 – 5.21 (1H, tm, J 6.9 Hz, H-2'''), 5.68 (1H, s, OH-3''), 6.72 (1H, d, J 8.8 Hz, H-5'), 6.86 (1H, d, J 8.4 Hz, H-5''), 7.12 (1H, dd, J 2.0, 8.4 Hz, H-6'), 7.27 (1H, d, J 2.0 Hz, H-2''), 7.42 (1H, d, J 15.7 Hz, H-2), 7.60 (1H, d, J 8.8 Hz, H-6'), 7.65 (1H, d, J 15.7 Hz, H-3); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 18.10 (s, CH₃), 23.01 (s, CH₂), 26.00 (s, CH₃), 56.05 (s, OCH₃), 56.24 (s, OCH₃), 63.27 (s, OCH₃), 106.56 (s, CH), 110.75 (s, CH), 113.35 (s, CH), 122.69 (s, CH), 122.89 (s, CH), 124.14 (s, C), 124.85 (s, CH), 126.49 (s, C), 129.12 (s, C), 129.83 (s, CH), 131.83 (s, C), 143.27 (s, CH), 146.06 (s, C), 148.74 (s, C), 159.17 (s, C), 161.68 (s, C), 191.77 (s, CO); m/z (API ES+) 383 (MH⁺, 100%); HRMS found [MH]⁺ 383.18547 for C₂₃H₂₇O₅, C₂₃H₂₇O₅ requires [MH]⁺ 383.18530.

1-(4-Methoxy-2-(methoxymethoxy)phenyl)ethanone **374**³⁵⁴



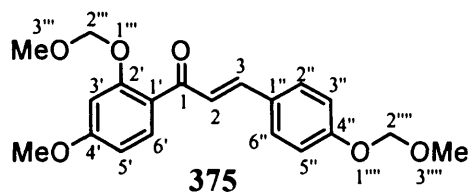
374

To a stirring solution of 2-hydroxy-4-methoxyacetophenone (1.66g, 10.0 mmol) in anhydrous tetrahydrofuran (3.5 cm³) at 0 °C under argon, sodium hydride (0.60 g, 15.0 mmol, 60% dispersion in mineral oil) was added. The reaction was then stirred for 5 minutes at 0 °C, before adding Chloromethyl methyl ether (1.14 cm³, 15.0 mmol) to the mixture. The reaction was then stirred for a further 10 minutes at 0 °C. The reaction was then heated to 65 °C for 18 hours. The reaction was then allowed to cool to room temperature; it was then tipped into water (5 cm³) and extracted with ethyl acetate (3 × 10 cm³). The organic fraction was then washed with brine (2 × 10 cm³) and dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:4) afforded **374** as a pale yellow oil (1.37 g, 6.5 mmol, 65%); R_f 0.50 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 2944, 2840, 1664, 1596, 1573, 1499, 1449, 1217, 1153, 1134, 1064, 984, 921, 833; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.61 (3H, s, H-2), 3.52 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 5.28 (2H, s, H-2''), 6.58 (1H, dd, J 2.4, 8.8 Hz, H-5'), 6.70 (1H, d, J 2.4 Hz, H-3'), 7.82 (1H, d, J 8.8 Hz, H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 31.98 (s, CH₃), 55.76 (s, OCH₃), 56.67 (s, OCH₃), 94.69(s, OCH₂O), 101.09 (s, CH), 107.10 (s, CH), 121.93 (s, C), 132.63 (s, CH), 158.82 (s, C), 164.49 (s, C), 197.94 (s, CO); m/z (API

Chapter 6 – Experimental

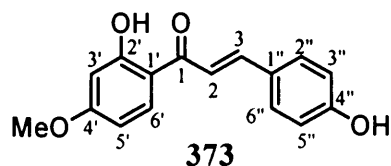
ES+) 211 (MH⁺, 100%), 233 (MNa⁺); HRMS found [MH]⁺ 211.09654 for C₁₁H₁₅O₄, C₁₁H₁₅O₄ requires [MH]⁺ 211.09649.

(E)-1-(4-Methoxy-2-(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **375**



The chalcone **375** was obtained by following Protocol F. Using *1*-(4-methoxy-2-(methoxymethoxy)phenyl)ethanone **374** (0.100 g, 0.48 mmol), 4-(methoxymethoxy)benzaldehyde **332** (0.079 g, 0.47 mmol), and aqueous sodium hydroxide solution (0.96 cm³, 2M) in methanol (1.0 cm³). The mixture was extracted with ethyl acetate (3 × 5 cm³). Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:19 to 3:7) afforded **375** as a yellow oil (0.129 g, 0.36 mmol, 62%); R_f 0.23 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 2936, 2828, 1687, 1650, 1598, 1572, 1508, 1443, 1422, 1238, 1210, 1150, 1076, 920, 830, 807; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.48 (3H, s, OCH₃), 3.50 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 5.21 (3H, s, H-2'''), 5.25 (3H, s, H'''), 6.63 (1H, dd, *J* 2.3, 8.8 Hz, H-5'), 6.73 (1H, d, *J* 2.3 Hz, H-3'), 7.05 (2H, d, *J* 8.6 Hz, H-3'' & H-5''), 7.38 (1H, d, *J* 16.0 Hz, H-2), 7.53 (2H, d, *J* 8.6 Hz, H-2'' & H-6''), 7.63 (1H, d, *J* 16.0 Hz, H-3), 7.70 (1H, d, *J* 8.8 Hz, H-6'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.79 (s, OCH₃), 56.39 (s, OCH₃), 56.72 (s, OCH₃), 94.44 (s, OCH₂O), 95.24 (s, OCH₂O), 101.86 (s, CH), 107.35 (s, CH), 116.72 (s, CH), 123.41 (s, C), 125.63 (s, CH), 129.33 (s, C), 130.09 (s, CH), 132.58 (s, CH), 142.41 (s, CH), 157.94 (s, C), 159.10 (s, C), 163.92 (s, C), 191.29 (s, CO); *m/z*(API ES+) 358 (MH⁺, 100%); HRMS found [MH]⁺ 359.14959 for C₂₀H₂₃O₆, C₂₀H₂₃O₆ requires [MH]⁺ 359.14891.

(E)-1-(2-Hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **373**³⁵⁵

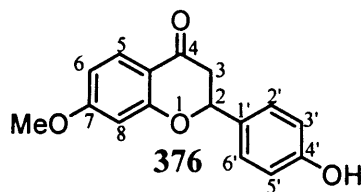


The method adopted was that of Wang²³⁰ *et al.* To a stirring solution of *(E)*-1-(4-methoxy-2-(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **375** (0.039 g, 0.14 mmol) in methanol (3 cm³) under argon was added hydrochloric acid (1.0 cm³, 3M). The reaction was then heated to reflux at 75 °C for 45 minutes. The reaction was cooled to room temperature and tipped into water (3 cm³), it was then extracted with chloroform (3 ×

Chapter 6 – Experimental

10 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Purification of the crude residue by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:4) afforded **373** as a yellow solid (0.012 g, 0.04 mmol, 41%); m.p. 151 - 156 °C; R_f 0.18 (SiO₂, ethyl acetate:hexane 1:19 to 1:3); ν_{\max} (cm⁻¹) 3227, 2922, 2851, 1626, 1607, 1589, 1543, 1505, 1456, 1441, 1417, 1368, 1280, 1215, 1163, 1128, 1021, 997, 959, 790; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.87 (3H, s, OCH₃), 4.99 (1H, s, OH-4''), 6.47 – 6.51 (2H, m, H-3' & H-5'), 6.89 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.46 (1H, d, *J* 15.4 Hz, H-2), 7.58 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.83 (1H, d, *J* 9.2 Hz, H-6'), 7.86 (1H, d, *J* 15.4 Hz, H-3), 13.54 (1H, s, OH-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 55.83 (s, OCH₃), 101.31 (s, CH), 107.93 (s, CH), 114.36 (s, C), 116.25 (s, CH), 118.15 (s, CH), 127.98 (s, C), 130.83 (s, CH), 131.38 (s, CH), 144.47 (s, CH), 158.24 (s, C), 166.34 (s, C), 166.84 (s, C), 192.19 (s, CO); *m/z*(API-ES+) 271 (MH⁺, 100%), 293 (MNa⁺, 10%); HRMS found [MH]⁺ 271.09700 for C₁₆H₁₅O₄, C₁₆H₁₅O₄ requires [MH]⁺ 271.09649.

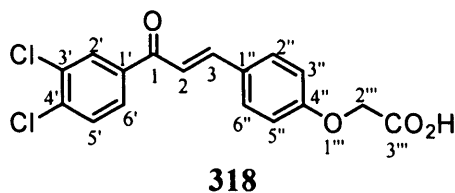
2,3-Dihydro-2-(4-hydroxyphenyl)-7-methoxychromen-4-one **376**²⁸¹



2,3-Dihydro-2-(4-hydroxyphenyl)-7-methoxychromen-4-one **376** was also isolated from the column chromatography of (*E*)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **373**. Purification by column chromatography (SiO₂, ethyl acetate:hexane 1:19 to 1:3) afforded **376** as yellow cube crystals (0.010 g, 0.04 mmol, 34%); m.p. 181 - 182 °C; R_f 0.16 (SiO₂, ethyl acetate:hexane 1:3); ν_{\max} (cm⁻¹) 3253, 2923, 2850, 1652, 1594, 1573, 1516, 1441, 1251, 1200, 1161, 1115, 834, 815; δ ¹H NMR (400 MHz; CDCl₃; ppm) 2.81 (1H, dd, *J* 2.9 Hz, *J* 16.9 Hz, H-3), 3.06 (1H, dd, *J* 13.2, 16.9 Hz, H-3), 3.83 (3H, s, OCH₃), 5.41 (1H, dd, *J* 2.9, 13.2 Hz, H-2), 5.50 (1H, s, OH-4'), 6.48 (1H, d, *J* 2.4 Hz, H-8), 6.62 (1H, dd, *J* 2.4, 8.7 Hz, H-6), 6.90 (2H, d, *J* 8.4 Hz, H-3' & H-5'), 7.35 (2H, d, *J* 8.4 Hz, H-2' & H-6'), 7.87 (1H, d, *J* 8.7 Hz, H-5); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 44.14 (s, CH₂), 55.65 (s, OCH₃), 79.73 (s, CH), 100.92 (s, CH), 110.26 (s, CH), 114.80 (s, C), 115.66 (s, CH), 127.98 (s, CH), 128.78 (s, CH), 131.02 (s, C), 156.07 (s, C), 163.63 (s, C), 166.24 (s, C), 190.94 (s, CO); *m/z*(API-ES+) 271 (MH⁺, 100%).

Chapter 6 – Experimental

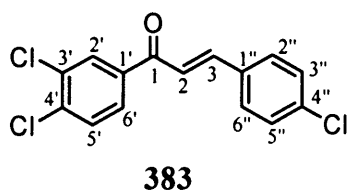
(E)-3-(4-Carboxymethoxyphenyl)-1-(3,4-dichlorophenyl)prop-2-en-1-one **318**²⁵³



318

To a stirring solution of 3,4-dichloroacetophenone (0.95 g, 5.0 mmol), and 4-formyl phenoxyacetic acid (0.90 g, 5.0 mmol) in methanol (10 cm³), was added an aqueous sodium hydroxide solution (2 cm³, 10M) and the mixture stirred at room temperature overnight. The mixture was then acidified with hydrochloric acid (until pH = 1, 1M), and extracted with ethyl acetate (3 × 100 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from methanol afforded **318** as a yellow crystals (1.16 g, 3.3 mmol, 66%); m.p. 208 – 209 °C; R_f 0.18 (SiO₂, ethyl acetate:methanol 19:1); $\nu_{\max}(\text{cm}^{-1})$ 2560, 1732, 1702, 1659, 1594, 1574, 1509, 1433, 1421, 1290, 1243, 1202, 1176, 987, 820, 681; δ ¹H NMR (400 MHz; d₆-DMSO; ppm) 4.76 (2H, s, H-2'''), 6.99 (2H, d, *J* 8.6 Hz, H-3'' & H-5''), 7.74 (1H, d, *J* 15.4 Hz, H-2), 7.81 (1H, d, *J* 8.5 Hz, H-5'), 7.83 (1H, d, *J* 15.4 Hz, H-3), 7.87 (2H, d, *J* 8.6 Hz, H-2'' & H-6''), 8.08 (1H, dd, *J* 2.1, 8.5 Hz, H-6'), 8.37 (1H, d, *J* 2.1 Hz, H-2'), 13.08 (1H, broad s, CO₂H); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 65.22 (s, OCH₂O), 115.61 (s, CH), 119.79 (s, CH), 128.31 (s, C), 129.13 (s, CH), 131.00 (s, CH), 131.74 (s, CH), 131.77 (s, CH), 132.59 (s, C), 136.48 (s, C), 138.63 (s, C), 145.74 (s, CH), 160.78 (s, C), 170.55 (s, CO₂H), 187.52 (s, CO).

(E)-1-(3,4-Dichlorophenyl)-3-(4-chlorophenyl)prop-2-en-1-one **383**³⁵⁶



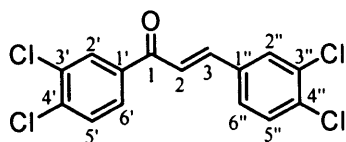
383

The chalcone **383** was obtained following Protocol A using 3,4-dichloroacetophenone (0.38 g, 2.0 mmol), 4-chlorobenzaldehyde (0.28 g, 2.0 mmol) and aqueous sodium hydroxide solution (1 cm³, 4M) in methanol (2 cm³). Recrystallization from methanol afforded **383** as light yellow crystals (0.41 g, 1.3 mmol, 66%); m.p. 141 - 143 °C; R_f 0.83 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max}(\text{cm}^{-1})$ 1659, 1601, 1577, 1552, 1490, 1459, 1405, 1207, 1049, 1030, 1012, 984, 830, 736; δ ¹H NMR (400 MHz; CDCl₃; ppm) 7.41 (2H, d, *J* 8.6 Hz, H-3'' & H-5''), 7.42 (1H, d, *J* 15.6 Hz, H-2), 7.59 (2H, d, *J* 8.6 Hz, H-2'' & H-6''), 7.59 (1H, d, *J* 8.5 Hz, H-5'), 7.79 (1H, d, *J* 15.6 Hz, H-3), 7.85 (1H, dd, *J* 2.1, 8.5 Hz, H-6'), 8.09 (1H, d, *J* 2.1 Hz, H-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 121.48 (s, CH), 127.72 (s, CH), 129.60

Chapter 6 – Experimental

(s, CH), 129.98 (s, CH), 130.68 (s, CH), 131.05 (s, CH), 133.19 (s, C), 133.61 (s, C), 137.18 (s, C), 137.74 (s, C), 137.81 (s, C), 144.75 (s, CH), 187.91 (s, CO).

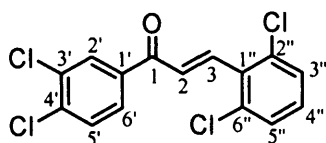
(E)-1,3-Bis(3,4-dichlorophenyl)prop-2-en-1-one **384**³⁵⁷



384

The chalcone **384** was obtained following Protocol A using 3,4-dichloroacetophenone (0.38 g, 2.0 mmol), 3,4-dichlorobenzaldehyde (0.35 g, 2.0 mmol) and aqueous sodium hydroxide solution (1 cm³, 4M) in methanol (2 cm³). Recrystallization from methanol afforded **384** as cream crystals (0.57 g, 1.6 mmol, 83%); m.p. 161 – 163 °C; R_f 0.56 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max}(\text{cm}^{-1})$ 3061, 1660, 1604, 1578, 1553, 1472, 1394, 1310, 1206, 1027, 989, 768; $\delta^1\text{H NMR}$ (400 MHz; CDCl₃; ppm) 7.43 (1H, d, *J* 15.2 Hz, H-2), 7.47 (1H, dd, *J* 2.0, 8.4 Hz, H-6''), 7.51 (1H, d, *J* 8.4 Hz, H-5''), 7.60 (1H, d, *J* 8.5 Hz, H-5'), 7.72 (1H, d, *J* 15.2 Hz, H-3), 7.74 (1H, d, *J* 2.0 Hz, H-2''), 7.85 (1H, dd, *J* 2.1, 8.5 Hz, H-6'), 8.10 (1H, d, *J* 2.1 Hz, H-2'); $\delta^{13}\text{C NMR}$ (100 MHz; CDCl₃; ppm) 122.50 (s, CH), 127.73 (s, CH), 127.88 (s, CH), 130.12 (s, CH), 130.70 (s, CH), 131.10 (s, CH), 131.28 (s, CH), 133.69 (s, C), 134.72 (s, C), 135.12 (s, C), 137.52 (s, C), 137.96 (s, C), 143.30 (s, CH), 187.54 (s, CO).

(E)-3-(2,6-Dichlorophenyl)-1-(3,4-dichlorophenyl)prop-2-en-1-one **385**



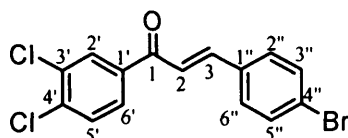
385

The chalcone **385** was obtained following Protocol A using 3,4-dichloroacetophenone (0.38 g, 2.0 mmol), 2,6-dichlorobenzaldehyde (0.35 g, 2.0 mmol) and aqueous sodium hydroxide solution (1 cm³, 4M) in methanol (2 cm³). Recrystallization from methanol afforded **385** as fluffy light yellow crystals (0.46g, 1.3 mmol, 66%); m.p. 100 – 101 °C; R_f 0.57 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max}(\text{cm}^{-1})$ 3088, 1668, 1609, 1580, 1553, 1466, 1437, 1426, 1298, 1205, 1027, 732; $\delta^1\text{H NMR}$ (400 MHz; CDCl₃; ppm) 7.24 (1H, t, *J* 8.1 Hz, H-4''), 7.40 (2H, d, *J* 8.1 Hz, H-3'' & H-5''), 7.59 (1H, d, *J* 16.0 Hz, H-2), 7.60 (1H, d, *J* 8.5 Hz, H-5'), 7.85 (1H, dd, *J* 2.0, 8.5 Hz, H-6'), 7.88 (1H, d, *J* 16.0 Hz, H-3), 8.10 (1H, d, *J* 2.0 Hz, H-2'); $\delta^{13}\text{C NMR}$ (100 MHz; CDCl₃; ppm) 127.94 (s, CH), 129.18 (s, CH), 129.69 (s, CH), 130.41 (s, CH), 130.93 (s, CH), 131.08 (s, CH), 132.44 (s, C), 133.67 (s, C), 135.48 (s, C), 137.43 (s,

Chapter 6 – Experimental

C), 137.96 (s, C), 139.25 (s, CH), 188.14 (s, CO); m/z (API-ES +) 345 ($[MH]^+$, 75%), 347 ($[MH]^+$, 100%), 349 ($[MH]^+$, 50%); HRMS found $[MH]^+$ 344.94037 for $C_{15}H_{19}Cl_4O$, $C_{15}H_{19}Cl_4O$ requires $[MH]^+$ 344.94020.

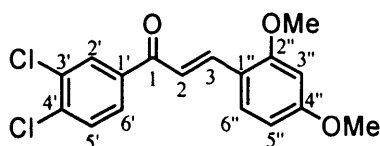
(E)-3-(4-Bromophenyl)-1-(3,4-dichlorophenyl)prop-2-en-1-one **386**



386

The chalcone **386** was obtained following Protocol A using 3,4-dichloroacetophenone (0.38 g, 2.0 mmol), 4-bromobenzaldehyde (0.37 g, 2.0 mmol) and aqueous sodium hydroxide solution (1 cm³, 4M) in methanol (2 cm³). Recrystallization from methanol afforded **386** as cream crystals (0.40 g, 1.1 mmol, 56%); m.p. 154 - 155 °C; R_f 0.77 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 1660, 1600, 1577, 1553, 1486, 1464, 1401, 1209, 1030, 984, 829, 735; δ ¹H NMR (400 MHz; CDCl₃; ppm) 7.43 (1H, d, J 15.6 Hz, H-2), 7.51 (2H, d, J 8.6 Hz, H-2'' & H-6''), 7.57 (2H, d, J 8.6 Hz, H-3'' & H-5''), 7.59 (1H, d, J 8.5 Hz, H-5'), 7.77 (1H, d, J 15.6 Hz, H-3), 7.84 (1H, dd, J 2.1 Hz, J 8.5 Hz, H-6'), 8.09 (1H, d, J 2.1 Hz, H-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 121.58 (s, C), 125.58 (s, C), 127.73 (s, CH), 130.18 (s, CH), 130.69 (s, CH), 131.05 (s, CH), 132.56 (s, CH), 133.62 (s, C), 137.76 (s, C), 137.79 (s, C), 144.81 (s, CH), 187.90 (s, CO); m/z (API-ES +) 355 ($[MH]^+$, 65%), 357 ($[MH]^+$, 90%), 359 ($[MH]^+$, 45%); HRMS found $[MH]^+$ 354.92885 for $C_{15}H_{10}BrCl_2O$, $C_{15}H_{10}BrCl_2O$ requires $[MH]^+$ 354.92866.

(E)-1-(3,4-Dichlorophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one **387**



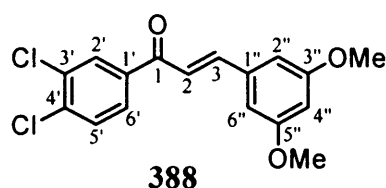
387

The chalcone **387** was obtained following Protocol A using 3,4-dichloroacetophenone (0.38 g, 2.0 mmol), 2,4-dimethoxybenzaldehyde (0.33 g, 2.0 mmol) and aqueous sodium hydroxide solution (1 cm³, 4M) in methanol (2 cm³). Recrystallization from methanol afforded **387** as fluffy bright yellow crystals (0.48 g, 1.4 mmol, 72%); m.p. 129 - 130 °C; R_f 0.47 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 2836, 1662, 1589, 1578, 1504, 1472, 1434, 1413, 1300, 1280, 1209, 807; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.87 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 6.48 (1H, d, J 2.4 Hz, H-3''), 6.54 (1H, dd, J 2.4, 8.7 Hz, H-5''), 7.45 (1H, d, J 15.6

Chapter 6 – Experimental

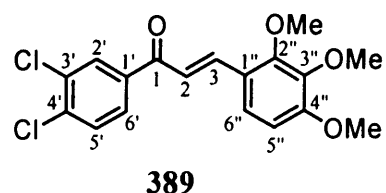
Hz, H-2), 7.56 (1H, d, J 8.7 Hz, H-6''), 7.57 (1H, d, J 8.1 Hz, H-5'), 7.82 (1H, dd, J 2.0, 8.1 Hz, H-6'), 8.06 (1H, d, J 15.6 Hz, H-3), 8.08 (1H, d, J 2.0 Hz, H-2'); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 55.76 (s, OCH_3), 55.82 (s, OCH_3), 98.68 (s, CH), 105.78 (s, CH), 117.00 (s, C), 119.43 (s, CH), 127.71 (s, CH), 130.65 (s, CH), 130.80 (s, CH), 131.52 (s, CH), 133.27 (s, C), 136.89 (s, C), 138.72 (s, C), 142.04 (s, CH), 160.86 (s, C), 163.67 (s, C), 188.86 (s, CO); m/z (API-ES +) 337 ($[\text{MH}]^+$, 100%), 339 ($[\text{MH}]^+$, 65%); HRMS found $[\text{MH}]^+$ 337.03974 for $\text{C}_{17}\text{H}_{15}\text{Cl}_2\text{O}_3$, $\text{C}_{17}\text{H}_{15}\text{Cl}_2\text{O}_3$ requires $[\text{MH}]^+$ 337.03928.

(*E*)-1-(3,4-Dichlorophenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one **388**



The chalcone **388** was obtained following Protocol A using 3,4-dichloroacetophenone (0.38 g, 2.0 mmol), 3,5-dimethoxybenzaldehyde (0.33 g, 2.0 mmol) and aqueous sodium hydroxide solution (1 cm^3 , 4M) in methanol (2 cm^3). Recrystallization from methanol afforded **388** as fluffy yellow crystals (0.47g, 1.4 mmol, 70%); m.p. 114 – 115 °C; R_f 0.60 (SiO_2 , ethyl acetate:hexane 1:3); ν_{max} (cm^{-1}) 2963, 1661, 1590, 1557, 1466, 1449, 1425, 1283, 1160, 1065, 978, 834, 825, 806, 667; δ ^1H NMR (400 MHz; CDCl_3 ; ppm) 3.85 (6H, s, OCH_3), 6.55 (1H, t, J 2.3 Hz, H-4''), 6.77 (2H, d, J 2.3 Hz, H-2'' & H-6''), 7.38 (1H, d, J 15.6 Hz, H-2), 7.58 (1H, d, J 8.4 Hz, H-5'), 7.74 (1H, d, J 15.6 Hz, H-3), 7.84 (1H, dd, J 2.0, 8.4 Hz, H-6'), 8.09 (1H, d, J 2.0 Hz, H-2'); δ ^{13}C NMR (100 MHz; CDCl_3 ; ppm) 55.75 (s, OCH_3), 103.33 (s, CH), 106.75 (s, CH), 121.63 (s, CH), 127.76 (s, CH), 130.70 (s, CH), 130.99 (s, CH), 133.538 (s, C), 136.56 (s, C), 137.58 (s, C), 137.93 (s, C), 146.30 (s, CH), 161.34 (s, C), 188.22 (s, CO); m/z (API-ES +) 337 ($[\text{MH}]^+$, 100%), 339 ($[\text{MH}]^+$, 65%); HRMS found $[\text{MH}]^+$ 337.04019 for $\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{O}_3$, $\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{O}_3$ requires $[\text{MH}]^+$ 337.03928.

(*E*)-1-(3,4-Dichlorophenyl)-3-(2,3,4-trimethoxyphenyl)prop-2-en-1-one **389**

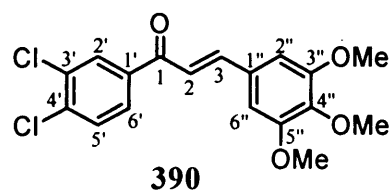


The chalcone **389** was obtained following Protocol A using 3,4-dichloroacetophenone (0.38 g, 2.0 mmol), 2,3,4-trimethoxybenzaldehyde (0.39 g, 2.0 mmol) and aqueous sodium hydroxide solution (1 cm^3 , 4M) in methanol (2 cm^3). Recrystallization from methanol

Chapter 6 – Experimental

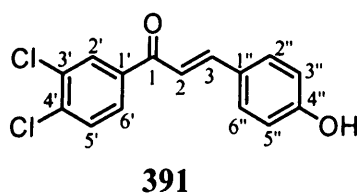
afforded **389** as fluffy white crystals (0.60 g, 1.8 mmol, 90%); m.p. 121 – 122 °C; R_f 0.47 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max}(\text{cm}^{-1})$ 2946, 1631, 1592, 1552, 1498, 1460, 1418, 1299, 1281, 1255, 847, 797; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.90 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.73 (1H, d, J 8.6 Hz, H-5''), 7.40 (1H, d, J 8.6 Hz, H-6''), 7.47 (1H, d, J 16.0 Hz, H-2), 7.58 (1H, d, J 8.3 Hz, H-5'), 7.84 (1H, dd, J 2.0, 8.3 Hz, H-6'), 8.02 (1H, d, J 16.0 Hz, H-3), 8.09 (1H, d, J 2.0 Hz, H-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.35 (s, OCH₃), 61.17 (s, OCH₃), 61.68 (s, OCH₃), 107.85 (s, CH), 120.39 (s, CH), 121.82 (s, C), 124.39 (s, CH), 127.73 (s, CH), 130.67 (s, CH), 130.89 (s, CH), 133.37 (s, C), 137.16 (s, C), 138.44 (s, C), 141.69 (s, CH), 142.70 (s, C), 154.23 (s, C), 156.45 (s, C), 188.64 (s, CO); m/z (API-ES +) 367 ([MH]⁺, 100%), 369 ([MH]⁺, 70%); HRMS found [MH]⁺ 367.04975 for C₁₈H₁₇Cl₂O₄, C₁₈H₁₇Cl₂O₄ requires [MH]⁺ 367.04984.

(*E*)-1-(3,4-Dichlorophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **390**



The chalcone **390** was obtained following Protocol A using 3,4-dichloroacetophenone (0.38 g, 2.0 mmol), 2,3,4-trimethoxybenzaldehyde (0.39 g, 2.0 mmol) and aqueous sodium hydroxide solution (1 cm³, 4M) in methanol (2 cm³). Recrystallization from methanol afforded **390** as yellow crystals (0.35 g, 0.9 mmol, 48%); m.p. 129 - 130 °C; R_f 0.40 (SiO₂, ethyl acetate:hexane 1:3); $\nu_{\max}(\text{cm}^{-1})$ 3086, 2941, 2836, 1662, 1597, 1579, 1551, 1503, 1465, 1453, 1435, 1417, 1284, 1207, 1131, 1033, 976, 770; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.92 (3H, s, OCH₃), 3.94 (6H, s, OCH₃), 6.87 (2H, s, H-2'' & H-6''), 7.31 (1H, d, J 15.6 Hz, H-2), 7.59 (1H, d, J 8.5 Hz, H-5'), 7.75 (1H, d, J 15.6 Hz, H-3), 7.85 (1H, dd, J 2.1, 8.5 Hz, H-6'), 8.09 (1H, d, J 2.1 Hz, H-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.51 (s, OCH₃), 61.27 (s, OCH₃), 106.11 (s, CH), 120.43 (s, CH), 127.76 (s, CH), 130.14 (s, C), 130.65 (s, CH), 130.99 (s, CH), 133.49 (s, C), 137.48 (s, C), 138.10 (s, C), 141.10 (s, C), 146.51 (s, CH), 153.77 (s, C), 188.21 (s, CO); m/z (API-ES +) 367 ([MH]⁺, 100%), 369 ([MH]⁺, 70%); HRMS found [MH]⁺ 367.04985 for C₁₈H₁₇Cl₂O₄, C₁₈H₁₇Cl₂O₄ requires [MH]⁺ 367.04984.

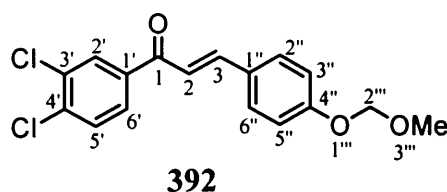
(*E*)-1-(3,4-Dichlorophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **391**²²⁴



Chapter 6 – Experimental

The method adopted was that of Wang²³⁰ *et al.* To a stirring solution of (*E*)-1-(3,4-dichlorophenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **392** (0.100 g, 0.30 mmol) in methanol (6 cm³) under argon was added hydrochloric acid (1.2 cm³, 3M). The reaction was then heated to reflux at 75 °C for 45 minutes. The reaction was cooled to room temperature and tipped into water (6 cm³), it was then extracted with dichloromethane (3 × 10 cm³). The organic fraction was then dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Purification of the crude residue by column chromatography (SiO₂, ethyl acetate:hexane 1:9 to 1:4) afforded **391** as a bright yellow solid (0.09g, 0.30mmol, 99%); m.p. 193 - 196 °C; R_f 0.31 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3220, 2924, 1646, 1591, 1559, 1544, 1516, 1444, 1286, 1219, 1170, 1051, 1029, 988; δ ¹H NMR (400 MHz; CD₃OD; ppm) 6.75 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.45 (1H, d, *J* 15.4 Hz, H-2), 7.55 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.60 (1H, d, *J* 8.4 Hz, H-5'), 7.69 (1H, d, *J* 15.4 Hz, H-3), 7.90 (1H, dd, *J* 1.6, 8.4 Hz, H-6'), 8.10 (1H, d, *J* 1.6 Hz, H-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 116.51 (s, CH), 118.42 (s, CH), 126.33 (s, C), 129.05 (s, CH), 130.91 (s, CH), 131.74 (s, CH), 132.10 (s, CH), 132.54 (s, C), 136.32 (s, C), 138.77 (s, C), 146.40 (s, CH), 161.16 (s, C), 187.39 (s, CO).

(*E*)-1-(3,4-Dichlorophenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **392**

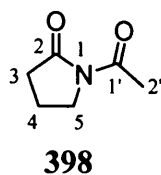


The chalcone **392** was obtained following Protocol A using 3,4-dichloroacetophenone (0.57 g, 3.0 mmol), 4-(methoxymethoxy)benzaldehyde **332** (0.50 g, 3.0 mmol) and aqueous sodium hydroxide solution (3 cm³, 2M) in methanol (7 cm³). Recrystallization from methanol afforded **392** as yellow needles (0.67 g, 2.0 mmol, 66%); m.p. 109 – 110 °C; R_f 0.50 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 1661, 1594, 1572, 1509, 1425, 1211, 1174, 1155, 1070, 1038, 981, 728; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.50 (3H, s, OCH₃), 5.24 (2H, s, H-2'''), 7.09 (2H, d, *J* 8.8 Hz, H-3'' & H-5''), 7.34 (1H, d, *J* 15.4 Hz, H-2), 7.59 (1H, d, *J* 8.4 Hz, H-5'), 7.61 (2H, d, *J* 8.8 Hz, H-2'' & H-6''), 7.81 (1H, d, *J* 15.4 Hz, H-3), 7.84 (1H, dd, *J* 2.1, 8.4 Hz, H-6'), 8.09 (1H, d, *J* 2.1 Hz, H-2'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 56.45 (s, OCH₃), 94.43 (s, OCH₂O), 116.83 (s, CH), 119.26 (s, CH), 127.68 (s, CH), 128.47 (s, C), 130.60 (s, CH), 130.64 (s, CH), 130.93 (s, CH), 133.46 (s, C), 137.32 (s, C), 138.28 (s, C), 145.99 (s, CH), 159.83 (s, C), 188.20 (s, CO); *m/z*(API-ES +) 337 ([MH]⁺, 100%), 339

Chapter 6 – Experimental

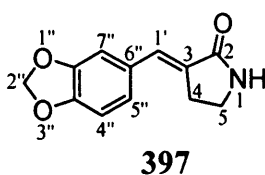
([MH]⁺, 60%), 359 ([MNa]⁺, 35%), 361 ([MNa]⁺, 20%); HRMS found [MH]⁺ 337.03980 for C₁₇H₁₅O₃Cl₂, C₁₇H₁₅O₃Cl₂ requires [MH]⁺ 337.03928.

1-Acetylpyrrolidin-2-one **398**³²³



To a stirring solution of 2-pyrrolidinone (2.50 cm³, 32.9 mmol) in anhydrous tetrahydrofuran (20 cm³) at -20 °C under argon was slowly added a butyllithium solution in hexane (13.30 cm³, 2.5 M). The mixture was then stirred at -20 °C for a further 20 minutes, and then chilled to -78 °C before adding acetyl chloride (2.56 cm³, 36.0 mmol) in anhydrous tetrahydrofuran (30 cm³). The mixture was then left stirring overnight allowing it to warm to room temperature. It was then tipped into water (100 cm³) and extracted with ethyl acetate (6 × 30 cm³). The organic fraction was dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. To give a yellow oil (2.97 g, 23.4 mmol, 71%); R_f 0.52 (SiO₂, ethyl acetate:hexane 1:1); ν_{max}(cm⁻¹) 2986, 2901, 1736, 1687, 1485, 1463, 1419, 1359, 1245, 1228, 1191; δ ¹H NMR (400 MHz; CDCl₃; ppm) 1.97 (2H, quintet, *J* 7.7 Hz, H-4), 2.43 (3H, s, H-2'), 2.54 (2H, t, *J* 8.1 Hz, H-3), 3.74 (2H, t, *J* 7.2 Hz, H-5); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 17.13 (s, CH₂), 24.92 (s, CH₃), 33.54 (s, CH₂), 45.30 (s, CH₂), 171.37 (s, CO), 175.58 (s, CO).

(E)-3-((Benzo[*d*][1,3]dioxol-6-yl)methylene)pyrrolidin-2-one **397**³²¹

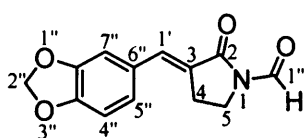


The method adopted was that of Zimmer³²¹ *et al.* To a stirring solution of sodium hydride (4.82 g, 120.5 mmol, 60% dispersion in mineral oil) in anhydrous tetrahydrofuran (50 cm³) at 0 °C under argon, a mixture of piperonal (5.94 g, 39.6 mmol) and 1-acetylpyrrolidin-2-one **398** (5.09 g, 40.1 mmol) in anhydrous tetrahydrofuran (50 cm³) was added slowly drop wise over an hour. The reaction was then stirred at 0 °C for a further hour. Methanol (15 cm³) was then added to the mixture at 0 °C to quench any remaining sodium hydride. The mixture was then tipped into ice, acidified with Sulfuric acid (6M), and extracted with chloroform (10 × 30 cm³). The organic fraction was dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. Recrystallization from 2-propanol afforded **397** as a cream powder

Chapter 6 – Experimental

solid (2.32 g, 10.7 mmol, 27%); m.p. 246 – 248 °C; R_f 0.23 (SiO₂, ethyl acetate); ν_{\max} (cm⁻¹) 3174, 3051, 2898, 1678, 1639, 1601, 1495, 1444, 1266, 1240, 1038, 810; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.12 (2H, td, J 6.5, 2.8 Hz, H-4), 3.56 (2H, t, J 6.5 Hz, H-5), 6.01 (2H, s, H-2''), 6.45 (1H, br. s, H-1), 6.86 (1H, d, J 7.9 Hz, H-4''), 6.97 – 7.02 (2H, m, H-5'' & H-7''), 7.28 (1H, t, J 2.8 Hz, H-1'); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 26.36 (s, CH₂), 39.38 (s, CH₂), 102.01 (s, CH₂), 109.29 (s, CH), 109.42 (s, CH), 124.99 (s, CH), 128.37 (s, CH), 130.63 (s, C), 131.10 (s, C), 148.00 (s, C), 148.35 (s, C), 171.39 (s, CO); m/z (AP⁺) 218 (MH⁺, 100%); HRMS found [MH]⁺ 218.0812 for C₁₂H₁₂O₃N, C₁₂H₁₂O₃N requires [MH]⁺ 218.0812.

(*E*)-3-((Benzo[*d*][1,3]dioxol-5-yl)methylene)-2-oxopyrrolidine-1-carbaldehyde **396**



396

The method adopted was that of Rigo³²² *et al.* A mixture of (*E*)-3-((benzo[*d*][1,3]dioxol-6-yl)methylene)pyrrolidin-2-one **397** (1.64 g, 7.6 mmol) in neat formic acid (27 cm³) was heated to reflux at 105 °C for 18 hours. The mixture was then cooled to room temperature and evaporated *in vacuo*. Purification of the crude residue by column chromatography (SiO₂, ethyl acetate:CHCl₃ 1:4) afforded **396** as a white solid (1.02 g, 4.2 mmol, 55%); m.p. 197 – 198 °C; R_f 0.56 (SiO₂, ethyl acetate:CHCl₃ 1:4); ν_{\max} (cm⁻¹) 2915, 1724, 1684, 1634, 1600, 1505, 1493, 1451, 1440, 1342, 1310, 1229, 1039, 718; δ ¹H NMR (400 MHz; CDCl₃; ppm) 3.09 (2H, dt, J 2.8 Hz J 7.3 Hz, H-4), 3.82 (2H, t, J 7.3 Hz, H-5), 6.03 (2H, s, H-2''), 6.88 (1H, d, J 8.0 Hz, H-4''), 7.00 (1H, d, J 1.6 Hz, H-7''), 7.06 (1H, dd, J 1.6, 8.0 Hz, H-5''), 7.53 (1H, t, J 2.8 Hz, H-1'), 9.24 (1H, s, H-1'''); δ ¹³C NMR (100 MHz; CDCl₃; ppm) 23.78 (s, CHCH₂NH), 39.59 (s, CHCH₂NH), 101.78 (s, OCH₂O), 108.93 (s, CH), 109.36 (s, CH), 126.22 (s, C), 126.67 (s, CH), 128.94 (s, C), 136.06 (s, CH), 148.34 (s, C), 149.31 (s, C), 161.21 (s, CHO), 170.17 (s, CO); m/z (AP⁺) 246 (MH⁺, 100%); HRMS found [MH]⁺ 246.0758 for C₁₃H₁₂O₄N, C₁₃H₁₂O₄N requires [MH]⁺ 246.0761.

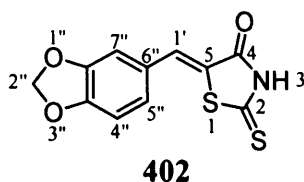
Protocol H

The method adopted was that of Zimmer³²¹ *et al.* To a stirring solution of sodium hydride (0.36 g, 9.0 mmol, 60% dispersion in mineral oil) in anhydrous tetrahydrofuran (5 cm³) at 0 °C under argon, a mixture of benzaldehyde and rhodanine in anhydrous tetrahydrofuran was added slowly drop wise over 20 minutes. The reaction was then stirred at 0 °C for a further hour. Methanol (3 cm³) was then added to the mixture at 0 °C to quench any remaining

Chapter 6 – Experimental

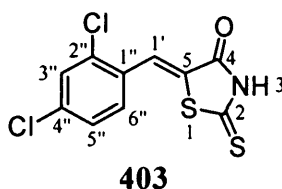
sodium hydride. The mixture was then tipped into ice, acidified with Sulfuric acid (10 cm³, 1M). The precipitate was then filtered off and recrystallized from acetone.

(Z)-5-((Benzo[d][1,3]dioxol-6-yl)methylene)-2-thioxothiazolidin-4-one **402**³⁵⁸



The KNK analogue **402** was obtained following Protocol H using piperonal (0.45 g, 3.0 mmol) and rhodanine (0.40 g, 3.0 mmol) in anhydrous tetrahydrofuran (5 cm³). Recrystallization from acetone afforded **402** as a yellow powder (0.52 g, 1.95 mmol, 65%); m.p. 292 - 293°C; R_f 0.10 (SiO₂, ethyl acetate:hexane 1:1); ν_{max} (cm⁻¹) 3158 (broad m), 3051 (m), 2913 (m), 2833, 1691, 1582, 1501, 1431, 1371, 1264, 1223, 1035, 920, 803, 665; δ¹H NMR (400 MHz; d₆-DMSO; ppm) 6.13 (2H, s, H-2''), 7.08 (1H, d, *J* 8.0 Hz, H-4''), 7.11 (1H, d, *J* 1.9 Hz, H-7''), 7.15 (1H, dd, *J* 1.9 Hz, *J* 8.0 Hz, H-5''), 7.56 (1H, s, H-1'), 13.81 (1H, br. s, NH-3); δ¹³C (100MHz; d₆-DMSO; ppm) 102.84 (s, CH₂), 110.01 (s, CH), 110.19 (s, CH), 123.57 (s, C), 127.39 (s, CH), 127.84 (s, C), 132.60 (s, CH), 149.00 (s, C), 150.36 (s, C), 170.05 (s, CO), 196.04 (s, CS).

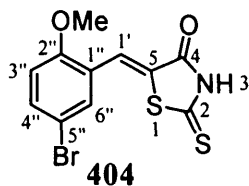
(Z)-5-(2,4-Dichlorobenzylidene)-2-thioxothiazolidin-4-one **403**³⁵⁹



The KNK analogue **403** was obtained following Protocol H using 2,4-dichlorobenzaldehyde (0.53 g, 3.0 mmol) and rhodanine (0.40 g, 3.0 mmol) in anhydrous tetrahydrofuran (5 cm³). Recrystallization from acetone afforded **403** as a light orange solid (0.38 g, 1.3 mmol, 44%); m.p. 230 – 231 °C; R_f 0.37 (SiO₂, ethyl acetate:hexane 1:3); ν_{max} (cm⁻¹) 3100, 2924, 2847, 1734, 1592, 1578, 1467, 1444, 1412, 1234, 1153, 1108, 803, 773, 734, 684, 669; δ¹H NMR (400 MHz; d₆-DMSO; ppm) 7.51 (1H, d, *J* 8.7 Hz, H-6''), 7.58 (1H, dd, *J* 1.9, 8.7 Hz, H-5''), 7.64 (1H, s, H-1'), 7.82 (1H, d, *J* 1.9 Hz, H-3''); δ¹³C (100MHz; d₆-DMSO; ppm) 125.38 (s, CH), 129.17 (s, CH), 130.53 (s, C), 130.60 (s, C), 130.70 (s, CH), 131.08 (s, CH), 136.30 (s, C), 136.38 (s, C), 169.99 (s, CO), 196.04 (s, CS).

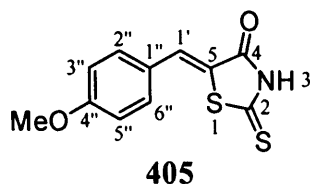
Chapter 6 – Experimental

(Z)-5-(5-Bromo-2-methoxybenzylidene)-2-thioxothiazolidin-4-one **404**



The KNK analogue **404** was obtained following Protocol H using 5-bromo-2-methoxybenzaldehyde (0.64 g, 3.0 mmol) and rhodanine (0.40 g, 3.0 mmol) in anhydrous tetrahydrofuran (5 cm³). Recrystallization from acetone afforded **404** as orange flakes (0.51 g, 1.6 mmol, 52%); m.p. 262 - 263°C; R_f 0.22 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3140, 3029, 2857, 1694, 1577, 1478, 1454, 1253, 1229, 1186, 815; δ ¹H NMR (400 MHz; d₆-DMSO; ppm) 3.90 (3H, s, OCH₃), 7.13 (1H, d, *J* 8.4 Hz, H-3''), 7.48 (1H, d, *J* 2.5 Hz, H-6''), 7.62 – 7.68 (2H, m, H-4'' & H-1'') 13.85 (1H, br s, NH-3); δ ¹³C (100MHz; d₆-DMSO; ppm) 56.82 (s, CH₃), 113.5 (s, C), 114.99 (s, CH), 124.35 (s, C), 125.77 (s, CH), 128.15 (s, C), 132.37 (s, CH), 135.55 (s, CH), 157.76 (s, C), 170.24 (s, CO), 196.52 (s, CS); *m/z*(API-ES+) 330 (MH⁺, 100%), 332 (MH⁺, 100%); HRMS found [MH]⁺ 329.92554 for C₁₁H₉BrNO₂S₂, C₁₁H₉BrNO₂S₂ requires [MH]⁺ 329.92526.

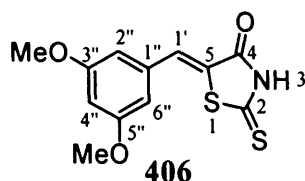
(Z)-5-(4-Methoxybenzylidene)-2-thioxothiazolidin-4-one **405**^{324,360}



The KNK analogue **405** was obtained following Protocol H using *p*-anisaldehyde (0.34 g, 3.0 mmol) and rhodanine (0.40 g, 3.0 mmol) in anhydrous tetrahydrofuran (5 cm³). Recrystallization from acetone afforded **405** as light orange crystals (0.50 g, 2.0 mmol, 66%); m.p. 254 – 255 °C; R_f 0.22 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3127, 2923, 2851, 1683, 1582, 1563, 1507, 1443, 1421, 1258, 1237, 1197, 1168, 821; δ ¹H NMR (400 MHz; d₆-DMSO; ppm) 3.83 (3H, s, OCH₃), 7.11 (2H, d, *J* 9.0 Hz, H-3'' & H-5''), 7.56 (2H, d, *J* 9.0 Hz, H-2'' & H-6''), 7.61 (1H, s, H-1''), 13.75 (1H, br. s, NH-3); δ ¹³C (100MHz; d₆-DMSO; ppm) 56.25 (s, CH₃), 115.78 (s, CH), 122.97 (s, C), 126.17 (s, C), 132.52 (s, CH), 133.37 (s, CH), 162.01 (s, C), 170.21 (s, CO), 196.24 (s, CS).

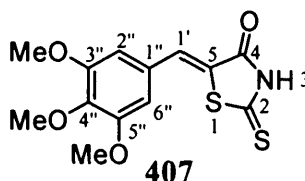
Chapter 6 – Experimental

(Z)-5-(3,5-Dimethoxybenzylidene)-2-thioxothiazolidin-4-one **406**



The KNK analogue **406** was obtained following Protocol H using 3,5-dimethoxybenzaldehyde (0.50 g, 3.0 mmol) and rhodanine (0.40 g, 3.0 mmol) in anhydrous tetrahydrofuran (5 cm³). Recrystallization from acetone afforded **406** as a orange solid (0.53 g, 1.9 mmol, 63%); m.p. 257 – 259°C; R_f 0.22 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3190, 3140, 3065, 2833, 1712, 1597, 1482, 1439, 1204, 1156, 981, 740; δ ¹H NMR (400 MHz; d₆-DMSO; ppm) 3.79 (6H, s, OCH₃), 6.63 (1H, t, *J* 2.1 Hz H-4''), 6.71 (2H, d, *J* 2.1 Hz, H-2'' & H-6''), 7.55 (1H, s, H-1'), 13.82 (1H, br. s, NH-3); δ ¹³C (100MHz; d₆-DMSO; ppm) 56.15 (s, CH₃), 103.48 (s, CH), 108.74 (s, CH), 126.95 (s, C), 132.17 (s, CH), 135.52 (s, C), 161.56 (s, C), 170.16 (s, CO), 196.36 (s, CS); *m/z*(API-ES+) 282 (MH⁺, 100%); HRMS found [MH]⁺ 282.02487 for C₁₂H₁₂NO₃S₂, C₁₂H₁₂NO₃S₂ requires [MH]⁺ 282.02531.

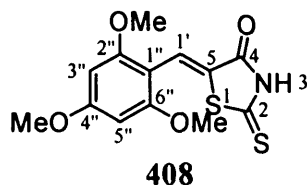
(Z)-5-(3,4,5-Trimethoxybenzylidene)-2-thioxothiazolidin-4-one **407**³⁶¹



The KNK analogue **407** was obtained following Protocol H using 3,4,5-trimethoxybenzaldehyde (0.59 g, 3.0 mmol) and rhodanine (0.40 g, 3.0 mmol) in anhydrous tetrahydrofuran (5 cm³). Recrystallization from acetone afforded **407** as orange crystals (0.37 g, 1.2 mmol, 40%); m.p. 192 – 194 °C; R_f 0.15 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3140, 2923, 2853, 1687, 1595, 1571, 1498, 1454, 1416, 1299, 1216, 1128, 1067, 991, 823; δ ¹H NMR (400 MHz; d₆-DMSO; ppm) 3.71 (3H, s, OCH₃), 3.82 (6H, s, OCH₃), 6.88 (2H, s, H-2'' & H-6''), 7.57 (1H, s, H-1'), 13.80 (1H, br. s, NH-3); δ ¹³C (100MHz; d₆-DMSO; ppm) 56.71 (s, CH₃), 60.91 (s, CH₃), 108.62 (s, CH), 125.04 (s, C), 129.13 (s, C), 132.69 (s, CH), 140.41 (s, C), 153.94 (s, C), 170.03 (s, CO), 196.19 (s, CS).

Chapter 6 – Experimental

(Z)-5-(2,4,6-Trimethoxybenzylidene)-2-thioxothiazolidin-4-one **408**



The KNK analogue **408** was obtained following Protocol H using 2,4,6-trimethoxybenzaldehyde (0.59 g, 3.0 mmol) and rhodanine (0.40 g, 3.0 mmol) in anhydrous tetrahydrofuran (10 cm³). Recrystallization from acetone afforded **408** as a light orange/brown solid (0.76 g, 2.4 mmol, 81%); m.p. 260 – 262 °C; R_f 0.12 (SiO₂, ethyl acetate:hexane 1:3); ν_{max}(cm⁻¹) 3055, 2977, 2935, 2852, 1704, 1682, 1607, 1561, 1461, 1440, 1202, 1179, 1157, 1121; δ¹H NMR (400 MHz; d₆-DMSO; ppm) 3.84 (3H, s, OCH₃), 3.86 (6H, s, OCH₃), 6.29 (2H, s, H-3'' & H-5''), 7.79 (1H, s, H-1'), 13.37 (1H, br. s, NH-3); δ¹³C (100MHz; d₆-DMSO; ppm) 56.51 (s, CH₃), 91.77 (s, CH), 104.4 (s, C), 123.02 (s, C), 126.47 (s, CH), 160.67 (s, C), 165.65 (s, C), 170.61 (s, CO), 197.92 (s, CS); m/z(API-ES+) 312 (MH⁺, 100%); HRMS found [MH]⁺ 312.03590 for C₁₃H₁₄NO₄S₂, C₁₃H₁₄NO₄S₂ requires [MH]⁺ 312.03588.

Chapter 7 – Reference

- 1 J. T. Patterson, *The Dread Disease : Cancer and Modern American Culture*, Harvard University Press, Cambridge, Massachusetts, 1987.
- 2 Cancer Research UK. **September 2005**. *CancerStats Mortality - UK*. [WWW] <URL: http://www.cancerresearchuk.org/aboutcancer/statistics/statsmisc/pdfs/cancerstats_mortality.pdf> [Accessed 4 th November 2005], .
- 3 R. Doll and R. Peto, *J. Natl. Cancer Inst.*, **1981**, 66, 1191-308.
- 4 J. Mann, *The elusive magic bullet : the search for the perfect drug*, Oxford University Press, New York, 1999.
- 5 R. E. McGrew, *Encyclopedia of Medical History*, McGraw-Hill, New York, 1985.
- 6 C. P. Bryan, *Ancient Egyptian medicine : the Papyrus Ebers*, Ares Publishers, Chicago, 1974.
- 7 J. Hill, *Cautions Against the Immoderate Use of Snuff. Founded on the Known Qualities of the Tobacco Plant; And the Effects it Must Produce when this Way Taken into the Body: And Enforced by Instances of Persons who have Perished Miserably of Diseases, Occasioned, or Rendered Incurable by its Use*, R. Baldwin and J. Jackso, London, 1761.
- 8 P. Pott, "Chirurgical Observations Relative to the Cataract, The Polypus of the Nose, The Cancer of the Scrotum, The Different Kinds of Ruptures, and The Mortification of the Toes and Feet. Ch. III". In *"The Chirurgical Works."* Hawes, W. Clarke, and R. Collins, London, 1775, pp. 60-68.
- 9 K. Yamagiwa and K. Ichikawa, *Mitt. Med. Fak. Kaiserl. Univ. Tokio*, **1915**, 15, 295-344.
- 10 R. D. Passey and J. Carter-Braine, *J. Pathol. and Bacteriol.*, **1925**, 28, 133-44.
- 11 O. Muntsch, *Klinische Wochenschrift*, **1934**, 13, 482-5.
- 12 I. Berenblum, *J. Pathol. and Bacteriol.*, **1935**, 40, 549-58.
- 13 A. Gilman and F. S. Philips, *Science*, **1946**, 103, 409-15,436.
- 14 L. Clarke and D. J. Waxman, *Cancer Res.*, **1989**, 49, 2344-50.
- 15 A. Paci, T. Martens and J. Royer, *Bioorg. Med. Chem. Lett.*, **2001**, 11, 1347-1349.
- 16 B. Rosenberg, L. Van Camp and T. Krigas, *Nature*, **1965**, 205, 698 - 9.
- 17 B. Rosenberg, L. Van Camp, E. B. Grimley and A. J. Thomson, *J. Biol. Chem.*, **1967**, 242, 1347-52.
- 18 F. Coste, J.-M. Malinge, L. Serre, W. Shepard, M. Roth, M. Leng and C. Zelwer, *Nucleic Acids Res.*, **1999**, 27, 1837-1846.
- 19 S. Farber, E. C. Cutler, J. W. Hawkins, J. H. Harrison, E. C. Peirce, II and G. G. Lenz, *Science*, **1947**, 106, 619-21.
- 20 S. Farber, L. K. Diamond, R. D. Mercer, R. F. Sylvester, Jr. and J. A. Wolff, *N. Engl. J. Med.*, **1948**, 238, 787-93.
- 21 D. R. Seeger, J. M. Smith, Jr. and M. E. Hultquist, *J. Am. Chem. Soc.*, **1947**, 69, 2567.
- 22 M. Kompis Ivan, K. Islam and L. Then Rudolf, *Chem. Rev.*, **2005**, 105, 593-620.
- 23 W. H. Gmeiner, *Curr. Med. Chem.*, **2005**, 12, 191-202.
- 24 G. B. Elion, *Science*, **1989**, 244, 41-7.
- 25 J. D. Watson and F. H. Crick, *Nature*, **1953**, 171, 737-8.
- 26 C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Plevan and J. Scheiner, *Nature*, **1957**, 179, 663-6.
- 27 R. Duschinsky, E. Plevan and C. Heidelberger, *J. Am. Chem. Soc.*, **1957**, 79, 4559-60.
- 28 R. R. Ellison, J. F. Holland, M. Weil, C. Jacquillat, M. Boiron, J. Bernard, A. Sawitsky, F. Rosner, B. Gussoff, R. T. Silver, A. Karanas, J. Cuttner, C. L. Spurr, D. M. Hayes, J. Blom, L. A. Leone, F. Haurani, R. Kyle, J. L. Hutchison, R. J. Forcier and J. H. Moon, *Blood*, **1968**, 32, 507-23.
- 29 R. L. Noble, C. T. Beer and J. H. Cutts, *Ann. N.Y. Acad. Sci.*, **1958**, 76, 882-94.
- 30 R. L. Noble, C. T. Beer and J. H. Cutts, *Biochem. Pharmacol.*, **1958**, 1, 347-57.
- 31 I. S. Johnson, J. G. Armstrong, M. Gorman and J. P. Burnett, Jr., *Cancer Res.*, **1963**, 23, 1390-1427.
- 32 G. Ma, S. I. Khan, J. Mustafa, L. A. Walker and I. A. Khan, *Lipids*, **2005**, 40, 303-308.
- 33 C. J. Thomas, N. J. Rahier and S. M. Hecht, *Bioorg. Med. Chem.*, **2004**, 12, 1585-1604.
- 34 M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail and G. A. Sim, *J. Am. Chem. Soc.*, **1966**, 88, 3888-90.
- 35 Y. H. Hsiang, R. Hertzberg, S. Hecht and L. F. Liu, *J. Biol. Chem.*, **1985**, 260, 14873-8.
- 36 A. Fleming, *Brit. J. Exp. Pathol.*, **1929**, 10, 226-36.
- 37 S. Farber, G. D'Angio, A. Evans and A. Mitus, *Ann. N.Y. Acad. Sci.*, **1960**, 89, 421-5.

Chapter 7 – Reference

- 38 S. A. Waksman and F. J. Gregory, *Antibiot. & Chemo.*, **1954**, *4*, 1050-6.
- 39 S. Wakaki, H. Marumo, K. Tomioka, G. Shimizu, E. Kato, H. Kamada, S. Kudo and Y. Fujimoto, *Antibiot. & Chemo.*, **1958**, *8*, 228-40.
- 40 D. V. Lefemine, M. Dann, F. Barbatschi, W. K. Hausmann, V. Zbinovsky, P. Monnikendam, J. Adam and N. Bohonos, *J. Am. Chem. Soc.*, **1962**, *84*, 3184-5.
- 41 N. M. Nguy, I. C. Chiu and H. Kohn, *J. Org. Chem.*, **1987**, *52*, 1649-55.
- 42 G. S. Kumar, R. Lipman, J. Cummings and M. Tomasz, *Biochemistry*, **1997**, *36*, 14128-14136.
- 43 F. Arcamone, G. Franceschi, P. Orezzi, G. Cassinelli, W. Barbieri and R. Mondelli, *J. Am. Chem. Soc.*, **1964**, *86*, 5334-5.
- 44 G. A. Leonard, T. W. Hambley, K. McAuley-Hecht, T. Brown and W. N. Hunter, *Acta crystallogr. D.*, **1993**, *49*, 458-67.
- 45 G. T. Beatson, *Lancet*, **1896**, *148*, 162-65.
- 46 E. C. Dodds, L. Golberg, W. Lawson and R. Robinson, *Nature*, **1938**, *142*, 34.
- 47 Anonymous, *Lancet*, **1992**, *339*, 1-15.
- 48 V. C. Jordan, *J. Natl. Cancer Inst.*, **1998**, *90*, 967-971.
- 49 H. Liu, E.-S. Lee, C. Gajdos, S. T. Pearce, B. Chen, C. Osipo, J. Loweth, K. McKian, A. De Los Reyes, L. Wing and V. C. Jordan, *J. Natl. Cancer Inst.*, **2003**, *95*, 1586-1597.
- 50 A. M. Brzozowski, A. C. Pike, Z. Dauter, R. E. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G. L. Greene, J. A. Gustafsson and M. Carlquist, *Nature*, **1997**, *389*, 753-8.
- 51 R. Capdeville, E. Buchdunger, J. Zimmermann and A. Matter, *Nat. Rev. Drug Discov.*, **2002**, *1*, 493-502.
- 52 S. S. Clark, W. M. Crist and O. N. Witte, *Annu. Rev. Med.*, **1989**, *40*, 113-122.
- 53 T. G. Lugo, A. M. Pendergast, A. J. Muller and O. N. Witte, *Science*, **1990**, *247*, 1079-1082.
- 54 G. Q. Daley, R. A. Vanetten and D. Baltimore, *Science*, **1990**, *247*, 824-830.
- 55 M. A. Kelliher, J. McLaughlin, O. N. Witte and N. Rosenberg, *Proc. Natl. Acad. Sci. U. S. A.*, **1990**, *87*, 6649-6653.
- 56 N. Heisterkamp, G. Jenster, J. Tenhove, D. Zovich, P. K. Pattengale and J. Groffen, *Nature*, **1990**, *344*, 251-253.
- 57 J. Zimmermann, E. Buchdunger, H. Mett, T. Meyer and N. B. Lydon, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 187-192.
- 58 T. Schindler, W. Bornmann, P. Pellicena, W. T. Miller, B. Clarkson and J. Kuriyan, *Science*, **2000**, *289*, 1938-1942.
- 59 M. E. M. Noble, J. A. Endicott and L. N. Johnson, *Science*, **2004**, *303*, 1800-1805.
- 60 B. D. Gromperts, I. M. Kramer and P. E. R. Tatham, *Signal Transduction*, Elsevier Academic Press, London, **2002**.
- 61 Q. Li and H. L. Sham, *Expert Opin. Ther. Patents*, **2002**, *12*, 1663-1702.
- 62 A. Jordan, J. A. Hadfield, N. J. Lawrence and A. T. McGown, *Med. Res. Rev.*, **1998**, *18*, 259-296.
- 63 M. A. Jordan and L. Wilson, *Nat. Rev. Cancer*, **2004**, *4*, 253-265.
- 64 J. J. Correia, *Pharmacol. Ther.*, **1991**, *52*, 127-47.
- 65 L. C. Morejohn and D. E. Fosket, *Pharmacol. Ther.*, **1991**, *51*, 217-30.
- 66 D. L. Sackett and J. K. Varma, *Biochemistry*, **1993**, *32*, 13560-5.
- 67 R. B. G. Ravelli, B. Gigant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel and M. Knossow, *Nature*, **2004**, *428*, 198-202.
- 68 T. J. Fitzgerald, *Biochem. Pharmacol.*, **1976**, *25*, 1383-7.
- 69 J. A. Beutler, J. H. Cardellina, C. M. Lin, E. Hamel, G. M. Cragg and M. R. Boyd, *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 581-584.
- 70 J. J. Lichius, O. Thoison, A. Montagnac, M. Pais, F. Guerittevoegelein, T. Sevenet, J. P. Cosson and A. H. A. Hadi, *J. Nat. Prod.*, **1994**, *57*, 1012-1016.
- 71 Q. Shi, K. Chen, L. P. Li, J. J. Chang, C. Autry, M. Kozuka, T. Konoshima, J. R. Estes, C. M. Lin, E. Hamel, A. T. McPhail, D. R. McPhail and K. H. Lee, *J. Nat. Products*, **1995**, *58*, 475-482.
- 72 M. L. Edwards, D. M. Stermerick and P. S. Sunkara, *J. Med. Chem.*, **1990**, *33*, 1948-1954.

Chapter 7 – Reference

- 73 S. Ducki, R. Forrest, J. A. Hadfield, A. Kendall, N. J. Lawrence, A. T. McGown and D. Rennison, *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 1051-1056.
- 74 N. J. Lawrence, D. Rennison, A. T. McGown, S. Ducki, L. A. Gul, J. A. Hadfield and N. Khan, *J. Comb. Chem.*, **2001**, *3*, 421-426.
- 75 M. Cushman, D. Nagarathnam, D. Gopal, H. M. He, C. M. Lin and E. Hamel, *J. Med. Chem.*, **1992**, *35*, 2293-2306.
- 76 D. L. Sackett, *Pharmacol. Ther.*, **1993**, *59*, 163-228.
- 77 G. R. Pettit, *J. Nat. Prod.*, **1996**, *59*, 812-821.
- 78 G. G. Dark, S. A. Hill, V. E. Prise, G. M. Tozer, G. R. Pettit and D. J. Chaplin, *Cancer Res.*, **1997**, *57*, 1829-1834.
- 79 K. Ohsumi, R. Nakagawa, Y. Fukuda, T. Hatanaka, Y. Morinaga, Y. Nihei, K. Ohishi, Y. Suga, Y. Akiyama and T. Tsuji, *J. Med. Chem.*, **1998**, *41*, 3022-3032.
- 80 G. R. Pettit, M. D. Minardi, H. J. Rosenberg, E. Hamel, M. C. Bibby, S. W. Martin, M. K. Jung, R. K. Pettit, T. J. Cuthbertson and J. C. Chapuis, *J. Nat. Prod.*, **2005**, *68*, 1450-1458.
- 81 K. G. Pinney, M. P. Mejia, V. M. Villalobos, B. E. Rosenquist, G. R. Pettit, P. Verdier-Pinard and E. Hamel, *Bioorg. Med. Chem.*, **2000**, *8*, 2417-2425.
- 82 G. R. Pettit, C. R. Anderson, D. L. Herald, M. K. Jung, D. J. Lee, E. Hamel and R. K. Pettit, *J. Med. Chem.*, **2003**, *46*, 525-531.
- 83 N. J. Lawrence, L. A. Hepworth, D. Rennison, A. T. McGown and J. A. Hadfield, *J. Fluor. Chem.*, **2003**, *123*, 101-108.
- 84 G. R. Pettit, M. R. Rhodes, D. L. Herald, E. Hamel, J. M. Schmidt and R. K. Pettitt, *J. Med. Chem.*, **2005**, *48*, 4087-4099.
- 85 M. Medarde, A. B. S. Maya and C. Perez-Melero, *J. Enzyme Inhib. Med. Chem.*, **2004**, *19*, 521-540.
- 86 A. B. S. Maya, B. del Rey, R. P. L. de Clairac, E. Caballero, I. Barasoain, J. M. Andreu and M. Medarde, *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 2549-2551.
- 87 A. B. S. Maya, C. Perez-Melero, C. Mateo, D. Alonso, J. L. Fernandez, C. Gajate, F. Mollinedo, R. Pelaez, E. Caballero and M. Medarde, *J. Med. Chem.*, **2005**, *48*, 556-568.
- 88 M. E. Janik and S. L. Bane, *Bioorg. Med. Chem.*, **2002**, *10*, 1895-1903.
- 89 J. Kaffy, R. E. Pontikis, J. C. Florent and C. Monneret, *Org. Biomol. Chem.*, **2005**, *3*, 2657-2660.
- 90 O. Provot, A. Giraud, J. F. Peyrat, M. Alami and J. D. Brion, *Tetrahedron Lett.*, **2005**, *46*, 8547-8550.
- 91 J. A. Hadfield, K. Gaukroger, N. Hirst, A. P. Weston, N. J. Lawrence and A. T. McGown, *Eur. J. Med. Chem.*, **2005**, *40*, 529-541.
- 92 C. Borrel, S. Thoret, X. Cachet, D. Guenard, F. Tillequin, M. Koch and S. Michel, *Bioorg. Med. Chem.*, **2005**, *13*, 3853-3864.
- 93 L. Wang, K. W. Woods, Q. Li, K. J. Barr, R. W. McCroskey, S. M. Hannick, L. Gherke, R. B. Credo, Y. H. Hui, K. Marsh, R. Warner, J. Y. Lee, N. Zielinski-Mozng, D. Frost, S. H. Rosenberg and H. L. Sham, *J. Med. Chem.*, **2002**, *45*, 1697-1711.
- 94 G. C. Tron, F. Pagliai, E. Del Grosso, A. A. Genazzani and G. Sorba, *J. Med. Chem.*, **2005**, *48*, 3260-3268.
- 95 B. Coggiola, F. Pagliai, G. Allegrone, A. A. Genazzani and G. C. Tron, *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 3551-3554.
- 96 G. R. Pettit, B. Toki, D. L. Herald, P. Verdier-Pinard, M. R. Boyd, E. Hamel and R. K. Pettit, *J. Med. Chem.*, **1998**, *41*, 1688-1695.
- 97 J. P. Liou, C. W. Chang, J. S. Song, Y. N. Yang, C. F. Yeh, H. Y. Tseng, Y. K. Lo, Y. L. Chang, C. M. Chang and H. P. Hsieh, *J. Med. Chem.*, **2002**, *45*, 2556-2562.
- 98 G. R. Pettit, M. P. Grealish, D. L. Herald, M. R. Boyd, E. Hamel and R. K. Pettit, *J. Med. Chem.*, **2000**, *43*, 2731-2737.
- 99 J. P. Liou, J. Y. Chang, C. W. Chang, C. Y. Chang, N. Mahindroo, F. M. Kuo and H. P. Hsieh, *J. Med. Chem.*, **2004**, *47*, 2897-2905.
- 100 R. Romagnoli, P. G. Baraldi, M. K. Jung, M. A. Iaconinoto, M. D. Carrion, V. Remusat, D. Preti, M. A. Tabrizi, F. Francesca, E. De Clercq, J. Balzarini and E. Hamel, *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 4048-4052.

Chapter 7 – Reference

- 101 J. P. Liou, Y. L. Chang, F. M. Kuo, C. W. Chang, H. Y. Tseng, C. C. Wang, Y. N. Yang, J. Y. Chang, S. J. Lee and H. P. Hsieh, *J. Med. Chem.*, **2004**, *47*, 4247-4257.
- 102 B. L. Flynn, P. Verdier-Pinard and E. Hamel, *Org. Lett.*, **2001**, *3*, 651-654.
- 103 B. L. Flynn, G. P. Flynn, E. Hamel and M. K. Jung, *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2341-2343.
- 104 B. L. Flynn, E. Hamel and M. K. Jung, *J. Med. Chem.*, **2002**, *45*, 2670-2673.
- 105 E. Hamel, H. H. Ho, G. J. Kang and C. M. Lin, *Biochem. Pharmacol.*, **1988**, *37*, 2445-2449.
- 106 S. M. Kupchan, R. Britton, W., M. Ziegler, F., C. Gilmore, J., R. J. Restivo and R. F. Bryan, *J. Am. Chem. Soc.*, **1973**, *95*, 1335 - 1336.
- 107 D. B. M. Wickramaratne, T. Pengsuparp, W. Mar, H. B. Chai, T. E. Chagwedera, C. W. W. Beecher, N. R. Farnsworth, A. D. Kinghorn, J. M. Pezzuto and G. A. Cordell, *J. Nat. Prod.*, **1993**, *56*, 2083-2090.
- 108 R. W. J. Wang, L. I. Rebhun and S. M. Kupchan, *Cancer Res.*, **1977**, *37*, 3071-3079.
- 109 F. Cortese, B. Bhattacharyya and J. Wolff, *J. Biol. Chem.*, **1977**, *252*, 1134-1140.
- 110 P. B. Schiff, A. S. Kende and S. B. Horwitz, *Biochem. Biophys. Res. Commun.*, **1978**, *85*, 737-746.
- 111 F. Zavala, D. Guenard, J. P. Robin and E. Brown, *J. Med. Chem.*, **1980**, *23*, 546-549.
- 112 J. K. Batra, L. Jurd and E. Hamel, *Biochem. Pharmacol.*, **1986**, *35*, 4013-4018.
- 113 W. R. Leopold, W. L. Elliot, S. A. Prysbronowsky and R. A. Wand, *Proc. Am. Assoc. Cancer Res.*, **1993**, *34*, 296.
- 114 K. Vogel, J. Sterling, Y. Herzig and A. Nudelman, *Tetrahedron*, **1996**, *52*, 3049-3056.
- 115 Z. Ji, H. K. Wang, K. F. Bastow, X. K. Zhu, S. J. Cho, Y. C. Cheng and K. H. Lee, *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 607-612.
- 116 H. Mujagic, B. M. Conger, C. A. Smith, S. J. Occhipinti, W. H. Schuette and S. E. Shackney, *Cancer Res.*, **1983**, *43*, 3598-3603.
- 117 S. S. Rai and J. Wolff, *J. Biol. Chem.*, **1996**, *271*, 14707-14711.
- 118 R. Rahmani and X. J. Zhou, *Cancer Surv.*, **1993**, *17*, 269-281.
- 119 H. J. Anderson, J. E. Coleman, R. J. Andersen and M. Roberge, *Cancer Chemother. Pharmacol.*, **1997**, *39*, 223-226.
- 120 G. R. Pettit, Z. A. Cichacz, C. L. Herald, F. Gao, M. R. Boyd, J. M. Schmidt, E. Hamel and R. L. Bai, *J. Chem. Soc.-Chem. Commun.*, **1994**, 1605-1606.
- 121 Y. Hirata and D. Uemura, *Pure Appl. Chem.*, **1986**, *58*, 701-710.
- 122 G. R. Pettit, C. L. Herald, M. R. Boyd, J. E. Leet, C. Dufresne, D. L. Doubek, J. M. Schmidt, R. L. Cerny, J. N. A. Hooper and K. C. Rutzler, *J. Med. Chem.*, **1991**, *34*, 3339-3340.
- 123 G. R. Pettit, R. Tan, F. Gao, M. D. Williams, D. L. Doubek, M. R. Boyd, J. M. Schmidt, J. C. Chapuis, E. Hamel, R. Bai, J. N. A. Hooper and L. P. Tackett, *J. Org. Chem.*, **1993**, *58*, 2538-2543.
- 124 M. H. G. Munro, J. W. Blunt, E. J. Dumdei, S. J. H. Hickford, R. E. Lill, S. X. Li, C. N. Battershill and A. R. Duckworth, *J. Biotechnol.*, **1999**, *70*, 15-25.
- 125 M. J. Towle, K. A. Salvato, J. Budrow, B. F. Wels, G. Kuznetsov, K. K. Aalfs, S. Welsh, W. J. Zheng, B. M. Seletsky, M. H. Palme, G. J. Habgood, L. A. Singer, L. V. DiPietro, Y. Wang, J. J. Chen, D. A. Quincy, A. Davis, K. Yoshimatsu, Y. Kishi, M. J. Yu and B. A. Littlefield, *Cancer Res.*, **2001**, *61*, 1013-1021.
- 126 T. D. Aicher, K. R. Buszek, F. G. Fang, C. J. Forsyth, S. H. Jung, Y. Kishi, M. C. Matelich, P. M. Scola, D. M. Spero and S. K. Yoon, *J. Am. Chem. Soc.*, **1992**, *114*, 3162-3164.
- 127 W. R. Gamble, N. A. Durso, R. W. Fuller, C. K. Westergaard, T. R. Johnson, D. L. Sackett, E. Hamel, J. H. Cardellina and M. R. Boyd, *Bioorg. Med. Chem.*, **1999**, *7*, 1611-1615.
- 128 J. E. Coleman, E. D. Desilva, F. M. Kong, R. J. Andersen and T. M. Allen, *Tetrahedron*, **1995**, *51*, 10653-10662.
- 129 R. L. Bai, G. F. Taylor, Z. A. Cichacz, C. L. Herald, J. A. Kepler, G. R. Pettit and E. Hamel, *Biochemistry*, **1995**, *34*, 9714-9721.
- 130 F. M. Uckun, C. Mao, S. T. Jan, H. Huang, A. O. Vassilev, C. S. Navara and R. K. Narla, *Curr. Pharm. Design*, **2001**, *7*, 1291-1296.
- 131 G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald, M. R. Boyd, J. M. Schmidt and J. N. A. Hooper, *J. Org. Chem.*, **1993**, *58*, 1302-1304.

Chapter 7 – Reference

- 132 M. Takahashi, S. Iwasaki, H. Kobayashi, S. Okuda, T. Murai and Y. Sato, *Biochim. Biophys. Acta*, **1987**, *926*, 215-223.
- 133 K. H. Lee, H. Nozaki, I. H. Hall, R. Kasai, T. Hirayama, H. Suzuki, R. Y. Wu and H. C. Huang, *J. Nat. Prod.*, **1982**, *45*, 509-510.
- 134 A. R. Safa, E. Hamel and R. L. Felsted, *Biochemistry*, **1987**, *26*, 97-102.
- 135 K. Ootsu, Y. Kozai, M. Takeuchi, S. Ikeyama, K. Igarashi, K. Tsukamoto, Y. Sugino, T. Tashiro, S. Tsukagoshi and Y. Sakurai, *Cancer Res.*, **1980**, *40*, 1707-1717.
- 136 R. L. Bai, R. E. Schwartz, J. A. Kepler, G. R. Pettit and E. Hamel, *Cancer Res.*, **1996**, *56*, 4398-4406.
- 137 Y. Li, H. Kobayashi, Y. Hashimoto, R. Shirai, A. Hirata, K. Hayashi, Y. Hamada, T. Shioiri and S. Iwasaki, *Chem.-Biol. Interact.*, **1994**, *93*, 175-183.
- 138 E. M. Tonsing, P. S. Steyn, M. Osborn and K. Weber, *Eur.J. Cell Biol.*, **1984**, *35*, 156-164.
- 139 M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon and A. T. McPhail, *J. Am. Chem. Soc.*, **1971**, *93*, 2325-&.
- 140 P. B. Schiff, J. Fant and S. B. Horwitz, *Nature*, **1979**, *277*, 665-667.
- 141 K. C. Nicolaou, Z. Yang, J. J. Liu, H. Ueno, P. G. Nantermet, R. K. Guy, C. F. Claiborne, J. Renaud, E. A. Couladouros, K. Paulvannan and E. J. Sorensen, *Nature*, **1994**, *367*, 630-634.
- 142 R. A. Holton, C. Somoza, H. B. Kim, F. Liang, R. J. Biediger, P. D. Boatman, M. Shindo, C. C. Smith, S. C. Kim, H. Nadizadeh, Y. Suzuki, C. L. Tao, P. Vu, S. H. Tang, P. S. Zhang, K. K. Murthi, L. N. Gentile and J. H. Liu, *J. Am. Chem. Soc.*, **1994**, *116*, 1597-1598.
- 143 R. A. Holton, H. B. Kim, C. Somoza, F. Liang, R. J. Biediger, P. D. Boatman, M. Shindo, C. C. Smith, S. C. Kim, H. Nadizadeh, Y. Suzuki, C. L. Tao, P. Vu, S. H. Tang, P. S. Zhang, K. K. Murthi, L. N. Gentile and J. H. Liu, *J. Am. Chem. Soc.*, **1994**, *116*, 1599-1600.
- 144 S. J. Danishefsky, J. J. Masters, W. B. Young, J. T. Link, L. B. Snyder, T. V. Magee, D. K. Jung, R. C. A. Isaacs, W. G. Bornmann, C. A. Alaimo, C. A. Coburn and M. J. DiGrandi, *J. Am. Chem. Soc.*, **1996**, *118*, 2843-2859.
- 145 P. A. Wender, N. F. Badham, S. P. Conway, P. E. Floreancig, T. E. Glass, J. B. Houze, N. E. Krauss, D. S. Lee, D. G. Marquess, P. L. McGrane, W. Meng, M. G. Natchus, A. J. Shuker, J. C. Sutton and R. E. Taylor, *J. Am. Chem. Soc.*, **1997**, *119*, 2757-2758.
- 146 E. Nogales, S. G. Wolf, I. A. Khan, R. F. Luduena and K. H. Downing, *Nature*, **1995**, *375*, 424-427.
- 147 I. Ringel and S. B. Horwitz, *J. Natl. Cancer Inst.*, **1991**, *83*, 288-291.
- 148 D. T. Hung, J. Chen and S. L. Schreiber, *Chem. Biol.*, **1996**, *3*, 287-293.
- 149 E. terHaar, R. J. Kowalski, E. Hamel, C. M. Lin, R. E. Longley, S. P. Gunasekera, H. S. Rosenkranz and B. W. Day, *Biochemistry*, **1996**, *35*, 243-250.
- 150 D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides and C. M. Woods, *Cancer Res.*, **1995**, *55*, 2325-2333.
- 151 J. D. Winkler and P. H. Axelsen, *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 2963-2966.
- 152 G. M. Tozer, C. Kanthou, C. S. Parkins and S. A. Hill, *Int. J. Exp. Pathol.*, **2002**, *83*, 21-38.
- 153 G. M. Tozer, C. Kanthou and B. C. Baguley, *Nat. Rev. Cancer*, **2005**, *5*, 423-435.
- 154 J. Folkman, *Semin. Cancer Biol.*, **2003**, *13*, 159-167.
- 155 E. Hamel, *Pharmacol. Ther.*, **1992**, *55*, 31-51.
- 156 C. Kanthou and G. M. Tozer, *Blood*, **2002**, *99*, 2060-2069.
- 157 G. M. Tozer, V. E. Prise, J. Wilson, M. Cemazar, S. Q. Shan, M. W. Dewhirst, P. R. Barber, B. Vojnovic and D. J. Chaplin, *Cancer Res.*, **2001**, *61*, 6413-6422.
- 158 V. E. Prise, D. J. Honess, M. R. L. Stratford, J. Wilson and G. M. Tozer, *Int. J. Oncol.*, **2002**, *21*, 717-726.
- 159 N. J. Lawrence and A. T. McGown, *Curr. Pharm. Design*, **2005**, *11*, 1679-1693.
- 160 S. Ducki, J. A. Hadfield, N. J. Lawrence, X. G. Zhang and A. T. McGown, *Planta Med.*, **1995**, *61*, 586-587.
- 161 S. Ducki, J. A. Hadfield, N. J. Lawrence, C. Y. Liu, A. T. McGown and X. G. Zhang, *Planta Med.*, **1996**, *62*, 185-186.
- 162 S. Ducki, J. A. Hadfield, X. G. Zhang, N. J. Lawrence and A. T. McGown, *Planta Med.*, **1996**, *62*, 277-278.

Chapter 7 – Reference

- 163 B. Qian, In "*Clinical Effects of Anticancer Chinese Medicine*", Shanghai Translation Publishing House, 1987, pp. 6-7.
- 164 C. Minyi, *Anticancer Medicinal Herbs*, Hunan Science and Technology Publishing House, Changsha, 1992.
- 165 J. M. Edmondson, L. S. Armstrong and A. O. Martinez, *Meth. Cell Sci.*, 1988, 11, 15-17.
- 166 S. Ducki, J. A. Hadfield, L. A. Hepworth, N. J. Lawrence, C. Y. Liu and A. T. McGown, *Bioorg. Med. Chem. Lett.*, 1997, 7, 3091-3094.
- 167 L. Jayasinghe, B. Balasooriya, W. C. Padmini, N. Hara and Y. Fujimoto, *Phytochem*, 2004, 65, 1287-1290.
- 168 M. Sato, H. Tsuchiya, M. Akagiri, S. Fujiwara, T. Fujii, N. Takagi, N. Matsuura and M. Iinuma, *Lett. Appl. Microbiol.*, 1994, 18, 53-55.
- 169 L. M. Ni, C. Q. Meng and J. A. Sikorski, *Expert Opin. Ther. Patents*, 2004, 14, 1669-1691.
- 170 H. H. Ko, L. T. Tsao, K. L. Yu, C. T. Liu, J. P. Wang and C. N. Lin, *Bioorg. Med. Chem.*, 2003, 11, 105-111.
- 171 S. F. Nielsen, M. Larsen, T. Boesen, K. Schonning and H. Kromann, *J. Med. Chem.*, 2005, 48, 2667-2677.
- 172 R. S. Li, G. L. Kenyon, F. E. Cohen, X. W. Chen, B. Q. Gong, J. N. Dominguez, E. Davidson, G. Kurzban, R. E. Miller, E. O. Nuzum, P. J. Rosenthal and J. H. McKerrow, *J. Med. Chem.*, 1995, 38, 5031-5037.
- 173 J. N. Dominguez, C. Leon, J. Rodrigues, N. G. de Dominguez, J. Gut and P. J. Rosenthal, *J. Med. Chem.*, 2005, 48, 3654-3658.
- 174 R. Edenharder and X. Tang, *Food Chem. Toxicol.*, 1997, 35, 357-372.
- 175 R. Edenharder, I. Vonpetersdorff and R. Rauscher, *Mutat. Res.*, 1993, 287, 261-274.
- 176 N. J. Lawrence, A. T. McGown, S. Ducki and J. A. Hadfield, *Anti-Cancer Drug Des.*, 2000, 15, 135-141.
- 177 S. Ducki, *PhD Thesis*, UMIST, Manchester, UK, 1997.
- 178 E. P. Kohler and H. M. Chadwell, *Org. Synth*, 1922, 2, 1-3.
- 179 L. P. Li, H. K. Wang, S. C. Kuo, T. S. Wu, A. Mauger, C. M. Lin, E. Hamel and K. H. Lee, *J. Med. Chem.*, 1994, 37, 3400-3407.
- 180 M. Cushman, D. Nagarathnam, D. Gopal, A. K. Chakraborti, C. M. Lin and E. Hamel, *J. Med. Chem.*, 1991, 34, 2579-88.
- 181 A. Kendall, *MPhil Thesis*, UMIST, Manchester, UK, 1998.
- 182 K. A. Rashid, C. A. Mullin and R. O. Mumma, *Mutat. Res.*, 1986, 169, 71-79.
- 183 R. Forrest, *Undergraduate Project Report*, UMIST, Manchester, UK, 1996.
- 184 Rabinovi.D, *J. Chem. Soc. B*, 1970, 11-&.
- 185 Rabinovi.D and Z. Shakked, *Acta Crystallogr. Sect. B: Struct. Sci.*, 1974, B 30, 2829-2834.
- 186 Rabinovi.D, G. M. J. Schmidt and Z. Shaked, *J. Chem. Soc.-Perkin Trans. 2*, 1973, 33-37.
- 187 J. C. Wallet, E. Molins and C. Miravittles, *Acta Crystallogr. Sect. C-Cryst. Struct. Commun.*, 1995, 51, 123-125.
- 188 M. Bolte, G. Schutz and H. J. Bader, *Acta Crystallogr. Sect. C-Cryst. Struct. Commun.*, 1996, 52, 2807-2809.
- 189 J. P. Declercq, G. Germain, M. Vanmeerssche and G. Labbe, *B. Soc. Chim. Belg.*, 1978, 87, 239-240.
- 190 J. Alvarezbuilla, J. L. Novella, E. Galvez, P. Smith, F. Florencio, S. Garciablanco, J. Bellanato and M. Santos, *Tetrahedron*, 1986, 42, 699-708.
- 191 D. Rennison, *PhD Thesis*, UMIST, Manchester, UK, 2001.
- 192 N. J. Lawrence, D. Rennison, A. T. McGown and J. A. Hadfield, *Bioorg. Med. Chem. Lett.*, 2003, 13, 3759-3763.
- 193 L. Huang, M. E. Wall, M. C. Wani, H. Navarro, T. Santisuk, V. Reutrakul, E. K. Seo, N. R. Farnsworth and A. D. Kinghorn, *J. Nat. Prod.*, 1998, 61, 446-550.
- 194 D. M. Fitzgerald, J. F. O'Sullivan, E. M. Philbin and T. S. Wheeler, *J. Chem. Soc.*, 1955, 860 - 862.
- 195 E. McDonald and P. Smith, *J. Chem. Soc.-Perkin Trans. 1*, 1980, 837-842.

Chapter 7 – Reference

- 196 N. J. Lawrence, D. Rennison, S. Ducki, M. Woo, A. T. McGown and J. A. Hadfield, In Combretastatin A4 derivatives having antineoplastic activity, *PCT Int. Appl.* (2003), 89 pp. WO 0340077, Chem. Abs., 2003, 138, 385212., pp. .
- 197 G. Bose, E. Mondal, A. T. Khan and M. J. Bordoloi, *Tetrahedron Lett.*, **2001**, 42, 8907-8909.
- 198 A. Marsura, L.-D. Cuong and G. Gellon, *Synthesis*, **1985**, 537-41.
- 199 W. C. Black, C. Brideau, C.-C. Chan, S. Charleson, N. Chauret, D. Claveau, D. Ethier, R. Gordon, G. Greig, J. Guay, G. Hughes, P. Jolicoeur, Y. Leblanc, D. Nicoll-Griffith, N. Ouimet, D. Riendeau, D. Visco, Z. Wang, L. Xu and P. Prasit, *J. Med. Chem.*, **1999**, 42, 1274-1281.
- 200 S. Mull Eric, J. Sattigeri Viswajanani, L. Rodriguez Alice and A. Katzenellenbogen John, *Bioorg. Med. Chem.*, **2002**, 10, 1381-98.
- 201 C. W. Shoppee and R. J. Hart, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 2369-73.
- 202 L. S. Hegedus, "Palladium in Organic Synthesis". In "*Organometallics in Synthesis : A Manual*", John Wiley & Sons, Chichester, **1994**, pp. 448.
- 203 M. Woo, *Design and synthesis of new anticancer agents*, Umist, MPhil Thesis, **2001**.
- 204 C. Borrel, S. Thoret, X. Cachet, D. Guénard, F. Tillequin, M. Koch and S. Michel, *Bioorg. Med. Chem.*, **2005**, 13, 3853-3864.
- 205 A. Hosoda, E. Nomura, K. Mizuno and H. Taniguchi, *J. Org. Chem.*, **2001**, 66, 7199-7201.
- 206 S. Nahm and S. M. Weinreb, *Tetrahedron Lett.*, **1981**, 22, 3815-3818.
- 207 J. P. Liou, C. W. Chang, J. S. Song, Y. N. Yang, C. F. Yeh, H. Y. Tseng, Y. K. Lo, Y. L. Chang, C. M. Chang and H. P. Hsieh, *J. Med. Chem.*, **2002**, 45, 2556-2562.
- 208 Z. Chen, V. P. Mocharla, J. M. Farmer, G. R. Pettit, E. Hamel and K. G. Pinney, *J. Org. Chem.*, **2000**, 65, 8811-8815.
- 209 K. L. Habermas, S. E. Denmark and T. K. Jones, *Org. React.*, **1994**, 45, 1-158.
- 210 H. Pellissier, *Tetrahedron*, **2005**, 61, 6479-6517.
- 211 A. J. Frontier and C. Collison, *Tetrahedron*, **2005**, 61, 7577-7606.
- 212 D. B. Bruce, A. J. S. Sorrie and R. H. Thomson, *J. Chem. Soc.*, **1953**, 2403 - 2406.
- 213 W. S. Murphy and S. Wattanasin, *J. Chem. Soc.-Perkin Trans. 1*, **1980**, 1555-1566.
- 214 J. M. Allen, K. M. Johnston, J. F. Jones and R. G. Shotton, *Tetrahedron*, **1977**, 33, 2083-2087.
- 215 X. H. Gu, H. Yu, A. E. Jacobson, R. B. Rothman, C. M. Dersch, C. George, J. L. Flippen-Anderson and K. C. Rice, *J. Med. Chem.*, **2000**, 43, 4868-4876.
- 216 S. Caddick, *Tetrahedron*, **1995**, 51, 10403-10432.
- 217 M. Nuchter, U. Muller, B. Ondruschka, A. Tied and W. Lautenschlager, *Chem. Eng. Technol.*, **2003**, 26, 1207-1216.
- 218 F. Mavandadi and P. Lidstrom, *Curr. Top. Med. Chem.*, **2004**, 4, 773-792.
- 219 M. Nuchter, B. Ondruschka, W. Bonrath and A. Gum, *Green Chem.*, **2004**, 6, 128-141.
- 220 C. O. Kappe, *Angew. Chem.-Int. Edit.*, **2004**, 43, 6250-6284.
- 221 M. J. Gronnow, R. J. White, J. H. Clark and D. J. Macquarrie, *Org. Process Res. Dev.*, **2005**, 9, 516-518.
- 222 M. E. Zwaagstra, H. Timmerman, M. Tamura, T. Tohma, Y. Wada, K. Onogi and M. Q. Zhang, *J. Med. Chem.*, **1997**, 40, 1075-1089.
- 223 W. L. F. Armarego and D. D. Perrin, *Purification of Laboratory Chemicals*, Butterworth Heinemann, Oxford, **1996**.
- 224 J. R. Dimmock, N. M. Kandepu, M. Hetherington, J. W. Quail, U. Pugazhenthii, A. M. Sudom, M. Chamankhah, P. Rose, E. Pass, T. M. Allen, S. Halleran, J. Szydlowski, B. Mutus, M. Tannous, E. K. Manavathu, T. G. Myers, E. De Clercq and J. Balzarini, *J. Med. Chem.*, **1998**, 41, 1014-1026.
- 225 W. M. Clark, A. M. Tickner-Eldridge, G. K. Huang, L. N. Pridgen, M. A. Olsen, R. J. Mills, I. Lantos and N. H. Baine, *J. Am. Chem. Soc.*, **1998**, 120, 4550-4551.
- 226 R. Beugelmans, M. Boischoussy and Q. Tang, *J. Org. Chem.*, **1987**, 52, 3880-3883.
- 227 C. P. Ridley, M. V. R. Reddy, G. Rocha, F. D. Bushman and D. J. Faulkner, *Bioorg. Med. Chem.*, **2002**, 10, 3285-3290.
- 228 L. Crombie and S. V. Jamieson, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 7, 1467 - 1475.
- 229 M. E. Jung and M. A. Lyster, *J. Org. Chem.*, **1977**, 42, 3761-3764.
- 230 Y. Q. Wang, W. F. Tan, W. D. Z. Li and Y. L. Li, *J. Nat. Prod.*, **2001**, 64, 196-199.

Chapter 7 – Reference

- 231 S. V. Ley, I. R. Baxendale, R. N. Bream, P. S. Jackson, A. G. Leach, D. A. Longbottom, M. Nesi, J. S. Scott, R. I. Storer and S. J. Taylor, *J. Chem. Soc., Perkin Trans. 1*, **2000**, 3815-4195.
- 232 M. L. Go, X. Wu and X. L. Liu, *Curr. Med. Chem.*, **2005**, *12*, 483-499.
- 233 H. K. Hsieh, L. T. Tsao, J. P. Wang and C. N. Lin, *J. Pharm. Pharmacol.*, **2000**, *52*, 163-171.
- 234 H. K. Hsieh, T. H. Lee, J. P. Wang, J. J. Wang and C. N. Lin, *Pharm. Res.*, **1998**, *15*, 39-46.
- 235 M. L. Go, M. Liu, P. Wilairat, P. J. Rosenthal, K. J. Saliba and K. Kirk, *Antimicrob. Agents Chemother.*, **2004**, *48*, 3241-3245.
- 236 R. J. Anto, K. Sukumaran, G. Kuttan, M. N. A. Rao, V. Subbaraju and R. Kuttan, *Cancer Lett.*, **1995**, *97*, 33-37.
- 237 N. H. Nam, Y. Kim, Y. J. You, D. H. Hong, H. M. Kim and B. Z. Ahn, *Eur. J. Med. Chem.*, **2003**, *38*, 179-187.
- 238 N. H. Nam, D. H. Hong, Y. J. You, Y. Kim, S. C. Bang, H. M. Kim and B. Z. Ahn, *Arch. Pharmacol. Res.*, **2004**, *27*, 581-588.
- 239 N. H. Nam, H. T. T. Huong, H. M. Kim and B. Z. Ahn, *Kor. J. Pharmacogn.*, **2000**, *31*, 77-81.
- 240 N. H. Nam, Y. J. You, Y. Kim, D. H. Hong, H. M. Kim and B. Z. Ahn, *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 1173-1176.
- 241 C. Pouget, F. Lauthier, A. Simon, C. Fagnere, J. P. Basly, C. Delage and A. J. Chulia, *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 3095-3097.
- 242 Y. K. Rao, S. H. Fang and Y. M. Tzeng, *Bioorg. Med. Chem.*, **2004**, *12*, 2679-2686.
- 243 L. Li, H. K. Wang, S. C. Kuo, T. S. Wu, D. Lednicer, C. M. Lin, E. Hamel and K. H. Lee, *J. Med. Chem.*, **1994**, *37*, 1126-1135.
- 244 Y. Xia, Z. Y. Yang, P. Xia, K. F. Bastow, Y. Nakanishi and K. H. Lee, *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 699-701.
- 245 J. R. Dimmock, S. K. Raghavan, B. M. Logan and G. E. Bigam, *Eur. J. Med. Chem.*, **1983**, *18*, 248-254.
- 246 J. R. Dimmock, D. W. Elias, M. A. Beazely and N. M. Kandepu, *Curr. Med. Chem.*, **1999**, *6*, 1125-1149.
- 247 J. A. Benvenuto, T. H. Connor, D. K. Monteith, J. L. Laidlaw, S. C. Adams, T. S. Matney and J. C. Theiss, *J. Pharm. Sci.*, **1993**, *82*, 988-991.
- 248 G. Saydam, H. H. Aydin, F. Sahin, O. Kucukoglu, E. Erciyas, E. Terzioglu, F. Buyukkececi and S. B. Omay, *Leuk. Res.*, **2003**, *27*, 57-64.
- 249 G. M. D. Cunha, J. B. Fontenele, H. V. N. Junior, F. C. M. de Sousa, E. R. Silveira, N. A. P. Nogueira, M. O. de Moraes, G. S. B. Viana and L. V. Costa-Lotufo, *Phytother. Res.*, **2003**, *17*, 155-159.
- 250 Y. Kimura and K. Baba, *Int. J. Cancer*, **2003**, *106*, 429-437.
- 251 Y. Kimura, M. Taniguchi and K. Baba, *Planta Med.*, **2004**, *70*, 211-219.
- 252 R. Stoll, C. Renner, S. Hansen, S. Palme, C. Klein, A. Belling, W. Zeslawski, M. Kamionka, T. Rehm, P. Muhlhahn, R. Schumacher, F. Hesse, B. Kaluza, W. Voelter, R. A. Engh and T. A. Holak, *Biochemistry*, **2001**, *40*, 336-344.
- 253 S. K. Kumar, E. Hager, C. Pettit, H. Gurulingappa, N. E. Davidson and S. R. Khan, *J. Med. Chem.*, **2003**, *46*, 2813-2815.
- 254 T. Okuyama, M. Takata, J. Takayasu, T. Hasegawa, H. Tokuda, A. Nishino, H. Nishino and A. Iwashima, *Planta Med.*, **1991**, *57*, 242-246.
- 255 K. Baba, M. Taniguchi and K. Nakata, *Foods Food Ingredients J. Jpn.*, **1998**, *178*, 52-60.
- 256 S. Murakami, H. Kijima, Y. Isobe, M. Muramatsu, H. Aihara, S. Otomo, K. Baba and M. Kozawa, *J. Pharm. Pharmacol.*, **1990**, *42*, 723-726.
- 257 T. Fujita, S. Sakuma, T. Sumiya, H. Nishida, Y. Fujimoto, K. Baba and M. Kozawa, *Res. Commun. Chem. Path.*, **1992**, *77*, 227-240.
- 258 E. Shimizu, A. Hayashi, R. Takahashi, Y. Aoyagi, T. Murakami and K. Kimoto, *Journal of Nutr. Sci. Vitaminol.*, **1999**, *45*, 375-383.
- 259 M. Matsuura, Y. Kimura, K. Nakata, K. Baba and H. Okuda, *Planta Med.*, **2001**, *67*, 230-235.
- 260 T. Akihisa, H. Tokuda, M. Ukiya, M. Iizuka, S. Schneider, K. Ogasawara, T. Mukainaka, K. Iwatsuki, T. Suzuki and H. Nishino, *Cancer Lett.*, **2003**, *201*, 133-137.

Chapter 7 – Reference

- 261 H. Ogawa, M. Ohno and K. Baba, *Clin. Exp. Pharmacol. Physiol.*, **2005**, *32*, 19-23.
- 262 A. S. R. Anjaneyulu and U. V. Mallavadhani, *J. Chem. Soc.-Perkin Trans. 1*, **1988**, 623-628.
- 263 J. J. Romano and E. Casillas, *Tetrahedron Lett.*, **2005**, *46*, 2323-2326.
- 264 A. Furstner, *Angew. Chem.-Int. Edit.*, **2000**, *39*, 3013-3043.
- 265 R. H. Grubbs, *Handbook of Metathesis*, Wiley-VCH, Weinheim, Germany, **2003**.
- 266 K. J. Ivin and J. C. Mol, *Olefin Metathesis and Metathesis Polymerization*, Academic Press, San Diego, **1997**.
- 267 R. R. Schrock, J. S. Murdzek, G. C. Bazan, J. Robbins, M. Dimare and M. Oregan, *J. Am. Chem. Soc.*, **1990**, *112*, 3875-3886.
- 268 G. C. Bazan, E. Khosravi, R. R. Schrock, W. J. Feast, V. C. Gibson, M. B. Oregan, J. K. Thomas and W. M. Davis, *J. Am. Chem. Soc.*, **1990**, *112*, 8378-8387.
- 269 G. C. Bazan, J. H. Oskam, H. N. Cho, L. Y. Park and R. R. Schrock, *J. Am. Chem. Soc.*, **1991**, *113*, 6899-6907.
- 270 P. Schwab, M. B. France, J. W. Ziller and R. H. Grubbs, *Angew. Chem.-Int. Edit. Engl.*, **1995**, *34*, 2039-2041.
- 271 P. Schwab, R. H. Grubbs and J. W. Ziller, *J. Am. Chem. Soc.*, **1996**, *118*, 100-110.
- 272 T. R. Belderrain and R. H. Grubbs, *Organometallics*, **1997**, *16*, 4001-4003.
- 273 M. Scholl, S. Ding, C. W. Lee and R. H. Grubbs, *Org. Lett.*, **1999**, *1*, 953-956.
- 274 M. S. Sanford, J. A. Love and R. H. Grubbs, *J. Am. Chem. Soc.*, **2001**, *123*, 6543-6554.
- 275 M. M. Vasbinder and S. J. Miller, *J. Org. Chem.*, **2002**, *67*, 6240-6242.
- 276 A. K. Chatterjee, D. P. Sanders and R. H. Grubbs, *Org. Lett.*, **2002**, *4*, 1939-1942.
- 277 A. K. Chatterjee, T. L. Choi and R. H. Grubbs, *Synlett*, **2001**, 1034-1037.
- 278 A. K. Chatterjee and R. H. Grubbs, *Org. Lett.*, **1999**, *1*, 1751-1753.
- 279 J. Elaridi, W. R. Jackson and A. J. Robinson, *Tetrahedron: Asymmetry*, **2005**, *16*, 2025-2029.
- 280 K. Baba, K. Nakata, M. Taniguchi, T. Kido and M. Kozawa, *Phytochem*, **1990**, *29*, 3907-3910.
- 281 C. Pouget, C. Fagnere, J. P. Basly, A. E. Besson, Y. Champavier, G. Habrioux and A. J. Chulia, *Pharm. Res.*, **2002**, *19*, 286-291.
- 282 M. Artico, R. Di Santo, R. Costi, E. Novellino, G. Greco, S. Massa, E. Tramontano, M. E. Marongiu, A. De Montis and P. La Colla, *J. Med. Chem.*, **1998**, *41*, 3948-3960.
- 283 L. T. Vassilev, *J. Med. Chem.*, **2005**, *48*, 4491-4499.
- 284 C. Klein and L. T. Vassilev, *Br. J. Cancer*, **2004**, *91*, 1415-1419.
- 285 M. Oren, *Cell Death Differ.*, **2003**, *10*, 431-442.
- 286 A. J. Levine, *Cell*, **1997**, *88*, 323-331.
- 287 B. Vogelstein, D. Lane and A. J. Levine, *Nature*, **2000**, *408*, 307-310.
- 288 M. Y. Li, C. L. Brooks, F. Wu-Baer, D. L. Chen, R. Baer and W. Gu, *Science*, **2003**, *302*, 1972-1975.
- 289 T. Juven-Gershon and M. Oren, *Mol. Med.*, **1999**, *5*, 71-83.
- 290 J. Momand, D. Jung, S. Wilczynski and J. Niland, *Nucleic Acids Res.*, **1998**, *26*, 3453-3459.
- 291 M. Hollstein, D. Sidransky, B. Vogelstein and C. C. Harris, *Science*, **1991**, *253*, 49-53.
- 292 P. Hainaut and M. Hollstein, "p53 and human cancer: The first ten thousand mutations". In *Advances in Cancer Research, Vol 77*", Advances in Cancer Research, **77**, **2000**, pp. 81-137.
- 293 M. R. Arkin and J. A. Wells, *Nat. Rev. Drug Discov.*, **2004**, *3*, 301-317.
- 294 P. H. Kussie, S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A. J. Levine and N. P. Pavletich, *Science*, **1996**, *274*, 948-953.
- 295 L. D'Silva, P. Ozdowy, M. Krajewski, U. Rothweiler, M. Singh and T. A. Holak, *J. Am. Chem. Soc.*, **2005**, *127*, 13220-13226.
- 296 L. H. Chen, H. Yin, B. Farooqi, S. Sebti, A. D. Hamilton and J. D. Chen, *Mol. Cancer Ther.*, **2005**, *4*, 1019-1025.
- 297 D. A. Sharp, S. A. Kratowicz, M. J. Sank and D. L. George, *J. Biol. Chem.*, **1999**, *274*, 38189-38196.
- 298 J. van der Zee, *Ann. Oncol.*, **2002**, *13*, 1173-1184.
- 299 L. F. Fajardo, *Cancer Res.*, **1984**, *44*, 4826-4835.
- 300 H. S. Reinhold and B. Endrich, *Int. J. Hyperther.*, **1986**, *2*, 111-137.

Chapter 7 – Reference

- 301 C. Streffer, "Molecular and cellular mechanisms of hyperthermia." In "*Thermoradiotherapy and Thermochemotherapy Volume 1*", Springer Verlag, Berlin, **1995**, pp. 47-74.
- 302 S. Yokota, M. Kitahara and K. Nagata, *Cancer Res.*, **2000**, *60*, 2942-2948.
- 303 C. W. Song, A. Shakil, R. J. Griffin and K. Okajima, *Semin. Oncol.*, **1997**, *24*, 626-632.
- 304 H. H. Kampinga and E. Dikomey, *Int. J. Radiat. Biol.*, **2001**, *77*, 399-408.
- 305 F. A. Stewart and J. Denekamp, *Brit. J. Radiol.*, **1978**, *51*, 307-316.
- 306 C. Marino and A. Cividalli, *Int. J. Hyperther.*, **1992**, *8*, 771-781.
- 307 O. Dahl, "Interaction of heat and drugs *in vitro* and *in vivo*." In "*Thermoradiotherapy and Thermochemotherapy Volume 1*", Springer Verlag, Berlin, **1995**, pp. 103-121.
- 308 E. W. Gerner and M. J. Schneider, *Nature*, **1975**, *256*, 500-502.
- 309 F. F. Liu, N. Miller, W. Levin, B. Zanke, B. Cooper, M. Henry, M. D. Sherar, M. Pintilie, J. W. Hunt and R. P. Hill, *Int. J. Hyperther.*, **1996**, *12*, 197-208.
- 310 S. Lindquist and E. A. Craig, *Annu. Rev. Genet.*, **1988**, *22*, 631-677.
- 311 J. Landry, D. Bernier, P. Chretien, L. M. Nicole, R. M. Tanguay and N. Marceau, *Cancer Res.*, **1982**, *42*, 2457-2461.
- 312 D. D. Mosser, A. W. Caron, L. Bourget, C. DenisLarose and B. Massie, *Mol. Cell. Biol.*, **1997**, *17*, 5317-5327.
- 313 Y. Q. Wei, X. Zhao, Y. Kariya, K. Teshigawara and A. Uchida, *Cancer Immunol. Immunother.*, **1995**, *40*, 73-78.
- 314 L. M. Larocca, F. O. Ranelletti, N. Maggiano, S. Rutella, E. O. LaBarbera, C. Rumi, F. Serra, M. T. Voso, M. Piantelli, L. Teofili and G. Leone, *Int. J. Cancer*, **1997**, *73*, 75-83.
- 315 K. T. Riabowol, L. A. Mizzen and W. J. Welch, *Science*, **1988**, *242*, 433-436.
- 316 R. I. Morimoto, A. Tissières and C. Georgopoulos, *The biology of heat shock proteins and molecular chaperones*, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY, USA, **1994**.
- 317 R. Voellmy, *Crit Rev Eukaryot Gene Expr.*, **1994**, *4*, 357-401.
- 318 M. Koishi, S. Yokota, T. Mae, Y. Nishimura, S. Kanamori, N. Horii, K. Shibuya, K. Sasai and M. Hiraoka, *Clin. Cancer Res.*, **2001**, *7*, 215-219.
- 319 N. Hosokawa, K. Hirayoshi, A. Nakai, Y. Hosokawa, N. Marui, M. Yoshida, T. Sakai, H. Nishino, A. Aoike, K. Kawai and K. Nagata, *Cell Struct. Funct.*, **1990**, *15*, 393-401.
- 320 N. Hosokawa, K. Hirayoshi, H. Kudo, H. Takechi, A. Aoike, K. Kawai and K. Nagata, *Mol. Cell. Biol.*, **1992**, *12*, 3490-3498.
- 321 H. Zimmer, D. C. Armbruster and L. J. Trauth, *J. Heterocycl. Chem.*, **1965**, *2*, 171-175.
- 322 B. Rigo, C. Lespagnol and M. Pauly, *J. Heterocycl. Chem.*, **1988**, *25*, 49-57.
- 323 H. Sasaki, Y. Mori, J. Nakamura and J. Shibasaki, *J. Med. Chem.*, **1991**, *34*, 628-633.
- 324 A. I. Khodair, *J. Heterocycl. Chem.*, **2002**, *39*, 1153-1160.
- 325 J. S. Isaacs, W. P. Xu and L. Neckers, *Cancer Cell*, **2003**, *3*, 213-217.
- 326 R. Bagatell and L. Whitesell, *Mol. Cancer Ther.*, **2004**, *3*, 1021-1030.
- 327 L. Neckers, *Curr. Med. Chem.*, **2003**, *10*, 733-739.
- 328 M. P. Goetz, D. Toft, J. Reid, M. Ames, B. Stensgard, S. Safgren, A. A. Adjei, J. Sloan, P. Atherton, V. Vasile, S. Salazaar, A. Adjei, G. Croghan and C. Erlichman, *J. Clin. Oncol.*, **2005**, *23*, 1078-1087.
- 329 J. L. Grem, G. Morrison, X. D. Guo, E. Agnew, C. H. Takimoto, R. Thomas, E. Szabo, L. Grochow, F. Grollman, J. M. Hamilton, L. Neckers and R. H. Wilson, *J. Clin. Oncol.*, **2005**, *23*, 1885-1893.
- 330 J. Y. Zou, Y. L. Guo, T. Guettouche, D. F. Smith and R. Voellmy, *Cell*, **1998**, *94*, 471-480.
- 331 R. I. Morimoto, *Genes Dev.*, **1998**, *12*, 3788-3796.
- 332 R. Bagatell, G. D. Paine-Murrieta, C. W. Taylor, E. J. Pulcini, S. Akinaga, I. J. Benjamin and L. Whitesell, *Clin. Cancer Res.*, **2000**, *6*, 3312-3318.
- 333 F. Guo, K. Rocha, P. Bali, M. Pranpat, W. Fiskus, S. Boyapalle, S. Kumaraswamy, M. Balasis, B. Greedy, E. S. M. Armitage, N. Lawrence and K. Bhalla, *Cancer Res.*, **2005**, *65*, 10536-10544.
- 334 B. A. Bhat, K. L. Dhar, S. C. Puri, A. K. Saxena, M. Shanmugavel and G. N. Qazi, *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 3177-3180.
- 335 C. D. Gutsche, E. F. Jason, R. S. Coffey and H. E. Johnson, *J. Am. Chem. Soc.*, **1958**, *80*, 5767 - 5772.
- 336 I. J. S. Fairlamb, A. R. Kapdi and A. F. Lee, *Org. Lett.*, **2004**, *6*, 4435-4438.

Chapter 7 – Reference

- 337 M. T. Bogert and R. M. Isham, *J. Am. Chem. Soc.*, **1914**, *36*, 514 - 530.
- 338 T. Biftu, B. G. Hazra and R. Stevenson, *J. Chem. Soc., Perkin Trans. 1*, **1979**, 2276-81.
- 339 Y. H. Kuo and S. T. Lin, *Chem. Pharm. Bull.*, **1993**, *41*, 1507-1512.
- 340 V. J. Harding, *J. Chem. Soc.*, **1914**, *105*, 2790 - 2800.
- 341 F. Herencia, M. L. Ferrandiz, A. Ubeda, J. N. Dominguez, J. E. Charris, G. M. Lobo and M. J. Alcaraz, *Biocrg. Med. Chem. Lett.*, **1998**, *8*, 1169-1174.
- 342 S. Khabnadideh, D. Pez, A. Musso, R. Brun, L. M. R. Perez, D. Gonzalez-Pacanowska and I. H. Gilbert, *Bioorg. Med. Chem.*, **2005**, *13*, 2637-2649.
- 343 V. S. Parmar, S. C. Jain, K. S. Bisht, N. K. Sharma, Himanshu and S. Gupta, *Indian J. Chem. Sect B- Org. Chem. Incl. Med. Chem.*, **1998**, *37*, 628-643.
- 344 E. Maccarone, G. Cuffari, A. Passerini and F. Raymo, *J. Chem. Res. Miniprint*, **1991**, *9*, 2401-2413.
- 345 K. Yamada, T. Kurokawa, H. Tokuyama and T. Fukuyama, *J. Am. Chem. Soc.*, **2003**, *125*, 6630-6631.
- 346 F. E. Ziegler, I. Chliwner, K. W. Fowler, S. J. Kanfer, S. J. Kuo and N. D. Sinha, *J. Am. Chem. Soc.*, **1980**, *102*, 790-798.
- 347 V. Lisowski, S. Leonce, L. Kraus-Berthier, J. S. D. Santos, A. Pierre, G. Atassi, D. H. Caignard, P. Renard and S. Rault, *J. Med. Chem.*, **2004**, *47*, 1448-1464.
- 348 F. Effenberger and J. Jager, *J. Org. Chem.*, **1997**, *62*, 3867-3873.
- 349 T. Giannopoulos, J. R. Ferguson, B. J. Wakefield and G. Varvounis, *Tetrahedron*, **2000**, *56*, 447-453.
- 350 C. Fruit, A. Turck, N. Ple, L. Mojovic and G. Queguiner, *Tetrahedron*, **2001**, *57*, 9429-9435.
- 351 D. Kaliakoudas, C. H. Eugster and P. Ruedi, *Helv. Chim. Acta*, **1990**, *73*, 48-62.
- 352 M. Takasugi, S. Nagao, T. Masamune, A. Shirata and K. Takahashi, *Chemistry Letters*, **1980**, 1573-1576.
- 353 A. Sakai, T. Aoyama and T. Shioiri, *Heterocycles*, **2000**, *52*, 643-659.
- 354 J. A. N. Augustyn, B. C. B. Bezuidenhout and D. Ferreira, *Tetrahedron*, **1990**, *46*, 2651-2660.
- 355 N. C. Veitch, P. S. E. Sutton, G. C. Kite and H. E. Ireland, *J. Nat. Prod.*, **2003**, *66*, 210-216.
- 356 R. Correa, M. A. S. Pereira, D. Buffon, L. dos Santos, V. Cechinel, A. R. S. Santos and R. J. Nunes, *Archiv Der Pharmazie*, **2001**, *334*, 332-334.
- 357 M. Artico, R. Disanto, R. Costi, S. Massa, A. Retico, G. Apuzzo, G. Simonetti and V. Strippoli, *J. Med. Chem.*, **1995**, *38*, 4223-4233.
- 358 W. H. Burton, W. L. Budde and C. C. Cheng, *J. Med. Chem.*, **1970**, *13*, 1009-1012.
- 359 F. C. Brown, C. K. Bradsher, S. M. Bond and M. Potter, *J. Am. Chem. Soc.*, **1951**, *73*, 2357 - 2359.
- 360 P. M. Chakrabarti, N. B. Chapman and K. Clarke, *Tetrahedron*, **1969**, *25*, 2781-2785.
- 361 E. Campaigne and W. E. Kreighbaum, *J. Org. Chem.*, **1961**, *26*, 1326 - 1327.

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

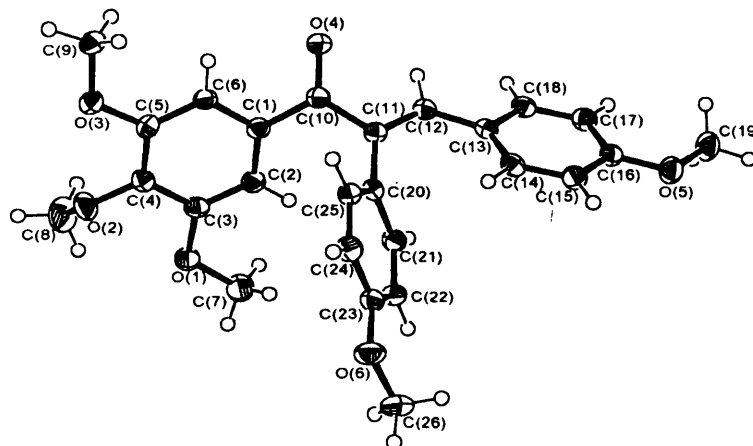


Table 1. Crystal data and structure refinement for 03Lawrence4 (173)

Identification code	03Lawrence4
Empirical formula	C ₂₆ H ₂₆ O ₆
Formula weight	434.47
Temperature	150(2) K
Wavelength	0.71073
Crystal system	Monoclinic
Space Group	P2 (1) / c
Unit cell dimensions	a = 15.8047 (4) Å alpha = 90 deg. b = 6.8725 (2) Å beta = 103.7263 (10) deg. c = 21.6295 (8) Å gamma = 90 deg.
Volume	2282.25 (12) Å ³
Z	4
Density (calculated)	1.264 Mg/m ³
Absorption coefficient	0.089 mm ⁻¹
F (000)	920
Crystal size	0.30 x 0.10 x 0.08 mm
Theta range for data collection	3.12 to 27.52 deg.
Index ranges	-20 ≤ h ≤ 20, -8 ≤ k ≤ 8, -27 ≤ l ≤ 28
Reflection collected	16881
Independent reflections	5191 [R(int) = 0.0746]
Absorption correction	'multi-scan'
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	5191 / 0 / 295
Goodness-of-fit on F ²	1.039
Final R indices [I > 2σ(I)]	R1 = 0.0517, wR2 = 0.1221
R indices (all data)	R1 = 0.0885, wR2 = 0.1400
Extinction coefficient	0.0203 (19)
Largest diff. peak and hole	0.236 and -0.221 e.Å ⁻³

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

Table 3. Bond length [Å] and angles [deg] for 03Lawrence4 (173)

O(1)-C(3)	1.370 (2)	O(1)-C(7)	1.434 (2)
O(2)-C(4)	1.370 (2)	O(2)-C(8)	1.428 (3)
O(3)-C(5)	1.367 (2)	O(3)-C(9)	1.431 (2)
O(4)-C(10)	1.227 (2)	O(5)-C(16)	1.366 (2)
O(5)-C(19)	1.427 (2)	O(6)-C(23)	1.371 (2)
O(6)-C(26)	1.425 (2)	C(1)-C(2)	1.393 (2)
C(1)-C(6)	1.396 (2)	C(1)-C(10)	1.494 (2)
C(2)-C(3)	1.384 (2)	C(3)-C(4)	1.398 (2)
C(4)-C(5)	1.401 (2)	C(5)-C(6)	1.386 (2)
C(10)-C(11)	1.500 (2)	C(11)-C(12)	1.354 (2)
C(11)-C(20)	1.488 (2)	C(12)-C(13)	1.464 (2)
C(13)-C(18)	1.399 (2)	C(13)-C(14)	1.405 (2)
C(14)-C(15)	1.373 (3)	C(15)-C(16)	1.405 (2)
C(16)-C(17)	1.380 (3)	C(17)-C(18)	1.385 (3)
C(20)-C(21)	1.391 (2)	C(20)-C(25)	1.397 (2)
C(21)-C(22)	1.389 (2)	C(22)-C(23)	1.381 (3)
C(23)-C(24)	1.396 (3)	C(24)-C(25)	1.382 (2)
C(3)-O(1)-C(7)	116.90 (14)	C(4)-O(2)-C(8)	116.20 (16)
C(5)-O(3)-C(9)	116.42 (14)	C(16)-O(5)-C(19)	117.08 (15)
C(23)-O(6)-C(26)	117.31 (15)	C(2)-C(1)-C(6)	120.55 (16)
C(2)-C(1)-C(10)	121.02 (15)	C(6)-C(1)-C(10)	118.35 (15)
C(3)-C(2)-C(1)	119.87 (16)	O(1)-C(3)-C(2)	124.66 (15)
O(1)-C(3)-C(4)	115.11 (15)	C(2)-C(3)-C(4)	120.19 (15)
O(2)-C(4)-C(3)	122.16 (15)	O(2)-C(4)-C(5)	118.13 (15)
C(3)-C(4)-C(5)	119.53 (16)	O(3)-C(5)-C(6)	124.72 (15)
O(3)-C(5)-C(4)	114.85 (15)	C(6)-C(5)-C(4)	120.43 (15)
C(5)-C(6)-C(1)	119.41 (16)	O(4)-C(10)-C(1)	119.80 (15)
O(4)-C(10)-C(11)	120.68 (15)	C(1)-C(10)-C(1)	119.52 (14)
C(12)-C(11)-C(20)	124.88 (16)	C(12)-C(11)-C(10)	116.32 (15)
C(20)-C(11)-C(10)	118.80 (14)	C(11)-C(12)-C(13)	130.34 (16)
C(18)-C(13)-C(14)	116.87 (16)	C(18)-C(13)-C(12)	118.16 (16)
C(14)-C(13)-C(12)	124.79 (15)	C(15)-C(14)-C(13)	121.83 (16)
C(14)-C(15)-C(16)	119.91 (17)	O(5)-C(16)-C(17)	125.12 (15)
O(5)-C(16)-C(15)	115.40 (16)	C(17)-C(16)-C(15)	119.47 (16)
C(16)-C(17)-C(18)	119.95 (16)	C(17)-C(18)-C(13)	121.97 (17)
C(21)-C(20)-C(25)	117.84 (16)	C(21)-C(20)-C(11)	121.41 (15)
C(25)-C(20)-C(11)	120.76 (15)	C(22)-C(21)-C(20)	121.85 (16)
C(23)-C(22)-C(21)	119.27 (16)	O(6)-C(23)-C(22)	124.82 (16)
O(6)-C(23)-C(24)	115.11 (16)	C(22)-C(23)-C(24)	120.07 (16)
C(25)-C(24)-C(23)	119.92 (16)	C(24)-C(25)-C(20)	121.03 (16)

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

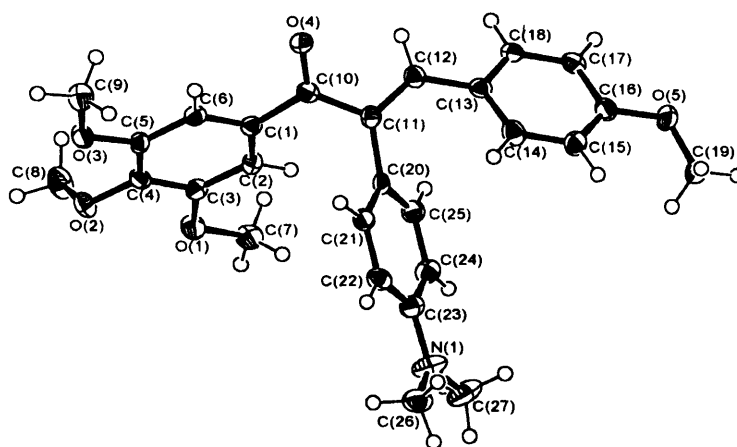


Table 1. Crystal data and structure refinement for 03Lawrence2 (175)

Identification code	s92
Empirical formula	C ₂₇ H ₂₉ N O ₅
Formula weight	447.51
Temperature	150(2) K
Wavelength	0.71073
Crystal system	Orthorhombic
Space Group	P2 (1) 2 (1) 2 (1)
Unit cell dimensions	a = 7.2683 (2) Å alpha = 90 deg. b = 13.9317 (4) Å beta = 90 deg. c = 22.9055 (7) Å gamma = 90 deg.
Volume	2319.41 (12) Å ³
Z	4
Density (calculated)	1.282 Mg/m ³
Absorption coefficient	0.088 mm ⁻¹
F (000)	952
Crystal size	0.38 x 0.35 x 0.30 mm
Theta range for data collection	2.92 to 27.49 deg.
Index ranges	-7 ≤ h < 9, -17 ≤ k < 18, -29 ≤ l < 29
Reflection collected	12133
Independent reflections	5078 [R(int) = 0.0743]
Max. and min. transmission	0.9741 and 0.9673
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	5078 / 0 / 305
Goodness-of-fit on F ²	1.021
Final R indices [I > 2σ(I)]	R1 = 0.0564, wR2 = 0.1164
R indices (all data)	R1 = 0.0919, wR2 = 0.1308
Absolute structure parameter	-0.1(12)
Extinction coefficient	0.0111 (15)
Largest diff. peak and hole	0.227 and -0.272 e.Å ⁻³

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

Table 3. Bond length [Å] and angles [deg] for 03Lawrence2 (175)

O(1)-C(3)	1.370 (3)	O(1)-C(7)	1.432 (3)
O(2)-C(4)	1.373 (3)	O(2)-C(8)	1.438 (3)
O(3)-C(5)	1.369 (3)	O(3)-C(9)	1.437 (3)
O(4)-C(10)	1.227 (3)	O(5)-C(16)	1.374 (3)
O(5)-C(19)	1.434 (3)	N(1)-C(23)	1.383 (4)
N(1)-C(26)	1.432 (4)	N(1)-C(27)	1.438 (4)
C(1)-C(6)	1.389 (3)	C(1)-C(2)	1.395 (3)
C(1)-C(10)	1.493 (3)	C(2)-C(3)	1.392 (3)
C(3)-C(4)	1.399 (3)	C(4)-C(5)	1.394 (3)
C(5)-C(6)	1.394 (3)	C(10)-C(11)	1.497 (3)
C(11)-C(12)	1.347 (3)	C(11)-C(20)	1.497 (3)
C(12)-C(13)	1.472 (3)	C(13)-C(18)	1.393 (3)
C(13)-C(14)	1.399 (4)	C(14)-C(15)	1.381 (4)
C(15)-C(16)	1.385 (4)	C(16)-C(17)	1.385 (4)
C(17)-C(18)	1.383 (4)	C(20)-C(21)	1.385 (4)
C(20)-C(25)	1.399 (3)	C(21)-C(22)	1.379 (4)
C(22)-C(23)	1.407 (4)	C(23)-C(24)	1.408 (4)
C(24)-C(25)	1.371 (4)		
C(3)-O(1)-C(7)	117.1 (2)	C(4)-O(2)-C(8)	114.5 (2)
C(5)-O(3)-C(9)	116.5 (2)	C(16)-O(5)-C(19)	116.7 (2)
C(23)-N(1)-C(26)	120.4 (2)	C(23)-N(1)-C(27)	120.3 (2)
C(26)-N(1)-C(27)	119.0 (2)	C(6)-C(1)-C(2)	120.4 (2)
C(6)-C(1)-C(10)	118.2 (2)	C(2)-C(1)-C(10)	121.2 (2)
C(3)-C(2)-C(1)	120.0 (2)	O(1)-C(3)-C(2)	124.8 (2)
O(1)-C(3)-C(4)	115.3 (2)	C(2)-C(3)-C(4)	119.9 (2)
O(2)-C(4)-C(5)	118.6 (2)	O(2)-C(4)-C(3)	121.6 (2)
C(5)-C(4)-C(3)	119.8 (2)	O(3)-C(5)-C(4)	115.8 (2)
O(3)-C(5)-C(6)	123.9 (2)	C(4)-C(5)-C(6)	120.3 (2)
C(1)-C(6)-C(5)	119.7 (2)	O(4)-C(10)-C(1)	119.4 (2)
O(4)-C(10)-C(11)	120.1 (2)	C(1)-C(10)-C(11)	120.4 (2)
C(12)-C(11)-C(20)	123.7 (2)	C(12)-C(10)-C(11)	116.4 (2)
C(20)-C(11)-C(10)	119.9 (2)	C(11)-C(12)-C(13)	128.1 (2)
C(18)-C(13)-C(14)	117.5 (2)	C(18)-C(13)-C(12)	119.5 (2)
C(14)-C(13)-C(12)	123.1 (2)	C(15)-C(14)-C(13)	121.7 (2)
C(14)-C(15)-C(16)	119.6 (2)	O(5)-C(16)-C(15)	123.8 (2)
O(5)-C(16)-C(17)	116.3 (2)	C(15)-C(16)-C(17)	119.9 (2)
C(18)-C(17)-C(16)	120.0 (2)	C(17)-C(18)-C(13)	121.3 (2)
C(21)-C(20)-C(25)	117.2 (2)	C(21)-C(20)-C(11)	122.3 (2)
C(25)-C(20)-C(11)	120.5 (2)	C(22)-C(21)-C(20)	121.8 (3)
C(21)-C(22)-C(23)	121.0 (3)	N(1)-C(23)-C(22)	121.7 (2)
N(1)-C(23)-C(24)	121.1 (2)	C(22)-C(23)-C(24)	117.2 (2)
C(25)-C(24)-C(23)	120.6 (3)	C(24)-C(25)-C(20)	122.1 (3)

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

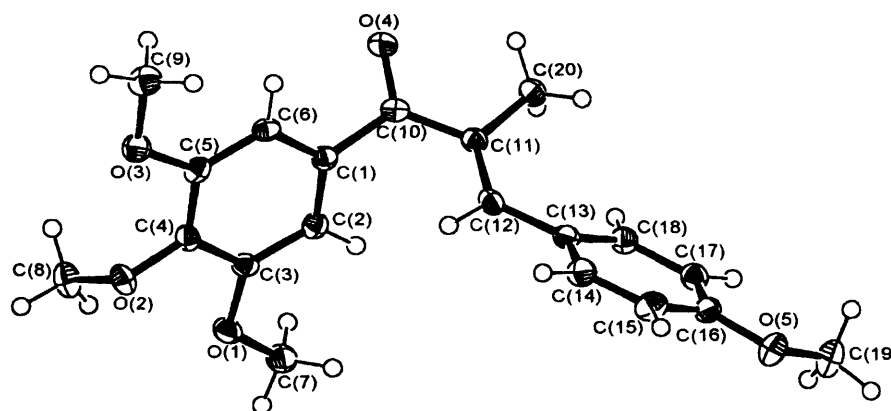


Table 1. Crystal data and structure refinement for 03Lawrence1 (182)

Identification code	s92
Empirical formula	C ₂₇ H ₂₉ N O ₅
Formula weight	447.51
Temperature	150(2) K
Wavelength	0.71073
Crystal system	Orthorhombic
Space Group	P2 (1) / c
Unit cell dimensions	a = 11.5106 (3) Å alpha = 90 deg. b = 14.2603 (4) Å beta = 93.263 (2) deg. c = 10.5117 (3) Å gamma = 90 deg.
Volume	1722.64 (8) Å ³
Z	4
Density (calculated)	1.320 Mg/m ³
Absorption coefficient	0.094 mm ⁻¹
F (000)	728
Crystal size	0.22 x 0.20 x 0.18 mm
Theta range for data collection	2.93 to 27.46 deg.
Index ranges	-14 ≤ h < 14, -18 ≤ k < 18, -13 ≤ l < 13
Reflection collected	18706
Independent reflections	3905 [R(int) = 0.0828]
Max. and min. transmission	0.9832 and 0.9675
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parametres	3905 / 0 / 232
Goodness-of-fit on F ²	1.019
Final R indices [I > 2sigma (I)]	R1 = 0.0486, wR2 = 0.1178
R indices (all data)	R1 = 0.0784, wR2 = 0.1352
Extinction coefficient	0.019 (2)
Largest diff. peak and hole	0.263 and -0.326 e.Å ⁻³

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

Table 3. Bond length [Å] and angles [deg] for 03Lawrence1 (182)

O(1)-C(3)	1.366 (2)	O(1)-C(7)	1.429 (2)
O(2)-C(4)	1.382 (2)	O(2)-C(8)	1.420 (2)
O(3)-C(5)	1.370 (2)	O(3)-C(9)	1.428 (2)
O(4)-C(10)	1.230 (2)	O(5)-C(16)	1.375 (2)
O(5)-C(19)	1.421 (2)	C(1)-C(2)	1.393 (2)
C(1)-C(6)	1.394 (2)	C(1)-C(10)	1.507 (2)
C(2)-C(3)	1.393 (2)	C(3)-C(4)	1.398 (2)
C(4)-C(5)	1.393 (2)	C(5)-C(6)	1.391 (2)
C(10)-C(11)	1.479 (2)	C(11)-C(12)	1.345 (2)
C(11)-C(20)	1.512 (2)	C(12)-C(13)	1.466 (2)
C(13)-C(18)	1.394 (2)	C(13)-C(14)	1.406 (2)
C(14)-C(15)	1.378 (3)	C(15)-C(16)	1.390 (2)
C(16)-C(17)	1.393 (2)	C(17)-C(18)	1.389 (2)
C(3)-O(1)-C(7)	117.84 (13)	C(4)-O(2)-C(8)	114.29 (13)
C(5)-O(3)-C(9)	117.87 (13)	C(16)-O(5)-C(19)	118.19 (14)
C(2)-C(1)-C(6)	120.94 (15)	C(2)-C(1)-C(10)	120.43 (15)
C(6)-C(1)-C(10)	118.30 (14)	C(3)-C(2)-C(1)	119.17 (15)
O(1)-C(3)-C(2)	124.91 (15)	O(1)-C(3)-C(4)	114.89 (14)
O(2)-C(3)-C(4)	120.20 (15)	O(2)-C(4)-C(5)	119.09 (15)
O(2)-C(4)-C(3)	120.77 (15)	C(5)-C(4)-C(3)	120.05 (14)
O(3)-C(5)-C(6)	124.92 (15)	O(3)-C(5)-C(4)	115.07 (14)
C(6)-C(5)-C(4)	120.01 (15)	C(5)-C(6)-C(1)	119.56 (15)
O(4)-C(10)-C(11)	120.60 (15)	O(4)-C(10)-C(1)	117.80 (15)
C(11)-C(10)-C(1)	121.60 (14)	C(12)-C(11)-C(10)	119.05 (14)
C(12)-C(11)-C(20)	124.93 (16)	C(10)-C(11)-C(20)	115.41 (14)
C(11)-C(12)-C(13)	127.94 (15)	C(18)-C(13)-C(14)	116.92 (16)
C(18)-C(13)-C(12)	124.32 (15)	C(14)-C(13)-C(12)	118.72 (15)
C(15)-C(14)-C(13)	121.72 (16)	C(14)-C(15)-C(16)	120.20 (16)
O(5)-C(16)-C(15)	115.79 (15)	O(5)-C(16)-C(17)	124.41 (16)
C(15)-C(16)-C(17)	119.77 (16)	C(18)-C(17)-C(16)	119.33 (16)
C(17)-C(18)-C(13)	122.17 (15)		

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

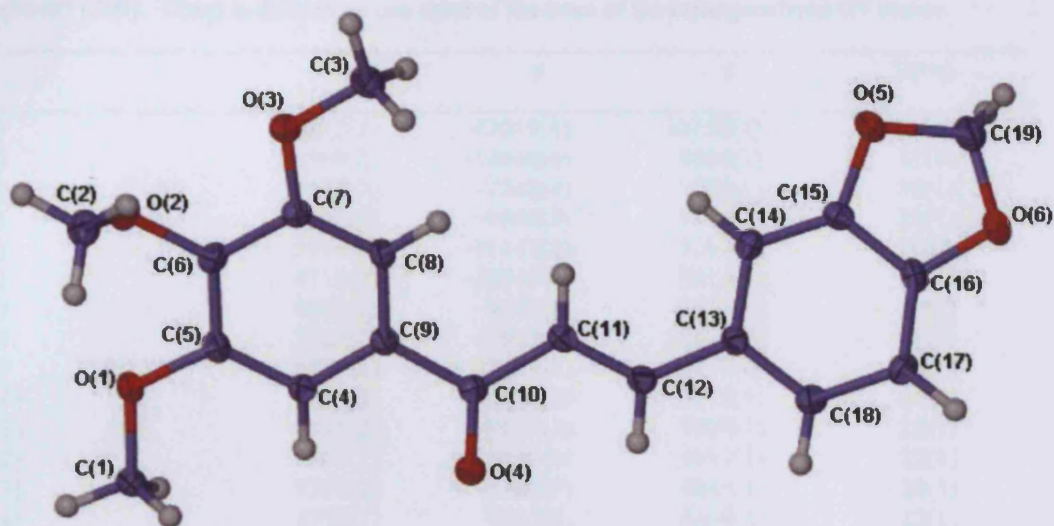


Table 1. Crystal data and structure refinement for njl0403 (209).

Identification code	njl0403	
Empirical formula	C ₁₉ H ₁₈ O ₆	
Formula weight	342.33	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 2 ₁ /n	
Unit cell dimensions	a = 10.1965(4) Å	α = 90°.
	b = 6.5167(2) Å	β = 101.484(2)°.
	c = 24.6468(9) Å	γ = 90°.
Volume	1604.93(10) Å ³	
Z	4	
Density (calculated)	1.417 Mg/m ³	
Absorption coefficient	0.106 mm ⁻¹	
F(000)	720	
Crystal size	0.18 x 0.18 x 0.10 mm ³	
Theta range for data collection	2.92 to 27.50°.	
Index ranges	-13 ≤ h ≤ 12, -7 ≤ k ≤ 8, -31 ≤ l ≤ 31	
Reflections collected	11803	
Independent reflections	3664 [R(int) = 0.0911]	
Completeness to theta = 27.50°	99.1 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9895 and 0.9812	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	3664 / 0 / 229	
Goodness-of-fit on F ²	1.014	
Final R indices [I > 2σ(I)]	R1 = 0.0615, wR2 = 0.1411	
R indices (all data)	R1 = 0.1124, wR2 = 0.1664	
Largest diff. peak and hole	0.313 and -0.312 e.Å ⁻³	

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

Table 2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for njl0403 (209). $U(\text{eq})$ is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
C(1)	6317(3)	-13019(4)	10158(1)	26(1)
C(2)	2599(3)	-12466(4)	8804(1)	31(1)
C(3)	3583(3)	-7202(4)	7729(1)	34(1)
C(4)	6556(2)	-9606(3)	9496(1)	21(1)
C(5)	5619(2)	-11147(3)	9322(1)	20(1)
C(6)	4712(2)	-10948(3)	8819(1)	20(1)
C(7)	4682(2)	-9139(3)	8513(1)	21(1)
C(8)	5629(2)	-7612(3)	8678(1)	21(1)
C(9)	6576(2)	-7862(3)	9170(1)	20(1)
C(10)	7647(2)	-6315(3)	9375(1)	21(1)
C(11)	7916(2)	-4637(3)	9009(1)	23(1)
C(12)	8863(2)	-3252(3)	9187(1)	22(1)
C(13)	9230(2)	-1483(3)	8885(1)	20(1)
C(14)	8779(2)	-1253(3)	8309(1)	23(1)
C(15)	9119(2)	511(3)	8074(1)	22(1)
C(16)	9869(2)	2047(3)	8378(1)	24(1)
C(17)	10333(3)	1878(4)	8938(1)	25(1)
C(18)	10004(2)	70(4)	9184(1)	24(1)
C(19)	9539(3)	2941(4)	7490(1)	35(1)
O(1)	5510(2)	-12898(2)	9612(1)	25(1)
O(2)	3857(2)	-12544(2)	8624(1)	25(1)
O(3)	3696(2)	-9050(2)	8044(1)	26(1)
O(4)	8312(2)	-6488(2)	9848(1)	27(1)
O(5)	8771(2)	1112(2)	7524(1)	32(1)
O(6)	10025(2)	3669(3)	8039(1)	34(1)

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

Table 3. Bond lengths [Å] and angles [°] for njl0403 (209).

C(1)-O(1)	1.433(3)
C(1)-H(1A)	0.9800
C(1)-H(1B)	0.9800
C(1)-H(1C)	0.9800
C(2)-O(2)	1.438(3)
C(2)-H(2A)	0.9800
C(2)-H(2B)	0.9800
C(2)-H(2C)	0.9800
C(3)-O(3)	1.425(3)
C(3)-H(3A)	0.9800
C(3)-H(3B)	0.9800
C(3)-H(3C)	0.9800
C(4)-C(5)	1.393(3)
C(4)-C(9)	1.394(3)
C(4)-H(4)	0.9500
C(5)-O(1)	1.363(3)
C(5)-C(6)	1.397(3)
C(6)-O(2)	1.380(3)
C(6)-C(7)	1.397(3)
C(7)-O(3)	1.374(3)
C(7)-C(8)	1.390(3)
C(8)-C(9)	1.400(3)
C(8)-H(8)	0.9500
C(9)-C(10)	1.498(3)
C(10)-O(4)	1.231(3)
C(10)-C(11)	1.478(3)
C(11)-C(12)	1.330(3)
C(11)-H(11)	0.9500
C(12)-C(13)	1.460(3)
C(12)-H(12)	0.9500
C(13)-C(18)	1.399(3)
C(13)-C(14)	1.412(3)
C(14)-C(15)	1.362(3)
C(14)-H(14)	0.9500
C(15)-C(16)	1.385(3)
C(15)-O(5)	1.387(3)
C(16)-C(17)	1.372(3)
C(16)-O(6)	1.376(3)
C(17)-C(18)	1.396(3)
C(17)-H(17)	0.9500
C(18)-H(18)	0.9500
C(19)-O(6)	1.426(3)
C(19)-O(5)	1.438(3)
C(19)-H(19A)	0.9900
C(19)-H(19B)	0.9900
O(1)-C(1)-H(1A)	109.5
O(1)-C(1)-H(1B)	109.5
H(1A)-C(1)-H(1B)	109.5
O(1)-C(1)-H(1C)	109.5
H(1A)-C(1)-H(1C)	109.5
H(1B)-C(1)-H(1C)	109.5
O(2)-C(2)-H(2A)	109.5
O(2)-C(2)-H(2B)	109.5
H(2A)-C(2)-H(2B)	109.5
O(2)-C(2)-H(2C)	109.5
H(2A)-C(2)-H(2C)	109.5
H(2B)-C(2)-H(2C)	109.5
O(3)-C(3)-H(3A)	109.5
O(3)-C(3)-H(3B)	109.5
H(3A)-C(3)-H(3B)	109.5

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

O(3)-C(3)-H(3C)	109.5
H(3A)-C(3)-H(3C)	109.5
H(3B)-C(3)-H(3C)	109.5
C(5)-C(4)-C(9)	119.9(2)
C(5)-C(4)-H(4)	120.1
C(9)-C(4)-H(4)	120.1
O(1)-C(5)-C(4)	124.4(2)
O(1)-C(5)-C(6)	115.7(2)
C(4)-C(5)-C(6)	119.9(2)
O(2)-C(6)-C(5)	119.94(19)
O(2)-C(6)-C(7)	120.3(2)
C(5)-C(6)-C(7)	119.8(2)
O(3)-C(7)-C(8)	124.5(2)
O(3)-C(7)-C(6)	115.0(2)
C(8)-C(7)-C(6)	120.5(2)
C(7)-C(8)-C(9)	119.3(2)
C(7)-C(8)-H(8)	120.3
C(9)-C(8)-H(8)	120.3
C(4)-C(9)-C(8)	120.4(2)
C(4)-C(9)-C(10)	116.6(2)
C(8)-C(9)-C(10)	123.0(2)
O(4)-C(10)-C(11)	120.8(2)
O(4)-C(10)-C(9)	119.0(2)
C(11)-C(10)-C(9)	120.16(19)
C(12)-C(11)-C(10)	120.8(2)
C(12)-C(11)-H(11)	119.6
C(10)-C(11)-H(11)	119.6
C(11)-C(12)-C(13)	127.7(2)
C(11)-C(12)-H(12)	116.2
C(13)-C(12)-H(12)	116.2
C(18)-C(13)-C(14)	119.3(2)
C(18)-C(13)-C(12)	118.6(2)
C(14)-C(13)-C(12)	122.1(2)
C(15)-C(14)-C(13)	117.2(2)
C(15)-C(14)-H(14)	121.4
C(13)-C(14)-H(14)	121.4
C(14)-C(15)-C(16)	122.7(2)
C(14)-C(15)-O(5)	128.0(2)
C(16)-C(15)-O(5)	109.2(2)
C(17)-C(16)-O(6)	127.9(2)
C(17)-C(16)-C(15)	121.9(2)
O(6)-C(16)-C(15)	110.2(2)
C(16)-C(17)-C(18)	116.1(2)
C(16)-C(17)-H(17)	121.9
C(18)-C(17)-H(17)	121.9
C(17)-C(18)-C(13)	122.8(2)
C(17)-C(18)-H(18)	118.6
C(13)-C(18)-H(18)	118.6
O(6)-C(19)-O(5)	108.01(19)
O(6)-C(19)-H(19A)	110.1
O(5)-C(19)-H(19A)	110.1
O(6)-C(19)-H(19B)	110.1
O(5)-C(19)-H(19B)	110.1
H(19A)-C(19)-H(19B)	108.4
C(5)-O(1)-C(1)	116.55(18)
C(6)-O(2)-C(2)	114.03(17)
C(7)-O(3)-C(3)	117.37(18)
C(15)-O(5)-C(19)	104.84(17)
C(16)-O(6)-C(19)	105.08(18)

Symmetry transformations used to generate equivalent atoms:

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

Table 4. Anisotropic displacement parametres ($\text{\AA}^2 \times 10^3$) for njl0403 (209). The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^{*2} U^{11} + \dots + 2 h k a^* b^* U^{12}]$

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
C(1)	30(2)	24(1)	22(1)	3(1)	2(1)	-3(1)
C(2)	25(2)	35(1)	35(2)	-1(1)	9(1)	-7(1)
C(3)	41(2)	29(1)	29(1)	8(1)	-4(1)	0(1)
C(4)	20(1)	21(1)	21(1)	0(1)	4(1)	0(1)
C(5)	21(1)	17(1)	25(1)	1(1)	11(1)	3(1)
C(6)	19(1)	19(1)	24(1)	-4(1)	7(1)	-1(1)
C(7)	20(1)	22(1)	21(1)	-1(1)	5(1)	2(1)
C(8)	25(1)	17(1)	22(1)	1(1)	7(1)	0(1)
C(9)	21(1)	20(1)	20(1)	-1(1)	6(1)	2(1)
C(10)	20(1)	20(1)	21(1)	0(1)	4(1)	2(1)
C(11)	24(1)	22(1)	22(1)	3(1)	5(1)	1(1)
C(12)	24(1)	22(1)	20(1)	0(1)	5(1)	1(1)
C(13)	18(1)	20(1)	23(1)	2(1)	5(1)	0(1)
C(14)	20(1)	22(1)	26(1)	-2(1)	4(1)	-2(1)
C(15)	21(1)	24(1)	21(1)	3(1)	4(1)	2(1)
C(16)	24(1)	19(1)	30(1)	5(1)	9(1)	-1(1)
C(17)	26(1)	22(1)	27(1)	-3(1)	3(1)	-3(1)
C(18)	23(1)	26(1)	23(1)	-1(1)	4(1)	1(1)
C(19)	33(2)	35(2)	33(1)	10(1)	1(1)	-10(1)
O(1)	28(1)	20(1)	26(1)	4(1)	3(1)	-5(1)
O(2)	24(1)	21(1)	30(1)	-4(1)	7(1)	-4(1)
O(3)	28(1)	24(1)	23(1)	3(1)	-2(1)	-1(1)
O(4)	28(1)	27(1)	24(1)	4(1)	-1(1)	-4(1)
O(5)	37(1)	31(1)	24(1)	9(1)	1(1)	-8(1)
O(6)	43(1)	26(1)	32(1)	8(1)	6(1)	-8(1)

Chapter 8 – Appendix A – Supplementary X-ray Crystal Data

Table 5. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parametres ($\text{\AA}^2 \times 10^3$) for njl0403 (209).

	x	y	z	U(eq)
H(1A)	7264	-13088	10133	38
H(1B)	6075	-14251	10344	38
H(1C)	6164	-11800	10370	38
H(2A)	2760	-12521	9209	47
H(2B)	2046	-13638	8650	47
H(2C)	2135	-11187	8675	47
H(3A)	3449	-6043	7966	51
H(3B)	2818	-7304	7419	51
H(3C)	4404	-6987	7587	51
H(4)	7179	-9743	9836	25
H(8)	5633	-6410	8460	26
H(11)	7407	-4547	8642	27
H(12)	9358	-3436	9553	26
H(14)	8259	-2285	8094	27
H(17)	10848	2931	9147	30
H(18)	10319	-113	9570	29
H(19A)	10298	2632	7307	42
H(19B)	8972	3998	7269	42

