

Discovery of therapies for lymphomas and leukaemia: Synthesis and antiproliferative action of novel ethanoanthracenes



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Declaration

This thesis has not been submitted as an exercise for a degree at this, or any other university. The work presented herein is entirely my own unless otherwise stated. I agree that the library may lend or copy this thesis upon request.

James Mc Keown

Abstract

Cancer is a broad class of diseases responsible for up to 9.6 million deaths worldwide in 2018, accountable for approximately every one in six deaths globally. Cancer is second only in all-cause mortality to circulatory system diseases such as ischemic heart disease (IHD). By 2025, 20 million new cancer cases are expected to be diagnosed in the Western world.

Globally, chronic lymphocytic leukaemia (CLL) is the most common leukaemic disease in developed countries, primarily affecting the elderly. CLL is classed as a clonal disorder of mature B-lymphocytes and its clinical patient prognoses is affected mainly by the mutational status of the immunoglobulin G heavy chain variable region (IGHV) and distinct chromosomal abnormalities such as trisomy of chromosome 12. Patients with unmutated IGHV have a better prognosis than those with the wild type variant.

Structures related to the tetracyclic anti-depressant maprotiline demonstrate potent selective antiproliferative and pro-apoptotic effects *in vitro* in B-cell malignancies, namely Burkitt's Lymphoma (BL) cell lines DG-75 and MUTU-1. Based on these preliminary studies, libraries of structurally related nitrostyrene and chalcone-based ethanoanthracenes were designed, based on the proven effectiveness of a nitrostyrene core structure and chalcone moieties in leukaemic cell lines. These ethanoanthracenes were synthesised using Henry-Knoevenagel condensation, Claisen-Schmidt condensation and Diels–Alder cycloaddition reactions. The products were structurally arranged into nine series and antiproliferative activity was determined using the alamar Blue assay on the two CLL cell lines HG-3 (unmutated IGHV) and PGA-1 (mutated IGHV). Lead compounds from these series were discovered to elicit potent antiproliferative activity, e.g. for compound (**24**) IC₅₀ values 0.048 μ M (HG-3) and 0.061 μ M (PGA-1) which were superior to the clinically used chemotherapeutic agent for CLL, fludarabine. Pro-apoptotic cell death in both HG-3 and PGA-1 cell lines was observed. Low to moderate cytotoxicity was noted in CLL cell lines when tested close to the determined compound IC₅₀ values. This finding was also supported by the low toxicity of representative nitrostyrene and chalcone ethanoanthracene lead compounds in peripheral blood mononuclear cells (PBMCs), indicating selective toxicity for chronic lymphocytic leukaemia. Analysis of the structure-activity relationships (SARs) identified the lead nitrostyrene and chalcone ethanoanthracene compounds from this study as potential agents for progression to preclinical drug development for the treatment of CLL.

This thesis is dedicated to the memory of those who accompanied me on my life's journey so far; provided friendship, support, welcome company and most of all cherished memories, who have left this world well before their time.

It is not the years in your life but the life in your years that counts.

Adlai E. Stevenson (1954)

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Abbreviations

ACN	Acetonitrile
ACS	American Cancer Society
ADCC	Antibody dependent cell mediated cytotoxicity
APCI	Atmospheric-pressure chemical ionization
B.C.	Before Christ
BL	Burkitt's Lymphoma
BOC	tert-Butoxycarbonyl protecting group
CHOP	Cyclophosphamide, doxorubicin, vincristine (oncovin), and prednisone
CLL	Chronic Lymphocytic Leukaemia
CML	Chronic Myeloid Leukaemia
CODOX-M/IVAC	Cyclophosphamide, doxorubicin, methotrexate/isofosfamide, etoposide and high dose cytarabine
CSR	Class switch recombination
CVAD	cyclophosphamide, vincristine, doxorubicin (also known as Adriamycin), dexamethasone
DA	Diels Alder
DAT	Dopamine reuptake transporter
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EBNA	Epstein Barr Virus nuclear antigen
eBL	Endemic Burkitt's Lymphoma
EBV	Epstein Barr Virus
EC₅₀	Concentration at which a drug provides 50% maximal response
ESI	Electrospray Ionisation
EWG	Electron Withdrawing Group
FACS	Fluorescence activated cell sorting
FADH	Flavin adenine dinucleotide
FBS	Foetal Bovine Serum
FCR	Fludarabine Cyclophosphamide Rituximab

FISH	Fluorescence in situ hybridization
FT-IR	Fourier-Transform infrared spectroscopy
GTP	Guanine Triphosphate
H2AFX	H2A histone family member X
HBA	Hydrogen Bond Acceptor
HEK	Human Embryonic activity
HIV	Human immunodeficiency virus
HL	Hodgkin's Lymphoma
HPV	Human Papilloma Virus
HRMS	High resolution mass spectrometry
HTA	High Throughput Analysis
iBL	Immunodeficiency Burkitt's Lymphoma
IARC	International Agency for Research on Cancer
IGHV	Immunoglobulin heavy chain variable region
LDL	Low density lipoprotein
MOE	Molecular Operating Environment
NAC	<i>N</i> -acetyl cysteine
NAD(P)H	Nicotinamide adenine dinucleotide (phosphate)
NCD	Non-communicable disease
NCI	National Cancer Institute
NET	Noradrenaline reuptake transporter
NHL	Non-Hodgkin's Lymphoma
NSRI	Noradrenaline selective reuptake inhibitor
PBMC	Peripheral blood mononuclear cell
TLR	Toll-like receptor
TPSA	Total Polar Surface Area
QOL	Quality of Life
RPMI	Roswell Park Memorial Institute
ROS	Reactive oxygen species
sBL	Sporadic Burkitt's Lymphoma
SEM	Standard Error Measurement
SERT	Serotonin reuptake transporter

SIB	Swiss Institute of Bioinformatics
TLC	Thin Layer Chromatography
UV	Ultra Violet
WHO	World Health Organisation
¹³C NMR	Carbon nuclear magnetic resonance
¹H NMR	Proton nuclear magnetic resonance

Chapter 1: Literature Review

1.1 Cancer-A Brief Introduction

Cancer is by definition, when normal cells lose their ability to effectively manage their regulatory mechanisms responsible for growth and proliferation, hence resulting in uncontrolled cell expansion. According to the WHO (World Health Organisation), cancer is one of the leading causes of morbidity and mortality worldwide, accounting for 9.6 million deaths globally in 2018, second only to cardiovascular and cerebrovascular diseases such as ischaemic heart disease and stroke¹. In the next two decades, according to GLOBOCAN 2018 (Global Cancer Observatory), incidence of cancerous diseases is estimated to increase by approximately 60% , resulting in a projected figure of 29.5 million cases by 2040 (Figure 1)².

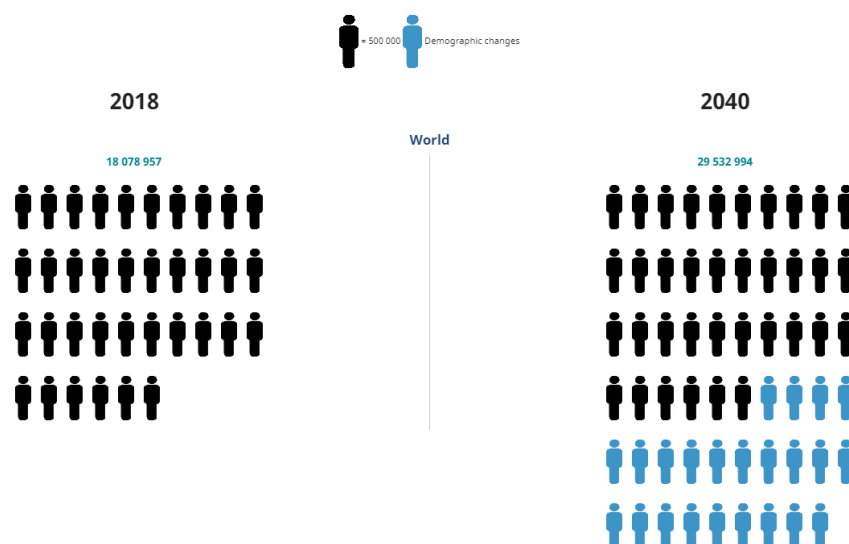


Figure 1-GLOBOCAN 2018 estimated cancer incidence from 2018 to 2040 (all cancers, all ages) ²

Approximately 8987 people die each year in Ireland as a result of malignancies, with the cumulative lifetime risk of developing cancer currently being 1 in 3, reflecting increasing worldwide cancer incidence³. Akin to many other developed, industrialised countries in the Western World, cancer presents a challenging healthcare burden, arising from many varied factors, including levels of economic and socioeconomic development (often cited through the HDI-Human Development Index measure)⁴. The most significant and preventable are behavioural and lifestyle associated issues including high BMI (Body Mass Index), low physical activity, low vegetable and fruit intake in diet as well as tobacco and alcohol consumption, accounting for up to one third of global cancer cases^{1, 5, 6}. In terms of all-cancer prevalence in the Irish population, breast cancer (30% of cases

in women) and prostate cancer (29% of cases in men) are the most commonly diagnosed cancers, excluding NMSC (non-melanoma skin cancer), (Figure 2).

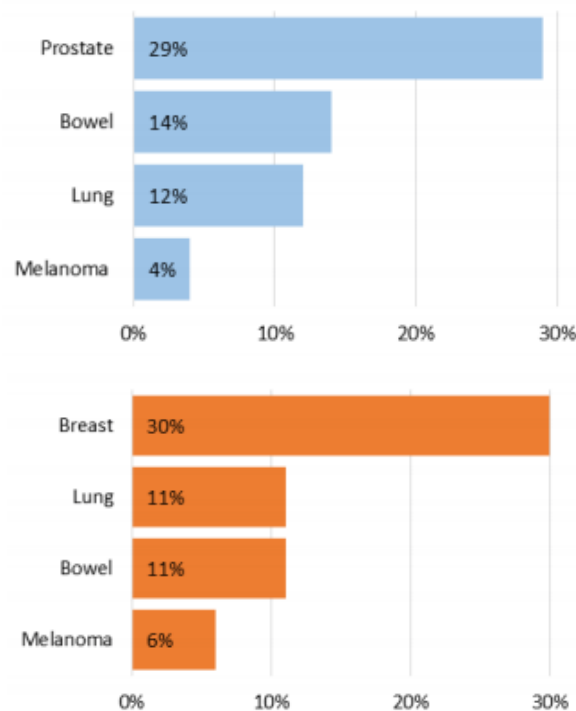


Figure 2: Top four invasive cancer incidence for males (blue) and females (orange) in Ireland (2016-2018), excluding non-melanoma skin cancer (NMSC)⁷

Cancer is an umbrella term for a multitude of heterogeneous diseases that can manifest in and spread to any part of the body. The central feature of cancer is the rapid proliferation of abnormal cells beyond normal physiological boundaries, often becoming invasive in nature and devastating multiple organ systems, ultimately leading to death as the clinical outcome in the majority of patient cases³. Furthermore, with 30-50% cancers being preventable, the WHO aims to help reduce premature mortality from the global burden of cancer and other non-communicable disease by at least 25% by 2025 by the implementation of a comprehensive cancer control strategy. This policy consists of a four pronged approach which consists of:

Primary prevention- Considered to be most valuable method in relation to public health along with being the most sustainable and cost-effective strategy for reducing disease burden e.g. reduced tobacco consumption or increased physical activity^{8, 9, 10}.

Secondary prevention- Also known as early detection, this consists of adequate and prompt access of symptomatic individuals to timely medical diagnoses, available screening services for at-risk population groups and public dissemination of basic disease

education. Such measures currently in place prevent the development of greater than 30% of potential new cancer cases¹¹.

Diagnosis and treatment- Through sufficient clinical assessment and treatment, patient survival and quality of life can be greatly improved, particularly in developing countries where research, medical facilities and treatment access can be sub-optimal, leading to greater proportions of advanced and end stage cancers¹².

Palliation- This is the holistic patient treatment route taken where advanced stage cancers no longer respond to available therapeutics and/or clinical procedures. The primary goal shifts towards ensuring that both patients and their families have the highest quality of life (psychologically, spiritually and physically) as the patient's clinical condition deteriorates¹³.

As a whole, by observing and monitoring disease through patient occurrence, presentation, treatment outcomes as well as other epidemiological variables in an iterative process, improvements in cancer care can be focussed on current patient needs based on current scientific and clinical evidence in the context of each country's current healthcare resources¹⁴.

1.1.2 History of Cancer

The origin of the term “cancer” is derived from the Greek *carcinos* and *carcinoma* (meaning crab), describing the physical appearance of specific tumours spreading throughout of patient's body tissue¹⁵, taking on a crab-like guise. The coining of this idiom is synonymous with the “Father of modern medicine” Hippocrates (460-370 B. C.) but was later translated into its more common Latin vernacular “cancer” by the Roman physician Celsus (28-50 B. C.), (Figure 3).

Neoplastic diseases are very ancient maladies, dating back as early as the Palaeozoic Era (approximately 542-251 million years ago)^{16, 17}, with the first well documented case of cancer and subsequent metastasis being the skeletal remains of an *Allosaurus fragilis* discovered in Utah (USA)¹⁸. Even though such pathopaleological evidence strongly suggests cancer existence predates human appearance, other studies have established the relative scarcity of evidence of cancer in early humans, supporting the theory that age,

diet and environmental factors substantially influence cancer incidence in humans¹⁹. Even though the argument exists that short lifespans of individuals in ancient times prevented neoplasm development, there has been an increasing trend of cancer prevalence documented in modern man over the past century. This high prevalence is mainly attributed to the increased life expectancy associated with modern societal advances (healthcare, sanitation, etc.), whereby our environments are for the most part synthetic or artificial and our behaviours largely sedentary, exposing us to numerous carcinogens and risk factors on a daily basis, to which an estimated 75% of all human cancer are related²⁰.



Figure 3: Roman physician Celsus (left), depiction of Carcinos (middle), Greek physician Hippocrates (right)²¹⁻²³

1.1.3 Biology of Cancer

Cancer develops from a single population of normal body cells by means of genetic cellular mutation²⁴, allowing them to circumvent conventional regulatory and preservation mechanisms related to growth and replication²⁵. As the human body contains on average 10^{14} cells²⁶, such mutations occur consistently at a constant rate but by virtue of DNA (deoxyribonucleic acid) cellular repair mechanisms (e.g. DNA ligase), tumour suppressor genes (PTEN) and protective tumour suppression systems such as p53 (protein 53), they are neutralised before aberrant cell populations take hold. However, once such mutations accumulate through abnormal cell proliferation and replication, the likelihood of gaining a selective evolutionary advantage increases. These cells will then follow the same principles of evolution that have dictated the development of known life for billions of years, the law of natural selection and the genetic drift phenomenon²⁷. Aside from selective mutational pressures, non-mutational epigenetic factors are thought to play a significant role in carcinogenesis.

Once this process is set in motion, cells begin to lose their defining niche characteristics (loss of differentiation)²⁵ and divide to form collective growths comprised of a single

progenitor cell line referred to as a neoplasm or tumour. It is from these “tumour cells” that the two main categories of neoplasm can develop. If the primary tumour remains localized as a single cluster, the growth is classified as benign and can be successfully treated/and or cured by surgical resection and removal²⁶. Conversely, if tumour cells become invasive and begin to aggressively overrun adjacent and/or distant tissues, it is categorised as malignant and officially defined in medical terms as a cancer²⁸. The major threat with malignancies is their ability to exploit the circulatory and lymphatic systems as transport highways to begin the metastatic cascade and if the appropriate physiological conditions are met, numerous secondary tumours can be established in remote biological sites such as breast cancer propagating in neural tissue. Once this process of metastasis occurs, the disease becomes much harder to treat and subsequently eradicate, with the majority of deaths being attributed to this process as opposed to the influences of the primary cell mass itself ²⁶ (Figure 4).

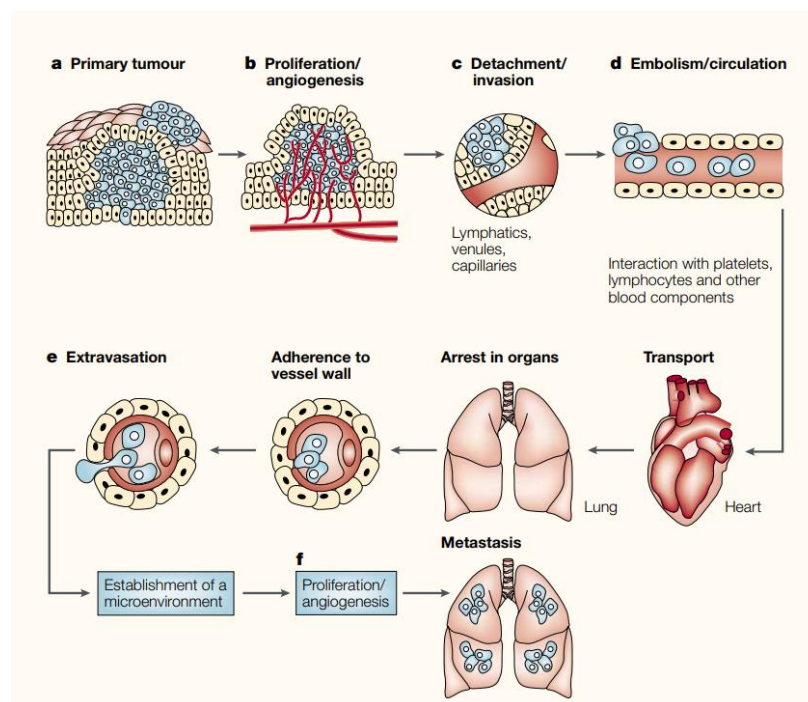


Figure 4: Main steps of the metastatic process from the establishment of the primary tumour to the seeding of secondary tumour sites²⁹

In terms of classification, cancers are subdivided according to their tissue of origin with carcinomas (derived from epithelial cells) being the most widespread, accounting for >80% of all cancers (lung, gastrointestinal, breast, skin). The next major cancer group are sarcomas, which evolve from connective or muscular tissue e.g. chondrosarcoma. Both groups fall under the broad category of solid-state cancers. The remaining types are

then generalised under haematological malignancies of leukaemias and lymphomas (non-solid cancers) which could be myeloid (blood cell progenitor) or lymphatic in origin²⁴. Tumorigenesis is a multi-factorial process thought to arise from countless interactions between environmental traits and individual genetic predispositions, developing first as a precancerous abnormally replicating body cell before eventually progressing to malignancy²⁴. The core preventable factors (which are subject to change) that can predispose any human being to an increased susceptibility to cancer development are as follows:

Chemical - such as tobacco, (defined as one the most destructive of the chemical carcinogens), pharmaceutical drug treatments, environmental pollution, asbestos

Biological - Diet and lifestyle including lack of adequate physical exercise, alcohol consumption, high fat-low nutrient diets, infectious diseases and introduction of viral oncogenic sequences e.g. HPV-1 (Human papillomavirus) are associated with cervical cancer incidence

Physical - Ultraviolet (normally causing DNA base changes) and ionizing radiation

In addition, non-preventable factors (not subject to change or constant) relating to the individual's inherited genetic makeup and family history e.g. Hereditary Breast and Ovarian Cancer Syndrome (HBOC) form the other main group of influences which determines an individual's lifetime probability of developing a certain cancer type³⁰.

In 1964, the WHO carried out a comprehensive study into cancer-related mortality paying particular attention to lifestyle and environmental factors such as daily occupational exposure to carcinogens and dietary deficiencies³¹. Both risks could be modified by the individual on a daily basis and therefore deemed preventable. Doll and Peto later concluded through international cancer epidemiology studies that variation in cancer rates could be partly attributed to each country's environmental and behavioural factors including smoking, reproductive behaviour, diet, sexual behaviour, occupational exposure and contracted infections³². Three factors which have gained widespread recognition in recent times are tobacco use, obesity/physical inactivity and communicable diseases (e.g. chronic infections)³³. Tobacco is currently a major global epidemic, with over 8 million deaths ascribed to its use per annum, approximately 1.2 million of which are indirect second-hand smoke exposure³⁴. Furthermore, epidemiological studies have

established causal relationships between tobacco consumption and at least 14 cancer types^{35, 36}. With smoking cessation, lung cancer risk in former smokers decreases to half that of active smokers within 10 years after quitting, along with concomitant increases in life expectancy and decreased risk of cardio and cerebrovascular disease^{37, 38}. Despite Ireland ranking first in the European tobacco control scale³⁹, implementation of preventative and control measures to reduce smoking among the population have had an appreciable impact since. This is evident with 7% decrease in smokers aged 15 years and over in 2016 compared with 2007 and a 0.5% current per annum decrease in current smokers in 2017⁴⁰. These measures included raising tax on tobacco products by at least 10% (shown by American Cancer Society (ACS) to reduce the consumption of cigarettes by 3-5%⁴¹) as well as the implementation of Tobacco Free Ireland government strategy which aims to see Ireland tobacco free (defined as <5% active smokers) by 2025^{40, 42}. In relation to the obesity epidemic sweeping across western society (more than doubling in prevalence worldwide since 1980⁴³), the IARC (International Agency For Research on Cancer) Handbook of Cancer Prevention –Absence of Excess Body Fat cites that excess energy intake, the nature of our built environments and lack of adequate physical activity were considered among some of the main drivers of obesity⁴⁴. These contributions are estimated to cause 1/5th to 1/3rd of the most prevalent cancers, with diet coming second only to tobacco use as a theoretically preventable cancer cause^{45, 46}. Hence, obesity is widely considered as a core risk factor to cancer development. This is especially evident with breast and colon cancer being two of the most common malignancies in Western Europe and United States. Moreover, the capability of exercise to influence cancer risk is independent of level/degree of obesity, outlining not only why healthy eating/social behaviours and moderate physical activity are key in obesity and cancer prevention.

In recent years, greater consideration has been given to the role of communicable pathogens and infections in carcinogenesis, with approximately 15% of all cancers being associated with contraction of infectious agents^{8,19}. Many of these are strongly associated with *H.pylori* (gastrohelicosis-precancerous stage and gastric cancer)^{47, 48}, HPV (human papilloma virus types 16 and 18-cervical cancer and cervical dysplasia-precancerous stage)⁴⁹, along with Hepatitis B and C (responsible for hepatocellular carcinoma development)^{24, 36, 50}. With the development of the HPV vaccine, its successful administration to at-risk individuals⁴⁶ and continuous cervical screening programmes⁵¹,

prevention rather than cancer treatment is always the preferred course of action as opposed to pharmacotherapy of already established disease⁵².

Carcinogens (also referred to as mutagens) induce DNA damage and/or genetic mutations at the level of DNA replication as well as impeding normal cellular differentiation. As these discrepancies accumulate in cell progeny, there is increased risk of carcinogenesis through genetic sequence variation (genetic) and incomplete / inaccurate DNA replication through dysregulation in gene expression or gene silencing, while the core DNA construct remains intact (epigenetic)²⁵. Consequently, defective cell regulatory mechanisms result, predominantly in proto-oncogene and tumour suppression gene management, two critical components of cellular growth and proliferation⁵³. Proto-oncogenes are genes which code for proteins involved in typical cell growth and division, driving cellular replication. If genetic changes occur favouring overactivity, overexpression or mutation (gain of function) enabling cancer formation, they become known as an oncogenes^{25, 54}.

Three major routes through which this can occur are:

- Chromosome movement/translocation causing genes to occupy new loci
- Amplification of the expression of a particular gene sequence leading to respective protein overexpression
- Point mutations / SNPs (single nucleotide polymorphisms) within the gene involving either the promoter (or other control components) or intra-gene disparity

The most commonly mutated oncogene is the ras family of proto-oncogenes present in approximately 27% of all human cancers⁵⁵. These GTPases are involved in the cell signalling cascade acting as molecular switches for cell cycle stimulation through protein kinase interaction⁵⁶. Typically activated in response to growth co-factor binding to the epidermal growth factor receptor (EGFR), the mutated protein continues to promote cell cycle progression in the absence of growth factor stimulus through loss of self-inhibition and inability to cleave GTP^{25, 54, 57}. Therefore, the end result is excessive cellular division.

On the other end of the spectrum are tumour suppressor genes, which act in opposition to oncogenes by preventing unregulated and uncontrolled cellular growth in the cell cycle. They exert their effect through four general classes of regulatory proteins:

- DNA repair enzymes e.g. Endonucleases, DNA ligase
- Pro-apoptotic proteins e.g. Bcl-2
- Intracellular proteins acting as cell cycle regulators such as p16 cyclin-kinase
- Inhibitory hormone activated receptors e.g. tumour derived growth factor β ^{26, 57}

By detecting faults in DNA replication/cell division and delaying stepwise progression of the cell cycle, they allow for damaged DNA to be repaired before it can be passed onto daughter cells. If this cannot be facilitated, these cellular enforcers induce programmed cell death through apoptosis or controlled cell suicide²⁵. TP53 and the protein regulator it generates (p53) is one of the most prominent tumour suppressors involved in carcinogenesis. Once their negative/ suppressive control is abolished or diminished, this confers cell immortality through unrestricted proliferation, resulting from the multifactorial control which this transcription factor elicits in terms of DNA checking, repair and apoptosis induction. Hence, if such barriers were suboptimal in any respect, unfettered cell growth would arise, such as in chronic myeloid leukaemia (CML)²⁷.

Such aberrant cells have characteristic hallmarks which include (Figure 5):

- Abnormal cellular signalling pathways
- Evasion of programmed cell death (PCD)- apoptosis
- Cell immortality through boundless cell division
- Angiogenesis facilitating the generation of new blood vessels to allow for (a) nutritional sustenance of the dividing tumour cells e.g. amino acids, carbohydrates and cell respiration, (b) provision of a medium through which cells from the primary tumour mass can migrate⁵⁸
- Evasion of growth suppressors
- Initiation of tissue invasion and metastasis
- Genetic mutations and instability
- Deregulation of cellular energetics and metabolism
- Cellular immortality
- Immune system evasion
- Pro-tumorigenic inflammatory environment



Figure 5-The hallmarks in cancer cell proliferation and survival⁵⁹

1.1.4 Treatment of Cancer

Generally cancer treatment stems from the three traditional treatment modalities- surgery, radiotherapy and systemic chemotherapy. However, with the advent of biologic agents in disease pharmacotherapy, immunotherapeutics and stem cell treatments have become more widely available and used clinically^{60, 61}. The selection of which treatment approach chosen should be based on current best practice guidelines and evidence-based medicine for the cancer subset involved as well as individual patient circumstances and presentation.

Surgery has been used since Greek times¹⁹ in helping to diagnose and treat cancer and has been a cornerstone of solid-cancer treatment for the past 100 years. Currently, its applicability has expanded with the advent of modern medicine with increasingly complex procedures, especially for metastatic or recurrent disease. Three of surgery's major functions are the prophylaxis, diagnosis and treatment of neoplastic disease. The goal of prophylactic surgery is the removal of precancerous tissue before it can gain malignant properties⁶². This option is only taken where high disease risk exists (>50%) and predisposition centres around genetic susceptibility, for example the removal of colonic polyps to prevent colonic cancer⁶³.

Diagnostic surgery is used as a medium through which a biopsy sample can be obtained (biological specimen of the tissue of interest) to facilitate histological examination and definitive diagnosis of cancer type^{24, 62}, an example being a laparoscopy for diagnosis of

hepatocellular carcinoma. This approach subsequently informs clinical treatment course going forward⁶⁴. Cancer treatment can also involve reduction or curative surgery through the excision of all or the vast majority of tumour tissue. This can either cure or halt progression of the cancer based on its nature (malignant or benign)^{62,65}.

Radiotherapy involves exposing of malignant cells in cancerous tissue to high frequency energy waves which induce DNA damage, preventing them from replicating by virtue of their increased mitotic rate and in most cases, killing them outright⁶⁶.

Three main types of radiotherapy delivery exist:

- external beam therapy where direct high energy x-ray beams are passed momentarily through the tissue of interest (using either photons or electrons)
- internal therapy (referred to as brachytherapy) whereby agents labelled with radioisotopes deliver high radiation “payloads” selectively to cancer cells through being implanted into the tumour mass, minimizing the impact on healthy cell population
- injectable radioisotopes- such as strontium 89 used for metastatic bone cancer⁶⁷,

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Chemotherapy has been at the forefront of cancer treatment for decades and continues to be utilized as one of the main anticancer treatment forms in malignant conditions, often being used in conjunction with other treatment modalities. Due to the fact that neoplasms arise from normal somatic body cells, exploitation of selective molecular signals / targets can be troublesome, hence many traditional anti-proliferative agents are non-specific in their mode of action, affecting both cell types due to rapid mitotic division²⁵. As a result, many chemotherapeutic regimens are limited through their narrow therapeutic indexes and the biological limitations of healthy body tissues, primarily bone marrow and gastrointestinal mucosa cells which undergo accelerated division^{24, 69}. Pronounced side-effects can leave patients immunocompromised, increasing the risk of secondary infection as well as causing further adverse side effects due to their non-discriminatory nature²⁵. Examples of these side-effects include the need for hydration with chloride containing fluid necessary in cisplatin treatment to prevent renal toxicity, along with alopecia, nausea, sterility and teratogenicity. Chemotherapy can additionally be used to shrink tumours (adjuvant use) or even eliminate residual tumour cells post-treatment

(neo-adjuvant use) being administered orally, subcutaneously, intramuscularly and intravenously, with the majority of such services being delivered in outpatient clinics⁷⁰.

Some examples of typical chemotherapeutic agents are:

- **Antimetabolites**- these directly interact with DNA to inhibit its functions and the enzymes required for the creation of DNA nucleotides, thus leading to abnormal DNA and induction of apoptosis e.g. cytarabine, a DNA polymerase inhibitor.
- **Alkylating agents**- these highly electrophilic compounds interact with the bases of DNA, forming strong, irreversible covalent bonds and may lead to cross linking between dsDNA (double stranded), inhibiting transcription and DNA replication e.g. ifosfamide.
- **Anti-neoplastic antibiotics**- these anthracycline molecules act by inhibition of DNA topoisomerase 2 and intercalation of same strand DNA base pairs e.g. doxorubicin.
- **Targeted therapeutics**- tyrosine kinase inhibitors e.g. lepatinib are available for specific cancer types such as HER-2 positive breast cancer^{24, 71}.
- **Immunotherapeutics**- This is the use of human monoclonal antibodies raised against specific cancer cell receptors such as Rituxumab in Non-Hodgkin's lymphoma or Chimeric Antigen Receptor (CAR) T-cell therapy^{72, 73}.
- **Stem-cell therapies-Autologous or allogeneic HSC transplantation (HSCT)**- This can be used to renew the haemopoetic system⁶¹.

1.2 Burkitt's Lymphoma

Lymphomas are cancers which arise from abnormal proliferation of lymphoid tissue and are sub-classified based on the histological appearance into either Hodgkin's (HL) or non-Hodgkin's lymphoma (NHL)²⁴. Lymphomas are a broad class of malignancy that account for approximately 3.5% of invasive cancers worldwide, with up to 80% of these cases comprising of non-Hodgkin's lymphoma⁷⁴. Non-Hodgkin's lymphoma are malignant tumours of the lymphatic system which result from unregulated clonal expansion of either B or T-lymphocytes. This type of cancer can occur at any age and is characterised by the presentation of lymphadenopathy (swelling of the lymph nodes- usually most prominent in the jaw, abdomen, neck), fever, weight loss and subversion of

the bone marrow (leukaemia)²⁴. These classes of disease usually occur by the common mechanism of multistep tumourigenesis due to the accumulation of several cellular aberrations during cellular differentiation. Among the various defects which can predispose people to lymphoma are CSR (class switch recombination), which is responsible for inducing DNA breaks, somatic hypermutation (leading to antigen hyperactivity and subsequent deletions / insertions responsible oncogenic translocation) or chromosomal translocation, which is the case in Burkitt's Lymphoma (BL)^{75, 76}. The particular case of Burkitt's lymphoma, consists of aggressive rapidly dividing mature germinal or post-germinal B-cells, having the fastest tumourigenesis of any human cancer (approximately 24-48 hours)^{77, 78}.



Figure 6: Lymphoma belt-East and Central Africa^{79, 80}

BL was discovered by esteemed Trinity College medical graduate Dr Dennis Parsons Burkitt, who in the late 1950's served as a medical surgeon in the Second World War in Kampala, Uganda. Here Burkitt observed a rapidly developing facial and abdominal tumours which had a high incidence in young children of 5-12 years of age across equatorial Africa⁸¹, accounting for one quarter to one half of all paediatric cancers in tropical Africa (East, Central and West of the continent)⁸². The area of highest childhood incidence were noted by Burkitt to be 15° either side of the equator in Central Africa dubbed the "lymphoma belt", within which 70% of all childhood lymphomas are caused by BL (Figure 6). Outside of Africa, the highest incidence of BL occurs in Papua New Guinea, where it accounts for a large percentage (19%) of all registered cancers on

average⁸⁰. In Ireland, BL accounts for approximately 2 in every 100 new lymphoma cases diagnosed annually⁶.

1.2.1 Characteristics of Burkitt's lymphoma

Burkitt's lymphoma (BL) as a WHO non-communicable disease (NCD) can be divided into three clinical presentations: sporadic (sBL), endemic (eBL) and immunodeficiency-related (iBL)⁸³. The sporadic variant occurs predominantly in developed nations e.g. North America, where it can account for upwards of 30% of NHL (Non-Hodgkin's lymphoma) childhood cases^{33, 82}. Unlike its endemic or immunodeficiency subtypes, sporadic cases seem to occur independently of climactic condition or geographical location and are only associated with the presence of EBV (Epstein Barr Virus) on rare occasion (association rate < 20%)⁸⁴. It is also quite rare in incidence with a 0.01 per 100,000 population frequency compared to its endemic variant whose incidence rate is 1-20 per 100,000, being up to 100-2000 times more prevalent⁸⁴. Manifestations of sBL are inclined to be nodal in origin; with the most common being abdominal tumours but colonization of neuronal or bone tissue is not uncommon upon diagnosis.

The defining associations of endemic BL are its connection and prevalence in high humidity areas of brought about by reasonably high average temperatures (>15°C) and annual rainfall (>50 cm) as documented by Haddow et al.⁸⁵. This connection then led to the detailed study of geographical BL incidence areas by Wright in Uganda, from which Burkitt and Kafuko deduced the linkage between endemic malarial incidence and BL, which is now established as a disease co-factor⁸⁴.

In 1964, Anthony Epstein and Yvonne Barr were first to discern the presence of a previously unknown herpes-like virus released by BL tumour cell cultures. This virus later came to be referred to as the Epstein-Barr virus. In later studies by both Epstein and Barr⁸⁶ established that upwards of 90% of all Burkitt's lymphoma cases in the lymphoma belt were virus seropositive, with some countries such as Uganda having a 97% correlation between infection and disease. Infection with EBV usually occurs during infancy due to the gradual decrease in passive maternal antibodies contained in the colostrum reaching the end of their circulatory life⁸⁷. The clinical presentation of symptoms in children tend to be unusual or even atypical but appear to mimic those of infectious mononucleosis

(fever, headache, sore throat, lymphadenopathy and general malaise)²⁴. Although incidences of splenomegaly, pronounced fatigue/physical weakness, hyperlymphocytosis and raised transaminase levels have also been noted in patient presentations^{81, 88}. Conversely in developed countries, EBV exposure occurs at a later stage, with only 50%-70% of adults/adolescents being described as EBV⁺ serotypes.

The main transmission route for the EBV is through saliva and subsequent infection of the B-cells which reside in the oropharyngeal epithelium, gaining entrance into the lymphatic system. Rochford et al. report that childhood exposure in Central Africa to holoendemic malaria (*via Plasmodium falciparum*) is a major pathogenic contributor, deduced from the fact that the positive EBV titres discovered in variable geographical regions were within a uniform range and that incidence in populations that lived beyond 5000 ft. above sea level was non-existent (due to unfavourable climate conditions for *P. falciparum* breeding)⁸⁸. Moreover, in areas where malarial transmission was hyper or mesoendemic, BL frequency was higher, providing evidence that EBV was not the only causative factor in lymphoblastic lymphomas observed. In light of this discovery, latent EBV viral infection is still thought to play a role in the progression of malignant B-cell development as viral infection rates are still markedly higher than in countries where BL remains unidentified. This is especially pertinent with the infection rate in the <5 yr age category rising above 90%. Hence, it is established that the Epstein-Barr virus, along with the principal mitogen *Plasmodium falciparum* (proven through induced B-cell propagation studies⁸⁹) are responsible for eBL incidence.

This relationship has been investigated thoroughly in the past few decades, beginning with the work of Moss et al.⁹⁰ and that repeated malarial infection in holoendemic regions from *P. falciparum* could impair specific T-cell response to EBV contraction. Consequently, this led to the concept of memory phenome, whereby the response of cytotoxic T-lymphocytes (CTL) is dictated by the frequency and intensity of past exposure to antigenically heterologous infections as documented by Liu et al⁹¹. This then causes long-lasting decreased EBV specific T-cell immunity and allows for the expansion of the latently infected B-cell pool through proliferation (mediated by the expression of latent genes such as EBNA-1) with subsequent colonisation of the germinal centres and production of infected memory B-cells^{77, 84}. *P. falciparum* antigens are hypothesized to stimulate TLR-9 (toll-like receptor-9) on memory B cells increasing the viral load through B-cell proliferative stimulation or induced differentiation from latent to lytic

state⁸⁴. This leads to an increasing malignancy risk due to EBV promoting genetic instability, DNA damage, inefficient repair (measured *via* H2AX biomarker) and telomere lengthening as a result of telomerase deregulation in the cell cycle⁹².

The third subtype is iBL, associated primarily with HIV infected patients presenting with high CD4⁺ blood counts. With severe immunosuppression of CD4 T-helper cells, the body's immune system becomes unable to fight disease and hence cannot eliminate EBV, leading to carcinogenesis and malignant cell generation as per the mechanism discussed in EBV and malarial association⁹³. Unlike other types, iBL has a worldwide distribution, with approximately 30% incidence in Europe and the United States.

Histologically, BL cells have a “starry sky” appearance due to the presence of infiltrating benign histiocytes engulfing apoptotic lymphoma cells (Figure 7). The defining genomic characteristics that remain constant across all BL subtypes are the reciprocal chromosomal translocation of the proto-oncogene *c-myc* from chromosome 8 to chromosome 14 (Figure 8)⁹⁴ (essential for cell cycle control) in greater than 80% of cases. Previously reported by Burkitt and Kafuko⁹⁵ and Rochford et al.⁸⁴, malaria and EBV are merely disease cofactors. Furthermore, due to its B-cell lineage (CD 19, 20, 22, 79) and germinal centre origin (CD 10, BCL-6), BL displays characteristic cell surface markers and protein expression patterns, with a lack of BCL-2 expression^{96, 97}. This process involves supraphysiological expression of *c-myc* by numerous mechanisms, the most common being through the interaction between DNA coding regions in *c-myc* protein and Ig (immunoglobulin) enhancer elements. In this way, chromosomal B-cell specific transcription elements can be activated up to 500 kbp (kilo-base pairs) from the binding site. The translocation itself is between *c-myc* and the IgH locus in 80% of BL cases, leading to aberrant cellular growth and invasive behaviour. Normally, up-regulation of *c-myc* proto-oncogene is prevented through the cellular maintenance and repair systems of pro-apoptotic p53 and BIM (*bcl-2*-interacting mediator of cell death)⁹⁸.

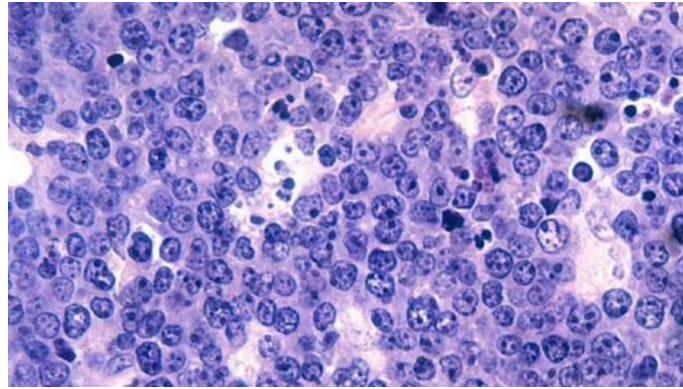


Figure 7: Histological appearance of Burkitt's Lymphoma⁹⁹

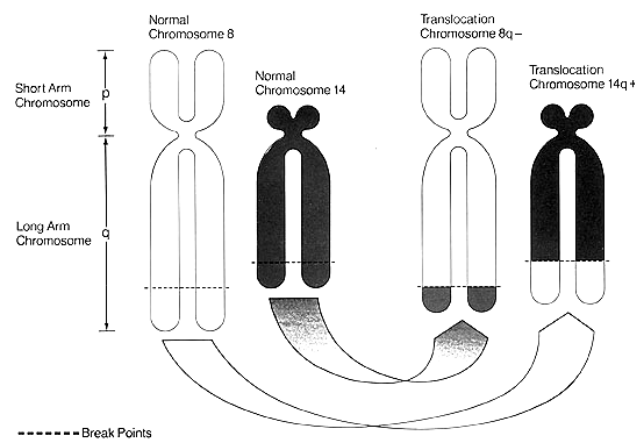


Figure 8: Chromosomal translocation t(8:14)-characteristic of Burkitt's Lymphoma

In EBV⁺ BL cell lines, nuclear viral proteins EBNA3A and EBNA3C are intricately involved in the undermining of the BIM (Bcl-like interacting mediator of cell death), a major factor in B-cell lymphoproliferative disease. Through inhibiting programmed cell death through this tumour suppression gene, an increased chance of B-cell lymphomagenesis exists. Proliferation of these overexpressing cells can also occur as a result of widespread mutations in TP53, a tumour suppressor gene, which can supersede normal apoptotic mechanisms, allowing the defect to be carried to further generations, fostering the conditions needed for carcinogenesis^{25, 100}. EBV therefore acts as an enabler through cellular immortalisation and facilitating further pro-cancerous changes such as telomerase inhibition⁹² (Figure 8).

1.2.2 Treatment of Burkitt's lymphoma

The common standard chemotherapeutic treatment for large diffuse B-cell lymphoma is a high dose regimen referred to as the CHOP regimen which consists of cyclophosphamide (alkylating agent), hydroxydaunorubicin (intercalating agent), vincristine (Oncovin™ anti-tubulin agent) and prednisolone (steroid) and is currently the clinical preference for the management of advanced NHL patients¹⁰¹. Despite the relative success of the regimen in the treatment of the vast majority of B-cell lymphomas, it has been found to generate subclinical responses in BL patients, leading to higher rates of relapse^{102, 103}. With the absence of a “gold-standard” therapy established through randomized controlled trials, treatment for BL regimens were adapted from paediatric clinical trials or from regimens of related haemato-oncological conditions such as Acute Lymphoblastic Leukaemia (ALL)¹⁰⁴. The use of multiple cytotoxic combination therapies specifically targeted at BL was investigated with remarkable success, with an average 2-year patient survival between 49-89%¹⁰³. One of the most promising of these therapies was developed by Magrath et al. in conjunction with the US National Cancer Institute which consisted of a short intensive treatment with cure rates approaching 90%¹⁰⁵. It was named the CODOX-M/IVAC regimen which consists of cyclophosphamide (alkylating agent), vincristine (antitubulin agent), methotrexate (dihydrofolate reductase inhibitor-cytotoxic) and doxorubicin (intercalating agent), delivered concomitantly with cyclical dosing regimens of etoposide, ifosfamide, cytarabine and intrathecal methotrexate/ cytarabine (Figure 9).

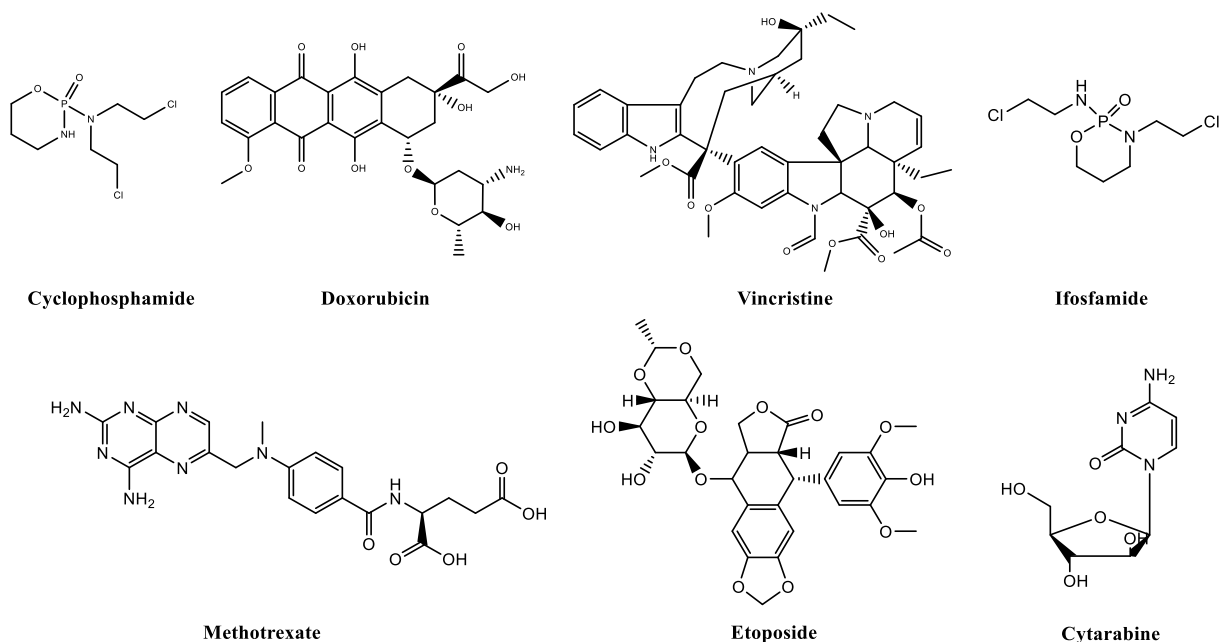


Figure 9: Drug components of the CODOX-M/IVAC regimen¹⁰⁵

Due to the associated cytotoxicities (mainly neurotoxic and myelosuppressive in nature) and treatment-related deaths, modified CODOX-M regimens were explored, by means of dose and schedule changes, dramatically reducing treatment related ADRs (Adverse Drug Reaction), with no correlated patient deaths¹⁰⁶. This preserved efficacy is also accompanied by high 2 year patient survival rate of approximately 71%¹⁰³.

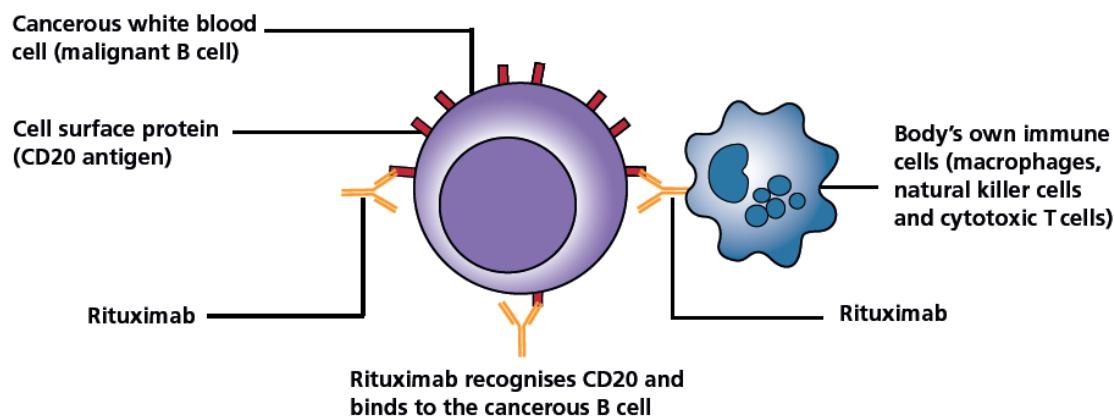


Figure 10:Rituximab mechanism of action¹⁰⁷

Monoclonal antibodies such as Rituximab (chimeric monoclonal antibody against CD20) are also used in modern regimens to selectively target B-cell line cancer cells by means of CD20 surface antigen protein expressed exclusively on B-cells (Figure 10), allowing the body's own immune cells to eradicate the malignancy¹⁰⁷. It has been found to increase overall survival rates in B-cell lymphoma patients through increasing malignant cell

sensitisation to existing chemotherapeutic regimens, fewer relapses and increased overall response and remission when compared to standard chemotherapy regimens^{108, 109}. Rituximab is also currently being used in the treatment of advanced stage (3 and 4) follicular lymphoma, maintenance of cancer remission and the application of radio immunotherapy, allowing for direct irradiation of lymphoma cells. Rituximab's practicality in combination chemotherapeutic regimens for BL can also be observed through the amendment of Thomas et al.¹¹⁰ hyper-CVAD regimen (cyclophosphamide, vincristine, doxorubicin and dexamethasone) with cyclical administration of methotrexate. Pre-rituximab addition, the overall response rate was 81% with survival rates for patients aged over 60 years averaging 17%, conversely with Mab stimulation, both observed response and survival rate increased by 8% and 12% respectively^{110, 111} even with significant toxicity experienced and maintenance of no treatment-related deaths. Further studies into the treatment of HIV associated-lymph proliferative diseases have also yielded positive and optimistic results. Baue et al. found that with the co-administration of rituximab with the standard CHOP regimen in HIV positive patients, high cure rates of 77% as well as 2 years survival rates of 75% were noted. In addition to this, Noy et al. reported in the AMC 048 Phase 2 trial that a similar modification to the CODOX-M/IVAC regimen could achieve high rates of long term disease remission along with minimal drug-toxicity increase¹¹². In the treatment of iBL, high CD4+ cell count and absence of disease progression were cited as indicators of survival¹¹³.

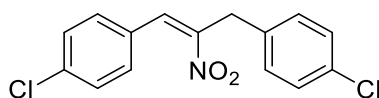


Figure 11: Example structure of 1,3-bis(aryl)-2-nitro-1-propenes

There is on-going research in the development of more selective, potent and economical treatment options for BL, for example, the work that has been conducted in TCD by Mc Namara et al.^{114, 115, 116} and Cloonan et al.^{117, 118}. This research demonstrated the antiproliferative potential of a lead compound from a library of 1,3-bis(aryl)-2-nitro-1-propenes in chemosensitive MUTU-1 and chemoresistant DG-75 BL cell lines with pEC₅₀ values of 4.3-6 μ M across both cell lines (Figure 11). Due to the high antitumour and pro-apoptotic potency of simple nitrostyrene analogues in the same study and the evidence base of past analyses^{119, 120}, these structures were incorporated into on-going studies within the remit of this project, investigating the antidepressant mediated

programmed cell death in BL, specifically that of maprotiline and its equivalents^{117, 118}. The development of select nitrostyrene based adducts are explored and investigated in this thesis as possible therapeutic agents for CLL.

1.3 CLL (Chronic Lymphocytic Leukaemia)

Leukaemia is a broad term for a specific class of haematological malignancy which begins in the bone marrow or other blood forming tissues as genetically aberrant white blood cells (Figure 12). This malignant cellular phenotype is driven by the presence of key chromosome abnormalities (translocations and/or loci deletions) as well as accumulated genetic mutations which result in the subsequent epigenetic changes that result in condition pathogenesis¹²¹. The two main epigenetic outcomes that are considered central to leukaemogenesis are changes to DNA methylation mechanisms and atypical histone modifications patterns¹²². With leukaemia, there are two main broad specifications based on progressive nature of the disease; these are acute and chronic leukaemias respectively. Acute leukaemias occur through the accumulation of detrimental mutations in the early haematopoietic precursor, resulting in rapid and uncontrollable expansion of deviant immature white blood cells. This results in significant metabolic changes in the body system, leading to oxygen deprivation and healthy cell crowding with subsequent poor immune response and haemostasis. While curable, prompt effective treatment such as bone marrow transplantation or targeted chemotherapy regimens is essential. Younger and/or stronger individuals often have better outcomes due to treatment intensity and significant biological toll on the patient¹²³.

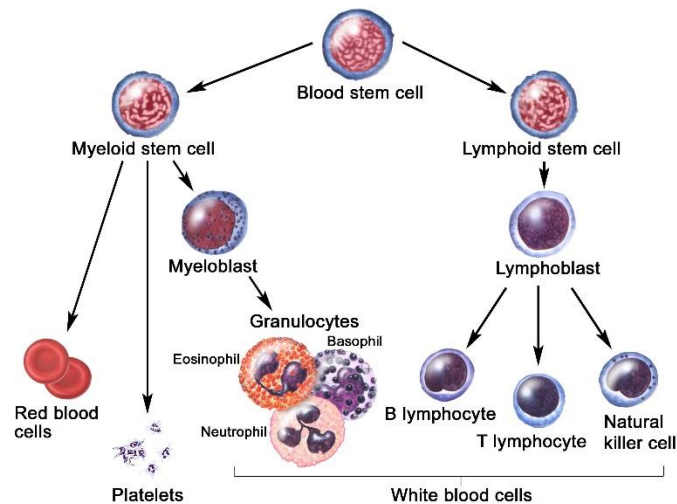


Figure 12: Haematopoietic stem cell differentiation ¹²⁴

Chronic leukaemias are more indolent and insidious in nature as they consist of the gradual accumulation of abnormal white blood cells over a prolonged period of time through dysregulation of the normal cell cycle death mechanisms such as apoptosis. Although with a comparatively longer median survival time relative to similar malignancies and the existence of many methods of disease treatment and management, these subclasses of leukaemia remain practically incurable and are prone to relapse after remission is induced¹²⁵⁻¹²⁸. Within these designations, the origin or progenitor cell lineage helps to further group these related, yet distinct neoplasms. Myeloid leukaemias develop from myeloid stem cells (responsible for generation of red blood cells, platelets and granulocytes) and are characterised by the accumulation of cancerous myeloid cells in the blood e.g. basophils (Figure 12). Furthermore, lymphoid leukaemias arise from lymphoid progenitor cells and are characterised by the accumulation of cancerous lymphoid cells, such as B cells, in the blood. Based on these two means of cross-classifying leukaemias, four main classes of leukaemia exist. These are:

- **Acute Myeloid Leukaemia (AML)**- this is a relatively rare cancer type that occurs mainly in elderly individuals aged > 60 years and relatively uncommon under the age of 45 years. Incidence is slightly higher in males than females¹²⁹.
- **Acute Lymphoblastic Leukaemia (ALL)**- this is the most common childhood cancer, representing up to 80% of cancer incidence in children¹³⁰.
- **Chronic Myeloid Leukaemia (CML)**- this is a relatively rare malignancy. It can manifest across any age however, it is most prevalent from mid-old age (approx.

40-60 years or greater), with a slight increase in individuals that were subjected to radiation exposure^{126, 131}.

- **Chronic Lymphocytic Leukaemia (CLL)**- this is a slow growing cancer that involves the clonal expansion of mature like B-lymphocytes (blast cells).

As CLL is the malignancy at the core of this thesis, it will be discussed in greater detail from this point onwards. Chronic Lymphocytic Leukaemia is the most common leukaemia of the global adult population in developed countries and largely a disease of the elderly^{132, 133}. This is evident as greater than 70% of patients are >65 years at time of clinical diagnosis (with the median age being 72 years). However, over the past decade, manifestation has become increasingly common in younger patients with approximately 15% of patients being 55 years of age or younger¹³⁴. In addition, men have a higher predisposition to disease development than women with a 1.5-2 fold risk¹³⁵. According to a study by Redaelli et al., Ireland (along with Australia, Italy and the US) was found to be among the highest incidence rates for CLL worldwide¹³⁶. This is reinforced by a recent epidemiological investigation that describes Ireland as having a CLL age-adjusted incidence of approximately 4.5 per 100,000 in males and 2 per 100,000 in females¹³⁷.

1.3.1 Characteristics of Chronic Lymphocytic Leukaemia

CLL can be defined as a clinically heterogeneous lymphoproliferative disorder that originates from a single, round and mature B-lymphocyte phenotype (typically CD5⁺) by clonal expansion, usually involving the bone marrow, spleen, lymph nodes and peripheral blood (Figure 13)¹³⁸⁻¹⁴⁰. The pathogenesis occurs through proliferation in the lymph node compartment where the malignant cells establish a favourable tumour microenvironment communicating with cellular machinery such as stromal cells and B-cell receptors (considered a core driver of CLL survival and proliferation). Furthermore, the initial leukaemogenic cascade is thought to involve a self-renewing and multipotent haematopoietic stem cell population¹⁴¹. The most common form of disease presentation is the discovery of lymphocytosis through routine assessment of complete blood counts for other clinical reasons. Additionally, another common presentation is through lymphadenopathy or splenomegaly found as a result of unrelated medical assessments;

with cytopenias and other more B-cell specific symptoms (such as fatigue, increased infection frequency) presenting seldomly¹⁴⁰.

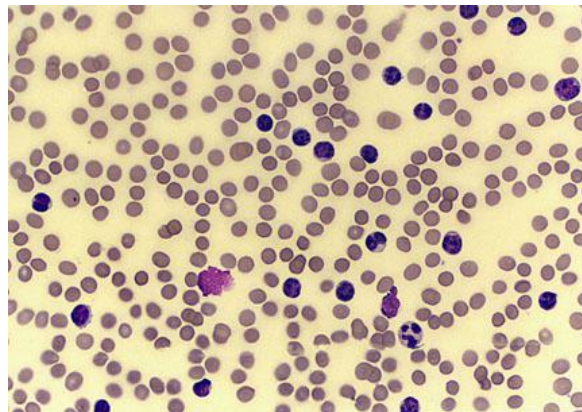


Figure 13: Microscope image of characteristic CLL cell phenotype (dark purple) and smudge cells (pink irregular shape) among normal red blood cells

The diagnostic criteria for CLL requires that greater than 5000 monoclonal B lymphocytes/ μ L are consistently documented in peripheral blood samples over a 3 month period via flow cytometry with less than 55% prolymphocytes¹³³. This is accompanied by the presence of scant intracellular cytoplasm in lymphocytes coupled with the appearance of smear/smudge cells (damaged lymphocyte artefacts). However, even though past studies have referenced an inversely proportional relationship with disease severity and smudge cell presence, this has since been found to be greatly influenced by slide preparation techniques and hence is not extremely reliable^{142, 143}. A key criteria by which CLL identification is confirmed is by the observation of a characteristic cell immunophenotype comprising of CD19, CD20, CD23 and CD5 co-expression (B-cell and T-cell markers respectively) (Figure 14). This is coupled with decreased IgM, IgD and CD 79b, typical of mature, active B-lymphocytes^{132, 144}. In addition to these techniques, improved karyotyping of CLL cells through interphase fluorescence *in situ* hybridization (FISH) allows for increasingly reliable and reproducible identification of chromosomal aberrations which can adversely impact on patient prognosis. Such abnormalities include deletions in the short arm of chromosome 17 (del(17p)), trisomy of chromosome 12 and deletions in the long arm of (del(13q)) (the most commonly identified chromosome defect)^{145, 146}. While this particular assessment is used on an individual patient basis, greater evidence is needed before it is introduced into routine clinical practice¹⁴⁷.

In a small number of patients, this characteristic malignancy can be alternatively classified as either small lymphocytic leukaemia (SLL) or monoclonal B-cell lymphocytosis (MBL), depending on presentation. SLL can be defined as the same disease however, it is locally confined to lymphoid or other body organs without bone marrow and/or blood involvement and is confirmed via tissue biopsy¹⁴⁸. With monoclonal B-cell lymphocytosis, there is only blood and/or bone marrow involvement with no other tissues being affected. Moreover, the cell count for MBL is below the CLL diagnostic threshold^{149, 150}.

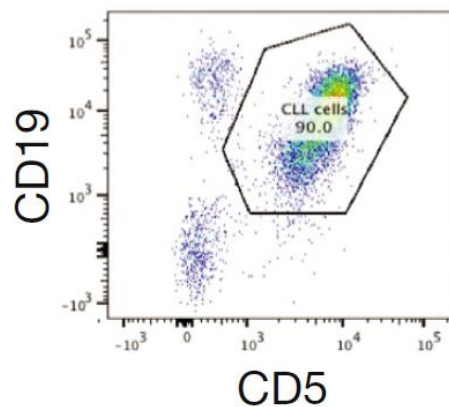


Figure 14: Flow cytometry dotplot output showing a characteristic CLL population which are positive for both CD 19 and CD 5^{140, 147}

1.3.2 Staging of CLL

Once a diagnosis of CLL has been definitively established, patients undergo risk stratification through clinical staging systems, two of the most common being the Rai and Binet systems (Table 1). The Rai classification was published in 1975 and was the first prognostic system that helped to associate clinical course and symptom severity with patient outcomes¹⁵¹. It consists of five distinct stages as detailed in Table 1 below, each with its own clinical criteria. In 1977, Binet and colleagues reported a modified classification scheme which again used clinical disease progression and symptomatic criteria as a predictor of patient prognosis, but sought to simplify the criteria to create three main stages (A,B,C)¹⁵².

Stage	Clinical features
Binet:	
– A	<3 areas of lymphadenopathy [†]
– B	>3 areas of lymphadenopathy [†]
– C	Anemia (<10 g/dl) ± thrombocytopenia (<100 × 10 ⁹ /l)
Rai:	
– 0	Lymphocytosis in peripheral blood marrow only
– I/II	Lymphadenopathy [†] ± hepatosplenomegaly
– III/IV	Anemia (<11 g/dl) ± thrombocytopenia (<100 × 10 ⁹ /l)

[†]Nodal ares: cervical, axillary, inguinal, spleen and liver.

Table 1: Rai and Binet staging in CLL¹⁵³

With CLL, the various clinical outcomes are determined predominately by the mutational profile of the heavy variable region immunoglobulin (IGHV) genes representing two distinct disease subtypes: IGHV-mutated (IGHV-M) and IGHV-unmutated (IGHV-UM). IGHV-M is typically representative of good clinical prognosis in CLL patients, while IGHV-UM is characteristic of poorer clinical prognosis, accounting for 60% and 40% respectively of total diagnosed cases¹⁵⁴. This stark difference is thought to be due a number of factors (Figure 15):

- **B-cell receptor (BCR) reactivity-** In IGHV-UM cells, the BCR exhibits low affinity and is associated with poly or self-reactivity. This is where adjacent BCRs mutually interact in the absence of exogenous antigens. They are also referred to as being “cell-autonomous”. In contrast, IGHV-M CLL BCRs are only mono or oligo-reactive and they respond relatively poorly to somatic IgM stimulation¹⁵⁵.
- **Clonal Evolution-** With IGHV-UM cells, rapid clonal expansion takes place, involving an iterative process of expansion, genetic divergence and clonal selection, yielding rapidly evolving cells that can actively adapt to intrinsic and extrinsic cancer cell death mechanisms. On the other hand, IGHV-M CLL cells have a relatively low level ability to adjust to such stimuli in the microenvironment¹⁵⁶.

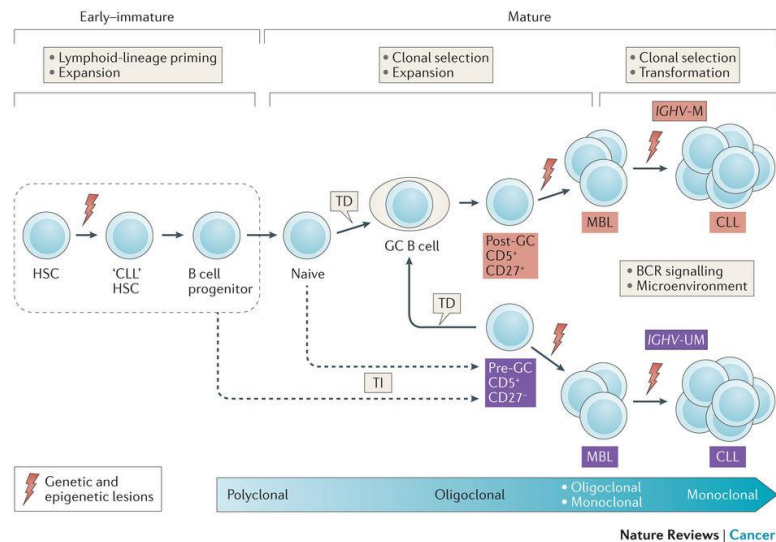


Figure 15: Developmental cascade and clonal evolution of CLL^{155, 156}

- **Genetic Lesions-** Higher risk genetic lesions are observed in IGHV-UM cells, which lead to more aggressive and invasive disease variant, while IGHV-M CLL are found to have lower risk genetic insults for which the outcomes are not as detrimental to the body's organ systems.
- **Binding Antigens-** IGHV-M CLL cells only respond to high affinity auto-antigens such as β -(1, 6) glucans or rheumatoid factors (RF), the binding of which decreases the strength of the corresponding signal cascade. Moreover, with IGHV-UM cells, a wider range of auto-antigens can bind such as ssDNA, dsDNA, oxLDL as well as microbial antigens like lipopolysaccharides (LPS). In doing so, the signal generated is amplified and the magnitude of downstream transcription factor activation is greatly increased¹⁵⁷.

Other prognostic markers which complement existing staging systems include:

- TP 53-Mutations or deletions of the gene for this regulatory tumour suppressor protein¹⁵⁸.
- Serum β_2 microglobulin concentration-this is an extracellular protein part of the human leukocyte antigen class 1 complex (MHC Class 1) which level rises in active leukaemia¹⁵⁹.
- ZAP 70 (Zeta-chain associated protein kinase 70)-This is a signalling protein that is part of a T-cell selectively expressed on CLL cells, having a 77-95% correlation with IGHV mutational status^{160, 161}.

- NOTCH 1-this gene located on chromosome 9b and is involved in the expression of a transmembrane protein in the regulation of haematopoietic stem cell development with up to 30% of refractory/relapsed patients carrying this gene mutation¹⁶².
- SF3B1 (Splicing factor 3B1)-this gene is responsible for encoding for subunit 1 of the splicing factor 3b protein complex involved in mRNA specific splice site recognition and is mutated in up to 15% of patients^{163, 164}.

1.3.3 Treatment of Chronic Lymphocytic Leukaemia

As CLL is a heterogeneous disease in nature, disease treatment is individualised. Newly diagnosed patients are clinically observed at 1-3 month intervals (even if the presence of high risk genetic drivers are confirmed) if they are asymptomatic in what is collectively known as the “watch and wait” approach (Rai stage 0, Binet stage A). This is supported by numerous clinical trials and meta-analyses citing no benefit of beginning treatment during this period and increased risk of drug-related harm^{147, 165}. This approach is usually taken until disease progression is confirmed.

Traditionally, the most common initial clinical treatments were alkylating agents such as chlorambucil and cyclophosphamide for progressive and late-stage CLL, either as single agents or used in combination with corticosteroids¹⁶⁶ (Figure 16). The overall response rate (ORR) for chlorambucil at 15 mg/ day was found to be 89% by Jaksic et al. , which is consistent with other similar studies¹⁶⁷. Having a similar mechanism of action to chlorambucil, cyclophosphamide is occasionally used as a substitute single agent when patients have poor tolerance of chlorambucil with daily dosing ranging from 2–3 mg/kg/day orally or 20 mg/kg intravenously every 2–3 weeks. Compared to combination therapies (which have been used successfully in other related haematological malignancies such as Non-Hodgkin’s Lymphoma (NHL) ^{168, 169} of cyclophosphamide, vincristine, doxorubicin and prednisone (CHOP, CVD, CVP), chlorambucil and prednisone in combination was found not to be clinically inferior¹⁷⁰⁻¹⁷².

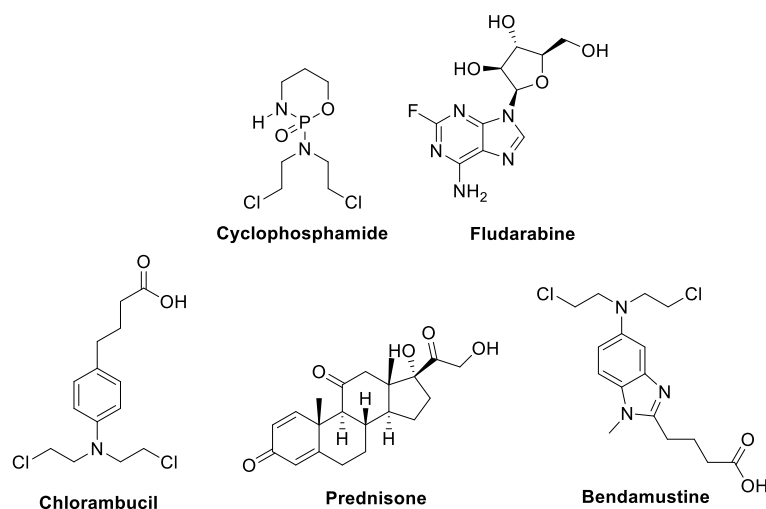


Figure 16: Structures of commonly used chemotherapeutic drugs for CLL treatment

Currently, purine nucleoside analogues (PNAs) are the preferred first-line agents for the treatment of CLL as available clinical evidence shows that previously untreated CLL patients have more beneficial outcomes in terms of time of disease progression and response¹⁷³. One of the most employed is fludarabine, which can be used as a single agent or in combination with other chemotherapeutic agents such as cyclophosphamide (FC regimen)¹⁷⁴ (Figure 16). When the FC treatment was compared to fludarabine alone across numerous studies on advanced CLL in patients under 66 years reported by Flinn et al.¹⁷⁵, the FC combination was observed to have statistically higher ORR (Overall Relative Response) (e.g. 94% vs 83%) and progression-free survival (PFS) (e.g. 40 months vs 20 months).

In younger patients (<65 yrs) who are otherwise fit and healthy with low-risk prognostic factors and who have not received previous treatment, fludarabine, cyclophosphamide and rituximab (FCR) are used in combination as a first-line treatment and was the first chemotherapy regimen that impacted on overall patient survival in CLL^{158, 176}. In addition, Hallek et al. found from Phase 3 clinical trial that fludarabine in combination with cyclophosphamide and rituximab yielded greater complete response (CR) (45% vs 23%) and progression free-survival (PFS) (43 months vs 32 months) in comparison to the FC regimen in advanced CLL¹³². Rituximab, as described previously, is an anti-CD 20 chimeric monoclonal antibody with leukaemic cell action mediated by a variety of mechanisms such as apoptotic induction and complement protein cell lysis¹³⁸. In CLL, while it has shown great promise in combination with PNA agents, its efficacy is poor as a monotherapy at the established dosage regimen (325 mg/m² weekly)¹⁷⁷. Elderly patients

(>65yrs and who may have existing comorbidities) have been shown not to respond to FCR treatment well, mainly due to the main adverse effect of myelosuppression leading to infection or other complications necessitating treatment cessation¹⁷⁸. Eichhorst et al., in the CLL10 comparative study, observed that bendamustine plus rituximab (BR) combination therapy in elderly patients resulted in fewer grade 4 adverse drug reactions and / or hospitalisations (41% vs 71%) and fewer incidences of treatment related myelodysplastic syndrome compared to the FCR regimen¹⁷⁰. Hence, such chemoimmunotherapy combinations are preferred as frontline treatment options for those who cannot tolerate FCR therapy.

Alemtuzumab is a humanized anti-CD 52 monoclonal antibody which is thought to act through antibody-dependent cell-mediated cytotoxicity¹⁷⁹. The successful use of alemtuzumab for CLL as a single agent (30 mg i. v. three times a week-max. 12 weeks) versus chlorambucil (40 mg/m² orally every 28 days up to a maximum of 12 cycles) has been shown by Hillmen et al.¹⁸⁰, with objective response rate (ORR) of 83% and 55% respectively. Combination therapy with other cytotoxic agents has been studied extensively, yielding favourable results. One such regimen was the combination of alemtuzumab and fludarabine, cyclophosphamide and rituximab in advanced CLL cases with the resultant ORR (94%) and CR (69%) being higher than previous chemotherapy combinations tested in the disease.

Ongoing clinical trials are currently taking place in the quest for effective leukaemia treatment with greater patient regimen tolerance and reduced side-effect profiles. One such trial taking place in Ireland is CLL13, a Phase 3 multicenter randomized, prospective, open-label trial of standard chemoimmunotherapy (fludarabine, cyclophosphamide, rituximab or bendamustine, rituximab) versus 3 combination therapies which consist of:

1. rituximab plus venetoclax
2. obinutuzumab plus venetoclax
3. obinutuzumab plus ibrutinib plus venetoclax¹⁸¹ (Figure 17)

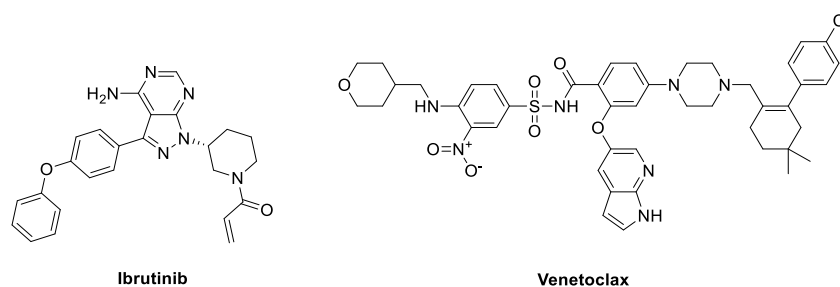


Figure 17: Chemical structures of modern small molecule therapies in current use

Obinutuzumab (GA-101) is a glycoengineered type 2 humanized monoclonal antibody targeted at the CD-20 surface antigen expressed on B-lymphocytes and is approved for CLL treatment in combination with chlorambucil in elderly patients with comorbidities¹⁸². It carries out its therapeutic effect through three main mechanisms of action:

1. Recruitment of immune effector cell pathways such as antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis
2. Activation of the complement protein cascade
3. Directly activating intracellular cell death signalling pathways

While sharing the same epitope target as rituximab, obinutuzumab induces greater ADCC *in vitro* as a result of reduced fucose content and a higher affinity for FcγRIIIa (a CD-16 receptor subtype responsible for natural killer cell activation) and displays a greater propensity to induce direct cell death and B-cell depletion than rituximab as a monotherapy¹⁸³⁻¹⁸⁵ (Figure 18). While neutropenia and infusion-related reactions were found to be more common than with rituximab, this did not lead to an increased infection risk¹⁸⁶.

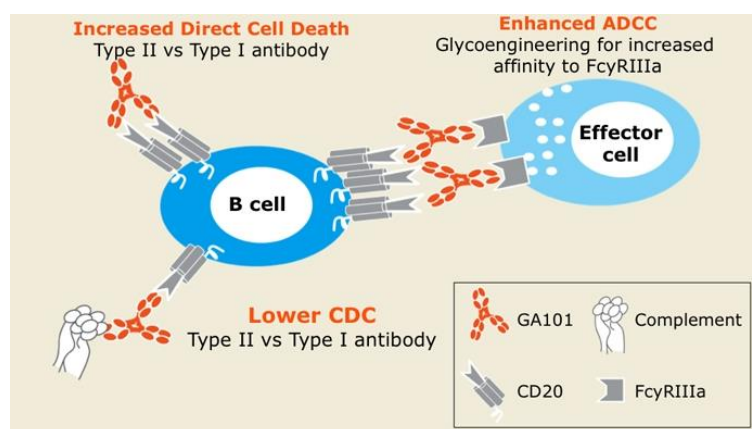


Figure 18: Mechanisms of action for Obinutuzumab¹⁸⁶

Venetoclax is a highly specific inhibitor for B-cell lymphoma 2 (BCL-2) anti-apoptotic protein, which when overexpressed in CLL promotes malignant cell survival through facilitation of resistance development (Figure 19). Binding to the hydrophobic BH3 domain of the Bcl-2 protein (pockets P₂: 4-chlorophenyl group, P₄: piperazine and azaindole groups), the drug acts specifically through the displacement of pro-apoptotic activators such as BIM (Bcl-2-like protein 11) to induce programmed cell death such as apoptosis (Figure 19). The main drawback of Venetoclax in clinical use is the propensity for tumour lysis syndrome to develop in treated patients with rapid apoptosis and reduction in patient tumour burden within hours of dosing¹⁸⁷⁻¹⁸⁹. Venetoclax (Venclexta™) was developed in a joint collaboration by Abbvie and Genentech and was approved by the US Food and Drug Administration (FDA) in 2016 for the treatment of CLL¹⁹⁰. Venetoclax was originally synthesised *via* a 14 step reaction method however, due to raw material availability, financial feasibility and lengthy manufacturing times among other reasons, a more efficient and cost-effective route is currently used on a production scale (Figure 20)¹⁹¹⁻¹⁹⁴.

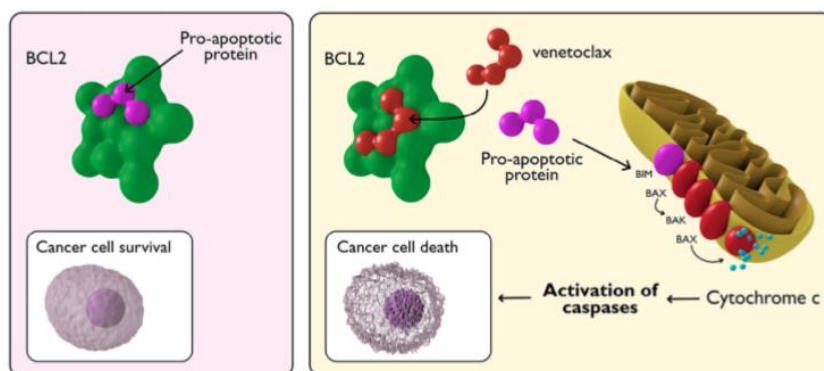


Figure 19: Mechanism of action of Venetoclax through BCL-2 interaction inhibition

Firstly, 1-bromo-3-fluoro-4-iodobenzene undergoes an iodine-magnesium exchange with *i*-PrMgCl, leading to the formation of a highly functionalised Grignard reagent. This activated compound is then allowed to react with Boc₂O to form an ester intermediate. The ester generated then undergoes nucleophilic aromatic substitution with the aza-indole compound with an excellent yield of 86%, to produce the one of three major reactants for Venetoclax formation (Figure 20). Secondly, the intermediate sulfonamide is obtained through the reaction of the methanamine with 4-chloro-3-nitrobenzenesulfonamide via nucleophilic aromatic substitution yielding sulfonamide intermediate (B) (Figure 21). The last of the key reagents, intermediate (C), is synthesised allowing 3,3-

dimethylcyclohexanone to react with POCl₃ in DMF (dimethylformamide), resulting in the vinyl chloride derivative. Next, this then undergoes Suzuki coupling with 4-chlorophenylboronic acid to form the respective aldehyde, which undergoes subsequent reductive amination with N-Boc piperazine, in 87% yield. The piperazine group is then deprotected, yielding the piperazine bis-HCl salt. Palladium-based C-N coupling (Buchwald–Hartwig amination) allows for intermediate (C) formation, following cysteine-mediated palladium removal. The final synthetic step then involves ester hydrolysis and sulfonamide coupling, resulting in Venetoclax formation with a 71% yield, (Figure 22).

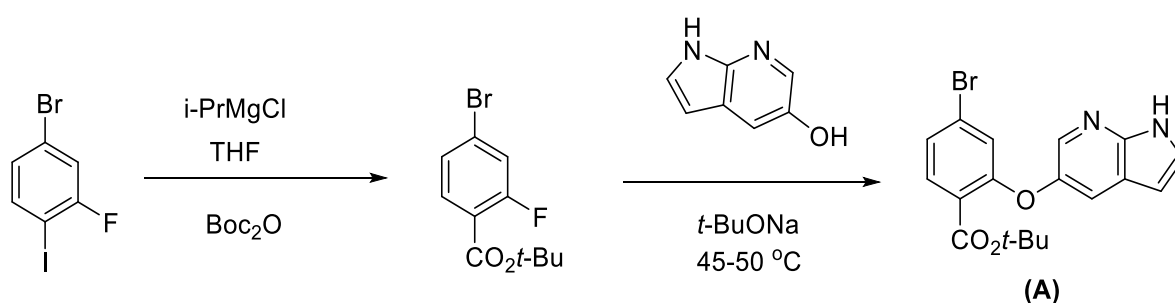


Figure 20: Formation of Intermediate (A) in Venetoclax synthesis

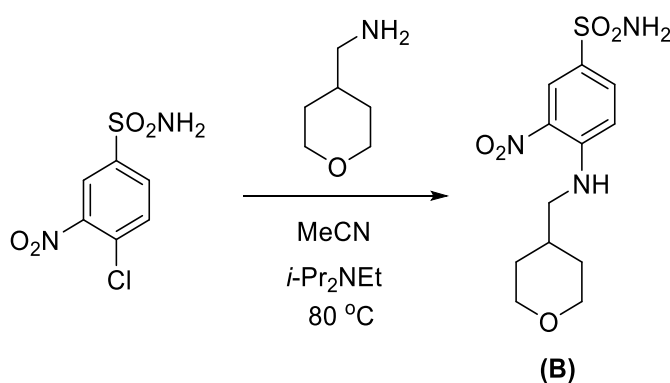


Figure 21: Formation of sulfonamide intermediate (B)

Ibrutinib is an irreversible, selective inhibitor of Bruton Tyrosine Kinase (BTK), a key effector in the B-cell receptor signalling pathway and works by covalently binding to the cysteine-481 residue of the protein *via* a Michael addition reaction (Figure 23). BTK is an essential tyrosine kinase central to the B-cell receptor (BCR) signalling, the activation of which promotes CLL cell growth and maturation. Due to upregulated constitutive activity and sensitivity of BCRs to activation *via* external or auto-antigens in CLL, it represents a valid and effective clinical target for CLL treatment. This conjugation then

prevents the phosphorylation of Tyr 223 residue and hence, BTK activation¹⁹⁵ (Figure 23). The main clinical adverse drug events that can occur with clinical use include hypertension, neutropenia and pneumonia¹⁹⁶⁻¹⁹⁸. Ibrutinib has been found to be of benefit for poor prognosis patients and has shown promise in the treatment of older CLL patients^{199, 200}.

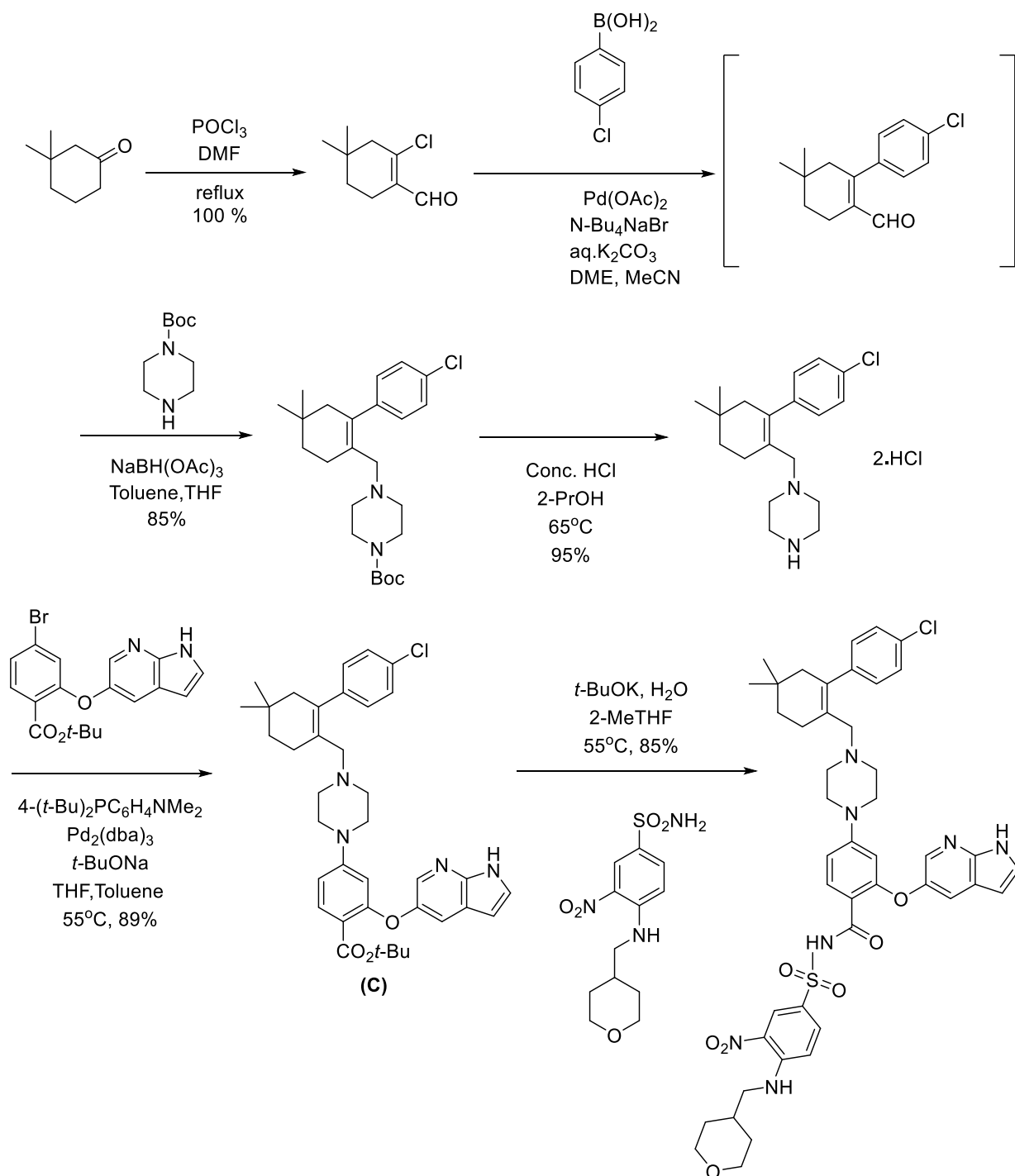


Figure 22: Synthesis of intermediate (C) and subsequent couplings of intermediates to yield Venetoclax

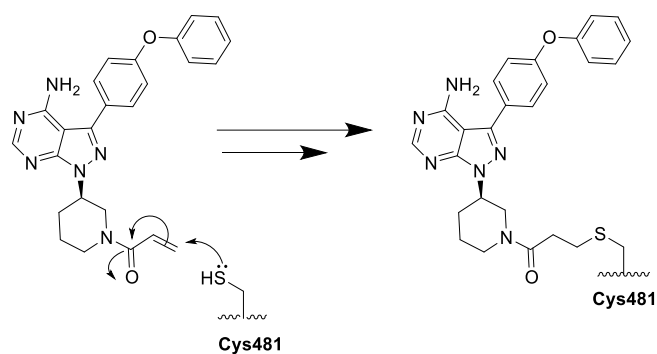


Figure 23: Ibrutinib mechanism of action ¹⁹⁵

Discovered by Celera Pharmaceuticals, the drug was developed as a clinical agent in conjunction with the Janssen Pharmaceutical Division (Johnson and Johnson). Since its discovery, numerous synthetic routes exist with the most commercially viable being the route described briefly^{201, 202} (Figure 24). Firstly, commercially available 4-phenoxybenzoyl chloride was condensed with malonitrile, following a quench with aqueous sulfuric acid, the resultant alkene alcohol group underwent *O*-methylation via dimethyl sulfate yielding the dicyano vinyl derivative (**B**) in excellent yield (84%). Following this, the aminopyrazole intermediate (**C**) was formed through the condensation with hydrazine hydrate in refluxing ethanol. Subsequently, treatment with neat formamide at elevated temperatures led to the pyrimidopyrazole compound (**D**). This product then underwent selective *N*-alkylation at the pyrazole nitrogen by piperidinyl tosylate with a modest yield of 32% (**E**). The final synthetic steps involved amine regeneration and subsequent amide formation using acryloyl chloride to generate ibrutinib (**F**) with a 50% yield²⁰³.

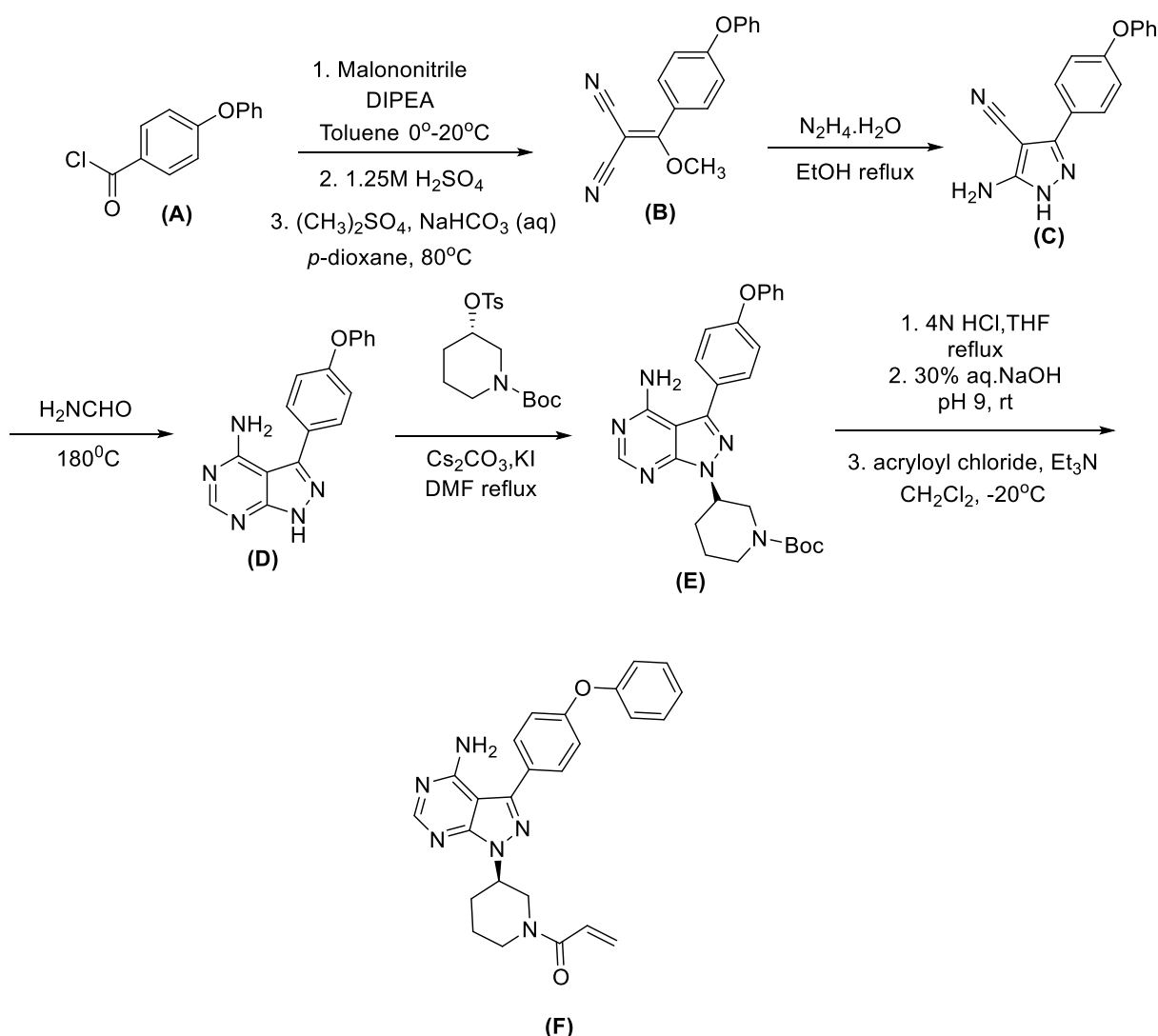


Figure 24: Synthesis of Ibrutinib

Another widely used targeted clinical agent in the treatment of CLL is the first-in-class small molecule Idelalisib, which exerts its therapeutic activity through the reversible, selective ATP-competitive antagonist of delta isoform of catalytic subunit p110 of phosphoinositol-3-kinase (PI3K δ). The binding mode of this interaction is illustrated in Figure 25. In B-cell haematological malignancies, (PI3K δ) hyperactivity is characteristic, with its primary physiological function being associated with B-cell growth, activation, antigen signalling, chemotaxis and antibody production, among other important humoral immune functions²⁰⁴⁻²⁰⁸. Idelalisib is currently approved for use in combination with an anti-CD 20 antibody (e.g. rituximab) for the treatment of adult CLL predominately as a second-line therapy, however can be used as first-line clinical therapy if the presence of deleterious genetic markers are confirmed such as TP53 mutations and 17p chromosome deletions²⁰⁹.

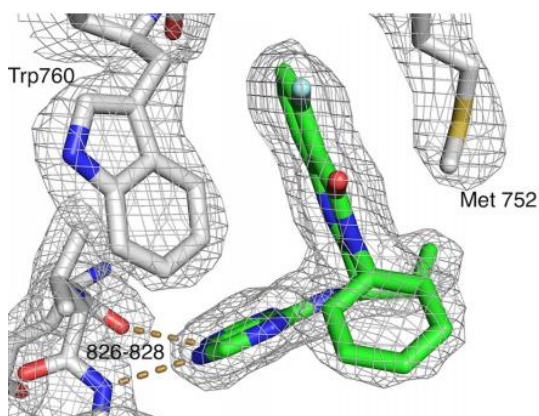


Figure 25: Binding mode of Idelalisib x-ray crystal structure to PI3K δ binding pocket

Discovered by ICOS, Idelalisib was developed as a clinical agent by Calistoga Pharmaceuticals, which was subsequently acquired by Gilead Sciences. The original synthesis route for Idelalisib will now be discussed. The initial synthetic step involves the condensation of 2-fluoro-6-nitro benzoic acid with aniline, following treatment with oxalyl chloride (to form the acid chloride derivative *in situ* in the presence of catalytic amount of DMF), affording 2-fluoro-6-nitro-*N*-phenylbenzamide. This *N*-phenyl benzamide product then undergoes amide bond formation in the presence of oxalyl chloride (to allow the formation of the acid chloride intermediate) with *N*-Boc-*L*-2-aminobutyric acid, resulting in formation of the butyrate adduct. Next, the *N*-Boc-*L*-aminobutyrate intermediate is reduced through the use of zinc in the presence of acetic acid to produce the Boc protected quinazolinone compound. Removal of the protective Boc group and subsequent arylation with 6-bromopurine affords Idelalisib with a moderate yield of 50%^{210, 211}. There have been many modifications trialled to help improve synthesis yield while maintaining enatiopurity with one of the more notable efforts being reported by Mekala et al., achieving a very good yield of 60% while maintaining high enatiopurity of 99.9% through methodical step-by-step reaction optimisation (Figure 26)²¹¹.

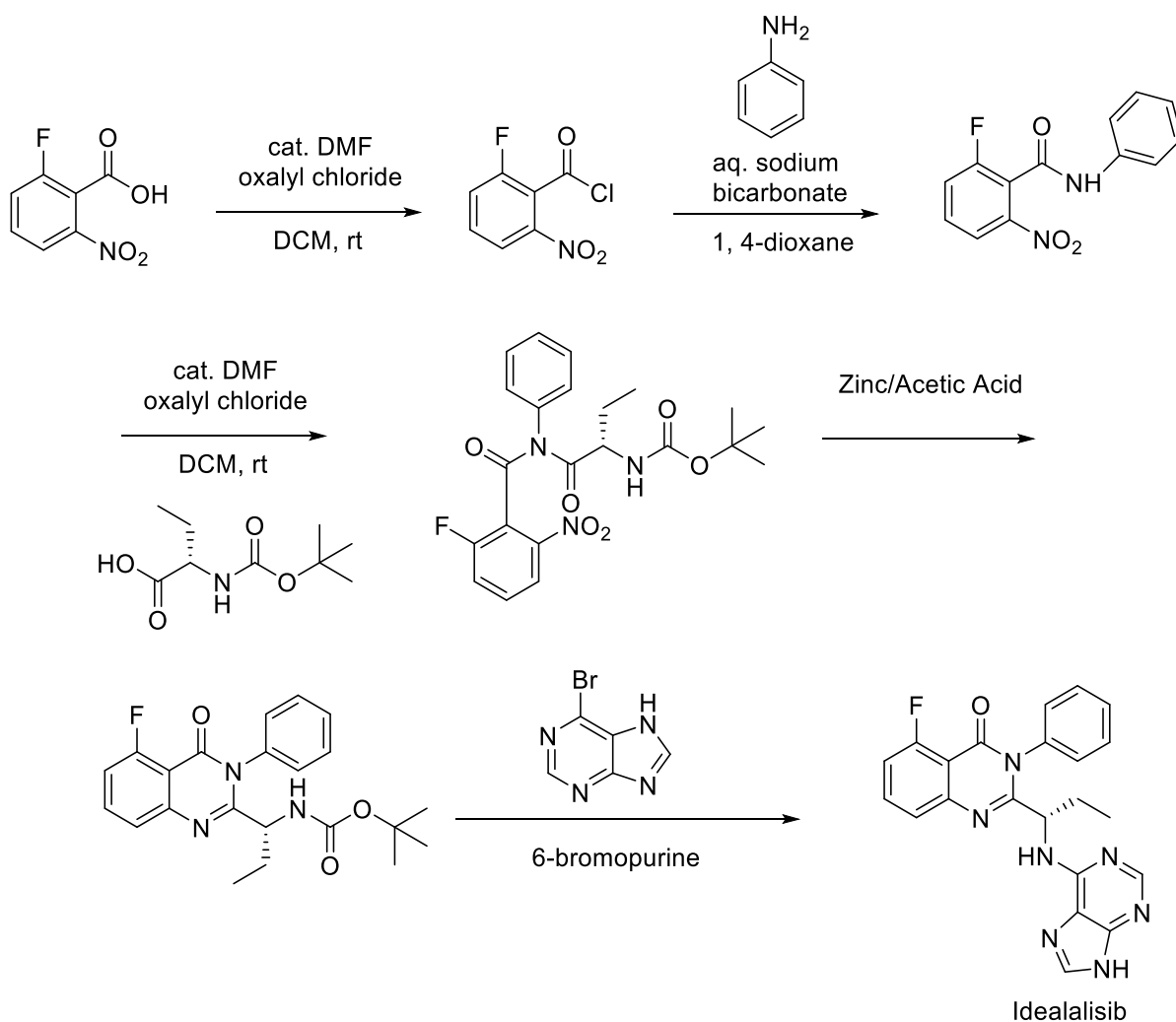


Figure 26: Original synthetic route of Idelalisib^{208, 210}

In terms of preclinical development of other CLL-directed small molecule therapies an interesting example is the development of novel 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) inhibitors²¹², for example the imadazole styryl benzamide (Figure 27). Previous genomic and proteomic studies have shown that the vitamin D receptor (VDR) is highly expressed and upregulated in CLL compared to that of normal lymphocyte populations (B-cells, T-cells)²¹³. Furthermore, it has been demonstrated that administration of vitamin D analogues such as calcitriol to primary CLL cells *in vitro* has a selective, cytotoxic effect through a p53-independent mechanism²¹⁴. Ferla et al. reported potent activity of the mentioned CYP24A1 inhibitors in primary CLL cells and observed low nanomolar enzyme inhibition and LD₅₀ values compared to ketoconazole and fludarabine respectively²¹². Significant upregulation of VDR target gene mRNAs GADD45 α (Growth arrest and DNA damage protein 45 α) and CDKN1A (cyclin

dependent kinase inhibitor 1A) were also noted, which play a key role in cellular growth inhibition and apoptosis and cell-cycle regulation respectively^{215, 216}.

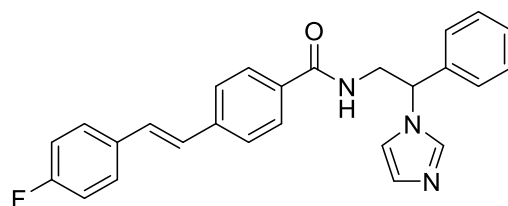


Figure 27: Imidazole styrylbenzamide example which targets CLL through CYP24A1 inhibition

Another noteworthy example of experimental therapy development is research conducted by Zhu et al. into development of an autologous CLL vaccine²¹⁷. Early work in this research space showed the ability of immune cells to have an impact on CLL population viability through observed beneficial graft vs. leukaemia (in reduced-intensity conditioned allogeneic stem cell transplantation patients) and spontaneous disease remission post-viral infection or post-inflammatory cytokine exposure. In addition to this, the existence of CLL active T-cells were observed in patients²¹⁸⁻²²¹. Utilizing the nature of activated B-lymphocytes as antigen presenting cells, CLL cells were activated through pretreatment with ionomycin and imiquimod and were coated with a non-depleting anti-CD200mAb (a transmembrane type Ia glycoprotein documented in CLL and other haematological malignancies such as acute leukaemia, observed to have immunosuppressive function). A potent cytotoxic effect *in vitro* and *in vivo* (in CLL-injected mice) was observed due to the successful immunization of peripheral blood lymphocytes (PBL) and production of CD8⁺ cytotoxic T-lymphocytes²²²⁻²²⁴, showing the potential of CLL to be selectively targeted by vaccine-based technology.

In terms of current agents in clinical trials, Novartis Pharmaceuticals are currently running a Phase 1b open-label clinical trial investigating the effect of co-administration of ianalumab (VAY736) and ibrutinib in CLL patients²²⁵. Ianalumab is a novel human IgG1/κ mAb designed to target aberrant mature and immature B cells through two main mechanisms, one being the classical mAb mediated ADCC to while the other is direct, competitive blockade of BAFF-R signalling (BAFF [B-cell activating factor of the TNF-family] receptor-a key pro-survival factor in B-cells), leading to apoptosis and interruption of downstream survival pathways^{226, 227}. Considering the information examined up to this point, the origin and background of the research project will now be discussed.

1. 3. 4 Project basis and background

Maprotiline is an atypical tetracyclic anti-depressant, related to typical tricyclic antidepressants e.g. amitriptyline, displaying similarity in terms of pharmacological action through monoamine neurotransmitter reuptake inhibition from the neuronal synapse (Figure 28). However, it displays more selectivity against norepinephrine and has only a marginal effect on serotonin, targeting the noradrenaline transporter (NET) over the serotonin reuptake transporter (SERT) (KD 11. 1 nM versus KD >5000 nM), hence being referred to as an NSRI (noradrenaline selective reuptake inhibitor)^{228, 229}. As well as traditional antidepressant effects, maprotiline acts as a competitive acetylcholinergic muscarinic receptor antagonist, anti-histamine and also exerts modest influence on adrenoceptors²³⁰⁻²³².

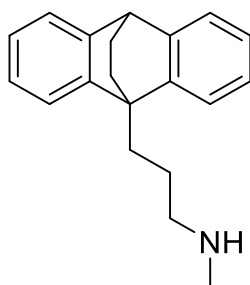


Figure 28: Maprotiline (3-(9, 10-ethanoanthracen-9-yl)-N-methylpropan-1-amine)

Maprotiline was first synthesised and patented in 1969 by Wilhelm and Schmidt (Ciba-Geigy)²³³. A number of synthetic routes for maprotiline and various other dibenzo [b,e]bicyclo[2,2,2] octadienes were documented^{233, 234}. The initial step involves a Michael addition of anthrone (**A**) to acrylonitrile using potassium tert-butoxide as a basic catalyst. *In situ* nitrile hydrolysis is then facilitated using hydrochloric acid to yield 3-(10-oxo-9,10-dihydroanthracen-9-yl) propanoic acid (**B**) (Figure 29). Zinc mediated reduction in aqueous ammonia then follows resulting in the reduction of the ketone to form 3-(9-anthracenyl) propanoic acid (**C**). The 9,10-dihydroanthracen-9-yl is then introduced *via* a high pressure Diels–Alder reaction with ethylene to yield 3-(9,10-dihydro-9,10-ethanoanthracen-9-yl) propanoic acid (**D**). This acid is then treated with oxalyl chloride to produce an acid chloride and the resultant compound then reacts with methylamine to produce 3-(9,10-dihydro-9,10-ethanoanthracen-9-yl)-N-methylpropanamide (**E**). The product is then reduced with lithium aluminium hydride to

produce 3-(9,10-dihydro-9,10-ethanoanthracen-9-yl)-*N*-methylpropan-1-amine (maprotiline) (**F**).

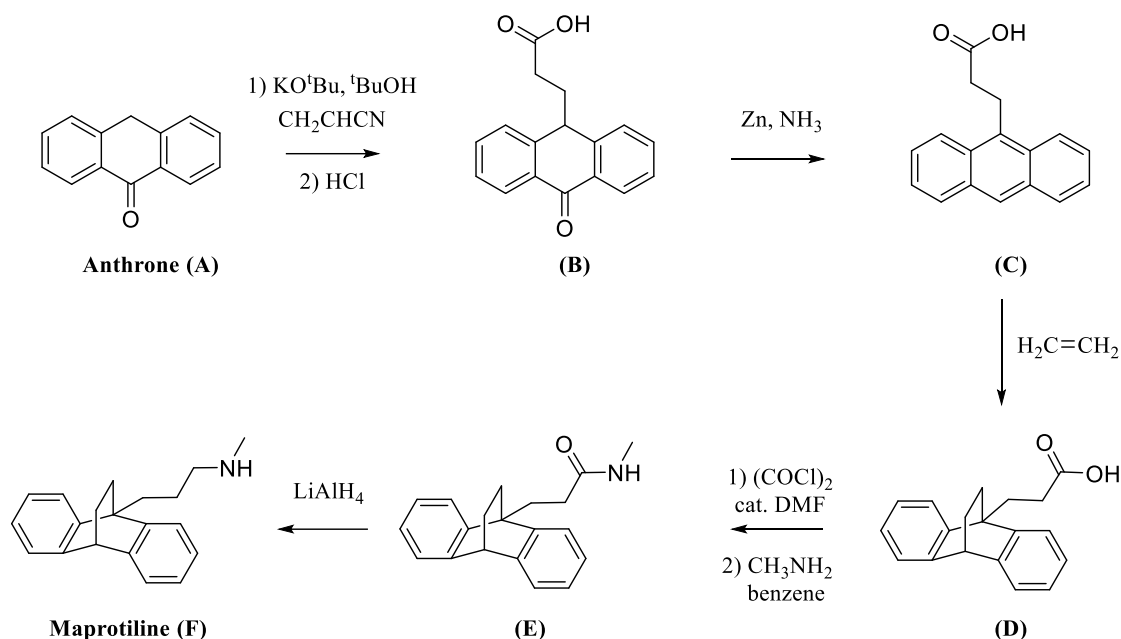


Figure 29: Synthesis of maprotiline²³³

Currently, maprotiline is not used as a conventional clinical treatment for depression due to the emergence of more selective and tolerable SSRIs (selective serotonin reuptake inhibitors). Despite this, recent discoveries into alternative pharmacological actions and drug repurposing have been investigated. Studies conducted have demonstrated potential analgesic, anti-inflammatory and, of primary interest, anti-MDR (multi drug resistance) effects of maprotiline in *Plasmodium falciparum* and various cancer cell lines^{232, 235, 236}. MDR and chemotherapeutic resistance is an evolving paradigm facing cancer research at present with local changes in tumour microenvironment dictated by epigenetic heterogeneity and subsequent mutations such as in P-glycoprotein cellular efflux pump. Such up-regulated selective expression renders cancer cells and parasites alike, immune to pharmacotherapy; however the resistance threshold of both diseases to conventional treatments has been shown to be significantly lessened by the use of maprotiline and its analogues^{237, 238}. Previously reported by the Meegan group, the common link between promising 9,10-dihydro-9,10-ethano/ethenoanthracene derivatives based on maprotiline was thought to be initially attributed to the presence of proton-accepting nitrogen atom, polyaromatic ring presence and high lipophilicity²³⁵.

Further research by the group observed that minor variations introduced onto the side chain consisting of a two carbon bridge facilitated optimization of the anti-MDR potency. Moreover, an optimal distance of 6.6-6.7 angstroms between the hydrogen bond acceptors (HBA) on the bridgehead group of the ethano derivatives was also found to be important for anti-MDR activity, determined by a later SAR study in 2003²³⁸. These maprotiline analogues were able to downregulate MDR activity in MDR leukaemic cell lines (L5178), allowing increased intracellular accumulation of chemotherapeutic agents measured by rhodamine 123 (used as a model for efflux inhibition). The compounds which conferred the highest chemosensitive response were those containing sizable diphenylphosphamide and diphenylphosphonate groups as bridge substituents, achieving 100% sensitization at 2.0 μM and 0.6 μM respectively.

Previous work in School of Pharmacy and Pharmaceutical Sciences, in collaboration with the School of Biochemistry and Immunology Trinity College Dublin, showed promising antiproliferative activity of maprotiline in Burkitt's lymphoma cell lines²³⁹. The most important finding was caspase-independent type-2 autophagic cell death induced in the chemoresistant DG-75 cells, which exhibited low EC_{50} values in the ranges of 5.9-15.3 μM and 5.9-15.6 μM when treated with maprotiline and fluoxetine respectively over 72 h period^{117, 118}. This precise pro-autophagic pathway was not observed with other NSRIs such as nisooxetine or NET ligands such as norepinephrine despite being present in excess. Hence, the autophagy induced by this selective NET molecule is thought to be transporter independent, supported by the absence of death in SERT overexpressing DG-75 cell lines treated with fluoxetine and maprotiline¹¹⁷.

Chapter 2: Synthesis of anthracene and ethanoanthracene based compounds

2.1 Objectives of the project

Despite the recent advancements in the clinical treatment of Chronic Lymphocytic Leukaemia (CLL), there still remains a requirement for the discovery and development of novel therapeutic agents to combat emerging disease resistance and provide a curative treatment, as opposed to maintenance alone. The objectives of this study are to design, synthesise and evaluate the antiproliferative activity of a series of novel 9,10-dihydro-9,10-ethanoanthracene compounds structurally related to the antidepressant maprotiline for use in treatment of CLL (Figure 30). In order to accomplish this objective the following compound libraries were prepared for investigation:

- **Series 1:** (*E*)-9-(2-Nitrovinyl)anthracenes
- **Series 2:** (*E*)-9-(2-Nitrovinyl)anthracene-maleimide adducts
- **Series 3:** Anthracene chalcones and related compounds
- **Series 4:** Ethanoanthracene chalcone-maleic anhydride adducts
- **Series 5-9:** Ethanoanthracene chalcone-maleimide adducts and related compounds

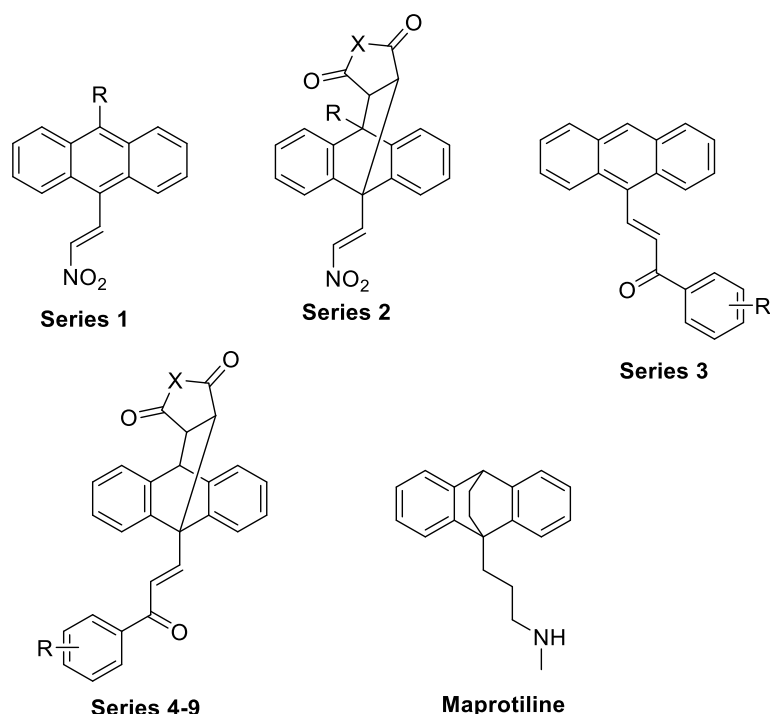


Figure 30: Representative scaffolds of main compound classes prepared and maprotiline: (top left) (*E*)-9-(2-nitrovinyl)anthracene, (top centre) (*E*)-9-(2-nitrovinyl)anthracene-maleimide adducts, (top right) anthracene chalcones, (bottom left) ethanoanthracene chalcone-maleimide adducts and maprotiline (bottom right)

Preliminary *in vitro* evaluations of these compounds listed above were completed to quantify potential antiproliferative activity in representative CLL cell lines.

2.2 General introduction to project chemistry

The following reaction scheme briefly outlines the reactions utilized to generate the series of compounds discussed in this thesis (Figure 31):

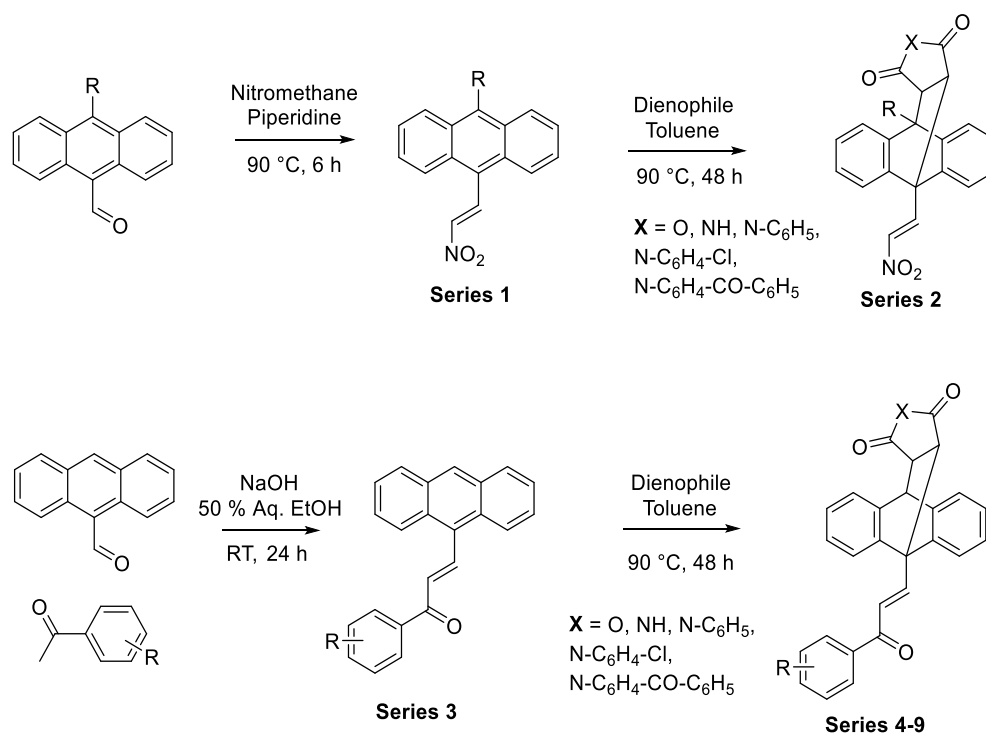


Figure 31: General reaction scheme for ethanoanthracene syntheses

2.2.1 Series 1-Nitrostyrenes

Nitrostyrenes are a highly versatile class of chemical structure; a well-known variant of the most basic unit represented by the structure of (*E*)-(2-nitrovinyl)benzene (Figure 32). These nitroolefin compounds of the general structure $R_2C=C(R)NO_2$ are an extremely useful family of electrophilic alkenes which have the capacity to participate in a wide variety of chemical reactions (such as Michael additions, aldol condensations and Mannich reactions), due to susceptibility to nucleophilic attack resulting in covalent bond formation. Furthermore, diverse functional group manipulation of the nitro group itself makes them useful pharmaceutical intermediates leading to greater reaction flexibility²⁴⁰⁻

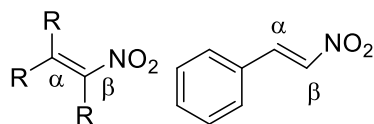


Figure 32: General nitroalkene structure and (*E*)-(2-nitrovinyl)benzene

β -Nitrostyrenes have been found to display numerous biological activities. These include not only antiproliferative but also antibacterial activity^{120, 244} and potent tyrosine kinase/platelet inhibition. Kim et al. describe such derivatives as acting through highly selective telomerase inhibition *in vitro*²⁴⁵ expressing high potency, whereby the core nitrostyrene moiety is essential for the induction of apoptotic programmed cell death (Figure 33). Derivatives of this pharmacophore have shown great promise with ascorbic acid analogues achieving LC₅₀ values of 10-25 μ M, with no significant reductions in efficacy or potency in rat pituitary GH3 tumour cell lines overexpressing the MDR-1 glycoprotein¹¹⁹.

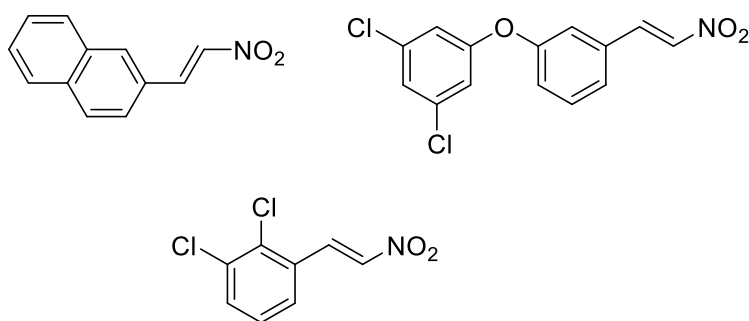


Figure 33: (*E*)-2-(2-Nitrovinyl)naphthalene- a representative example of nitrostyrene based telomerase inhibitors²⁴⁵

Furthermore, nitrostyrene derivatives have also been documented as effective phospholipase A₂ inhibitors, hence having the potential to impact on availability of substrates for enzymes central to the inflammatory process (e.g. arachidonic acid and cyclooxygenase enzymes). As a result, excessive levels of pro-inflammatory mediator production such as prostaglandins and leukotrienes can be modulated, potentially impacting on treatment of pathological conditions including cardio-respiratory diseases, cancer and CNS disorders²⁴⁶⁻²⁴⁹. The nitrostyrene derivatives tested were observed to have potent Km values as low as 35 nM. The proposed mechanism through which enzyme inhibition is thought to occur involves the formation of charge transfer complexes between phospholipase A₂ (PLA₂) and the nitrostyrene compounds, where those with lower energy highest occupied molecular orbitals (HOMO) are more susceptible to attack from the electrons of the PLA₂ binding site.

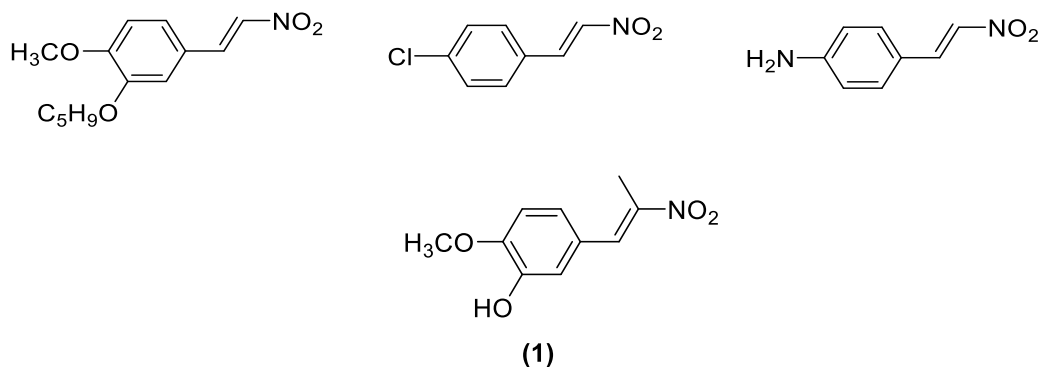


Figure 34: Examples of nitrostyrene derivatives capable of phospholipase A₂ enzyme inhibition, (*E*)-2-methoxy-5-(2-nitroprop-1-en-1-yl)phenol-synthetic β -nitrostyrene (**1**)^{248, 250}

Modifications to the original nitrostyrene structure to improve cancer chemotherapeutic properties have been moderately successful in enhancing the pro-apoptotic cascade. Among them are substitutions on positions 2 and 3 of the aromatic hydrocarbon core along with methyl substitutions at positions 3, 4 and 5 producing a dramatic 2-3 fold increase in phosphatase inhibition activity²⁵¹ (Figure 34). This is relevant as protein-tyrosine phosphatases such as SHP-1 are overexpressed in many cancer types including prostate, ovarian and breast cancer. Another study of note was carried out by Tsai et al., investigating the anti-tumour effect of the synthetic β -nitrostyrene derivative (**1**) as a potential lung cancer therapeutic. It was found to have potent *in vitro* activity (IC₅₀ 2.12-3.28 μ g/mL) in human lung cancer cell lines as well as *in vivo* with a H520 murine xenograph model (Figure 34). The compound was observed to exert its cytotoxicity through causing G₂/M phase cell-cycle arrest in addition to growth suppression as a result of glutathione (GSH) depletion and reactive oxygen species (ROS)-associated pathway²⁵⁰.

Pettit et al., through their consideration of the molecular structures of (*E*)-combretastatin and (*E*)-resveratrol and subsequent generation of derived β -(*E*)-nitrostyrenes, demonstrated not only their ability as cancer cell growth inhibitors *in vitro*, but also the ability to function as potent tubulin polymerization inhibitors. For example, compound (**2**) (Figure 35) displayed a promising mean IC₅₀ value of 2.5 μ M in tubulin polymerization assays and was thought to act through a non-competitive binding site as evidenced by enzyme kinetic studies conducted as part of the same work. This was further supported by the absence of mitotic arrest when β -nitrostyrenes were dosed at cytotoxic concentrations, a behaviour which is a typical hallmark of tubulin inhibitors acting

through the colchicine binding site of β -tubulin^{120, 252}. However, this unique binding behaviour was found to be structure dependent, with a 3,4,5-trimethoxyphenyl moiety similar to that of the colchicine A ring allowing cell cycle arrest at the G₂/M phase and interference with α,β -tubulin microtubule assembly. In addition, compound (2) has the potential to undergo non-competitive binding at the colchicine β -tubulin hydrophobic pocket in a similar binding mode, explored through molecular docking simulations of the most promising nitrostilbenes, compounds (3) and (4)²⁵³ (Figure 35).

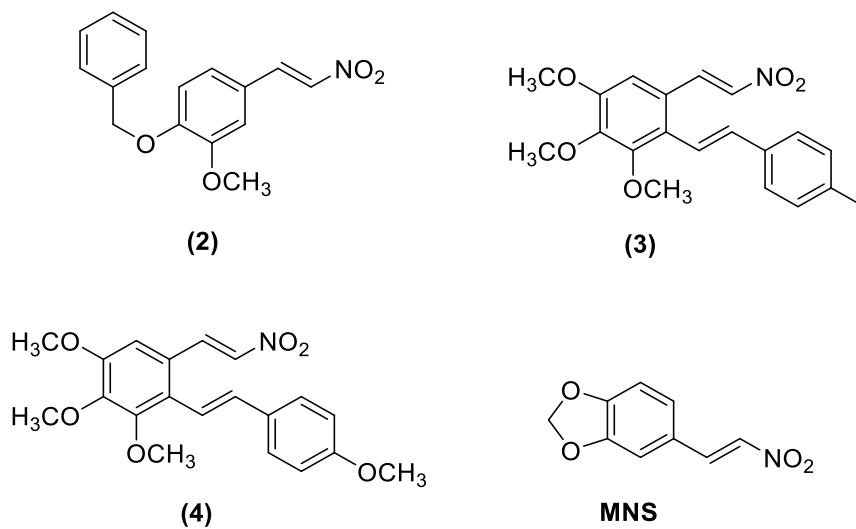


Figure 35: Examples of nitrostilbene-based antimitotic drugs-(top left) compound (2), (top right) compound (3) (bottom left) compound (4), (bottom right) 3,4-methylenedioxy-nitrostyrene (MNS)

Apart from widely demonstrated antiproliferative activities of this pharmacophore, He et al. demonstrated the ability of 3,4-methylenedioxy-nitrostyrene (MNS) (Figure 35) to inhibit NLRP3 inflammasome activation through blockade of the protein complex assembly and the prevention of NLRP3 ATPase activity. As constitutive activation of the NLRP3 inflammasome has been associated with a number of chronic, non-communicable diseases (Alzheimer's disease, Type 2 diabetes and atherosclerosis), the growing body of literature supports the development of NLRP3 selective inhibitors as therapeutics to reduce the disproportionate inflammatory response which drives the pathogenesis and disease progression²⁵⁴. A moderately potent NLRP3 inhibitor with an inhibitory IC₅₀ value of 2 μ M, MNS is highly selective for the NLRP3 inflammasome and is thought to act through its ability to undergo a Michael addition with cysteine residues present in the NACHT (a specific nucleotide-binding domain) and LRR (leucine-rich repeat) domains of the protein complex. This theory is supported through loss of activity on reduction of the nitrovinyl alkene bond and the introduction of competitive Michael acceptor

compounds in NLRP3 inflammasome binding assays²⁵⁵. First explored in the 1940s, β -nitrostyrene molecules exhibit both antibacterial and antifungal properties^{256, 257}, with their effectiveness being more pronounced in Gram-positive than Gram-negative bacteria and fungi²⁵⁸. For example, previous studies such as Milhazes et al. and Ajiboye et al. have shown their usefulness as potential antibacterial agents and adjuvant therapies for existing treatments for clinically relevant pathogens like *E.coli*, *S.aureus* and *Enterococcus spp*^{244, 259}. Despite the exact mode of action remaining elusive, a proposed mechanism of action theorises that such compounds act as tyrosine mimetics, inhibiting protein tyrosine phosphatases and thus disrupting microbe homeostasis and normal cellular processes. It is thought that nitrostyrenes act through covalent inhibition of protein tyrosine phosphatase 1B (PTB1B) via nucleophilic attack of the cysteine residue present at the active site. This is supported by the potential formation of hydrogen bonds between the nitro group and terminal amine amino acid residues, allowing the correct molecular conformation for inhibition to occur²⁶⁰ (Figure 36).

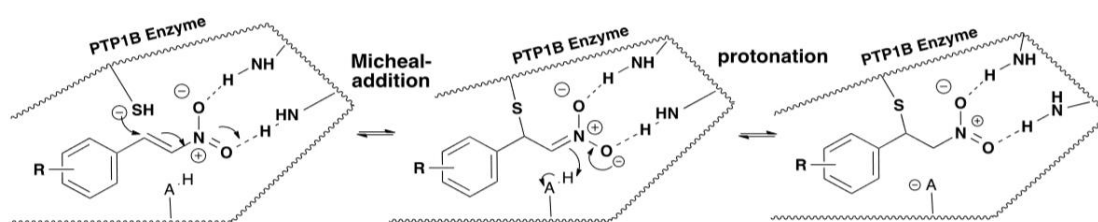


Figure 36: Proposed antimicrobial mechanism of action of β -nitrostyrenes ($R=H, Br, Cl, F$)²⁶⁰

2.2.2 Henry-Knoevenagel condensation reaction

2.2.2.1 The Knoevenagel condensation reaction

From previous research carried out within the group in relation to antiproliferative activity in Burkitt's Lymphoma, we were interested in exploring the same in CLL. Hence, in order to investigate this, the Henry-Knoevenagel reaction was used for synthesis of the required β -nitrostyrenes. Therefore, the Knoevenagel and Henry reactions along with their applications to this work will now be discussed.

The Knoevenagel condensation reaction (Figure 37) was first documented by the German organic chemist Emil Knoevenagel in 1889²⁶¹. The reaction consists of amine catalysed mixed aldol condensation between carbonyl containing structures lacking α -hydrogens such as an aldehyde and a compound containing an activated methylene group which is acidic in nature (diethyl malonate predicted pKa 12.36) due the influence of attached electron withdrawing substituents.

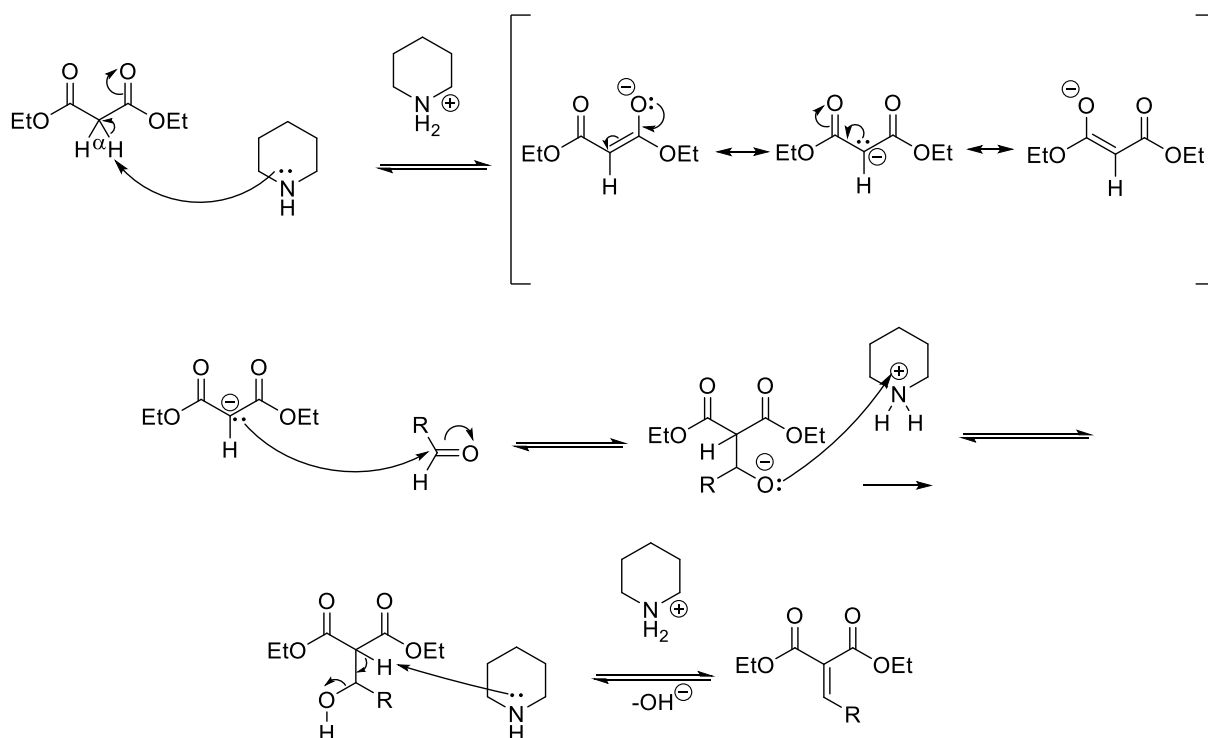


Figure 37: General Knoevenagel condensation mechanism using piperidine as a base and diethyl malonate as the activated methylene source (R=H, Alkyl, Aryl)²⁶²

2.2.2.2 The Henry reaction

Following this, the Henry reaction was first discovered by Louis Henry in 1895²⁶³ and is noted as a classical C-C bond forming reaction. It involves linkage of an aliphatic nitro compound (containing acidic α -hydrogen atoms) with a carbonyl containing moiety such as an aldehyde or ketone²⁶⁴. The reaction is referred to a nitroaldol condensation and is base catalysed (usually using primary/secondary amines), leading to deprotonation of the acidic α -hydrogens of the nitroalkane to produce a nitronate anion²⁶⁵. This acts as a nucleophile attacking electrophilic carbon of the carbonyl functional group, leading to β -nitroalcohol product generation along with catalytic base renewal²⁶³, (Figure 38).

Subsequent dehydration of the β -nitroalcohol intermediate affords the nitrostyrene product. By virtue of their acidity (nitromethane pK_a 10.21), nitroalkenes can also be formed by the reaction of an appropriate aldehyde and nitroalkane using bases such as piperidine or basic aluminium oxide²⁶⁶.

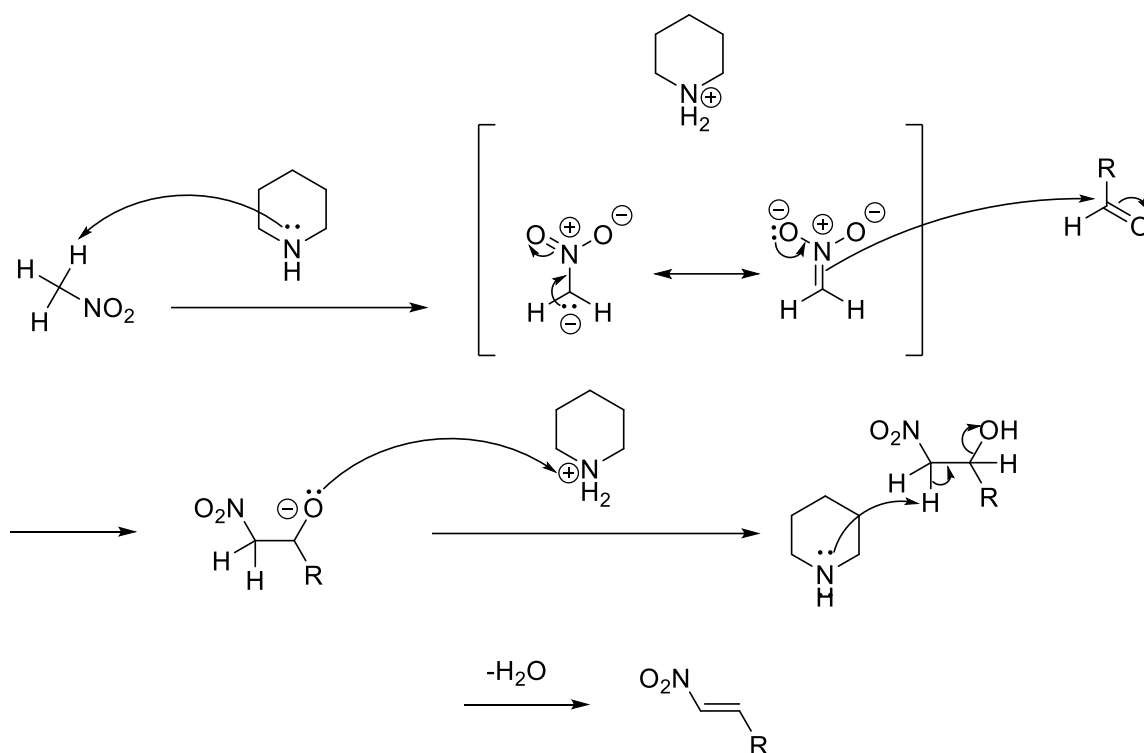


Figure 38: General Henry nitroaldol reaction mechanism using piperidine as a base and nitromethane as a nitroalkane reactant ($\text{R}=\text{H}$, Alkyl, Aryl)²⁶⁷

In terms of Henry reaction applications, the resultant β -nitroalcohol provides a valuable synthetic intermediate²⁶⁸ required for the synthesis of numerous pharmacologically important agents, for example, novel adrenergic antagonists for the treatment of diabetes and obesity²⁶⁹. Moreover, in cancer research and the continuous development of the classic Henry reaction by organic chemists such as Shibasaki, highly efficient and enantioselective synthesis of both synthetic and natural molecules continue to revolutionise organic synthesis. An excellent example is the synthesis of the C-13 side chain of Taxol using the asymmetric Shibasaki method and the optically active transition metal catalyst La-(R)-binaphthol ²⁷⁰ where production yields and time were increased and decreased respectively²⁷¹ (Figure 39).

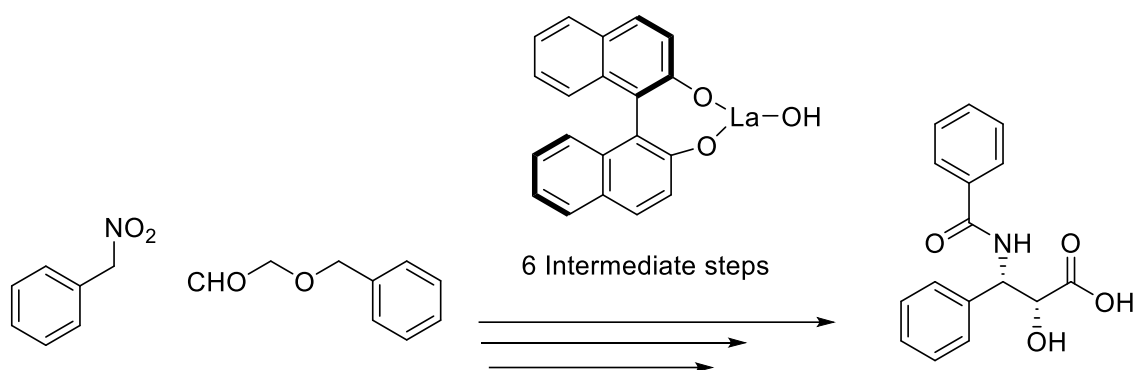


Figure 39: Reaction scheme for the synthesis of C-13 side chain of Taxol using the optically active transition metal catalyst La-(*R*)-binaphthol^{270, 271}

Another notable development using the Henry reaction is the discovery of environmentally sustainable catalysts to allow formation of β -nitroalcohol derivatives at comparatively mild conditions (room temperature [RT] and relatively non-toxic organic solvents such as dimethyl sulfoxide [DMSO]) in comparison to those currently used in classic organic synthesis. A contemporary example of this is the use of alginate hydrogel (AHG) beads cross-linked using calcium as the metallic gelling agent. Yields of up to 88% were obtained and >99% reagent conversion (using substituted benzaldehydes and nitromethane), with the catalyst being recycled in three consecutive synthetic runs without loss of activity²⁷² (Figure 40).

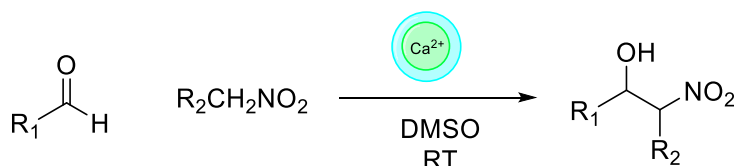


Figure 40: Ca^{2+} -AHG-catalyzed Henry reaction in DMSO²⁷²

In general, the Henry–Knoevenagel reaction generates an enolate anion (using either a base or acid catalyst²⁶⁵) due to the nitro group inductive effect and subsequent generation of a carbanion nucleophile. This nucleophile not only permits the generation of satisfactory quantities of nucleophilic enolates by weak bases but also plays a role in the dehydration reaction²⁷³. The enolate anion then attacks the iminium ion formed, ultimately leading to dehydration and formation of the unsaturated alkene product (Figure 38). In summary, the principal difference between these organic reactions is that in the Henry reaction, (the nucleophilic attack on the carbonyl containing moiety) is carried out by nitroalkanes with α -active methylene groups (facilitated by the presence of a weak

base). Whereas in the case of the Knoevenagel condensation, nucleophilic attack is facilitated by any active methylene compound (usually by virtue of two adjacent electron withdrawing groups [EWGs]), not being limited solely to nitroalkanes. As this current work contains the key elements of both reactions, it is referred to as a Henry–Knoevenagel reaction.

Since Knoevenagel forged the foundations of modern aminocatalysis more than 130 years ago²⁶¹ various catalytic modifications have been developed such as the use of bicyclic guanidines, phosphotranes and lithium aluminium hydride in THF²⁶⁸. Moreover, greener synthetic routes have been explored, for example Meuldijk et al. who created a novel, environmentally friendly, solvent-free condensation replacing pyridine and piperidine catalysts with relatively benign amines [e.g. benzylamine, 2-aminoethanol] and ammonium salts [e.g. ammonium sulfate, ammonium bicarbonate]²⁷⁴. Reaction efficiency (measured in % conversion), yield and purity remained extremely high (up to 100%) despite the modifications, representing more sustainable production methods than those currently used. In industrial environments, the reaction is employed for stereoselective preparation of pharmaceuticals and their intermediates e.g. the generation of HIV protease inhibitor Amprenavir²⁷⁵.

2.3 Series 1: Synthesis of (*E*)-9-(2-nitrovinyl) anthracenes

In the present work, synthesis of (*E*)-9-(2-nitrovinyl) anthracenes (**5**) and (**6**) was achieved using a piperidine-catalysed Henry-Knoevenagel condensation reaction of 9-anthraldehyde with nitromethane (Figure 41).

This synthetic method was described by Parker et al.²⁶⁶ and afforded excellent yields of 71% and 99% for (**5**) and (**6**) respectively. Both compounds were obtained as solids following recrystallisation from methanol and diethyl ether as a two-solvent system. An additional series of previously synthesised nitrostyrene compounds (**7-20**) were evaluated (chapter 3 biochemical study)

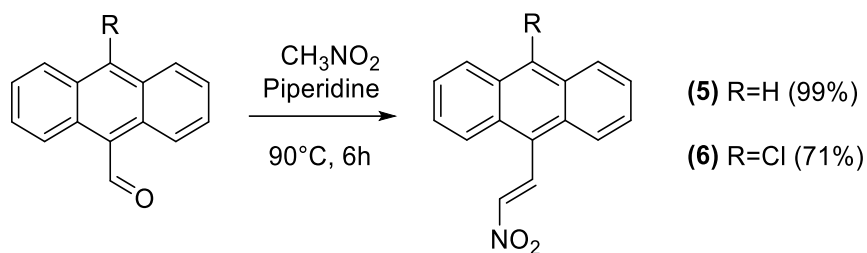


Figure 41: General reaction scheme for the synthesis of (*E*)-9-(2-nitrovinyl)anthracenes (Series 1)

2.3.1 Structural characterisation of (*E*)-9-chloro-10-(2-nitrovinyl)anthracene (**6**)

(*E*)-9-Chloro-10-(2-nitrovinyl)anthracene (**6**) was chosen as a representative example of Series 1 of the (*E*)-9-(2-nitrovinyl)anthracenes and ^1H NMR and ^{13}C NMR spectra will now be discussed in detail. The numbering of the compound (**6**) was generated in ACD/ChemSketch version 12.01. The ^1H NMR spectrum (400 MHz, CDCl_3) of compound (**6**) (Figure 42) shows a doublet signal at 7.49 ppm ($J=14$ Hz, 1H), a coupling constant typical of the *trans* configuration of double bonds²⁷⁶. This signal was assigned to H17 of the double bond. The multiplet observed at 7.64 ppm integrating for four protons (two overlapping signals) was assigned to H4, H5, H11 and H12. Further downfield, the doublet at 8.17 ppm ($J=8.5$ Hz) integrating for two protons account for H6 and H10, while the doublet at 8.59 ppm ($J=8.5$ Hz) also integrating for two protons can be appointed to H3 and H13. This is due to the latter proton pair being in close proximity to the chlorine atom at C1 of the central anthracene core ring. The remaining doublet signal at 8.93 ppm ($J=14$ Hz, 1H) is that of H16 of the alkene bond resonating further downfield than H17.

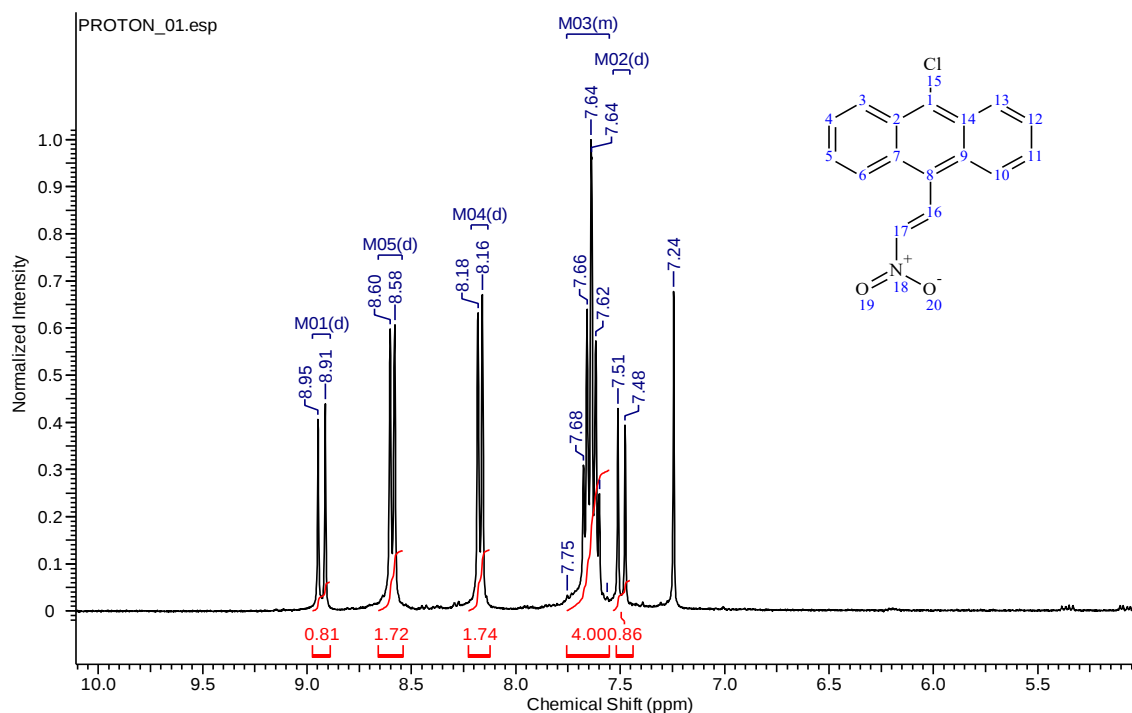


Figure 42: ^1H NMR spectrum of (E)-9-chloro-10-(2-nitrovinyl)anthracene (**6**)

The ^{13}C NMR spectrum (101 MHz, CDCl_3) of compound (**6**) (Figure 43) is presented below. The signal at 135.4 ppm was assigned to C1 while the furthest downfield signal at 143.2 ppm was assigned to C17, due to the deshielding effect of the electron-withdrawing nitro functional group. The signal appearing at 130.1 ppm corresponds to the quaternary carbons C7 and C9 on the central ring of the anthracene-derived core. The quaternary carbon C8 was identified as the weak signal at 122.9 ppm, while the halogen bearing carbon C1 was observed at 128.5 ppm, due to the influence of the chlorine atom through the conjugated polyaromatic system. C5 and C11 are represented by the signal at 127.6 ppm, while the signal at 127.1 ppm accounts for C4 and C12 due to experiencing similar chemical environments along the upper area of the anthracene core structure. The remaining two signals at 125.7 ppm and 124.8 ppm represent the atom pairs C6, C10 and C3, C13 respectively.

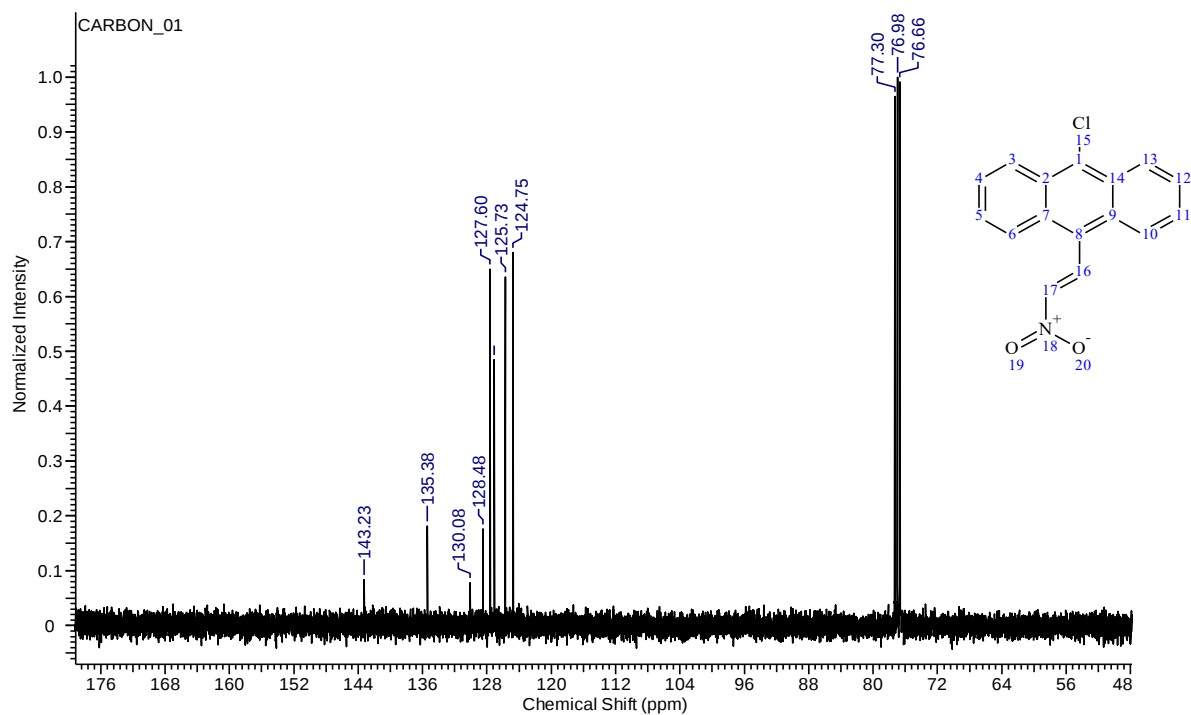


Figure 43: ^{13}C NMR spectrum of (*E*)-9-chloro-10-(2-nitrovinyl)anthracene (6)

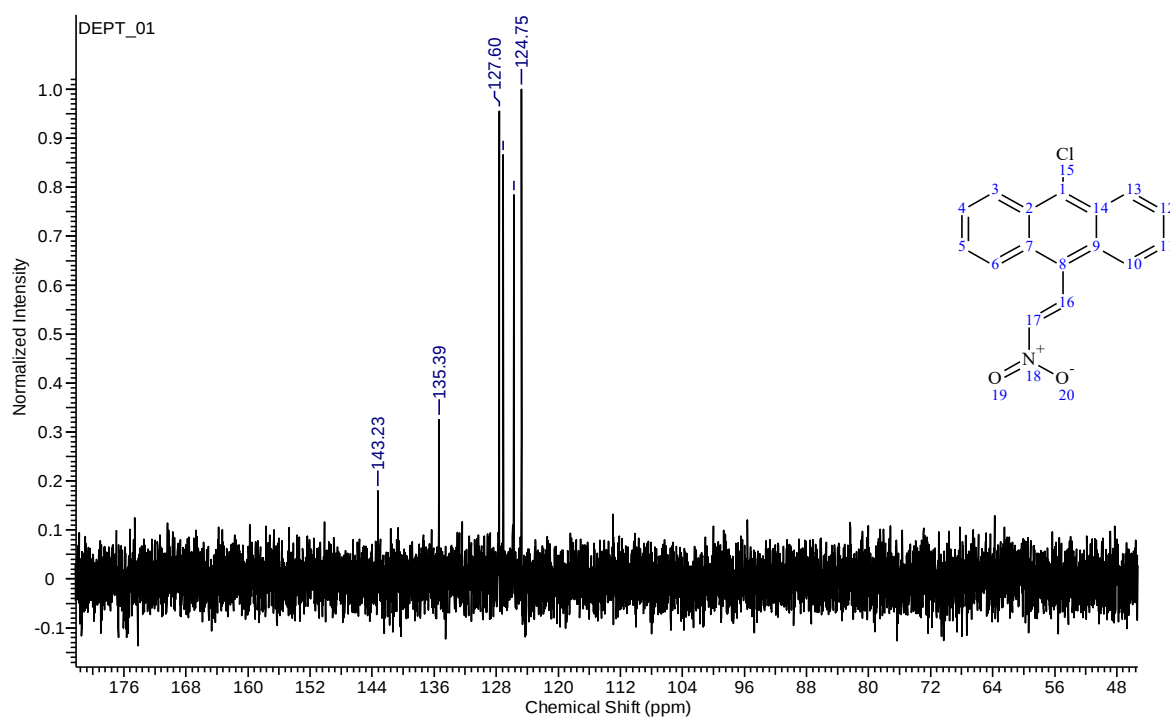


Figure 44: DEPT-90 spectrum of (*E*)-9-Chloro-10-(2-nitrovinyl)anthracene (6)

The DEPT-90 spectrum (Figure 44) was used to confirm the identity of the nitrovinyl carbons of the double bond (C16, C17) and was also used in conjunction with the

HMBC spectrum (Figure 46) to correctly assign the quaternary carbons in the structure (C7 and C9) at 130.1 ppm. The HSQC spectrum (Figure 45) was used to confirm the correct assignment of proton signals to their respective adjacent carbon atoms such as H (4, 5, 11, 12).

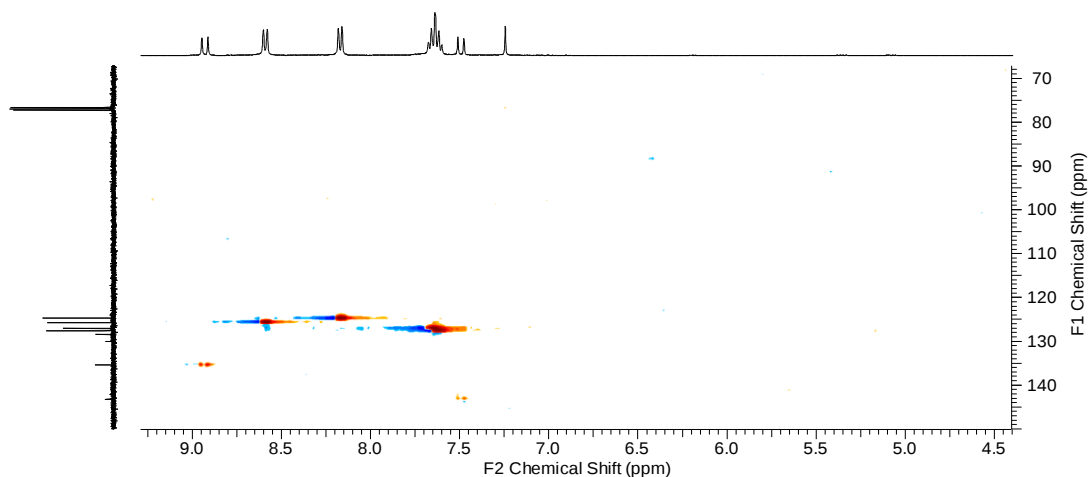


Figure 45: HSQC spectrum of (*E*)-9-chloro-10-(2-nitrovinyl)anthracene (6)

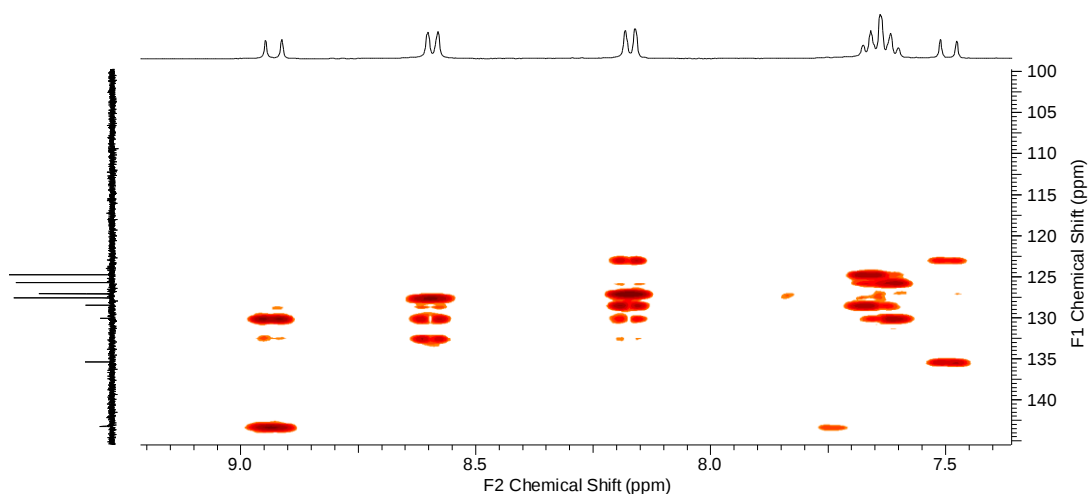


Figure 46: HMBC spectrum of (*E*)-9-chloro-10-(2-nitrovinyl)anthracene (6)

2.4 Series 2 : Ethanoanthracenes

Ethanoanthracenes are a class of organic compound that generally consist of three main functional regions that make up the molecule as a whole. These are an anthracene-derived core (a fused three member-ringed polyaromatic hydrocarbon, illustrated in blue), an aliphatic two carbon bridge (which may bear substituents at R^2 and R^3 , illustrated in red)

that spans between carbon atoms 9 and 10 of the centre ring of the anthracene core and a functionalised “tail” chain extending from the 9 position (illustrated in black at R¹). One example of particular interest being maprotiline as it is the initial lead compound on which this work is based (containing a methylaminopropyl substituent at position 9) (Figure 47).

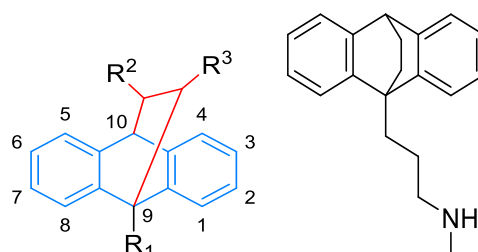


Figure 47: (left) General structure of ethanoanthracene compounds illustrating the anthracene core (blue), aliphatic carbon bridge (red) and the side-chain at position 9 of the anthracene core centre ring (black), (right) maprotiline ethanoanthracene-based scaffold

2.4.1 Biological activities of ethanoanthracenes

Such structures have been explored for a variety of disease conditions and medical applications due to their diverse biological activities apart from malignancy treatment. Maprotiline (see Chapter 1), a tetracyclic antidepressant molecule has been previously shown to elicit potent antiproliferative effects on leukaemia and lymphoma cell lines (particularly Burkitt’s lymphoma) through apoptotic caspase dependent cell death¹¹⁶. In addition it has been shown to be very effective in a variety of multi-drug resistant cell lines (e.g. parental HL-60 overexpressing either P-glycoprotein efflux pumps [P-gp] or breast cancer resistance protein [BCRP])²³⁹. Bova et al. reported vasorelaxant effects of similar compounds (with the aim of developing new cardiovascular agents), mediated by voltage and frequency dependent competitive binding to the pore-forming subunit of preferentially inactivated L-type Ca²⁺ channels²⁷⁷ (Figure 48).

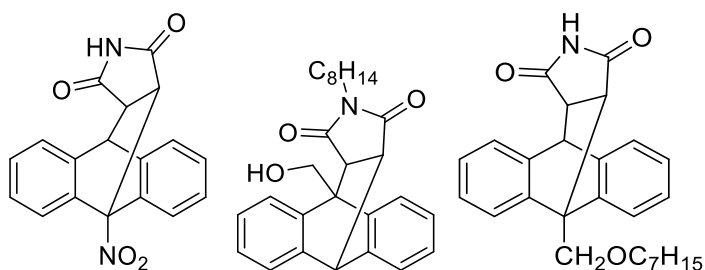


Figure 48: Example structure of compounds with a vasorelaxant effect *via* L-type Ca^{2+} channels (left) and antibiotic activity against reference and clinical isolates of *S.aureus* (centre), potential endocannabinoid regulators through binding to the cannabinoid type-2 receptor (right)²⁷⁷⁻²⁷⁹

Potent antimicrobial activity was also observed by Bonvicini et al. in related derivatives when assessed in *Staphylococcus aureus*. They displayed promising antimicrobial activity against pan-antibiotic resistant clinical isolates as well as *S.aureus* reference strains with IC_{50} and minimum inhibitory concentration (MIC) as low as 4.2 μM and 6.25 μM respectively. The mechanism by which this effect was exerted is thought to be related to interference with bacterial membrane functionality through inhibition of enzymes associated with bacterial energetics and redox-homeostasis²⁷⁸ (Figure 48).

Furthermore, lipophilic chain analogues were investigated by Bisi et al. as novel endocannabinoid system modulators. The compounds were found to have significant selectivity for the cannabinoid type-2 (CB2) receptor along with good affinity and sub-micromolar activity being observed. They were found to be acting through a non-competitive antagonism of the CB2 receptor and have the potential to be used in diseases associated with endocannabinoid dysregulation such as chronic pain, atherosclerosis and cancer (including leukaemias)²⁷⁹ (Figure 48).

Apart from the diverse array of biological activities highlighted, these polycyclic scaffolds exhibit significant anticancer activity across many different cancer types²⁸⁰. In contrast to their ability to elicit potent anti-tumour responses in BL, related structures have been noted to act as effective agents in pancreatic cancer treatment. The mechanism of action through which this activity operates was confirmed to be the inhibition of the binding interaction of S100P (a calcium-binding protein highly expressed in early stage pancreatic cancer) and the receptor for advanced glycation end-products (RAGE- an important mediator of cell proliferation, survival and cell growth). This involves prevention of S100P signalling and translocation across the cellular membrane where polymerization in the presence of high Ca^{2+} and Zn^{2+} ion concentrations facilitates RAGE binding and activation^{281, 282} (Figure 49).

Dihydroethanoanthracene compounds such as the compound in Figure 49 (right) have also been explored as non-cytotoxic chemosensitizers which reverse multidrug resistance (MDR) to established anti-cancer treatments through inhibition of P-gp mediated drug efflux²³⁸.

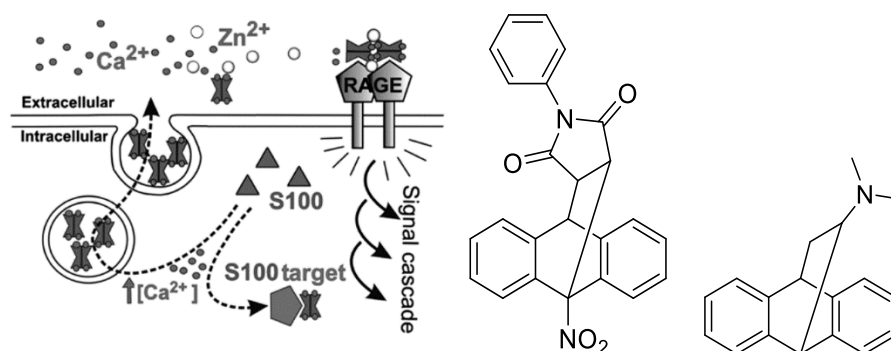


Figure 49: (left) Mechanism of S100P and RAGE interaction example of an anthracene-based S100P/RAGE interaction inhibitor, (centre) an example of a dihydroethanoanthracene based MDR reversal agent (right)^{238, 281, 282}

2.4.2 Diels–Alder cycloaddition reaction for the synthesis of Series 2 ethanoanthracenes

In the present work, the preparation of the initial (*E*)-9-(2-nitrovinyl)-9,10,11,15-tetrahydro-9,10-[3,4]furanoanthracene-12,14-dione compound library required Diels–Alder reaction of the substituted anthracenes with the appropriate dienophiles. This reaction is classified as a pericyclic reaction, a one-step reaction where no charged intermediates are formed and proceeds *via* a high-energy cyclic transition state (Figure 50).

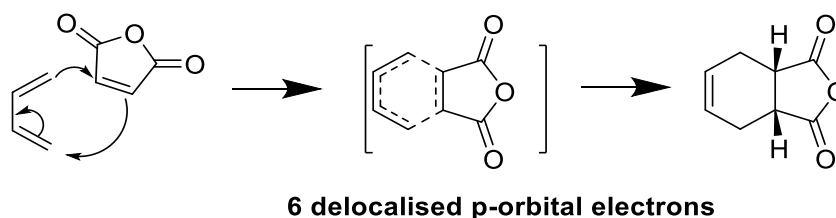


Figure 50: The general reaction mechanism for Diels–Alder reaction

The Diels–Alder reaction is one of the most widely utilized reactions in organic chemistry and was discovered by Otto Diels and his research student Kurt Alder in 1928 in the University of Kiel. For their innovative work, they received the Nobel Prize in 1950²⁸³.

The reaction occurs between a conjugated diene and a π component (usually an alkene) called a dienophile which donates its two π -electrons to the formation of two new C-C bonds and a new cyclic system. This bond formation is facilitated by the perfect orientation overlap of p -orbitals in parallel planes and is known as a [4+2] cycloaddition reaction,^{264, 265} [4+2] accounting for the electron rich diene and electron poor dienophile contribution respectively²⁸⁴. Cycloadditions involve the reaction of two or more contributing molecules, resulting in the formation of a cyclic product, without the elimination of any other chemical species²⁸⁵ (Figure 50). This symmetrical interaction between the orbitals of carbons (1 and 4) of the diene and two carbons of the dienophile facilitates stabilisation, typically between the HOMO (Highest Occupied Molecular Orbital) in the diene and the LUMO (Lowest Unoccupied Molecular Orbital) in the dienophile²⁷³ as this yields the strongest intermolecular communication.

The rate of reaction can be accelerated by the presence of electron donating substituents in the diene and electron-withdrawing substituents in the dienophile. For the reaction to take place the less thermodynamically favourable but more reactive *s-cis* (*cisoid*) configuration in the diene is required for concerted reaction, whereby the double bonds are orientated close enough to enable reaction with the alkene double bond of the dienophile^{265, 286}. This is observed especially in the case of cyclical dienes in permanent *s-trans* configurations where the reaction does not take place as the product would have an impossible *trans* double bond in the 6-membered ring²⁶⁵. The versatility of the reaction in synthetic applications lies in the elegant stereo and regioselectivity^{287, 288}, where the stereochemistry of both diene and dienophile are preserved in the cyclization process (*E/Z* configurations). This stereospecific characteristic allows the prediction of product configurations through the application of Frontier Molecular Orbital (FMO) theory. Electron deficient dienophiles have low energy LUMOs and dienes have high energy HOMOs and consequently a better orbital overlap is achieved due to the smaller energy gap than that of the reverse situation. The reactants in which the carbons have the highest FMO coefficients initiate the bonding process²⁶⁴. The mechanism of the Diels–Alder cycloaddition of anthracene nitrostyrene-type diene with a maleic anhydride or maleimide dienophile is illustrated in Figure 51.

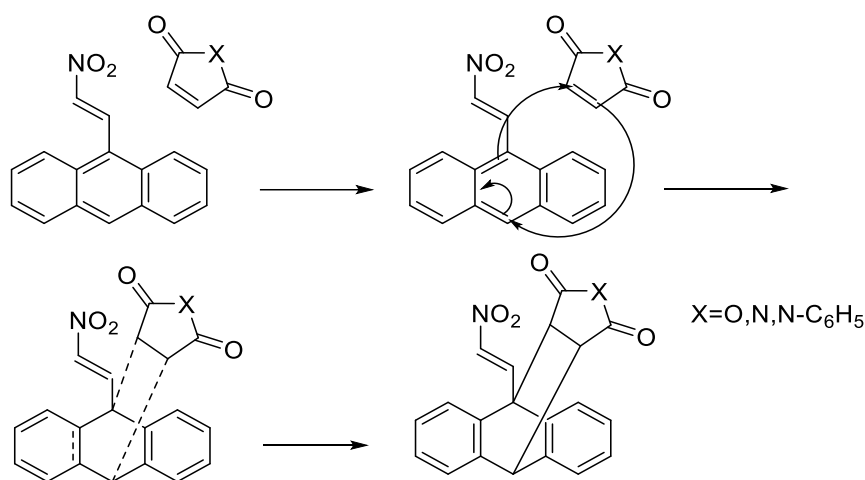


Figure 51: Mechanism of Diels–Alder cycloaddition of anthracene diene core with dienophile system [(*E*)-9-(2-nitrovinyl)anthracene used as representative example]

Normally, there are two relative stereochemical orientations of reactants in the transition state (TS) in relation to the diene, *endo* and *exo*²⁷³ (Figure 52). In the *exo* configurations the dienophile substituent is orientated towards the molecular orbital (MO) of the diene. Its formation is thermodynamically favoured due to steric factors, known from the study of reverse Diels–Alder reactions, where the one carbon atom bridge link in the resulting adduct covers the anhydride ring^{265, 273, 285}. In the greater part of classic Diels–Alder reactions, the *endo* product is to be expected when an electron withdrawing group is part of the dienophile following the empirical Alder rule²⁸⁹. This can be used by chemists as a predictive guide of the stereochemistry of the Diels–Alder (DA) reaction²⁷³. The *endo* TS is more sterically impeded than its *exo* form but is the predominant kinetic product of the reaction as a result of the interaction between the dienophile electron withdrawing carbonyl groups (EWG's) of the dienophiles used and electrons of the double bond being created, lowering the energy of the TS through secondary orbital interactions. However, dipolar and van der Waals interactions are also thought to be involved^{265, 273}.

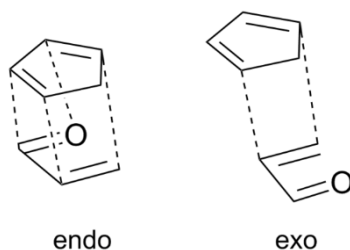


Figure 52: Representation of *endo* and *exo* products that can result from the Diels–Alder reaction of project specific dienes and dienophiles using cyclopentadiene (diene) and acrolein (dienophile) as an example

Apart from the classical thermal approach in various aromatic-based organic solvents such as toluene and xylene, a number of other synthetic modifications for Diels–Alder reactions have been described in the literature. The rate at which the cycloaddition occurs (as well as regio and stereoselectivity) can be accelerated through the use of Lewis acids (e.g. aluminium chloride) in the presence of oxygen-chelating or phosphorous-containing ligands²⁹⁰. To date, use of copper-based complexes and chiral sulfur-containing ligands provide the most selective and efficient Lewis acid catalysed reaction.

However, the use of such reagents in Diels–Alder reaction modification is somewhat limited by the possibility of promoting reactant polymerisation²⁹¹. Furthermore, reaction rates can be increased through the utilization of higher pressures^{292, 293}. Apart from organometallic mediated approaches to the reaction, there has been increasing interest in the past two decades surrounding the discovery and development of enzymatic methods of reaction catalysis, named Diels–Alderase (DAases). Such potential enzymes were first discovered through the biosynthetic studies of Ichicara et al. into phytopathogenic fungi and their respective phytotoxins²⁹⁴. Examples in nature of these enzymes include lovastatin nonketide synthase, which facilitates the intramolecular Diels–Alder reaction to produce lovastatin (a fungal metabolite capable of lowering cholesterol through (3-hydroxy-3-methylglutaryl-coenzyme A reductase [HMG CoA reductase inhibition])). However, despite the directed evolution of selected, catalytic ribozymes and antibodies as a means of Diels–Alder biocatalysis, a *bona fide* Diels–Alderase that has developed through the process of natural selection has yet to be identified to date^{295, 296}.

2.4.3 Synthesis of the maleimide dienophiles

The dienophiles acrylonitrile, maleimide, maleic anhydride, phenylmaleimide and dimethyl acetylenedicarboxylate chosen for the Diels–Alder cycloaddition are commercially available reagents (Figure 53). The *p*-chloro (**22**) and *p*-benzophenone (**23**) based dienophile derivatives (Figure 55) were directly synthesised from 2,5-furandione via an amic acid intermediates as shown in the scheme below using *p*-chloroaniline and (4-aminophenyl)(phenyl)methanone (Figure 54). (**22**) and (**23**) were characterised by ¹H NMR, ¹³C NMR, IR spectroscopy, HRMS and melting point. They were obtained with

fair-good yields of 29% and 55% respectively. These dienophiles were then chosen for use in the subsequent Diels–Alder reactions.

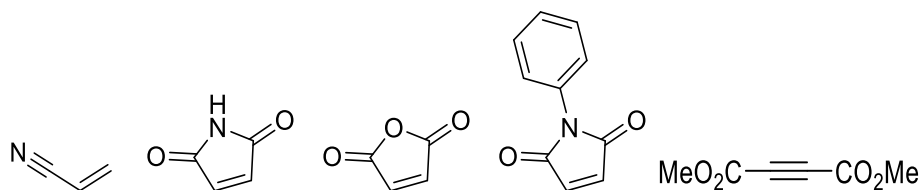


Figure 53: Commercially available dienophiles: acrylonitrile, maleimide, maleic anhydride, N-phenylmaleimide, dimethyl acetylenedicarboxylate

The route of synthesis used to generate the maleimide derivatives required for the subsequent cycloaddition reaction involved the generation of corresponding amic acid intermediates from the respective parent amines in diethyl ether. These intermediates were then allowed to react with sodium acetate and acetic anhydride, resulting in the formation of the related maleimide compound through dehydration and subsequent ring closure. This method of maleimide synthesis was established by American chemist Norman Searle in 1941²⁹⁷ (Figure 54).

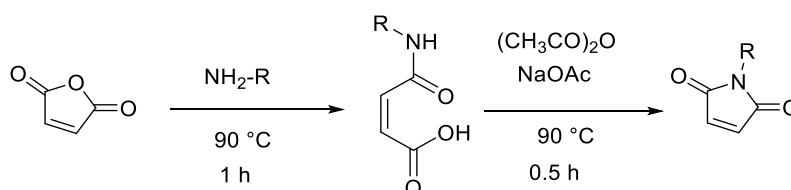


Figure 54: Maleimide derivative synthesis scheme²⁹⁷

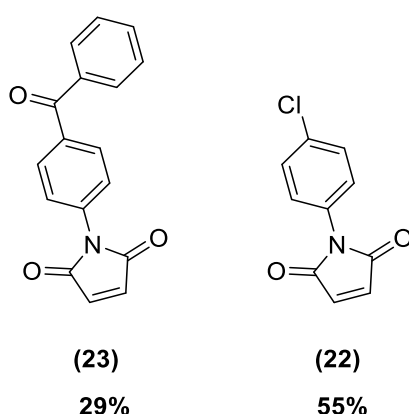


Figure 55: Maleimide dienophile derivatives synthesised and yields obtained

The mechanism for their production (Figure 56) involves a two-step synthetic process. The initial step (Step 1) comprises of nucleophilic attack by an appropriately

substituted amine on the electrophilic charge on the carbonyl group of the maleic anhydride. This results in ring opening of the maleic anhydride molecule, facilitated by the protonation of the oxygen atom on the central ring, with the subsequent formation of a hydroxyl functional group. The corresponding amic acid intermediate is generated.

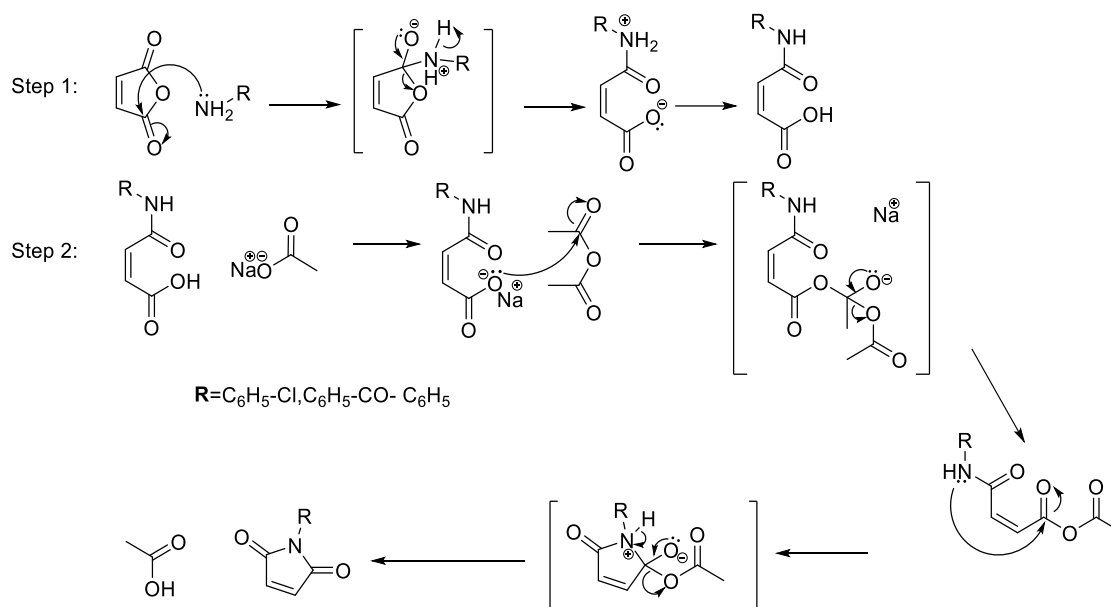


Figure S6: Mechanism for the synthesis of 1H-pyrrole-2,5-dione derivatives from 2,5-furandione (maleic anhydride)

The following step (Step 2) involves the coordination of the sodium ion from sodium acetate with the electronegative oxygen of the acidic functional group, forming an amic acid salt. The net negative charge on the terminal oxygen of the salt then carries out nucleophilic attack on one of the two carbonyl groups present on acetic anhydride, displacing the sodium ion and forming an acetate intermediate. On formation of this intermediate, sodium acetate is then regenerated through an acetate anion leaving group elimination and its consequent association with the free sodium ion. Following the formation of the equivalent acetate derivative, the amine of the amide functional group acts as a nucleophile, attacking the carbonyl closest in proximity to the diene double bond (due to the presence of a favourable leaving group). This results in ring closure, cleaving another acetate group in the process, regenerating the ketone functionality, giving the corresponding maleimide derivative.

Other simple and efficient methods of maleimide generation have also been reported in scientific literature. One such example by Deshpande et al. is the use of phase transfer catalyst tetrabutylammonium bromide (TBAB) in the presence of sodium carbonate and

dimethyl sulfate with excellent yields up to 95%²⁹⁸. Another interesting method is radical mediated dehydration by Garad et al. using ammonium persulfate–dimethyl sulfoxide (APS–DMSO), yielding maleimide products upwards of 90% yield²⁹⁹.

2.4.4 Synthesis of Series 2 ethanoanthracenes by Diels–Alder reaction: (*E*)-9-(2-nitrovinyl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

Based on previous research within the Meegan group with (*E*)-9-(2-nitrovinyl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones in Burkitt's lymphoma (BL) (Chapter 1), a focussed series of the most potent compounds was synthesised for further evaluation and testing in related B-cell malignancy CLL (Table 2). The dienophiles (CC-1A, CC-1B and those commercially available, Figure 53, 55) were reacted with the Series 1: (*E*)-9-(2-nitrovinyl) anthracenes (**5**) and (**6**) generated previously, as outlined in the previous scheme (Figure 51). This afforded the cycloaddition products 9-(*E*)-2-nitrovinyl-9,10-dihydro-9,10-[3,4]furanoanthracene-12,14-diones (**24–28**) in good yields of 30–51% (Table 2), (Figure 57). The products were obtained as solids on isolation and recrystallisation from toluene.

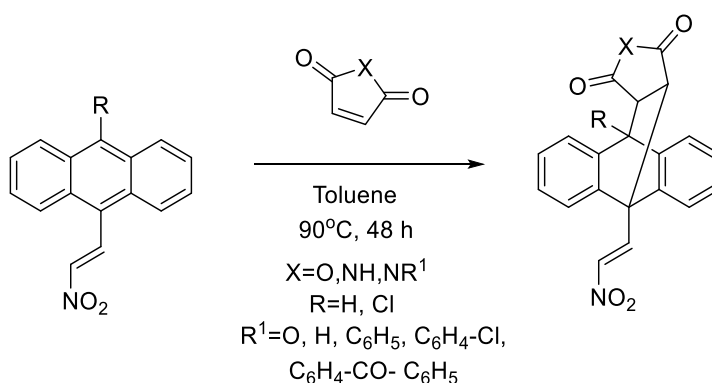


Figure 57: General reaction scheme for the synthesis of (*E*)-9-(2-nitrovinyl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (Series 2)

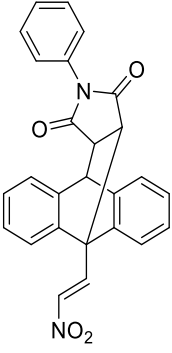
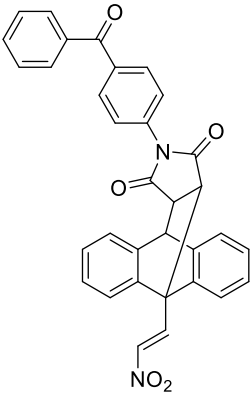
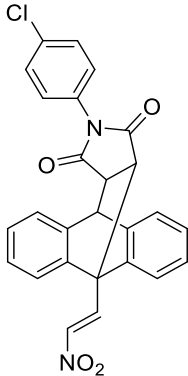
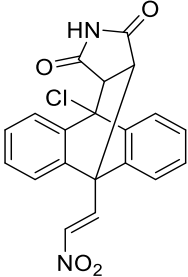
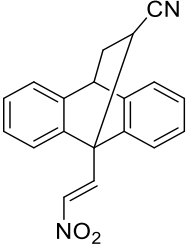
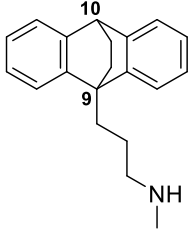
 (24) 30%	 (28) 40%	 (25) 51%
 (27) 50%	 (26) 30%	 Maprotiline

Table 2: (*E*)-9-(2-Nitrovinyl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones synthesised (24-28) (Series 2) with respective chemical yields and maprotiline structure (positions 9 and 10 annotated)

These lead compounds from BL studies bear structural similarity to the tetracyclic antidepressant maprotiline, containing the three main characteristics of an electrophilic functional group chain at position 9 of anthracene-based core, the polyaromatic core itself and the two carbon aliphatic bridge structure linking positions 9 and 10 of the centre ring (Table 2).

2.4.4 Structural characterisation of (*E*)-9-(2-nitrovinyl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**24**)

(*E*)-9-(2-Nitrovinyl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**24**) was chosen as a representative example and the ^1H NMR and ^{13}C NMR spectra will now be discussed in detail. The numbering of the compound (**24**) was generated in ACD/ChemSketch version 12.01. Furthest upfield in the above spectrum (Figure 58), H8 of the ethanoanthracene bridge appears as a double doublet at 3.52 ppm (1H, $J=8.2, 2.7$ Hz). The next signal is a doublet at 3.91 ppm (1H, $J=8.5$ Hz) representing H3, adjacent to the carbonyl functional group at position 2. The remaining proton at 4.93 ppm (1H, $J=2.4$ Hz) was assigned to H7, in the aliphatic region on the centre ring of the anthracene-like core. In the aromatic region, the multiplet observed at 6.39 ppm accounts for the proton pair H21 and H25 of the phenyl ring of the maleimide substituent due to the inductive and electronegative effect of conjugation to nitrogen and two carbonyl groups. The multiplet signal at 7.29 ppm (10H) accounts for the four proton pairs of the anthracene core (H13, H18), (H14, H17), (H15, H16) and (H12, H19). In addition, the remaining proton pair of the maleimide phenyl ring substituent (H22, H24) is accounted for within this signal. The doublet signal at 7.58 ppm (1H, $J=6.7$ Hz) accounts for H23 at the *para* position of the maleimide nitrogen. The doublet downfield at 8.08 ppm (1H, $J=14$ Hz) corresponds to H28 of the nitrovinyl double bond, while the most downfield signal is a doublet at 8.29 ppm (1 H, $J=14$ Hz) representing H27, which suffers greater proton deprotection due to the strong electron withdrawing effect of the nitro functional group. The corresponding coupling constant supports the retention of the (*E*)/*trans* configuration on the nitrovinyl double bond in product formation, which has a typical range of (12-16 Hz)²⁷⁶.

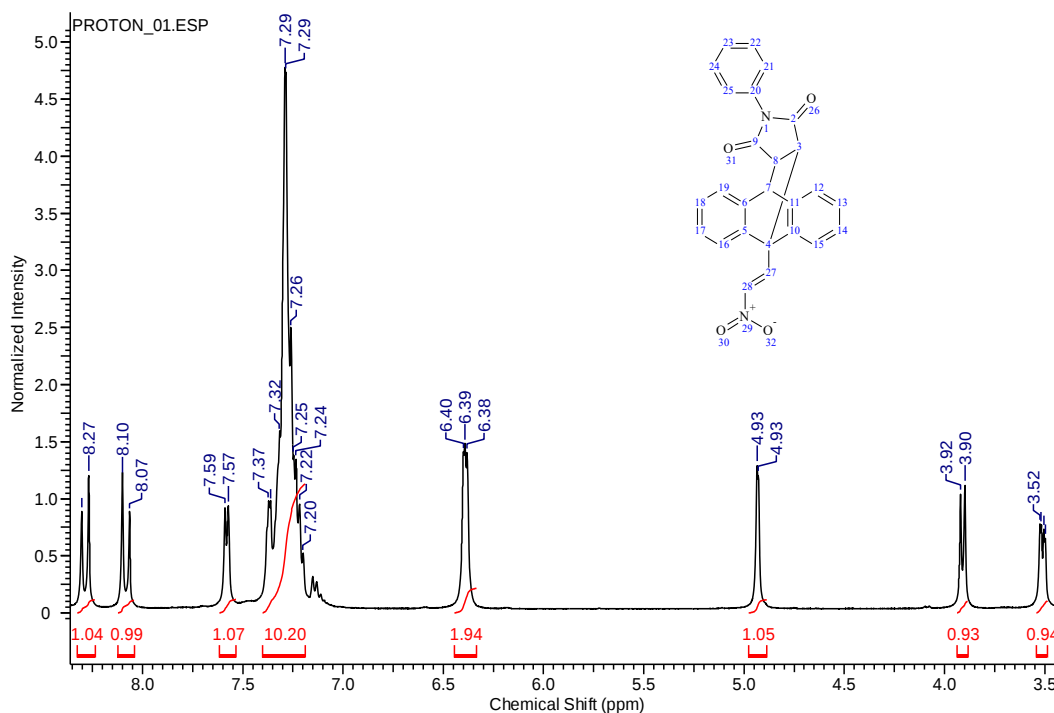


Figure 58: ^1H NMR spectrum of *(E)*-9-(2-nitrovinyl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**24**)

In the ^{13}C NMR spectrum of **(24)** (Figure 59), the signals that appear at 39.9–40.6 ppm are due to DMSO solvent signal. The furthest upfield signal at 45.2 ppm represents C8 of the aliphatic carbon bridge. The signal at 48.1 ppm corresponds to C7 on the centre ring of the anthracene core, while the other remaining carbon of the ethanoanthracene bridge C3 resonates at 48.4 ppm. The remaining signal at 50.1 ppm represents the quaternary carbon C4 on the centre ring of the polycyclic core. Signals at 145.7 and 137.7 ppm are observed for C28 and C27 alkene carbons respectively. C23 is represented by signal at 124.9 ppm due to being on the *para*-substituted phenyl ring of the maleimide substituent. Quaternary carbon C20 was assigned to the signal at 131.9 ppm. The quaternary carbons C5 and C10 were assigned to the signals at 139 ppm. The remaining quaternary carbons C6 and C11 were assigned to 141.4 ppm and 141.7 ppm respectively. The signals at 123.5 ppm and 123.9 ppm correspond to C16 and C13 respectively. The signals at 124.9 ppm and 125.7 ppm are assigned to C12 and C19. The broad signal at 126.9 ppm represents the two carbon pairs of the aromatic rings of the polycyclic core (C14, C17) and (C13, C18). The signals 127.4 ppm and 127.6 ppm were assigned to C21 and C25, while the signal at 127.8 ppm was assigned to C23, in the *para* position to the nitrogen atom of the maleimide group. The final aromatic C-H carbon signals at 129.0 ppm and 129.3 ppm corresponded to C22 and C24 of the maleimide phenyl ring. The

signals appearing at 175.17 ppm and 175.52 ppm represent the carbonyl carbons C2 and C9 respectively.

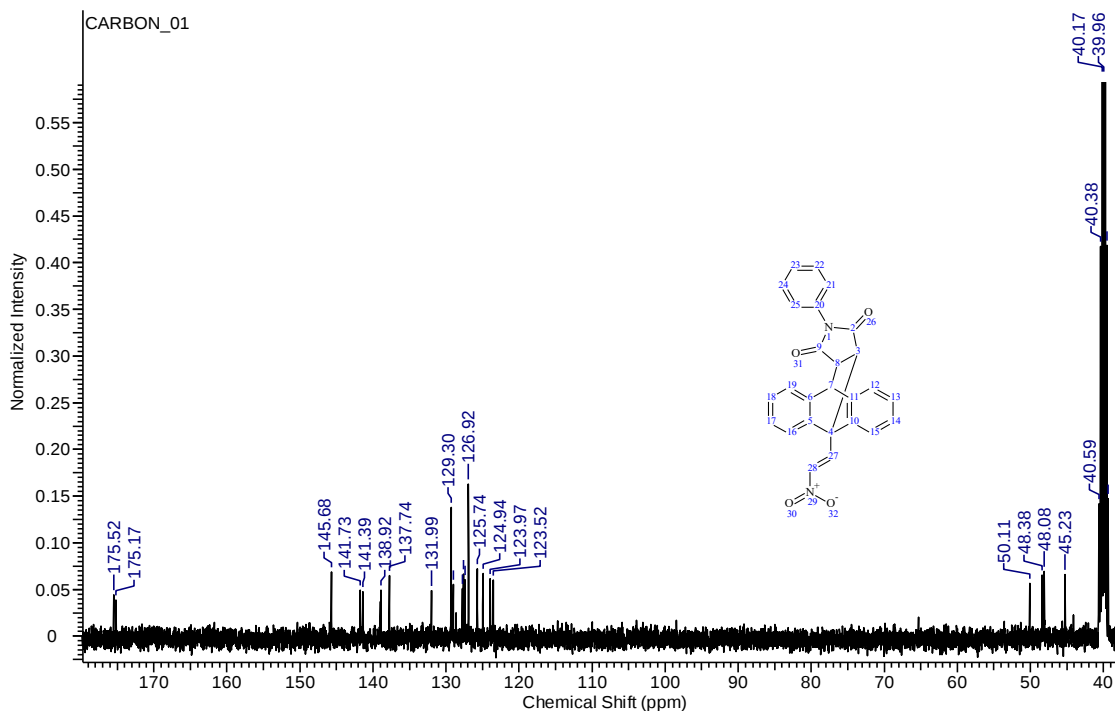


Figure 59: ^{13}C NMR spectrum of (*E*)-9-(2-nitrovinyl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**24**)

The DEPT 90 spectrum (Figure 60) was used in the assignment of the nitrovinyl functional group carbons (C27, C28), the carbons of the ethanoanthracene bridge as well as the aromatic (C-H) carbons of the molecule (C3, C7, C8). In conjunction with the HSQC spectrum (Figure 61), the assignment of each proton signal to its respective carbon was confirmed, along with allowing the identification of quaternary carbon signals (cross-referencing with the DEPT 90 spectrum) (e.g. C4, C20). The assignment of these quaternary carbons was then completed using the crosspeaks from the HMBC spectrum (Figure 62).

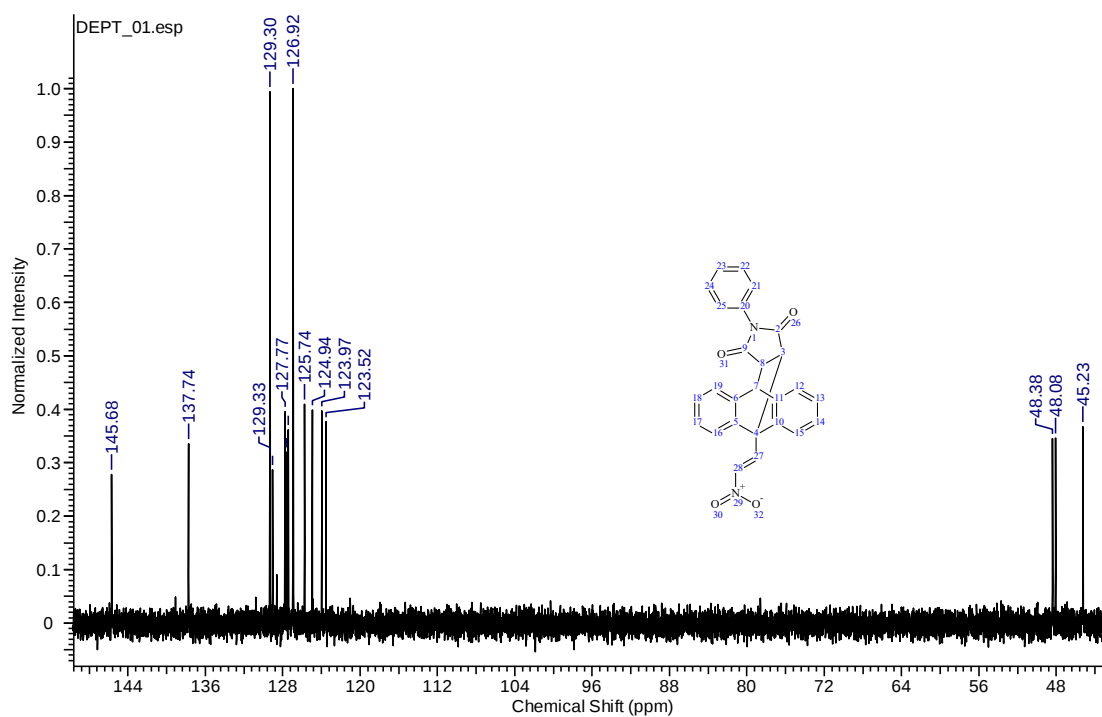


Figure 60: DEPT 90 spectrum of (E)-9-(2-nitrovinyl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (24)

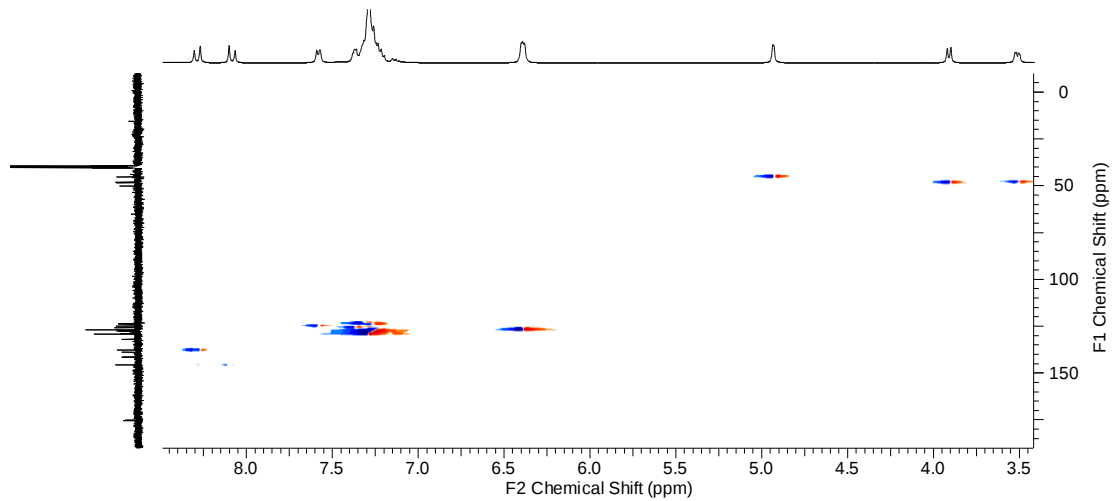


Figure 61: HSQC spectrum of (E)-9-(2-nitrovinyl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (24)

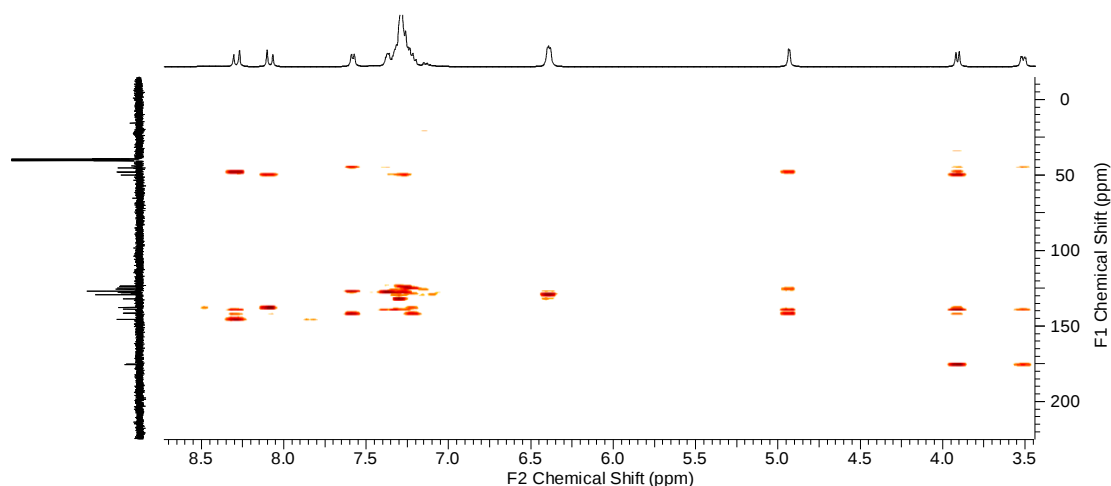


Figure 62: HMBC spectrum of *(E)*-9-(2-nitrovinyl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**24**)

All of our resultant products retain the *trans/E* configuration (see X-ray details of compound (**91**) for confirmation, Figure 108) in adduct form, resulting in high isomeric purity. Hence, chromatographic separation of the products is not required. In addition, due to the symmetrical diene system, the *endo/exo* designation does not apply. Under conventional reaction conditions (e.g heat, agitation), dienophile addition occurs across positions 9 and 10 due to the high localization of π electrons present. However, Smith et al. have conducted *ab initio* molecular dynamics simulations of DA reactions in single-walled carbon nanotubes, suggesting that it would be possible for either 1,4-*endo* or 1,4-*exo* addition products to predominate (dependent on variable such as reactant size and nanotube diameters)³⁰⁰.

2.5 Series 3 : Chalcones

Chalcone-based molecules are an invaluable scaffold structure in medicinal and synthetic chemistry, being extensively studied over the past decade³⁰¹. Their usefulness in traditional medicine dates back hundreds of years with many vegetables (potatoes, bean sprouts), fruits (apples, citruses) and roots such as liquorice which contains isoliquiritigenin being used for the management of chronic conditions e.g.cancer and diabetes^{302, 303}. Coming from the broad biosynthetic family of phenolic compounds, chalcones are the biosynthetic precursor to all flavonoids^{167, 304}. Such useful secondary

plant metabolites occur in nature through the sequential action of the shikimic acid, pentose phosphate and phenylpropanoid pathways³⁰⁵.

Chalcones (1,3-diphenyl-2-propen-1-ones) can exist in one of two main configurations either *trans* (*E*) or *cis* (*Z*). This depends on the geometry of the two aromatic rings in relation to the alkene double bond of the characteristic three carbon α,β -unsaturated ketone linker between them (Figure 63). The *E* isomer tends to be the most thermodynamically favourable of the two possible configurations, due to the often strong steric forces at play in the *Z* isomers^{304, 306}. As a compound class, they are known to exhibit a wide variety of biological activities, some of the foremost being antioxidant, anti-inflammatory, anti-cancer, anti-microbial and anti-malarial^{307, 308}.

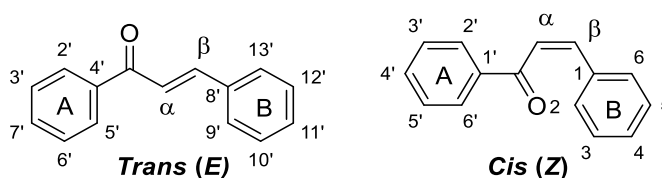
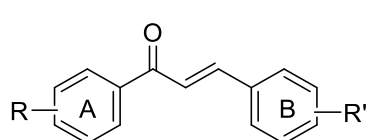


Figure 63: General structure and notation of chalcones (1,3-diphenyl-2-propen-1-ones)

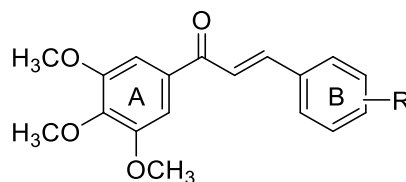
2.5.1 Biological activity of Chalcones

Salum et al. describes the antiproliferative and antimitotic activity of colchicine-based chalcone compounds based on two distinct molecular modelling structures using the Protein Data Bank X-ray crystal structures of tubulin-colchicine and tubulin-ABT751 complexes (PDB IDs 1SA0 and 3HKC). Type 1 where substitution was varied on both rings A and B; Type 2 where ring A retained a 3,4,5-trimethoxy substitution and only ring B varied (Figure 64). A range of cancer cell lines *in vitro* including L1210 (murine acute lymphoblastic leukaemia cells), human acute lymphoblastic leukaemia cells (REH and JURKAT), human umbilical vein endothelial cells (HUVEC) and NIHT3 fibroblast cells were examined together with tubulin assembly inhibition³⁰⁹. Analogues containing the 3,4,5-trimethoxy substitution pattern on ring A adjacent to the carbonyl group were among the most potent in both biochemical assays.



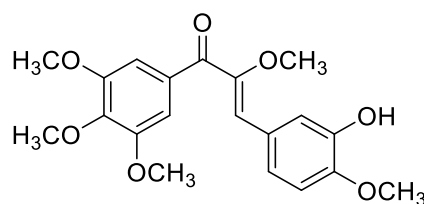
Type 1 chalcones

R=R'=H
 R=3-OH, -OCH₃, Ring B=2-naphthyl
 R=(OCH₃)₃, Ring B=2-naphthyl
 R=(-O-CH₂-O-), R'=3,4,5-(OCH₃)₃



Type 2 chalcones

R=4-N(CH₃)₂, 3,4-Cl₂, 4-Cl, 4-CH₃-Ph, 2,6-Cl₂,
 4-Br, 4-NO₂, 4-F, 4-O(CH₂)₃CH₃, 3-NO₂, 4-OCH₃,
 2,4,6-(OCH₃)₃, 3,4,5-(OCH₃)₃, 2,4,5-(OCH₃)₃,
 C₅H₆O, 3-CF₃-4-Cl, 2,4-Cl₂, 3,5-Cl₂



SD400

Figure 64: Cytotoxic chalcone structures type 1 (left-where R and R' represent substitution on the aromatic rings A and B) and type 2 (right-where ring A has a fixed 3,4,5-trimethoxyphenyl moiety, with only ring B being subject to variation in functional groups (top); antiproliferative chalcone variant compound SD400 (bottom))³⁰⁹⁻³¹²

Furthermore, Ducki et al, reported the successful design and synthesis of potent nanomolar antiproliferative chalcone-variants such as compound SD400 (Figure 64), with antiproliferative IC₅₀ activity as low as 0.23 nM being observed in the chronic myeloid leukaemia (CML) K-562 cell line³¹⁰. Such compounds were found to arrest cell mitosis in the G₂/M phase and noted to operate through competitive inhibition of tubulin assembly through tritium-labelled colchicine binding experiments^{311, 313}. Similar success was also observed on retention of the 3,4,5-trimethoxyphenyl of ring A and substitution of the *N,N*-dimethylamino group on ring B, resulting in cytotoxicity IC₅₀ values as low as 0.5 μM and microtubulin arrest concentration of 2.8 μM with selective pharmacological toxicity (peripheral blood mononuclear cells [PBMC] cytotoxic IC₅₀ 92.5 μM)³⁰⁹. Interestingly, a recent study by Dong et al. identified that benzochalcone derivatives [including compounds (29) and (30)] (Figure 65), using α-naphthoflavone (ANF) (a 7,8-benzoflavone derivative) as a lead scaffold, could exhibit dual activity as both CYP1B1 selective inhibitors (with IC₅₀ values as low as 4.8 nM). They displayed promising cytotoxicity in both wild type (MCF-7, MDA-MB-231) (IC₅₀ range 1.8-48.1 μM) and drug-resistant breast cancer cell lines (LCC6/P-gp and MCF-7/1B1) with IC₅₀ values ranging from 3.9->100 μM (Figure 65). The main objective of this study was to

reduce the susceptibility of protocarcinogens being activated through CYP450 oxidation, limiting the risk of drug-drug interactions through inhibition of liver enzyme function and enable selective targeting of CYP1B1 and P-glycoprotein overexpressing tumour cells (which have been extensively documented as two main mechanisms of treatment resistance in multiple cancers)³¹² (Figure 65).

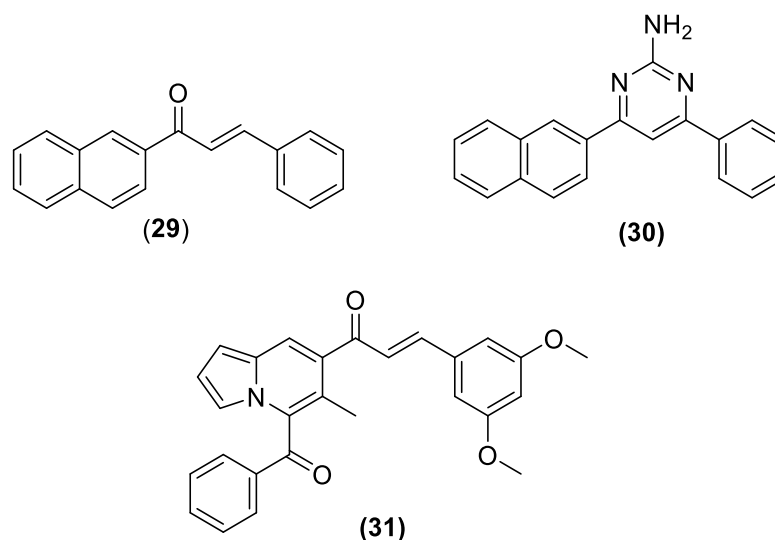


Figure 65: CYP1B1 selective inhibitor structures compound (29) (top left) and compound (30) (top right), Indazoline-chalcone hybrid anticancer agent compound (31) (bottom)³¹⁴

Similarly, this approach of molecular hybridization (combination of known, key pharmacophore elements with the view of creating a beneficial, synergistic clinical effect beyond the reach of either pharmacophore alone) with chalcone based functional groups to exploit multiple pharmacological targets and/or synergise anticancer activity can be observed in research reported by Park et al., where indazoline (an isomer of indole and core structure of the chemotherapy drug camptothecin) is used to synthesise novel indolizine-chalcone hybrid (Figure 65) **(31)**. Of the potent compounds observed, with collective IC_{50} values $< 2 \mu\text{M}$, compound **(31)** shown above was chosen as a potential anticancer lead scaffold. Annexin V/ PI staining in conjunction with caspase 3/7 assays supported a mechanism of caspase-dependent apoptosis in U937 lymphoma cells treated with compound **(31)**³¹⁴ (Figure 65). Furthermore, chalcone-based molecular structures have been shown to have promising activity as anti-inflammatory agents and potential therapies for inflammation mediated disease pathogenesis and progression such as in atherosclerosis and Alzheimer's disease^{301, 315}. Pandey et al. describe butein (a tetrahydrochalcone) is capable of $\text{Nf-}\kappa\text{B}$ inhibition and attenuation of $\text{Nf-}\kappa\text{B}$ related gene expression through interaction with the IKK [inhibitor of nuclear factor- κB (I κB)

kinase] complex, which serves as the master regulator of the inflammation cascade^{316, 317}. Chalcone-like structures exert this effect through the covalently modifying these inflammatory regulator proteins, which is thought to occur through Michael addition of the Cys residue 179 to the α,β -unsaturated ketone group. Furthermore, Cai et al. found that a similar anti-inflammatory response could be elicited in BV2 microglial cells *via* IKK inhibition and nuclear translocation in the Nf- κ B signalling pathway by chalcone adducts such as **(32)**³¹⁸ (Figure 66).

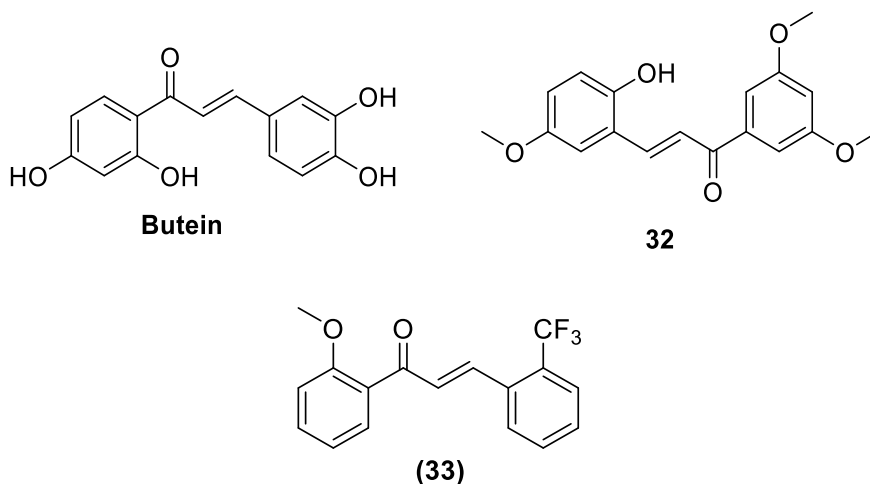


Figure 66: (top left) Chemical structures of Butein, (top right) **(32)** and (bottom) compound **(33)**-examples of chalcone-based Nrf2 modulators^{316, 318}

Moreover, the potential of chalcones to influence various diseases through disruption of constitutively activated pathological inflammatory responses *via* the Keap1-ARE-Nrf2 (Kelch-like-ECH associated protein 1 pathway-Antioxidant Response Element-Nuclear factor erythroid 2 related factor 2) has also been observed. Electrophilic agents have been documented to induce release of Nrf2 through covalent modification of the negative regulator Keap1 cysteine residues, subsequent protein complex conformational change and Keap1 degradation^{319, 320}. One such example reported by Kumar et al. is a series of novel fluorochalcones acting as potent Nrf2 activators both *in vitro* and *in vivo* in mice and human lung epithelial cells, the lead compound being compound **(33)** (Figure 66), leading to an approximate 5-fold up-regulation of Nrf2-dependent antioxidant genes such as NQ1(NAD(P)H:quinone oxidoreductase 1) and GCLM (glutamate-cysteine ligase modifier subunit)³²¹. Other potential targets proposed for chalcones as anticancer agents include receptor tyrosine kinase (RTK) inhibition mediated through VEGF and EGFR along with aldosereductase (ALR2) inhibition, preventing sorbitol synthesis in glucose metabolic pathway³²².

2.5.2 Chalcone synthesis

Claisen–Schmidt condensation

The Claisen–Schmidt condensation was first documented by German chemists Rainer Ludwig Claisen and J.G. Schmidt who independently published on this discovery in 1881³²³. The general classification to which this specific chemical reaction belongs is known as the aldol reaction, a classic C-C bond forming method where the α -carbon of one aldehyde or ketone molecule reacts with the carbonyl carbon of another (Figure 67).

The initial step of the reaction is the generation of a finite concentration of nucleophilic enolate from the aldehyde or ketone reagent. Its formation is normally catalysed by base addition (hydroxide being the most widely employed); however, acid catalysed versions also exist e.g. TiCl_4 or HCl gas^{324, 325}, the former utilised by Mahrwald et al., with alcohols such as ethanol (diluted or as the sole solvent) employed as the reaction medium (Figure 67). It can also be regarded as a cross-condensation as it involves the formation of C-C bond between two different molecular species³²⁶. Due to the presence of excess non-enolized aldehyde/ketone and thus intact carbonyl groups, the enolate will undergo nucleophilic addition to the electrophilic carbonyl carbon, resulting in the generation of the corresponding alkoxide anion. The negative charge on the resultant alkoxide is then protonated by a water molecule or other protic solvent (created from aldehyde/ketone enolization) to form the final “aldol” or β -hydroxy aldehyde/ketone product, with this step also being subject to dynamic equilibrium. This is so-called due to the presence of a hydroxyl group at the β -position relative to the carbonyl group on the molecule^{276, 327}. In addition, this product can then undergo dehydration easily in basic media to yield its respective α,β -unsaturated carbonyl form, partly facilitated by the conjugation of the carbonyl group with the $\text{C}=\text{C}$ double bond, resulting in a favourable hydroxide leaving group (Figure 67).

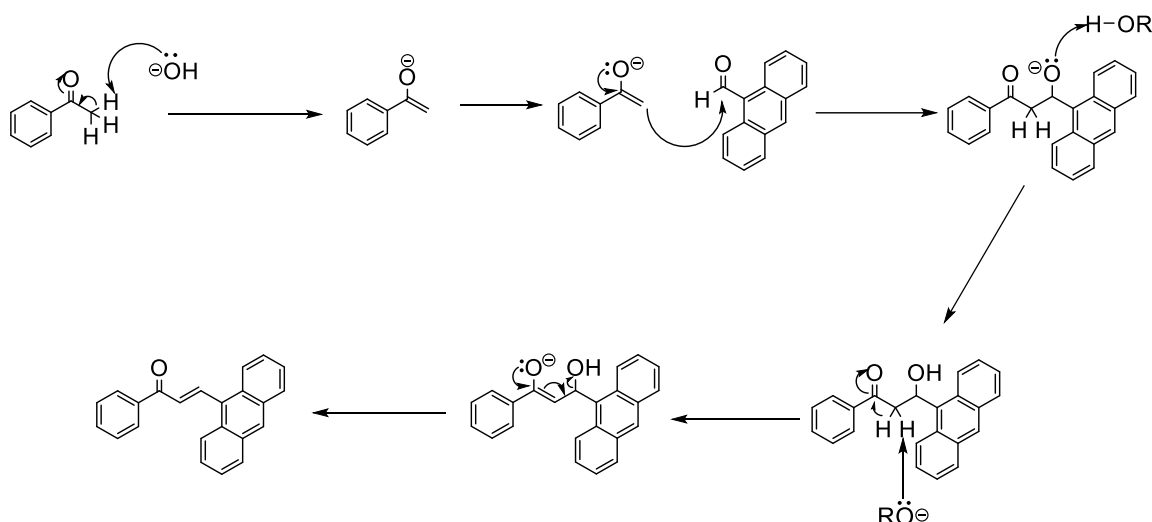


Figure 67: Mechanism of Claisen-Schmidt reaction to synthesise (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-one

The transition from this stabilised anionic intermediate is defined as the rate-limiting step, with the elimination being unimolecular (typical of E1 mechanisms) and from the conjugate base (cB) as opposed to the molecule's original state; the process is known as an E1cB elimination reaction^{328, 329}. Thus, it represents a viable route to the production of corresponding α,β -unsaturated aldehyde and ketone products.

In general, aldol reaction condensation side-products can occur from self-condensation of both aldehydes and ketones due to the presence of multiple α -hydrogens. However, due to only one partner compound being enolisable and aldehydes being more electrophilic than ketones, cross-condensation is preferred over self-condensation and no by-products are observed²⁷⁶. Furthermore, such mixed aldol reactions drive equilibrium to the right-hand side or product formation (through non-reversible dehydration reaction), making them a feasible means of generating α,β -unsaturated ketones³²⁶. Due to the potential for such difficulties mentioned above, alternative cross-coupling reactions have been explored for chalcone synthesis such as Suzuki coupling, Wittig reaction, Julia-Kocienski as well as Friedel-Crafts acylation³³⁰⁻³³³. A select few examples will now be briefly outlined.

Suzuki-cross coupling (Figure 68) is a powerful palladium catalysed carbon coupling reaction and was first discovered by Nobel Prize winning chemist Akira Suzuki in 1979 whereby two potential synthetic approaches leading to the same chemical product can be taken (each variant having a respective pair of an organoborate and a halide)^{330, 334}. With optimum reaction conditions such as McCarthy's conditions [anhydrous toluene,

tetrakis(triphenylphosphine) palladium; CeCO_3], yields of approximately 90% can be achieved with high (*E*) selectivity³³⁵.

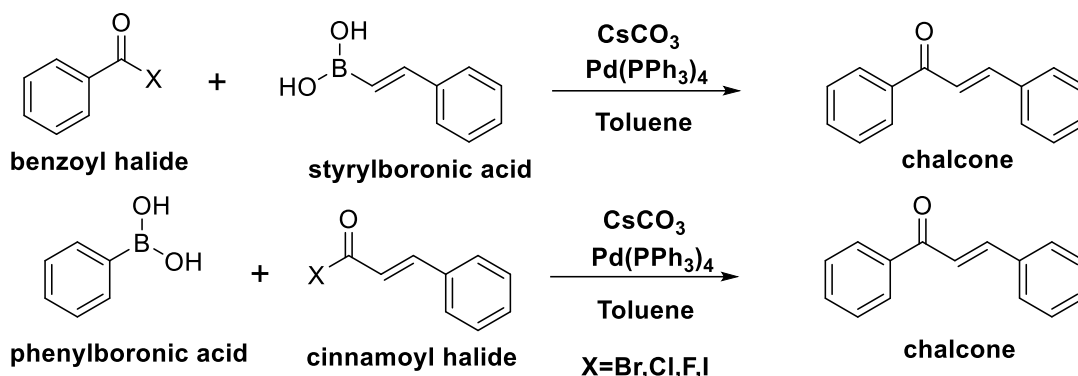


Figure 68: General reaction scheme for the Suzuki reaction for chalcone synthesis

The Wittig reaction (Figure 69) is another useful method for chalcone structure generation. Discovered in 1954 by Nobel Prize winning chemist Georg Wittig, the Wittig reaction allows for the formation of an alkene from the reaction of an aldehyde or ketone with a phosphonium salt, facilitated by a basic environment (e.g. NaH , NEt_3).^{301, 336} The alkene configuration is predominantly governed by the reactivity of the respective ylide formed, with stable ylides favouring *E* isomer formation³³⁷.

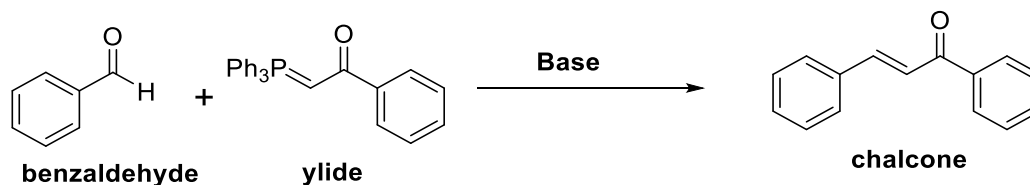


Figure 69: General reaction scheme for the Wittig reaction for chalcone synthesis

2.5.3 Series 3: Synthesis of (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-ones

Based on the previous research conducted within our laboratory and a thorough examination of available academic literature, it was decided to explore chalcone alternatives to the nitrovinyl group (located at C9 of the anthracene core) of our initial lead compounds (Series 1 and 2) principally due to potential nitrovinyl group genotoxicity and mutagenicity arising from biological metabolites³³⁸. In doing so, chalcones represented a viable option due to conservation of the majority of the SAR identified by McNamara et al.¹¹⁶, ease of synthesis and modification, previously

documented activity in leukaemia cell lines³⁰⁷ in addition to supporting molecular modelling studies using MOE v2016.08 (Figure 70).

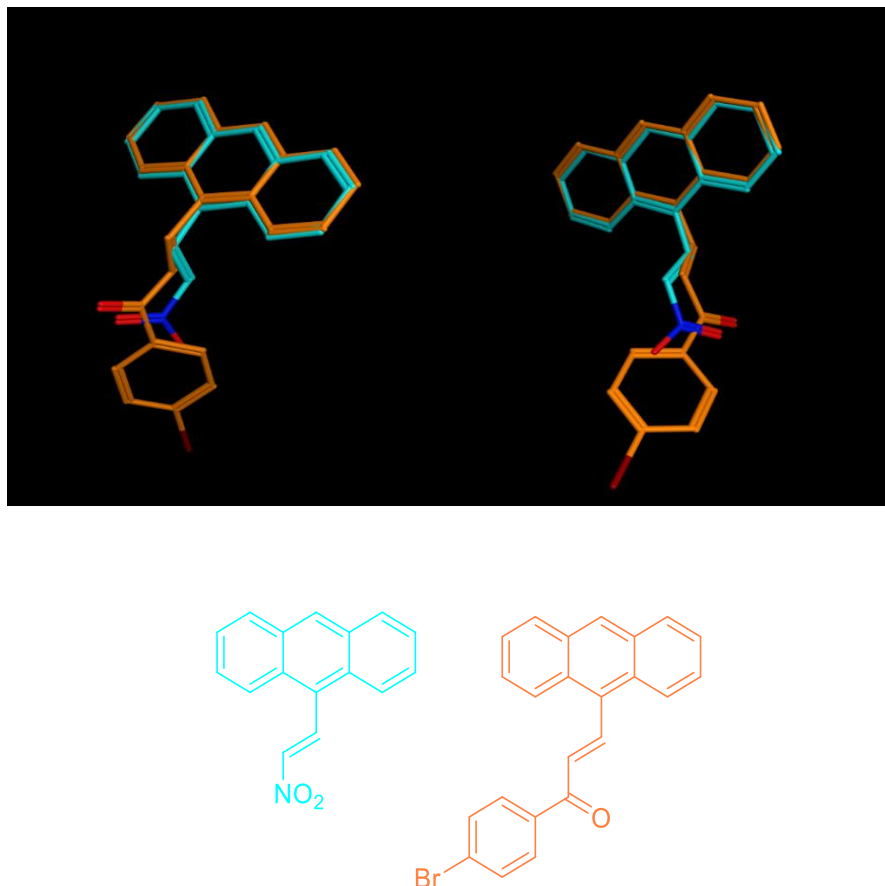


Figure 70: Flexible alignment of (*E*)-9-(2-nitrovinyl)anthracene (**1**) and (*E*)-3-(anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (**34**); atom colours: oxygen (red), nitrogen (blue), bromine (brown)

The lowest energy conformation of (*E*)-9-(2-nitrovinyl) anthracene (**1**) and an example of the anthracene chalcones (*E*)-3-(anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (**34**) were flexibly aligned to demonstrate the similarities between both compounds respective molecular features. Good complementarity can be seen between the planar anthracene core and vinyl bond structures. The spatial projection of the nitro and carbonyl groups is also shown to correlate well, retaining the electron withdrawing inductive effect and hydrogen-bond acceptor (HBA) on the wider conjugated structure, noted as a key factor in selective targeting of CLL over healthy white blood cells, represented experimentally using Peripheral Blood Mononuclear Cells (PBMCs)³³⁹.

In the present work, a series of (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-ones (Series 3) (**34-50**) was prepared by means of a Claisen–Schmidt condensation reaction between 9-anthraldehyde and a series of substituted aromatic and heterocyclic acetophenone derivatives in the presence of sodium hydroxide (Figure 71, Tables 3,4 and 5). The reaction was optimised by increasing the scale of the reaction from 2.5 mmol to 5 mmol, allowing for good to excellent yields in addition to easy purification through recrystallisation from ethanol (adapted from methods used by Hassan et al., Larionov et al. and Arshad et al.)³⁴⁰⁻³⁴².

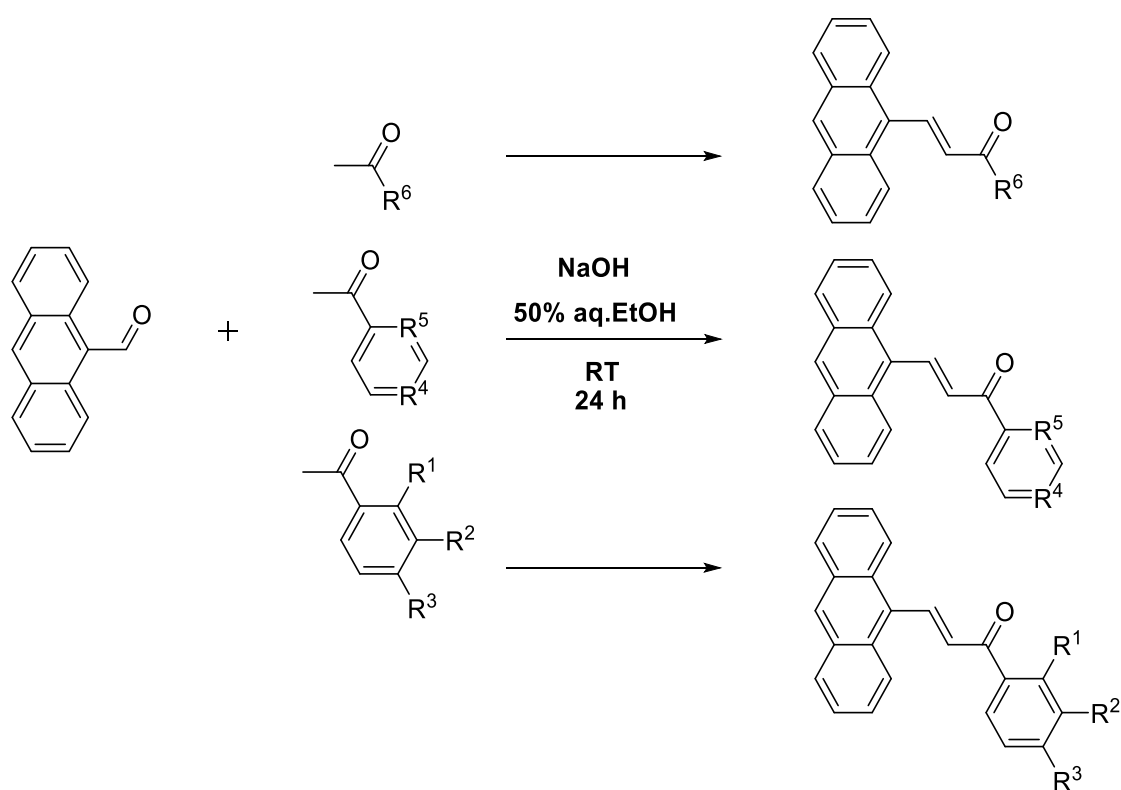


Figure 71: General reaction scheme for the synthesis of (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-ones

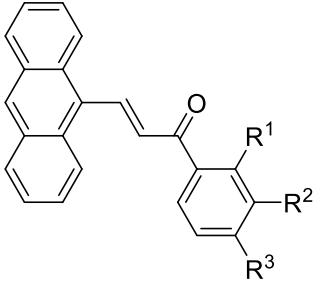
												
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)	No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(34)	H	H	Br	-	-	65	(40)	H	H	I	-	70
(35)	H	H	NO ₂	-	-	78	(41)	H	H	F	-	88
(36)	H	H	CH ₂ CH ₃	-	-	61	(42)	H	H	OCH ₃	-	58
(37)	Cl	H	Cl	-	-	75	(43)	H	H	CH ₃	-	61
(38)	H	OCH ₃	OCH ₃	-	-	85	(44)	OCH ₃	OCH ₃	OCH ₃	-	86
(39)	H	H	H	-	-	44	(45)	H	H	Cl	-	65

Table 3: Type 1 chalcones (34-45) (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-one library (Series 3)

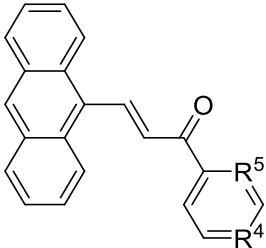
						
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)
(46)	-	-	-	N	H	48
(47)	-	-	-	H	N	58

Table 4: Type 2 chalcones (46, 47) (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-one library (Series 3)

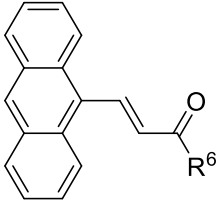
					
No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(48)	-	-	-	C ₄ H ₃ O (furan)	67
(49)	-	-	-	C ₁₀ H ₇ (Naphthyl)	38
(50)	-	-	-	C ₄ H ₃ S (thiophene)	86

Table 5: Type 3 chalcones (48-50) (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-one library (Series 3)

Three main structural types of acetophenones were used in the synthesis of the Series 3 chalcones. The first structural class (type 1) (Table 3) contained diverse substitution of the aryl ring in both position and atom type including halogens (fluorine, bromine, chlorine), alkyl (methyl, ethyl), methoxy groups and unsubstituted benzoyl ring. The second class (type 2) (Table 4) had 2 and 4-pyridyl rings. The third class (type 3) (Table 5) included five membered heterocycles which were introduced using the relevant acetyl compounds in the case of the furan and thiophene structures. In addition, a 2-naphthyl structure, introduced by the use of its corresponding acetyl derivative. Moderate to very good yields of 38-88% were recorded in the synthesis of the (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-one library with a view to their main use as diene intermediates for corresponding ethanoanthracene derivative synthesis *via* Diels–Alder reaction (Tables 3-5). A lower yield of 38% with the naphthyl chalcone derivative could have resulted from the steric bulk of the naphthyl functional group impacting on the reaction rate.

2.5.4 Structural characterisation of (E)-3-(Anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (34)

(E)-3-(Anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (**34**) was chosen as a representative structure for this series and its ^1H NMR and ^{13}C NMR spectra will now be discussed. The numbering of the compound (**34**) was generated in ACD/ChemSketch version 12.01. The ^1H NMR (400 MHz, CDCl_3) spectrum of (**34**) (Figure 72) firstly shows a distinctive doublet signal at 8.80 ppm furthest downfield integrating for one proton. This corresponds to H15 of the alkene functional group, resonating higher than its adjacent counterpart H16 due to the inductive effect of the carbonyl at position 17, increasing its electrophilicity. The coupling constant measured confirms the presence of *E* /*trans* alkene configuration ($J=15.8$ Hz), given the typical *E* configuration coupling constant range of 12-16 Hz²⁷⁶. The lone singlet at 8.46 ppm integrating for a single proton was assigned to the uncoupled proton H10. This occurs due to lack of directly adjacent protons. The following doublet signal at 8.27 ppm integrating for two protons was assigned to the protons H3 and H11. The multiplet signal appearing at 8.02 ppm integrating for two protons was assigned to protons H6 and H14. The doublet signal observed further upfield at 7.93 ppm, integrating for two protons represents the protons of the benzoyl aromatic ring, H20 and H24, having a higher chemical shift due to the inductive effect of the adjacent carbonyl group. The multiplet appearing at 7.64 ppm, integrating for two protons was assigned to the remaining two protons of the acetophenone-derived functional group, H21 and H23. The remaining multiplet signal centred at 7.50 ppm integrating for five protons, four assigned to the remaining protons on the anthracene core H1, H2, H12 and H13, while the remaining proton accounts for H16 adjacent to the carbonyl group. These appear among the aromatic protons due to the partial negative charge on the carbon atom C16, allowing greater shielding of H16.

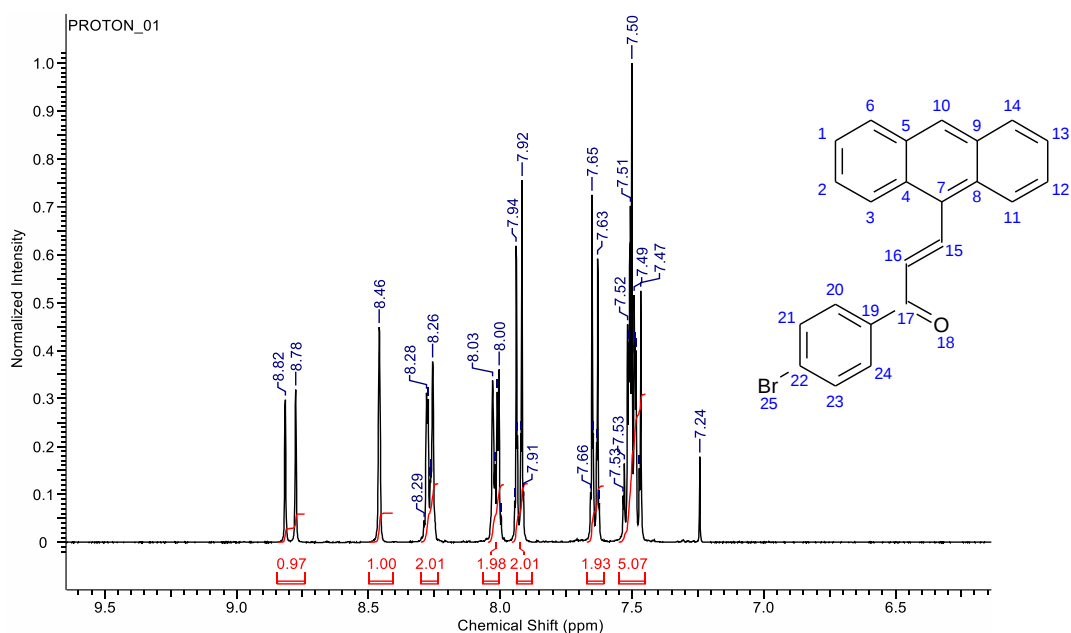


Figure 72: ^1H NMR spectrum of (E)-3-(anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (**34**)

The ^{13}C NMR (101 MHz, CDCl_3) spectrum (Figure 73) for the compound (**34**) showed a signal at 142.5 ppm which corresponds to C15 of the bond, resonating further downfield due to the influence of the electron withdrawing carbonyl group. In contrast, C16 is observed at 130.4 ppm due to the electropositive nature caused by the carbonyl carbon dipole. Hence, the electrons of the bond shield C16 to a greater degree. C10 of the centre anthracene ring of the polycyclic core is represented by 128.6 ppm. The signal at 125.2 ppm denotes the carbon pair (C3, C11) while the signal 128.9 ppm represents the carbons (C6, C14) at the unsubstituted end of the anthracene core. The signal at 130.2 ppm denotes carbons C20 and C24 of the 4-bromoacetophenone-derived functional group, shifted downfield due to the strong inductive effect of the carbonyl group. The remaining carbons C21 and C23 of the benzoyl ring adjacent to the bromine atom correspond to the signal at 132.1 ppm due to both the inductive effect of carbonyl group and the halogen atom at the *para* position of the aromatic ring. The remaining C-H carbon pairs (C1, C13) and (C2, C12) of the anthracene core are represented by signals at 125.5 ppm and 126.5 ppm respectively.

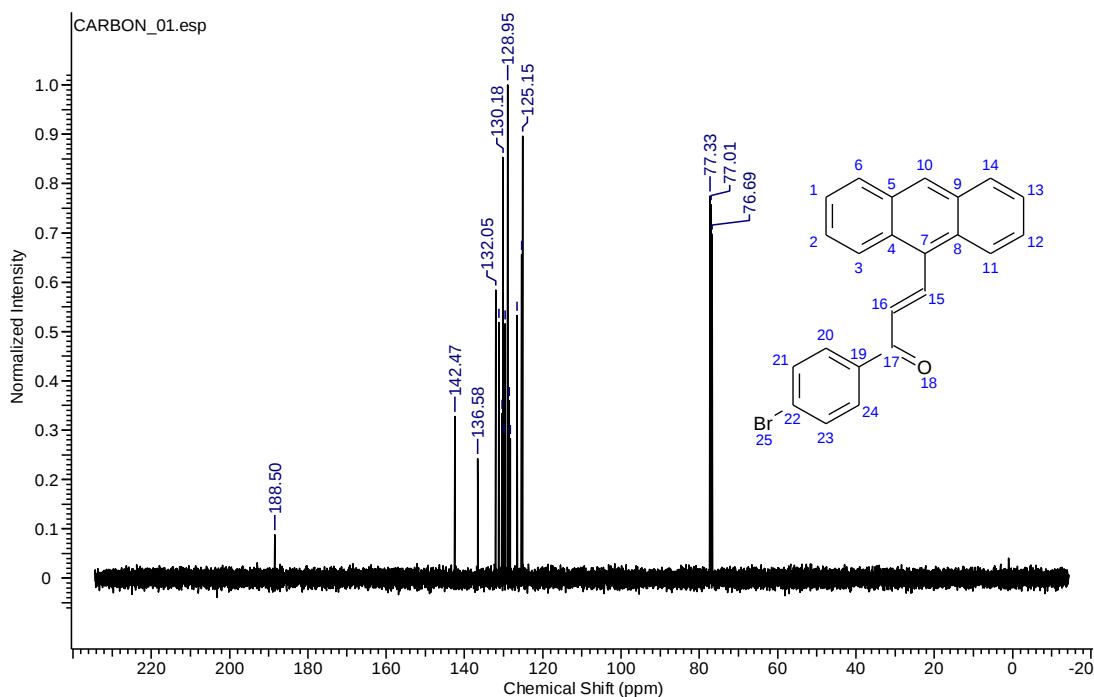


Figure 73: ^{13}C NMR spectrum of (*E*)-3-(anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (34)

C22 adjacent to the bromine atom is represented by the signal at 128.3 ppm and C19 is observed at 136.6 ppm due to being directly adjacent to the carbonyl carbon. The quaternary carbons C4 and C8 correspond to 129.6 ppm on the substituted side of the anthracene core. The carbon atoms C5 and C9 were assigned to the signal at 131.3 ppm. The most downfield signal at 188.5 ppm represents the carbonyl carbon C17. The assignment of the alkene signals, the polycyclic anthracenene core and the benzoyl aromatic ring were assisted through the use of the DEPT 90 and HSQC spectra (Figures 74 and 75). The HMBC spectrum was utilized for the assignment of quaternary carbons as well as confirming previous signal designations of protons and associated carbons (Figure 76).

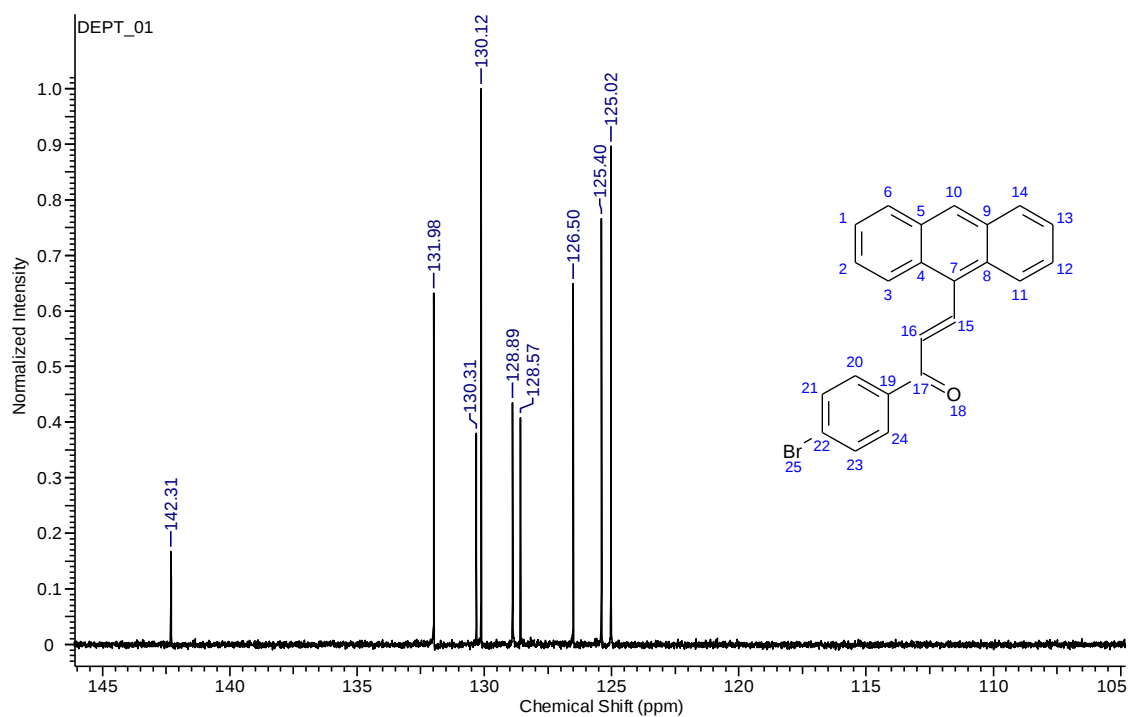


Figure 74: DEPT 90 spectrum of (E)-3-(anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (34)

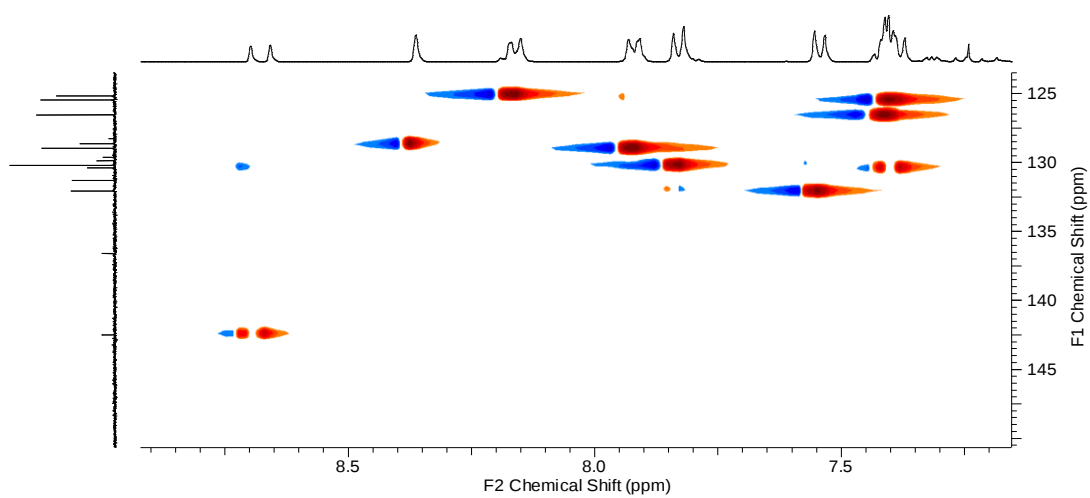


Figure 75: HSQC spectrum of (E)-3-(anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (34)

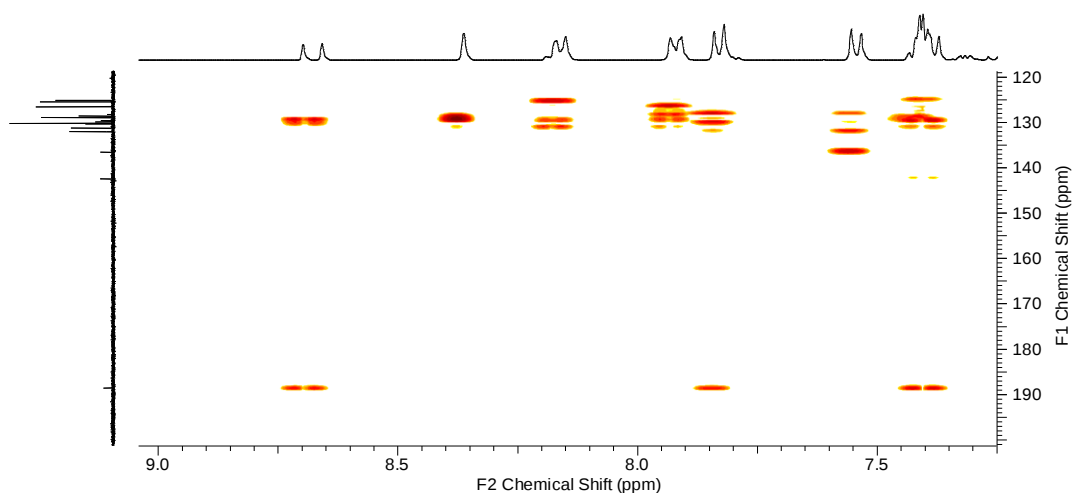


Figure 76: HMBC spectrum of (*E*)-3-(anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one (**34**)

2.5.5 X-ray crystallography of chalcones

In the present work the formation of *E* / *trans* configuration alkene double bond in the chalcone products (**34-50**) was confirmed by NMR structural elucidation by determining the associated coupling constants (discussed in structural characterisation example) and by X-ray crystallography of (**44**) [(*E*)-3-(anthracen-9-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one], a representative compounds of the series (Figure 77, Table 6). The crystal packing was found to be monoclinic in nature. The bond length of C₁₅-C₁₆ was consistent with that of an alkene double bond (1.336 Å) and the bond length of C₁₇-O₁₈ was consistent with a carbonyl functional group (1.228 Å)^{343, 344}. The presence of the alkene double bond is also supported through the bond angles of C₁₅-C₁₆-C₁₇ and C₁-C₁₅-C₁₆ which were 120.1° and 124.6° respectively, indicating the trigonal-planar nature expected of such a functional group side-chain.

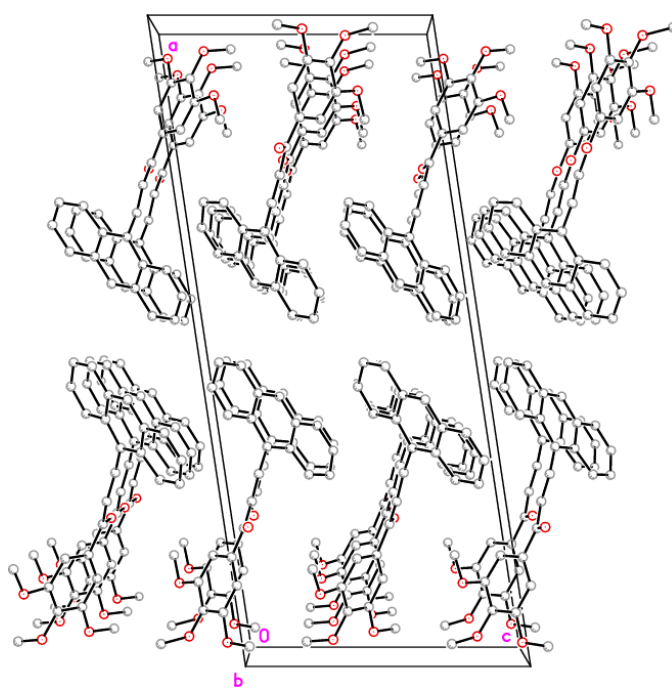
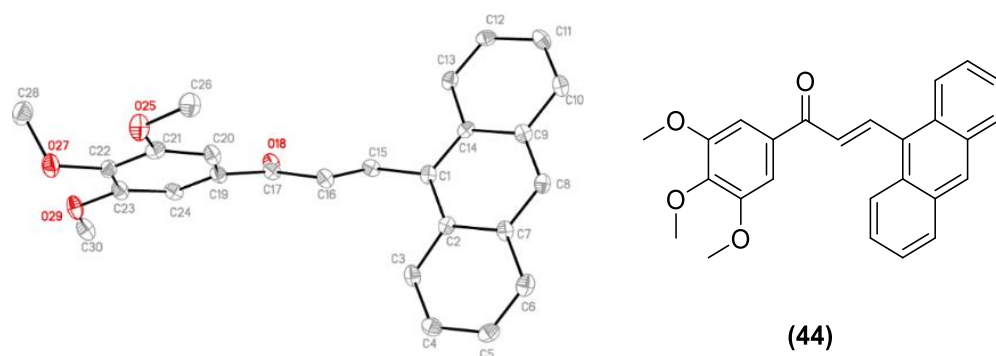


Figure 77: X-ray structure of (*E*)-3-(anthracen-9-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**44**) and corresponding crystal packing arrangement

Empirical formula	C ₂₆ H ₂₂ O ₄
Formula weight	398.43
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2 ₁ /c
Unit cell dimensions	a = 29.099(3) Å b = 5.4348(5) Å c = 12.6086(11) Å $\alpha = 90^\circ$ $\beta = 98.582(3)^\circ$ $\gamma = 90^\circ$
Volume	1971.7(3) Å ³
Z	4
Density (calculated)	1.342 Mg/m ³
Absorption coefficient	0.090 mm ⁻¹
F(000)	840
Crystal size	0.26 x 0.13 x 0.04 mm ³
Theta range for data collection	2.832 to 25.596°.
Index ranges	-35 ≤ h ≤ 35, -6 ≤ k ≤ 6, -15 ≤ l ≤ 15
Reflections collected	18897
Independent reflections	3698 [R(int) = 0.0772]
Completeness to theta = 25.596°	99.4 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7452 and 0.5974
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	3698 / 0 / 274
Goodness-of-fit on F²	1.122
Final R indices [I > 2σ(I)]	R1 = 0.0754, wR2 = 0.1683
R indices (all data)	R1 = 0.1046, wR2 = 0.1803
Largest diff. peak and hole	0.282 and -0.334 e.Å ⁻³

Table 6: Crystal data details and structural refinement for (44) (*E*)-3-(anthracen-9-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one

Interestingly, on slow evaporation over a number of weeks from ethanol, one anthracene chalcone was observed to undergo conversion of the alkene double bond to the *Z* / *cis* configuration. This phenomenon was captured with compound (*Z*)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-one (*cis* 40) from single crystal X-ray analysis (Figure 78, Table 7). The crystal packing structure was orthorhombic in nature. Evidence for the alkene double bond was confirmed by the observed bond length C₁₁-C₁₀ (1.332 Å) (within the range of similar structures reported previously³⁴⁵) and the bond angles of C₁₂-C₁₁-C₁₀

and C₁₁-C₁₀-C₈ which were 128.5° and 124.1° respectively indicating the trigonal-planar nature expected of such a functional group side-chain. The crystal data details and structural refinement are presented in report below (Figure 78).

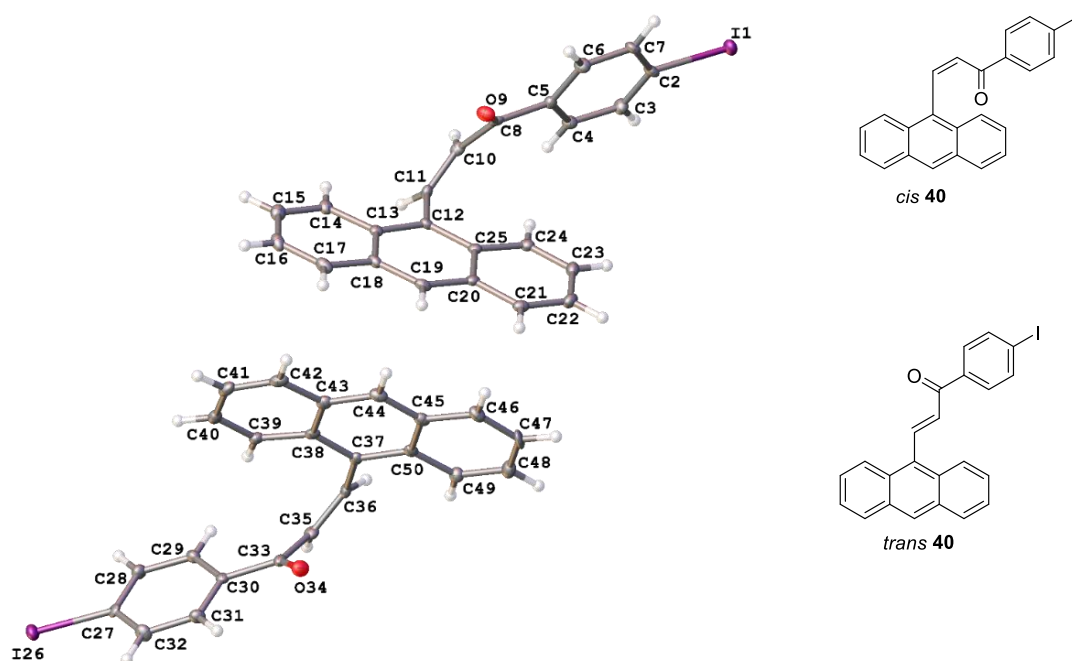


Figure 78: X-ray structure of (Z)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-one and corresponding crystal packing arrangement; structures of *cis* 40 and *trans* 40

In addition, the beginning of the isomerisation process can be noted from the above ¹H NMR spectrum of (E)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-one (**40**) (Figure 79) after approximately two days in ethanol (exposed to sunlight). Evidence of the beginning of this process can be observed with the appearance of a weak doublet signal at 8.33 ppm (*J*=8.7 Hz), typical in both chemical shift and coupling constant of a *cis*/*Z* chalcone structure²⁷⁶, while the predominant configuration is *trans*/*E* with the presence of a strong doublet signal at 8.80 ppm (*J*=15.8 Hz).

Empirical formula	C ₂₃ H ₁₅ IO
Formula weight	434.25
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	Pca2 ₁
Temperature	100(2) K
Unit cell dimensions	a = 10.8908(4) Å b = 10.4764(4) Å c = 30.3908(12) Å α = 90° β = 90° γ = 90°
Volume	3467.5(2) Å ³
Z	8
Density (calculated)	1.664 Mg/m ³
Absorption coefficient	1.854 mm ⁻¹
F(000)	1712
Crystal size	0.29 x 0.22 x 0.19 mm ³
Theta range for data collection	2.698 to 27.182°
Independent reflections	7641 [R(int) = 0.0217]
Completeness to theta = 25.242°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7455 and 0.6848
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	7641 / 1 / 446
Goodness-of-fit on F²	1.123
Final R indices [I>2σ(I)]	R1 = 0.0187, wR2 = 0.0467
R indices (all data)	R1 = 0.0195, wR2 = 0.0472
Absolute structure parameter	0.323(18)
Largest diff. peak and hole	0.846 and -0.579 e.Å ⁻³
Independent reflections	7641 [R(int) = 0.0217]
Completeness to theta = 25.242°	99.7 %
Absorption correction	Semi-empirical from equivalents

Table 7: Crystal data details and structural refinement for (Z)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-one (*cis* 40)

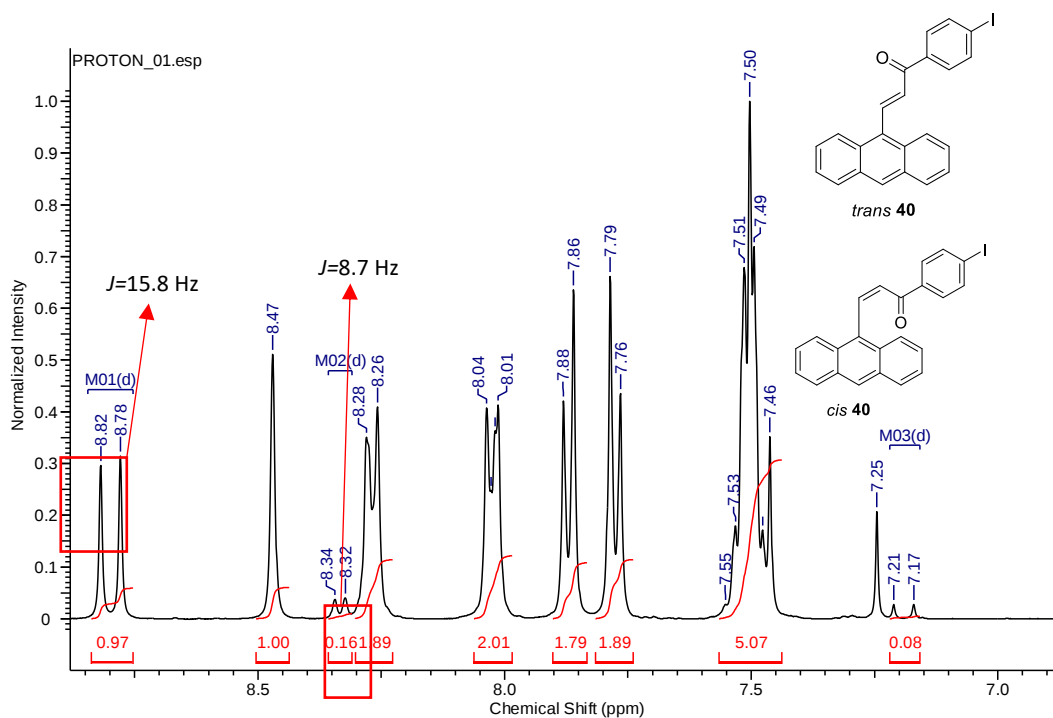


Figure 79: ^1H NMR spectrum of *(E)*-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-one (**40**)

As *trans* chalcones are more thermodynamically and sterically stable, few studies exist on the synthesis and characterisation of *cis*-chalcones. However, Yoshizawa et al. used the generation of siloxyallene intermediates at -78°C and subsequent treatment with sulfuric acid and dimethoxyethane to synthesize *cis* chalcones at both high yields (up to 85%) and excellent diastereomeric purity (99% *cis*/ 1% *trans*)^{301, 346}.

2.5.6 Reduction of chalcones via Luche reduction: Synthesis of *(E)*-3-(anthracen-9-yl)-1-phenylprop-2-en-1-ols

The reduction of selected chalcones was next examined. The basis of this chemical transformation was to investigate whether a change to the electrophilicity of the chalcone-like moiety (through the reduction of the carbonyl group) would have an appreciable effect on biological activity in CLL.

In the present work, a sodium borohydride-mediated reduction of the previously synthesised chalcone products (**34** [Br], **40** [I] and **40** [F]), using the lanthanide salt cerium (III) chloride to favour the 1,2-reduction of the carbonyl group of the enone over the 1,4-reduction of the chalcone double bond in an alcohol solvent. This modification of the traditional carbonyl reduction is known as a Luche reduction. This specific reaction

method was chosen as traditional borohydride mediated reductions of α,β -unsaturated ketones tend to produce a mixture of 1,2 and 1,4-reduction products^{276, 347}.

The Luche reduction was first reported in 1978 by Jean Louis Luche, a French chemist renowned for his contributions to both synthetic organic and sonochemistry³⁴⁸. Through the use of this selective Lewis-acid catalyst, the acceleration of the rate of borohydride methanolysis and subsequent *in situ* formation of multiple sodium methoxyborohydrides was achieved. With these “harder” reduction agents, [following from the HSAB (hard and soft acids and bases)/Pearson acid-base concept], more selective 1,2-reduction is facilitated with high regioselectivity and reported conversion yields up to 100%³⁴⁹. This Luche reduction provides excellent product yields and selective reduction of the carbonyl functional group, leaving the alkene double bond intact^{350, 351}.

The mechanistic basis for the Luche reduction can be divided into two main sequences. The initial step involves the coordination of the cerium cation to the lone pairs of electrons on the alcohol hydroxyl group. In doing so, the resulting inductive effect creates a electrophilic charge on the hydroxyl proton, hence making it more electrophilic in nature (Figure 80, 81). The borohydride anion then captures the proton, leaving a localised negative charge on the alcohol oxygen atom. Due to the instability of the resultant product, hydrogen gas is then released and the normal valency of the boron atom restored through the generation of borane BH_3 . The coordinated alkoxide then carries out nucleophilic attack of the borane, regenerating the free cerium cation in the process, leading to three possible alkoxyborohydrides due to the extent of alkyloxy attack on the borane molecule, resulting in mono, di or tri-alkoxyborohydrides^{350, 351} (Figure 80, 81).

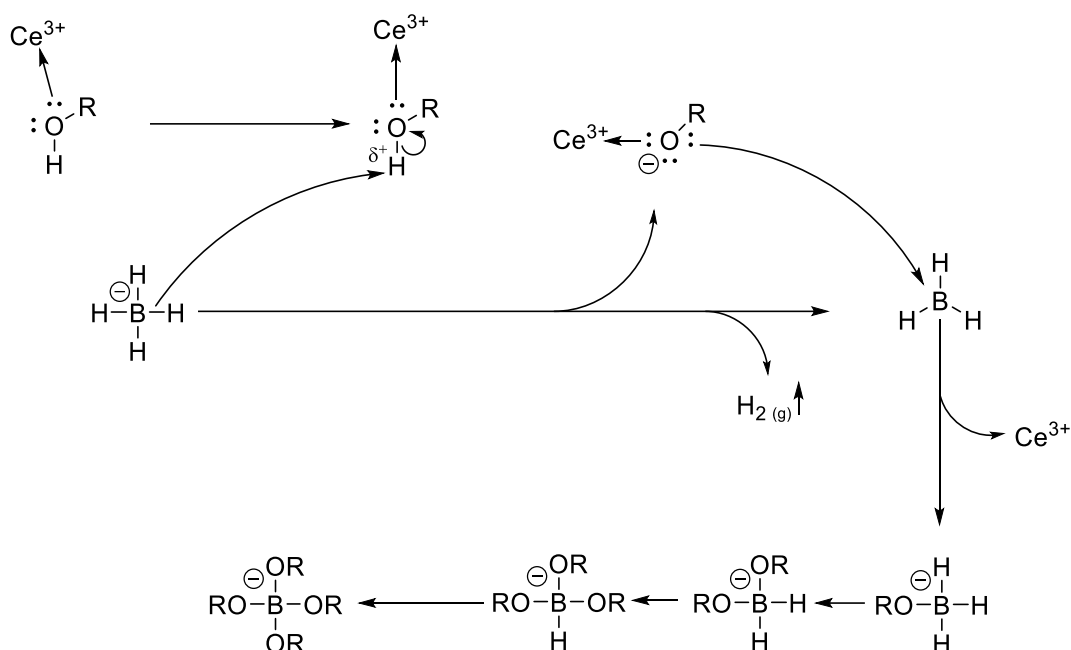


Figure 80: Mechanism for the formation of the alkoxyborohydride reducing agents^{350, 351}

With the generation of the alcohol-lanthanide coordination reagent, the electrophilic charge on the proton of the molecule undergoes nucleophilic attack from a lone pair of electrons of the enone carbonyl oxygen. The removal of the proton results in the alkoxy coordination intermediate and leads to a positive charge on the carbonyl oxygen due to oxygen valency being exceeded (Figure 80, 81). In order to neutralise this positive charge, electrons from the carbonyl π -bond move up onto the oxygen, making the carbonyl carbon highly electrophilic and facilitating its nucleophilic attack by the negative charge of the alkoxyborohydrides present in the reaction medium. This allows the carbon to maintain its valency through the addition of a proton adjacent to the now hydroxyl group, allowing for the formation of stable alcohol derivative. With this, the alkoxyborane by-product of the reaction then reacts with the coordinated alkoxide to regenerate the free cerium cation and one of the three active alkoxyborohydride reducing agents. A fourth inactive structure can also be formed in both steps of the reaction, whereby all possible covalent bond sites are occupied by alkyloxy groups, hence it cannot contribute to the reaction as a hydride source.

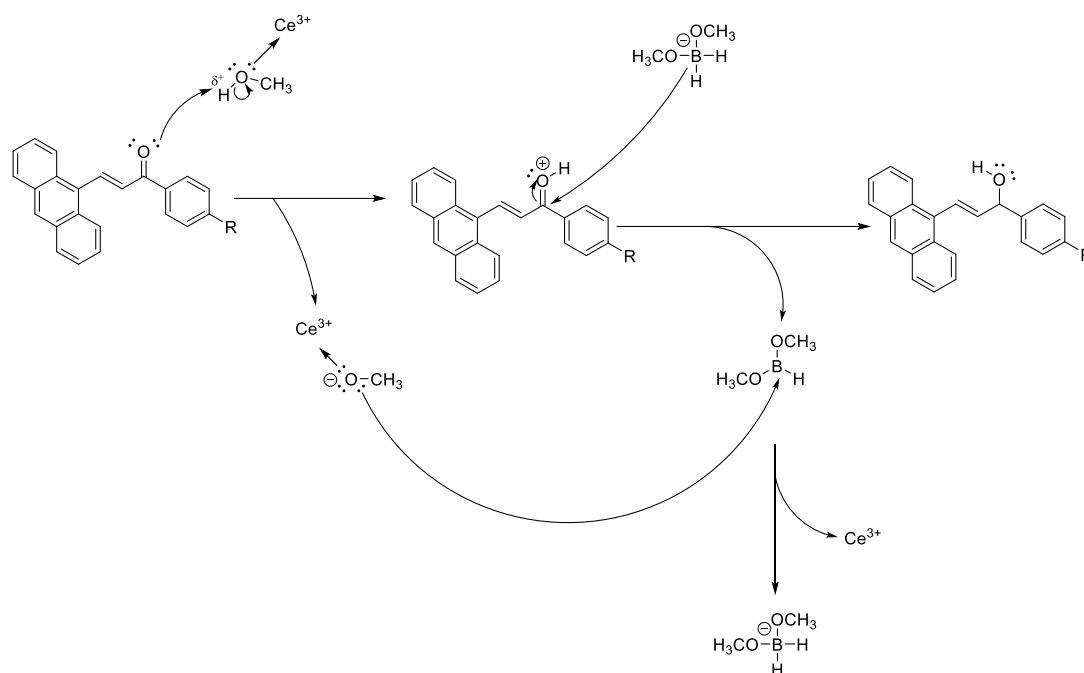


Figure 81: Mechanism for the synthesis of *(E)*-3-(anthracen-9-yl)-1-phenylprop-2-en-1-ol derivatives

Other documented variations on carrying out the Luche reduction include the use of partially rehydrated alumina by Jones-Mensah et al. resulting in high regio and stereoselectivity of up to 90% for the 1,2-reduced enone product with a view to reducing reaction complexity and cost³⁵². Forkel et al. have also used an alternative route through the use of calcium mediated reduction of α,β -epoxy ketones, with excellent yields of up to 85% and very good diastereoselectivity³⁵³. The use of zinc as a means of reaction catalyst has also been reported by Nakata et al., resulting in high stereoselectivity and product yields up to 87%³⁵⁴. Such stereoselective generation of functionalized alcohols plays a valuable role in producing easily modifiable intermediates for both natural product and synthetic organic chemistry³⁵⁵.

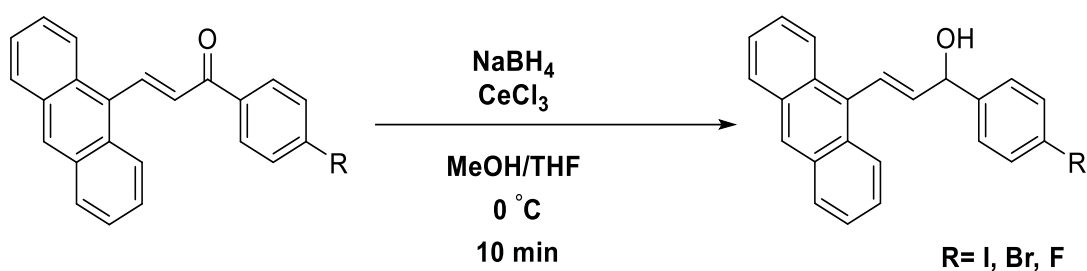


Figure 82: Reaction scheme of enone reduction *via* the Luche reduction mechanism

Compound	R	(%) Yield
(51)*	Br	78%
(52)*	F	73%
(53)*	I	96%

Table 8: (*E*)-3-(anthracen-9-yl)-1-phenylprop-2-en-1-ol derivatives synthesised (where * denotes a novel compound)

Figure 82 shows the general reaction scheme for the reduction and Table 8 shows the structures of the alcohol products produced, all of which are novel compounds. Excellent yields ranging from 73-96% were obtained and were obtained as solids without the need for further purification.

2.5.7 Structural characterisation of (*E*)-3-(Anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-ol (53)

(*E*)-3-(Anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-ol (**53**) was chosen as a representative sample of the series and its ^1H NMR (Figure 83) and ^{13}C NMR spectra (Figure 84) will now be discussed. The numbering of the compound (**53**) was generated in ACD/ChemSketch version 12.01. The spectrum of (**53**) shows the presence of an OH (H9) doublet at 2.20 ppm, integrating for one proton which is coupled to (H8). The

multiplet at 5.62 ppm was assigned to the H8, attached to the alcohol carbon, integrating for one proton. The double doublet signal at 6.21 ppm ($J=16.2, 6.2$ Hz, 1H) was assigned to the vinyl proton (H10), resonating further upfield than H11 due to the electron donating effect of the hydroxy group. The resulting coupling constant also supports the preservation of the *E* configuration of the double bond through the carbonyl reduction reaction²⁷⁶. The next doublet signal was assigned to H3 and H5 on the aryl-ring 7.33 ppm ($J=8.3$ Hz, 2H). The multiplet signal at 7.44 ppm (5H) accounts for the four proton signals of H15, H17, H21 and H24 at the top and bottom of the anthracene core and the remaining vinyl proton signal H11. The doublet signal at 7.75 ppm ($J=8.3$ Hz, 2H) represents the proton pair (H2, H6) on the aryl substituent, resonating further downfield due to the inductive effect of the adjacent iodine atom. The remaining multiplets at 7.98 ppm (2H) and 8.18 ppm (2H) correspond to the proton pairs (H15, H23) and (H16, H22) respectively. The most downfield signal of the spectrum, a broad singlet at (1H) at 8.37 ppm, representing H19 on the anthracene core.

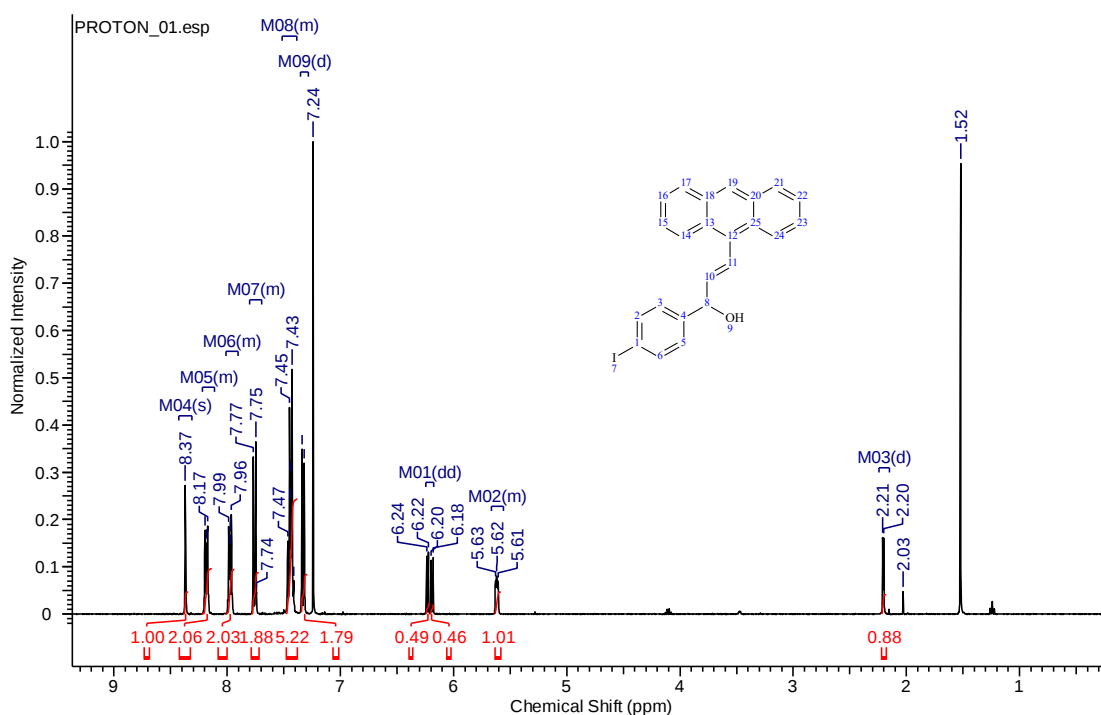


Figure 83: ¹H NMR spectrum of (*E*)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-ol (**53**)

The ¹³C NMR (101 MHz, CDCl₃) spectrum for compound (*E*)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-ol (**53**) showed a signal at 74.9 ppm which corresponds to C8

adjacent to the aliphatic hydroxyl group. The signal at 131.3 ppm refers to the quaternary carbon C12 on the central anthracene ring. The signal of the carbon pair (C2, C6) of the acetophenone ring resonates at 128.3 ppm due to the effect of the adjacent iodine atom. In contrast, C3 and C5 appear at 126.6 ppm. The signal resonating at 126.6 ppm refers to C19 of the central ring of the anthracene core. The carbon pair (C16, C22) corresponds to the signal at 125.6 ppm while the signal at 128.67 ppm refers to the carbon pair (C15, C23) on each side of the polycyclic core. The quaternary carbon C1 appears at 93.4 ppm due to the effect of direct covalent bonding with the iodine atom. The signal of the quaternary carbon pair (C18, C20) appear at 131.5 ppm, while the remaining quaternary carbons C13 and C25 resonate at 129.4 ppm. The signals at 129.4 ppm and 125.1 ppm represent the carbon pairs (C17, C21) and (C14, C24). The highest resonating carbon signal appears at 142.4 ppm and represents the quaternary carbon C4 on the acetophenone-derived aromatic ring. Disappearance of the carbonyl carbon signal at approx. 170 ppm confirms that a successful reduction was achieved (Figure 84).

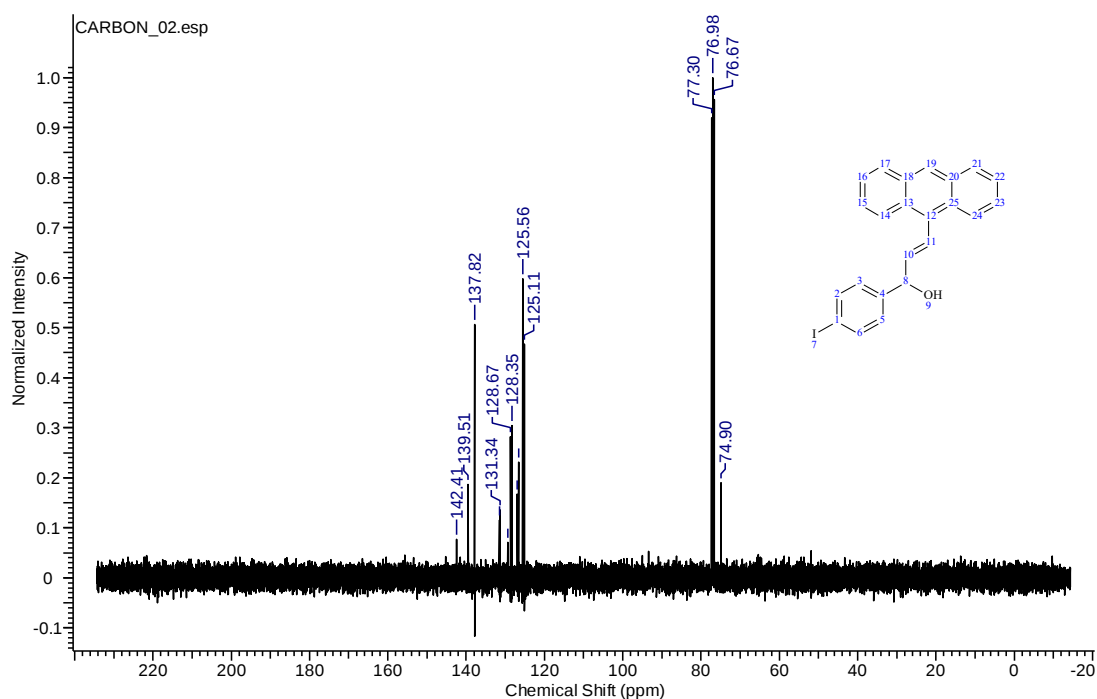


Figure 84: ^{13}C NMR spectrum of (E)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-ol (53)

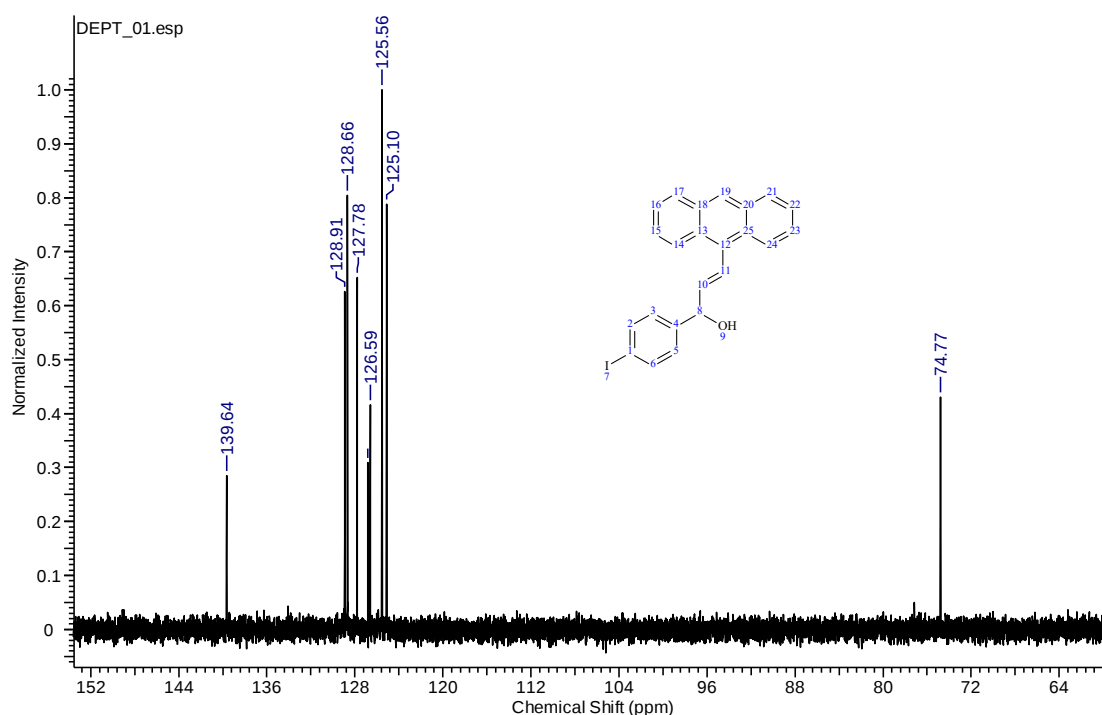


Figure 85: DEPT 90 spectrum of (*E*)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-ol (**53**)

In the structural assignment of the compound, the DEPT 90 spectrum (Figure 85) was used to identify the corresponding carbon signals of previous assigned protons from the ^1H NMR spectrum, the most notable being adjacent to the hydroxyl group and one of two alkene signals. Following this, their assignment was confirmed through the HSQC spectrum (Figure 86) and assignment of remaining the H-C signals was carried out. Quaternary carbons were then allocated to the outstanding signals using the HMBC spectrum (Figure 87). In addition, the mass of (**53**) was confirmed by low resolution mass spectrometry LRMS (APCI) and the required molecular ion was calculated to be $\text{C}_{23}\text{H}_{17}\text{IO} [\text{M}^+]$: 436.03 and found as 436.3.

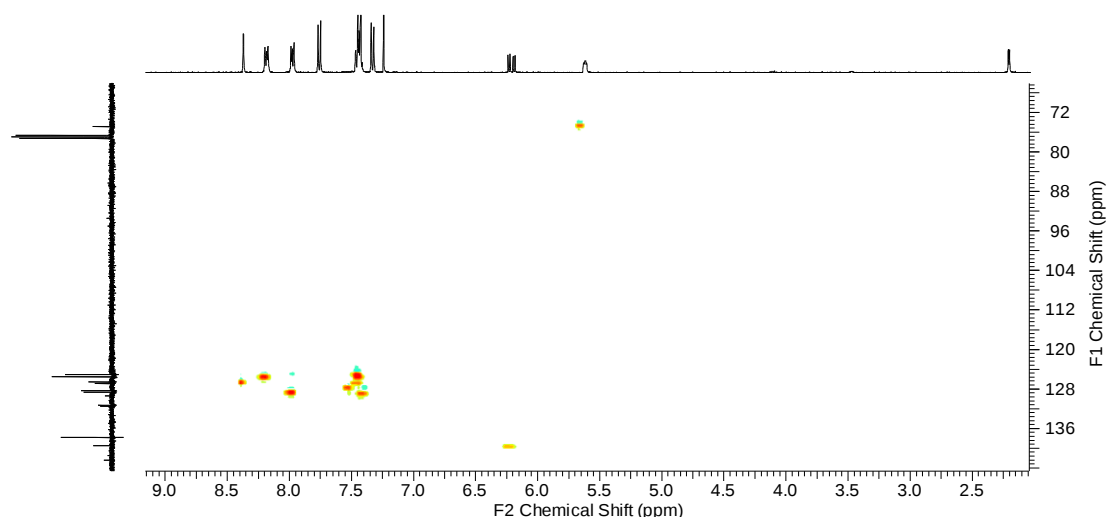


Figure 86: HSQC spectrum of (*E*)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-ol (**53**)

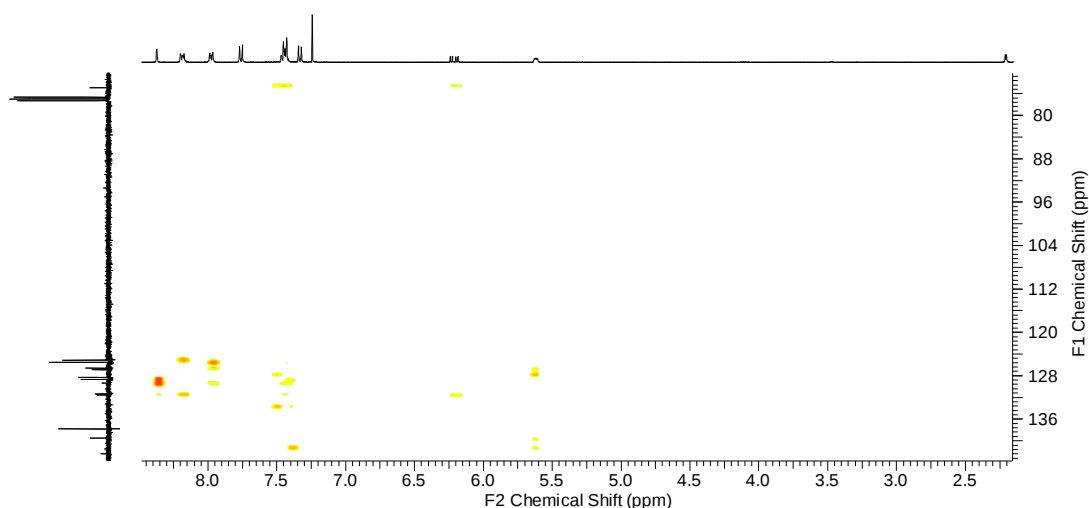


Figure 87: HMBC spectrum of (*E*)-3-(anthracen-9-yl)-1-(4-iodophenyl)prop-2-en-1-ol (**53**)

2.5.8 Series 3: Synthesis of 5-(anthracen-9-yl)-3-phenyl-4,5-dihydro-1*H*-pyrazoles

Another interesting and widely-utilized chalcone transformation that was undertaken as part of this work was the formation of a pyrazoline heterocycle from the α,β -unsaturated ketone functional group. Pyrazolines (Figure 88) have a characteristic five membered heterocycle, whereby three carbon atoms and two nitrogen atoms are in adjacent positions to one another. The term pyrazole was first coined by renowned German chemist named Ludwig Knorr³⁵⁶, with Emil Fischer and Oskar Knoevenagel when synthesizing and characterising 2-pyrazolines in 1877.

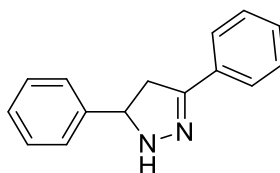


Figure 88: General pyrazoline structure

2.5.9 Biological activity of pyrazolines

Pyrazolines are regarded (similar to chalcones) as a privileged structure in medicinal chemistry, being part of many useful medicines and biochemical agents with a diverse biological activities including antiproliferative, antiinflammatory, antimalarial and MAO-B inhibitory activity acting as potential Parkinson's and Alzheimer's disease agents, examples of which will now be briefly outlined^{357, 358}. In terms of anticancer activity, George et al. reported the antiproliferative activity of a novel series of 3,5 - diarylpyrazoline and 6-phenylpyradazine hybrid agents against A549 (lung), HepG-2 (liver), CaCo-2 (intestinal) and MCF-7 (breast) cancer cell lines. Promising biological activity was observed with IC₅₀ values as low as 1.67 μ M with compounds such as compound **(54)**, causing cell death through caspase-dependent apoptosis³⁵⁹. Furthermore, selective and potent novel antileukaemic pyrazoles were reported by Stefanec et al. The two most active compounds **(55)** and **(56)** were observed to induce cell cycle arrest at G₀/G₁ phase and were found to induce both intrinsic and extrinsic apoptotic cell death in K562 (CML), Jurkat (BL) and AL patient samples. In addition, the compounds were found to be selective for neoplastic cells over both Peripheral Blood Mononuclear Cells (PBMCs) and Red Blood Cells (RBCs)³⁶⁰ (Figure 89).

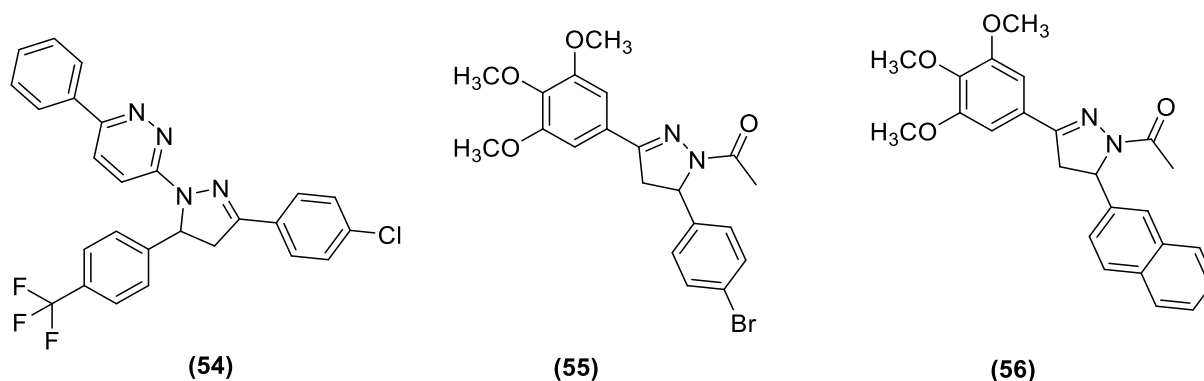


Figure 89: Pyrazoline-based scaffolds exhibiting antiproliferative activity³⁶⁰

Moreover, anti-inflammatory activity of pyrazoline derivatives have been noted by Fioravanti et al. where *N*-substituted-3,5-diphenyl-2-pyrazoline compounds were evaluated for COX (cyclooxygenase) activity (Figure 90). The most promising compounds **(57)** and **(58)** were found to be COX-2 selective, with low IC₅₀ values of 6.77 μ M and 3.20 μ M. These biological results were confirmed through molecular modelling studies of compounds **(57)** and **(58)** in COX-1 and COX-2 active sites computed using AutoDock Vina software³⁶¹. Another example of anti-inflammatory and analgesic potential of pyrazolines was the *in vivo* screening of a series of fluoro-pyrazoline derivatives using the carrageenan induced paw edema method and tail flick method in rats by Jadhav et al. Compounds **(59)** and **(60)** were found to have a more potent anti-inflammatory effect than diclofenac, while compounds **(61)** and **(62)** were found to have analgesic activity similar to the aspirin control (110 % inhibition vs 119% inhibition)³⁶².

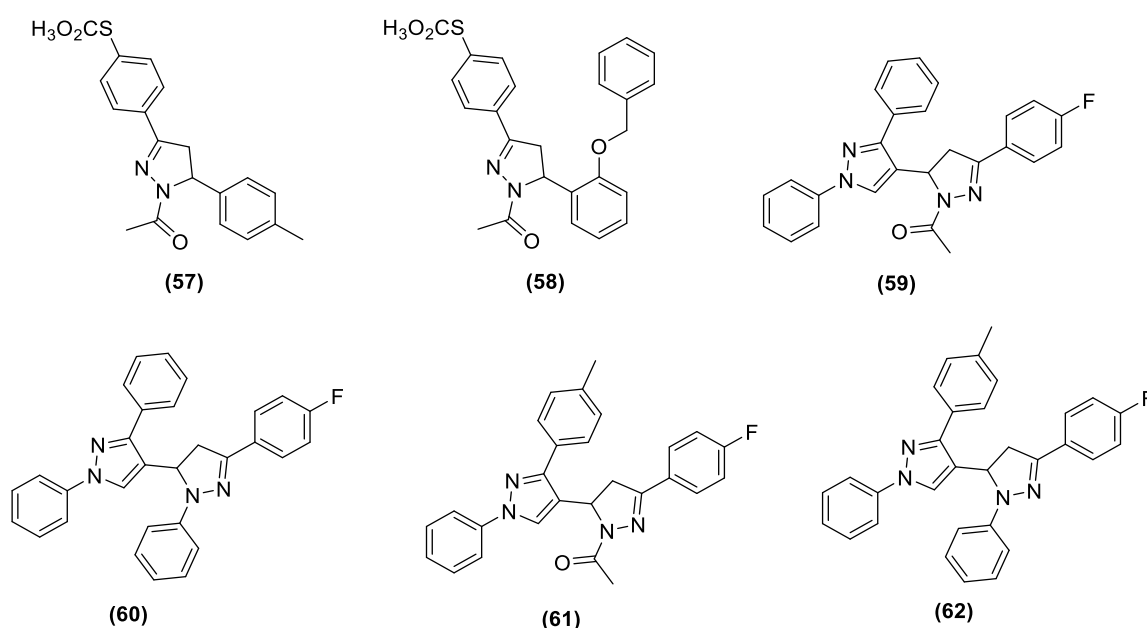


Figure 90: Structures of anti-inflammatory and analgesic pyrazoline agents

Kumar et al. documented the promising antimalarial activity of a series of pyrazole-pyrazolines substituted with benzenesulfonamide both *in vitro* and *in vivo* (Figure 91). Several compounds such as compound **(63)** exhibited low EC₅₀ values of 1.31-1.38 μ M in both chloroquine (CQ) sensitive (3D7) and CQ resistant (RKL-9) strains of *P. falciparum*. In addition, compound **(63)** showed a 83% reduction in parasitaemia (day 4) in *P. berghei* mouse model³⁶³. Interestingly, Unsal-Tan et al. synthesised and biologically assessed novel 2-pyrazoline compounds in an effort to create multifunctional

agents to target the multifactorial nature of Alzheimer's disease. Two compounds (**64**) and (**65**) (Figure 91) were identified as potential leads. Compound (**64**) was shown to be a potent, highly selective butylcholinesterase (BChE) inhibitor (IC_{50} 0.5 μ M) as well as acting a strong anti-aggregation agent for $A\beta_{1-42}$ aggregation (83.4% inhibition). Compound (**65**) exhibited dual ChE activity ([BChE] and Acetylcholinesterase [AChE], IC_{50} = 6.0 and 6.5 μ M respectively), along with $A\beta_{1-42}$ anti-aggregation activity (72.6%) and neuroprotective effects on both $A\beta_{1-42}$ and H_2O_2 treated SH-SY5Y cells³⁶⁴.

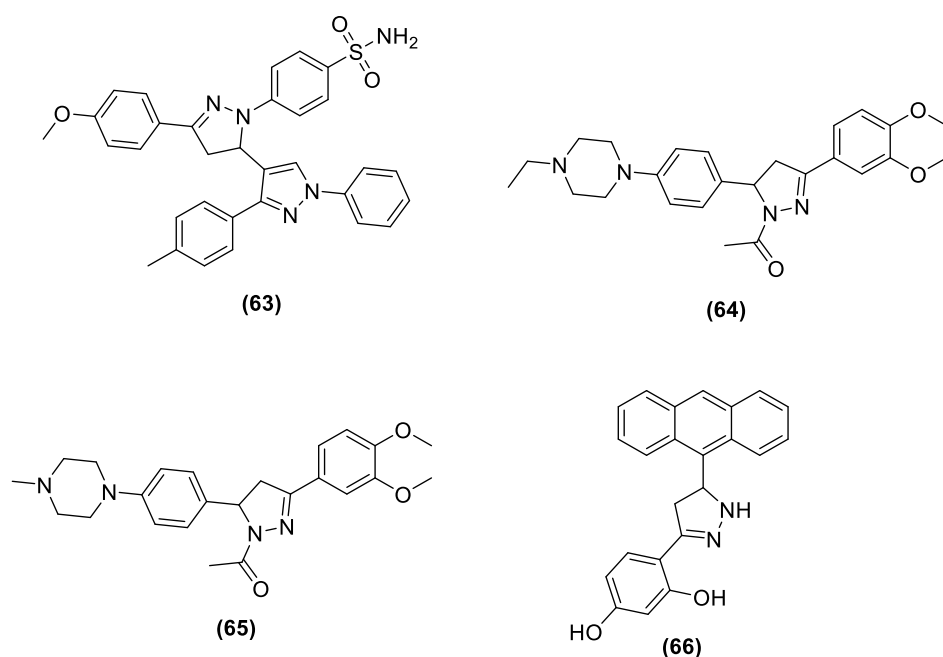


Figure 91: Pyrazoline compounds with antimalrial activity and beneficial activity against Alzheimer's and Parkinson's disease^{363, 364}

In addition, Mishra et al. identified anthracene-based pyrazolines as potential selective neurodegenerative therapeutics against the disease progression of Parkinson's and Alzheimer's disease through impairment of neurotransmitter metabolism (in this case, by monoamine oxidase [MAO]). Selective MAO-B inhibitory activity in both rat and human MAO-A/MAO-B enzymes were observed with the compounds tested (e.g. **66**) ranging from 10-383 fold more effective in rat samples and 15-177 fold more effective in human samples (Figure 91). Furthermore, this inhibitory effect was exerted at treatment concentrations as low as 0.45 nM (rat) and 0.31 nM (human), showing at least 100 fold greater potency against current clinical MAO-B inhibitor selegiline³⁵⁸ (Figure 91).

2.5.10 Synthesis of pyrazolines

The most commonly used method for the synthesis of pyrazolines is an α,β -unsaturated ketone from a chalcone and hydrazine hydrate in acidic solution. The mechanism for the formation of 2-pyrazolines (Figure 92) begins with acid mediated activation of the α,β -unsaturated ketone carbonyl group of the anthracene chalcone molecule. Due to the polarised alkene double bond (resulting from the carbonyl dipole) and need to balance the positive charge on the carbonyl oxygen, the β carbon of the double bond becomes highly electrophilic. This then undergoes nucleophilic attack by the lone pair of electrons of hydrazine hydrate nitrogen to form a covalent bond and intermediate **(A)**. Deprotonation of **(A)** at the hydrazine functional group and protonation of the alkene double bond as a result of the addition of a proton from the reaction environment to the π electrons, leading to intermediate **(B)**. The electrophilic carbonyl carbon then undergoes nucleophilic attack by remaining lone pair of electrons on the hydrazine functional group, resulting in the formation of 5-membered ring system, leading to intermediate **(C)**. The final synthetic step involves the dehydration of intermediate **(C)**, resulting in the 2-pyrazoline product.

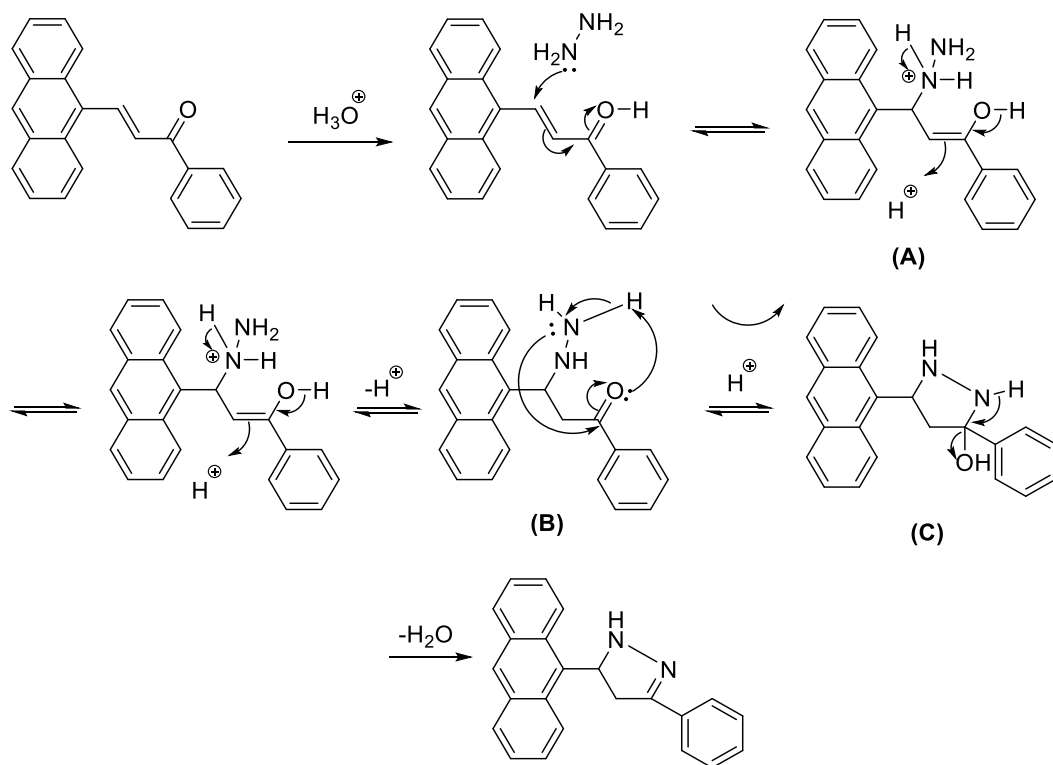


Figure 92: Mechanism for the synthesis of anthracene-based 2-pyrazolines³⁶⁵

The reaction conditions used in the current work to generate the focussed library below involved the formation of pyrazoline adducts of selected anthracene chalcone products using hydrazine-hydrate and conc.HCl in EtOH. Poor to fair yields (3-30%) resulted from the described method (Figure 93, Table 9). Optimisation of reaction should be undertaken in the future whether by conventional or microwave means (reaction time, temperature, acid catalyst type) to ensure higher yields are obtained.

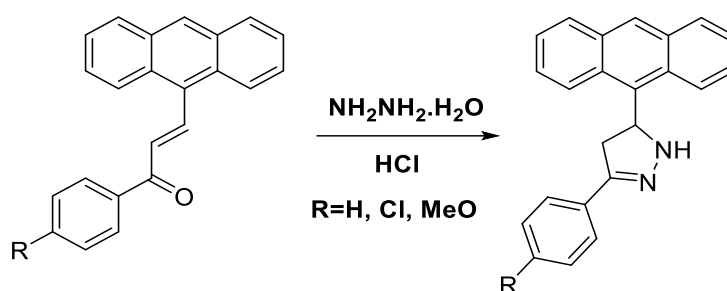


Figure 93: General reaction scheme for the synthesis of 5-(anthracen-9-yl)-3-phenyl-4,5-dihydro-1H-pyrazoles

Compound	R	(%) Yield
(67)	H	21
(68)	Cl	30
(69)	MeO	3

Table 9: 5-(Anthracen-9-yl)-3-phenyl-4,5-dihydro-1H-pyrazoles synthesised and associated yields

2.5.11 Structural characterisation of 5-(anthracen-9-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole (68)

5-(Anthracen-9-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole (**68**) was chosen as the representative structure for this series and its ^1H NMR spectrum will now be discussed. The numbering of the compound (**68**) was generated in ACD/ChemSketch version

12.01. The ^1H NMR spectrum (400 MHz, CDCl_3) (Figure 94) shows a singlet at 8.46 ppm (s, 1H) assigned to H19. The multiplet at 8.38 – 8.43 ppm (m, 2H) corresponds to the protons H14 and H24 of the anthracene core, closest to the heterocyclic functional group. The multiplet at 8.01– 8.07 ppm refers to H17 and H21 of the unsubstituted side of the polycyclic core. The doublet peak at 7.69 ppm (d, $J=8.29$ Hz, 2H) corresponds to H7 and H11 of the *p*-chloro substituted aromatic ring. The multiplet signal at 7.47 ppm (m, $J=6.43$, 2.70 Hz, 4H) assigned to H15, H23, H16 and H22. The doublet at 7.40 ppm (d, $J=8.29$ Hz) was assigned to H8 and H10. The triplet at 6.50 ppm (t, $J=1.00$ Hz, 1H) refers to the proton H1. The multiplet at 3.67 – 3.75 ppm (m, 1H) corresponds to the proton H5. The remaining H4 signal of the CH_2 of the 5-membered pyrazole ring is observed as a multiplet at 3.58 – 3.65 ppm (m, 2H). Absence of characteristic *trans* alkene double bond proton doublets supports the successful conversion of chalcone to the pyrazoline group.

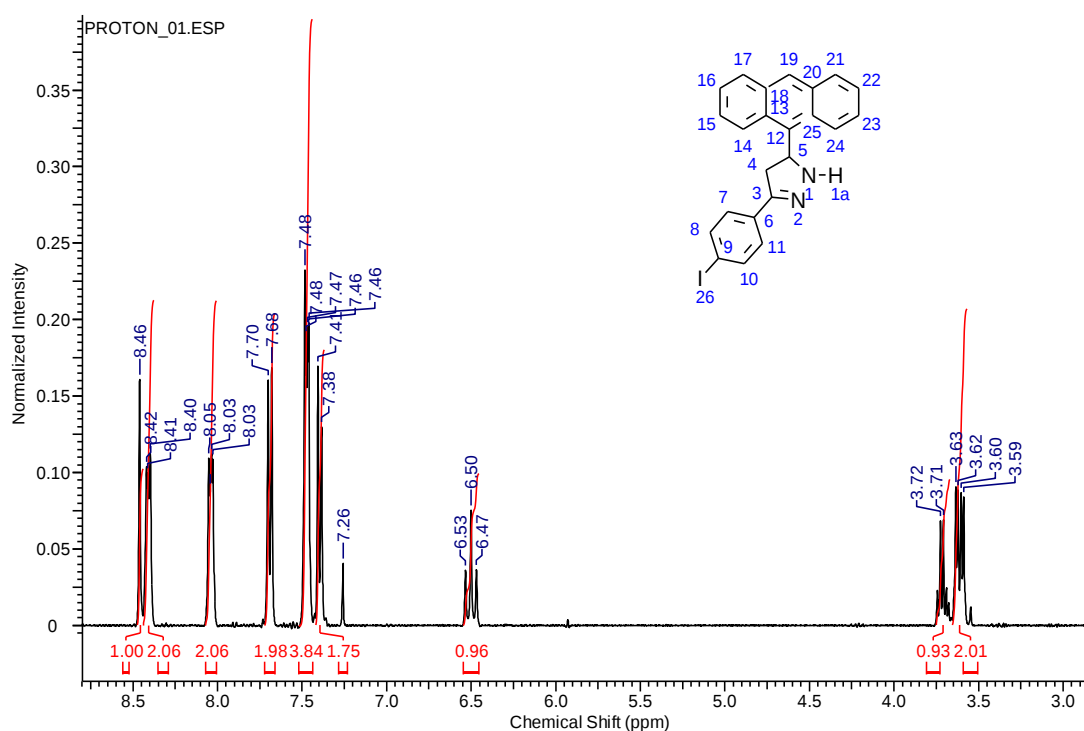


Figure 94: ^1H NMR spectrum of 5-(anthracen-9-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole (68)

Prior to further studies on such pyrazoline structures, full spectroscopic characterisation will have to be undertaken.

2.5.12 Synthesis of ethanoanthracene-maleic anhydride adducts and related compound series

Following the generation and assembly of the previously described chalcone and maleic anhydride / maleimide focussed libraries, the ethanoanthracene compounds afforded through the application of a Diels–Alder reaction were arranged into five groups according to the dienophiles used (maleic anhydride, maleimide, *N*-phenylmaleimide, *p*-chlorophenyl maleimide and *p*-benzophenone maleimide) series 4-9.

2.5.13 Series 4: Synthesis of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

The synthesis of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (**70-85**) was carried out using the described Diels–Alder reaction with the substituted chalcone structures acting as a diene system and maleic anhydride as the respective dienophile (Figure 96, Tables 10-13). Very good yields of 30% - 99% were noted for the compound series and they were obtained as solids following recrystallisation from toluene. Previously optimised reaction conditions used in the synthesis of ethanoanthracene nitrostyrenes were retained for the series²³⁹ e.g. (**24**) (Figure 57, Table 2). Products obtained had three distinct structural features consisting of a dihydrofuran-2,5-dione bridgehead, anthracene-derived central core and an α,β -unsaturated ketone with diverse R substitutions (detailed in Tables 10-13). The products were subdivided according to the type of chalcone used (detailed previously, see synthesis of chalcones) i.e. type 1, type 2 or type 3.

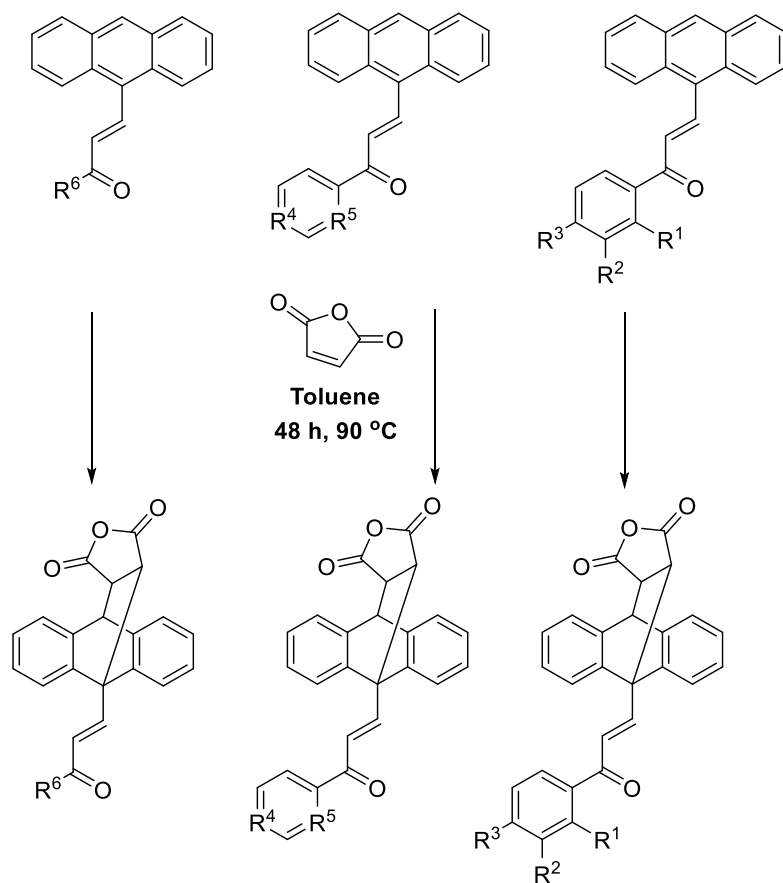


Figure 95: General reaction scheme for the synthesis of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

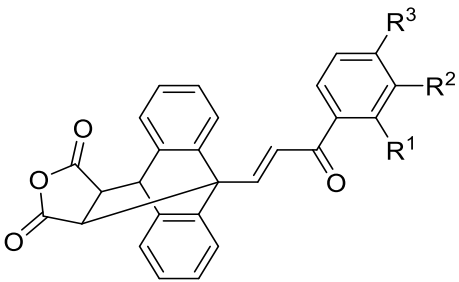
												
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)	No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(70)	H	H	Br	-	-	86	(76)	H	H	I	-	81
(71)	H	H	NO ₂	-	-	77	(77)	H	H	F	-	79
(72)	H	H	CH ₂ CH ₃	-	-	30	(78)	H	H	OCH ₃	-	30
(73)	Cl	H	Cl	-	-	76	(79)	H	H	CH ₃	-	73
(74)	H	OCH ₃	OCH ₃	-	-	83	(80)	OCH ₃	OCH ₃	OCH ₃	-	78
(75)	H	H	H	-	-	91	(81)	H	H	Cl	-	89

Table 10: Type 1 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (70-81)

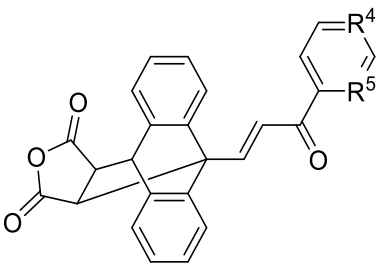
						
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)
(82)	-	-	-	N	H	99
(83 A)	-	-	-	H	N	81

Table 11: Type 2 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (82, 83 A)

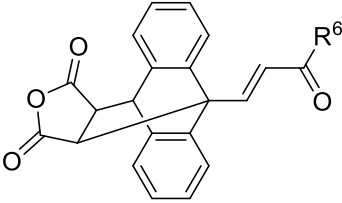
					
No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(83 B)	-	-	-	C ₄ H ₃ O (furan)	81
(84)	-	-	-	C ₁₀ H ₇ (Naphthyl)	86
(85)	-	-	-	C ₄ H ₃ S (thiophene)	31

Table 12: Type 3 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (83 B-85)

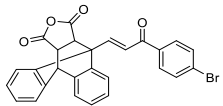
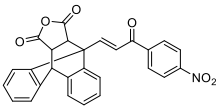
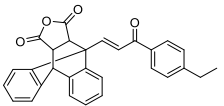
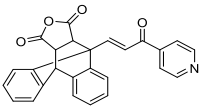
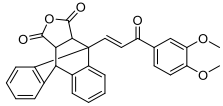
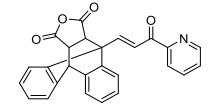
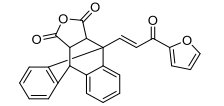
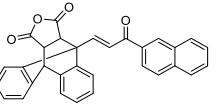
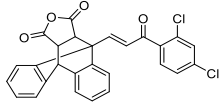
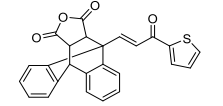
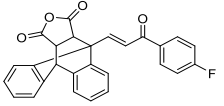
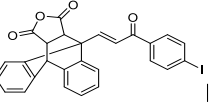
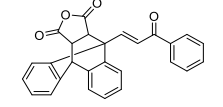
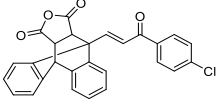
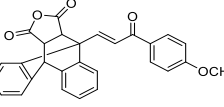
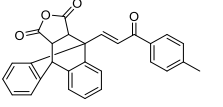
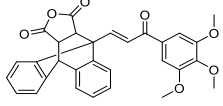
Maleic anhydride adducts			
			
(70) (86 %)	*(71) (77 %)	*(72) (70 %)	*(82) (99 %)
			
*(74) (83 %)	*(83 A) (70 %)	*(83 B) (81 %)	*(84) (86 %)
			
*(73) (76 %)	*(85) (76 %)	*(77) (79 %)	*(76) (81 %)
			
*(75) (91%)	*(81) (89%)	*(78) (30%)	*(79) (73%)
			
*(80) (78%)			

Table 13: Structures of the (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione series and yields (where * denotes novel compounds, previously unreported)

2.5.13 Structural characterisation of (E)-3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10 [3,4]furanoanthracene-12,14-dione (**74**)

(E)-3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10

[3,4]furanoanthracene-12,14-dione (**74**) was chosen as a representative example for this series and its ^1H -NMR (Figure 97) and ^{13}C NMR (Figure 98) spectra will now be discussed. The numbering of the compound (**74**) was generated in ACD/ChemSketch version 12.01. The ^1H NMR (400 MHz, $\text{DMSO-}d_6$) spectrum (Figure 97) of (**74**) shows a double doublet at 3.83 ppm (dd, $J=9.1, 3.3$ Hz), corresponding to H8. The next signal observed is a doublet at 4.29 ppm (d, $J=9.1$ Hz, 1H), representing the proton H3. The signals appearing at 3.87 ppm and 3.89 ppm denote the protons of the two methoxy groups at positions 33 and 35 (2x s, 6H), which are non-equivalent signals. The doublet at 4.98 ppm ($J=3.3$ Hz, 1H) represents the proton H7 of the central ring of the ethanoanthracene core. The doublet that appears at 7.17 ppm (d, $J=8.7$ Hz, 1H) denotes the proton H30 of the acetophenone aromatic ring. The multiplet at 7.27 ppm (m, 6H) represents the six aromatic protons of the anthracene core H12, H13, H14, H15, H16 and H19. H18 and H17 appear as multiplet signals at 7.44 ppm (m, 1H) and 7.34 ppm (m, 1H) respectively.

The doublet at 7.58 ppm (d, $J=7$ Hz, 1H) represents H27 of the benzoyl aromatic ring. The doublet appearing at 7.70 ppm (d, $J=1.7$ Hz, 1H) represents H30 of the aromatic ring. The doublet that appears at 7.77 ppm (d, $J=16.6$ Hz, 1H) is assigned to H22 of the alkene double bond, directly adjacent to the carbonyl functional group. This coupling constant value of >12 Hz was consistent with previously reported *trans* alkene bonds. The multiplet that appears at 7.90 ppm denotes the proton H31 on the acetophenone-derived aromatic ring and H21 of the alkene double bond, appearing at a higher chemical shift due to the inductive effect of the carbonyl group on the conjugated double bond.

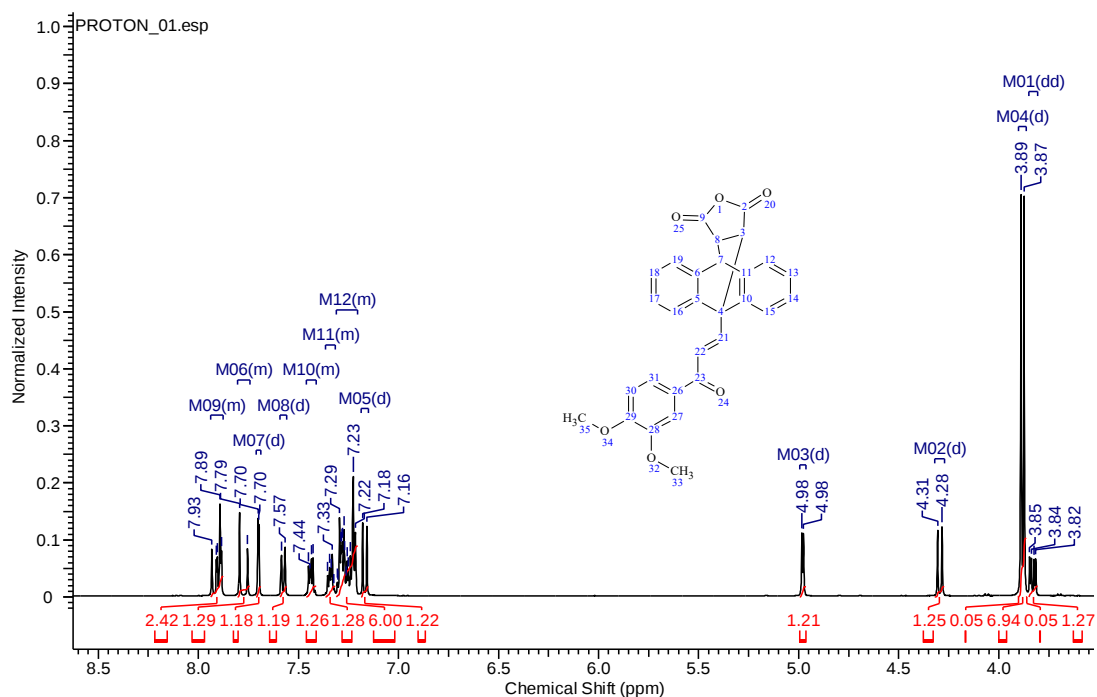


Figure 96: ^1H NMR spectrum of (*E*)-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10 [3,4]furanoanthracene-12,14-dione (**74**)

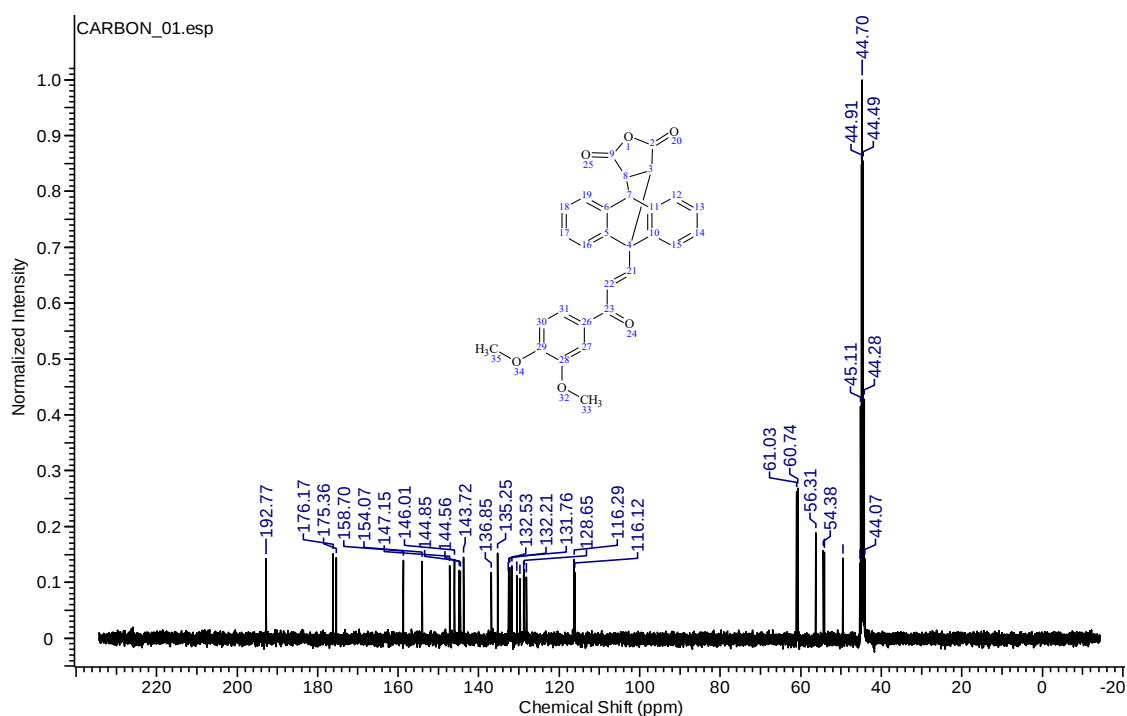


Figure 97: ^{13}C NMR spectrum of (*E*)-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10 [3,4]furanoanthracene-12,14-dione (**74**)

The ^{13}C NMR spectrum of (**74**) (101 MHz, $\text{DMSO-}d_6$) (Figure 98) shows C8 as a signal at 54.1 ppm, while C3 appears as a signal at 54.3 ppm. C7 of the centre anthracene ring

resonates at 49.4 ppm. The quaternary carbon C4 of the centre ring of the polycyclic core corresponds to the signal at 56.3 ppm. The methyl signals of the two methoxy group carbons C33 and C35 resonate at 60.7 ppm and 61.0 ppm respectively. C27 of the benzoyl aromatic ring refers to the signal at 116.2 ppm, while C30 appears as a signal at 116.1 ppm. C17, C18 and C19 appear as signals at 128.1 ppm, 130.5 ppm and 129.7 ppm respectively due to the possible carbonyl deshielding cone interaction and potential hydrogen bond formation with the maleic anhydride structure due to the configuration above the aromatic ring. For the remaining aromatic C-H carbons of the aromatic ring, C16 appears at 128.6 ppm, while C12, C13, C14 and C15 show as signals at 132.2 ppm, 132.6 ppm, 132.5 ppm and 131.7 ppm. The quaternary carbons C28 and C29 attached to the methoxy groups of the benzoyl ring resonate at 154.0 ppm and 158.7 ppm. C26 adjacent to the carbonyl group of the α,β -unsaturated ketone moiety shows a signal at 135.21 ppm. The remaining quaternary aromatic carbons of the anthracene core C6, C5, C11 and C10 show signals at 143.7 ppm, 146.0 ppm, 144.8 ppm and 147.1 ppm. The alkene double bond carbons of the C21 and C22 resonate at 136.8 ppm and 144.5 ppm respectively. The non-equivalent carbonyl groups of the maleic anhydride functional group C2 and C9 are assigned to the signals at 175.4 ppm and 176.2 ppm. The highest resonating signal in the spectrum corresponds to the carbonyl group of the α,β -unsaturated ketone functional group C23 at 192.8 ppm.

The DEPT 90 spectrum (Figure 99) was used to help identify the alkene double bond signals C21 (136.8 ppm) and C22 (144.5 ppm) as well as the carbons of the ethanoanthracene bridge C3 (54.3 ppm), C7 (49.4 ppm) and C8 (54.1 ppm). The HSQC spectrum (Figure 100) was used to assign the corresponding carbon signals to the protons allocated in the ^1H NMR spectrum (Figure 97) and in conjunction with the HMBC spectrum (Figure 101) was used to identify and assign quaternary carbons such as C4 (56.2 ppm) in the ^{13}C spectrum.

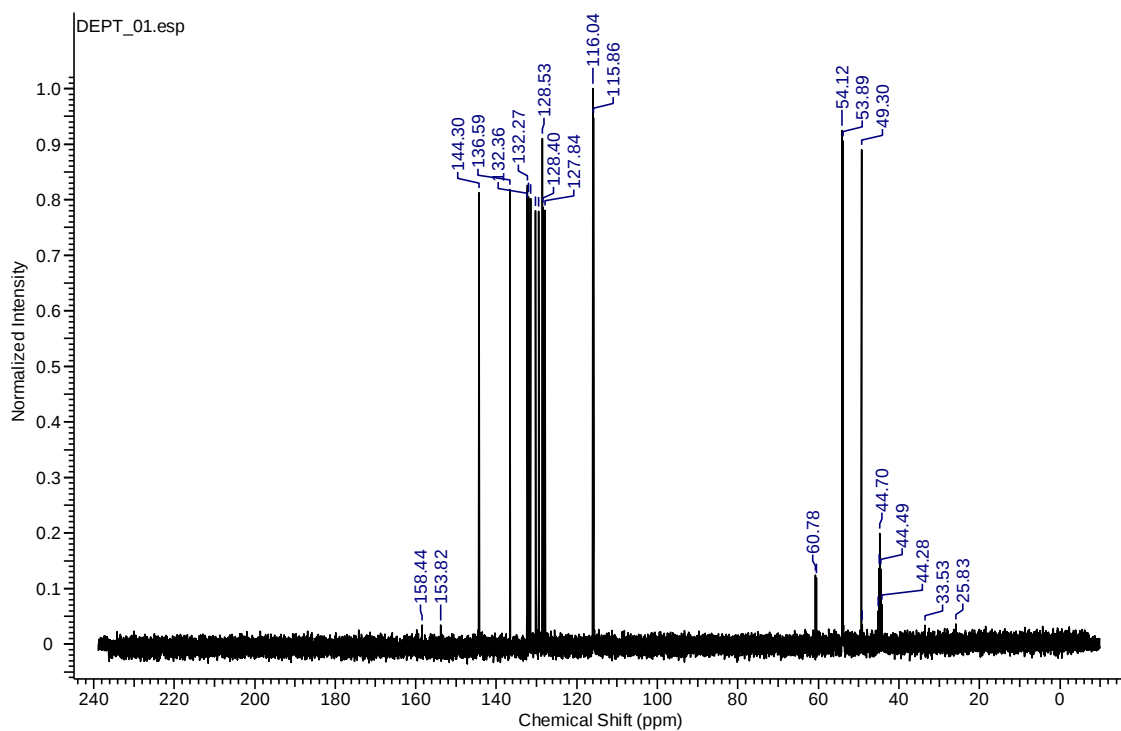


Figure 98: DEPT 90 of (*E*)-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10 [3,4]furanoanthracene-12,14-dione (74)

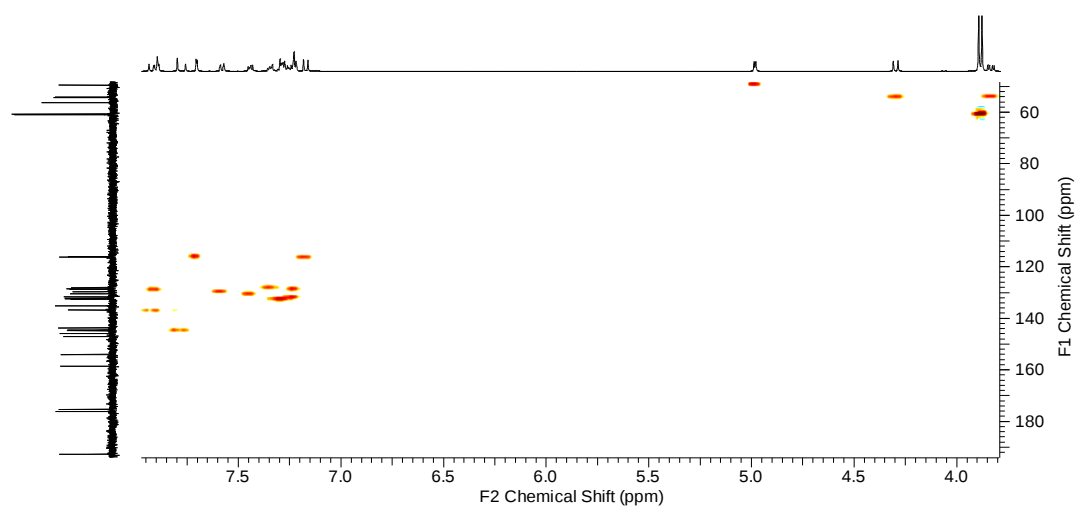


Figure 99: HSQC spectrum of (*E*)-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10 [3,4]furanoanthracene-12,14-dione (74)

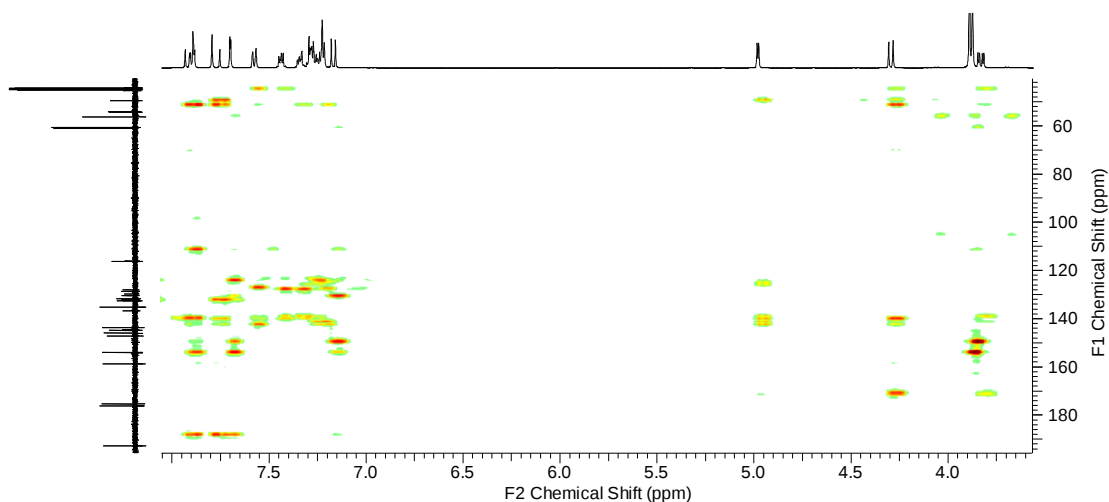


Figure 100: HMBC spectrum of *(E)*-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10[3,4]furanoanthracene-12,14-dione (**74**)

The IR spectrum (Figure 102) confirmed the structure of (**74**) and shows bands at 1669.07 cm^{-1} (C=O of α,β -unsaturated ketone), 1774.29 cm^{-1} (C=O groups of the maleic anhydride moiety), 2970.65, 2934.18 cm^{-1} (aromatic C-H stretch), 1635.22, 1592.71, 1511.42, 1466.16 cm^{-1} (aromatic C=C stretch), 1850.60 cm^{-1} (trans C=C stretch), 1310.73 cm^{-1} , 1128.99 cm^{-1} (C-O of aryl methyl ether), 1267.34, 1153.02 cm^{-1} (C-O of anhydride) and 949.47 cm^{-1} (aromatic out of plane C-H bend).

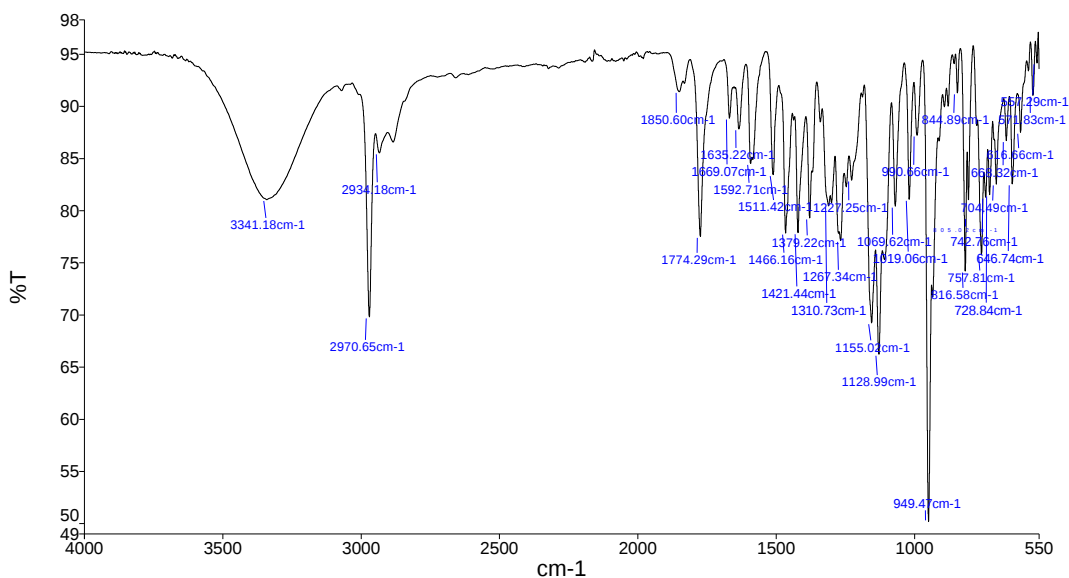


Figure 101: IR spectrum of *(E)*-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10[3,4]furanoanthracene-12,14-dione (**74**)

Series 5: Synthesis of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

The synthesis of a series of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (**86-102**) was carried out utilizing the described Diels–Alder reaction with the previously discussed substituted chalcone structures acting as a diene system and maleimide as the dienophile. Very good yields of 46-100 % were obtained for the compounds which were obtained as solids after recrystallisation from toluene (Figure 103, Tables 14-17). The products contained three distinct structural features consisting of a pyrrolidine-2,5-dione bridgehead, anthracene-derived central core and an α,β -unsaturated ketone with diverse R substitutions (detailed in Table 14).

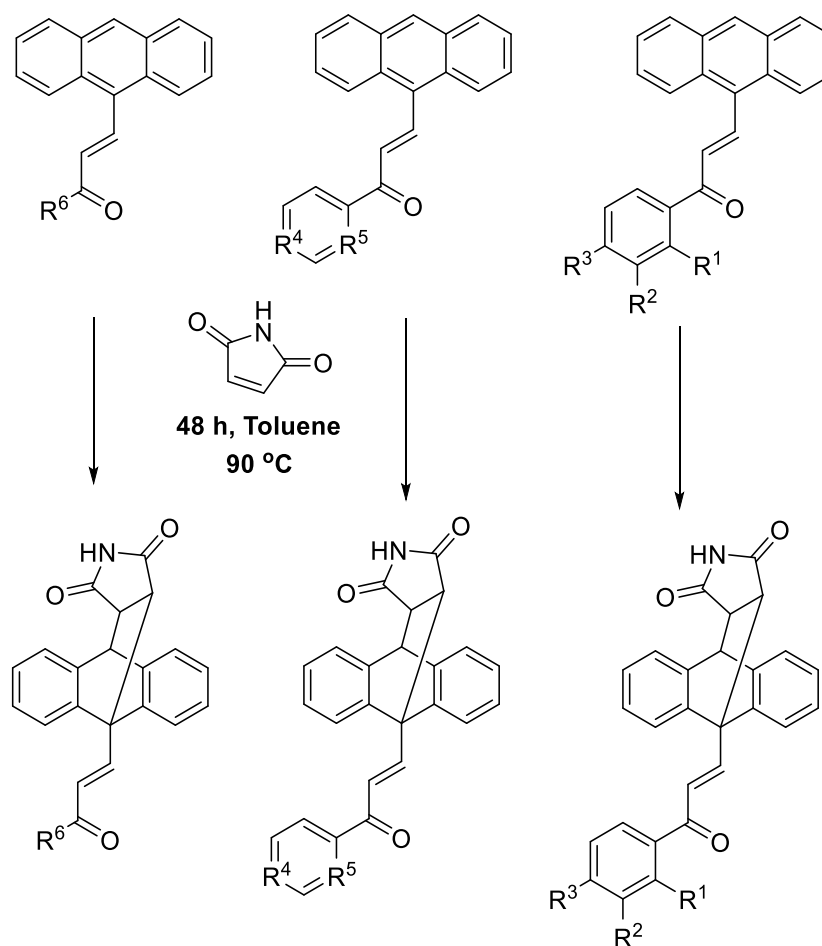


Figure 102: General reaction scheme for the synthesis of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

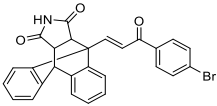
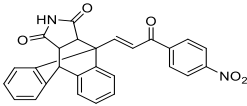
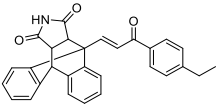
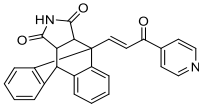
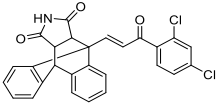
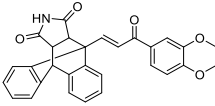
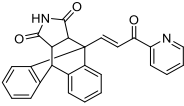
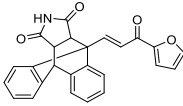
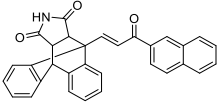
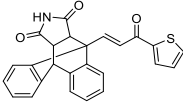
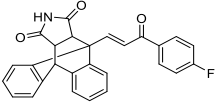
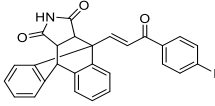
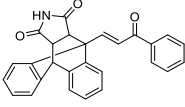
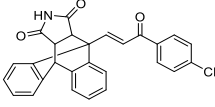
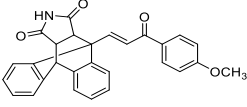
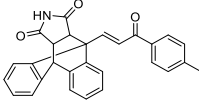
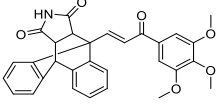
Maleimide derivatives			
			
(86)* (91 %)	(87)* (87 %)	(88)* (82%)	(89)* (100 %)
			
(90)* (82 %)	(91)* (83 %)	(99)* (72%)	(95)* (81 %)
			
(96)* (80 %)	(97)* (80 %)	(99)* (100 %)	(98)* (93 %)
			
(93)* (61%)	(94)* (65%)	(100)* (70%)	(101)* (46%)
			
(102)* (53%)			

Table 14: Structures of the (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (Series 5) and yields (where * denotes novel compounds previously unreported)

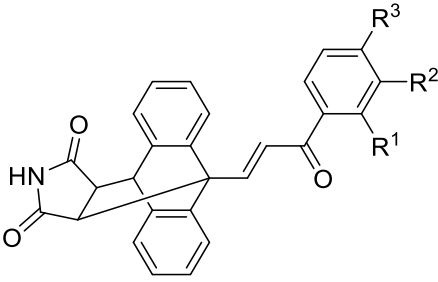
												
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)	No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(86)	H	H	Br	-	-	91	(92)	H	H	I	-	93
(87)	H	H	NO ₂	-	-	87	(93)	H	H	F	-	100
(88)	H	H	CH ₂ CH ₃	-	-	82	(94)	H	H	OCH ₃	-	70
(89)	Cl	H	Cl	-	-	82	(95)	H	H	CH ₃	-	46
(90)	H	OCH ₃	OCH ₃	-	-	83	(96)	OCH ₃	OCH ₃	OCH ₃	-	53
(91)	H	H	H	-	-	61	(97)	H	H	Cl	-	65

Table 15: Type 1 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (86-97)

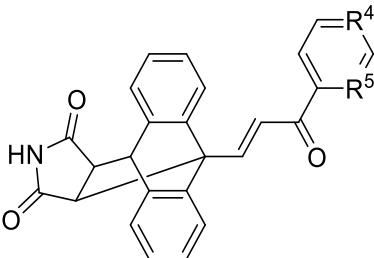
						
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)
(98)	-	-	-	N	H	100
(99)	-	-	-	H	N	72

Table 16: Type 2 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (86-97)

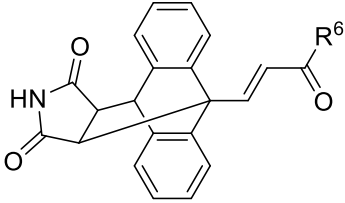
					
No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(100)	-	-	-	C ₄ H ₃ O (furan)	81
(101)	-	-	-	C ₁₀ H ₇ (Naphthyl)	86
(102)	-	-	-	C ₄ H ₃ S (thiophene)	80

Table 17: Type 3 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

2.5.14 Structural characterisation of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**86**)

(*E*)-9-(3-(4-Bromophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**86**) was chosen as a representative example for this series and its ¹H-NMR (Figure 104) and ¹³C NMR spectra (Figure 105) will now be discussed. The numbering of the compound (**86**) was generated in ACD/ChemSketch version 12.01.

The ¹H NMR spectrum (400 MHz, CDCl₃) spectrum of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**86**) shows a multiplet signal at 3.32 ppm (1H, CH) which corresponds to H8 of the ethanoanthracene bridge. The doublet signal at 3.82 ppm (1H, *J*=8.71 Hz, CH) refers to the H3 proton of the carbon bridge of the anthracene centre ring, while the other respective doublet signal in the aliphatic region of the spectrum at 4.81 ppm (1H, *J*=3.32 Hz, CH) denotes H7. The aromatic proton H19 corresponds to the doublet signal at 7.51 ppm (1H, *J*=7.05 Hz). The apparent triplet signal at 7.33 ppm (1H, *J*= 4.98 Hz x2) corresponded to H18, whereas the other apparent triplet signal resonating at 7.26 ppm (1H, *J*= 4.15 Hz x2) refers to H17. The highest resonating signal in the spectrum was H1 directly attached to the maleimide nitrogen, resonating at 10.87 ppm.

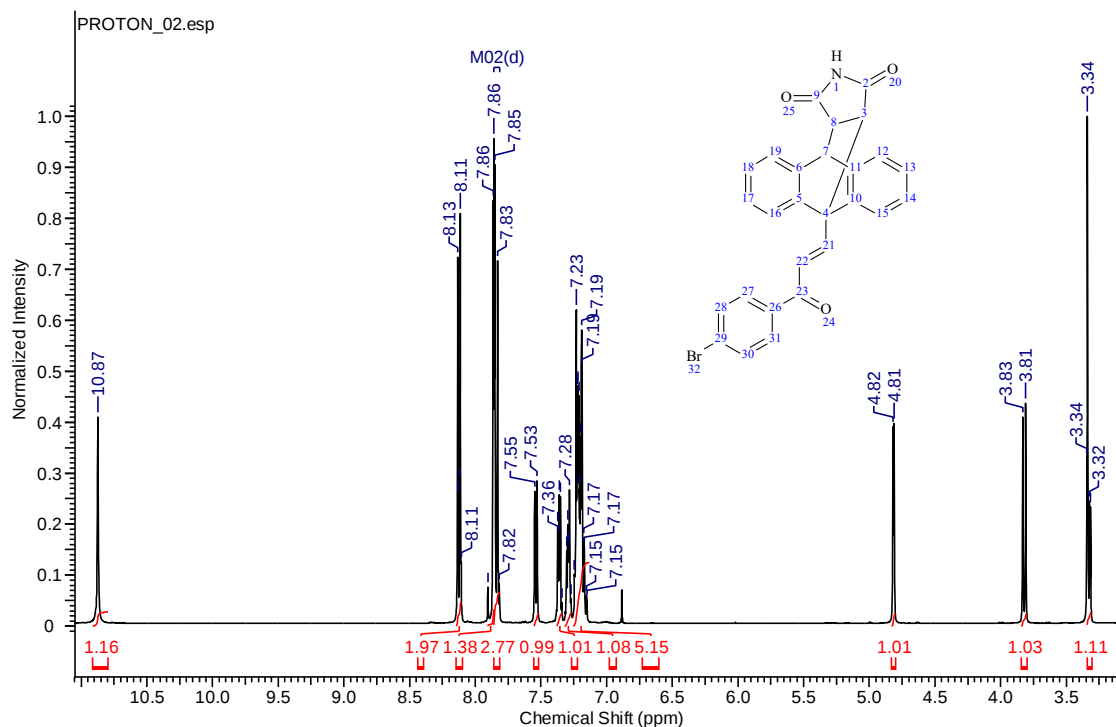


Figure 103: ^1H -NMR spectrum of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**86**)

The ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$) spectrum (Figure 105) of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**86**) shows C8 of the ethanoanthracene bridge at 49.3 ppm, while the other corresponding carbon C3 is observed at 49.1 ppm. The signal at 45.0 ppm represents C7 of the anthracene core of the centre ring of the polycyclic core. The carbon C4 is represented by the signal at 51.7 ppm. The carbon pair (C6, C11) corresponds to the signal at 139.1 ppm which are two quaternary carbons that are present on the anthracene core of the molecule. The other two quaternary carbons C5 and C10 of the core structure were observed at 140.2 ppm. C29, the carbon adjacent to the bromine atom of the acetophenone-derived moiety of the tail-like side chain is represented by the signal at 128 ppm. The two carbonyl groups (C2, C9) present on the maleimide functional group corresponded to the signals at 177.5 ppm and 177.7 ppm respectively. The most downfield signal at 189.1 ppm represented C23, the carbonyl group of the α,β -unsaturated ketone functional group. C19 signal appears at 124.6 ppm, with the signals for C18 and C17 appear at 125.7 ppm and 123.3 ppm respectively. Furthermore, C16 resonates at 123.65 ppm. The signal at 131.1 ppm represents the carbon pair (C27, C31) on the benzoyl functional group aromatic ring while C28 and C30 resonate at 132.5 ppm. The

carbons of the alkene bond C21 and C22 are observed at 143.1 ppm and 131.5 ppm respectively due to the inductive effect of the dipole of the carbonyl functional group. For the remaining aromatic ring of the anthracene polycyclic core structure, C12 corresponded to the signal at 126.6 ppm, C13 was represented by the signal observed at 127.3 ppm. C14 was anoted to resonate at 127.0 ppm and the signal for C15 was noted at 123.6 ppm.

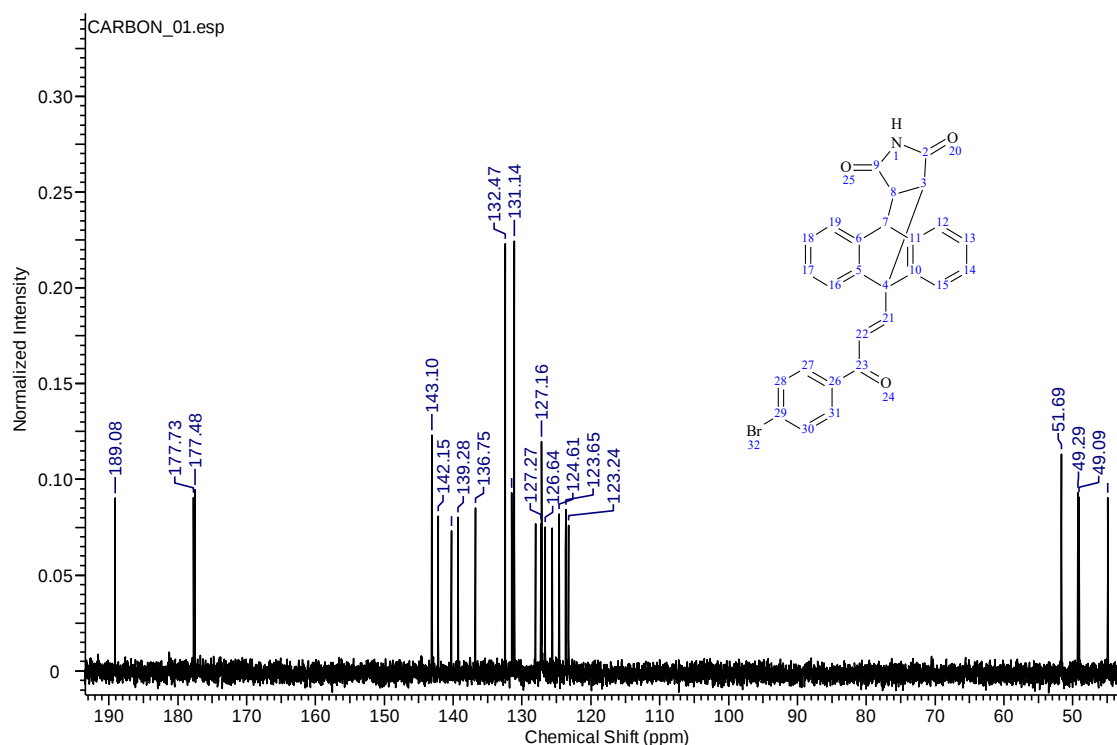


Figure 104: ^{13}C NMR spectrum of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**86**)

A potential explanation for the differential resonance of the aromatic core scaffold ring signals (e.g. C16, C17, C18) would be due to the orientation of the maleimide directly over one of the aromatic rings of the anthracene core. This orientation could allow for the formation of hydrogen bonds between the nitrogen and the respective aromatic ring hydrogens, causing them to appear more deshielded than normal. In support of this, in the present work, the crystal structure of (**91**) (Figure 108) showed evidence of both imide (NH) and aromatic hydrogen interaction as well as π - π stacking. This could also be compounded by the fact that a cyclic imide as opposed to the cyclic anhydride described previously would cause a greater polarisation of the the respective carbonyl groups, thus

increasing the effect of cone mediated shielding / deshielding based on proton/carbon orientation.

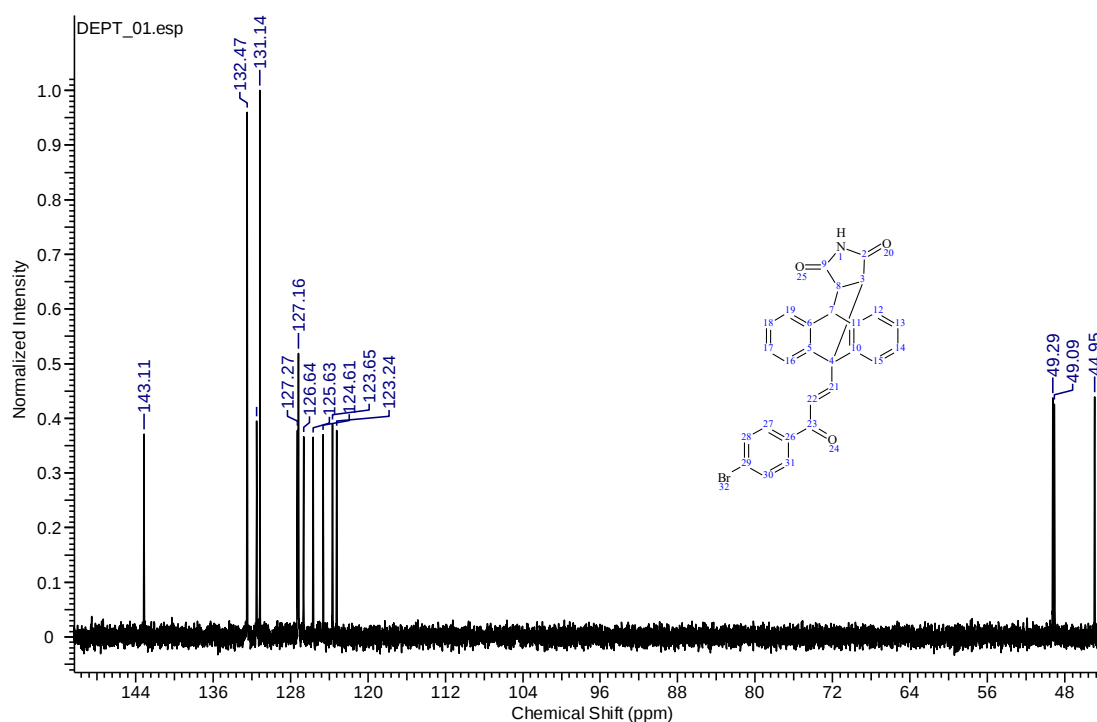


Figure 105: DEPT 90 of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**86**)

The DEPT 90 spectrum (Figure 106) was used in the assignment of primary, secondary and tertiary carbons, in particular the alkene carbons of the α,β -unsaturated ketone group C21 (143.1 ppm) and C22 (131.5 ppm). In addition, it was used in the identification of the ethanoanthracene aliphatic bridge carbons C3 (49.1 ppm), C8 (49.3 ppm) and C7 (45 ppm) (Figure 105).

The IR spectrum (Figure 107) confirmed the structure of (**86**) and shows bands at 1717.93 cm^{-1} ($\text{C}=\text{O}$ of α, β -unsaturated ketone), 1777.23 cm^{-1} ($\text{C}=\text{O}$ groups of the maleimide moiety), 3338.02 cm^{-1} (secondary amide NH stretch), 1675.39 cm^{-1} (secondary amide NH bend), 1583.1 cm^{-1} (C-N stretch), 2928.47, 3064.98 cm^{-1} (aromatic C-H stretch), 1425.56, 1633.78 cm^{-1} (aromatic C=C stretch), obscured by signals in region 1660-1600 cm^{-1} (*trans* C=C stretch) and at 1005.47 cm^{-1} (aryl Br). Furthermore, the mass of (**86**) was confirmed through high-resolution mass spectrometry (HRMS) [calculated (m/z) 482.039729, found (m/z) 482.039159].

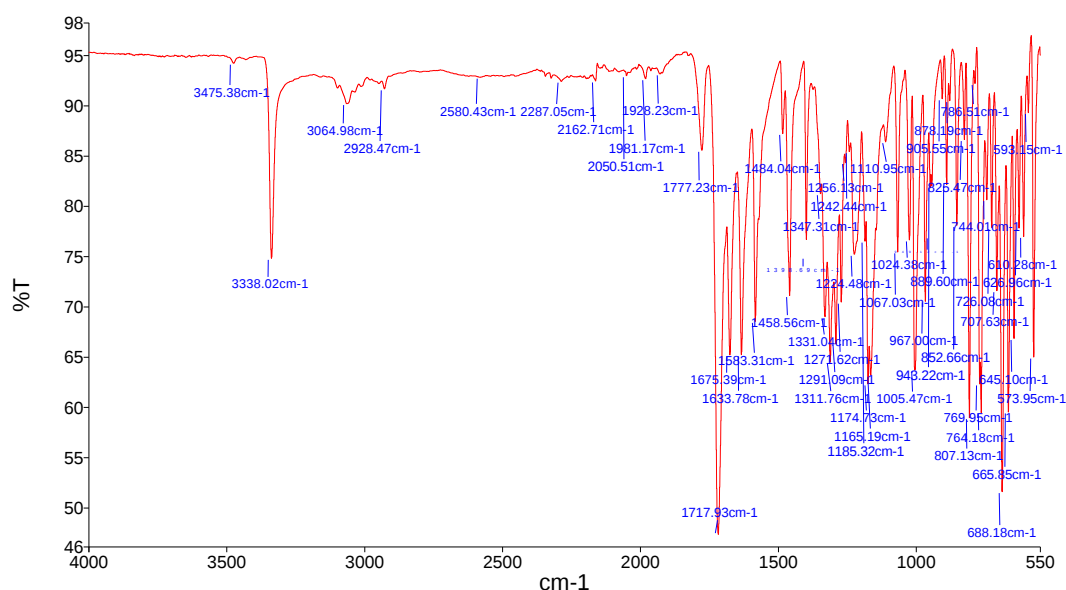


Figure 106: IR spectrum of *(E)*-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**86**)

2.5.15 X-ray crystallography of *(E)*-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**91**)

The retention of *E* / *trans* configuration alkene double bond of the ethanoanthracene chalcone products together with the structure of (**91**) was determined and confirmed by X-ray crystallography of *(E)*-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**91**) (Figure 108, Table 18). The crystal was found to be monoclinic, with the crystal packing arrangement shown in Figure 108. The bond length of C6-C7 was consistent with that of an alkene double bond (1.321 Å) and the bond length of O3-C8 consistent with a carbonyl functional group (1.219 Å) in similar literature compounds^{344, 345}. The presence of the alkene double bond is also supported through the bond angles of C5-C6-C7 and C6-C7-C8 which were 128.06° and 120.82° respectively, indicating the trigonal-planar nature expected of such a functional group side-chain. The presence of the imide functional group was supported by the bond lengths of O1-C1 and O2-C2 both measuring 1.205 Å, typical of carbonyl functional groups. In addition, observation of N1-C1 and N1-C2 bond lengths of 1.375 Å and 1.380 Å respectively support imide presence as these bond lengths are typical of C-N imide bonds³⁶⁶.

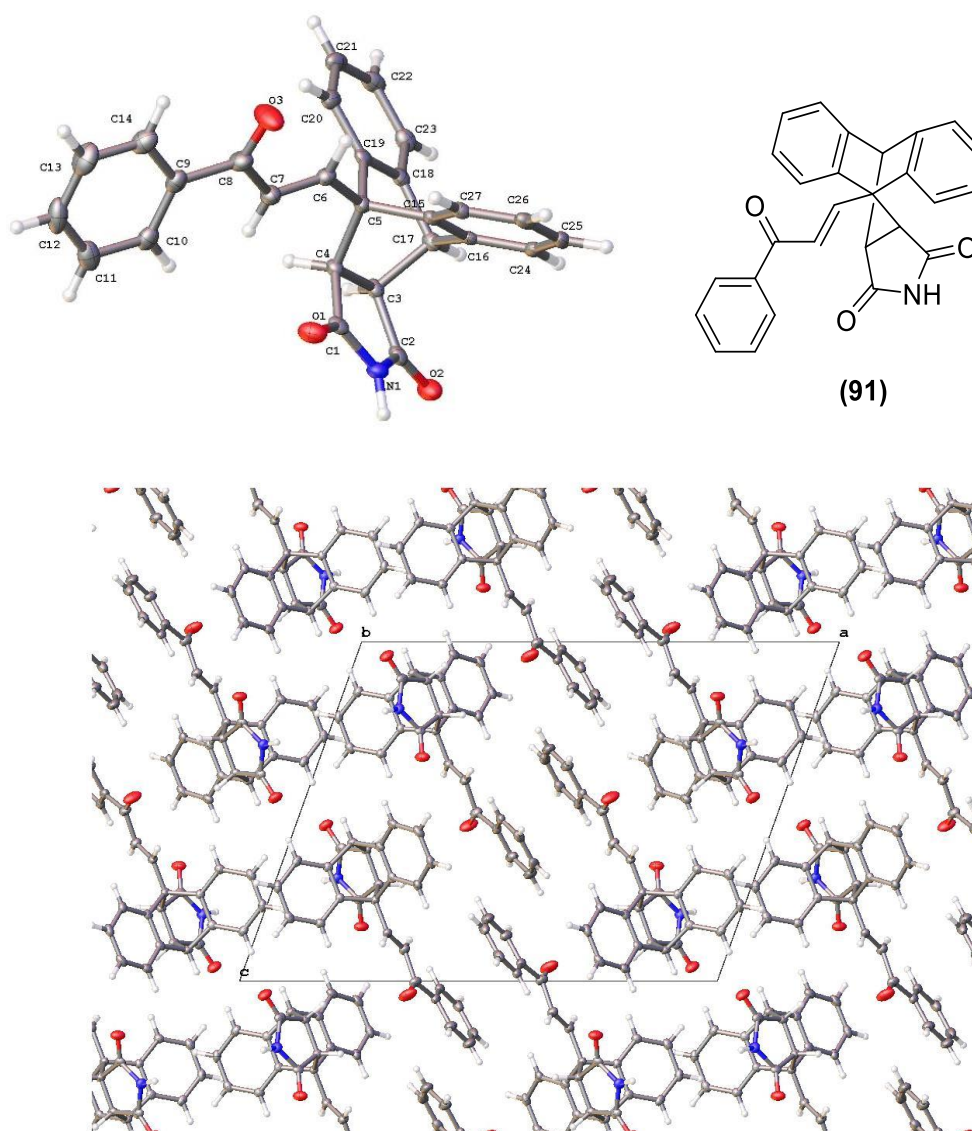


Figure 107: X-ray structure of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (91) and its corresponding crystal packing arrangement

In addition to the points previously mentioned, examination of the X-ray crystal showed the presence of these compounds as racemic mixtures, with the two stereocentres being present at the ethanoanthracene carbon bridge being in the (*R,R*) and (*S,S*) stereochemical configuration. While not explored further in this work, an interesting avenue for future studies would be the chiral separation of the resultant enantiomers and characterisation of their respective physicochemical and biological traits in comparison to the corresponding racemate.

Empirical formula	C ₂₇ H ₁₉ NO ₃
Formula weight	405.43
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2 ₁ /c
Unit cell dimensions	a = 20.1992(16) Å b = 6.5975(5) Å c = 15.2217(11) Å $\alpha = 90^\circ$ $\beta = 109.811(2)^\circ$ $\gamma = 90^\circ$
Volume	1908.5(3) Å ³
Z	4
Density (calculated)	1.411 Mg/m ³
Absorption coefficient	0.092 mm ⁻¹
F(000)	848
Crystal size	0.22 x 0.132 x 0.063 mm ³
Theta range for data collection	2.678 to 27.252°
Index ranges	-26 ≤ h ≤ 25, -8 ≤ k ≤ 8, -19 ≤ l ≤ 19
Reflections collected	20477
Independent reflections	4272 [R(int) = 0.0765]
Completeness to theta = 25.242°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7455 and 0.6960
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	4272 / 1 / 280
Goodness-of-fit on F²	1.043
Final R indices [I > 2σ(I)]	R1 = 0.0508, wR2 = 0.1051
R indices (all data)	R1 = 0.0872, wR2 = 0.1223
Largest diff. peak and hole	0.234 and -0.296 e.Å ⁻³

Table 18: Crystal data details and structural refinement for (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (91)

2.5.16 Series 6: Synthesis of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

The synthesis of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (Series 6) (**103-119**) was carried out utilizing the described Diels–Alder reaction with the previously discussed substituted chalcone structures acting as a diene system and N-phenylmaleimide as the dienophile. Fair to excellent yields of 20%-93% were obtained for the compound series and were obtained as solids after recrystallisation from toluene (Figure 109, Tables 19-22). The products contained had three distinct structural features consisting of a 1-phenylpyrrolidine-2,5-

dione bridgehead, anthracene-derived central core and an α,β -unsaturated ketone with diverse R substitutions (detailed in Table 22).

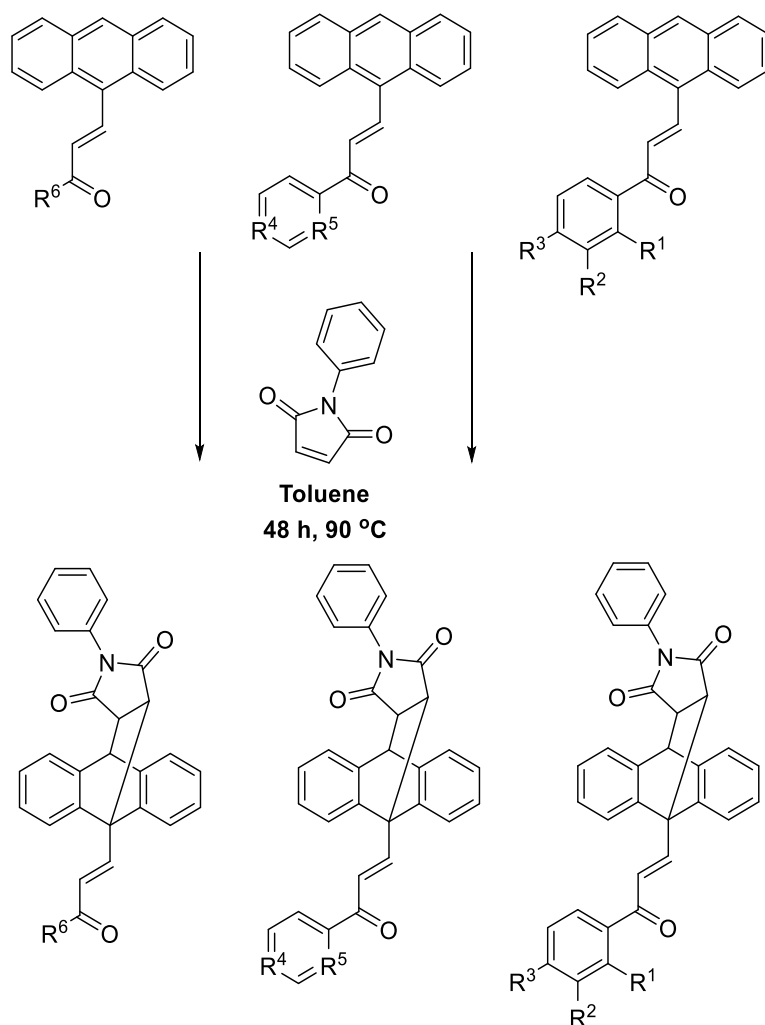


Figure 108: General reaction scheme for the synthesis of (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

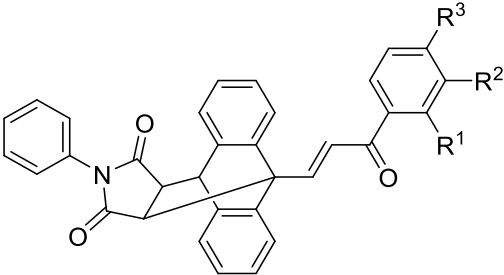
												
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)	No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(103)	H	H	Br	-	-	84	(109)	H	H	I	-	
(104)	H	H	NO ₂	-	-	86	(110)	H	H	F	-	
(105)	H	H	CH ₂ CH ₃	-	-	90	(111)	H	H	OCH ₃	-	
(106)	Cl	H	Cl	-	-	75	(112)	H	H	CH ₃	-	
(107)	H	OCH ₃	OCH ₃	-	-	93	(113)	OCH ₃	OCH ₃	OCH ₃	-	
(108)	H	H	H	-	-	42	(114)	H	H	Cl	-	

Table 19: Type 1 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (103-114)

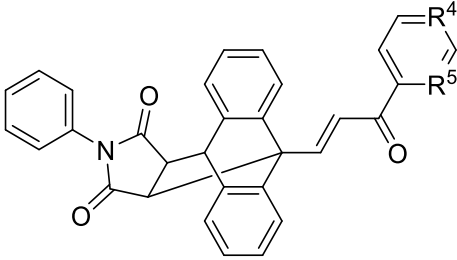
						
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)
(115)	-	-	-	N	H	82
(116)	-	-	-	H	N	91

Table 20: Type 2 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (115, 116)

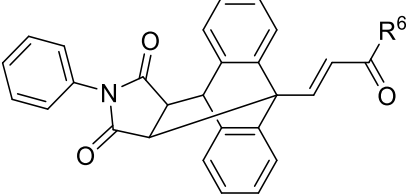
					
No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(117)	-	-	-	C ₄ H ₃ O (furan)	87
(118)	-	-	-	C ₁₀ H ₇ (Naphthyl)	65
(119)	-	-	-	C ₄ H ₃ S (thiophene)	81

Table 21: Type 3 (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (117-119)

N-phenymaleimide adducts			
(103)* (84 %)	(104)* (86 %)	(105)* (90 %)	(115)* (82 %)
(106)* (75 %)	(107)* (93 %)	(116)* (91 %)	(117)* (87%)
(118)* (65 %)	(119)* (81 %)	(110)* (88 %)	(109)* (20 %)
(108)* (42%)	(114)* (20%)	(111)* (56%)	(112)* (51%)
(113)* (62%)			

Table 22: Structures of the (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione series and yields (where * denotes novel compounds synthesised)

2.5.17 Structural characterisation of (*E*)-9-(3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**111**)

(*E*)-9-(3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**111**) was chosen as the representative example for this series and its ^1H NMR (Figure 110) and ^{13}C NMR spectra (Figure 111) will now be discussed. The numbering of the compound (**111**) was generated in ACD/ChemSketch version 12.01.

The ^1H NMR (400 MHz, $\text{DMSO-}d_6$) spectrum of (**111**) shows a double doublet signal at 3.55 ppm ($J=5.3, 3.3$ Hz, 1H) which corresponds to H8 of the ethanoanthracene bridge. The singlet signal at 3.85 ppm (3H) refers to H39 of the methoxy benzoyl substituent. The doublet signal at 3.99 ppm (1H, $J=8.55$ Hz) refers to the proton of the carbon bridge of the anthracene centre ring (H3), while the respective doublet signal in the aliphatic region of the spectrum at 4.94 ppm (1H, $J=3.68$ Hz) denotes H7. The multiplet at 6.41 ppm (2H) corresponds to the proton pair (H34, H36) of the benzoyl aromatic ring due to the inductive influence of the 4-methoxy functional group. The remaining proton pair (H33, H37) appear as the highest resonating signal as a doublet at 8.15 ppm ($J=9.2$ Hz, 2H) due to the combined influence of both the carbonyl and methoxy group. The aromatic multiplet at 7.30 ppm (m, 6H) corresponds to H17 as well as the five protons of the phenylmaleimide aromatic ring (H21, H22, H23, H24, H25). The lower resonating aromatic multiplet signal at 7.23 ppm refers to H16 and the four other proton signals on the opposite aromatic ring of the polycyclic core (H12, H13, H14, H15). H19 shows as a doublet signal at 7.59 ppm ($J=7.2$ Hz, 1H), while H18 appears as an apparent triplet signal at 7.38 ppm ($J=4.8$ Hz). The doublet at 7.81 ppm ($J=16.27$ Hz, 1H) refers to H28 of the α, β -unsaturated ketone, while H27 resonates as a doublet at 7.89 ppm ($J=16.27$ Hz, 1H) due to the inductive influence of the conjugated carbonyl group at position 27. The alkene coupling constant supports the retention of the *trans* configuration of the double bond (>12 Hz)²⁷⁶.

The ^{13}C NMR (400 MHz, $\text{DMSO-}d_6$) spectrum (Figure 111) of (*E*)-9-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**111**) shows a signal at 45.4 ppm which corresponds C7 of the centre ring of the polycyclic core. C3 and C8 of the aliphatic ethanoanthracene bridge appear at 48.2 ppm and 48.3 ppm respectively. The carbon C4 appears as a signal at 52.04 ppm. The methyl group (C39) of the *p*-methoxyaryl substituent refers to the signal at 56.0 ppm. The alkene carbons (C27, C28) of the double bond refer to the signals at 141.3 ppm and 132.1 ppm respectively due to the inductive effect of the conjugated carbonyl group and the resultant partial charges created.

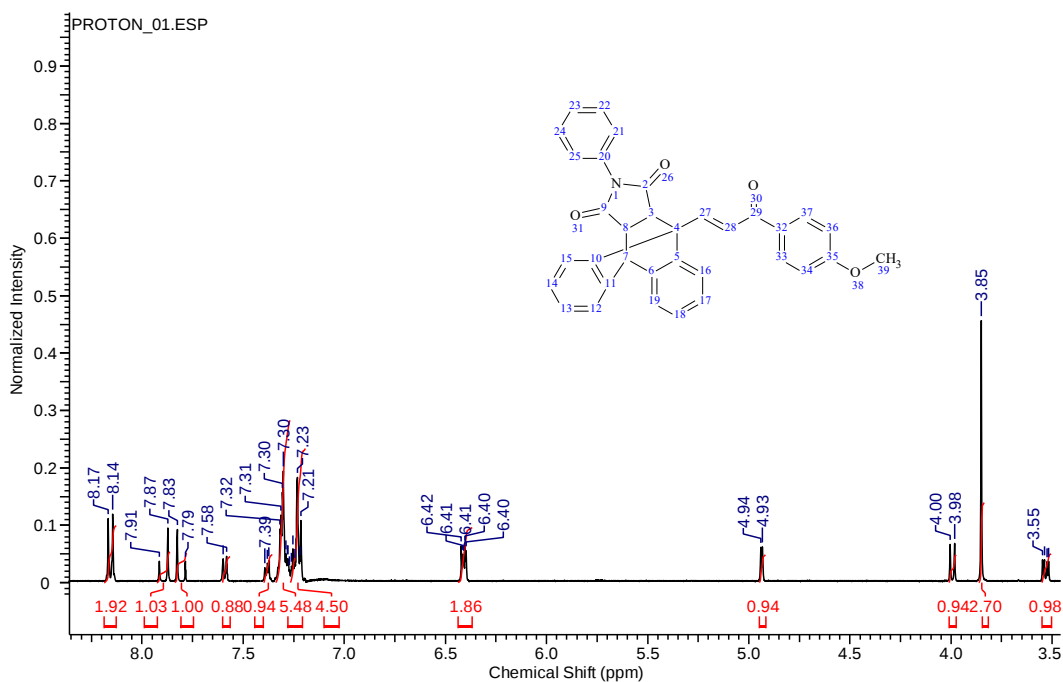


Figure 109: ^1H NMR spectrum of *(E)*-9-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (111)

The quaternary carbon C20 for the phenylmaleimide aromatic ring is represented by the signal at 130.5 ppm. C23 resonates at 129.1 ppm, while the adjacent carbon pair (C22, C24) are observed at 129.3 ppm. C21 and C25 correspond to the signal at 124.5 ppm, while C17 appears as a signal at 123.3 ppm. The remaining CH carbons of the anthracene core C12, C13, C14 and C15 refer to the signals at 127.4 ppm, 127 ppm, 126.9 ppm and 123.8 ppm. The carbon pair (C33, C37) are observed at 131.5 ppm, while C34 and C36 appear as a signal at 127 ppm, while the quaternary carbon C32 corresponds to the signal at 132.1 ppm. C35 of the acetophenone-derived aromatic ring directly connected to the methoxy group resonates at 163.9 ppm due to the inductive effect of the adjacent oxygen atom, in addition to the influence of the conjugated carbonyl group. The carbonyl of the α,β -unsaturated ketone functional group is the highest resonating signal in the spectrum at 188.3 ppm, while the carbonyl signals of the phenylmaleimide (C2, C9) appear at 175.3 and 175.9 respectively. The remaining quaternary carbon pairs of the polycyclic core (C5, C6) and (C10, C11) were assigned to the signals (142.8 ppm, 141.7 ppm) and (140.2 ppm, 139.2 ppm) respectively.

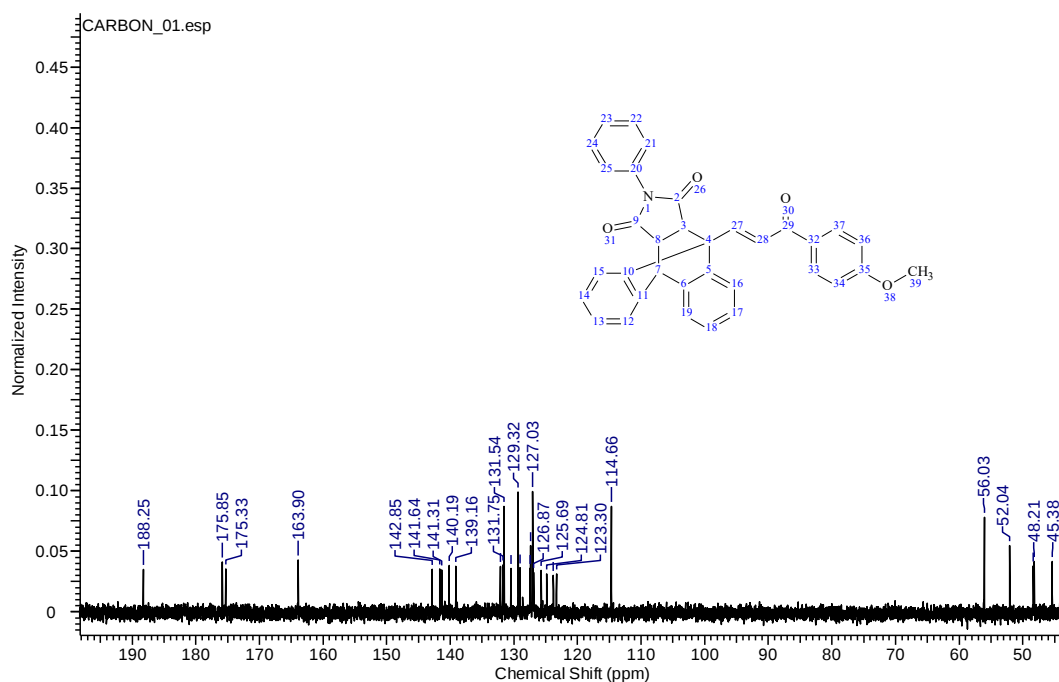


Figure 110: ^{13}C NMR spectrum of (*E*)-9-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**111**)

The DEPT 90 spectrum (Figure 112) of compound (**111**) was used in the assignment of the alkene signals C27 (141.3 ppm) and C28 (132.1 ppm). In addition, the spectrum was also used for the assignment of methyl of the methoxyaryl functional group C39 (56 ppm). The HSQC spectrum (Figure 113) was used in the identification of the corresponding carbon signals for the protons of the ethanoanthracene aliphatic bridge (C3, C7, C8) at 48.2 ppm, 45.3 ppm and 48.3 ppm respectively. The HMBC spectrum (Figure 114) was used in the assignment of quaternary carbons (C5, C6) and (C10, C11) appearing at (142.8 ppm, 141.7 ppm) and (140.2 ppm, 139.2 ppm), which were identified on comparison of ^{13}C NMR (Figure 111) and DEPT 90 (Figure 112).

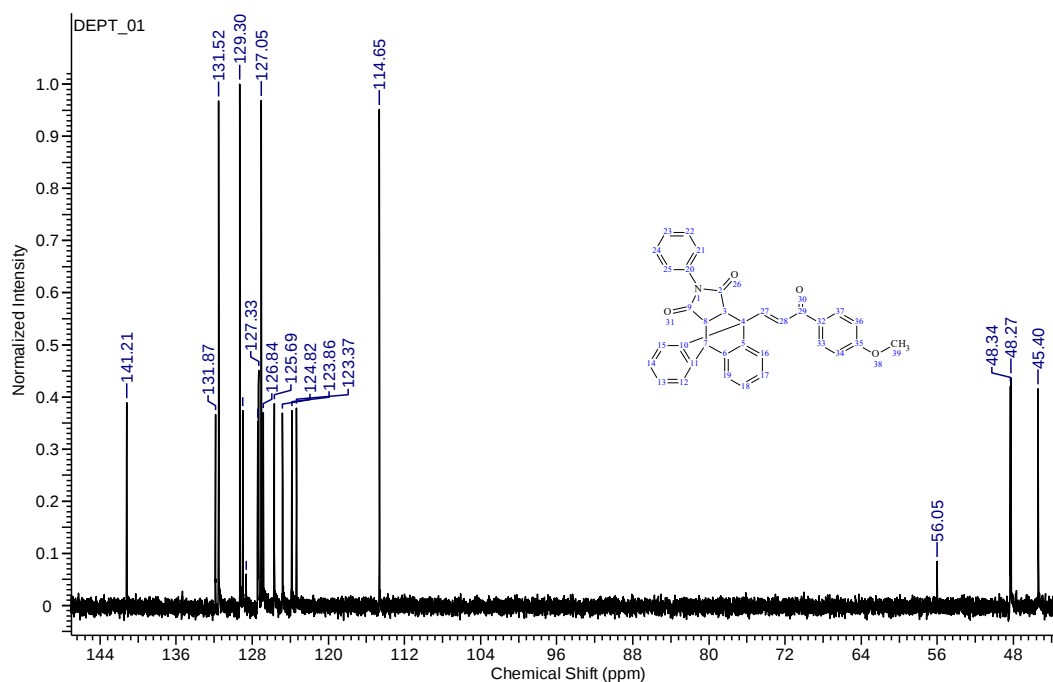


Figure 111: DEPT 90 spectrum of (*E*)-9-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (111)

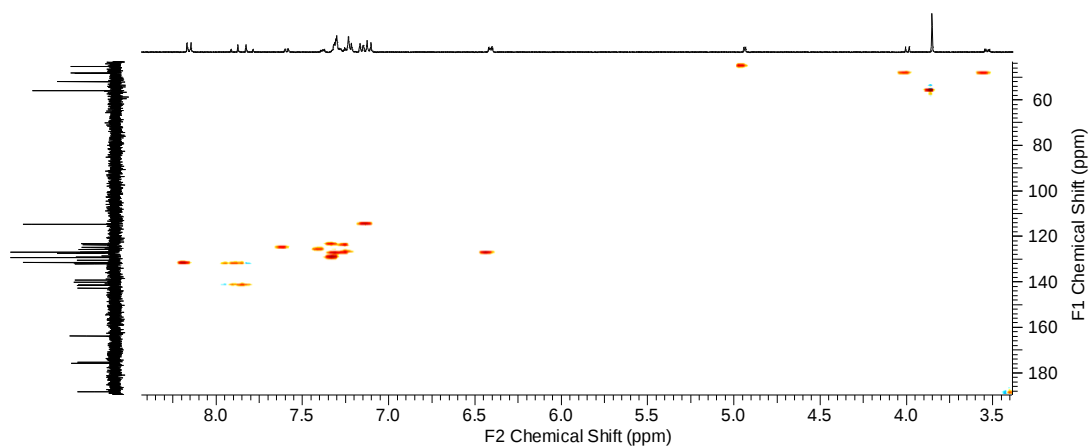


Figure 112: HSQC spectrum of (*E*)-9-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (111)

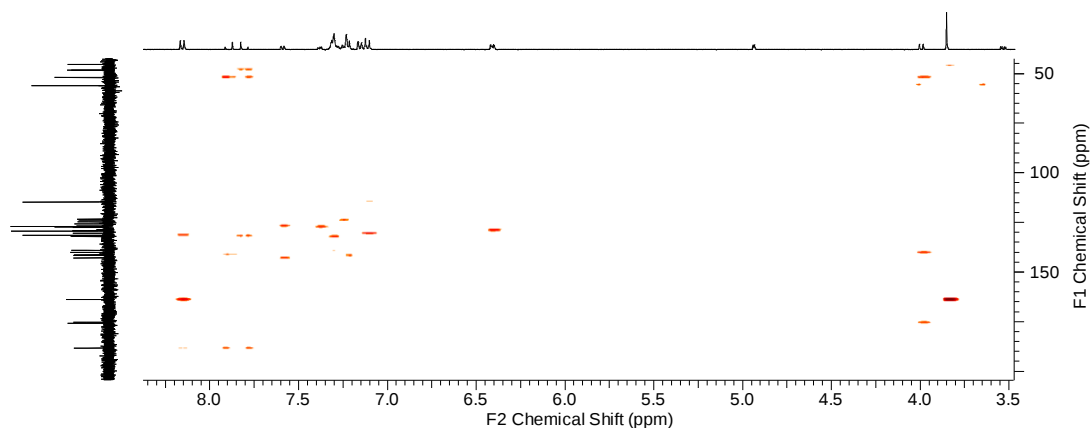


Figure 113: HMBC spectrum of of (*E*)-9-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**111**)

The IR spectrum (Figure 115) confirmed the structure of (**111**) and shows bands at 1712.75 cm^{-1} (C=O of α,β -unsaturated ketone), 1777.01 cm^{-1} (C=O groups of the maleimide moiety), 3337.59 cm^{-1} (secondary amide NH stretch), 1673.00 cm^{-1} (secondary amide NH bend), 1584.03 cm^{-1} (C-N stretch), 2928.53, 2965.89, 3064.98 cm^{-1} (aromatic C-H stretch), 1458.12, 1634.37 cm^{-1} (aromatic C=C stretch), 1602.78 cm^{-1} (*trans* C=C stretch) and 1174.75, 999.53 cm^{-1} (aryl C-O signal).

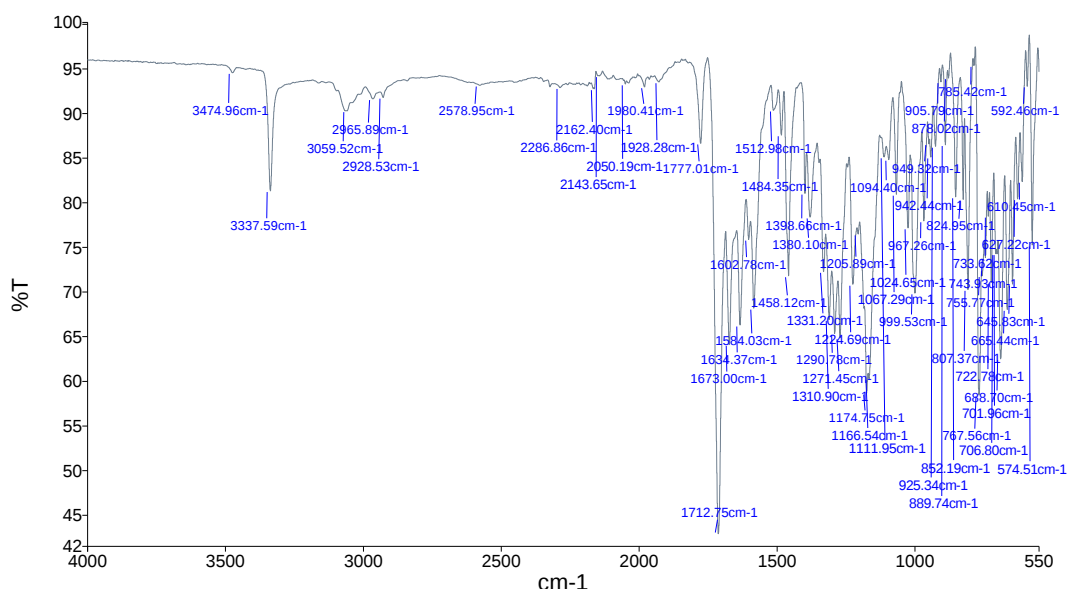


Figure 114: IR spectrum of (*E*)-9-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**111**)

2.5.18 X-ray crystallography of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (103)

Additional evidence for the retention of the vinyl bond stereochemistry and the *trans*/*E* configuration was obtained from the X-ray crystallography of a representative ethanoanthracene maleimide structure (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**103**) which clearly confirms the molecular structure and shows the *trans* alkene bond of the α,β -unsaturated ketone group (Figure 116).

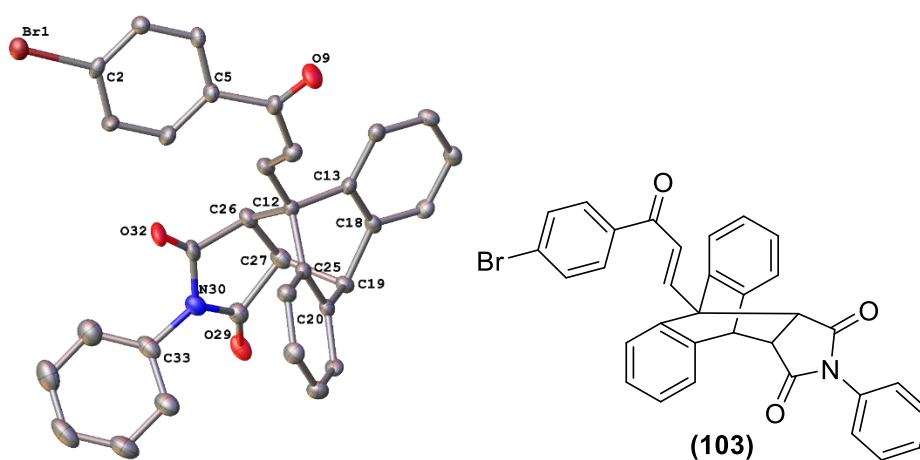


Figure 115: X-ray structure of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**103**)

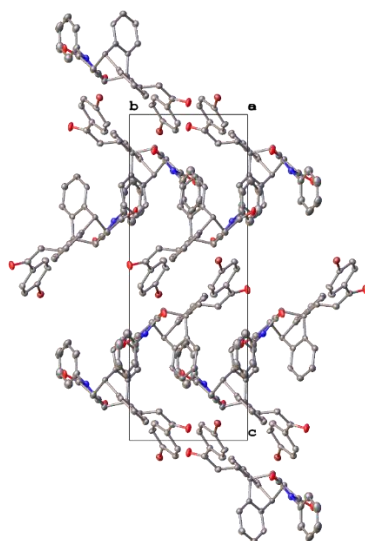


Figure 116: Packing arrangement of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**103**)

The crystal was found to be monoclinic with a sinusoidal packing nature (Figure 116-118). The bond length of C₁₀-C₁₁ was consistent with that of an alkene double bond (1.335 Å) and the bond length of O₉-C₈ consistent with a carbonyl functional group (1.227 Å). The presence of the alkene double bond is also supported through the bond angles of C₁₀-C₁₁-C₁₂ and C₈-C₁₀-C₁₁ which were 123.9° and 125.6° respectively, indicating the trigonal-planar nature expected of such a functional group side-chain^{344, 345}. The presence of the maleimide functional group was supported by the bond lengths of O₃₂-C₃₁ and O₂₉-C₂₈ measuring 1.18 Å and 1.19 Å, typical of carbonyl functional groups. Furthermore, N₃₀-C₃₃ was observed to be 1.36 Å, within the expected range of conjugated C-N bonds³⁶⁶.

Empirical formula	C ₃₃ H ₂₂ BrNO ₃
Formula weight	560.42
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2 ₁ /c
Unit cell dimensions	a = 13.5908(4) Å b = 8.0761(2) Å c = 23.2409(6) Å α = 90° β = 106.0230(10)° γ = 90°
Volume	2451.83(11) Å ³
Z	4
Density (calculated)	1.518 Mg/m ³
Absorption coefficient	1.715 mm ⁻¹
F(000)	1144
Crystal size	0.23 x 0.15 x 0.12 mm ³
Theta range for data collection	2.965 to 26.141°
Index ranges	-16 ≤ h ≤ 16, -10 ≤ k ≤ 10, -28 ≤ l ≤ 28
Reflections collected	65657
Independent reflections	4872 [R(int) = 0.0860]
Completeness to theta = 25.242°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7453 and 0.6766
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	4872 / 1044 / 566
Goodness-of-fit on F²	1.063
Final R indices [I > 2σ(I)]	R1 = 0.0388, wR2 = 0.0758
R indices (all data)	R1 = 0.0622, wR2 = 0.0832
Largest diff. peak and hole	0.351 and -0.453 e.Å ⁻³

Figure 117: Crystal data details and structural refinement for (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (103)

The synthesis of an example of the series (**103**) was also achieved by reacting the corresponding maleic anhydride chalcone adduct (**75**) with aniline to form (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**103**) through acid catalysed dehydration and subsequent cyclisation of the amic acid intermediate (Figure 119). A lower yield with this method (72%) was obtained compared to direct Diels–Alder cycloaddition of the chalcone (**39**) and N-phenylmaleimide (84%). However, it had the advantage of avoiding recrystallisation and the affording of a highly pure product. The product was identical to the previously obtained sample, confirmed by ^1H NMR and ^{13}C NMR (see Chapter 5: Experimental).

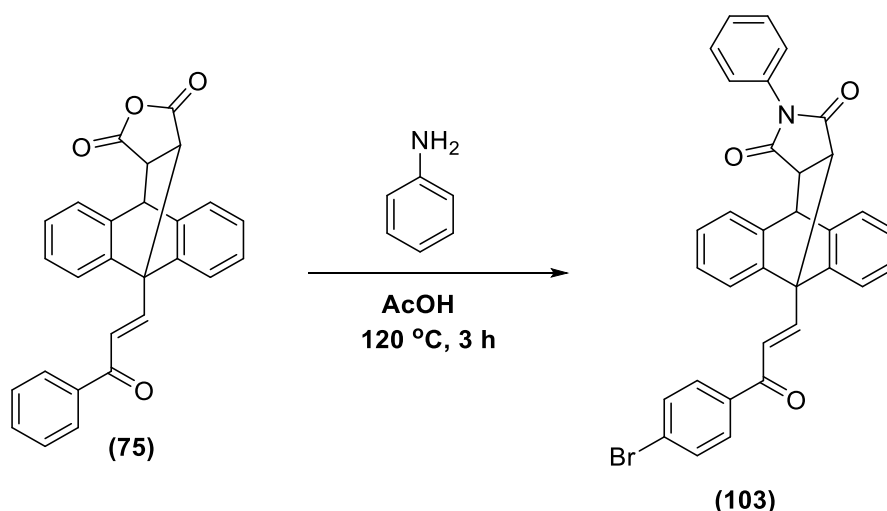


Figure 118: General reaction scheme for the synthesis of (*E*)-9-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-13-phenyl-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**103**)

The mechanism (Figure 120) by which the reaction occurs firstly involves the nucleophilic attack of the maleic anhydride group carbonyl group, leading to acid-mediated ring cleavage and the formation of the corresponding amic acid intermediate. The lone pair of electrons on the amic acid then act as a nucleophile to attack carbonyl carbon of acetic acid. Dehydration subsequently takes place leading to an amic acetate intermediate. The α -carbon closest to the acetate functional group undergoes nucleophilic attack by the lone pair of electrons on the nitrogen of the secondary amide. This results in cyclisation of bridgehead structure to generate a substituted maleimide head group, in addition to the regeneration of the acetic acid catalyst.

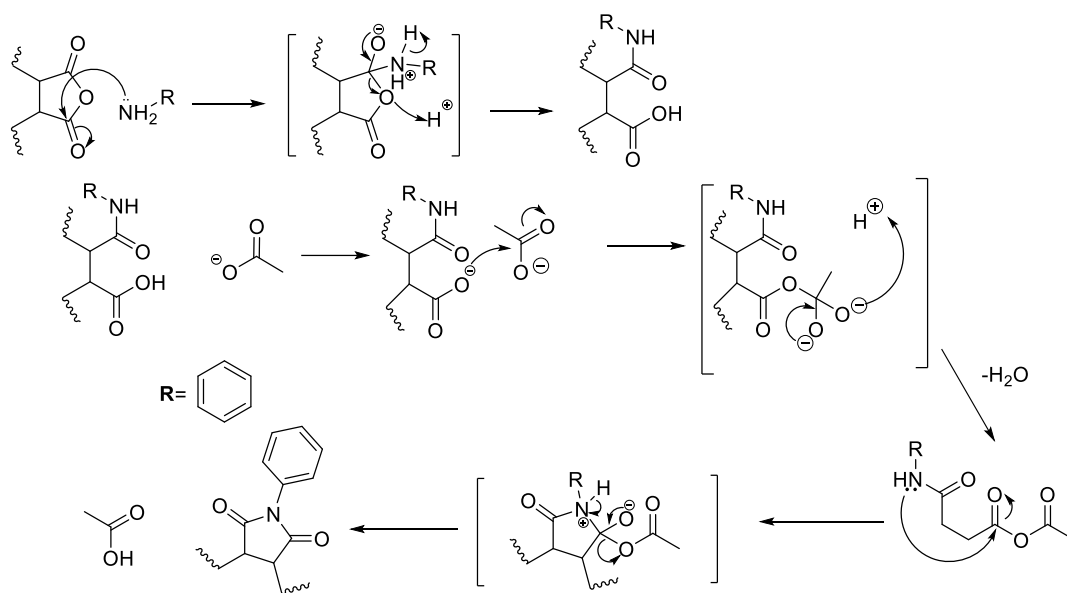


Figure 119: Proposed mechanism for the synthesis of (103) using aniline-maleic anhydride method

2.5.20 Series 7: Synthesis of (*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

The synthesis of the (*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (Series 7) (**120-136**) was carried out utilizing the described Diels-Alder reaction with the previously discussed substituted chalcone structures acting as a diene system and *p*-chlorophenylmaleimide as the dienophile. Poor to excellent yields of 3-95 % were obtained for the compound series and were obtained as solids after recrystallisation from toluene (Figure 121, Tables 23-26). The products contained three structurally distinct regions consisting of a 1-(4-chlorophenyl)pyrrolidine-2,5-dione bridgehead, the anthracene-derived central core and an α,β-unsaturated ketone with diverse R substitutions (detailed in Table 26).

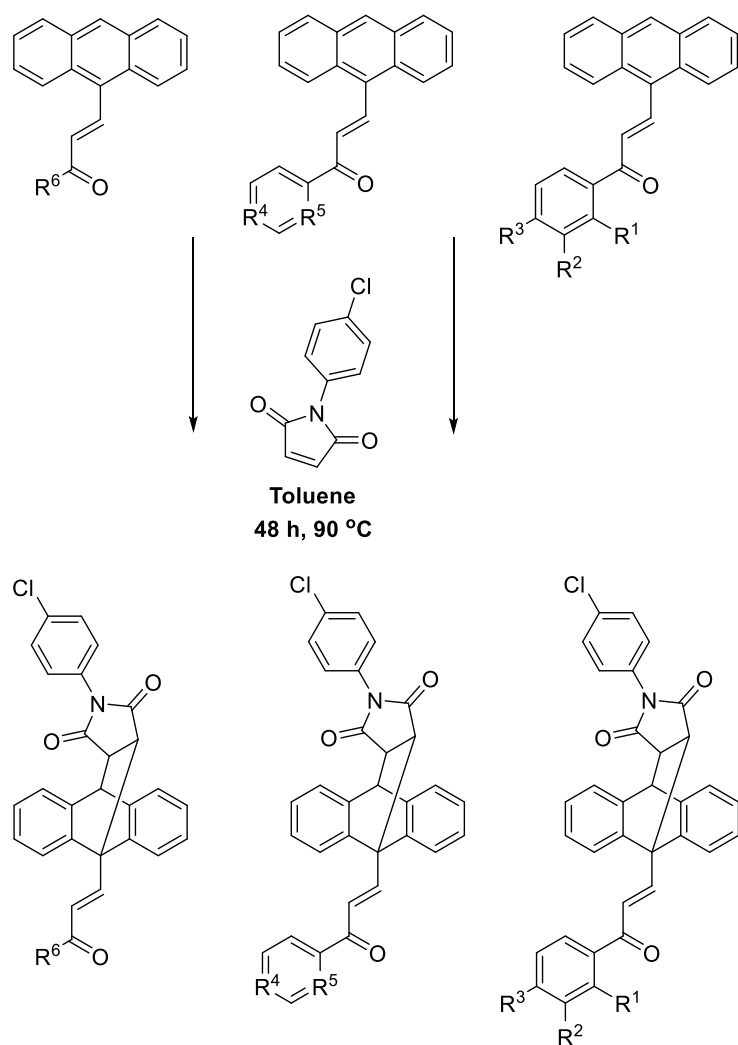


Figure 120: General reaction scheme for the synthesis of (*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

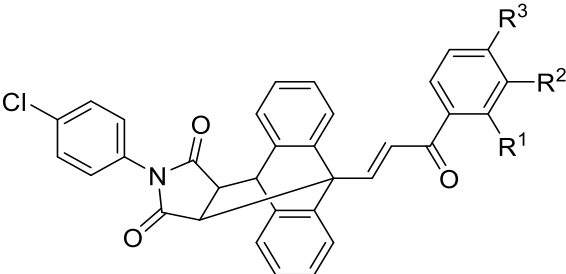
												
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)	No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(120)	H	H	Br	-	-	95	(126)	H	H	I	-	92
(121)	H	H	NO ₂	-	-	82	(127)	H	H	F	-	88
(122)	H	H	CH ₂ CH ₃	-	-	3	(128)	H	H	OCH ₃	-	63
(123)	Cl	H	Cl	-	-	87	(129)	H	H	CH ₃	-	60
(124)	H	OCH ₃	OCH ₃	-	-	4	(130)	OCH ₃	OCH ₃	OCH ₃	-	35
(125)	H	H	H	-	-	48	(131)	H	H	Cl	-	39

Table 23: Type 1 (*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione series (120-131)

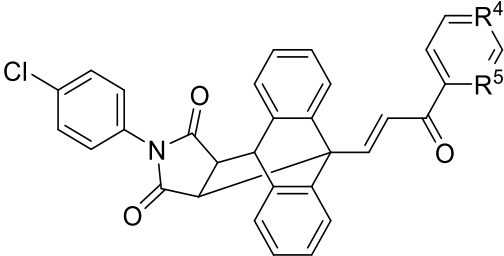
						
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)
(132)	-	-	-	N	H	
(133)	-	-	-	H	N	

Table 24: Type 2 (*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (132, 133)

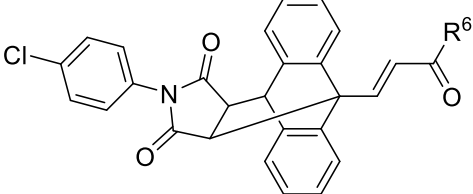
					
No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(134)	-	-	-	C ₄ H ₃ O (furan)	
(135)	-	-	-	C ₁₀ H ₇ (Naphthyl)	
(136)	-	-	-	C ₄ H ₃ S (thiophene)	

Table 25: Type 3 (*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (134-136)

<i>p</i>-Chlorophenymaleimide adducts			
(120)* (95%)	(121)* (82%)	(122)* (3%)	(132)* (92%)
(123)* (87%)	(124)* (4%)	(133)* (57%)	(134)* (46%)
(135)* (88%)	(136)* (70%)	(127)* (88%)	(126)* (92%)
(125)* (48%)	(131)* (39%)	(128)* (63%)	(129)* (60%)
(130)* (35%)			

Table 26: Structures of the (*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrruloanthracene-12,14-dione series and yields (where * denotes novel compounds synthesised)

2.5.19 Structural characterisation of (*E*)-13-(4-Chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**130**)

(*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**130**) was chosen as the representative example for this series and its ^1H NMR (Figure 122) and ^{13}C NMR spectra (Figure 123) will now be discussed. The numbering of the compound (**130**) was generated in ACD/ChemSketch version 12.01.

The ^1H NMR (400 MHz, $\text{DMSO-}d_6$) spectrum of (*E*)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**130**) (Figure 122) shows a double doublet signal at 3.53 ppm ($J=5, 3.3$ Hz, 1H), which corresponds to H8 of the ethanoanthracene bridge. The singlet signal of H42 (3H, CH_3) appears at 3.76 ppm, which are on 4 position of the trimethoxy aryl substituted ring. The two methyl groups H40 and H44 of the methoxy moieties at positions 3 and 5 of the benzoyl substituent resonate at 3.85 ppm (6H, $2 \times \text{CH}_3$). The remaining proton of the carbon bridge H3 is represented by the doublet signal at 4.00 ppm ($J=8.3$ Hz, 1H). H7 on the centre ring of the polycyclic anthracene core is observed as a doublet signal at 4.94 ppm ($J=3.3$ Hz, 1H). The broad signal at 7.47 ppm (2H) corresponds to the proton pair of H34 and H38 of the acetophenone-derived aromatic ring which appears as a singlet due to the absence of neighbouring protons.

H22 and H24 are represented by the doublet signal at 6.47 ppm ($J=8.7$ Hz, 2H), appearing lower than expected for an aromatic signal due to the deactivating and electron donating effect of the chlorine atom in the *ortho* position relative to the protons. The signals of the alkene double bond of the α,β -unsaturated ketone functional group are represented by H26 and H27 which resonate as doublets at 7.80 ppm ($J=16.2$ Hz, 1H), and 7.88 ppm ($J=16$ Hz, 1H) respectively, with H27 appearing at a higher chemical shift due to the inductive effect of the carbonyl carbon. The corresponding coupling constant supports the retention of the *E/trans* configuration on the alkene double bond in product formation, which has a typical range of (12-16 Hz)²⁷⁶.

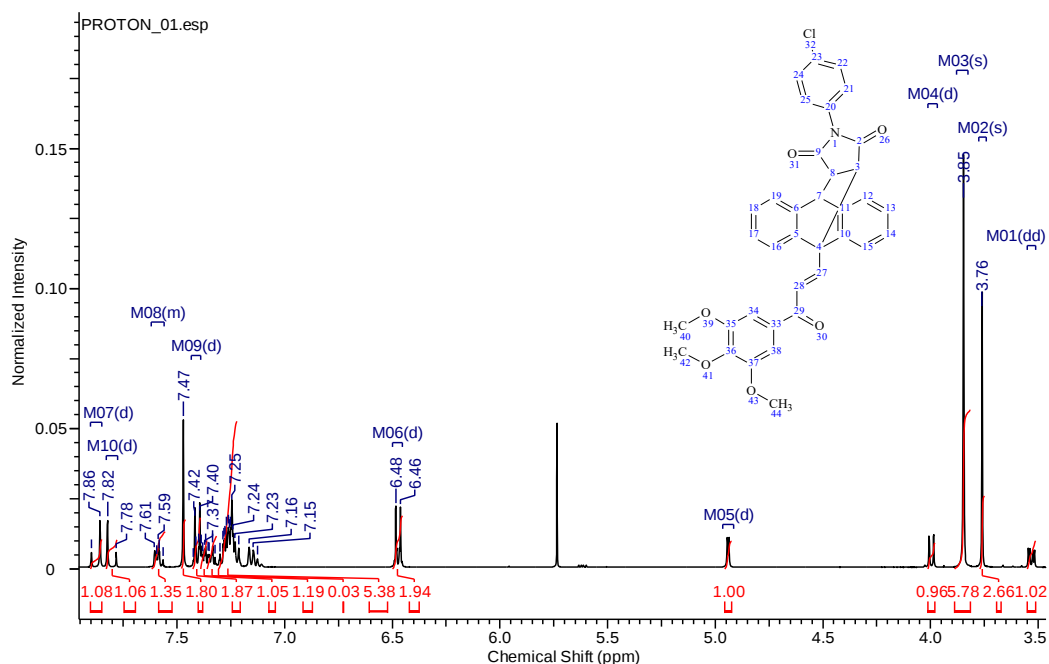


Figure 121: ^1H NMR spectrum of *(E)*-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**130**)

The ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) spectrum of *(E)*-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**130**) (Figure 123) shows C8 of the ethanoanthracene bridge resonates at 45.4 ppm, while C3 is observed at a higher chemical shift of 48.3 ppm. C7 of the central aliphatic ring of the polycyclic core appears at 48.6 ppm. The signal at 52.1 ppm corresponds to the two methyl group carbons C40 and C44, while C42 is represented by the signal at 60.7 ppm. The carbon pair (C34, C38) resonate at 106.9 ppm due to the electron donating effect of the three trimethoxy groups on the aromatic ring, hence leading to greater shielding from the applied magnetic field. The most downfield signal in the spectrum is C29 at 189.1 ppm, representing the carbonyl group of the α,β unsaturated ketone functional group. The carbons of the alkene chalcone structure (C27, C26) were assigned to 141.5 ppm and 132.5 ppm respectively, being observed at a high chemical shift due to the inductive effect of the carbonyl on the conjugated double bond. The signal observed at 125.7 ppm corresponds to the carbon pair (C21, C25) of the N-phenylmaleimide substituent aromatic ring. C22 and C24 are represented by the signal at 129.5 ppm. The remaining carbon pair on the substituted phenyl ring (C21, C25) resonate at 128.6 ppm. C23 adjacent to the chlorine atom in the *para* position of the ring appears at 133.5 ppm and the signal at 130. ppm corresponds to C20 attached to the nitrogen of the cyclic imide, both resonating

higher than typical aromatic signals due to the electronegative influence of chlorine and nitrogen respectively.

The quaternary carbons C35 and C37 appear at 142.6 ppm and 142.8 ppm, with C36 resonating at 153.4 ppm due to the electron withdrawing effect of the alkene carbonyl on the acetophenone aromatic ring. C33 showed as a signal at 133.1 ppm. C19 was observed at 124.9 ppm, while C18, C17 and C16 were noted at 125.7 ppm, 126.9 ppm and 123.3 ppm respectively. C12 was assigned to the signal at 129.7 ppm. The quaternary carbons on the central ring of the anthracene core C5 and C10 were observed at 142.6 ppm and 142.8 ppm respectively, while C6 and C11 corresponded to the signals at 139.2 ppm and 140.1 ppm. C13, C14 and C15 were observed as 127.4 ppm, 127.5 ppm and 124 ppm.

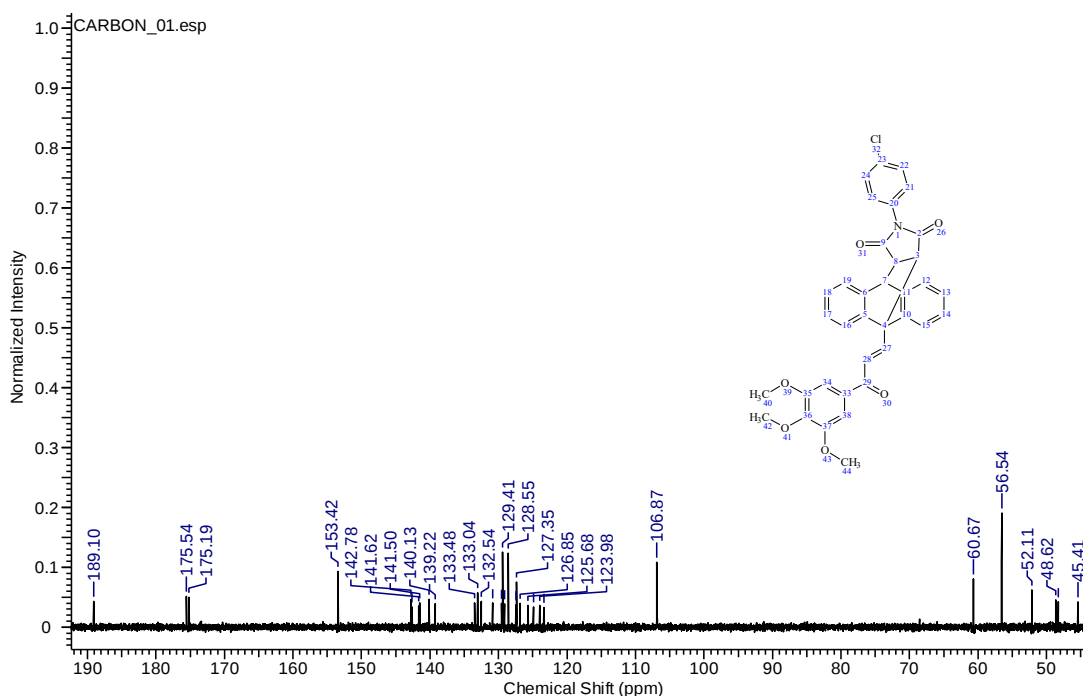


Figure 122: ^{13}C NMR spectrum of *(E)*-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**130**)

The DEPT 90 spectrum (Figure 124) was used in the assignment of the aromatic CH tertiary carbons (e.g. C21, C25) as well as the alkene signals of the double bond (C27, C26) and the ethanoanthracene bridge on the centre ring (C3, C7 and C8). Initial proton signal assignment was confirmed using the HSQC spectrum (Figure 125) and was also (in conjunction with the DEPT 90 spectrum) used to establish the presence of quaternary carbons e.g. C20. The assignment of carbon signals (in particular quaternary carbon signals) were confirmed *via* HMBC spectrum (Figure 126) of the molecule.

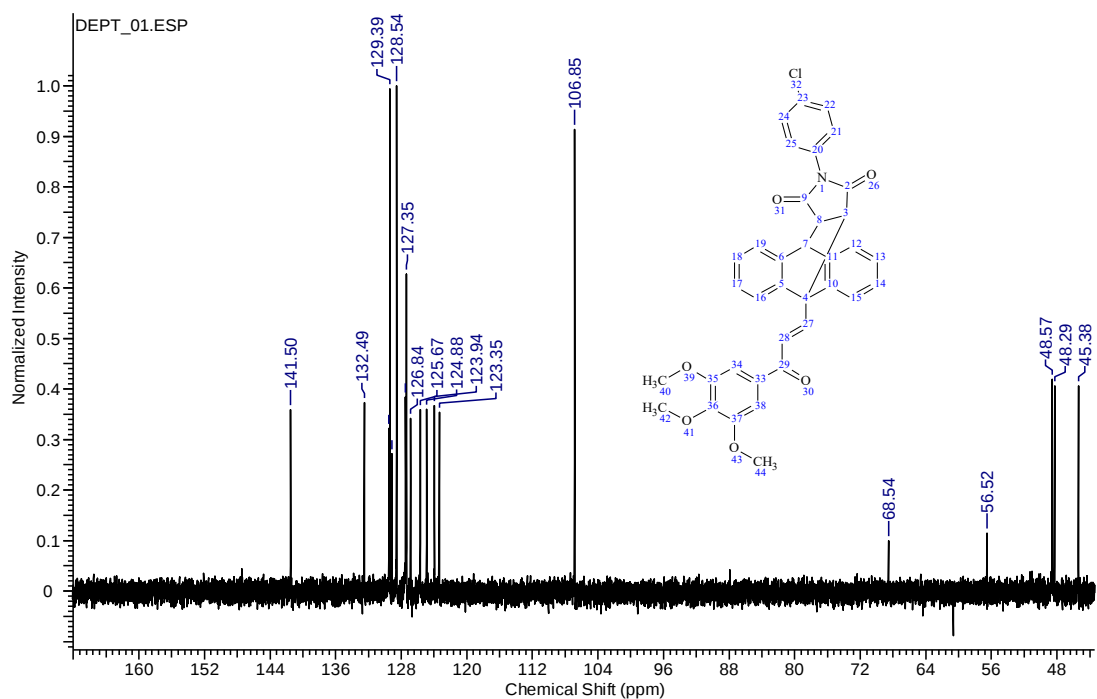


Figure 123: DEPT 90 spectrum of (E)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (130)

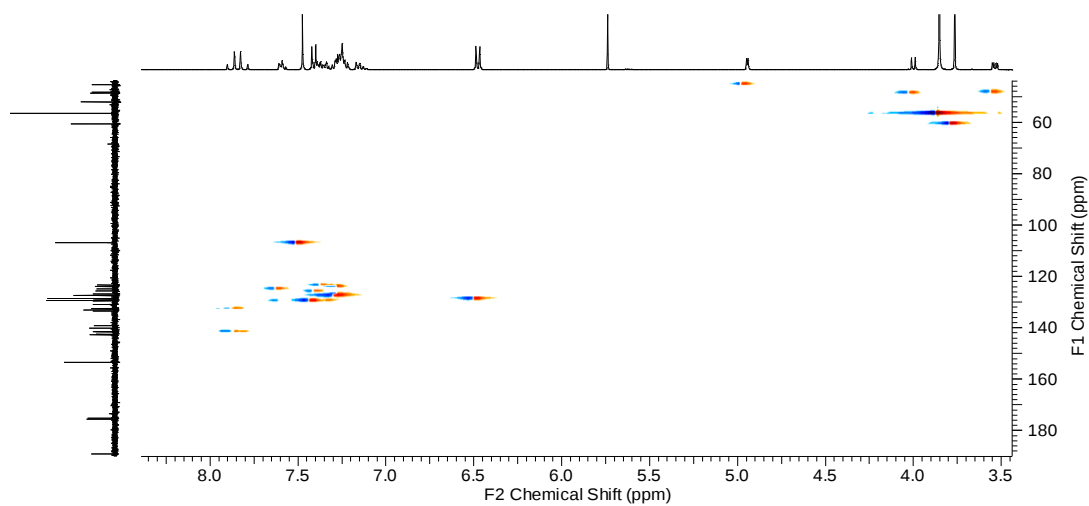


Figure 124: HSQC spectrum of (E)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (130)

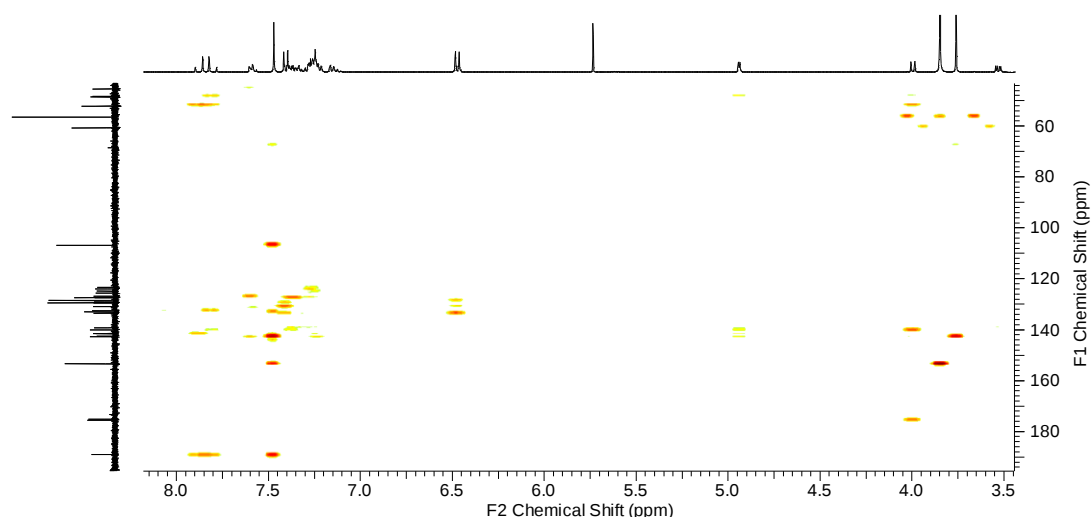


Figure 125: HMBC spectrum of (E)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**130**)

The IR spectrum below (Figure 127) confirmed the structure of (**130**) and shows bands at 1711.53 cm^{-1} (C=O of α,β -unsaturated ketone), 1778.30 cm^{-1} (C=O groups of the maleimide moiety), 3337.27 cm^{-1} (secondary amide NH stretch), 1672.90 cm^{-1} (secondary amide NH bend), 1583.00 cm^{-1} (C-N stretch), 2839.73 , 2967.31 , 3065.70 cm^{-1} (aromatic C-H stretch), 1458.17 , 1634.70 cm^{-1} (aromatic C=C stretch), 1604.27 cm^{-1} (*trans* C=C stretch), 1121.50 cm^{-1} (aryl C-Cl) and 1175.27 , 1003.70 cm^{-1} (aryl C-O).

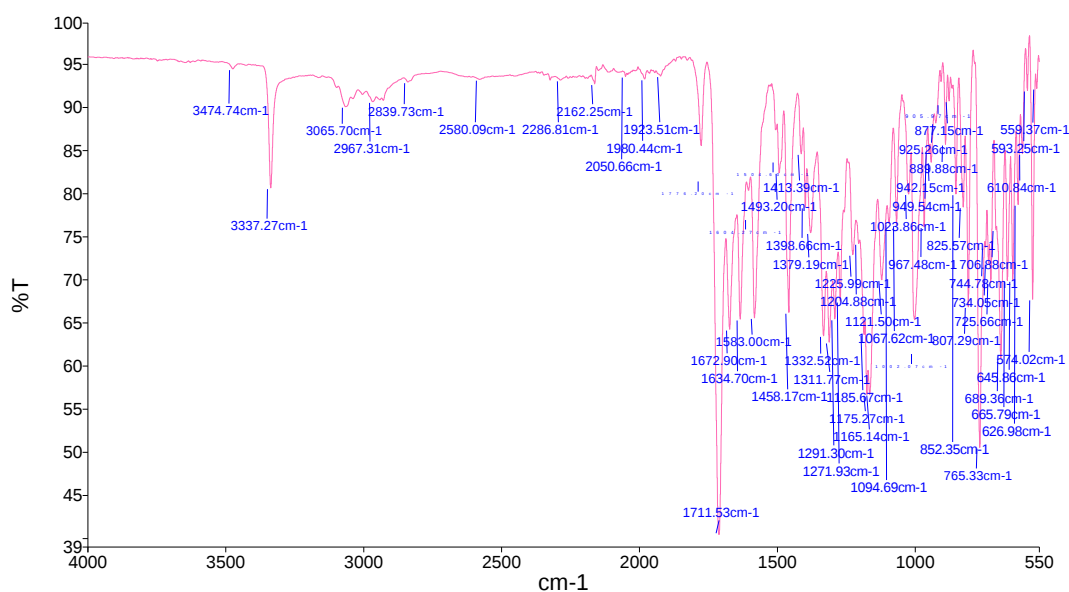


Figure 126: IR spectrum of (E)-13-(4-chlorophenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**130**)

Series 8: Synthesis of (*E*)-13-(4-Benzoylphenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

The synthesis of the (*E*)-13-(4-benzoylphenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (**137-153**) was carried out utilizing the described Diels–Alder reaction with the previously discussed substituted chalcone structures acting as a diene system and *p*-benzophenonemaleimide as the dienophile. Fair to excellent yields of 30-85% were obtained for the compound series and were obtained as solids after recrystallisation from toluene (Figure 128, Tables 27-30). The structure of this series of compounds was distinct from previous series discussed due to the steric bulk of the benzophenone-substituted maleimide as opposed to an aryl substituted maleimide, unsubstituted maleimide or anhydride functional groups being present in the heterocyclic ring scaffold e.g. series 4-7. The products obtained demonstrated three distinct structural regions consisting of a 1-(4-benzoylphenyl)pyrrolidine-2,5-dione bridgehead, the anthracene-derived central core and an α,β -unsaturated ketone with diverse R substitutions (detailed in Tables 27-30).

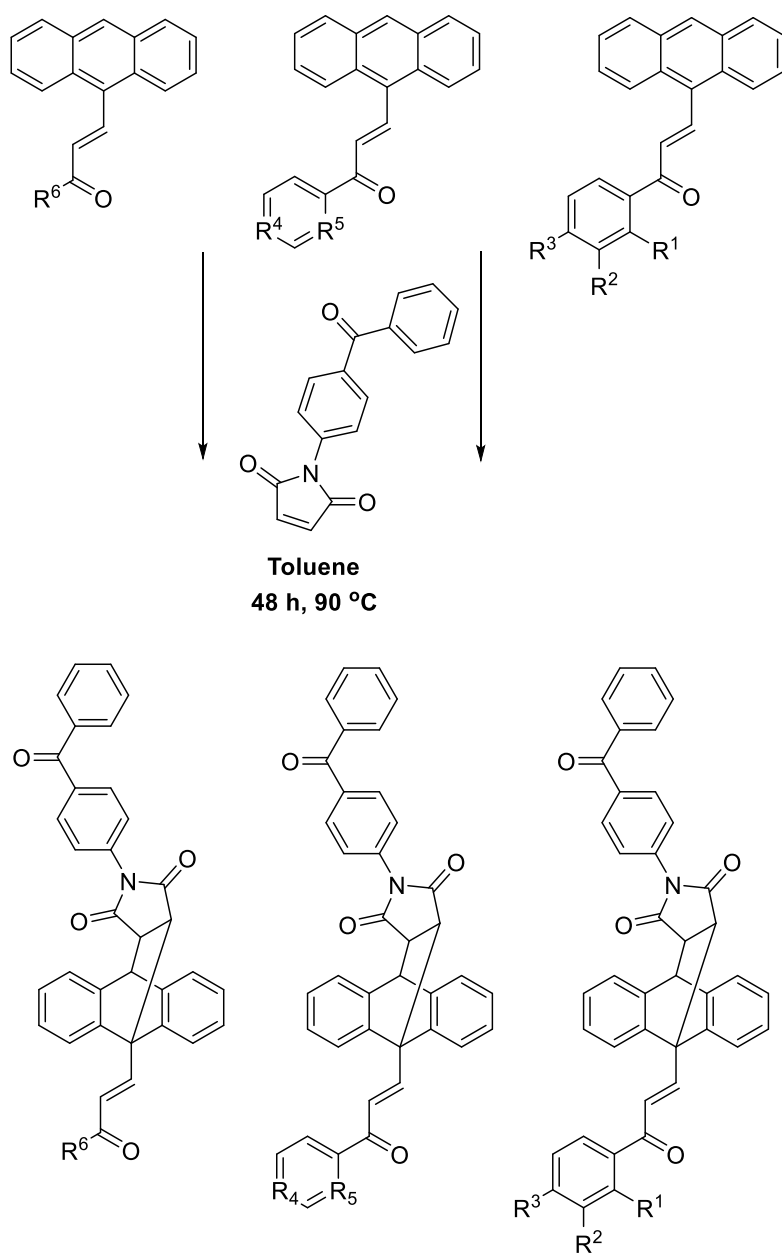


Figure 127: General reaction scheme for the synthesis of *(E)*-13-(4-benzoylphenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones

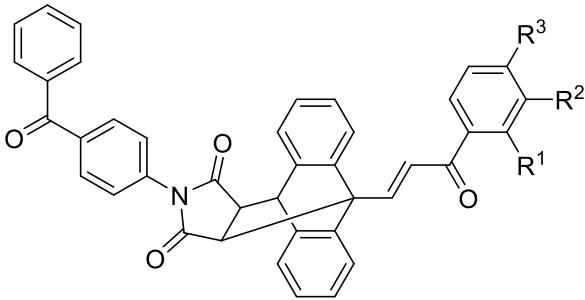
												
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)	No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(137)	H	H	Br	-	-	71	(143)	H	H	I	-	85
(138)	H	H	NO ₂	-	-	75	(144)	H	H	F	-	77
(139)	H	H	CH ₂ CH ₃	-	-	76	(145)	H	H	OCH ₃	-	50
(140)	Cl	H	Cl	-	-	63	(146)	H	H	CH ₃	-	59
(141)	H	OCH ₃	OCH ₃	-	-	72	(147)	OCH ₃	OCH ₃	OCH ₃	-	44
(142)	H	H	H	-	-	49	(148)	H	H	Cl	-	30

Table 27: Type 1 (*E*)-13-(4-benzoylphenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (137-148)

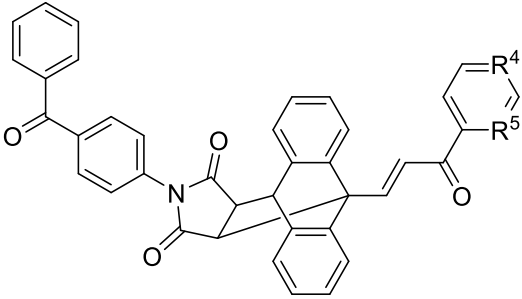
						
No.	R ¹	R ²	R ³	R ⁴	R ⁵	Yield (%)
(149)	-	-	-	N	H	62
(150)	-	-	-	H	N	75

Table 28: Type 2 (E)-13-(4-benzoylphenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (149, 150)

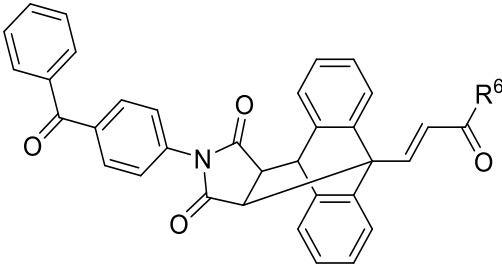
					
No.	R ¹	R ²	R ³	R ⁶	Yield (%)
(151)	-	-	-	C ₄ H ₃ O (furan)	60
(152)	-	-	-	C ₁₀ H ₇ (Naphthyl)	55
(153)	-	-	-	C ₄ H ₃ S (thiophene)	51

Table 29: Type 3 (E)-13-(4-benzoylphenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (151-153)

<i>p</i>-Benzophenonemaleimide derivatives			
(137)* (71%)	(138)* (75%)	(139)* (76%)	(149)* (62%)
(140)* (63%)	(141)* (72%)	(150)* (75%)	(151)* (60%)
(152)* (55%)	(153)* (51%)	(144)* (77%)	(143)* (85%)
(142)* (49%)	(148)* (30%)	(145)* (50%)	(146)* (59%)
(147)* (44%)			

Table 30: Structures of the (*E*)-13-(4-benzoylphenyl)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-diones (where * denotes novel compound synthesised)

2.5.20 Structural characterisation of (*E*)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**148**)

(*E*)-13-(4-Benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**148**) was chosen as a representative example for this series and its ^1H -NMR (Figure 129) and ^{13}C NMR spectra (Figure 130) will now be discussed. The numbering of the compound (**148**) was generated in ACD/ChemSketch version 12.01.

The ^1H NMR spectrum of (*E*)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**148**) (Figure 129) shows a double doublet signal at 3.58 ppm ($J=4.6, 3.3$ Hz, 1H) corresponding to H8 of the ethanoanthracene carbon bridge. H3 appears as a doublet signal at 4.04 ppm ($J=8.3$ Hz, 1H), while H7 of the centre ring of the polycyclic core resonates at 4.97 ppm as a doublet signal ($J=2.9$ Hz, 1H). The proton signals of the α,β -unsaturated ketone group (H27, H28) show as doublet signals at 7.91 ppm ($J=15.8$ Hz, 1H) and 7.88 ppm ($J=15.8$ Hz, 1H) respectively. This is due to the inductive effect of the conjugated carbonyl group at C29 of the molecule. In addition, the large coupling constants of the doublet signals (>12 Hz) support that the *E/ trans* configuration of the alkene double bond has been maintained throughout product synthesis²⁷⁶.

The doublet signal that appears at 8.17 ppm refers to H36 and H38 ($J=8.3$ Hz, 1H) of the acetophenone functional group aromatic ring closest to the carbonyl of the α,β -unsaturated ketone side chain. The remaining proton signals of this aromatic ring (H35, H39) appear as a doublet further upfield at 6.66 ppm ($J=8.7$ Hz, 1H). The triplet at 7.35 ppm ($J=4.56$ Hz $\times 2$, 1H) corresponds to H18, while H17 appears as a triplet at 7.40 ppm ($J=4.56$ Hz $\times 2$, 1H). The remaining aromatic proton signals of the anthracene core appear as a large multiplet at 7.25 ppm (6H) representing H12, H13, H14, H15, H16 and H19. This unexpected resonance potentially occurs due to the effect of the deshielding cones of the imide carbonyl groups and the possible formation of hydrogen bonding between the above mentioned carbonyl functional group oxygen atoms and the protons H17 and H18.

The proton pair (H42, H44) resonate as an apparent triplet at 7.53 ppm ($J=7.3$ Hz x2, 2H) on the outermost aromatic ring of the benzophenone moiety. H43 of the same aromatic ring appears as a multiplet at 7.62 ppm (1H). The remaining benzophenone aromatic protons appear as a large multiplet signal at 7.67 ppm (6H).

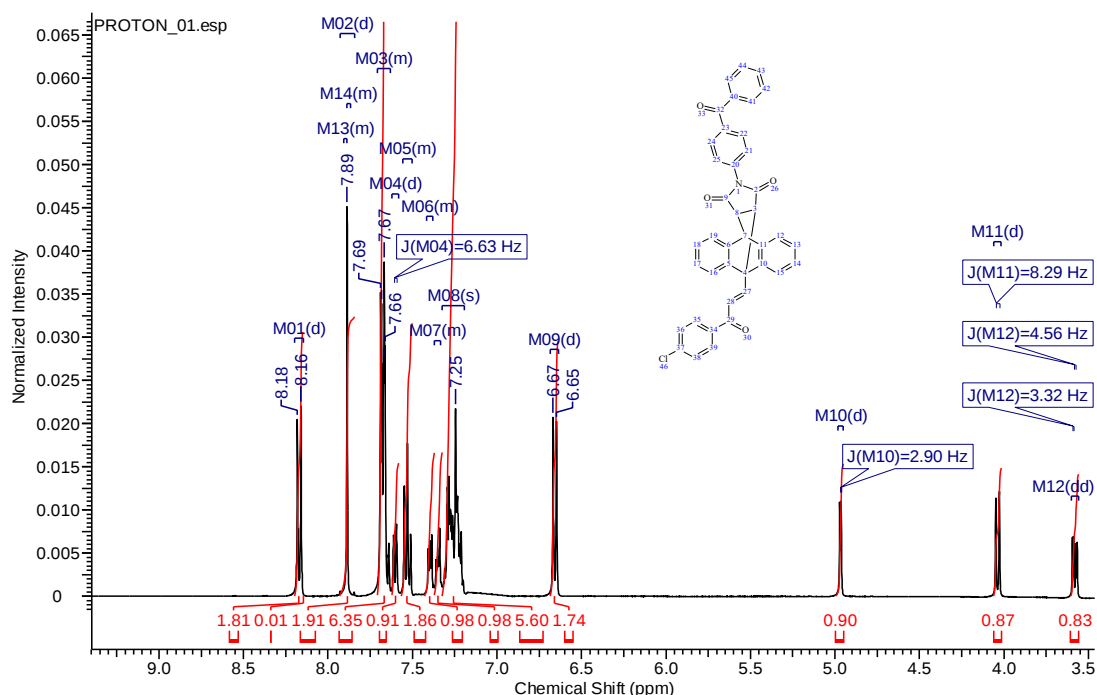


Figure 128: ^1H NMR spectrum of (E)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**148**)

The ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) spectrum of (E)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**148**) (Figure 130) shows the signals of the carbon ethanoanthracene bridge (C3, C8) at 45.5 ppm and 45.5 ppm respectively. The carbon signal at 45.3 ppm corresponds to C7 of the centre ring of the polycyclic core. C35 and C39 of the benzoyl aromatic ring appear at 131.0 ppm, while C36 and C38 at 127.0 ppm. The quaternary carbon C37 resonates at 138.9 ppm due to the adjacent chlorine atom and the remaining quaternary carbon of the acetophenone-derived aryl ring C34 corresponds to the signal at 137.4 ppm, due to the effect of the adjacent carbonyl group. C17 and C18 appear at 123.4 ppm and 125.8 ppm respectively, while the remainder of the anthracene core aromatic carbons (H12, H13, H14, H15, and H16 H19) resonate collectively at 124.0 ppm, 125.7 ppm, 126.8 ppm, 126.9 ppm, 127.0 ppm and 127.4 ppm. The carbon pair (C42, C44) of the benzophenone moiety correspond to the signal at 128.6 ppm, while C43 appears at

124.9 ppm. The remaining aromatic carbon signals of the benzophenone moiety (C20, C21, C22, C24, C25, C41, C45) correspond to the signals at 129.1 ppm, 129.3 ppm, 129.6 ppm, 130.1 ppm, 130.6 ppm and 133.4 ppm collectively.

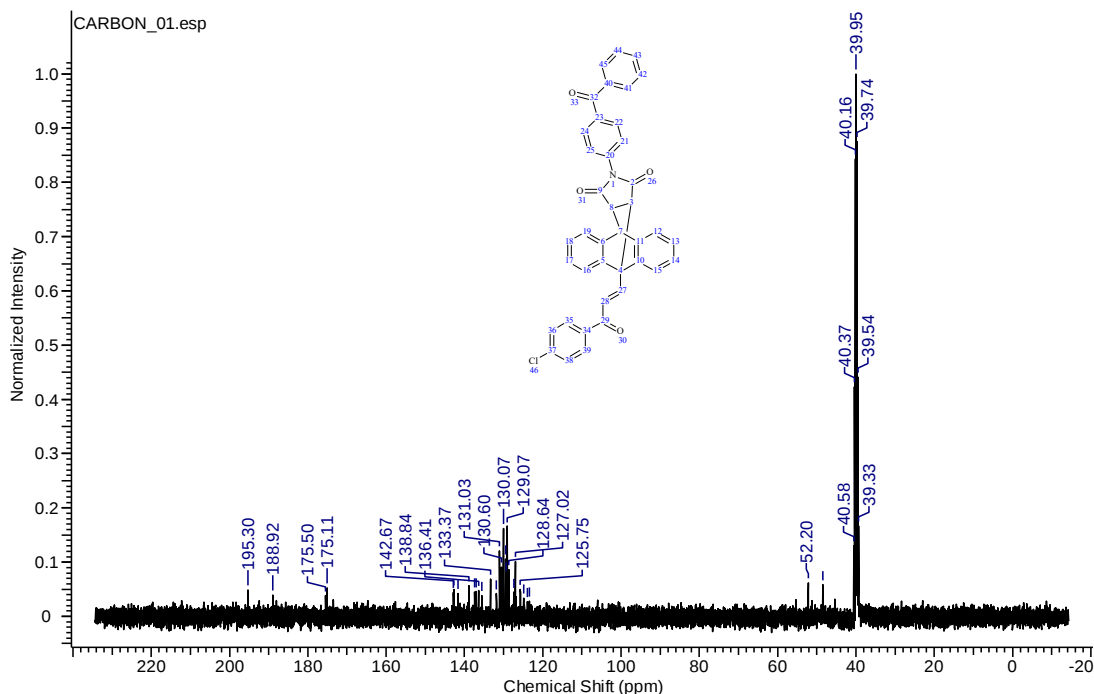


Figure 129: ^{13}C NMR spectrum of (E)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (148)

The alkene bond carbon signals (C27, C28) resonate at 142.8 ppm and 131.8 ppm, due to the inductive effect of the carbonyl dipole of the α,β -unsaturated ketone group. The maleimide carbonyl carbons C2 and C9 were represented by the signals at 175.1 ppm and 175.5 ppm respectively. The carbonyl group of α,β -unsaturated ketone group C29 shows at 188.9 ppm. The most downfield carbon signal in the spectrum at 195.3 ppm corresponds to C32 the carbonyl of the benzophenone-maleimide moiety, due to the effect of the adjacent aromatic rings.

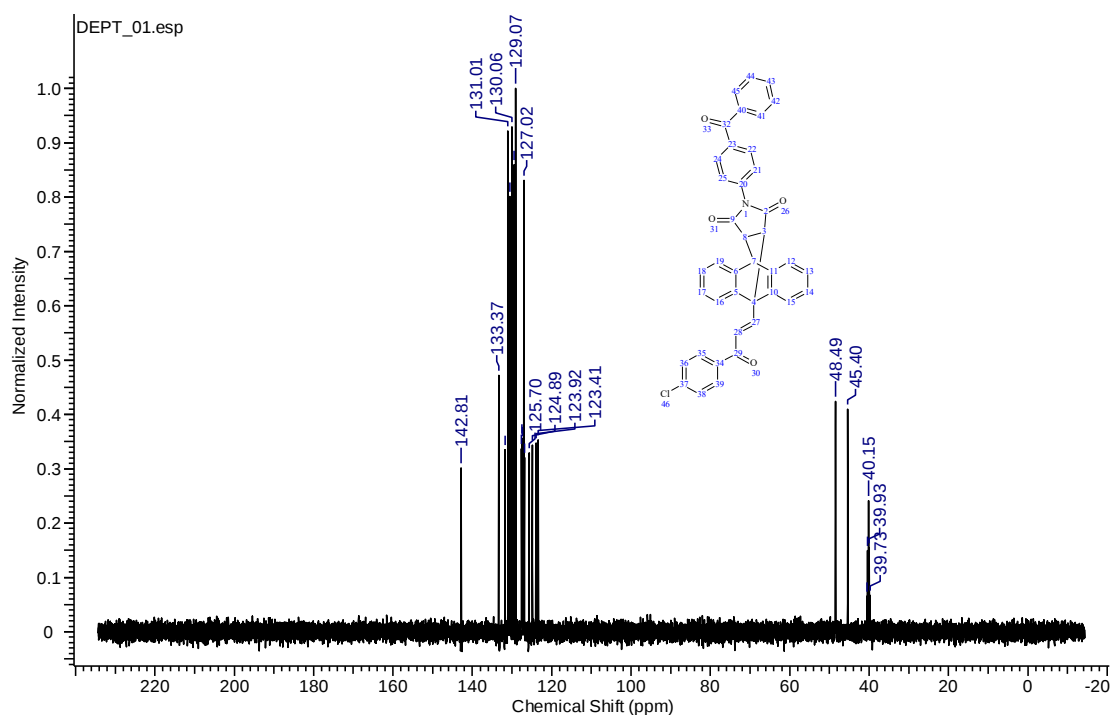


Figure 130: DEPT 90 spectrum of (E)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (148)

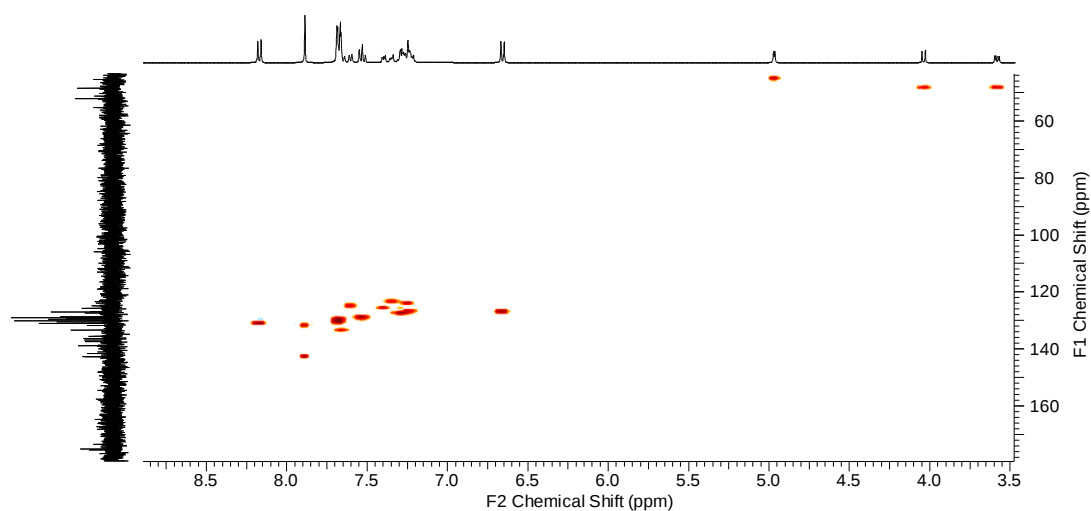


Figure 131: HSQC spectrum of (E)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (148)

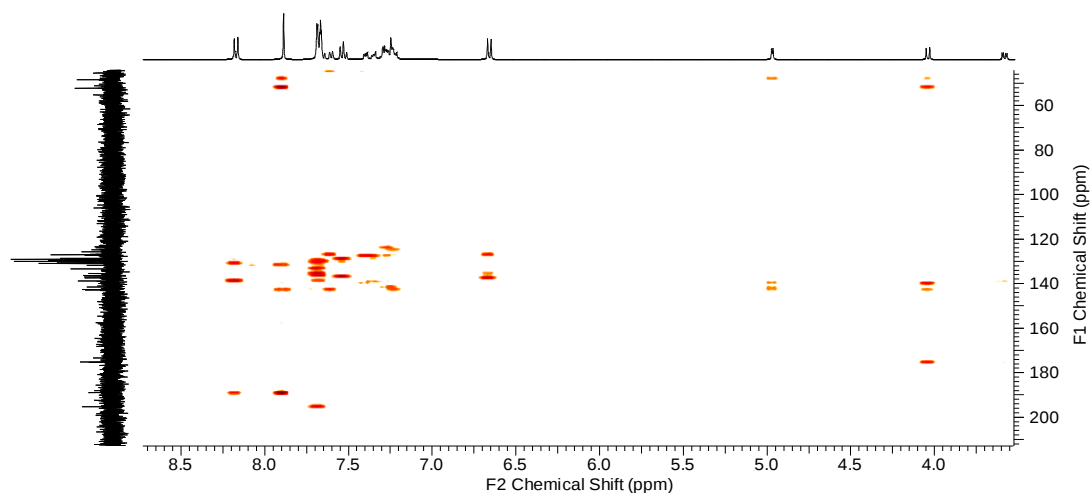


Figure 132: HMBC spectrum of (*E*)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**148**)

The DEPT 90 spectrum (Figure 131) was used to help identify the alkene double bond signals (C27, C28) as well as the C-H aromatic signals of the chalcone-based core (e.g.C12, C13) and benzophenone moiety of the maleimide portion of the molecule (e.g.C42, C44).These assignments were then confirmed by the HSQC spectrum (Figure 132), helping also to identify the remaining quaternary carbon signals such as C23 and C40.The assignment of the remaining quaternary carbon signals was then confirmed through the HMBC spectrum (Figure 133).

The IR spectrum confirmed the structure of (**148**) (Figure 134) and shows absorption bands at 1714.51 cm^{-1} (C=O of α,β -unsaturated ketone), 1777.65 cm^{-1} (C=O groups of the maleimide moiety), 3336.63 cm^{-1} (secondary amide NH stretch), 1673.10 cm^{-1} (secondary amide NH bend), 1584.43 cm^{-1} (C-N stretch), 2927.85, 2965.99, 3066.98 cm^{-1} (aromatic C-H stretch), 1458.28, 1634.80 cm^{-1} (aromatic C=C stretch), 1604.08 cm^{-1} (*trans* C=C stretch) and 1144.75 cm^{-1} (aryl C-Cl).

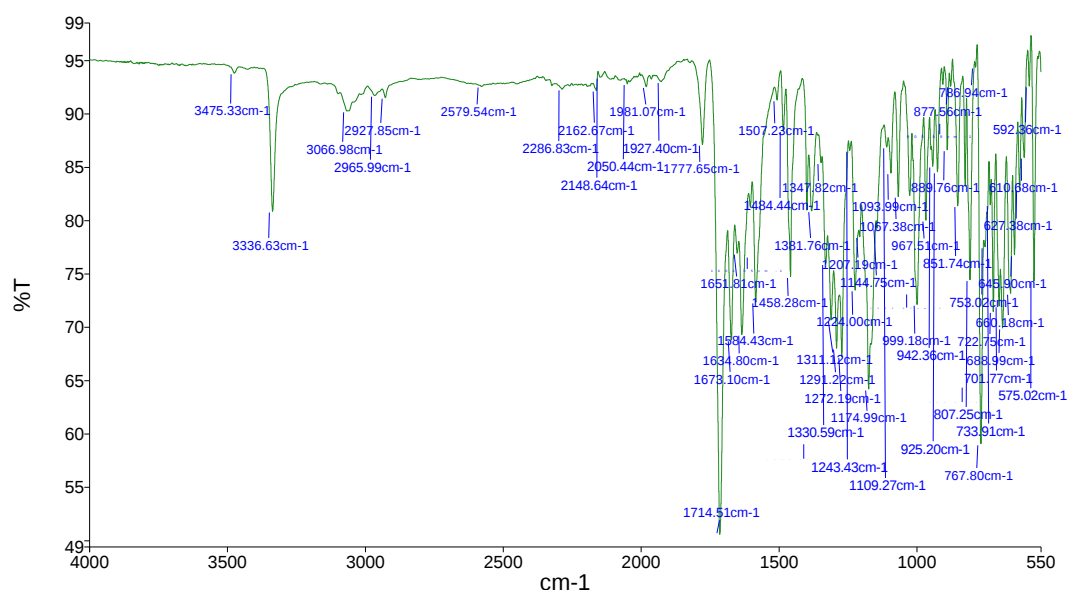


Figure 133: IR spectrum of (*E*)-13-(4-benzoylphenyl)-9-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione (**148**)

2.5.22 Series 8: Synthesis and characterisation of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**)

The synthesis of the dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**) (Figure 134) was carried out utilizing the described Diels–Alder reaction with the previously discussed acetophenone chalcone (**39**) acting as a diene system and dimethyl acetylenedicarboxylate as the dienophile. Acetylene derivatives have been successfully used in Diels–Alder reactions e.g by Dastoorani for the synthesis of new dibenzofuran compounds³⁶⁷. Compound (**154**) allowed for further investigation of the structure-activity relationship of the ethanoanthracene scaffold in CLL. The compound was isolated as a solid after recrystallisation from methanol. Compound (**154**) differs from previous Diels–Alder adducts with a double bond being present on the central carbon bridge structure of the adduct in addition to the presence of two ester functional groups from the bridge head structure. This is in contrast to the heterocyclic ring that is present in series 4-8. The product was obtained as a yellow/orange crystalline solid in a good yield of 62%.

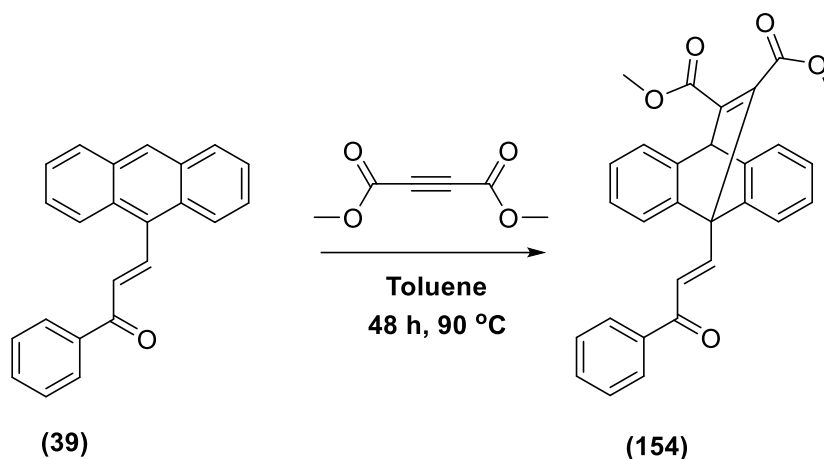


Figure 134: General reaction scheme for the synthesis of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**)

2.5.21 Structural characterisation of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**)

The ^1H NMR (Figure 136) and ^{13}C NMR spectra (Figure 137) will now be discussed. The numbering of the compound (**154**) was generated in ACD/ChemSketch version 12.01.

The ^1H NMR spectrum of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**) (400 MHz, $\text{DMSO}-d_6$) (Figure 136) shows two singlet signals at 3.64 ppm (3H) and 3.72 ppm (3H) which correspond to the methyl ester groups of the ethanoanthracene bridgehead (H21, H23). The next downfield singlet signal at 5.80 ppm refers to H4 of the centre ring of the polycyclic core. The multiplet signal at 7.11 ppm (4H) represents the four protons on the sides of the anthracene core (H8, H9, H14, H15). The multiplet signal at 7.48 ppm (2H) refer to the protons H7 and H16. The multiplet at 7.55 ppm (3H) corresponds to the protons H10 and H13 of the polycyclic core as well as H18 of alkene double bond. The triplet signal appearing at 7.61 ppm ($J = 7.72 \times (2) \text{ Hz}$, 2H) is assigned to the protons H29 and H31 of the acetophenone phenyl ring. The remaining proton of the phenyl ring appears as a doublet signal at 7.71 ppm ($J = 10.88 \text{ Hz}$, 1H), while the adjacent doublet signal corresponds to H17 of the alkene double bond ($J = 16.12 \text{ Hz}$, 1H). The coupling constant indicates conservation of the (*E*) configuration of the double bond ($J > 12 \text{ Hz}$)²⁷⁶. The highest resonating signal in the spectrum is a doublet at 8.08 ppm ($J = 7.8 \text{ Hz}$, 2H) which

corresponds to the proton pair (H28, H32), due to the inductive influence of the conjugated carbonyl functional group.

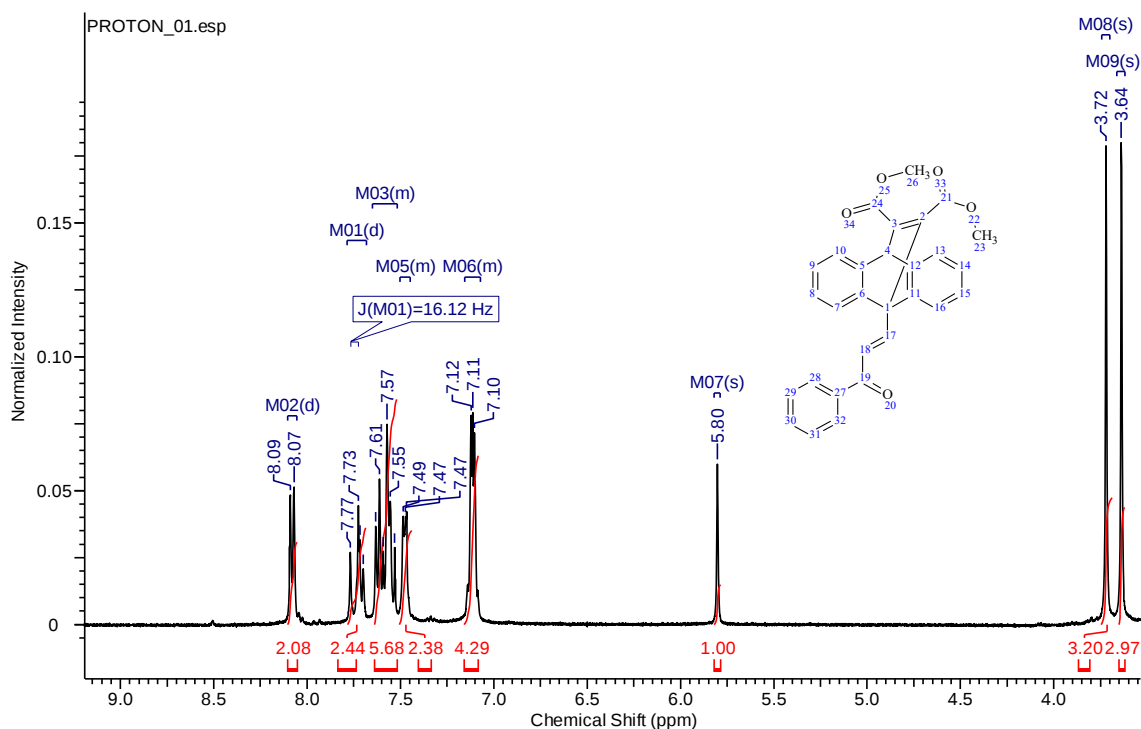


Figure 135: ^1H NMR spectrum of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**)

The ^{13}C NMR (101 MHz, DMSO- d_6) (Figure 137) spectrum of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**) (Figure 112) displays a signal at 49.9 ppm which represents C1 adjacent to the α,β -unsaturated ketone functional group. The signal at 52.9 ppm in the aliphatic region of the spectrum accounts for C4 of the polycyclic core central ring. The remaining two signals in the aliphatic region 53.2 ppm and 57.8 ppm correspond to C2 and C3 of the bridgehead double bond. The carbons C17 and C18 of the α,β -unsaturated ketone refer to the signals 142.6 ppm and 133.4 ppm respectively. The two ester carbonyl groups present at the bridge structure of the molecule are represented by the signals at 163.6 ppm and 167.0 ppm. The most downfield signal at 189.2 ppm corresponds to the carbonyl group of the α,β -unsaturated ketone.

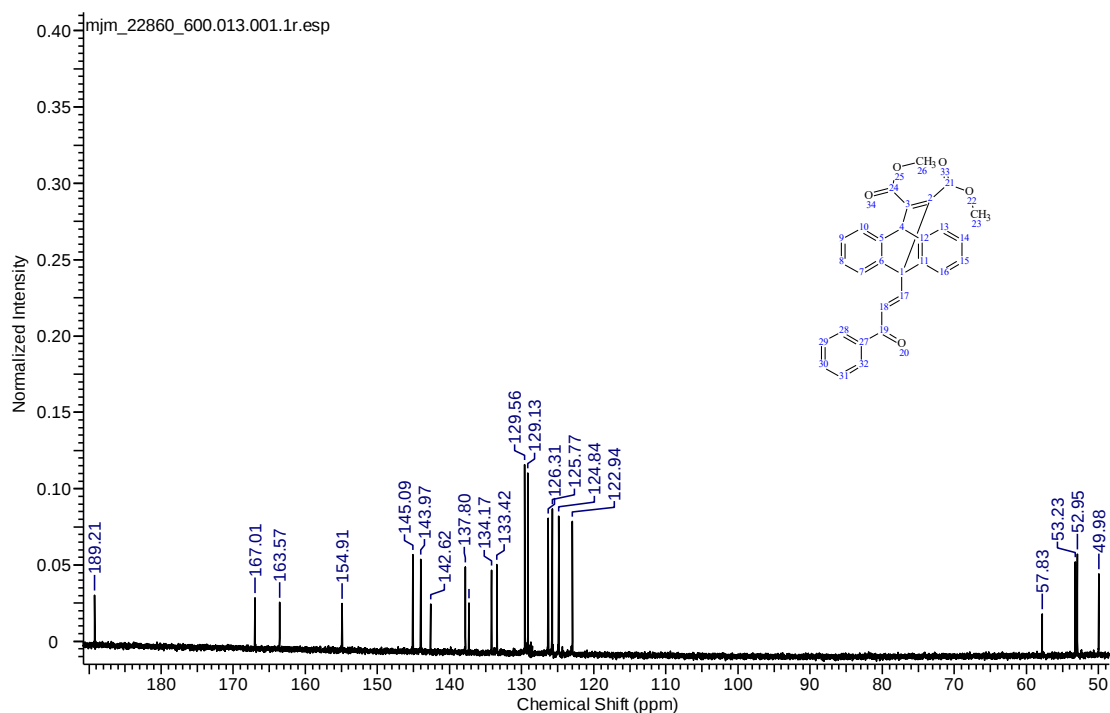


Figure 136: ^{13}C NMR spectrum of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**)

2.5.23 X-ray crystallography analysis of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**)

The formation of *E*/ *trans* configuration alkene double bond of the ethanoanthracene chalcone products was confirmed by NMR structural analysis through associated coupling constants (discussed in structural characterisation example) and by X-ray crystallography of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**). The crystal was found to be monoclinic, with the crystal packing arrangement shown in Figure 138, Figure 139 and Table 31. The bond length of C15-C16 was consistent with that of an alkene double bond (1.3269 Å) and the bond length of O28-C27 consistent with a carbonyl functional group (1.2263 Å)^{344, 345}. The presence of the alkene double bond is also supported through the bond angles of C14-C25-C26 and C27-C26-C25 which were 126.47° and 120.01° respectively, indicating the trigonal-planar nature expected of such a functional group side-chain. The presence of the ester functional groups was supported by the bond lengths of O18-C17 and O22-C21 measuring 1.2073 Å and 1.2021 Å, typical of carbonyl functional groups. In addition, the presence of O23-C24 and O19-C20 measuring 1.45 Å and 1.4464 Å

respectively support ester presence as these bond lengths are typical of C-O bonds. The existence of a double bond at the ethanoanthracene bridgehead reinforced by the bond length of C15-C16 being reported as 1.34 Å, commonly associated with alkene bonds²⁷⁶.

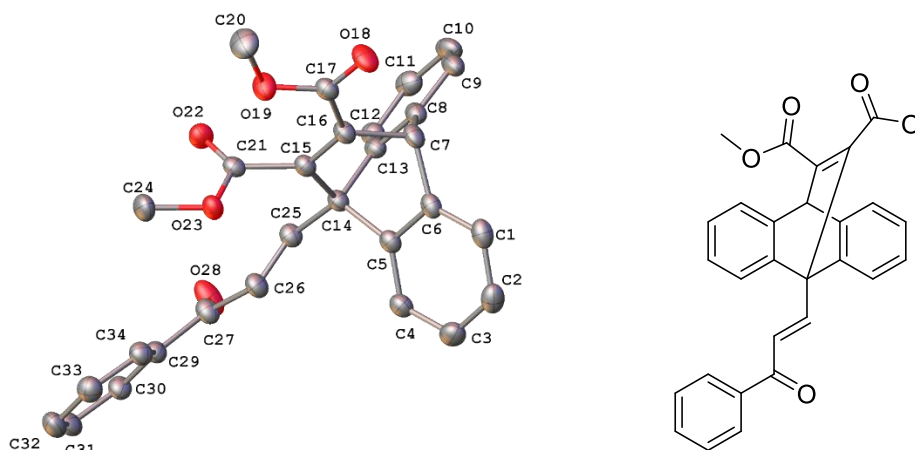


Figure 137: X-ray crystal structure of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (154)

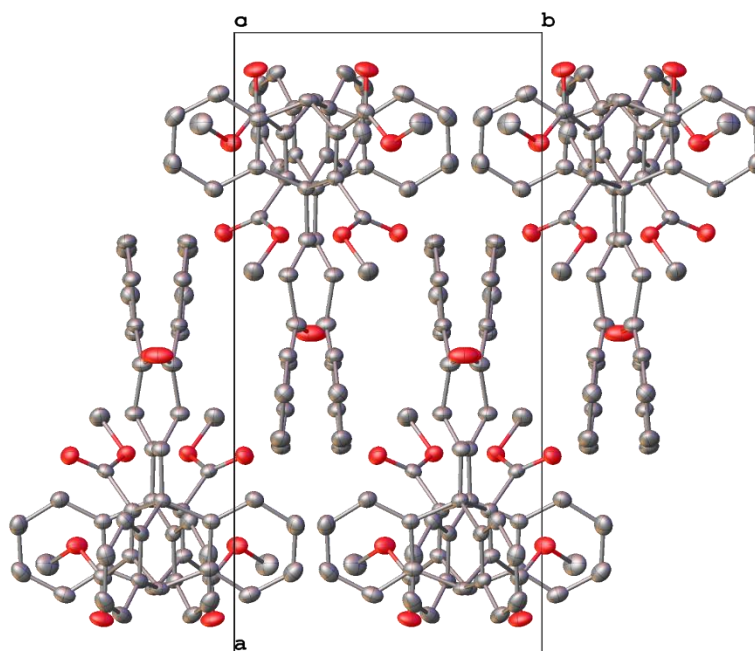


Figure 138: Packing arrangement of dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (154)

Empirical formula	C ₂₉ H ₂₂ O ₅
Formula weight	450.46
Temperature	100(2) K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	P2 ₁ /c
Unit cell dimensions	a = 18.9744(16) Å b = 8.7405(7) Å c = 14.7391(12) Å $\alpha = 90^\circ$ $\beta = 111.108(3)^\circ$ $\gamma = 90^\circ$
Volume	2280.4(3) Å ³
Z	4
Density (calculated)	1.312 Mg/m ³
Absorption coefficient	0.726 mm ⁻¹
F(000)	944
Crystal size	0.36 x 0.17 x 0.14 mm ³
Theta range for data collection	2.496 to 70.208°.
Index ranges	-22 ≤ h ≤ 22, -10 ≤ k ≤ 10, -17 ≤ l ≤ 17
Reflections collected	27728
Independent reflections	4310 [R(int) = 0.0438]
Completeness to theta = 67.679°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7533 and 0.6341
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	4310 / 0 / 310
Goodness-of-fit on F²	1.057
Final R indices [I > 2σ(I)]	R1 = 0.0373, wR2 = 0.1007
R indices (all data)	R1 = 0.0395, wR2 = 0.1027
Extinction coefficient	0.0024(2)
Largest diff. peak and hole	0.218 and -0.215 e.Å ⁻³
Index ranges	-22 ≤ h ≤ 22, -10 ≤ k ≤ 10, -17 ≤ l ≤ 17
Reflections collected	27728
Independent reflections	4310 [R(int) = 0.0438]

Table 31: Crystal data details and structural refinement for dimethyl (*E*)-9-(3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**154**)

2.5.23 Series 9: Synthesis and characterisation of 13,13'-(ethane-1,2-diyl)bis(9-((*E*)-3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione) (**155**)

The dimer 13,13'-(ethane-1,5-diyl)bis(9-((*E*)-3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione) (**155**) (Series 9) (Figure 140) was synthesised utilizing the described Diels–Alder reaction with the previously discussed anthracene chalcone (**39**) (1 Eq.) acting as a diene system and 1,1'-(ethane-1,5-diyl)bis(1*H*-pyrrole-2,5-dione (2 Eq.) as the dienophile (in collaboration with Maria Vera, Universidad de Murcia, Spain). The compound was obtained as a solid after

washing with toluene and diethyl ether on isolation, with no recrystallisation necessary and a very good yield of 76% was obtained. **(155)** was confirmed as a novel compound and has three distinct structural regions consisting of a 1,1'-(ethane-1,2-diyl)bis(pyrrolidine-2,5-dione) bridgehead linker between the two anthracene-derived central cores, which bear an α,β -unsaturated ketone with an unsubstituted aryl ring.

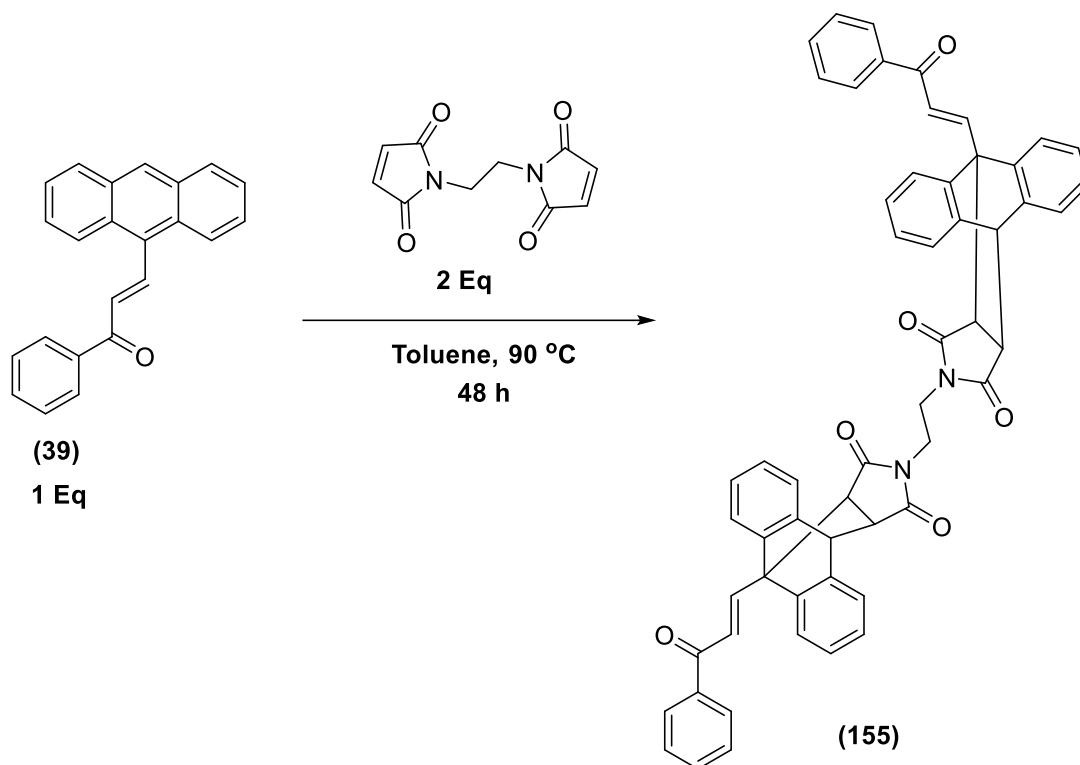


Figure 139: General reaction scheme for the synthesis of 13,13'-(ethane-1,2-diyl)bis(9-((*E*)-3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione) (**155**)

2.5.22 Structural characterisation of 13,13'-(ethane-1,2-diyl)bis(9-((*E*)-3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione) (**155**)

The ^1H -NMR (Figure 141) and ^{13}C NMR spectra (Figure 142) will now be discussed. The numbering of the compound (**155**) was generated in ACD/ChemSketch version 12.01. The ^1H NMR spectrum for 13,13'-(ethane-1,2-diyl)bis(9-((*E*)-3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione) (**155**) (Figure 140) shows a triple doublet at 3.28 ppm ($J=2.93, 1.47$ Hz, 2H), accounting for the protons H8 and H29 of the ethanoanthracene bridge. H3 and H24 of the ethanoanthracene bridge structure appear as a doublet at 3.47 ppm ($J=8.1$ Hz). The proton pair (H7, H28) correspond to the double doublet at 4.77 ppm ($J=2.9$ Hz, 2H)

located on the central ring of the respective polycyclic cores of the dimer molecule. The multiplets that appear at 7.15 ppm (m, 8H) and 7.27 ppm (m, 8H) account for the protons (H13, H14, H17, H18, H34, H35, H38 and H39) of the aromatic rings of the polycyclic cores and the protons (H12, H15, H16, H19, H33, H36, H37 and H40) that occupy the remaining aromatic positions on the rings mentioned. The pair of doublets present at 7.39 ppm (d, $J = 8.07$ Hz, 4H) correspond to H55, H57, H61 and H63 of the acetophenone aromatic ring. The apparent double triplet at 7.55 ppm represents the protons H56 and H62 (m, $J = 4.77, 7.34$ Hz). The double doublet signal at 7.80 ppm ($J = 16.1, 8.1$ Hz) accounts for H53 and H62, *para* to the carbonyl attached to the aromatic ring of the acetophenone functional group. The doublet signal at 7.97 ppm (16.1 Hz, 2H) accounts for H42 and H48 of the alkene double bond groups of the α,β -unsaturated ketone, with the coupling constant being typical of *E* configuration molecules²⁷⁶. The aromatic proton signals of the benzoyl functional group adjacent to the carbonyl group appear as a double doublet at 8.16 ppm ($J = 12.1, 7.7$ Hz, 4H). This represents the most downfield signal of the spectrum due to the inductive influence of the carbonyl group on the aromatic protons, leading to a significant deshielding effect.

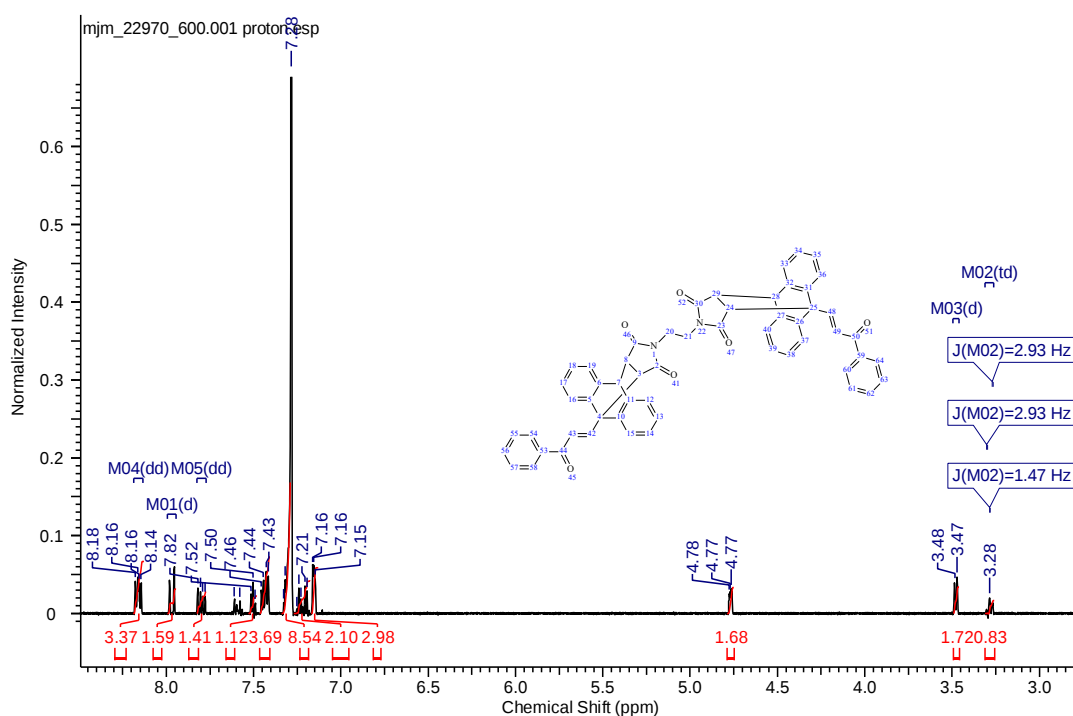


Figure 140: ^1H NMR spectrum of 13,13'-(ethane-1,2-diyl)bis(9-((*E*)-3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione) (155)

The ^{13}C NMR spectrum for 13,13'-(ethane-1,2-diyl)bis(9-((*E*)-3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione) (Figure 142) (**155**) shows C20 and C21 of the ethane dimer linker as a signal at 45.6 ppm, while C8 and C29 of the ethano bridgehead correspond to the signal at 47.9 ppm. The remaining carbons of the ethano structure (C3, C24) appear as a signal at 48.4 ppm. The signal at 48.4 ppm in the spectrum's aliphatic region is that of C7 and C28, which are located on the central ring of the polycyclic anthracene core. The remaining signal at 52.1 ppm is C4, the quaternary carbon on the central ring, adjacent to the chalcone side-chain. The carbon signals of the alkene double bond (C43, C49) and (C42, C48) are observed as signals at 133.2 ppm and 139.3 ppm respectively. The carbonyl groups of the maleimide moieties (C2, C9, C23, C30) are represented by the signals appearing at 174.9 ppm, 175.2 ppm, 175.9 ppm and 176.0 ppm. The signals at 190.1 and 190.0 ppm represent the carbonyls (C44, C50) on the α,β -unsaturated ketone functional groups, resonating the furthest downfield due to the strong inductive effect of the carbonyl dipoles. In addition, the mass of (**155**) was also confirmed with HRMS (MALDI) and the required molecular ion was calculated to be $\text{C}_{56}\text{H}_{40}\text{N}_2\text{O}_6 \text{ Na}$ [$\text{M}^+ + \text{Na}$]: 859.2784 and found as 859.2796.

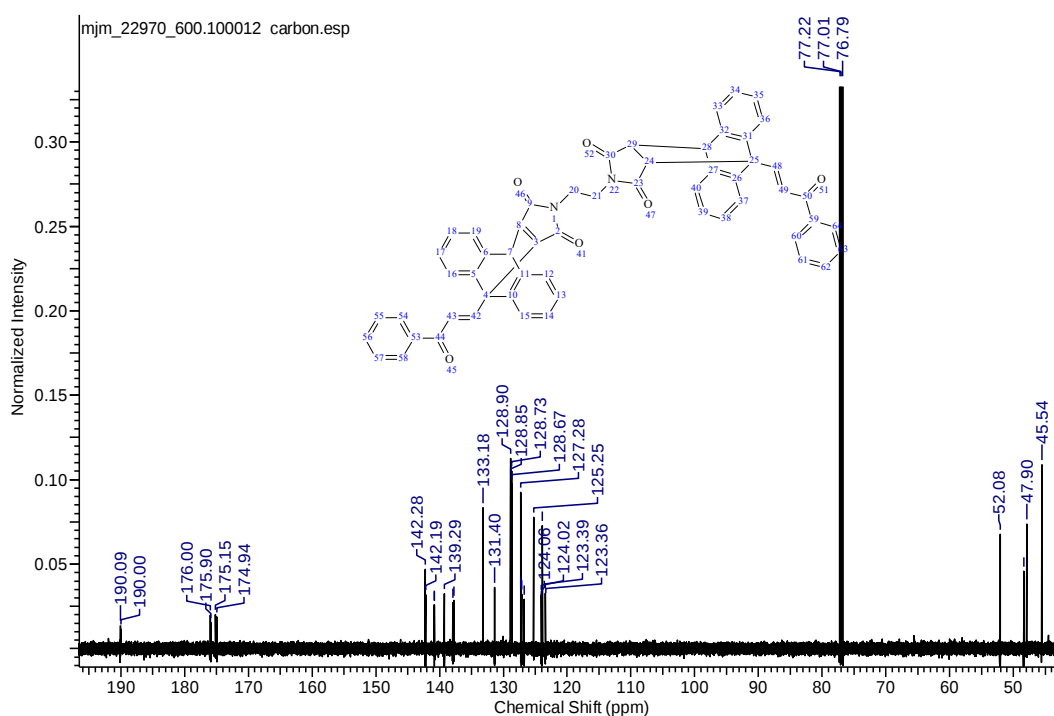


Figure 141: ^{13}C NMR spectrum of 13,13'-(ethane-1,2-diyl)bis(9-((*E*)-3-oxo-3-phenylprop-1-en-1-yl)-9,10-dihydro-9,10-[3,4]epipyrroloanthracene-12,14-dione) (**155**)

2.6 Drug Stability

The stability of representative examples of the compounds synthesised in the previous section was now investigated. Stability is defined by the ability of a given drug product or drug substance to remain within strictly established parameters of potency, identity and purity during a specified time period [for example-shelf life or t_{90} -the time taken for the original potency of the active principle (100%) to drop by 10%]³⁶⁸. Apart from chemical stability (dealing with the impact drug potency), therapeutic (ability to provide therapeutic efficacy), toxicological (potential for toxic degradant development), microbial (resistance to microbial growth) and physical stability (uniformity of formulation/drug characteristics e.g.dissolution) are also of great importance in the guarantee and maintenance of adequate product quality³⁶⁹.

The guidelines used in relation to drug substance/product manufacture in Europe, US and Japan are provided by the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). With specific reference to the stability assessment of new drug substances and products, ICH Q1A (R2) details the relevant methods, validation, acquisition and subsequent data analysis standards necessary to meet regulatory requirements. The singular goal of these tests is to establish the effect of various environmental conditions e.g.temperature, humidity on the quality of the product as well as the establishment of core practical parameters such as the shelf-life³⁷⁰.

2.6.1 HPLC method development

HPLC method development is a key step required in any drug development/discovery analytical process involving the variation of components, conditions and parameters to find the most suited, streamlined method and optimal conditions for individual product detection and analysis³⁷¹. This involves exploration of the three main variable components of any HPLC method: sample preparation (chemical nature of sample, pH, % organic solvent), HPLC conditions (flow rate, % organic solvent, temperature, column specifications) and standardization (peak integration, detection wavelengths, standard

concentrations). In addition, other important considerations in method development include run speed, specificity for intended application and resolution power³⁷².

For this particular analysis, a classic isocratic method of method screening was used (acetonitrile/water), beginning with 100% acetonitrile (ACN) as the mobile phase and subsequently reducing the proportion of acetonitrile by 5%, while increasing the proportion of 0.1% trifluoroacetic acid (TFA) in water by the same amount. The separation was assessed at each isocratic step and the optimal mobile phase, run time and flow rate identified for each respective compound class.

2.6.2 Linearity determination

In terms of method validation, one of the major steps is to ensure method linearity for the analyte of interest. Linearity is defined as the ability of an analytical system to determine the analyte concentration in a test sample where concentration is directly proportional to the response detected (usually only applicable within a certain concentration range)³⁷³.

This validation parameter is verified through the use of a calibration curve whereby a series of known concentrations of the analyte are graphed on the x-axis while the detection response of the instrument is graphed on the y-axis. These should also consist of zero sample to rule out contamination and ensure an accurate detection response to increasing analyte concentration. From the resulting points, a best-fit line is determined via linear regression and a subsequent coefficient of determination is calculated, giving a specific R^2 as a result.

In this study the stability of the products **(24)** and **(99)** were determined first by plotting calibration curves (Figure 143, 144) with the concentration of sample (mg/mL) on the x-axis and peak area (arbitrary units) on the y-axis. A stock solution of 1mg/mL in ACN for compounds **(24)** and **(99)** are diluted to various concentrations of 0.1mg/mL, 0.2mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL, 0.6 mg/mL, 0.7 mg/mL, 0.8 mg/mL and 0.9 mg/mL. On consideration of the R^2 values of both calibration curves (0.9936, 0.9954), the values were sufficiently high to confirm an acceptable fit of the experimental data to the calculated regression line for progression of the developed HPLC method into stability studies. These studies were conducted in triplicate.

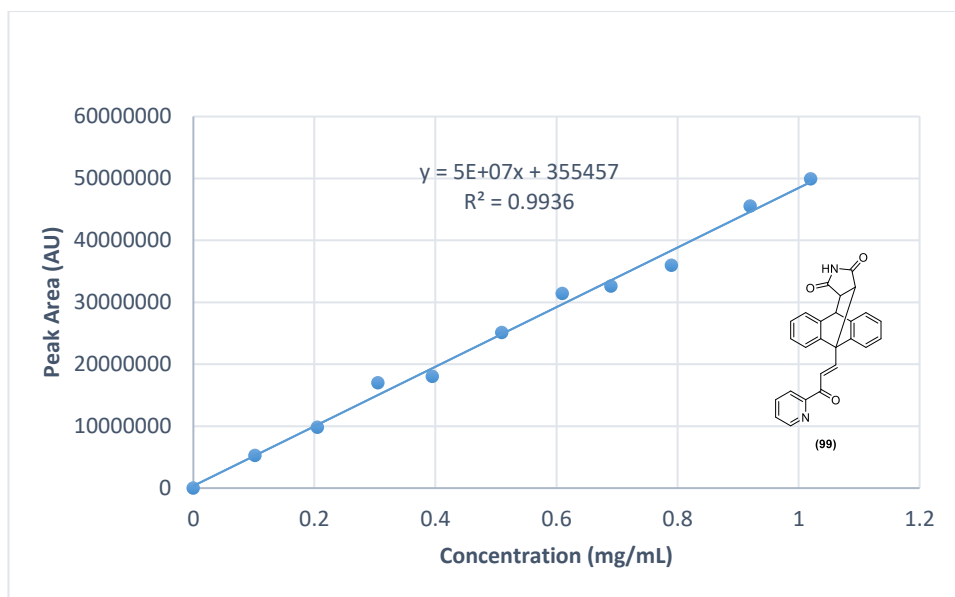


Figure 142: Compound (99) Calibration curve

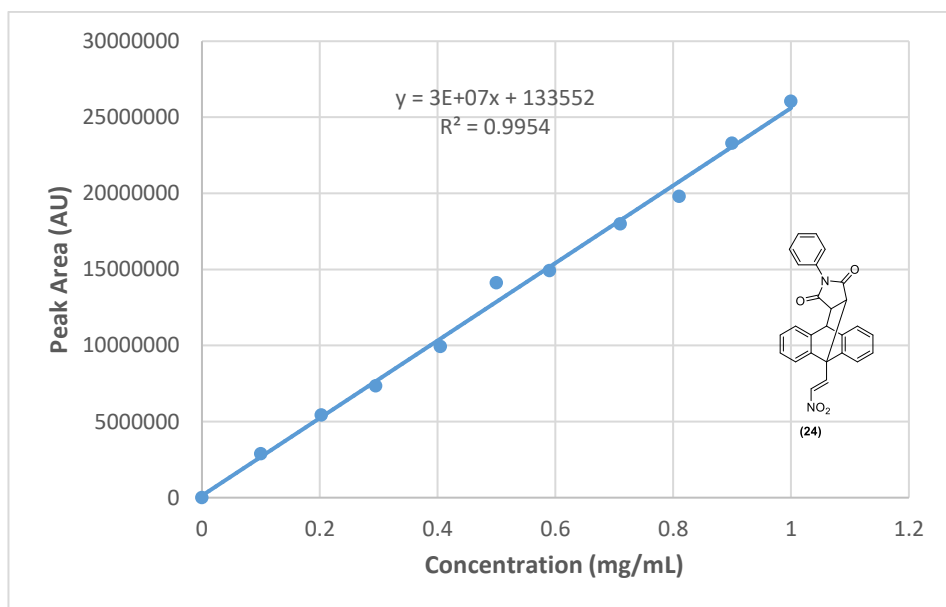


Figure 143: Compound (24) Calibration curve

2.6.3 Stability study of compounds (24) and (99) in phosphate buffers

Compounds (24) and (99) (Figure 147) were used for stability studies and were subjected to different pH conditions at a physiologically relevant temperature of 37 °C for a period of 24 hours. The pH chosen for each of the phosphate buffers are characteristic pH values which would be found in the body. In mimicking these pH values using a selection of specific phosphate buffers, compound stability *in vivo* can be predicted. The pH values

chosen were pH 4, pH 7.4 and pH 9 as this range mimics the pH conditions of the stomach (pH 2-4), intestine (pH 8-10) and blood (pH 7.4), giving a valuable insight into how a given compound may behave in each body compartment. As varying pH conditions are used spanning from acidic to basic, varying degradation pathways can occur depending on both the compound and the buffer used. This could be seen through the appearance and growth of other unidentified peaks as the study progressed, inferring the formation of degradants. These products were not investigated further.

In conducting these studies the chemical stability of the tested drugs were assessed and this information could be utilized in pre-clinical formulation development allowing for minimization of degradation at specific pH values, promotion of drug stability and optimum pharmacological activity, without being too robust as to affect the excretion process. The 24 h stability study was carried out in triplicate in order to ensure accurate results. The results will now be discussed according to buffer pH and compound (Figure 145, 146).

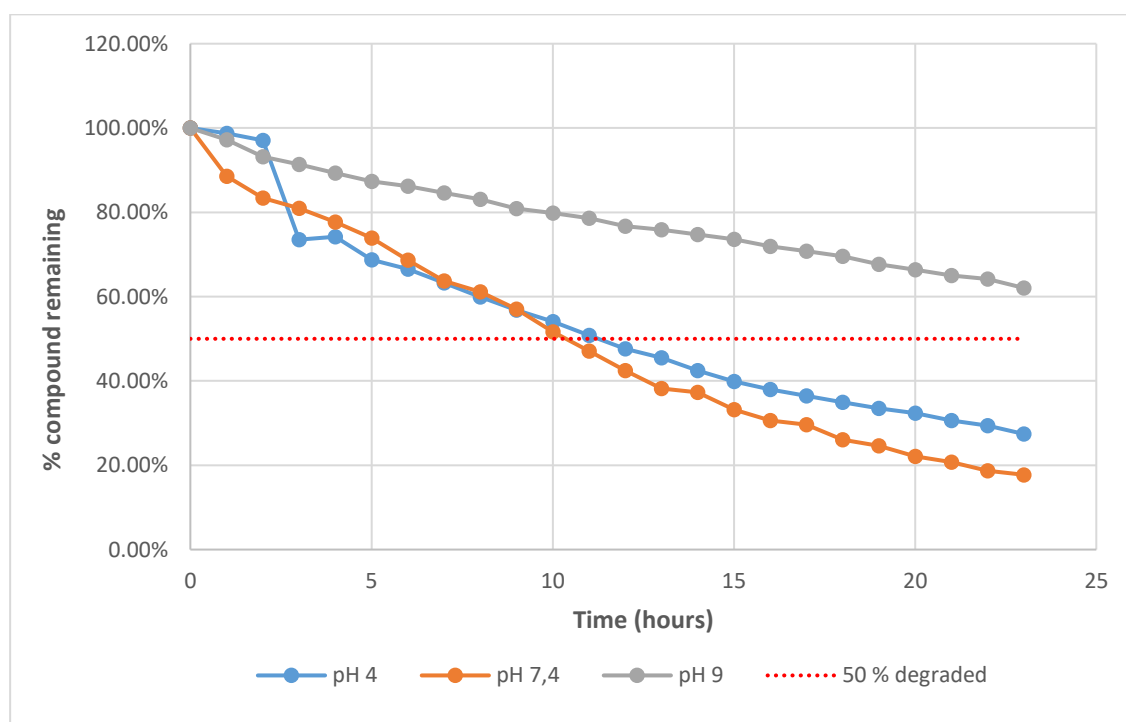


Figure 144: Stability testing of compound (24) at pH 4.0, 7.4 and 9.0 over 24 h

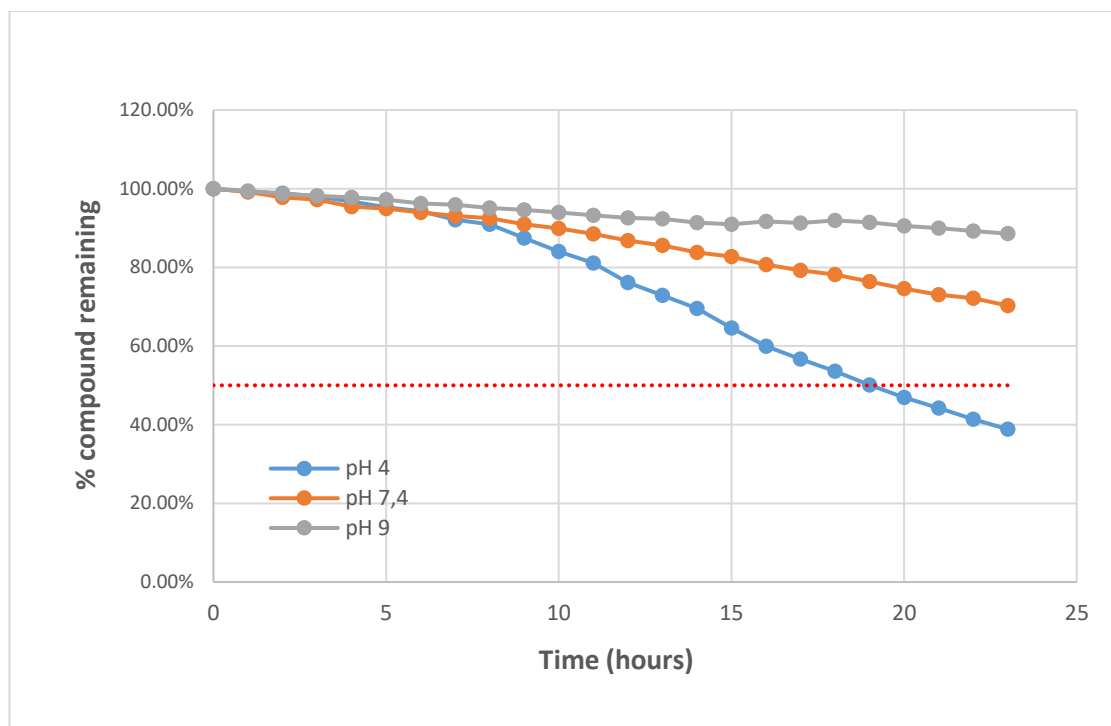


Figure 145: Stability testing of compound (99) at pH 4.0, 7.4, and 9.0 over 24 h

Compound (24)

At pH 4.0, compound (24) (Figure 147) shows two discernible plateaus in degradation (Figure 145). The first occurs from approximately 0-2 h where minimal breakdown of the drug substance occurs. Between hours 2 and 3, a relatively rapid loss of 20% of original drug content was noted, after which the stability plateaus for a second time between hours 3 and 4. Beyond hour 4, a stable and gradual decreasing trend was observed with the half-life of compound (24) being 11 hours at pH 4. At pH 7.4, (24) was observed to undergo relatively consistent degradation, maintaining a steady downwards trend throughout the test. The resultant half-life of (24) noted at pH 7.4 was 10 hours. At pH 9.0, the rate at which the degradation of (24) occurred was noticeably slower than the other two pH values, evidenced by the shallow slope of the reaction's trend-line. The half-life of (24) at pH 9.0 was unable to be determined within the 24 hour test period, hence it was estimated to be at least >24 hours.

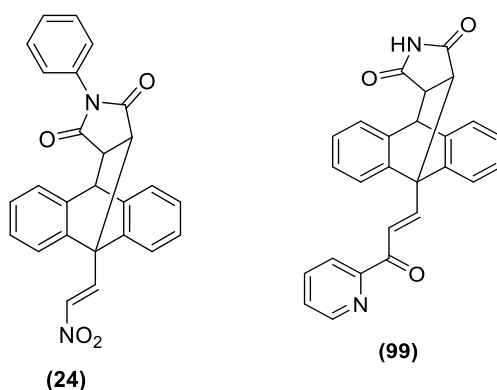


Figure 146: Chemical structures of compounds (24) and (99)

Compound (99)

At pH 4.0, the rate at which compound **(99)** (Figure 147) degrades is minimal up to hour 8, beyond this point degradation occurs as a steady decrease, with the half-life of 19 hours (Figure 146). At pH 7.4, the degradation rate is similar to that of pH 4.0, with a small quantity being converted within the first 8 hours. However, beyond this point, degradation takes place at a far slower rate at pH 7.4, with a more gradual trend downwards in % compound remaining. The half-life of **(99)** at pH 7.4 was unable to be determined within the 24 hour test period, hence it was estimated to be at least >24 hours, extending moderately beyond this point given its more gradual reaction course. At pH 9.0, the rate at which breakdown occurs is again similar to the other pH environments, however, from hour 4 onwards, the reaction departs from the other two, remaining more stable, the difference becoming very noticeable beyond hour 9. The total % drop in compound concentration over the course of 24 hours only amounts to approximately 10%. Therefore, determination of the reaction half-life for **(99)** at pH 9.0 was not possible within 24 h due to the gradual reaction rate at which the drug degraded. Furthermore, judging by the time needed for a 10% decrease in compound concentration, coupled with the reaction rate, the half-life would far exceed 24 hours.

In summary, for compound **(99)**, it was concluded that it was most stable in pH 9.0 (62.1 % drug remaining) as opposed to 27.4 % remaining at pH 4.0 and 17.7 % remaining at pH 7.4 after 24 hours. Similarly, compound **(99)** was found to be the most stable in pH 9.0 (88.6% drug remaining) in comparison with 70.3% remaining at pH 7.4 and 38.9% remaining at pH 4.0 after 24 hours. Compounds **(24)** and **(99)** both contain the substituted pyrrolidine-2,5-dione heterocyclic structure. In addition, compounds **(24)** contains a nitrovinyl functional group at C9 of the anthracene core, while compound **(99)** contains

an α,β -unsaturated ketone system located at C9 of the anthracene core, derived from the chalcone. Some potential degradation reactions will now be briefly discussed.

A possible degradation route for both compounds **(24)** and **(99)** could be through the hydrolysis of cyclic imide of the maleimide based functional group initiated through nucleophilic attack of the carbonyl δ^+ carbon, leading to the resultant amic acid product³⁷⁴ (Figure 148). This could then potentially degrade further to liberate a dicarboxylic acid and corresponding primary amine. Degradation products of nitrostyrene containing compounds such as **(24)** have also been reported^{375, 376}. For chalcone compound **(99)** acid or base catalysed retro-aldol reactions are possible³⁷⁷. The base catalysed retro-aldol degradation of compound **(99)** leading to the formation of its corresponding aldehyde and ketone shown in (Figure 149).

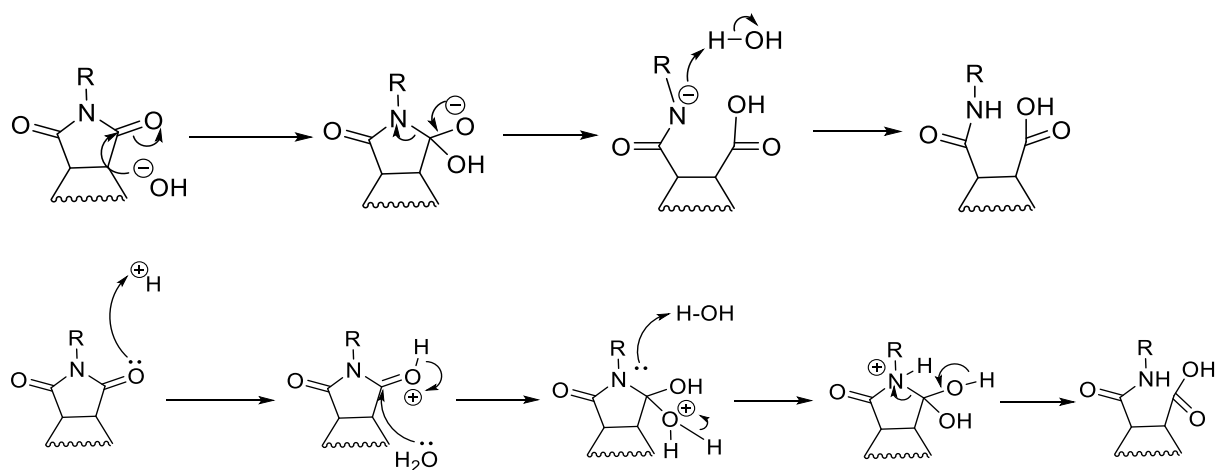


Figure 147: Potential base (top) and acid (bottom) catalysed hydrolysis of the pyrrolidine-2,5-dione in **(24)** and **(99)**

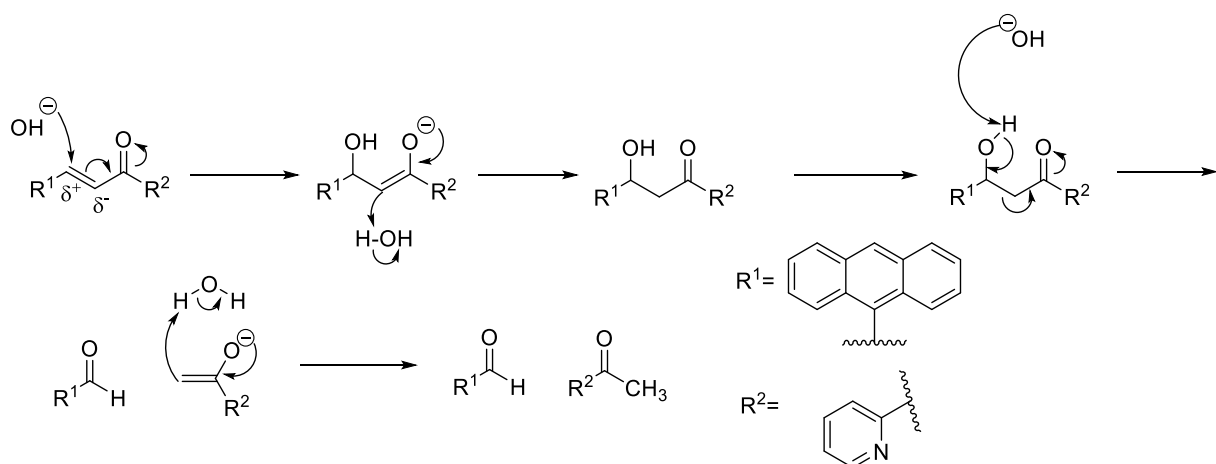


Figure 148: Base retro-aldol degradation of chalcone-based compound **(99)**